Lipase-Catalyzed Resolution of Glycerol 2,3-Carbonate

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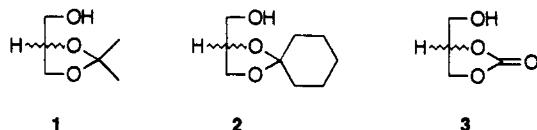
Received October 26, 1993 (Revised Manuscript Received January 28, 1994®)

Glycerol 2,3-carbonate was resolved *via Pseudomonas fluorescens* lipase catalyzed acetylation of the racemate and subsequent alcoholysis of the optically enriched acetate product in the presence of the same enzyme. The two reactions, performed in $CHCl_3$, gave (*R*)- and (*S*)-glycerol 2,3-carbonate respectively in high chemical and optical yields.

Introduction

Optically active 2,3-O-substituted glycerols are extensively used as chiral synthons to prepare a large variety of enantiomerically pure compounds of great biological interest.[1] Their pronounced versatility is due to the presence, on a C_3 unit, of the primary OH group and protected vicinal diol functionality.

Whereas optically active 2,3-*O*-isopropylideneglycerol (1) and 2,3-*O*-cyclohexylideneglycerol (2) have been widely investigated and used, there are few reports on optically active glycerol 2,3-carbonate (3). The use of this chiral building block is limited to those preparations that involve selective removal of the diol protection under basic conditions because of the presence of an acid-sensitive moiety. An example of such a strategy is the synthesis of the enantiomeric trityl derivatives of glycerol, intermediates in the preparation of unsaturated lipids.[2]



Likely, the use of this chiral synthon is limited by poor availability. In fact, both enantiomers of 3 are obtained from "Chiral Pool" through tedious multistep processes.[2-6] On the contrary, the racemic carbonate is readily accessible from glycerol by treatment with diethyl carbonate in the presence of a catalytic amount of NaOH.[7]

On this basis we have considered the resolution of the racemate the method of choice for the preparation of both (*S*)-3 and (*R*)-3. Quite surprisingly this classic approach had not been investigated up to now. Previously we had found an easy procedure to obtain (*R*)-1, (*S*)-1, (*R*)-2, and (*S*)-2 by resolution of the corresponding hydrogen phthalates with (*S*)- and (*R*)-1-methylbenzylamine (MBA).[8] Prompted by those results, we have considered the possibility of applying the same methodology to the resolution of 3. Indeed optically active MBA has proved effective in resolving the racemic hydrogen phthalate, prepared by reaction of 3 with phthalic anhydride in pyridine. (*S*)-3 and (*R*)-3, however, could not be recovered from the corresponding resolved hydrogen phthalates, the ester function not being selectively removable. The unexpected obstacle led us to rely on the use of enzymes. After dropping the initial project of removing the phthalic portion by enzyme-catalyzed hydrolysis, we adopted an alternative strategy, based on direct enzymatic resolution of 3.

Recently, lipases have been used as catalysts in kinetic resolutions because they are readily available, stable, quite inexpensive, and require no cofactor. Prompted by the reports on resolutions of racemic alcohols and esters affected by these enzymes in organic solvents *via* enantioselective transesterification in the presence of an acyl donor and *via* enantioselective alcoholysis respectively,[9-12] we planned to use lipases for partially transesterifing 3 and, after separation of the residual unreacted substrate, reconverting the ester product to the starting alcohol. The two combined reactions, transesterification and alcoholysis, can act in a complementary way for the final resolution, allowing us to obtain both optically pure enantiomers of 3 in high yields only on the following conditions: sufficiently high enantioselectivity ($E \geq 10$) of both processes and preferential reaction of the enzyme with the same enantiomer in both transesterification and alcoholysis.

It is to be noted that whereas the transesterification is accomplished on racemic alcohol 3 and the fraction of interest is the unreacted alcohol, in the alcoholysis the fraction of interest is the product, whose optical purity drastically decreases as conversion increases. The reconversion into alcohol, however, can be extended without pronounced expense of optical yield, since the substrate is already optically enriched, resulting from the enantioselective transesterification.

Results and Discussion

Racemic glycerol 2,3-carbonate (3) was readily synthesized from glycerol by a standard method.[7] Initial attempts at enantioselective acylation were performed, exposing 3

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® Abstract published in *Advance ACS Abstracts*, March 1, 1994.

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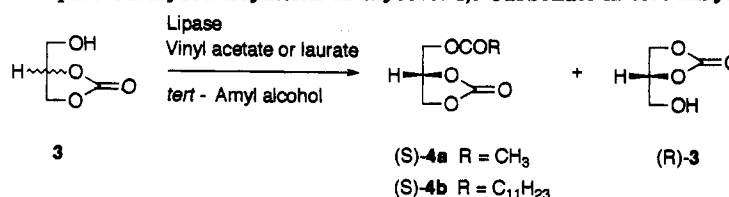
0022-3263/94/1959-1751$04.50/0 © 1994 American Chemical Society

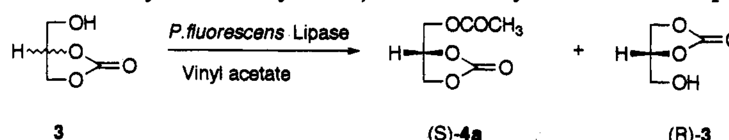
Table 1. Lipase-Catalyzed Acylation of Glycerol 2,3-Carbonate in *tert*-Amyl Alcohol[a]



entry	acylating agent	lipase source	recovered substrate yield,[c] %	$[\alpha]^{20}_D$,[d] deg	op,[e] %	abs conf	E[b]
1	vinyl acetate	*M. miehei*[f]	46	+10.6	24.3	*R*	1.9
2	vinyl acetate	porcine pancreas[g]	40	+18.5	42.4	*R*	2.6
3	vinyl acetate	*C. cylindracea*[h]	0				
4	vinyl acetate	*R. arrhizus*[i]	0				
5	vinyl acetate	*P. fluorescens*[j]	40	+31.2	71.6	*R*	5.8
6	vinyl laurate	porcine pancreas[k]	48	+4.8	11	*R*	1.4
7	vinyl laurate	*P. fluorescens*[l]	46	+2.8	6.4	*R*	1.2
8	vinyl laurate	*M. miehei*[m]	42	+3.3	7.6	*R*	1.2

[a] Conditions: substrate (8.5 mmol), vinyl acetate or laurate (28.3 mmol), lipase, *tert*-amyl alcohol (30 mL). [b] Calculated according to ref 13 and assuming optical purity as ee and (1 − yield) as *c*. [c] Isolated yield resulting from flash chromatography (entries 1–5) or hexane–water extraction (entries 6–8). [d] *c* 1.9, dioxane. [e] Optical purity (op) calculated by comparison with the maximum specific rotation $[\alpha]^{20}_D$ +43.6 given in the literature for the *R* isomer (see ref 2). [f] Lypozime TM (NOVO): 100 mg; 40 °C for 3 h and then 25 °C for 15 h. [g] Lipase from Sigma: 5 mg (supported on 15 mg of Celite); 8 °C for 2 h. [h] Lipase from Fluka: 270 mg; 5 °C for 3 h, then gradually raised to 40 °C during 24 h. TLC analyses show the only unreacted substrate. [i] Lipase from Fluka: 2.4 g; conditions and TLC responses as in the above note. [j] Lipase from Fluka: 200 mg; 5 °C for 1.5 h. [k] Lipase from Sigma (see note *g*): 5 mg; 5 °C for 1 h. [l] Lipase from Fluka: 200 mg; 5 °C for 1 h. [m] Lypozyme TM: 100 mg; 25 °C for 1 h.

Table 2. Acetylation of Glycerol 2,3-Carbonate by *P. fluorescens* Lipase[a]



entry	solvent	product yield,[d] %	product ee,[e] %	product abs conf	recovered substrate yield,[d] %	$[\alpha]^{20}_D$,[f] deg	op,[g] %	abs conf	c[b]	*E*[c]
1	THF	24	66.0	*S*	70	+11.2	26	*R*	28	6.2
2	i-Pr_2O	52	60.0	*S*	40	+33.3	76.4	*R*	56	8.8
3	$CHCl_3$	55	62.4	*S*	40	+41.0	94	*R*	60	14.3
4	$CHCl_3$	54	65.0	*S*	40	+40.6	93	*R*	59	15.7
5	$CHCl_3$	51	65.4	*S*	39	+39.4	90.4	*R*	58	14.3

[a] Conditions: substrate (8.5 mmol), vinyl acetate (28.3 mmol), lipse from *Pseudomonas fluorescens* (200 mg), solvent (30 mL), 4 °C for 1 h (THF and i-PrO_2) or 7 h ($CHCl_3$). [b] Calculated from the optical purity of the recovered substrate and the enantiomeric excess of the product according to ref 13. [c] Calculated from the computed *c* value and the enantiomeric excess of the product according to ref 13. [d] Isolated yield after flash chromatography. [e] Determined by ^1H NMR in the presence of Eu(hfc)$_3$. [f] *c* 1.9, dioxane. [g] Optical purity calculated by comparison with the maximum specific rotation $[\alpha]^{20}_D$ +43.6 given in the literature for the *R* isomer (see ref 2).

to five different lipases in *tert*-amyl alcohol in the presence of vinyl acetate. As shown by the results compiled in Table 1, very low enantioselectivities were observed with the exception of the *Pseudomonas fluorescens* lipase (PFL) catalyzed reaction (entry 5). The screened enzymes preferentially utilized the *S* enantiomer. Consequently the ester product was enriched in the *S* form and the remaining alcohol in the *R* form. In the presence of lipases from *Candida cylindracea* and *Rihzopus arrhizus* (entries 3 and 4) the reaction did not proceed as well as in control experiments performed without the enzyme.

To separate the unreacted alcohol (*R*)-**3** from the ester more easily, vinyl acetate was replaced by vinyl laurate (entries 6–8). Indeed the resulting dodecylate (*S*)-**4b** could be isolated by simple extraction in hexane. The residual (*R*)-**3**, however, showed very low optical activity in spite of conversion degrees (*c*) greater than 50%.

PFL, which had exhibited the highest enantioselectivity in preliminary experiments, was selected for further evaluation. In addition to initially employed *tert*-amyl alcohol (ϵ = 5.82; log *P* = 0.9), we have considered the use of other solvents in the acetylation reaction: THF (ϵ = 7.58; log *P* = 0.5), diisopropyl ether (ϵ = 3.88; log *P* = 1.9), and $CHCl_3$ (ϵ = 4.81; log *P* = 2). The choice of these four solvents was made on the basis of the following features: (a) a moderate polarity (as indicated by the ϵ values[14]), suitable for a complete or partial dissolution of the substrate, (b) a lipophilicity (represented by the partition coefficients between 1-octanol and water, log *P*[15]) compatible with enzymatic activity, (c) the representativeness for different classes of solvents (alcohols, ethers, and halogenated solvents). As shown by the results listed in Table 2, replacement of *tert*-amyl alcohol with THF and diisopropyl ether produced only slight increases in enantioselectivity (entries 1 and 2). On the contrary, the acetylation in $CHCl_3$ proceeded with a significantly enhanced enantioselectivity (*E* = 14.3; entry 3).

To confirm the above result and more precisely evaluate the degree of enantioselectivity, the acetylation was repeated twice in the same solvent (entries 4 and 5). *c* values were calculated from the optical purity of residual alcohol and the enantiomeric excess of the ester product,

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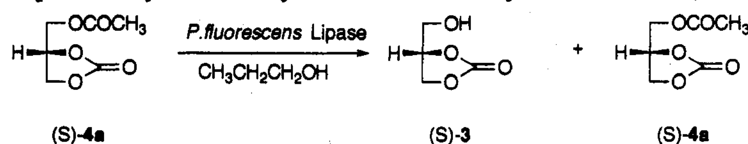
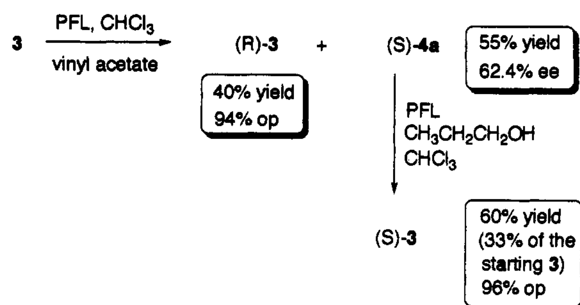
Table 3. Lipase Catalyzed Alcoholysis of *S*-Enriched Glycerol 1-Acetate 2,3-Carbonate[a]



entry	solvent	lipase source	ee,[b] %	abs conf	product yield,[d] %	product $[\alpha]^{20}_D$,[e] deg	product op,[f] %	product abs conf	recovered substrate yield,[d] %	recovered substrate ee,[g] %	recovered substrate abs conf	E[c]
1	*tert*-amyl alcohol	*M. miehei*[h]	65	*S*	71	−34.4	79	*S*	25.5	40.4	*S*	2.6
2	*tert*-amyl alcohol	*P. fluor.*[i]	62.4	*S*	87.5	−31.1	71.3	*S*	10.4	5.2	*S*	2.3
3	$CHCl_3$	*P. fluor.*[j]	62.4	*S*	60	−41.8	96	*S*	37	13	*S*	19

[a] Conditions: substrate (3 mmol), *n*-propanol (1.35 mL), solvent (25 mL). [b] Enantiomeric excess of the substrate before the alcoholysis determined by ^{1}H NMR in the presence of Eu(hfc)$_3$. [c] Calculated from *c*, the initial ee of the ester and the optical purity of the product according to ref 13 and assuming the product yield as *c* and the optical purity as the ee of the product. [d] Isolated yield after flash chromatography. [e] *c* 1.9, dioxane. [f] Optical purity calculated by comparison with the maximum specific rotation $[\alpha]^{20}_D$ +43.6 given in the literature for the *R* isomer (see ref 2). [g] Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$. [h] Lipozyme TM: 170 mg; 25 °C for 24 h. [i] Lipase from Fluka: 90 mg; 25 °C for 4 h. [j] Lipase from Fluka: 90 mg; 25 °C for 16 h.

Scheme 1



determined *via* ^{1}H NMR in the presence of Eu(hfc)$_3$ as a chiral shift reagent. The computed *c* values were consistent with the isolated yields of (*R*)-**3** and the resulting enantioselectivities were fairly constant (entries 3–5).

Enriched (*S*)-**4a** was then reconverted into the corresponding alcohol **3** through enzymatic alcoholysis in the presence of an excess of *n*-propanol. Again, lipases reacted preferentially with the *S* enantiomer, allowing us to obtain **3** in the *S* form. In particular, the reaction catalyzed by PFL in $CHCl_3$ proceeded with the highest enantioselectivity (E = 19; entry 3), consistent with the trend observed for the acetylation of **3**, giving (*S*)-**3** with 96% ee and in 60% yield (33% of the starting **3**).

In summary, this paper describes an efficient enzymatic approach to the simultaneous preparation of (*R*)-**3** and (*S*)-**3** in high optical and chemical yields (Scheme 1). In fact, this two-step resolutive process allows us to obtain (*R*)-**3** and (*S*)-**3** from readily available **3** through PFL-catalyzed transesterification and succesive alcoholysis in 40% and 33% chemical yield and 94% and 96% optical purity, respectively. In both transformations the enzyme shows similar moderate enantioselectivities and unchanged stereochemical preference; the above-mentioned conditions for a successful recycling strategy are so fulfilled. Furthermore, the present results confirm the importance of a proper choice of the lipase, the acylating agent, and the solvent. In particular, the latter plays a crucial role in determining the enantioselectivity of lipase-catalyzed reactions. A precise explanation of this influence cannot be given at present for lack of a theoretical basis. A recently reported study[16] on the influence of the solvent on the enantioselectivity of PFL-catalyzed transesterifications shows the dependence of E on both the hydrophobicity (represented by log P) and the polarity (as indicated by ϵ) of the solvent. In this context our results, although obtained with a limited series of solvents, suggest that the favorable influence of chloroform may be due to its peculiar property of high hydrophobicity combined with a good polarity.

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Experimental Section

^{1}H NMR spectra were recorded at 200 MHz. Lipases from *P. fluorescens*, *R. arrhizus*, and *C. cylindracea* were purchased from Fluka. Lipase from *Mucor miehei* (Lipozyme TM) and porcine pancreatic lipase were purchased from Novo and Sigma, respectively.

***P. fluorescens* Lipase-Catalyzed Acetylation of 3 in $CHCl_3$.** A mixture of **3** (1 g, 8.5 mmol), vinyl acetate (2.6 mL, 28.3 mmol), and *P. fluorescens* lipase (SAM-2) (200 mg) in $CHCl_3$ (30 mL) was stirred for 7 h at 4 °C. After filtration the solvent was removed in vacuo. Flash chromatography (hexane–ethyl acetate 4:6) of the residue afforded (*S*)-**4a** (0.75 g, 55%) and unreacted (*R*)-**3** (0.4 g, 40%) as colorless oils. **(*R*)-3**: $[\alpha]^{20}_D$ +41.0° (*c* 1.9, dioxane) [lit.[2] $[\alpha]_D$ +43.6° (*c* 1.9, dioxane)]; ^{1}H NMR (DMSO) δ 3.55 (m, 1 H), 3.7 (m, 1 H), 4.3 (dd, 1 H), 4.55 (pseudo t, 1 H), 4.85 (m, 1 H), 5.3 (t, 1 H). **(*S*)-4a**: 62.4% ee [determined by ^{1}H NMR in $CDCl_3$ in the presence of Eu(hfc)$_3$]; ^{1}H NMR (DMSO) δ 2.1 (s, 3 H), 4.2–4.4 (m, 3 H), 4.6 (pseudo t, 1 H), 5.1 (m, 1 H).

***P. fluorescens* Lipase-Catalyzed Alcoholysis of (*S*)-4a in $CHCl_3$.** A mixture of (*S*)-**4a** (480 mg, 3 mmol; 62.4% ee), *n*-propanol (1.35 mL), and *P. fluorescens* (SAM-2) (90 mg) in $CHCl_3$ (25 mL) was stirred at 25 °C for 16 h. After filtration the solvent was removed in vacuo and the resulting residue chromatographed on silica gel (hexane–ethyl acetate 4:6), obtaining unreacted (*S*)-**4a** (177 mg, 37%) and (*S*)-**3** (212 mg, 60%) as colorless oils. **(*S*)-4a**: 13% ee [determined by ^{1}H NMR in the presence of Eu(hfc)$_3$]. **(*S*)-3**: $[\alpha]^{20}_D$ −41.8° (*c* 1.9, dioxane). ^{1}H NMR spectra of (*S*)-**4a** and (*S*)-**3** were identical with those of (*S*)-**4a** and (*R*)-**3** resulting from the acetylation of **3**.

***P. fluorescens* Lipase-Catalyzed Acetylation of 3 in *tert*-Amyl Alcohol, THF, and *i*-Pr_2O.** The reactions were performed under the conditions reported in Table 1 (entry 5) and Table 2 (entries 1 and 2) footnotes and worked up as described for PFL-catalyzed acetylation in $CHCl_3$.

***M. miehei* and Porcine Pancreatic Lipase-Catalyzed Acetylations of 3 in *tert*-Amyl Alcohol.** The reactions were performed under the conditions reported in Table 1 (entries 1 and 2) footnotes and worked up as described for PFL-catalyzed acetylation in $CHCl_3$.

Lipase-Catalyzed Acylation of 3 with Vinyl Laurate. A mixture of the selected lipase, **3** (1 g, 8.5 mmol), and vinyl laurate (7.4 mL, 28.3 mmol) in *tert*-amyl alcohol (30 mL) was stirred under the conditions reported in Table 1 (entries 6–8) footnotes. After filtration the solvent was removed in vacuo. The resulting residue was worked up by the addition of water and washing with hexane. The aqueous phase was successively added with

NaCl and extracted with ethyl acetate. The organic extract was concentrated under reduced pressure, obtaining (*R*)-**3**. (*S*)-**4b** was recovered by evaporation of hexane and flash chromatography (hexane–ethyl acetate 7:3) of the resulting residue. (*S*)-**4b**: ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.25 (br s, 16 H), 1.6 (m, 2 H), 2.4 (t, 2 H), 4.2–4.4 (m, 3 H), 4.55 (pseudo t, 1 H), 4.9 (m, 1 H).

***M.* miehei and *P. fluorescens* Lipase-Catalyzed Alcoholyses of (*S*)-4a in *tert*-Amyl Alcohol.** The reactions were performed under the conditions reported in Table 3 (entries 1 and 2) footnotes and worked up as described for PFL-catalyzed alcoholysis in $CHCl_3$.

Acknowledgment. This work was supported by the Ministero dell'Università della Ricerca Scientifica e Tecnologica of Italy.

Supplementary Material Available: ^1H NMR spectra for compounds (*S*)-**4a** (product of acetylation), (*R*)-**3**, (*S*)-**4b**, (*S*)-**4a** (unreacted substrate of alcoholysis), and (*S*)-**3** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.