Synthesis of (-)-(*S*)-2-Hydroxymethyl-2,3-dihydro-1,4-benzodioxine by Enzyme-Catalyzed Resolution in Organic Media

David Mauleón*, Cinta Lobato and Germano Carganico

R & D Department, Laboratorios Menarini S.A. Alfonso XII, 587,
08912 Badalona, Spain

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The chiral primary alcohol 2-hydroxymethyl-2,3-dihydro-1,4-benzodioxine (**1**) has been resolved by enantioselective acetylation in vinyl acetate solution, using *Pseudomonas sp.* Amano PS lipase. Enantiomers (-)-*S*-**1** (99% ee) and (+)-*R*-**1** (72% ee) were obtained in 45 and 65% yield, respectively.

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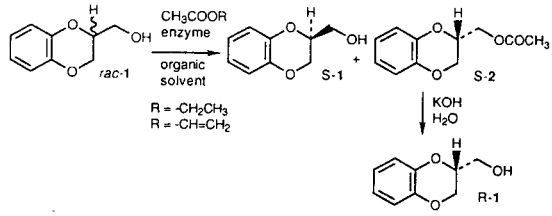
The synthesis of homochiral compounds containing a 2-substituted-2,3-dihydro-1,4-benzodioxine nucleus (also named 1,4-benzodioxane) usually starts from optically pure 2-hydroxymethyl-2,3-dihydro-1,4-benzodioxine (**1**). The enantiomers of this alcohol **1** have been prepared starting from D-mannitol [1], chiral epihalohydrins [2], and glycidol derivatives [3]. These methods have limited preparative usefulness for multi-gram preparations of *S*-**1** or *R*-**1**, due to either the large number of steps required or the use of expensive reagents. Since racemic benzodioxane **1** can be easily obtained from epichlorohydrin and catechol, resolution of **1** would constitute a cost-effective procedure for the synthesis of its enantiomers. However, classical resolution methods such as diastereomeric crystallization are difficult to apply to neutral compounds. Very recently, enzymatic resolution with Amano P-30 lipase in aqueous medium has been described [4] for the 8-methoxy derivative of compound **1**; however, this method gave poor results when applied to the synthesis of *S*-**1** (62% ee). It has been shown that several biocatalytic reactions of water-insoluble substrates can proceed with high enantioselectivity when carried out in organic media [5,6]. Lipases are the most suitable enzymes for this kind of reactions, being very stable and active in apolar organic solvents. Resolution of racemic alcohols has been carried out by lipase-catalyzed transesterification with both simple alkyl esters [7-10] and activated esters [11-16]. To make the enzymatic transesterification irreversible in organic media, enol esters have been used as the acylating agent [17]. In this case, the lipases isolated from *Pseudomonas* microorganisms usually show the highest enantioselectivity [18-21].

In the present work this methodology has been applied to the synthesis of (-)-(*S*)-2-hydroxymethyl-2,3-dihydro-1,4-benzodioxine (*S*-**1**) from the corresponding racemic alcohol. This biocatalytic transesterification was performed in anhydrous media with ethyl acetate or vinyl acetate as the acylating agents (Scheme I).

Different enzymes were tested, comprising an esterase (PLE) and six lipases (Table I). Reactions were carried out under several conditions, using either an inert organic solvent (diisopropyl ether or hexane) or the acylating agent itself as the solvent. The most reactive enantiomer was *R*-**1**, the remaining substrate being enriched in *S*-**1**, except in the case of the transesterification with *Candida rugosa* lipase, which showed opposite enantioselectivity. The enantiomeric excess of acetate *S*-**2** (or *R*-**2** with *Candida* lipase) was determined by stereospecific hplc, using a Chiralcel OJ column. Since the enantiomers of alcohol **1** were not resolved under these hplc conditions, the enantiomeric excess of *R*-**1** (or *S*-**1**) could not be directly measured. However, in those cases where a high ee was calculated for *S*-**1**, a sample of the crude reaction product was treated with benzoyl chloride and the resulting mixture of acetate *S*-**2** and benzoate *R*-**3** was analyzed by chiral hplc (four separate peaks), comparing them with authentic samples of these esters.

Scheme I



All lipases tested were able to catalyze efficiently the transesterification of *rac*-**1** with ethyl and vinyl acetates, whereas pig liver esterase showed little activity (less than 10% conversion). Some of these biotransformations were very fast, reaching conversions greater than 50% in few minutes. In most cases the stereoselectivity was very poor, giving only moderate enantiomers excess for *S*-**2**. However, use of the lipase Amano PS from *Pseudomonas cepacea* allowed us to obtain the enantiomers *S*-**1** in acceptable yield and with excellent optical purity. Thus, *rac*-**1** (25 mmole scale) was treated for 30 minutes with an excess of vinyl acetate and 5 g of lipase PS until a conversion of 75% into the acetate *S*-**2** (35% ee). The unreacted alcohol (-)-*S*-**1** was then isolated by column chromatography in 45% yield (yields are calculated as the percentage of the theoretical 50% yield based on *rac*-**1**), showing an optical purity of 99% (measured after conversion into the acetate). On the other hand, treatment of *rac*-**1** with lipase

Table I

Transesterification Reactions of *rac*-**1** in Organic Media

Enzyme [a] (mg/mmole of **1**)	R	Reaction conditions [b]	time (hours)	c (%)	ee S-**1** [c]	ee S-**2** [d]	E [e]
CL (100)	CH_2CH_3	A	22	23	15 (R)	50 (R) [f]	4
	$CH{=}CH_2$	B	22	28	12 (R)	33 (R) [f]	2
PPL (100)	CH_2CH_3	A	22	48	34	37	3
	$CH{=}CH_2$	B	22	40	10	15	2
AP-10 (520)	CH_2CH_3	A	96	29	24	58	5
	$CH{=}CH_2$	B	21	33	20	38	3
	$CH{=}CH_2$	C	1.5	39	37	58	5
Lipozyme (200)	CH_2CH_3	A	21	30	32	76	10
	$CH{=}CH_2$	B	3.5	10	7	61	5
	$CH{=}CH_2$	C	3	46	58	68	10
	$CH{=}CH_2$	D	0.33	78	95	26	5
PS (200)	CH_2CH_3	A	3.5	36	40	72	9
	CH_2CH_3	A	21	66	80	41	6
	$CH{=}CH_2$	B	29	11	7	57	4
	$CH{=}CH_2$	C	0.25	70	99 [g]	43	16
	$CH{=}CH_2$	C [h]	1	74	99 [g]	35	14
	$CH{=}CH_2$	D	0.16	78	99 [g]	27	10
AP-6 (2100)	CH_2CH_3	A	168	42	0	0	1
	$CH{=}CH_2$	B	21	44	4	5	1
	$CH{=}CH_2$	C	2	43	12	16	2
PLE (1400)	CH_2CH_3	A	72	3	2	57	4
	$CH{=}CH_2$	B	168	5	3	58	4
	$CH{=}CH_2$	C	22	8	3	38	2
	$CH{=}CH_2$	D	6	5	3	45	3

[a] For enzyme sources and abbreviations see Experimental. [b] After addition of the powdered enzyme and stirring at 37° for the indicated period of time, conversion was measured by gas chromatography. A: Ethyl acetate as solvent; B: 1 equivalent of vinyl acetate in diisopropyl ether; C: Vinyl acetate as solvent; D: 10 equivalents of vinyl acetate in diisopropyl ether. [c] Calculated from the conversion and the ee of ester **2**. [d] Determined by chiral hplc (Chiralcel OJ™). [e] Calculated as described by Chen *et al*. [5]. [f] Enantiomers *R*-**1** and *R*-**2** are obtained. [g] Measured by chiral hplc after conversion into the benzoate. [h] Half of the enzyme amount was used.

PS in ethyl acetate for 3.5 hours resulted in a 36% conversion into acetate *S*-**2**, having 72% ee. After chromatographic separation and alkaline hydrolysis of this ester, a 65% yield of the benzodioxane (+)-(*R*)-**1** was obtained. The enantiomeric excess of this alcohol was also of 72%, thus indicating that no racemization took place during the hydrolysis. Optical purities for the above enantiomers of benzodioxane **1** were confirmed by comparison with samples prepared by the method previously described [3].

In conclusion, transesterification of racemic benzodioxanylmethanol **1** with acetate esters, catalyzed by the lipase PS from *Pseudomonas cepacea*, constitutes a useful and short alternative to the stereocontrolled synthesis for the preparation of the enantiomers of **1**. In the case of *S*-**1** high ee values could be obtained.

EXPERIMENTAL

Apparatus.

Melting points were measured in a MFB 595.010M Gallenkamp apparatus and are uncorrected. The nmr spectra were recorded on Varian XL-200 and Gemini-300 spectrometers; chemical shifts (δ) are reported in ppm from TMS. Gas chromatography was performed on a Perkin Elmer 8600 apparatus, with a flame detector and a BP1 capillary column of 12 m and 0.22 mm diameter. The hplc analysis were performed in a Hewlett-Packard HP 1090 apparatus, with an uv detector (λ = 254 nm), using a Chiralcel OJ™ column (Daicel, Japan; cellulose *m*-dinitrophenylcarbonate on silica gel) of 25 x 0.45 cm and eluting with mixtures of hexane and isopropanol (from 5:1 to 4:1). Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Reagents and Enzymes.

Vinyl acetate was purchased from Fluka Chemie AG, Buchs, Switzerland and used without further purification. All solvents were analytical grade. Racemic 2-hydroxymethyl-2,3-dihydro-1,4-benzodioxine (**1**) was synthesized in 65% yield from catechol and epichlorohydrin and purified by distillation (120-125°, 0.5 Torr). CL: *Candida rugosa* lipase Type VII was obtained from Sigma Chemical Co., St. Louis, MO, USA. PPL: porcine pancreatic lipase Type II was obtained from Sigma. AP-10: *Mucor miehei* lipase was obtained from Amano Pharmaceutical Co., Nagoya,

Japan. Lipozyme: *Mucor miehei* lipase was obtained from NOVO Nordisk A/S, Bagsvaerd, Denmark. PS: *Pseudomonas cepacea* lipase was obtained from Amano. AP-6: *Aspergillus niger* lipase was obtained from Amano. PLE: porcine liver esterase was obtained from Boehringer Mannheim Gmbh, Mannheim, Germany.

(-)-(*S*)-2-Hydroxymethyl-2,3-dihydro-1,4-benzodioxine (*S*-**1**).

A mixture of 4.15 g (25 mmoles) of racemic **1** and 5 g of lipase Amano PS in 100 ml of vinyl acetate (dried over calcium chloride) was heated to 37°. After stirring at this temperature for 30 minutes, the suspension was centrifuged and the supernatant was filtered to remove all the enzyme. The clear solution was evaporated to dryness at reduced pressure and the residue was purified by column chromatography on silica gel, eluting with a mixture of hexane and ethyl acetate (7:3). The alcohol (-)-(*S*)-**1** was crystallized from diethyl ether, obtaining 0.94 g (45% yield) of a solid, mp 73-74° (lit [21] 74-74.5°); $[\alpha]_D^{25}$ = -34.1° (C = 0.6, ethanol) (lit [1] $[\alpha]_D^{25}$ = -34°, c = 0.1, ethanol, and [22] $[\alpha]_D$ = -33°, c = 0.7, ethanol); ^{1}H-nmr (300 MHz, deuteriochloroform): δ ppm 2.19 (bs, 1H, OH), 3.78-3.91 (ddd, 2H, CHC*H*$_2$OH), 4.07 (dd, 1H, C^{3}Hax), 4.19-4.29 (m, 2H, C^{2}H + C^{3}He), 6.77-6.89 (m, 4H, Ar); ^{13}C-nmr (75.5 MHz, deuteriochloroform): δ ppm 62.12 (CH_2OH), 65.49 (C^3H_2), 73.81 (C^2H), 117.79 and 117.86 (C^{5}H and C^{8}H), 122.17 and 122.25 (C^{6}H and C^{7}H), 143.42 and 143.61 (C^{4a} and C^{8a}).

An analytical sample of this alcohol *S*-**1** was converted into the corresponding acetate *R*-**2** by reaction with 1.2 equivalents of acetyl chloride and 1.2 equivalents of triethylamine in anhydrous dichloromethane (77% yield); ^{1}H-nmr (200 MHz, deuteriochloroform): δ ppm 2.08 (s, 3H, COC*H*$_3$), 4.02 (dd, J = 4.61, 7.61 Hz, C^{3}Hax), 4.23-4.36 (m, 4H, C^{3}He + C^2*H*C*H*$_2$O-), 6.70-6.80 (m, 4H, Ar). This acetate was analyzed by chiral hplc, showing an enantiomeric excess of 99%.

(+)-(*R*)-2-Hydroxymethyl-2,3-dihydro-1,4-benzodioxine (*R*-**1**).

a) *Enzymatic Transesterification.*

A mixture of 4.15 g (25 mmoles) of racemic **1** and 5 g of lipase Amano PS in 100 ml of ethyl acetate was heated to 37° and then stirred for 3.5 hours. The reaction was stopped by filtration of the enzyme, the filtrate was evaporated to dryness at reduced pressure and the residue was chromatographed on silica gel. On elution with a mixture of hexane and ethyl acetate (85:15), 1.8 g of the acetate *S*-**2** was obtained as a clear oil; ^{1}H-nmr: identical to that above described; ^{13}C-nmr (50.4 MHz, deuteriochloroform): δ ppm: 20.97 (CO*C*H$_3$), 62.94 (*C*H$_2$OCO), 65.40 (C^3H_2), 71.25 (C^2H), 117.76 and 117.98 (C^{5}H and C^{8}H), 122.10 and 122.39 (C^{6}H and C^{7}H), 143.37 and 143.58 (C^{4a} and C^{8a}), 171.44 (C=O). Chiral hplc analysis indicated an enantiomeric excess of 72% for this acetate *S*-**2**.

b) *Hydrolysis to R*-**1**.

The above acetate *S*-**2** was dissolved in 20 ml of methanol and added to 20 ml of a 5 *N* aqueous solution of potassium hydroxide. The resulting mixture was heated to reflux for 1 hour, cooled, and evaporated to a volume of 20 ml. The aqueous phase was extracted with diethyl ether (3 x 15 ml), the organic layers were dried over sodium sulphate and evaporated to give 1.34 g of the alcohol (+)-(*R*)-**1** (93%; 65% overall yield), mp 71-72°; $[\alpha]_D^{25}$ = +23.9° (c = 0.6, ethanol); ^{1}H-nmr and ^{13}C-nmr spectra identical to those found for *S*-**1**. A sample of this product was acetylated as above and the resulting ester showed an enantiomeric excess of 72% by chiral hplc.

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