Enzymatic Kinetic Resolution of Methyl 3-Phenylglycidate by Transesterification with Amino Alcohols

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Abstract: Kinetic resolution of racemic methyl *trans*-3-(4-methoxyphenyl)glycidate, a key intermediate for the synthesis of the well-known drug diltiazem hydrochloride, has been accomplished by transesterification reactions with suitable amino alcohols catalyzed by Novozym 435 in organic solvents.

Keywords: amino alcohols; biotransformations; enantioselectivity; enzymatic resolution; kinetic resolution

Introduction

The benzothiazepinone derivative **1**, known as diltiazem hydrochloride, is a coronary vasodilator and a calcium channel blocker (for anti-anginal and antihypertensive actions)[1] and is produced worldwide on a multi-ton scale per year.[2]

This molecule possesses two stereogenic carbons with the (*S*)-absolute configuration (specifically, 2*S*,3*S*) and therefore the synthetic protocols for its preparation require the introduction of the correct stereocenters either by asymmetric synthesis or by resolution of a racemic intermediate. Conventional resolution procedures applied to the synthesis of **1** consist either in the transformation of the racemate into a diastereomeric mixture by interaction with an enantiomerically pure resolving agent[3] or in the selective crystallization of one of the enantiomer from a supersaturated solution.[4] Alternatively, the enzymatic kinetic resolution of a suitable intermediate ester can also be exploited.[5]

Obviously, resolutions performed at an initial step of the process are economically more convenient, as usually the value of the compound subjected to resolution is lower and, as a consequence, the discharged isomer does not represent a relevant loss. For this reason the biocatalyzed kinetic resolution is applied to the racemic methyl ester of *trans*-3-(4-methoxyphenyl)-glycidate [MePGA, *trans*-(±)-**2**], in turn obtained by Darzens condensation between 4-methoxybenzaldehyde and methyl chloroacetate (Scheme 1). Early patents describe the efficient and selective hydrolysis of the "wrong" (2*S*,3*R*)-enantiomer of MePGA by action of a lipase in a buffer solution or in a biphasic system to give the unstable acid (2*S*,3*R*)-**3**.[2,5] The Tanabe process is currently "the biocatalyzed process for the production of enantiomerically pure (2*R*,3*S*)-**2**."

Enzymatic transesterifications in organic solvents have also been investigated (for recent reviews on this methodology see[6]): two patents describe the stereoselective exchange of the methyl ester *trans*-(±)-**2** with aliphatic alcohols (**4a**) catalyzed by different lipases and esterases (Scheme 2).[7,8] However, as the isolation of the single isomers has to be performed by chromatography or by fractional distillation, this approach is well-suited for the laboratory scale but is not easily applied at the industrial level.

To overcome this limitation, researchers at Synthelabo suggested a protocol based on the insolubilization of one of the enantiomers: enzymatic transesterification of *trans*-(±)-**2** in toluene in the presence of sodium 4-hydroxybutyrate (**4b**, Scheme 2) led to the formation of the insoluble carboxyester of the

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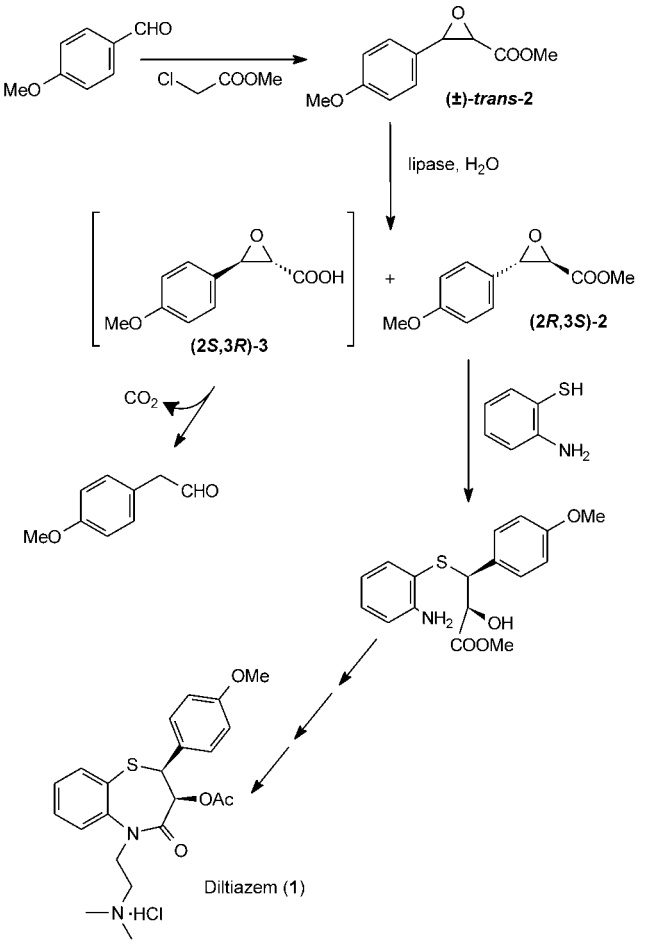
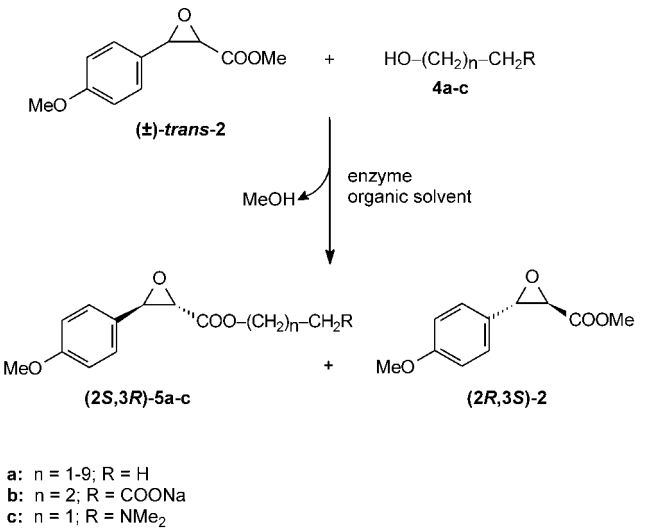


Scheme 1. Chemo-enzymatic synthesis of diltiazem.[5]



a: n = 1-9; R = H
b: n = 2; R = COONa
c: n = 1; R = NMe_2

Scheme 2, Enzymatic kinetic resolution of *trans*-(±)-**2** by transesterification with aliphatic alcohols.

"wrong" (2*S*,3*R*)-enantiomer [(2*S*,3*R*)-**5b**], a compound that could be eliminated by filtration.[9] However, the toluene solution proved to be quite "thick" due to the presence, in addition to the insoluble enzyme, of the product and of the excess of sodium hydroxybutyrate, both as solids, making the reaction work-up difficult. To improve the filtration properties of the suspension it was necessary to work using diluted solutions, under conditions that are unsuitable for an industrial process.

To our knowledge, a process for the enzymatic kinetic resolution of *trans*-(±)-**2** by transesterification with suitable amino alcohols (i.e., **4c**, Scheme 2) has never been described in the literature. In this paper we report on the simple process that we have developed using amino alcohols of industrial applicability, a process that allows the separation of the reacted enantiomer from the unreacted one by simple extraction with an acid medium.[10]

Results and Discussion

We started our investigation by screening the selectivity of several commercially available lipases. Transesterification reactions between MePGA [*trans*-(±)-**2**, 0.1 M) and 2-(dimethylamino)ethanol (**4c**, 2 M) were performed in *tert*-butyl methyl ether (an arbitrarily chosen solvent), monitoring the conversion and the enantiomeric excess of the unreacted substrate by HPLC. The enantiomeric ratio "E" was calculated according to the well-known formula[11] and, of the more than 20 different hydrolases tested, the best results were obtained with the lipases from *Humicola lanuginosa* (lipase CE-5), porcine pancreas, *Pseudomonas cepacia* (lipase PS), and *Candida antarctica* (Novozym 435) (Table 1). With these enzymes the (2*S*,3*R*)-enantiomer of MePGA was preferentially transesterified, while the enantiomer useful for diltiazem synthesis remained mainly unchanged. For practical reasons, related to enzyme availability in a well-defined immobilized form, we decided to focus our attention on the lipase from *Candida antarctica*, a biocatalyst that is also widely appreciated and used for its stability and reproducibility of action.

The next step was the so-called "medium engineering"[12]: transesterification reactions were performed in different solvents and the results are reported in

Table 1. Kinetic resolution of racemic *trans*-MePGA [*trans*-(±)-**2**] by transesterification with 2-(dimethylamino)ethanol (**4c**) catalyzed by different commercially available lipases in *tert*-butyl methyl ether.

Enzyme	Conversion	$ee_{substrate}$	E[a]
Novozym 435	52.11	72.68	10.70
Lipase CE-5	34.88	44.86	17.56
Lipase PS	25.39	20.95	5.14
Porcine pancreatic lipase	17.09	17.23	13.24

[a] Calculated as described in ref.[11]

Table 2. Influence of solvent on the enantioselectivity of Novozym 435.

Enzyme	Conversion	$ee_{substrate}$	E
tert-butyl methyl ether	49.22	69.60	12.51
toluene	44.90	64.16	16.16
xylene	42.60	60.44	18.00
2-methyl-2-butanol	49.53	68.92	11.61
tert-butanol	57.07	78.57	8.98
tetrahydrofuran	35.08	43.19	13.66
dioxane	22.31	24.81	17.44
acetonitrile	31.70	36.55	11.99
acetone	21.73	23.96	17.17

Table 2. "E" values higher than those in *tert*-butyl methyl ether were obtained in various solvents and, for reasons related to the overall industrial process used for the production of diltiazem, the choice was toluene.

Scaling-up of this biotransformation obviously required a significant increase of substrate concentration [K_M values for *trans*-(±)-**2** and for **4c** were determined to be 0.29 M (≈ 60 mg/mL) and 0.39 M (≈ 40 μL/mL), respectively]. It was possible to solubilize *trans*-(±)-**2** up to 15% w/v (0.72 M) but, as a consequence, the amount of enzymatically released methanol was not negligible anymore, and the reverse transesterification reaction caused the expected significant and deleterious decrease in the ee of the recovered MePGA.[13] This problem was resolved either by trapping the released methanol with suitable molecular sieves or by continuous azeotropic distillation under reduced pressure (30 torr) of the quaternary mixture methanol/methylcyclohexane/toluene/2-(dimethylamino)ethanol. Both solutions made the enzymatic transesterification virtually irreversible and, after 4 h, the unreacted substrate could be recovered enriched in (2*R*,3*S*)-**2** up to 80% ee.

We also investigated the behavior of other aliphatic (**6, 7**) and alicyclic (**8, 9**) alcohols carrying a tertiary amino group and, as shown in Table 3, all of them were accepted as nucleophiles by the enzyme. The "E" values obtained with **6 – 9** (Figure 1) were lower than the one obtained with 2-(dimethylamino)ethanol and therefore this latter nucleophile was the reagent of choice.

Table 3. Kinetic resolution of racemic *trans*-MePGA [*trans*-(±)-**2**] catalyzed by Novozym 435 in toluene using different amino alcohols.

Amino alcohols	Reaction time [h]	Conversion [%]	$ee_{substrate}$	E
4c	2	44.90	64.16	16.16
6	5	51.60	60.40	6.51
7	3	59.85	76.84	6.94
8	3	55.16	78.52	10.52
9	3	51.96	65.28	7.76

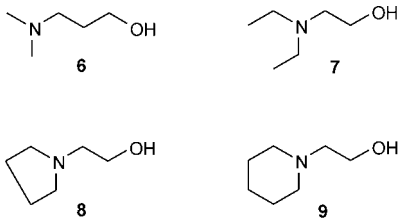


Figure 1. Alcohols **6 – 9** tested.

Table 4. Kinetic resolution of racemic *trans*-MePGA [*trans*-(±)-**2**] using recycled Novozym 435.

Cycle	Conversion [%][a]	$ee_{substrate}$ [a]	E
II	50.46	71.28	11.87
III	49.60	70.49	12.56
IV	49.62	70.68	12.67
V	48.78	70.13	13.64
VI	47.47	67.14	13.50

[a] Determined after 4.5 h.

As azeotropic distillation appeared more suitable for a large-scale application of this biotransformation, the final step was an investigation of the performances of the immobilized enzyme under these operative conditions. As shown in Table 4, the steady values of the extent of conversion and of the ee of the residual substrate, measured in subsequent reaction cycles, confirmed the high stability of Novozym 435 using this reaction protocol (toluene, high concentration of the substrate and of the alcoholic nucleophile).

As detailed in the Experimental Section, the optimized kinetic resolution of *trans*-(±)-**2** followed by selective crystallization allowed the isolation of (2*R*,3*S*)-**2** with 98% ee and 37.5% yield from racemic *trans*-(±)-**2**.

Conclusion

The use of mild reaction conditions, the availability of a particularly stable enzyme – handy and recoverable at the end of the reaction – the use of simple amino alcohols, and the remarkably simplified final procedure for isolation of the (2*R*,3*S*)-enantiomer of MePGA with good yields and high ee make the protocol described in this paper suitable for a large-scale application.

Additionally, this methodology is of more general applicability, as has been shown by the data reported in Table 3 (different amino alcohols investigated) and by the preliminary results obtained with other racemic methyl carboxylates. A more detailed investigation of the kinetic resolution of these new compounds is currently in progress and the results will be reported in due course.

Experimental Section

Materials

Racemic *trans*-MePGA [(±)-*trans*-**2**] and an authentic sample of (2*R*,3*S*)-**2** were furnished by Zambon. The amino alcohols **4c**, **6 – 9** were obtained from Aldrich. Novozym 435 (an immobilized lipase from *Candida antarctica*, E.C. 3.1.1.3) was obtained from Novo Nordisk. The other lipases were obtained from Sigma (porcine pancreas lipase) and from Amano (lipase PS and lipase CE-5). Molecular sieves (5 Å) were obtained from Fluka.

HPLC Analysis

HPLC analyses were performed using a Jasco 880/PU instrument equipped with a Jasco 875 UV/VIS detector (reading was done at 260 nm).

A silica column (Whatman Partisil 5) was used for the determination of the extent of conversion, eluting with petroleum ether/ethyl acetate/2-(dimethylamino)ethanol, 95:5:0.5 (flow rate 1.2 mL/min). Retention times: *trans*-(±)-**2**, 10.15 min; (2*S*,3*R*)-**5c**, 19.10 min.

A chiral column (Chiralcel OD, Daicel) was used for the determination of the enantiomeric excess of residual **2**, eluting with petroleum ether/propan-2-ol, 93:7 (flow rate 0.5 mL/min). Retention times: (2*R*,3*S*)-**2**, 20.80 min; (2*S*,3*R*)-**2**, 28.90 min. A solution of pure (2*R*,3*S*)-**2** was used to assign the retention times to the correct enantiomers.

Screening of Lipases for the Kinetic Resolution of *trans*-(±)-2 (Table 1)

A sample of the lipase (10 – 100 mg) was added to a *tert*-butyl methyl ether solution (1 mL total volume, 0.8 mL solvent) containing 2-(dimethylamino)ethanol (**4c**; 0.2 mL, 2 mmol) and *trans*-(±)-**2** (21 mg, 0.1 mmol). The suspension was stirred at room temperature for a definite time and then the enzyme was removed by filtration. A part of the solution (20 μL) was diluted by adding 200 μL of a mixture of petroleum ether:ethyl acetate (95:5) and the conversion was determined by HPLC analysis using the silica column. The remaining part of the solution was washed four times with 5 mL of a 0.05 M acetate buffer, pH 5, the remaining organic phase was dried over $MgSO_4$ and the ee of the residual substrate was determined by HPLC analysis using the chiral column.

Influence of Organic Solvents on the Enantioselectivity of Novozym 435 (Table 2)

A sample of Novozym 435 (10 mg) was added to a solution (1 mL total volume, 0.8 mL of one of the organic solvents listed in Table 2) containing 2-(dimethylamino)ethanol (**4c**; 0.2 mL, 2 mmol) and *trans*-(±)-**2** (21 mg, 0.1 mmol). The suspension was stirred at room temperature for 2 h and then the enzyme was removed by filtration. A part of the solution (20 μL) was diluted by adding 200 μL of a mixture of petroleum ether:ethyl acetate (95:5) and the conversion was determined by HPLC analysis using the silica column. The remaining part of the solution was washed four times with 5 mL of a 0.05 M acetate buffer, pH 5, the remaining organic phase was dried over $MgSO_4$ and the ee of the residual substrate was determined by HPLC analysis using the chiral column.

Kinetic Resolution of *trans*-(±)-2 in the Presence of Molecular Sieves

trans-(±)-**2** (10 g, 48.1 mmol) was dissolved in toluene (40 mL) and 2-(dimethylamino)ethanol (**4c**; 9.8 mL, 97.7 mmol): molecular sieves 5 Å (15 g) and Novozym 435 (1 g) were successively added to the solution. The suspension was kept under stirring at room temperature for 4 h (*c* 52.8%) and then filtered under vacuum. The enzyme and the molecular sieves were washed with toluene (23 mL) and the toluene phases collected. Water cooled at 0 °C (100 mL) was added to the toluene solution and kept under stirring for 30 minutes. After phases separation, water cooled at 0 °C (80 mL) and, slowly under stirring, water (20 mL) and a 85% solution of phosphoric acid up to pH 6.8 (1.7 mL) were added to the organic phase. The solution was kept under stirring for 1 h, maintaining the pH value at 6.8 by subsequent addition of the previously prepared phosphoric acid solution. This washing step was repeated by adding water cooled to 0 °C (80 mL) and the phosphoric acid solution (to keep the solution at pH 6.8, approximately 23 mL) to the toluene phase. Following stirring for 3 h, the phases were separated and the aqueous phase was extracted with toluene.

The collected toluene phases contained the unreacted *trans*-(±)-**2** (4.56 g, 21.9 mmol), with an enantiomeric ratio of 90:10 in favor of the 2*R*,3*S* enantiomer [yield in (2*R*,3*S*)-**2** equal to 41% from racemic *trans*-(±)-**2**].

The toluene solution was evaporated under reduced pressure, recovering 4.7 g of crude product. Fresh toluene (6 mL) was added, warmed at 50 °C up to complete dissolution of the solid, then the crystallization was started by addition of a few crystals of pure (2*R*,3*S*)-**2**.

The mixture was cooled at 0 °C in 2 h and kept at this temperature for 1 h. The precipitate was filtered, washed with toluene pre-cooled at 0 °C (2 mL) and dried in an oven at 60 °C under vacuum for 4 h giving 3.1 g (14.9 mmol) of (2*R*,3*S*)-**2** with 98% ee (overall yield after crystallization equal to 37.5%).

Performances of Different Amino Alcohols for the Kinetic Resolution of (±)-*trans*-2 (Table 3)

A sample of Novozym (20 mg) was added to a toluene solution (1 mL total volume, 0.8 mL solvent) containing *trans*-(±)-**2** (200 mg, 0.96 mmol) and one of the amino alcohols listed in Table 3 (0.2 mL). Following the addition of 5 Å molecular sieves (500 mg), the suspensions were stirred at room temperature for 3 – 5 h and then the enzyme was removed by filtration. A part of the solution (20 μL) was diluted by adding 200 μL of a mixture of petroleum ether:ethyl acetate (95:5) and the conversion was determined by HPLC analysis using the silica column. The remaining part of the solution was washed four times with 5 mL of a 0.05 M acetate buffer, pH 5, the remaining organic phase was dried over $MgSO_4$ and the ee of the residual substrate was determined by HPLC analysis using the chiral column.

Resolution of *trans*-(±)-2 by Enzymatic Transesterification with 2-(Dimethylamino)-ethanol (4c) under Azeotropic Distillation Conditions

2-(Dimethylamino)ethanol (**4c**; 12 mL, 119.6 mmol), Novozym 435 (1 g) and methylcyclohexane (10 mL) were added to a toluene solution (58 mL) of *trans*-(±)-**2** (12 g, 57.7 mmol). The suspension was stirred at 25 °C for 4 h, removing the azeotrope by continuous vacuum distillation (P = 30 torr) and replacing the evaporated solvents and 2-(dimethylamino)ethanol by subsequent additions (toluene 6 mL; methylcyclohexane 24 mL; 2-(dimethylamino)ethanol 12 mL, 3 mL/h). The enzyme was removed by filtration, washed with toluene and recycled as described in Table 4.

The isolation of the desired product was carried out as previously described, yielding the (2*R*,3*S*)-**2** with comparable yield and enantiomeric excess (36.5 and 98%, respectively).

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