

Lipase-Catalyzed Kinetic Resolution of (\pm)-Mandelonitrile under Conventional Condition and Microwave Irradiation

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A resolução cinética da (\pm)-mandelonitrila foi realizada utilizando a lipase de *Candida antarctica* sob condição convencional (agitação orbital) e irradiação de micro-ondas em tolueno, produzindo o acetato de (*S*)-mandelonitrila com elevada seletividade (92-98% ee, excesso enantiomérico). O álcool remanescente da (*R*)-mandelonitrila sob irradiação de micro-ondas e agitação orbital foi parcialmente convertido em benzaldeído via equilíbrio químico espontâneo. O acetato de (*S*)-mandelonitrila foi produzido com 92% ee e rendimento de 35% em 8 h de reação sobre irradiação de micro-ondas. A transesterificação da (\pm)-mandelonitrila em agitador orbital produziu a (*R*)-mandelonitrila (51% ee) remanescente e o acetato da (*S*)-mandelonitrila (98% ee) de acordo com a regra Kazlauskas com 184 h de reação.

The kinetic resolution of (\pm)-mandelonitrile was carried out using lipase from *Candida antarctica* under conventional condition (orbital shaker) and microwave irradiation in toluene, producing the (*S*)-mandelonitrile acetate with high selectivity (up to >98% ee, enantiomeric excess). The unreacted (*R*)-mandelonitrile under microwave irradiation and conventional condition was partially converted into benzaldehyde by spontaneous chemical equilibrium. The (*S*)-mandelonitrile acetate under microwave irradiation was produced with 92% ee and 35% yield for 8 h of reaction. Conventional transesterification of (\pm)-mandelonitrile in an orbital shaker produced unreacted (*R*)-mandelonitrile (51% ee) and (*S*)-mandelonitrile acetate (98% ee) in accordance with Kazlauskas rule for 184 h of reaction.

Keywords: kinetic resolution, *Candida antarctica* lipase, mandelonitrile, microwave irradiation, orbital shaker

Introduction

Enantiomerically pure cyanohydrins and their esters are important building blocks in organic chemistry.¹ Several biocatalytic methods have been developed with the aim of obtaining enantiopure (*R*)- and/or (*S*)-mandelonitrile. For example, the hydroxynitrile lyase from *Hevea brasiliensis* is a versatile catalyst for the highly enantioselective synthesis of (*S*)-cyanohydrins.²

One particularly elegant approach is the dynamic kinetic resolution of cyanohydrins formed *in situ* from an aldehyde and acetone cyanohydrin, the cyanide source. The utilization of an enantioselective lipase for catalyzing the esterification leads to high yield and enantiomeric purity of the formed cyanohydrin ester.³

The (\pm)-mandelonitrile under acidic conditions is stable, and in basic conditions, the racemization of

enantiopure mandelonitrile occurs.³ The racemization of mandelonitrile by CALB (*Candida antarctica* lipase type B) and amberlite *in situ* promoted a dynamic kinetic resolution yielding the (*S*)-mandelonitrile acetate with high selectivity. However, the reaction was slow, with a poor yield for enantiopure (*S*)-mandelonitrile acetate to the long reaction times (3-10 days).^{3,4}

Recently, the enzymatic kinetic resolution of a set of aromatic and aliphatic cyanohydrins in organic media has been investigated. The behavior of potential lipases, molecular sieves, acyl reagent, reaction temperature and organic solvents on the kinetic resolution was studied. As also, the influence of substrate structure, steric and electronic nature and position of the aryl substituent on the enantioselectivity was discussed.⁵

Optically active, mandelonitrile was obtained by the kinetic resolution (KR) in solvent-free system by lipase from *Alcaligenes* sp. and the reaction occurred within a short period of time at 5 h. This study showed that

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benzaldehyde and benzoic acid could inhibit the lipase activity.⁶ Other methods have also been investigated to obtain enantiopure mandelonitrile derivatives by nitrilases.⁷⁻⁹

To the best of our knowledge, the direct esterification of (\pm)-mandelonitrile (**1**) under conventional condition and microwave irradiation has not been reported by lipase from *Candida antarctica*.

Microwave irradiation has been reported to be a very attractive tool for chemical applications in organic syntheses. Previous studies of lipase-catalyzed kinetic resolutions of secondary alcohols under microwave irradiation have in several cases shown enhancements in both rate and/or enantioselectivity.¹⁰ For immobilized lipases, the reactivities and enantioselectivities in microwave and oil bath experiments were identical to the kinetic resolution of 1-(\pm)-phenylethanol.¹¹ CALB catalyzed the resolution of (*R,S*)-2-octanol with vinyl acetate as the acyl donor under microwave irradiation and conventional heating. Under optimum conditions, (*S*)-2-octanol was obtained at 50.5% conversion with 99% enantiomeric excess in 2 h under microwave irradiation.¹² The immobilized lipase from *C. antarctica* used for transesterification reaction has shown to be an efficient biocatalyst with good thermal stability.^{13,14} Microwave-assisted lipase-catalyzed transesterification has emerged as a useful tool in organic synthesis. To date, no reports are available for the kinetic resolution of (\pm)-mandelonitrile (**1**) by microwave irradiation.

The purpose of this study is to show the use of two methods in the kinetic resolution (\pm)-mandelonitrile (**1**) by conventional condition (orbital shaker) and microwave irradiation.

Experimental

General methods

¹H and ¹³C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC-200 spectrometer (¹H at 200 MHz and ¹³C at 50 MHz). The spectra were taken in chloroform-*d* (CDCl₃) and the chemical shifts were given in ppm using tetramethylsilane (TMS) as internal standard. Near-IR spectrum was recorded on a Bomem MB-102 spectrometer. The spectroscopic data are in agreement with those reported in the literature.¹⁵

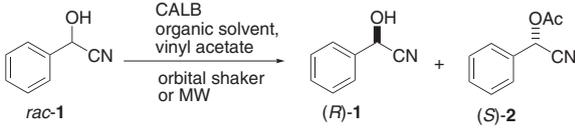
(\pm)-Mandelonitrile (**1**) was purchased from Sigma-Aldrich and contained (less than 0.5%) benzaldehyde. Anhydride acetic, pyridine and benzaldehyde were purchased from Synth. Column chromatography separation was carried out using silica flash (400-230 mesh) and hexane/ethyl acetate mixtures as eluent.

Enzymatic KR under conventional condition was carried out using a Tecnal TE-421 orbital shaker. Enzymatic reaction was analyzed using a Shimadzu GC 2010 gas chromatograph equipped with an AOC 20i auto injector, a flame ionization detector (FID) and a Varian chiral column CP-Chiralsil-DEX (β -cyclodextrin) (25 m \times 0.25 mm \times 0.39 μ m). The conditions employed in the gas chromatographic analyses were the following: nitrogen (60 kPa) as carrier gas, injector temperature of 200 °C, injector split ratio of 1:20 and detector temperature of 200 °C. The oven temperature was programmed at 100 °C followed by a linear increase of 10 °C min⁻¹ to 175 °C for 2 min, and a linear increase of 5 °C min⁻¹ to 180 °C for 5 min, time analysis of 15.5 min. The enantiomeric excess (ee) of (*S*)-mandelonitrile acetate (**2**) was determined by gas chromatographic analyses with chiral stationary phase employing the retention times obtained for both enantiomers of **2**: (*R*)-enantiomer = 7.54 min and (*S*)-enantiomer = 7.82 min. The *rac*-**1** did not show the enantioseparation on GC-FID analysis. The enantiomeric excess of the unreacted (*R*)-mandelonitrile (**1**) was determined after its derivatization with Ac₂O/py (acetic anhydride/pyridine). The enzymatic reaction was also analyzed by liquid chromatography (LC) performed on a Shimadzu liquid chromatographic system (Kyoto, Japan) using a high pressure pump LC-10AD and a SPD-M10Avp photodiode array detector (DAD) system coupled with a CBM 20A interface. Data collection was performed using LC Solution software. The photodiode array UV detector was recorded between 220 and 254 nm to acquire chromatograms. The LC analyses were conducted with a Chiralcel OD-H column (0.46 \times 25 cm), a detector at 254 and 220 nm and a mixture of hexane and *iso*-propanol (9:1). The flow rate was 1.0 mL min⁻¹. Retention times were: benzaldehyde (5.04 min), (*R*)-mandelonitrile **1** (9.15 min), (*S*)-mandelonitrile **1** (9.69 min), (*R*)-mandelonitrile acetate **2** (11.40 min) and (*S*)-mandelonitrile acetate **2** (12.58 min). (\pm)-Mandelonitrile acetate (**2**) was obtained by acetylation of (\pm)-mandelonitrile (**1**) using Ac₂O/py.

Kinetic resolution of (\pm)-mandelonitrile

Lipase-catalyzed acylation on conventional condition (orbital shaker)

Vinyl acetate (0.5 mL, 5.4 mmol), lipase (160 mg) and the (\pm)-mandelonitrile (**1**) (80 μ L, 0.67 mmol) were added to 10 mL organic solvent (hexane, ethyl ether or toluene) in 50 mL Erlenmeyer flasks. The reaction mixture was stirred in a rotary orbital shaker at 32°C and 130 rpm. The reaction progress was monitored by collecting samples (0.1 mL) according to the time indicated in

Table 1. Kinetic resolution of (\pm)-mandelonitrile (**1**) using lipase from *Candida antarctica*^a


entry	solvents	time / h	c ^f [yield] / % ^g	c [yield] / % 2	ee / % 2 ^h
Conventional condition					
1	hexane	48	65	35	> 98
2		72	69	38	> 98
3		120	56	44	95
4	ethyl ether	48	77	23	> 98
5		72	70	30	> 98
6		120	66	34	95
7	toluene	48	67	33	> 98
8		72	65	35	> 98
9		120	63	37	> 98
10		168	54	46	> 98
11		184	51 [15] ^b	48 [25] ^c	> 98
Microwave condition					
12	toluene	1	82	18	> 98
13		2	75	25	94
14		3	71	29	94
15		4	68	32	94
16		5	65	36	93
17		6	63	38	93
18		7	62	39	93
19		8	60 [8] ^d	40 [35] ^e	92

^aThe reactions were carried out in quadruplicate; the results presented were obtained from these values; ^bisolated yield of (*R*)-**1** (15%, 51% ee); ^cisolated yield of (*S*)-**2** (25%, 98% ee); ^disolated yield of (*S*)-**2** (8%, 89% ee); ^eisolated yield of (*S*)-**2** (35%, 92% ee); ^fc: conversion determined by GC-FID analyses; ^gconversion of **1** and benzaldehyde; ^henantiomeric excess (ee) determined by GC-FID analysis

Table 1, which were analyzed by gas chromatography with chiral stationary phase. An additional experiment was performed, as described above, using the toluene as solvent for isolation and purification of the biocatalyzed (*S*)-mandelonitrile acetate (**2**). After the reaction was completed, the immobilized lipase was filtered off. The toluene was evaporated under reduced pressure. The residue was purified by column chromatography on silica flash using 8:2 hexane/ethyl acetate as eluent. The enzymatic kinetic resolution was carried out in quadruplicate and the results presented in Table 1 were obtained from these values. Activity of CALB is 10,000 units *per g* (1 unit will catalyze the formation of 1 μ mol butyric acid from tributyrin at 40 °C and pH 7.5). In preliminary studies, 80 mg of CALB were used, and no product was detected, applying conventional condition for 148 h.

Lipase-catalyzed acylation on microwave irradiation

The microwave irradiation experiment was performed using a Discover System from CEM Corporation. 10 mL toluene, vinyl acetate (0.5 mL, 5.4 mmol), lipase (160 mg) and the *rac*-mandelonitrile (**1**) (80 μ L, 0.67 mmol) were added to a 50 mL bottom flask. The whole reaction mixture was placed in the microwave oven and irradiated for 10 s at a 2.45 GHz frequency, a power output of about 200 W. The reaction progress was monitored by collecting samples (0.1 mL) according to the time indicated in Table 1, which were analyzed by gas chromatography with chiral stationary phase. After the irradiation of 8 h, the bottom flask was quickly removed and the temperature of the sample reaction mixture was around 80 °C. After the reaction was completed, the immobilized lipase was filtered off. The toluene was evaporated under reduced pressure. The residue was purified by column chromatography on silica flash using 8:2 hexane/ethyl acetate as eluent. The kinetic enzymatic resolution was carried out in quadruplicate and the results presented in Table 1 were obtained from these values.

Assignment of absolute configuration

The optical rotation value was measured with a Perkin-Elmer 241 polarimeter (Waltham, MA, USA) and the data were determined using the sodium D line (589 nm) and a 1 dm cuvette. The absolute configuration for the enantiomerically (*S*)-mandelonitrile (**2**) was determined comparing their specific optical rotation value with those reported in the literature.^{15,16}

(*R*)-(+)-2-hydroxy-2-phenylacetoneitrile (**1**)

Experimental data: $[\alpha]_D^{20} +19.1$ (*c* 1.15; CHCl₃), 51% ee; $[\alpha]_D^{20} +40.6$ (*c* 1.0; CHCl₃), 92% ee.¹⁶

(*S*)-(-)-cyano(phenyl)methyl acetate (**2**)

Experimental data: $[\alpha]_D^{20} -3.95$ (*c* 0.82; CHCl₃), 98% ee; $[\alpha]_D^{20} -5.6$ (*c* 0.58; CHCl₃), 98.3% ee.¹⁵

Results and Discussion

Lipase-catalyzed esterification with vinylacetate ester as acylating agent has been shown to be an effective method for preparing chiral alcohols, resulting in the formation of acetaldehyde after the acyl transfer process. In order to investigate the solvent effect on the transesterification reaction of (\pm)-mandelonitrile (**1**), different organic solvents were used. The reactions were conducted in an orbital shaker catalyzed by CALB. Hydrophobic solvent toluene gave excellent optical purity (up to 98% ee) and conversion to the (*S*)-mandelonitrile acetate (**2**) (entries 7-11, Table 1). Although the use of hexane and ethyl ether influenced

the KR with high selectivity, the results showed that the activity and selectivity were dependent on the solvents. The reaction in hexane and ethyl ether resulted in minor selectivity of (*S*)-**2** at 120 h (95% ee, entries 3 and 6, Table 1). Moreover, the enzymatic reaction conducted in toluene under orbital shaker resulted in a long period of time to be completed (168-184 h, entries 10 and 11, Table 1).

In addition, the solvent choice is important for use in microwave irradiation, as it can influence in the enantioselectivity of enzymatic reaction. The toluene has higher boiling point, it appear to be an excellent solvent for the investigation of microwave-assisted kinetic resolution.¹⁰

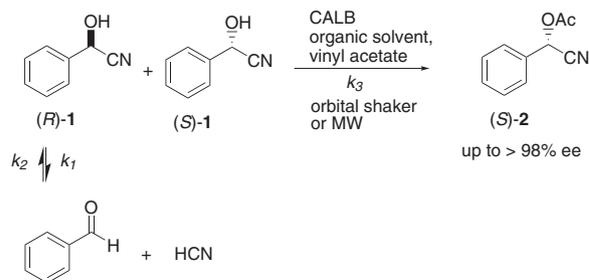
The kinetic resolution of (\pm)-mandelonitrile (**1**) yielded the (*S*)-mandelonitrile (**2**) with good conversion and optical purity (c 38-40% and 92-93% ee, entries 17-19, Table 1) under microwave irradiation. The kinetic resolution under conventional system occurred with a longer time (c 46-48%, > 98% ee, 168-184 h, entries 10 and 11, Table 1). The temperature effect on the activity and enantioselectivity of CALB under microwave irradiation was observed at 60 and 80 °C. When the reaction was realized in toluene at 60 °C, under microwave irradiation, no product was detected between 1 to 8 h. However, when the reaction was carried out at 80 °C, the collision chances between the enzyme and substrate molecules increased and this fact might help the formation of "enzyme-substrate" complex, and then, the reaction rate was also favored (entries 12-19, Table 1).¹² On the other, the reaction realized in toluene at 32 °C applying conventional condition occurred slowly at 48 and 184 h (entries 7-11, Table 1).

Due to the coincidence of the retention times of mandelonitrile (**1**) and benzaldehyde in chromatographic analyses by GC-FID, the reactions were analyzed by HPLC (see Supplementary Information (SI) section). The (*S*)-mandelonitrile acetate (**2**) was obtained with high enantiomeric excess (98% ee) and (*R*)-mandelonitrile (**1**) in minor optical purity (54% ee by HPLC; 51% ee by GC-FID) under conventional condition (SI section). The poor yield of (*R*)-**1** was suggested by the partially decomposition of mandelonitrile **1** to benzaldehyde under microwave irradiation and conventional condition. HPLC analyses were performed after purification of the reaction (184 h) with silica flash column chromatography for kinetic resolution in on orbital shaker. The absolute configuration of (*S*)-mandelonitrile acetate (**2**) was attributed by comparing the rotation signal measured with that reported in the literature.^{15,16} Therefore, it is expected that the unreacted enantiomer **1** present *R*-configuration.

When the KR was conducted on microwave irradiation, the (*S*)-mandelonitrile acetate (**2**) was obtained with

minor optical purity (92% ee), but in a shorter time (8 h) by GC-FID analysis. Surprisingly, the unreacted mandelonitrile (**1**), after derivatization with anhydride acetic and pyridine, was agreed for the (*S*)-enantiomer (**1**), which would be incoherent for a typical KR (GC-FID analysis).

In accordance to GC-FID and HPLC analyses, it is concluded that lipase-catalyzed highly enantioselective kinetic resolution of the (\pm)-mandelonitrile (**1**). Conventionally, the (*S*)-mandelonitrile (**1**) was selectively esterificated to (*S*)-mandelonitrile acetate (**2**) in accordance with Kazlauskas rule, under orbital shaker and microwave irradiation. However, the (*R*)-mandelonitrile (**1**) was preferentially transformed to benzaldehyde by spontaneous chemical equilibrium by microwave irradiation and orbital shaker. For this kinetic resolution, it is suggested that the rate esterification of (*S*)-mandelonitrile (**1**) (k_3 , Scheme 1) occurred more slowly than the decomposition of (*R*)-mandelonitrile (**1**) to benzaldehyde (k_1 , Scheme 1) under microwave irradiation. Therefore, under microwave irradiation, the (*R*)-mandelonitrile (**1**) that was not acetylated by lipase was partially converted to benzaldehyde, and after completed the reaction to 8 h, was enriched with the (*S*)-enantiomer **1**.



Scheme 1. Pathway for kinetic enzymatic resolution and the spontaneous transformation of (*R*)-mandelonitrile (**1**) to benzaldehyde.

Finally, the hypothesis described in Scheme 1 was confirmed by GC-FID and HPLC analyses of the reaction products under conventional condition and microwave irradiation (see SI section).

Conclusions

In conclusion, this study investigated the first efficient kinetic resolution of (\pm)-mandelonitrile (**1**) by lipase from *C. antarctica* under conventional condition and microwave irradiation, producing the (*S*)-mandelonitrile acetate (**2**) with high selectivity (up to > 98% ee). The unreacted (*R*)-mandelonitrile (**1**) was partially converted to benzaldehyde from spontaneous chemical equilibrium under microwave irradiation and conventional condition.

Acknowledgements

A. L. M. Porto gratefully acknowledges Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for their financial support. J. R. Oliveira and S. S. Ribeiro thank FAPESP for the scholarships. The authors also wish to thank Novozymes (Curitiba-PR, Brazil) for donating the immobilized CALB (Novozym 435®) and wish to thank Prof. Timothy John Brockson (Universidade Federal de São Carlos) for optical rotation measurements.

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbq.org.br> as a PDF file.

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Submitted: March 3, 2012

Published online: June 26, 2012

FAPESP has sponsored the publication of this article.

Supplementary Information

Lipase-Catalyzed Kinetic Resolution of (\pm)-Mandelonitrile under Conventional Condition and Microwave Irradiation

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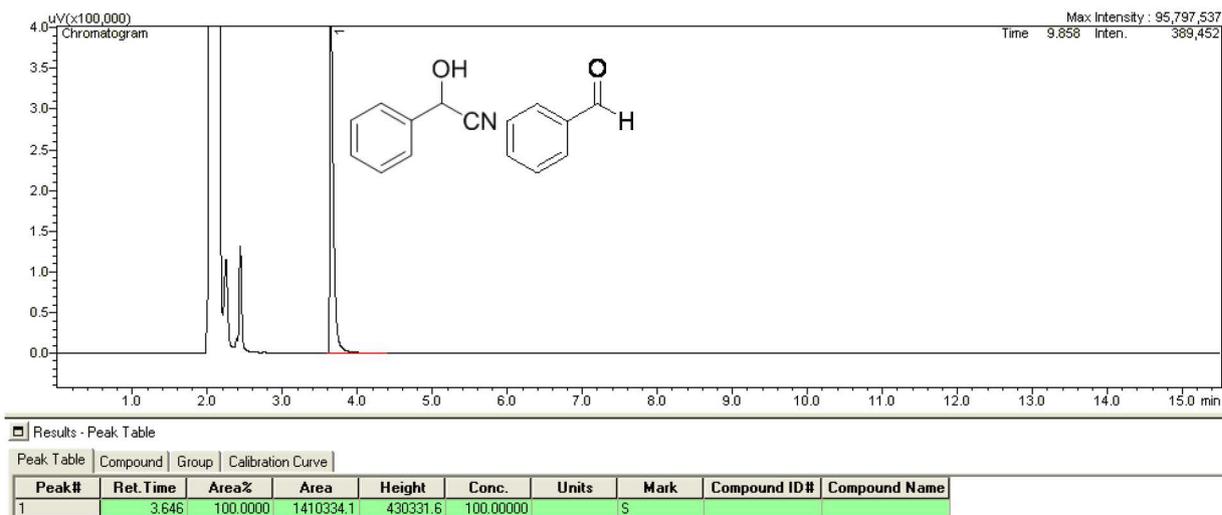


Figure S1. GC-FID chromatogram of (\pm)-mandelonitrile and benzaldehyde.

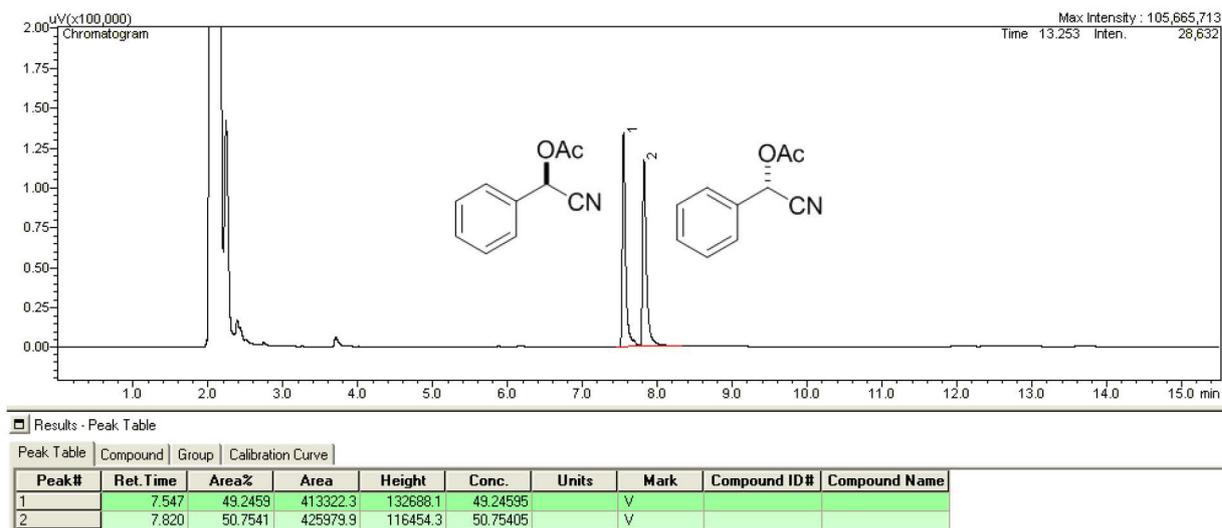


Figure S2. GC-FID chromatogram of (\pm)-mandelonitrile acetate.

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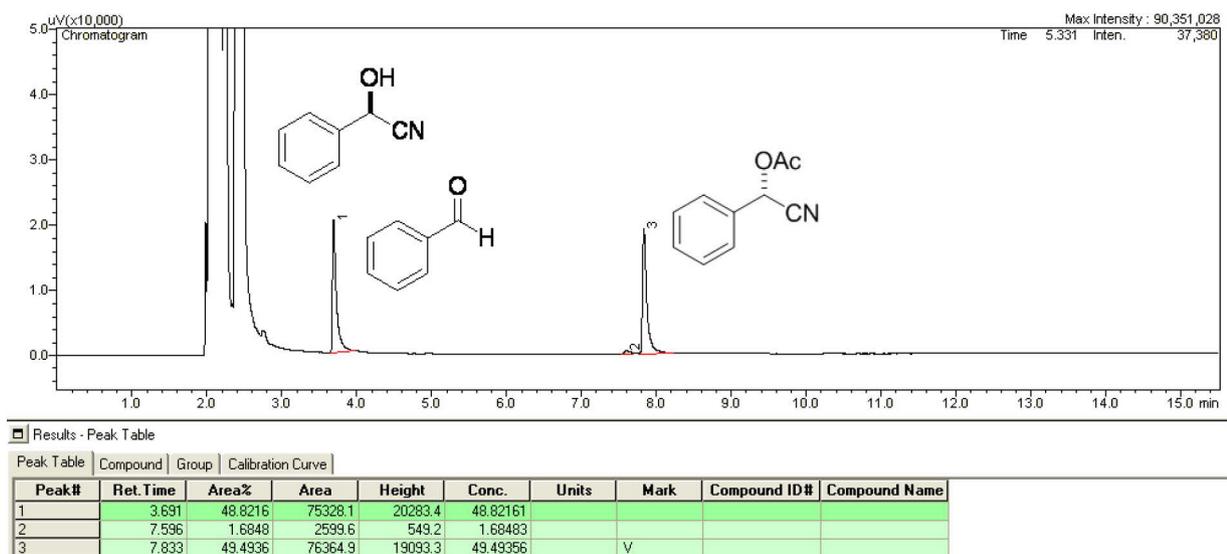


Figure S3. GC-FID chromatogram of (*S*)-mandelonitrile acetate (92% ee) and unreacted mandelonitrile/benzaldehyde obtained by lipase CALB under microwave irradiation (8 h, 80 °C) in toluene. This chromatogram reports a union of the four reactions.

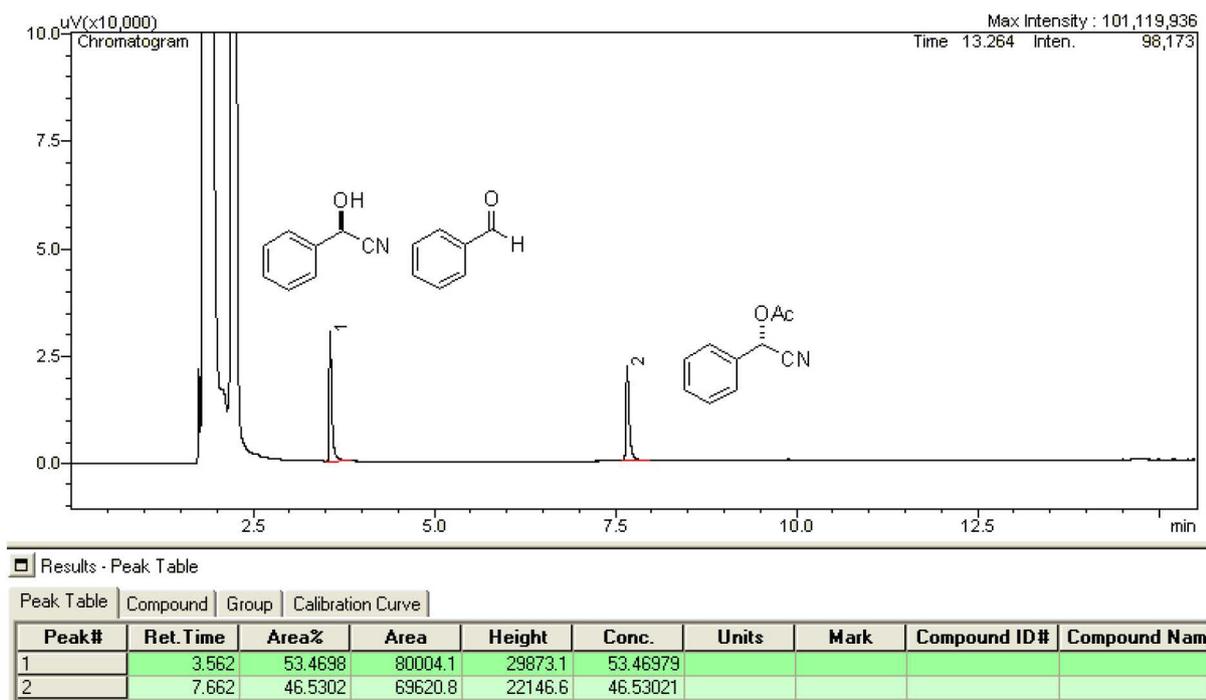


Figure S4. GC-FID chromatogram of (*S*)-mandelonitrile acetate (98% ee) and unreacted (*R*)-mandelonitrile/benzaldehyde obtained by lipase CALB under conventional condition on an orbital shaker in toluene (130 rpm, 168 h, 32 °C).

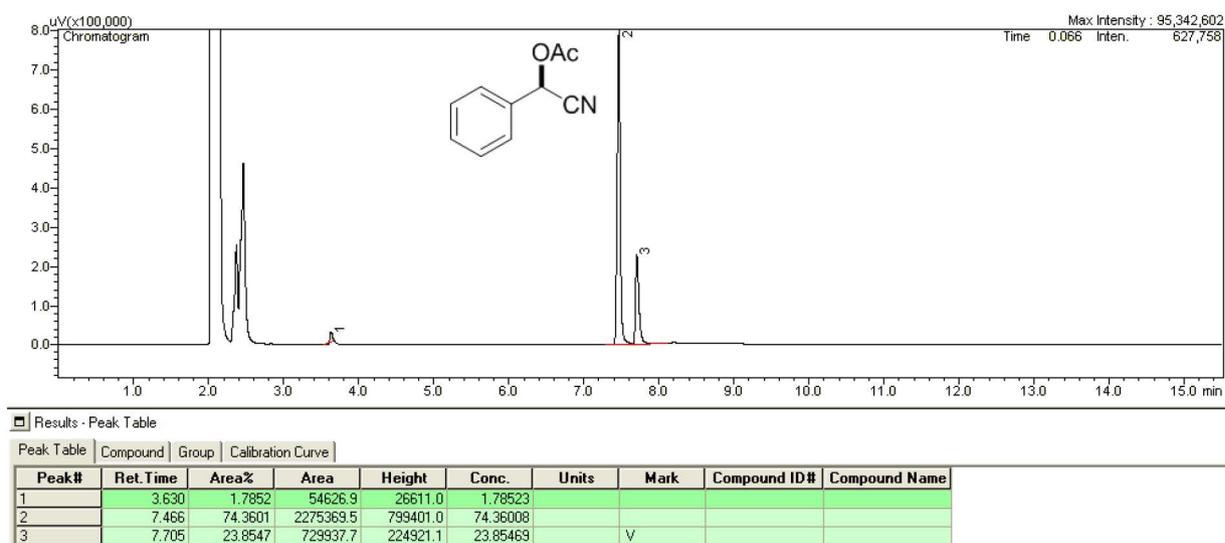


Figure S5. GC-FID chromatogram of (*R*)-mandelonitrile acetate (51% ee) obtained after derivatization with $\text{Ac}_2\text{O}/\text{py}$ of the unreacted (*R*)-mandelonitrile by CALB under conventional condition on an orbital shaker in toluene (130 rpm, 184 h, 32 °C). This chromatogram reports a union of the four reactions.

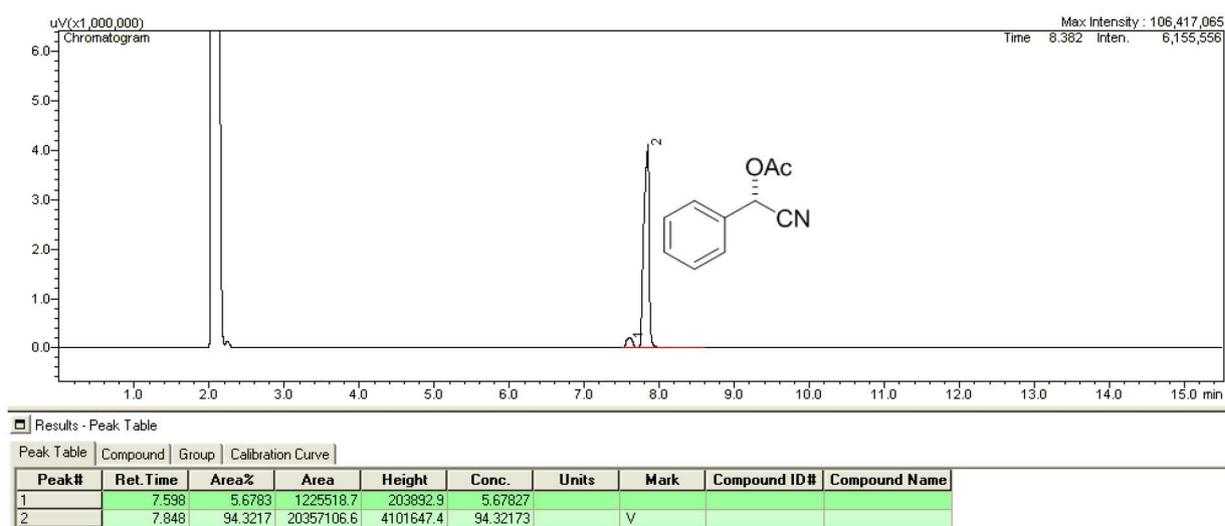


Figure S6. GC-FID chromatogram of (*S*)-mandelonitrile acetate (89% ee) obtained after derivatization with $\text{Ac}_2\text{O}/\text{py}$ of the unreacted mandelonitrile by CALB on microwave irradiation (8 h, 80 °C). This chromatogram reports a union of the four reactions.

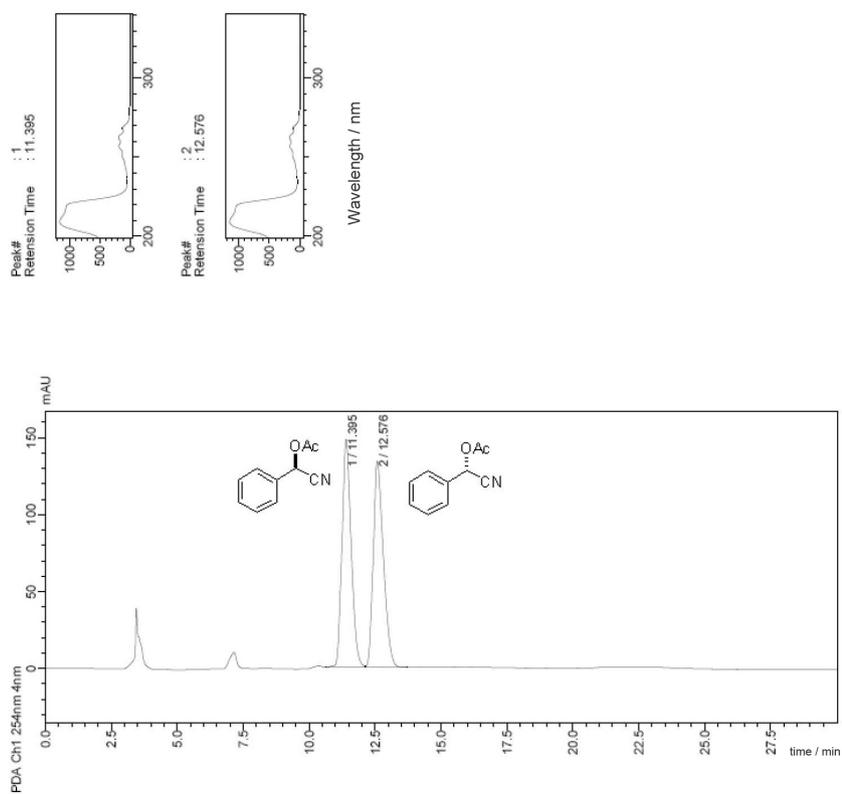


Figure S7. UV spectra and HPLC chromatogram of (\pm)-mandelonitrile acetate.

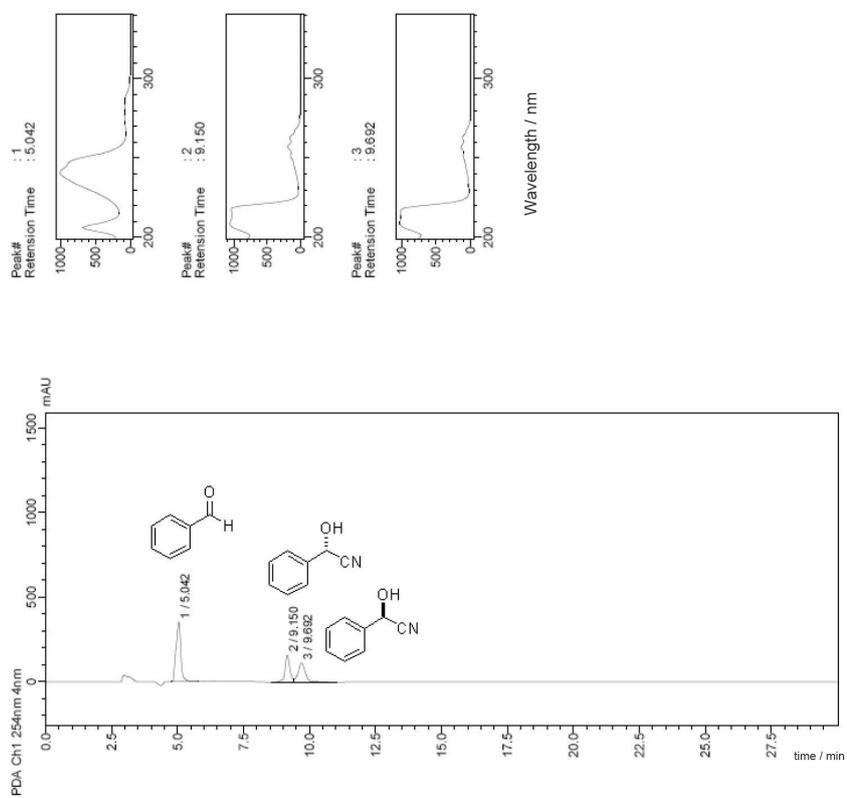


Figure S8. UV spectra and HPLC chromatogram of (\pm)-mandelonitrile and benzaldehyde.

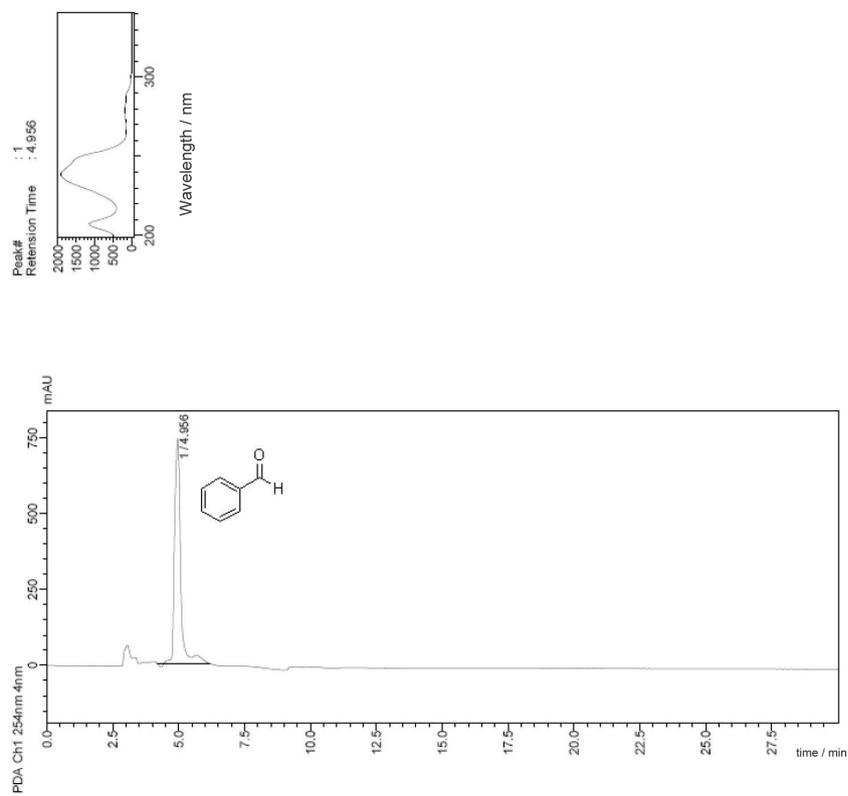


Figure S9. UV spectrum and HPLC chromatogram of benzaldehyde (retention time 4.96 min)

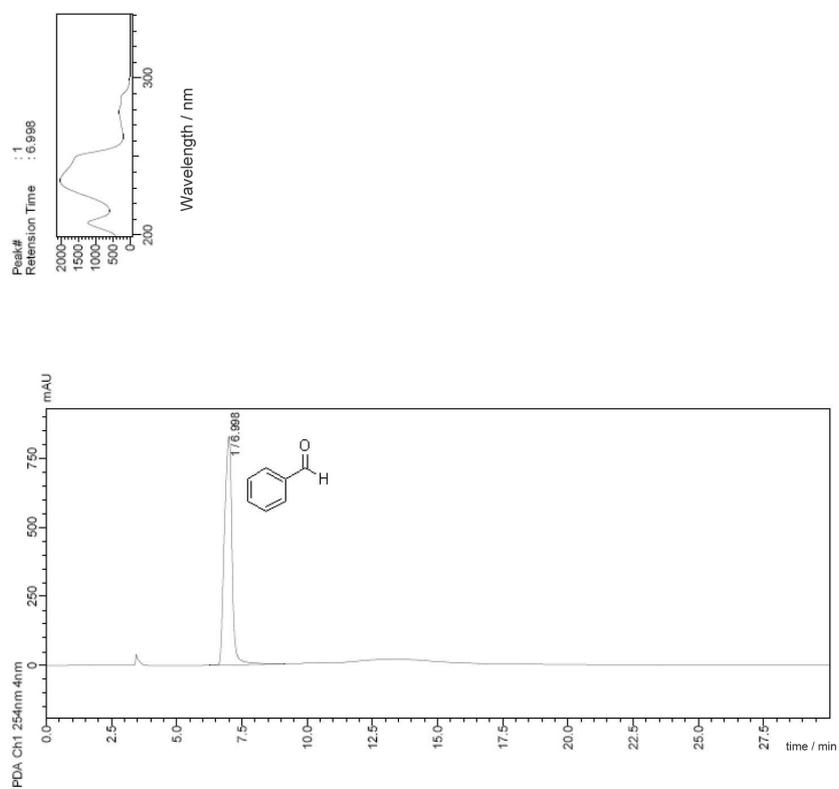


Figure S10. UV spectrum and HPLC chromatogram of benzaldehyde (retention time 6.99 min)

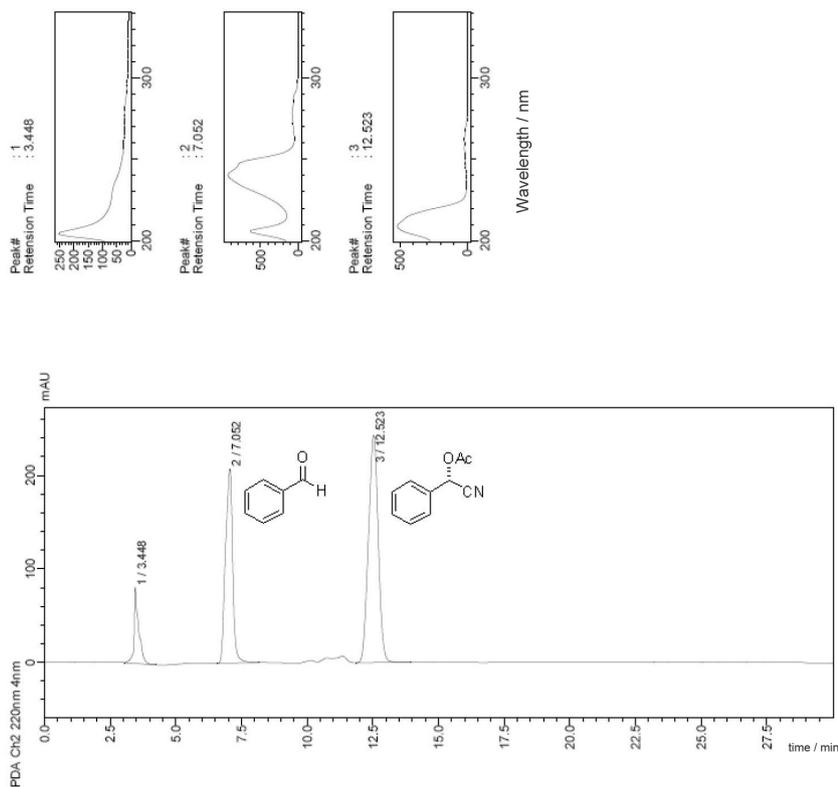


Figure S11. UV spectra and HPLC chromatogram of (*S*)-mandelonitrile acetate (98% ee) separated by column chromatographic. Reaction obtained by lipase CALB under conventional condition on an orbital shaker (130 rpm, 184 h, 32 °C).

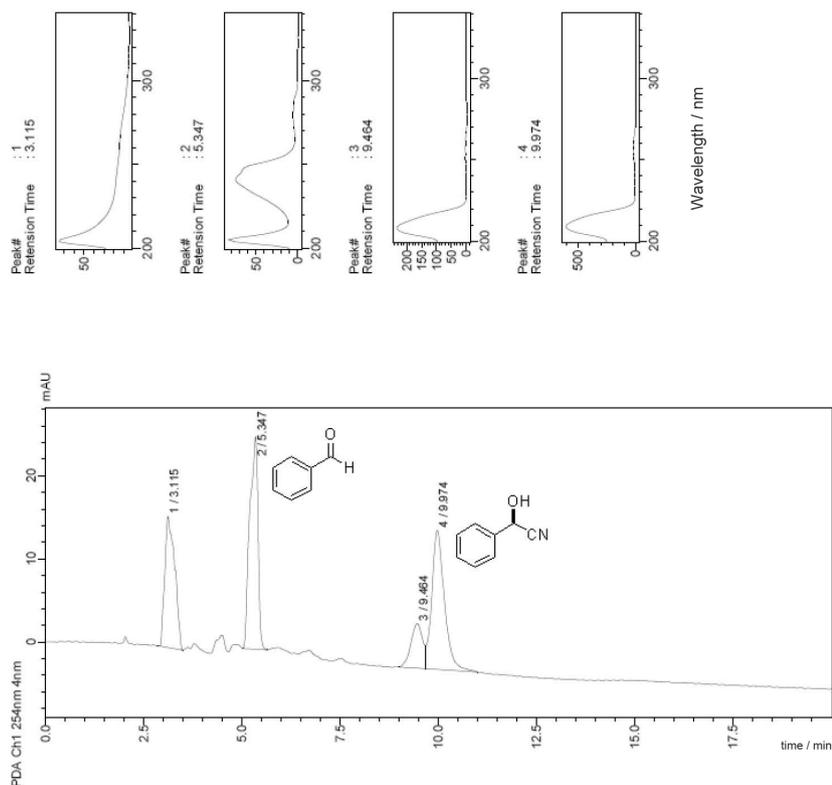


Figure S12. UV spectra and HPLC chromatogram of (*R*)-mandelonitrile (54% ee) separated by column chromatographic. Reaction obtained by lipase CALB under conventional condition on an orbital shaker (130 rpm, 184 h, 32 °C).