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Study of the enantioselectivity of the CAL-B-catalysed transesterification of α-substituted α-propylmethanols and α-substituted benzyl alcohols

Eduardo García-Urdiales, Francisca Rebolledo and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33006 Oviedo, Spain

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Abstract—A study of the enantioselectivity exhibited by the lipase B from *Candida antarctica* in the transesterification of different α-substituted α-propylmethanols with vinyl acetate is shown. The best results are obtained when the large-sized (L) substituent of the alcohol is either a phenyl group or more especially a cyclohexyl group, although the reaction rates are lower than when linear or slightly branched groups are present. It is also found that ramification at the β-position of the L substituent has a deleterious effect on both lipase activity and enantioselectivity. Moreover, some α-substituted benzyl alcohols bearing medium-sized (M) substituents larger than an ethyl and smaller than a propyl group are resolved by means of this methodology with moderate-good enantioselectivities ($E=46$–57) and similar reaction rates. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Lipases have been widely employed as catalysts for the resolution of racemic secondary alcohols.[1–3] Among them, lipase B from *Candida antarctica* (CAL-B) has proven to be a particularly efficient biocatalyst in these processes,[4,5] the stereochemical outcome of the reaction being reliably predictable by Kazlauskas' rule.[6] Moreover, substrate mapping studies have shown the structural requirements that a secondary alcohol needs in order to be successfully resolved by CAL-B. Thus, alcohols bearing a medium-sized (M) substituent larger than an ethyl group or a large-sized (L) substituent smaller than an *n*-propyl group lead to poor enantiomeric ratio (E)[7] values. The same behaviour is observed if the M substituent of the alcohol contains chlorine or bromine atoms.[8]

However, there are reported examples in the literature of highly selective CAL-B-catalysed resolutions of some secondary alcohols which do not fulfil these requirements.[9,10] In this sense, a previous study carried out in our research group has shown that CAL-B catalyses the resolution of (±)-1-phenylbutan-1-ol (M substituent larger than an ethyl group) with moderate enantioselectivity ($E=19$).[11] Moreover, this value is higher than the one reported for the resolution of (±)-decan-4-ol ($E=10$).[8] Both substrates differ only in their L substituents, which are made up of the same number of carbon atoms. In order to shed light on the influence that the different arrangements adopted by the L substituent can have on both the activity and the enantioselectivity of CAL-B. Herein, we wish to report our results concerning the resolution of some α-substituted α-propylmethanols bearing a number of six carbon groups as the L substituent. Likewise, we report the resolution of a number of racemic α-substituted benzyl alcohols bearing M substituents which are larger than an ethyl group, in order to establish more accurate parameters concerning the largest size of this substituent that can be efficiently accepted by this lipase.

2. Results and discussion

Transesterifications of alcohols (±)-**1b**–**h** (Table 1) were carried out using lipase B from *C. antarctica* as catalyst (Novozym 435), vinyl acetate as acylating agent and 1,4-dioxane as solvent. In order to avoid the competitive enzymatic hydrolysis of the involved esters, all reagents and solvents were dried prior to use, a nitrogen atmosphere was employed and 4 Å molecular sieves were added to ensure low water activity in the reaction medium. For each transesterification, six aliquots were

* Corresponding author. Tel./fax: +34 985 10 34 48; e-mail: vgs@sauron.quimica.uniovi.es

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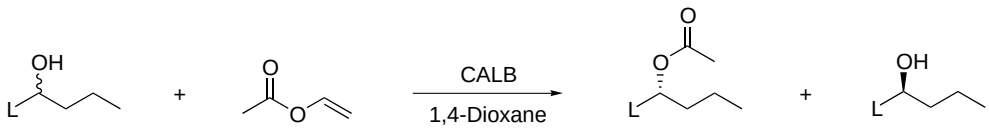
Table 1. CAL-B-catalysed transesterification of alcohols (±)-**1a–e**



(±)-**1a-e** (*R*)-**2a-e** (*S*)-**1a-e**

Entry	Alcohol	L	*t* (h)	*c* (%)	*E*
1	(±)-**1a**	Ph	163	17.3	19.4±0.4
2	(±)-**1b**	*n*-C_6H_{13}	96	33.7	9.0±0.2
3	(±)-**1c**	*i*-C_6H_{13}	160 (96)	44.1 (33.9)	11.5±0.1
4	(±)-**1d**	2-Ethylbutyl	163	5.5	3.6±0.1
5	(±)-**1e**	Cyclohexyl	168	5.9	65.1±2.8

withdrawn at regular intervals and analysed by means of chiral GC. Thus, the enantiomeric excesses of the substrates **1** (e.e.$_s$) and the products **2** (e.e.$_p$) were measured during all the process. For each transesterification reaction four parameters were determined: *E*, *c*, c_{FRE} and c_{SRE}. Enantiomeric ratio (*E*) values were calculated by fitting the e.e.$_s$ and e.e.$_p$ values to the equation developed by Rakels et al.[12] Conversion values (*c*) were determined from the e.e.$_s$ and e.e.$_p$ values according to Sih et al.[13] Finally, conversion values for the fast- and slow-reacting enantiomers of the substrates (c_{FRE} and c_{SRE}) were determined by means of the degree of conversion (*c*) and the ratio of the peak areas corresponding to each enantiomer of the product **2** measured for each aliquot.

2.1. Resolution of α-substituted α-propylmethanols

We first compared the result previously reported for the resolution of (±)-1-phenylbutan-1-ol [(±)-**1a**][11] with the ones obtained for alcohols (±)-**1b–e** under the same experimental conditions (Table 1). All substrates possess an L substituent made up of six carbon atoms and in all cases, the enzyme follows Kazlauskas' rule; thereby esters (*R*)-**2a–e** were obtained as the major enantiomers. As can be seen in Table 1, the reaction rate and the enantioselectivity vary greatly according to the structure of the L group. The presence of ramification (branching) at the α- or β-positions of the L group has a negative influence on the reaction rate. Thus, for alcohols (±)-**1b**,**c** (entries 2 and 3) the degree of conversion reached is higher than for alcohols (±)-**1a** (entry 1) and, especially, (±)-**1e** (entry 5) and (±)-**1d** (entry 4). With regard to the enantioselectivity, the higher values are obtained with (±)-**1a** and (±)-**1e** (entries 1 and 5), that is, with substrates bearing a conformationally rigid L substituent. Again, ramification at the β-position in (±)-**1d** (entry 4) has a negative influence on the enantioselectivity.

Phenyl and cyclohexyl rings have been described as bulkier groups than the corresponding linear or slightly branched aliphatic moieties made up of the same number of carbon atoms.[14] According to Kazlauskas' rule, this would mean that the differences in size between the M and the L substituents would be greater for alcohols (±)-**1a**,**e** than (±)-**1b**,**c**. This fact could account for the lower *E* values obtained for the latter.

Kazlauskas' rule has been traditionally translated into an active site model for lipases consisting of two 'pockets' of different size (Fig. 1).[15–17] When a secondary alcohol is resolved by a lipase, the fast-reacting enantiomer binds to the active site in the manner shown in Fig. 1. However, when the other enantiomer reacts with the lipase, its large-sized substituent has to be accommodated in the smallest pocket (the stereoselectivity pocket). Thus, the steric repulsions between this substituent and the stereoselectivity pocket account for the lower reaction rate for this enantiomer. As the enantiomeric ratio reflects the ratio of reactivity between the fast- and the slow-reacting enantiomers of a racemic mixture,[7] the differences in the *E* values measured for the transesterifications of alcohols (±)-**1a–e** could be ascribed to different rates of conversion for their slow-reacting enantiomers, their fast-reacting enantiomers or both of them. For irreversible processes, the degree of conversion is related to the reaction rate and so, to the activation energy of the reaction. The degree of conversion of each enantiomer of alcohols (±)-**1a–e** has to be related to the energy of the diastereomeric transition states that CAL-B forms with them. In Figs. 2 and 3, the conversions of each enantiomer of alcohols (±)-**1a–e** (c_{FRE} and c_{SRE}) are plotted against the reaction time. As can be seen, c_{FRE} and c_{SRE} values considerably vary with the L substituent of the substrates, thereby show-

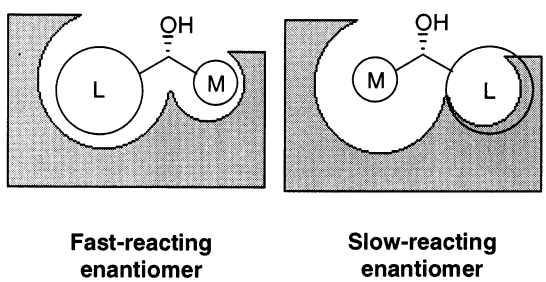


Figure 1. Active site model for lipases derived from Kazlauskas' rule and applied to the resolution of racemic secondary alcohols.

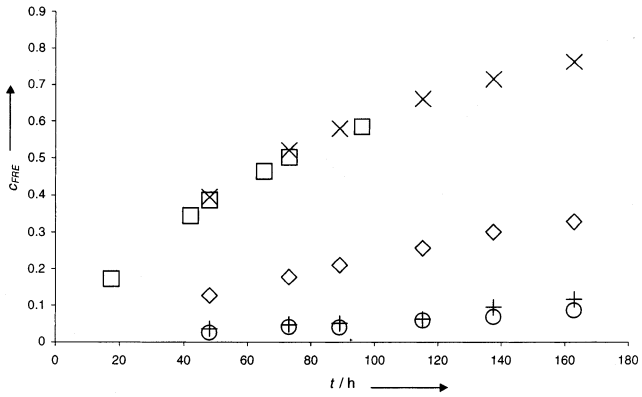


Figure 2. Degrees of conversion of the fast-reacting enantiomers of alcohol's **1a–f**: (*R*)-**1a** (◇), (*R*)-**1b** (□), (*R*)-**1c** (X), (*R*)-**1d** (○) and (*R*)-**1e** (+).

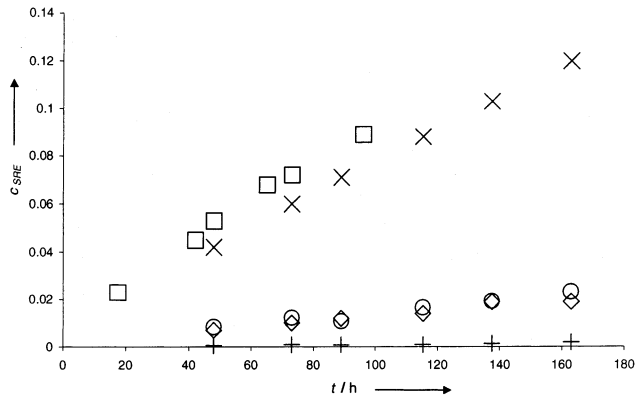


Figure 3. Degrees of conversion of the slow-reacting enantiomers of alcohols **1a–f**: (*S*)-**1a** (◇), (*S*)-**1b** (□), (*S*)-**1c** (X), (*S*)-**1d** (○) and (*S*)-**1e** (+).

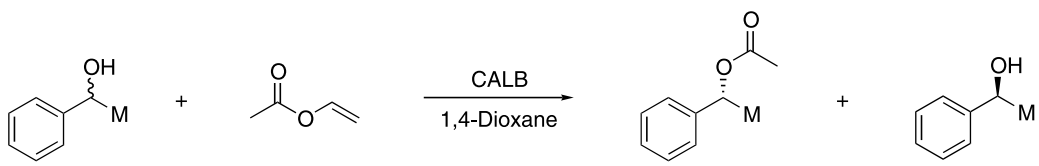
ing that the structure of this substituent greatly affects its interaction energy with the two pockets of the active site of this lipase. More specifically, both enantiomers of alcohols **1b** and **1c** react faster than the corresponding enantiomers of the other alcohols tested (**1a**, **1d** and **1e**). This situation can be ascribed to the fact that both pockets of the CAL-B active site can better accommodate the *n*-C_6H_{13} and the *i*-C_6H_{13} groups than the other L substituents collected in Table 1, thus corroborating the fact that linear or slightly branched substituents can be regarded as less bulky groups than more branched or cyclic moieties made up of the same number of carbon atoms.

2.2. Resolution of α-substituted benzyl alcohols

Because (±)-**1a** is converted with moderate enantioselectivity and conversion, we chose some benzyl alcohols, (±)-**1f–h** in order to investigate the influence of the M substituent. The results obtained in the CAL-B-catalysed transesterification of (±)-**1f–h** and that for (±)-**1a**[11] are collected in Table 2. In all cases, the enzyme follows Kazlauskas' rule, esters (*R*)-**2g** and **2h** and (*S*)-**2f** being obtained as the major enantiomers. Although CAL-B reacts at approximately the same rate with all the substrates, the enantioselectivity exhibited by the enzyme varied depending on the M substituent of the alcohol. Thus, the *E* values decreased as follows: (±)-**1g**, **1h**>(±)-**1f**>(±)-**1a**.

For substrates (±)-**1a** and **1f–h**, the L substituent is a phenyl group. Taking into account the active site model for lipases (Fig. 1), the larger the M substituent is, the lower *E* values should be obtained. If we consider the molecular volume of M as the criterion to estimate its size, Kazlauskas' rule can explain the different enantioselectivity displayed by CAL-B during the resolution of (±)-**1a**, **1f** and **1h** (Table 2, entries 1, 2 and 4). However, when alcohol (±)-**1g** (Table 2, entry 3) is included the rule fails. The analysis of the degrees of conversion obtained for each enantiomer of the substrates (Figs. 4 and 5) reveals some characteristic of the two pockets. As it is shown in Fig. 5 (*S*)-**1a** is converted faster than the other (*S*)-enantiomers, which means that the propyl moiety is the best accommodated group in the largest pocket, probably due to a better steric interaction. From Fig. 4, the order of accomodation in the smallest pocket is deduced as follows: allyl (83

Table 2. CAL-B-catalysed transesterification of alcohols (±)-**1a** and **1f–h**



(±)-**1a,f-h** (*R*)-**2a,g,h** (*S*)-**2f** (*S*)-**1a,g,h** (*R*)-**1f**

Entry	Alcohol	M	*c* (%)[a]	*E*	*V* (Å^3)[b]
1	(±)-**1a**	*n*-Propyl	17.3	19.4±0.4	90
2	(±)-**1f**	Methoxymethyl	17.4	45.8±3.9	78
3	(±)-**1g**	Allyl	23.1	55.1±0.7	83
4	(±)-**1h**	Cyanomethyl	20.6	56.5±0.8	66

[a] Reaction time: 163 h.
[b] Values calculated with PC MODEL 5.13 (Serena Software, USA).

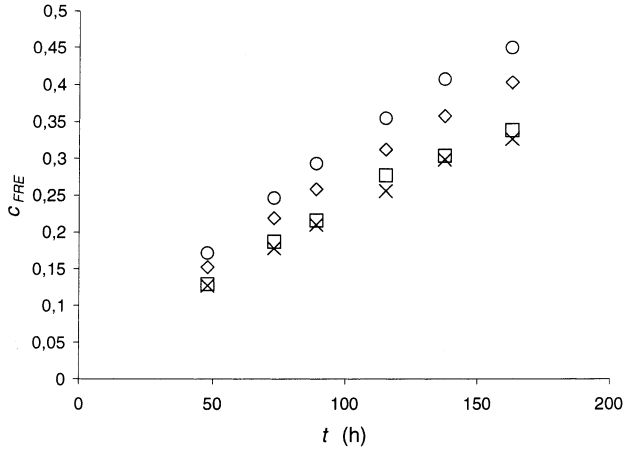


Figure 4. Degree of conversion of the fast-reacting enantiomers of alcohols **1a** and **1f–h**: (*R*)-**1a** (X), (*S*)-**1f** (□), (*R*)-**1g** (○) and (*R*)-**1h** (◇).

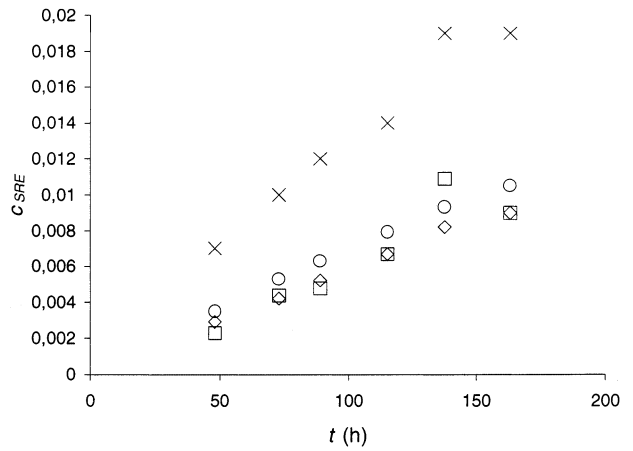


Figure 5. Degree of conversion of the slow-reacting enantiomers of alcohols **1a** and **1f–h**: (*R*)-**1a** (X), (*S*)-**1f** (□), (*R*)-**1g** (○) and (*R*)-**1h** (◇).

Å^3)>cyanomethyl (66 Å^3)>methoxymethyl (78 Å^3) and propyl (90 Å^3), which evidence that the steric repulsions are not the only interactions responsible for CAL-B enantioselectivity. In this sense, the distinct ability of the M substituents to adopt different conformations in the active site of the enzyme and the existence of electronic interactions could also play a key role during CAL-B enantiorecognition process. The traditional model, which only takes into account steric interactions on the basis of the size of the substituents, is inappropriate to explain CAL-B enantioselectivity towards these types of alcohols.

3. Conclusions

We have shown that lipase B from *C. antarctica* can be an efficient catalyst for the resolution of secondary alcohols bearing medium-sized substituents larger than an ethyl group but smaller than an *n*-propyl group. Results have been discussed on the basis of the model for lipase enantioselectivity derived from Kazlauskas' rule and some limitations to it have been put forward. Previous results concerning the resolution of (±)-1-phenylbutan-1-ol[11] led us to expect that solvent engineering will allow acceleration of the rate of these reactions, thus widening the range of substrates that can be successfully resolved by this lipase for synthetic applications.

4. Experimental

4.1. General

Lipase B from *C. antarctica*, Novozym 435, was a gift from Novo Nordisk co. and was employed without any previous treatment. All reagents were purchased from Aldrich Chemie, Avocado or Janssen. (±)-Decan-4-ol (±)-**1b**, (±)-1-phenyl-2-methoxyethanol (±)-**1f** and (±)-3-hydroxy-3-phenylpropanenitrile (±)-**1h** were obtained by standard reduction ($NaBH_4$, MeOH) of the corresponding commercially available ketones. (±)-6-Ethyloctan-4-ol (±)-**1d** and (±)-1-phenylbut-3-en-1-ol (±)-**1g** were prepared by addition of 2-ethylbutan-1-yl magnesium bromide to butanal and allyl magnesium bromide to benzaldehyde, respectively. (±)-8-Methylnonan-4-ol (±)-**1c** was prepared by addition of *n*-propyl magnesium bromide to 5-methyl-*N*-methyl-*N*-methoxyhexanamide, which was obtained by addition of *N*-methoxymethanamine to the 5-methylhexanoyl chloride (5-methylhexanoic acid, oxalyl chloride, $DMF_{(cat.)}$, THF). 1,4-Dioxane was distilled over sodium and stored under nitrogen. Flash chromatography was performed with Merck silica gel 60 (230–240 mesh). Optical rotations were measured by means of a Perkin–Elmer 1720-X FT IR spectrometer. Mass spectra were recorded on a VG Autospec. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX 300 (^{1}H 300 MHz and ^{13}C 75.5 MHz) spectrometer using TMS as internal standard. CG analyses were performed in a Hewlett–Packard 5890 Series II chromatograph equipped with a FID detector, using the capillary columns RtβDEXse (30 m×0.25 mm; Restek) and Chiraldex-BPH (30 m×0.25 mm; Astec) as chiral stationary phases and nitrogen as carrier gas (110 kPa).

4.2. Typical procedure for the enzymatic transesterification of alcohols (±)-1b–h

To a solution of the corresponding alcohol (1.0 mmol) in 1,4-dioxane (10 mL), vinyl acetate (3.0 mmol), molecular sieves (250 mg) and CAL-B (200 mg) were added. The resulting mixture was shaken at 30°C and 250 rpm for the time shown in Table 1. Six aliquots (10 μL) were withdrawn at regular intervals and analysed through chiral GC. For each enzymatic reaction a control experiment without enzyme was carried out and no non-enzymatic reaction was detected through GC analysis. For transesterifications of alcohols (±)-**1b**, **1c** and **1f–h**, after the reaction time shown in Table 1 (entries 2 and 3) and Table 2 (entries 2–4) the enzyme and the molecular sieves were filtered off and washed with dichloromethane and the organic solvents were

evaporated. Flash chromatography of the residue (eluent: dichloromethane/hexane gradient) yielded the corresponding substrates and products.

4.2.1. (*R*)-Decan-4-yl acetate (*R*)-2b. Colourless liquid; yield, 90% (34% *c*); $[\alpha]_D^{22}$ +4.7 (*c* 0.82, $CHCl_3$, 74% e.e.); IR (neat) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 0.88 (m, 6H, $2CH_3$), 1.26 (m, 10H, $5CH_2$), 1.49 (m, 4H, $2CH_2$), 2.03 (s, 3H, CH_3), 4.87 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 13.9 (CH_3), 14.0 (CH_3), 18.5 (CH_2), 21.2 (CH_3), 22.5 (CH_2), 25.2 (CH_2), 29.1 (CH_2), 31.7 (CH_2), 34.1 (CH_2), 36.2 (CH_2), 74.1 (CH), 170.9 (C=O); MS (70 eV) *m*/*z* (%) 157 (18), 140 (56), 115 (80), 97 (80), 43 (100).

4.2.2. (*R*)-2-Methylnonan-6-yl acetate (*R*)-2c. Colourless liquid; yield, 85% (44% *c*); $[\alpha]_D^{21}$ +5.9 (*c* 1.07, $CHCl_3$, 73% e.e.); IR (neat) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 0.85 (d, 6H, $^3J_{HH}=6.5$ Hz, $2CH_3$), 0.90 (t, 3H, $^3J_{HH}=7.5$ Hz, CH_3), 1.16 (m, 2H, CH_2), 1.30 (m, 4H, 2 CH_2), 1.49 (m, 5H, 2 CH_2, CH), 2.03 (s, 3H, CH_3), 4.87 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 13.9 (CH_3), 18.5 (CH_2), 21.2 (CH_3), 22.4 (CH_3), 22.5 (CH_3), 23.0 (CH_2), 27.8 (CH), 34.3 (CH_2), 36.2 (CH_2), 38.7 (CH_2), 74.1 (CH), 170.9 (C=O); MS (70 eV) *m*/*z* (%) 157 (7), 140 (17), 115 (53), 97 (67), 43 (100).

4.2.3. (*S*)-1-Phenyl-2-methoxyethyl acetate (*S*)-2f. Colourless liquid; yield, 92% (17% *c*); $[\alpha]_D^{22}$ +84.2 (*c* 1.19, $CHCl_3$, 95% e.e.); IR (neat) 1739 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 2.13 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 3.56 (dd, 1H, $^2J_{HH}=10.9$ Hz, $^3J_{HH}=3.9$ Hz, C*H*H), 3.73 (dd, 1H, $^2J_{HH}=10.9$ Hz, $^3J_{HH}=8.0$ Hz, CH*H*), 5.97 (dd, 1H, $^3J_{HH}=8.0$ Hz, $^3J_{HH}=3.9$ Hz, CH), 7.35 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 21.2 (CH_3), 59.1 (CH_3), 74.2 (CH), 75.2 (CH_2), 126.6 (CH), 128.2 (CH), 128.4 (CH), 137.4 (C), 170.2 (C=O); MS (70 eV) *m*/*z* (%) 162 (40), 149 (100).

4.2.4. (*R*)-1-Phenylbut-3-en-1-yl acetate (*R*)-2g. Colourless liquid; yield, 87% (23% *c*); $[\alpha]_D^{22}$ +58.4 (*c* 0.81, $CHCl_3$, 95% e.e.); IR (neat) 1739 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 2.08 (s, 3H, CH_3), 2.60 (m, 2H, CH_2), 5.07 (m, 2H, CH_2), 5.71 (m, 1H, CH), 5.81 (dd, 1H, $^3J_{HH}=6.3$ Hz, $^3J_{HH}=7.7$ Hz, CH), 7.34 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 21.2 (CH_3), 40.7 (CH_2), 75.0 (CH), 118.0 (CH_2), 126.4 (CH), 127.9 (CH), 128.3 (CH), 133.2 (CH), 139.9 (C), 170.2 (C=O); MS (70 eV) *m*/*z* (%) 190 (M^+, <1), 149 (80), 107 (100).

4.2.5. (*R*)-2-Cyano-1-phenylethyl acetate (*R*)-2h. White solid; yield, 94% (21% *c*); mp 123–124°C; $[\alpha]_D^{22}$ +71.7 (*c* 1.21, $CHCl_3$, 95% e.e.); IR (neat) 2252, 1739 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 2.16 (s, 3H, CH_3), 2.91 (dd, 2H, $^2J_{HH}=1.0$ Hz, $^3J_{HH}=6.2$ Hz, CH_2), 5.98 (dd, 1H, $^3J_{HH}=6.3$ Hz, $^3J_{HH}=6.0$ Hz, CH), 7.40 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.9 (CH_3), 25.5 (CH_2), 70.4 (CH), 116.0 (CN), 126.0 (CH), 128.9 (CH), 129.1 (CH), 137.1 (C), 169.5 (C=O); MS (70 eV) *m*/*z* (%) 189 (M^+, 55), 162 (59), 149 (38), 147 (74), 130 (48), 120 (53), 107 (100).

4.3. Determination of the enantiomeric excesses

The e.e. of alcohols **1b–h** and esters **2b–h** were determined by means of chiral GC. Only for the case of compounds **1c**, **1d**, **1g** and **2e** direct analyses were not possible. Thus, alcohols **1c** and **1d** were transformed into their propanoyl ester derivatives (*O*-Prp-**1c**, **1d**), alcohol **1f** into its trifluoroacetyl derivative (*O*-TFA-**1f**) and ester **2e** into its corresponding alcohol (**1e**) prior to analysis. Conditions are as follows. **1b**: RtβDEXse, 80°C, 30 min hold, 80–120°C, 2°C/min, 10 min hold, t_R1 (*R*) = 46.3 min, t_R2 (*S*) = 47.1 min, Rs 1.0; *O*-Prp-**1c**: Chiraldex-BPH, 50°C, 5 min hold, 50–100°C, 1°C/min, 30 min hold, t_R1 (*S*) = 36.9 min, t_R2 (*R*) = 37.6 min, Rs 1.2; *O*-Prp-**1d**: Chiraldex-BPH, 50°C, 5 min hold, 50–100°C, 1°C/min, 10 min hold, t_R1 (*S*) = 32.5 min, t_R2 (*R*) = 33.3 min, Rs 1.9; **1e**: RtβDEXse, 100°C, 25 min hold, t_R1 (*S*) = 18.9 min, t_R2 (*R*) = 20.6 min, Rs 2.1; *O*-TFA-**1f**: RtβDEXse, 80°C, 20 min hold, 80–120°C, 2°C/min, 30 min hold, t_R1 (*R*) = 32.0 min, t_R2 (*S*) = 32.4 min, Rs 1.0; **1g**: RtβDEXse, 80°C, 5 min hold, 80–150°C, 2°C/min, 10 min hold, t_R1 (*S*) = 34.3 min, t_R2 (*R*) = 34.6 min, Rs 1.8; **1h**: RtβDEXse, 170°C, 17 min hold, t_R1 (*R*) = 13.6 min, t_R2 (*S*) = 14.5 min, Rs 3.9; **2b**: RtβDEXse, 80°C, 30 min hold, 80–120°C, 2°C/min, 10 min hold, t_R1 (*S*) = 47.8 min, t_R2 (*R*) = 48.3 min, Rs 0.8; **2c**: Chiraldex-BPH, 50°C, 5 min hold, 50–100°C, 1°C/min, 30 min hold, t_R1 (*S*) = 30.4 min, t_R2 (*R*) = 31.2 min, Rs 1.2; **2d**: Chiraldex-BPH, 50°C, 5 min hold, 50–100°C, 1°C/min, 10 min hold, t_R1 (*S*) = 26.7 min, t_R2 (*R*) = 27.8 min, Rs 2.1; **2f**: RtβDEXse, 80°C, 20 min hold, 80–120°C, 2°C/min, 30 min hold, t_R1 (*R*) = 50.5 min, t_R2 (*S*) = 51.5 min, Rs 1.7; **2g**: RtβDEXse, 80°C, 5 min hold, 80–150°C, 2°C/min, 10 min hold, t_R1 (*S*) = 32.0 min, t_R2 (*R*) = 32.6 min, Rs 2.3; **2h**: RtβDEXse, 170°C, 17 min hold, t_R1 (*S*) = 9.7 min, t_R2 (*R*) = 9.9 min, Rs 1.7.

4.4. Determination of the absolute configuration

The absolute configuration of alcohols **1f** and **1g** was determined by comparison of the sign of their optical rotations with the data published in the literature for their (*R*)-enantiomers.[18,19] The absolute configuration of alcohol **1h** was determined by comparison of the elution times of their peaks in the GC chromatogram with those published for both enantiomers of this compound.[20] Finally, the absolute configuration of esters **2b–e** was assigned assuming that the enzyme followed Kazlauskas' rule[6] during the transesterfication processes. This assumption is in accordance with the fact that the elution times of the peaks corresponding to the major enantiomers of esters **2b–d** and the one published for ester **2a**[9] are always higher than the ones corresponding to the minor enantiomers of these esters, no matter which stationary phase is employed.

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