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Solvent role in the lipase-catalysed esterification of cinnamic acid and derivatives. Optimisation of the biotransformation conditions

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ABSTRACT

The esterification of cinnamic acid has been deeply investigated using ethanol as nucleophile and *Candida antarctica* lipase type B (CAL-B) as suitable biocatalyst. Special attention has been paid to the role that the solvent plays in the production of ethyl cinnamate. Therefore, volatile organic solvents and deep eutectic mixtures were employed in order to find optimal reaction conditions. Once that hexane was selected as the solvent of choice, other parameters that affect the enzyme activity were investigated in order to produce ethyl cinnamate with excellent yield. The CAL-B loading, nucleophile equivalents, temperature and reaction time have been identified as key parameters in the enzyme efficiency, and the potential of lipase-catalysed esterification has been finally exploited to produce a series of ethyl esters with different pattern substitutions on the aromatic ring.

1. Introduction

Cinnamic acid (Fig. 1, **1a**) is a key intermediate in the biosynthesis of a wide number of biologically active compounds including coumarins, flavonoids, isoflavonoids, phenylpropopanoids and stilbenes among many others, finding the *trans*-isomer as its most common form in nature [1,2]. The importance of cinnamic acid and derivatives is demonstrated by the presence of this structural motif in numerous antibacterial, antifungal, anti-inflammatory, antioxidant and antitumor agents [3–5], making them attractive targets for the pharmaceutical industry. Interestingly, the importance of other cinnamic acid derivatives [6,7], including for instance the odorous cinnamaldehyde and ethyl cinnamate (**2a**), has not gone unnoticed for the production of agrochemicals, polymers cosmetics and fragrances, expanding the potential of cinnamic acid derivatives to other industrial sectors. In addition, hydroxycinnamic acids, such as ferulic (**1b**) and caffeic acid (**1c**), are currently gaining great importance because of their presence in numerous food ingredients, their esterified forms displaying remarkable bioactivity values since their solubilisation properties is favoured when considering different formulations [8].

Biocatalysis is currently considered a sustainable methodology for the production of valuable organic compounds based on the exquisite selectivity displayed by enzymes [9–13]. Among the enzyme classes, hydrolases are when possible the selection of choice, since they can work in a wide range of experimental conditions (i.e.: temperature, solvent, substrate concentrations …) [14]. This is especially feasible when immobilised forms are employed, providing higher biocatalyst stabilities and the possibility to reuse the enzyme [15–18]. In this context, probably *Candida antarctica* lipase type B (CAL-B) is the model enzyme to start with a screening due to its well-known application in aminolysis, ammonolysis, esterification, perhydrolysis, thiolysis or transesterification reactions [19–22].

Focusing on the esterification of cinnamic and phenolic acids, a few examples have been described in the literature (Scheme 1), resulting CAL-B, *Bacillus lincheniformis* lipase or *Thermomyces lanuginosus* lipase as efficient enzymes when combined with alkyl alcohols (methanol, ethanol, *n*-propanol, isopropanol, butanol, pentanol, 2-ethylhexanol, octanol, cyclohexanol, benzyl alcohol, lauryl alcohol, *cis*-9-octadecen-1-ol …) [23–36]. Remarkably, along the last three decades the solvent role has been deeply study, finding hydrophobic ones such as hexane, cyclohexane, cyclooctane, 1-octane or isooctane as the best solutions [25,26,29,33,35], while the design of solvent-free reactions [24,34] and the selection of DMSO [30], ionic liquids [23,27,28,31] or alcohols [32,36] appear as suitable alternatives, especially due to their ability to solubilise the starting carboxylic acid in order to achieve higher conversions.

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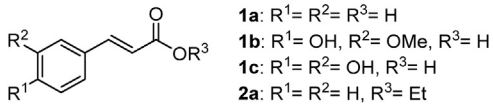
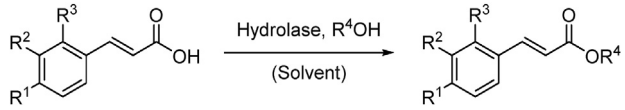


Fig. 1. Chemical structure of cinnamic acid, ferulic acid, caffeic acid and ethyl cinnamate (**1a**, **1b**, **1c** and **2a**, respectively).



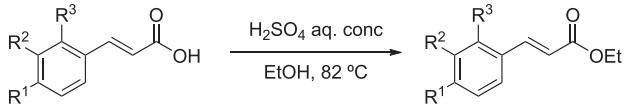
Scheme 1. Hydrolase-catalysed esterification of cinnamic acid and other carboxylic acid derivatives.

Disappointingly, all these enzymatic processes required high temperatures (45–80 °C) and sometimes also reduce pressures, so herein, we have tried to provide a deeper understanding of the esterification of cinnamic acid and derivatives using CAL-B. For that reason, a comprehensive study has been performed, paying special attention to the possibility of using volatile organic solvents including bio-based solvents, but also novel deep eutectic mixtures, which could improve the sustainability of the process and lead to a higher solubilisation and reactivity of these α,β-unsaturated carboxylic acids, respectively.

2. Results and discussion

Prior to the development of the enzymatic study, the synthesis of ethyl cinnamate (**2a**) was carried out by refluxing cinnamic acid in ethanol (EtOH) in the presence of concentrated sulphuric acid. After monitorisation of the chemical process by TLC analyses, observing the disappearance of the carboxylic acid, the ester **2a** was recovered in excellent purity and 86% yield after liquid-liquid extraction (Table 1, entry 1). Similarly, other cinnamic acids bearing different substitutions in the aromatic ring were converted into the corresponding ethyl esters (2–24 h, 75–98% yield) including ethyl ferulate and ethyl caffeate (**2b** and **2c**, entries 2 and 3) and compounds monosubstituted at the C-4 (entries 4–6), C-3 (entries 7–10) or C-2 position (entries 11–14) of the aromatic ring.

Table 1
Chemical esterification of **1a**-**n** using ethanol and sulphuric acid.



Entry	Acid	R^1	R^2	R^3	Time (h)	Yield **2a-n** (%)[a]
1	**1a**	H	H	H	2	86
2	**1b**	OH	OCH_3	H	24	94
3	**1c**	OH	OH	H	24	96
4	**1d**	NO_2	H	H	24	94
5	**1e**	CF_3	H	H	22	98
6	**1f**	CH_3	H	H	24	96
7	**1g**	H	CH_3	H	24	87
8	**1h**	H	OCH_3	H	22	98
9	**1i**	H	F	H	2	75
10	**1j**	H	NO_2	H	22	93
11	**1k**	H	H	NO_2	24	95
12	**1l**	H	H	CF_3	22	79
13	**1m**	H	H	Br	24	98
14	**1n**	H	H	OCH_3	24	95

[a] Yields of ethyl esters **2a**-**n** were calculated after isolation by liquid-liquid extraction.

2.1. Screening of solvents for lipase-catalysed experiments

An initial test was performed searching for the most adequate organic solvent, selecting the esterification of cinnamic acid as the benchmark reactions. Under enzymatic standard conditions for this type of lipase-catalysed reactions: the required solvent for a 100 mM concentration of **1a** in spite that most of the solvent could not solubilized the substrate, an excess of EtOH (3 eq) as nucleophile, a substrate:CAL-B 1:0.75 (*w/w*) ratio, 24 h and 30 °C, the reaction was monitorised over the time by TLC analyses. The set of organic solvent tested has been disclosed in Fig. 2 and organised depending on their logP values. The choice includes mostly hydrocarbons (heptane, hexane and pentane) and alkyl ethers such as cyclopentyl methyl ether (CPME), *tert*-butyl methyl ether (TBME), 2-methyl tetrahydruran (2-MeTHF) and tetrahydrofuran (THF), but also other media like ethyl acetate (EtOAc), acetonitrile (MeCN), Cyrene™ (dihydrolevoglucosenone) or the own EtOH as both nucleophile and solvent. It must be highlighted that this broad selection was motivated in some cases for the possibility to develop more sustainable processes by replacing traditional petroleum solvents by bio-based ones such as CPME [37,38], 2-MeTHF [39] and Cyrene™ [40].

As expected, hexane and heptane led to the higher conversions in these experimental conditions (17–18%), while only TBME (13%) seems to be a practical alternative although limited by its low boiling point for further optimisation. None of the bio-based solvents led to good results, so the focus was moved from there to the use of deep eutectic mixtures as solvents [41–45]. Interestingly, this class of neoteric solvents have gained recent attention as emerging environmentally friendly media when exploring the synthetic potential of enzymes [46], mainly hydrolases [47–49] and redox enzymes [50,51]. Initially, combinations of choline chloride (ChCl) as hydrogen bond acceptor (HBA) with either urea (U) or glycerol (Gly) as hydrogen bond donors (HBDs) were considered in 1:2 mol/mol ratio. Unfortunately, none of them gave appreciable conversion values in the esterification of **1a** under the reaction conditions attempted with the volatile organic solvents.

In spite of these discouraging initial results, but considering the importance of the water presence in enzymatic processes involving deep eutectic solvents (DES) [52,53], the ChCl:urea mixture was selected for further investigations since in addition it does not contain polyalcohols in its structure, avoiding thus the occurrence of competitive reactions. The DES was prepared again but drying firstly both hydrophilic components prior to obtain the eutectic mixture, and then a few ratios of DES:water were attempted in the esterification of cinnamic acid under previously set-up conditions (Table 2). Interestingly a maximum lipase activity was found when using a 10% of water, even higher than the one previously found with hexane, although long reaction times (72 h) and higher temperatures (60 °C) did not improve the conversion value.

2.2. Optimisation of the reaction conditions

Once selected hexane as the best solvent for the production of ethyl cinnamate (**2a**), other parameters were considered to achieve higher conversions including the use of different enzyme loadings (1:0.75 or 1:1, *w/w*), nucleophile excess (3 or 6 eq of EtOH), temperature (30, 45 or 55 °C) and reaction time (24 or 72 h). A summary of this research is depicted in Table 3. Starting from the experimental conditions used in the solvent screening, the reaction was prolonged from 1 to 3 days, increasing the conversion from 18

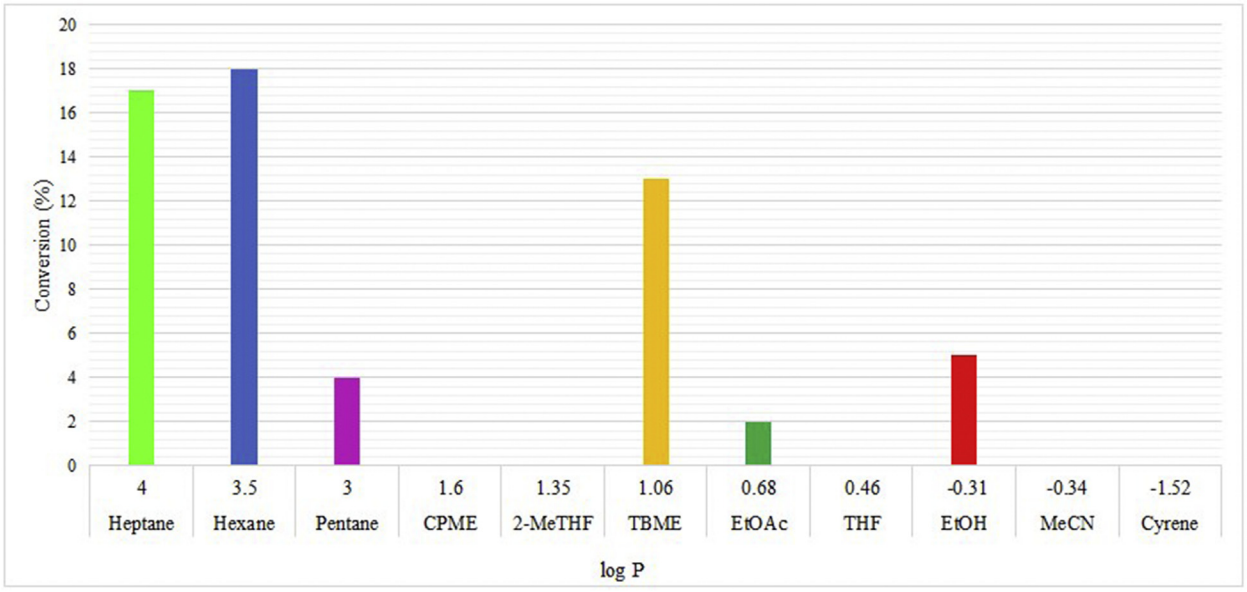


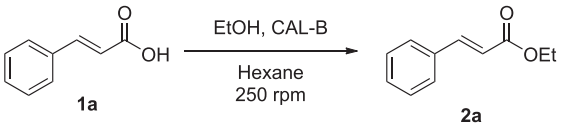
Fig. 2. Influence of the solvent in the esterification of cinnamic acid (**1a**) catalysed by CAL-B using 3 equivalents of ethanol for 24 h at 45 °C and 250 rpm.

Table 2
Lipase-catalysed esterification of cinnamic acid (**1a**) using ethanol (3 eq) as nucleophile and ChCl:urea (1:2 mol/mol) as reaction medium in the presence of water after 24 h at 45 °C and 250 rpm.

Entry	DES:H_2O	Conversion **2a** (%)[a]
1	100:0	<3
2	90:10	27
3	75:25	10
4	50:50	<3
5	25:75	<3

[a] Conversion values were calculated through ^{1}H NMR analyses of the reaction crudes after enzyme filtration and liquid-liquid extraction.

Table 3
Esterification of **1a** with EtOH using CAL-B and hexane as biocatalyst and solvent, respectively.



1a **2a**

Entry	T (°C)	t (h)	EtOH (eq)	**1a**: CAL-B (w/w)	Conversion **2a** (%)[a]
1	30	24	3	1:0.75	18
2	30	72	3	1:0.75	40
3	45	72	3	1:0.75	82
4	45	72	6	1:0.75	58
5	45	72	3	1:1	91
6	55	72	3	1:1	92

[a] Percentage of product **2a** was calculated through ^{1}H NMR analyses of the reaction crudes after enzyme filtration and solvent evaporation.

to 40% (entries 1 and 2). Therefore, the reaction temperature was increased to 45 °C, finding a 82% conversion after 72 h (entry 3), while an increase in the amount of EtOH led to a considerable loss of activity (58% conversion, entry 4). The reactivity was further improved by increasing the loading of enzyme to a 1:1 CAL-B: **1a** ratio (91% conversion, entry 5), while the use of a higher temperature (55 °C, entry 6) provided similar results.

The enzyme reusability was finally explored, finding that a significant loss of the activity was found after the third use of CAL-B to produce ethyl cinnamate in 66% conversion. This gradual enzyme deactivation can be explained based on the high temperature (45 °C) and long reaction times (72 h) required for reaching high conversion values.

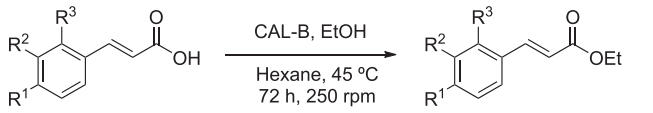
2.3. Reaction scope

Under optimised reaction conditions for **1a**, the esterification of a series of cinnamic acids **1b**-**n** was explored, those bearing a variety of pattern substitutions on the aromatic ring as already discussed in Table 1. Therefore, employing a 100 mM substrate concentration in hexane, 3 equivalents of ethanol and using CAL-B (1:1 w/w substrate/enzyme ratio), the reactions were left for 72 h at 45 °C and 250 rpm (Table 4). On the one hand, in some cases, and because of the high insolubility of the substrates, even at 45 °C, no conversion was found for ferulic acid (**1b**: R^1 = OH, R^2 = OCH_3 and R^3 = H), caffeic acid (**1c**: R^1 = R^2 = OH and R^3 = H) and 2-nitrocinnamic acid (**1d**: R^1 = NO_2 and R^2 = R^3 = H), or almost a negligible one was observed for 4-nitrocinnamic acid (**1e**, entry 4). On the other hand, substrates with electron withdrawing (entries 5 and 9–13, or electron donating (entries 6–8 and 14) groups at different positions of the aromatic ring were tested, finding complete conversions for **1i** and **1m**, which possess a halogen atom directed linked to the aromatic ring. In general, moderate to good isolated yields were obtained after column chromatography purification, attaining the lower conversions when considering selected electron donating groups in the phenyl ring (**1f** and **1n**).

3. Conclusions

The esterification of cinnamic acid and other related carboxylic acids have been explored using ethanol and two independent strategies, the traditional Fischer esterification using sulphuric acid as catalyst and the alternative biocatalytic approach with *Candida antarctica* lipase type B as biocatalyst. Good to excellent yields were attained using sulphuric acid and refluxing ethanol, while for the enzymatic transformation more sustainable conditions were attempted. Special attention has been paid to the solvent nature in

Table 4
CAL-B catalysed esterification of carboxylic acids **1a**-**n** using 3 equivalents of ethanol and hexane as solvent for 72 h at 45 °C and 250 rpm.



Entry	Acid	R^1	R^2	R^3	Conversion **2a-n** (%)[a]
1	**1a**	H	H	H	91 (79)
2	**1b**	OH	OCH_3	H	<3
3	**1c**	OH	OH	H	<3
4	**1d**	NO_2	H	H	<3
5	**1e**	CF_3	H	H	73 (36)
6	**1f**	CH_3	H	H	14
7	**1g**	H	CH_3	H	97 (90)
8	**1h**	H	OCH_3	H	78 (65)
9	**1i**	H	F	H	100 (73)
10	**1j**	H	NO_2	H	76 (69)
11	**1k**	H	H	NO_2	6
12	**1l**	H	H	CF_3	78 (67)
13	**1m**	H	H	Br	100 (90)
14	**1n**	H	H	OCH_3	19

[a] Conversion values were calculated through ^{1}H NMR analysis of the reaction crudes after enzyme filtration and solvent evaporation. Isolated yields after column chromatography purification appear in parentheses.

order to investigate new sustainable media solutions for the enzymatic production of alkyl esters, although the higher conversions were found with petroleum-derived solvents such as heptane and hexane. The use of bio-based solvents such as cyclopentyl methyl ether, 2-methyltetrahydrofurane or Cyrene™ does not seem to be a practical alternative, although when exploring the use of deep eutectic solvents in combination with water, significant conversions were achieved, which open the window for the esterification of highly insoluble compounds.

Optimisation of the reaction conditions in terms of CAL-B loading, ethanol equivalents, temperature and reaction time have allowed the production of a series of ethyl esters, some of them with complete conversion, and many of them with good isolated yields after column chromatography purification on silica gel. Overall, an alternative strategy to the traditional Fischer esterification was deeply studied, presenting the use of CAL-B in esterification reactions as a complementary approach to traditional chemical methods or the use of lipase-catalysed transesterification reactions starting from another ester instead of a carboxylic acid as starting material [54,55].

4. Experimental section

All chemical reagents were obtained from Sigma-Aldrich and used as received. The corresponding DES were prepared by heating the corresponding hydrogen bond donor (HBD, urea or glycerol) and acceptor (ABD, ChCl) overnight (ChCl:Gly 1:2 mol/mol at 60 °C; ChCl:urea 1:2 mol/mol at 100 °C). Initial experiments were performed with the chemicals as received but since especially ChCl is highly hygroscopic, urea and ChCl were dried in a high vacuum pump for a few days prior to be mixed under nitrogen atmosphere (ChCl at 60 °C and urea at room temperature). *Candida antarctica* lipase type B (CAL-B, Novozym® 435, 7300 PLU/g) was obtained from Novozymes.

Thin layer chromatographies (TLCs) were conducted with silica gel precoated plates and visualised with UV and potassium permanganate stain. Column chromatographies were performed using silica gel (230–400 mesh). NMR spectra were recorded on a 300 MHz spectrometer for the measurement of conversion values and characterisation of carboxylic acids and esters. All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. IR spectra were recorded on a FT/IR-4700 type A spectrophotometer, and the frequency (ν) of the most representative absorption bands are listed.

The performance of ^{1}H NMR for carboxylic acids **1a**-**n** and ethyl esters **2a**-**n** has allowed us the calculation of conversion values for enzymatic experiments, so experiments in the adequate deuterated solvent ($CDCl_3$, MeOH-d_4 and DMSO-d_6) were performed, assuring the complete solubility of both class of organic compounds to achieve reliable results.

4.1. General procedure for the chemical esterification of phenolic acids (*1a*-*n*)

A suspension of the corresponding phenolic acid **1a-n** (500 mg) in ethanol (EtOH, 3.8 mL, 65.1 mmol) was dissolved by the addition of a 98% aqueous solution of sulphuric acid (0.5 mL). The solution was magnetically stirred at 82 °C and monitorised by TLC analysis (20–60% EtOAc/hexane). After the disappearance of the starting carboxylic acid, the reaction was cooled to room temperature, and water (10 mL) was added. The resulting ethyl ester was extracted with EtOAc (3 × 15 mL), combining the organic phases, that were dried over Na_2SO_4. In the reactions with phenolic acids **1b** and **1c**, an additional washing step of the combined organic phases was performed, first with an $NaHCO_3$ aqueous saturated solution (15 mL) and later with brine (20 mL). After filtration and solvent evaporation under reduced pressure, the corresponding ethyl esters were recovered with high purity (**2a**-**n**, 75–98%, see Table 1).

Ethyl cinnamate (2a). Colourless liquid (86% isolated yield). R_f (60% EtOAc/hexane): 0.90. IR (ν): 1737, 1577, 1254, 1072 cm^{-1}. ^{1}H NMR δ (300.13 MHz, $CDCl_3$): 7.69 (d, 1H, J = 16.0 Hz), 7.57–7.48 (m, 2H), 7.42–7.35 (m, 3H), 6.44 (d, 1H, J = 16.3 Hz), 4.27 (q, 2H, J = 7.1 Hz), 1.34 (t, 3H, J = 7.1 Hz) ppm. ^{13}C RMN δ (75.5 MHz, $CDCl_3$): 167.1 (C), 144.7 (CH), 134.6 (C), 130.3 (CH), 129.0 (2 CH), 128.2 (2 CH), 118.4 (CH), 60.6 (CH_2), 14.4 (CH_3) ppm.

Ethyl ferulate (2b). Dark yellow colour solid (94% isolated yield). Melting point 64–65 °C. R_f (50% EtOAc/hexane): 0.74. IR (ν): 3528, 1737, 1510, 1252, 1031 cm^{-1}.^{1}H NMR δ (300.13 MHz, MeOD-d_4): 7.60 (d, 1H, J = 16.0 Hz), 7.18 (d, 1H, J = 2.0 Hz), 7.06 (dd, 1H, J = 8.3, 2.0 Hz), 6.80 (d, 1H, J = 8.2 Hz), 6.35 (d, 1H, J = 16.0 Hz), 4.22 (q, 2H, J = 7.1 Hz), 3.89 (s, 3H), 1.31 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 169.3 (C), 150.6 (C), 149.3 (C), 146.6 (CH), 127.7 (C), 124.0 (CH), 116.4 (CH), 115.6 (CH), 111.6 (CH), 61.4 (CH_2), 56.4 (CH_3), 14.6 (CH_3) ppm.

Ethyl caffeate (2c). Pale yellow colour solid (96% isolated yield). Melting point 144–148 °C. R_f (40% EtOAc/hexane): 0.37. IR (ν): 3450, 1658, 1278, 1041 cm^{-1}. ^{1}H NMR δ (300.13 MHz, MeOD-d_4): 7.53 (d, 1H, J = 15.9 Hz), 7.03 (d, 1H, J = 2.00 Hz), 6.93 (dd, 1H, J = 8.2, 2.0 Hz), 6.77 (d, 1H, J = 8.2 Hz), 6.24 (d, 1H, J = 15.9 Hz), 4.21 (q, 2H, J = 7.1 Hz), 1.30 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 169.3 (C), 149.5 (2 C), 146.7 (CH), 127.7 (C), 122.9 (CH), 116.4 (CH), 115.2 (CH), 115.0 (CH), 61.4 (CH_2), 14.6 (CH_3) ppm.

Ethyl 4-nitrocinnamate (2d). Scaly yellow solid (94% isolated yield). Melting point 137–140 °C. R_f (30% EtOAc/hexane): 0.82. IR (ν): 1708, 1644, 1390, 1110 cm^{-1}. ^{1}H NMR δ (300.13 MHz, DMSO-d_6): 8.23 (d, 2H, J = 8.8 Hz), 8.01 (d, 2H, J = 8.8 Hz), 7.75 (d, 1H, J = 16.1 Hz), 6.85 (d, 1H, J = 16.1 Hz), 4.21 (q, 2H, J = 7.1 Hz), 1.27 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, DMSO-d_6): 165.7 (C), 148.1 (C), 141.8 (CH), 140.5 (C), 129.5 (2 CH), 123.9 (2 CH), 122.5 (CH), 60.5 (CH_2), 14.2 (CH_3).

Ethyl 4-trifluoromethylcinnamate (2e). Colourless liquid (98% isolated yield). R_f (30% EtOAc/hexane): 0.79. IR (ν): 1705, 1631, 1397,

1067 cm $^{-1}$. 1H NMR δ (300.13 MHz, MeOD-d_4): 7.72–7.56 (m, 5H), 6.56 (d, 1H, J = 16.0 Hz), 4.22 (q, 2H, J = 7.1 Hz), 1.29 (t, 3H, J = 7.1 Hz). ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 168.0 (C), 144.0 (CH), 139.5 (C), 129.7 (2 CH), 127.2 (C), 126.8 (q, 2 CH, J = 3.8 Hz), 123.6 (q, C, J = 271.3 Hz), 121.9 (CH), 61.9 (CH_2), 14.6 (CH_3) ppm. ^{19}F NMR δ (282 MHz, MeOD-d_4): 64.4 ppm.

Ethyl 4-methylcinnamate (2f). Colourless liquid (96% isolated yield). R_f (20% EtOAc/hexane): 0.70. IR (ν): 2872, 1705, 1634, 1095 cm^{-1}. ^{1}H NMR δ (300.13 MHz, MeOD-d_4): 7.64 (d, 1H, J = 16.0 Hz), 7.47 (d, 2H, J = 8.1 Hz), 7.21 (d, 2H, J = 8.00 Hz), 6.45 (d, 1H, J = 16.0 Hz), 4.23 (q, 2H, J = 7.1 Hz), 2.35 (s, 3H), 1.32 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 168.8 (C), 146.1 (CH), 142.1 (C), 133.0 (C), 130.6 (CH), 129.2 (CH), 117.8 (CH), 61.5 (CH_2), 21.4 (CH_3), 14.6 (CH_3) ppm.

Ethyl 3-methylcinnamate (2g). Colourless liquid (87% isolated yield). R_f (30% EtOAc/hexane): 0.90. IR (ν): 2870, 1705, 1635, 1093 cm^{-1}. ^{1}H NMR δ (300.13 MHz, $CDCl_3$): 7.69 (d, 1H, J = 16.0 Hz), 7.35–7.19 (m, 4H), 6.45 (d, 1H, J = 16.0 Hz), 4.29 (q, 2H, J = 7.1 Hz), 2.38 (s, 3H), 1.36 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, $CDCl_3$): 167.1 (C), 144.8 (CH), 138.5 (C), 134.3 (C), 131.1 (CH), 128.8 (CH), 128.7 (CH), 125.3 (CH), 118.0 (CH), 60.5 (CH_2), 21.3 (CH_3), 14.4 (CH_3) ppm.

Ethyl 3-methoxycinnamate (2h). Colourless liquid (98% isolated yield). R_f (30% EtOAc/hexane): 0.87. IR (ν): 1704, 1635, 1094 cm^{-1}. ^{1}H NMR δ (300.13 MHz, $CDCl_3$): 7.65 (d, 1H, J = 16.0 Hz), 7.30 (d, 1H, J = 7.9 Hz), 7.11 (dt, 1H, J = 7.6, 1.3 Hz), 7.03 (t, 1H, J = 2.1 Hz), 6.92 (ddd, 1H, J = 8.2, 2.6, 1.0 Hz), 6.42 (d, 1H, J = 16.0 Hz), 4.26 (q, 2H, J = 7.1 Hz), 3.81 (s, 3H), 1.33 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, $CDCl_3$): 167.1 (C), 160.0 (C), 144.6 (CH), 135.9 (C), 130.0 (CH), 120.8 (CH), 118.6 (CH), 116.2 (CH), 113.0 (CH), 60.6 (CH_2), 55.4 (CH_3), 14.4 (CH_3) ppm.

Ethyl 3-fluorocinnamate (2i). Colourless liquid (75% isolated yield). R_f (30% EtOAc/hexane): 0.90. IR (ν): 1706, 1640, 1392, 1076 cm^{-1}. ^{1}H NMR δ (300.13 MHz, MeOD-d_4): 7.62 (d, 1H, J = 16.0 Hz), 7.44–7.30 (m, 3H), 7.16–7.07 (m, 1H), 6.51 (d, 1H, J = 16.0 Hz), 4.23 (q, 2H, J = 7.1 Hz), 1.31 (t, 3H, J = 7.1 Hz). ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 166.0 (C), 163.0 (d, C, J = 413.3 Hz), 144.5 (d, CH, J = 2.5 Hz), 138.1 (d, C, J = 7.8 Hz), 131.8 (CH, d, J = 7.9 Hz), 125.4 (d, CH, J = 3.2 Hz), 120.6 (CH), 118.1 (d, CH, J = 21.6 Hz), 115.2 (d, CH, J = 23.0 Hz), 61.8 (CH_2), 14.6 (CH_3) ppm. ^{19}F NMR δ (282 MHz, MeOD-d_4): 114.8 ppm.

Ethyl 3-nitrocinnamate (2j). Scaly white solid (98% isolated yield). Melting point 73–76 °C R_f (30% EtOAc/hexane): 0.84. IR (ν): 1712, 1643, 1392, 1100 cm^{-1}. ^{1}H NMR δ (300.13 MHz, $CDCl_3$): 8.37 (t, 1H, J = 2.0 Hz), 8.22 (ddd, 1H, J = 8.2, 2.3, 1.1 Hz), 7.82 (d, 1H, J = 8.0 Hz), 7.70 (d, 1H, J = 16.0 Hz), 7.58 (t, 1H, J = 8.0 Hz), 6.55 (d, 1H, J = 16.0 Hz) 4.28 (q, 2H, J = 7.1 Hz),1.34 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, $CDCl_3$): 166.2 (C), 148.8 (C), 141.8 (CH), 136.3 (C), 133.7 (CH), 130.1 (CH), 124.6 (CH), 122.5 (CH), 121.6 (CH), 61.0 (CH_2), 14.4 (CH_3) ppm.

Ethyl 2-nitrocinnamate (2k). Brown liquid (93% isolated yield). R_f (30% EtOAc/hexane): 0.80. IR (ν): 1711, 1638, 1392, 1095 cm^{-1}. ^{1}H NMR δ (300.13 MHz, DMSO-d_6): 8.08 (dd, 1H, J = 8.1, 1.4 Hz), 8.00–7.86 (m, 2H), 7.78 (td, 1H, J = 7.7, 1.4 Hz), 7.68 (td, 1H, J = 7.7, 1.5 Hz), 6.63 (d, 1H, J = 15.8 Hz), 4.22 (q, 2H, J = 7.1 Hz), 1.26 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, DMSO-d_6): 165.5 (C), 148.3 (C), 139.5 (CH), 133.9 (CH), 131.0 (CH), 129.3 (CH), 129.2 (C), 124.7 (CH), 122.6 (CH), 60.5 (CH_2), 14.1 (CH_3) ppm.

Ethyl 2-trifluoromethylcinnamate (2l). Pale yellow liquid (79% isolated yield). R_f (30% EtOAc/hexane): 0.81. IR (ν): 1714, 1632, 1393, 1061 cm^{-1}. ^{1}H NMR δ (300.13 MHz, MeOD-d_4): 8.00 (dq, 1H, J = 15.8, 2.3 Hz), 7.89–7.81 (m, 1H), 7.75–7.48 (m, 3H), 6.52 (d, 1H, J = 15.8 Hz), 4.25 (q, 2H, J = 7.1 Hz), 1.31 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 167.6 (C), 140.9 (CH), 134.3 (C), 133.7 (CH), 131.1 (CH), 129.8 (C), 129.4 (C), 129.2 (CH), 127.2 (q, CH, J = 5.8 Hz), 123.5 (CH), 61.9 (CH_2), 14.5 (CH_3) ppm. ^{19}F NMR δ (282 MHz, MeOD-d_4): 60.2 ppm.

Ethyl 2-bromocinnamate (2m). Colourless liquid (98% isolated yield). R_f (30% EtOAc/hexane): 0.9. IR (ν): 1708, 1634, 1094, 593 cm^{-1}. ^{1}H NMR δ (300.13 MHz, $CDCl_3$): 8.04 (d, 1H, J = 15.9 Hz), 7.59 (dt, 2H, J = 7.8, 1.8 Hz), 7.31 (tdd, 1H, J = 7.9, 1.3, 0.6 Hz), 7.21 (td, 1H, J = 7.7, 1.7 Hz), 6.38 (d, 1H, J = 15.9 Hz), 4.27 (q, 2H, J = 7.1 Hz), 1.34 (t, 3H, J = 7.1 Hz). ^{13}C NMR δ (75.5 MHz, $CDCl_3$): 166.5 (C), 143.0 (CH), 134.6 (C), 133.5 (CH), 131.3 (CH), 127.9 (CH), 127.8 (CH), 125.4 (C), 121.2 (CH) 60.8 (CH_2), 14.4 (CH_3) ppm.

Ethyl 2-methoxycinnamate (2n). Colourless liquid (95% isolated yield). R_f (20% EtOAc/hexane): 0.51. IR (ν): 1703, 1629, 1095 cm^{-1}. ^{1}H NMR δ (300.13 MHz, MeOD-d_4): 7.96 (d, 1H, J = 16.2 Hz), 7.55 (dd, 1H, J = 7.7, 1.6 Hz), 7.41–7.33 (m, 1H), 7.07–6.91 (m, 2H), 6.53 (d, 1H, J = 16.2 Hz), 4.22 (q, 2H, J = 7.1 Hz), 3.89 (s, 3H), 1.31 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR δ (75.5 MHz, MeOD-d_4): 169.2 (C), 159.8 (C), 141.3 (CH), 132.9 (CH), 129.7 (CH), 124.2 (C), 121.8 (CH), 119.1 (CH), 112.4 (CH), 61.5 (CH_2), 56.0 (CH_3), 14.6 (CH_3) ppm.

*4.2. General procedure for the esterification of carboxylic acids **1a-n** using CAL-B*

Ethanol (87.6 μL, 1.5 mmol) was added to a suspension of the corresponding carboxylic acid **1a-n** (0.5 mmol: 74.1 mg for **1a**, 97.1 mg for **1b**, 90.1 mg for **1c**, 96.6 mg for **1d, 1j** and **1k**, 108.1 mg for **1e** and **1l**, 81.1 mg for **1f** and **1g**, 89.1 mg for **1h** and **1n**, 83.1 for **1i**, and 113.5 mg for **1m**) and CAL-B (1:1 substrate:enzyme *w/w* ratio) in the desired solvent (5 mL, 100 mM of **1a-n**, see Fig. 2). The mixture was shaken in an orbital shaker for 72 h at 45 °C and 250 rpm. After this time, the enzyme was filtered-off and washed with ethyl acetate (2 × 5 mL). The solvent was distilled under reduced pressure, affording a reaction crude that was analysed by ^{1}H NMR to calculate the conversion of the lipase-catalysed esterification reaction. For those reactions leading to high conversion values, chromatography purification on silica gel were performed to isolated the corresponding ethyl esters and compared with the standards produced by chemical esterification (see Tables 1 and 4). Column chromatographies on silica gel were made using 10.0 g SiO_2 and mixtures of ethyl acetate and hexane as eluents (10–30% EtOAc/hexane).

*4.3. General procedure for the esterification of cinnamic acid (**1a**) using CAL-B in DES as reaction media*

Ethanol (35.0 μL, 0.6 mmol) and CAL-B (29.6 mg, 1:1 substrate:enzyme *w/w* ratio) was added to a mixture of cinnamic acid (**1a**, 29.6 mg, 0.2 mmol) and the corresponding eutectic mixture (2 mL). For these experiments involving the influence of water, the total volume of the (DES:H_2O) mixture was 2 mL for a final 100 mM cinnamic acid concentration (see Table 2). The mixture was shaken in an orbital shaker for 24 h at 45 °C and 250 rpm. After this time, the enzyme was filtered-off and washed with ethyl acetate (5 × 5 mL), finding two phases that were shaken in a vortex stirrer (3 × 10 s). The upper organic phase with the organic compounds **1a** and **2a** dissolved in EtOAc were collected, and the solvent distilled under reduced pressure. The resulting reaction crude was analysed by ^{1}H NMR to measure the reaction conversion.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.tet.2020.131873. These data include NMR spectra, MOL files and InChiKeys of the most important compounds described in this article.

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