Tetrahedron: *Asymmetry* 19 (2008) 2378–2384

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Effect of alkaloids on the activity and selectivity of *Candida rugosa* lipase in the kinetic resolution of 2-hydroxymethyl-1-phenylthioferrocene with planar chirality

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ARTICLE INFO

Article history:
Received 20 August 2008
Accepted 9 October 2008
Available online 20 November 2008

ABSTRACT

The use of the *o*-(4-chlorobenzoyl) hydroquinine as an additive in the enzymatic transesterification in the presence of *Candida rugosa* lipase (CRL) of a planar chiral molecule, such as 2-hydroxymethyl-1-phenylthioferrocene, shows a large enhancement of the reactivity and selectivity of this lipase towards this primary alcohol. An *E*-value of 143 could be reached at 53% conversion with vinyl acetate as the acylating agent in toluene.

1. Introduction

The synthesis of enantioenriched molecules is still a challenging task, which finds wide applications in various areas of industry.[1] Enzymes are very useful synthetic tools, which integrate numerous methods of organic transformations in a large variety of catalytic reactions. They offer access to a wide range of transformations, and, their utility has also been reinforced by the possibility to combine enzymatic and homogeneous catalysis. Furthermore, their use also contributes to the development of greener processes for the production of enantioenriched intermediates for organic synthesis.[2] In this field of research, enzymatic kinetic resolutions of racemic substrates such as secondary alcohols are one of the most exploited for their efficiency and versatility.[3] Hydrolytic enzymes[4] usually display high efficiencies and functional specificities, and are thus among some of the most exploited enzymatic catalysts used for biotransformations.

When optimising a chemical transformation involving an enzyme, various parameters have to be taken into account for they often control both the reactivity and the enantioselectivity in reactions such as kinetic resolutions. For instance, when dealing with the kinetic resolution of a racemic alcohol with a lipase, basic parameters such as the nature of the enzyme,[5] the acylating agent,[6] and the solvent,[7] as well as the amount of residual water in the reaction media[8] are often screened during the optimisation process. However, studies on the influence of additives on the outcome of enzymatic reactions are less frequent in the literature, despite the fact that some striking observations have been already reported for some transformations.[9]

Indeed, the catalytic activity and/or the enantioselectivity of a kinetic resolution can be strongly influenced by additives such as chiral and achiral amines,[10] crown ethers[11] and cyclodextrines.[12] Some of these additives are also used for the immobilisation, lyophilisation and mutagenesis of some enzymes. Some of us have already reported the influence of crown ethers on the issue of the *Candida cylindracea*[13]-catalysed acylation of secondary benzylic alcohols.

We then decided to focus our efforts on the effect of various additives on the transesterification enzymatic resolution of a primary α-substituted ferrocenyl alcohol, using *Candida rugosa* lipase (CRL, type VII) as a catalyst. Ferrocene derivatives bearing planar chirality through substitution at the α-position have been widely used for the design of chiral ligands with high efficiencies in various catalytic transformations.[14] Some derivatives have also been shown to display antitumoural properties,[15] and thus there is an increasing need for efficient synthetic methods which will allow a straightforward access to such enantiomerically enriched substrates.

2. Results and discussion

The racemic substrate **4** was easily prepared on a multigram scale, starting from the commercially available DMAF-**1** (Scheme 1). Ortholithiation of **1** was carried out following the literature procedure[16] with *tert*-butyl lithium in diethyl ether. The ortholithiated intermediate was then captured by diphenyldisulfide, and provided the racemic sulfide **2** in 92% yield. The amino group was then replaced by a hydroxyl by first reacting **2** in acetic anhydride.

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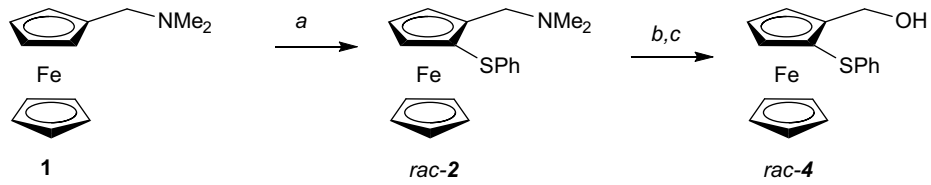
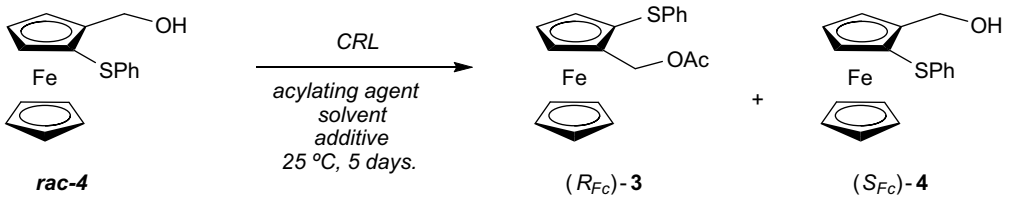
doi:10.1016/j.tetasy.2008.10.009



Scheme 1. Reagents and conditions: (a) (i) 1.1 equiv *t*-BuLi, Et_2O, rt, 15 min; (ii) 1.5 equiv PhSSPh, rt, 12 h (92%); (b) Ac_2O, 80 °C, 12 h (95%); (c) K_2CO_3, MeOH, 1 h (95%).



Scheme 2.

The racemic acetate **3** was then deprotected by base-catalysed methanolysis, and the target alcohol *rac*-**4** was isolated in excellent yield.

The first kinetic resolution experiments were carried out on a 1 mmol scale of starting alcohol **4** with 3 equiv of acylating agents (vinyl acetate VA or isopropenyl acetate IA) and 50 mg of commercially available *C. rugosa* type VII lipase (with an activity of 1170 U/mg) in 6 mL of anhydrous *t*-butyl methyl ether (TBME). Each reaction was run in duplicate and in one of the flasks; a known quantity of an additive was added. The course and selectivity of the kinetic resolution were checked by sampling the reaction mixture and quantification by HPLC on a *Chiralpak* AD column (Scheme 2). The observations on the effect of selected additives and acylating agents are collected in Table 1.

The first observations show that the reactivity of the enzyme is enhanced when vinyl acetate is used as the acylating agent; an acceleration of a factor of 3 (ratio = conversion/reaction time = 7) was reached with this reagent, albeit without any noticeable change in the selectivity. We also checked the influence of the presence of residual water in the reaction media. The effect of water on the reactivity and selectivity of enzymes in organic media is well documented,[9,13] and it can vary according to the choice of the acylating agent.[7,15] The influence of water was also studied by carrying out experiments with the addition of molecular sieves, or with the addition of hydrophilic co-solvents (Table 1). For both acylating agents, we observed that the beneficial effect of molecular sieves as the reaction was accelerated in both cases, and a slight improvement in the selectivity factor was also measured (entries 2 and 9 vs entries 1 and 8).

In all cases, the addition of water or ethylene glycol (entries 4 and 5) inhibits the reaction totally, and no conversion of the substrate was observed after 5 days. DMSO and DFM gave similar results (entries 6 and 7) but the addition of molecular sieves, used as a water sequestrate, not only restored the activity, but also gave increased conversions for both acylating agents (entries 2 and 9). However, complete inhibition of the reaction was again observed when the amount of molecular sieves was increased (entry 3).

From these experiments, we were able to conclude that the quantity of residual water in the reaction media has a strong influence on both the activity and the selectivity of the CRL lipase for the acylation of alcohol **4**. It was then decided to control this factor in the following experiments by using strictly anhydrous solvents and molecular sieves.

Commercially available *o*-(4-chlorobenzoyl)-hydroquinine **A1** was first selected as an additive, and the acylation experiments of alcohol **4** with CRL were carried out under optimised conditions with both acylating agents. The activation threshold with this additive was first checked by carrying out experiments with increasing amounts of **A1**. The measured data for this series of experiments are collected in Table 2.

The data from Table 2 show an unambiguous enhancement of both reactivity and enantioselectivity when **A1** was used as an additive; this effect was observed with both acylating agents.

Table 1
Influence of water and polar solvents on the CRL-catalysed acylation of **4**

Entry	Acylating agent[a]	Additive	ee_S[b]	Yield[d] (%)	ee_P[b]	Yield[d] (%)	C[c] (%)	E[c]
1		—	9.8	45	88	35	10	17
2		Molecular sieves 50 mg	34.7	42	88	35	28	22
3		Molecular sieves 150 mg	NR[f]	95	NR[f]	—	NR[f]	NR[f]
4	**IA**	H_2O[e]	NR[f]	70	NR[f]	—	NR[f]	NR[f]
5		Glycol ethylene[e]	NR[f]	75	NR[f]	—	NR[f]	NR[f]
6		DFM[e]	0.3	80	7	Nd[g]	4	1
7		DMSO[e]	NR[f]	80	NR[f]	—	NR[f]	NR[f]
8	**VA**	—	44.8	45	82.5	30	35	16
9		Molecular sieves[e] 50 mg	56.7	45	87	40	40	25

[a] Each experiment was carried out on 1 mmol of racemic **4**, 3 mmol of acylating agent, 6 mL of TBME and 50 mg CRL (58,500 U).
[b] Measured by HPLC on a *Chiralpak* AD column.
[c] Conversion:[17] $C = ee_S/ee_P + ee_S$; selectivity:[17] $E = \mathrm{Ln}[(1 - C)(1 - ee_{(S)})]/\mathrm{Ln}[(1 - C)(1 + ee_{(S)})]$.
[d] Isolated yield.
[e] 0.025% of the final solvent volume.
[f] No reaction.
[g] No evaluated.

Table 2
Influence of the amount of additive **A1** on the kinetic resolution of **4**

Entry	Acylating agent[a]	Additive (mol %)	ee_S[b]	Yield[c] (%)	ee_P[b]	Yield[c,d] (%)	C[b] (%)	E[b]
1		0	34.7	42	88	42	28	22
2		5	9	45	88.7	30	9	18
3		10	46.7	40	88	30	35	25
4	**IA**	15	34.8	40	89.7	32	28	26
5		20	34.1	40	89.7	30	28	26
6		30	67.4	40	82.8	40	45	21
7		50	68.4	40	86.2	40	44	28
8		0	56.7	47	87	45	40	25
9		5	99.5	45	80	42	55	52
10		10	97	45	87.3	45	53	61
11	**VA**	15	99.1	46	85.7	45	54	69
12		20	99.9	45	86.2	47	54	100
13		30	69.2	47	88.7	45	44	34
14		50	99.9	47	84.4	45	54	87

[a] Each experiment was carried out with 1 mmol of racemic **4**, 3 mmol of acylating agent, 6 mL of TBME and 50 mg CRL (58,500 U).
[b] Measured by HPLC on a *Chiralpak* AD column.
[c] Conversion:[17] $C = ee_S/ee_P + ee_S$; selectivity:[17] $E = \mathrm{Ln}[(1 - C)(1 - ee_{(S)})]/\mathrm{Ln}[(1 - C)(1 + ee_{(S)})]$.
[d] Isolated yield.

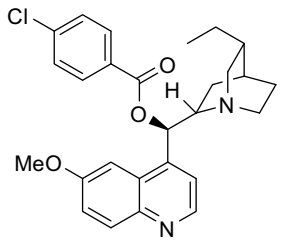
When isopropenyl acetate was used for the acylation, a threshold was obtained with the addition of 30 mol % of the additive as the conversion reached $C = 45\%$ (against $C = 28\%$ when no additive was used, entry 6 vs entry 1). A slight improvement in the selectivity was also observed with higher amounts of the additive, albeit without any noticeable change in the reactivity. A more significant effect was observed with vinyl acetate as only a 5 mol % of the additive was required to reach a 50% conversion. We were also pleased to note the beneficial effect of this additive on the enantioselectivity of both remaining alcohol **4** and acetate **3** as the selectivity was increased to $E = 87$ with 50 mol % of the additive (against $E = 25$ without additive, entry 14 vs entry 8). It should be noted that such an effect has not been seen before with this lipase, and we have thus determined a threshold of 5 mol % for this additive when vinyl acetate was used as the acylating agent ($C = 55\%$ and $E = 52$, entry 9).

We next studied the influence of the structure of cinchona alkaloids on the reactivity and selectivity of the reaction with both acylating agents. Each experiment was also carried out in two different hydrophobic solvents: toluene ($\mathrm{Log}P = 2.5$) and *t*-butyl methylether ($\mathrm{Log}P = 1.35$). The data obtained for this series of experiments are shown in Table 3.

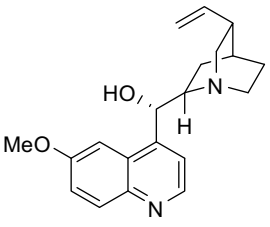
The data from Table 4 show an important effect on the course of the reaction when an alkaloid is added to the reaction media. In all cases, we observed an increase of both selectivity and reactivity of the CRL, these effects being tampered by the nature of the solvent and the acylating agent. The best selectivity ($E = 143$, entry 26) was reached at 53% conversion when **A1** was used with vinyl acetate in toluene.

We indeed observed that the use of toluene allowed a strong improvement in the catalytic activity of the lipase, as only 24 h was required against five days when TBME was used as the solvent. Without any additive, both reactivity and selectivity are better in toluene as E reaches 45 when $C = 21\%$ after 24 h; whereas in TBME, the selectivity reaches only 21 when $C = 40\%$ after five days at room temperature (entries 17 and 25). The stronger hydrophobicity of this solvent has thus a positive effect on the activity of the lipase.

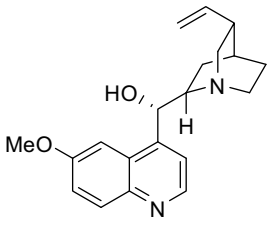
In toluene, a strong acceleration of the reactivity was observed for the acceleration with isopropenyl acetate as the acylating agent, and both selectivity and reactivity were improved with the entire alkaloid additives tested in this reaction. The best selectivities (up to $E = 67$–69) were reached with quinine **A5** and cinchonine **A7** at half conversion of the substrate (entries 13 and



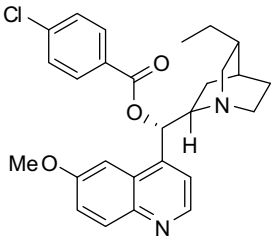
O-(4-chlorobenzoyl)-hydroquinine **A1**



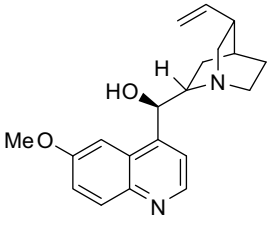
quinidine **A2**



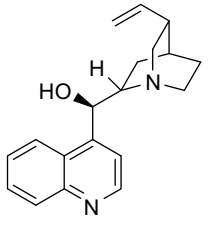
hydroquinidine **A3**



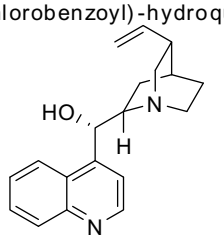
O-(4-chlorobenzoyl)-hydroquinidine **A4**



quinine **A5**



cinchonidine **A6**



cinchonine **A7**

Table 3
Screening of several additives on the kinetic resolution of **4**

Entry	Acylating agent	Solvent	Time	Additive	ee_S[c]	Yield[d] (%)	ee_P[c]	Yield[d] (%)	C[c] (%)	E[c]
1				—	34.7	42	88.1	42	28	22
2				**A1**	67.4	40	82.8	40	45	21
3				**A4**	35.7	45	87.7	32	29	22
4		TBME	5 days	**A2**	37.7	42	86.1	35	30	19
5				**A5**	39.7	45	82.4	35	32	15
6				**A6**	26.4	45	85.9	36	23	17
7				**A7**	22.5	47	89.6	30	22	23
8				**A3**	76.8	40	70.9	40	52	13
9	**IA**[a]			—	13.5	40	87.4	30	13	17
10				**A1**	79.1	45	90	42	47	46
11				**A4**	56.6	46	88.1	35	39	28
12		Toluene	24 h	**A2**	99.9	46	63.8	45	61	31
13				**A5**	99.9	46	80.8	47	55	69
14				**A6**	80.9	40	85.8	42	48	33
15				**A7**	96.6	40	88.5	40	52	67
16				**A3**	99.9	42	65.3	41	60	33
17				—	56.7	47	86.9	42	40	25
18				**A1**	99.5	45	79.9	42	55	52
19				**A4**	99.9	45	76.6	40	57	54
20		TBME	5 days	**A2**	99.9	46	82.2	45	55	75
21				**A5**	99.9	40	79.6	42	56	64
22				**A6**	99.9	40	85.2	40	54	93
23				**A7**	99.9	41	76.1	36	57	53
24				**A3**	99.9	45	71.4	40	58	43
25	**VA**[b]			—	25.6	47	94.5	45	21	45
26				**A1**	99.9	45	90.1	42	53	143
27				**A4**	63	45	91	40	41	40
28		Toluene	24 h	**A2**	86.1	40	88.9	40	49	47
29				**A5**	97.5	42	88.7	42	52	73
30				**A6**	98.1	45	91.4	40	52	103
31				**A7**	99.9	45	86.2	41	54	100
32				**A3**	78.4	45	87.7	40	47	36

[a] Each experiment was carried out with 1 mmol of racemic **4**, 3 mmol of acylating agent, 6 mL of solvent and 50 mg CRL (58,500 U) with 30% of additive.
[b] Each experiment was carried out with 1 mmol of racemic **4**, 3 mmol of acylating agent, 6 mL of solvent and 50 mg RCL (58,500 U) with 5% of additive.
[c] Measured by HPLC on a *Chiralpak* AD column; Conversion:[17] $C = ee_S/ee_P + ee_S$; selectivity:[17] $E = \mathrm{Ln}[(1 - C)(1 - ee_{(S)})]/\mathrm{Ln}[(1 - C)(1 + ee_{(S)})]$.
[d] Isolated yield.

Table 4
Effect on simple Lewis base additives on the kinetic resolution of **4**

Entry	Acylating agent	Solvent	Additive	ee_S[a]	Yield[c] (%)	ee_P[a]	Yield[c] (%)	C[b] (%)	E[b]
1			—	56.7	42	86.9	42	40	25
2			Et_3N	49.3	40	91.4	35	35	36
3		TBME	Pyridine	46.1	40	90.2	40	34	31
4			DMAP	NR[d]	—	NR[d]	—	NR[d]	NR[d]
5	**VA**		DABCO	45.1	40	86.2	36	34	21
6			—	25.6	47	94.5	45	21	45
7			Et_3N	32.4	42	90.8	35	26	28
8		Toluene	Pyridine	34.8	45	93	36	27	39
9			DMAP	NR[d]	—	NR[d]	—	NR[d]	NR[d]
10			DABCO	78.5	42	88.5	40	47	39

[a] Each experiment was carried out with 1 mmol of racemic **4**, 3 mmol of acylating agent, 6 mL of solvent and 50 mg RCL (58,500 U) with 5% of additive.
[b] Measured by HPLC on a *Chiralpak* AD column; Conversion:[17] $C = ee_S/ee_P + ee_S$; selectivity:[17] $E = \mathrm{Ln}[(1 - C)(1 - ee_{(S)})]/\mathrm{Ln}[(1 - C)(1 + ee_{(S)})]$.
[c] Isolated yield.
[d] No reaction.

15). With TBME as a solvent, the best reactivity was reached with vinyl acetate, and a strong influence of the alkaloid additives on the course of the reaction was again observed with a selectivity ranging from 43 to 93 with this acylating agent, the best selectivity was obtained with cinchonidine **A6**. A weaker influence was observed for isopropenyl acetate, the best results being obtained with additive **A1** which gave a selectivity of 21 at 45% conversion (entry 2).

Vinyl acetate also proved to be the best acylating agent when toluene was used as a solvent for the reaction. A strong effect was reached with the additives, giving selectivities as high as 143 (**A1**, entry 26), $E = 103$ (cinchonidine **A6**, entry 30) and $E = 100$ (cinchonine **A7**, entry 31).

It is however difficult to truly rationalise these observations and the impact of the structure of the alkaloid additive on the selectivity of the reaction. Indeed, even if all these additives display a common basic skeleton, they also bear some differences related to functional group differentiations and stereochemical differences. For instance, analogies such as the presence of a vinyl group on the bicyclic tertiary amine skeleton can be found in **A5**, **A6** and **A7**, albeit not present in **A1**. Therefore, locating interaction sites which might be responsible for better enantioselection between the enzyme and the additives is not simple. We also checked that no reaction took place when the experiments were carried out in the presence of an additive, an acylating agent and without any

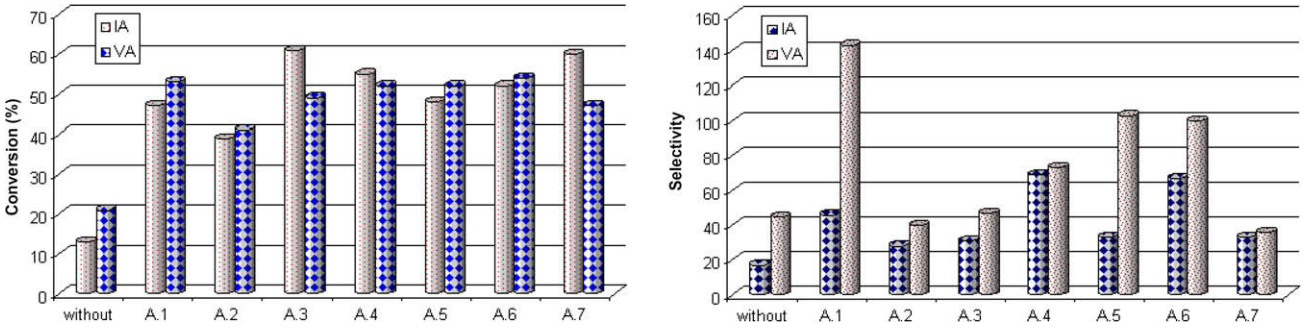


Figure 1. Comparison of reactivity and selectivity of the lipase in TBME for additives **A1**–**A7**.

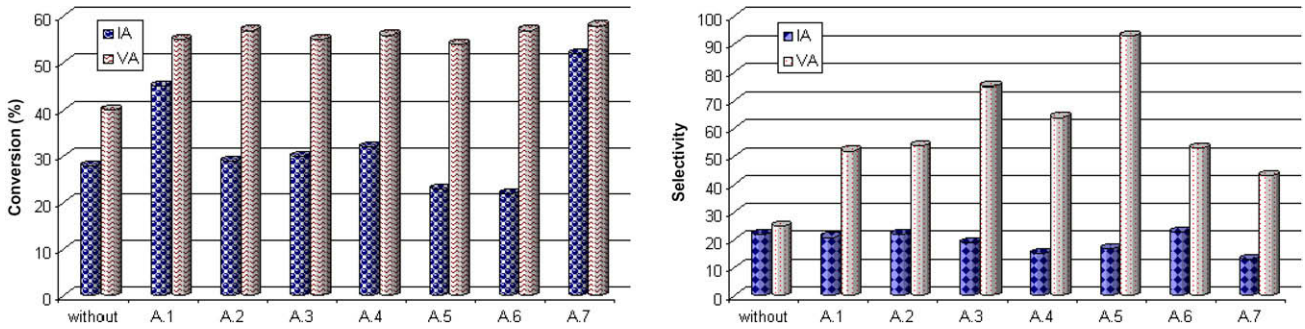


Figure 2. Comparison of reactivity and selectivity of the lipase in toluene for additives **A1**–**A7**.

addition of the enzyme. We, thus presumed that the interaction of the additive and the lipase is responsible for both reactivity and enantioselectivity of the catalytic system. Figures 1 and 2 illustrate the comparison of the reactivity and the selectivity of the lipase in TBME and toluene for additives **A1**–**A7**.

2.1. Effect of simple Lewis base additives

In order to obtain some information on the effect of the alkaloids on the outcome of the reaction, we have selected simple organic bases as additives in order to check the influence of a simple Lewis base on the reactivity of our system. Alkaloids can indeed be considered as Lewis bases as the non-bonding electron pair on the nitrogen can be used for complexation, and have some significant effect on the catalysis of the acylation. We thus selected triethylamine, pyridine, DMAP and DABCO, which were tested as additives in the catalytic acylation of alcohol **4**. Vinyl acetate was previously shown to be the best acylating agent as was selected, and reactions were carried out in toluene and TBME for comparison. The observed effects of these additives are given in Table 4.

The use of triethylamine and pyridine as additives gave very little differences with the reference reactions. Slight modifications were observed with these additives, but cannot be considered as fully representative of the real influence on the catalysis of the reaction. However, when a stronger Lewis base (DMAP) was used as an additive, complete inhibition of the reaction was observed in both toluene and TBME. Interestingly, a significant increase in the enantioselectivity and selectivity of $E = 39$ was observed with DABCO in toluene. This particular additive was initially selected for its structural similarity with the aza-bicyclic framework of the cinchona alkaloids; it could be postulated that this fragment on the alkaloid might intervene in the catalytic cycle to facilitate the access of the substrate to the active site of the enzyme. However, more studies would be required to confirm this preliminary observation.

3. Conclusion

We have shown the influence of the addition of water on the reactivity and the selectivity of the *C. rugosa* lipase (CRL) in the acylation of hydroxymethyl-1-phenylthioferrocene **4** in organic solvents, and our observations show the influence of hydrophobic interactions on the catalytic activity of this lipase. The use of cinchona alkaloids and their derivatives as additives allowed us to improve both the selectivity and the enzymatic reactivity of this enzyme, and we have shown that this effect was also strongly dependant on the nature of the solvent, the presence of residual water and on the nature of the acylating agent. After optimisation, a selectivity of $E = 143$ at $C = 53\%$ conversion could be reached, yielding both the remaining substrate and product with excellent enantioselectivities. Thus, the use of additives such as alkaloids might offer a useful alternative in poorly selective enzymatic acylation reactions.

4. Experimental

4.1. General

NMR spectra were recorded on Brucker spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm from tetramethylsilane with the solvent resonance as the internal standard for ^{1}H NMR and chloroform-*d* (δ 77.0 ppm) for ^{13}C NMR. Coupling constants (*J*) are given in Hertz. Following abbreviations classify the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal. The mass spectra were obtained from the mass-service at Université catholique de Louvain (FINNIGAN-MAT TSQ 7000 and FINNIGAN-MAT LQC spectrometers). IR spectra were recorded on Shimadzu FTIR-8400S spectrometer. Optical rotations were determined using a Perkin–Elmer 241 Polarimeter at room temperature using a cell

of 1 dm length and $\lambda = 589$ nm. The enantiomeric excesses were measured by a chiral stationary phase HPLC on Chiralpak® AD column. Retention times are reported in minutes.

4.2. 2-*N*,*N*-Dimethylaminomethyl-1-phenylthio-ferrocene *rac*-2

A dry Schlenck tube was loaded under argon with 10 g of DMAF **1** (41 mmol), and diluted with 50 mL of freshly distilled diethyl ether. 31.5 mL of a 1.5 M *tert*-butyl lithium solution (1.15 equiv, 47.3 mmol) was added dropwise, and the resulting brick red solution was stirred at room temperature for a further 15 min after the end of the addition. Diphenyl disulfide (13.5 g, 61.5 mmol, 1.5 equiv) in 40 mL of diethyl ether was added dropwise at room temperature, and the brown solution was kept at this temperature for a further 1 h before careful quenching with brine. The aqueous phase was extracted with ether, and the organic phase was dried over magnesium sulfate, filtered and concentrated in vacuo. The crude brown oil was purified by flash chromatography on silica gel (80:20:1 cyclohexane/ethyl acetate/triethylamine) to give the pure sulfide **2** as a brown solid in a 92% yield (13.3 g). R_f: 0.7 (5:5:1 cyclohexane/EtOAc/Et_3N); mp: 70 °C, IR (film, cm^{-1}): ν = 690.4; 736.7; 817.7; 1026; 1080; 1103.2; 1176.5; 1261.3; 1477.3; 1581.5; 2765.7; 2812; 2939.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.02 (s, 6H, N(CH_3)$_2$); 3.37 and 3.42; 3.43 and 3.47 AB (dd, J = 13.3 Hz, 2H, CH_2N(CH_3)$_2$); 4.6 (s, 5H, C_P); 4.32 (s, 1H, C_P), 4.46 and 4.50 (d, 2H, J = 13.6 Hz, C_P); 7.01–7.11 (m_a, 5H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 45.13; 57; 69.14; 70.36; 71.3; 75.62; 76; 88; 124.84; 126.18; 128.49; 140. MS (D-APCI; m/z): 242.18 ([$FcCH_2NMe_2$]$^+$, 100%); 307 ([$FcSPhCH_2$]$^+$, 49%); 350.08 ([M−H]$^+$, 35%); 351 ([M]$^+$, 24%); 352.04 ([M+H]$^+$, 6%).

4.3. 2-Acetoxymethyl-1-phenylthioferrocene *rac*-3

Thirteen grams of sulfide *rac*-**2** (37 mmol) is dissolved in 30 mL of acetic anhydride, and the solution was refluxed for 15 h. After filtration on Celite and concentration in vacuo, the crude reaction mixture was purified by flash chromatography on silica gel (80:20 cyclohexane/ethyl acetate). 12.8 g of the pure acetate (95%) is isolated as deep orange crystals. Chiral HPLC analysis (*Chiralpak* AD column) (hexane/EtOH: 99:1; 1 mL/min): rt_1 = 9.5 min, rt_2 = 12.8 min. P_f: 128 °C. IF (film, cm^{-1}): ν = 690.4; 744.4; 817.7; 952.7; 999; 1022.2; 1238.2; 1369.3; 1577.6; 1728.1 (ν C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.75 (s, 3H, O=C–CH_3); 4.27 (s, 5H, C_P); 4.41 (s, 1H, C_P), 4.52 and 4.55 (d, 2H, J = 10.17 Hz, C_P); 4.90 and 4.94 (d, J = 11.38 Hz, 1H) and 5.07–5.11 (d, J = 12.39 Hz, 1H) CH_2OH; 7.04–7.2 (m_a, 5H, aromatiques). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.61; 61; 69.62; 70.21; 71.83; 76.17; 85; 124.95; 126.17; 128.48; 141.5; 172. MS (EI; m/z): 43.9 ([Ac]$^+$, 86%); 148.8 ([Fc−H]$^+$, 71%); 185.8 ([Fc]$^+$, 53%); 366 ([M]$^+$, 100%); 367.1 ([M+H]$^+$, 24%).

4.4. 2-Hydroxymethyl-1-phenylthioferrocene *rac*-4

At first, 12 g of racemic acetate **3** (32.7 mmol) was dissolved in 50 mL of dry methanol and stirred overnight with 50 g of potassium carbonate. After filtration on Celite and concentration, the residue is taken up in dichloromethane and washed with water. After standard work up, the pure alcohol *rac*-**4** (10 g, 95%) was isolated as yellow-orange crystals. Chiral HPLC analysis (*Chiralpak* AD column) (hexane/EtOH: 97:3; 1 mL/min): rt_1 = 30.6 min, rt_2 = 34.1 min. P_f: 127 °C. IF (film, cm^{-1}): ν = 690.4; 740.6; 817.7; 987.4; 1006.7; 1076.2; 1107; 1249.7; 1481.2; 1581.5; 3244 (ν OH). ^{1}H NMR (300 MHz, $CDCl_3$): 1.510 (s, OH); 4.270 (s, 5H, C_P); 4.41 (s, 1H, C_P); 4.37 (m, 2H, C_P), 4.53 (m, 1H, CP + 2H, CH_2OH); 7.06 (m, 3H, Ph); 7.17 (m, 2H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 59; 96.2; 70.1; 70.6; 75.6; 91; 125.2; 125.8; 128.9; 140. MS (EI; m/z): 185.8 ([Fc]$^+$, 100%); 323.9 ([M]$^+$, 100%).

4.5. General procedure for the acylation of racemic alcohol 4 with *C. rugosa* lipase

A dry Schlenck tube was charged with 1 mmol of the racemic alcohol **4** and 6 mL of solvent. After dissolution of the alcohol, the *C. rugosa* lipase, the additive, the molecular sieves and the acylating agent (3 mmol) were added, and the suspension was stirred at room temperature for the indicated time. The reaction mixture was filtered on Celite and concentrated in vacuo. The acetate **3** and the remaining alcohol were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate: 80:20) and analysed by chiral HPLC.

The absolute configuration of both compounds was determined by polarimetry, by comparison with literature data: (R_{Fc})-2-hydroxymethyl-1-phenylthioferrocene $[\alpha]_D$ = +51.2 (c 1, CH_2Cl_2); ee = 90–91%. (S_{Fc})-2-hydroxymethyl-1-phenylthio-ferrocene **4**: $[\alpha]_D$ = −56.2 (c 1, CH_2Cl_2); ee = 99% (lit.[18] $[\alpha]_D$ = −50 (c 1.14, $CHCl_3$); ee = 95%).

Acknowledgements

Algerian ministry of education and scientific research (FNR 2000 and PNR), Université catholique de Louvain and FNRS are gratefully acknowledged for financial support of this work. UCL is acknowledged for a grant 'cooperation au développement'. Shimadzu Benelux is acknowledged for financial support for the acquisition of a FTIR-8400S spectrometer.

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