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Enantiomeric resolution by lipase-catalysed esterification of a *trans*-5,6-dihydro-1,10-phenanthroline possessing helical and central chirality

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Abstract—Lipase-catalysed enantioselective esterification was carried out to obtain the kinetic resolution of (±)-*trans*-5-hydroxy-6-methoxy-1,10-phenanthroline (±)-**1b**. Different lipases from *Candida cylindracea*, *Candida antarctica*, *Rhizomucor miehei*, *Pseudomonas fluorescens* and *Pseudomonas cepacia* were tested in an unusual methanol/vinyl acetate (8:92 v/v) reaction mixture *P. fluorescens* lipase showed good enantiodifferentiation ($E = 48$) and allowed us to prepare both enantiomers (+)-(5*S*,6*S*,*M*)-**1b** and (−)-(5*R*,6*R*,*P*)-**1b** with an enantiomeric excess of >97%.

1. Introduction

The high efficiency of lipase catalysis in organic synthesis to prepare fine chiral chemicals is well recognised. This methodology has been broadened by extending their use to organic media, particularly with the possibility of applying it to hydrophobic substrates, as well as catalysing reverse esterification reactions.[1] Moreover, the potential of biocatalysis in non-aqueous medium has been improved by modulating the enzymatic action using additives,[2] co-solvents[3] or new organic media, such as room temperature ionic liquids (RTILs).[4] The main use of this technology concerns the resolution of racemic molecules possessing central chirality, but the capacity of these enzymes for the stereoselective differentiation of asymmetric planes[5] and axes[6] has permitted us to exploit lipases in order to also access stereochemically complex and rigid molecular systems in enantiopure form.

Functionalised bipyridines and phenanthrolines, such as Ru(II) and Cu(I) complexes, respectively, have attracted increasing attention in the field of supramolecular and macromolecular chemistry as well as in nanoscience, largely because of their electronic, photophysical, acid–base and luminescence properties.[7] Moreover, chiral 2,2′-bipyridyls and structurally similar 1,10-phenanthroline derivatives are very attractive systems as chiral ligands in catalytic reactions, due to their metal chelating capacity to form stable complexes with transition metals. For example, 2,2′-bipyridine metal ion complexes are used in asymmetric reactions, such as the addition of diethylzinc to aldehydes, cyclopropanation of alkenes and the hydrogenation of ketones,[8] as well as 1,10-phenanthrolines, which are useful in the reduction of ketones[8c] and the enantioselective hydrolysis of amino acid esters.[9]

Most chiral 2,2′-bipyridines are C_2-symmetric and their synthesis in enantiopure form can be achieved by coupling two halo-pyridines containing the desired homochiral centres, while the asymmetric 2,2′-bipyridines are obtained by hetero coupling, cyclisation reactions of enantiopure building blocks, or by introducing chiral groups, principally at the 6 and/or 6′ positions.[8a,c] There are very few examples regarding the synthesis of 3,3′-disubstituted-2,2′-bipyridines, usually obtained through oxidative cleavage of 1,10-phenanthroline by aqueous $KMnO_4$ or by coupling of 3-substituted halo-pyridines.[10] Among them, *trans*-5,6-dihydro-1,10-phenanthrolines are interesting 3,3′-disubstituted bipyridine derivatives because of their greater rigidity due to the presence of the bridge. Racemic *trans*-5,6-diol-, -halohydrin- and -aminoalcohols-1,10-phenanthroline derivatives have already been obtained;[11] however, asymmetric syntheses or resolution methodologies are not

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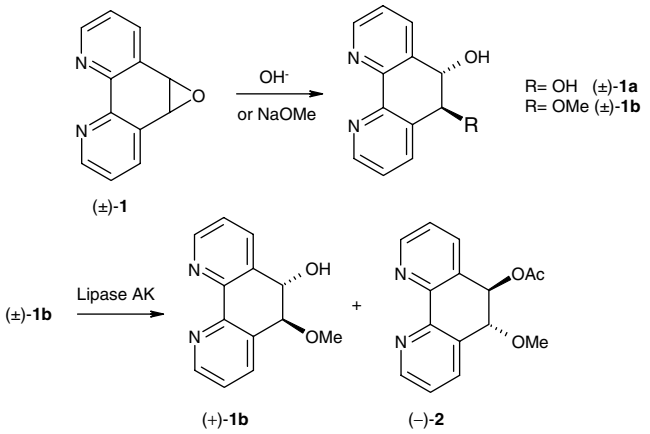
currently available. Therefore, we envisaged obtaining enantiopure *trans*-5,6-dihydro-1,10-phenanthroline derivatives by enzymatic resolution in organic solvent.

2. Results and discussion

Recently, we have focussed on using lipases as biocatalysts in organic medium for the enantioselective synthesis of chiral atropisomeric molecules. As part of this project, the resolution of 3,3′-bis(hydroxymethyl)-2,2′-bipyridine *N*,*N*-dioxide has been obtained, proving that a bipyridine skeleton can be suitable for lipase acceptance.[6a] Following positive results, this biocatalytic method has been investigated in the resolution of *trans*-5,6-dihydroxy-1,10-phenanthroline (±)-**1a**, easily obtainable from the epoxide ring opening of commercially available phenanthroline epoxide (±)-**1**.



Scheme 1.

Due to its structural features, (±)-**1a** was insoluble in common organic solvents, meaning that in order to investigate lipase-mediated enantioselective esterification of our substrate, it was necessary to operate in a methanol/vinyl acetate mixture, an unusual reaction medium but successfully utilised in previous work.[6a] As a preliminary experiment, a reaction without the lipase was performed dissolving (±)-**1a** in 20% (v/v) methanol in vinyl acetate. After 24 h incubation at 40 °C, HPLC investigation revealed an almost total conversion of (±)-**1a** in mono- and diacetyl derivatives in a 3:2 ratio, respectively. As a result of this, in order to achieve the resolution of (±)-**1a**, the complementary enzymatic alcoholysis of the diacetyl derivative was considered. To this end, the substrate was dissolved in a 20% methanol/*t*-buthylmethyl ether mixture and different experiments were performed in the presence of two lipases in free form, from *Candida cylindracea* and *Pseudomonas fluorescens* (lipase AK), respectively, and three immobilised PS-D (from *Pseudomonas cepacia*), Lipozyme IM (from *Rhizomucor miehei*) and Novozym 435 (from *Candida antarctica*). Under these conditions, all the lipases employed did not catalyse the desired reaction and no alcoholysis products were detected by HPLC analysis even when prolonging the reaction time. This result is probably due to the steric hindrance arising from the presence of the two acetyl groups.

As the results were not encouraging, we decided to use the 5,6-dihydro-1,10-phenanthroline architecture in the enantiomeric form using methoxyl derivative (±)-**1b** as a possible substrate for the lipase catalysis. Compound (±)-**1b** was easily obtained in high chemical yield, and as a racemate, from (±)-**1** by epoxide ring opening reaction in the presence of sodium methoxide as catalyst in methanol.[11b] Due to the better solubility of (±)-**1b**, 8% of methanol was sufficient enough to dissolve it in vinyl acetate, and a preliminary blank experiment was performed under these conditions to show any possible spontaneous acetylation reaction. After 10 days of incubation, only 5% of the corresponding racemic acetyl derivative was detected; thus, this reaction medium system was used in the further enzymatic esterification experiments (Scheme 1).

Preliminary screening was carried out using all the above considered lipases, while the reactions were monitored by HPLC chiral analysis to obtain simultaneous information on enantiomeric excesses and conversion values. Among the enzymes tested, lipase AK from *P. fluorescens* was found to catalyse the enantioselective esterification of (±)-**1b** more efficiently, giving a 45% substrate conversion and an $E = 48$ after 10 days.

To reduce the possible negative effects of methanol on the lipase, a parallel esterification reaction of (±)-**1b** was carried out using the more acceptable 2-propanol (8%) in vinyl acetate. No significant advantage was observed, giving lipase AK in 12 days, a 45% substrate conversion and an $E = 30$ value. Taking into account the better *E*-value obtained, a preparative resolution of (±)-**1b** was performed using methanol as co-solvent. Under these conditions, after 11 days of reaction, chiral HPLC analysis of reaction mixture showed a 48% conversion, unreacted (+)-**1b** with 82% ee and acetyl derivative (−)-**2** with 89% ee. Attempts to improve the ee value of (−)-**2** by resorting to crystallisations failed; therefore, ester (−)-**2** was hydrolysed by conventional chemical hydrolysis and the alcohol obtained resubjected to enzymatic resolution in the previous conditions adopted. After 4 days of reaction, 85% of substrate conversion was detected, and hydrolysis of the new (−)-**2** produced furnished the desired (−)-**1b** with 98% ee.

In a second preparative reaction, esterification of (±)-**1b** was prolonged until 60% conversion and the unreacted (+)-**1b** was recovered with 97% ee.

The absolute configuration of (+)-**1b** can be defined considering its chiroptical properties, assuming that the conformation of the C-5 and C-6 substituents is known.[12]

In this context, analysis of the CD spectrum of (+)-**1b** showed a negative CD band at 226 nm ($\Delta\varepsilon$ −0.62), and a positive CD band at 241 nm ($\Delta\varepsilon$ +2.88), in good agreement with the bands at 228 and 265 nm reported for the biphenyl chromophore in the *trans*-(9,10)-dihydroxy-phenanthrene (9*S*,10*S*)-**3** (see Fig. 1), indicating a *M* helicity of the bipyridyl system.[13]

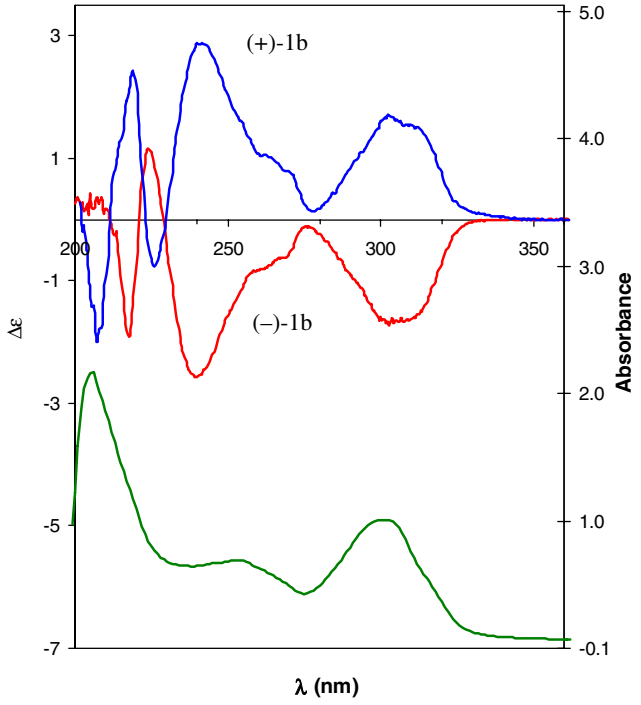
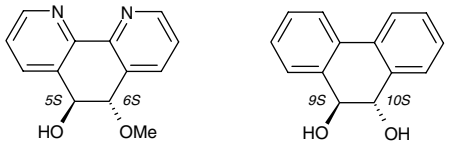


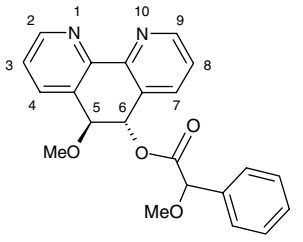
Figure 1. CD and UV spectra of (+)-**1b** and (−)-**1b**.



(+)-**1b** (*S*,*S*)-**3**

The 7.2 Hz value for J_{5-6} measured in the ^{1}H NMR spectrum of (+)-**1b**, recorded in methanol, showed a pseudoaxial relationship for H-5 and H-6 protons, thus, indicating (5*S*,6*S*)-configuration for the carbon atoms at the 5- and 6-positions (see Fig. 2).

The absolute configuration of the benzylic carbons in (+)-**1b**, was also confirmed by NMR analysis comparing the differences in chemical shifts ($\Delta\delta^{RS}$) of signals in proton spectra of diastereoisomeric methoxyphenylacetic ester derivatives **4a** and **4b**.[14]



4a, (*R*)-MPA ester
4b, (*S*)-MPA ester

For each diastereoisomer, a complete assignment of the proton resonances was obtained by extensive 2D-NMR homo- and hetero-nuclear experiments. A $\Delta\delta^{RS} > 0$ was measured for resonances relative to the H_5, H_4, H_3 and H_2 positions, while $\Delta\delta^{RS} < 0$ was measured for proton resonances of H_7, H_8 and H_9. Although this procedure has so far been reported to determine only alcohols with protons on both substituents flanking the stereogenic centre bearing the hydroxyl group, with these data in hand it seems reasonable to assign a (5*S*,6*S*)-absolute configuration to (+)-**1b**. Consequently, (5*R*,6*R*)-configuration must be assigned to ester (−)-**2**, and this confirms the (*R*)-enantiopreference that lipase AK has in the recognition of secondary alcohols.

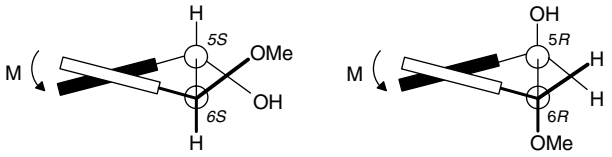


Figure 2.

3. Conclusion

The single enantiomers (+)-**1b** and (−)-**1b** of helical *trans*-5,6-dihydro-5-hydroxy-6-methoxy-1,10-phenanthroline, were obtained by an enzyme-catalysed esterification procedure. Lipase AK from *P. fluorescens* showed good efficiency in the esterification of (±)-**1b**, working in an unusual methanol/vinyl acetate mixture, furnishing (5*S*,6*S*)-(+)-**1b** with an *M* helicity, with ee 97% and the acetyl derivative (−)-**2** with 98% ee, which by chemical hydrolysis gave the homochiral (5*R*,6*R*)-(−)-**1b**. The absolute configuration of alcohol (+)-**1b** was determined using CD and NMR analyses. Since 3,3′bridged 2,2′-bipyridines are a stereochemically interesting class of derivatives, further biocatalytic procedures are currently under investigation in our laboratory in order to access other derivatives.

4. Experimental

4.1. Materials and methods

Immobilised lipase PS-D (from *P. cepacia)* and Lipase AK (*P. fluorescens*) were purchased from Aldrich, Lipozyme IM (immobilised lipase from *Mucor miehei*) was from Fluka while Novozyme 435 (immobilised lipase from *C. antarctica*) and *C. cylindracea* were obtained from Sigma. The racemic (±)-**1b** was obtained by epoxide ring opening of commercial (Aldrich) racemic 1,10-phenanthroline 5,6-epoxide following the reported procedure.[11c]

Thin-layer chromatography (TLC) was carried out on Merck silica gel 60-F_{254} precoated glass plates eluting with 8:1:1 (v:v:v) ethyl acetate–2-propanol–NH_4OH. Preparative liquid chromatography was performed using Silica gel® Si 60 (0.04–0.063 mm) from Merck.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS and coupling constants (J) in Hz. Optical

rotations were recorded on a DIP 135 JASCO instrument using a ϕ 3.5 × 100 mm cell.

Enantiomeric excesses and substrate conversions were determined by chiral HPLC analysis using a Chiralcel® OD (Daicel Chemical Industries) column eluting with 50:50 *n*-hexane–2-propanol mixture at 0.5 mL/min flow rate at 23 °C with simultaneous detection at 220, 250, 266 and 275 nm: t_R/min 11.32 for (5*R*,6*R*)-enantiomer and 17.17 for (5*S*,6*S*)-enantiomer of **1b** while t_R/min 13.43 and 32.21, respectively, for the (5*S*,6*S*)- and (5*R*,6*R*)- enantiomers of acetyl derivative **2**.

CD spectra were registered at room temperature in CH_3OH (1 cm cell length) on a JASCO J-815 spectropolarimeter. The UV spectrum of **1b** was recorded in CH_3OH on an Agilent 8453 UV–visible spectrophotometer.

4.2. Enzymatic resolution of (±)-1b

In a general enzymatic esterification, 20 mg (0.088 mmol) of (±)-**1b** was dissolved in 5 mL of solvent mixture containing 8% (v/v) of alcohol in vinyl acetate, then 100 mg of lipase was added. The reaction was shaken at 290 rpm and 45 °C until about 50% substrate conversion, as determined by chiral HPLC analysis.

4.3. Preparative lipase AK catalysed esterification of (±)-1b

Substrate of 400 mg (1.75 mmol) was dissolved in 8 mL of MeOH; 92 mL of vinyl acetate and 2 g of lipase AK were added to the clear solution. The reaction mixture was shaken for 12 days, after which the enzyme was filtered off and the solvent evaporated in vacuo. The dark brown precipitate obtained was chromatographed on silica gel, eluting with 8:0.5:0.5 (v:v:v) ethyl acetate–2-propanol–NH_4OH. The acetyl derivative (−)-**2** was obtained as a first eluted product (230 mg, yield 48%) ee 89%; ^{1}H NMR in $CDCl_3$ δ 2.01 (s, 3H), 3.41 (s, 3H), 4.49 (d, 1H, $J = 4.8$ Hz), 6.17 (d, 1H, $J = 4.8$ Hz), 7.36 (m, 2H,), 7.78 (d, 1H, $J = 7.5$ Hz), 7.82 (d, 1H, $J = 7.6$ Hz), 8.82 (d, 1H, $J = 4.6$ Hz), 8.86 (d, 1H, $J = 4.6$ Hz); ^{13}C NMR in $CDCl_3$ δ 20.9, 57.6, 70.5, 77.9, 123.8, 124.1, 129.2, 129.8, 137.2, 137.8, 150.4, 150.6, 150.8, 150.9, 170.1.

The alcohol residue (+)-**1b** was obtained as a second eluted product (192 mg, yield 48%) ee 82%.

4.4. Improvement of the enantiomeric excess of alcohol (+)-1b

Alcohol (±)-**1b** (200 mg, 0.87 mmol) was dissolved in 50 mL of vinyl acetate containing 8% of methanol, after which the reaction was started by the addition of 2 g of lipase AK. After 7 days of reaction at 290 rpm and 45 °C, a 60% of substrate conversion was detected by HPLC analysis. The enzyme was filtered off and the mixture was chromatographed on silica gel column to obtain alcohol (+)-**1b** with an ee 97% (yield 35%); $[\alpha]_D^{25} = +93.2$ (*c* 0.43, CH_3OH). CD: λ_{ext} 219 ($\Delta\varepsilon$ +2.43), 221 ($\Delta\varepsilon$ +1.21), 225 ($\Delta\varepsilon$ −0.75), 231 ($\Delta\varepsilon$ +0.50), 241 ($\Delta\varepsilon$ +2.87), 253 ($\Delta\varepsilon$ +1.66), 277 ($\Delta\varepsilon$ +0.14), 291 ($\Delta\varepsilon$ +0.95), 304 ($\Delta\varepsilon$ +1.66), 324 ($\Delta\varepsilon$ +0.30).

4.5. Improvement of the enantiomeric excess of ester (−)-1b

Acetyl derivative (−)-**2** (54 mg, 0.2 mmol, ee 89%) was dissolved in CH_3OH (2 mL) after which aq NH_4OH (4 mL) was added. The reaction was stirred at 45 °C overnight and then the solvent was evaporated. Hydrolysis product (−)-**1b** was acetylated by an enzymatic procedure reported above to obtain an 85% substrate conversion after 4 days. The acetylated product (−)-**2** was isolated by chromatographic column in 81% yield and ee 98%, $[\alpha]_D^{25} = -163.8$ (*c* 0.4, CH_3OH). Chemical hydrolysis of (−)-**2** gave (−)-**1b** with 100% yield and 98% ee: $[\alpha]_D^{25} = -94.7$ (*c* 0.4, CH_3OH). CD: λ_{ext} 211 ($\Delta\varepsilon$ +0.06), 217 ($\Delta\varepsilon$ −1.92), 220 ($\Delta\varepsilon$ −0.34), 224 ($\Delta\varepsilon$ +1.15), 230 ($\Delta\varepsilon$ −0.62), 239 ($\Delta\varepsilon$ −2.56), 258 ($\Delta\varepsilon$ −0.86), 275 ($\Delta\varepsilon$ −0.11), 303 ($\Delta\varepsilon$ −1.61), 344 ($\Delta\varepsilon$ +0.01).

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