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Kinetic resolution of leishmanicidal *meta* and *para* (±)-2-[Hydroxy(nitrophenyl)methyl]acrylonitrile catalyzed by CALB: *In vitro* evaluations of separated *meta* (R), (S) and (R/S) adducts

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ABSTRACT

The acyl derivatives enantiomers of the Morita–Baylis–Hillman adduct (±)-2-[Hydroxy(*m*-nitrophenyl)methyl]acrylonitrile (**1**) and (±)-2-[Hydroxy(*p*-nitrophenyl)methyl]acrylonitrile (**2**) were efficiently separated by kinetic resolution catalyzed by lipase B from *Candida antarctica* giving (R)-1 and (R)-**2** in very high enantioselectivity (>99% e.e.). These absolute configurations were elucidated by Mosher methodology. The opposite enantiomers (S)-**1** and (S)-**2** (86.8% e.e. and 97.5% e.e., respectively) were prepared through the hydrolysis of the corresponding unreacted esters. Antileishmanial activities for the adduct (±)-**1** and their separated enantiomers (R)-**1** and (S)-**1** were evaluated for the first time. The results showed that the racemic compound is more potent than each separated enantiomers.

1. Introduction

Morita–Baylis–Hillman adducts (MBHA) are a promising new class of bioactive compounds that exhibit several bioactivities, *i.e.* anti-leishmania, anti-malaria, antibacterial, antifungal, herbicide and that are actives against some human tumour cell lines. The bioactivities of MBHA were recently reviewed [1]. This class of compounds are efficiently prepared from Morita–Baylis–Hillman one-pot reaction [2]. The Morita–Baylis–Hillman reaction (MBHR) is a relatively recent method of C-C bond formation [1,2]. This reaction occurs between an electrophilic sp^2 carbon (*i.e.*, aldehydes, ketones or imines) and the α position of an alkene (or alkyne) connected to an electron-attractors groups (EAG), under tertiary amines as nucleophilic catalyst. The 1,4-diazabicyclo[2.2.2]octane (DABCO) is widely used as MBHR catalyst (Scheme 1) [3]. MBHA preparation can be performed efficiently in a single synthetic step depending on the additives and reaction conditions. This fact provides high chemoselectivity in short reactions times, employing ecofriendly synthetic protocols [4].

On the other hand, kinetic resolution represents a key tool in the synthesis of optically active MBHA. The use of lipases to catalyze the kinetic resolution of MBHA is one of the most useful and convenient methods described in the literature [5]. It is mainly used when vinyl acetate is the acyl donor in organic solvents [6]. Other methods quite versatile are enzymatic hydrolysis of acetates or nitrile group of the racemic MBHA [7]. Basavaiah et al. [8] have employed *pig liver* acetone powder (PLAP) for the hydrolysis of acetate group of the racemic MBHA. However, the alcohols were obtained with moderate to low enantiomeric excess.

It should be noted that the enantioselective synthesis of MBHA has been reported in a significant number of studies [3]. Curiously, none of them evaluate the biological activities of enantiomerically pure MBHA.

The MBHA (±)-2-[Hydroxy(*m*-nitrophenyl)methyl]acrylonitrile (±)-**1**, (±)-2-[Hydroxy(*p*-nitrophenyl)methyl]acrylonitrile (±)-**2** (Scheme 2) and the corresponding *ortho* nitro isomers have proven to be the most efficient antiparasitic, *i.e.* anti leishmania [1,9] and anticancer [10] compounds until now. However, the uses of only racemates in biological evaluations represent a limitation to the

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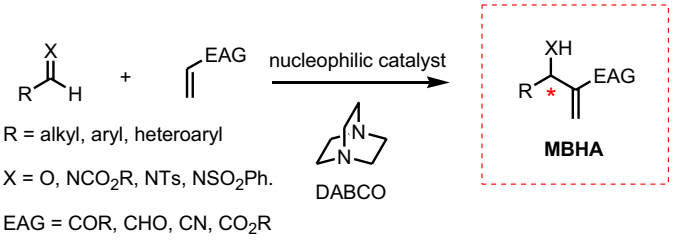


Scheme 1. The Morita–Baylis–Hillman reaction.

development of new drugs. Therefore, in this paper we report the first efficient bio-resolution of the MBHA racemates (±)-**1** and (±)-**2** employing the immobilized *Candida antarctica* Lipase B. Besides, MTPA Mosher's procedure was used to determine the absolute configurations of these compounds. Finally, we report in this work the investigation of the *in vitro* antileishmanicidal activities of enantiomers (S)-**1**, (R)-**1** and (±)-**1** against promastigote form of *Leishmania (V.) braziliensis* [11,12].

2. Results and discussion

The racemic MBHA (±)-**1** and (±)-**2** were synthetized in high yields (99%) through a one-pot free-solvent reaction (0 °C, 30 min.) previously described by us [4]. In a second step, the MBHA (±)-**1** and (±)-**2** were reacted with acetyl chloride to form the esters (±)-**3** (82%) and (±)-**4** (>99%), respectively (Scheme 2) [13].

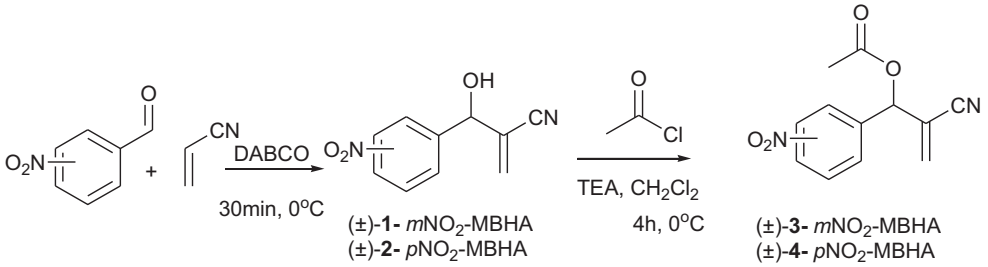
Since biocatalytic resolution is based on the hydrolysis of esters (±)-**4** to optimize a methodology for the efficient kinetic resolution of (±)-**2** (Scheme 3). Thus, four different lipases were tested and the obtained results are summarized in Table 1.

The data from Table 1 showed that the lipases from *Aspergillus niger* (Entry 1) and *Candida rugosa* (Entry 2) converted the ester (±)-**4** to alcohol (±)-**2** (35% and 48% yields respectively). However, no enantiomeric excess of alcohol **2** was observed. On the other hand, the moderated molecular recognition by lipase from *Pseudomonas cepacia* (52% e.e., Entry 3) and high molecular recognition by CALB (37% isolated yield and >99% e.e., Entry 4) resulted mainly in the (R)-**2** alcohol. The unreacted (−)-(S)-**4** ester were separated by flash chromatography (40.6% isolated yield) followed by the hydrolysis with K_2CO_3 resulting in the (−)-(S)-**2** alcohol with 97.5% enantiomeric excess (Fig. 1 and Scheme 3).

The lipase from *C. antarctica* B (Entry 4) was found to be the best enzyme for the resolution of (±)-**4**. It was subsequently applied in the hydrolysis of ester (±)-**3** which resulted in the alcohol (+)-(R)-**1** with 43% of isolated yield and 99.7% enantiomeric excess (Fig. 2). The unreacted ester (−)-3S was isolated with 47.6% yield and quantitatively hydrolysed producing alcohol (−)-(S)-**1** with 86.8% of enantiomeric excess. The chromatograms of racemic (±)-**1** and the purified enantiomers are showed in Fig. 2.

Furthermore, the highly stereospecificity found in the hydrolysis of (±)-**3** and (±)-**4** was maintained even when reactions have been subjected for 4–10 days (analyzing once daily) under CALB catalysis. The structure of MBHA is also important for the hydrolysis reaction success. No reaction was observed when the ester showed the nitro group in the *ortho* position of aromatic moiety, even when the reaction time was increased to several days by CALB and others lipases in Table 1.

The absolute configuration of alcohol (R)-**1** ($[\alpha]_D^{25}$ = +37 (C = 1.6, $CHCl_3$)) (Scheme 2) and (R)-**2** ($[\alpha]_D^{25}$ = +30 (C = 1.6, $CHCl_3$)) was determinate through the MTPA Mosher's protocol [14,15]. These results are in accordance with the Kazlauskas's rule [16]. The structures of the double derivatization of MBHA (+)-(R)-**1** e (+)-(R)-**2** with (S) and (R) MTPA (**5**–**8**) are showed in Fig. 3. The aromatics protons of *m*-NO_2Ph moiety of **6** are deshielded ($\Delta\delta$ = 0.15 ppm) by the (S)-MTPA, in accordance to the R chiral carbon to the alcohol moiety (Fig. 3). On the other hand, the use of (R)-MTPA shields ($\Delta\delta = -0.06\,ppm$) the aromatics protons of *m*-NO_2Ph moiety of **5**, also in accordance to the R chiral carbon to the alcohol moiety (Fig. 3). The same tendency of chemical shift values were obtained for the corresponding *p*-NO_2Ph regioisomers **7** and **8**



Scheme 2. Preparations of esters (±)-**3** and (±)-**4**.

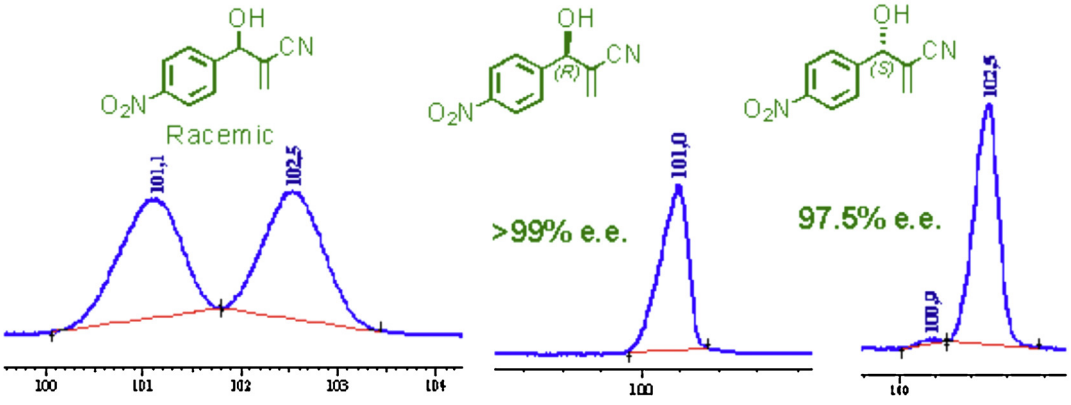


Fig. 1. Chromatograms of racemic (±)-**2** and the corresponding isolated enantiomers.