Applied Catalysis A: General 492 (2015) 76–82

Contents lists available at ScienceDirect

Applied Catalysis A: General

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

Chemoenzymatic synthesis of rasagiline mesylate using lipases

Thiago de Sousa Fonseca, Marcos Reinaldo da Silva, Maria da Conceição Ferreira de Oliveira, Telma Leda Gomes de Lemos, Ricardo de Araújo Marques, Marcos Carlos de Mattos*

Departamento de Química Orgânica e Inorgânica, Laboratório de Biotecnologia e Síntese Orgânica (LABS), Universidade Federal do Ceará, Campus do Pici, Caixa Postal 6044, 60455-970 Fortaleza, CE, Brazil

ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 4 December 2014
Accepted 7 December 2014
Available online 12 December 2014

Keywords:
Rasagiline mesylate
Chemoenzymatic synthesis
Kinetic enzymatic resolution
Lipases
Mitsunobu reaction
Indanol
Pseudomonas fluorescens
Amano AK
Thermomyces lanuginosus

ABSTRACT

A straightforward chemoenzymatic synthesis of rasagiline mesylate has been developed. The key steps for the introduction of chirality involved kinetic enzymatic resolution with lipases via acetylation of *rac*-indanol and an inversion configuration Mitsunobu reaction of the produced (*S*)-indanol. Immobilized lipase from *Thermomyces lanuginosus* proved to be a robust biocatalyst in the kinetic resolution, leading to (*S*)-indanol with high selectivity (*e.e.* > 99%, *E* > 200) in just 15 min, at 35 °C, in hexane, being reused for ten-times without significant loss of the activity and selectivity.

1. Introduction

Rasagiline mesylate (***R*-7**) (Fig. 1), also known as *R*-(+)-*N*-propargyl-1-aminoindan mesylate, is a commercially marketed pharmaceutically active substance, under the brand name Azilect®, indicated for treatment of Parkinson's disease (PD) being effective both as monotherapy in early PD and as adjunctive in patients with advancing PD and motor fluctuations [1–9]. This chiral compound is a potent second-generation propargylamine pharmacophore that selectively and irreversibly inhibits the B-form of the monoamine oxidase enzyme (MAO-B) over type A by a factor of fourteen [3–10]. European drug-regulatory authorities approved this potent MAO-B inhibitor in February 2005 and the US FDA in May 2006 [11]. Although the *S*-(−)-enantiomer of *N*-propargyl-1-aminoindane still exerts some neuroprotective properties, the potency of *R*-(+)-enantiomer against the MAO-B enzyme is approximately 1000-fold higher [12].

In the last years, many efforts have been focused on developing procedures to introduce chirality in the target molecule (***R*-7**) by the synthesis of *R*-1-aminoindan or *R*-*N*-propargyl-1-aminoindan (***R*-6**). Some examples are the strategies based on classical kinetic resolution with chiral acids [4,12–22], hydrosilylation in the presence of chiral rhodium [23] or ruthenium [24] catalysts, asymmetric synthesis using chiral auxiliaries [25–28] or asymmetric induction using chiral oxazaborolidine [29]. Biocatalytic routes include deracemization with cyclohexylamine oxidase (CHAO) [30], kinetic enzymatic resolution in the presence of subtilisin [31,32], ω-transaminase [33], lipase from *Candida antarctica* B [34] and more recently dynamic kinetic resolution by *C. antarctica* B and Pd nanocatalyst [35]. Although less employed, an alternative approach to the introduction of chirality in rasagiline mesylate (***R*-7**) is the synthesis of the intermediate (*S*)-indanol followed by conversion of the hydroxyl group into a leaving group, and subsequent reaction with an appropriate nucleophile to promote inversion of configuration [36,37].

Chemists have found great difficulties in the kinetic resolution of small molecules via nonenzymatic systems, *rac*-indanol being historically one of the most challenging alcohols to enrich via the aforementioned methodology [38–41]. On the other hand, enzymatic processes have been utilized successfully in resolving both enantiomers of *rac*-indanol with high selectivity, especially in the presence of lipases [42–50].

* Corresponding author. Tel.: +55 8533669144; fax: +55 8533669782.
E-mail address: mcdmatto@ufc.br (M.C. de Mattos).

http://dx.doi.org/10.1016/j.apcata.2014.12.015
0926-860X/

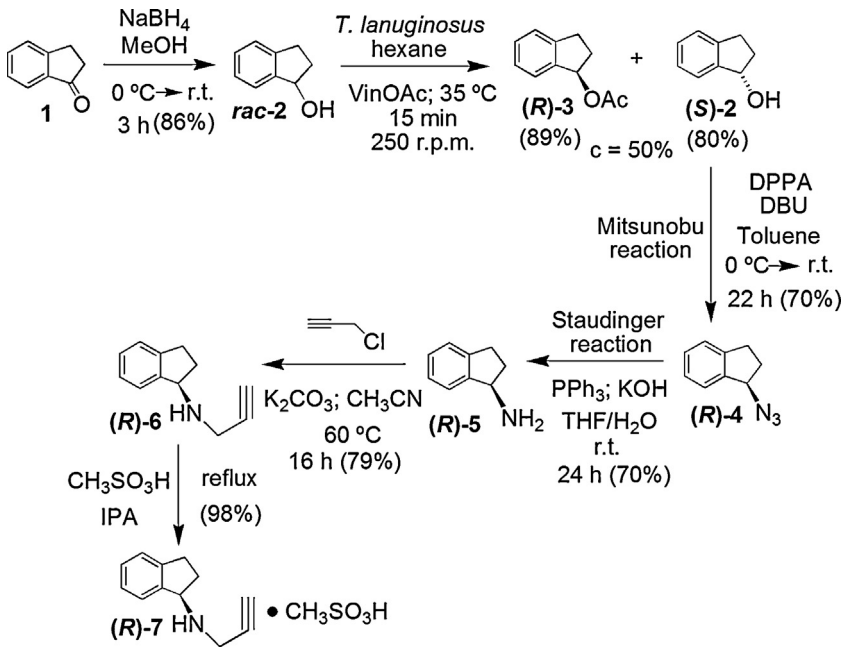

Fig. 1. Chemoenzymatic synthesis of rasagiline mesylate.

Particularly, lipases (EC 3.1.1.3) have largely demonstrated their powerful utility as a versatile tool for the synthesis of enantiomerically pure compounds of great importance, and are the most employed enzymes in the industrial process in the last three decades. This fact is mainly based on their wide availability in nature, their broad substrate acceptance and low cost, besides the maintenance of high activity and selectivity in organic solvents, and the fact of not requiring the addition of cofactors during the biocatalytic process [51,52].

Herein, we wish to report the chemoenzymatic synthesis of rasagiline mesylate (***R*-7**) which had the introduction of the chirality in the target molecule achieved by lipase-mediated kinetic resolution of *rac*-indanol (***rac*-2**) followed by Mitsunobu reaction. We have especially focused on the optimization of the reaction conditions including the enzymes recycling outcome.

2. Experimental

2.1. Enzymes

(i) Immobilized lipases: *C. antarctica* lipase type B immobilized on acrylic resin (CAL-B, Novozym 435, 7300.0 U/g) and *Rhizomucor miehei* lipase immobilized on anionic resin (RML, 150.0 U/g) were purchased from Novozymes®. *Thermomyces lanuginosus* lipase immobilized on immobead-150 (TLL, 250.0 U/g) and *Rhizopus oryzae* lipase immobilized on immobead-150 (ROL, 340.0 U/g) were acquired from Sigma-Aldrich®. (ii) Free lipases: *Pseudomonas fluorescens* lipase (AK, 22,100.0 U/g), *Penicillium camemberti* lipase (G, 50.0 U/g), *Aspergillus melleus* lipase (Acylase I, 200.0 U/g) were acquired from Sigma-Aldrich®. Porcine pancreas lipase (PPL, 46.0 U/g solid), and *Candida rugosa* lipase (CRL, 1.4 U/g) were obtained from Sigma®.

2.2. Chemical materials

Chemical reagents were purchased from different commercial sources and used without further purification. Methanol, hexane, ethyl acetate and dichloromethane were acquired from Synth®. Toluene and diethyl ether were obtained from Vetec®. Acetonitrile and hexane, HPLC grade, were purchased from TEDIA® and tetrahydrofuran was acquired from Sigma-Aldrich®. Solvents used in the reaction of biocatalysis were distilled over an adequate desiccant under nitrogen. Analytical TLC analyses were performed on aluminum sheets pre-coated with silica gel 60 F254 (0.2-mm thick) from Merck®. Flash chromatographies were performed using silica gel 60 (230–240 mesh).

2.3. Analysis

Melting points of the *rac*-indanol (***rac*-2**) and (*R*)-*Rasagiline Mesylate* (***R*-7**) were determined in open capillary tube Mettler Toledo model FP62 and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1720-X F7 using NaCl plates or KBr pellets in. ^{1}H, ^{13}C NMR, and DEPT were obtained using Spectrometers Bruker model Avance DPX 300 and Avance DRX-500, operating at frequencies of 300 and 500 MHz for hydrogen and frequencies of 75 and 125 MHz for carbon, respectively. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). Measurement of the optical rotation was done in a Perkin-Elmer 241 polarimeter. Gas chromatograph (GC) analysis were carried out in a Shimadzu chromatograph model GC 2010 with a flame ionization detector using a chiral column CP-chirasil-dex (25 m × 0.25 mm × 0.25 μm, 0.5 bar N_2). For the following of the reaction time courses: 110 °C; 0.5 °C/min 130 °C (hold 15 min); 5.0 °C/min 140 °C (hold 5 min). Retention times were: (*S*)-acetate (***S*-3**) 32.68 min; (*R*)-acetate (***R*-3**) 33.17 min; (*S*)-alcohol (***S*-2**) 37.95 min; (*R*)-alcohol (***R*-2**) 38.98 min.

2.4. Calculation of enantiomeric excess, conversion and enantiomeric ratio

The efficiency of kinetic resolution was evaluated based on the optical purity of the compounds, expressed in terms of enantiomeric excess of the substrate ($e.e._s$) and product ($e.e._p$), using the following Eqs. (1) and (2):

$$e.e._s = \frac{A-B}{A+B} \quad (1)$$

$$e.e._p = \frac{A-B}{A+B} \quad (2)$$

A denote the majority enantiomer and *B* denote the minority enantiomer represented by the chromatographic peak areas.

The conversion (*c*) is calculated using Eq. (3):

$$c = \frac{e.e._s}{e.e._s + e.e._p} \quad (3)$$

Enantioselectivity was expressed as enantiomeric ratio (*E*) and calculated by Eq. (4):

$$E = \frac{\ln[1 - c(1 + e.e._p)]}{\ln[1 - c(1 - e.e._p)]} \quad (4)$$

The results of the $e.e._s$ and $e.e._p$ are expressed in a percentage using Eqs. (5) and (6):

$$e.e._s = \frac{A - B}{A + B} \times 100 \quad (5)$$

$$e.e._p = \frac{A - B}{A + B} \times 100 \quad (6)$$

Additionally, the conversion (*c*) is also calculated in a percentage, using (5) and (6).

2.5. Synthesis of rac-indanol (*rac-2*)

To a solution of indanone (**1**) (1000 mg, 7.50 mmol) in methanol (75.5 mL), sodium borohydride (1142.3 mg, 30.20 mmol) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then for 2.5 h at room temperature, after which the solvent was evaporated under reduced pressure. The resulting suspension was acidified with 10 mL of 1N HCl and extracted with EtOAc (3 × 50 mL). Organic phases were combined and dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure, and the resulting crude purified after flash chromatography (10–90% EtOAc/hexane) to afford *rac*-indanol (***rac*-2**) as a white solid in 86% yield.

2.6. Synthesis of rac-indanyl acetate (*rac-3*)

DMAP (143.8 mg, 1.25 mol) and acetic anhydride (1185.6 μL, 12.48 mmol) were added to a *rac*-indanol (***rac*-2**), 500 mg, 4.16 mmol) solution in dichloromethane (40 mL). The reaction was stirred at room temperature during 4 h and after that time, the solvent was evaporated under reduced pressure. The resulting crude was purified by flash chromatography on silica gel (5–95% EtOAc/hexane) to afford the desired *rac*-indanyl acetate (***rac*-3**) as a yellow liquid in 80% yield.

2.7. Synthesis of (R)-azidoindane (*R-4*)

Over a solution under a nitrogen atmosphere of (*S*)-indanol (***S*-2**) (500 mg, 3.70 mmol) in dry toluene (37 mL), DPPA (956 μL, 4.44 mmol) was added for 10 min at 0 °C, after DBU (663.0 μL, 4.44 mmol) was added dropwise at 0 °C for 2 h and the resulting mixture was stirred at room temperature for 20 h. After this time, the solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography on silica gel (hexane) to afford (*R*)-azidoindane (***R*-4**) as a yellow liquid in 70% yield.

2.8. Synthesis of (R)-indanamine (*R-5*)

A suspension of (*R*)-azidoindane (***R*-4**) (200 mg, 1.26 mmol), PPh_3 (366.8 mg, 1.40 mmol) and KOH (70.56 mg, 1.26 mmol) in THF (9.45 mL) and water (3.15 mL) was stirred at room temperature for 24 h. After this time, the solvent was evaporated under reduced pressure and the crude product was diluted with 5 mL of a 25% HCl solution to pH 2. The resulting aqueous solution was washed with Et_2O (3 × 50 mL) and treated with 8 mL of 25% NaOH solution to pH 14. After, the aqueous solution was extracted with Et_2O (3 × 50 mL) and the organic phases were combined and washed with brine (30 mL), dried with Na_2SO_4, filtered and the solvent evaporated under reduced pressure affording (*R*)-indanamine (***R*-5**) as a brown oil in 70% yield.

2.9. Synthesis of (R)-N-propargyl-1-aminoindan (*R-6*)

A suspension of (*R*)-indanamine (***R*-5**) (70 mg, 0.53 mmol), K_2CO_3 (76 mg, 0.53 mmol) and propargyl chloride (39.4 μL, 0.53 mmol) in acetonitrile (5.3 mL) was stirred at 60 °C for 16 h. After this time, the solvent was evaporated under reduced pressure and the crude product was diluted with 10 mL of a 10% NaOH solution. The resulting aqueous solution was extracted with CH_2Cl_2 (3 × 50 mL) and the organic phases were combined and dried with Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography on silica gel (40–60% EtOAc/hexane) affording the corresponding (*R*)-*N*-propargyl-1-aminoindan (***R*-6**) as an orange liquid in 79% yield.

2.10. Synthesis of (R)-rasagiline mesylate (R-*7*)

A suspension of (*R*)-*N*-propargyl-1-aminoindan (***R*-6**) (50 mg, 0.29 mmol) in 5 mL of isopropanol and 3 μL of methanesulfonic acid was heated to reflux for 1 h. After, the mixture was allowed to cool to 5 °C. The obtained suspension was filtered, and the collected solid was washed with 1.5 mL of isopropanol, affording the corresponding optically active (*R*)-rasagiline mesylate (***R*-7**) as a white solid in 98% yield.

2.11. General procedure for the lipase-catalyzed hydrolysis of rac-indanyl acetate (*rac-3*) (screening)

A suspension of *rac*-indanyl acetate (***rac*-3**) (30 mg, 0.17 mmol) and lipase (ratio 2:1 in weight respect to the ***rac*-3**) in a mixture of phosphate buffer 100 mM pH 7.0/THF (80/20, v/v) was shaken at 30 °C and 250 rpm for 24 h. After this time, the products were extracted with EtOAc (3 × 10 mL) and the organic phases were combined and dried with Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The reaction crude was purified by flash chromatography on silica gel (5–95% EtOAc/hexane), yielding (*S*)-indanyl acetate (***S*-3**) and (*R*)-indanol (***R*-2**) being their enantiomeric excess determined by GC.

2.12. General procedure for the lipase-catalyzed acetylation of rac-indanol (*rac-2*)

To a suspension of the *rac*-**2** (30 mg, 0.22 mmol) and lipase (15 mg) in dry organic solvent (2.2 mL) under nitrogen atmosphere, vinyl acetate (103 μL, 1.10 mmol) was added, and the reaction was shaken at temperatures ranging from 30 to 50 °C and 250 rpm. Aliquots were regularly analysed by GC analysis and after the adequate time, the reaction was stopped and the enzyme filtered off and washed with the respective solvent (50 mL). The solvent was evaporated under reduced pressure and the reaction crude purified by flash chromatography on silica gel (5–95% EtOAc/hexane), yielding (*S*)-indanol (***S*-2**) and (*R*)-indanyl acetate (***R*-3**), being their enantiomeric excess determined by GC.

rac-Indanol (***rac*-2**): Solid. R_f (10% EtOAc/hexane): 0.25. m.p.: 52–55 °C. IR υ_{max} (cm^{-1}): 3209, 1475, 1455, 1326, 1053, 760 and 738 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ (ppm): 1.9 (m, 1H), 2.4 (m, 1H), 2.8 (m, 1H), 3.0 (m, 1H), 5.1 (t, 1H, J = 6.2 Hz), 7.2 (m, 3H) and 7.4 (dd, 1H, J = 6.0 Hz and 2.6 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 29.9 (CH_2), 35.9 (CH_2), 76.4 (CH), 124.3 (CH), 125.0 (CH), 126.8 (CH), 128.4 (CH), 143.4 (C) and 145.1 (C).

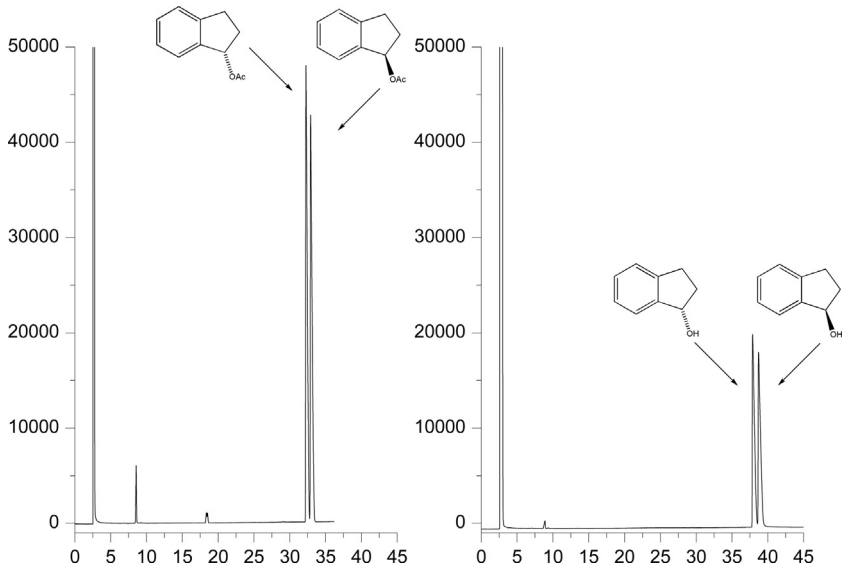

Fig. 2. GC chromatograms of *rac*-indanyl acetate t_R 32.68 min. (*S*) t_R 33.17 min. (*R*) and *rac*-indanol t_R 37.95 min. (*S*) t_R 38.98 min. (*R*).

rac-Indanyl acetate (***rac*-3**): Liquid. R_f (5% EtOAc/hexane): 0.53. IR υ_{max} (cm^{-1}): 1730, 1232, 1479, 1462 and 751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.0 (s, 3H), 2.1 (m, 1H), 2.5 (m, 1H), 2.9 (m, 1H), 3.1 (m, 1H), 6.2 (t, 1H, *J* = 6.8 Hz), 7.2 (m, 3H) and 7.4 (dd, 1H, *J* = 7.9 and 2.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 21.5 (CH_3), 30.4 (CH_2), 32.5 (CH_2), 78.6 (CH), 125.0 (CH), 125.7 (CH), 126.9 (CH), 129.1 (CH), 141.3 (C), 144.6 (C) and 171.3 (C).

(*R*)-azidoindane (***R*-4**): Liquid. R_f (hexane): 0.55. IR υ_{max} (cm^{-1}): 2086, 1479, 1478, 1235 and 751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.1 (m, 1H), 2.4 (m, 1H), 2.9 (m, 1H), 3.0 (m, 1H), 4.9 (dd, 1H, *J* = 9.0 Hz and *J* = 4.9 Hz), 7.3 (m, 3H) and 7.4 (dd, 1H, *J* = 6.5 Hz and 2.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 30.5 (CH_2), 32.5 (CH_2), 65.9 (CH), 124.6 (CH), 125.1 (CH), 126.9 (CH), 128.9 (CH), 140.7 (C) and 143.7 (C). $[\alpha]_D^{20}$ = +28.7 (*c* 1.5, hexane) for 95% *e.e.* of the (*R*)-enantiomer. Lit $[\alpha]_D^{20}$ = +29.3 (*c* 1.61, hexane) for 94.1% *e.e.* of the (*R*)-enantiomer [53].

(*R*)-indanamine (***R*-5**): Oil. R_f (10% MeOH/CH_2Cl_2): 0.25. IR υ_{max} (cm^{-1}): 2920, 2849, 1476, 1457, 1059 and 740 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.7 (m, 1H), 1.8 (s, 2H), 2.5 (m, 1H), 2.8 (m, 1H), 2.9 (m, 1H), 4.4 (t, 1H, *J* = 7.4 Hz), 7.2 (m, 3H) and 7.3 (dd, 1H, *J* = 9.0 Hz and 2.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 30.3 (CH_2), 37.5 (CH_2), 57.4 (CH), 123.5 (CH), 124.9 (CH), 126.7 (CH), 127.4 (CH), 143.7 (C) and 147.6 (C). $[\alpha]_D^{20}$ = −19.6 (*c* 1.0, $CHCl_3$) for 92% *e.e.* of the (*R*)-enantiomer. Lit $[\alpha]_D^{20}$ = −20.0 (*c* 1.0, $CHCl_3$) for 96% *e.e.* of the (*R*)-enantiomer [27].

(*R*)-*N*-propargyl-1-aminoindan (***R*-6**) Liquid. R_f (40% EtOAc/hexane): 0.40. IR υ_{max} (cm^{-1}): 3290, 2926, 2848, 1477, 1456 and 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.7 (s, 1H), 1.85 (m, 1H), 2.25 (s, 1H), 2.4 (m, 1H), 2.8 (m, 1H), 3.1 (m, 1H), 3.5 (s, 2H), 4.4 (t, 1H, *J* = 6.2 Hz), 7.2 (m, 3H), 7.4 (dd, 1H, *J* = 7.9 and 2.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 30.7 (CH_2), 33.5 (CH_2), 36.4 (CH_2), 62.1 (CH), 71.6 (CH), 82.7 (C), 124.4 (CH), 125.1 (CH), 126.5 (CH), 127.8 (CH), 144.0 (C) and 144.7 (C). $[\alpha]_D^{20}$ = +17.6 (*c* 1.0, $CHCl_3$) for 92% *e.e.* of the (*R*)-enantiomer. Lit $[\alpha]_D^{25}$ = +18.8 (*c* 1.7, $CHCl_3$) for 94% *e.e.* of the (*R*)-enantiomer [54].

Rasagiline mesylate (***R*-7**): Solid. Mp: 156–158 °C. IR υ_{max} (cm^{-1}): 3292, 3050 2920, 2847, 1471, 1458, 1351, 1180, 985, 890, 752, 740, 518 and 492 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm): 2.0 (s, 1H), 2.3 (s, 1H), 2.5 (m, 1H), 2.6 (s, 3H), 2.7 (m, 1H), 2.9 (m, 1H), 3.1 (m, 1H), 3.8 (s, 2H), 4.0 (t, 1H, *J* = 6.2 Hz), 7.1 (m, 3H), 7.45 (dd, 1H, *J* = 7.8 Hz and 3.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 30.0 (CH_2), 33.1 (CH_2), 37.2 (CH_2), 38.0 (CH_3), 62.8 (CH), 80.7 (CH), 84.0 (C), 124.6 (CH), 125.0 (CH), 126.8 (CH), 128.0 (CH), 144.8 (C) and 145.1 (C). $[\alpha]_D^{20}$ = +19.0 (*c* 2.0, EtOH) for 92% *e.e.* of the (*R*)-enantiomer. Lit $[\alpha]_D^{20}$ = +21.46 (*c* 2.0, EtOH) for 99% *e.e.* of the (*R*)-enantiomer [55].

3. Results and discussion

The straightforward chemoenzymatic synthesis of rasagiline mesylate (***R*-7**) is depicted in Fig. 1. The key steps for the introduction of the *R*-configuration in the target molecule (*R*-**7**) were the kinetic enzymatic resolution of racemic indanol (***rac*-2**), and subsequent Mitsunobu reaction in the obtained (*S*)-indanol (***S*-2**).

3.1. Synthesis of racemic indanol (*rac*-2) and its acetate (*rac*-3)

Initially, the reduction of indanone (**1**) was carried out by using sodium borohydride in MeOH to yield the racemic alcohol (***rac*-2**) in 86% yield after flash chromatography [56]. Next, the chemical acetylation (Ac_2O/DMAP/CH_2Cl_2) of ***rac*-2** at room temperature allowed the preparation of the corresponding racemic acetate (***rac*-3**) with 80% isolated yield. Adequate chiral GC analyses were developed for both alcohol and acetate in order to achieve a reliable method to measure the enantiomeric excess values of both remaining substrate and the final product from the lipase-catalyzed resolution, Fig. 2.

3.2. Kinetic resolution of rac-indanyl acetate (*rac*-3) via hydrolysis reaction

For initial screening, nine commercially available lipases (ratio 2:1 in weight respect to ***rac*-3**) were tried in the resolution of ***rac*-3** at 30 °C and 24 h of time reaction. This hydrolytic approach is depicted in Fig. 3 (Path a) and all the experimental data are summarized in Table 1.

As result, lipases from *R. oryzae* immobilized on immobead-150, *C. antarctica* type B immobilized on acrylic resin, *A. melleus* and *R. miehei* immobilized on anionic resin led to low values of enantiomeric ratio (E 1–14), conversion (*c* 3–18%) and enantiomeric excess of the substrate ($e.e._s$ 1–15%) and product ($e.e._p$ 14–86%), respectively (Table 1 entries 3, 5, 8 and 9). Meanwhile, lipases from *P. camemberti* and porcine pancreas led to high values of enantiomeric excess of the product ($e.e._p$ 96% and 89%), but low values of

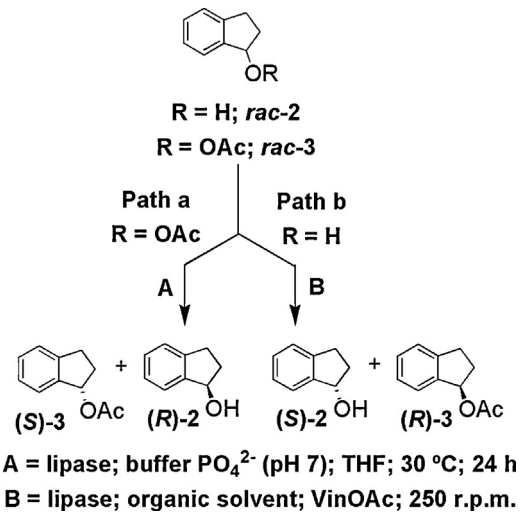

Fig. 3. (Path a) Kinetic enzymatic resolution of ***rac*-3** via hydrolysis reaction; (Path b) kinetic enzymatic resolution of ***rac*-2** via acetylation reaction.

Table 1
Kinetic resolution via hydrolysis of *rac*-indanyl acetate using lipases.[a]

Entry	Lipase	$e.e._s$[b] (%)	$e.e._p$[b] (%)	c[c] (%)	E[d]
1	*P. camemberti*	40	96	29	76
2	*P. fluorescens*	90	94	49	111
3	*R. oryzae* immobilized on immobead-150	15	78	16	9
4	*T. lanuginosus* immobilized on immobead-150	93	96	49	167
5	CAL-B immobilized on acrylic resin	3	14	18	1
6	*C. rugosa*[e]	82	86	51	28
7	Porcine pancreas	21	89	19	23
8	Acylase I from *A. melleus*	1	37	3	2
9	*R. miehei* immobilized on anionic resin	6	86	7	14

[a] Conditions: 30 °C, 24 h and lipase:***rac*-3** (2:1) at 250 r.p.m.
[b] Determined by GC.
[c] Conversion, $c = e.e._s/(e.e._s + e.e._p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + e.e._p)]/\ln[1 - c(1 - e.e._p)]$.
[e] Conversion of 51% reached at 14 h.

enantiomeric excess of the substrate ($e.e._s$ 40% and 21%), low conversion values (c 29 and 19%) and moderate to low enantiomeric ratio (E 76 and 23), respectively (Table 1, entries 1 and 7). Conversion value of 60% was achieved in the presence of lipase from *C. rugosa*, after 24 h of reaction. With this result, enantiomeric excess values of both substrate and product could not be analyzed. Thus, we monitored the reaction until a conversion of 51% in 14 h was reached. As a result enantiomeric excess values of substrate and product of 82% and 86%, respectively, and enantiomeric ratio (E) of 28, were obtained (Table 1, entry 6).

The most effective lipases in the kinetic resolution of indanyl acetate (***rac*-3**) were lipase from *P. fluorescens* Amano AK and the lipase from *T. lanuginosus*, with conversion close to 50%, enantiomeric excess values of the substrate ($e.e._s$ 90% and 93%) and product ($e.e._p$ 94% and 96%), respectively and the enantiomeric ratio (E) > 100 (Table 1, entries 2 and 4).

3.3. Kinetic resolution of indanol (*rac*-2) via acetylation reaction

With the aim of improving enzymatic kinetic resolution and produce indanol with the desired *S*-configuration (***S*-2**), we turned our attention to the acetylation reaction in organic solvents by using the most efficient enzymes (ratio 2:1 in weight respect to ***rac*-2**) from the previous screening, Fig. 3 (Path b). The acetylation reactions were carried out in the presence of vinyl acetate (VinOAc) as acyl donor and organic solvents as acetonitrile (MeCN), toluene, tetrahydrofuran (THF) and hexane at temperatures ranging from 30 to 50 °C. All the experimental data are summarized in Table 2.

The kinetic resolution of *rac*-indanol (***rac*-2**) in the presence of lipase Amano AK from *P. fluorenscens* was more efficient using hexane at 30 °C in 15 min of reaction, obtaining the ***S*-2** with 98% enantiomeric excess and the remaining acetate ***R*-3** with 97% enantiomeric excess, conversion of 50%, and enantiomeric ratio (E) > 200 (Table 2, entry 7). A slightly better enantiopreference was observed when the reaction was carried out in the presence of lipase from *T. lanuginosus* in hexane, attaining 50% conversion after 15 min at 35 °C, and yielding both *S*-indanol (***S*-2**) and *R*-indanyl acetate (***R*-3**) with enantiomeric excess > 99%, and enantiomeric ratio (E) > 200 (Table 2, entry 16). It is noteworthy that the kinetic resolution in the presence of *T. lanuginosus* in hexane at 30 °C reached a conversion of 50% after 6 h, but an increase in temperature of 5 °C (35 °C) was responsible for achieving 50% conversion in just 15 min of reaction. Low or moderate activity and selectivity were observed in

Table 2
Kinetic resolution via acetylation of *rac*-indanol using lipases in organic solvents.[a]

Lipase	Entry	Solvent	T (°C)	Time (min)	$e.e._s$ (%)[b]	$e.e._p$ (%)[b]	c (%)[c]	E[d]
Lipase AK from *P. fluorescens*	1	MeCN	30	360	94	96	49	>200
	2	MeCN	50	60	92	94	49	116
	3	Toluene	30	360	82	>99	45	>200
	4	Toluene	50	360	51	>99	34	>200
	5	THF	30	360	3	>99	3	>200
	6	THF	50	360	71	>99	42	>200
	7	Hexane	30	15	98(78)	97(85)	50	>200
	8	MeCN	30	360	32	>99	24	>200
T. lanuginosus lipase immobilized on immobead-150	9	MeCN	50	360	77	>99	44	>200
	10	Toluene	30	360	9	>99	8	>200
	11	Toluene	50	360	17	>99	14	>200
	12	THF	30	360	3	>99	3	>200
	13	THF	50	360	1	>99	1	>200
	14	Hexane	30	360	>99	96	51	>200
	15	Hexane	50	60	>99	92	52	126
	16	Hexane	35	15	>99(80)	>99(89)	50	>200

[a] Conditions: 5 equiv. of vinyl acetate and lipase:***rac*-2** (2:1) at 250 r.p.m.
[b] Determined by GC and isolated yields in brackets.
[c] Conversion, $c = e.e._s/(e.e._s + e.e._p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + e.e._p)]/\ln[1 - c(1 - e.e._p)]$.

the presence of solvents with higher dielectric constant as MeCN (37.5), THF (7.5) and toluene (2.4) when compared with hexane (1.9) even at higher temperatures. In fact, Fitzpatric and Klibanov [57] showed that the dielectric constant of a solvent maintains an inverse relationship with enantioselectivity of an enzyme. According to these authors, the increase of the dielectric constant increases the flexibility of the enzyme and with this, the steric constraints become more forgiving, thereby allowing for a great reactivity of both enantiomers and, in turn, relaxed enantioselectivity. In addition, it is known that the structure of a solvent exerts some influence in the enantioselectivity. Generally, linear solvents are more effective than those with a branched chain in transesterification mediated by a lipase [58]. Besides dielectric constant and the structure of solvent, 1-octanol–water partition constant (log *P*) is an important solvent property that was proposed as a quantitative measure of solvent polarity. In general, the enzyme activity for lipase catalyzed reactions increases with increasing the log *P* value of the solvent. Polar solvents strips-off the essential hydration layer on the enzyme surface and thus caused the enzyme inactivation, whereas apolar solvents preserves the microaqueous layer around the enzyme, thereby retaining the activity [59,60]. Thus, *n*-hexane (log *P* 3.5) was more effective in comparison with solvents with lower log *P* such as toluene (2.5), THF (0.46) and MeCN (−0.34). It is noteworthy that the relationship between log *P* of the solvent and the ability of a lipase to mediate a specific reaction is not so straightforward suggesting an in-depth analysis of the reaction system is required taking into account an appropriate juxtaposition of polarities of substrate, product, solvent, and enzyme–solvent interphase [61,62].

3.4. Optimization of enzyme:substrate ratio

Based on the results achieved with lipase from *T. lanuginosus*, this enzyme was chosen for subsequent experiments. An attempt was also made to improve the enzyme loading (enzyme:substrate). Consequently, a set of preliminary experiments were performed with decreasing amount of enzyme (1:1, 0.5:1 and 0.25:1), which led to identical results when 1:1 and 0.5:1 ($e.e._s$ and $e.e._p > 99\%$, $c = 50\%$) enzyme:substrate ratios were used. A decrease in the enzymatic activity and selectivity ($e.e._s = 75\%$; $e.e._p > 99\%$ and $c = 43\%$) was observed with the ratio of 0.25:1.

3.5. Reuse of the immobilized enzyme

The next step was to study the recycling of the lipases from *T. lanuginosus* immobilized on immobead-150 by performing the acetylation of *rac*-indanol (***rac*-2**) with vinyl acetate in hexane at 35 °C and using a ratio of 0.5:1 (enzyme:substrate, w/w) for 15 min of reaction. Reproducible results were observed, over ten reuse cycles, in terms of enantioselectivity ($E > 200$), conversion (50–51%) and enantiomeric excess values (97% to >99%).

3.6. Synthesis of rasagiline mesylate (*R*-7)

Once the potential of the kinetic enzymatic resolution of *rac*-indanol via acetylation reaction was demonstrated to obtain enantiomerically pure ***S*-2**, we turned our attention to the synthesis of *rasagiline mesylate* (***R*-7**). Starting from ***S*-2**, a modified Mitsunobu reaction [63] was performed, which yielded (*R*)-azidoindane (***R*-4**) in 70%. This compound was subjected to Staudinger reaction in a similar protocol described by Hanessian *et al.* [64], in the presence of PPh_3 and THF/H_2O, and afforded (*R*)-indanamine (***R*-5**) in moderate yield (45%). While searching for suitable reaction conditions [65,66], we found that adding base (KOH) in the reaction medium and using saturated NaCl solution (brine) in the extraction step improves the yield of (*R*)-indanamine (***R*-5**) to 70%.

Finally, reaction between *R*-indanamine (***R*-5**) and propargyl chloride afforded (*R*)-*N*-propargyl-1-aminoindan (***R*-6**) in 79% yield. Subsequent treatment of ***R*-6** with methanesulfonic acid afforded rasagiline mesylate (***R*-7**) in 98% yield.

4. Conclusion

In summary, we have successfully developed a chemoenzymatic synthesis of rasagiline mesylate (***R*-7**). The key steps for introduction of chirality in the target molecule with *R*-configuration were achieved by lipase-mediated kinetic resolution via acetylation reaction of *rac*-indanol (***rac*-2**) followed by Mitsunobu reaction. The most effective lipases in kinetic resolution of *rac*-indanol were Amano AK from *P. fluorenscens* and lipase from *T. lanuginosus*. The latter proved to be a robust biocatalyst since in only 15 minutes of reaction, a ratio enzyme:substrate (0.5:1), under mild conditions, led to the desired enantiomer (*S*)-indanol (***S*-2**) with *e.e.* > 99% at full conversion of 50%. Finally, this synthetic strategy to obtain rasagiline mesylate can be considered environmentally benign, since a commercially available, low cost, stable, reusable for multiple reaction cycles and highly enantioselective biocatalyst was used.

Acknowledgements

The authors thank to the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Ensino Superior (CAPES) for fellowships and financial support.

References

[1] S.B.D. Winter, E.D. Lopez, M.B. Velez, US Patent 0 041 920 (2010), to K&L Gates LLP.
[2] M.S. Sudhir, R.V. Nadh, Drug Invent. Today 5 (2013) 133–138.
[3] J.J. Chen, D.M. Swope, K. Dashtipour, Clin. Ther. 29 (2007) 1825–1849.
[4] S.K. Kapubalu, T.R. Kovvuri, J.M.R. Gunnam, O. Gudaparthi, P.K. Dubey, Int. J. Pharm. Sci. Rev. Res. 12 (2012) 161–163.
[5] G. Marras, M. Rasparini, R. Tufaro, G. Castaldi, D. Ghisleri, EP 2 181 980 2010, to Chemo Ibérica S.A.
[6] M. Fernández, S. Negro, K. Slowing, A. Fernández-Carballido, E. Barcia, Int. J. Pharm. 419 (2011) 271–280.
[7] S. Hoy, G. Keating, Drugs 72 (2012) 643–669.
[8] S. Perez-Lloret, O. Rascol, Expert Opin. Drug Saf. 10 (2011) 633–643.
[9] O. Weinreb, T. Amit, P. Riederer, M.B.H. Youdim, S.A. Mandel, in: B.H.Y. Moussa, D. Peter (Eds.), International Review of Neurobiology, Academic Press, New York, 2011, p. p281.
[10] M. Fernández, E. Barcia, A. Fernández-Carballido, L. Garcia, K. Slowing, S. Negro, Int. J. Pharm. 438 (2012) 266–278.
[11] D.R.P. Guay, Am. J. Geriatr. Pharmacother. 4 (2006) 330–346.
[12] L. Selic, WO Patent 064 216 (2011), to LEK Pharmaceuticals D.D.
[13] M.B.H. Youdim, J.P.M., Finberg, R. Levy, J. Sterling, D. Lerner, T. Berger-Paskin, H. Yellin, A. Veinberg, US Patent 5 532 415 (1996), to Teva Pharmaceutical Industries, Ltd. and Technion Research and Development Foundation Ltd.
[14] R. Lidor, E. Bahar, WO Patent 21 640 (1996), to Teva Pharmaceutical Industries, Ltd. and Lemmon Company.
[15] R. Lidor, E. Bahar, O. Zairi, G. Atili, D. Amster, Org. Prep. Proc. Int. 29 (1997) 701–706.
[16] A.L. Gutman, I. Zaltzman, V. Ponomarev, M. Sotrihim, G. Nisnevich, WO Patent 068 376 (2002), to ISP Finetech Ltd.
[17] C.-H. Yao, T.-T. Chen, US Patent 0 218 361 (2011), to Everlight USA, Inc.
[18] A.A. Thanedar, S.A. Deshmukh, S.S. Zope, L.M. Kelkar, J.P. Koilpillai, J.M. Gajera, WO Patent 048 612 (2011), to Glenmark Generics Limited.
[19] P. Cherukupally, V.R. Vajrala, V.K. Adla, S. Rangineni, S. Kanniah, WO Patent 059 913 (2010), to Dr. Reddy's Laboratories Ltd. and Dr. Reddy's Laboratories, Inc.
[20] P. Allegrini, B.G. Romano, E. Attolino, M. Artico, D. Rossi, US Patent 0 029 987 (2010), to Dipharma Francis S.r.l.
[21] M.B.H. Youdim, J.P.M. Finberg, R. Levy, J. Sterling, D. Lerner, T. Berger-Paskin, H. Yellin, A. Veinberg, WO Patent 11 016 (1995), to Teva Pharmaceutical Industries Ltd. and Technion Research and Development Foundation, Ltd.
[22] M.B.H. Youdim, J.P.M. Finberg, R. Levy, J. Sterling, D. Lerner, T. Berger-Paskin, EP Patent 0 436 492 (1991), to Teva Pharmaceutical Industries Ltd. and Technion Research and Development Foundation, Ltd.
[23] H. Brunner, R. Becker, S. Gauder, Organometallics 5 (1986) 739–746.
[24] I. Takei, Y. Nishibayashi, Y. Ishii, Y. Mizobe, S. Uemura, M. Hidai, Chem. Commun. (2001) 2360–2361.

[25] A.L. Gutman, M. Etinger, G. Nisnevich, F. Polyak, Tetrahedron Asymm. 9 (1998) 4369–4379.
[26] E. Juaristi, J.L. Léon-Romo, A. Reyes, J. Escalante, Tetrahedron Asymm. 10 (1999) 2441–2495.
[27] P.G.H. Uiterweerd, M. van der Sluis, B. Kaptein, B. de Lange, R.M. Kellogg, Q.B. Broxterman, Tetrahedron Asymm. 14 (2003) 3479–3485.
[28] J.T. Colyer, N.G. Andersen, J.S. Tedrow, T.S. Soukup, M.M. Faul, J. Org. Chem. 71 (2006) 6859–6862.
[29] M.M. Pakulski, S.K. Mahato, M.J. Bosiak, M.P. Krzeminski, M. Zaidlewicz, Tetrahedron Asymm. 23 (2012) 716–721.
[30] H. Leisch, S. Grosse, H. Iwaki, Y. Hasegawa, P.C.K. Lau, Can. J. Chem. 90 (2012) 39–45.
[31] A.L. Gutman, E. Meyer, E. Kalerin, F. Polyak, J. Sterling, Biotechnol. Bioeng. 40 (1992) 760–767.
[32] A.L. Gutman, E. Shkolnik, E. Meyer, F. Polyak, D. Brenner, A. Boltanski, Ann. N. Y. Acad. Sci. 799 (1996) 620–632.
[33] M.S. Malik, E-S. Park, J-S. Shin, Green Chem. 14 (2012) 2137–2140.
[34] M. Päiviö, P. Perkiö, L.T. Kanerva, Tetrahedron Asymm. 23 (2012) 230–236.
[35] G. Ma, Z. Xu, P. Zhang, J. Liu, X. Hao, J. Ouyang, P. Liang, S. You, X. Jia, Org. Process Res. Dev. 18 (2014) 1169–1174.
[36] L.T. Boulton, I.C. Lennon, E. Bahar, WO Patent 120 577 (2006), to Teva Pharmaceutical Industries Ltd.
[37] K.T. Reddy, K.S. Kumar, G. Omprakash, P.K. Dubey, Pharma Chem. 3 (2011) 110–115.
[38] B. Clapham, C.-W. Cho, K.D. Janda, J. Org. Chem. 66 (2001) 868–873.
[39] A.C. Spivey, D.P. Leese, F. Zhu, S.G. Davey, R.L. Jarvest, Tetrahedron 60 (2004) 4513–4525.
[40] H. Shiigi, H. Mori, T. Tanaka, Y. Demizu, O. Onomura, Tetrahedron Lett. 49 (2008) 5247–5251.
[41] C.I. Sheppard, J.L. Taylor, S.L. Wiskur, Org. Lett. 13 (2011) 3794–3797.
[42] Y. Kita, Y. Takebe, K. Murata, T. Naka, S. Akai, J. Org. Chem. 65 (2000) 83–88.
[43] M-J. Kim, H.M. Kim, D. Kim, Y. Ahn, J. Park, Green Chem. 6 (2004) 471–474.
[44] N. Bouzemi, H. Debbeche, L. Aribi-Zouioueche, J.-C. Fiaud, Tetrahedron Lett. 45 (2004) 627–630.
[45] N. Bouzemi, L. Aribi-Zouioueche, J.-C. Fiaud, Tetrahedron Asymm. 17 (2006) 797–800.
[46] X-M. Wu, J-Y. Xin, W. Sun, C-G. Xia, Chem. Biodiversity 4 (2007) 183–188.
[47] L. Ou, Y. Xu, D. Ludwig, J. Pan, J.H. Xu, Org. Process Res. Dev. 12 (2008) 192–195.
[48] A.F. Fois, A. Yap, A.F. Masters, Th. Maschmeyer, Top. Catal. 48 (2008) 153–157.
[49] H. Debbeche, M. Toffano, J.-C. Fiaud, L. Aribi-Zouioueche, J. Mol. Catal. B: Enzym. 66 (2010) 319–324.
[50] M. Päiviö, D. Mavrynsky, R. Leino, L.T. Kanerva, Eur. J. Org. Chem. (2011) 1452–1457.
[51] A. Ghanem, Tetrahedron 63 (2007) 1721–1754.
[52] V. Gotor-Fernández, V. Gotor, in: V. Gotor, I. Alfonso, E. Garcia-Urdiales (Eds.), Asymmetric Organic Synthesis with Enzymes, Wiley-VCH, Weinheim, Germany, 2008, p. p171.
[53] E. Kuliszewska, M. Hanbauer, F. Hammerschmidt, Chem. Eur. J. 14 (2008) 8603–8614.
[54] R.P. Reddy, H.M.L. Davies, Org. Lett. 8 (2006) 5013–5016.
[55] J.B. i Llado, M.C.B. Montero, J.M. Llinas, US Patent 7 781 616 (2010), to Medichem, S.A.
[56] P.A. Kraakman, E.T.J. Nibbering, W.H. de Wolf, F. Bickelhaupt, Tetrahedron 43 (1987) 5109–5124.
[57] P.A. Fitzpatrick, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 3166–3171.
[58] K. Nakamura, M. Kinoshita, A. Ohno, Tetrahedron 51 (1995) 8799–8808.
[59] L.T. Kanerva, J. Vihanto, M.H. Halme, J.M. Loponen, E.K. Euranto, Acta Chem. Scand. 44 (1990) 1032–1035.
[60] S. Gogoi, M.G. Pathak, A. Dutta, N.N. Dutta, Ind. J. Biochem. Biophys. 45 (2008) 192–197.
[61] B. Yang, S-J. Kuo, P. Hariyadi, K.L. Parkin, Enzyme Microb. Technol. 16 (1994) 577–583.
[62] S-J. Kuo, K.L. Parkin, J. Am. Oil Chem. Soc. 73 (1996) 1427–1433.
[63] R. Fernández, A. Ros, A. Magriz, H. Dietrich, J.M. Lassaletta, Tetrahedron 63 (2007) 6755–6763.
[64] S. Hanessian, R. Margarita, A. Hall, S. Johnstone, M. Tremblay, L. Parlanti, J. Am. Chem. Soc. 124 (2002) 13342–13343.
[65] K. Chow, W.K. Fang, E.G. Corpuz, D.W. Gil, M.E. Garst, US Patent 0 255 239 (2008), to Allergan, INC.
[66] F.L. Lin, H.M. Hoyt, H. van Halbeek, R.G. Bergman, C.R. Bertozzi, J. Am. Chem. Soc. 127 (2005) 2686–2695.