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Biocatalytic synthesis of chiral *N*-(2-hydroxyalkyl)-acrylamides

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Abstract
The preparation of a series of novel chiral *N*-(2-hydroxylalkyl)-acrylamides through a lipase-catalyzed resolution of racemic alkanolamines is described. The absolute stereochemistry and enantiomeric excess of the products were determined by a modified Mosher's method. The method was validated for this particular case by the synthesis of an enantiomerically pure product. Moreover, the stereoselective behavior of the lipase in this reaction is discussed.

Keywords: *Candida antarctica lipase B,* N-*(hydroxyalkyl)-acrylamides, stereoselectivity, Mosher's method, primary alcohol*

Introduction

Lipases, working in non-aqueous media, have been widely used for several synthetic reactions (Bommarius & Riebel 2004; Carrea & Riva 2008). They are well known for their high enantioselectivity (Faber 2000; Buchholz et al. 2005), a property that has found them widespread application in the synthesis of enantiomerically pure compounds (Gotor et al. 2007). They have been employed for resolving racemic mixtures of several highly functionalized chiral molecules such as amino acids (Forró & Fülöp 2010) and amino alcohols (Turcu et al. 2010).

We have applied lipases as catalysts in a variety of reactions such as esterification, transesterification, aminolysis and even polymerization, which produced various steroid and terpenoid derivatives, substituted amides, polyacrylamides and polyamidoamines with pharmacological properties and biomedical applications (Rustoy & Baldessari 2005; Rustoy et al. 2007; Monsalve et al. 2008, 2009, 2010; Quintana & Baldessari 2009). Among them, lipase-catalyzed aminolysis of ethyl acrylate under controlled reaction conditions allowed us to obtain a series of *N*-hydroxyalkylacrylamides, which are useful starting materials for the production of polymer matrices employed in electrophoresis (Rustoy & Baldessari 2006). Similarly, Gotor's group reported the enzymatic synthesis of *N*-(3-dimethylaminopropyl)-acrylamide (Torre et al. 2005).

Chiral acrylamides are useful compounds in organic synthesis. They can act as chiral inductors in controlling the stereochemistry of cycloadditions (Nyerges et al. 2005) and the tacticity of chiral polymeric matrices applied in stereoselective separations (Tian et al. 2010), as well as monomers in the synthesis of chiral thermoresponsive amphiphilic co-networks (Tobis et al. 2010).

Considering these potential applications and extending our work on this subject, in the present paper we describe the enzymatic synthesis and stereochemical characterization of a series of chiral *N*-(2-hydroxyalkyl)-acrylamides (**2a**–**2d**). For this purpose, we performed a lipase-catalyzed aminolysis of ethyl acrylate with the corresponding racemic 2-hydroxyalkyamines (**1**) (Scheme 1).

Previous work in this area includes the studies of Puertas et al. (1993) describing the enantioselective conversion of racemic amines to (*R*)-acrylamides using *Candida antarctica* lipase B.

Among the methods available to determine the absolute stereochemistry of chiral compounds, NMR spectroscopy using chiral derivatizing agents such

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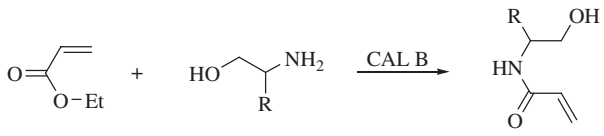


1 **2**

a: R: -CH_3; **b**: R: -CH_2CH_3; **c**: R: -$(CH_2)_2CH_3$; **d**: R: -$(CH_2)_3CH_3$

Scheme 1. Lipase-catalyzed synthesis of **2a**–**2d**.

as 2-methoxy-2-(trifluoromethyl)-phenylacetic acid (MTPA) has been widely used in the case of stereogenic centers bearing either hydroxyl or amine groups (Pehk et al. 1993). More recently, this methodology has been applied for the derivatization of chiral primary alcohols, thus determining the absolute configuration of stereogenic centers at a two-bond distance from the hydroxyl group that reacts with MTPA (Akiyama et al. 2003; Galman & Hailes 2009). Moreover, the modified Mosher's method has been used to determine the absolute configuration of primary alcohols with chiral methyl groups at C-2 (Czuba et al. 2003).

In this work we used the modified Mosher's method for determining the absolute configuration and enantiomeric ratio of the enzyme-catalyzed reaction products. We also synthesized a Mosher's ester of an enantiomerically pure *N*-(2-hydroxyalkyl)-acrylamide in order to test the reliability of the method.

Materials and methods

General remarks

Candida antarctica lipase B (CALB) (Novozym® 435; 7400 PLU/g) was purchased from Codexis, Inc. (Pasadena, CA, USA). The enzyme was used 'straight from the bottle'. Chemical reagents and solvents were sourced from Aldrich (Buenos Aires, Argentina), Sigma (Buenos Aires, Argentina), Fluka (Buenos Aires, Argentina), or J.T. Baker (Tampa, FL, USA). Enzymatic reactions were carried out in an Innova 4000 digital incubator shaker (New Brunswick Scientific Co.) (Edison, NJ, USA) at 200 rpm. Reactions under microwave irradiation were performed using a Discovery monomode microwave reactor (CEM Corporation) (Matthews, NC, USA). Reactions were followed by TLC on Merck (Buenos Aires, Argentina) silica gel 60F-254 aluminum sheets (0.2 mm thickness). For flash chromatography Merck silica gel 60 (60–230 mesh) was used. Optical rotations were measured with a Perkin Elmer (Waltham, MA, USA) 343 polarimeter (solvents are indicated). FTIR spectra were obtained on a Shimadzu (Columbia, MD, USA) FTIR-8300 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ as solvent using a Bruker (Billerica, MA, USA) AC-200 spectrometer operating at 200.13 MHz and 50.32 MHz for ^{1}H and ^{13}C, respectively, and a Bruker AM-500 NMR instrument operating at 500.14 MHz and 125.76 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are reported in δ units (ppm) relative to TMS set at 0 ppm, and coupling constants are given in Hertz. High-resolution mass spectra (HRMS) were recorded on a Bruker microTOF-Q II operating in electrospray ionization mode.

Enzymatic synthesis of N-(2-hydroxyalkyl)-acrylamides 2a to 2d under standard conditions

Hydroxyalkylacrylamides were prepared as follows: 0.9 mmol of the corresponding *rac*-2-amino alcohol was dissolved together with 0.9 mmol of ethyl acrylate in 7.5 mL of diisopropylether. CALB (100 mg) was added to the solution and then the reaction was allowed to proceed in an orbital shaker (33°C, 200 rpm) and monitored by TLC. Once the reaction reached its maximum conversion, the enzyme was filtered off and the solvent evaporated. The resulting hydroxyalkylacrylamide was purified by flash chromatography (acetone).

Synthesis of 2a under microwave irradiation

Substrate **1a** was dissolved in diisopropylether and CALB was added as described for the enzymatic synthesis of **2a** under standard conditions. The reaction was allowed to proceed under microwave irradiation with magnetic stirring in a closed vessel. The temperature was fixed to 30°C by automated control of irradiation power. The vessel was continuously refrigerated with an air current at 0°C during the reaction. The product **2a** was isolated as described (83% yield, racemic).

(S)-N-(2-hydroxy-1-methylethyl)-acrylamide, (S)-2a

Yellowish oil, 42% yield, 32% ee (determined by integration of H-1′a and H-1′b proton signals of its (*R*)-MTPA derivative, (*S*)-**3a**). $[\alpha]_D^{25}$: –22.0° (*c* = 1, $CHCl_3$). IR (film)/(cm^{-1}): 3292 br (OH), 2971, 1650 (C = O). ^{1}H NMR (200.13 MHz, $CDCl_3$), δ 1.19 (dd, 3H, $\mathcal{J}$ = 6.2 Hz, –CH_3), 3.17 (m, 1H, –CH_2–OH), 3.50 (m, 1H, –CH_2–OH), 3.93 (m, 1H, –CH(NH)–), 5.60 (dd, 1H, $\mathcal{J}$ = 9.4, 1.7 Hz, = CH_2), 6.15 (dd, 1H, $\mathcal{J}$ = 16.9, 9.4 Hz, = CH(CO)), 6.29 (m, 1H, $\mathcal{J}$ = 16.9, 1.7 Hz, = CH), ^{13}C NMR (50.32 MHz; $CDCl_3$), δ 20.83 (CH_3), 47.07 (CH), 67.16 (CH_2OH), 126.87 (= CH_2), 130.56 (= CH–), 169.47 (C = O); *m/z* (HRMS) ($[M+H]^+$) calcd. for $C_6H_{12}NO_2$, 130.0868; found 130.0864.

*(S)-*N*-[1-(hydroxymethyl)-propyl]-acrylamide, (S)-**2b***

Yellowish oil, 60% yield, 35% ee (determined by integration of H1′a and H1′b proton signals of its (*R*)-MTPA derivative, (*S*)-**3b**). $[\alpha]^{25}_{D}$: –23.3° (*c* = 1, $CHCl_3$). IR (film), ν (cm^{-1}): 3300 br (OH), 2970, 1650 (C = O). ^{1}H NMR (200.13 MHz, $CDCl_3$), δ (ppm): 0.90 (3H, t, $\mathcal{J}$ = 7.4 Hz, $–CH_3$); 1.50 (2H, m, $CH_3–CH_2–$); 3.52 (2H, m, $–CH_2–OH$); 3.87 (1H, m, –CH(NH)–); 5.60 (dd, $\mathcal{J}$ = 8.4 and 1.8 Hz, = CH_2); 6.15 (dd, $\mathcal{J}$ = 16.9 and 8.4 Hz, = CH(CO)–); 6.25 (m, $\mathcal{J}$ = 16.9 and 1.8 Hz, = CH). ^{13}C NMR (50.32 MHz, $CDCl_3$), δ (ppm): 10.55 (CH_3), 24.10 (CH_2), 53.26 (CHNH), 64.42 (CH_2OH), 126.55 (= CH_2), 130.90 (= CH), 166.47 (C = O). HRMS, *m/z*: 144.1019. ($[M+H]^+$). Calcd for $C_7H_{14}NO_2$: 144.1025.

(S)-N-[1-(hydroxymethyl)-butyl]-acrylamide, (S)-2c

Yellowish oil, 65% yield, 51% ee (determined by integration of H1′a and H1′b proton signals of its (*R*)-MTPA derivative, (*S*)-**3c**). $[\alpha]^{25}_{D}$: –29.2° (*c* = 1, $CHCl_3$). IR (film), ν (cm^{-1}): 3302 br (OH), 2969, 1650 (C = O). ^{1}H NMR (200.13 MHz, $CDCl_3$), δ (ppm): 0.88 (3H, t, $\mathcal{J}$ = 6.9 Hz, $–CH_3$); 1.35 (2H, m, $CH_3–CH_2–$); 1.48 (2H, m, $–CH_2–$CH(NH)–); 3.58 (2H, m, $–CH_2–OH$); 3.99 (1H, m, –CH(NH)–); 5.60 (dd, $\mathcal{J}$ = 9.2 and 2.2 Hz, (CO)–CH_2 =); 6.14 (dd, $\mathcal{J}$ = 16.9 and 9.2 Hz, = CH_2); 6.22 (m, $\mathcal{J}$ = 16.9 and 2.2 Hz, = CH(CO)–). ^{13}C NMR (50.32 MHz, $CDCl_3$), δ (ppm): 13.94 (CH_3), 19.30 (CH_3CH_2), 33.28 (CH_2), 51.58 (CHNH), 64.95 (CH_2OH), 126.55 (= CH_2), 130.88 (= CH), 166.36 (C = O). HRMS, *m/z*: 158.1176 ($[M+H]^+$). Calcd for $C_8H_{16}NO_2$: 158.1181.

(S)-N-[1-(hydroxymethyl)-pentyl]-acrylamide, (S)-2d

Yellowish oil, 72% yield, 67% ee (determined by integration of H1′a and H1′b proton signals of its (*R*)-MTPA derivative, (*S*)-**3d**). $[\alpha]^{25}_{D}$: –45.9° (*c* = 1, $CHCl_3$); IR (film), ν (cm^{-1}): 3308 br (OH), 2974, 1650 (C = O). ^{1}H NMR (200.13 MHz; $CDCl_3$), δ 0.89 (t, 3H, $\mathcal{J}$ = 6.6 Hz, $CH_3–$); 1.32 (m, 4H, $CH_3–(CH_2)_2–$), 1.54 (m, 2H, $–CH_2–$CH(NH)–), 3.62 (m, 2H, $CH_2–OH$), 3.99 (m, 1H, –CH(NH)–), 5.65 (dd, 1H, $\mathcal{J}$ = 9.5, 2.2 Hz, (CO)–CH_2=), 6.14 (dd, 1H, $\mathcal{J}$ = 17.2, 9.5 Hz, CH_2=), 6.22 (m, 1H, $\mathcal{J}$ = 17.2, 2.2 Hz, =CH(CO)); ^{13}C NMR (50.32 MHz, $CDCl_3$), δ 13.94 (CH_3), 19.30 (CH_3CH_2), 33.28 (CH_2CHNH), 51.58 (CHNH), 64.95 (CH_2OH), 126.55 (CH_2=), 130.88 (=CH), 166.36 (C = O); *m/z* (HRMS) $[M+H]^+$ calcd. for $C_9H_{18}NO_2$, 172.1338; found 172.1339.

Synthesis of MTPA derivatives

The reaction was carried out under anhydrous conditions. To a stirred solution of the acrylamide (0.40 mmol) in CH_2Cl_2 (5 mL), triethylamine (54 µL, 0.40 mmol) and MTPA chloride (100 mg, 0.40 mmol) in CH_2Cl_2 (2 mL) were added and the reaction mixture was stirred for 12 h at room temperature. The solvent was evaporated and the product was purified using flash chromatography (hexane–EtOAc, 1:2, v/v).

(R)-N-[1-(hydroxymethyl)-propyl]-acrylamide, (R)-2b and (2R,2′S)-2′-(acrylamido)-propyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (2R,2′S)-3a

Colorless oil, 60% yield, 32% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3a** and (*S*)-**4a**), $[\alpha]^{25}_{D}$: –24.4 (*C* = 1, $CHCl_3$); IR (KBr) (cm^{-1}) 3290br, 2938s, 1749s, 1663s; ^{1}H NMR (500.14 MHz; $CDCl_3$), δ 1.40 (3H, dd, $\mathcal{J}$ = 6.3, 6.4 Hz, $–CH_3$), 3.49 (3H, ds, OCH_3) 4.30 (1H, m, CHNH) 4.33 (0.36H, dd, $\mathcal{J}$ = 4.0, 11.2 Hz, CHO (2*R*,2′*R*)), 4.37 (1.28H, d, $\mathcal{J}$ = 4.0, 4.1 Hz (2*R*,2′*S*)), 4.46 (0.36H, dd, $\mathcal{J}$ = 4.1, 11.2 Hz, CHO (2*R*,2′*R*)), 5.63 (1H, ddd, $\mathcal{J}$ = 1.1, 1.2, 10.3, 10.4 Hz, = CH), 5.98 (1H, ddd, $\mathcal{J}$ = 10.3, 10.4, 16.9, 17.0 Hz, = CH), 6.22 (1H, ddd, $\mathcal{J}$ = 1.1, 1.2, 16.9, 17.0 Hz, = CH(CO)), 7.41 (3H, m, Ph); 7.50 (2H, m, Ph); ^{13}C NMR (125.76 MHz; $CDCl_3$), δ 16.3 (CH_3), 44.1 and 44.4 (CHNH), 54.5 and 54.7 (CH_3O), 68.0 and 68.2 (CH_2O), 84.4 and 84.6 (CPh), 123.4 (q, $\mathcal{J}_{CF}$ = 287.9 Hz), 126.8 (CH_2=), 127.3 (Ph), 128.6 (Ph), 129.7 (Ph), 130.4 (CH=), 132.0 (Ph), 165.3 (C = O, amide), 166.5 and 166.6 (C = O, ester); *m/z* HRMS: $[M+H]^+$ calcd. for $C_{16}H_{19}F_3NO_4$, 346.1266; found 346.1260.

(2S,2′S)-2′-(acrylamido)-propyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2S,2′S)-4a

Colorless oil, 58% yield 32% ed (from integrations of H1′a and H1′b 1H NMR signals from (*S*)-**3a** and (*S*)-**4a**); $[\alpha]^{25}_{D}$: +32.1° (*c* = 1, $CHCl_3$). ^{1}H NMR (500.14 MHz, $CDCl_3$), δ 1.40 (3H, dd, $\mathcal{J}$ = 6.3, 6.4 CH_3), 3.49 (3H, ds, OCH_3), 4.30 (1H, m, CHNH), 4.33 (0.68H, dd, $\mathcal{J}$ = 4.0, 11.2 Hz, CHO (2*S*,2′*S*)), 4.37 (0.32H, d, $\mathcal{J}$ = 4.0, 4.1 Hz (2*S*,2′*R*)), 4.46 (0.68H, dd, $\mathcal{J}$ = 4.1 and 11.2 Hz, CHO (2*S*,2′*S*)), 5.63 (1H, ddd, $\mathcal{J}$ = 1.1, 1.2, 10.3, 10.4 Hz, CH =), 5.98 (1H, ddd, $\mathcal{J}$ = 10.3, 10.4, 16.9, 17.0 Hz, = CH), 6.22 (1H, $\mathcal{J}$ = 1.1, 1.2, 16.9, 17.0 Hz, = CH(CO)), 7.41 (3H, m, Ph); 7.50 (2H, m, Ph). ^{13}C NMR (125.76 MHz; $CDCl_3$): δ 16.3 (CH_3), 44.1 and 44.4 (CHNH), 54.5 and 54.7 (CH_3O), 68.0 and 68.2 (CH_2O), 84.4 and 84.6 (CPh), 123.4 (q, $\mathcal{J}_{CF}$ = 287.9

Hz), 126.8 (CH_2=), 127.3 (Ph), 128.6 (Ph), 129.7 (Ph), 130.4 (CH=), 132.0 (Ph), 165.3 (C = O, amide), 166.5 and 166.6 (C = O, ester).

*(2*R*,2′*S*)-2′-(acrylamido)-butyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2*R*,2′*S*)-**3b***

Colorless oil, 70% yield, 35% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3b** and (*S*)-**4b**). $[\alpha]_D^{25}$: –27.3° (*c* = 1, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3300 br, 2933 s, 1752 s, 1659 s. ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.93 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, –CH_3); 1.54 (2H, m, CH_2); 3.52 (3H, ds, OCH_3); 4.30 (1H, m, –CH(NH)–); 4.33 (0.34H, dd, $\mathcal{J}$ = 3.9 and 11.2 Hz, CHO (2*R*,2′*R*)); 4.37 (1.34H, dd, $\mathcal{J}$ = 3.9 and 4.8 Hz (2*R*,2′*S*)); 4.46 (0.34H, dd, $\mathcal{J}$ = 4.8 and 11.2 Hz, CHO (2R,2′R)); 5.63 (1H, $\mathcal{J}$ = 1.1, 1.2, 10.3 and 10.4 Hz, = CH); 5.98 (1H, $\mathcal{J}$ = 10.3. 10.4, 16.9 and 17.0 Hz, = CH); 6.23 (1H, $\mathcal{J}$ = 1.1, 1.2, 16.9 and 17.0 Hz, = CH(CO)); 7.41 (3H, m, Ph); 7.50 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 10.2 (CH_3), 24.5 and 24.6 (CH_2), 49.5 and 49.6 (CHNH), 54.4 and 54.5 (CH_3O), 67.0 and 67.1 (CH_2O), 84.6 and 84.8 (CPh), 124.0 (q, $\mathcal{J}_{CF}$ = 288.0 Hz), 127.0 (= CH_2), 127.2 (Ph), 128.9 (Ph), 129.9 (Ph), 131.0 (= CH), 133.0 (Ph), 165.5 (C = O, amide), 166.5 (C = O, ester). HRMS, *m*/*z*: 360.1417 ($[M+H]^+$). Calcd for $C_{17}H_{21}F_3NO_4$: 360.1423.

*(2*S*,2′*S*)-2′-(acrylamido)-butyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2*S*,2′*S*)-**4b***

Colorless oil, 65% yield, 35% ed (determined by integration of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3b** and (*S*)-**4b**). $[\alpha]_D^{25}$: +35.7° (*c* = 1, $CHCl_3$). ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.93 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, –CH_3); 1.54 (2H, m, CH_2); 3.52 (3H, ds, OCH_3); 4.30 (1H, m, –CH(NH)–); 4.33 (0.52H, dd, $\mathcal{J}$ = 3.9 and 11.2 Hz, CHO (2*S*,2′*S*)); 4.37 (0.52H, d, $\mathcal{J}$ = 3.9 and 4.8 Hz (2*S*,2′*R*)); 4.46 (0.74H, dd, $\mathcal{J}$ = 4.8 and 11.2 Hz, CHO (2*S*,2′*S*)); 5.63 (1H, $\mathcal{J}$ = 1.1, 1.2, 10.3 and 10.4 Hz, = CH); 5.98 (1H, $\mathcal{J}$ = 10.3. 10.4, 16.9 and 17.0 Hz, = CH); 6.23 (1H, $\mathcal{J}$ = 1.1, 1.2, 16.9 and 17.0 Hz, = CH(CO)); 7.41 (3H, m, Ph); 7.50 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 10.2 (CH_3), 24.5 and 24.6 (CH_2), 49.5 and 49.6 (CHNH), 54.4 and 54.5 (CH_3O), 67.0 and 67.1 (CH_2O), 84.6 and 84.8 (CPh), 124.0 (q, $\mathcal{J}_{CF}$ = 288.0 Hz), 127.0 (= CH_2), 127.2 (Ph), 128.9 (Ph), 129.9 (Ph), 131.0 (= CH), 133.0 (Ph), 165.5 (C = O, amide), 166.5 (C = O, ester).

*(2*R*,2′*S*)-2′-(acrylamido)-pentyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2*R*,2′*S*)-**3c***

Colorless oil, 72% yield, 45% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3c** and (*S*)-**4c**). $[\alpha]_D^{25}$: –31.2° (*c* = 1, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3310 br, 2913 s, 1764 s, 1655 s. ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.90 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, CH_3); 1.45–1.62 (4H, m, $(CH_2)_2$); 3.54 (3H, ds, OCH_3); 4.31 (1H, m, –CH(NH)–); 4.35 (0.30H, dd, $\mathcal{J}$ = 4.4 and 11.8 Hz, CHO (2*R*,2′*R*)); 4.37 (1.40H, d, $\mathcal{J}$ = 4.2 and 4.4 Hz (2*R*,2′*S*)); 4.46 (0.30H, dd, $\mathcal{J}$ = 11.8 and 4.2 Hz, CHO (2*R*,2′*R*)); 5.60 (1H, ddd, $\mathcal{J}$ = 1.1, 10.3 and 10.4 Hz, = CH); 6.01 (1H, ddd, $\mathcal{J}$ = 10.3. 10.4, 16.7 and 17.0 Hz, = CH); 6.27 (1H, ddd, $\mathcal{J}$ = 1.1, 16.7 and 17.0 Hz, = CH(CO)); 7.39 (3H, m, Ph); 7.55 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 11.8 (CH_3), 17.3 (CH_2), 30.5 and 30.7 (CH_2), 50.2 and 50.3 (CHNH), 54.9 and 55.1 (CH_3O), 67.8 and 68.0 (CH_2O), 84.8 and 84.9 (CPh), 124.7 (q, $\mathcal{J}_{CF}$ = 287.0 Hz), 127.7 (= CH_2), 128.0 (Ph), 128.9 (Ph), 129.7 (Ph), 130.8 (= CH), 133.4 (Ph), 165.1 (C = O, amide), 166.8 (C = O, ester). HRMS *m*/*z*: 374.1601 ($[M+H]^+$). Calcd for $C_{18}H_{23}F_3NO_4$: 374.1579.

*(2*S*,2′*S*)-2′-(acrylamido)-pentyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2*S*,2′*S*)-**4c***

Colorless oil, 70% yield, 45% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3c** and (*S*)-**4c**); $[\alpha]_D^{25}$: +37.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.89 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, –CH_3); 1.44–1.61 (4H, m, $(CH_2)_2$); 3.53 (3H, ds, OCH_3); 4.30 (1H, m, –CH(NH)–); 4.34 (0.76H, dd, $\mathcal{J}$ = 4.4 and 11.8 Hz, CHO (2*S*,2′*S*)); 4.36 (0.48H, d, $\mathcal{J}$ = 4.2 and 4.2 Hz (2*R*,2′*S*)); 4.45 (0.76H, dd, $\mathcal{J}$ = 4.2 and 11.8 Hz, CHO (2*S*,2′*S*)); 5.59 (1H, $\mathcal{J}$ = 1.1, 10.3 and 10.4 Hz, = CH); 6.00 (1H, $\mathcal{J}$ = 10.3, 10.4, 16.7 and 17.0 Hz, = CH); 6.26 (1H, $\mathcal{J}$ = 1.1, 16.7 and 17.0 Hz, = CH(CO)); 7.38 (3H, m, Ph); 7.54 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 11.8 (CH_3), 17.3 (CH_2), 30.5 and 30.7 (CH_2), 50.2 and 50.3 (CHNH), 54.9 and 55.1 (CH_3O), 67.8 and 68.0 (CH_2O), 84.8 and 84.9 (CPh), 124.7 (q, $\mathcal{J}_{CF}$ = 287.0 Hz), 127.7 (= CH_2), 128.0 (Ph), 128.9 (Ph), 129.7 (Ph), 130.8 (= CH), 133.4 (Ph), 165.1 (C = O, amide), 166.8 (C = O, ester).

*(2*R*,2′*S*)-2′-(acrylamido)-hexyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (2*R*,2′*S*)-**3d***

Colorless oil, 75% yield, 67% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3d** and

(*S*)-**4d**). $[\alpha]_D^{25}$: –30.5° (*c* = 1, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3300 br, 2908 s, 1767 s, 1653 s. ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.89 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, CH_3); 1.32 (4H, m, $(CH_2)_2$); 1.51 (4H, m, CH_2); 3.53 (3H, ds, OCH_3); 4.33 (1H, m, –CH(NH)–); 4.35 (0.15H, dd, $\mathcal{J}$ = 4.3 and 11.2 Hz, CHO (2*R*,2′*R*)); 4.36 (1.70H, d, $\mathcal{J}$ = 4.1 and 4.3 Hz (2*R*,2′*S*)); 4.45 (0.15H, dd, $\mathcal{J}$ = 4.1 and 11.2 Hz, CHO (2*R*,2′*R*)); 5.61 (1H, $\mathcal{J}$ = 1.2, 10.1 and 10.5 Hz, = CH); 6.08 (1H, $\mathcal{J}$ = 10.1. 10.5, 16.9 and 17.3 Hz, = CH); 6.26 (1H, $\mathcal{J}$ = 1.2, 16.9 and 17.3 Hz, = CH(CO)); 7.31 (3H, m, Ph); 7.57 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 13.8 (CH_3), 22.4 (CH_2), 27.9 (CH_2), 31.2 (CH_2), 48.0 and 48.1 (CHNH), 55.4 and 55.5 (CH_3O), 67.3 and 67.4 (CH_2O), 84.4 and 84.8 (CPh), 124.5 (q, $\mathcal{J}_{CF}$ = 288.0), 126.9 (= CH_2), 127.8 (Ph), 128.5 (Ph), 129.7 (Ph), 130.6 (= CH), 132.4 (Ph), 165.0 (C = O, amide), 166.5 and 166.7 (C = O, ester). HRMS *m/z*: 388.1700 ($[M+H]^+$). Calcd for $C_{19}H_{25}F_3NO_4$: 388.1736.

*(2*S*,2′*S*)-2′-(acrylamido)-hexyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (2*S*,2′*S*)-**4d***

Colorless oil, 75% yield, 67% ed (from integrations of H1′a and H1′b ^{1}H NMR signals from (*S*)-**3d** and (*S*)-**4d**). $[\alpha]^{25}{}_D$: +41.1° (*c* = 1, $CHCl_3$). ^{1}H NMR (500.14 MHz, $CDCl_3$), δ (ppm): 0.89 (3H, dt, $\mathcal{J}$ = 7.3 and 7.5 Hz, –CH_3); 1.32 (4H, m, $(CH_2)_2$); 1.51 (4H, m, CH_2); 3.53 (3H, ds, OCH_3); 4.33 (1H, m, –CH(NH)–); 4.35 (0.82H, dd, $\mathcal{J}$ = 4.3 and 11.2 Hz, CHO (2*S*,2′*S*)); 4.36 (0.18H, d, $\mathcal{J}$ = 4.1 and 4.3Hz (2*S*,2′*R*)); 4.45 (0.82H, dd, $\mathcal{J}$ = 4.1 and 11.2 Hz, CHO (2*S*,2′*S*)); 5.61 (1H, $\mathcal{J}$ = 1.2, 10.1 and 10.5 Hz, = CH); 6.08 (1H, $\mathcal{J}$ = 10.1. 10.5, 16.9 and 17.3 Hz, = CH); 6.26 (1H, $\mathcal{J}$ = 1.2, 16.9 and 17.3 Hz, = CH(CO)); 7.31 (3H, m, Ph); 7.57 (2H, m, Ph). ^{13}C NMR (125.76 MHz, $CDCl_3$), δ (ppm): 13.8 (CH_3), 22.4 (CH_2), 27.9 (CH_2), 31.2 (CH_2), 48.0 and 48.1 (CHNH), 55.4 and 55.5 (CH_3O), 67.3 and 67.4 (CH_2O), 84.4 and 84.8 (CPh), 124.5 (q, $\mathcal{J}_{CF}$ = 288.0 Hz), 126.9 (= CH_2), 127.8 (Ph), 128.5 (Ph), 129.7 (Ph), 130.6 (= CH), 132.4 (Ph), 165.0 (C = O, amide), 166.5 and 166.7 (C = O, ester).

*(2*S*,2′*R*)-2′-(acrylamido)-butyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (2*S*,2′*R*)-**4b***

Commercial (*R*)-2-amino-1-butanol was treated as described for the synthesis of *N*-(2-hydroxyalkyl)-acrylamides **2a** to **2d** under standard conditions. The resulting acrylamide (*R*)-**2b** was treated as described for the synthesis of MTPA derivatives.

Colorless oil, 57% yield. >99% ee (determined by integration of H1'a and H1'b proton signals). $[\alpha]_{25}^{D}$: +133.1 (C = 1, $CHCl_3$); ^{1}H NMR (500.14 MHz; $CDCl_3$) δ 0.92 (3H, t, J= 7.5 Hz, CH_3), 1.52 (2H, m, CH_2) 3.51 (3H, ds, OCH_3), 4.27 (1H, m, CHNH), 4.36 (2H, dd, J= 3.9, 4.8 Hz, CHO), 5.61 (1H, ddd, J = 1.4, 10.3 Hz, CH=), 5,99 (1H, dd, J = 10.3, 17.2 Hz, CH=), 6.22 (1H, dd, J = 1.4, 17.2 Hz, =CH(CO)), 7.41 (3H, m, Ph), 7.49 (2H, m, Ph); ^{13}C NMR (125.76 MHz; $CDCl_3$): δ 10.2(CH_3), 24.5 (CH_3), 49.6 (CHNH), 54.4 (CH_3O), 67.0 (CH_2O), 84.8 (CPh), 124.0 (q, J_{CF} = 288.0 Hz, (CF_3)), 127.0 (CH_3=), 127.2 (Ph), 128.9 (Ph), 129.9 (Ph), 131.0 (=CH), 133.0 (Ph), 165.5 (C = O, amide), 166.5(C = O, ester).

Results and discussion

The lipase-catalyzed aminolysis reaction for the production of the chiral *N*-(2-hydroxyalkyl)-acrylamides (**2a**–**2d**) was studied. To start the work, *rac*-**2a** was chosen as a model substrate in order to optimize the reaction conditions using the lipase from *C. antarctica* B as biocatalyst, based on the good results previously reported using this enzyme in aminolysis reactions (Puertas et al. 1993; Rustoy & Baldessari 2005, 2006; Torre et al. 2005).

We performed several experiments varying reaction parameters such as enzyme/substrate ratio (E/S) between 0.1 and 5, ethyl acrylate/**2a** ratio (A/S) between 0.5 and 1.2, reagent concentrations from 0.012 M to 1.2 M, temperature (33°C and 55°C) and solvent (diisopropylether, hexane and toluene). As a result we chose as standard conditions: diisopropylether as solvent, 33°C, an E/S ratio and A/S ratio of 1, and a concentration 0.12 M of both ethyl acrylate and hydroxyalkanolamine **2a**.

Either higher substrate concentration or higher temperature favored the formation of aza-Michael products. The same results were observed when hexane was used as solvent. This side reaction had previously been observed in other examples of lipase-catalyzed aminolysis and it was necessary to find optimal conditions in each particular case to minimize it (Torre et al. 2005; Monsalve et al. 2010).

Finally, the synthesis of the products **2a**–**2d** was performed under the optimized conditions described. Table I (column 3) shows that the chiral hydroxyalkylacrylamides were obtained in moderate to good yields. An increase in yield was observed as the chain length of the alkyl group R in the stereocenter of the alkanolamine increased.

Optical rotation measurements of **2a**–**2d** showed that the lipase-catalyzed preparation of *N*-(2-hydroxyalkyl)-acrylamides was stereoselective. We also

Table I. Enzyme-catalyzed synthesis, absolute configuration and enantiomeric excess of **2a**–**2d**.

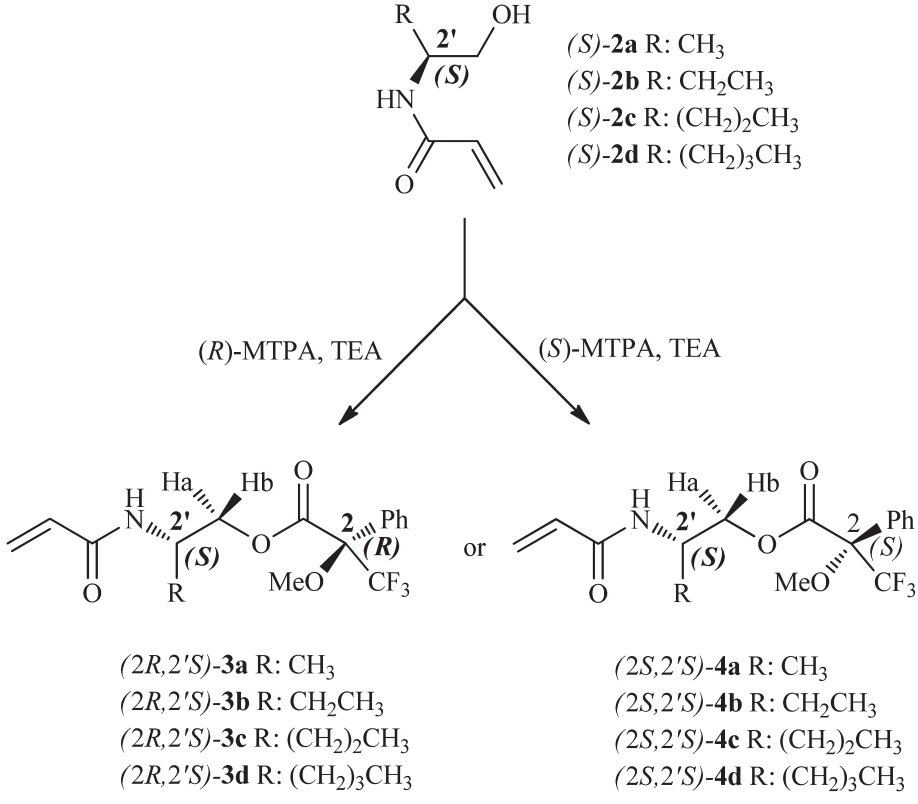
Product	R	% yield	MTPA amido ester	% ee **2a**–**2d**[a]
2a	CH_3	42	(2*R*,2′*S*)-**3a** (2*S*,2′*S*)-**4a**	32% (*S*)
2a[b]	CH_3	83	– –	0
2b	CH_3CH_2–	60	(2*R*,2′*S*)-**3b** (2*S*,2′*S*)-**4b**	35% (*S*)
2c	$CH_3(CH_2)_2$–	65	(2*R*,2′*S*)-**3c** (2*S*,2′*S*)-**4c**	45% (*S*)
2d	$CH_3(CH_2)_3$–	72	(2*R*,2′*S*)-**3d** (2*S*,2′*S*)-**4d**	67% (*S*)

Reactions were performed under standard conditions, time: 12 h.
[a]Determined from the integration of ^{1}H NMR signals for H1′a and H1′b from both (*R*)- and (*S*)-MTPA amido esters.
[b]Performed under microwave irradiation, time: 1 h

performed the preparation of **2a** under microwave irradiation, which resulted in a dramatic decrease in reaction time and an increase in product yield but the complete loss of stereoselectivity. Without lipase, under microwave irradiation, the substrate did not react at all.

The stereoselective behavior of lipases during hydrolysis of esters of chiral secondary alcohols has been extensively studied. Kazlauskas' rule is intended to predict the behavior of lipases in such cases and, according to this rule, lipases tend to hydrolyze esters of chiral secondary alcohols having absolute configuration (*R*) faster than their enantiomer (Kazlauskas et al. 1991). Lipases also show the same stereochemical preference in transesterification and aminolysis reactions (Puertas et al. 1993). This model takes into consideration that the substituent priority can be correlated to substituent size, although this is not always the case. The enormous diversity of substrates accepted by lipases and reaction conditions applied showed that this rule is not met in some circumstances (González-Sabín et al. 2005; Xia et al. 2009). Therefore, we considered it important to investigate the stereoselective behavior of CALB in the aminolysis reaction carried out in this work and to determine whether the results could be correlated with Kazlauskas' rule.

With this in mind, we prepared the (*R*)- and (*S*)-MTPA esters of the products **2a**–**2d** in order to assign their absolute stereochemistry and the degree of stereoselectivity achieved in each case (Scheme 2). The derivatives were obtained in good yield (58–75%) by treatment of each hydroxyalkylacrylamide **2a**–**2d** with MTPA chloride in triethylamine.



Scheme 2. MTPA esters (2*R*,2′*S*)-**3a**–**d** and (2*S*,2′*S*)-**4a**–**d**.

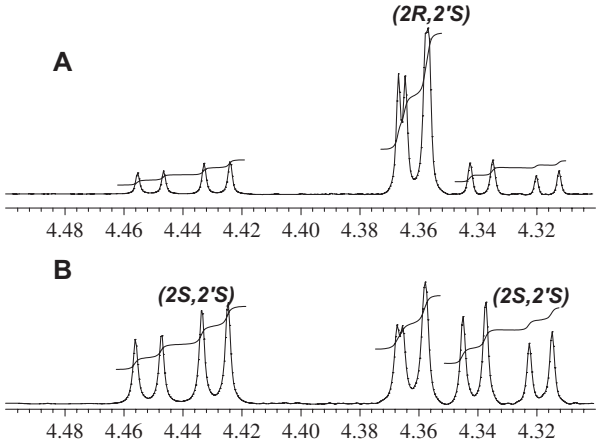


Figure 1. ^{1}H NMR signals for diastereotopic protons H1′a and H1′b in the (*R*)-MTPA amido ester (2*R*,2′*S*)-**3b** (spectrum A) and the (*S*)-MTPA amido ester (2*S*,2′*S*)-**4b** (spectrum B).

After purification, the MTPA esters were analyzed by ^{1}H NMR spectroscopy in $CDCl_3$. The results observed for compounds (2*R*,2′*S*)-**3b** and (2*S*,2′*S*)-**4b** were taken as examples and showed significant differences in the chemical shift for many signals. Particularly, signals for the diastereotopic protons H-1'a and H-1'b of the (*R*)-MTPA amidoester (2*R*,2′*S*)-**3b** for the major isomer were at $\delta = 4.36$ and 4.37 ppm and for the minor isomer at $\delta = 4.33$ and 4.44 ppm (Figure 1, spectrum A). For the (*S*)-MTPA ester (2*S*,2′*S*)-**4b** these signals were reversed, so that those corresponding to the major isomer are observed at $\delta = 4.33$ and 4.44 ppm and the signals for the minor isomer at $\delta = 4.36$ and 4.37 ppm (Figure 1, spectrum B). The integration of the above mentioned signals in both cases gave the same 35% ee value for **2b**.

The same procedure was applied to study the enantiomeric purity of the other three products **2a**, **2c** and **2d**. Table 1 (columns 4 and 5) shows these results.

This pattern for the oxymethylene protons of chiral primary alcohols esterified with MTPA agrees with previously published results on the determination of the absolute stereochemistry of a series of chiral primary alcohols with a methyl group at the C-2 position (Akiyama et al. 2003) and chiral 1,3-dihydroxyketones (Galman & Hailes 2009). In these studies the absolute configurations of stereogenic centers were assigned by considering the $\Delta\delta$ between oxymethylene protons. Larger $\Delta\delta$ values of the (*R*)-MTPA derivative were diagnostic for *R* stereochemistry on the carbon vicinal to oxymethylene whereas smaller $\Delta\delta$ values of the (*R*)-MTPA derivative were diagnostic for the opposite stereochemistry (*S*). Accordingly, the (*S*)-MTPA derivatives of (*R*)-primary alcohols showed smaller $\Delta\delta$ values for their oxymethylene proton signals than those prepared from (*S*)-primary alcohols.

For the (*R*)-MTPA derivatives of chiral *N*-(2-hydroxyalkyl)-acrylamides reported here, we found $\Delta\delta = 0.01$ ppm for the major isomer and $\Delta\delta = 0.11$ ppm for the minor isomer. This indicates that the absolute stereochemistry of the major isomer is (2*R*,2'*S*) and therefore that (*S*) seems to be the absolute configuration of the products **2a**–**2d**.

However, as this result was difficult to rationalize in terms of Kazlauskas' rule, which predicted an (*R*)-configuration, we sought additional confirmation. Therefore the stereochemical outcome of the enzymatic aminolysis of ethyl acrylate may need to be re-examined. Previous studies on the enzyme-catalyzed hydrolysis and transesterification of esters (Francalanci et al. 1987) and hydrolysis of amide (Fadnavis et al. 1999) derivatives of such compounds showed enantioselectivity towards (*R*)- or (*S*)-enantiomers depending on the catalyst and the reaction conditions. For instance, Carrea and co-workers (1993) studied the hydrolysis of a series of butyrates of racemic primary alcohols and found that *Pseudomonas cepacia* lipase showed the same stereochemical behavior as observed in the present work, producing the (*S*)-alcohol.

In order to support our findings, we also prepared (2*S*,2′*R*)-2′-(acrylamido)-butyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate, (2*S*,2′*R*)-**4b**. First, we used as substrate the pure enantiomer (*R*)-2-amino-1-butanol in the enzymatic synthesis of hydroxyalkylacrylamide (*R*)-**2b** and then (*R*)-**2b** was treated with MTPA chloride following the previously described procedure. ^{1}H NMR analysis of (2*S*,2′*R*)-**4b** showed the oxymethylene proton signals at $\delta = 4.36$ and 4.37 ppm, which confirms our stereochemical assignment based on the previous results employing MTPA esters from β-disubstituted chiral primary alcohols.

Thus, these results do not accord with Kazlauskas' rule, which is usually reliable with substrates having substituents which differ significantly in size. In the acylation reaction of every chiral alkanolamine used in this work the enzyme showed an enantioselective behavior opposite to that described by Kazlauskas' rule, selectively giving the product from reaction of alkanolamine with (*S*) configuration instead of the (*R*) predicted by the rule. In **1a** this could be explained considering that the hydroxymethyl group is clearly larger than the methyl group, but in **1b** ethyl and hydroxymethyl substituents are about the same size and compounds **1c** and **1d** have larger alkyl substituent than their hydroxyalkyl substituent.

The opposite configuration observed between the experimental results and that predicted by the rule is possibly due to electronic effects (i.e. substituents

having a remarkably different polarity) rather than to a difference in substituent size. Therefore the chemo- and enantioselectivity of the lipase-catalyzed *N*-acylation of chiral alkanolamines may be driven by the presence of the hydroxyl group, which seems to accommodate preferably in the less hindered side of the alcohol-binding site of the lipase. According to recent research on the enantioselectivity of CALB (Juhl et al. 2009) the residues that determine the stereoselectivity of the reaction are larger and hydrophobic at one side of Ser105 (Trp104, Ile189) and smaller and more polar at the opposite side (Gln106, Gly39, Thr40). This would accord with our results if we assume that the hydroxyl group of alkanolamines **1a**–**1d** tends to accommodate closer to the most polar region of the binding site prior to aminolysis.

Conclusions

In this study we have prepared a series of novel chiral *N*-hydroxyalkylacrylamides by enzymatic aminolysis of ethyl acrylate with racemic chiral alkanolamines. CALB was employed as catalyst and showed moderate to good yield, chemo- and enantioselectivity towards the formation of (*S*)-acrylamides. An increased rate and yield was observed in the synthesis of **2a** by combining microwave irradiation and lipase catalysis, although no stereoselectivity could be achieved under these conditions. The enzymatic approach provides a simple and mild alternative method for the synthesis of this type of compound, which are difficult to obtain by traditional synthetic procedures. The products were completely identified by spectroscopic methods and their absolute configuration and enantiomeric excess could be successfully determined through ^{1}H NMR analysis of their respective MTPA esters. Moreover, determination of the absolute configuration of β-disubstituted chiral primary alcohols using MTPA derivatives was verified through the chemoenzymatic synthesis of the acrylamide and its MTPA derivative (*R*)-**4b** using (*R*)-4-amino-1-butanol as substrate.

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