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Combined batch and continuous flow procedure to the chemo-enzymatic synthesis of biaryl moiety of Odanacatib

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ABSTRACT

The development of chemo-enzymatic reaction under continuous flow conditions is an emerging area where biotechnology and organic chemistry can joint efforts in order to develop better process. Here in we report our effort on the development of a continuous flow approach to the synthesis of Odanacatib intermediate using a combined batch and continuous flow apparatus arriving on the desired molecule in excellent yields and selectivity.

1. Introduction

Osteoporosis is a pathological condition characterized by increased bone turnover, low bone mass and an increased risk of fracture. The bone loss results from an imbalance between bone resorption and formation. Osteoporosis therapies fall into two classes: antiresorptive drugs, which slow down bone resorption (*e.g.* cathepsin K inhibitors) and anabolic drugs, which stimulate bone formation [1].

The cathepsins are a family of cysteine and aspartic proteases with collagenolytic activity. Cathepsin K, a cysteine protease, can efficiently degrade type I and II collagen, both of which are major matrix components of bone and cartilage. Cathepsin K is the most abundant cysteine protease expressed in osteoclasts and plays a central role in mediating bone resorption. During the bone resorption, cathepsin K is secreted by osteoclasts and accumulates in the acidified resorption lacunae where it degrades matrix proteins [2–6].

Odanacatib (**1**) (Scheme 1) is a potent, orally-active selective cathepsin K inhibitor, which is currently being developed for the treatment of postmenopausal osteoporosis [7–9]. Considering the important role of Odanacatib (**1**) for the osteoporosis treatment, the aim of this work is the development of a chemo-enzymatic synthesis for the chiral biaryl unit **2**, by combining enzymatic reduction and continuous flow methodology, or alternatively batch microwave chemistry. The retrosynthetic analysis of Odanacatib (**1**) shows two main building blocks, the chiral biaryl unit **2** and the fluorinated amino acid **3** (Scheme 1). Both building blocks have already been subject of research of several groups [8,10–12], and our approach consists on the development of a chemo enzymatic cascade reaction starting with the asymmetric bioreduction of 4′-bromo-2,2,2-trifluoroacetophenone (**4**) to the desired (*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**), followed by a continuous flow Suzuki coupling to afford the desired biaryl unit **2**.

2. Experimental

2.1. Chemicals and materials

The ketone 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) was purchased from commercial sources. The racemic alcohol 1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) was synthesized

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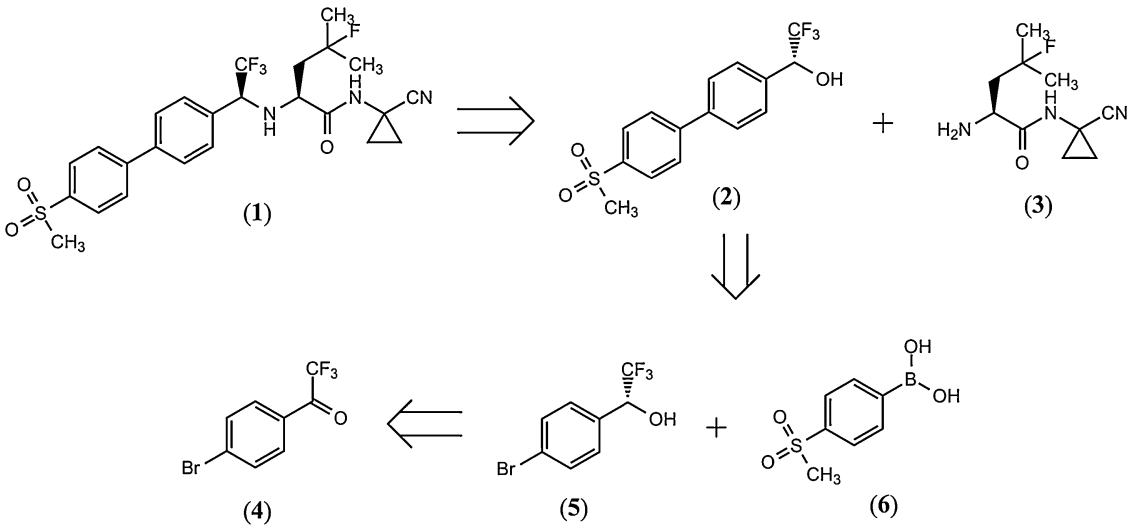


Scheme 1. Retrosynthetic analysis of Odanacatib (**1**).

by conventional reduction of the 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) ($NaBH_4$, THF, room temperature) [13]. ^1H NMR and ^{13}C NMR spectra were obtained by using a DPX-300 spectrometer (^1H NMR: 300.13 MHz, ^{13}C NMR: 75.5 MHz). Melting points were determined on a Stuart™ SMP3 melting point apparatus. Microwave heating experiments were carried out using a Monowave 300 single-mode microwave reactor from Anton Paar GmbH (Graz, Austria). The experiments were performed in a 10 mL Pyrex microwave process vial equipped with a magnetic stirring bar at a rate of 600 rpm. Reaction times refer to hold times at the temperatures indicated and not to total irradiation times. Flow experiments was performed in an ASIA 110 series microreactor (Syrris Ltd, Royston, UK), employing a two-feed microreactor stainless steel coil [14].

2.2. GC–MS analysis

GC–MS analysis was performed on a Trace-GC Ultra – DSQ II-MS system (ThermoElectron, Waltham, MA, USA). The GC conditions were as follows: HP-5 MS column (30 m × 0.25 mm ID, 0.25 μm film, Agilent, Waldbronn, Germany), carrier gas helium 5.0, flow 1 mL/min, temperature gradient identical to GC-FID. The MS conditions were as follows: positive EI ionization, ionization energy 70 eV, ionization source temperature 280 °C, emission current 100 μA, full-scan-mode.

2.3. GC-FID analysis

GC-FID analysis was performed on a Trace-GC (ThermoFisher) with a flame ionization detector using a HP5 column (30 m × 0.250 mm × 0.25 μm). The detector gas for the flame ionization is H_2 and compressed air (5.0 quality).

2.4. Synthesis of rac-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) [13]

A solution of 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) (0.5 g, 2 mmol, 1 equiv.) in 2 mL of tetrahydrofuran was prepared and cooled in an ice bath. Then, the sodium borohydride (0.037 g, 1 mmol, 0.5 equiv.) was slowly added and the reaction mixture was stirred at room temperature about 1 h, when the end of reaction was observed by TLC (Rf = 0.66, eluent: petroleum ether: ethyl acetate 20%, UV in 254 nm). After the solvent volume reduction under reduced pressure, the isolation was done through the adding water and extraction with ethyl acetate (4 × 5 mL). The organic layer was dried (Na_2SO_4) and evaporated at reduced pressure to give the racemic alcohol **5** as a white powder in 91% yield, mp 52.5–53.6 °C (mp 55–56 °C) [9].

^1H NMR (300 MHz, $CDCl_3$, TMS) δ (ppm): 2.78 (s, 1H, OH), 5.00 (q, 1H, J = 6 Hz, CHOHCF_3), 7.37 (d, 2H, J = 9 Hz, H3 and H5), 7.56 (m, 2H, H2 and H6).

^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ (ppm): 72.5 (q, J = 31.5 Hz, CHOH), 118.6, 122.3, 126.0 e 129.8 (q, J = 280.5 Hz, CF_3), 128.0 (C4), 129.3 (C2 e C6), 132.1 (C3 e C5), 133 (d, C1).

GC-FID: after 1 min at 50 °C the temperature was increased in 25 °C min^{-1} steps up to 300 °C and kept at 300 °C for 4 min. Retention time: t_R (ketone **4**) = 5.4 min, t_R (alcohol **5**) = 6.5 min.

2.5. Asymmetric enzymatic reduction by LB-ADH [15]

Lyophilized *L. brevis* cells containing the overexpressed ADH (LB-ADH) (10 mg) were rehydrated in sodium phosphate buffer (950 μL, 50 mM, pH 7.5) and NADPH (0.74 mg, 1 mM) for 30 min at 30 °C while shaking. Then, 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) (12.6 mg, 50 mM) and 2-propanol (50 μL) were added to the mixture. Reactions were shaken at 30 °C and 700 rpm for 24 h. Then, the reaction was stopped by extraction with ethyl acetate (4 × 3 mL). The organic layer was separated by centrifugation (10 min, 5000 rpm) and dried (Na_2SO_4). Conversions and enantiomeric excesses of the corresponding alcohols were determined by GC.

Chiral GC, Chirasil DEX CB column, 140 °C to 160 °C (1 °C min^{-1}), t_R [(*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol] (**5**) = 10.63 min, t_R [(*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol] (**5**) = 11.25 min.

2.6. Asymmetric enzymatic reduction by ADH-A [15]

Lyophilized *E. coli* cells containing the overexpressed ADH-A (10 mg or 20 mg) were rehydrated in sodium phosphate buffer (850 μL, 50 mM, pH 7.5) and NADH (0.71 mg, 1 mM) for 30 min at 30 °C while shaking. Then, 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) (12.6 mg, 50 mM) and 2-propanol (150 μL) were added to the mixture. Reactions were shaken at 30 °C and 700 rpm for different times. Then, the reaction was stopped by extraction with ethyl acetate (4 × 3 mL). The organic layer was separated by centrifugation (10 min, 5.000 rpm) and dried (Na_2SO_4). Conversions and enantiomeric excesses were determined by GC.

The product (*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) was obtained in 98% yield in 2 h by using 20 mg of ADH-A.

$[\alpha]_D^{20}$ −27.5 (*c* 1.06, EtOH) [9].

Chiral GC, DEX-CB column, 140 °C to 160 °C (1 °C min^{-1}), t_R [(*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol] (**5**) = 10.63 min, t_R [(*S*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol] (**5**) = 11.25 min.

2.7. Suzuki–Miyaura coupling loading of 0.2 mol% of palladium under microwave conditions [16]

Reaction optimization on small scale was performed in a Monowave 300 (Anton Paar GmbH) in 10 mL Pyrex microwave process vials using standard procedures in temperature control mode. Suzuki reactions under microwave conditions were carried out adding 1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) (95.1 mg, 0.375 mmol, 1 equiv.), 4-(methanesulfonyl)phenylboronic acid (**6**) (75 mg, 0.375 mmol, 1 equiv.), potassium carbonate (103.6 mg, 0.75 mmol, 2 equiv.), tetrakis(triphenylphosphine)palladium(0) (0.84 mg, 0.00075 mmol, 0.2 mol%) and a solution of distilled water and isopropanol (3 mL, 1:1). All the reactions were performed in closed vessels. After that, the solvent was evaporated and the mixture was extracted with ethyl acetate (4 × 5 mL). The organic layer was dried (Na_2SO_4). Conversions were determined by GC-FID and enantiomeric excesses were determined by HPLC. The product **2** was obtained as a white powder in 92% yield in 5 min at 110 °C, mp 171.9–172.8 °C.

^{1}H NMR (300 MHz, DMSO-d_6, TMS) δ (ppm): 3.27 (s, 3H, $SO_2C\underline{H}_3$), 5.25–5.29 (m, 1H, $C\underline{H}OHCF_3$), 6.95 (d, *J* = 4.2 Hz, 1H, CHO$\underline{H}$), 7.65 (d, *J* = 8.4 Hz, 2H, H3 e H5), 7,80 (d, *J* = 8.4 Hz, 2H, H2 e H6), 7.95–8.04 (m, 4H, H2′, H6′, H3′ e H5′).

^{13}C NMR (75 MHz, DMSO-d_6, TMS) δ (ppm): 43.6 ($SO_2\underline{C}H_3$), 70.1 (q, *J* = 30.7 Hz, $\underline{C}HOHCF_3$), 123.2 e 126.9 (d, *J* = 280.5 Hz, $CHOH\underline{C}F_3$), 127.1 (C2 and C6), 127.7 (C2′, C6′, C3 and C5), 128.4 (C3′ and C5′), 136.3 (C1′), 138.9 (C1), 139.8 (C4), 144.6 (C4′).

2.8. Determination of ee by means of chiral HPLC measurement

Column: Chiralpak AD (Chiracel), length = 25 cm, internal diameter = 4.6 mm, isocratic flow with 1.0 mL/min, eluent: n-heptane/2-PrOH = 85:15. t_R *R*-enantiomer (**2**) = 22.80 min, t_R *S*-enantiomer (**2**) = 26.89 min. Determination of conversion by means of achiral GC-FID measurement: after 1 min at 50 °C the temperature was increased in 25 °C min^{-1} steps up to 300 °C and kept at 300 °C for 4 min. Retention time: t_R [1-(4-bromophenyl)-2,2,2-trifluoroethanol] (**5**) = 6.5 min, t_R [2,2,2-trifluoro-1-(4′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl)ethanol] (**2**) = 11.6 min.

2.9. Suzuki–Miyaura coupling in continuous flow loading of 0.2 mol% of palladium [16]

A sample of 4-(methanesulfonyl)phenylboronic acid (**6**) (250 mg, 1.25 mmol, 1 equiv.) and potassium carbonate (345 mg, 2.5 mmol, 2 equiv.) was dissolved in 5 mL of distilled water. In an additional flask, a sample of *rac*-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) (317 mg, 1.25 mmol, 1 equiv.) and tetrakis(triphenylphosphine)palladium(0) (2.8 mg, 0.0025 mmol, 0.2 mol%) was dissolved in 5 mL of isopropanol. Each reaction mixture was pumped through the coil reactor (4 mL PFA-coil, 5 min residence time, flow rate 0.8 mL/min) using a static BPR (7 bar, 100 PSI) in a BPR holder made of steel and heated at 110 °C. The complete reaction mixture was collected for 10 min and the solvent was evaporated. The mixture was extracted with ethyl acetate (4 × 10 mL). The organic layer was dried (Na_2SO_4) and conversions were determined by GC-FID. The isolated yield was 87% (racemic alcohol **2**) and 84% (*R* enantiomer **2**).

2.10. Asymmetric enzymatic reduction and Suzuki–Miyaura coupling loading of 5 mol% of palladium under microwave conditions toward an one-pot process [17]

Lyophilized *E. coli* cells containing the overexpressed ADH (ADH-A′) (40 mg) were added to sodium phosphate buffer (1700 μL, 50 mM, pH 7.5) and NADH (1.42 mg, 1 mM). Then, 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**4**) (25.2 mg, 50 mM) was dissolved in 2-propanol (300 μL) and the solution was added to the mixture. The reaction was shaken at 30 °C and 700 rpm for 2 h. After this time, the supernatant was separated by centrifugation (10 min, 5000 rpm) and then 4-(methanesulfonyl)phenylboronic acid (**6**) (20 mg, 0.1 mmol, 1 equiv.), potassium carbonate (27.6 mg, 0.2 mmol, 2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (0.23 mg, 0.0002 mmol, 5 mol%) dissolved in isopropanol were added to the 10 mL Pyrex microwave vial. The reaction was heated by microwave treatment at 110 °C for 5 min. After cooling, the solvent was evaporated and the crude reaction mixture was extracted with ethyl acetate (4 × 10 mL). The organic layer was dried (Na_2SO_4) and conversions were determined by GC-FID. The conversion in GC-FID was higher than 99% for the product **2**.

2.11. Suzuki–Miyaura coupling in continuous flow with catalyst loading of 5 mol% [16]

An aqueous solution (10 mL) containing 4-(methanesulfonyl) phenylboronic acid (**6**) (500 mg, 2.5 mmol, 1 equiv.) and potassium carbonate (690 mg, 5.0 mmol) and a solution of isopropanol (5 mL) containing 1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) (317 mg, 1.25 mmol) and tetrakis(triphenylphosphine)palladium(0) (70 mg, 0.2 mol%) were pumped from separate feeds through a coil reactor (16 mL PFA-coil, 5 min residence time, flow rate 3.2 mL/min) using a static BPR (7 bar, 100 PSI) in a BPR holder made of steel and heated at 110 °C. The aqueous solution was used in excess to flush the system before and after the reaction. The complete reaction mixture was collected for 10 min and the solvent was evaporated to give a black residue which was suspended in acetone and filtered. The filtrate was evaporated and water (20 mL) was added. The mixture was extracted with ethyl acetate (3 × 20 mL) and the organic layer was dried (Na_2SO_4) and evaporated to give a light yellow product in 73% crude yield. Further purification by column chromatography furnished the product in 45% yield. Conversions were determined by GC-FID.

2.12. Synthesis of acetophenones **7**, **8** and **10** [16]

Reactions were performed in a Monowave 300 (Anton Paar GmbH) in 10 mL Pyrex microwave process vials using standard procedures in temperature control mode. Suzuki reactions under microwave conditions were carried out adding 1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) (95.1 mg, 0.375 mmol, 1 equiv.), corresponding phenylboronic acid (0.375 mmol, 1 equiv.), potassium carbonate (103.6 mg, 0.75 mmol, 2 equiv.), tetrakis(triphenylphosphine)palladium(0) (0.84 mg, 0.00075 mmol, 0.2 mol%) and a solution of distilled water and isopropanol (3 mL, 1:1). All the reactions were performed in closed vessels for 5 min at 110 °C. After that, the solvent was evaporated and the mixture was extracted with ethyl acetate (4 × 5 mL). The organic layer was dried (Na_2SO_4). Conversions were determined by GC-FID.

The product 4′-(4-methylsulfonylphenyl)-2,2,2-trifluoroacetophenone (**7**) was obtained as a white powder in 92% yield, mp

165.7–166.8 °C. The melting point is in agreement with previous report (mp 157.9–158.8 °C) [18].

^{1}H NMR (300 MHz, DMSO-d_6, TMS) δ (ppm): 3.27 (s, 3H, $SO_2C\underline{H}_3$), 7.73–7.83 (m, 4H, hydrogen atoms of the aromatic ring), 7.96–8.04 (m, 4H, hydrogen atoms of the aromatic ring).

^{13}C NMR (75 MHz, DMSO-d_6, TMS) δ (ppm): 43.6 ($SO_2\underline{C}H_3$), 92.5 (q, J_{C-F} = 30.7 Hz, C1), 123.5 (d, J_{C-F} = 287.2 Hz, $\underline{C}F_3$), 126.6 (C2 e C6), 127.7 (C3′ e C5′), 128.2 (C3 e C5);139.0 (C2′ e C6′), 139.1 (C1′), 139.9 (C4′), 144.5 (C4).

The product 4′-(4-methylthiophenyl)-2,2,2-trifluoroacetophenone (**8**) was obtained as a yellow powder in 98% yield, mp 107.2–108.4 °C. The melting point is in agreement with previous report (mp 114.6–116.2 °C [18].

^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ (ppm): 2.55 (s, 3H, $SC\underline{H}_3$), 7.36 (d, 2H, J = 8.55, H3′ e H5′), 7.59 (d, 2H, J = 8.58, H2′ e H6′), 7.75 (d, 2H, J = 8.73, H2 e H6), 8.15 (d, 2H, J = 8.64, H3 e H5).

^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ (ppm): 15.7 (s, $SC\underline{H}_3$), 117 (d, J_{C-F} = 289 Hz, $CO\underline{C}F_3$), 126.9 (C3′ e C5′), 127.4 (C2′ e C6′), 127.9 (C3 e C5), 128.6 (C4), 131.1 (d, J_{C-F} = 1.5 Hz, C2 e C6), 135.7 (C1′), 140.5 (C4′), 147.7 (C1), 180.3 (d, J_{C-F} = 35.5 Hz, $\underline{C}OCF_3$).

The product 4′-phenil-2,2,2-trifluoroacetophenone (**10**) was obtained as a white powder in 62% yield, mp 49.8–50.9 °C. The melting point is in agreement with previous report (mp 49–50 °C) [19].

^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ (ppm): 7.46–7.55 (m, 3H, H3′, H5′ e H4′), 7.64–7.68 (m, 2H, H2′ e H6′), 7.76–7.80 (m, 2H, H2 e H6), 8.16–8.19 (m, 2H, H3 e H5).

^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ (ppm): 117.0 (d, J = 289.5 Hz, $CO\underline{C}F_3$), 127.6 (C4′), 127.9 (C2′ e C6′), 128.8 (C4), 129.2 (C2 e C2′), 129.4 (C3′ e C5′), 131.0 (C3 e C5), 139.4 (C1′), 148.5 (C1), 180.1 (q, J = 35.2 Hz, $\underline{C}OCF_3$).

2.13. Asymmetric enzymatic reduction of acetophenone 7, 8 and 10 by ADH-A [15]

The asymmetric enzymatic reduction of acetophenones **7**, **8** and **10** to corresponding alcohols **2**, **9** and **11** was performed as previous described in Section 2.6.

For the compound (*R*)-2,2,2-trifluoro-1-(4′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl)ethanol (**2**), see Section 2.7.

For the compound (*R*)-2,2,2-trifluoro-1-(4′-(methylthio)biphenyl-4-yl)ethanol (**9**):

^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ (ppm): 2.54 (s, 3H, $SC\underline{H}_3$), 5.08 (q, 1H, $C\underline{H}OHCF_3$), 7.33–7.36 (m, 2H, H3′ e H5′), 7.52–7.56 (m, 4H, H2, H6, H3 e H5), 7.64–7.61 (m, 2H, H2′ e H6′).

^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ (ppm): 16 (s, $SC\underline{H}_3$), 72.8 (q, J_{C-F} = 32.2 Hz, $\underline{C}HOHCF_3$), 124.5 (d, J_{C-F} = 285 Hz, $CHOH\underline{C}F_3$), 127.1 (C3′ e C5′), 127.2 (C2′ e C6′), 127.7 (C2 e C6), 128.2 (C3 e C5), 133.0 (C4), 137.3 (C1′), 138.5 (C4′), 142.0 (C1).

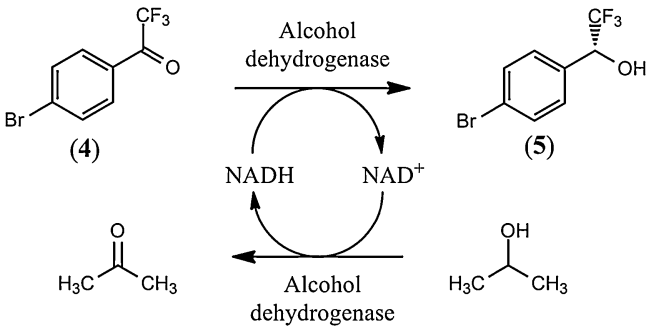
For the compound (*R*)-1-(biphenyl-4-yl)-2,2,2-trifluoroethanol (**11**)

^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ (ppm): 5.07 (q, 1H, $C\underline{H}OHCF_3$), 7.37–7.66 (m, 9H, H2, H6, H3, H5, H2′, H6′, H3′, H5′ e H4′).

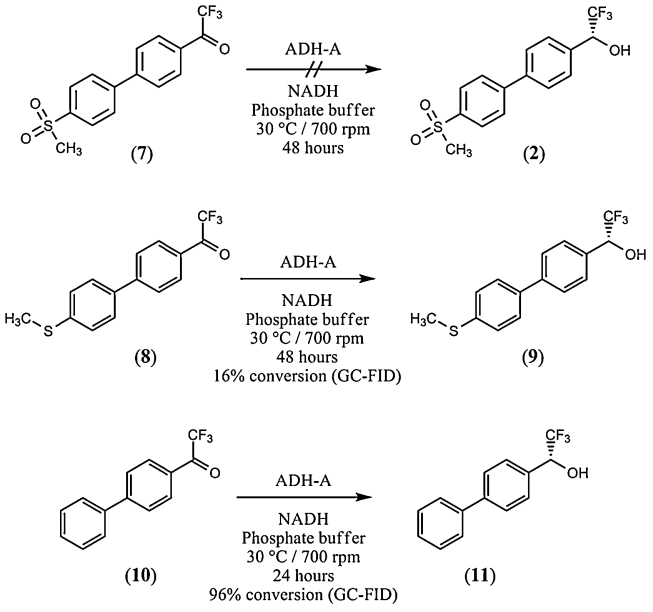
^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ (ppm): 72.8 (q, J = 31.5 Hz, $\underline{C}HOHCF_3$), 124.5 (d, J = 285 Hz, $CHOH\underline{C}F_3$), 127.4 (C4′), 127.6 (C3 e C5), 127.9 (C2′ e C6′), 128.1 (C2 e C6), 129.1 (C3′ e C5′), 133.3 (C4), 140.6 (C1), 142.6 (C1′).

3. Results and discussion

We started our work studying the enzymatic reduction of **4** employing a recombinant alcohol dehydrogenase from *Rhodococcus ruber* (ADH-A). The enzymatic reduction was carried out under substrate-coupled cofactor regeneration with isopropanol as reducing agent. ADH-A catalyzes not only the reduction of the ketone to the desired alcohol, but also the oxidation of the co-substrate isopropanol to acetone, thus regenerating the required cofactor NAD(P)H, which then can be used in catalytic amount (Scheme 2) [15].



Scheme 2. Asymmetric reduction of 4′-bromo-2,2,2-trifluoroacetophenone (**4**) to (*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) by using a single alcohol dehydrogenase (ADH-A) and isopropanol as reducing agent to regenerate the cofactor.



Scheme 3. Asymmetric reduction of 50 mM of acetophenones **7**, **8** and **10** to the alcohol **2**, **9** and **11**, respectively, by using ADH-A and isopropanol as reducing agent to regenerate the cofactor. Conversions were determined by GC-FID.

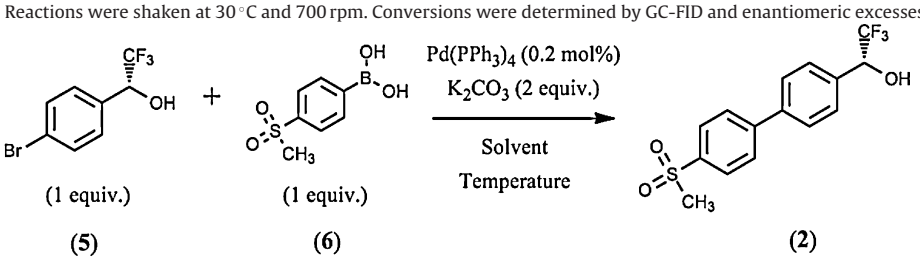
First, the ADH-A preparation (lyophilized *Escherichia coli* cells containing the overexpressed ADH) were rehydrated in sodium phosphate buffer in the presence of the cofactor NADH. Then, 4′-bromo-2,2,2-trifluoroacetophenone (**4**) dissolved in isopropanol was added and shaken at 30 °C at 700 rpm from 30 min to 6 h. Afterwards, the reaction was stopped by extraction with ethyl acetate and the organic layer was separated by centrifugation [15]. The GC analysis showed conversions ≥99% and an enantiomeric excesses of 98% for the *R* enantiomer could be obtained after 2 h of reaction (Table 1). The same reaction profile was also evaluated using 10 mg of lyophilized cells and similar results were obtained for longer reaction times (5 h). Isolated yields as high as 98% for the reduction were achieved. However, increasing the concentration of the ketone **4** to 100 mM, a slight decrease in conversion was observed (91% after 24 h).

Other related substrates were also evaluated in the enzymatic reduction, including: 2,2,2-trifluoro-1-(4′-(methylsulfonyl) biphenyl-4-yl)ethanone (**7**) and 2,2,2-trifluoro-1-(4′-(methylthio) biphenyl-4-yl)ethanone (**8**). The results indicate low reactivity for

Table 1
Asymmetric reduction of a 50 mM solution of 4′-bromo-2,2,2-trifluoroacetophenone (**4**) by using ADH-A preparation (lyophilized cells of *E. coli* containing overexpressed enzyme – 20 mg).

Entry	Reaction time (h)	Conv. (%) GC-FID	e.e. (%) GC-FID
1	0.5	74	98 (*R*)
2	1	94	98 (*R*)
3	2	>99	98 (*R*)
4	4	>99	99 (*R*)
5	6	>99	99 (*R*)

Reactions were shaken at 30 °C and 700 rpm. Conversions were determined by GC-FID and enantiomeric excesses were determined by GC-FID.



these substrates (Scheme 3), in contrast to 1-(biphenyl-4-yl)-2,2,2-trifluoroethanone (**10**), which however provided a 96% conversion by GC-FID, using the same conditions.

Once the bioreduction was successfully achieved, the next step was the Suzuki–Miyaura coupling between (*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**, 1 equiv.) and 4-(methanesulfonyl)phenylboronic acid (**6**, 1 equiv.) under controlled microwave heating using potassium carbonate (2 equiv.) as base, tetrakis(triphenylphosphine)palladium(0) (0.2 mol%) as catalyst and a solution of distilled water/isopropanol (1:1) as solvent (Scheme 4) [16] in order to use the same solvent system used in the bioreduction step, aiming to perform a cascade reaction. Conversions ≥99% toward the desired product were achieved in 5 min at 110 °C and provided a 92% isolated yield (Table 2, entries 1–6). Higher conversions were obtained using microwave heating compared to reactions at room temperature (Table 2, entries 6–7). The Suzuki–Miyaura coupling was also efficient using different proportions of water and isopropanol and in the absence of isopropanol (Table 2, entries 8–10). All reactions occurred without racemization, therefore product **2** was obtained in the same optical purity as achieved in the previous bioreductive step.

The optimized reactions under microwave heating were translated to a continuous flow environment (Asia - Syrris) [20]. Reactants were pumped in separate lines to a T mixer and then through a PFA-Coil reactor at 110 °C (4 mL PFA-*Coil*, 5 min residence time, combined flow rate 0.8 mL/min). The reaction mixture was collected for 10 min, the solvent was evaporated and extracted with ethyl acetate to give the product in a crude yield of 84%. No racemization was observed (Scheme 4).

Table 2
Suzuki–Miyaura coupling between (*R*)-1-(4-bromophenyl)-2,2,2-trifluoroethanol (**5**) and 4-(methanesulfonyl)phenylboronic acid (**6**) under microwave heating and at room temperature.

Entry	Temperature	Solvent	Reaction time	Conv. (%) GC-FID
1	110 °C (MW)		5 min	>99
2	110 °C (MW)		1 min	93
3	80 °C (MW)		10 min	98
4	80 °C (MW)	iPrOH: H_2O (1:1)	5 min	95
5	80 °C (MW)		1 min	85
6	r.t.		3 h	60
7	r.t.		7 h	61
8		H_2O	5 min	98
9	110 °C (MW)	H_2O:iPrOH (3:2)		>99
10		H_2O:iPrOH (4:1)		>99

Conversions were determined by GC-FID.

Table 3
Suzuki–Miyaura coupling under microwave irradiation at 110 °C for 5 min by adding whole cells, cofactor (NADH), phosphate buffer or phosphate buffer and NADH.

Entry	Biotransformation reagent	Conv. (%) GC-FID
1	*E. coli* cells only	0%
2	NADH	>99%
3	Phosphate buffer	>99%
4	Phosphate buffer and NADH	>99%

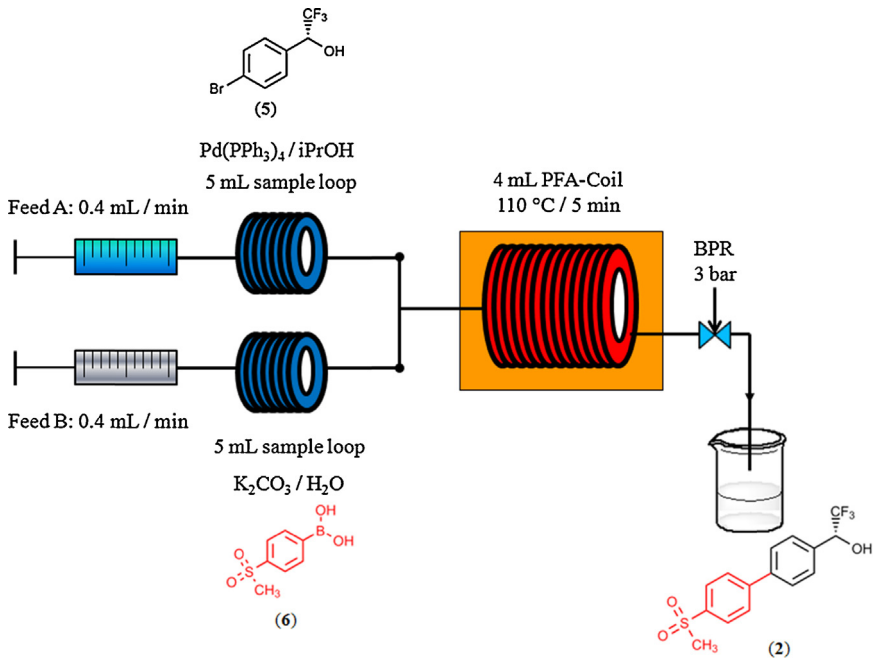
Conversions were determined by GC-FID.

In an effort to improve the overall process efficiency by decreasing the required number of work-up and purification steps we attempted to develop a one-pot process consisted on enzymatic reduction followed by Suzuki–Miyaura coupling [17]. The asymmetric enzymatic reduction was performed as described previously and after the end of the first step, 4-(methanesulfonyl)phenylboronic acid (**6**, 1 equiv.), potassium carbonate (2 equiv.), tetrakis(triphenylphosphine)palladium(0) (0.2 mol%) and isopropanol were added to the reaction mixture in the 10 mL Pyrex microwave vial. The reaction was then heated by microwave irradiation at 110 °C for 5 min. After cooling, the solvent was evaporated and the crude reaction mixture was extracted with ethyl acetate, leading to a GC-FID conversion of only 2% for the desired Suzuki–Miyaura coupling product (Scheme 5).

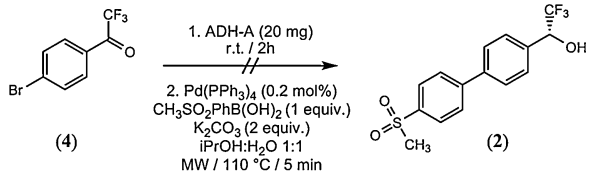
In order to find out which factor was disturbing the Suzuki–Miyaura coupling, the reaction was carried out in the presence of each one of the biotransformation reagents separately (Table 3). From those experiments we could conclude that the whole cells used in the biocatalytic step, but not phosphate buffer or NADH, were in some way preventing the Suzuki–Miyaura coupling reaction (Table 3).

In an attempt to solve this problem, we carried out a centrifugation after the biocatalytic step, followed by addition of Suzuki–Miyaura reactants to the supernatant (Scheme 6). Unfortunately, this approach needs high catalyst loadings (≥3 mol%), to achieve high conversions under microwave heating (Table 4, entries 1–2). When the Suzuki–Miyaura coupling step was performed at room temperature, however, lower conversions and longer reaction times were observed even with 5 mol% of palladium catalyst (Table 4, entry 3 and 4).

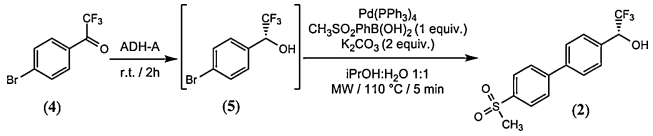
The final step was to translate this procedure to a continuous flow environment. Biocatalytic reduction was performed as described previously and followed by centrifugation and addition of the Pd catalyst to the supernatant. The supernatant and an aqueous solution containing the boronic acid and potassium carbonate were pumped in separate lines to a T-mixer and then through



Scheme 4. Suzuki–Miyaura coupling in continuous flow. Concentration of alcohol in feed A: 0.25 M. Concentration of boronic acid in feed B: 0.25 M.



Scheme 5. Asymmetric enzymatic reduction using ADH-A and Suzuki–Miyaura coupling toward an one-pot process.



Scheme 6. Asymmetric enzymatic reduction using lyophilized cells from *Escherichia coli* (ADH-A′), centrifugation and Suzuki–Miyaura coupling using the supernatant.

the PFA-coil reactor at 110 °C (16 mL, 5 min residence time, combined flow rate 3.2 mL/min). The reaction mixture was collected for 10 min. Full conversion was observed by GC–MS and the product was obtained in 73% crude yield and high optical purity.

For comparison purposes, a single Suzuki–Miyaura coupling reaction with 5 mol% of palladium catalyst was carried out under continuous flow without performing the previous biocatalytic step. An aqueous solution containing the boronic acid and base and a solution of isopropanol containing the halide and metal catalyst were pumped from separate feeds through a PFA-coil reactor at 110 °C (4 mL PFA-coil, 5 min residence time, combined flow rate 0.8 mL/min). The reaction mixture was collected for 10 min and full conversion was observed by GC–MS. The product was obtained in 73% crude yield. Further purification by column chromatography furnished the product in 45% yield.

Table 4
Asymmetric enzymatic reduction, centrifugation (5000 rpm for 10 min) and Suzuki–Miyaura coupling under microwave heating (110 °C for 5 min) and at room temperature.

Entry	$Pd(PPh_3)_4$	Temperature	Reaction time	Conv. (%) GC-FID
1	4 mol%	110 °C (MW)	5 min	98
2	5 mol%	110 °C (MW)	5 min	>99
3	5 mol%	r.t.	3.5 h	60
4	5 mol%	r.t.	7 h	61

Conversions were determined by GC-FID.

4. Conclusions

In conclusion we presented an enantioselective two step synthesis of an important intermediate for the new cathepsin K inhibitor Odanacatib (**1**), based on asymmetric biocatalytic reduction followed by Suzuki–Miyaura cross coupling. It was demonstrated that a system composed by alcohol dehydrogenase with substrate-coupled cofactor regeneration leads to the desired alcohol **5** in excellent yields and enantiomeric excess. In addition, a Suzuki–Miyaura reaction between **5** and **6** was also achieved in high yields and translated to a continuous flow process. The coupling of the biocatalytic reduction with the continuous-flow Suzuki–Miyaura reaction was developed, leading to the desired product **2** in 73% overall yield, without isolation of any intermediate.

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