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Asymmetric synthesis of arylselenoalcohols by means of the reduction of organoseleno acetophenones by whole fungal cells

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Abstract

A series of novel organoseleno acetophenones (**3a–f)** have been synthesized. The microbial reduction of the seleno ketones (**3)** has been evaluated using whole cells of *Rhizopus oryzae* CCT 4964, *Aspergillus terreus* CCT 3320, *A. terreus* CCT 4083 and *Emericella nidulans* CCT 3119. These microorganisms showed Prelog and anti-Prelog stereoselectivity, leading to the arylselenoalcohols in moderate to high enantiomeric excesses. The organoselenium compounds were compatible with the biocatalytic conditions employed.

Keywords: Microbial reduction; Whole fungal cells; Ketones; Selenium; Alcohols

1. Introduction

In recent years chiral organoselenium compounds have been prepared and some of them were applied in asymmetric synthesis [1]. This subject constitutes a new trend in this field of organoelemental chemistry. In this way, the search for efficient methods to prepare organoselenium compounds with high enantiomeric purities is an important goal in this area of chemistry. In this context, biocatalysis has attracted much attention for being an important method in the preparation of optically active compounds with high stereoselectivity under environmentally friendly conditions [2,3]. Notwithstanding the intense activity in the field of organoselenium chemistry along the last three decades, to our knowledge, few synthetic studies on the application of the biocatalysis to prepare enantiopure selenium compounds were published to date [4].[1] Recently, we and others have explored new microorganism strains native from the Brazilian rain forest for several synthetic purposes, for instance in the oxidation of sulfides [5], hydrolysis of epoxides [6], reduction of fluoroacetophenones and deracemization of fluorophenylethanols [7].

In this paper, we combine our longstanding interest in the chemistry of selenium compounds [8] with our biotransformation program, with the aim of synthesizing selenium containing chiral building blocks, which could be used to assembly more complex chiral compounds by means of the well known selenium organic chemistry [9].

In this way, the present study was aimed at the synthesis of several organoseleno acetophenones (**3a–f)** and screening for microorganisms that provide their microbial reduction into the corresponding chiral arylselenoalcohols (Scheme 1). The main purpose of this study is to determine the behavior of the selenium containing molecules towards biocatalytic conditions.

2. Experimental

2.1. General methods

The microorganisms *Rhizopus oryzae* CCT 4094, *Aspergillus terreus* CCT 3320, *A. terreus* CCT 4083 and *Emericella nidulans* CCT 3119 were purchased from the culture collection of the André Tosello Foundation (Brazil) [10].[2] Lipase B from *Candida antarctica*, Novozym 435, was a gift

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[1] The attempted preparation of chiral selenoxides by fungal oxidation of selenides was reported. However, the chiral selenoxides were not obtained [4b].

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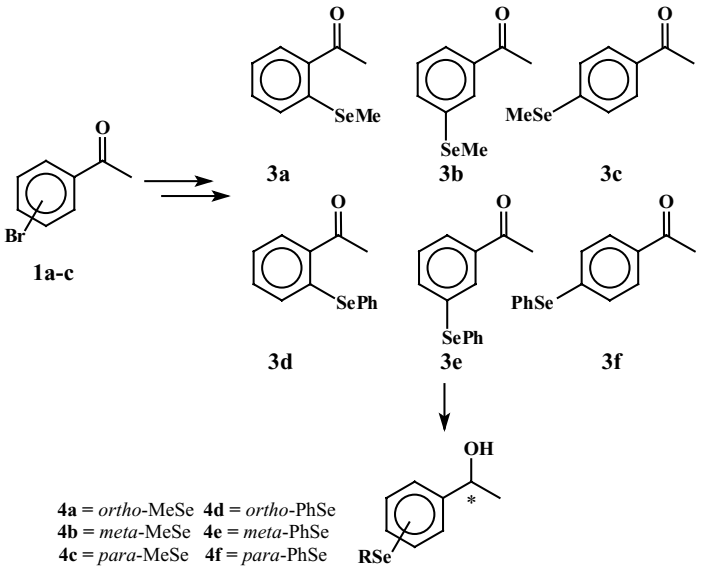


Scheme 1.

from Novozymes A/S, and was employed without any previous treatment. All chemical reactions were carried out under dry nitrogen atmosphere. Solvents were purified by standard procedures. All other reagents are commercially available and were used without further purification. Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed using precoated plates (aluminum foil, silica gel 60 F_{254} Merck, 0.25 mm). Merck 60 silica gel (230–400 mesh) was used for flash chromatography. GC analyses were performed in a Shimadzu GC-17A instrument with a FID detector, using hydrogen as a carrier gas (100 kPa). Mass spectra were recorded on a Shimadzu GC–MS P5050A (70 eV) spectrometer. The fused silica capillary columns used were either a J&W Scientific DB-5 (30 m × 0.25 mm) or a chiral column Chirasil-Dex CB β-cyclodextrin (25 m × 0.25 mm) for determination of the enantiomeric excesses. Optical rotations were determined on a JASCO DIP-378 polarimeter. Infrared spectra were recorded on a Perkin-Elmer 1750-FT-IR spectrometer. NMR spectra were recorded on Bruker DPX 500 or DPX 300 instrument. For ^{1}H (instrument operating at 500.13 or 300 MHz) δ values are referenced to Me_4Si (0 ppm) and for ^{13}C (instrument operating at 125.77 or 75.5 MHz) δ values are referenced to $CDCl_3$ (77.0 ppm). Chemical shifts are given in ppm and coupling constants are given in Hertz. Microanalyses were performed by Central Analítica, Instituto de Química, USP, Brazil.

2.2. General procedure for the preparation of organoseleno acetophenones [1-(organylseleno-phenyl)-ethanones (**3a–f**)]

2.2.1. Synthesis of the ketals (**2a–c**)

The preparation of these ketals from bromo-acetophenones was carried out according to the usual method [11].

2.2.2. Synthesis of the methylseleno acetophenones (**3a–c**)

To a solution of the ketal **2** (0.726 g, 3 mmol) in dry THF (30 ml) under nitrogen atmosphere, cooled to −76 °C, *t*-BuLi (6.34 ml of a 0.52 M solution in pentane, 3.3 mmol) was slowly added. After warming up and stirring for 30 min at 0 °C, selenium powder (0.260 g, 3.3 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 3 h, then methyl iodide (0.468 g, 3.3 mmol) was added. After further 15 min, the work-up was done with saturated aqueous solution of NH_4Cl (20 ml) and the aqueous layer was extracted with ethyl acetate (3 × 30 ml). The organic phases were combined and the solvent was removed in vacuum. The residue was dissolved in acetone (20 ml) and the hydrolysis of the ketal group was carried out with an aqueous solution of 1N hydrochloric acid (15 ml) under reflux for 2 h. The mixture was extracted with ethyl acetate (3 × 50 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (9:1) to afford the desired methylseleno acetophenones **(3a–c)**.

1-(2-Methylseleno-phenyl)-ethanone **(3a)** [12]. Yield: 30%. Oil: IR (film) cm^{-1}: 3050, 2995, 1654, 1582, 1553, 1458, 1429, 1360, 1289, 1260, 1025, 959, 762, 599. ^{1}H NMR (300 MHz) δ: 7.97 (d, J = 7.57 Hz, 1H), 7.47 (d, J = 3.9 Hz, 1H), 7.31–7.24 (m, 2H), 2.66 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (75 MHz) δ: 198.8, 139.3, 134.8, 132.7, 132.1, 127.7, 124.3, 27.3, 6.54. MS: *m/z* (relative intensity) 214 (M^+, 31), 212 (15), 199 (100), 169 (5), 156 (9), 130 (3), 117 (6), 91 (94), 77 (15), 63 (12), 43 (74).

1-(3-Methylseleno-phenyl)-ethanone **(3b)**. Yield: 30%. Oil: IR (film) cm^{-1}: 3057, 3003, 2928, 1684, 1567, 1470, 1416, 1356, 1252, 1070, 961, 907, 787, 686, 591. ^{1}H NMR (500 MHz) δ: 7.99 (dt, J = 1.7 and 0.3 Hz, 1H), 7.76 (ddd,

J = 7.7, 1.7 and 1.1 Hz, 1H), 7.60 (ddd, J = 7.7, 1.7 1.1 Hz, 1H), 7.35 (dt, J = 7.7, 0.4 Hz, 1H), 2.61 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (125 MHz) δ: 197.6, 137.7, 134.6, 132.8, 129.7, 129.1, 126.1, 26.6, 7.2. MS: *m/z* (relative intensity) 214 (M^+, 98), 212 (49), 210 (18), 201 (18), 199 (92), 197 (46), 195 (18), 184 (11), 171 (34), 169 (28), 156 (18), 153 (5), 130 (5), 117 (8), 91 (99), 76 (20), 63 (18), 50 (30), 43 (100).

1-(4-Methylseleno-phenyl)-ethanone **(3c)**. Yield: 27%. mp = 71 °C. IR (KBr) cm^{-1}: 2996, 2959, 2922, 1669, 1564, 1424, 1394, 1356, 1273, 1184, 1081, 955, 911, 812, 745, 589. ^{1}H NMR (300 MHz) δ: 7.85 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 2.59 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (75 MHz) δ: 197.3, 140.8, 134.4, 128.6, 128.5, 26.4, 6.7. MS: *m/z* (relative intensity) 214 (M^+, 74), 212 (37), 199 (100), 184 (21), 171 (15), 156 (14), 130 (3), 117 (6), 91 (61), 76 (13), 63 (13), 43 (50). (Found: C, 50.75; H, 4.58. Calculated for $C_9H_{10}OSe$: C, 50.72; H, 4.73.)

2.2.3. Synthesis of the phenylseleno acetophenones (3d–f)

To a solution of the ketal **2** (0.726 g, 3 mmol) in dry THF (9 ml) under nitrogen atmosphere, cooled to −76 °C, *t*-BuLi (6.34 ml of a 0.52 M solution in pentane, 3.3 mmol) was slowly added. After warming up and stirring for 30 min at 0 °C, a solution of diphenyl diselenide (1.03 g, 3.3 mmol) in THF (2 ml) was added. The reaction mixture was allowed to warm up to room temperature and then it was stirred for an additional period (3 h). The work-up was done with saturated aqueous solution of NH_4Cl (4 ml) and the aqueous layer was extracted with ethyl acetate (3 × 30 ml). The organic phases were combined and the solvent was removed in vacuum. The residue was dissolved in acetone (20 ml) and the hydrolysis of the ketal group was carried out with an aqueous solution of 1N hydrochloric acid (15 ml) under reflux for 2 h. The mixture was extracted with ethyl acetate (3 × 50 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (9:1) to afford the desired phenylseleno acetophenones **(3d–f)**.

1-(2-Phenylseleno-phenyl)-ethanone **(3d)**. Yield: 56%. mp = 60 °C. IR (KBr) cm^{-1}: 3058, 3002, 2964, 1664, 1582, 1556, 1456, 1432, 1358, 1297, 1250, 1137, 1026, 957, 747, 695, 598, 473. ^{1}H NMR (500 MHz) δ: 7.96 − 7.93 (m, 1H), 7.71 − 7.67 (m, 2H); 7.47 − 7.37 (m, 3H), 7.23 − 7.17 (m, 2H), 6.99 − 6.95 (m, 1H), 2.67 (s, 3H). ^{13}C NMR (125 MHz) δ: 198.7, 140.4, 137.3, 133.8, 132.4, 131.5, 129.6, 129.5, 129.4, 128.9, 124.6, 27.2. MS: *m/z* (relative intensity) 276 (M^+, 100), 274 (46), 272 (18), 261 (39), 259 (20), 232 (37), 230 (21), 201 (13), 199 (69), 197 (35), 181 (14), 152 (52), 126 (4), 115 (6), 102 (6), 91 (27), 77 (37), 63 (10). (Found: C, 61.14; H, 4.66. Calculated for $C_{14}H_{12}OSe$: C, 61.10; H, 4.39.)

1-(3-Phenylseleno-phenyl)-ethanone **(3e)**. Yield: 55%. Oil: IR (film) cm^{-1}: 3056, 3003, 2961, 1686, 1570, 1474, 1435, 1413, 1356, 1252, 1066, 997, 765, 739, 689, 590, 451. ^{1}H NMR (500 MHz) δ: 8.03 (t, J = 1.7 Hz, 1H), 7.82 (ddd, J = 7.7, 1.7 and 1.1 Hz, 1H), 7.59 (ddd, J = 7.7, 1.7 and 1.1 Hz, 1H); 7.51 − 7.47 (m, 2H), 7.34, (t, J = 7.7 Hz, 1H), 7.32 − 7.27 (m, 3H), 2.54 (s, 3H). ^{13}C NMR (125 MHz) δ: 197.4, 137.9, 136.8, 133.5, 132.4, 132.1, 130.0, 129.5, 129.4, 127.8, 126.9, 26.5. MS: *m/z* (relative intensity) 276 (M^+, 69), 274 (34), 261 (26), 91 (61), 76 (13), 63 (13), 43 (50). (Found: C, 61.36; H, 4.72. Calculated for $C_{14}H_{12}OSe$: C, 61.10; H, 4.39.)

1-(4-Phenylseleno-phenyl)-ethanone **(3f)** [13]. Yield: 66%. mp = 57 °C (lit. [13], 61 °C). IR (KBr) cm^{-1}: 3055, 2995, 1677, 1580, 1468, 1433, 1392, 1268, 1057, 1004, 955, 819, 747, 691, 599, 475. ^{1}H NMR (500 MHz) δ: 7.80 − 7.76 (m, 2H), 7.59 − 7.56 (m, 2H), 7.40 − 7.32 (m, 5H), 2.54 (s, 3H). ^{13}C NMR (125 MHz) δ: 197.3, 140.2, 135.1, 135.0, 130.2, 129.6, 128.8, 128.5, 128.4, 26.4. MS: *m/z* (relative intensity) 276 (M^+, 100), 274 (46), 261 (77), 259 (38), 196 (1), 181 (68), 154 (19), 153 (23), 129 (20), 115 (7), 102 (4), 77 (46), 63 (10), 43 (81).

2.3. General procedure for the preparation of the stereoisomeric mixtures of organoseleno-α-methylbenzyl alcohols [(R,S)-1-(organylseleno-phenyl)-ethanols (4a–f)]

To a solution of organoseleno acetophenones **(3a–f)** (1 mmol) in ethanol (5 ml) was added $NaBH_4$ (1.1 mmol, 42 mg), and the mixture was stirred at room temperature for 4 h. After work-up with saturated aqueous solution of NH_4Cl (4 ml), the aqueous layer was extracted with methylene dichloride (3 × 20 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (4:1) to afford compounds (*R*,*S*)-**4a–f**.

(*R*,*S*)-1-(2-methylseleno-phenyl)-ethanol **(4a)**. Yield: 92%. Oil: IR (film) cm^{-1}: 3380, 3059, 2971, 2926, 1587, 1568, 1464, 1432, 1369, 1290, 1197, 1129, 1088, 1006, 900, 785, 458. ^{1}H NMR (300 MHz) δ: 7.51 (dd, J = 1.9 and 5.5 Hz, 1H), 7.39 (dd, J = 1.9 and 5.7 Hz, 1H), 7.26 − 7.11 (m, 2H), 5.23 (quart., J = 6.3 Hz, 1H), 2.78 (s, 1H), 2.33 (s, 3H), 1.48 (d, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz) δ: 145.6, 130.0, 129.9, 127.8, 126.5, 125.2, 68.6, 23.8, 7.4. MS: *m/z* (relative intensity) 216 (M^+, 40), 214 (19), 210 (21), 199 (14), 183 (32), 157 (19), 117 (4), 105 (100), 91 (34), 77(52), 65 (10), 51 (28), 43 (77). (Found: C, 50.51; H, 5.56. Calculated for $C_9H_{12}OSe$: C, 50.24; H, 5.62.)

(*R*,*S*)-1-(3-methylseleno-phenyl)-ethanol **(4b)**. Yield: 95%. Oil. IR (film) cm^{-1}: 3375, 3054, 2972, 2926, 2872, 1590, 1571, 1474, 1420, 1368, 1333, 1273, 1202, 1110, 1072, 1011, 905, 785, 700, 442. ^{1}H NMR (500 MHz) δ: 7.46 − 7.42 (m, 1H), 7.31 (dt, J = 7.6 and 1.5 Hz, 1H),

7.26 – 7.21 (m, 1H), 7.19 – 7.16 (m, 1H), 4.85 (quart., J = 6.5 Hz, 1H), 2.35 (s, 3H) 1.88 (s, 1H), 1.48 (d, J = 6.5 Hz, 3H). ^{13}C NMR (75 MHz) δ: 146.7, 132.1, 129.3, 129.1, 127.3, 123.2, 70.1, 25.1, 7.1. MS: *m/z* (relative intensity) 216 (M^+, 49), 201 (20), 199 (12), 184 (2), 173 (17), 157 (40), 155 (20), 121 (3), 99 (11), 91 (26), 78 (43), 77 (34), 65 (8), 51 (21), 43 (100).

(*R*,*S*)-1-(4-methylseleno-phenyl)-ethanol **(4c)**. Yield: 95%. Oil: IR (film) cm^{-1}: 3362, 2971, 2927, 2871, 1721, 1595, 1493, 1419, 1368, 1273, 1113, 1087, 900, 791, 581. ^{1}H NMR (500 MHz) δ: 7.40 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 4.85 (quart., J = 6.3 Hz, 1H), 2.34 (s, 3H), 1.47 (d, J = 6.3 Hz, 3H), 1.30 (s, 1H). ^{13}C NMR (125 MHz) δ: 143.9, 130.5, 130.4, 126.1, 70.0, 25.1, 7.3. MS: *m/z* (relative intensity) 216 (M^+, 57), 214 (29), 201 (71), 183 (5), 173 (10), 157 (44), 153 (10), 121 (4), 100 (20), 91 (28), 78 (56), 51 (22), 43 (100). (Found: C, 50.51; H, 5.86. Calculated for $C_9H_{12}OSe$: C, 50.24; H, 5.62.)

(*R*,*S*)-1-(2-phenylseleno-phenyl)-ethanol **(4d)**. Yield: 96%. Oil: IR (film) cm^{-1}: 3374, 3059, 2971, 2923, 1576, 1468, 1438, 1368, 1337, 1291, 1196, 1081, 1010, 898, 739, 691, 476. ^{1}H NMR (500 MHz), δ: 7.58 (dd, J = 7.7 and 1.5 Hz, 1H), 7.40 – 7.35 (m, 3H), 7.33 (dt, J = 6.9 and 1.3 Hz, 1H), 7.27 – 7.22 (m, 3H), 7.14 (dt, J = 7.6 and 1.5 Hz, 1H), 5.33 (quart., J = 6.4 Hz, 1H), 1.89 (s, 1H), 1.44 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz) δ: 147.08, 134.85, 132.52, 131.32, 129.42, 129.14, 128.47, 128.25, 127.25, 125.94, 69.32, 24.17. MS: *m/z* (relative intensity) 278 (M^+, 37), 245 (11), 200 (9), 185 (21), 157 (21), 105 (100), 91 (13), 77 (67), 65 (8), 51 (30), 43 (75). (Found: C, 60.72; H, 5.07. Calculated for $C_{14}H_{14}OSe$: C, 60.66; H, 5.09.)

(*R*,*S*)-1-(3-phenylseleno-phenyl)-ethanol **(4e)**. Yield: 95%. Oil. IR (film) cm^{-1}: 3354, 3055, 2972, 2925, 2871, 1573, 1473, 1440, 1416, 1370, 1298, 1200, 1069, 1015, 901, 789, 738, 695, 472. ^{1}H NMR (500 MHz) δ: 7.50 – 7.44 (m, 3H), 7.34 (dt, J = 7.2 and 1.6 Hz, 1H), 7.29 – 7.22 (m, 5H), 4.84 (quart., J = 6.4 Hz, 1H), 2.85 (s, 1H), 1.46 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz) δ: 146.99, 133.06, 131.87, 131.38, 130.38, 129.88, 129.44, 129.34, 127.40, 124.39, 70.06, 25.17. MS: *m/z* (relative intensity) 278 (M^+, 70), 263 (9), 245 (2), 232 (6), 185 (18), 157 (74), 130 (28), 115 (4), 105 (27), 91 (9), 77 (72), 65 (9), 51 (53), 43 (100). (Found: C, 60.41; H, 5.10. Calculated for $C_{14}H_{14}OSe$: C, 60.66; H, 5.09.)

(*R*,*S*)-1-(4-phenylseleno-phenyl)-ethanol **(4f)**. Yield: 97%. Oil: IR (film) cm^{-1}: 3355, 3064, 2972, 2925, 2874, 1576, 1478, 1439, 1401, 1370, 1296, 1201, 1084, 1069, 1013, 898, 825, 737, 691, 544. ^{1}H NMR (500 MHz) δ: 7.47 – 7.43 (m, 4H), 7.29 – 7.24 (m, 5H), 4.87 (quart., J = 6.4 Hz, 1H), 1.57 (s, 1H), 1.48 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz) δ: 145.10, 133.21, 132.87, 131.16, 129.94, 129.31, 127.31, 126.43, 70.02, 25.15. MS: *m/z* (relative intensity) 278 (M^+, 71), 263 (61), 232 (7), 185 (27), 157 (79), 130 (15), 121 (5), 105 (13), 91 (8), 77 (77), 65 (10), 51 (56), 43 (100). (Found: C, 60.49; H, 5.37. Calculated for $C_{14}H_{14}OSe$: C, 60.66; H, 5.09.)

2.4. General procedures for cultures and biotransformation

The fungi from slants were cultivated in 100 ml of malt extract medium (ME; 20 g/L) in 250 ml Erlenmeyer flasks at 32 °C on a orbital shaker (170 rpm) for 96 h. The fungal cells were harvested by filtration and used in the bioreduction reaction. Sterile material was used to perform the experiments and the microorganisms were manipulated in a laminar flow cabinet.

2.4.1. Small scale reactions

The biotransformation was performed by re-suspending the wet cells of the appropriate fungi (4 g) in a phosphate buffer solution (50 ml, pH 7.0, 0.1 M) in a 125 ml Erlenmeyer flask. To these cell suspensions, solutions of the seleno ketones **(3a–f)** (20 mg) in ethanol (0.5 ml) were added and the mixtures were incubated in a orbital shaker (170 rpm) at 32 °C for the times indicated in Table 1. The progress of the biotransformation was monitored by GC.

2.4.2. Preparative-scale reduction reaction

The reactions using 100 mg of ketone **(3)** were performed in 500 ml Erlenmeyer flasks containing whole cells of *R. oryzae* (15 g) in 200 ml of a phosphate buffer solution (pH 7.0, 0.1 M) at 32 °C on a orbital shaker (170 rpm). After the appropriate conversion time (Table 1), the mixture was filtered and the biomass was washed with brine (100 ml) and ethyl acetate (100 ml). The aqueous phase was extracted with ethyl acetate (4 × 100 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (4:1) as eluent to afford compounds **4**. Organoseleno-α-methylbenzyl alcohol = ***4***b: oil, yield = 55%, $[\alpha]_D^{22}$ −30.4° (*c* 1.05, $CHCl_3$), e.e. = 94%; **4c**: oil, yield = 50%, $[\alpha]_D^{22}$ −56.8° (*c* 0.47, $CHCl_3$), e.e. = 96%; **4e**: oil, yield = 27%, $[\alpha]_D^{22}$ −21.8° (*c* 0.27, $CHCl_3$), e.e. = 87%; **4f**: oil, yield = 25%, $[\alpha]_D^{22}$ −34.1° (*c* 0.41, $CHCl_3$), e.e. = 71%.

2.5. GC analysis for determination of the enantiomeric excess (e.e.)

The reaction progress was monitored every 24 h by collecting 2 ml samples. These samples were extracted by stirring with ethyl acetate (0.5 ml) followed by centrifugation (6000 rpm, 5 min). The organic phase was analyzed by GC (1 μl) in a fused silica chiral capillary column. The products of the biocatalyzed reactions were compared with a racemic mixture previously obtained from chemical reduction (see Section 2.3). GC conditions: compounds **4a–c**: injector 220 °C; detector 220 °C; 150 °C 30 min hold, 1 °C/min

Table 1
Asymmetric bioreduction of seleno ketones (**3a–f)** using whole fungal cells

Entry	Substrate	Whole fungal cells	Time (days)	*c* (%)	Product **4** e.e.[a] (%) configuration[b]
1	**3a**	*R. oryzae* CCT 4964	7	n.c.	–
2	**3b**	*R. oryzae* CCT 4964	2	99	94 (*S*)
3	**3c**	*R. oryzae* CCT 4964	2	91	96 (*S*)
4	**3d**	*R. oryzae* CCT 4964	7	n.c.	–
5	**3e**	*R. oryzae* CCT 4964	7	90	87 (*S*)
6	**3f**	*R. oryzae* CCT 4964	7	85	71 (*S*)
7	**3a**	*A. terreus* CCT 4083	7	n.c.	–
8	**3b**	*A. terreus* CCT 4083	2	76	90 (*S*)
9	**3c**	*A. terreus* CCT 4083	2	86	55 (*R*)
10	**3d**	*A. terreus* CCT 4083	7	n.c.	–
11	**3e**	*A. terreus* CCT 4083	9	21	47 (*S*)
12	**3f**	*A. terreus* CCT 4083	9	12	45 (*S*)
13	**3a**	*A. terreus* CCT 3320	7	n.c.	–
14	**3b**	*A. terreus* CCT 3320	2	75	86 (*S*)
15	**3c**	*A. terreus* CCT 3320	10	75	95 (*R*)
16	**3d**	*A. terreus* CCT 3320	7	n.c.	–
17	**3e**	*A. terreus* CCT 3320	7	41	99 (*S*)
18	**3f**	*A. terreus* CCT 3320	7	n.c.	–
19	**3a**	*E. nidulans* CCT 3119	7	n.c.	–
20	**3b**	*E. nidulans* CCT 3119	3	9	67 (*S*)
21	**3c**	*E. nidulans* CCT 3119	5	99	99 (*R*)
22	**3d**	*E. nidulans* CCT 3119	7	n.c.	–
23	**3e**	*E. nidulans* CCT 3119	10	n.c.	–
24	**3f**	*E. nidulans* CCT 3119	7	n.c.	–

c: conversion determined by GC; n.c.: no conversion.
[a] Compounds **4a–f**: determined by GC analysis using a chiral column (Chirasil-Dex CB β-cyclodextrin 25 m × 0.25 mm).
[b] See Section 2.

(until 180 °C) retention time for **4a** (enantiomers = 10.8 min and 12.4 min), **4b** (*R* = 11.0 min; *S* = 11.4 min) and **4c** (*R* = 12.4 min; *S* = 13.3 min). Compounds **4d–f**: injector 220 °C; detector 220 °C; 150 °C 2 °C/min (until 180 °C) retention time for **4d** (enantiomers = 21.2 and 22.9 min), **4e** (*R* = 25.9 min; *S* = 26.4 min) and **4f** (*R* = 32.4 min; *S* = 33.4 min).

2.6. Determination of the absolute configurations: general procedure

2.6.1. Synthesis of (S)-3- and (S)-4- bromo-α-methylbenzyl alcohol [(S)-5a and (S)-5b]

(*R*,*S*)-3- and (*R*,*S*)-4-bromo-α-methylbenzyl alcohol (**5a**,**b**) were obtained by standard reduction of the corresponding commercially available ketones.

To a solution of the appropriate alcohol (1 g; 5 mmol) in *tert*-butyl methyl ether (15 ml), vinyl acetate (0.25 ml) and Novozym 435 (Lipase B from *C. antarctica*, 250 mg) were added. The resulting mixture was magnetically stirred at room temperature. The reaction progress was monitored by GC analysis. The enzyme was removed by filtration and washed with ethyl acetate. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent to afford compounds (*S*)-3- and (*S*)-4-bromo-α-methylbenzyl alcohol, respectively.

(*S*)-*3-bromo-α-methylbenzyl alcohol* [(*S*)-**5a**]: reaction time = 3 h, yield: 56%. e.e. = 79%. $[\alpha]_D^{22}$ −19.3° (*c* 2.0, EtOH) (lit. [14] $[\alpha]_D^{26}$ −28.6° (*c* 1.78, EtOH).

(*S*)-*4-bromo-α-methylbenzyl alcohol* [(*S*)-**5b**]: reaction time = 1 h, yield: 40%. e.e. = 98%. $[\alpha]_D^{22}$ −45.1° (*c* 5.2, $CHCl_3$) (lit. [14] $[\alpha]_D^{23}$ −37.9° (*c* 1.13, $CHCl_3$).

2.6.2. Synthesis of (S)-1-(3-methylseleno-phenyl)-ethanol (4b) and (S)-1-(4-methylseleno-phenyl)-ethanol (4c) from (S)-3- and (S)-4-bromo-α-methylbenzyl alcohols [(S)-5a and (S)-5b], respectively

To a solution of the (*S*)-3- or (*S*)-4-bromo-α-methylbenzyl alcohol [(*S*)-**5a** or (*S*)-**5b**] (0.200 g, 1 mmol) in dry THF (5 ml) under nitrogen atmosphere, cooled to −76 °C, *t*-BuLi (6.34 ml of a 0.52 M solution in pentane, 3.3 mmol) was slowly added. After warming up and stirring for 30 min at 0 °C, selenium powder (0.087 g, 1.1 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred for additional 3 h, then methyl iodide (0.156 g, 1.1 mmol) was added. After further 15 min, the work-up was done with saturated aqueous solution of NH_4Cl (4 ml) and the aqueous layer was extracted with ethyl acetate (3 × 30 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and a pale yellow oil was obtained, which was used without further purification as a standard for chiral GC analyses (GC conditions: injector 220 °C; detector 220 °C; 150 °C 30 min

hold, 14°C/min until 180 °C). t_R (min): (*S*)-**4b**, 11.4 and (*S*)-**4c**, 13.3.

*2.6.3. Synthesis of (S)-1-(3-phenylseleno-phenyl)-ethanol (**4e**) and (S)-1-(4-phenylseleno-phenyl)-ethanol (**4f**) from (S)-3- and (S)-4- bromo-α-methylbenzyl alcohols [(S)-**5a** and (S)-**5b**], respectively*

To a solution of the (*S*)-3- or 4- bromo-α-methylbenzyl alcohol [(*S*)-**5a** or (*S*)-**5b**] (0.200 g, 1 mmol) in dry THF (5 ml) under nitrogen atmosphere, cooled to −76 °C, *t*-BuLi (6.34 ml of a 0.52 M solution in pentane, 3.3 mmol) was slowly added. After warming up and stirring for 30 min at 0 °C, a solution of diphenyl diselenide (0.343 g, 1.1 mmol) in THF (2 ml) was added. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 3 h. The work-up was done with saturated aqueous solution of NH_4Cl (4 ml) and the aqueous layer was extracted with ethyl acetate (3 × 30 ml). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and a pale yellow oil was obtained, which was used without further purification as a standard for chiral GC analyses (GC conditions: injector 220 °C; detector 220 °C; 150 °C and 2 °C/min until 180 °C). t_R (min): (*S*)-**4e**, 26.4 and (*S*)-**4f**, 33.4.

3. Results and discussion

3.1. Synthesis of the organoseleno acetophenones (3a–f)

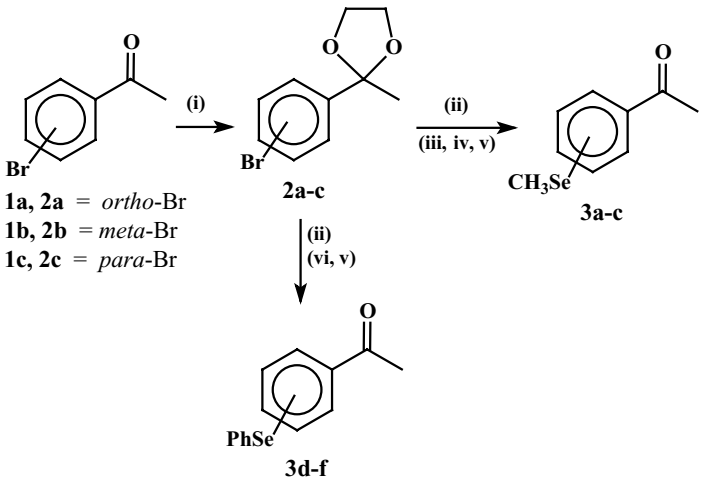
The organoseleno acetophenones (**3a–f)** were prepared from commercially available *ortho*-, *meta*- and *para*-bromoacetophenones (**1a–c)** as described in Scheme 2.

Initially, the ketone carbonyl group was protected according to the usual method [11]. Ketals (**2a–c)** in THF were then treated with *t*-BuLi followed by addition of selenium powder according to Wirth's protocol [15] in the case of compounds **3a–c**. After the insertion reaction was completed, as indicated by consumption of the gray selenium powder, with the formation of a clear yellow solution, the lithium arylselenolate was alkylated with methyl iodide. Finally, hydrolysis of the ketal with an aqueous solution of 1N hydrochloric acid gave the desired methylseleno acetophenones (**3a–c)** in 27–30% overall yield. The phenylseleno acetophenones (**3d–f)** were similarly prepared (Scheme 2). In this case, after the bromo-lithium exchange step, a solution of diphenyl diselenide in THF was added to the aryllithium solution. Hydrolysis of the ketal group with aqueous solution of 1N hydrochloric acid gave the phenylseleno acetophenones (**3d–f)** in 55–60% overall yield.

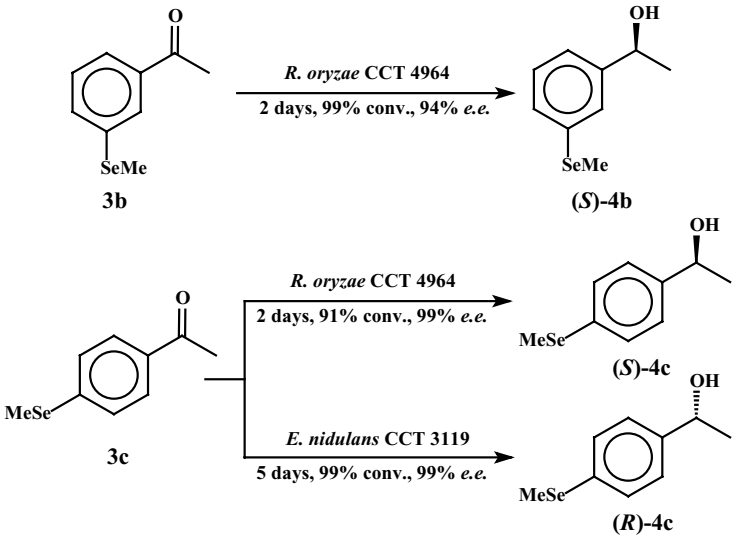
3.2. Bioreduction of the organoseleno acetophenones (3a–f)

With the organoseleno acetophenones (**3a–f)** in hand, we screened for microorganisms which are able to perform the bioreduction of the carbonyl group (Table 1). The strains of microorganisms were selected according to our previous assays with fluoroacetophenones [7]. The microbial reduction was performed by re-suspending the wet cells of the appropriate fungi in a phosphate buffer solution. To these cells suspension, solutions of the ketones **3a-f** in ethanol were added and the mixtures were incubated in rotary shaker at 32 °C. The progress of the reaction was monitored by GC analysis. The results are summarized in Table 1.

As can be observed, two of the six seleno ketones (**3)**, namely *ortho*-methylseleno acetophenone (**3a**) and *ortho*-phenylseleno acetophenone (**3d**), were unreactive towards the four fungi investigated under the bioreduction conditions employed. The reaction was stopped after 7 days and no conversion was observed. It is worth mentioning that these fungi under identical conditions performed the



Scheme 2. Reagents and conditions: (i) C_6H_6, *p*-TsOH, ethylene glycol, reflux, 4 h; (ii) *t*-BuLi, THF, −76 °C, 30 min; (iii) selenium powder, −76 °C → r.t. 3 h; (iv) MeI, r.t. 15 min; (v) acetone/aq. 1N HCl, reflux, 2 h; (vi) PhSeSePh, THF, −76 °C → r.t. 2 h.



Scheme 3.

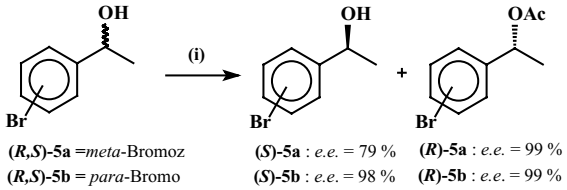
reduction of *ortho*-fluoroacetophenone [7]. This different behavior of the *ortho*-organoseleno acetophenones could be attributed to steric constraint due to intramolecular non-bonded Se···Y (Y = carbonyl oxygen) interactions producing a pseudo-high-valent selenium species [16], which could lead to a rigid array hindering the formation of the enzyme-substrate complex. This assumption is supported by the reduction of the *meta*-methylseleno acetophenone (**3b**) by the four fungi employed (entries 2, 8, 14 and 20, Table 1), *R. oryzae* CCT 4964 being the most effective, leading to the *meta*-methylseleno-α-methylbenzyl alcohol (**4b**) in good conversion (99%) and enantiomeric excess (94%). Similar reactivity was presented by *para*-methylseleno acetophenone (**3c**) towards the fungi tested (entries 3, 9, 15 and 21, Table 1) which was reduced to the *para*-methylseleno-α-methylbenzyl alcohol (**4c**) with good conversion rates and enantiomeric excess. The *para*-phenylseleno acetophenone (**3f**) gave low conversion rates and e.e. with all fungi tested, except with *R. oryzae* CCT 4964 which transformed **3f** into **4f** in 85% conversion and 71% e.e. When the reduction of the seleno ketones (**3)** were carried out with cells of *R. oryzae* CCT 4964 the alcohols obtained showed the same absolute configuration, independently from the organoseleno substituent on the phenyl ring. In all these cases, the enantioselectivity is in accordance with Prelog's rule [17]. Interestingly, the reduction of **3c** using cells of *A. terreus* CCT 4083, *A. terreus* CCT 3320 and *E. nidulans* CCT 3119 (entries 9, 15 and 21, respectively, Table 1) showed the anti-Prelog stereoselectivity. Scheme 3 summarizes the best results obtained in this study (Scheme 3). Work is in progress to scale-up the reactions according to the described methods which are used for this purpose [2].

As can be observed in this scheme, *meta*- and *para*-methylseleno-α-methylbenzyl alcohols **4b** and **4c** are obtained with good conversion and enantiomeric excesses through the bioreduction of the corresponding methylseleno-acetophenones. In the case of the *para*-isomer, both enantiomers can be obtained with high e.e. by using different fungi. This method to prepare chiral organoseleno-α-methylbenzyl alcohol complements the *ortho*-metalation method developed to prepare chiral selenium compounds [18].

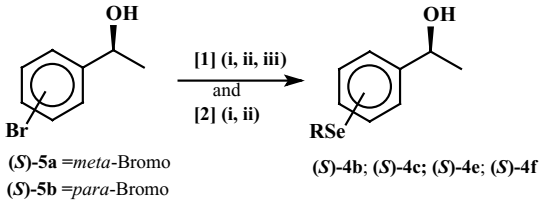
3.3. Determination of the absolute configurations

The absolute configurations were attributed by chiral GC correlation with standards (*S*)-**4b**–(*S*)-**4f** prepared from (*S*)-3- to (*S*)-4-bromo-α-methylbenzyl alcohol **5a** and **5b**, respectively (see details in Section 2). (*S*)-**5a** and (*S*)-**5b** were obtained by enzymatic kinetic resolution of the appropriate mixture of *meta*- or *para*-bromo-α-methylbenzyl alcohol [(*R*,*S*)-**5a** or (*R*,*S*)-**5b**] mediated by Novozym 435 (CAL-B) in *tert*-butyl methyl ether as the solvent and vinyl acetate as the acetyl donor (Scheme 4).

(*S*)-**5a** and (*S*)-**5b** were separated from (*R*)-**6a** to (*R*)-**6b** by silica gel column chromatography. With (*S*)-**5a** and (*S*)-**5b** in hands, compounds (*S*)-**4b**–(*S*)-**4f** were prepared by treatment of the aromatic bromides with *t*-BuLi (three equivalents) at −78 °C and then with selenium powder at room temperature followed by alkylation with methyl iodide to



Scheme 4. Reagents and conditions: [(*R*,*S*)-**5a** or (*R*,*S*)-**5b** (5 mmol), *tert*-butyl methyl ether (15 ml), CAL-B (250 mg), vinyl acetate (0.25 ml). Reaction time: 3 and 1 h, respectively.



Scheme 5. Reagents and conditions: [1] (i) (*S*)-**5a** or (*S*)-**5b** (1 mmol), *t*-BuLi (3 mmol), N_2, THF, −76 °C, (ii) 0 °C, 30 min, then selenium powder, r.t. 3 h, (iii) MeI, r.t. 15 min; [2] (i) (*S*)-**5a** or (*S*)-**5b** (1 mmol), *t*-BuLi (3 mmol), N_2, THF, −76 °C, (ii) PhSeSePh, THF, −76 °C → r.t. 3 h.

obtain compounds **4b** and **4c**, or with diphenyl diselenide to obtain compounds **4e** and **4f** (Scheme 5).

4. Conclusion

In conclusion, we have successfully synthesized several organoseleno acetophenones and a number of these ketones was reduced with whole fungal cells originated from the Brazilian rain forest, affording the corresponding chiral arylselenoalcohols. The organoselenium compounds were compatible with the biocatalytic conditions employed. To the best of our knowledge, this is the first biotransformation of selenium ketones for synthetic purposes. Further studies with new fungal strains and the synthetic application of the chiral arylselenoalcohols are in progress in our research group.

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