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Published in: Tetrahedron

DOI: 10.1016/j.tet.2012.09.087

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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2012

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chemoenzymatic approaches to obtain chiral-centered selenium compounds

Patrícia B. Brondani, Nathalie M.A.F. Guilmoto, Hanna M. Dudek, Marco W. Fraaije, Leandro H. Andrade

Abstract

The synthesis of chiral-centered selenium compounds is presented. Enantioselective oxidations of these organoselenium compounds were performed using a wide range of biocatalysts, including Baeyer–Villiger monooxygenases, oxidoreductases-containing Aspergillus terreus, and lipase (Cal-B) in the presence of oxidants. Finally, efficient synthesis of enantiopure organoselenium compounds using a kinetic resolution approach mediated by Cal-B was achieved.

1. Introduction

Organoselenium compounds are very useful in organic synthesis, and they are regularly employed in the preparation of many organic compounds including natural products. They can be used in nucleophilic, electrophilic, and radical reactions due to their chemical properties and can be incorporated into a variety of substrates under mild conditions. Additionally, organoselenium compounds have shown biological activities like antioxidant, antitumor, antimicrobial, and antiviral activities. Including these activities, we can mention that chiral and achiral organoselenanes can inactivate protein tyrosine phosphatases (PTPs) and cysteine proteases (cathepsins S and V) thereby acting as enzyme inhibitors.

Very few studies on the enzymatic reactions of selenium compounds for synthetic purposes have been published to date. Enzymatic processes, due to their wide applications under mild conditions, are environmentally friendly reactions. From the synthetic organic point of view the enzyme-catalyzed reactions present a vast opportunity to selenium chemistry regarding selectivity, including regio-, enantio-, and chemoselectivity.

In the search for novel chiral-centered selenium compounds to be used in our studies for the development of protease inhibitors, we encountered a lack of synthetic methodologies to obtain such compounds. We here describe a study about the application of different chemoenzymatic approaches to obtain chiral-centered selenium compounds, see Fig. 1. An oxidative approach was applied to racemic organoselenium compounds in order to perform their kinetic resolution in enzyme-mediated reactions (Fig. 1a). The second approach involved kinetically resolved precursor 1-phenylethanol by lipases, and their transformation to the desired chiral organoselenium compounds using selenium chemistry (Fig. 1b).
2. Results and discussion

2.1. Synthesis of chiral-centered selenium compounds: kinetic resolution using a selenium oxidation approach

Initially, we synthesized phenylselenium derivatives (1–3) in which the selenium atom is attached to a phenyl and a 1-phenylethyl group. The protocol was based on the inversion of configuration of the phenylethanol derivatives by their reactions with phenylselenocyanide and tributylphosphine (Scheme 1).

Next, we performed the synthesis of benzylselenides derivatives (4–6). Initially, mesylated phenylethanol derivatives were prepared in situ from their corresponding alcohols and mesyl chloride (MsCl). Then, benzyl selenoate was prepared from benzyl bromide (BnBr), elemental selenium, and NaBH₄, which was then transferred to the mesylated derivatives to give the benzylselenides (4–6) (Scheme 1).

It is noteworthy that the addition of DMF is relevant for the success of the reaction. In fact, DMF improves the yield by solubilizing the selenolate anion, which is otherwise known to exist as boron complex possessing diminished nucleophilicity.

2.1.1. Oxidative kinetic resolution of chiral selenides mediated by BVMOs. Baeyer–Villiger monooxygenases (BVMOs) are flavin-containing and NAD(P)H-dependent enzymes that catalyze the incorporation of one oxygen atom into organic substrates. BVMOs usually mediate reactions with high enantio- and regioselectivity and are known to be excellent alternatives for performing the oxidation of aldehydes and ketones to their corresponding esters, the oxygenation of heteroatoms (sulfur, nitrogen, phosphorus, boron, and selenium) and even epoxidation reactions.

For the selenium oxidation approach using BVMOs, we first selected the phenylselenide derivative 1 as model substrate and PAMO as biocatalyst. This compound was selected to evaluate the influence of a phenyl group directly attached to the selenium atom on the oxidation reaction. We also employed a cofactor regenerating system with phosphite dehydrogenase (PTDH) and a sacrificial substrate (sodium phosphite). In this test we observed that the oxidation reaction occurred with good enantioselectivity (84% ee, Table 1, entry 1). Next, we increased the amount of phosphite in the reaction medium leading to higher enantioselectivity (98% ee, Table 1, entry 2). By applying the same reaction conditions to compound 4 (with the benzyl group attached to the selenium atom and no substituent in the aromatic ring) lower enantioselectivity was observed (14% ee, Table 1, entries 6 and 7).

As hydrogen peroxide can be produced by BVMOs, we also evaluated the use of catalase, an enzyme that degrades hydrogen peroxide into dioxygen and water. However, we observed several by-products even with no PAMO in the reaction medium (control reactions). These results could be due to the low purity of commercial catalase that can contain small quantities of other oxidoreductases.

In an effort to investigate other BVMOs, we performed reactions using M446G PAMO, CHMO, and HAPMO as biocatalysts. When M446G PAMO mediated the reactions, good conversions but low enantioselectivity were observed (Table 1, entries 3 and 8). When CHMO or HAPMO was employed, no reaction was observed (Table 1, entries 4, 5, 9, and 10).

By using appropriate reaction condition with PAMO, we decided to explore the oxidative kinetic resolution of several other selenides (2, 3, 5, and 6, Table 1, entries 11–14). In all cases, the selenium oxidation was observed but with low or no enantioselectivity.

In an ideal oxidative kinetic resolution of selenides, a β-elimination reaction should be observed with one enantiomer, which would produce RSeOH and styrene derivative (RCH=CH₂), and the other enantiomer would be untouched. After assigning the absolute configuration we concluded that, when the selenium oxidation occurs, the (R)-selenide undergoes the oxidation faster than the (S)-selenide.

2.1.2. Oxidative kinetic resolution of (RS)-phenylselenide 1 mediated by Aspergillus terreus. We have recently described that A. terreus
strains (URM 3571 and CCT 3320) produce oxidoreductases, which can mediate Baeyer–Villiger reactions, deracemization of several phenylethanol derivatives, and selenium oxidations.9d,15

We tested these fungal strains for the oxidation of the phenylethanol derivative 1, which was the best substrate in the previous reactions with BVMOs. The reactions were carried out using buffer, substrate, and fungal cells, which were incubated at 32 °C for 1, 3, and 5 days. All bio-oxidations were followed by a control reaction in the absence of fungal cells. However, even in 5 days, the phenylethanol derivative 1 remained in the racemic form. This lack of reactivity can be attributed to the presence of a steric hindrance near the selenium atom. As (RS)-phenylethanol derivative 1 has two bulky moieties surrounding the selenium atom, it can be hindering the oxidation mediated by enzymes from A. terreus. In a previous study with fungi and organoselenium alcohols, we observed a similar result, in which selenium oxidation is very slow or does not occur at all.16

2.1.3. Oxidative kinetic resolution of (RS)-phenylethanol derivative 1 mediated by Candida antarctica lipase B (CAL-B) in the presence of oxidants. Lipases are also known to catalyze oxidations in the presence of oxidizing species such as H₂O₂ or peracids, which constitutes an alternative method for bio-oxidations. They can be used in epoxidation reactions or, by lipase-mediated in situ formation of peroxy acids, even in Baeyer–Villiger reactions. Among the lipases studied so far, Novozym 435, a commercial preparation of lipase from C. antarctica, has been shown the best results in oxidative transformations becoming the most popular lipase for this purpose.16

In this context, we decided to explore the use of lipase in the presence of oxidants (H₂O₂, NaIO₄, m-CPBA) in order to perform enantioselective oxidation of organoselenium compounds. Therefore, the lipase-induced enantioselective oxidation reactions were carried out at 0 °C and room temperature in organic solvent. Control reactions in the absence of lipase and/or oxidant were also studied (Table 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>BVMO</th>
<th>Phosphate (mM)</th>
<th>Time (h)</th>
<th>Conv. (%)</th>
<th>ee (%)</th>
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<td>1</td>
<td>1</td>
<td>PAMO</td>
<td>20</td>
<td>5</td>
<td>52</td>
<td>84</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>PAMO</td>
<td>50</td>
<td>5</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>M446G</td>
<td>24</td>
<td>5</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>HAPMO</td>
<td>50</td>
<td>24</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>CHMO</td>
<td>50</td>
<td>24</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>PAMO</td>
<td>20</td>
<td>5</td>
<td>53</td>
<td>(−)²</td>
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<tr>
<td>7</td>
<td>4</td>
<td>PAMO</td>
<td>50</td>
<td>5</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>M446G</td>
<td>50</td>
<td>5</td>
<td>51</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>HAPMO</td>
<td>50</td>
<td>24</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>CHMO</td>
<td>50</td>
<td>24</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
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<td>2</td>
<td>PAMO</td>
<td>50</td>
<td>8</td>
<td>51</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>PAMO</td>
<td>50</td>
<td>8</td>
<td>54</td>
<td>(−)²</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>PAMO</td>
<td>50</td>
<td>5</td>
<td>49</td>
<td>(−)²</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>PAMO</td>
<td>50</td>
<td>5</td>
<td>47</td>
<td>49</td>
</tr>
</tbody>
</table>

a Tris buffer 50 mM pH 7.5, DMSO (1%), substrate (10 mM), phosphate (20 or 50 mM), PTDH (1 μM), NADPH (0.2 mM), and BVMO (8 μM).
b Conv. = conversion based on selenide consumption using HPLC analysis.
c Enantiomeric excess measured by HPLC.
d No reaction was observed.
e No ee was observed.

Table 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidant</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Conv. (%)</th>
<th>ee (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂O₂</td>
<td>Hexane</td>
<td>20</td>
<td>0</td>
<td>90</td>
<td>(−)²</td>
</tr>
<tr>
<td>2</td>
<td>H₂O₂</td>
<td>Hexane</td>
<td>25</td>
<td>0.5</td>
<td>100</td>
<td>(−)²</td>
</tr>
<tr>
<td>3</td>
<td>NaIO₄</td>
<td>Hexane</td>
<td>0</td>
<td>2</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
<td>4</td>
<td>NaIO₄</td>
<td>Hexane/MeOH (9:1)</td>
<td>25</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>NaIO₄</td>
<td>Hexane/MeOH (1:1)</td>
<td>25</td>
<td>2</td>
<td>3</td>
<td>(−)²</td>
</tr>
<tr>
<td>6</td>
<td>NaIO₄</td>
<td>Hexane/TEGAc (9:1)</td>
<td>25</td>
<td>2</td>
<td>(−)¹</td>
<td>(−)²</td>
</tr>
<tr>
<td>7</td>
<td>NaIO₄</td>
<td>Hexane/MeOH (9:1)</td>
<td>25</td>
<td>15</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>m-CPBA</td>
<td>Hexane</td>
<td>25</td>
<td>2</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>9</td>
<td>m-CPBA</td>
<td>Hexane/MeOH (9:1)</td>
<td>25</td>
<td>2</td>
<td>100</td>
<td>(−)²</td>
</tr>
<tr>
<td>10</td>
<td>m-CPBA</td>
<td>Hexane/TEGAc (9:1)</td>
<td>25</td>
<td>2</td>
<td>90</td>
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<td>m-CPBA</td>
<td>Toluene</td>
<td>25</td>
<td>2</td>
<td>100</td>
<td>(−)²</td>
</tr>
</tbody>
</table>

a Substrate (0.25 mM), solvent (1 mL), CAL-B (20 mg), oxidant (3 equiv), 0 or 25 °C, 2–15 h.
b Conv. = conversion based on selenide consumption using HPLC analysis.
c Enantiomeric excess measured by HPLC.
d No reaction was observed.
e No ee was observed.
f 2 equiv.

In most cases, no enantioselectivity (Table 2, entries 1, 2, 7, 9, 10, and 11) or no reaction was observed (Table 2, entries 3 and 6). In control reactions, we observed oxidation reaction without lipase, which can be the reason for the low enantioselectivity. However, the best system used was m-CPBA in n-hexane at room temperature (Table 2, entry 8), which resulted in enantioselective oxidation of (R)-1. In fact, these results demonstrate the potential of peracids and lipase for enantioselective selenium oxidation, without precedent until now.
2.2. Synthesis of chiral-centered selenium compounds: kinetic resolution using a transesterification approach

Another approach was evaluated to obtain both enantiomers of chiral selenides 1–6. Kinetic resolution of phenylethanol derivatives through a lipase-catalyzed transesterification reaction was the protocol of choice. This process was performed with vinyl acetate as acyl donor and Cal-B as biocatalyst (Scheme 2).17 We obtained the corresponding (R)-acetate and the untouched (S)-alcohol in high ee, >99%.

After column chromatography and ester hydrolysis, both enantiomerically pure phenylethanol derivatives were achieved. Then, these alcohols were transformed into the chiral selenides 1–6 using the same protocol described in the Section 2.1. As the insertion of the selenium moiety involved a Mitsunobu reaction or a nucleophilic substitution reaction with a selenolate species, we noticed that an (S)-alcohol will provide an (R)-selenide while an (R)-alcohol will provide an (S)-selenide.

3. Conclusion

Among the BVMOs tested, PAMO showed the best results for oxidation of chiral selenides. PAMO was able to oxidize phenylselenium-containing compound with no substituent in the aromatic ring with great enantioselectivity (98% ee). The mutant M446G showed a similar substrate acceptance, but it displayed lower enantioselectivity. The BVMOs, HAPMO, and CHMO were not able to oxidize any of the tested substrates. When whole cells of A. terreus were used, no significant selenium oxidation was observed. By using another approach, Cal-B in the presence of m-CPBA as oxidant species, enantioselective conversion was observed (45% ee). By applying a different approach, lipase-catalyzed transesterification reactions provided us the enantiomerically pure alcohols, which were transformed into the desired chiral benzyl and phenylselenides.

4. Experimental

4.1. General

Recombinant phenylacetone monooxygenase (PAMO) from Thermobifida fusca, M446G PAMO mutant, 4-hydroxyacetoephene monooxygenase (HAPMO) from Pseudomonas fluorescens, and cyclohexanone monooxygenase (CHMO) from Acinetobacter sp. were overexpressed and purified as previously described.18 Phosphite dehydrogenase was obtained as previously described.19 Both sodium phosphate and NADPH were purchased from Sigma–Aldrich. Gas chromatography (GC) analyses were performed on a Shimadzu GC-17A instrument equipped with a flame ionization detector. High performance liquid chromatography (HPLC) analyses were performed on a Shimadzu LC-10AD or Shimadzu SPD-M1OA liquid chromatograph equipped with a variable wavelength UV detector (deuterium lamp 190–600 nm). Sterile materials were used to perform the experiments involving microorganisms. A laminar flow cabinet Fisher Hamilton (Class II Biological Safety Cabinet) was used to handle microorganisms.

4.2. General procedure for synthesis of chiral organoselenium compounds (1–3)

The enantiomerically pure forms of 1-phenylethanol derivatives were prepared by lipase-catalyzed transesterification reactions of their racemic forms with vinyl acetate according to the methodology described in literature.17 Each enantiomer of organoselenium compounds (1–3) was synthesized from 1-phenylethanol derivatives by their reactions with phenylselenocyanide and tributylphosphate as described in literature.10

4.2.1. Phenyl(1-phenylethyl)selane (1). Yellow oil (82%), 1H NMR (200 MHz, CDCl3): δ 1.73 (d, J=7.0 Hz, 3H), 4.43 (q, J=7.0 Hz, 2H) 7.05–7.60 (m, 10H). 13C NMR (50 MHz, CDCl3): δ 22.1, 42.4, 127.1, 127.7, 128.3, 128.8, 129.1, 131.4, 135.4, 141.6, 143.5. LRMS (El) m/z (%): 262 (M+), 156 (8), 105 (100), 79 (10), 78 (9), 77 (14), 51 (7). FT-IR (film, cm−1) νmax: 3057, 2961, 2918, 1576, 1474, 1436, 1021, 769, 691. [α]D25 +87 (c 1.0, CH2Cl2) for (S)-enantomer (ee>99%) and [α]D25 −70 (c 1.0, CH2Cl2) for (R)-enantomer (ee>99%).

The enantiomeric excess was analyzed by HPLC equipped with a Chiralcel OJ-H column. Eluent—mixture of n-heptane and isopropanol or n-hexane and iso-propanol (99:1). Flow rate=0.5 mL/min; UV detector=254 nm; retention times: ts=21.55 min (S) and 31.88 min (R) or ts=16.01 min (S) and 31.13 min (R).
4.2.2. Phenyl-[1-(4-tolylyl)]selane (2). Yellow oil (87%), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 1.73\) (d, \(J=7.0\) Hz, 3H), 2.30 (s, 3H), 4.44 (q, \(J=7.2\) Hz, 1H), 7.04–7.63 (m, 9H), \(^13\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 21.1, 22.3, 42.2, 127.0, 127.7, 129.1, 131.4, 135.2, 136.6, 140.5, 144.5\). 

4.2.3. General procedure for synthesis of chiral organoselenium compounds (4–6)\(^{11}\)

To a 50 mL round-bottomed flask (I) were added the enantio-
merically pure forms of phenylethyl derivative (1 mmol, pre-
pared as described in Section 4.2), CH\(_2\)Cl\(_2\) (50 mL), and DMAP
(2 mmol, 0.244 g). After 15 min, MsCl (1.5 mmol, 0.12 mL) was
added slowly. The reaction mixture was stirred overnight at
room temperature.

In a second 50 mL round-bottomed flask (II) were added sele-
nium (1 mmol, 0.080 g), Et\(_2\)O (3 mL), DMF (2 mL), and NaBH\(_4\)
(1 mmol, 0.037 g). The mixture was stirred for 15 min and then
BnBr (1 mmol, 0.119 mL) was added. After stirring 3 h at room
temperature, additional amount of NaBH\(_4\) (1.5 mmol, 0.055 g) was
added. The reaction mixture was stirred over more 20 min. The
content of flask (I) was added slowly to the flask (II) and the
resulting mixture was stirred at room temperature for 3 h.

After that, the ethanol/DMF was removed in vacuum, and
H\(_2\)O (5 mL) was added into the crude material. The resulting
aqueous mixture was extracted with Et\(_2\)O (2 × 5 mL) and the
organic extract was dried over MgSO\(_4\). The solvent was removed
in vacuum and the mixture was purified by flash column chroma-
tography (pentane as eluent) providing the pure product
(50–75% yield).

4.3.1. Benzyl-[1-(phenethyl)]selane (4). Yellow oil (65%), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 1.69\) (d, \(J=7.0\) Hz, 3H), 3.70 (s, 2H), 4.07 (q, \(J=7.0\) Hz, 1H), 7.19–7.28 (m, 10H), \(^13\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 22.5, 37.7, 53.0, 126.6, 127.3, 128.3, 128.8, 139.0, 144.1\). 

4.3.2. Benzyl-[1-(4-tolylyl)]selane (5). Yellow oil (50%), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 1.69\) (d, \(J=7.0\) Hz, 3H), 2.31 (s, 3H), 3.80 (s, 2H), 3.99 (q, \(J=7.0\) Hz, 1H), 7.12–7.25 (m, 9H), \(^13\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 21.1, 32.5, 41.0, 52.9, 127.1, 128.3, 128.9, 132.9, 137.0, 139.1, 140.0, 144.8\). 

LRMS (EI) m/z (\%): 290 (M\(^+\), 3), 207 (4), 182 (3), 120 (11), 99 (10), 117 (14), 91 (55), 77 (8), 65 (10), 51 (6). FT-IR (film, cm\(^{-1}\)) \(\nu_{max}\): 3024, 2917, 1655, 1510, 1429, 829, 715, 695, 591, 492. \([\alpha]^{D}\) \(+63\) (c 1.0, CH\(_2\)Cl\(_2\)) for (S)-enantio-
mer (ee-99%) and \([\alpha]^{D}\) \(-76\) (c 1.0, CH\(_2\)Cl\(_2\)) for (R)-enantio-
mer (ee-99%).

The enantiomeric excess was analyzed by HPLC equipped with a Chiracel \(\alpha\)-OH column. Eluent=–n-heptane. Flow rate=0.5 mL/min; UV detector=220 nm; retention times: \(t\_R=37.74\) min (S) and 41.54 min (R).

4.3.3. Benzyl-[1-(4-fluorophenyl)]ethyl-selane (6). Yellow oil (43%), \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta 1.64\) (d, \(J=7.2\) Hz, 3H), 3.69 (s, 2H), 3.80 (q, \(J=7.2\) Hz, 1H), 6.97–7.2 (m, 9H), 7.38–7.43 (m, 2H), \(^13\)C NMR (50 MHz, CDCl\(_3\)): \(\delta 22.9, 41.6, 114.8, 115.2, 127.9, 128.6, 128.8, 135.6, 161.6\) (d, \(J=64.4\) Hz). 

LRMS (EI) m/z (%): 280 (M\(^+\), 4), 262 (4), 105 (100), 91 (41), 77 (9), 65 (7), 51 (4). FT-IR (film, cm\(^{-1}\)) \(\nu_{max}\): 3070, 2965, 2864, 1602, 1508, 1223, 834, 741, 692, 533, 469. \([\alpha]^{D}\) \(+180\) (c 1.0, CH\(_2\)Cl\(_2\)) for (S)-enantio-
mer (ee-99%) and \([\alpha]^{D}\) \(+168\) (c 1.0, CH\(_2\)Cl\(_2\)) for (R)-enantio-
mer (ee-99%).

The enantiomeric excess was analyzed by HPLC equipped with a Chiracel \(\alpha\)-OH column. Eluent=–n-heptane. Flow rate=0.5 mL/min; UV detector=254 nm; retention times: \(t\_R=27.88\) min (S) and 32.61 min (R).

4.4. General procedure for synthesis of chiral organoselenium compounds (4–6)\(^{11}\)

To a 2 mL Eppendorf\(^{\circ}\) tube containing the solution of the racemic selenide compounds 1–6 (1 M in DMSO, 5 μL) were added Tris/HCl buffer at pH 7.5 (50 mM, 440 μL), phosphate solution (500 mM, 20 or 50 μL), NADPH (100 mM, 10 μL), PTDH (100 μM, 5 μL), and BVMO (100 μM, 20 or 40 μL). Reaction mixture was shaken at 200 rpm and 30 °C (PAMO and M446G PAMO mutant) or 150 rpm and 25 °C (HAPMO and CHMO) for the appropriate time (Table 1). The reaction mixture was extracted with EtOAc (3 × 0.5 mL) and the organic extract was dried over Na\(_2\)SO\(_4\) and analyzed by HPLC. Control experiments in absence of enzyme were performed for all substrates, and no selenium oxidation was observed.

4.5. General procedure for selenium oxidation mediated by A. terreus URM 3571 and CCT 3320

Erlenmeyers flasks (250 mL) containing 100 mL of culture medium (malt extract, 20 g/L) were inoculated with Aspergillus spores. Growth was carried out in an orbital shaker (160 rpm) at 32 °C for 5 days. The fungal cells were harvested by filtration.

The biotransformation was performed by re-suspending the fungal cells (1 g) with Tris/HCl (50 mM, pH 7.5, 50 mL) in an Erlenmeyer flask (125 mL). To this cell suspension, sedanene (1) (0.1 mmol) was added and incubated in an orbital shaker (160 rpm) at 32 °C for 1–5 days. Then reaction mixture was filtered and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The organic extract was dried over Na\(_2\)SO\(_4\) and analyzed by chiral HPLC. Control experiments in absence of fungi were performed.

4.6. General procedure for selenium oxidation reactions mediated by CAL-B

To a flask (2 mL, Eppendorf\(^{\circ}\) tube) containing the sedanene (1) (0.25 mmol, 65 μg) were added the organic solvent (1 mL) and CAL-B (20 mg). After 5 min, the oxidant (2 or 3 equiv, 0.5 or
0.75 mmol) was added. Reaction mixture was shaken at room temperature or 0 °C for the appropriate time (Table 2). The enzyme was filtered and the solution was washed with distilled water and the aqueous layer was extracted with n-hexane (2×1 mL). The organic extract was dried over Na2SO4 and analyzed by HPLC. Control experiments in absence of enzyme and/or oxidant were performed.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.09.087.

References and notes