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ORIGINAL RESEARCH PAPER

Stereoselective yeast-mediated reduction of *trans*-5-(1′-oxo-3′-methylbutyl)-3-methyldihydrofuran-2-one: production of chiral intermediates for the synthesis of β-secretase inhibitors

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Abstract
Objective To investigate the usefulness of yeast strains to the production of chiral hydroxylactones, important synthons in the synthesis of β-secretase inhibitors.
Results Strains *Rhodotorula glutinis* KCh 242, *Rhodotorula rubra* KCh 82, *Rhodotorula marina* KCh 77 and *Saccharomyces cerevisiae* KCh 464 reduced racemic *trans*-5-(1′-oxo-3′-methylbutyl)-3-methyldihydrofuran-2-one (**4**) according to the Prelog's rule to afford preferentially the stereoisomers with *S* configurations at C-1′. *R. marina* KCh 77 and *R. glutinis* KCh 242 exhibited selectivity towards 3*S*, 5*R* enantiomer of the substrate, whereas *R. rubra* KCh 82 and *S. cerevisiae* KCh 464 preferred the reduction of its antipode. The highest yield of 3*R*,5*S*,1′*S*-hydroxylactone **3c** (78 %) was obtained with *R. rubra* KCh 82.
Conclusions Different stereoisomers of *trans*-5-(1′-hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one and its 5-substituted analogues are produced as important intermediates in the synthesis of drugs for the therapy of Alzheimer's disease.

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Introduction

Stereospecific reduction of a carbonyl group leading to enantiomerically pure alcohols is catalyzed by both the isolated enzymes and whole-cell systems of yeasts, filamentous fungi or plants (Goldberg et al. 2007a, b; Homann et al. 2004; Mączka and Mironowicz 2004). The majority of bioreductions are catalyzed by dehydrogenases that follow Prelog's rule delivering the hydride ion from the *re*-face of a prochiral ketone which, in most cases, leads to (*S*)-alcohols. Bioreductions have a wide application in the production of chiral intermediates or final products used in pharmacy or agrochemistry (Rodrigues et al. 2004; Fardelone et al. 2011). Among them, hydroxylactones represent one of the most common group of biologically active compounds with cytotoxic, antifeedant and antifungal properties (Murcia et al. 2010; Gliszczyńska et al. 2011; Grudniewska et al. 2015). They can be easily accessed from the corresponding oxolactones by the reduction of a carbonyl group (Ribeiro et al. 2006, 2008).

The synthesis of β-secretase inhibitors that may be used in the therapy of Alzheimer's disease has been presented (Sussman et al. 2011). The starting material was D-glucono-1,5-lactone which was transformed to

the key chiral intermediate hydroxylactone, **I**. The latter was treated with methanesulfonylchloride to afford corresponding mesylate which was subsequently reacted with sodium azide. Stereoselective alkylation of lactone ring at C-2 with lithium diisopropylamide followed by treating with methyl iodide yielded azidolactone, **II**, (Scheme 1).

In this paper we propose a modification of this synthetic pathway in which the intermediate is chiral α-methylhydroxylactone, **3c**, obtained by yeast-mediated reduction of α-methyl-γ-(1-oxo-3-methylbutyl)-γ-butyrolactone (**4**). This approach was inspired by the results of Ribeiro et al. (2006, 2008). They used different fungal and yeast strains (*Aspergillus niger*, *Geotrichum candidum* and *Hansenula* sp.) for the stereospecific reduction of the carbonyl group in α-acetyl-γ-butyrolactones to produce chiral hydroxylactones used, that is as GHB receptor ligands.

Materials and methods

Chemical and biological materials

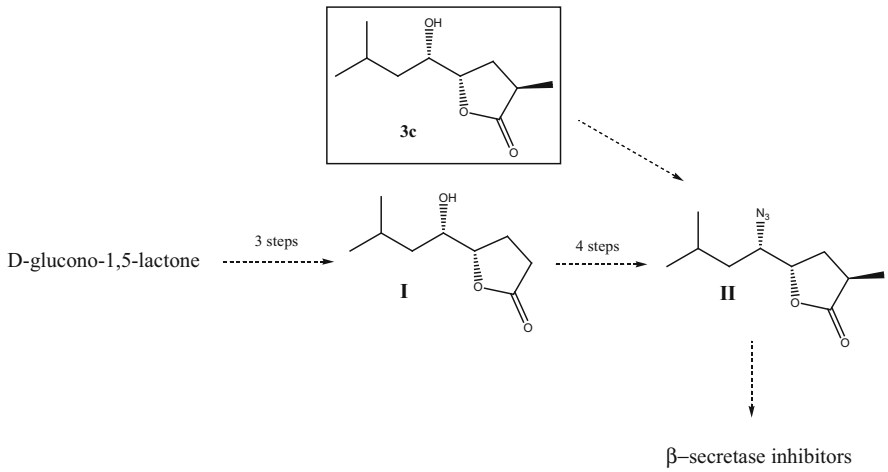
Racemic 5-methylhex-1-en-2-ol (**1**) was obtained by Chojnacka et al. (2007) as the product of reaction of acrolein with isobutyl magnesium bromide. Triethyl orthopropionate (97 %) and *m*-chloroperbenzoic acid (*m*-CPBA, 77 %) were purchased from Sigma-Aldrich. Other chemicals and reagents were of analytical grade. Yeast strains: *Rhodotorula glutinis* KCh 242, *Rhodotorula rubra* KCh 82, *Rhodotorula marina* KCh 77 and *Saccharomyces cerevisiae* KCh 464 came from the collection of the Department of Chemistry of Wrocław University of Environmental and Life Sciences. The strains were cultivated on a Sabouraud agar consisting of 5 g Aaminobac l^{-1}, 5 g peptone K l^{-1}, 40 g glucose l^{-1}, and 15 g agar l^{-1} at 28 °C, pH 5.5.

Analysis

Gas chromatography was carried out using HP-5 column (cross-linked methyl silicone gum, 30 m × 0.32 mm × 0.25 μm) and an FID detector. The enantiomeric compositions of bioreduction products were determined on a Varian CP Chirasil-DEX CB column (25 m × 0.25 mm × 0.25 μm) using the program: injector 250 °C, detector (FID) 280 °C, column: 80–150 °C (25 °C min^{-1}), 150 °C (25 min hold), 150–200 °C (30 °C min^{-1}), 200 °C (2 min hold). Spectroscopic measurements were carried out for $CDCl_3$ solutions on a Bruker Avance AMX 300 spectrometer. IR spectra were determined using Mattson IR 300 Thermo Nicolet spectrophotometer. Optical rotations were measured in CH_2Cl_2 on a Jasco P-2000-Na digital polarimeter with intelligent Remote Module (iRM) controller.

Synthesis of racemic (*E*)-ethyl 2,7-dimethylocta-4-enoate (**2**)

The alcohol **1** (2.1 g, 18 mmol) was subjected to the reaction with triethyl orthopropionate according to the standard protocol of Johnson–Claisen rearrangement. The crude product was purified by silica gel column



Scheme 1 Fragment of the synthesis of β-secretase inhibitors (Sussman et al. 2011) and hydroxylactone **3c** as an alternative intermediate in the synthetic pathway

chromatography (hexane/acetone, 19:1, v/v) to give ester **2** (3.3 g, 90 % isolated yield): ^{1}H NMR δ: 0.85 (d, J = 6.6 Hz, 6H, ($C\underline{H}_3$)$_2$CH–), 1.13 (d, J = 7.0 Hz, 3H, CH_3-2), 1.24 (t, J = 7.1 Hz, 3H, –OCH$_2$C$\underline{H}_3$), 1.57 (m, 1H, (CH_3)$_2$C$\underline{H}$–), 1.86 (m, 2H, CH_2-6), 2.12, 2.34 (two dt, J = 13.9, 7.0 Hz, 2H, CH_2-3), 2.46 (m, H-2), 4.11 (q, J = 7.1 Hz, 2H, –OC$\underline{H}_2$CH$_3$), 5.32 (dt, J = 15.2, 7.0 Hz, 1H, H-4), 5.44 (dt, J = 15.2, 7.0 Hz, 1H, H-5), ^{13}C NMR δ: 14.24 (–OCH$_2$C$\underline{H}_3$), 16.50 (CH_3-2), 22.20 (($\underline{C}H_3$)$_2$CH–), 28.34 ((CH_3)$_2$-C$\underline{H}$–), 36.76 (C-3), 39.78 (C-2), 41.91 (C-6), 60.11 (–O$\underline{C}$H$_2$CH$_3$), 127.82 (C-4), 131.74 (C-5), 176.31 (C-1), IR (film, cm^{-1}): 1737, 1453, 1375, 1177, 970.

Synthesis of hydroxylactones **3a,b**

The ester **2** (3 g, 15 mmol) was epoxidized by *m*-CPBA in a standard manner. The resulting crude mixture of epoxyesters was stirred in the solution of THF/H_2O/$HClO_4$ (10:5:0.5 by vol.) for 72 h. The products were extracted with diethyl ether and the extracts were washed with $NaHCO_3$, brine and dried. Pure lactones **3a** and **3b** were isolated by column chromatography (hexane/acetone/ethyl acetate/2-propanol/methylene chloride/diethyl ether, 45:0.2:0.2:3:0.2:0.2 by vol.).

*(±)-cis-5-(1′-Hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one (**3a**)*

Yield 1.33 g (45 %), ^{1}H NMR δ: 0.93, 0.96 (two d, J = 6.6 Hz, 6H, ($C\underline{H}_3$)$_2$CH–), 1.16 (ddd, J = 13.8, 9.0, 3.3 Hz, 1H, one of CH_2-2′), 1.27 (d, J = 7.2 Hz, 3H, CH_3-3), 1.37 (ddd, J = 13.8, 9.9, 5.1 Hz, 1H, one of CH_2-2′), 1.80-1.87 (m, 2H, (CH_3)$_2$CH– and OH), 1.93 (td, J = 12.0, 10.2 Hz, 1H, one of CH_2-4), 2.28 (ddd, J = 12.0, 9.0, 6.0 Hz, 1H, one of CH_2-4), 2.69 (ddq, J = 12.0, 9.0, 7.2 Hz, 1H, H-3), 4.05 (dt, J = 9.9, 3.3 Hz, 1H, H-1′), 4.28 (ddd, J = 10.2, 6.0, 3.3 Hz, 1H, H-5), ^{13}C NMR δ: 15.03 (CH_3-3), 21.76, 23.46 ($\underline{C}H_3$)$_2$CH–), 24.38 ((CH_3)$_2$C$\underline{H}$–), 29.51 (C-4), 35.46 (C-3), 40.32 (C-2′), 68.37 (C-1′), 80.81 (C-5), 179.47 (C-2), IR (film, cm^{-1}): 3448, 1768, 1456, 1379, 1201, 1017.

*(±)-trans-5-(1′-Hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one (**3b**)*

Yield 1.48 g (53 %), ^{1}H NMR δ: 0.92, 0.96 (two d, J = 6.6 Hz, 6H, ($C\underline{H}_3$)$_2$CH–), 1.16 (ddd, J = 13.8, 9.0, 3.3 Hz, 1H, one of CH_2-2′), 1.27 (d, J = 7.8 Hz, 3H, CH_3-3), 1.39 (ddd, J = 13.8, 10.2, 5.4 Hz, 1H, one of CH_2-2′), 1.77–1.84 (m, 2H, (CH_3)$_2$C$\underline{H}$– and one of CH_2-4), 2.19 (s, 1H, –OH), 2.48 (ddd, J = 12.6, 9.6, 4.8 Hz, 1H, one of CH_2-4), 2.80 (dq, J = 9.6, 7.8 Hz, 1H, H-3), 3.99 (dt, J = 10.2, 3.3 Hz, 1H, H-1′), 4.37 (ddd, J = 8.4, 4.8, 3.3 Hz, 1H, H-5), ^{13}C NMR δ: 16.48 (CH_3-3), 21.76, 23.48 ($\underline{C}H_3$)$_2$CH–), 24.42 ((CH_3)$_2$C$\underline{H}$–), 29.36 (C-4), 34.54 (C-3), 40.94 (C-2′), 69.87 (C-1′), 80.92 (C-5), 180.61 (C-2), IR (film, cm^{-1}): 3451 (s), 1765, 1456, 1381, 1204, 1014.

Oxidation of hydroxylactone **3b**

Following a standard procedure, hydroxylactone **3b** (1.4 g, 7.5 mmol) was oxidized by a solution of $Na_2Cr_2O_7$ (1.96 g, 7.5 mmol) in H_2SO_4 to afford 1.27 g (92 % yield) of (±)-*trans*-5-(1′-oxo-3′-methylbutyl)-3-methyldihydrofuran-2-one (**4**): ^{1}H NMR δ: 0.92, 0.93 (two d, J = 6.6 Hz, 6H, ($C\underline{H}_3$)$_2$CH–), 1.28 (d, J = 6.9 Hz, 3H, CH_3-3), 2.04–2.24 (two m, 2H, one of CH_2-4 and (CH_3)$_2$C$\underline{H}$–), 2.40 (dd, J = 17.1, 6.6 Hz, 1H, one of CH_2-2′), 2.50 (dd, J = 17.1, 6.9 Hz, 1H, one of CH_2-2′), 2.49-2.62 (m, one of CH_2-4 and H-3), 4.77 (dd, J = 9.3, 3.3 Hz, 1H, H-5), ^{13}C NMR δ: 15.43 (CH_3-3), 22.45 (($\underline{C}H_3$)$_2$CH–), 23.86 ((CH_3)$_2$C$\underline{H}$–), 32.49 (C-4), 32.93 (C-3), 47.77 (C-2′), 79.73 (C-5), 178.81 (C-2), 207.30 (C-1′), IR (film, cm^{-1}): 1779, 1722, 1456, 1370, 1269, 1162, 1026.

Isolation of products obtained after biotransformation of oxolactone **4** catalyzed by *R. glutinis* KCh 242

After 7 days of transformation (Scheme 3) biomass was centrifuged and the products were extracted from the liquid medium with CH_2Cl_2 and separated by column chromatography (hexane/acetone/ethyl acetate/2-propanol/methylene chloride/diethyl ether, 60:0.2:0.2:3:0.2:0.2 by vol.) to afford pure products.

*(−)-trans-(3R,5S,1′S)-5-(1′-Hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one (**3c**)*

Yield 0.069 g (43 %), *e.e.* = 48 %, $[\alpha]_D^{20} = -2.4$ (*c* 3.9, CH_2Cl_2); ^{1}H NMR δ: 0.92, 0.95 (two d, J = 6.9 Hz, 6H, ($C\underline{H}_3$)$_2$CH–), 1.24 (m, 1H, one of CH_2-2′), 1.28 (d, J = 7.5 Hz, 3H, CH_3-3), 1.54 (ddd, J = 13.8, 9.6, 4.8 Hz, 1H, one of CH_2-2′), 1.77–1.88

(two m, 2H, $(CH_3)_2CH$– and OH), 1.95 (ddd, $J = 12.9$, 9.3, 7.5 Hz, 1H, one of CH_2-4), 2.33 (ddd, $J = 12.9$, 9.3, 4.8 Hz, 1H, one of CH_2-4), 2.80 (dq, $J = 9.3$, 7.5 Hz, 1H, H-3), 3.65 (dt, $J = 9.6$, 4.8 Hz, 1H, H-1′), 4.36 (dt, $J = 9.3$, 4.8 Hz, 1H, H-5), ^{13}C NMR δ: 15.03 (CH_3-3), 21.76, 23.46 ($CH_3)_2CH$–), 24.38 (($CH_3)_2CH$–), 29.51 (C-4), 35.46 (C-3), 40.32 (C-2′), 68.37 (C-1′), 80.81 (C-5), 179.47 (C-2), IR (film, cm^{-1}): 3448, 1768, 1456, 1379, 1201, 1017.

*(−)-trans-(3S,5R,1′S)-5-(1′-Hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one (**3d**)*

Yield 0.085 g (52 %), *e.e.* = 90 %, $[\alpha]_D^{20} = -6.2$ (*c* 2.6, CH_2Cl_2); spectroscopic data of in accordance with those of racemic lactone **3b**.

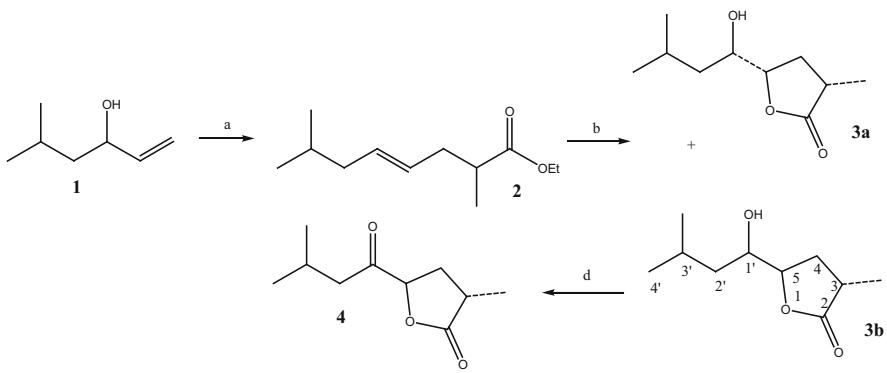
Results and discussion

The synthetic pathway leading to opically active isomers of *trans*-5-(1′-hydroxy-3′-methylbutyl)-3-methyldihydrofuran-2-one (Scheme 2) started with the Johnson–Claisen rearrangement of racemic allyl alcohol **1** to γ,δ-unsaturated ester **2** in the reaction of **1** with triethyl orthopropionate. Ester **2** was further transformed in two-step procedure into the mixture of diastereoisomeric hydroxylactones **3a** and **3b** involving epoxidation of double bond with *m*-CPBA followed by acidic lactonization. Isomer *trans* (**3b**) was separated from the products mixture by column chromatography and oxidized to the corresponding oxolactone **4** with sodium dichromate.

Lactone **4** was subjected to biotransformation using four yeast strains from our culture collection. They are known for their ability to reduce chalcones, prophiophenones and flavanones (Janeczko et al. 2013, 2014; Janeczko and Kostrzewa-Susłow 2014). Two diastereoisomeric hydroxylactones (**3c**, **3d**) were formed as a result of the reduction of the carbonyl group at C-1′. The substrate conversion and a composition of the products mixture depended on a strain. *R. marina* KCh 77 the reduced substrate only to the isomer **3d** which started to accumulate after 4 days to reach 52 % on the 10th day (Fig. 1c). *S. cerevisiae* KCh 464 preferred the reduction of opposite enantiomer of substrate and after 10 days the conversion reached 30 % and isomer **3c** made up 25 % of the products mixture (Fig. 1d). In the experiments with *R. rubra* KCh 82 and *R. glutinis* KCh 242 (Fig. 1a, b), the complete conversions of substrate **4** were achieved after 10 days. In both cases until 4th day isomer **3c** predominated but in the case of *R. glutinis* KCh 242 from that time the opposite enantiomer of substrate was reduced faster and in 7th day of transformation hydroxylactone **3d** was the major product (Fig. 1a). For *R. rubra* KCh 82 the tendency was maintained and after 10 days hydroxylactone **3c** significantly prevailed over isomer **3d** (78 vs. 22 %) (Fig. 1b).

Since biotransformation of oxolactone **4** with *R. glutinis* KCh 242 afforded both hydroxylactones **3c** and **3d** in significant yields, in this experiment they were isolated, purified and identified (Scheme 3).

Spectroscopic measurements confirmed the *S* configuration at C-1′ in both products as a result of stereospecific reduction of a carbonyl group following the Prelog's rule. The same stereochemical preference by the strains of *R. glutinis* has been demonstrated in the course of reduction of different phenones (Homann et al. 2004; Zilbeyaz and Kurbanoglu 2010) and also



Scheme 2 Reagents: (*a*) $EtC(OEt)_3$, H^+, 140 °C (*b*) *m*-CPBA, CH_2Cl_2, rt, 24 h (*c*) $THF/H_2O/HClO_4$, rt, 72 h (*d*) $Na_2Cr_2O_7/H_2SO_4$, Et_2O, rt, 48 h

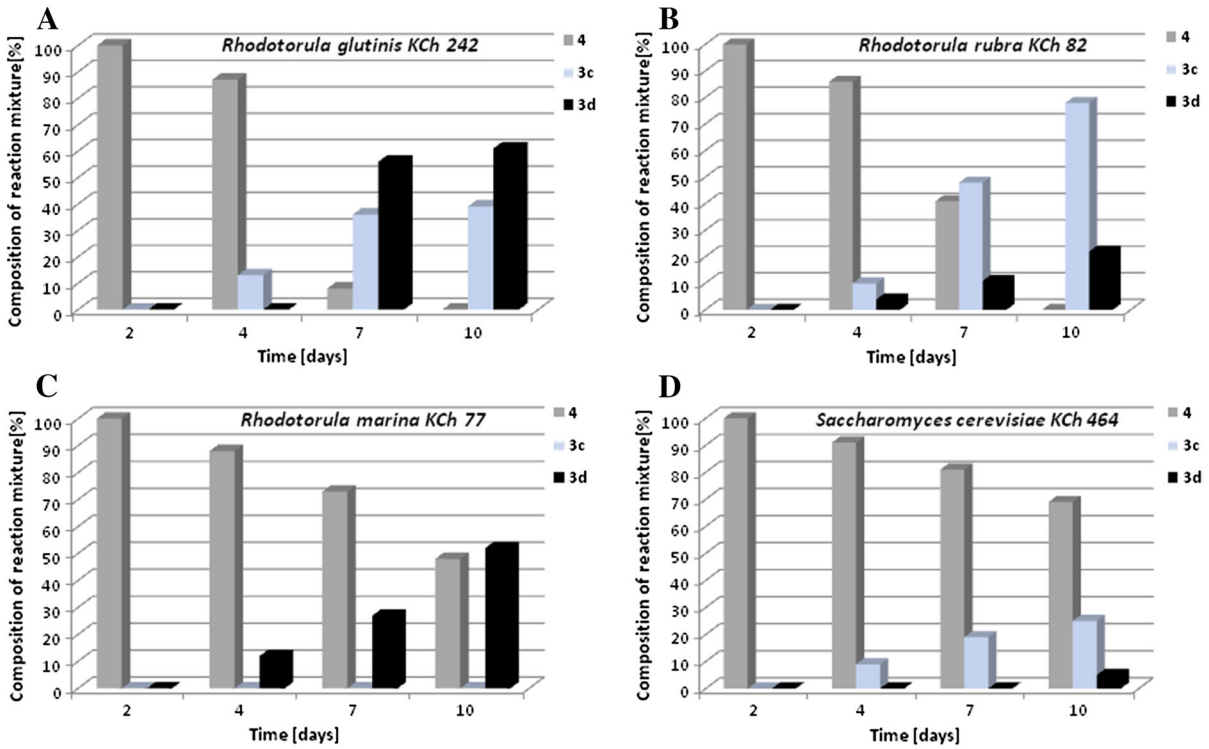
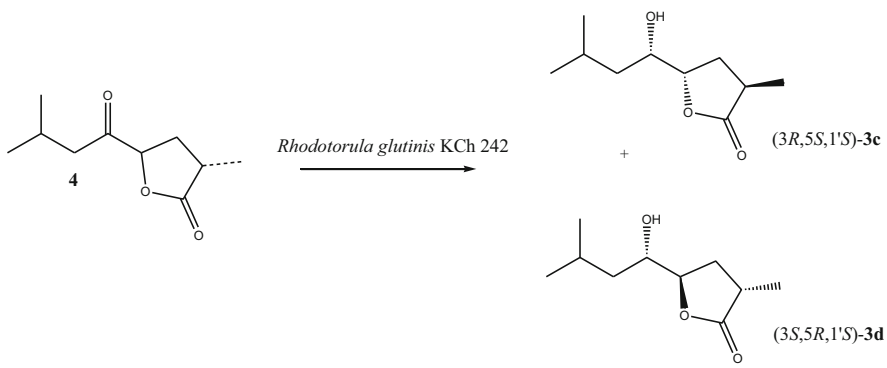


Fig. 1 Time course of biotransformation of oxolactone **4** in yeast cultures under the following conditions: Yeasts were cultivated for 3 days with shaking (150 rpm) at 25 °C in 50 ml medium (glucose 30 g l^{-1}, peptone 10 g l^{-1}), followed by addition of lactone **4** (in acetone 10 mg ml^{-1}). For the analysis of reaction progress the samples were extracted with CH_2Cl_2, the extracts were dried over $MgSO_4$, concentrated and analyzed by GC

Scheme 3 Bioreduction of oxolactone **4** by *R.glutinis* KCh 242: substrate 160 mg, 25 °C, 150 rpm, 7 days



during the reduction of propiophenone and dihydrochalcone catalyzed by *R. glutinis* KCh 242 studied herein (Janeczko et al. 2013; Janeczko and Kostrzewa-Susłow 2014). On the contrary, formation of two diastereoisomers (**3c, 3d**) was the result of different enantioselectivity of strains towards the substrate. Enantiomer 3*R*, 5*S* was reduced faster by *R. rubra* KCh 82 and *S. cerevisiae* KCh 464 to afford (3*R*,5*S*,1′*S*)-hydroxylactone **3c**. *R. marina* KCh 77 and *R. glutinis* 242 favoured the reduction of enantiomer.

3*S*, 5*R* and (3*S*,5*R*,1′*S*)-hydroxylactone **3d** as was the only or the major isomer produced (Fig. 1a c, respectively).

The presented chemoenzymatic strategy is a useful way to produce chiral hydroxylactones as key

intermediates for the synthesis of β-secretase inhibitors. The application of orthopropionate in the Johnson–Claisen rearrangement of **1** avoids the methylation step in synthetic pathway presented in Scheme 1. The stereospecificity of yeast dehydrogenases and different enantioselectivity of reduction observed for particular strains expands the scope of synthesized stereoisomers. The starting materials, easily accessed by Grignard reaction, may be also the analogs of 5-methylhex-1-en-2-ol with various alkyl substituent at the hydroxy group which makes the presented methodology a general route to introduce a variety of alkyl side chain groups at P1/P1′ fragment of peptides which may be studied as potential β-secretase inhibitors.

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