Tetrahedron: *Asymmetry* 27 (2016) 389–396

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Efficient methodology to produce a duloxetine precursor using whole cells of *Rhodotorula rubra*

Isabella Rimoldi [a], Giorgio Facchetti [a], Donatella Nava [a], Martina Letizia Contente [b], Raffella Gandolfi [a,*]

[a] *Dipartimento di Scienze Farmaceutiche, Sez. Chimica Generale e Organica 'A. Marchesini', Università degli Studi di Milano, Via Venezian 21, 20133 Milano, Italy*
[b] *Dipartimento di Scienze per gli Alimenti, la Nutrizione, l'Ambiente, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy*

ARTICLE INFO

Article history:
Received 11 March 2016
Accepted 11 April 2016
Available online 20 April 2016

ABSTRACT

Different types of yeasts were employed as biocatalysts in the reduction of β-ketonitriles. The red microorganism, *Rhodotorula rubra*, was selected as the best performing catalyst in the reduction of different substituted ketonitriles giving total stereoselectivity in most cases (90–99% ee). In particular, its use as fresh and lyophilised cells was expanded to a semi-preparative scale for the production of the duloxetine precursor **1a**. *R. rubra* was screened in the reduction of alkylation products in comparison with *Pichia henricii* for assignment of configuration of products **2a** and **11a** after derivatisation with *S*-MPA.

1. Introduction

β-Hydroxy ketones are important building blocks for the synthesis of chiral 1,3-amino alcohols, 1,3 diamines, 1,3-hydroxy acids used for the preparation of naturally occurring molecules and those of pharmaceutical interest as well.[1–3] Indeed, they provide the scaffold for the synthesis of different classes of drugs such as fluoxetine, duloxetine, atomoxetine, atorvastatin, ezetimibe and selective inhibitors of the serotonin and norepinephrine reuptake.[4] These drugs, in the absence of a valid alternative, are bound to retain a vast sales volume on pharmaceutical market with about 11.9 $ billions sold in 2011 and with a projected sale of about 13.4 $ billions for 2018.[5]

Different synthetic approaches have been optimized to produce β-hydroxynitriles relying on the asymmetric transfer hydrogenation of β-ketonitriles with the employment of chiral organic and inorganic catalysts yielding the corresponding aminoalcohols after a simple Pd/C reduction of the nitrile moiety.[6–9]

Unfortunately, high production costs along with an environmentally hazardous metal toxicity have hampered the use of this type of catalyst especially for the synthesis of products useful to the pharmaceutical industry.

A valid alternative emerging for the production of these useful intermediates is the use of biocatalysts, i.e. the kinetic resolution starting from racemic mixtures of β-hydroxynitriles catalyzed by lipases and nitrilases[10–12] or the enantioselective reduction of β-ketonitriles catalyzed by ketoreductases.[13–15] Over the last decade different isolated carbonyl reductases have been employed in the reduction of these valuable precursors although their application was correlated to the presence of redox cofactors thus inevitably introducing the need for fine tuning the appropriate cofactor recycle system.[16–19] In contrast, the use of whole cells could overcome this unprofitable drawback.

In this regard the use of bakers' yeasts in the reduction of β-ketonitriles allows products with high enantiomeric excesses in an (*S*)-configuration to be obtained, but in low yields due to the competitive non-stereoselective reductive α-alkylation secondary reaction leading to the insertion of an ethyl group at the β-position; the formation of this by-product is due to the presence of acetaldehyde deriving from microorganism metabolism.[20–23] The reaction equilibrium could be shifted in favour of the reduction products over the alkylation ones by using a biphasic system or by a precise monitoring of the reaction temperature, albeit with a decrease of reaction rates and yields.[23]

Dehli et al. had previously studied the reduction of β-ketonitriles employing fungus *Curvularia lunata* whole cells achieving the *S* configuration of the product in 77% yield and also in this case the formation of the alkylation reaction products.[24] Moreover, this biocatalyst was able to reduce in a diastereo- and enantioselective manner the corresponding alkylation products, in exactly the opposite way from *Saccharomyces cerevisiae*, and thus allowing chiral substituted β-hydroxynitriles to be obtained simply by modulating growth and biotransformation reaction conditions. Conversely, alkylation products were totally lacking when recombinant *Escherichia coli* whole cells were employed. This type of cell, in fact, were proved to over express ketoreductases allowing the isolation of both enantiomers of the product.[16]

* Corresponding author. Tel.: +39 02 503 14609; fax: +39 02 503 14615.
E-mail address: raffaella.gandolfi@unimi.it (R. Gandolfi).

http://dx.doi.org/10.1016/j.tetasy.2016.04.002
0957-4166/

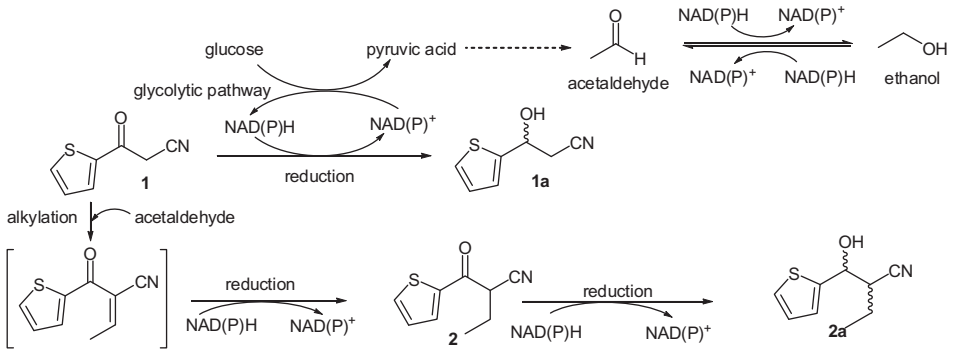
Indeed, in the literature the most relevant attempts relative to the obtainment of these valuable scaffolds in an enantiopure form appeared to be focused on the stereoselective reduction of benzoylacetonitrile precursors. For these reasons the selection of new biocatalysts able to selectively reduce β-keto nitriles bearing different aromatic heterocyclic moieties appears extremely appealing, especially those ones that avoid the use of cofactor recycle systems for accomplishing the desired products in higher yields.

2. Results and discussion

The use of whole cells in the reduction reactions is attractive as it gives the possibility of exploiting the microbial metabolism for the redox cofactor recirculation. On the other hand, the production of acetaldehyde as a by-product generally results in low yields of the desired product due to the competitive reductive alkylation reaction occurring, especially with substrates that are very activated as in the case of β-ketonitriles (Scheme 1).

Herein selected aerobic and facultative anaerobic yeasts were evaluated for their ability to reduce a series of substituted β-ketonitriles. The preliminary screening was realized only with the aim to address the reaction in favour of the reduction product **1a**, using as standard substrate the 3-oxo-3-(thiophen-2-yl)propanenitrile **1**, whose reduction product is the valuable precursor of duloxetine. All the reactions were conducted in presence of glucose (50 g/L) as co-substrate. The results obtained after 48 h of biotransformation are reported in Table 1.

Similarly to what has been reported in the literature,[20] many of the yeasts screened in this work were able to transform substrate **1** in the reductive alkylation product **2** (entries 1–13), although different behaviours were highlighted. In the case of *S. cerevisiae* and of other anaerobic yeasts the substrate **1** is totally converted into the product **2** as evidenced by entries 1–7. In the other cases the formation of reduction products **1a** and **2a** was observed as in biotransformation by *Curvularia lunata* CECT 2130, studied by Dehli and Gotor,[13] obtaining an enantiomerically enriched product nevertheless with poor diastereomeric excesses of product **2a** (entries 8–13). Despite the low molar conversion, *P. henricii* was the only yeast able to give a good enantiomeric excess in favour of diastereomers in with (*R,R*)- and (*S,R*)-configurations of substrate **2** (entry 10). Only the red yeasts, known to be aerobic ones, allowed the reduction product **1a** to be obtained with an (*S*)-configuration (entries 14–17) with moderate-to-high enantiomeric excess (62–98%) and without the formation of by-products. Alcohol **1a** was instead obtained with the (*R*)-configuration using as biocatalyst *Tourolopsis magnoliae*, still in good yield and ee (entry 12). On substituting the glucose as co-substrate with ethanol, which is easily oxidised into acetaldehyde, a general increase in the reductive alkylation product **2** was observed. This concurrent reaction was particularly evident when red yeasts were employed,



Scheme 1. Biotransformation of β-ketonitriles with whole-cells.

Table 1
Biotransformation of 3-oxo-3-(thiophen-2-yl)propanenitrile **1** using glucose as co-substrate

Entry	Microorganisms	1a		2	2a			
		(%)[a]	ee (%)[b]	(%)[a]	(%)[a]	de (%)[a]	ee *anti* (%)[c]	ee *syn* (%)[c]
1	*Saccharomyces marzianus* CBS397	–		100	–			
2	*Pichia pini* CBS 744	–		100	–			
3	*Pichia pastoris* CBS 2612	–		100	–			
4	*Saccharomyces cerevisiae Zeus*	–		100	–			
5	*Sporobolomyces holsaticus* NCYC 420	–		97	–			
6	*Sporobolomyces tsugae* CBS 503	–		100	–			
7	*Kluyveromyces marxianus* CBS1553	–		91	–			
8	*Lindnera fabianii* CBS 5640	18	58 (*S*)	79	–			
9	*Pichia glucozyma* CBS 5766	–		89	12			
10	*Pichia henricii CBS* 5765	12	61 (*R*)	80	8	39 *syn*	97 *RR*	95 *SR*
11	*Pichia etchelsii*	19	58 (*R*)	70	11	1 *anti*	14 *SS*	37 *SR*
12	*Torulopsis magnoliae* IMAP 4425	87	67 (*R*)	0	13	2 *syn*	38 *RR*	46 *SR*
13	*Torulopsis molischiana CBS 837*	34	29 (*S*)	0	66	18 *anti*	65 *SS*	46 *RS*
14	*Sporidiobolus pararoseus SD2*	53	62 (*S*)	0	–			
15	*Sporobolomyces salmonicolor MIM*	100	73 (*S*)	0	–			
16	*Rhodotorula rubra MIM146*	100	97 (*S*)	0	–			
17	*Rhodotorula rubra MIM147*	100	98 (*S*)	0	–			

[a] Molar conversion and de% were calculated by ^{1}H NMR analysis.
[b] Ee% was determined by HPLC analysis using OJ-H Chiralcel as chiral column (hexane:2-propanol = 90:10, flow = 1.0 mL/min, λ = 230 nm).
[c] Ee% was determined by HPLC analysis in accordance to literature conditions.[14]

thus affording just a small quantity of product **2a**, with good enantiomeric excess in favour of *SS* diastereomer (Table 2).

Taking into consideration the high enantioselectivity shown by *R. rubra* MIM 147 in the reduction of 3-oxo-3-(thiophen-2-yl) propanenitrile **1** (Table 1, entry 3), the scope of its particularly interesting biocatalytic performance was expanded to the reduction of different substituted aryl β-ketonitriles. In particular, the use of resuspended cells that were grown for 48 h was compared to the use of lyophilised cells at a concentration of 20 g L^{-1} (dry weight) successively resuspended in 0.1 M phosphate buffer at pH = 7 or resuspended in tap water (Table 3).

The reduction of the carbonyl group, independent of the reaction media, proceeded with a high reaction rate in 24 h by employing resuspended cells and afforded the reduction product only in the (*S*)-configuration in all cases. The only exception was observed in the case of a deactivated substrate as **6** (49%, entry 5). When the biocatalyst was employed in a lyophilised form a general decrease in the reaction rate was seen, probably as a consequence of a possible partial inactivation by the dehydrogenases. As an exception, a total molar conversion after 72 h of the substrate can be observed in the case of the unsubstituted aryl **4** (entry 3) or with the heterocyclic aromatic substrates **1** and **3** (entries 2 and 3) where the heteroatom is in the α-position to the carbonyl group. When the aromatic ring is differently substituted, the best results in terms of conversion were seen in the presence of a *para* activating substituent (entries 6 and 8) whereas the presence of a deactivating group in the *meta* (substrates **6** and **10**, entries 5 and 9) or in the *para* position (substrates **5** and **8**, entries 4 and 7), lowered the molar conversion Anyway, the ee% of the desired alcohols is very high (94–99%) in all cases, confirming that *R. rubra* is a useful catalyst in the reduction of activated ketonitriles. With the aim of evaluating the possibility to produce semi-preparative amounts of (*S*)-3-hydroxy-3-(thiophen-2-yl)propanenitrile, a precursor of duloxetine, biotransformations were carried out using different substrate concentrations: 2, 4, 6 or 10 g L^{-1} in tap water. Substrate inhibition was not observed either at lower or higher concentrations up to 10 g L^{-1}, even if, as expected, a decrease in molar conversion was highlighted starting from 6 g L^{-1} (complete conversion in 48 h). In fact after 12 h the biotransformation was complete for 2 g L^{-1} concentration and in 24 h for 4 g L^{-1} concentration. After chromatographic purification starting from 1 g of **1**, 786 mg of **1a** were obtained. When 10 g L^{-1} substrate concentration was added, 80% yield was achieved in 96 h and the reaction didn't proceed further. In all cases stereoselectivity in favour of the (*S*)-enantiomer remained constant (98–99%).

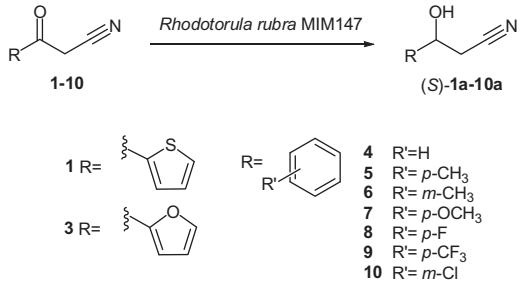
As the results obtained in this preliminary study highlighted that using ethanol as co-substrate and *R. rubra* MIM 147 as the biocatalyst the reductive alkylation product **2** is preferentially obtained (Table 2, entry 3), product **2a** is not formed. Substrates **2**, **11** and **12** were synthesised choosing *Saccharomyces cerevisiae*,

Table 2
Biotransformations of 3-oxo-3-(thiophen-2-yl)propanenitrile **1** using ethanol as co-substrate

Entry	Microorganisms	**1a**		**2**	**2a**			
		(%)[a]	ee (%)[b]	(%)[a]	(%)[a]	de (%)[a]	ee *anti* (%)[c]	ee *syn* (%)[c]
1	*Sporobolomyces salmonicolor* MIM	38	61 *S*	42	20	71 *anti*	77 *SS*	8 *RS*
2	*Rhodotorula rubra* MIM146	17	97 *S*	78	5	50 *anti*	68 *SS*	12 *RS*
3	*Rhodotorula rubra* MIM147	12	98 *S*	88	0			
4	*Sporidiobolus pararoseus* SD2	21	82 *S*	74	5	41 *anti*	73 *SS*	52 *RS*

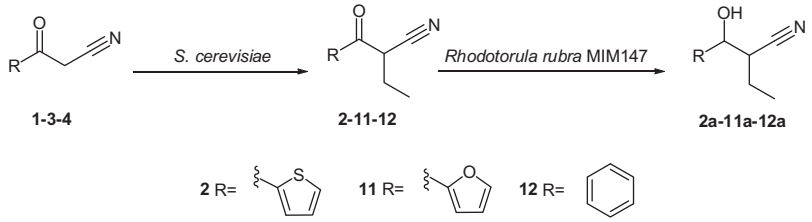
[a] Molar conversion and de% were calculated by ^{1}H NMR analysis.
[b] Ee% was determined by HPLC analysis using OJ-H Chiralcel as chiral column (hexane:2-propanol = 90:10, flow = 1.0 mL/min, λ = 230 nm).
[c] Ee% was determined by HPLC analysis in accordance to literature conditions.[14]

Table 3
Reductive biotransformation of different aryl substituted β-ketonitriles using *R. rubra* as biocatalyst



Entry	Substrate	Resuspended cells		Lyophilised cells	
		Molar conversion (%)[a]	ee (% of *S*)	Molar conversion (%)[b]	ee (% of *S*)
1	**1**	100	97	100	>99
2	**3**	100	97	100	98
3	**4**	100	97	100	98
4	**5**	100	97	31	96
5	**6**	49	94	51	90
6	**7**	100	99	100	99
7	**8**	97	96	15	99
8	**9**	95	96	90	98
9	**10**	100	96	3	94

[a] Molar conversion (%) after 24 h was calculated by ^{1}H NMR analysis.
[b] Molar conversion (%) after 72 h. Ee% was determined by HPLC analysis using OJ-H Chiralcel as chiral column (hexane:2-propanol = 90:10, flow 1 mL/min, λ = 216 nm).



Scheme 2. Biotransformation by *S. cerevisiae* affording substrates **2**, **11** and **12.**

Table 4
Reductions of different *rac*-2-arylbutanenitrile using *R. rubra* MIM147 as biocatalyst

Entry	Substrate	de[a] *anti* (%)	ee[b] *anti* (% of *S,S*)	ee[b] *syn* (% of *R,S*)
1	**2**	66	97	50
2	**11**	76	95	0
3	**12**	90	98	28

[a] de% were calculated from ^{1}H NMR analysis.
[b] Ee% was determined by HPLC analysis using Phenomenex Cellulose 4 as chiral column (hexane:2-propanol = 90:10, flow 1 mL/min, λ = 230 nm).

a catalyst reported in literature for its ability to produce this type of substrates under suitable reaction conditions (Scheme 2).

These substrates are particularly interesting to study due to the presence of additional stereogenic centre[14] so the second part of this research work focused on the capability to reduce and resolve the diastereomers of the corresponding reduction products. The results are summarized in the following table (Table 4).

As reported in our previous work[25] for substrate **12**, after 48 h the biotransformations were realized using a substrate concentration of 1 g L^{-1} for all employed substrates. In all cases the enantiomeric excess for the *anti*-diastereomers was very high (95–98%) although the diastereomeric excess was influenced by the different aryl group in α position to the carbonyl.

2.1. Assignment of configuration for 2a and 11a

The configuration for diastereomeric mixture of **12a** was reported in literature.[9,14] The assignment of the configuration for diastereomers of **2a** and **11a** was realised using *R. rubra* MIM147 as biocatalyst, able to preferentially form the *anti*-(*S, S*)-diastereomer (Table 4) and *Pichia henricii* CBS 5765, forming the *anti*-(*R,R*)-diastereomer and one *syn*-(*S,R*)-diastereomer with high ee% (Table 1, entry 10). Employing directly compounds **2** and **11** as substrate for biotransformations the reduction reaction conducted with *P. henricii* compared to *R. rubra* proceeded slowly but when substrate concentrations was equal to 1 g L^{-1} and concentration resuspended was 40 g L^{-1} (dry weight), an increase of diastereomeric excess for **2a** was observed (63% de *syn*). In the case of substrate **11** a diastereomeric excess of 85% *syn* was achieved. The reactions were completed after 72 h.

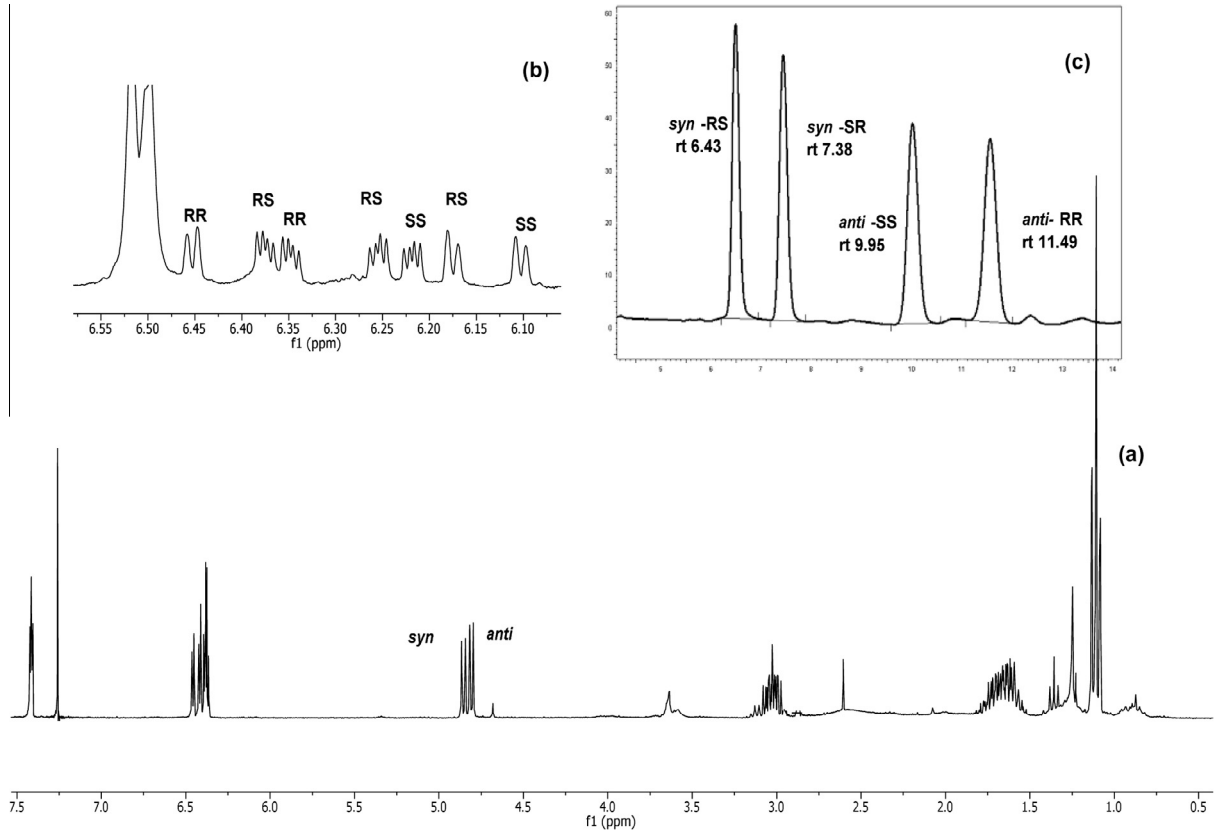


Figure 1. Comparison among ^{1}H NMR spectra of racemic mixture (a), *S*-MPA derivatives (b) and HPLC chromatogram (c) for **11a**.

The configurations for **2a** and **11a** were determined by sequential comparison of: ^{1}H NMR spectra with the corresponding ones obtained after derivatisation with (*S*)-MPA (α-methoxy-phenyl acetic acid),[26] HPLC profiles of racemic mixture respectively of **2a** and **11a** (Figs. 1 and 3) and those ones achieved by reduction with *R. rubra* and *P. henricii* (Figs. 2 and 4).

As reported in Figure 1 a racemic mixture of **11a** obtained by reduction with $NaBH_4$ was analyzed by ^{1}H NMR spectroscopy before (Fig. 1a) and after reaction with *S*-MPA in presence of 4-DMAP and DCC (Fig. 1b). In Figure 1c the corresponding HPLC analysis was reported using chiral column Phenomenex Lux Cellulose 4 (hexane:2-propanol = 90:10, flow 1 mL/min, λ = 230 nm).

From ^{1}H NMR spectra, the biotransformation with *R. rubra*, an enantiomeric excess of 95% in favour of diastereomers with an *anti*-configuration was obtained based on the literature configuration assignment.[14] On the contrary on reduction with *P. henricii* the *syn* diastereomers were predominant (63% de). By comparison of HPLC chromatograms and ^{1}H NMR spectra of MPA-derivatives it was possible to assign the configurations to all the four diastereomers (Fig. 2a and b).

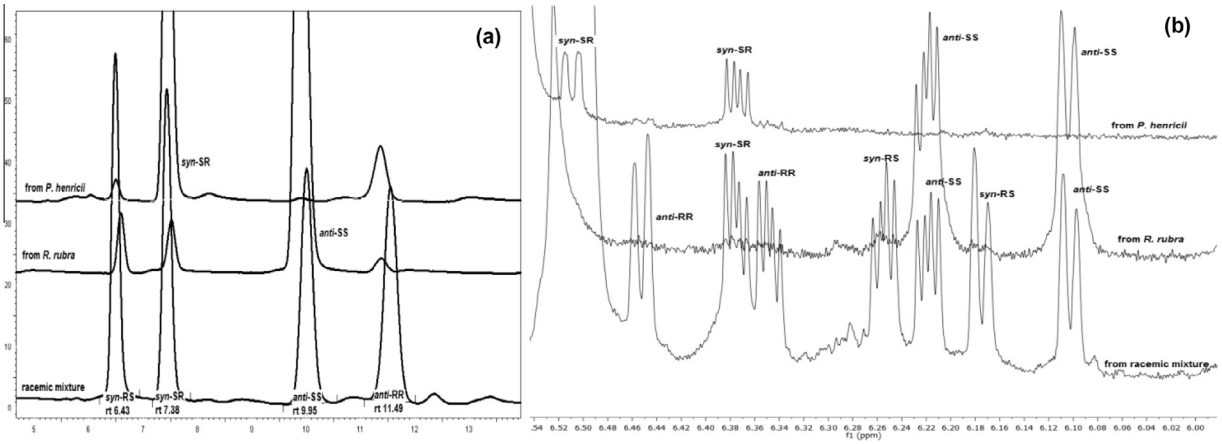


Figure 2. HPLC chromatograms for racemic mixture of **11a**, reduction products from *R. rubra* and from *P. henricii* (a) compared with the corresponding ^{1}H NMR spectra of MPA-derivatives (b).

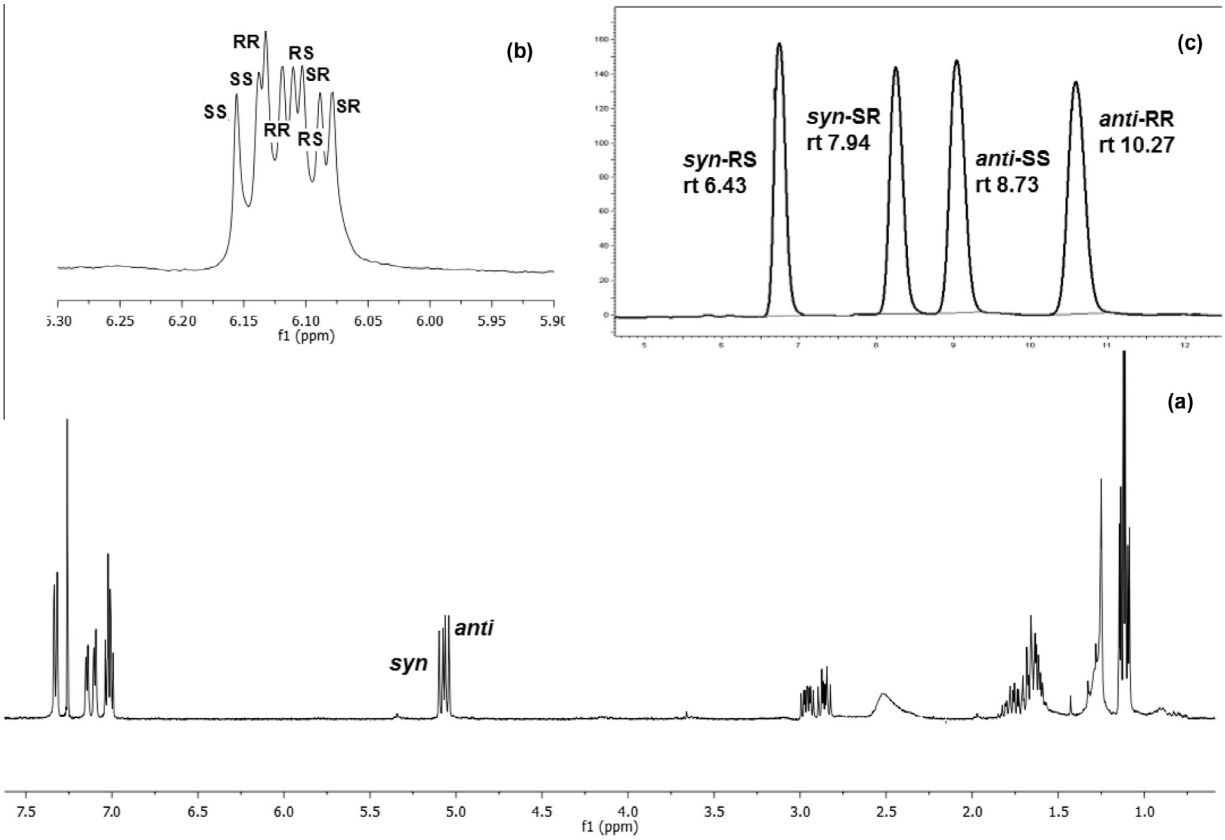


Figure 3. Comparison among ^{1}H NMR spectra of racemic mixture (a), derivatives with MPA (b) and HPLC chromatogram (c) for **2a**.

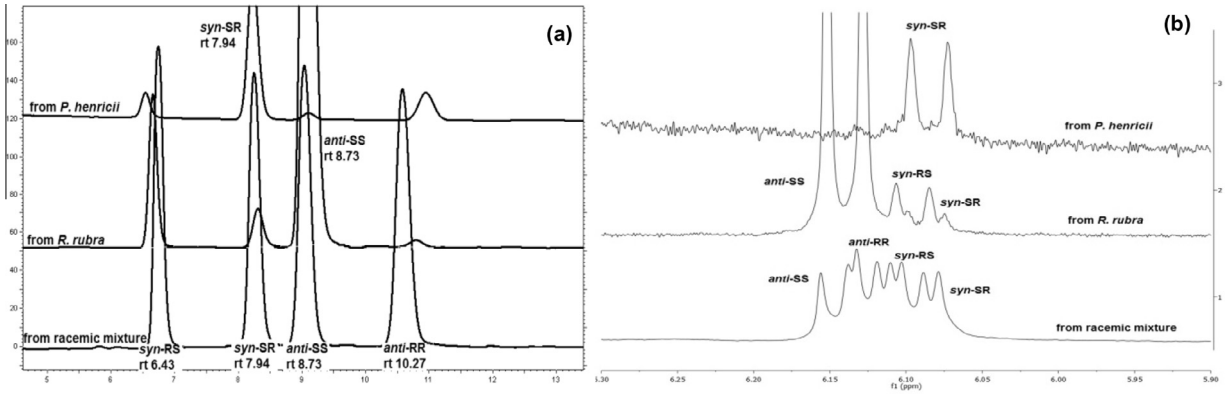


Figure 4. HPLC chromatograms for racemic mixture of **2a**, reduction products from *R. rubra* and from *P. henricii* (a) compared with corresponding ^{1}H NMR spectra of MPA-derivatives (b).

The same approach was used to assign the configurations for the four diastereomers obtained by reduction of **2a** as reported in the following Figures 3 and 4.

3. Conclusion

In conclusion the strategy to use the aerobic yeasts allows the amount of alkylation product during biotransformation of ketonitriles to be reduced. Thus, in the presence of ethanol, that was directly converted into acetaldehyde, a major production of alkylated product was seen. *Rhodotorula rubra* was revealed as a valid biocatalyst for the reduction of different substituted ketonitriles using either fresh whole cells or lyophilised ones. The semipreparative production of dulexetine's (*S*)-precursor in very good enantiomeric was realized using tap water as reaction solvent in 4 g/L substrate concentration. The assignment of configuration of the four diastereomeric reduction products of **2a** and **11a** was easily realised thanks to the possibility to obtain the enriched products by *Rhodotorula rubra* versus *Pichia henricii*.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker Avance 300 MHz equipped with a non-reverse probe. Chemical shifts (in ppm) were referenced to residual solvent proton/carbon peak. Polarimetry analyses were carried out on Perkin Elmer 343 Plus equipped with Na/Hal lamp. MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electronspray ionisation source and an 'Ion Trap' mass analyser. The MS spectra were obtained by direct infusion of a sample solution in MeOH under ionisation, ESI positive. Catalytic reactions were monitored by HPLC analysis with Merck-Hitachi L-7100 equipped with Detector UV6000LP and chiral column (OJ-H Chiralcel, OD-H Chiralcel or Phenomenex Lux Cellulose 4). FTIR spectra were collected by using a Perkin Elmer (MA, USA) FTIR Spectrometer 'Spectrum One' in a spectral region between 4000 and 450 cm^{-1} and analysed by transmittance technique with 32 scansions and 4 cm^{-1} resolution. Commercially reagent grade solvents were dried according to standard procedures and freshly distilled under nitrogen before use.

4.2. Microorganisms: culture conditions

Strains from official collections or from our collection were routinely maintained on a malt extract (8 g L^{-1} agar 15 g L^{-1}, pH = 5.5). In order to obtain cells for the biocatalytic activity tests, the microorganisms were grown on solid medium for 72 h at 28 °C and then they were cultured in 1000 mL Erlenmeyer flasks containing 100 mL of the medium (OD_{530nm} mL^{-1} = 0.1 at t_0).The microorganisms were incubated for 48 h at 28 °C on a reciprocal shaker (100 rpm). The yeasts were grown on malt extract with 5 g L^{-1} Difco yeast extract pH = 5.6, excepting for *Sporidiobolus pararoseus* SD2 that grown on YPD medium (10 g L^{-1} Difco yeast extract, 10 g L^{-1} peptone, 20 g L^{-1} glucose). Fresh cells from submerged cultures were centrifuged (4000×*g* for 15 min at 4 °C) and washed with tap water before using. In the case of *Rhodotorula rubra* MIM147 the cells were also lyophilised. The cells used in the screening biotransformations were concentrated in ratio 1:2.

4.3. General procedure of biotransformation

The biotransformation screening was carried out in 10 ml-screw capped test tubes re-suspending the yeast cells in 5 mL of 0.1 M phosphate buffer pH = 7 containing 50 g L^{-1} of glucose (or ethanol 10 g L^{-1}) and adding 2 g $L^{-1}$of substrate **1** dissolved in DMSO (1%). The reaction mixtures were magnetically stirred at 28 °C for 48 h. The product was extracted with diethyl ether (2 × 5 mL), dried with Na_2SO_4 and the solvent was removed in vacuo. The samples were analysed by ^{1}H NMR to determinate the molar conversion and the diastereomeric excess while the enantiomeric excess was recorded by HPLC equipped with OD-H Chiralcel, eluent: hexane:2-propanol = 95:5, flow = 0.8 mL/min, λ = 230 nm; rt: **1a**: (*S*) = 49.9 min, (*R*) = 52.2 min; **2** = 23.9 min; **2a**: (*S,S*) = 26.5 min, (*R,S*) = 28.8 min, (*S,R*) = 34.3 min, (*R,R*) = 36.4 min.[14]

1a: ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.13 (t, *J* = 7.3 Hz, 3H), 2.88 (d, *J* = 5.9 Hz, 2H), 5.27–5.34 (m, 1H), 7.00 (t, *J* = 4.8 Hz, 1H), 7.10 (d, *J* = 3.3 Hz, 1H), 7.32 (d, *J* = 4.7 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 28.43, 66.38, 117.33, 124.93, 125.95, 127.33, 144.75 ppm. IR ν = 3429, 2922, 2254, 1413, 1040, 850, 709 cm^{-1}. MS (ESI) of C_7H_7NOS (*m/z*): calcd 153.2, found 175.8 [M+Na^+].

2: ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.14 (t, *J* = 7.3 Hz, 3H), 1.99–2.15 (m, 2H), 4.11–4.18 (d, *J* = 6.2, *J* = 1.4 Hz, 1H), 7.15–7.20 (m, 1H), 7.75 (d, *J* = 7.6 Hz, 1H) 7.84 (d, *J* = 6.7 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 11.82, 24.36, 42.62, 117.37, 128.92, 133.86, 136.82, 141.16, 183.62 ppm. IR ν = 3094, 2974, 2934, 2248, 1671, 1413, 1247, 1206, 1064, 731 cm^{-1}. MS (ESI) of C_9H_9NOS (*m/z*): calcd 179.2, found 201.8 [M+Na^+].

2a: ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.09 (t, *J* = 7.4 Hz, 3H, *anti*), 1.12 (t, *J* = 7.4 Hz, 3H, *syn*), 1.51–1.68 (m, 2H, *anti*), 1.69–1.8 (m, 2H, *syn*), 1.58 (br, *-OH*), 2.82–2.92 (m, 2H, *anti*), 2.94–2.99

(m, 2H, *syn*), 5.05 (d, J = 6.3 Hz, 1H, *anti*), 5.08 (d, J = 6.5 Hz, 1H, *syn*), 6.99–7.10 (m, 1H, *syn* + *anti*), 7.11 (d, J = 3.6 Hz, 1H, *anti*), 7.13 (d, J = 3.1 Hz, 1H, *syn*), 7.32 (d, J = 5.2 Hz, 1H, *anti* + *syn*) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 11.77 (*syn*), 11.83 (*anti*), 21.69 (*syn*), 22.69 (*anti*), 42.81 (*syn*), 43.23 (*anti*), 69.87 (*syn*), 70.16 (*anti*), 120.01 (*syn*), 120.14 (*anti*), 125.55 (*anti*), 125.7 (*syn*), 125.97 (*syn*), 126.02 (*anti*), 127.2 (*anti* + *syn*), 143.71 (*syn*), 144.05 (*anti*) ppm. IR ν = 3435, 2970, 2933, 2245, 1711, 1461, 1032, 706 cm^{-1}. MS (ESI) of $C_9H_{11}NOS$ (m/z): calcd 181.27, found 204.8 [M+Na$^+$].

4.4. Screening of different aryl ketonitriles with *Rhodotorula rubra* MIM147

Fresh cells and lyophilised cells of *Rhodotorula rubra* MIM147 were used 20 g L^{-1} dry weight re-suspended in phosphate buffer or in tap water with 50 g L^{-1} of glucose; then the substrates **1**, **3**–**10** were dissolved in DMSO and added at 2 g L^{-1} concentration. For substrate **1**, biotransformations were also carried out at 4, 6 or 10 g L^{-1} concentration.

HPLC conditions:[6,8] OJ-H Chiralcel, eluent: hexane:2-propanol = 90:10, flow = 1.0 mL/min, λ = 216 nm.

Compound **1a**: (*S*) = 32.1 min, (*R*) = 38.3 min; compound **3a** rt (*S*) = 22.2 min, (*R*) = 25.8 min; compound **4a** rt (*S*) = 23.4 min, (*R*) = 29.7 min; compound **5a** rt (*S*) = 20.6 min, (*R*) = 24.4 min; compound **6a** rt (*S*) = 18.5 min, (*R*) = 22.0; compound **10a** rt (*S*) = 23.7 min, (*R*) = 28.6 min; compound **7** rt (*S*) = 47.7 min, (*R*) = 49.5 min; compound **8a** rt (*S*) = 21 min, (*R*) = 27.7 min; compound **9** rt (*S*) = 15.5 min, (*R*) = 17.9 min.

4.5. Enzymatic reduction of *rac*-2-(thiophene-2-carbonyl) butanenitrile 2, *rac*-2-(furan-2-carbonyl)butanenitrile 11 and *rac*-2-benzoylbutanenitrile 12

Commercial Baker's yeast (50 g L^{-1}) was suspended in a phosphate buffer (200 mL, 0.1 M, pH = 7) containing 50 g L^{-1} of glucose and 5 g L^{-1} of the substrate (**1**, **3** or **4)** was added. The biotransformation system was shaken with mechanic stirrer at 28 °C. When the total conversion was achieved, the cells were separated by centrifugation. Both the aqueous phases and the cells mixture were extracted with diethyl ether (3x50 mL), dried with Na_2SO_4 and the solvent was removed in vacuo.

2: The crude product was purified by flash chromatography (cyclohexane/diisopropyl ether = 7:3) to give 1.04 g of **2** (88% yield).

11: The crude product was purified by flash chromatography (CH_2Cl_2/cyclohexane/ethyl acetate = 4:1:1) to give 929 mg of **11** (77% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.14 (t, J = 7.4 Hz, 3H), 2.02–2.09 (m, 2H), 4.11–4.16 (q, J = 6.2, 4.3 Hz, 1H), 6.61–6.64 (dd, J = 3.6 Hz, J = 1.9 Hz, 1H), 7.40 (d, J = 4.4 Hz, 1H) 7.68 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 11.73, 23.66, 41.91, 113.45, 117.06, 119.93, 147.88, 150.57, 179.74 ppm. IR ν = 3135, 2964, 2933, 2249, 1681, 1567, 1464, 1394, 1261, 1021, 771 cm^{-1}. MS (ESI) of $C_9H_9NO_2$ (m/z): calcd 163.2, found 185.8 [M+Na$^+$].

12: The crude product was purified by flash chromatography (CH_2Cl_2/hexane/ethyl acetate = 4:1:1) to give 1.02 g of **12** (86% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.16 (t, J = 7.7 Hz, 3H), 2.02–2.15 (m, 2H), 4.30 (dd, J = 6.2, 4.3 Hz, 1H), 7.49–7.56 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H) 7.95 (d, J = 6.7 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 11.71, 23.77, 41.69, 117.41, 128.92–134.63, 170.91, 190.97 ppm. IR ν = 3467, 2975, 2936, 2249, 1694, 1597, 1449, 1265, 1233, 1208, 1000, 696 cm^{-1}. MS (ESI) of $C_{11}H_{11}NO$ (m/z): calcd 173.2, found 196.1 [M+Na$^+$].

4.6. Synthesis of *rac*-2-(hydroxy(thiophen-2-yl)methyl)butanenitrile 2a, *rac*-2(furan-2-yl(hydroxyl)methyl)butanenitrile 11a and *rac*-2-(hydroxyl(phenyl)methyl)butanenitrile 12a

The *rac*-**2a**, *rac*-**11a** and *rac*-**12a** were obtained by reduction of the substrates **2**, **11** and **12** (1 equiv) in ethanol with $NaBH_4$ (1.5 equiv). The reactions were magnetically stirred at room temperature for 4 h and extracted with water and ethyl acetate (3 × 50 mL), dried with Na_2SO_4 and the solvent was removed in vacuo.

2a: HPLC conditions: column: Phenomenex Lux Cellulose 4, eluent: hexane:2-propanol = 90:10, flow = 1 mL/min, λ = 230 nm; rt: (*R,S*) = 6.43 min, (*S,S*) = 8.73 min, (*S,R*) = 7.94 min, (*R,R*) = 10.27 min.

11a: ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.10 (t, J = 7.4 Hz, 3H, *anti*), 1.11 (t, J = 7.4 Hz, 3H, *syn*), 1.54–1.76 (m, 2H, *anti* + *syn*), 2.97–3.08 (m, 2H, *anti* + *syn*), 4.80 (d, J = 6.3 Hz, 1H, *anti*), 4.85 (d, J = 6.8 Hz, 1H, *syn*), 6.36–6.39 (m, 2H, *anti*), 6.41 (d, J = 3.3 Hz, 1H, *syn*), 6.45 (d, J = 3.3 Hz, 1H, *syn*), 7.41–7.43 (m, 1H, *anti* + *syn*) ppm;^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 11.71 (*syn*), 11.82 (*anti*), 21.67 (*syn*), 22.65 (*anti*), 40.09 (*syn*), 40.49 (*anti*), 67.64 (*syn*), 67.92 (*anti*), 108.35 (*anti*) 108.50 (*syn*), 110.79 (*syn* + *anti*), 119.81 (*syn*), 119.99 (*anti*), 143.06 (*syn* + *anti*), 152.78 (*syn* + *anti*) ppm. IR ν = 3434, 2971, 2936, 2244, 1730, 1462, 1149, 745 cm^{-1}. MS (ESI) of $C_9H_{11}NO_2$ (m/z): calcd 165.3, found 187.9 [M+Na$^+$]. HPLC conditions: column: Phenomenex Lux Cellulose 4, eluent: hexane:2-propanol = 90:10, flow = 1 mL/min, λ = 216 nm; rt: (*R,S*) = 6.43 min, (*S,S*) = 9.95 min, (*S,R*) = 7.38 min, (*R,R*) = 11.49 min.

12a: ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.09 (t, J = 7.7 Hz, 3H, *anti*), 1.17 (t, J = 7.6 Hz, 3H, *syn*), 1.51–1.69 (m, 2H), 2.76–2.83 (m, 2H, *anti*), 2.87–2.95 (m, 2H, *syn*), 4.79 (d, J = 6.2 Hz, 1H, *anti*), 4.83 (d, J = 6.6 Hz, 1H, *syn*), 7.33–7.56 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 9.84 (*syn*), 10.38 (*anti*), 24.86 (*anti*), 25.87 (*syn*), 41.43 (*anti*), 42.51 (*syn*), 76.86 (*syn*), 77.46 (*anti*), 127.04 (*syn* + *anti*), 128.05 (*anti*), 128.22 (*syn*), 128.55 (*anti*), 128.69 (*syn*), 140.61 (*anti*), 141.42 (*syn*) ppm. FTIR ν = 3390, 2964, 1494, 1453, 160, 1103, 1038, 702 cm^{-1}. MS (ESI) of $C_{11}H_{11}NO$ (m/z): calcd 175.1, found 198.3 [M+Na$^+$]. HPLC conditions: column: OD-H Chiralcel, eluent: hexane:2-propanol = 95:5, flow = 0.8 mL/min, λ = 216 nm. rt: (*R,S*) = 25.6 min, (*S,S*) = 26.5 min, (*S,R*) = 34.6 min, (*R,R*) = 36.0 min.

4.7. 2-(Hydroxy(thiophen-2-yl)methyl)butanenitrile 2a and 2 (furan-2-yl(hydroxyl)methyl)butanenitrile 11a derivatisation

The enantiomerically enriched mixtures of **2a** and **11a** were obtained by biotransformation using fresh cells of *Rhodotorula rubra* MIM 147 (20 g L^{-1} dry weight) and *Pichia henricii* CBS 5765 (40 g L^{-1} dry weight) suspended in phosphate buffer 0.1 M pH = 7 containing 50 g L^{-1} of glucose. Compounds **2** or **11** were dissolved in DMSO and added to the biotransformation system to a final substrate concentration of 1 g L^{-1} in presence of 1% solvent. After 72 h the reactions were extracted with diethyl ether (3 × 50 mL), dried with Na_2SO_4 and the concentrated at reduce pressure. The crude products **2a** and **11a** were purified by column chromatography on silica gel (CH_2Cl_2/cyclohexane/ethyl acetate = 4:1:1).

^{1}H NMR of the corresponding derivatives with MPA (α-methoxy-phenyl acetic acid): To a solution of *rac*-**2a** and *rac*-**11a** and the enriched products obtained by biotransformation (1 equiv) in $CDCl_3$ (0.75 mL), *S*-(+)-MPA (1 equiv), 4-DMAP (0.5 equiv) and DCC (1.5 equiv) were added.[26]

^{1}H NMR for **2a** ($CDCl_3$, 300 MHz, 25 °C) δ = 6.08 (d, J = 7.1 Hz, 1H, (*S,R*)-*syn*), 6.10 (d, J = 7.1 Hz, 1H, (*R,S*)-*syn*), 6.14 (d, J = 6.8 Hz, 1H, (*R,R*)-*anti*), 6.15 (d, J = 6.8 Hz, 1H, (*S,S*)-*anti*) ppm. ^{1}H NMR for **11a** ($CDCl_3$, 300 MHz, 25 °C) δ = 6.10 (d, J = 3.3 Hz, 1H, (*S,S*)-*anti*),

6.17 (d, J = 3.3 Hz, 1H, (*R,S*)-*syn*), 6.20–6.23 (m, 1H, (*S,S*)-*anti*), 6.24–6.26 (m, 1H, (*R,S*)-*syn*), 6.20–6.23 (m, 1H, (*R,R*)-*anti*), 6.34–6.36 (m, 1H, (*S,R*)-*syn*), 6.44 (d, J = 3.3 Hz, 1H, (*R,R*)-*anti*) ppm.

4.8. Synthesis of (*S*)-3-hydroxy-3-(thiophen-2-yl)propanenitrile 1a with *Rhodotorula rubra* MIM 147

Cells obtained by centrifugation (4000×*g* for 15 min at 4 °C) of the culture broth (500 mL) were washed with tap water (3 × 200 mL) and re-suspended in 250 mL of tap water containing 50 g L^{-1} of glucose. 1 g of **1** was dissolved in DMSO and added to the biotransformation system to give 4 g L^{-1} of substrate concentration and 1% of solvent. The biotransformation system was shaken with mechanic stirrer at 28 °C for 24 h. The cells were separated by centrifugation and the two fractions were extracted with diethyl ether (3 × 100 mL), the combined organic layer was dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash chromatography (ethyl acetate/cyclohexane = 7:3) to give 786 mg of (*S*)-**2**a (78% yield). (*S*)-**1a**: All characterization data are in agreement with previously reported. $[\alpha]_D^{20}$ = −42.1 (*c* 0.8, $CHCl_3$).

References

1. Simon, R. C.; Mutti, F. G.; Kroutil, W. *Drug Discovery Today: Technol.* **2013**, *10*, e37–e44.
2. Müller, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 362–365.
3. Kamal, A.; Khanna, G. B. R.; Krishnaji, T.; Ramu, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 613–615.
4. Soni, P.; Banerjee, U. C. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 771–777.
5. Every-Palmer, S.; Howick, J. *J. Eval. Clin. Pract.* **2014**, *20*, 908–914.
6. Soltani, O.; Ariger, M. A.; Vázquez-Villa, H.; Carreira, E. M. *Org. Lett.* **2010**, *12*, 2893–2895.
7. Vázquez-Villa, H.; Reber, S.; Ariger, M. A.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2011**, *50*, 8979–8981.
8. Chen, C.; Kong, L.; Cheng, T.; Jin, R.; Liu, G. *Chem. Commun.* **2014**, 10891–10893.
9. Zerla, D.; Facchetti, G.; Fuse, M.; Pellizzoni, M.; Castellano, C.; Cesarotti, E.; Gandolfi, R.; Rimoldi, I. *Tetrahedron: Asymmetry* **2014**, *25*, 1031–1037.
10. Träff, A.; Lihammar, R.; Bäckvall, J.-E. *J. Org. Chem.* **2011**, *76*, 3917–3921.
11. Kamal, A.; Khanna, G. B. R.; Ramu, R. *Tetrahedron: Asymmetry* **2002**, *13*, 2039–2051.
12. Kamila, S.; Zhu, D.; Biehl, E. R.; Hua, L. *Org. Lett.* **2006**, *8*, 4429–4431.
13. Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3693–3700.
14. Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 1485–1492.
15. Zhu, D.; Yang, Y.; Hua, L. *J. Org. Chem.* **2006**, *71*, 4202–4205.
16. Xu, G.-C.; Yu, H.-L.; Zhang, Z.-J.; Xu, J.-H. *Org. Lett.* **2013**, *15*, 5408–5411.
17. Nowill, R. W.; Patel, T. J.; Beasley, D. L.; Alvarez, J. A.; Jackson Iii, E.; Hizer, T. J.; Ghiviriga, I.; Mateer, S. C.; Feske, B. D. *Tetrahedron Lett.* **2011**, *52*, 2440–2442.
18. Zhu, D.; Ankati, H.; Mukherjee, C.; Yang, Y.; Biehl, E. R.; Hua, L. *Org. Lett.* **2007**, *9*, 2561–2563.
19. Hammond, R. J.; Poston, B. W.; Ghiviriga, I.; Feske, B. D. *Tetrahedron Lett.* **2007**, *48*, 1217–1219.
20. Smallridge, A. J.; Ten, A.; Trewhella, M. A. *Tetrahedron Lett.* **1998**, *39*, 5121–5124.
21. Itoh, T.; Takagi, Y.; Fujisawa, T. *Tetrahedron Lett.* **1989**, *30*, 3811–3812.
22. Fuganti, C.; Pedrocchi-Fantoni, G.; Servi, S. *Tetrahedron Lett.* **1990**, *31*, 4195–4198.
23. Florey, P.; Smallridge, A. J.; Ten, A.; Trewhella, M. A. *Org. Lett.* **1999**, *1*, 1879–1880.
24. Gotor, V.; Dehli, J. R.; Rebolledo, F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 307–309.
25. Facchetti, G.; Gandolfi, R.; Fuse, M.; Zerla, D.; Cesarotti, E.; Pellizzoni, M.; Rimoldi, I. *New J. Chem.* **2015**.
26. Feske, B. D.; Kaluzna, I. A.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 9654–9657.