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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 1325–1330

Synthesis of chiral cycloalkanols using yeast whole cell bioreactors

Marie Zarevúcka,[a] Zdeněk Wimmer,[a,*] David Šaman,[a] Kateřina Demnerová[b] and Martina Macková[b]

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences Czech Republic, Department of Natural Products, Flemingovo náměstí 2, CZ-16610 Prague 6, Czech Republic*
[b]*Institute of Chemical Technology, Technická 5, CZ-16628 Prague 6, Czech Republic*

Received 10 February 2004; revised 2 March 2004; accepted 8 March 2004

Abstract—*Geotrichum ludwigii*, *Geotrichum candidum*, and *Saccharomyces cerevisiae* whole cells were used for bioreduction of ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}carbamate **1**, an insect juvenile hormone bioanalog, to produce the stereoisomers of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate **2**–**5** with as high as possible enantiomeric purity (>99% ee for **2** and **3** in several cases). Hydrophobic polymer Amberlite XAD-7 was used for potential modification of the reaction course mediated by isoenzymes present in the yeast whole cell bioreactors. Only stereoisomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate were obtained as major products of the studied enzymic process mediated by selected microorganisms.

1. Introduction

Prochiral cycloalkanones have been convenient substrates for asymmetric reduction toward enantiomerically pure cycloalkanol derivatives.[1] Enzymes and microorganisms represent valuable chiral biocatalysts in this synthesis, because they usually enable achieving products with high enantiomeric excess.[2–4] When compared with employing chiral reagents, most enzymic or microorganism-mediated reductions are less laborious processes. There are some limitations in the application of microbial whole cell systems: the stereoselectivity of the reduction process can be lower in comparison with the process in which pure enzyme is used due to the existence of different types of alcohol dehydrogenases (reductases; EC 1.1.1.1) showing different stereoselectivity. Several methods have been described to increase the stereoselectivity of reduction processes mediated by microorganisms.[2,4–7]

Using specific strategies to circumvent the inhibitory effects of the substrates on the biocatalysts, preference of either the (*S*)-enzyme [the enzyme affording the (*S*)-alcohol by reduction of parent prochiral ketone] or the (*R*)-enzyme (the enzyme affording the (*R*)-alcohol by reduction of parent prochiral ketone) can be achieved.[5,7] One such strategy is to adsorb the substrate onto a hydrophobic polymer such as the Amberlite XAD-type resin.[5,7] The method is based on the finding that an adsorbed hydrophobic organic compound is partitioned between the solid organic phase and the aqueous phase. The equilibrium process of this partitioning results in having present the major quantity of the organic substrate in the solid organic phase. Hydrophobic polymer can act as a pool for the organic substrate. It supplies an amount of the substrate into the aqueous phase on the basis of the equilibrium process. The substrate is the subject of enzymic transformation, and the hydrophobic polymer assists in maintaining its concentration in the aqueous phase. The product of the enzymic process, which is released by the enzyme, is continuously extracted from the aqueous phase and cell surface by the same hydrophobic polymer. Such a process assists in preventing enzyme inhibition by both the substrate and product concentration.

Based on this finding, a possible synthesis of both enantiomers of the chiral cycloalkanol derivatives starting from parent prochiral ketone should be executable in principle.

It has been reported recently that the strain IFO 5767 from *Geotrichum candidum* is able to reduce a number of

* Corresponding author. Tel.: +42-0220-183-281; fax: +42-0224-310-090; e-mail: wimmer@uochb.cas.cz

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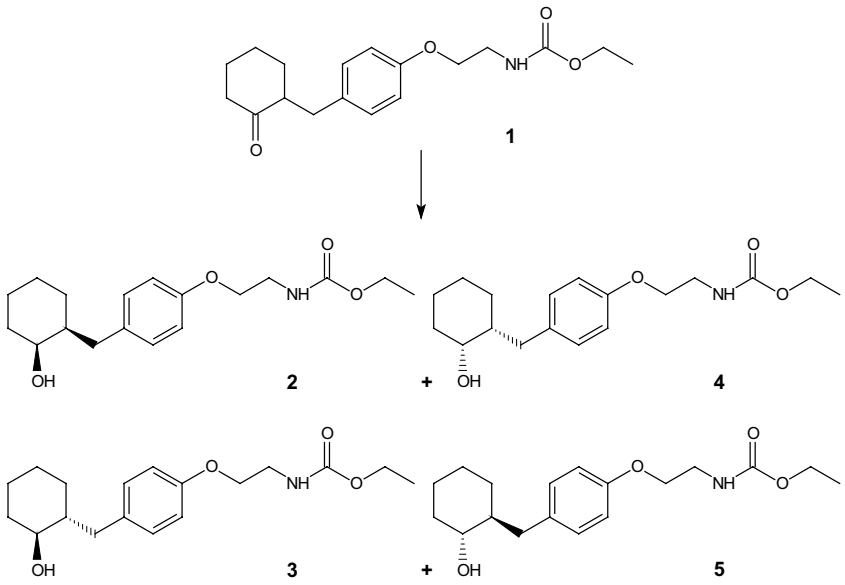
doi:10.1016/j.tetasy.2004.03.006



Scheme 1.

prochiral compounds containing a carbonyl functionality, and to produce both enantiomers of the products bearing the hydroxyl functionality.[5] This particular strain of *G. candidum* was not available for our screening study, however, a certain number of other strains of *Saccharomyces cerevisiae* have been previously used for the reduction of 2-substituted cyclohexanones.[1] Moreover, different techniques and different microorganisms have been used recently for the reduction of ketones to chiral alcohols.[2,4,6,7]

A number of experiments with different strains of *S. cerevisiae*[1] performed by us resulted in the conclusion that the preferred course of the reduction mediated by the tested strains of these two yeasts led to the synthesis of 2-substituted (1*S*)-cycloalkanol derivatives.[1] However, asymmetric reductions of prochiral ketones with a single microorganism under modified conditions have also been described,[4–7] using selected modification of the reaction course by addition of reaction auxiliaries. One of those approaches used the addition of hydrophobic polymer Amberlite XAD-7.[5,7] If this method[5,7] worked with the substrates used as synthetic intermediates for the synthesis of target compounds of interest in our current projects, it would eliminate the need for laborious enzymic resolution processes, through which we synthesize the (1*R*)-enantiomers of target chiral 2-substituted cycloalkanols, which are not accessible by alternative reductions (enzymic or chemical) of parent ketones with high enantiomeric excess.[8]

An interesting question arises in connection with the above-summarized results[4–7] achieved by different research groups: can any of the strains of *Geotrichum* species or *S. cerevisiae*, available to us, be used for the bioreduction of 2-substituted cyclohexanones selectively to all possible stereoisomers of 2-substituted cyclohexanols? One of the biologically active compounds, an insect juvenile hormone bioanalog of primary importance,[8,9] ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}carbamate **1**, was used as substrate in this study. Two of the four possible stereoisomers of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}-carbamate **2** and **3** are easily accessible by the reduction of the ketone **1** by *S. cerevisiae* (Scheme 1), however, the opposite stereoisomeric pair **4** and **5** of the products are still hard to synthesize easily and efficiently.[8] Therefore, this partial investigation was performed to learn, if any of the previously and currently studied strains of *G. ludwigii* (*Dipodascus magnusii*), *G. candidum*, and *S. cerevisiae*[10] can produce the desired enantiomers **4** and **5** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate (Scheme 1). Based on our recent results, only the most active strain of *S. cerevisiae* was employed in this investigation together with one strain from *G. ludwigii* and three different strains of *G. candidum*.

2. Results and discussion

Using a strain DBM 48 of *G. ludwigii* together with three different strains from *G. candidum* (DBM 4012, DBM 4013, and DBM 4166), and performing the bioreduction process either in the absence or in the presence of hydrophobic polymer Amberlite XAD-7, it has been generally found that the major enantiomers of the product, ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]-phenoxy}ethyl}carbamate, bear the absolute configurations (1*S*,2*S*)-**2** and (1*R*,2*S*)-**3**. With this finding, it should be noted that the addition of hydrophobic polymer Amberlite XAD-7 resulted in no kinetic preference for the (*R*)-enzyme in any of the employed strains of the tested *Geotrichum* species by controlling the concentrations of both the substrate **1** and the products **2**–**5**. Based on the results (Table 1), exchange of the cultivation medium for water resulted in an increase of the enantiomeric excess of the major enantiomers **2** and

Table 1. Reduction of *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}carbamate **1** by whole cells of different yeasts

Entry (no)	Yeast[a] (strain)	Experimental conditions	Yield (mol%)		Ee[b] (major product; %)	
			Products **2/4**	Products **3/5**	Product **2**	Product **3**
1	GL (DBM 48)	Method A	5	17	75	55
2	GL (DBM 48)	Method B	6	18	80	61
3	GL (DBM 48)	Method C	10	13	31	25
4	GL (DBM 48)	Method D	11	16	>99	75
5	GC (DBM 4012)	Method A	22	32	85	81
6	GC (DBM 4012)	Method B	25	34	87	82
7	GC (DBM 4012)	Method C	9	14	95	68
8	GC (DBM 4012)	Method D	8	12	66	56
9	GC (DBM 4013)	Method A	16	19	64	50
10	GC (DBM 4013)	Method B	17	21	68	55
11	GC (DBM 4013)	Method C	7	8	34	42
12	GC (DBM 4013)	Method D	8	10	40	49
13	GC (DBM 4166)	Method A	37	54	>99	>99
14	GC (DBM 4166)	Method B	39	55	>99	>99
15	GC (DBM 4166)	Method C	9	13	79	64
16	GC (DBM 4166)	Method D	11	15	97	83
17	SC (DBM 2115)	Method A	45	48	90	>99
18	SC (DBM 2115)	Method B	46	48	93	>99
19	SC (DBM 2115)	Method C	40	44	87	>99
20	SC (DBM 2115)	Method D	40	45	89	>99

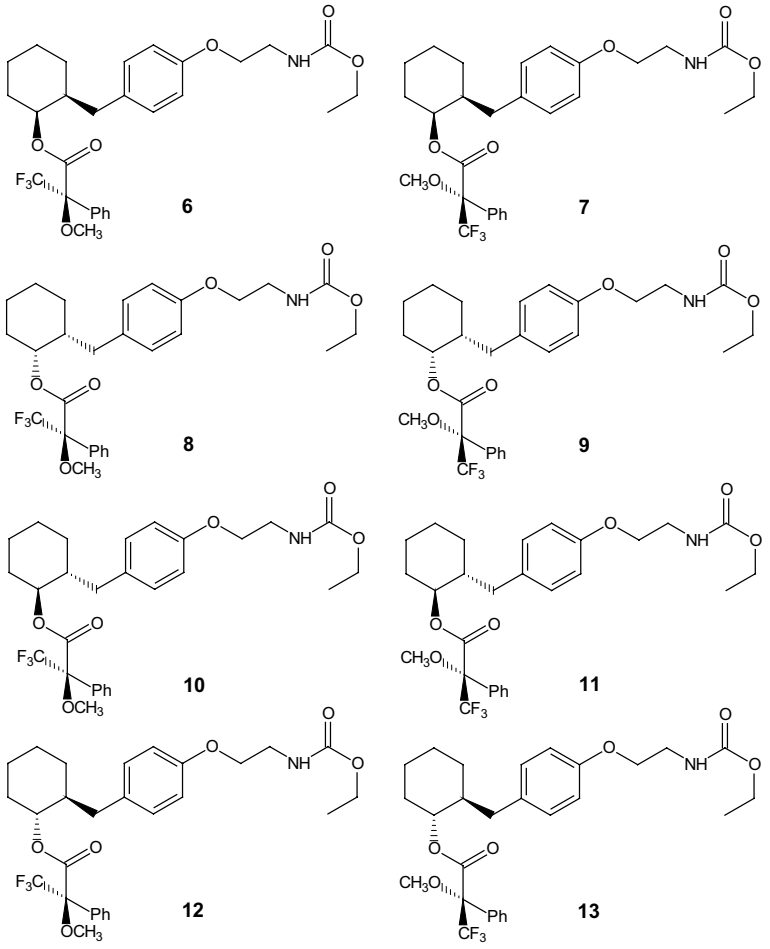
[a] GL = *G. ludwigii*, GC = *G. candidum*, SC = *S. cerevisiae*.
[b] Ee = enantiomeric purity.

3 of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate, when the strains DBM 48 (*G. ludwigii*), DBM 4013, and DBM 4166 (*G. candidum*) were used as whole cell bioreactors (entries 1–4 and 9–16 in Table 1). The chemical yields of the products of the enzymic reduction of the ketone **1** by the strains DBM 48 (*G. ludwigii*), DBM 4012, and DBM 4013 (*G. candidum*) were rather too low to have a synthetic importance at the moment (entries 1–12 in Table 1). In turn, the strain DBM 4166 (*G. candidum*) produced the products **2/4** and **3/5** in acceptable chemical yield and with high enantiomeric excess of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}-ethyl}carbamate only in the absence of hydrophobic polymer Amberlite XAD-7 (entries 13 and 14 in Table 1).

A reduction process mediated by *S. cerevisiae*, strain DBM 2115 (entries 17–20 in Table 1), resulted in (a) much higher chemical yields of the products **2/4** and **3/5**, and (b) in very high enantiomeric purity of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate in most of the bioreduction modifications used (entries 17–20 in Table 1), when compared with the results obtained by using whole cells of *G. ludwigii* and *G. candidum* as bioreactors. It should be stressed that the addition of hydrophobic polymer Amberlite XAD-7, which assisted in controlling the concentrations both of the substrate and of the products, resulted in no effect on the possible preference of the (*R*)-enzyme through the reaction kinetics of the studied enzymic process, and, therefore, this modification of the described enzymic reduction of the ketone **1** resulted in the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate with undesired absolute configuration being obtained (Scheme 1).

Determination of the enantiomeric purity of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate was done by two ways. The first method consists in a synthesis of diastereoisomeric derivatives **6**–**13** of the respective products **2/4** and **3/5**. The second method consists in application of a chiral stationary phase of a cyclodextrin type to separate enantiomers **2/4** and **3/5** of the respective alcohols using chiral HPLC analysis. Combination of both methods is convenient to have two independent analytical methods for assigning the absolute configuration of the major products **2** and **3**.

Convenient diastereoisomeric derivatives are the diastereoisomeric esters of the alcohols **2/4** and **3/5** with enantiomerically pure (*S*)-(+)- or (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (MTPCl, Mosher's chloride).[11] The diastereoisomeric mixtures of esters **6**–**13** (Scheme 2) can be separated by analytical HPLC and the content of each of the enantiomers **2**–**5** of the alcohol present in samples is calculated, based on the respective peak areas of the esters **6**–**13** in the HPLC chromatograms. The absolute configuration of the esters **6**–**13** can be assigned by measuring their ^{1}H and ^{19}F NMR spectra.[12] Enantiomeric purity and the absolute configuration of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}-carbamate were determined by applying of this approach. Assigning of the absolute configuration to the C(2) stereogenic center was based on the differences of the chemical shifts of the signals of both hydrogen atoms of the CH_2–Ar (benzyl) group, which are not equivalent due to their chiral environment. An up-field shift of the signals of benzyl hydrogen atoms in the ^{1}H NMR spectra of the diastereoisomeric esters **6**–**13**, when comparing the esters derived from (*S*)-(−)-MTPA (**7**, **9**, **11**, and **13**) with the esters derived from (*R*)-(+)-MTPA



Scheme 2.

(**6**, **8**, **10**, and **12**) is consistent only with the (*S*)-absolute configuration at the C(2) stereogenic center of **2** and **3**.[12] The evaluation of the ^{19}F NMR data of the diastereoisomeric esters **6**–**13** led to the same conclusion. A downfield shift of the signal of the CF_3 group in the (*R*)-MTPA esters **6**, **8**, **10**, and **12** in comparison with the same shift observed in the (*S*)-MTPA esters **7**, **9**, **11**, and **13** is in agreement with the expected displacement of the CF_3 group from the eclipsed arrangement with the carbonyl group in consequence of a steric interaction of the bulkier groups, that is, of the phenyl group of the MTPA part of the esters **6**–**13**, and of the benzyl substituent at C(1) of the alcoholic part of the esters **6**–**13**. The selected data are given in Table 2.

The second method, based on the chromatographic behaviour of the enantiomeric mixtures **2/4** and **3/5** of the alcohols using a chiral HPLC Nucleodex β-OH column, filled with a chiral β-cyclodextrin-based stationary phase, was applied to confirm the above-described structure assignments. The results obtained from the chiral HPLC analysis were in good agreement with those obtained by analyzing the diastereoisomeric esters **6**–**13** of the studied enantiomers **2/4** and **3/5** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}-ethyl}carbamate.

Table 2. The ^1H and ^{19}F NMR based assignment of the absolute configuration of the 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA) esters **6**–**13**

Compound	δ [H–CH′Ar][a]	δ [H′–CHAr] [a]	δ (CF_3)[b]	AC[c]
6	2.25	2.45	−67.13	1*S*,2*S*
7	2.33	2.50	−67.29	1*S*,2*S*
8	2.33	2.52	−67.31	1*R*,2*R*
9	2.25	2.45	−67.12	1*R*,2*R*
10	2.07	2.70	−67.44	1*R*,2*S*
11	2.17	2.87	−67.54	1*R*,2*S*
12	2.17	2.89	−67.55	1*S*,2*R*
13	2.07	2.69	−67.46	1*S*,2*R*

[a] ^1H NMR data based on the chemical shifts of the signals of the hydrogen atoms in the benzyl (CH_2Ar) group of the alcohol part of the MTPA esters **6**–**13**.

[b] ^{19}F NMR data based on the chemical shifts of the signals of the fluorine atoms in the CF_3 functionality of the acid part of the MTPA esters **6**–**13**.

[c] AC = absolute configuration of **2** and **3**, the major enantiomers of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate.

3. Conclusion

To summarize the results of this investigation, four conclusive remarks should be given:

(a) The presence of hydrophobic polymer Amberlite XAD-7 (entries 3, 4, 7, 8, 11, 12, 15, 16, 19, and 20 in Table 1) resulted in no affect on the reaction kinetics of the enzymic reduction. The (*S*)-enzymes were found to be the responsible mediating factors in whole cells of all microorganisms used (*G. ludwigii*, *G. candidum*, and *S. cerevisiae*) for reduction of ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}-ethyl}carbamate **1**, and their biocatalytic activity resulted in the production of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)-methyl]phenoxy}ethyl}carbamate bearing the undesired absolute configurations with different enantiomeric purity (Table 1).

(b) Using the strains DBM 48 (*G. ludwigii*), DBM 4012, DBM 4013, and DBM 4166 (*G. candidum*), the chemical yields of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]-phenoxy}ethyl}carbamate were low (entries 1–12 and 15–16 in Table 1). Higher enantiomeric excesses of the major enantiomers **2** and **3** of the alcohol were achieved in the presence of hydrophobic polymer Amberlite XAD-7 only when the strain DBM 48 (*G. ludwigii*) was employed (entry 4 in Table 1). Using the strain DBM 4166 (*G. candidum*) in the absence of hydrophobic polymer Amberlite XAD-7 (entries 13 and 14 in Table 1) resulted in achieving chemical yields of the synthetically interesting products **2/4** and **3/5** being achieved, and with the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate being obtained with >99% enantiomeric purity.

(c) Using the strain DBM 2115 of *S. cerevisiae* (entries 17–20 in Table 1), both the chemical yields of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate, and their enantiomeric excess, were high enough for having a synthetic importance. No effect of hydrophobic polymer Amberlite XAD-7 on the reaction course mediated by the strain DBM 2115 of *S. cerevisiae* was observed.

(d) Application of hydrophobic polymer Amberlite XAD-7 for assisting in changing the reaction course of the studied enzymic processes with the employed microorganisms resulted only in slight differences proved by different enantiomeric excesses of the major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}carbamate, and it has only limited synthetic impact.

4. Experimental

4.1. General

A Unimax 1010 incubator (Heidolph, Germany) equipped with controlled heating and shaking plate for up to 5 shake-flasks (250 mL size) was used for performing the reactions mediated by microorganisms. The ^{1}H NMR and the ^{13}C NMR spectra were recorded on a Varian UNITY 500 spectrometer (in a FT mode) at, respectively, 499.8 and 125.7 MHz in deuteriochloroform using tetramethylsilane (δ 0.0) as internal reference. The ^{19}F NMR spectra were recorded at 470.3 MHz in deuteriochloroform using hexafluorobenzene as external reference (δ −162.9). IR spectra were recorded in chloroform on a Bruker IFS 88 instrument. MS (EI) were recorded on a VG analytical 70–250 SE mass spectrometer, ZAB-EQ (BEQQ configuration) at 70 eV. Preparative column chromatography was performed on a silica gel type 60 (particle size 0.04–0.063 mm; Fluka, Switzerland). TLC was performed on aluminum sheets precoated with silica gel 60 (Merck, Germany). Analytical HPLC was carried out on a TSP (Thermoseparation Products, USA) instrument equipped with a ConstaMetric 4100 Bio pump and a SpectroMonitor 5000 UV DAD. The analyses of the chiral products were performed on a chiral Nucleodex β-OH column (150 × 4 mm; Macherey-Nagel, Germany) using a methanol/water (4:1, v/v) as mobile phase at 0.3 mL min^{-1}. The eluate was monitored at 220, 254, and 275 nm and UV spectra were run from 200 to 300 nm.

4.2. Microorganisms

G. ludwigii, strain DBM 48 and *G. candidum*, strains DBM 4166, DBM 4012, and DBM 4013 were obtained from the Institute of Chemical Technology (Prague, Czech Republic). *S. cerevisiae*, strain DBM 2115, was obtained from the Research Institute of Fermentation Industry (Prague, Czech Republic). All microorganisms (yeasts) were grown on medium consisted of glucose (30 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), corn steep (10 g L^{-1}), KH_2PO_4 (1 g L^{-1}), K_2HPO_4 (1 g L^{-1}), $NaNO_3$ (2 g L^{-1}), KCl (0.5 g L^{-1}), and $FeSO_4 \cdot 7H_2O$ (0.02 g L^{-1}).

4.3. Enzymic reduction of ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}carbamate 1

The yeasts were cultivated in the cultivation medium (100 mL) at 27 ± 1 °C for 48 h. Ethyl *N*-{2-{4-[(2-oxocyclohexyl)methyl]phenoxy}ethyl}carbamate **1** (120 mg; 0.376 mmol) dissolved in acetone (0.5 mL) was added to the yeast cells using four different modifications of the experiment. Method A: The substrate **1** was added directly to the original suspension of the yeast cells in the cultivation medium. Method B: The substrate **1** was added to the centrifuged yeast cells, which were suspended in water (90 mL) before the substrate **1** was added. Method C: The substrate **1** and the hydrophobic polymer Amberlite XAD-7 (2 g per flask) were added directly to the original suspension of the yeast cells in the cultivation medium. Method D: The substrate **1** and the hydrophobic polymer Amberlite XAD-7 (2 g per flask) were added to the centrifuged yeast cells, which were suspended in water (90 mL) before the substrate **1** and the hydrophobic polymer Amberlite XAD-7 were added. Then the reaction was maintained under shaking at 27 ± 1 °C for 7 days in 250 mL shake-flasks using a Unimax incubator. Thereafter, the organic compounds were extracted with ether (4 × 100 mL). The extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was applied on the

top of a column filled with silica gel, and the respective diastereoisomeric products **2/4** and **3/5** were separated. Their chemical yields and enantiomeric purity values are given in Table 1.

Product **2/4**: ^{1}H NMR ($CDCl_3$): 1.24 (t, $J = 7.2$, 3H), 1.26–1.83 (m, 9H), 2.48 (dd, $J = 7.5$, 13.5, 1H), 2.65 (dd, $J = 7.3$, 13.5, 1H), 3.56 (br q, $J = 5.4$, 2H), 3.78 (dt, $J = 2.5$, 2.5, 4.3, 1H), 4.00 (t, $J = 5.1$, 2H), 4.12 (q, $J = 7.2$, 2H), 5.20 (br s, 1H), 6.80 (m, 2H), 7.10 (m, 2H); ^{13}C NMR ($CDCl_3$): 14.32 (q), 20.21 (t), 25.07 (t), 26.10 (t), 33.00 (t), 37.46 (t), 40.35 (t), 43.51 (d), 60.70 (t), 66.73 (t), 68.28 (d), 114.05 (d), 129.80 (d), 133.51 (s), 156.47 (s), 156.63 (s); IR (CCl_4): 3630, 3465, 1729, 1516, 1246, 979 cm^{-1}; MS (EI): m/z 321 (M^+, 6), 116 (100), 88 (32), 44 (6); $[\alpha]_D^{24} = +12.15$ (c 0.52, $CHCl_3$).

Product **3/5**: ^{1}H NMR: 1.24 (t, $J = 7.1$, 3H), 1.28–1.61 (m, 9H), 2.33 (dd, $J = 9.0$, 13.5, 1H), 3.08 (dd, $J = 4.0$, 13.5, 1H), 3.27 (dt, $J = 4.4$, 9.5, 9.5, 1H), 3.56 (br q, $J = 5.4$, 2H), 4.00 (t, $J = 5.1$, 2H), 4.11 (q, $J = 7.1$, 2H), 5.12 (br s, 1H), 6.80 (m, 2H), 7.08 (m, 2H); ^{13}C NMR: 14.62 (q), 24.84 (t), 25.36 (t), 29.86 (t), 35.76 (t), 37.96 (t), 40.65 (t), 47.00 (d), 60.92 (t), 66.93 (t), 74.36 (d), 114.15 (d), 130.26 (d), 133.20 (s), 156.56 (s), 156.62 (s); IR (CCl_4): 3625, 3465, 1726, 1513, 1243, 1069, 1052 cm^{-1}; MS (EI): m/z 321 (M^+, 6), 116 (100), 88 (24); $[\alpha]_D^{24} = +16.35$ (c 0.65, $CHCl_3$).

4.4. Synthesis of 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid esters 6–13

A general procedure used for the synthesis of the (*R*)- and (*S*)-MTPA (3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid; Mosher's acid) esters on a milligram scale starting from the (*S*)-(+)- or (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (MTPCl, Mosher's chloride) was already described in details.[11] The esters **6–13** (Scheme 1) were obtained in quantitative yields. Their selected ^{1}H and ^{19}F NMR data, which are important for assignment of the absolute configuration of the parent major enantiomers **2** and **3** of ethyl *N*-{2-{4-[(2-hydroxycyclohexyl)methyl]phenoxy}ethyl}-carbamate, are given in Table 2 and discussed in Section 2.

Acknowledgements

This study was supported by the grants 203/02/0166 (GACR) and S4055104 (GAAVCR). The authors thank Mrs. M. Wimmerová for her skillful technical assistance.

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