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Chemoenzymatic Synthesis of "α-Bichiral" Synthons. Application to the Preparation of Chiral Epoxides

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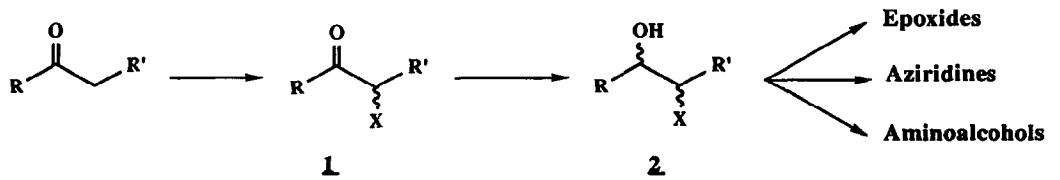
Abstract : Microbiological reduction of 3-bromo-2-octanone and 3-azido-2-octanone led to all the stereoisomers of 3-bromo-2-octanol and 3-azido-2-octanol. Chiral 2,3-epoxyoctanes were prepared from the 3-bromo-2-octanols.

Optically pure hydroxylated α-substituted compounds are particularly useful synthons for stereospecific synthesis. By using different substituents, they can afford chiral epoxides, aziridines or aminoalcohols.

To obtain all the stereoisomers of "bichiral" synthons of this type, we used the results of microbiological reductions studied previously. Reduction experiments with numerous carbonyl compounds including monoketones[1], β-diketones[2], α-diketones[3], propargyl ketones[4] and β-ketosulfones[5] showed that both enantiomers of the corresponding alcohols could be obtained by using appropriate microorganism and bioconversion conditions.

With different substituents α to the hydroxyl (X = Br, N_3 or O), various diastereoisomers of bichiral synthons **2** can be obtained by the chemoenzymatic sequence shown below, in which the key step is the microbiological reduction of ketone **1**.



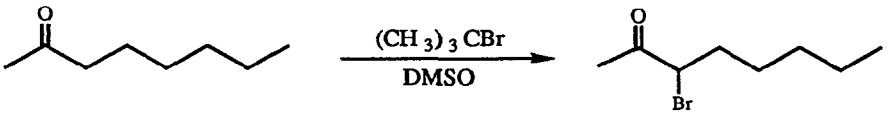
1 **2**

We describe here the preparation of the stereoisomers of chiral bromohydrins **2** (X = Br) and azidoalcohols **2** (X = N_3) obtained from 2-octanone (R = CH_3, R' = n-C_5H_{11}), together with the synthesis of all the chiral isomers of 2,3-epoxyoctane from these bromohydrins.

I - Synthesis of Chiral 3-Bromo-2-Octanols

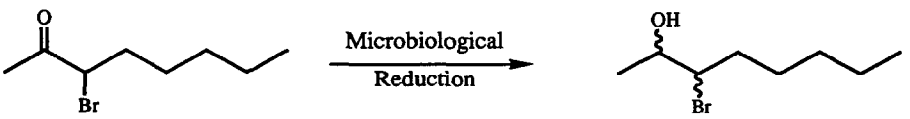
First we set out to synthesize all the isomers of the α-bromoalcohols by microbiological reduction of the corresponding α-bromoketones.

By this means we prepared 3-bromo-2-octanone **3** from 2-octanone by the method of Armani *et al.*[6] as shown below, with a yield of 80 % :



3

On the basis of our previous results for the microbiological reduction of carbonyl compounds, we screened our strains to identify those which together would afford all the isomers of 3-bromo-2-octanol **4**.



4

We selected : Bakers' yeast and the fungi *Aspergillus niger*, *Beauveria sulfurescens*, *Geotrichum candidum* and *Mortierella isabellina*. We used the bacterium *Lactobacillus kefir* as it was reported by Wong *et al*[7] to reduce ketones to alcohols with R configuration. The yeast was used freeze-dried under non-fermenting conditions, *i.e.* suspended in water without added sugar. Bioconversions with the other microorganisms were carried out using washed resting cells. All the microorganisms reduced 3-bromo-2-octanone after 24 h reaction with varied chemical and optical yields. The results are collected in Table I.

Table I : Microbiological Reduction of 3-Bromo-2-Octanone

	syn Bromhydrin			*anti* Bromhydrin			
	$[\alpha]_J^{25}$	e.e.	Conf.	$[\alpha]_J^{25}$	e.e.	Conf.	Yield
Freeze-dried Bakers' yeast	- 38	>98 %	(2S,3S)	+ 40	>98%	(2S,3R)	50 % (50/50)
Mortierella isabellina	- 38	>98 %	(2S,3S)	+ 39	97 %	(2S,3R)	40 % (55/45)
Beauveria sulfurescens	- 29	77 %	(2S,3S)	+ 32	80 %	(2S,3R)	62 % (30/70)
Aspergillus niger	+ 38	>98 %	(2R,3R)	-	-	-	48%
Geotrichum candidum	+ 26	70 %	(2R,3R)	- 32	80 %	(2R,3S)	51 % (60/40)
Lactobacillus kefir	+ 38	>98 %	(2R,3R)	- 37	92 %	(2R,3S)	40 % (40/60)

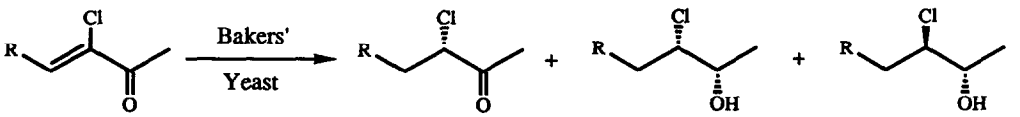
All the reactions described are enantiogenic, a single alcohol configuration was obtained in each case. Generally, they yielded two diastereoisomers of the four possible bromoalcohols. These were readily separated by chromatography on silica column. The yields recorded in the last column of Table I are overall yields of diastereoisomers after work-up. The proportions of each diastereoisomer are given in brackets.

With the yeast and *M. isabellina*, the (2S) alcohol formed gave equivalent amounts of *syn* (2S,3S) et *anti* (2S,3R) diastereoisomers with very high enantiomeric excesses. With *B. sulfurescens*, the *anti* (2S,3R) isomer predominated (70 %) over the *syn* (2S,3S) isomer and the enantiomeric excess of neither isomer was not very high. The result obtained with *A. niger* was noteworthy, regardless of the reaction time, only the *syn* (2R,3R) diastereoisomer was obtained, with a very high enantiomeric excess. Unreacted optically active bromoketone was also recovered. With *G. candidum* and *L. kefir* the alcohol formed had the (2R) configuration; a mixture of (2R,3R) and (2R,3S) diastereoisomers was obtained in which the predominant isomer (60 %) was *syn* with *G. candidum* and *anti* with *L. kefir*. The highest enantiomeric excess for the (2R,3S) isomer was obtained with *L. kefir*.

The enantiomeric excesses were determined from the esters obtained by the reaction of each bromohydrin with the chloride of (+)-(S)-O-acetyllactic acid by gas phase chromatography. The proportion of each diastereoisomeric ester was measured and the enantiomeric excess calculated for each bromohydrin.

The optically active 3-bromo-2-octanols have not been described in the literature before. We assigned their absolute configurations by analogy with published data and by chemical correlation:

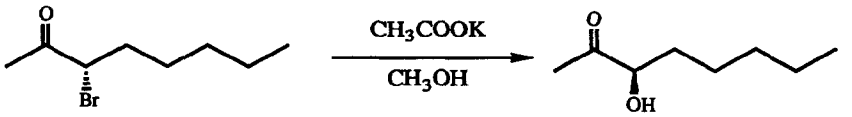
- We found that the *syn* and *anti* 3-bromo-2-octanols isolated from the reduction with bakers' yeast had the same structure and the same optical rotation sign as the *syn* and *anti* chlorohydrins described by Japanese authors[8] studying the reduction of unsaturated α-chloroketones by the same microorganism. These authors described the saturated (3S) chloroketone and the diastereoisomeric *syn* (2S,3S) and *anti* (2S,3R) chlorohydrins for R = n-C_5H_{11}.



(3S) [α]D = - 36.8 (2S,3S) [α]D = - 33.4 (2S,3R) [α]D = +26.6

By comparison, we therefore tentatively assigned the (2S,3S) configuration to the *syn* bromohydrin and the (2S,3R) configuration to the *anti* bromohydrin obtained with bakers' yeast, and the opposite configurations to the enantiomeric bromohydrins.

- By chemical correlation, we determined the absolute configuration of optically active 3-bromo-2-octanone recovered unchanged after reduction with *A.niger*. Treatment of this bromoketone with potassium acetate in the presence of methanol according to Burgstahler and Nordin[9] gave optically active 3-hydroxy-2-octanone with a negative optical rotation. In previous work[10] we described the synthesis of (3S)-3-hydroxy-2-octanone obtained by the microbiological reduction of 2,3-octanedione by *B. sulfurescens*, which has a positive optical rotation. The 3-hydroxy-2-octanone formed here from the bromoketone must therefore have (3R) configuration.

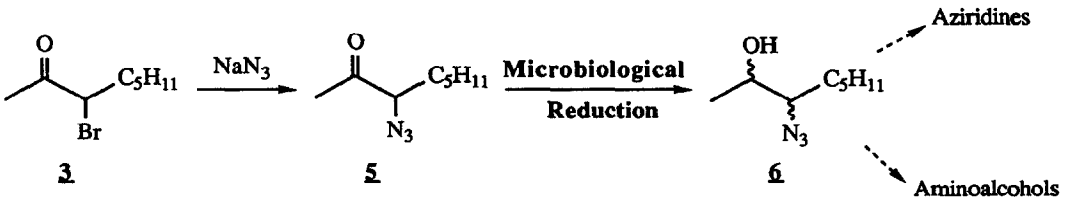


As the conversion of the bromoketone to the hydroxyketone takes place with a change of configuration, the 3-bromo-2-octanone, which did not react with *A. niger*, must therefore have (3S) configuration, which confirms that only (3R)-3-bromo-2-octanone is reduced by this microorganism and that 3-bromo-2-octanol obtained with *A. niger* has (2R,3R) absolute configuration. Thus by successive comparisons the configurations of all the bromohydrins isolated with the various microorganisms used can be confirmed.

These results show that by an appropriate choice of microorganism all four stereoisomers of 3-bromo-2-octanol can be obtained with very high enantiomeric excesses. The best results were obtained for the *syn* (2S,3S) and (2R,3R) isomers with respectively *M. isabellina* and *A. niger*, and for the *anti* (2S,3R) and (2R,3S) isomers with respectively bakers' yeast and *L. kefir*.

II - Synthesis of Chiral 3-Azido-2-Octanols

We went on to study the microbiological reduction of α-azidoketones **1** (X = N_3), with a view to converting these into chiral aziridines or aminoalcohols. We carried out the synthesis of 3-azido-2-octanols **6** by microbiological reduction of 3-azido-2-octanone **5** obtained by the action of sodium azide on 3-bromo-2-octanone **3** according to Boyer and Straw[11].



3 **5** **6**

The microbiological reduction of azidoketone **5** was studied with the same bioconversion methods and the same microorganisms as those used for 3-bromo-2-octanone. Strain screening afforded microorganisms able to produce all the stereoisomers of 3-azido-2-octanol **6** with the best chemical and optical yields.

The results are collected in Table II. Here also, as in the case of the bromoketone, the reduction of the carbonyl function was enantiogenic, and in all cases the reaction yielded two diastereoisomers of 3-azido-2-octanol which could be separated and purified by column chromatography. The yields recorded in the last column of Table II are overall yields of pure diastereoisomers. The proportions of each stereoisomer are given in brackets.

The enantiomeric excesses were determined by gas phase chromatography either from esters after reaction of the azidooctanol with the chloride of (+)-(S)-O-acetyllactic acid, or by analysis of the azidooctanols on a chiral column.

No optically active 3-azido-2-octanol has been described in the literature. First of all, we assigned the absolute configuration of each isomer by analogy with the results observed with each microorganism for the reduction of 3-bromo-2-octanone.

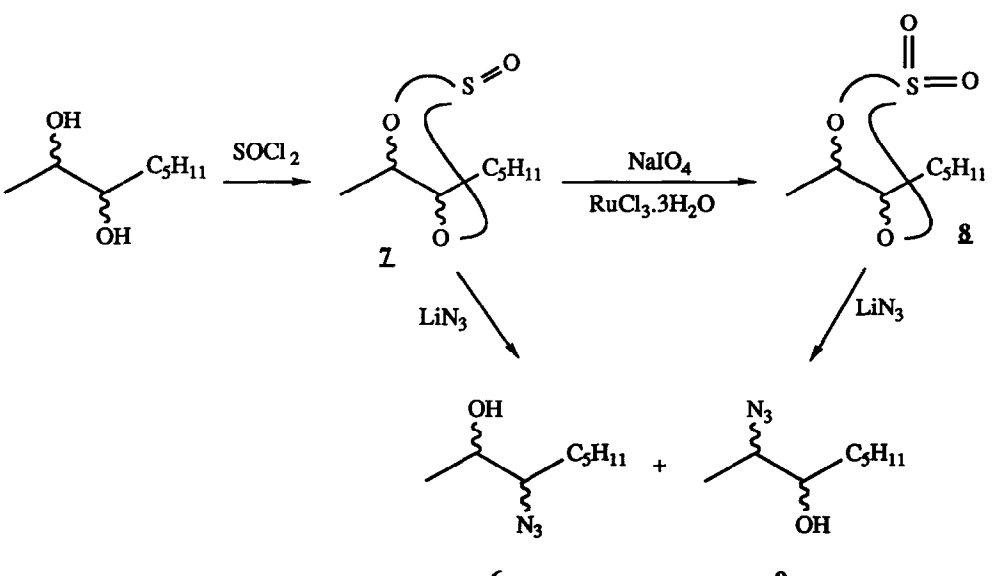
Table II : Microbiological Reduction of 3-Azido-2-Octanone

	syn			*anti*			Yield
	$[\alpha]_D^{25}$	e.e.	Conf.	$[\alpha]_D^{25}$	e.e.	Conf.	
Freeze-dried Bakers' yeast	+ 19	95 %	(2S,3S)	+ 8	> 98 %	(2S,3R)	55 % (50/50)
Mortierella isabellina	+ 19	95 %	(2S,3S)	+ 7.5	96 %	(2S,3R)	53 % (50/50)
Beauveria sulfurescens	+ 20	97 %	(2S,3S)	+ 5	68 %	(2S,3R)	50 % (70/30)
Aspergillus niger	- 17	82 %	(2R,3R)	- 7	88 %	(2R,3S)	55 % (50/50)
Lactobacillus kefir	- 20	97 %	(2R,3R)	- 8	> 98 %	(2R,3S)	48 % (50/50)

With bakers' yeast, *M. isabellina* and *B. sulfurescens*, alcohol (2S) was formed; the best enantiomeric excesses were obtained with *B. sulfurescens* (ee = 97 %) for the *syn* isomer with (2S,3S) configuration and bakers' yeast (ee > 98 %) for the *anti* isomer with (2S,3R) configuration.

With *A. niger*, both enantiomers of the azidoketone were reduced with predominant formation of the (2R) alcohol, but the enantiomeric excesses of the isomeric azidoalcohols were not excellent (82% and 88% respectively for *syn* and *anti*). The best enantiomeric excesses for these two diastereoisomers were obtained with bacterium *L. kefir*, 97 % for the (2R,3R) and 98 % for the (2R,3S).

We confirmed the assignments of the absolute configurations by stereospecific synthesis of chiral isomers of 3-azido-2-octanol. This involved converting chiral α-diols of known absolute configuration into azidoalcohols *via* the formation of cyclic sulfites or cyclic sulfates by the method of Sharpless *et al*[12,13].



The cyclic sulfite 7 was obtained quantitatively by the action of thionyl chloride on 2,3-octanediol. It was then oxidized to sulfate 8 by a mixture of ruthenium chloride and sodium periodate[12]. An attempt to cleave sulfate 8 with lithium azide in the conditions of Sharpless[13] (reflux overnight in THF) was unsuccessful and the sulfate was recovered unchanged. However, we succeeded in cleaving the cyclic sulfate using the conditions of Lohray and Ahuja[14] (overnight at 115-120°C in dimethylformamide) to obtain the azidooctanols. Under these conditions, DMF has to be removed under reduced pressure, and the resulting lithium salts have to be acid-hydrolysed. If the cyclic sulfite 7 is cleaved in DMF, the mixture of azidooctanols is obtained directly. The cleavage reaction, of the sulfite or the sulfate, takes place at both the ring carbons, giving a mixture of 3-azido-2-octanol 6 and 2-azido-3-octanol 9 which are separated and purified by column chromatography. The absolute configuration of the azidooctanols formed in this way is deduced from that of the starting α-diol with inversion of the configuration on the carbon bearing the azido group as shown by Sharpless *et al*[13] and Lohray and Ahuja[14].

In previous work[3], we studied the microbiological reduction of acyclic α-diones and we described the preparation of all the isomers of the chiral 2,3-octanediols with very high enantiomeric excesses. The results obtained for the transformation of two isomers of 2,3-octanediol into azidoalcohols are collected in Table III. The yields recorded in the last column of Table III are overall yields of pure azidooctanols calculated from the α-diols. Enantiomeric excesses were determined under the same conditions as for the 3-azido-2-octanols.

Table III : Synthesis of Azidooctanols from α-Diols

2,3-Octanediol	2-Azido-3-Octanol			3-Azido-2-Octanol			
Configuration	Configuration	$[\alpha]_D^{25}$	ee	Configuration	$[\alpha]_D^{25}$	ee	Yield
(2S,3S)	(2R,3S)	- 51	> 98 %	(2S,3R)	+ 8	> 98 %	80 % (50/50)
(2S,3R)	(2R,3R)	- 50	> 98 %	(2S,3S)	+ 21	> 98 %	80 % (50/50)

We first carried out the reactions on the (+)-(2S,3R)-2,3-octanediol obtained by the reduction of 2,3-octanedione with bakers' yeast[3]. After cleavage of the cyclic sulfite we obtained two *syn* azidooctanols as shown by gas phase chromatography. After purification, it was found that the isomer with the longest retention time had the same physical constants (^1H and ^{13}C NMR, IR, optical rotation) as those observed for the *syn* azidooctanol from the reduction of 3-azido-2-octanone by bakers' yeast, *M.isabellina* or *B. sulfurescens*. As we started with diol (2S,3R) and since there is an inversion of configuration at carbon 3, this isomer must be (2S,3S)-3-azido-2-octanol. The second azidooctanol must therefore be 2-azido-3-octanol with (2R,3R) absolute configuration . Both azidooctanols were obtained with very high enantiomeric excesses (ee > 98 %).

We also used as starting material (-)-(2S,3S)-2,3-octanediol obtained by reduction of 2,3-octanedione by *B. sulfurescens*[3]. After cleavage of the cyclic sulfite we isolated two *anti* azidooctanols. The isomer with the

longest retention time had the same physical constants (^1H and ^{13}C NMR, IR, optical rotation) as *anti* 3-azido-2-octanol obtained by reduction of azidooctanone by bakers' yeast, *M. isabellina* or *B. sulfurescens*. Since we started with a (2S,3S) diol and since there is an inversion of configuration at carbon 3, this isomer must be (2S,3R)-3-azido-2-octanol. The second azidooctanol must therefore be *anti* 2-azido-3-octanol with (2R,3S) configuration . Both azidoalcools were also obtained with very good enantiomeric excesses.

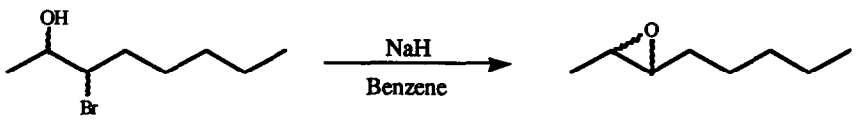
These two reactions confirm the absolute configuration of the 3-azido-2-octanols obtained by direct microbiological reduction. In addition, they afforded a synthesis of two optically pure isomers of 2-azido-3-octanol.

Hence the microbiological reduction of 3-azido-2-octanone gave access to all the stereoisomers of 3-azido-2-octanol ; the highest enantiomeric excesses were obtained with *B. sulfurescens* for the (2S,3S) isomer and with bakers' yeast for the (2S,3R) isomer. For the (2R,3R) and (2R,3S) isomers of these azidoalcohols the highest optical yields were obtained with the bacterium *L. kefir* .

III - Synthesis of Chiral Isomers of 2,3-Epoxyoctane

Once the chemoenzymatic synthesis of two types of α-bichiral synthons had been achieved, these were used as precursors of chiral compounds interesting in organic synthesis.

The first application was the synthesis of chiral isomers of 2,3-epoxyoctane **10** from the isomers of 3-bromo-2-octanol **4**. These were treated with sodium hydride in benzene according to Furstoss *et al*[15] to give the epoxides directly.



4 **10**

The results of these reactions are collected in Table IV. The yields were calculated for the purified epoxide and the enantiomeric excesses were determined by gas phase chromatography on a chiral column.

The absolute configuration of each isomer of 2,3-epoxyoctane was deduced from that of the starting 3-bromo-2-octanol. The coupling constant between the two epoxide protons was 4.2 Hz for the epoxides derived from the *syn* bromhydrins and 2.1 Hz for those obtained from the *anti* bromhydrins. These values agree with those given in the literature for the *cis* and *trans* epoxides[16]. As the Japanese authors[7] showed for the chlorohydrins, we observed that a *syn* bromohydrin gave a *cis* epoxide while an *anti* bromohydrin gave a *trans* epoxide. Epoxide formation takes place with inversion of configuration at the 3-carbon bearing the bromine atom.

Table IV : Synthesis of chiral 2,3-epoxyoctanes

Bromhydrin		2,3-Epoxyoctane			
Conf.	From	Yield	$[\alpha]_{\gamma}^{25}$	e.e.	Conf.
(2S,3S)	*Mortierella isabellina*	60 %	+ 8	> 98 %	(2S,3R)
(2R,3R)	*Aspergillus niger*	58 %	- 8	> 98 %	(2R,3S)
(2S,3R)	Freeze-dried Bakers' yeast	61 %	+ 5	> 98 %	(2S,3S)
(2R,3S)	*Lactobacillus kefir*	60 %	- 4,5	92 %	(2R,3R)

Thus, we were able to synthesize all the isomers of 2,3-epoxyoctane from 2-octanone in three steps with good yields and very high enantiomeric excesses.

Our successful preparation of chiral 2,3-epoxyoctanes relied on biological catalysts to carry out the key step in the synthetic route, namely the enantiogenic reduction of a carbonyl function.These results illustrate the usefulness of bioconversion reactions in organic synthesis.

We are presently studying the conversion of azidooctanols to chiral aziridines and aminoalcohols, together with the synthesis of novel aromatic bromohydrins and azidoalcohols.

EXPERIMENTAL SECTION

1 - GENERAL METHODS

CHROMATOGRAPHY: Gas chromatography (GC) was performed using an instrument equipped with a flame ionisation detector and a 50 m x 0.32 mm capillary column coated with Carbowax 20 M for analytical analysis or a 25 m x 0.25 mm capillary column coated with Lipodex E (modified γ-cyclodextrine) for determination of enantiomeric excesses. The carrier gas was hydrogen at 65 kPa. Oven temperature was 90°C for 5 min and then 90°C to 170°C at 4°C/min for bromooctanols and azidooctanols, and 70°C for epoxides. Column chromatography was performed on silica gel 60 Merck (70-230 µm). Eluents varied and are indicated in each case.

SPECTROSCOPY AND ANALYTICAL METHODS: After bioconversion, crude mixtures were analysed by GC and the retention times of the reduction products or epoxides were compared with those of chemically obtained racemates. Optical rotations of the compounds were determined at 25°C for the mercury J line (λ = 578 nm, c in g/mL). Enantiomeric excesses were determined by GC for epoxides and azidooctanols using the Lipodex E column and for esters of bromo- or azidooctanols, obtained after reaction with the chloride of (+)-(S)-O-acetyl-lactic acid according to[17], using the Carbowax column. NMR analyses were carried out on purified compounds in $CDCl_3$. For ^1H (300.13 MHz) and ^{13}C (75.47 MHz) NMR spectra, the chemical shifts were relative to chloroform. High Resolution Mass Spectrometry (HRMS) and microanalysis were performed by the Service Central d'Analyses du CNRS Vernaison (France).

MICROBIOLOGICAL METHODS: The microorganisms were all laboratory-grown except freeze-dried bakers' yeast which was a commercial product (ANCEL S.A. Strasbourg). Preculture and culture conditions for fungi *Aspergillus niger* ATCC 9142, *Beauveria sulfurescens* ATCC 7159, *Mortierella isabellina* NRRL 1757 and *Geotrichum candidum* CBS 233-76 have already been described elsewhere[3]. *Lactobacillus kefir* DSM 20587 was grown as recommended by ATCC on lactobacillus MRS broth, available from DIFCO.

BIOCONVERSION CONDITIONS:

. General case: Bioconversions with microorganisms in metabolic resting phase were performed as previously described[3] using 5g of biomass to convert 50 µL of substrate.

. Commercial freeze-dried bakers' yeast: 1 g of freeze-dried bakers' yeast was placed in a 500 mL conical flask containing 50 mL of distilled water and 50 µL of substrate. After incubation at 27°C on a rotating table set at 200 rpm, the mixture was spun for ten minutes at 8000 rpm. The liquor was then continuously extracted with ether for 24 h. The ether phase was dried on $MgSO_4$ and the solvent evaporated off under vacuum.

. *Lactobacillus kefir:* After 24 h cultivation at 30°C under nitrogen, the culture was spun and the cells washed with an aqueous solution of NaCl (8g/L) and recovered (5g/L). The bioconversions were carried out with 6 g of wet cells in 50 mL of water and 50 µL of substrate; work-up was as in the general case.

2 - SUBSTRATES

2-Octanone was a commercial product (Lancaster Synthesis Ltd.).

3-bromo-2-octanone **3** : To a mixture of 5.2 g of 2-octanone and 6.2 g of anhydrous dimethylsulfoxide was added 22.6 g of *t* -butylbromide The mixture was stirred and heated at 60-65°C for 12 h. The reaction mixture was diluted with water and extracted three times with Et_2O. The organic phase was washed with water and dried on $MgSO_4$. After purification by column chromatography on silicagel (300 g) (eluent: pentane / ether 98/2 v/v), 6.7 g of pure 3-bromo-2-octanone was obtained (yield: 80 %).

Retention time: 680 s. ^1H NMR (300.13 MHz) δ: 0.88 (t, 3H, J = 6 Hz); 1.20 to 1.55 (m, 6H); 1.82 to 2.08 (m, 2H); 2.32 (s, 3H); 4.21 (t, 1H, J = 7.5 Hz). ^{13}C NMR (75.47 MHz) δ: 13.9 (C-8); 22.4 (C-7); 26.0 (C-1); 27.0 (C-6); 31.2 (C-5); 33.6 (C-4); 54.5 (C-3); 202.0 (C-2). Anal. Calcd for $C_8H_{15}BrO$: C: 46.39; H: 7.30; Br: 38.58. Found: C: 46.04; H: 7.38; Br: 38.40.

3-azido-2-octanone 5 : To a mixture of 20.7 g of 3-bromo-2-octanone and 12 mL of acetic acid dissolved in 70 mL of ethanol, with stirring and in an ice-bath, was added 13 g of sodium azide. The mixture was stored in a refrigerator for 24 hours and stirred occasionally. The ethanol was evaporated off under vacuum and the residue was diluted with water and extracted three times with Et_2O. The organic phase was washed with saturated aqueous $NaHCO_3$ and dried on $MgSO_4$. After evaporation of the solvent, we obtained 15.2 g of pure 3-azido-2-octanone (yield: 96 %).

Retention time: 720 s. ^1H NMR (300.13 MHz) δ: 0.91 (t, 3H, J = 6 Hz); 1.24 to 1.52 (m, 6H); 1.60 to 1.86 (m, 2H); 2.23 (s, 3H); 3.75 to 3.86 (dd, 1H, J = 5 Hz and J = 3.5 Hz). ^{13}C NMR (75.47 MHz) δ: 13.9 (C-8); 22.4 (C-7); 25.5 (C-6); 26.8 (C-1); 30.7 (C-5); 31.4 (C-4); 69.0 (C-3); 205.3 (C-2). Anal. Calcd. for $C_8H_{15}N_3O$: C: 56.78; H: 8.93; N: 24.83. Found: C: 56.70; H: 8.92; N: 25.01.

3 - MICROBIOLOGICAL REDUCTIONS OF 3-BROMO-2-OCTANONE 3

Incubation time was 24 hours. The products of the residues were separated on a silica gel column, the eluent was pentane / ether 90/10. In each case, the yields are overall yields for diastereoisomers after work-up.

Bakers' yeast: The residue from fifteen flasks consisted of: 20% 3-bromo-2-octanone, 40% (-)-(2S,3S)-3-bromo-2-octanol and 40% (+)-(2S,3R)-3-bromo-2-octanol. Yield: 50%

(-)-(2S,3S)-3-bromo-2-octanol (0.190 g)

Retention time: 1100 s. ^1H NMR (300.13 MHz) δ: 0.90 (t, 3H, J = 6 Hz); 1.28 (d, 3H, J = 6.5 Hz); 1.40 to 1.63 (m, 6H); 1.88 (q, 2H, J = 6.5 Hz); 2.00 (s, 1H, exchangeable with D_2O); 3.75 (q, 1H, J = 6.5 Hz); 3.95 to 4.05 (m, 1H). ^{13}C NMR (75.47 MHz) δ: 14.0 (C-8); 21.3 (C-1); 22.5 (C-7); 27.5 (C-6); 31.2 (C-5); 35.5 (C-4); 66.6 (C-3); 70.3 (C-2). $[\alpha]_J^{25}$ = -38 (c = 0.04, $CHCl_3$); ee > 98 %. HRMS Calcd for (M-OH): 191.0435; Found: 191.0453.

(+)-(2S,3R)-3-bromo-2-octanol (0.190 g)

Retention time: 1160 s. ^1H NMR (300.13 MHz) δ: 0.90 (t, 3H, J = 6.5 Hz); 1.27 (d, 3H, J = 6.5Hz); 1.29 to 1.63 (m, 6H); 1.75 (q, 2H, J = 7 Hz); 2.10 (s, 1H, exchangeable with D_2O); 3.78 (q, 1H, J = 6.5 Hz); 4.10 to 4.21 (m, 1H). ^{13}C NMR (75.47 MHz) δ: 15.3 (C-8); 20.9 (C-1); 24.1 (C-7); 29.0 (C-6); 32.9 (C-5); 35.6 (C-4); 67.5 (C-3); 71.8 (C-2). $[\alpha]_J^{25}$ = +40 (c = 0.02, $CHCl_3$); ee > 98%. HRMS Calcd for (M-OH): 191.0435; Found: 191.0448.

***Mortierella isabellina*:** The residue from fifteen flasks consisted of: 55% (-)-(2S,3S)-3-bromo-2-octanol and 45% (+)-(2S,3R)-3-bromo-2-octanol. Yield: 40%

(-)-(2S,3S)-3-bromo-2-octanol (0.165 g)

$[\alpha]_J^{25}$ = -38 (c = 0.04, $CHCl_3$); ee > 98%.

(+)-(2S,3R)-3-bromo-2-octanol (0.135 g)

$[\alpha]_J^{25}$ = +39 (c = 0.02, $CHCl_3$); ee = 97%.

Beauveria sulfurescens: The residue from ten flasks consisted of: 30 % (-)-(2S,3S)-3-bromo-2-octanol and 70 % (+)-(2S,3R)-3-bromo-2-octanol. Yield: 62 %.

(-)-(2S,3S)-3-bromo-2-octanol (0.090 g)

$[\alpha]_J^{25}$ = -29 (c = 0.01, $CHCl_3$); ee = 77 %.

(+)-(2S,3R)-3-bromo-2-octanol (0.220 g)
$[\alpha]_J^{25}$ = +32 (c = 0.02, $CHCl_3$); ee = 80 %.

Aspergillus niger: The residue from twenty flasks consisted of: 50 % 3-bromo-2-octanone and 48 % (+)-(2R,3R)-3-bromo-2-octanol and 2 % (-)-(2R,3S)-3-bromo-2-octanol.
(-)-(3S)-3-bromo-2-octanone (0.500 g)
Same NMR spectra and retention time as observed for the racemic 3-bromo-2-octanone.
$[\alpha]_J^{25}$ = -23 (c = 0.05, $CHCl_3$).
(-)-(2R,3R)-3-bromo-2-octanol (0.480 g)
Same NMR spectra and retention time as observed for the (2S,3S)-3-bromo-2-octanol.
$[\alpha]_J^{25}$ = +38 (c = 0.03, $CHCl_3$); ee > 98 %.

Geotrichum candidum: The residue from fifteen flasks consisted of: 60 % (+)-(2R,3R)-3-bromo-2-octanol and 40 % (-)-(2R,3S)-3-bromo-2-octanol. Yield: 51 %.
(+)-(2R,3R)-3-bromo-2-octanol (0.230 g)
$[\alpha]_J^{25}$ = +26 (c = 0.02, $CHCl_3$); ee = 70 %.
(-)-(2R,3S)-3-bromo-2-octanol (0.150 g)
Same NMR spectra and retention time as observed for the (2S,3R)-3-bromo-2-octanol.
$[\alpha]_J^{25}$ = -32 (c = 0.02, $CHCl_3$); ee = 80 %.

Lactobacillus kefir: The residue from seven flasks contained: 40 % (+)-(2R,3R)-3-bromo-2-octanol and 60 % (-)-(2R,3S)-3-bromo-2-octanol. Yield: 40 %.
(+)-(2R,3R)-3-bromo-2-octanol (0.055 g)
$[\alpha]_J^{25}$ = +38 (c = 0.02, $CHCl_3$); ee > 98 %.
(-)-(2R,3S)-3-bromo-2-octanol (0.085 g)
$[\alpha]_J^{25}$ = -37 (c = 0.03, $CHCl_3$); ee = 92 %.

4 - SYNTHESIS OF (3R)-3-HYDROXY-2-OCTANONE

A mixture of 0.200 g of (3S)-3-bromo-2-octanone and 0.250 g of potassium acetate dissolved in 14 mL of methanol was refluxed for 5 hours. After cooling, methanol was evaporated under vacum, the residue was diluted with water and extracted with Et_2O. The organic phase was dried on $MgSO_4$. After evaporation of ether, 0.2 g of pure 3-hydroxy-2-octanone was obtained (yield: 90 %).
(3R)-3-hydroxy-2-octanone. ^1H NMR (300.13 MHz) δ: 0.90 (t, 3H, J = 6 Hz); 1.20 to 1.60 (m, 6H); 1.75 to 1.90 (m, 2H); 2.20 (s, 3H); 3.47 (s, 1H, exchangeable with D_2O); 4.15 to 4.24 (m, 1H). $[\alpha]_J^{25}$ = -40 (c = 0.03, $CHCl_3$). Lit[10] (3S)-3-hydroxy-2-octanone $[\alpha]_J^{25}$= +92 (c = 0.03, $CHCl_3$); ee = 92 %.

5 - MICROBIOLOGICAL REDUCTIONS OF 3-AZIDO-2-OCTANONE 5

Incubation time was 24 h. The products of the residues were separated on a silica gel column; the eluent was pentane / ether 90/10. In each case, the yields are overall yields for diastereoisomers after work-up.

Bakers' yeast: The residue from fifteen flasks consisted of: 50 % (+)-(2S,3S)-3-azido-2-octanol and 50 % (+)-(2S,3R)-3-azido-2-octanol. Yield: 55 %.

(+)-(2S,3S)-3-azido-2-octanol (0.205 g)

Retention time: 1230 s. ^{1}H NMR (300.13 MHz) δ: 0.91 (t, 3H, J = 7Hz); 1.24 (d, 3H, J = 7 Hz); 1.28 to 1.55 (m, 6H); 1.57 to 1.67 (m, 2H); 1.95 (s, 1H, exchangeable with D_2O); 3.09 to 3.21 (m, 1H); 3.65 to 3.81 (m, 1H). ^{13}C NMR (75.47 MHz) δ: 13.9 (C-8); 20.0 (C-1); 22.5 (C-7); 25.8 (C-6); 30.7 (C-5); 31.6 (C-4); 68.7 (C-3); 69.7 (C-2). IR: 2110 cm^{-1} (C-N_3). $[\alpha]_J^{25}$ = +19 (c = 0.02, $CHCl_3$); ee = 95 %. Anal. Calcd for $C_8H_{17}N_3O$: C: 56.11; H: 10.01; N: 24.54; Found C: 56.05; H: 9.96; N: 24.62.

(+)-(2S,3R)-3-azido-2-octanol (0.205 g)

Retention time: 1280 s. ^{1}H NMR (300.13 MHz) δ: 0.91 (t, 3H, J = 7 Hz); 1.22 (d, 3H, J = 7 Hz); 1.25 to 1.42 (m, 6H); 1.46 to 1.62 (m, 2H); 1.77 (s, 1H, exchangeable with D_2O); 3.30 to 3.41 (m, 1H); 3.81 to 3.92 (m, 1H). ^{13}C NMR (75.47 MHz) δ: 13.9 (C-8); 18.2 (C-1); 22.5 (C-7); 26.2 (C-6); 30.1 (C-5); 31.6 (C-4); 68.3 (C-3); 70.0 (C-2). IR: 2110 cm^{-1} (C-N_3). $[\alpha]_J^{25}$ = +8 (c = 0.01, $CHCl_3$); ee > 98 %. Anal. Calcd for $C_8H_{17}N_3O$: C: 56.11; H: 10.01; N: 24.54; Found C: 56.11; H: 10.05; N: 24.42.

Mortierella isabellina: The residue from six flasks consisted of: 50 % (+)-(2S,3S)-3-azido-2-octanol and 50% (+)-(2S,3R)-3-azido-2-octanol. Yield: 53 %.

(+)-(2S,3S)-3-azido-2-octanol (0.080 g)

$[\alpha]_J^{25}$ = +19 (c = 0.02, $CHCl_3$); ee = 95 %.

(+)-(2S,3R)-3-azido-2-octanol (0.080 g)

$[\alpha]_J^{25}$ = +7.5 (c = 0.01, $CHCl_3$); ee = 96 %.

Beauveria sulfurescens: The residue from six flasks consisted of: 20 % 3-azido-2-octanone, 55 % (+)-(2S,3S)-3-azido-2-octanol and 25 % (+)-(2S,3R)-3-azido-2-octanol. Yield: 50 %.

(+)-(2S,3S)-3-azido-2-octanol (0.105 g)

$[\alpha]_J^{25}$ = +20 (c = 0.02, $CHCl_3$); ee = 97 %.

(+)-(2S,3R)-3-azido-2-octanol (0.045 g)

$[\alpha]_J^{25}$ = +5 (c = 0.01, $CHCl_3$); ee = 68 %.

***Aspergillus niger*:** The residue from fifteen flasks consisted of: 40 % 3-azido-2-octanone, 30 % (-)-(2R,3R)-3-azido-2-octanol and 30% (-)-(2R,3S)-3-azido-2-octanol. Yield: 55 %.

(-)-(2R,3R)-3-azido-2-octanol (0.205 g)

Same NMR spectra and retention time as observed for the (2S,3S)-3-azido-2-octanol.

$[\alpha]_J^{25}$ = -17 (c = 0.01, $CHCl_3$); ee = 82 %.

(-)-(2R,3S)-3-azido-2-octanol (0.205 g)

Same NMR spectra and retention time as observed for the (2S,3R)-3-azido-2-octanol.

$[\alpha]_J^{25}$ = -7 (c = 0.01, $CHCl_3$); ee = 88 %.

Lactobacillus kefir: The residue from nine flasks consisted of: 50 % (+)-(2R,3R)-3-azido-2-octanol and 50 % (-)-(2R,3S)-3-azido-2-octanol. Yield: 48 %

(-)-(2R,3R)-3-azido-2-octanol (0.110 g)
$[\alpha]_J^{25}$ = -20 (c = 0.01, $CHCl_3$); ee = 97 %.

(-)-(2R,3S)-3-azido-2-octanol (0.110 g)
$[\alpha]_J^{25}$ = -8 (c = 0.01, $CHCl_3$); ee > 98 %.

6 - SYNTHESES OF AZIDOOCTANOLS (6, 9) FROM 2,3-OCTANEDIOLS

a - From (-)-(2S,3S)-2,3-octanediol

(-)-(2S,3S)-2,3-octanediol was prepared by microbiological reduction of 2,3-octanedione by *Beauveria sulfurescens* as previously described[3].

To 0.275 g of (-)-(2S,3S)-2,3-octanediol dissolved in 3 mL of CCl_4 was added 0.2 mL of thionyl chloride. The mixture was refluxed for 0.5 hours. After cooling, the mixture was diluted with ether and the organic phase was washed with saturated aqueous $NaHCO_3$ and dried on $MgSO_4$. After evaporation of the solvent, we obtained 0.320 g of cyclic sulfite (yield: 90%).

To 0.320 g of cyclic sulfite dissolved in 15 mL of dimethylformamide was added 0.2 g of lithium azide; the mixture was stirred and maintained at 115-120°C overnight. After cooling, the mixture was diluted with 20 mL of water and extracted with Et_2O. The organic phase was washed successively with saturated aqueous $NaHC0_3$ and water, and dried on $MgSO_4$. After evaporation of ether, the residue was analysed by GC; it contained 50 % 2-azido-3-octanol and 50 % 3-azido-2-octanol. The azidooctanols were separated on a silica gel column. Eluent: pentane/ ether 90/10. Overall yield : 80 %.

(+)-(2S,3R)-3-azido-2-octanol (0.130 g)
Retention time: 1280 s. NMR spectra were identical to those observed with (2S,3R)-3-azido-2-octanol obtained by microbiological reduction. $[\alpha]_J^{25}$ = +8 (c = 0.01, $CHCl_3$); ee > 98 %.

(-)-(2R,3S)-2-azido-3-octanol (0.125 g)
Retention time: 1240 s. ^1H NMR (300.13 MHz) δ: 0.91 (t, 3H, J = 7.5 Hz); 1.27 (d, 3H, J = 7.5 Hz); 1.29 to 1.38 (m, 6H); 1.39 to 1.58 (m, 2H); 1.87 (s, 1H, exchangeable with D_2O); 3.45 to 3.56 (m, 1H); 3.57 to 3.67 (m, 1H). ^{13}C NMR (75.47 MHz) δ: 13.3 (C-8); 14.0 (C-1); 22.6 (C-7); 25.6 (C-6); 31.6 (C-5); 32.7 (C-4); 61.9 (C-2); 74.0 (C-3).$[\alpha]_J^{25}$ = -51 (c = 0.02, $CHCl_3$); ee > 98 %. Anal. Calcd for $C_8H_{17}N_3O$: C: 56.11; H: 10.01; N: 24.54. Found C: 56.18; H: 9.99; N: 24.63.

b - From (+)-(2S,3R)-2,3-octanediol

(+)-(2S,3R)-2,3-octanediol was prepared by microbiological reduction of 2,3-octanedione by bakers' yeast as previously described[3].

The corresponding cyclic sulfite was prepared by the same procedure as above from 0.430 g of (2S,3R)-2,3-octanediol in 4 mL of CCl_4 and 0.3 mL of thionyl chloride. We obtained 0.508 g of cyclic sulfite (yield 90 %). The crude cyclic sulfite (0.508 g) dissolved in 20 mL of dimethylformamide was treated with 0.4 g of lithium azide as above. The residue was analysed by GC; it contains: 50 % 2-azido-3-octanol and 50 % 3-azido-2-octanol. The azidooctanols were purified as above. Overall yield : 80 %.

(+)-(2S,3S)-3-azido-2-octanol (0.200 g)
Retention time: 1230 s. NMR spectra were identical to those observed with (2S,3S)-3-azido-2-octanol obtained by microbiological reduction. $[\alpha]_J^{25}$ = +21 (c = 0.03, $CHCl_3$); ee > 98 %.

(-)-(2R,3R)-2-azido-3-octanol (0.200 g)
Retention time: 1190 s. ^1H NMR (300.13 MHz) δ: 0.90 (t, 3H, J = 7.5 Hz); 1.17 to 1.39 (m, 6H); 1.31 (d, 3H, J = 7.5 Hz); 1.40 to 1.57 (m, 2H); 1.97 (s, 1H, exchangeable with D_2O); 3.34 to 3.53 (m, 2H). ^{13}C NMR (75.47 MHz) δ: 14.0 (C-8); 15.8 (C-1); 22.6 (C-7); 25.2 (C-6); 31.8 (C-5); 33.8 (C-4); 62.3 (C-2); 74.6 (C-3).$[\alpha]_J^{25}$= -50 (c = 0.03, $CHCl_3$); ee > 98 %. Anal. Calcd for $C_8H_{17}N_3O$: C: 56.11; H: 10.01; N: 24.54. Found C: 56.02; H: 9.99; N: 24.55.

7 - PREPARATION OF CHIRAL 2,3-EPOXYOCTANES 10

General methods: To 0.1 g of 3-bromo-2-octanol dissolved in 15 mL of anhydrous benzene was added 0.06 g of sodium hydride. The mixture was stirred overnight at room temperature. After filtration, the solvent was evaporated off under vacuum and the residue diluted with water and extracted four times with Et_2O. The organic phase was dried on $MgSO_4$ and the solvent evaporated off. The residue was purified on a silica gel column, eluent pentane/ ether 95/5.

- From 0.100 g of (-)-(2S,3S)-3-bromo-2-octanol obtained by microbiological reduction with *M. isabellina*, 0.04 g of (+)-(2S,3R)-2,3-epoxyoctane was prepared (yield: 60 %).
(+)-(2S,3R)-2,3-epoxyoctane
Retention time: 370 s. ^1H NMR (300.13 MHz) δ: 0.91 (t, 3H, J = 6 Hz); 1.27 (d, 3H, J = 6 Hz); 1.30 to 1.64 (m, 8H); 2.82 to 2.94 (m, 1H, J = 4.2 Hz); 3.00 to 3.09 (m, 1H, J = 4.2 Hz). $[\alpha]_J^{25}$ = +8 (c = 0.02, pentane); ee > 98 %. HRMS Calcd: 128.1201; Found: 128.1201.

- From 0.150 g of (+)-(2R,3R)-3-bromo-2-octanol obtained by microbiological reduction with *A. niger*, 0.053 g of (-)-(2R,3S)-2,3-epoxyoctane was prepared (yield: 58 %).
(-)-(2R,3S)-2,3-epoxyoctane
Same NMR spectrum and retention time as its (2S,3R) enantiomer. $[\alpha]_J^{25}$ = -8 (c = 0.04, pentane); ee > 98 %.

- From 0.100 g of (+)-(2S,3R)-3-bromo-2-octanol obtained by microbiological reduction with freeze-dried bakers' yeast, 0.04 g of (+)-(2S,3S)-2,3-epoxyoctane was prepared (yield: 61 %).
(+)-(2S,3S)-2,3-epoxyoctane
Retention time: 310 s. ^1H NMR (300.13 MHz) δ: 0.90 (t, 3H, J = 6 Hz); 1.30 (d, 3H, J = 5.5 Hz); 1.25 to 1.35 (m, 6H); 1.38 to 1.58 (m, 2H); 2.60 to 2.70 (m, 1H, J = 2.1 Hz); 2.71 to 2.75 (m, 1H, J = 2.1 Hz). $[\alpha]_J^{25}$ = +5 (c = 0.03, pentane); ee > 98 %. HRMS Calcd: 128.1201; Found: 128.1198.

- From 0.100 g of (-)-(2R,3S)-3-bromo-2-octanol obtained by microbiological reduction with *L. kefir*, 0.04 g of (-)-(2R,3R)-2,3-epoxyoctane was prepared (yield: 60 %).
(-)-(2R,3R)-2,3-epoxyoctane
Same NMR spectrum, retention time as its (2S,3S) enantiomer. $[\alpha]_J^{25}$ = -4.5 (c = 0.01, pentane); ee = 92 %.

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