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Chemoenzymatic synthesis of α-halogeno-3-octanol and 4- or 5-nonanols. Application to the preparation of chiral epoxides

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Abstract

A study of the microbiological reduction of different α-halogenoketones (4-chloro-3-octanone, 4-chloro-5-nonanone, 5-bromo-4-nonanone and 5-chloro-4-nonanone) with several strains of microorganism showed great difficulty in reducing ketone functions located in the middle of carbon chains. However, by choosing the appropriate microorganism, several enantiomerically pure diastereoisomers of the corresponding halohydrins have been obtained and were transformed into chiral epoxides.

1. Introduction

Enantiomerically pure epoxides are important chiral building blocks in organic synthesis and can be used as key intermediates in the synthesis of more complex enantiopure bioactive compounds due to their ability to react with a broad variety of nucleophiles. In recent years, many chemical and biological methods have been developed.[1,2] Among the most used techniques, the Sharpless epoxidation of olefins[3] is limited to allylic alcohols, and Jacobsen's catalysts[4] give mainly high enantiomeric excesses with some *cis*-substituted olefins. Recently, Jacobsen[5] reported a kinetic resolution of racemic primary epoxides via catalytic hydrolysis using chiral cobalt based complexes.

Among the biocatalytic methods,[6] two main direct pathways have been studied: the stereoselective microbiological epoxidation of prochiral olefins and the enzymatic resolution of racemic epoxides using hydrolytic enzymes. In the first method, the reaction is mostly catalyzed by monooxygenases (cytochromes P450,[7,8] ω-hydroxylases,[9] methane monooxygenases,[10,11] etc.). The preparative scale is difficult to perform with isolated enzymes due to their complex nature and their dependance of a redox

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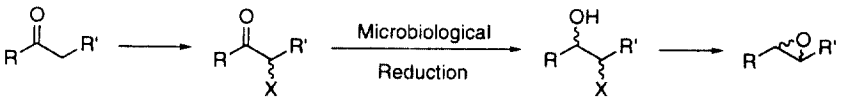
cofactor, but whole-cell microorganisms can be used instead. Chloroperoxidase from *Caldaryomyces fumago* is also able to transform alkenes into epoxides.[12,13] However, as in the case of monooxygenases, only the *cis* olefins yield epoxides with high enantiomeric excesses.

Hydrolytic enzymes may be more promising as enantioselective biocatalysts, because they are cofactor independent.[14] Although their mammalian origin has been known for a long time (mEH and cEH), they have mainly been investigated during detoxification studies. More recently, several groups have started a search for epoxide hydrolases from microbial sources: Furstoss and his co-workers were the first to use the fungi *Aspergillus niger*[15] and *Beauveria bassiana*[16] to hydrolyze racemic epoxides; then Faber's group used bacteria, in particular *Rhodococcus*.[17] More recently, Weijers found the same activity in the yeast *Rhodotorula glutinis*.[18] The drawback of this method is the theoretical limited yield in epoxide (and in diol) of 50%. Although some improvements have been made to obtain a 100% yield in enantiomerically pure diol,[19] methods for obtaining a 100% yield in epoxide have not yet been realized.

Indirect strategies for the obtention of enantiomerically pure epoxides can also been planned, starting from enantiomerically pure α-halohydrins for example. In 1993,[20] we described a three-step chemoenzymatic synthesis of chiral 2,3-epoxides from α-halogenoketones. The microbiological reduction of these ketones yielded, by choosing the appropriate microorganism, halohydrins with excellent enantiomeric excesses. They were then chemically converted into epoxides (Scheme 1). Several α-halogenoketones were tested but the R group was always a methyl or a phenyl group.



Scheme 1.

In this work, we wanted to know if our method could be generalized whatever the size of the R group. We have chosen four aliphatic α-halogenoketones, each with a long carbon chain and with the keto function at different positions on the chain: 4-chloro-3-octanone **1**, 5-chloro-4-nonanone **2**, 4-bromo-5-nonanone **3** and 4-chloro-5-nonanone **4** (Fig. 1). We have studied their reduction with several strains of microorganism in order to obtain enantiomerically pure halohydrins and then chiral 3,4- or 4,5-epoxides.

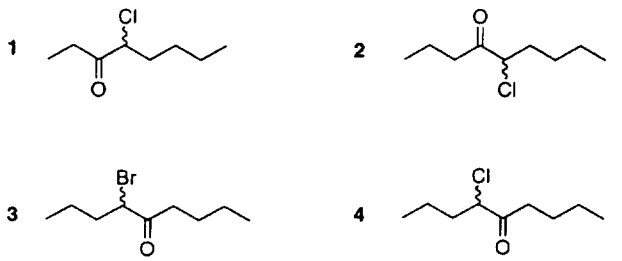


Figure 1.

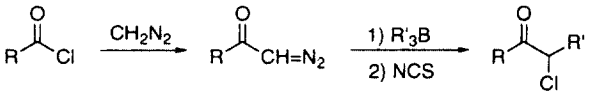
2. Results and discussion

2.1. Synthesis of the substrates

Two different synthetic routes were used according to the symmetrical or unsymmetrical character of the α-halogenoketone we wanted to test. The synthesis of 5-chloro-4-nonanone **2** has already beeen

described by Barluenga et al.[21] The reaction of a carboxylic acid ester with bromochloromethyllithium, and further treatment with lithium dibutylcuprate led, after hydrolysis, to compound **2** with 87% yield. However, in spite of several attempts under different conditions, we did not succeed in obtaining **2** in greater than 20% yield.

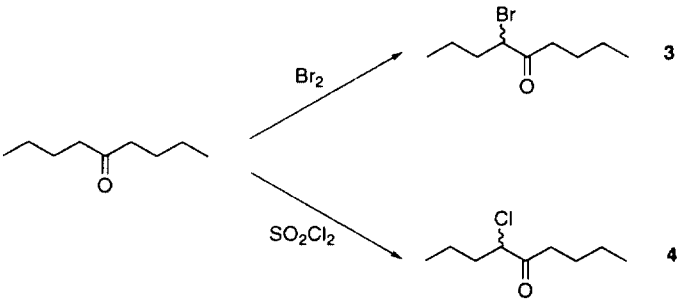
4-Chloro-3-octanone **1** and 5-chloro-4-nonanone **2**, the unsymmetrical α-halogenoketones of our study, were prepared in two steps via the formation of an intermediate diazoketone. The first step consisted in the reaction of diazomethane with the appropriate acid chloride to yield a diazoketone.[22] The reaction of this diazoketone with trialkylborane first and then with *N*-chlorosuccinimide (NCS)[23] provided the corresponding α-haloketone (Scheme 2). The overall yields were around 30%.



1 R = - CH_2-CH_3 R' = - C_4H_9 **2** R = - C_3H_7 R' = - C_4H_9

Scheme 2.

The other haloketones, 4-bromo-5-nonanone **3** and 4-chloro-5-nonanone **4**, were both obtained from 5-nonanone after reaction with bromine[24] and sulfuryl chloride,[25] respectively (Scheme 3).



Scheme 3.

2.2. *Microbiological reductions of the substrates synthesized*

The strains of microorganism selected were as follows: baker's yeast and the yeast *Rhodotorula glutinis*, the fungi *Aspergillus niger*, *Beauveria bassiana*, *Cunninghamella echinulata* var. *elegans*, *Geotrichum candidum*, *Mortierella isabellina* and the bacterium *Lactobacillus kefir*. All these microorganisms have already shown their abilities to reduce several 2-halogeno-3-ketones to yield enantiomerically pure α-halohydrins.[20,26] The yeast was used freeze-dried under non-fermenting conditions. Bioconversions with the other microorganisms were carried out using washed resting cells. Each microorganism was assayed for a 6, 24 or 48 h incubation time and with each substrate. The results are collected in Table 1.

Most of the microorganisms tested were able to reduce the α-haloketones **1**, **2**, **3** and **4**. However, the GC analyses of some samples showed the presence of many by-products, some of them in large quantities, particularly in the case of the reduction of **2**, **3** and **4**. Two by-products were identified as 4- or 5-nonanone and as the corresponding alcohols, but the others, which sometimes represented more than 50% of the mixture, have not been isolated. All the microorganisms which did not lead to at least 50% of halohydrins, were not used further.

The next step consisted of quantitative assays in order to determine the absolute configuration and the enantiomeric excess of each halohydrin formed. The results are given in Table 2. The chemical yields

Table 1
Microbiological reductions of **1**, **2**, **3** and **4**

Substrate	Microorganisms	Incubation time (h)	Relative ratio determined by GC		
			Halogenoketone	Halohydrin	By-products
1	Bakers' yeast	24	0	75	25
	Rhodotorula glutinis	6	0	100	0
	Aspergillus niger	24	20	80	0
	Mortierella isabellina	6	0	80	20
	Lactobacillus kefir	24	10	80	10
2	Bakers' yeast	24	15	70	15
	Rhodotorula glutinis	6	0	90	10
	Aspergillus niger	48	0	69	31
	Beauveria bassiana	24	0	100	0
	Cunninghamella echinulata	6	5	10	85
	Geotrichum candidum	24	6	10	84
	Mortierella isabellina	24	0	70	30
	Lactobacillus kefir	24	7	62	31
3	Bakers' yeast	24	13	56	31
	Rhodotorula glutinis	48	20	60	20
	Aspergillus niger	24	10	10	80
	Beauveria bassiana	24	37	31	32
	Cunninghamella echinulata	24	84	12	4
	Geotrichum candidum	24	28	50	22
	Mortierella isabellina	6	0	100	0
	Lactobacillus kefir	24	61	39	0
4	Bakers' yeast	24	10	80	10
	Rhodotorula glutinis	24	0	90	10
	Aspergillus niger	24	60	0	40
	Beauveria bassiana	24	11	62	27
	Cunninghamella echinulata	6	30	20	50
	Geotrichum candidum	24	20	28	52
	Mortierella isabellina	6	0	70	30
	Lactobacillus kefir	24	30	60	10

recorded in the last column of Table 2 are the overall yields of diastereoisomers after work-up. The proportions of each isomer are given in brackets.

The first comment concerned the low yields of halohydrins obtained whatever the substrate. They are much lower than those obtained during the reduction of 3-bromo-2-octanone[20] for example (50%). The first explanation is the formation of many by-products, as described previously. We have also noticed that the percentage in weight of the residue obtained after bioconversion and extraction was relatively low (40%) compared to the initial weight of haloketone (loss of organic matter). The products of the reaction were either totally degraded into H_2O and CO_2, or stocked in the cells. After bioconversion, the cells were broken, suspended in ethanol and stirred for 24 h. From this ethanolic suspension, only

Table 2
Microbiological reductions of **1**, **2**, **3** and **4**: quantitative assays

Microorganism		*Syn* halohydrin			*Anti* halohydrin			Yield
		$[\alpha]_D^{25}$	e.e.	Abs. Conf.	$[\alpha]_D^{25}$	e.e.	Abs. Conf.	
Bakers' Yeast	**1**	- 35	≥ 98 %	(3S,4S)	+ 25	≥ 98 %	(3S,4R)	13 % (50 / 50)
	2	- 36	≥ 98 %	(4S,5S)	+ 14	≥ 98 %	(4S,5R)	15 % (57 / 43)
	3	+ 7	40 %	(4R,5R)	-	-	-	9 % (90 /10)
	4	- 6	17 %	(4S,5S)	+ 13	≥ 98 %	(4R,5S)	28 % (85 / 15)
Rhodotorula glutinis	**1**	- 35	≥ 98 %	(3S,4S)	+ 25	≥ 98 %	(3S,4R)	26 % (48 / 52)
	2	- 36	≥ 98 %	(4**S**,5S)	- 14	≥ 98 %	(4**R**,5S)	8 % (50 / 50)
	3	- 17	≥ 98 %	(4S,5S)	+ 17	≥ 98 %	(4R,5S)	18 % (50 / 50)
	4	- 32	≥ 98 %	(4S,5S)	+ 10	78 %	(4R,5S)	14 % (50 / 50)
Aspergillus niger	**1**	+ 13	38 %	(3R,4R)	- 25	≥ 98 %	(3R,4S)	10 % (30 / 70)
	2	- 23	65 %	(4**S**,5S)	- 10	71 %	(4**R**,5S)	8 % (60 / 40)
Mortierella isabellina	**1**	- 18	53 %	(3S,4S)	+ 14	56 %	(3S,4R)	15 % (50 / 50)
	2	- 11	30 %	(4S,5S)	-	-	-	5 % (85 / 15)
	3	- 12	70 %	(4S,5S)	+ 12	70 %	(4R,5S)	18 % (50 / 50)
	4	- 4	12 %	(4S,5S)	+ 3	23 %	(4R,5S)	52 % (50/50)
Lactobacillus kefir	**1**	+ 35	≥ 98 %	(3R,4R)	- 9	34 %	(3R,4S)	18 % (30 / 70)
	2	- 27	75 %	(4**S**,5S)	- 14	≥ 98 %	(4**R**,5S)	6 % (40 / 60)
	4	- 32	≥ 98 %	(4S,5**S**)	- 13	≥ 98 %	(4S,5**R**)	20 % (40 / 60)

NB : a) Quantitative assays were realized with *Beauveria bassiana*, but the residue obtained after reduction was so low that no purification was carried out.

b) The microbiological reduction by *Geotrichum candidum* of **3** yielded the (4R,5S)-4-chloro-5-nonanol with a 70% ee and the (4R,5S)-4-chloro-5-nonanol with a 62% ee.

haloketone was isolated in a low yield (about 10% of the starting material). Therefore the haloketone or the products formed are probably further metabolized. This hypothesis was confirmed by the presence of several peaks on the GC chromatograms, a phenomenon that can explain the low yield of halohydrin obtained.

The comparison of the results obtained for the four substrates shows that the further the position of the ketone from the halide, the more difficult the microbiological reduction. The reduction of 4-chloro-3-octanone **1** led to the four enantiomerically pure diastereoisomers: *syn* (3*S*,4*S*) and *anti* (3*S*,4*R*)-4-chloro-3-octanol were obtained with baker's yeast or *R. glutinis*. With *A. niger*, only the *anti* (3*R*,4*S*) diastereoisomer was enantiomerically pure, whereas *L. kefir* gave only the *syn* (3*R*,4*R*) diastereoisomer.

In the case of 5-chloro-4-nonanone **2**, only three diastereoisomers were obtained enantiomerically pure: the (4*S*,5*S*) and (4*S*,5*R*) with baker's yeast, and the (4*R*,5*S*) with *L. kefir*. That is also the case for 4-chloro-5-nonanone **4**: the (4*S*,5*S*) and (4*S*,5*R*) diastereoisomers were obtained with *L. kefir* and the (4*R*,5*S*) with baker's yeast. In the end, only two diastereoisomers were obtained enantiomerically pure for **3**: the (4*S*,5*S*) and the (4*S*,5*R*) with *R. glutinis*. In contrast to the case of 1-phenyl-2-bromo-1-propanone and 1-phenyl-2-chloro-1-propanone,[26] where the change of the nature of the halide atom improved the percentage of reduction, here changing the bromine atom into a chlorine has not greatly modified the reduction of the haloketone. The same microorganisms led to the same isomers. Only the yield is better in the case of **4**.

Another surprising phenomenon in these results concerns the absolute configuration of the halohydrins obtained with some microorganisms: *R. glutinis, A. niger, L. kefir* for **2**, and *L. kefir* for **4**. Indeed, in the case of *L. kefir* with **4** for example, the *syn* diastereoisomer has an alcohol with an (*S*) configuration, whereas the absolute configuration of the alcohol in the *anti* diastereoisomer is the opposite one (*R*), both of them presenting excellent enantiomeric excesses. The reactions of reduction are not enantiogenic at all. Moreover, the carbon atom bearing the halogen has, in both cases, the (*S*) configuration. The question is where has the (*R*)-enantiomer of the haloketone gone. One hypothesis could be that, regarding the very low yield in halohydrins (20% in our example), the isolated ones are not representative of all the reactions that have taken place in the microorganism. Many by-reactions are observed (elimination of the halogen, etc.) and we suppose that some isomers of the halohydrin can be preferentially metabolized or further transformed.

2.3. *Determination of the enantiomeric excesses of the halohydrins formed*

Several methods were used to measure the enantiomeric excess of each isomer of the halohydrins formed. For the products obtained by microbiological reduction of **1** and **4**, enantiomeric excesses were determined after converting each diastereoisomer of the racemic and of the optically active chlorohydrin into the corresponding MTPA esters by Mosher's method,[27] either from ^{13}C and ^{19}F NMR spectra for **1**, or from ^{1}H NMR spectra for **4** (see Experimental section).

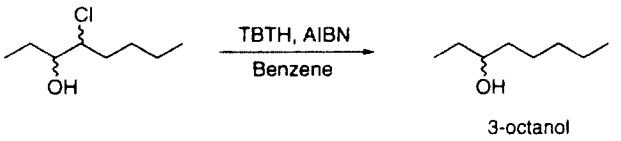
For the halohydrins coming from the reduction of **2** and **3**, the enantiomeric excesses were determined by gas phase chromatography (see Experimental section). Two methods were used: (a) each isomer of the halohydrin was analyzed directly using a chiral phase column coated with modified γ-cyclodextrins (Lipodex E) (this method was used for the *syn* and *anti* diastereoisomers of **2** and for the *syn* isomer of **3**); (b) the *anti* diastereoisomer of **3** was treated with (*S*)-*O*-acetyllactic chloride[28] and the derivative obtained was analyzed on a non-chiral column.

2.4. *Determination of the absolute configuration*

None of the optically active halohydrins isolated from the reductions of **1**, **2**, **3** or **4** have been described in the literature before. For each substrate, we assigned the absolute configuration of the halohydrins by analogy with published data and by chemical correlations.

We have already studied the reduction of 3-bromo-2-octanone.[20] With baker's yeast an equivalent mixture was obtained of (−)-(2*S*,3*S*) and (+)-(2*S*,3*R*)-3-bromo-2-octanol. Moreover, the diastereoisomer which was first eluted from the chromatography column, and which exhibited the lower chemical shifts for the CH–OH and the CH–Cl protons on the ^{1}H NMR spectrum, corresponded to the *syn* (2*S*,3*S*)-3-bromo-2-octanol. By comparison of the ^{1}H NMR chemical shifts and of the sign of the specific rotation of each isomer of 4-chloro-3-octanol, obtained by microbiological reduction with baker's yeast, with

those of the isomers of 3-bromo-2-octanol, we tentatively assigned the (−)-(3*S*,4*S*) configuration to the first eluted diastereoisomer and the (+)-(3*S*,4*R*) to the second one. To give evidence of the absolute configuration proposed, a chemical correlation was assayed, corresponding to the dechlorination of each diastereoisomer of the chlorohydrin (Scheme 4).



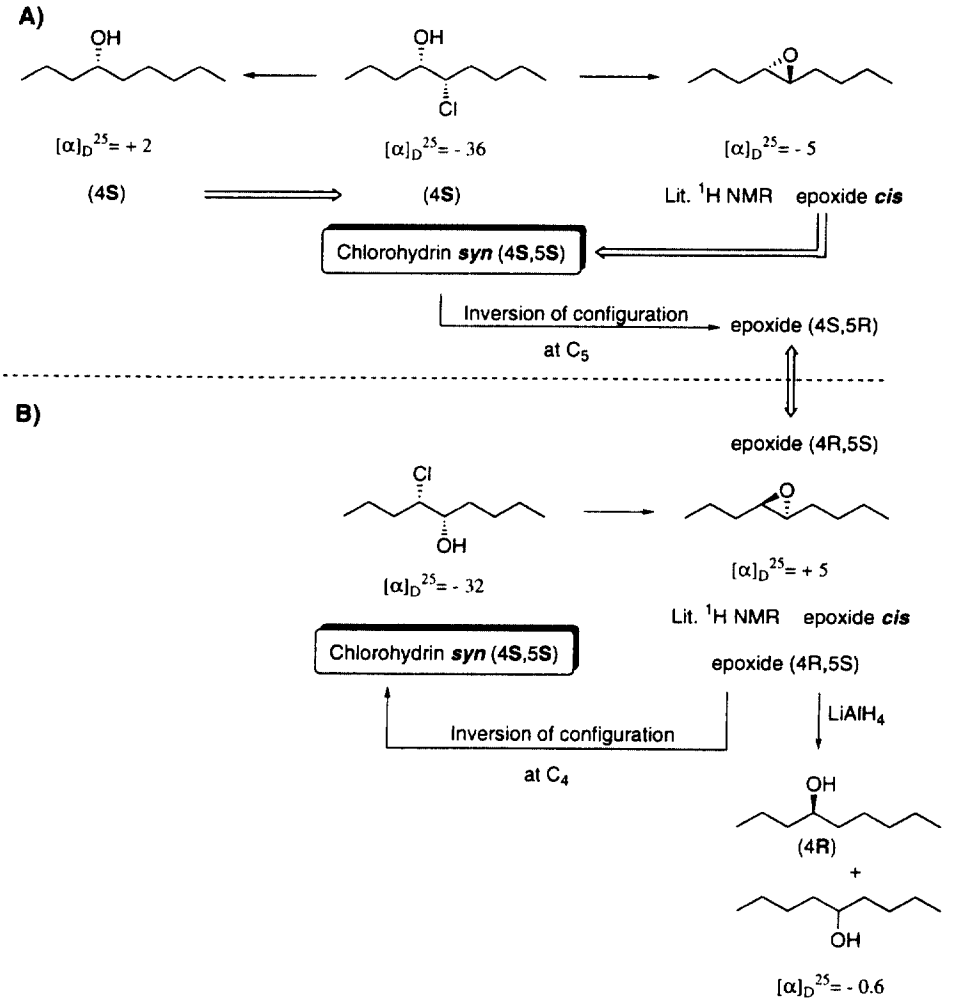
Scheme 4.

The treatment of each diastereoisomer of the chlorohydrin obtained with baker's yeast with tributyltin hydride in benzene, in the presence of 2,2′-azobisisobutyronitrile (AIBN) as catalyst,[29] afforded the 3-octanol in high yield. The comparison of the sign of the specific rotation of the 3-octanol obtained by dechlorination of the *syn* and *anti* 4-chloro-3-octanol, obtained with baker's yeast, with that reported in the literature[30] allowed us to check the absolute configuration of the C-3 stereogenic centre. In both cases, the dechlorination reaction yielded the (3*S*)-3-octanol and so the assignments of (−)-(3*S*,4*S*) for the diastereoisomer first eluted and of (+)-(3*S*,4*R*) for the second one were confirmed.

The methods used to determine the absolute configuration of the chlorohydrins obtained after the microbiological reduction of 5-chloro-4-nonanone **2** and 4-chloro-5-nonanone **4** by baker's yeast and *Rhodotorula glutinis*, respectively, are described in Scheme 5 for one of the diastereoisomers obtained in each case.

For the chlorohydrin, first eluted after column chromatography from the products of the reduction of 5-chloro-4-nonanone **2** by baker's yeast, the same dechlorination reaction as that described previously was first carried out (Scheme 5A). The 4-nonanol obtained exhibits the (*S*) configuration (comparison of the sign of its specific rotation with the data given in the literature[31]). So the asymmetric carbon C_4 of the chlorohydrin has an (*S*) configuration. In order to determine the absolute configuration of the other asymmetric carbon (C_5), a chemical correlation was made. The chlorohydrin was converted into the corresponding epoxide by treatment with potassium carbonate.[32] The *cis* or *trans* character can be assigned to the epoxide formed by comparing its ^{1}H NMR spectra with the data given in the relevant literature[33] for *cis* [δ=2.93 ppm (centred)] and *trans* [δ=2.68 ppm (centred)] racemic 4,5-epoxynonane. In our case, we obtained a *cis* epoxide. Utaka et al.[34] reported that a *syn* chlorohydrin gives a *cis* epoxide while an *anti* chlorohydrin gives a *trans* epoxide. Moreover, the epoxide formation takes place with inversion of configuration at the chlorine-bearing carbon atom. We can then deduce that this isomer corresponds to the *syn* (4*S*,5*S*)-5-chloro-4-nonanol. The same reactions were carried out on the other isomer of the 5-chloro-4-nonanol obtained with baker's yeast. These reactions led to the formation of (4*S*)-4-nonanol and *trans* 4,5-epoxynonane. This isomer corresponds to the *anti* (4*S*,5*R*)-5-chloro-4-nonanol.

The absolute configurations of the diastereoisomers obtained from the reduction of **4** by *Rhodotorula glutinis* were determined by the synthesis of the corresponding 4,5-epoxynonanes. The chlorohydrin eluted first by column chromatography was converted into the epoxide. By comparing its ^{1}H NMR spectrum with data given in the literature,[33] we can deduce the *cis* character of the epoxide formed. The sign of its specific rotation is opposite (+5) to that of the *cis*-(4*S*,5*R*)-4,5-epoxynonane obtained previously by cyclization of (4*S*,5*S*)-5-chloro-4-nonanol (Scheme 5A and B). Its absolute configuration is then *cis*-(4*R*,5*S*)-4,5-epoxynonane. The epoxide formation takes place with inversion of configuration at the chlorine-bearing carbon atom, so we can deduce that the chlorohydrin, eluted first by column chromatography and obtained from the microbiological reduction of **4** with *R. glutinis*, corresponds to



Scheme 5.

the *syn* (4*S*,5*S*)-4-chloro-5-nonanol. The same cyclization was carried out on the other isomer of 4-chloro-5-nonanol obtained with *R. glutinis*, with formation of *trans* 4,5-epoxynonane. This epoxide presented the same sign of specific rotation as the *trans* (4*S*,5*S*)-4,5-epoxynonane obtained from (4*S*,5*R*)-5-chloro-4-nonanol. The second isomer is therefore the *anti* (4*R*,5*S*)-4-chloro-5-nonanol.

These assignments were checked by another chemical correlation. Each diastereoisomer of epoxide was opened by $LiAlH_4$ under reflux[35] to yield a mixture of 4- and 5-nonanol. As the 5-nonanol is inactive, the sign of the specific rotation of the mixture corresponds to that of 4-nonanol. By comparing it with the data given in the literature,[31] we assign the (4*R*) configuration to the alcohol coming from the *cis* 4,5-epoxynonane (Scheme 5B) and the (4*S*) configuration to the alcohol coming from the *trans* 4,5-epoxynonane. As the reaction of epoxidation takes place with inversion of configuration of the C_4 carbon, we confirm the assignment of the absolute configuration of the carbon C_4 of both diastereoisomers of 4-chloro-5-nonanol.

The absolute configuration of each diastereoisomer of the bromohydrin was assigned by analogy of the ^1H NMR spectra and of the sign of specific rotation between 4-bromo-5-nonanol and 4-chloro-5-nonanol.

As the yields of the reduction of **3** were very low and as only two enantiomerically pure diastereoisomers were obtained, further chemical correlations have not been investigated.

2.5. *Synthesis of chiral epoxides*

Our aim was to prepare the corresponding chiral epoxides. We were able to obtain the four isomers of 4,5-epoxynonane enantiomerically pure, by treating with potassium carbonate the appropriate chlorohydrins arising from the reduction of 5-chloro-4-nonanone **2** or 4-chloro-5-nonanone **4**[29] (Table 3).

Table 3
Synthesis of chiral 4,5-epoxynonane

Starting	Chlorohydrin			Epoxides		Yield
Substrate	Conf.	obtained from	$[\alpha]_D^{25}$	e.e.	Conf.	reaction
2	(4S,5S)	Bakers' yeast	- 5	≥ 98 %	(4S,5R)	50%
	(4S,5R)	Bakers' yeast	- 32	≥ 98 %	(4S,5S)	70 %
	(4R,5S)	*Lactobacillus kefir*	+ 32	≥ 98 %	(4R,5R)	72 %
4	(4S,5S)	*Rhodotorula glutinis*	+ 5	≥ 98 %	(4R,5S)	48 %

The yields of epoxidation are quite good. The enantiomeric excesses of the epoxides were directly deduced from those of the chlorohydrins. We have shown previously that no racemization was observed during the cyclization.[20,26] By the same method, it would be possible to prepare three isomers of 3,4-epoxyoctane enantiomerically pure.

3. Conclusion

All these results show the possibility of reducing a ketone function even in the middle position of a long carbon chain (contrary to what is reported by Faber[36]), and to obtain, by choosing the appropriate microorganism, several diastereoisomers of the corresponding halohydrin enantiomerically pure. However, the shift of the ketone position along the carbon chain implies a large decrease in the yield of the reduction, mostly because of the formation of large quantities of by-products. This last phenomenon leads to a limitation of the microorganisms that can be used to reduce this type of compound.

This work was carried out to validate the generality of our three-step chemoenzymatic synthesis of chiral epoxides, shown before only on 2,3-epoxides: synthesis and microbiological reduction of an α-halogenoketone, cyclization into epoxide from the diastereoisomers of chlorohydrins obtained previously optically pure. This method is still applicable, but the synthesis of the 3,4- or 4,5-epoxides via this route is less interesting than in the case of 3-halogeno-2-ketone, because of the low yield obtained.

4. Experimental section

4.1. *General methods*

Gas chromatography (GC) was performed using an instrument equipped with a flame ionization detector and a 50 m×0.32 mm capillary column coated with Carbowax 20M for analytical analysis or

a 25 m×0.25 mm capillary column coated with Lipodex E (modified γ-cyclodextrin) for determination of enantiomeric excesses. The carrier gas was hydrogen at 65 kPa. Oven temperature: P1 (40°C for 5 min and then 40 to 200°C at 8°C/min) or P2 (80°C for 5 min and then 80 to 200°C at 5°C/min). For ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) NMR spectra, the chemical shifts were relative to chloroform. For ^{19}F (376.48 MHz), they were relative to $CFCl_3$. Microanalyses were performed by the Service Central d'Analyses du CNRS, Vernaison (France).

Microbiological methods: The microorganisms were all laboratory-grown except for freeze-dried baker's yeast which was a commercial product (VAHINE Monteux). Preculture and culture conditions for the fungi *Aspergillus niger* ATCC 9142, *Beauveria bassiana* ATCC 9142, *Cunninghamella elchinulata* var. *elegans* ATCC 9245, *Geotrichum candidum* CBS 233-76 and *Mortierella isabellina* NRRL 1757, for the bacterium *Lactobacillus kefir* DSM 20587 and for the yeast *Rhodotorula glutinis* NRRL Y 1091 have already been described elsewhere.[26]

4.2. Syntheses of the substrates

4.2.1. Synthesis of 4-chloro-3-octanone 1

4.2.1.1. *Synthesis of diazoketone:* $CH_3–CH_2–CO–CH=N_2$. To a solution of diazomethane (2.9 g; 6.9 mmol; 3 equiv.) in diethyl ether, placed in an ice bath, was added dropwise 2.15 g (2.3 mmol; 1 equiv.) of propionyl chloride. After the end of the addition, the solution was stirred at room temperature until no nitrogen bubbling was observed. Diethyl ether was then removed on a steam bath. The residue was purified by column chromatography on silica gel (eluent: pentane:ether 85:15). 1.45 g of pure diazoketone was obtained (yield: 63%).

Retention time: 390 s (P1). ^{1}H NMR (400.13 MHz) δ: 1.05 (t, 3H, J=7.5 Hz); 2.26 (q, 2H, J=7.5 Hz); 5.25 (s, 1H). ^{13}C NMR (100.61 MHz) δ: 8.9 (CH_3); 33.9 (CH_2); 53.7 (CH); 195.8 (C=O).

4.2.1.2. *Synthesis of 4-chloro-3-octanone 1.* To a stirred solution of tributylborane (24 mL; solution 1 M in THF) was added dropwise a solution of 2.4 g (24.5 mmol) of diazoketone in 20 mL of dry THF while maintaining the temperature at −40°C. After stirring for an additional hour at room temperature, the solution was cooled to 0°C. *N*-Chlorosuccinimide (3.23 g; 25 mmol) was added in small portions to maintain the temperature at 0°C. The solution was stirred at 0°C for an additional 15 min. 20 mL of cold 3 N NaOH solution was then added. The solution was stirred vigorously (15 min), then poured into cold saturated brine solution (200 mL), and extracted with ether (three 50 mL portions). The residue remaining after concentration (rotatory evaporator) of the dried ($MgSO_4$) extract was purified by chromatography (eluent: pentane:ether 98:2). 1.75 g of chloroketone was obtained (yield: 45%). Retention time: 450 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.92 (t, 3H, J=7.1 Hz); 1.09 (t, 3H, J=7.2 Hz); 1.25–1.35 (m, 2H); 1.35–1.52 (m, 2H); 1.77–1.89 (m, 1H); 1.89–2.00 (m, 1H); 2.68 (q, 2H, J=7.2 Hz); 4.21 (dd, 1H, J=5.5 Hz, J=8.4 Hz). ^{13}C NMR (100.61 MHz) δ: 7.8 (C-1); 13.8 (C-8); 22.1 (C-7); 28.2 (C-6); 31.9 (C-2); 33.6 (C-5); 63.6 (C-4); 206.2 (C-3). Anal. calcd for $C_8H_{15}ClO$: C, 59.07; H, 9.30; O, 9.84. Found: C, 59.06; H, 9.17; O, 9.88.

4.2.2. Synthesis of 5-chloro-4-nonanone 2

4.2.2.1. *Synthesis of diazoketone:* $CH_3–CH_2–CH_2–CO–CH=N_2$. The same procedure as that described previously for the synthesis of **1** was used. Butyryl chloride was added instead of propionyl chloride. Yield: 66%. Retention time: 480 s (P1). ^{1}H NMR (400.13 MHz) δ: 0.85 (t, 3H, J=6.9 Hz); 1.55 (sex, 2H, J=6.9 Hz); 2.15–2.35 (m, 2H); 5.25 (s, 1H). ^{13}C NMR (100.61 MHz) δ: 13.4 (CH_3); 18.5 (CH_2); 42.7 (CH_2); 54.0 (CH); 195.1 (C=O).

4.2.2.2. *Synthesis of 5-chloro-4-nonanone 2.* Same procedure as that described previously for the synthesis of **1**. Yield: 50%. Retention time: 480 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.91 (t, 3H, *J*=7.3 Hz); 0.93 (t, 3H, *J*=7.4 Hz); 1.25–1.40 (m, 3H); 1.40–1.52 (m, 1H); 1.63 (sex, 2H, *J*=7.3 Hz); 1.75–1.90 (m, 1H); 1.90–2.00 (m, 1H); 2.62 (t, 2H, *J*=7.2 Hz); 4.18 (dd, 1H, *J*=5.6 Hz, *J*=8.4 Hz). ^{13}C NMR (100.61 MHz) δ: 13.6, 13.9 (C-1, C-9); 17.1 (C-2); 22.1 (C-8); 28.2 (C-7); 33.5 (C-3); 40.4 (C-6); 63.8 (C-5); 205.6 (C-4). Anal. calcd for $C_9H_{17}ClO$: C, 61.18; H, 9.70; O, 9.06. Found: C, 61.15; H, 9.61; O, 8.99.

4.2.3. Synthesis of 4-bromo-5-nonanone 3

To a vigorously stirred solution of 4 g of 5-nonanone (28 mmol) in 40 mL glacial acetic acid was added dropwise bromine (1.4 mL, 28 mmol) at room temperature. The mixture was then stirred for 1 h. 10 mL of water was then added to the mixture, which was extracted three times with ether. The organic layer was dried over $MgSO_4$. The residue was purified by column chromatography (eluent: pentane:ether 98:2). Yield: 50%. Retention time: 440 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.91 (t, 3H, *J*=7.3 Hz); 0.94 (t, 3H, *J*=7.4 Hz); 1.27–1.39 (m, 3H); 1.41–1.52 (m, 1H); 1.56 (qu, 2H, *J*=7.4 Hz); 1.84–2.01 (m, 2H); 2.58–2.75 (m, 2H); 4.22 (dd, 1H, *J*=6.4 Hz, *J*=8.1 Hz). ^{13}C NMR (100.61 MHz) δ: 13.5; 13.9 (C-1, C-9); 20.7 (C-2); 22.2 (C-8); 26.1 (C-6); 35.4 (C-7); 38.7 (C-3); 53.6 (C-4); 204.5 (C-5). Anal. calcd for $C_9H_{17}BrO$: C, 48.88; H, 7.75; O, 7.23. Found: C, 48.81; H, 7.79; O, 7.26.

4.2.4. Synthesis of 4-chloro-5-nonanone 4

In a 50 mL round-bottomed flask was stirred a solution of 5-nonanone (2 g; 14 mmol) in 10 mL of CCl_4. To this solution was added dropwise 1.90 g (1.15 mL; 15 mmol) of sulfuryl chloride. After stirring for an additional hour, the solution was washed with water and the organic layer was dried over $MgSO_4$. The residue, obtained after solvent evaporation, was purified on a silica gel column (eluent: pentane:ether 98:2). 1.5 g of pure chloroketone was obtained. Yield: 60%. Retention time: 490 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.87 (t, 3H, *J*=7.2 Hz); 0.90 (t, 3H, *J*=6.7 Hz); 1.28 (sex, 2H, *J*=7.5 Hz); 1.34–1.41 (m, 1H); 1.41–1.49 (m, 1H); 1.54 (qu, 2H, *J*=7.5 Hz); 1.70–1.80 (m, 1H); 1.80–1.92 (m, 1H); 2.60 (t, 2H, *J*=7 Hz); 4.16 (dd, 1H, *J*=5.4 Hz, *J*=8.4 Hz). ^{13}C NMR (100.61 MHz) δ: 13.3; 13.7 (C-1, C-9); 19.3 (C-2); 22.1 (C-8); 25.7 (C-6); 35.6 (C-7); 38.2 (C-3); 63.4 (C-4); 205.3 (C-5). Anal. calcd for $C_9H_{17}ClO$: C, 61.18; H, 9.70; O, 9.06. Found: C, 61.09; H, 9.61; O, 9.08.

4.3. Microbiological reductions

Incubation times varied and are indicated for each microorganism. The products from the residues were separated on a silica gel column, the eluent was pentane:ether 95:5. In each case, the yields are overall yields for diastereoisomers after work-up.

4.3.1. Microbiological reductions of 4-chloro-3-octanone 1

Baker's yeast: Incubation time: 24 h. The residue from 10 flasks consisted of 50% of (−)-(3*S*,4*S*)-4-chloro-3-octanol and 50% of (+)-(3*S*,4*R*)-4-chloro-3-octanol. Yield: 13%.

(−)-(3*S*,4*S*)-4-Chloro-3-octanol: Retention time: 695 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.90 (t, 3H, *J*=6.5 Hz); 0.95 (t, 3H, *J*=6.5 Hz); 1.15–1.30 (m, 1H); 1.30–1.45 (m, 2H); 1.45–1.55 (m, 1H); 1.55–1.70 (m, 2H); 1.80 (qu, 2H, *J*=6.5 Hz); 1.98 (s, 1H, exchangeable with D_2O); 3.48–3.55 (m, 1H); 3.88 (td, 1H, *J*=3.5 Hz, *J*=7.1 Hz). ^{13}C NMR (100.61 MHz) δ: 10.1 (C-1); 14.0 (C-8); 22.3 (C-7); 27.6 (C-6); 28.9 (C-2); 34.7 (C-5); 68.6 (C-4); 75.3 (C-3). Anal. calcd for $C_8H_{17}ClO$: C, 58.35; H, 10.40; O, 9.72. Found: C, 58.36; H, 10.63; O, 9.76. $[\alpha]_D^{25}$ −35 (*c* 0.03, $CHCl_3$); *ee*≥98%.

(+)-(3*S*,4*R*)-4-Chloro-3-octanol: Retention time: 765 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.90 (t, 3H, *J*=7 Hz); 1.00 (t, 3H, *J*=7 Hz); 1.25–1.40 (m, 3H); 1.40–1.55 (m, 1H): 1.55–1.70 (m, 2H); 1.70–1.80 (m, 2H); 2.38 (s, 1H, exchangeable with D_2O); 3.60 (dt, 1H, *J*=3.6 Hz, *J*=8.7 Hz); 3.94 (dt, 1H, *J*=3.6 Hz, *J*=10.0 Hz). ^{13}C NMR (100.61 MHz) δ: 10.3 (C-1); 14.0 (C-8); 22.3 (C-7); 25.4 (C-6); 29.0 (C-2); 32.3 (C-5); 68.8 (C-4); 76.3 (C-3). Anal. calcd for $C_8H_{17}ClO$: C, 58.35; H, 10.40; O, 9.72. Found: C, 57.96; H, 10.36; O, 9.67. $[\alpha]_D^{25}$ +25 (*c* 0.04, $CHCl_3$); *ee*≥98%.

Rhodotorula glutinis: Incubation time: 6 h. The residue from 20 flasks consisted of 48% of (−)-(3*S*,4*S*)-4-chloro-3-octanol and 52% of (+)-(3*S*,4*R*)-4-chloro-3-octanol. Yield: 26%.

(−)-(3*S*,4*S*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ −35 (*c* 0.029, $CHCl_3$); *ee*≥98%.

(+)-(3*S*,4*R*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ +25 (*c* 0.032, $CHCl_3$); *ee*≥98%.

Aspergillus niger: Incubation time: 24 h. The residue from 20 flasks consisted of 20% of **1**, 24% of (+)-(3*R*,4*R*)-4-chloro-3-octanol and 56% of (−)-(3*R*,4*S*)-4-chloro-3-octanol. Yield: 10%.

(+)-(3*R*,4*R*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ +13 (*c* 0.01, $CHCl_3$); *ee*=38%.

(−)-(3*R*,4*S*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ −25 (*c* 0.05, $CHCl_3$); *ee*≥98%.

Mortierella isabellina: Incubation time: 6 h. The residue from 10 flasks consisted of 50% of (−)-(3*S*,4*S*)-4-chloro-3-octanol and 50% of (+)-(3*S*,4*R*)-4-chloro-3-octanol. Yield: 15%.

(−)-(3*S*,4*S*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ −18 (*c* 0.02, $CHCl_3$); *ee*=53%.

(+)-(3*S*,4*R*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ +14 (*c* 0.02, $CHCl_3$); *ee*=56%.

Lactobacillus kefir: Incubation time: 24 h. The residue from 12 flasks consisted of 10% of **1**, 27% of (+)-(3*R*,4*R*)-4-chloro-3-octanol and 63% of (−)-(3*R*,4*S*)-4-chloro-3-octanol. Yield: 18%.

(+)-(3*R*,4*R*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ +35 (*c* 0.01, $CHCl_3$); *ee*≥98%.

(−)-(3*R*,4*S*)-4-Chloro-3-octanol: $[\alpha]_D^{25}$ −9 (*c* 0.01, $CHCl_3$); *ee*=34%.

4.3.2. Microbiological reductions of 5-chloro-4-nonanone **2**

Baker's yeast: Incubation time: 24 h. The residue from 16 flasks consisted of 15% of **2**, 48% of (−)-(4*S*,5*S*)-5-chloro-4-nonanol and 37% of (+)-(4*S*,5*R*)-5-chloro-4-nonanol. Yield: 15%.

(−)-(4*S*,5*S*)-5-Chloro-4-nonanol: Retention time: 750 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.92 (t, 3H, *J*=7.2 Hz); 0.94 (t, 3H, *J*=7.1 Hz); 1.24–1.46 (m, 4H); 1.46–1.62 (m, 4H); 1.81 (q, 2H, *J*=7.2 Hz); 1.96 (s, 1H, exchangeable with D_2O); 3.58–3.68 (m, 1H); 3.90 (dt, 1H, *J*=3.7 Hz, *J*=6.7 Hz). ^{13}C NMR (100.61 MHz) δ: 13.9; 14.0 (C-1, C-9); 19.1 (C-2); 22.4 (C-8); 28.9 (C-7); 34.7 (C-6); 37.0 (C-3); 69.2 (C-5); 73.7 (C-4). Anal. calcd for $C_9H_{19}ClO$: C, 60.49; H, 10.72; O, 8.95. Found: C, 59.83; H, 10.70; O, 8.83. $[\alpha]_D^{25}$ −36 (*c* 0.02, $CHCl_3$); *ee*≥98%.

(+)-(4*S*,5*R*)-5-Chloro-4-nonanol: Retention time: 810 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.93 (t, 3H, *J*=7.1 Hz); 0.97 (t, 3H, *J*=7.0 Hz); 1.28–1.42 (m, 4H); 1.42–1.67 (m, 4H); 1.69–1.78 (m, 2H); 2.38 (s, 1H, exchangeable with D_2O); 3.72–3.80 (m, 1H); 4.01 (dt, 1H, *J*=3.7 Hz, *J*=9.6 Hz). ^{13}C NMR (100.61 MHz) δ: 14.0, 14.1 (C-1, C-9); 19.2 (C-2); 22.3 (C-8); 29.1 (C-7); 32.3 (C-6); 34.6 (C-3); 69.3 (C-5); 74.6 (C-4). Anal. calcd for $C_9H_{19}ClO$: C, 60.49; H, 10.72; O, 8.95. Found: C, 60.05; H, 11.07; O, 8.78. $[\alpha]_D^{25}$ +14 (*c* 0.04, $CHCl_3$); *ee*≥98%.

Rhodotorula glutinis: Incubation time: 6 h. The residue from 11 flasks consisted of 50% of (−)-(4*S*,5*S*)-5-chloro-4-nonanol and 50% of (−)-(4*R*,5*S*)-5-chloro-4-nonanol. Yield: 8%.

(−)-(4*S*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −36 (*c* 0.01, $CHCl_3$); *ee*≥98%.

(−)-(4*R*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −14 (*c* 0.01, $CHCl_3$); *ee*≥98%.

Aspergillus niger: Incubation time: 48 h. The residue from 15 flasks consisted of 60% of (−)-(4*S*,5*S*)-5-chloro-4-nonanol and 40% of (−)-(4*R*,5*S*)-5-chloro-4-nonanol. Yield: 8%.

(−)-(4*S*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −23 (*c* 0.02, $CHCl_3$); *ee*=65%.

(−)-(4*R*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −10 (*c* 0.01, $CHCl_3$); *ee*=71%.

Mortierella isabellina: Incubation time: 24 h. The residue from 10 flasks consisted of 85% of (−)-(4*S*,5*S*)-5-chloro-4-nonanol and 15% of *anti* 5-chloro-4-nonanol. Yield: 5%.

(−)-(4*S*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −11 (*c* 0.01, $CHCl_3$); *ee*=30%.

Lactobacillus kefir: Incubation time: 24 h. The residue from 12 flasks consisted of 10% of **2**, 36% of (−)-(4*S*,5*S*)-5-chloro-4-nonanol and 54% of (−)-(4*R*,5*S*)-5-chloro-4-nonanol. Yield: 6%.

(−)-(4*S*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −27 (*c* 0.01, $CHCl_3$); *ee*=75%.

(−)-(4*R*,5*S*)-5-Chloro-4-nonanol: $[\alpha]_D^{25}$ −14 (*c* 0.02, $CHCl_3$); *ee*≥98%.

4.3.3. *Microbiological reductions of 4-bromo-5-nonanone* **3**

Baker's yeast: Incubation time: 24 h. The residue from 12 flasks consisted of 19% of **3**, 73% of (+)-(4*R*,5*R*)-4-bromo-5-nonanol and 8% of *anti* 4-bromo-5-nonanol. Yield: 9%.

(+)-(4*R*,5*R*)-4-Bromo-5-nonanol: Retention time: 890 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.91 (t, 3H, *J*=7.1 Hz); 0.94 (t, 3H, *J*=7.3 Hz); 1.20–1.34 (m, 2H); 1.34–1.45 (m, 3H); 1.45–1.60 (m, 3H); 1.72–1.83 (m, 2H); 1.90–2.02 (m, 1H); 3.44–3.53 (m, 1H); 3.98 (td, 1H, *J*=4.0 Hz, *J*=8.7 Hz). ^{13}C NMR (100.61 MHz) δ: 13.5; 14.1 (C-1, C-9); 21.1 (C-2); 22.7 (C-8); 27.8 (C-7); 35.6 (C-6); 37.9 (C-3); 65.3 (C-5); 73.9 (C-4). Anal. calcd for $C_9H_{19}BrO$: C, 48.44; H, 8.58; O, 7.17. Found: C, 48.39; H, 8.63; O, 7.10. $[\alpha]_D^{25}$ +7 (*c* 0.024, $CHCl_3$); *ee*=40%.

Rhodotorula glutinis: Incubation time: 48 h. The residue from 12 flasks consisted of 25% of 3, 38% of (−)-(4*S*,5*S*)-4-bromo-5-nonanol and 37% of (+)-(4*R*,5*S*)-4-bromo-5-nonanol. Yield: 18%.

(−)-(4*S*,5*S*)-4-Bromo-5-nonanol: $[\alpha]_D^{25}$ −17 (*c* 0.02, $CHCl_3$); *ee*≥98%.

(+)-(4*R*,5*S*)-4-Bromo-5-nonanol: Retention time: 810 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.90 (t, 3H, *J*=7.1 Hz); 0.94 (t, 3H, *J*=7.0 Hz); 1.28–1.42 (m, 4H); 1.48–1.62 (m, 3H); 1.62–1.90 (m, 3H); 2.38 (s, 1H, exchangeable with D_2O); 3.65–3.75 (m, 1H); 4.21 (td, 1H, *J*=3.2 Hz, *J*=10.4 Hz). ^{13}C NMR (100.61 MHz) δ: 13.5, 14.1 (C-1, C-9); 21.3 (C-2); 22.7 (C-8); 28.2 (C-7); 32.9 (C-6); 35.2 (C-3); 65.2 (C-5); 74.9 (C-4). Anal. calcd for $C_9H_{19}BrO$: C, 48.44; H, 8.58; O, 7.17. Found: C, 48.41; H, 8.65; O, 7.21. $[\alpha]_D^{25}$ +17 (*c* 0.02, $CHCl_3$); *ee*≥98%.

Mortierella isabellina: Incubation time: 6 h. The residue from 10 flasks consisted of 50% of (−)-(4*S*,5*S*)-4-bromo-5-nonanol and 50% of (+)-(4*R*,5*S*)-4-bromo-5-nonanol. Yield: 18%.

(−)-(4*S*,5*S*)-4-Bromo-5-nonanol: $[\alpha]_D^{25}$ −12 (*c* 0.01, $CHCl_3$); *ee*=70%.

(+)-(4*R*,5*S*)-4-Bromo-5-nonanol: $[\alpha]_D^{25}$ +12 (*c* 0.01, $CHCl_3$); *ee*=70%.

4.3.4. *Microbiological reductions of 4-chloro-5-nonanone* **4**

Baker's yeast: Incubation time: 24 h. The residue from 10 flasks consisted of 10% of **4**, 77% of (−)-(4*S*,5*S*)-4-chloro-5-nonanol and 13% of (+)-(4*R*,5*S*)-4-chloro-5-nonanol. Yield: 28%.

(−)-(4*S*,5*S*)-4-Chloro-5-nonanol: Retention time: 837 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.94 (t, 3H, *J*=7.1 Hz); 0.99 (t, 3H, *J*=7.2 Hz); 1.25–1.36 (m, 2H); 1.36–1.52 (m, 3H); 1.52–1.65 (m, 3H); 1.65–1.85 (m, 2H); 2.15 (s, 1H, exchangeable with D_2O); 3.55–3.67 (m, 1H); 3.92 (td, 1H, *J*=4.1 Hz, *J*=8.8 Hz). ^{13}C NMR (100.61 MHz) δ: 13.5, 14.0 (C-1, C-9); 19.9 (C-2); 22.6 (C-8); 27.9 (C-7); 34.3 (C-6); 36.9 (C-3); 68.7 (C-4); 74.0 (C-5). Anal. calcd for $C_9H_{19}ClO$: C, 60.49; H, 10.72; O, 8.95. Found: C, 60.53; H, 10.71; O, 8.93. $[\alpha]_D^{25}$ −6 (*c* 0.04, $CHCl_3$); *ee*=17%.

(+)-(4*R*,5*S*)-4-Chloro-5-nonanol: Retention time: 899 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.92 (t, 3H, *J*=7.1 Hz); 0.95 (t, 3H, *J*=7.3 Hz); 1.24–1.42 (m, 4H); 1.48–1.58 (m, 3H); 1.62–1.78 (m, 3H); 1.95 (s, 1H, exchangeable with D_2O); 3.70–3.78 (m, 1H); 4.03 (td, 1H, *J*=3.8 Hz, *J*=9.5 Hz). ^{13}C NMR (100.61 MHz) δ: 13.6, 14.0 (C-1, C-9); 20.1 (C-2); 22.7 (C-8); 28.1 (C-7); 32.2 (C-6); 34.6 (C-3); 68.9 (C-4); 74.9 (C-5). Anal. calcd for $C_9H_{19}ClO$: C, 60.49; H, 10.72; O, 8.95. Found: C, 60.72; H, 10.77; O, 9.00. $[\alpha]_D^{25}$ +13 (*c* 0.02, $CHCl_3$); *ee*≥98%.

Rhodotorula glutinis: Incubation time: 24 h. The residue from 10 flasks consisted of 50% of (−)-(4*S*,5*S*)-4-chloro-5-nonanol and 50% of (+)-(4*R*,5*S*)-4-chloro-5-nonanol. Yield: 14%.

(−)-(4*S*,5*S*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ −32 (*c* 0.03, $CHCl_3$); *ee*≥98%.

(+)-(4*R*,5*S*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ +10 (*c* 0.01, $CHCl_3$); *ee*=78%.

Mortierella isabellina: Incubation time: 6 h. The residue from 15 flasks consisted of 50% of (−)-(4*S*,5*S*)-4-chloro-5-nonanol and 50% of (+)-(4*R*,5*S*)-4-chloro-5-nonanol. Yield: 52%.

(−)-(4*S*,5*S*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ −4 (*c* 0.02, $CHCl_3$); *ee*=12%.

(+)-(4*R*,5*S*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ +3 (*c* 0.03, $CHCl_3$); *ee*=23%.

Lactobacillus kefir: Incubation time: 24 h. The residue from 14 flasks consisted of 30% of **4**, 28% of (−)-(4*S*,5*S*)-4-chloro-5-nonanol and 42% of (−)-(4*S*,5*R*)-4-chloro-5-nonanol. Yield: 20%.

(−)-(4*S*,5*S*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ −32 (*c* 0.02, $CHCl_3$); *ee*≥98%.

(−)-(4*S*,5*R*)-4-Chloro-5-nonanol: $[\alpha]_D^{25}$ −13 (*c* 0.05, $CHCl_3$); *ee*≥98%.

4.4. Determination of the enantiomeric excesses

4.4.1. Diastereoisomers of 4-chloro-3-octanol

The enantiomeric excesses were determined by ^{13}C or ^{19}F NMR after converting an aliquot of each diastereoisomer of the product to the corresponding α-methoxy-α-trifluoromethylphenyl acetate (MPTA ester), synthesized according to Dale et al.,[27] and was then purified by column chromatography (eluent: pentane:ether 75:25). Yield: 90%.

Diastereoisomer 1 (*syn*): ^1H NMR (400.13 MHz) δ: 0.85 (t, 3H, *J*=7.1 Hz); 0.90 (t, 3H, *J*=7.1 Hz); 1.25–1.43 (m, 3H); 1.48–1.60 (m, 1H); 1.60–1.90 (m, 4H); 3.60 (s, 3H); 3.97 (td, 1H, *J*=3.4 Hz, *J*=9.8 Hz); 5.19 (td, 1H, *J*=3.4 Hz, *J*=7.7 Hz); 7.35–7.50 (m, 3H); 7.55–7.67 (m, 2H). ^{13}C NMR (100.61 MHz) δ [italic shifts correspond to the (3*S*,4*S*) diastereoisomer]: *9.4*, 9.8 (CH_3); 13.9 (CH_3); 22.2 (CH_2); 23.7, *23.9* (CH_2); *28.7*, 28.8 (CH_2); 33.6, *33.9* (CH_2); 55.7 (OCH_3); 62.0, *62.4* (CH–O); 79.3, *79.5* (CH–Cl); 122.0 (CF_3); 124.8 (C–CF_3); 127.4, *127.6* (Ar); 128.5 (Ar); 129.7 (Ar); 132.0 (Ar); 166.3 (C=O). ^{19}F NMR (376.48 MHz) δ: −0.001 (diastereoisomer *R*,*R*); 0.259 (diastereoisomer *S*,*S*).

Diastereoisomer 2 (*anti*): ^1H NMR (400.13 MHz) δ: 0.83 (t, 3H, *J*=7 Hz); 0.93 (t, 3H, *J*=7 Hz); 1.18–1.43 (m, 3H); 1.50–1.80 (m, 4H); 1.80–1.95 (m, 1H); 3.61 (s, 3H); 4.11 (td, 1H, *J*=3.5 Hz, *J*=10.2 Hz); 5.15 (td, 1H, *J*=3.5 Hz, *J*=8.1 Hz); 7.35–7.50 (m, 3H); 7.50–7.58 (m, 1H); 7.58–7.70 (m, 1H). ^{13}C NMR (100.61 MHz) δ [italic shifts correspond to the (3*S*,4*R*) diastereoisomer]: *9.5*, 9.6 (CH_3); 13.9 (CH_3); 22.2 (CH_2); *22.7*, 23.4 (CH_2); 28.6, *28.8* (CH_2); *33.1*, 33.3 (CH_2); 36.2 (CH_2); 55.6, *55.7* (OCH_3); 62.3, *62.8* (CH–O); 80.0, *80.3* (CH–Cl); 122.0 (CF_3); 124.8 (C–CF_3); *127.5*, 127.6 (Ar); 128.5 (Ar); 129.7 (Ar); 132.0, *132.2* (Ar); 166.3, *166.5* (C=O). ^{19}F NMR (376.48 MHz) δ: 0.099 (diastereoisomer *R*,*S*); 0.342 (diastereoisomer *S*,*R*).

4.4.2. Diastereoisomers of 5-chloro-4-nonanol

The enantiomeric excesses were determined by gas chromatography on a chiral capillary column (Lipodex E). Oven temperature: 80°C. The retention times are as follows: 1316 s [diastereoisomer (4*S*,5*S*)] and 1352 s [diastereoisomer (4*R*,5*R*)]; 1600 s [diastereoisomer (4*S*,5*R*)] and 1773 s [diastereoisomer (4*R*,5*S*)].

4.4.3. Diastereoisomers of 4-bromo-5-nonanol

The enantiomeric excesses were determined by gas chromatography: either on a Carbowax 20M after derivatization of the *syn* diastereoisomer with lactic chloride[28] [oven temperature: 100°C. Retention

times: 4870 s (4*R*,5*R*) and 5315 s (4*S*,5*S*)] or on a chiral capillary column (Lipodex E) for the *anti* diastereoisomer: oven temperature: 90°C. Retention times: 1679 s (4*S*,5*R*) and 1800 s (4*R*,5*S*).

4.4.4. Diastereoisomers of 4-chloro-5-nonanol

The enantiomeric excesses were determined by ^{1}H NMR after converting an aliquot of each diastereoisomer of the product to the corresponding MTPA-ester. Purification by column chromatography (eluent: pentane:ether 95:5). Yield: 90%.

Diastereoisomer 1 (***syn***): ^{1}H NMR (400.13 MHz) δ (italic shifts correspond to the (4*S*,5*S*) diastereoisomer): 0.84, *0.85* (t, 3H, *J*=7.0 Hz); *0.91*, 0.92 (t, 3H, *J*=7.3 Hz); 1.05–1.21 (m, 1H); 1.21–1.45 (m, 4H); 1.45–1.56 (m, 1H); 1.62–1.79 (m, 4H); 3.58, *3.60* (s, 3H); 3.93–4.02 (m, 1H); 5.17–5.29 (m, 1H); 7.35–7.45 (m, 3H); 7.55–7.65 (m, 2H). ^{13}C NMR (100.61 MHz) δ: 13.4 (CH_3); 13.9 (CH_3); 19.8 (CH_2); 22.4 (CH_2); 27.0, *27.4* (CH_2); 30.0, *30.3* (CH_2); 35.7, *36.1* (CH_2); 55.7 (OCH_3); 61.8, *62.0* (CH–O); 78.1, *78.2* (CH–Cl); 121.9 (CF_3); 124.8 (C–CF_3); 127.4, *127.5* (Ar); 127.7 (Ar); 129.7 (Ar); 132.0 (Ar); 166.3 (CO).

Diastereoisomer 2 (***anti***): ^{1}H NMR (400.13 MHz) δ [italic shifts correspond to the (4*R*,5*S*) diastereoisomer]: 0.88 (t, 3H, *J*=7.0 Hz); 0.93 (t, 3H, *J*=7.1 Hz); 1.09–1.22 (m, 1H); 1.22–1.48 (m, 4H); 1.48–1.55 (m, 1H); 1.55–1.80 (m, 3H); 1.80–1.91 (m, 1H); 3.57, *3.63* (s, 3H); 4.13 (td, 1H, *J*=3.8 Hz, *J*=9.0 Hz); 5.10 (td, 1H, *J*=3.8 Hz, *J*=10.0 Hz); 7.35–7.45 (m, 3H); 7.55–7.65 (m, 2H). ^{13}C NMR (100.61 MHz) δ: 13.4 (CH_3); 14.1 (CH_3); 19.7, *19.9* (CH_2); 22.3 (CH_2); 27.1, *27.2* (CH_2); 29.1, *29.9* (CH_2); 35.3, *35.5* (CH_2); 55.6 (OCH_3); 62.4, *62.9* (CH–O); 78.7, *78.8* (CH–Cl); 121.9 (CF_3); 124.8 (C–CF_3); 127.5 (Ar); 127.6 (Ar); 129.7 (Ar); 132.3 (Ar); 166.4 (CO).

4.5. Determination of the absolute configuration

4.5.1. Dechlorination of the chlorohydrins

This chemical correlation was used for both the diastereoisomers of 4-chloro-3-octanol and 5-chloro-4-nonanol.

From 4-chloro-3-octanol: A solution of 50 mg (0.3 mmol) of each diastereoisomer of 4-chloro-3-octanol, obtained by microbiological reduction with baker's yeast, in 3 mL of benzene, was treated with tributyltin hydride (90 μL, 35.6 mmol) and azoisobutyronitrile (AIBN 4 mg) under reflux for 2 h. The solvent was evaporated off. The crude residue was chromatographed on silica (pentane:ether 90:10) to afford 3-octanol. Yield: 90%. Retention time: 350 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.91 (t, 3H, *J*=7.1 Hz); 0.94 (t, 3H, *J*=7.1 Hz); 1.20–1.60 (m, 10H); 1.85 (s, 1H, exchangeable with D_2O); 3.45–3.60 (m, 1H). Same ^{1}H and ^{13}C NMR spectra as those described by Bonini.[30]

- from the diastereoisomer *syn*: $[\alpha]_D^{25}$ +11.5 (*c* 0.01, $CHCl_3$). Lit.:[30] (−)-(3*R*)-3-octanol: $[\alpha]_D^{25}$ −12.5 (*c* 1.29, $CHCl_3$).
- from the diastereoisomer *anti*: $[\alpha]_D^{25}$ +11.1 (*c* 0.02, $CHCl_3$). Lit.:[30] (−)-(3*R*)-3-octanol: $[\alpha]_D^{25}$ −12.5 (*c* 1.29, $CHCl_3$).

From 5-chloro-4-nonanol: The same procedure as that described above was used. The starting material was each diastereoisomer of 5-chloro-4-nonanol, obtained by the reduction with baker's yeast, and the final compound was 4-nonanol. Retention time: 408 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.91 (t, 3H, *J*=7.0 Hz); 0.95 (t, 3H, *J*=7.0 Hz); 1.26–1.34 (m, 8H); 1.34–1.56 (m, 4H); 1.75 (s, 1H, exchangeable with D_2O); 3.55–3.67 (m, 1H). Same ^{1}H and ^{13}C NMR spectra as those described by Ohta.[31]

- from the diastereoisomer *syn*: $[\alpha]_D^{25}$ +2 (*c* 0.05, $CHCl_3$). Lit.:[31] (+)-(4*S*)-4-nonanol: $[\alpha]_D^{25}$ +0.57 (*c* 5.8, hexane), *ee*=60%.

– from the diastereoisomer *anti*: $[\alpha]_D^{25}$ +2 (*c* 0.03, $CHCl_3$). Lit.:[31] (+)-(4*S*)-4-nonanol: $[\alpha]_D^{25}$ +0.57 (*c* 5.8, hexane), *ee*=60%.

4.5.2. Synthesis of the chiral epoxides

General method: To a solution of 1 mmol of chlorohydrin in 5 mL of DMF was added 93 μL of distilled water and 3 mmol of K_2CO_3. The mixture was stirred at room temperature for 2 days. Water was then added and the mixture was extracted three times with ether. The organic layer was dried over $MgSO_4$. The solvent was removed on a water bath and the residue was chromatographed (eluent: pentane:ether 90:10).

From the *syn* (4*S*,5*S*) and *anti* (4*S*,5*R*)-5-chloro-4-nonanol obtained with baker's yeast:

***cis* (4*S*,5*R*)-4,5-Epoxynonane**: Yield: 50%. Retention time: 550 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.93 (t, 3H, *J*=7.2 Hz); 0.99 (t, 3H, *J*=7.2 Hz); 1.33–1.45 (m, 4H); 1.45–1.60 (m, 6H); 2.87–2.96 (m, 2H). ^{13}C NMR (100.61 MHz) δ: 14.1 (C-1, C-9); 20.0 (C-2); 22.7 (C-8); 27.6 (C-7); 28.8 (C-6); 29.9 (C-3); 57.1; 57.2 (C-4, C-5). $[\alpha]_D^{25}$ −5 (*c* 0.02, pentane).

***trans* (4*S*,5*S*)-4,5-Epoxynonane**: Yield: 70%. Retention time: 530 s (P2). ^{1}H NMR (400.13 MHz) δ: 0.90 (t, 3H, *J*=7.1 Hz); 0.95 (t, 3H, *J*=7.1 Hz); 1.24–1.45 (m, 4H); 1.45–1.60 (m, 6H); 2.61–2.70 (m, 2H). ^{13}C NMR (100.61 MHz) δ: 14.1 (C-1, C-9); 19.4 (C-2); 22.6 (C-8); 28.2 (C-7); 31.9 (C-6); 34.3 (C-3); 58.8; 58.9 (C-4, C-5). $[\alpha]_D^{25}$ −32 (*c* 0.02, pentane).

From the *anti* (4*R*,5*S*)-5-chloro-4-nonanol obtained with *Lactobacillus kefir*:

***trans* (4*R*,5*R*)-4,5-Epoxynonane**: Yield: 72%. Same retention time and NMR spectra as those described above. $[\alpha]_D^{25}$ +32 (*c* 0.05, pentane).

From the *syn* (4*S*,5*S*) and *anti* (4*R*,5*S*)-4-chloro-5-nonanol obtained with *Rhodotorula glutinis*:

***cis* (4*R*,5*S*)-4,5-Epoxynonane**: Yield: 48%. Same retention time and NMR spectra as those described above. $[\alpha]_D^{25}$ +5 (*c* 0.04, pentane).

***trans* (4*S*,5*S*)-4,5-Epoxynonane**: Yield: 71%. Same retention time and NMR spectra as those described previously. $[\alpha]_D^{25}$ −25 (*c* 0.05, pentane).

4.5.3. Opening of 4,5-epoxynonane

General method: A solution of 1 mmol of epoxide in 6 mL dry ether was added to a suspension of 0.3 mmol of $LiAlH_4$ in dry ether. The mixture was refluxed for 2 h, then decomposed with water and extracted three times with ether. After evaporation of the solvent, the residue was chromatographed, eluent: pentane:ether 70:30, and a mixture of 4- and 5-nonanol was obtained.

From *cis* (4*R*,5*S*)-4,5-epoxynonane: Yield: 60%. $[\alpha]_D^{25}$ −0.6 (*c* 0.02, $CHCl_3$). Lit.:[31] (+)-(4*S*)-4-nonanol: $[\alpha]_D^{25}$ +0.57 (*c* 5.8, hexane), *ee*=60%.

From *trans* (4*S*,5*S*)-4,5-epoxynonane: Yield: 65%. $[\alpha]_D^{25}$ +0.4 (*c* 0.02, $CHCl_3$). Lit.:[31] (+)-(4*S*)-4-nonanol: $[\alpha]_D^{25}$ +0.57 (*c* 5.8, hexane), *ee*=60%.

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