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Lipase-catalysed preparation of enantiomerically enriched odorants

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Abstract

This review describes the use of lipase-mediated reactions to prepare enantiomerically enriched chiral fragrances for the evaluation of the odour properties of single stereoisomers.

Keywords: Lipase; Enantiomer; Chiral fragrances; Odour; Resolution

1. Introduction

The need of single enantiomer compounds is growing rapidly. Enantiopure chiral drugs represent the 36% of the global market, and the top two drugs in terms of global sales—Lipitor and Zocor—are single enantiomer molecules [1]. Natural flavours are usually single enantiomers, and the synthesis of the corresponding natural-identical compounds requires stereoselective synthetic approaches. In the field of fragrance chemistry, the first stereoisomerically enriched synthetic fragrances are now being commercialised. Kharismal®, Hedione HC® and Super Cepionate® are methyl dihydrojasmonates with a high content of *cis* diastereoisomer; Paradisone® is optically active (+)-*cis*-methyl dihydrojasmonate; Sandranol®, Dartanol®, Sanjinol®, and Levosandol® are optically active (−)-2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol. An increasing concern for human health, and also for the environment preservation, has favoured the trend of employing single enantiomer chemicals in those products that are to interact with human beings.

Nowadays, catalytic enantioselective synthesis is highly preferred in the preparation of enantiopure compounds, even if classical resolution via diastereoisomeric salts is still widely employed. When a new method is to be established, catalysis is first investigated, because a minor amount of chemicals is immobilized in the reaction vessel, and waste is reduced.

Both chemocatalysis and biocatalysis are possible. Chemocatalysis is dominated by metal catalysis, but recent work has also involved organocatalysis [2–4] and Lewis acid catalysis in enantioselective transformations. Several biocatalysed processes are currently running on an industrial scale producing up to hundreds of tons of optically pure intermediates for drug industry and agrochemistry [5–7]. According to a recent survey appeared in *Chemical & Engineering News* [1], biological routes to chiral small molecules will have “a phenomenal growth”. The following reasons are given: (i) Getting the right enzyme in the right quantities is not longer as rate limiting as it used to be. (ii) When a really good enzyme is available, the development of a biocatalytic method is generally characterised by lower costs than those involved in metal-catalysed syntheses. (iii) After an enzyme has been developed, the running cost is low. (iv) Many companies are currently trying to gain expertise in biotransformations even through collaboration with a partner. Biocatalysis is poised for a wider industrial use ranging from resolution to enantioselective synthesis [8]. Since 2000, more than 400 patents on use of microorganisms or enzymes to produce higher purity specialty chemicals have been issued [9].

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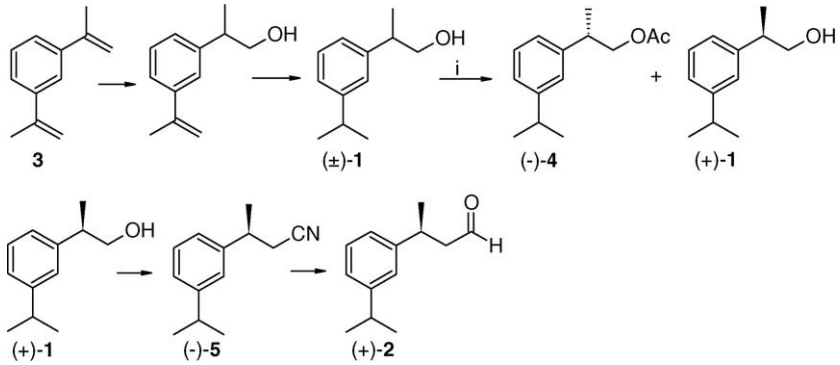


Scheme 1. (i) PPL, *t*-butyl methyl ether, vinyl acetate; column chromatography.

In biocatalysis, enzymes, in particular lipases (E.C. 3.1.1.3), dominate. Lipases generally show remarkable chemoselectivity, regioselectivity and enantioselectivity towards a broad range of substrates. They are readily available in large quantities, because many of them can be produced in high yields by gene expression in an appropriate microorganism, such as a fungi, yeast or bacteria. They do not require cofactors nor catalyse side reactions [10–12]. They remain enzymatically active in organic solvents [13] with great advantages as for the dissolution of the substrates and the recovery of the final products. New promising solvents have been found for lipase-catalysed reactions, such as ionic liquids [14,15] and supercritical carbon dioxide [16,17].

Lipases are employed to catalyse hydrolysis or acylation reactions under mild conditions: common organic solvents, atmospheric pressure and usually, room temperature. No specific reaction apparatus is needed. The handling of lipases is safe for the operator and the environment. In most cases, the enzyme can be recovered and employed again, without loss of activity. The main applications in the field of catalytic stereoselective synthesis involve simple and dynamic kinetic resolutions of racemates [18].

This review deals with the recent applications of lipase-catalysed reactions in the synthesis of the enantiomerically enriched steroisomers of chiral fragrances.

The search of the effective odour vector of chiral fragrances is very challenging. All the possible stereoisomers of a certain chiral odorant have to be prepared in enantiopure form. Their odour properties have to be evaluated and the corresponding odour threshold determined.

This review will show that lipase-catalysed kinetic resolution is a general and efficient method to prepare all the enantiomers of a certain fragrance in high optical purity. An alcoholic function is needed in a key intermediate or in the final product. The OH moiety can be easily created, and transformed in a variety of other functional groups. Thus, the technique of lipase-mediated resolution can be successfully applied to a great number of chiral odorants.

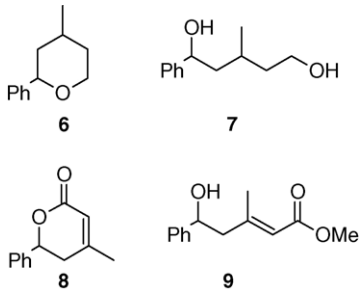
1.1. Lipase-mediated acetylation of primary alcohols and hydrolysis of primary alcohol esters

Porcine pancreatic lipase (PPL) acetylation of the primary alcohol **1**, in *t*-butyl methyl ether solution, in the presence of vinyl acetate as an acyl donor, was the key step [19] in the preparation of both the enantiomers of the odorant Florhydral® **2**. This latter is widely employed in perfumery to convey a fresh marine and ozonic touch [20]. Racemic alcohol **1** was prepared from commercial *m*-diisopropenyl benzene **3** in two reaction steps: hydroboration, followed by quenching with $NaOH\text{-}H_2O_2$, and subsequent catalytic reduction (Scheme 1).

Treatment of (±)-**1** with PPL was afforded after 96 h acetate (−)-**4** (ee = 60%) and unreacted alcohol (+)-**1** (ee = 55%, c[1] = 48%, E = 8.5). The enantiomeric excess of (+)-**1** was increased by repeating the enzymic transesterification. After 120 h reaction time, alcohol (+)-**1** was recovered with ee > 99%. Acetate (−)-**4** (ee = 60%) was hydrolysed with KOH in methanol, and the corresponding alcohol (−)-**1** was submitted to enzymatic acetylation. After 48 h, acetate (−)-**4** with ee > 99% was obtained. Alcohols (+)- and (−)-**1** were then employed as starting materials for the preparation of Florhydral enantiomers. Treatment with *p*-toluenesulphonyl chloride in pyridine, followed by cyanide displacement (Scheme 1) afforded compounds (−)- and (+)-**5**, which were reduced with diisobutylaluminum hydride in THF at −10 °C, to afford enantiopure (+) and (−)-**2**, respectively.

The two enantiopure samples of (+)- and (−)-**2** were submitted to Givaudan perfumers for the olfactory evaluation and the following descriptions were obtained. (+)-**2** was found to be floral, watery, green, yet with acidic touch, even in the dry down note. In comparison with racemic **2**, this enantiomer is more green, a bit less watery, and more powerful (odour threshold = 0.035 ng/l air). Enantiomer (−)-**2** has a typical Florhydral smell, floral, fresh, green, muguet-like, but

[1] Conversion (c) and enantiomeric ratio (E) were calculted according to C.-S. Chen, Y. Fujimoto, G. Girdaukas, C.H. Sih, J. Am. Chem. Soc. 104 (1982) 7294.



Scheme 2.

more marine, and more plastic (odour threshold = 0.88 ng/l air). We also prepared the best enantiomer of Florhydral by a baker's yeast (BY)-mediated enantiospecific synthesis [19].

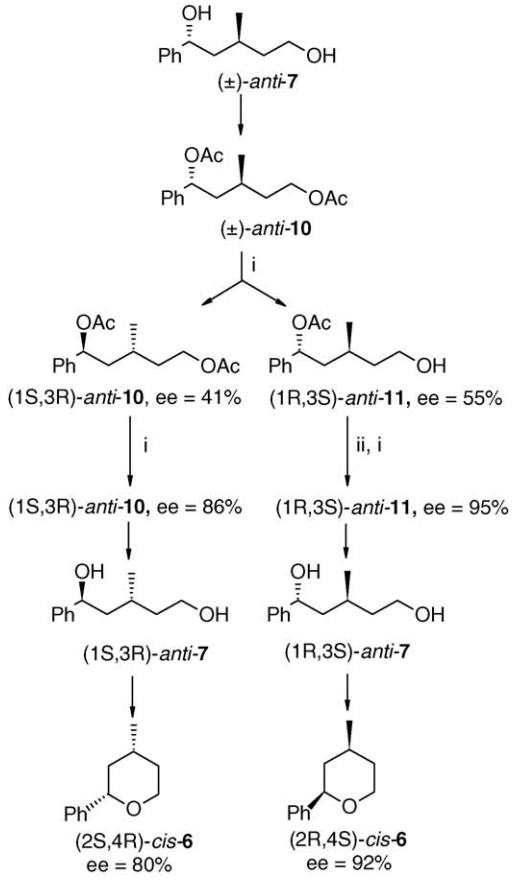
Doremox® (**6**) is a substantive rose odorant which is very useful in the preparation of perfumes and concentrated perfuming bases, as well as of a variety of articles, such as soaps, bath or cosmetic formulations, air and body deodorants, detergents, fabric softeners, and household products [20].

We prepared all the four possible stereoisomers of Doremox® in enantiomerically enriched form by taking advantage of the chemo- and enantioselective hydrolysis of a primary alcohol acetate ester, in the presence of a secondary alcohol acetate ester [21]. For this purpose, we synthesised the two racemic diols *anti*-**7** and *syn*-**7** by $LiAlH_4$ reduction of lactone **8** and hydroxy ester **9** (Scheme 2).

Racemic diols *anti*-**7** (de > 99%) and *syn*-**7** (de = 83%) were acetylated with Ac_2O/Py, and the corresponding acetyl derivatives *anti* and *syn*-**10** were submitted separately to enzymic saponification, at pH 7.8 in the presence of Lipase PS. As for diacetate *anti*-**10** (Scheme 3), the first enzymic saponification afforded monoacetate (1*R*,3*S*)-*anti*-**11** showing 55% ee (c = 43%, E = 5.1). This latter was acetylated and submitted again to lipase-mediated hydrolysis to give (1*R*,3*S*)-*anti*-**11** with ee = 95%. Treatment with KOH in methanol gave (1*R*,3*S*)-*anti*-**7** (ee = 95%). Diacetate (1*S*,3*R*)-*anti*-**10** (ee = 41%) recovered unreacted from the first saponification, was depleted of the (1*R*,3*S*) enantiomer as much as possible by prolonged enzymic reaction. Finally, it afforded diol (1*S*,3*R*)-*anti*-**7** with ee = 86% by reaction with KOH in methanol.

The same procedure was applied to diacetate *syn*-**10** (Scheme 4, first saponification c = 43%, E = 4.1). (1*S*,3*S*)-*syn*-**7** (ee = 70%, de = 70%) and (1*R*,3*R*)-*syn*-**7** (ee = 50%, de = 79%) were obtained from monoacetate *syn*-**11** and survived diacetate *syn*-**10**, respectively.

The four enantiomerically enriched diols were treated with tosyl chloride and pyridine, then with sodium methylate in methanol to promote ring closure and give the four stereoisomers of Doremox® (Schemes 3 and 4). These latter samples were submitted to olfactory evaluation, and (2*S*,4*R*)-*cis*-**6** was found to be the nicest and the most powerful isomer of the series. We also optimised an enantiospecific and completely

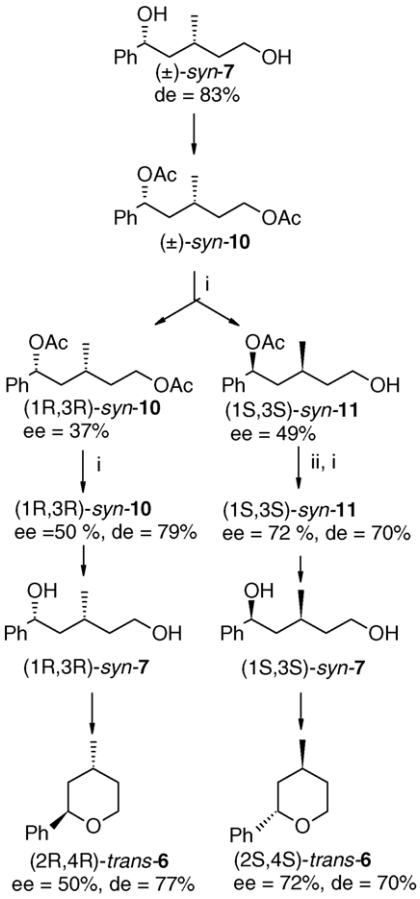


Scheme 3. (i) Lipase PS, THF-water. pH 7.8, NaOH 0.025 M; (ii) acetic anhydride, pyridine.

diastereoselective synthesis of the best isomer (2*S*,4*R*)-*cis*-**6** (ee > 99%, de = 98%) mediated by BY [21].

We exploited the chemoselective PPL-mediated hydrolysis (pH 7.8, THF-water, NaOH 0.5 M) of the primary alcohol acetate ester of enantiopure intermediate (3*S*,4*R*)-**12** in the synthesis of (4*S*,5*S*)-Aerangis lactone (**13**) [22], the main odour component of the African "moth orchids" *Aerangis confusa* J. Stewart and *Aerangis kirkii* (Rolfe) Schltr [23] (Scheme 5). Diacetate (3*S*,4*R*)-**12** was obtained as a single enantiomer and diastereoisomer by BY reduction of keto acid **14** to (3*S*,4*R*)-cognac lactone (**15**), followed by $LiAlH_4$ reduction and chemical acetylation. Mono alcohol mono acetate **16** was converted through a four step route into (4*S*,5*S*)-**13**.

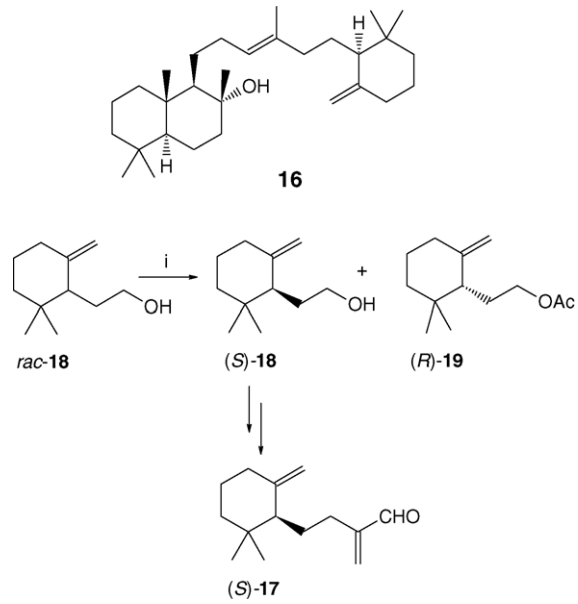
The legendary amber (Fr. ambergris, grey amber) is a pathological metabolite of the sperm whale, probably arising from injuries in its intestines as a result of certain food intakes. The tricyclic triterpene (+)-ambrein is one of its main constituents (Scheme 6). When the excreted chunks of amber are exposed to sunlight and air at the surface of the sea, a number of oxidation products are gradually formed from ambrein. These compounds have a pronounced odour, highly valued in perfumery since antiquity. Amber aldehyde or γ-coronal (**17**) is one of these ambrein's odorous metabolites. Mori and



Scheme 4. (i) Lipase PS, THF-water, pH 7.8, NaOH 0.025 M; column chromatography; (ii) acetic anhydride, pyridine.

co-workers prepared the most precious (*S*)-enantiomer by enzymatic resolution of γ-cyclohomogeraniol (**18**) (Scheme 6) [24].

Acetylation of racemic **18** with vinyl acetate in the presence of lipases was found to give optically active (*R*)-(−)-acetate **19** (ee = 49%), leaving optically active (*S*)-(+)-**18** (ee = 41%, *c* = 45%, *E* = 4.2). Investigation of 13 different enzymes, revealed lipase AK to be the enzyme of choice when used at 0–4 °C with 1.5 eq. of vinyl acetate in hexane in the presence of molecular sieves 4 Å. In preparative experiments,

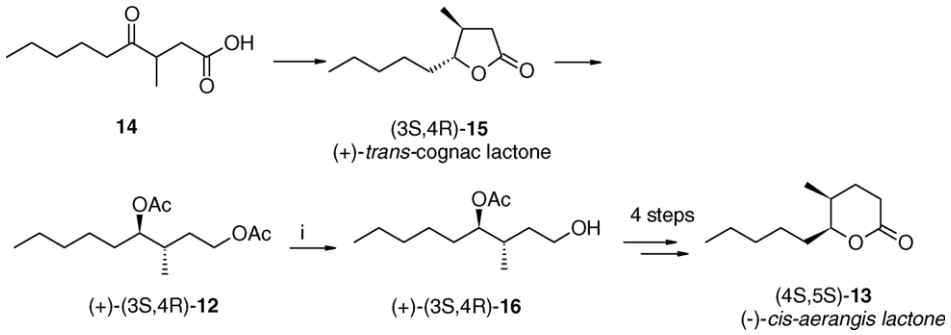


Scheme 6. (i) Lipase AK, vinyl acetate, hexane, MS 4 Å, 0–4 °C.

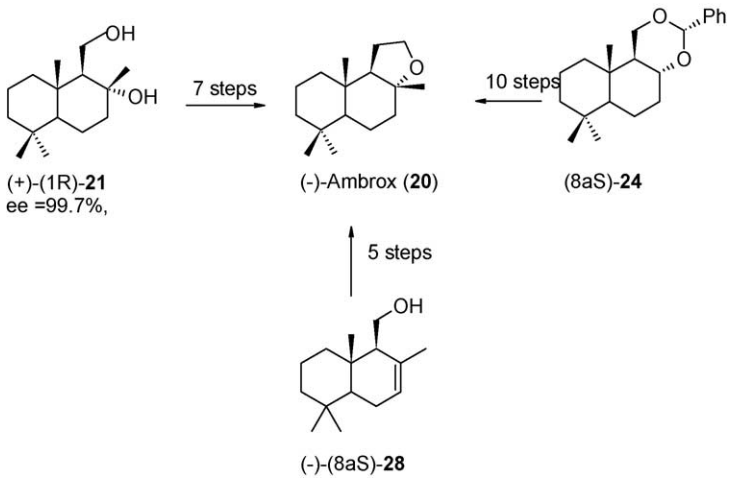
partially resolved (*S*)-(+)-**18** was further acetylated twice enzymatically, to give (*S*)-(+)-**18** showing ee = 97.8%. The partially resolved (*R*)-(−)-**19** was again hydrolysed with sodium hydroxide, and the resulting (*R*)-(−)-**18** was again enzymatically acetylated to give (*R*)-(−)-**19** of increased enantiomeric purity. This enantiomerically enriched acetate (*R*)-(−)-**4** was hydrolysed to give (*R*)-(−)-**18** of 98% ee. Although tedious, this procedure afforded both the enantiomer of **18** in gram-scale. (*S*)-**18** was then converted into (*S*)-γ-coronal through five reaction steps.

Another precious amber odorant is (−)-Ambrox®(**20**). Some interesting bio-catalysed routes to (−)-Ambrox® have been optimised in the last years. The key step of all these approaches (Scheme 7) involve the lipase-mediated acetylation of a suitable primary alcohol intermediate.

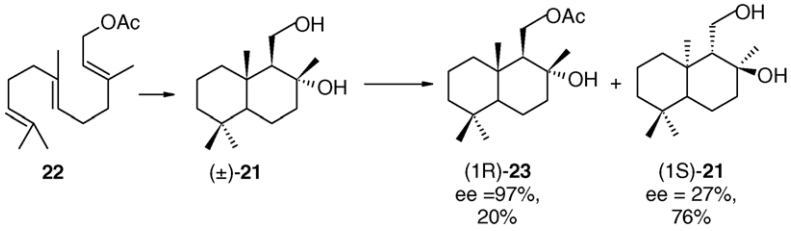
The preparation of (1*R*)-**21** was described by Tanimoto and Oritani [25]. Racemic derivative **21**, readily obtained by cyclisation of farnesyl acetate (**22**) with chlorosulphonic acid and subsequent saponification, was submitted to acetylation in the presence of Lipase PS-30 (*Pseudomonas* sp.,



Scheme 5. (i) PPL, THF-water, pH 7.8, NaOH 0.5 M.



Scheme 7. Lipase-mediated approaches to (−)-Ambrox.

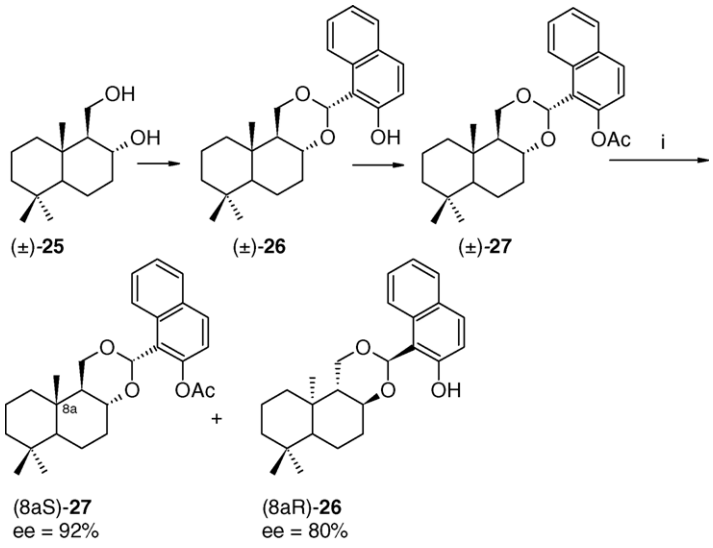


Scheme 8. (i) chlorosulphonic acid; (ii) saponification; (iii) Lipase PS-30, vinyl acetate, 2 days.

Amano) (Scheme 8). Acetate (1*R*)-**23** (ee = 97%) and diol (1S)-**21** (ee = 27%, *c* = 22%, *E* = 85.8) were obtained. (1*R*)-**23** was deacetylated by $LiAlH_4$ reduction, and crystallised, to give enantiomerically pure (1*R*)-**21** (ee = 99.7%). Repeated acetylations of the recovered substrate (1*S*)-**21** brought its enantiomeric excess to the value of 89%. Recrystallisation from diisopropyl ether then afforded enantiomerically pure (1*S*)-**21**. Diol (1*R*)-**21** was then converted into (−)-Ambrox through seven reaction steps. The same authors employed the chiral building block (1*R*)-**21** even for the preparation of (+)-ambrein [26], in order to satisfy the request of fragrance companies, which were interested in the release of ambergris scent by degradation of synthetic ambrein.

Intermediate (8a*S*)-**24** was prepared by Akita et al. [27]. Racemic diol **25** was first submitted to enzyme-mediated acetylation with lipase "Godo E-4" from *Pseudomonas* sp. in the presence of isopropenyl acetate to obtain a mixture of four different optically active compounds [28]. The authors overcome the problem of the high reactivity of the primary OH group and of the presence of two reaction sites by treating racemic diol **25** with 2-hydroxynaphthaldehyde to give the phenolic acetal **26** as a single diasteroisomer (Scheme 9). The corresponding acetyl derivative **27** was exposed to acylase I (no. A-2156) from *Aspergillus melleus*, to give hydrolised product (8a*R*)-**26** (ee 80%) and unreacted (8a*S*)-**27** (ee 92%, *c* = 53%, *E* = 27.5). The enantiomeric excess of both materials was improved by crystallisation, to give enantiomerically pure (8a*R*)-**26** and (8a*S*)-**27**. Treatment of (8a*S*)-**25**, obtained by hydrogenolysis of (8a*S*)-**27** (H_2, 20% $Pd(OH)_2$-C/AcOEt), with benzaldehyde in the presence of a catalytic amount of conc. sulphuric acid gave only (8a*S*)-**24** in 98% yield (Scheme 8). This latter was converted into (−)-**20** in 10 reaction steps.

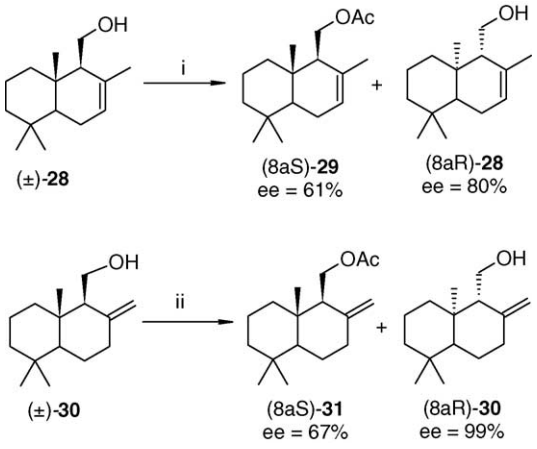
Enantiopure (−)-(8a*S*)-drimenol (**28**) has been recently used to prepare (−)-Ambrox® [29]. Racemic **28** was treated with PL-266 in the presence of isopropenyl acetate in diisopropyl ether at 33 °C, to afford acetate (8a*S*)-**29** (ee = 61%) and unreacted (8a*R*)-**28** (ee = 80%, *c* = 57%, *E* = 9.9) (Scheme 10). The ee of (8a*R*)-**28** was improved to 88% by repeating the enzymatic acetylation. On the other hand, (8a*S*)-**29** (ee = 61%) was treated with $LiAlH_4$, to give (8a*S*)-**28**, which was submitted to enzymatic acetylation in order to obtain (8a*S*)-**29** with increased ee (ee = 85%). The authors found that enantiomerically pure (8a*S*)-drimenol **28** could be obtained in 90% yield also by treatment of albicanol (8a*S*)-**30** with $BF_3{\cdot}Et_2O$. This latter derivative could be prepared by lipase-mediated acetylation. Racemic Albicanol (**30**) was treated with lipases in diisopropyl ether solution at 33 °C in the presence of isopropenyl acetate. OF-360 (from *Candida rugosa*) and MY-30 (from *C. rugosa*) gave acetate (8a*R*)-**31** and unreacted (8a*S*)-**30**, while Amano P (from *Pseudomonas* sp.) and PL-266 (from *Alcaligenes* sp.) provided acetate (8a*S*)-**31** and alcohol (8a*R*)-**30**. Whereas, in the presence of PL-266, acetate (8a*S*)-**31** and (8a*R*)-**30** were obtained with ee = 67% and ee > 99%, respectively (*c* = 59%, *E* = 19.5). Then, optically active (8a*S*)-**31** with ee = 67% was



Scheme 9. (i) Acylase I from *Aspergillus melleus* in H_2O saturated $(i\text{-}Pr)_2O$.

reduced with $LiAlH_4$ to give albicanol (8a*S*)-**30**, which was submitted again to PL-266-catalysed transesterification, affording enantiomerically pure albicanyl acetate (8a*S*)-**31** in 53% yield. This latter was deacetylated by reduction and converted into drimenol (8a*S*)-**28** in order to prepare (−)-Ambrox (five reaction steps).

A combination of lipase-catalysed acetylation and hydrolysis of a primary alcoholic function was exploited by Kiyota et al., in order to prepare the enantiomerically enriched enantiomers of *cis*-α irone (**32**) and *cis*-γ-irone (**33**), which are key components of Iris root oil [30]. Lipase-mediated transesterification of racemic alcohol **34** was investigated in the presence of vinyl acetate as an acyl donor. PPL (Sigma), Lipase 2G (Nagase), and CHIRAZYME® L-9 exhibited moderate enantioselectivity. Hydrolysis of the corresponding racemic acetate **35** at pH 7 phosphate buffer gave good results when PPL was employed: alcohol (*S*)-**34** showing ee = 89% (*c* = 38%, *E* = 29.6) was recovered. To obtain enantiomerically enriched derivative **34**, these reactions were combined: the transesterification of racemic **34**, followed by hydrolysis of the intermediary acetate (−)-**35** (76% ee) gave (*S*)-**34** (96% ee). In the same work, classical resolution of 2,2,4-trimethylcyclohex-3-ene carboxylic acid with 1-phenylethylamine was performed, in order to prepare not only (*S*)-**34** but also its (*R*)-enantiomer. (*R*)- and (*S*)-**34** were then converted, according to two different synthetic routes, into (+)- and (−)-*cis*-α-irone and into (+)- and (−)-*cis*-γ-irone (Scheme 11).



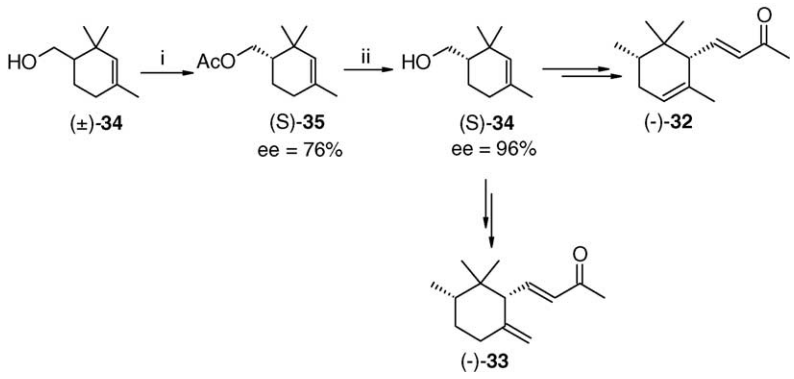
Scheme 10. (i) Lipase PL-266, isopropenyl acetate, $(i\text{-}Pr)_2O$, 33 °C; (ii) Lipase PL-266, isopropenyl acetate, $(i\text{-}Pr)_2O$, 33 °C.

1.2. Lipase-mediated Acetylation of Secondary Alcohols

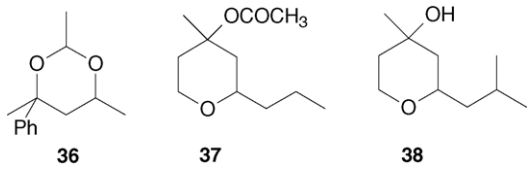
We successfully employed the bio-catalysed resolution of β-hydroxy ketones for the preparation of all the stereoisomers of the commercial floral odorants Floropal (**36**) [31], Clarycet (**37**), and Florol (**38**) (Scheme 12) [32].

Floropal is a grapefruit fragrance with *fruity rhubarb undertones* [20]. It has been employed as a mixture of two diastereoisomers (**36a** and **36b**) for more than 20 years as an aromatic substance (Vertacetal® and Floropal/Corps. 717). In 2000, Pickenhagen and co-workers [33] reported the odour properties of the two diastereoisomers **36a** and **36b**. Ketal **36a** was described as *strong, herbal-fresh, green, and typical grapefruit*; **36b** was found to be *very weak, chemical solvent-like*, and to have a detracting influence upon the sensory properties of the mixture. They also developed a procedure, based either on fractional distillation or on reaction with $BF_3{\cdot}Et_2O$, to enrich the mixture into the more valuable derivative **36a**.

We submitted racemic hydroxy ketone **39**, obtained by aldolic condensation of acetophenone and acetaldehyde, to lipase-mediated esterification in *t*-butyl methyl ether solution, in the presence of vinyl acetate. Acetate (*R*)-**40** (ee = 97%) and unreacted alcohol (*S*)-**39** (ee = 93%) were recovered (*c* = 49%, *E* = 226). Both (*R*)-**40** and (*S*)-



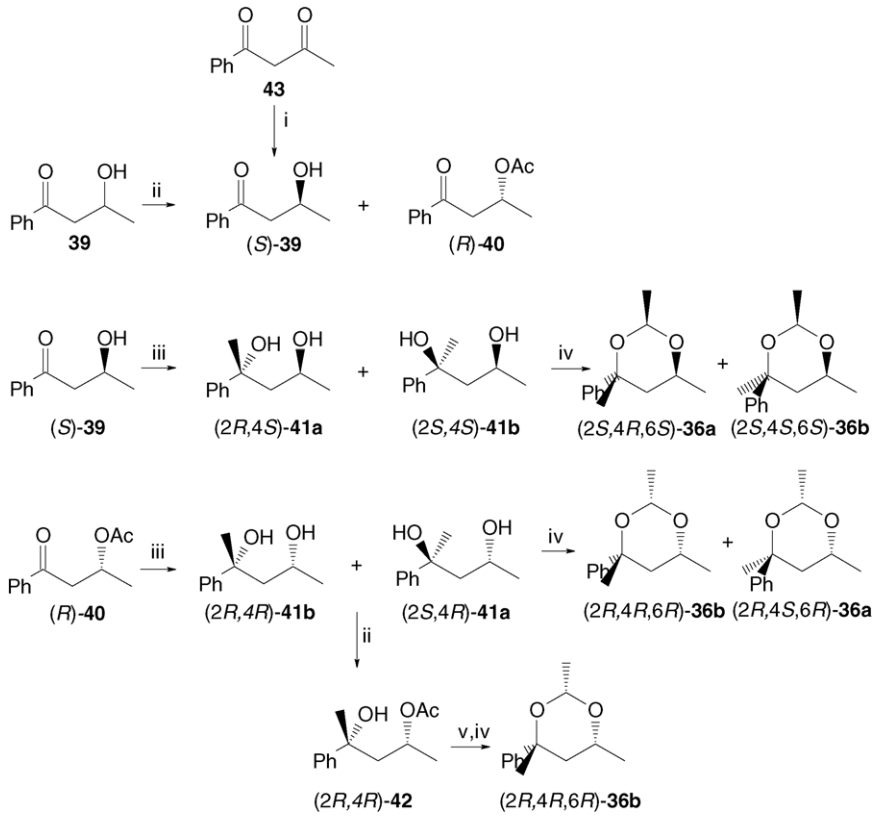
Scheme 11. (i) PPL, Vinyl acetate; (ii) PPL, toluene, pH 7.



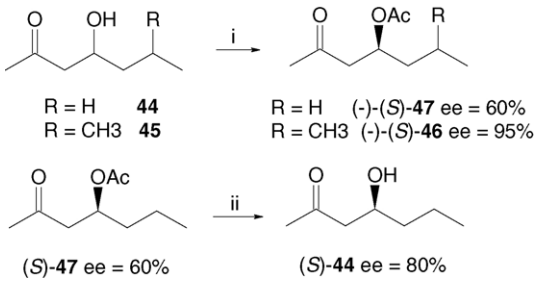
Scheme 12.

39 derivatives were submitted to methyl magnesium iodide treatment, followed by reaction with acetaldehyde, to afford two mixtures, one of enantiopure (2*R*,4*R*,6*R*)-**36b** (66%) and (2*R*,4*S*,6*R*)-**36a** (33%), and the other of enantiopure (2*S*,4*R*,6*S*)-**36a** (33%) and (*2S*,4*S*,6*S*)-**36b** (66%) (Scheme 13).

The mixture of diol derivatives (2*S*,4*R*)-**41a** and (2*R*,4*R)*-**41b**, prepared by reaction of methyl magnesium iodide with (*R*)-**40**, was submitted to enzyme-mediated transesterification in the usual conditions, and a diastereoselective acetylation of (2*R*,4*R*)-**41b** was observed, affording acetate (2*R*,4*R*)-**42**. This latter was hydrolysed with potassium hydroxide in methanol to provide (2*R*,4*R)*-**41b** (ee > 99%; de = 86%, i.e. (2*R*,4*R*)-**41b**/(2*S*,4*R*)-**41a** 93:7), which was soon converted into Floropal (2*R*,4*R*,6*R*)-**36b** (ee > 99%; de = 76%, i.e. (2*R*,4*R*,6*R*)-**36b**/(2*R*,4*S*,6*R*)-**36a** 88:12).



Scheme 13. (i) Baker's Yeast; (ii) Lipase PS, *t*-butyl methyl ether, vinyl acetate; (iii) CH_3Mgl, Et_2O; (iv) CH_3CHO, CH_2Cl_2, *p*-toluenesulphonic acid; (v) KOH, MeOH.



Scheme 14. (i) *t*-butyl methyl ether, vinyl acetate, lipase; column chromatography; (ii) THF-water, pH 7.8, CCL.

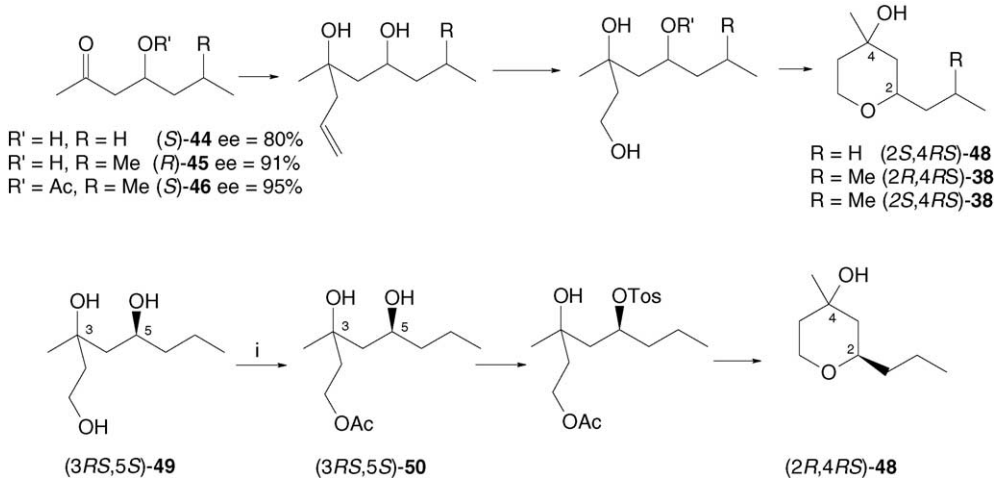
The two mixtures of enantiopure diastereoisomers **36a** and **36b** were enriched in the most valuable diastereoisomer by treatment with $BF_3{\cdot}Et_2O$, according to reference 33. All the samples of Floropal isomers were submitted to olfactory evaluations (Givaudan and Dragoco perfumers).

The most relevant results were the following: (2*S*,4*R*,6*S*)-(−)-**36a** was described to be the most interesting of the enantiomers of the valuable diastereoisomer **36a**. Its presence conferred to the mixture (2*S*,4*R*,6*S*)-**36a**/(2*S*,4*S*,6*S*)-**36b** (1:2) a nice character, in spite of the fact that the major component was (2*S*,4*S*,6*S*)-**36b**. (2*R*,4*R*,6*R*)-(+)-**36b** was found to have a pronounced floral character, quite different from that of racemic **36b**. We also obtained the starting hydroxy ketone with 92% optical purity by BY fermentation of diketone **43**.

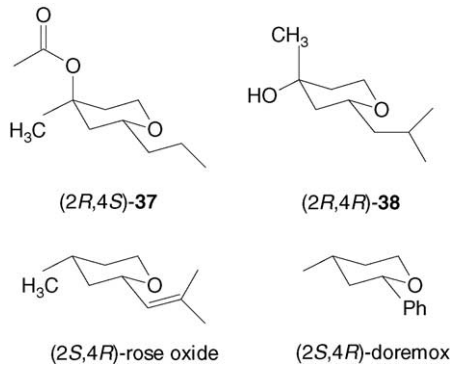
Clarycet® (**37**) and Florol® (**38**) are two widely used floral fragrances commercialised as mixtures of two racemic diastereoisomers. Clarycet® (IFF) is described to have a *herbal, floral, rosy odour with a dried fruitiness and a suggestion of clary sage.* Florol® (Firmenich) is *fresh, soft, with a natural floral note; it can be used in almost all perfume types where it gives elegant floral diffusion without changing the character of the fragrance.* We prepared all the steroisomers of Clarycet® and Florol® by a bio-catalysed route, based on the kinetic resolution of hydroxy ketones **44** and **45** (Scheme 14). Lipase PS catalysed the enantioselective acetylation of derivative **45** in *t*-butyl methyl ether and in the presence of vinyl acetate, affording the corresponding acetate (*S*)-**46** (30% yield) with ee = 95% (Scheme 14). A rather slow enrichment of the starting alcohol allowed us to recover hydroxy ketone (*R*)-**45** showing ee = 91% (35% yield). Different results were obtained with racemic **44**. Only *C. rugosa* lipase (CRL, ex *Candida cylindracea* lipase) was found to promote the acetylation of compound **44** with low enantioselectivity. Acetate (*S*)-**47** showed ee = 60%. A further enrichment was obtained by submitting (*S*)-**47** (ee = 60%) to bio-catalysed hydrolysis in water-THF solution at pH 7.8 in the presence of CRL. Alcohol (*S*)-**44** (67% yield) was recovered with ee = 80%. A striking influence of the structure on the steric course of lipase acetylation was thus observed.

Enantiomerically enriched hydroxy ketones (*S*)-**44** (ee = 80%), (*R*)-**45** (ee = 91%) and acetate (*S*)-**46** (ee = 95%) were converted into the pyrane derivatives (2*S*,4*RS*)-**48**, (2*R*,4*RS*)-**38**, and (2*S*,4*RS*)-**38**, respectively (Scheme 15). The diastereoisomers of each mixture could be separated by column chromatography to afford the following products: (2*S*,4*R*)-**48** (de > 99%, ee = 80%), (2*S*,4*S*)-**48** (de > 99%, ee = 80%), (2*R*,4*R*)-**38** (de > 99%, ee = 91%), (2*R*,4*S*)-**38** (de = 96%, ee = 91%), (2*S*,4*R*)-**38** (de > 99%, ee = 95%), and (2*S*,4*S*)-**38** (de > 99%, ee = 95%). Treatment of (2*S*,4*R*)-**48** and (2*S*,4*S*)-**48** in refluxing acetic anhydride in the presence of sodium acetate gave (2*S*,4*R*)-**37** and (2*S*,4*S*)-**37**, respectively.

The CRL-mediated acetylation of (±)-**44** was too slow to allow the recovery of the enantiomerically enriched isomer (*R*)-**44**, thus a different route to (2*R*)-Clarycet® diastereoisomers had to be found (Scheme 15). Chemoselective acetylation of the primary alcohol moiety of the two diastereoisomeric triols (3*RS*,5*S*)-**49** was achieved by treatment with Lipase PS in *t*-butyl methyl ether and vinyl acetate. The two diastereoisomeric monoacetates (3*RS*,5*S*)-**50** (78% yield) were treated with *p*-toluenesulphonyl chloride in pyridine, then submitted to saponification with 10% NaOH in ethanol. Ring closure occurred with inversion of configuration at the carbon



Scheme 15. (i) Lipase PS, *t*-butyl methyl ether, vinyl acetate.



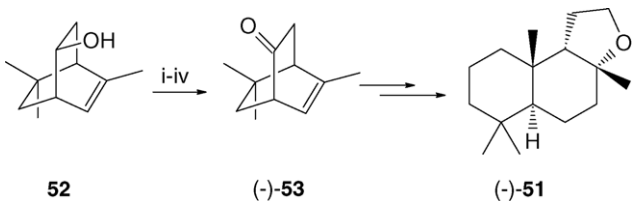
Scheme 16.

atom in position 2 and a mixture of the two diastereoisomers (2*R*,4*RS*)-**48** was obtained. The two diasteroisomers were separated by column chromatography and submitted separately to acetylation in refluxing acetic anhydride in the presence of sodium acetate. The two enantiomerically enriched Clarycet® isomers (2*R*,4*S*)-**37** (69% yield) and (2*R*,4*R*)-**37** (64% yield) were recovered.

All the samples of Clarycet and Florol stereoisomers were evaluated by Givaudan perfumers. The following conclusions could be drawn. As for Clarycet®, the single enantiomer (2*R*,4*S*)-**37** has a nice floral odour, which makes it distinguished from the other stereoisomers. This latter can be related to the best isomers of other tetrahydropyranil fragrances, such as (2*S*,4*R*)-rose oxide and (2*S*,4*R*)-Doremox®, which show the methyl group at C(4) and the substituent at C(2) in *cis* diequatorial arrangement, just as (2*R*,4*S*)-**37** (Scheme 16).

A gradual variation of odour intensity has been noticed in the four Florol® isomers, from 1.21 ng/l of (2*R*,4*R*)-**38** to more than 600 ng/l shown by (2*S*,4*R*)-**38**. The two enantiomers (2*R*,4*R*) and (2*S*,4*S*)-**38** of the diastereoisomer bearing the OH group and the isobutyl chain in equatorial positions are decidedly the most intense, and are responsible of the odour of commercial Florol®.

The growing demand for ambergris odorants has stimulated an intense search for this type of odorous compounds. In one of these investigations [34], (−)-9a-epi-Ambrox (**51**) was found to possess a strong amber scent and a low threshold value (0.15 ppb). A lipase-catalysed route to (−)-**51** was optimised by Paquette and Maleczka (Scheme 17) [35]. Racemic alcohol **52** was converted into its chloroacetate and subjected



Scheme 17. (i) $ClCH_2COCl$, pyridine, THF; (ii) Lipase P-30; (iii) NaOH 15%, THF; (iv) PDC, CH_2Cl_2.

to hydrolysis with Lipase P-30 (Scheme 17). By carrying out the enzymatic reaction to 60% completion and saponifying the unreacted ester, the authors were able to obtain (−)-**52** of high optical purity (92%). This latter was oxidised to ketone (−)-**53**, which was then converted in 8 reaction steps into (−)-**51**.

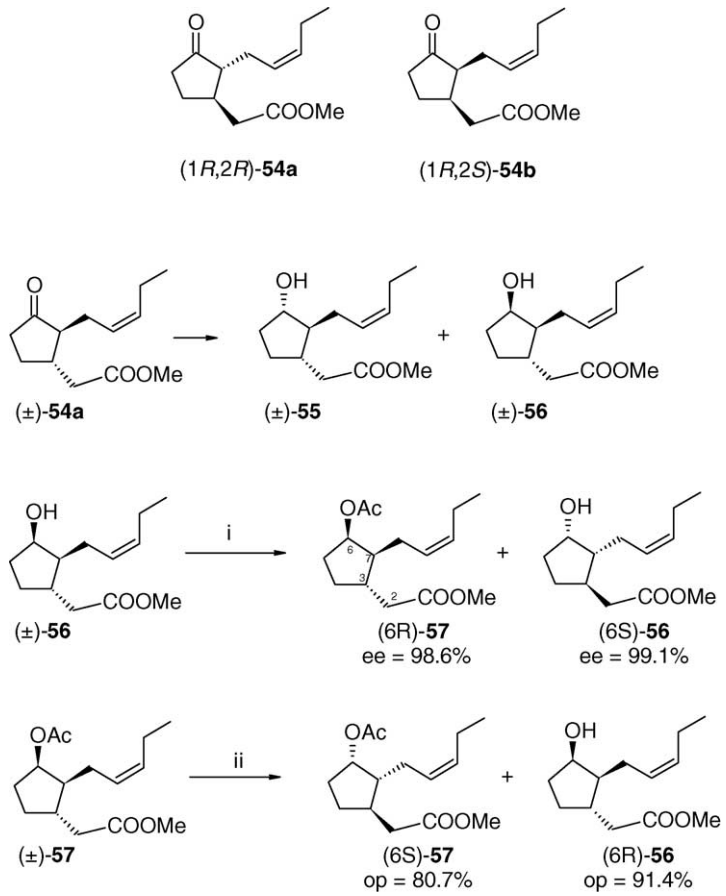
(1*R*,2*R*)-(−)-Methyl *(Z)*-jasmonate ((1*R*,2*R*)-**54a**) and its diastereoisomer (+)-(1*R*,2*S*)-**54b** occur in nature in the proportions of 97:3 as the odorous principle of jasmin flower oil (*Jasminium grandiflorum* L.) [36–38] whereas their enantiomers are nearly odourless.

A lipase-mediated approach to the enantiomers of **54a** was reported by Kiyota et al. (Scheme 18) [39]. Racemic methyl (*Z*)-jasmonate **54a** was reduced to give the two separable diastereoisomeric racemic alcohols **55** and **56**. Transesterification of **55** with vinyl acetate or vinyl chloroacetate gave very modest results with the enzymes tested: lipase MY (Meito); P, PS30 Amano; P, 2G and Rhilipase® (Nagase); immobilized lipase (Toyobo); Chirazyme® L-2 cf. C2 (Roche). On the contrary, Lipase P (Amano) acetylation of **56** in the presence of vinyl acetate was characterised by good selectivity ($E = 370$). Acetate (6*R*)-**57** and unreacted alcohol (6*S*)-**56** were found to be enantiomerically pure ($c = 50\%$). Hydrolysis of acetate **57** was also investigated, and the following results were obtained. Lipase P (Amano)-catalysed the hydrolysis of **57** in hexane and phosphate buffer (pH 7), to give unreacted acetate (6*S*)-**57** (op = 80.7%) and alcohol (6*R*)-**56** (op = 91.4%) ($c = 47\%$, $E = 52.7$). (−)- and (+)-**54a** were obtained by Dess-Martin periodinane oxidation of the enantiomerically pure alcohols (6*S*)- and (6*R*)-**56**.

(*R*)-Muscone (**58**) [40,41] is the odoriferous principle of the dried solidified secretion from a preputial follicle of the male musk deer (*Moschus moschiferus* L.). It belongs to the so-called class of *macrocyclic musks*. The musk note of natural (*R*)-**58** was described as *very nice musky note, rich, and powerful*, that of the (*S*)-enantiomer was found *poor, and less strong* [42]. The odour thresholds measured in water of the two enantiomers were 61 and 233 ppb, respectively. Lipase-mediated acetylation of 3-methylcyclopentadecanol (**59**) was exploited for the preparation of enantiopure muscone (Scheme 19) [43,44]. Transesterification of racemic **59** (mixture of two racemic diastereoisomers) with isopropenyl acetate in isopropyl ether solution, mediated by lipase QL (*Alcaligenes* sp.) gave, after hydrolysis and CrO_3 oxidation, (*R*)-muscone with ee = 88% and good chemical yields.

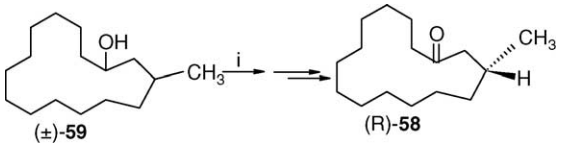
1-(2,2,6-Trimethyl-cyclohexyl)-3-hexanol **60** is a synthetic fragrance [45] with *a powdery-woody odour with animal, steroid-type undertones* known with the commercial name of Timberol®. The commercial mixture is mainly composed by four racemic diastereoisomers. It was established that the woody animal note was due to the *anti*-diastereoisomers (**60a**,**b**) [46], so the 1:1 mixture of the two racemic *anti*-diastereoisomers **60a** and **60b** was introduced into the market with the brand name of Norlimbanol®.

The single enantiomers of **60a** and **60b** were synthesised, starting from (1*R*,6*S*)- and (1*S*,6*R*)-dihydrocyclocitral and



Scheme 18. (i) Lipase P (Amano), vinyl acetate, $(i\text{-Pr})_2O$, 25 °C, $E = 370$; (ii) Lipase P (Amano), hexane, phosphate buffer pH 7, 25 °C, $E = 41$.

(*R*)- and (*S*)-2-propyloxirane [47] and the olfactory evaluation of (+)- and (−)-**60a** and of (+)- and (−)-**60b** gave the following results. (1′*R*,3*S*,6′*S*)-(+)-**60a** was described as the best of the series, *powerful and longlasting, with a very nice woody-ambery note*. (1′*S*,3*S*,6′*R*)-(−)-**60b** showed *an odour note resembling that of*(1*R*,3*S*,6′*S*)-(+)-**60a**,*but less powerful and decidedly inferior*. The (3*S*) isomers were *devoid of the animalic character and very weak* [47,48]. A few years ago, we submitted the mixture of the four stereoisomers of **60a**,**b** to enzymic acetylation (Scheme 20) [49]. (3*R*)-acetate **61a** was first obtained (24 h reaction time, 19%). After 7 days, (3*R*)-acetate **61b** was recovered (18%). Hydrolysis of (3*R*)-**61a** and (3*R*)-**61b** gave (3*R*)-**60a** and (3*R*)-**60b**, respectively. Inversion of the configuration by acetate displacement, followed by saponification, afforded the corresponding odorous (3*S*)-isomers.

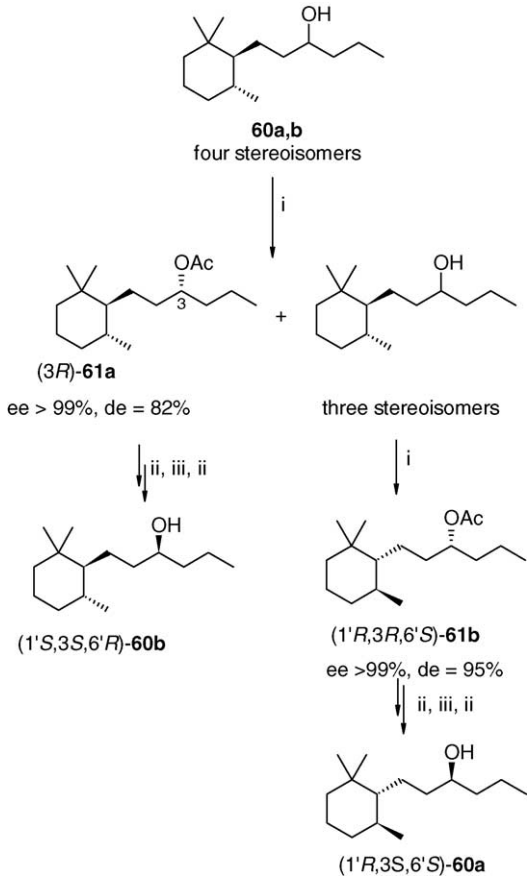


Scheme 19. (i) Lipase QL, isopropenyl acetate, $(i\text{-Pr})_2O$.

1.3. Lipase-mediated acetylation of 1,3 diols

Magnolan is a *substantive floral, rosy odorant* which is employed *to convey the freshness of rose flower dew* in perfume compositions [20]. It is prepared by Prins reaction of α-methyl styrene with acetaldehyde [50] and it is commercialised as a mixture of two diastereoisomers (**62a** and **62b**).

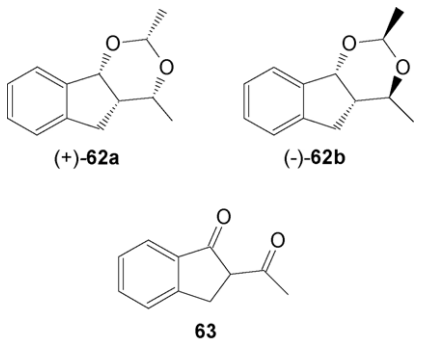
We optimised a bio-catalysed approach to the enantiomers of **62a** and **62b** [51]. $NaBH_4$ reduction of diketone **63** (Scheme 21), prepared according to the literature [52], gave a mixture which mainly contained (80%) the same diols **64a** and **64b**, obtained by hydrolysis of commercial Magnolan®. Diols **64a** and **64b** were separated by column chromatography, and submitted separately to lipase-mediated acetylation, using Lipase PS and CRL as catalysts. Different regiochemistry and enantioselectivity were observed with the two different enzymes (Scheme 22). When racemic diol **64a** was treated with CRL, diacetate (1*R*,2*S*,1′*R*)-**65a** (ee > 99%, 12%), mono acetate (1*R*,2*S*,1′*R*)-**66a** (ee = 92%, 16%), and diol (1*S*,2*R*,1′*S*)-**64a** (ee = 77%, 12%) were recovered. Treatment of racemic **64a** with Lipase PS promoted the acetylation of the OH group in position 1′ of the opposite enantiomer, affording (1*S*,2*R*,1′*S*)-**67a** (ee = 87.5%, 24%), and unreacted (1*R*,2*S*,1′*R*)-**64a** (ee = 93%, 31%). A different be-



Scheme 20. (i) Lipase PS, *t*-butyl methyl ether, vinyl acetate; (ii) KOH, MeOH; (iii) *p*-TosCl, pyridine; AcONa in DMF.

haviour was observed when racemic diol **64b** was submitted to enzyme-mediated transesterification. CRL treatment provided diacetate (1*S*,2*R*,1′*R*)-**65b** (ee > 99%, 11%), monoacetate (1*S*,2*R*,1′*R*)-**66b** (ee = 89%, 15%), and diol (1*R*,2*S*,1′*S*)-**64b** (ee = 86%, 18%). Lipase PS promoted the acetylation of **64b** with the same enantioselectivity and different regiochemistry, to afford monoacetate (1*S*,2*R*,1′*R*)-**67b** (ee = 93%, 39%) and diol (1*R*,2*S*,1′*S*)-**64b** (ee = 93%, 32%).

Monoacetate derivatives (1*S*,2*R*,1′*S*)-**67a** (ee = 87.5%) and (1*S*,2*R*,1′*R*)-**67b** (ee = 93%) were hydrolysed, to afford diols (1*S*,2*R*,1′*S*)-**64a** and (1*S*,2*R*,1′*R*)-**64b**. Both the enantiomers of **64a** and **64b** were converted into Magnolan® stereoisomers (Scheme 23) by reaction with acetaldehyde in methylene chloride in the presence of pyridium *p*-toluenesulphonate as a catalyst. The four samples of Magnolan® stereoisomers were evaluated by Givaudan perfumers, together with the corresponding racemic mixtures.



Scheme 21.

As for the comparison of the two racemic diastereoisomers **62a** and **62b**, constituents of commercial Magnolan, a difference in their odour response was found, just as it was reported for the diastereoisomers **36a** and **36b** of Floropal. The most appreciated isomer **62b** shows the phenyl ring linked to C(4a) in axial arrangement, resembling the axial phenyl group of **36a**.

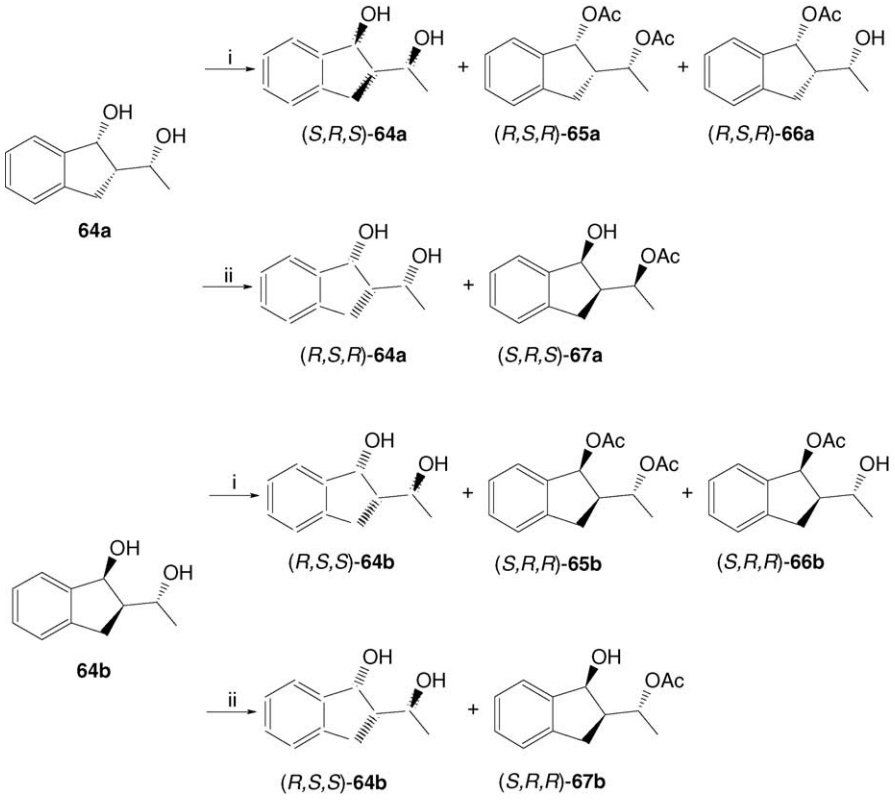
(−)-**62b** is the most interesting enantiomer of diastereoisomer **62b**. The configuration of the three stereocentres is the same observed in (−)-**36a**, the most appreciated of **36a** enantiomers (Scheme 23).

1.4. Lipase-mediated acetylation of allylic alcohols

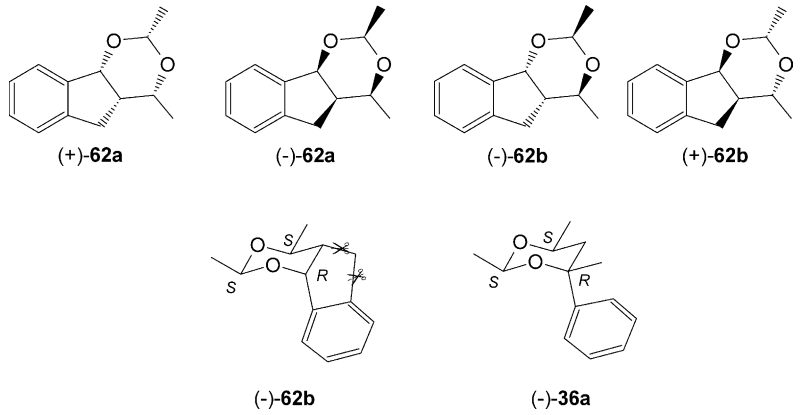
We extensively employed lipase-mediated acetylation of allylic alcohols in the preparation of the enantiomers of α-ionone, the odoriferous principle of violet oil, γ-ionone, and of all the 10 isomers of irone, the main constituents of iris oil. All this work has been already collected in recent specific reviews [53,54]. Some other interesting example are as follows.

Kinetic resolution of allylic alcohol **68** and **69** was exploited by Fehr and Galindo, to prepare the best isomer of jasmonate **54** and of its corresponding dihydro derivative **70** (Scheme 24). Methyl dihydrojasmonate (**70**) is also a valuable compound for the manufacture of perfumes (trade names: Cepionate®, Hedione®) due to its fruity flower fragrance. The *cis* diastereosiomer **70b** is much more powerful than the *trans* diastereoisomer **70a**, and the odour of **70b** is mainly due to the (1*R*,2*S*) enantiomer. Racemic allylic alcohols **68** and **69** were submitted to enzymatic kinetic resolution, by treatment with dimethyl malonate at 40 °C and reduced pressure (8 torr), in the presence of catalytic amounts of Novozym 435 (immobilized *Candida antarctica* from Novo Nordisk) and of $KHCO_3$ (5 mol%). The corresponding malonates (*R*)-**71** (ee = 100%, *c* = 49%, *E* = 751) and (*R*)-**72** (ee = 97%, 48%, *E* = 200) were obtained after 80 min reaction time. The unconverted alcohol derivatives (*S*)-**68** and (*S*)-**69** were racemised under acidic conditions and recycled, thus improving the efficiency of the enzymatic process. Malonates (*R*)-**71** and (*R*)-**72** were converted into the desired enantiomerically pure (1*R*,2*S*)-**70b** and (1*R*,2*S*)-**54b**, according to the same five step synthetic route.

α-Damascone (**73**) smells floral-fruity green, apple-like with a harsh camphoraceous cork-note. This cork-note is due to the (R)-enantiomer, while (S)-**73** is linear, clear and more intense, with a pleasant wine-like note [55]. Great efforts have been devoted to the enantioselective synthesis of (S)-**73**. Mori et al. reported on a biocatalytic approach to (S)-damascone in 1993 [56], exploiting the chiral building block (R)-**74**, he



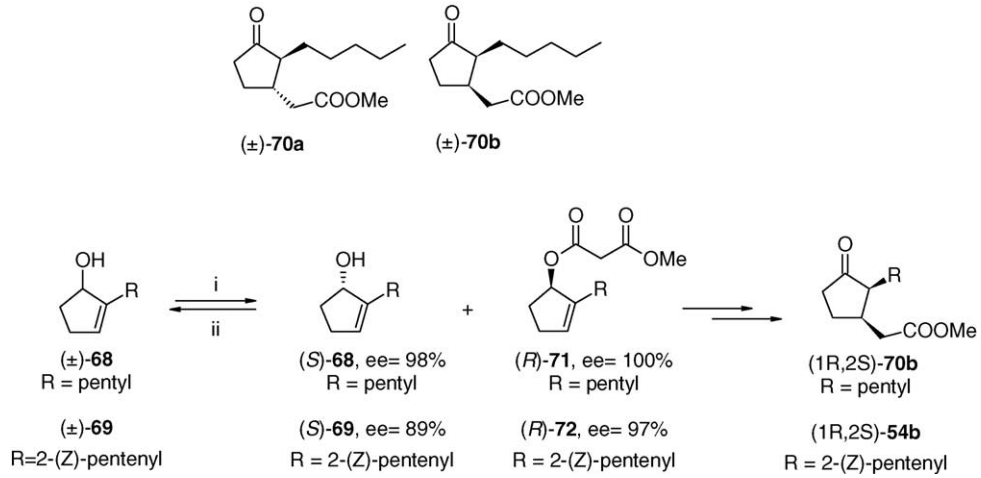
Scheme 22. (i) CCL, *t*-butyl methyl ether, vinyl acetate; (ii) Lipase PS, *t*-butyl methyl ether, vinyl acetate.



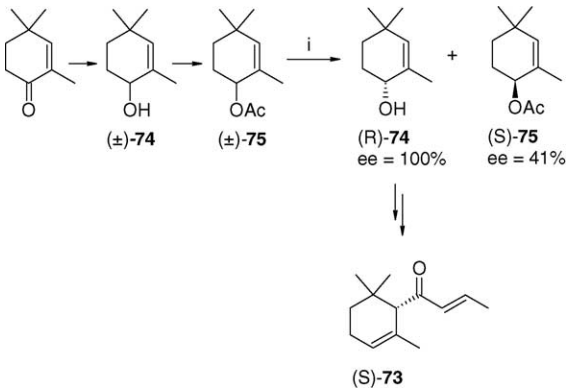
Scheme 23.

had previously prepared (Scheme 25) [57]. 2,4,4-Trimethyl-2-cyclohexenone was reduced with $NaBH_4/CeCl_3$ to provide (±)-**74**, which was acetylated to (±)-**75**, and submitted to enzymatic hydrolysis. PLE treatment in 0.1 M phosphate buffer with 20% MeOH at pH 7.5 afforded (R)-**74** (100% ee) and (S)-**75** (41% ee) after 65.5 h at –10 °C (c = 29%, E = 296). Two different routes were then optimised to obtain (S)-**73** from (R)-**74**.

We have recently reported on a lipase-mediated approach to all the four stereoisomers of Rhubafuran (**76**), a *grapefruit fragrance with rhubarb undertones* [20]. Allylic alcohol (*E*)-**77** was submitted to Lipase PS acetylation, and after 72 h, acetate (*R*,*E*)-**78** (ee > 99%) and alcohol (*S*,*E*)-**77** (ee > 99%, c = 50%, E = 1057) were recovered by column chromatography. (*R*,*E*)- and (*S*,*E*)-**77** were converted into diastereoisomeric diols (2*S*,4*RS*)- and (2*R*,4*RS*)-**79**, respectively (Scheme 26). Each mixture of diastereoisomers was submitted to column chromatography, and the four isomers of diol **79** were obtained as pure compounds. Unfortunately, when the diastereoisomerically pure diols **79** were treated with triphenyl phosphine and NBS, or reacted with *p*-toluenesulphonyl chloride and pyridine, mixtures of Rhubafuran® diastereoisomers were obtained. The steric hindrance of the stereogenic carbon atom next to the primary



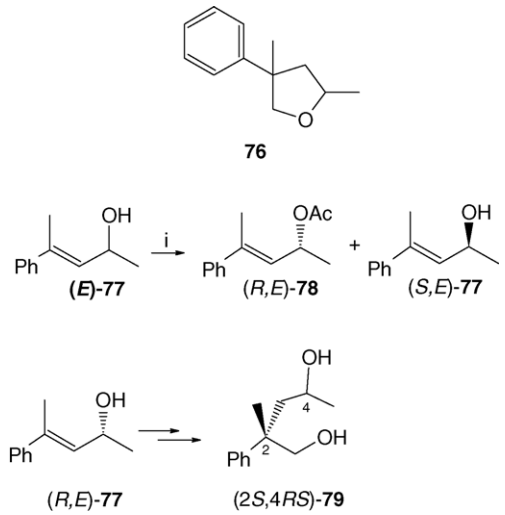
Scheme 24. (i) dimethyl malonate, $KHCO_3$, Novozym 435, 40 °C, 8 torr, 80 min; (ii) aqueous H_2SO_4, THF, 20 °C, 24 h.



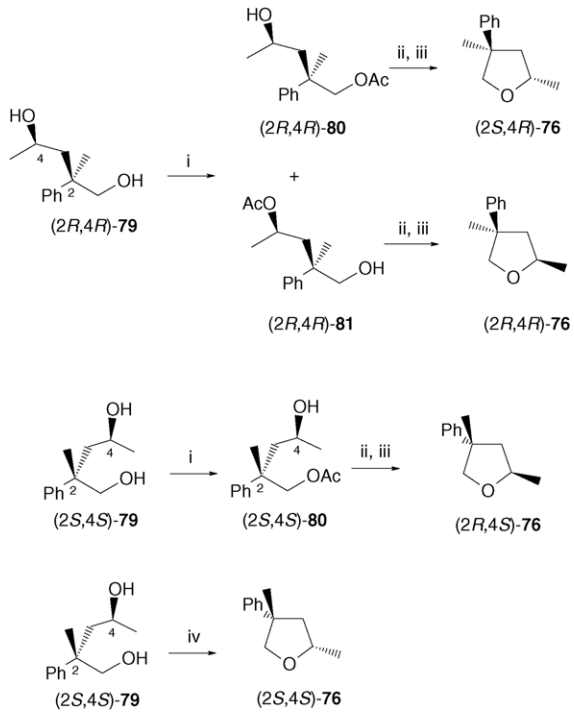
Scheme 25. (i) PLE, phosphate buffer, 20% MeOH, pH 7.5, 65.5 h, −10 °C.

hydroxylic group seems to make its reaction with NBS or *p*-TosCl quite difficult, and the reaction of the secondary OH group becomes competitive. As in the case of Clarycet® isomers, we tried to selectively convert the primary OH group into an acetate ester by submitting the four enantiopure steroisomers of diol **79** to enzymatic-mediated acetylation in the presence of Lipase PS in separate experiments. The following results were obtained (Scheme 27).

Diols (2*R*,4*S*)-**79** and (2*S*,4*R*)-**79** after 8 days gave a 1:1 mixture of the two possible monoacetates that could not be separated by column chromatography. Diols (2*R*,4*R*)-**79** and (2*S*,4*S*)-**79** gave after 5 days, respectively, a 1:1 mixture of the two monoacetates (2*R*,4*R*)-**80** and (2*R*,4*R*)-**81** (which could be separated by column chromatography), and the single monoacetate (2*S*,4*S*)-**80**. Even enzymes did not show a definite preference for the hindered primary OH



Scheme 26. (i) Lipase PS, *t*-butyl methyl ether, vinyl acetate; column chromatography.



Scheme 27. (i) Lipase PS, *t*-butyl methyl ether, vinyl acetate; (ii) *p*-TosCl, pyridine; (iii) KOH, MeOH; (iv) PPh_3, NBS, CH_2Cl_2.

moiety of this kind of substrate. Reaction with *p*-TosCl in pyridine, followed by saponification with NaOH 10% in ethanol, afforded the following samples of Rhubafuran®: from (2*R*,4*R*)-**81**: (2*R*,4*R*)-**76** de = 84%, ee > 99%; from (2*R*,4*R*)-**80**: (2*S*,4*R*)-**76** de = 66%, ee > 99%; from (2*S*,4*S*)-**80**: (2*R*,4*S*)-**76** de = 78%, ee > 99%. The preparation of the fourth isomer was accomplished by reaction of (2*S*,4*S*)-**79** with NBS and PPh_3 (de = 60% and ee > 99%, 67% yield). All the samples were submitted to Givaudan perfumers. (2*R*,4*S*)-**76** was found to be the most pleasant one, floral, linalool-like, rhubarb and citrus, green, slightly eucalyptus. (2*R*,4*S*)-**76**, followed by its enantiomer, are the real odour vectors of commercial Rhubafuran®.

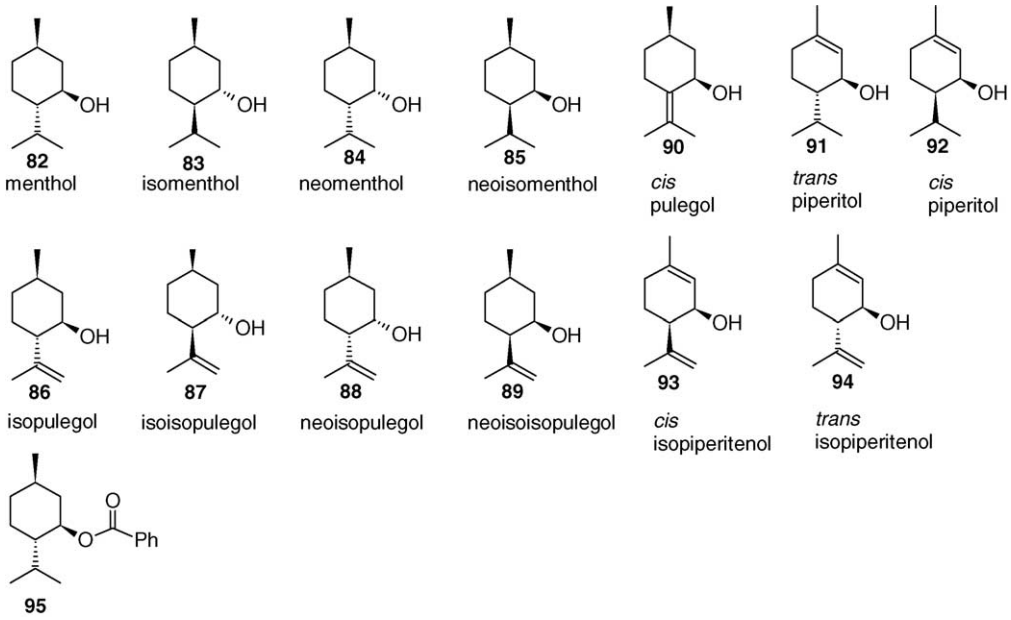
1.5. Lipase-mediated acetylation of p-menthan-3-ol derivatives

The 3-oxygenated monoterpenes **82**–**94** (Scheme 28) of *p*-menthane family are important natural compounds. They are widespread in nature and are used extensively in flavour and fragrance industries and as pharmaceuticals, cosmetics, agrochemicals and cooling substances. Menthol **82** is the most relevant compound of this class. Several examples of lipase-mediated acetylation of racemic menthol are described in the literature (see for example [58]). Only some relevant examples will be herein reported.

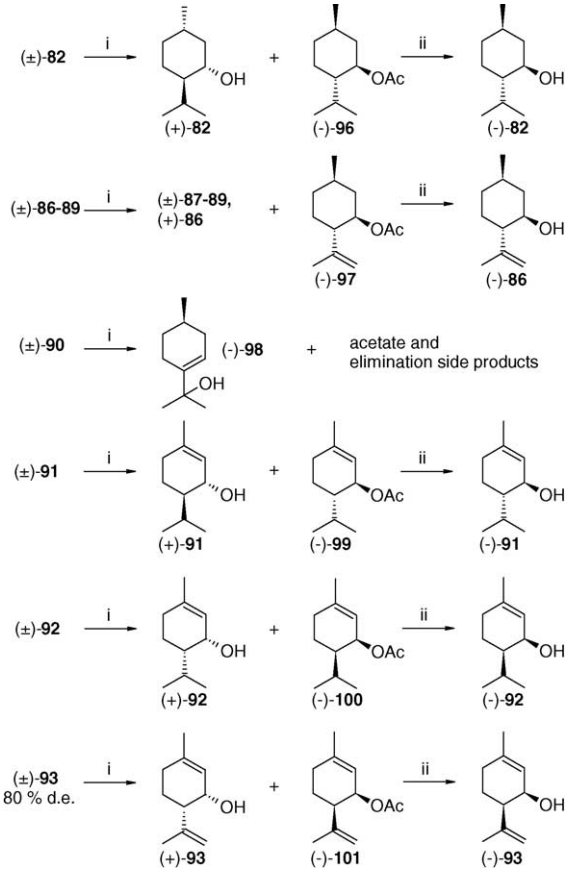
Researchers at CSIR Bio/Chemtek developed a process to produce L-menthol from the readily available raw material *m*-cresol. Alkylation of *m*-cresol gave thymol, which was submitted to hydrogenation to afford four pairs of racemic diastereomers: menthol (**82**), isomenthol (**83**), neomenthol (**84**), and neoisomenthol (**85**). Acylation of this mixture using Lipase AK (Amano) afforded L-menthyl acetate showing 96% enantiomeric excess (ee). L-Menthyl acetate was separated from the unreacted isomers by distillation, and hydrolyses to give (−)-menthol. Enzyme activity was shown to be retained even after 2000 h of operation. Furthermore, isomerization/racemization of the unreacted isomers regenerated the initial mixture of diastereomers, which was routed again to enzyme resolution. Over several cycles, thymol is almost fully converted to L-menthol. This process is the subject of a 2002 patent assigned to AECI [59].

Racemic menthyl benzoate **95** is the starting compound to produce L-menthol in thousand tons per year through the so-called Haarman & Reimer process, which involves a fractional crystallisation promoted by crystal seeds of optically pure menthyl benzoate [60]. Vorlova et al. [61] optimised the enantioselective hydrolysis of this key industrial starting compound under enzymatic catalysis. Starting from the results of a preliminary screening of commercially available lipases and esterases they decided to focus on the heterologously expressed *C. rugosa* lipase. The possibility to obtain this enzyme in a pure form, non-contaminated with other hydrolytic enzymes, enabled the production of L-menthol at an optical purity not achievable with the commercial preparations. The authors were able to produce in large amounts and high purity the recombinant CRL which exhibited remarkably high enantioselectivity ($E > 100$) in the kinetic resolution of **95**. Thus, after 8 h at 40 °C, 50% (−)-menthol of excellent optical purity (>99.9% ee) was obtained This high selectivity was not restricted to menthyl benzoate, but also allowed efficient resolution of other menthyl esters.

We recently investigated the behaviour of the whole class of *p*-menthan-3-ols **82**–**94** under lipase-catalysed acetylation [62].



Scheme 28. The most common *p*-menthan-3-ols.



Scheme 29. (i) Lipase, vinyl acetate *t*-butyl methylether, (ii) KOH, MeOH; (iii) MnO_2,CH_2CL_2.

Porcine pancreatic lipase did not catalyse the acetylation reaction of these substrates and only a trace of acetate was detected in a few cases (**82** and **83**). Both *C. rugosa* lipase and lipase PS were found to be good catalysts for the same reaction, although the latter enzyme seemed to be superior in terms of efficiency and enantioselectivity (Scheme 29).

Racemic menthol **82** was converted by CRL and lipase PS into the (−)-menthyl acetate **96** with good enantioselectivity (ee = 94–97%) though the ee of the recovered alcohol was moderate (ee = 60–78%) (Schema 29, c = 39–44%, E = 59.7–152). The mixture of the isopulegol isomers **86**–**89** was acetylated very slowly and both CRL and lipase PS afforded only (−)-isopulegol acetate **97** with good enantioselectivity (ee = 95–98%) and complete diastereoselectivity. Hydrolysis of the latter acetate gave (−)-isopulegol **86** with high enantiomeric purity. This fact is noteworthy since (−)-**86** is a relevant product for industrial processes, fine chemical production and for its cooling activity.

Isomeric monounsaturated alcohols **90**–**92** showed the following behaviour. Racemic **90** was slowly converted in its acetate, but with concomitant isomerization to tertiary alcohol **98** and its acetate. Isolation gave only alcohol **98** with very low enantiomeric enrichment (8% ee by lipase PS and 0% ee by CRL). *trans*-Piperitol **91** and *cis*-piperitol **92** were converted by lipase PS in enantiopure acetate (−)-**99** and (−)-**100**, respectively. The unreacted (+)-**91** and (+)-**92** show good enantiomeric purity (ee = 81 and 91%, respectively). The same reactions performed with CRL gave analogous results although with inferior selectivity.

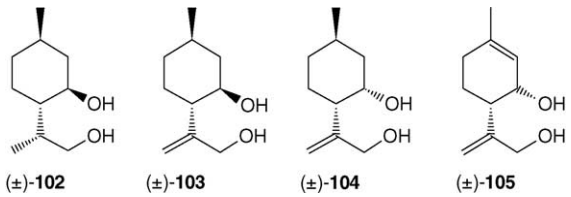
Racemic isopiperitenol **93** was converted by CRL and lipase PS into acetate (−)-**101** with good to excellent enantioselectivity (89 and 99% ee, respectively) whereas recovered alcohol (+)-**93** showed moderate to good enantioenrichment by CRL (55% ee) and lipase PS (92% ee), respectively. The starting material (±)-**93** was not a single diastereoisomer (90% of *cis* derivative) the isolated products showed comparable values of de, confirming that both lipases are not diastereoselective. This study of the enzyme-mediated resolution of racemic *p*-menthan-3-ols **82**–**94** allowed us to draw the following conclusions. *Porcine pancreatic* lipase is not a catalyst for the acetylation reaction of these substrates. On the contrary, CRL and lipase PS afforded the acetates in good to excellent enantioselectivity, with the single exception of *cis*-pulegol **90**. The reaction was found to be also diastereoselective for the *p*-menthan-3-ols **82** and **86**–**89** without a double bond in the cyclohexanic ring. Complete distereoselectivity was found for the enzymic process performed on the racemic mixture of the eight-isopulegol isomers. The relevant (−)-isopulegol **86** was obtained exclusively in enantiopure form.

Alcohols **91**–**94** showing one unsaturation on C(1) are acetylated without diastereoselectivity and with an enantiodiscrimination depending exclusively on the C(3) configuration.

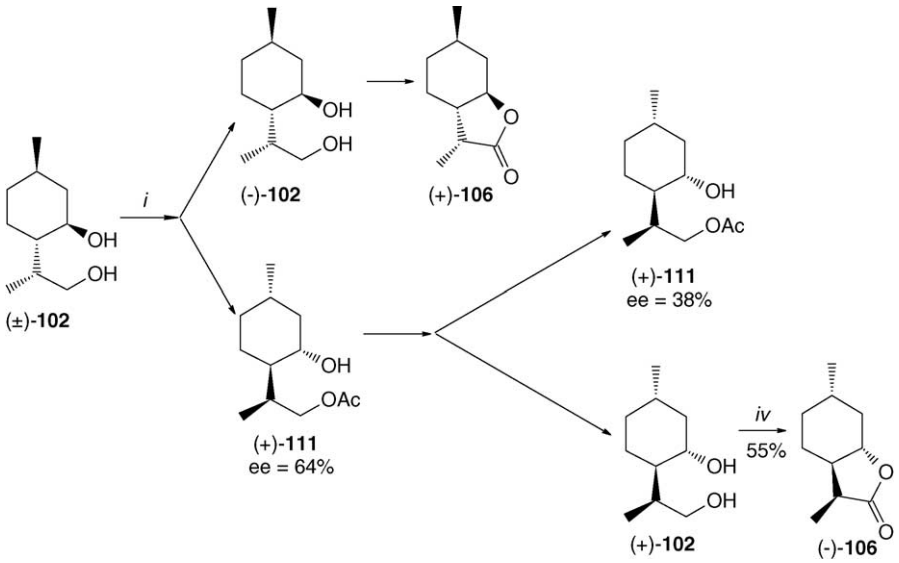
Dienic *cis*-isopiperitenol **93** could be also resolved by this procedure. We also investigate the behaviour under lipase acetylation of the structurally related *p*-menthane-3,9-diols **102**–**105**, precursors of various *p*-menthane odorants, a class of compounds showing interesting organoleptic properties [63] (Scheme 30).

We prepared the four racemic and diastereoisomerically pure diols **102**–**105** and tested their reactivity towards the irreversible acetylation-catalysed by three different lipases (lipase PS, CRL, and PPL). We found that both the kinetics and enantioselectivity of the acetylation step were strongly affected by the enzyme used and by the relative configuration of the substrates.

Each of the four diastereoisomerically pure diols **102**–**105** was treated with vinyl acetate in *t*-butyl methyl ether solution in the presence of lipases (lipase PS, CRL, and PPL). All these lipases-mediated the acetylation of the primary alcohol functions, but, when these groups were in allylic position (compounds **103**–**105**), the reaction was very fast (less



Scheme 30.



Scheme 31. (i) Vinyl acetate, *t*-BuOMe, lipase PS; (ii) KOH, MeOH, reflux; (iii) Vinyl acetate, *t*-BuOMe, CCL; (iv) $KMnO_4/CuSO_4{\cdot}5H_2O$, CH_2Cl_2; (v) MnO_2, CH_2Cl_2; (vi) 10% Ag_2CO_3/celite, benzene, ruflux; (vii) $(Ph_3P)_4RhH$ cat., toluene, reflux.

then 36 h) and no enantioselectivity was observed in this step.

The saturated diol **102** reacted slowly, and after 4 days, ca. 50% of the starting diol was acetylated. The enantioselectivity of this step was dependent on the kind of the type of lipase. Lipase PS showed the highest selectivity with a preference for the conversion of the (+)-isomer. PPL showed the lowest selectivity, and CRL, though with poor selectivity, converted the (−)-isomer. The monoacetylated compounds obtained were not further acetylated by the above-mentioned enzymes, even after long reaction time.

As for the acetylation of the secondary-alcohol group, diols **103**–**105** show a different behaviour. Compounds **103**, and **105** were slowly converted into the enantiomerically pure diacetates (99% ee) and into the enantiomerically enriched (92 and 94% ee, respectively) monoacetylated derivatives only when lipase PS was used as a catalyst. Derivative **104**, which differed from **105** only in the *cis* relative configuration at C(3) and C(4) was not converted by any of the enzymes used.

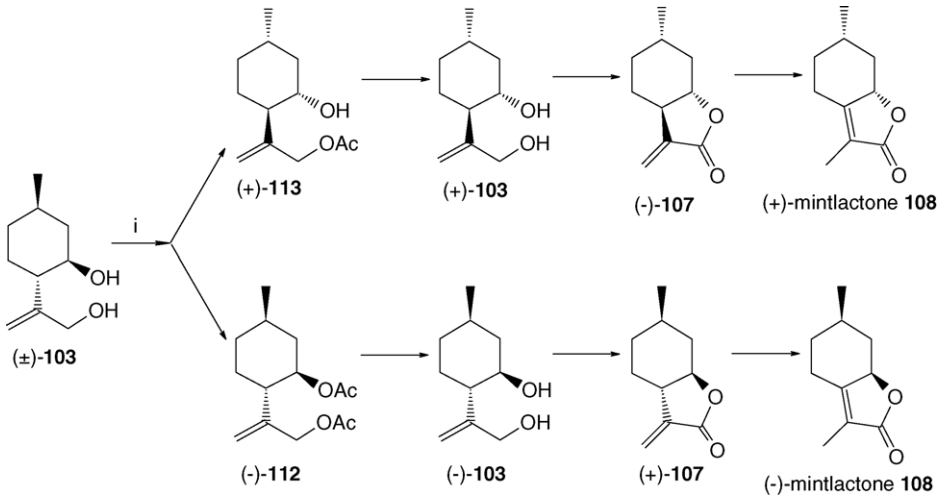
We devised a large-scale method for the preparation of all the enantiomeric forms of diols **102**, **103** and **105** and then converted them into the following products: (+)- and (−)-**106**, (+)- and (−)-**107**, (+)- and (−)-**108**, (+)- and (−)-**109**, and (+)- and (−)-**110**. We first treated **102** with lipase PS in the presence of vinyl acetate and *t*-butyl methyl ether as a solvent allowing the reaction to proceed over 50% conversion (Scheme 31). Thus, the unreacted diol (−)-**102** was isolated in satisfactory enantiomer purity (92% ee), whereas acetylated (+)-**111** showed lower purity (64% ee). To obtain enantiomerically pure (+)-**102**, we exploited the reverse selectivity of CRL toward acetylation of **102**. Accordingly, (+)-**111** was hydrolysed and the crude diol obtained submitted to CRL-mediated acetylation forcing again the reaction to >50% of conversion. The isolation procedure provided, besides (+)-**111** (38% ee), the unreacted diol (+)-**102** in good enantiomer purity (94% ee). The oxidation of diols (+)- and (−)-**102** was accomplished by means of $KMnO_4/CuSO_4{\cdot}5H_2O$ in CH_2Cl_2 solution, to afford natural ethers (−)- and (+)-**106**, respectively.

The enantiomeric forms of **103** and **105** were prepared by a more direct pathway. Treatment of (±)-**103** with lipase PS in the presence of vinyl acetate and *t*-butyl methyl ether as solvent gave diacetate (−)-**112** in very high enantiomer purity (99% ee) and the monoacetylated (+)-**113** also in good enantiomer purity (92% ee) (Scheme 32). Thus, KOH hydrolysis of the acetate moieties provided (−)- and (+)-**103**, respectively. The conversion of the latter diols into *trans-p*-menthene lactones **107** was performed according to a two-step procedure reported in the literature [63]. The reaction of (+) and (−)-**107** with $[RhH(Ph_3P)_4]$ in refluxing toluene afforded enantiopure (−)- and (+)-mintlactone **108**.

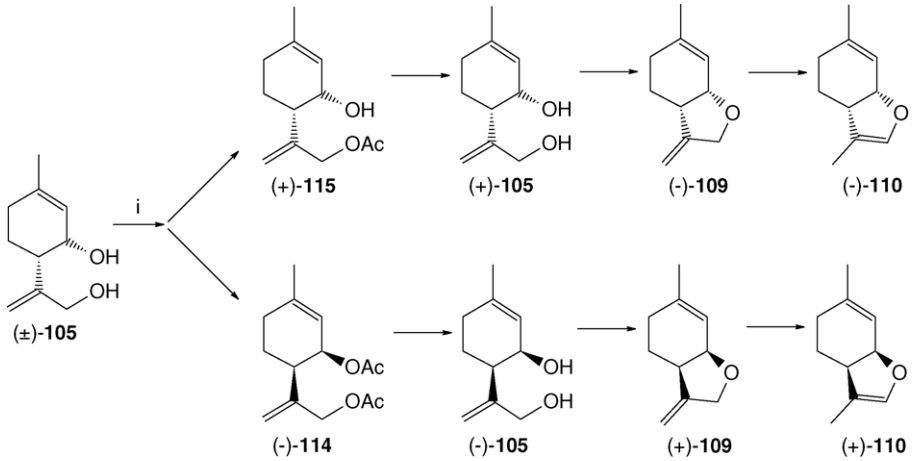
Treatment of (±)-**105** with lipase PS in presence of vinyl acetate and *t*-butyl methyl ether as solvent gave the diacetate (−)-**114** in very high enantiomer purity (99% ee) and the monoacetylated (+)-**115** also in good enantiomer purity (94% ee) (Scheme 33). The subsequent KOH hydrolysis of the acetate moieties provided (−)- and (+)-**105**, respectively. The reaction of the latter diols with a catalytic amount of 5% aqueous HCl solution in Et_2O as solvent smoothly provided the ethers (+)- and (−)-**109**, respectively, in very good yields. By means of $[RhH(Ph_3P)_4]$ catalyst they were converted into (+)- and (−)- lactones **110**.

1.6. Asymmetrisation of meso-diols and meso-diesters

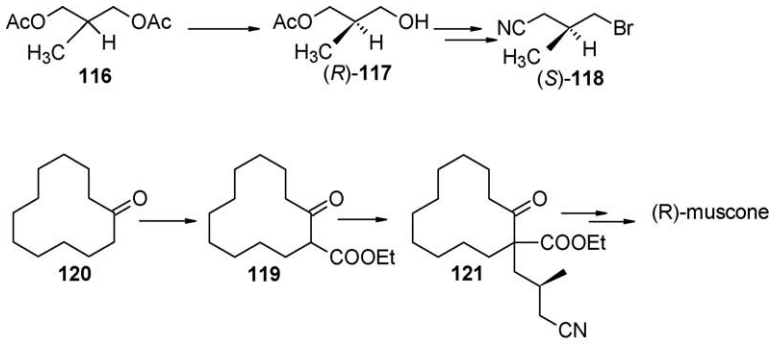
An interesting enzyme-mediated approach to enantiopure muscone took advantage of asymmetrisation of *meso*-diacetate **116** (Scheme 34) [64,65]. This latter was submitted



Scheme 32. (i) Vinyl acetate, *t*-BuOMe, lipase PS.



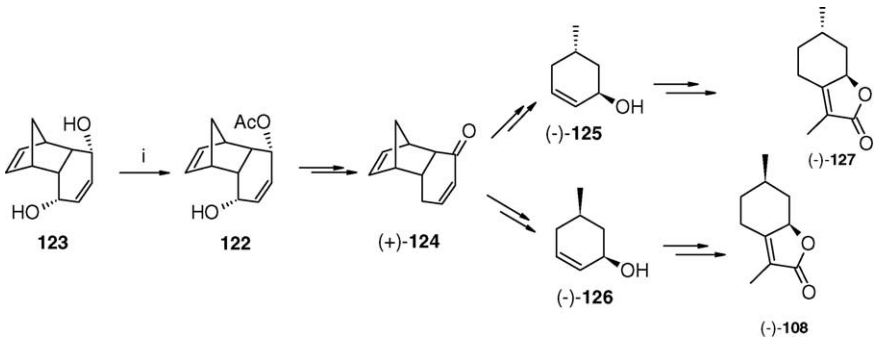
Scheme 33. (i) Vinyl acetate, *t*-BuOMe, lipase PS.



Scheme 34. (i) *Pseudomonas fluorescens* lipase, pH 7, 1.5 h, 25 °C.

to hydrolysis with *Pseudomonas fluorescens* in 0.5N phosphate buffer at pH 7. After 1.5 h at 25 °C, monoacetate (R)-**117** was obtained with ee > 99% in 33% yield, with the recovery of 66% of starting material. Compound (R)-**112** was converted into bromo derivative (*S*)-**118**, which was employed to alkylate derivative **119** (prepared by reaction of commercially available **120** with ethyl cyanoformate), in order to obtain **121**. This latter was transformed according to a three step reaction route into (*R*)-muscone (**58**).

Ogasawara and co-workers employed enantiopure monoacetate **122** as a precursor of (−)-mintlactone and (−)-isomintlactone [66,67] (Scheme 35). Treatment of *meso*-diol **123** with two equivalents of vinyl acetate in acetonitrile in the presence of lipase PS (Amano) gave



Scheme 35. (i) Lipase PS, vinyl acetate, CH_3CN, 28 °C, 16 days.

monoacetate **122** in 79% and ee > 99%. Monoacetate **122** was converted into the (+)-enantiomer of bicyclic ketone **124**, which was employed to prepare both (−)-**125** and (−)-**126**. These two diastereoisomers were used as starting materials for the preparation of (−)-isomintlactone (**127**) and (−)-mintlactone (**108**).

2. Conclusions

Some interesting qualitative conclusions may be drawn from this survey of recent literature. The lipase-mediated acetylation of primary alcohols and hydrolysis of primary alcohol acetates are highly chemoselective reactions: the primary function is preferentially converted in the presence of a secondary function (see hydrolysis of *anti*-**10**, *syn*-**10**, (3*S*,4*R*)-**12**, and acetylation of triol **49**). These reactions are very fast, and generally characterised by modest enantiomeric excesses. However, repeated lipase-catalysed acetylation/hydrolysis reactions can lead to satisfactory enantiomeric enrichment.

Lipase-mediated acetylation of secondary alcohols usually shows high enantioselectivity, even when a carbonyl group is present in β position (see for instance substrates **39** and **45**) or when the alcoholic function is in allylic position. The presence of the carbonyl and of the double bond can be exploited for further derivatisation of the substrate. Besides the examples herein reported, we employed the lipase-mediated kinetic resolution of various 3-hydroxyketones to obtain starting materials for the synthesis of dioxane odorants analogous to Floropal [68]. In the case of allylic alcohols, electrocyclic rearrangements have been successfully employed to convert allylic alcohols after lipase resolution (see for example substrates (*R*)-**71**, (*R*)-**72**, (*R*)-**74**, (*S*)-**77** and (*R*)-**78**)).

The stereochemical course of lipase-mediated acetylation of 1,3-diols and of *p*-menthan-3-ol derivatives was found to be dependent on the relative configuration of the substrate and on the enzyme employed

The examples herein described clearly demonstrate the usefulness of lipase-mediated reactions in the synthesis of the single stereoisomers of chiral fragrances. Odorous compounds are usually scarcely functionalised molecules belonging to the functional groups of aldehydes, ketones, ethers, cyclic ketals and acetals, and esters. Thus, it is seldom possible to apply the methods of classical resolution. The optimisation of chiral metal catalysts for the synthesis of all the single stereoisomers of chiral fragrances is very expensive and time-consuming, especially when the final aim is the screening of the odour properties of each isomer. The use of the compounds of the pool of chirality can be exploited only in some specific cases. The chemo and stereoselectivity shown by lipases make their use flexible and well suited for the synthesis of fragrant molecule. The only structural requirement is the presence of an alcohol as an intermediate in the synthetic pathway.

Acknowledgement

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