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Baker's yeast-mediated approach to (−)-*cis*- and (+)-*trans*-Aerangis lactones

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Abstract—The first enantioselective synthesis of natural (−)-*cis*-Aerangis lactone (−)-**1a** and its (+)-*trans*-diastereoisomer (+)-**1b** is described. The key steps in the synthesis are: (i) the enantiospecific and 100% diastereoselective baker's yeast reduction of 1,4-keto acid **2**, to afford enantiopure *trans*-cognac lactone (+)-**10**; (ii) the regioselective PPL-mediated hydrolysis of the primary acetate moiety of diacetate (+)-(3*S*,4*R*)-**3**, obtained from (+)-**10**. Chain elongation by one carbon atom via cyanide substitution, and inversion of the configuration of C(5) in nitrile derivative (+)-**21a** are also required to complete the synthetic route to (−)-**1a**.
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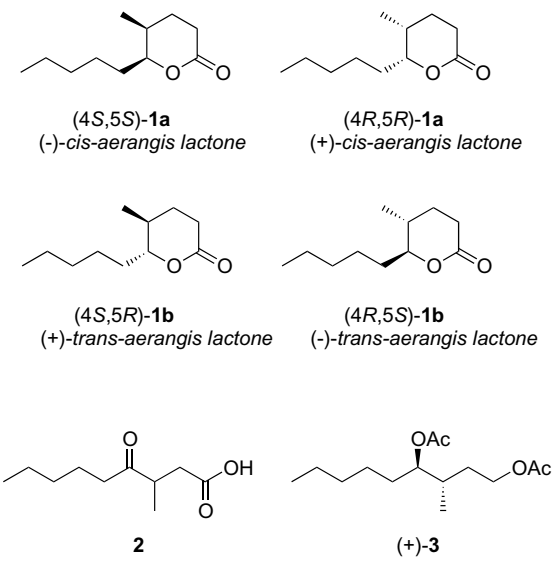
1. Introduction

cis-Aerangis lactone **1a** (*cis*-4-methyl-5-decanolide) was described by Kaiser in 1993 as the main odour component of the African 'moth orchids' *Aerangis confusa* J. Stewart and *Aerangis kirkii* (Rolfe) Schltr.[1] (±)-**1a** was first obtained as a 1:1 mixture with the *trans*-diastereoisomer **1b** by hydrogenation of dihydrojasmone and subsequent Baeyer–Villiger oxidation.[2] The two diastereoisomers were separated by preparative GC and characterised. The *cis* isomer was found to have a very pleasant and room-filling odour, reminiscent both of the smell of tuberose and gardenia, and of the fragrance of caramel, condensed milk and coconut.[2] The *trans* isomer exhibited similar, but somewhat weaker, olfactory properties. In 1995, Mosandl, Kaiser et al.[3] obtained samples of all four stereoisomers of Aerangis lactone, (4*S*,5*S*)- and (4*R*,5*R*)-**1a** and (4*S*,5*R*)- and (4*R*,5*S*)-**1b**, as homochiral compounds by chiral high performance liquid chromatography. These latter derivatives were reduced to the corresponding 1,5-diols, in order to derive their absolute configuration from the ^{1}H NMR spectra of the corresponding di-MTPA esters, according to Mosher's procedure. Optical rotation values for the four lactones were not reported in the work. By means of enantioselective multi-dimensional capillary GC the authors found that (4*S*,5*S*)-*cis*-4-methyl-5-decanolide **1a** was the sole stereoisomer of Aerangis lactone present in the scent of living, white flowering orchids (*Aerangis confusa*). The fragrance of (4*S*,5*S*)-**1a** was described[3] to be typical for the lactonic odour of *A. confusa* and *A. kirkii*, and identical to the olfactory qualities of natural Aerangis lactone. Its enantiomer (4*R*,5*R*)-**1a** was found to be reminiscent of δ-decalactone and cocos, and with a fragrance intensity much lower than (4*S*,5*S*)-**1a**.



In the present work we describe the first enantioselective synthesis of natural Aerangis lactone (−)-**1a**, and of

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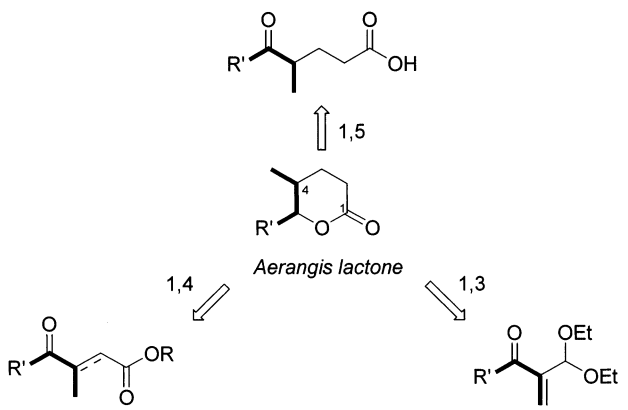
its epimer (+)-**1b**. Key steps are the enantiospecific and completely diastereoselective reduction of keto acid **2** mediated by Baker's yeast (BY), and the regioselective lipase-catalysed hydrolysis of the primary acetate moiety of derivative (+)-**3**, followed by suitable chain homologation by one carbon atom via cyanide substitution. This work on Aerangis lactone gave us the chance to carry out a study of the mode of BY reduction of various 1,5-, 1,4- and 1,3- difunctionalised synthons. The results of this investigation are reported herein.

2. Results and discussion

With the aim of finding the most convenient and useful access to natural **1a** and eventually achieving the synthesis of the other stereoisomers, we investigated the BY reductions of various 1,5-, 1,4-, and 1,3-difunctionalised synthons (Scheme 1). The C(5)-O fragment of Aerangis lactone could most likely be derived from the enantioselective reduction of a carbonyl group, while the C(4) centre, bearing the methyl group, could be involved in a double bond in the chosen precursor.

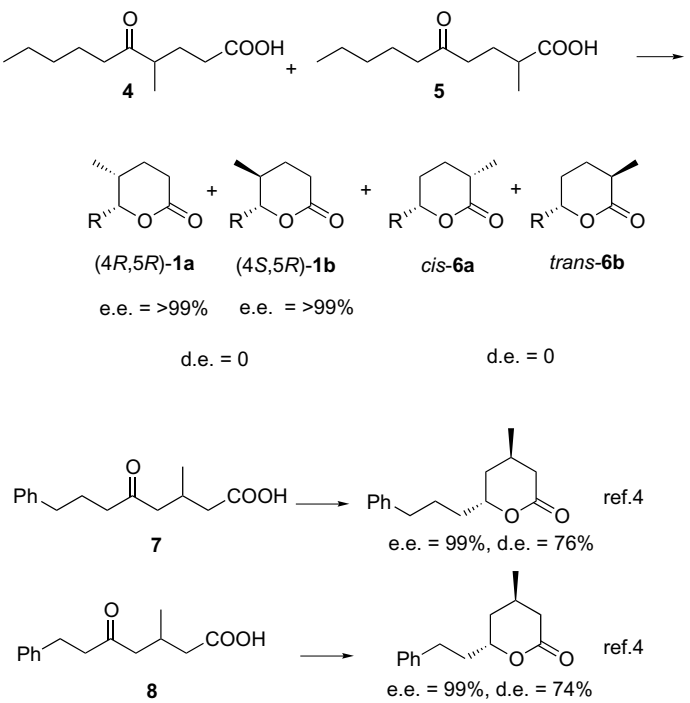


Scheme 1. 1,5-, 1,4- and 1,3-Difunctionalised synthons studied in this work as precursors of Aerangis lactone.

2.1. 1,5-Keto acid synthons

In the past we had been involved in the study of the mode of BY reduction of methyl substituted γ- and δ-keto acids.[4] We had noticed that the outcome of the reaction was almost unpredictable: the enzymatic system of baker's yeast was so complex, that it could transform structurally similar substrates in different ways. Nonetheless, one of the main results of that work was that enantiopure methyl substituted δ-lactones, showing high diastereoisomeric excess (d.e.), could be generally obtained by baker's yeast reduction of the corresponding keto acids. We thus tried to exploit this approach for the synthesis of natural Aerangis lactone (4*S*,5*S*)-**1a**, starting from a 1,5-difunctionalised synthon, such as derivative **4** (Scheme 2).

For a preliminary investigation of the behaviour of keto acid **4** under BY treatment, we prepared it through a simple and quick route, starting from commercial 2-methyl-glutaric acid, and affording even regioisomer **5**. When the 2:1 mixture (GC/MS) of substrates **4** and **5** was submitted to BY reduction, a 1:3 mixture of Aerangis lactones and 2-methyl regioisomers was obtained. Derivative **5** was readily and efficiently converted into the corresponding diastereoisomeric lactones *cis*-**6a** and *trans*-**6b** (1:1 ratio, GC/MS). Reduction of derivative **4** was much slower, and gave



Scheme 2. BY reductions of 1,5-difunctionalised substrates.

enantiopure (chiral GC) diastereoisomeric lactones *cis*-(4*R*,5*R*)-**1a** and *trans*-(4*S*,5*R*)-**1b** in a 1:1 ratio. The carbon bearing the hydroxyl function in all four lactones was tentatively assigned (*R*)-configuration on the basis of previous experience.[4] This assumption was confirmed for Aerangis lactones **1a** and **1b** by chiral GC analysis at the end of the synthesis.

We thus found that the steric course of the reaction was different from the one we had described in the past for BY treatment of structurally similar 1,5-keto acids **7** and **8**, which were reduced to afford the corresponding *trans*-enantiopure lactones with good d.e.s (Scheme 2).

2.2. 1,4-Keto acid synthons

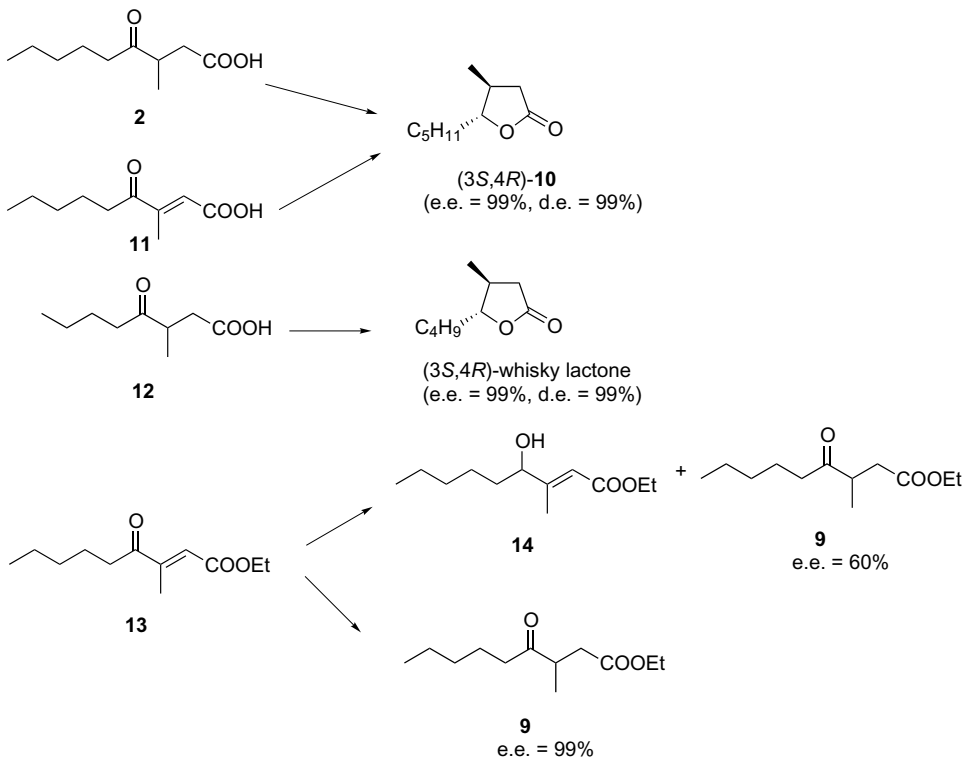
The absolute lack of diastereoselectivity in the BY reduction of **4** led us to investigate the behaviour of 1,4-keto acid **2**, having one carbon atom less, in order to find an efficient route to the desired Aerangis lactone (Scheme 3). Derivative **2** was prepared by saponification of the corresponding ethyl ester **9**, obtained by reaction of *n*-hexanal with methyl crotonate in the presence of benzoyl peroxide.[5] Upon BY reduction keto acid **2** afforded the substituted γ-lactone **10**, known as 'cognac lactone', in good yields after 48 h. This derivative was found to be the single diastereoisomer, (+)-(3*S*,4*R*)-**10**.[6a–h] The assignment of *trans* relative stereochemistry was based on the ^{1}H NMR spectrum: δ_H C(4)H$_{trans}$ = 4.01 ppm,[6b] C(4)H$_{cis}$ = 4.44 ppm.[7] The absolute configuration was established by the sign of the optical rotation, $[\alpha]_D^{20} = +72$, $c = 2.4$, CH_2Cl_2 (lit.[6a–h]). The enantiomeric and diastereoisomeric purity of (+)-*trans*-cognac lactone **10** was confirmed by chiral GC analysis, using a sample of racemic *cis*- and *trans*-cognac lactone (prepared by $NaBH_4$ reduction and saponification of ester **9**) as a reference. While we were preparing this work a paper appeared in this journal describing, among other results, the preparation of (+)-*trans*-cognac lactone through the same synthetic procedure.[6a]

(+)-*trans*-cognac lactone was also obtained by BY reduction of unsaturated 1,4-keto acid **11**. The same behaviour was observed for 1,4-keto acid **12**, affording a single whisky lactone diastereoisomer, namely (+)-(3*S*,4*R*)-*trans*-whisky lactone.[5]

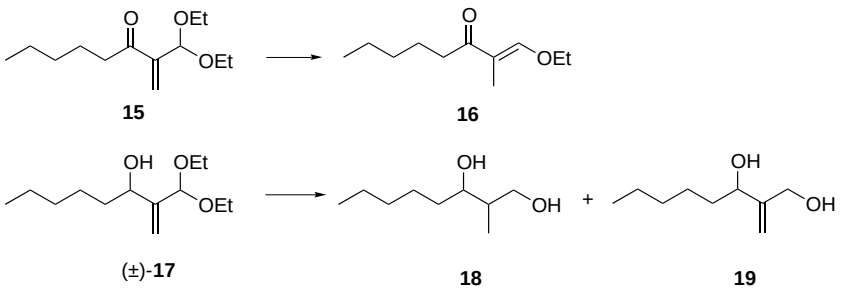
An exception to this general rule of 1,4-synthons was found when the unsaturated 1,4-keto ester **13** was treated with yeast, in order to generate the stereocentre at C(5) under the control of BY. A 7:3 mixture of the allylic alcohol **14** and saturated keto ester **9** was obtained. The enantiomeric excess of this latter derivative was established by means of chiral shift reagents, and e.e. of 60% was evaluated. No allylic alcohol was formed when the same reaction was carried on in the presence of non-polar resins. A 3:1 mixture of starting material **13** and of chiral derivative **9** was recovered after 48 h. The keto ester **9** resulted to be enantiomerically pure (chiral shift reagents), but recovery yields were too low to allow a practical application of the procedure.

2.3. 1,3-Keto acid synthons

Our investigation was then directed to 1,3-difunctionalised synthons of the type **15** (Scheme 4). This choice



Scheme 3. BY reductions of 1,4-difunctionalised substrates.



Scheme 4. BY reductions of 1,3-difunctionalised substrates.

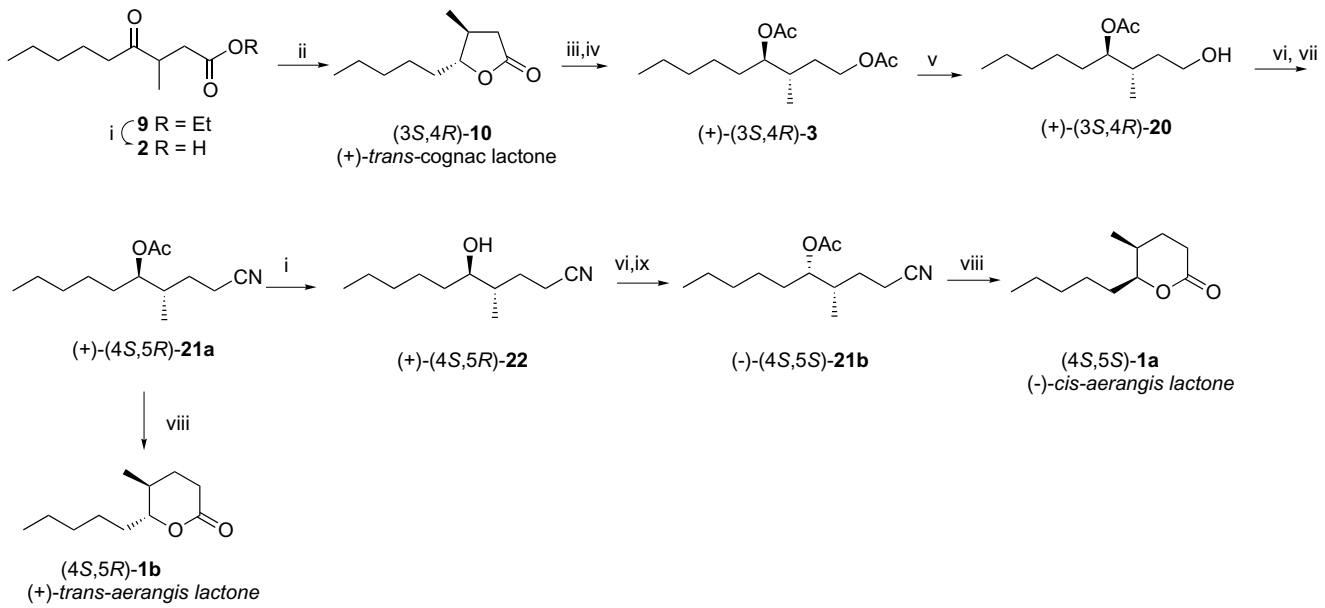
was based on some literature reports, describing the BY reduction of the double bond of structurally similar substrates, such as 2-substituted acrolein acetals,[8] 4,4′-dimethoxy crotonate derivatives,[9] and α-methylene ketones.[10] Unfortunately, yeast treatment of our substrate gave vinyl ethyl ether derivative **16**. The formation of this compound could be justified by loss of ethanol from the primary product of reduction of the double bond. Once again, a subtle modification of the substrate structure induces a great difference in the reaction outcome.

We then submitted alcohol derivative (±)-**17** to yeast treatment, with the aim of performing a form of kinetic resolution by BY reduction. A 1:1 mixture of saturated diol **18** and unsaturated derivative **19** was obtained. However, diol **18** was found to be a 2:1 mixture of two possible *trans* and *cis* diastereoisomers (GC/MS). Treatment with tosyl chloride and chain elongation by one carbon atom via cyanide reaction afforded, after saponification, a 4:1 mixture of (+)-*trans*-cognac lactone (e.e. = 65%) and (±)-*cis*-cognac lactone (chiral GC).

2.4. Synthesis of natural Aerangis lactone (Scheme 5)

After this investigation we decided that the most convenient approach to natural Aerangis lactone was that based on the BY reduction of 1,4-keto acid **2** to lactone (+)-**10**. (+)-*trans*-Cognac lactone was then employed as starting material for the preparation of both (+)-**1b**, and (−)-**1a**.

Reduction of (+)-**10** with lithium aluminum hydride gave, after treatment with acetic anhydride in pyridine, diacetate (+)-(3*R*,4*S*)-**3**. This was submitted to regioselective hydrolysis in water–THF in the presence of *Porcine pancreatic* lipase, keeping the pH constant at 7.8 by addition of 0.5 M aqueous NaOH. Mono alcohol mono acetate (+)-**20** was obtained, and converted, via tosylate derivatisation and cyanide treatment, into the nitrile derivative (+)-**21a**. Basic hydrolysis of (+)-**21a** in ethylene glycole with 10% KOH gave directly (+)-*trans* Aerangis lactone, after acid work-up (Scheme 5). The configuration of C(5) in (+)-**21a** was inverted via acetate displacement: hydrolysis of (+)-**21a** with KOH in methanol gave alcohol (+)-**22** which was converted



Scheme 5. (i) KOH, MeOH; (ii) Baker's yeast; (iii) $LiAlH_4$, THF; (iv) Ac_2O, pyridine; (v) PPL, THF–water, pH 7.8, NaOH 0.5 M; (vi) tosyl chloride, pyridine; (vii) NaCN, DMSO; (viii) KOH 10%, ethylene glicole; (ix) AcONa, DMF.

into the corresponding tosylate derivative. This latter was treated with sodium acetate in DMF, to afford nitrile (−)-**21b**. The diastereoisomeric purity of this derivative was determined via GC/MS: d.e. = 73%, t_R **21a** = 19.12 min, t_R **21b** = 18.81 min. Nucleophile substitution of acetate anion on the tosylate derivative did not proceed totally with configuration inversion: a partial contribution of a mechanism other than S_N2 should be hypothesised. Nitrile (−)-**21b** was submitted to basic hydrolysis to afford natural Aerangis lactone (−)-**1a**: e.e. = 99%, d.e. = 70%. Both e.e. and d.e. values were determined by chiral GC analysis, as we could find the conditions for a baseline-peak separation of the four stereoisomers of Aerangis lactone on a β-cyclodextrine based column. Following the same route, a reference sample of 1:1 (±)-**1a** and (±)-**1b** was prepared from a 1:1 mixture of the two possible racemic diastereoisomers of diacetate **3**.

3. Conclusions

The first enantioselective synthesis of enantiopure natural Aerangis lactone (−)-**1a** and the *trans*-diastereoisomer (+)-**1b** is reported. We took advantage of the BY reduction of 1,4-keto acid **2**, affording a single enantiopure diastereoisomer of *trans*-cognac lactone, (+)-**10**. This substrate was then manipulated through unexceptional reactions, to afford directly (+)-**1b** and, after inversion of configuration, (−)-**1a**.

This work on Aerangis lactone gave us the chance to further investigate the BY mode of reduction of various 1,5-, 1,4- and 1,3-difunctionalised synthons, we found that:

(i) Reduction of the carbonyl function of 1,5-keto acids in substrates **4** and **5** was enantiospecific, but barely diastereoselective. A kinetic preference for the reduction of the less hindered carbonyl in derivative **5** was also noticed. Structurally similar substrates, such as **7** and **8**, were reduced with higher diastereoselectivity[4] (d.e. = 76 and 74%, respectively).

(ii) Reduction of 1,4-keto acids, such as **2** and **12**, took an unexpectedly different steric course: only one diastereoisomer of the corresponding enantiopure *trans*-γ-lactone was recovered. In the presence of a conjugated double bond, such as in 1,4-keto ester **13**, the double bond and the carbonyl moiety were reduced separately, to afford a mixture of **9** and **14**. In the presence of non-polar resins, the double bond was preferentially reduced, and the corresponding keto ester **9** was obtained in enantiopure form.

(iii) Yeast treatment of the 1,3-difunctionalised derivative **15** did not effect reduction of the double bond, as expected on the base of literature reports.[8–10] The achiral enol ether **16** was obtained. Double bond reduction was observed when racemic alcohol derivative **17** was submitted to treatment with BY: the reaction proceeded with low diastereoselectivity and modest enantioselectivity.

Besides the successful synthesis of natural Aerangis lactone and of its *trans*-diastereoisomer (+)-**1b**, we have reported in this work the results of our broader research on the BY treatment of various substrates, which underline once again the unpredictability of BY reactions.

4. Experimental

Porcine pancreatic lipase (PPL type II, Sigma) was employed in this work. Chiral GC analyses of whisky and cognac lactones were performed on a Chirasil DEX CB, 25 m×0.25 mm column (Chrompack), installed on a DANI HT 86.10 gas chromatograph, with the following temperature program: 70°C (3′)–3.5°C/min–140°C–8°C/min–180°C (1′). Chiral GC analyses of Aerangis lactone were performed on a DeTBuSiBETA (086) 25 m×0.25 mm column (Mega, Italy), installed on a DANI GC 1000 gas chromatograph, with the following temperature program: 120°C (5′)–0.8°C/min–180°C (5′). GC–MS analyses were performed on a HP 6890 gas–chromatograph equipped with a 5973 mass-detector, using a HP-5MS column (30 m×0.25 mm×0.25 μm). The following temperature program was employed: 60°C (1 min)/6°/min/150° (1 min)/12°/min/280° (5 min). ^{1}H NMR spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise stated, on a Bruker AC-250 spectrometer (250 MHz ^{1}H). The chemical shift scale was based on internal tetramethylsilane. Coupling constant (J) values are in Hz. Optical rotations were measured on a Dr. Kernchen Propol digital automatic polarimeter. TLC analyses were performed on Merck Kieselgel 60 F_{254} plates. All the chromatographic separations were carried out on silica gel columns.

4.1. Synthesis of Aerangis lactones (−)-1a and (+)-1b

4.1.1. Ethyl 3-methyl-4-oxononanoate 9.[6a] Derivative **9** (22.5 g, 70%) was prepared from ethyl crotonate (17.1 g, 0.15 mol) and hexanal (90 g, 0.9 mol), in the presence of benzoyl peroxide (29.0 g, 0.12 mol) according to the procedure described by Günther and Mosandl for the preparation of 3-methyl-4-oxooctanoate ethyl ester.[5] ^{1}H NMR: δ 4.08 (2H, q, J = 7, COOCH_2), 2.98 (1H, m, H-C(3)), 2.75 (1H, dd, J = 17, 9, H-C(2)), 2.50 (2H, m, 2H-C(5)), 2.25 (1H, dd, J = 17, 5.4, H-C(2)), 1.57 (2H, quintuplet, J = 7.3, 2H-C(6)), 1.27 (4H, m, 2CH_2), 1.22 (3H, s, COOCH$_2$CH_3), 1.10 (3H, d, J = 7.3, CH_3-C(3)), 0.87 (3H, t, J = 7, CH$_2$CH_3); GC/MS t_R = 16.04 min; m/z: 55 (17), 71 (70), 99 (100), 112 (65), 158 (52), 169 (50), 214 (0.1)

4.1.2. 3-Methyl-4-oxononanoic acid 2.[6a] Ester **9** (21.4 g, 0.10 mol) was hydrolysed with KOH (8.40 g, 0.15 mol) in methanol (100 mL) to afford acid **2** (17.7 g, 95%): GC/MS t_R = 16.09 min m/z: 55 (13), 71 (55), 99 (100), 130 (30), 168 (5), 186 (5).

4.1.3. (+)-(3*S*,4*R*)-3-Methyl-4-nonanolide ((+)-*trans*-cognac lactone) (+)-10.[6a–h] Keto acid **2** (17.0 g, 0.091 mol) was submitted to baker's yeast according to the general procedure reported hereafter, to afford lactone

(+)-**10** (6.03 g, 39%): $[\alpha]_D^{20}=+72$ (*c* 2.4, CH_2Cl_2) (lit. Ref. 6a $[\alpha]_D^{20}=+73$ (*c* 0.2, MeOH); Ref. 6b $[\alpha]_D^{20}=+82.2$ (*c* 0.71, MeOH); Ref. 6d $[\alpha]_D^{20}=+83.2$ (*c* 0.69, MeOH); Ref. 6e $[\alpha]_D^{20}=+78$ (*c* 1.12, CH_2Cl_2); Ref. 6f $[\alpha]_D^{20}=+48.3$ (*c* 0.79, CH_2Cl_2); Ref.6g $[\alpha]_D^{20}=+72$ (*c* 1.0, CH_2Cl_2); Ref. 6h $[\alpha]_D^{20}=+79.5$ (CH_2Cl_2)); e.e. and d.e. = >99% chiral GC $t_R=24.72$ min; d.e. = >99% by GC/MS $t_R=14.76$ min; ^{1}H NMR: δ 4.00 (1H, m, H-C(4)), 2.67 (1H, m), 2.18 (2H, m), 1.75–1.25 (8H, m), 1.12 (3H, d, $J=6.4$, CH_3C(3)), 0.86 (3H, t, $J=6.8$, CH_3CH_2); *m*/*z* (GC/MS): 71 (28), 83 (11), 99 (100), 128 (8), 142 (4), 170 (1).

A 1:1 mixture of (±)-*trans*- and *cis*-cognac lactones,[7] to be used as a reference sample, was prepared by $NaBH_4$ reduction and saponification of keto ester **9**: GC/MS t_R (*trans*-cognac lactone) = 14.76 min, t_R (*cis*-cognac lactone) = 15.44 min, *m*/*z* (*cis*): 71 (25), 83 (20), 99 (100), 128 (8), 142 (8), 170 (1).); chiral GC: t_R (+)-**10** = 24.72 min, t_R (−)-**10** = 23.31 min, t_R *cis*-cognac lactone = 25.82 min, t_R *cis*-cognac lactone = 25.98 min.

4.1.4. (+)-(3*S*,4*R*)-1,4-Dihydroxy-3-methylnonane diacetate (+)-3. *trans*-Cognac lactone (+)-**10** (6.0 g, 0.035 mol) was treated with lithium aluminium hydride (1.99 g, 0.053 mol) in THF (50 mL). After the usual work-up, the diol was treated with acetic anhydride and pyridine in excess, to afford (+)-**3** (6.59 g, 73%): $[\alpha]_D^{20}=4.16$ (*c* 2.55, CH_2Cl_2); de >99% (GC/MS) $t_R=19.29$ min; ^{1}H NMR: δ 4.78 (1H, m, H-C(4)), 4.09 (2H, m, CH_2OAc), 2.03 (3H, s, CH_3COO), 2.01 (3H, s, CH_3COO), 1.78 (2H, m), 1.57–1.34 (3H, m), 1.26 (6H, m), 0.90 (3H, d, $J=6.4$, CH_3CH), 0.86 (3H, t, $J=6.8$, CH_3CH_2); *m*/*z* (GC/MS): 56 (86), 72 (83), 85 (100), 98 (60), 155 (44), 172 (0.08), 215 (0.04).

A 1:1 mixture of racemic (3*SR*,4*RS*) and (3*RS*,4*RS*) diacetates, to be used as a reference sample, was prepared by $LiAlH_4$ reduction of keto ester **2** and acetylation with Ac_2O and pyridine: GC/MS t_R (3*SR*,4*RS*) diastereosiomer = 19.29 min, t_R (3*RS*,4*RS*) diastereoisomer = 19.19 min, *m*/*z* (3*RS*,4*RS*) diastereoisomer: 56 (82), 72 (76), 85 (100), 98 (56), 155 (44), 172 (1.5), 215 (0.03). The ^{1}H NMR spectrum was similar to that of the single diastereoisomer (+)-**3**.

4.1.5. (+)-(3*S*,4*R*)-4-Acetoxy-3-methylnonan-1-ol (+)-20. A solution of diacetate (+)-**3** (6.50 g, 0.026 mol) in THF (5 mL) was added to a suspension of PPL (3 g) in water (100 mL) at pH 7.8. The reaction was monitored with a pH-stat with continuous addition of aqueous 0.5 M NaOH. After 24 h, the enzyme was removed by filtration and the filtrate was extracted with ethyl acetate. The organic phase was dried (Na_2SO_4), and concentrated under reduced pressure to give a residue, which was chromatographed on a silica gel column, using hexane/ethyl acetate 4:1. Derivative (+)-**20** (4.41 g, 81%) was obtained: $[\alpha]_D^{20}=+9.6$ (*c* 1, CH_2Cl_2); ^{1}H NMR: δ 4.81 (1H, dt, $J=5.6$, 7.2, H-C(4)), 3.69 (2H, m, CH_2OH), 2.06 (3H, s, CH_3COO), 1.88 (1H, m), 1.65 (1H, m), 1.57–1.19 (9H, m), 0.91 (3H, d, $J=7.1$, CH_3CH), 0.88 (3H, t, $J=7.1$, CH_3CH_2); GC/MS $t_R=17.25$ min *m*/*z*: 56 (78), 85 (100), 155 (14), 173 (10).

According to the same procedure a sample of a 1:1 mixture of the two racemic (3*SR*,4*RS*)- and (3*RS*,4*RS*)-diastereoisomers was prepared from the corresponding mixtures of diacetates. The two diastereoisomers could not be distinguished by GC/MS, but had different signals in the ^{1}H NMR spectra: (3*RS*,4*RS*)-diastereoisomer 4.88 (1H, m, H-C(4)), 2.05 (3H, s, CH_3COO), 0.92 (3H, d, $J=7.1$, CH_3CH).

4.1.6. (+)-(4*S*,5*R*)-5-Acetoxy-4-methylnonanonitrile (+)-21a. To a solution of alcohol (+)-**20** (4.40 g, 0.020 mol) in pyridine (20 mL), 4-toluenesulphonyl chloride (5.81 g, 0.030 mol) was added at 0°C. After the usual work-up, the residue was dissolved in DMSO (20 mL) and NaCN (1.47 g, 0.030 mol) was added. The mixture was heated at 50°C for 3 h, then diluted with water and extracted with methylene chloride. The organic phase was dried, and concentrated. The residue was purified by columnn chromatography, eluting with hexane/ethyl acetate 9:1, to afford (+)-**21a** (3.38 g, 75%): $[\alpha]_D^{20}=+7.4$ (*c* 1.4, CH_2Cl_2); d.e. = >99% GC/MS $t_R=19.12$ min; ^{1}H NMR: δ 4.79 (1H, m, H-C(5)), 2.36 (2H, m, CH_2CN), 2.06 (3H, s, CH_3COO), 1.88 (2H, m), 1.50 (4H, m), 1.27 (5H, m), 0.92 (3H, d, $J=7.1$, CH_3CH), 0.87 (3H, t, $J=7.1$, CH_3CH_2); *m*/*z* GC/MS: 55 (75), 83 (100), 113 (57), 154 (68), 182 (23).

According to the same procedure a sample of a 1:1 mixture of the two racemic diastereoisomers (4*SR*,5*RS*)- **21a** and (4*RS*,5*RS*)-**21b** was prepared from the corresponding mixtures of alcohols. Derivatives **21a** and **21b** could be distinguished by GC/MS: t_R (**21b**) = 18.81 min.

4.1.7. (+)-(4*S*,5*R*)-4-Methyl-4-decanolide (+)-1b.[2,3] A solution of nitrile (+)-**21a** (0.800 g, 3.56 mmol) in ethylene glycole (10 mL) in the presence of KOH 10% (2 mL) was heated at 100°C for 8 h. The reaction mixture was diluted with water, acidified with HCl 10%, and extracted with ethyl acetate. The residue was bulb to bulb distilled to afford (+)-**1a** (0.445 g, 68%): $[\alpha]_D^{20}=+45$ (*c* 0.3, CH_2Cl_2); e.e. and d.e. = >99% by chiral GC $t_R=27.00$ min; d.e. = >99% by GC/MS $t_R=17.68$ min; ^{1}H NMR: δ 3.93 (1H, m, H-C(5)); 2.61 (1H, ddd, $J=17$, 7, 5, C*H*HCOO), 2.44 (1H, ddd, $J=17$, 10, 7, C*H*HCOO), 1.89 (1H, m), 1.70 (2H, m), 1.63–1.26 (8H, m), 1.00 (3H, d, $J=6.5$, CH_3-C(4)), 0.89 (3H, t, $J=7$, CH_3CH_2). *m*/*z* GC/MS: 56 (100), 84 (69), 113 (90), 128 (12), 184 (0.1).

According to the same procedure, a sample of a 1:1 mixture of the two racemic diastereoisomers **1a** and **1b** was prepared from the corresponding mixtures of nitriles. Derivatives **1a** and **1b** could be distinguished by GC/MS: t_R **1a** = 18.15 min, t_R **1b** = 17.68 min. The four stereoisomers could be separated by chiral GC analysis: t_R (+)-**1b** = 27.00 min, t_R (−)-**1b** = 27.31 min, t_R (−)-**1a** = 29.43 min, t_R (+)-**1a** = 30.22 min.

4.1.8. (+)-(4*S*,5*R*)-5-Hydroxy-4-methylnonanonitrile (+)-22. A solution of nitrile (+)-**21a** (2.50 g, 0.011 mol) was treated with KOH (0.933 g, 0.017 mol) in methanol (20 mL). After the usual work-up, the residue was purified

by column chromatography, eluting with hexane/ethyl acetate 7:3, and alcohol (+)-**22** (1.85 g, 92%) was obtained. $[\alpha]_D^{20}=2$ (*c* 1.1, CH_2Cl_2); ^1H NMR: δ 3.42 (1H, m, H-C(5)); 2.45 (1H, ddd, $J=16$, 8, 6, C*H*HCN), 2.34 (1H, dt, $J=16$, 8, C*H*HCN), 1.91 (1H, m), 1.69–1.21 (10H, m), 0.94 (3H, d, $J=6.5$, CH_3-C(4)), 0.89 (3H, t, $J=7$, CH_3CH_2); GC/MS $t_R=17.63$ min *m*/*z*: 55 (95), 83 (100), 101 (60), 112 (32), 141 (18), 182 (0.01).

4.1.9. (−)-(4*S*,5*S*)-5-Acetoxy-4-methylnonanonitrile (−)-21b. Alcohol (+)-**22** (1.80 g, 0.01 mol) was converted into the tosylate derivative, according to the procedure described for (+)-**20**. This was dissolved in DMF (20 mL), and AcONa (1.23 g, 0.015 mol) was added. The reaction mixture was stirred for 3 h, diluted with water and extracted with ethyl acetate. The residue was chromatographed, eluting with hexane/ethyl acetate 9:1, to give a 1:1 mixture of acetate (−)-**21b** and of the corresponding (4*S*,5*S*) formate derivative (1.71 g); $[\alpha]_D^{20}=-6$ (*c* 0.7, CH_2Cl_2); d.e. = 73% by GC/MS, t_R (−)-**21b** = 18.81 min, t_R (formate ester) = 18.27 min; ^1H NMR: δ 8.11 (1H, s, C*H*O), 5.02 (1H, m, H-C(5)), 4.88 (1H, m, H-C(5)), 2.40 (4H, m, CH_2CN of acetate and formate), 2.06 (3H, s, CH_3COO), 1.96–1.40 (22H, m), 0.97 (3H, d, $J=7$, CH_3CH), 0.95 (3H, d, $J=7$, CH_3CH), 0.89 (6H, t, $J=7$, CH_3CH_2); *m*/*z* (GC/MS) **21b**: 55 (68), 83 (100), 113 (55), 154 (60), 182 (23); *m*/*z* (GC/MS) formate ester: 55 (90), 83 (100), 101 (64), 141 (32), 166 (8), 182 (2).

4.1.10. (−)-(4*S*,5*S*)-4-Methyl-4-decanolide (−)-1a.[2,3] According to the procedure described for the preparation of (+)-**1b**, the 1:1 mixture of nitrile (−)-**21b** and of the corresponding formate (1.71 g) was converted in natural Aerangis lactone (−)-**1a** (1.05 g, 57% from alcohol (+)-**22**): $[\alpha]_D^{20}=-35$ (*c* 0.9, CH_2Cl_2); e.e. >99%, d.e. = 70% by chiral GC $t_R=29.43$ min; ^1H NMR: δ 4.27 (1H, m, H-C(5)); 2.53 (2H, t, $J=7$, CH_2COO), 2.03 (3H, m), 1.67 (2H, m), 1.60–1.20 (6H, m), 0.95 (3H, d, $J=6.5$, CH_3-C(4)), 0.89 (3H, t, $J=7$, CH_3CH_2). *m*/*z* GC/MS: 56 (100), 84 (62), 113 (52), 128 (7), 184 (0.1).

4.2. Synthesis of the substrates

4.2.1. 4-Methyl-5-oxo-decanoic acid 4 and 2-methyl-5-oxo decanoic acid 5. The 2:1 mixture of derivatives **4** and **5** was prepared from 2-methyl glutaric acid, according to the procedure already reported in Ref. 4. The two corresponding methyl esters could be distinguished by GC/MS: methyl ester of **4** $t_R=16.92$ min, *m*/*z*: 55 (35), 71 (58), 99 (100), 126 (51), 158 (31), 183 (24), 214 (4); methyl ester of **5** $t_R=17.05$ min, *m*/*z*: 55 (66), 71 (79), 99 (100), 126 (95), 158 (77), 183 (38), 214 (0.5).

4.2.2. 3-Methyl-4-oxo-octanoic acid 12. This derivative was prepared (16.5 g, 96%) by saponification with KOH (8.4 g, 0.15 mol) in methanol (100 mL) of the corresponding ethyl ester (20 g, 0.1 mol) prepared according to Ref. 5.

4.2.3. Ethyl 3-methyl-4-hydroxy-2-nonenoate 14. To a solution of pentyl magnesium bromide, prepared from pentyl bromide (15.1 g, 0.10 mol) and magnesium (2.28 g, 0.095 mol), in THF (300 mL) a solution of zinc(II) bromide (10.7 g, 0.048 mol) in THF (30 mL) was added under nitrogen, maintaining the temperature below 20°C. To the resulting greyish mixture a solution of 3-formyl crotonic acid ethyl ester (14.2 g, 0.10 mol) was added at 20°C. The reaction mixture was quenched with ice, acidified with diluted HCl, and extracted with diethyl ether. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed eluting with hexane/ethyl acetate 9:1, to afford derivative **14** (12.4 g, 61%): ^1H NMR: δ 5.91 (1H, s; H-C(2)), 4.16 (2H, q, $J=7$, COOCH_2), 4.08 (1H, m, H-C(4)), 2.11 (3H, s, CH_3-C(3)), 1.65–1.31 (8H, m), 1.28 (3H, s, COOCH$_2CH_3$), 0.89 (3H, t, $J=7$, CH_2CH_3); *m*/*z* GC/MS $t_R=18.87$ min: 69 (25), 87 (50), 115 (100), 143 (63), 169 (17), 196 (2).

4.2.4. Ethyl 3-methyl-4-oxo-2-nonenoate 13. A mixture of allylic alcohol **14** (12.0 g, 0.056 mol) and manganese(IV) oxide (1.5 equiv.) in methylene chloride (200 mL) was stirred under reflux for 4 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed, eluting with hexane:ethyl acetate 19:1, to give keto ester **13** (9.14 g, 77%): ^1H NMR: δ 6.52 (1H, m; H-C(2)), 4.23 (2H, q, $J=7$, COOCH_2), 2.68 (2H, t, $J=7.3$, CH_2CO), 2.22 (3H, d, $J=1.5$, CH_3-C(3)), 1.62 (2H, quintuplet, $J=7.3$, CH_2CH_2CO), 1.32 (7H, m+t, $J=7.3$, 2 CH_2+COOCH$_2CH_3$), 0.89 (3H, t, $J=7$, CH_2CH_3); *m*/*z* GC/MS $t_R=17.67$ min: 85 (31), 110 (100), 139 (100), 167 (40), 183 (4).

4.2.5. 2-Diethoxymethyl-oct-1-en-3-ol 17. Derivative **17** was prepared (55.2 g, 80%) from hexanal (30 g, 0.30 mol) and 2-bromo-3,3-diethoxy-propene (81.5 g, 0.39 mol) according to the literature:[11] ^1H NMR: δ 5.17 (2H, s, C=CH_2), 4.81 (1H, s, C*H*(OEt)$_2$), 4.04 (1H, t, $J=6.2$, C*H*OH), 3.66–3.32 (4H, m, 2OCH_2CH$_3$), 1.58–1.14 (14H, m), 0.90 (3H, t, $J=7$, CH_2CH_3); *m*/z GC/MS $t_R=16.36$ min: 85 (65), 103 (100), 113 (83), 185 (23).

4.2.6. 2-Diethoxymethyl-oct-1-en-3-one 15. Derivative **15** was prepared (25.8 g, 65%) from alcohol **17** (40.0 g, 0.17 mol) by Swern oxidation:[11] ^1H NMR: δ 5.97 (1H, m, C=C*H*), 5.92 (1H, m, C=C*H*), 5.19 (1H, s, C*H*(OEt)$_2$), 3.65–3.35 (4H, m, 2OCH_2CH$_3$), 2.59 (2H, t, $J=7$, CH_2CO), 1.68–1.21 (6H, m), 1.17 (6H, t, $J=7$, 2OCH_2CH_3), 0.92 (3H, t, $J=7$, CH_2CH_3); *m*/z GC/MS $t_R=15.48$ min: 85 (65), 103 (100), 113 (83), 185 (23).

4.3. Yeast reduction

A suspension of baker's yeast (2.5 kg) and D-glucose (2.0 kg) in tap water (7.5 l) was stirred for 30 min at 32°C. A solution of the substrate (0.15 mol) in ethanol (20 mL) was then added. After stirring the mixture for 48 h at rt Celite (1 kg) was added, and the reaction mixture filtered, washing the Celite pad with ethyl

acetate. The filtrate was adjusted to pH 4 with aqueous 2N HCl, and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on a silica gel column.

4.4. Reduction products

4.4.1. BY reduction of derivatives 4 and 5. The 2:1 mixture of **4** and **5** (20.0 g, 0.10 mol) gave a mixture (GC/MS) of 4-methyl decanolides and of the 2-methyl regioisomers (7.72 g, 42%). The mixture was analysed by GC/MS: *cis*-**6a** and *trans*-**6b** $t_R = 17.27$ and 17.56 min (not assigned), t_R **1b** = 17.68 min, t_R **1a** = 18.15 min Aerangis lactones were separated from the 2-methyl regioisomers by column chromatography, eluting with hexane/ethyl acetate 9:1. The two 1:1 Aerangis lactones were identified by chiral GC/MS: (+)-**1b** $t_R = 27.00$ min, e.e. = >99%; (+)-**1a** $t_R = 30.22$ min, e.e. = >99%.

4.4.2. BY reduction of keto acid 12. Keto acid **12** (16.0 g, 0.094 mol) gave (+)-(3*S*,4*R*)-3-methyl-4-octanolide[5] ((+)-*trans*-whisky lactone) (5.57 g, 38%): $[\alpha]_D^{20} = +90$ (*c* 0.5, MeOH) (lit. Ref. 5 $[\alpha]_D^{20} = 96$ (*c* 1, MeOH); e.e. and d.e. = >99% chiral GC $t_R = 21.64$ min; d.e. = >99% by GC/MS $t_R = 12.47$ min; ^{1}H NMR: δ 4.00 (1H, m, H-C(4)), 2.66 (1H, m), 2.18 (2H, m), 1.70–1.20 (6H, m), 1.14 (3H, d, $J = 6.4$, CH_3C(3)), 0.92 (3H, t, $J = 6.8$, CH_3CH_2); *m*/*z* (GC/MS): 71 (25), 87 (15), 99 (100), 114 (1), 156 (0.5).

A 1:1 mixture of racemic *trans*- and *cis*-whisky lactones, to be used as a reference sample, was prepared by $NaBH_4$ reduction of keto acid **12**: GC/MS t_R (*trans*-whisky lactone) = 12.47 min, t_R (*cis*-whisky lactone) = 13.10 min, *m*/*z* (*cis*): 69 (23), 87 (20), 99 (100), 114 (7), 156 (3); chiral GC: t_R (+)-*trans*-whisky lactone = 21.64 min, t_R (−)-*trans*-whisky lactone = 22.77 min, t_R *cis*-whisky lactone = 23.35 min, t_R *cis*-whisky lactone = 23.53 min.

4.4.3. BY reduction of derivative 13. Unsaturated keto ester **13** (9.0 g, 0.042 mol) gave a 7:3 mixture (GC/MS) of allylic alcohol **14** and keto ester **9** (3.5 g). The desired keto ester **9** was isolated by column chromatography, eluting with hexane:ethyl acetate 19:1. Keto ester **9** (1.12 g, 12%): $[\alpha]_D^{20} = 23.8$, *c* 1.9, CH_2Cl_2; e.e. = 60% by ^{1}H NMR in the presence of chiral shift reagents ($Eu(hfc)_3$): signal of the major enantiomer 4.25 ppm (q, $COOCH_2$); signal of the minor enantiomer 4.26 ppm, $\Delta\delta = 0.01$ ppm.

When derivative **13** (9.0 g, 0.042 mol) was reduced by BY in the presence of resin XAD 1180,[12] a 3:1 mixture (^{1}H NMR) of starting material **13** and saturated keto ester **9** was obtained (4.32 g). The two derivatives could not be separated by column chromatography. Keto ester **9** (impure of **13**): $[\alpha]_D^{20} = 27.8$, *c* 2.8, CH_2Cl_2; e.e. = 95% by ^{1}H NMR in the presence of chiral shift reagents.

4.4.4. BY reduction of derivative 15. Reduction of **15** (7 g, 0.021 mol) gave derivative **16** (2 g, 52%): ^{1}H NMR: δ 7.15 (1H, m, C=C*H*), 4.03 (2H, q, $J = 7$, OCH_2CH_3), 2.39 (2H, t, $J = 7$, CH_2CO), 1.64 (3H, d, $J = 1.25$, CH_3-C=), 1.65–1.20 (9H, m), 0.92 (3H, t, $J = 7$, CH_2CH_3); *m*/z GC/MS $t_R = 15.48$ min: 55 (55), 69 (100), 83 (92), 101 (40), 140 (5).

4.4.5. BY reduction of derivative 17. Reduction of **17** (10 g, 0.043 mol) gave a 1:1 mixture of derivatives **18** and **19** (2.65 g). In order to recover the desired unsaturated diol **18**, the reaction mixture was oxidised with MnO_2 in methylene chloride. After the usual work-up, the residue was chromatographed, using hexane:ethyl acetate 7:3 as an eluent, to recover derivative **18** (1.5 g, 22%) as a 2:1 mixture (GC/MS) of the two possible *trans* and *cis*-diastereoisomer: GC/MS t_R (*trans*) = 13.19 *m*/*z*: 55 (60), 71 (35), 83 (100), 101 (65); t_R (*cis*) = 13.12 *m*/*z*: 55 (67), 71 (39), 83 (100), 101 (65); ^{1}H NMR: δ 3.68 (3H, m), 1.89–1.08 (12H, m), 0.89 (3H, t, $J = 7$, CH_2CH_3).

The relative configuration of the diastereoisomers and their enantiomeric purity was assessed converting diol **18** into cognac lactone, via tosylate derivatisation, cyanide substitution, and alkaline hydrolysis. A 4:1 mixture of *trans*- and *cis*-cognac lactones was obtained (GC/MS). Chiral GC analysis: (+)-*trans*-cognac e.e. = 65% and (±)-*cis*-cognac lactone.

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