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Stereochemistry of terpene derivatives. Part 6: Chemoenzymatic synthesis of chiral bicyclo[3.1.0]hexane derivatives with olfactory properties ☆

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ABSTRACT

Starting from (+)-3-carene **1**, several chiral fragrant compounds with the bicyclo[3.1.0]hexane system were synthesized. These compounds were used as substrates for the biotransformation with lipases as biocatalysts. Pure diastereoisomers were obtained and their absolute configuration was confirmed by X-ray crystallography. The olfactory properties of new compounds were determined.

1. Introduction

Biocatalysis is an effective tool for the structural modification of bioactive, natural, and synthetic compounds. Enzymes, especially lipases (EC 3.1.1.3), are very useful in the preparation of a broad range of compounds in their optically active forms. These enzymes possess wide substrate specificity, generally high chemo-, regio-, and stereoselectivity, and in many cases perform under mild conditions.[2] Moreover, lipases remain enzymatically active in organic solvents, which makes them the ideal tools in organic synthesis. Their ability to catalyze hydrolysis as well as transesterifications is well recognized and has been described in a number of papers on lipases.[3]

The optically active monoterpene (+)-3-carene **1**, a major component of Polish turpentine obtained from *Pinus sylvestris* (L.), is a natural, inexpensive, and widely available raw material. Easily obtained derivatives of this monoterpene have been used as chiral auxiliaries in enantioselective transformations.[4] Therefore, this natural product is a convenient substrate in the synthesis of chiral derivatives with the bicyclo[3.1.0]hexane moiety, displaying interesting olfactory properties.[5] Moreover, (+)-3-carene **1** was used as a starting material in the synthesis of chiral, biologically active products such as β-amino acids[6] and sulfides.[7]

Since 1961, when Ohloff published the results of his research on the enantioselective perception of chiral odorants, it is well known that stereochemistry plays an important role in odor properties.[8] Enantiomers of one compound can both differ in intensity and elicit different odor sensations.[9] Thus, there is a strong emphasis on receiving products in a single stereoisomer form. Nowadays, biotransformation is a method highly preferred in the preparation of enantiomerically pure compounds.[10]

Herein, we report lipase-catalysed kinetic resolution as an efficient method for gaining the fragrant diastereoisomers of *gem*-dimethylbicyclo[3.1.0]hexane derivatives with high enantiomeric purity. Our goal was to obtain pure diastereoisomers of the secondary allylic alcohol (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo-[3.1.0]hex-2-en-2-yl)]ethanol and their esters and to determinate absolute configuration of newly formed stereogenic center. These results enabled us to evaluate and compare the odor characteristics of pure diastereoisomers with their mixtures.

2. Results and discussion

The key compound, secondary allylic alcohol (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*RS*,1*S*,5*R*)-**4**, was synthesized in a three-step procedure from the monoterpene hydrocarbon (+)-3-carene **1**. Ozonolysis of **1** followed by intramolecular aldol condensation of ketoaldehyde **2** afforded bicyclic enone[11] **3**, which was reduced with lithium aluminum hydride to alcohol (*RS*,1*S*,5*R*)-**4**. The authors of this procedure[12] did not observe that the reduction of enone **3** proceeded with asymmetric induction, leading to the formation of a diastereoisomeric mixture in an 85:15 ratio (CGC) (Scheme 1).

The excess of one diastereoisomer can be explained by the presence of a *gem*-dimethylcyclopropane moiety as a steric hindrance.

Acetate (*RS*,1*S*,5*R*)-**5** was obtained from a mixture of alcohol (*RS*,1*S*,5*R*)-**4** in one step reaction in pyridine medium with the addition of acetyl chloride (Scheme 1).

Secondary allylic alcohol (*RS*,1*S*,5*R*)-**4** was a substrate for enzymatic transesterifications in anhydrous conditions. In these reactions lipases from *Burkholderia cepacia*, *Pseudomonas fluorescence*, and *Candida rugosa* were used in the presence of vinyl acetate as an acyl donor (Scheme 2).[13]

☆ Part 5 see Ref. 1.

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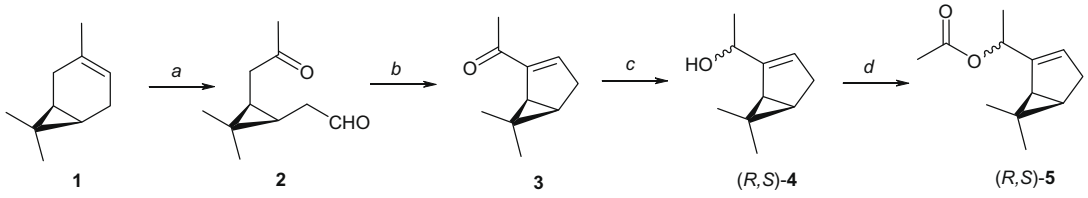
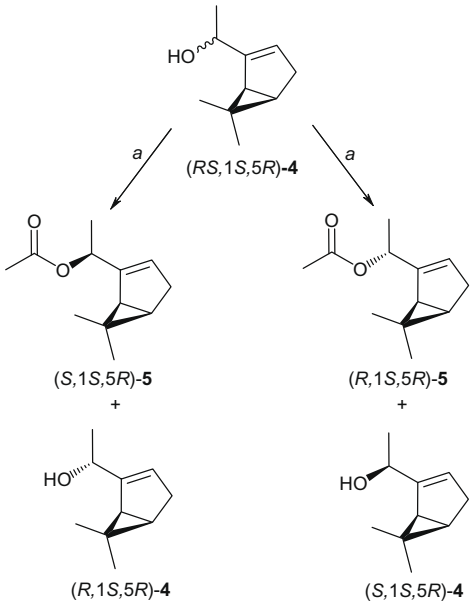


Scheme 1. Reagents: (a) (1) O_3; (2) Zn/AcOH; (b) 5% NaOH; (c) $LiAlH_4$; (d) AcCl, Py.



Scheme 2. Reagents: (a) lipase, $AcOC_2H_3$, *i*-Pr_2O, powdered molecular sieves 3 Å.

Table 1
Results of enzymatic transesterification of (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*RS*,1*S*,5*R*)-**4**

Lipase	Time (h)	ee of acetate (%)	
		37 °C	45 °C
Amano PS from *Burkholderia cepacia*	3	62.9	59.1
	7	68.4	71.7
	24	85.6	84.8
	48	86.8	85.2
AK from *Pseudomonas fluorescence*	3	42.7	51.8
	7	76.2	74.9
	24	82.1	83.2
	48	83.4	84.1
AYS from *C. rugosa*	3	7.6	7.1
	7	7.5	9.4
	24	12.3	19.3
	48	15.7	24.6

The influence of temperature and reaction time was also investigated (Table 1). The best results for biotransformations were obtained for Amano PS lipase from *B. cepacia* at 37 °C for 24 h. Under these conditions the enzyme was able to convert only one form of alcohol **4**. It led us to obtain a pure diastereoisomer of acetate (*R*,1*S*,5*R*)-**5**[14] and a mixture of alcohol (*S*,1*S*,5*R*)-**4** (which was not a substrate for this enzyme) with unreacted alcohol (*R*,1*S*,5*R*)-**4** in an 89:11 ratio.

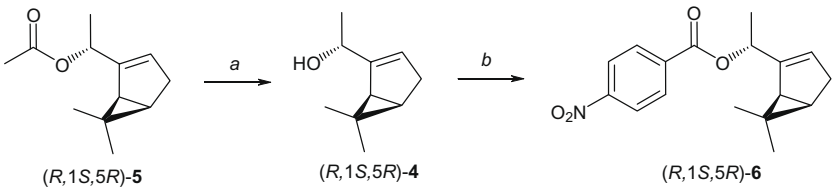
Diastereopure acetate (*R*,1*S*,5*R*)-**5**, obtained by enzymatic transesterification in anhydrous conditions, was reduced with lithium aluminum hydride to the secondary alcohol (*R*,1*S*,5*R*)-**4**,[15] which was transformed to the crystalline *p*-nitrobenzoate derivative (*R*,1*S*,5*R*)-**6** (Scheme 3).

The newly formed stereogenic center in the side chain at C-9 was determined by X-ray crystallography as having an (*R*)-configuration and was assigned on the basis of the known absolute configuration at the C-1 and C-5 carbon atoms in the *gem*-dimethylbicyclo [3.1.0]hex-2-ene system (Fig. 1).

The second strategy for obtaining pure diastereoisomeric compounds was the enzymatic hydrolysis of (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethyl acetate (*RS*,1*S*,5*R*)-**5**. The biotransformations were carried out at room temperature with shaking (150 rpm) in a biphasic system consisting of 0.05 M phosphate buffer, pH 7, and a mixture of diisopropyl ether and hexane with one of the lipases from *B. cepacia*, *P. fluorescence*, and *C. rugosa* (Scheme 4).[16] All enzymes tested gave no acceptable results; biocatalysts did not show stereospecificity when hydrolyzing acetate (*RS*,1*S*,5*R*)-**5**, giving a mixture of (*R*)- and (*S*)-isomers of alcohol **4** with an unreacted mixture of acetate (*RS*,1*S*,5*R*)-**5**.

All of the obtained compounds, pure diastereoisomeric forms and their mixtures, exhibited various interesting fragrances.[17] The comparative analysis of the odoriferous properties of alcohols (*RS*,1*S*,5*R*)-**4**, (*R*,1*S*,5*R*)-**4**, (*S*,1*S*,5*R*)-**4** and esters (*RS*,1*S*,5*R*)-**5**, (*R*,1*S*,5*R*)-**5** showed that all diastereoisomers are medium intensive with fruity or herbal-balsamic note. Odor characteristics of the newly obtained pure diastereoisomers in comparison with diastereoisomeric mixture (*RS*,1*S*,5*R*)-**4** and (*RS*,1*S*,5*R*)-**5** are given in Table 2.

The search for the effective odor vector with chiral fragrance is very challenging. All of the possible stereoisomers of a certain chiral odorants have to be prepared in a diastereopure form and the odoriferous properties have to be evaluated.[9] Herein, we have shown that biocatalysis is an easy way to obtain chiral fragrant compounds and it is a good alternative to a chemical synthesis.



Scheme 3. Reagents: (a) $LiAlH_4$; (b) *p*-NBCl, Py.

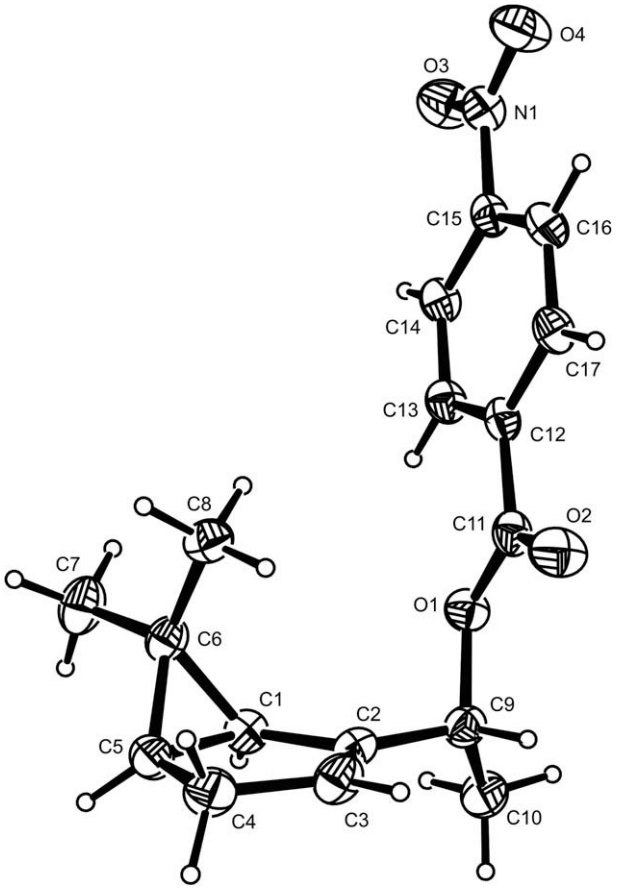


Figure 1. Crystal structure of (*R*,1*S*,5*R*)-**6**.

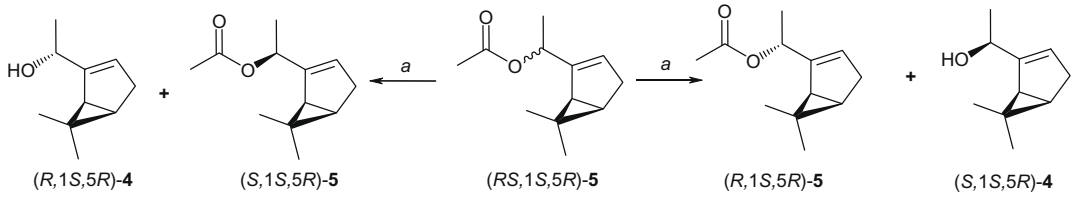
The examples described herein demonstrate the usefulness of the lipase-mediated reaction in the synthesis of single stereoisomers. The only limitation is finding a well-fitting biocatalyst for a given compound.

3. Experimental

(+)-3-Carene was purchased from Acros Organics ($[\alpha]_D^{25} = +15.3$ (neat); $n_D^{26} = 1.4697$, d = 0.864 g/cm^3, bp = 170–171 °C, ee = 100%). The sources of lipases were Amano PS from *B. cepacia* (Aldrich), lipases AK from *P. fluorescence* (Amano), lipases AYS from *C. rugosa* (Amano). All materials were obtained from commercial suppliers: Sigma, Aldrich, Fluka, POCh, Serva, and used without purification. The course of all the reactions, composition of products, and their purities were checked by thin-layer chromatography (TLC) and gas chromatography (GC). The enantiomeric excess was determined by chiral gas chromatography (CGC). TLC was carried out on Silica Gel 60 F_{254} 0.2 mm (Merck). Plates were developed in a mixture of hexane, diethyl ether, ethyl acetate, and acetone in various ratios and visualized with 20% ethanolic H_2SO_4, containing 0.1% of anisaldehyde. Preparative column chromatography was carried out on silica gel (230–400 mesh, Merck) with a mixture of hexane, diethyl ether, ethyl acetate, and acetone (various ratios) as an eluent. Analytical GC was performed on Hewlett Packard 5890 (series II) instrument using capillary column HP-5 (length 25 m, temperature 120–180 °C). Melting points were determined on a Boetius apparatus. IR spectra were taken from liquid films or in KBr on Perkin–Elmer 621 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard on a Bruker Avance™ DRX 300 or Bruker Avance™ DRX 600 instrument. Chemical shifts (δ) are reported in ppm and coupling constants (J) are given in hertz. ^{13}C–^{1}H substitution was determined with DEPT-135 experiments. The names of the compounds are compatible with IUPAC nomenclature. Numbering of carbon atoms in all the compounds was changed to simplify interpretation of ^{1}H and ^{13}C NMR spectra. Optical rotation measurements were obtained on a PolAAr-31 automatic polarimeter (Optical Activity Ltd). X-ray data were collected at ambient temperature using a KM4CCD κ-axis diffractometer with graphite-monochromated Mo Kα radiation. The data were corrected for Lorentz and polarization effects. Data collection, reduction, and analysis were carried out with CRYSALIS CCD and CRYSALIS RED programs.[18] The structure was solved by direct methods and refined by the full matrix least-squares method on all F^2 data using SHELXS97 and SHELXL97 programs.[19] Non-hydrogen atoms were refined with anisotropic displacement parameters. Methyl hydrogen atoms were located in ΔF maps and refined as part of rigid riding and rotating groups; the remaining hydrogen atoms were positioned geometrically and treated as riding. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited to the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 766355.

3.1. (+)-1-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.0]hex-2-en-2-yl)]-ethanol (*RS*,1*S*,5*R*)-4

Bicyclic enon **3** (4.00 g, 26.6 mmol) in anhydrous diethyl ether (80 ml) was dropped to the solution of $LiAlH_4$ (1.40 g, 36.9 mmol)



Scheme 4. Reagents: (a) lipase, *i*-Pr_2O/hexane, phosphate buffer pH 7.

Table 2
Odor characteristics of pure isomers and their mixture

Compound	Odor characteristics
(*RS*,1*S*,5*R*)-**4**	Medium intensive, fruity with peppermint note
(*RS*,1*S*,5*R*)-**5**	Medium intensive, sweet with resinous note
(*R*,1*S*,5*R*)-**4**, ee = 100%	Medium intensive, floral-woody with gerbera and apple note
(*S*,1*S*,5*R*)-**4**, ee = 78%	Medium intensive, fruity with chemical-camphor note
(*R*,1*S*,5*R*)-**5**, ee = 100%	Medium intensive, woody-balsamic with honey note

in anhydrous diethyl ether (80 ml). The mixture was stirred at −10 °C until TLC monitoring showed the absence of substrate **3** (about 1 h). Then, distilled water was dropped carefully until white precipitate was observed. Next, the solution was decanted and dried over anhydrous $MgSO_4$. After removing the solvent, the crude product **4** was purified by column chromatography (eluent: hexane–ethyl acetate 7:1) to give mixture of alcohol (*RS*,1*S*,5*R*)-**4** (3.0 g, 19.7 mmol, 74% yield). Chiral gas chromatography showed two diastereoisomers of alcohol (*RS*,1*S*,5*R*)-**4** in a ratio of 85 to 15. $[\alpha]_D^{28} = +87.0$ (*c* 1.0, $CHCl_3$); $n_D^{28} = 1.4783$; IR (film, cm^{-1}): 3354 (vs); 3019 (s); 2867 (s); 1638 (w); 1449 (s); 1373 (vs); 1065 (vs); 802 (vs); ^1H NMR ($CDCl_3$, 300 MHz): 0.68 (s, 3H at C-7 or at C-8, *S*-isomer); 0.70 (s, 3H at C-7 or at C-8, *R*-isomer); 0.93 (s, 3H at C-7 or at C-8, *S*-isomer); 0.95 (s, 3H at C-7 or at C-8, *R*-isomer); 1.16 (d, *J* = 6.5 Hz, 3H at C-10); 1.23 (t, *J* = 7.1 Hz, 1H at C-5); 1.53 (dd, *J* = 6.5, 3.0 Hz, 1H at C-1, *R*-isomer); 1.63 (dd, *J* = 6.6, 3.0 Hz, 1H at C-1, *S*-isomer); 1.97 (d, *J* = 16.0 Hz, 1H at C-4); 2.32 (dd, *J* = 18.1, 8.2 Hz, 1H at C-4, *R*-isomer); 2.51–2.52 (m, 1H at C-4, *S*-isomer); 4.11 (q, *J* = 6.5 Hz, 1H at C-9, *S*-isomer); 4.26 (q, *J* = 6.3 Hz, 1H at C-9, *R*-isomer); 5.24 (s, 1H at C-3); ^{13}C NMR ($CDCl_3$, 75 MHz): 13.10 (C-7 or C-8, *S*-isomer); 13.18 (C-7 or C-8, *R*-isomer); 19.40 (C-6); 21.54 (C-10, *S*-isomer); 21.96 (C-10, *R*-isomer); 26.33 (C-7 or C-8); 29.22 (C-5, *R*-isomer); 29.82 (C-5, *S*-isomer); 31.06 (C-4, *S*-isomer); 31.71 (C-4, *R*-isomer); 36.73 (C-1); 66.85 (C-9, *S*-isomer); 67.30 (C-9, *R*-isomer); 124.42 (C-3, *R*-isomer); 126.62 (C-3, *S*-isomer); 148.32 (C-2). Anal. Calcd for $C_{10}H_{16}O$: C, 78.90; H, 10.59. Found: C, 78.69; H, 10.67.

3.2. (+)-1-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.0]hex-2-en-2-yl)]-ethyl acetate (*RS*,1*S*,5*R*)-5

The mixture of alcohol (*RS*,1*S*,5*R*)-**4** (0.80 g, 5.2 mmol) in anhydrous diethyl ether (60 ml) and anhydrous pyridine (0.64 ml, 7.9 mmol) was intensively stirred at an ice bath temperature. Next, the solution of fresh distilled acetyl chloride (0.56 ml, 7.9 mmol) in anhydrous diethyl ether (60 ml) was added carefully. The reaction was carried out at room temperature and stopped when TLC analysis showed the absence of substrate **4**, and then the reaction mixture was diluted with diethyl ether (120 ml). To the obtained slightly acidic environment a solution of dilute HCl was added. The aqueous phase was extracted three times with diethyl ether. All ether phases were washed with 2% H_2SO_4 solution, 10% $NaHCO_3$ solution, and saturated NaCl solution. The organic phase was dried over $MgSO_4$ and after removing the solvent, the crude product (*RS*,1*S*,5*R*)-**5** was obtained (0.88 g, 4.5 mmol, 86% yield). Chiral gas chromatography showed two diasteroisomers of acetate (*RS*,1*S*,5*R*)-**5** in a ratio of 85 to 15. $[\alpha]_D^{28} = +225.0$ (*c* 1.0, $CHCl_3$), $n_D^{26} = 1.4578$; IR (film, cm^{-1}): 2868 (s); 1736 (vs); 1448 (m); 1371 (m); 1242 (vs); 1062 (m); 800 (w); ^1H NMR ($CDCl_3$, 300 MHz): 0.69 (s, 3H at C-7 or at C-8, *R*-isomer); 0.71 (s, 3H at C-7 or at C-8, *S*-isomer); 0.96 (s, 3H at C-7 or at C-8, *S*-isomer); 0.98 (s, 3H at C-7 or at C-8, *R*-isomer); 1.24 (d, *J* = 6.6 Hz, 3H at C-10); 1.25 (t, *J* = 7.9 Hz, 1H at C-5); 1.58 (dd, *J* = 6.5, 3.1 Hz, 1H at C-1, *R*-isomer); 1.61 (dd, *J* = 7.0, 3.4 Hz, 1H at C-1, *S*-isomer); 1.97 (s, 3H at C-12); 2.02 (d, *J* = 16.1 Hz, 1H at C-4); 2.36 (dd, *J* = 18.3, 7.8 Hz, 1H at C-4, *R*-isomer); 2.51–2.53 (m, 1H at C-4, *S*-isomer); 5.22 (q, *J* = 6,4 Hz, 1H at C-9, *S*-isomer); 5.30 (s, 1H at C-3); 5.36 (q, *J* = 6,1 Hz, 1H at C-9, *R*-isomer);^{13}C NMR ($CDCl_3$, 75 MHz): 13.07 (C-7 or C-8); 19.43 (C-6); 21.30 (C-10, *R*-isomer); 21.70 (C-10, *S*-isomer); 26.32 (C-7 or C-8); 29.11 (C-5); 30.40 (C-4, *S*-isomer); 31.78 (C-4, *R*-isomer); 35.93 (C-1, *S*-isomer); 36.69 (C-1, *R*-isomer); 43.73 (C-12); 66.83 (C-9, *S*-isomer); 69.33 (C-9, *R*-isomer); 124.40 (C-3, *S*-isomer); 125.34 (C-3, *R*-isomer); 143.61 (C-2); 170.35 (C-11). Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.11; H, 9.46.

3.3. Enzymatic hydrolysis of (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo-[3.1.0]hex-2-en-2-yl)]ethyl acetate (*RS*,1*S*,5*R*)-5

Enzymatic hydrolysis of (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo-[3.1.0]hex-2-en-2-yl)]ethyl acetate **5** was carried out in a biphasic system (3.8 ml) consisting of 0.05 M phosphate buffer, pH 7 (3.0 ml), and a mixture of diisopropyl ether (0.2 ml) with *n*-hexane (0.6 ml). After the addition of the substrate (0.60 g, 3.1 mmol) and 100 mg of suitable lipase the reactions were carried out at room temperature with shaking (150 rpm). The reactions were stopped after certain periods of time, and the product was extracted twice with 15 ml of ethyl acetate and the organic phase was dried over anhydrous $MgSO_4$. After filtration, the solvent was removed by evaporation and the obtained products were purified by column chromatography and analyzed by GC and CGC.

3.4. Enzymatic transesterification of (+)-1-[(1*S*,5*R*)-6,6-di-methyl bicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*RS*,1*S*,5*R*)-4; (+)-(1*S*)-1-[(1*S*, 5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*S*,1*S*,5*R*)-4; (+)-(1*R*)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)] ethyl acetate (*R*,1*S*,5*R*)-5

Enzymatic transesterification of (+)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*RS*,1*S*,5*R*)-**4** was carried out in diisopropyl ether (6 ml) with the addition of 40 mg of powdered molecular sieves (3 Å mesh). 2.00 g (13.1 mmol) of the substrate; 1600 mg of suitable lipase and vinyl acetate (3.60 ml, 39.1 mmol) were added. The reactions were carried out at 37 °C in a shaker (150 rpm). The reaction was stopped after certain periods of time by filtration followed by evaporation of the organic layer. The resulting product was purified by column chromatography (eluent: hexane–acetone from 40:1 to 10:1) to give alcohol **4** and acetate **5**.

Alcohol (*S*,1*S*,5*R*)-**4**: ee = 78%; $[\alpha]_D^{29} = +12.0$ (*c* 1.0, $CHCl_3$); $n_D^{30} = 1.4717$; IR (film, cm^{-1}): 3354 (vs); 3019 (s); 2867 (s); 1638 (w); 1449 (s); 1373 (vs); 1065 (vs); 802 (vs); ^1H NMR ($CDCl_3$, 300 MHz): 0.68 (s, 3H at C-7 or at C-8); 0.93 (s, 3H at C-7 or at C-8); 1.16 (d, *J* = 6.5 Hz, 3H at C-10); 1.23 (t, *J* = 7.1 Hz, 1H at C-5); 1.63 (dd, *J* = 6.6, 3.0 Hz, 1H at C-1); 1.97 (d, *J* = 16.0 Hz, 1H at C-4); 2.51–2.52 (m, 1H at C-4); 4.11 (q, *J* = 6.5 Hz, 1H at C-9); 5.24 (s, 1H at C-3); ^{13}C NMR ($CDCl_3$, 75 MHz): 13.10 (C-7 or C-8); 19.40 (C-6); 21.54 (C-10); 26.33 (C-7 or C-8); 29.82 (C-5); 31.06 (C-4); 36.73 (C-1); 66.85 (C-9); 126.62 (C-3); 148.32 (C-2). Anal. Calcd for $C_{10}H_{16}O$: C, 78.90; H, 10.59. Found: C, 78.64; H, 10.74.

Acetate (*R*,1*S*,5*R*)-**5**: $[\alpha]_D^{26} = +180.0$ (*c* 1.0, $CHCl_3$); $n_D^{28} = 1.4597$; IR (film, cm^{-1}): 3021 (w); 2940 (m); 1737 (s); 1641 (w); 1448 (m); 1371 (m); 1242 (vs); 1048 (m); ^1H NMR ($CDCl_3$, 600 MHz): 0.77 (s, 3H at C-7 or at C-8); 1.05 (s, 3H at C-7 or at C-8); 1.32 (d, *J* = 6.4 Hz, 3H at C-10); 1.33 (t, *J* = 7.4 Hz, 1H at C-5); 1.66 (dd, *J* = 7.1, 3.2 Hz, 1H at C-1); 2.05 (s, 3H at C-12); 2.07 (d, *J* = 18.1 Hz, 1H at C-4); 2.43 (dd, *J* = 18.1, 7.7 Hz, 1H at C-4); 5.38 (s, 1H at C-3); 5.43 (q, *J* = 6,5 Hz, 1H at C-9); ^{13}C NMR ($CDCl_3$, 75 MHz): 14.95 (C-7 or C-8); 18.70 (C-10); 19.13 (C-6); 21.41 (C-7 or C-8); 27.94 (C-5); 28.88 (C-4); 29.98 (C-1); 46.73 (C-12); 75.15 (C-9); 108.31 (C-3); 148.53 (C-2); 170.86 (C-11). Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.11; H, 9.46.

3.5. (+)-(1*R*)-1-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol (*R*,1*S*,5*R*)-4

Acetate (obtained by enzymatic transesterification in anhydrous conditions) (*R*,1*S*,5*R*)-**5** (1.20 g, 6.17 mmol) in anhydrous diethyl ether (60 ml) was dropped to a solution of $LiAlH_4$ (0.480 g, 12.6 mmol) in anhydrous diethyl ether (80 ml). The mixture was stirred at room temperature until TLC monitoring showed the absence of substrate **5** (about 1 h). Then, distilled water was dropped carefully until a white precipitate was observed. Next,

the solution was decanted and dried over $MgSO_4$. After removing the solvent, the crude product was purified by column chromatography (eluent: hexane–ethyl acetate 7:1) to give pure alcohol (*R*,1*S*,5*R*)-**4** (0.61 g, 4.0 mmol, 65% yield): $[\alpha]_D^{29} = +69.0$ (*c* 1.0, $CHCl_3$); $n_D^{28} = 1.4828$; IR (film, cm^{-1}): 3360 (s); 3078 (m); 2972 (vs); 2932 (s); 1645 (m); 1451 (s); 1372 (m); 1085 (s); 1069 (s); ^1H NMR ($CDCl_3$, 600 MHz): 0.81 (s, 3H at C-7 or at C-8); 1.06 (s, 3H at C-7 or at C-8); 1.28 (d, *J* = 6,5 Hz, 3H at C-10); 1.34 (t, *J* = 7.1 Hz, 1H at C-5); 1.65 (dd, *J* = 6.4, 3.0 Hz, 1H at C-1); 2.08 (d, *J* = 18.1 Hz, 1H at C-4); 2.43 (dd, *J* = 18.2, 7.6 Hz, 1H at C-4); 4.38 (q, *J* = 6.3 Hz, 1H at C-9); 5.35 (s, 1H at C-3); ^{13}C NMR ($CDCl_3$, 151 MHz): 13.18 (C-7 or C-8); 19.41 (C-6); 21.98 (C-10); 26.33 (C-7 or C-8); 29.23 (C-5); 31.71 (C-4); 36.69 (C-1); 67.27 (C-9); 122.87 (C-3); 148.33 (C-2). Anal. Calcd for $C_{10}H_{16}O$: C, 78.90; H, 10.59. Found: C, 78.82; H, 10.68.

3.6. (+)-(1*R*)-1-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.0]hex-2-en-2-yl)ethyl *p*-nitrobenzoate (*R*,1*S*,5*R*)-6

Alcohol ((+)-1-[(1*S*,5*R*,9*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol) (*R*,1*S*,5*R*)-**4** (0.35 g, 2.3 mmol) was dissolved in anhydrous pyridine (8.0 ml). *p*-Nitrobenzoic chloride (0.48 g, 2.6 mmol) was added to the solution in portions and the mixture was stirred overnight. Next, the reaction was warmed up to 60 °C and stirred for 2 h more. After this time, the mixture was diluted with water (160 ml), a saturated $NaHCO_3$ solution (56 ml) was added, and the product was extracted with diethyl ether. The combined organic layers were washed with a 5% H_2SO_4 solution, then with brine, and dried over anhydrous $MgSO_4$. The solvent was removed by evaporation and the crude product (0.48 g, 1.6 mmol, 69% yield) was purified by crystallization from methanol giving a pure crystalline product (*R*,1*S*,5*R*)-**6**: $[\alpha]_D^{28} = +15.2$ (*c* 1.0, $CHCl_3$); mp = 63–66 °C (methanol); IR (KBr, cm^{-1}): 3112 (m); 3054 (m); 2942 (s); 2906 (s); 1725 (vs); 1606 (m); 1529 (vs); 134 (s); 1272 (vs); 1119 (s); ^1H NMR ($CDCl_3$, 600 MHz): 0.67 (s, 3H at C-7 or at C-8); 0.98 (s, 3H at C-7 or at C-8); 1.30 (t, *J* = 7.1 Hz, 1H at C-5); 1.42 (d, *J* = 6.5 Hz, 3H at C-10), 1.67 (dd, *J* = 6.5, 2.9 Hz, 1H at C-1); 2.04 (d, *J* = 18.5 Hz, 1H at C-4); 2.40 (dd, *J* = 18.4, 7.8 Hz, 1H at C-4); 5.41 (s, 1H at C-3); 5.66 (q, *J* = 6.5 Hz, 1H at C-9); 8.15–8.22 (m, 4H, at C-13 and C-14 and C-16, and C-17); ^{13}C NMR ($CDCl_3$, 151 MHz): 13.17 (C-7 or C-8); 19.16 (C-10); 19.56 (C-6); 26.33 (C-7 or C-8); 29.12 (C-5); 31.84 (C-4); 36.49 (C-1); 71.28 (C-9); 123.54 (C-13 and C-17); 126.23 (C-3); 130.65 (C-14 and C-16); 136.22 (C-12); 143.00 (C-15); 150.49 (C-2); 163.93 (C-11). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.63; H, 6.48; N, 4.76. Crystal data: $C_{17}H_{19}NO_4$, M_r = 301.33, *T* = 299 K, Mo Kα radiation, monoclinic, space group $P2_1$, *a* = 10.2474(19) Å, *b* = 7.4649(9) Å, *c* = 11.480(2) Å, *β* = 114.12(2)°, *V* = 801.5(2) $Å^3$, *Z* = 2, D_c = 1.249 Mg m^{-3}, *R* = 0.0475, *wR* = 0.1077 (for 1524 all data). CCDC 766355.

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