Pheromones 109[+]

(2*S*,4*R*,5*S*)-2,4-Dimethyl-5-hexanolide: Ants of Different Species *Camponotus* Can Distinguish the Absolute Configuration of Their Trail Pheromone

Hans Jürgen Bestmann,* Bernd Liepold, Anita Kress, and Andreas Hofmann[a]

Abstract: The absolute configuration of 2,4-dimethyl-5-hexanolide, which was found on a nanogram-scale in different *Camponotus* species as a trail pheromone, is determined by the combination of synthetic methods with electrophysiological and behavior tests. In five different species the same absolute configuration (2*S*,4*R*,5*S*) **3** was found.

Keywords: electrophysiology · lactones · behavior tests · Wittig reactions

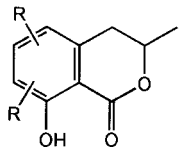
Introduction

It is likely that ants have lived on Earth for more than 100 million years. There are about 9500 known species of ants. Chemical communication plays an important role in the organization of ant populations. When, for example, a new food source is discovered, the successful scout marks the way back to the nest with a trail pheromone. Nestmates follow this odor to the newly discovered resource, a process which leads to the well known ant-streets. The structures of the trail pheromones are only known for about 15–20 ant species.[2]

For some years, we have been working on the structure elucidation and biosynthesis of the trail pheromones of different species of the subfamily Formicinae. We have identified two main active trail-following compounds: dihydroisocumarines (**1**)[3a, d] and chiral δ-lactones **2**[3b, c, e–h] in the rectal bladders of these species. The biosynthesis of **1** and **2** takes the fatty acid pathway with incorporation of acetate and propionate.[4]

Whereas there is only one chiral center in **1**, the absolute configuration of which can be considered as known,[3e, h] there are at least three chiral centers in **2**. Herein we report the determination of the absolute configuration of 2,4-dimethyl-5-hexanolide **3**.

We found hexanolide **3** in five different *Camponotus* species: *C. herculeanus*,[3b] *C. socius*,[3f] *C. ligniperdus*,[3f] *C. vagus*,[3f] *C. pennsylvanicus*,[3f] but we had no evidence whether the trail pheromones have the same absolute configuration in all the rectal bladders of these species. In all these ant species there are only picogram to nanogram amounts of the trail pheromones, therefore it was clear from the beginning of this investigation that the determination of the absolute configuration of **3** would only be possible by a combination of partial enantioselective synthesis and biological tests. We started this investigation with *C. herculeanus*, in which we first identified **3**.[3b] Figure 1 shows all possible stereoisomers of **3**; the heats of formation calculated by the semiempirical AM1 method are shown below the formulae.



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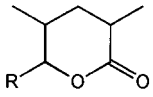

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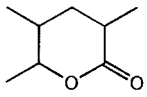

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Results and Discussion

Because of the difficulty of synthesizing all eight stereoisomers of **3** stereospecifically, a concept was developed that

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[+] Part 108: Ref. [1a], Part 107: Ref. [1b].

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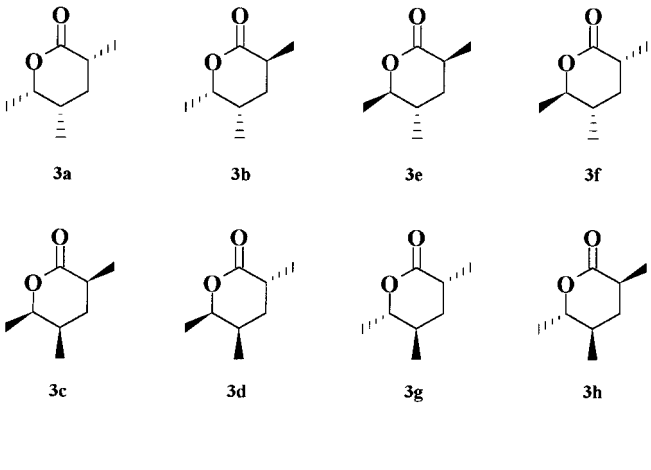


ΔH_f = -110.72 kcal mol^{-1}	ΔH_f = -109.77 kcal mol^{-1}	ΔH_f = -110.69 kcal mol^{-1}	ΔH_f = -111.29 kcal mol^{-1}

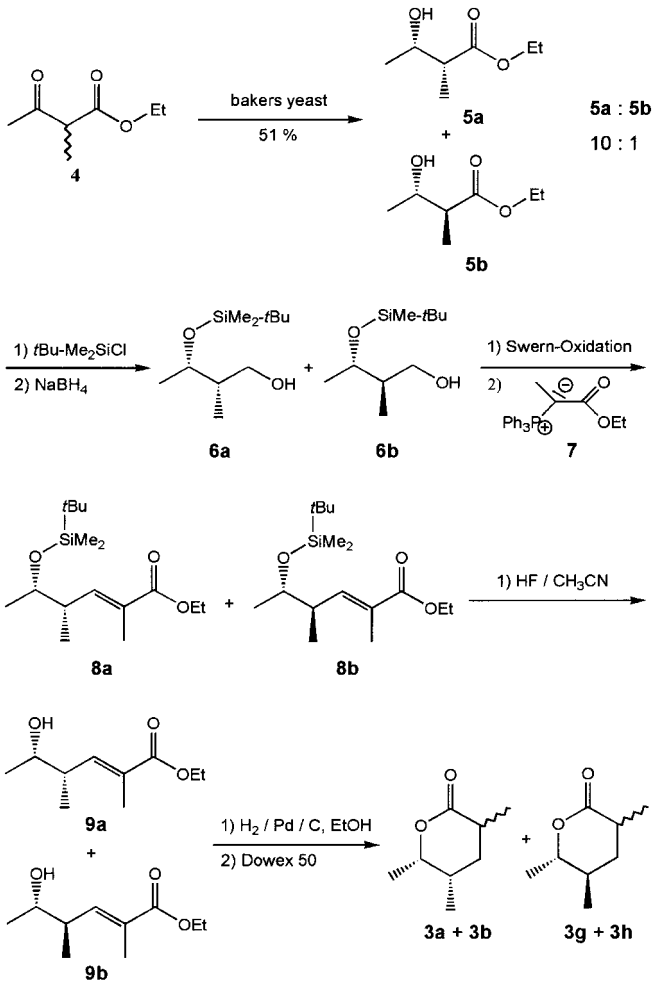
Figure 1. All possible stereoisomers of **3** with heats of formation, calculated by the AM-1 methods.

allowed the stereospecific combination of two stereogenic centers, while the third remained racemic. In this way the number of structures to be synthesized was reduced. We expected an antennogramm test[3a, 5] on the antenna of *C. herculeanus* after the first synthesis to exclude a number of the stereoisomers of **3** (Figure 1) as biologically active compounds.

For the synthesis, we needed a flexible approach that allowed a broad variation in the stereogenic centers. We decided to use ethyl 3-hydroxy-2-methylbutanoate (**5**) as the central building block, as this compound is easily accessible in all stereoisomeric forms and allows the construction of two of the three stereogenic centers stereospecifically in the target compounds. We started with the enzymatic reduction of ethyl 2-methyl-3-oxobutanoate (**4**) with bakers yeast. According to the literature,[6] the (2*R*,3*S*)-*syn*-diastereoisomer of **5a** is formed preferentially. The conditions used led to a 10:1 mixture of **5a** and the *anti*-diastereoisomer **5b** (2*S*,3*S*-*anti*) as determined by GC. This mixture could not be separated preparatively.

Reaction of the mixture **5a**+**5b** with *tert*-butyl-dimethylsilyl chloride followed by a reduction of the ester group with $NaBH_4$ led to **6a** (2*S*,3*R*-*syn*) and **6b** (2*R*,3*R*-*anti*). GC and NMR analysis showed that the ratio did not change during the reaction of the stereoisomers. The next step was a Swern oxidation to the corresponding aldehydes which were treated without purification with (carboethoxyethylidene)triphenylphosphorane **7** to give **8a**+**8b**. The reaction was strongly

Abstract in German: *Die absolute Konfiguration von 2,4-Dimethyl-5-hexanolid* ***3*** *das als Spurpheromon im ng-Maßstab in verschiedenen Camponotus*-Arten gefunden wurde, konnte durch eine Kombination von synthetischen Methoden und die Anwendung von elektrophysiologischen Meßmethoden und Verhaltenstesten aufgeklärt werden. Es zeigte sich, daß die 5 verschiedenen untersuchten Ameisenarten die selbe absolute Konfiguration nämlich 2*S*, 4*R*, 5*S* von **3** als Spurpheromon verwenden.



Scheme 1. Synthesis of **3a**+**3b** and **3g**+**2h.**

E-selective and the ratio of the diastereoisomers **8a**+**8b** remained unchanged. Finally, the silyl protecting group was eliminated with HF in acetonitrile to **9a**+**9b**, followed by double-bond hydrogenation with Pd/C in ethanol. During this reaction we already found partial lactonization to **3**, which could be made complete by changing the solvent to THF and addition of Dowex 50. The total yield of the reaction sequence in which the main product is the mixture of **3a**+**3b** (*syn*) and the by-product **3g**+**3h** (*anti*) is 86%. The GC (Figure 2 lower part) shows that the main fraction **3a**+**3b** could not be separated on an SE54 capillary column, but separation was possible for the *anti* isomers **3g**+**3h**.

The first results with respect to the absolute configuration of **3** could be obtained by coupling the GC with an electroantennogram (EAG) detector, which we have developed continuously over the years.[5] The upper trace in Figure 2 shows the peaks from the biological detector. The mixture **3a**+**3b** showed no biological activity, but one of the two *anti* products **3g**+**3h** was shown to have biological activity. We could thus exclude the *syn* isomers as candidates for the absolute configuration of the natural trail pheromone because they showed no biological activity and their retention times on the GC did not fit those of the natural product. We then had to decide between the *anti* isomers **3e**+**3f** and **3g**+**3h** and at this stage we postulated **3g**+**3h** as candidates for the natural trail

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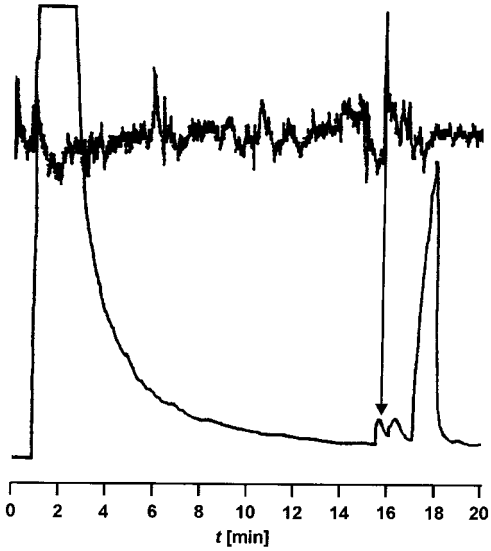
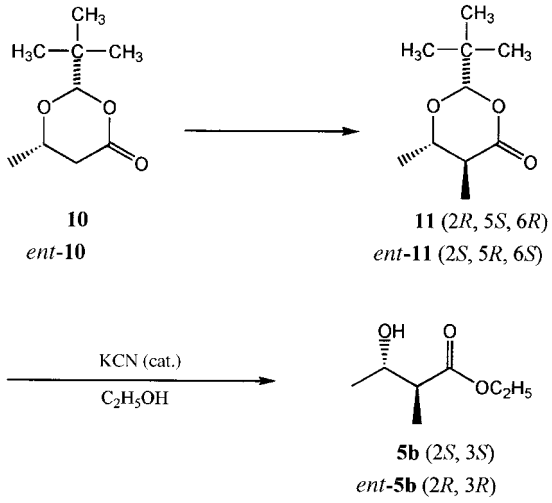


Figure 2. GC-EAD of *Camponotus herculeanus* with the product of the synthesis described in Scheme 1.



Scheme 2. Synthesis of **5b** and *ent*-**5b**.

pheromone because one of the two shows biological activity in the EAG-GC.

For the synthesis of the *anti* isomers of **3**, we started with the enantiomers of ethyl 3-hydroxybutanoate, which were converted to **10** and *ent*-**10** using Seebach's procedure[7] followed by enolate methylation to **11**, which was isolated in an *anti*/*syn* diastereoselectivity of 95:5. The *syn* product could be removed by recrystallization from pentane, which is more effective than the column chromatographic procedure described in the literature.[8] For the transformation of **11** to **5b** and *ent*-**5b** without epimerization, we found the best procedure to be to stir the compound in methanol in the presence of catalytic amounts of KCN at room temperature. The transformation of **5b** into the mixture **3e**+**3f** or **3g**+**3h** is described above.

With the two mixtures **3g**+**3h** and **3e**+**3f** as well as the pure *syn* mixture **3a**+**3b**, which is obtained by column chromatography of the synthesis product described above, comparative antennogram tests were carried out. The results are shown in Figure 3. Clearly the mixture **3g**+**3h** gives the strongest electrophysiological response over the whole range of the stimulus source loading.[5]

EAG curves pointed to a biological activity, but did not give any information about the behavior. We therefore carried out behavior tests with mixtures of the diastereoisomers that had been tested in the EAG as follows. Two connected circles were drawn on thick paper as shown in Figure 4. A fine glass pipette was used to lay synthetic trails of dilute solutions (2 mL at a concentration of $1\,\mu mL^{-1} = 1\,ng\,cm^{-1}$) of diastereomers **3g**+**3h**, **3a**+**3b**, and **3e**+**3f** in pentane. The trails were drawn in semicircles that alternated in their directions. The trail on the horizontal connecting lines consisted of the mixtures

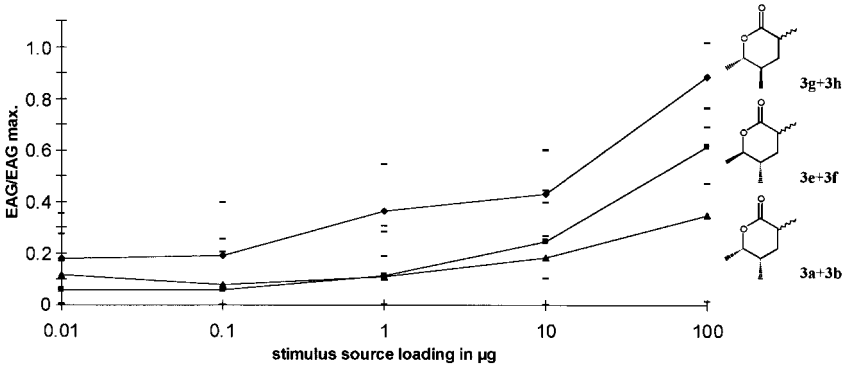


Figure 3. Dose response curve constructed from responses in EAG on the antenna of *Camponotus herculeanus* with the three epimer mixtures **3g**+**3h**, **3e**+**3f**, and **3a**+**3b**.

	3g +3h	3e +3f	3a +3b
Test1	10	0	0
Test2	10	0	0
Test3	10	0	0

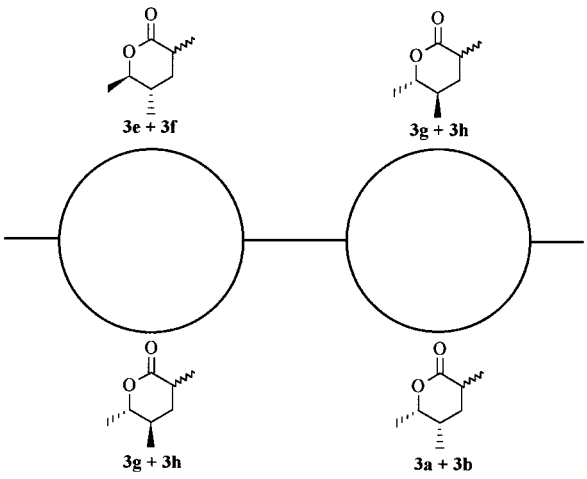


Figure 4. Trail test. A mixture of the C-2 isomers is placed on the horizontal paths, but only one set of epimers in each of the semicircular paths (as shown). Therefore the ants must choose between epimer mixtures at the junction of the semicircular paths.

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3g+**3h**, **3a**+**3b**, and **3e**+**3f**. The ants must choose which trail (that is, which isomer **3g**+**3h**, **3a**+**3b**, or **3e**+**3f**) they want to follow at the connecting points of the horizontal lines and the circles. The paper with the artificial trails was placed on a glass plate supported by wooden blocks in a basin. The ants were placed on a further wooden block and left alone for five hours. The test was started by the connection of the block with the ants with the thick paper containing the test trails using an additional piece of paper, which the ants then used as a bridge to gain access to the synthetic trails. The behavior of the ants on contact with the trail was observed and recorded. Only cases in which the ants followed the entire trail were counted. Ten such runs were counted per test.

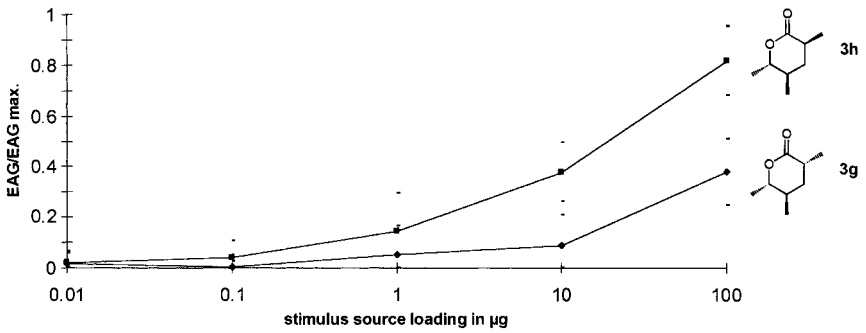


Figure 6. Dose response curves constructed from response in EAGs on the antenna of *Camponotus herculeanus* with separated epimers **3g** and **3h**.

The results are shown in the table of Figure 4. The tests showed an exclusive preference for the epimeric mixture **3g**+**3h**, which confirmed the results of the EAG tests. This leads to the conclusion that either **3g** or **3h** is the natural pheromone. The separation of these two stereoisomers was possible by preparative GC (Fraktovap 2400, Carlo Erba, packed column carbovax).[9] The first isomer eluted showed the following effects in the DIF-NOE-NMR experiment. The NOE-amplification effect between H2 and H4 shows clearly that these protons are situated on the same side of the „ring plane“ in axial positions (Figure 5). Thus the relative stereochemistry of the two methyl groups on C2 and C4 in an equatorial position is evident. This is confirmed by the NOE effect of the two diastereotopic protons on C3. Proton H_e shows NOE amplification to H2 as well as to H4. Proton H_a only exerts an effect to H5, demonstrating the axial position of these two protons and the equatorial position of the methyl group on C5. Because the absolute configuration on the stereogenic centers C4 and C5 is known, the absolute configuration of the first eluted stereoisomer is that of **3h**, that is (2*S*,4*R*,5*S*) and the second compound is then **3g** (2*R*,4*R*,5*S*).

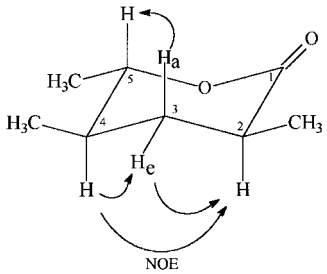


Figure 5. NOE effects on **3h**.

The final decision as to which of the two diastereoisomers is the trail pheromone had to be made by the EAG tests and last but not least by the ant itself, that is by the trail-following test. Figure 6 showed that in the EAG **3h** was more active than **3g**. This was confirmed by the behavior test in which **3h** and **3g** were offered to the ants. Compound **3h** was strongly preferred (Figure 7). In this way the absolute configuration of the trail pheromone of the ant *Camponotus herculeanus* has been determined as (2*S*,4*R*,5*S*)-2,4-dimethyl-5-hexanolide (**3h**) by the ants themselves.

	3g	**3h**
Test1	0	10
Test2	0	10
Test3	0	10

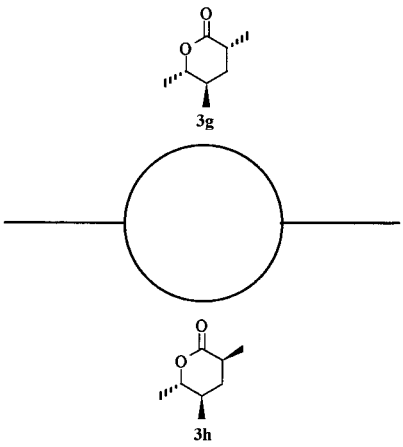


Figure 7. Trail-drive-test in the form of a circle. Both stereoisomers **3g** and **3h** are placed on the horizontal paths, but only one (as shown) with diethyl ether on the upper or lower semicircular paths.

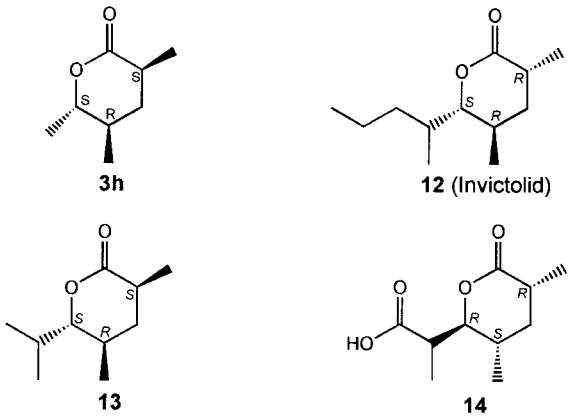
We mentioned above that we found 2,4-dimethyl-5-hexanolide (**3**) not only as a trail pheromone in *C. herculeanus* but also in other *Camponotus* species. The question of the absolute configuration of **3** in these species had not been answered. With the four other species we carried out EAG tests first with the mixtures **3g**+**3h** and **3e**+**3f** as well as control experiments with **3a**+**3b**. In all cases **3g**+**3h** was the most active mixture followed by **3e**+**3f**. Also in the comparative tests between **3g** and **3h** in the EAG **3h** was more active than **3g** for every ant species. Finally, with all these species the trail-following behavior test was conducted. However, with *C. ligniperdus* because of the small number of animals which we had in our hands, and because of their very aggressive and nervous behavior, we could not make a significant statement. In all other cases the ants followed the trail of **3h** (Table 1).

Table 1. Results of circular trail-drive-test for *C. socius*, *C. pennsylvanicus*, and *C. vagus*.

	2*R*,4*R*,5*S* (**3g**)[%]	2*S*,4*S*,5*R* (**3h**)[%]
C. socius	0	100
C. pennsylvanicus	0	100
C. vagus	4	96

These experiments show that the *Camponotus* species *C. herculeanus*, *C. socius*, *C. vagus*, and *C. pennsylvanicus* all use the 2,4-dimethyl-5-hexanolide with the same absolute configuration (**3h**) as a trail pheromone. Because of the clear results in the EAG test, we are sure that we can also include *C. ligniperdus* in this number. A comparison of the heats of formation calculated for the different diastereoisomeric forms of **3** (listed in Figure 1) show that, interestingly, Nature uses the thermodynamically most stable isomer of **3** as a trail pheromone.

The absolute configurations of three analogously substituted δ-lactones have been investigated and should be compared with **3h**: invictolide **12** has been discussed as the queen pheromone of the ant *Solenopsis invicta*,[10] with the absolute configuration shown in the formula.[11] It differs from **3h** in the α-position. We note that a compound **3** was recently detected in the heads of *Lasius niger*.[12] A stereospecific synthesis was described by Mori and the synthesized compound has been correlated with invictolide.[13] We therefore believe that the absolute configuration of this compound was **3g**. [α] value is also consistent with this conclusion (see Experimental Section).



Compound **13** is the sexual pheromone of the wasp *Macrocentrus grandii*, a parasite of the European cornborer *Ostrinia nubilalis* and has the same absolute configuration of all three stereogenic centers as **3h**.[14] None of these three natural products correspond, with respect to the position of the substituents at the lactone ring, to the absolute configuration of the Prelog–Djerassi lactone **14**.[15]

Experimental Section

IR spectra were recorded with Beckmann A1, A3, and A4 instruments. NMR spectra were recorded on JEOL JNMPMX60, JNMPS100, and JNMGX400 spectrometers with tetramethylsilane as internal standard. ^{31}P NMR: 85% H_3PO_4 as external standard. Unless otherwise mentioned, spectra were recorded at 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR), or 162 MHz (^{31}P NMR), solvent $CDCl_3$. Mass spectra were measured with a Varian MATCH4B (EFO-4B source, direct admission, 70 eV). GC-MS were recorded with a Finnigan MAT90 with a Varian 3400, (He 2 mL min^{-1}, injector temperature 220 °C). GC were measured on a Hewlett-Packard GC with a HP 3394 integrator, N_2 2 mL min^{-1} injector temperature 220 °C, and detector temperature 260 °C. The temperature program was 4 min 60 °C, 6 °C min^{-1} to 260 °C, 10 min 260 °C. Elemental analysis were measured with a Heraeus Mikromat CHa. Polarimeter: Schmidt and Haensch-Digital polarimeter.

The reactions were conducted with exclusion of oxygen and moisture. The solvents were dried by standard procedures. M.p. and b.p. were not corrected.

Ethyl (2*R*,3*S*)-3-hydroxy-2-methylbutanoate (**5a**): To a suspension of bakers yeast (100 g) in water (5 L) at 30 °C, sugar (15 g) was added and the mixture stirred for 30 min. With a light stream of air bubbling through the suspension, ethyl 2-methyl-3-oxobutanoate (**4**) (5 g) and sugar (20 g) were added continuously over a period of 24 h, and the temperature was maintained at 30 °C. The suspension was extracted with diethyl ether in a Kutscher–steudel extractor, and the diethyl ether solution was dried over $MgSO_4$. The solvent was evaporated and the residue distilled by Kugelrohr. B.p. 70–80 °C (bath temperature).[16] Yield 2.58 g colorless oil. The product contains about 8% diastereoisomers (GC). $[\alpha]_D = +14$ ($c = 1$, $CHCl_3$), Ref.[16]: $[\alpha]_D = +10$ ($c = 5.6$, MeOH); ^{1}H NMR: $\delta = 1.18$–1.20 (2d, 6H, CH_3), 1.28 (t, 3H, $J = 7.2$ Hz, CH_2-CH_3), 2.48 (m, 1H, CH-2), 2.78 (sh 1H, OH), 3.99 (m, 1H, CH-3), 4.18 (m, 2H, OCH_2); ^{13}C NMR: $\delta = 11.1$, 14.2 (CH_3), 19.8 (C-4), 45.5 (C-2), 60.6 (OCH_2), 68.0 (C-3), 176.0 (C1); MS: *m/z* (%): 131 ([$M^+ - CH_3$]), 103 (21), 102 (79), 85 (20), 74 (100), 56 (33).

Ethyl (2*R*,3*R*)- and (2*S*,3*S*)-3-hydroxybutanoate (5b and *ent*-5b, respectively): To a stirred solution of Dioxanone **10** (prepared according to ref. [8], then recrystallized in petroleum ether) in EtOH was added a catalytic amount of KCN (<5 mg). After 20 min the solvent was evaporated. The residue was taken up with diethyl ether (20 mL), washed with water (5 mL), and dried over $MgSO_4$. After removal of the solvent in vacuo, the crude product was purified by chromatography on silica gel with diethyl ether to give **5b** or *ent*-**5b**. Colorless oil **5b**. $[\alpha]_D^{20} = +24$ ($c = 1.05$, $CHCl_3$); *ent*-**5b**: $[\alpha]_D^{20} = -30$ ($c = 1.9$, $CHCl_3$); ^{1}H NMR: $\delta = 1.18$ (d, 3H, $J = 7.7$ Hz, CH_3), 1.22 (d, 3H, $J = 6.1$ Hz, CH_3), 1.28 (t, 3H, $J = 7.2$ Hz, CH_3), 2.45 (dd, 1H, CH), 2.88 (d, 1H, $J = 5.5$ Hz, OH), 3.89 (m, 1H, CH), 4.18 (q, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR: $\delta = 14.1$, 14.2, 20.7, 47.0, 60.6, 69.4, 176.0; MS: *m/z* (%): 131([$M^+ - CH_3$]); $C_7H_{14}O_3$ (146.2): calcd C 57.51, H 9.65; found C 57.24, H 9.68.

(2*S*,3*R*)- and (2*R*,3*S*)-3-[(-*O*-*tert*-Butyldimethylsilyl)oxy]-2-methylbutan-1-ol (6b and *ent*-6b, respectively): To a solution of imidazole (940 mg, 13.8 mmol) in DMF was added *tert*-butyldimethylsilyl chloride (1.1 g, 6.9 mmol). After 20 min a solution of **5a** (800 mg, 5.5 mmol) in DMF (2 mL) was added and stirring was continued for 24 h at room temperature. The mixture was quenched with a dilute aqueous solution of NaCl, extracted three times with diethyl ether, and dried over $MgSO_4$. The solvent was removed in vacuo and the remaining oil passed through silica (15 g) and eluted with pentane/AcOEt (5:1) to yield the silylated hydroxy ester, which was taken up in toluene and added to a suspension of $LiBH_4$ (150 mg, 6.9 mmol) in diethyl ether (10 mL). After 5 h the temperature was gradually raised and the solvents removed under reduced pressure. The solid residue was hydrolyzed with dilute aqueous HCl until a pH of 6 was reached, then the solution was saturated with K_2CO_3 and extracted three times with diethyl ether. After drying over $MgSO_4$, the solvent was removed in vacuo and the crude product purified by chromatography (petroleum ether/ethyl acetate (5/1)). Yield 740 mg (88%) colorless oil. **6b**: $[\alpha]_D^{20} = +23$ ($c = 1.0$, $CHCl_3$), Ref.[17]: $[\alpha]_D = -25$ ($c = 0.2$, $CHCl_3$); *ent*-**6b**: $[\alpha]_D^{20} = -25$ ($c = 1.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.07$, 0.08 (2s, 6H, CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.97 (d, 3H, $J = 7$ Hz, CH_3), 1.22 (d, 3H, $J = 6$ Hz, CH_3), 1.60 (m, 1H, CH), 2.91 (s, 1H, OH), 3.55 (m, 1H, CH), 3.80 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): $\delta = -4.2$, 1.0, 14.6, 17.9, 22.2, 26.0, 41.7, 65.9, 74.1; IR (film): $\nu =$ 3400, 2980, 2950, 2910, 2880, 1465, 1260, 1105, 1075, 1030, 830, 770, 725 cm^{-1}; MS: *m/z* (%): 203 (<1) [$M^+ - CH_3$], 189 (14), 161 (32) [$M^+ - C_4H_9$], 159 (21), 147 (41), 119 (22), 75 (100), 73 (42), 28 (64); $C_{11}H_{26}O_2Si$ (218.4): calcd C 60.49, H 12.0; found C 60.28, H 11.89.

(2*S*,3*S*)-3-[(*O*-*tert*-Butyldimethylsilyl)oxy]-2-methylbutan-1-ol (6a): Compound **6a** was synthesized according to the procedure for **6b** or *ent*-**6b**

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starting from **5b** or *ent*-**5b**. Colorless oil, contains **5a**, 8% enantiomers. $[\alpha]_D = +14$ ($c = 1.0$, $CHCl_3$); ^{13}C NMR: $\delta = -4.5$, 1.0, 12.3, 17.9, 18.3, 25.8, 40.8, 65.6, 72.2; MS: *m/z* (%): 203 ($[M^+ - CH_3]$, <1%); $C_{11}H_{26}O_2Si$ (218.4): calcd C 60.49, H 12.0; found C 60.22, H 12.15.

Ethyl (4*S*,5*R*)- and (4*R*,5*S*)-5-[(*O*-*tert*-butyldimethylsilyl)oxy]-2,4-dimethylhex-2-enoate (8b and *ent*-8b, respectively): To a stirred solution of oxalyl chloride (0.43 mL, 5 mmol) in CH_2Cl_2 (25 mL) at $-78\,^\circ C$ was added a solution of DMSO (0.53 mL, 7.5 mmol) in CH_2Cl_2 (8 mL). After 10 min a solution of **6a** (520 mg, 2.39 mmol) in CH_2Cl_2 (10 mL) was added and the mixture was stirred for 30 min with the temperature maintained at $-78\,^\circ C$. Triethylamine (3 mL, 21 mmol) was added slowly and then the mixture was allowed to warm up to $0\,^\circ C$. (Carboethoxyethylene)triphenylphosphorane (**7**) (1.04 g, 2.9 mmol) was added, and the mixture was stirred and heated under reflux overnight. The solvent was evaporated in vacuo and the residue diluted with diethyl ether, hydrolyzed with water, and extracted with diethyl ether. After drying over $MgSO_4$ and concentrating in vacuo, the product was purified by chromatography on silica gel with pentane/ethyl acetate (5/1) to yield 510 mg (71%) of **8b** as a colorless oil. **8b**. $[\alpha]_D^{20} = +18$ ($c = 0.5$, $CHCl_3$); *ent*-**8b**: $[\alpha]_D^{20} = -19$ ($c = 1.1$, $CHCl_3$); ^1H NMR: $\alpha = 0.01$ (s, 3H, CH_3), 0.04 (s, 3H, CH_3), 0.84 (s, 9H, $C(CH_3)_3$), 0.95 (d, 3H, $J = 6.8$ Hz, CH_3), 1.06 (d, 3H, $J = 6.1$ Hz, CH_3), 1.26 (t, 3H, $J = 7.1$ Hz, CH_3), 1.81 (s, 3H, CH_3), 2.47 (m, 1H, CH), 3.68 (m, 1H, CH), 4.15 (m, 2H, CH_2), 6.65 (d, 1H, $J = 10.3$ Hz, CH); ^{13}C NMR ($CDCl_3$): $\alpha = -4.8$, -4.3, 12.6, 14.3, 16.0, 18.0, 21.4, 25.8, 41.1, 60.3, 71.7, 127.4, 145.0, 168.4; IR (film): $\tilde{\nu} =$ 2960, 2930, 2900, 2860, 1710, 1650, 1455, 1370, 1240, 1080, 1025, 820, 760 cm^{-1}; MS: *m/z* (%): 185 (1) $[M^+ - CH_3]$, 256 (4), 243 $[M^+ - C_4H_9]$, 159 (94), 147 (100), 115 (21), 103 (50), 73 (93); $C_{16}H_{32}O_3Si$ (300.51): calcd C 63.95, H 10.73; found C 63.79, H 10.86.

Ethyl (4*S*,5*S*)-5-[(*O*-*tert*-butyldimethylsilyl)oxy]-2,4-dimethylhex-2-enoate (8a): Compound **8a** was synthesized according to the procedure described for **8b** and *ent*-**8b** starting from **6a**, colorless oil, contains about 8% enantiomers. $[\alpha]_D = -140$ ($c = 1.2$, $CHCl_3$); ^{13}C NMR: $\delta = -4.9$, -4.3, 12.7, 14.3, 15.3, 18.1, 21.9, 25.9, 41.3, 60.1, 71.6, 127.0, 145.0, 168.4; MS: *m/z* (%): 285 ($[M^+ - CH_3]$); $C_{16}H_{32}O_5Si$ (300.5): calcd C 63.95, H 10.73; found C 63.64, H 10.97.

Ethyl (4*S*,5*R*)- and (4*R*,5*S*)-5-hydroxy-2,4-dimethylhex-2-enoate (9b and *ent*-9b respectively): To a solution of **8b** or *ent*-**8b** (45 mg, 1.5 mmol) in CH_3CN was added HF (2 mL, 50% aqueous solution). After stirring for 2 h at room temperature, the mixture was diluted with diethyl ether (80 mL), extracted twice with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The solvent was evaporated in vacuo and the crude product purified by chromatography on silica gel with pentane/ethyl acetate (5/1) $\rightarrow$(1/1) to yield 250 mg (89%) of **9b**. Colorless oil. **9b**: $[\alpha]_D^{20} = +34$ ($c = 1.05$, $CHCl_3$); *ent*-**9b** $[\alpha]_D = -35$ ($c = 1.05$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 1.03$ (d, 3H, $J = 6.6$ Hz, CH_3), 1.20 (d, 3H, $J = 6.4$ Hz, CH_3), 1.30 (t, 3H, $J = 7.1$ Hz, CH_3), 1.78 (br s, 1H, OH), 1.88 (s, 3H, CH_3), 2.53 (m, 1H, CH), 3.70 (m, 1H, CH), 4.20 (q, 2H, $J = 7.2$ Hz, CH_2), 6.66 (d, 1H, $J = 10$ Hz, CH); ^{13}C NMR ($CDCl_3$): $\delta = 12.7$, 14.2, 16.0, 20.6, 40.9, 60.5, 71.3, 129.0, 143.5, 168.7; IR (film): $\tilde{\nu} = 3440$, 2980, 1710, 1645, 1290, 1260, 1180, 1100, 760 cm^{-1}; MS: *m/z* (%): 171 (<1) $[M^+ - CH_3]$, 142 (100), 114 (47), 96 (60), 69 (40), 45 (22); $C_{10}H_{18}O_3$: calcd C 64.49, H 9.74; found C 64.57, H 9.82.

Ethyl (4*S*,5*S*)-5-hydroxy-2,4-dimethylhex-2-enoate (9a): Compound **9a** was synthesized according to the procedure described for **9b** and *ent*-**9b** starting from **8a**. Colorless oil contains 8% of enantiomers. $[\alpha]_D^{20} = -21$ ($c = 1.0$, $CHCl_3$); ^{13}C NMR: $\delta = 12.8$, 14.3, 15.7, 21.1, 40.9, 60.6, 71.4, 128.0, 143.6, 168.3.

2,4-Dimethyl-5-hexanolide: A solution of compound **9** (100 mg) (different mixture of epimers) in diethyl ether was hydrogenated with Pd/C/10% catalyst. Dowex 50 was added during this procedure and stirred for 3 h. The progress of the hydrogenation was monitored by TLC. If the lactonization after this time was not finished (GC monitor) the solvent was distilled and the residue dissolved in THF (15 mL), 10 mg Dowex 50 was then added, and this suspension stirred for 30 min. The solvent was evaporated and the residue chromatographed on silica gel in ether. Starting from **9b**, **3g**+**3h** were obtained. The stereoisomers can be separated by preparative GC. (Fractowap 2400, Carlo Erba). Column CV 74 Carbovax 20M, N_2 1.6 bar, injection temperature $240\,^\circ C$, detector temperature $225\,^\circ C$. Compound **3h** had a retention time of 44 min, and **3g** had a retention time of 46 min.

3h: Retention time analytic 13.07 min. $[\alpha]_D = -23$ ($c = 0.175$, $CHCl_3$); ^1H NMR: $\delta = 0.92$ (d, 3H, $J = 6.6$ Hz, H_3CC-4), 1.21 (d, 3H, $J = 7.1$ Hz, H_3CC-2), 1.27 (m, 1H, CH_a-3), 1.29 (d, 3H, $J = 6.4$ Hz, H_3CC-5), 1.63 (m, 1H, CH-4), 1.84 (ddd, 1H, CH_3-3), 2.45 (m, 1H, CH-2), 3.97 (dd, 1H, CH-5); ^{13}C NMR: $\delta = 17.2$, 17.3, 20.1, 35.8, 36.3, 37.6, 83.6, 174.4; IR (film): $\tilde{\nu} =$ 2980, 2940, 2890, 1730, 1460, 1380, 1250, 1220 cm^{-1}; MS: *m/z* (%): 142 ($[M^+]$) 127 ($[M^+ - CH_3]$), 100, 98 ($[M^+ - CO_2]$, 18), 70 (14), 56 (100); $C_8H_{14}O_2$ (142.2): calcd C 67.57, H 9.92; found C 67.39, H 9.98.

3g: Retention time analytic 13.28 min. $[\alpha]_D = -83$ ($c = 0.2$, $CHCl_3$), Ref.[13]: $[\alpha]_D = -87.1$ ($c = 0.2$, $CHCl_3$); ^1H NMR: $\delta = 0.94$ (d, 3H, $J = 6.4$ Hz, H_3CC-4), 1.15 (d, 3H, $J = 6.8$ Hz, H_3CC-2), 1.23 (m, 1H, CH_2-3), 1.28 (d, 3H, $J =$ 6.1 Hz, H_3CC-5), 1.54–1.74 (m, 12, CH_2-3, CH-4), 2.58 (m, 1H, CH-2), 4.0 (m, 1H, CH-5); ^{13}C NMR: $\delta = 16.4$, 17.0, 19.4, 32.6, 33.4, 35.2, 83.7, 176.4; $C_8H_{14}O_2$: calcd C 67.57, H 9.92; found C 67.63, H 9.99.

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