Pergamon

Tetrahedron, Vol. 53, No. 1, pp. 145-160, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)00976-3

Chemoenzymatic Synthesis of Marine Brown Algae Pheromones

Jacques Lebreton, Véronique Alphand and Roland Furstoss*

*Groupe "Biocatalyse et Chimie Fine"**, *ERS 157 associée au CNRS, Faculté des Sciences de Luminy, Case 901, 163 av. de Luminy, F-13288 Marseille Cedex 9, France*

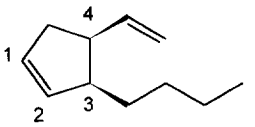
Abstract: (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1**, (+)-(*Z*)-(3*S*,4*S*)-Multifidene **2**, (+)-(*E*)-(3*S*,4*S*)-Multifidene **3**, (+)-(3*R*,4*S*)-Viridiene **4** and (+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene **5**, constituents of various brown algae pheromones, were synthesized from racemic bicycloheptenone **7** *via* a novel microbiological Baeyer-Villiger oxidation performed using the fungus *Cunninghamella echinulata*. The total synthesis of these pheromone constituents was achieved by using, as a key step, a one-pot Swern oxidation and a Wittig or Julia-Lythogoe olefination in order to perform the stereocontrolled construction of the C-3 (E) or (Z) double bond located on the side chain.

INTRODUCTION

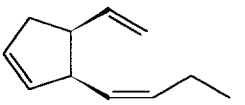
Brown algae are living in the coastal zones of all continents and use for their reproduction a unique and fascinating system of communication.[1] Thus, during the sexual reproduction, female and male plants release motile unicellular gametes into the surrounding sea water. Female cells secrete a characteristic and complex mixture of olefinic hydrocarbons to attract their corresponding androgametes in the vicinity. As soon as one male gamete has fused with the female, the zygote formed looses its attraction for the other male gametes. In general, this pheromonal bouquet is composed of one major product as a specific pheromone, accompanied by a few minor constituents in the range of 1 to 15%. Most of the isolated pheromones to date are acyclic or cyclic highly volatile and hydrophobic hydrocarbons.[2] Thus, (+)-(Z)-(3*S*,4*S*)-Multifidene **2** is the major and most active pheromone of the algae *Cutleria multifida* and *Chorda tomentosa*,[3] (+)-(3*R*,4*S*)-Viridiene **4** was isolated as a major component in the algae *Desmarestia aculeata* and *D. viridis*,[4] (+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene **5** is the major pheromone found in *Perithalia caudata*[5] whereas (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1** and (+)-(E)-(3*S*,4*S*)-Multifidene **3** were isolated as minor components in *Dictyopteris acrostichoides*.[2] (Scheme 1).

** Fax : +(33) 91 26 91 45, E-mail : furstoss@lac.gulliver.fr

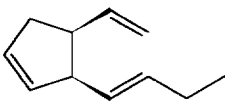
SCHEME 1



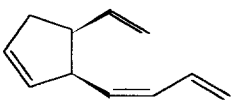
(+)-(3S,4S)-3-*n*-butyl-4-vinylcyclopent-1-ene **1**



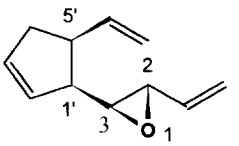
(+)-(Z)-(3S,4S)-Multifidene **2**



(+)-(E)-(3S,4S)-Multifidene **3**



(+)-(3*R*,4*S*)-Viridiene **4**



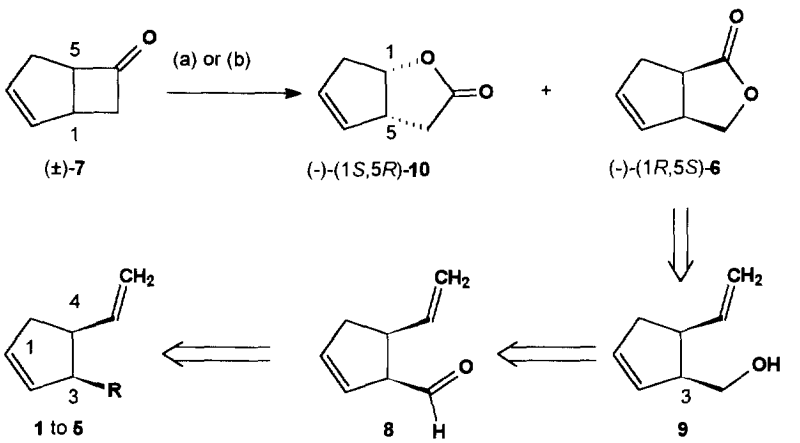
(+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene **5**

The biosyntheses of these compounds have been studied by Boland and coll., who have suggested that α-linolenic acid (eicosa-5,8,11,14,17-pentaenoic acid), present in the phospholipids of the female gamete plasma membrane, seems to be the precursor of all C_{11} hydrocarbons in brown algae.[6] The total synthesis of these compounds, either in racemic or optically active form, have been achieved by several groups.[7-11] However, these syntheses imply quite lengthy reaction schemes. As part of our ongoing effort to combine chemical synthesis and enzymatic transformations for stereoselective synthesis of natural products and biological active substances[12], we have recently focused our attention on the synthesis of these various compounds in enantiomerically pure form and report here our results.[13]

RESULTS

All these pheromones were synthesized starting from a single enantiopure building block, i.e. the (-)-(1*R*,5*S*) lactone **6** (Scheme 2). This was prepared using enantioselective Baeyer-Villiger oxidation of the commercially available racemic bicyclo[3.2.0]hept-2-en-6-one **7**. As illustrated, the most direct route to reach our targets from lactone **6** was to use aldehyde **8** as a key intermediate to achieve, using either Wittig or Julia-Lythogoe olefination, the stereocontroled construction of the (E) or (Z) double bond implied in the C-3 side chain of these molecule. However, two different reports from the literature mentioned the failure to use aldehyde **8** without isomerization (to its conjugated isomer or its epimer) in the presence of either stabilized or non-stabilized phosphonium ylides.[8a,10a] In order to overcome this drawback, we decided to prepare this unstable aldehyde at low temperature and to trap it *in situ* with different reagents. The major problem raised by this approach was to find a mild oxidation method - allowing to prepare aldehyde **8** from **9** - which would also be compatible with the reagent used to build up, in the second step, the C-3 side chain of the target.

SCHEME 2



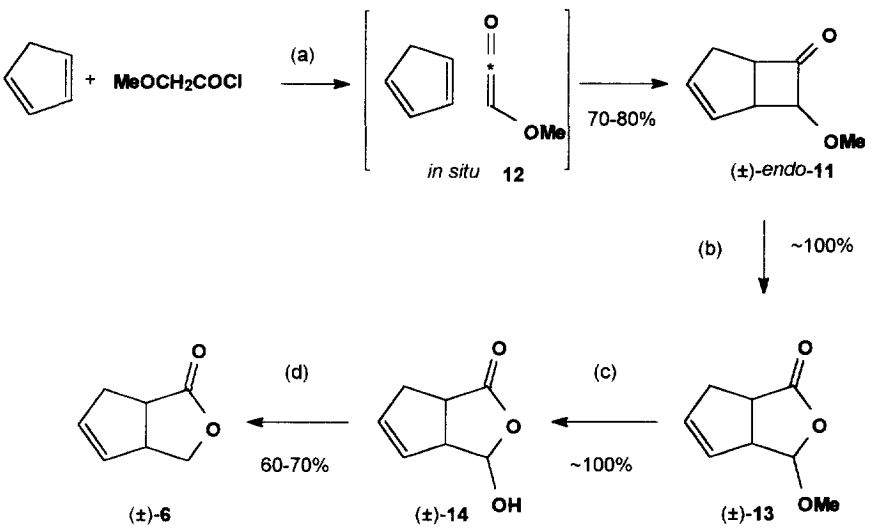
Conditions: (a) Culture of *Acinetobacter*. (b) Culture of *C. echinulata*.

Preparation of racemic and optically active lactone 6.

We have previously described that oxidation of ketone **7**, using an *Acinetobacter* strain, led to an enantio- and regioselective differentiation of the enantiomers of this racemic ketone, leading - in an enantiodivergent fashion - to (-)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-ene-2-one **6** on the one hand and to its (-)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-ene-3-one isomer **10** on the other hand. Both these products were thus obtained in a state of high enantiomeric purity (i.e. ee > 95% for **6**; ee > 95% for **10**).[14] However, this approach necessitates the chromatographic separation of the two lactones, which may be cumbersome when used on large scale quantities. We recently have discovered that this problem could be overcome by performing this Baeyer-Villiger oxidation using the fungus *Cunninghamella echinulata* NRRL 3655 which leads, in 30-35% yield, to one major product, i.e. the enantiopure (ee ≥ 95%) (-)-(1*R*,5*S*)-**6** enantiomer. This approach was thus used further on in order to prepare several gram-scale quantities of this starting material. However, in order to investigate the following synthetic steps, and to save this enantiopure building block, we decided to set up a preparative approach to racemic lactone **6**. To the best of our knowledge, only two syntheses of this racemic material have been previously reported.[15] However we decided to develop a new and more efficient method for the preparation of racemic **6**. Thus, as presented in Scheme 3, we prepared this lactone *via* the α-methoxy substitued bicyclic ketone **11**, which could itself be synthesized using a [2+2] cycloaddition between cyclopentadiene and α-methoxyketene **12**.[16] The choice of this α-substituted ketone intermediate **11** was based on the fact that its chemical Baeyer-Villiger oxidation would lead to incorporation of the oxygen atom into the C(6)-C(7) electron deficient bond, thus leading to the desired precursor of lactone **6**.[17] It is to emphasize that this [2+2]

cycloaddition has been described to only afford a 5-10% yield of the desired cycloaddition product **11**.[18] In order to improve this yield, we carried out an extensive study in order to determine the optimum experimental conditions. We thus found that (a) the reaction mixture had to be refluxed for a short period of time (30-40 minutes) before work-up and (b) that the starting methoxyacetyl chloride had to be freshly distilled just before use. Using these precautions, ketone **11** was obtained in 70-80% yield after work-up and distillation. Only traces of the *exo* cycloadduct were detected (^{1}H NMR) in the crude reaction mixture.

SCHEME 3



Conditions (a) 5.5 eq. cyclopentadiene, 1.0 eq. methoxyacetyl chloride, CH_2Cl_2, -78°C then 1.1 eq., Et_3N, -78°C to rt, 12h; 1h reflux. (b) 1.0 eq., m-CPBA, 2.0 eq., $NaHCO_3$, rt, 2-3h. (c) Cat.: concentrated HCl, THF/H_2O (3/5), rt, 3 days. (d) 1.0 eq. $NaBH_4$, EtOH, rt, 3h.

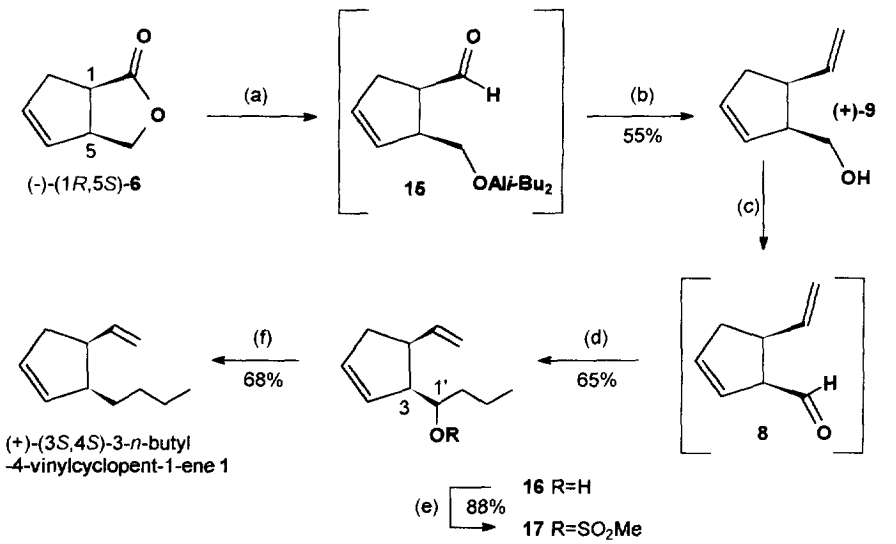
As expected, treatment of ketone **11** with *meta*-chloroperbenzoic acid (m-CPBA) in the presence of sodium hydrogenocarbonate (CH_2Cl_2; rt) yielded, in an essentially quantitative yield, the desired α-methoxylactone **13** as a single isomer. No trace of any by-product, i.e. neither regioisomeric lactone nor epoxide (resulting from oxidation of the double bond) was formed. Crude **13** could thus be used without purification for hydrolysis (concentrated HCl in THF/H_2O) which afforded **14** in high yield. Finally, reduction of this crude product with sodium borohydride in ethanol at rt led to racemic **6**, which could be purified by simple distillation. It is to emphasize that this methodology allowed us to prepare 10-20 gram-scale quantities of racemic **6**, without using any tedious (and yield lowering) chromatographic separation at any synthetic step. This afforded **6** in only four steps from commercial material, with an overall 50% preparative yield.

Synthesis of the target molecules.

1) Synthesis of (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene 1

According to our strategy, the key-building block of our syntheses - i.e. lactone **6** (either in its racemic form for exploratory studies or in enantiopure form) - had to be transformed into the corresponding alcohol **9**, the real precursor of aldehyde **8**. This was achieved by reduction of **6** with diisobutylaluminium hydride (DIBAL-H) in toluene at low temperature, followed by *in situ* condensation of the intermediate aldehyde **15** with triphenyl(methylidene)phosphorane (formed from the phosphonium bromide using BuLi as a base) (scheme 4). This afforded **9** in an acceptable yield (50 to 55%).[19] Attempts to improve this yield by changing either the nature of the base used to form the ylide [*t*BuOK, sodium bis(trimethylsilyl)-amide (NaHMDS)] and/or the solvent used in the reduction step (THF instead of toluene) were unsuccessful. Nevertheless, ^{1}H NMR analysis of the crude product indicated that no by-product was formed in this step. Thus, it seems reasonable to consider that this moderate yield is essentially due to the high volatility of the hydroxyolefin **9**. At this stage, an efficient method allowing for oxidation of **9** into the sensitive aldehyde **8** (without risk of migration of the endocyclic

SCHEME 4



Conditions (a) 1.3 eq. DIBAL-H, Tol., -78°C, 1h. (b) 2.3 eq. $Ph_3P{=}CH_2$, THF, -78°C to rt, 12h. (c) 1.3 eq. $(ClCO)_2$, 2.6 eq. DMSO, THF, -78°C, 3h, then 6.1 eq. Et_3N, -78° to 0°C, 1h. (d) ~3.6 eq. PrMgBr, -78°C to rt, 12h. (e) 1.3. eq. Et_3N, 1.2 eq. MsCl, cat. DMAP, CH_2Cl_2, 0°C to rt, 2h. (f) 1.7 eq. $LiAlH_4$, THF, rt.

double bond and/or epimerization at C-3) was needed. For this purpose, the well-known Swern oxidation[20] was particularly attractive since it can be performed in THF, thus offering the possibility to trap the intermediate aldehyde *in situ* with an appropriate reagent. Depending on the nature of this reagent, the synthesis could thus be directed towards the specific targets of our syntheses.

As underlined previously, our first target was (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1**. Thus, **9** was oxidized in THF using a typical Swern oxidation procedure and the resulting solution of **8** was treated at -78°C by slow addition of a 3 M solution of *n*PrMgBr which led to **16** in 65% yield. This was shown by GC and ^{1}H NMR to be a 85 to 15 mixture of epimers at C-1' (Scheme 4). The ^{1}H NMR spectrum of **16** exhibited two sets of signals characteristic for the two olefinic protons of the cycle, a proof that the endocyclic double bond stayed in place in both cases. On the other hand, the *cis*-stereochemistry of the substituents - and therefore the absolute configuration at C-3 - was proven by the conversion of **16** into (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1**. It is to emphasize that this satisfactory result indicated that aldehyde **8** was stable in the presence of a weakly basic, nucleophilic and coordinating reagent, and should allow further syntheses using other similar types of reagents.

The mixture of alcohols **16**, upon exposure to methanesulfonyl chloride in presence of triethylamine and a catalytic amount of 4-dimethylaminopyridine (DMAP), gave the corresponding mesylates **17** which were treated with lithium aluminium hydride. GC and ^{1}H NMR analysis of the crude product indicated that no epimerization and/or isomerization took place during this set of transformations and that **1** was more than 98% stereochemically pure. After purification by thin layer chromatography, we were gratified to isolate (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1** as a single isomer in 55% yield (2 mmol scale). The spectral ^{1}H and ^{13}C NMR data for (+)-**1** were identical with those previously reported[2,7b] and its enantiomeric purity was checked by chiral GC analysis which indicated an ee value of 98%.

2) Synthesis of (+)-(Z)-(3*S*,4*S*)-Multifidene 2

The approach used for the side chain construction of (+)-(Z)-(3*S*,4*S*)-Multifidene **2** relies on a Wittig olefination, as described in Scheme 5. However, although the one pot (two-steps) Swern-Wittig condensation has been largely used with stabilized ylides (*vide supra*), only one example has been reported to our knowledge with non-stabilized intermediates.[21] Also, it was clear that elaboration of the C-3 chain using a strongly basic and non-coordinating reagent, like a non-stabilized ylide, might promote decomposition of aldehyde **8**, as described in the literature.[8e,10a] However, we were pleased to find out that, upon quenching the solution of **8** at -78°C by slow addition of an excess triphenyl(propylidene)phosphorane solution [formed from the phosphonium bromide using sodium bis(trimethylsilyl)-amide (NaHMDS) as a base] we isolated Multifidene **2** as a single isomer in 74% yield. This product was shown by GC analysis to be more than 98% pure, indicating that very high (Z) side-chain selectivity did occur (some attempts made to perform the same transformation in similar conditions using the

Dess-Martin reagent[23] as oxidant led to a 7:3 mixture of **2** and of its C-3 epimer in 50-60% yield.[24]). Once again, no epimerization and/or isomerization of **8** did occur during this transformation. The spectral ^{1}H and ^{13}C NMR data for **2** were identical with those previously reported.[8] These experiments were conducted on a 2 mmol scale using enantiopure **6** as starting material. This afforded (+)-(*Z*)-(3*S*,4*S*)-**2**, which enantiomeric purity was checked by chiral GC analysis indicating an ee value of 98%. Its absolute configuration, which results from the absolute configuration of the starting lactone chiron, was ascertained by the positive sign of its optical rotation.[22]

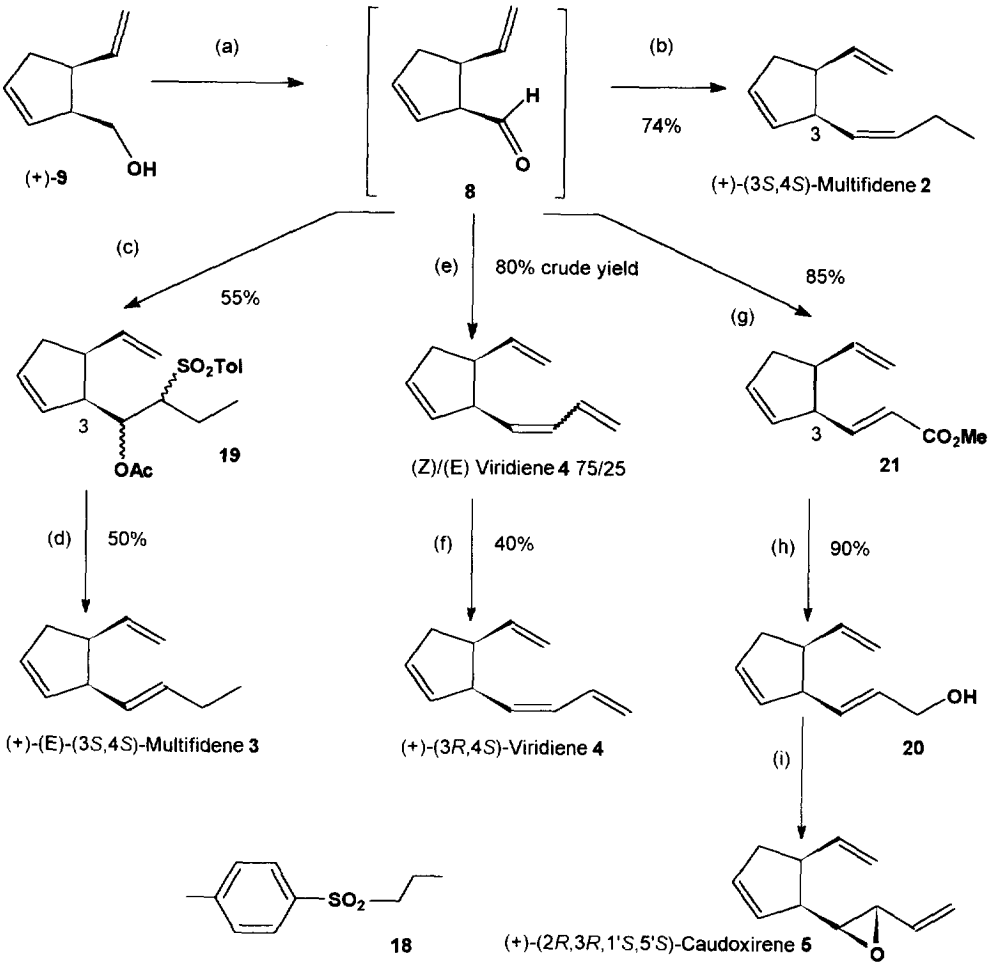
3) Synthesis of (+)-(E)-(3*S*,4*S*)-Multifidene 3

Owing to our preceeding success using the Wittig reaction, the most attractive option to synthesize **3** was based upon the Julia-Lythogoe methodology to build up the (E) side chain selectively.[25] The α-sulfonyl carbanion was thus reacted with an aldehyde or ketone, and the intermediate adduct was quenched *in situ* with acetic anhydride. Independently to the *threo* and *erythro* stereochemistry of the β-acetoxy sulfone intermediate, the reductive elimination produced predominantly the corresponding (E) olefins. The required alkyl sulfone **18** was prepared from *n*-propyl bromide and sodium *p*-tolyl sulphinate salt in high yield.[25b] The α-sulfonyl carbanion of **18**, generated by addition of *n*BuLi, was slowly added to a solution of **8** at -78°C (Scheme 5). After warming the reaction mixture to rt overnight, an excess of acetic anhydride was added at 20°C leading, in 50-60% isolated yield, to the corresponding β-acetoxy sulfone **19** as a mixture of *threo* and *erythro* diastereomers. Finally, treatment of this mixture with sodium amalgam in methanol at -20°C provided, after purification, (E)-Multifidene **3** in 50% yield as a 95:5 mixture with its (Z) isomer **2** (GC analysis ratio). The spectral ^{1}H NMR data of (+)-**3** were in excellent agreement with those previously described [9] and its enantiomeric purity was shown by chiral GC analysis to be higher than 97%.

4) Synthesis of (+)-(3*R*,4*S*)-Viridiene 4

A similar Swern/Wittig sequence was applied to the synthesis of the natural enantiomer of (+)-(3*R*,4*S*)-Viridiene **4** *via* the olefinic alcohol **9**, obtained from enantiopure (-)-(1*R*,5*S*)-**6**. Thus, a triphenyl(propenylidene)phosphorane solution was slowly added to the aldehyde solution at -100°C.[26] After work-up, this afforded a 75/25 mixture of the (Z) and (E)-isomers of **4** in 70-80% crude yield. Purification of this mixture of isomers was easily accomplished using 4-phenyl-2,3,4-triazoline-3,5-dione as selective dienophile which reacted exclusively with the (E) isomer.[27] Thus, stereochemically pure (+)-(3*R*,4*S*)-Viridiene **4** was obtained in 40% yield. The spectral ^{1}H NMR data of (+)-**4** were in good agreement with those previously reported[10] and its enantiomeric purity was shown by chiral GC analysis to be higher than 98%.

SCHEME 5



Conditions: (a) 1.3 eq. $(ClCO)_2$, 2.3 eq. DMSO, THF, -78°C, 3h, then 3.6 eq. Et_3N, -78° to 0°C, 1h. (b) ~8 eq. Ph_3P=CH-Et, -78°C to rt, 12h.(c) ~1.9 eq. Lithium α-sulfonyl carbanion of **18**, -78°C to rt, 12h, then at -20°C ~4.0 eq. Ac_2O, -20°C to rt, 3h. (d) 5% Na(Hg), NaH_2PO_4, MeOH, -20°C, 1-2 h. (e) ~7.8 eq. Ph_3P=CH-CH=CH_2, -100°C to rt, 12h. (f) 4-phenyl-2,3,4-triazoline-3,5-dione, THF, rt, 5 min. (g) ~3 eq. Ph_3P=CH-CO_2Me, -78°C to rt, 12h. (h) 2.5 eq. DIBAL-H, CH_2Cl_2, -78°C to rt, 2h. (i) See ref. 11a.

5) Synthesis of (+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene 5

As a last target, we turned our attention to the allylic alcohol **20**, which was used by Boland and coll. in the total synthesis of (+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene **5**. The addition of methyl (triphenylphosphoranylidene) acetate (Ph_3P=CH-CO_2Me) to a solution of **8** at low temperature afforded the (E)-α,β-unsaturated methyl ester **21**, in 85% isolated yield. The E/Z ratio was shown by ^1H NMR to be as high as 95/5 (Scheme 5). Reduction of

21 with DIBAL-H in CH_2Cl_2 at low temperature afforded the allylic alcohol **20** (90% yield), characterized by comparison of its spectroscopic properties and optical rotation with those published in the literature.[11a] This sequence allowing preparation of the allylic alcohol **20**, precursor of (+)-(2*R*,3*R*,1'*S*,5'*S*)-Caudoxirene **5**, is an interesting improvement of the previous synthesis[11a] (16% overall yield in 5 steps). Indeed the overall yield is of about 70% and the number of steps is reduced to three.

CONCLUSION

In conclusion, we have achieved in this work the short, efficient, and stereoselective syntheses of some constituents of brown algae pheromones, using combination of a biocatalytic step and of chemical transformations. These various targets were obtained in good yields as compared to the previously described approaches. Our strategy implied the synthesis of a common intermediate using a novel enzyme-catalyzed oxidation of a racemic commercial ketone, which occurred enantioselectively to afford a single - chemically unexpected - lactone regioisomer (in 30% yield) in high enantiomeric purity. From this chiral building-block, our target molecules were obtained *via* a one/pot two steps Swern oxidation/Wittig or Julia-Lythogoe olefination to construct the C-3 chain, as a key-step. It is to be emphasized that, since we performed the synthesis of the aldehyde **8** without any isomerization / epimerization, this allowed us to prepare our various targets in high stereochemical integrity. This was the most important point of our strategy because, in our experience, no purification could have been carried out later on in the reaction scheme owing to the low polarity and high volatility of the various products.

We are currently investigating other applications offered by combining a biotransformation methodology with chemical synthesis.

Acknowledgements. This work has been achieved in the context of an European Community "Human Capital and Mobility" Program. The authors thank Dr. C. Mazzini for fruitful discussions and Dr. M. Campredon for NOES experiments.

EXPERIMENTAL SECTION

All reactions involving anhydrous conditions were conducted in flame-dried glassware under a positive nitrogen (oxygen-free) atmosphere. Solvents were distilled under nitrogen immediately prior use. THF, ethyl ether and toluene were dried by distillation from sodium benzophenone ketyl, triethylamine and dimethyl sulfoxide (at reduced pressure) from calcium hydride, CH_2Cl_2 from phosphorous pentoxide and methanol from magnesium methoxide. Commercial propyl magnesium bromide in ether and *n*-butyllithium in hexane were titrated prior to use with 2-butanol using 1,10-phenantroline.[28] Organic layers containing volatile products were stripped in a rotavap (water aspirator-reduced pressure) and a water bath at 10-20°C. Preparative TLC were conducted using pre-coated silica gel 60 F_{254} plates, (layer thickness 2 mm, Merck). Methoxyacetyl chloride was prepared as

described in the literature (Bp. 108-112°C)[29]. Cyclopentadiene was obtained by thermal cracking of dicyclopentadiene.[30] Sodium amalgam was freshly prepared following ref. 31. The ^{1}H NMR (250 MHz) and ^{13}C NMR (62.5 MHz) were recorded in $CDCl_3$ and chemical shifts are given in ppm referring to TMS as internal standard. Coupling constants are in hertz. IR spectra were obtained from neat oils and absorption maxima are given in cm^{-1}. Ees were determined by chiral GC analysis using a 25 m capillary column (6-*O*-methyl-2,3-di-*O*-pentyl)-β-cyclodextrine and a racemic sample as reference. Retention times (t_R) were checked by coinjection of racemic/chiral material. A GC capillary column (BP-10, 25 m × 0.32 mm × 0,25 μm) was used to determine the ratio of isomers. Separations by flash chromatography were performed using 60H silica gel (Merck).

(±)-7-*endo*- and (±)-7-*exo*-Methoxy-bicyclo[3.2.0]hept-2-en-6-one (11). A solution of methoxyacetyl choride (109 g, 1.0 mol) in dry CH_2Cl_2 (300 mL) was added dropwise over 1 h to a stirred solution of cyclopentadiene (365 g, 5.5 mol) and triethylamine (150 mL, 1.08 mol) in dry CH_2Cl_2 (1.5 L) at -78°C under nitrogen. After 1 h stirring at -78°C, the reaction mixture was allowed to warm up to rt overnight, and was then refluxed for 1 h. Most of the solvent was stripped off and the brown residue was suspended in ether (1.3 L). The triethylamonium hydrochloride salt was removed by filtration through a short pad of Celite and washed with ether (2 x 250 mL). The combined filtrates were successively washed with water, 5% aqueous solution of HCl, water and brine. The ether layer was dried ($MgSO_4$) and evaporated, the residue was purified by distillation to furnish **11** (120 g, 78%), bp. 59-64°C/0.01 mmHg. The *endo/exo* ratio was determined to be > 95/5 by GC analysis (oven 120°C): *exo* **11** t_R = 4.7 min; *endo* **11** t_R = 7.1 min. An analytical sample was purified by silica gel chromatography (linear gradient 0 to 5% ethyl acetate/pentane) to afford successively the *exo* (minor) and the *endo* (major) enantiomers of **11**. *exo* **11**: IR (neat) 3060, 2990, 2931, 2849, 1778, 1449, 1355, 1114, 949. ^{1}H NMR: 2.76 (*m*, 2H); 3.26 (*ddd*, J = 7.3, 7.3 and 3.6, 1H); 3.44 (*ddd*, J = 7.3, 7.3, 2.6, 1H); 3.51 (*s*, 3H); 5.20 (*s*, 1H); 5.68 (*m*, 1H); 5.83 (*m*, 1H). ^{13}C NMR: 36.6 (C4), 40.9 (C1), 54.0 (C5), 56.5 (C8), 107.1 (C7), 128.1 (C2 or C3), 133.2 (C3 or C2), 180.3 (C6). Anal. Calcd for $C_8H_{10}O_2$: C, 69.48; H, 7.24; O, 23.17. Found: C, 69.53; H, 7.20; O, 23.27 . *endo*-**11**: IR (neat) 3058, 2988, 2931, 2950, 2831, 1774, 1444, 1360, 1219, 1140, 1049. ^{1}H NMR: 2.40 (*m*, J = 16.8 and 8.5, 1H); 2.62 (*m*, J = 16.8, 1H); 3.38 (*s*, 3H); 3.44 (*m*, J = 3.2, 1H); 3.75 (*m*, 1H); 4.62 (*dd*, J = 8.5 and 3.2, 1H); 5.68 (*m*, 1H); 5.80 (*m*, 1H). ^{13}C NMR: 34.7 (C4), 45.7 (C1), 54.0 (C5), 58.3 (C8), 92.0 (C7), 128.0 (C2 or C3), 135.3 (C3 or C2). (These data are comparable to those previously reported).[16]

(±)-4-*endo*- and (±)-4-*exo*-Methoxy-3-oxabicyclo[3.3.0]oct-6-en-2-one (13). To a stirred solution of **11** (31.0 g, 0.20 mol) in CH_2Cl_2 (500 mL) in the presence of sodium hydrogen carbonate (35 g, 0.42 mol) at rt was added dropwise over 3-4 h a solution of *meta*-chloroperbenzoic acid (52 g, 0.21 mol based on 70% purity) in CH_2Cl_2 (500 mL). After stirring overnight at rt, the reaction mixture was poured into a 10% aqueous solution of sodium hydrogen sulfite (250 mL) and stirred for 20 min, then the whole was extracted twice with CH_2Cl_2. The combined extract were washed twice with saturated aqueous solution of sodium carbonate, twice with water and finally with brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the desired lactone **13** (34.0 g, 100%) as a colorless oil. This was used directly without further purification. An analytic sample was purified by bulb to bulb distillation (oven at 120-130°C/0.1 mmHg): IR (neat) 3059, 2938, 2858, 1772, 1449, 1362, 1216, 1177, 1146, 1109, 955, 922. ^{1}H NMR: 2.69 (*m*, J = 16.8 and 8.5, 1H); 2.87 (*m*, J = 16.8, 1H); 3.26 (pseudo *dt*, J = 2.0 and 8.6, 1H); 3.58 (*s*, 3H); 3.71 (*m*, 1H); 5.56 (*d*, J = 6.0, 1H); 5.71 (*m*, 1H); 5.87 (*m*, 1H). ^{13}C NMR: for 36.4 (C8), 43.8 (C1), 50.8 (C5), 58.4 (MeO), 106.7 (C4), 127.3 (C6 or C7), 133.3 (C7 or C6), 178.3 (C2). Mass spectrum CI *m/z* 172 ($M+NH_4^+$), 125 (M^+-Me). Anal. Calcd for $C_8H_{10}O_3$: C, 62.3; H, 6.5; O, 31.1. Found: C, 62.45; H, 6.61; O, 30.94.

(±)-4-*endo*- and (±)-4-*exo*-Hydroxy-3-oxabicyclo[3.3.0]oct-6-en-2-one (14). A stirred solution of **13** (34.0 g, 0.20 mol) in a water/THF mixture(500 mL/300 mL) at rt was flushed with nitrogen and concentrated solution of HCl (2 mL) was added in once. After stirring 3 days at rt under nitrogen, the reaction mixture was concentrated under reduced pressure to the half. The resultant mixture was extracted 4 times with CH_2Cl_2 and the combined extracts were washed twice with water, 3 times with brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the hydroxyl lactone **14** (28.0 g, 100%) as colorless oil which was used in the following reaction without further purification. An analytic sample was purified by bulb to bulb distillation (kugelrohr) oven at 120-130°C/0.1 mmHg: IR (neat) 3378, 3060, 2943, 2860, 1760, 1443, 1360, 1225, 1172, 1143, 1108, 949, 931. ^{1}H NMR: 2.6-2.9 (*m*, 2H); 3.3-3.5 (*m*, 2H); 5.6-6.1 (*m*, 4H). ^{13}C NMR: for the major C4-

OH epimer 36.7 (C8), 41.6 (C1), 55.0 (C5), 101.8 (C4), 128.5 (C6 or C7), 133.2 (C7 or C6), 182.3 (C2), for the minor C4-OH epimer 36.9 (C8), 41.6 (C1), 51.8 (C5), 100.9 (C4), 126.9 (C6 or C7), 133.6 (C7 or C6), 180.4 (C2). Mass spectrum *m/z* 123 (M^+-OH), 119 (M^+-OH-CH_2).

(±)-3-Oxabicyclo[3.3.0]oct-6-en-2-one (6). Sodium borohydride (8 g, 0.21 mol) was added in small portions over 1.5-2 h to a stirred solution of **14** (28.0 g, 0.20 mol) in absolute ethanol (150 mL) at rt. After 5 h, the thick white suspension was cooled at 0°C and water (15 mL) was carefully added, then the reaction mixture was concentrated under reduced pressure. The residue was diluted with ether (260 mL) and water (30 mL), 5% aqueous HCl solution was added until pH 2-3 and the whole heterogeneous mixture was stirred overnight. The organic layer was separated and the aqueous phase was extracted twice with ether (200 mL). The combined organic phases were washed with water and brine and dried ($MgSO_4$). After evaporation the residue was purified by distillation to afford the lactone **6** (19 g, 68%), bp. 66-68°C/0.01 mmHg. The spectroscopic data obtained for this racemic material were identical with those reported for the chirale (-)-(1*R*,5*S*) lactone **6** (see next).

(-)-(*1R,5S*)-3-Oxabicyclo[3.3.0]oct-6-en-2-one (6). Spores of *C. echinulata* NRRL 3655 (2.10^7 spores in 0.5% Tween 80 solution) were used to inoculate a 5 L complex medium in a 7 L Setric fermentor (complex medium composition: 100 g Corn Steep Liquor, 20 g glucose, 5 g KH_2PO_4, 10 g K_2HPO_4, 10 g $NaNO_3$, 2.5 g KCl, 2.5 g $MgSO_4$, 0.1 g $FeSO_4$, 1 mL Pluronic PE 8100 (BASF), 0.25 mL Antifoam Silicon 426R (Prolabo)). Cells were grown for 60 h at 27°C (450 rpm, 60 L/h air) then harvested by filtration and washed with water (5-7 g/L dry weight) before to be suspended in 5 L phosphate buffer (5 g KH_2PO_4, 10 g K_2HPO_4, pH 6.9). This cell suspension was partitioned in 2 L and 3 L baffled flasks filled at 1/5th volume and incubated with ketone **7** (5.2 g overall, 48 mmol. solubilized in 50 mL EtOH) on a giratory shaker (27° C, 150 rpm). Biotransformation was monitored by periodic sampling of aliquots (1 mL) which were extracted by 1 mL ethylacetate solution (containing 0.5 g/L tridecane as an internal standard). These were analysed by chiral gc at 80°C: (*1S,4R*)-**7** t_R= 2.7 min, (*1R,4S*)-**7**: t_R= 3.0 min, *endo*-bicyclo[3.2.0]hept-2-en-6-ol t_R= 5.2 and 5.3 min, *exo*-bicyclo[3.2.0]hept-2-en-6-ol: t_R = 6.4 and 6.9 min, (-)-(*1R,5S*)-**6** : t_R = 19.5 min, (*1S,5R*)-**6** t_R = 24.4 min, (*1R,5S*)-2-oxabicyclo[3.3.0]oct-6-en-3-one: t_R = 20 min, (*1S,5R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one t_R= 22.8 min. *Endo*- and *exo*-bicyclo[3.2.0]hept-2-en-6-ols were formed transiently.[31] The reaction was stopped after 24-30 h. Biotransformation was quenched by addition of a HCl solution until pH 2. The medium was then continuously extracted with CH_2Cl_2 for 48 h. After drying over $MgSO_4$, purification by flash chromatography over silica gel with pentane/ether gradient afforded (+)-(*1S,4R*)-**7** [32] (1.7 g, 33% yield, 86% ee), a mixture of bicyclo[3.2.0]hept-2-en-6-ols (*endo/exo*:3/2, 0.5 g, 9% yield) and (-)-(*1R,5S*)-**6** (2g, 34% yield, ≥ 95% ee [14]). ^{1}H NMR: 2.7-2.8 (*m*, 2H); 3.13 (*dd*, J = 7.7, 7.7 and 2.8, 1H); 3.6 (*m*, 1H); 4.23. (*dd*, J = 7.7 and 1.5, 1H); 4.42 (*ddd*, J = 9.1 and 7.7, 1H); 5.67 (*m*, 1H); 5.87 (*m*, 1H); ^{13}C NMR: 36.6 (C8), 41.7 (C1), 46.5 (C5), 71.6 (C4), 130.8 (C6 or C7), 132.3 (C7 or C6), 181.0 (C2).

(+)-(1*S*,5*S*)-5-Vinyl-2-cyclopenten-1-methanol (9). To a stirred suspension of methyltriphenylphosphonium bromide (6.7 g, 18.7 mmol) in THF (10 mL) at -78°C under nitrogen was added dropwise a 1.32 M solution of *n*-butyllithium (14.2 mL, 18.7 mmol) in hexane. The cooling bath was removed, and the reaction mixture was allowed to warm up to rt and stirred 1 h. To a solution of (-)-(1*R*,5*S*)-**6** (1.01 g, 8.1 mmol) in dry toluene (3 mL) at -78°C under nitrogen was added dropwise over a period of 10 min, a 1.0 M solution of diisobutylaluminium hydride in hexane (10.6 mL, 10.6 mmol). After stirring 1 h at -78°C, the later solution was rapidly transferred *via* a double-hipped needle to the methylidene(triphenyl)phosphorane solution at -78°C. The reaction mixture (allowed to warm up to rt overnight) was then diluted with ether (100 mL) and acidified at 0°C to pH 2-3 with an 2% aqueous solution of HCl. The organic layer was washed with water, brine and dried over $MgSO_4$. Removal of the solvent and chromatography of the crude product on silica gel(linear gradient of 1 to 5% ethyl acetate/pentane) yielded **9** (550 mg, 55%). The ee of (+)-**9** was higher than 98%: GC analysis (oven at 40°C for 20 min then 5°C /min): (+)-(1*S*,5*S*)-**9** t_R = 37.6 min and (-)-(1*R*,5*R*)-**9** t_R = 38.4 min $[\alpha]_{578}^{18}$ = +167 (c = 2.1, CH_2Cl_2) (literature[10a] for (-)-(3*R*,4*R*)-**9** $[\alpha]_{578}^{20}$ = -185.4 (c = 1.634, CH_2Cl_2)).: ^{1}H NMR: 1.6 (broad *s*, 1H); 2.20 (*m*, J = 16.4, 8.2 and 3.5, 1H); 2.42 (*dd*, J = 16.4 and 8.2, 1H); 2.84 (*m*, 1H); 2.96 (*quint.*, 8.3, 1H); 3.52 (*d*, J = 5.3, 2H); 5.01 (*dd*, J = 10.0 and 2.2, 1H); 5.06 (*dd*, J = 17.0 and 2.2, 1H); 5.58 (*m*, 1H); 5.82 (*m*, 1H); 5.96

(*ddd*, J = 17.0, 10.0 and 9.0, 1H). ^{13}C NMR: 38.3 (t), 44.9 (d), 51.6 (d), 63.0 (t), 115.5 (t), 131.2 (d), 132.5 (d), 139.8 (d). These data were identical with those previously reported for (+)-**9**.[19]

(3*S*,4*S*)-3-[(1'*R*)-But-1'-ol)]- and (3*S*,4*S*)-3-[(1'*S*)-but-1'-ol)] -4-vinylcyclopent-1-ene (16). To a stirred solution of oxalyl chloride (0.3 mL, 3.3 mmol) in THF (10 mL) at -78°C under nitrogen was added dropwise (over 10 min) a solution of DMF (0.36 mL, 4.6 mmol) in THF (3 mL). After 30 min a solution of **9** (250 mg, 2 mmol) in THF (7 mL) was added (over 10-15 min). The reaction mixture was stirred for 3 h and then treated with triethylamine (2.1 mL, 12.2 mmol). The cooling bath was removed, replaced by an ice-water bath, and the reaction mixture was allowed to warm up to 0°C over 30 min. After 10 min at 0°C, the reaction mixture was cooled again at -78°C and treated with and excess of a propyl magnesium bromide 3 M solution (2.4 mL, 7.2 mmol) in ether. The reaction mixture was allowed to warm up to rt overnight, quenched with water and concentrated under reduce pressure. The residue was then diluted with ether (50 mL), acidified at 0°C to pH 4 with a saturated aqueous solution of sodium dihydrogen phosphate and extracted twice with ether. The combined organic extracts were washed twice with water, brine and dried ($MgSO_4$). Removal of the solvent and chromatography of the crude product on silica gel (linear gradient 1 to 5% ethyl acetate/pentane) yielded **16** (205 mg, 65%) as a 85/15 mixture of isomers: GC analysis (oven at 60°C): major isomer of **16** t_R = 34.0 min. minor isomer of **16**: t_R = 34.6 min. IR (neat) 3427, 3060, 2996, 2919, 2872, 1460, 1378, 1119, 908. ^{1}H NMR: for the major isomer **16**: 0.87 (*t*, J = 7.5, 3H); 1.0-1.5 (*m*, 6H), 2.0-2.4 (*m*, 2H); 2.78 (broad *s*, 1H); 2.80 (*quint.*, J = 8.6, 1H); 3.52 (broad *s*, 1H); 5.0-5.2 (*m*, 2H); 5.70 (*m*, 1H); 5.94 (*m*, 1H); 6.04 (*ddd*, J = 17.2, 10.4 and 8.6, 1H). The minor isomer of **16** could only be distinguished by the following three chemical shifts: δ(ppm). 3.60 (m); 5.52 (*m*); 5.73 (*m*). ^{13}C NMR for the major isomer **16**: 14.0 (q), 19.3 (t), 38.4 (t), 38.5 (t), 45.7 (d), 54.3 (d), 71.2 (d), 115.2 (t), 128.7 (d), 134.0 (d), 140.1 (d). For the minor isomer **16** 14.1 (q), 18.6 (t), 37.2 (t), 38.3 (d), 45.0 (d), 56.1 (d), 71.0 (d), 115.1 (t), 130.9 (d), 131.2 (d), 140.5 (d). Mass spectrum *m/z* 174 ($M+NH_4^+$), 167 ($M+H^+$) 149 (M^+-H_2O). Anal. Calcd for $C_{11}H_{18}O$: C, 79.52; H, 10.84; O, 9.64. Found: C, 79.62; H, 10.70; O, 9.68.

(3*S*,4*S*)-3-[(1'*R*)-But-1'-methanesulfonate)]- and (3*S*,4*S*)-3-[(1'*S*)-but-1'-methanesulfonate)]-4-vinylcyclopent-1-ene (17). A solution of **16** (270 mg, 1.6 mmol) in CH_2Cl_2 (5 mL) in an ice-water bath under nitrogen was treated sequentially with triethylamine (0.28 mL, 2 mmol) and methanesulfonyl chloride (0.15 mL, 1.9 mmol), followed by a catalytic amount of 4-(dimethylamino)pyridine (~20 mg). After stirring 1 h at 0°C, the reaction mixture was diluted with ether (25 mL), quenched with water, and decanted. The organic layer was washed with 2% HCl, twice with water, brine and dried ($MgSO_4$). Removal of the solvent and chromatography of the crude product on silica gel (linear gradient 0 to 5% ethyl acetate/pentane) yielded the mesylate **17** (342 mg, 88%). IR (neat) 3060, 2996, 2931, 2859, 1631, 1460, 1337, 1166, 908. ^{1}H NMR: 0.86 (*t*, J = 7.4, 3H); 1.38 (*sext.*, J = 7.4, 2H), 1.79 (*m.*, 2H), 2.18 (*m*, J = 16.0, 1H); 2.43 (*ddd*, J = 16.0, 7.4 and 3.4, 1H); 2.9-3.0 (m, 2H), 2.00 (*s*, 3H); 4.73 (*dt*, J = 6.9 and 6.5, 1H); 5.05 (*m*, J = 10.3, 1H); 5.07 (*m*, J = 16.8 0, 1H); 5.78 (m, 1H); 5.88 (*m*, 1H); 5.90 (*ddd*, J = 16.8, 10.3 and 9.0, 1H). ^{13}C NMR for the major isomer **17**: 14.1 (q), 18.4 (t), 35.6 (t), 38.5 (t), 39.1 (t), 39.4 (q), 45.6 (d), 84.5 (d), 116.1 (t), 129.9 (d), 133.3 (d), 138.6 (d). For the minor isomer **17**: 14.0 (q), 18.6 (t), 33.9 (t), 37.9 (d), 39.0(q), 44.6 (d), 56.1 (d), 85.0 (d), 116.3 (t), 129.8 (d), 133.3 (d), 138.2. (d). Mass spectrum *m/z* 268 ($M+NH_4^+$), 149 (M^+-MeSO_3H).

(+)-(3*S*,4*S*)-3-*n*-Butyl-4-vinylcyclopent-1-ene (1). To an ice-cold stirred suspension of $LiAlH_4$ (80 mg, 2 mmol) in dry ether (5 mL) under nitrogen was added dropwise a solution of **17** (290 mg, 1.2 mmol) in ether (3 mL). After stirring for 2 h at rt, the mixture was cooled in ice bath and water was cautiously added followed by a 2% HCl solution until the aqueous phase was slightly acidic. After 3 times extraction with pentane, the combined organic extracts were washed twice with water, brine and dried ($MgSO_4$). Careful removal of the solvent and purification using preparative silica gel TLC (pentane) led to (+)-(3*S*,4*S*)-3-*n*-butyl-4-vinylcyclopent-1-ene **1** (115 mg, 68%). The enantiomeric purity of (+)-**1** was higher than 98%: GC analysis (oven at 32°C): (+)-(3*S*,4*S*)-**1** t_R = 35.0 min and (-)-(3*R*,4*R*)- **1**: t_R = 35.6 min. $[\alpha]_{578}^{18}$ = +186 (c = 3.0, CH_2Cl_2) (literature[2] for the unnatural (-)-(3*R*,4*R*)-**1** $[\alpha]_{578}^{20}$ = -170.9 (c = 5.27, CH_2Cl_2, ee~92%)). The spectral ^{1}H NMR data for (+)-**1** were identical with those previously reported[7b]: ^{1}H NMR: 0.88 (*t*, J = 6.4, 3H);1.0-1.5 (*m*, 6H), 2.18 (*m*, J = 15.8, 1H); 2.43 (*m*, J = 15.8, and 7.9, 1H); 2.61 (broad *s*, 1H); 2.88 (*quint.*, J = 7.8, 1H); 4.94 (*dd*, J = 10.3 and 2.0, 1H); 5.05

(*dd*, J = 17.2 and 2.0, 1H); 5.68 (*m*, 1H) 5.78 (*m*, 1H); 5.90 (*ddd*, J = 17.2, 10.3 and 8.2, 1H). ^{13}C NMR: 14.1 (q), 23.0 (t), 30.3 (t), 30.4 (t), 37.6 (t), 46.4 (d), 48.4 (d), 114.1 (t), 129.1 (d), 135.1 (d), 140.2 (d).

(+)-(3*S*,4*S*)-3-[(1Z)-But-1-enyl)]-4-vinylcyclopent-1-ene: Multifidene (2). To a stirred suspension of propyltriphenylphosphonium bromide (6.2 g, 16.1 mmol) in THF (10 mL) at -78°C under nitrogen was added dropwise a 2.0 M solution of sodium bis(trimethylsilyl)amide in THF (8 mL, 16.0 mmol). The cooling bath was removed, and the reaction mixture was allowed to warm up to rt and stirred for 1 h. To a stirred solution of oxalyl chloride (0.3 mL, 3.3 mmol) in THF at -78°C under nitrogen was added dropwise in 10 min a solution of DMF (0.36 mL, 4.6 mmol) in THF (2 mL). After 30 min a solution of **9** (256 mg, 2 mmol) in THF (6 mL) was added in 10-15 min; the reaction mixture was stirred 3 h and then treated with triethylamine (1.0 mL, 7.2 mmol). The cooling bath was removed, replaced by ice-water bath, and the reaction mixture was allowed to warm up to 0°C in 30 min. After 15 min at 0°C, the reaction mixture was recooled at -78°C and the solution of propylidene (triphenyl)phosphorane at rt was added *via* cannula (3.0 mL of THF rinse). The reaction mixture was allowed to warm up to rt overnight, quenched with water and then diluted with pentane (20 mL), acidified at 0°C to pH 4 with a saturated aqueous solution of sodium dihydrogen phosphate and extracted 3 times with pentane (20 ml x 3). The combined organic extracts were washed twice with water, brine and dried ($MgSO_4$). Careful removal of the solvent and purification by preparative TLC (silica gel) with pentane as eluent gave (+)-(Z)-Multifidene **2** (207 mg, 74%). The Z/E ratio was higher than 98%: GC analysis (oven at 40°C): (+)-(3*S*,4*S*)-(E)-Multifidene **3** t_R = 14.2 min and (+)-(3*S*,4*S*)- Multifidene **2** t_R =15.6 min. The ee of (+)-**2** was higher than 98%: GC analysis (oven at 35°C): (+)-(Z)-(3*S*,4*S*)-Multifidene **2**: t_R = 27.6 min and (-)-(Z)-(3*R*,4*R*)-Multifidene **2**: t_R = 27.9 min. $[\alpha]_{578}^{18}$ = +259 (c = 1, CCl_4) (literature[9a] $[\alpha]_{578}^{20}$ = + 261 (c = 0.83, CCl_4)). The ^{1}H and ^{13}C NMR data for (+)-**2** were identical with those previously reported[9a]: ^{1}H NMR: 0.98 (*t*, J = 7.5, 3H); 2.08 (*dquint.*, J = 7.5 and 1.1, 2H); 2.28 (*m*, J = 16.4, 8.2 and 3.3, 1H); 2.45 (*m*, J = 16.4 and 8.2, 1H); 2.99 (*quint.*, J = 8.2, 1H); 3.63 (*pseudo t* (broad), J = 8.3, 1H); 4.96 (*m*, J = 10.2, 1H); 4.99 (*m*, J = 16.9, 1H); 5.14 (*tt*, J = 10.6 and J = 1.1, 1H); 5.41 (*dt*, J = 10.6 and J = 7.6, 1H); 5.53 (*m*, 1H); 5.77 (*m*, 1H); 5.88 (*ddd*, J = 16.9, 10.2 and 8.5, 1H). ^{13}C NMR: 14.3 (q), 20.7 (t), 37.0 (t), 46.7 (d), 46.8 (d), 113.9 (t), 128.3 (d), 129.9 (d), 131.9 (d), 134.4 (d), 140.1 (d).

(+)-(3*R*,4*S*)-3-[But-1-acetate-2-*p*-toluenesulfonate]-4-vinylcyclopent-1-ene (19). To a stirred solution of **18** (1.03 g, 5.2 mmol), prepared following the literature[25b], in THF (10 mL) at -78°C under nitrogen was added dropwise over 10 min a 1.55 M solution of *n*-butyllithium in hexane (3.5 mL, 5.4 mmol). After 30 min, the bright yellow clear solution was allowed to warm up to rt. A solution of oxalyl chloride (0.3 mL, 3.3 mmol) in THF (5 mL) was treated with a solution of dimethyl sulfoxide (0.34 mL, 4.7 mmol) in THF (2.2 mL), after 30 min, a solution of **9** (355 mg, 2.86 mmol) in THF (8.2 mL) was added in 10-15 min. The reaction mixture was stirred 3 h and then treated with triethylamine (1.0 mL, 7.1 mmol). The solution of lithium propyl *p*-toluenesulfonate at rt was rapidly cannulated *via* a double-hipped needle (2.0 mL of THF rinse) to the solution of **8** at -78°C. The mixture was allowed to warm up to rt overnight, cooled to-78°C and treated with and excess of acetic anhydride (0.98 mL, 11.0 mmol). The reaction mixture was allowed to warm to 0°C over a period of 2-3 h, quenched at 0°C with water and then diluted with ether (50 mL). The organic layer was washed with water, twice with a saturated aqueous solution of sodium dihydrogen phosphate, water, brine and then dried ($MgSO_4$). Removal of the solvent and chromatography of the crude mixture on silica gel (linear gradient 1 to 15% ethyl acetate/pentane) afforded an unseparable mixture of diastereoisomers of **19** (570 mg, 55%). $[\alpha]_{578}^{18}$ = +66 (c = 2.0, CH_2Cl_2). IR (neat) 3048, 2966, 2919, 2849, 1737, 1590, 1454, 1366, 1320, 1226, 1143, 1020. ^{1}H NMR: 0.8-1.3 (*m*, 3H); 1.6-3.2 (*m*, 5H); 2.1 and 2.0 (*s*, 3H); 2.5 (*s*, 3H); 3.4 (*m*, 1H); 3.8 (*m*, 1H); 4.6-5.8 (*m*, 6H); 7.3 (*m*, 2H); 7.6 (*m*, 2H). Mass spectrum *m/z* 363 ($M+H^+$), 303 (M^+-AcOH).

(+)-(3*S*,4*S*)-3-[(1E)-But-1-enyl)]-4-vinylcyclopent-1-ene: (E)-Multifidene (3). To a vigorously stirred mixture of **19** (398 mg, 1.1 mmol) and disodium hydrogen phosphate (1.84 g, 13 mmol) in methanol (10 mL) at -20°C under nitrogen were slowly added three portions (approximately 900 mg each, 6 mmol) of freshly prepared ~5% sodium amalgam. After stirring at 0°C for 2 h, the mixture was triturated with pentane (20 mL) and water (3 mL) was added. The organic phase was washed twice with water, brine and dried ($MgSO_4$). Careful removal of the solvent and purification by preparative TLC (silica gel, pentane as eluent) gave (+)-(E)-Multifidene **3** (76 mg, 50%). The E/Z ratio was determined to be higher than 95/5: GC analysis (oven at 40°C):

(+)-(3*S*,4*S*)-(E)-Multifidene **3** t_R = 14.8 min. and (+)-(Z)-(3*S*,4*S*)-Multifidene **2**: t_R = 16.2 min. The ee of (+)-**3** was higher than 97%: GC analysis (oven at 35°C): (+)-(3*S*,4*S*)-(E)-Multifidene **3**: t_R = 25.8 min. and (-)-(3*R*,4*R*)-(E)-Multifidene **3**: t_R = 26.9 min. $[\alpha]_{578}^{18}$ = +269 (c = 2.7, $CHCl_3$) (literature[2] for the unnatural (-)-(3*R*,4*R*)-(E)-Multifidene **3** $[\alpha]_{578}^{20}$ = -246 (c = 1.76, $CHCl_3$, ee~80%)). ^{1}H NMR: 0.98 (*t*, J = 7.0, 3H); 2.00 (*dquint.*, J = 7.0 and 1.3, 2H); 2.18 (*m*, J = 16.4, 8.2 and 3.3, 1H); 2.35 (*m*, J = 16.4 and 8.2, 1H); 2.83 (*quint.*, J = 8.2, 1H); 3.18 (*pseudo t* (broad), J = 8.2, 1H); 4.96 (*m*, J = 10.2, 1H); 4.99 (*m*, J = 16.9, 1H); 5.23 (*ddt*, J = 15.1, 8.3 and J = 1.3, 1H); 5.44 (*dt*, J = 15.1 and J = 6.2, 1H); 5.65 (*m*, 1H); 5.77 (*m*, 1H); 5.84 (*ddd*, J = 16.9, 10.2 and 8.0, 1H). ^{13}C NMR: 36.9 (t), 46.8 (d), 47.1 (d), 114.4 (t), 117.4 (t), 129.2 (d), 130.4 (d), 131.7 (d), 132.2 (d), 133.5 (d), 139.6 (d). These ^{1}H and ^{13}C NMR data were identical with those previously described.[9b]

(+)-(3*R*,4*S*)-3-[(1Z)-1,3-Butadienyl)]-4-vinylcyclopent-1-ene: Viridiene (4). This compound was synthesized following a similar procedure as described above for the preparation of (+)-(Z)-Multifidene **2**. A suspension of allyltriphenylphosphonium bromide (6.7 g, 17.5 mmol) in THF (15 mL) was treated with a 2.0 M solution of sodium bis(trimethylsilyl)amide in THF (8.6 mL, 17.2 mmol). A solution of oxalyl chloride (0.31 mL, 3.5 mmol) in THF (6 mL) was treated with a solution of DMSO (0.39 mL, 5.0 mmol) in THF (2.5 mL), after 30 min, a solution of **9** (267 mg, 2.2 mmol) in THF (8 mL) was added over 10-15 min. The mixture was stirred 3 h and then treated with triethylamine (1.0 mL, 7.0 mmol). The solution of **8** was treated with an excess of the solution of propylidene (triphenyl)phosphorane at -100°C. After workup and careful removal of the solvent, the crude product was filtered through a short pad of silica gel with pentane to afford Viridiene **4** (~210 mg) as a 75/25 mixture of (Z)/(E) isomers. This Z/E ratio was determined by integration of the C-3 proton signal in the ^{1}H NMR spectrum of the crude mixture and by GC analysis:(oven at 37°C). (+)-(3*R*,4*S*)-Viridiene **4**: t_R = 26.5 min and (E)-Viridiene **4**: t_R = 26.0 min. This mixture was diluted in THF (1 mL) and was treated at rt by addition of an approximately one molar solution of 4-phenyl-1,2,4-triazoline-3,5-dione until the red color of the reagent persisted. The solution was concentrated under reduced pressure to the half and purified on preparative TLC (silica gel, pentane) to afford (+)-(3*R*,4*S*)-Viridiene **4** (120 mg, 40%). Its ee was higher than 98% .GC analysis (oven at 35°C): (+)-(3*R*,4*S*)-Viridiene **4**: t_R = 37.1 min and (-)-(3*S*,4*R*)-Viridiene **4** t_R = 38.2 min. $[\alpha]_{578}^{18}$ = + 239 (c = 2.1, pentane); literature[10a] $[\alpha]_{578}^{18}$ = + 228 (c = 0.224, pentane). The ^{1}H NMR data of (+)-**2** were in good agreement with those previously reported[10c]: ^{1}H NMR: 2.30 (*m*, J = 16.3, 8.2 and 3.2, 1H); 2.48 (*m*, J = 16.2 and 8.2, H); 3.03 (*qi*, J = 8.0 Hz, 1H); 3.80 (broad *t*, J = 9.2 Hz, 1H); 4.98 (*m*, J = 10.6, 1H); 5.00 (*m*, J = 17.6, 2H); 5.11 (d, J = 10.6, 1H); 5.18 (*d*, J = 17.0, 1H); 5.25 (*dd*, J = 10.6 and 10.6, 1H); 5.60 (*m*, 1H); 5.80-5.90 (*m*, 1H); 6.01 (*dd*, J = 10.6 and 10.6, 1H); 6.68 (*ddd*, J = 17.0, 10.6 and 10.6, 1H). ^{13}C NMR: 36.9 (t), 46.8 (d), 47.1 (d), 114.4 (t), 117.4 (t), 129.2 (d), 130.4 (d), 131.7 (d), 132.2 (d), 133.5 (d), 139.6 (d). E-Viridiene **4**: on the ^{1}H NMR spectrum of the crude mixture the E-Viridiene **4** could only be distinguished from Viridiene **4** by the two following chemical shifts: δ(ppm) 3.30 (br *t*, J = 8.1 Hz, 1H (C-3)); 6.25 (*ddd*, J = 17.0, 10.5 and 10.5, 1H). ^{13}C NMR: 37.1 (t), 47.2 (d), 51.7 (d), 114.3 (t), 115.2 (t), 130.7 (d), 130.9 (d), 133.1 (d), 134.6 (d), 137.0 (d), 139.9 (d).

(+)-(1'*R*,5'*S*, 2E)-3-(5'-Vinylcyclopent-2'-enyl)prop-2-en-1-oic methyl ester (21). This product was obtained following a procedure similar to the one described above for the preparation of (+)-(Z)-Multifidene **2**. A solution of oxalyl chloride (0.35 mL, 3.9 mmol) in THF (10 mL) was treated with a solution of DMSO (0.42 mL, 5.3 mmol) in THF (2 mL), after 30 min, a solution of **9** (351 mg, 2.8 mmol) in THF (5 mL) was added over 10-15 min. The mixture was stirred for 3 h and then treated with triethylamine (1.2 mL, 9.0 mmol). The solution of the **8** at -78°C was treated with an excess of Methyl (triphenylphosphoranylidene) acetate (2.7 g, 8.0 mmol) added at once as a solid. After workup and removal of the solvent, the crude residue was chromatographied on silica gel (linear gradient 1 to 5% ethyl acetate/pentane) and yielded the α,β-unsaturated methyl ester **21** (423 mg, 85%) as a 95/5 mixture of (E)/(Z)isomer. This Z/E ratio was determined by GC analysis (oven at 80°C). (+)-(1'*R*,5'*S*, 2E)-**21**: t_R = 25.8 min and (1'*R*,5'*S*, 2Z)-**21**: t_R = 28.9 min. $[\alpha]_{578}^{18}$ = +266 (c = 2.0, CH_2Cl_2). IR (neat) 3048, 2942, 2919, 2848, 1719, 1642, 1437, 1267, 914, 738. ^{1}H NMR: 2.29 (*m*, J = 16.2, 8.2 and J = 3.2, 1H); 2.48 (*m*, J = 16.2 and 8.2, 1H); 3.09 (*quint.*, J = 8.2, 1H); 3.46 (*m*, J = 8.3, 1H); 3.72 (*s*, 3H); 5.00 (*m*, J = 10.2, 1H); 5.05 (*m*, J = 17.3, 1H); 5.62 (*m*, 1H); 5.78 (*ddd*, J = 17.3, 10.2 and 8.2, 1H); 5.79 (*d*, J = 15.2, 1H); 5.91

(*m*, 1H); 6.83 (*dd*, J = 15.2 and 8.2, 1H). ^{13}C NMR: for the (E) isomer: 29.7 (t), 37.2 (t), 47.3 (d), 51.3 (q), 115.4 (t), 120.8 (d), 131.2 (d), 132.6 (d), 138.8 (d), 149.0 (d), 166.9 (s). For the (Z) isomer: 36.5 (t), 46.6 (d), 47.7 (d), 51.1 (q), 115.0 (t), 118.9 (d), 131.7 (d), 132.5 (d), 139.1 (d), 149.4 (d). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.16; H, 7.86; O, 17.98. Found: C, 74.31; H, 7.70; O, 17.99.

(+)-(1'*R*,5'*S*, 2E)-3-(5'-Vinylcyclopent-2'-enyl)-prop-2-en-1-ol (20). To a solution of **21** (337 mg, 1.9 mmol) in dry toluene (3 mL) at -78°C under nitrogen was added dropwise (over 5 min) a 1.0 M solution of diisobutylaluminium hydride in hexane (4.6 mL, 4.6 mmol). After stirring 1 h at -78°C, the reaction mixture was allowed to warm up to rt overnight, then diluted with ether (100 mL) and acidified at 0°C to pH 2-3 with an 2% aqueous HCl. solution. The organic layer was washed with water, brine and dried ($MgSO_4$). Removal of the solvent and chromatography of the crude product on silica gel (linear gradient 5 to 25% ethyl acetate/pentane) yielded the allylic alcohol **20** (255 mg, 90%) as a 93/7 mixture of (E)/(Z)isomer. This Z/E ratio was determined by GC analysis (oven at 60°C). (+)-(1'*R*,5'*S*, 2E)-**20**: t_R =28.9 min and (1'*R*,5'*S*, 2Z)-**20**: t_R = 29.7 min. The ee of (+)-**21** was higher than 98%: GC analysis (oven at 100°C): (+)-(1'*R*,5'*S*, 2E)-**21**: t_R = 8.9 min and (-)-(1'*S*,5'*R*, 2E)-**21**: t_R = 9.4 min. $[\alpha]_{578}^{18}$ = +266 (c = 2.0, CH_2Cl_2) (lit[11a] $[\alpha]_{578}^{20}$ = +352.2 (c = 3.01, CH_2Cl_2). IR (neat) 3342, 3055, 2926, 2852, 1676, 1638, 1443, 1090, 993, 912. The ^1H and ^{13}C NMR data of this product were identical with those reported in the literature.[11a] ^1H NMR: 1.4 (broad *s*, 1H); 2.26 (*m*, J = 16.0, 8.0 and 2.0, 1H); 2.44 (*m*, J = 16.0, 8.0, 1H); 2.98 (*quint.*, J = 8.2, 1H); 3.33 (pseudo *t*, J = 8.2, 1H); 4.10 (*d*, J = 5.0, 1H); 4.9-5.1 (*m*, 2H); 5.5-5.7 (*m*, 3H); 5.79 (*m*, H); 5.79 (*ddd*, J = 17.0, 10.5 and 7.8, 1H). ^{13}C NMR: for the (E) isomer 37.4 (t), 47.1 (d), 51.3 (d), 63.7 (t), 114.6 (t), 129.6 (d), 131.0 (d), 132.6 (d), 133.4 (d), 140.2 (d). For the (Z) isomer: 29.9 (t), 47.3 (d), 47.4 (d), 58.7 (t), 114.9 (t), 129.0 (d), 131.8 (d), 132.7 (d), 133.8 (d), 140.3 (d).

REFERENCES AND NOTES

1. For a general review on sexual pheromones in algae, see: Maier I., Müller D. G. *Biol. Bull.*, **1986**, *170*, 145-175.
2. Wirth, W.; Fischer-Lui, I.; Boland, W.; Icheln, D.; Runge, T.; König, W. A.; Phillips, J.; Clayton, M. *Helv. Chim. Acta.* **1992**, 75, 734-744 and literature cited.
3. Maier, I.; Müller, D. G.; Gassmann, G.; Boland, W.; Marner, F.-J.; Jaenicke, L. *Naturwissenschaften* **1984**, 71, 48-49.
4. Müller, D. G.; Peters, A.; Gassmann, G.; Boland, W.; Marner, F.-J.; Jaenicke, L. *Naturwissenschaften* **1982**, 69, 290-291.
5. Wirth, D.; Boland, W. *Helv. Chim. Acta.* **1990**, 73, 916-921.
6. Boland, W.; Pohnert, G.; Maier, I. *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 1602-1604. Panke-Böcker, S.; Pohnert, G.; Fhisher-Lui, I.; Boland, W.; Peters, A. F. *Tetrahedron* **1995**, 51, 7927-7936. Pohnert, G.; Boland, W. *Tetrahedron* **1994**, 50, 10235-10244. Stratmann, K.; Boland, W.; Müller, D. G. *Tetrahedron* **1993**, 49, 3755-3766. Stratmann, K.; Boland, W.; Müller, D. G. *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 1246-1248.
7. For synthesis of nonatural (-)-**1** see: a) ref. 2. For racemic **1** see: b) Boland, W.; K. Mertes *Helv. Chim. Acta.* **1984**, 67, 616-624.
8. For synthesis of (+)-**2** see: a) Hemamalini, S.; Scheffold, R. *Helv. Chim. Acta.* **1995**, 78, 447-451. For (+)-**2** and (-)-**2** see: b) Boland, W.; Jaenicke, L.; Müller, D. G. *Liebigs Ann. Chem.* **1981**, 2266-2271. For (-)-**2** see: c) Kramp, P.; Helmchen, G.; Holmes, A. B. *J. Chem. Soc., Chem. Commun.* **1993**, 551-552. For racemic **2** see: d) Randall M. L., Tallario J. A., Snapper M. L. *J. Am. Chem. Soc.*, **1995**, 117, 9610-9611. e) Burks, J. E.; Crandall, J. K. *J. Org. Chem.* **1984**, 49, 4663-4670. f) Crouse, G. D.; Paquette, L. A. *J. Org. Chem.* **1981**, 46, 4272-4274. g) Boland, W.; Jaenicke, L. *J. Org. Chem.* **1979**, 44, 4819-4824. h) Boland, W.; Jaenicke, L. *Chem. Ber.* **1978**, 111, 3262-3275.

9. For synthesis of nonatural (-)-**3** see: a) ref. 2. For racemic **3** see: b) ref. 8h.

10. For synthesis of (+)-**4** see: a) Boland, W.; Jaenicke, L.; Niedermeyer, U.; Jaenicke, L.; Görish, H. *Helv. Chim. Acta.* **1985**, 68, 2062-2073. For racemic **2** see: b) ref. 8d. c) Boland, W.; Jakoby, K.; Jaenicke, L. *Helv. Chim. Acta.* **1982**, 65, 2355-2362.

11. For synthesis of (+)-**5** see: a) Wirth, D.; Boland, W.; Müller, D. G. *Helv. Chim. Acta.* **1992**, 75, 751-758. For racemic **3** see: b) ref. 5.

12. For an excellent overview of biotransformations in organic syntheses, see: Azerad, R. *Bull. Soc. Chim. Fr.* **1995**, 132, 17-51 and literature cited.

13. A preliminary note of some of this work has been published: Lebreton, J.; Alphand, V.; Furstoss, R. *Tetrahedron Lett.* **1996**, 37, 1011-1014.

14. Alphand, V.; Archelas, A.; Furstoss, R. *Tetrahedron Lett.* **1989**, 30, 3663-3664. Alphand, V.; Furstoss, R. *J. Org. Chem.* **1992**, 57, 1306-1309.

15. a) Au-Yeung, B.-W.; Wang, Y. *J. Chem. Soc., Chem. Commun.* **1985**, 825-827. b) Govindan, S. V.; Hudlicky, T.; Koszyk, F. J. *J. Org. Chem.* **1983**, 48, 3581-3583.

16 DoMinh, T.; Strausz, O.P. *J. Am. Chem. Soc.* **1970**, 92, 1766.

17. a) Genicot, C.; Gobeau, B.; Ghosez, L. *Tetrahedron Lett.* **1991**, 32, 3827-3830. b) Chida, N.; Tobe, T.; Ogawa, S. *Tetrahedron Lett.* **1994**, 35, 7249-7252.

18. Rey, M.; Roberts, S.; Dieffenbacher, Dreiding, A. S. *Helv. Chim. Acta.* **1970**, 53, 417-432.

19. Boland, W.; Ney, P.; Jaenicke, L. *Synthesis* **1980**, 1015-1017.

20. For a recent ref. see: a) Ahman, J.; Somfai, P. *Tetrahedron Lett.* **1995**, 36, 303-306. For a review on Swern oxidation see: b) Tidwell, T. T. *Synthesis* **1990**, 857-870.

21. Ireland, R.E.; Norbeck, D. W. *J. Org. Chem.* **1985**, 50, 2198-2202.

22. Boland, W.; Mertes, K.; Jaenicke, L.; Müller, D. G.; Fölster, E. *Helv. Chim. Acta.* **1983**, 66, 1905-1913.

23. Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, 59, 7549-7552.

24. Chemical shifts of the protons at 3.31 ppm (C-3, m (*t*-like)) and at 2.92 ppm (C-3, *qi.*, J = 8.2 Hz) on the ^{1}H NMR (250 MHz, $CDCl_3$) spectrum of the crude mixture are in agreement with the previous data reported in the literature (see ref. 8h) for the C-3 epimer of Multifidene **2**.

25. a) For a review of sulfone-based olefination, see: Simpkins, N. S. *In Sulphones in Organic Synthesis*; Baldwin J. E., Magnus P. D., Eds.; Pergamon Press: Oxford, **1993**; 254-262. b) For recent ref., see: Keck, G. E.; Savin, K. A.; Weglarz, M. A. *J. Org. Chem.* **1995**, 60, 3194-3204.

26. Addition of a triphenyl(propenylidene)phosphorane solution to the reaction mixture at -78°C gave rise to a nearly 1/1 mixture of (+)-Viridiene **4** (Z isomer) and of its E isomer in low yield.

27. Cookson, R.C.; Gilani, S. S. H.; Stevens, I. D. R. *J. Chem. Soc. (C)* **1967**, 1906-1909.

28. Walton, S. C.; Eastham, J. F. *J. Orgomet. Chem.* **1967**, 9, 165.

29. Jones, M. *Synthesis* **1984**, 727.

30. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Pratical Organic Chemistry 5th Ed.*; John Wiley and Sons, Inc.: New York, **1989**, p 1122.

31. Renfrow, W. B.; Hauser, C. R. *Oganic Syntheses*; John Wiley and Sons, Inc.: New York, **1943**; Vol. II, p. 607-609.

32. More detailed aspects of this biotransformation will be published elsewhere.

33. Fantin G., Fogagnolo M., Medici A., Pedrini P., Rosini G.; *Tetrahedron: Asymmetry,* **1994**, *5*, 1635-1638.

(*Received in Belgium* 5 *July* 1996; *accepted* 24 *October* 1996)