Structure elucidation of hypocreolide A by enantioselective total synthesis†

Katharina Götz,[a] Johannes C. Liermann,[a] Eckhard Thines,[b] Heidrun Anke[b] and Till Opatz*[a]

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The nonenolide hypocreolide A was isolated from culture filtrates of the ascomycete *Hypocrea lactea*. It exhibits moderate antimicrobial activity against various tested fungi and bacteria. Since neither the relative nor the absolute stereochemistry of the compound could be initially assigned, a stereochemically flexible total synthesis was developed. The two stereogenic centers were formed in high enantioselectivity and yield using transition metal catalyzed asymmetric reactions. While attempts to construct the ten-membered lactone in a ring-closing olefin metathesis gave disappointing results, a combination of cross metathesis and macrolactonization provided the title compound in nine steps and 12% overall yield.

Introduction

During our ongoing search for biologically active fungal metabolites, a nonpolar compound with a molecular weight of 268 g mol^{-1} and moderate antifungal, antibacterial and cytotoxic activity was isolated from culture filtrates of the ascomycete *Hypocrea lactea* IBWF 02002. Structure elucidation by spectroscopic methods revealed this compound (**1**, $C_{16}H_{28}O_3$) to be a nonenolide with close resemblance to members of the putaminoxin family (**2**)[1–3] as well as to aspinolide A (**3**)[4] and Stagonolide F (**4**) (Fig. 1).[5]

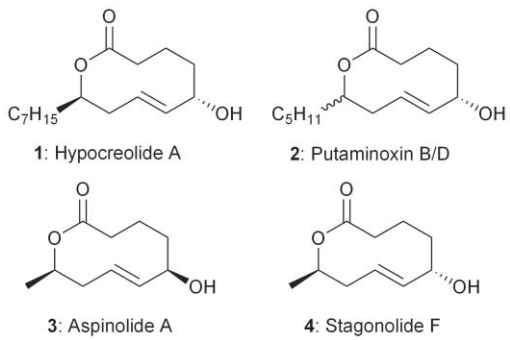
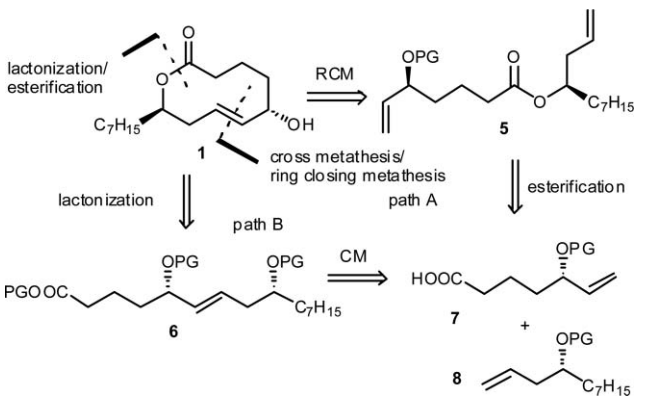


Fig. 1 Structures of some nonenolides.

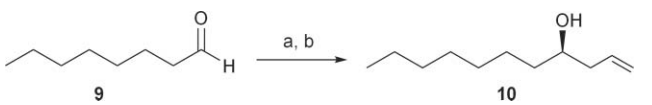
Nonenolides are known as secondary metabolites from microorganisms, plants and animals and exhibit various biological activities including antibacterial, antifungal, phytotoxic or enzyme-inhibitory effects.[1–13] Despite the high structural similarity of the compounds depicted in Fig. 1, the absolute configuration of the carbinol center is inconsistent. To determine both relative and absolute configuration of lactone **1** which was later named hypocreolide A, a synthesis providing access to all four stereoisomers was envisaged. Disconnection of the ester bond and the olefin indicate that the formation of the 10-membered ring would be feasible using either an esterification/ring closing metathesis (path A) or a cross metathesis/macrolactonization sequence (path B, Scheme 1).[14–22]



Scheme 1 Retrosynthetic analysis for hypocreolide A. RCM: Ring closing metathesis, CM: cross metathesis.

Results and discussion

The southwestern fragment, homoallylic alcohol (**10**), could be prepared in high yield and 97% enantiomeric excess (GC) from octanal and allyltributylstannane in a Maruoka-Keck allylation using a preformed Ti-BINOL complex (Scheme 2).[23,24]



Scheme 2 Synthesis of key intermediate **10**: a) $TiCl_4$, $Ti(O^iPr)_4$, Ag_2O, (*S*)-1,1′-binaphthol, CH_2Cl_2; b) allyl tributylstannane, CH_2Cl_2,–15 °C → 0 °C. 94%, ee (GC) 97%. *Ent*-**10**: 84%, ee (GC) 96%.

The northeastern fragment **14** was obtained in an overall yield of 52% starting from the reaction of glutaric acid methyl ester chloride with bis(trimethylsilyl)acetylene in the presence of $AlCl_3$.[25,26] The alkynone **12** was reduced to the alkynol *via*

[a]*Universität Hamburg, Institut für Organische Chemie, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany. E-mail: opatz@chemie.uni-hamburg.de; Fax: +49 40 428383834; Tel: +49 40 428384239*
[b]*Institut für Biotechnologie und Wirkstoff-Forschung (IBWF), Erwin-Schrödinger-Straße 56, D-67663 Kaiserslautern, Germany*
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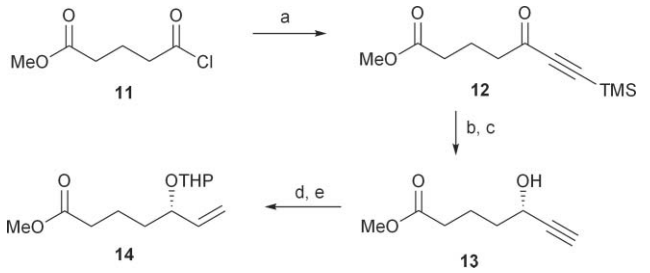
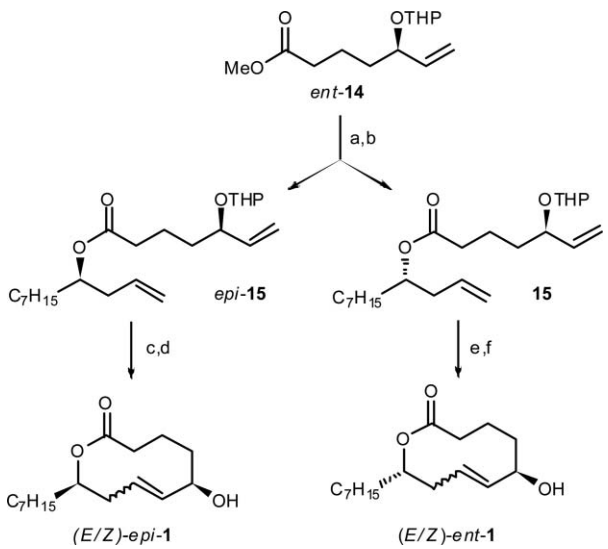
asymmetric transfer hydrogenation using Noyori's Ru-diamine catalyst.[27,28] Desilylation afforded the alkyne **13** (98% ee, GC).[29] Lindlar hydrogenation and THP protection furnished fragment **14**.(Scheme 3).



Scheme 3 Sythesis of key intermediate **14** by asymmetric transfer hydrogenation: a) $AlCl_3$, bis(trimethylsilyl)acetylene, CH_2Cl_2, 0 °C, 69%; b) [Ru((*S*,*S*)-Ts-DPEN-Cl(*p*-cymene)] (4 mol%), ^{i}PrOH; c) KF, DMF–H_2O, 82% from **12**, ee (GC) 98%, *ent*-**13** 87% from **12**, ee (GC) 96%; d) H_2, Pb-poisoned Pd/$CaCO_3$, quinoline, MeOH; e) PPTS, 3,4-dihydro-2*H*-pyran, CH_2Cl_2, 90% from **13**. *Ent*-**14** 62% from *ent*-**13**.

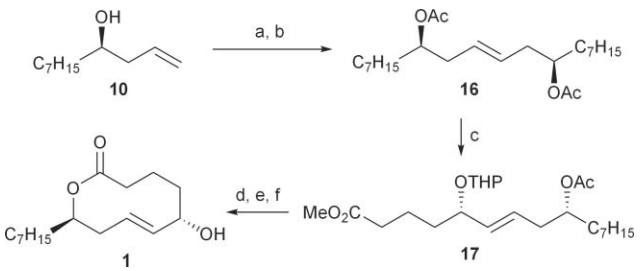
Attempts to prepare hypocreolide A according to path A (Scheme 1) by means of saponification of methyl ester *ent*-**14**, Yamaguchi esterification[30] with alcohol **10**, and subsequent ring closing metathesis using Grubbs' second generation catalyst gave a 3 : 2-mixture of the (*Z*)- and the (*E*)-configured lactones *epi*-**1** (Scheme 4). Comparison of the products with the natural lactone revealed however that hypocreolide A was not present. In particular, the coupling pattern of H-5 in the (*E*)-isomer differed significantly while its ^{1}H chemical shifts were roughly in accordance with those of **1**.



Scheme 4 Ring closing metathesis: a) LiOH, THF–MeOH–H_2O (2/1/1), 0 °C; b) 2,4,6-trichlorobenzoyl chloride, NEt_3, **10** or *ent*-**10**, DMAP, THF, **15**: 64% from *ent*-**14**; *epi*-**15**: 72% from *ent*-**14** c) [$RuCl_2$(IMes)(PCy_3)=CHPh], CH_2Cl_2, reflux, d) PPTS, *p*-TsOH, EtOH, 44% (crude) from *epi*-**15**; e) PPTS, MeOH; f) [$RuCl_2$(IMes)(PCy_3)=CHPh], CH_2Cl_2, reflux, 26% from **15**

Thus, the absolute configuration of the alcohol component **10** was inverted in order to obtain the correct diastereomer. The outcome of the RCM of **15** proved to depend critically on the catalyst. While Fürstner's (PCy_3)$_2Cl_2$–Ru(3-phenylindenylid-1-ene) complex[31] gave no reaction, the second generation Hoveyda-Grubbs catalyst[32] did produce a roughly equimolar *E*/*Z*-mixture of *ent*-**1**. In contrast, Grubbs' second generation metathesis catalyst[33] furnished the (*Z*)-isomer exclusively. The yields and selectivities observed for the RCM approach were disappointing and it was eventually decided to attempt the cross metathesis route (path B, Scheme 1) instead.

Homoallylic alcohol **10** was acetylated and dimerized to bisacetate **16** using Grubbs' second generation metathesis catalyst[33] according to a protocol by Blackwell *et al.*[34] A higher loading of the same catalyst was then required to effect the cross metathesis with ester **14** which furnished the (*E*)-configured seco-precursor **17** in 66% yield. The synthesis of **1** was completed by simultaneous saponification and deacetylation followed by Yamaguchi lactonization[30] and acidolytic removal of the THP group. Unfortunately, the yield of 37% could not be increased and all other tested lactonization protocols gave inferior results (Scheme 5).



Scheme 5 Synthesis of hypocreolide A (**1**) by macrolactonization: a) pyridine, Ac_2O; b) [$RuCl_2$(IMes)(PCy_3)=CHPh] (0.7 mol%), CH_2Cl_2, MW, 95% from **10**; c) [$RuCl_2$(IMes)(PCy_3)=CHPh] (15 mol%), **14**, 1,2-dichloroethane, microwave irradiation, 66%; d) LiOH, THF–MeOH–H_2O (2/1/1); e) 2,4,6-trichlorbenzoyl chloride, NEt_3, THF, then DMAP, toluene, reflux; f) PPTS, *p*TsOH, EtOH, 37% from **17**.

Fortunately, not only the spectroscopic data but also the optical rotation of the synthetic material matched those of natural **1**. In addition, a sample of racemic **1** was prepared from *rac*-**10** and *rac*-**14** according to the same procedure. Comparison by chiral GC proved the synthetic material to be enantiopure and identical to hypocreolide A.

Conclusions

In summary, a short catalytic asymmetric synthesis of hypocreolide A from simple starting materials has been developed and allowed the determination of its relative and absolute configuration. Both chiral catalysts are available in either enantiomeric form and the procedure is likely to permit the synthesis of other members of the nonenolide class.

Experimental section

General methods

All anhydrous reactions were carried out avoiding moisture by standard procedures under argon atmosphere. Commercially available reagents were used as received. The solvents were dried by distillation from appropriate drying agents.[35] The petroleum

ether used had a bp range of 60–90 °C. Reactions were monitored by TLC using TLC glass plates (silica gel 60 F_{254}, E. Merck). Flash chromatography was carried out on silica gel (32–63 μm, 60 Å, Acros). ^{1}H-, ^{13}C-, and ^{2}D-NMR spectra were recorded on a Bruker AMX 400, AV 400, or DRX 500 spectrometer. The chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane and are referenced to $CHCl_3$ (7.26 ppm) for ^{1}H NMR and $CDCl_3$ (77.16 ppm) for ^{13}C NMR. The optical rotations were measured with a Krüss Optronic P8000 polarimeter at 25 °C. IR spectra were recorded on a Bruker ALPHA-P FT-IR spectrometer. High resolution mass spectra (HRMS) and mass spectra (MS) were obtained on sector field or quadrupole-TOF mass spectrometers. The ee value determination was carried out by chiral GC using 50% Heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV-1701 as the stationary phase and an FID detector. Meting points were measured on an Apotec melting point apparatus and are uncorrected.

Fermentation and isolation

Microorganisms. Strain IBWF 02002 was isolated from fruiting bodies collected near Kaiserslautern, Germany. It was grown and kept on YMG-medium consisting of glucose 1%, malt extract 1% (Difco Laboratories, Detroit), and yeast extract 0.4% (Hartge Ingredients, Hamburg), in tap water. For solid media, 1.5% agar was added. It is deposited in the strain collection of the IBWF (Institute of Biotechnology and Drug Research, Kaiserslautern).

Fermentation. Fermentations in 500 mL Erlenmeyer flasks containing 200 mL YMG medium were inoculated with four pieces (1 cm × 1 cm) cut from agar slants. The flasks were incubated on a rotary shaker at 120 rpm and 20 °C.

Fermentations on a larger scale were carried out in a Biostat (Braun, Melsungen, Germany) containing 20 L of YMG medium with stirring (120 rpm) and aeration (3 L air per minute) at 20 °C. To prevent foaming, silicone antifoam (Merck, Darmstadt) was added. The fermentor was inoculated with 200 mL of a well grown culture in the same medium. Daily samples were withdrawn and assayed for pH, glucose and maltose content as well as for biological activity towards *Nematospora coryli*. After 4 days when the biological activity had reached a peak and the glucose was almost used up, the fermentation was stopped. Prolonged incubation resulted in decrease of the biological activity. The culture broth was separated by filtration. Mycelia contained no active compounds and were discarded. The culture broth of daily samples was extracted with EtOAc, the organic phase dried with Na_2SO_4, concentrated *in vacuo* and the residue dissolved in MeOH to a concentration of 10 mg mL^{-1} and used for biological assays as well as HPLC analysis.

Isolation of the compound. The broth from two fermentations (30 L) was extracted twice with 12 L EtOAc. The organic phases were combined, dried with Na_2SO_4 and evaporated to yield 4.0 g of an oily crude extract. This was further fractionated by chromatography on silica gel in cyclohexane–EtOAc (column size: 220 × 45 mm; silica gel 60, 63–200 μm particle size; Merck, Darmstadt, Germany). Upon elution with cyclohexane-EtOAc (70 : 30), an antifungal product (70 mg) was obtained which was purified by preparative HPLC with a Jasco modular HPLC system (Groß-Umstadt, Germany) consisting of two binary pumps (PU-1586) and the multi-wavelength detector UV-1570M with a LiChrospher 100 RP-18 column (250 × 25 mm, 5 μm particle size; Merck) and elution with 80% MeCN in water at a flow rate of 7.5 mL min^{-1}. and yielded 24 mg of compound **1**.

Table 1 Antimicrobial activity of compound **1** in the serial dilution test

Organism	MIC/μg mL^{-1}
Fungi	
Candida albicans	50
Nematospora coryli	15
Magnaporthe grisea	25
Mucor miehei	25
Paecilomyces variotii	50
Penicillium notatum	50
Phytophthora infestans	25
Bacteria	
Bacillus brevis	50
B. subtilis	50
Escherichia coli K12	50
Enterobacter dissolvens	50
Micrococcus luteus	50
Proteus vulgaris	50
Pseudomonas fluorescens	50
Staphylococcus aureus	50

Biological evaluation. Antimicrobial activity was determined in the serial dilution test. Bacteria were tested in nutrient broth (Difco), yeasts and fungi in YMG medium. Cytotoxic activity was assayed as described previously[36] with slight modifications. HeLa S3 (ATCC CCL 2.2) and Hep G2 (DSMZ ACC 180) cells were grown in D-MEM (GIBCO, BRL), supplemented with 10% fetal calf serum (GIBCO, BRL), 65 μg mL^{-1} of penicillin G and 100 μg mL^{-1} of streptomycin sulfate. The assays were conducted with 1×10^5 cells mL^{-1} medium.

Nematicidal tests with the plant parasitic *Meloidogyne incognita* and the saprophytic *Caenorhabditis elegans* were carried out as described.[37]

Biological activities

The antimicrobial activities are shown in Table 1. Antifungal activity was weak but more pronounced than the antibacterial activity. Phytotoxic activity was not observed (up to 300 μg mL^{-1}) using *Setaria italica* and *Lepidium sativum* seeds. While *Meloidogyne incognita* was not affected up to 100 μg mL^{-1}, *Caenorhabditis elegans* larvea were killed by 50 μg mL^{-1} of compound **1**. Cytotoxic effects towards human cells started at 5 μg mL^{-1}. For Hep G2 cells and HeLa S3 cells 10 μg mL^{-1} were lethal to more than 90% of the cells.

Structure elucidation

The structure of hypocreolide A (**1**) was elucidated using a combination of spectroscopic techniques. ESI-HRMS indicated a molecular formula of $C_{16}H_{28}O_3$, requiring three double bond equivalents. Since only one C=C double bond and one carbonyl group were contained as judged by NMR, the molecule must contain a single ring. Two-dimensional homo- and heteronuclear NMR experiments (COSY, HSQC, HMBC) revealed a ten-membered lactone with an (*E*)-configured double bond.

Analytical data for 1

Weakly yellow crystals, mp. 28.5–29.5 °C; $[\alpha]_D^{25}$ –23.7 (*c* 0.45, $CHCl_3$); UV (MeOH): No significant absorption above 220 nm; ν_{max} (KBr)/cm^{-1} 3437 (br), 2929 (sh), 2858, 1731 (sh), 1639, 1437 (sh), 1368, 1336, 1256, 1222, 1158, 1107, 1069, 1028, 976, 932, 874, 846; ^{1}H NMR, COSY, TOCSY (400 MHz, $CDCl_3$) δ 5.55 (dddd, *J* 15.7, 10.4, 4.8, 2.3 Hz, 1H, H-7), 5.43 (dd, *J* 15.7, 1.6 Hz, 1H, H-6), 5.01 (m_c, 1H, H-9), 4.41 (br s, 1H, H-5), 2.50–2.39 (m, 2H, H_a-2, H_a-8), 2.18–1.88 (m, 4H, H_b-2, H_a-3, H_a-4, H_b-8), 1.84 (br s, 1H, OH), 1.72–1.49 (m, 4H, H_b-3, H_b-4, H_2-10), 1.43–1.23 (m, 10H, H_2-11, H_2-12, H_2-13, H_2-14, H_2-15), 0.88 (t, *J* 6.9 Hz, 3H, H_3-16) ppm; ^{13}C NMR, HSQC, HMBC (100.6 MHz, $CDCl_3$): δ 176.60 (C-1), 136.76 (C-6), 126.45 (C-7), 76.93 (C-9), 68.59 (C-5), 40.90 (C-8), 36.79 (C-4), 35.89 (C-2), 34.35 (C-10), 31.91 (C-14), 29.51 (C-12), 29.30 (C-13), 26.09 (C-11), 22.78 (C-15), 17.96 (C-3), 14.23 (C-16) ppm; APCI-MS (pos.): *m/z* 269.2 (24%, $[M+H]^+$), 251.2 (100%, $[M–OH]^+$); APCI-MS (neg.): *m/z* 267.2 (18%, $[M-H]^-$), 249.1 (100%, $[M–H_3O]^-$); ESI-HRMS: *m/z* calcd. for $[C_{16}H_{28}O_3+Na]^+$: 291.1931, found: 291.1932.

Synthetic procedures, compound characterization

(*R*)-Undec-1-en-4-ol 10. $Ti(O^iPr)_4$ (0.29 mL, 0.97 mmol, 0.15 eq) was added at 0 °C to a stirred solution of $TiCl_4$ (1M in CH_2Cl_2, 0.32 mL, 0.32 mmol) in CH_2Cl_2 (7 mL). The solution was allowed to warm to room temperature and was stirred for 1 h. Under exclusion of direct light, Ag_2O (0.15 g, 0.65 mmol, 0.10 eq) was added. After stirring for 5 h the mixture was diluted with CH_2Cl_2 (14 mL) and treated with (*S*)-1,1′-binaphthol (0.37 g, 1.30 mmol, 0.20 eq) for 2 h to furnish the chiral catalyst.[23] The suspension was treated with octanal (1.01 mL, 6.47 mmol, 1 eq) and allyl(tributyl)stannane (2.20 mL, 7.12 mmol, 1.1 eq) at –15 °C. The mixture was allowed to warm to 4 °C and was stirred for 16 h. The reaction mixture was diluted with Et_2O (50 mL) and washed with saturated $NaHCO_3$ solution (2 × 30 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (PE/ethyl acetate 6 : 1) affording a yellowish oil (1.04 g, 6.11 mmol, 94%). Enantiomeric excess (GC): 97%.

R_f (PE/ethyl acetate 4 : 1): 0.45; $[\alpha]_D^{25}$ +6.6 (*c* 1.0, CH_2Cl_2); (Lit:[38] $[\alpha]_D^{25}$ +6.51 (*c* 1.04, $CHCl_3$); ν_{max}(film)/cm^{-1} 3358, 3077, 2956, 2925, 2855, 1641, 1465, 993, 911; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.89–5.78 (m, 1H, H-2), 5.17–5.10 (m, 2H, H-1), 3.64 (m_c, 1H, H-4), 2.30 (m_c, 1H, H-3a), 2.18–2.09 (m, 1H, H-3b), 1.50–1.40 (m, 2H, H-5), 1.34–1.22 (m, 10H, H-6/H-7/H-8/H-9/H-10), 0.88 (t, ^3J 6.9 Hz, 3H, H-11) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 135.08 (C-2), 118.22 (C-1), 70.84 (C-4), 42.09 (C-3), 36.98 (C-5), 31.97 (C-9), 29.77/29.42 (C-7/C-8), 25.83 (C-6), 22.80 (C-10), 14.25 (C-11) ppm.

(*S*)-Undec-1-en-4-ol *ent*-10. Octanal (1.01 mL, 6.47 mmol) and allyl(tributyl)stannane (2.20 mL, 7.12 mmol, 1.1 eq) were added at –15 °C to the catalyst, which was prepared from dried $Ti(O^iPr)_4$ (0.29 mL, 0.97 mmol, 0.15 eq), $TiCl_4$ (1M in CH_2Cl_2, 0.32 mL, 0.32 mmol), Ag_2O (0.15 g, 0.65 mmol, 0.10 eq) and (*R*)-1,1′-binaphthol (0.37 g, 1.30 mmol, 0.20 eq). Following the same procedure, the title compound was obtained as a yellowish oil (923 mg, 5.42 mmol, 84%). Enantiomeric excess (GC): 96%. $[\alpha]_D^{25}$ –7.6 (*c* 1.0, CH_2Cl_2).

5-Oxo-7-trimethylsilylhept-6-ynoic acid methyl ester 12[26]. A solution of bis(trimethylsilyl)acetylene (1.82 mL, 8.02 mmol, 1.1 eq) and glutaric acid methyl ester chloride (1.01 mL, 7.29 mmol) in CH_2Cl_2 (12 mL) was added dropwise to a suspension of $AlCl_3$ (1.17 g, 8.75 mmol, 1.2 eq) in CH_2Cl_2 (10 mL) at 0 °C and stirred for 30 min. The reaction mixture was quenched with ice and saturated aq. citric acid (20 mL) and extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The brown residue was purified by column chromatography (PE/ethyl acetate 4 : 1) on silica gel to afford the title compound (1.14 g, 5.04 mmol, 69%) as a yellowish oil.

R_f (PE/ethyl acetate 4 : 1): 0.61; ν_{max}(film)/cm^{-1} 2957, 1737, 1675, 1252, 1112, 841, 761; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 3.68 (s, 3H, OMe), 2.64 (t, ^3J 7.2 Hz, 2H, H-4), 2.37 (t, ^3J 7.3 Hz, 2H, H-2), 1.97 (m_c, 2H, H-3), 0.24 (s, 9H, $SiMe_3$) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 186.83 (C-5), 173.60 (C-1), 101.97 (C-6), 98.28 (C-7), 51.75 (OMe), 44.31 (C-4), 32.95 (C-2), 19.09 (C-3), –0.64 ($SiMe_3$) ppm; the data are in accordance with reported values.[26]

(*S*)-5-Hydroxy-7-trimethylsilylhept-6-ynoic acid methyl ester. A mixture of $((RuCl_2(\eta^6\text{-}p\text{-cymene}))_2)$ (54 mg, 88 μmol), (*S*,*S*)-TsDPEN (65 mg, 175 μmol) and KOH (80 mg, 1.4 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 10 min. The solution was treated with water (5 mL) and the color changed from orange to deep purple. The organic layer was washed with water (2 × 5 mL), dried over CaH_2 and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (1 mL) and added to a solution of **12** (1.00 g, 4.42 mmol) in degassed isopropanol (25 mL) at room temperature. After stirring for 2 h, the solution was concentrated *in vacuo* and the residue was filtered on silica gel (PE/ethyl acetate 4 : 1) to afford the title compound (969 mg, 4.24 mmol, 96%) as a yellowish oil.

R_f (PE/ethyl acetate 4 : 1): 0.37; ν_{max} 3300, 2956, 1738, 1248, 838; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 4.37 (dt, ^3J 5.8 Hz, 5.4 Hz, 1H, H-5), 3.68 (s, 3H, OMe), 2.38 (t, ^3J 7.1 Hz, 2H, H-2), 1.88 (d, ^3J 5.4 Hz, 1H, OH), 1.85–1.69 (m, 4H, H-3/H-4), 0.17 (s, 9H, $SiMe_3$) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 174.06 (C-1), 106.46 (C-6), 89.89 (C-7), 62.56 (C-5), 51.72 (OMe), 37.06 (C-4), 33.70 (C-2), 20.69 (C-3), –0.01 ($SiMe_3$) ppm; ESI-HRMS *m/z* calcd. for $[C_{11}H_{20}O_3Si+Na]^+$: 251.1079, found: 251.1089.

(*R*)-5-Hydroxy-7-trimethylsilylhept-6-ynoic acid methyl ester. A solution of **12** (1.00 g, 4.42 mmol) in degassed isopropanol (5 mL) was treated with Noyori's catalyst (175 μmol, 4 mol%), which was prepared from $[(RuCl_2(\eta^6\text{-}p\text{-cymene}))_2]$ (54 mg, 88 μmol), (*R*,*R*)-TsDPEN (65 mg, 175 μmol) and KOH (80 mg, 1.4 mmol) in CH_2Cl_2 (5 mL). The title compound (981 mg, 4.30 mmol, 97%) was isolated as a yellowish oil.

(*S*)-5-Hydroxyhept-6-ynoic acid methyl ester 13. (*S*)-5-Hydroxy-7-trimethylsilylhept-6-ynoic acid methyl ester (0.98 g, 4.3 mmol) was dissolved in DMF (15 mL) and treated with a solution of KF (0.50 g, 8.6 mmol, 2.0 eq) in water (2 mL) at room temperature. After 30 min, 1 M hydrochloric acid (15 mL) was added and the product was extracted with Et_2O (3 × 30 mL). The

combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE/ethyl acetate 1 : 1) affording (*S*)-5-hydroxy-hept-6-ynoic acid methyl ester (0.57 g, 3.6 mmol, 85%) as a yellowish oil. Yield over two steps from **12**: 82%. Enantiomeric excess (GC): 98%.

R_f (PE/ethyl acetate 1 : 1): 0.57; $[\alpha]_D^{25}$ −14.4 (*c* 1.0, $CDCl_3$); (lit:[39] $[\alpha]_D^{20}$ −18.6, *c* 1.01 in CCl_4); ν_{max}(film)/cm^{-1} 3436, 3286, 2954, 2924, 1731, 1437, 1159 ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 4.39 (dt, ^3J 6.2 Hz, ^4J 2.1 Hz, 1H, H-5), 3.67 (s, 3H, OMe), 2.46 (d, ^4J 2.1 Hz, 1H, H-7), 2.37 (t, ^3J 7.1 Hz, 2H, H-2), 1.88–1.67 (m, 4H, H-3/H-4) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 174.00 (C-1), 84.64 (C-6), 73.32 (C-7), 61.97 (C-5), 51.74 (OMe), 36.96 (C-4), 33.47 (C-2), 20.54 (C-3) ppm; GC-MS: *m*/*z* (%): 102 (13), 101 (24), 97 (5), 96 (9), 95 (8), 83 (6), 79 (54), 78 (7), 77 (16), 74 (39), 70 (31), 68 (9), 67 (6), 65 (7), 59 (28), 55 (38), 53 (23), 52 (59), 51 (25), 50 (21), 45 (5), 44 (7), 43 (34), 42 (100), 41 (40), 40 (13).

(*R*)-5-Hydroxyhept-6-ynoic acid methyl ester *ent*-13. Treatment of (*R*)-5-hydroxy-7-trimethylsilylhept-6-ynoic acid methyl ester (1.70 g, 7.44 mmol) with KF (0.87 g, 15.0 mmol, 2 eq) in DMF–H_2O (4 mL/0.5 mL) gave the title compound (1.05 g, 6.72 mmol, 90%) as a yellowish oil. Yield over two steps from **12**: 87%. Enantiomeric excess (GC): 96%.

$[\alpha]_D^{25}$ +12.1 (*c* 0.5, $CDCl_3$).

(*S*)-5-Hydroxyhept-6-enoic acid methyl ester. (*S*)-5-Hydroxyhept-6-ynoic acid methyl ester (0.50 g, 3.2 mmol) and quinoline (0.30 mL, 2.5 mmol) were dissolved in methanol (10 mL). Lindlar's catalyst (5% Pd on $CaCO_3$, poisoned with Pb, 55 mg) was added and the reaction mixture was stirred at room temperature under H_2 atmosphere until 1 eq. was absorbed. After stirring for 30 min under nitrogen atmosphere, the catalyst was filtered through Celite® and washed with hot methanol (3 × 70 mL). The filtrate was concentrated, treated with 1 M hydrochloric acid (10 mL), and extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE/ethyl acetate 10 : 1) affording the title compound (0.49 g, 3.1 mmol, 97%) as a yellowish oil.

R_f (PE/ethyl acetate 2 : 1): 0.54; $[\alpha]_D^{25}$ +3.2 (*c* 0.1, $CDCl_3$); ν_{max}(film)/cm^{-1} 3435, 2952, 2872, 1734, 1437; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.86 (ddd, $^3J_{trans}$ 16.9 Hz, $^3J_{cis}$ 10.5 Hz, ^3J 6.2 Hz, 1H, H-6), 5.24 (pseudo-d, J_{app} ~ 17.2 Hz, 1H, H-7$_{trans}$), 5.12 (pseudo-d, J_{app} ~ 10.4 Hz, 1H, H-7$_{cis}$), 4.11 (m_c, 1H, H-5), 3.67 (s, 3H, OMe), 2.35 (t, ^3J 7.3 Hz, 2H, H-2), 1.78–1.65 (m, 2H, H-3), 1.64–1.53 (m, 2H, H-4) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 174.18 (C-1), 141.02 (C-6), 115.05 (C-7), 72.87 (C-5), 51.68 (OMe), 36.41 (C-4), 33.94 (C-2), 20.88 (C-3) ppm; EI-MS: *m*/*z* (%) 127 (8), 126 (11), 102 (27), 101 (8), 99 (6), 98 (29), 85 (5), 83 (10), 81 (14), 74 (100), 71 (6), 70 (9), 59 (19), 57 (39), 55 (28), 44 (7), 43 (37), 42 (20), 41 (21), 40 (31), 39 (15); ESI-HRMS *m*/*z* calcd. for $[2C_8H_{14}O_3–CH_3OH+Na]^+$: 307.1521, found: 307.1516.

(*R*)-5-Hydroxyhept-6-enoic acid methyl ester. (*R*)-5-Hydroxyhept-6-ynoic acid methyl ester (502 mg, 3.21 mmol) was treated with quinoline (0.30 mL, 2.5 mmol), Lindlar's catalyst (5% Pd on $CaCO_3$, poisoned with Pb, 55 mg) and H_2 in methanol (10 mL). The catalyst was washed with cold methanol (1 × 50 mL) yielding the title compound as a yellowish oil (336 mg, 2.12 mmol, 66%).

(5*S*)-5-(Tetrahydropyran-2′-yloxy)-hept-6-enoic acid methyl ester 14. (*S*)-5-Hydroxy-hept-6-enoic acid methyl ester (42 mg, 0.27 mmol) was dissolved in CH_2Cl_2 (3 mL), treated with PPTS (6.5 mg, 26 μmol, 0.10 eq) and 3,4-dihydro-2*H*-pyran (DHP) (0.60 mL, 0.66 mmol, 2.5 eq), and stirred at room temperature for 16 h. Saturated aq. $NaHCO_3$ (10 mL) was added, followed by extraction with Et_2O (2 × 15 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 4 : 1), affording the title compound (60 mg, 0.25 mmol, 93%, 90% from **13**, diastereomeric mixture 1 : 1) as a yellow oil.

R_f (cyclohexane/ethyl acetate 1 : 1): 0.80; $[\alpha]_D^{25}$ −25 (*c* 0.4, $CDCl_3$); ν_{max}(film)/cm^{-1} 2941, 2870, 1738; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.86 (ddd, $^3J_{trans}$ 17.2 Hz, $^3J_{cis}$ 10.5 Hz, ^3J 6.7 Hz, 1H, H-6^A), 5.62 (ddd, $^3J_{trans}$ 17.6 Hz, $^3J_{cis}$ 9.9 Hz, ^3J 7.8 Hz, 1H, H-6^B), 5.28–5.08 (m, 4H, H-7^{A,B}), 4.71–4.67 (m, 1H, H-2′^{A}), 4.66–4.63 (m, 1H, H-2′^{B}), 4.15–3.99 (m, 2H, H-5^{A,B}), 3.93–3.82 (m, 2H, H-6$_a$′^{A,B}), 3.66 (s, 6H, OMe), 3.60–3.37 (m, 2H, H-6$_b$′^{A,B}), 2.39–2.28 (m, 4H, H-2^{A,B}), 1.91–1.46 (m, 20H, H-3^{A,B}/H-4^{A,B}/H-3′^{A,B}/H-4′^{A,B}/H-5′^{A,B}) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 174.19/174.12 (C-1^{A,B}), 139.51/138.36 (C-6^{A,B}), 117.73/115.34 (C-7^{A,B}), 97.94/95.25 (C-2′^{A,B}), 77.78/76.17 (C-5^{A,B}), 62.74/62.50 (C-6′^{A,B}), 51.64/51.62 (OMe), 35.10/34.15/34.10/34.06 (C-2^{A,B}/C-4^{A,B}), 31.03/30.90 (C-3′^{A,B}), 25.71/25.60 (C-5′^{A,B}), 21.17/20.67 (C-3^{A,B}), 19.83/19.75 (C-4′^{A,B}) ppm; ESI-HRMS: *m*/*z* calcd. for $[C_{13}H_{22}O_4+Na]^+$: 265.1416, found: 265.1425.

(5*R*)-5-(Tetrahydropyran-2′-yloxy)-hept-6-enoic acid methyl ester *ent*-14. (*R*)-5-Hydroxy-hept-6-enoic acid methyl ester (59 mg, 0.37 mmol) was treated with PPTS (9.5 mg, 38 μmol, 0.1 eq) and DHP (87 μL, 0.95 mmol, 2.5 eq) in CH_2Cl_2 (15 mL). Following the same procedure, the title compound (85 mg, 0.35 mmol, 94%, 62% from *ent*-**13**) was obtained as a yellow oil.

(5*R*)-5-(Tetrahydropyran-2′-yloxy)-hept-6-enoic acid. Methyl ester *ent*-**14** (120 mg, 495 μmol) was dissolved in THF–MeOH–H_2O (2 : 1 : 1, 4 mL) at 0 °C and LiOH (40 mg, 1.5 mmol, 3 eq) was added. After 1 h, the ice bath was removed and the reaction mixture was stirred for another 16 h at room temperature. The reaction was stopped by adding saturated aq. KH_2PO_4 until pH 5 was reached and the acid was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue (110 mg) was used in the next step without further purification.

(5*R*,4″*S*)-5-(Tetrahydropyran-2′-yloxy)-hept-6-enoic acid undec-1″-en-4″-yl ester 15. 2,4,6-Trichlorobenzoyl chloride (73 μL, 482 μmol, 1.0 eq) and triethylamine (71 μL, 506 μmol, 1.1 eq) were added to a solution of (5*R*)-5-(tetrahydropyran-2′-yloxy)-hept-6-enoic acid (110 mg, 482 μmol) in THF (4 mL). After 30 min, a solution of (*S*)-**10** (97 mg, 570 μmol, 1.2 eq) and *N*,*N*-dimethylaminopyridine (DMAP, 61 mg, 506 μmol, 1.1 eq) in THF (2 mL) was added. The reaction was terminated after 20 h by quenching with saturated aq. NH_4Cl (5 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, and concentrated *in vacuo*.

The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 20 : 1) to afford the title compound (120 mg, 315 μmol, 64% from *ent*-**14**) as a yellowish oil, d.r. ~ 1 : 1.

R_f (cyclohexane/ethyl acetate 5 : 1): 0.71; $[\alpha]_D^{25}$ +10.2 (*c* 1.0, $CDCl_3$); ^{1}H NMR, COSY (500 MHz, $CDCl_3$) δ 5.86 (ddd, $^3J_{trans}$ 17.2 Hz, $^3J_{cis}$ 10.4 Hz, ^3J 6.7 Hz, 1H, H-6^A), 5.74 (ddt, $^3J_{trans}$ 17.1 Hz, $^3J_{cis}$ 10.1 Hz, ^3J 7.1 Hz, 2H, H-2″^{A,B}), 5.66–5.57 (m, 1H, H-6^B), 5.26–5.09 (m, 4H, H-7^{A,B}), 5.09–5.01 (m, 4H, H-1″^{A,B}), 4.92 (pseudo-q, J_{app} ~ 6.2 Hz, 2H, H-4″^{A,B}), 4.71–4.67 (m, 1H, H-2′^{A}), 4.67–4.64 (m, 1H, H-2′^{B}), 4.12–4.06 (m, 1H, H-5^B), 4.06–4.02 (m, 1H, H-5^A), 3.92–3.83 (m, 2H, H-6$_a$′^{A,B}), 3.52–3.43 (m, 2H, H-6$_b$′^{A,B}), 2.36–2.26 (m, 8H, H-2^{A,B}, H-3″^{A,B}), 1.89–1.46 (m, 24H, H-3^{A,B}, H-4^{A,B}, H-3′^{A,B}, H-4′^{A,B}, H-5′^{A,B}, H-5″^{A,B}), 1.35–1.19 (m, 20H, H-10″^{A,B}, H-9″^{A,B}, H-8″^{A,B}, H-7″^{A,B},H-6″^{A,B}), 0.87 (t, ^3J 6.9 Hz, 6H, H-11″^{A,B}) ppm; ^{13}C NMR, HSQC, HMBC (126 MHz, $CDCl_3$) δ 173.41/173.34 (C-1), 139.55/138.41 (C-6), 134.04 (C-2″), 117.68/117.66 (C-7/C-1″), 97.99/95.03 (C-2′), 76.11 (C-5), 73.39/73.24 (C-4″), 62.74/62.31 (C-6′), 38.85 (C-3″), 35.14 (C-4), 34.13 (C-2), 33.77 (C-5″), 31.93 (C-9″), 30.86 (C-3′), 29.57/29.33 (C-8″/C-7″), 25.73/25.60 (C-5′), 25.46 (C-6″), 22.36 (C-10″), 21.36 (C-3), 19.82/19.62 (C-4′), 14.23 (C-11″) ppm; GC-MS: *m*/*z* (%) 127 (54), 109 (10), 97 (7), 86 (6), 85 (100), 84 (11), 83 (11), 81 (15), 79 (5), 69 (19), 67 (20), 57 (13), 56 (10), 55 (35), 54 (16), 53 (6), 43 (24), 42 (15), 41 (40), 39 (16); ESI-MS: *m*/*z* 403.30 (100%, $[M+Na]^+$); ESI-HRMS: *m*/*z* calcd. for $[C_{23}H_{40}O_4+Na]^+$: 403.2824, found: 403.2835.

(5*R*,4″*R*)-5-(Tetrahydropyran-2′-yloxy)-hept-6-enoic acid undec-1″-en-4″-yl ester *epi*-15. was prepared accordingly from (5*R*)-5-(tetrahydropyran-2′-yloxy)-hept-6-enoic acid (18 mg, 74 μmol) and (*R*)-**10** (15 mg, 89 μmol) to afford the title compound (20 mg, 53 μmol, 72%) as a yellowish oil, d.r. ~ 1 : 1.

R_f (cyclohexane/ethyl acetate 5 : 1): 0.70; $[\alpha]_D^{25}$ +38.4 (*c* 1.0, $CDCl_3$); ν_{max}(film)/cm^{-1} 2925, 2856, 1732; ^{1}H NMR, COSY (500 MHz, $CDCl_3$) δ 5.86 (ddd, $^3J_{trans}$ 17.2 Hz, $^3J_{cis}$ 10.4 Hz, ^3J 6.7 Hz, 1H, H-6^A), 5.74 (ddt, $^3J_{trans}$ 17.2 Hz, $^3J_{cis}$ 10.1 Hz, ^3J 7.1 Hz, 2H, H-2″^{A,B}), 5.62 (ddd, $^3J_{trans}$ 17.7 Hz, $^3J_{cis}$ 9.8 Hz, ^3J 7.8 Hz, 1H, H-6^B), 5.26–5.09 (m, 4H, H-7^{A,B}), 5.09–5.01 (m, 4H, H-1″^{A,B}), 4.95–4.88 (m, 2H, H-4″^{A,B}), 4.70–4.63 (m, 2H, H-2′^{A,B}), 4.16–4.00 (m, 2H, H-5^{A,B}), 3.94–3.82 (m, 2H, H-6$_a$′^{A,B}), 3.54–3.42 (m, 2H, H-6$_b$′^{A,B}), 2.37–2.22 (m, 8H, H-2^{A,B}, H-3″^{A,B}), 1.88–1.45 (m, 24H, H-3^{A,B}, H-4^{A,B}, H-3′^{A,B}, H-4′^{A,B}, H-5′^{A,B}, H-5″^{A,B}), 1.37–1.17 (m, 20H, H-10″^{A,B}, H-9″^{A,B}, H-8″^{A,B}, H-7″^{A,B},H-6″^{A,B}), 0.87 (t, ^3J 6.9 Hz, 6H, H-11″^{A,B}) ppm. ^{13}C-NMR, HSQC, HMBC (126 MHz, $CDCl_3$) δ 173.56 (C-1), 138.40 (C-6), 134.03 (C-2″), 117.71/117.69 (C-7/C-1″), 97.99/95.02 (C-2′), 77.37/76.10 (C-5), 73.30/73.25 (C-4″), 62.31 (C-6′), 38.85 (C-3″), 35.12 (C-4), 34.66/34.62/33.76 (C-2/C-5″), 31.92 (C-9″), 31.03 (C-3′), 29.56/29.33 (C-8″/C-7″), 25.73/25.60/25.47 (C-5′/C-6″), 22.78 (C-10″), 21.36 (C-3), 19.83/19.62 (C-4′), 14.24 (C-11″) ppm. ESI-HRMS: *m*/*z* calcd. for $[C_{23}H_{40}O_4+Na]^+$: 403.2824, found: 403.2820.

(5*R*,4′*S*)-5-Hydroxyhept-6-enoic acid undec-1′-en-4′-yl ester. A solution of THP ether **15** (100 mg, 263 μmol) in ethanol (12 mL) was treated with PPTS (7.0 mg, 28 μmol, 0.1 eq) and *p*-TsOH monohydrate (5.0 mg, 26 μmol, 0.1 eq). After 16 h, saturated $NaHCO_3$ solution (5 mL) was added and the mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 17 : 1) affording the title compound (59 mg, 199 μmol, 76%) as a yellow oil.

R_f (cyclohexane/ethyl acetate 3 : 1): 0.42; $[\alpha]_D^{25}$ −18.7 (*c* 1.0, $CDCl_3$); ν_{max}(film)/cm^{-1} 3054, 2927, 2857, 1726, 1422, 1264, 733, 703; ^{1}H NMR, COSY (500 MHz, $CDCl_3$) δ 5.86 (ddd, $^3J_{trans}$ 16.9 Hz, $^3J_{cis}$ 10.4 Hz, ^3J 6.2 Hz, 1H, H-6), 5.74 (ddt, $^3J_{trans}$ 17.2 Hz, $^3J_{cis}$ 10.1 Hz, ^3J 7.1 Hz, 1H, H-2′), 5.23 (pseudo-d, J_{app} ~ 16.9 Hz, 1H, H-7$_{trans}$), 5.11 (pseudo-d, J_{app} ~ 10.4 Hz, 1H, H-7$_{cis}$), 5.09–5.02 (m, 2H, H-1′), 4.98–4.87 (pseudo-quin, J_{app} ~ 6.4 Hz, 1H, H-4′), 4.11 (m_c, 1H, H-5), 2.36–2.23 (m, 4H, H-2/H-3′), 1.80–1.64 (m, 2H, H-3), 1.64–1.47 (m, 4H, H-4/H-5′), 1.34–1.21 (m, 10H, H-10′/H-9′/H-8′/H-7′/H-6′), 0.87 (t, ^3J 6.9 Hz, 3H, H-11′) ppm; ^{13}C NMR, HSQC, HMBC (126 MHz, $CDCl_3$) δ 173.43 (C-1), 141.03 (C-6), 134.02 (C-2′), 117.69 (C-1′), 115.01 (C-7), 73.40 (C-4′), 72.87 (C-5), 38.84 (C-3′), 36.43 (C-4), 34.43 (C-2), 33.77 (C-5′), 31.92 (C-9′), 29.55/29.33 (C-8′/C-7′), 25.47 (C-6′), 22.78 (C-10′), 20.95 (C-3), 14.23 (C-11′) ppm; ESI-HRMS: *m*/*z* calcd. for $[C_{18}H_{32}O_3+Na]^+$: 319.2249, found: 319.2259.

(5*R*,6*Z*,9*S*)-5,9-Dihydroxyhexadec-6-enoic acid-ϑ-lactone (*Z*)-*ent*-1. A solution of (5*R*,4′*S*)-5-hydroxyhept-6-enoic acid undec-1′-en-4′-yl ester (20 mg, 67 μmol) in CH_2Cl_2 (20 mL) was treated with Grubbs 2nd generation catalyst[33] ([$RuCl_2$(IMes)(PCy_3)=CHPh], 5.0 mg, 8.0 μmol) and irradiated with microwaves for 40 min (100 W, 40 min, 50 °C, reflux). Another portion of the catalyst (1 mg) was added and irradiation was repeated under identical conditions for 20 min. The reaction mixture was concentrated *in vacuo* and the residue was purified by preparative layer chromatography (cyclohexane/ethyl acetate 3 : 1) to afford the title compund (4.7 mg, 18 μmol, 26%) as a colorless oil.

R_f (cyclohexane/ethyl acetate 3 : 1): 0.26; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (dt, J_t ~ 10 Hz, J_d 6.8 Hz, 1H, H-7), 5.51 (t, *J* 10.2 Hz, 1H, H-6), 4.90 (m_c, 1H, H-6), 4.67 (dt, J_t 9.9 Hz, J_d 3.5 Hz, 1H, H-5), 2.43 (m_c, 1H), 2.26–2.36 (m, 2H), 2.13 (ddd, *J* 13.2, 6.7, 2.0 Hz, 1H), 1.93–2.08 (m, 2H), 1.50–1.74 (m, 4H), 1.22–1.38 (m, 10H, H_2-11, H_2-12, H_2-13, H_2-14, H_2-15), 0.88 (t, *J* 6.8 Hz, 3H, H_3-16) ppm; The sample contained impurities and was not further characterized.

(5*R*,9*R*)-5,9-Dihydroxyhexadec-6-enoic acid-ϑ-lactone, *epi*-hypocreolide A *epi*-1. To a solution of *epi*-**15** (12 mg, 30 μmol) in CH_2Cl_2 (20 mL), Grubbs 2nd generation catalyst[33] ([$RuCl_2$(IMes)(PCy_3)=CHPh], 1.0 mg, 2.0 μmol) was added and the mixture was refluxed for 20 h. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography over silica gel (cyclohexane/ethyl acetate 20 : 1). The isolated product was dissolved in ethanol (1 mL) and PPTS (1.0 mg, 6.0 μmol) was added, followed by stirring at room temperature for 16 h. Since THP deprotection was incomplete, *p*-TsOH monohydrate (2.0 mg, 12 μmol) was added and stirring was continued for 16 h. After addition of water and saturated $NaHCO_3$ solution, the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to yield the title compound (3.5 mg, crude yield 44%) as a brown oil. *E*/*Z*-ratio 2 : 3.

^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.65 (td, *J* 10.8, 6.3, 1H, H-7^Z), 5.62–5.49 (m, 2H, H-7^E, H-6^Z), 5.32 (dd, *J* 15.3, 9.4, 1H, H-6^E), 5.01 (dddd, *J* 11.4, 8.1, 4.7, 3.2 Hz, 1H, H-9^E), 4.83 (dtd,

J 9.1, 5.4, 1.3 Hz, 1H, H-9^{Z}), 4.42 (td, *J* 10.1, 4.1 Hz, 1H, H-5^{Z}), 4.01 (td, *J* 9.4, 3.4 Hz, 1H, H-5^{E}), 2.91 (ddd, *J* 14.5, 10.8, 5.4, 1H, H-8$_{a}^{Z}$), 2.54 (ddd, *J* 14.8, 7.2, 2.3 Hz, 1H, H-2$_{a}^{Z}$), 2.49–1.19 (m, 38H, H-8^{E}, H-2^{E}, H-8$_{b}^{Z}$, H-2$_{b}^{Z}$, H-3, H-4, H-10, H-11, H-12, H-13, H-14, H-15), 0.88 (t, *J* 6.9 Hz, 3H, H-16^{Z}), 0.88 (t, *J* 6.9 Hz, 3H, H-16^{E}) ppm; the sample contained impurities and was not further characterized.

(*R*)-Undec-1-en-4-yl acetate. To (*R*)-Undec-1-en-4-ol **10** (150 mg, 881 µmol), pyridine (94 µL, 1.17 mmol, 1.3 eq) and acetic anhydride (93 µL, 0.98 mmol, 1.1 eq) were added and the resulting solution was stirred at room temperature for 16 h. The reaction mixture was diluted with Et_2O (20 mL) and washed with 1 M hydrochloric acid (2 × 5 mL) and brine (10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give the title compound (181 mg, 852 µmol, 97%) as a yellowish oil.

R_f (PE/ethyl acetate 3 : 1): 0.81; $[\alpha]_D^{25}$ +21.4 (*c* 1.0, $CDCl_3$); ν_{max}(film)/cm^{-1} 2926, 2857, 1738, 1235; ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.75 (ddt, $^3J_{trans}$ 17.1 Hz, $^3J_{cis}$ 10.1 Hz, ^3J 7.0 Hz, 1H, H-2), 5.10–5.02 (m, 2H, H-1), 4.91 (pseudo-quin, J_{app} ~ 6.3 Hz, 1H, H-4), 2.35–2.24 (m, 2H, H-3), 2.03 (s, 3H, Me), 1.56–1.48 (m, 2H, H-5), 1.33–1.19 (m, 10H, H-6/H-7/H-8/H-9/H-10), 0.87 (t, ^3J 6.8 Hz, 3H, H-11) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 170.94 (C=O), 134.01 (C-2), 117.66 (C-1), 73.54 (C-4), 38.79 (C-3), 33.75 (C-5), 31.93 (C-9), 29.57/29.32 (C-7/C-8), 25.45 (C-6), 22.78 (C-10), 21.37 (OCO*C*H$_3$), 14.22 (C-11) ppm; ESI-MS: *m*/*z* 235.17 ([M+Na]$^+$, 100%); the spectroscopic data match those reported in the literature.[40]

(8*R*,13*R*)-8,13-Bis(acetoxy)eicos-10-ene 16. To a solution of (*R*)-undec-1-en-4-yl acetate (76.6 mg, 361 µmol) in CH_2Cl_2 (1 mL), Grubbs second generation catalyst[33] ([$RuCl_2$(IMes)(PCy_3)=CHPh], 2.0 mg, 2.4 µmol, 0.7 mol-%) was added and the mixture was irradiated with microwaves (100 W, 50 °C) for 1 h. The reaction mixture was concentrated *in vacuo*. The oily residue was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 20 : 1) affording the title compound (70.3 mg, 177 µmol, 98%) as a brownish oil.[34] *E*/*Z*-ratio 3.3 : 1 (^{1}H NMR).

R_f (cyclohexane/ethyl acetate 3 : 1): 0.78; $[\alpha]_D^{25}$ +55 (*c* 1.0, $CDCl_3$); ν_{max}(film)/cm^{-1} 1380, 902, 722; ^{1}H NMR, COSY (*E*-isomer, 400 MHz, $CDCl_3$) δ 5.41 (m_c, 2H, H-10/H-11), 4.85 (m_c, 2H, H-8/H-13), 2.38–2.17 (m, 4H, H-9/H-12), 2.03 (s, 6H, OCOCH$_3$), 1.59–1.43 (m, 4H, H-7/H-14), 1.33–1.21 (m, 20H, H-2/H-3/H-4/H-5/H-6/H-5/H-15/H-16/H-17/H-18/H-19), 0.88 (t, ^3J 6.9 Hz, 6H, H-1/H-20) ppm; ^{13}C NMR, HSQC, HMBC (*E*-isomer, 101 MHz, $CDCl_3$) δ 170.89 (C=O), 128.49 (C-10/C-11), 73.84 (C-8/C-13), 37.44 (C-9/C-12), 33.62 (C-7/C-14), 31.93 (C-3/C-18), 29.60/29.35 (C-4/C-5/C-16/C-17), 25.46 (C-6/C-15), 22.78 (C-2/C-19), 21.39 (OCO*C*H$_3$), 14.22 (C-1/C-20) ppm; ESI-MS: *m*/*z* 419.31 ([M+Na]$^+$, 100%); ESI-HRMS: *m*/*z* calcd. for [$C_{24}H_{44}O_4$+Na]$^+$: 419.3137, found: 419.3146; characteristic data of the *Z*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (m_c, 2H, H-10/H-11), 4.87 (m_c, 2H, H-(/H-13) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 127.29 (C-10/C-11), 73.97 (C-8/C-13), 33.87 (C-7/C-14), 32.26 (C-3/C-18), 25.56 (C-6/C-15) ppm;

(5*S*,6*E*,9*R*)-9-Acetoxy-5-(tetrahydropyran-2′-yloxy)-hexadec-6-enoic acid methyl ester 17. Diacetate **16** (34 mg, 86 µmol, 1.6 eq), ester **14** (13 mg, 54 µmol) and Grubbs 2nd generation catalyst ([$RuCl_2$(IMes)(PCy_3)=CHPh], 7.0 mg, 8.2 µmol, 15 mol-%) were dissolved in 1,2-dichloroethane (2 mL) and irradiated in microwave (100 W, 100 °C) for 40 min. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 15 : 1) affording the title compound (15 mg, 35 µmol, 66%) as a brownish oil.

R_f (cyclohexane/ethyl acetate 10 : 1): 0.10; $[\alpha]_D^{25}$ +9.9 (*c* 1.0, $CDCl_3$); ^{1}H NMR, COSY (400 MHz, $CDCl_3$) δ 5.63–5.49 (m, 4H, H-6^{A,B}/H-7^{A,B}), 4.88 (m_c, 2H, H-9^{A,B}), 4.69–4.65 (m, 2H, H-2′^{A,B}), 4.09–3.97 (m, 2H, H-5^{A,B}), 3.88–3.81 (m, 2H, H-6′$_{a}^{A,B}$), 3.66 (s, 6H, OMe^{A,B}), 3.51–3.40 (m, 2H, H-6′$_{b}^{A,B}$), 2.37–2.25 (m, 8H, H-2^{A,B} /H-8^{A,B}), 2.02 (s, 6H, OCOCH$_3^{A,B}$), 1.88–1.44 (m, 24H, H-3′^{A,B} /H-4′^{A,B} /H-5′^{A,B} /H-10^{A,B} /H-3^{A,B} /H-4^{A,B}), 1.35–1.18 (m, 20H, H-15^{A,B} /H-14^{A,B} /H-13^{A,B} /H-12^{A,B} /H-11^{A,B}), 0.87 (t, ^3J 6.8 Hz, 6H, H-16^{A,B}) ppm; ^{13}C NMR, HSQC, HMBC (101 MHz, $CDCl_3$) δ 174.2‡ (COOMe), 170.9‡ (O*C*OCH$_3$), 134.45/133.32/129.47/126.80 (C-6/C-7), 97.65/94.93 (C-2′), 77.08/75.40 (C-5), 73.72/73.53 (C-9), 62.62/62.52 (C-6′), 51.63 (OMe), 37.13/37.04 (C-8), 35.29/34.29/34.15/34.11/33.77/33.71 (C-2/C-4/C-3′), 31.93 (C-14), 30.97 (C-10), 29.86/29.59/29.34/29.33 (C-13/C-12), 25.72/25.64/25.45/25.41 (C-11/C-5′), 22.78 (C-15), 21.37 (OCO*C*H$_3$), 21.27/20.78 (C-3), 19.82/19.77 (C-3), 14.23 (C-16) ppm; ESI: *m*/*z* 449.29 ([M+Na]$^+$, 100%); ESI-HRMS: *m*/*z* calcd. for [$C_{24}H_{42}O_6$+Na]$^+$: 449.2879, found: 449.2890.

(5*S*,6*E*,9*R*)-5,9-Dihydroxyhexadec-6-enoic acid-ϑ-lactone, Hypocreolide A 1. Ester **17** (15 mg, 35 µmol) was dissolved in a mixture of THF–MeOH–H_2O (2 : 1 : 1, 2 mL), treated with LiOH (4.0 mg, 0.17 mmol, 4.8 eq), and stirred at room temperature. After 5 h, the solution was diluted with Et_2O (10 mL) and washed with saturated aq. KH_2PO_4 (5 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue (18 mg) was taken up in THF (3 mL) and stirred for 50 min with 2,4,6-trichlorobenzoyl chloride (30 µL, 0.20 mmol, 5.6 eq) and triethylamine (30 µL, 0.21 mmol, 6.1 eq). The reaction mixture was diluted with toluene (7 mL), filtered and added dropwise over a period of 2.5 h to a refluxing solution of DMAP (30 mg, 0.25 mmol, 7 eq) in toluene (20 mL). After stirring for another 30 min, the reaction mixture was allowed to cool to room temperature and was washed with 1 M hydrochloric acid (10 mL), saturated aq. $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in ethanol (3 mL), treated with catalytic amounts of PPTS and stirred for 16 h at room temperature. The reaction mixture was diluted with ethyl acetate (15 mL) and washed with ice cooled saturated $NaHCO_3$-solution (5 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude cyclization product was dissolved in ethanol (3.0 mL) and PPTS (5.0 mg, 20 µmol) as well as *p*-TsOH·H_2O (1.0 mg, 5 µmol) were added. The solution was stirred for 18 h at room temperature. After addition of ice water (10 mL) and saturated aq. $NaHCO_3$ (5 mL), the product was extracted with ethyl acetate (2 × 10 mL). Drying over Na_2SO_4 and concentration

‡ determined from HMBC

in vacuo afforded a crude product which was purified by column chromatography on silica gel (cyclohexane/ethyl acetate 10 : 1) to afford the title compound (3.5 mg, 13 μmol, 37%) as a colorless oil.

R_f (cyclohexane/ethyl acetate 3 : 1): 0.26; $[\alpha]_D^{25}$ –29 (*c* 0.35, $CDCl_3$); spectroscopic data were identical to those of the natural product (*vide supra*).

Racemic Hypocreolide A *rac*-1. An analytical sample of *rac*-**1** was prepared from *rac*-**10** and *rac*-**14** according to the procedure given for **1**. The undesired diastereomer was removed by column chromatography on silica gel (cyclohexane/ethyl acetate 10 : 1). Comparison of natural and synthetic **1** with *rac*-**1** by chiral GC confirmed the identical absolute configuration for both samples.

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