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Pheromone synthesis. Part 250: Determination of the stereostructure of CH503, a sex pheromone of male *Drosophila melanogaster*, as (3*R*,11*Z*,19*Z*)-3-acetoxy-11,19-octacosadien-1-ol by synthesis and chromatographic analysis of its eight isomers☆

Yasumasa Shikichi [a], Kazuaki Akasaka [b], Shigeyuki Tamogami [c], Shruti Shankar [d,e], Joanne Y. Yew [d,e], Kenji Mori [a,*]

[a] *Photosensitive Materials Research Center, Toyo Gosei Co., Ltd, 4-2-1 Wakahagi, Inzai-shi, Chiba 270-1609, Japan*
[b] *Shokei Gakuin University, 4-10-1 Yurigaoka, Natori-shi, Miyagi 981-1295, Japan*
[c] *Technical Research Institute, T. Hasegawa Co., Ltd, 29-7 Kariyado, Nakahara-ku, Kawasaki-shi, Kanagawa 211-0022, Japan*
[d] *Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Singapore*
[e] *Department of Biological Sciences, 14 Science Drive 4, National University of Singapore, Singapore 117546, Singapore*

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ABSTRACT

All the eight stereoisomers of 3-acetoxy-11,19-octacosadien-1-ol (**1**), the male sex pheromone (CH503) of *Drosophila melanogaster*, were synthesized from two acetylenic starting materials and the enantiomers of 3,4-epoxy-1-butanol PMB ether. Complete separation of the eight isomers of **1** by reversed phase HPLC at −20 °C was achieved after their esterification with (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid (**27**), and the natural CH503 was found to be (3*R*,11*Z*,19*Z*)-**1**.

1. Introduction

A new pheromone named CH503 was isolated in 2009 by Yew et al. from the male fruit fly *Drosophila melanogaster*, and identified as 3-acetoxy-11,19-octacosadien-1-ol (**1**, Scheme 1).[2] The pheromone **1** is transferred from males to females during mating, remains on the surface of females for at least ten days, and inhibits male courtship. In 2010, we synthesized the enantiomers of (11*Z*,19*Z*)-**1**, and found (3*S*,11*Z*,19*Z*)-**1** to be bioactive, while (3*R*,11*Z*,19*Z*)-**1** was only slightly bioactive.[3] We therefore tentatively concluded that the naturally occurring CH503 might be (3*S*,11*Z*,19*Z*)-**1**, especially because (*Z*)-alkenes, such as (7*Z*,11*Z*)-pentacosadiene[4] and (*Z*)-11-octadecenyl acetate (vaccenyl acetate)[5] were already known as pheromones of *D. melanogaster*.

In pheromone science, it is well established that bioactivity depends on the correct stereochemistry of pheromones.[6–8] In order to definitively determine the stereostructure of the natural CH503, we have now completed the synthesis of all the eight stereoisomers of **1**, established their separation method by HPLC after derivatization, and concluded that the natural CH503 is (3*R*,11*Z*,19*Z*)-**1**.

2. Results and discussion

2.1. Synthesis of the remaining six stereoisomers of CH503 (1)

*2.1.1. Retrosynthetic analysis of CH503 (**1**).* As shown in Scheme 1, the geometries of the double bonds at C-11 and C-19 of **1** can be established by employing either lithium aluminum hydride reduction to give an (*E*)-alkene structure from an alkyne or Lindlar hydrogenation to give the (*Z*)-isomer, and the chirality at C-3 can be fixed by employing Jacobsen's hydrolytic kinetic resolution (HKR)[9–11] of an epoxide intermediate.

Accordingly, the retrosynthetic analysis of the remaining six stereoisomers of **1** as shown in Scheme 1 is similar to that used in

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* Corresponding author. Tel.: +81 3 3816 6889; fax: +81 3 3813 1516; e-mail address: kjk-mori@arion.ocn.ne.jp (K. Mori).

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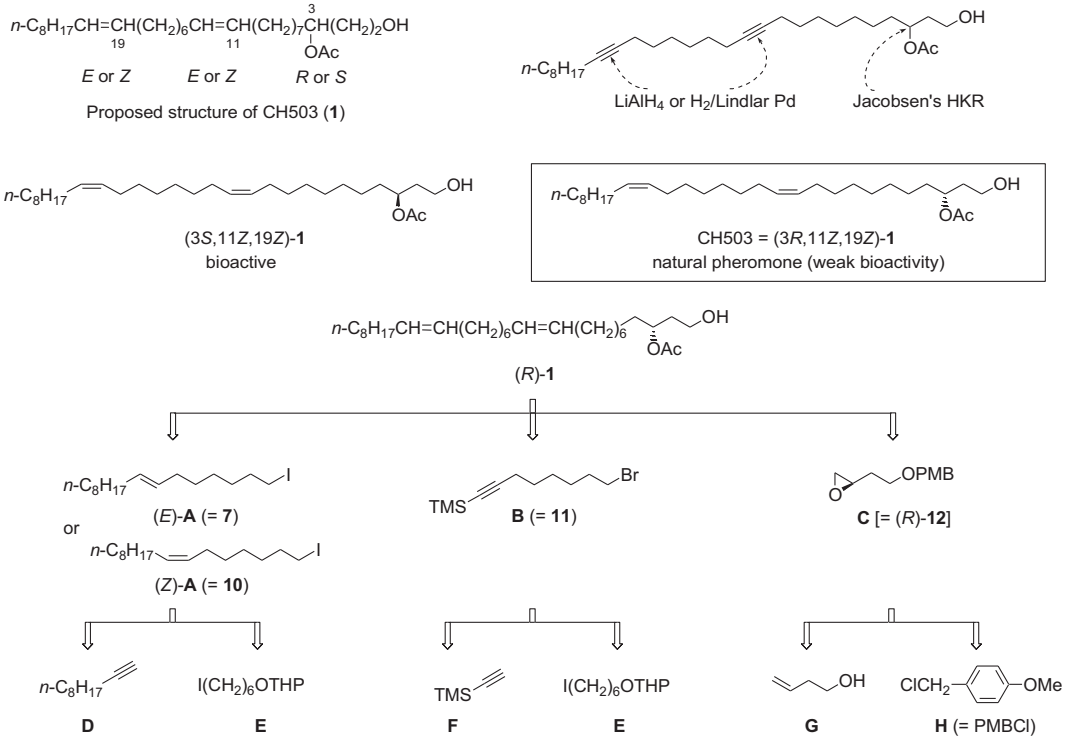
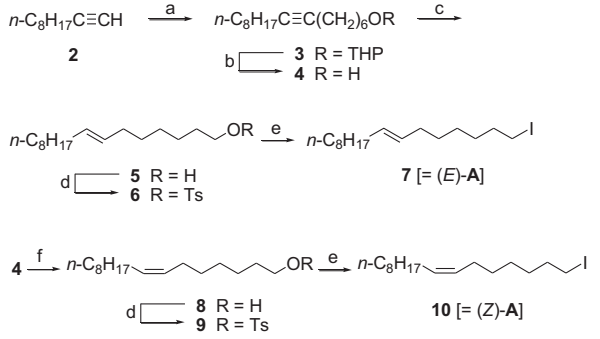


Scheme 1. Structure of CH503, the male sex pheromone of *D. melanogaster*, and retrosynthetic analysis of (*R*)-**1**.

our previous synthesis of (3*R*,11*Z*,19*Z*)- and (3*S*,11*Z*,19*Z*)-**1**,[3] although hydroboration–protonolysis is not employed in the present synthesis. The target molecule **1** can be dissected into three building blocks **A**, **B**, and **C**. The alkenyl building block **A** would be prepared from alkyne **D** and iodide **E**, while the alkynyl building block **B** would be synthesized from trimethylsilyl(TMS)acetylene **F** and iodide **E**. The optically active epoxide **C** would be prepared from 3-buten-1-ol **G** and *p*-methoxybenzyl chloride (PMBCl) **H**. This plan was realized as detailed below.

*2.1.2. Synthesis of (E)- and (Z)-7-hexadecenyl iodides **7** and **10**.* Scheme 2 summarizes the synthesis of the alkenyl building blocks (*E*)- and (*Z*)-**A** (=**7** and **10**, respectively). Commercially available 1-decyne (**2**) was converted to the known 7-hexadecyn-1-ol (**4**) via **3**.[3] Reduction of **4** to (*E*)-7-hexadecen-1-ol (**5**) was executed by heating **4** and lithium aluminum hydride in diglyme and THF.[12] Tosylation of **5** was followed by treatment of the resulting tosylate **6** with sodium iodide in DMF to give the desired iodide **7** [=(*E*)-**A**]. The overall yield of **7** based on **2** was 68% (five steps).



Scheme 2. Synthesis of the building blocks (*E*)-**A** (=**7**) and (*Z*)-**A** (=**10**). Reagents: (a) *n*-BuLi, I(CH$_2$)$_6$OTHP, THF, HMPA; (b) *p*-TsOH, MeOH (82%, two steps); (c) LiAlH$_4$, THF, diglyme (97%); (d) *p*-TsCl, C$_5$H$_5$N (96% for **6**; 87% for **9**); (e) NaI, DMF (89% for **7**; 97% for **10**); (f) H$_2$, Lindlar's Pd cat., quinoline, cyclohexane (99%).

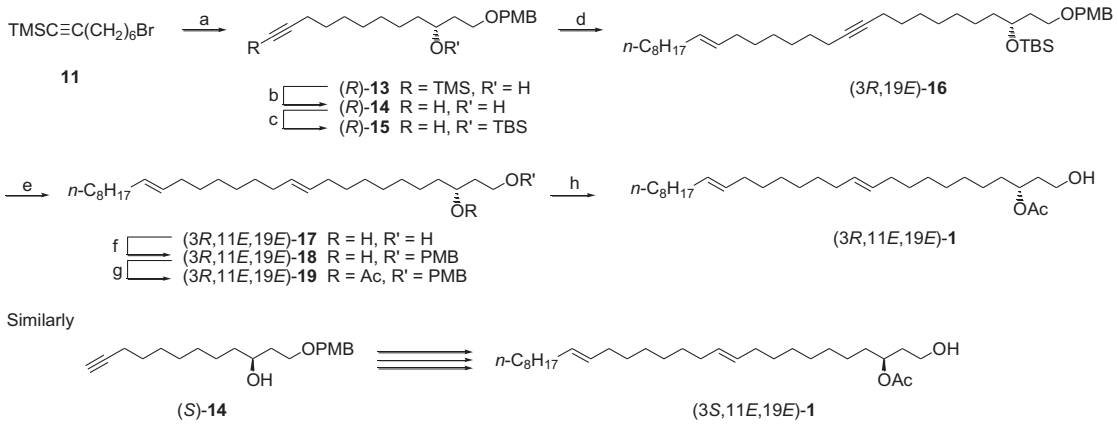
For the synthesis of (*Z*)-7-hexadecenyl iodide (**10**), the alkynol **4** was hydrogenated over Lindlar's palladium catalyst[13] in the presence of quinoline in cyclohexane to give (*Z*)-hexadecen-1-ol (**8**), which was converted to **10** via tosylate **9**. The overall yield of **10** based on **2** was also 68% (five steps). The stereoselectivity of reductions of these alkynes to alkenes will be discussed later in Section 2.4.

*2.1.3. Synthesis of the enantiomers of (11E,19E)-**1**.* The coupling of the three building blocks **A**, **B**, and **C** is summarized in Scheme 3 to give the enantiomers of (11*E*,19*E*)-**1**. 8-Trimethylsilyl-7-octynyl bromide (**11**) was converted to the corresponding Grignard reagent, which was coupled with (*S*)-3-epoxy-1-butanol PMB ether (**12**) in the presence of copper(I) bromide to give (*R*)-**13** as reported previously.[3] Treatment of (*R*)-**13** with potassium carbonate in methanol furnished the known alcohol (*R*)-**14**.[3] The hydroxyl group at C-3 of (*R*)-**14** was protected as *tert*-butyldimethylsilyl(TBS) ether to give (*R*)-**15**. Alkylation of (*R*)-**15** with *n*-butyllithium and (*E*)-**10** afforded (3*R*,19*E*)-**16**.

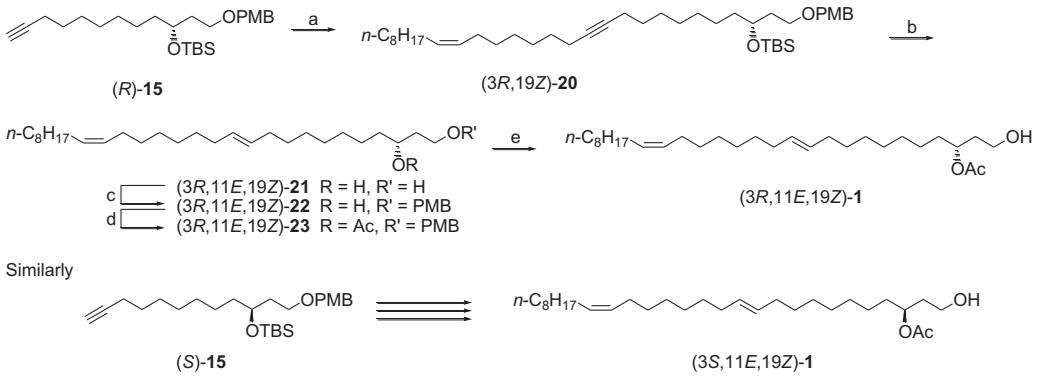
Reduction of (3*R*,19*E*)-**16** with lithium aluminum hydride in diglyme and THF gave crystalline diol (3*R*,11*E*,19*E*)-**17** in 88% yield. Reductive removal of the PMB protective group took place due to the present drastic reduction conditions (140 °C, 48 h). Since it was necessary to block the C-1 hydroxy group prior to the acetylation of the C-3 hydroxy group, (3*R*,11*E*,19*E*)-**17** was reprotected as 1-PMB ether by treatment with potassium *tert*-butoxide and PMBCl to give (3*R*,11*E*,19*E*)-**18** in 64% yield. Acetylation of (3*R*,11*E*,19*E*)-**18** furnished (3*R*,11*E*,19*E*)-**19**, whose treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and water[14] furnished (3*R*,11*E*,14*E*)-**1** as an oil. Its IR and ^{13}C NMR spectra indicated the presence of (*E*)-double bonds (see Section 2.2).

Similarly, (*S*)-**14** was synthesized by employing (*R*)-3-epoxy-1-butanol PMB ether (**12**) as one of the starting materials. It was then converted to the oily (3*S*,11*E*,19*E*)-**1**. The overall yields of the enantiomers of (11*E*,19*E*)-**1** were 16% for the (*R*)-isomer and 13% for the (*S*)-isomer, respectively, based on **11** (eight steps).

*2.1.4. Synthesis of the enantiomers of (11E,19Z)-**1**.* Scheme 4 shows the conversion of (*R*)-**15** into (3*R*,11*E*,19*Z*)-**1**, which could be



Scheme 3. Synthesis of (3*R*,11*E*,19*E*)- and (3*S*,11*E*,19*E*)-**1**. Reagents: (a) Mg, THF, CuBr, (*S*)- or (*R*)-**12**; (b) K_2CO_3, MeOH [83% for (*R*)-**14**; 82% for (*S*)-**14**, two steps]; (c) TBSCl, imidazole, DMF [98% for (*R*)-**15**; 97% for (*S*)-**15**]; (d) *n*-BuLi, **7**, THF, HMPA [89% for (*R*)-**16**; 84% for (*S*)-**16**]; (e) $LiAlH_4$, THF, diglyme [88% for (*R*)-**17**; 92% for (*S*)-**17**]; (f) *t*-BuOK, PMBCl, THF, DMF [64% for (*R*)-**18**; 57% for (*S*)-**18**]; (g) Ac_2O, DMAP, C_5H_5N [95% for (*R*)-**19**; 94% for (*S*)-**19**]; (h) DDQ, CH_2Cl_2, H_2O [41% for (3*R*,11*E*,19*E*)-**1**; 40% for (3*S*,11*E*,19*E*)-**1**].



Scheme 4. Synthesis of (3*R*,11*E*,19*Z*)- and (3*S*,11*E*,19*Z*)-**1**. Reagents: (a) *n*-BuLi, **10**, THF, HMPA [89% for (*R*)-**20**; 91% for (*S*)-**20**]; (b) $LiAlH_4$, THF, diglyme [96% for (*R*)-**21**; quant. for (*S*)-**21**]; (c) *t*-BuOK, PMBCl, THF, DMF [35% for (*R*)-**22**; 31% for (*S*)-**22**]; (d) Ac_2O, DMAP, C_5H_5N [98% for (*R*)-**23**; 95% for (*S*)-**23**]; (c) DDQ, CH_2Cl_2, H_2O [54% for (3*R*,11*E*,19*Z*)-**1**; 49% for (3*S*,11*E*,19*Z*)-**1**].

achieved in a manner similar to that of (*R*)-**15** to (3*R*,11*E*,19*E*)-**1**. Accordingly, (*R*)-**15** was alkylated with *n*-butyllithium and (*Z*)-7-hexadecenyl iodide (**10**) to give (3*R*,19*Z*)-**20**, which was reduced with lithium aluminum hydride to furnish crystalline diol (3*R*,11*E*,19*Z*)-**21**. Protection of its terminal hydroxy group as PMB ether afforded (3*R*,11*E*,19*Z*)-**22** as an oil, whose acetylation gave oily (3*R*,11*E*,19*Z*)-**23**. Finally, removal of its PMB protective group furnished the desired (3*R*,11*E*,19*Z*)-**1** as an oil in 13% overall yield based on **11** (eight steps).

In the same manner, (*S*)-**15** was converted to oily (3*S*,11*E*,19*Z*)-**1** in 10% overall yield based on **11** (eight steps).

*2.1.5. Synthesis of the enantiomers of (11Z,19E)-**1**.* Scheme 5 summarizes the conversion of (3*R*,19*E*)-**16** to (3*R*,11*Z*,19*E*)-**1**. Removal of the TBS protective group of (3*R*,19*E*)-**16** afforded crystalline (3*R*,19*E*)-**24**, whose Lindlar hydrogenation gave an oily (3*R*,11*Z*,19*E*)-**25** with the intact PMB protective group. The corresponding acetate (3*R*,11*Z*,19*E*)-**26** was treated with DDQ and water to furnish (3*R*,11*Z*,19*E*)-**1** as an oil in 33% overall yield based on **11** (eight steps). Since the PMB protective group was kept intact in the course of the Lindlar hydrogenation, the overall yield was better than in other cases.

Similarly, (3*S*,19*E*)-**16** afforded (3*S*,11*Z*,19*E*)-**1** as an oil in 29% overall yield based on **11** (eight steps).

Because the enantiomers of (11*Z*,19*Z*)-**1** had been prepared in 2010,[3] all the eight possible stereoisomers of CH503 (**1**, 100–200 mg each) were in our hands (K.M. and Y.S.) ready for further analytical and biological studies.

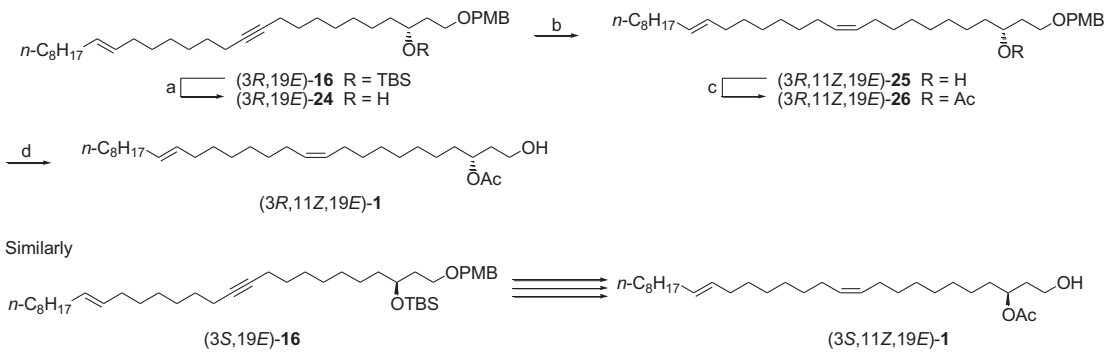
2.2. Comparison of the spectroscopic and gas chromatographic properties of the stereoisomers of CH503 (1)

Comparison of the spectroscopic and chromatographic properties of a natural product and its synthetic counterpart was traditionally an acceptable method for its structure confirmation. The scope and limitations of that comparison method were critically examined recently.[15,16] In the present case of CH503 (**1**), however, due to the scarce amount obtained from *D. melanogaster*, only its mass spectrum (MS) was available to us. Fig. 1 shows the MS of (3*R*,11*Z*,19*Z*)-**1**. The MS of all the stereoisomers of **1** were found to be identical.

We further compared the spectral properties (see Supplementary data) of the four *E*/*Z*-isomers of (*R*)- or (*S*)-**1** (by K.M. and Y.S.). A distinct difference could be observed between the IR spectrum of (11*Z*,19*Z*)-**1** and that of (11*E*,19*E*)-**1**. In the former, no absorption was observed at 966 cm^{-1} due to (*E*)-double bonds, while in the latter there is a strong absorption at 966 cm^{-1}. In the cases of (11*E*,19*Z*)- and (11*Z*,19*E*)-**1**, both exhibited an absorption of medium intensity at 966 cm^{-1}, and they showed similar IR spectra.

The four *E*/*Z*-isomers of (*R*)-**1** exhibited somewhat different ^1H NMR spectra in the olefinic region. Especially, (11*Z*,19*Z*)-**1** could be easily distinguished from (11*E*,19*E*)-**1** due to the difference in δ_H: around 5.35 for (11*Z*,19*Z*)-**1** and around 5.38 for (11*E*,19*E*)-**1**. However, the (11*E*,19*Z*)- and (11*Z*,19*E*)-isomers showed very similar signal patterns around δ_H=5.32–5.40.

In the ^{13}C NMR spectra of the *E*/*Z*-isomers of (*S*)-**1**, their olefinic carbons also showed slight differences in their δ_C. Here again, (11*Z*,19*Z*)-**1** could be readily distinguished from (11*E*,19*E*)-**1**:



Scheme 5. Synthesis of (3*R*,11*Z*,19*E*)- and (3*S*,11*Z*,19*E*)-**1**. Reagents: (a) TBAF, THF [91% for (*R*)-**24**; 85% for (*S*)-**24**]; (b) H_2, Lindlar's Pd cat., quinoline, cyclohexane [97% for (*R*)-**25**; 98% for (*S*)-**25**]; (c) Ac_2O, DMAP, C_5H_5N [96% for (*R*)-**26**; 97% for (*S*)-**26**]; (d) DDQ, CH_2Cl_2, H_2O [54% for (3*R*,11*Z*,19*E*)-**1**; 53% for (3*S*,11*Z*,19*E*)-**1**].

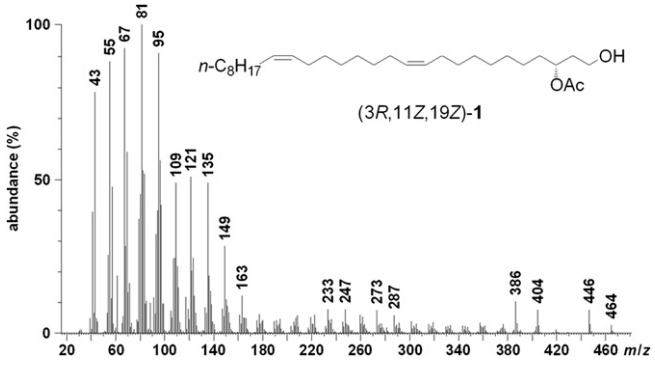


Fig. 1. Mass spectrum (70 eV, EI) of (3*R*,11*Z*,19*Z*)-**1**.

δ_C=129.6–129.8 for (11*Z*,19*Z*)-**1** and 130.3–130.4 for (11*E*,19*E*)-**1**. The differences in the ^{13}C NMR spectra of (11*E*,19*Z*)-**1** and (11*Z*,19*E*)-**1** were very small. These known spectroscopic methods were therefore unsuitable for the determination of the double bond geometries of the natural CH503.

Finally, GC analysis of the four *E*/*Z*-isomers of (*S*)-**1** was examined (by S.T.) employing three different stationary phases: nonpolar TC-1, TC-5 with medium polarity, and polar Pure-Wax. The four isomers exhibited slightly different retention times (see Supplementary data). The retention times (t_R) of the isomers were (11*Z*,19*Z*)-**1**<(11*Z*,19*E*)-**1**<(11*E*,19*Z*)-**1**<(11*E*,19*E*)-**1** in the cases of TC-1 and TC-5. The differences in their retention times, however, were so small that no base-peak separation of the isomers was possible due to the tailing and broadening of the peaks.

Since no chiroptical information of the natural CH503 was available, the absolute configuration at C-3 of CH503 could not be determined by chiroptical methods.

2.3. Complete separation of the eight stereoisomers of CH503 (1) by Ohrui–Akasaka's derivatization-reversed phase HPLC method

We then examined Ohrui–Akasaka's derivatization-reversed phase HPLC method.[17–19] They recently designed chiral and fluorescent derivatizing reagents, such as (1*R*,2*R*)- and (1*S*,2*S*)-2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylic acid (**27**, Fig. 2; commercially available from Tokyo Kasei: TCI-A1657). Due to the fluorescent nature of the reagent, detection of the derivatives is possible at 10^{-15} mol levels. Ohrui–Akasaka's method was employed by Mori et al. to determine the absolute configuration and enantiomeric composition of a marine natural product plakoside A,[20] the New World screwworm fly pheromone,[21,22] and tribolure, the pheromone of the red flour beetle.[23,24] At present, this HPLC method is the most powerful one to determine the absolute configuration of a stereogenic center far separated from a hydroxy or carboxy group. It occurred to K.M. that the method might be useful also in determining the *E*/*Z*-geometry of a double bond far separated from a functional group, because the successful separation of isomers is most probably due to the interaction of the lengthy hydrocarbon chain with the anthracene ring of **28**.

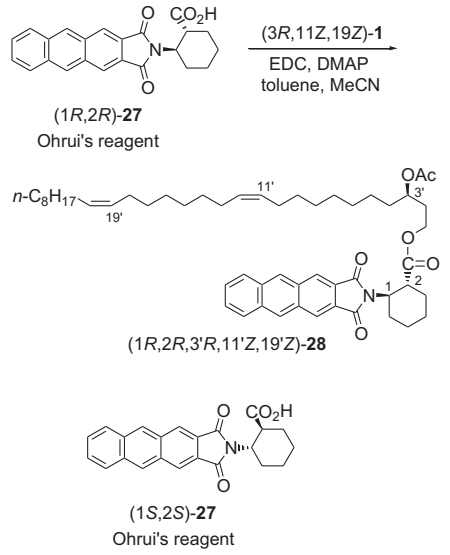


Fig. 2. Preparation of derivative **28** for HPLC analysis.

Derivatization (by K.A.) of the eight stereoisomers of **1** with (1*R*,2*R*)-**27** in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) afforded **28**, whose eight stereoisomers were completely separable on a Develosil column (C30-UG-3, 3 μm, 4.6 mm i.d.×15 cm, Nomura Chemical Co., Aichi, Japan) at −20 °C by elution with a mixture of methanol/acetonitrile/THF/hexane (202:215:150:55, v/v/v/v) at a flow rate of 0.4 mL/min (Fig. 3A). The observed good separation guaranteed the successful determination of the stereostructure of the natural CH503 by the present HPLC analysis.

2.4. Determination of the stereochemical purity of the synthetic stereoisomers of CH503 by Ohrui–Akasaka's HPLC method

Prior to the determination of the stereostructure of the natural CH503 (**1**), the present HPLC method was applied to the determination of the stereochemical purity of our synthetic stereoisomers of **1**. Since the enantiomeric purity at C-3 of the synthetic **1** was determined as 92–94% ee by analyzing the

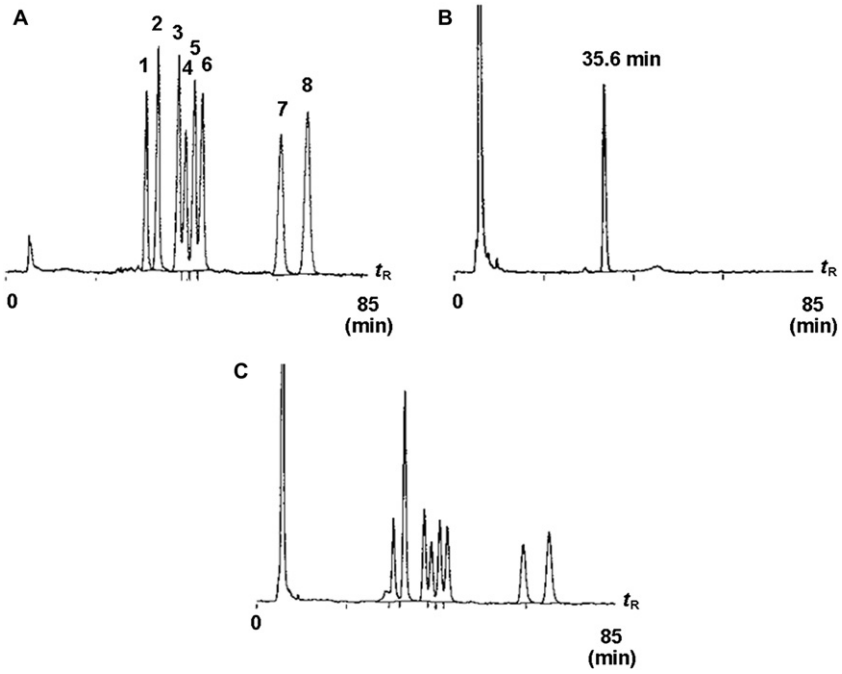


Fig. 3. HPLC chromatograms of (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylate (**28**) of (A) a mixture of the eight synthetic isomers of CH503 (**1**); (B) the natural CH503 from *D. melanogaster*; (C) co-injection of the natural **28** and the synthetic mixture of **28**. HPLC conditions: column, Develosil C30-UG-3 (4.6×150 mm); mobile phase, MeOH/MeCN/THF/hexane (202:215:150:55, v/v/v/v); flow rate, 0.4 mL/min; column temperature, −20 °C; detection, Ex. 298 nm, Em. 462 nm. Peaks (t_R) are: 1 (32.8 min): (3*S*,11*Z*,19*Z*)-**1**; 2 (35.5 min): (3*R*,11*Z*,19*Z*)-**1**; 3 (40.3 min): (3*S*,11*E*,19*Z*)-**1**; 4 (42.0 min): (3*S*,11*Z*,19*E*)-**1**; 5 (44.1 min): (3*R*,11*E*,19*Z*)-**1**; 6 (45.9 min): (3*R*,11*Z*,19*E*)-**1**; 7 (64.3 min): (3*S*,11*E*,19*E*)-**1**; 8 (70.5 min): (3*R*,11*E*,19*E*)-**1**.

enantiomeric purity of the epoxide **12**,[3,7] the HPLC analysis was employed to determine the ratio of *E*/*Z*-isomers of the synthetic **1**.

The synthetic eight stereoisomers were slightly impure as shown in Table 1. The enantiomers of (11*Z*,19*Z*)-**1** were prepared by reducing the two triple bonds simultaneously by hydroboration–protonolysis, which was believed to give (*Z*)-alkene stereospecifically. The HPLC analysis, however, revealed that the undesired (11*E*,19*Z*)- and (11*Z*,19*E*)-isomers were generated substantially (2–6%), although the desired (11*Z*,19*Z*)-**1** was the major product (88–93%).

Table 1
E/*Z*-Isomeric ratio of the synthetic samples of the stereoisomers of CH503 (**1**) as determined by the reversed-phase HPLC of the corresponding **28**

Synthetic sample	Isomeric ratio (% ratio of the peak areas)			
	(11*Z*,19*Z*)	(11*E*,19*Z*)	(11*Z*,19*E*)	(11*E*,19*E*)
(3*S*,11*Z*,19*Z*)-**1**	88.44	5.94	5.61	
(3*S*,11*E*,19*Z*)-**1**	4.31	88.37		7.31
(3*S*,11*Z*,19*E*)-**1**	3.21		94.50	2.29
(3*S*,11*E*,19*E*)-**1**		3.84	4.95	91.21
(3*R*,11*Z*,19*Z*)-**1**	93.39	4.29	2.38	
(3*R*,11*E*,19*Z*)-**1**	8.56	86.89		4.55
(3*R*,11*Z*,19*E*)-**1**	2.41		95.70	1.89
(3*R*,11*E*,19*E*)-**1**		3.45	3.07	93.47

Both (11*Z*,19*E*)- and (11*E*,19*Z*)-**1** were prepared by Lindlar hydrogenation of either the triple bond at C-11 or that at C-19. In the case of (11*Z*,19*E*)-**1**, about 2% of (11*E*,19*E*)-**1** was detected, while in the case of (11*E*,19*Z*)-**1**, 4–7% of (11*E*,19*E*)-**1** could be detected. Presumably in the latter case, thermal isomerization of (19*Z*)-double bond took place in the course of the reduction of (19*Z*)-**20** to (11*E*,19*Z*)-**21**.

Reduction of the triple bond with lithium aluminum hydride was not highly stereoselective either, giving 2–8% of (*Z*)-isomers. These analytical results indicated that reduction of triple bonds by hydroboration–protonolysis, Lindlar hydrogenation, and lithium aluminum hydride in diglyme were not so highly stereoselective as previously thought to be.

2.5. Determination of the stereostructure of CH503 as (3*R*,11*Z*,19*Z*)-1 by Ohrui–Akasaka's HPLC method

The final stage of the present work was the clarification of the stereostructure of CH503 as (3*R*,11*Z*,19*Z*)-**1** by the above described Ohrui–Akasaka's HPLC analysis.

A pheromone extract containing about 12.5 μg of CH503 was prepared (by S.S. and J.Y.Y.) from 700 males and females of *D. melanogaster*, and derivatized with (1*R*,2*R*)- and (1*S*,2*S*)-**27** (by K.A.). The retention time (35.6 min, Fig. 3B) of the natural CH503 derivatized with (1*R*,2*R*)-**27** agreed with that (35.5 min) of (1*R*,2*R*,3′*R*,11′*Z*,19′*Z*)-**27**.

Co-injection of (1*R*,2*R*)-**28** derived from the natural CH503 with a mixture of all the eight isomers of (1*R*,2*R*)-**28** definitively confirmed the stereostructure of the natural CH503 as 3*R*,11*Z*,19*Z* (Fig. 3C). The retention time (32.8 min) of **28** derived from the natural CH503 and (1*S*,2*S*)-**27** also agreed with that (32.8 min) of (1*S*,2*S*,3′*R*,11′*Z*,19′*Z*)-**28**. No peak derived from other stereoisomers of CH503 could be detected in the chromatograms of the natural pheromone derivatized with either (1*R*,2*R*)- or (1*S*,2*S*)-**27**. The stereostructure of the natural CH503 was therefore concluded as 3*R*,11*Z*,19*Z*. This conclusion was supported by the co-injection experiment of (1*R*,2*R*)-**28** derived from the natural CH503 and (1*R*,2*R*)-**28** derived from (3*R*,11*Z*,19*Z*)-**1** (Fig. 4).

3. Conclusion

The stereostructure of natural CH503 was established as pure (3*R*,11*Z*,19*Z*)-3-acetoxy-11,19-octacosadien-1-ol (**1**), which had been shown in 2010 to be less bioactive than (3*S*,11*Z*,19*Z*)-**1**.[3] Ohrui–Akasaka's derivatization-reversed phase HPLC analysis was again shown to be a powerful tool in determining the stereochemistry of scarce biofunctional molecules including both

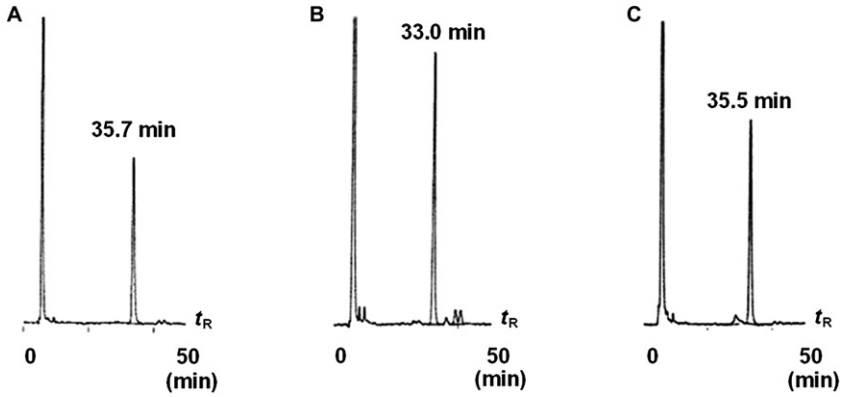


Fig. 4. HPLC chromatograms of (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylate (**28**) of (A) synthetic (3*R*,11*Z*,19*Z*)-**1**; (B) synthetic (3*S*,11*Z*,19*Z*)-**1**; (C) co-injection of **28** derived from the natural CH503 and **28** derived from (3*R*,11*Z*,19*Z*)-**1**. HPLC conditions are the same as those described in Fig. 3.

enantiomerism and *E*/*Z*-isomerism. Details of the pheromone activity of each of the stereoisomers of **1** as well as biological implication thereof will be published separately in due course (by J.Y.Y.).

4. Experimental

4.1. General

Boiling and melting points are uncorrected values. Refractive indices (n_D) were measured with an Atago DR-M2 refractometer. Melting points were measured with a SII EXSTAR DSC 6220. Optical rotations were measured with a Jasco P-1020 polarimeter. IR spectra were measured with a Jasco FT/IR-410 spectrometer. ^{1}H NMR spectra (400 MHz, TMS at δ=0.00 as internal standard) and ^{13}C NMR spectra (100 MHz, $CDCl_3$ at δ=77.0 as internal standard) were recorded by a Jeol JNM-AL 400 spectrometer. GC–MS were measured with an Agilent Technologies 5975 inert XL instrument. HRMS were recorded on an Agilent Technologies 6530 Accurate Mass Q-TOF LC/MS. DART (Direct analysis in Real Time) mass spectrometry was performed using an atmospheric pressure ionization time of flight mass spectrometer (AccuTOG-DART; JEOL USA, Inc.) equipped with a DART interface.[25] Column chromatography was carried out on Merck Kieselgel 60 Art 1.07734.

4.2. (*E*)-7-Hexadecen-1-ol (5)

A solution of 7-hexadecyn-1-ol (**4**, 15.0 g, 62.9 mmol) in dry diglyme (25 mL) was added dropwise to a stirred and ice-cooled suspension of $LiAlH_4$ (8.83 g, 233 mmol) in dry THF (18 mL) and dry diglyme (115 mL) at 10–20 °C. The mixture was stirred and heated under Ar. THF was removed when the mixture was heated at 130 °C. The stirred mixture was then heated at 140 °C for 16 h. The excess $LiAlH_4$ was destroyed by dropwise addition of water to the stirred and ice-cooled mixture, and hexane was added simultaneously to the mixture to dilute the suspension. It was then poured into ice-dil HCl, and extracted with hexane. The hexane solution was successively washed with water, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give 14.6 g (97%) of **5** as a colorless oil, which solidified in a deep freezer. n_D^{22} 1.4564; ν_{max} (film): 3342 (br, O–H), 2925 (s), 2854 (s), 1464 (m), 1378 (w), 1056 (m), 966 (m), 722 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.18–1.44 (19H, br), 1.52–1.62 (2H, m), 1.90–2.05 (4H, br), 3.59–3.70 (2H, br, C*H*$_2$OH), 5.33–5.44 (2H, m, CH=CH). HRMS of **5** [Ionization: APCI; polarity: positive; corona current: 6 μA; nebulizer: N_2 (50 psi); drying gas: N_2 (5 L/min, 350 °C); vaporizer: 350 °C; capillary current: 3500 V; eluant: 0.01 M HCO_2H aq/MeOH (10/90)]: calcd for $C_{16}H_{33}O$ [$(M+H)^+$]: 241.2526, found: 241.2522.

4.3. (*E*)-7-Hexadecenyl tosylate (6)

Tosyl chloride (17.3 g, 90.7 mmol) was added portionwise to a stirred and ice-cooled solution of **5** (14.6 g, 60.7 mmol) in dry pyridine (80 mL) at 0–5 °C. After stirring for 4 h at 0–5 °C, water was added to the mixture until the precipitates were dissolved and stirring was continued for 5 min. It was then diluted with water and extracted with EtOAc. The EtOAc solution was successively washed with water, dil $CuSO_4$ solution, water, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give 23.0 g (96%) of **6** as a colorless oil, which solidified in a deep freezer. n_D^{22} 1.4921; ν_{max} (film): 2925 (s), 2854 (s), 1599 (m, arom. C=C), 1464 (m), 1364 (s, SO_2), 1177 (s, SO_2), 1098 (s), 966 (s), 815 (m), 664 (m), 555 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.15–1.38 (18H, br), 1.55–1.68 (2H, m), 1.86–2.00 (4H, m), 2.50 (3H, s, $C_6H_4CH_3$), 4.02 (2H, t, *J* 6.4, C*H*$_2$OTs), 5.26–5.42 (2H, m, CH=CH), 7.34 (2H, d, *J* 8.4, arom. H), 7.79 (2H, d, *J* 8.4, arom. H). This was employed for the next step without further purification.

4.4. (*E*)-7-Hexadecenyl iodide (7)

Powdered sodium iodide (22.3 g, 149 mmol) was added portionwise to a solution of **6** (22.9 g, 58.0 mmol) in DMF (100 mL) at room temperature. After exothermic reaction, the mixture was stirred and heated at 80 °C for 5 h. After cooling, the mixture was diluted with water, and extracted with Et_2O. The Et_2O solution was successively washed with water, dil $Na_2S_2O_3$ solution, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (19.4 g) was chromatographed over SiO_2 (200 g). Elution with hexane/EtOAc (100:1) gave 18.0 g (89%) of **7** as a colorless oil, n_D^{23} 1.4893; ν_{max} (film): 2925 (s), 2853 (s), 1462 (m), 1377 (w), 1198 (w), 966 (m), 722 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.18–1.45 (18H, br), 1.77–1.87 (2H, m), 1.90–2.05 (4H, br), 3.19 (2H, t, *J* 6.8, CH_2I), 5.31–5.45 (2H, m, CH=CH). GC–MS [column: HP-5MS 5% phenylmethylsiloxane, 30 m×0.25 mm i.d.; press: 52.7 kPa; temp: 50–160 °C (+10 °C/min), then 160–220 °C (+4 °C/min)]: t_R 32.77 min [**10**=(*Z*)-isomer, 0.8%], t_R 33.12 min (**7**, 98.5%). MS of **7** (70 eV, EI): *m*/*z*: 350 (23) [M^+, $C_{16}H_{31}I$], 294 (1), 280 (2), 266 (8), 252 (11), 238 (5), 224 (6), 210 (7), 196 (17), 183 (9), 169 (3), 155 (13), 139 (3), 125 (9), 111 (27), 97 (67), 83 (88), 69 (100), 55 (81), 41 (45). HRMS of **7** [same conditions as for **5**]: calcd for $C_{16}H_{32}I$ [$(M+H)^+$]: 351.1543, found: 351.1547.

4.5. (*Z*)-7-Hexadecen-1-ol (8)

Lindlar's Pd catalyst on $CaCO_3$ with Pb^{2+} (Aldrich, 60 mg) and quinoline (120 μL) were added to a solution of **4** (3.00 g, 12.6 mmol)

in cyclohexane (30 mL). The mixture was stirred under an H_2 atmosphere (gas cylinder, 1 atm) at room temperature for 2 h. The mixture was then filtered through Celite and the catalyst and Celite were washed with Et_2O. The filtrate and washings were diluted with Et_2O, and successively washed with dil HCl, water, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo to give 2.99 g (99%) of **8** as a colorless oil, which solidified in a deep freezer. n_D^{24} 1.4581; ν_{max} (film): 3333 (br, O–H), 3005 (w), 2925 (s), 2854 (s), 1464 (m), 1378 (w), 1057 (m), 722 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20–1.43 (19H, br), 1.52–1.62 (2H, m), 1.93–2.09 (4H, m), 3.64 (2H, t, *J* 6.8, C*H*$_2$OH), 5.30–5.40 (2H, m, CH=CH). HRMS of **8**[same conditions as for **5**]: calcd for $C_{16}H_{33}O$ [$(M+H)^+$]: 241.2526, found: 241.2526.

4.6. (*Z*)-7-Hexadecenyl tosylate (9)

In the same manner as described above for **6**, **8** (3.08 g, 12.8 mmol) in dry pyridine (20 mL) was treated with tosyl chloride (3.58 g, 18.8 mmol) to give 4.40 g (87%) of **9** as a colorless oil, n_D^{23} 1.4937; ν_{max} (film): 3004 (w), 2925 (s), 2854 (s), 1599 (m, arom. C=C), 1464 (m), 1364 (s, SO_2), 1177 (s, SO_2), 1098 (m), 962 (m), 815 (m), 664 (m), 555 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.18–1.38 (18H, br), 1.58–1.68 (2H, m), 1.92–2.04 (4H, m), 2.50 (3H, s, $C_6H_4CH_3$), 4.02 (2H, t, *J* 6.4, C*H*$_2$OTs), 5.26–5.39 (2H, m, CH=CH), 7.34 (2H, d, *J* 8.0, arom. H), 7.79 (2H, d, *J* 8.2, arom. H). This was employed for the next step without further purification.

4.7. (*Z*)-7-Hexadecenyl iodide (10)

In the same manner as described above for **7**, **9** (3.54 g, 8.97 mmol) in DMF (15 mL) was treated with sodium iodide (3.44 g, 23.0 mmol) to give 3.04 g (97%) of **10** as a colorless oil, n_D^{24} 1.4906; ν_{max} (film): 3004 (w), 2925 (s), 2853 (s), 1459 (m), 1377 (w), 1198 (w), 722 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.19–1.47 (18H, br), 1.77–1.88 (2H, m), 1.95–2.09 (4H, m), 3.19 (2H, t, *J* 7.0, CH_2I), 5.29–5.41 (2H, m, CH=CH). GC–MS [same conditions as for **7**]: t_R 32.90 min (**10**, 96.2%), t_R 32.97 min [**7**=(*E*)-isomer, 3.3%]. MS of **10** (70 eV, EI): *m*/*z*: 350 (24) [M^+, $C_{16}H_{31}I$], 294 (1), 280 (3), 266 (9), 252 (10), 238 (6), 224 (7), 210 (7), 196 (16), 183 (11), 169 (3), 155 (14), 139 (3), 125 (10), 111 (29), 97 (70), 83 (89), 69 (100), 55 (84), 41 (47). HRMS of **10** [same conditions as for **5**]: calcd for $C_{16}H_{32}I$ [$(M+H)^+$]: 351.1543, found: 351.1536.

4.8. 3-*tert*-Butyldimethylsilyloxy-11-dodecyn-1-ol PMB ether (15)

4.8.1. (R)-Isomer. Imidazole (4.04 g, 59.3 mmol) and TBSCl (4.47 g, 29.7 mmol) were added to a stirred solution of (*R*)-**14** (4.72 g, 14.8 mmol) in dry DMF (30 mL) at room temperature. The solution was stirred at room temperature for 3 h. It was then diluted with water, and extracted with Et_2O. The Et_2O solution was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (8.01 g) was chromatographed over SiO_2 (120 g). Elution with hexane/EtOAc (30:1) gave 6.29 g (98%) of (*R*)-**15** as a colorless oil, n_D^{23} 1.4867; $[\alpha]_D^{27}$ −8.21 (*c* 4.08, hexane); ν_{max} (film): 3311 (m, C≡C–H), 2931 (s), 2856 (s), 2117 (w), 1613 (m, arom. C=C), 1586(w), 1514 (s), 1463 (m), 1249 (s), 1097(s), 1039 (s) 835 (s), 774 (s), 633 (m); δ_H ($CDCl_3$): 0.03 (3H, s, CH_3Si), 0.04 (3H, s, CH_3Si), 0.87 (9H, s, CH_3C), 1.23–1.33 (6H, br), 1.33–1.46 (4H, br), 1.48–1.56 (2H, m),1.65–1.79 (2H, m),1.93 (1H, t, *J* 2.8, C≡CH), 2.15–2.20 (2H, m), 3.50 (2H, t, *J* 6.8), 3.77–3.85 (1H, br), 3.80 (3H, s, OCH_3), 4.37–4.45 (2H, m, OCH_2Ar), 6.87 (2H, d, *J* 8.4, arom. H), 7.25 (2H, d, *J* 8.4, arom. H). HRMS of (*R*)-**15** [Ionization: ESI; polarity: positive; fragmentor: 150 V; nebulizer: N_2 (50 psi); drying gas: N_2 (10 L/min, 350 °C); capillary current: 3500 V; eluant: 0.01 M HCO_2H aq/MeCN (10/90)]: calcd for $C_{26}H_{45}O_3Si$ [$(M+H)^+$]: 433.3132, found: 433.3143.

4.8.2. (S)-Isomer. In the same manner, (*S*)-**14** (3.38 g, 10.6 mmol) gave 4.45 g (97%) of (*S*)-**15** as a colorless oil, n_D^{22} 1.4870; $[\alpha]_D^{27}$ +8.31 (*c* 4.01, hexane). Its IR and ^{1}H NMR spectra were identical to those of (*R*)-**15**. HRMS of (*S*)-**15**: calcd for $C_{26}H_{45}O_3Si$ [$(M+H)^+$]: 433.3132, found: 433.3135.

4.9. (*E*)-3-*tert*-Butyldimethylsilyloxy-19-octacosen-11-yn-1-ol PMB ether (16)

4.9.1. (R)-Isomer. A solution of *n*-BuLi in hexane (1.6 M, 9.9 mL, 16 mmol) was added dropwise to a stirred and cooled solution of (*R*)-**15** (6.19 g, 14.3 mmol) in dry THF (18 mL) and HMPA (5 mL) at −65 to −40 °C under Ar. The mixture was warmed to −10 °C for 10 min, and then cooled again to −65 °C. A solution of **7** (6.03 g, 17.2 mmol) in dry THF (5 mL) was added dropwise to the stirred and cooled solution at −65 °C, and the temperature was gradually raised to room temperature. After stirring for 1 h at room temperature, the mixture was stirred and heated at reflux for 2 h. After cooling, the mixture was diluted with water and extracted with hexane. The hexane solution was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (11.4 g) was chromatographed over SiO_2 (200 g). Elution with hexane/EtOAc (100:1–30:1) gave 8.34 g (89%) of (*R*)-**16** as a colorless oil, n_D^{24} 1.4853; $[\alpha]_D^{19}$ −5.86 (*c* 4.11, hexane); ν_{max} (film): 2928 (s), 2855 (s), 1613 (m, arom. C=C), 1586 (w), 1514 (s), 1463 (m), 1249 (s), 1098 (s), 1040 (s), 967 (m), 835 (s), 774 (m); δ_H ($CDCl_3$): 0.03 (3H, s, CH_3Si), 0.04 (3H, s, CH_3Si), 0.82–0.93 (12H, m), 1.16–1.52 (32H, br), 1.62–1.77 (2H, m), 1.89–2.00 (4H, br), 2.10 (4H, t, *J* 6.8, 2× C≡CCH_2), 3.47 (2H, t, *J* 6.8), 3.72–3.82 (1H, br), 3.77 (3H, s, OCH_3), 4.34–4.42 (2H, m, OCH_2Ar), 5.29–5.43 (2H, br, CH=CH), 6.84 (2H, d, *J* 8.8, arom. H), 7.22 (2H, d, *J* 7.6, arom. H). HRMS of (*R*)-**16**: calcd for $C_{42}H_{75}O_3Si$ [$(M+H)^+$]: 655.5480, found: 655.5486.

4.9.2. (S)-Isomer. In the same manner, (*S*)-**15** (5.32 g, 12.3 mmol) gave 6.77 g (84%) of (*S*)-**16** as a colorless oil, n_D^{24} 1.4854; $[\alpha]_D^{22}$ +5.94 (*c* 4.14, hexane). Its IR and ^{1}H NMR spectra were identical to those of (*R*)-**16**. HRMS of (*S*)-**16**: calcd for $C_{42}H_{75}O_3Si$ [$(M+H)^+$]: 655.5480, found: 655.5487.

4.10. (11*E*,19*E*)-11,19-Octacosadiene-1,3-diol (17)

4.10.1. (R)-Isomer. A solution of (*R*)-**16** (3.34 g, 5.10 mmol) in dry diglyme (10 mL) was added dropwise to a stirred and ice-cooled suspension of $LiAlH_4$ (1.16 g, 30.6 mmol) in dry THF (4 mL) and dry diglyme (23 mL) at 10–20 °C. The mixture was then stirred vigorously and heated at 140 °C for 48 h under Ar. The excess $LiAlH_4$ was destroyed by dropwise addition of water to the stirred and ice-cooled mixture, and Et_2O was added simultaneously to the mixture to dilute the suspension. It was then poured into ice-dil HCl, and extracted with EtOAc. The EtOAc solution was successively washed with water, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (4.52 g) was chromatographed over SiO_2 (50 g). Elution with hexane/EtOAc (5:1–0:100) gave 1.91 g (88%) of (*R*)-**17** as a colorless solid. Mp 57–58 °C; $[\alpha]_D^{21}$ +0.60 (*c* 4.51, $CHCl_3$); ν_{max} (film): 3309 (br, O–H), 2922 (s), 2850 (s), 1468 (s), 1118 (m), 1049 (m), 964 (s), 720 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.10–1.55 (32H, br), 1.62–1.77 (2H, m), 1.86–2.04 (8H, br), 2.27–2.40 (2H, m), 3.79–3.93 (3H, m), 5.33–5.43 (4H, m, 2× CH=CH). HRMS of (*R*)-**17**: calcd for $C_{28}H_{55}O_2$ [$(M+H)^+$]: 423.4197, found: 423.4198.

4.10.2. (S)-Isomer. In the same manner, (*S*)-**16** (3.50 g, 5.34 mmol) gave 2.07 g (92%) of (*S*)-**17** as a colorless solid. Mp 56–57 °C; $[\alpha]_D^{23}$ −0.55 (*c* 4.04, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical to

those of (*R*)-**17**. HRMS of (*S*)-**17**: calcd for $C_{28}H_{55}O_2$ [$(M+H)^+$]: 423.4197, found: 423.4197.

4.11. (11*E*,19*E*)-11,19-Octacosadiene-1,3-diol 1-PMB ether (18)

4.11.1. (R)-Isomer. A solution of (*R*)-**17** (1.77 g, 4.19 mmol) in dry THF (23 mL) and dry DMF (5 mL) was added to powdered *t*-BuOK (615 mg, 5.03 mmol) at room temperature under Ar. The mixture was stirred for 0.5 h at room temperature, and then PMBCl (0.62 mL, 4.6 mmol) was added dropwise to the stirred mixture. After stirring for 1 h at room temperature, the mixture was stirred and heated at 50 °C for 4 h. After cooling, the mixture was diluted with water and extracted with EtOAc. The EtOAc solution was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (2.38 g) was chromatographed over SiO_2 (70 g). Elution with hexane/EtOAc (10:1–5:1) gave 1.45 g (64%) of (*R*)-**18** as a colorless solid and (3*R*,11*E*,19*E*)-11,19-octacosadiene-1,3-diol 3-PMB ether (262 mg, 12%) as a colorless solid. Subsequent elution with EtOAc afforded 286 mg (16%) of recovered diol (*R*)-**17**. Properties of (*R*)-**18**: mp 41–42 °C; $[\alpha]_D^{21}$ +6.56 (*c* 4.18, hexane); ν_{max} (film): 3500 (br, O–H), 2925 (s), 2849 (s), 1613 (m, arom. C=C), 1585 (w), 1516 (s), 1468 (s), 1252 (s), 1124 (m), 1093 (m), 1034 (s), 963 (s), 818 (s), 720 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.08–1.52 (32H, br), 1.69–1.75 (2H, m), 1.89–2.05 (8H, br), 2.88 (1H, d, *J* 2.9), 3.58–3.72 (2H, m), 3.72–3.86 (1H, br), 3.80 (3H, s, OCH_3), 4.45 (2H, s, OCH_2Ar), 5.32–5.43 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.5, arom. H), 7.25 (2H, d, *J* 8.8, arom. H). HRMS of (*R*)-**18**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4772.

4.11.2. (S)-Isomer. In the same manner, (*S*)-**17** (2.00 g, 4.73 mmol) gave 1.47 g (57%) of (*S*)-**18** as a colorless solid and 395 mg (20%) of recovered diol (*S*)-**17**. Properties of (*S*)-**18**: mp 37–38 °C; $[\alpha]_D^{27}$ −5.91 (*c* 4.02, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**18**. HRMS of (*S*)-**18**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4760.

4.12. (11*E*,19*E*)-3-Acetoxy-11,19-octacosadien-1-ol PMB ether (19)

4.12.1. (R)-Isomer. Acetic anhydride (3 mL) and DMAP (10 mg) were added to a solution of (*R*)-**18** (1.18 g, 2.17 mmol) in dry pyridine (6 mL). The mixture was stirred overnight at room temperature. It was then diluted with ice-water and extracted with Et_2O. The Et_2O solution was successively washed with water, dil $CuSO_4$ solution, water, $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (1.23 g) was chromatographed over SiO_2 (25 g). Elution with hexane/EtOAc (20:1) gave 1.21 g (95%) of (*R*)-**19** as a colorless oil, n_D^{24} 1.4864; $[\alpha]_D^{27}$ −13.0 (*c* 4.15, hexane); ν_{max} (film): 2925 (s), 2853 (s), 1738 (s, C=O), 1613 (m), 1586 (w), 1514 (s), 1464 (m), 1371 (m), 1245 (s), 1097 (m), 1038 (m), 967 (m), 820 (m), 723 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20–1.38 (30H, br), 1.48–1.58 (2H, br), 1.77–1.87 (2H, m), 1.90–2.04 (8H, br), 2.00 (3H, s, $COCH_3$), 3.40–3.50 (2H, m), 3.80 (3H, s, OCH_3), 4.40 (2H, s, OCH_2Ar), 4.98–5.05 (1H, m), 5.32–5.43 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.8, arom. H), 7.25 (2H, d, *J* 9.5, arom. H). HRMS of (*R*)-**19**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4875.

4.12.2. (S)-Isomer. In the same manner, (*S*)-**18** (1.09 g, 2.01 mmol) gave 1.10 g (94%) of (*S*)-**19** as a colorless oil, n_D^{25} 1.4860; $[\alpha]_D^{22}$ +13.1 (*c* 4.16, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**19**. HRMS of (*S*)-**19**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4875.

4.13. (11*E*,19*E*)-3-Acetoxy-11,19-octacosadien-1-ol (CH503) [(11*E*,19*E*)-1]

4.13.1. (R)-Isomer. DDQ (285 mg, 1.26 mmol) was added to a stirred and ice-cooled solution of (*R*)-**19** (522 mg, 0.892 mmol) in CH_2Cl_2 (18 mL) and water (1.8 mL) at 0–5 °C. The mixture was stirred for 15 min at 0–5 °C and then for 2.5 h at room temperature. The reaction was quenched with satd $NaHCO_3$ solution and the mixture was extracted with Et_2O. The Et_2O solution was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (545 mg) was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (15:1–10:1) gave 172 mg (41%) of (3*R*,11*E*,19*E*)-**1** as an oil, which solidified in a deep freezer. n_D^{24} 1.4681; $[\alpha]_D^{28}$ −7.11 (*c* 1.01, hexane); ν_{max} (film): 3460 (br, O–H), 2925 (s), 2853 (s), 1739 (s, C=O), 1464 (m), 1374 (m), 1245 (s, C–O), 1055 (m), 1023 (m), 966 (m), 722(w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.17–1.40 (30H, br), 1.48–1.71 (3H, m), 1.79–1.88 (1H, m), 1.91–2.01 (8H, br), 2.08 (3H, s, $COCH_3$), 2.46–2.54 (1H, br), 3.49–3.58 (1H, br, CH*H*OH), 3.58–3.68 (1H, br, C*H*HOH), 4.97–5.06 (1H, m, CHOAc), 5.32–5.43 (4H, m, 2× CH=CH); δ_C ($CDCl_3$): 14.1, 21.1, 22.7, 25.5, 29.03, 29.05, 29.07, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.60, 32.63, 34.6, 37.5, 58.6, 71.6, 130.27, 130.32, 130.40, 130.41, 172.0; GC–MS [column: HP-5MS 5% phenylmethylsiloxane, 30 m×0.25 mm i.d.; press: 52.7 kPa; temp: 50–325 °C (+15 °C/min)]: t_R 23.65 min (94.4%). MS of (3*R*,11*E*,19*E*)-**1** (70 eV, EI): *m*/*z*: 464 (3) [M^+, $C_{30}H_{56}O_3$], 446 (8), 404 (8), 386 (11), 374 (2), 357 (3), 287 (5), 273 (8), 259 (5), 247 (9), 233 (8), 219 (5), 207 (6), 194 (5), 177 (6), 163 (13), 149 (28), 135 (52), 121 (53), 109 (49), 95 (92), 81 (100), 67 (92), 55 (91), 43 (79). HRMS of (3*R*,11*E*,19*E*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4312.

4.13.2. (S)-Isomer. In the same manner as described above, (*S*)-**19** (513 mg, 0.877 mmol) was treated with DDQ (280 mg, 1.23 mmol) in CH_2Cl_2 (18 mL) and water (1.8 mL) to gave 165 mg (40%) of (3*S*,11*E*,19*E*)-**1** as an oil, which solidified in a deep freezer. n_D^{26} 1.4674; $[\alpha]_D^{28}$ +6.91 (*c* 1.08, hexane). Its IR, ^1H and ^{13}C NMR spectra were identical to those of (3*R*,11*E*,19*E*)-**1**. GC–MS [same conditions as for (3*R*,11*E*,19*E*)-**1**]: t_R 23.64 min (95.5%). MS of (3*S*,11*E*,19*E*)-**1** (70 eV, EI): *m*/*z*: 464 (3) [M^+, $C_{30}H_{56}O_3$], 446 (7), 404 (8), 386 (11), 374 (2), 357 (3), 287 (6), 273 (7), 259 (6), 247 (8), 233 (8), 219 (5), 207 (6), 194 (5), 177 (6), 163 (12), 149 (27), 135 (51), 121 (51), 109 (50), 95 (91), 81 (100), 67 (90), 55 (84), 43 (77). HRMS of (3*S*,11*E*,19*E*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4300.

4.14. (*Z*)-3-*tert*-Butyldimethylsilyloxy-19-octacosen-11-yn-1-ol PMB ether (20)

4.14.1. (R)-Isomer. In the same manner as described above for (*R*)-**16**, (*R*)-**15** (3.37 g, 7.79 mmol) in dry THF (10 mL) and HMPA (2.5 mL) was treated with *n*-BuLi in hexane (1.6 M, 5.1 mL, 8.2 mmol) and **10** (3.00 g, 8.56 mmol) in dry THF (2.5 mL) to give 4.55 g (89%) of (*R*)-**20** as a colorless oil, n_D^{25} 1.4852; $[\alpha]_D^{20}$ −5.80 (*c* 4.02, hexane); ν_{max} (film): 3003 (w), 2928 (s), 2855 (s) 1613 (m, arom. C=C), 1586 (w), 1514 (m), 1463 (m), 1249 (s), 1099 (m), 1040 (m), 835 (m), 774 (m); δ_H ($CDCl_3$): 0.03 (3H, s, CH_3Si), 0.04 (3H, s, CH_3Si), 0.85–0.92 (12H, m), 1.20–1.54 (32H, br), 1.65–1.80 (2H, m), 1.96–2.06 (4H, br), 2.10–2.16 (4H, t-like, 2× C≡CCH_2), 3.50 (2H, t, *J* 6.8), 3.75–3.85 (1H, br), 3.80 (3H, s, OCH_3), 4.37–4.45 (2H, m, OCH_2Ar), 5.30–5.39 (2H, m, CH=CH), 6.87 (2H, d, *J* 8.5, arom. H), 7.25 (2H, d, *J* 7.3, arom. H). HRMS of (*R*)-**20**: calcd for $C_{42}H_{75}O_3Si$ [$(M+H)^+$]: 655.5480, found: 655.5481.

4.14.2. (S)-Isomer. Similarly, (*S*)-**15** (2.00 g, 4.62 mmol) afforded 2.75 g (91%) of (*S*)-**20** as a colorless oil, n_D^{26} 1.4850; $[\alpha]_D^{20}$ +5.83 (*c* 4.37, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**20**. HRMS of (*S*)-**20**: calcd for $C_{42}H_{75}O_3Si$ [$(M+H)^+$]: 655.5480, found: 655.5487.

4.15. (11*E*,19*Z*)-11,19-Octacosadiene-1,3-diol (21)

4.15.1. (R)-Isomer. In the same manner as described above for (*R*)-**17**, (*R*)-**20** (3.50 g, 5.34 mmol) in dry diglyme (10 mL) was treated

with $LiAlH_4$ (1.22 g, 32.1 mmol) in dry THF (4 mL) and dry diglyme (23 mL) to give 2.16 g (96%) of (*R*)-**21** as a colorless solid. Mp 37–38 °C; $[\alpha]_D^{22}$ +0.51 (*c* 4.04, $CHCl_3$); ν_{max} (film): 3305 (br, O–H), 3002 (m), 2916 (s), 2850 (s), 1467 (s), 1118 (m), 1049 (m), 963 (m), 721 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.18–1.56 (32H, br), 1.61–1.77 (2H, m), 1.89–2.08 (8H, m), 2.49–2.79 (2H, br), 3.78–3.94 (3H, br), 5.29–5.44 (4H, m, 2× CH=CH). HRMS of (*R*)-**21**: calcd for $C_{28}H_{55}O_2$ [$(M+H)^+$]: 423.4197, found: 423.4197.

4.15.2. (S)-Isomer. In the same manner, (*S*)-**20** (2.57 g, 3.92 mmol) gave 1.66 g (quant.) of (*S*)-**21** as a colorless solid. Mp 36–38 °C; $[\alpha]_D^{20}$ −0.58 (*c* 3.88, $CHCl_3$). Its IR and ^1H NMR spectra were identical to those of (*R*)-**21**. HRMS of (*S*)-**21**: calcd for $C_{28}H_{55}O_2$ [$(M+H)^+$]: 423.4197, found: 423.4196.

4.16. (11*E*,19*Z*)-11,19-Octacosadiene-1,3-diol 1-PMB ether (22)

4.16.1. (R)-Isomer. In the same manner as described above for (*R*)-**18**, (*R*)-**21** (2.00 g, 4.73 mmol) in dry THF (25 mL) and dry DMF (5 mL) was treated with PMBCl (0.71 mL, 5.2 mmol) and *t*-BuOK (694 mg, 5.68 mmol) to give 889 mg (35%) of (*R*)-**22** as a colorless oil, which solidified in a deep freezer. Subsequent elution with EtOAc afforded 900 mg (45%) of recovered diol (*R*)-**21**. Properties of (*R*)-**22**: n_D^{25} 1.4922; $[\alpha]_D^{26}$ +5.74 (*c* 2.80, hexane); ν_{max} (film): 3445 (br, O–H), 3002 (w), 2925 (s), 2853 (s), 1613 (m, arom. C=C), 1586 (w), 1514 (s), 1464 (m), 1249 (s), 1092 (m), 1039 (m), 967 (m), 822 (m), 722 (m); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.08–1.54 (32H, br), 1.68–1.78 (2H, m), 1.90–2.09 (8H, m), 2.82–2.94 (1H, br), 3.58–3.72 (2H, m), 3.74–3.87 (1H, br), 3.80 (3H, s, OCH_3), 4.45 (2H, s, OCH_2Ar), 5.30–5.44 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.6, arom. H), 7.25 (2H, d, *J* 8.3, arom. H). HRMS of (*R*)-**22**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4776.

4.16.2. (S)-Isomer. Similarly, (*S*)-**21** (1.55 g, 3.67 mmol) afforded 621 mg (31%) of (*S*)-**22** as a colorless oil, which solidified in a deep freezer and 686 mg (44%) of recovered (*S*)-**21**. Properties of (*S*)-**22**: n_D^{23} 1.4911; $[\alpha]_D^{27}$ −5.72 (*c* 2.86, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**22**. HRMS of (*S*)-**22**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4764.

4.17. (11*E*,19*Z*)-3-Acetoxy-11,19-octacosadien-1-ol PMB ether (23)

4.17.1. (R)-Isomer. In the same manner as described above for (*R*)-**19**, (*R*)-**22** (375 mg, 0.691 mmol) gave 394 mg (98%) of (*R*)-**23** as a colorless oil, n_D^{25} 1.4856; $[\alpha]_D^{27}$ −12.0 (*c* 3.00, hexane); ν_{max} (film): 3002 (w), 2925 (s), 2854 (s), 1738 (s, C=O), 1613 (m, arom. C=C), 1586 (w), 1514 (m), 1464 (m), 1371 (m), 1246 (s), 1098 (m), 1038 (m), 967 (m), 821 (m), 724 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.17–1.38 (30H, br), 1.45–1.60 (2H, br), 1.77–1.87 (2H, m), 1.90–2.07 (8H, m), 2.00 (3H, s, $COCH_3$), 3.40–3.51 (2H, m), 3.80 (3H, s, OCH_3), 4.40 (2H, s, OCH_2Ar), 4.96–5.05 (1H, m), 5.30–5.43 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.8, arom. H), 7.25 (2H, d, *J* 8.8, arom. H). HRMS of (*R*)-**23**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4879.

4.17.2. (S)-Isomer. Similarly, (*S*)-**22** (348 mg, 0.641 mmol) afforded 355 mg (95%) of (*S*)-**23** as a colorless oil, n_D^{23} 1.4855; $[\alpha]_D^{22}$ +12.5 (*c* 2.70, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**23**. HRMS of (*S*)-**23**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4871.

4.18. (11*E*,19*Z*)-3-Acetoxy-11,19-octacosadien-1-ol (CH503) [(11*E*,19*Z*)-1]

4.18.1. (R)-Isomer. In the same manner as described above for (3*R*,11*E*,19*E*)-**1**, (*R*)-**23** (366 mg, 0.626 mmol) was treated with DDQ (199 mg, 0.877 mmol) in CH_2Cl_2 (13 mL) and water (1.3 mL) to give 158 mg (54%) of (3*R*,11*E*,19*Z*)-**1** as an oil, which solidified in a deep freezer. n_D^{25} 1.4679; $[\alpha]_D^{26}$ −7.03 (*c* 1.07, hexane); ν_{max} (film): 3447 (br, O–H), 3004 (w), 2925 (s), 2853 (s), 1739 (s, C=O), 1464 (m), 1375 (m), 1245 (s, C–O), 1055 (m), 1022 (m), 966 (m), 721(w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.07–1.39 (30H, br), 1.47–1.71 (3H, m), 1.78–1.88 (1H, br), 1.88–2.07 (8H, m), 2.07 (3H, s, $COCH_3$), 2.42–2.54 (1H, br), 3.48–3.58 (1H, br, CH*H*OH), 3.58–3.68 (1H, br, C*H*HOH), 4.97–5.07 (1H, br, CHOAc), 5.29–5.43 (4H, m, 2× CH=CH); δ_C ($CDCl_3$): 14.0, 21.1, 22.7, 25.4, 27.1, 27.2, 29.0, 29.1, 29.26, 29.32, 29.47, 29.56, 29.57, 29.69, 29.72, 31.9, 32.52, 32.54, 34.6, 37.5, 58.5, 71.6, 129.8, 129.9, 130.2, 130.3, 171.9; GC–MS [same conditions as for (3*R*,11*E*,19*E*)-**1**]: t_R 23.62 min (92.7%). MS of (3*R*,11*E*,19*Z*)-**1** (70 eV, EI): *m*/*z*: 464 (3) [M^+, $C_{30}H_{56}O_3$], 446 (8), 404 (7), 386 (11), 374 (2), 357 (3), 287 (5), 273 (7), 259 (6), 247 (8), 233 (7), 219 (5), 207 (5), 194 (5), 177 (6), 163 (13), 149 (28), 135 (49), 121 (52), 109 (49), 95 (87), 81 (100), 67 (89), 55 (85), 43 (75). HRMS of (3*R*,11*E*,19*Z*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4303.

4.18.2. (S)-Isomer. Similarly, (*S*)-**23** (313 mg, 0.535 mmol) afforded 123 mg (49%) of (3*S*,11*E*,19*Z*)-**1** as an oil, which solidified in a deep freezer. n_D^{24} 1.4681; $[\alpha]_D^{27}$ +7.36 (*c* 1.02, hexane). Its IR, ^1H and ^{13}C NMR spectra were identical to those of (3*R*,11*E*,19*Z*)-**1**. GC–MS [same conditions as for (3*R*,11*E*,19*E*)-**1**]: t_R 23.61 min (90.4%). MS of (3*S*,11*E*,19*Z*)-**1** (70 eV, EI): *m*/*z*: 464 (3) [M^+, $C_{30}H_{56}O_3$], 446 (7), 404 (7), 386 (10), 374 (2), 357 (3), 287 (5), 273 (7), 259 (6), 247 (8), 233 (7), 219 (5), 207 (7), 194 (5), 177 (6), 163 (13), 149 (27), 135 (50), 121 (53), 109 (48), 95 (90), 81 (100), 67 (89), 55 (87), 43 (81). HRMS of (3*S*,11*E*,19*Z*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4303.

4.19. (*E*)-19-Octacosen-11-yne-1,3-diol 1-PMB ether (24)

4.19.1. (R)-Isomer. A solution of TBAF in THF (1.0 M, 5 mL, 5 mmol) was added to a solution of (*S*)-**16** (2.50 g, 3.82 mmol) in dry THF (5 mL). After stirring overnight at room temperature, an additional amount of TBAF in THF (1.0 M, 5 mL, 5 mmol) was added to the mixture to complete the reaction. The mixture was stirred for 2 d at room temperature. It was then diluted with ice-water and extracted with Et_2O. The Et_2O solution was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue (2.41 g) was chromatographed over SiO_2 (50 g). Elution with hexane/EtOAc (5:1) gave 1.88 g (91%) of (*R*)-**24** as a colorless solid. Mp 32–34 °C; $[\alpha]_D^{24}$ +6.17 (*c* 4.12, hexane); ν_{max} (film): 3507 (br, O–H), 2924 (vs), 2850 (s), 1613 (m, arom. C=C), 1586 (w), 1517 (m), 1464 (s), 1252 (s), 1126 (m), 1091 (m), 1035 (m), 963 (m), 813 (s), 723 (m); δ_H ($CDCl_3$): 0.88 (3H, t, 6.8), 1.17–1.53 (32H, br), 1.67–1.76 (2H, m), 1.90–2.03 (4H, br), 2.13 (4H, t, *J* 6.8, 2× C≡CCH_2), 2.89 (1H, d, *J* 3.2), 3.57–3.65 (1H, m), 3.65–3.73 (1H, m), 3.73–3.84 (1H, br), 3.80 (3H, s, OCH_3), 4.45 (2H, s, OCH_2Ar), 5.31–5.44 (2H, m, CH=CH), 6.88 (2H, d, *J* 8.4, arom. H), 7.25 (2H, d, *J* 8.4, arom. H). HRMS of (*S*)-**24**: calcd for $C_{36}H_{61}O_3$ [$(M+H)^+$]: 541.4615, found: 541.4613.

4.19.2. (S)-Isomer. In the same manner, (*S*)-**16** (2.50 g, 3.82 mmol) gave 1.76 g (85%) of (*S*)-**24** as a colorless solid. Mp 32–34 °C; $[\alpha]_D^{24}$ −6.23 (*c* 3.97, hexane). Its IR and ^1H NMR spectra were identical to those of (*S*)-**24**. HRMS of (*R*)-**24**: calcd for $C_{36}H_{61}O_3$ [$(M+H)^+$]: 541.4615, found: 541.4592.

4.20. (11*Z*,19*E*)-11,19-Octacosadiene-1,3-diol 1-PMB ether (25)

4.20.1. (R)-Isomer. In the same manner as described above for **8**, (*R*)-**24** (1.73 g, 3.20 mmol) was stirred with Lindlar's Pd catalyst on $CaCO_3$ with Pb^{2+} (Aldrich, 35 mg) and quinoline (69 μL) in cyclohexane (17 mL) under an H_2 atmosphere for 3 h to gave 1.69 g (97%)

of (*R*)-**25** as a colorless oil, which solidified in a deep freezer. n_D^{22} 1.4937; $[\alpha]_D^{25}$ +6.36 (*c* 4.09, hexane); ν_{max} (film): 3445 (br, O–H), 3002 (w), 2924 (s), 2853 (s), 1613 (m, arom. C=C), 1586 (w), 1514 (s), 1464 (m), 1248 (s), 1094 (m), 1038 (m), 967 (m), 822 (m), 722 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.08–1.53 (32H, br), 1.68–1.78 (2H, m), 1.89–2.12 (8H, m), 2.88 (1H, d, *J* 3.2), 3.57–3.65 (1H, m), 3.65–3.73 (1H, m), 3.73–3.89 (1H, br), 3.80 (3H, s, OCH_3), 4.45 (2H, s, OCH_2Ar), 5.29–5.45 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.6, arom. H), 7.25 (2H, d, *J* 9.0, arom. H). HRMS of (*R*)-**25**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4770.

4.20.2. (S)-Isomer. Similarly, (*S*)-**24** (1.68 g, 3.11 mmol) afforded 1.66 g (98%) of (*S*)-**25** as a colorless oil, which solidified in a deep freezer. n_D^{23} 1.4934; $[\alpha]_D^{25}$ −6.38 (*c* 3.96, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**25**. HRMS of (*S*)-**25**: calcd for $C_{36}H_{63}O_3$ [$(M+H)^+$]: 543.4772, found: 543.4773.

4.21. (11*Z*,19*E*)-3-Acetoxy-11,19-octacosadien-1-ol PMB ether (26)

4.21.1. (R)-Isomer. In the same manner as described above for (*R*)-**19**, (*R*)-**25** (1.57 g, 2.89 mmol) afforded 1.62 g (96%) of (*R*)-**26** as a colorless oil, n_D^{25} 1.4866; $[\alpha]_D^{22}$ −13.3 (*c* 4.05, hexane); ν_{max} (film): 3002 (w), 2925 (s), 2853 (s), 1738 (s, C=O), 1613 (m, arom. C=C), 1586 (w), 1514 (m), 1464 (m), 1371 (m), 1246 (s), 1097 (m), 1038 (m), 967 (m), 821 (m), 723 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.20–1.40 (30H, br), 1.48–1.58 (2H, br), 1.78–1.88 (2H, m), 1.90–2.07 (8H, m), 2.00 (3H, s, $COCH_3$), 3.40–3.51 (2H, m), 3.80 (3H, s, OCH_3), 4.40 (2H, s, OCH_2Ar), 4.97–5.05 (1H, m), 5.29–5.44 (4H, m, 2× CH=CH), 6.87 (2H, d, *J* 8.8, arom. H), 7.25 (2H, d, *J*=9.5, arom. H). HRMS of (*R*)-**26**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4877.

4.21.2. (S)-Isomer. Similarly, (*S*)-**25** (1.57 g, 2.89 mmol) afforded 1.64 g (97%) of (*S*)-**26** as a colorless oil, n_D^{27} 1.4858; $[\alpha]_D^{25}$ +13.4 (*c* 4.09, hexane). Its IR and ^1H NMR spectra were identical to those of (*R*)-**26**. HRMS of (*S*)-**26**: calcd for $C_{38}H_{65}O_4$ [$(M+H)^+$]: 585.4877, found: 585.4880.

4.22. (11*Z*,19*E*)-3-Acetoxy-11,19-octacosadien-1-ol (CH503) [(11*Z*,19*E*)-1]

4.22.1. (R)-Isomer. In the same manner as described above for (3*R*,11*E*,19*E*)-**1**, (*R*)-**26** (513 mg, 0.877 mmol) was treated with DDQ (279 mg, 1.23 mmol) in CH_2Cl_2 (18 mL) and water (1.8 mL) to give 222 mg (54%) of (3*R*,11*Z*,19*E*)-**1** as an oil, which solidified in a deep freezer. n_D^{26} 1.4673; $[\alpha]_D^{27}$ −7.33 (*c* 1.07, hexane); ν_{max} (film): 3460 (br, O–H), 3005 (w), 2925 (s), 2853 (s), 1738 (s, C=O), 1464 (m), 1375 (m), 1245 (s, C–O), 1055 (m), 1023 (m), 966 (m), 722 (w); δ_H ($CDCl_3$): 0.88 (3H, t, *J* 6.8), 1.18–1.42 (30H, br), 1.47–1.71 (3H, m), 1.78–1.88 (1H, m), 1.91–2.06 (8H, m), 2.08 (3H, s, $COCH_3$), 2.32–2.39 (1H, m), 3.49–3.58 (1H, m, CH*H*OH), 3.58–3.68 (1H, m, C*H*HOH), 4.98–5.06 (1H, m, CHOAc), 5.29–5.44 (4H, m, 2× CH=CH); δ_C ($CDCl_3$): 14.1, 21.1, 22.7, 25.5, 27.20, 27.22, 29.07, 29.18, 29.19, 29.33, 29.37, 29.42, 29.5, 29.64, 29.68, 29.74, 31.9, 32.60, 32.63, 34.7, 37.5, 58.6, 71.6, 129.8, 130.0, 130.3, 130.4, 172.0; GC–MS [same conditions as for (3*R*,11*E*,19*E*)-**1**]: t_R 23.61 min (95.8%). MS of (3*R*,11*Z*,19*E*)-**1** (70 eV, EI): *m*/*z*: 464 (2) [M^+, $C_{30}H_{56}O_3$], 446 (8), 404 (7), 386 (11), 374 (1), 357 (3), 287 (6), 273 (8), 259 (5), 247 (8), 233 (8), 219 (5), 207 (6), 194 (4), 177 (6), 163 (13), 149 (29), 135 (50), 121 (49), 109 (49), 95 (91), 81 (100), 67 (90), 55 (86), 43 (78). HRMS of (3*R*,11*Z*,19*E*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4300.

4.22.2. (S)-Isomer. Similarly, (*S*)-**26** (515 mg, 0.880 mmol) afforded 215 mg (53%) of (3*S*,11*Z*,19*E*)-**1** as an oil, which solidified in a deep freezer. n_D^{24} 1.4673; $[\alpha]_D^{27}$ +7.33 (*c* 1.03, hexane). Its IR, ^1H and ^{13}C NMR spectra were identical to those of (3*R*,11*Z*,19*E*)-**1**. GC–MS [same conditions as for (3*R*,11*E*,19*E*)-**1**]: t_R 23.54 min (92.5%). MS of (3*S*,11*Z*,19*E*)-**1** (70 eV, EI): *m*/*z*: 464 (3) [M^+, $C_{30}H_{56}O_3$], 446 (8), 404 (7), 386 (9), 374 (2), 357 (4), 287 (6), 273 (7), 259 (6), 247 (8), 233 (9), 219 (5), 207 (8), 194 (5), 177 (6), 163 (12), 149 (27), 135 (50), 121 (49), 109 (50), 95 (92), 81 (100), 67 (90), 55 (86), 43 (80). HRMS of (3*S*,11*Z*,19*E*)-**1**: calcd for $C_{30}H_{57}O_3$ [$(M+H)^+$]: 465.4302, found: 465.4302.

4.23. HPLC analysis

4.23.1. Analytical HPLC instruments. The HPLC pumps used was Tosoh DP-8020 equipped with Rheodyne 7125 sample injector with a 100 μL sample loop. The fluorescence detector was Jasco FP-920 with a 16 μL flow cell. The integrator was Chromatocorder 21 (System Instrument, Tokyo, Japan). Cryocool CC100-II was used to control the temperature of the second column.

4.23.2. Sample preparation procedure for analytical HPLC. The pheromone extract sample (containing about 12.5 μg of CH503) was dissolved in about 1 mL of toluene/acetonitrile (1:1). The excess amounts of the (1*R*,2*R*)- or (1*S*,2*S*)-**27**, DMAP, and EDC were added to the sample solution (about 0.3 mL). The solution was left to stand for over 1 h at room temperature. An aliquot was then loaded onto a silica gel TLC (7 cm in length, silica gel 60 F254, Art-5554, Merck) and developed with hexane/EtOAc (4:1, v/v). The target spot (R_f=0.44) detected by fluorescence was collected, packed in a Pasteur pipette, and eluted with EtOAc/MeOH (4:1, v/v). The eluate containing **28** was used for an HPLC analysis.

4.23.3. HPLC separation. The derivatives **28** were separated on a reversed-phase column (Develosil C30-UG-3, 3 μm, 4.6 mm i.d.× 150 mm, Nomura Chemical Co., Aichi, Japan) by eluting with a mixture of MeOH/MeCN/THF/hexane (202/215/150/55, v/v/v/v) at a rate of 0.4 mL/min at −20 °C. The detection was carried out by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm).

4.23.4. Analytical results. It was possible to separate eight derivatives of CH503 stereoisomers by the C-30 reversed phase column at −20 °C (Fig. 3A).

The retention time of the natural pheromone derivatized with (1*R*,2*R*)-**27** (35.6 min) was almost identical with that of the (1*R*,2*R*)-reagent-derivative of the authentic (3*R*,11*Z*,19*Z*)-**1** (35.5min) (Fig. 3B). Fig. 4C shows the chromatogram of the co-injection of **28** derived from the natural CH503 and (1*R*,2*R*,3′*R*,11′*Z*,19′*Z*)-**28**. The t_R of the natural pheromone derivatized with (1*S*,2*S*)-**27** also almost agreed with that of the (1*R*,2*R*)-reagent-derivative of the authentic(3*S*,11*Z*,19*Z*)-**1** (32.8 min), which was the enantiomer of the (1*S*,2*S*)-reagent-derivative of the (3*R*,11*Z*,19*Z*)-**1**. Since enantiomeric derivatives have the same retention time, the t_R of the (1*S*,2*S*)-reagent-derivative of the natural product also agreed with that of the (1*S*,2*S*)-reagent- derivative of the (3*R*,11Z,19Z)-**1**. Moreover, in the natural sample no peak derived from stereoisomers of CH503 was detected in both chromatograms. Therefore, both results mean that the stereochemistry of the natural product is 3*R*,11*Z*,19*Z*.

4.24. Preparation of a sample containing the natural CH503

The HPTLC fraction containing ca. 12.5 μg of CH503 was prepared twice as follows. Flies (700 males and females of *D. melanogaster*) were extracted with hexane (4 mL) for 20 min at room temperature. The extract was concentrated using a stream of N_2. The residue was dissolved in hexane (200 μL), and subjected to SiO_2 HPTLC (Merck, Catalog No. 1.05633.0001) separation by developing with hexane/EtOAc/AcOH (66:33:1, v/v/v). One lane of the HPTLC plate was loaded

with synthetic (3*R*,11*Z*,19*Z*)- or (3*R*,11*E*,19*E*)-**1** to determine the position of the natural CH503. The position was visualized under UV illumination after spraying with a primuline (Direct Yellow 59) solution (0.1% in 20% acetone). Based on the position of the synthetic standard, the SiO_2 of corresponding positions on unstained parallel bands was scraped off and eluted with hexane. The hexane solution was evaporated with a stream of N_2 to leave the residue containing ca. 12.5 μg of CH503.[3] The synthetic standard was loaded last onto the HPTLC plate to avoid the possibility of cross-contamination. In addition, the lane containing the synthetic standard was cut off from the rest of the plate prior to scraping off the portion containing natural CH503. The major component of the residue was confirmed as CH503 (**1**) by Direct Analysis in Real Time (DART) mass spectrometry.[25,26] The DART interface was operated in positive-ion mode with He with the gas heater set to 200 °C. The glow discharge needle potential was set to 3.5 kV. Electrode 1 was set to +150 V, and electrode 2 (grid) was set to +250 V.

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Supplementary data

Supplementary data, spectral data of all the stereoisomers of CH503 (**1**), can be found in the online version at doi:10.1016/j.tet.2012.03.002.

References and notes

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