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Synthesis of the Four Stereoisomers of 6-Acetoxy-19-methylnonacosane, the Most Potent Component of the Female Sex Pheromone of the New World Screwworm Fly, with Special Emphasis on Partial Racemization in the Course of Catalytic Hydrogenation

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Synthesis of the Four Stereoisomers of 6-Acetoxy-19-methylnonacosane, the Most Potent Component of the Female Sex Pheromone of the New World Screwworm Fly, with Special Emphasis on Partial Racemization in the Course of Catalytic Hydrogenation[‡]

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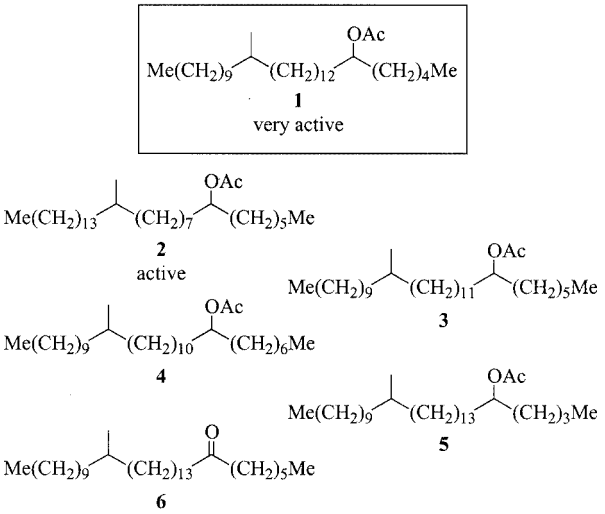
Starting from the enantiomers of citronellal and 1-octyn-3-ol, all four stereoisomers of 6-acetoxy-19-methylnonacosane were synthesized with sufficiently high stereochemical purities (more than 90% *ee* at C-6; about 97% *ee* at C-19) for their biological testing as the female sex pheromone of the screwworm fly (*Cochliomyia hominivorax*). All four isomers showed strong pheromone activity even at 1 μg, and no significant difference was observed in their potency. Adams' platinum oxide was found to cause partial racemization of enantiomerically pure secondary propargylic alcohols in the course of their catalytic hydrogenation to saturated and secondary alcohols, while palladium-charcoal was less potent in causing partial racemization. A new HPLC-based discrimination of chiral and secondary alcohols proved to be useful in following such a subtle partial racemization.

Introduction

The New World screwworm fly (*Cochliomyia hominivorax*) is a serious pest to livestock in Central and South America, and infestation of animal wounds by screwworm larvae results in a condition called myiasis.[1] In order to eradicate this fly, the sterile male release program was executed successfully in North and Central America. Possible incompatibility of the sex pheromones in released colony flies versus wild flies, however, may cause problems that would render a release program unsuccessful. Clarification of the pheromone system of *C. hominivorax* is therefore important and urgent. In 1993, Pomonis et al. reported the identification of sixteen compounds in a pheromonally active HPLC fraction extracted from the females of *C. hominivorax*.[1] Unfortunately, however, they were unable to isolate and identify the individual compounds responsible for the pheromone activity.

In 2001, we started a joint project to identify the pheromonally active compounds among Pomonis' sixteen candidates. One of us (D. A. C.) selected five compounds, **1, 2, 3, 4** and **6** (see Scheme 1), as the plausible pheromone candi-



Scheme 1. Structures of the pheromone candidates of the screwworm fly

[‡] Pheromone Synthesis, CCXXV. Part CCXXIV: T. Tashiro, S. Kurosawa, K. Mori, *Biosci. Biotechnol. Biochem.*, in press.

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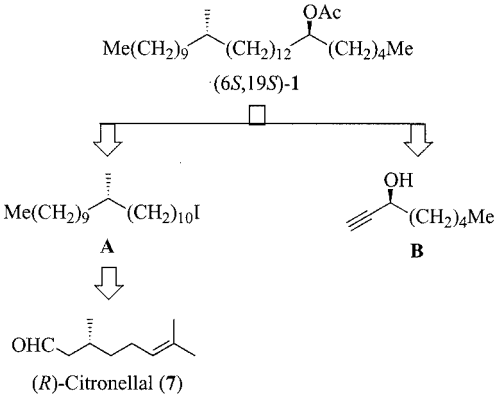
dates, and these were synthesized in 2002 as racemic and diastereomeric mixtures by K. M. and co-workers.[2] Subsequently, **5** was also proposed by D.A.C. as a pheromone candidate. K.M. then synthesized a racemic and diastereomeric mixture of **5** as well as the enantiomers of **6**.[3] Bioassay of all the synthetic products on *C. hominivorax* revealed **1** and **2** to be active as the sex pheromone.[4] Because **1** was more potent than **2** in pheromone activity, we undertook the synthesis of all the possible stereoisomers of **1** for biological evaluation. An important aspect of our project was the precise estimation of the stereochemical purity of the synthetic stereoisomers of **1**. Owing to the long distance between the two stereogenic centers of **1**, its stereochemical purity was difficult to estimate. The only reliable method for that purpose was Ohrui's analytical protocol for chiral alcohols employing fluorescent and chiral labelling reagents.[5] This paper describes the results of our joint work culminating in the successful synthesis (K.M.), analysis (T.O. and H.O.) and bioassay (D.R.B. and D.A.C.) of the four stereoisomers of 6-acetoxy-19-methylnonacosane (**1**), all of which showed similar pheromone activity.

Results and Discussion

Since our purpose was to prepare all stereoisomers of **1** with high enantiomeric purity, the synthesis utilized a so-called "chiral pool" approach. Scheme 2 shows the retrosynthetic analysis of (6*S*,19*S*)-**1**. The acetate (6*S*,19*S*)-**1** can be constructed by combining two building blocks **A** and **B**, the latter being commercially available (Aldrich, 98% *ee*). The iodide **A** can be prepared from (*R*)-citronellal (**7**, Takasago, 97% *ee*).

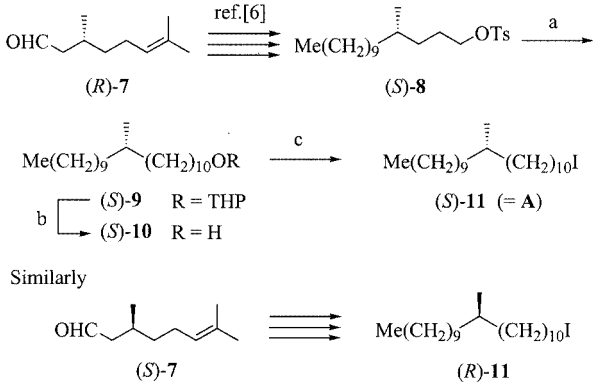


Scheme 2. Retrosynthetic analysis of (6*S*,19*S*)-**1**

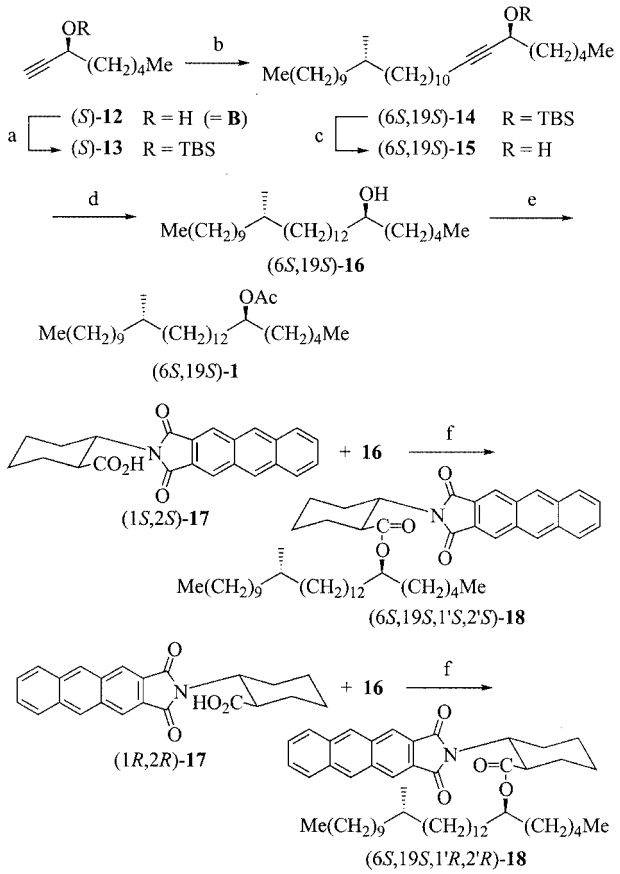
Synthesis and Analysis of (6*S*,19*S*)-19-Methylnonacosan-6-ol (16)

For the successful synthesis of the four stereoisomers of **1**, we had to develop a route which minimizes the danger of partial racemization in the course of the synthesis. We therefore first synthesized (6*S*,19*S*)-19-methylnonacosan-6-ol (**16**) as summarized in Schemes 3 and 4, and analyzed its stereochemical purity as shown in Scheme 4 and Figure 1.

Preparation of the methyl-branched alkyl iodide **11** is shown in Scheme 3. (*R*)-Citronellal (**7**) was converted into tosylate (*S*)-**8** as reported previously.[6] Under Schlosser conditions in the presence of dilithium tetrachlorocuprate,[7] (*S*)-**8** was coupled with tetrahydropyranyl(THP)oxyheptyl-



Scheme 3. Synthesis of the building blocks (*R*)- and (*S*)-**11**: reagents: (a) $THPO(CH_2)_7MgBr$, Li_2CuCl_4, THF; (b) TsOH, MeOH (69%, 2 steps); (c) i) TsCl, C_5H_5N; ii) NaI, DMF (77%, 2 steps)



Scheme 4. Synthesis of (6*S*,19*S*)-**1**; reagents: (a) TBSCl, imidazole, DMF (95%); (b) *n*BuLi, THF/HMPA, then (*S*)-**11**; (c) TBAF, THF (48%, 2 steps); (d) H_2, PtO_2, hexane (69%, after SiO_2 chromatography); (e) Ac_2O, DMAP, C_5H_5N, CH_2Cl_2 (64%, after SiO_2 chromatography); (f) EDC, DMAP, toluene, MeCN, room temp., >10 h (quant.)

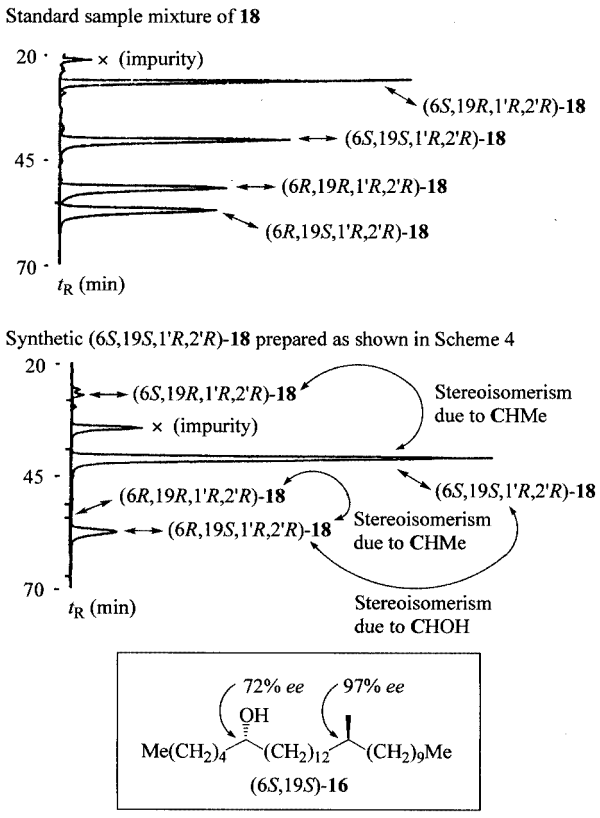


Figure 1. HPLC separation of the derivatized alcohol (for detailed analytical conditions see Exp. Sect.)

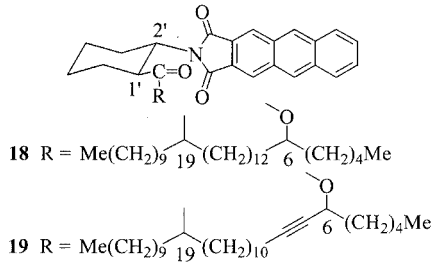
magnesium bromide to give (*S*)-**9**. Removal of the THP protective group of (*S*)-**9** afforded the crystalline alcohol (*S*)-**10**. Tosylation of (*S*)-**10** was followed by treatment of the resulting tosylate with sodium iodide to give (*S*)-**11** in 54% overall yield based on (*S*)-**8**. Similarly, (*R*)-**11** was prepared from (*S*)-**7**.

Another building block, (*S*)-1-octyn-3-ol (**12**), was first converted into the corresponding *tert*-butyldimethylsilyl (TBS) ether (*S*)-**13** as shown in Scheme 4.

Treatment of (*S*)-**13** in THF/HMPA with 1.2 equivalents of *n*-butyllithium generated the alkynide anion, which was alkylated with (*S*)-**11** to give (6*S*,19*S*)-**14**, whose TBS protective group was removed by treatment with tetra(*n*-butyl)-ammonium fluoride (TBAF) in THF. The acetylenic alcohol (6*S*,19*S*)-**15** was obtained in 48% yield based on (*S*)-**11** (2 steps). Hydrogenation of (6*S*,19*S*)-**15** over Adams' platinum oxide in hexane at room temperature for 2.5 h afforded (6*S*,19*S*)-19-methylnonacosan-6-ol (**16**) as a low-melting solid, m.p. 35.0−36.5 °C. Acetylation of (6*S*,19*S*)-**16** furnished (6*S*,19*S*)-**1**, one of the desired target molecules, as an oil.

Needless to say, the product (6*S*,19*S*)-**1** should have high stereochemical purity if reliable bioassay data is to be obtained. It was therefore important to estimate the stereochemical purity of (6*S*,19*S*)-**1** in order to check the overall preservation of the stereochemistry in the course of the synthetic sequence. Ohrui's analytical method for chiral acids and alcohols was most appropriate for that purpose, as evidenced by our recent application of that method for determination of the absolute configuration of plakoside A, a marine sphingolipid.[8] Accordingly, (6*S*,19*S*)-**16**, the precursor of (6*S*,19*S*)-**1**, was treated with Ohrui's chiral and fluorescent derivatizing reagents (1*S*,2*S*)- and (1*R*.2*R*)-**17** [2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid] to give the esters (6*S*,19*S*,1′*S*,2′*S*)-and (6*S*,19*S*,1′*R*,2′*R*)-**18**, respectively.[5] As shown in Figure 1, the derived esters **18** were not homogeneous, indicating that the synthetic alcohol (6*S*,19*S*)-**16** was stereochemically impure. Table 1 shows the HPLC retention times of the four stereoisomers of **18** and also those of **19**. They were clearly separated on a tandem series of two reversed-phase columns (Develosil C30-UG-3) cooled to −30 °C (for **18**) or −40 °C (for **19**). HPLC analysis of the esters **18** derived from (6*S*,19*S*)-**16** allowed us to estimate the enantiomeric purity of the starting alcohol **16** as 72% *ee* at C-6 and 97% *ee* at C-19.

Table 1. HPLC retention times of the stereoisomers of **18** and **19**



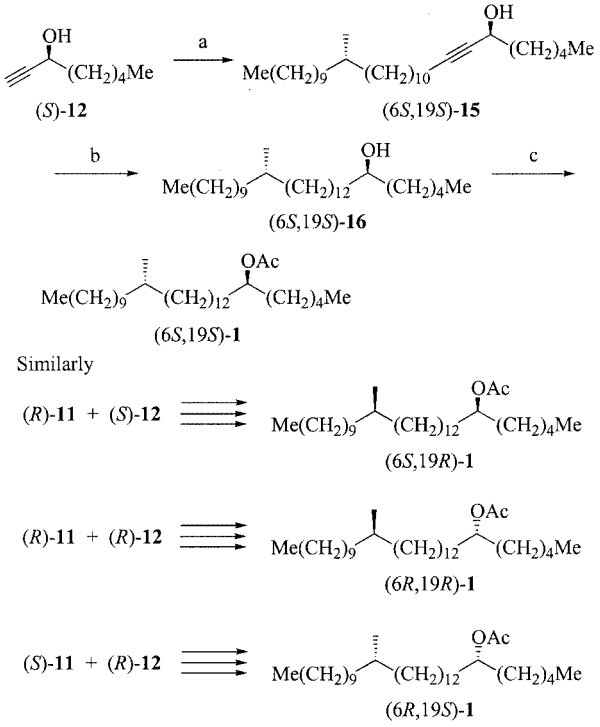
Stereoisomers	Retention times[a] (min)
(6*S*,19*R*,1′*R*,2′*R*)- and (6*R*,19*S*,1′*S*,2′*S*)-**18**	64.4[b]
(6*S*,19*S*,1′*R*,2′*R*)- and (6*R*,19*R*,1′*S*,2′*S*)-**18**	130.5[b]
(6*R*,19*R*,1′*R*,2′*R*)- and (6*S*,19*S*,1′*S*,2′*S*)-**18**	157.8[b]
(6*R*,19*S*,1′*R*,2′*R*)- and (6*S*,19*R*,1′*S*,2′*S*)-**18**	177.9[b]
(6*R*,19*S*,1′*R*,2′*R*)- and (6*S*,19*R*,1′*S*,2′*S*)-**19**	169.0[c]
(6*R*,19*R*,1′*R*,2′*R*)- and (6*S*,19*S*,1′*S*,2′*S*)-**19**	175.5[c]
(6*S*,19*S*,1′*R*,2′*R*)- and (6*R*,19*R*,1′*S*,2′*S*)-**19**	224.7[c]
(6*S*,19*R*,1′*R*,2′*R*)- and (6*R*,19*S*,1′*S*,2′*S*)-**19**	242.1[c]

[a] Column: Develosil C30-UG-3 (4.6 mm i.d. × 150 mm) × 2. [b] Column temp.: −30 °C; Eluent: MeCN/THF/hexane/MeOH, 40:210:120:80, flow rate: 0.2 mL/min. [c] Column temp.: −40 °C; Eluent: MeCN/THF/hexane/MeOH, 60:80:30:60, flow rate: 0.3 mL/min.

The low enantiomeric purity at C-6 must be the result of partial racemization at a certain stage in converting (*S*)-**13** (98% *ee*) to (6*S*,19*S*)-**16**. We thought that the racemization was caused by addition of a small excess of *n*-butyllithium to (*S*)-**13** to generate the alkynide anion. The excess base might have abstracted the proton at C-3 of (*S*)-**13**, which would have led to partial racemization. Another possible cause of racemization was the hydrogenation step (**15** → **16**), but at this stage we assumed it to be less important than the effect of the excess base.

Synthesis of the Four Stereoisomers of 6-Acetoxy-19-methylnonacosane (1)

In order to avoid racemization at C-3 of (*S*)-**13**, we decided to employ the dianion derived from (*S*)-**12** for its alkylation with (*S*)-**11**. Under the conditions for formation of the dianion of (*S*)-**12** by its treatment with *n*-butyllithium, there must be no anion formation at C-3 of (*S*)-**12** to give a trianion. We therefore prepared the four stereoisomers of acetylenic alcohol **15** by dianion alkylation as shown in Scheme 5. Hydrogenation of **15** over Adams' platinum oxide was allowed to continue for only 15 min, and the product **16** was acetylated to give the desired four stereoisomers of the acetate **1**. It should be noted that (6*S*,19*R*)-**16** and (6*R*,19*S*)-**16** showed higher melting points (56−57 °C and 55−56°C, respectively) than those (37−38 °C and 34−35 °C, respectively) of (6*S*,19*S*)-**16** and (6*R*,19*R*)-**16**. The stereoisomers of **1** or **16** could not be distinguished from each other by spectroscopic methods including 300 MHz ^{1}H NMR, because their two stereogenic centers are separated by twelve carbon atoms.



Scheme 5. Synthesis of the four stereoisomers of **1**; reagents: (a) 2.2 equiv. *n*BuLi, THF/HMPA, then (*S*)-**11** (68%); (b) H_2, PtO_2, EtOAc (15 min at room temp.; 85%); (c) Ac_2O, DMAP, C_5H_5N, CH_2Cl_2 (98%)

Table 2. Results of HPLC determination of stereochemical purities of the four stereoisomers of 19-methylnonacosan-6-ol (**16**) prepared as shown in Scheme 5 (after their derivatization to **18**)

Stereoisomers	Enantiomeric purity (% *ee* ±0.5%) at	
	C-6	C-19
(6*S*,19*S*)-**16**	93.5	97
(6*S*,19*R*)-**16**	97.5	98.5
(6*R*,19*R*)-**16**	90	96.5
(6*R*,19*S*)-**16**	99.5	98.5

lation are shown in Table 2. The results indicated that both (6*S*,19*R*)-and (6*R*,19*S*)-**16** were virtually pure at both stereogenic centers. The other two isomers (6*S*,19*S*)- and (6*R*,19*R*)-**16**, however, were partially racemized at C-6 to give products of 93.5% *ee* and 90% *ee* at C-6, respectively. Since the dianion alkylation method for preparation of **16** cannot accompany racemization at C-6, 3 to 5% inversion at C-6 of (6*S*,19*S*)- and (6*R*,19*R*)-**16** must have been brought about by partial racemization in the course of hydrogenation.

This possibility was examined by comparing the results of the HPLC analysis of **19** derived from acetylenic alcohols **15** and that of **18** derived from the hydrogenation products **16** (Table 3). While 5% palladium-charcoal (Wako Pure Chemicals Co.) did not cause any appreciable racemization at C-6, Adams' platinum oxide (Kojima Chemical Co.) caused partial racemization after 3 h of hydrogenation at room temperature. When the reaction time with platinum catalyst was shortened to 15 min, the degree of racemization at C-6 became almost negligible.

Table 3. Partial racemization at C-6 in the course of catalytic hydrogenation of 19-methylnonacos-7-yn-6-ol (**15**) to 19-methylnonacosan-6-ol (**16**) as analyzed by HPLC after derivatization[a]

Condition of hydrogenation[b]	Stereoisomers		Enantiomeric purity (% *ee* ±0.5%) at	
			C-6	C-19
1) H_2, PtO_2, EtOAc, room temp., 15 min	precursor	(6*S*,19*R*)-**15**	98.5	98
	product	(6*S*,19*R*)-**16**	97.5	98.5
2) H_2, PtO_2, EtOAc, room temp., 3 h	precursor	(6*S*,19*R*)-**15**	98.5	98
	product	(6*S*,19*R*)-**16**	65	98
3) H_2, 5% Pd-C, EtOAc, room temp., 15 min	precursor	(6*R*,19*S*)-**15**	100	98.5
	product	(6*R*,19*S*)-**16**	99.5	98
4) H_2, 5% Pd-C, EtOAc, room temp., 3 h	precursor	(6*R*,19*R*)-**15**	99.5	97.5
	product	(6*R*,19*R*)-**16**	99	98

[a] HPLC analysis was done after derivatizing **15** and **16** with (1*S*,2*S*)-**17** and (1*R*,2*R*)-**17** to give **18** and **19**. [b] PtO_2: Kojima Chemical Co. 5% Pd-C: Wako Pure Chemical Co.

Platinum Oxide-Catalyzed Hydrogenation Caused Partial Racemization at C-6

The results of the HPLC analysis of the esters **18** derived from the four stereoisomers of **16** prepared by dianion alky-

It is widely accepted that platinum oxide catalyst is less prone than palladium catalyst to cause partial racemization at branched-chain stereogenic centers in the course of hydrogenation.[9] Our results, however, suggest that plati-

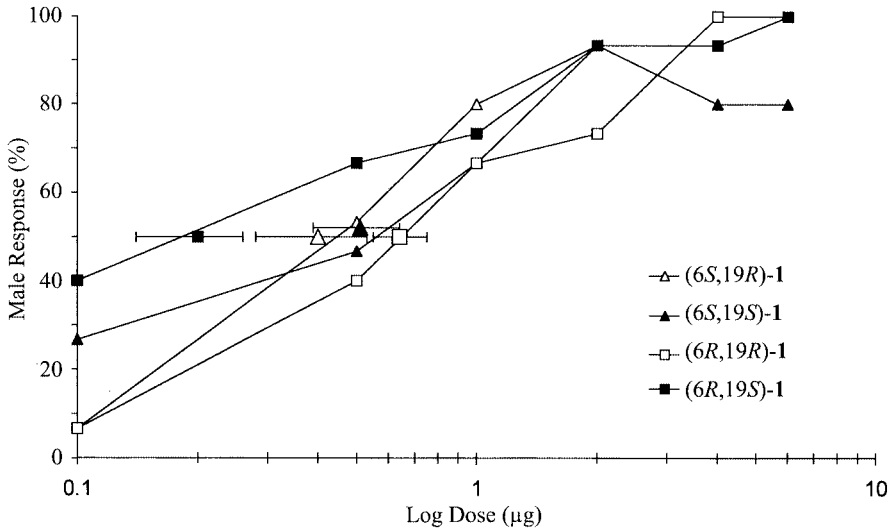


Figure 2. Dose−behavioral response curves of P-95 strain of *C. hominivorax* males to the four stereoisomers of 6-acetoxy-19-methylnonacosane ($N = 15$). The percentage of males that gave full copulatory responses to treated decoys, dead females and solvent controls was recorded. Horizontal error bars indicate 95% fiducial limits for the 50% response levels to each of the stereoisomers determined from probit analysis using all treatments [fiducial limits to (6*S*,19*S*)-**1** are shown separated for convenience]. The symbol in the middle of the fiducial limits line marks the 50% response of the predicted response curves

num oxide should be avoided or used only for a short period to keep the possible racemization to a minimum.

Bioassay and Conclusion

The four stereoisomers of 6-acetoxy-19-methylnonacosane (**1**) were bioassayed in dose−response tests at 0.1 to 6 μg against male screwworm flies (Figure 2). The activities of the isomers were statistically indistinguishable. Full pheromone activity was generally 66% and above at 1 μg and above 73% at 2 μg, with some isomers having 100% responses at 4 μg and 6 μg. Many responses above 2 μg were 93%. The pheromone activity of the synthetic stereoisomers of **1** was therefore quite good, yielding 50% response at 0.5 μg. The stereochemistry of **1**, however, was not significant for bioactivity, indicating that the stereochemistry was not critical for the recognition of **1** by the pheromone receptors. In this regard, some other examples are known,[10] especially in the case of 3,11-dimethylnonacosan-2-one, the female sex pheromone of the German cockroach (*Blattella germanica*).[11] The absolute configuration of the naturally occurring **1** remains obscure. Detailed biological results will be published separately by one of us (D.A.C.).

Another practically important conclusion is the danger of using platinum oxide as a catalyst for hydrogenation of unsaturated and optically active secondary alcohols. The platinum catalyst will diminish the enantiomeric purity at the asymmetric carbon atom bearing a hydroxy group.

Experimental Section

General: IR: Horiba FT-720. ^{1}H NMR: Varian Mercury-300 (300 MHz) (TMS at δ = 0.00 pm or $CHCl_3$ at δ = 7.26 ppm as internal standard). ^{13}C NMR: Varian Mercury-300 (75 MHz) ($CDCl_3$ at δ = 77.0 ppm as internal standard). HRMS: Jeol JMS-SX102A. Melting points: Uncorrected values, Yanaco MP-S3. n_D: Atago DNT-1. $[\alpha]_D$: Jasco DIP-320. CC: Merck Kieselgel 60 Art 1.07734.

(*S*)-11-Methylhenicosan-1-ol [(*S*)-10]: A solution of $THPO(CH_2)_7Br$ (7.8 g, 25 mmol) in dry THF (30 mL) was added to Mg (800 mg, 33 mmol) and a small piece of I_2 (ca. 5 mg) with stirring under Ar to make a Grignard reagent. The generated Grignard reagent was added dropwise to a stirred and cooled solution of (*S*)-**8** [ca. 3.4 g; prepared from (*S*)-4-methyltetradecan-1-ol (1.8 g, 7.9 mmol)] in THF (40 mL) at −78 °C under Ar. A solution of Li_2CuCl_4 in THF (0.1 M, 1.0 mL) was added to the mixture. The stirring was continued overnight with gradual warming to room temperature (22 °C). The mixture was poured into ice and satd. NH_4Cl solution, and extracted with hexane. The hexane solution was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give a crude oil (7.9 g) containing (*S*)-**9**. This was dissolved in MeOH (80 mL), and acidified with *p*-TsOH·H_2O (150 mg, 0.8 mmol). After stirring for two days at room temperature (22 °C), the mixture was neutralized with $NaHCO_3$ aq. solution, and concentrated in vacuo to remove MeOH. The residue was extracted with Et_2O. The organic extract was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo to give a crude oil (5.2 g). This was chromatographed over SiO_2 (60 g). Elution with hexane/EtOAc (60:1) gave (*S*)-**10** (1.8 g, 69%) as an oil, n_D^{24} = 1.4520. $[\alpha]_D^{24}$ = +0.55 (c = 2.2, hexane). IR (film): $\tilde{\nu}$ = 3350 (m, OH), 1055 (m, C−O), 720 (m) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.83 (d, J = 6.3 Hz, 3 H, 11-CH_3), 0.87 (t, J = 6.6 Hz, 3 H, 21-H_3), 1.00−1.18 (m, 2 H), 1.18−1.39 (br., 33 H), 1.44 (s, 1 H, OH), 1.56 (m, 2 H, 2-H_2), 3.62 (t, J = 6.9 Hz, 2 H, 1-H_2) ppm. $C_{22}H_{46}O$ (326.6): calcd. C 80.90, H 14.20; found C 80.41, H 13.58.

(*R*)-11-Methylhenicosan-1-ol [(*R*)-10]: Applying the same method to (*R*)-4-methyltetradecan-1-ol (3.2 g, 14 mmol) yielded 1.8 g (39%) of (*R*)-**10** as an oil, n_D^{23} = 1.4511. $[\alpha]_D^{23}$ = −0.40 (c = 2.3, hexane). Its IR and NMR spectra were identical with those of (*S*)-**10**. $C_{22}H_{46}O$ (326.6): calcd. C 80.90, H 14.20; found C 80.45, H 14.01.

(*S*)-1-Iodo-11-methylhenicosane [(*S*)-11]: *p*-TsCl (0.9 g, 4.7 mmol) was added in one portion to a stirred and ice-cooled solution of (*S*)-**10** (1.2 g, 3.7 mmol) in dry pyridine (6.0 mL). The mixture was

left to stand overnight in a refrigerator, poured into ice/water and extracted with hexane/Et_2O. The extract was washed with dil. HCl, H_2O, $NaHCO_3$ satd. solution and brine, dried ($MgSO_4$) and concentrated in vacuo to give 1.5 g of the tosylate as an oil. This was dissolved in DMF (15 mL), and stirred with NaI (4.0 g, 27 mmol) for 2 h at 50 °C. The mixture was then diluted with H_2O and extracted with hexane/Et_2O. The extract was washed with H_2O and brine, dried ($MgSO_4$) and concentrated in vacuo to give 1.64 g of crude (*S*)-**11** as an oil. This was chromatographed over SiO_2 (20 g). Elution with hexane gave (*S*)-**11** as an oil (1.2 g, 77%), n_D^{24} = 1.4735. $[\alpha]_D^{24}$ = +0.74 (*c* = 3.0, hexane). IR (film): $\tilde{\nu}$ = 720 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.83 (d, *J* = 6.3 Hz, 3 H, 11-CH_3), 0.88 (t, *J* = 6.6 Hz, 3 H, 21-H_3), 1.00−1.15 (m, 2 H), 1.20−1.48 (br. 33 H), 1.82 (seemingly quint., *J* = 7.2 Hz, 2 H, 2-H_2), 3.18 (t, *J* = 7.2 Hz, 2 H, 1-H_2) ppm. $C_{22}H_{45}I$ (436.5): calcd. C 60.54, H 10.39; found C 60.30, H, 10.24.

(*R*)-1-Iodo-11-methylhenicosane [(*R*)-11]: Applying the same method to (*R*)-**10** (1.1 g, 3.4 mmol) yielded 0.8 g (54%) of (*R*)-**11** as an oil, n_D^{21} = 1.4765. $[\alpha]_D^{21}$ = −0.30 (*c* = 3.4, hexane). Its IR and ^1H NMR spectra were identical with those of (*S*)-**11**. $C_{22}H_{45}I$ (436.5): calcd. C 60.54, H 10.39; found C 61.02, H 10.51.

(*S*)-3-*tert*-Butyldimethylsilyloxy-1-octyne [(*S*)-13]: TBSCl (7.2 g, 48 mmol) and imidazole (8.2 g, 140 mmol) were added to a stirred and ice-cooled solution of (*S*)-**12** (Aldrich, 98% *ee;* 5.0 g, 40 mmol) in dry DMF (50 mL). The mixture was left to stand overnight at room temperature, diluted with H_2O and extracted with Et_2O. The extract was washed with H_2O and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 9.0 g (95%) of (*S*)-**13** as an oil, bp 75−78 °C/3 Torr. n_D^{26} = 1.4326. $[\alpha]_D^{22}$ = −42.4 (*c* = 1.01, hexane). This was used in the next step without further purification.

(6*S*,19*S*)-19-Methylnonacos-7-yn-6-ol [(6*S*,19*S*)-15] via (6*S*,19*S*)-14: A solution of *n*BuLi in hexane (1.6 M, 1.8 mL, 2.9 mmol) was added to a stirred and cooled solution of (*S*)-**13** (673 mg, 2.8 mmol) in dry THF (5 mL) and HMPA (1 mL) at −40 °C under Ar. The mixture was stirred at 0 °C for 30 min, and then cooled to −40 °C. To this was added with stirring and cooling at −40 °C a solution of (*S*)-**11** (510 mg, 1.2 mmol) in THF (5 mL). The mixture was stirred overnight at room temperature, poured into ice/NH_4Cl solution and extracted with hexane/Et_2O. The extract was washed with H_2O and brine, dried ($MgSO_4$) and concentrated in vacuo to give 570 mg of crude (6*S*,19*S*)-**14** as an oil, which was dissolved in THF (4 mL). TBAF (1 M in THF, 4 mL) was added to the solution above and the mixture was left to stand for three days at room temperature. The mixture was diluted with H_2O and extracted with hexane. The hexane solution was washed with H_2O and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (50:1) gave 320 mg (48%) of (6*S*,19*S*)-**15** as an oil, $n_D^{23.5}$ = 1.4620. $[\alpha]_D^{20}$ = −5.0 (*c* = 1.02, hexane). The oil solidified in a refrigerator to give a waxy solid. IR (film): $\tilde{\nu}$ = 3365 (m, OH), 2370 (w, C≡C), 1020 (m, C−O), 720 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.83 (d, *J* = 6.3 Hz, 3 H, 19-CH_3), 0.88 (t, *J* = 7.0 Hz, 3 H, 29-H_3), 0.89 (t, *J* = 7.0 Hz, 3 H, 1-H_3), 1.00−1.18 (m, 2 H), 1.20−1.60 (br., 41 H), 1.60−1.75 (m, 4 H, 5-H_2, 10-H_2), 2.19 (dt, *J* = 2.1, 6.9 Hz, 2 H, 9-H_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0, 14.1, 18.7, 19.7, 22.6, 22.7, 24.9, 27.1, 28.7, 28.8, 29.1, 29.4, 29.5, 29.6, 29.65, 29.67, 29.69, 29.70, 29.72, 30.0, 31.5 (x, 2), 31.6, 31.9, 32.7, 37.1, 38.2, 62.8, 81.3, 85.6 ppm. $C_{30}H_{58}O$ (434.8): calcd. C 82.87, H 13.45; found C 82.66, H 13.46.

(6*S*,19*S*)-19-Methylnonacosan-6-ol [(6*S*,19*S*)-16]: Hydrogenation of (6*S*,19*S*)-**15** with H_2 and PtO_2 was first done in hexane, later in EtOAc. PtO_2 (40 mg) was added to a solution of (6*S*,19*S*)-**15** (120 mg, 0.28 mmol) in hexane (5 mL), and the suspension was vigorously stirred for 2.5 h under H_2 at room temperature. Pt black finally precipitated by coagulation. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (5 g). Elution with hexane/EtOAc (50:1) gave 83 mg (69%) of **16** as a solid, m.p. 35−36.5 °C. $[\alpha]_D^{24}$ = −0.5 (*c* = 0.52, hexane). IR (nujol): $\tilde{\nu}$ = 3325 (m, OH), 1130 (m, C−O), 720 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.83 (d, *J* = 6.3 Hz, 3 H, 19-CH_3), 0.88 (t, *J* = 6.0 Hz, 3 H, 1-H_3 or 29-H_3), 0.89 (t, *J* = 6.0 Hz, 1-H_3 or 29-H_3), 1.00−1.18 (m, 2 H), 1.20−1.60 (br., 50 H), 3.58 (m, 1 H, 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0, 14.1, 19.7, 22.6, 22.7, 25.3, 25.7, 27.1, 29.4, 29.63, 29.64, 29.67, 29.69, 29.71, 29.72, 29.73, 30.0, 31.6, 31.9, 32.7, 37.1, 37.4, 37.5, 72.0 ppm. $C_{30}H_{62}O$ (438.8): calcd. C 82.11, H 14.24; found C 82.07, H 14.44.

(6*S*,19*S*)-6-Acetoxy-19-methylnonacosane [(6*S*,19*S*)-1]: Acetic anhydride (0.6 mL, 6.4 mmol) and DMAP (100 mg, 0.8 mmol) were added to a stirred and ice-cooled solution of (6*S*,19*S*)-**16** (75 mg, 0.17 mmol) in CH_2Cl_2 (1 mL) and pyridine (1 mL). After stirring for 30 min at room temperature, the mixture was poured into ice/water and extracted with hexane. The hexane solution was washed with dil. HCl, $NaHCO_3$ aq. solution and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue (84 mg) was chromatographed on SiO_2 (2 g). Elution with hexane/EtOAc (70:1) gave 53 mg (64%) of (6*S*,19*S*)-**1** as a colorless oil, n_D^{25} = 1.4501. $[\alpha]_D^{24}$ = −0.7 (*c* = 0.84, hexane). IR (film): $\tilde{\nu}$ = 1740 (s, C=O), 1240 (s, C−O), 1020 (m), 720 (m) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.83 (d, *J* = 6.3 Hz, 3 H, 19-CH_3), 0.88 (t, *J* = 6.0 Hz, 6 H, 1-H_3, 29-H_3), 1.00−1.18 (m, 2 H), 1.18−1.40 (br., 45 H), 1.40−1.56 (m, 4 H, 5-H_2, 7-H_2), 2.03 (s, 3 H, $OCOCH_3$), 4.85 (seemingly quint., *J* = 6.0 Hz, 1 H, 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0, 14.1, 19.7, 21.3, 22.5, 22.65, 22.69, 22.70, 25.0, 25.3, 27.1, 29.4, 29.54, 29.58, 29.61, 29.64, 29.66, 29.67, 29.70, 29.72, 30.0, 31.6, 31.7, 31.92, 31.93, 32.7, 34.05, 34.11, 37.1, 74.5, 171.0 ppm. $C_{32}H_{64}O_2$ (480.9): calcd. C 79.93, H 13.42; found C 79.89, H 13.31.

19-Methylnonacos-7-yn-6-ol (15) via the Dianion of 12. (i) (6*S*,19*S*)-15: The dianion of (*S*)-**12** was prepared by addition of a solution of *n*BuLi in hexane (1.6 M, 8.5 mL, 12 mmol) to a stirred and cooled solution of (*S*)-**12** (760 mg, 6 mmol) in THF (7.5 mL) and HMPA (1.5 mL) at −40 °C under Ar. The mixture was stirred at 0 °C for 20 min. The stirred solution was then cooled to −78 °C. To this mixture was added dropwise a solution of (*S*)-**11** (720 mg, 1.6 mmol) in THF (7.5 mL) with stirring and cooling at −78 °C. After stirring at −78 °C for 20 min, the stirred mixture was left to stand overnight with gradual warming to room temperature (22 °C). It was then poured into ice/NH_4Cl aq. solution and extracted with hexane/Et_2O. The extract was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (20 g). Elution with hexane/EtOAc (50:1) afforded 493 mg (68%) of (6*S*,19*S*)-**15** as an oil, n_D^{21} = 1.4645. $[\alpha]_D^{21}$ = −4.7 (*c* = 1.9, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**15** prepared via (6*S*,19*S*)-**14**.

(ii) (6*S*,19*R*)-15: In the manner described for (6*S*,19*S*)-**15**, (*R*)-**11** (370 mg, 0.85 mmol) and (*S*)-**12** (Aldrich, 98% *ee*; 412 mg, 3.4 mmol) gave 110 mg (30%) of (6*S*,19*R*)-**15**, as an oil, n_D^{21} = 1.4634. $[\alpha]_D^{21}$ = −4.0 (*c* = 1.0, hexane). Its IR and NMR spectra were virtually identical with those of (6*S*,19*S*)-**15**.

(iii) (6*R*,19*R*)-15: In the manner described for (6*S*,19*S*)-**15,** (*R*)-**11** (370 mg, 0.85 mmol) and (*R*)-**12** (410 mg, 3.4 mmol) gave 150 mg (41%) of (6*R*,19*R*)-**15** as oil, n_D^{21} = 1.4640. $[\alpha]_D^{22}$ = +4.2 (*c* = 1.1,

hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**15**.

(iv) (6*R*,19*S*)-15: In the manner described for (6*S*,19*S*)-**15,** (*S*)-**11** (655 mg, 1.5 mmol) and (*R*)-**12** (760 mg, 6 mmol) gave 261 mg (40%) of (6*R*,19*S*)-**15** as an oil, n_D^{21} = 1.4608. $[\alpha]_D^{21}$ = +4.3 (*c* = 2.9, hexane). Its IR and NMR spectra were virtually identical with those of (6*S*,19*S*)-**15**.

19-Methylnonacosan-6-ol (16). (i) (6*S*,19*S*)-16: Adams' catalyst (PtO_2, 50 mg) was added to a solution of (6*S*,19*S*)-**15** (120 mg, 0.28 mmol) in EtOAc (5 mL). The suspension was vigorously stirred under H_2 at room temperature (20 °C) for 15 min. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was chromatographed over SiO_2 (4 g). Elution with hexane/EtOAc (50:1) gave 102 mg (85%) of (6*S*,19*S*)-**16** as a solid. Recrystallization from acetone yielded pure (6*S*,19*S*)-**16**, m.p. 37−38 °C. $[\alpha]_D^{24}$ = −0.6 (*c* = 0.64, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**16** prepared via (6*S*,19*S*)-**14**. HRMS (EI) [$M^+ - H_2O$] ($C_{30}H_{60}$): calcd. 420.4695; found 420.4693.

(ii) (6*S*,19*R*)-16: In the manner described for (6*S*,19*S*)-**16**, (6*S*,19*R*)-**15** (100 mg, 0.23 mmol) in EtOAc (5 mL) was hydrogenated over PtO_2 (40 mg) for 15 min to give 63 mg (63%) of (6*S*,19*R*)-**16**, m.p. 56−57 °C, after SiO_2 chromatography and recrystallization from acetone. $[\alpha]_D^{21}$ = −0.5 (*c* = 0.63, hexane). Its IR and NMR spectra were almost identical with those of (6*S*,19*S*)-**16**. HRMS (EI) [$M^+ - H_2O$] ($C_{30}H_{60}$): calcd. 420.4695; found 420.4672,

(iii) (6*R*,19*R*)-16: In the manner described for (6*S*,19*S*)-**16**, (6*R*,19*R*)-**15** (110 mg, 0.25 mmol) in EtOAc (5 mL) was hydrogenated over PtO_2 (45 mg) for 15 min to give 58 mg (53%) of (6*R*,19*R*)-**16**, mp. 34−35 °C, after SiO_2 chromatography and recrystallization from acetone. $[\alpha]_D^{21}$ = +1.0 (*c* = 0.75, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**16**. HRMS (EI) [$M^+ - H_2O$]: calcd. 420.4695; found 420.4707.

(iv) (6*R*,19*S*)-16: In the manner described for (6*S*,19*S*)-**16**, (6*R*,19*S*)-**15** (212 mg, 0.49 mmol) in EtOAc (5 mL) was hydrogenated over PtO_2 (50 mg) for 15 min to give 140 mg (66%) of crystalline (6*R*,19*S*)-**16**, m.p. 55−56 °C, after SiO_2 chromatography and recrystallization from acetone. $[\alpha]_D^{21}$ = +0.7 (*c* = 1.43, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*R*)-**16**. HRMS (EI) [$M^+ - H_2O$]: calcd. 420.4695; found 420.4701.

6-Acetoxy-19-methylnonacosane (1). (i) (6*S*,19*S*)-1: Acetic anhydride (0.5 mL, 5.3 mmol) and DMAP (10 mg, 0.08 mmol) were added to a stirred and ice-cooled solution of (6*S*,19*S*)-**16** (50 mg, 0.11 mmol) in CH_2Cl_2 (1 mL) and pyridine (1 mL). The mixture was left to stand at room temperature (about 22 °C) for three days. The usual workup and chromatography over SiO_2 afforded 54 mg (98%) of (6*S*,19*S*)-**1** as a colorless oil, n_D^{21} = 1.4530. $[\alpha]_D^{22}$ = −1.3 (*c* = 0.53, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**1** prepared via (6*S*,19*S*)-**14**. $C_{32}H_{64}O_2$ (480.9): calcd. C 79.93, H 13.42; found C 80.11, H 13.55.

(ii) (6*S*,19*R*)-1: In the manner described for (6*S*,19*S*)-**1**, (6*S*,19*R*)-**16** (48 mg, 0.11 mmol) afforded 47 mg (89%) of (6*S*,19*R*)-**1** as an oil, n_D^{21} = 1.4520. $[\alpha]_D^{21}$ = −1.2 (*c* = 0.47, hexane). Its IR and NMR spectra were virtually identical with those of (6*S*,19*S*)-**1**. $C_{32}H_{64}O_2$ (480.9): calcd. C 79.93, H 13.42; found C 79.70, H 13.45.

(iii) (6*R*,19*R*)-1: In the manner described for (6*S*,19*S*)-**1**, (6*R*,19*R*)-**16** (50 mg, 0.11 mmol) afforded 54 mg (98%) of (6*R*,19*R*)-**1** as an oil, n_D^{21} = 1.4521. $[\alpha]_D^{21}$ = +1.9 (*c* = 0.58, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*S*)-**1**. $C_{32}H_{64}O_2$ (480.9): calcd. C 79.93, H 13.42; found C 79.50, H 13.21.

(iv) (6*R*,19*S*)-1: In the manner described for (6*S*,19*S*)-**1**, (6*R*,19*S*)-**16** (118 mg, 0.27 mmol) furnished 128 mg (99%) of (6*R*,19*S*)-**1** as an oil, n_D^{21} = 1.4510. $[\alpha]_D^{21}$ = +1.1 (*c* = 1.22, hexane). Its IR and NMR spectra were identical with those of (6*S*,19*R*)-**1**. $C_{32}H_{64}O_2$ (480.9): calcd. C 79.93, H 13.42; found C 80.18, H 13.41.

Sample Preparation Procedure for Analytical HPLC: (1*S*,2*S*)- or (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid (**17**, 1.3 mg, 10 equiv. to **16** or **15**) and DMAP (a catalytic amount) were added to each stereoisomer of **16** or **15** (0.15 mg) in a mixture of toluene and MeCN (1:1, 0.15 mL). After addition of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride [EDC, Tokyo Kasei (TCI), 2.0 mg, 30 equiv. to **16** or **15**], the mixture was kept at room temperature for more than 10 h. An aliquot was then loaded onto a silica gel TLC plate (10 cm length, Silicagel 60 F_{254}, Art − 5744, Merck) and developed with hexane/EtOAc (4:1, v/v). The target spot **18** or **19** detected by fluorescence was collected, packed in a Pasteur pipette and eluted with EtOAc/EtOH (4:1, v/v). After evaporation of the solvent with a N_2 gas stream, the residue was dissolved in MeOH and used for an HPLC analysis. For preparation of **17**, which is not yet commercially available, see ref.[12].

HPLC Separation: The derivatives **18** or **19** were separated on a reversed-phase column (Develosil C30-UG-3, 3μm, (4.6 mm I. D. × 150 mm) × 2, Nomura Chemical Co., Aichi, Japan). The detection was carried out by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm). The separation was performed with either a mixture of MeCN/THF/hexane/MeOH (40:210:120:80) at a flow rate of 0.2 mL/min or with a mixture of MeCN/THF/hexane/MeOH (60:80:30:60) at a flow rate of 0.3 mL/min. The column temperature was kept at −30 °C or at −40 °C. The temperature of the sample solution was gradually raised to room temperature by using a loop, and detection was carried out at room temperature. The results of HPLC separation are discussed in the text.

Behavioral Dose−Response Studies: Insects were resting at the start of each behavioral assay. The percentage of males responding increased with the dose of the test stimulus. (6*R*,19*S*)-**1** elicited 50% response level at the lowest calculated treatment (≈ 0.20 μg) using all treatments, and reached an upper plateau at about 2 μg as did responses to all stereoisomers (Figure 2). Males did not respond to solvent-washed decoys. Responses over all concentrations of the stereoisomers were not significantly different ($x2 = 0.71$, $df = 3$, $P > 0.05$). However, using ProcProbit for all treatments, the 95% fiducial limits (FL) for the 50% response level of (6*R*,19*S*)-**1** was 0.20 μg (0.14−0.26 μg) that did not overlap with the fiducial limits of (6*S*,19*R*)-**1** (0.28−0.53 μg), (6*S*,19*S*)-**1** (0.39−0.54 μg), or (6*R*,19*R*)-**1** (0.55−0.75 μg), but there was overlap between treatments (6*S*,19*R*)-**1**, (6*S*,19*S*)-**1** and (6*R*,19*R*)-**1**.

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