Synthesis and Inhibitory Properties of Pheromone Analogues for the Epoxide Hydrolase of the Gypsy Moth

Steven M. Graham† and Glenn D. Prestwich*

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794-3400

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A series of analogues of disparlure, the gypsy moth (*Lymantria dispar*) sex attractant, was synthesized, and the potency of these inhibitors in suppressing the metabolism of disparlure by the *L. dispar* epoxide hydrolase (EH) was determined. The analogues substituted at the 6-position (6-hydroxy-, 6-oxo-, and 6,6-difluorodisparlure; (±)-*threo,cis*-**11**, (±)-**13**, and (±)-**17**, respectively), along with 9,9-difluorodisparlure [(±)-**26**], were the most potent inhibitors (IC_{50} values of 4–9 μM). Two other 9-substituted analogues, 9-hydroxydisparlure [(±)-*threo,cis*-**21**] and 9-oxodisparlure [(±)-**22**], were slightly less potent (IC_{50} values of 18 and 30 μM, respectively). Analogues substituted at both the 6- and 9-positions (*threo,erythro*-6,9-dihydroxy-, *threo,threo*-6,9-dihydroxy-, and 6,9-dioxodisparlure; (±)-*threo,erythro*-**32**, (±)-*threo,threo*-**32**, and (±)-**33**, respectively) were generally the least potent inhibitors (IC_{50} values of 27–200 μM). On the basis of a model of the EH active site, a hypothesis is advanced to rationalize the higher potencies of the 6-substituted analogues. Pheromone metabolism plays a key role in pheromone perception, and the potential consequences of inhibition of pheromone metabolism are discussed.

Introduction

Insects possess substrate-specific catabolic enzymes that are important in the clearance of pheromone from their sensory tissues,[1] but the precise role of these enzymes in signal transduction is poorly understood. It appears that the major role of these catabolic enzymes is to convert stimulatory pheromones to nonstimulatory products, thus preventing sensory adaptation. In the Lepidoptera, the male monitors a pheromone plume emitted by the female and locates her by chemically-stimulated upwind flight.[2] Wind tunnel studies have shown that the ability of a male to follow a pheromone plume can be impaired by the presence of high pheromone concentrations. Saturation of the sensory neuron receptors is proposed to be responsible for this impairment.[3] In principle, inhibition of the catabolic breakdown of pheromone could lead to mating disruption, via prolonging high pheromone levels within the peripheral sensory system. Herein we describe the synthesis of a series of pheromone analogues and the assay of these analogues as inhibitors of the major pheromone-catabolizing enzyme for the gypsy moth, *Lymantria dispar*.

Since its accidental release in Massachusetts in 1869,[4] the gypsy moth has become a major forest pest in the United States. *L. dispar* females appear to emit a single pheromone, (+)-(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane [(+)-disparlure, Figure 1].[5–7] No mention of the absolute stereochemistry of disparlure was made at the time of its isolation.[8] Once the separate (+)-(7*R*,8*S*)- and (−)-(7*S*,8*R*)-enantiomers of disparlure (Figure 1) were synthesized,[9] two key discoveries were made. First, the (+)-enantiomer was found to be attractive to *L. dispar* males while a combination of (+)- and (−)-disparlure inhibited attraction of males to females.[5] Second, there were separate sensory neurons responsive to (+)-disparlure and to (−)-disparlure within each sensory hair, which is the basic structural unit of the male antennae.[5] Only (+)-responsive cells were depolarized when exposed to the pheromone extracts of *L. dispar* females; this result has been interpreted to show that *L. dispar* females emit only (+)-disparlure.[5]

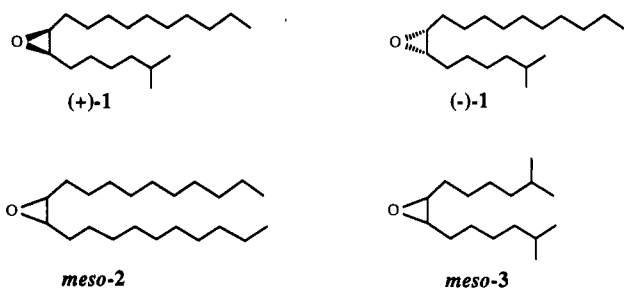

Figure 1. Structure of disparlure (1) and the disparlure analogues *meso*-**2** and *meso*-**3**.

Our initial studies[10] in the field of pheromone perception and metabolism in *L. dispar* were concerned with identification of the metabolites of disparlure. A single compound, *threo*-2-methyl-7,8-octadecanediol, was identified as the major metabolite produced by an epoxide hydrolase (EH). EH activity was found in homogenates

* To whom correspondence should be addressed.

† Present address: Department of Chemistry, Oklahoma State University, Stillwater, OK 74078.

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(1) (a) *Pheromone Biochemistry*; Prestwich, G. D., Blomquist, G. J., Eds.; Academic: New York, 1987. (b) *Behavior Modifying Chemicals for Insect Pest Management*; Ridgway, R. L., Silverstein, R. M., Inscoe, M. N., Eds.; Dekker: New York, 1990.

(2) Baker, T. C. *Experientia* **1989**, *45*, 248–262.

(3) Cardé, R. T. In ref 1b, p 51.

(4) Forbush, E. H.; Fernald, C. H. *The Gypsy Moth. Porthetria dispar (Linn.) A report of the work of destroying the insect in the Commonwealth of Massachusetts, together with an account of its history and habits both in Massachusetts and Europe*; Massachusetts State Board of Agriculture: Boston, MA, 1896.

(5) Hansen, K. *Physiol. Entomol.* **1984**, *9*, 9 and references cited therein.

(6) Plimmer, J. R.; Leonhardt, B. A.; Webb, R. E.; Schwalbe, C. P. In *Insect Pheromone Technology: Chemistry and Applications*; Leonhardt, B. A., Beroza, M., Eds.; ACS Symposium Series 190; American Chemical Society: Washington, DC, 1982; pp 231–233.

(7) Kolodny-Hirsch, D. M.; Schwalbe, C. P. In ref 1b, p 363.

(8) Bierl, B. A.; Beroza, M.; Collier, C. W. *Science* **1970**, *170*, 87–89.

(9) Mori, K.; Ebata, T. *Tetrahedron Lett.* **1986**, *42*, 3471 and references cited therein.

(10) Prestwich, G. D.; Graham, S. McG.; Kuo, J.-W.; Vogt, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 636–642.

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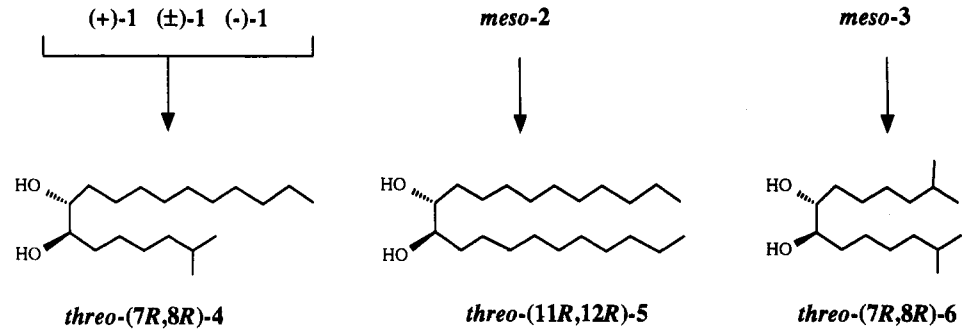


Figure 2. Hydration of any enantiomer of disparlure or of the disparlure analogues *meso*-**2** or *meso*-**3** by the *L. dispar* EH gives only the *threo*-(*R*,*R*)-diol.

of male and female antennae and male and female legs. Using male antennal homogenates, it was shown that labeled (+)-**1**, (−)-**1**, (±)-**1**, and a *meso*-analogue **2** were converted to diol at significantly different rates, decreasing in the order (+)-**1** > (±)-**1** > (−)-**1** > *meso*-**2**.

We then turned our attention to determining the stereochemical course of the hydration reaction.[11] Substrates (+)-**1**, (−)-**1**, (±)-**1**, and the two *meso*-analogues **2** and **3** were all converted stereoselectively to their respective *threo*-(*R*,*R*)-diols (Figure 2) in high enantiomeric excess using the *L. dispar* tissues mentioned above as the EH source.

From these data, three conclusions were drawn: First, the same EH was present in each of the *L. dispar* tissues studied. Second, this EH was the predominant disparlure EH present in each tissue. Third, this EH required a specific orientation of the epoxide in its epoxide binding site, but could accept *either* alkyl chain in either of the two proposed lipophilic binding sites. Thus, some variability in the alkyl chains of disparlure was permitted. Given this information, a series of disparlure analogues was synthesized and we show that these analogues inhibit the antennal EH of *L. dispar* in vitro.

Results

Synthesis of EH Inhibitors. 1. Synthesis of C-6 Functionalized Disparlure Analogues. All analogues were synthesized as racemic mixtures. The syntheses of 6-hydroxy- (**11**), 6-oxo- (**13**), and 6,6-difluorodisparlure (**17**) are shown in Scheme 1. Oxidation[12] of 5-methyl-1-hexanol (**7**)[13] gave aldehye **8**, which was then condensed with the lithium salt[14] of 1-dodecyne in THF to give alkynol **9** in 72% yield. Alkynol **9** was a common intermediate to both the oxygen- and fluorine-containing analogues. Thus, **9** was semihydrogenated to give allylic alcohol **10** as a 87:13 7*Z*:7*E* mixture. Chromatography on silica gel failed to separate completely the *Z*- and *E*-allylic alcohols **10**, and resolution of the *Z*/*E* mixture was deferred until the next step; the epoxy alcohols **11** were more readily separated than were the allylic alcohols. Epoxidation of the 87:13 7*Z*:7*E* mixture with *m*-CPBA[15] gave 6,7-*threo*-7,8-*cis*-**11** (*threo,cis*-**11**; 6-hydroxydisparlure) as the major product after chromatography on silica gel in 73% isolated yield. Throughout this discussion, *threo* and *erythro* refer to the orientation of a hydroxyl group and the vicinal epoxide oxygen. Other recovered minor products consisted of the *threo,trans*-**11**, *erythro,cis*-**11**, and *erythro,trans*-**11** isomers.

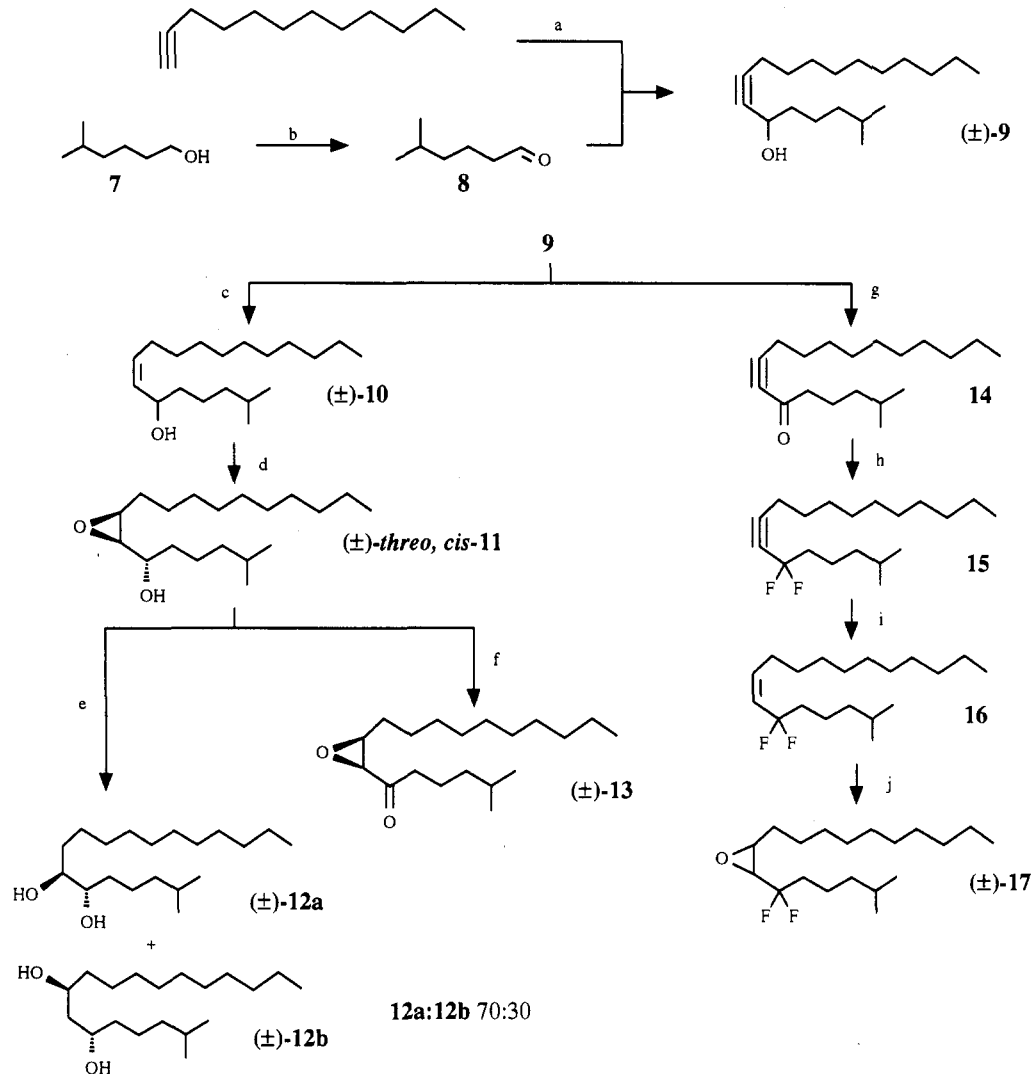
The *threo,cis* configuration of major product **11** was established by its method of synthesis and confirmed by conversion to a diol with $LiAlH_4$. Rossiter[15] found that treatment of a related *Z*-allylic alcohol, (*Z*)-3-penten-2-ol, with *m*-CPBA gave the corresponding *threo,cis* epoxy alcohol in 90% diastereomeric excess; therefore, (*Z*)-**10** was also expected to give the corresponding *threo,cis* epoxy alcohol as the major product upon *m*-CPBA oxidation.[16] More rigorous proof of the *threo* configuration was obtained by conversion of *threo,cis*-**11** to its diol. When treated with $LiAlH_4$, epoxy alcohol *threo,cis*-**11** gave a quantitative yield of diols **12a** and **12b** in ca. a 7:3 ratio. The ^1H NMR of the major product **12a** was virtually identical to that of the known *threo*-disparlure diol (±)-**4**, especially the chemical shift of the carbinol protons (**12a** = 3.41 ppm; **4** = 3.38 ppm).[10] No *erythro* diol (δ = ca. 3.58 ppm) was observed. Finally, 6-hydroxydisparlure (*threo,cis*-**11**) was converted to the target 6-oxodisparlure (**13**) by oxidation with pyridinium dichromate.

Synthesis of 6,6-difluoro analogue **17** was achieved as follows. Oxidation of alkynol **9** to ketone **14**, followed by treatment with neat diethylamidosulfur trifluoride (DAST)[17,18] at 55 °C, gave 6,6-difluoroalkyne **15**. Semihydrogenation of alkyne **15** using 5% $Pd/BaSO_4$ to give alkene **16** failed initially but proceeded in 89% yield if the catalyst was removed and the reaction mixture reduced using a fresh batch of catalyst. This phenomenon also occurred in the synthesis of the 9,9-difluoro analogue (*vide infra*) and can be attributed to poisoning of the $Pd/BaSO_4$ catalyst by a sulfur-containing impurity from the previous DAST reaction.[18] Difluoro alkene **16** was converted to the target 6,6-difluorodisparlure (**17**) in 75% yield by treatment with *m*-CPBA in CH_2Cl_2 at 40 °C for 24 h.

Synthesis of EH Inhibitors. 2. Synthesis of C-9 Functionalized Disparlure Analogues. The syntheses

(11) (a) Prestwich, G. D.; Graham, S. McG.; Kõnig, W. A. *J. Chem. Soc., Chem. Commun.* **1989**, 575–577. (b) Graham, S. M., Prestwich, G. D. *Experientia* **1992**, *48*, 19–21.
(12) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *35*, 399–402.
(13) Graham, S. M. Ph.D. Dissertation, State University of New York at Stony Brook, 1990.
(14) Midland, M. M. *J. Org. Chem.* **1975**, *40*, 2250–2252.
(15) Rossiter, B. E. Ph.D. Dissertation, Massachusetts Institute of Technology, 1981.
(16) Further proof of the *threo,cis* configuration of **11** was obtained by synthesizing all four possible diastereomers of **11** (see Experimental Section) using the less selective $VO(acac)_2$/*tert*-butyl hydroperoxide system to epoxidize an approximate 46:54 mixture of (*Z*)- and (*E*)-**10**. The diagnostic H-7 ^1H NMR chemical shift and coupling patterns and the relative proportions of each diastereomer of **11** formed were in good agreement with that predicted by Rossiter (ref 15), thus confirming that the stereochemistry of *threo,cis*-**11** had been assigned correctly.
(17) (a) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574–578. (b) Sun, W.-C.; Ng, C.-S.; Prestwich, G. D. *J. Org. Chem.* **1992**, *57*, 132–137.
(18) Sun, W.-C. Ph.D. Dissertation, State University of New York at Stony Brook, 1990.

Scheme 1. Synthesis of Racemic 6-Substituted Disparlure Analogue[a]



[a] Reagents: (a) *n*-BuLi, THF, −40 °C, 72%; (b) PDC, CH_2Cl_2, 100%; (c) H_2–Pd/$BaSO_4$–pyridine, 20 h, 76%; (d) *m*-CPBA, CH_2Cl_2, 73%; (e) $LiAlH_4$, Et_2O, 99%; (f) PDC, CH_2Cl_2, 61%; (g) PDC, CH_2Cl_2, 68%; (h) neat DAST, 55 °C, 92 h, 50%; (i) H_2-Pd/$BaSO_4$–pyridine, 24 h, 89%; (j) *m*-CPBA, CH_2Cl_2 40 °C, 24 h, then room temperature, 24 h, 75%.

of 9-hydroxy- (**21**), 9-oxo- (**22**), and 9,9-difluorodisparlure (**26**) are shown in Scheme 2. The same methodology was used as in the synthesis of C-6 functionalized analogues. Thus, alkynylation of decanal with the lithium salt of 7-methyl-1-octyne (**18**)[13] gave alkynol **19**. This alkynol was then semihydrogenated and epoxidized to give the target 8,9-*threo*-7,8-*cis*-**21** (*threo,cis*-**21**; 9-hydroxydisparlure), a portion of which was converted to the desired 9-oxodisparlure (**22**). Alternatively, alkynol **19** was oxidized, difluorinated, semihydrogenated, and epoxidized as described above to give 9,9-difluoro analogue **26**.

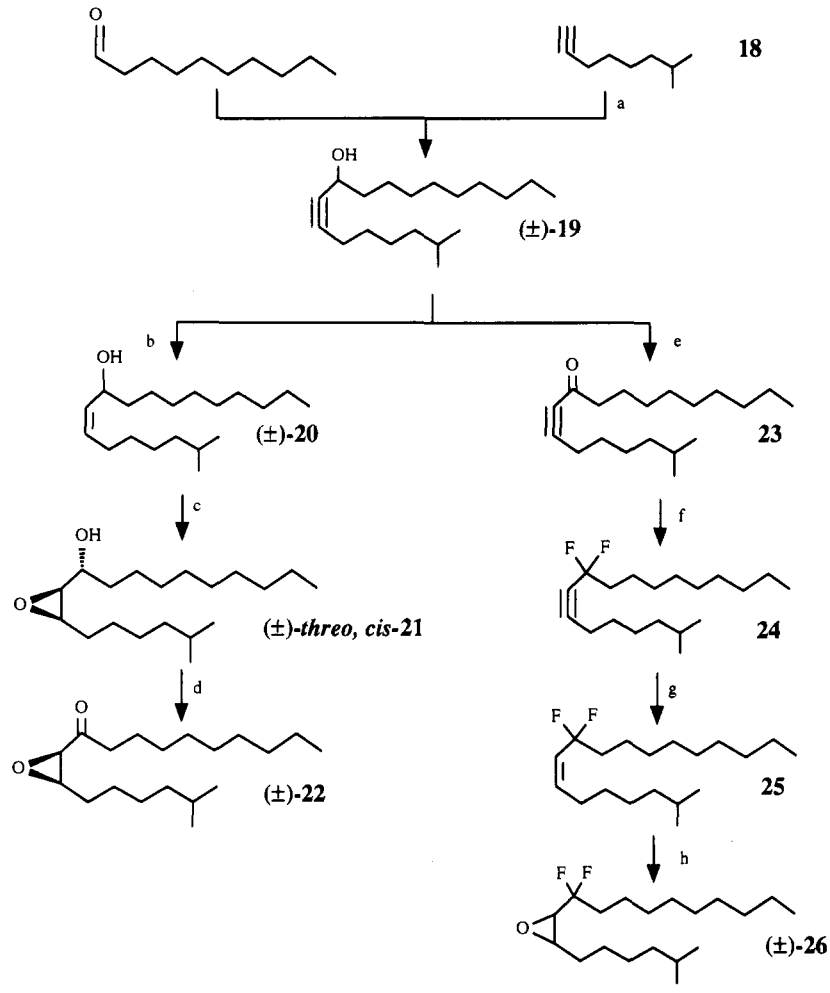
Synthesis of Potential EH Inhibitors. 3. Synthesis of C-6,9 Difunctionalized Disparlure Analogues. The syntheses of 6,7-*threo*-8,9-*erythro*-dihydroxydisparlure (*threo,erythro*-**32**), 6,7-*threo*-8,9-*threo*-dihydroxydisparlure (*threo,threo*-**32**), and 6,9-dioxodisparlure (**33**) are presented in Scheme 3. The general method of the C-6 and C-9 functionalized cases was used with only minor modifications.

Reaction of monolithium acetylide[14] with decanal and subsequent protection[19] of alkynol **27** gave silyl ether **28**. After treatment of the lithium salt of **28** with 5-methylhexanal (**8**), the resultant diastereomeric mixture of monoprotected alkyne diols **29** was semihydrogenated and epoxidized as described previously. The ^1H- and ^{13}C-NMR spectra for compound **29** indicated that the two (racemic) diastereomers were formed in an approximate 1:1 ratio. After epoxidation of monoprotected diol **30**, four (racemic) diastereomers of **31** are possible. Only two (racemic) compounds were observed in the ^1H- and ^{13}C-NMR spectra of **31**, again in an approximate 1:1 ratio, suggesting that the *m*-CPBA epoxidation was highly selective for one face of the double bond of each diastereomer of **30** (more below). The diastereomers of compounds **29**, **30**, and **31** could not be readily separated on TLC.

Conversion of monoprotected epoxy diol **31** to diol **32** gave two products with an R_f difference of 0.32 on TLC. These compounds were separated by chromatography on silica gel and could be assigned the structures shown in Scheme 3 as described below. The more mobile component was *threo,erythro*-**32** (50%), and the less mobile epoxy diol was *threo,threo*-**32** (47%). By analogy with the C-6 and C-9 hydroxydisparlure syntheses, the free hydroxyl (as opposed to the silylated hydroxyl) appeared to control

(19) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190–1691.

Scheme 2. Synthesis of Racemic 9-Substituted Disparlure Analogues[a]



[a] Reagents: (a) *n*-BuLi, THF, −40 °C, 71%; (b) H_2–Pd/$BaSO_4$–pyridine, 24 h, 81%; (c) *m*-CPBA, CH_2Cl_2, 72%; (d) PDC, CH_2Cl_2, 67%; (e) PDC, CH_2Cl_2, 79%; (f) neat DAST, 55 °C, 4 days, 55%; (g) H_2–Pd/$BaSO_4$–pyridne, 16 h, 73%; (h) *m*-CPBA, CH_2Cl_2, 68 h rt, then 27 h, 40 °C, 41%.

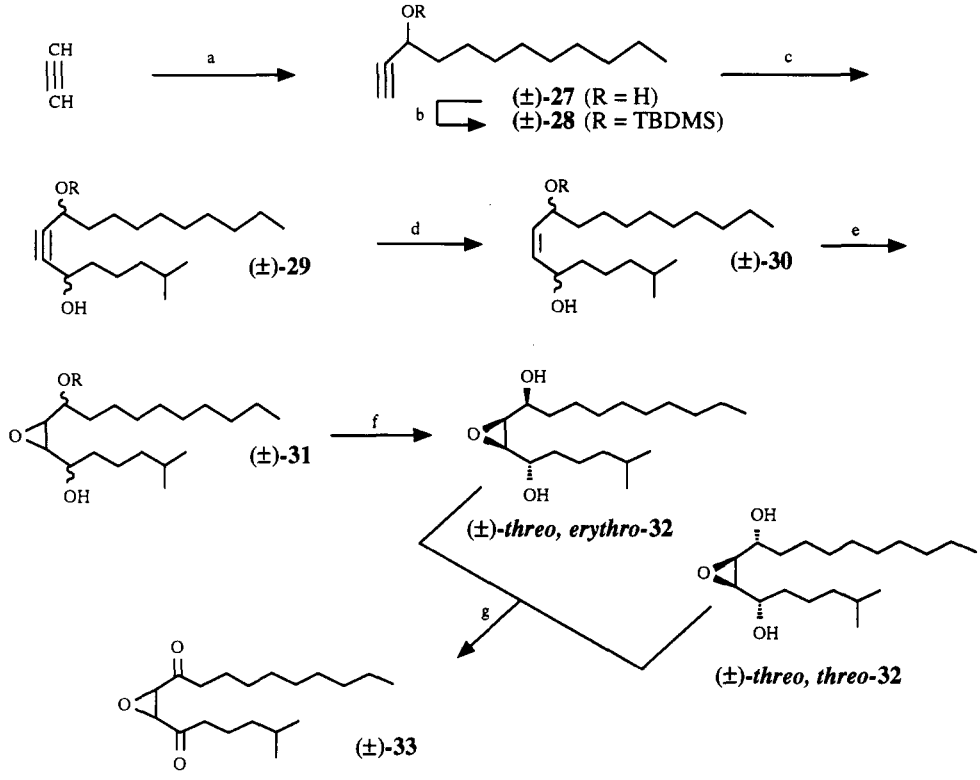
the approach of the incoming oxygen during the *m*-CPBA reaction, leading to the assignment of the 6,7-oxygens as *threo*. Given this, the assignment of the 8,9-oxygen configuration was most easily made using ^{13}C NMR. One of the 6,9-dihdroxydisparlures showed only one signal in the epoxide carbon region and it showed only one signal in the carbinol carbon region (Note: the other 6,9-dihdyroxydisparlure showed two carbon signals in each of these regions). This suggested that the former compound had a higher degree of symmetry relative to the latter compound; therefore, the former is *threo,threo*-**32** and the latter is *threo,erythro*-**32**. Finally, both diols could be oxidized to 6,9-dioxodisparlure (**33**).

Purification of [$^3H_3$]Disparlure. The radiolabeled (+)-disparlure used in this study was synthesized at the National Tritium Labeling Facility in Berkeley, CA, in 1986.[10] The (+)-disparlure was prepared by tritiation of an optically active 5,6-alkenyl epoxide with no-carrier-added T_2 gas using $(Ph_3P)_3RhCl$ as catalyst, giving (+)-[5,6-$^3H_2$]disparlure at a nominal specific activity of 58 Ci/mmol (One triton = 29 Ci/mmol). Radiolabeled disparlure was stored in 1:1 heptane:toluene at −20 °C at a concentration of ca. 100 mCi/mL and was still usable after 5 years. Due to the method of synthesis, ca. 10–20% of 2-methyloctadecan-8-one was present in the (+)-disparlure, caused by the rhodium-induced rearrangement of the precursor alkenyl epoxide during the tritiation.[10] This ketone impurity had an R_f on silica gel that was very similar to disparlure and, therefore, was most conveniently removed by reduction to its corresponding alcohol with $NaBH_4$ followed by silica gel chromatography. After several years in storage, however, substantial primary and secondary radiolytic decomposition had occurred,[20] as evidenced by TLC/fluorography of aliquots. Thus, prior to use in biochemical studies, (+)-[$^3H_2$]disparlure was purified by a three-step process consisting of crude purification by preparative TLC, reduction of ketone impurities with $NaBH_4$, and then a second round of preparative TLC. The purified (+)-[$^3H_2$]disparlure thus produced was homogeneous according to radioimaging TLC scanning.

Biochemical Studies. K_M, V_{max}, and IC_{50} Determinations. In order to acquire kinetic data for the inhibitors, an estimate of the apparent K_M and V_{max} for the *L. dispar* male EH was required. The rates of hydration of (+)-[5,6-$^3H_2$]disparlure using homogenates of whole male antennae as a function of substrate concentration were determined. The results are shown in a Lineweaver–Burk plot (Figure 3), giving an apparent

(20) (a) Evans, E. A. *Self-Decomposition of Radiochemicals: Principles, Control, Observation and Effects*; Amersham Corp.: Arlington Heights, 1975. (b) Prestwich, G. D. In ref 1a, Chapter 14.

Scheme 3. Synthesis of Racemic 6,9-Disubstituted Disparlure Analogues[a]



[a] Reagents: (a) *n*-BuLi, THF, −78 °C, then decanal, 77%; (b) TBDMS–Cl, imidazole, DMF, 73%; (c) *n*-BuLi, THF, −40 °C, then 5-methylhexanal (8), 71%; (d) H_2–Pd/$BaSO_4$–pyridine, 24 h, 82%; (e) *m*-CBPA, CH_2Cl_2, 83%; (f) $Bu_4N^+F^-$, THF, 97%; (g) PDC, CH_2Cl_2, then CrO_3·(pyr)$_2$, CH_2Cl_2, 59%.

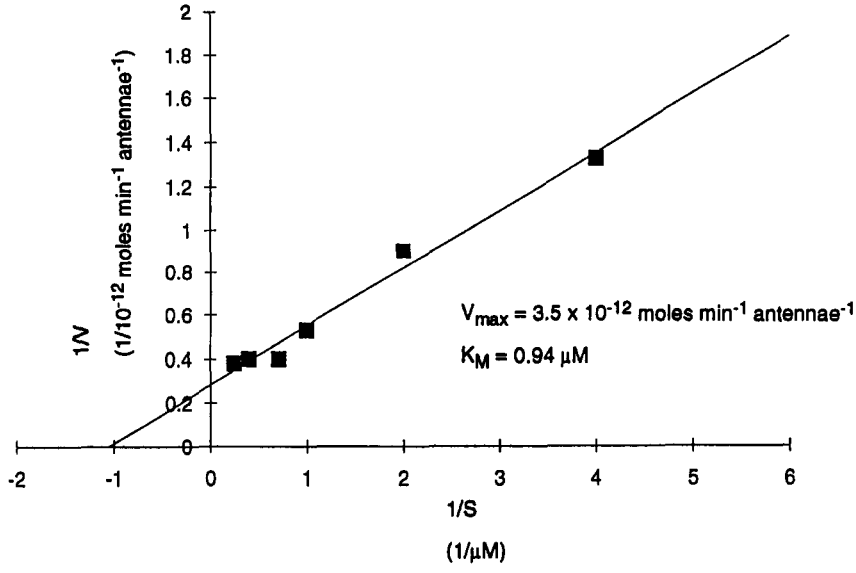


Figure 3. Double reciprocal plot for the hydration of (+)-disparlure by the *L. dispar* male antennal EH.

K_M of 0.94 μM and a maximal rate of hydration (V_{max}) of 3.5 pmol disparlure min^{-1} $antenna^{-1}$.

The results of the inhibition of *L. dispar* male EH by racemic disparlure analogues *threo,cis*-**11**, **13**, **17**, *threo,cis*-**21**, **22**, **26**, *threo,erythro*-**32**, *threo,threo*-**32**, and **33** are shown in Table 1. In general, the 6-substituted analogues (*threo,cis*-**11**, **13**, and **17**) showed the highest inhibitory potency (IC_{50} values all under 10 μM); the 9,9-difluoro analogue (**26**) also was among this group (IC_{50} < 4 μM). The remaining inhibitors had IC_{50} values in the 18–49 μM range, except for the *threo,threo*-6,9-dihydroxy analogue **32** (IC_{50} ~200 μM).

Discussion

Epoxide hydrolases are known enzymes of xenobiotic detoxification and normal biosynthesis in plants,[21] insects,[22] and vertebrates[23] and may occur in either microsomal[23,24] or soluble[23,24] forms. Purification[25] and inhibition[26] of the vertebrate cytosolic EH has been extensively studied, and a variety of inhibitory motifs have

(21) Blée, E.; Schuber, F. *J. Biol. Chem.* **1992**, *267*, 11881–11887.
(22) (a) Touhara, K.; Prestwich, G. D. *J. Biol. Chem.* **1993**, *268*, 19604–19609. (b) Hammock, B. D.; Roe, R. M. In *Methods in Enzymology*; Law, J. H., Rilling, H. C., Eds.; Academic: Orlando, 1985; Vol. 111, pp 487–494.

Table 1. IC_{50} Values for the Racemic Disparlure Analogues

inhibitor	IC_{50} (μM)
(±)-*threo,cis*-**11** (6-hydroxy)	9
(±)-**13** (6-oxo)	4
(±)-**17** (6,6-difluoro)	5
(±)-*threo,cis*-**21** (9-hydroxy)	18
(±)-**22** (9-oxo)	30
(±)-**26** (9,9-difluoro)	<4
(±)-*threo,erythro*-**32** (*t,e*-6,9-dihydroxy)	49
(±)-*threo,threo*-**32** (*t,t*-6,9-dihydroxy)	~200
(±)-**33** (6,9-dioxo)	27

been discerned for this class of enzymes, including the α-keto epoxides (chalcone oxides). For the microsomal insect EH, which hydrates juvenile hormone, a novel series of α-hydroxy epoxides (glycidols) has been described.[27] In this study of the *L. dispar* EH, we have incorporated the α-keto, α-hydroxy, and α,α-difluoro epoxide modifications into the disparlure skeleton. Our dual purpose was to test for the generality of these inhibitory motifs in a novel, nonenantiospecific enzyme[10,11] and to prepare a disparlure analogue capable of blocking pheromone perception by adult male *L. dispar* moths.[20b]

Discussions of structure–activity relationships among disparlure analogues are complicated by the unusual fact that the *L. dispar* EH can bind and hydrate both enantiomers of this chiral pheromone.[10,11] This behavior would also be expected to apply to both enantiomers of the racemic inhibitors. On the basis of a previous model of the active site developed for inhibition of animal cytosolic EH,[26a] a protonated amino acid would activate the epoxide ring toward hydration, while a basic residue would activate a water molecule for addition to the epoxide. In this model, binding of the epoxide oxygen to the protonated amino acid represents normal substrate binding, whereas binding of the ketone oxygen to this electrophilic site leads to inhibition. This model is consistent with our previous results of disparlure hydration by the *L. dispar* EH, for which a strict requirement for the epoxide orientation was observed, while a smaller but significant preference for orientation of the alkyl side chains in their respective binding pockets was found.

Interestingly, Blée and Schuber[21] recently discovered a similar requirement for epoxide orientation with a flexible stereoselectivity for side chain orientation for the hydration of the enantiomers of *cis*-9,10-epoxystearic acid by a plant EH. Their ^{18}O-labeling studies confirmed that attack at either C-9 or C-10 could occur, *depending on which carbon possessed the S configuration*, to produce the 9*R*,10*R* diol in both cases. This result is exactly analogous to our model proposed for disparlure hydration[10,11] based on chiral GC analysis of product mixtures. Indeed, Blée and Schuber independently proposed an explanation identical to ours to rationalize this lack of enantiomeric selectivity.[21]

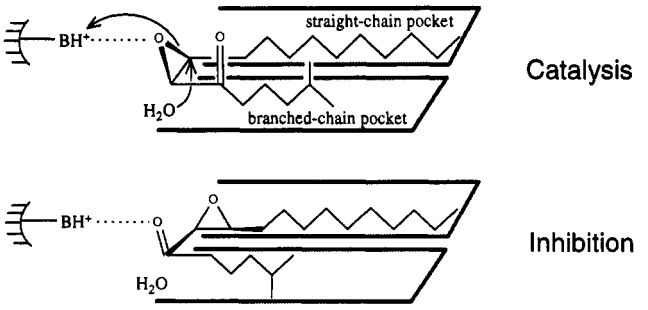


Figure 4. Proposed mechanism of inhibition of the *L. dispar* antennal EH by an epoxy ketone. The (+)-enantiomer of (±)-**13** is shown. The protonated amino acid activates the epoxide ring toward nucleophilic attack by water (catalysis). Alternatively, the ketone oxygen can bind to the protonated amino acid, which effectively deactivates the epoxide ring toward nucleophilic attack (inhibition).

For example, consider a model of a possible active site binding orientation for the antipode of 6-oxo analogue **13** with the same epoxide stereochemistry as (+)-disparlure ("(+)"-6-oxo, Figures 4 and 5A). In order for the "(−)"-6-oxo antipode to bind with the epoxide ring "up", the side chains must exchange binding pockets, which means the carbonyl oxygen must also exchange pockets (Figure 5A). Moving the carbonyl oxygen to the other hydrophobic binding pocket as in the "(−)"-6-oxo case reduces inhibitory potency. Similarly, both the carbonyl and epoxide oxygens of the "(−)"-9-oxo antipode could adopt the same orientation as seen in the "(+)"-6-oxo antipode, but this requires placing the branched chain of disparlure in the "straight chain" pocket (Figure 5B). Inhibitor binding to *L. dispar* EH thus balances preferential binding of the carbonyl oxygen with preferential side chain binding. Therefore, the "(+)"-6-oxo antipode has both side chain and carbonyl oxygen positions optimized for inhibition. This explains the generally higher inhibitory potencies shown by the 6-substituted series. The 6,9-disubstituted analogues (*threo,erythro*-**32**, *threo,threo*-**32**, and **33**) were designed to reassert the normal preferences for side chain binding, but their poor IC_{50} values indicated that the additional functional groups prevented effective binding.

In conclusion, we have shown that a process important in pheromone perception, namely pheromone metabolism, can be inhibited by substrate analogues. It is worth noting that in the test system the abundant pheromone binding protein (PBP) is present to solubilize *both* enantiomers of the pheromone[28] and perhaps also the inhibitors. This protein, which has recently been cloned,[29] may play a key role in pheromone clearance as proposed for PBP-esterase interactions in other insects.[30] The importance of binding protein–carrier protein interactions has been recently demonstrated for the juvenile hormone (JH) specific EH and JH BP in the larval stages in the Lepidopteran *M. sexta*.[31] Experiments to test the activity of the analogues

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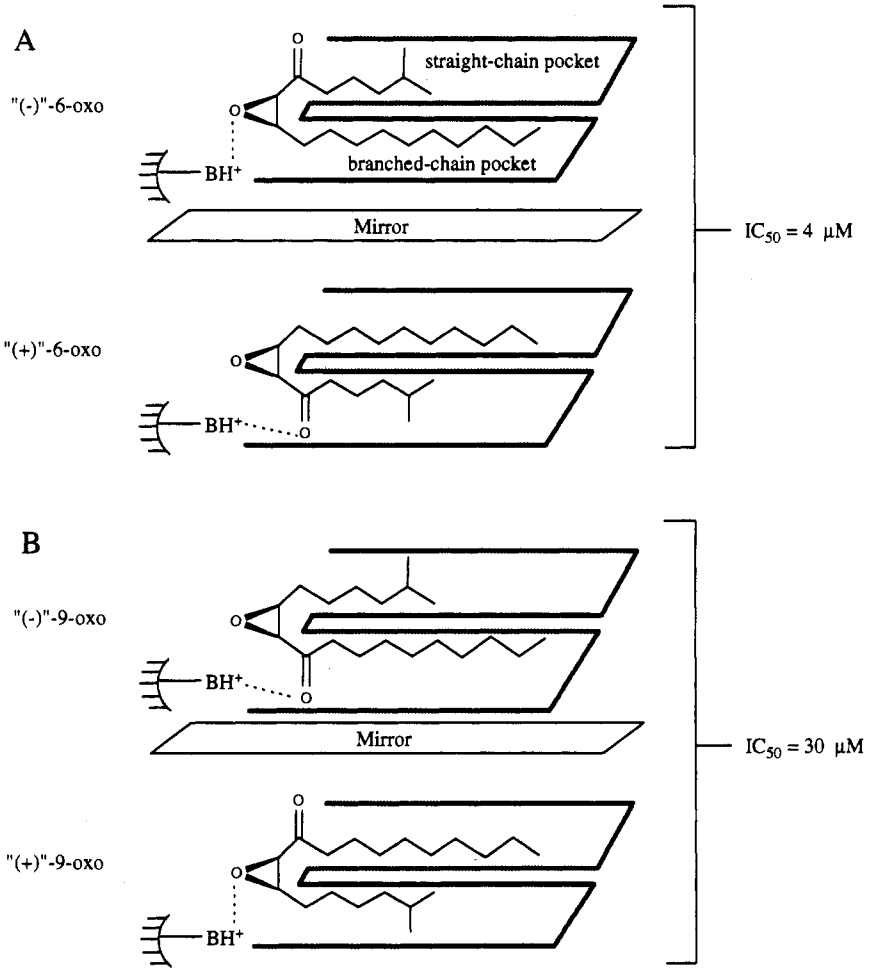

Figure 5. Possible binding orientations for the enantiomers of (±)-**13** and (±)-**22** in the active site of the *L. dispar* EH. The mirror plane shown is intended only to reflect the substrate antipodes, not the EH itself.

in modifying flight behavior by *L. dispar* males are in progress.

Experimental Methods

General. Ether and THF were distilled from sodium/benzophenone ketyl; methylene chloride was distilled from calcium hydride. DMF and acetonitrile were dried over molecular sieves. Pyridine was distilled from potassium hydroxide. Small (<5 mL) quantities of CH_2Cl_2 and benzene were dried by filtration through activated alumina. Aldehydes were distilled prior to use. Imidazole was crystallized from CH_2Cl_2. *tert*-Butyl hydroperoxide (TBHP) was dried as a CH_2Cl_2 solution over $MgSO_4$; *m*-CPBA was washed with pH = 7 phosphate buffer. Other reagents were purified as necessary or were used as obtained from the supplier.

The phrase "dried" refers to drying with $MgSO_4$ and filtering; "concentration" generally refers to removal of solvent by rotary evaporation. Chromatography on silica gel was performed as by Still.[32] TLC was performed on silica TLC plates using hexane:ethyl acetate (H:EA) as eluant. Gas chromatography (GC) was performed using Hewlett-Packard 25-m × 0.32-mm (1-μm film) HP-1 or HP-5 capillary columns. For GC, a splitless glass injector (250–280 °C) and a flame ionization detector (280–320 °C) were used. Column conditions are reported as follows: (initial temperature in °C, initial time/program rate in °C per minute/final temperature in °C, column type).

Unless stated otherwise, 300-MHz ^1H NMR and 75-MHz ^{13}C NMR spectra were obtained in $CDCl_3$ solution and referenced to either the residual $CHCl_3$ peak or to internal tetramethylsilane. The ^{13}C NMR assignments were made on the basis of the attached proton test and/or by calculation[33] of expected values.

Electron impact GC/mass spectra (EI GC/MS) were obtained at 70 eV; chemical ionization GC/MS (CI GC/MS) were obtained using isobutane as the ionization gas. High-resolution mass spectra (HRMS) were also electron impact. Only selected major and diagnostic ions are reported for all mass spectra.

Except where noted, final compounds tested as inhibitors were characterized by 300-MHz ^1H NMR, 75-MHz ^{13}C NMR, CI GC/MS and/or EI GC/MS, and HRMS.

Radioimaging TLC scanning (RTLCS) were performed on a Bioscan System 500 imaging scanner. Liquid scintillation counting (LSC) of radioactive samples was performed in a liquid scintillation counter using Fisher Scintiverse II scintillation cocktail. Counting efficiency was 57–61% for tritium, and counts-per-minute (cpm) data were corrected using the external standard ratio method.

Synthesis of 6-Functionalized Disparlure Analogues. 5-Methylhexanal (8). To a stirred mixture of pyridinium dichromate (PDC, 1.425 g, 3.79 mmol) in 5 mL of CH_2Cl_2 was added 5-methyl-1-hexanol[13] (**7**, 310.0 mg, 2.67 mmol) in 1 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 21 h, diluted with 30 mL of pentane, and filtered to remove solids. The filtrate was loaded onto a 20-mm diameter pad of 15 mm of silica gel and 15 mm of $MgSO_4$ (silica on top, $MgSO_4$ on bottom) and eluted with 50 mL of 80:20 pentane:ether. The resultant clear filtrate was carefully concentrated to give a quantitative yield of the desired aldehyde **8** as a clear liquid.

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Prior to use in subsequent reactions, the aldehyde was distilled at reduced pressure, bp = 67–70 °C (83 mm). TLC: R_f = 0.40 (90:10 H:EA). ^{1}H NMR: δ 9.767 (t, J = 1.8 Hz, 1H); 2.411 (td, J = 7.3, 1.8 Hz, 2H); 1.70–1.50 (m, 3H); 1.35–1.20 (m, 2H); 0.892 (d, J = 6.6 Hz, 6H). ^{13}C NMR: δ 202.631; 43.903; 38.159; 27.619; 22.209; 19.734. EI GC/MS: no M$^+$; 96 (37); 86 (11); 81 (49); 71 (68); 70 (55); 55 (93); 44 (77); 43 (100).

(±)-2-Methyloctadec-7-yn-6-ol [(±)-9]. To a stirred solution of 1-dodecyne (567.2 mg, 3.41 mmol) in 8 mL of THF at −40 °C under N_2 was added 1.6 M n-BuLi in hexanes (2.1 mL, 3.4 mmol). The resultant solution was stirred for 45 min at −40 °C, followed by the addition of 5-methyl-1-hexanal (8, 345 mg, 3.02 mmol) in 3 mL of THF. The reaction mixture was stirred for 40 min at −40 °C and then quenched by the addition of moist ether (10 mL) and 5 mL of saturated NH_4Cl. The quenched reaction was diluted with 75 mL of hexane; the organic layer was washed with saturated NH_4Cl (2 × 50 mL) and saturated NaCl (50 mL), and dried. Chromatography (90:10 H:EA) gave 612.5 mg (2.18 mmol, 72%) of the desired alkynol **9** as a clear liquid. TLC: R_f = 0.33 (90:10 H:EA). ^{1}H NMR: δ 4.326 (tt, J = 6.5, 1.7 Hz, 1H); 2.175 (td, J = 7.0, 1.9 Hz, 2H); 2.053 (bs, 1H); 1.70–1.55 (m, 2H); 1.55–1.30 (m, 5H); 1.243 (bs, 16H); 0.87–0.85 (m, 9H). ^{13}C NMR: δ 84.851; 81.412; 62.271; 38.466; 38.168; 31.750; 29.450; 29.413; 29.183; 29.021; 28.711; 28.575; 27.734; 22.912; 22.512; 22.375; 22.327; 18.507; 13.878. EI GC/MS: no M$^+$; 265 (2); 237 (4); 195 (20); 139 (37); 43 (100).

(±)-(*Z*)-2-Methyloctadec-7-en-6-ol [(±)-10]. A mixture of alkynol (±)-**9** (254.7 mg, 0.918 mmol), 5% palladium/$BaSO_4$ (24.7 mg), and pyridine (1 mL) was degassed with aspirator vacuum, flushed with H_2 three times, and then stirred under H_2 for 24 h. The reaction mixture was diluted with 5 mL of hexane and filtered through a small plug of silica. The plug was eluted with several milliliters of 80:20 H:EA, and the combined eluates were concentrated. The last traces of pyridine were removed at the vacuum pump, and the reamining pale yellow oil was chromatographed twice (95:5 H:EA) to give a total of 195.7 mg (0.69 mmol, 76%) of products. The fraction most enriched in desired product contained 135.6 mg of allylic alcohol **10**, a clear liquid, as a 87:13 Z:E mixture. TLC: R_f = 0.31 (90:10 H:EA). GC (50,1/20/220, HP-5): 15.88 min (Z, 87%); 16.45 min (E, 13%). ^{1}H NMR: δ 5.58–5.48 (m, 7*E* isomer); 5.396 (dt, J = 11.0, 7.2 Hz, 1H); 5.299 (dd, J = 10.8, 8.9 Hz, 1H); 4.352 (app. q, J = 6.9 Hz, 1H); 2.27 (bs, 1H); 2.025 (q, J = 7.1 Hz, 2H); 1.60–1.40 (m, 3H); 1.22 (bs, 20H); 0.83–0.81 (m, 9H). ^{13}C NMR: δ 132.757; 131.666; 67.427; 38.896; 37.716; 31.857; 29.713; 29.603; 29.495; 29.308; 27.898; 27.679; 23.169; 22.629; 22.489; 14.014. EI GC/MS: no M$^+$; 197 (58); 123 (35); 113 (19); 95 (93); 57 (100).

(±)-6,7-*threo*-7,8-*cis*-7,8-Epoxy-2-methyloctadecan-6-ol [(±)-6-Hydroxydisparlure, (±)-*threo,cis*-11]. To a solution of allylic alcohol (±)-**10** (106.0 mg, 0.375 mmol, as a 87:13 7*Z*;7*E* mixture) in 2 mL of CH_2Cl_2 at room temperature was added purified m-CPBA (101.6 mg, 0.59 mmol). The reaction mixture was stirred 4 h at room temperature, followed by removal of most of the solvent. To the resultant white semisolid was added 2 mL of 10% (w/v) aqueous Na_2SO_3 (ca. 1.8 mmol SO_3^{2-}), 2 mL of saturated aqueous $NaHCO_3$, and 5 mL of hexane. After the mixture was stirred for 10 min, the homogeneous aqueous layer was extracted with 3 × 5 mL of hexane. The combined organic layers were dried and concentrated to give an essentially quantitative yield of epoxy alcohols as a clear liquid. This liquid was chromatographed (90:10 H:EA) to give at least three compounds by TLC. The structure of the major product (R_f = 0.19, 79.2 mg, 0.275 mmol, 73%) was assigned as the (±)-*threo,cis*-isomer (*threo,cis*-**11**) shown in Scheme 1. The minor products were subsequently assigned as the (±)-*erythro,trans*-isomer (*erythro,trans*-**11**, R_f = 0.26, 3.1 mg, 3%); the R_f = 0.22 material (6.7 mg, 6%) was an approximate 1:1 mixture of the (±)-*threo,trans*- and (±)-*erythro,cis*-isomers (*threo,trans*-**11** and *erythro,cis*-**11**, respectively). These assignments were confirmed using data from the $VO(acac)_2$ and $LiAlH_4$ experiments described below. **[(±)-*threo,cis*-11].** TLC: R_f = 0.19 (95:5 H:EA). GC (50,1/20/220, HP-5): 20.24 min (≥97.2%). ^{1}H NMR: δ 3.433 (td, J = 7.9, 4.0 Hz, 1H); 2.997 (bq, J = 4.6 Hz, 1H); 2.863 (dd, J = 8.1, 4.3 Hz, 1H); 2.79 (bs, 1H, exch. with D_2O); 1.60–1.40 (m, 5H); 1.22 (bs, 20H); 0.84–0.81 (m, 9H). ^{13}C NMR: δ 69.619; 60.893; 58.146; 38.884; 34.125; 31.815; 29.461; 29.398; 29.233; 28.293; 27.837; 26.724; 22.793; 22.569; 22.485; 13.965. EI GC/MS: no M$^+$; 297 (0.2); 128 (9.2); 110 (14.4); 95 (27.1); 82 (100). CI GC/MS: 299 (MH$^+$, 27); 281 (100).

(±)-6,7-*threo*-7,8-*cis*-, (±)-6,7-*threo*-7,8-*trans*-, (±)-6,7-*erythro*-7,8-*cis*-, and (±)-6,7-*erythro*-7,8-*trans*-7,8-Epoxy-2-methyloctadecan-6-ol [(±)-*threo,cis*-, (±)-*threo,trans*-, (±)-*erythro,cis*-, and (±)-*erythro,trans*-11]. To allylic alcohols (±)-(*E*)- and (*Z*)-**10** (4.8 mg, 17 μmol, as a 46:54 Z:E mixture) and $VO(acac)_2$ (0.07 mg, 0.3 μmol) was added 100 μL (300 μmol) of 3 M TBHP in CH_2Cl_2. After being stirred for 3.5 h at room temperature, the reaction mixture was diluted with 1.5 mL of hexane, washed with 2 × 1.5 mL of saturated aqueous $NaHCO_3$, 1.5 mL of saturated NaCl, dried, and concentrated to give a residue. Traces of remaining TBHP were removed at the vacumm pump (≥3 h) to give 4.5 mg (ca. 94%) of epoxy alcohols as a clear liquid. TLC analysis showed at least three products; 300-MHz ^{1}H NMR showed four products. TLC: R_f = 0.31, 0.23, 0.19 (95:5 H:EA). Selected H-7 ^{1}H NMR data: δ *cis* compounds *threo* (33%), 2.89 (dd, $J_{6,7}$ = 8.1, $J_{7,8}$ = 4.3 Hz, H-7); *erythro* (13%), 2.84 (dd, $J_{6,7}$ = 7.9, $J_{7,8}$ = 4.1 Hz, H-7); *trans* compounds *erythro* (40%), 2.77 (t, $J_{6,7}$ = $J_{7,8}$ = 2.8 Hz, H-7); *threo* (14%), 2.72 (dd, $J_{6,7}$ = 5.0, $J_{7,8}$ = 2.3 Hz, H-7).

(±)-*threo*-2-Methyl-6,7-octadecanediol (12a) and (±)-*threo*-2-Methyl-6,8-octadecanediol (12b). In order to confirm the structure of epoxy alcohol *threo,cis*-**11** as (±)-*threo,cis*, a small sample was converted to its diol. Thus, to a stirred suspension of $LiAlH_4$ (20.4 mg, 0.54 mmol) in 1 mL of diethyl ether was added neat epoxy alcohol (±)-*threo,cis*-**11** (29.7 mg). The reaction mixture was stirred for 45 min at room temperature and then quenched with 22 μL of water, 22 μL of 15% (w/w) NaOH, and 75 μL of water. The white precipitate was filtered off and the filtrate dried over $MgSO_4$. GC analysis (HP-5) at this point showed essentially two components, in an approximate 70:30 ratio. Chromatography (95:5 → 60:40 H:EA) gave a total of 29.6 mg (99% yield) of material which was pooled into three separate samples and analyzed by GC (HP-5). The first sample (12.6 mg, 42%) was a 97:3 mixture of 6,7-diol:6,8-diol. The second sample (12 mg, 40%) was a 49:51 mixture of 6,7-diol:6,8-diol. The third sample (5.0 mg, 17%) was a 39:61 mixture of 6,7-diol:6,8-diol. (±)-*threo*-2-Methyl-6,7-octadecanediol (**12a**). TLC: R_f = 0.22 (80:20 H:EA). GC (50, 1/20/220, HP-5): 22.48 min (6,7-diol, 97%); 23.74 min (6,8-diol, 3%). ^{1}H NMR: δ 3.41 (bs, 2H); 2.09 (bs, 2H, exch. with D_2O); 1.60–1.40 (m, 5H); 1.4–1.2 (bs, 22H); 0.89–0.87 (m, 9H). The *threo* structure was confirmed by the value of H-6,H-7 chemical shift, 3.41 ppm in this case. A typical value for *erythro* diols would be 3.6 ppm.[10] The major component in the ^{1}H NMR spectrum of the third sample was consistent with the structure of the diol **12b**. (±)-*threo*-2-Methyl-6,8-octadecanediol (**12b**). TLC: R_f = 0.17 (80:20 H:EA). GC (50, 1/20/220, HP-5): 22.44 min (6,7-diol, 39%); 23.63 min (6,8-diol, 61%). ^{1}H NMR: δ 3.95 (bs, 2H, H-6 and H-8); 3.41 (*threo*-6,7-diol **12a**, H-6 and H-7); 2.26 (bs, 2H, exch. with D_2O, 6-OH and 8-OH); 1.99 (*threo*-6,7-diol **12a**, exch. with D_2O); 1.60 (t, J ≈ 7 Hz, 2H, H-7); 1.60–1.40 (m); 1.4–1.2 (bs); 0.89–0.87 (m).

(±)-*cis*-7,8-Epoxy-2-methyloctadecan-6-one [(±)-6-Oxodisparlure, (±)-13]. To a stirred mixture of PDC (351.1 mg, 933.3 μmol) in 1 mL of CH_2Cl_2 was added epoxy alcohol (±)-*threo,cis*-**11** (33.8 mg, 113 μmol) in 0.5 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 7 days and poured into 5 mL of hexane. The reaction vessel was washed with 1:1 pentane:ether (3 × 3 mL) and the combined mixture filtered through a small pad of silica gel. The pad was eluted with 20 mL of 90:10 H:EA, and the resultant clear filtrate was concentrated and chromatographed (95:5 H:EA) to give 20.5 mg (69.1 μmol, 61%) of the desired 6-oxodisparlure **13**, a clear liquid. TLC: R_f = 0.38 (90:10 H:EA). ^{1}H NMR: δ 3.576 (d, J = 4.7 Hz, 1H); 3.196 (m, 1H); 2.500 (t, J = 7.4 Hz, 2H); 1.67–1.40 (m, 5H); 1.32–1.25 (bs, 16H); 1.22–1.16 (m, 2H); 0.90–0.85 (m, 9H). ^{13}C NMR: δ 206.295; 58.591; 58.480; 41.242; 38.521; 31.884; 29.532; 29.485; 29.425; 29.282; 27.833; 27.623; 26.330; 22.627; 22.399; 21.133; 13.987. EI GC/MS: 296 (M$^+$, 1.6); 155 (21); 95 (100). CI GC/MS: 297 (MH$^+$, 100); 281 (82). HRMS: calcd for $C_{19}H_{36}O_2$ 296.2715, found 296.2708.

2-Methyloctadec-7-yn-6-one (14). To a stirred mixture of PDC (673.5 mg, 1.79 mmol) in 2 mL of CH_2Cl_2 was added alkynol (±)-**9** (251.6 mg, 0.897 mmol) in 1 mL of CH_2Cl_2. The reaction

mixture was stirred at room temperature for 20 h and poured into 25 mL of pentane. The reaction vessel was washed with 1:1 pentane:ether (3 × 10 mL) and the combined mixture filtered through a small pad of silica gel. The pad was eluted with 50 mL of 1:1 pentane:ether, and the resultant clear filtrate was concentrated and chromatographed (98:2 H:EA) to give 169.6 mg (0.609 mmol, 68%) of the desired 6-oxoalkyne **14**, a clear liquid. TLC: R_f = 0.50 (95:5 H:EA). ^{1}H NMR: δ 2.399 (t, J = 7.4 Hz, 2H); 2.255 (t, J = 7.0 Hz, 2H); 1.62–1.53 (m, J = 7.6 Hz, 2H); 1.51–1.41 (m, J = 6.7 Hz, 1H); 1.40–1.15 (bs, 16H); 1.15–1.07 (m, 2H); 0.80–0.78 (m, 9H). ^{13}C NMR: δ 187.808; 93.693; 80.679; 45.475; 37.966; 31.678; 29.327; 29.246; 29.093; 28.825; 28.627; 27.554; 22.450; 22.182; 21.810; 18.659; 13.832. EI GC/MS: no M$^+$; 263 (24); 235 (58); 193 (80); 137 (56); 43 (100).

6,6-Difluoro-2-methyloctadec-7-yne (15). A solution of oxoalkyne **14** (169.6 mg, 0.609 mmol) in neat DAST (0.8 mL) was stirred for 92 h at 55 °C in sealed screwcap vial. The reaction mixture was poured into 50 mL of hexane and then neutralized cautiously with solid $NaHCO_3$ and saturated aqueous $NaHCO_3$ until the pH of the aqueous layer was ≥8. The hexane layer was washed with water (20 mL), 1 M HCl (2 × 20 mL), water (20 mL), and saturated NaCl (20 mL) and then dried. Concentration and chromatography (hexane) gave 92.3 mg (0.307 mmol, 50%) of the desired 6,6-difluoroalkyne **15** as a clear liquid. TLC: R_f = 0.51 (hexane). ^{1}H NMR: δ 2.264 (tt, J = 7.0, 5.1 Hz, 2H); 2.04–1.87 (m, 2H); 1.63–1.49 (m, 5H); 1.41–1.19 (m + bs, 16H); 0.93–0.86 (m, 9H). ^{13}C NMR: δ 115.085 (t, J = 231 Hz); 88.554 (t, J = 6.4 Hz); 74.103 (t, J = 39.8 Hz); 39.700 (t, J = 26.3 Hz); 38.206; 31.912; 29.562; 29.488; 29.325; 29.052; 28.780; 27.841; 27.790; 22.688; 22.417; 20.847; 18.351; 14.069. EI GC/MS: no M$^+$; 195 (2); 165 (2); 159 (7); 139 (13); 43 (100). CI GC/MS: no MH$^+$; 281 (28); 261 (50); 109 (100).

(*Z*)-6,6-Difluoro-2-methyloctadec-7-ene (16). A stirred suspension of 5% Pd–$BaSO_4$ (7.3 mg) in 0.5 mL of pyridine was vacuum-degassed/H_2-flushed three times. The catalyst was stirred under H_2 for 15 min and changed from brown to black during this time. A solution of difluoroalkyne **15** (77.8 mg, 0.259 mmol) in 0.5 mL of pyridine was then added. The reaction mixture was stirred for 24 h under H_2 (1 atm); analysis of an aliquot by GC (HP-5) showed no progress. The reaction mixture was filtered through a small plug of $MgSO_4$ and the filtrate added to a second batch of catalyst (24.6 mg in 0.5 mL pyridine) prepared as described above. After being stirred for 24 h under H_2 (1 atm), the reaction mixture was diluted with hexane (5 mL) and filtered through $MgSO_4$. Solvent was removed and the residue chromatographed (hexane) to give 70.0 mg (0.231 mmol, 89%) of the desired 6,6-difluoroalkene **16**, a clear liquid, as a 93:7 *Z*:*E* mixture. TLC: R_f = 0.57 (hexane). GC (50,1/20/200, HP-5): 15.65 min (*Z*, 93%); 15.91 min (*E*, 7%). ^{1}H NMR: δ 5.698 (dtt, J = 13.7, 7.8, 1.6 Hz, 1H); 5.444 (br. q of t, J ≈ 14.0, 1.5 Hz, 1H); 2.27–2.21 (m, 2H); 1.96–1.80 (m, 2H); 1.60–1.47 (m, 1H); 1.56–1.36 (m, 4H); 1.36–1.26 (bs, 14H); 1.25–1.17 (bq, J = 7.5 Hz, 2H); 0.91–0.85 (m, 9H). ^{13}C NMR: δ 137.970 (t, J = 5.8 Hz); 124.716 (t, J = 27.3 Hz); 122.748 (t, J = 239.0 Hz); 39.128; 38.610 (t, J = 13.4 Hz); 31.931; 29.618; 29.477; 29.366; 29.271; 28.332; 27.837; 22.701; 22.461; 20.220; 14.097. EI GC/MS: 302 (M$^+$, 1.5); 282 (1.0); 267 (0.5); 177 (2); 152 (7); 109 (15); 43 (100). CI GC/MS: no MH$^+$; 283 (10); 263 (100). HRMS: calcd for $C_{19}H_{36}F_2$ 302.2787, found 302.2781.

(±)-6,6-Difluoro-*cis*-7,8-epoxy-2-methyloctadecane [(±)-6,6-Difluorodisparlure, (±)-17]. To a solution of difluoroalkene **16** (62.6 mg, 0.207 mmol, as a 93:7 *7Z*:*7E* mixture) in 1 mL of CH_2Cl_2 at room temperature was added purified *m*-CPBA (74.0 mg, 0.43 mmol). The reaction mixture was stirred at 40 °C for 24 h and at room temperature for 24 h. Most of the solvent was evaporated and the resultant white residue stirred with a mixture of 10% (w/v) aqueous Na_2SO_3 (1 mL) and saturated $NaHCO_3$ (1 mL). After being stirred for 5 min, the resultant homogeneous solution was extracted with 4 × 2 mL portions of hexane. The combined organic layers were dried and concentrated to give a clear liquid. This liquid was chromatographed (98:2 H:EA) to give 49.7 mg (0.156 mmol, 75%) of the desired 6,6-difluorodisparlure **17** as a 94:6 *cis*:*trans* mixture. TLC: R_f = 0.43 (95:5 H:EA). GC (50,1/20/220, HP-5): 15.03 min (5.6%, *trans*); 15.38 min (94.4%, *cis*). ^{1}H NMR: δ 3.075 (ddd, J = 12.4, 10.0, 4.1 Hz, 1H); 3.02–3.00 (m, 1H); 1.99–1.82 (m, 2H); 1.75–1.68 (m, 2H); 1.57–1.45 (m, 3H); 1.30–1.24 (bs, 16H); 1.23–1.17 (m, 2H); 0.90–0.85 (m, 9H). ^{13}C NMR: δ 121.765 (t, J = 243.4 Hz); 57.547; 56.349 (dd, J = 36.2, 29.9 Hz); 38.548; 36.409 (t, J = 24.8 Hz); 31.890; 29.562; 29.508; 29.472; 29.370; 29.324; 27.769; 27.523; 27.005; 22.668; 22.419; 19.279; 14.074. EI GC/MS: no M$^+$; 255 (0.7); 219 (0.8); 205 (0.6); 191 (0.4); 183 (6); 177 (10); 163 (17); 143 (6); 43 (100). CI GC/MS: 319 (MH$^+$, 100); 299 (47); 279 (13). HRMS: calcd for $C_{19}H_{36}F_2O$ 318.2736, found 318.2733.

Synthesis of 9-Functionalized Disparlure Analogues. (±)-2-Methyloctadec-7-yn-9-ol [(±)-19]. Prepared in a fashion analogous to (±)-**9**, the desired alkynol **19** (2.08 mmol, 71%, pale yellow liquid) was obtained by reaction of the lithium acetylide of 7-methyl-1-octyne[13] with decanal. TLC: R_f = 0.33 (90:10 H:EA). ^{1}H NMR: δ 4.308 (bt, J = 6.5 Hz, 1H); 2.168 (td, J = 7.0, 1.8 Hz, 2H); 1.70–1.55 (m, 2H); 1.55–1.30 (m, 4H); 1.23 (bs, 18H); 0.86–0.82 (m, 9H). ^{13}C NMR: δ 85.230; 81.382; 62.579; 38.354; 38.110; 31.837; 29.498; 29.267; 28.848; 27.792; 26.550; 25.169; 22.611; 22.500; 18.622; 14.020. EI GC/MS: no M$^+$; 279 (0.6); 237 (2.4); 209 (10.5); 181 (64.7); 153 (24.7); 97 (100).

(±)-(*Z*)-2-Methyl-7-octadecen-9-ol [(±)-20]. This compound was prepared by the general method of (±)-**10**. This crude product was chromatographed twice (90:10 H:EA) to give a total of 201.3 mg (0.713 mmol, 81%) of product. The fraction most enriched in desired product contained 146 mg of allylic alcohol **20**, a clear liquid, as a 86:14 *7Z*:*7E* mixture. TLC: R_f = 0.34 (90:10 H:EA). GC (50,1/20/220, HP-5): 15.84 min (*Z*, 86%); 16.42 min (*E*, 14%). ^{1}H NMR: δ 5.66–5.56 (m, *7E* isomer); 5.464 (dt, J = 11.0, 7.2 Hz, 1H); 5.343 (ddt, J = 10.8, 8.8, 1.3 Hz, 1H); 4.409 (bq, J ~ 7.0 Hz, 1H); 4.05–3.98 (bq, *7E* isomer); 2.072 (bq, J ≈ 7.0 Hz, 2H); 1.60–1.45 (m, 4H); 1.40–1.20 (bs, 18H); 1.20–1.10 (m, 2H); 0.90–0.84 (m, 9H). ^{13}C NMR: δ 132.640; 132.216; 67.674; 38.812; 37.513; 31.869; 29.970; 29.593; 29.303; 27.909; 27.732; 27.040; 25.383; 22.589; 14.059. EI GC/MS: no M$^+$; 239 (2); 197 (2); 183 (12); 155 (49); 137 (24); 127 (6); 81 (100).

(±)-8,9-*threo*-7,8-*cis*-7,8-Epoxy-2-methyloctadecan-9-ol [(±)-9-Hydroxydisparlure, (±)-*threo,cis*-21]. Prepared in a fashion analogous to (±)-**11**, the desired (±)-*threo,cis*-epoxy alcohol **21** (0.314 mmol, 72%), a clear liquid, was obtained after chromatography (90:10 H:EA). The purity was greater than 98.8% by GC. TLC: R_f = 0.40 (80:20 H:EA). GC (50,1/20/220, HP-5): 20.14 min (≥98.8%). ^{1}H NMR: δ 3.439 (td, J = 8.0, 4.0 Hz, 1H); 2.993 (bq, J ≈ 4.6 Hz, 1H); 2.862 (dd, J = 8.2, 4.5 Hz, 1H); 1.61–1.40 (m, 6H); 1.38–1.22 (bs, 18H); 1.17–1.12 (m, 2H); 0.86–0.82 (m, 9H). ^{13}C NMR: δ 69.608; 60.832; 58.095; 38.852; 34.077; 31.823; 29.589; 29.462; 28.373; 27.843; 27.139; 27.018; 24.988; 22.542; 22.461; 13.875. EI GC/MS: no M$^+$; 155 (2); 152 (3); 142 (2); 124 (16); 96 (53); 82 (100). CI GC/MS: 299 (MH$^+$, 31); 281 (100).

(±)-*cis*-7,8-Epoxy-2-methyloctadecan-9-one [(±)-9-Oxodisparlure, (±)-22]. This compound was prepared by the general method of (±)-**13**. After chromatography (95:5 H:EA) 27.5 mg (92.8 μmol, 67%) of the desired 9-oxodisparlure **22**, a clear liquid, was obtained. TLC: R_f = 0.41 (90:10 H:EA). ^{1}H NMR: δ 3.500 (d, J = 4.9 Hz, 1H); 3.15–3.09 (m, 1H); 2.443 (t, J = 7.4 Hz, 2H); 1.56–1.51 (m, 2H); 1.49–1.35 (m, 3H); 1.28–1.17 (bs, 16H); 1.12–1.04 (m, 2H); 0.805 (t, J = 6.8 Hz, 3H); 0.787 (d, J = 6.6 Hz, 6H). ^{13}C NMR: δ 206.365; 58.513; 41.033; 38.737; 31.816; 29.360; 29.323; 29.196; 27.828; 27.507; 27.035; 26.558; 23.192; 22.611; 22.550; 22.481; 14.016. EI GC/MS: 296 (M$^+$, 3.1); 197 (33); 155 (37); 123 (25); 95 (30). CI GC/MS: 297 (MH$^+$, 20); 281 (100); 279 (10). HRMS: calcd for $C_{19}H_{36}O_2$ 296.2715, found 296.2708.

2-Methyloctadec-7-yn-9-one (23). Prepared in a fashion analogous to **14**, the desired oxoalkyne **23** (202.9 mg, 0.729 mmol, 79%, clear liquid) was obtained after chromatography (98:2 H:EA). TLC: R_f = 0.46 (95:5 H:EA). ^{1}H NMR: δ 2.457 (t, J = 7.5 Hz, 2H); 2.306 (t, J = 7.0 Hz, 2H); 1.601 (m, J = 7.2 Hz, 2H); 1.508 (m, J = 7.1 Hz, 1H); 1.40–1.12 (m, 18H); 0.84–0.80 (m, 9H). ^{13}C NMR: δ 187.855; 93.797; 80.970; 45.437; 38.246; 31.756; 29.275; 29.127; 28.918; 27.946; 27.740; 26.518; 24.139; 22.508; 22.386; 18.832; 13.854. EI GC/MS: no M$^+$; 263 (1); 235 (3); 207 (11); 193 (24); 179 (42); 151 (65); 137 (22); 123 (87); 109 (79); 95 (49); 81 (100).

9,9-Difluoro-2-methyloctadec-7-yne (24). To a stirred solution of oxoalkyne **23** (160.6 mg, 0.577 mmol) in 0.5 mL of CH_2Cl_2 was added DAST (added neat using a glass micropipet, 200 μL, 1.5 mmol). After 41 h at room temperature, GC (HP-5)

indicated only 11% conversion to product. The reaction mixture was diluted with 1 mL of hexane and heated to 55 °C for 8 h; GC analysis at this point showed 14% conversion to product. The reaction mixture was cooled to room temperature, 0.75 mL of DAST was added, and the mixture stirred for 24 h at 55 °C (33% conversion). The reaction mixture was poured into 50 mL of hexane, washed cautiously with 50 mL of water, 50 mL of 1 M HCl, and 2 × 50 mL of saturated $NaHCO_3$, and dried. Removal of solvent gave 159 mg (ca. 99%) of an orange oil which was chromatographed (98:2 H:EA) to give 38.2 mg (0.127 mmol, 22%) of the desired product as a clear liquid. Also recovered was 97.2 mg (0.349 mmol, 60%) of starting material. The recovered starting material was dissolved in 0.75 mL of DAST and stirred for 4 days at 55 °C. Workup as above gave an additional 35.0 mg (0.117 mmol, 33%) of product, for a total combined yield of 55%. TLC: R_f = 0.67 (95:5 H:EA). ^{1}H NMR: δ 2.267 (tt, J = 7.0, 5.0 Hz, 2H); 2.06–1.91 (m, 2H); 1.60–1.46 (m, 5H); 1.45–1.20 (m + bs, 16H); 0.94–0.86 (m, 9H). ^{13}C NMR: δ 115.145 (t, J = 232 Hz); 88.512 (t, J = 5.5 Hz); 74.152 (t, J = 39.8 Hz); 39.496 (t, J = 26.4 Hz); 38.318; 31.873; 29.394; 29.263; 29.008; 28.065; 27.839; 26.528; 22.987; 22.655; 22.521; 18.362; 14.053. EI GC/MS: no M^+; 285 (2); 238 (1); 237 (1); 223 (1); 217 (2); 195 (2); 181 (4); 161 (6); 153 (11); 123 (18); 43 (100). CI GC/MS: no MH^+; 281 (100); 261 (50).

(*Z*)-9,9-Difluoro-2-methyloctadec-7-ene (25). Prepared in a fashion analogous to **16**, 70.1 mg (0.232 mmol, 73%) of the desired difluoroalkene **25**, a clear liquid, was obtained as an 89:11 *Z*:*E* mixture. The *Z*:*E* ratio varied from run to run; a ratio of 95:5 *Z*:*E* was obtained occasionally. TLC: R_f = 0.54 (hexane). GC (50,1/20/200, HP-5): 15.68 min (*Z*, 89%); 15.95 min (*E*, 11%). ^{1}H NMR: δ 5.695 (dtt, J = 11.8, 7.7, 1.8 Hz, 1H); 5.437 (tdt, J = 14.5, 11.8, 1.6 Hz, 1H); 2.29–2.20 (m, 2H); 1.97–1.81 (m, 2H); 1.57–1.43 (m, J = 6.9 Hz, 1H); 1.35–1.24 (bs, 18H); 1.20–1.12 (m, 2H); 0.88–0.85 (m, 9H). ^{13}C NMR: δ 137.926 (t, J = 6.4 Hz); 124.734 (t, J = 27.4 Hz); 122.783 (t, J = 238.5 Hz); 38.803; 35.573 (t, J = 26.7 Hz); 31.896; 29.724; 29.445; 29.308; 28.360; 27.953; 27.035; 22.678; 22.608; 22.391; 14.097. EI GC/MS: 302 (M^+, 0.5); 282 (0.8); 267 (0.3); 135 (5); 110 (12); 56 (100). CI GC/MS: no MH^+; 283 (21); 263 (62).

(±)-9,9-Difluoro-*cis*-7,8-epoxy-2-methyloctadecane [(±)-9,9-Difluorodisparlure (±)-26]. To a solution of difluoroalkene **25** (2.06 mg, 6.81 μmol, as a 95:5 7*Z*:7*E* mixture) in 100 μL of CH_2Cl_2 at room temperature was added purified *m*-CPBA (1.84 mg, 10.7 μmol). The reaction was allowed to sit for 68 h at room temperature in a sealed screw-cap vial; GC (HP-5) indicated ca. 60% conversion to product. An additional 2 mg of *m*-CPBA was added and the reaction heated to 40 °C for 27 h. After workup and chromatography as in (±)-**17**, 0.9 mg (2.8 μmol, 41%) of the desired 9,9-difluorodisparlure **26** was obtained. TLC: R_f = 0.37 (95:5 H:EA). GC (50,1/20/220, HP-5): 15.02 min (*trans*, 4.9%); 15.39 min (*cis*, 95.1%). ^{1}H NMR: δ 3.101 (ddd, J = 10.2, 4.1, 2.4 Hz, 1H); 3.05–3.00 (m, 1H); 2.01–1.84 (m, 2H); 1.75–1.66 (m, 2H); 1.59–1.44 (m, 3H); 1.39–1.27 (bs, 16H); 1.23–1.17 (m, 2H); 0.89–0.86 (m, 9H). EI GC/MS: no M^+; 219 (10); 141 (6); 123 (30); 95 (46); 69 (100). CI GC/MS: 319 (MH^+, 100); 299 (23). HRMS: calcd for $C_{19}H_{36}F_2O$ 318.2736, found 318.2745.

Synthesis of 6,9-Difunctionalized Disparlure Analogues. (±)-1-Dodecyn-3-ol [(±)-27]. A solution of monolithium acetylide was prepared as follows: After passage through two −78 °C cold traps to remove acetone, a stream of acetylene with a measured flow of 70–80 mL/min was bubbled into 20 mL of stirred THF at −78 °C for 10 min (700–800 mL total), yielding a solution which contained ca. 29–33 mmol of acetylene. To this solution at −78 °C was added 1.6 M *n*-BuLi in hexanes (5.1 mL, 8.2 mmol) over several minutes. The resultant slightly cloudy solution was stirred for 30 min at −78 °C under N_2, followed by the addition of a solution of decanal (1.20 g, 7.68 mmol) in 3 mL of THF. The reaction was stirred at −78 °C for 5 min and then allowed to warm to room temperature over 30 min, after which it was quenched by the addition of 3 mL of water. Anhydrous K_2CO_3 was added until the aqueous layer thickened. The organic layer was decanted and the aqueous layer extracted with 3 × 40 mL portions of ether. The combined organic layers were dried and concentrated. Chromatography (90:10 H:EA) gave 1.073 g (5.88 mmol, 77%) of the desired alkynol **27** as a clear liquid. TLC: R_f = 0.23 (90:10 H:EA). ^{1}H NMR: δ 4.339 (td, J = 6.6, 2.1 Hz, 1H); 2.58–2.48 (bs, 1H); 2.432 (d, J = 2.4 Hz, 1H); 1.75–1.60 (m, 2H); 1.50–1.35 (m, 2H); 1.30–1.20 (bs, 12H); 0.853 (t, J = 6.8 Hz, 3H). ^{13}C NMR: δ 85.065; 72.687; 62.186; 37.573; 31.833; 29.475; 29.247; 29.213; 24.990; 22.614; 14.030. EI GC/MS: no M^+; 181 (0.3); 153 (0.8); 135 (3.9); 121 (12.0); 55 (100).

(±)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-dodecyne [(±)-28]. A solution of *tert*-butyldimethylsilyl chloride (539.2 mg, 3.58 mmol) in 1.5 mL of DMF was added to a solution of alkynol (±)-**27** (555.8 mg, 3.05 mmol) and imidazole (511.6 mg, 7.51 mmol) in 0.5 mL of DMF. The biphasic mixture was stirred at room temperature for 20 h under N_2 and then diluted with hexane (50 mL) and washed with water (3 × 50 mL). The hexane layer was dried and concentrated to give a yellow oil. The oil was chromatographed (98:2, 95:5 H:EA) to give 656.3 mg (2.21 mmol, 73%) of the desired silyl ether **28** as a clear liquid. TLC: R_f = 0.47 (98:2 H:EA). ^{1}H NMR: δ 4.326 (td, J = 6.5, 2.1 Hz, 1H); 2.358 (d, J = 2.2 Hz, 1H); 1.70–1.63 (m, 2H); 1.43–1.35 (m, 2H); 1.268 (bs, 12H); 0.91–0.88 (m, 12H); 0.132 (s, 3H); 0.107 (s, 3H). ^{13}C NMR: δ 85.835; 71.826; 62.832; 38.640; 31.929; 29.557; 29.314; 25.812; 25.147; 22.694; 18.232; 14.076; −4.528; −5.043. EI GC/MS: no M^+; 281 (1); 239 (11); 221 (9); 169 (9); 113 (97); 75 (100).

(±)-9-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-7-octadecyn-6-ol [(±)-29]. This compound was prepared by the general method of (±)-**9**, which gave 530.1 mg (1.29 mmol, 71%) of the desired monoprotected alkyne diol **29**, a yellow liquid, as a mixture of diastereomers. TLC: R_f = 0.47 (90:10 H:EA). ^{1}H NMR: δ 4.40–4.33 (m, 2H); 1.97–1.93 (bs); 1.70–1.61 (m, 4H); 1.58–1.48 (m, 1H); 1.46–1.40 (m, 4H); 1.27–1.23 (bs, 12H); 1.23–1.15 (m, 2H); 0.95–0.85 (m, 18H); 0.12 (s, 3H); 0.09 (s, 3H). ^{13}C NMR: δ 86.694; 84.914; 62.942; 62.493; 38.669; 38.561; 38.171; 38.056; 31.892; 31.592; 29.557; 29.306; 29.268; 27.958; 27.883; 25.772; 25.263; 22.965; 22.665; 22.525; 22.352; 18.215; 14.074; −4.398; −4.566; −4.928; −5.125. EI GC/MS: no M^+; 353 (5); 335 (1); 283 (5); 257 (14); 145 (14); 75 (100).

(±)-*syn*- and *anti*-(7*Z*)-9-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-7-octadecen-6-ol [(±)-*syn*- and *anti*-30]. The general method of compound **10** was used, which gave 244.8 mg (0.593 mmol, 82%) of product. TLC, GC, and ^{1}H and ^{13}C NMR indicated the product was a 50:50 mixture of the diastereomeric *syn*-**30** and *anti*-**30** allylic diols. TLC: R_f = 0.44, 0.40 (90:10 H:EA). GC (50,1/20/240, HP-5): 20.88 min (50.1%); 21.31 min (49.9%). ^{1}H NMR: δ 5.470 (dd, J = 11.0, 8.3 Hz), 5.453 (dd, J = 10.8, 8.3 Hz), 5.329 (dd, J = 11.3, 9.0 Hz), 5.321 (dd, J = 11.4, 8.5 Hz) (2H, H-7, H-8); 4.44–4.35 (m, 2H); 1.82–1.76 (bs, 1H); 1.60–1.30 (m, 5H); 1.30–1.22 (bs, 16H); 1.22–1.15 (m, 2H); 0.88–0.85 (m, 18H); 0.063 (s), 0.044 (s), 0.036 (s), 0.013 (s) (6H). ^{13}C NMR: δ 136.578, 135.416, 131.713, 131.444 (C-7, C-8); 69.196, 68.884, 67.997, 67.825 (C-6, C-9); 39.041; 38.921; 38.730; 38.093; 38.005; 31.902; 29.619; 29.302; 27.977; 25.892; 25.346; 23.225; 22.633; 22.531; 18.161; 13.984; −4.211; −4.590; −4.637. EI GC/MS: 20.88-min isomer, no M^+; 355 (47); 327 (3); 285 (50); 257 (3); 75 (100); 21.31-min isomer, no M^+; 355 (38); 337 (2); 285 (59); 257 (3); 75 (100).

(±)-6,7-*threo*-8,9-*threo*- and (±)-6,7-*threo*-8,9-*erythro*-9-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-*cis*-7,8-epoxyoctadecan-6-ol [(±)-*threo,threo*-31 and (±)-*threo,erythro*-31]. The general method of (±)-**11** was used. After workup and chromatography (95:5 H:EA), 195.5 mg (0.456 mmol, 83%) of the desired monoprotected epoxy diols *threo,threo*-**31** and *threo,erythro*-**31** were obtained. Both the GC (HP-5) and ^{13}C NMR indicated an approximate 1:1 mixture of diastereomers. TLC: R_f = 0.35, 0.36 (90:10 H:EA). GC (50,1/20/260, HP-5): 19.01 min, 19.72 min. ^{1}H NMR: δ [3.77–3.71 (m), 3.742 (bq, J ≈ 5.6 Hz), 3.527 (td, J = 8.3, 2.7 Hz), 3.48–3.42 (m), (2H, H-6, H-9)]; 3.00–2.89 (m, 2H); [2.341 (s), 2.329 (s), (1H, −OH)]; 1.61–1.31 (m, 5H); 1.31–1.15 (bs + m, 18H); 0.91–0.84 (m, 18H); [0.116 (s), 0.034 (s), 0.030 (s), (6H)]. ^{13}C NMR: δ 71.276, 69.100, 68.981, 68.774 (C-6, C-9); 62.445, 60.637, 60.554 (C-7, C-8); 38.955; 38.845; 35.797; 34.691; 34.581; 31.850; 29.774; 29.508; 29.260; 27.869; 25.811; 24.930; 24.314; 22.990; 22.616; 22.482; 18.134; 18.030; 14.038; −4.195; −4.318; −4.955. EI GC/MS: 19.01-min isomer, no M^+; 353 (4); 313 (2); 279 (4); 271 (5); 243 (100); 95 (33); 75 (44); 19.72 min isomer, no M^+; 353 (9); 313 (8); 271 (23); 243 (23); 201 (88); 95 (58); 75 (100).

(±)-6,7-*threo*-8,9-*threo*- and (±)-6,7-*threo*-8,9-*erythro,cis*-7,8-Epoxy-2-methyloctadecane-6,9-diol [(±)-*threo,threo*- and (±)-threo,erythro-6,9-Dihdyroxydisparlure, (±)-*threo,threo*-

and (±)-*threo,erythro*-32]. A solution of 1.0 M tetrabutylammonium fluoride (0.38 mL, 0.38 mmol) in THF was added to an approximate 1:1 mixture of the monoprotected epoxy diols (±)-*threo,threo*-**31** and (±)-*threo,erythro*-**31** (52.1 mg, 121 μmol). The reaction mixture was stirred at 0 °C for 30 min and room temperature for 45 min and then diluted with 2 mL of hexane and washed with 3 × 1 mL of water. The organic layer was dried and concentrated. Chromatography of the crude product (80:20, 60:40 H:EA) effectively separated the desired *threo,threo*-**32** and *threo,erythro*-**32**, for a combined yield of 37.2 mg (118 μmol, 97%). (±)-*threo,erythro*-**32**. White solid, 19.2 mg (61.0 μmol, 50%). TLC: R_f = 0.62 (60:40 H:EA). ^{1}H NMR: δ 3.77–3.71 (m, 1H, H-9); 3.66–3.60 (m, 1H, H-6); 2.978 (t, J = 4.3 Hz, 1H); 2.954 (t, J = 4.4 Hz, 1H); 2.40–1.90 (br, 2H, –OH); 1.74–1.47 (m, 5H); 1.36–1.17 (bs, 18H); 0.89–0.85 (m, 9H). ^{13}C NMR: δ 69.503, 69.376 (C-6, C-9); 60.772, 60.390 (C-7, C-8); 38.873; 35.707; 34.506; 31.860; 29.528; 29.283; 27.915; 25.092; 22.985; 22.626; 22.514; 14.066. CI GC/MS: 315 (MH^+, 43); 297 (93); 279 (95). (±)-*threo,threo*-**32**. Clear liquid, 18.0 mg (57 μmol, 47%). TLC: R_f = 0.30 (60:40 H:EA). ^{1}H NMR: δ 3.62–3.56 (m, 2H, H-6, H-9); 3.07–3.03 (m, 2H, H-7, H-8); 2.78–2.76 (br, 2H, –OH); 1.61–1.43 (m, 5H); 1.35–1.23 (bs, 16H); 1.23–1.14 (m, 2H); 0.89–0.85 (m, 9H). ^{13}C NMR: δ 69.224 (C-6, C-9); 61.952 (C-7, C-8); 38.834; 34.342; 34.106; 31.858; 29.530; 29.291; 27.883; 25.102; 22.900; 22.628; 22.525; 14.049. CI GC/MS: no MH^+; 297 (47); 279 (64).

(±)-*cis*-7,8-Epoxy-2-methyloctadecane-6,9-dione [(±)-6,9-Dioxodisparlure, (±)-33]. To a stirred mixture of PDC (321.6 mg, 855 μmol) in 1 mL of CH_2Cl_2 was added a mixture of the epoxy diols (±)-*threo,threo*-**32** and (±)-*threo,erythro*-**32** (23.5 mg, 74.7 μmol) in 1 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 14 days and then filtered through a small plug of silica. ^{1}H NMR of the crude product showed an approximate 3:1 ratio of 6,9-dione to hydroxy ketone. To complete the reaction, CrO_3·(pyr)$_2$ in CH_2Cl_2 was prepared as follows: CrO_3 (44.8 mg, 0.448 mmol) was added to a stirred solution of pyridine (78.2 mg, 0.989 mmol) in 1.5 mL of CH_2Cl_2 at 0 °C. The reagent was stirred for 30 min at 0 °C and 15 min at room temperature and the crude product described above added in 1 mL of CH_2Cl_2. After being stirred for 90 min at room temperature, the reaction mixture was decanted into 1 mL of hexane. The reaction flask was washed with 3 × 2 mL of 1:1 hexane:ether, and the combined organic layers were washed with 3 × 5 mL of saturated $NaHCO_3$, 5 mL of water, 3 × 5 mL of saturated $CuSO_4$, 5 mL of water, and 5 mL of saturated NaCl and dried. Concentration and chromatography of the residue (95:5 H:EA) gave 13.6 mg (43.8 μmol, 59%) of the desired epoxy diketone **33**. TLC: R_f = 0.22 (90:10 H:EA). ^{1}H NMR: δ 3.636 (s, 2H, H-7, H-8); 2.62–2.41 (m, 4H); 1.59–1.40 (m, 5H); 1.23–1.20 (bs, 12H); 1.19–1.05 (m, 2H); 0.85–0.79 (m, 9H). ^{13}C NMR: δ 204.565 (C-6, C-9); 58.475 (C-7, C-8); 41.257; 41.072; 38.131; 31.790; 29.310; 29.267; 29.178; 28.984; 27.728; 22.795; 22.582; 22.346; 20.652; 14.000. EI GC/MS: 310 (M^+, 1.0); 281 (2.4); 240 (2.6); 197 (10); 155 (28); 95 (100). CI GC/MS: 311 (MH^+, 66); 295 (7.3); 293 (3.8); 283 (4.5). HRMS: calcd for $C_{19}H_{34}O_3$ 310.2509, found 310.2513.

Purification of Disparlure. The (+)-[5,6-$^3H_2$]disparlure used in this study was synthesized at the National Tritium Labeling Facility in Berkeley, CA, in 1986,[10] and then was stored in 1:1 heptane:toluene at –20 °C at a concentration of ca. 100 mCi/mL. Due to the method of synthesis, ca. 10–20% of 2-methyloctadecan-8-one was present in the (+)-[5,6-$^3H_2$]-disparlure, caused by the rhodium-induced rearrangement of the precursor alkenyl epoxide during tritiation with carrier-free T_2 gas.[10] Substantial primary and secondary radiolytic decomposition of the (+)-[5,6-$^3H_2$]disparlure had occurred,[20] as evidenced by TLC/fluorography of aliquots. Prior to use, the (+)-5,6-$^3H_2$]-disparlure was purified by the following a three-step process. Stored samples of (+)-[5,6-$^3H_2$]disparlure were combined, and the heptane/toluene was removed by rotary evaporation. The sample was dissolved in 500 μL of 98:2 hexane:ethyl acetate (H:EA) and applied as a 10-cm band to a 20-cm × 20-cm tapered preparative silica TLC plate. The plate had been prescored 3 cm from each vertical edge to create marker lanes; each marker lane was then spotted with nonradiolabeled disparlure. Following development in 98:2 H:EA, the marker lanes were broken off and visualized with vanillin stain. The corresponding radiolabeled region was then scraped off, eluted with 50:50 H:EA, and evaporated to dryness. The sample was then dissolved in 1000 μL of ethanol, $NaBH_4$ (1–2 mg) was added, and the reaction was stirred overnight at room temperature. The ethanol was evaporated, 10 drops of 1 M HCl were added, and the products extracted into 1000 μL of hexane. The crude product was dried over $MgSO_4$, filtered, evaporated, redissolved in 1000 μL of 1:1 hexane:benzene, and purified by preparative TLC as before. After extraction from the silica gel, the purified radiolabeled (+)-disparlure (3.0 mCi, 51 nmol) was evaporated and dissolved in 540 μL of ethanol to give the 94 μM stock solution used in the K_M, V_{max}, and inhibitor studies.

Determination of K_M and V_{max}. Homogenates of *L. dispar* male antennae were used. Briefly,[10] 12 whole antennae from newly emerged (day 0 or day 1) *L. dispar* adult males were homogenized in 10 mM Tris·HCl (pH 7) buffer to give a final concentration of 2 antenna equivalents (a.e.)/mL. The homogenate was centrifuged at 12000*g* at 0 °C for 10 min, and the supernatant was stored at 4 °C until use. Starting with the 94 μM stock solution of radiolabeled (+)-disparlure, 50, 25, 14, and 10 μM stocks were prepared by serial dilution with ethanol. Next, to each of eight tubes at 4 °C was added 300-μL aliquots (0.6 a.e.) of the antennal homogenate, followed by 3–12.8-μL aliquots of the appropriate radiolabeled (+)-disparlure stock so that initial substrate concentrations of 4.0, 2.5, 1.4, 1.0, 0.25, 0.14, and 0.10 μM were obtained *and* that the ethanol concentration in each tube not exceed 4% (v/v). The tubes were incubated at 27 °C for 20 min, with vortexing every 5 min, after which the reaction was stopped by the addition of 300 μL of ethyl acetate followed by vortexing. Three-μL aliquots of the ethyl acetate layer were spotted on a TLC plate, and the plate was developed; the ratio of disparlure to its diol, expressed as percent conversion, was determined as previously described.[10] Percent conversions ranged from 2.6% to 12%. Percent recoveries under these conditions were 50–67%. The percent conversions were transformed into rates and are shown in the Lineweaver–Burk plot of Figure 3.

Determination of IC_{50} Values. Stock solutions of 50, 20, 4, 2, and 0.4 mM in ethanol (except for 9,9-difluoro analogue **26**, which was 10, 4, 2, and 0.4 mM) were prepared from each of the nine racemic inhibitors (*threo,cis*-**11**, **13**, **17**, *threo,cis*-**21**, **22**, **26**, *threo,erythro*-**32**, *threo,threo*-**32**, and **33**). Using the male antennal homogenates described above, 3 μL of each inhibitor stock was added to 300 μL of homogenate at 27 °C, giving inhibitor concentrations of 500, 200, 40, 20, and 4 μM (except for the 9,9-difluoro analogue **26**, which gave 100, 40, 20, and 4 μM). Each tube was incubated for 5 min at 27 °C, followed by the addition of 4 μL of the 94 μM radiolabeled (+)-disparlure stock, giving an initial disparlure concentration of 1.2 μM. Each tube was incubated at 27 °C for 20 min and then quenched with ethyl acetate and the percent conversion of disparlure to diol, and thus the rate of conversion was determined as described above. The rate data were plotted as a function of inhibitor concentration (not shown); the inhibitor concentration required to reduce the rate to one-half of V_{max} was determined by extrapolation, and these values are shown in Table 1.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra for the nine inhibitors (*threo,cis*-**11**, **13**, **17**, *threo,cis*-**21**, **22**, **26** (^{1}H NMR only), *threo,erythro*-**32**, *threo,threo*-**32**, and **33**) (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.