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Synthesis and biological activity of conformationally restricted gypsy moth pheromone mimics

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ABSTRACT

The design and synthesis of a series of conformationally constrained mimics of gypsy moth sex pheromone, (+)-disparlure (7*R*,8*S*)-2-methyl-7,8-epoxyoctadecane, are described. The core structure of the mimics is derived from 5-(2′-hydroxyethyl)cyclopent-2-en-1-ol. Substituent optimization of the analogs was accomplished through the synthesis of mini-libraries and pure individual compounds, followed by electrophysiological experiments with male gypsy moth antennae. The electroantennogram results show that the analogs elicited weak to no antennal responses themselves. There was a clear structure–activity pattern for odorant activity, with ethyl substituents being best. Further, when puffed simultaneously with the pheromone, some of the compounds gave a significant enhancement of the antennal depolarization, indicating an additive or synergistic effect. A pure pheromone stimulus following a mixed compound/pheromone stimulus was generally not affected, with two exceptions: one compound enhanced and another inhibited a subsequent stimulus. The compounds also prolonged the stimulation of the antenna, which manifested itself in widened electroantennogram peaks. We tested the hypothesis that this prolonged stimulation may be due to the stabilization of a particular conformer of the pheromone-binding protein (PBP). Compounds that caused PBP2 to adopt a similar conformation than in the presence of pheromone also caused peak widening. This was not the case with PBP1.

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1. Introduction

The gypsy moth *Lymantria dispar* L. is a defoliator of forest and shade trees in Europe, Asia, and North America.[1] The females of this species produce and release a sex-attractant pheromone, which they disperse into the wind. The males follow a pheromone plume upwind to the source or until they lose the plume.[2,3] In a landmark effort, the structure of this attractant pheromone was determined to be (+) *cis*-(7*R*,8*S*)-epoxy-2-methyloctadecane ((+)-disparlure) (+)-**1** (Fig. 1), by isolation of the active compound from an extract of $\sim 10^5$ female gypsy moths.[4,5] The moths are highly selective towards this compound, with respect to chain length, position of the methyl group and of the epoxide moiety.[4,6,7] Further research provided synthetic routes to the enantiomers of disparlure,[8–19] which were tested against the antennae of male moths[20–23] and in field trapping experiments,[23–25] and these tests showed that (+)-**1** is the main active sex-attractant pheromone of *L. dispar*. The enantiomer, (−)-**1** has been identified as a major component of the pheromone mixture released by the nun moth *Lymantria monacha*, a closely related species.[20,21] The (−) enantiomer is not attractive by itself to either species, but inhibits upwind flight behavior in the gypsy moth, when simultaneously presented with (+)-**1**. The nun moth itself also uses (+)-**1**, as well as the non-branched analog *cis*-(7*R*,8*S*)-7,8-epoxyoctadecane, as its major attractant pheromone. Enantiomer (−)-**1** has no behavioral effect on the nun moth by itself.[20,21] This discrimination between blends of enantiomers[20,21] and other components[20,21,26] has been proposed as one mechanism for reproductive species isolation.[20,21,27] Electrophysiological studies with gypsy moth antennae have revealed that the moth has separate populations of sensory hairs that respond either to (+)-**1** or to (−)-**1**, but not to both.[22] This means that the moth detects both enantiomers of **1** and is able to distinguish them by the population of sensilla that responds. A consequence of this enantiomeric discrimination in practical trapping applications is that the number of moths caught in pheromone-baited traps is highest when lures of (+)-**1** of high enantiomeric purity ($\geqslant$98% ee) are used.[23]

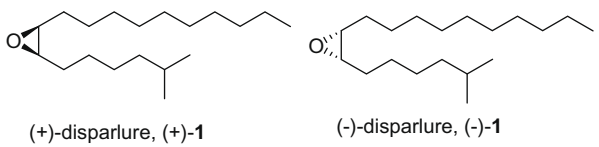

Figure 1. Two enantiomers of naturally occurring disparlure.

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The sensory hairs on moth antennae are hollow exoskeleton structures, into which the dendrites of 2–3 olfactory nerve cells protrude.[28] The space between the exoskeleton and nerve cell dendrite contains a protective solution, the sensillar lymph, which contains high levels of the odorant-binding protein, a secreted protein. Sensory hairs that specialize on pheromone detection contain specialized versions of the odorant-binding protein, the pheromone-binding protein (PBP).[29–31] The gypsy moth has two such binding proteins: PBP1 and PBP2.[32] Binding studies have revealed that both proteins bind both enantiomers of **1**, but PBP1 binds (−)-**1** more strongly and PBP2 binds (+)-**1** more strongly. Interestingly, both proteins bind racemic **1** more weakly than either enantiomer.[33,34] Such synergistic/antagonistic blend effects have been described for gypsy moth PBPs with other ligands.[35] Other molecular recognition proteins in the sensory hairs of moths have been identified for silk moths (*Bombyx mori*, *Antheraea polyphemus*) and for pest moths (*Heliothis virescens*), as well as for fruit flies (*Drosophila melanogaster*): olfactory receptors (ORs)[36,37] and the sensory neuron membrane protein (SNMP).[38,39] The ORs are expressed on the dendrite and have been shown to be selectively activated by various ligands, in behavioral,[40] genetic/physiological[41–43] and in vitro[36,37] studies. All the proteins (PBPs, ORs, and SNMP) are necessary to elicit a pheromone response by the nerve cell housed in a sensory hair.[38,40–42,44,45]

We hypothesize that it should be possible to design compounds that compete with the pheromone for the PBPs or the membrane components, but that bind to one or more components and alter the response of the system to the pheromone. Further, we hypothesize that the pheromone only has few active conformations (among millions of possible conformers), and that there will be a set of conformers that bind to at least one of the protein components. Analogs of pheromones and active conformations have been studied previously for the European corn borer[46,47] and for the turnip moth.[48–53] We aim to mimic the set of conformers that allosterically compete for binding sites in the olfactory system and that alter the olfactory response, by introducing conformational restrictions and replacing CH_2 groups with oxygen in the structure. Compounds that interfere with pheromone detection could find practical use in mating disruption. Compounds that enhance pheromone detection could find use in monitoring/trapping programs.

Herein, we describe the synthesis and biological activity of conformationally restricted mimics of (+)-**1** and (−)-**1**, which are derived from 5-(2′-hydroxyethyl)-cyclopent-2-en-1-ol (**2**) (Scheme 1). The chain-length optimization of the mimics, through the synthesis of mini-libraries and pure compounds, followed by electrophysiological experiments with male gypsy moth antennae is presented. Four properties of the compounds were measured: (1) odorant activity of the compounds by themselves, (2) simultaneous (short-term) agonism or antagonism when the compound is mixed with the pheromone, (3) long-term inhibition of the response to a pure pheromone stimulus, ~1 min after exposure of the antenna to the compound, and (4) widening of the electroantennogram (EAG) peak relative to a peak of pure (+)-**1** when the compound is present.

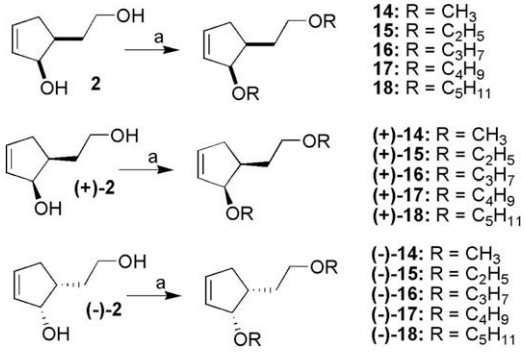
2. Results and discussion

2.1. Synthesis

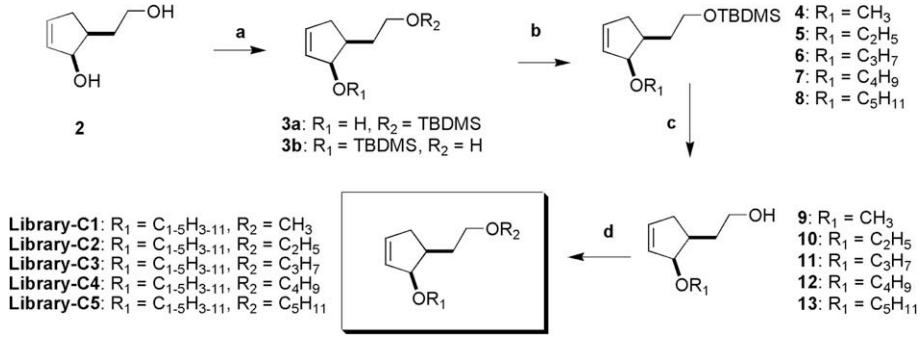
We chose to synthesize small sets of compounds and individual members because the chemical sensing system in insects is nonlinear. The sensation and subsequent behavior of the insect depend on both the concentration of a chemical signal and blend composition. Conversely, testing of many individual compounds is very laborious. To minimize the number of assays and of confounding blend effects, we have developed a screening strategy, in which systematically varied small libraries of alkoxy benzenes (with 4–5 compounds) are screened as insect olfaction or gustation inhibitors.[54] Here, we employed the same strategy for the screening of conformationally constricted gypsy moth pheromone mimics prepared from framework **2**.

The parent diol (**2**) was synthesized and resolved as described in our previous work.[55] Diol (**2**) was used as the parent intermediate for the targeted compounds in Schemes 1 and 2.

For the synthesis of mini-libraries, the selective protection of the primary hydroxyl group in racemic diol **2** with a *tert*-butyldimethylsilyl (TBDMS) group was achieved in the presence triethylamine and catalytic *N*,*N*-dimethylaminopyridine (DMAP). The mono-protection resulted in regioisomers of **3a** and **3b**, in a 9:1 ratio and 90% yield. The secondary hydroxyl group of pure **3a** was



Scheme 2. Synthesis of homologous diethers. Reagents and conditions: (a) KH, RX, THF, 0 °C to room temperature; X = Br or I.



Scheme 1. Synthesis of five mini-libraries. Reagents and conditions: (a) DMAP, NEt_3, TBDMSCl, CH_2Cl_2, room temperature; (b) KH, R_1X, THF, 0 °C to room temperature; (c) TBAF, THF, rt; (d) KH, R_2X, THF, 0 °C to room temperature.

alkylated with the appropriate alkyl halide using KH as the base in THF. The mono-alkylated diols **9–13** were obtained in quantitative yield upon treating **4–8** with *t*-butylammonium fluoride (TBAF) in THF at room temperature. Equimolar mixtures of racemic mono-alkoxy compounds **9–13** were then alkylated with one alkyl halide, to generate five mini-libraries **library-C1–C5**, each with five racemic members. The reactions were done in THF, with KH as the base. The reaction progress was monitored by gas chromatography (GC), and it proceeded at similar rates for all components, generating the crude dialkylated set of products in high purity (over 90% by GC, total dialkylated material) and no significant bias. However, the more volatile dialkoxy members such as $R^1 = R^2 = CH_3$ evaporated more than other library members, introducing some biases during purification. The identity of the members in each library was confirmed by GC–mass spectrometry (GC–MS) analyses. The increment of one methylene group between members in each library was reflected in very well-resolved peaks in the GC analyses. Members in each library have a common alkyl group, and a variable second alkyl group, **C1–C5**. Each library contains one member with identical alkyl groups (**14–18**). These members were synthesized as individual compounds and fully characterized (^1H, ^{13}C NMR, and MS). These individual members are useful during the biological assays, to obtain information about the molecular mass range and enantiomers that are best for biological activity.

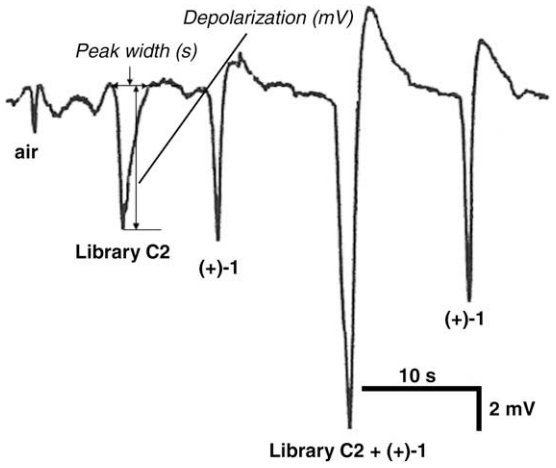


Figure 2. A typical electroantennogram (EAG) experiment trace for odorant **library-C2** (Scheme 1). The order of stimuli is: air, the test compound/set (100 μg on the cartridge), (+)-disparlure, (+)-**1**, (100 ng), the test compound /set (100 μg) + (+)-**1** (100 ng), and (+)-**1** (100 ng).

2.2. Electroantennogram (EAG) bioassay

All diether individual analogs and five mini-libraries were tested in this study. Each test consisted of five puffs: (i) air, (ii) the compound or library, (iii) pure (+)-**1**, (iv) a mixture of (+)-**1** and the compound or library, and (v) pure (+)-**1**. The pure (+)-**1** puffs gave expected depolarizations, in the neighborhood of 10 mV (Fig. 2).

2.2.1. Depolarization data

The libraries by themselves did not significantly depolarize the antenna (compared to air), on average. The exception was the ethyl library (**Lib-C2**), which showed a significant EAG depolarization, compared to the air control (Table 1), suggesting that this mixture is detectable by the moth antenna. Individual compounds **15–17** gave borderline significant depolarizations by themselves ($P = 0.1$) and showed a structure–activity relationship. Compound **15** (diethyl substituted) was a stronger odorant than **16** (dimethyl) or **17** (dipropyl). Compound **18** (dibutyl) was not an odorant. The results with **Lib-C2** and compound **15** suggest that the ethyl group was optimal for odorant activity.

For the short-term effect (Table 1), mixtures of **Lib-2**, **Lib-4**, **Lib-5**, (±)-**17**, (±)-**18** or (−)-**18** with (+)-**1** showed a simple additive effect. However, the mixture of compound (+)-**18** with (+)-**1** depolarized more than one would expect from the addition of the individual depolarizations, suggesting a synergistic effect.

Generally, significant long-term effects were not detected. On average, none of the compounds showed a significant difference (enhancement or suppression of the depolarization) between the pure (+)-**1** stimulus preceding the mixed stimulus and the one following it.

2.2.2. EAG prolongation (peak widening)

Most of the libraries and compounds significantly lengthened the EAG signal, compared to air and (+)-**1** controls (Table 2). Compound (±)-**16** gave the most significant activity by itself and mixed with (+)-**1**. The peak widening was an immediate response to the test compounds; no peak widening was observed in long term tests. The cases such as **Lib-C3**, **Lib-C4**, and **16** presented here are interesting in that they generally gave wider EAG peaks by themselves than when mixed with the pheromone (+)-**1**. This suggests that there may be a competition between the peak-broadening compound and the pheromone when they are mixed. The enantioselectivity for pairs of **16** and **18** was not significant. A SAR pattern was seen for this activity: methyl groups were too small, propyl groups were best and pentyl groups were too large.

Table 1
EAG data for alternating stimuli of (+)-**1** and various test compounds (by measurement of depolarization in mV)

Test compound	Treatment 1[a,b] (air)	Treatment 2 (mimics)[a,b] (CAE)	Treatment 3 [(+)-**1**][a,b]	Treatment 4 (mix)[a,b] (STE)	Treatment 5 [(+)-**1**][a,b] (LTE)
Lib-C1	1.7 ± 0.7 (B)	4.9 ± 1.1 (A/B)	12.7 ± 3.2 (A)	14.2 ± 2.0 (A)	10.7 ± 4.5 (A/B)
Lib-C2	1.5 ± 0.5 (C)	5.7 ± 0.7 (B)	9.5 ± 2.8 (B)	13.8 ± 2.4 (A)	9.6 ± 3.7 (B)
Lib-C3	1.3 ± 0.5 (A)	4.6 ± 0.5 (A)	9.6 ± 4.8 (A)	9.7 ± 1.8 (A)	8.5 ± 4.5 (A)
Lib-C4	1.3 ± 0.5 (C)	3.2 ± 0.3 (B/C)	8.2 ± 2.0 (A/B)	9.8 ± 1.7 (A)	7.1 ± 2.0 (A/B)
Lib-C5	1.5 ± 0.5 (B)	2.5 ± 0.5 (B)	9.6 ± 3.9 (A/B)	13.2 ± 2.6 (A)	6.5 ± 2.1 (A/B)
(±)-**14**	1.3 ± 0.2 (A)	4.4 ± 1.4 (A)	12.4 ± 5.4 (A)	19.3 ± 6.5 (A)	10.2 ± 4.3 (A)
(±)-**15**	1.1 ± 0.3 (B)	9.5 ± 1.7 (A/B)	7.7 ± 3.2 (A/B)	14.7 ± 2.0 (A)	10.8 ± 5.4 (A/B)
(±)-**16**	1.0 ± 0.4 (B)	4.9 ± 0.5 (A/B)	9.6 ± 3.8 (A)	8.3 ± 2.6 (A)	8.4 ± 3.9 (A)
(±)-**17**	1.0 ± 0.3 (B)	4.4 ± 1.3 (A/B)	7.3 ± 3.9 (A/B)	14.5 ± 3.0 (A)	8.7 ± 4.4 (A/B)
(±)-**18**	1.2 ± 0.4 (B)	2.0 ± 0.6 (B)	9.5 ± 3.7 (A/B)	18.7 ± 5.1 (A)	9.6 ± 4.8 (B)
(+)-**16**	2.3 ± 0.1 (C)	6.1 ± 0.9 (B/C)	14.9 ± 3.7 (A)	14.3 ± 2.2 (A)	12.1 ± 3.9 (A/B)
(−)-**16**	2.6 ± 0.1 (B)	5.4 ± 0.4 (A/B)	14.9 ± 5.0 (A)	12.1 ± 1.7 (A/B)	11.3 ± 1.1 (A/B)
(+)-**18**	3.0 ± 0.2 (B)	3.1 ± 0.5 (B)	11.7 ± 3.2 (B)	26.4 ± 6.1 (A)	11.5 ± 2.9 (B)
(−)-**18**	2.8 ± 0.2 (B)	4.4 ± 1.8 (B)	13.6 ± 3.6 (B)	22.9 ± 5.1 (A)	13.0 ± 4.0 (B)

[a] Data shown is the mean ± SE for four replicates.
[b] Values in the same row followed by different capital letters differ significantly (ANOVA, $P < 0.05$).

Table 2
EAG data for alternating stimuli of (+)-**1** and various test compounds (by measurement of peak width in second)

Test compound	Treatment 1[a,b] (air)	Treatment 2 (mimics)[a,b] (CAE)	Treatment 3 [(+)-**1**][a,b]	Treatment 4 (mix)[a,b] (STE)	Treatment 5 [(+)-**1**][a,b] (LTE)
Lib-C1	0.69 ± 0.12 (C)	1.00 ± 0.19B/C	1.25 ± 0.08 (B)	1.75 ± 0.16 (A)	1.33 ± 0 (B)
Lib-C2	0.67 ± 0 (C)	2.42 ± 0.37 (A)	1.50 ± 0.10 (B)	2.00 ± 0.24 (A/B)	1.33 ± 0 (B)
Lib-C3	0.67 ± 0 (C)	2.33 ± 0.19 (A)	1.33 ± 0 (B)	2.17 ± 0.0.17 (A)	1.33 ± 0 (B)
Lib-C4	0.75 ± 0.08 (B)	2.42 ± 0.37 (A)	1.33 ± 0 (B)	2.09 ± 0.21 (A)	1.33 ± 0 (B)
Lib-C5	0.67 ± 0 (C)	2.17 ± 0.42 (A)	1.25 ± 0.08 (B/C)	1.67 ± 0.14 (A/B)	1.25 ± 0.08 (B/C)
(±)-**14**	0.67 ± 0 (C)	1.33 ± 0 (B)	1.33 ± 0 (B)	1.75 ± 0.16 (A)	1.25 ± 0.08 (B)
(±)-**15**	0.59 ± 0.09 (B)	1.92 ± 0.46 (A)	1.33 ± 0 (A/B)	1.75 ± 0.16 (A)	1.33 ± 0 (A/B)
(±)-**16**	0.59 ± 0.09 (D)	2.50 ± 0.22 (A)	1.30 ± 0.03 (C)	2.08 ± 0.08 (B)	1.33 ± 0 (C)
(±)-**17**	0.59 ± 0.09 (B)	1.17 ± 0.17 (A)	1.25 ± 0.08 (A)	1.42 ± 0.09 (A)	1.25 ± 0.08 (A)
(±)-**18**	0.67 ± 0 (B)	1.25 ± 0.48 (B)	1.33 ± 0 (A)	1.59 ± 0. 09 (A)	1.33 ± 0.14 (A)
(+)-**16**	0.67 ± 0 (B)	2.67 ± 0.36 (A)	1.33 ± 0 (B)	2.25 ± 0.25 (A)	1.33 ± 0 (B)
(−)-**16**	0.67 ± 0 (D)	2.42 ± 0.16 (A)	1.25 ± 0.09 (C)	2.00 ± 0 (B)	1.30 ± 0.03 (C)
(+)-**18**	0.75 ± 0.08 (B)	0.67 ± 0 (B)	1.25 ± 0.08 (A)	1.42 ± 0.08 (A)	1.33 ± 0 (A)
(−)-**18**	0.67 ± 0 (B)	0.84 ± 0.17 (B)	1.33 ± 0 (A)	1.42 ± 0.09 (A)	1.33 ± 0 (A)

[a] Data shown is the mean ± SE for four replicates.
[b] Values in the same row followed by different capital letters differ significantly (ANOVA, P <0.05).

The activity order was: Me < Et ⩽ Pr > Bu ≈ Pent. This result is consistent with enantiomers of **16** (dipropyl) being more active than enantiomers of **18** (dipentyl) (Table 2). There was no difference between enantiomers for peak widening.

2.2.3. Percentage changes in depolarization relative to the first (+)-1 signal

To account for individual antennal variation, we have analyzed the percentage changes of the air-corrected signals, relative to the first pure (+)-**1** puff (Table 3). The libraries or compounds by themselves showed that **Lib-C2** (ethyl) gave the strongest odorant effect compared with air; compound **15** (diethyl) was the strongest odorant overall. Longer or shorter substituents gave lower activities compared to puff iii of pure (+)-**1** (=100%). The mixtures of libraries or compounds with (+)-**1** generally showed values >100%, mostly due to additive effects (see above). The enantiomers of **18** both doubled the depolarization of the mixture relative to pure (+)-**1**, but did not give significant activity by themselves, again confirming a synergistic effect of this compound with (+)-**1**. Compound (+)-**16** also doubled the depolarization of the mixture, compared to pure (+)-**1**, but (−)-**16** did not. Interestingly, the activity of (±)-**16** was the average of the two enantiomer activities. This suggests that the enantiomers of **16** do not antagonize or synergize with each other.

Table 3
EAG data for alternating stimuli of (+)-**1** and various test compounds (by measurement of depolarization in percentage)

Test compound	Treatment 2 (mimics)[a,b] (CAE)	Treatment 4 (mix)[a,b] (STE)	Treatment 5 [(+)-**1**][a,b] (LTE)
Lib-C1	48 ± 26 (A)	148 ± 46 (A)	78 ± 20 (A)
Lib-C2	81 ± 21 (A)	192 ± 38 (A)	111 ± 25 (A)
Lib-C3	78 ± 27 (A)	174 ± 58 (A)	92 ± 13 (A)
Lib-C4	37 ± 14 (B)	135 ± 19 (A)	87 ± 15 (A/B)
Lib-C5	37 ± 10 (B)	190 ± 43 (A)	78 ± 25 (B)
(±)-**14**	25 ± 4 (B)	220 ± 49 (A)	65 ± 6.8 (B)
(±)-**15**	173 ± 47 (A)	283 ± 69 (A)	165 ± 13 (A/B)
(±)-**16**	84 ± 38 (A)	155 ± 65 (A)	115 ± 39 (A)
(±)-**17**	54 ± 19 (A)	276 ± 98 (A)	112 ± 6 (A)
(±)-**18**	18 ± 11 (B)	248 ± 58 (A)	98 ± 28 (B)
(+)-**16**	35 ± 5 (B)	250 ± 64 (A)	76 ± 22 (B)
(−)-**16**	37 ± 5 (A)	109 ± 31 (A)	111 ± 43 (A)
(+)-**18**	14 ± 12 (B)	226 ± 26 (A)	79 ± 30 (B)
(−)-**18**	6 ± 10 (B)	220 ± 57 (A)	118 ± 58 (A/B)

[a] Data shown is the mean ± SE for four replicates.
[b] Values in the same row followed by different capital letters differ significantly (ANOVA, P <0.05).

2.3. Assays with gypsy moth PBP1 and PBP2

PBPs are hypothesized to be involved in both odorant signal activation[56] and in odorant scavenging.[35] We have developed a method to examine the local environment around the conserved tryptophan residue (Trp37) on PBPs, by fluorescence quenching.[57] Trp37 is predicted to be on the α2/3 loop of the protein, which has been suggested to assist ligand uptake[58] or peripheral ligand binding.[56] We have used the method to examine the hypothesis that different PBP conformers (with a different environment around Trp37) may be involved in the lengthening (peak widening) of the EAG signals, when particular compounds are present.

For Trp fluorescence quenching, we have employed two types of quenchers: neutral acrylamide and anionic iodide, to assess the overall environment around Trp37. As shown in Eq. 1, where F is the fluorescence intensity in the presence of quencher and F_0 is the intensity in the absence of quencher, the degree of quenching is linearly related to the quenching constant, the Stern–Volmer constant (K_{SV}) (Eq. 1).

$$\frac{F_0}{F} = 1 + K_{sv}\,[\mathrm{Q}] \tag{1}$$

F and F_0 refer to the fluorescence intensities of the quenched and original (non-quenched samples), respectively, and [Q] is the concentration of the quenchers.

In a protein, acrylamide and iodide may have different accessibility to the tryptophan residue due to steric and/or electrostatic effects, and therefore they quench tryptophan fluorescence differently. Therefore, the ratio of the Stern–Volmer constants of the two quenchers, K_{SVac}/K_{SVI^-}, should reflect the overall environment around the quenched residue. Previous tests have shown that different ligands induce different local conformational changes manifested in different ratios of the Stern–Volmer constants.[57]

Racemic compounds **14–18** were tested and compared to (+)-**1** (see Supplementary data S2). The strongest peak-widening compounds had the same K_{SV} ratios as (+)-**1** for PBP2, within limits of error and consistent with another study (Gong, Gries and Plettner, unpubl.). This pattern was not seen for PBP1. By stabilizing a conformation similar to the complex of PBP2 and (+)-**1**, compounds that broaden the EAG peak could either prolong stimulation of the olfactory response or inhibit the recovery of the signaling cascade after the end of the stimulus.

2.4. Discussion

Previous studies have focused on analogs that stimulate the antenna on their own and/or elicit a behavior. In particular, studies

with the turnip moth (*Agrotis segetum*) and the European corn borer (*Ostrinia nubilalis*) have revealed that moth pheromones with one *Z* double bond adopt a bent *cisoid* conformation, with the three odotopes (the structural recognition elements at the two ends and in the middle of the chain) are arranged at a specific distance[48,51,53,59] and configuration.[46,48,50,60,61] For *A. segetum*, this active conformation was found to be one of the energy minima.[53,59] Many homologs and analogs with conformational restrictions followed a clear pattern: the greater the energy cost for the analog to adopt the bioactive conformation, the lower the activity in single-cell recordings.[48,60,62] A similar picture emerges for gypsy moth, if the data from a previous study[7] are subjected to a similar evaluation (see Supplementary data S1). According to this analysis, the gypsy moth pheromone also adopts a *cisoid* conformation, with recognition of three odotopes: the relative lengths of the two chains from the epoxy moiety, the position of the methyl branch relative to the epoxy group and the epoxy group itself (Fig. 3). The activity of many analogs and homologs of **1** correlates well with the energy cost required to adopt a conformation that fits the recognition model (Fig. 3 C). The greater the geometrical deviation of the constrained analog from the average *cisoid* conformation of **1**, the lower the activity observed.

In a study with a lactone pheromone from the sugarcane borer (*Eldana saccharina*), a fluorinated pheromone analog that adopted a similar distribution of conformers to the natural pheromone was active, while analogs that adopted a different distribution of conformers were inactive.[63] These results imply that the olfactory system (in particular the selective components: OBP and OR) is more likely to be activated by the most common set of conformers than by minor, high energy conformers. The total recognition space (Fig. 3A) is most likely defined by the combined selectivities of the OR and the PBPs).

The steric and electronic properties of odotopes in a pheromone are also important in recognition. For example, the replacement of the methylene group in molecules with an oxygen atom could alter the biological activity positively or negatively.[49,51,64] Mechanistically, if there is a functional group present, it could contribute to binding/recognition interactions through dipoles and hydrogen bonding.[22,33,35,59] Our design was based on the *cisoid* conformation of disparlure (Fig. 3). In the present work, we have chosen 5-(2′-hydroxyethyl)cyclopent-2-en-ol (**2**) as a core framework to mimic odotopes of disparlure. The cyclopentene ring with two *cis*-substituents at position-1 and 5 is the key structural character for the required restricted *cisoid* conformation. A conformational analysis of the compounds with $R_1 = R_2$ has revealed that compound **15**, which gave the strongest odorant response, also fits best into the active space of disparlure (Fig. 4).

The incorporation of ether linkages in the mimics not only simplified the preparation but also had a positive effect on the biological properties. In previous studies, ether analogs of pheromones were more water soluble than the pheromone itself, elicited EAG responses[59,64] and bound to a PBP.[64] In this study we also found that the mimics were more water soluble than (+)-**1**, bound to the PBPs and showed structure–activity relationships with respect to four different biological activities tested here.

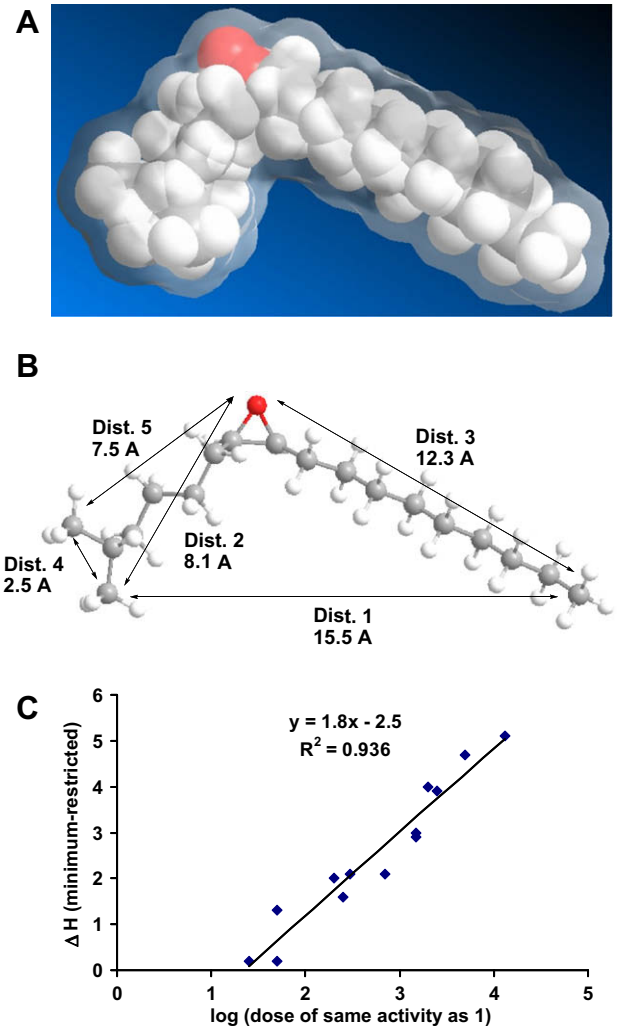


Figure 3. Conformational modeling of (+)-disparlure (+)-**1**. (A) Space delineated by two superimposed *cisoid* conformers of (+)-**1**. (B) Distances between odotopes in the *cisoid* minima. (C) Analysis of disparlure homologs and positional isomers with respect to their electroantennogram responses relative to the response to racemic **1**. The activity data is from Ref. 7 and was collected on individual sensory hairs that responded to 1. The log of the dose of a homolog or isomer required to elicit the same response as racemic 1 is plotted on the *X* axis. The compounds were minimized, and then constrained into the dimensions shown in B. The energy difference (kcal/mol) between the minimum and the restricted conformer is plotted along the *Y* axis.

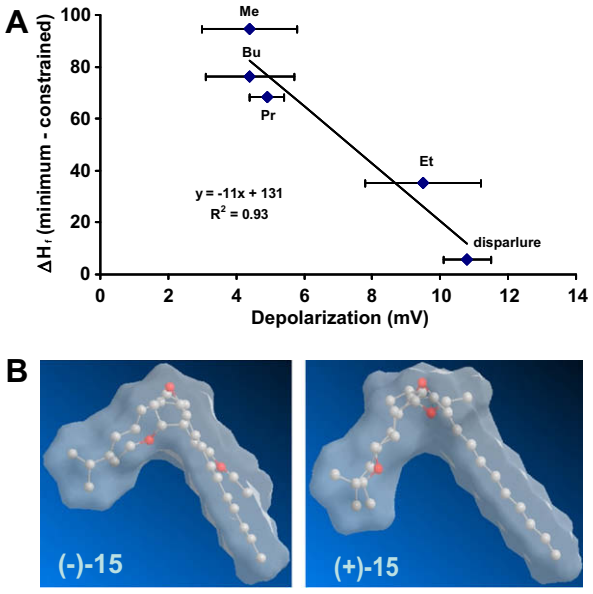


Figure 4. (A) Conformational modeling of compounds (±)-**14**, (±)-**15**, (±)-**16**, and (±)-**17**. As for Fig. 3, the compounds were minimized, then constrained such that the cyclopentene ring overlaps with C6–C9 of (+)-**1** in the *cisoid* conformation. Each enantiomer was modeled individually, and the average energy penalty was taken as an estimate for the racemate. Compound (±)-**18** was also modeled, but its substituent chains are too large to fit into the branched-chain pocket. (B) Superpositions of (+)-**1** (*cisoid* conformation) and the enantiomers of compound **15**.

Chiral recognition by insect antennae has been well observed in a number of studies.[20,21,49] The enantiomerically pure diols are potential chiral synthons for the synthesis of a wide variety of biologically interesting molecules. In previous work,[55] we have developed an efficient protocol for the synthesis of both enantiomers. The enantiomerically pure analogues were used to probe the enantioselectivity of the pheromone olfactory system toward the *cisoid* mimics of disparlure. Of the four activities tested, only one (the short-term blend effect) showed enantioselectivity for the compound with the optimal chain length: (+)-**16** enhanced the response of the antenna to a mixed plume with (+)-**1**, but (−)-**16** did not.

All the compounds elicited wider EAG peaks than (+)-**1**, by themselves or when mixed with (+)-**1**. To determine whether the pheromone-binding proteins (PBPs) play a role in peak widening, a fluorescent assay, that is, sensitive to the protein conformation was done with PBP1 and PBP2. The strongest peak-widening compounds elicited the same conformational parameter (K_{SV} ratios) for PBP2 as (+)-**1**, within limits of error. This pattern was not observed for PBP1, which binds (+)-**1** less well than PBP2.[34] The PBPs have been implicated in both activation and termination of the signaling cascade, most likely via different ligand binding states or conformers.[56,57] The most parsimonious interpretation of our results is that compounds that cause peak widening stabilize a conformer that is activating (i.e., mimics a conformer with (+)-**1**) and thereby destabilize or deplete a signal termination mode of the PBP.

3. Conclusions

A series of conformationally constrained mimics of **1** was designed and synthesized. The electroantennogram results showed that the mimics elicited weak or no antennal responses themselves. The compound with the medium sized ethyl chain showed odorant activity; compounds with smaller or larger groups did not. Further, when presented simultaneously with the pheromone of the gypsy moth (+)-**1**, some of the tested compounds significantly enhanced the antennal responses. Compounds with longer chains such as butyl or pentyl gave stronger activity. Long-term effects of mixed compound/pheromone stimuli on subsequent pure pheromone stimuli were subtle, for two compounds (**14** and **15**, Table 3). The peak-widening activity was observed most strongly for compounds **15** and **16**, and was correlated with the stabilization of a particular 'active' conformer of PBP2.

4. Experimental

4.1. Chemistry: general

Commercial grade solvents were distilled under nitrogen prior to use and reagents were used without further purification with the following exceptions: triethylamine was distilled and stored over NaOH. CH_2Cl_2 was distilled over $CaCl_2$ and stored over molecular sieves 3 Å. Dried THF was obtained from a MBRAUN LTS 350 solvent purification system. GC was run on a Hewlett Packard 5890 using a SPB column (Supelco, 30 m, 0.25 mm i.d., 0.25 μm film), programmed 100 °C (5 min), 10 °C/min, 200 °C (4.0 min), 15 °C/min, 250 °C (14.0 min). The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker 400, 500 and 600 MHz spectrometers. The reference for NMR chemical shifts was the residue peaks of solvents. Data are given as chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz). HRMS and ESI-MS were recorded on a 6210 Series Time-of-Flight LC/MS System. Infrared spectra (IR) were recorded on a Perkin Elmer 599B IR spectrophotometer using NaCl plates. Flash column chromatography was performed with Kieselgel 60 (230–400 mesh ASTM).

4.2. (±)-*cis*-5-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]cyclopent-2-enol 3

A solution of diol **2** (261 mg, 2.3 mmol), triethylamine (0.35 mL, 2.51 mmol), DMAP (11.2 mg, 0.09 mmol), and *tert*-butyldimethylsilyl chloride (381 mg, 2.53 mmol) in dry CH_2Cl_2 was stirred at 0 °C for 12 h. The reaction mixture was diluted with dichloromethane and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (ethyl acetate/hexane 3:97) to afford pure alcohol **3** as colorless oil (450 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87–5.90 (m, 1H), 5.79–5.81 (m, 1H), 4.56–4.58 (m, 1H), 3.72–3.76 (td, J = 4.2, 10.0 Hz, 1H), 3.54–3.59 (dt, J = 10.0, 3.0 Hz, 1H), 3.07 (d, J = 3.2, –OH), 2.06–2.1 (m, 1H), 2.01–2.06 (m, 1H), 1.82–1.89 (dtd, J = 3.9, 10.0, 14.1 Hz, 1H), 1.56–1.62 (ddd, J = 4.3, 7.4, 14.6 Hz, 1H), 0.82 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.8, 132.7, 75.9, 63.5, 42.2, 37.6, 31.6, 25.8.18.2, −5.5, −5.6; MS *m/z* (relative intensity): 241 (M^+−H, 2%), 111 (10%), 93 (100%); Anal. Calcd for $C_{13}H_{26}O_2Si$: C, 64.41; H, 10.81. Found: C, 64.20; H, 11.13; HRMS calcd for $C_{13}H_{26}NaO_2Si$ (M+Na): 265.1594; found: 265.1602; IR (neat): 3397, 3062, 2929, 2857, 1471, 1090, 835, 775, 719 cm^{-1}.

4.3. General procedure for preparation of compounds 4–8

To a solution of compound **2** (1 mmol) in 5 mL of dry THF was added dropwise to a suspended solution of KH (1.1 mmol) in 20 mL of dry THF at 0 °C. The mixture was stirred at 0 °C for 30 min. The alkylating reagents (alkyl halides) (2.2 mmol) were added dropwise at 0 °C. After completion of addition, the reaction mixture was warmed to room temperature and kept stirring for another 12 h. The reaction was quenched with water–saturated diethyl ether, which was washed with saturated NH_4Cl and brine solution. The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 6:1) to give the title compound.

4.3.1. (±)-*cis*-*tert*-Butyl-dimethyl-[2-(2-methoxy-cyclopent-3-enyl)-ethoxy]silane 4

Compound **3** (484 mg, 2 mmol) was treated with KH (294 mg, 1.1 mmol) and iodomethane (624 mg, 4.4 mmol), according to the general method described in Section 4.3, to give pure product **4** (colorless oil, 474 mg, 93%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.06 (m, 1H), 6.00 (m, 1H), 4.14 (dt, J = 6.6, 1.8 Hz, 1H), 3.69 (m, 2H), 3.32 (s, 3H), 2.37 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.30 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 2.13 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 1.88 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 1.61 (m, 1H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 136.35, 130.42, 85.24, 62.39, 56.63, 38.62, 37.27, 31.99, 25.97, 18.34, −5.29; HRMS calcd for $C_{14}H_{29}O_2Si$ (M+H): 257.1931; found 257.1930; $C_{14}H_{28}$ (M+Na): 279.1751; found: 279.1747; IR (neat): 3058, 2955, 2855, 1461, 1090, 835, 774 cm^{-1}.

4.3.2. (±)-*cis*-*tert*-Butyl-dimethyl-[2-(2-methoxy-cyclopent-3-enyl)-ethoxy]silane 5

Compound **3** (484 mg, 2 mmol) was treated with KH (294 mg, 1.1 mmol) and iodoethane (686 mg, 4.4 mmol), according to the general method described in Section 4.3, to give pure product **5** (colorless oil, 495 mg, 92%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.94 (m, 1H), 4.25 (dt, J = 7.2, 1.8 Hz, 1H), 3.68 (m, 2H), 3.53 (m, 1H), 3.46 (m, 1H), 2.35 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.29 (ddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 2.14 (m, 1H), 1.88 (ddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.60 (m, 1H), 1.17 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.42, 131.14, 83.89, 64.71, 62.45, 38.44, 37.26, 32.13, 25.98, 18.34, 15.64, −5.29; HRMS calcd for $C_{15}H_{31}O_2Si$ (M+H): 271.2088; found

271.2085; $C_{15}H_{30}NaO_2Si$ (M+Na): 293.1907; found: 293.1904. IR (neat): 3057, 2955, 2857, 1463, 1090, 835, 774 cm^{-1}.

4.3.3. (±)-*cis*-*tert*-Butyl-dimethyl-[2-(2-propyloxy-cyclopent-3-enyl)-ethoxy]silane 6

Compound **3** (726 mg, 3 mmol) was treated with KH (882 mg, 1.1 mmol) and bromopropane (738 mg, 6 mmol), according to the general method described in Section 4.3, to give pure product **6** (colorless oil, 864 mg, 101%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.23 (dt, J = 7.2, 1.8 Hz, 1H), 3.69 (m, 2H), 3.44 (m, 1H), 3.32 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.30 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 2.14 (m, 1H), 1.89 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.53–1.63 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.47, 131.34, 83.99, 71.20, 62.47, 38.52, 37.29, 32.14, 25.98, 23.38, 18.35, 10.68, −5.29; HRMS calcd for $C_{16}H_{32}NaO_2Si$ (M+Na): 307.2064; found: 307.2063; IR (neat): 3060, 2955, 2857, 1460, 1090, 835, 770 cm^{-1}.

4.3.4. (±)-*cis*-*tert*-Butyl-dimethyl-[2-(2-butyloxy-cyclopent-3-enyl)-ethoxy]silane 7

Compound **3** (726 mg, 3 mmol) was treated with KH (882 mg, 1.1 mmol) and bromobutane (822 mg, 6 mmol), according to the general method described in Section 4.3, to give pure product **7** (colorless oil, 890 mg, 99%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.22 (dt, J = 7.2, 1.8 Hz, 1H), 3.68 (m, 2H), 3.47 (m, 1H), 3.38 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.30 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.14 (m, 1H), 1.88 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.46–1.62 (m, 3H), 1.31–1.41 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.46, 131.12, 83.97, 69.22, 62.46, 38.53, 37.29, 32.31, 32.15, 25.99, 19.41, 18.35, 13.95, −5.26; HRMS calcd for $C_{17}H_{34}NaO_2Si$ (M+Na): 321.2220; found: 321.2217; IR (neat): 3059, 2955, 2857, 1460, 1093, 837, 774 cm^{-1}.

4.3.5. (±)-*cis*-*tert*-Butyl-dimethyl-[2-(2-pentyloxy-cyclopent-3-enyl)-ethoxy]silane 8

Compound **3** (726 mg, 3 mmol) was treated with KH (882 mg, 1.1 mmol) and iodopentane (1200 mg, 6 mmol), according to the general method described in Section 4.3, to give pure product **8** (colorless oil, 930 mg, 99%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.22 (dt, J = 7.2, 1.8 Hz, 1H), 3.69 (m, 2H), 3.47 (m, 1H), 3.38 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.30 (dddd, J = 14.2, 7.2, 7.2, 7.2 Hz, 1H), 2.14 (m, 1H), 1.89 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 1.83 (m, 1H), 1.46–1.63 (m, 4H), 1.31–1.41 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.46, 131.13, 83.99, 69.56, 62.47, 38.52, 37.28, 32.16, 29.89, 28.04, 25.99, 22.55, 18.35, 14.05, −5.26; HRMS calcd for $C_{18}H_{36}NaO_2Si$ (M+Na): 335.2377; found: 335.2371; IR (neat): 3057, 2955, 2857, 1460, 1090, 835, 774 cm^{-1}.

4.4. General procedure for preparation of compounds 9–13

To a solution of compounds **9–13** (1 mmol) in 15 mL of THF was added *tert*-butyl ammonium fluoride (TBAF) (2 mmol) at rt. After 20 h, the reaction mixture was diluted with diethyl ether, which was then washed with saturated NH_4Cl and brine solutions. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 6:1) to the title compound.

4.4.1. (±)-*cis*-2-(2-Methoxy-cyclopent-3-enyl)-ethanol 9

Compound **4** (430 mg, 1.7 mmol) was treated with TBAF (3.4 mL, 3.4 mmol), according to the general method described in Section 4.9, to give pure product **9** (colorless oil, 200 mg, 85%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.07 (m, 1H), 6.00 (m, 1H), 4.22 (dt, J = 6.6, 1.8 Hz, 1H), 3.73 (m, J = 10.8, 6 Hz, 1H), 3.65 (m, J = 7.8, 5.4 Hz, 1H), 3.31 (s, 3H), 2.39 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.31 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.15 (m, 1H), 1.90 (m, 1H), 1.70 (m, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 136.64, 129.88, 84.84, 62.16, 56.09, 40.05, 37.48, 31.83; HRMS calcd for $C_8H_{15}O_2$ (M+H): 143.1067 found: 143.1062; $C_8H_{14}NaO_2$ (M+Na): 165.0886; found: 165.0885; IR (neat): 3372, 3057, 2954, 2871, 1461, 1073, 724 cm^{-1}.

4.4.2. (±)-*cis*-2-(2-Ethoxy-cyclopent-3-enyl)-ethanol 10

Compound **5** (450 mg, 1.7 mmol) was treated with TBAF (3.7 mL, 3.7 mmol), according to the general method described in Section 4.9, to give pure product **10** (colorless oil, 200 mg, 69%). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.04 (m, 1H), 5.97 (m, 1H), 4.35 (dt, J = 6.6, 1.8 Hz, 1H), 3.74 (m, J = 10.8, 6 Hz, 1H), 3.66 (m, J = 7.8, 5.4 Hz, 1H), 3.57 (m, 1H), 3.46 (m, 1H), 2.41 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.35 (m, 1H), 2.18 (m, 1H), 1.93 (m, 1H), 1.73 (m, 1H), 1.19 (t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.88, 130.49, 83.49, 64.15, 62.17, 40.15, 37.48, 31.97, 15.40; HRMS calcd for $C_9H_{16}NaO_2$ (M+Na): 179.1043; found: 179.1043; IR (neat): 3372, 3057, 2954, 2871, 1461, 1073, 724 cm^{-1}.

4.4.3. (±)-*cis*-2-(2-Propyloxy-cyclopent-3-enyl)-ethanol 11

Compound **6** (860 mg, 3.0 mmol) was treated with TBAF (6 mL, 6 mmol), according to the general method described in Section 4.9, to give pure product **11** (colorless oil, 394 mg, 77%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.03 (m, 1H), 5.96 (m, 1H), 4.32 (dt, J = 7.2, 1.8 Hz, 1H), 3.73 (m, 1H), 3.65 (m, 1H), 3.45 (m, 1H), 3.33 (m, 1H), 2.39 (m, 1H), 2.33 (m, 1H), 2.16 (m, 1H), 1.93 (m, 1H), 1.72 (m, 1H), 1.56 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.88, 130.52, 83.54, 70.54, 62.18, 40.19, 37.49, 31.96, 23.19, 10.59; HRMS calcd for $C_{10}H_{18}NaO_2$ (M+Na): 193.1199; found: 193.1200; IR (neat): 3372, 3057, 2954, 2871, 1461, 1073, 724 cm^{-1}.

4.4.4. (±)-*cis*-2-(2-butyloxy-cyclopent-3-enyl)-ethanol 12

Compound **7** (890 mg, 2.99 mmol) was treated with TBAF (6 mL, 6 mmol), according to the general method described in Section 4.9, to give pure product **12** (colorless oil, 500 mg, 91%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.04 (m, 1H), 5.97 (m, 1H), 4.23 (br d, J = 7.2 Hz, 1H), 3.75 (m, 1H), 3.66 (m, 1H), 3.50 (m, 1H), 3.38 (m, 1H), 2.40 (m, 1H), 2.35 (ddd, J = 20.4, 20.4, 7.2 Hz, 1H), 2.17 (m, 1H), 1.94 (m, 1H), 1.73 (m, 1H), 1.56 (m, 2H), 1.36 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.95, 130.51, 83.53, 68.60, 62.24, 40.29, 37.52, 32.09, 31.99, 19.34, 13.88; HRMS calcd for $C_{11}H_{21}O_2$ (M+H): 181.1536; found: 181.1535; $C_{11}H_{20}NaO_2$ (M+Na) 207.1356; found: 207.1357; IR (neat): 3372, 3057, 2954, 2871, 1461, 1073, 724 cm^{-1}.

4.4.5. (±)-*cis*-2-(2-butyloxy-cyclopent-3-enyl)-ethanol 13

Compound **8** (936 mg, 3 mmol) was treated with TBAF (6 mL, 6 mmol), according to the general method described in **4.9**, to give pure product **13** (colorless oil, 379 mg, 64%). ^1H NMR ($CDCl_3$, 600 MHz) δ 6.03 (m, 1H), 5.96 (m, 1H), 4.32 (d, J = 6.0 Hz, 1H), 3.73 (m, J = 10.8, 5.4 Hz, 1H), 3.65 (m, 1H), 3.48 (m, 1H), 3.37 (m, 1H), 2.37 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.34 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.17 (m, 1H), 1.93 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.71 (m, 1H), 1.55 (m, 2H), 1.30 (m, 4H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.89, 130.52, 83.54, 68.94, 62.20, 40.24, 37.50, 31.98, 29.67, 28.28, 22.46, 13.98; HRMS calcd for $C_{12}H_{23}O_2$ (M+H): 199.1693; found: 199.1693; $C_{12}H_{23}NaO_2$ (M+Na) 221.1512; found: 221.1513; IR (neat): 3372, 3057, 2954, 2871, 1461, 1073, 724 cm^{-1}.

4.5. General procedure for mini-library synthesis

To a solution of compounds **9–13** (1 mmol in 1:1:1:1:1 molar ratio) in 5 mL of dry THF was added dropwise to a suspended solution of KH (2 mmol) in 20 mL of dry THF at 0 °C. The mixture was stirred at 0 °C for 30 min. The alkylating reagents (alkyl halides) (2 mmol) were added dropwise at 0 °C. After completion of the addition, the reaction mixture was warmed to room temperature and kept stirring for another 12 h. The reaction was quenched with water–saturated diethyl ether, which was washed with saturated NH_4Cl and brine solution. The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by a silica gel plug (washed with hexanes/EtOAc 6:1) to give the desired library.

4.5.1. Library-C1

A mixture of **9** (40 mg, 0.2817 mmol), **10** (43.94 mg, 0.2817 mmol), **11** (47.89 mg, 0.2817 mmol), **12** (51.83 mg, 0.2817 mmol), and **13** (55.21 mg, 0.2817 mmol) was treated with KH (337 mg, 2.817 mmol) and iodomethane (400 mg, 2.817 mmol), according to the general method described in Section 4.15, to give **library-C1** (colorless oil, 260 mg, 100%). ESI-MS *m/z* (M+Na): 179.1050, 193.1209, 207.1365, 221.1518, and 235.1675.

4.5.2. Library-C2

A mixture of **9** (40 mg, 0.2817 mmol), **10** (43.94 mg, 0.2817 mmol), **11** (47.89 mg, 0.2817 mmol), **12** (51.83 mg, 0.2817 mmol), and **13** (55.21 mg, 0.2817 mmol) was treated with KH (337 mg, 2.817 mmol) and iodoethane (439 mg, 2.817 mmol), according to the general method described in Section 4.15, to give **library-C2** (colorless oil, 280 mg, 100%). ESI-MS *m/z* (M+Na): 193.1206, 207.1363, 221.1516, 235.1674, and 249.1831.

4.5.3. Library-C3

A mixture of **9** (40 mg, 0.2817 mmol), **10** (43.94 mg, 0.2817 mmol), **11** (47.89 mg, 0.2817 mmol), **12** (51.83 mg, 0.2817 mmol), and **13** (55.21 mg, 0.2817 mmol) was treated with KH (337 mg, 2.817 mmol) and brompropane (347 mg, 2.817 mmol), according to the general method described in Section 4.15, to give **library-C3** (colorless oil, 300 mg, 100%). ESI-MS *m/z* (M+Na): 207.1359, 221.1513, 235.1670, 249.1828, and 263.1986.

4.5.4. Library-C4

A mixture of **9** (40 mg, 0.2817 mmol), **10** (43.94 mg, 0.2817 mmol), **11** (47.89 mg, 0.2817 mmol), **12** (51.83 mg, 0.2817 mmol), and **13** (55.21 mg, 0.2817 mmol) was treated with KH (337 mg, 2.817 mmol) and bromobutane (386 mg, 2.817 mmol), according to the general method described above, to give **library-C4** (colorless oil, 316 mg, 99%). ESI-MS *m/z* (M+Na): 221.1515, 235.1671, 249.1832, 263.1988, and 277.2143.

4.5.5. Library-C5

A mixture of **9** (40 mg, 0.2817 mmol), **10** (43.94 mg, 0.2817 mmol), **11** (47.89 mg, 0.2817 mmol), **12** (51.83 mg, 0.2817 mmol), and **13** (55.21 mg, 0.2817 mmol) was treated with KH (337 mg, 2.817 mmol) and iodopentane (558 mg, 2.817 mmol), according to the general method described above, to give **library-C5** (colorless oil, 340 mg, 100%). ESI-MS *m/z* (M+Na): 235.1679, 249.1863, 263.1991, 277.2146, and 291.2305.

4.6. General procedure for preparation of compounds (±)-14–18, (+)-14–15, (−)-14–18

To a solution of diol **2**/(+)-**2**/(−)-**2** (1 mmol) in 5 mL of dry THF was added dropwise to a suspended solution of KH (2.2 mmol) in 20 mL of dry THF at 0 °C. The mixture was stirred at 0 °C for 30 min. The alkylating reagents (alkyl halides) (4 mmol) were added dropwise at 0 °C. After completion of the addition, the reaction mixture was warmed to room temperature and kept stirring for another 12 h. The reaction was quenched with water–saturated diethyl ether, which was washed with saturated NH_4Cl and brine solution. The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 6:1) to give the title compound.

4.6.1. Methoxy-(±)-*cis*-5-(2-methoxy-ethyl) cyclopent-2-enol 14

(±) Diol **2** (256 mg, 2 mmol) was treated with KH (588 mg, 4.4 mmol) and iodomethane (1136 mg, 8 mmol), according to the general method described in Section 4.21, to give pure product **14** (colorless oil, 281 mg, 91%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.05 (m, 1H), 6.00 (m, 1H), 4.14 (dt, J = 6.6, 2.0 Hz, 1H), 3.45 (m, 2H), 3.34 (s, 3H), 3.30 (s, 3H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.12 (m, 1H), 1.91 (dddd, J = 13.6, 6.8, 6.8, 6.8 Hz, 1H), 1.65 (m, 1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 136.25, 130.45, 85.12, 72.02, 58.53, 56.69, 38.95, 37.19, 28.7; HRMS calcd for $C_9H_{16}NaO_2$ (M+Na): 179.1043; found: 179.1040. IR (neat): 3052, 2955, 2857, 1460, 1094, 713 cm^{-1}.

4.6.2. Ethoxy-(±)-*cis*-5-(2-ethoxyethyl)cyclopent-2-enol 15

(±) Diol **2** (256 mg, 2 mmol) was treated with KH (588 mg, 4.4 mmol) and iodoethane (1248 mg, 8 mmol), according to the general method described in Section 4.21, to give pure product **15** (colorless oil, 378 mg, 103%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.93 (m, 1H), 4.23 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 6H), 2.35 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 13.2, 6.6, 6.6, 6.6 1H), 2.13 (m, 1H), 1.92 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 1.65 (dddd, J =13, 6.5, 6.5, 6.5 Hz, 1H), 1.19 (t, J = 7.2 Hz, 3H), 1.16 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.39, 131.11, 83.76, 69.85, 66.01, 64.77, 38.80, 37.16, 29.01, 15.61, 15.24; HRMS calcd for $C_{11}H_{20}NaO_2$ (M+Na): 207.1356; found: 207.1361; IR (neat): 3052, 2955, 2857, 1460, 1094, 713 cm^{-1}.

4.6.3. Propyloxy-(±)-*cis*-5-(2-propyloxyethyl)cyclopent-2-enol 16

(±) Diol **2** (256 mg, 2 mmol) was treated with KH (588 mg, 4.4 mmol) and bromopropane (984 mg, 8 mmol), according to the general method described in Section 4.21, to give pure product **16** (colorless oil, 388 mg, 92%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.94 (m, 1H), 4.21 (td, J=6.7, 2.0 Hz, 1H), 3.48 (m, 2H), 3.43 (m, 1H), 3.37 (t, J = 6.7 Hz, 2H), 3.33 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.29 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.14 (m, 1H), 1.94 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.52–1.70 (m, 5H), 0.92 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 135.55, 131.11, 83.86, 72.57, 71.22, 70.04, 38.94, 37.23, 29.02, 23.37, 22.95, 10.67, 10.61; HRMS calcd for $C_{13}H_{24}NaO_2$ (M+Na) 235.1669; found: 235.1674; IR (neat): 3052, 2955, 2857, 1460, 1094, 713 cm^{-1}.

4.6.4. Butyloxy-(±)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol 17

(±) Diol **2** (256 mg, 2 mmol) was treated with KH (588 mg, 4.4 mmol) and bromobutane (1096 mg, 8 mmol), according to the general method described in Section 4.21, to give pure product **17** (colorless oil, 378 mg, 103%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.20 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.49, 131.11, 83.85, 70.64, 70.08, 69.24, 38.95, 37.24, 32.30, 31.88, 29.05, 19.38, 13.92, 13.91; HRMS calcd for $C_{15}H_{28}NaO_2$ (M+Na): 263.1982; found: 263.1985; IR (neat): 3052, 2955, 2857, 1460, 1094, 713 cm^{-1}.

4.6.5. Pentyloxy-(±)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol 18

(±) Diol **2** (128 mg, 1 mmol) was treated with KH (267 mg, 4.4 mmol) and iodopentane (792 mg, 8 mmol), according to the general method described in Section 4.21, to give pure product **18** (colorless oil, 262 mg, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.21 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 8H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.48, 131.11, 83.87, 70.96, 70.08, 69.58, 38.94, 37.24, 29.88, 29.48, 29.02, 28.38, 22.54, 14.03; ^{1}HRMS calcd for $C_{17}H_{32}NaO_2$: 291.2295; found: 291.2299; IR (neat): 3052, 2955, 2857, 1460, 1094, 713 cm^{-1}.

4.6.6. Methoxy-(+)-*cis*-5-(2-methoxy-ethyl)cyclopent-2-enol (+)-14

(+) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and iodomethane (89 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (+)-**14** (colorless oil, 19 mg, 79%). $[\alpha]_D^{20} = +84.8$ (0.003, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.05 (m, 1H), 5.99 (m, 1H), 4.13 (dt, J = 6.6, 2.0 Hz, 1H), 3.43 (m, 2H), 3.34 (s, 3H), 3.30 (s, 3H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 15.6, 7.8, 7.8, 7.8 MHz, 1H), 2.12 (m, 1H), 1.91 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.64 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H).

4.6.7. Methoxy-(−)-*cis*-5-(2-methoxy-ethyl)cyclopent-2-enol (−)-14

(−) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and iodomethane (89 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (−)-**14** (colorless oil, 18 mg, 79%). $[\alpha]_D^{20} = -80.5$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.05 (m, 1H), 5.99 (m, 1H), 4.13 (dt, J = 6.6, 2.0 Hz, 1H), 3.43 (m, 2H), 3.34 (s, 3H), 3.30 (s, 3H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 15.6, 7.8, 7.8, 7.8 MHz, 1H), 2.12 (m, 1H), 1.91 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.64 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H).

4.6.8. Ethoxy-(+)-*cis*-5-(2-ethoxyethyl)cyclopent-2-enol (+)-15

(+) Diol **2** (20 mg, 0.156 mmol) was treated with KH (42 mg, 0.313 mmol) and iodoethane (97 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (+)-**15** (colorless oil, 18 mg, 63%). $[\alpha]_D^{20} = +80.7$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.93 (m, 1H), 4.23 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 6H), 2.35 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 13.2, 6.6, 6.6, 6.6 1H), 2.13 (m, 1H), 1.92 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 1.65 (dddd, J = 13, 6.5, 6.5, 6.5 Hz, 1H), 1.19 (t, J = 7.2 Hz, 3H), 1.16 (t, J = 7.2 Hz, 3H).

4.6.9. Ethoxy-(−)-*cis*-5-(2-ethoxyethyl)cyclopent-2-enol (−)-15

(−) Diol **2** (20 mg, 0.156 mmol) was treated with KH (42 mg, 0.313 mmol) and iodoethane (97 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (−)-**15** (colorless oil, 17 mg, 63%).%). $[\alpha]_D^{20} = -78.9$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.93 (m, 1H), 4.23 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 6H), 2.35 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 2.13 (m, 1H), 1.92 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 1.65 (dddd, J = 13, 6.5, 6.5, 6.5 Hz, 1H), 1.19 (t, J = 7.2 Hz, 3H), 1.16 (t, J = 7.2 Hz, 3H).

4.6.10. Propyloxy-(+)-*cis*-5-(2-propyloxyethyl)cyclopent-2-enol (+)-16

(+) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and bromopropane (77 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (+)-**16** (colorless oil, 25 mg, 77%). $[\alpha]_D^{20} = +85.8$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.94 (m, 1H), 4.21 (td, J = 6.7, 2.0 Hz, 1H), 3.48 (m, 2H), 3.43 (m, 1H), 3.37 (t, J = 6.7 Hz, 2H), 3.33 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.29 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.14 (m, 1H), 1.94 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.52–1.70 (m, 5H), 0.92 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H).

4.6.11. Propyloxy-(−)-*cis*-5-(2-propyloxyethyl)cyclopent-2-enol (−)-16

(−) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and bromopropane (77 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (−)-**16** (colorless oil, 30 mg, 88%). $[\alpha]_D^{20} = -81.8$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.00 (m, 1H), 5.94 (m, 1H), 4.21 (td, J = 6.7, 2.0 Hz, 1H), 3.48 (m, 2H), 3.43 (m, 1H), 3.37 (t, J = 6.7 Hz, 2H), 3.33 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.29 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.14 (m, 1H), 1.94 (dddd, J = 13.2, 6.6, 6.6, 6.6 Hz, 1H), 1.52–1.70 (m, 5H), 0.92 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H).

4.6.12. Butyloxy-(+)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol (+)-17

(+) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and bromobutane (86 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (+)-**17** (colorless oil, 30 mg, 81%). $[\alpha]_D^{20} = +83.8$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.20 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H).

4.6.13. Butyloxy-(−)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol (−)-17

(−) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and bromobutane (86 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (−)-**17** (colorless oil, 32 mg, 86%). $[\alpha]_D^{20} = -79.6$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.20 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H).

4.6.14. Pentyloxy-(+)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol (+)-18

(+) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and iodopentane (124 mg, 0.624 mmol), according to the general method described in Section 4.21, to give pure product (+)-**18** (colorless oil, 20 mg, 48%). $[\alpha]_D^{20} = +83.7$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.21 (dt, J = 6.7, 1.9 Hz, 1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 8H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H).

4.6.15. Pentyloxy-(−)-*cis*-5-(2-butyloxyethyl)cyclopent-2-enol (−)-18

(−) Diol **2** (20 mg, 0.156 mmol) was treated with KH (63 mg, 0.468 mmol) and iodopentane (124 mg, 0.624 mmol), according to the general method described above, to give pure product (−)-**18** (colorless oil, 28 mg, 67%). $[\alpha]_D^{20} = -80.2$; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.01 (m, 1H), 5.95 (m, 1H), 4.21 (dt, J = 6.7, 1.9 Hz,

1H), 3.47 (m, 3H), 3.37 (m, 2H), 3.35 (m, 1H), 2.36 (dddd, J = 16.2, 9.0, 2.4, 1.8 Hz, 1H), 2.27 (dddd, J = 14.4, 7.2, 7.2, 7.2 Hz, 1H), 2.13 (m, 1H), 1.93 (dddd, J = 12.8, 6.4, 6.4, 6.4 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 4H), 1.36 (m, 8H), 0.91 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H).

4.7. Bioassay

Electroantennogram (EAG) analysis was performed on a Syntech EAG system (Hilversum, The Netherlands), fitted with a CS-05 stimulus air controller and an AUTO *SPIKE* I DAC-2/3 amplification system and recording program. Isolated antennae from 1 to 3 days old gypsy moths were used. Stimuli were delivered on small filter papers in cartridges, fashioned out of a 5.5′ Pasteur pipette. Antennae were detached from the moth and cut at the tip. Each antenna was connected with the base of the antenna attached to the reference electrode and the tip attached to the recording electrode. The electrodes were Ag/AgCl, housed in glass capillaries, which were filled with EAD ringer solution.[61]

In this study, a competitive electroantennogram (EAG) analysis was employed, in which the odorant activity of the compounds or mini-libraries by themselves and their effect on the antennal responses to (+)-**1** were measured. For the EAG assays, a responsive antenna from a male gypsy moth was mounted between electrodes and subjected to specific chemical stimuli. The stimuli were delivered as puffs that passed through a cartridge with the stimulus and merged with an air stream passing over the antennal preparation. The pheromone was kept constant at 100 ng/cartridge and five puffs were recorded for each replicate (Fig. 3): (a) clean air; (b) the test compound/set alone (100 μg on the cartridge); (c) pure (+)-**1** (100 ng on the cartridge); (d) the test compound/set and (+)-**1**; (e) pure (+)-**1**. The strength of EAG response was recorded by measuring following parameters (Fig. 3): compound alone effect (CAE), short-term effect (STE), and long-term effect (LTE). Two features of the EAG signals were used: depolarization (mV) and peak width (s). The STE and LTE (percentages) were calculated relative to the value of the depolarization or peak width from the second puff [pure (+)-**1**].

For assays with gypsy moth PBP1 and PBP2, portions of 5 mM quencher (potassium iodide or acrylamide) stock solutions were added consecutively to 500 μL 2 μM protein samples with 10 μM compounds. The PBP and ligand mixtures were incubated for at least 2 h before each test. Three independent samples under each condition were used. Samples were excited at 295 nm and data was collected from 310 to 350 nm on a PTI fluorimeter equipped with 814 photomultiplier detection system at 20 °C. Compounds tested were: (+)-**1**, (±)-**14**, **15**, **16**, **17**, and **18**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.061.

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