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Design and synthesis of bombykol analogues for probing pheromone-binding protein–ligand interactions

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ABSTRACT

Mono-, difluorinated, and thioanalogues of *Bombyx mori* female sex pheromones (bombykol, **1**) were designed according to the ab initio calculations. These rationally designated analogues were synthesized using hydroboration and Sonogashira coupling strategy via (5*E*,7*Z*)-undecadien-1-ol (**10**) as a common intermediate. A new simplified binding assay based on nanoLC-linear ion trap ESI-MS for quantifying complexation of the *B. mori* pheromone-binding protein (BmPBP) with native (**1**) and prepared analogues was developed. The binding properties of native **1** and thioanalogue **4** with PBP were studied in detail. The dissociation constant (K_D) of **1** and **4** was determined to be 2.1×10^{-6} M and 2.4×10^{-6} M, respectively. The similar values for both ligands correlated with ab initio calculations. The new binding assay could be used to determine the K_D of other PBPs.

1. Introduction

Since Butenandt[1] identified bombykol as a sex pheromone of the silkworm moth, *Bombyx mori* (L.), considerable progress has been made in understanding its pheromone chemistry and biology.[2,3] The mechanism suggests that various proteins act collaboratively, including pheromone receptors, pheromone-binding proteins (PBPs), and pheromone-degrading enzymes, during the olfactory process.[4] The molecular structure of the proteins is already firmly established; even the crystal structure of the pheromone-binding protein (PBP) with the native ligand is known.[5] However, the limited information available on the kinetic parameters of individual *peri*-receptor events in insect antennae has slowed construction of a permanent model, despite Kaissling's proposals.[6,7] The role of individual proteins in sex pheromone perception and degradation needs to be investigated further to understand how insects recognize individual pheromone molecules.[8]

Determining binding constants of lipophilic pheromone ligands in aqueous solutions of proteins is not a trivial task, as the water-immiscible ligand is difficult to be kept in solution at the higher concentrations needed for NMR or calorimetric assays. The competitive assay for radioactive substrate analogues developed by Prestwich[9] requires specific conditions for measurement and does not provide binding constants of the expected magnitudes. Mass spectrometric methods were employed to measure binding of bombykol (**1**) to *B. mori* PBP (BmPBP).[10] Leal's group developed an assay based on filtering off the non-bound pheromone from the incubation solution containing PBP at appropriate pH and ionic strength; the ligand was then determined by gas chromatography (GC) and the PBP by electrospray mass spectrometry (ESI-MS).[11] Other researchers use competitive binding studies with fluorescence readouts[12] to study the pheromone binding indirectly. Fluorescence probes, which are expected to bind to the PBP binding site, are used (1-aminoanthracene), and the release of the fluorescence probe is measured by fluorescence spectroscopy. Because the probe is not structurally related to sex pheromone structures, the binding can be nonspecific.

To improve assay accuracy, we may need to use an internal standard that is structure like native bombykol but with a different mass. This will allow the binding constants of diverse ligands to be determined in a standardized way. We have recently been able to identify amino acid residues that contribute significantly to the enthalpy of binding of bombykol ligands in the BmPBP–Bom complex.[13] Here, using a similar computation method, we characterize the energetic binding changes that occur when an atom is isosterically replaced in the original ligand. Those calculations were the basis of the set of pheromone analogues we designed. In the following sections we describe the synthesis of the newly designed pheromone analogues and evaluate their binding constants by simultaneously determining amounts of both ligand and PBP using

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LC–ESI-MS. The approach speeds evaluation of biological assays and minimizes sample handling.

2. Results and discussion

2.1. Quantum chemical calculation

2.1.1. Interaction energy change of the ligand versus Phe12 and Phe118 in the BmPBP

The most important interacting partners for the middle, saturated part of the ligand are aromatic side chains, namely Phe12, Phe118 residues.[13] We estimated the effect of pheromone structure isosteric change on the interaction energy for the Phe12 and Phe118 residues. Using the X-ray structure of the BmPBP–bombykol complex, the particular CH_2 groups were replaced by CF_2 or S and the structures of pheromone and the two phenylalanine benzene rings were optimized for the positions of hydrogen and fluorine atoms. We selected sites distant from the terminal alcohol group and from conjugated double bonds, evaluating the C4, C5, C6, C7, and C16 positions in the carbon chain. We conclude (Table 1) that none of the modifications resulted in a large value of repulsion or attraction; however, several positions seem to be preferential.

2.1.2. Model interactions with an aromatic ring

In order to elucidate the orientation preference of these functional groups toward the aromatic residues, we considered the interactions of four model molecules (2-fluoropropane, 2,2-difluoropropane, and dimethylsulfane were compared with propane) with benzene; three different orientations were studied: above the ring center, above the carbon atom of the benzene ring, and above the ring plane shifted 2 Å out of the ring (see Fig. 1). Dissociation curves were calculated using RI-MP2/aug-cc-pVDZ ab initio method for each complex. In addition, unrestrained optimizations of the structures corresponding to the calculated optimum benzene ligand distance were performed. The calculated interaction energies are shown in Table 2.

Table 1
The effect of the substitution of CF_2 or S group for the CH_2 group in bombykol (**1**)

Position	C4	C5	C6	C7	C16
CF_2	−0.4	0.9	−0.2	0.2	0.0
S	−0.6	−0.3	−0.3	−0.6	0.0

The interaction energies (kcal/mol) of pheromone versus Phe12 and Phe118 in native crystallographic conformation. Values are relative to the native pheromone interactions.

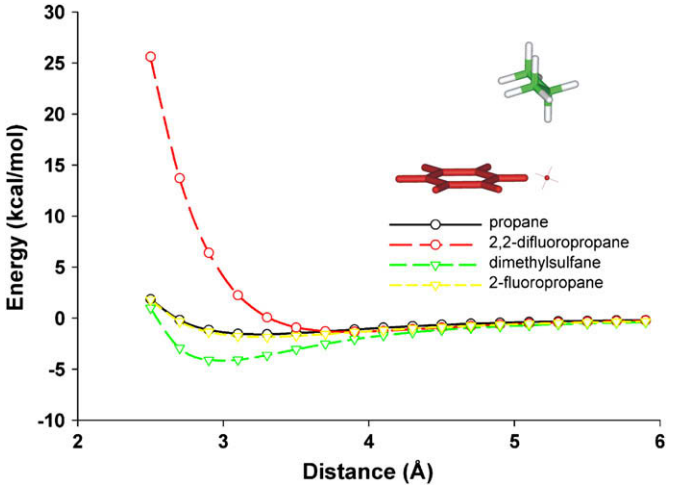


Figure 1. Four model molecules (2-fluoropropane, 2,2-difluoropropane, and dimethylsulfane were compared with propane) were considered to interact with benzene in three different orientations (above the ring center, above a carbon atom of the benzene ring, and above ring shifted 2 Å out of ring).

Table 2
Unrestrained optimization of the structures corresponding to the calculated optimum benzene ligand distances

Starting orientation	Propane	2,2-Difluoro-propane	2-Fluoro-propane	Dimethyl-sulfane
Above center	−3.1	−3.4[a]	−3.5	−2.6
Above, 2 Å away	−2.9	−3.3[a]	−3.4	−3.8

Interaction values of optimized structures (in kcal/mol).
[a] The optimization from both orientations ended in a similar conformation.

Propane and 2-fluoropropane behave similarly, interacting more strongly above the center of benzene and less strongly as the molecule moves from the ring center. The dimethylsulfane molecule interacts more strongly if above the ring plane, shifted 2 Å sideways (Fig. 1).

The effect of solvation has been assessed using a continuum solvation model. The results confirmed our assumption that the CH_2 group replacement by more polar groups systematically enhances solubility of the ligand. The ΔG_{solv} change ranged between −2.2 and −3 kcal/mol (Table 3).

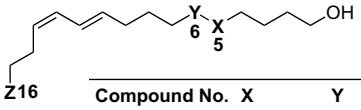
In order to gain similar or stronger affinity in comparison to the native ligand bombykol, the modified ligands need to compensate for the unfavorable effect of solvation (Table 3). The compensation may arise from the enthalpic contribution to the energy of ligand–protein interaction. The calculations on model molecules show that the considered functional groups may enhance the enthalpic contribution (Table 2). It was shown that the three model molecules interact more strongly than propane. Interaction energies calculated by unrestrained optimization, are surprisingly similar (Table 2), though the resulting conformations are different. In addition, the difference in behavior of 2-fluoropropane and 2,2-difluoropropane is also remarkable (Fig. 1), indicating that the interaction enthalpy increases in response to contact with the aromatic ring and the C–H bond polarized by the adjacent C–F group. This led us to consider testing the racemic mixture of monofluorinated bombykol. We have chosen to test the following functional groups in the following positions in the bombykol molecule (Fig. 2): Y (C6) for –CF_2– and for the racemic mixture of –CFH–, Z (C16) for –CH2F–, and X (C5) for –S–.

2.2. Synthesis of the bombykol ligands

A simple and efficient synthetic approach was designed based on Pd-catalyzed Sonogashira coupling, hydroboration, and hydrolysis steps. The synthesis of bombykol analogues **2**, **3**, and **4** shares a common dienol intermediate.

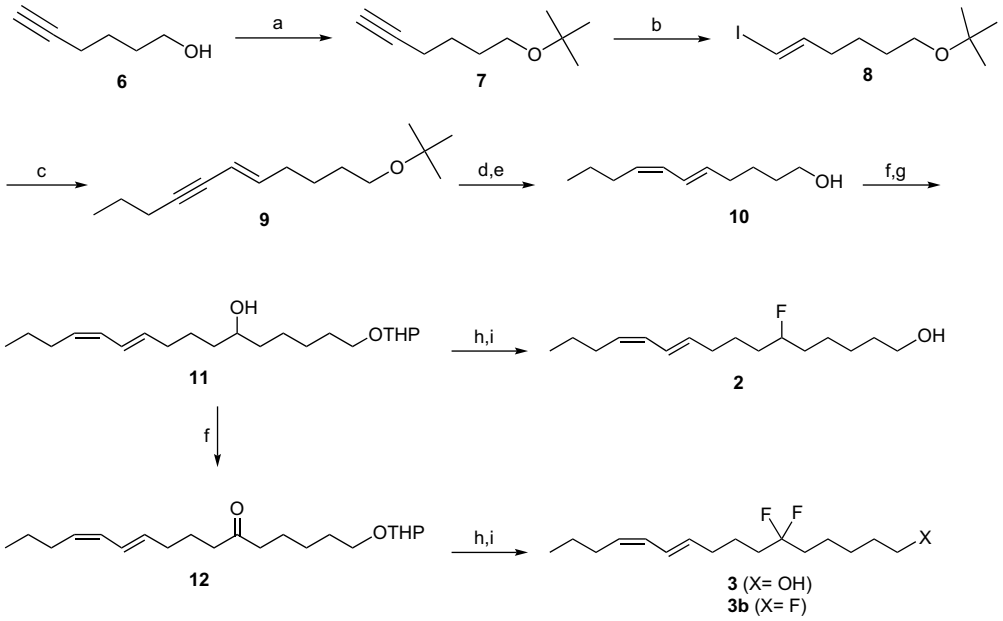
Table 3
The $\Delta\Delta G_{solv}$ for replacement of C(n) atoms in bombykol (**1**)

Ligand	Bombykol	C(5)H_2_S	C(6)H_2_C(6)F_2	C(6)H_C(6)HF	CH_3_CH_2F
E (kcal/mol)	0	−2.7	−3.0	−2.3	−2.2



Compound No.	X	Y	Z
1	CH_2	CH_2	CH_3
2	CH_2	CHF	CH_3
3	CH_2	CF_2	CH_3
4	S	CH_2	CH_3
5	CH_2	CH_2	CH_2F

Figure 2. Chemical structures of rationally designed bombykol (**1**) analogues.



Scheme 1. (a) 2-Methylpropene; (b) DIBAL-H, I_2; (c) 1-pentyne, $Pd(PPh_3)_4$, CuI; (d) Cy_2BH/AcOH; (e) Ac_2O, $FeCl_3$, then NaOH; (f) PCC; (g) $BrMgC_5H_{10}OTHP$; (h) DAST; (i) PTSA.

The synthesis of both mono- and difluoro ligands **2** and **3** started from protected (*E*)-iodohexan-6-ol (**8**, Scheme 1),[14] which was prepared from the corresponding alkynol **6**.

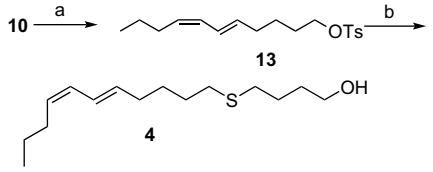
Only *tert*-butyl ether of the alcohol functionality was found to protect (*E*)-iodohex-1-en-1-ol **8** at more than 90% yield. Moreover, the *tert*-butoxy protecting group is highly recommended in the chemistry of organolithium or Grignard reagents, because of the bulk of the *tert*-butyl group, which efficiently impedes any chelation of the metal (Li or Mg) by the oxygen of the *tert*-butyl ether functionality.[15] Pd-catalyzed Sonogashira coupling reaction with pent-1-yne led to enyne **9** in a good yield. The selective reduction of triple bond was performed with dicyclohexylborane, which is known to be a mild reagent for cis-isomer formation.[16] The isomeric purity of the prepared (5*E*,7*Z*)-undecadienol **10** was higher than 96% (using NMR and GC on DB5 phase). After deprotection, the terminal hydroxyl group in **10** was oxidized, and the obtained aldehyde reacted with Grignard reagent, prepared from THP-protected 1-bromopentan-5-ol. For 6-fluoro ligand **2**, the formed OH-group in **11** was directly fluorinated using DAST in 80% yield. THP protection was removed in the presence of *p*-toluenesulfonic acid, resulting in an overall yield of ligand **2** of 16%. For 6,6′-difluorinated compound, alcohol **11** was converted to the corresponding ketone (**12**) and then fluorinated as described above. It turned out that the usual fluorination procedure was not efficient enough for the keto-group; however, the DAST needed for the complete fluorination of **12** led to the cleavage of THP-protecting group and subsequently a similar level of fluorination was observed in the terminal OH-function. Unfortunately, 1,6,6-trifluorodiene side product (17%) was an isolated product; the expected 6,6′-difluoroalcohol **3** was obtained only in a trace amount insufficient to fully characterize the compound to the question of how specific are PBP–pheromone interactions.

For sulfur-containing ligands, alcohol **10** was tosylated and treated with 4-mercaptobutan-1-ol (Scheme 2). The absence of an *O*-protecting group in mercaptobutanol does not affect the reaction and does not impair the yields. Thus, thioanalogue **4** was obtained from **10** in 87% isolated yield.

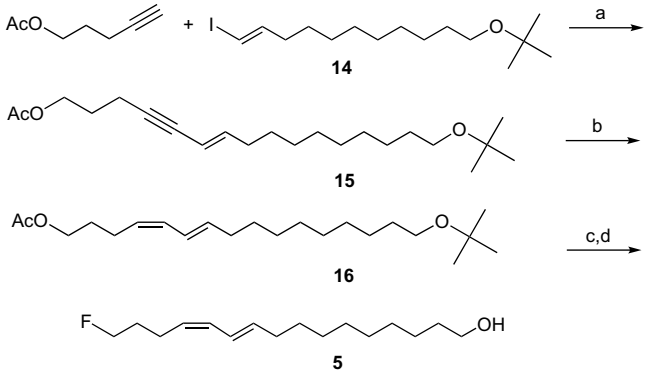
16-Fluoro compound **5** was prepared using the same strategy described above for fluoro ligands starting from pentynol acetate and iodoalkene **14** in 24% overall yield (Scheme 3).

2.3. Binding assay of ligands to BmorPBP

The molecular interactions of bombykol with its binding partner PBP have recently[11] been studied in an assay in which the pheromone is incubated with the PBP under physiological conditions in plastic vials equipped with molecular filter membranes; subsequently the complex is separated from the unreacted ligand by centrifugation. However, the GC–MS analysis of the pheromone requires hexane to be extracted from the aqueous buffer, and the additional LC–MS analysis of the protein increases the complexity of the analysis, possibly leading to more experimental errors or extended analysis times. These could be eliminated by detecting both ligands and PBPs



Scheme 2. (a) TsCl; (b) HSC_4H_8OH.



Scheme 3. (a) $Pd(PPh_3)_4$, CuI; (b) Cy_2BH; (c) MeONa; (d) DAST; Ac_2O, $FeCl_3$, MeONa.

in aqueous solutions at conditions similar to those of the binding assay. In our approach, descriptions of how the binding energy changes when atoms or functional groups are replaced in the pheromone may help to clarify the mechanisms of the *peri*-receptor events in insect olfaction and might provide answers to the question of how specific are PBP–pheromone interactions.

To determine the binding constants of BmPBP with bombykol and its modified isomers, we used an assay based on a centrifugal filter device that separated bound and free ligands.[11] Only one analytical method (LC–ESI-MS) was used to determine the amount of PBP and ligand before and after incubation. The method is more sensitive than earlier methods and does not require large amounts of protein/ligand as was necessary in GC quantification[11] of free ligand or native gel assays.[17] Three filtration columns were tested, and Microcon filters were found to recover most of the PBPs (91%). To compare, using the ZEBA column to isolate BmBPB–pheromone complex led to 29% recovery of the PBP, using the VIVASPIN column led to 10%. The binding of thio ligand **4** to BmPBP was studied and results were compared with the binding constants for PBP–bombykol complex. A detailed description of the method will be published elsewhere. The assays were repeated three times. Analyses were performed within several hours after the binding assay as ligands are notoriously unstable when stored for long periods (24 h).

A baseline separation observed in Figure 3 for the interacting species on a reversed-phase *n*-butyl-silica column with a well-defined PBP peak at 17.5 min and the hydrophobic bombykol eluting shortly thereafter at 21 min demonstrate the usefulness of the approach. The selected ion chromatogram plotted at 239.2 and 1588.5 Da using a 1 Da window, for the molecular peaks of **1** and for the $[M+10H]^{10+}$ PBP multiplet, respectively (selected to maintain comparable protein/ligand signal ratios), enables sensitive quantitation in the picomolar concentration range to be performed (2.5 pmol PBP and 5 pmol **1** on column). The ESI spectra of **1** display an intense protonated molecular peak at 239.1 Da and an additional dimer $[2M+H]^+$ peak (inset Bol). The purity of the recombinant protein is demonstrated in inset BmPBP by a full protein envelope of multiplet peaks carrying charges in the range of 8–14 protons.

The association binding constants (K_D) for sulfo ligand **4** and bombykol **1** determined from these assays were 2.4×10^{-6} M and 2.1×10^{-6} M, respectively. From the previously published K_D, the value of our association constant is closer to 1.1×10^{-6} M, determined using the fluorescence assay.[12] The previously published value using a 'cold binding assay'[11] was 0.105×10^{-6} M. The observed differences could be explained by different experimental setups. If we consider the calculated binding enthalpy (ca. 20 kcal/mol)[13] and the difficulty of observing the binding of **1** with BmPBP in native electrospray experiments,[10] the nanomolar value for K_D determined by Leal[11] seems to be too low. Based on our native electrospray experiments, the dissociation of **1** from PBP should be comparable with the dissociation of heme from hemoglobin[18] with $K_D=6.2\times10^{-6}$ M. The similar value of K_D correlates with the predicted effects of isosteric replacement determined by ab initio calculations.

The uses of the developed assay could be further extended to evaluate binding constants for similar assays of other PBPs. Constants of ligand binding for the lipocaline family[4] functionally related to PBPs might be measured by this approach after minor modifications.

3. Experimental

3.1. General

All reactions were carried out under an inert atmosphere (Ar). All reported yields are of isolated pure products. The NMR spectra

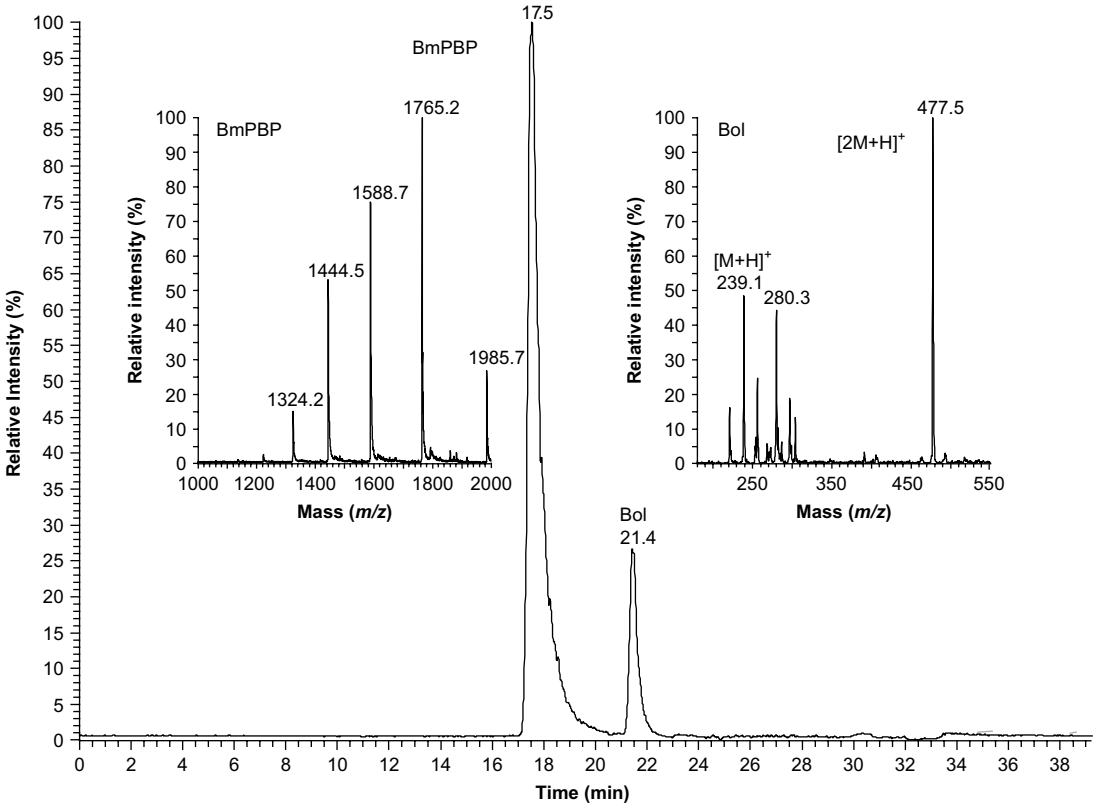


Figure 3. A section of total ion current trace from LC–MS separation of a mixture after binding assay of bombykol (**1**) with BmPBP protein. Insets show average mass spectra under peaks of BmPBP and (**1**), respectively.

were recorded on Bruker AVANCE DR 400 spectrometer. All spectra were measured in $CDCl_3$. Chemical shifts δ are given in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Mass spectra were obtained on MassSpec 2 or Quattro II (both Micromass, Manchester, UK).

3.1.1. Computational details

3.1.1.1. Methods. All structures were first optimized using the density functional theory (DFT) method. The B3LYP functional[19] was used in conjunction with the 6-31G** basis set.[20] The unrestrained optimization of the model complexes was performed at the second order Møller Plesset (MP2) perturbation method level using the aug-cc-pVDZ basis set.[21] Interaction energies were computed at the MP2/aug-cc-pVDZ level of theory. MP2 calculations were sped up by expanding the Coulomb interactions in an auxiliary basis set, the resolution-of-identity (RI) approximation.[22] The solvation effect was described using the COSMO model of an aqueous solvent implemented in the Turbomole 5.7 program suite[23] with the default parameters.

3.1.1.2. Model. The model for native interaction of pheromone and the residues Phe12, Phe118 was built by truncating the X-ray structure [Ref. 5, 1DQE]. Whole pheromone and benzene rings of the phenylalanines were considered. The coordinates of the heavy atoms were fixed at the original crystallographic positions; hydrogen atom positions were optimized.

3.1.2. BmPBP preparation and purification

The recombinant protein was prepared as follows.[24,25] Briefly, the pheromone-binding protein from *B. mori* (BmPBP) was expressed in *Escherichia coli* periplasm. The recombinant pET22b(+)/BmPBP plasmid (obtained from J. Krieger, Hohenheim University, Germany) was transformed on *E. coli* BL21(DE3) expression hosts (Novagen) and the expression was performed by growing the no induced cells at 30 °C in an LB medium (2-L flasks with 700 mL suspension culture) supplemented with 50 mg/L ampicillin. The culture was grown until A_{600} values were greater than 2. The cells were harvested by centrifugation and the periplasmic fraction was obtained by the osmotic shock procedure described in the pET System manual (Novagen). The periplasmic fraction was loaded onto a DEAE-Toyopearl 650S (Tosoh, Tokyo, Japan) column (diameter 2.3 cm, length 15 cm) equilibrated with 10 mM Tris–HCl, pH 8.0. The proteins were eluted with a very slow linear gradient of 0–200 mM NaCl in 10 mM Tris–HCl, pH 8.0. The fractions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the fractions containing pure BmPBP were pooled. The BmPBP was precipitated using trichloroacetic acid and stored at −80 °C. Prior to use, the fatty acids were removed by delipidation.[26] The obtained solution was aliquoted, vacuum-dried, and stored at −20 °C prior to use.

3.1.3. Binding assay and LC–MS analysis

A 1 μL aliquot of 10 μM ethanolic ligand solution was added to 99 μL of 5 μM delipidated protein solution in 10 mM buffer (ammonium acetate, pH 7) in a glass vial insert silanized by dimethylsilyl dichloride. The sample was incubated in a shaker (300 rpm) at 27 °C for 1 h. An aliquot (5 μL) of the reaction mixture was taken for assay A and the rest was transferred to a washed Microcon YM-10 10 kDa cut-off membrane column (Millipore, Billerica, MA, USA) and centrifuged (17,950 g/4 °C/10 min). The filter was washed with another 95 μL of buffer, pH 7. The retentate was weighed and diluted to total volume 95 μL. Part of the retentate solution (5 μL) was diluted with 45 μL of an eluent solution (acetonitrile–0.1% formic acid 1:10 v/v) and analyzed by LC–MS. The experiments were performed with three assay replicates each using three analytical determinations. The binding constants were calculated from ligand peak areas integrated in selected ion chromatograms (SIMs) displayed each time as molecular ion intensities with 0.5 Da mass window and average area values of three consecutive retentate analyses. The SIM peak areas of the PBP (at a mass of 1588.5 Da, corresponding to a 10-fold protonated protein) were used for normalizing the yield of the centrifugation and extraction of the sample from the membrane columns.

The assay samples were analyzed using a nanoHPLC–MS system consisting of an Agilent 1100 series autosampler and a micro-LC pump (Agilent, Palo Alto, CA, USA) and of a linear ion trap mass spectrometer (LTQ, Thermo-Finnigan Inc., San Jose, CA, USA). The injected samples were first loaded onto a Nanoease trap column (240 mm×180 μm I.D.) and then eluted onto a reversed-phase Nanoease nano-LC analytical column (100 mm×75 μm I.D., both packed with Symmetry 300 C4 3.5 μm particles, both Waters, Milford, MA, USA). The sample was injected from 10% MeCN–0.1% FA at a flow rate of 12 μL/min for 5 min and eluted by a linear 10–90% acetonitrile gradient in 10 min at a flow rate of approximately 300 μL/min after a passive split. The possible hydrophobic contaminants were then removed by an additional 90% MeCN–0.1% FA step and the column was equilibrated at 10% MeCN–0.1% FA for additional 12 min. The column outlet was coupled to the nanoelectrospray ionization source of the LTQ linear ion trap mass spectrometer (Thermo-Finnigan Inc.) using a liquid junction and an uncoated fused silica nanoelectrospray needle (120 mm×30 μm I.D., Picotip, New Objective, Woburn, MA, USA).

The mass spectrometer operated with 1.7 kV set on the emitter, 42 V on the ion transfer capillary and 140 V tube lens voltages, and 140 °C capillary temperature using an alternating three full mass scan events (100–2000 Da followed by an SIM scan of the BmPBP and of the ligand). The binding constants were calculated based on Eq. 1:

$$K_D = \left[PBP_{total}\right] \times \left[PBP_{free}\right] / \left[PBP_{complex}\right] \quad (1)$$

3.1.4. Synthesis of ligands

*3.1.4.1. 1-tert-Butoxyhex-5-yne (**7**).* Amberlyst (1.25 g) was added to the reaction mixture of 5-hexyn-1-ol (**6**, 5 g, 0.05 mol) in hexanes (25 mL) at 0 °C. 2-Methylpropene was bubbled into the mixture for 14 h at room temperature. The resin was filtered off, K_2CO_3 (1 g) was added, and the solvent was evaporated. Flash chromatography (FC) on silica gel, eluting with CH_2Cl_2, provided the product (7.8 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.28 (t, 2H, 1-CH_2), 2.14 (m, 2H, 4-CH_2), 1.86 (t, 1H, 6-CH), 1.54 (m, 4H, 2-CH_2, 3-CH_2), 1.11 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=84.9 (C-5), 72.8 (C_{quad}), 68.6 (C-6), 61.3 (C-1), 30.1 (C-2), 27.9 (3×CH_3), 25.8 (C-3), 18.7 (C-4). EIMS, *m*/*z* 154, $[M]^{\bullet+}$.

*3.1.4.2. (E)-6-tert-Butoxy-1-iodohex-1-ene (**8**).* A solution of alkyne **7** (4 g, 26 mmol) in hexanes (30 mL) was treated with DIBAL-H (40 mL, 1 M in hexanes) at 55 °C for 5 h and cooled till −45 °C, and a solution of iodine (8 g, 1.1 equiv) in THF (30 mL) was added over 60 min. The resulting mixture was allowed to warm to room temperature and stirred at those conditions for 14 h, then poured into 6 M NaOH, and stirred until the brown color disappeared. The organic layer was separated, and the aqueous layer was extracted with hexanes (3×10 mL). The combined organic extracts were washed with water (3×10 mL) and dried over Na_2SO_4. FC on silica gel, eluted with CH_2Cl_2–hexanes (1:3), gave **8** (6.74 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.43 (m, 1H, 2-CH), 5.91 (dt, 1H, 1-CH, J_{12}=14.3 Hz, J_{13}=1.4 Hz), 3.26 (m, 2H, 6-CH_2), 2.01 (m, 2H, 3-CH_2), 1.45 (m, 4H, 4-CH_2, 5-CH_2), 1.11 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=146.9 (C-2), 74.9 (C-1), 72.8 (C_{quad}), 61.5 (C-6), 36.3 (C-3), 30.3 (C-5), 28.0 (3×CH_3), 25.8 (C-4). EIMS, *m*/*z* (rel int.): 280 (5, $[M]^{\bullet+}$), 207 (75, $[M-OPg]^+$).

*3.1.4.3. (E)-11-tert-Butoxy-undec-6-en-4-yne (**9**).* Vinyliodide **8** (2.2 g, 8 mmol) was injected at room temperature into a solution of $Pd(MeCN)_2Cl_2$ (450 mg, 5 mol %) in anhydrous piperidine (30 mL) under argon atmosphere. The resulting mixture was stirred for an additional hour. Then a solution of pentyne (2 mL, 2 equiv) in piperedine (5 mL) and CuI (350 mg, 10 mol %) as solid were added. After 18 h stirring under argon, the mixture was poured into water (20 mL) and extracted into ether (2×20 mL). Extracts were washed with aq HCl (1 M, 10 mL), satd aq $NaHCO_3$ (10 mL), brine (2×20 mL), and water (2×10 mL), and dried over Na_2SO_4. FC on silica gel, eluting with CH_2Cl_2–hexanes (1:5), gave **9** (1.6 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.95 (m, 1H, 7-CH), 5.40 (d, 1H, 6-CH, J_{67}=14.0 Hz), 3.25 (m, 2H, 11-CH_2), 2.18 (m, 2H, 3-CH_2), 2.01 (m, 2H, 4-CH_2), 1.46 (m, 6H, 3×CH_2), 1.10 (br s, 9H, 3×CH_3), 0.91 (m, 3H, 1-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=143.4 (C-7), 110.4 (C-6), 88.9 (C-4), 79.7 (C-5), 72.8 (C_{quad}), 61.7 (C-11), 34.0 (C-8), 30.3 (C-10), 27.9 (3×CH_3), 26.3 (C-9), 23.0 (C-2), 22.7 (C-3), 13.9 (C-1). EIMS, *m*/*z* (rel int.): 222 (5, $[M]^{\bullet+}$), 149 (75, $[M-OPg]^+$).

*3.1.4.4. (5E,7Z)-Undecadien-1-ol (**10**).* A dicyclohexylborane suspension was prepared in THF (15 mL) from borane–DMS complex (2 M, 3 mL) and cyclohexene (488 mg, 2.2 equiv). The obtained white suspension was treated at 0 °C with a solution of enyne **9** (600 mg, 2.7 mmol) in THF (5 mL). The mixture was warmed to room temperature and stirred for 4 h. The formed vinylborane was hydrolyzed with glacial AcOH (2.5 mL) at room temperature for 14 h; the reaction mixture was then neutralized with NaOH (20%, 5 mL), and carefully treated with aq H_2O_2 (30%, 2.5 mL). The crude product was extracted with hexanes (3×50 mL) and dried over Na_2SO_4. FC on silica gel, eluted with CH_2Cl_2–hexanes (1:3), gave *O*-*tert*-butoxy-undec-5-(*E*)-7-(*Z*)-dien-1-ol (560 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.23 (dd, 1H, 7-CH, J_{76}=15.0 Hz, J_{78}=11.0 Hz), 5.88 (t, 1H, 6-CH, J_{65}=15.0 Hz), 5.58 (m, 1H, 8-CH), 5.21 (m, 1H, 5-CH), 3.26 (m, 2H, 1-CH_2), 2.05 (m, 2H, 9-CH_2), 1.77 (m, 2H, 4-CH_2), 1.40 (m, 6H, 3×CH_2), 1.10 (br s, 9H, 3×CH_3), 0.81 (m, 3H, 11-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=134.6 (C-5), 130.1 (C-6), 129.1 (C-7), 126.2 (C-8), 72.7 (C_{quad}), 61.7 (C-1), 31.9 (C-4), 30.3 (C-2), 27.9 (3×CH_3), 26.0 (C-9), 25.9 (C-10), 23.0 (C-3), 14.4 (C-11). EIMS, *m*/*z* (rel int.): 224 (4, $[M]^{\bullet+}$), 167 (63, $[M-Pg]^+$), 151 (70, $[M-OPg]^+$).

To a solution of protected dienol (2 g, 9 mmol) in ether (45 mL), acetic anhydride (4.5 mL) and $FeCl_3$ (167 mg) were added under argon. The dark brown solution was stirred at room temperature for 23 h and allowed to settle. Na_2HPO_4 (16 mL) was added, and the mixture was stirred for an additional 2 h. The organic phase was separated; the aqueous one was washed with ether (3×20 mL). The combined extracts were washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The dark brown residue was dissolved in methanol (30 mL), and NaOH (1.6 g) in water (9 mL) was added. The resulting mixture was stirred at room temperature overnight, then poured into the ether (3×20 mL). The combined organic extracts were washed with water (20 mL) and brine (15 mL), and dried over Na_2SO_4. FC on silica gel, eluting with CH_2Cl_2, gave **10** (1.14 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.24 (dd, 1H, 7-CH, J_{76}=15.0 Hz, J_{78}=11.0 Hz), 5.88 (t, 1H, 6-CH, J_{65}=15.0 Hz), 5.57 (m, 1H, 8-CH), 5.24 (m, 1H, 5-CH), 3.57 (m, 2H, 1-CH_2), 2.06 (m, 3H, 9-CH_2, OH), 1.50 (m, 2H, 4-CH_2), 1.35 (m, 6H, 3×CH_2), 0.85 (m, 3H, 11-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=134.3 (C-5), 130.5 (C-6), 129.0 (C-7), 126.4 (C-8), 63.1 (C-1), 32.9 (C-4), 32.6 (C-2), 30.1 (C-9), 25.9 (C-10), 23.2 (C-3), 14.1 (C-11). EIMS, *m*/*z* (rel int.): 168 $[M]^{\bullet+}$.

3.1.4.5. 2-(5-Bromopentyloxy)-tetrahydro-2H-pyran. 5-Bromopentan-1-ol (1 g, 6 mmol), prepared from 1,5-pentandiol,[27] was protected with DHP (0.54 mL, 1 equiv). FC on silica gel, eluted with CH_2Cl_2–hexanes (2:3), provided the product (1.5 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.50 (m, 1H, CH-2′), 3.86 (m, 2H, CH_2-6′), 3.60 (m, 4H, CH_2-1, CH_2-5), 1.55 (m, 12H, 6×CH_2). ^{13}C (100 MHz, $CDCl_3$): δ=98.9 (C-2′), 67.4 (C-1), 62.4 (C-6′), 33.7 (C-5), 32.6 (C-4), 30.8 (C-2), 28.9 (C-3′), 25.5 (C-3), 25.0 (C-5′), 19.7 (C-4′). GC–MS, *m*/*z* (rel int.): 252, 250 $[M]^{\bullet+}$.

*3.1.4.6. (10E,12Z)-1-(Tetrahydro-2H-pyran-2-yloxy)hexadeca-10,12-dien-6-ol (**11**).* Alcohol **10** (850 mg, 5.1 mmol) in CH_2Cl_2 (5 mL) was added to the suspension of PCC (1.3 g, 1.2 equiv) and anhydrous NaOAc (98 mg) in CH_2Cl_2 (6 mL). The resulting mixture was stirred at room temperature for 90 min, then poured into ether (200 mL), and filtered through the column with sand/Celite/coal/sand to give (5*E*,7*Z*)-undecadien-1-al (630 mg, 76%), which was used in the next step without additional purification. ^{13}C (100 MHz, $CDCl_3$): δ=201.4 (CHO), 131.7 (C-5), 129.7 (C-6), 127.5 (C-7), 125.3 (C-8), 42.2 (C-2), 31.1 (C-9), 24.7 (C-10), 21.9 (C-3), 20.8 (C-4), 14.1 (C-11).

One small crystal of iodine was added to a dry Mg (273 mg) and the mixture was stirred under heat till the glass walls turned violet. After cooling, THF (2 mL) was added followed by 1/5 of the 5-*O*-tetrahydropyranoxy-1-bromopentane solution (1.4 g, 5.6 mmol, dissolved in 4 ml THF). The resulting mixture was stirred at 65 °C for 30 min. Then the rest of 5-*O*-tetrahydropyranoxy-1-bromopentane was added carefully, and the mixture was stirred under refluxing for an additional 2.5 h. The reaction was allowed to cool; the reaction solution was added dropwise by syringes into a mixture of aldehyde (630 mg, 3.8 mmol) in THF (6 mL) cooled till 0 °C. After 15 min the ice-bath was removed, and the resulting mixture was stirred at room temperature for 19 h. Ice-cold NH_4Cl (10 mL) was added, the organic phase was separated, and the aqueous one was extracted into pentane and ether (1:1, 100 mL). The combined organic extracts were washed with NH_4Cl (3×50 mL) and water (20 mL), and dried over Na_2SO_4. FC on silica gel, eluted with CH_2Cl_2–pentane (1:3→100%), furnished **11** (810 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.83 (t, 1H, 11-CH, J_{1110}=15.0 Hz), 5.52 (m, 1H, 13-CH), 5.20 (m, 1H, 10-CH), 4.45 (m, 1H, CH-THP), 3.74 (m, 1H, C*H*H-THP), 3.60 (m, 1H, CH*H*-THP), 3.50 (m, 1H, 6-CH), 3.36 (m, 1H, 1-C*H*H), 3.28 (m, 1H, 1-CH*H*), 2.00 (m, 4H, 14-CH_2, 9-CH_2), 1.55 (m, 2H, CH_2), 1.29 (m, 18H, 9×CH_2), 0.79 (m, 3H, 16-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=134.0 (C-10), 130.1 (C-11), 128.7 (C-12), 126.0 (C-13), 98.9 (C-2′), 71.7 (C-6), 67.5 (C-1), 62.4 (C-6′), 37.4 (C-7), 37.0 (C-5), 32.8 (C-14), 30.8 (C-9), 29.7 (2C, C-15, 3′) 26.3, 25.4 (3C), 22.9 (C-2, 8, 4, 3, 5′), 19.7 (C-4′), 13.7 (C-16). EIMS *m*/*z* (rel int.): 338 (47, $[M]^{\bullet+}$), 320 (40, $[M-H_2O]^+$).

*3.1.4.7. (10E,12Z)-6-Fluoro-undeca-10,12-dien-1-ol (**2**).* Alcohol **11** (160 mg, 0.47 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and cooled to −78 °C. Then DAST (76 μL, 1.2 equiv) was added and the mixture was stirred for 1 h, then poured into water (20 mL), and extracted with CH_2Cl_2 (20 mL). After the mixture was dried, a small amount of silica was added, and the solvents were evaporated. The residue was applied on top of a column prepacked with silica gel. Elution with CH_2Cl_2–pentane (4:1) provided the fluorinated THP protected **2** (128 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.30 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.99 (t, 1H, 11-CH, J_{1110}=15.0 Hz), 5.68 (dt, 1H, 13-CH, J_{1312}=11.0 Hz), 5.48 (m, 1H, 10-CH), 4.60 (m, 1H, CH-THP), 4.49 (m, 1H, 6-CHF, J_{HF}=44 Hz), 3.89 (m, 1H, C*H*H-THP), 3.75 (m, 1H, CH*H*-THP), 3.53 (m, 1H, 1-C*H*H), 3.42 (m, 1H, 1-CH*H*), 2.15 (m, 4H, 9-CH_2, 14-CH_2), 1.73 (m, 2H, CH_2), 1.35–1.67 (m, 18H, 9×CH_2), 0.94 (m, 3H, 16-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=133.7 (C-10), 130.2 (C-11), 128.6 (C-12), 126.2 (C-13), 98.9 (C-1′), 94.3 (d, C-6, J_{CF}=165 Hz) 67.5 (C-1), 62.4 (C-5′), 35.1 (d, C-7, J_{CF}=21 Hz), 34.6 (d, C-5, J_{CF}=21 Hz) 32.6 (C-14), 30.8 (C-9), 29.7 (C-2′), 29.6 (C-14), 26.2, 25.5, 24.9, 25.0 (d, J_{CF}=4 Hz) (d, J_{CF}=4 Hz) 22.9 (C-2, 15, 8, 4, 3, 4′), 19.7 (C-3′), 13.7 (C-16). EIMS, *m*/*z* (rel int.): 340 (25, $[M]^{\bullet+}$), 322 (35, $[M-H_2O]^{\bullet+}$).

To a solution of THP ether (100 mg, 0.29 mmol) in methanol (2 mL) PTSA monohydrate (76 mg, 2 equiv) was added, and resulting mixture was stirred at room temperature for 1 h, then diluted with

ether (10 mL), washed with water (3×10 mL), and dried over Na_2SO_4. FC on silica gel, eluted with CH_2Cl_2, furnished **2** (70 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.25 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.89 (t, 1H, 11-CH, J_{1110}=15.0 Hz), 5.58 (dt, 1H, 13-CH, J=11.0 Hz), 5.27 (m, 1H, 10-CH), 4.45 (m, 1H, 6-CHF, J_{CF}=40 Hz) 3.58 (m, 2H, 1-CH_2), 3.37 (s, 1H, OH), 2.06 (m, 4H, 9-CH_2, 14-CH_2), 1.32 (m, 14H, 7×CH_2), 0.85 (m, 3H, 16-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=134.1 (C-10), 130.6 (C-11), 129.0 (C-12), 126.6 (C-13), 94.6 (d, C-6, J_{CF}=166 Hz) 63.3 (C-1), 35.2 (C-7), 35.0 (C-5), 33.0 (2C) 31.5, 30.9, 30.1, 26.0, 23.3 (C-2, 3, 4, 8, 9, 14, 15), 19.7 (C-16). EIMS, *m*/*z* (rel int.): 256 $[M]^{\bullet+}$.

*3.1.4.8. (10E,12Z)-1-(Tetrahydro-2H-pyran-2-yloxy)hexadeca-10,12-dien-6-on (**12**).* Alcohol **11** (200 mg, 0.59 mmol) was oxidized as described for **10**. Filtration through sand/Celite/charcoal/sand furnished **12** (180 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.20 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.88 (t, 1H, 11-CH, J_{1110}=15.0 Hz), 5.54 (m, 1H, 13-CH), 5.27 (m, 1H, 10-CH), 4.49 (m, 1H, CH-THP), 3.79 (m, 1H, C*H*H-THP), 3.66 (m, 1H, CH*H*-THP), 3.40 (m, 1H, 1-C*H*H), 3.30 (m, 1H, 1-CH*H*), 2.05 (m, 4H, 9-CH_2, 14-CH_2), 1.51 (m, 2H, CH_2), 1.29 (m, 18H, 9×CH_2), 0.85 (m, 3H, 16-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=211.4 (C-6), 133.6 (C-10), 130.8 (C-11), 128.9 (C-12), 126.9 (C-13), 99.3 (C-2′), 66.2 (C-1), 62.8 (C-6′), 37.4 (C-7), 37.0 (C-5), 32.8 (C-9), 30.8 (C-3′) 29.7 (2C, C-2, 14) 26.3, 25.4, 22.9 (3C) (C-3, 4, 8, 15, 5′) 19.7 (C-4′), 14.1 (C-16). EIMS *m*/*z* (rel int.): 336 (35, $[M]^+$), 318 (100, $[M-H_2O]^{\bullet+}$).

*3.1.4.9. (10E,12Z)-6,6-Difluoro-undeca-10,12-dien-1-ol (**3**).* To a solution of ketone **12** (180 mg, 0.54 mmol) in CH_2Cl_2 (4 mL) DAST (1 g, 10 equiv) was added; the mixture was stirred under reflux for 62 h, then poured into water (20 mL), and extracted with CH_2Cl_2 (20 mL). After the mixture was dried, a small amount of silica was added, and the solvents were evaporated. The residue was applied on top of a column packed with silica gel. Elution with CH_2Cl_2–pentane (4:1) provided trifluorinated by-product 1,6,6-trifluoro-(10*E*,12*Z*)-undecadiene (**3b**) (30 mg, 17%) and traces of deprotected **3** (0.5 mg, 2%). ^{1}H NMR (400 MHz, $CDCl_3$) for **3b**: δ=6.20 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.89 (dt, 1H, 11-CH_2, J_{1110}=15.0 Hz), 5.54 (m, 1H, 13-CH), 5.27 (m, 1H, 10-CH), 4.36 (dt, 2H, 1-CH2, J_{HF}=47 Hz, J_{12}=7 Hz) 2.05 (m, 4H, 9-CH_2, 14-CH_2), 1.29-1.75 (m, 14H, 7×CH_2), 0.85 (m, 3H, 16-CH_3). EIMS *m*/*z* 276 $[M]^{\bullet+}$. ^{1}H NMR (400 MHz, $CDCl_3$) for **3**: δ=6.20 (dd, 1H, 12-CH, J_{1211}=15.0 Hz, J_{1213}=11.0 Hz), 5.89 (dt, 1H, 11-CH_2, J_{1110}=15.0 Hz), 5.54 (m, 1H, 13-CH), 5.27 (m, 1H, 10-CH), 3.62 (m, 3H, 1-CH_2, OH), 2.05 (m, 4H, 9-CH_2, 14-CH_2), 1.10-1.75 (m, 14H, 7×CH_2), 0.85 (m, 3H, 16-CH_3). EIMS *m*/*z* (rel int.): 274 $[M]^{\bullet+}$.

*3.1.4.10. 1-Tosyl-(5E,7Z)-undeca-5,7-diene (**13**).* The mixture of alcohol **10** (110 mg, 0.65 mmol), tosyl chloride (187 mg, 2 equiv), and triethylamine (34 μL) was stirred in pyridine (1.3 mL) at room temperature for 20 h. The reaction mixture was poured into cold water (5 mL) and extracted with ether (2×5 mL). The combined extracts were washed with HCl (2 M, 3 mL), water (5 mL), $NaHCO_3$ (5 mL), water (2×5 mL), and brine (3 mL), dried over Na_2SO_4, and concentrated in vacuo. FC on silica gel, eluted with CH_2Cl_2–pentane (1:3), gave **13** (95 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.71 (d, 2H, Ph, J=8.0 Hz), 7.26 (d, 2H, Ph, J=8.0 Hz), 6.20 (dd, 1H, 7-CH, J_{76}=15.0 Hz, J_{78}=11.0 Hz), 5.85 (t, 1H, 6-CH, J_{65}=15.0 Hz), 5.49 (m, 1H, 8-CH), 5.26 (m, 1H, 5-CH), 3.96 (m, 2H, 1-CH_2), 2.35 (br s, 3H, Ph-CH_3), 2.00 (m, 4H, 9-CH_2, 4-CH_2), 144.6 (Ph), 1.48 (m, 6H, 3×CH_2), 0.85 (m, 3H, 11-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=144.6 (Ph),133.3 (C-5), 133.1 (Ph), 130.4 (C-6), 129.7 (2C, Ph), 128.5 (C-7), 127.7 (2C, Ph), 126.4 (C-8), 70.4 (C-1), 31.6 (C-4), 30.0 (C-2), 28.3 (C-9), 25.1 (C-10), 22.6 (C-3), 21.6 (Ph-Me), 14.1 (C-11). EIMS, *m*/*z* (rel int.): 322 (10, $[M]^{\bullet+}$), 151 (50, $[M-OTs]^+$).

*3.1.4.11. 4-((5E,7Z)-Undeca-5,7-dienylthio)butan-1-ol (**4**).* To a suspension of NaH (14 mg, 2 equiv) in THF (1 mL) tosylate **14** (90 mg, 0.28 mmol) and mercaptobutanol (35 μL, 1.5 equiv) in THF (1 mL) were added at 0 °C. The suspension was stirred at 0 °C for 1 h, and at room temperature for 19 h, then poured into the ice-cold water (5 mL), and extracted into the ether (3×5 mL). The combined organic phases were washed with HCl (0.5 M, 3 mL) and water (3×3 mL), and dried over Na_2SO_4. FC on silica gel, eluting with CH_2Cl_2–pentane (1:3), furnished **4** (85 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.21 (m, 1H, 7′-CH), 5.88 (t, 1H, 6′-CH, J_{65}=15.0 Hz), 5.57 (m, 1H, 8′-CH), 5.26 (m, 1H, 5′-CH), 3.60 (m, 2H, 1-CH_2), 2.45 (m, 4H, 4-CH_2, 1′-CH_2), 2.06 (m, 4H, 4′-CH_2, 9′-CH_2), 1.54 (2H, CH_2), 1.42 (2H, CH_2), 1.34 (m, 4H, 2×CH_2), 1.20 (m, 2H, CH_2), 0.85 (m, 3H, 11′-CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=133.8 (C-5′), 130.1 (C-6′), 128.6 (C-7′), 126.1 (C-8′), 62.4 (C-1), 32.6 (C-4′), 32.6 (C-2′), 32.6 (C-9′), 31.9 (C-4, C-1′), 29.7 (CH_2), 28.6 (CH_2), 25.6 (C-10′), 22.9 (C-3′), 13.8 (C-11′). EIMS, *m*/*z* (rel int.): 256 $[M]^{\bullet+}$.

*3.1.4.12. (E)-11-tert-Butoxy-1-iodoundec-1-ene (**14**).* Iodide **14** was prepared by hydroalumination–iodination sequence starting from commercially available undecen-10-yn-1-ol in 94% overall yield as described for iodide **8**. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.41 (m, 1H, 2-CH), 5.90 (dt, 1H, 1-CH, J_{1110}=14.0 Hz), 3.24 (m, 2H, 11-CH_2), 1.96 (m, 2H, 3-CH_2), 1.43 (m, 4H, 2×CH_2), 1.19 (m, 10H, 5×CH_2), 1.01 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=147.0 (C-2), 74.4 (C-1), 72.6 (C_{quad}), 61.9 (C-11), 36.3 (C-3), 31.8, 31.0, 29.7, 29.5, 29.1 (4×CH_2), 27.8 (3×CH_3), 26.5, 22.8 (2×CH_2). EIMS, *m*/*z* (rel int.): 352 (10, $[M]^{\bullet+}$), 279 (70, $[M-OPg]^+$).

*3.1.4.13. (E)-16-tert-Butoxyhexadec-6-en-4-ynyl acetate (**15**).* Acetate **15** was prepared from iodide **14** and 1-acetyl-pent-4-yne via Sonogashira coupling as described for enyne **9** in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.97 (m, 1H, 6-CH), 5.38 (d, 1H, 7-CH, J_{1110}=14.0 Hz), 4.09 (t, 2H, 1-CH_2), 3.25 (t, 2H, 16-CH_2), 2.32 (m, 2H, 3-CH_2), 1.98 (m, 5H, Ac-Hs, 13-CH_2), 1.78 (m, 2H, CH_2) 1.48 (m, 4H, 2×CH_2), 1.20 (m, 10H, 5×CH_2), 1.11 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=171.5 (CO), 144.3 (C-7), 109.9 (C-6), 87.2 (C-5), 80.4 (C-4), 72.8 (C_{quad}), 63.6 (C-1), 62.0 (C-16), 33.4 (C-8), 31.1, 29.9, 29.8, 29.8, 29.5, 29.2, 28.3, 28.0 (3×CH_3), 26.6, 26.0, 19.6. EIMS, *m*/*z* (rel int.): 350 (15, [M].$^+$), 281 (65, $[M-OPg]^+$).

*3.1.4.14. (4Z,6E)-16-tert-Butoxyhexadeca-4,6-dienyl acetate (**16**).* Acetate **16** was prepared by hydroboration–hydrolysis of enyne **15** as described for **10** in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.00 (m, 1H, 12-CH), 5.75 (t, 1H, 11-CH, J_{1110}=14.0 Hz), 5.46 (dt, 1H, 10-CH, J_{1011}=14.0 Hz), 5.01 (m, 1H, 13-CH), 3.84 (t, 2H, 16-CH_2), 3.08 (t, 2H, 1-CH_2), 2.01 (m, 2H, 14-CH_2), 1.85 (m, 2H, 4-CH_2), 1.81 (s, 3H, Ac-Hs), 1.50 (m, 4H, 2×CH_2), 1.20 (m, 14H, 7×CH_2), 1.10 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=171.4 (CO), 135.8 (C-7), 130.1 (C-6), 128.2 (C-5), 125.6 (C-4), 72.7 (C_{quad}), 64.2 (C-1), 62.0 (C-16), 33.4 (C-8), 31.1, 29.9, 29.8, 29.7, 29.6, 28.9 (CH_2), 27.9 (3×CH_3), 26.6, 26.0, 24.4, 21.6 (CH_2). EIMS, *m*/*z* (rel int.): 352 (15, $[M]^{\bullet+}$), 279 (70, $[M-OPg]^+$).

*3.1.4.15. (10E,12Z)-16-Fluorohexadeca-10,12-dien-1-ol (**5**).* The deprotection of acetate **16** was performed by standard procedure using NaOH (4 equiv) and H_2O in methanol in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.20 (m, 1H, 12-CH), 5.91 (t, 1H, 11-CH, J_{1110}=14.0 Hz), 5.62 (dt, 1H, 10-CH, J_{1110}=14.0 Hz), 5.25 (m, 1H, 13-CH), 3.59 (t, 2H, 16-CH_2), 3.25 (t, 2H, 1-CH_2), 2.19 (m, 2H, 14-CH_2), 2.01 (m, 2H, 4-CH_2), 1.59 (m, 2H, CH_2), 1.44 (m, 4H, 2×CH_2), 1.21 (m, 10H, 5×CH_2), 1.11 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=135.7 (C-10), 129.8 (C-11), 129.1 (C-12), 125.7 (C-13), 72.8 (C_{quad}), 62.8 (C-16), 62.1 (C-1), 33.2 (C-9), 33.0, 32.0, 31.1, 29.9 (2C), 29.8, 28.0 (3×CH_3), 26.6, 24.4, 23.0. EIMS, *m*/*z* (rel int.): 236 (75, $[M-O^tBu].^+$), 253 (25, $[M-^tBu]^+$), 295 (40, $[M-CH_3]$), 310 (35, $[M]^+$). The fluorination of terminal hydroxy group in deacetylated **16** was performed with DAST as described for **2** in 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (m, 1H, 12-CH), 5.93 (t, 1H, 11-CH, J_{1110}=14.0 Hz), 5.64 (dt, 1H, 10-CH, J_{1110}=14.0 Hz), 5.25 (m, 1H,

13-CH), 4.39 (dt, 2H, 16-CH2, J_{HF}=47 Hz, J_{1516}=6 Hz) 3.25 (t, 2H, 1-CH2, J_{12}=7 Hz), 2.23 (m, 2H, 14-CH_2), 2.02 (m, 2H, 4-CH_2), 1.68 (m, 4H, 2×CH_2), 1.49 (m, 4H, 2×CH_2), 1.21 (m, 8H, 4×CH_2), 1.11 (br s, 9H, 3×CH_3). ^{13}C (100 MHz, $CDCl_3$): δ=136.0 (C-10), 130.2 (C-11), 128.1 (C-12), 125.6 (C-13), 83.8 (d,C-16, J_{CF}=164 Hz) 72.8 (C_{quad}), 62.0 (C-1), 33.8 (C-9), 33.3, 31.1, 30.9, 30.7, 29.9, 29.6, 28.0 (3×CH_3), 26.6, 25.0, 23.7. EIMS, *m*/*z* (rel int.): 312 (35, $[M]^{\bullet+}$), 297 (10, $[M-CH_3^{\bullet}]^+$), 255 (25, $[M-{}^t\mathrm{Bu}]^+$).

The deprotection of (4*Z*,6*E*)-16-*tert*-butoxy-1-fluorohexadeca-4,6-diene was performed in two steps as described for **10** in 51% overall yield. Compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (m, 1H, 12-CH), 5.93 (t, 1H, 11-CH, J_{1110}=14.0 Hz), 5.64 (dt, 1H, 10-CH, J_{1110}=14.0 Hz), 5.20 (m, 1H, 13-CH), 4.39 (dt, 2H, 16-CH2, J_{HF}=47 Hz, J_{1516}=7 Hz) 3.57 (t, 2H, 1-CH_2), 2.22 (m, 2H, 14-CH_2), 2.02 (m, 2H, 4-CH_2), 1.65 (m, 2H, CH_2), 1.49 (m, 4H, 2×CH_2), 1.22 (m, 10H, 5×CH_2). ^{13}C (100 MHz, $CDCl_3$): δ=135.9 (C-10), 130.2 (C-11), 128.1 (C-12), 125.6 (C-13), 83.8 (d, C-16, J_{CF}=164 Hz) 63.5 (C-1), 33.2 (2C), 29.9, 29.8 (3C), 29.7, 29.6, 26.1, 23.5 EIMS, *m*/*z* (rel int.): 256 $[M]^{\bullet+}$.

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References and notes

1. Butenandt, A.; Beckmann, R.; Stamm, D.; Hecker, E. *Naturwissenschaften B* **1959**, *14*, 283–284.
2. *Insect Pheromone Biochemistry and Molecular Biology: The Biosynthesis and Detection of Pheromones and Plant Volatiles*; Blomquist, G. J., Vogt, R., Eds.; Elsevier Academic: San Diego, CA, 2003.
3. Tegoni, M.; Campanacci, V.; Cambillau, C. *Trends Biochem. Sci.* **2004**, *29*, 257–264.
4. Krieger, J.; Brier, H. Transduction Mechanism of Olfactory Sensory Receptors. In *Insect Pheromone Biochemistry and Molecular Biology*; Blomquist, G. J., Vogt, R., Eds.; Elsevier Academic: London, 2003.
5. Sandler, B. H.; Nikonova, L.; Leal, W. S.; Clardy, J. *Chem. Biol.* **2000**, *7*, 143–151.
6. Kaissling, K. E. *Chem. Senses* **2004**, *29*, 529–531 Erratum 747.
7. Kaissling, K. E.; Rospars, J. P. *Chem. Senses* **2001**, *26*, 125–150.
8. Kaissling, K. E.; Priesner, E. *Naturwissenschaften* **1970**, *57*, 23–28.
9. Du, G.; Prestwich, G. D. *Biochemistry* **1995**, *34*, 8726–8732.
10. Oldham, N. J.; Krieger, J.; Breer, H.; Fischedick, A.; Hoskovec, M.; Svatoš, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 4341–4343.
11. Leal, W. S.; Chen, A. M.; Ishida, Y.; Chiang, V. P.; Erickson, M. L.; Morgan, T. I.; Tsuruda, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5386–5391.
12. Campanacci, V.; Krieger, J.; Bette, S.; Sturgis, J. N.; Lartigue, A.; Cambillau, C.; Breer, H.; Tegoni, M. *J. Biol. Chem.* **2001**, *276*, 20078–20084.
13. Klusák, V.; Havlas, Z.; Rulíšek, L.; Vondrášek, J.; Svatoš, A. *Chem. Biol.* **2003**, *10*, 331–340.
14. Hoskovec, M.; Šaman, D.; Svatoš, A. *Collect. Czech. Chem. Commun.* **2001**, *66*, 1682–1690.
15. Alexakis, A.; Gardette, M.; Colin, S. *Tetrahedron Lett.* **1988**, *29*, 2951–2954.
16. Bown, H. C.; Korytnyk, W. *J. Am. Chem. Soc.* **1966**, *82*, 3866–3870.
17. Vogt, R. G.; Riddiford, L. M. *Nature* **1981**, *293*, 161–163.
18. Rose, M. Y.; Olson, J. S. *J. Biol. Chem.* **1983**, *258*, 4298–4303.
19. Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100; Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789; Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
20. Hehre, W. J.; Radom, L.; Schleyer, P. V.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley-Interscience: New York, NY, 1986.
21. Woon, D. E.; Dunning, T. H., Jr. *J. Chem. Phys.* **1993**, *98*, 1358–1371.
22. Eichkorn, K.; Weigen, F.; Treutler, O.; Ahlrichs, R. *Theor. Chim. Acta* **1997**, *97*, 119–124.
23. Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kölmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165.
24. Mohl, C.; Breer, H.; Krieger, J. *Invert. Neurosci.* **2002**, *4*, 165–174.
25. Wojtasek, H.; Leal, W. S. *J. Biol. Chem.* **1999**, *274*, 30950–30956.
26. Oldham, N. J.; Krieger, J.; Breer, H.; Svatoš, A. *Chem. Senses* **2001**, *26*, 529–531.
27. Kang, S. K.; Kim, W. S.; Moon, B. H. *Synthesis* **1985**, *12*, 1161–1162.