pheromone production

transmission

perception

processing

behavioural responses

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The Influence of Fluorinated Molecules (Semiochemicals and Enzyme Substrate Analogues) on the Insect Communication System

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Can the introduction of fluorine atoms affect the bioactivity of natural semiochemicals? Can fluorine contribute in the creation of specific enzyme inhibitors to interrupt or disrupt the insect communication system? The first step for the bioactivity of a molecule is interaction with the biological sensor. Hydrogen and fluorine are almost bioisosteric and the receptor site of the enzyme can still recognize and accept the fluoro analogue of its natural substrate. However, the peculiar electronegativity of the fluorine atom can affect the binding, absorption, and transport of the molecule. The differences in the molecule's electronic properties can lead to differences in the chemical interactions between the receptor and the fluorinated substrate. Fluorine introduction can modify the metabolic stability and pathway of the semiochemicals in many different ways. Fluorinated analogues can show synergism, inhibition, or hyperagonism effects on insect behaviors, that is, the activity of the nonfluorinated parent compounds can be mimicked, lost, or increased. In any case, the fluorinated molecules can interact with the bioreceptors in a new and disrupting way. The semiochemicals are olfactory substances: fluorine can affect their volatility or smell. Production of semiochemicals from exogenous substances, perception at antennal receptors, and processing of biological responses are the main steps of communication among insects. In the production step, the fluorinated molecules can interact with enzymes that catalyze the biosynthesis of the natural pheromones. In the perception step, fluorinated semiochemicals can interact with the olfactory receptor cells; this often leads to totally unpredictable behaviors. Fluorinated molecules have been developed as probes to elucidate the complex chemorecognition processes of insects. Many of these molecules have been tested to find highly effective behavior-modifying chemicals. New analogues have been synthesized to investigate the metabolic pathway of a pheromone molecule and many of them are promising disrupting agents. Despite such titanic research efforts, the results have often been random, rational trends in the induced behaviors have sometimes been impossible to find, and practical applications of the fluorinated semiochemicals are still uncertain.

1 Introduction

Humans and insects are opponents in food and health wars.[1] The humans try to interfere with the communication systems of the insects to disturb or kill them. Promising weapons are behavior-modifying chemicals[2] that can reduce or alleviate the ecological complications[3] associated with the use of pesticides and that are fundamental components of the integrated pest-management approach.[4] The semiochemicals (from the Greek: *semeon*, signal) are olfactory or contact behavior-modifying chemicals divided into allelochemicals (*allelon*, of each other) for interspecific communication and pheromones (*phereum*, to carry; *horman*, to stimulate) for intraspecific communication.[5] Can fluorine help humans to create more efficient and selective weapons to overcome the insect enemy? Fluorinated pheromones belong to the general class of halogenated parapheromones, "chemical compounds not known to exist in nature but structurally related to natural pheromone components that in some way affect physiologically or behaviorally the insect communication system."[6] A look at the specialized review *Insect Parapheromones in Olfaction Research and Semiochemical-Based Pest Control Strategies*[6] shows that 59 references are devoted to literature concerning fluorinated analogues and 59 to literature concerning all other possible pheromone modifications: fluorine must play an important role,[7] at least in scientists' minds.

Fluorine mimics hydrogen from a steric point of view but leads to a large perturbation in the electronic distributions; as a consequence, the natural substrate perception could be altered by competitive fluoro analogues binding with specific pheromone receptors.[8] So, fluorinated pheromones are expected to act as agonists, mimics, synergists, hyperagonists, antagonists, antipheromones, and inhibitors.[6] Moreover, fluorine can affect the volatility, thermal and/or oxidative stability, and lipophilicity, all of which are closely related to the pheromonal communication efficiency.[9] Finally, fluorinated pheromones are expected to interfere directly with the communication or to act as valid probes for the investigation of the communication mechanisms.

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The synergistic or inhibiting capability concepts have been nicely defined:[10] "When pheromone analogues that mimicked the biological activity of the natural one at a low dose were co-evaporated at a high dose along with it, male response to the natural pheromone was not inhibited (synergism). However, when analogues that lacked pheromone quality were evaporated at high dose with natural pheromones, male response was inhibited. The inhibitory analogues bind to the pheromone receptors without provoking behavioral response but are not cleared from the receptor as efficiently as the pheromone or as pheromonally active analogues, which are more effectively recognized and removed by substrate-specific catabolic enzymes."

This review presents some examples of interference in the communication system of insects elicited by fluoro analogues of bioactive compounds (pheromones and/or enzymatic substrates), it tries to outline the rationale followed by scientists in projecting the selective fluorine insertion and their efforts in rationalizing the results, and it tries to provide evidence of the trends (if any) in the behavior of fluorinated pheromones.

2 The Pheromonal Communication System of Insects

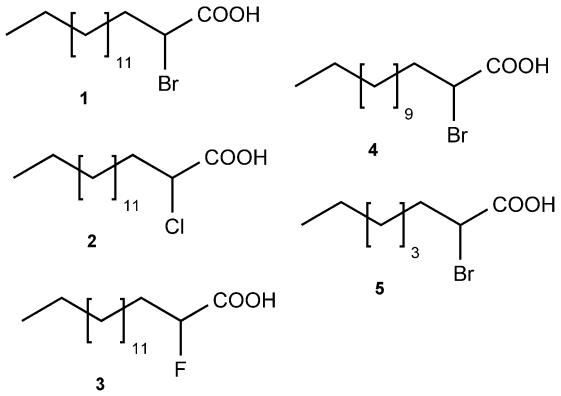
Six main steps can be recognized: 1) specific chemical production, 2) emitter–receiver transmission, 3) receptor-site perception, 4) signal processing, 5) behavioral response, and 6) chemical catabolism. Interference in any of these steps can disturb or destroy the communication. Fluorinated compounds could achieve this aim, especially as a) pheromone-biosynthesis inhibitors, b) probes of the pheromone–receptor interaction, c) pheromone-catabolism inhibitors, d) delayed-action toxicants, or e) metabolic pathway tracers.

2.1 Pheromone-biosynthesis inhibitors

The production step is based on evolved chemical reactions in secretory cells converting exogenous materials into pheromone precursors.[11] Most of the enzymes and effectors involved in this complex pheromone-producing system are still uncharacterized.[134] In many moths, biosynthesis of the female sex pheromone is controlled by the pheromone-biosynthesis-activating neuropeptide,[12] which acts at different steps depending on the species. 2-Bromohexadecanoic acid (**1**) is a nonspecific inhibitor of the membrane enzymes involved in lipid metabolism in mammals.[13] Since the fatty acyl reductases are membrane-bound enzymes, **1** might impair the production of the natural pheromones in those species in which the reduction of the acyl moieties is the neuropeptide-controlled step.[14] Other α-halo fatty acids, **2**–**5**, were evaluated, among which 2-fluorohexadecanoic acid (**3**), the fluoro analogue of palmitic acid[15] was found to be considerably less active than the bromo analogues **4** and **5**. Bromo substitution seemed to be very important for inhibitory action and the chain length seemed to be of secondary importance (Scheme 1).

β oxidation[16] is the first step in the synthesis of *Z*9,*E*11–14:Ac[135] (**6**, Scheme 2), the sex-pheromone component of the Egyptian army worm *Spodoptera littoralis*.[17]

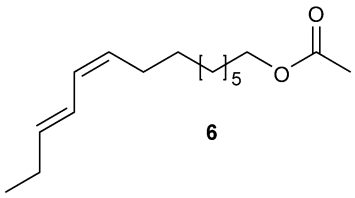
The inhibition of pheromone biosynthesis can occur at any stage between the initial transformation of the substrate pal-



Scheme 1. *Halogenated substrates **1**–**5** for pheromone-biosynthesis inhibition.*

Cristina Pesenti was born in Legnano (Italy) in 1971. She studied chemistry at the University of Milano. She graduated in 1996. Her first job was in the PRASSIS-Research Institute of Sigma Tau (Settimo Milanese) working on to the synthesis of new potentially active pharmacological compounds. In 1997 she moved to Prof. Pierfrancesco Bravo's laboratories where she applied for the School of Specialization in Organic Synthesis and worked on the synthesis of fluorinated analogues of GABOB and pepstatine, as well as on the preparation of fluoro analogues of pheromonally active compounds. In the same research group, she is completing her PhD thesis based on the study of fluorinated analogues of HIV-1 protease inhibitors.

Fiorenza Viani was born in Brescia (Italy) in 1959. She studied industrial chemistry at the University of Milano. She graduated in 1984. In the same year she moved to Prof. Pierfrancesco Bravo's laboratories where she applied for the School of Specialization in Organic Synthesis. She worked on the preparation of fluorinated analogues of natural compounds. Her field of interest involved the synthesis of fluoro analogues of nucleosides as potentially antiviral agents, of fluorinated alkaloids and amino acids, and of fluorinated analogues of semiochemicals. She is still working in the same research group and bioorganic chemistry is her main field of interest.



Scheme 2. *Natural pheromone (Z,E)-9,11-tetradecadienyl acetate (Z9,E11–14:Ac,* ***6****).*

mitic acid into its coenzyme A (CoA) ester and the final conversion into acetyl-CoA (Figure 1).[18]

The model compound (2-bromopalmitic acid **1**, Scheme 1) is known to be a good inhibitor of palmitoyl-CoA oxidation after its conversion into 2-bromopalmitoyl-CoA.[16b]

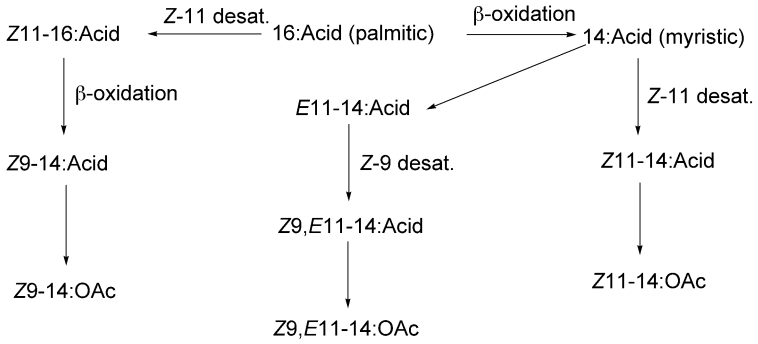
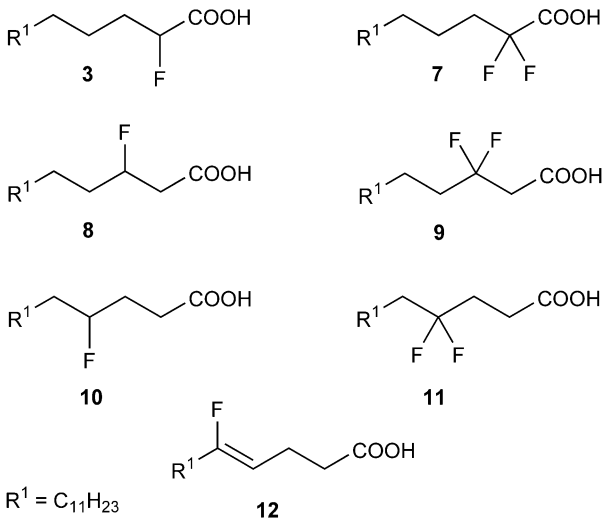


Figure 1. *Biosynthesis of the natural pheromone* ***6*** *from palmitic acid through β oxidation.*

The incorporation of fluorine in the 2- (compounds **3** and **7**, Scheme 3) and/or 3-position (compounds **8** and **9**) could complicate the possible abstraction of any proton at these sites by the acyl-CoA dehydrogenase (enzymatic oxidation blockage).

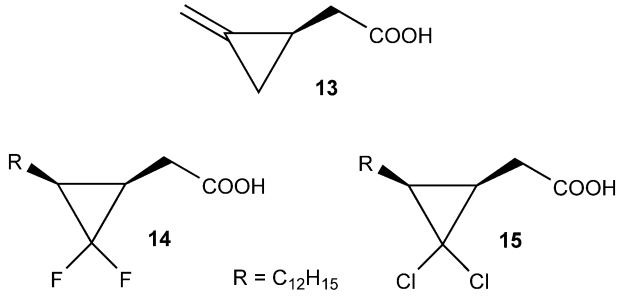


Scheme 3. *Fluorinated substrates* ***3*** *and* ***7–12****, analogues of 2-bromopalmitic acid (****1****) for blockage of β oxidation.*

Moreover, fluorine(s) in the 4- (compounds **10** and **11**) and 5-positions (compound **12**) could increase, through electron-withdrawing effects, the electrophilicity of the carbon atom at the 3-position and the β-oxidation product could be attacked by an enzyme nucleophilic site, thereby causing a "suicide" enzymatic inhibition.[19] 3-Fluorohexadecanoic acid (**8**) turned out to be the most promising inhibitor, while the introduction of a second fluorine at the 2-position (**7**) did not affect the inhibitory potency and the introduction of a second fluorine at the 3-position (**9**) decreased the inhibitory potency.

cis-(2,2-Difluoro-3-dodecylcyclopropyl)acetic acid (**14**, Scheme 4) and its dichloro analogue **15**[20] were evaluated as potential biosynthetic inhibitors due to their structural similarities to (methylenecyclopropyl)acetic acid **13**,[21] whose CoA ester is believed to inhibit the oxidation of fatty acids in vivo and in vitro.[22]

The generated carbanionic species for **14** and **15** could open the cyclopropane ring to form an activated halodiene, a potentially good Michael acceptor capable of irreversibly alkylating a nucleophilic active site of the enzyme. Nevertheless, no activity was found for **14**, whilst **15** showed some interesting data. The striking differences in the activities of **14** and **15** (inhibition potencies of 5.7 and 61.9%, respectively) were rationalized in terms of the donating ability of the fluorine atom when located on an sp^2 carbon (on β and γ carbons):[23] the fluorinated diene-CoA ester could not be as good a Michael acceptor as expected and was less active than its chlorinated analogue.



Scheme 4. *Cyclopropyl halogenated substrates* ***14*** *and* ***15****, analogues of (methylenecyclopropyl)acetic acid* ***13*** *for biosynthesis inhibition.*

2.2 Transmission from the emitter to the receiver

The transmission step is based on odorant molecules with relatively low molecular weight[24] or on molecules that interact through physical contact, that is, contact pheromones in the cockroach species.[25] Most of their physical properties (water solubility, vapor pressure, polarity, lipophilicity, and surface activity), as well as their reactivity, can be affected by the introduction of fluorine.

2.3 Pheromone–receptor interaction probes

2.3.1 Pheromone binding and transport

The odorant-binding proteins are a class of proteins, 13–17 kDa in size, that facilitate the passage of hydrophobic odorant molecules from the environment to the surface of the olfactory receptor neurons. They are present in the sensillar lymph at enormous concentrations (10–20 mM). In Lepidoptera, there are two distinct classes of such proteins: the pheromone-binding proteins, devoted to sex-pheromone recognition, and the general odorant-binding proteins, specialized in general odorant recognition.[26]

2.3.2 Pheromone–receptor interactions

For Lepidoptera pheromones, a three-part static model was at first invoked:[27] 1) the polar functional group, involved in hydrogen bonding and/or electrostatic interactions, 2) the carbon–carbon double bond, involved in electrostatic interactions, and 3) the terminal alkyl group, involved in weak dispersion forces, are recognized by the corresponding three "active sites" on the receptor surface. Then, dynamic models were considered where the substrate molecule is bound stepwise to the receptor:[28] the polar group is responsible for the substrate–receptor primary recognition, while the other parts of the molecule are responsible for excitation functions. The same three pharmacophore elements, alkylic chain, double bond(s), and polar group, are usually the main targets in fluoro-substitution projects for structure–activity relationship studies.

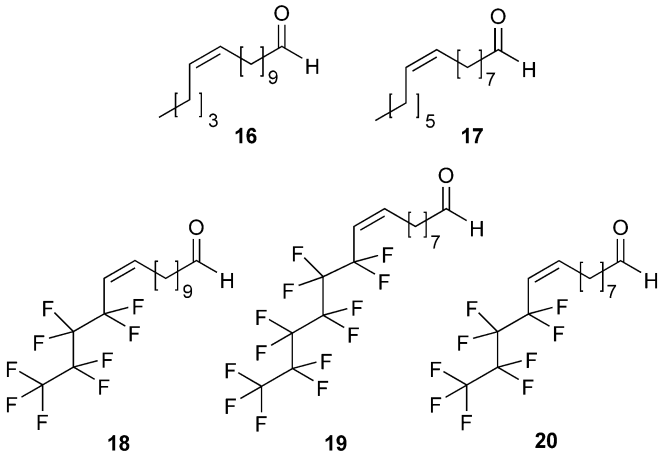
2.3.3 Behavioral responses

Wind-tunnel assays, field-test experiments, and actographic analyses detect external male responses. Each olfactory receptor cell is a very efficient bipolar neuron where a few hundred molecules of pheromone can be detected from the environment.[29] The inner nervous responses to the stimuli can be detected through a single sensillum recording (SSR), related to the electronic activity at a single receptor cell, or through an electroantennogram apparatus (EAG) that reflects the combined activity of many olfactory cells on the antennae.[30] The dose-response EAG curves display the inner/outer responses following two simple rules: a linear dose–activity relationship suggests a direct receptor–molecule interaction and shape similarities for two different chemicals indicate that they are interacting with the same receptor. There are three important kinetic parameters that can give an evaluation of the extent and/or the kind of interaction between a pheromone/parapheromone and a receptor. The electroantennographic peak amplitude (PA, in mV) is proportional to the intrinsic activity, the depolarization time (the time to reach 4/5 of the depolarization, 4/5DT, in ms) is linked to the kinetics of the interaction with the receptor and is correlated to the receptor–substrate fit, and finally, the repolarization time (the time to reach 2/3 of the repolarization, 2/3RT, in ms) is linked to the kinetics of substrate removal from the receptor. The intrinsic activity, that is, the molecule–receptor interaction capabilities of a pheromone analogue, is evaluated when tested alone; the inhibition or the synergistic activities are evaluated from mixtures with the corresponding natural pheromone(s).

2.3.4 Chain modifications

Perfluorination: Perfluorination at the hydrocarbon end of a molecule imparts a dualistic polar–nonpolar nature to the molecule and reduces its solubility in both aqueous (hydrophilic) and lipid (hydrophobic) environments.[31] At a macroscopic level, the perfluoroalkyl compounds have larger dipole moments and they are more volatile, less lipophilic, and more stable; at a molecular level, the introduction of perfluoroalkyl chains on a natural pheromone molecule increases the bulkiness, reduces the chain flexibility, and reduces the chain hydrophobicity.[32]

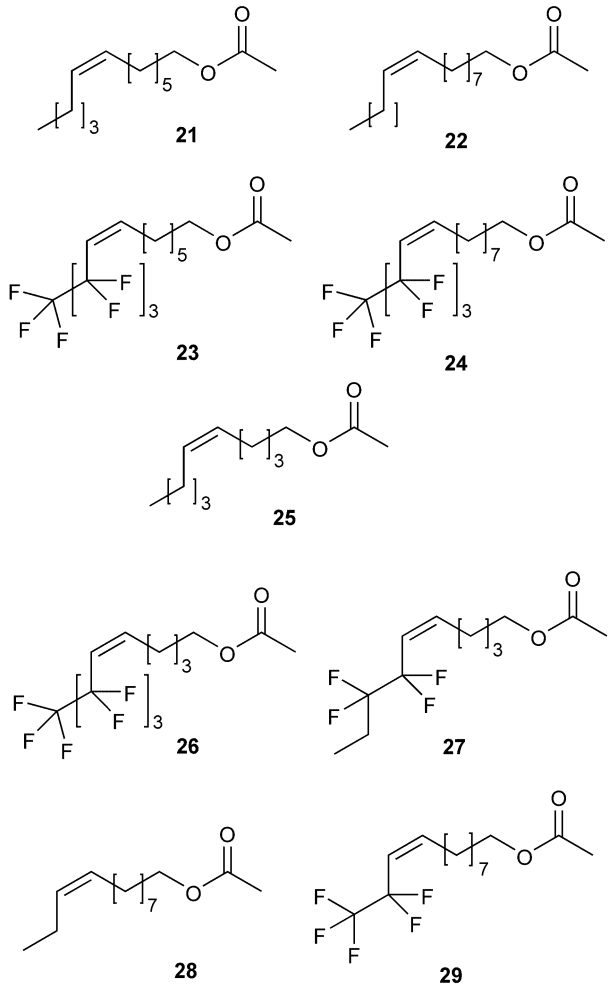
The major components of the pheromone blend of the earworm moth *Heliothis zea*,[33] *Z*11–16:Ald (**16**), and of the southern corn borer *Diatraea grandiosella*,[34] *Z*9–16:Ald (**17**),[31] were targeted (Scheme 5). The fluoro analogues **18**–**20** were all



Scheme 5. *Aldehyde pheromones (Z11–16:Ald (**16**) and Z9–16:Ald (**17**)) and the corresponding polyfluorinated aldehyde parapheromones Pfb-Z11–16:Ald (**18**), Pfh-Z9–16:Ald (**19**), and Pfb-Z9–14:Ald (**20**).*

more volatile than the corresponding molecules and the pheromone receptor neurons (by SSR measurements) still responded to **18**–**20**, albeit at higher concentrations.

EAG and wind-tunnel activity studies[35a–d] were run on the major components of the pheromone blend of the cabbage looper *Trichopulsia ni*,[36] *Z*7–12:Ac (**21**) and *Z*9–14:Ac (**22**), of the turnip moth *Agrotis segetum*,[37] *Z*5–10:Ac (**25**), and of the grape berry moth *Eupoecilia ambiguella*,[38] *Z*9–12:Ac (**28**, Scheme 6).[35e] Both **23** and **24** were required in 1000–10000 times higher concentrations than the natural pheromones for equal output, **24** was inactive alone but elicited a synergistic activity (mimicking the pheromone action) when mixed with **21** and **22** in traps, **26** gave no response, **27** was 100-fold less active than **25**, and **29** was only weakly active, that is, it



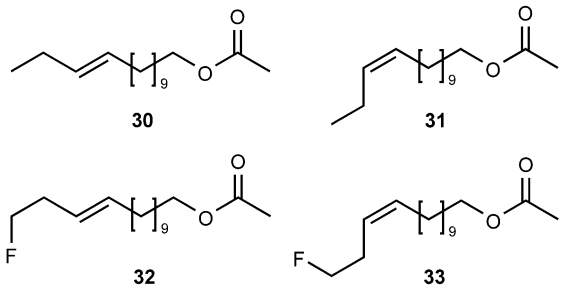
*Scheme 6. Acetate natural pheromones Z7–12:Ac (**21**) and Z9–14:Ac (**22**) with their corresponding fluoro analogues Pfb-Z7–12:Ac (**23**) and Pfb-Z9–14:Ac (**24**); pheromone Z5–10:Ac (**25**) with its fluoro analogues Pfb-Z5–10:Ac (**26**) and 7,7,8,8-Ttf-Z5–10:Ac (**27**); pheromone Z9–12:Ac (**28**) and its fluoro analogue Pfe-Z9–12:Ac (**29**).*

evoked slow activation and take-off but low attraction to the source. However, it showed an unexpected synergistic effect with an enhancement in trap catches when it was mixed with **28**.

The fluoro analogues give hardly rationalizable effects and no general trend can be outlined. The fluorinated chain is less hydrophobic than that of the corresponding natural molecules and, probably, the alteration of the hydrophobicity of the terminal alkyl group leads to a reduction in the receptor affinity. However, the functionality and the chain-length are still recognized.[28]

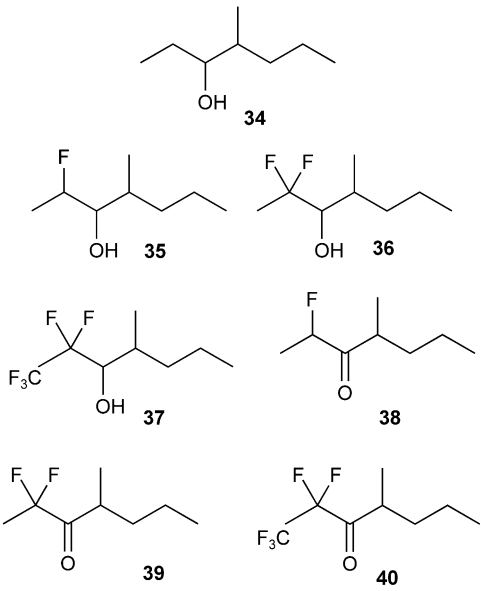
The terminal part of the chain: A single fluorine is not sterically demanding and this is true whether it is placed in the terminal or in the middle part of the chain, but a trifluoromethyl group has to be considered a bulky substituent and it could seriously affect the biological properties of a fluoro analogue.[7a]

The sex-pheromone blend of the European corn borer *Ostrinia nubilalis*[39] is produced in two genetically distinct pheromonal types with different *E*/*Z* proportions of **30** (*E*) and **31** (*Z*, Scheme 7) depending on the family: insects of *E*-Type respond to a 3:97 *Z*/*E* mixture, those of *Z*-Type respond to a 97:3 *Z*/*E* mixture.[40] The stimulating and inhibiting properties of fluorinated molecules on male behaviors were determined by testing *anti*-**32** against *E*-Type and *syn*-**33** against *Z*-Type families of insects.[41] The fluoro analogues **32** and **33** showed pheromonal activities, in wind-tunnel analyses as well as in the field, that were indistinguishable from those of the corresponding natural pheromones.



*Scheme 7. Monofluorinated analogues 14F,E11–14:Ac (**32**) and 14F,Z11–14:Ac (**33**) of pheromones E11–14:Ac (**30**) and Z11–14:Ac (**31**).*

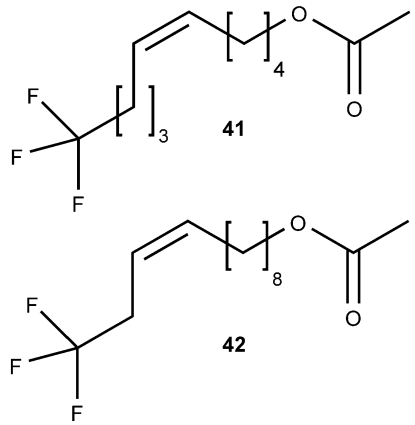
4-Methyl-3-heptanol (**34**) is one component of the aggregation pheromone of the European elm bark beetle *Scolytus multistriatus,* a nonflying insect.[42] The fluoro analogues **35**–**40** (Scheme 8) were tested through a laboratory walking-beetle assay.[43] The activity (**34** ≈ **35** > **36** > **37** for alcohols; and **38** > **39** > **40** for the less active ketones) decreased with an increasing number of fluorine atoms in the chain. The alcoholic oxygen atom is thought to link to the receptor in a noncovalent interaction (hydrogen bond) as a proton acceptor; this



*Scheme 8. Natural pheromone 4-methyl-3-heptanol (**34**) and its fluorinated alcohol (**35**–**37**) and keto analogues (**38**–**40**).*

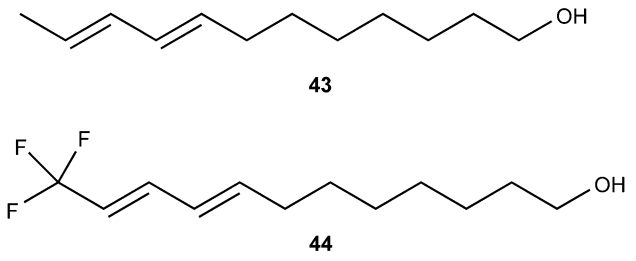
would explain the low activity of the keto analogues. The lower activity of the parapheromones with respect to that of the natural pheromone can be interpreted by considering the presence of fluoroalkyl electron-withdrawing groups that decrease the basicity of the hydroxy group and, consequently, the bond strength to the proton donor.

A terminal trifluoro-substitution process was performed on *Z*5–10:Ac (**25**)[35c] and on the major component of the pheromone blend of the grape berry moth *Eupoecilia ambiguella*, *Z*9–12:Ac (**28**).[35a] Reduced biological activity was observed for analogue **41** (Scheme 9, tenfold less active than **25**), but in a lesser extent than that observed for the already cited corresponding perfluorinated analogue **26**. The other fluoro analogue **42** was inactive.[44]



Scheme 9. *10,10,10-Tf-Z5–10:Ac (**41**) and 12,12,12-Tf-Z9–12:Ac (**42**), fluoro analogues of Z5–10:Ac (**25**) and Z9–12:Ac (**28**).*

The main sex-pheromone component of the codling moth *Cydia pomonella*,[45] codlemone (**43**, Scheme 10), turned out to be satisfactory for pest monitoring but less reliable for pest control due to its chemical degradation and/or isomerization under field conditions. Actually, after 150 days, more than half of the compound was lost due to its chemical decomposition.



Scheme 10. *Codlemone (E8,E10–12:Ac, **43**) and its trifluoro analogue E8,E10,12,12,12-Tf-12:Al (**44**).*

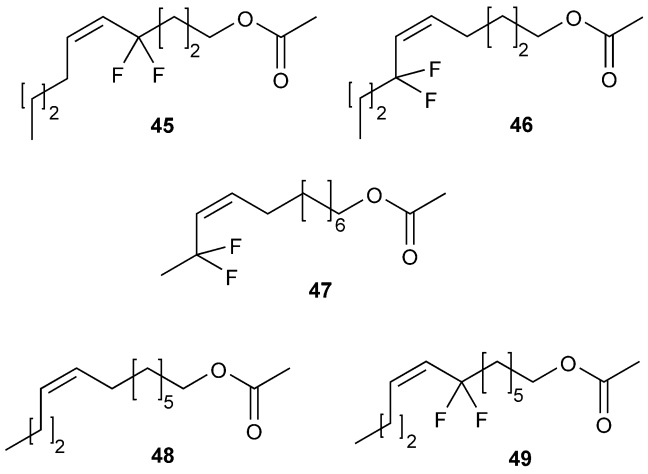
In the search for more stable analogues, **44** was synthesized,[46] but the insertion of three fluorine atoms on the terminal carbon atom of the chain dramatically decreased the electroantennographic activity.[47]

The bioactivity of the fluoro analogues seems negatively correlated with the number of fluorine atoms in the terminal methyl group. Reduction in the absorption and transport of the parapheromones in vivo cannot fully explain the loss of electroantennographic activity, since the trifluoromethyl group is among the most lipophilic substituents. A lower affinity for the receptor sites is probably involved.[31]

2.3.5 Double-bond modification

Fluorine(s) adjacent to double bonds: A difluoromethylene group adjacent (α) to a double bond may affect both the conformational chain flexibility and the double-bond reactivity. The strong permanent dipole of the carbon–fluorine bond may interfere with proper binding to the receptor site.[48]

Electroantennographic and wind-tunnel activity tests were run[49] on the difluoro analogues (Scheme 11) of the major components of the pheromone blend of *Agrotis segetum* (**25**), *Eupoecilia ambiguella* (**28**), and the oriental fruit moth *Grapholita molesta*[50] (**48**). As a general trend, difluoro substitution decreased the volatility. Analogues **45** and **46** were 100-times less active than the natural molecule **25**, whereas **47** was equipotent with the pheromone **28** and **49** elicited a synergistic activity (that is, the natural pheromone action was mimicked) when mixed with the natural pheromone **48**.

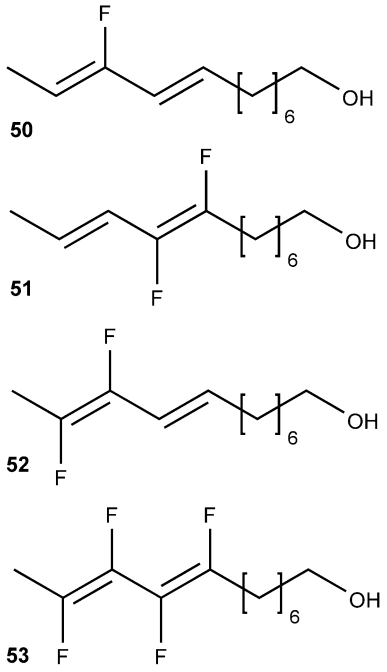


Scheme 11. *Difluorinated analogues (**45**–**47** and **49**) with fluorine atoms adjacent to the double bonds of the natural pheromones Z5–10:Ac (**25**), Z9–12:Ac (**28**), and Z8–12:Ac (**48**).*

In selected species, fluorination at the allylic position enhances, or at least preserves, the pheromonal activity. In all cases, the maintenance of the geometry of the double bond appears to be essential for the bioactivity.

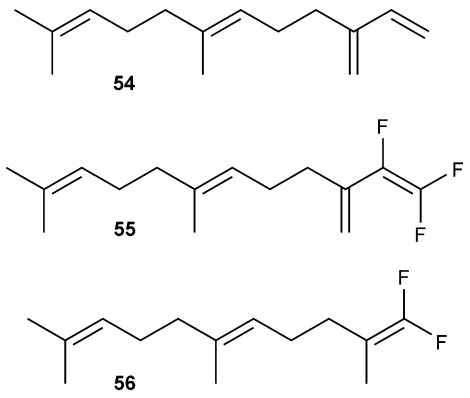
Fluorine on double bonds: The introduction of a single fluorine atom on the double bonds of a pheromone molecule is expected to affect the chemical stability more than the biological activity of the fluoro analogue.[51]

"The first fluorine-containing pheromone ever synthesized was presumably (8*E*,10*Z*)-10-fluoro-8,10-dodecadien-1-ol [**50**]. It was found in antennographic studies to behave identically to codlemone [**43**] whereas a 10-methyl-branched analogue proved completely inactive."[7a] Different analogues[52] of codlemone **43** elicited different activities (Scheme 12). The "terminal" difluoro analogue **52** showed single sensillar and electroantennographic responses similar to those of the natural pheromone, while the "inner" analogue **51** elicited weaker responses and the complete fluorination of the diene system (**53**) resulted in loss of the electrophysiological activities. The interesting higher activity elicited in field tests by **52** in comparison with that elicited by the natural pheromone was not due to differences in volatility, since **52** and **43** showed similar vapor pressure. The higher chemical stability of **52** might explain its better performance under external conditions.[47]



Scheme 12. *Monofluoro (10F,E8,Z10–12:Al (**50**)), difluoro (8,9-Df-E8,E10–12:Al (**51**) and 10,11-Df-E8,E10–12:Al (**52**)), and tetrafluoro (8,9,10,11-Ttf-E8,E10–12:Al (**53**)) analogues of codlemone (**43**).*

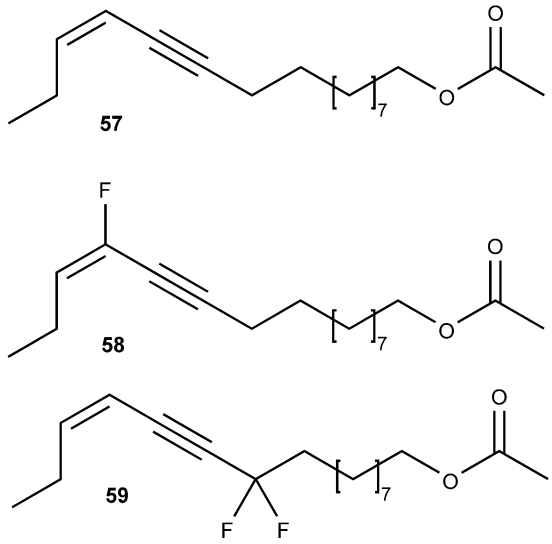
The alarm pheromone of aphids *Myzus persicae* and *Lipaphis erysmi*,[53] farnesene (**54**), enhances the effectiveness of the contact insecticide permethrin against the aphids in laboratory bioassays (Scheme 13). Two fluoro analogues, **55** and **56**, gave exciting results and showed higher activity than farnesene itself. Analogue **55** moved almost all of the population of *Myzus persicae* and 90% of *Lipaphis erysmi*. Analogue **56** moved 98% of all aphids in comparison to the 84% moved by the natural pheromone.[54] On the other hand, fluorine atoms



Scheme 13. *Natural pheromone (E)-β-farnesene (**54**), trifluoro analogue **55**, and difluoro parapheromone **56**.*

on the conjugated diene system of farnesene did not improve the stability to air and light: as a matter of fact, **55** turned out to be less stable (half life = 6 h) than **54** (half life = 21 h). Compound **56**, only partially structurally related and only prepared because of difficulties in the chemical synthesis of **55**, showed an interesting stability to ultraviolet irradiation in air (half life = 220 h) as well as a preserved bioactivity.

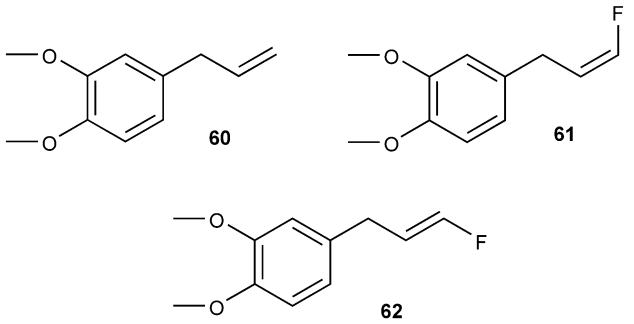
Scheme 14 shows the fluoro analogues[55] of pityolure **57**, the main component of the sex-pheromone blend of the pine processionary moth, *Thaumetopoea pityocampa*.[56] In field tests, **58** was slightly active alone and showed significant inhibition of the pityolure activity. Analogue **59** was essentially inactive alone; after preexposure of the males to its vapors, a 24% reduction of the antennographic response of the insects to pityolure and a 49% decrease in male catches were produced. The loss of activity of **58** and **59** was thought to be mainly a consequence of the profound electronic changes, rather than of the small differences in volume. Actually, the ol-



Scheme 14. *Natural pheromone pityolure (**57**) and its fluoro analogues (Z)-13-fluoro-13-hexadecen-11-ynyl acetate (**58**) and (Z)-10,10-difluoro-13-hexadecen-11-ynyl acetate (**59**).*

factory receptor can still recognize the analogues (the inhibition of the pityolure activity comes from the tolerated steric perturbation of the analogues), while the signal transduction is blocked (the absence of intrinsic activity of the analogues comes from the electronic changes in the molecules).

The eugenol methyl ether (**60**, Scheme 15) is a potent and specific natural plant kairomone, attractant for the oriental fruit fly *Bactrocera dorsalis*.[57] The fluorine isosteric replacement was studied with the aim of enhancing the metabolic or chemical stability and reducing the kairomone toxicity. As a matter of fact, the fluoro olefins **61** and **62** were tested as inhibitors



Scheme 15. *Natural kairomone (eugenol methyl ether, **60**) and its fluoro analogues **61** and **62** as enzyme inhibitors.*

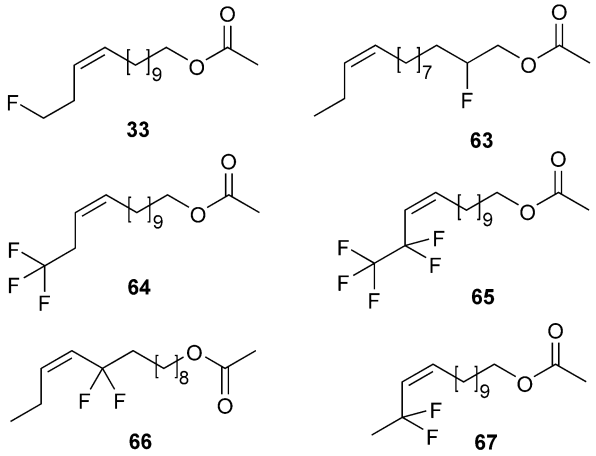
of the enzymes that catalyze the oxidation of the allylic methylene to the toxic species. Analogue **62** was as active as **60** in field tests, a mixture of **62** and **61** was appreciably active, while **61** showed substantially lower activity.[58]

Computational studies: In the absence of crystallographic data for the pheromone-binding site, computational studies were performed with the aim of rationalizing the effects of the insertion of the fluorine atom on the alkenylic chain.[59]

A structure–activity relationship study on the pheromone of the *Z*-Type European corn borer, (*Z*)-11-tetradecen-1-ol acetate (**31**, Scheme 16), was published.[60] Selected fluorinated molecules of known bioactivity were chosen: monofluoro (**33**, **63**) and trifluoro (**64**) analogues that mimicked the natural pheromone activity together with inactive analogues having the fluorine atom adjacent to the double bond (**65**–**67**).

The molecular volume and surface area of the lowest energy conformation of **31** and of active and inactive fluoro analogues were too similar to be important structure–activity parameters. Correlations between the dipole moment and activity were not evidenced but the differences in the extreme values of the electrostatic potential map for the natural and the fluorinated pheromones could reflect acceptor-interaction mechanisms. As a matter of fact, the local maxima for the protons of the *Z* double bond (**31**, **33**, **63**–**67**) were consistently different between the active and the inactive fluoro analogues.

The absence of fluorine atoms on the carbon atoms adjacent to the double bond seems essential to maintain the pheromonal activity.



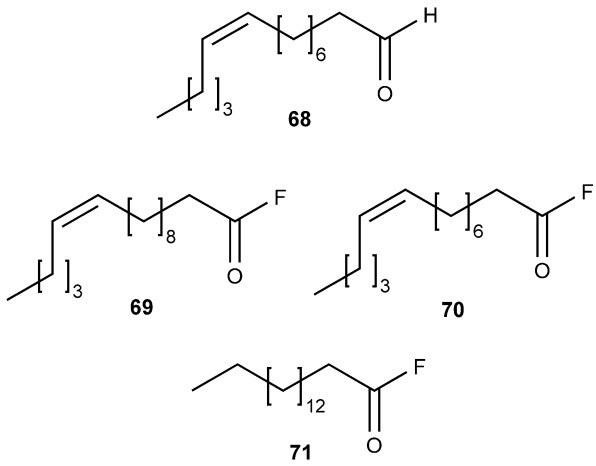
Scheme 16. *Fluorinated molecules (**33**, **63**–**67**) included in the computational studies.*

2.3.6 Polar-group modification

Strict requirements of shape and electronic distribution (dipole-moment directions) are expected for a successful interaction with the olfactory receptor cells.[27, 28a, 61]

Aldehyde: Retinoyl fluoride is a retinal mimic[62] that gives specific inactivation of bovine opsin through irreversible formation of a covalent amide adduct with the primary amino group of the enzyme.[63] An acyl fluoride whose structure is related to that of the pheromone could act in a similar manner with the olfactory receptor, thus leading to a severe disturbance of the behavioral responses of insects.

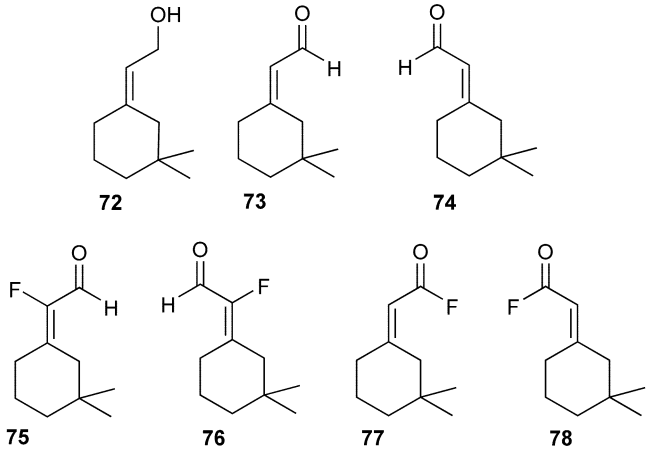
Pheromones **16** and **68** are the major components (in 15:1 relative ratio) of the sex-pheromone blend of the tobacco budworm Noctuidae *Heliothis virescens*[64] (Scheme 17). The acyl fluoride analogues (**69**–**71**) were tested as disrupting agents. An equimolar mixture of compounds **69** and **70** gave an ab-



Scheme 17. *Acyl fluorides (Z11–16:Acf (**69**), Z9–14:Ald (**70**), and 16:Acf (**71**)) as fluoro analogues of the pheromones Z11–16:Ald (**16**) and Z9–14:Ald (**68**).*

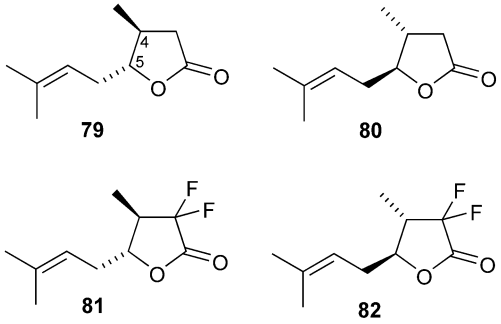
normal male response during daylight, with production of aberrant aphrodisiac-like sexual responses (hyperagonism). The nonstructurally related **71** was totally inactive. Unfortunately, the chemical stabilities of **69** and **70**, with half-lives of 2.5 h in air and of 2–20 days in anhydrous organic solvents prior to giving the acid, were not compatible with their practical use.[65]

Acyl fluorides and α-fluoro aldehyde analogues[66] of the components of the aggregation pheromone blend of the boll weevil *Anthonomus grandi*[67] were tested (Scheme 18). Electroantennographic assays showed no activity for the acyl fluorides **77** and **78**, whilst the α-fluoro aldehydes **75** and **76** gave a 100- and 1000-fold lower activity than the respective natural pheromones (**73** and **74**). In a field test, the amounts of insects captured by the pheromone alone and by mixtures of the pheromone and fluoro analogues **75** and **76** were nearly identical, with no inhibitory effect shown. Moreover, the male/female ratio of the collected insects didn't show any fluctuation. The higher electrophilicity of the carbonyl group in the acyl fluorides **77** and **78** presumably reduces the ability of the analogues to stimulate the receptor and causes inactivity, while the lower electrophilicity of the same carbonyl group in the α-fluoro aldehydes **75** and **76** creates a potent agonist and explains the pheromone-mimicking behavior. This example was the first reported for fluorinated pheromones showing the same activity as the natural ones in Coleoptera and led to the preparation of experimental traps baited with **75** and/or **76**.



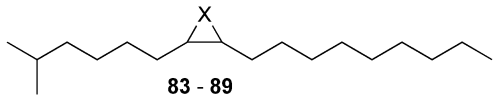
Scheme 18. *Pheromones **72**–**74**, α-fluoro aldehydes **75** and **76**, and acyl fluorides **77** and **78** as disrupting agents.*

Lactones: The fluoro analogues **81** and **82** of the sex pheromone of the African sugarcane borer[68] *Eldana saccharina* were recently studied (Scheme 19).[69] The difluoromethylene moiety is believed to act similarly to an ether oxygen atom in vivo and interesting biological activities are known for α,α-difluorinated analogues of biomolecules.[48,70] Although the size of the fluorine atom minimizes the difluoromethylene steric hindrance, the electronic effects of the *gem*-difluorination event are unpredictable. Surprisingly, the electroantennographic dose-response curves of the fluorinated molecules **81** and **82** were very similar to those of the natural pheromones **79** and **80** and, moreover, at higher doses **82** gave higher antennographic measurement values than the more active pheromone **79**. The introduction of the difluoromethylene group on the polar moiety seems not to disturb its perception at the receptor and the promising fluoro analogues may be used as efficient probes to investigate the eldanolide–receptor interaction.



Scheme 19. *Natural pheromones (+)-(4*S*,5*R*)- and (−)-(4*R*,5*S*)-eldanolide (**79** and **80**, respectively) and the fluoro analogues (+)-(4*R*,5*R*)-3,3-difluoroeldanolide (**81**) and (−)-(4*S*,5*S*)-3,3-difluoroeldanolide (**82**).*

Epoxides: The oxygen atom of the polar group of the disparlure sex pheromones (+)-(7*R*,8*S*)-**83** and (−)-(7*S*,8*R*)-**84** from the gipsy moth *Lymantria dispar*[71] was chosen for some selective modifications (Scheme 20) and the resulting effects were investigated by using electroantennogram assays.[72] The epoxide group of disparlure was replaced by various three-membered rings in the analogues: racemic mixtures of (7*R*,8*S*)-/(7*S*,8*R*)-1,1-difluoro-2-(5-methylhexyl)-3-nonylcyclopropanes (difluorocyclopropyl analogues **85**), of (7*R*,8*S*)-/(7*S*,8*R*)-1,1-dichloro-2-(5-methylhexyl)-3-nonylcyclopropanes (dichlorocyclopropyl analogues **86**), and of (7*R*,8*S*)-/(7*S*,8*R*)-1-(5-methylhexyl)-2-nonylcyclopropanes (cyclopropyl analogues **87**) were tested. The aziridinyl analogues, (7*R*,8*S*)- and (7*S*,8*R*)-2-(5-methylhexyl)-3-nonylaziridine (**88** and **89**, respectively) were investigated either as enantiopure compounds or as a racemic mixture.



Scheme 20. *Disparlure and its analogues. Active (7*R*,8*S*)-(+)-disparlure (**83**, X = O) and inhibitor (7*S*,8*R*)-(−)-disparlure (**84**, X = O); three-membered ring analogues: difluorocyclopropyl **85** (X = CF_2), dichlorocyclopropyl **86** (X = CCl_2), cyclopropyl **87** (X = CH_2), (7*R*,8*S*)-aziridinyl **88** (X = NH), and (7*S*,8*R*)-aziridinyl **89** (X = NH).*

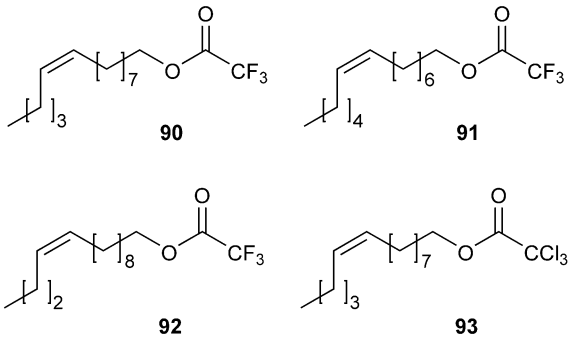
It is known[73] that separate sensory neurons respond to (+)- and (−)-disparlure (**83** and **84**, respectively). The (+) enantiom-

er elicits attractiveness to *Lymantria dispar* males while a blend of the enantiomers inhibits the attraction of the males to the females. The authors found that the antennal receptors of males were twofold more responsive to (+)-disparlure (**83**) than to (−)-disparlure (**84**). Among the racemic series, the antennographic responses of the difluorocyclopropyl analogues **85** and the dichlorocyclopropyl analogues **86** were greater than that of the cyclopropyl analogues **87**. These results might be explained by taking into account the inductive effects of the fluorine and chlorine atoms that mimic the electron-withdrawing effect of the oxygen atom in the natural pheromone. The greater value for the difluorocyclopropyl analogues **85**, as compared to that of the dichlorocyclopropyl analogues **86**, might indicate less steric hindrance. Actually, the difluoromethylene group is considered an isostere of the oxygen atom. The greater antennographic activity of (+)-(7*R*,8*S*)-aziridine analogue **88** relative to that elicited by (−)-(7*S*,8R)-**89** correlates with the relationship of these analogues with the two enantiomers of disparlure, (+)-(7*R*,8*S*)-**83** and (−)-(7*S*,8*R*)-**84**. An intermediate response was elicited by the **88**/**89** racemate. In all the analogues, the antennographic responses were dramatically lower than those of the less responsive disparlure enantiomer **84**, a result indicating that the steric and the polar requirements for the interactions with the receptor must be stringent. In field experiments, the difluorocyclopropyl analogue **85** was found unattractive alone and had no significant effect on the number of male moths responding to (+)-**83** or to racemic (±)-disparlure (**83**/**84**); this reflects the specificity of the receptors for (+)-disparlure (**83**) and (−)-disparlure (**84**).

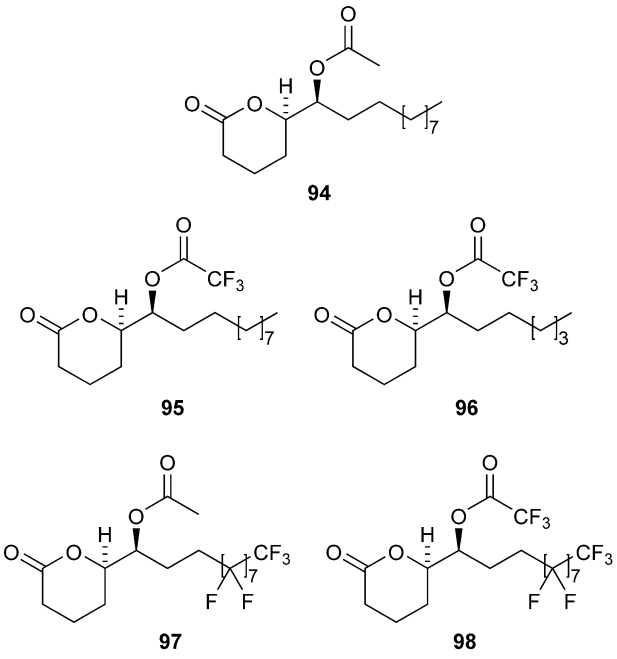
Esters: In the very first example of halogenated analogues tested as positive antipheromone compounds,[74] some analogues were chosen because of their structural similarities to (*Z*)-11-hexadecenal (**16**), the major components of the seven-component sex-pheromone blend of the tobacco budworm moth *Heliothis virescens*.

The trifluoroacetyl analogue **90** (Scheme 21) inhibited most insect mating (89%) for a few days after its foliar application, **91** showed a very low inhibition (5%), **92** gave no inhibition (1%), and the chloro analogue **93** elicited the strongest inhibitory effect (93%). Specific information on the nature of the receptor was (and still is) absent, so the observed totally reversible inhibition was tentatively explained as being due to an associative–dissociative mechanism between the fluorinated molecules and the antennal receptor. The strong inhibitory effects of **90** and **93** were rationalized on the basis of the ability of the trifluoro- and trichloromethyl electron-withdrawing groups to increase the electrophilic nature of the carbonyl moiety.[61b] The absence of inhibitory activity of **91** and **92** was explained by invoking different receptors, because the corresponding alkenylic chains are different.

Fluorine was introduced in the polar group (trifluoroacetyl analogues **95** and **96**), in the alkylic chain (pentafluoro analogue **97**), and in both moieties (pentafluoroalkyl-trifluoroacetyl analogue **98**)[56,75] of the oviposition pheromone **94** of the mosquito *Culex quinquefasciatus*[76] (Scheme 22). Only **98** was inactive; **95**–**97** elicited a marked egg-deposition enhancement



Scheme 21. *The first family of halogenated analogues, Z9–14:Tfm-Ac (**90**), Z8–14:Tfm-Ac (**91**), Z10–14:Tfm:Ac (**92**), and Z9–14:Tclm-Ac (**93**), tested as antipheromones of the natural pheromone, Z11–16:Ald (**16**).*

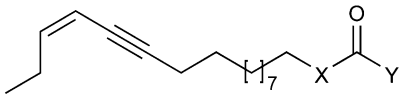


Scheme 22. *Hexadecanolide analogues (**95**–**98**) of natural pheromone (−)-(5R,6S)-6-acetoxy-5-hexadecanolide (**94**).*

in comparison with that observed for the untreated experiment. The effects on the volatility were impressive: whilst the natural pheromone **94** was relatively involatile with an attraction range of only few centimetres, all the active analogues showed higher vapor pressures (**95**, fifty-seven times; **96**, five times; **97**, four times more volatile than **94**). Compounds **95** and **97** were hydrolyzed in water but their bioactivities were retained. On the other hand, **98** lost its activity just because of water hydrolysis. Surprisingly, in spite of the chain shortening from ten to six carbon atoms, **96** retained a marked activity.

The trifluoromethyl ketone **102** was chosen as a bioisosteric analogue of pityolure **57** where the ether oxygen atom has

been replaced by the methylene group.[77] Chlorinated para-pheromones (**103**–**105**) were selected to study the effects of higher spatial volume and lower polarity in comparison with the fluoro analogues **99**–**101**. No iodo/bromo derivatives were included, because of their higher bulkiness (Scheme 23). All

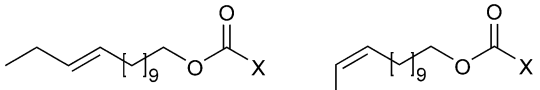


99 - 105

Scheme 23. *Structurally related halogenated analogues (**99**–**105**) of pheromone pityolure (**62**): (Z)-13-hexadecen-11-ynyl fluoroacetate (**99**, X = O, Y = CH_2F), difluoroacetate **100** (X = O, Y = CHF_2), trifluoroacetate **101** (X = O, Y = CF_3), trifluoro-16-nonadecen-14-yn-2-one (**102**, X = CH_2, Y = CF_3), 13-hexadecen-11-ynyl chloroacetate (**103**, X = O, Y = CH_2Cl), dichloroacetate **104** (X = O, Y = $CHCl_2$), and trichloroacetate **105** (X = O, Y = CCl_3).*

the analogues showed poor antennographic activity (20% or less of the natural-pheromone value) with a peculiar behavior for **101** that enhanced the pheromone responses at lower doses and behaved as a good antagonist at higher doses. The order of inhibitory activity (**99** > **100** > **101** > **103** > **104**) could be explained if the steric size plays a primary role over the polarity of modified group. The trifluoromethyl ketone **102** was active in wind-tunnel assays with a peak of full inhibition (100%) at a dose of 10 μg.

Wind-tunnel experiments were run on the European corn borer males testing, as usual, *syn*-fluoro analogues against *Z*-Type and *anti*-fluoro analogues against *E*-Type (Scheme 24).[41]

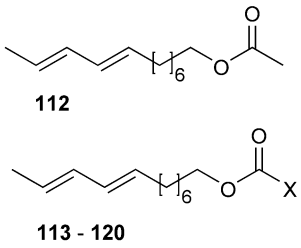


106 - 108 **109 - 111**

Scheme 24. *Fluoroacetate analogues of* E*-Type (natural pheromone* E*11–14:Ac (**30**)):* E*11–14:Fm-Ac (**106**, X = CH_2F),* E*11–14:Dfm-Ac (**107**, X = CHF_2),* E*11–14:Tfm-Ac (**108**, X = CF_3); and of* Z*-Type (natural pheromone* Z*11–14:Ac (**31**)): Z11–14:Fm-Ac (**109**, X = CH_2F), Z11–14:Dfm-Ac (**110**, X = CHF_2), Z11–14:Tfm-Ac (**111**, X = CF_3).*

The trifluoromethyl acetate *E*11–14:TfmAc (**108**) had no pheromonal activity, with 5% or less upwind flight responses, but possessed a high inhibitory activity with 83% inhibition of the upwind flight responses. The corresponding *Z*11–14:TfmAc (**111**) had a lower inhibitory (60%) and a higher pheromonal (20%) activity. *E*11–14:FmAc (**106**) Had a very high pheromonal (80%) but weak inhibitory activity (30%). The monofluoro-, difluoro-, and trifluoromethyl analogues **109**–**111** showed similar weak pheromonal activities and comparable moderate inhibitory actions.

The intrinsic and inhibitory activities of the halogenated analogues[78] of the sex attractant of the alfalfa moth *Cydia medicaginis*[79] were evaluated (Scheme 25). The fluorinated molecules **113**–**115** showed decreased but still retained activity, while the chloro- (**116**–**118**) and bromo (**119**, **120**) analogues elicited



112

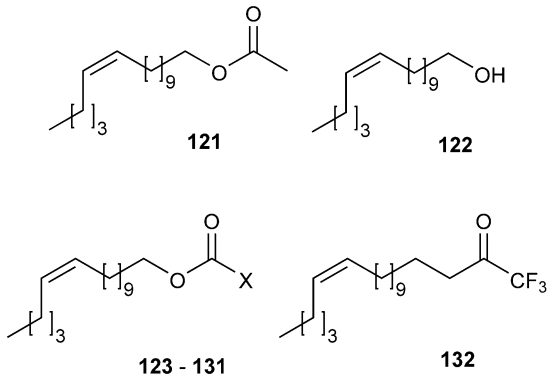
113 - 120

Scheme 25. *Halogenated analogues of natural pheromone* E*8,*E*10–12:Ac (**112**): FmAc **113** (X = CH_2F), DfmAc **114** (X = CHF_2), TfmAc **115** (X = CF_3), ClmAc **116** (X = CH_2Cl), DclmAc **117** (X = $CHCl_2$), TclmAc **118** (X = CCl_3), BrmAc **119** (X = CH_2Br), and DbrmAc **120** (X = $CHBr_2$).*

much smaller electroantennographic responses. Mixtures of **112** and fluorinated analogues always gave lower antennographic responses than **112** alone, a result indicating an inhibitory action in all of the examples. The total number of captured males was evaluated in field experiments for the fluoro analogues alone (attractants) and in a mixture with the natural pheromone (inhibitors). The monofluoroacetyl analogue **113** was the most potent field attractant (55% of the attractiveness of the natural pheromone; order of activity: **113** > **114** > **115**) and the weaker inhibitor (order of inhibitory activity: **113** < **114** < **115**). The steric size correlates well to the pheromonal activity inside the fluorinated series and furnishes a nice explanation for the higher intrinsic activity of fluoro versus chloro and bromo analogues. On the other hand, the order of the inhibitory potencies correlates well to the nucleophilic character of the carbonyl moiety.

The effects of mono-, di-, and trihalogenated acetates[80] were also evaluated on the diamond black *Plutella xylostella*[81] and on the corn stalk borer *Sesamia nonagrioides*.[82] The common components of this sex-pheromone blend are the acetate *Z*11–16:Ac (**121**), the alcohol *Z*11–16:Al (**122**), and the aldehyde *Z*11–16:Ald (**16**). The trifluoromethyl ketone **132** was included as a steric and electronic analogue of the natural pheromone **121** (Scheme 26).

The two insect species gave similar antennographic responses and such behavior is easily understandable because they share the same receptor. None of the examined analogues showed higher intrinsic activity than the natural pheromone **121**: the monofluoro acetate **123** was the most potent (about 45% of the response of **121**) in both families and the trifluoromethyl ketone **132** was one of the least active (less than 20% of the response given by **121** in both families). The fluoroacetate **123** showed an interesting but unexplained high antennographic inhibition activity (60%) at low doses (1 μg) and a linear dose-response relationship at higher doses. The difluoro and trifluoro analogues (**124** and **125**, respectively) always displayed a linear dose-response relationship. The chloro ana-



Scheme 26. *Halogenated analogues of pheromone Z11–16:Ac (**121**): FmAc **123** (X = CH_2F), DfmAc **124** (X = CHF_2), TfmAc **125** (X = CF_3), ClmAc **126** (X = CH_2Cl), DclmAc **127** (X = $CHCl_2$), TclmAc **128** (X = CCl_3), BrmAc **129** (X = CH_2Br), DbrmAc **130** (X = $CHBr_2$), TbrmAc **131** (X = CBr_3), and Z12–17:Tfmk (**132**).*

logues **126**–**128** and the bromo analogue **129** elicited a lower activity (less than 20% in both the species) as well as a modest and nonlinear inhibition. The keto analogue **132** was weakly active on both families and was also the poorest inhibitor with some activity shown only at high concentrations. In the field, **132** turned out to be a good synergist for the action of the natural pheromone and also a disruptant of its activity.

The "fluorine for hydrogen" replacement retains some bioactivity, probably and qualitatively due to a certain similarity in bulkiness. In fact, the fluoro analogues **123**–**125** are better inhibitors than the chloro and bromo ones (**126**–**131**). Within the same three series (fluorinated, chlorinated, and brominated molecules), the differences are so small that a comparison is not meaningful.

As a general trend, the structural requirements necessary for the inhibition of the pheromonal response seem far less restrictive than those necessary for the elicitation of the same response. Actually, the pheromonal activity order ($FAc \geq F_2Ac \geq F_3Ac$) indicates that the monofluoroacetate analogues, which are more similar in size to the natural pheromone, are better attractants than the difluoro- and trifluoroacetate analogues; the trifluoro analogues, which are the bulkiest ones, are the worst attractants of the series. The inhibitory activity ($F_3Ac \geq F_2Ac \geq FAc$) suggests that the fluoro analogues interact with the pheromone receptor without provoking behavioral responses and are possibly degradated on the antennae more slowly than the natural pheromone. The trifluoromethyl ketone **132** (as well as **102**) belongs to a different chemical family and its inability to inhibit the pheromone responses may be attributed to its structure, which makes it a bad substrate for the olfactory receptor.

As already outlined, the real nature of the interactions between the polar group and the receptor site is still unknown; as a consequence, no rigorous analysis of the observed effects is possible.

A computational model was proposed, on Lepidoptera *Agrotis segetum*, to investigate the interactions between the sex pheromone *Z*5–10:Ac (**25**) and its receptor.[51] Such a model was based on the modification of the polar group by the introduction of a number of isosteric functionalities that mimic some aspects of the electrostatic properties of the original polar group. It turned out that the carbonyl group was crucial for the pheromone–receptor interaction, but both oxygen atoms of the ester moiety were important for full biological activity.

2.4 Processing and signal transduction

After the activation of the receptor sites in the receptor cell membrane, concatenated events follow and lead to the scotophase period in which the males are responsive to the pheromones. In these steps, fluoro analogues can not really be helpful since, it is commonly accepted, that they act on prior steps of the transduction cascade.[83]

2.5 Pheromone catabolism

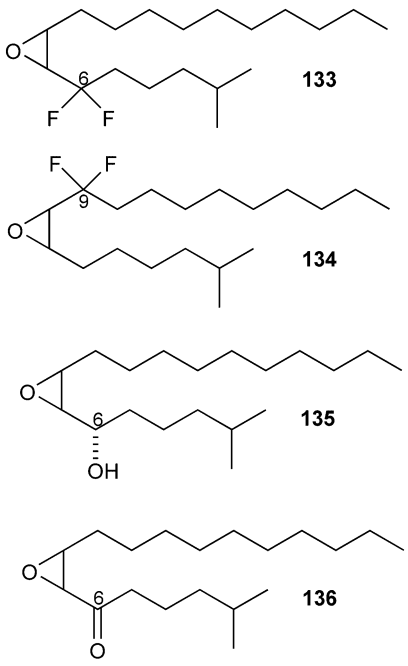
The catabolism step converts stimulatory pheromones into nonstimulatory products, thus avoiding the hyperstimulation due to the persistence of the odorants at the receptor. The inactivation or the removal of the substrates must occur before the next plume comes because "saturated" neurons lead to behavioral disruptions. Different families of catabolic enzymes are specifically involved in the cleaning operations. It has been determined that the pheromone-binding proteins facilitate the removal of the pheromone metabolites[84] and perhaps deliver pheromones and non-pheromonal molecules to the sensillar enzymes for their inactivation.[85]

2.5.1 Pheromone-catabolism inhibitors

Inhibition of the catabolic breakdown of pheromones could lead to mating disruption but, although the importance of the catabolic activity in the olfaction seems undisputed, the precise role of the substrate-specific catabolic enzymes in the signal transduction is still poorly understood.[9]

Fluoro analogues of epoxides: An epoxide hydrolase is the pheromone-catabolizing enzyme of *Lymantria dispar* that converts active epoxides into inactive diols by hydration reactions. Although the enzyme requires a specific orientation of the epoxide inside its receptor binding site, the epoxide hydrolase appears not to discriminate between the two alkyl side chains in either of the two proposed lipophilic pockets: thus, some variability in the alkyl chains of disparlure is permitted and both enantiomers can be hydrolyzed, even if at different ratios. The α-keto- (**136**), α-hydroxy- (**135**), and α,α-difluoroepoxide (**133** and **134**) modifications (Scheme 27) were incorporated into the disparlure skeleton and, due to the low enantioselectivity of the enzyme, the molecules were evaluated only in racemic form.[86] The authors claimed a dual purpose: testing for the generality of the inhibitors in a nonenantiospecific enzyme and preparing a disparlure analogue capable of blocking pheromone perception by adult males.

A model was proposed (Figure 2), on the basis of the one developed for the inhibition of the animal cytosolic epoxide



Scheme 27. *Disparlure difluoro analogues (±)-6,6- and (±)-9,9-difluorodisparlure (**133** and **134**, respectively) and oxygenated parapheromones (±)-*threo*-6-hydroxydisparlure (**135**) and (±)-6-oxodisparlure (**136**).*

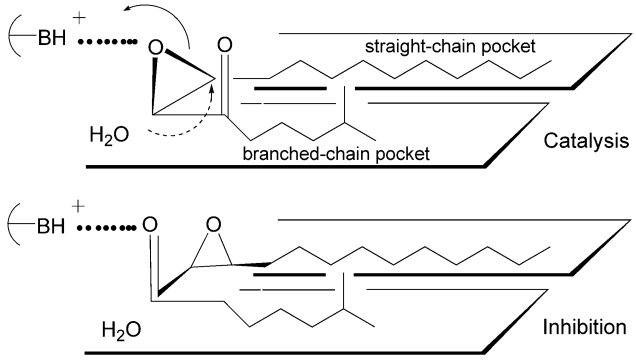


Figure 2. *Proposed mechanism of catalysis (catabolism) and inhibition of the* Lymantria dispar *antennal epoxide hydrolase by the (+)-enantiomer of (±)-**136**.*

hydrolase.[87] A protonated amino acid binds the oxygen atom of the epoxide ring, thus activating it towards hydration: this represents the normal way to catalysis. On the other hand, the noncovalent binding of the ketone oxygen atom to the same electrophilic site deactivates the epoxide ring towards nucleophilic attack, thus leading to inhibition.

The inhibitory potency of the racemic analogues on the enzyme was determined by using the homogenates of whole male antennae and measuring the rates of hydration of (+)-[5,6-$^3H_3$]-disparlure. In general, the highest inhibitory potencies were shown by the analogues substituted at the 6-position (**133**, **135**, and **136**, Scheme 27), whereas 9-hydroxy, 9-oxo, 6,9-dihydroxy, and 6,9-dioxo analogues (not shown) showed weak potencies. The 9,9-difluoro analogue **134** was also among the group of good inhibitors. The inhibitory behaviors of the analogues were tentatively explained by invoking possible binding orientations, but only for the 6-oxo analogue **136** (as well as for the noninhibitory 9-oxo analogue) was such a behavior tentatively rationalized by a model. Compound **136** was supposed to have both side chains in the optimized positions for eliciting inhibition through either good fit to the enzyme receptor or spatially favored interaction between the keto moiety and the protonated amino acid (BH^+) of the enzyme (inhibitory interaction). On the other hand, the 9-oxo analogue could adopt the same orientation as the 6-oxo compound only by placing the branched chain of disparlure in the "straight-chain" pocket (see Figure 2). So, the 9- or 6,9-disubstituted analogues were supposed to be bad enzyme receptor substrates, due to the steric perturbations offered by the additional functional groups.

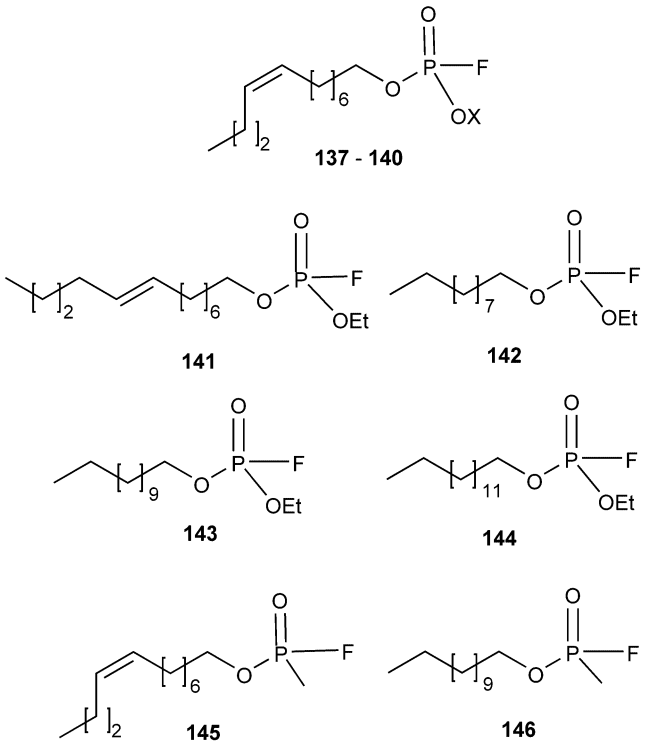
The authors did not comment on the high inhibitory activity of the 6,6-difluoro-substituted analogue **133** nor on the even more surprisingly high inhibitory activity of the 9,9-difluoro-substituted analogue **134**. Both behaviors suggest a noncovalent interaction between the protonated electrophilic amino acid site (BH^+) and the fluorine atom(s) that leads to a deactivation of the epoxide ring towards nucleophilic attack of water (inhibition). Probably, the small steric size of fluorine induces a low steric hindrance in the fluorinated straight chain of the nonfavored 9,9-difluoro analogue **134**, thereby allowing binding to the receptor site of the enzyme.

Phosphorofluoridates and methylphosphonofluoridates: (*Z*)-8-Dodecenyl acetate carboxyl esterase is the enzyme that degrades the natural pheromone **48** of the fruit moth *Grapholita molesta*. Organophosphorus esters were tested as inhibitors,[88] since the esterase-inhibiting properties of certain phosphate and phosphonate esters are well known.[89]

Only those compounds containing the same (*Z*)-8-dodecenyl moiety (**137**–**140** and **145**, Scheme 28) present in the structure of the natural pheromone elicited a disruptant activity: **138** and **140** were the most potent analogues. After preexposure of the males to **138** and **140**, the number of responding individuals to the pheromone plumes decreased dramatically to 20% for **138** and to 10% for **140**. The disrupting activity of **140** was close to that of the pheromone itself, which gave no responding males after pre-exposure under the same conditions. Analogues **138** and **140** probably act through the inactivation of the pheromone esterase.

Trifluoromethyl ketones (Tfmks): The experiments of Abeles and co-workers first showed that fluoro ketones, fluoromethyl ketones, and trifluoromethyl ketones can inhibit a number of serine esterases and proteases, namely, acetyl cholinesterase, zinc metalloprotease, aspartylprotease, pepsin,[90a] α-chymotrypsin, porcine pancreatic elastase,[90b] and human carboxyl peptidase A.[90e] Further investigations involved human leukocyte elastase,[90f] juvenile hormone esterase,[90g,h] and mammalian carboxyl esterases,[90i] such as human liver carboxyl esterase.[90j]

The trifluoromethyl ketones were found to act as slow, tight-binding inhibitors, that is, inhibitors that slowly reach the equilibrium between enzyme and inhibitor (slow-binding) and that



Scheme 28. *Phosphorofluoridates (**137**, X = CH_3; **138**, X = CH_2CH_3; **139**, X = $CH(CH_3)_2$; **140**, X = (Z)-$(CH_2)_7CH{=}CH(CH_2)_2CH_3$; **141**–**144**) and phosphonofluoridates (**145**, **146**) as putative inhibitors.*

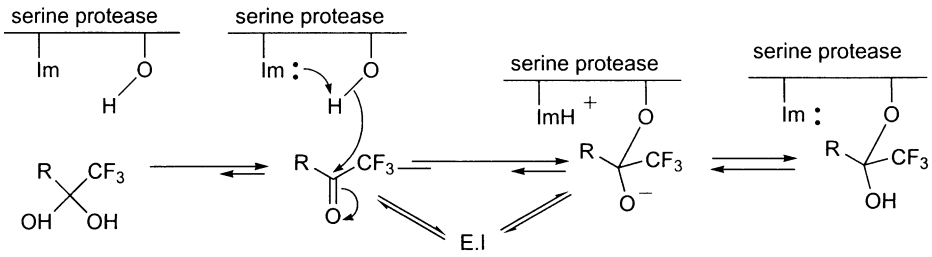
In Lepidoptera, the trifluoromethyl ketones are known to be good in vitro reversible inhibitors of the hydrolysis of the acetate group of the pheromone to the corresponding alcohol. Such a process is performed by the antennal esterases[10,80a,93] and is responsible for the catabolism of the pheromone molecules in the male olfactory tissues. The activity of these inhibitors arises from the small volume of the fluorine atom so that the receptor site of the enzyme can still accomodate the trifluoromethyl ketones and possibly form stable tetrahedral hemiketal intermediates in a reversible manner, by analogy with what is observed with other esterases and proteases (covalent reversible inhibition).

What kind of external behavior and kinetic parameter modifications should be expected? Any intrinsic activity should be absent, because the trifuoromethyl ketones are not supposed to be substrates for the receptor. The electroantennographic responses of the natural pheromones (in mixtures or after preexposure to the fluorinated molecules) should be modified, both in intensities (lower peak amplitude–habituation) and in kinetics (longer or absent repolarization time). Finally, the behavior should be disrupted with a prolonged activity of the males due to the persistence of the pheromone at the receptor site.

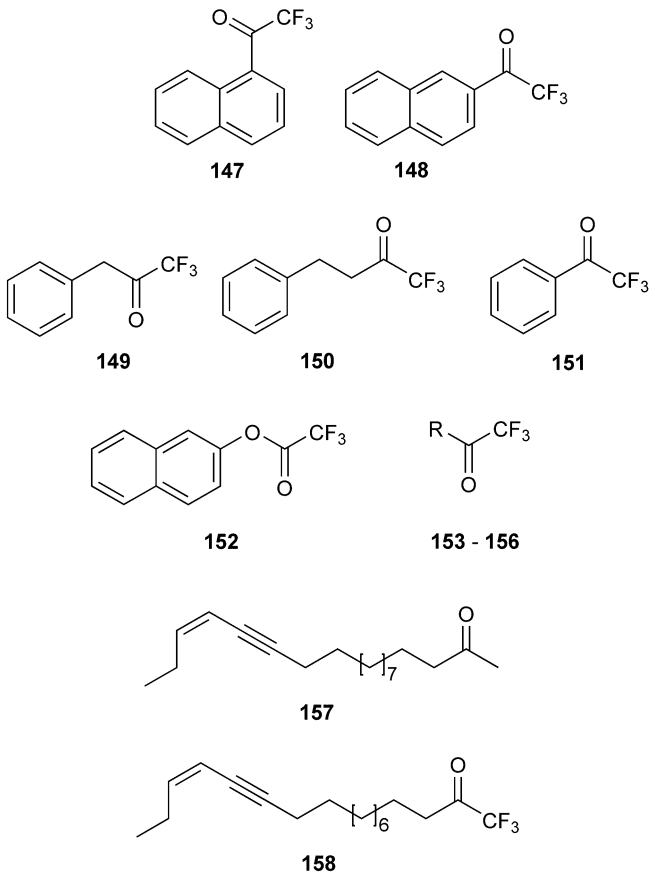
Some trifluoromethyl ketones (Scheme 30) were evaluated as inhibitors of male responses to the pheromone of the processionary moth *Thaumetopea pityiocampa*.[94] Aromatic trifluoromethyl ketones **147**–**151**, aromatic trifluoromethyl acetate **152** (fluorinated analogue of a good substrate for the sensillar esterases of *Antheraea polyphemus*[95]), long-chain alkylic trifluoromethyl ketones **153**–**156**, and the three parapheromones **102**, **157**, and **158** (formally derived from an isosteric replacement[136] of the alcoholic oxygen atom of pityolure (**57**) by a methylene group) were tested.[96] A notable blockage of the antennographic pheromone detection was displayed by **147**, **148**, **153**, and **102**. In the field, a remarkable disruptant effect (when mixed to the natural pheromone) was shown by **149**, **153**, **102**, **158**, and **152**. The nonfluorinated compound **157** was the most effective in laboratory bioassays and elicited attractant activity in field assays. The pityolure fluoro analogue **102** was a modest antagonist of **57** and exhibited a threefold-lower attractant activity than pityolure itself. In general, the compounds showed low specificity for the substrate and exhibited only modest or zero antennographic intrinsic activity.

give a substantial inhibition only when the concentrations of the inhibitor and the enzyme are comparable (tight-binding).[91]

The real chemical form (keto or hydrate) of the inhibitor continues to be the subject of discussion. In principle, since a trifluoromethyl ketone is in equilibrium with its predominant hydrate form in aqueous solution, the enzyme could bind either the keto or the hydrate form. If it binds the ketone, the formation of the hemiketal involves serine addition. If the enzyme binds the hydrate, it must first catalyze the dehydration of this species, before serine addition can occur. An X-ray crystallographic study[92] of the complex between porcine pancreatic elastase and an α,α-difluoro-β-keto amide clearly indicated a covalent bond between the active site of the serine residue (Ser195) and the carbonyl carbon atom of the ketone (hemiketal). The inhibitory action results from the fact that the adjacent fluorine atoms stabilize the formation of the tetrahedral intermediate, thus shifting the equilibrium between the noncovalent enzyme–inhibitor complex and the covalent hemiketal intermediate in favor of the latter, as shown in Scheme 29. According to this mechanism, the covalently bound, tightly bound inhibitor only dissociates when the active site imidazole moiety is protonated.[90f,k]



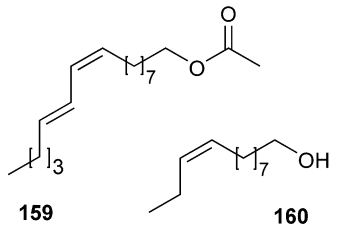
Scheme 29. *Hypothesis of mechanism for slow, tight-binding inhibition of trifluoromethyl ketones with serine proteases. Im = the imidazole moiety of the histidine residue, E.I = the noncovalent enzyme–inhibitor complex.*



Scheme 30. *Aromatic trifluoromethyl ketones (**147**–**151**), aromatic trifluoromethyl acetate (**152**), alkyl trifluoromethyl ketones (R = $C_{12}H_{25}$, **153**; R = $C_{13}H_{27}$, **154**; R = $C_{16}H_{33}$, **155**; R = $C_{17}H_{35}$, **156**), and analogues (**157**, **158**) of the natural pheromone.*

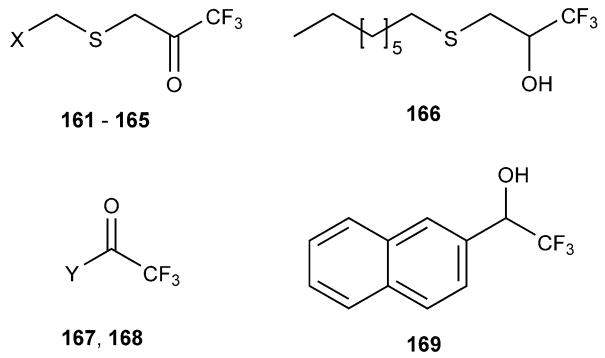
The results could not be correlated and the authors outlined the contradictory data reported for the activity of structurally related methyl ketone analogues of other sex pheromones.[28b, 74] Strict molecular and stereoelectronic requirements seem necessary for a successful recognition of the incoming signal by the antennal receptor.

Spodoptera littoralis,[97] *Mamestra brassicae*,[98] and *Eliothis zea* share pheromones[137] belonging to the different chemical families of esters (*Z*9,*E*11–14:Ac (**6**), *Z*9,*E*11–16:Ac (**159**, Scheme 31), and *Z*11–16:Ac (**121**)), alcohols (*Z*9–14:Al (**160**) and *Z*11–16:Al (**122**)), and aldehydes (*Z*11–16:Ald (**16**).



Scheme 31. *Z9,E11–16:Ac (**159**) and Z9–14:Al (**160**), components of the pheromone blend.*

The β-thio-trifluoromethyl ketones and alcohols (**161**–**166**, Scheme 32) were tested[99] along with the alkylic-chain trifluoromethyl ketone C12:Tfmk (**153**) and the aromatic trifluoromethyl ketone **148**. The authors evaluated either intrinsic activities (the percentage of the males landing at the source in wind-tunnel assays after preexposure to the volatile) or kinetic parameters (peak amplitude, depolarization time, repolarization time ratios before and/or during treatment with the natural pheromone and the putative inhibitors). The trifluoromethyl alcohol **169** elicited an intrinsic power higher than that of the pheromone blend with 80% catches for the former, compared to 70% for the latter. The trifluoromethylnonyl ketone **167** showed an activity similar to that of the natural pheromones, All other analogues were less active (40–60% of the activity of the pheromone blend). Only **162**, **163**, and **153** reversibly decreased the electroantennographic-peak amplitude value and increased the repolarization-time value, while the thio derivative **163** also increased the depolarization-time value. The aromatic trifluoromethyl ketone **148** reversibly decreased the peak amplitude value but it modified neither the depolarization- nor the repolarization-time values. Finally, **167**–**169** did not alter any of the three parameters. All the thio-trifluoromethyl ketones showed low toxicity in mammals, in the range of that elicited by the natural pheromones of the Lepidoptera.



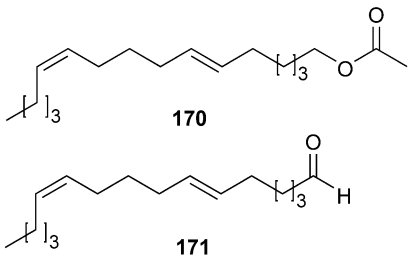
Scheme 32. *Fluorothio parapheromones: HTFP **161**, X = C_5H_{11}; OTFP **162**, X = C_7H_{15}; DTFP **163**, X = C_9H_{19}; DoTFP **164**, X = $C_{11}H_{23}$; PTFP **165**, X = $C_{14}H_{29}$; and OTFP:OH **166**; alkyl Tfmks C9:Tfmk **167**, Y = C_9H_{19}; and C15:Tfmk **168**, Y = $C_{15}H_{31}$; and aromatic trifluoromethyl ketone **169**. HTFP = hexylthiotrifluoropropan-2-one, OTFP = octylthiotrifluoropropan-2-one, DTFP = decylthiotrifluoropropan-2-one, DoTFP = dodecylthiotrifluoropropan-2-one, PTFP = pentadecylthiotrifluoropropan-2-one, OTFP:OH = 3-octylthio-1,1,1-trifluoro-propan-2-ol.*

A high lipophilicity seems to be important. In fact, the β-thio-trifluoromethyl ketones are better inhibitors than the corresponding alkyl analogues that are devoid of sulfur.

OTFP **162** was chosen for wind-tunnel assays where it induced erratic flights in the males and decreased the number of those contacting the source. Moreover, it showed the same effects on all three insect species (*Spodoptera littoralis*, *Mamestra brassicae*, and *Eliothis zea*). The promising OTFP **162** was submitted to actographic analysis[100] of the responses of one single male of *Mamestra brassicae*. The male, after stimulation with the natural-pheromone blend, became very active and performed fanning with intense locomotion in the direction of

the pheromone source. Surprisingly, after preexposure to **162** and stimulation by the same blend, instead of showing an enhancement of its activity, the insect showed a dose-dependent reduction of the in vivo responses.

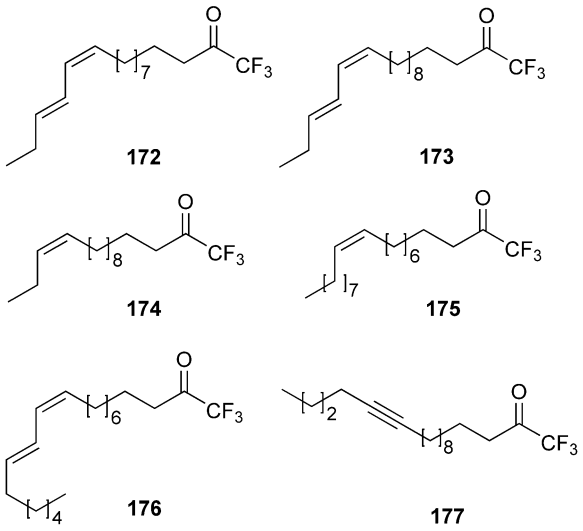
HTFP **161**, OTFP **162**, and DTFP **164** were evaluated[101] as sensillar esterase inhibitors in the silk moths *Anthereae polyphemus* and *Antereae pernyi*[102] whose pheromone blend is shown in Scheme 33. The electroantennographic and nerve impulse behaviors were investigated by running the test stimuli on the acetate **170**, an esterase substrate, and the aldehyde **171**, which is not degraded by the enzyme. In either insect species, after 30 seconds of preapplication of low doses of trifluoromethyl ketone, the peak amplitude of the response to the pheromones *E*6,*Z*11–16:Ac (**170**) and *E*6,*Z*11–16:Ald (**171**) was reversibly reduced, with the following activity order: DTFP ≫ HTFP > OTFP. Surprisingly, the responses of either type of receptor (acetate or aldehyde) were inhibited, with a just slightly stronger effect elicited on the first. The depolarization-time value (4/5DT) was not affected and the repolarization-time value (2/3RT) was prolonged. The result is surprising and unpredicted since the aldehyde **171** is not supposed to be an esterase substrate.



Scheme 33. *Natural pheromones* E6,Z11–16:Ac (***170***) *and* E6,Z11–16:Ald (***171***).

The results were in line with what was found for OTFP (peak amplitude decreased and repolarization time increased) tested on *Spodoptera littoralis*[103] and *Sesamia nonagrioides* species.[104] However, the inhibition of the pheromone deactivation, by whatever mechanism, would be expected to prevent the peak-amplitude parameter from declining at all.[105] Moreover, the slight prolongation of the repolarization time observed for **171** should not be due to its reduced degradation since the trifluoromethyl ketones are not expected to inhibit the sensillar oxidase responsible for the degradation of the aldehyde component **171**. Nuclear magnetic resonance studies, based on the fluorine nucleus, focused on OTFP in order to study more precisely the type of enzyme–inhibitor interaction. The trifluoromethyl ketone was hydrated in a mixture of buffered hydrochloric acid solution and water; this resulted in one sharp ^{19}F NMR spectroscopy signal. In the presence of the enzyme, the spectrum showed a much broader signal with a downfield shift, a result indicating that the binding of the inhibitor to the enzyme had occurred. The chemical-shift value was compatible with that of a species of tetrahedral geometry, a fact that points to the presence of a hydrate or hemiacetal. When OTFP was mixed with the esterase extract in the presence of an excess of an irreversible phosphorylating enzyme inhibitor (paraoxon), it was demonstrated that paraoxon irreversibly displaced the enzyme–OTFP complex. Since the phosphorylation process occurs specifically by binding a serine residue of the active site, it is probable that the inhibitor-enzyme interaction occurs through formation of a hemiacetal.

The in vitro inhibition bioassay of *Spodoptera littoralis*, with or without preincubation, was studied.[103] The authors employed a preparation of crude antennal esterase, obtained from the homogenization of frozen antennae, and measured the ratio of tritiated alcohol to the total radioactivity found. Compounds **172**–**174** (Scheme 34) turned to be potent inhibitors of the estrase in vitro.

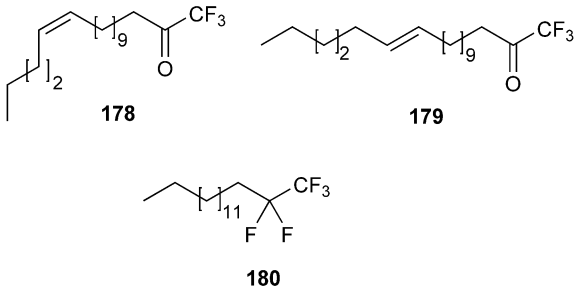


Scheme 34. *Natural pheromone analogues* ***172*** *and* ***173****, olefinic trifluoromethyl ketones* ***174*** *and* ***175****, diene trifluoromethyl ketone* ***176****, and acetylene trifluoromethyl ketone* ***177****.*

All trifluoromethyl ketones acted as tight slow-binding inhibitors, since they elicited a higher potency after preincubation. The most potent inhibitors without preincubation were the dienes **172** and **173**, whose structures closely mimicked the natural pheromone, *Z*9,*E*11–14:Ac (**6**). Also, the monoene **174** with the double bond of the chain at the same (11) position as the natural pheromone **6** displayed a remarkable activity, while the monoene **175**, with chain elongation at the nonpolar end of the molecule and a double-bond displacement to the centre of the chain, displayed a lower activity. The pityolure analogue **102** displayed a fair activity.

Since the best inhibitors (**177**–**179** and **102**), as well as the natural pheromone **6**, show the same $CH_3CH_2CH{=}CH$ functionality at the far end of the molecule, this group might play an important role in the esterase inhibitory activity.

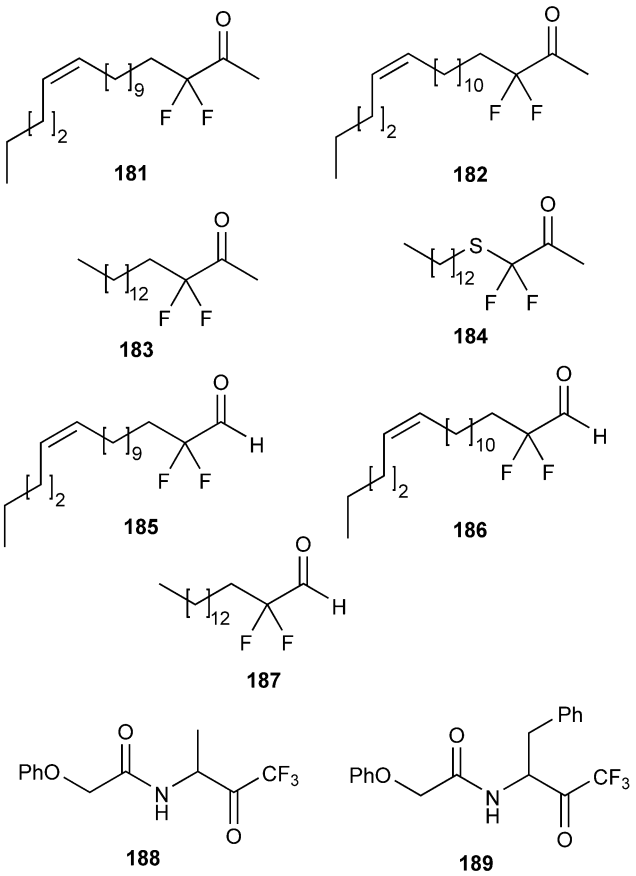
Are these data suggesting a direct trifluoromethyl ketone–receptor interaction? Only *Z*11–16:Tfmk (**178**, Scheme 35) turned out to be a promising substrate for the antennal esterase inhibition of *Sesamia nonagrioides*, whose natural pheromone is the acetate **121**. Compound **178** elicited a good in



Scheme 35. *Trifluoromethyl ketones Z11–16:Tfmk (**178**) and E11–16:Tfmk (**179**) and pentafluoro analogue **180**.*

vitro response and a true wind-tunnel disrupting activity,[106] while the *E* analogue **179** and the alkylic-chain analogue **180** were totally inactive. The natural pheromone **121** and the trifluoromethyl ketone **178** showed different intrinsic antennographic responses and the fluoro analogue was consistently less active. When applied as a background, it reduced the electroantennographic responses of the pheromone and significantly increased its repolarization time. The depolarization time was not affected. The strict structural analogies to the natural pheromone could make the fluoro analogue **178** a good substrate for receptor recognition and transduction processes. The same compound was submitted[107] to actographic investigation performed on *Mamestra brassicae* males. The actographic analysis showed that the latency of the response was proportional to the doses of the trifluoromethyl ketone and that the percentage of responding males was not affected.

OTFP (**162**), *Z*9,*E*11–14:Tfmk (**172**) and *Z*11–16:Tfmk (**178**) were included in an in vitro investigation focusing on the real active form (keto or hydrate) of some putative esterase inhibitors, α,α-difluoromethyl ketones (Dfmks)[108] and α,α-difluoro aldehydes (Dfas)[109] (Scheme 36),[110] on *Spodoptera littoralis* and *Sesamia nonagrioides*.[138] Compounds **162**, **172**, and **178** confirmed their esterase inhibitory activities (**162** > **172** > **178**) on either entomological line. Compound **181** showed activity only on *Sesamia nonagrioides* but the activity was comparable with that of **178**. Compounds **182**, **184**, **188**, and **189** were inactive on both insect species. All other difluoro parapheromones had activities 20–80 times lower than that of OTFP. An attempt to correlate the hydration constant and the inhibitory potency of the analogues was performed through ^{19}F NMR spectroscopy studies. The extent of hydration followed the order: α,α-difluoromethyl ketones < α,α-difluoro-β-thioalkylmethyl ketones < trifluoromethyl ketones < β-thio-trifluoromethyl ketones < α,α-difluoro aldehydes, but no clear correlation was found. The authors stated that their results were in contrast with what was already reported[111] on the correlation between the hydration constants and the potencies of some trifluoromethyl ketones as inhibitors of the juvenile hormone esterase. In another study, based on molecular modeling,[112] the same authors had demonstrated that β-substituted trifluoromethyl ketones showed an intramolecular hydrogen bond between the hydrate and the heteroatom located in β position



Scheme 36. *α,α-Difluoromethyl ketones **181**–**184**, α,α-difluoro aldehydes **185**–**187**, and parapheromones **188** and **189**.*

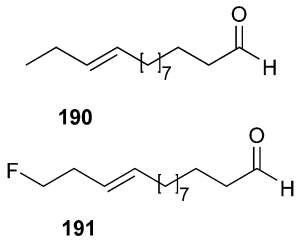
to the carbonyl group and that the strength of this hydrogen bond correlated well with the inhibitory activity.

The link between the anti-esterase activity of the trifluoromethyl ketones and the effects of these compounds on the pheromonal communication system is not straightforward and further physiological and behavioral investigations are necessary to determine to what extent the effects on male behavior are related to the inhibition of the sensillar esterases.

2.6 Delayed-action toxicants

The latent toxicity of the even-numbered fatty acids bearing a single fluorine substituent in the ω-position is due to the in vivo β oxidation to the fluoroacetate.[113] The fluorinated analogues can be seen as pro-insecticides[114] and the insect oxidative enzymes are recruited to generate the toxin which would block the citric acid cycle by fluorocitrate formation.[115] The potential utility of the fluoroacetate-releasing compounds is mitigated by their own low specificity and by their high toxicity for nontargeted species.

(*E*)-14-Fluorotetradec-11-en-1-al (**191**) is the fluoro analogue of (*E*)-11-tetradecenal (**190**, Scheme 37), the major component of the sex-pheromone blend of the western spruce budworm *Choristoneura occidentalis*.[116] The fluorinated analogue **191** eli-

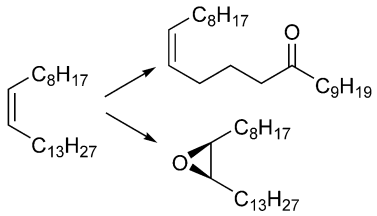


Scheme 37. *Natural pheromone* E*11–14:Ald (**190**) and its fluoro analogue 14F-*E*11–14:Ald (**191**).*

cited significant antennographic dose-dependent reductions of the peak-amplitude parameter but was equivalent to the natural pheromone in terms of long-range attraction of males.[117] An interesting comparison with the sensory-disruptant acyl fluorides **69** and **70**[61] evidenced how, in compound **191**, the ω-fluoro substitution preserved the biological activity of the aldehyde analogue. The data on the toxicity revealed that a high percentage of males (90%) and a lower but still significant percentage of females (70%) died when submitted to the action of **191**. It was also shown that antennectomized males continued to die under the action of the fluoro analogue, a fact demonstrating that a pheromone can enter the moth through body surfaces other than the antennae. It attracts and kills: the animal would be killed by its own metabolic talents.[9a]

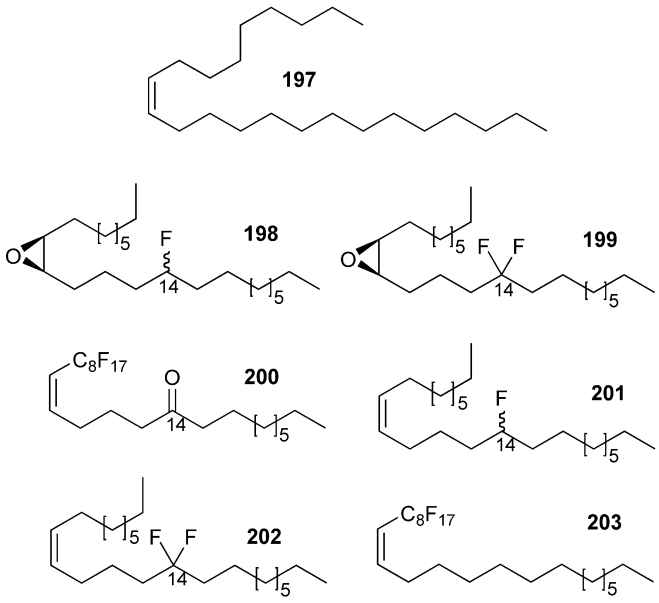
2.7 Metabolic pathway tracers

Common approaches to the study of the molecular mechanisms by which the pheromones activate transductory processes involve the use of radioligands or other pheromone analogues.[95,118] A significant example of a synthetic project based on fluoro analogues employed to elucidate metabolic pathways focused on the housefly *Musca domestica*,[119] whose pheromone, muscalure (**197**), is converted by the cuticle cytochrome P-450 monooxygenases into 9,10-epoxide and 14-keto metabolites (Scheme 38).



Scheme 38. *Oxidations of (Z)-9-tricosene (**197**) performed by the cuticular polysubstrate monooxygenase of the housefly.*

In order to investigate the metabolic processing and to probe the substrate requirements for the monooxygenase system, the 14-position of tricosene was blocked to give the fluoro analogues **198**, **199**, **201**, and **202** and the alkylic chain was modified to provide the fluoro analogues **200** and **203** (Scheme 39).[120]



Scheme 39. *Natural pheromone (Z)-9-tricosene (**197**), fluoro analogues **198–200** prepared to verify metabolic processing, and fluoro analogues **201–203** prepared for biochemical experiments.*

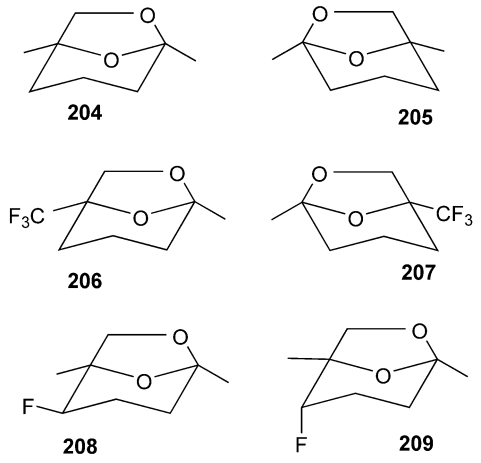
The 14-position was blocked with either one or two fluorine atoms, thereby altering the C–H oxidation, and a perfluorooctyl chain was used to replace the octyl chain to probe the importance of the hydrophobicity of the alkyl chain and of the nucleophilicity of the olefin in the epoxidation reaction. The authors synthesized and tested other nonfluorinated parapheromones (not shown in Scheme 39) bearing methyl, dimethyl, cyclopropyl, and hydroxy substituents at the 14-position of the pheromone chain.

Only three of the analogues of (*Z*)-9-tricosene tested (**201**, **202**, and 14-methyl-(*Z*)-9-tricosene) were metabolized to the corresponding epoxide. This suggests that only minimal structural change at the 14-position of **197** is allowed with retention of metabolic activity. The fluoro analogue **203** was not hydroxylated at the 14-position and none of the fluoro analogues were effective inhibitors in both male and female houseflies, with the polysubstrate monooxygenase thus showing very strict structural requirements for the substrate.

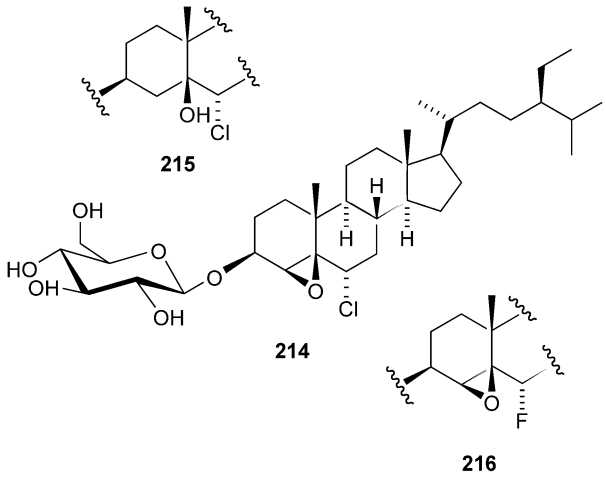
3 Fluorinated Parapheromones and Stereochemistry

Due to the importance of the stereochemistry of the chiral pheromones in the expression of the bioactivity,[121] the fluoro analogues of chiral pheromones should be prepared in a stereodefined manner. To our knowledge, there are still few examples of enantiopure chiral parapheromones where fluorine atoms or fluoroalkyl groups are placed on carbon stereocenters.

The aggregation pheromone blend of the southern pine beetle *Dendroctonus frontalis*[122] is composed of an 85:15 mixture of (−)-(1*S*,5*R*)- and (+)-(1*R*,5*S*)-frontalin (**204** and **205**, respectively). The fluoro analogues **206**–**209** (Scheme 40)[123]



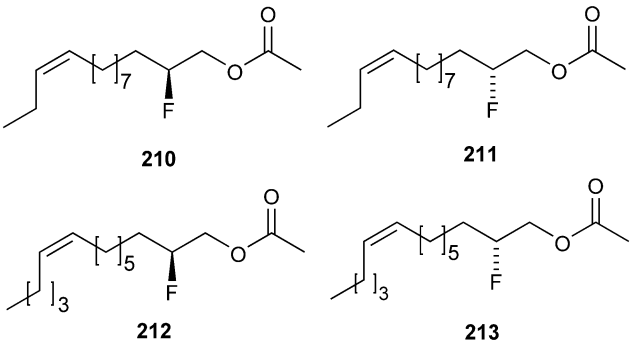
*Scheme 40. Natural pheromones (−)-(1S,5R)-frontalin (**204**) and (+)-(1R,5S)-frontalin (**205**), trifluoromethyl analogues **206** and **207**, and monofluoro analogues **208** and **209**.*



*Scheme 42. Natural pheromones Blattellastanoside A (**214**) and Blattellastanoside B (**215**) and the 6-fluoro analogue of A (**216**).*

were prepared and tested on the European coleopteran species *Dendroctonus micans*[124] through antennographic and field tests. Due to the unexpected absence of an infestation of coleopterans in the chosen wood, interesting but not conclusive data were collected. An outstanding activity of the axially fluorinated frontalin analogue (**209**) was detected with strong dose-dependent EAG responses and high attractiveness in wood on some Elateridae species.

The fluorinated analogues of the sex pheromones Z11–14:Ac (**31**) and Z9–14:Ac (**22**) of *Ostrinia nubilalis* and the beet army worm *Spodoptera exigua*,[125] respectively, were synthesized.[126] Preliminary assays showed that (*S*)-**210** and (*R*)-**211** (Scheme 41) displayed remarkably different behavior against *Ostrinia nubilalis*: while **210** showed a promising mimicking activity, **211** was totally inactive.



*Scheme 41. Enantiopure fluoro analogues (S)- and (R)-2F,Z11–14:Ac (**210** and **211**, respectively); (S)- and (R)-2F,Z9–14:Ac (**212** and **213**, respectively).*

The arrestant components A (**214**) and B (**215**, Scheme 42; A is 70 times more active than B) of German cockroach *Blatella germanica* L.[127] are the only chlorinated steroidal glucosidal contact pheromones known. The fluoro analogue **216**[128] was surprisingly more active than **214**. The steroidal side chain seemed to be strictly required for bioactivity, as was the sugar part. The amazingly high activity of the fluoro analogue in comparison to that of the natural pheromone has not yet been rationalized.

4 New Frontiers and Different Approaches

The pheromone–binding site interactions are still poorly understood and there is no complete information about which amino acids are involved.[8] Photoaffinity labeling of pheromone components,[26a] expression and purification of recombinant pheromone-binding proteins,[84b,129] NMR spectroscopy studies of α-helix proteins,[130] and, finally, crystallization studies[131] may fully elucidate the protein structures in the future and would allow us to understand the binding mechanism. The insect olfactory system is considered to be relatively simple in containing only thousands of receptor neurons. Neurobiologists are trying to improve knowledge of the mechanisms of the insect brain[132] and of the entire olfactory system. The olfactory neurons that insects use to locate potential mates or that help them to find food could be "turned off" through the identification of which odours interact with known individual receptors. Selectively disabling or overexpressing newly identified genes that code for the target proteins would change the odour sensitivities and the insect behaviors.

5 Summary and Outlook

In 1959 the first sex attractant was isolated and identified from half a million female silkworm (*Bombyx mori*) pheromone glands and after thirty years of classical chemical analyses: it was bombykol, (*E,Z*)-10,12-hexadecadien-1-ol.[133] The chemistry of semiochemicals was born. Now, half a century later, the semiochemicals are applied in integrated pest-management systems that take advantage of their environmental smooth impact (nontoxic, highly specific, and effective in low concen-

trations) in comparison to classical methods such as the employment of pesticides and insecticides. However, in depth knowledge of the targeted insects and high purity of the utilized pheromones are required, various technical difficulties and several problems of chemical degradation can arise, and high production costs are involved. Parapheromones should be designed as metabolically stable, active, cost-effective, and environmentally safe analogues. In this family, the fluorinated pheromones can influence many chemical, biological, and physical aspects of the corresponding natural ones, so enhancing the possibilities of a successful fight against pests. But, the simplistic notion that fluorine acts as a hydrogen isostere cannot explain the variety of often unpredictable effects observed.[34a] In fact, such effects can be different from one insect species to another because of the different kind of olfactory receptors involved in the studies. Moreover, in spite of the low quantities required for the chemical treatment of infested areas, these analogues are considered new drugs and the extensive toxicity tests necessary before their open-air employment result in additional costs. Fluorinated parapheromones can be excellent structure–activity relationship probes, but their future as large-scale pest-control chemicals remains doubtful.

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[134] The enzymes are only part of the chemistry involved at this stage. A more complex understanding of the biosynthesis would include studies of the structure of the enzymes, cofactors, corresponding mRNA, and genes.

[135] In the text, the chemical names of the compounds are almost exclusively indicated with abbreviations taken from the original papers. In the present case, (*Z*,*E*)-9,11-tetradecadienyl acetate (**6**) is indicated as *Z*9,*E*11–14:Ac. As a general rule, when double bond(s) is (are) present in the alkenylic chain, the number(s) which follow(s) the stereochemical indication(s) (*E* or *Z*) refer(s) to its (their) position(s) in the chain (9 indicates the position of the *Z* and 11 that of the *E* double bond in compound **6**), with the carbon atom closest to the polar functionality numbered as 1 (the acetate in **6**). The number after the line refers to the length of the chain and indicates the total number of carbon atoms (14 for **6**). The abbreviation at the end of the name indicates the chemical nature of the compound (Ac is for acetate in the example compound **6**). Other abbreviations for polar groups: Ald for aldehyde; Al for alcohol; Acfs for acyl fluorides; FmAc, ClmAc, and BrmAc for fluoro-, chloro-, and bromomethyl acetate, respectively; DfmAc, DclmAc, and DbrmAc for difluoro-, dichloro-, and dibromomethyl acetate, respectively; TfmAc, TclmAc, and TbrmAc for trifluoro-, trichloro-, and tribromomethyl acetate, respectively; Tfmk for trifluoromethyl ketone; Dfmk for difluoromethyl ketone; Dfa for difluoro aldehydes. Alkylic chains are indicated by a capital C followed by a number indicating the total number of carbon atoms in the alkylic framework. Other abbreviations: Pfe = perfluoroethyl, Pfb = perfluorobutyl, Pfh = perfluorohexyl, Df = difluoro, Tf = trifluoro, Ttf = tetrafluoro, Fm = fluoromethyl, Dfm = difluoromethyl, Tfm = trifluoromethyl.

[136] The replacement of the acetate group ($OCOCH_3$) of the natural pheromone by the trifluoroacyl moiety ($COCF_3$) is one of the most common strategies.

[137] Three receptor cells responding to the major compound of the pheromone blend, *Z*11–16:Ac, are known and different antagonists are used by heterospecific species (*Z*9–14:Ac and *Z*11–16:Ald).

[138] Some Dfmks are inhibitors of serine (ref. [95a]) and HIV-1 proteases. Some Dfmas are inhibitors of acetylcholinesterase (ref. [95c]) and of antennal-oxidizing enzymes in antennal tissues.

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