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New Fluorinated Derivatives as Esterase Inhibitors. Synthesis, Hydration and Crossed Specificity Studies

Carmen Quero,[a] Gloria Rosell,[b] Oscar Jiménez,[a] Sergio Rodriguez,[a] M. Pilar Bosch[a] and Angel Guerrero[a,*]

[a]*Department of Biological Organic Chemistry, Institute of Chemistry and Environmental Research (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain*

[b]*Department of Pharmacology and Therapeutic Chemistry, Unity Associated to CSIC, Faculty of Pharmacy, University of Barcelona, Avda. Diagonal, s/n. 08028 Barcelona, Spain*

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Abstract—A variety of new fluorinated chemicals have been prepared for the first time and tested as inhibitors of esterases, one of the main enzymes involved in pheromone catabolism, in two economically important pests, the Egyptian armyworm *Spodoptera littoralis* (*SL*) and the Mediterranean corn borer *Sesamia nonagrioides* (*SN*). Using the respective major component of the pheromone as substrate, the K_m and V_{max} of the antennal esterase of both insects resulted to be 5.66×10^{-4} M and 8.47×10^{-6} Mmin^{-1} for *SL* and 1.61×10^{-7} M and 1.25×10^{-7} Mmin^{-1} for *SN*, pointing out that *SN* esterase has a higher affinity for its corresponding substrate than *SL*. In general, the trifluoromethyl ketones (TFMKs) exhibited higher inhibitory potency than the corresponding difluoromethyl ketones (DFMKs) or difluoroaldehydes (DFAs). The compounds appeared to hydrate differently in aqueous solution, the extent of hydration following the order: α,α-DFMKs < α,α-difluoro-β-thioalkylmethyl ketones < TFMKs < β-thiotrifluoromethyl ketones < α,α-DFAs. No clear correlation has been found between the K_{hyd} and the inhibitory potency and no specificity has been found when the chemicals were assayed on extracts of both insects.

Introduction

The development of potent enzyme inhibitors is an area of pivotal importance in the pharmaceutical and agrochemical fields.[1] Illustrative of the significance of these compounds is, for example, the development of inhibitors of human neutrophil elastase for treatment of pulmonary emphysema,[2] or inhibitors of neuropathy target esterase, the target site of certain neurotoxic organophosphorus compounds.[3] Incorporation of polyfluoroketone moieties into inhibitors exhibiting close structural analogy to the substrates has proven to be a useful strategy for generating strong inhibitors of diverse serine hydrolases, many members of this type of enzymes being pharmacologically attractive targets. Among others, enzymes inhibited by fluoroketone inhibitors include acetylcholinesterase,[4] chymotrypsin,[5] trypsin,[6] juvenile hormone esterase,[7] human liver microsomal carboxylesterases,[8] or cytosolic human phospholipase A_2.[9] Inhibition studies carried out with these fluorinated derivatives may be therapeutically significant in different areas, f.i. arachidonoyl ethanolamide (anandamide) hydrolysis inhibitors in processes involving analgesia, mood, nausea, memory, and so on,[10] and renin or angiotensin-converting enzyme inhibitors in hypertension phenomena.[11] The fluorinated ketones function as transition-state analogues of the enzyme, with the inhibition activity arising by formation of an adduct of tetrahedral geometry between the serine residue, present at the active site of the enzyme, with the highly electrophilic carbonyl moiety.[12,13] A crystal structure has been obtained for the covalent complex of porcine pancreatic elastase and peptidyl α,α-difluoro-β-keto amide, wherein the inhibitor is bound at the active site as a hemiketal with a Ser-195.[14]

We have previously reported that trifluoromethyl ketones (TFMKs) are good antagonists of the pheromone action in insects, as a consequence, at least in part, of the inhibition of the pheromone catabolism

*Corresponding author. Tel.: +34-93-400-6120; fax: +34-93-204-5904; e-mail: agpqob@iiqab.csic.es

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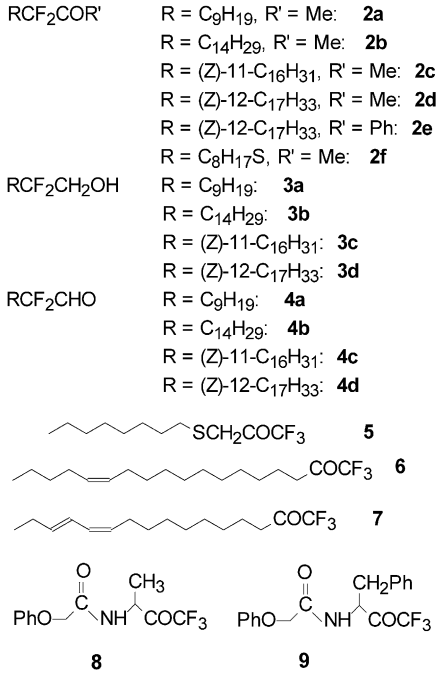
process.[13,15–20] We now report the synthesis, hydration and crossed specificity studies of new TFMKs, α,α-difluoromethyl ketones (DFMKs) and α,α-difluoroaldehydes (DFAs) (Scheme 1) as esterase inhibitors of two economically important pests, the Egyptian armyworm *Spodoptera littoralis* (*SL*) and the Mediterranean corn borer *Sesamia nonagrioides* (*SN*). TFMKs are known as potent inhibitors of the antennal esterase in male olfactory tissues.[15,21,22] Difluorinated ketones, in turn, have been reported to be good inhibitors of serine proteases[23] and HIV-1 proteases,[24] and difluoroaldehydes as inhibitors of acetylcholinesterase[4] and aldehyde-oxidizing enzymes of antennal tissues of *Helicoverpa (Heliothis) virescens*.[25]

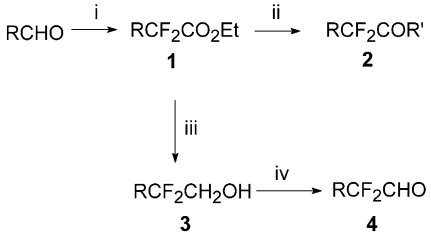
Results and Discussion

Synthesis

Introduction of a *gem*-difluoromethylene group in organic molecules has been recently reviewed.[26] For the synthesis of DFMKs many of the processes involve transformation of the more readily available short synthons chloro- or iododifluoromethyl ketones. Thus, Ishihara et al.[27] described the reaction of chlorodifluoromethyl ketones with Zn/Me_3SiCl in CH_3CN to produce the trimethylsilyl difluoroenol ethers intermediates, which were coupled with aldehydes and ketones to provide 3-hydroxydifluoromethyl ketones in 25–68% overall yields. The enol could be trapped 'in situ' by Lang and Schaub[28] with Zn/DMF and reacted with aldehydes in a one-step process. More recently, Médebielle et al. described the use of tetrakis(dimethylamino)ethylene as an alternative to Zn to generate the difluoracetyl anion in route to aromatic difluoroketones.[29] However, in order to prepare alkyl difluoromethyl ketones, reductive deoxygenation of the hydroxy group at the 3 position is required but this process is by no means straightforward. In fact, we experienced serious drawbacks when we attempted the related reductive deoxygenations of unsaturated 3-hydroxy-2,2-difluoroesters by a number of methods[30] since this process requires radical reduction conditions, non-compatible with radical sensitive groups (double and triple bonds) present in the substrate. Another approach for the synthesis of DFMKs was developed by Burton and Qiu,[31] who reported the radical addition of alkenes to iododifluoromethyl ketones in the presence of $Pd(PPh_3)_4$ followed by reductive deiodination with $Zn/NiCl_2.6H_2O$. Here, again, the procedure appears not to be appropriate for unsaturated substrates. A more elaborated approach was described in which difluoracylsilane intermediates suffered a Brook rearrangement when treated with CH_2N_2 to produce difluorinated trimethylsilyl enol ethers, which were converted by HF treatment to the expected DFMKs in good overall yields.[32,33] In our case, since we had previously developed an efficient synthesis of long-chain α,α-difluoroesters,[30] we decided to test the direct reaction of these compounds with methyllithium (Scheme 2). The process required 1.5 equiv of the nucleophile but a larger excess of the latter is not detrimental if the reaction is carried out at −78 °C in THF. At room temperature the reaction is not selective and proceeds further to give the corresponding trifluoromethyl alcohol. Surprisingly, only one precedent has been found in the literature on this type of reaction.[34] The reaction can be extended to other alkyllithiums (Table 1) resulting in a good, practical method of synthesis of α,α-difluoroketones **2a–2f**. For preparation of **2f**, the corresponding ester precursor **1f** was obtained by treatment of 1-octanethiol with NaH/DMF followed by reaction with ethyl bromodifluoroacetate.



Scheme 1. Structures of compounds **2–9** synthesized as potential inhibitors of esterases.



Scheme 2. Synthetic approach to DFMKs **2** and DFAs **4**.

Table 1. Synthesis of difluoromethyl ketones **2a–2f**

Ester	R	R'	Time (h)	Product	Yield (%)
1a	C_9H_{19}	Me	1.5	**2a**	76
1b	$C_{14}H_{29}$	Me	1	**2b**	44
1c	(*Z*)-11-$C_{16}H_{31}$	Me	4.75	**2c**	84
1d	(*Z*)-12-$C_{17}H_{33}$	Me	1	**2d**	44
1d	(*Z*)-12-$C_{17}H_{33}$	Ph	1	**2e**	47
1f	$C_8H_{17}S$	Me	3	**2f**	95

Reduction of difluoroesters **1** to the corresponding alcohols was easily carried out by treatment with lithium aluminum hydride (2.9 equiv) in ether at 0 °C in good to excellent yields (Table 2).

Synthesis of DFAs **4** was much more troublesome. Thus, direct reduction of **1a** or **1d** with diisobutylaluminum hydride in pentane at −55 °C[25] led to mixtures of the difluorinated alcohols **3a**–**3d** and aldehydes **4a**–**4d** in low yields. An apparently successful preparation of α,α-difluorononaldehyde hydrate has been reported by treatment of the ester with DIBAH in ether but the yield of the aldehyde or hydrate was not disclosed.[35] Therefore, a study of the oxidation of the fluorinated alcohols **3** was undertaken. Treatment of **3d** with PDC in CH_2Cl_2 yielded a mixture of the starting alcohol (25%) and the unexpected ester **10** (15%) (Scheme 3), resulting from an overoxidation to the acid followed by esterification, and other unidentified products.

Treatment of **3c** with TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy)/*N*-cholorosuccinimide/TBACl in a biphasic CH_2Cl_2-aqueous pH 8.6 buffer system[36] or with the Dess–Martin periodinane reagent[37] under different conditions led to mixtures of products and low yields of **4c** (ca. 15%). The best conditions found for the preparation of DFAs **4** were through Swern oxidation[38] using 3 equiv of $(COCl)_2$, 6 equiv DMSO and 12 equiv of Et_3N in CH_2Cl_2 at room temperature for 30 min (Scheme 2). Compounds **4** were obtained in 35–66% isolated yields (Table 2), as mixtures of the hydrate and aldehyde forms in ca. 4:1 ratio as determined by ^{19}F NMR spectroscopy.

Inhibition studies

The inhibition tests were carried out using a preincubation time of 10 min since we assumed that, in a similar manner to TFMKs, the new DFMKs and DFAs might exert also their inhibition effect through formation of a stable hemiacetal with a serine residue of the enzyme.[39] The inhibitory potency of the chemicals was determined by the relative decrease of the substrate (the major component of the pheromone of either insect species) hydrolysis in the presence of the inhibitor, with regard to mean values of hydrolysis obtained in control experiments (see Experimental). The TFMKs (**5**, **6**, **7**) exhibited higher inhibitory activity than the DFMKs (**2f**, **2c**, **2d**) and DFAs (**4b**, **4c**, **4d**) in either insect (Table 3). The inductive effect of the fluoro substituents substantially contributes to the potency of the inhibitors, the effect being higher with an increasing number of the halogen in the vicinity of the carbonyl to stabilize the hemiacetal adduct with the serine residue.[40]

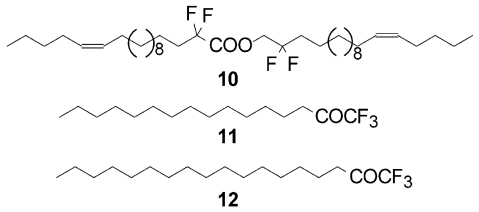
The highest inhibitory activity on both insects was shown by OTFP (**5**) with an IC_{50} 5.9 μM in *SL* and 16.3 μM in *SN* followed by compounds **7** (IC_{50} 64.5 μM in *SL* and 54.5 μM in *SN*) and **6** (IC_{50} 121.0 μM in *SL* and 123.7 μM in *SN*). Compound **7** is the TFMK analogue of (*Z,E*)-9,11-tetradecadienyl acetate, the major component of the pheromone of *SL*,[44] while **6** is the same analogue of (*Z*)-11-hexadecenyl acetate, the major component of the *SN* pheromone.[45] In both instances, the only modification was replacement of the acetate group of the parent pheromone by the trifluoroacyl moiety $COCF_3$. OTFP has been shown to act as a potent inhibitor of JHE,[41] and it is interesting to note that its closely related ketone **2f** elicited no activity. In this case, the much higher contents of hydrate in aqueous or organic solution of **5** (see below), in equilibrium with the free ketone, may account for this result. We have found by ^{19}F NMR that freshly prepared solutions of **5** in $CHCl_3$ appear to be a mixture of the hydrate and keto forms in 21:79 ratio, while in **2f** the hydrate form is practically undetectable. In this context, the activity of certain di- and trifluoro derivatives as inhibitors of acetylcholinesterase was postulated to be due to the interaction enzyme-unhydrated form of the inhibitor,[4] whereas the activity of difluorinated inhibitors of pepsin was attributed to binding of the hydrated form of the inhibitor to the enzyme.[43]

However, attribution of the higher activity of **5** with respect to **2f** to the different level of hydration in solution appears to be too simplistic since DFAs are also highly hydrated (the hydrate/keto form ratio found for freshly prepared **4d** was 80:20 in $CHCl_3$ soln., see below) but exert only moderate inhibitory activity. The real

Table 2. Synthesis of difluoroalcohols **3** and DFAs **4**

Ester	R	**3** (%)[a]	**4** (%)[a]
1a	C_9H_{19}	**3a** (75)	**4a** (66)
1b	$C_{14}H_{29}$	**3b** (65)	**4b** (35)
1c	(*Z*)-11-$C_{16}H_{31}$	**3c** (96)	**4c** (58)
1d	(*Z*)-12-$C_{17}H_{33}$	**3d** (69)	**4d** (42)

[a]DFAs were obtained as mixtures of the hydrate and keto forms.



Scheme 3. Structures of compounds **10**–**12**.

Table 3. Comparative inhibitory activity of compounds **2**–**9**

Compd	*S. littoralis* IC_{50} (μM)[a]	*S. nonagrioides* IC_{50} (μM)[a]
5	5.9 (±1.2)	16.3 (±0.4)
2f	N.A.[b]	N.A.[b]
2b	622.4 (±109.2)	850 (±56.4)
4b	513.1 (±95.5)	376.3 (±7.8)
2c	261.4 (±45.0)	264.2 (±73.3)
4c	260.3 (±22.4)	254.2 (±39.1)
2d	N.A.[b]	N.A.[b]
4d	N.A.[b]	164.9 (±26.3)
6	121.0 (±26.5)	123.7 (±39.0)
7	64.5 (±4.7)	54.5 (±7.8)
8	N.A.[b]	—
9	N.A.[b]	—

[a]IC_{50} values were calculated by least squares regression analyses in at least triplicate experiments considering five different concentrations of inhibitor and 2–3 controls for each replicate.
[b]Non active.

active form (keto or hydrate) responsible for esterase activity remains, therefore, an open question.

Compounds **8** and **9**, showing absolutely different structures than the pheromone, were completely inactive. Also, compounds with a longer chain than the main pheromone component, such as **2d** and **4d**, were ineffective as inhibitors except **4d** on *SN*. This compound is also a close analogue of the major component of the pheromone, which in addition contains (*Z*)-11-hexadecenol and *(Z)*-11-hexadecenal.[45] The activity of **4d** may be attributed to an additional inhibition on other oxidizing enzymes (oxidases, aldehyde dehydrogenases, etc.) required to perform a successful pheromone catabolism. In this context, inhibition of aldehyde dehydrogenases in antennal extracts of *H. virescens* by DFAs has been reported.[25] In *SL*, on the other hand, the pheromone complex is a mixture of dienic, monoenic and saturated acetates,[46] and, therefore, we could expect that in this insect esterases should be the major type of the catabolic proteins of the pheromone.

The calculated values of K_m and V_{max} of both esterase preparations were 5.66×10^{-4} M and 8.47×10^{-6} M min^{-1} for *SL* and 1.61×10^{-7} M and 1.25×10^{-7} M min^{-1} for *SN*, according to the double reciprocal plot of Lineweaver and Burk (Fig. 1). These results suggest that *SN* esterase has higher affinity for the substrate than that of *SL*, which agrees with the lower doses of the pheromone required (100 ng–1 μg) to elicit the complete behavioral sequence on *SN* males in comparison with *SL* (10 μg).[19]

Hydration studies

In order to establish a possible correlation between the hydration constant (K_{hyd}) and the inhibitory potency of the chemicals, we carried out for the first time studies directed to determine the K_{hyd} of these analogues by ^{19}F NMR. As cited above, several groups have established by spectrocopic means that the TFMKs inhibit several types of esterases by binding to the active serine residue as an ionized kemiketal.[12–14,47]

We followed a similar procedure to that reported by Linderman.[48] The selected compounds **2a**, **2f**, **4a**, **5**, **6** along with the saturated *n*-tetradecyl trifluoromethyl ketone (**11**) and *n*-hexadecyl trifluoromethyl ketone (**12**) (Scheme 3) were dissolved in H_2O/CH_3CN mixtures containing 0, 0.1, 0.24, 0.37, 0.5 and 0.75 mole fraction water. Except for compounds **2f** and **5**, the substrates were not completely soluble at $\chi_{H_2O}=0.75$ and, therefore, K_{hyd} app could not be readily determined at this molar fraction. On the other hand, for compound **4a** at $\chi_{H_2O}=0.1$ the K_{hyd} app was already 438, so lower mole fractions of water in the mixtures had to be considered. The keto and hydrate forms were allowed to equilibrate for 2.5 h, and the apparent K_{hyd} values were determined by the ratio of the areas of the two forms in the ^{19}F NMR spectra. The results are shown in Table 4. The K_{hyd} app and χ_{H_2O} values were plotted and adjusted to a straight line of equation

$$y = 1.07x + 2.46 \quad (r^2 = 0.96)$$

The interception point at the *Y* axis ($\chi_{H_2O}=0$) corresponded to the calculated K_{hyd} in pure water. It should be noted that the K_{hyd} app have only an approximate value since the study has been carried out following Linderman's assumptions for ideal solutions which obey Henry's or Raoult's law.[48] Our results show that there is considerable variation in the K_{hyd} values for the TFMKs, DFMKs, and DFAs tested. Thus, while DFMKs (f.i. **2a**) do not practically hydrate in solution, DFAs (f.i. **4a**) are completely hydrated. The extent of hydration follows the order: α,α-DFMKs < α,α-difluoro-β-thioalkylmethyl ketones < TFMKs < β-thiotrifluoromethyl ketones < α,α-DFAs. Comparison of the K_{hyd} with the IC_{50} values shows that although compounds with very low hydration, such as **2f**, are also inactive as inhibitors, no clear correlation between the two parameters was apparent among the active chemicals. Thus, the best inhibitor (compound **5**) showed a high K_{hyd} but DFA **4a** displayed a $K_{hyd} \cong 15\times$ higher being $\cong 16\times$ less active. Linderman et al.,[48] in contrast, reported a reasonable correlation of K_{hyd} and the potency of some TFMKs as inhibitors of JHE. Our results agree with those of the Raleigh group in that the most potent inhibitor (**5**) was also the most hydrated among the different TFMKs tested (**6**, **11**, **12**). In this context, it is worthy of note that in a molecular modeling study we have demonstrated that β-substituted TFMKs, like **5**, show an intramolecular hydrogen bond between the hydrate and the heteroatom (O, S, SO and SO_2) located in β position to the carbonyl and that the strength of this hydrogen bond correlates well with the inhibitory activity.[42]

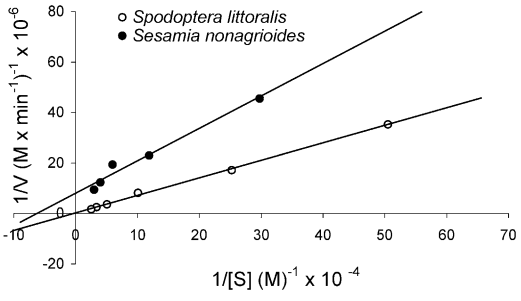


Figure 1. Lineweaver–Burk reciprocal plot of $1/[V]$ versus $1/[S]$ for determination of K_m and V_{max} of the antennal esterase of *SL* and *SN*.

Conclusions

In summary, new fluorinated derivatives have been synthesized as inhibitors of antennal esterases of two economically important pests. With the exception of compound **4d** in *SN*, the best esterase inhibitors found in both insects are the TFMKs structurally similar to the corresponding pheromone, the DFMKs and DFAs being less efficient inhibitors. The compounds appear to hydrate to different extent in aqueous solution, but no clear correlation has been found between the K_{hyd} and the inhibitory potency. The new type of compounds

Table 4. Equilibrium hydration constants of substrates **2a**, **2f**, **11**, **12**, **6**, **5** and **4a** by ^{19}F NMR spectroscopy in comparison with their inhibitory activity

Substrate	Mole fraction of H_2O in H_2O/CH_3CN mixtures								Calcd K_{hyd} (r^2)	IC_{50} (μM)
	0	0.025	0.05	0.1	0.24	0.37	0.5	0.75		
2a	0	—	—	0.0	0.1	0.2	0.3	—[a]	0.6 (0.97)	—
2f	0	—	—	0.1	0.6	0.9	1.0	2.0	2.5 (0.97)	N.A.
11	0	—	—	2.5	9.6	13.3	14.2	—[a]	31.3 (0.94)	—
12	0	—	—	3.5	10.0	12.4	16.7	—[a]	33.8 (0.98)	—
6	0	—	—	2.3	10.4	14.3	17.9	—[a]	37.5 (0.96)	123.7
5	0.3	—	—	24.0	72.7	91.2	109.7	249.0	299 (0.94)	16.3
4a	4.0	26.6	139.7	438.5	>999	>999	>999	—[a]	4499 (0.94)	254.2

[a]Substrate not completely soluble.

described may prove useful for further biochemical and basic studies in the search for new and more potent esterase inhibitors as tools in new approaches to pest control.

Experimental

Chemicals

The major components of the pheromone of *SL* and *SN*, (*Z,E*)-9,11-tetradecadienyl acetate and *(Z)*-11-hexadecenyl acetate, respectively, were purchased from Sigma (St. Louis, MO, USA) and found to be ≥98% pure by GC analysis. Compound **5** (OTFP) was prepared by alkylation of 1-octanethiol with 3-bromo-1,1,1-trifluoropropan-2-one in CH_2Cl_2 using Na_2CO_3 as base.[17] (*Z*)-11-hexadecenyl trifluoromethyl ketone (**6**) and (*E,Z*)-9,11-tetradecadienyl trifluoromethyl ketone (**7**) were prepared by *trans*-metallation reaction of the corresponding iodinated precursors[49] with *tert*-BuLi followed by treatment with ethyl trifluoroacetate.[50] Phenoxyacetyl-Ala-TFMK **8** and phenoxyacetyl-Phe-TFMK **9** had been prepared as inhibitors of metallo-β-lactamases[51] and were kindly supplied by Prof. C. J. Schofield.

General procedure for the synthesis of difluoromethyl ketones 2a–2f: (*Z*)-3,3-dfluoro-14-nonadecen-2-one (2c). Into a previously-flamed round-bottomed flask was introduced under Ar a soln of ethyl (*Z*)-2,2-difluoro-13-octadecenoate (21 mg, 0.06 mmol) in 1 mL of anhyd THF. The solution was cooled to −78 °C and then 77 μL of a 1.17 M soln of methyllithium in ether (0.09 mmol) were added dropwise. After stirring for 3 h 30 min at this temperature, the reaction was quenched by adding 1 mL of NH_4Cl satd soln. The solvent was stripped off and the organic material extracted with hexane (4×10 mL). The organic phases were washed with $NaHCO_3$ satd soln, brine and dried ($MgSO_4$). Evaporation of the solvent left a residue which was purified by column chromatography on silica gel 60 A (SDS, 35–70 μm) eluting with hexane/diethyl ether mixtures to give 16 mg (84%) of ketone **2c**. IR (film), ν: 2954, 2925, 2854, 1747, 1433, 1361, 1207, 1068, 968 cm^{-1}. ^1H NMR (300 MHz), δ: 5.32 (m, 2H, C*H*=C*H*), 2.29 (t, *J*=1.5 Hz, 3H, CH_3CO), 2.02–1.85 (c, 6H, CH_2CF$_2$, CH_2CH=CHCH_2), 1.44 (m, 2H, CH_2CH$_2$CF$_2$), 1.23 (bs, 18H, 9CH_2), 0.87 (t, *J*=6.6 Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −107.49 (t, *J*=17.5 Hz) ppm. ^{13}C NMR (75 MHz), δ: 199.1 (t, *J*=32 Hz, CF$_2$*C*O), 129.8 (*C*=*C*), 118.1 (t, *J*=250 Hz, *C*F$_2$), 32.3 (t, *J*=22.8 Hz, *C*H$_2$CF$_2$), 31.9, 29.7, 29.5, 29.4, 29.3, 29.26, 29.24, 27.1, 26.9, 24.1, 22.3, 21.2 (t, *J*=4.3 Hz, *C*H$_2$CF$_2$), 13.9 (*C*H$_3$) ppm. MS (EI), *m/z* (%): 316 (M^+, 0.6), 97 (33), 83 (50), 69 (73), 56 (62), 55 (100), 41 (98). Elem. Anal.: calcd for $C_{19}H_{34}OF_2$: C, 72.11; H, 10.82; F, 12.00. Found: C, 72.34; H, 10.93; F, 12.47.

3,3-Difluorododecan-2-one (2a). Yield 76%. IR (film), ν: 2958, 2927, 2856, 1747, 1467, 1361, 1207, 1083, 997, 806, 722 cm^{-1}. ^1H NMR (300 MHz), δ: 2.29 (t, *J*=1.5 Hz, 3H, CH_3), 1.93 (m, 2H, CH_2CF$_2$), 1.23 (bs, 14H, 7CH_2), 0.85 (t, *J*=6.9 Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −107.52 (t, *J*=17.2 Hz) ppm. ^{13}C NMR (75 MHz), δ: 199.08 (t, *J*=32.5 Hz, *C*O), 118.16 (t, *J*=249.6 Hz *C*F$_2$), 32.68, 32.38, 32.07, 31.83, 29.35, 29.28, 29.25, 29.22, 22.64, 21.25 (t, *J*=4.3 Hz, *C*H$_2$CF$_2$), 14.06 (*C*H$_3$) ppm. MS (EI), *m/z* (%): 220 (M^+,0.2%), 149 (1.5), 95(1.5), 94 (2), 57(8), 55(9), 43 (100). Elem. Anal.: calcd for $C_{12}H_{22}OF_2$: C, 65.42; H, 10.06; F, 17.25. Found: C, 65.55, H: 9.99, F,17.20.

3,3-Difluoroheptadecan-2-one (2b). (44% yield) IR (film), ν: 2925, 2956, 2854, 1747, 1427, 1467, 721 cm^{-1}. ^1H NMR (300 MHz), δ: 2.28 (t, *J*=1.5 Hz, 3H, CH_3), 1.96 (m, 2H, CH_2CF$_2$), 1.23 (bs, 24H, 12CH_2), 0.85 (t, *J*=6.9 Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −107.50 (t, *J*=18.6 Hz) ppm. ^{13}C NMR (75 MHz), δ: 198.9 (t, *J*=32.2 Hz, *C*O), 118.1 (t, *J*=249.6 Hz, *C*F$_2$), 32.37, 31.92, 29.68, 29.65, 29.62, 29.57, 29.40, 29.35, 29.29, 29.25, 23.99, 22.68, 21.2 (t, *J*=4.3 Hz, *C*H$_2$CF$_2$), 14.06 (*C*H$_3$) ppm. MS (EI), *m/z* (%): 290 (M^+, 0.5), 163 (1.5), 150 (1), 85 (5), 71 (9), 57 (15), 43 (100).

(*Z*)-3,3-Difluoro-15-eicosen-2-one (2d). (44% yield) IR (film), ν: 3004, 2956, 2925, 2856, 1747, 1465, 1074, 910, 734 cm^{-1}. ^1H NMR (300 MHz), δ: 5.32 (2dt, $J_1=J_2=4.5$ Hz, 2H, C*H*=C*H*), 2.29 (t, *J*=1.8 Hz, 3H, C(O)CH_3), 2.0 (m, 6H, CH_2CF$_2$, 2CH_2CH=CH), 1.24 (bs, 22H, 11CH_2), 0.87 (t, *J*=6.9 Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −107.49 (t, *J*=17.2 Hz) ppm. ^{13}C NMR (50 MHz), δ: 199.1 (t, *J*=33.5 Hz, *C*=O), 129.8 (*C*H=*C*H), 118.1 (t, *J*=250 Hz, *C*F$_2$), 32.35, 31.96, 29.76, 29.55, 29.39, 29.28, 27.19, 26.90, 24.10, 22.34, 21.23 (t, *J*=4.6 Hz, *C*H$_2$CF$_2$), 13.99 (*C*H$_3$) ppm. MS (EI), *m/z* (%): 330 (M^+, 3), 312 (3), 287 (3), 185 (3),

180 (2), 97 (23), 83 (32), 69 (41), 55 (70), 43 (100). Elem. Anal.: calcd for $C_{20}H_{36}F_2O$: C: 72.68; H: 10.97; F: 11.49. Found: C: 72.27; H: 11.13.

(*Z*)-1,1-Difluoro-13-octadecenyl phenyl ketone (2e). Yield 47%. IR (film), ν: 3002, 2925, 2854, 1703, 1598, 1450, 1178, 713 cm^{-1}. ^1H NMR (300 MHz), δ: 8.07 (m, 2H, arom.), 7.61 (m, 1H, arom.), 7.47 (m, 2H, arom.), 5.33 (2dt, $J_1=J_2=4.5$ Hz, 2H, C*H*=C*H*), 2.01 (m, 6H, CH_2CF_2, $2CH_2CH$=CH), 1.25 (bs, 22H,–CH_2–), 0.88 (t, $J=6.8$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −100.74 (t, $J=17.7$ Hz) ppm. ^{13}C NMR (50 MHz), δ: 189.5 (t, $J=31$ Hz, *C*=O), 134.1, 130.0, 128.5, 127.1 (C_6H_5), 129.84, 129.80 (*C*H=*C*H), 119.8 (t, $J=251$ Hz, CF_2), 31.96, 29.75, 29.56, 29.52, 29.41, 29.31, 29.20, 27.18, 26.90, 22.33, 21.3 (t, $J=4.3$ Hz, CH_2CF_2), 13.97 (CH_3) ppm. MS (EI), *m*/*z* (%): 392 (M^+, 1), 374 (3), 219 (2), 206 (4), 156 (3), 105 (100), 77 (15), 55 (11).

Ethyl 2,2-difluoro-2-octylthioacetate (1f). In a three-necked dry round-bottomed flask, equipped with magnetic stirrer and argon inlet, was placed sodium hydride (37 mg, 0.76 mmol) as a 55% dispersion in oil, which was washed with pentane. To the dry hydride was added DMF (0.6 mL) and 1-octanethiol (146 mg, 1 mmol) in DMF (1 mL) and the slurry was stirred until evolution of hydrogen ceased. Then, ethyl bromodifluoroacetate (156 mg, 0.76 mmol) was added and the reaction mixture stirred for 1 h. After the usual work up, the resulting reaction mixture was chromatographed on silica gel 60 A (SDS, 35–70 μm) eluting with hexane/ether mixtures to give the expected difluorinated ester **1f** (134 mg, 65%). IR ν: 2958, 2929, 2856, 1770, 1467, 1299, 1101, 987, 723 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 4.32 (q, $J=7.2$ Hz, 2H, CH_2CH_3), 2.83 (t, $J=7.5$ Hz, 2H, CH_2S), 1.63(qt, $J=7.80$ Hz, 2H, CH_2), 1.33 (t, $J=7.2$ Hz, 3H, CH_2CH_3), 1.24 (bs, 14H, $7CH_2$), 0.84 (t, $J=6.30$ Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$) δ: −83.35 (s). ^{13}C NMR ($CDCl_3$) δ: 161.88 (t, $J=32.88$ Hz, *C*O), 120.68 (t, $J=285.24$ Hz, CF_2), 63.47, 31.70, 29.54, 29.04, 28.92, 28.67 (t, $J=2.94$ Hz, CH_2S), 28.61, 22.57, 13.99, 13.80. Elem. Anal.: calcd for $C_{12}H_{22}F_2O_2S$: C: 53.70; H: 8.26; F: 14.16; S: 11.95. Found: C: 53.68; H: 8.42; F: 14.36; S: 11.77.

3,3-Difluoro-3-octylthiopropan-2-one (2f). (95% yield) IR (film), ν: 2956, 2927, 2856, 1747, 1467, 1087, 904 cm^{-1}. ^1H NMR (300 MHz), δ: 2.74 (t, $J=7.2$ Hz, 2H, CH_2S), 2.35 (t, $J=1.5$ Hz, 3H, $COCH_3$), 1.61(qt, $J=7.8$ Hz, 2H, CH_2), 1.28 (bs, 12H, $6CH_2$), 0.85(t, $J=6.60$ Hz, 3H, CH_3). ^{19}F NMR (282 MHz), δ: −87.62 (s). ^{13}C NMR (50 MHz), δ: 193.79 (t, $J=30.92$ Hz, *C*O), 123.16 (t, $J=288.11$ Hz, CF_2), 31.72, 29.53, 29.06, 28.93, 28.63, 28.53 (t, $J=3.24$ Hz, CH_2S), 23.35, 22.59, 14.03. Elem. Anal.: calcd for $C_{11}H_{20}F_2OS$: C, 55.43; H, 8.46; S, 13.45; F, 15.94. Found: C, 55.83; H, 8.22; S, 13.69; F, 15.96.

General procedure for the synthesis of difluoroalcohols 3a-3d: (*Z*)-2,2-Difluoro-13-octadecenol (3c). To a mixture of lithium aluminum hydride (21 mg, 0.52 mmol) in 3 mL of anhyd ether was added, at 0 °C under Ar, ethyl (*Z*)-2,2-difluoro-13-octadecenoate (63 mg, 0.18 mmol) in 1 mL of anhyd ether. The mixture was stirred at room temperature for 1 h, again cooled to 0 °C and quenched with 20 μL of water. After reaching room temperature, 1 mL of water and 2 mL of 0.5 N HCl were sequentially added. The organic material was extracted with ether (4×10 mL) and washed with brine and dried ($MgSO_4$). Evaporation of the solvent left a residue which was purified by column chromatoghraphy on silica gel eluting with hexane/diethyl ether mixtures to furnish alcohol **3c** (53 mg, 96%). Mp 47–50 °C. IR (film), ν: 3359, 2954, 2925, 2850, 1465, 908, 734 cm^{-1}. ^1H NMR (300 MHz), δ: 5.32 (m, 2H, C*H*=C*H*), 3.7 (dt, $J_1=13$ Hz, $J_2=6.9$ Hz, 2H, CH_2OH), 2.0–1.8 (c, 7H, CH_2CF_2, CH_2CH=$CHCH_2$, O*H*), 1.48 (m, 2H, $CH_2CH_2CF_2$), 1.24 (bs, 18H, $9CH_2$), 0.87 (t, $J=7.2$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −109.12 (tt, $J_1=17.6$ Hz, $J_2=13$ Hz) ppm. ^{13}C NMR (75 MHz), δ: 129.8 (*C*=*C*), 123.3 (t, $J=240$ Hz, CF_2), 64.0 (t, $J=32$ Hz, CF_2CH_2O), 32.2 (t, $J=23.7$ Hz, CH_2CF_2), 31.9, 29.7, 29.5, 29.48, 29.43, 29.36, 29.34, 29.2, 27.1, 26.8, 22.3, 21.7 (t, $J=4.5$ Hz), 13.9 (CH_3) ppm. MS (EI), *m*/*z* (%): 304 (M^+, 1.8), 83 (50), 69 (60), 55 (100), 43 (33). Elem. Anal.: calcd for $C_{18}H_{34}OF_2$: C, 71.01; H, 11.25; F, 12.48. Found: C, 71.36; H, 11.33; F, 12.31.

2,2-Difluoroundecanol (3a). Yield 75%. IR (film), ν: 3278, 2952, 2923, 2854, 1465, 1201, 1082, 908, 736 cm^{-1}. ^1H NMR (300 MHz), δ: 3.73 (dt, $J_1=12.9$ Hz, $J_2=6.3$ Hz, 2H, CH_2OH), 1.85 (m, 2H, CH_2CF_2), 1.24 (bs,14H, –CH_2–), 0.85 (t, $J=6.6$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −109.14 (tt, $J_1=17.2$ Hz, $J_2=12.4$ Hz) ppm. ^{13}C NMR (75 MHz), δ: 123.3 (t, $J=240$ Hz, CF_2), 64.0 (t, $J=31$ Hz, CH_2OH), 33.2 (t, $J=24$ Hz, CH_2CF_2), 31.84, 29.36, 29.25, 22.65, 21.7 (t, $J=4.4$ Hz, $CH_2CH_2CF_2$), 14.06 (CH_3) ppm. MS (EI), *m*/*z* (%): 208 (M^+, <1), 141 (1.6), 110 (5), 99 (13), 85 (43), 69 (27), 55 (45), 43 (100).

2,2-Difluorohexadecanol (3b). (65% yield) IR (film), ν: 3278, 2954, 2921, 2848, 1462, 1209, 1024, 908, 734 cm^{-1}. ^1H NMR (300 MHz), δ: 3.70 (dt, $J_1=12.9$ Hz, $J_2=4.2$ Hz, 2H, CH_2OH), 1.99 (m, 2H, CH_2CF_2), 1.23 (bs, 24H, $12CH_2$), 0.85 (t, $J=6.8$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −109.13 (tt, $J_1=17.2$ Hz, $J_2=12.7$ Hz) ppm. ^{13}C NMR (75 MHz), δ: 123.3 (t, $J=240$ Hz, CF_2), 64.0 (t, $J=32$ Hz, CH_2OH), 32.2 (t, $J=24$ Hz, CH_2CF_2), 31.91, 29.68, 29.66, 29.64, 29.60, 29.45, 29.37, 29.35, 22.68, 21.7 (t, $J=4.6$ Hz, $CH_2CH_2CF_2$), 14.10 (CH_3) ppm. MS (EI), *m*/*z* (%): 278 (M^+, <1), 258 (0.2), 240 (0.6), 212 (0.3), 85 (72), 71 (51), 57 (91), 43 (100).

(*Z*)-2,2-Difluoro-14-nonadecen-1-ol (3d). (69% yield) IR (film), ν: 3400, 3011, 2954, 2925, 2854, 1465, 1070, 908, 722 cm^{-1}. ^1H NMR (300 MHz), δ: 5.32 (m, 2H, C*H*=C*H*), 3.71 (dt, $J_1=12.6$ Hz, $J_2=4.8$ Hz, 2H, CH_2OH), 1.99 (m, 6H, CH_2CF_2, $2CH_2CH$=CH), 1.24 (bs, 22H, $11CH_2$), 0.87 (t, $J=7.2$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −109.14 (tt, $J_1=17.2$ Hz, $J_2=12.7$ Hz) ppm. ^{13}C NMR (75 MHz), δ: 129.87, 129.83 (*C*H=*C*H), 123.33 (t, $J=241$ Hz, CF_2), 64.07 (t, $J=32$ Hz, CH_2OH), 33,28 (t, $J=24$ Hz, CH_2CF_2), 31.96, 29.75, 29.58, 29.52, 29.44, 29.37, 29.34, 29.29, 27.18, 26.90, 22.33, 21.77 (t, $J=4.6$ Hz, $CH_2CH_2CF_2$), 13.98 (CH_3) ppm.

General procedure for the synthesis of difluoroaldehydes 4a–4d: (*Z*)-2,2-difluoro-13-octadecenal (4c). To a cold solution (−60 °C) of oxalyl chloride (25 μL, 0.295 mmol) in 0.2 mL of anhyd methylene chloride were added, under Ar and with vigorous stirring, anhyd DMSO (41 μL, 0.591 mmol) and anhyd methylene chloride (0.3 mL). The reaction mixture was stirred for 2 min and then a soln of (*Z*)-2,2-difluoro-13-octadecenol (3c) (30 mg, 0.098 mmol) in 0.2 mL of anhyd methylene chloride was added. The mixture was stirred for 30 min at −60 °C, then anhyd triethylamine (165 μL, 1.18 mmol) was added and the resulting mixture stirred at 0 °C for 30 min. After dilution with methylene chloride (5 mL), the reaction was quenched by addition of 1N HCl (2 mL). The aqueous layer was extracted with methylene chloride (4×5 mL), washed with $NaHCO_3$ sat. soln, brine and dried ($MgSO_4$). Removal of the solvent left a residue, which was purified by column chromatography on silica gel eluting with hexane/ether mixtures. The expected (*Z*)-2,2-difluoro-13-octadecenal (4c) was obtained (17 mg, 58%) as a mixture of the keto and hydrate forms in a ratio of 1:4. IR (film), ν: 3350, 3200, 3004, 2954, 2921, 2852, 1720, 1654, 1465, 1060, 734 cm^{-1}. ^1H NMR (300 MHz), δ: 9.48 (t, $J=1.2$ Hz, 1H, C*H*O), 5.32 (m, 2H, C*H*=C*H*), 4.97 (t, $J=6.9$ Hz, 1H, C*H*$(OH)_2$), 2.0–1.8 (c, 6H, CH_2CF_2, CH_2CH=CHCH_2), 1.24 (bs, 20H, 10CH_2), 0.87 (t, $J=7.2$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −111.2 (t, $J=17.4$ Hz, CF_2CHO), −118.3 (dt, $J_1=18$ Hz, $J_2=6.4$ Hz, $CH_2CF_2CH(OH)_2$) ppm. ^{13}C NMR (75 MHz), δ: 190.6 (t, $J=39$ Hz, *C*HO), 129.8 (*C*=*C*), 121.3 (t, $J=333$ Hz, *C*F_2), 89.6 (t, $J=31$ Hz, $CF_2CH(OH)_2$), 31.9, 29.7, 29.54, 29.50, 29.46, 29.43, 29.38, 29.34, 29.27, 29.25, 27.1, 26.8, 22.3, 21.1 (t, $J=4.2$ Hz), 13.9 (*C*H_3) ppm. MS (EI), *m*/*z* (%): 302 (M^+, 1.6), 97 (50), 83 (59), 69 (82), 57 (57), 56 (77), 55 (100), 41 (57). Exact Mass. calcd for $C_{18}H_{32}OF_2$: 302.2421. Found: 302.2420.

2,2-Difluoroundecanal (4a). Yield 66%. IR (film), ν: 3300, 3282, 2956, 2918, 2850, 1714, 1467, 1076, 1226, 1049, 736 cm^{-1}. ^1H NMR (300 MHz), δ: 9.48 (bs, 1H, C*H*O), 4.98 (t, $J=6.6$ Hz, 1H, C*H*$(OH)_2$), 3.10 (bs, 2H, CH(O*H*$)_2$), 1,90 (m, 2H, CH_2CF_2), 1.24 (bs, 24H, –CH_2–), 0.85 (t, $J=6.6$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −111.22 (t, $J=17.7$ Hz, CF_2CHO), −118.34 (dt, $J_1=17.7$ Hz, $J_2=7.0$ Hz, 2F, CF_2CH$(OH)_2$) ppm. ^{13}C NMR (75 MHz), δ: 190.63 (t, $J=39$ Hz, *C*HO), 121.4 (t, $J=244$ Hz, *C*F_2), 89.69 (t, $J=31$ Hz, *C*H$(OH)_2$), 31.85, 30.32, 29.41, 29.33, 29.25, 29.21, 22.66, 21.06 (t, $J=4.3$ Hz, *C*H_2CF_2), 14.08 (*C*H_3) ppm. MS (EI), *m*/*z* (%): 206 ($M^+<1$), 186 (1.5), 177 (1.6), 160 (2), 146(6), 135(39), 121 (28), 71 (27), 57 (50), 43(100).

2,2-Difluorohexadecanal (4b). (35% yield) IR (film), ν: 3100, 2956, 2918, 2850, 1714, 1471, 1076 cm^{-1}. ^1H NMR (300 MHz), δ: 9.48 (bs, 1H, C*H*O), 4.98 (t, $J=6.6$ Hz, 1H, C*H*$(OH)_2$), 3.02 (bs, 2H, CH(O*H*$)_2$), 1.90 (m, 2H, CH_2CF_2), 1.24 (bs, 24H, 12CH_2), 0.85 (t, $J=6.3$ Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −111.21 (t, $J=18.0$ Hz, CF_2CHO), −118.36 (dt, $J_1=18.6$ Hz, $J_2=7.0$ Hz, 2F, CF_2CH$(OH)_2$) ppm. ^{13}C NMR (75 MHz), δ: 190.61 (t, $J=36$ Hz, *C*HO), 121.38 (t, $J=244$ Hz, *C*F_2), 89.72 (t, $J=31$ Hz, *C*H$(OH)_2$), 31.92, 29.71, 29.64, 29.60, 29.55, 29.45, 29.27, 29.20, 22.68, 20.85 (t, $J=4.5$ Hz, *C*H_2CF_2), 14.11 (*C*H_3) ppm. MS (EI), *m*/*z* (%): 121(8), 97(20), 85(24), 71(46), 57(92), 43 (100).

(Z)-2,2-Difluoro-14-nonadecenal (4d). (42% yield) IR (film), ν: 3361, 3230, 3011, 2954, 2920, 2920, 2850, 1720, 1656, 1471, 1076, 736 cm^{-1}. ^1H NMR (300 MHz), δ: 9.48 (s, 1H, C*H*O), 5.32 (m, 2H, C*H*=C*H*), 4.97 (t, $J=7.0$ Hz, 1H, C*H*$(OH)_2$), 3.14 (bs, 2H, 2O*H*), 1.99 (m, 6H, CH_2CF_2, 2CH_2CH=CH), 1.24 (bs, 22H, 11CH_2), 0.87 (t, 7.2 Hz, 3H, CH_3) ppm. ^{19}F NMR (282 MHz), δ: −111.21 (t, $J=17.5$ Hz, CF_2CHO), −118.33 (dt, $J_1=18.3$ Hz, $J_2=6.7$ Hz, 2F, CF_2CH$(OH)_2$) ppm. ^{13}C NMR (75 MHz), δ: 190.6 (t, $J=39$ Hz, *C*HO), 129.87, 129.85 (*C*H=*C*H), 121,3 (t, $J=333$ Hz, *C*F_2), 89.6 (t, $J=31.3$ Hz, $CF_2CH(OH)_2$), 31.96, 29.75, 29.69, 29.57, 29.52, 29.44, 29.39, 29.35, 29.33, 29.27, 27.18, 26.90, 22.34, 21.11 (t, $J=4.5$ Hz, *C*H_2CF_2), 13.9 (*C*H_3) ppm. MS (EI), *m*/*z* (%): 316 (M^+, 24), 296 (1), 288 (1), 260 (2), 203 (8), 189 (7), 125 (8), 111 (16), 97 (29), 83 (32), 69 (41), 55 (100).

Determination of hydration constants

Compounds 2a, 2f, 4a, 5, 6, 11 and 12 were dissolved in water-acetonitrile mixtures, left for 2.5 h at a constant 20 °C temperature, and the ^{19}F NMR spectrum of the mixtures was recorded. The apparent K_{hyd} value was determined by simply integrating the area of the signal for the hydrate and keto forms and using these values to obtain the hydrate/keto ratio.[48] The actual K_{hyd} in pure water was obtained by extrapolation of the K_{hyd} app from a series of known mole fraction water (χ_{H_2O} 0.025, 0.05, 0.1, 0.24, 0.37, 0.5 and 0.75). The experiments were carried out in duplicate. The apparent K_{hyd} measured is a function of χ_{H_2O} and is related to the actual K_{hyd} by the activity of water (a_{H_2O}) in the solvent mixture. In the ideal case, the a_{H_2O} equals to χ_{H_2O} and therefore

$$K_{\text{hyd app}} = K_{\text{hyd}} \cdot \chi_{H_2O} = \frac{[\text{hydrate}]_{\text{equiv}}}{[\text{keto}]_{\text{equiv}}} \cdot \chi_{H_2O}$$

Insects

SL were reared in our laboratory on a slightly modified diet as compared to that reported previously.[52] Pupae were sexed and adults maintained at 25±1 °C on a 16 h:8 h L/D cycle until use. *SN* pupae proceeded from a culture maintained at the laboratories of the University of Lleida-IRTA (Lleida, Spain) following a modified diet from Poitout and Bues.[53] After reception, pupae were placed in a reverse photoperiod until emergence under similar conditions than *SL*.

Inhibition studies

Males of 1–2 days old were anesthetized with CO_2 and their antennae removed. These were immediately frozen

and kept at −80 °C until use. Antennal esterase preparations of *SL* were obtained by homogenizing batches of frozen antennae in 20 mM Tris–HCl buffer (pH = 7.4) on a variable speed Heidolph ZZR-2000 mixer for 5 min in an ice bath. The same protocol was used for *SN* esterase at pH = 8.0. Preliminary experiments had shown optimum enzymatic activity of extracts of the two insects at these pHs. The homogenate was sonicated at 40 w for 10 s and centrifuged at 14,000*g* for 2 min at 4 °C to remove the cuticular debris. The supernatant was adjusted with the required volume of the corresponding buffer solutions so that an aliquot of 100 μL was the equivalent of four antennae. For each assay, this aliquot was added to borosilicate tubes, previously treated with 1-decanol to prevent adsorption of the substrate to the glass surface.[54] The inhibitor solutions were prepared by concentration to dryness of an aliquot of the mother solution in hexane or ethyl acetate, followed by addition of ethanol so that 2 μL of the soln in 100 μL of the antennal preparation gave the desired final concentration of the inhibitor (1–800 μM). The inhibition tests were conducted by preincubation of 2 μL of the inhibitor solutions with one aliquot of the extract for 10 min at 28 °C for *SL* and 32 °C for *SN*. Then, the major component of the pheromone (2 μmoles for that of *SL* and 0.35 μmoles for that of *SN*) in 2 μL of ethanol was added to the mixture and incubated for 60 min more at the preincubation temperature for each insect. Incubation was stopped by addition of 160 μL of hexane. After vortexing for 1 min, the organic phase was separated and stored at −80 °C. Just before analysis, the solution was concentrated to a small volume (5–10 μL) and injected in GC. The extent of hydrolysis was calculated by the relative amount of the pheromone-derived alcohols with regard to the parent acetates on a GC fused silica capillary column (HP-FFAP 25 m×0.25 mm ID). For *SL* the GC conditions were injection at 80 °C hold for 1 min, followed by temperature program of 10 °C/min up to 230 °C and held at this temperature for 10 min. For *SN* the conditions were injection at 100 °C hold for 1 min, followed by temperature program of 5 °C/min up to 200 °C and then at 10 °C/min up to 230 °C and held at this temperature for an additional 10 min. Inhibitory potencies of the chemicals were determined by the relative decrease of substrate hydrolysis in the presence or not of inhibitor. IC_{50} of each compound was calculated by least squares regression analyses in at least triplicate experiments considering 5 different concentrations of the inhibitor and 2–3 controls for each replicate. An inhibitor was considered non active when no inhibition activity was found at 300 μM concentration.

Determination of kinetic parameters

For the determination of K_m and V_{max} of the enzyme of each species, four antennal equivalents in 100 μL of 20 mM Tris–HCl buffer (pH = 7.4 for *SL* or pH = 8.0 for *SN*) were added to borosilicate tubes, previously treated with 1-decanol as above. Then, 2 μL of a mother solution of the substrates in ethanol were added to the tubes to get a final concentration of the substrates from 1.5 to 50 μM. The mixture was incubated for 60 min at 28 °C for *SL* and 32 °C for *SN* and the process stopped by addition of 160 μL of hexane. A similar protocol was applied, and at least six replicates for each concentration were conducted.

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