Supporting information for the manuscript entitled:

Difluoromethylketones: potent inhibitors of WT and carbamate-insensitive G119S mutant *Anopheles gambiae* acetylcholinesterase

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Table of Contents

A. Procedures for biological assays

Reagents, materials and buffers for enzyme assays.
The Ellman reagent (5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), ≥98%), acetylthiocholine iodide (ATCh, ≥98%), bovine serum albumin (BSA, heat shock fractionated, A7030, lyophilized powder, ≥98%), dimethyl sulfoxide (DMSO, ≥99.9%) and recombinant *h*AChE (C1682) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant WT and G119S *Ag*AChE were prepared as previously described.[1] Propoxur (technical grade) was purchased from Mobay Chemical Corporation, Pittsburgh, PA, USA. Standard clear polystyrene 96-well microplates (8 rows A to H, by 12 columns 1 to 12 format; flat bottom wells, MPG-655101) and SealPlate adhesive films (LMT-SEAL-EX) were purchased from Phenix Research Products (Candler, NC, USA). Non-sealing polystyrene Costar corner notch lid covers (No. 3931 or 3099) for 96-well microplates were obtained from Corning Incorporated (Corning, NY, USA). Other chemicals and materials were purchased from Fisher Scientific Company LLC (Suwanee, GA, USA) or Sigma-Aldrich (St. Louis, MO, USA). Unless otherwise stated, the following buffers were used in this work: 1) buffer A is 0.1 M sodium phosphate buffer containing 0.02% NaN_3 (w/v), pH 7.7 at room temperature (23 ± 1°C), while 2) buffer B is buffer A containing 0.3% (v/v) Triton X-100, and 1 mg/mL bovine serum albumin (BSA), pH 7.7 at room temperature (23 ± 1°C).

Note: all solutions of fluorinated methyl ketones prepared for enzyme inhibition studies were

stored in a fume cupboard.

Enzyme inhibition assays.
Wild-type AChE enzymes (r*Ag*AChE-WT and *h*AChE) were diluted in ice-cold buffer B and observed using a modified Ellman assay[2] (described below) to give an approximate reaction rate of 40.0 mO.D./min (or ~0.0049 U/ml) at room temperature (23 ± 1°C), as previously reported.[1] Resistant enzyme (r*Ag*AChE-G119S) were diluted similarly and observed at a reaction rate of approximately 28.0 mO.D./min (or 0.0034 U/ml). All diluted enzymes were kept over ice prior to use. Aliquots of the diluted enzymes (10 μL) thus prepared were separately incubated, in duplicate, in a 96-well microplate with buffer A (150 μL), following the addition of an inhibitor solution (20 μL). The inhibitor solutions were prepared in buffer A with a DMSO concentration of 1% (v/v); the final assay concentration of DMSO was thus 0.1% (v/v). Note that inhibitor-free solvent controls substituted the inhibitor solution with 1% (v/v) DMSO in buffer A (20 μL). After waiting for the desired incubation time *t* at room temperature (23 ± 1°C), a freshly prepared solution of ATCh and DTNB (4 and 3 mM respectively, in buffer A, 20 μL), was added and mixed manually to start the enzymatic reaction. Thus, a total volume of 200 μL and optical pathlength of 0.60 cm was achieved in each well, with the following final concentrations: 0.015% (v/v) Triton X-100, 0.05 mg/mL BSA, 0.4 mM ATCh, 0.3 mM DTNB. Enzyme activity, in the absence and presence of inhibitor (v_0 and v, respectively), was monitored continuously at 405 nm at room temperature (23 ± 1°C) for up to 2.3 min on a DYNEX Triad microplate reader (DYNEX Technologies, Chantilly, VA, USA), analyzed using the Concert TRIAD Series Analysis Software (version 2.1.0.17), and corrected for spontaneous substrate hydrolysis. Inhibition potency of the fluoromethylketone inhibitors was typically assessed by measuring the IC_{50} values (nM) following a 10 and 60 min incubation of the enzymes in a 96-well microplate. A dose-response experiment was carried out with a series of inhibitor concentrations [I] ranging from 10^{-5} to 10^{-11} M in buffer A, starting with a 0.01 M inhibitor stock solution in DMSO, and two sets of inhibitor-free controls. At least ten inhibitor concentrations (in duplicate) were used and dispensed using a 12-multichannel pipette, with one set of inhibitor-free solvent control placed next to the highest [I] wells, and the other inhibitor-free solvent control set close to the lowest [I] wells. The enzyme residual activity (v/v_0) were determined and used to construct dose-response curves (Prism™ 5.0f software for Mac OS X, GraphPad Software Inc., San Diego, CA, USA, www.graphpad.com) according to the following nonlinear regression equation:

> log(inhibitor) versus normalized response (variable slope) equation:
> Y = 100 / (1 + 10ˆlogIC_{50}-X)*Hill slope);
> where X = log[I] and Y = percent of residual activity = $v/v_0 \times 100\%$.

Inhibitors assayed contained 0.1% DMSO (v/v) constant solvent in buffer A. For inhibitors that showed high volatility (see below), only the enzyme activity v_0 from the unaffected inhibitor–free solvent control was used. These were typically obtained from the inhibitor-free solvent controls that were distal from the highest [I] used on the microplate (next to the lowest [I] wells), and showed high enzyme activity compared to the other set of inhibitor-free solvent controls that were next to the highest [I] wells. The IC_{50} values (nM) were determined from at least two repeat experiments, using all the dose-response data collected. Standard error (SE) of the IC_{50} values are calculated from the 95% confidence interval according to the standard error formula SE =

(upper limit – lower limit)/(2×1.96).[3] Note that since the majority of trifluoromethyl ketone and difluoromethyl ketone inhibitors show respectively high to medium volatility, and comparatively high to medium vapor phase diffusion into the neighboring wells on the 96-well microplate (see below). The microplates for longer incubation dose-response experiments, at t = 60 min, were typically sealed with a SealPlate adhesive film to prevent and slow down evaporation.

Vapor phase diffusion experiments using *Ag*AChE-WT:
The enzyme activity assay and plate loading protocol for the vapor phase diffusion experiments were as described above with slight plate loading modification:
First, the enzyme-free background wells for spontaneous substrate hydrolysis (row H) were charged with buffer A (180 μL). Except for the center two wells D6-D7, wells in rows A to G were charged with buffer A (150 μL), aliquots of diluted *Ag*AChE-WT (10 μL) were added, followed by 1% (v/v) DMSO in buffer A (20 μL). An inhibitor solution (10^{-4} M, 20 μL) in buffer A with a DMSO concentration of 1% (v/v) was finally added to the center two wells D6-D7 for incubation and the microplate was loosely covered with a non-sealing polystyrene Costar corner notch lid. After the desired incubation time t at room temperature (23 ± 1°C), a freshly prepared solution of ATCh and DTNB (4 and 3 mM respectively, in buffer A, 20 μL) was added to each rows A to H (beginning at row D), and the enzyme activity was measured in the microplate reader. The final concentration of inhibitor in the center two wells D6-D7 was thus 10,000 nM in a total volume of 200 μL. Enzyme activity v for each well was monitored continuously at 405 nm at room temperature (23 ± 1°C) as described above, and corrected for spontaneous substrate hydrolysis (row H). The percent of residual activity ($v/v_0 \times 100\%$) for each well were determined using the highest average enzyme activity rates v_0 obtained from the inhibitor-free solvent controls from columns 1 or 12 of the microplate in rows A to G. Conditional formatting using Microsoft Excel for Mac 2011 was used to construct the microplate heat map displays. Three color formatting was chosen for the percent of residual activity, with the following color legend cutoffs: minimum: red = 10%; midpoint: yellow = 75%; maximum: green = 93%.

B. *Ag*AChE-WT residual activity data for Heat Map Figures 3 and 4, and Heat Map for dichlorvos (Figure S1).

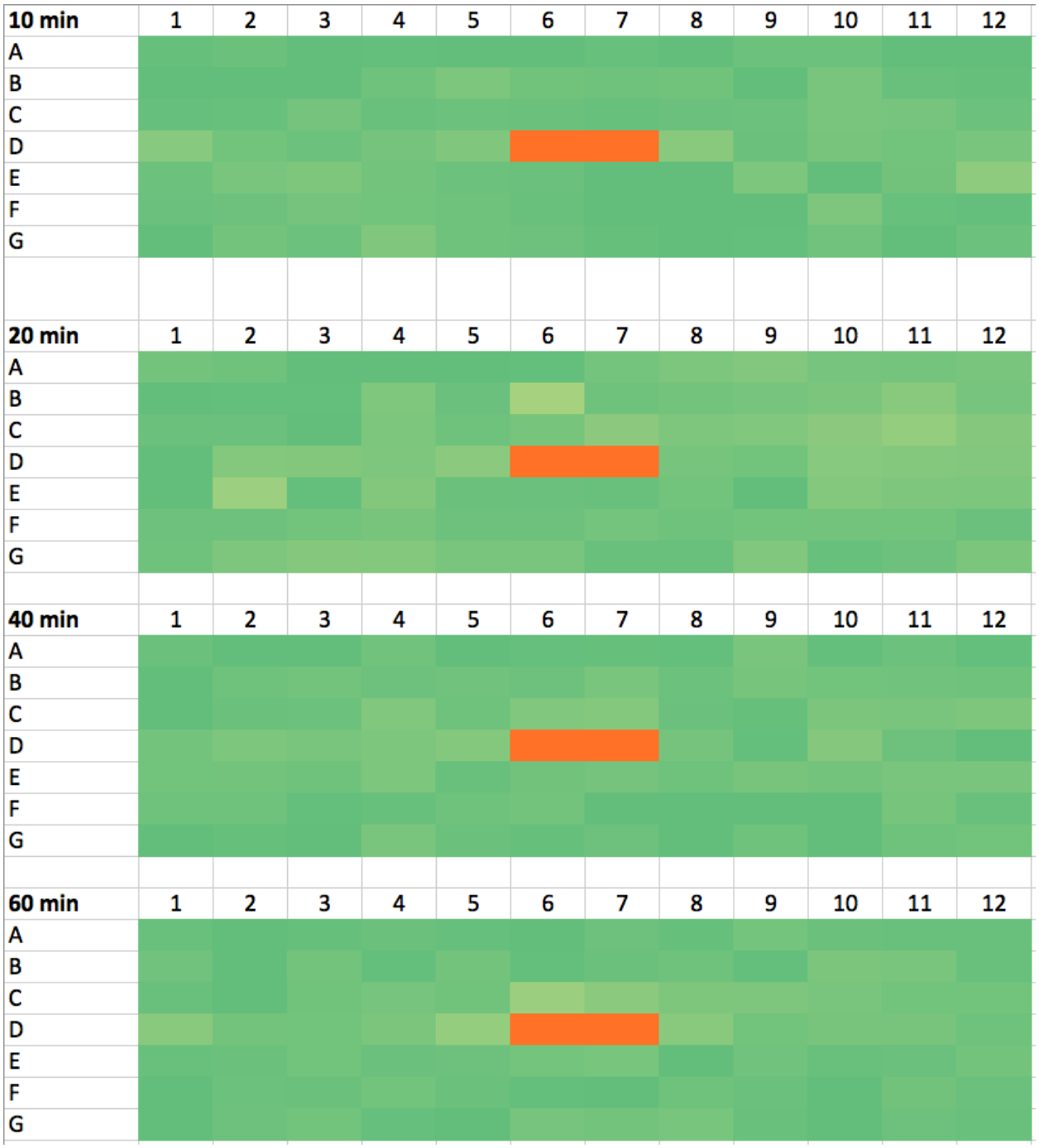
*Ag*AChE-WT residual activity data for Figure 3

10 min	1	2	3	4	5	6	7	8	9	10	11	12
A	98.0	99.1	98.0	93.1	86.5	79.2	81.6	91.0	94.5	97.7	99.2	102.6
B	101.0	94.6	90.7	79.2	66.4	58.8	63.6	68.7	86.9	94.5	99.7	99.4
C	97.5	95.2	87.5	69.0	42.6	25.3	28.0	50.0	78.0	94.0	97.3	100.1
D	97.4	93.2	85.9	63.0	29.8	-1.6	-2.7	50.6	82.2	95.3	98.8	98.2
E	98.7	94.5	85.0	68.4	46.1	30.9	37.9	69.7	92.0	99.1	100.7	99.3
F	100.6	95.3	90.6	83.3	74.9	73.5	78.4	91.0	97.6	98.6	99.3	98.7
G	106.8	98.3	97.2	96.3	89.7	86.3	92.2	98.0	98.3	100.5	99.2	99.0
20 min	1	2	3	4	5	6	7	8	9	10	11	12
A	98.7	95.5	90.9	76.2	62.7	53.2	57.5	71.9	87.2	95.4	101.3	101.7
B	102.6	94.4	81.3	61.8	38.3	23.4	30.5	46.8	71.6	91.6	96.6	99.1
C	98.7	90.9	71.3	40.3	15.8	3.8	4.4	21.5	55.2	86.0	95.5	98.5
D	99.2	88.6	71.6	44.4	11.9	-1.0	-1.3	21.8	62.0	87.8	97.4	98.9
E	98.3	90.2	73.4	43.9	20.1	10.6	10.3	27.0	65.6	92.3	98.5	99.4
F	100.9	94.7	84.2	67.8	51.0	46.2	48.1	62.7	86.7	96.9	99.0	99.7
G	101.7	98.9	94.1	86.5	83.1	79.8	83.0	91.1	94.8	99.1	99.9	100.6
40 min	1	2	3	4	5	6	7	8	9	10	11	12
A	97.1	94.0	86.1	65.8	48.8	33.2	37.5	55.3	77.5	92.0	99.4	100.6
B	99.6	92.8	73.9	44.3	23.0	11.3	9.3	20.6	51.9	79.7	99.6	101.9
C	95.7	86.2	60.6	24.3	4.7	0.8	1.0	4.6	23.7	64.8	93.1	101.3
D	96.4	84.0	49.1	13.1	0.5	-0.9	-0.8	1.1	29.3	64.5	93.0	97.0
E	97.7	85.9	54.7	19.5	1.0	-0.3	1.2	3.7	27.7	67.1	93.9	100.0
F	100.5	91.4	70.4	43.3	18.5	10.6	12.6	25.7	49.2	83.2	96.2	97.2
G	100.1	93.8	85.0	70.5	57.0	50.6	50.8	63.4	78.8	94.8	100.7	102.0
60 min	1	2	3	4	5	6	7	8	9	10	11	12
A	94.5	71.0	38.6	14.9	6.3	7.4	2.8	10.5	28.8	68.4	95.9	101.6
B	87.6	60.8	34.7	6.9	3.0	1.5	2.2	3.0	13.3	46.5	86.3	98.1
C	72.4	41.7	15.7	4.7	0.1	2.0	0.8	2.0	4.8	28.4	77.4	91.4
D	62.4	32.0	9.7	0.9	-0.9	-1.4	0.1	0.2	2.5	25.4	73.4	95.5
E	56.7	38.8	16.4	2.8	0.5	1.4	2.1	0.5	8.7	39.3	83.2	101.9
F	60.1	39.0	25.2	12.0	7.6	3.3	4.6	8.7	29.2	66.0	95.3	104.4
G	67.2	55.0	41.8	32.4	25.0	22.2	25.9	45.4	68.1	92.9	103.2	107.0

Enzyme Activity data for Figure 4

5g	1	2	3	4	5	6	7	8	9	10	11	12
A	98.0	99.1	98.0	93.1	86.5	79.2	81.6	91.0	94.5	97.7	99.2	102.6
B	101.0	94.6	90.7	79.2	66.4	58.8	63.6	68.7	86.9	94.5	99.7	99.4
C	97.5	95.2	87.5	69.0	42.6	25.3	28.0	50.0	78.0	94.0	97.3	100.1
D	97.4	93.2	85.9	63.0	29.8	-1.6	-2.7	50.6	82.2	95.3	98.8	98.2
E	98.7	94.5	85.0	68.4	46.1	30.9	37.9	69.7	92.0	99.1	100.7	99.3
F	100.6	95.3	90.6	83.3	74.9	73.5	78.4	91.0	97.6	98.6	99.3	98.7
G	106.8	98.3	97.2	96.3	89.7	86.3	92.2	98.0	98.3	100.5	99.2	99.0
9g	1	2	3	4	5	6	7	8	9	10	11	12
A	100.0	100.2	103.0	97.2	95.5	97.7	97.6	100.0	97.8	102.0	101.7	102.4
B	100.5	99.7	97.8	97.8	93.8	93.1	93.8	98.6	99.3	98.4	101.0	97.5
C	99.8	97.0	99.2	93.8	82.9	66.5	66.9	87.9	98.8	96.5	98.2	98.6
D	98.6	97.2	95.7	94.3	69.8	-1.7	-1.2	75.9	98.3	96.7	99.6	97.2
E	98.9	98.8	97.9	94.9	87.5	70.2	73.0	89.5	98.4	98.5	99.6	101.6
F	100.8	99.0	99.5	100.0	96.7	95.4	94.5	96.7	101.3	97.6	98.6	99.5
G	101.3	98.1	97.4	98.5	97.6	97.2	98.3	97.1	97.4	100.2	97.4	98.5
10g	1	2	3	4	5	6	7	8	9	10	11	12
A	100.2	99.6	100.5	101.3	101.1	100.7	101.7	98.1	100.1	102.2	97.5	100.2
B	101.1	100.5	101.3	100.5	99.2	98.4	99.9	99.6	97.3	101.2	97.9	101.6
C	100.2	98.6	95.9	99.5	98.3	98.9	99.9	100.2	99.0	98.8	97.3	98.1
D	97.1	99.4	96.1	94.6	98.0	1.3	1.8	98.0	98.7	96.9	97.0	99.4
E	100.2	97.5	101.0	100.7	100.2	99.4	102.2	100.2	101.1	99.9	99.0	100.0
F	101.6	99.5	100.1	100.4	98.7	101.1	101.2	98.8	98.9	96.7	98.8	97.5
G	99.5	97.5	99.6	98.1	100.4	99.0	100.5	96.8	97.1	99.2	99.6	97.9
Propoxur	1	2	3	4	5	6	7	8	9	10	11	12
A	101.4	101.5	101.1	100.3	99.1	100.4	100.4	100.8	98.9	98.6	99.4	100.3
B	100.8	100.4	97.5	95.7	96.8	97.1	99.5	96.5	98.2	99.9	98.2	97.6
C	98.4	97.6	98.2	96.5	97.4	97.1	98.7	98.1	96.8	96.8	97.5	98.5
D	99.5	98.5	96.7	97.2	96.3	1.2	0.3	99.2	99.1	97.5	97.5	99.5
E	99.6	99.0	98.9	97.5	98.5	100.8	97.2	98.9	98.0	97.9	98.1	97.7
F	101.5	97.7	98.4	97.5	97.5	97.6	98.5	98.4	98.1	95.5	97.9	97.1
G	98.9	95.6	98.5	94.1	98.5	98.0	97.4	97.4	95.7	98.4	98.9	98.7

Figure S1. Heat Map for dichlorvos. Microtiter plate heat maps of WT *Ag*AChE residual activity in which only wells D6-D7 of the microtiter plates were charged with 10,000 nM of the inhibitor **dichlorvos** for the indicated incubation time (10-60 min, 23 ± 1°C) before the addition of substrate. Data for Row H (enzyme-free background wells) are not shown. Color coding: red, ≤10% residual activity; yellow, ≤ 75% residual activity; green ≥ 93% residual activity. Progressive vapor phase diffusion of dichlorvos over 60 min is not evident.

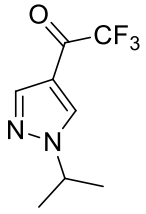


C. Synthesis and analytical characterization of tested compounds

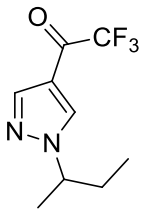
General

1-bromo-3-(*tert*-butyl)benzene and 1-bromo-3-(trimethylsilyl)benzene were purchased from Oakwood Chemicals and Sigma-Aldrich respectively and were used without purification, unless otherwise noted. Trifluoromethyl ketones **5b**[4] and **5c**[5], and *N*-alkyl-4-bromopyrazoles **11d**[6], **11e**[6], **11f**[7], **11g**[7], **11h**[8], **11i**[9] have been previously reported and characterized. ^{1}H NMR spectra were recorded at 400 or 500 MHz; the corresponding ^{13}C NMR resonance frequencies were 101 and 126 MHz; the corresponding ^{19}F NMR resonance frequencies were 376 and 470 MHz.

Trifluoromethylketones

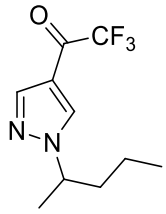


2,2,2-trifluoro-1-(1-isopropyl-1H-pyrazol-4-yl)ethanone (5d): A standard literature procedure for TFK synthesis was followed.[5] 4-bromo-1-isopropyl-1H-pyrazole (1.3 g, 7.1 mmol) **11d** was dissolved in dry THF (15 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 2.96 mL, 7.4 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, methyl trifluoroacetate (0.85 mL, 8.5 mmol) was added drop wise and stirred for 30 min at -78 ^{o}C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ether = 98:2) to yield the product as a liquid in 63% yield (0.922 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.08 (s, 1H), 4.63 – 4.52 (m, 1H), 1.57 (d, J = 6.7 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.42 (q, $^{2}J_{CF}$ = 37 Hz), 141.57, 131.65, 116.51 (q, $^{1}J_{CF}$ = 291 Hz), 115.94, 55.04, 22.55; ^{19}F NMR (471 MHz, $CDCl_3$) δ -78.00; HRMS (Mixed EIC) *m/z* calcd for $C_9H_{13}F_3N_3O$ $[M+H]^+$ 236.1011, found 236.0998.

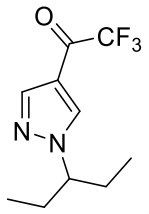


1-(1-(sec-butyl)-1H-pyrazol-4-yl)-2,2,2-trifluoroethanone (5e): 4-bromo-1-(sec-butyl)-1*H*-pyrazole (1.0 g, 5.0 mmol) was dissolved in dry THF (15 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 2.1 mL, 5.2 mmol) was added dropwise to the solution was stirred for 2 hours at -78 ^{o}C. After two hours, methyl trifluoroacetate (0.5 mL, 6.0 mmol) was added dropwise and stirred for 30 min at -78 ^{o}C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried

over $MgSO_4$. After concentration in vacuo (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ether = 98:2) to yield the product as a clear liquid in 67% yield (0.74 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.05 (s, 1H), 4.29 (h, J = 6.7 Hz, 1H), 1.98-1.94 (m, 1H), 1.88-1.80 (m, 1H), 1.55 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5 (q, $^2J_{CF}$ = 37 Hz), 141.7, 132.6, 115.8, 116.5 (q, $^1J_{CF}$ = 291 Hz), 61.1, 29.7, 20.5, 10.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ -77.99; HRMS (Mixed EIC) *m/z* calcd for $C_9H_{12}F_3N_2O$ $[M+H]^+$ 221.0901, found 221.0897.

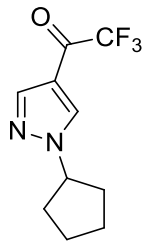


2,2,2-trifluoro-1-(1-(pentan-2-yl)-1H-pyrazol-4-yl)ethanone (5f): According to the procedure for **5d**, 4-bromo-1-(pentan-2-yl)-1H-pyrazole (3.5 g, 16.1 mmol) was dissolved in dry THF (15 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 6.7 mL, 16.9 mmol) was added drop wise to the solution was stirred for 2 hours at -78 ^{o}C. After two hours, methyl trifluoroacetate (1.94 mL, 19.3 mmol) was added drop wise and stirred for 30 min at -78 ^{o}C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried over $MgSO_4$. After concentration in vacuo (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ether = 98:2) to yield the product as a liquid in 60% yield (2.26 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 2H), 4.38 (h, J = 6.7 Hz, 1H), 1.97-1.90 (m, 1H), 1.79-1.72 (m, 1H), 1.54 (d, J = 6.8 Hz, 3H), 1.33 – 1.11 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6 (q, $^2J_{CF}$ = 37 Hz), 141.6, 132.5, 116.5 (q, $^1J_{CF}$ = 291 Hz), 115.9, 59.5, 38.7, 21.0, 19.2, 13.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ -77.99; HRMS (Mixed EIC) *m/z* calcd for $C_{10}H_{14}F_3N_2O$ $[M+H]^+$ 235.1058, found 235.1052.

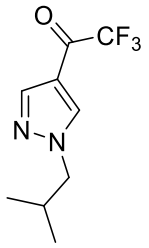


2,2,2-trifluoro-1-(1-(pentan-3-yl)-1H-pyrazol-4-yl)ethanone (5g): According to the procedure for **5d**, 4-bromo-1-(pentan-3-yl)-1H-pyrazole (1.23 g, 5.68 mmol) was dissolved in dry THF (15 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 2.38 mL, 5.96 mmol) was added drop wise to the solution was stirred for 2 hours at -78 ^{o}C. After two hours, methyl trifluoroacetate (0.68 mL, 6.81 mmol) was added drop wise and stirred for 30 min at -78 ^{o}C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried over $MgSO_4$. After concentration in vacuo (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (DCM: hexane = 20:80) to yield the product as a liquid in 67% yield (0.90 g). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 8.04 (s, 1H), 3.97 (tt, J = 9.4,

5.0 Hz, 1H), 1.99 – 1.81 (m, 4H), 0.79 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.7 (q, $^{2}J_{CF}$ = 36.7 Hz), 141.8, 133.7, 116.5 (q, $^{1}J_{CF}$ = 290.1 Hz), 115.6, 67.9, 27.9, 10.5. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -74.8. HRMS (ESI) *m/z* calcd for $C_{10}H_{13}F_3N_2O$ $[M+H]^+$ 235.1058, found 235.1059.



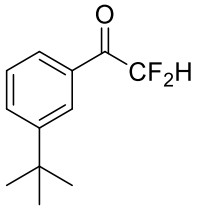
1-(1-cyclopentyl-1H-pyrazol-4-yl)-2,2,2-trifluoroethanone (5h): According to the procedure for **5d**, 4-bromo-1-cyclopentyl-1H-pyrazole (2.4 g, 16.4 mmol) was dissolved in dry THF (15 mL) at -78 °C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 4.6 mL, 17.5 mmol) was added drop wise to the solution was stirred for 2 hours at -78 °C. After two hours, methyl trifluoroacetate (1.31 mL, 19.9 mmol) was added drop wise and stirred for 30 min at -78 °C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried over $MgSO_4$. After concentration in vacuo (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ether = 98:2) to yield the product as a liquid in 52% yield (1.97 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.08 (s, 1H), 4.71 (p, J = 7.1 Hz, 1H), 2.29 – 2.17 (m, 2H), 2.11 – 1.99 (m, 2H), 1.97 – 1.86 (m, 2H), 1.82 – 1.70 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6 (q, $^{2}J_{CF}$ = 37 Hz), 141.7, 132.7, 115.3, 116.5 (q, $^{1}J_{CF}$ = 290 Hz), 64.0, 33.0, 24.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ -78.00; HRMS (Mixed EIC) *m/z* calcd for $C_{10}H_{12}F_3N_2O$ $[M+H]^+$ 233.0902, found 233.0895.



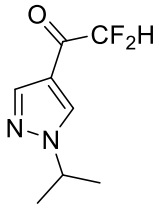
2, 2, 2-trifluoro-1-(1-isobutyl-1H-pyrazol-4-yl)ethan-1-one (5i): A standard literature procedure for TFK synthesis was followed. 4-bromo-1-(isobutyl)-1H-pyrazole (378 mg, 1.86 mmol) was dissolved in dry THF (7 mL) at -78 °C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.96 mL, 2.4 mmol) was added drop wise to the solution was stirred for 2 hours at -78 °C. After two hours, methyl trifluoroacetate (0.22 mL, 2.23 mmol) was added drop wise and stirred for 30 min at -78 °C. The solution was then allowed to warm up to room temperature and let stir for overnight. The mixture was then quenched with NH_4Cl (20 mL) and extracted with ether (50 mL), and dried over $MgSO_4$. After concentration in vacuo (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 80:20) to yield the product as a liquid in 43% yield (0.21 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 (s, 1H), 8.02 (s, 1H), 3.96 (d, J = 7.0 Hz, 2H), 2.25 (n, J = 7.0 Hz, 1H), 0.91 (d, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.5 (q, $^{2}J_{CF}$ = 36.7 Hz), 141.8 (q, $^{4}J_{CF}$ = 2.0 Hz), 134.4 (q,

$^4J_{CF}$ = 2.4 Hz), 116.4 (d, $^1J_{CF}$ = 290.3 Hz), 116.1, 60.3, 29.3, 19.7. HRMS (ESI) *m/z* calcd for $C_9H_{11}F_3N_2O$ $[M+H]^+$ 221.0902, found 221.0887.

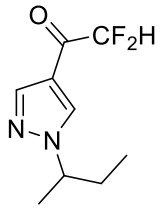
Difluoromethylketones



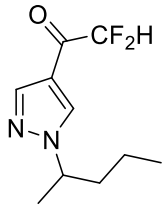
1-(3-(*tert*-butyl)phenyl)-2,2-difluoroethan-1-one (9c): 1-bromo-3-(*tert*-butyl)benzene (100 mg, 0.47 mmol) was dissolved in dry THF (4 mL) at -78 °C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.2 mL, 0.49 mmol) was added drop wise to the solution was stirred for 2 h at -78 °C. After two hours, ethyl difluoroacetate (0.05 mL, 0.56 mmol) was added drop wise and stirred for 10 min at -78 °C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 95:5) to yield the product as a yellow liquid in 47% yield (0.05 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (t, *J* = 2.1 Hz, 1H), 7.87 (dp, *J* = 7.7, 1.6 Hz, 1H), 7.70 (ddd, *J* = 7.9, 2.1, 1.1 Hz, 1H), 7.44 (t, *J* = 7.8, 0.4 Hz, 1H), 6.29 (t, $^2J_{HF}$ = 53.6 Hz, 1H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 188.0 (t, $^2J_{CF}$ = 25.1 Hz), 152.4, 132.3, 131.5 (t, $^4J_{CF}$ = 1.7 Hz), 128.8, 127.1 (t, $^3J_{CF}$ = 2.8 Hz), 126.5 (t, $^4J_{CF}$ = 1.9 Hz), 111.3 (t, $^1J_{CF}$ = 253.6 Hz), 35.1, 31.3. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -121.94 (d, $^2J_{FH}$ = 53.6 Hz). HRMS (ESI) *m/z* calcd for $C_{12}H_{14}F_2O$ $[M+Cl]^-$ 265.0807, found 265.0818.



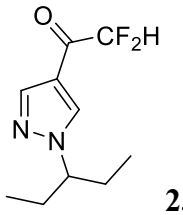
2,2-difluoro-1-(1-isopropyl-1H-pyrazol-4-yl)ethan-1-one (9d): 4-bromo-1-(isopropyl)-1H-pyrazole **11d** (200 mg, 1.06 mmol) was dissolved in dry THF (6 mL) at -78 °C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.44 mL, 1.11 mmol) was added drop wise to the solution was stirred for 2 h at -78 °C. After two hours, ethyl difluoroacetate (0.11 mL, 1.27 mmol) was added drop wise and stirred for 10 min at -78 °C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 80:20) to yield the product as a clear oil in 48% yield (0.1 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (s, 1H), 8.08 (s, 1H), 5.98 (t, $^2J_{HF}$ = 54.0 Hz, 1H), 4.54 (hept, *J* = 6.7 Hz, 1H), 1.54 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.4 (t, $^2J_{CF}$ = 26.6 Hz), 141.1 (t, $^4J_{CF}$ = 2.5 Hz), 131.1 (t, $^4J_{CF}$ = 3.5 Hz), 116.9, 111.1 (t, $^1J_{CF}$ = 253.4 Hz), 54.8, 22.5. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.56 (d, $^2J_{FH}$ = 53.9 Hz). HRMS (ESI) *m/z* calcd for $C_8H_{10}F_2N_2O$ $[M+H]^+$ 189.0840, found 189.0838.



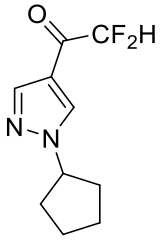
1-(1-(sec-butyl)-1H-pyrazol-4-yl)-2,2-difluoroethan-1-one (9e): 4-bromo-1-(*sec*-butyl)-1H-pyrazole (268 mg, 1.32 mmol) was dissolved in dry THF (5 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.55 mL, 1.39 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl difluoroacetate (0.14 mL, 1.58 mmol) was added drop wise and stirred for 10 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 40:60) to yield the product as a clear oil in 39% yield (0.11 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (t, J = 1.0 Hz, 2H), 5.96 (t, J = 54.0 Hz, 1H), 4.25 (h, J = 6.7 Hz, 1H), 2.06 – 1.68 (m, 2H), 1.48 (d, J = 6.8 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.5 (t, $^{2}J_{CF}$ = 26.6 Hz), 141.2 (t, $^{4}J_{CF}$ = 2.4 Hz), 132.0 (t, $^{4}J_{CF}$ = 3.5 Hz), 116.8 (t, $^{3}J_{CF}$ = 2.3 Hz), 111.1 (t, $^{1}J_{CF}$ = 252.9 Hz), 60.8, 29.7, 20.5, 10.3. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.35 (d, $^{2}J_{FH}$ = 54.2 Hz). HRMS (ESI) *m/z* calcd for $C_9H_{12}F_2N_2O$ $[M+H]^+$ 203.0996, found 203.0988.



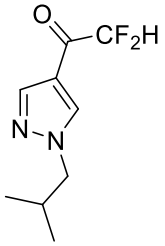
2,2-difluoro-1-(1-(pentan-2-yl)-1H-pyrazol-4-yl)ethan-1-one (9f): 4-bromo-1-(pentan-2-yl)-1H-pyrazole (500 mg, 2.03 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.96 mL, 2.40 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl difluoroacetate (0.24 mL, 2.76 mmol) was added drop wise and stirred for 10 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 50:50) to yield the product as a clear oil in 43% yield (0.21 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (s, 2H), 5.96 (t, $^{2}J_{HF}$ = 53.9 Hz, 1H), 4.42 (h, J = 6.7 Hz, 1H), 2.08 – 1.58 (m, 2H), 1.48 (d, J = 6.7 Hz, 3H), 1.32 – 1.03 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.5 (t, ^{2}JCF = 26.5 Hz), 141.1 (t, $^{4}J_{CF}$ = 2.6 Hz), 131.9 (t, $^{4}J_{CF}$ = 3.4 Hz), 116.8 (t, $^{3}J_{CF}$ = 2.3 Hz), 111.1 (t, $^{1}J_{CF}$ = 252.9 Hz), 59.2, 38.7, 20.9, 19.1, 13.5. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.52 (d, $^{2}J_{FH}$ = 53.8 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{14}F_2N_2O$ $[M+H]^+$ 217.1152, found 217.1149.



2,2-difluoro-1-(1-(pentan-3-yl)-1H-pyrazol-4-yl)ethan-1-one (9g): 4-bromo-1-(pentan-3-yl)-1H-pyrazole (500 mg, 2.30 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 1.03 mL, 2.42 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl difluoroacetate (0.25 mL, 2.76 mmol) was added drop wise and stirred for 10 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 50:50) to yield the product as a clear oil in 35% yield (0.17 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 8.04 (s, 1H), 5.99 (t, $^{2}J_{HF}$ = 54.0 Hz, 1H), 3.94 (tt, *J* = 9.4, 5.1 Hz, 1H), 2.10 – 1.73 (m, 4H), 0.77 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.6 (t, $^{2}J_{CF}$ = 26.6 Hz), 141.4 (t, $^{4}J_{CF}$ = 2.5 Hz), 133.1 (t, $^{4}J_{CF}$ = 3.5 Hz), 116.6, 111.2 (t, $^{1}J_{CF}$ = 253.1 Hz), 67.6, 28.0, 10.5. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.41 (d, $^{2}J_{FH}$ = 53.9 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{14}F_2N_2O$ $[M+H]^+$ 217.1152, found 217.1150.

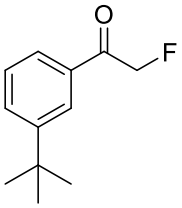


1-(1-cyclopentyl-1H-pyrazol-4-yl)-2,2-difluoroethan-1-one (9h): 4-bromo-1-(cyclopentyl)-1H-pyrazole (300 mg, 1.39 mmol) was dissolved in dry THF (10 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.58 mL, 1.46 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl difluoroacetate (0.18 mL, 1.67 mmol) was added drop wise and stirred for 10 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 50:50) to yield the product as a clear oil in 27% yield (0.1 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (s, 1H), 8.04 (s, 1H), 5.96 (t, $^{2}J_{HF}$ = 53.9 Hz, 1H), 4.69 (p, *J* = 7.1 Hz, 1H), 2.23 – 2.11 (m, 2H), 2.06 – 1.93 (m, 2H), 1.85 (m, 2H), 1.77 – 1.63 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.4 (t, $^{2}J_{CF}$ = 26.7 Hz), 141.3 (t, $^{4}J_{CF}$ = 2.5 Hz), 132.1 (t, $^{4}J_{CF}$ = 3.4 Hz), 116.9 (t, $^{3}J_{CF}$ = 2.4 Hz), 111.1 (t, $^{1}J_{CF}$ = 253.0 Hz), 63.8, 32.9, 24.1. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.46 (d, $^{2}J_{FH}$ = 53.9 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{12}F_2N_2O$ $[M+H]^+$ 215.0996, found 215.0996.

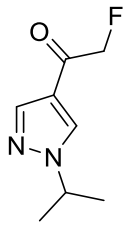


2,2-difluoro-1-(1-isobutyl-1H-pyrazol-4-yl)ethan-1-one (9i): 4-bromo-1-(isobutyl)-1H-pyrazole (260 mg, 1.28 mmol) was dissolved in dry THF (5 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.54 mL, 1.34 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl difluoroacetate (0.14 mL, 1.54 mmol) was added drop wise and stirred for 10 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: DCM = 40:60) to yield the product as a clear oil in 48% yield (0.12 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 (s, 1H), 8.02 (s, 1H), 5.97 (t, J = 53.9 Hz, 2H), 3.94 (d, J = 7.2 Hz, 2H), 2.26 (n, J = 7.0 Hz, 1H), 0.90 (d, J = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 182.5 (t, $^{2}J_{CF}$ = 26.6 Hz), 141.5 (t, $^{4}J_{CF}$ = 2.3 Hz), 133.9 (t, $^{4}J_{CF}$ = 3.7 Hz), 117.1, 111.2 ($^{1}J_{CF}$ = 254.3 Hz), 60.2, 29.3, 19.7. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -123.45 (d, $^{2}J_{FH}$ = 54.4 Hz). HRMS (ESI) *m/z* calcd for $C_9H_{12}F_2N_2O$ $[M+H]^+$ 203.0996, found 203.0971.

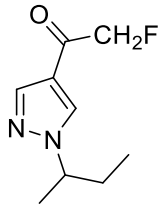
Fluoromethyl ketones



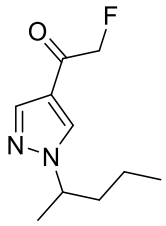
1-(3-(*tert*-butyl)phenyl)-2-fluoroethan-1-one (10c): 1-bromo-3-(*tert*-butyl)benzene (300 mg, 1.41 mmol) was dissolved in dry THF (5 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 2.0 mL, 4.9 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.16 mL, 1.69 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 90:10) to yield the product as a yellow liquid in 43% yield (0.12 g). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.88 (t, J = 1.8 Hz, 1H), 7.61 – 7.59 (m, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.19 (s, 1H), 5.47 (d, $^{2}J_{HF}$ = 47.0 Hz, 2H), 1.28 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 193.9 (d, $^{2}J_{CF}$ = 15.4 Hz), 152.4, 133.7, 131.5, 128.8. 125.2 (d, $^{4}J_{CF}$ = 2.6 Hz), 124.8 (d, $^{4}J_{CF}$ = 2.5 Hz), 83.7 (d, $^{1}J_{CF}$ = 182.5 Hz), 31.3. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -230.58 (t, $^{2}J_{FH}$ = 47.0 Hz). HRMS (Mixed EIC) *m/z* calcd for $C_{12}H_{15}FO$ $[2M+Na]^+$ 411.2112, found 411.2134.



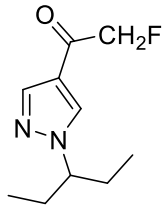
2-fluoro-1-(1-(isopropyl)-1H-pyrazol-4-yl)ethan-1-one (10d): 4-bromo-1-(isopropyl)-1H-pyrazole (551 mg, 2.91 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 1.45 mL, 3.06 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.33 mL, 3.49 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 80:20) to yield the product as a yellow solid in 20% yield (0.10 g); mp 158.5-159.8 ˚C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.06 (s, 1H), 7.98 (s, 1H), 5.07 (d, $^{2}J_{HF}$ = 47.5 Hz, 2H), 4.49 (hept, *J* = 6.7 Hz, 1H), 1.49 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 189.6 (d, $^{2}J_{CF}$ = 19.6 Hz), 140.4 (d, $^{4}J_{CF}$ = 5.3 Hz), 130.2 (d, $^{4}J_{CF}$ = 9.3 Hz), 119.5 (d, $^{3}J_{CF}$ = 3.1 Hz), 84.8 (d, $^{1}J_{CF}$ = 184.7 Hz), 54.6, 22.7. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -226.52 (t, $^{2}J_{FH}$ = 47.5 Hz). HRMS (ESI) *m/z* calcd for $C_8H_{11}FN_2O$ $[M+H]^+$ 171.0934, found 171.0928.



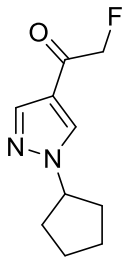
1-(1-(sec-butyl)-1H-pyrazol-4-yl)-2-fluoroethan-1-one (10e): 4-bromo-1-(*sec*-butyl)-1H-pyrazole (500 mg, 2.46 mmol) was dissolved in dry THF (5 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 1.0 mL, 1.34 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.29 mL, 2.95 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 70:30) to yield the product as a clear oil in 25% yield (0.12 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.03 (s, 1H), 8.01 (s, 1H), 5.09 (d, *J* = 47.5 Hz, 2H), 4.23 (h, *J* = 6.7 Hz, 1H), 2.02 – 1.70 (m, 2H), 1.49 (d, *J* = 6.8 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.6 (d, $^{2}J_{CF}$ = 19.5 Hz), 140.4 (d, $^{4}J_{CF}$ = 5.3 Hz), 131.1 (d, $^{4}J_{CF}$ = 9.3 Hz), 119.4 (d, $^{3}J_{CF}$ = 3.3 Hz), 84.7 (d, $^{1}J_{CF}$ = 184.6 Hz), 60.6, 29.8, 20.5, 10.4. HRMS (ESI) *m/z* calcd for $C_9H_{13}FN_2O$ $[M+H]^+$ 185.1090, found 185.1083.



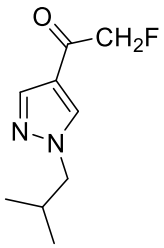
2-fluoro-1-(1-(pentan-2-yl)-1H-pyrazol-4-yl)ethan-1-one (10f): 4-bromo-1-(pentan-2-yl)-1H-pyrazole (504 mg, 2.32 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.97 mL, 2.43 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.27 mL, 2.78 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 70:30) to yield the product as a yellow oil in 28% yield (0.14 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (s, 1H), 8.03 (s, 1H), 5.11 (d, $^{2}J_{HF}$ = 47.5 Hz, 2H), 4.34 (h, *J* = 6.7 Hz, 1H), 1.94-1.86 (m, 1H), 1.75-1.67 (m, 1H), 1.50 (d, *J* = 6.8 Hz, 3H), 1.30 – 1.07 (m, 2H), 0.9 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.4 (d, $^{2}J_{CF}$ = 19.5 Hz), 140.2 (d, $^{4}J_{CF}$ = 5.2 Hz), 131.0 (d, $^{4}J_{CF}$ = 9.1 Hz), 119.3 (d, $^{3}J_{CF}$ = 3.1 Hz), 84.7 (d, $^{1}J_{CF}$ = 184.5 Hz), 58.9, 38.7, 21.0, 19.1, 13.5. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -226.25 (t, $^{2}J_{HF}$ = 46.8 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{15}FN_2O$ $[M+H]^+$ 199.1247, found 199.1241.



2-fluoro-1-(1-(pentan-3-yl)-1H-pyrazol-4-yl)ethan-1-one (10g): 4-bromo-1-(pentan-2-yl)-1H-pyrazole (525 mg, 2.41 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 1.01 mL, 2.53 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.28 mL, 2.89 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (DCM: ethyl acetate = 90:10) to yield the product as a yellow oil in 36% yield (0.17 g). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.09 (s, 1H), 8.07 (s, 1H), 5.16 (d, $^{2}J_{HF}$ = 47.5 Hz, 2H), 3.96 (tt, *J* = 9.4, 5.1 Hz, 1H), 2.03 – 1.81 (m, 4H), 0.81 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 189.6 (d, $^{2}J_{CF}$ = 19.6 Hz), 140.4 (d, $^{4}J_{CF}$ = 5.3 Hz), 130.2 (d, $^{4}J_{CF}$ = 9.3 Hz), 119.5 (d, $^{3}J_{CF}$ = 3.1 Hz), 84.7 (d, $^{1}J_{CF}$ = 184.7 Hz), 54.6, 22.7. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -226.26 (t, $^{2}J_{FH}$ = 47.6 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{15}FN_2O$ $[M+H]^+$ 199.1247, found 199.1231.



1-(1-cyclopentyl-1H-pyrazol-4-yl)-2-fluoroethan-1-one (10h): 4-bromo-1-(cyclopentyl)-1H-pyrazole (480 mg, 2.23 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 0.94 mL, 2.34 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.26 mL, 2.68 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (hexane: ethyl acetate = 70:30) to yield the product as a yellow oil in 30% yield (0.13 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (s, 1H), 8.02 (s, 1H), 5.10 (d, $^{2}J_{HF}$ = 47.5 Hz, 2H), 4.66 (p, *J* = 7.0 Hz, 1H), 2.30 – 2.11 (m, 2H), 2.09 – 1.95 (m, 2H), 1.95 – 1.80 (m, 2H), 1.80 – 1.65 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.4 (d, $^{2}J_{CF}$ = 19.5 Hz), 140.4 (d, $^{4}J_{CF}$ = 5.1 Hz), 131.2 (d, $^{4}J_{CF}$ = 9.1 Hz), 119.4 (d, $^{3}J_{CF}$ = 3.1 Hz), 84.7 (d, $^{1}J_{CF}$ = 184.4 Hz), 63.6, 32.9, 24.0. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -226.23 (t, *J* = 56.1 Hz). HRMS (ESI) *m/z* calcd for $C_{10}H_{13}FN_2O$ $[M+H]^+$ 197.1090, found 197.1087.



2-fluoro-1-(1-isobutyl-1H-pyrazol-4-yl)ethan-1-one (10i): 4-bromo-1-(isobutyl)-1H-pyrazole (500 mg, 2.46 mmol) was dissolved in dry THF (7 mL) at -78 ^{o}C under nitrogen and was stirred for 10 min. *n*-BuLi (2.5 M in hexanes, 1.03 mL, 2.59 mmol) was added drop wise to the solution was stirred for 2 h at -78 ^{o}C. After two hours, ethyl fluoroacetate (0.28 mL, 2.95 mmol) was added drop wise and stirred for 1 min at -78 ^{o}C upon which the mixture was then quenched with NH_4Cl (20 mL) and warmed to room temp. The mixture was then extracted with ether (50 mL), and dried over $MgSO_4$. After concentration *in vacuo* (note: remove shortly after the ether is removed, because the compound is quite volatile), the residue was purified with flash chromatography over silica gel (DCM: ethyl acetate = 95:5) to yield the product as a yellow oil in 27% yield (0.12 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (s, 2H), 5.10 (d, $^{2}J_{HF}$ = 47.5 Hz, 2H), 3.93 (d, *J* = 7.0 Hz, 2H), 2.23 (n, *J* = 7.0 Hz, 1H), 0.91 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.6 (d, $^{2}J_{CF}$= 19.8 Hz), 140.7 (d, $^{4}J_{CF}$= 5.3 Hz), 133.1 (d, $^{4}J_{CF}$= 10.3 Hz), 110.0, 84.8 (d, $^{1}J_{CF}$ = 184.7 Hz), 60.1, 29.3, 19.8. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -226.23 (tt, *J* = 47.6, 1.5 Hz). HRMS (ESI) *m/z* calcd for $C_9H_{13}FN_2O$ $[M+H]^+$ 185.1090, found 185.1095.

D. Protocol for determining the extent of hydration of fluorinated methyl ketones and ^{19}F NMR Spectra.

We followed the general approach of Nair et al.[10] to measure the ratio of fluorinated ketone and hydrate in aqueous solution. Briefly, 1 M solutions of the fluorinated methyl ketones were prepared in DMSO. This stock solution was diluted 100:1 with a 4:1 mixture of 0.1 M sodium phosphate buffer (pH 7.7) and D_2O; this gives a final concentration of 10 mM fluorinated methyl ketone and 1% v/v DMSO. The following resonances and integrals were observed after 24 h.

5c: ^{19}F δ (ppm) -72.2 (s, ketone, 12%), -84.3 (s, hydrate, 88%). Note that these chemical shifts are very similar to the chemical shifts reported for the ketone and hydrate forms of **5a** (-70.4 and -82.1 ppm) in a similar solvent.[10]
5g: ^{19}F δ (ppm) -74.8 (s, ketone, 33%), -85.6 (s, hydrate, 67%); note that the ^{19}F NMR spectrum of **5g** in $CDCl_3$ had ^{19}F δ (ppm) = -74.8.
9i: ^{19}F δ (ppm) -126.5 (d, $^2J_{HF}$ = 53.2 Hz, ketone, 78%), -132.8 (d, $^2J_{HF}$ = 55.6 Hz, hydrate, 22%); note that the ^{19}F NMR spectrum of **9i** in $CDCl_3$ had ^{19}F δ (ppm) = -123.5.
10i: ^{19}F δ (ppm) –230.8 (t, $^2J_{HF}$ = 46.2 Hz, ketone). No triplet corresponding to the hydrate could be identified; note that the ^{19}F NMR spectrum of **10i** in $CDCl_3$ had ^{19}F δ (ppm) = -226.2.

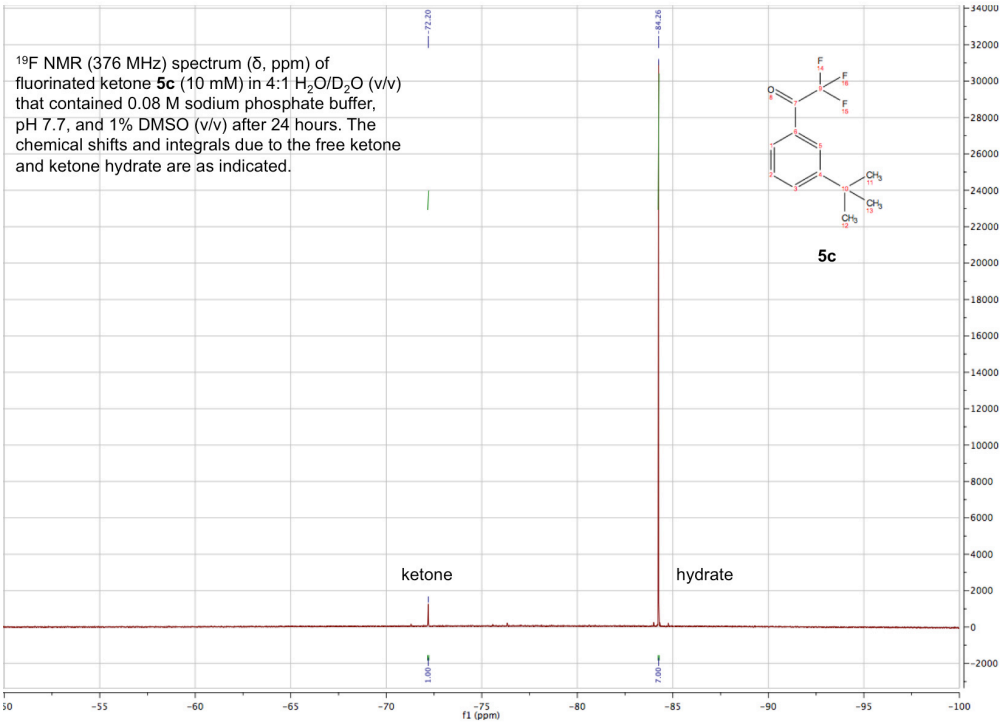


Figure S2. ^{19}F NMR (376 MHz) spectrum of **5c** in aqueous solution.

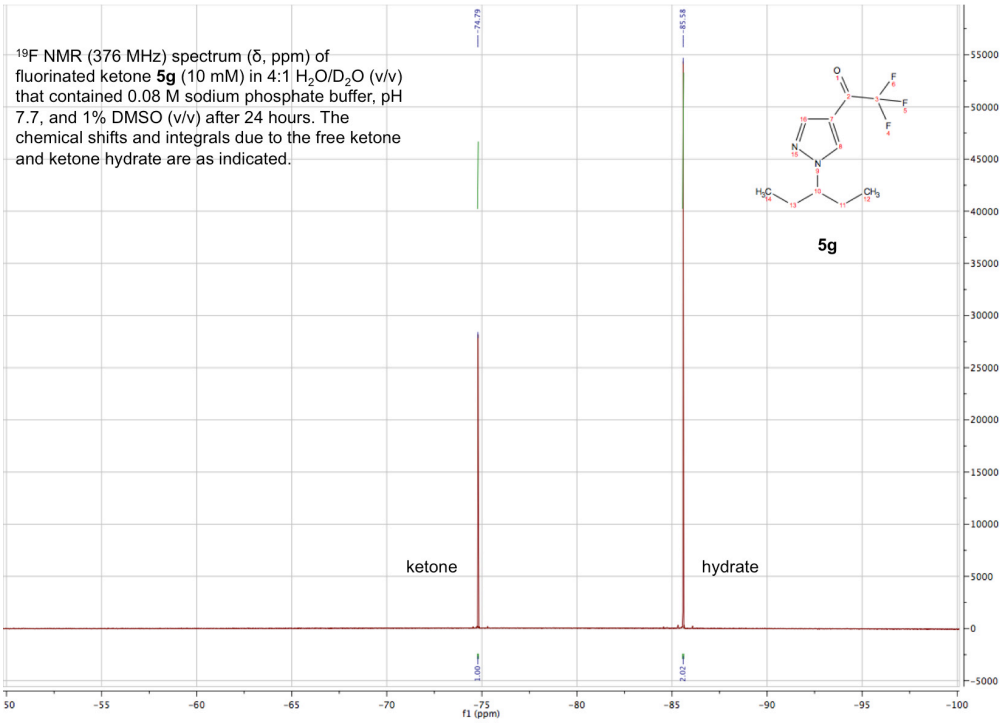


Figure S3. ^{19}F NMR (376 MHz) spectrum of **5g** in aqueous solution.

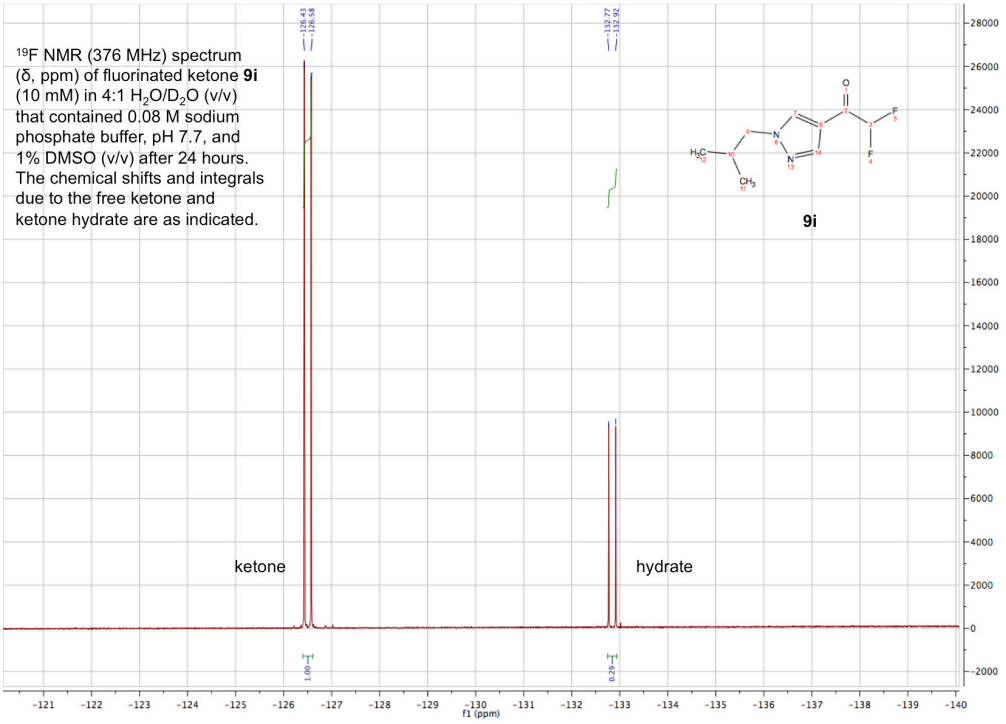


Figure S4. ^{19}F NMR (376 MHz) spectrum of **9i** in aqueous solution.

Figure S5. ^{19}F NMR (376 MHz) spectrum of **10i** in aqueous solution.

E. Mosquito toxicity assays

E.1 Mosquito rearing and tarsal contact toxicity assay

Anopheles gambiae eggs (G3 (MRA-112) and Akron (MRA-913) were obtained from MR4, and reared in tap water with fish food for larval sustenance (Tetra Fish, Blacksburg, VA, USA). Adult female non-blood-fed *An. gambiae* (G3 strains) 3-5 days old, were used for filter paper assay of tarsal contact toxicity, which were performed in exposure tubes according to the 2006 World Health Organization recommendations[11] with slight modifications. In brief, filter papers (15 x 12 cm) were treated with 2.0 mL of various concentrations of the fluorinated ketone in ethanol, are allowed to dry overnight. For the G3 strain, batches of 20-25 mosquitos (in triplicate) were transferred to a holding tube and allowed to adapt for one hour. Due to lower colony numbers, toxicity assays with the Akron strain used batches of 10-15 mosquitoes in duplicate. Mosquitoes were then transferred to the exposure tube (held horizontally) that contained a treated filter paper. Knockdown was noted after 1 h, and all mosquitoes were transferred back to the holding tube (held upright) and given free access to 10% (w/v) sugar water. Mortality was recorded at 24 h. Both during exposure and the 23 h period, mosquito tubes were kept in an environmental chamber at 24 ± 1˚ and 75 percent RH. To determine LC_{50} values typically 5-8 concentrations were examined and mortality data were used to probit analysis using Poloplus or SAS probit.[12]

E2. Fumigation toxicity assay

Fumigation toxicity was determined according to a published method.[13, 14] Briefly, 10 adult female non-blood-fed *An. gambiae* (G3 strain, 3-5 days old) were transferred to an exposure tube (used for tarsal contact toxicity in D.1. above), given free access to 10% (w/v) sugar water. and

the tube was transferred to a 1 L Mason jar. The test compound was dissolved in acetone, and 50 μL of the acetone solution was injected onto a piece of filter paper to deliver the desired dose. After a period of sixty seconds to allow the acetone vehicle to evaporate, the filter paper was placed in the Mason jar and the jar cap was screwed on to seal the vessel. Mortality was determined after 24 h.

E3. Injection toxicity assay

Fluorinated methyl ketones (**5g**, **9g**, and **10g**) were dissolved in acetone, which was then further diluted into a modified recipe of mosquito physiological saline (154 mM NaCl, 1.4 mM $CaCl_2$, 2.7 mM KCl, 4 mM HEPES, pH 6.9).[15] The final concentration of acetone in the physiological saline, which was injected into adult female *An. gambiae*, was 5% (v/v). Intrathoracic injections were performed using pulled glass capillary needles. The glass needles were prepared using a Model P-1000 Flaming/Brown Micropipette Puller (Sutter Instruments Co., Novato, CA). Pulled glass needles were prepared from thin wall glass capillary tubes (length 100 mm, 1 mm outer-diameter, 0.75 mm inner-diameter, no filament) obtained from World Precision Instruments (Sarasota, FL). The delivery of 100 nL (dose of 50 ng per mosquito) of test solution was performed with a Manual Microsyringe Pump (World Precision Instruments), according to the manufacturer's protocol for using mineral oil as a transfer medium. *An. gambiae* mosquitoes were briefly anesthetized using a 90-mm Petri dish on ice; special care was taken to keep cold exposure of mosquitoes to less than three minutes. Five adult female non-blood-fed *An. gambiae* (G3 strain, 3-5 days old) were injected per replicate, and a minimum of six replicates was obtained for each treatment. Mosquitoes were placed into an 85 mL test tube that was closed with a cotton ball containing a 10% sugar solution. The recovery of injected mosquitoes was observed within 1 hour after injection; typically, mosquitoes recovered from the injection within 3-5 minutes. Mortality of injected mosquitoes was determined at 24 hr. A 5% acetone in mosquito physiological saline control (negative control) was also injected; injection data were not considered valid in the event that control mortality exceeded 20%. Measured mortality for **5g**, **9g,** and **10g** were corrected for control mortality (11 ± 4%) by application of Abbott's modification.[16] Propoxur was administered in 200 nL injections using 5% (v/v) ethanol in water as vehicle, with doses of 0.00, 0.05, 0.10, 0.50, 1.0 and 5.0 ng. Ten adult female mosquitoes were injected with each dose, with two to three replicates per dose. There was no control mortality for these determinations.

F. References

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