Supporting Information

A Kinetic Isotope Effect and Isotope Exchange Study of the Non-enzymatic and Butyrylcholinesterase-Catalyzed Hydrolysis of Formylthiocholine.

Lori I. Robins, Kristen M. Meisenheimer, Emily J. Fogle, Cory A. Chaplan, Richard L. Redman, Joseph T. Vacca, Michelle R. Tellier, Brittney R. Collins, Dorothea H. Duong, Kathrin Schulz, and John F. Marlier*

Scheme S1. Synthetic route for FTC synthesis (see experimental section for more details).

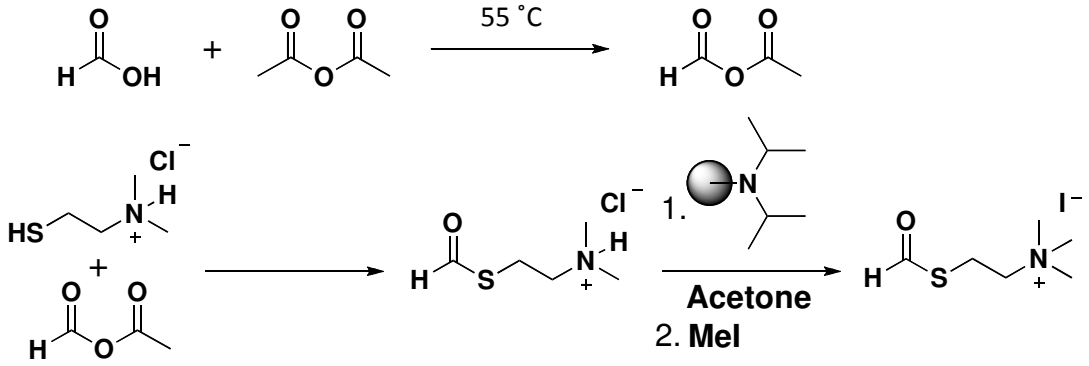
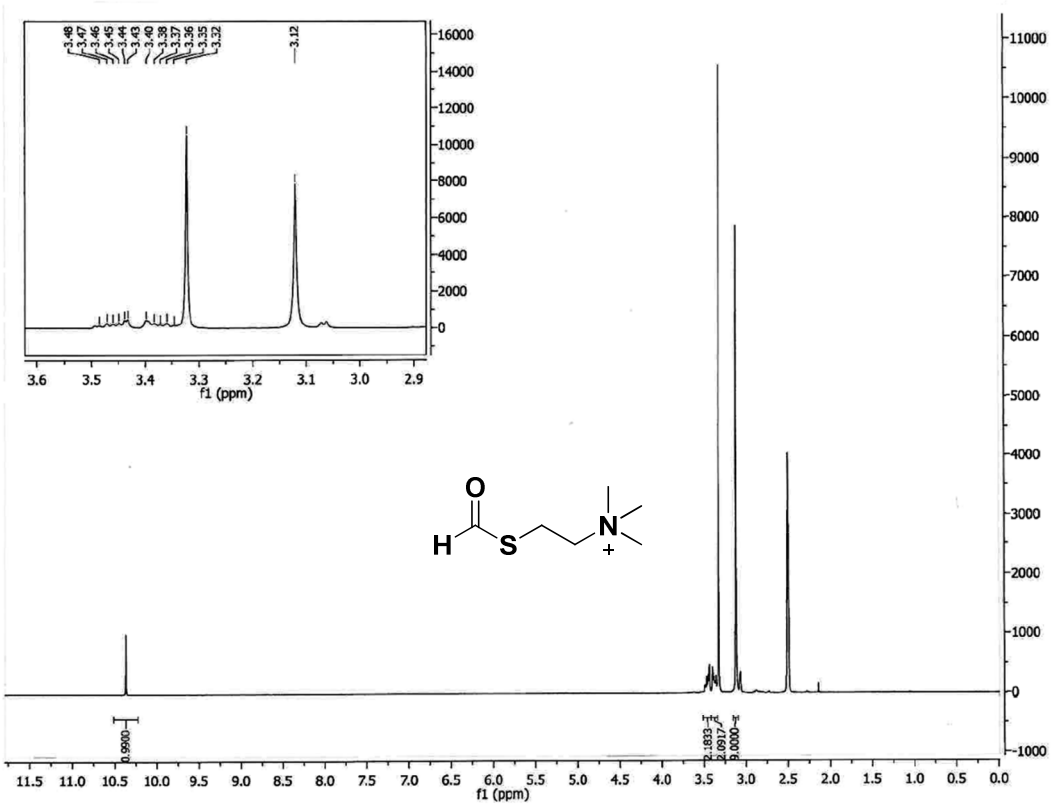
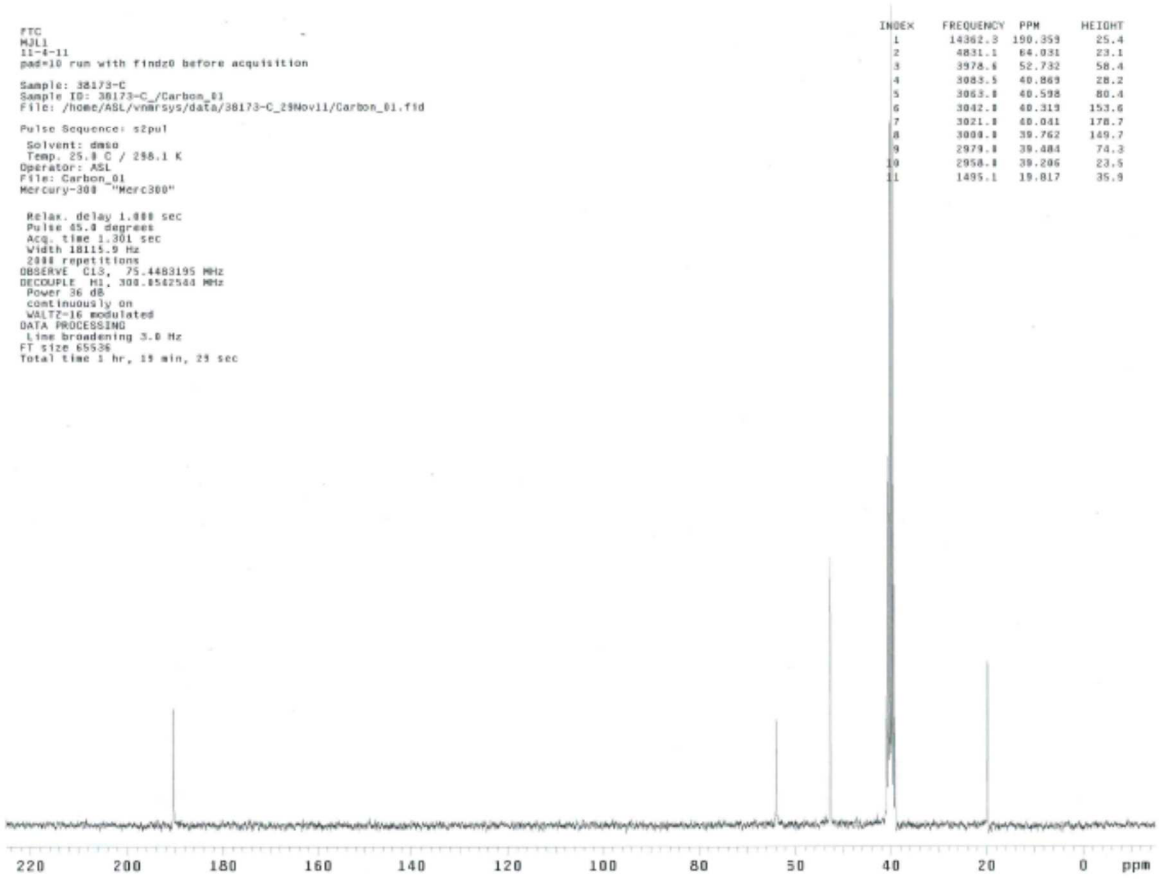


Figure S1. NMR spectra of FTC (a) ^{1}H-NMR of FTC, (b)^{13}C-NMR of FTC, (c) ^{1}H-NMR of D-FTC and (d) ^{1}H-NMR of 1-^{13}*C*-FTC.

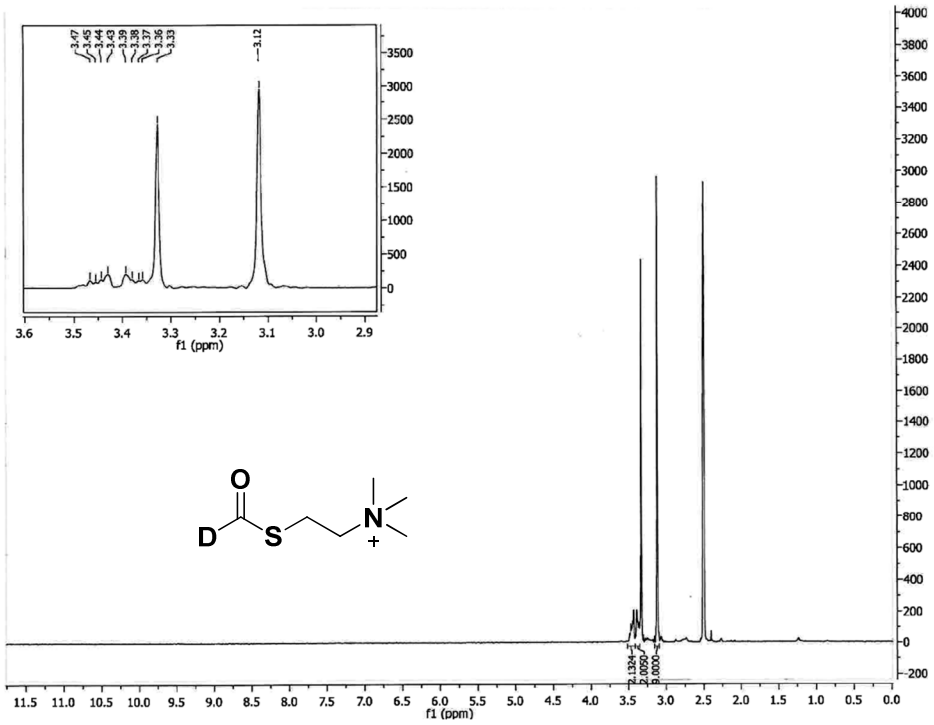
(a)



(b)


FTC
MJL1
11-4-11
pad=10 run with findz0 before acquisition
Sample: 38173-C
Sample ID: 38173-C_/Carbon_01
File: /home/ASL/vnmrsys/data/38173-C_29Nov11/Carbon_01.fid
Pulse Sequence: s2pul
Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: ASL
File: Carbon_01
Mercury-300 "Merc300"
Relax. delay 1.000 sec
Pulse 65.0 degrees
Acq. time 1.301 sec
Width 18115.9 Hz
2000 repetitions
OBSERVE C13, 75.4483195 MHz
DECOUPLE H1, 300.0542544 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 3.0 Hz
FT size 65536
Total time 1 hr, 15 min, 25 sec
INDEX FREQUENCY PPM HEIGHT
1 14362.3 150.359 25.4
2 4831.1 64.031 23.1
3 3978.6 52.732 58.4
4 3883.5 40.869 28.2
5 3863.8 40.598 80.4
6 3842.8 40.319 153.6
7 3821.8 40.041 178.7
8 3808.8 39.762 149.7
9 2979.8 39.484 74.3
10 2958.8 39.206 23.5
11 1495.1 19.817 35.9
220
200
180
160
140
120
100
80
50
40
20
0
ppm

(c)


3.47
3.45
3.44
3.43
3.39
3.38
3.37
3.36
3.33
3.12
f1 (ppm)
O
D
S
N
+
2.1324
2.0000
9.0000
f1 (ppm)

(d)

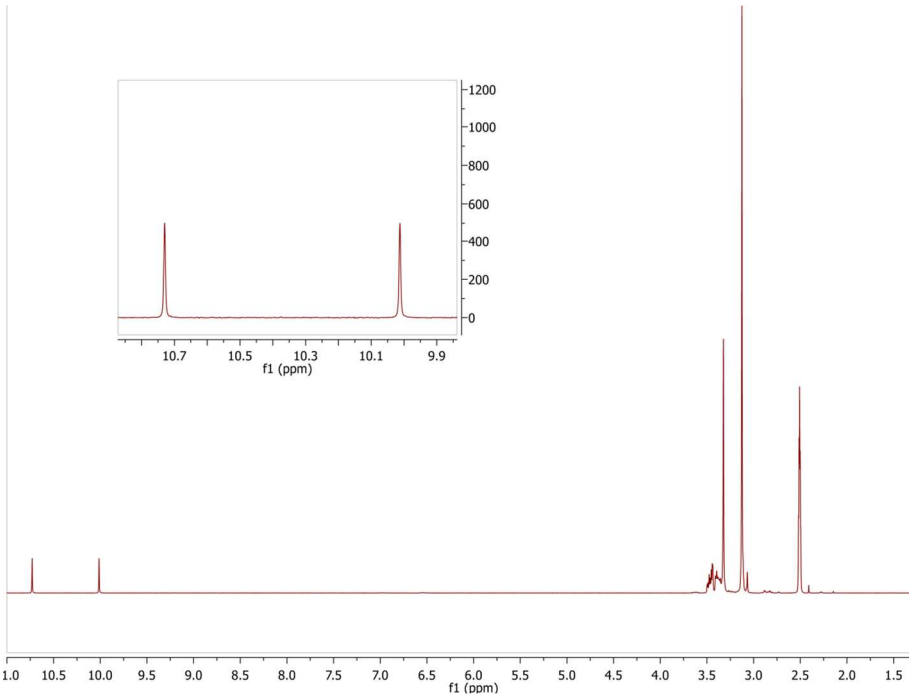
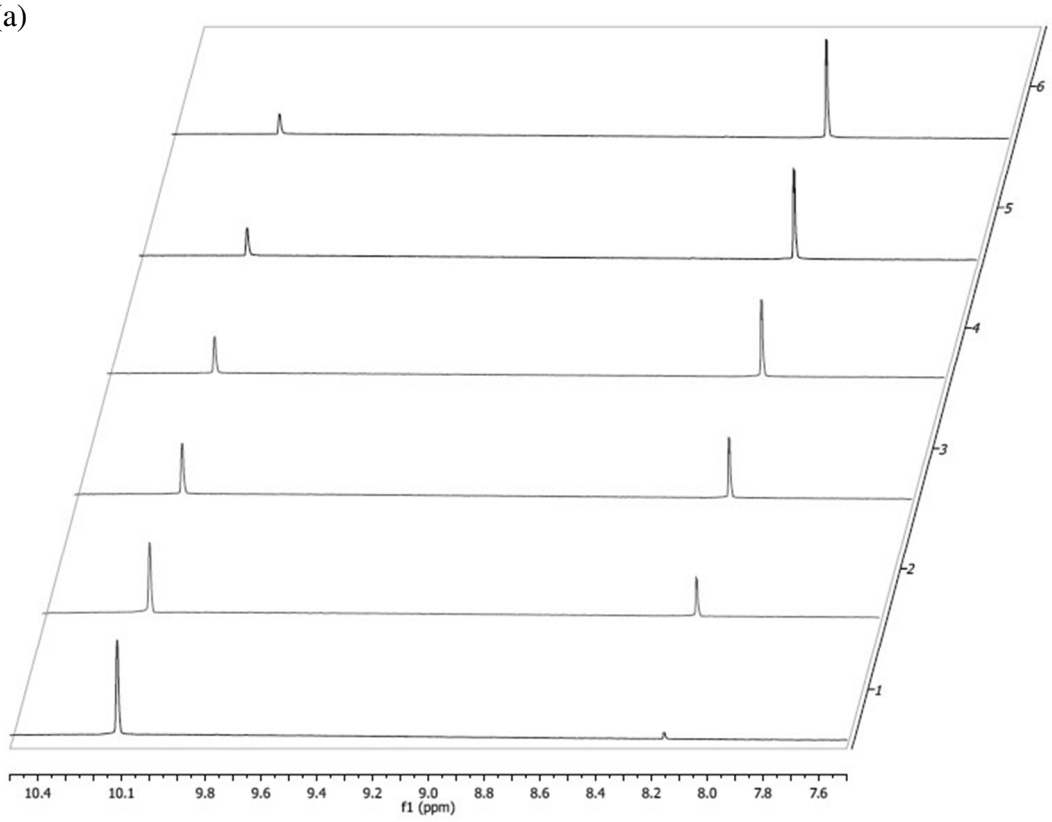

f1 (ppm)
f1 (ppm)

Figure S2. Sample Stacked Plots for the Solvent KIE on FTC Hydrolysis. (a) Hydrolysis of 50 mM FTC in 200 mM HCl. (b) Hydrolysis of 50 mM FTC 200 mM DCl.

(a)



(b)

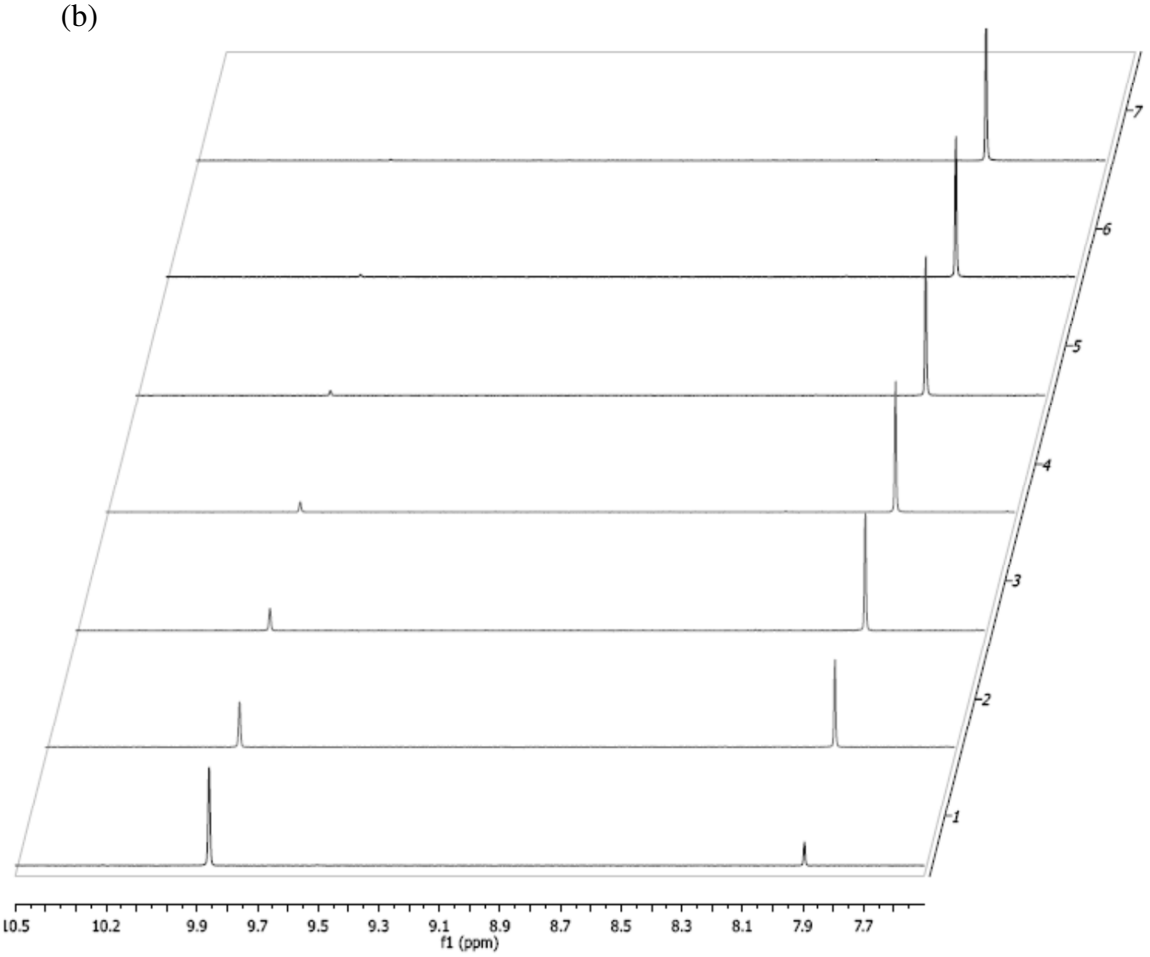
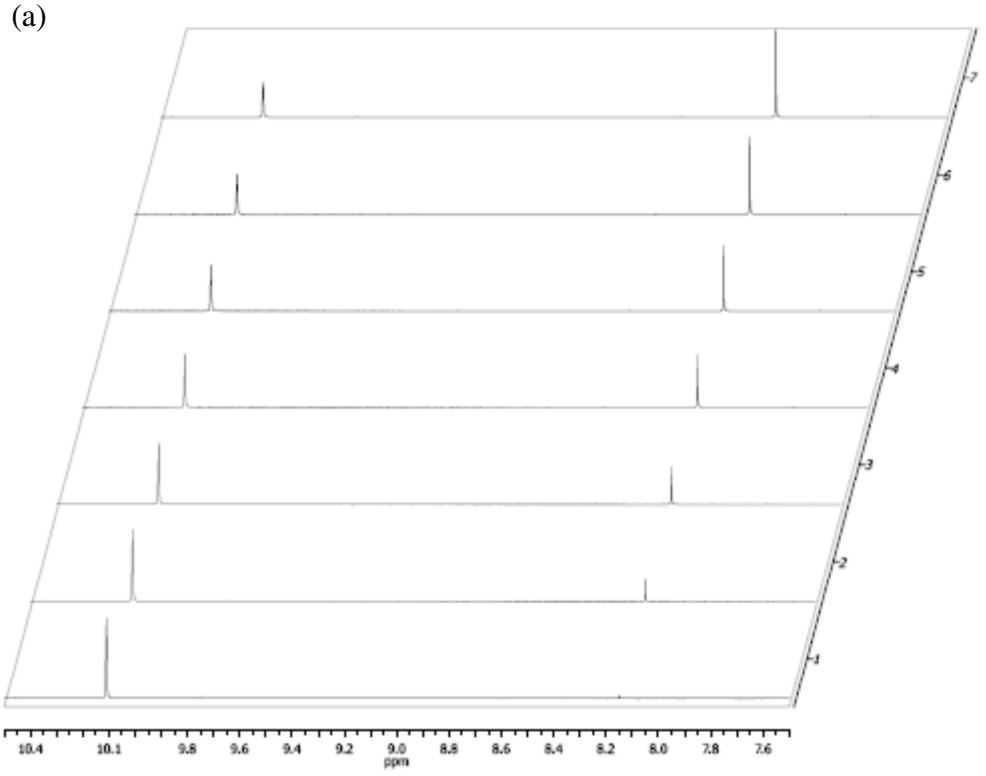


Figure S3. Sample Stacked Plots for the Formyl-H KIE. (a) Hydrolysis of H-FTC in 50 mM HCl/150 mM KCl. (b) Hydrolysis of D-FTC in 50 mM HCl/150 mM KCl.

(a)



(b)

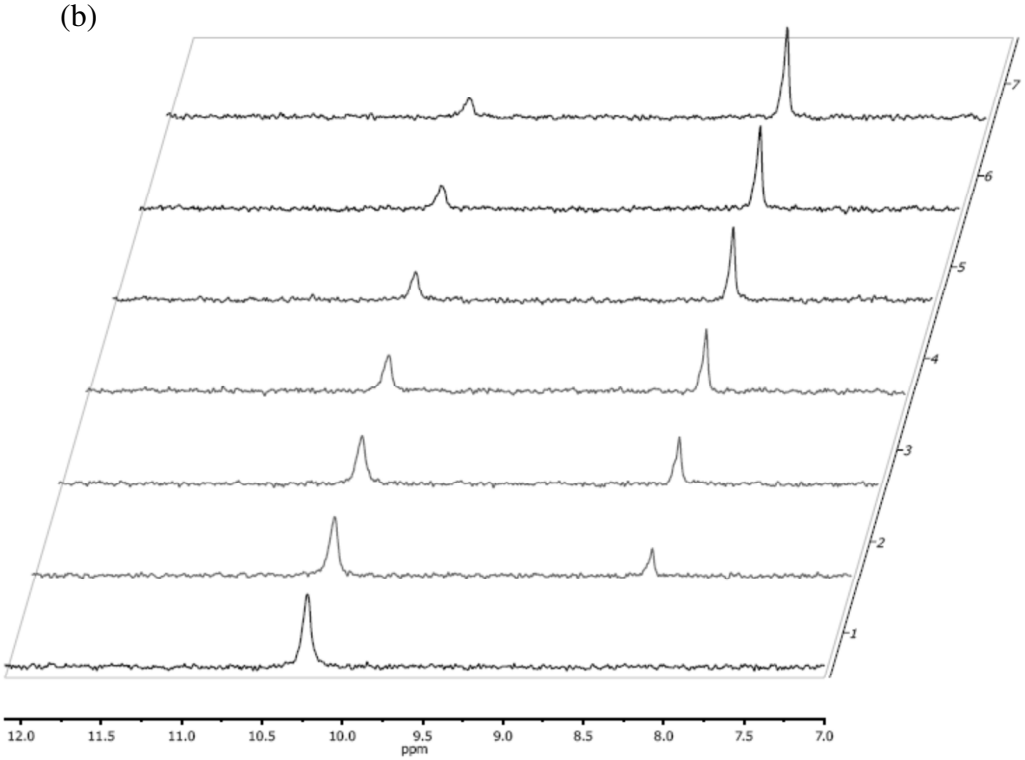


Figure S4. ^{18}O-Exchange (PIX) NMR spectra. (a) Butyrylcholinesterase-catalyzed hydrolysis of FTC in 100 mM MES in 90% ^{18}O water. (b) Acid-catalyzed hydrolysis – 200 mM HCl in 93% ^{18}O water. (c) Acid-catalyzed hydrolysis – 50 mM HCl in 93% ^{18}O water. (d) Base-catalyzed hydrolysis of FTC – 50 mM KOH in 93% ^{18}O water.

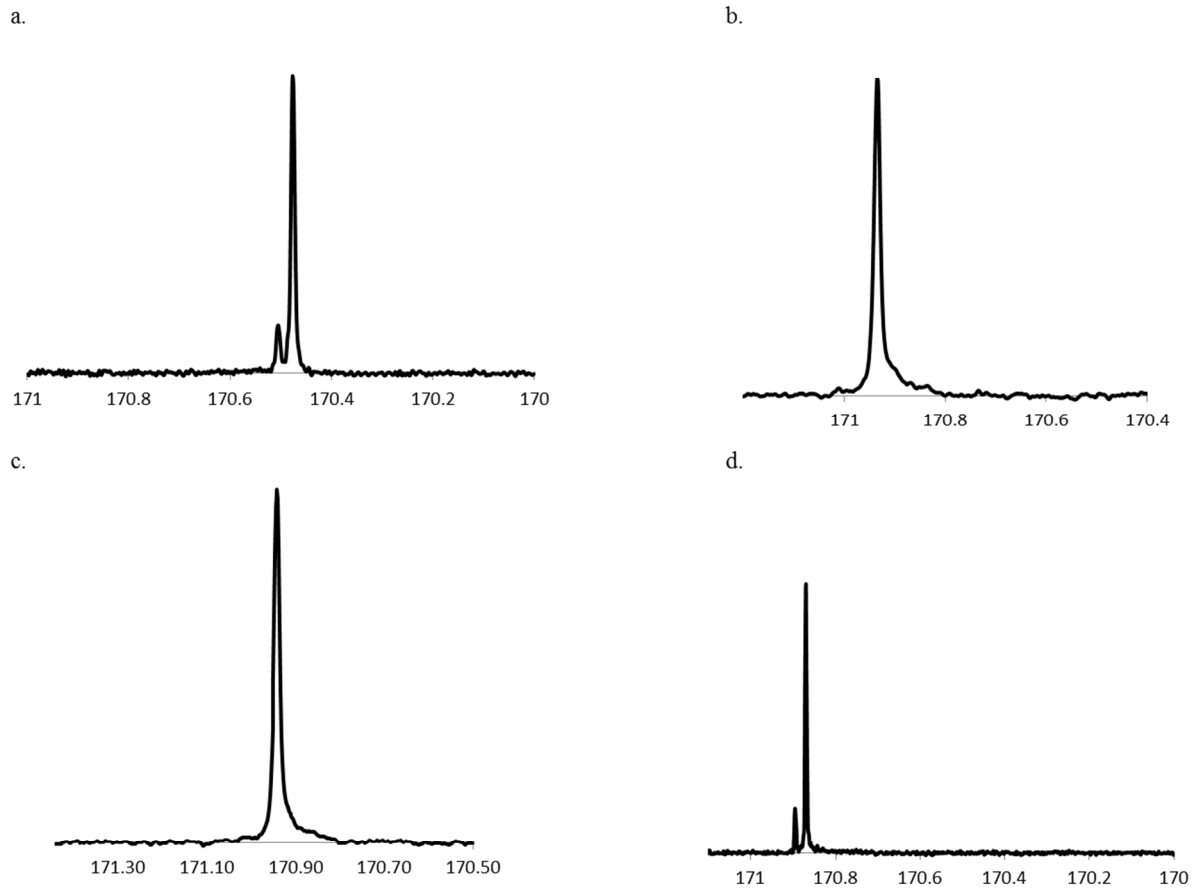


Table S1. Observed First-Order Rate Constants for H-FTC and D-FTC Hydrolysis in Acidic, Basic and Neutral Conditions at μ = 200 mM and 25°C.[a]

	Condition	k_{obs} (min^{-1})
FTC	200 mM HCl	0.0052 ± 0.0001
	200 mM DCl	0.026 ± 0.002
	50 mM HCl/150 mM KCl	0.0021 ± 0.0001
	50 mM DCl/150 mM KCl	0.0026 ± 0.0002
	H_2O/200 mM KCl	0.00135 ± 0.00007
	D_2O/200 mM KCl	0.00032 ± 0.00001
DFTC	200 mM HCl	0.00646 ± 0.00008
	50 mM HCl	0.00273 ± 0.00001
	H_2O/200 mM KCl	0.0018 ± 0.0001

[a]These rate constants were used for calculation of the KIEs by the direct method.

Table S2. Observed First-Order Rate Constants for FTC Hydrolysis at Various pH Values at μ = 200 mM and 25°C.[a]

Conditions	pH 1.0	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0
k_{obs} (min^{-1})	0.0023	0.00090	0.00088	0.00091	0.00084	0.00095	0.0013

[a]These rate constant, together with those for FTC in H_2O from Table 1 (above) were used to generate the log k_{obs} v. pH plot in the manuscript.