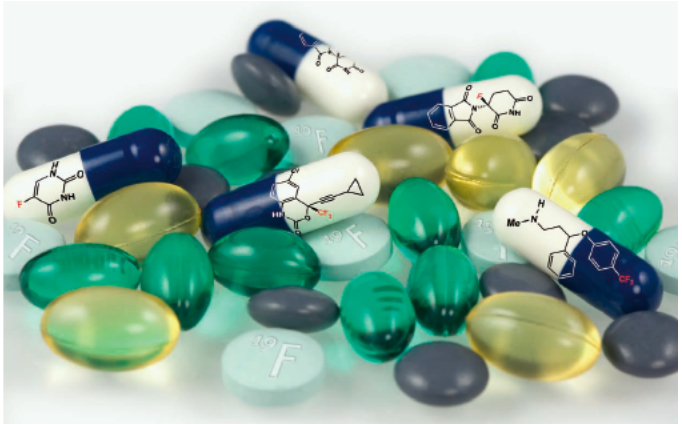
Biological Applications of Organofluorine Compounds



Anna Allen

MacMillan Group Meeting

November 5, 2009

Outline

Introduction to organofluorine chemistry and the C-F bond

Biological applications of organofluorine chemistry

- Metabolic stability
- Physicochemical changes
- Conformational changes
- Orthogonal reactivity
- Isosteres and notable discoveries

Lead references:

C–F bond fundamentals:

O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Biological applications:

Böhm, H.-J. *et al. ChemBioChem* **2004**, *5*, 637.

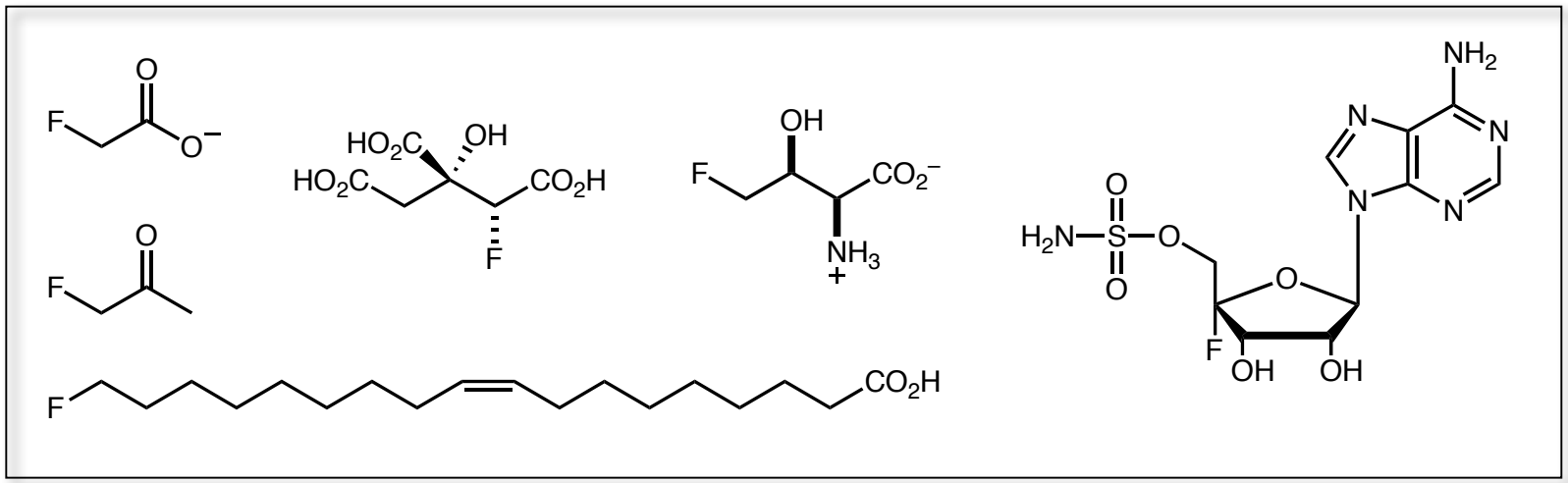
Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Kirk, K. L. *Curr. Top. Med. Chem.* **2006**, *6*, 1447.

Organofluorine Compounds

Only about 30 naturally occuring organofluorine compounds are known

- 13 biogenically produced, 8 of which are fatty acid derivatives
- one enzyme discovered (fluorinase) to catalyze C–F bond formation



Despite lack of organofluorines in nature

- 30% of agrochemicals contain C–F bonds
- 10% of pharmaceuticals contain C–F bonds

Why do we incorporate C–F bonds?

Harper, D. B.; O'Hagan, D.; Murphy, C. D. *Handbook of environmental chemistry* **2003**, *3*, 141.

Why are Organofluorine Compounds Important?

"Substitution of a C-H bond with a C-F bond can significantly change the properties of arenes; for example, fluorine substitution can increase the metabolic stability of pharmaceuticals."

Furuya, T.; Ritter, T. *Org. Lett.* **2009**, *11*, 2860.

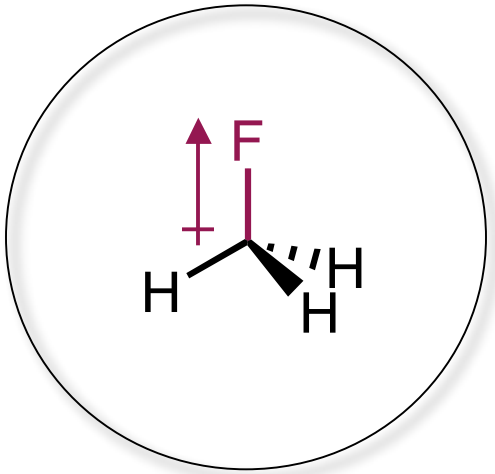
"Thus the trifluoromethyl unit is often present in synthetic drugs and agrochemicals, leading to altered physical and physiological behavior of these materials with respect to uptake, mode of action, and metabolism."

Eisenberger, P.; Gischig, S.; Togni, A. *Chem. Eur. J.* **2006**, *12*, 2579.

How does the C–F bond accomplish this?

Properties of the C-F Bond

Organofluorine properties governed by fluorine's electronegativity and size



- electronegativity, χ = 4.0
- Van der Waals radii 1.47 Å
  (hydrogen 1.20 Å, oxygen 1.57 Å)

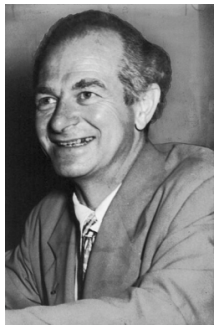
- Highly polarized bond
- Strongest single bond to carbon, 105 kcal/mol
  - Significant electrostatic attraction adds to strength
- Very low polarizability
- Tightly held lone pairs on fluorine

O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Hydrogen Bonding and the C–F Bond

"only the most electronegative atoms should form hydrogen bonds, and the strength of the bond should increase with increase in the electronegativity of the two bonded atoms… It is found empirically that fluorine forms very strong hydrogen bonds, oxygen weaker ones, and nitrogen still weaker ones."

- Linus Pauling, *The Nature of the Chemical Bond, 2nd Ed.,* **1939**



Dunitz, J. D. *ChemBioChem* **2004**, *5*, 614.

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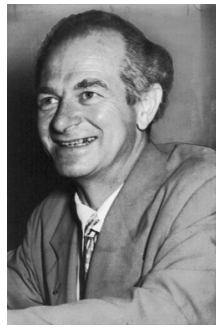
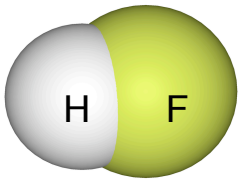


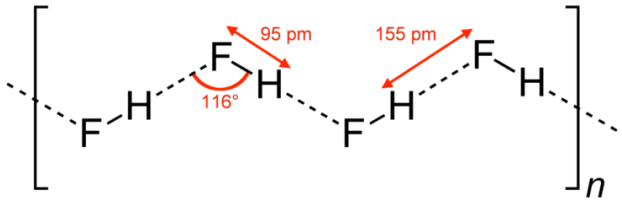


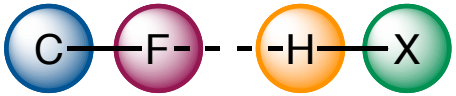
Hydrogen Fluoride

[F•••H•••F]
~ 40 kcal/mol

Organofluorine

C–F•••H–X
~ 1.5 – 2.4 kcal/mol





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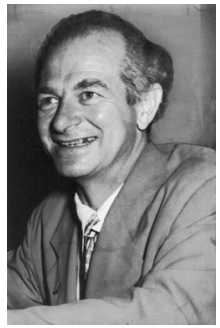
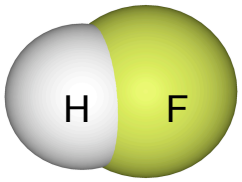


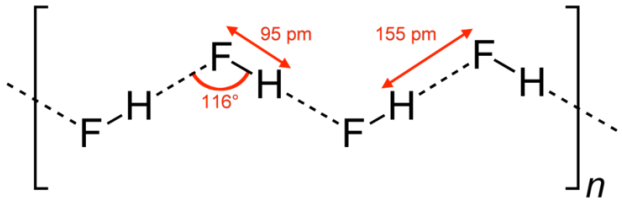


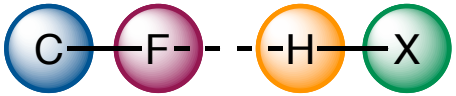
Hydrogen Fluoride

[F•••H•••F]
~ 40 kcal/mol

Organofluorine

C–F•••H–X
~ 1.5 – 2.4 kcal/mol





"It is interesting that in general fluorine atoms attached to carbon do not have significant power to act as proton acceptors in the formation of hydrogen bonds in the way that would be anticipated from the large difference in electronegativity of fluorine and carbon."

- Linus Pauling, *The Nature of the Chemical Bond, 3rd Ed.,* **1960**

Dunitz, J. D. *ChemBioChem* **2004**, *5*, 614.

Hydrogen Bonding and the C–F Bond

Organofluorine Hydrogen Bonds:

C(sp^3)–F ~ 2.38 kcal/mol

C(sp^2)–F ~ 1.48 kcal/mol

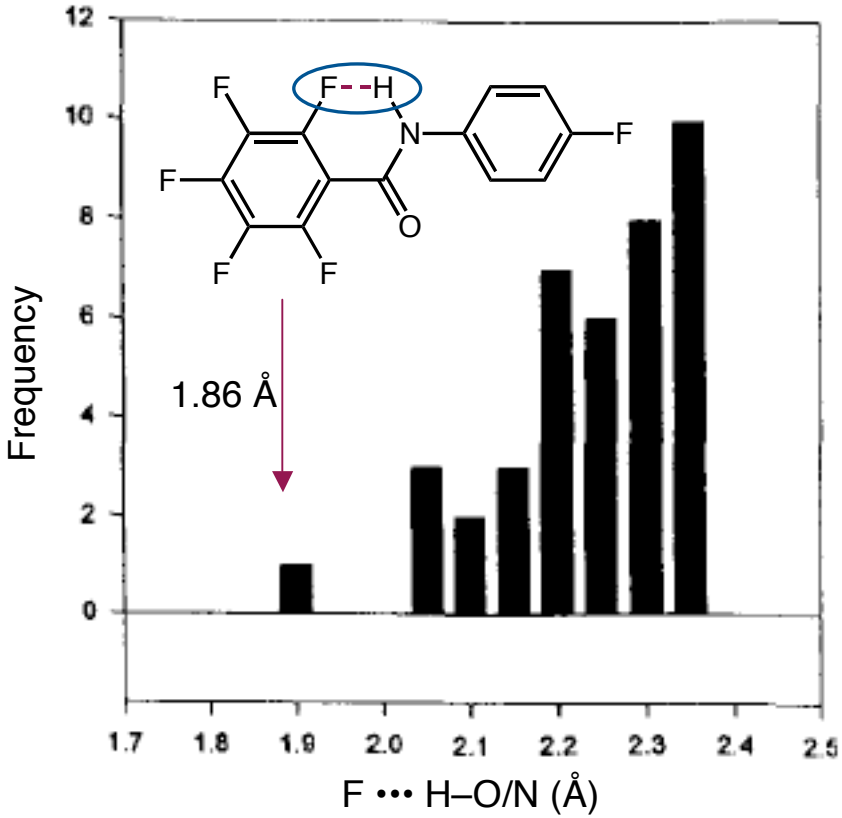
Shortest C–F•••H–X: 2.0 – 2.2 Å

Typical C–F•••H–X: 2.5 – 3.0 Å

(van der Waals distance: 2.65 Å)

Search of 146 272 entries in CSDS:

- 548 compounds with 1163 C–F bonds
- 166 contacts shorter than 2.35 Å
- 40 C–F•••H–O/N contacts
- 1 contact less than 2.0 Å



O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613.

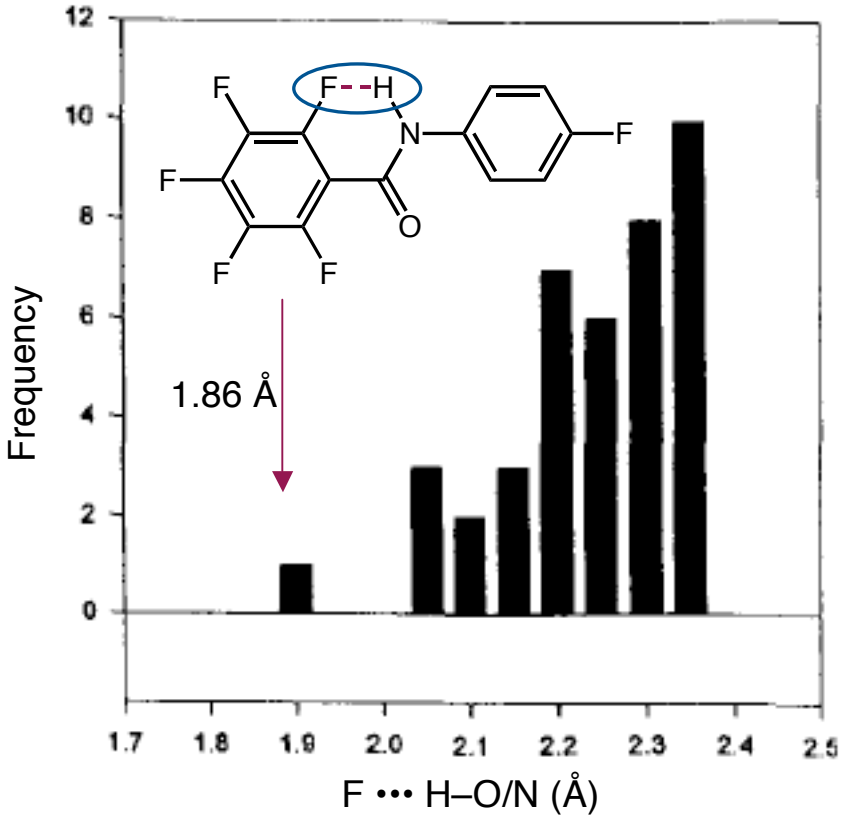
Hydrogen Bonding and the C–F Bond

Organofluorine Hydrogen Bonds:

Organofluorines only make weak “hydrogen bonding” interactions

- high electronegativity
- low polarizability
- low lying 2p electrons

Weak interactions often attributed to exclusively electrostatic attractions



O’Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.
Howard, J. A. K.; Hoy, V. J.; O’Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613.

Biological Applications of Organofluorine Chemistry

Organofluorine compounds have many biological applications:

- Enhance one or more properties of a target molecule
- As an investigative tool for biological mechanisms
- ^{18}F-Labeled radiopharmaceuticals/PET imaging
- Perfluorinated liquids in medicine
- Anesthetics

Increased Metabolic Stability of Organofluorine

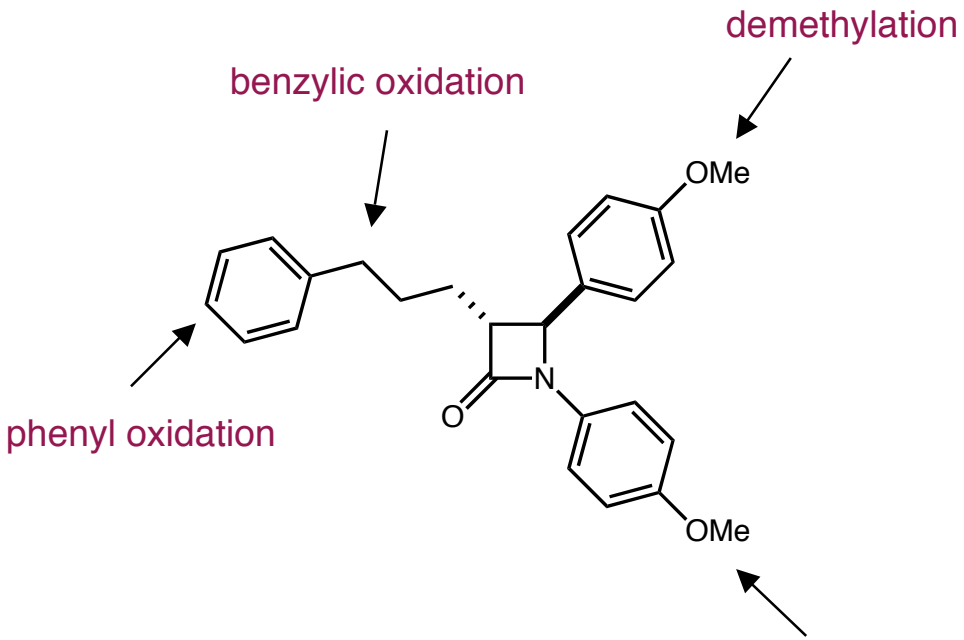
Problem: In mammals, lipophilic compounds have a tendency to be oxidized by liver enzymes, particularly Cytochrome P450

Solution: Several possible strategies available

- Make the compound more polar
  - Lower bioavailability
- Block the metabolically labile sites

Increased Metabolic Stability of Organofluorine

Lead compound for a chloesterol-absorption inhibitor: SCH 48461



Primary metabolic pathways:

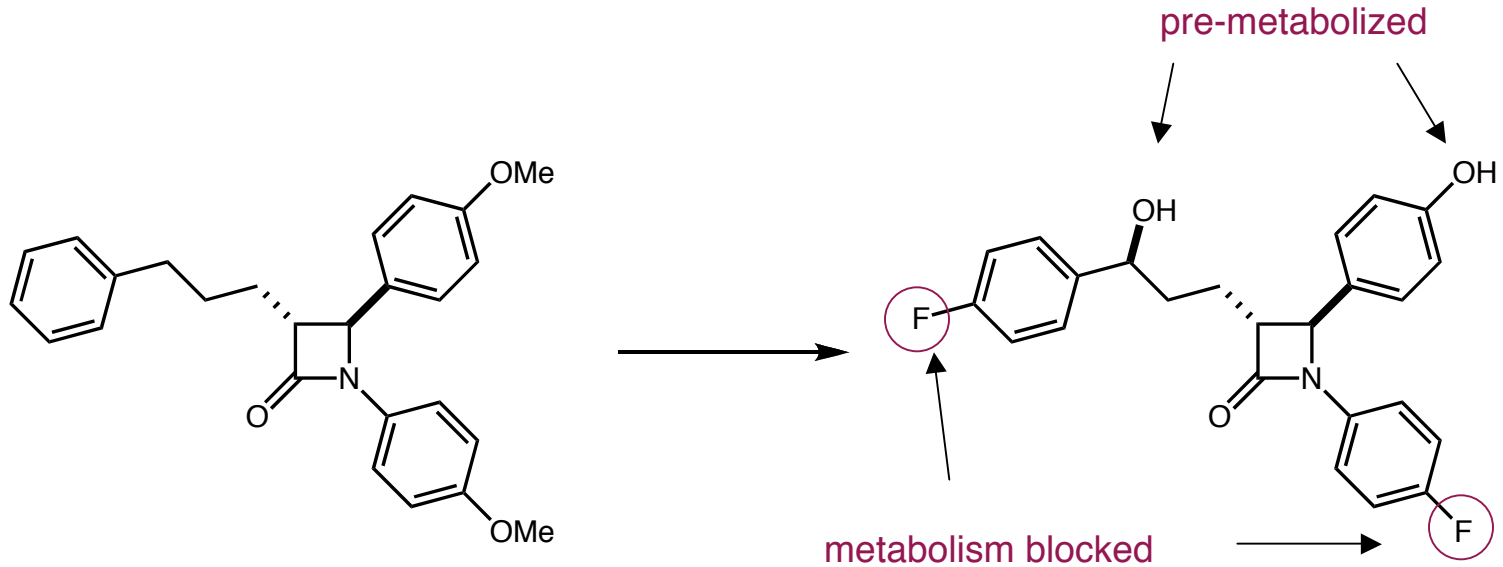
- dealkylation of anisyl groups
- *para* hydroxylation of phenyl
- benzylic oxidation

Leads to overall decreased potency

van Heek, M. *et al. J. Pharmacol. Exp. Therap.* **1997**, *283*, 157.
Clader, J. W. *J. Med. Chem.* **2004**, *47*, 1.

Increased Metabolic Stability of Organofluorine

Productive metabolism incorporated and non-productive blocked



SCH 48461

Ezetimibe
(400 times potency)

Why does incorporation of a fluorine block metabolically labile sites?

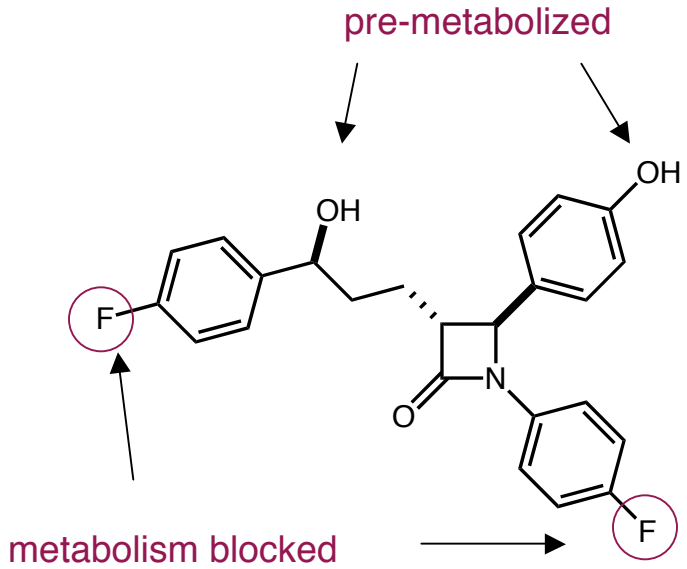
van Heek, M. *et al. J. Pharmacol. Exp. Therap.* **1997**, *283*, 157.
Clader, J. W. *J. Med. Chem.* **2004**, *47*, 1.

Increased Metabolic Stability of Organofluorine

Productive metabolism incorporated and non-productive blocked

- Fluorine inductively deactivates the phenyl groups towards oxidation

What about C–F bond strength?



Ezetimibe

Banks, R. E.; Smart, B. E.; Tatlow, J. C. *Organofluorine Chemistry: Principles and Commercial Applications*; Plenum Press: New York, 1994.

Increased Metabolic Stability of Organofluorine

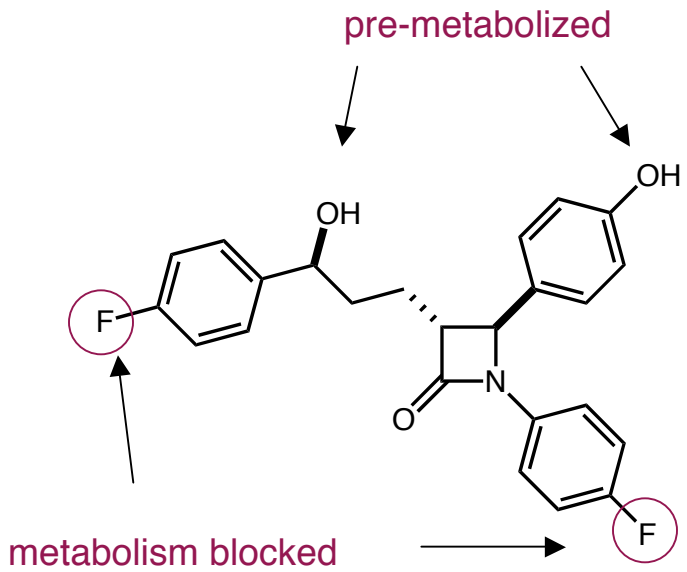
Productive metabolism incorporated and non-productive blocked

- Fluorine inductively deactivates the phenyl groups towards oxidation

What about C–F bond strength?

- Increased stability not due to greater strength of the C–F bond

- Biological oxidations do not involve isolated homolysis of C–H or C–F bond
  - strengths not directly related to oxidation rates



Ezetimibe

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Increased Metabolic Stability of Organofluorine

Bond energies and heats of formation are more relevant

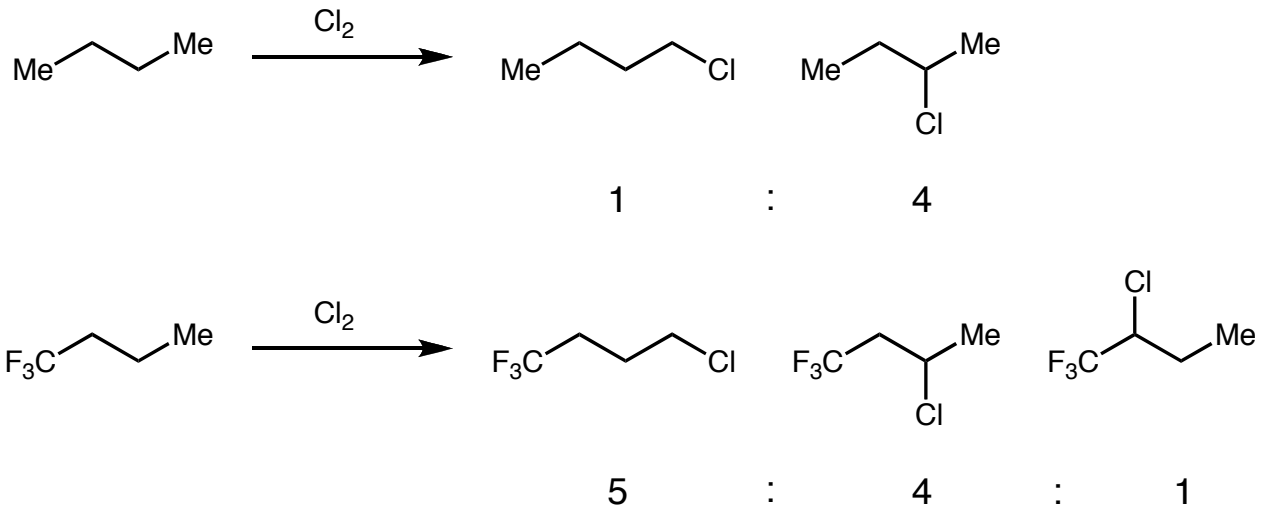
H–O	111 kcal/mol
C–O	85.5 kcal/mol
F–O	44.0 kcal/mol

The formation of F–O bonds unfavourable when compared to C–O and H-O so "attack" at fluorine is generally avoided.

Banks, R. E.; Smart, B. E.; Tatlow, J. C. *Organofluorine Chemistry: Principles and Commercial Applications*; Plenum Press: New York, 1994.

Increased Metabolic Stability of Organofluorine

Inductive effects of fluorine can provide protection as far as β hydrogens



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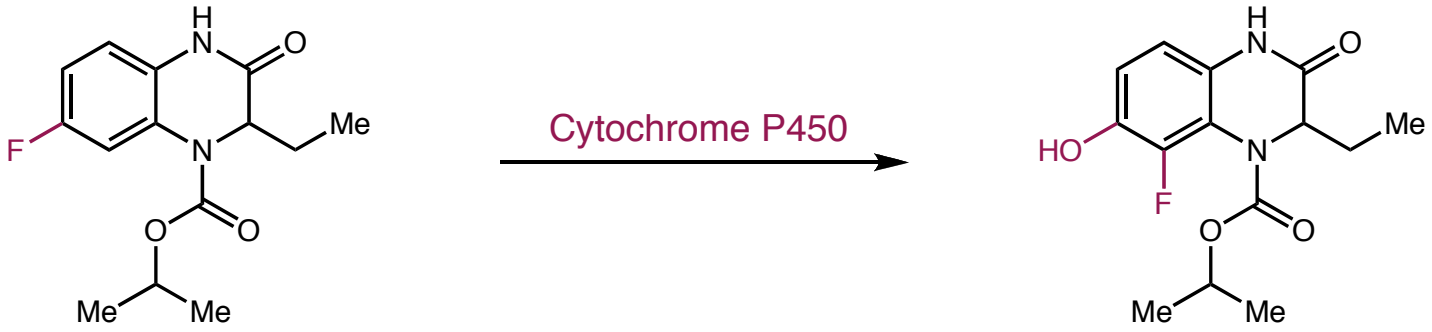
Increased Metabolic Stability of Organofluorine

Incorporating fluorine to reduce rate of aryl oxidation is the most common pharmaceutical application.



GW420867X
reverse transcriptase inhibitor

In a few cases, introduction of a fluorine substituent fails to block oxidation.

Park, K. B.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443.

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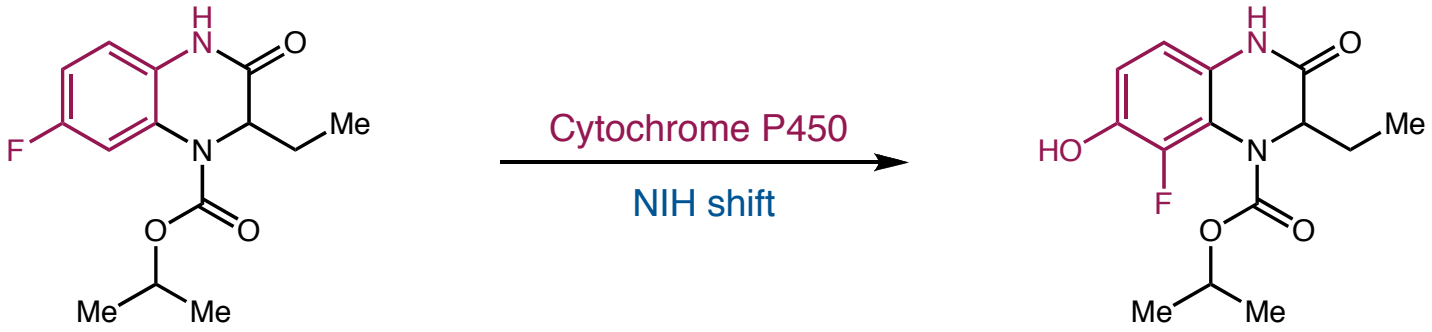

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reverse transcriptase inhibitor

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NIH shift is observed mostly in *p*-fluoroaniline or anilide structures.

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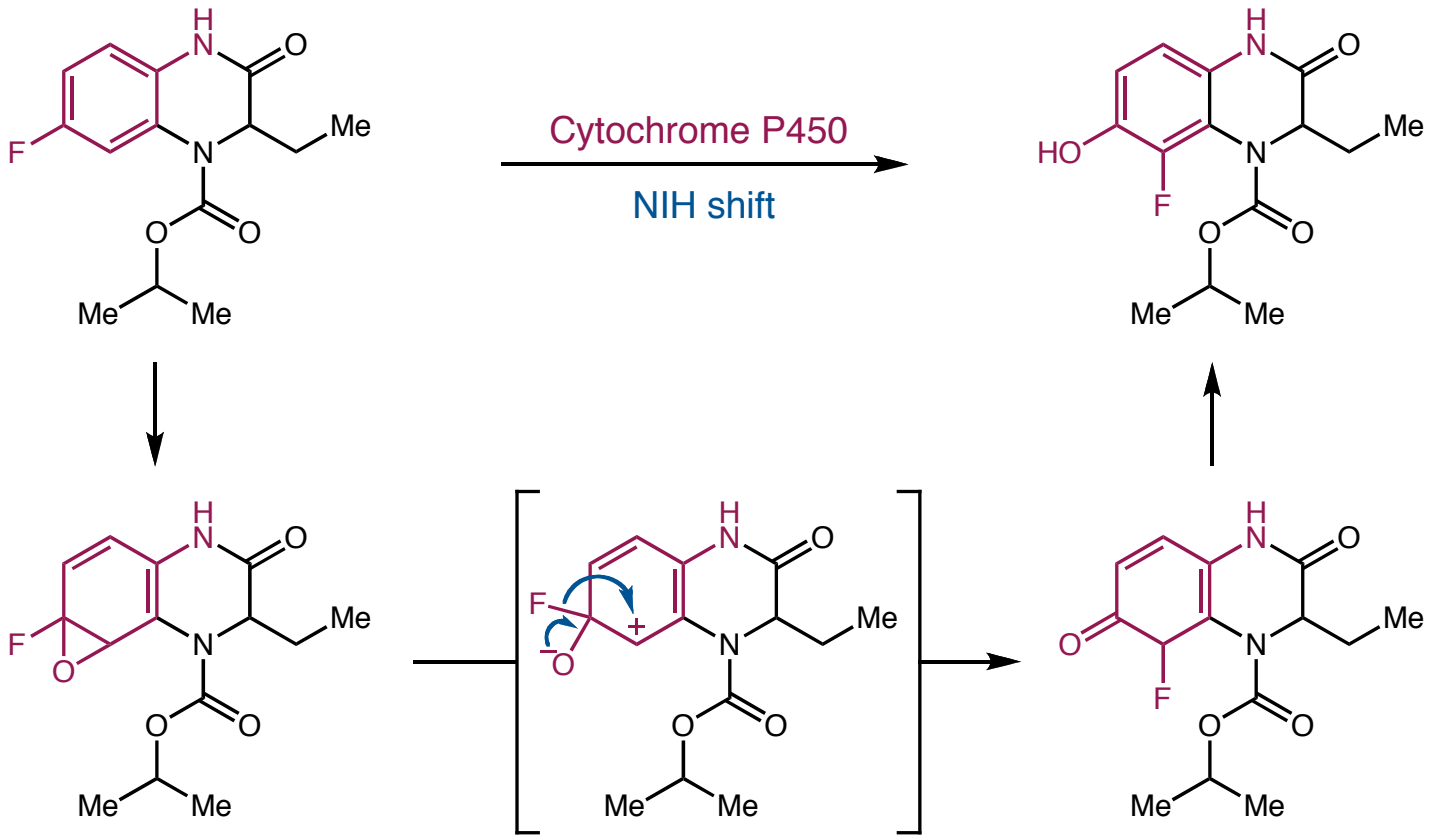

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Increased Metabolic Stability of Organofluorine

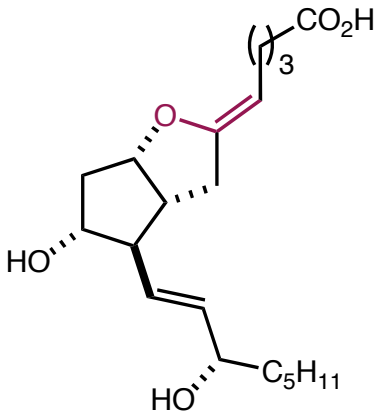
Problem: In mammals, lipophilic compounds have a tendency to be oxidized by liver enzymes, particularly Cytochrome P450

Solution: Several possible strategies available

- Make the compound more polar
  - Lower bioavailability
- Block the metabolically labile sites
- Deactivate metabolically labile sites without blocking

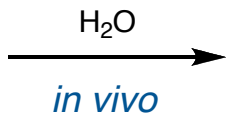
Increased Metabolic Stability of Organofluorine

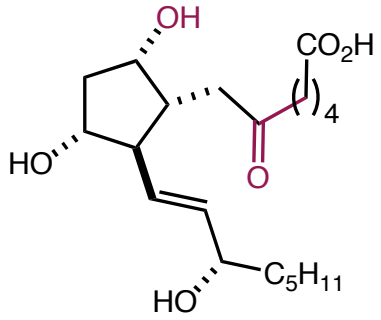
Hydrolytic stability can also be enhanced by fluorination



prostacyclin (PGI_2)
vasodilator
inhibitor of platelet aggregation

$T_{1/2}$ = 10 min
pH = 7.4





6-oxo-$PGF_{1\alpha}$
inactive metabolite

Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Increased Metabolic Stability of Organofluorine

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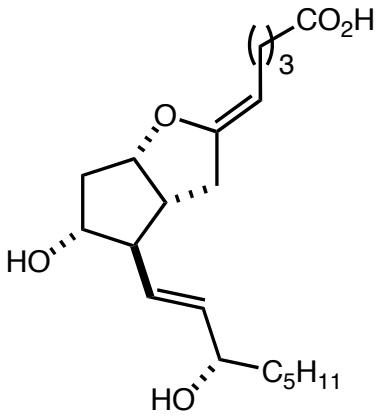
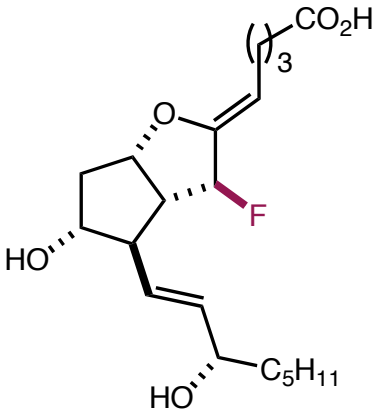


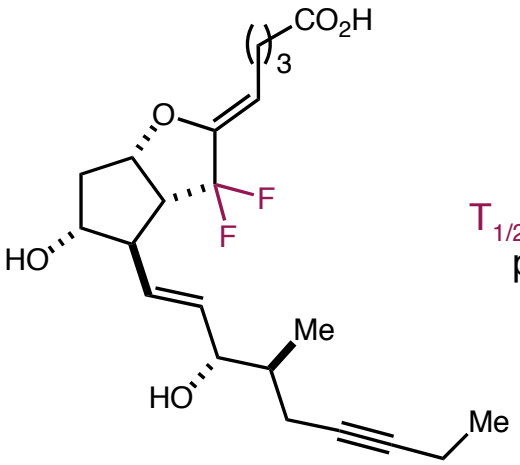
prostacyclin (PGI_2)
vasodilator
inhibitor of platelet aggregation

$T_{1/2}$ = 10 min
pH = 7.4

Rate of hydrolysis dramatically decreased through induction.



7-F-PGI_2
$T_{1/2}$ = >1 month
pH = 7.4



AFP-07
$T_{1/2}$ = 90 days
pH = 7.4

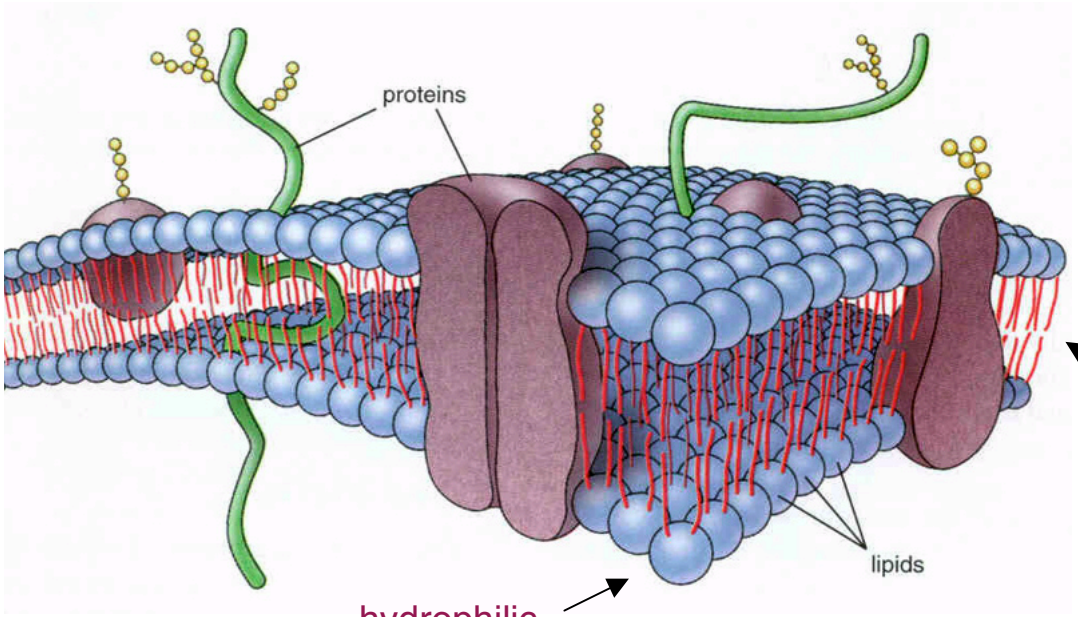
Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Modification of Physicochemical Properties

Most oral drugs are absorbed and distributed through passive transport

must be able to pass through the cell membrane.

Lipophilicity must be tuned to enter the lipid core but not become trapped



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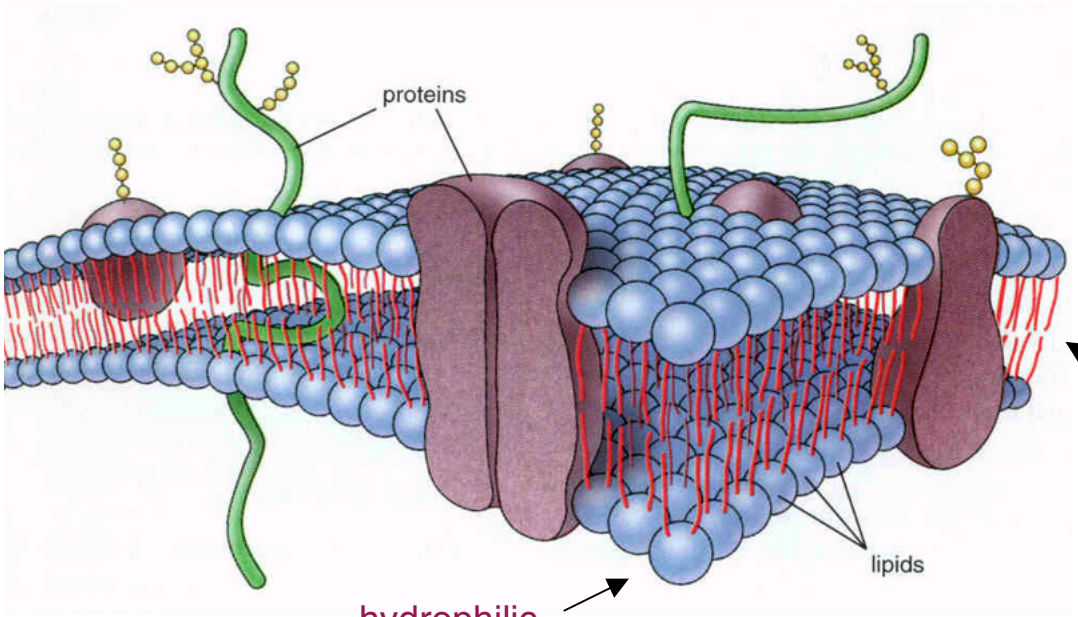


Fluorination can be used to modify lipophilicity

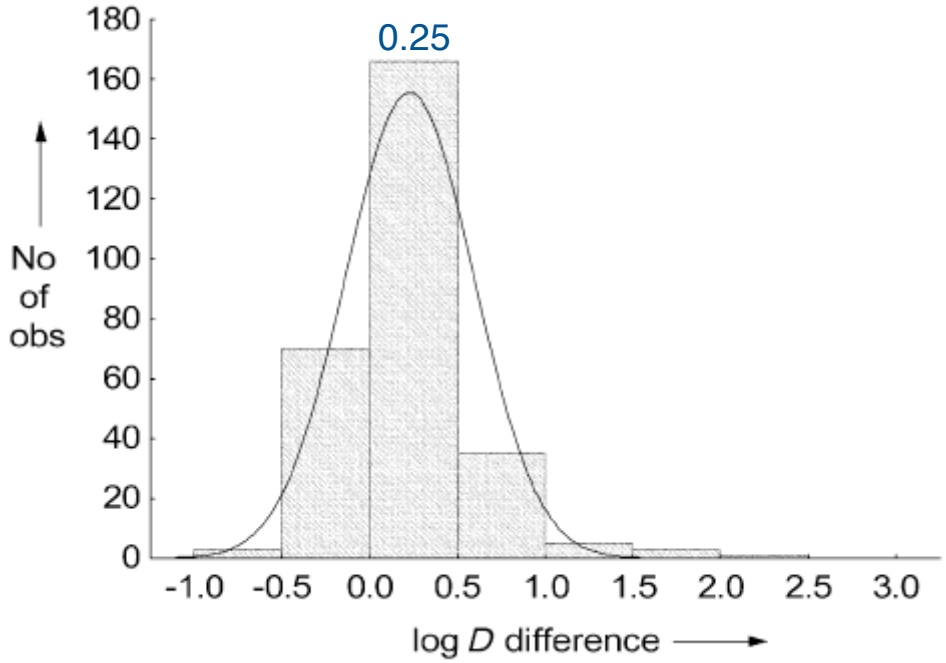
Modification of Physicochemical Properties

"Fluorination always increases lipophilicity"

Modification of Physicochemical Properties

"Fluorination usually increases lipophilicity"

common fluorination misconception



Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3.

Modification of Physicochemical Properties

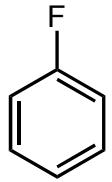
"Fluorination usually increases lipophilicity"

common fluorination misconception

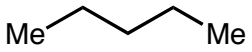
General Rules:

- Aromatic fluorination increases lipophilicity

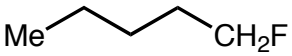

(2.13)


(2.27)

- Monofluorination and trifluoromethylation of saturated alkyl groups decreases lipophilicity



(3.11)



(2.33)

- Per/polyfluorination increases lipophilicity

reduce polarizability

- Fluorination adjacent to a basic functional group increases lipophilicity

Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3.

Modification of Physicochemical Properties

Fluorination adjacent to a basic functional group increases lipophilicity

induction from nearby fluorine decreases the pKa

10.7 8.97 7.52 5.7

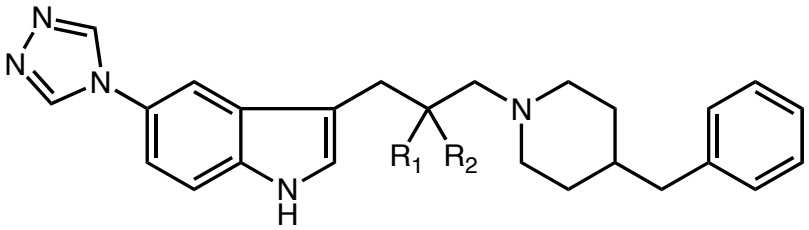
Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3.

Modification of Physicochemical Properties

Fluorination adjacent to a basic functional group increases lipophilicity
induction from nearby fluorine decreases the pKa

10.7 8.97 7.52 5.7

Decreasing pKa can increase bioavailability but decrease receptor binding



$5HT_{1D}$ agonists for migraine relief

$R_1 = R_2 = H$ pKa = 9.7
poor bioavailability, excellent binding

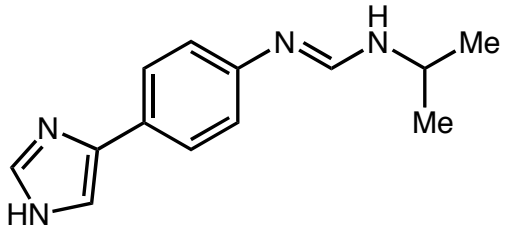
$R_1 = F$, $R_2 = H$ pKa = 8.7
good bioavailability, good binding

$R_1 = R_2 = F$ pKa = 6.7
excellent bioavailability, poor binding

Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3.
van Neil, M. B. *et al. J. Med. Chem.* **1999**, *42*, 2087.

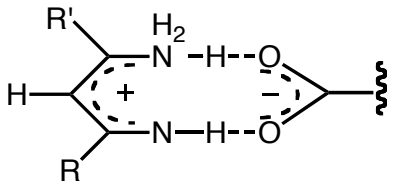
Modification of Physicochemical Properties

Altering the pKa can also increase drug activity: increase concentrations of the active form

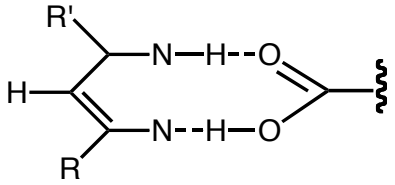


mifentidine
histamine H2 receptor antagonist

What is the active form?



protonated

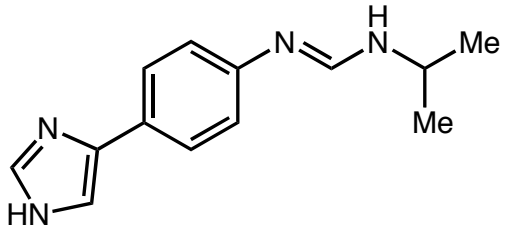


neutral

Donetti, A.; Cereda, E.; Ezhaya, A.; Micheletti, R. *J. Med. Chem.***1989**, *32*, 957.

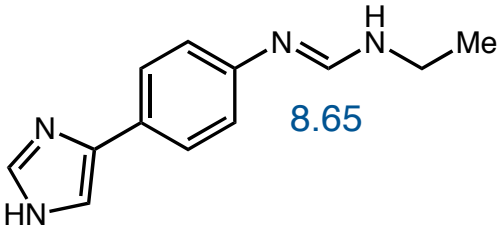
Modification of Physicochemical Properties

Altering the pKa can also increase drug activity: increase concentrations of the active form



mifentidine
histamine H2 receptor antagonist

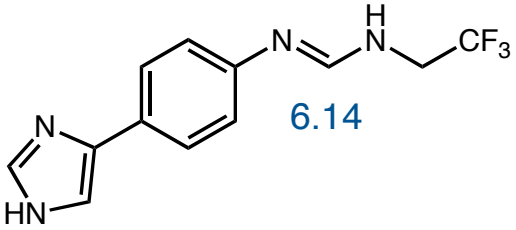
What is the active form?



8.65

5.57

K_D = 177 pM



6.14

4.45

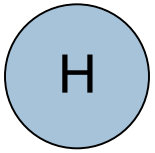
K_D = 7.6 pM

Increased activity of the fluorinated analog indicates the neutral species is active

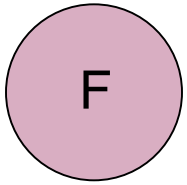
Donetti, A.; Cereda, E.; Ezhaya, A.; Micheletti, R. *J. Med. Chem.***1989**, *32*, 957.

Effects on Molecular Conformation

The large dipole of the C–F bond and the size of the fluorine atom play a significant role in the conformational behaviour of organofluorine compounds.

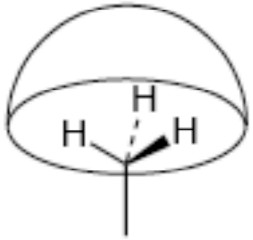


1.20 Å

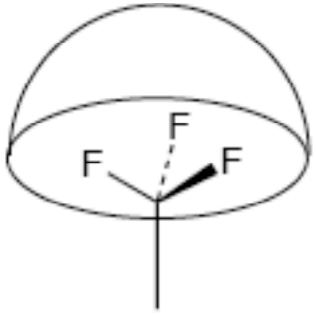


1.47 Å

single atom substitution usually imparts little steric demand



16.8 Å^3



42.6 Å^3

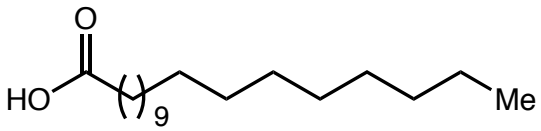
multi-atom substitution can have more drastic steric consequences

conformational changes can be subtle and sometimes difficult to predict

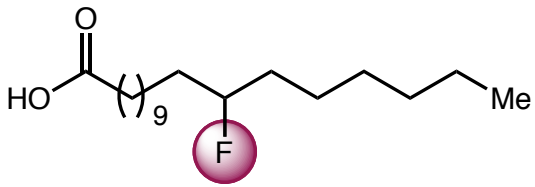
Seebach, D. *Angew. Chem. Int. Ed.* **1990**, *29*, 1320.

Effects on Molecular Conformation

Most common fluorine modification: Substituting C–H for C–F



stable monolayer in water



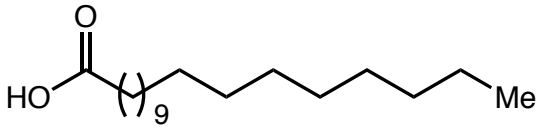
stable monolayer in water

Despite size difference, only small steric and geometric perturbations

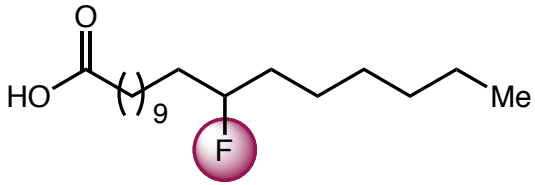
O'Hagan, D.; Rezpa, H. S. *J. Chem. Soc., Chem. Commun.* **1997**, 645.

Effects on Molecular Conformation

Most common fluorine modification: Substituting C–H for C–F



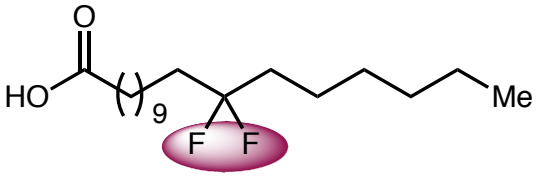
stable monolayer in water



stable monolayer in water

Despite size difference, only small steric and geometric perturbations

Substituting CH_2 with CF_2

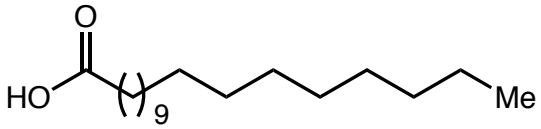


*un*stable monolayer
conformational disorder

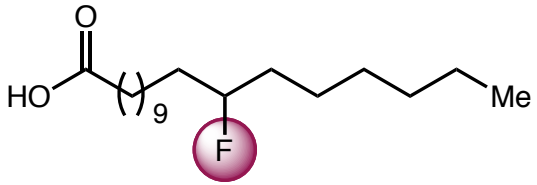
O'Hagan, D.; Rezpa, H. S. *J. Chem. Soc., Chem. Commun.* **1997**, 645.

Effects on Molecular Conformation

Most common fluorine modification: Substituting C–H for C–F



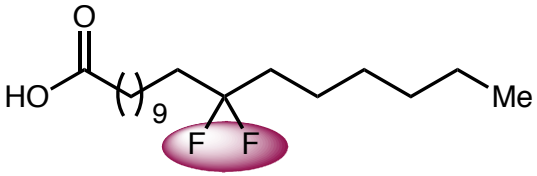
stable monolayer in water



stable monolayer in water

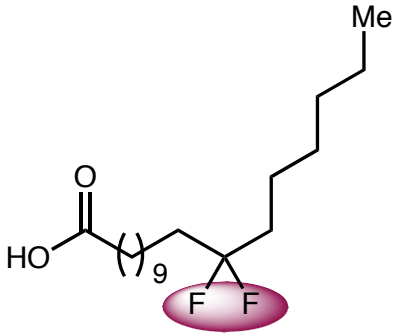
Despite size difference, only small steric and geometric perturbations

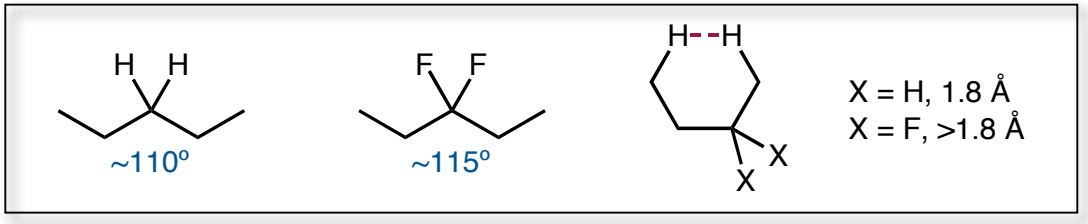
Substituting CH_2 with CF_2



*un*stable monolayer

conformational flexibility

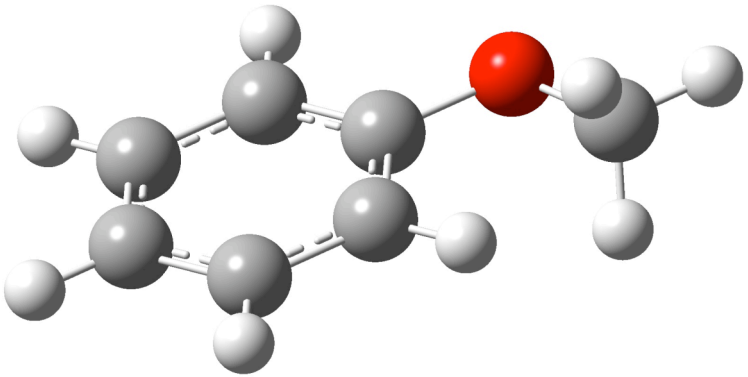


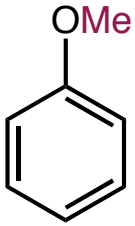


O'Hagan, D.; Rezpa, H. S. *J. Chem. Soc., Chem. Commun.* **1997**, 645.

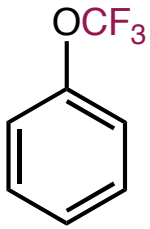
Effects on Molecular Conformation

Distortion Increases: methoxybenzene and trifluoromethoxybenzene do not adopt similar ground state conformations

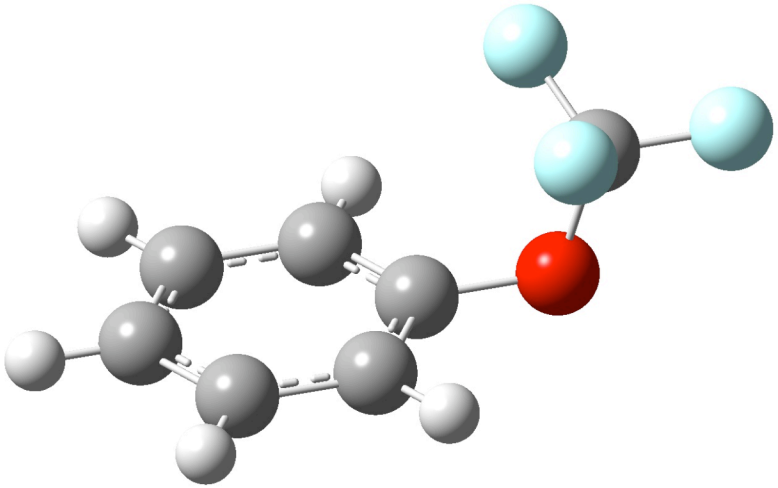




dihedral angle 0º
Me – 16.8 Å^3



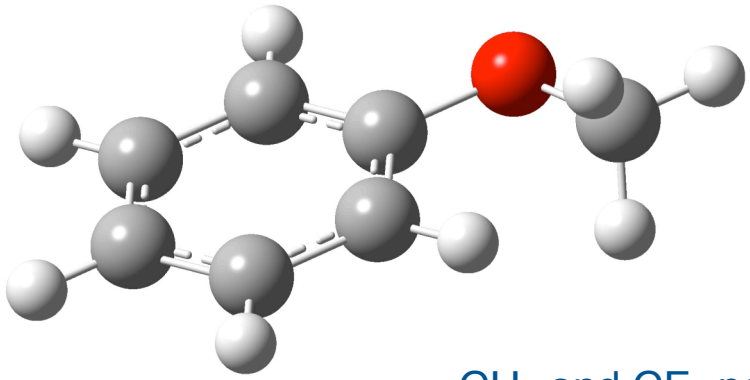
dihedral angle 90º
CF_3 – 42.6 Å^3

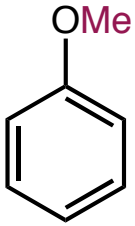


Klocker, J.; Karpfen, A.; Wolschann, P. *Chem. Phys. Lett.* **2003**, *367*, 566.

Effects on Molecular Conformation

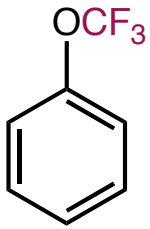
Distortion Increases: methoxybenzene and trifluoromethoxybenzene do not adopt similar ground state conformations



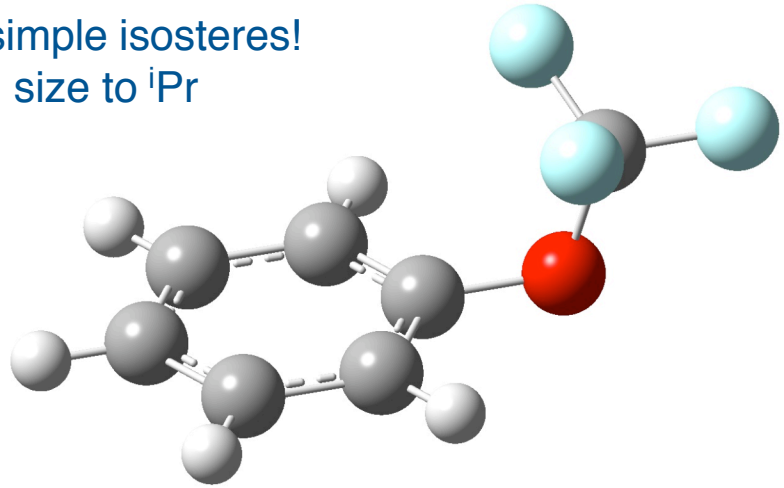


dihedral angle 0°
Me – 16.8 Å^3

CH_3 and CF_3 not simple isosteres!
CF_3 closer in size to ^{i}Pr



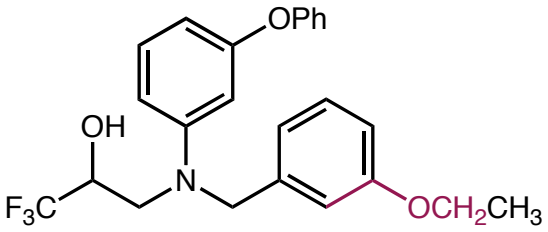
dihedral angle 90°
CF_3 – 42.6 Å^3



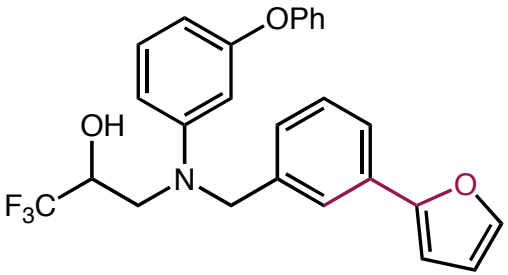
Klocker, J.; Karpfen, A.; Wolschann, P. *Chem. Phys. Lett.* **2003**, *367*, 566.

Effects on Molecular Conformation

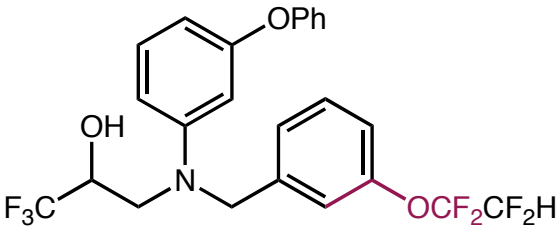
Effect of conformation on chloresteryl ester transfer protein inhibitors.



dihedral angle ~0°
IC_{50} = 1.6 μM

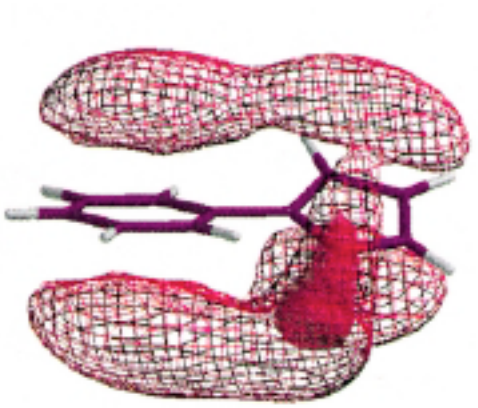


dihedral angle ~30°
IC_{50} = 0.48 μM



dihedral angle ~90°
IC_{50} = 0.2 μM

Out of plane orientation of phenyl substituent results in more efficient binding to target protein.



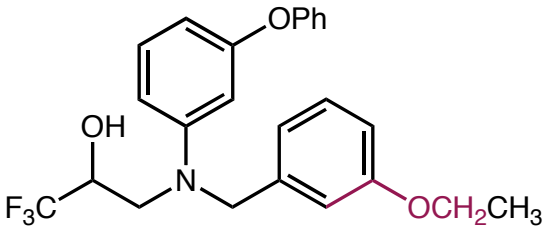
2-Ph furan

$PhOCF_2CF_2H$

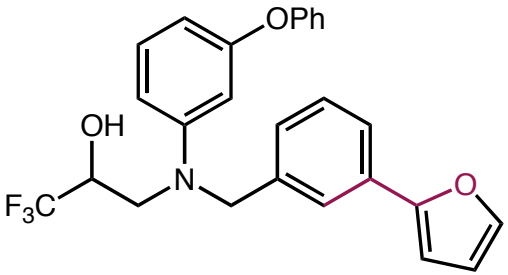
Massa, M. A. *et al. Bioorg. Med. Chem. Lett.* **2001**, *11*, 1625.

Effects on Molecular Conformation

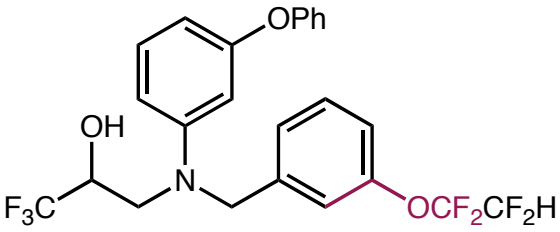
Effect of conformation on chloresteryl ester transfer protein inhibitors.



dihedral angle ~0°
IC_{50} = 1.6 μM

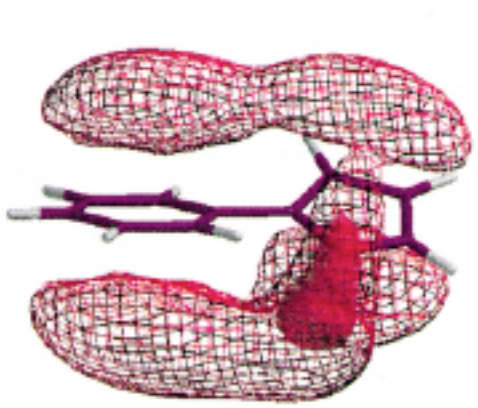


dihedral angle ~30°
IC_{50} = 0.48 μM



dihedral angle ~90°
IC_{50} = 0.2 μM

Fluorinated ethers possible isosteres for metabolically unstable 2-furyl compounds



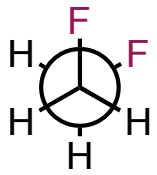
2-Ph furan

$PhOCF_2CF_2H$

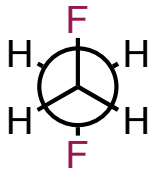
Massa, M. A. *et al. Bioorg. Med. Chem. Lett.* **2001**, *11*, 1625.

Effects on Molecular Conformation

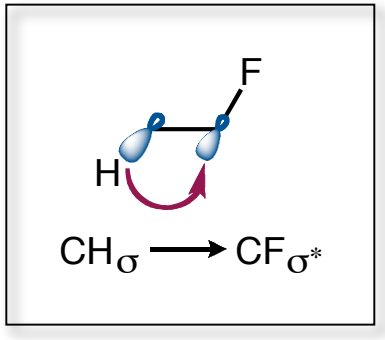
1,2-Fluorine bond attraction: the *gauche* effect



0.0 kcal/mol



0.7 kcal/mol

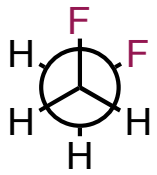


O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

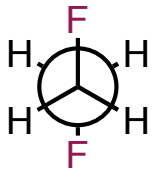
O'Hagan, D.; Bilton, C.; Howard, J. A. K.; Knight, .; Tozer, D, J. *J. Chem. Soc., Perkin Trans. 2* **2000**, 605.

Effects on Molecular Conformation

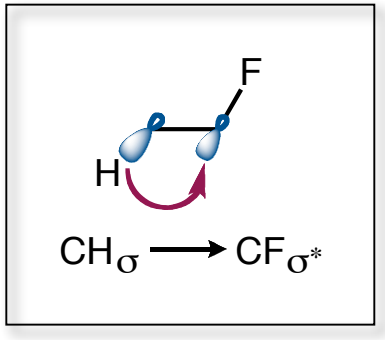
1,2-Fluorine bond attraction: the *gauche* effect



0.0 kcal/mol

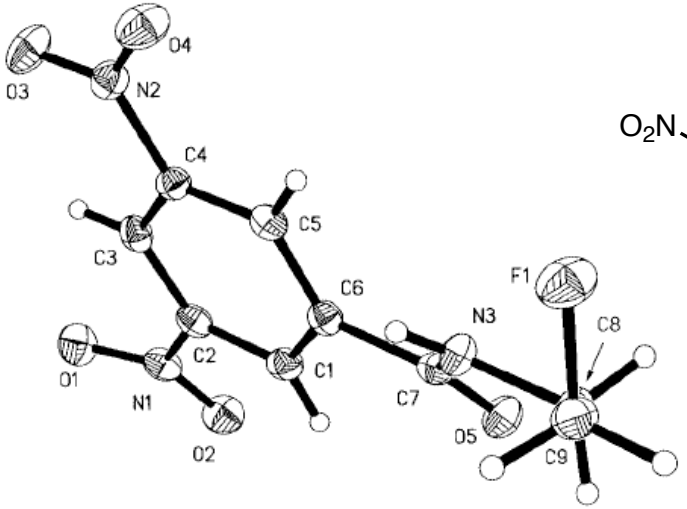


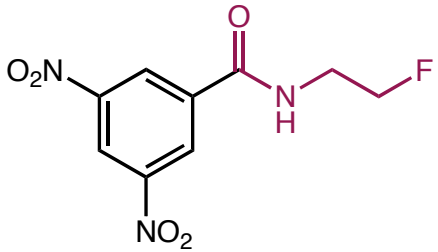
0.7 kcal/mol



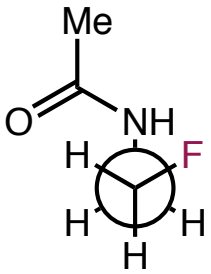
Gauche effect present in other heteroatom systems

- stabilization even greater for *N*-β-fluoroethylamides

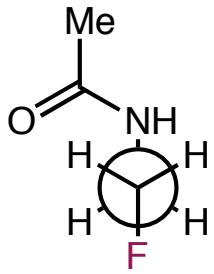




dihedral angle
63°



0.0 kcal/mol



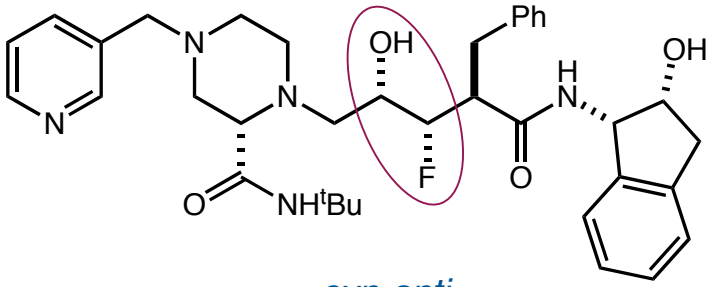
1.8 kcal/mol

O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

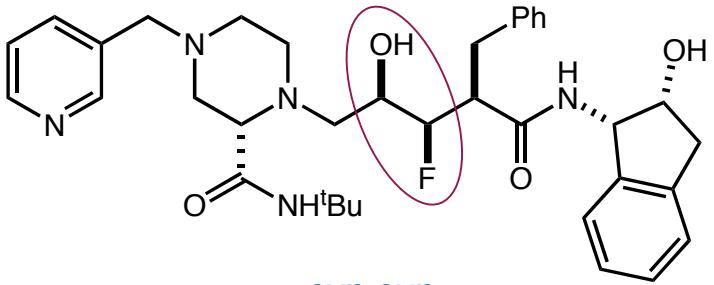
O'Hagan, D.; Bilton, C.; Howard, J. A. K.; Knight, .; Tozer, D, J. *J. Chem. Soc., Perkin Trans. 2* **2000**, 605.

Effects on Molecular Conformation

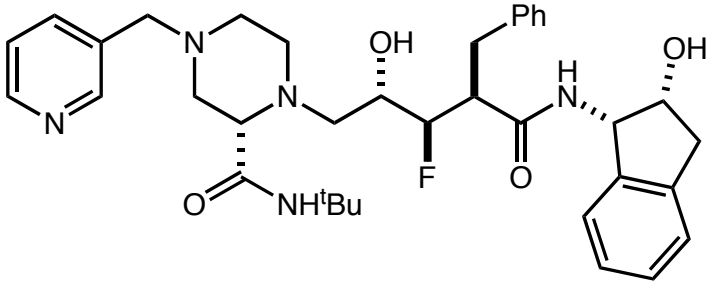
Fluorine vicinal to oxygen influences conformation in Indinavir analogs.



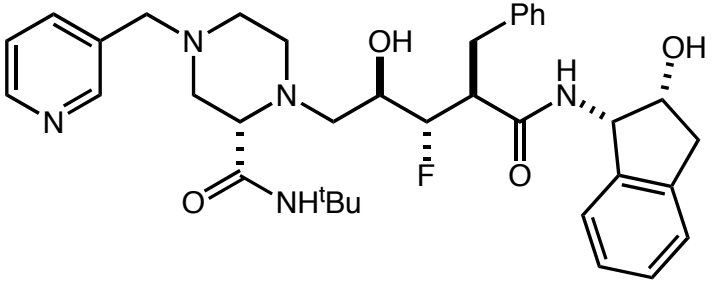
syn,anti
K_i = 20 nM



syn,syn
K_i = 2.0 nM



anti,syn
K_i = 5900 nM



anti,anti
K_i = 27 nM

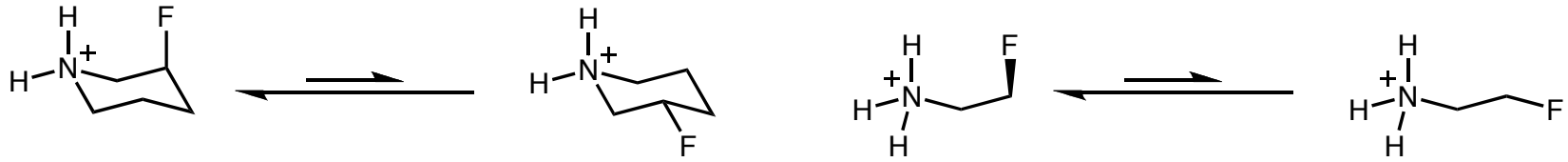
Syn fluorohydrin analogs maintain the required fully extended chain.

Myers, A. G.; Barbay, J.; K.; Zhong, B. *J. Am. Chem. Soc.* **2001**, *123*, 7202.

Effects on Molecular Conformation

Fluorine interacts with formal charges to induce conformational preferences

Charge-Dipole Interactions



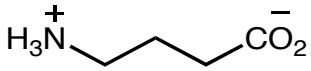
Large axial or *gauche* preference when a formal charge is present

Much larger energetic preference than *gauche* effect alone

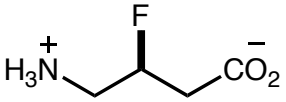
O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Effects on Molecular Conformation

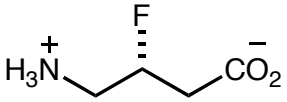
Charged-dipole interactions can probe protein-ligand interactions.



GABA



(*S*)-3F-GABA



(*R*)-3F-GABA

How does the neurotransmitter γ-aminobutyric acid (GABA) bind to proteins?

- In $GABA_A$ receptors (ligand-gated ion channels) (*S*)- and (*R*)-3F-GABA interacted similarly
- In GABA transaminase (metabolizing enzyme) (*S*)-3F-GABA has a much higher affinity

Deniau, G. *et al*. *ChemBioChem* **2007**, *8*, 2265.

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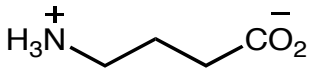
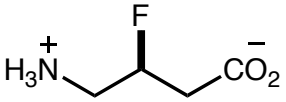


GABA



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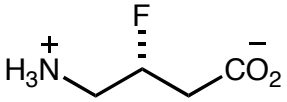

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Zwitterionic GABA has a protonated amine - charge-dipole interactions

Deniau, G. *et al. ChemBioChem* **2007**, *8*, 2265.

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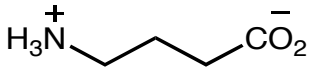
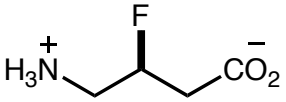


GABA



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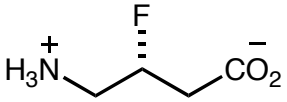
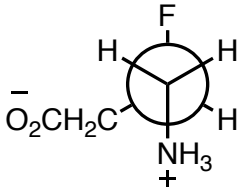
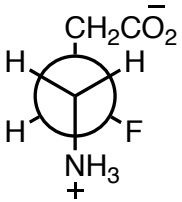
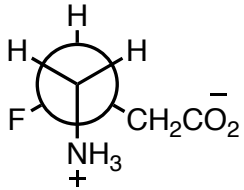


(*R*)-3F-GABA

Possible conformations of 3F-GABA







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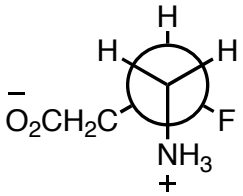

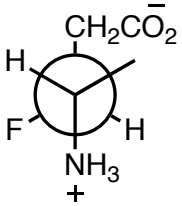

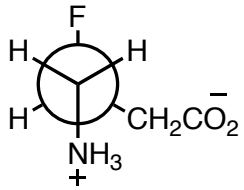

(*S*)-3F-GABA

Deniau, G. *et al. ChemBioChem* **2007**, *8*, 2265.

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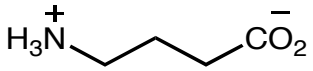
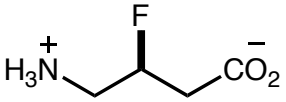


GABA



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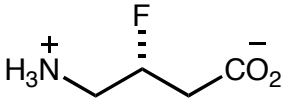
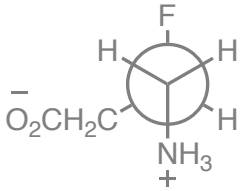
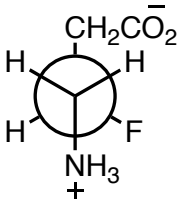


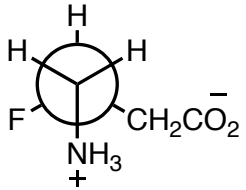
(*R*)-3F-GABA

Possible conformations of 3F-GABA

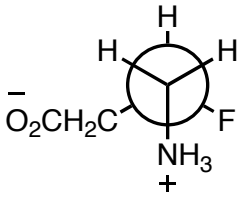


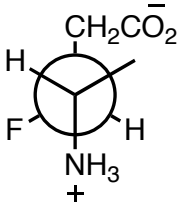
disfavoured

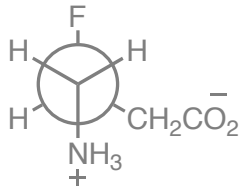




(*R*)-3F-GABA







disfavoured

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Deniau, G. *et al. ChemBioChem* **2007**, *8*, 2265.

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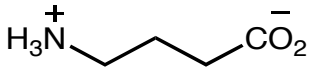

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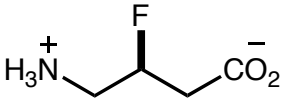

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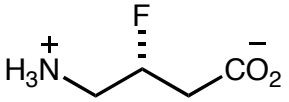
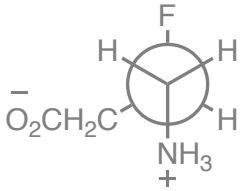


(*R*)-3F-GABA

$GABA_A$ receptors bind (*R*)- and (*S*)-3F-GABA equally



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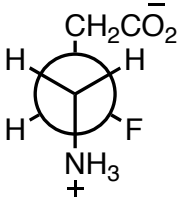
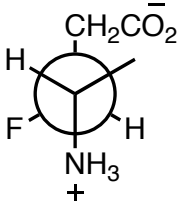
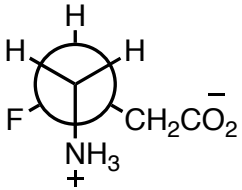


$GABA_A$ Binding

anti-zigzag





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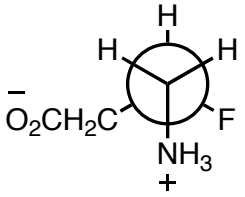

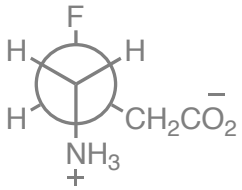

disfavoured

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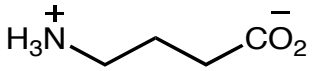

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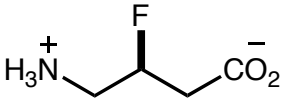

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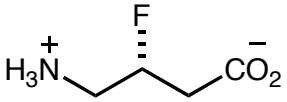
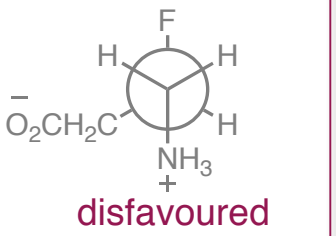


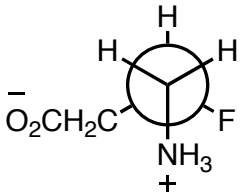
(*R*)-3F-GABA

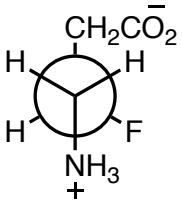
GABA transaminase has higher affinity for (*S*)-3F-GABA

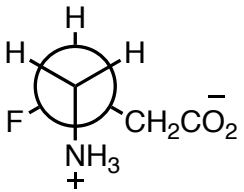


disfavoured

GABA transaminase binding







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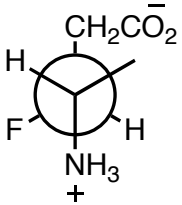
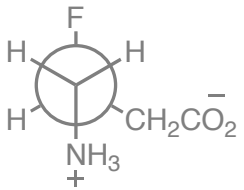




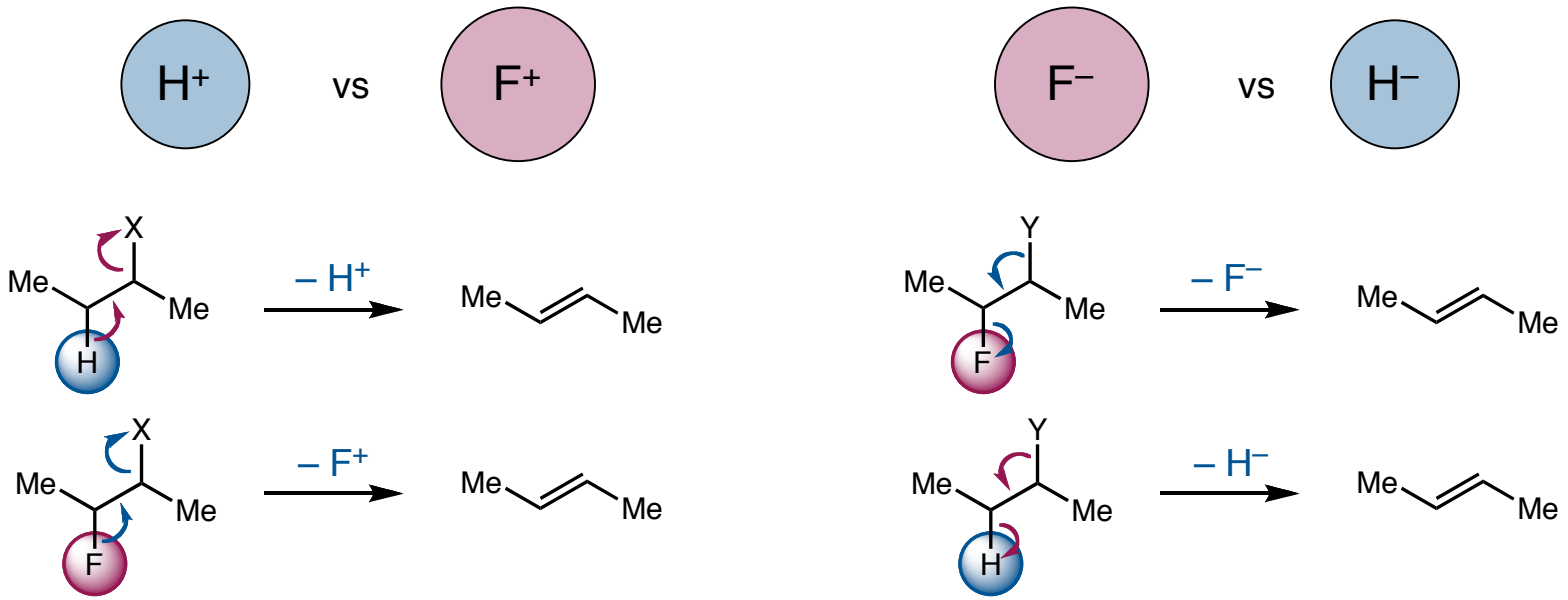
(*S*)-3F-GABA

disfavoured

Deniau, G. *et al. ChemBioChem* **2007**, *8*, 2265.

Exploitation of Orthogonal Reactivity

Hydrogen and fluorine have minor steric differences but show orthogonal reactivity



Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

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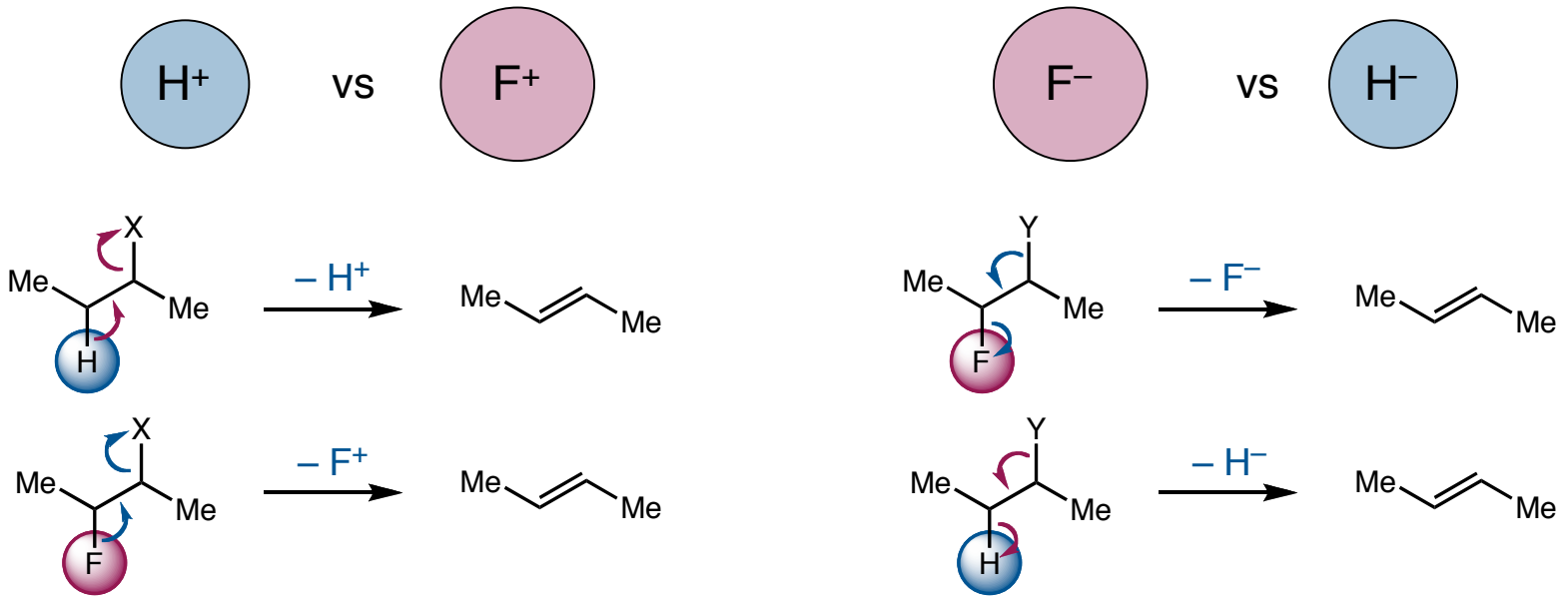

Prevent *in vivo* racemization

(*S*)-thalidomide
teratogenic effects

(*R*)-thalidomide
sedative effects

Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

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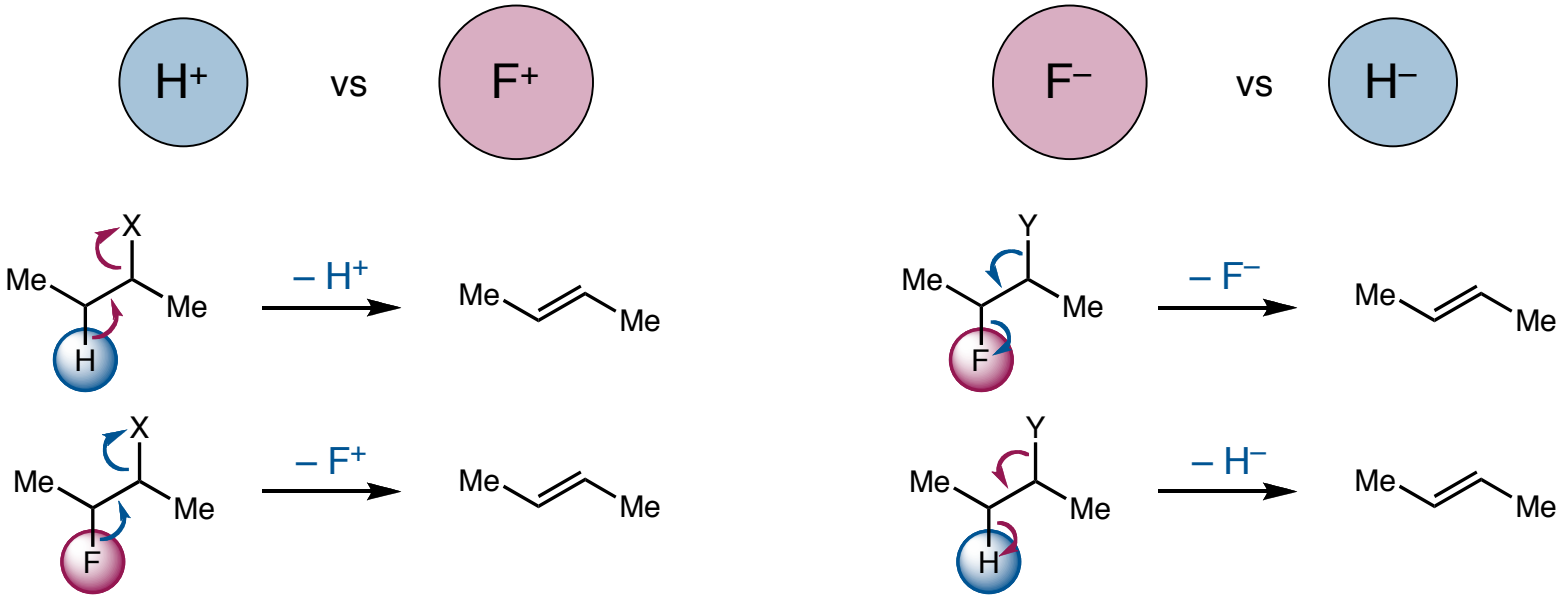

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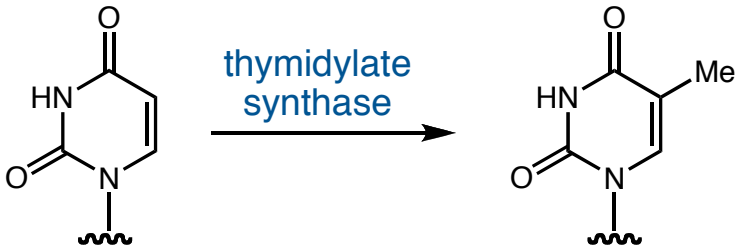
Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Exploitation of Orthogonal Reactivity

Hydrogen and fluorine have minor steric differences but show orthogonal reactivity

Exploit this inherent orthogonal reactivity to design enzyme inhibitors

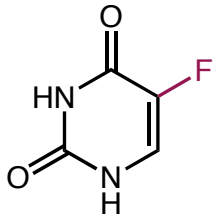


dUMP dTMP

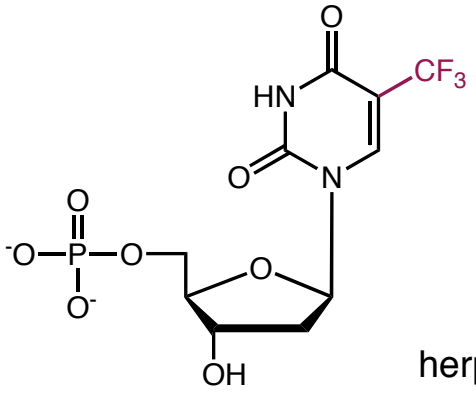
Thymidylate synthase (TS):
- converts dUMP to dTMP

dTMP required for DNA biosynthesis
Inhibition of TS causes apoptosis

Thymidylate synthase suicide inhibitors:



5-Fluorouracil
treatment of various cancers

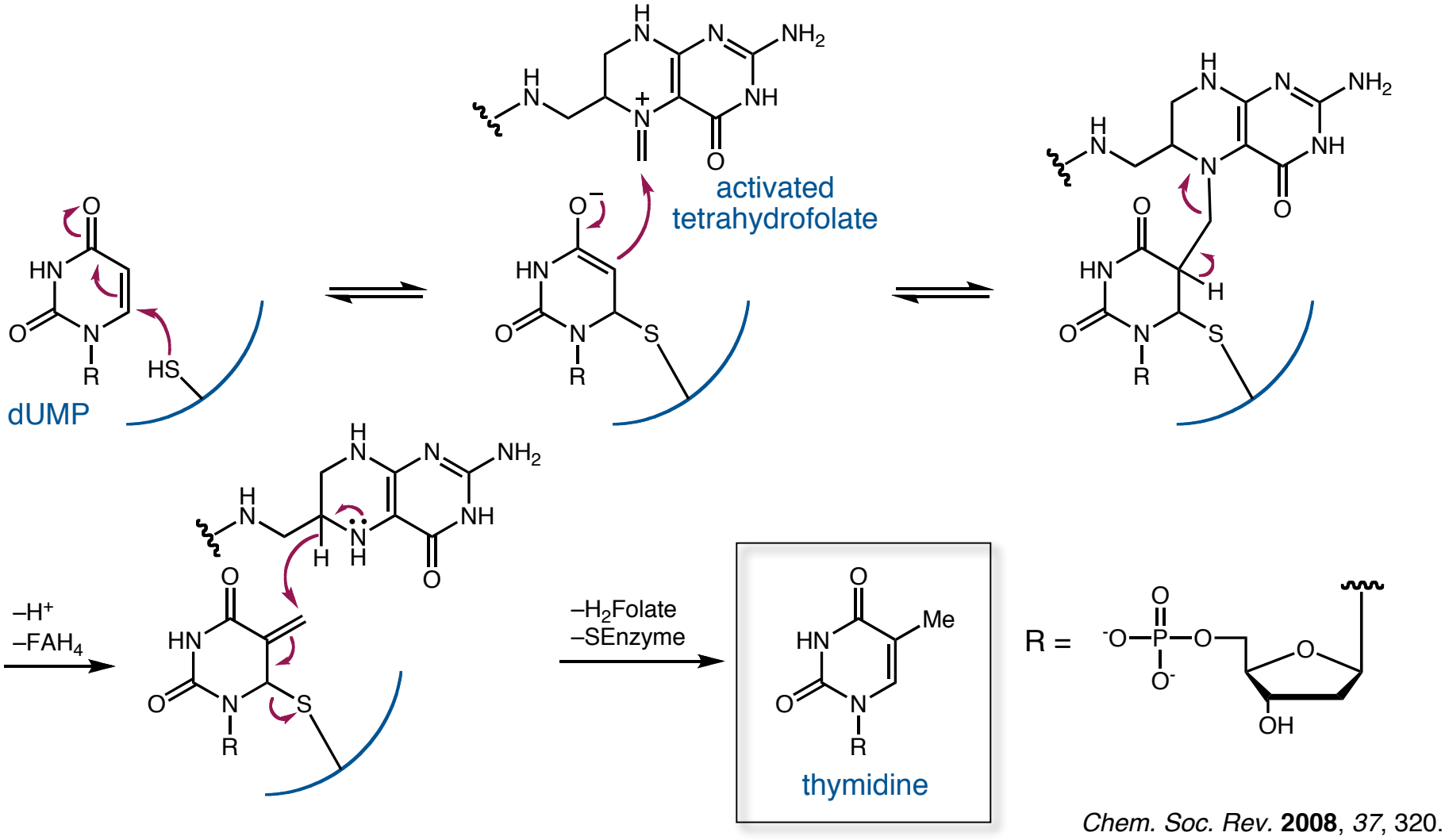


Trifluridine
herpes antiviral drug

Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.

Exploitation of Orthogonal Reactivity

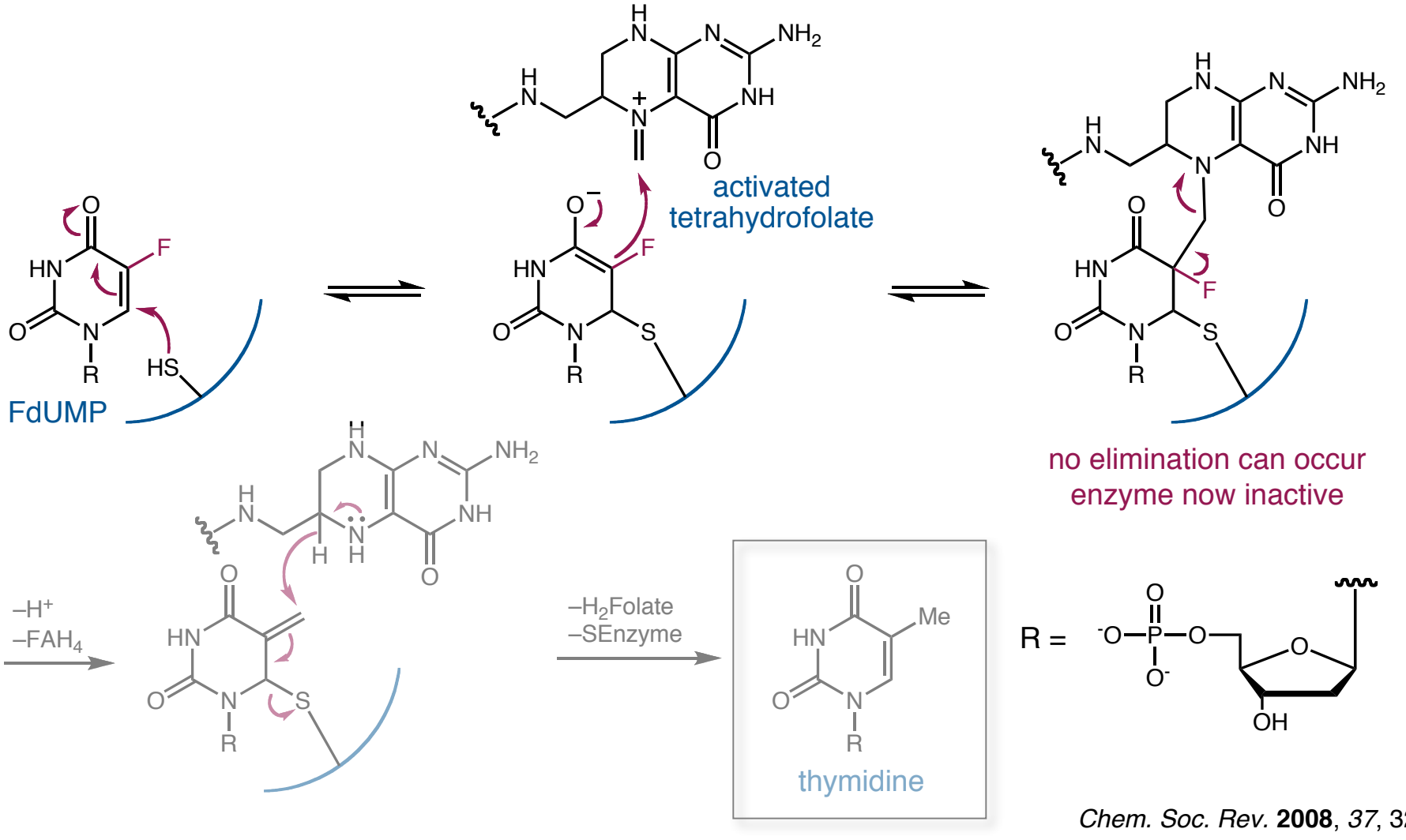
Thymidylate synthase mechanism:



Chem. Soc. Rev. **2008**, *37*, 320.

Exploitation of Orthogonal Reactivity

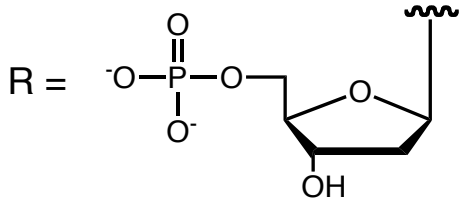
Thymidylate synthase inhibition using 5-Fluorouracil:

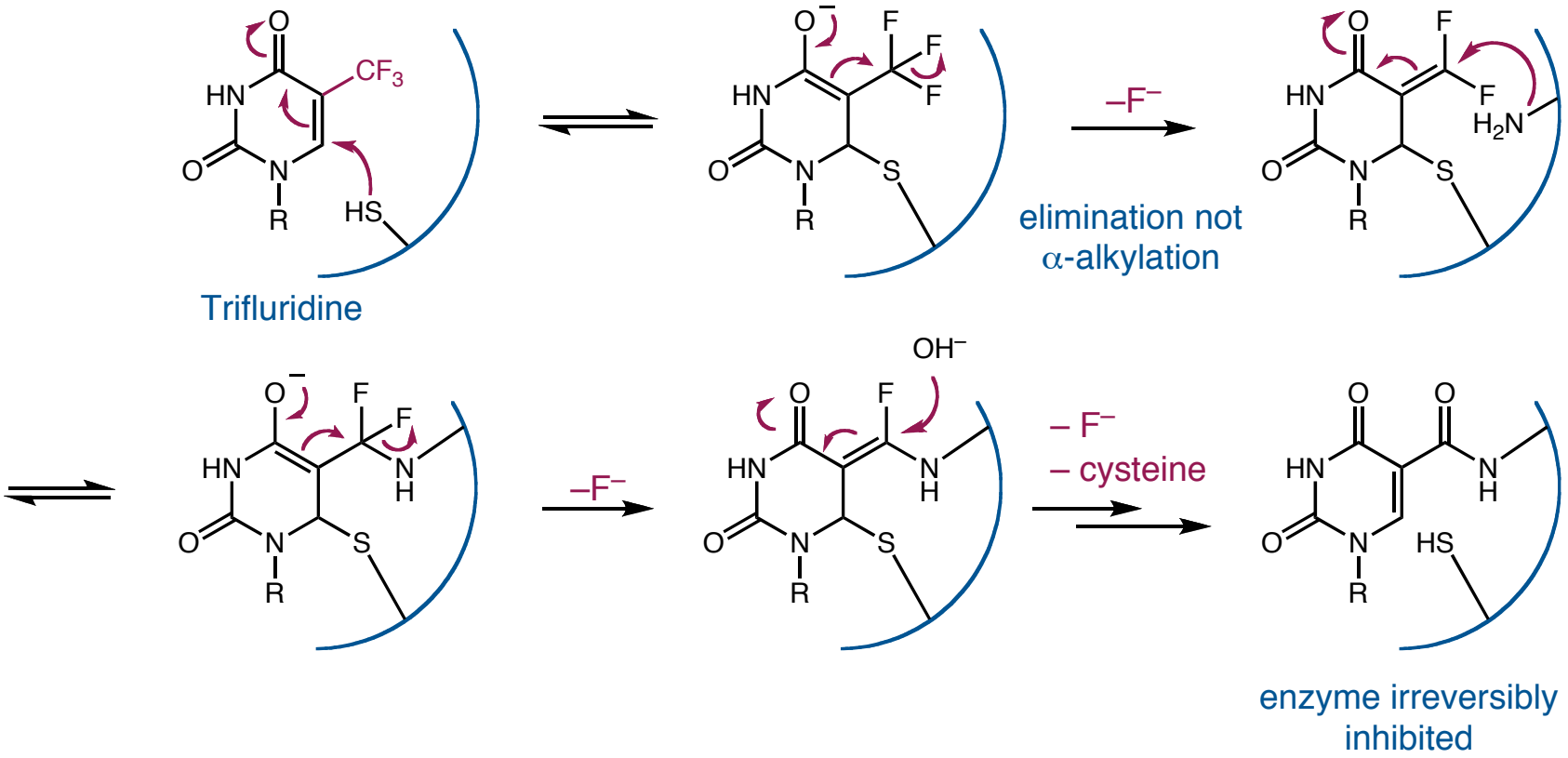


Chem. Soc. Rev. **2008**, *37*, 320.

Exploitation of Orthogonal Reactivity

Thymidylate synthase inhibition using Trifluridine:

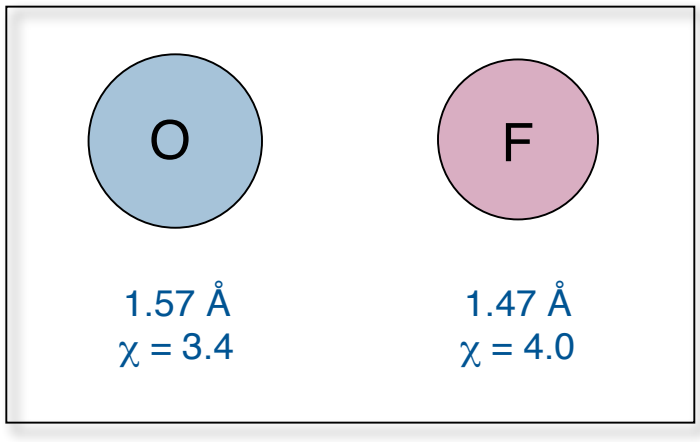




Chem. Soc. Rev. **2008**, *37*, 320.

Fluorinated Isosteres

Substituting C–OH with C–F



Sterically/electronically neutral change

- both electronegative atoms
- similar size match

Other considerations:

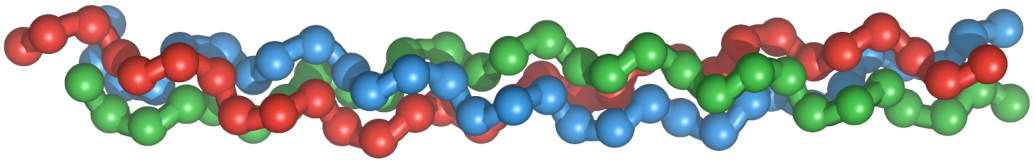
- loss of acidic hydrogen
- loss of hydrogen bond donor ability
- limited (or no) hydrogen bond acceptor ability

Use to explore roles of C–OH hydrogen bonding versus C–O bond polarity

O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine



Collagen:

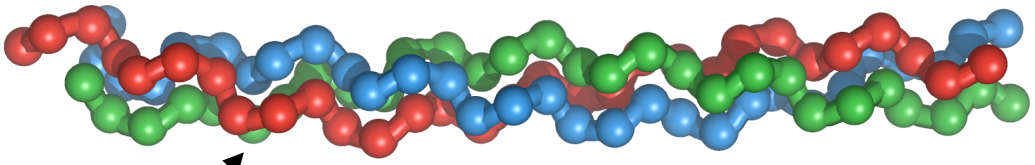
- Most abundant protein in animals
- Tight triple helix in connective tissue
- High tensile strength and thermal stability

What structural aspect of collagen causes its stability?

Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.

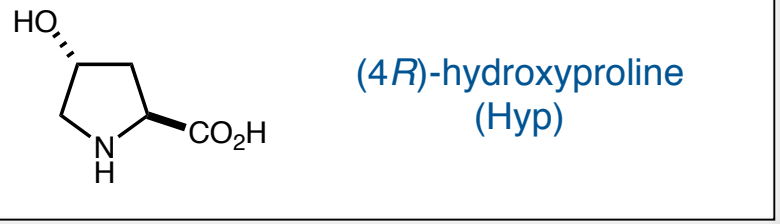
Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine

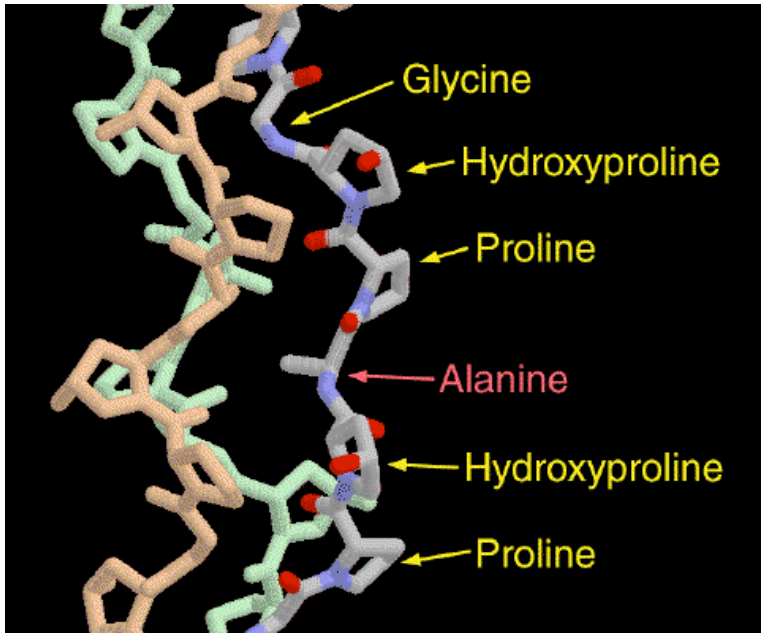


amino acid sequence
Xaa-Yaa-Gly

Xaa and Yaa usually proline or (4*R*)-hydroxyproline



(4*R*)-hydroxyproline
(Hyp)



Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.
Bella, J.; Brodsky, B.; Berman, H. M. *Science* **1994**, *266*, 75.

Fluorinated Isosteres

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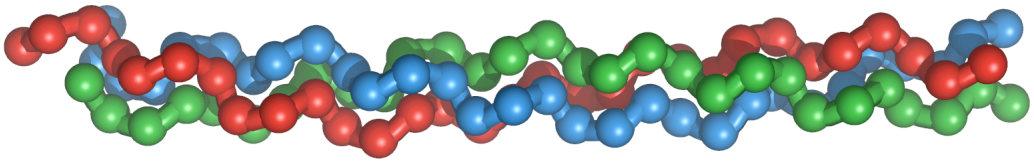

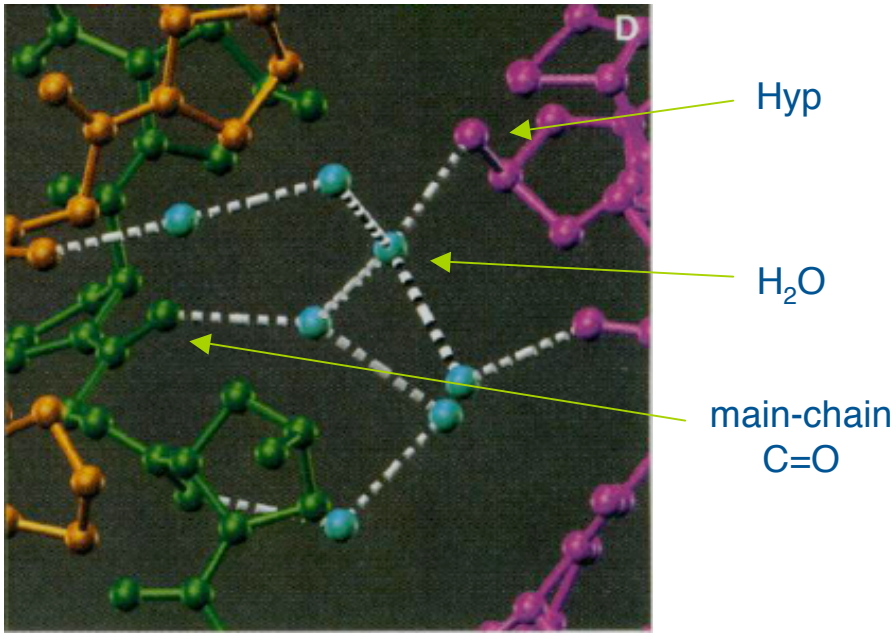

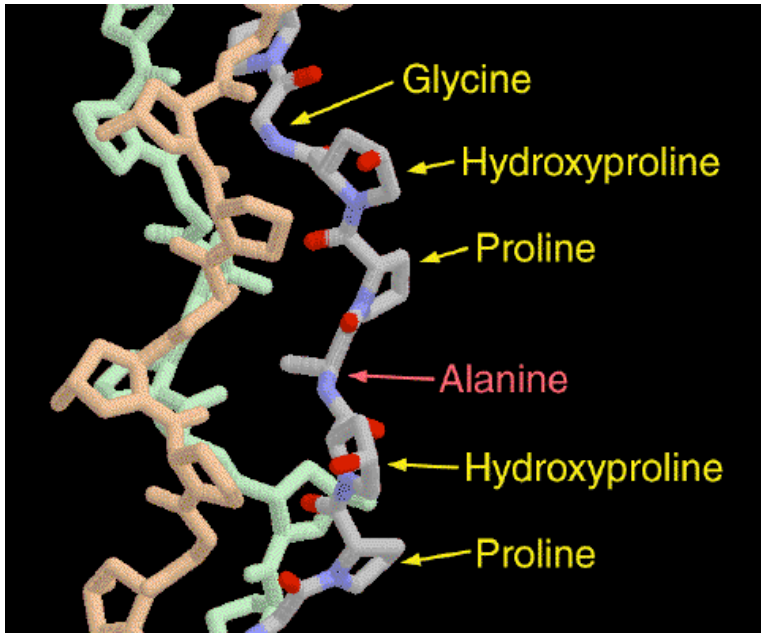

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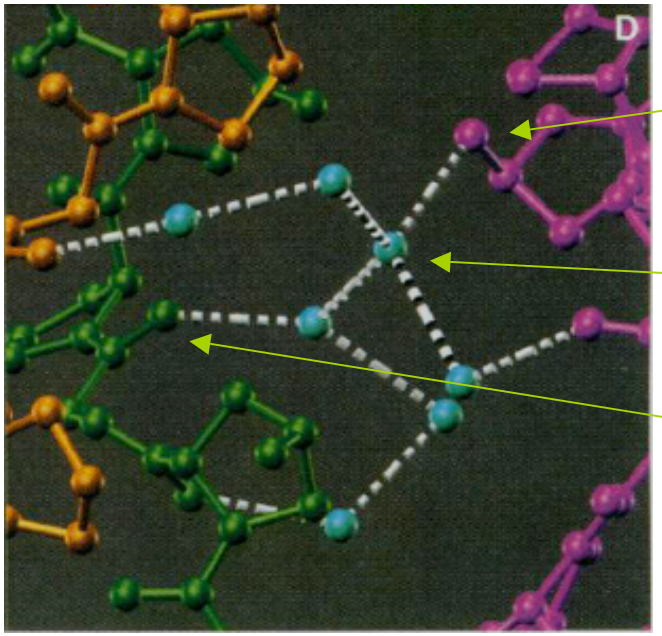
Bella, J.; Brodsky, B.; Berman, H. M. *Science* **1994**, *266*, 75.

Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine

Are the bridging water molecules the source of the stability?

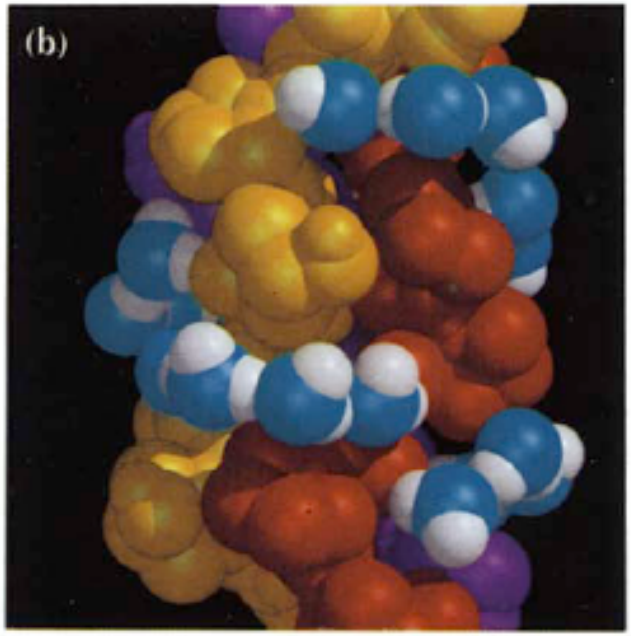
High entropic cost of immobilizing >500 water molecules per helix



Hyp

H_2O

main-chain
C=O



Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.
Bella, J.; Brodsky, B.; Berman, H. M. *Science* **1994**, *266*, 75.

Fluorinated Isosteres

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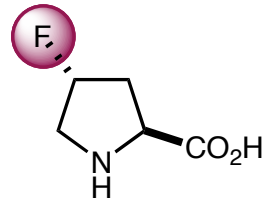
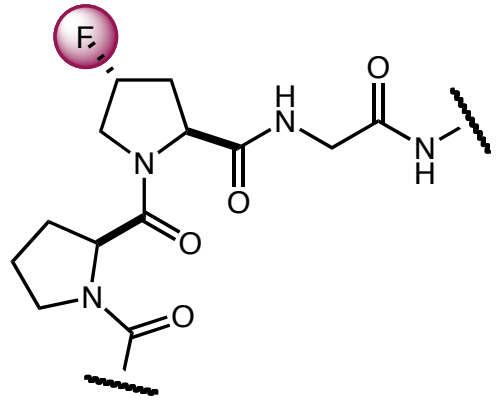
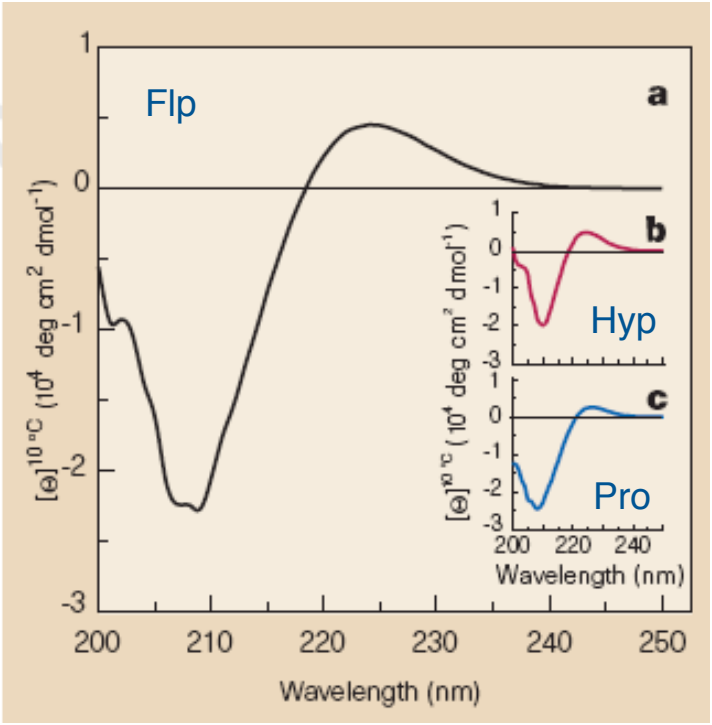


(4*R*)-fluoroproline
(Flp)

Synthesize $(ProFlpGly)_{10}$:





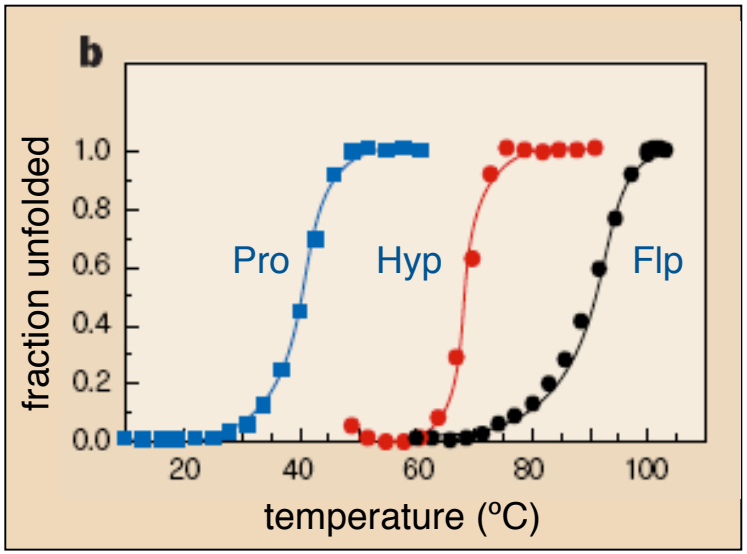
- weaken (or remove) hydrogen bonds
- retain polarity of the C–X bond

CD spectra of $(ProYaaGly)_{10}$

Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.

Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine



Stability:

$(ProProGly)_{10} < (ProHypGly)_{10} < (ProFlpGly)_{10}$

Stability of collagen relies on the polarized C–X bond and not water bridges.

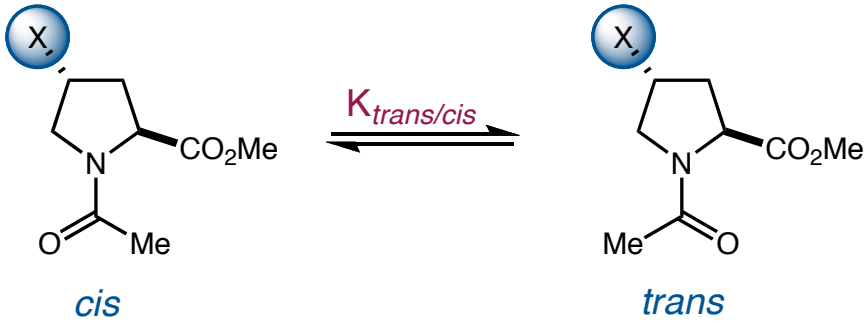
Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666.

Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine

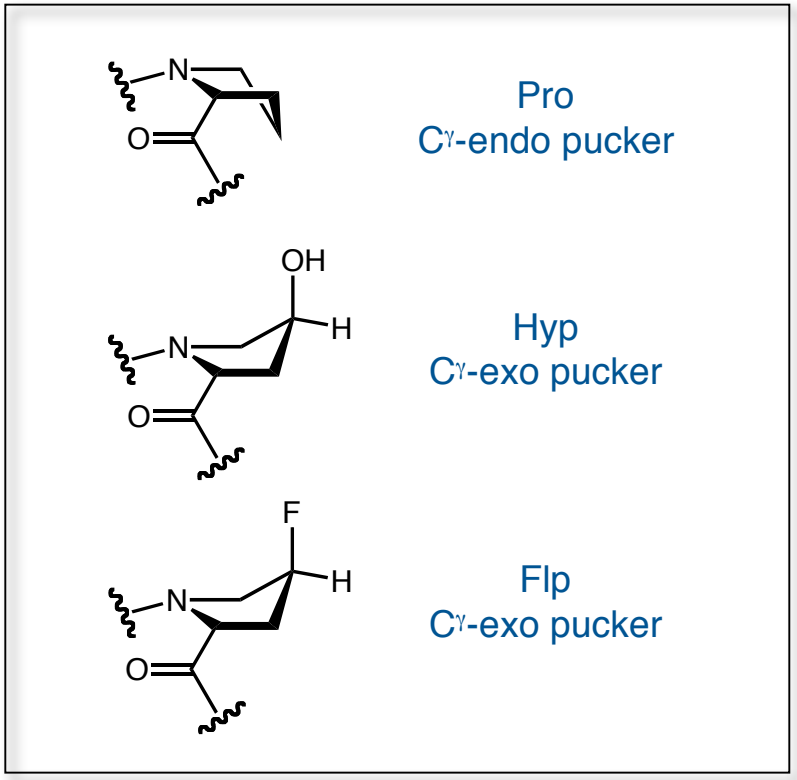
Collagen's triple helix requires *trans* peptide bonds favoured by Hyp and Flp

- Where does this preference come from?



cis *trans*

X	K
H	4.6
OH	6.1
F	6.7



Jenkins, C. L.; Lin, G.; Duo, J.; Rapolu, D.; Guzei, I. A.; Raines, R. T.; Krow, G. R. *J. Org. Chem.* **2004**, *69*, 8565.
Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777.

Fluorinated Isosteres

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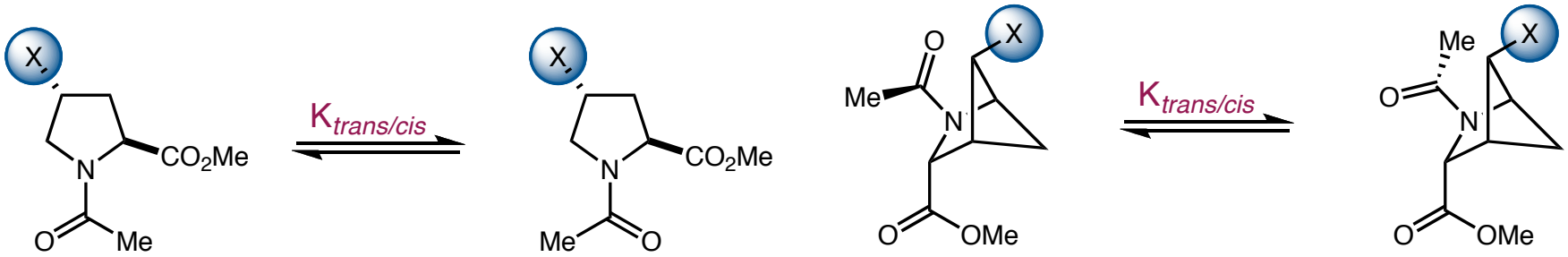

X	K
H	4.6
OH	6.1
F	6.7

X	K
H	3.5
OH	3.6
F	3.5

Jenkins, C. L.; Lin, G.; Duo, J.; Rapolu, D.; Guzei, I. A.; Raines, R. T.; Krow, G. R. *J. Org. Chem.* **2004**, *69*, 8565.
Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777.

Fluorinated Isosteres

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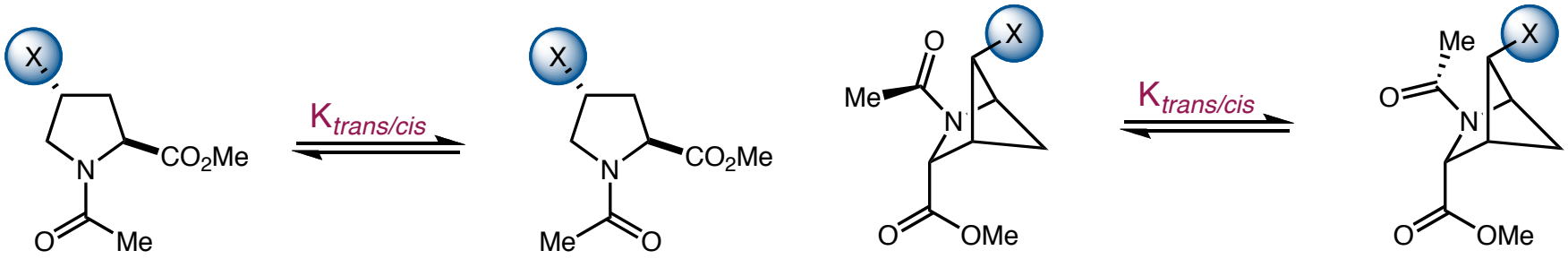


cis *trans* *cis* *trans*

X	K
H	4.6
OH	6.1
F	6.7

cis/trans ratio from conformation

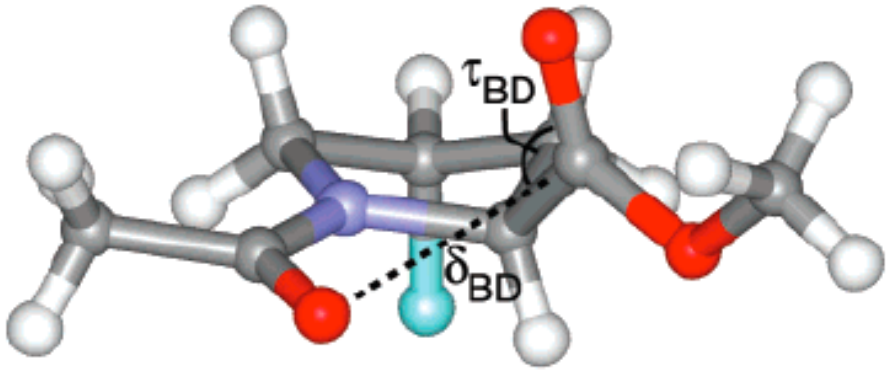
X	K
H	3.5
OH	3.6
F	3.5

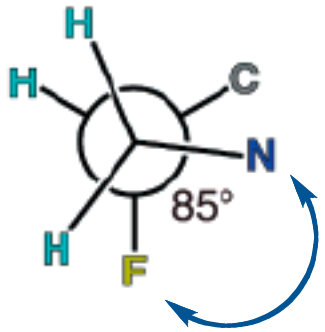
Jenkins, C. L.; Lin, G.; Duo, J.; Rapolu, D.; Guzei, I. A.; Raines, R. T.; Krow, G. R. *J. Org. Chem.* **2004**, *69*, 8565.
Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777.

Fluorinated Isosteres

Code for Collagen's Stability Deciphered - Using Fluorine

The *gauche* effect present between fluorine and nitrogen.





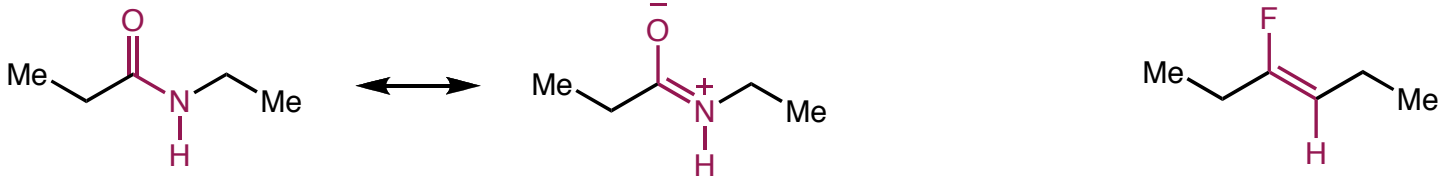
Favourable $O_0 \cdots C_1$ interaction stabilizes *trans* peptide bond.

Collagen stability stems from shape preference
not hydrogen bonded water network

Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777.

Fluorinated Isosteres

Vinylfluorides are steric and polar hydrophobic mimetics of amide bonds.



Polar hydrophobicity:

Maintaining the electrostatic charge distribution while decreasing overall polarizability.

O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.
Couve-Bonnaire, S.; Cahard, D.; Pannecoucke, X. *Org. Biomol. Chem.* **2007**, *5*, 1151.

Fluorinated Isosteres

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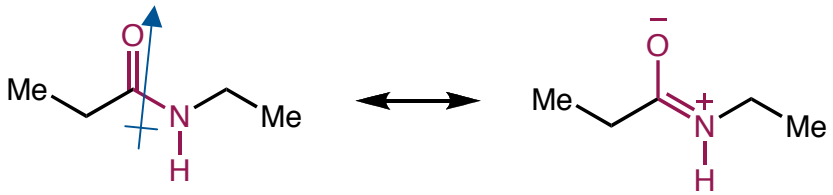
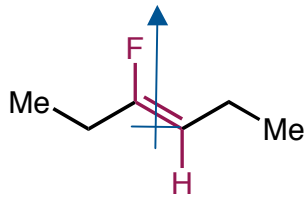
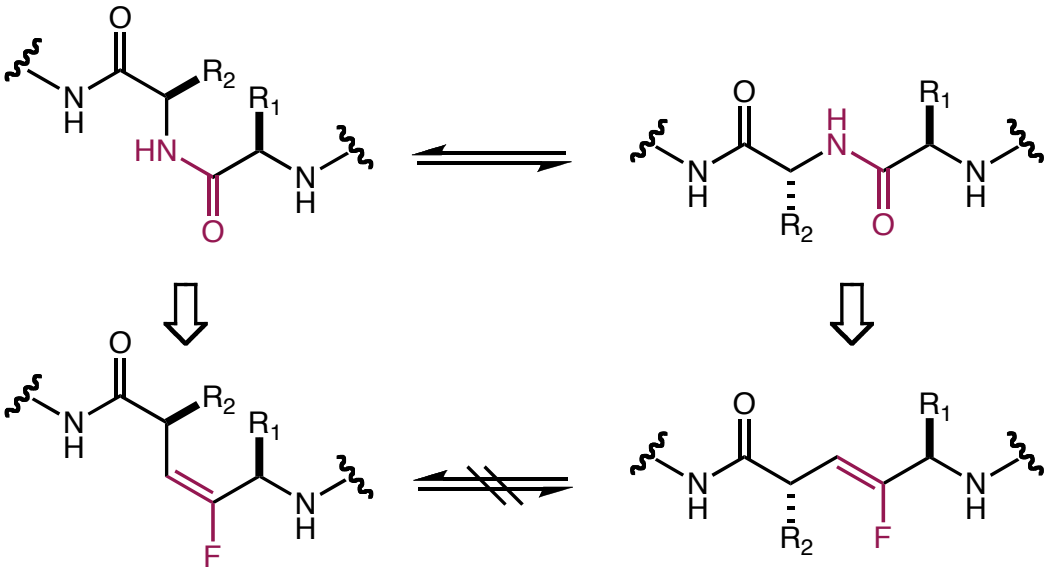


dipole ~3.7 D



dipole ~0.97 D

- Dipole orientation similar but weaker
- Limits (or removes) hydrogen bonding capacity
- Increases lipophilicity and possibily membrane penetration

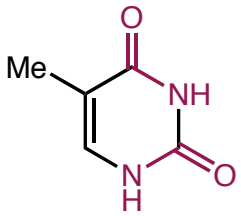


Used as metabolically stable and conformationally constrained peptide mimics

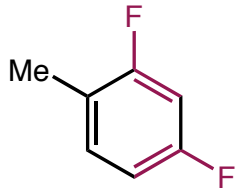
O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308.

Couve-Bonnaire, S.; Cahard, D.; Pannecoucke, X. *Org. Biomol. Chem.* **2007**, *5*, 1151.

Fluorinated Isosteres

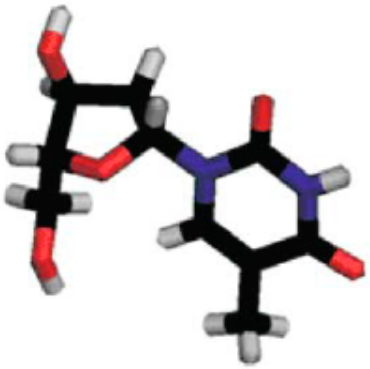


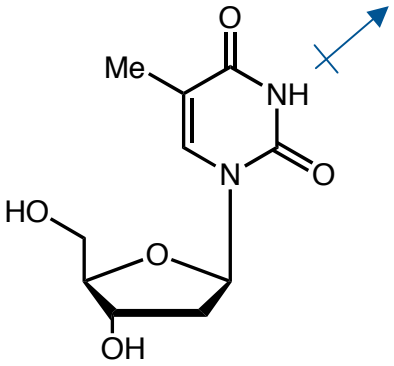
thymine (T)



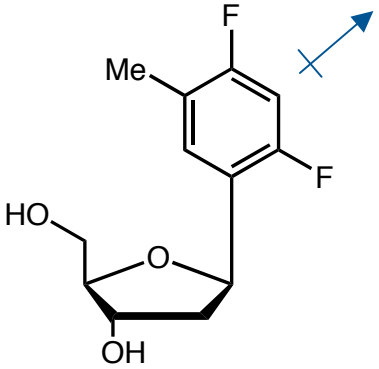
difluorotoluene (F)

difluorotoluene is a nearly perfect isostere for thymine

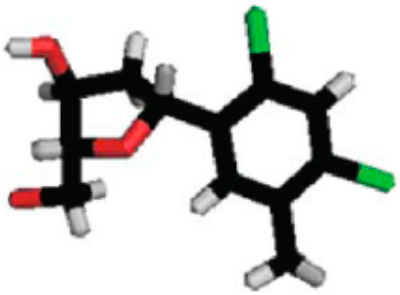




dipole ~4.19 D

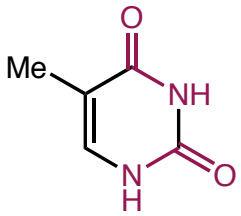


dipole ~1.84 D

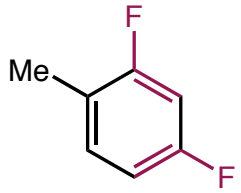


Kool, E. T.; Sintim, H. O. *Chem. Commun.* **2006**, 3663.

Fluorinated Isosteres



thymine (T)



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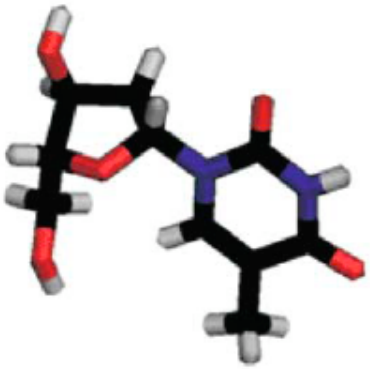
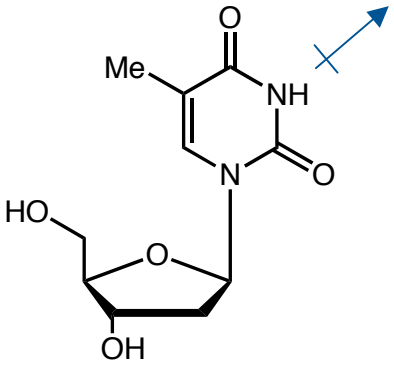
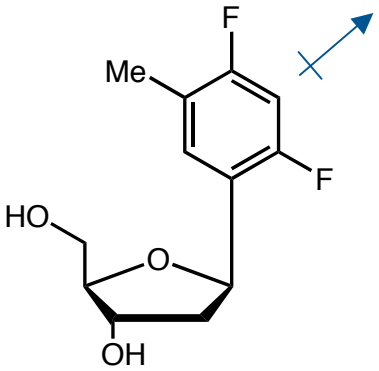
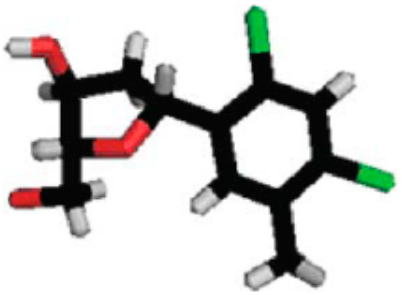




dipole ~4.19 D



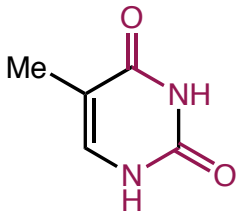
dipole ~1.84 D



no hydrogen bonds

Kool, E. T.; Sintim, H. O. *Chem. Commun.* **2006**, 3663.

Fluorinated Isosteres



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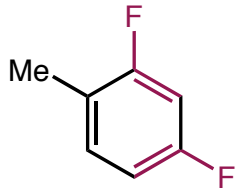

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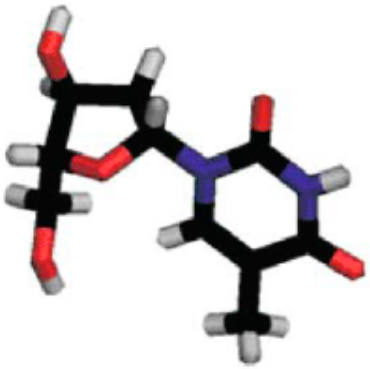
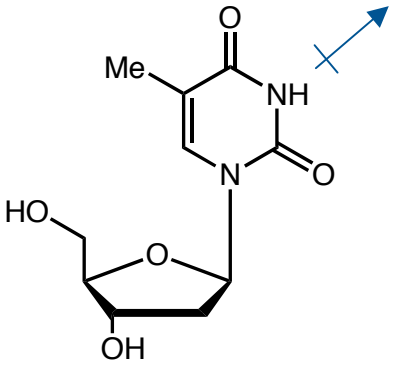
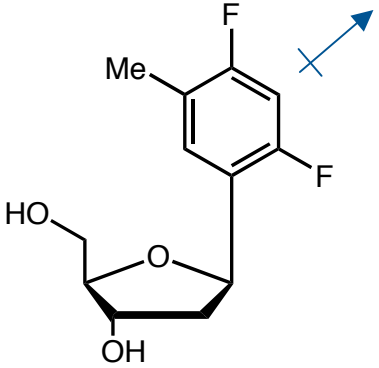
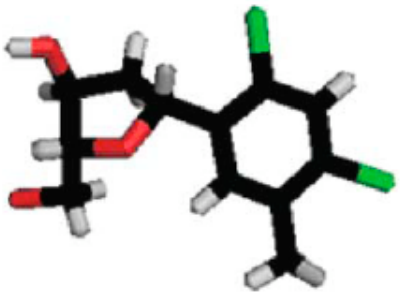
difluorotoluene is a nearly perfect isostere for thymine





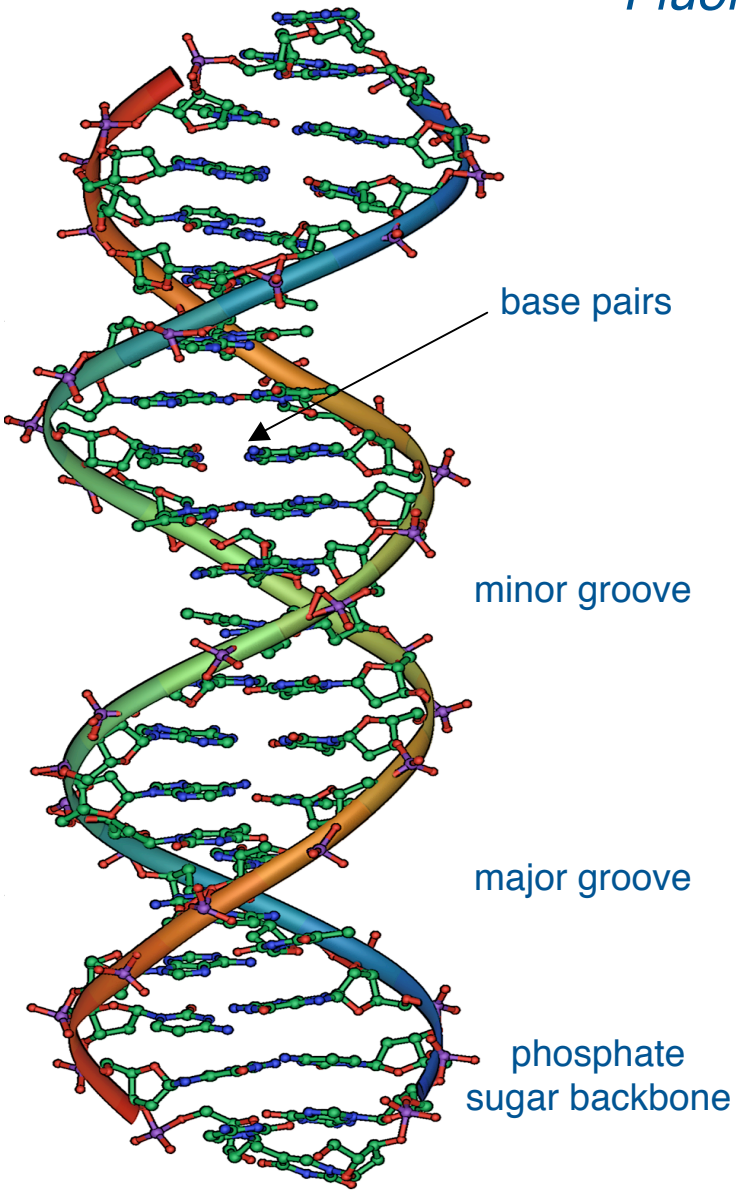




Probe function of Watson-Crick hydrogen bonds in DNA structure and replication.

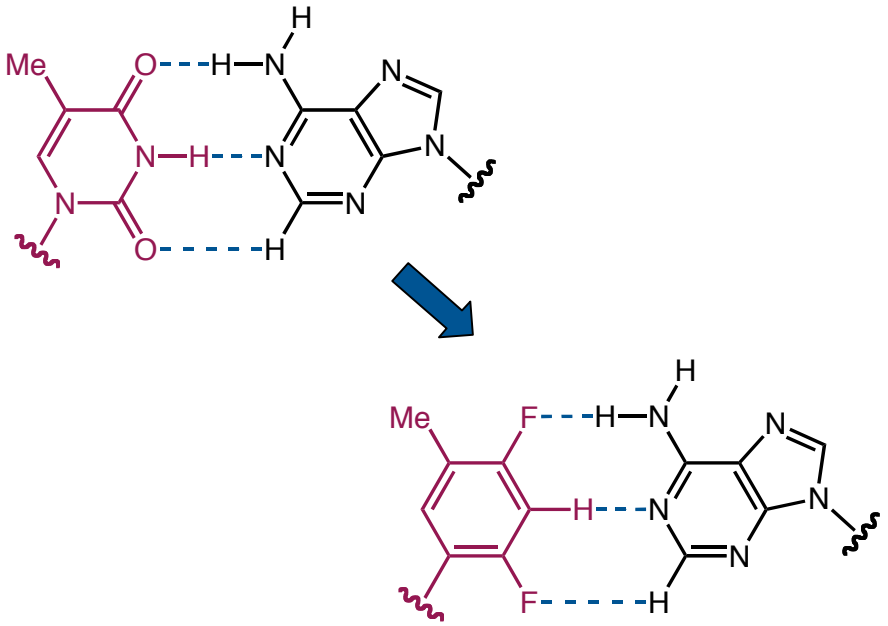
Kool, E. T.; Sintim, H. O. *Chem. Commun.* **2006**, 3663.

Fluorinated Isosteres



Factors may stabilize DNA structure:

- Watson-Crick hydrogen bonds
- Base stacking interactions
- Steric “fit” of base pairs
- Solvation of external backbone



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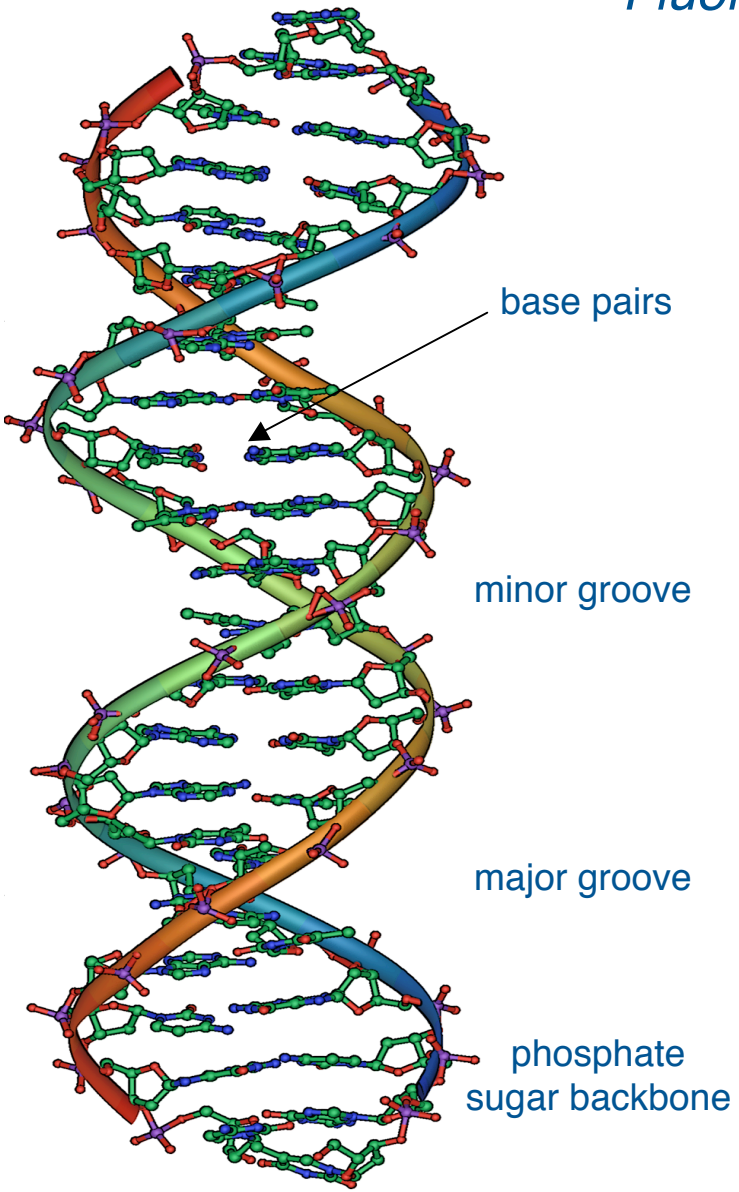

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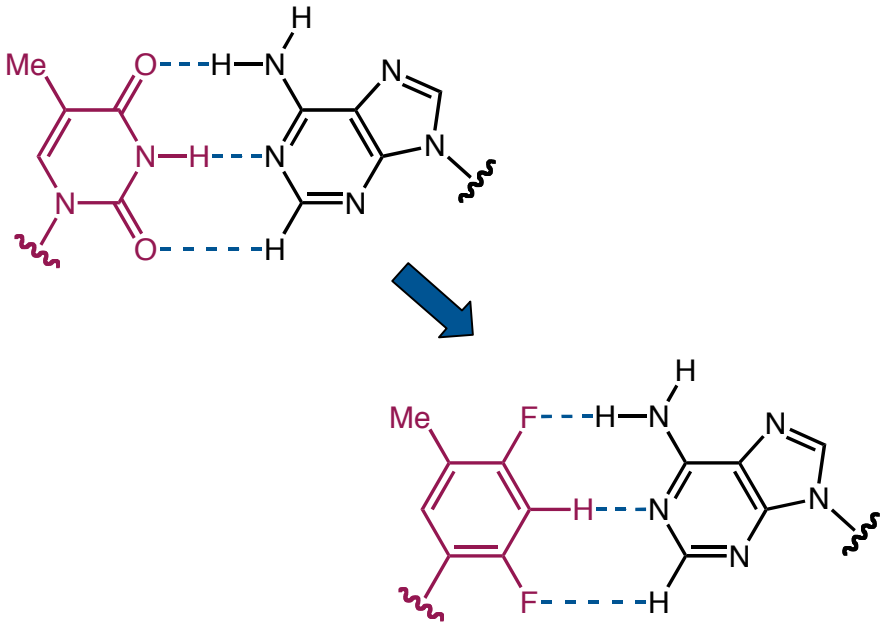
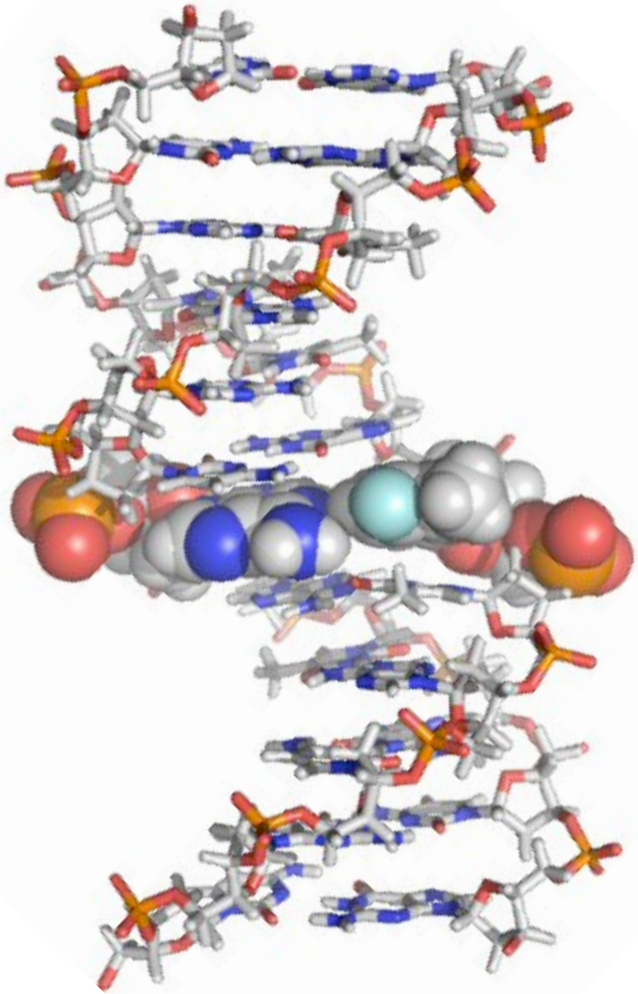
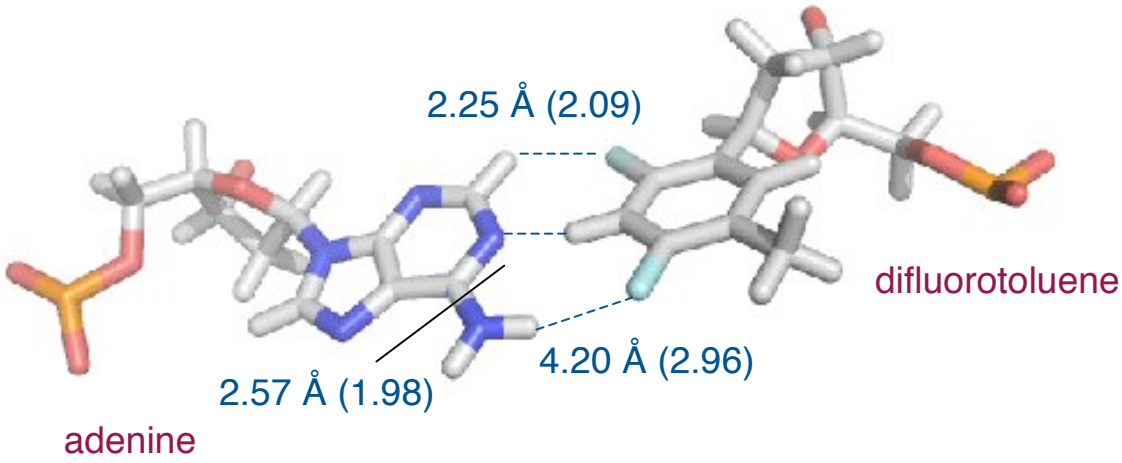


Fluorinated Isosteres

Substitution of difluorotoluene for thymine does not disrupt DNA structure





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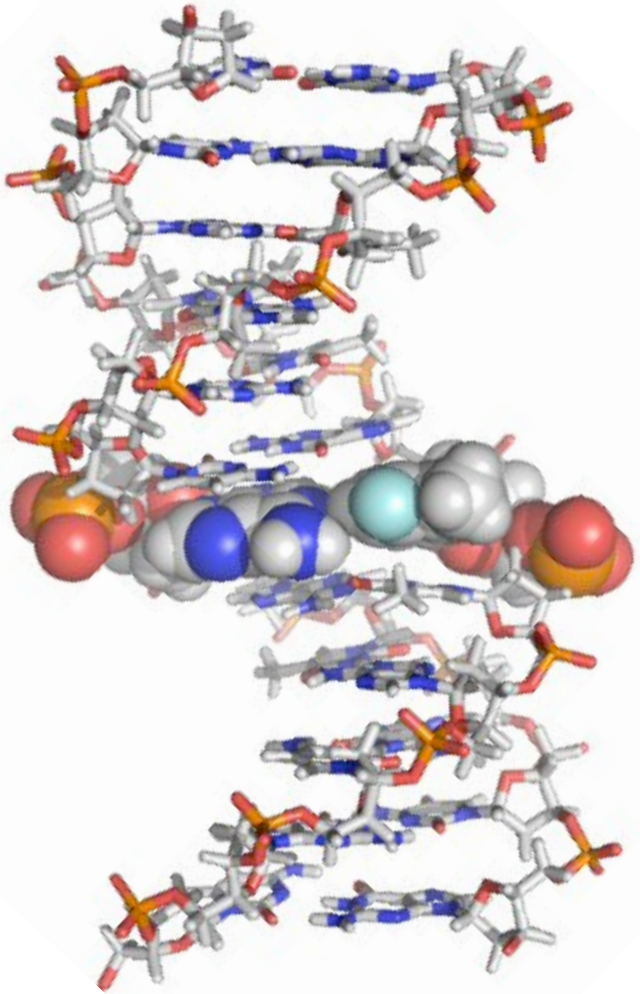

- Results in destabilized duplex (~3-4 kcal/mol)
- No selectivity for a natural base

	T_m(ºC)	ΔG(kcal)		T_m(ºC)	ΔG(kcal)
T.A	42.5	-9.7	F.A	26.2	-6.2
T.G	33.3	-7.5	F.G	23.6	-6.0
T.C	29.5	-6.6	F.C	23.7	-5.8
T.T	29.1	-6.8	F.T	24.0	-5.9

Watson-Crick hydrogen bonds contribute significantly to stabilization of DNA helix

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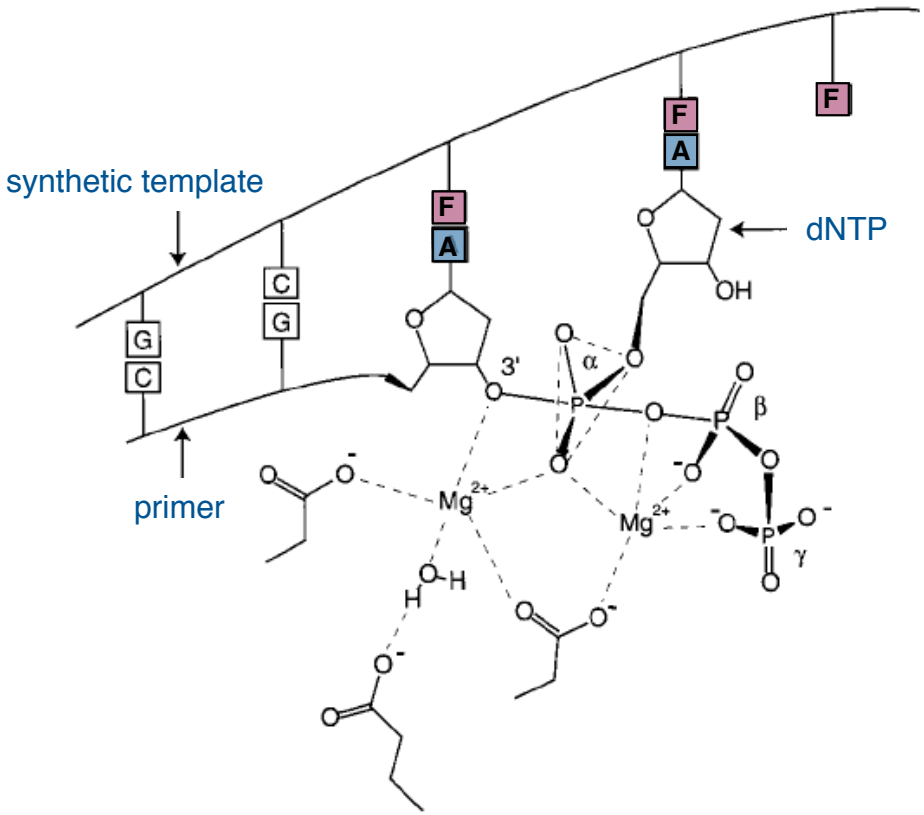
Fluorinated Isosteres

Are Watson-Crick hydrogen bonds required in DNA polymerase enzymes?



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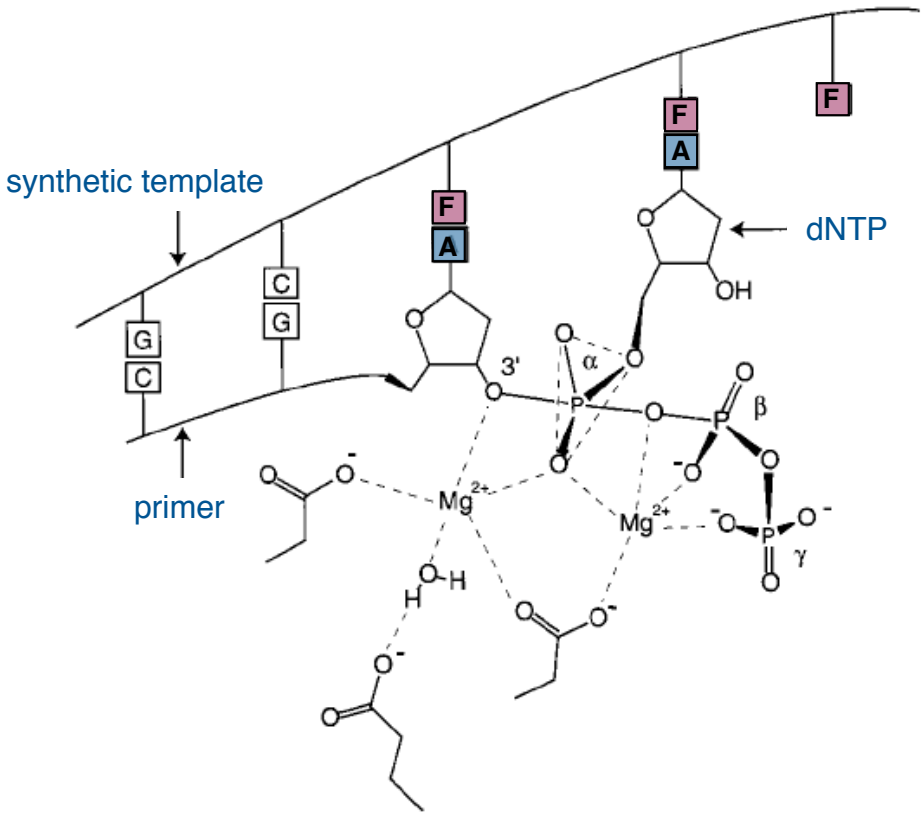
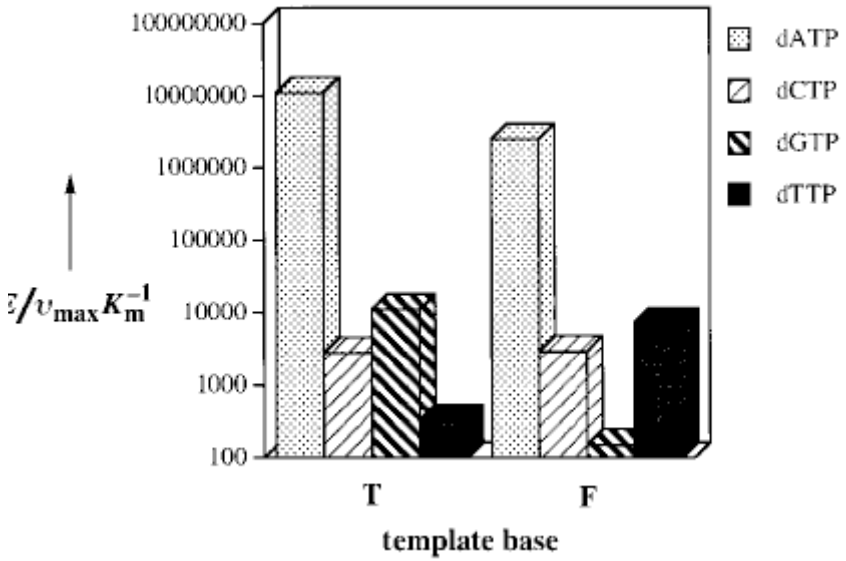


Difluorotoluene shows thymine-like polyermase activity

- similar efficiency of thymine
- similar selectivity for adenine



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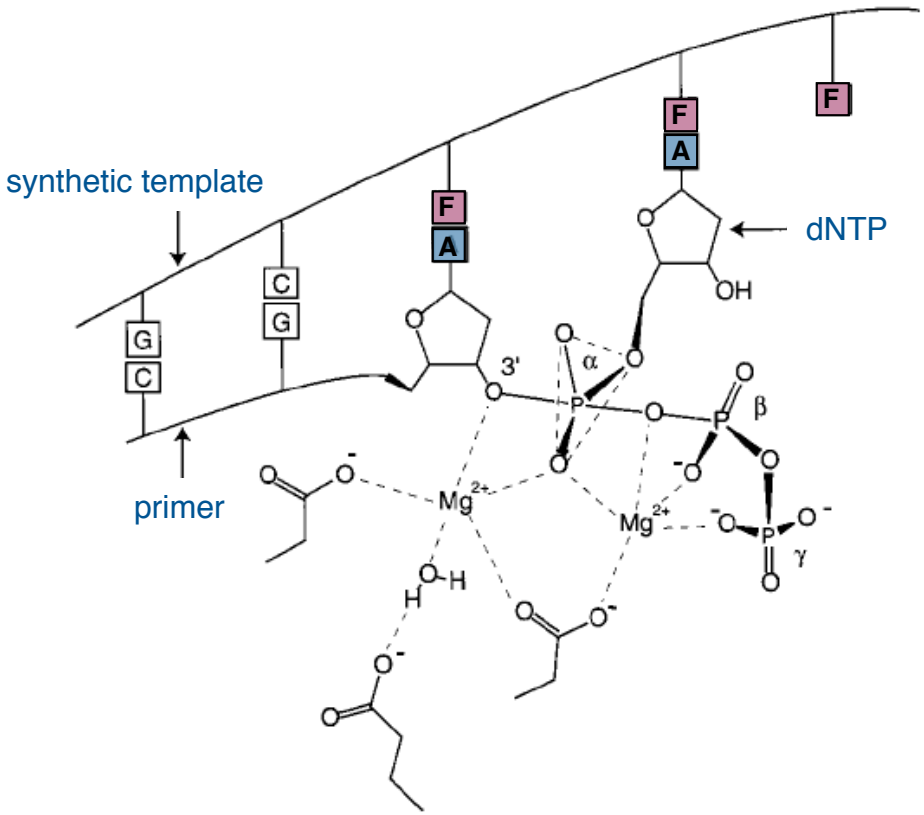

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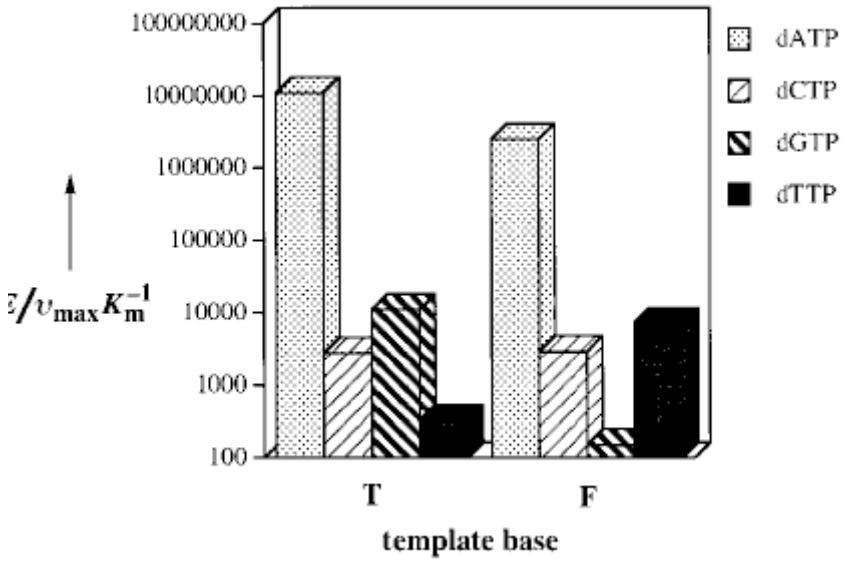

Could this be the A-Rule?

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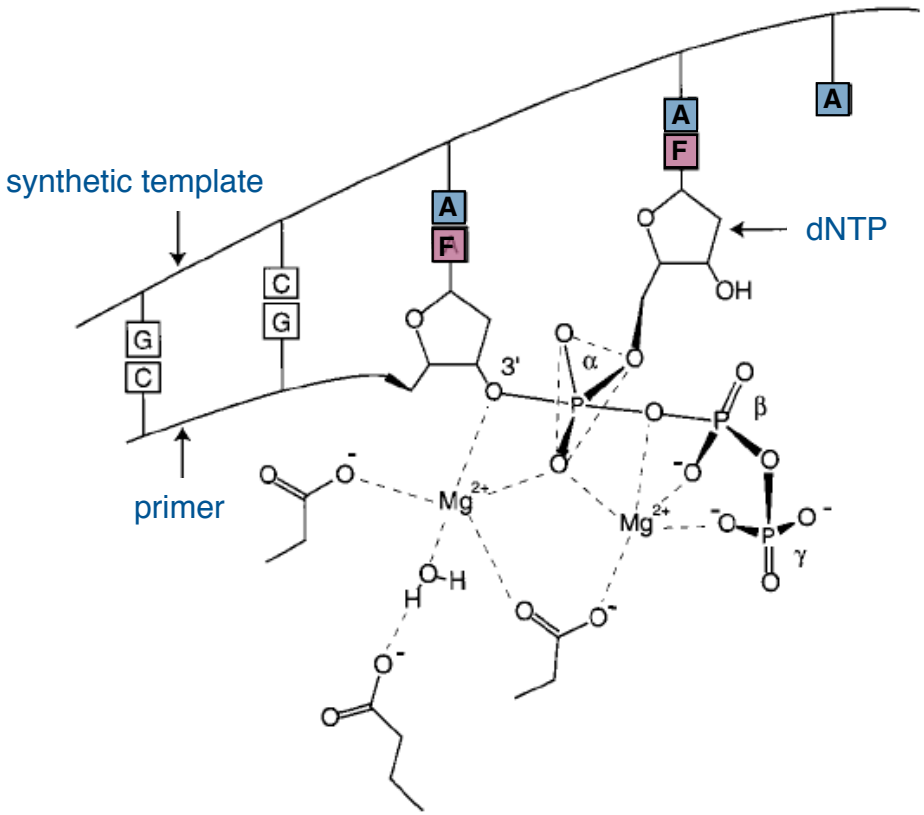

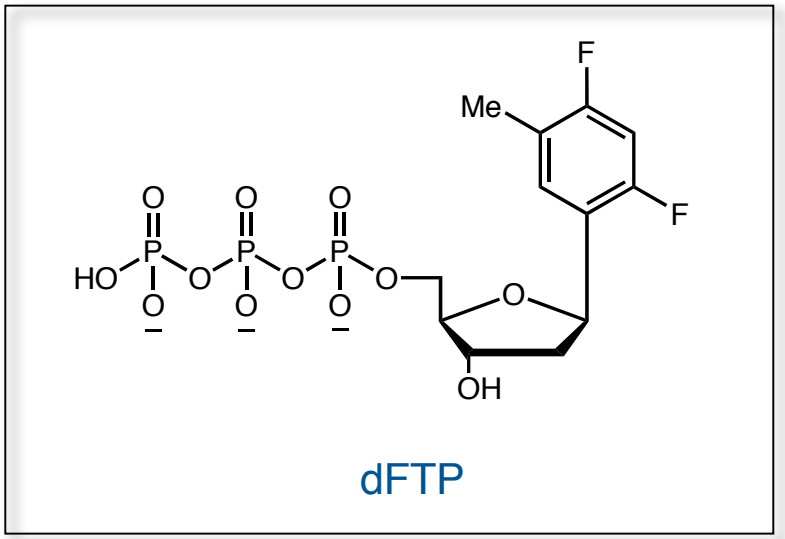

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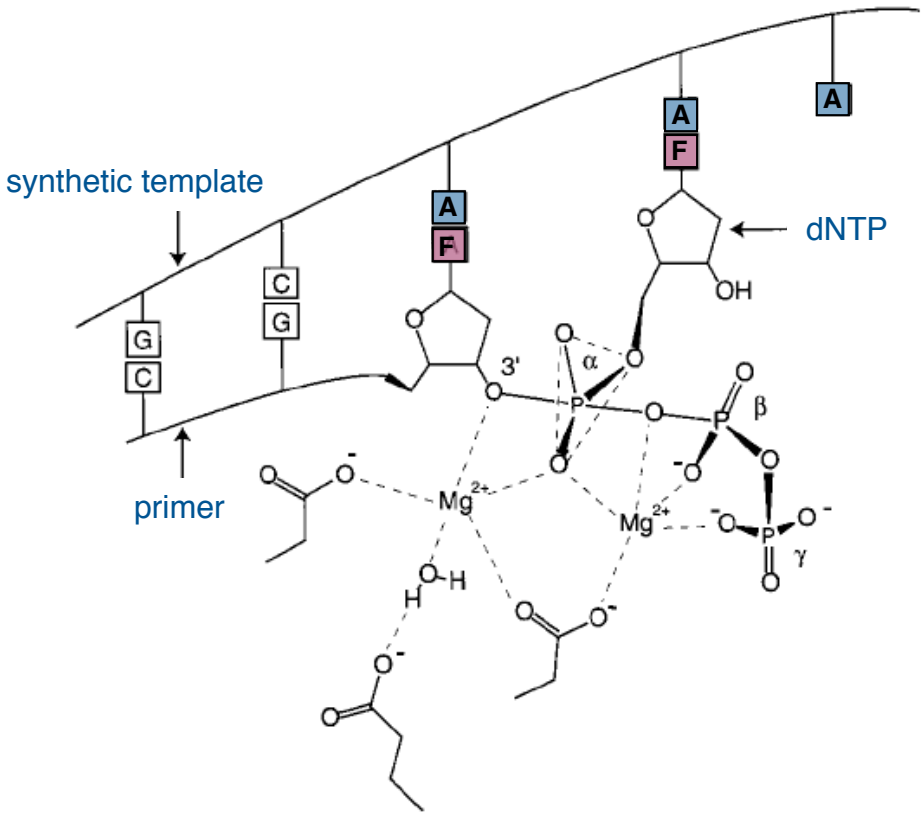
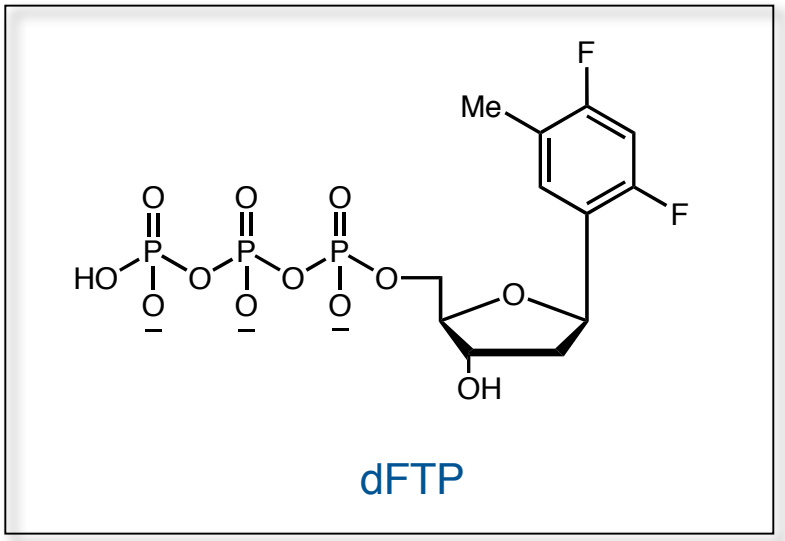


- dFTP successfully incorporated into the elongating primer
- F–A base pair synthesized with high efficiency and specificity



dFTP

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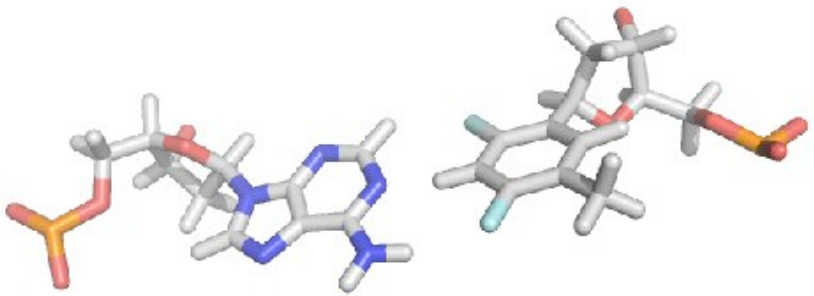
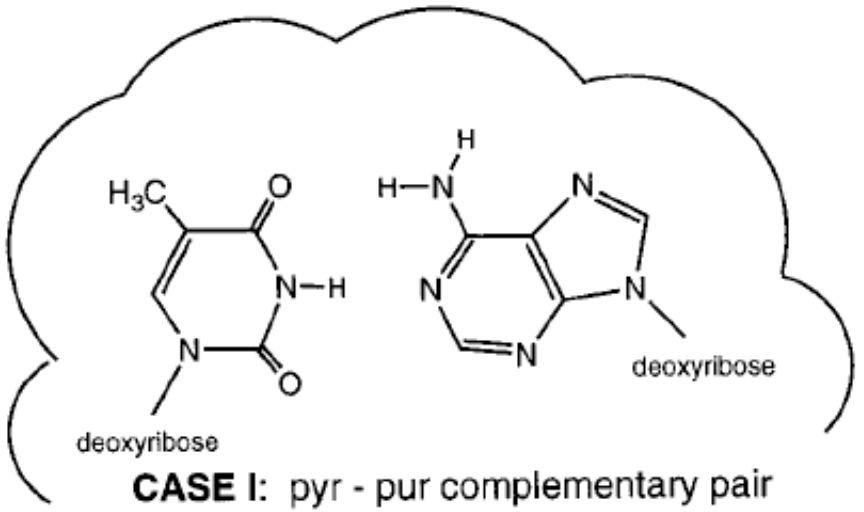
Fluorinated Isosteres

Conclusions:

- Replication of DNA base pairs can occur without Watson-Crick hydrogen bonds
- Steric effects are the main arbiters of DNA replication fidelity





Unanswered Question: Is this general over all classes of polymerases?

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Fluorinated Isosteres

Conclusions:

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- Steric effects are the main arbiters of DNA replication fidelity

The steric effect is being accepted as a key factor in replication fidelity.

L. Stryer, Biochemistry

4th Ed (1995): *"The likelihood of binding and making a phosphodiester bond is very low unless the incoming nucleotide forms a Watson-Crick base pair with the opposing nucleotide on the template."*

5th Ed (2001): *"The specificity of replication is dictated by hydrogen bonding and the complementarity of shape between bases."*

6th Ed (2007): *"The specificity of replication is dictated by complementarity of shape between bases."*

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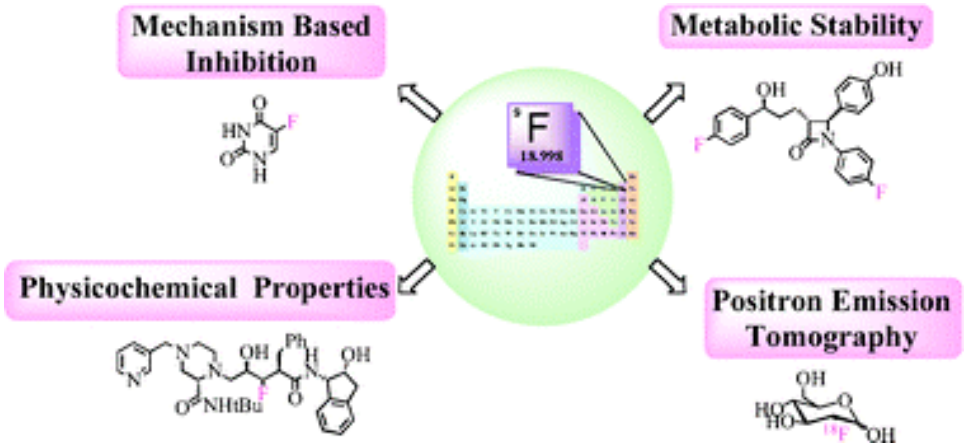
"*Fluorine leaves nobody indifferent; it inflames emotions be that affections or aversions. As a substituent, it is rarely boring, always good for a surprise, but often completely unpredictable.*"

- Manfred Schlosser

Angew. Chem. Int. Ed. **1998**, *110*, 1496.



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only sometimes

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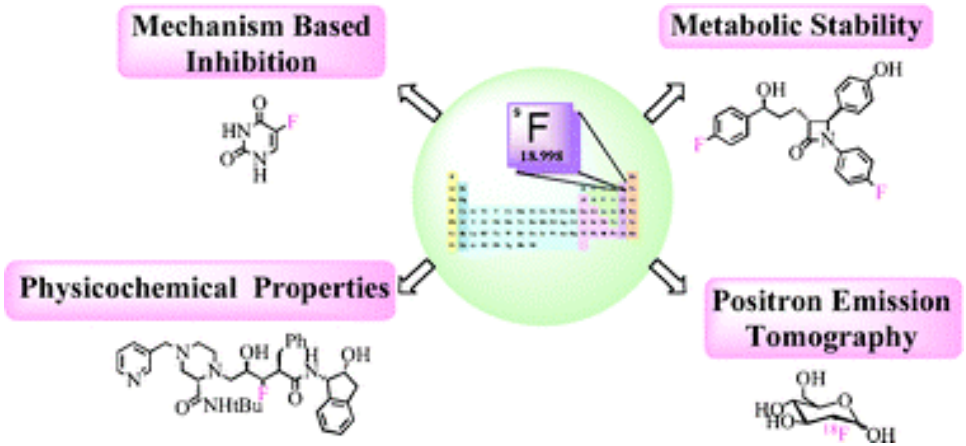

"Small atom with a big ego."