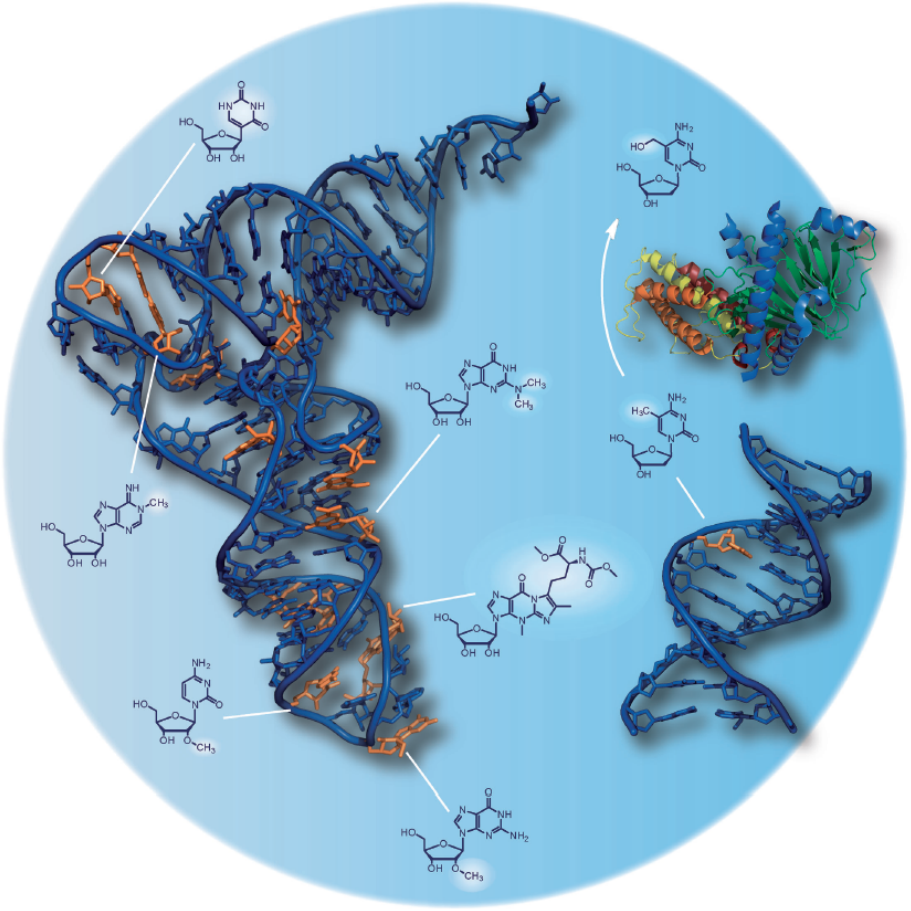
Noncanonical Nucleosides: Pseudouridine and 5-Methyldeoxycytidine



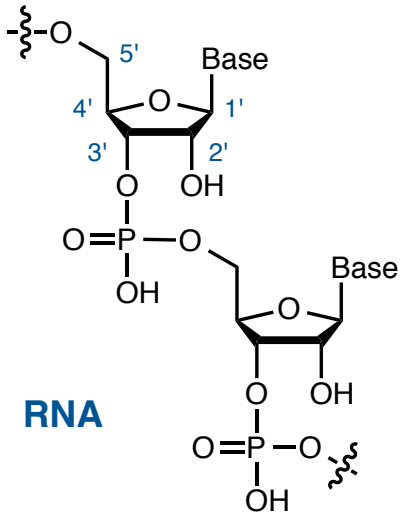
Valerie Shurtleff

MacMillan Group Meeting

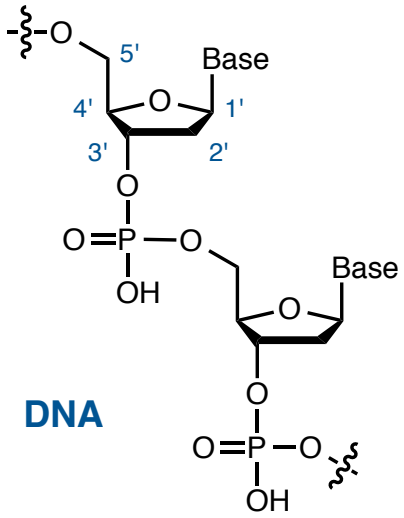
September 3, 2015

Nucleic Acids: Genetic Polymers

■ Ribonucleic acid and deoxyribonucleic acid

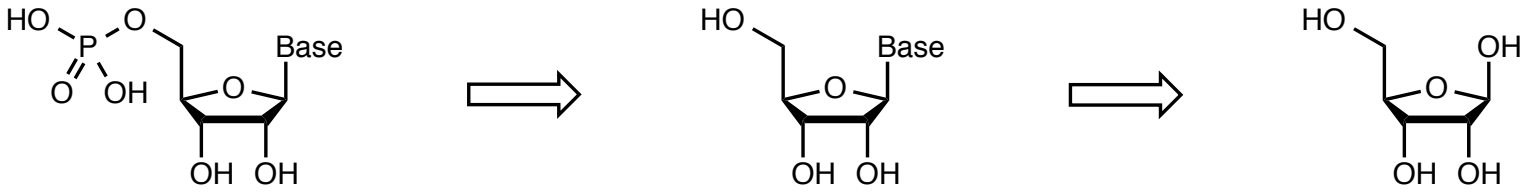


RNA



DNA

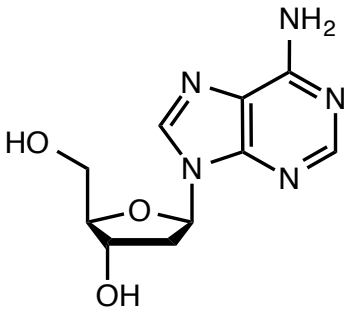
■ Monomeric building blocks



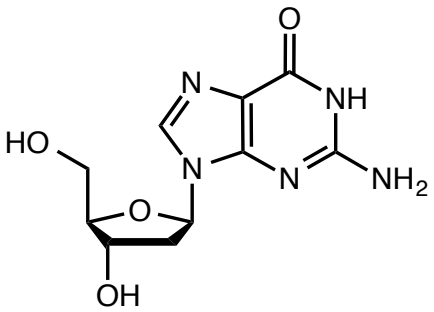
nucleotide **nucleoside** **ribose**

The Canonical Nucleosides

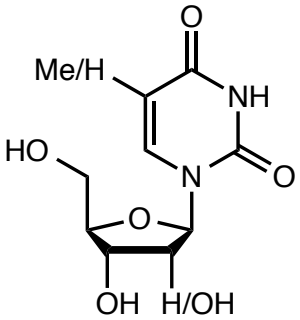
deoxyadenosine



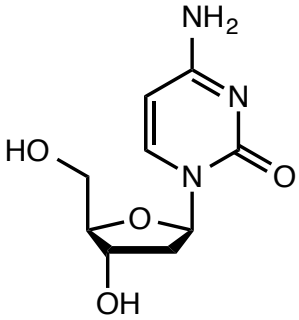
deoxyguanosine



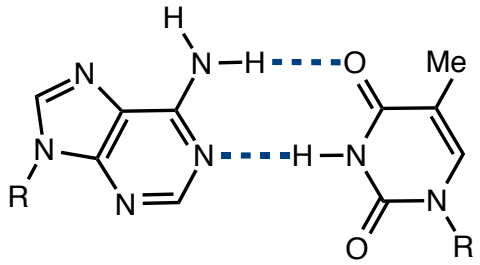
deoxythymidine/uridine



deoxycytidine

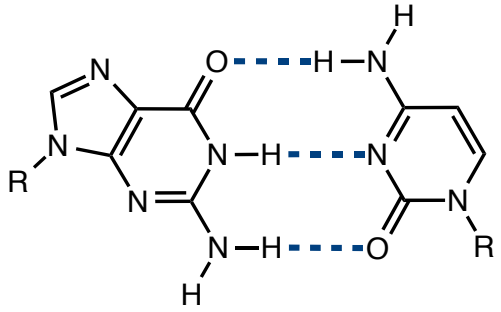


■ Watson–Crick base pairs



A adenine

T thymine



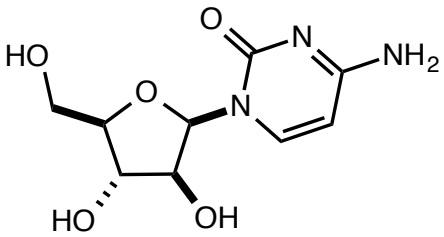
G guanine

C cytosine

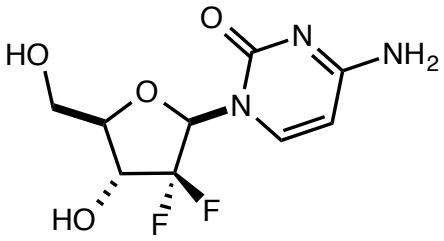
Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry*, 5th ed.; W.H. Freeman and Company: New York, 2008.

The Organic Chemist's View of Modified Nucleosides

Anticancer Agents
8 approved

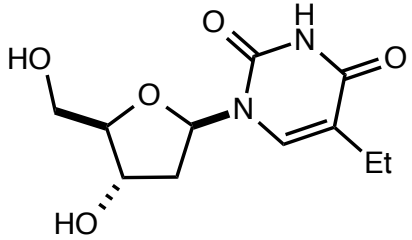


cytarabine
Pfizer, 1969

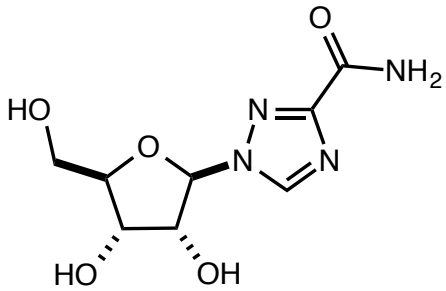


gemcitabine
Lilly, 1996

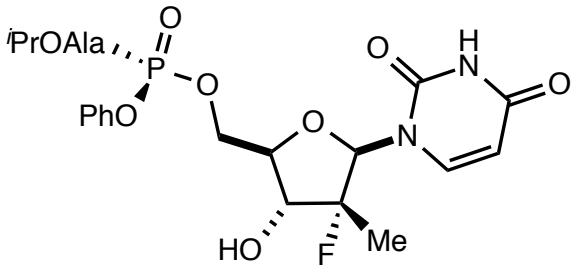
Antiviral Agents
12 approved



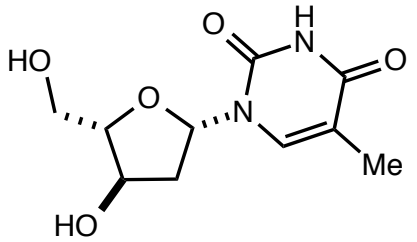
edoxudine
Upjohn, 1969



ribavirin
ICN, 1980



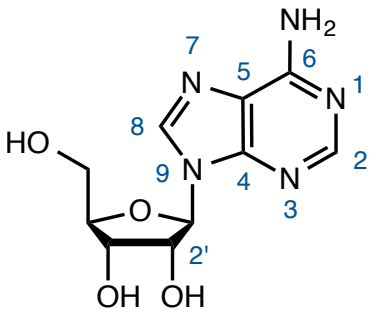
sofosbuvir
Gilead, 2013



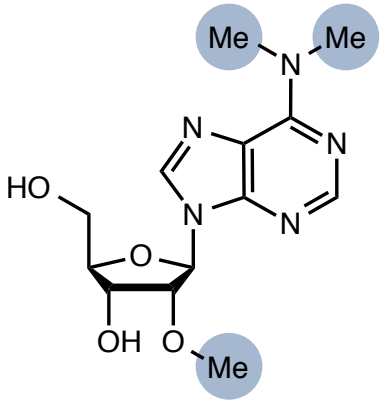
telbivudine
Novartis, 2006

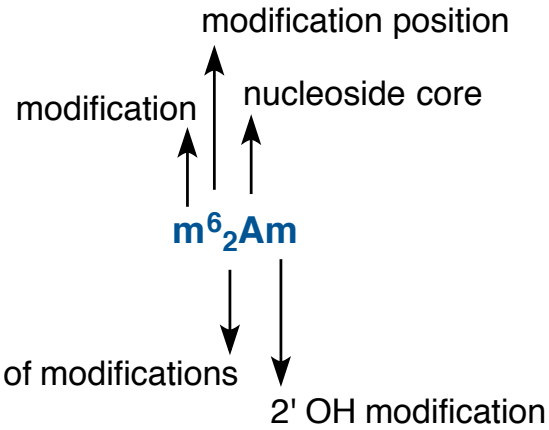
Naturally Occuring Noncanonical Nucleosides

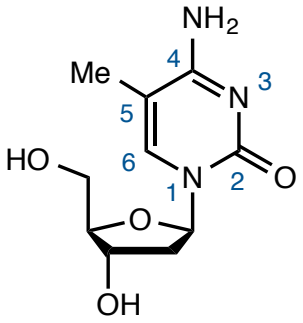
■ Nomenclature for describing nucleoside modifications



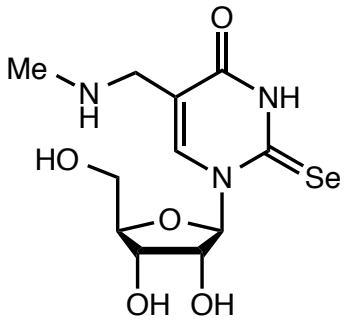
A





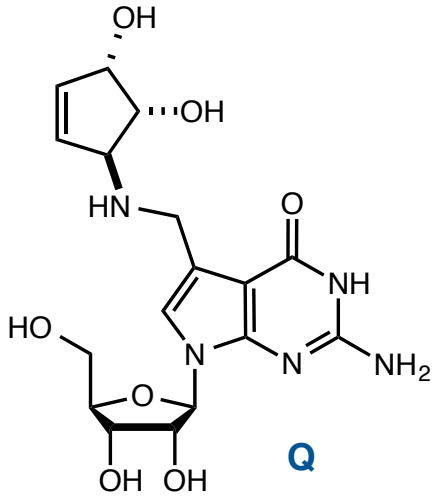


m^{5}dC



mnm^{5}se^{2}U

hypermodified nucleosides



Q

Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Nucleoside Modifications in RNA

many types of RNA with many functions ⟹ **many nucleoside modifications**

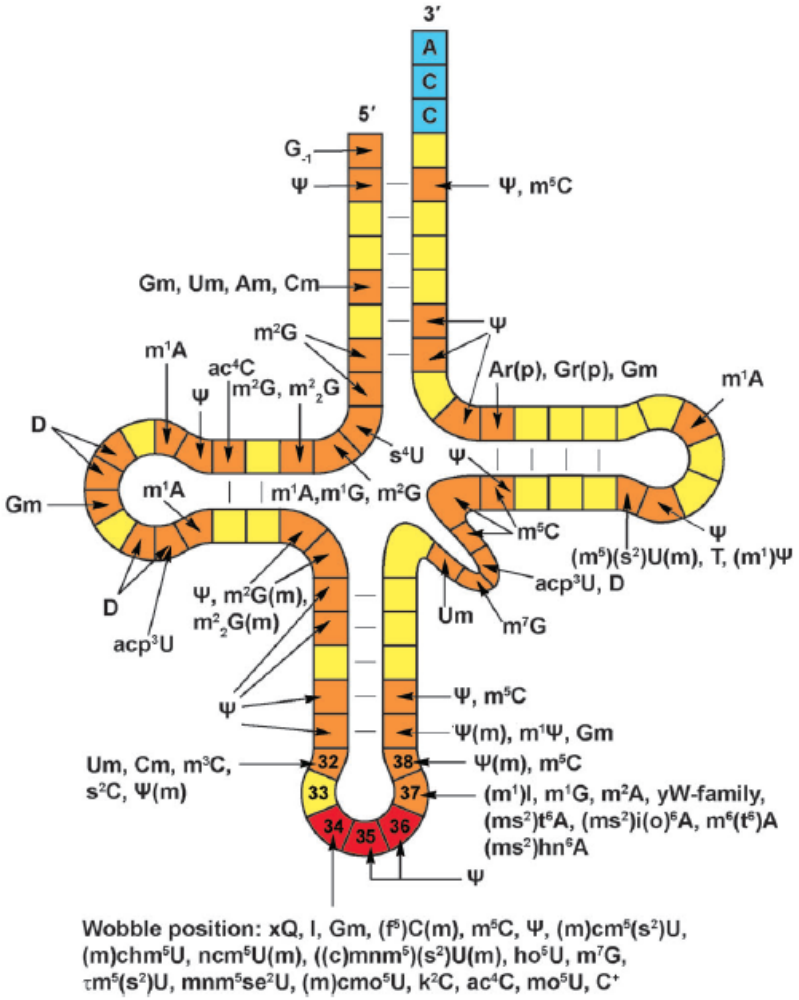
over 100 modified nucleosides known in RNA

Abbreviation	Full name	Function
tRNA	transfer RNA	mRNA decoding amino acid carrier
mRNA	messenger RNA	protein coding message
rRNA	ribosomal RNA	nucleic acid component of the ribosome
sncRNA	small noncoding RNA	
miRNA	micro RNA	regulating gene expression
piRNA	piwi-interacting RNA	regulating gene expression
siRNA	short interfering RNA	regulating gene expression
snRNA	small nuclear RNA	involved e.g. in intron splicing
snoRNA	small nucleolar RNA	involved in RNA modification
lncRNA	long noncoding RNA	nonprotein coding transcripts with regulatory or unknown function

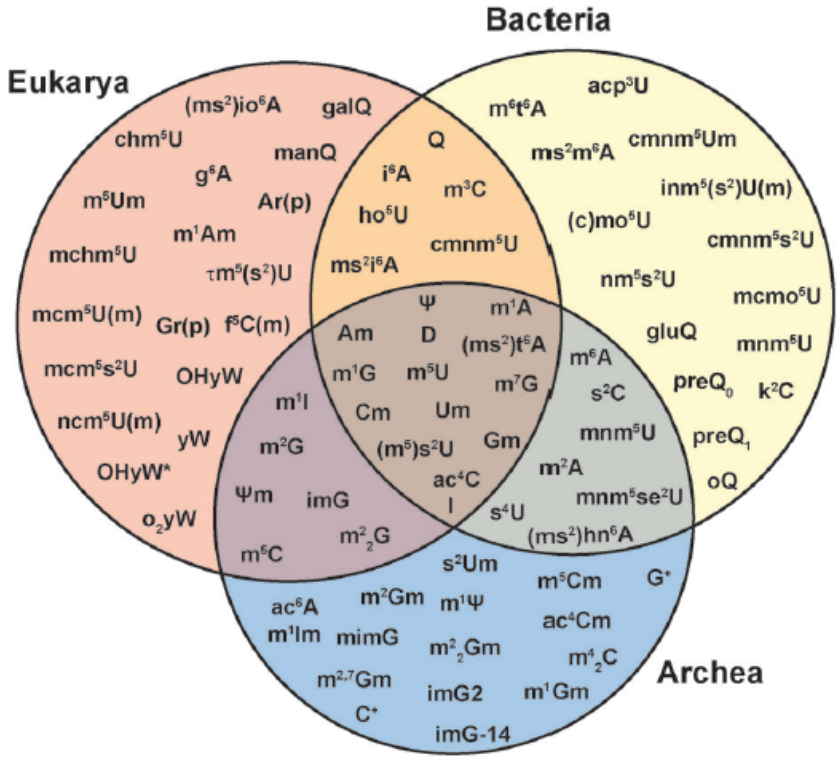
Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Nucleoside Modifications in RNA

sites of modification in a tRNA

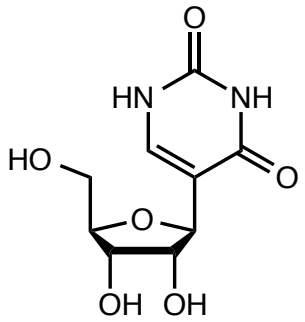


modified nucleosides in different domains



Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Pseudouridine: a C-Nucleoside Derived from Uridine

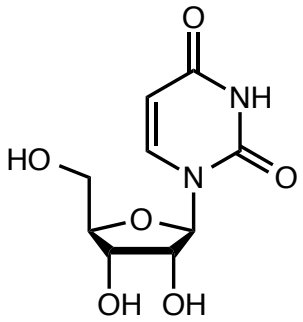


pseudouridine (Ψ)

- First modified nucleoside discovered in RNA
- Most prevalent of all natural modified nucleosides
- Present in almost all classes of RNA
- The "fifth nucleoside" in RNA

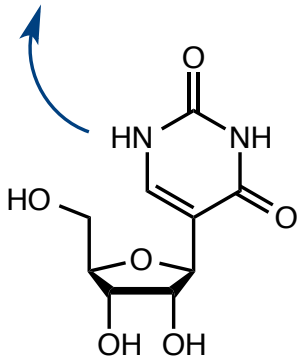
Charette, M.; Gray, M. W. *IUBMB Life* **2000**, *49*, 341.

The Function of Pseudouridine in RNA

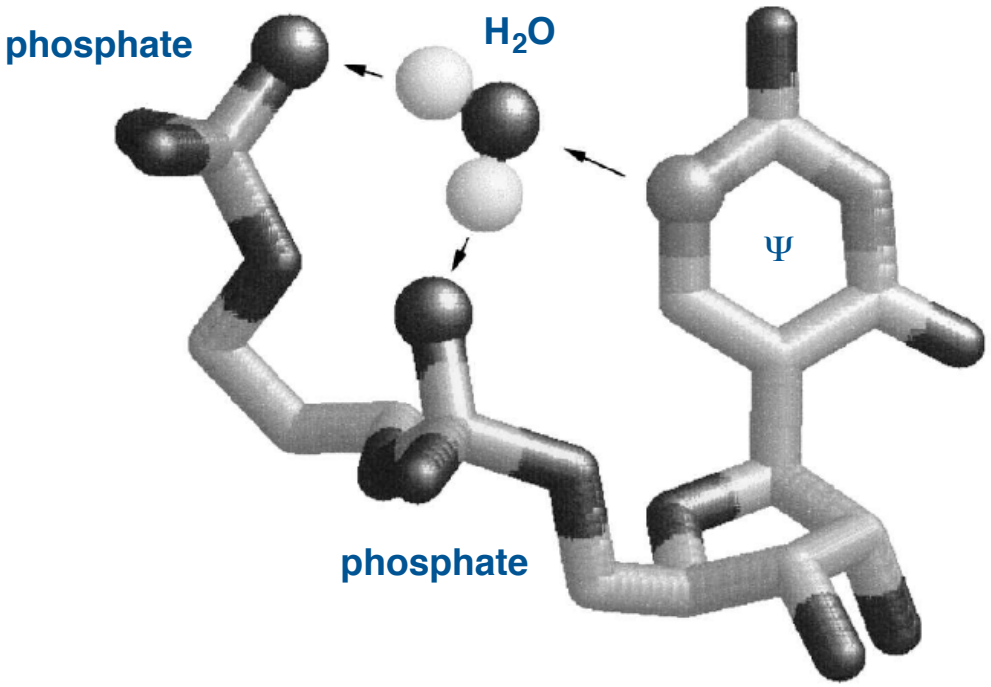


uridine (U)

additional H-bonding site



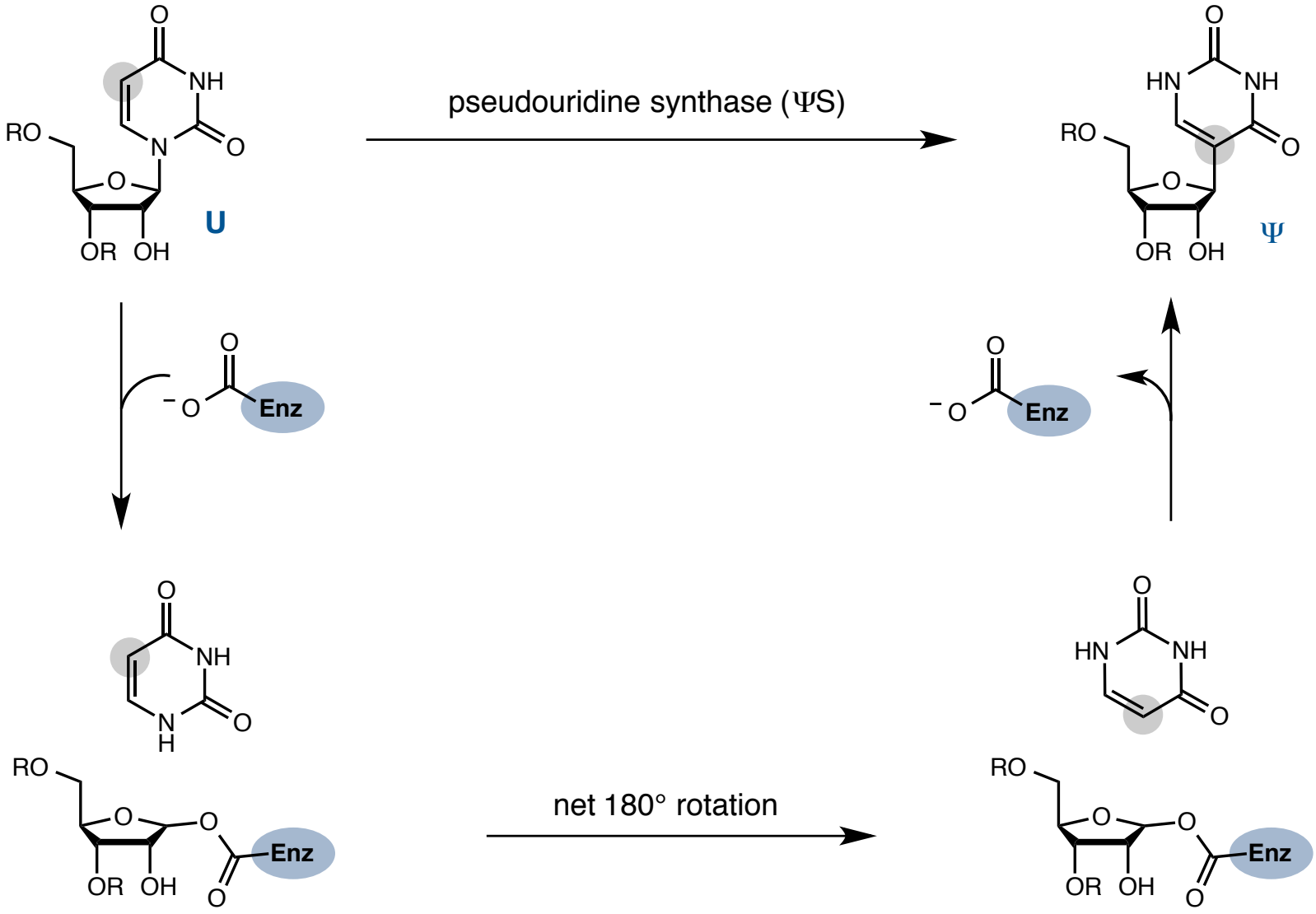
pseudouridine (Ψ)



- Unique H-bonding ability – coordination of water
- Ψ can serve to rigidify surrounding structure
- May stabilize complex 3D structure of RNA

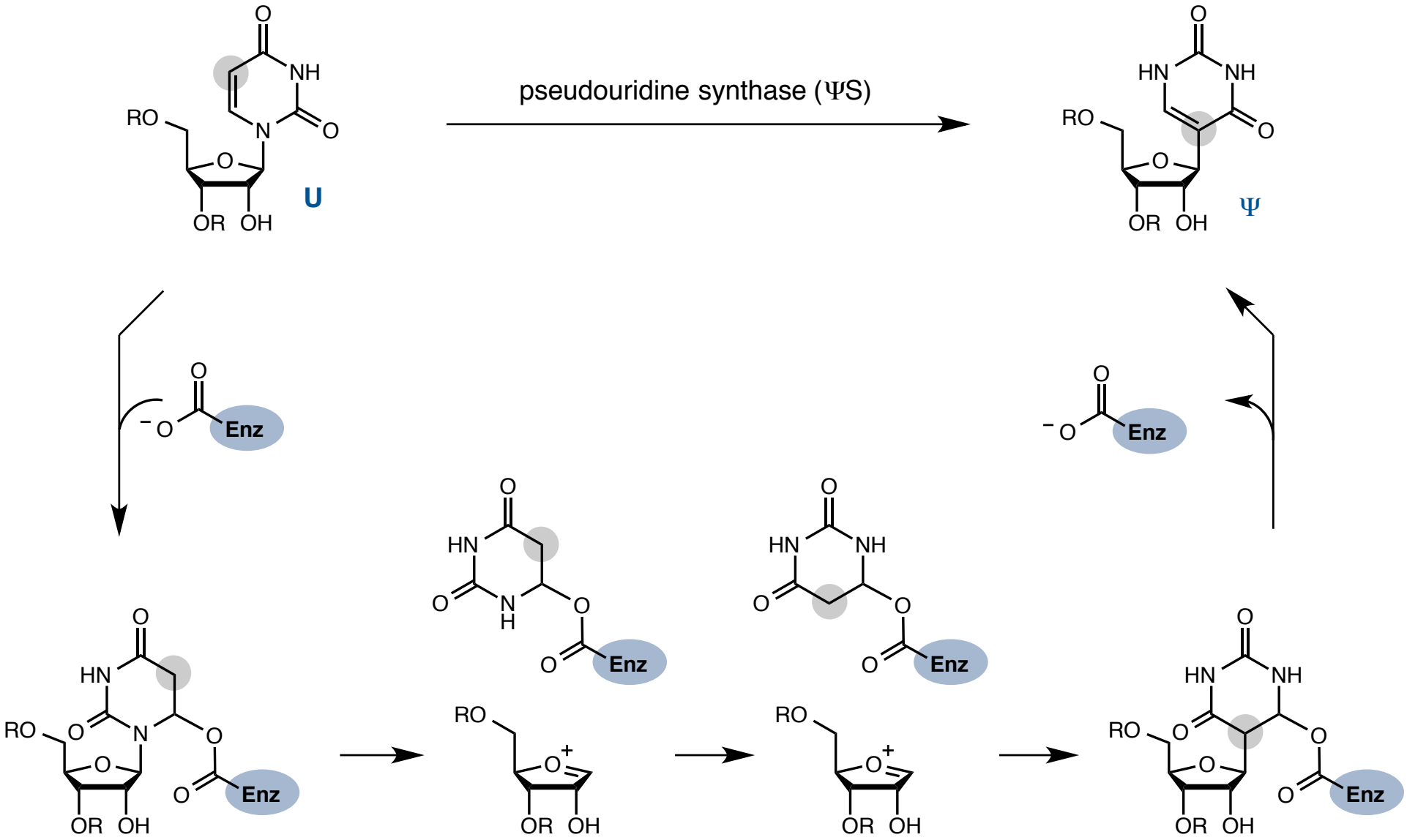
Charette, M.; Gray, M. W. *IUBMB Life* **2000**, *49*, 341.

"Acylal Mechanism" for the Biosynthesis of Pseudouridine



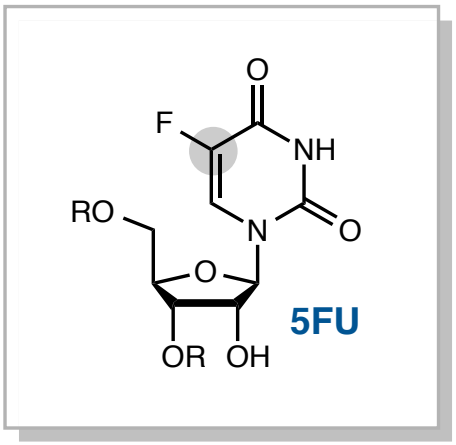
Gu. X.; Liu, Y.; Santi, D. V. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14270.

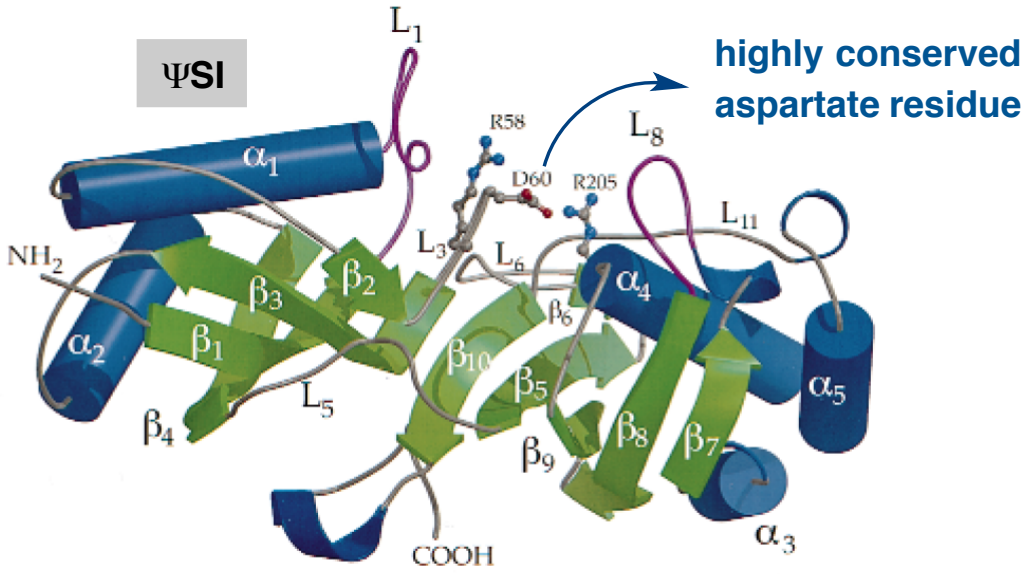
"Michael Mechanism" for the Biosynthesis of Pseudouridine

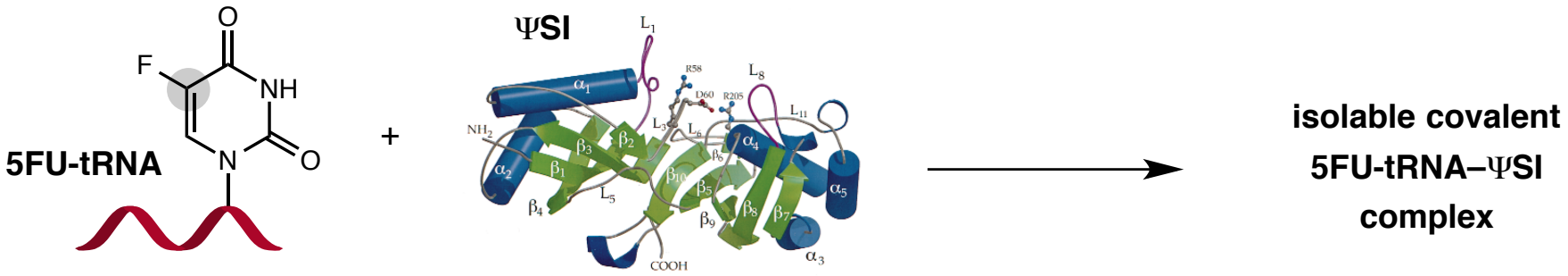


Gu. X.; Liu, Y.; Santi, D. V. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14270.

Evidence for the "Michael Mechanism"







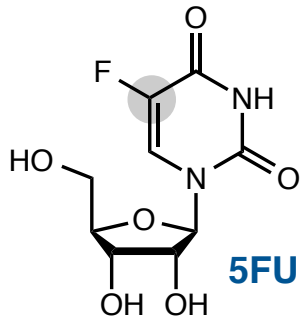
What is the identity of the 5FU-tRNA–ΨSI covalent complex?

Foster, P. G.; Huang, L.; Santi, D. V.; Stroud, R. M. *Nat. Struct. Biol.* **2000**, *7*, 23.

Gu. X.; Liu, Y.; Santi, D. V. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14270.

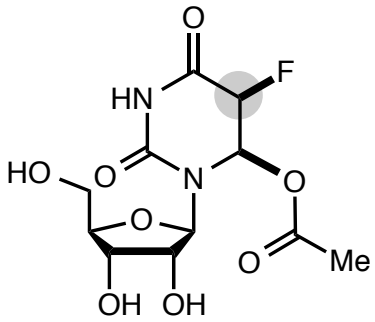
Evidence for the "Michael Mechanism"

model system



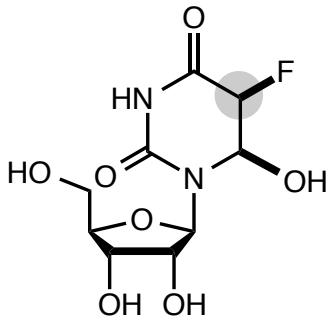
5FU

AcOH



"fairly stable" in H_2O at rt

H_2O, Δ

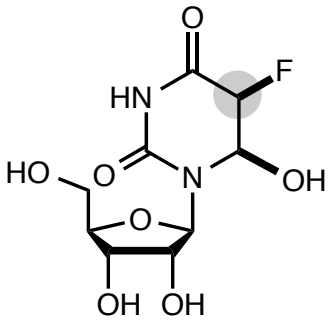


retention of configuration

---

isolable covalent 5FU-tRNA–ΨSI complex

digestion to nucleosides



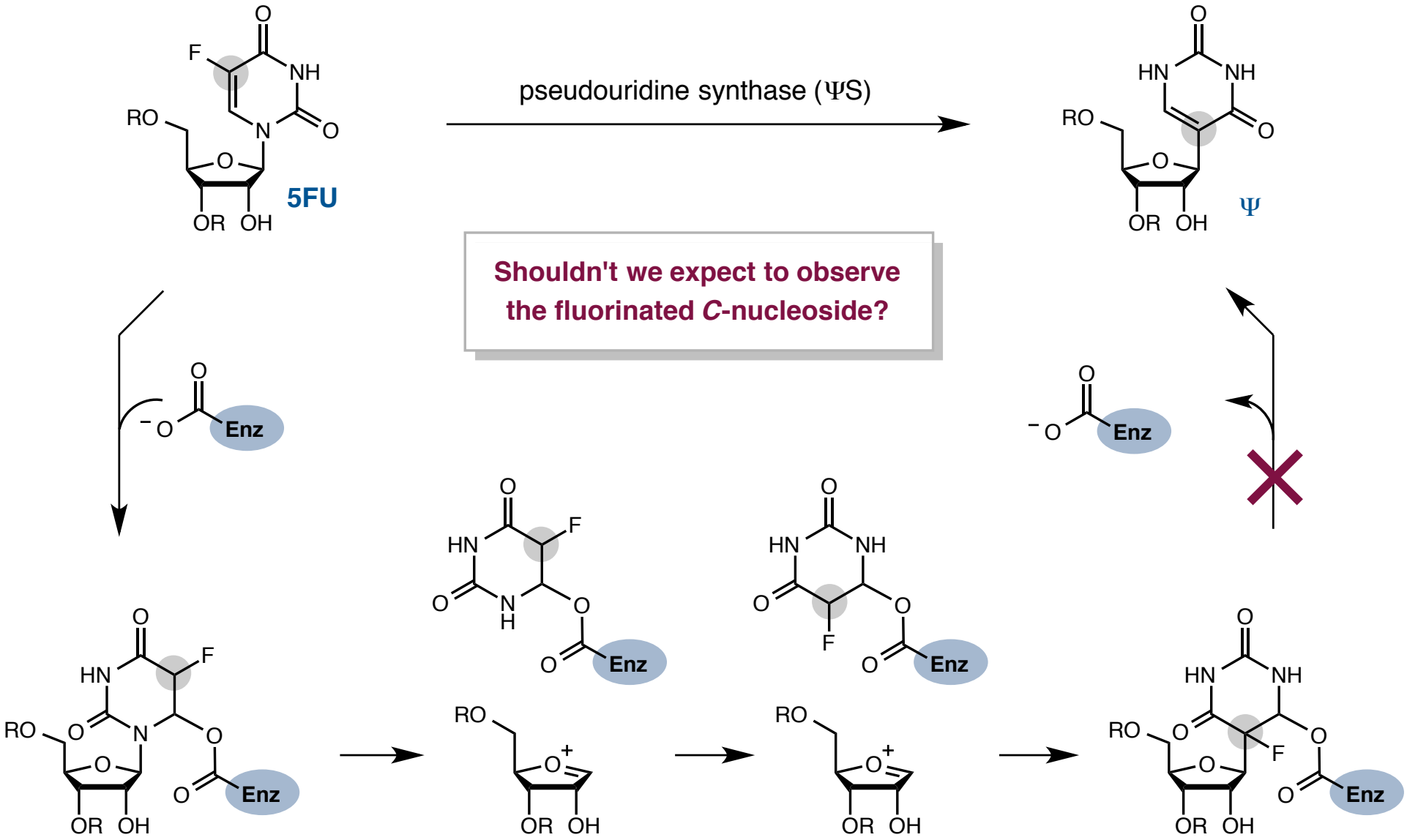
proposed product based on HPLC retention time

Proposal:

- **Asp-60 attacks C6 (1,4-addition)**
- **protonation from opposite face**
- **ester hydrolysis liberates pdt**

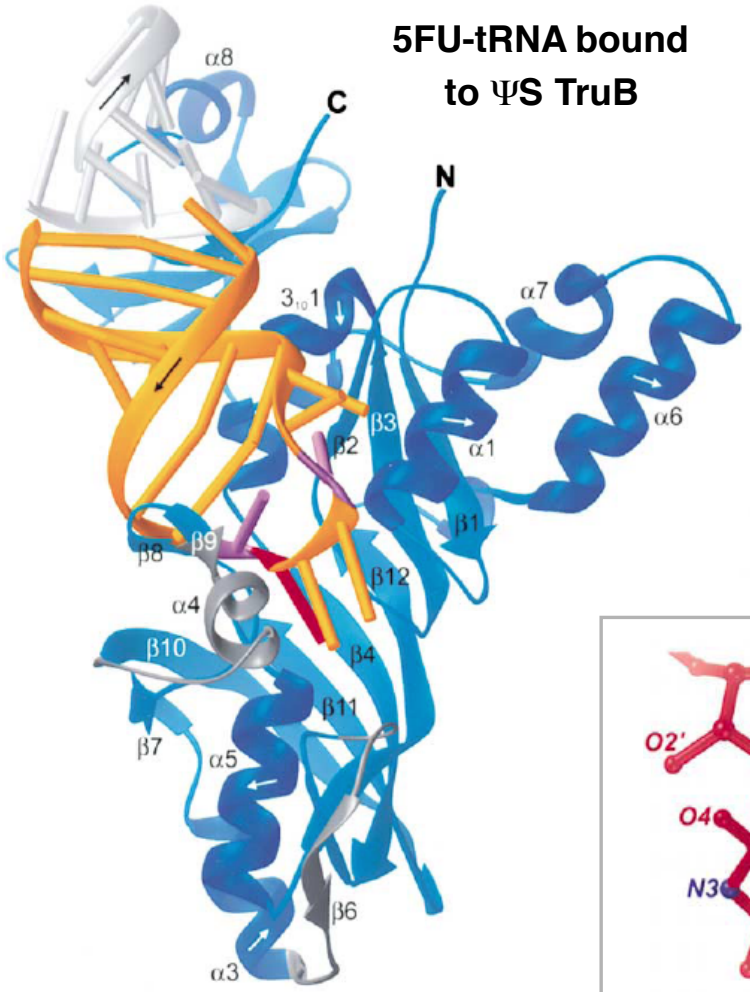
Gu. X.; Liu, Y.; Santi, D. V. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14270.

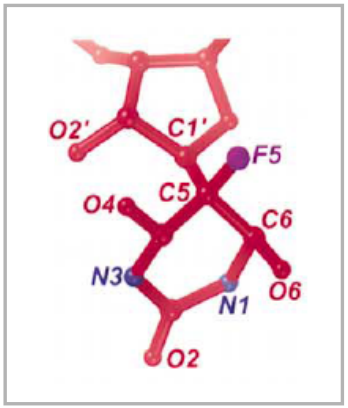
"Michael Mechanism" for the Biosynthesis of Pseudouridine



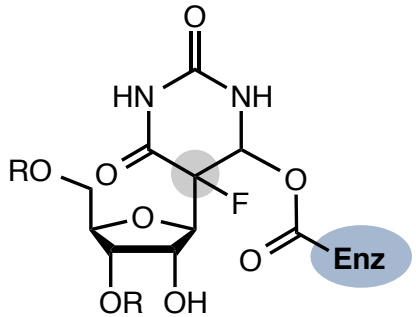
Gu. X.; Liu, Y.; Santi, D. V. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14270.

Rearrangement of 5FU by Pseudouridine Synthesis

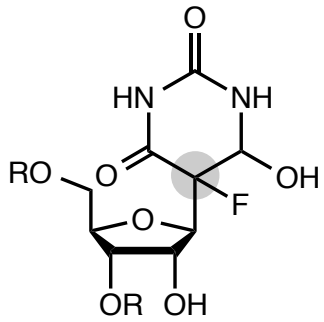




≡



ester
hydrolysis?



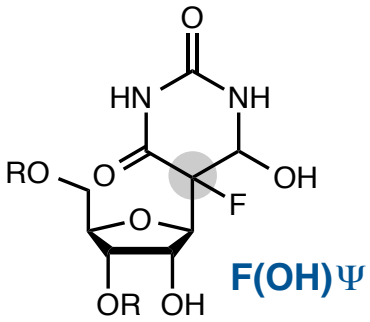
previously proposed product may have been misidentified

Hoang, C.; Ferré-D'Amaré, A. R. *Cell* **2001**, *107*, 929.

Revisiting the "Michael Mechanism"

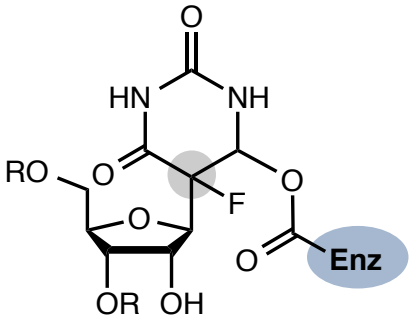
formation of F(OH)Ψ consistent with proposed Michael mech.

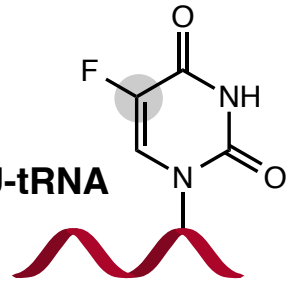
(not definitive proof – multiple pathways can be envisioned)



F(OH)Ψ

ester hydrolysis?



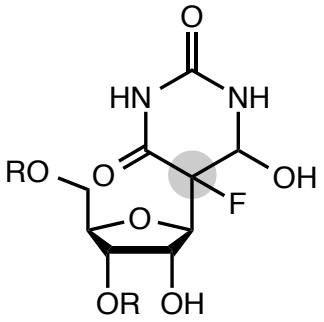


5FU-tRNA

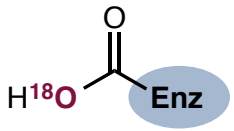
1. incubation with TruB in 50% $^{18}OH_2$ buffer

2. digestion

expected products from ester hydrolysis



^{18}O content reflects buffer content

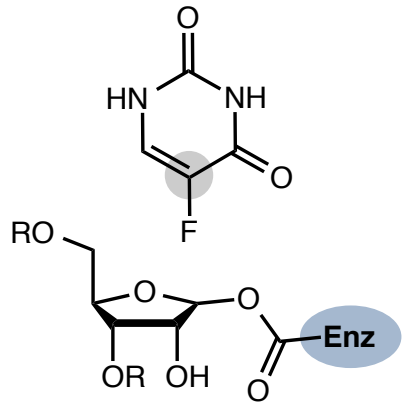


not observed

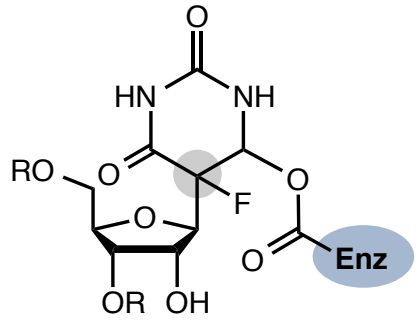
Spedaliere, C. J.; Ginter, J. M.; Johnston, M. V.; Mueller, E. G. *J. Am. Chem. Soc.* **2004**, *126*, 12758.

Possible Pathways for Formation of Observed Rearrangement Product

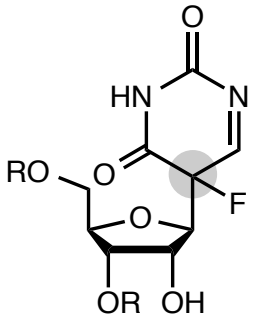
both mechanisms are consistent with data



via acylal mechanism

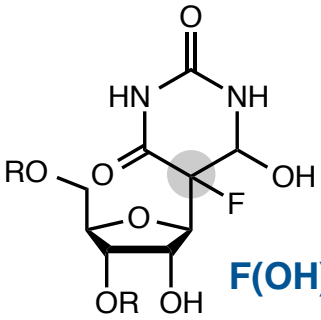


via Michael mechanism



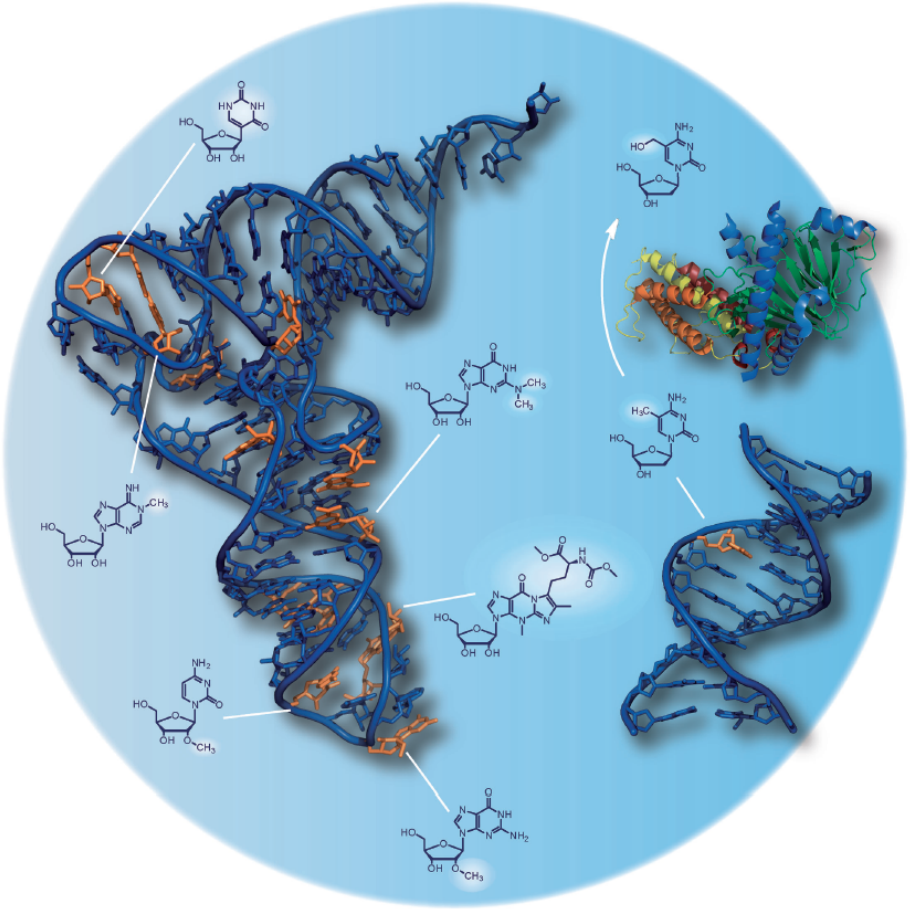
product release and
hydration in solution

Asp60-mediated hydration
(general base catalysis)



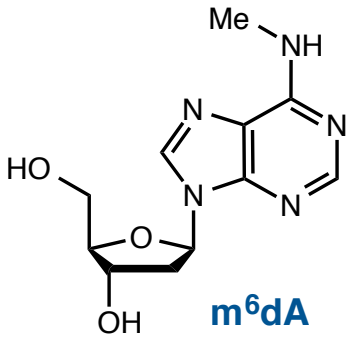
F(OH)Ψ

Spedaliere, C. J.; Ginter, J. M.; Johnston, M. V.; Mueller, E. G. *J. Am. Chem. Soc.* **2004**, *126*, 12758.

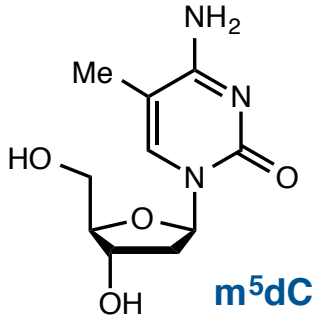


Noncanonical Nucleosides in DNA

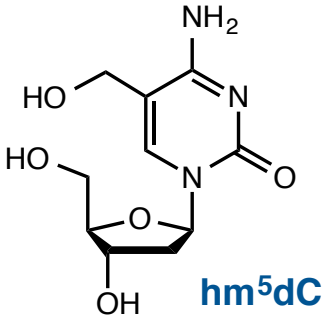
DNA: carrier of genetic information, critical but limited function ⟹ **few modifications observed**



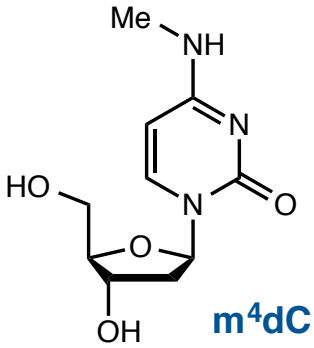
m^6dA



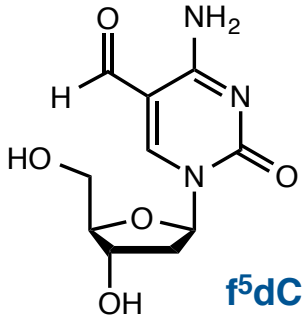
m^5dC (mC)



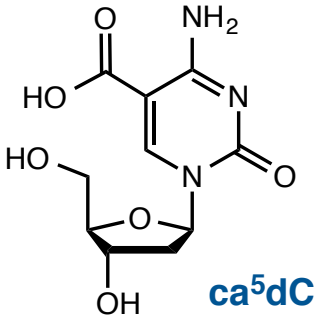
hm^5dC (hmC)



m^4dC



f^5dC (fC)



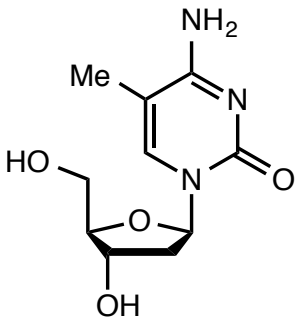
ca^5dC (caC)

various functions

epigenetic bases

Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Importance of 5-Methyldeoxycytidine

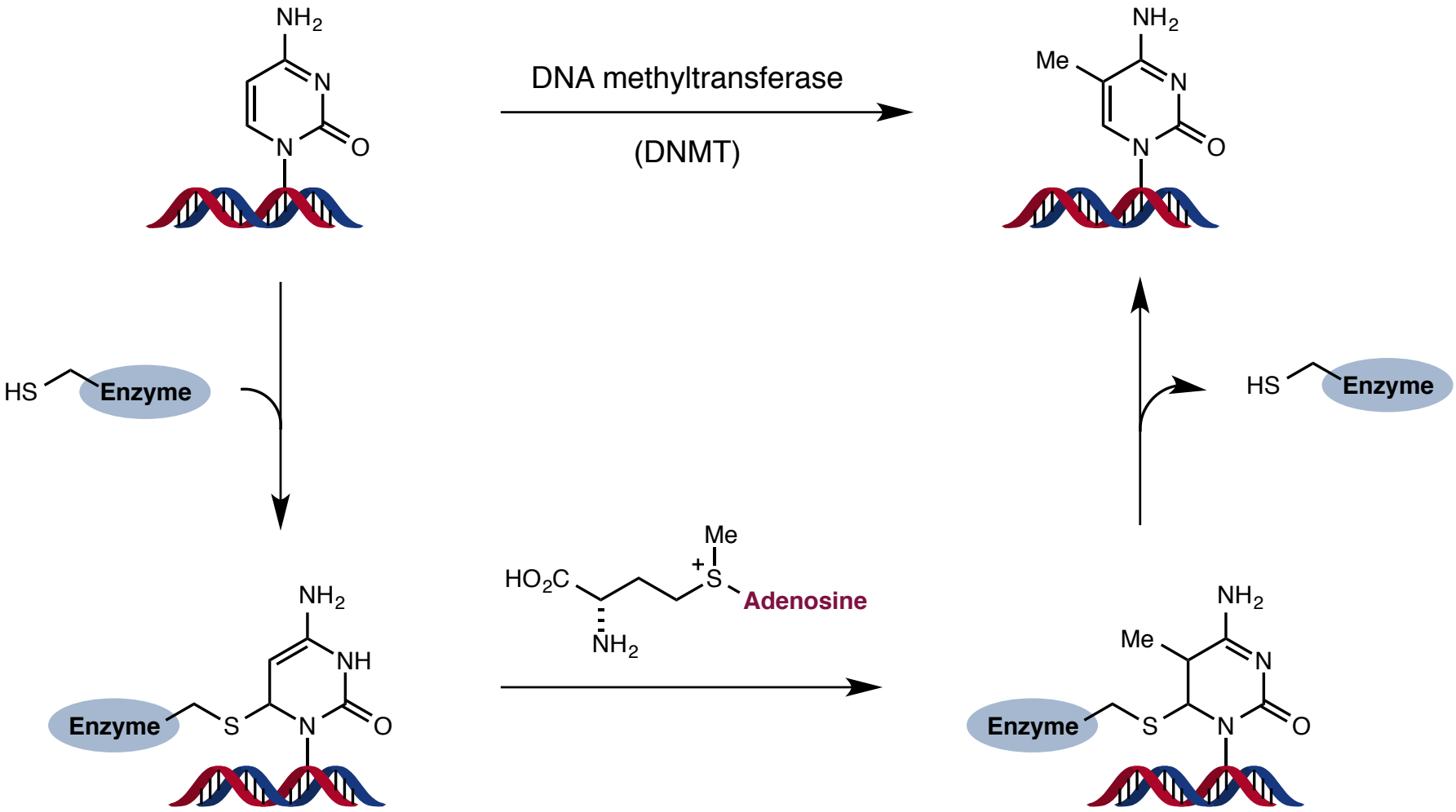


m^5dC (mC)

- Some plants: up to 25% methylated cytosine
- Human genome: ~5% methylated cytosine (up to 70% at CpG sites, often associated with promoter regions)

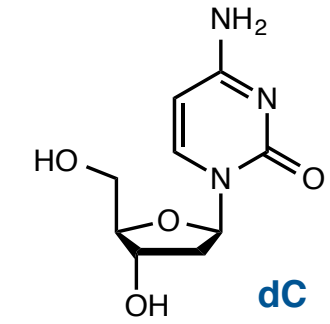
Jurkowski, T. P.; Jeltsch, A. *ChemBioChem* **2011**, *12*, 2543.

Biosynthesis of 5-Methyldeoxycytidine



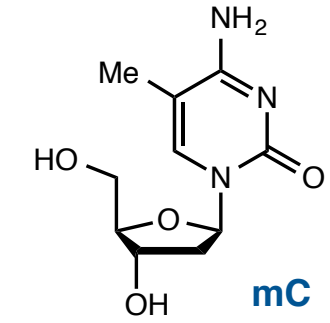
Jeltsch, A. *ChemBioChem* **2002**, *3*, 274.

5-Methyldeoxycytidine and Epigenetics



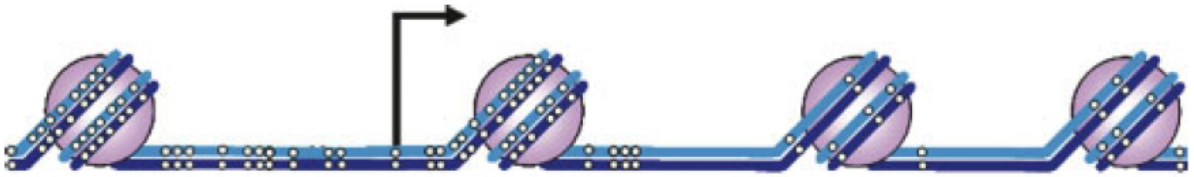
dC

DNA methyltransferase



mC

blocks binding of transcription factors
and/or induces chromatin remodeling



ACTIVE GENE

De novo methylation — **DNMTs**

SILENCED GENE

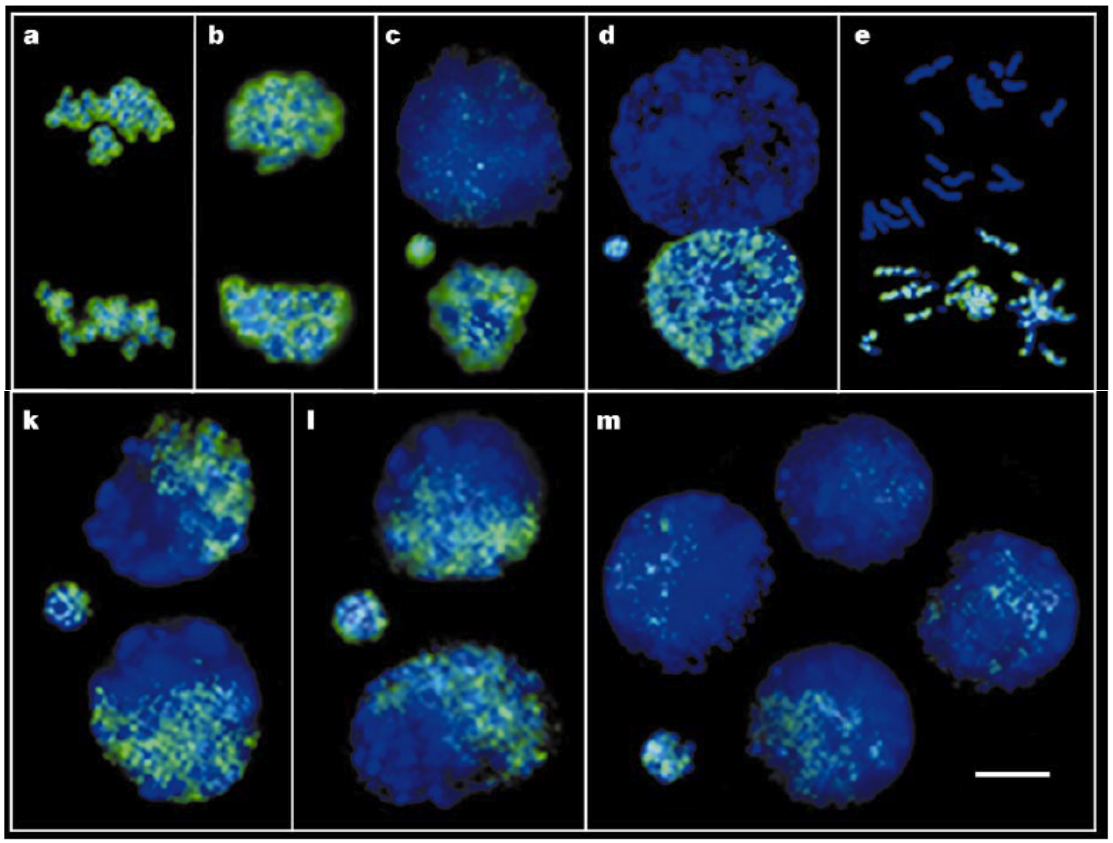
epigenetic modification, can be inherited by daughter cells

Miranda, T. B.; Jones, P. A. *J. Cell. Physiol.* **2007**, *213*, 384.

5-Methyldeoxycytidine and Epigenetics

■ Dramatic "genome reprogramming" events happen after fertilization and during development

examination of mouse zygote/proembryo after fertilization (anti-mC antibody stain, green)

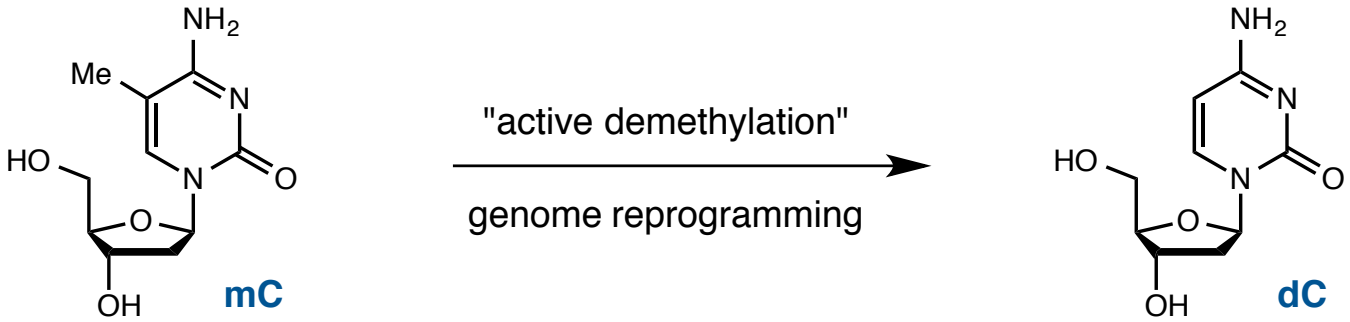


rapid, replication-independent loss of mC in paternal genome

gradual, replication-dependent loss of mC in maternal genome

Mayer, W.; Niveleau, A.; Walter, J.; Fundele, R.; Haaf, T. *Nature.* **2000**, *403*, 501.

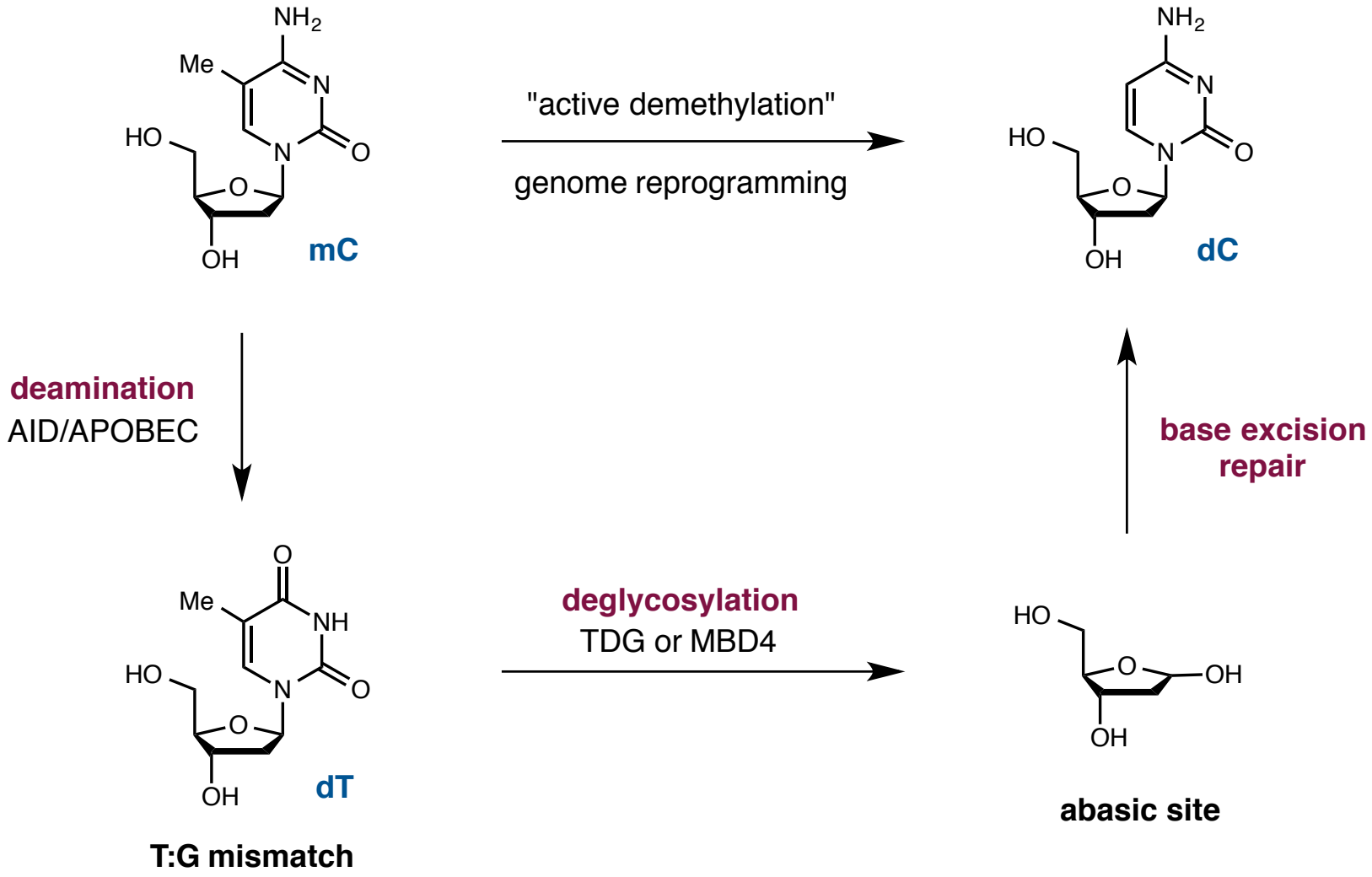
The Role of the Epigenetic Bases



What is the mechanism of "active demethylation?"

Possible Mechanisms of DNA Demethylation

■ Possible mechanism: deamination and mismatch repair

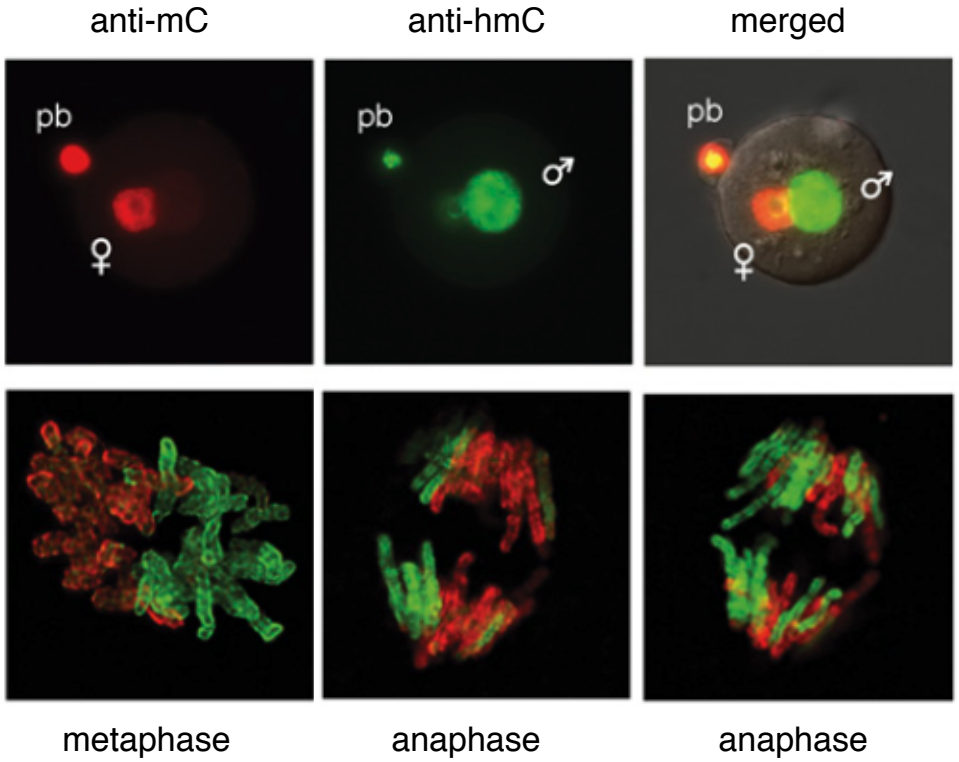


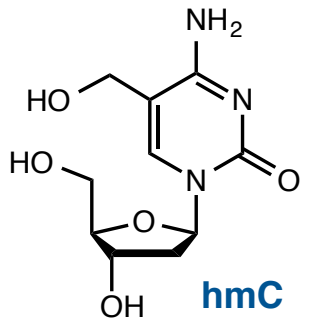
Branco, M. R.; Ficz, G.; Reik, W. *Nat. Rev. Genet.* **2012**, *13*, 7.

5-Methyldeoxycytidine and Epigenetics

■ Dramatic "genome reprogramming" events happen after fertilization and during development

examination of mouse zygote/proembryo using anti-mC and anti-hmC antibodies





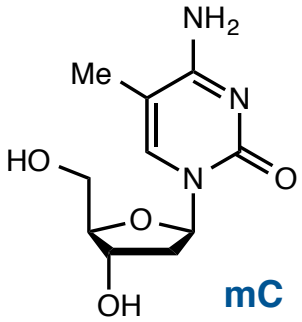
- **high levels of hmC in paternal genome**
- **anti-mC antibodies do not recognize hmC**
- **hmC persists through cell division**

depletion of mC signal in previous studies may be due to oxidation of mC to hmC (not genome-wide demethylation)

Iqbal, K.; Jin, S.-G.; Pfeifer, G. P.; Szabó, P. E. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 3642.

Possible Mechanisms of DNA Demethylation

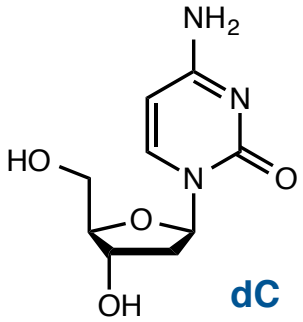
■ Recent discoveries suggest other possible mechanisms for demethylation



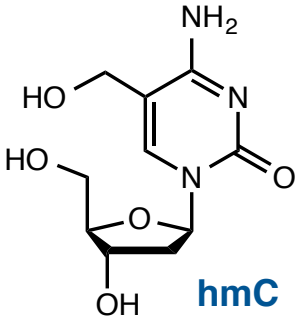
mC

"active demethylation"

genome reprogramming

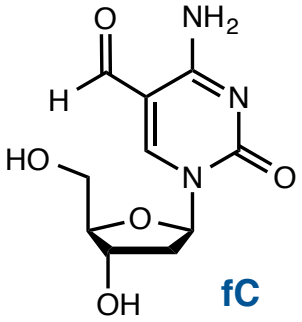


dC



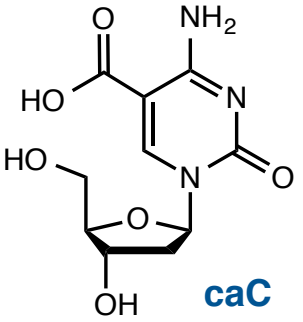
hmC

initial detection, 1972
renewed interest, 2009



fC

initial detection, 2011



caC

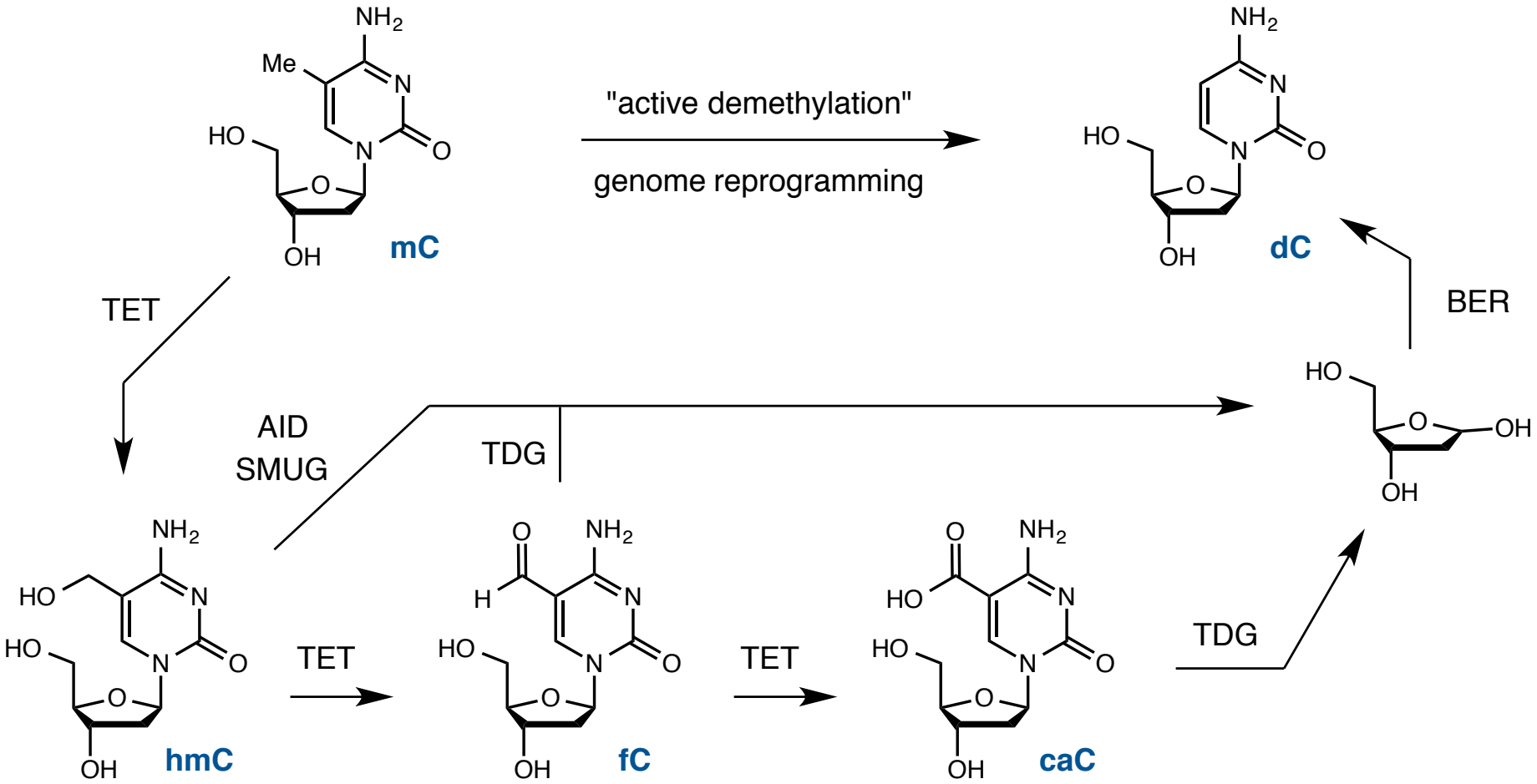
initial detection, 2011

Branco, M. R.; Ficz, G.; Reik, W. *Nat. Rev. Genet.* **2012**, *13*, 7.

Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Possible Mechanisms of DNA Demethylation

■ Possible mechanism: deglycosylation of oxidized intermediates

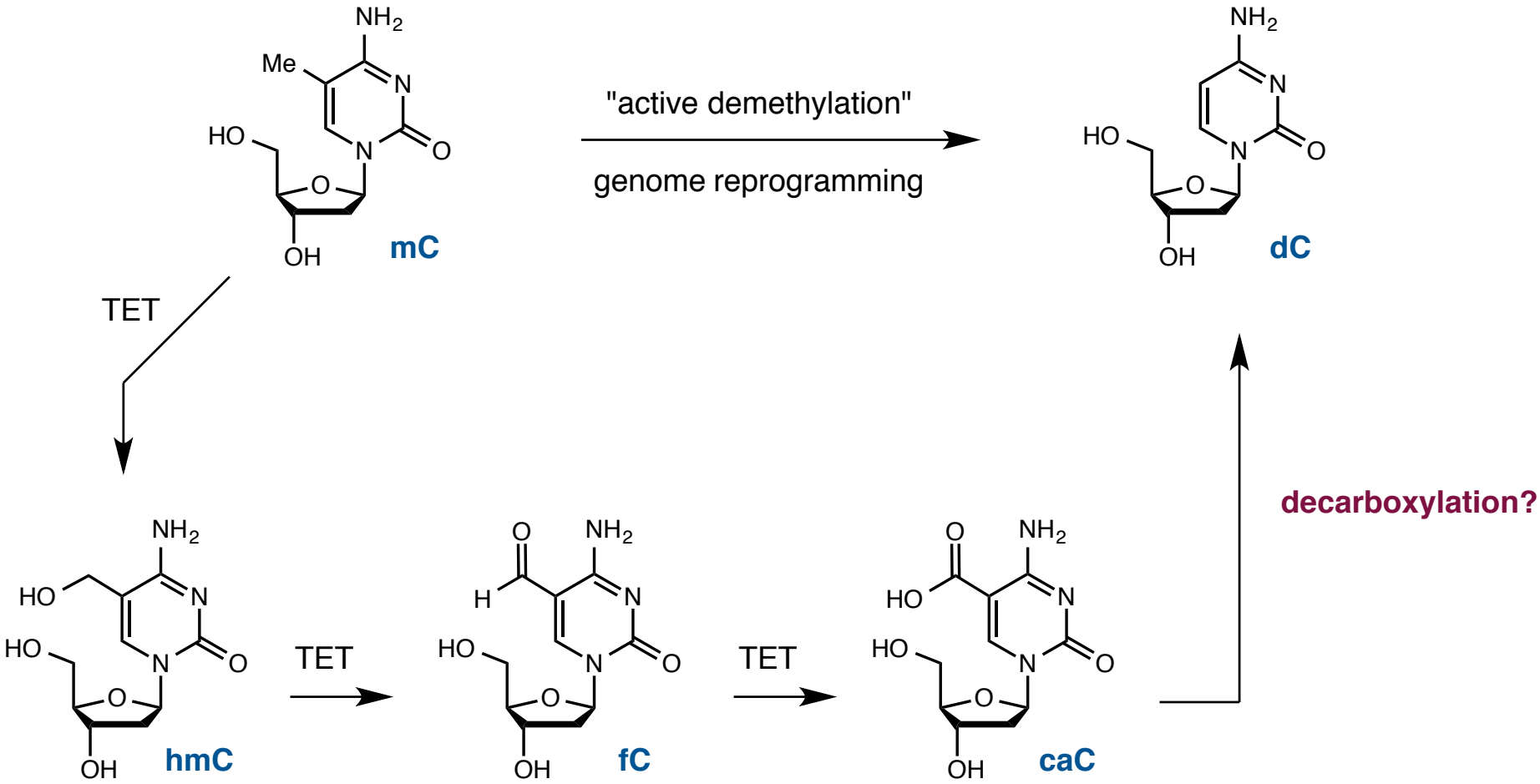


Branco, M. R.; Ficz, G.; Reik, W. *Nat. Rev. Genet.* **2012**, *13*, 7.

Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Possible Mechanisms of DNA Demethylation

■ Possible mechanism: decarboxylation

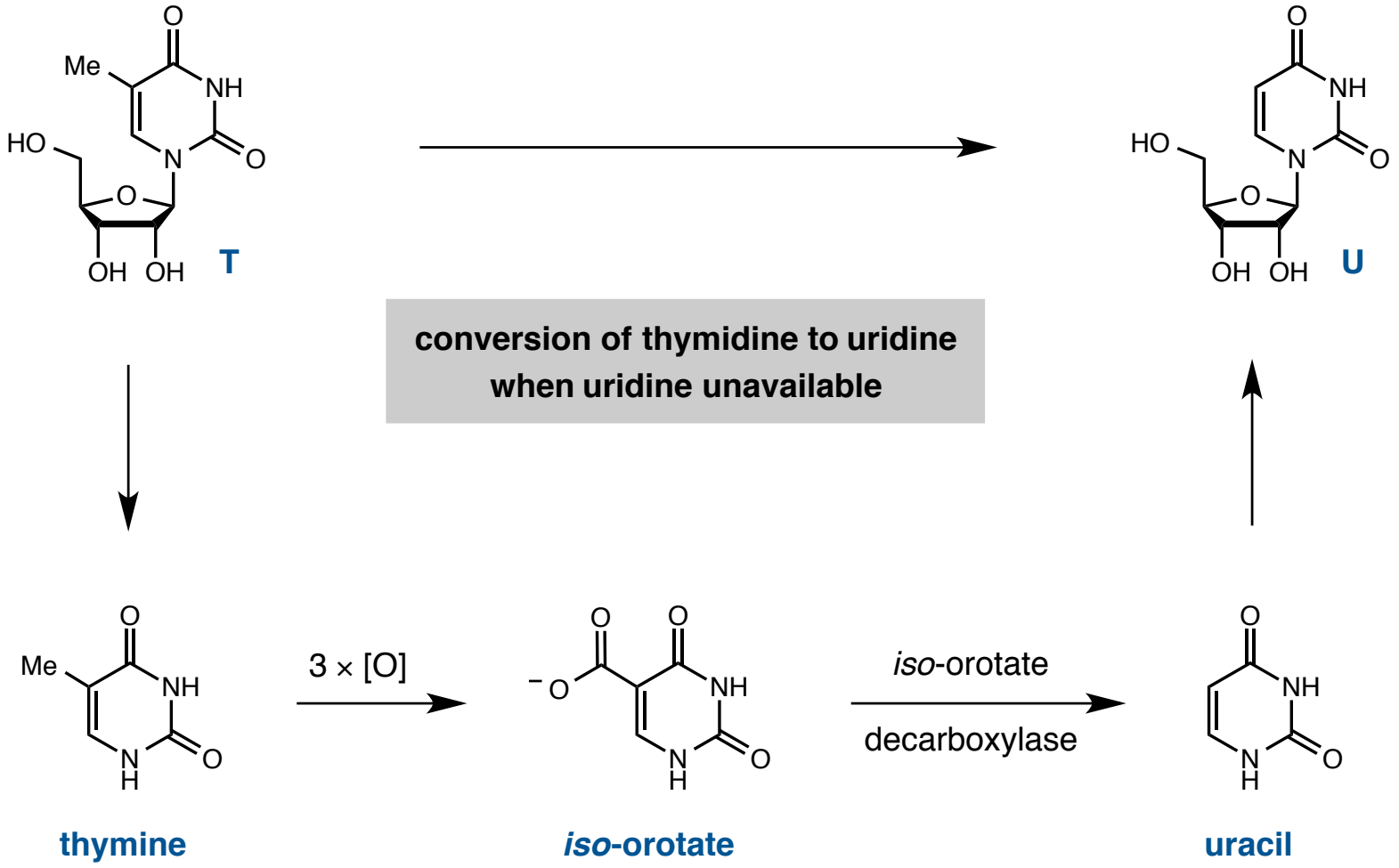


Branco, M. R.; Ficz, G.; Reik, W. *Nat. Rev. Genet.* **2012**, *13*, 7.

Carell, T.; Brandmayer, C.; Hienzsch, A.; Müller, M.; Pearson, D.; Reiter, V.; Thoma, I; et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 7110.

Evidence for the Feasibility of a Decarboxylative Mechanism

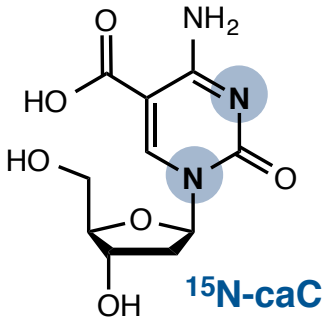
■ Fungal thymidine salvage pathway proceeds through oxidation/decarboxylation



Smiley, J. A.; Kundracik, M.; Landfried, D. A.; Barnes Sr., V. R.; Axhemi, A. A. *Biochim. Biophys. Acta Gen. Subj.* **2005**, *1723*, 256.

Evidence for the Feasibility of a Decarboxylative Mechanism

■ Demethylation of 5-methylcytidine with nuclear extract from mouse embryonic stem cells



5'-CCTTTCC--GAAGGGACGTTGACcaCGGATGCYC-3'
3'-GGAAAGGcaCTTCCCTGCAACTG--GCCTACGAG-5' D1

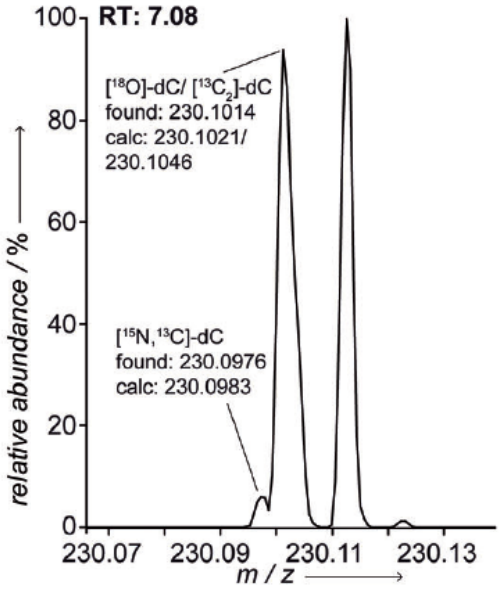
↓ incubation with mESC nuclear extract
re-isolation of the DNA duplex D1 using magnetic beads
total digest of D1

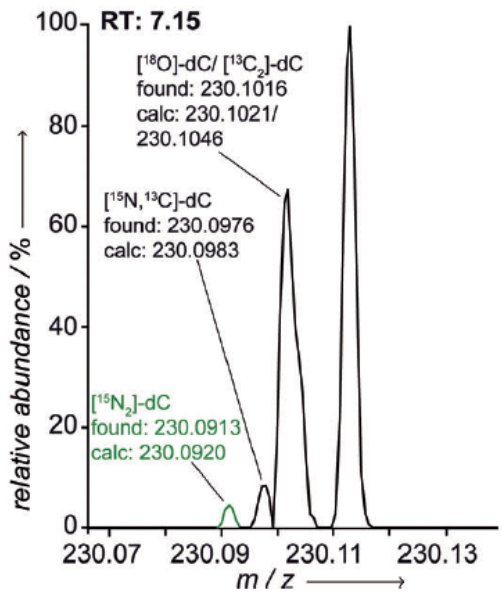
$[^{15}N_2]$-caC $[^{15}N_2]$-dC Y dA dT dG dC

no incubation with mESC

⇩

no ^{15}N-dC observed, no decarboxylation in absence of extract





after incubation with mESC

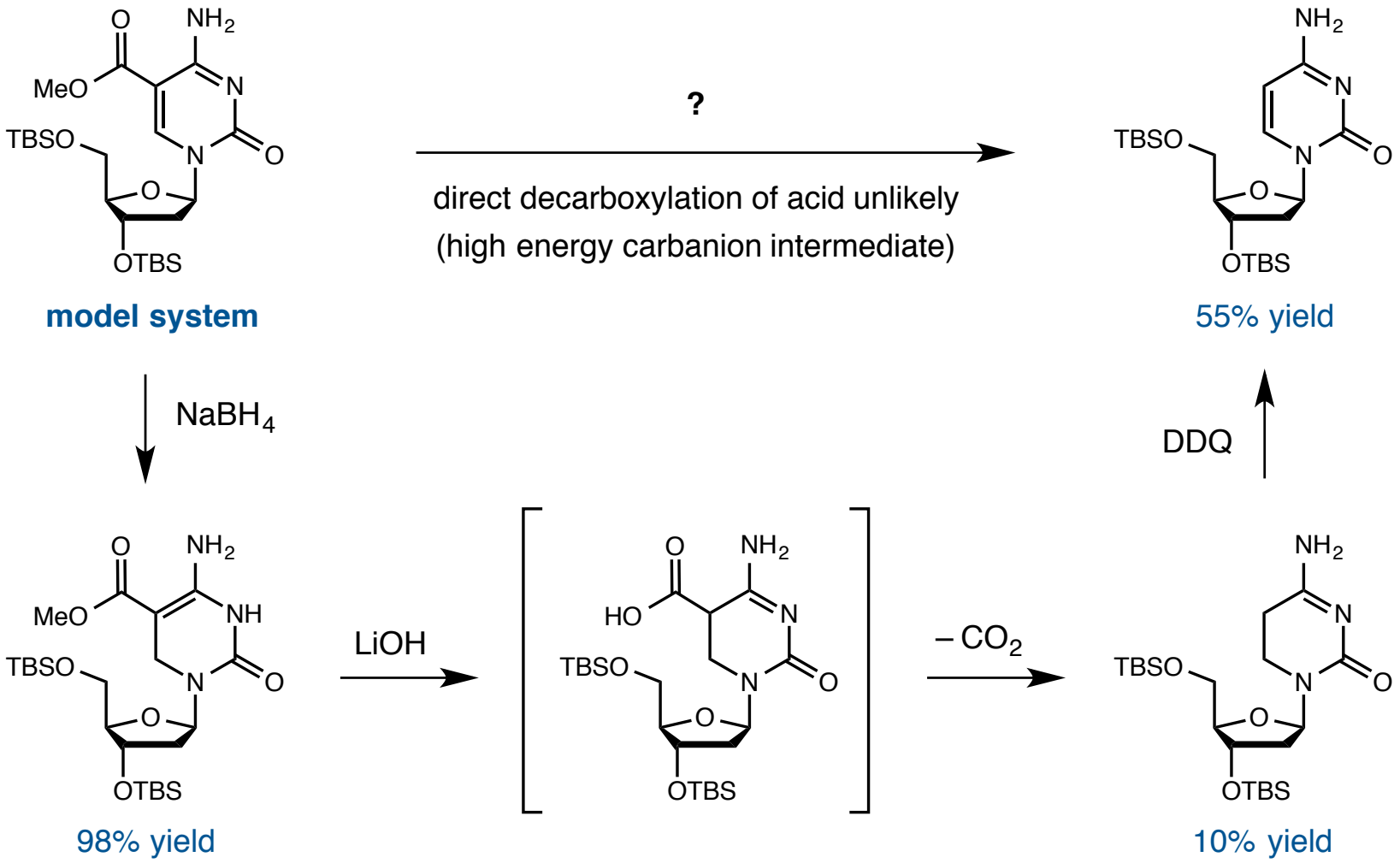
⇩

^{15}N-dC observed, decarboxylation of caC is possible

Schiesser, S.; Hackner, B.; Pfaffeneder, T.; Müller, M.; Hagemeier, C.; Truss, M. et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 6516.

Evidence for the Feasibility of a Decarboxylative Mechanism

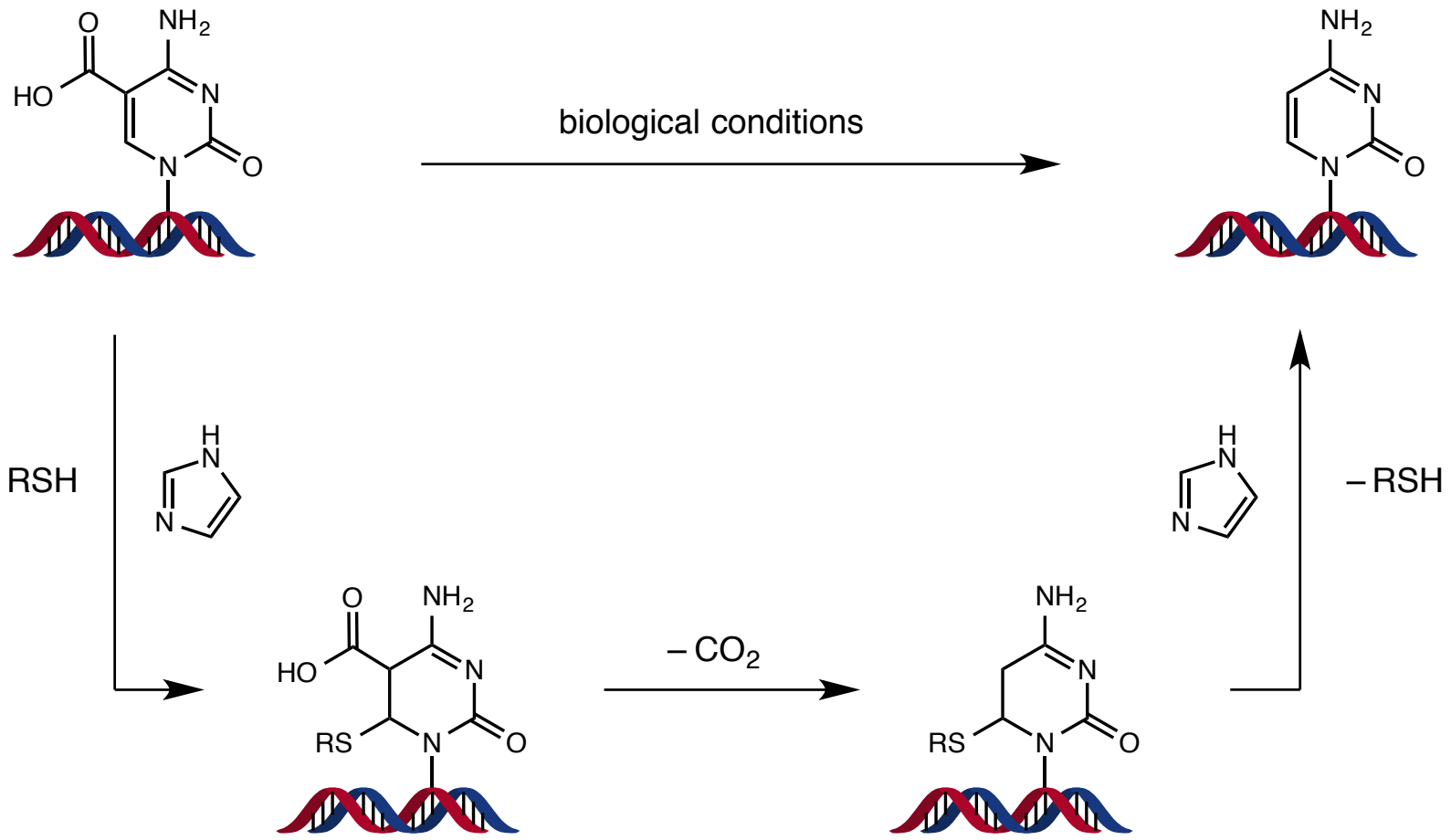
■ How would the decarboxylation occur on a molecular level?



Schiesser, S.; Hackner, B.; Pfaffeneder, T.; Müller, M.; Hagemeier, C.; Truss, M. et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 6516.

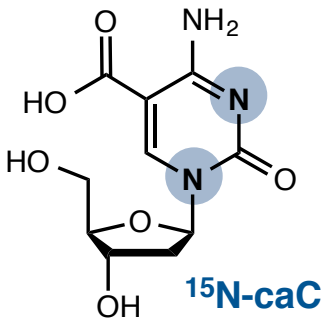
Evidence for the Feasibility of a Decarboxylative Mechanism

- But nature doesn't use $NaBH_4$ or DDQ...



Schiesser, S.; Hackner, B.; Pfaffeneder, T.; Müller, M.; Hagemeier, C.; Truss, M. et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 6516.

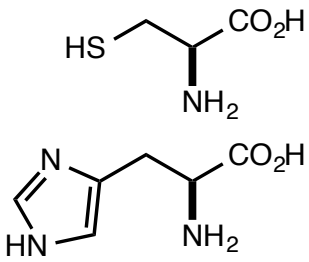
Evidence for the Feasibility of a Decarboxylative Mechanism



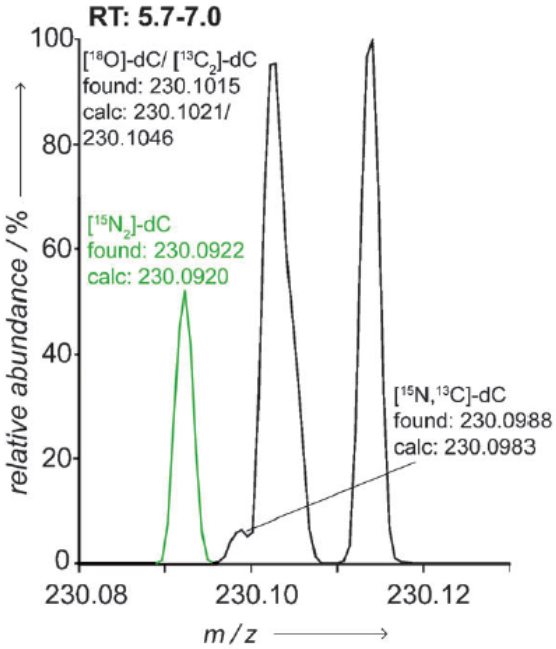
5'–GAGCATCCGGTCACCGTCCCTT**caC**GGAAAGG–3'

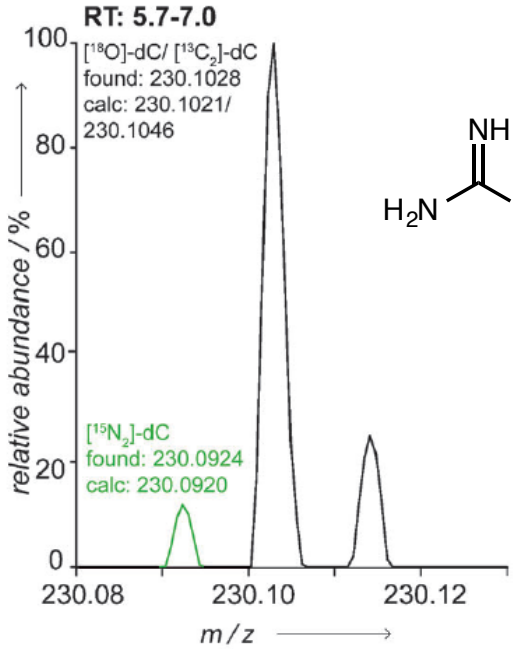
addition of amino acids

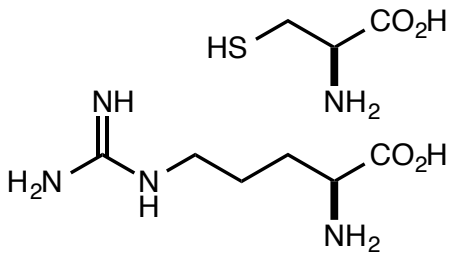
5'–GAGCATCCGGTCACCGTCCCTT**C**GGAAAGG–3' —digest→ dA, dG, dT, dC, [$^{15}N_2$]-dC, [$^{15}N_2$]-caC



decarboxylation observed





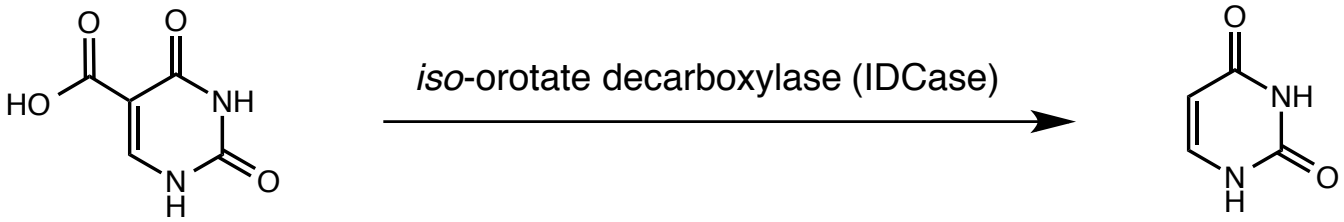


decarboxylation observed

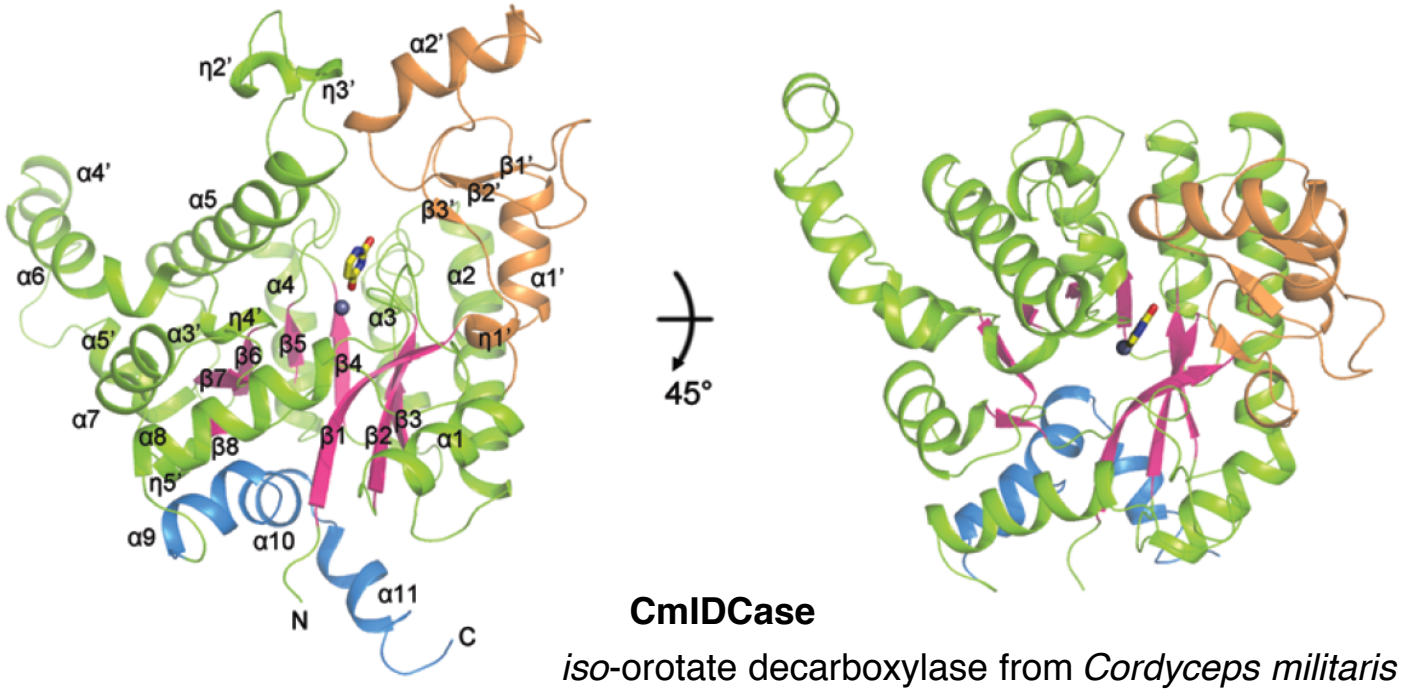
Schiesser, S.; Hackner, B.; Pfaffeneder, T.; Müller, M.; Hagemeier, C.; Truss, M. et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 6516.

Evidence for the Feasibility of a Decarboxylative Mechanism

■ Recall: decarboxylation of *iso*-orotate in thymidine salvage pathway



***iso*-orotate** **uracil**

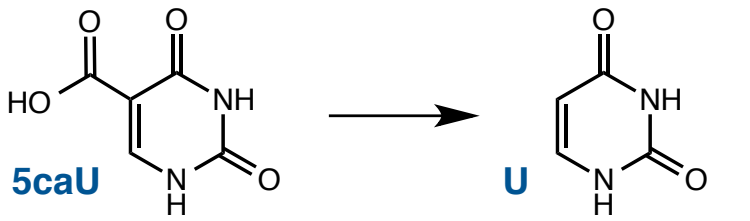


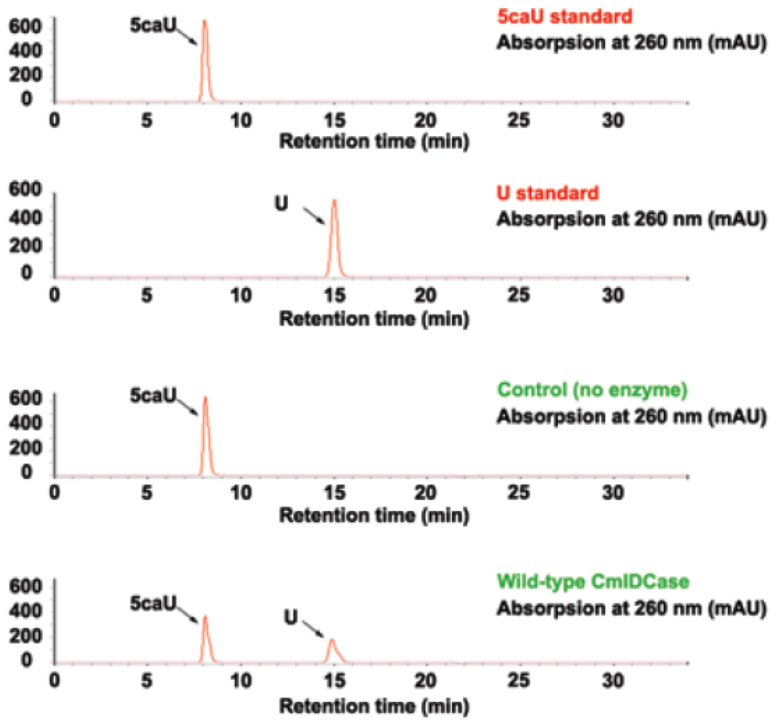
CmIDCase

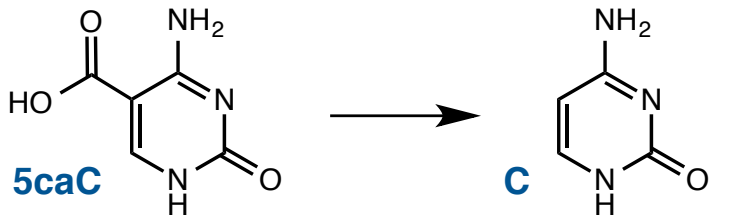
iso-orotate decarboxylase from *Cordyceps militaris*

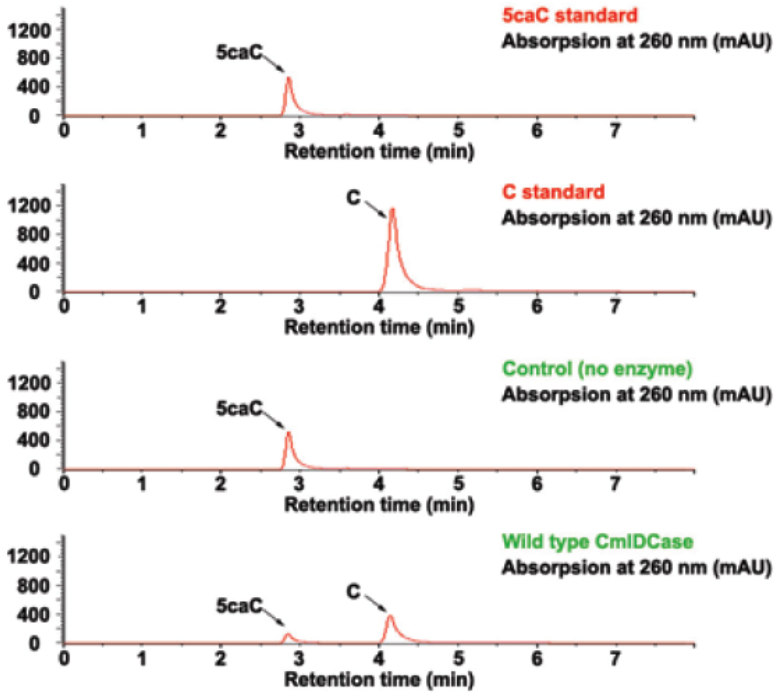
Xu, S.; Li, W.; Zhu, J.; Wang, R.; Li, Z.; Xu, G.-L.; Ding, J. *Cell Res.* **2013**, *23*, 1296.

Evidence for the Feasibility of a Decarboxylative Mechanism









A naturally occuring decarboxylase is capable of converting caC to C.

Xu, S.; Li, W.; Zhu, J.; Wang, R.; Li, Z.; Xu, G.-L.; Ding, J. *Cell Res.* **2013**, *23*, 1296.