

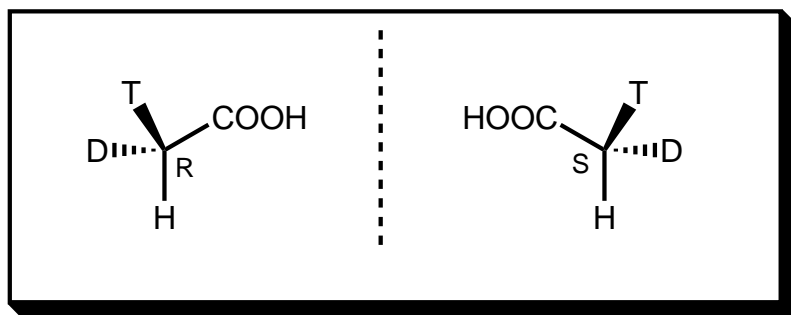
# Chiral Methyl Groups

Synthesis and Applications

Evans Group Seminar

January 12, 2001

Jason Burch

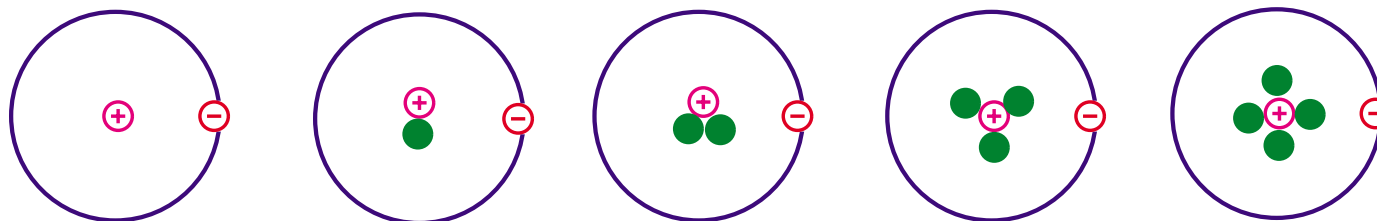


- I. Introduction and First Syntheses of Chiral Acetic Acid
- II. Determination of Configuration and Enantiomeric Excess
- III. Syntheses of Chiral Acetic Acid
  - (a) Nucleophilic Tritide
  - (b) Molecular Rearrangements
- IV. Biological Applications
  - (a) Methyl Transferases
  - (b) Methanogenesis
  - (c) Methane Monooxygenase

Leading References: Floss and Lee, *Acc. Chem. Res.*, **1993**, 26, 116.  
Evans, *Tritium and its Compounds*, 2<sup>nd</sup> ed., Wiley, New York, **1974**, pp. 1-13.

# Isotopes of Hydrogen

## Introduction



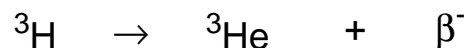
Chemical Symbol	${}^1_1\text{H}$	${}^2_1\text{H}$	${}^3_1\text{H}$	${}^4_1\text{H}$	${}^5_1\text{H}$
Common Name	Hydrogen	Deuterium	Tritium	—	—
Nuclear Spin	1/2	1	1/2	—	—
Radioactive?	No	No	Yes	Yes	Yes
Half Life	N / A	N / A	12.35 years	unknown	$10^{-1}$ s

Evans, *Tritium and its Compounds*, 2<sup>nd</sup>ed., Wiley, New York, **1974**, pp. 1-13.

# Isotopes of Hydrogen

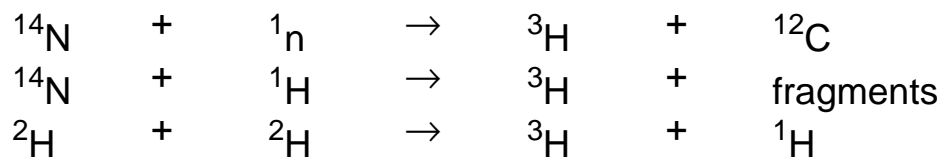
## Tritium

- radioactive decay

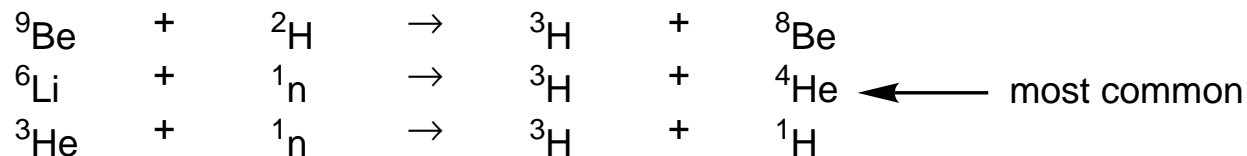


- natural occurrence

- following collisions induced by cosmic radiation in the upper atmosphere



- laboratory synthesis

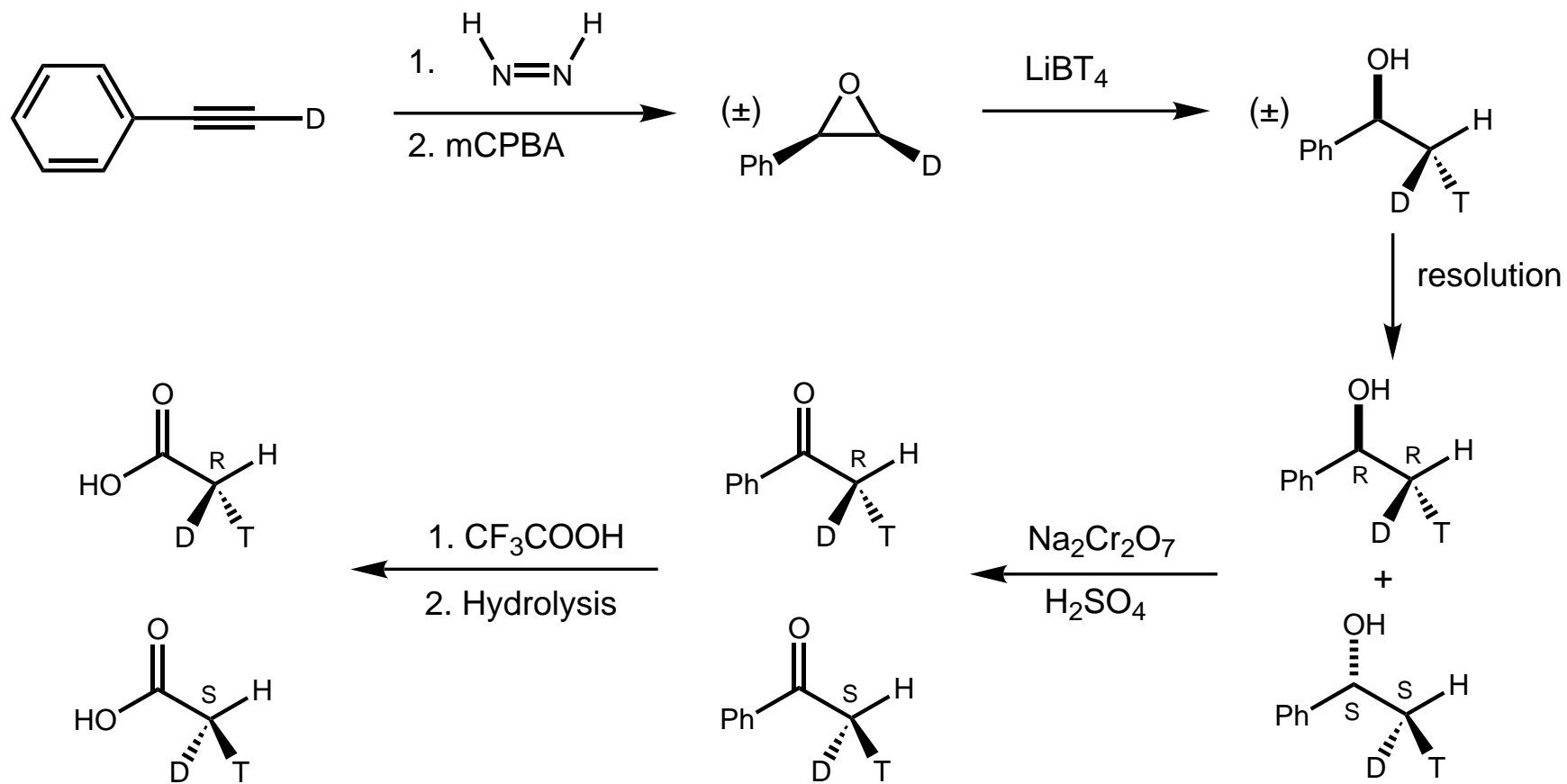


- before March 1954 (beginning of thermonuclear weapons testing) tritium content in rain water was < 5 "tritium units" (T.U. =  $1 \text{ } ^3\text{H} / 10^{18} \text{ } ^1\text{H}$ ); post-1954 this value rose to as high as 500 T.U.

Evans, *Tritium and its Compounds*, 2<sup>nd</sup> ed., Wiley, New York, **1974**, pp. 1-13.

# The First Syntheses of Chiral Acetic Acid

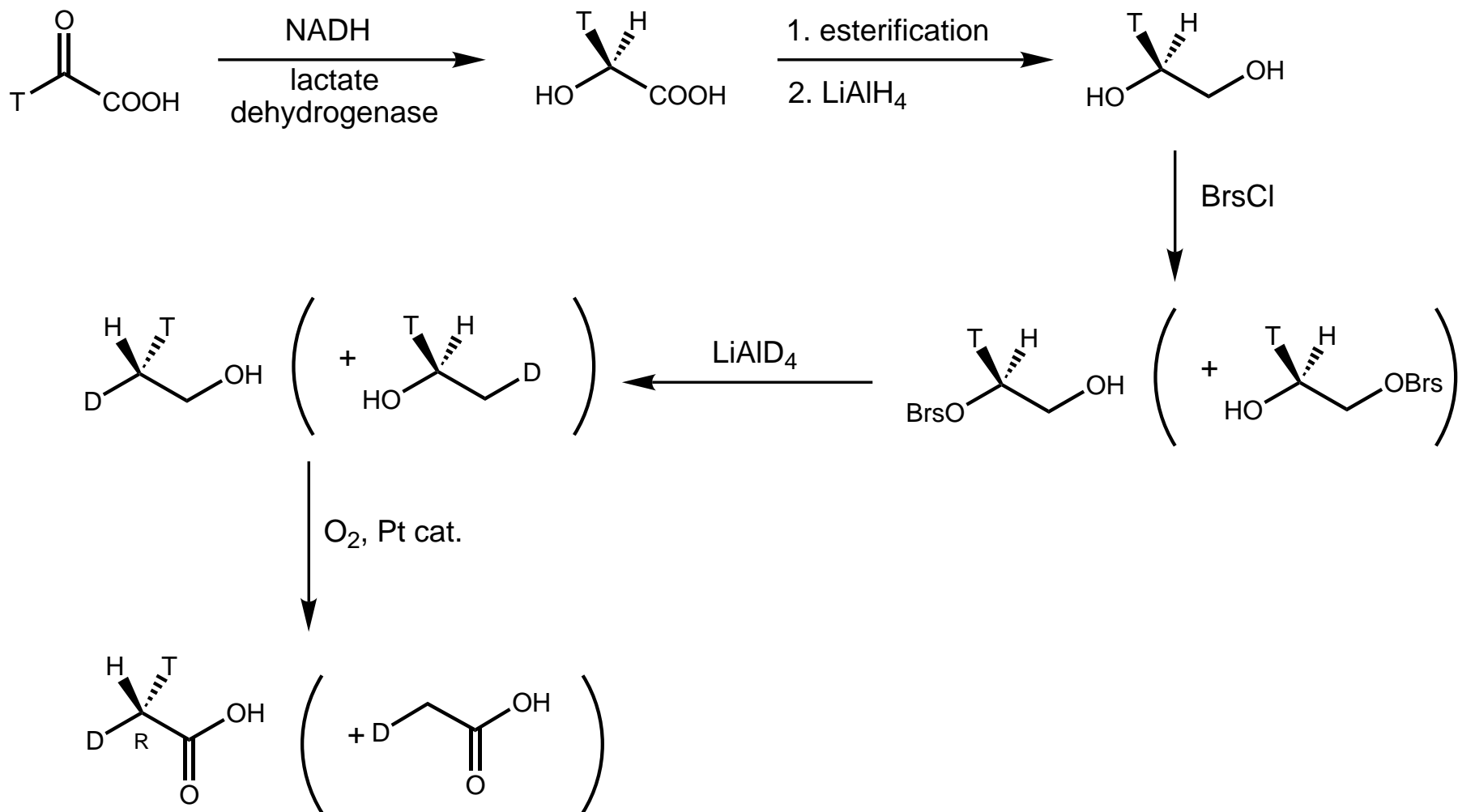
## Cornforth Epoxide Opening



Cornforth, *Nature* **1969**, 221, 1212.

# The First Syntheses of Chiral Acetic Acid

## Arigoni Enzyme-assisted Pathway

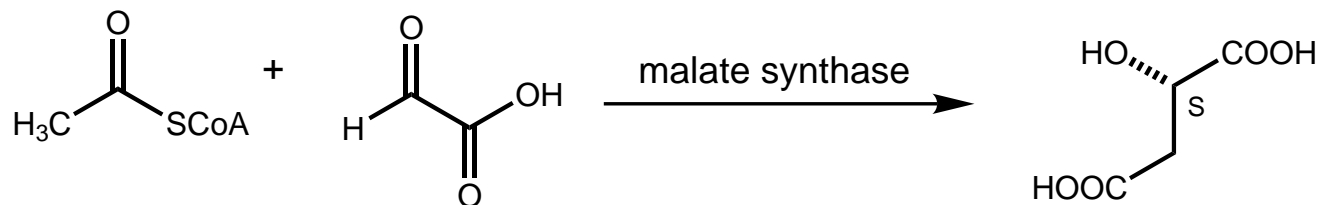


Arigoni, *Nature* **1969**, 221, 1213.

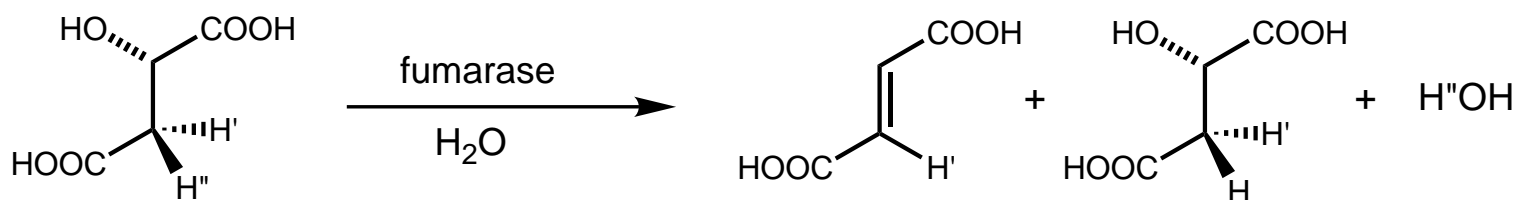
# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method

- basis



- known to exhibit isotope effect, favoring loss of H over D and T



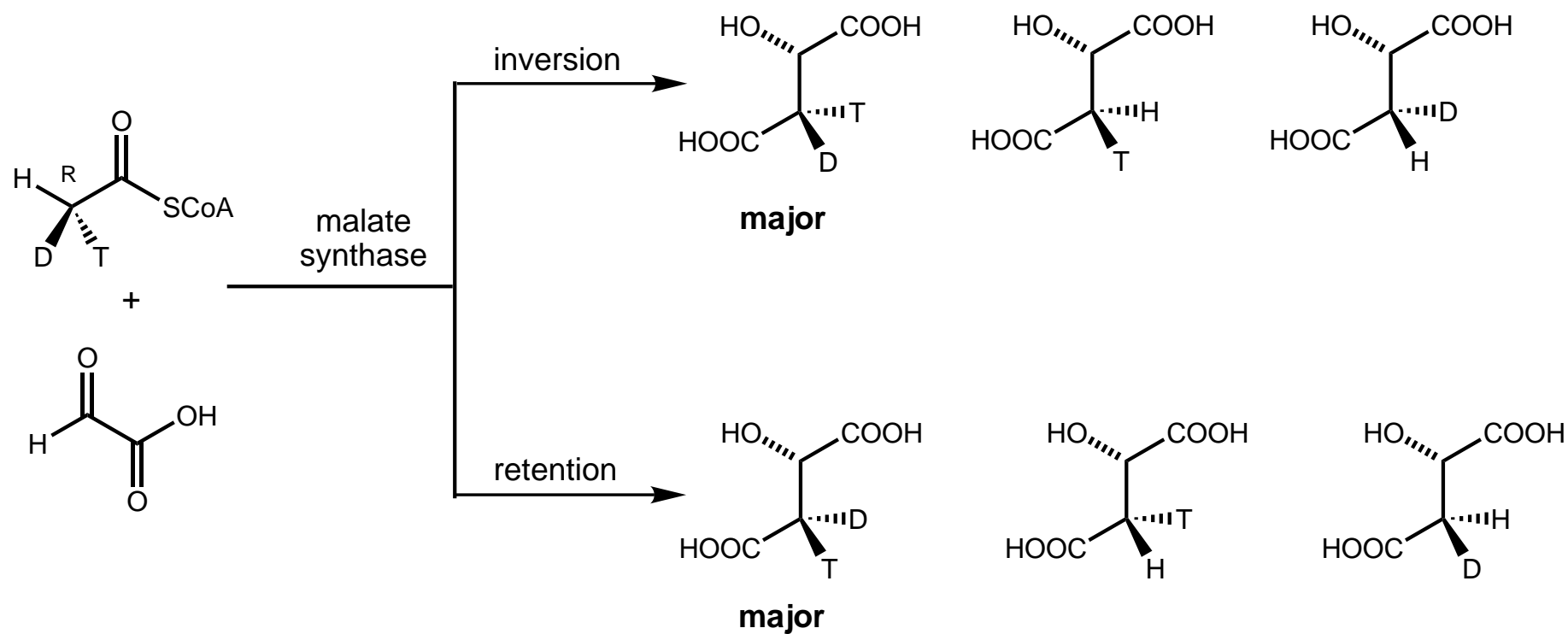
- only pro-R hydrogen is lost or exchanged with solvent

Cornforth, *Nature* **1969**, 221, 1212.  
Arigoni, *Nature* **1969**, 221, 1213.

# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method

Idea:

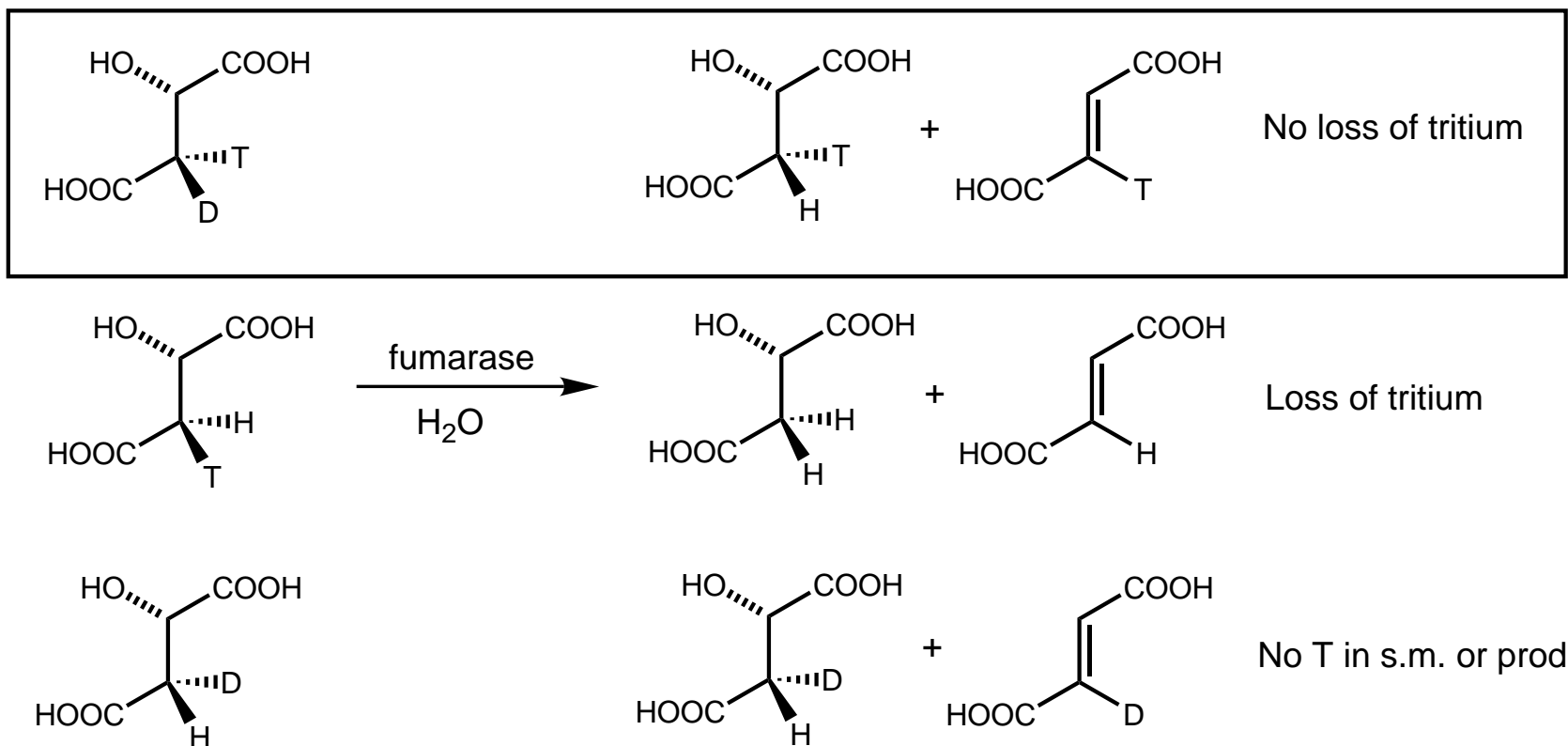


Cornforth, *Nature* **1969**, 221, 1212.  
Arigoni, *Nature* **1969**, 221, 1213.

# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method

If malate is formed with inversion:



$\therefore$  if inversion mode is favored, (R)-acetylSCoA  $\rightarrow$  >50% tritium retained

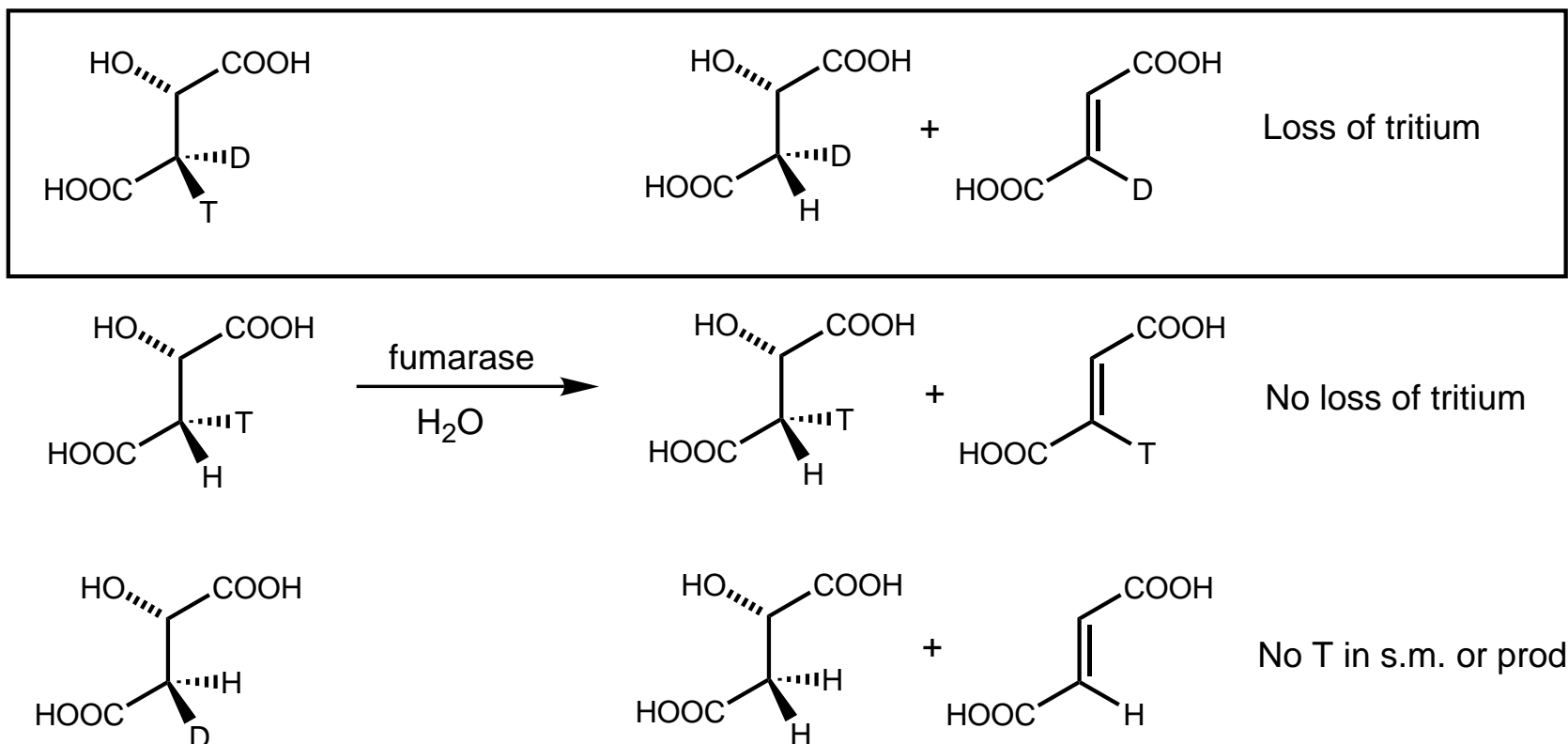
Cornforth, *Nature* **1969**, 221, 1212.  
Arigoni, *Nature* **1969**, 221, 1213.



# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method

If malate is formed with retention:

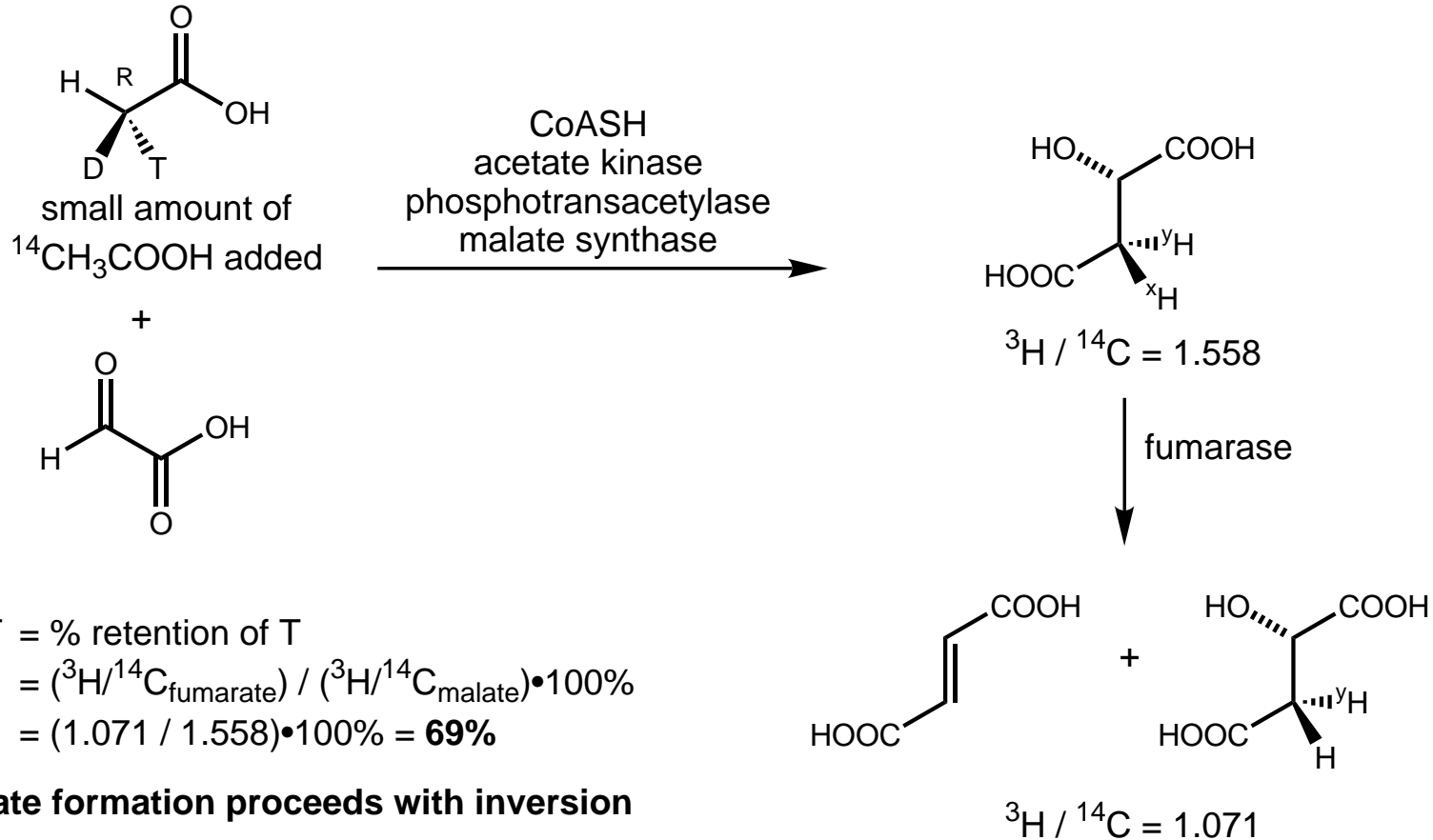


$\therefore$  if retention mode is favored, (R)-acetylSCoA  $\rightarrow$  <50% tritium retained

Cornforth, *Nature* **1969**, 221, 1212.  
Arigoni, *Nature* **1969**, 221, 1213.

# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method



Define:  $F = \% \text{ retention of T}$

$$= \left( \frac{^3\text{H}/^{14}\text{C}_{\text{fumarate}}}{^3\text{H}/^{14}\text{C}_{\text{malate}}} \right) \cdot 100\%$$

$$= (1.071 / 1.558) \cdot 100\% = \mathbf{69\%}$$

$\therefore$  malate formation proceeds with inversion

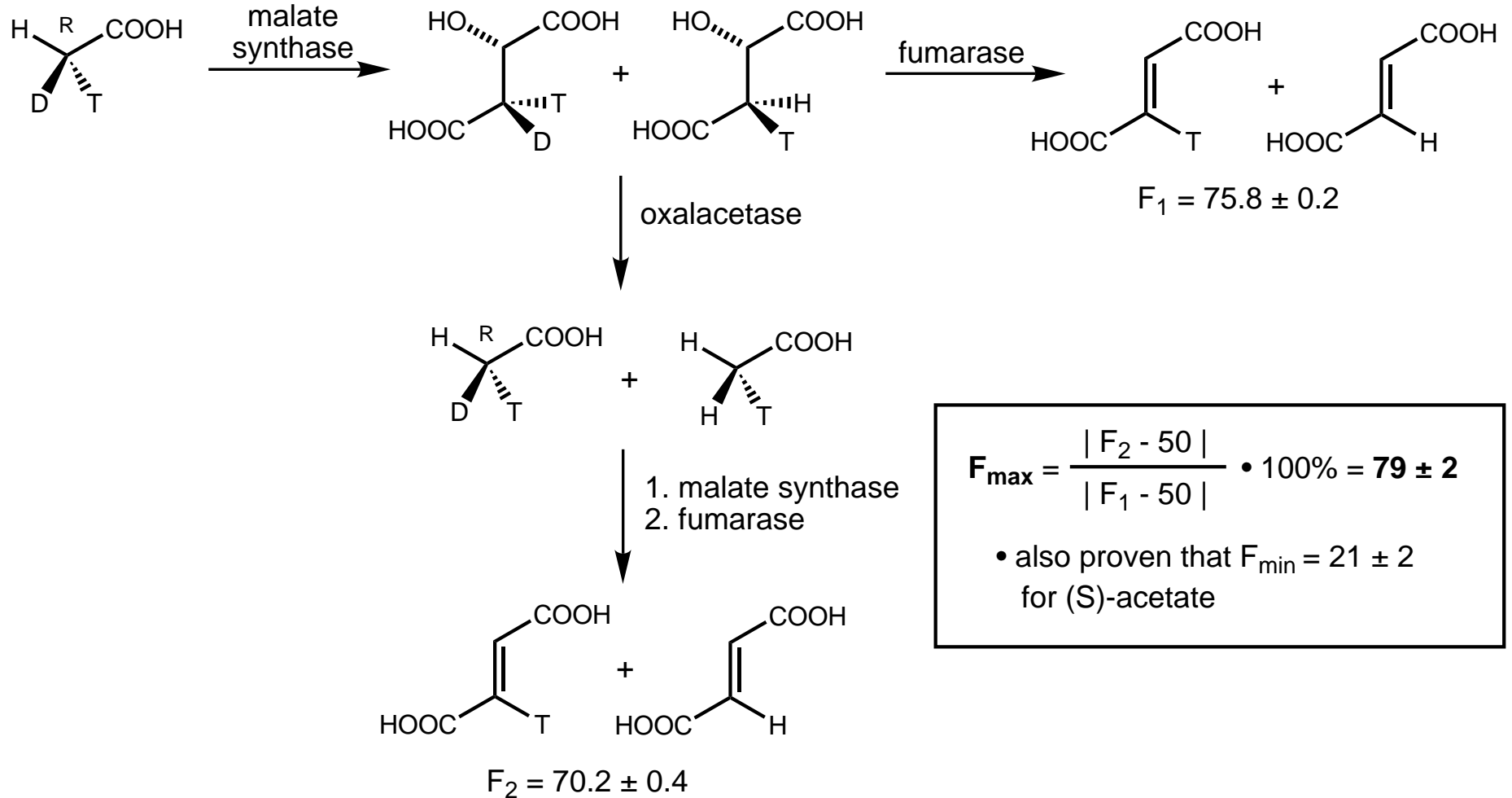
- if (S)-CHDTCOOH is used,  $F = 31\%$

**But what was %ee of starting acetate?**

Cornforth, *Nature* **1969**, 221, 1212.  
 Arigoni, *Nature* **1969**, 221, 1213.

# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method



$$F_{\max} = \frac{|F_2 - 50|}{|F_1 - 50|} \cdot 100\% = 79 \pm 2$$

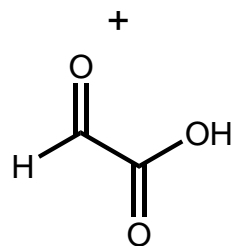
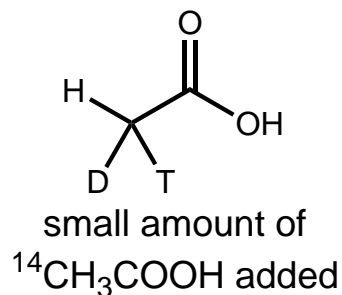
- also proven that  $F_{\min} = 21 \pm 2$  for (S)-acetate

Eggerer, *Eur. J. Biochem.* **1976**, *65*, 237.

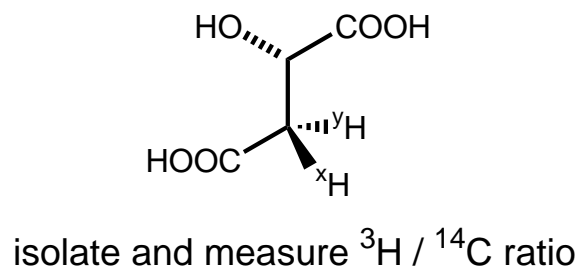
# Determination of Configuration and Enantiomeric Excess

## Cornforth / Arigoni Enzyme Method

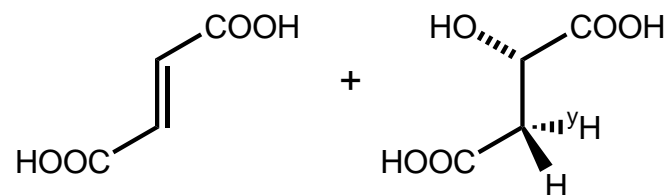
### • Overall Method



CoASH  
 acetate kinase  
 phosphotransacetylase  
 malate synthase



fumarase



$$F = \frac{{}^3\text{H} / {}^{14}\text{C} \text{ fumarate}}{{}^3\text{H} / {}^{14}\text{C} \text{ malate}} \cdot 100\%$$

- if  $F > 50\%$ , acetate was predominantly R config.
- if  $F < 50\%$ , acetate was predominantly S config.

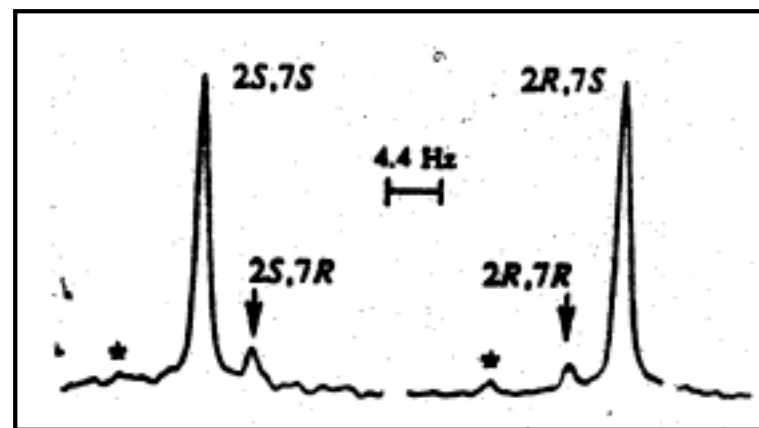
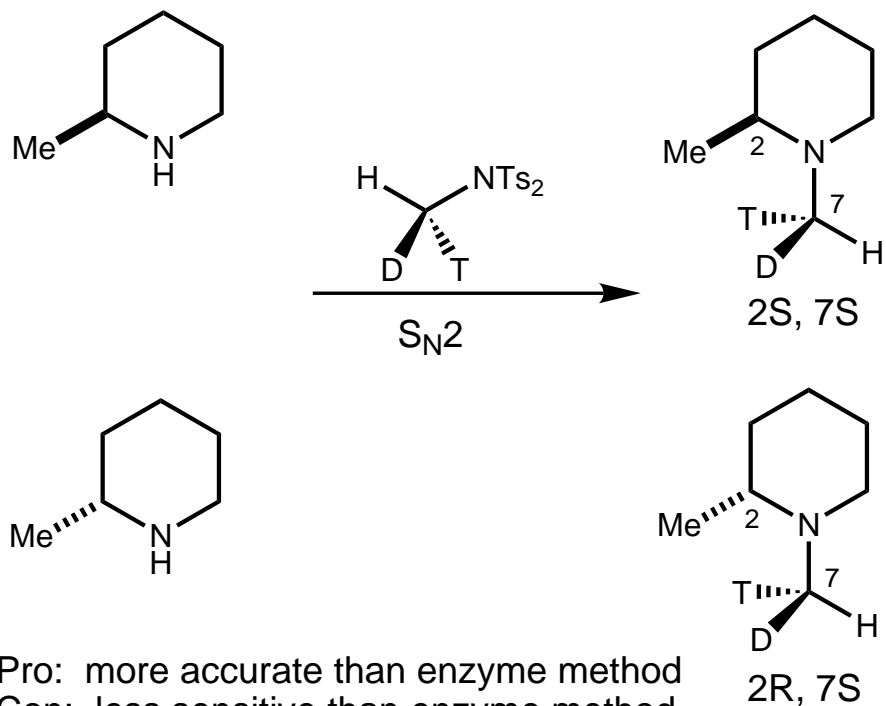
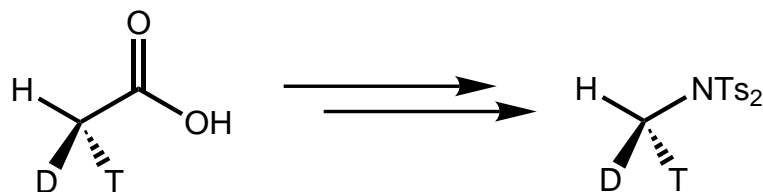
$$\%ee = \frac{|F - 50|}{29} \cdot 100\%$$

Cornforth, *Nature* **1969**, 221, 1212.  
 Arigoni, *Nature* **1969**, 221, 1213.

# Determination of Configuration and Enantiomeric Excess

## Anet's Direct Tritium NMR Method

Question: Are  $^3\text{H}$ -NMR shifts of diastereomeric methyl groups distinguishable?



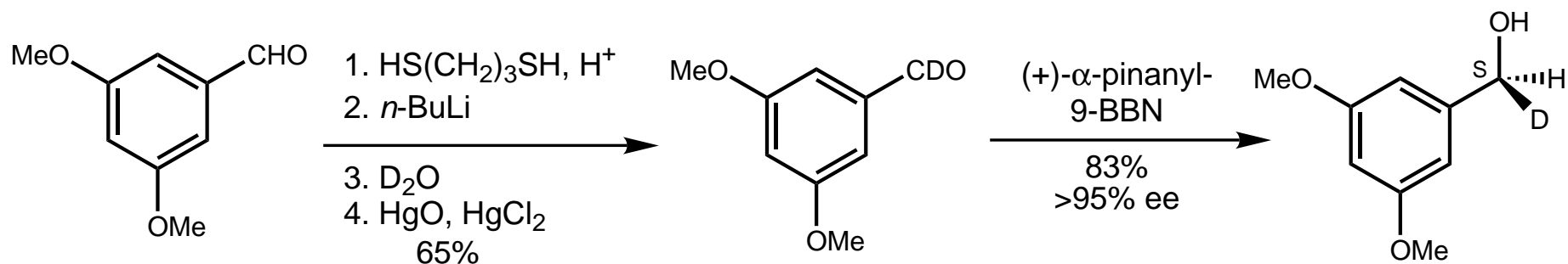
$^3\text{H}\{^1\text{H}, ^2\text{H}\}$ -NMR Spectra at 320 MHz

Pro: more accurate than enzyme method  
Con: less sensitive than enzyme method

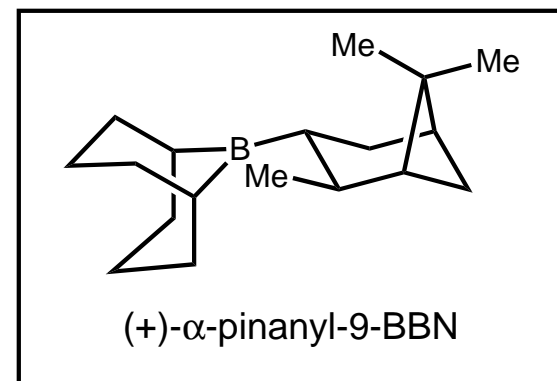
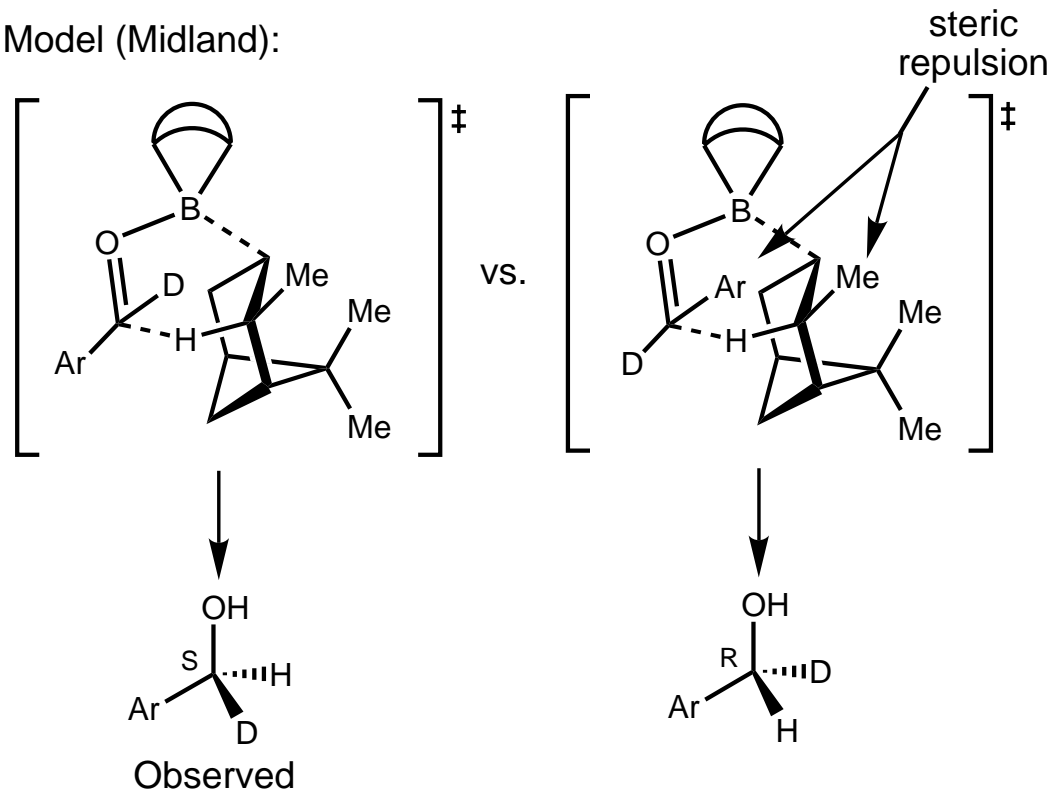
Anet and Floss, *J. Am. Chem. Soc.* **1989**, 111, 8935.

# Syntheses of Chiral Acetic Acid

## Floss Tosylate Displacement



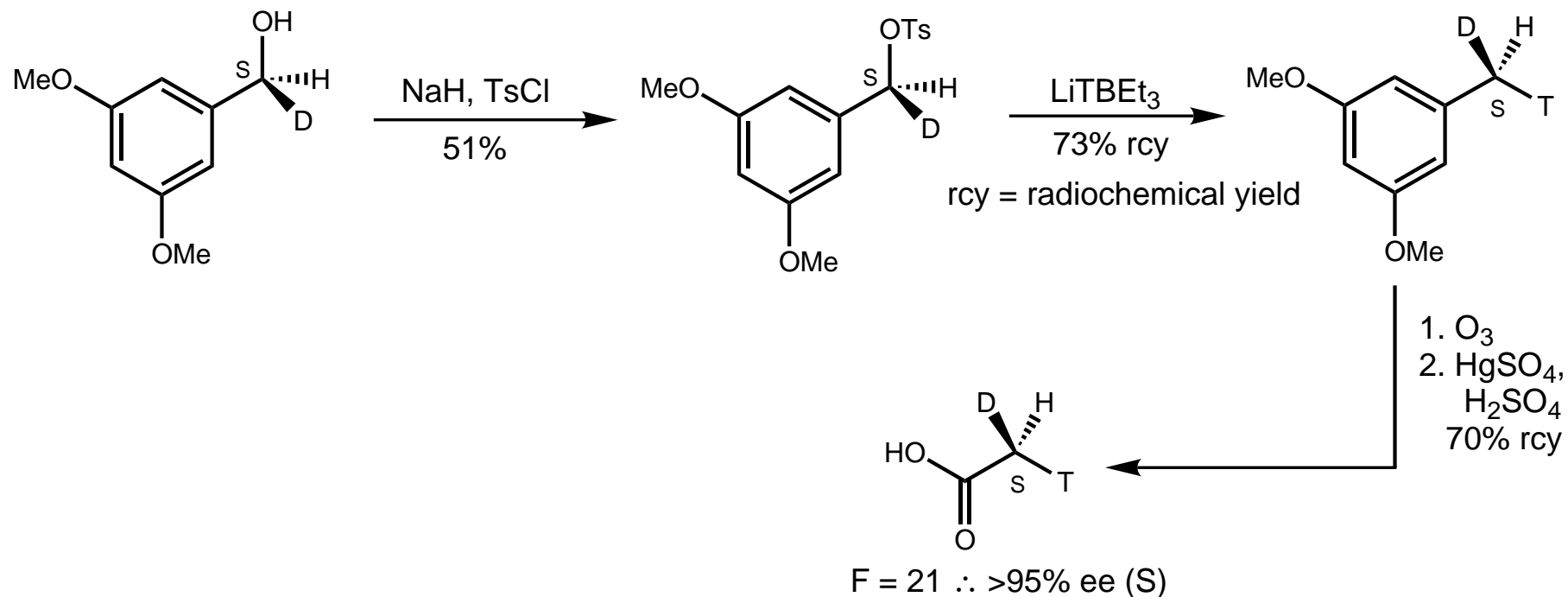
Model (Midland):



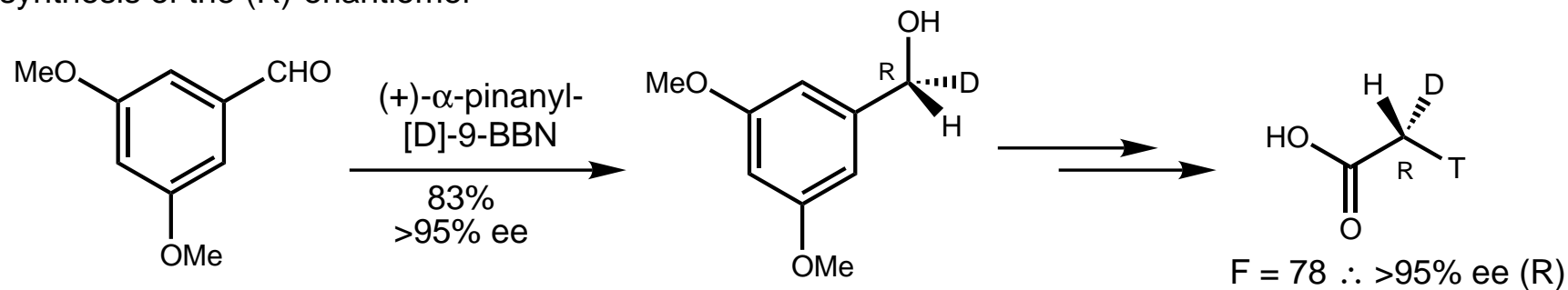
Floss, *J. Org. Chem.* **1983**, *48*, 3510.  
Midland, *J. Am. Chem. Soc.* **1979**, *101*, 2352.

# Syntheses of Chiral Acetic Acid

## Floss Tosylate Displacement



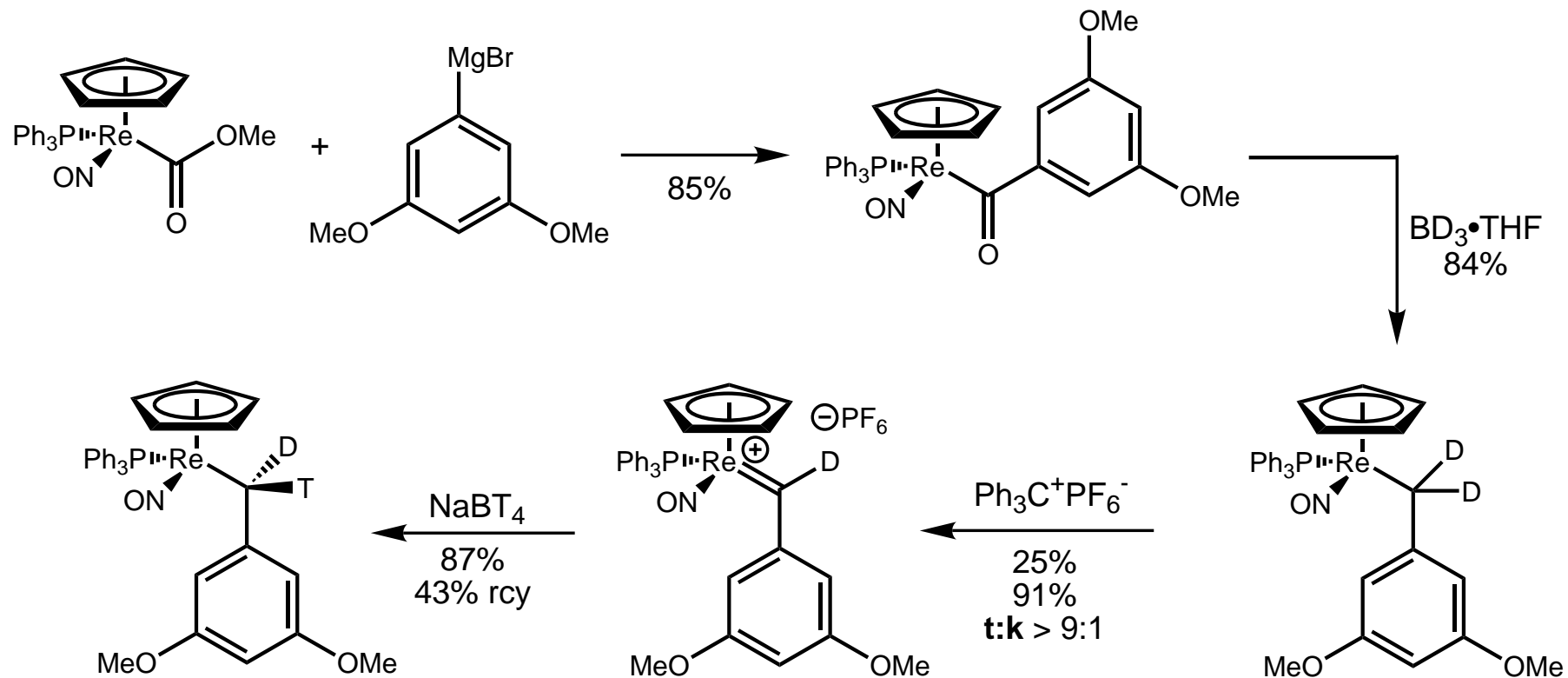
- synthesis of the (R)-enantiomer



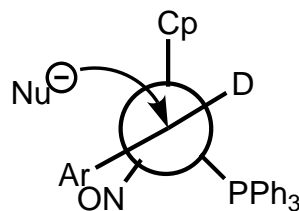
Floss, *J. Org. Chem.* **1983**, *48*, 3510.

# Syntheses of Chiral Acetic Acid

## Gladysz Rhenium-Carbon Bond Protonolysis



III

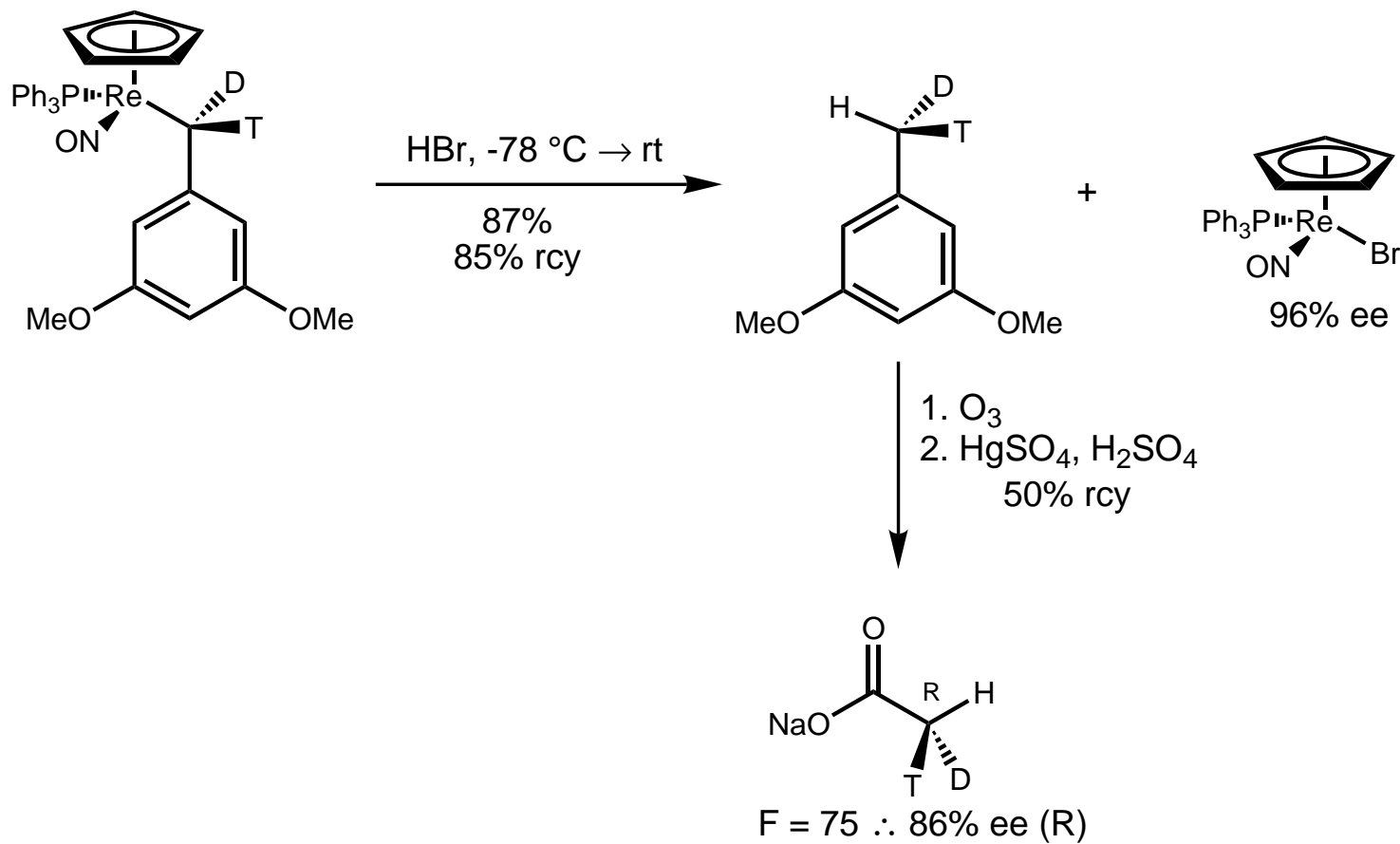


Gladysz and Floss, *J. Am. Chem. Soc.* **1987**, 109, 4837.



# Syntheses of Chiral Acetic Acid

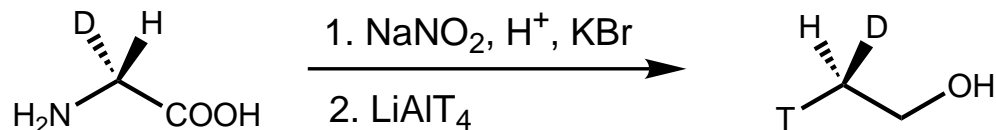
Gladysz Rhenium-Carbon Bond Protonolysis



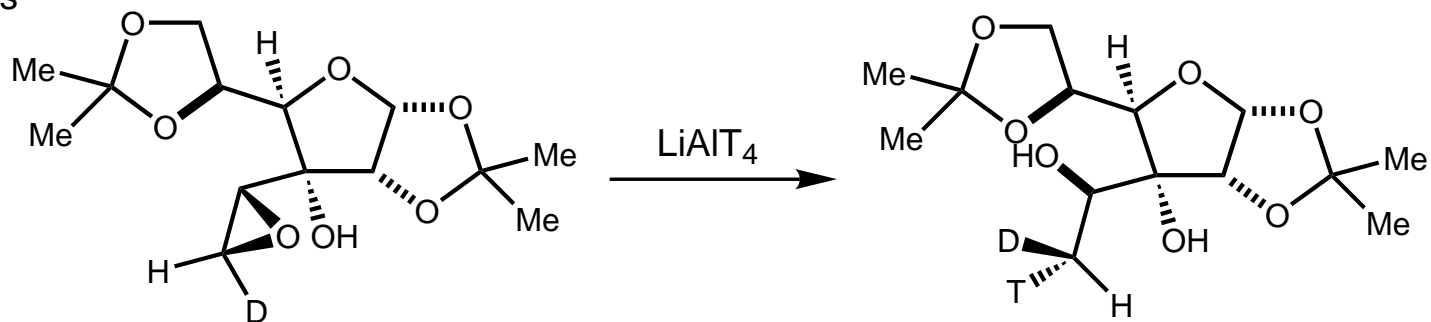
# Syntheses of Chiral Acetic Acid

## Other Nucleophilic Tritiide Examples

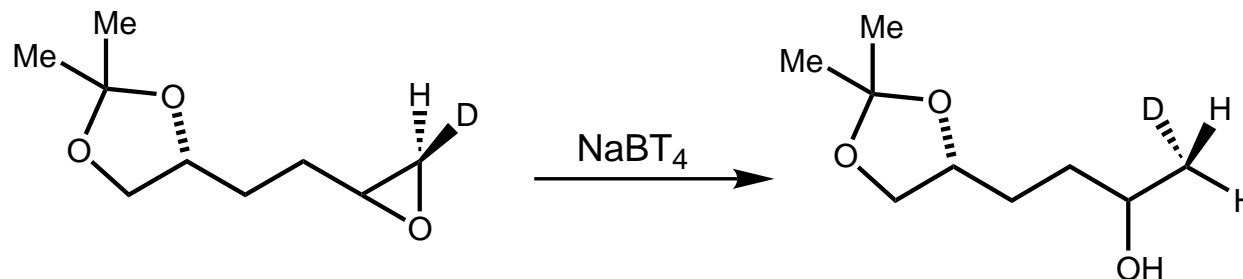
- Scott



- Floss



- Altman



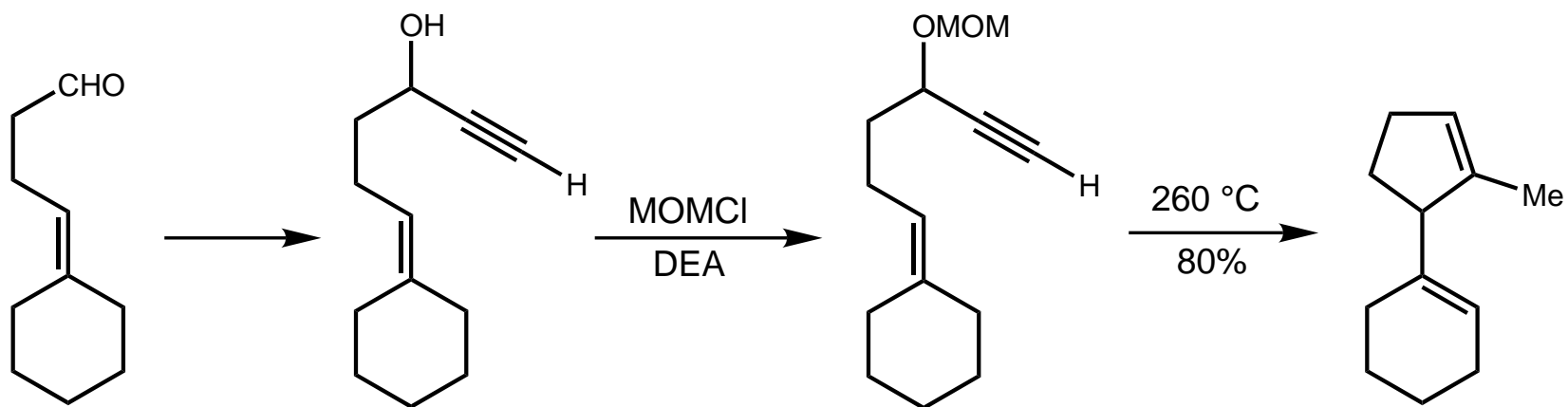
Scott, *Chem. Commun.* **1978**, 967.

Floss, *J. Org. Chem.*, **1984**, 49, 1290.

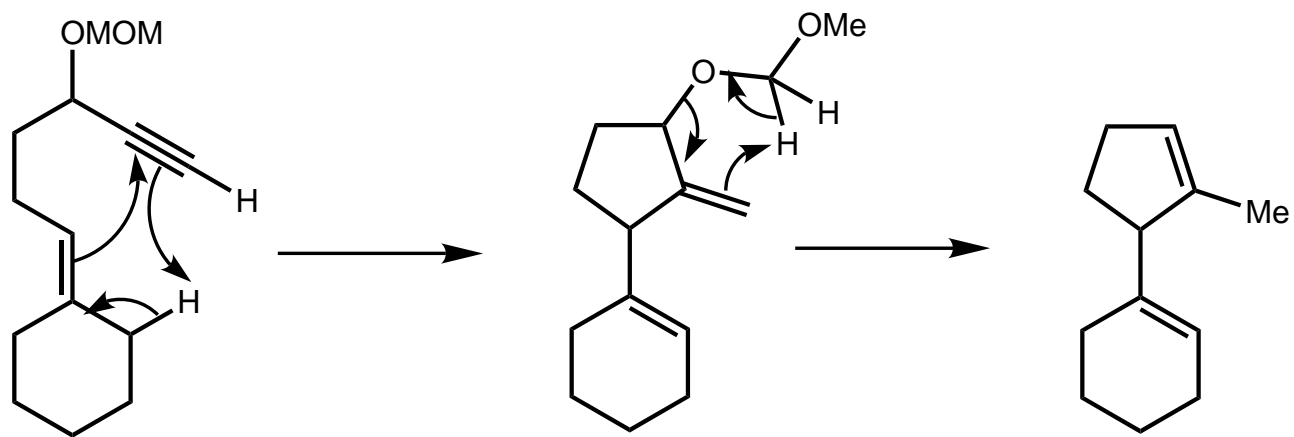
Altman, *J. Am. Chem. Soc.*, **1978**, 100, 3235.

# Syntheses of Chiral Acetic Acid

## Arigoni Ene Cascade - Racemic



Mechanism:

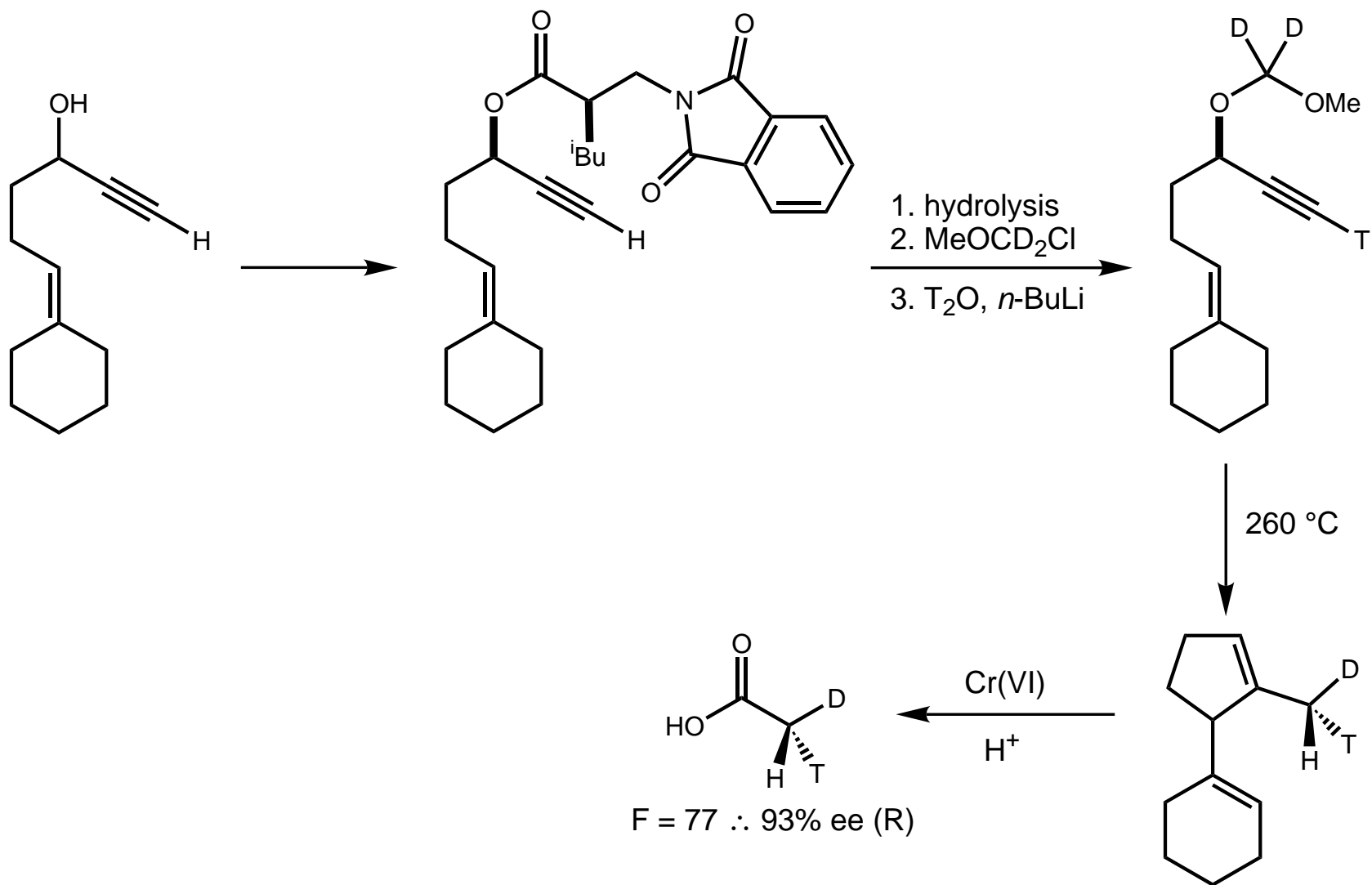


Observed by  
<sup>1</sup>H-NMR of an aliquot  
prior to completion

Arigoni, *Chem. Commun.* **1975**, 921.

# Syntheses of Chiral Acetic Acid

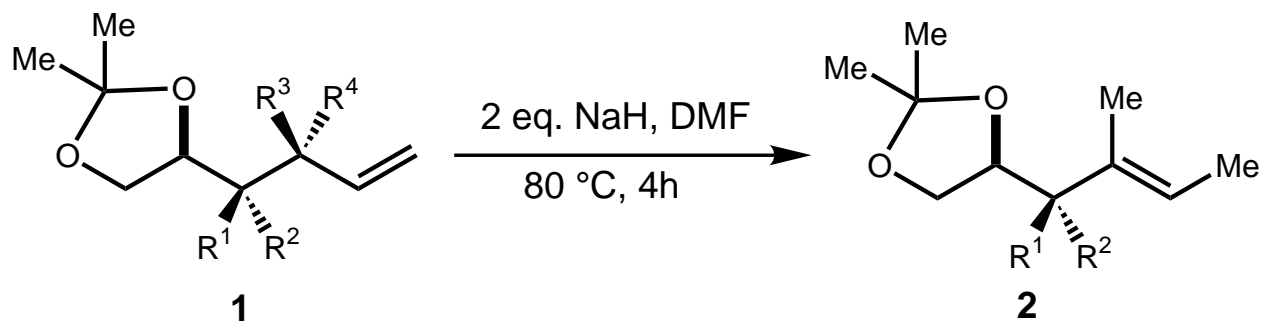
## Arigoni Ene Cascade - Non-racemic



Arigoni, *Chem. Commun.* **1975**, 921.

# Syntheses of Chiral Acetic Acid

Mulzer and Arigoni [1,3]-H-Shift

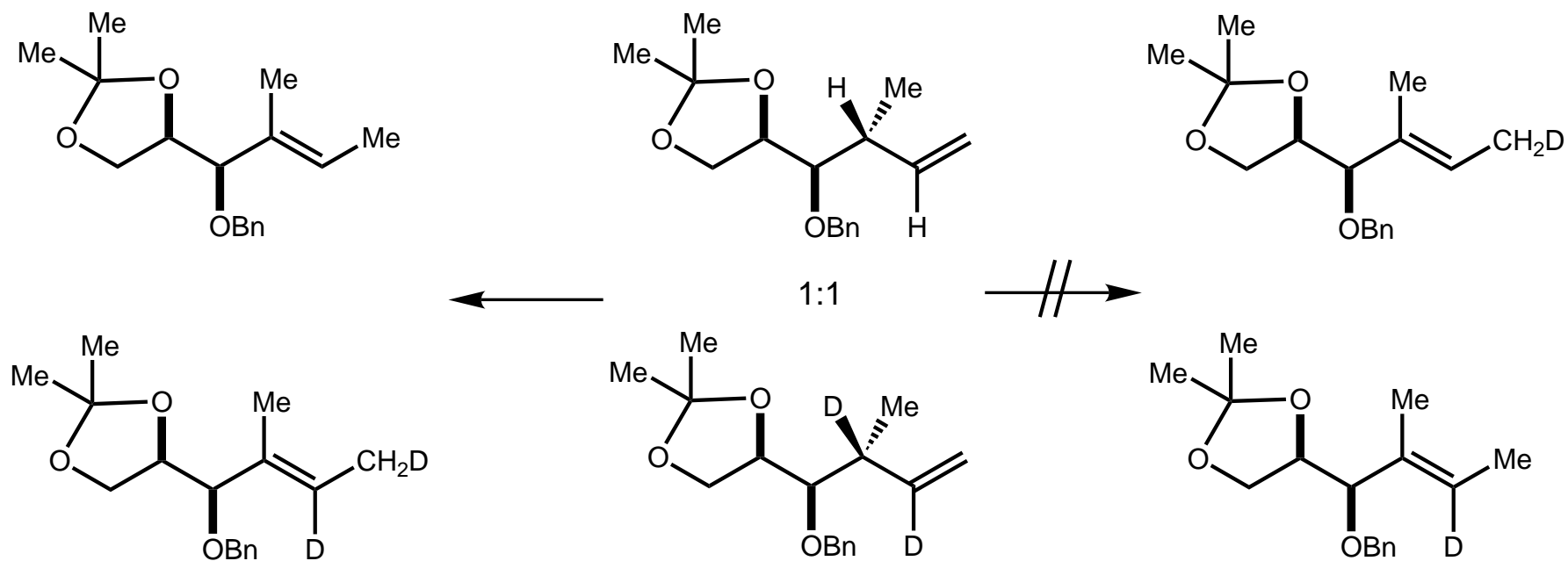


Educt	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	Product	Yield
1a	OBn	H	H	Me	2a	>95
1b	H	OBn	Me	H	2b	>95
1c	H	OBn	H	Me	2c	>90
1d	OPMB	H	H	Me	2d	67
1e	<i>Op</i> -ClBn	H	H	Me	2e	63
1f	OTHP	H	H	Me	2f	38
1g	OMe	H	H	Me	2g	3

# Syntheses of Chiral Acetic Acid

Mulzer and Arigoni [1,3]-H-Shift

Crossover experiment:

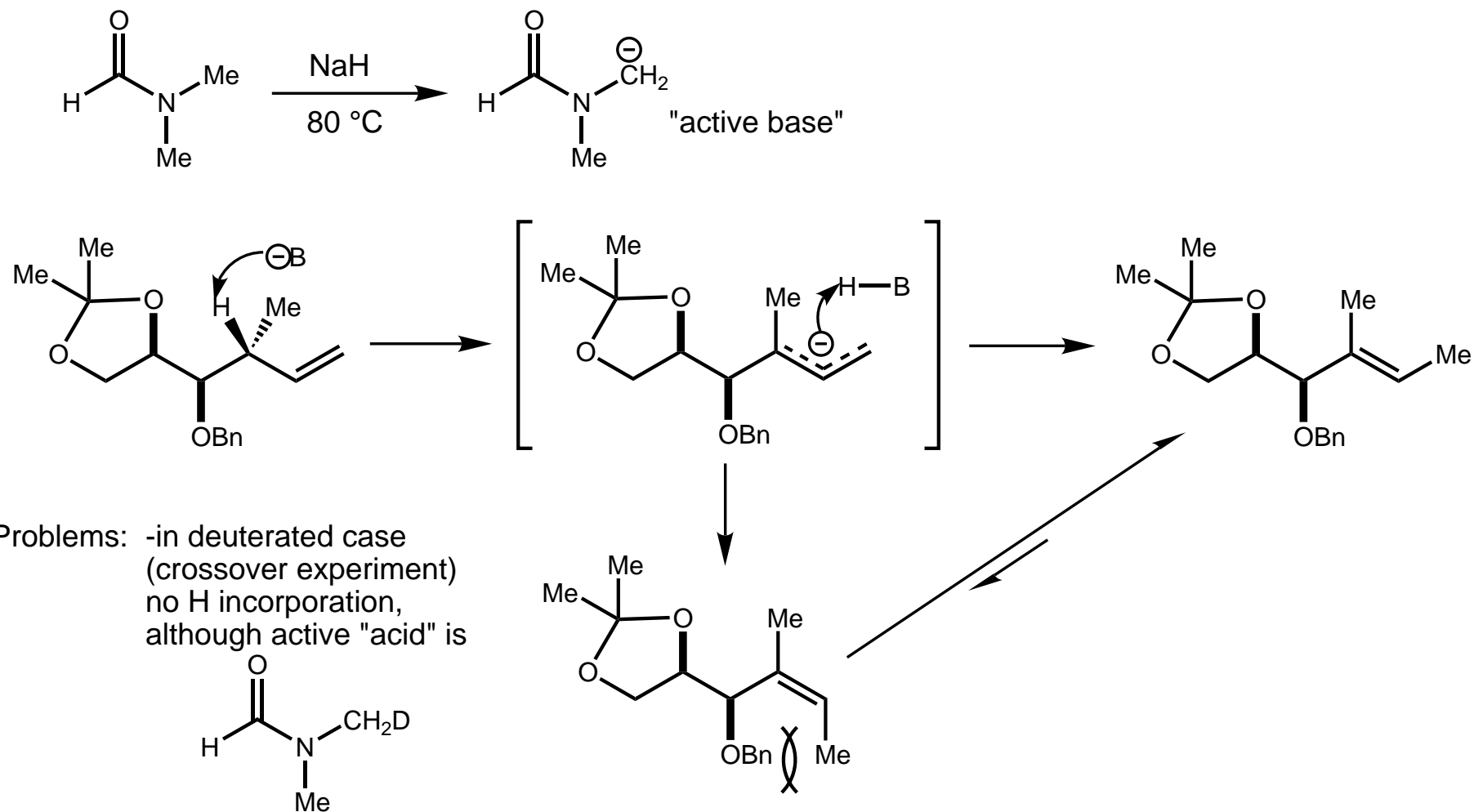


- appears to be exclusively an intramolecular [1,3]-H-shift

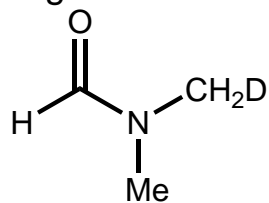
# Syntheses of Chiral Acetic Acid

Mulzer and Arigoni [1,3]-H-Shift

Mechanistic proposal:



Problems: -in deuterated case  
(crossover experiment)  
no H incorporation,  
although active "acid" is

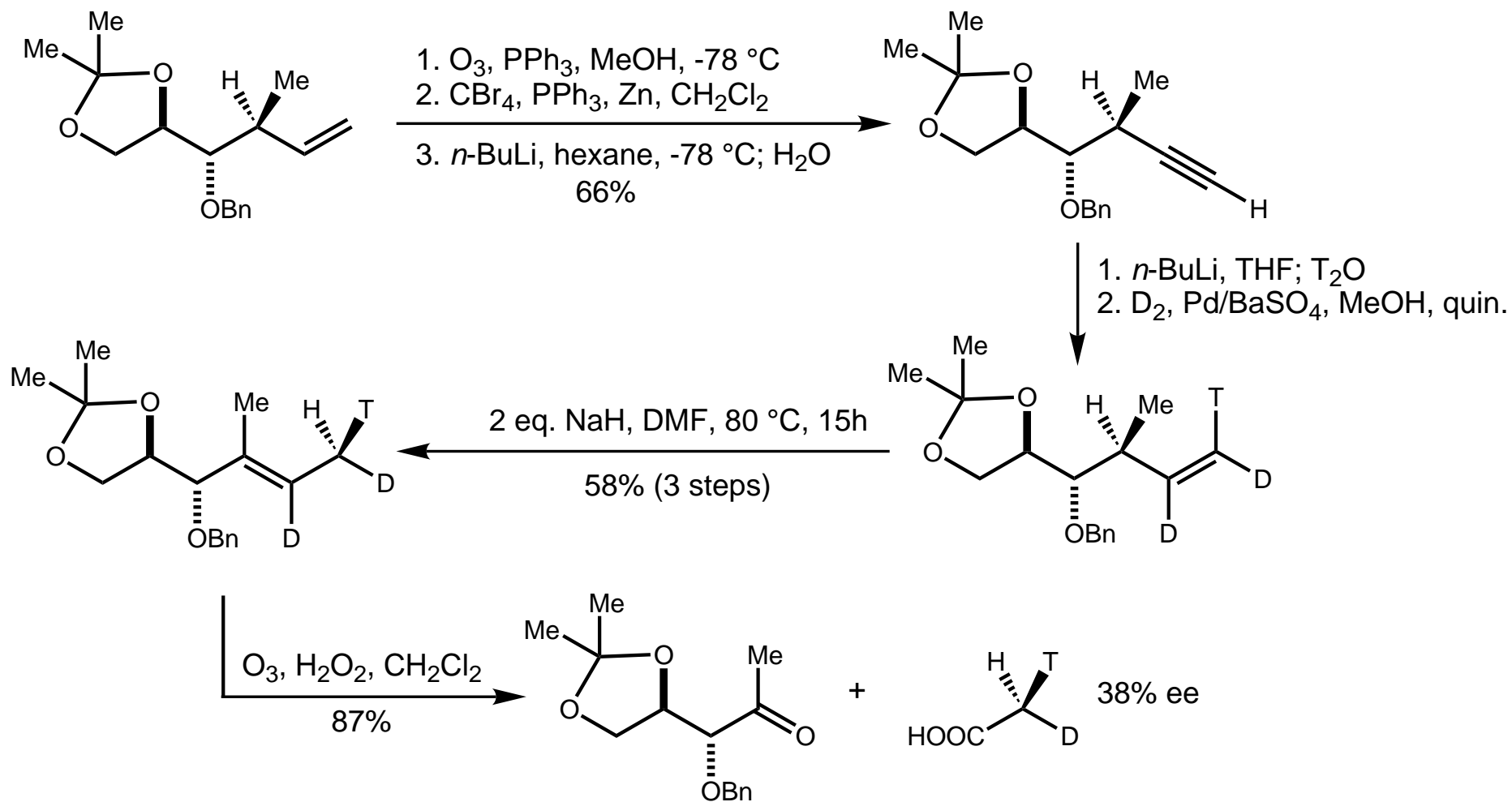


-necessity of benzyl not clear

Mulzer and Arigoni, *Tetrahedron Lett.* **1997**, 31, 5469.

# Syntheses of Chiral Acetic Acid

Mulzer and Arigoni [1,3]-H-Shift

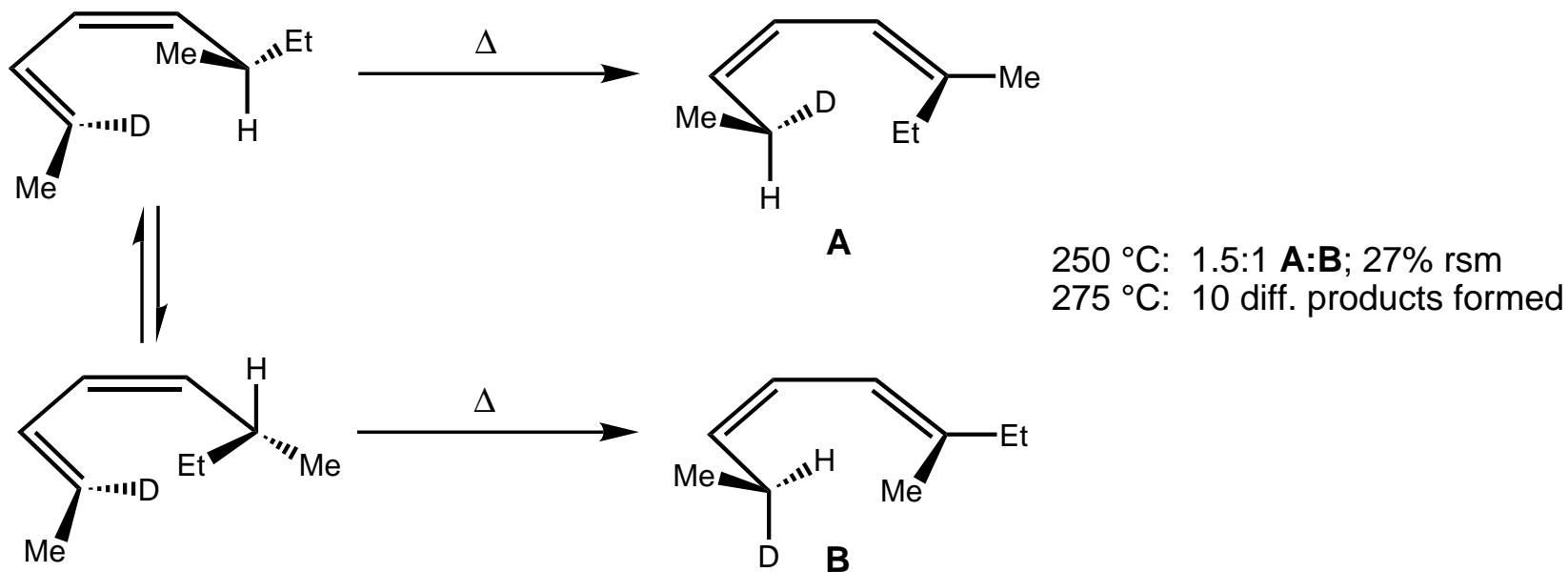




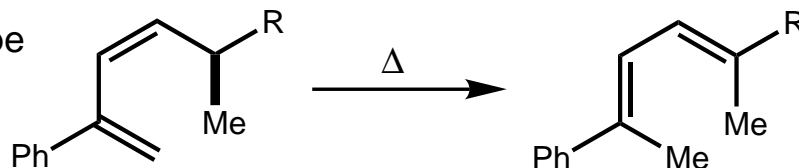
# Syntheses of Chiral Acetic Acid

## Mulzer and Floss [1,5]-H-Shift

- Roth's experiment:



Idea: use substrates of the type



- benefits: two disubstituted double bonds → two trisubstituted double bonds  
cross conjugated system → linear conjugated system  
phenyl group stabilizes toward polymerization and thermodimerization

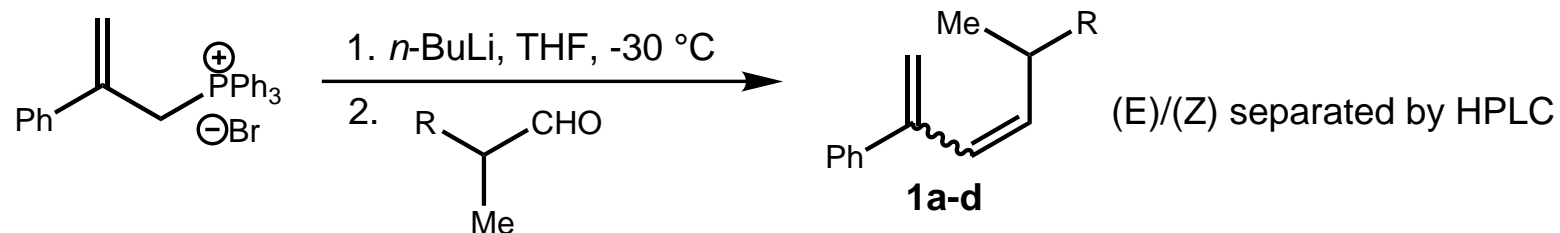
Roth, *Chem. Ber.* **1970**, 103, 426.

Mulzer and Floss, *J. Am. Chem. Soc.* **1999**, 121, 10848.

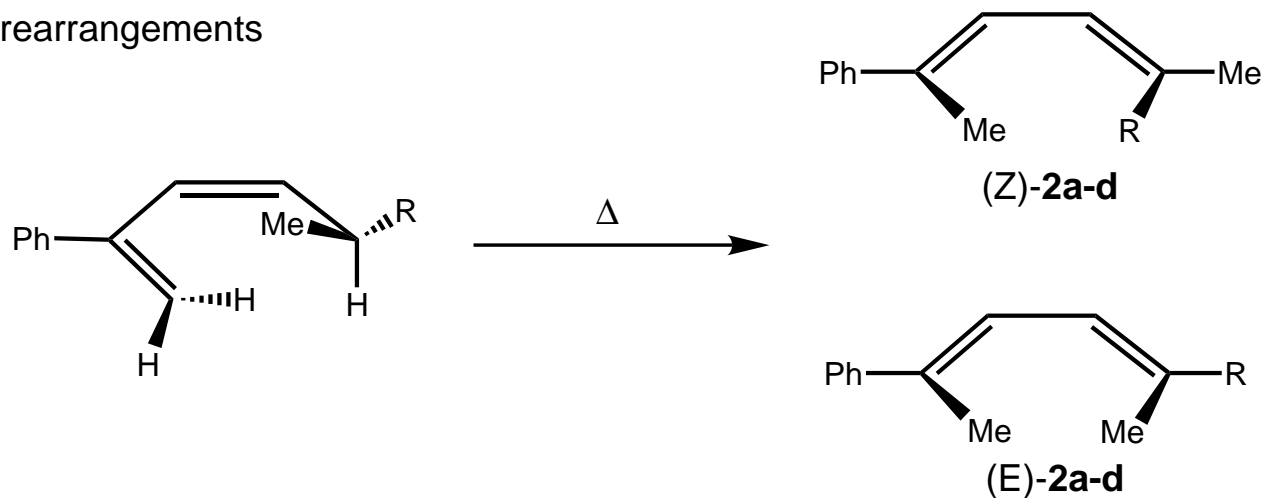
# Syntheses of Chiral Acetic Acid

Mulzer and Floss [1,5]-H-Shift

- synthesis of test substrates



- rearrangements

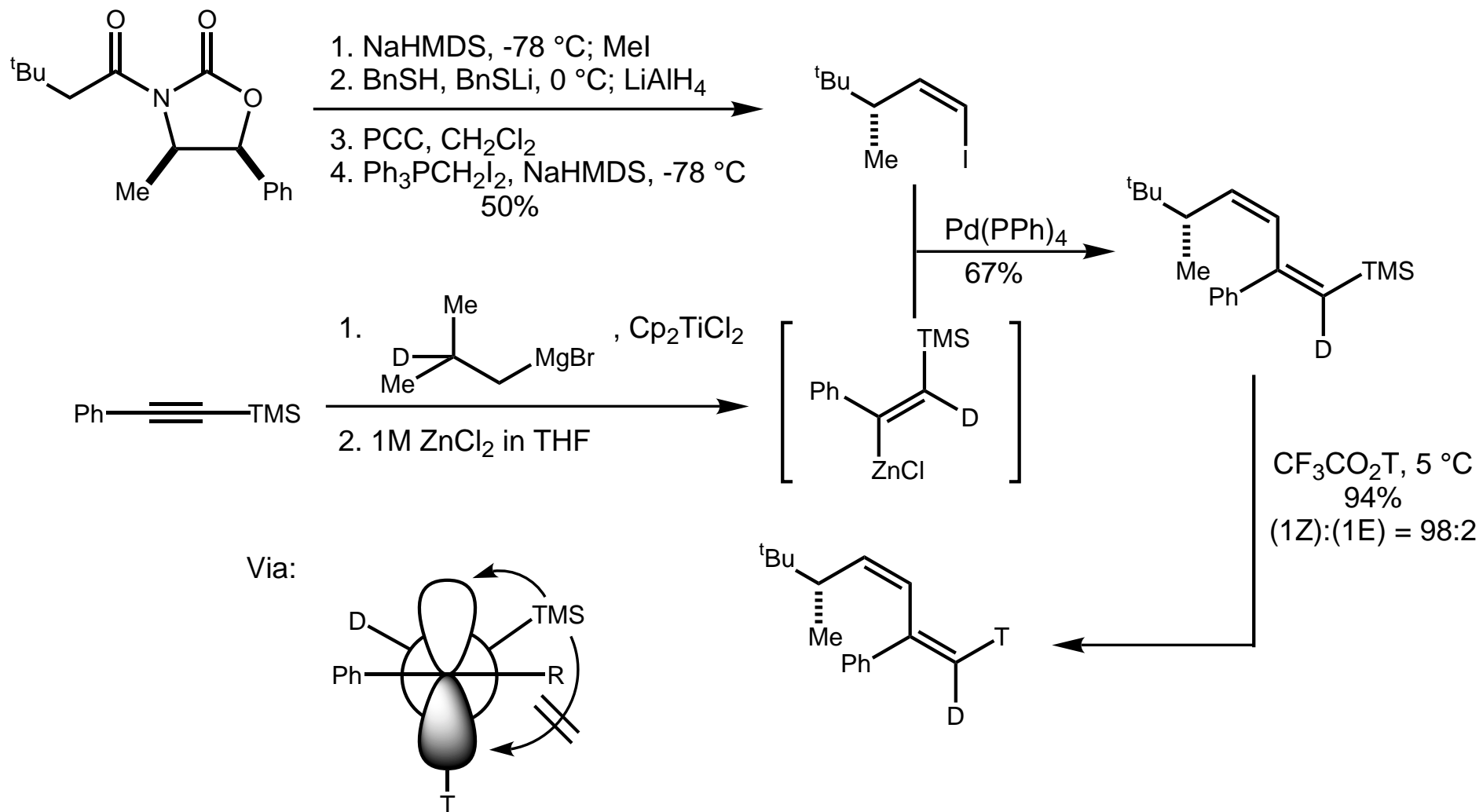


(Z)- <b>1 a</b>	R = Et	(E)/(Z)- <b>2 a</b>	= 1.5:1
<b>b</b>	= <sup>i</sup> Pr	<b>b</b>	= 2.5:1
<b>c</b>	= <sup>c</sup> Hex	<b>c</b>	= 3.2:1
<b>d</b>	= <sup>t</sup> Bu	<b>d</b>	= >100:1

Mulzer and Floss, *J. Am. Chem. Soc.* **1999**, 121, 10848.

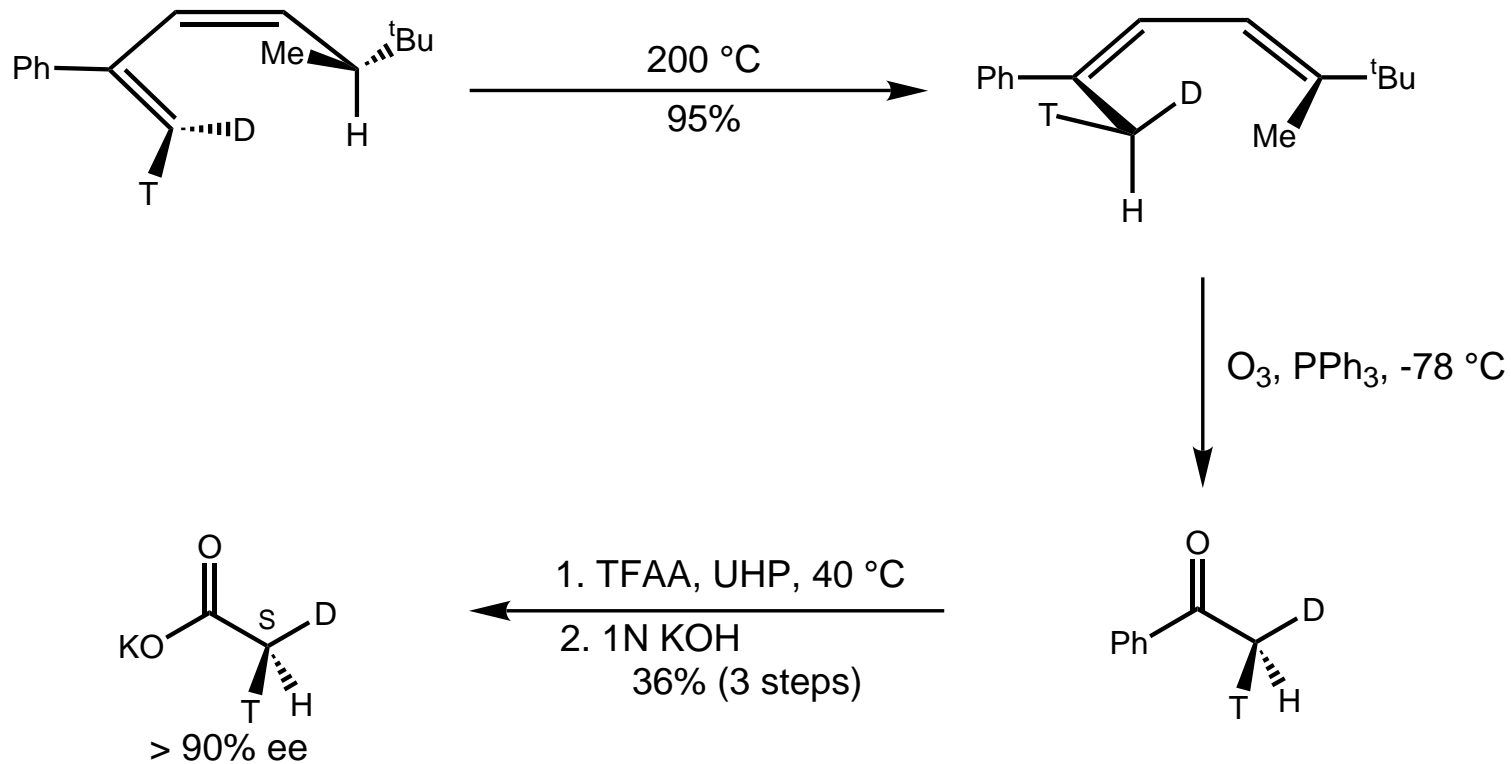
# Syntheses of Chiral Acetic Acid

Mulzer and Floss [1,5]-H-Shift



# Syntheses of Chiral Acetic Acid

Mulzer and Floss [1,5]-H-Shift

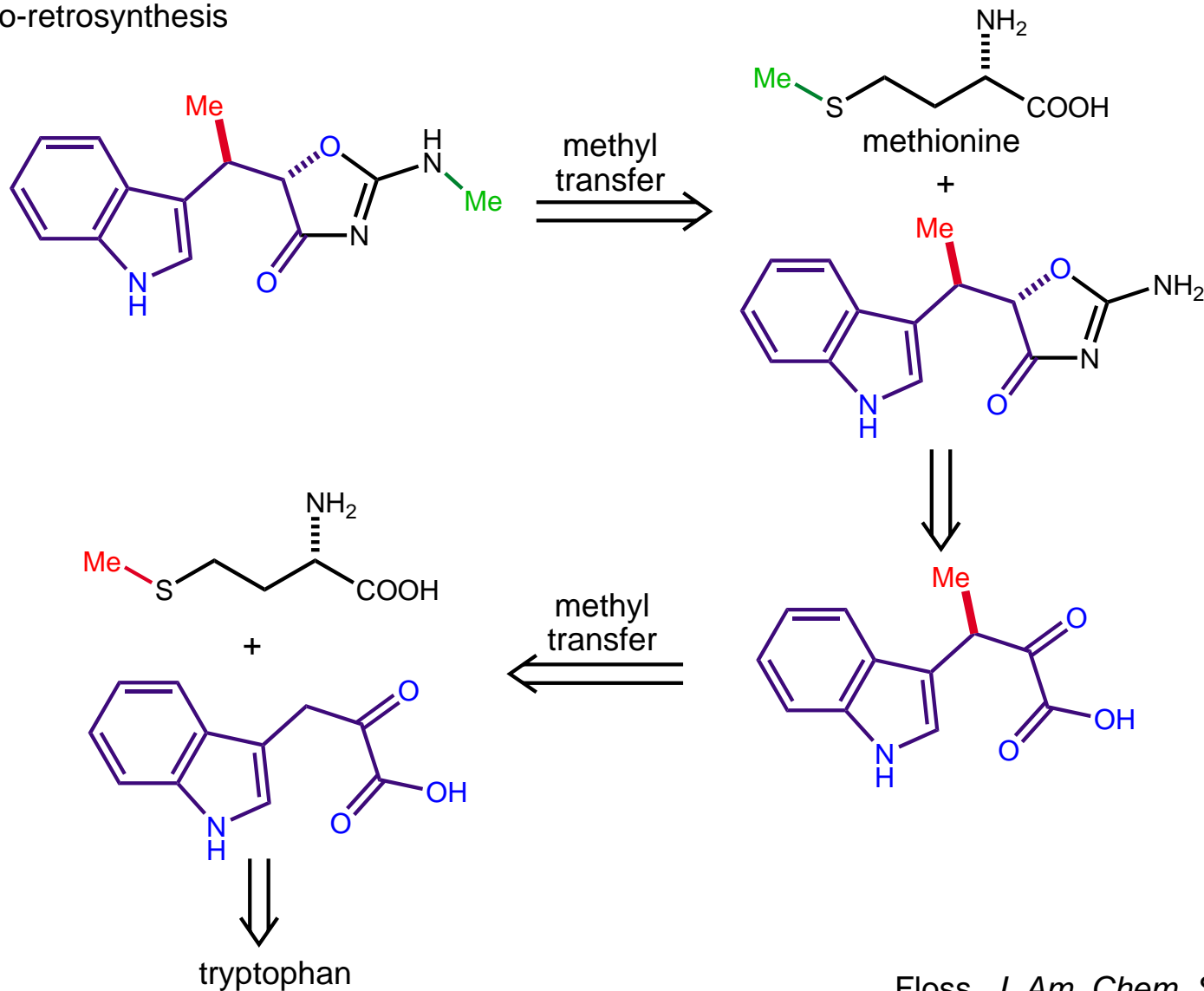


Mulzer and Floss, *J. Am. Chem. Soc.* **1999**, 121, 10848.

# Mode of Action of Methyl Transferases

## C- and N-Methylation in Indolmycin Biosynthesis

- Bio-retrosynthesis

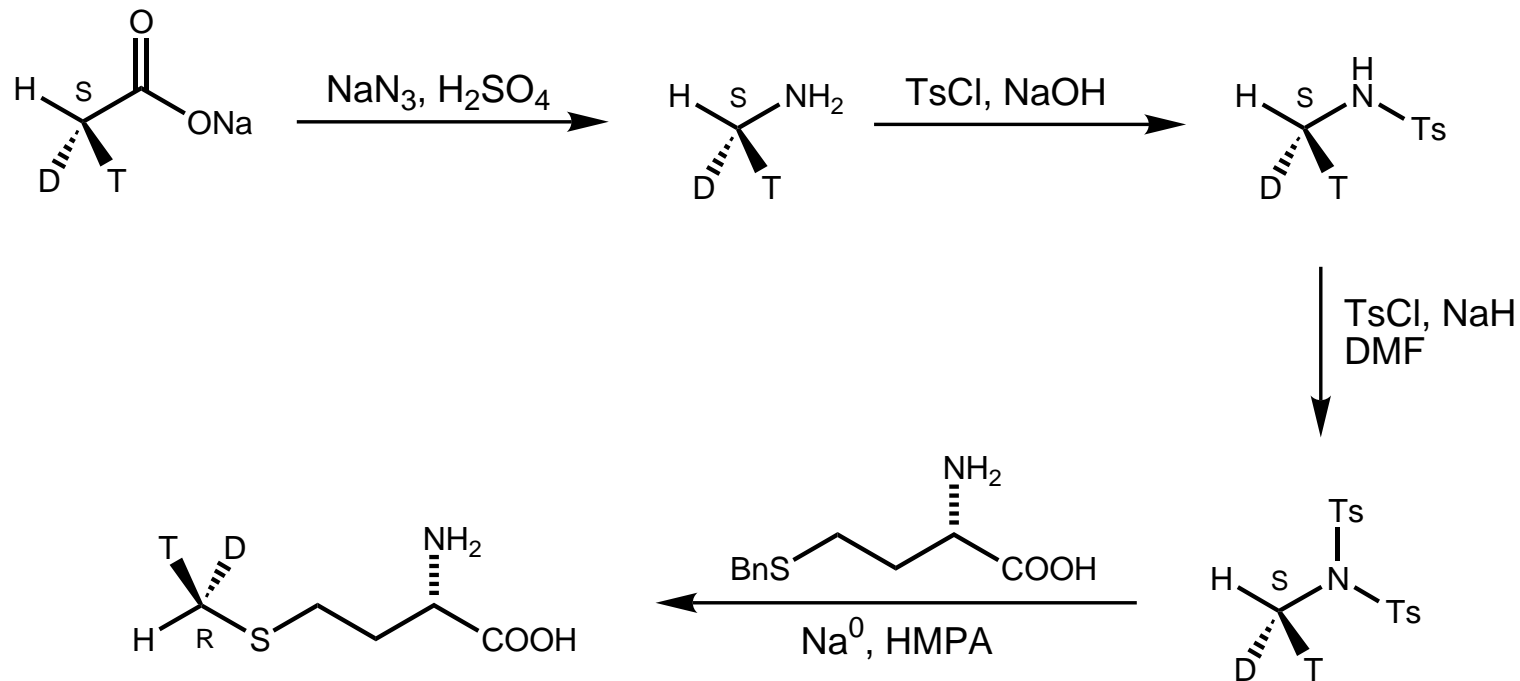


Floss, *J. Am. Chem. Soc.* **1971**, 93, 3029.  
Floss, *J. Biol. Chem.*, **1975**, 250, 7819.

# Mode of Action of Methyl Transferases

## C- and N-Methylation in Indolmycin Biosynthesis

- synthesis of methionine with a chiral methyl group

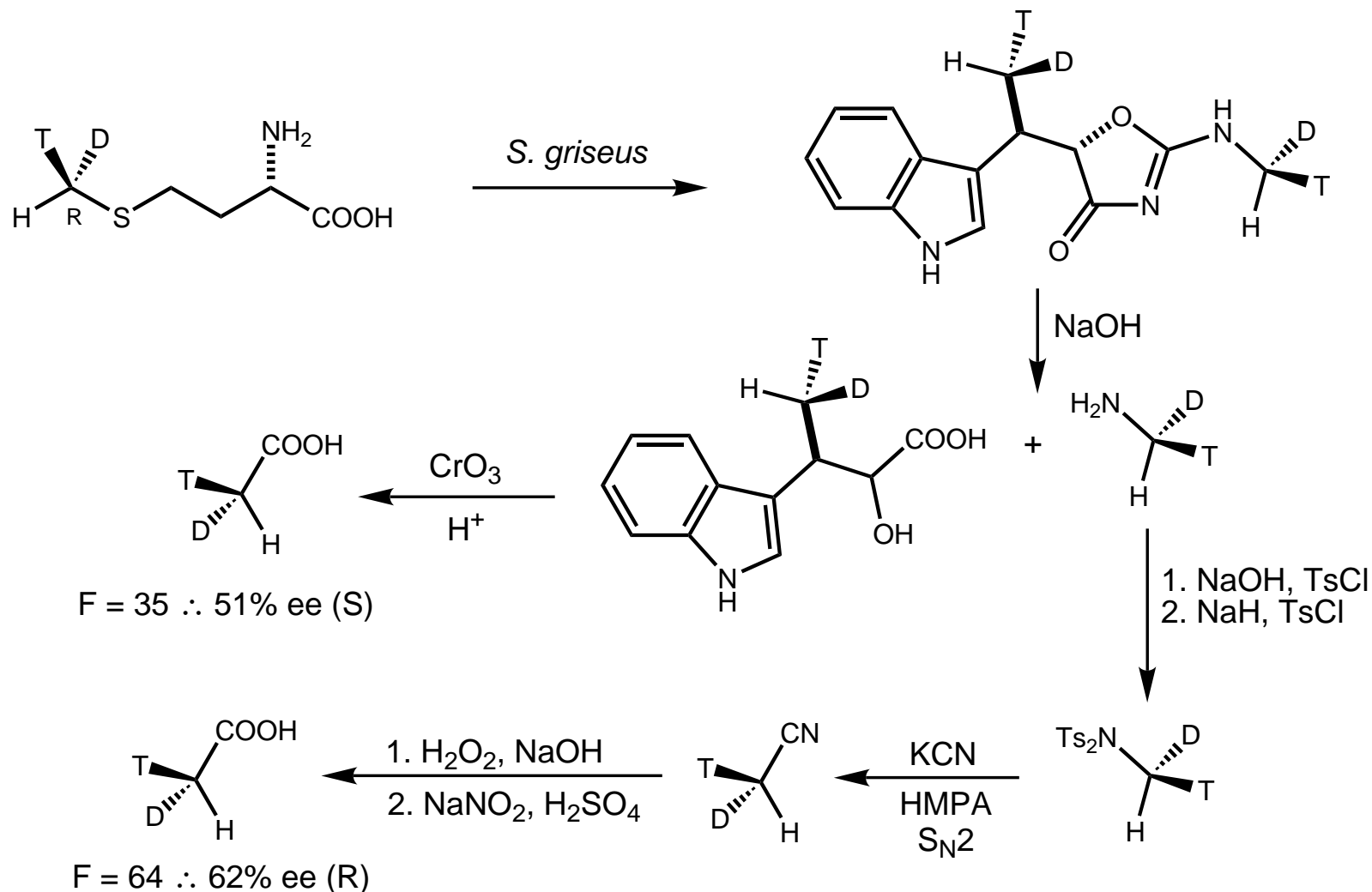


No appreciable change in  $^3\text{H} / ^{14}\text{C}$  ratio from acetate indicating insignificant proton exchange

Floss, *J. Am. Chem. Soc.* **1980**, *102*, 6314.

# Mode of Action of Methyl Transferases

## C- and N-Methylation in Indolmycin Biosynthesis



$\therefore$  Both methyl transfers proceed with inversion

Floss, *J. Am. Chem. Soc.* **1980**, *102*, 6314.

# Mode of Action of Methyl Transferases

## Examples of Methyl Transfers with Inversion

Enzyme or Product	Methylated Atom
vitamin B <sub>12</sub> (corrin ring)	carbon
loganin	oxygen
homocystein S-methyltransferase	sulfur
indolmycin	carbon
indolmycin	nitrogen
catechol O-methyltransferase	oxygen
phenylethanolamine N-methyltransferase	nitrogen
pectin	oxygen
histamine N-methyltransferase	nitrogen
asplasmomycin	carbon
4'-O-methylnorlaudanosoline 6-O-methyltransferase	oxygen
norreticulín N-methyltransferase	nitrogen
dimethylallyltryptophan N-methyltransferase	nitrogen
<i>EcoRI</i> DNA methyltransferase	nitrogen
<i>HhaI</i> DNA methyltransferase	carbon
t-RNA-uracil methyltransferase	carbon

"This may signify either that all these enzymes have a common evolutionary ancestor or that this mechanism is sufficiently superior to alternative ones to drive evolution."

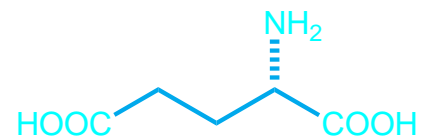
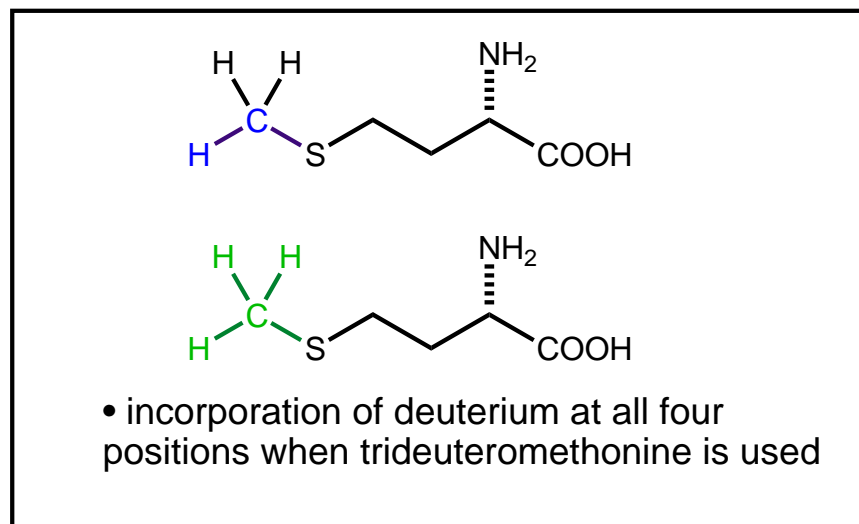
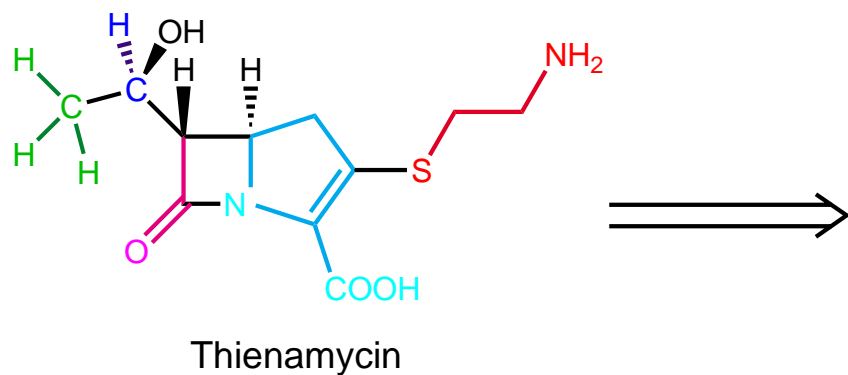
Floss, *Acc. Chem. Res.* **1993**, 26, 116.



# Mode of Action of Methyl Transferases

## Methyl Transfers with Retention - Thienamycin

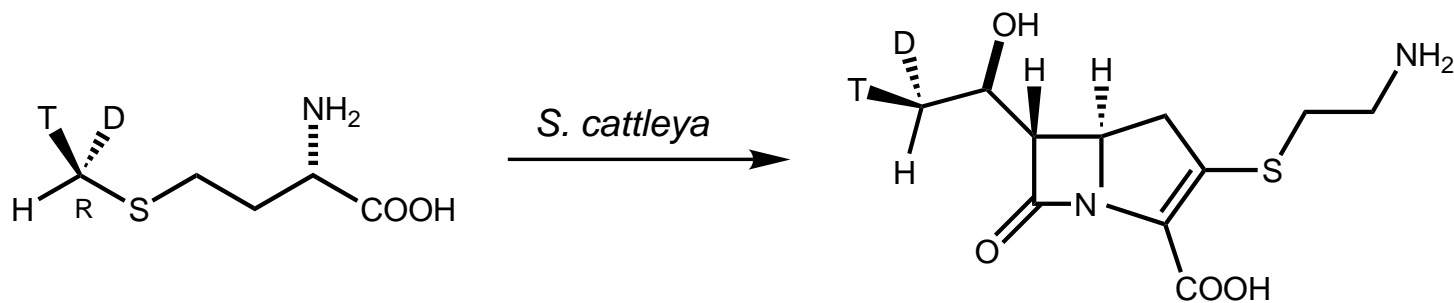
- Bio-retrosynthesis



Williamson, *J. Biol. Chem.* **1985**, *260*, 4637.

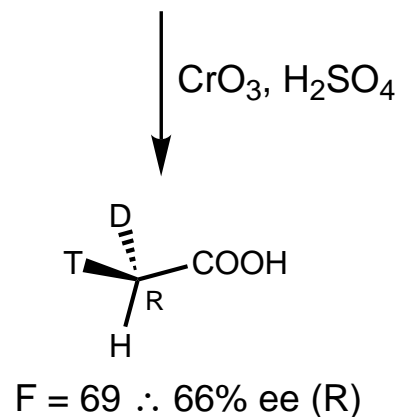
# Mode of Action of Methyl Transferases

## Methyl Transfers with Retention - Thienamycin



∴ **biosynthesis likely involves two methyl transfers**

- cobalt is required for thienamycin biosynthesis,  
so a corrin intermediate is possible



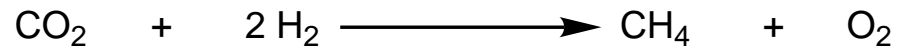
- a net retention has since been demonstrated in biosynthesis of thiostrepton as well

Floss, *J. Am. Chem. Soc.* **1986**, 108, 5635.  
Floss, *J. Am. Chem. Soc.* **1989**, 111, 7274.

# Steric Course of Methanogenesis

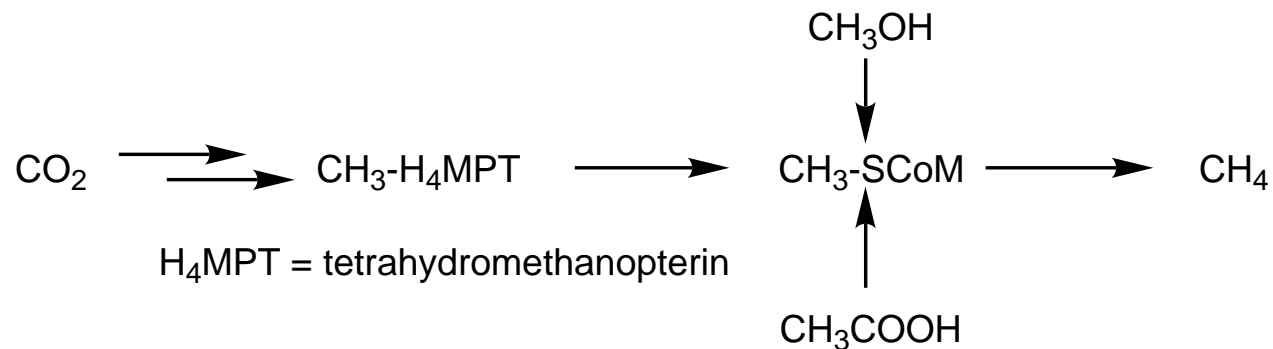
## Introduction

- methanogenesis: the conversion of carbon dioxide into methane



- methanogens: organisms capable of affecting this transformaton; *M. barkeri* is an example

- *M. barkeri* methanogenesis pathway:

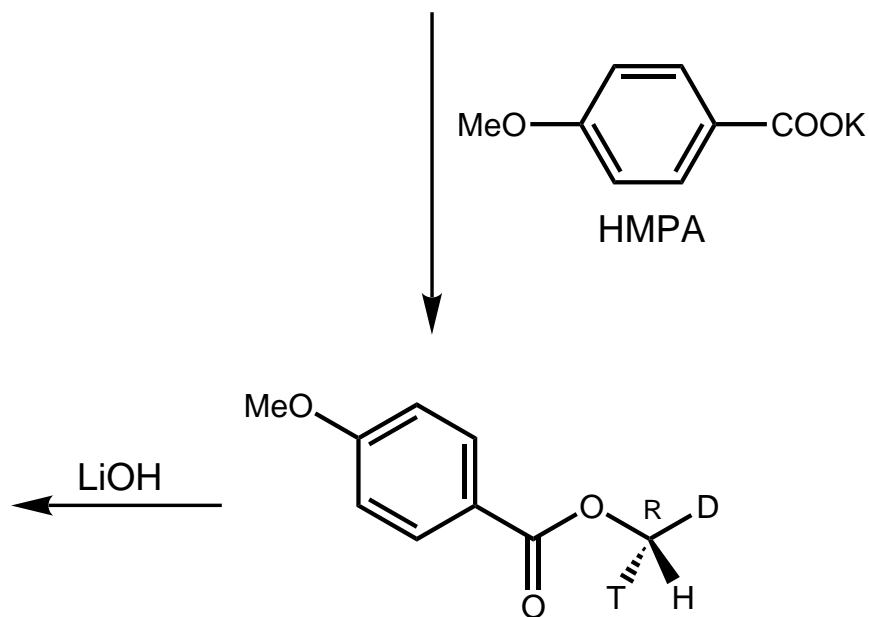
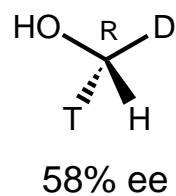
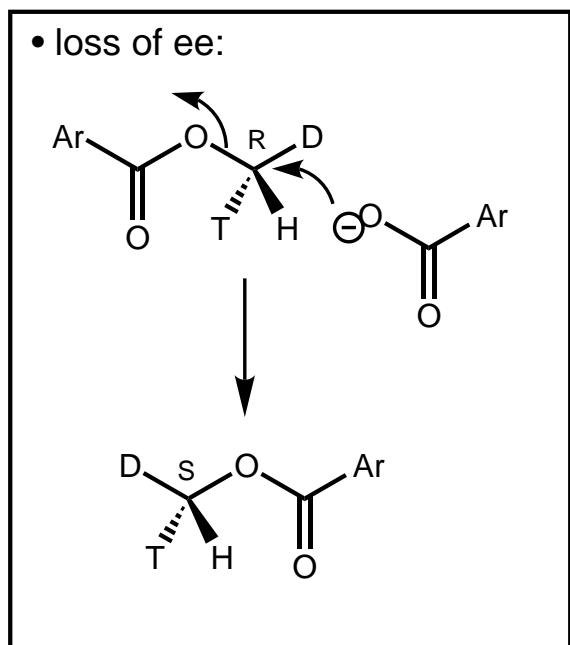
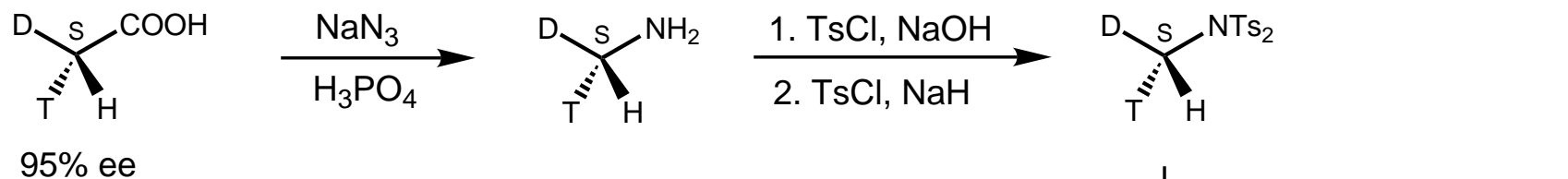


- also able to convert methanol and acetic acid to methane

Wolfe, *J. Biol. Chem.* **1988**, 263, 7913.  
Wolfe, *Annu. Rev. Biochem.* **1990**, 59, 355.

# Steric Course of Methanogenesis

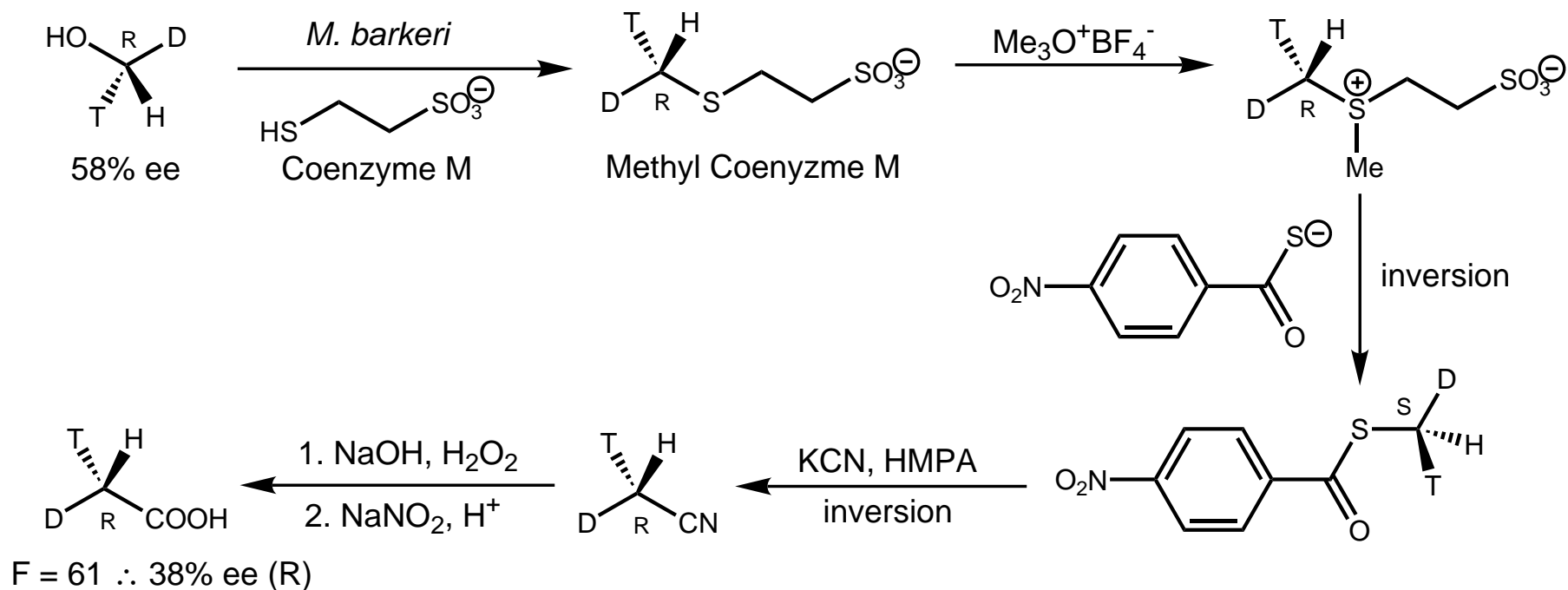
## Synthesis of Chiral Methanol



Floss, *J. Am. Chem. Soc.*, **1987**, 109, 7922.

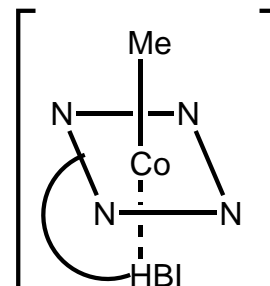
# Steric Course of Methanogenesis

## Methylation of Coenzyme M



- two inversions in chemical degradation ∴ same config. as methyl coenzyme M  
∴ **overall retention in methylation of coenzyme M**

- supports hypothesis that methylation proceeds via

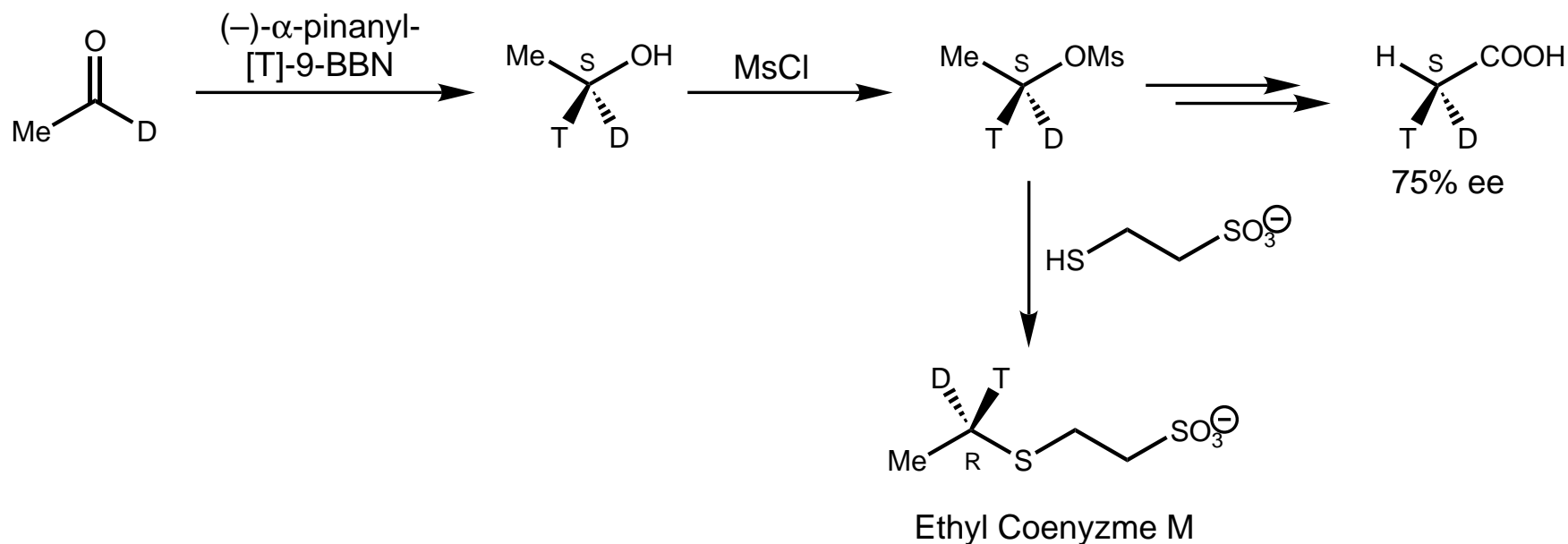


Floss, *J. Am. Chem. Soc.*, **1987**, 109, 7922.

# Steric Course of Methanogenesis

## Reduction of Alkyl Coenzyme M

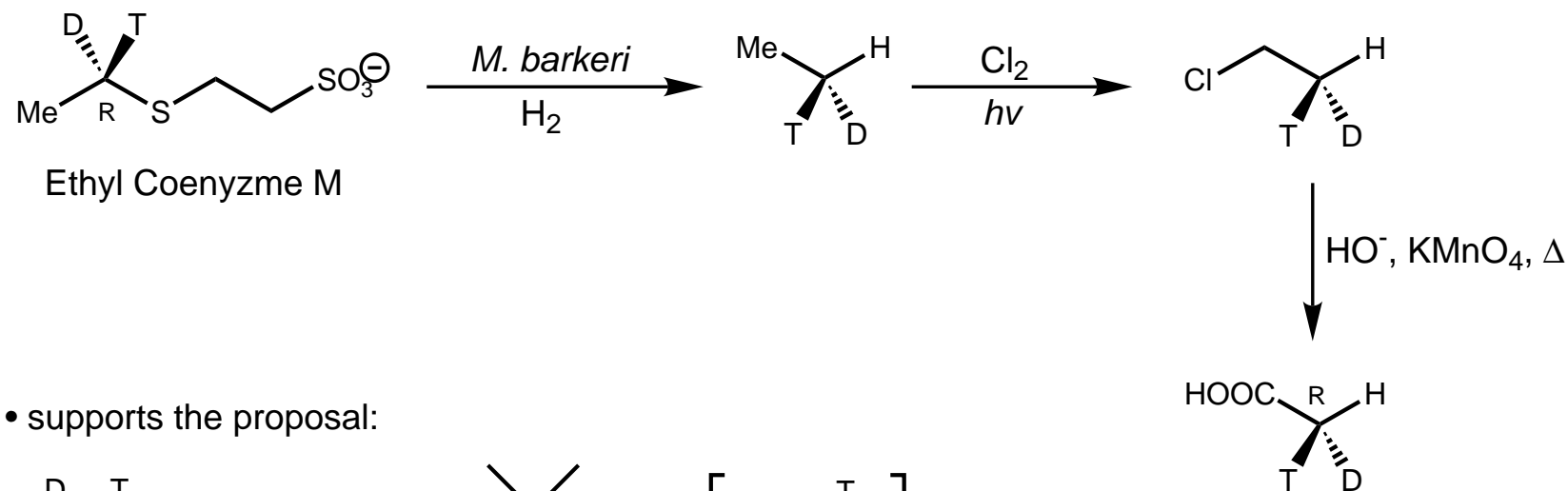
- Methyl Coenzyme M reduction product:  $\text{CH}_4$ ; can't study because no chiral center with only three H isotopes
- can study reduction of Ethyl Coenzyme M - *M. barkeri* known to reduce at 20% the rate of methyl
- synthesis of chiral Ethyl Coenzyme M:



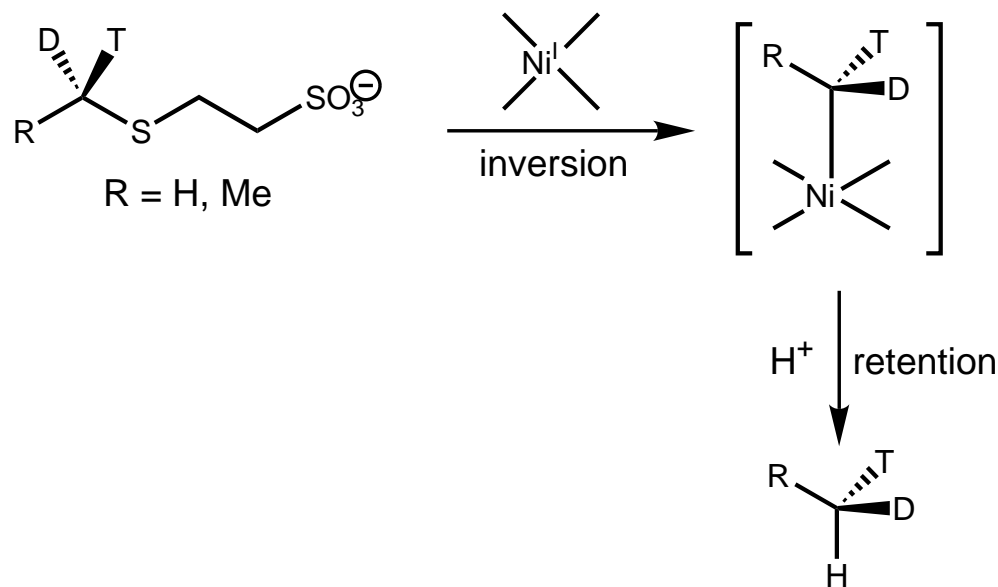
Floss, *J. Am. Chem. Soc.*, **1991**, 113, 4700.

# Steric Course of Methanogenesis

## Reduction of Alkyl Coenzyme M



• supports the proposal:



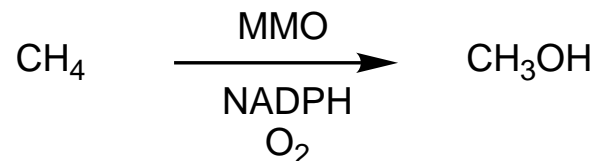
$F = 65 \therefore 51\% ee (R)$   
 $\therefore$  **net inversion of configuration**

Floss, *J. Am. Chem. Soc.*, **1991**, 113, 4700.

# Mode of Action of Methane Monooxygenase

## Introduction

- methane monooxygenase (MMO) catalyzes the NAD(P)H- and O<sub>2</sub>-dependant oxidation of methane to methanol



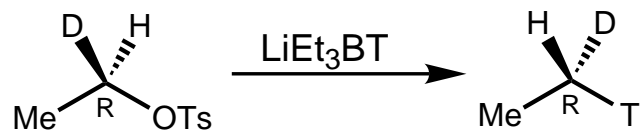
- two distinct types of MMO exist: soluble MMO (sMMO) and particulate MMO (pMMO)
  - sMMO is a non-heme iron-containing enzyme
  - pMMO is a copper-containing enzyme
  - pMMO is favored in organisms containing both forms so long as there is a sufficient concentration of copper ions
  - until recently, pMMO has been difficult to study due to isolation problems
- several mechanisms have been proposed:
  - radical intermediate
  - cation intermediate
  - concerted insertion into C-M bond

Floss and Lipscomb, *J. Am. Chem. Soc.* **1992**, *114*, 7561.  
Chan and Floss, *J. Am. Chem. Soc.* **1996**, *118*, 921.

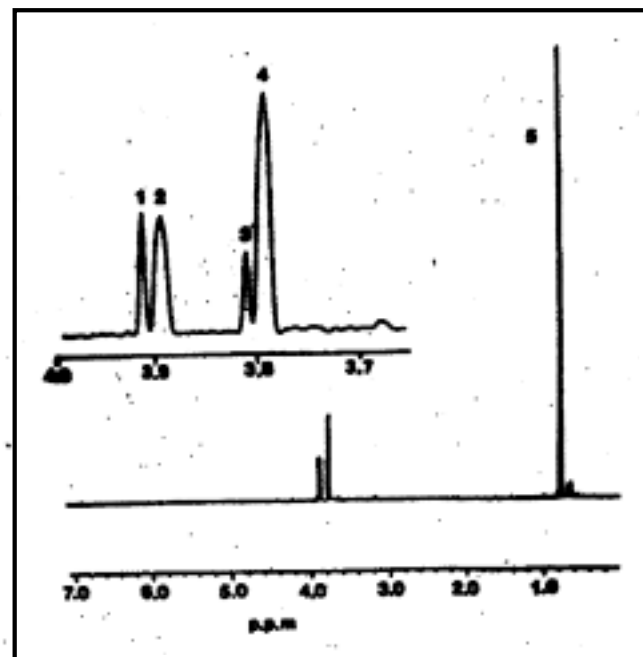
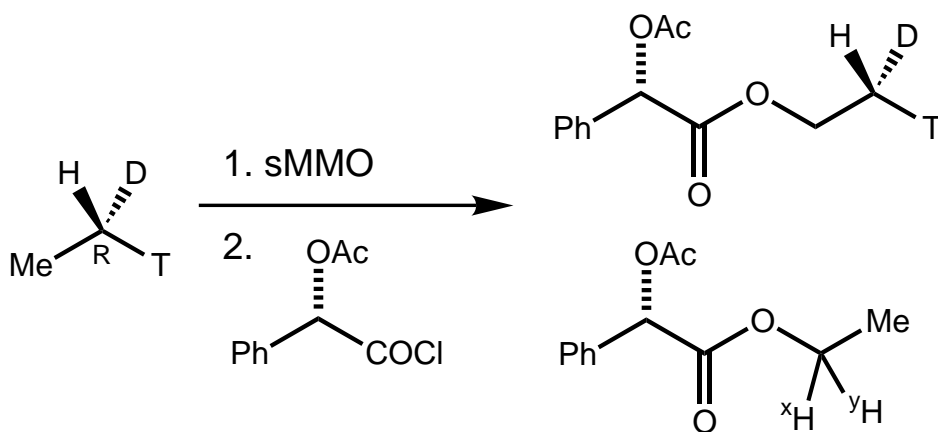


# Mode of Action of Methane Monooxygenase

## Synthesis and Oxidation of Chiral Ethane



• to date, this is the lowest molecular weight chiral molecule synthesised in enantioenriched form



$^3\text{H}\{^1\text{H}, ^2\text{H}\}$ -NMR Spectrum at 320 MHz

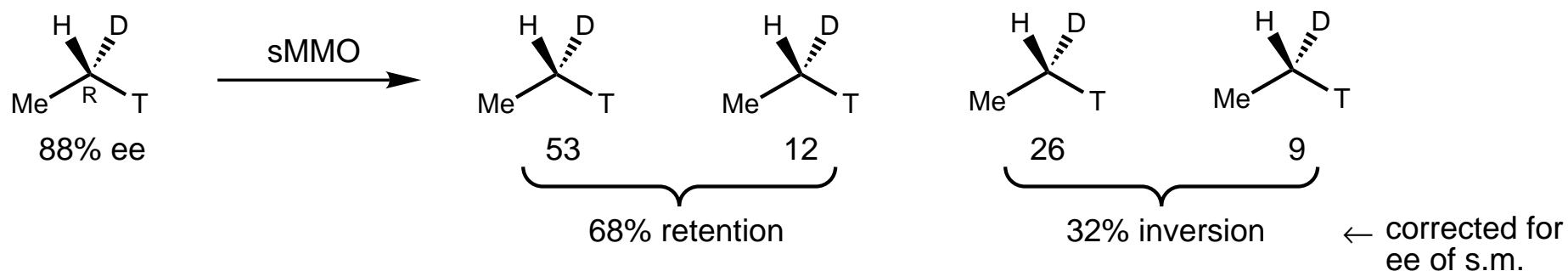
Floss and Lipscomb, *J. Am. Chem. Soc.* **1992**, *114*, 7561.

# Mode of Action of Methane Monooxygenase

## Evidence for a Radical Intermediate with sMMO

- Parker method can be used to assign configuration of each ester

- the results are (ignoring oxidation at unlabeled carbon):



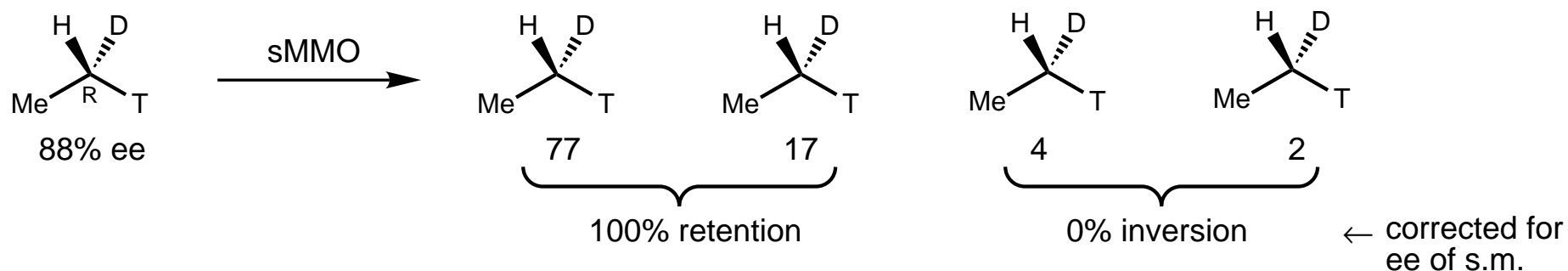
- supports the formation of an extremely short-lived radical species in mechanism
- barrier to bond rotation in ethyl radical = 0.15 kcal/mol  $\rightarrow$  rate for rotation =  $4.9 \times 10^{12} \text{ s}^{-1}$   
 $\therefore$  **estimated rate constant for oxidation =  $5.5 \times 10^{12} \text{ s}^{-1}$**

Floss and Lipscomb, *J. Am. Chem. Soc.* **1992**, *114*, 7561.

# Mode of Action of Methane Monooxygenase

## Evidence for a Concerted Mechanism with pMMO

- identical experiment to that used with sMMO was performed using pMMO



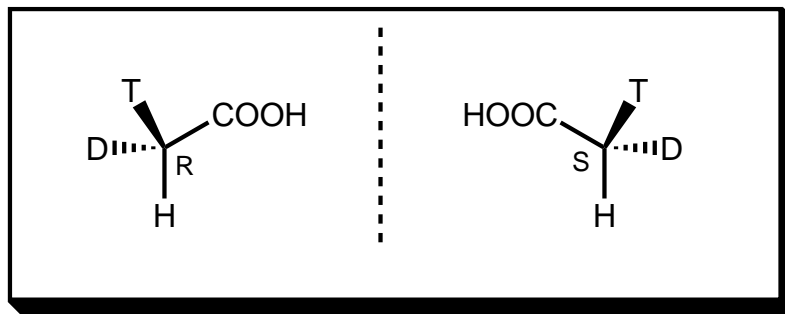
These results indicate that a radical intermediate is unlikely in this case, as it would need to have a lifetime of  $<10^{-14}$  s in order to exhibit complete retention

"Data instead point to a mechanism in which C-H bond cleavage is preceded by bond formation at the alkyl carbon, i.e., one proceeding through a pentacoordinated carbon species."

Chan and Floss, *J. Am. Chem. Soc.* **1996**, *118*, 921.

# Chiral Methyl Groups

## A Summary



- many syntheses of chiral acetic acid have been completed, ranging from displacements with nucleophilic tritide to elegant molecular rearrangements
- absolute configuration and enantiomeric excess can be determined using the Arigoni / Cornforth enzyme method (most sensitive) or using  $^3\text{H}$ -NMR of a chiral amine derivative (most accurate)
- chiral methyl groups can be used to determine the steric course of many biological reactions
- "At age [31] chiral methyl groups have made it through their teens [and twenties] and they still look beautiful."  
Floss, *Acc. Chem. Res.* **1993**, 26, 122.