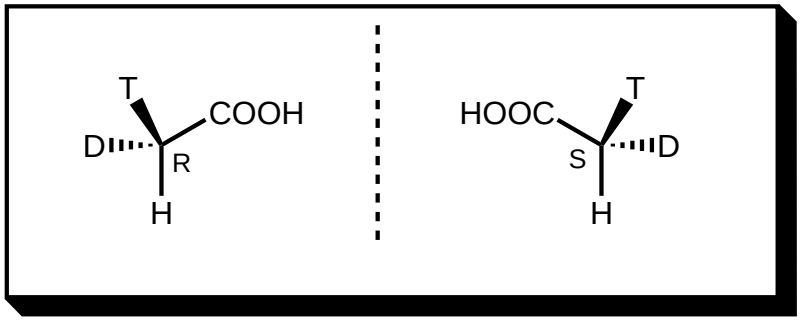
Chiral Methyl Groups

Synthesis and Applications

Evans Group Seminar

January 12, 2001

Jason Burch

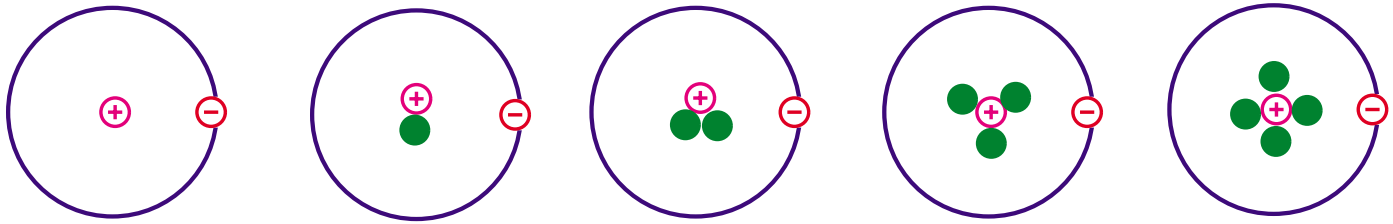


I. Introduction and First Syntheses of Chiral Acetic Acid
II. Determination of Configuration and Enantiomeric Excess
III. Syntheses of Chiral Acetic Acid
  (a) Nucleophilic Tritiide
  (b) Molecular Rearrangements
IV. Biological Applications
  (a) Methyl Transferases
  (b) Methanogenesis
  (c) Methane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

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

Isotopes of Hydrogen

Introduction



Chemical Symbol	$^{1}_{1}H$	$^{2}_{1}H$	$^{3}_{1}H$	$^{4}_{1}H$	$^{5}_{1}H$
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*, 2nd ed., Wiley, New York, **1974**, pp. 1-13.

Isotopes of Hydrogen

Tritium

• radioactive decay

$$^{3}H \rightarrow {}^{3}He + \beta^{-}$$

• natural occurrence

- following collisions induced by cosmic radiation in the upper atmosphere

$$^{14}N + {}^{1}n \rightarrow {}^{3}H + {}^{12}C$$
$$^{14}N + {}^{1}H \rightarrow {}^{3}H + \text{fragments}$$
$$^{2}H + {}^{2}H \rightarrow {}^{3}H + {}^{1}H$$

• laboratory synthesis

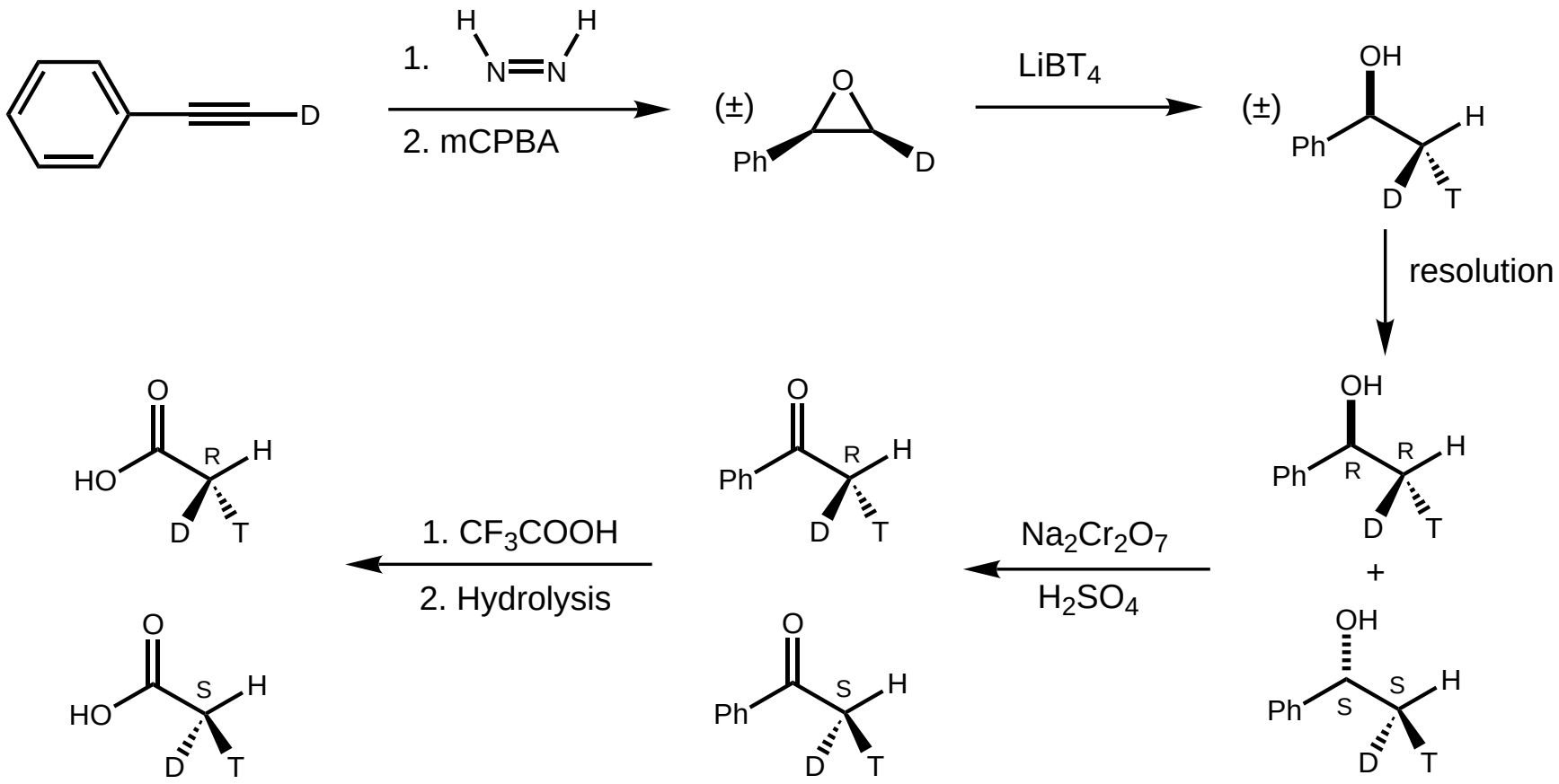
$$^{9}Be + {}^{2}H \rightarrow {}^{3}H + {}^{8}Be$$
$$^{6}Li + {}^{1}n \rightarrow {}^{3}H + {}^{4}He \leftarrow \text{most common}$$
$$^{3}He + {}^{1}n \rightarrow {}^{3}H + {}^{1}H$$

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

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

The First Syntheses of Chiral Acetic Acid

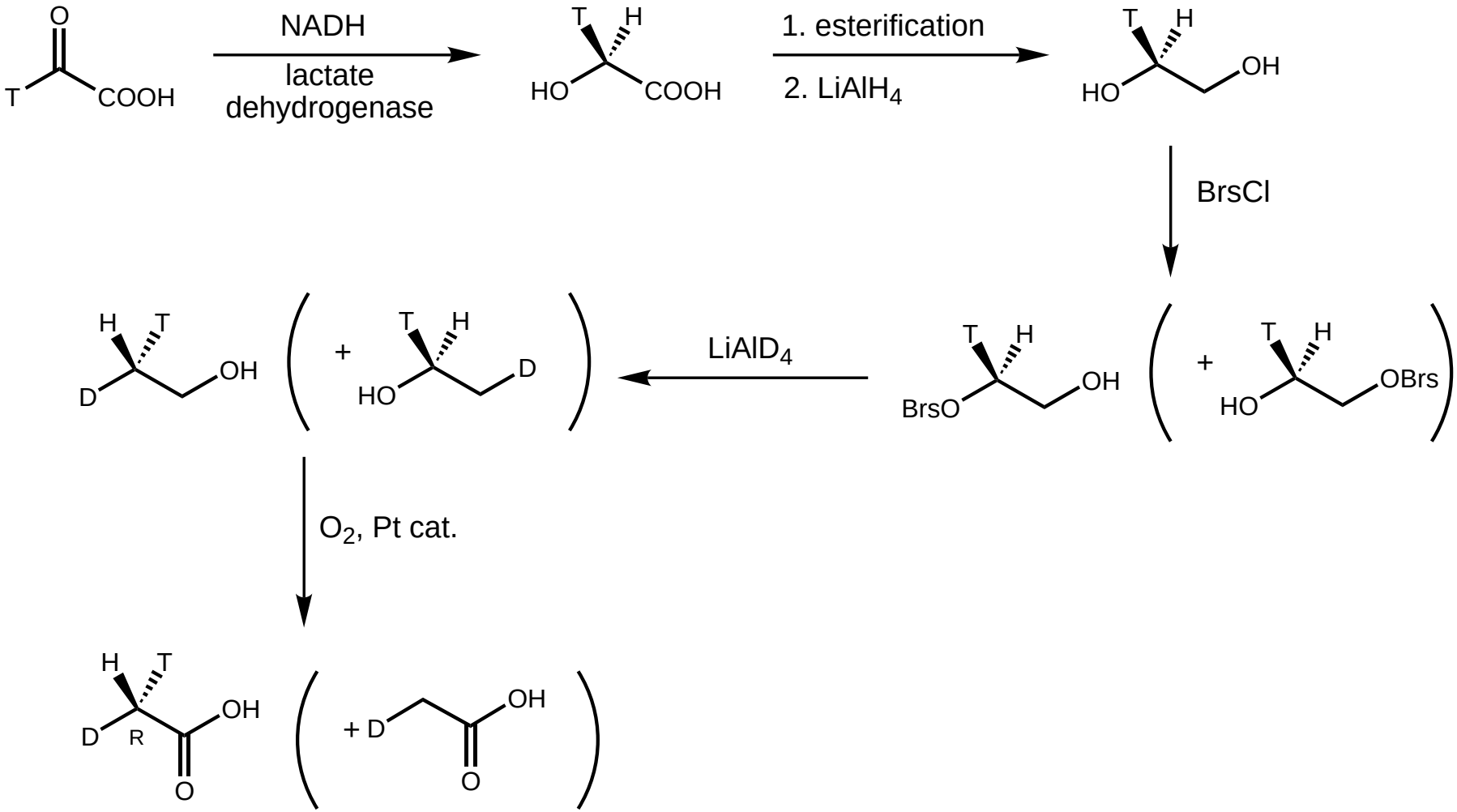
Cornforth Epoxide Opening



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

The First Syntheses of Chiral Acetic Acid

Arigoni Enzyme-assisted Pathway

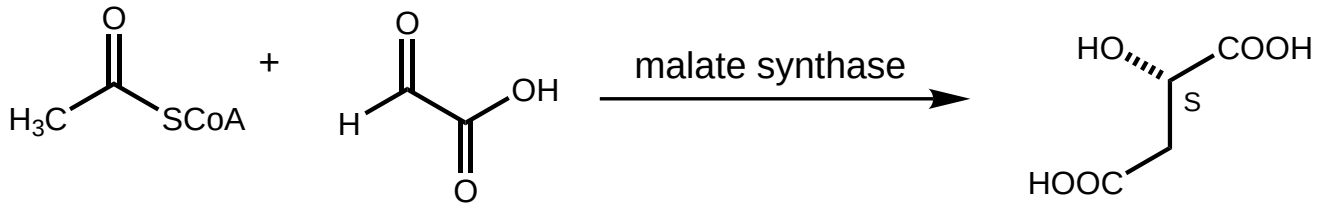


Arigoni, *Nature* **1969**, *221*, 1213.

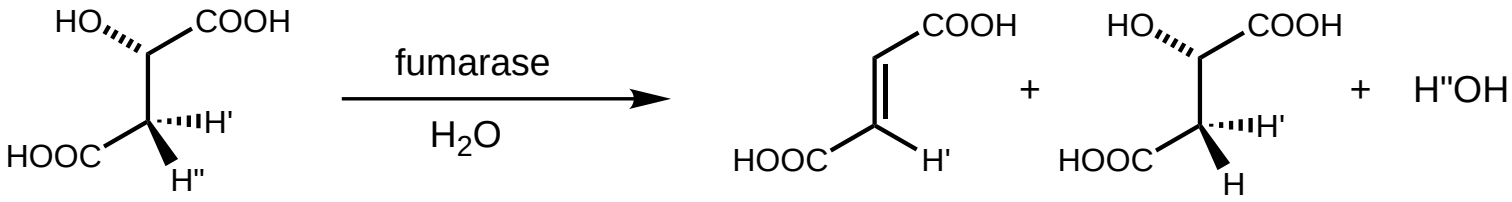
Determination of Configuration and Enantiomeric Excess

Cornforth / Arigoni Enzyme Method

- basis



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



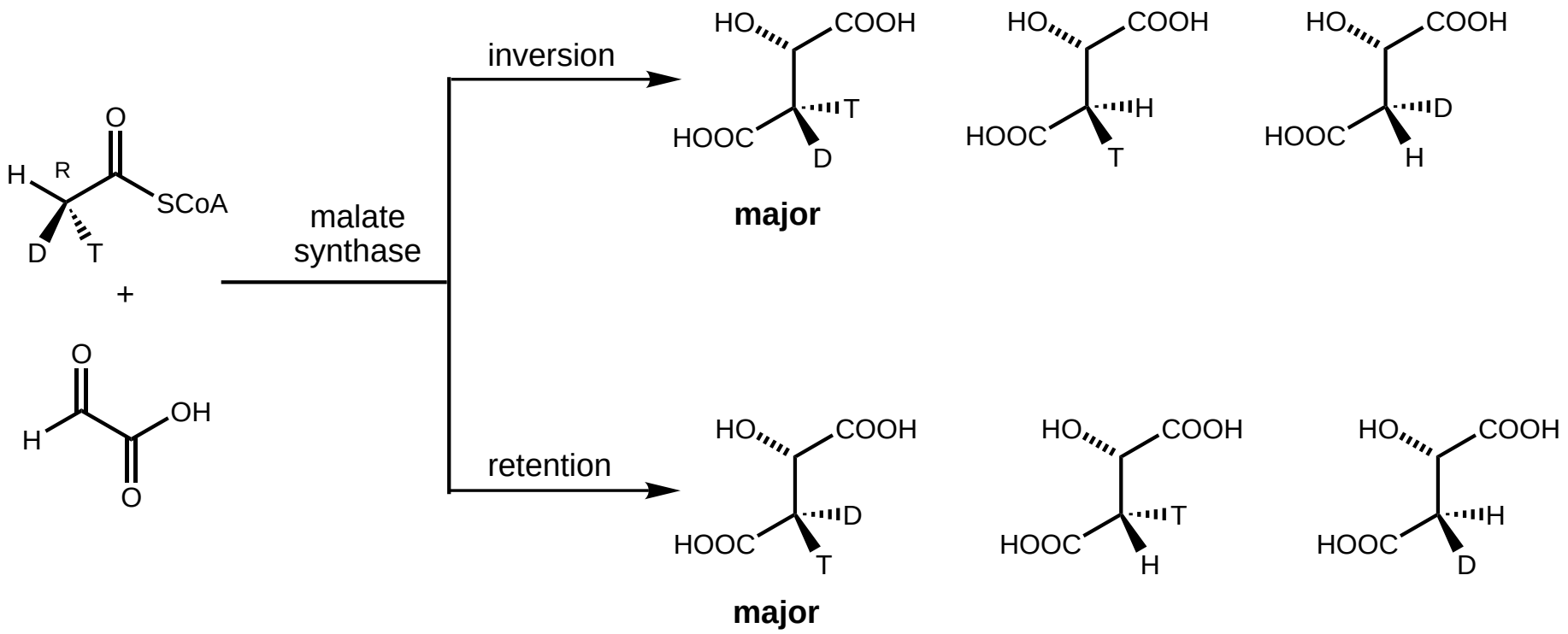
- only pro-R hydrogen is lost or exchanged with solvent

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

Determination of Configuration and Enantiomeric Excess

Cornforth / Arigoni Enzyme Method

Idea:

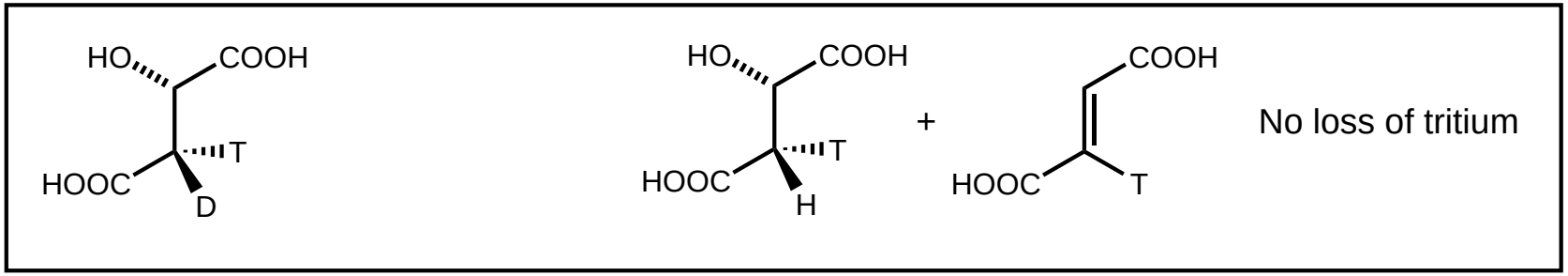


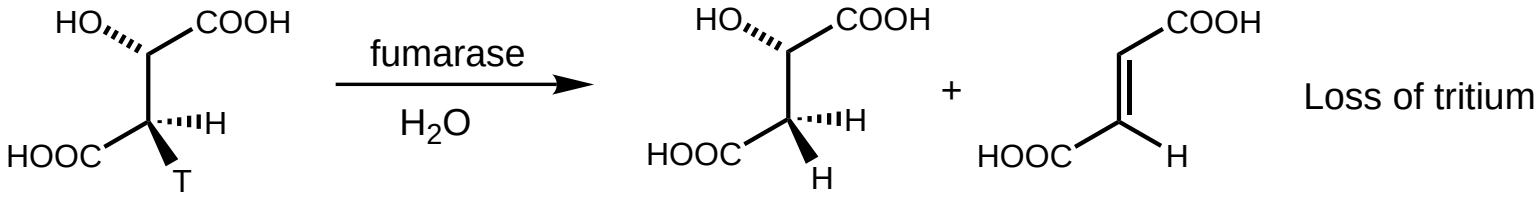
Cornforth, *Nature* **1969**, *221*, 1212.
Arigoni, *Nature* **1969**, *221*, 1213.

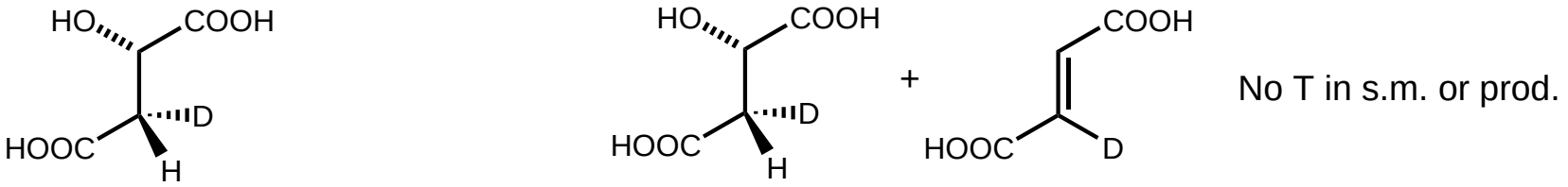
Determination of Configuration and Enantiomeric Excess

Cornforth / Arigoni Enzyme Method

If malate is formed with inversion:







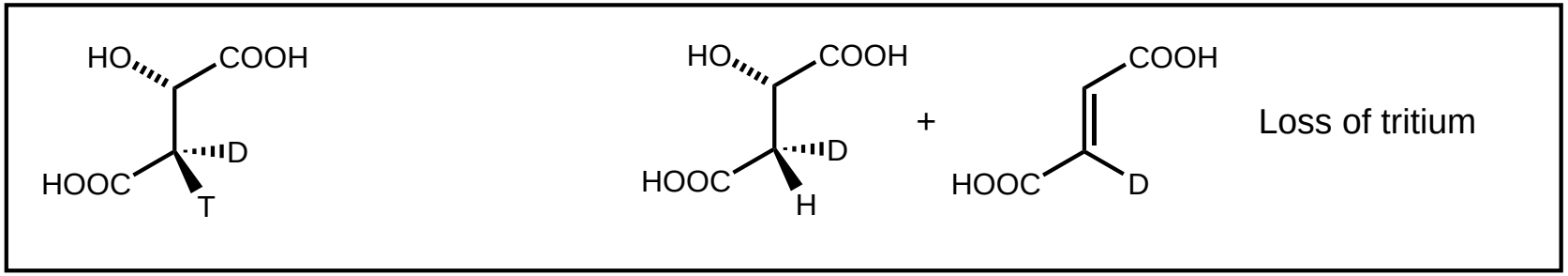
$\therefore$ if inversion mode is favored, (R)-acetylSCoA $\rightarrow$ >50% tritium retained

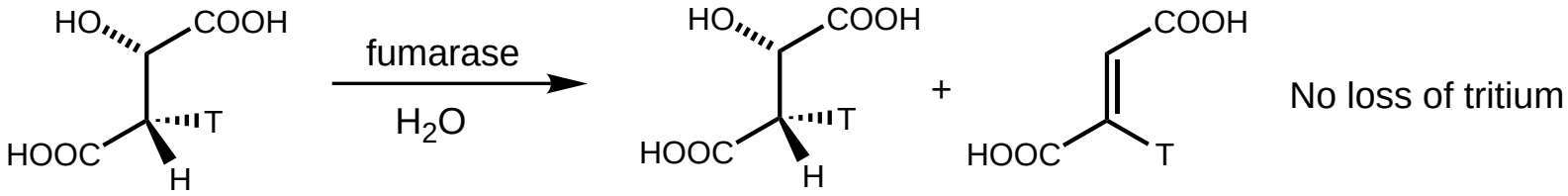
Cornforth, *Nature* **1969**, *221*, 1212.
Arigoni, *Nature* **1969**, *221*, 1213.

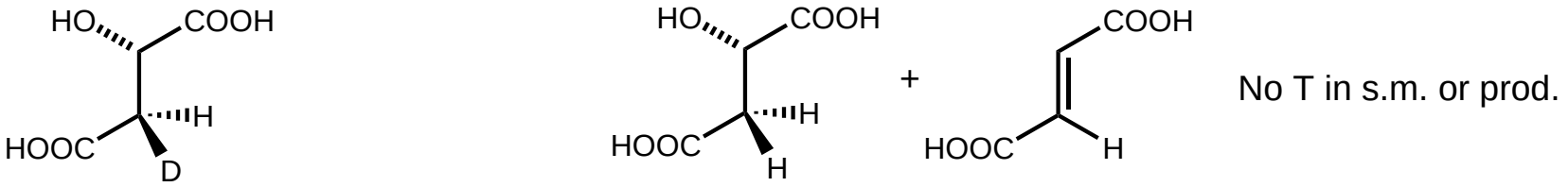
Determination of Configuration and Enantiomeric Excess

Cornforth / Arigoni Enzyme Method

If malate is formed with retention:





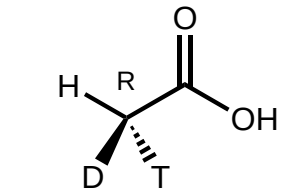


$\therefore$ if retention mode is favored, (R)-acetylSCoA $\rightarrow$ <50% tritium retained

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

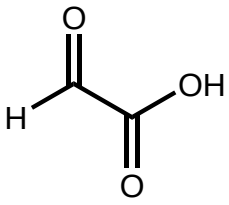
Determination of Configuration and Enantiomeric Excess

Cornforth / Arigoni Enzyme Method

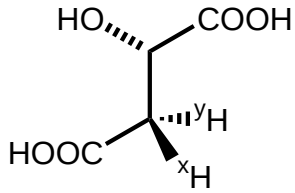


small amount of
$^{14}CH_3COOH$ added

+

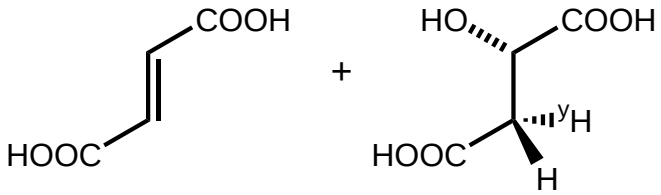


CoASH
acetate kinase
phosphotransacetylase
malate synthase



^{3}H / ^{14}C = 1.558

fumarase



^{3}H / ^{14}C = 1.071

Define: F = % retention of T

= $(^{3}H/^{14}C_{fumarate}) / (^{3}H/^{14}C_{malate})$•100%

= (1.071 / 1.558)•100% = **69%**

∴ **malate formation proceeds with inversion**

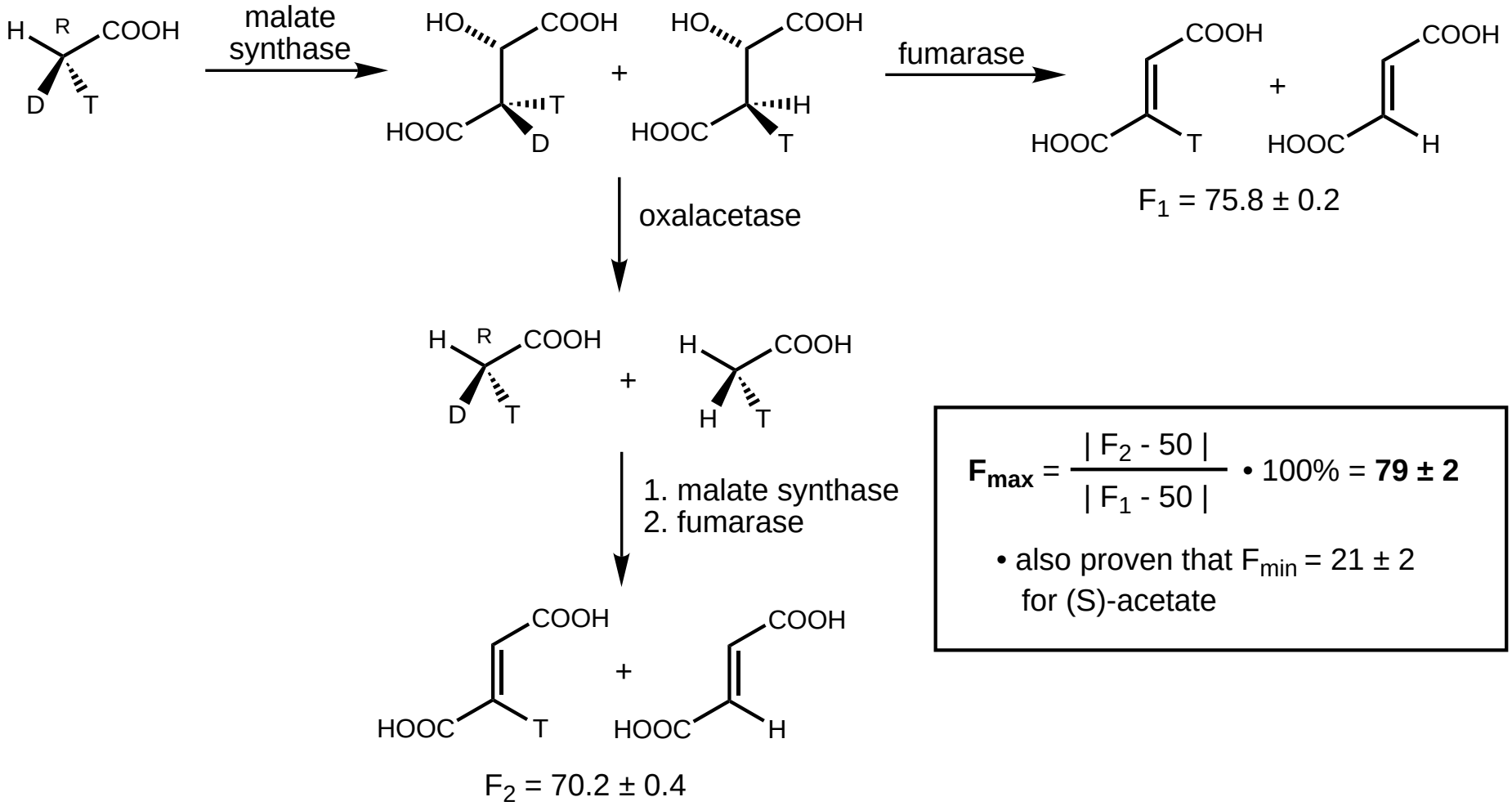
- if (S)-CHDTCOOH is used, F = 31%

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



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

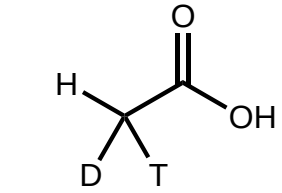
- also proven that $F_{min} = 21 \pm 2$ for (S)-acetate

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

Determination of Configuration and Enantiomeric Excess

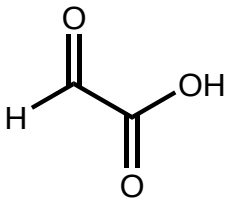
Cornforth / Arigoni Enzyme Method

- **Overall Method**

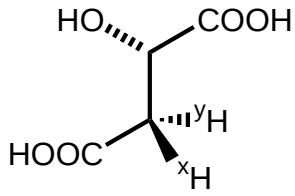


small amount of
$^{14}CH_3COOH$ added

+

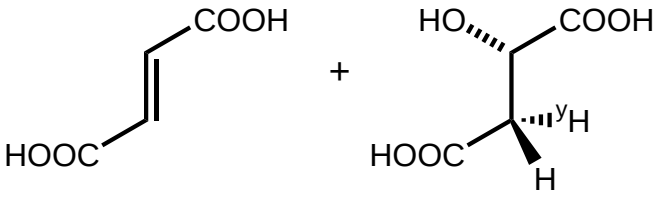


CoASH
acetate kinase
phosphotransacetylase
malate synthase



isolate and measure ^{3}H / ^{14}C ratio

fumarase



isolate and measure ^{3}H / ^{14}C ratio

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

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

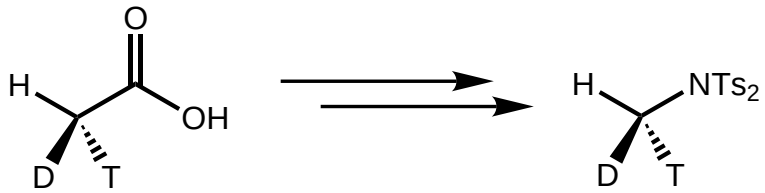
$$\%ee = \frac{|F - 50|}{29} \cdot 100\%$$

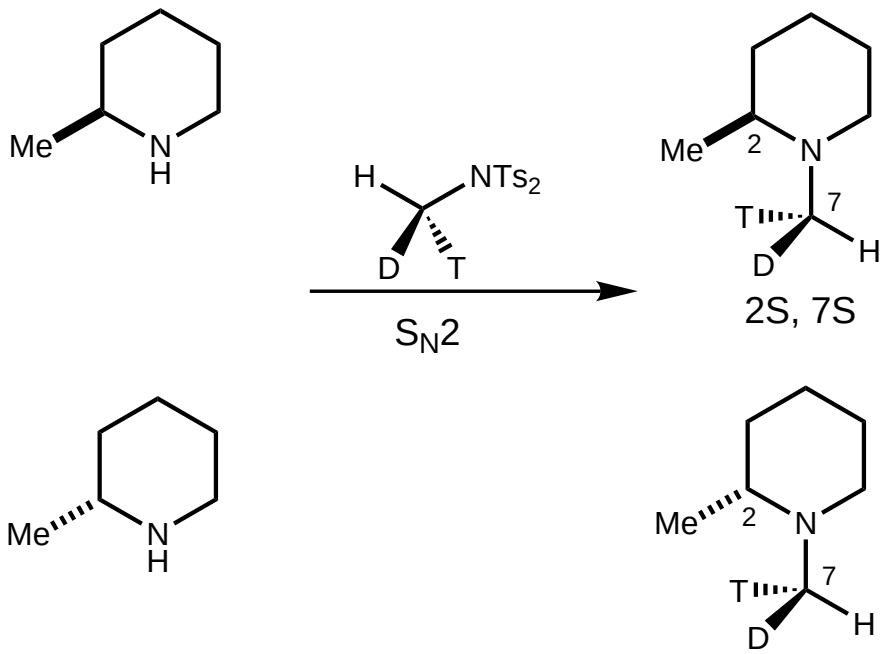
Cornforth, *Nature* **1969**, *221*, 1212.
Arigoni, *Nature* **1969**, *221*, 1213.

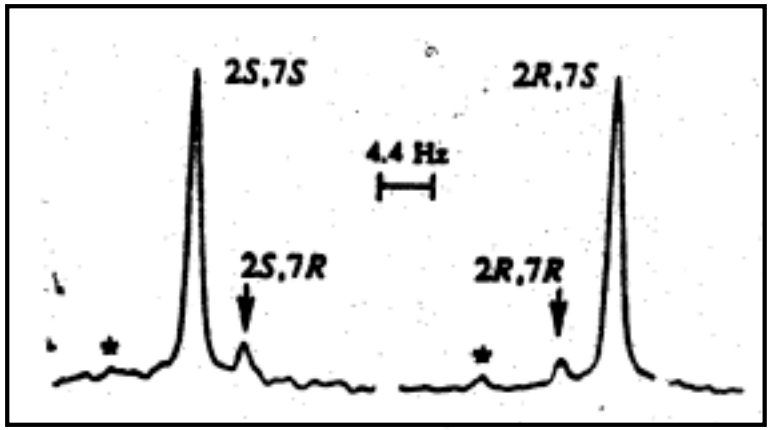
Determination of Configuration and Enantiomeric Excess

Anet's Direct Tritium NMR Method

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







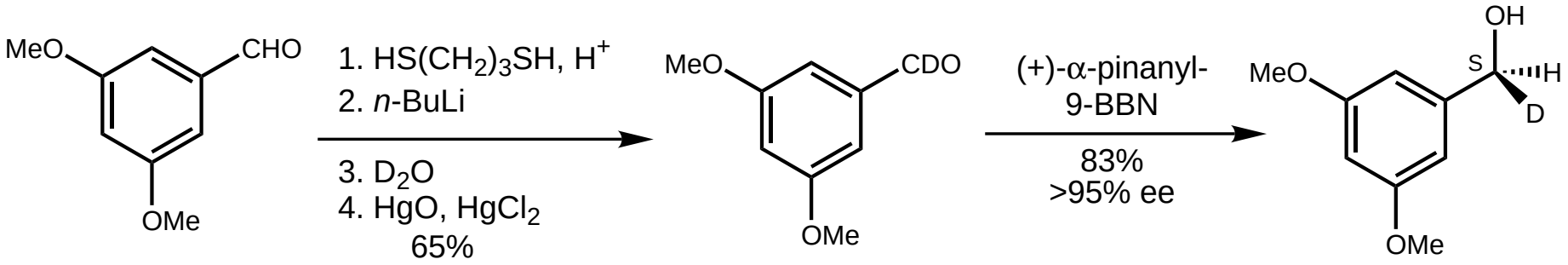
^{3} H{^{1}H,^{2}H}-NMR Spectra at 320 MHz

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

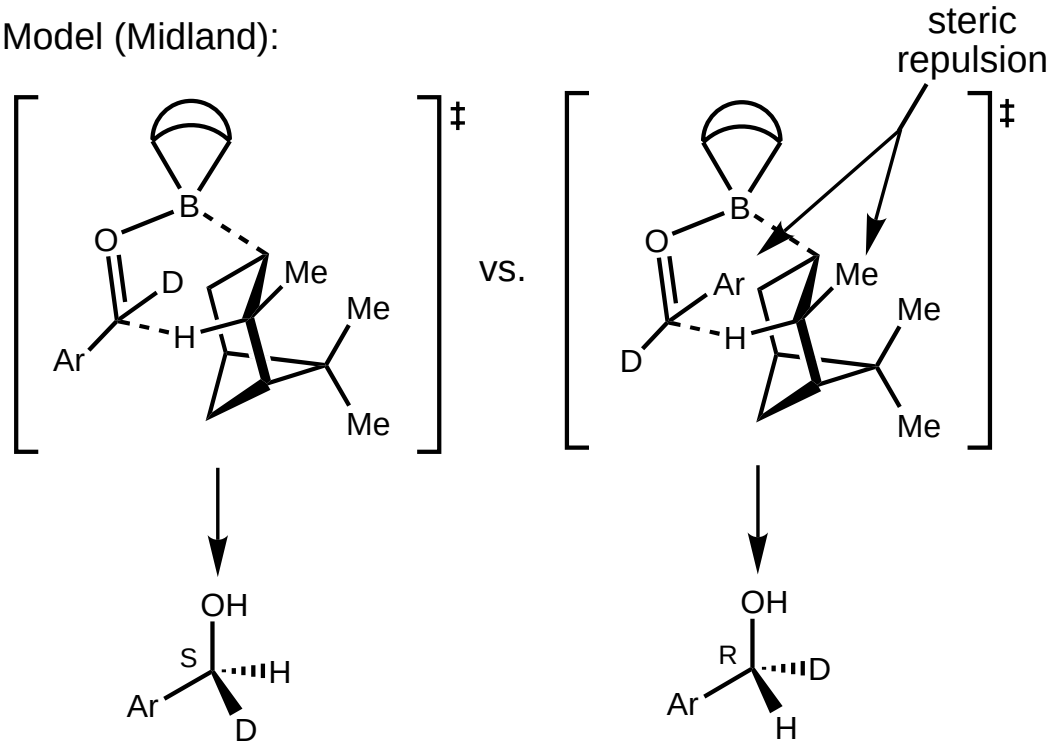
Anet and Floss, *J. Am. Chem. Soc.* **1989**, *111*, 8935.

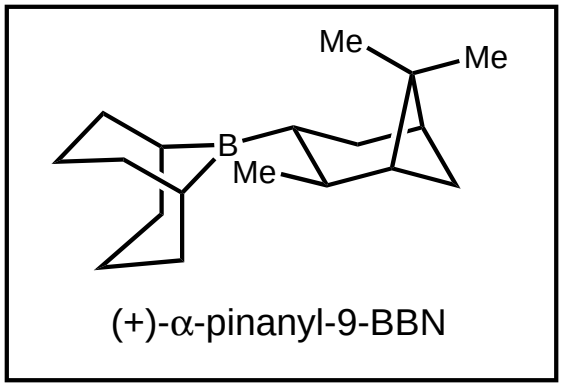
Syntheses of Chiral Acetic Acid

Floss Tosylate Displacement



Model (Midland):

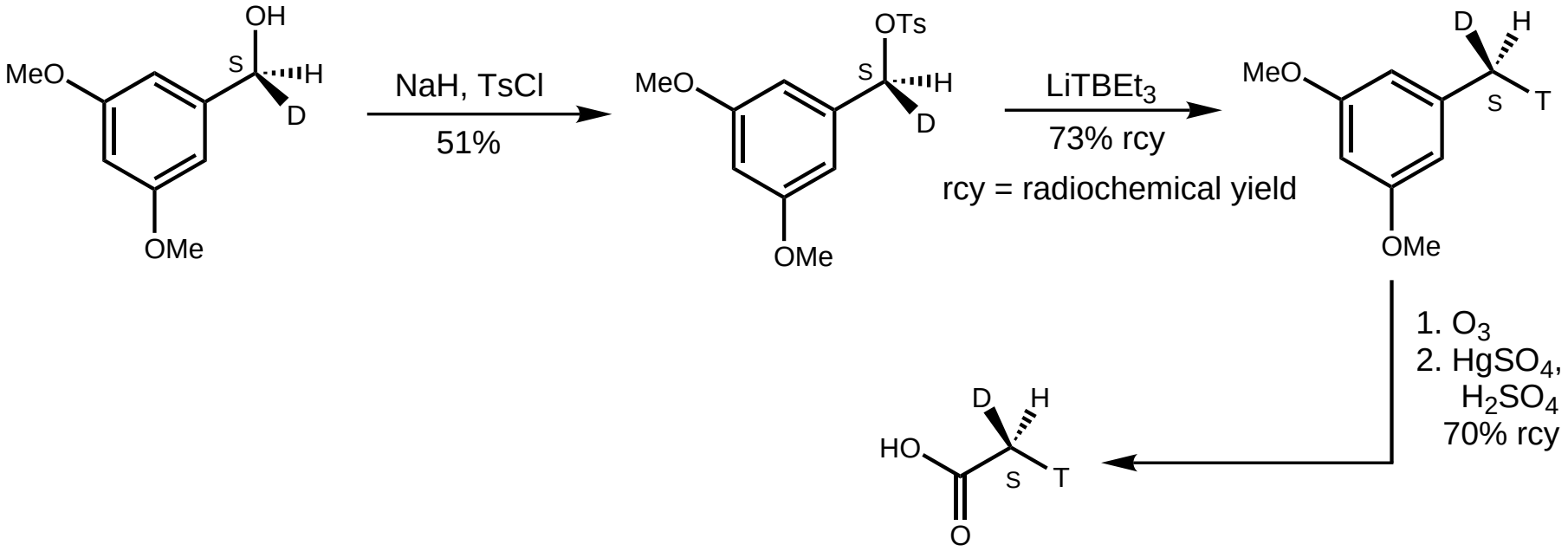




Floss, *J. Org. Chem.* **1983**, *48*, 3510.
Midland, *J. Am. Chem. Soc.* **1979**, *101*, 2352.

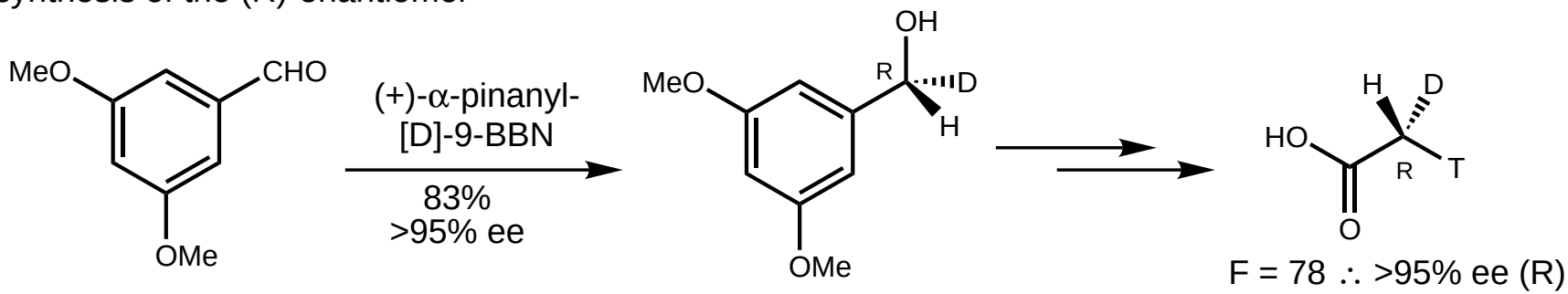
Syntheses of Chiral Acetic Acid

Floss Tosylate Displacement



F = 21 ∴ >95% ee (S)

- synthesis of the (R)-enantiomer

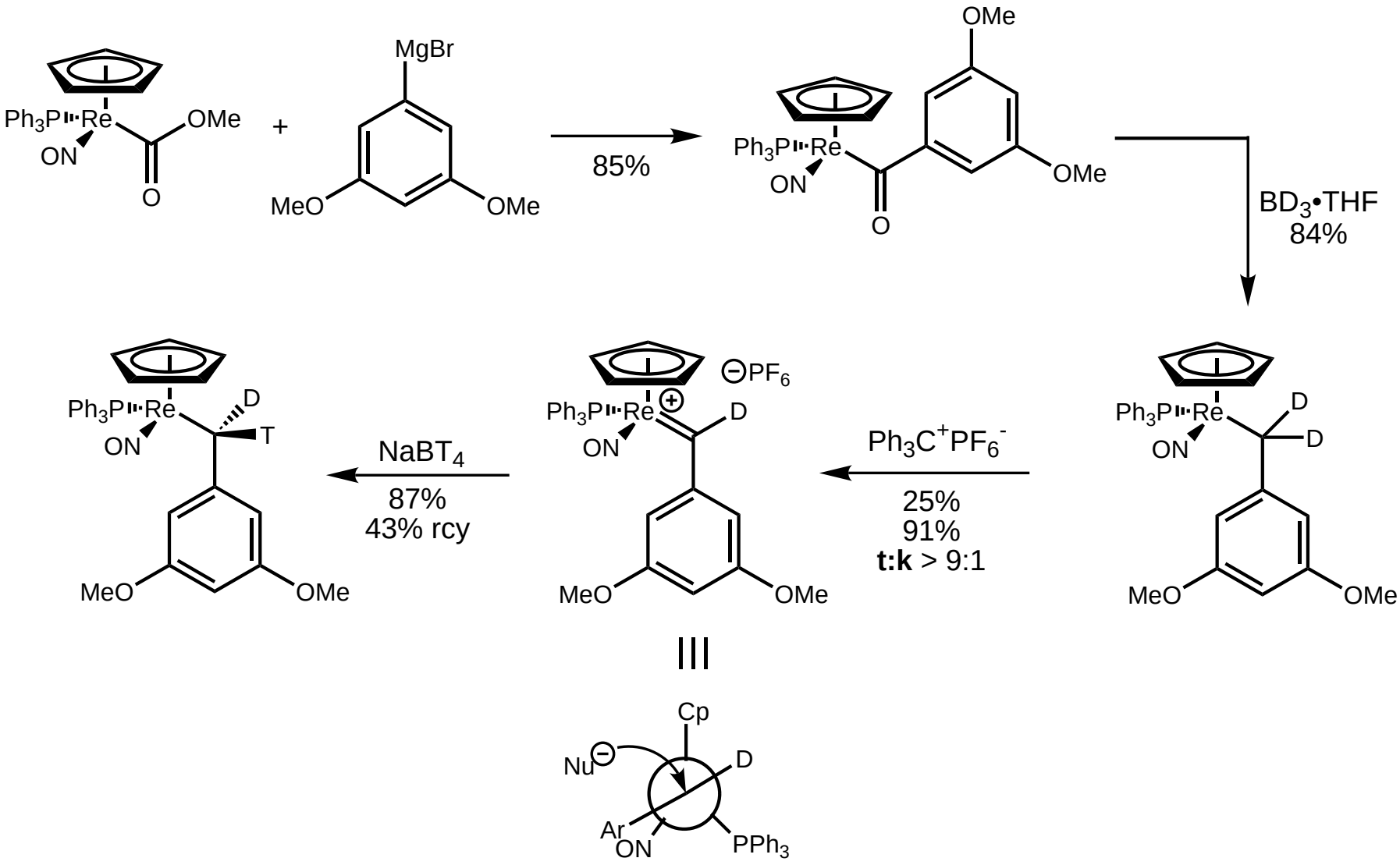


F = 78 ∴ >95% ee (R)

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

Syntheses of Chiral Acetic Acid

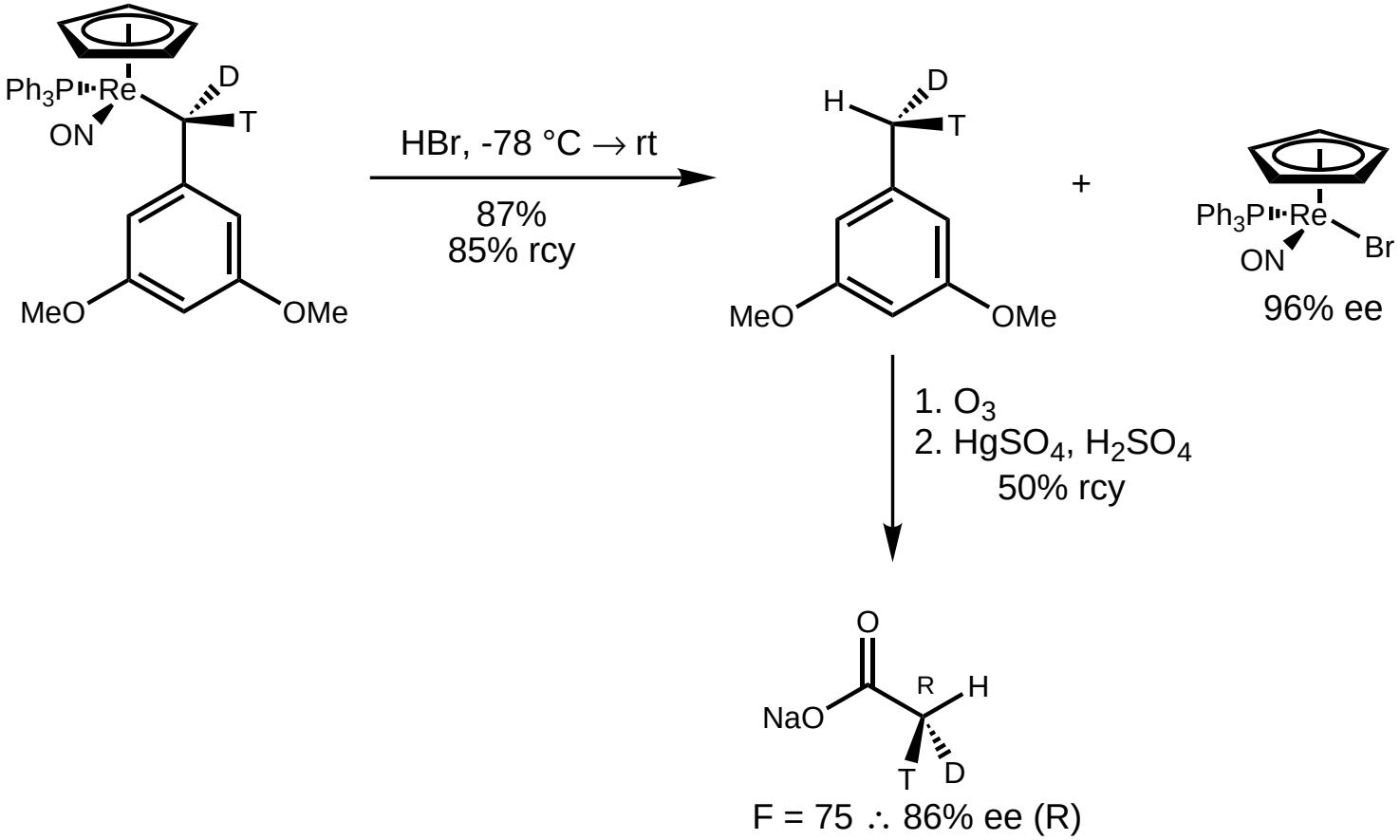
Gladysz Rhenium-Carbon Bond Protonolysis



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

Syntheses of Chiral Acetic Acid

Gladysz Rhenium-Carbon Bond Protonolysis

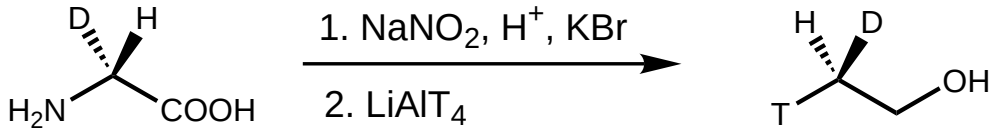


Gladysz and Floss, J. Am. Chem. Soc. 1987, 109, 4837.

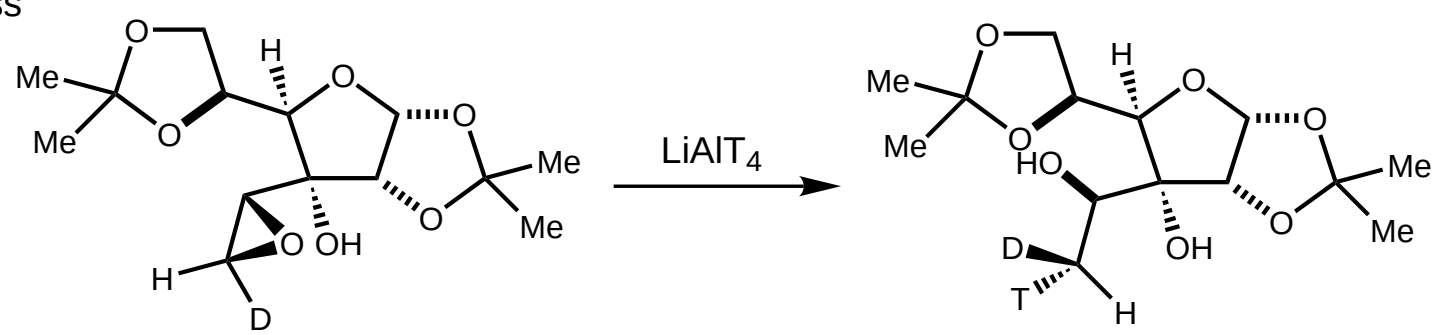
Syntheses of Chiral Acetic Acid

Other Nucleophilic Tritiide Examples

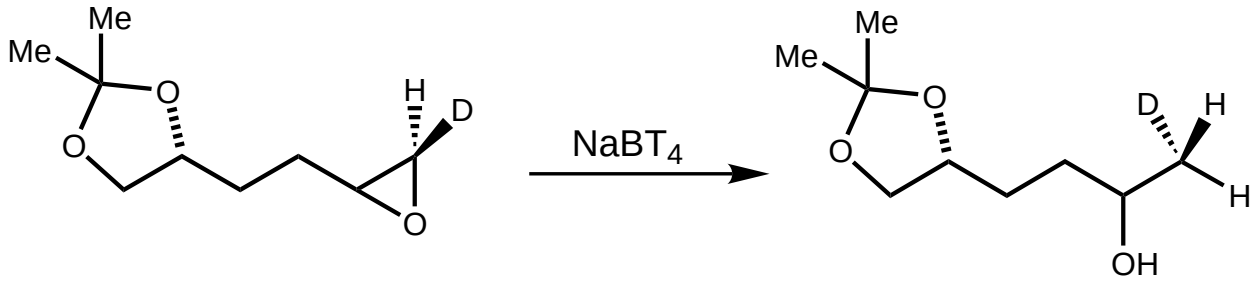
- Scott



- Floss



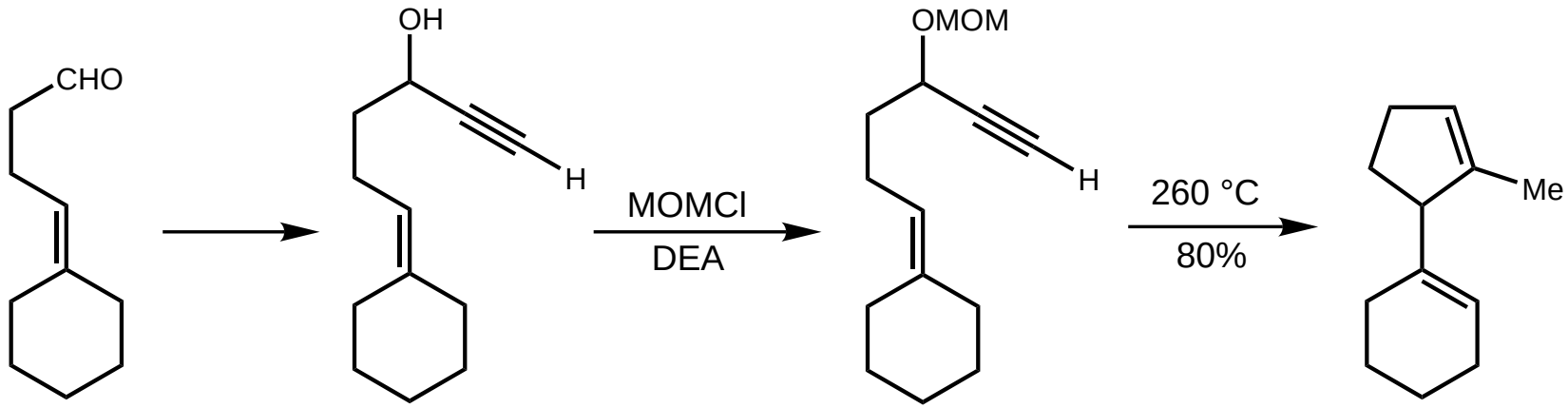
- Altman



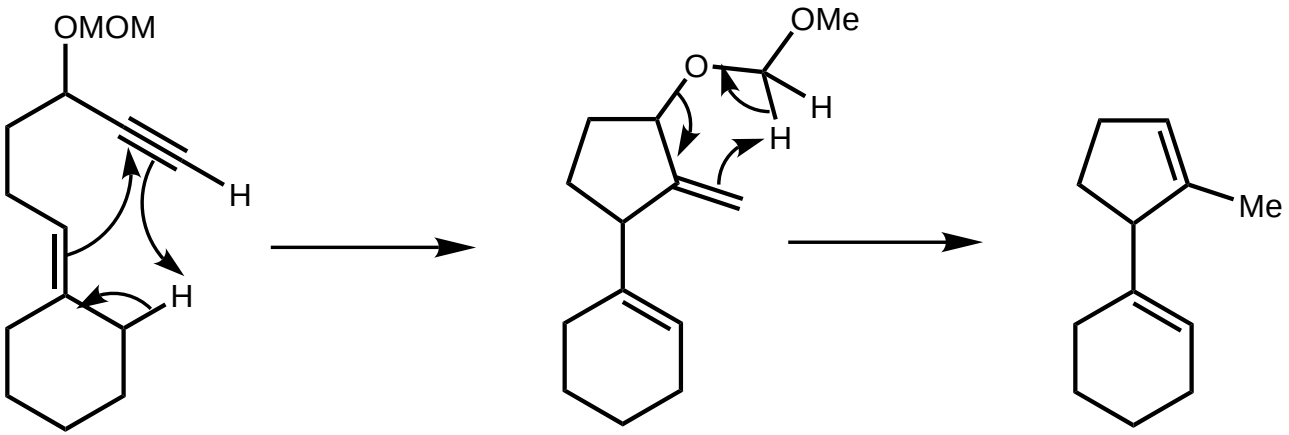
Scott, *Chem. Commun.* **1978**, 967.
Floss, *J. Org. Chem.*, **1984**, *49*, 1290.
Altman, *J. Am. Chem. Soc.*, **1978**, *100*, 3235.

Syntheses of Chiral Acetic Acid

Arigoni Ene Cascade - Racemic



Mechanism:

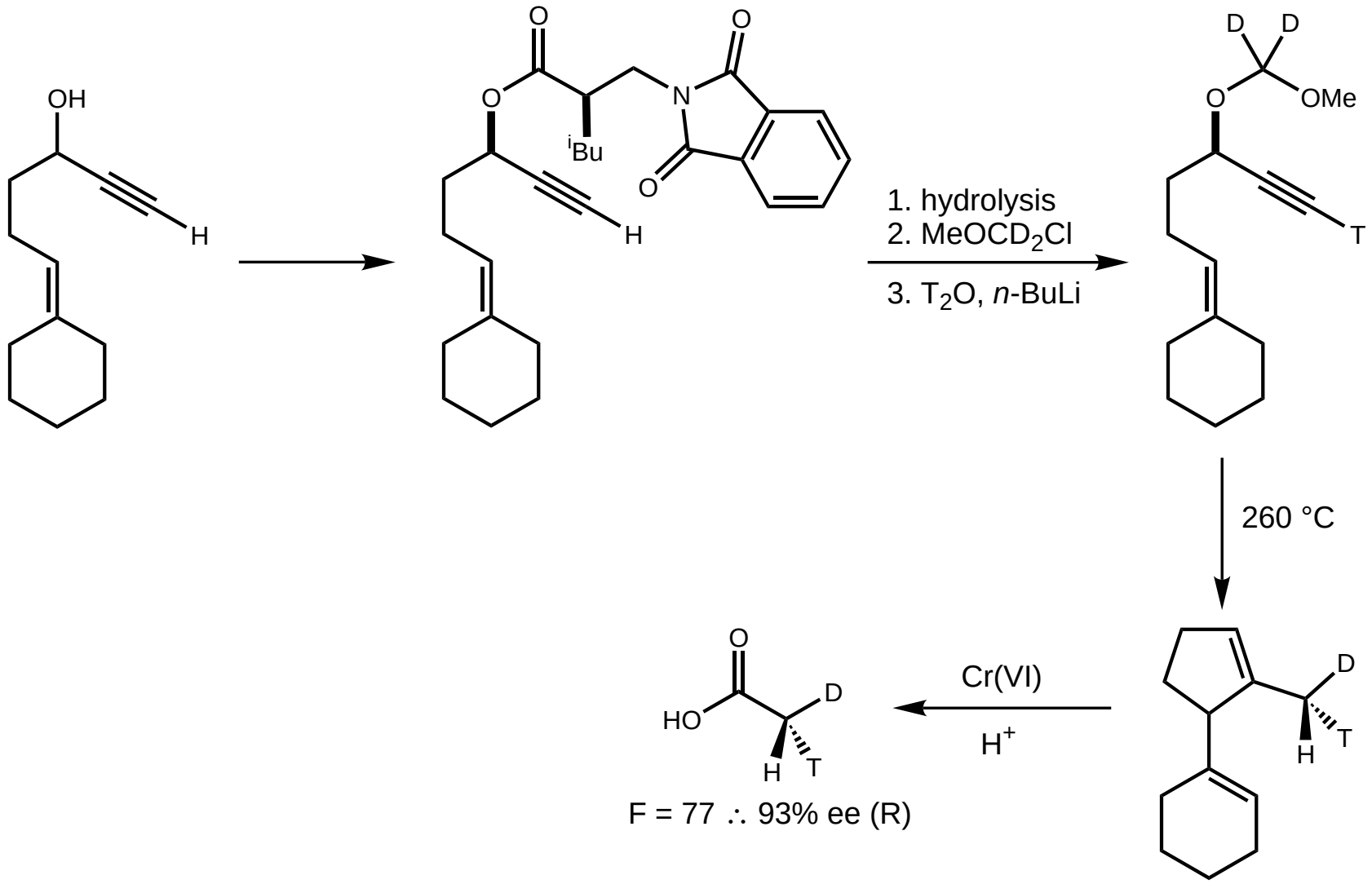


Observed by
^{1}H-NMR of an aliquot
prior to completion

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

Syntheses of Chiral Acetic Acid

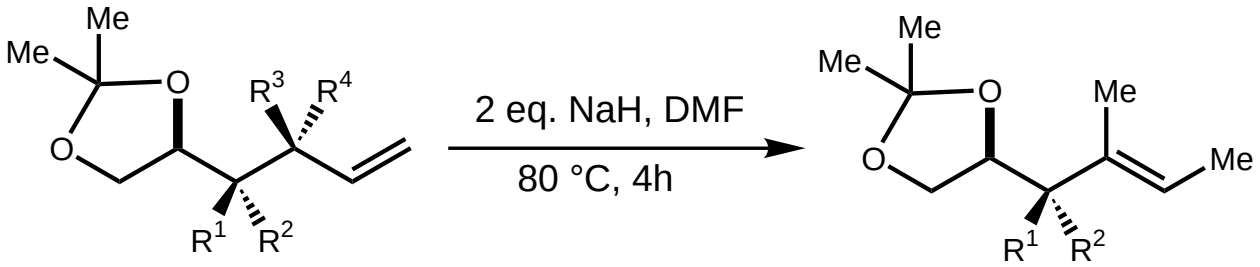
Arigoni Ene Cascade - Non-racemic



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

Syntheses of Chiral Acetic Acid

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



1 **2**

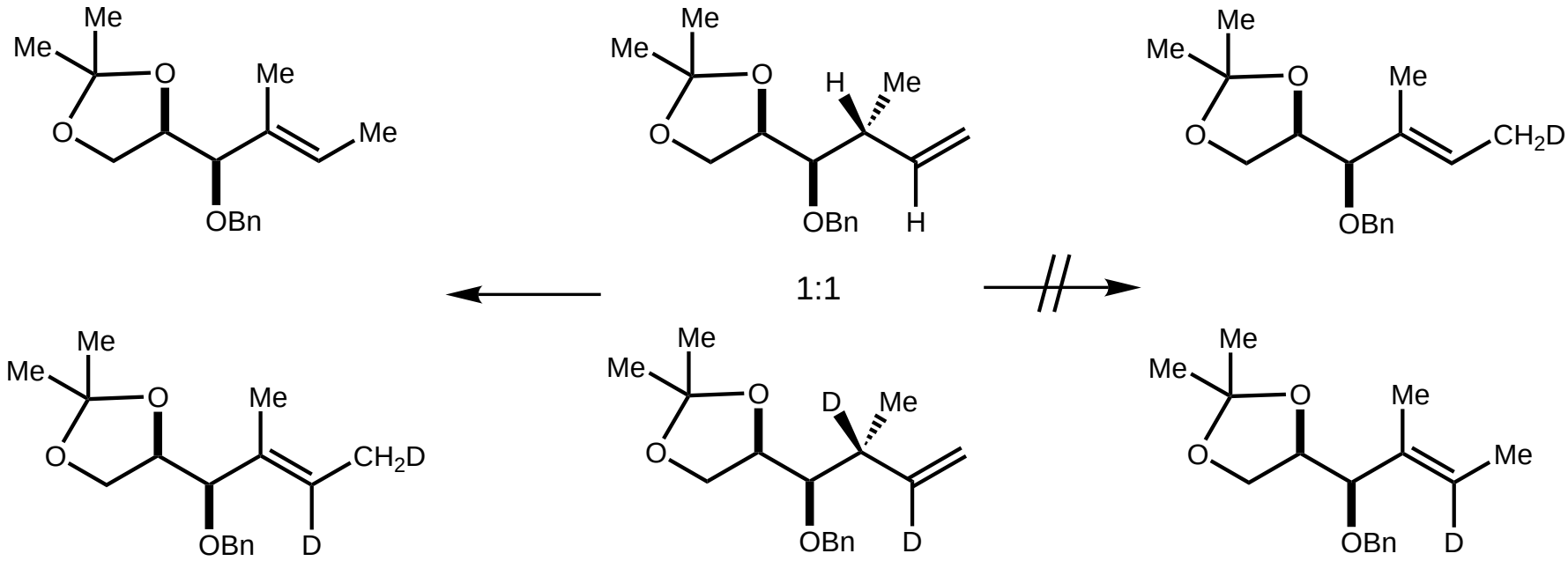
Educt	R^1	R^2	R^3	R^4	Product	Yield
1a	OBn	H	H	Me	2a	>95
1b	H	OBn	Me	H	2b	>95
1c	H	OBn	H	Me	2c	>90
1d	OPMB	H	H	Me	2d	67
1e	O*p*-ClBn	H	H	Me	2e	63
1f	OTHP	H	H	Me	2f	38
1g	OMe	H	H	Me	2g	3

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

Syntheses of Chiral Acetic Acid

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

Crossover experiment:



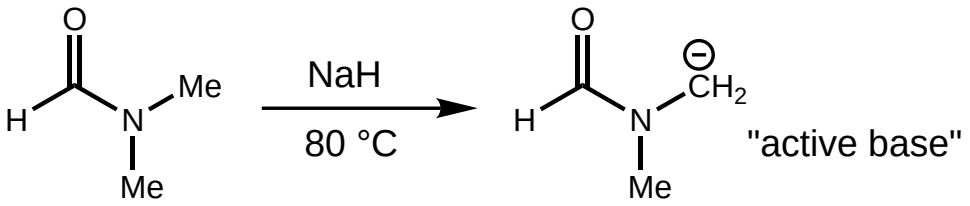
• appears to be exclusively an intramolecular [1,3]-H-shift

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

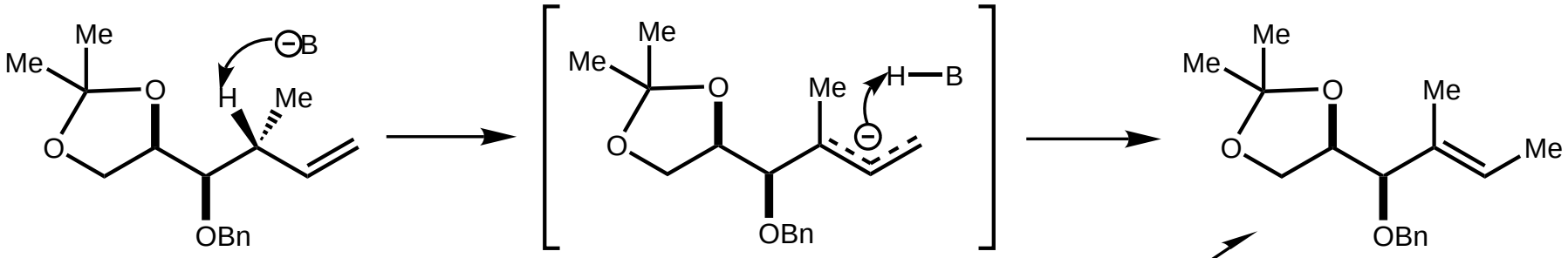
Syntheses of Chiral Acetic Acid

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

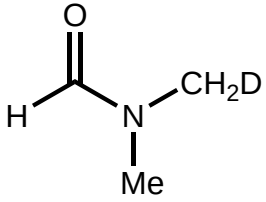
Mechanistic proposal:



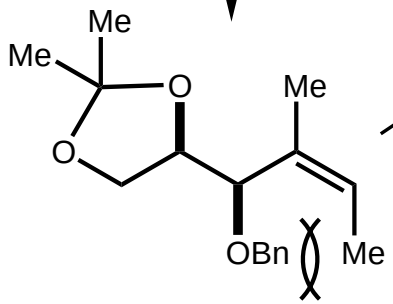
"active base"



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



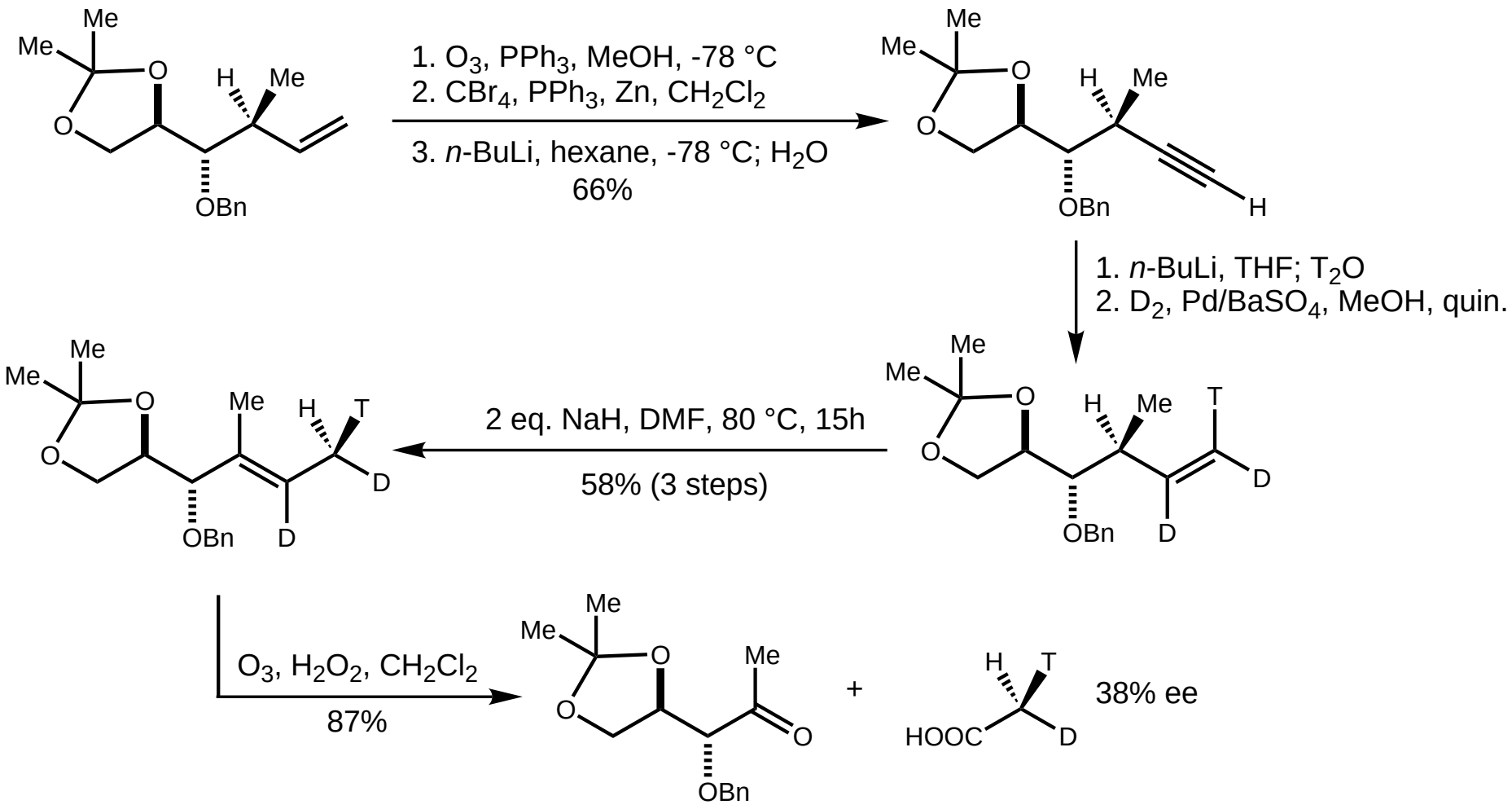
-necessity of benyzl not clear



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

Syntheses of Chiral Acetic Acid

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

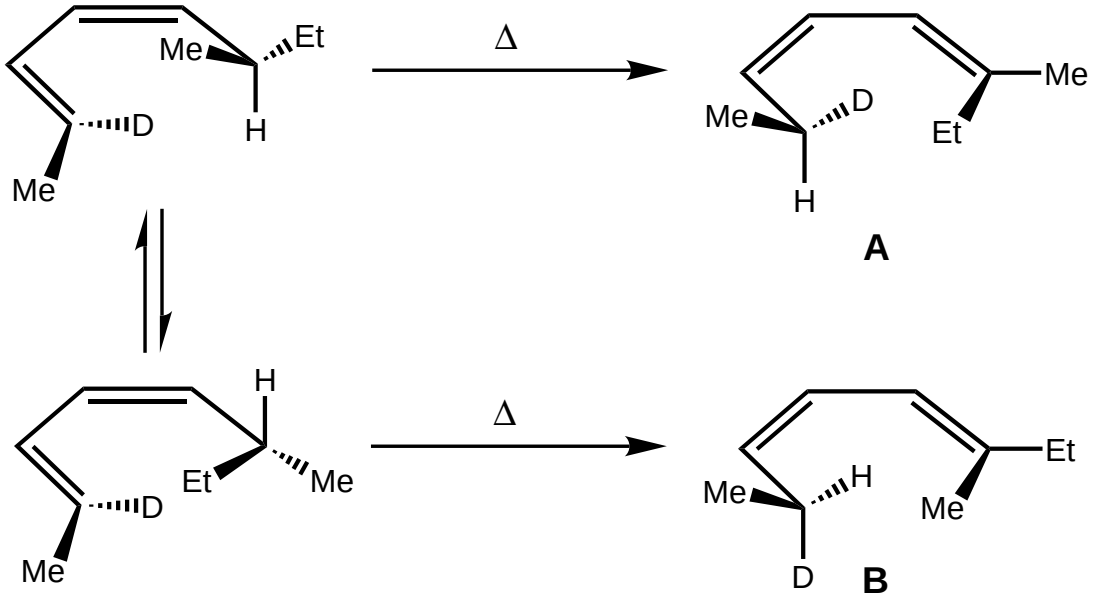


Mulzer and Arigoni, *Tetrahedron Lett.* **1997**, *31*, 5469.

Syntheses of Chiral Acetic Acid

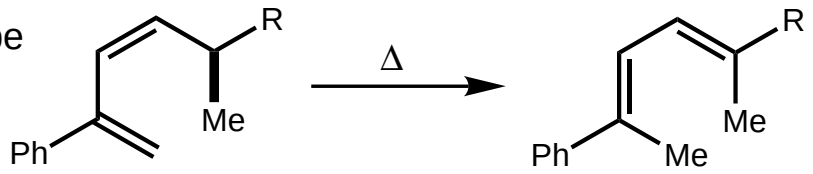
Mulzer and Floss [1,5]-H-Shift

- Roth's experiment:



250 °C: 1.5:1 **A:B**; 27% rsm
275 °C: 10 diff. products formed

Idea: use substrates of the type



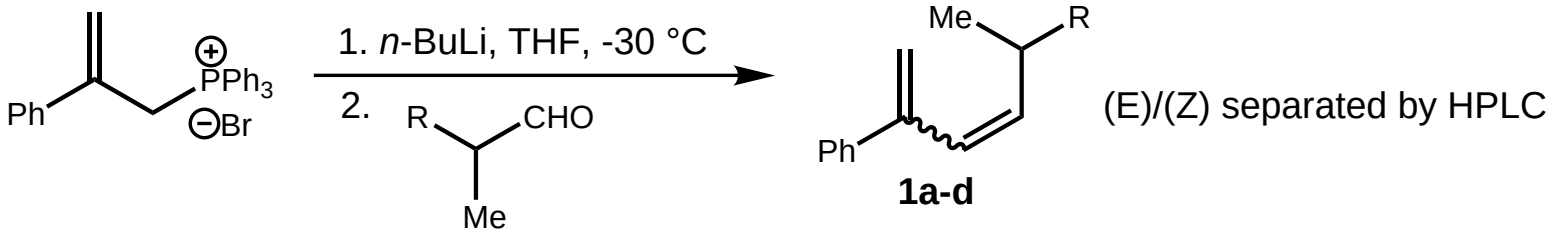
- benefits: two disubstituted double bonds → two trisubstituted double bonds
  cross conjugated system → linear conjugated system
  phenyl group stabilizes toward polymerization and thermodimerization

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

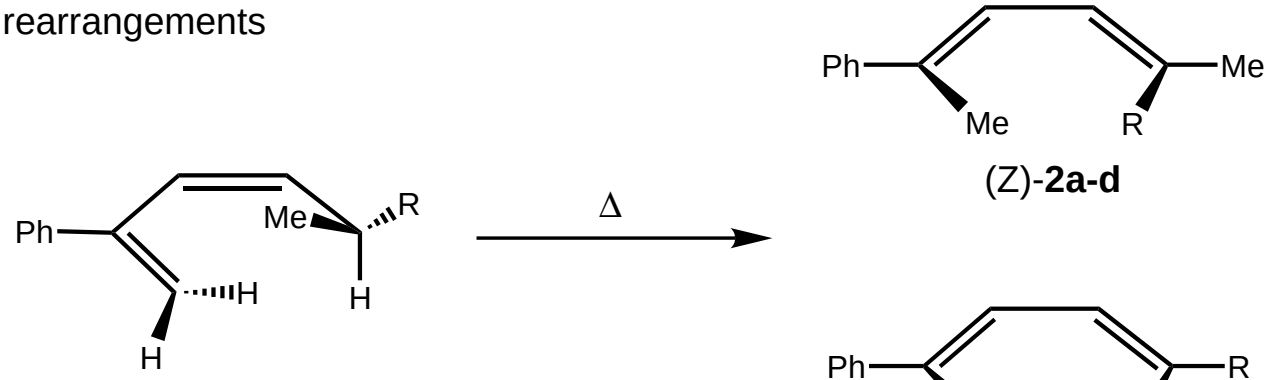
- synthesis of test substrates



1a-d

(E)/(Z) separated by HPLC

- rearrangements

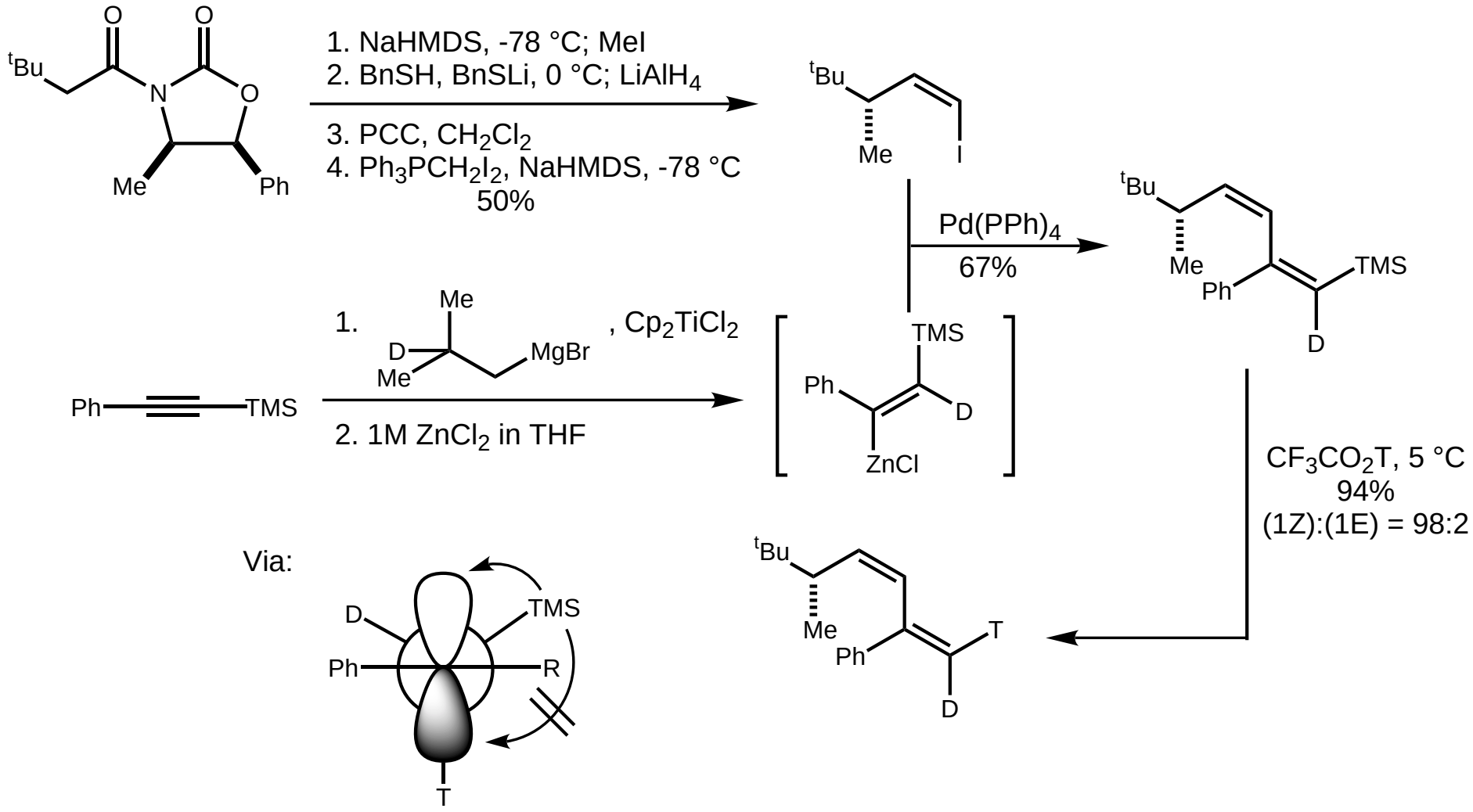


(Z)-**1 a**	R	= Et	(E)/(Z)-**2 a**	= 1.5:1
b		= ^{i}Pr	**b**	=2.5:1
c		= ^{c}Hex	**c**	=3.2:1
d		= ^{t}Bu	**d**	= >100:1

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

Syntheses of Chiral Acetic Acid

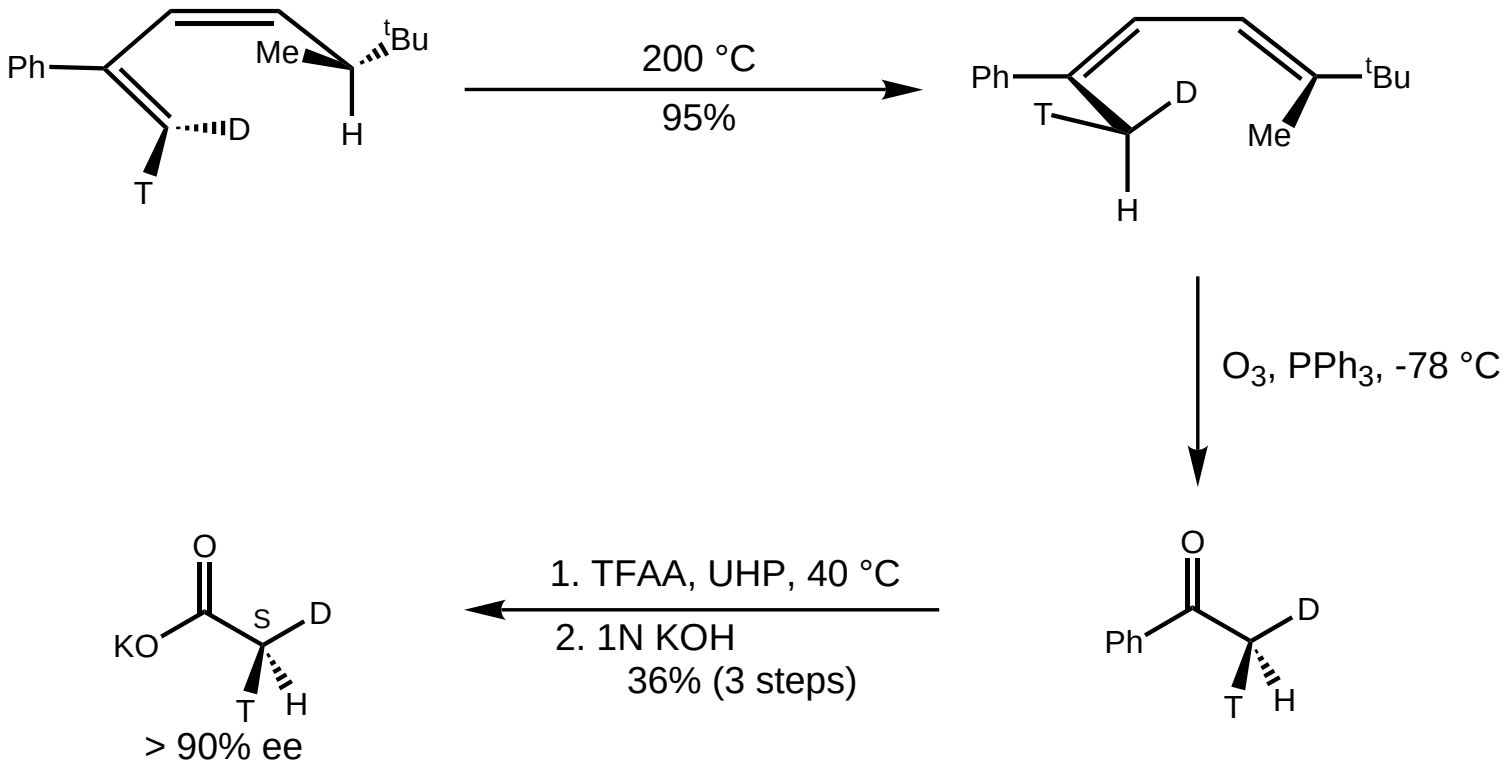
Mulzer and Floss [1,5]-H-Shift



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

Syntheses of Chiral Acetic Acid

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

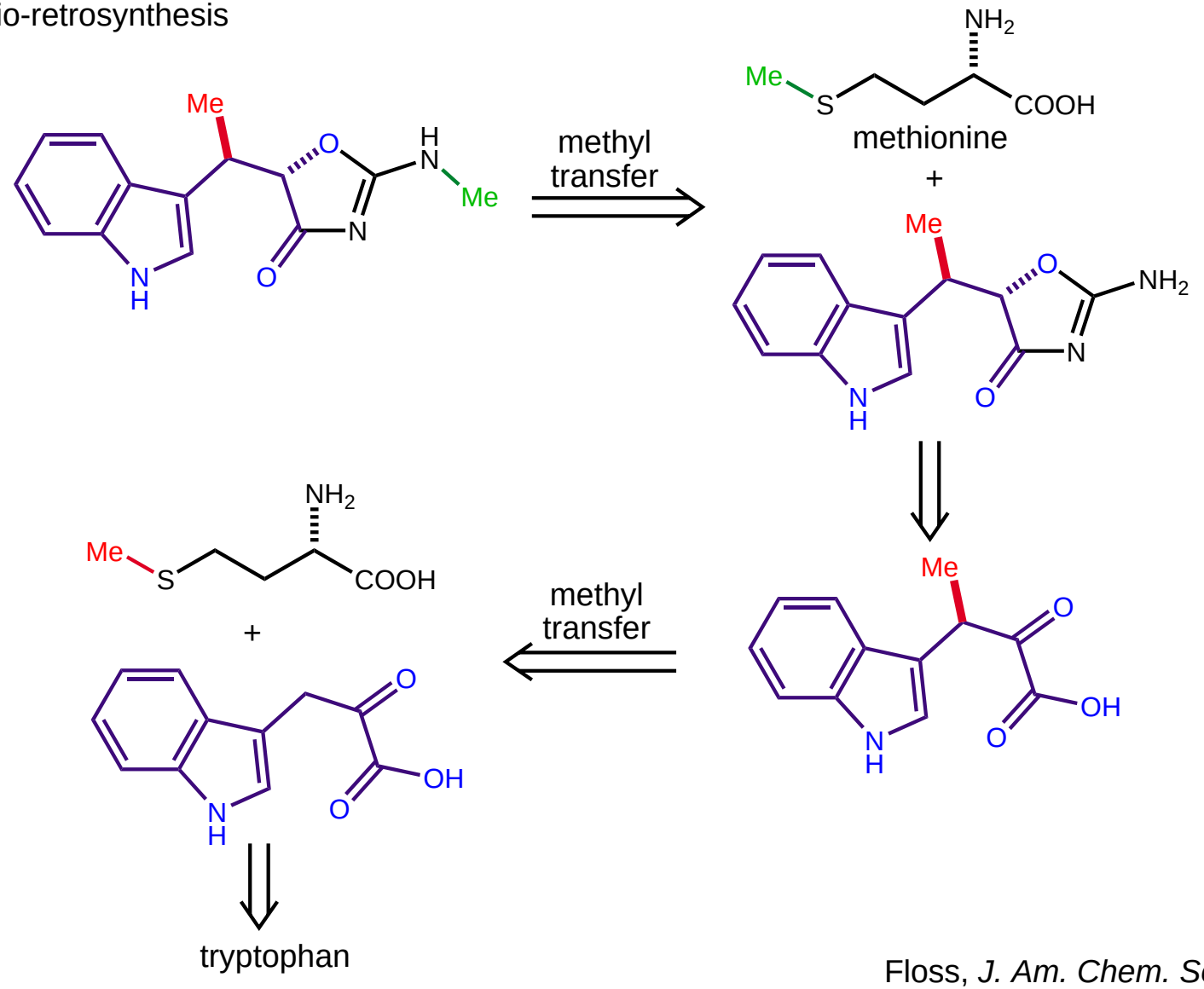


Mulzer and Floss, *J. Am. Chem. Soc.* **1999**, *121*, 10848.

Mode of Action of Methyl Transferases

C- and N-Methylation in Indolmycin Biosynthesis

- Bio-retrosynthesis

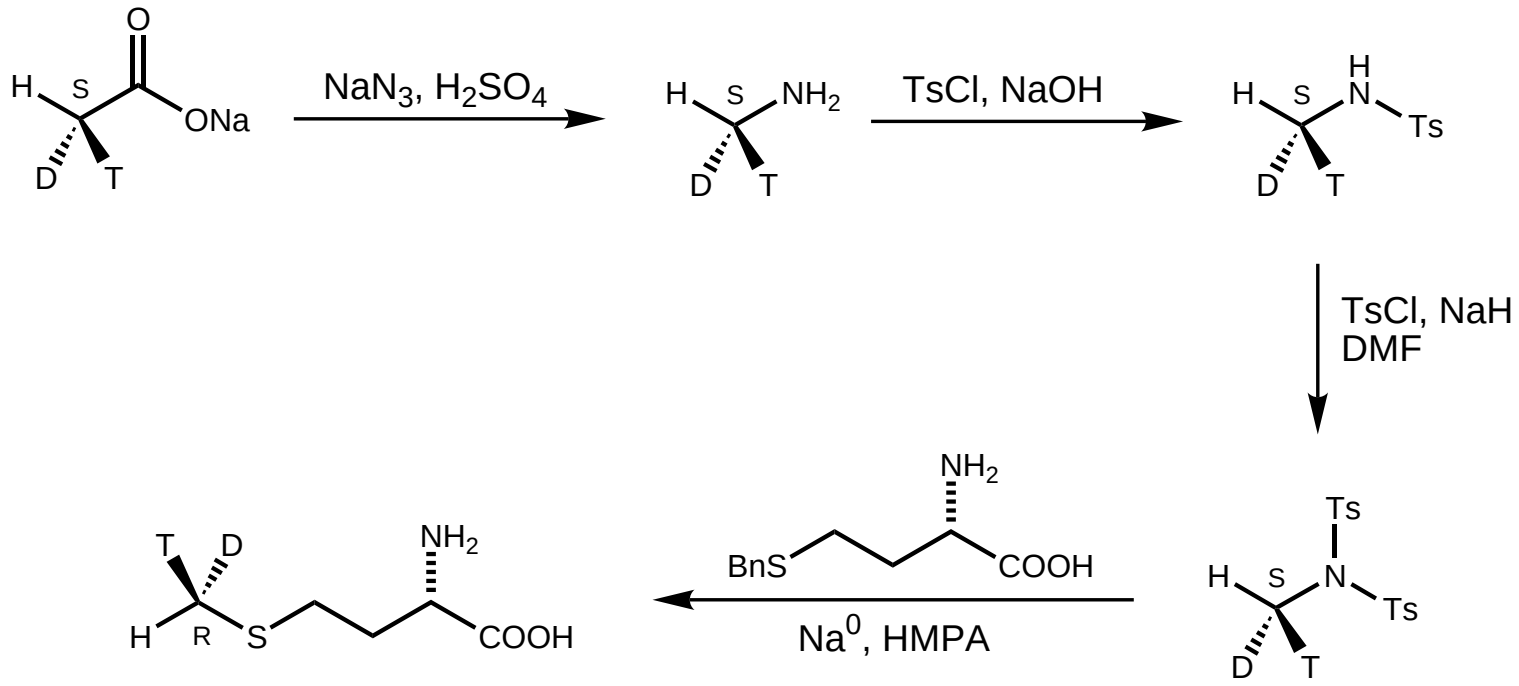


Floss, *J. Am. Chem. Soc.* **1971**, *93*, 3029.
Floss, *J. Biol. Chem.*, **1975**, *250*, 7819.

Mode of Action of Methyl Transferases

C- and N-Methylation in Indolmycin Biosynthesis

- synthesis of methionine with a chiral methyl group

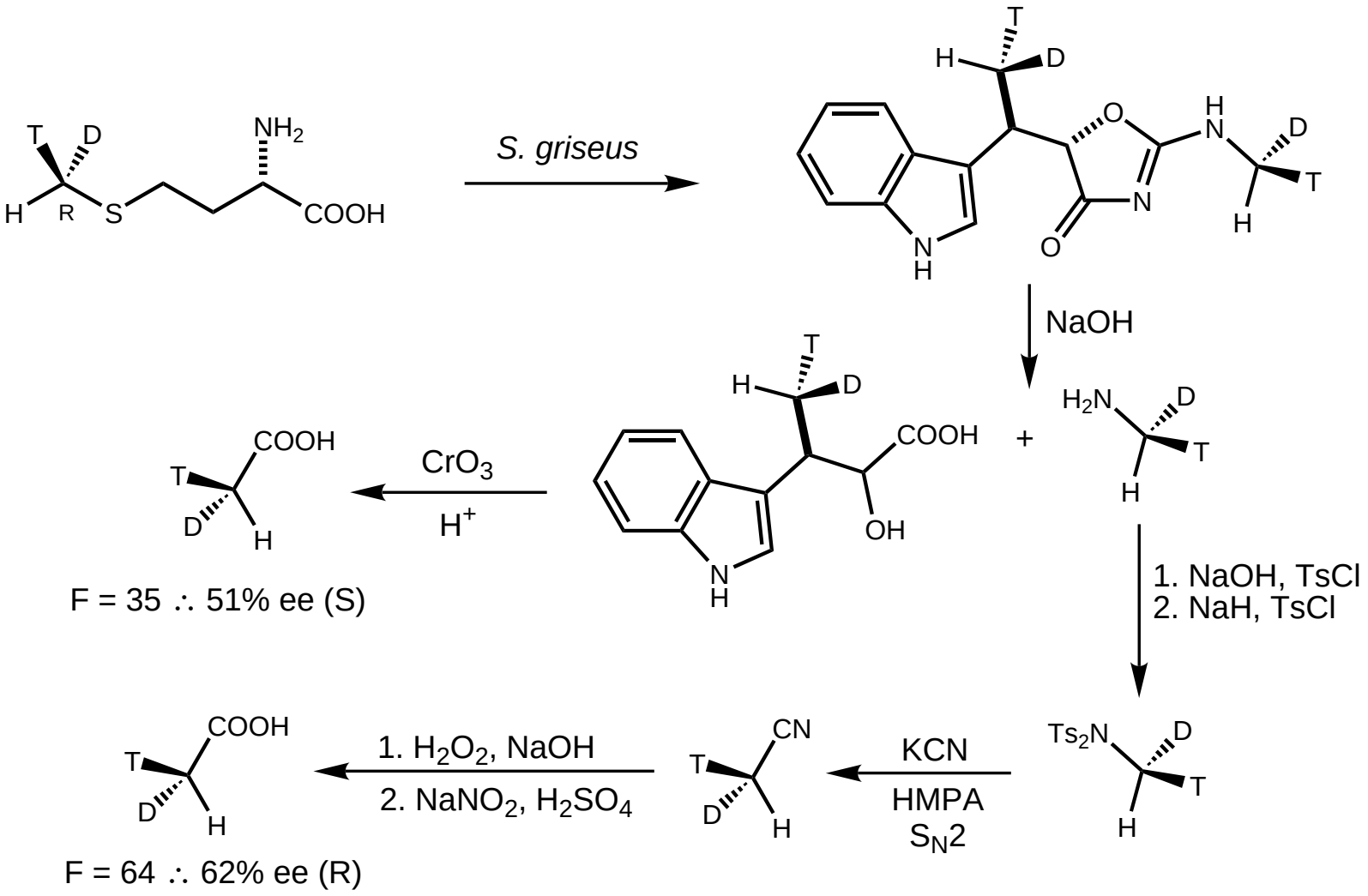


No appreciable change in
^{3}H / ^{14}C ratio from acetate indicating
insignificant proton exchange

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

Mode of Action of Methyl Transferases

C- and N-Methylation in Indolmycin Biosynthesis



∴ **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_{12} (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
norreticulin N-methyltransferase	nitrogen
dimethylallyltryptophan N-methyltransferase	nitrogen
*Eco*RI DNA methyltransferase	nitrogen
*Hha*I DNA methyltransferase	carbon
t-RNA-uracil methyltransferase	carbon

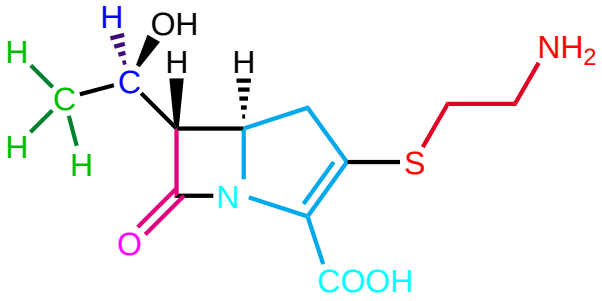
"This may signify either that all these enzymes have a common evolutionary ancestor or that this mechanism is sufficiently superior to alternative ones to drive evolution."

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

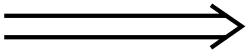
Mode of Action of Methyl Transferases

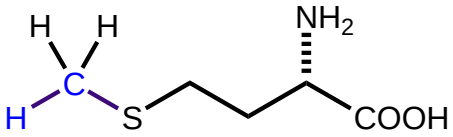
Methyl Transfers with Retention - Thienamycin

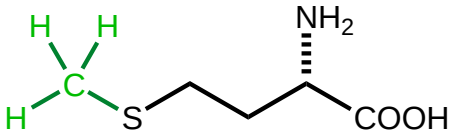
• Bio-retrosynthesis



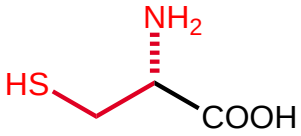
Thienamycin

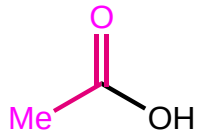


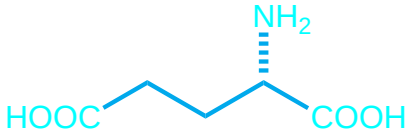




• incorporation of deuterium at all four positions when trideuteromethonine is used



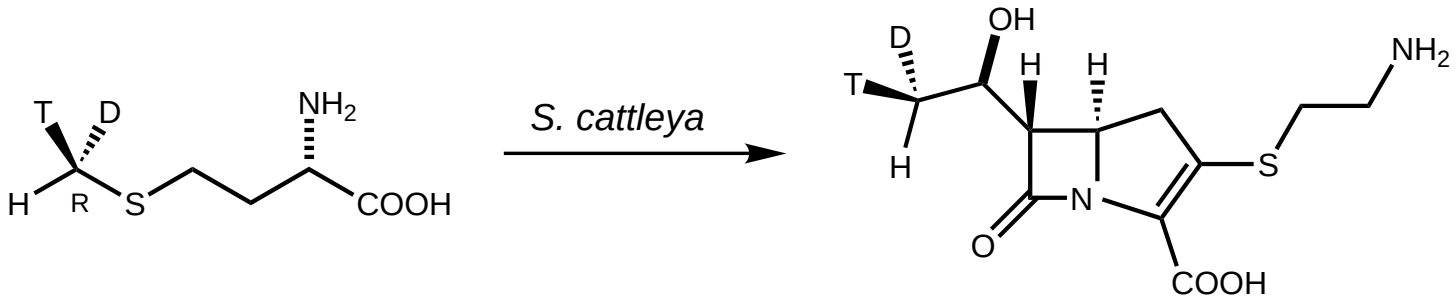


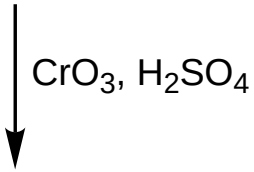


Williamson, *J. Biol. Chem.* **1985**, *260*, 4637.

Mode of Action of Methyl Transferases

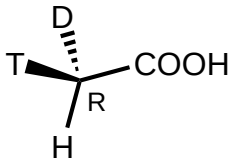
Methyl Transfers with Retention - Thienamycin





∴ **biosynthesis likely involves two methyl transfers**

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



F = 69 ∴ 66% ee (R)

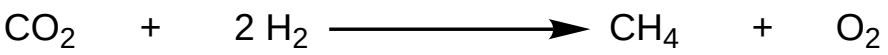
- a net retention has since been demonstrated in biosynthesis of thiostrepton as well

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

Steric Course of Methanogenesis

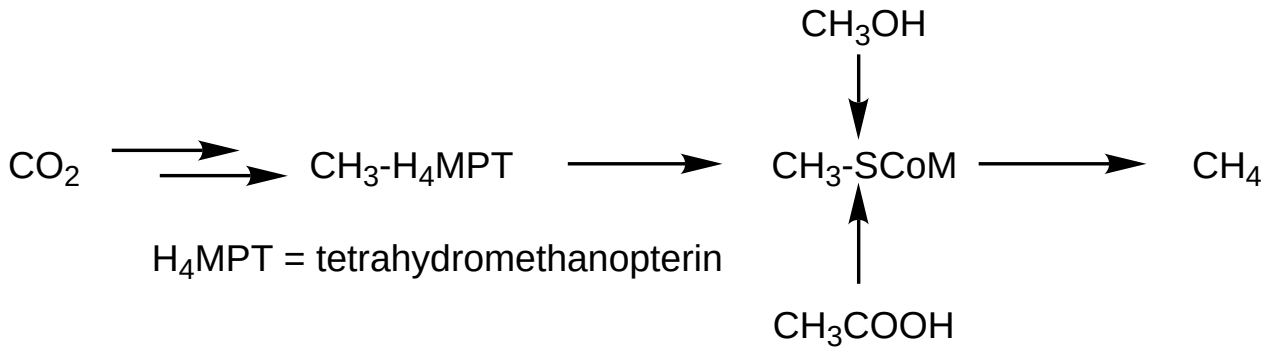
Introduction

• methanogensesis: the conversion of carbon dioxide into methane



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

• *M. barkeri* methanogenesis pathway:

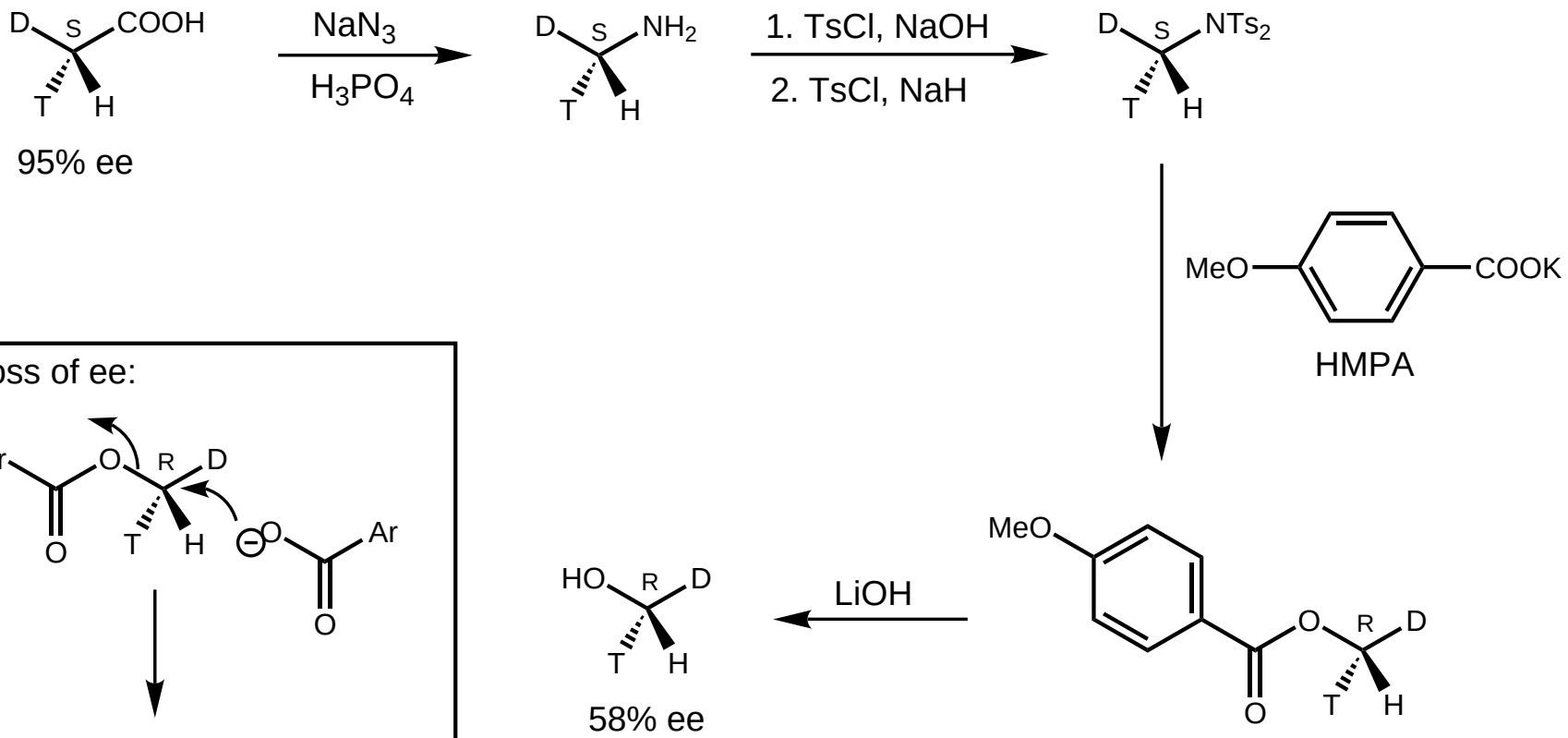


- also able to convert methanol and acetic acid to methane

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

Steric Course of Methanogenesis

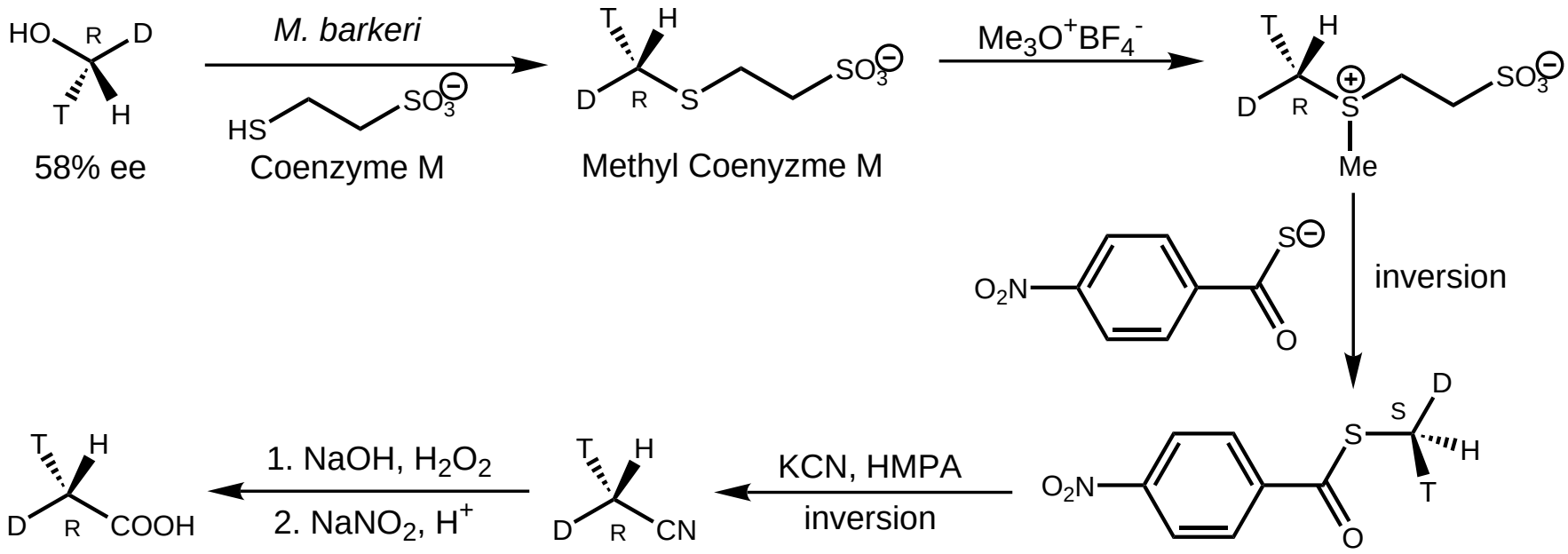
Synthesis of Chiral Methanol



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

Steric Course of Methanogenesis

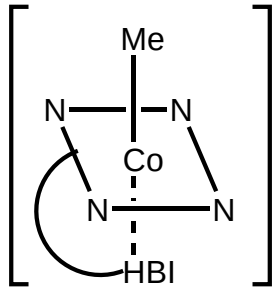
Methylation of Coenzyme M



F = 61 ∴ 38% ee (R)

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

- supports hypothesis that methylation proceeds via

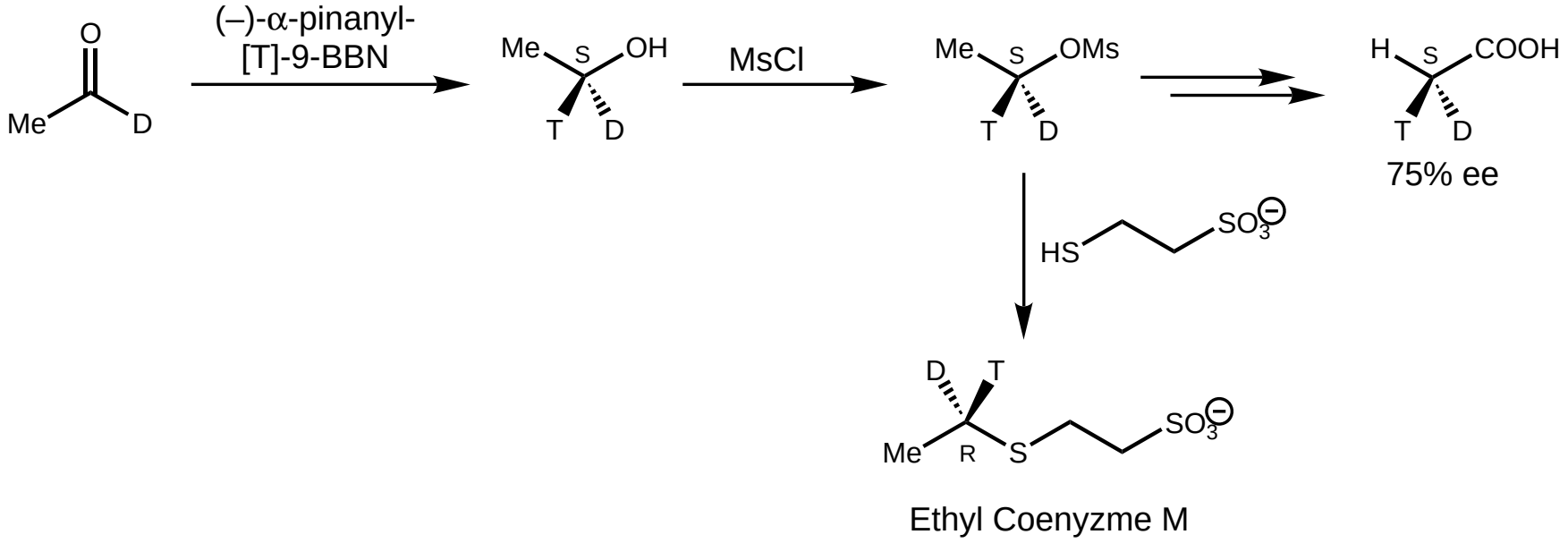


Floss, *J. Am. Chem. Soc.*, **1987**, *109*, 7922.

Steric Course of Methanogenesis

Reduction of Alkyl Coenzyme M

- Methyl Coenzyme M reduction product: 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:

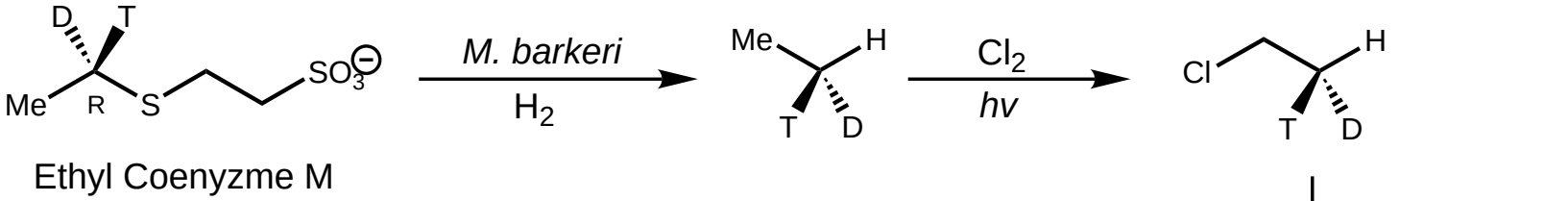


Ethyl Coenyzme M

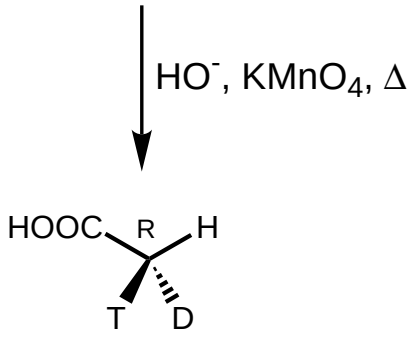
Floss, *J. Am. Chem. Soc.*, **1991**, *113*, 4700.

Steric Course of Methanogenesis

Reduction of Alkyl Coenzyme M



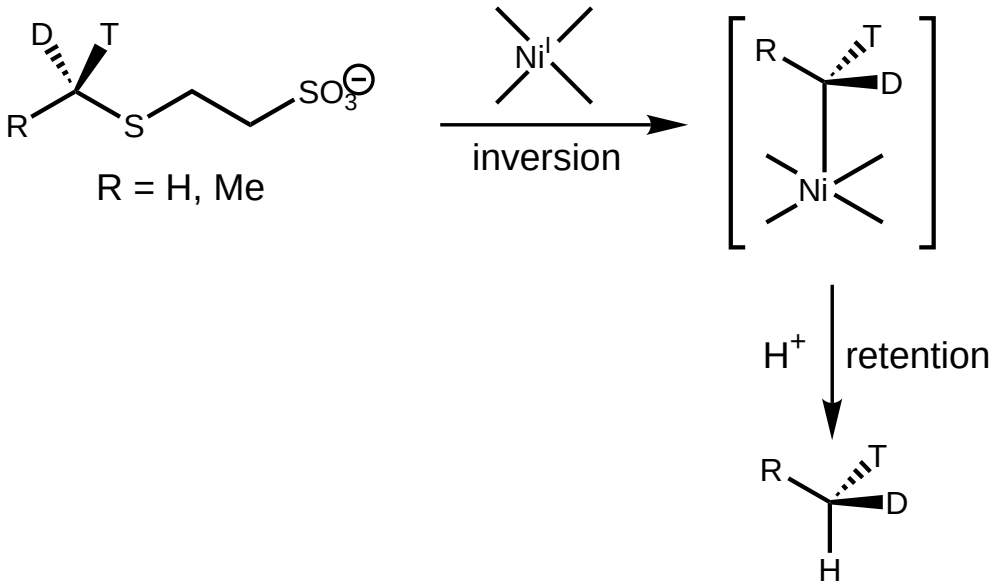
Ethyl Coenyzme M



F = 65 ∴ 51% ee (R)

∴ **net inversion of configuration**

• supports the proposal:

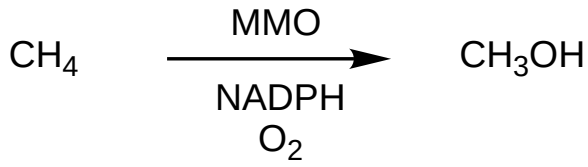


Floss, *J. Am. Chem. Soc.*, **1991**, *113*, 4700.

Mode of Action of Methane Monooxygenase

Introduction

• methane monooxygenase (MMO) catalyzes the NAD(P)H- and O_2-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

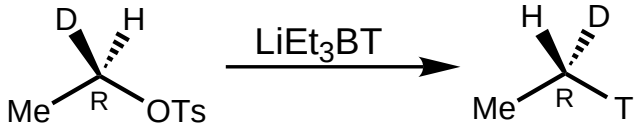
• several mechanisms have been proposed:

- radical intermediate
- cation intermediate
- concerted insertion into C-M bond

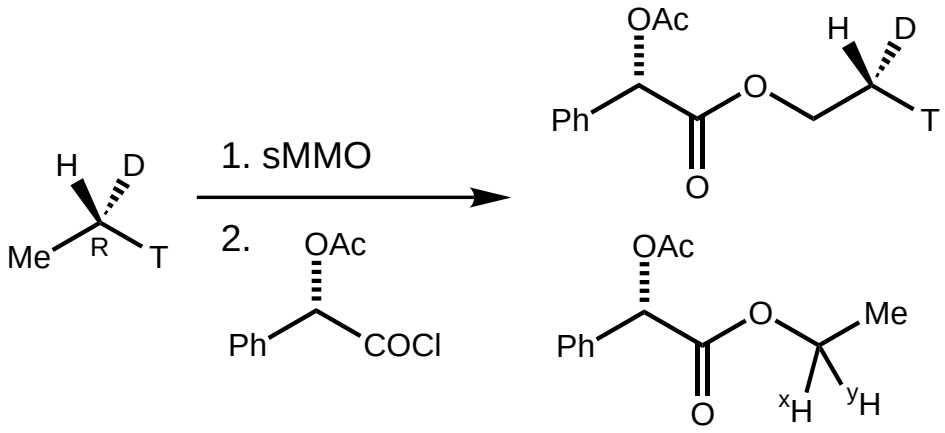
Floss and Lipscomb, *J. Am. Chem. Soc.* **1992**, *114*, 7561.
Chan and Floss, *J. Am. Chem. Soc.* **1996**, *118*, 921.

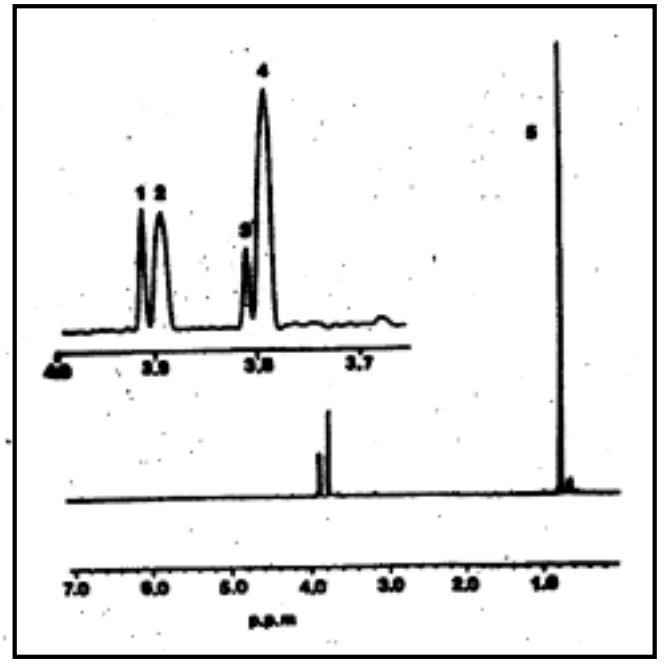
Mode of Action of Methane Monooxygenase

Synthesis and Oxidation of Chiral Ethane



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





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

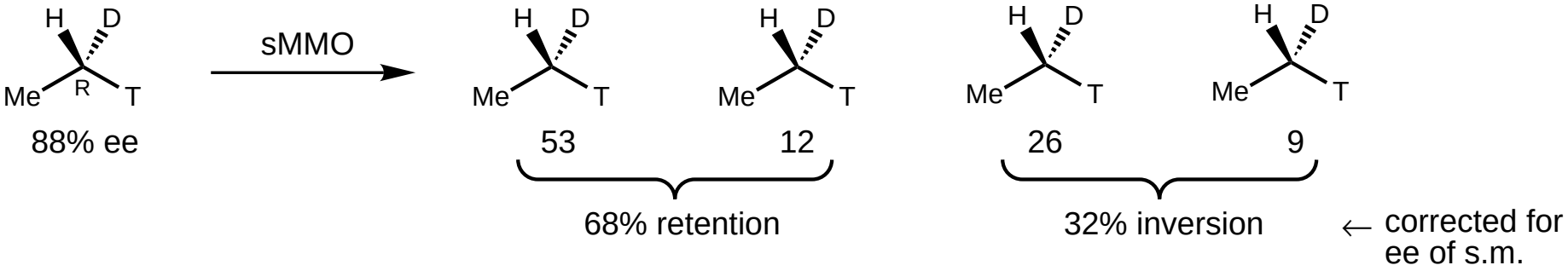
Floss and Lipscomb, *J. Am. Chem. Soc.* **1992**, *114*, 7561.

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 → rate for rotation = $4.9 \times 10^{12}\ s^{-1}$

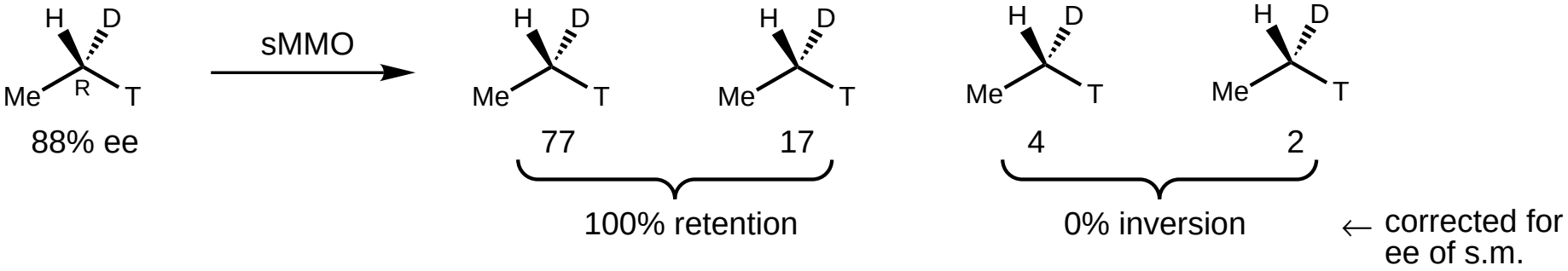
  ∴ **estimated rate constant for oxidation = $5.5 \times 10^{12}\ s^{-1}$**

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



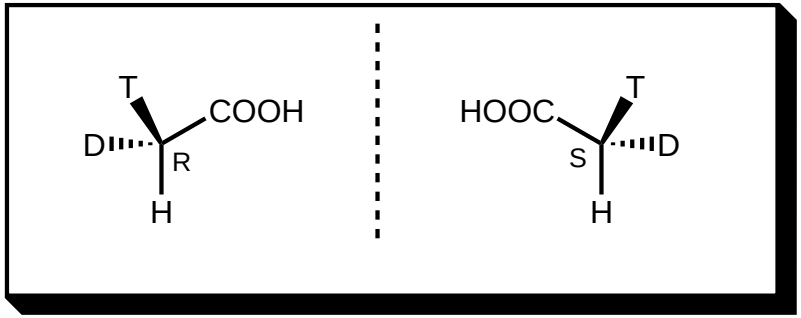
These results indicate that a radical intermediate is unlikely in this case, as it would need to have a lifetime of $<10^{-14}$ s in order to exhibit complete retention

"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 tritiide to elegant molecular rearrangements

• absolute configuration and enantiomeric excess can be determined using the Arigoni / Cornforth enzyme method (most sensitive) or using ^{3}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.