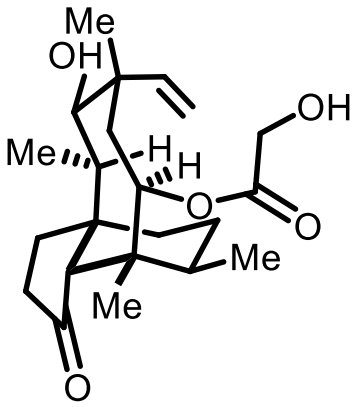
History of Pleuromutilin



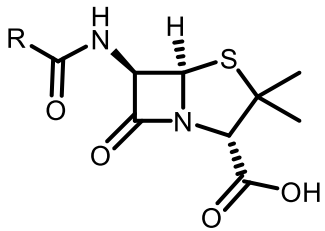
(+)-Pleuromutilin

GROUP MEETING

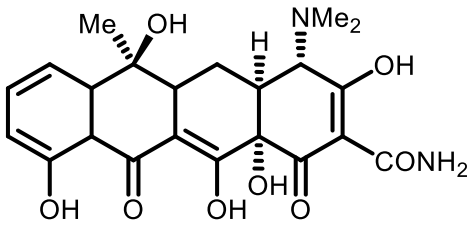
3/20/2018

KIMBERLY HILBY

Antibiotics: 1940-1970



R =Bn, penicillin
R= 2,6-methoxyphenyl, Methicillin



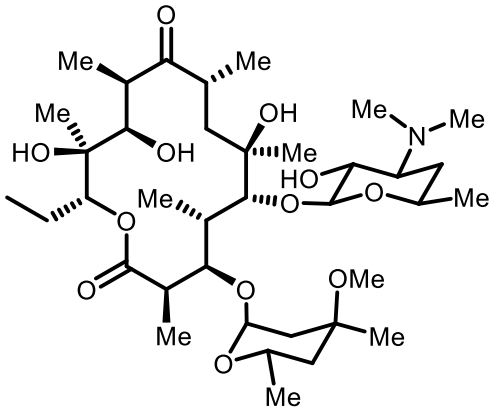
Tetracycline

Antibiotic Introduced

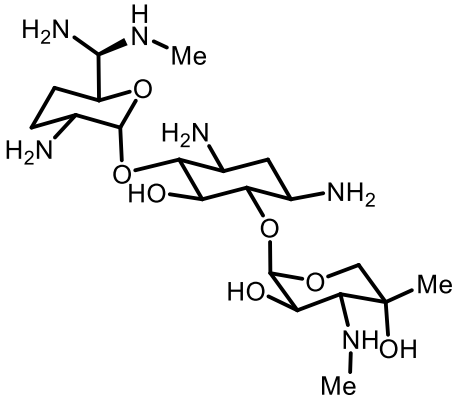
- 1943 penicillin
- 1950 tetracycline
- 1953 erthromycin
- 1960 methicillin
- 1967 gentamicin

Antibiotic Resistance Identified

- 1940 penicillin-R *Staphylococcus*
- 1959 tetracycline-R *Shigella*
- 1962 methicillin-R *Staphylococcus*
- 1965 penicillin-R *Pneumococcus*
- 1968 erthromycin-R *Streptococcus*



erthromycin

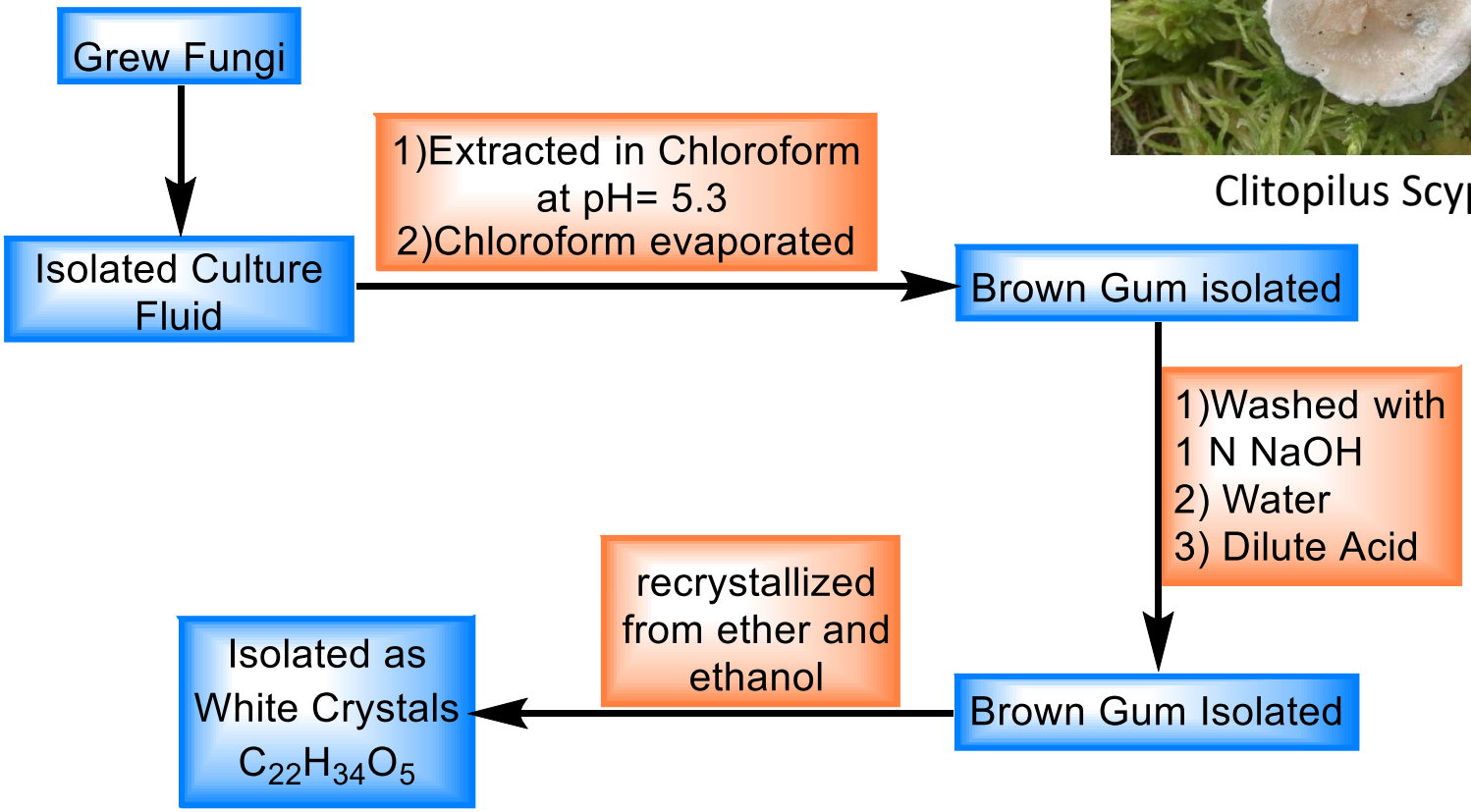


gentamicin

Isolation of Pleuromutilin

- Isolated from the Pleurotus Mutilus, an edible mushroom, in 1951

Clitopilus Scyphoides



KAVANAGH, F.; HARVEY, A.; ROBBINS, W.J. PROC. NATL. ACAD. SCI. U.S.A. 1951, 37, 570

Initial Bioactivity

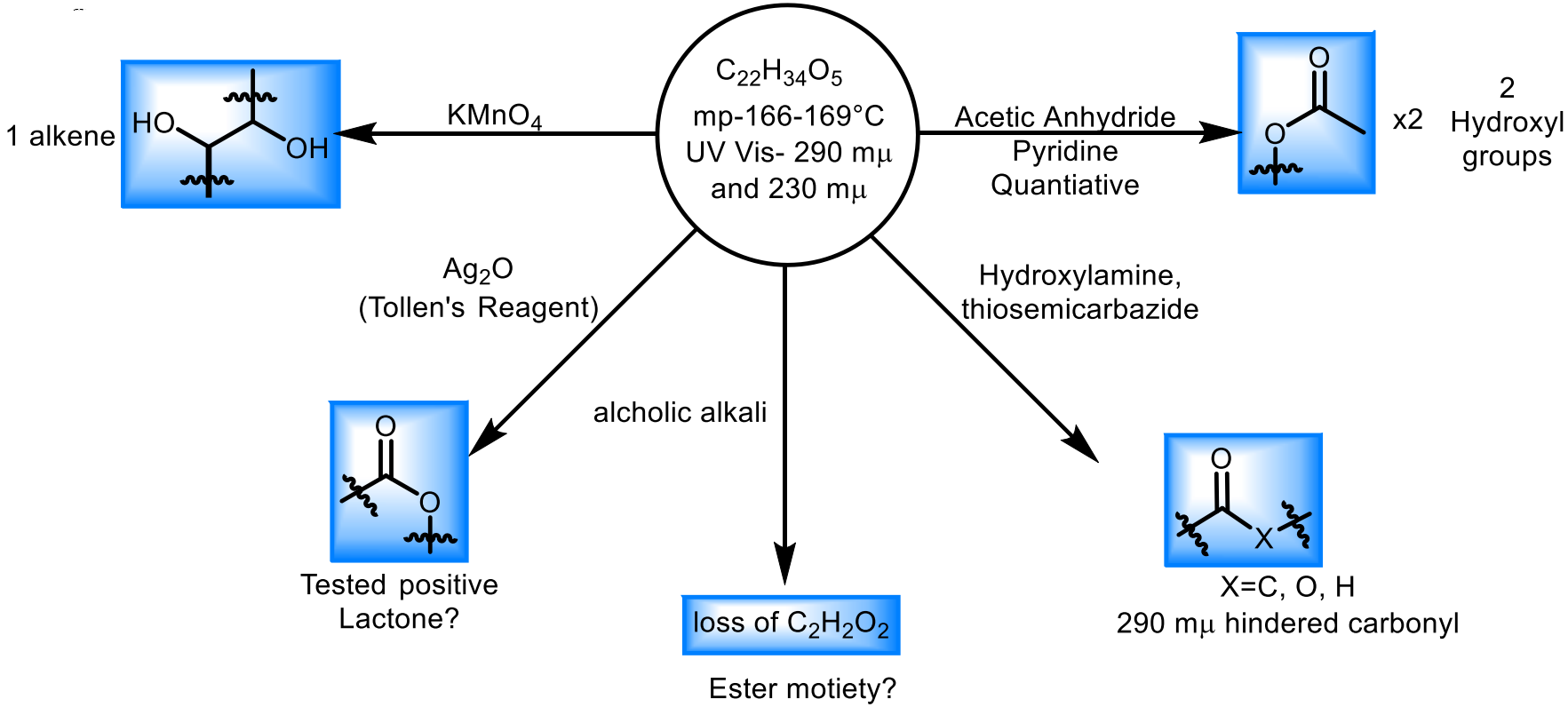
Bioactivity was shown to be moderate but effective against gram positive bacteria

Strain	Dilutions per ml
Bacillus mycoides	125
Bacillus subtilis	8
Escherichia coli	500
Klebsiella pnemoniae	1
Mycobacterium smegma	32
Pseudomonas aeruginoa	>1000
Staphylococcus aureus	0.25

Clitopilus Scyphoides

KAVANAGH, F.; HARVEY, A.; ROBBINS, W.J. PROC. NATL. ACAD. SCI. U.S.A. 1951, 37, 570

Initial Attempts of Characterization



Characterization

$C_{22}H_{34}O_5$ —alkaline hydrolysis→ $C_{20}H_{32}O_3$ —CrO_3→ $C_{20}H_{28}O_3$

Pleuromutilin
2 carbonyls
2 alcohol

Mutilin
1 carbonyl
2 alcohols

Trione
2 alcohols
were secondary

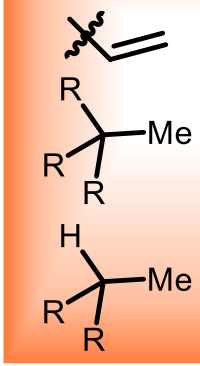
Kuhn-Roth Oxidation

$C_{20}H_{32}O_3$ —H_2CrO_4, H_2So_4, Δ→ Acetic Acid

Mutilin

No other violatiles- means methyls are all attached to ring system

PMR Spectrum



attached to quaternary carbon

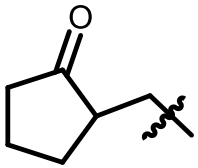
2 tertiary methyls

2 secondary methyls

IR for Compound showed a band 1735 cm^{-1} which is characteristic of a cyclopentanone

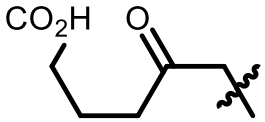
$C_{24}H_{36}O_5$ —1 Mole of O_2, KO^tBu→ $C_{24}H_{36}O_7$

Diacetylmutilin



Peak at 1735 cm^{-1}

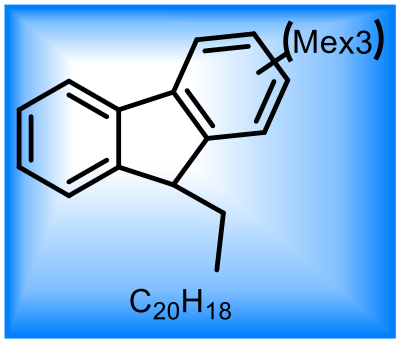
—1 Mole of O_2, KO^tBu→



Loss of 1735 cm^{-1}
Gain at 1689 cm^{-1}

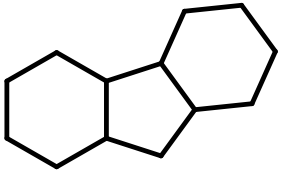
Structure of Pleuromutilin

$C_{20}H_{32}O_3$ → (Acetic Anhydride, Pyridine) → $C_{24}H_{36}O_7$ → (Se, 300°C) →

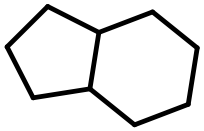


Mutilin

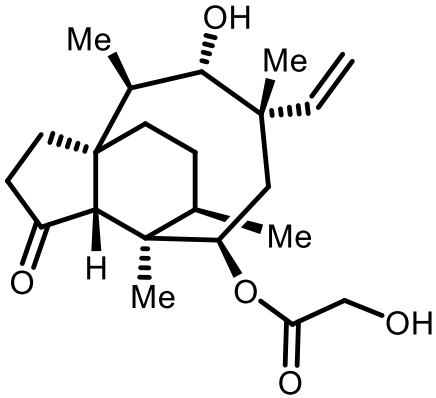
Matched by looking at Mass Spec Fragmentation



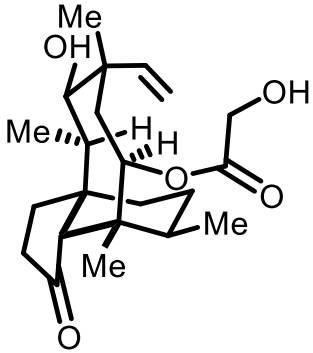
Hydrofluorene core was ruled out because IR showed evidence of Cyclopentanone ring



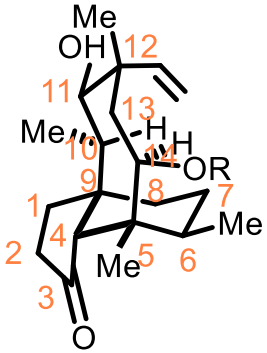
indane with some other ring can rearrange and cyclize to give the Fluorene



≡



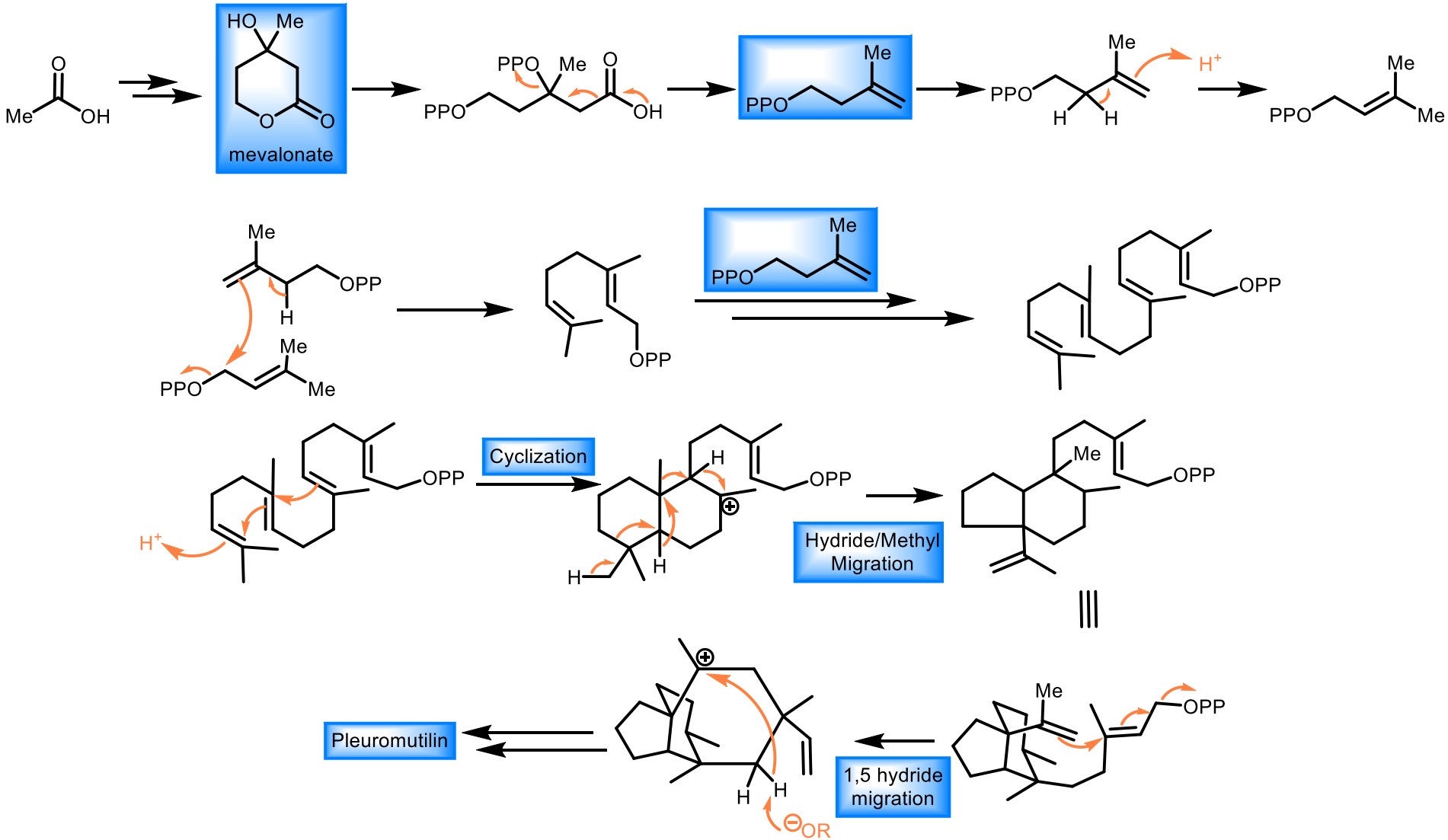
(+)-pleuromutilin



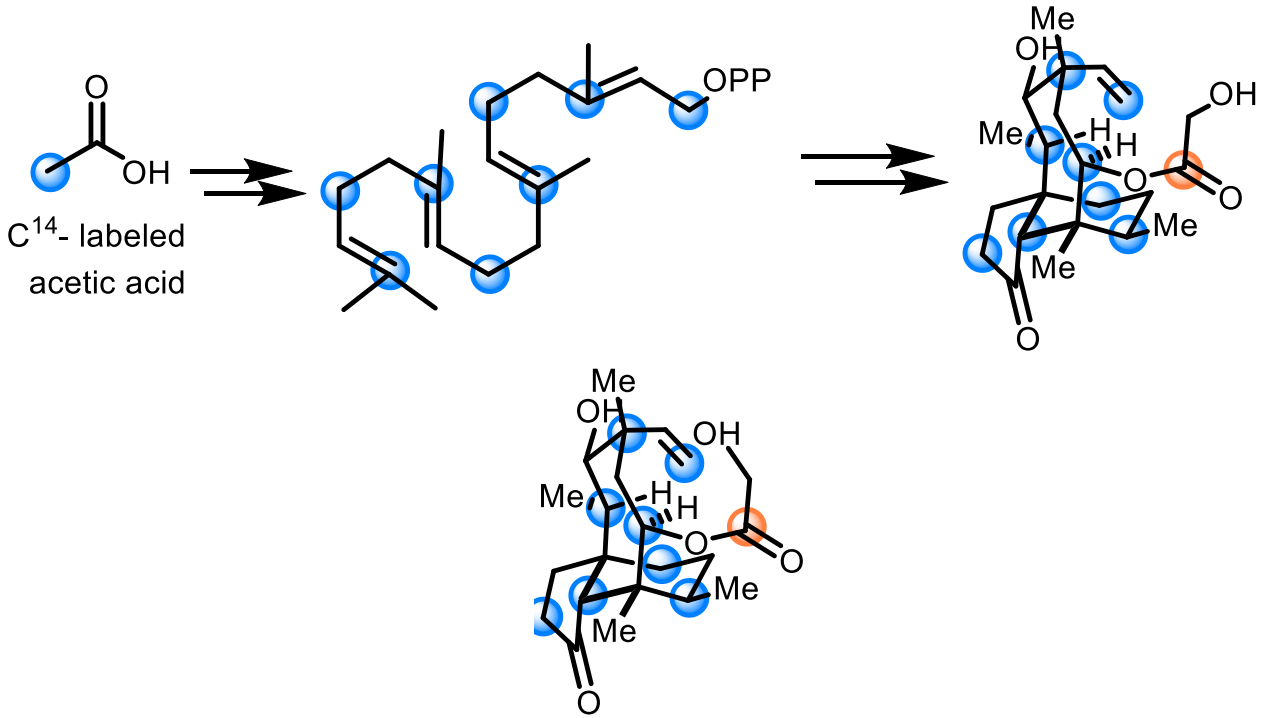
(+)-mutilin, R=H

(+)-pleuromutilin, R=$COCH_2OH$

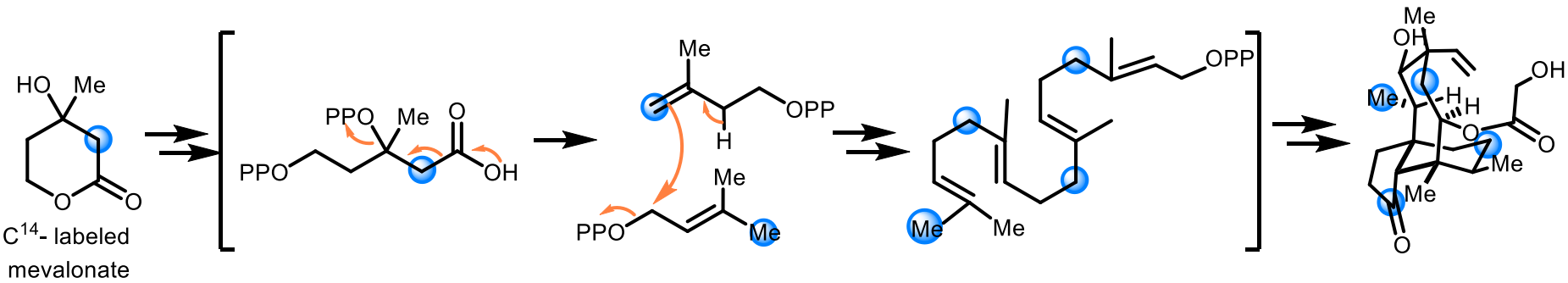
Hypothesized Biosynthesis



Evidence of terpene cyclization

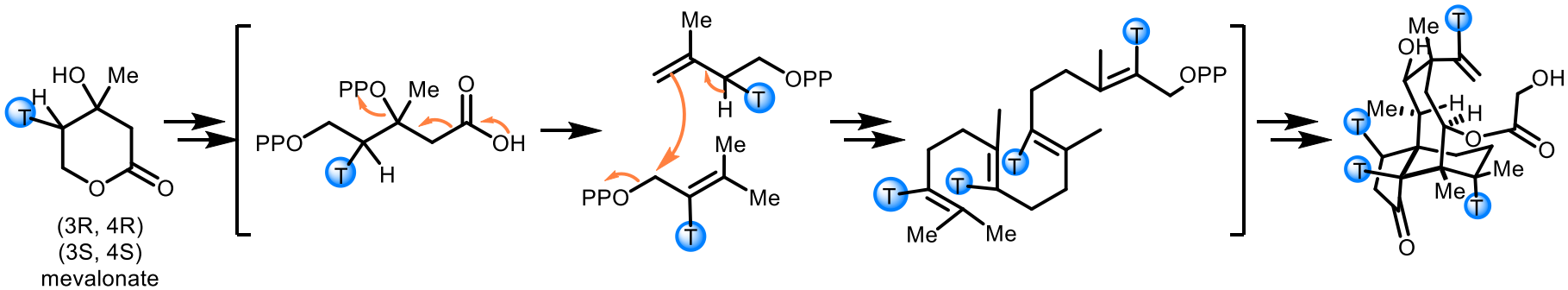


Biosynthesis of Pleuromutilin

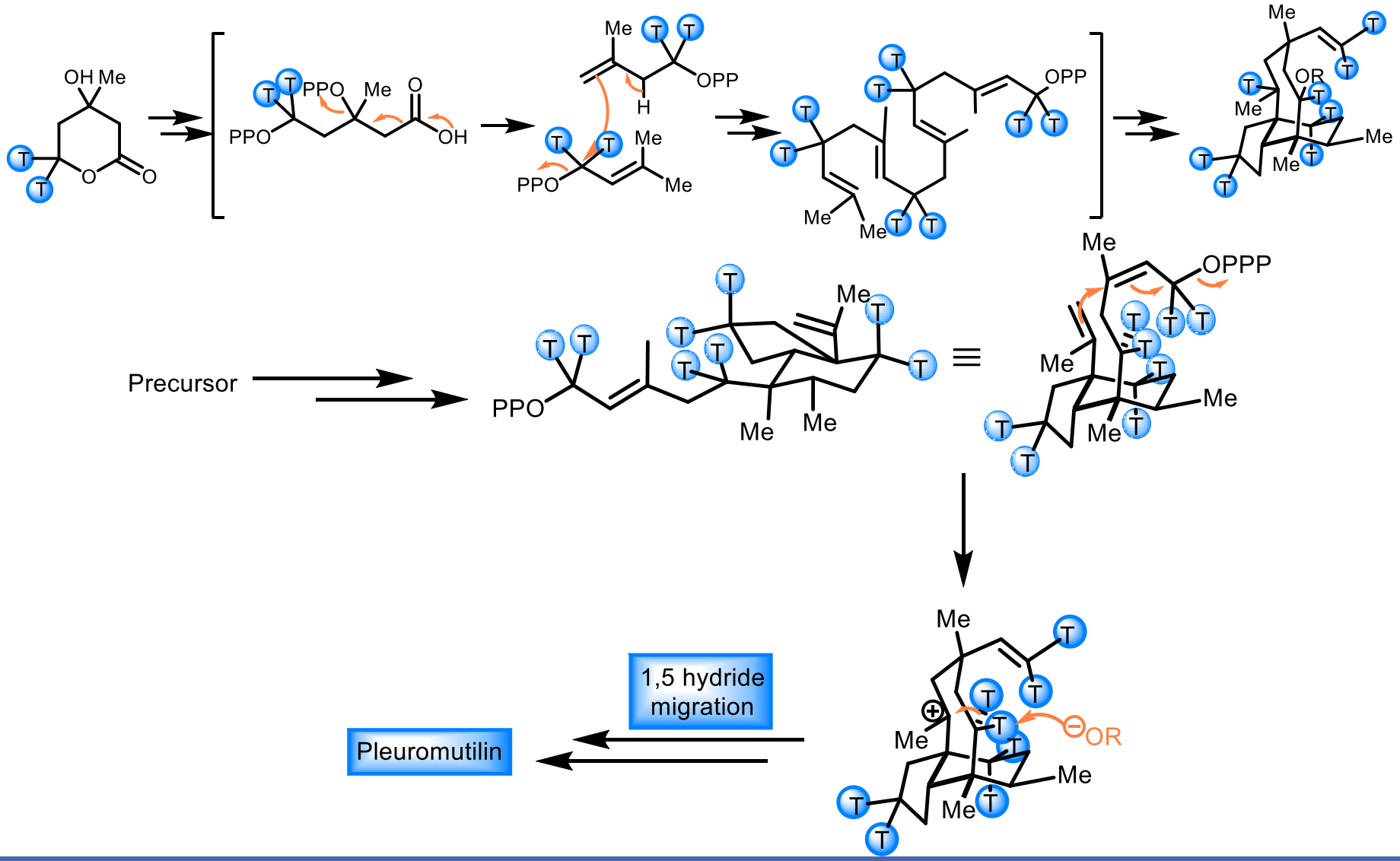


ARIGONI, D. PURE APP. CHEM. 1968, 17, 331-348

Evidence of Hydride/Methyl Shift

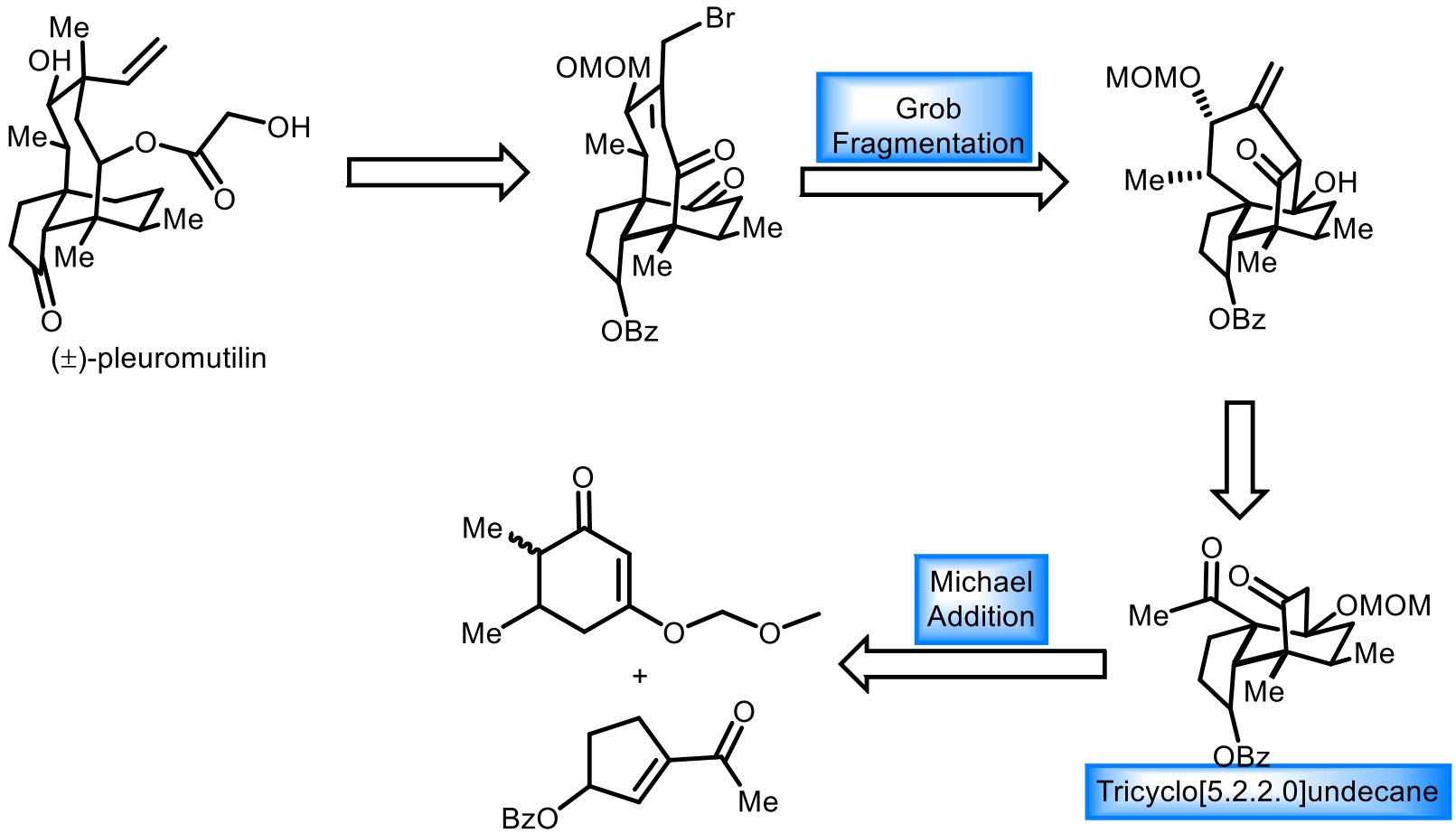


Evidence of 1,5-hydride transfer

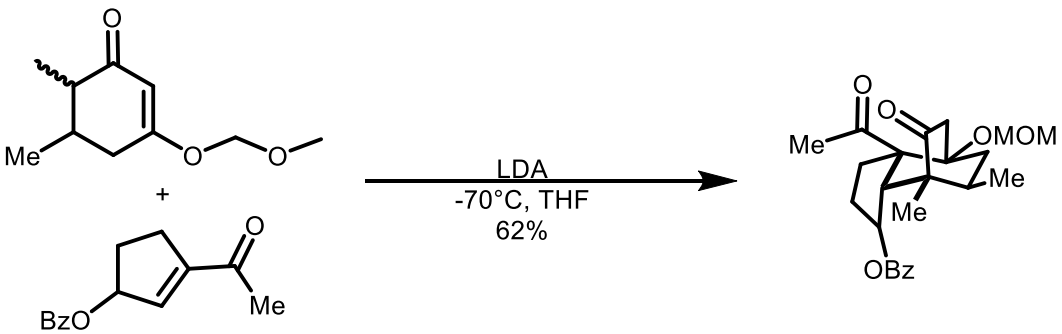


Gibbons's Synthesis

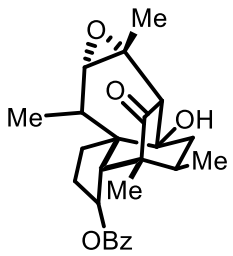
Dr. Grant Gibbons's synthesis was done at Harvard University in the labs of the late Professor R.B. Woodward



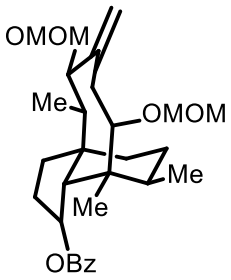
Gibbons's Synthesis of Tricycle

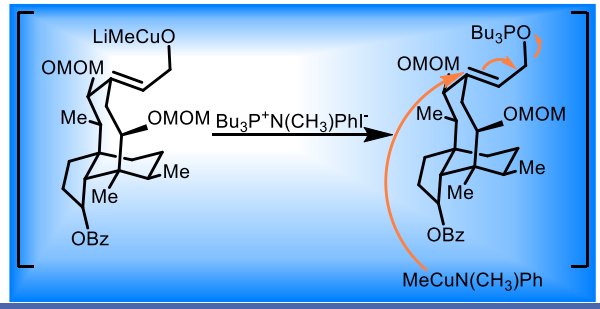


Gibbons's Synthesis to Core



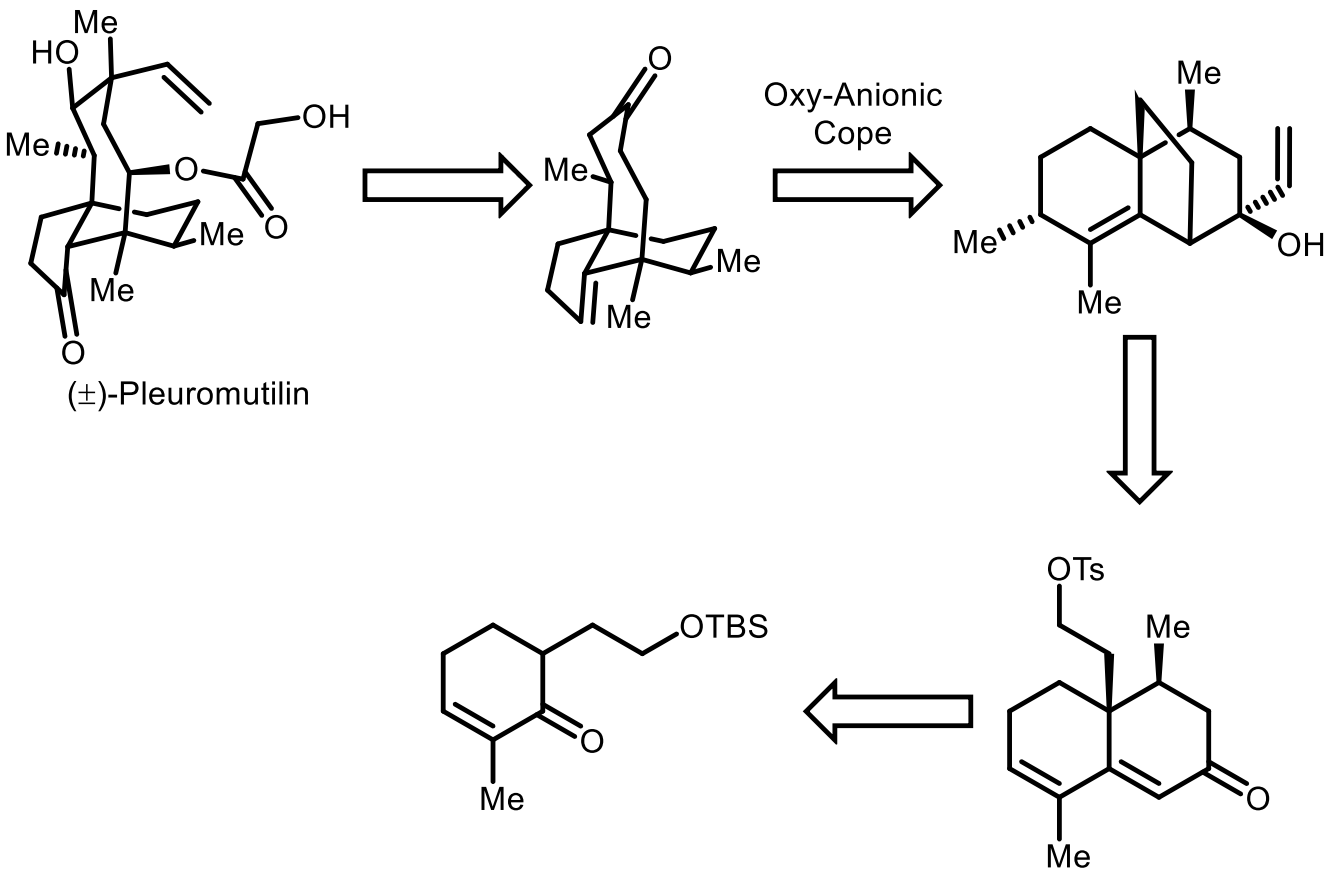
Gibbons's Elaboration of Ring





GIBBONS, G. J. AM. CHEM. SOC. 1982, 104, 1767-1769

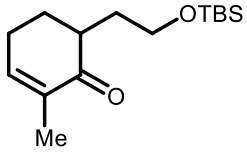
Boeckman Retrosynthetic Analysis



Professor Robert Boeckman from the University of Rochester

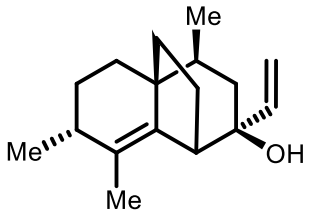
BOECKMAN, R.; SPRINGER, D.; ALESSI, T. J. AM. CHEM. SOC. 1989, 111, 8284-8286

Boeckman Racemic Synthesis



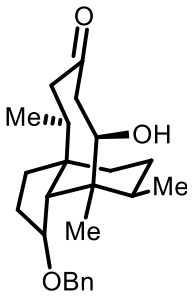
BOECKMAN, R.; SPRINGER, D.; ALESSI, T. J. AM. CHEM. SOC. 1989, 111, 8284-8286

Boeckman Racemic Synthesis



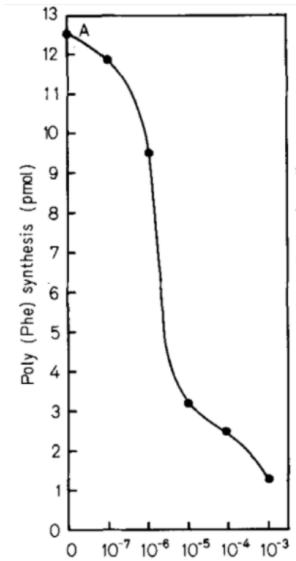
BOECKMAN, R.; SPRINGER, D.; ALESSI, T. J. AM. CHEM. SOC. 1989, 111, 8284-8286

Boeckman Racemic Synthesis

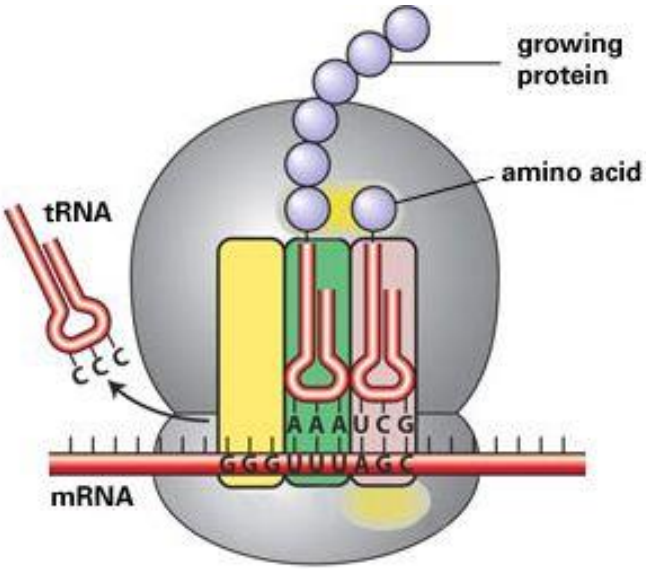


BOECKMAN, R.; SPRINGER, D.; ALESSI, T. J. AM. CHEM. SOC. 1989, 111, 8284-8286

Initial work on Mode of Action



Inhibits protein synthesis



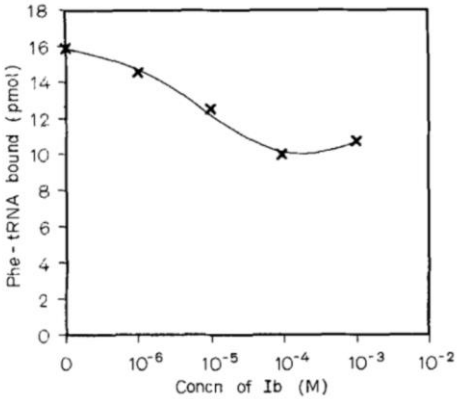


Fig. 5. *Effect of compound Ib on EF-T-catalyzed binding of Phe-tRNA to ribosomes in the presence of poly(U).* All other details are listed in Materials and Methods

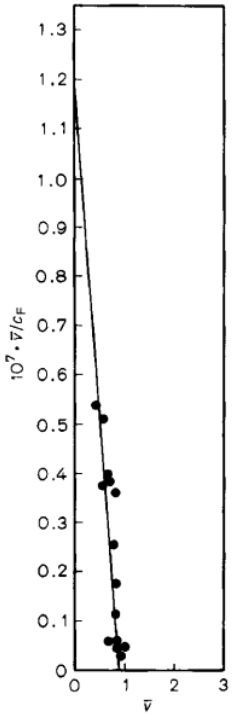
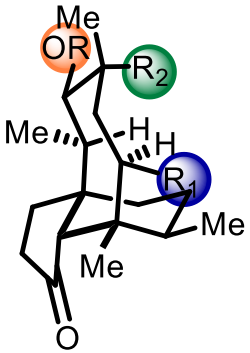


Fig. 1. *Scatchard plot of the equilibrium dialysis data from binding of compound II to ribosomes.* The ribosome concentration of 202 A_{260} units/ml was kept constant for all measurements

Does this by not allowing Phe-tRNA to bind to site A

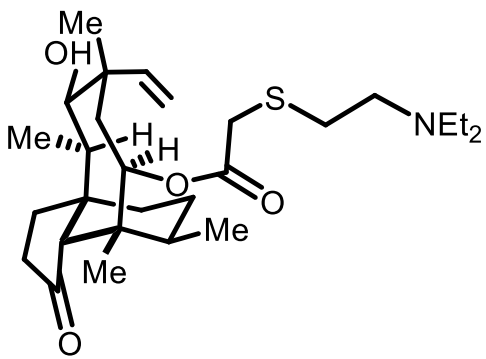
First SAR studies



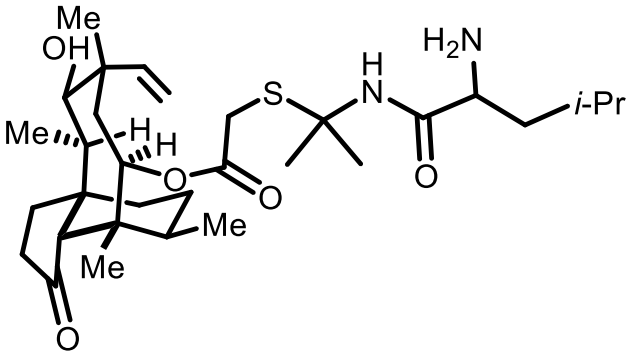
-OR	$-R_1$	$-R_2$	Staph. aureus	*Mycoplasma hominis*	*Mycoplasma Gallisepticum*	*Mycoplasma hyorhinis*
H	$COCH_2OH$	vinyl	.5	.3	.3	2
H	$COCH_2S(CH_2)_2NEt_2$	vinyl	0.05	0.01	0.006	0.3
H	$COCH_2NEt_2$	vinyl	1.4	5	5	3
H	$COCH_2S(CH_2)_2NEt_2$	ethyl	0.1	0.02	0.02	0.6
H	$COCH_2SC_6H_4COOH$	vinyl	1	>100	0.2	100
H	$COCH_2OAc$	vinyl	0.5	0.5	8	0.5
Ac	$COCH_2OAc$	vinyl	>10	>10	10	-

EGGER, H.; REINSHAGEN,H. J. ANTIBIO. 1976, 29, 923-927

New Drugs from these SAR Studies



Tiamulin



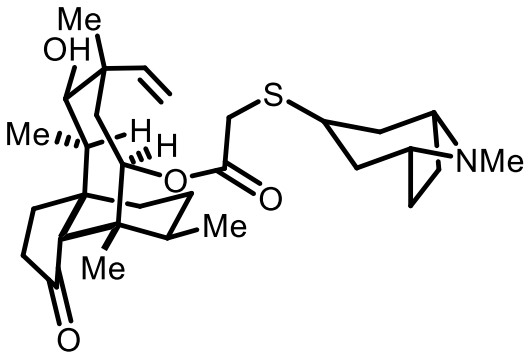
Valnemulin

- Tiamulin(1979) and Valnemulin(1999) are both used in veterinary medicine to treat pulmonary and intestinal infections in pigs

Why not more human drugs developed?

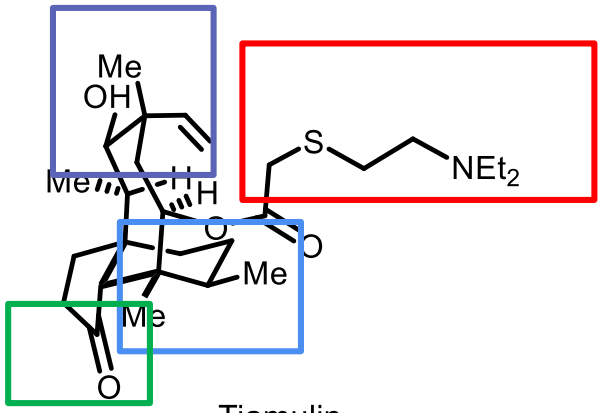
Rapidly and extensively metabolized by cytochrome P450 metabolism in vivo

GSK drug approved in 2007-only Pleuromutilin type drug approved for human use- topical antibiotic for skin infections

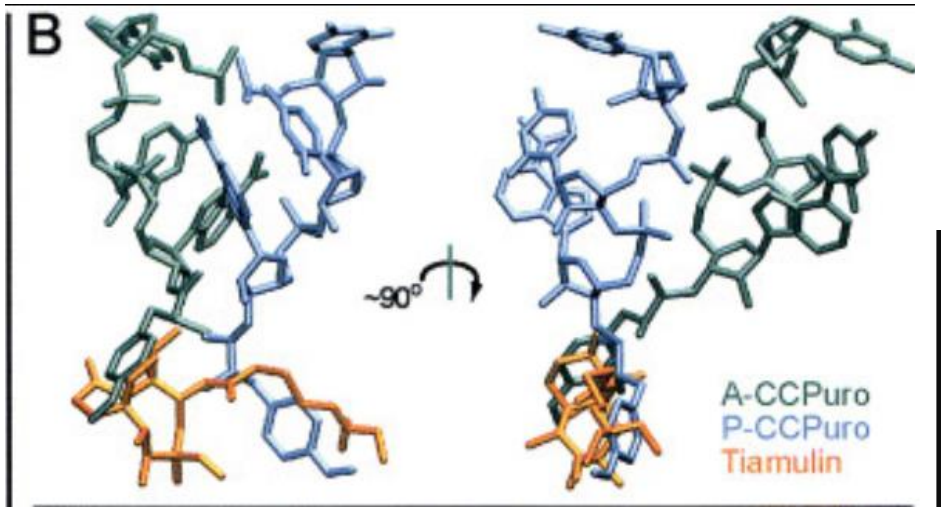


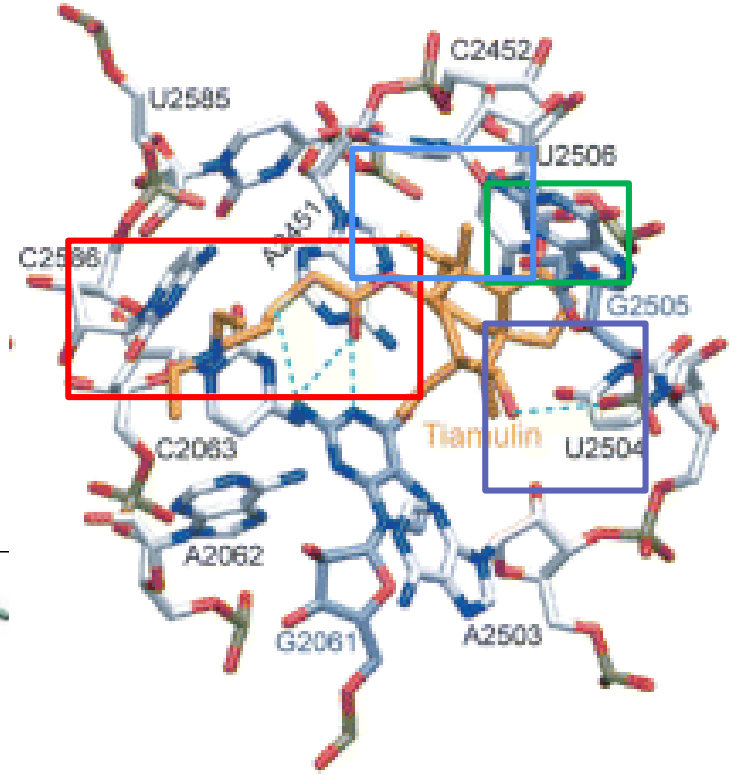
Retapamulin

Recent Work on Method of Action



Tiamulin

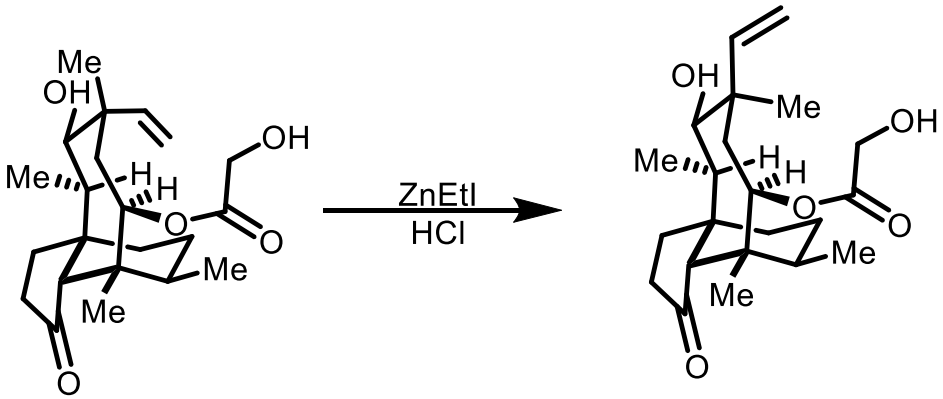




SCHLUNZEN, F.; PYETAN, E.; FUCINI, P.; YONATH, A.; HARMS, J.M. MOL. MICRO. 2004, 54, 1287-1294

Group Problem

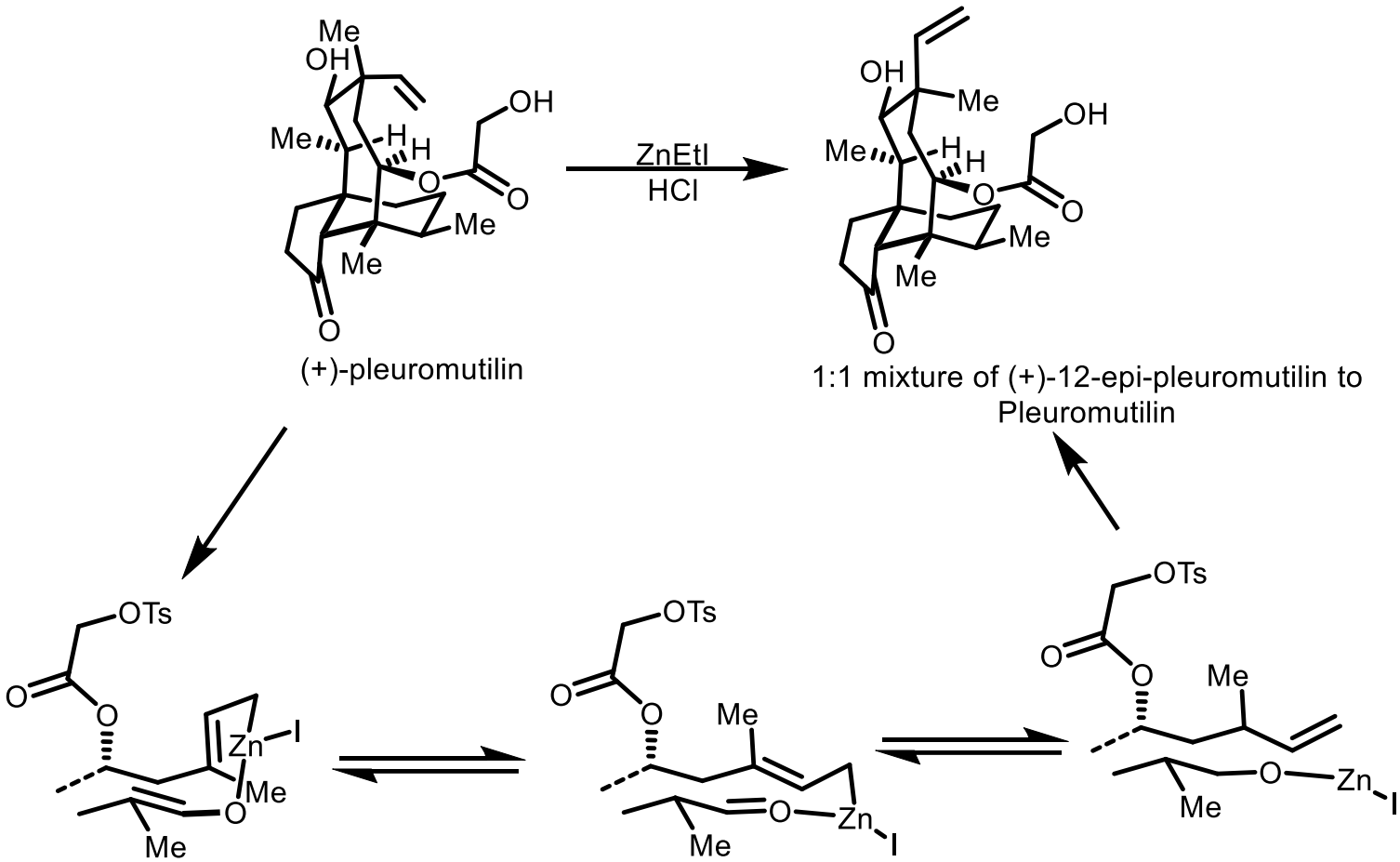
Propose a mechanism and model for the reaction



(+)-pleuromutilin

1:1 mixture of (+)-12-epi-pleuromutilin to (+)-pleuromutilin

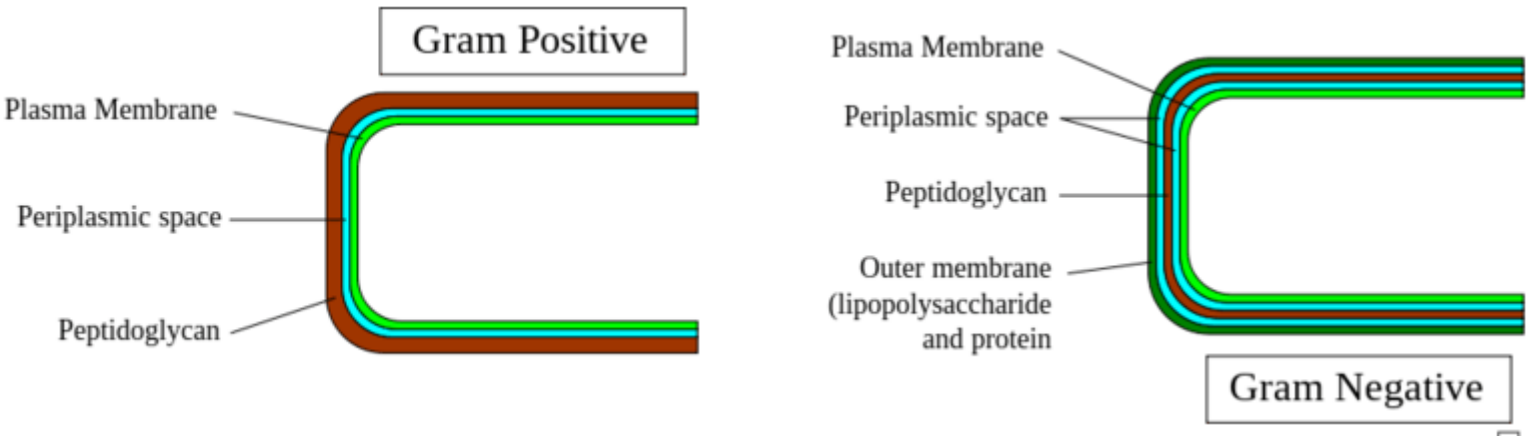
Group Problem



Discovery led to large quantities of 12-epi-pleuromutilin

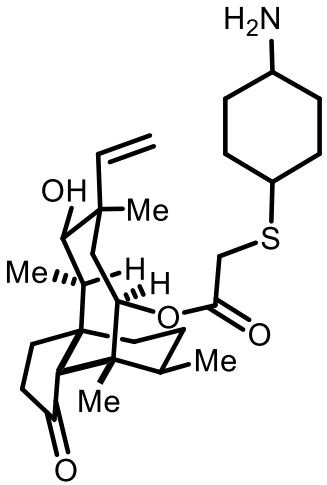
BERNER, H.; VYPLEL, H.; SCHULZ, G.; SCHNEIDER, H. MONATSH. CHEM. 1986, 117

Epi-pleuromutilin SAR

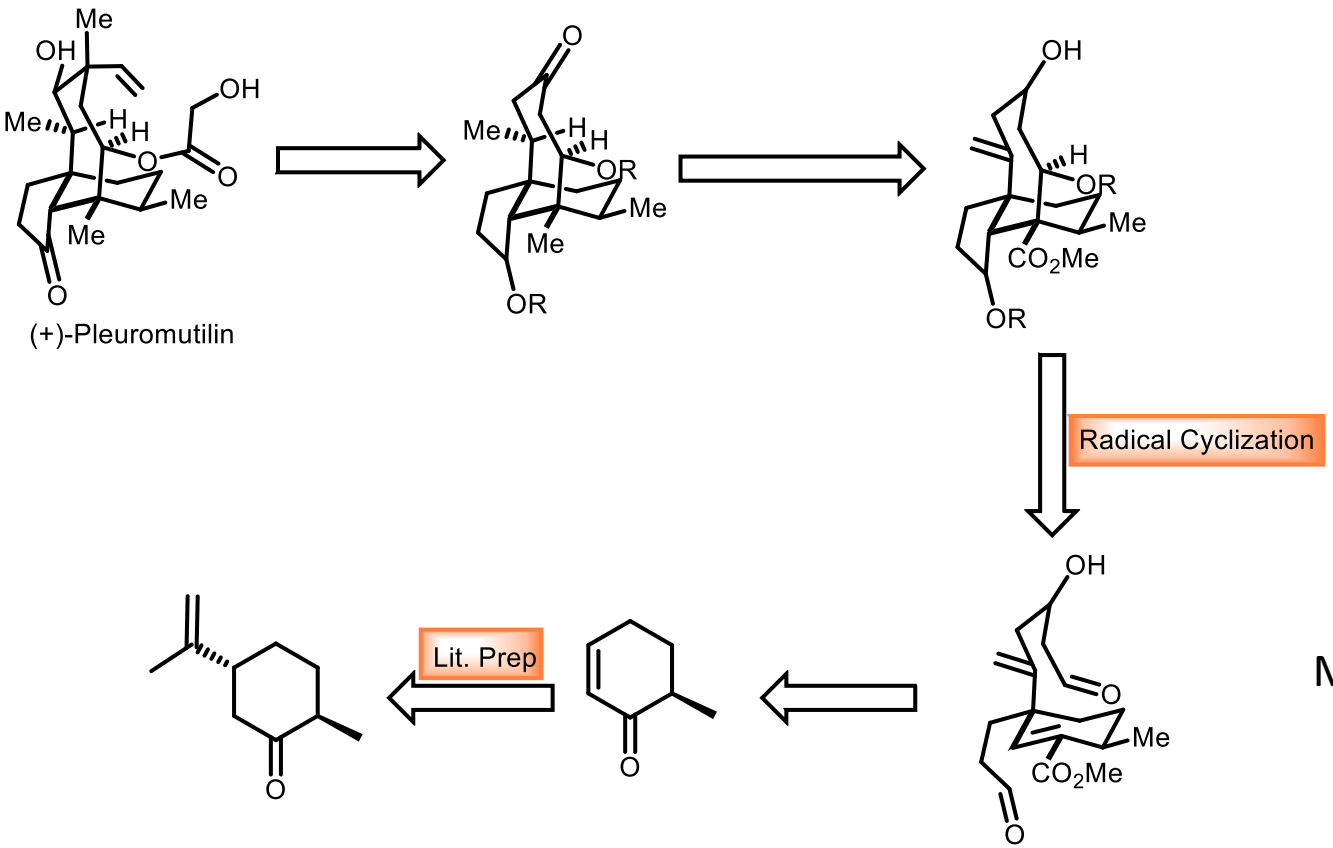


Gram negative bacteria:
-Channels to pump out harmful toxins to the bacteria
-Resistance is higher because of covering around the cell wall

In 2015 a patent was filed for a pleuromutilin derivative that was effective against gram negative bacteria

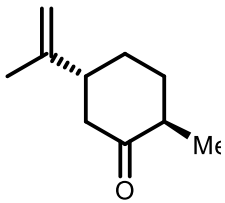


Procter Retrosynthetic Analysis



Professor David Procter at the University of Manchester reported the first non racemic synthesis of Pleuromutilin

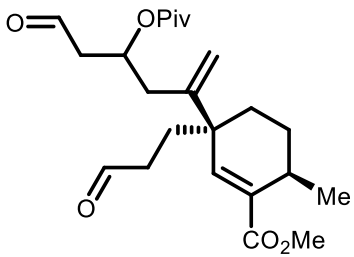
Procter Core Synthesis



(+)-trans-dihydrocarvone

FAZAKERLEY, N, HELM, M.; PROCTER, D. CHEM. EUR. J. 2013, 6718-6723

Procter Key Step



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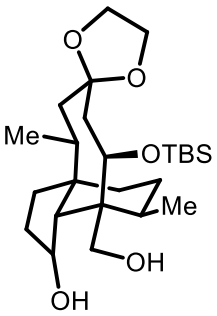
Procter Further Elaboration of Core



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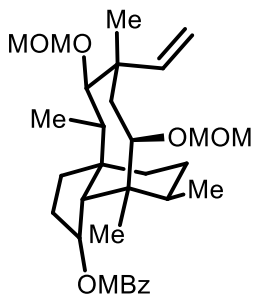
Procter End Game



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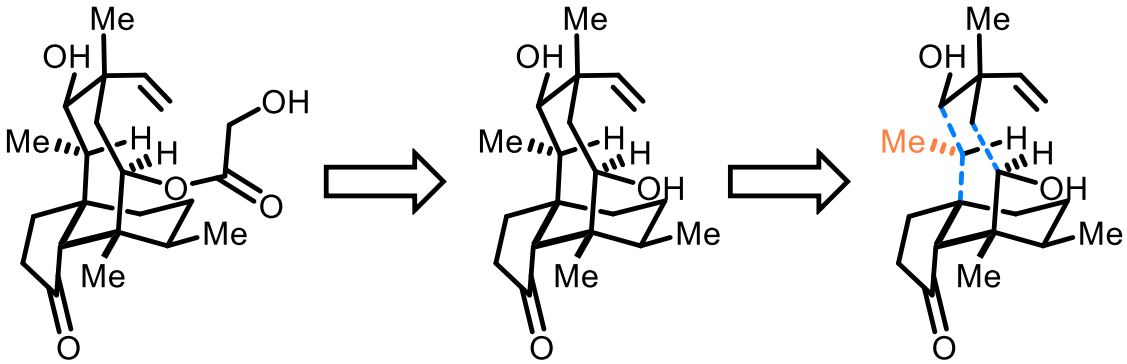
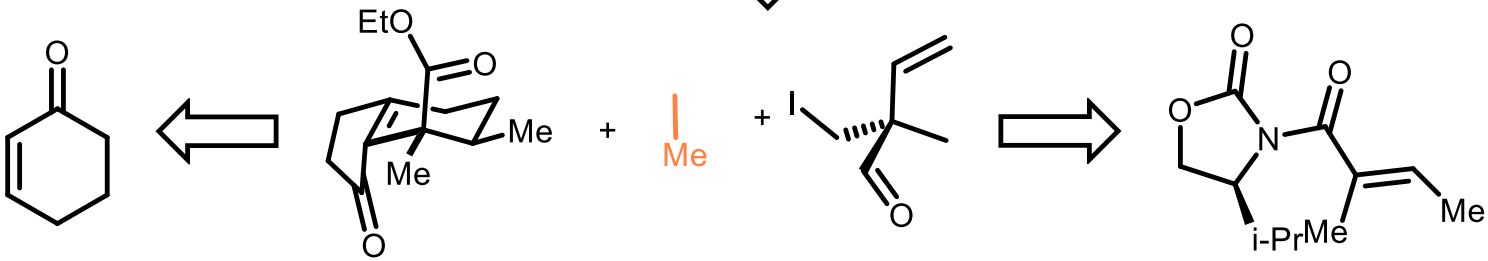
Herzon Retrosynthetic Analysis

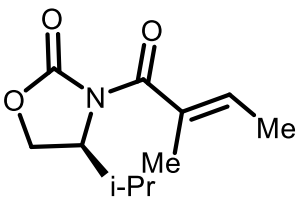


(+)-Pleuromutilin (+)-mutilin

Professor Seth Herzon from Yale University



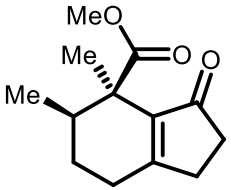
Herzon Synthesis Part 1



$Zn(CH_3)_2$,

3

Problems with Addition



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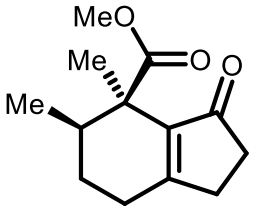
ZENG, M.; MURPHY, S.; HERZON, S. J. AM. CHEM. SOC. 16377-16388

1,4-Addition into the Enone



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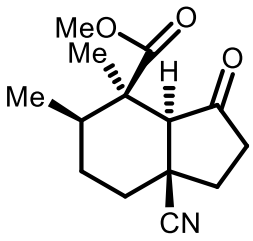
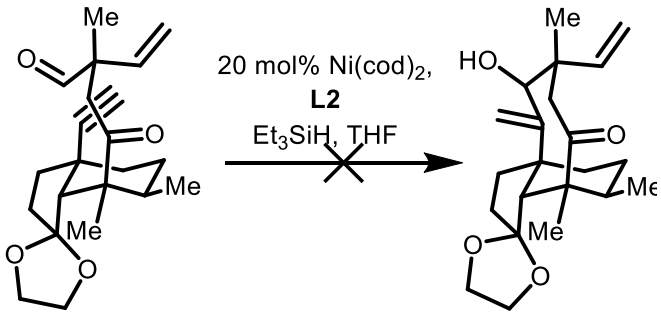
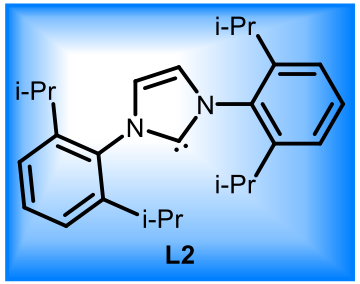
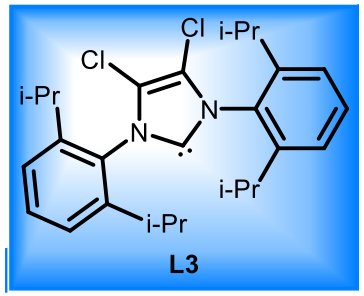
Solution to the Problem



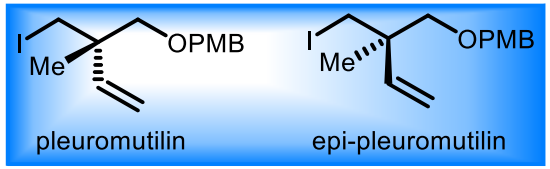
Alkyne and Aldehyde Coupling

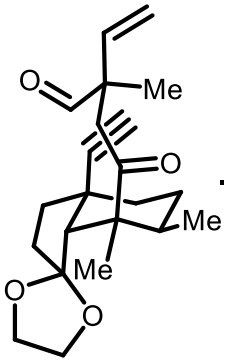




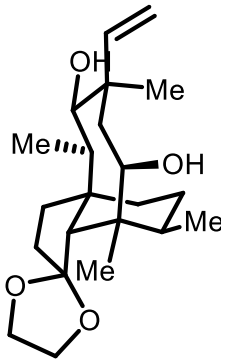


End Game

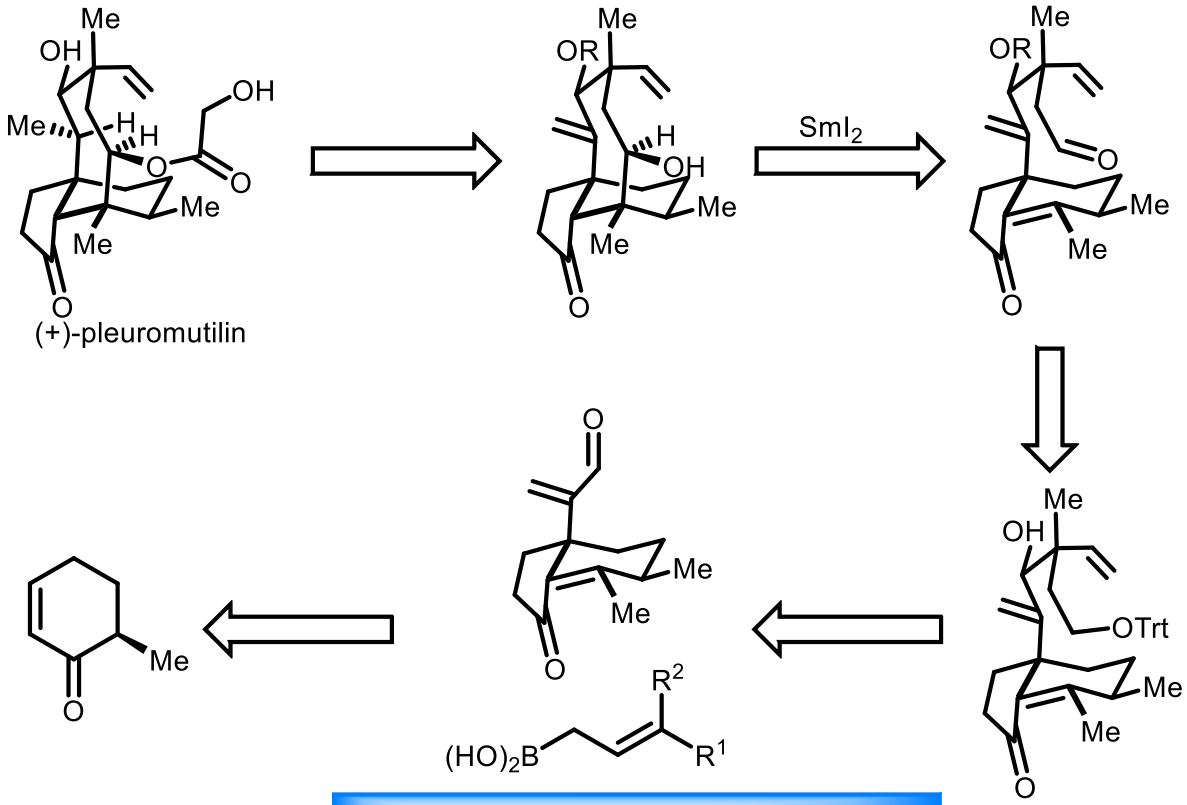

I
Me
OPMB
pleuromutilin
I
Me
OPMB
epi-pleuromutilin


O
Me
O
Me
Me
O
O

Final Step of Synthesis



Reisman Retrosynthetic Analysis



Z(pleuromutilin) :R^1=Me, R^2=CH_2CH_2OTrt
E(epi-pleuromutilin):R^1=CH_2CH_2OTrt, R^2=Me

Professor Sarah Reisman at Caltech University

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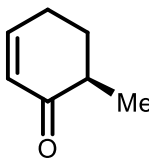
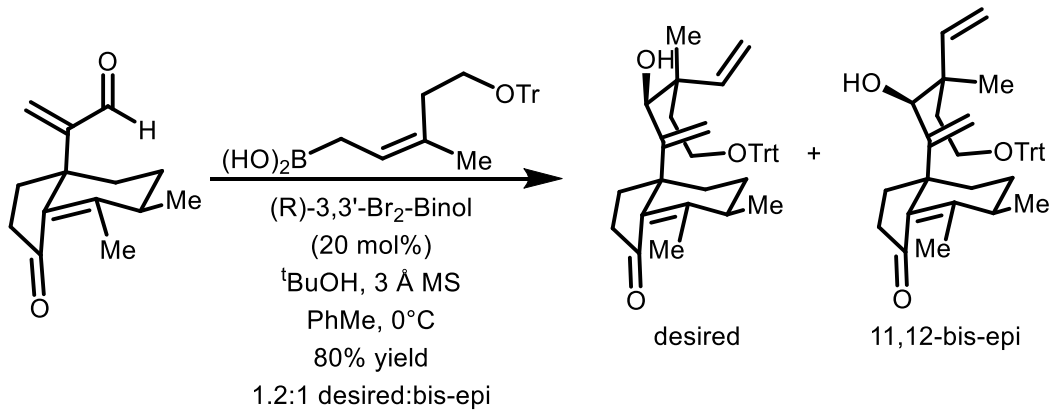
Reisman Total Synthesis



The troublesome step



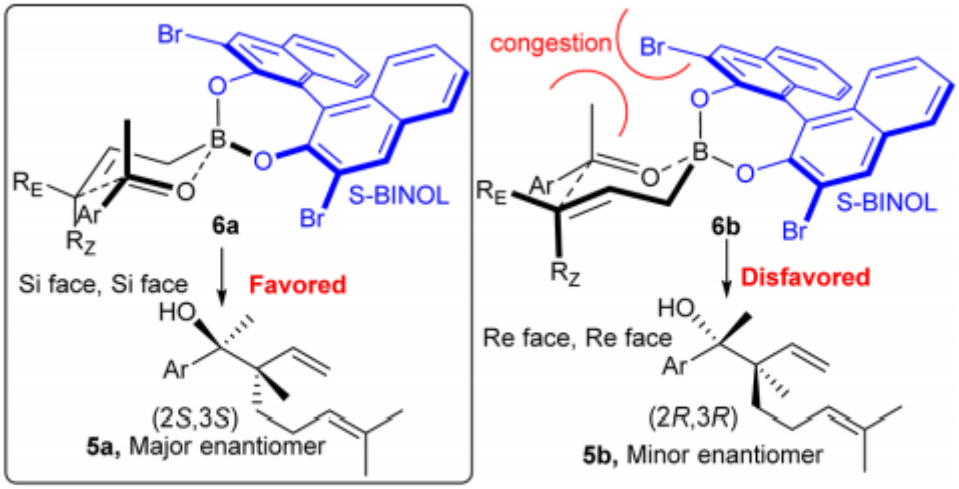
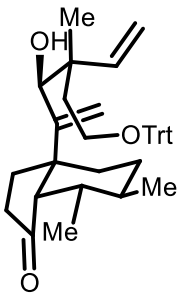
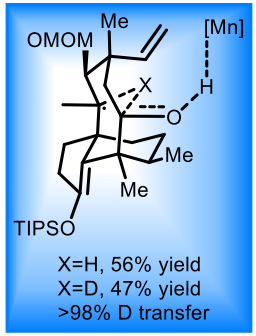


Figure 2. Plausible mechanism of the enantioselection.

End Game





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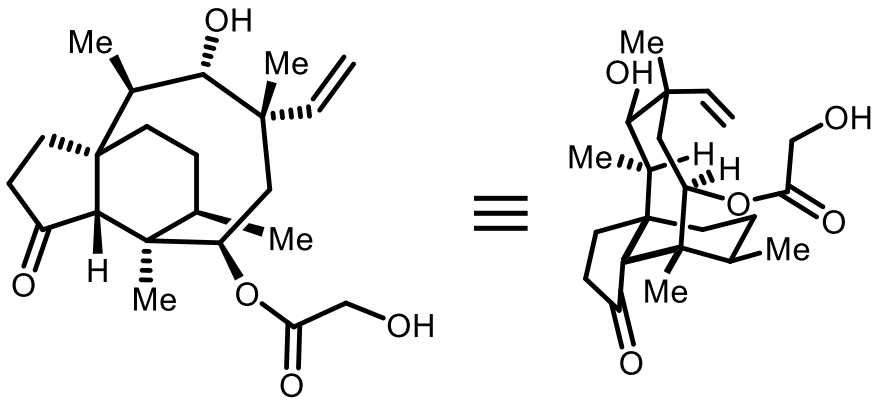
Conclusion



(+)-pleuromutilin

Author	Racemic/Non Racemic	Longest Linear Step	Overall Yield
Gibbons	Racemic	31	.7%
Boeckman	Racemic	27	.4%
Procter	Non Racemic	34	.7%
Herzon	Non Racemic	20	.7%
Reisman	Non Racemic	18	2.5%