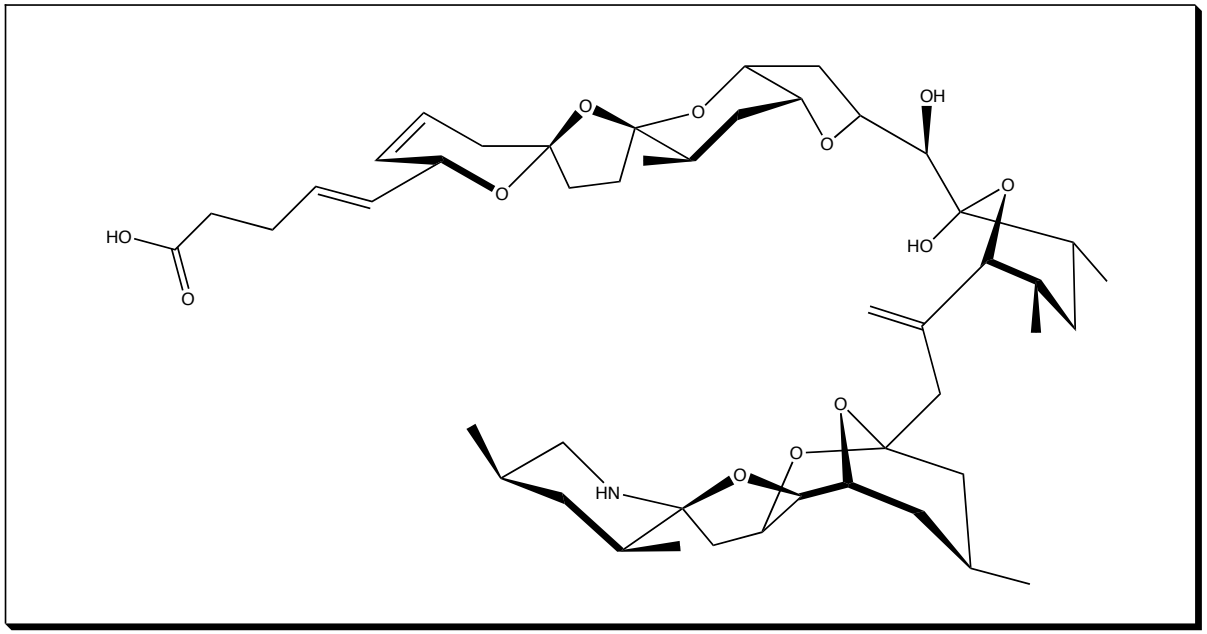
UNIVERSITY OF MINNESOTA-TWIN CITIES

SYNTHESIS OF THE ABCD AND FGHI DOMAINS OF AZASPIRACID

Son T. Nguyen, Ph.D.
Adviser: Professor Craig J. Forsyth



PhD research 2001- 2006

PRESENTATION OUTLINES

1- BACKGROUND

Structure, isolation, biological significance of azaspiracids

2- SYNTHESIS OF THE **FGHI** DOMAIN

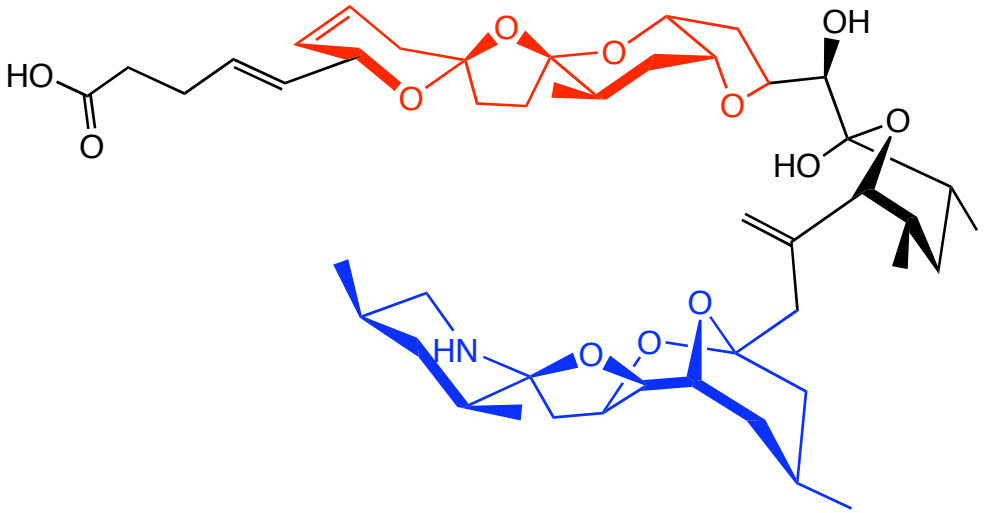
- Redesigned synthesis
- Development of an immunoassay based on the FGHI hapten

3- STRUCTURE REVISION AND SYNTHESIS OF THE **ABCD** DOMAIN

- Structural reassignment based on published NMR
- New synthesis

4- SUMMARY

5- ACKNOWLEDGEMENT



SHELLFISH

scallop

oyster

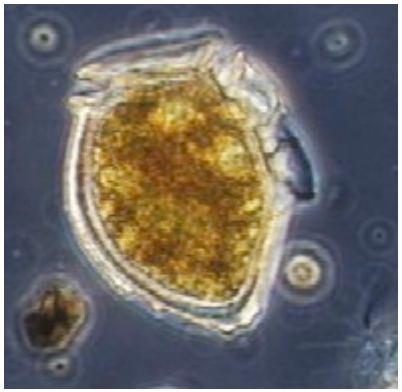
clam

mussel

Restaurant's special

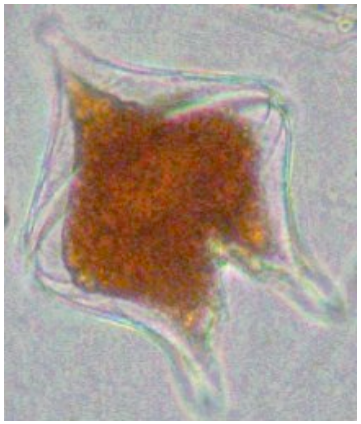
DINOFLAGELLATE - THE PRIMARY FOOD SOURCE

Definition: photosynthetic unicellular aquatic organisms



Dinophysis

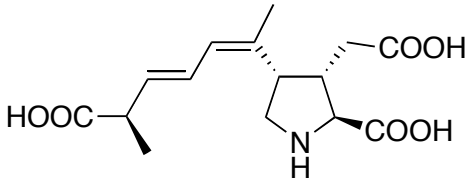
Gymnodinium



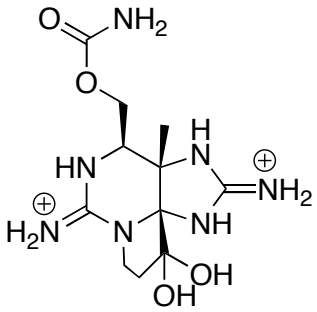
Protoperidinium

But they could be deadly…

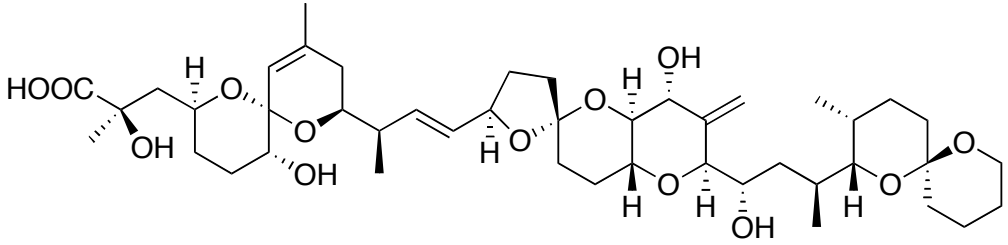
SHELLFISH POISONING FROM DINOFLAGELLATES



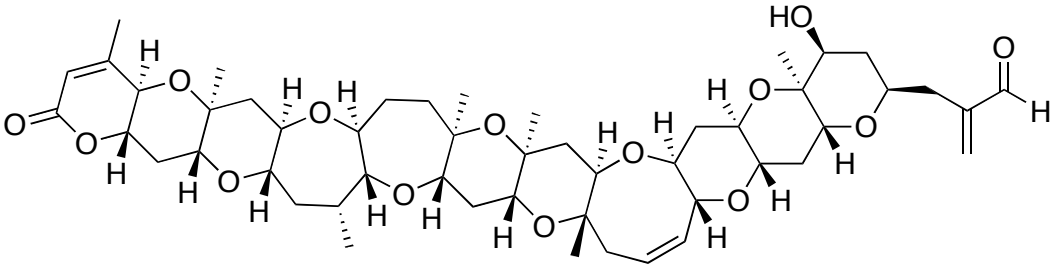
Domoic acid
Pseudonitzschia spp.
Amnesic Shellfish Poisoning (ASP)



Saxitoxin
Alexandrium, Gymnodinium, Pyrodinium spp.
Paralytic Shellfish Poisoning (PSP)



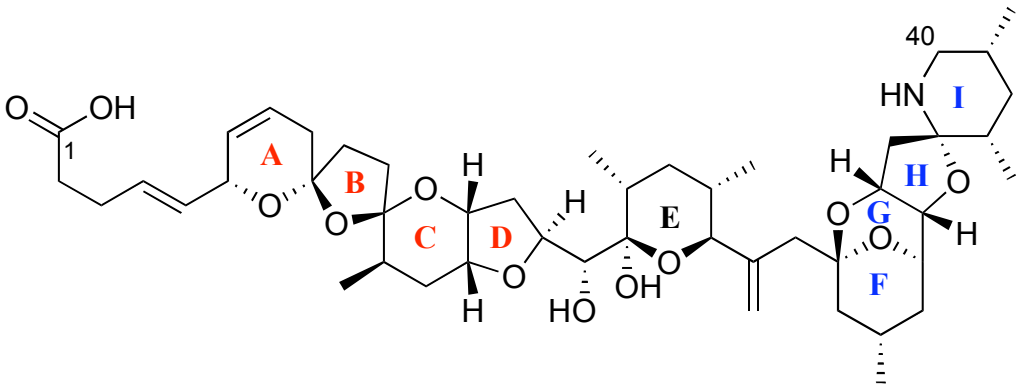
Okadaic acid
Dinophysis spp.
Diarrhetic Shellfish Poisoning (DSP)

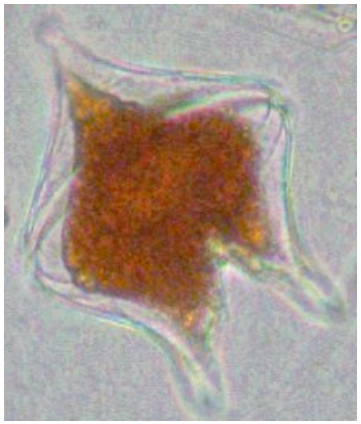


Brevetoxin B
Gymnodinium breve
Neurotoxic Shellfish Poisoning (NSP)

and AZASPIRACIDs --->

AZASPIRACID





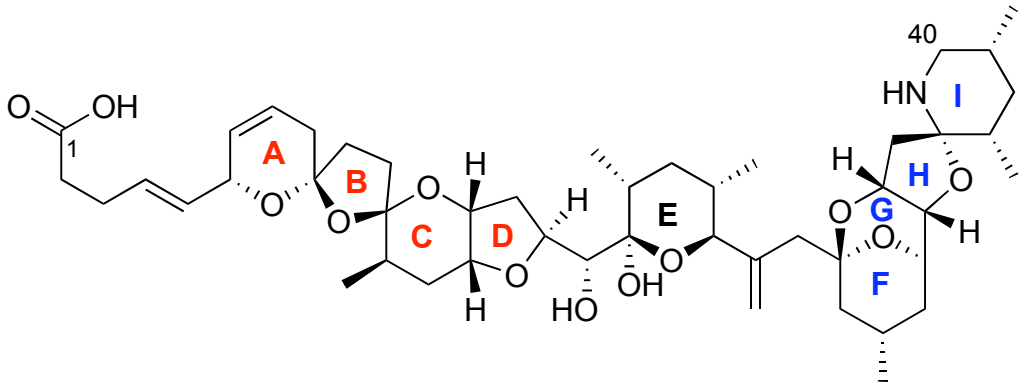
P. crassipes

- Azaspiracid poisoning (AZP) first recognized in 1995, the Netherlands
- Isolation from mussel *Mytilus edulis* and characterization first reported by Yasumoto and coworkers, 1998
- First synthesized with revised structure by Nicolaou group in 2004
- Originated from dinoflagellate *Protoperidinium crassipes*

Mytilus edulis
the blue mussel

Yasumoto *et al. J. Am. Chem. Soc*. **1998**, *120*, 9967-9968.
Nicolaou *et al. Angew. Chem. Int. Ed*. **2004**, *43*, 4312-4324.
Ito *et al*. *Toxicon*, **2000**, *38*, 917-930.

BIOLOGICAL SIGNIFICANCE OF AZASPIRACID



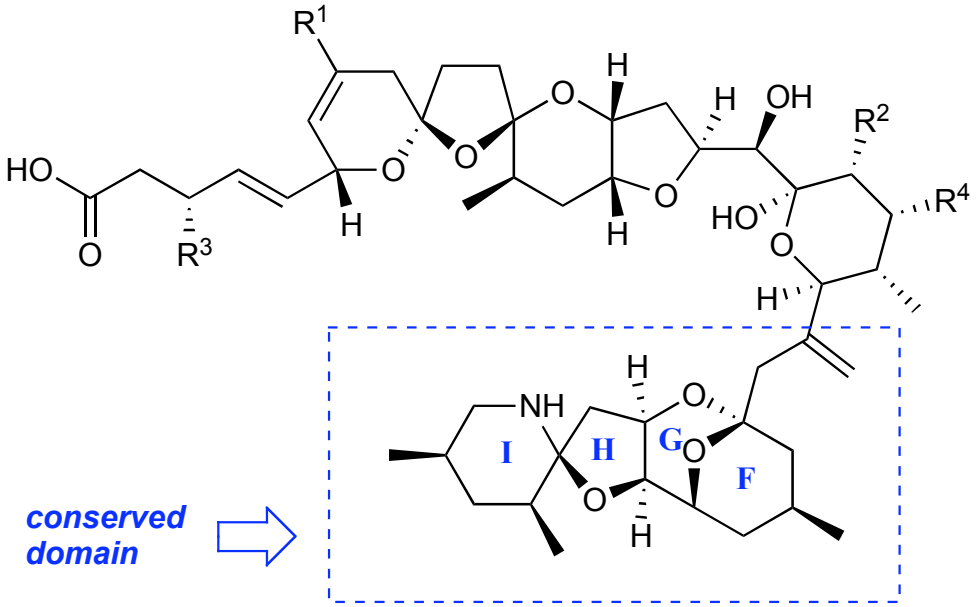
AZP symptoms: nausea, vomiting, severe diarrhea and stomach cramps; slow recovery

Toxicological studies in mice showed:

- lethal dose: 0.2 mg kg^{-1}
- serious damages to the intestine, pancreas, thymus, spleen, and liver
- sublethal doses promote lung tumors
- distinctive mode(s) of action

Ito *et al*. *Toxicon*, **2000**, *38*, 917-930.

NATURAL ANALOGS OF AZASPIRACID



Name	R^1	R^2	R^3	R^4
AZA1	H	Me	H	H
AZA2	Me	Me	H	H
AZA3	H	H	H	H
AZA4	H	H	OH	H
AZA5	H	H	H	OH
AZA6	Me	H	H	H
AZA7	H	Me	OH	H
AZA8	H	Me	H	OH
AZA9	Me	H	OH	H
AZA10	Me	H	H	OH
AZA11	Me	Me	OH	H

AZA1-AZA3 found in dinoflagellate *Protoperidinium crassipes*

AZA1-AZA11 found in mussel *Mytilus edulis*

The only method of detection at the present is HPLC-MS

Our goal: generate antibody probes specific to the FGHI domain to detect all azaspiracids

Ofuji *et al*. *Natural Toxins* **1999**, *7*, 99-102, and *Biosci. Biotech. & Biochem*. **2001**, *65*, 740-742.
James *et al*. *Toxicon* **2003**, *41*, 277-283.

THE CHALLENGES - THE OPPORTUNITIES

AZASPIRACIDS

1. STRUCTURE COMPLEXITY
9 RINGS, 20 STEREOCENTERS, 46 CARBONS

2. BIOLOGICAL SIGNIFICANCE
UNKNOWN MODES OF ACTION,
HIGHLY POTENT, MULTI-ORGAN DAMAGES

3. POOR MATERIAL SUPPLY
2mg/ 20 kg MUSSEL MEAT,
OCCURS SEASONALLY

4. SOCIAL IMPACTS
HARD ON SEAFOOD INDUSTRY,
SIMPLE METHODS OF DETECTION NEEDED

OUR TEAM

1. INTELLIGENCE, EXPERIENCE
CJF, STUDENTS, POSTDOCS, NIH

2. BIOCHEMISTS, TOXICOLOGISTS
VETERINARY INSTITUTE OF NORWAY
AGRESEARCH INC - NEWZEALAND

3. TOTAL SYNTHESIS
GOOD YIELD, RELIABLE SYNTHESIS,
WORKING FULL TIME ALL YEAR

4. RESEARCH COOPERATION
SYNTHETIC HAPTEN - ANTIBODY-
PORTABLE IMMUNOASSAY

Other synthetic groups with similar interests:
K. C. Nicolaou, D. A. Evans, R. G. Carter, M. Sasaki, S. Nishiyama.

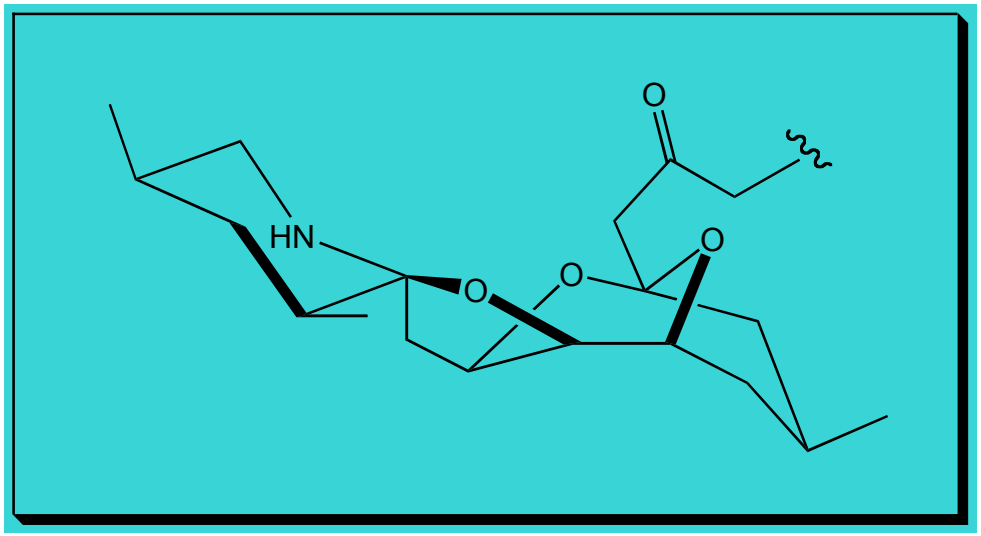
WORK PLAN

For the FGHI:
- Make both enantiomers using the same chemistry.
- Create antigens containing these fragments (the FGHI haptens).
- Raise antibodies specific to such antigens.
- Test binding affinity of the antibodies against the real azaspiracid.
- Our hypothesis is: only the correct enantiomer will give antibody that can recognize azaspiracid.
- Scale up the correct one.

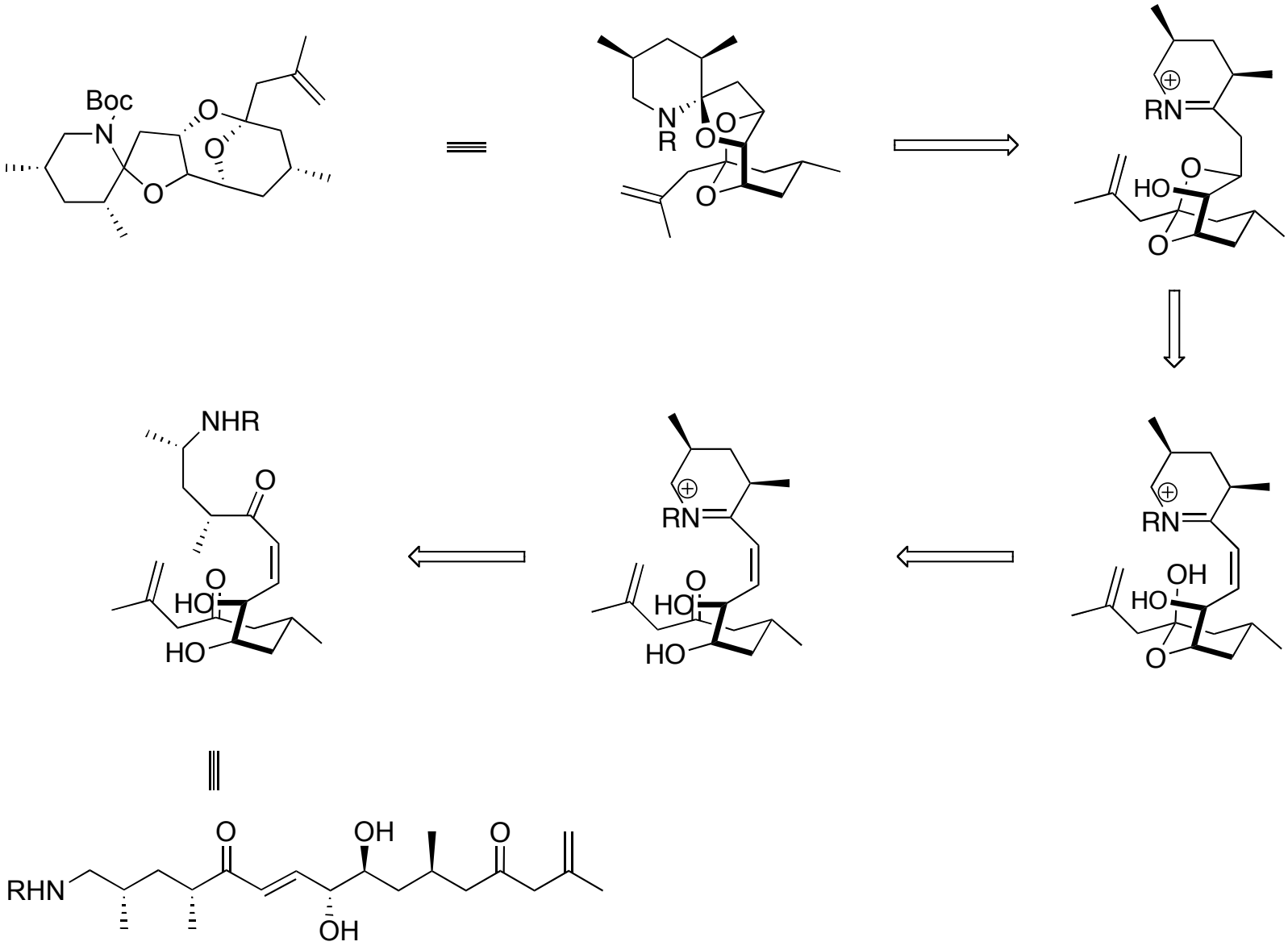
For the ABCD:
- Re-examine NMR data, propose alternative structures.
- Synthesize them in a way that is usable to make both enantiomers.
- Compare to NMR of azaspiracid.
- Assemble to E-FGHI to determine the absolute configuration.

PART ONE

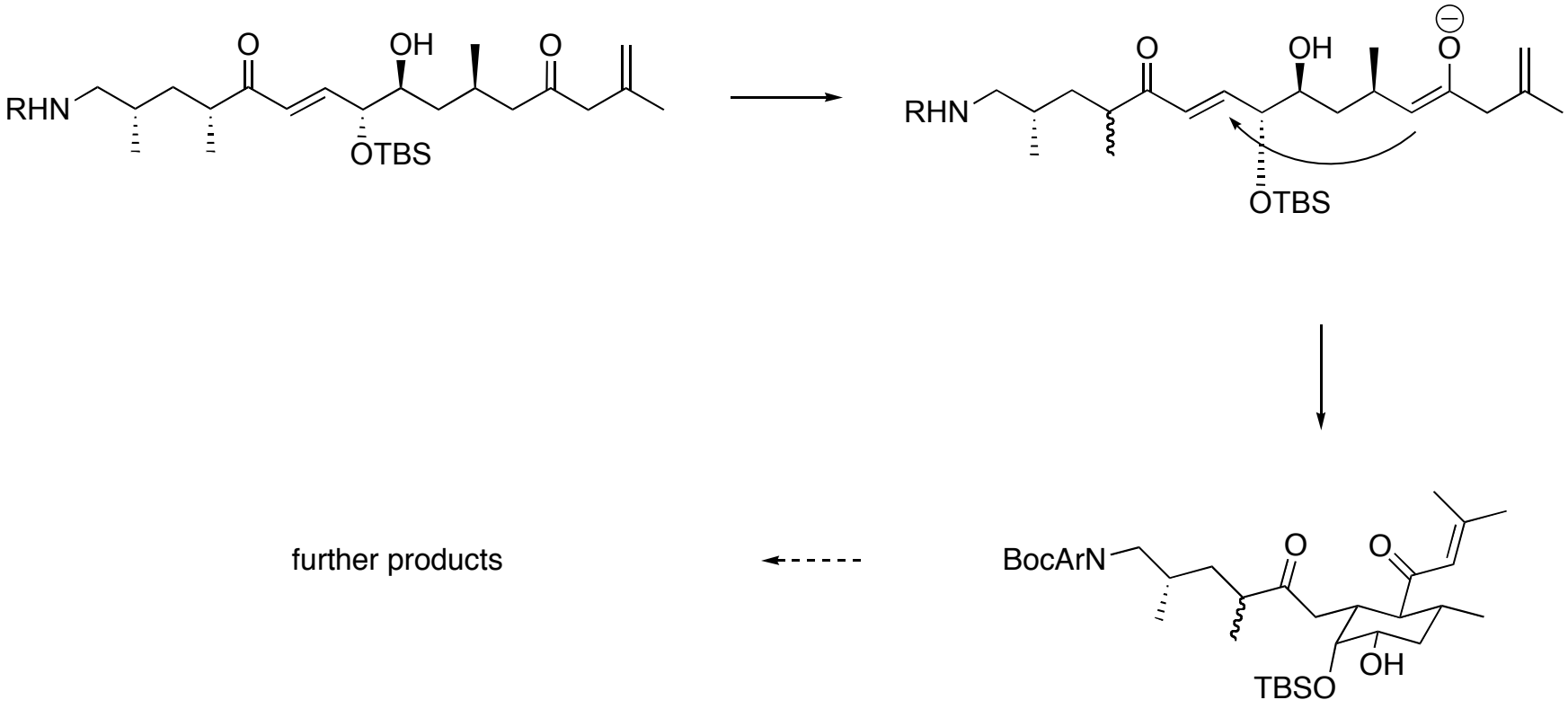
SYNTHESIS OF THE FGHI DOMAIN & THE HAPTEN FOR IMMUNIZATION EXPERIMENTS



BIOMIMETIC PROPOSAL

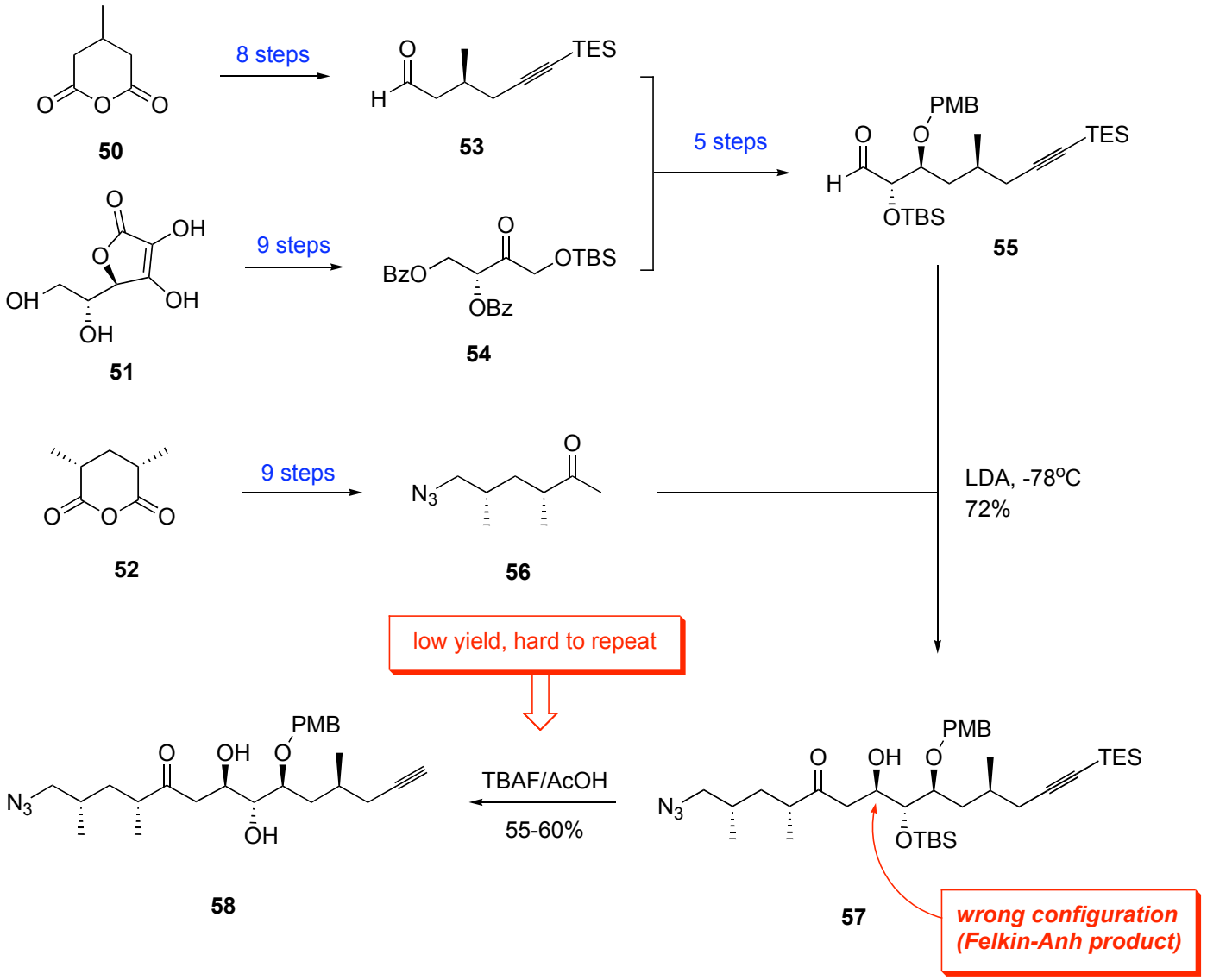


C-MICHEAL vs *O*-MICHEAL

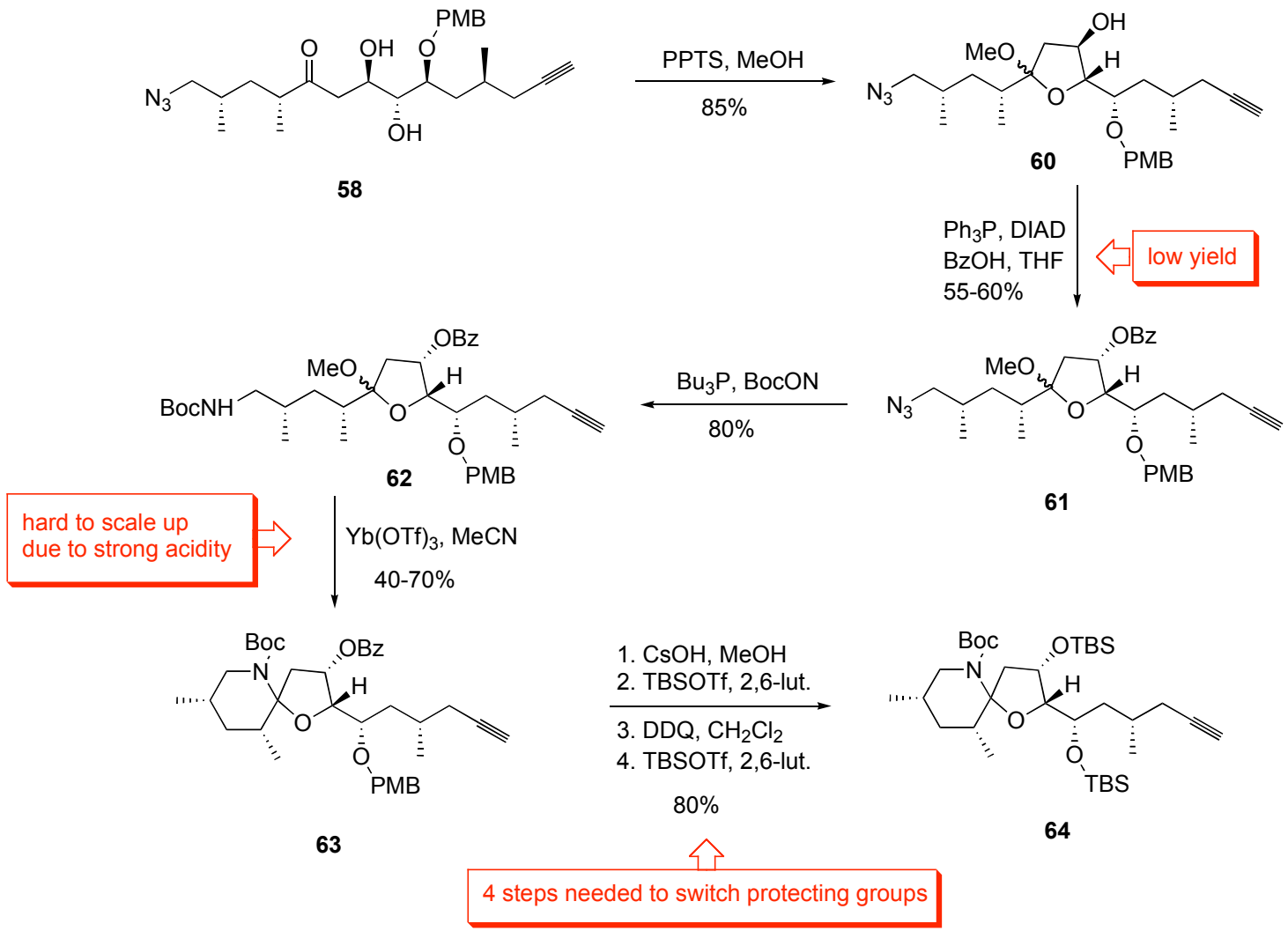


3 problems: C-Micheal, epimerization, double bond migration

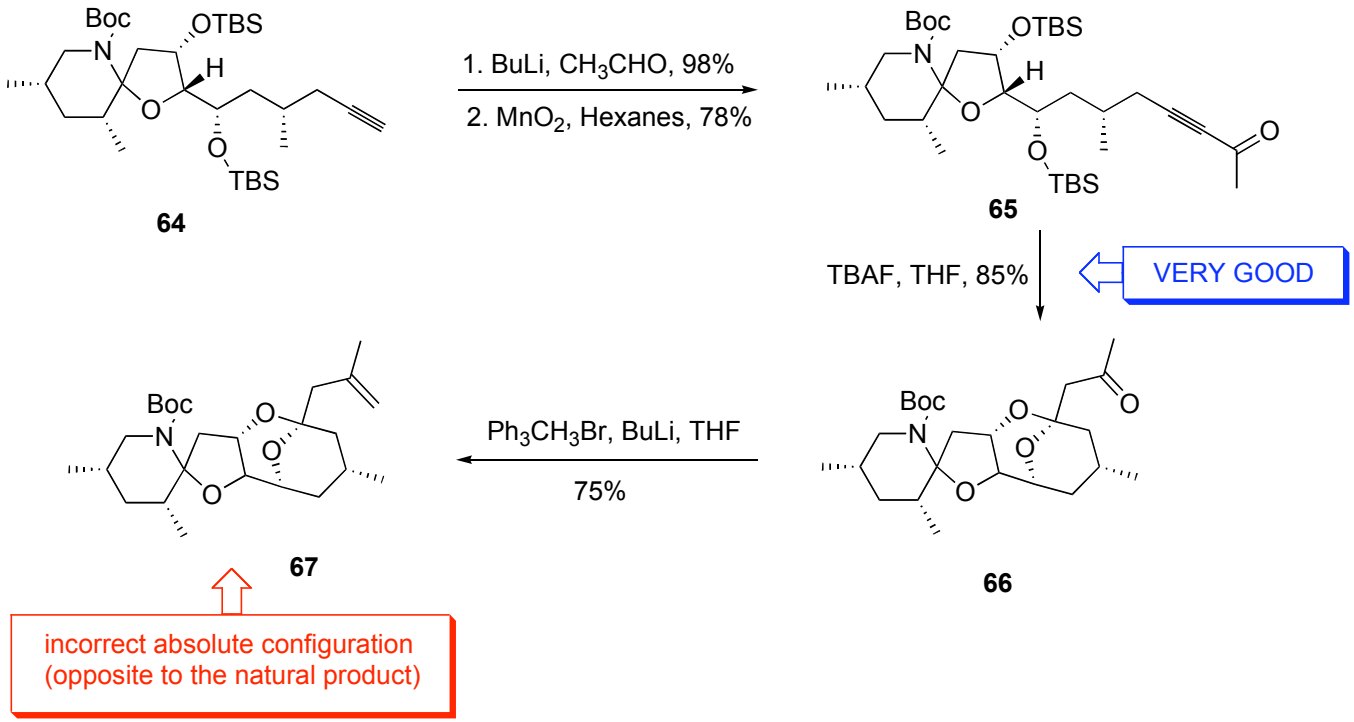
FIRST GENERATION SYNTHESIS OF THE FGHI DOMAIN - THE LINEAR PRECURSOR



FIRST GENERATION SYNTHESIS OF THE FGHI DOMAIN - THE SPIROAMINAL



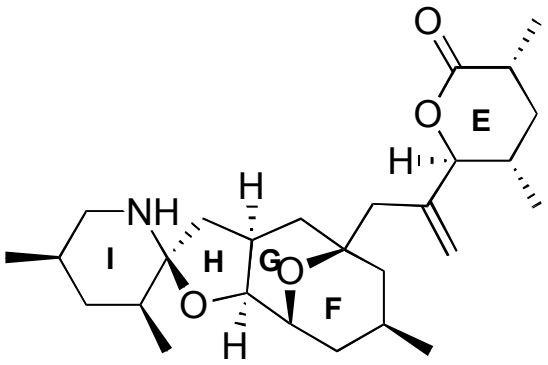
FIRST GENERATION SYNTHENSIS OF THE FGHI SYSTEM - THE 2,9-DIOXABICYCLO[3.3.1]NONANE



The formation of the FG ring system via Double Intramolecular Hetero-Michael Addition was unprecedented and superior to the traditional acid catalized ketalization.

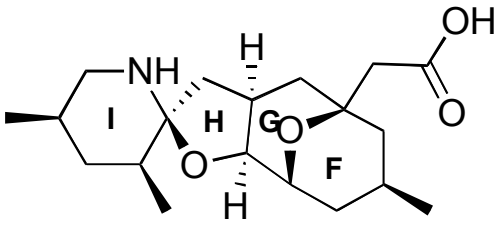
Forsyth, C. J. *et al. Angew. Chem. Int. Ed.* **2001**, *40*, 3663-3667.
Sasaki, M. *et al. Tetrahedron lett.* **2003**, *44*, 6199-6201.

NICOLAOU'S DETERMINATION OF THE E-FGHI ABSOLUTE CONFIGURATION



8

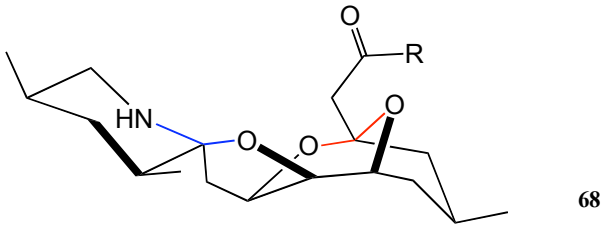
matched with the degradation product by ^{1}H NMR spectroscopy



9

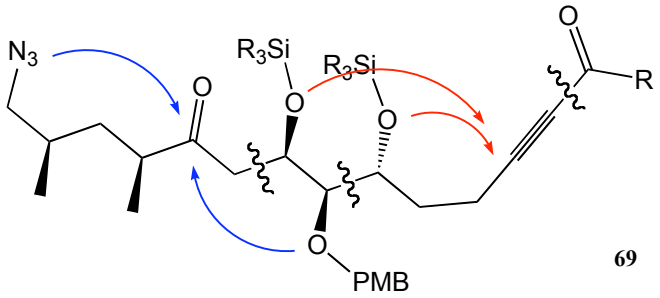
matched with the degradation product by ^{1}H NMR spectroscopy and optical rotation

NEW RETROSYNTHETIC PLAN FOR THE FGHI DOMAIN

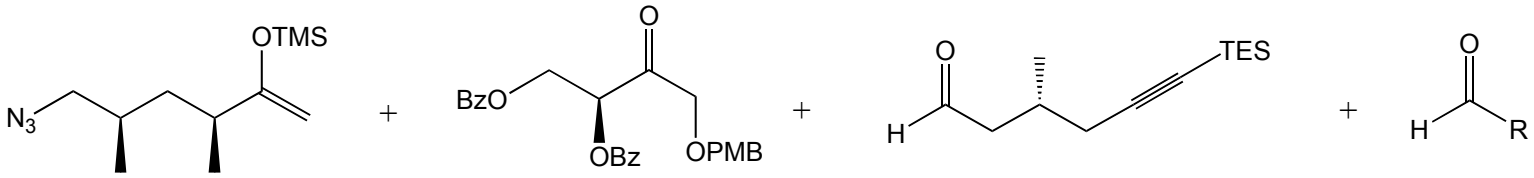


68

S-aW-Ad$_N$ DIHMA



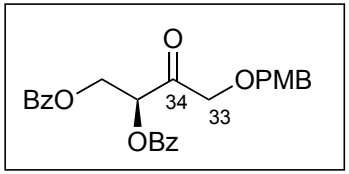
69

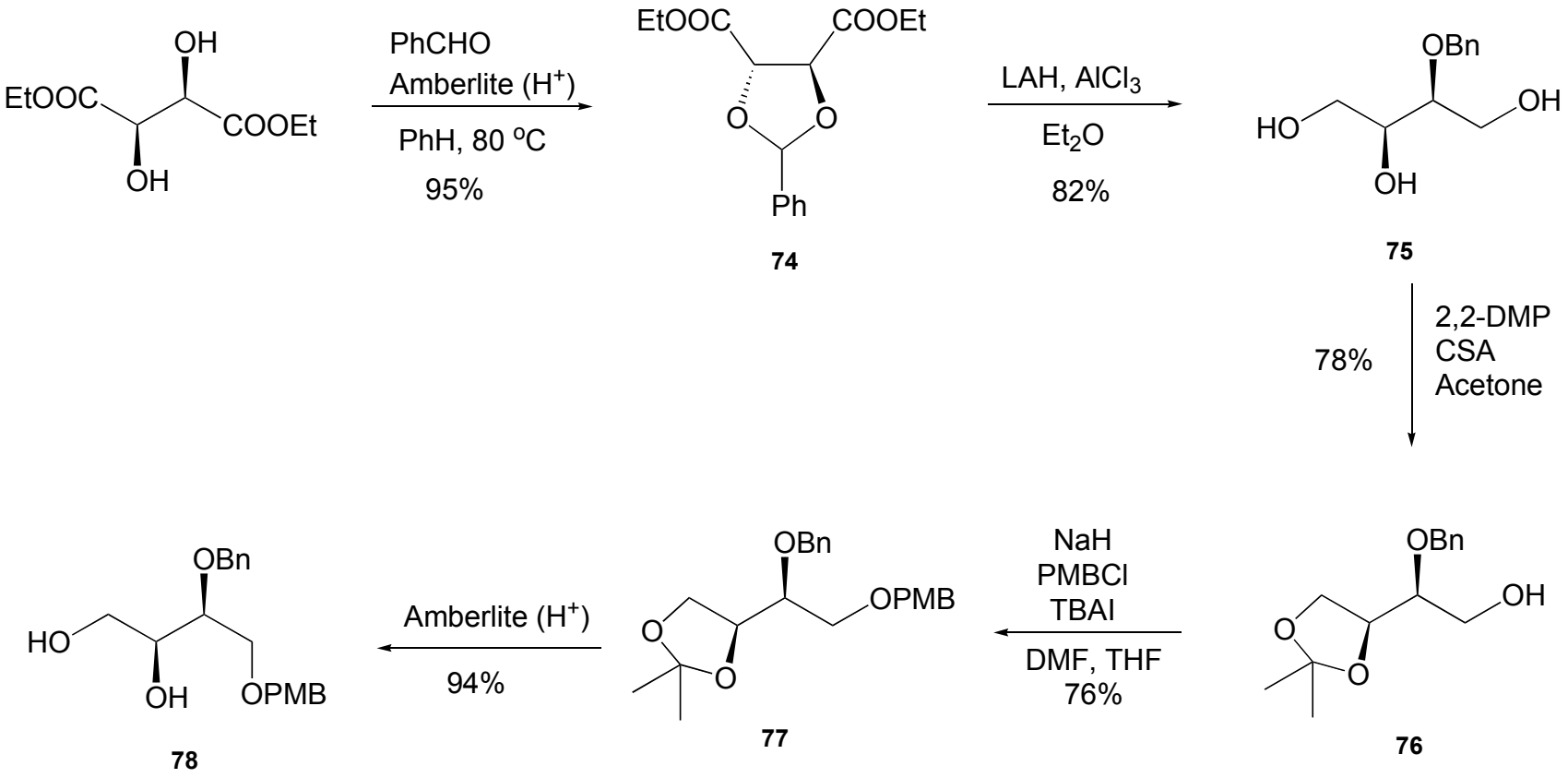


70 71 72 73

Mukaiyama aldol Paterson aldol NHK coupling

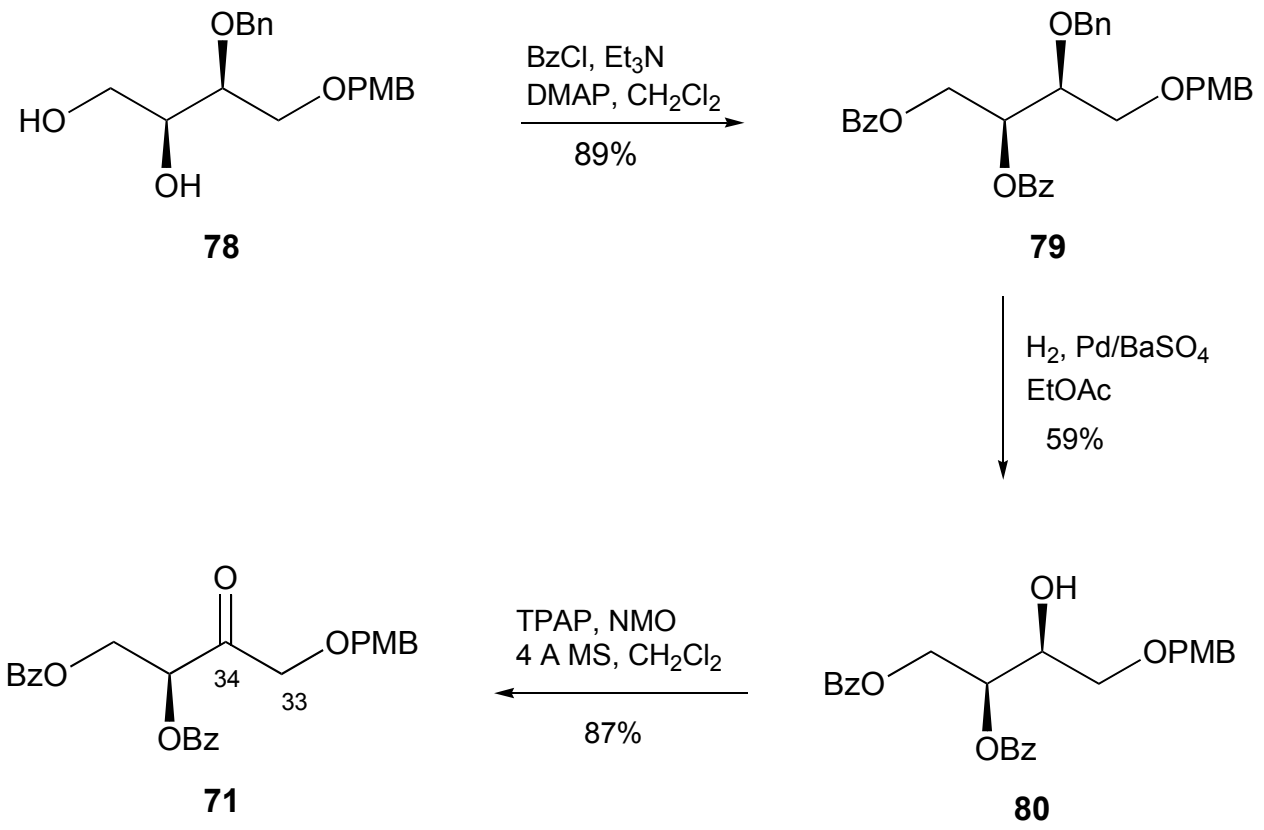
SYNTHESIS OF THE C33-C34 KETONE



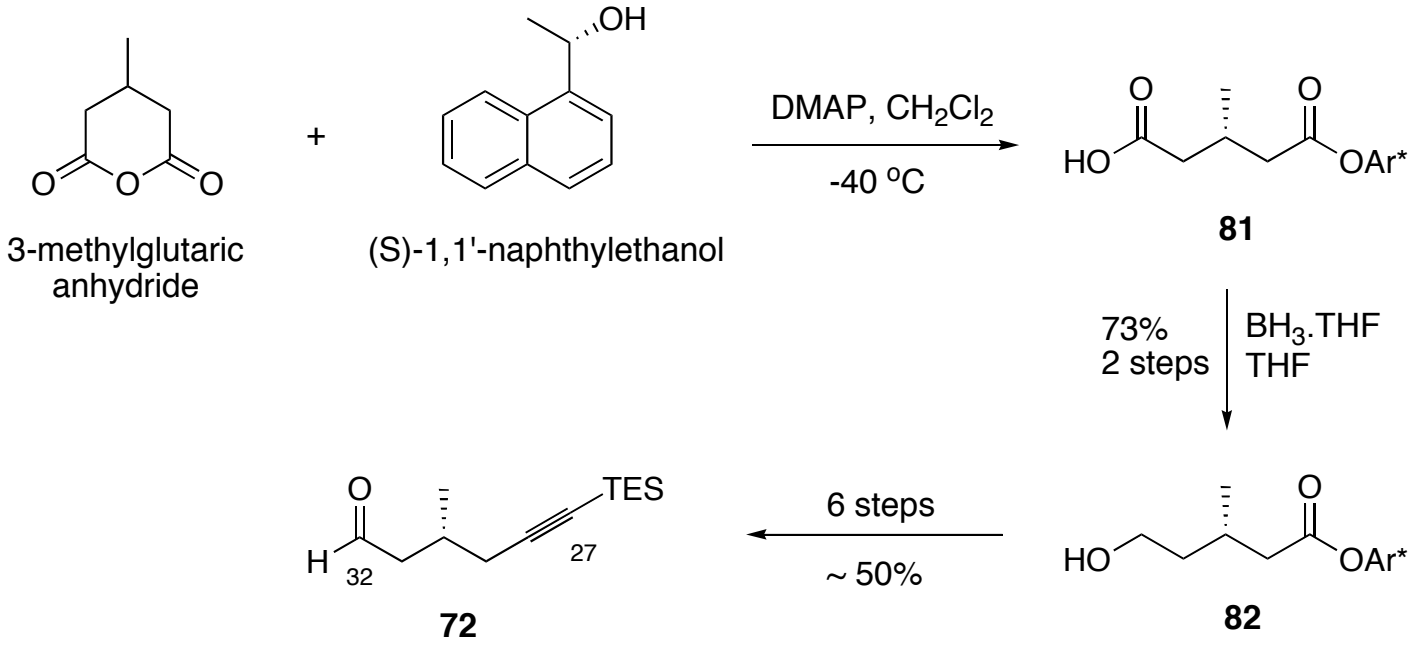


Ohno *et al*. *Chem. Pharm. Bull*. **1985**, *33*, 572-582.

SYNTHESIS OF THE C33-C34 KETONE (cont'd)



SYNTHESIS OF THE C27-C32 FRAGMENT



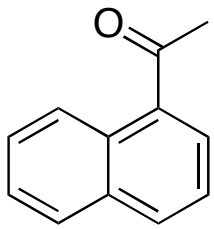
3-methylglutaric anhydride

(S)-1,1'-naphthylethanol

Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 142.

Preparation of (*S*)-(-)-1(1'-naphthyl)ethanol

a)

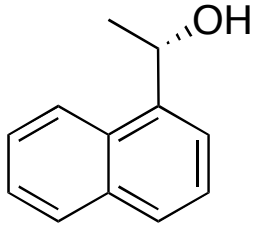


acetonaphthone

(R)-Me-CBS, BH_3.THF

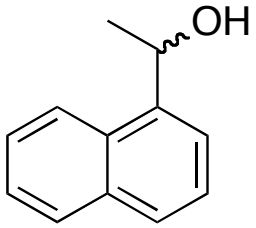
THF, -15 °C, 30 min

98 %



92 % ee

b)



racemic

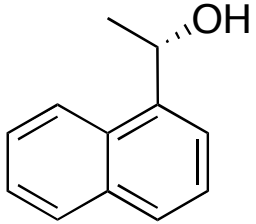
PPL

$C_3H_7CO_2CH_2CCl_3$

heptane, 60 °C

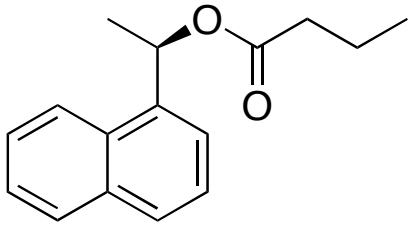
14 days

94%



(*S*)

+

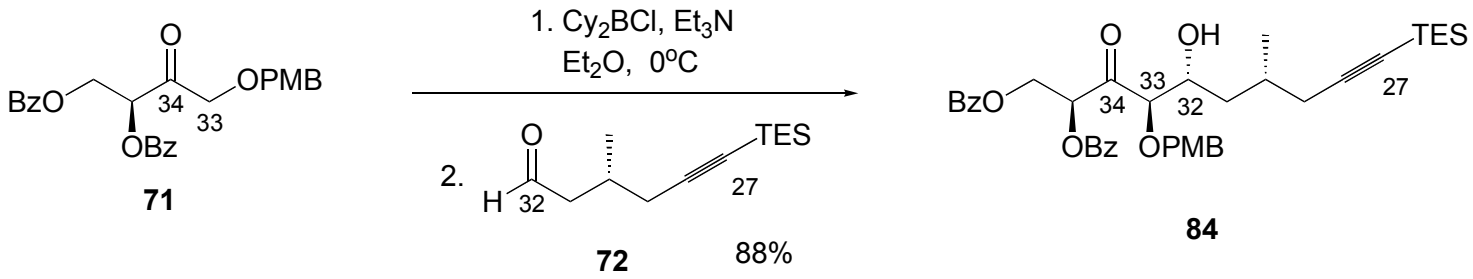


83

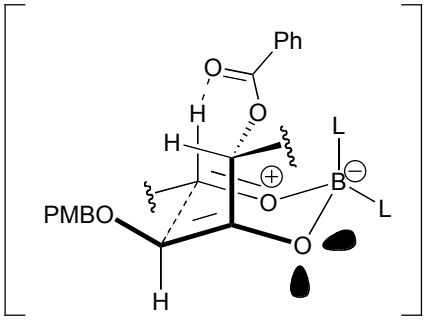
99.5 % ee

PPL: porcine pancreatic lipase

BORON MEDIATED ALDOL REACTION: FORMATION OF THE C32-C33 BOND



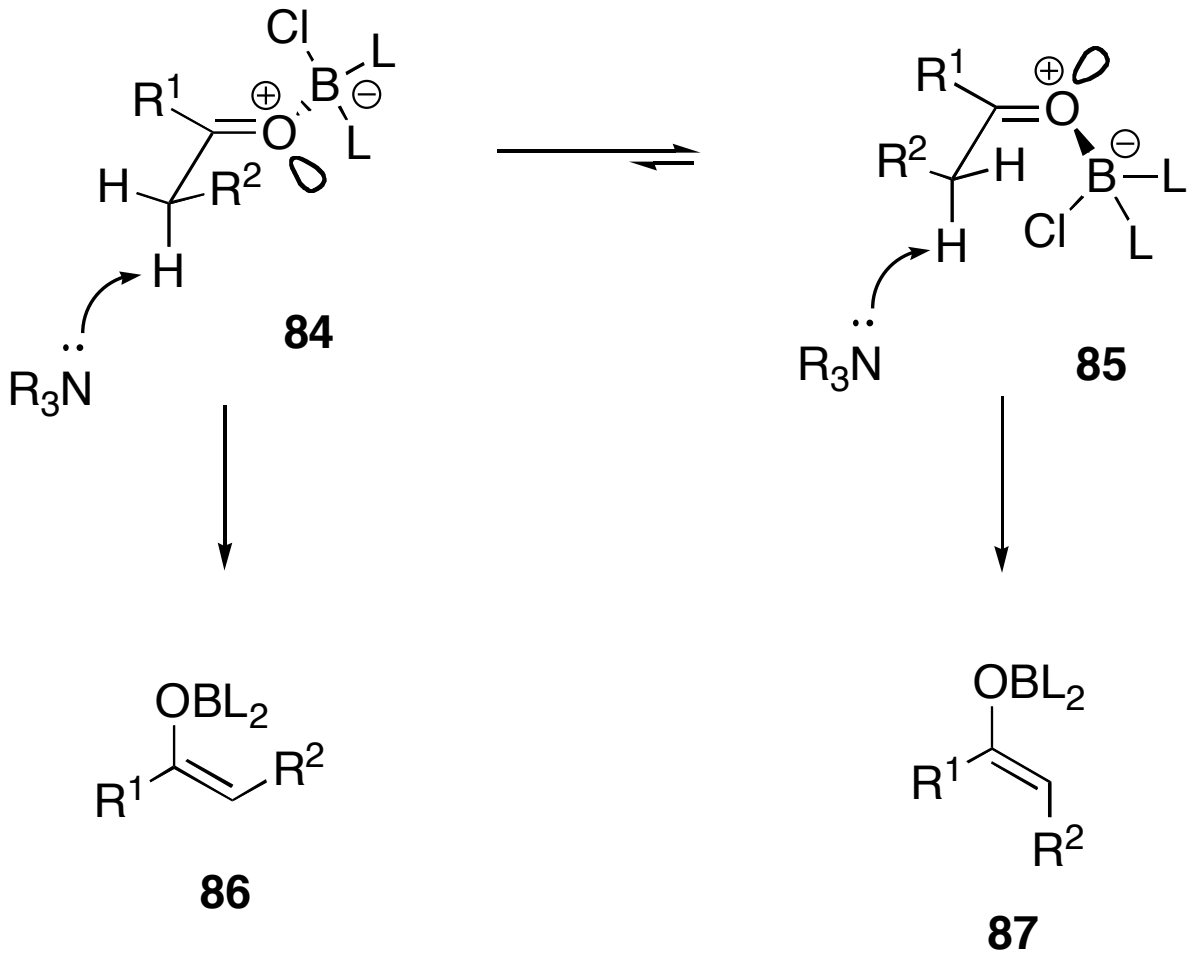
Proposed transition state



Paterson *et al*. *Synthesis*, **1998**, *special issue*, 639-652.

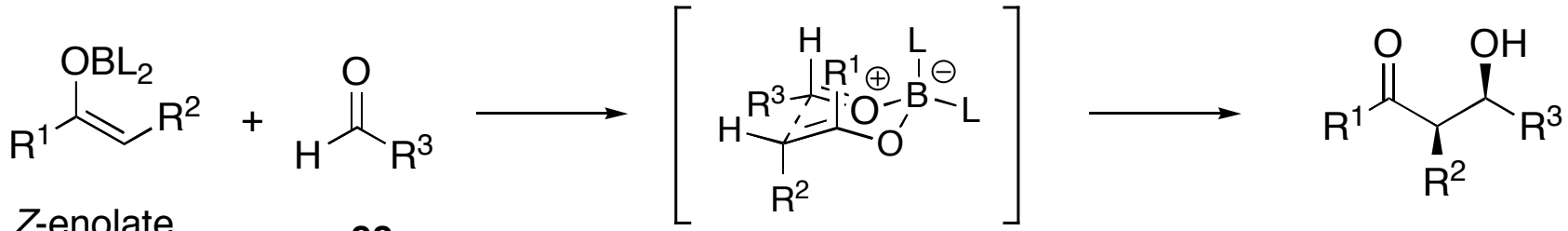
Yield improved by Kristin Glab

FORMATION OF BORON-ENOLATE



in which R^2 is smaller than R^1

CORELATION BETWEEN ENOLATE GEOMETRY AND PRODUCT'S STEREOCHEMISTRY



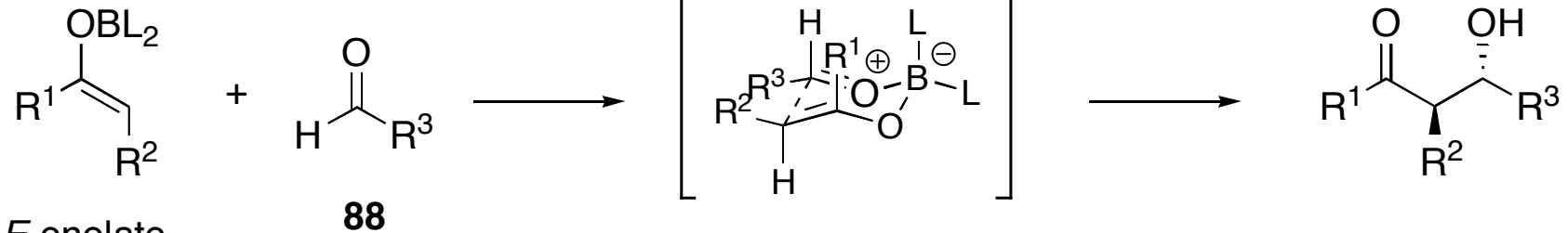
Z-enolate

86

88

1,2-*syn*

89



E-enolate

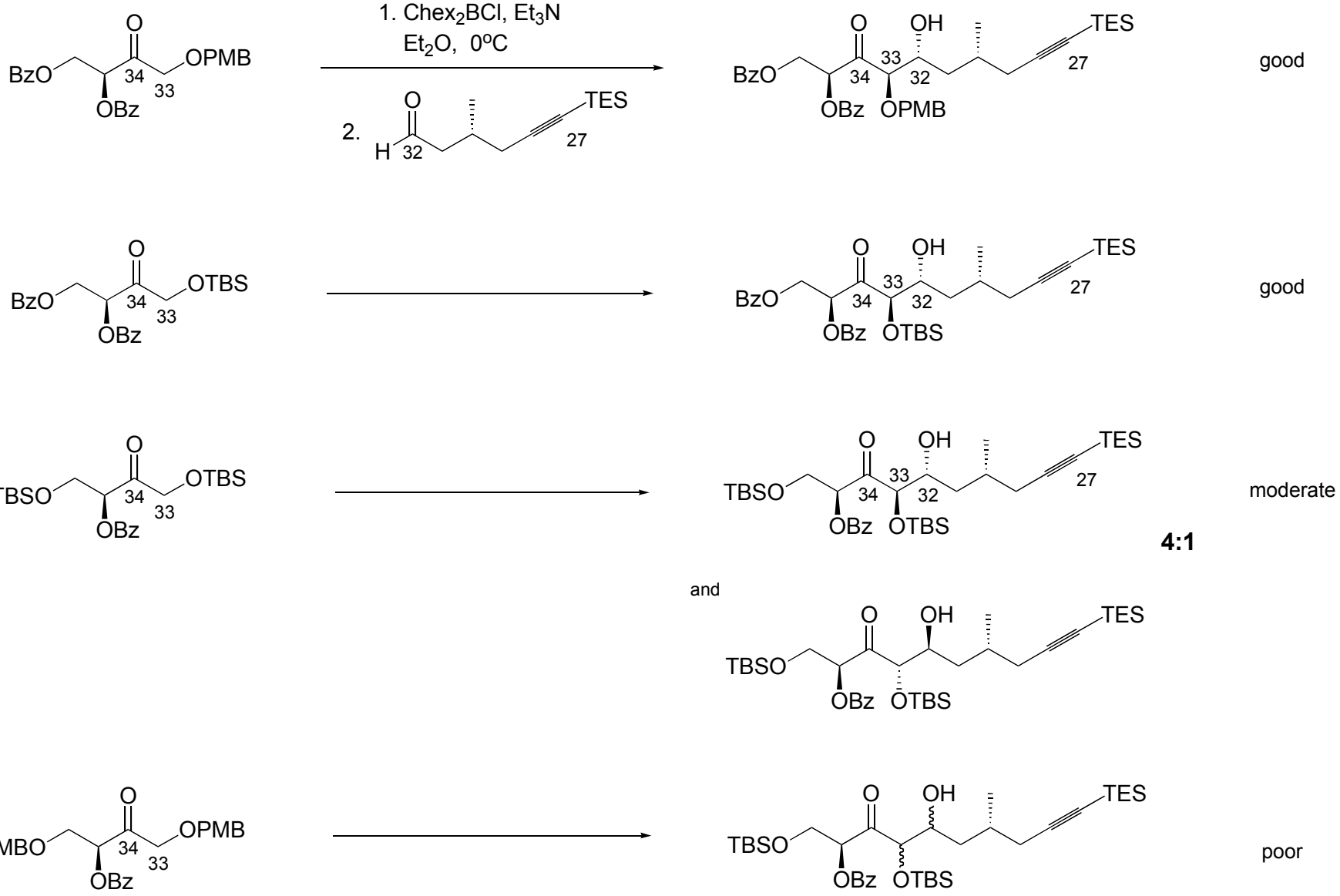
87

88

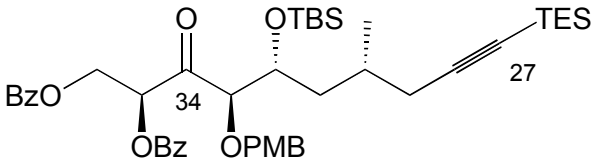
1,2-*anti*

90

RELATIONSHIP BETWEEN KETONE'S SUBSTITUENTS AND SELECTIVITY



SYNTHESIS OF THE C27-C34 FRAGMENT

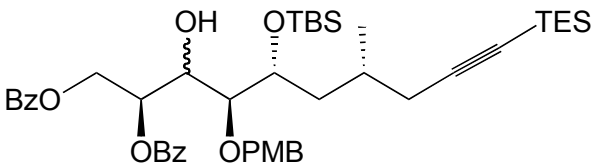


100

$NaBH_4$, MeOH

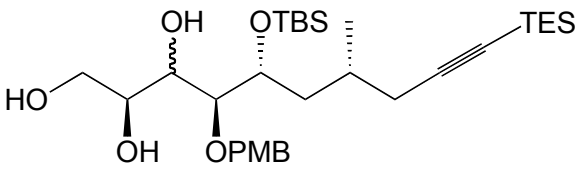
0 °C

98%



105

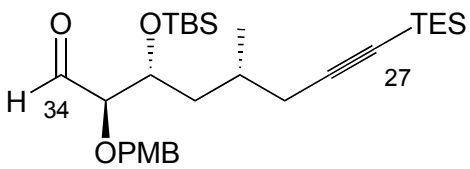
1. K_2CO_3, MeOH
2. AcOH to pH 7



101

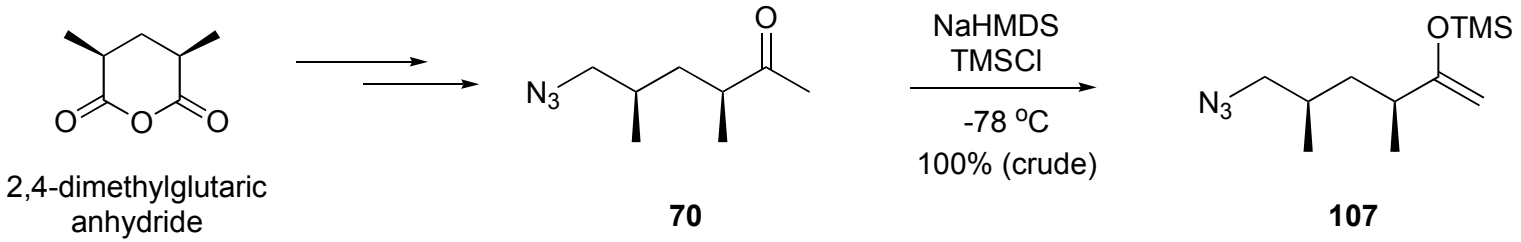
$NaIO_4$, MeOH

70%, 2 steps



106

PREPARATION OF THE C35-C40 FRAGMENT



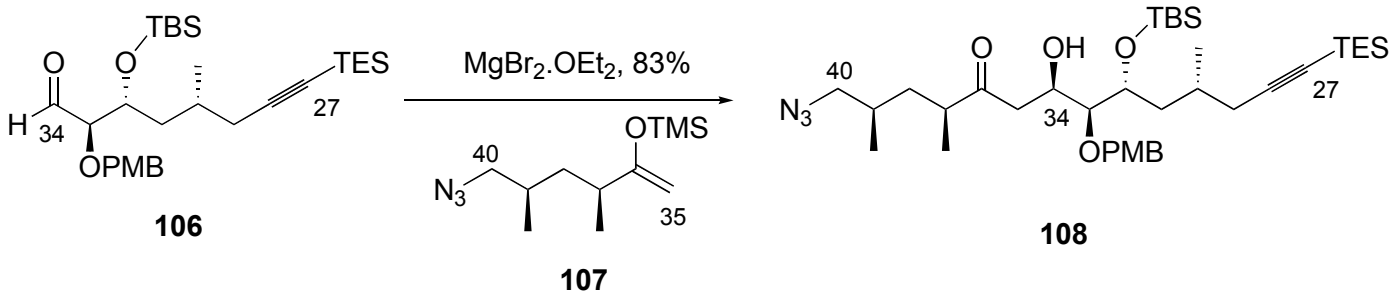
2,4-dimethylglutaric anhydride

70

107

Ketone provided by James Xu

MUKAIYAMA CHELATION CONTROL ALDOL: FORMATION OF C34-C35 BOND

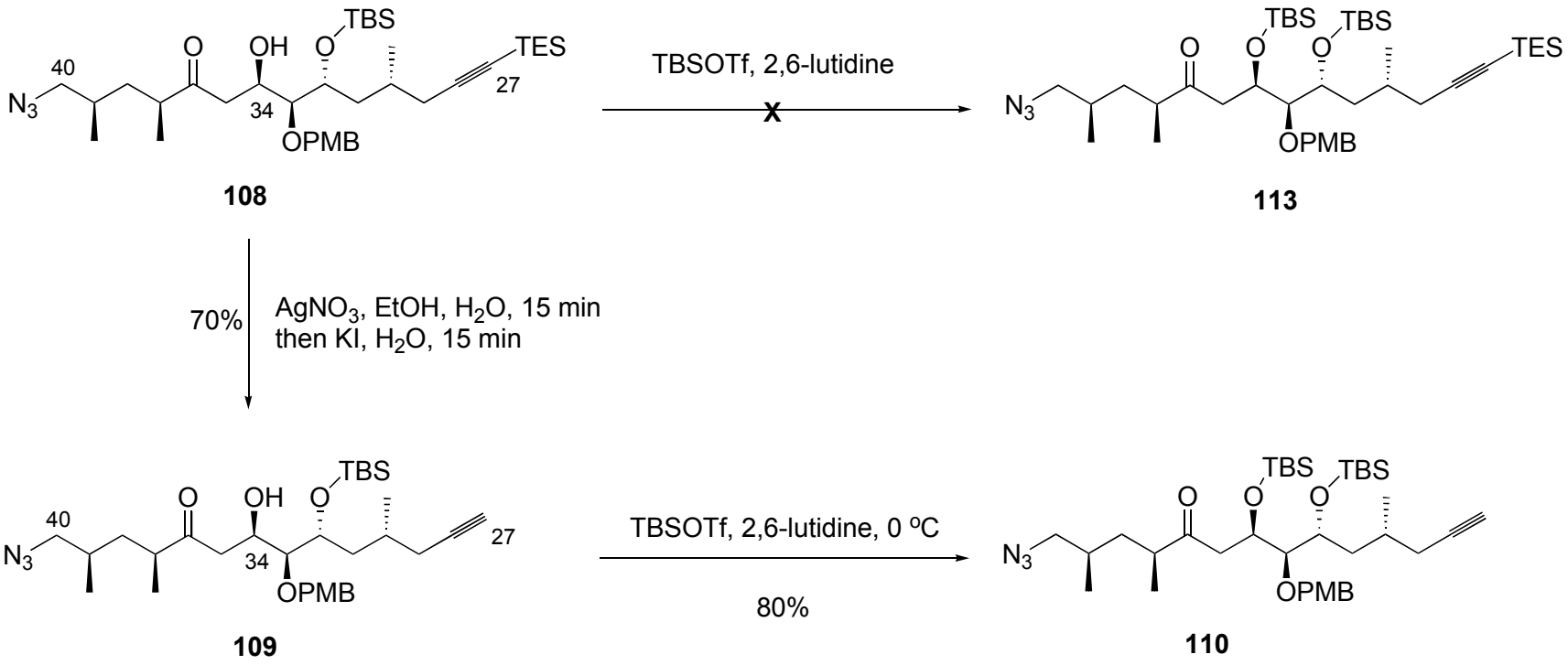


106 **107** **108**

Boger, D. L. *Modern Organic Synthesis*, 1999, 198.
Mukaiyama, T. *Angew. Chem., Int. Ed.*, 1977, 16, 817.

Scaled up and improved by Kristin Glab

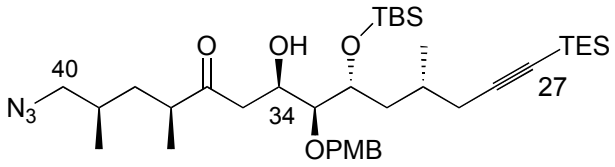
PROTECTING GROUP MANIPULATION



108 **113**

109 **110**

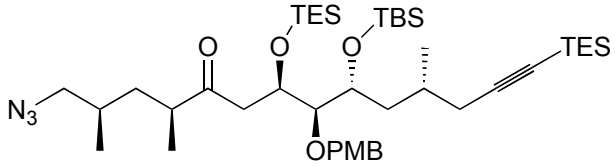
PREPARATION OF THE LINEAR PRECURSOR



108

TESOTf, 2,6-lutidine

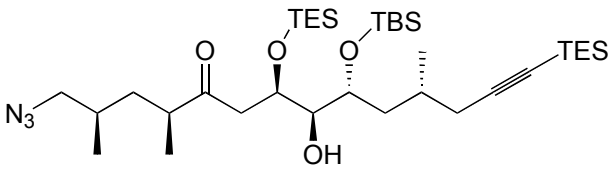
92%



108a

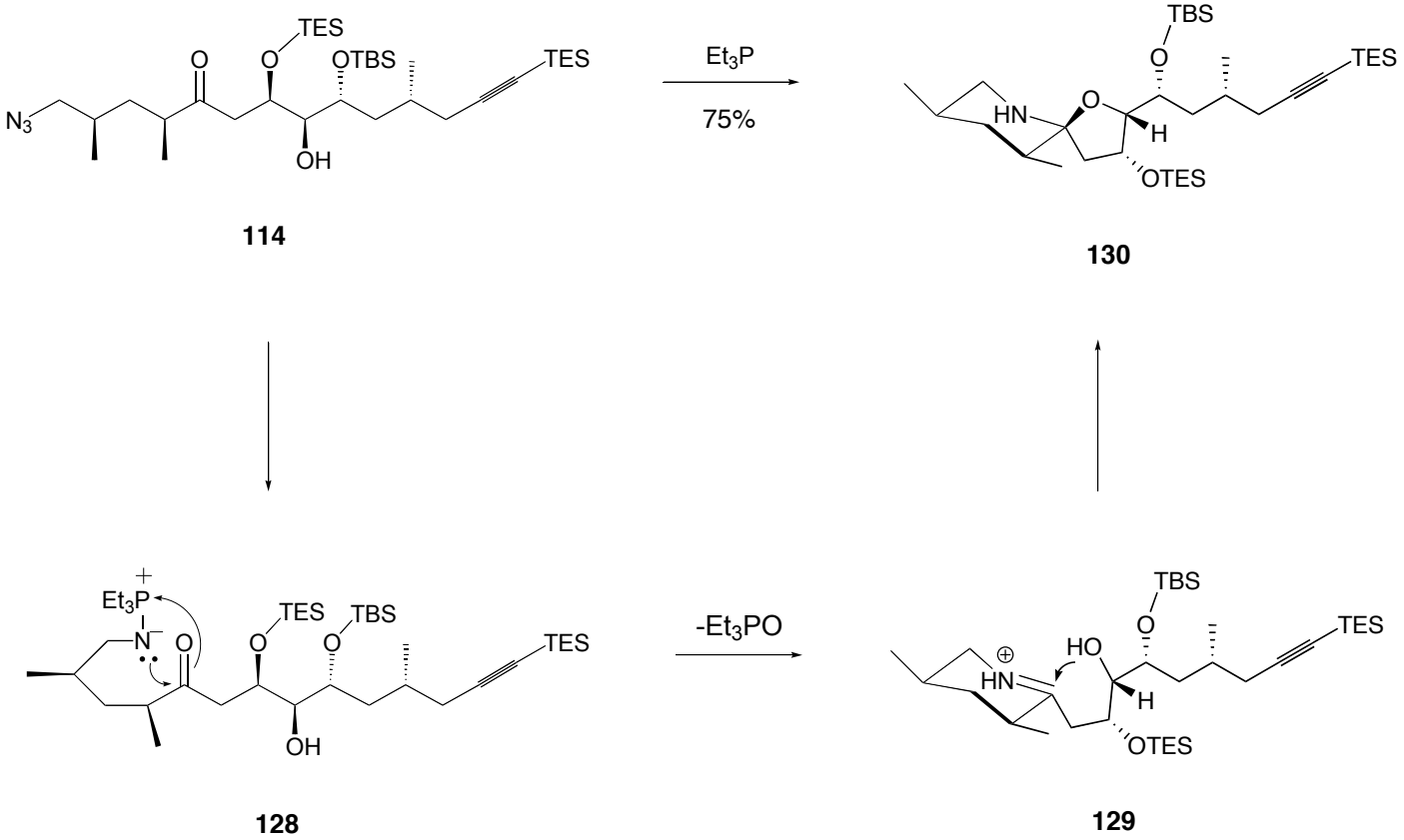
82%

DDQ, CH_2Cl_2, H_2O



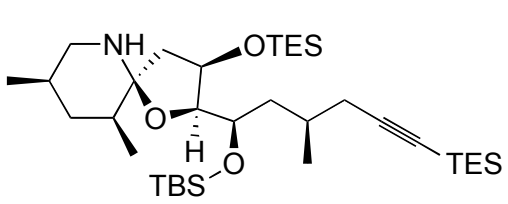
114

SPIROAMINAL FORMATION



Staudinger *et al. Helv. Chim. Acta.* **1919**, *2*, 612-635.
Gololobov *et al. Tetrahedron* **1981**, *37*, 437-472.

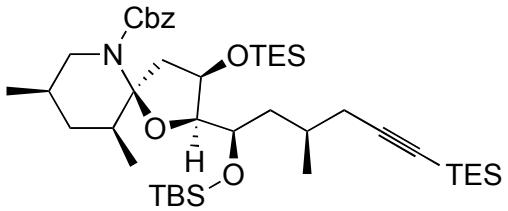
PREPARATION OF THE ALKYNYLIODIDE



130

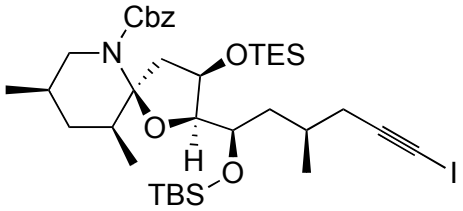
CbzCl
K_2CO_3
CH_2Cl_2

73%

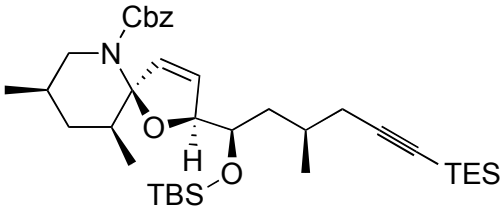


131

87% AgTFA, NIS DMF



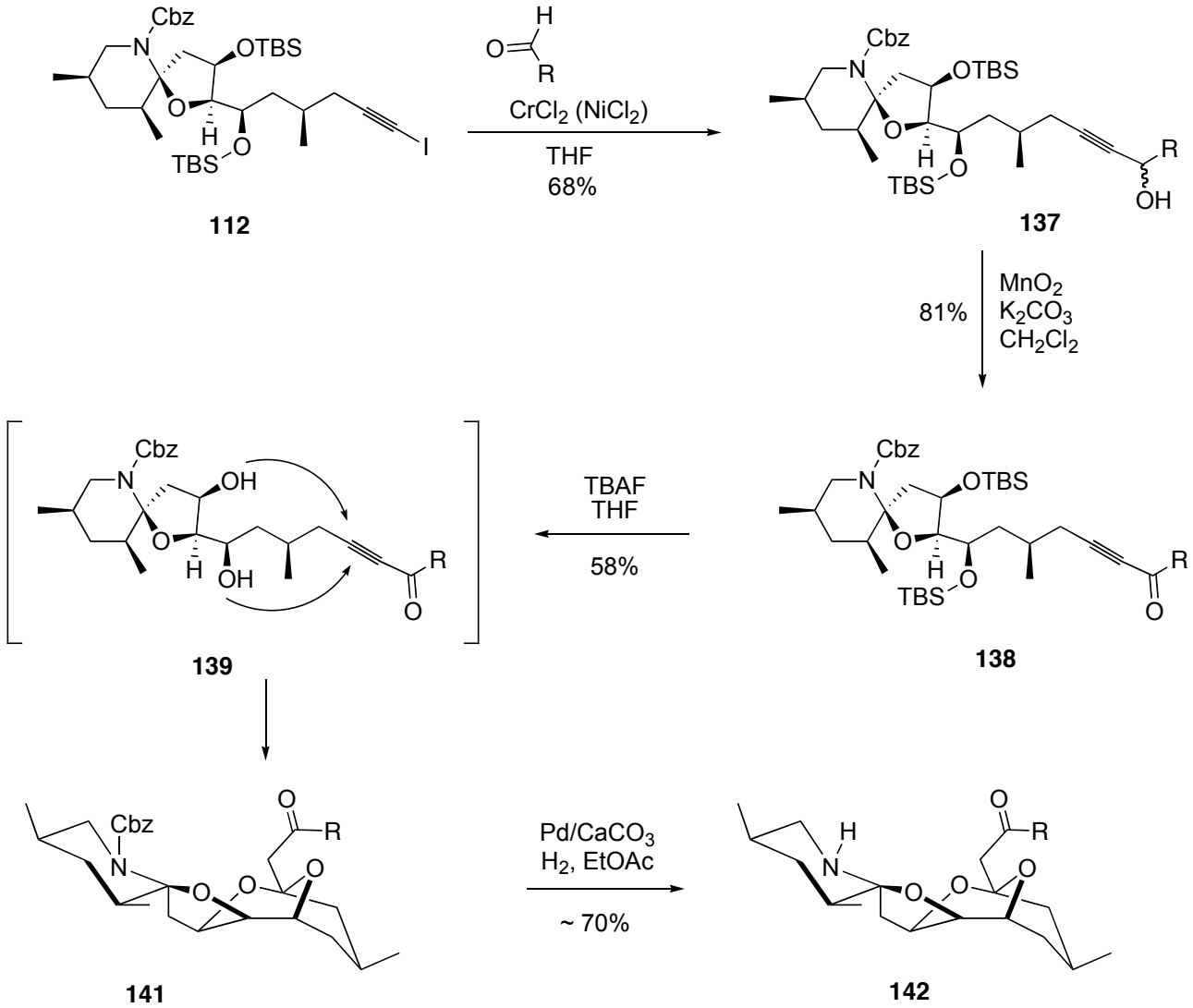
133



132

undesired product

SYNTHESIS OF THE HAPTEN



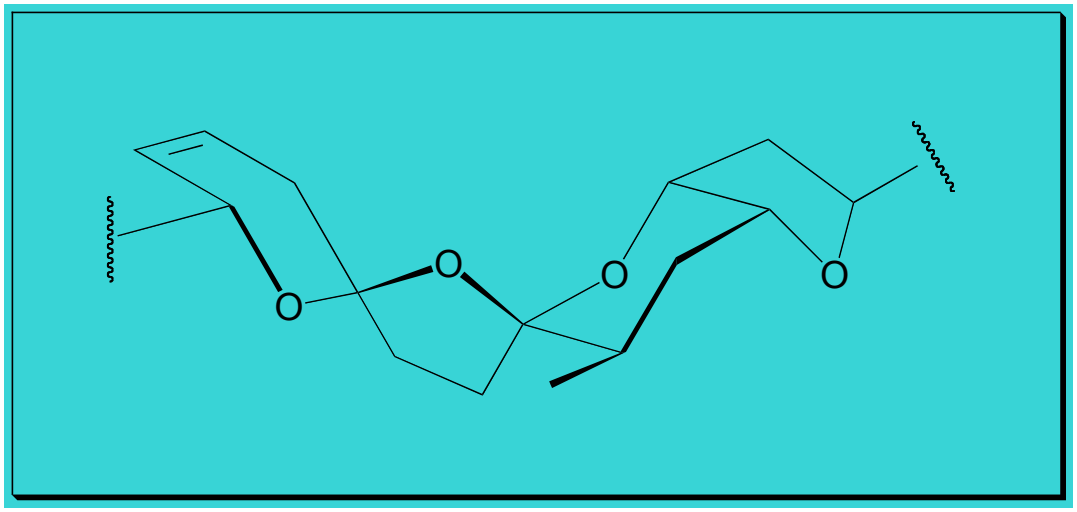
Unpublished data

COMPARISION BETWEEN THE FIRST AND SECOND GENERATION SYNTHESES OF THE FGHI DOMAIN

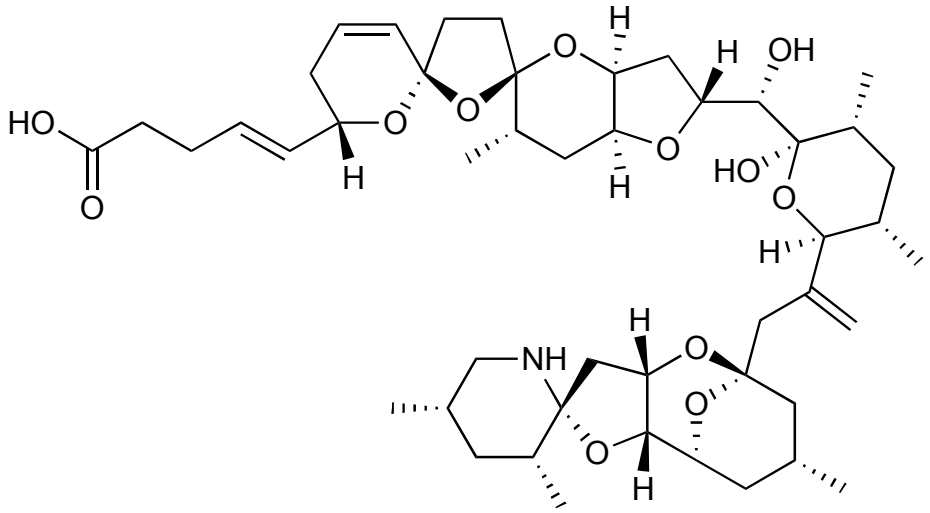
First generation	Second generation
o Wrong stereochemistry in the aldol product (C34)	o Correct stereochemistry by chelation control
o Lengthy protecting group manipulation	o Correct protecting groups from the beginning
o Non-selective condition for the removal of the triethylsilyl group using fluoride	o Chemoselective removal of the triethylsilyl group without using fluoride
o Hard to scale up the spiroaminal formation due to strong acidic conditions	o Novel spiroaminal formation, neutral condition, very scalable

PART TWO

STRUCTURE REVISION &
SYNTHESIS OF THE ABCD DOMAIN



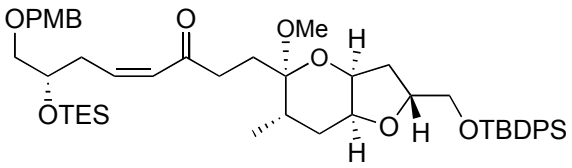
ORIGINALLY PROPOSED STRUCTURE OF AZASPIRACID



6

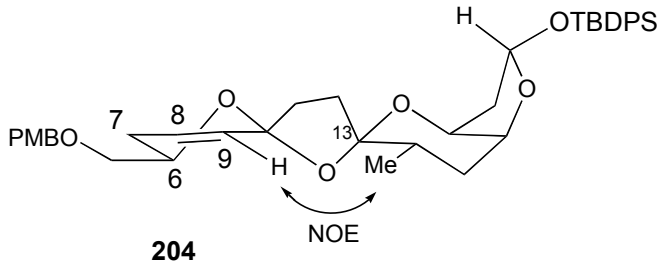
Yasumoto *et al. J. Am. Chem. Soc.,* **1998***, 120,* 9967-68

ATTEMPT TOWARDS THE ORIGINALLY PROPOSED STRUCTURE



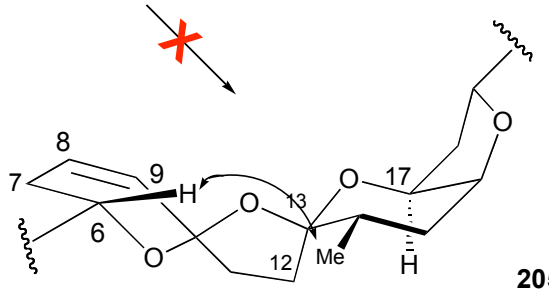
203

H^+ or L.A.



204

C13 anomeric, A/C cis
undesired configuration



205

C13 unanomeric, A/C trans
original target

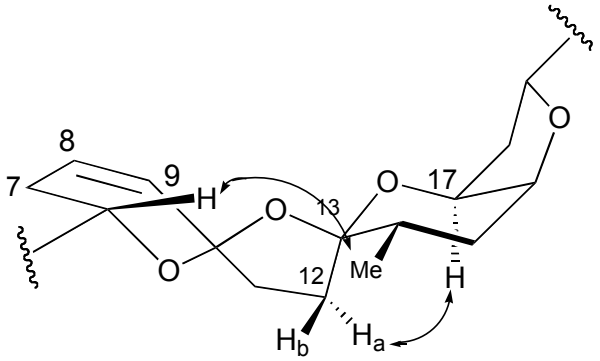
Nicolaou group: 2003 reported NMR spectrum of the synthetic sample of the proposed structure did NOT match with the natural product's NMR

Forsyth, C. J. *et al. Org. Lett.* **2001**, *3*, 975.
Nicolaou, K. C. *et al. Angew. Chem. Int. Ed.* **2003**, *42*, 3643

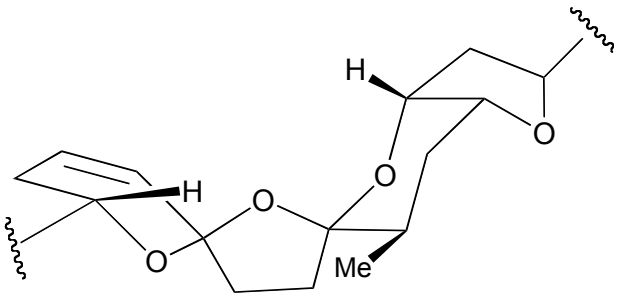
Amy Dounay, 2001

SON'S ANALYSIS & FINAL PROPOSAL

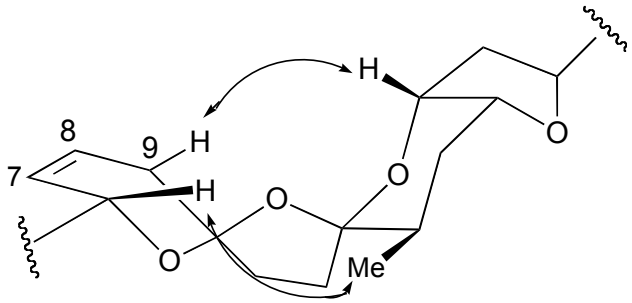
originally PUBLISHED structure



SON NGUYEN'S initial proposed structure

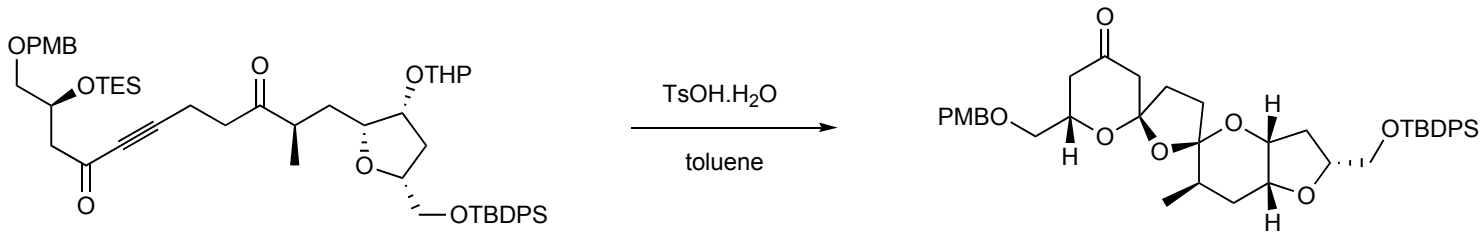


and the FINAL proposed structure

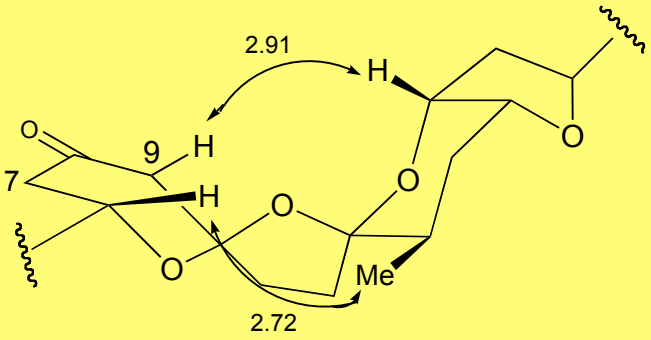


WE WON NIH GRANT FOR AZASPIRACD THE 2ND TIME!

QUICK SYNTHESIS OF THE TRIOXADISPIROKETAL SYSTEM - CONFIRMATION OF THE NOE's



214 **215**

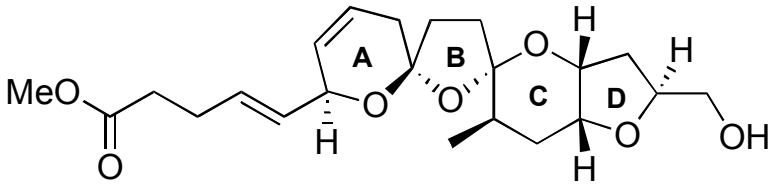


Observed NOE's corresponds well with the model

Forsyth, C. J. *et al. Org. Lett.* **2004**, *6*, 4159.

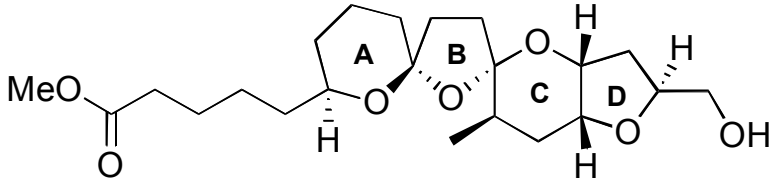
Lisa Geisler

NICOLAOU'S STRUCTURAL DETERMINATION OF THE ABCD DOMAIN



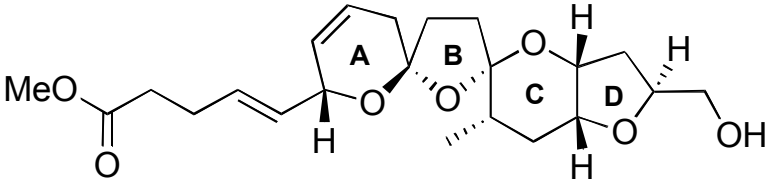
10

first revised structure-the C7-C8 alkene



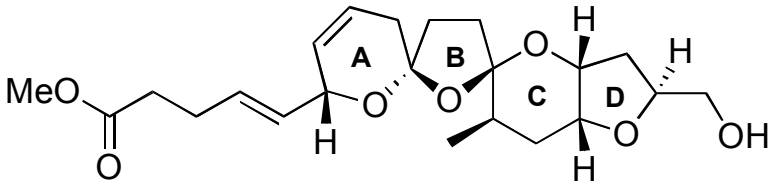
11

did not match with the hydrogenated degradation product



12

second revised structure-the C14-epi

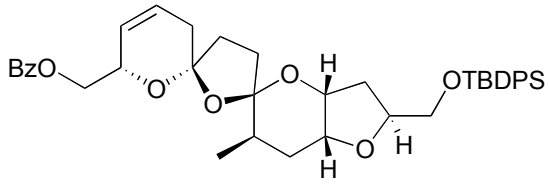


13

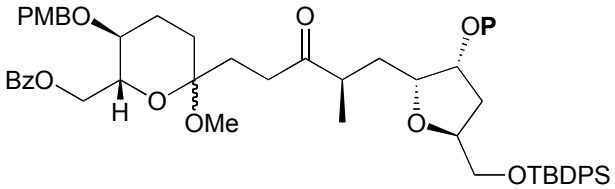
final revised structure-C6, C10, C13 reversed identical to degradation product by NMR spectroscopy

Absolute configuration remained unknown untill the total synthesis is done

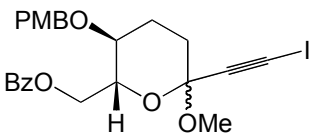
FIRST RETROSYNTHETIC PLAN FOR THE ABCD DOMAIN



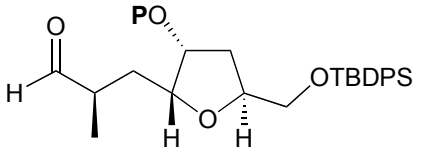
acid



NHK



+

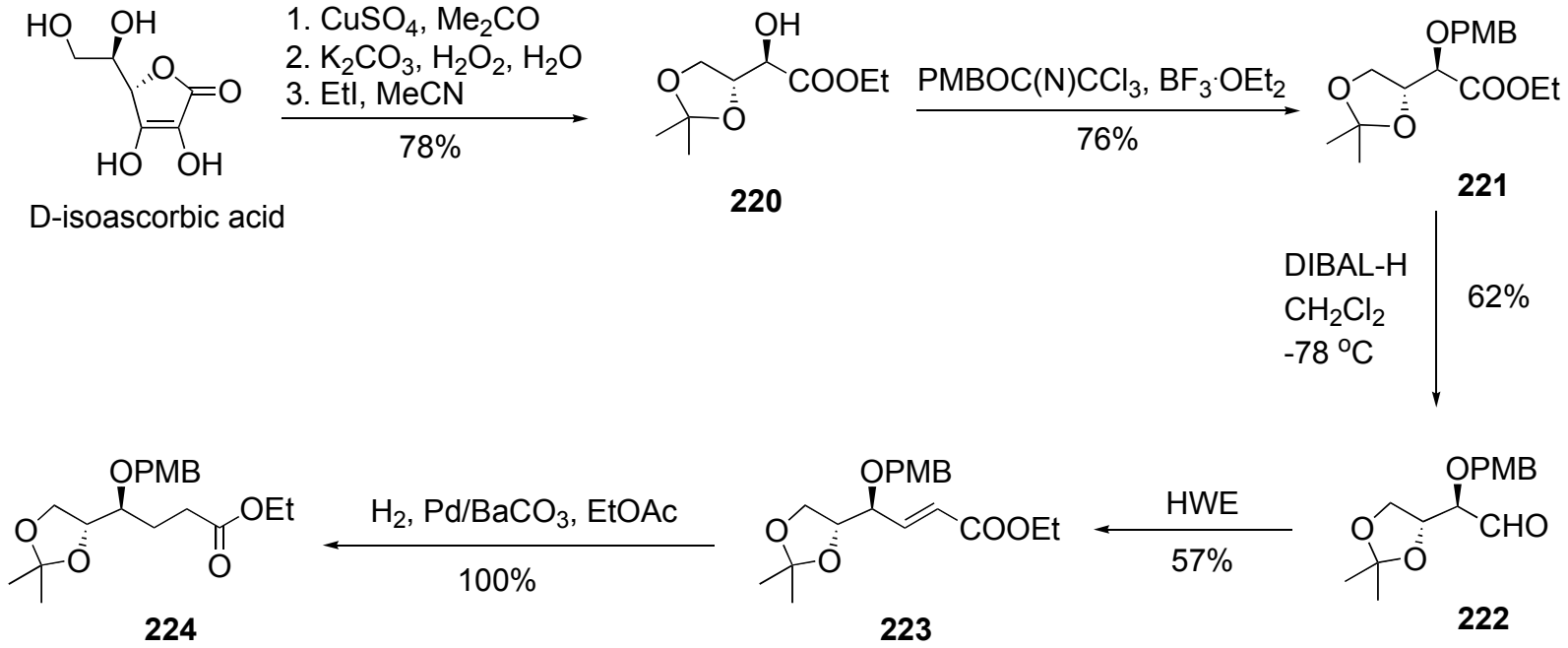


D-isoascorbic acid

L-galactonolactone

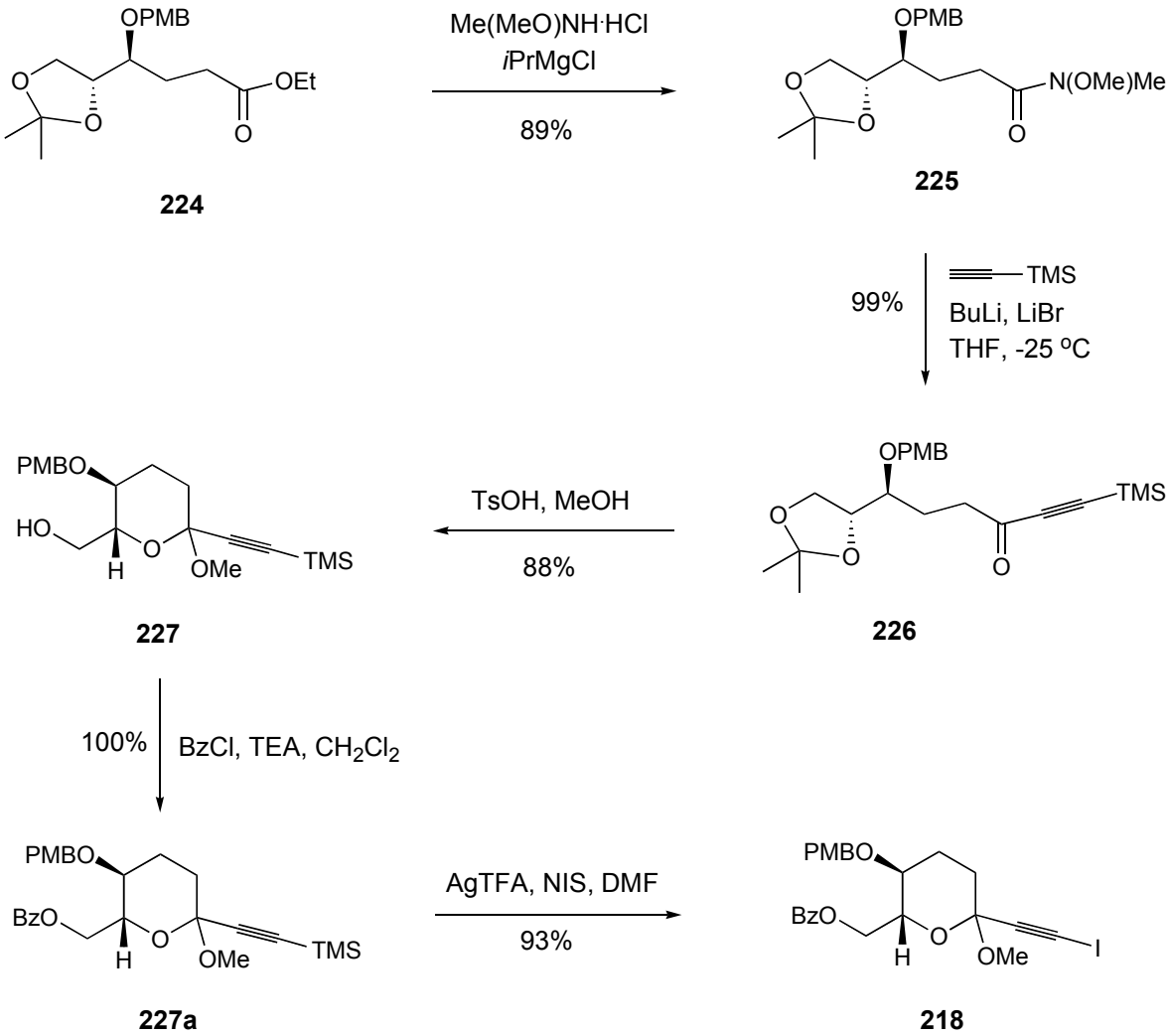
P=?

SYNTHESIS OF THE A RING PRECURSOR

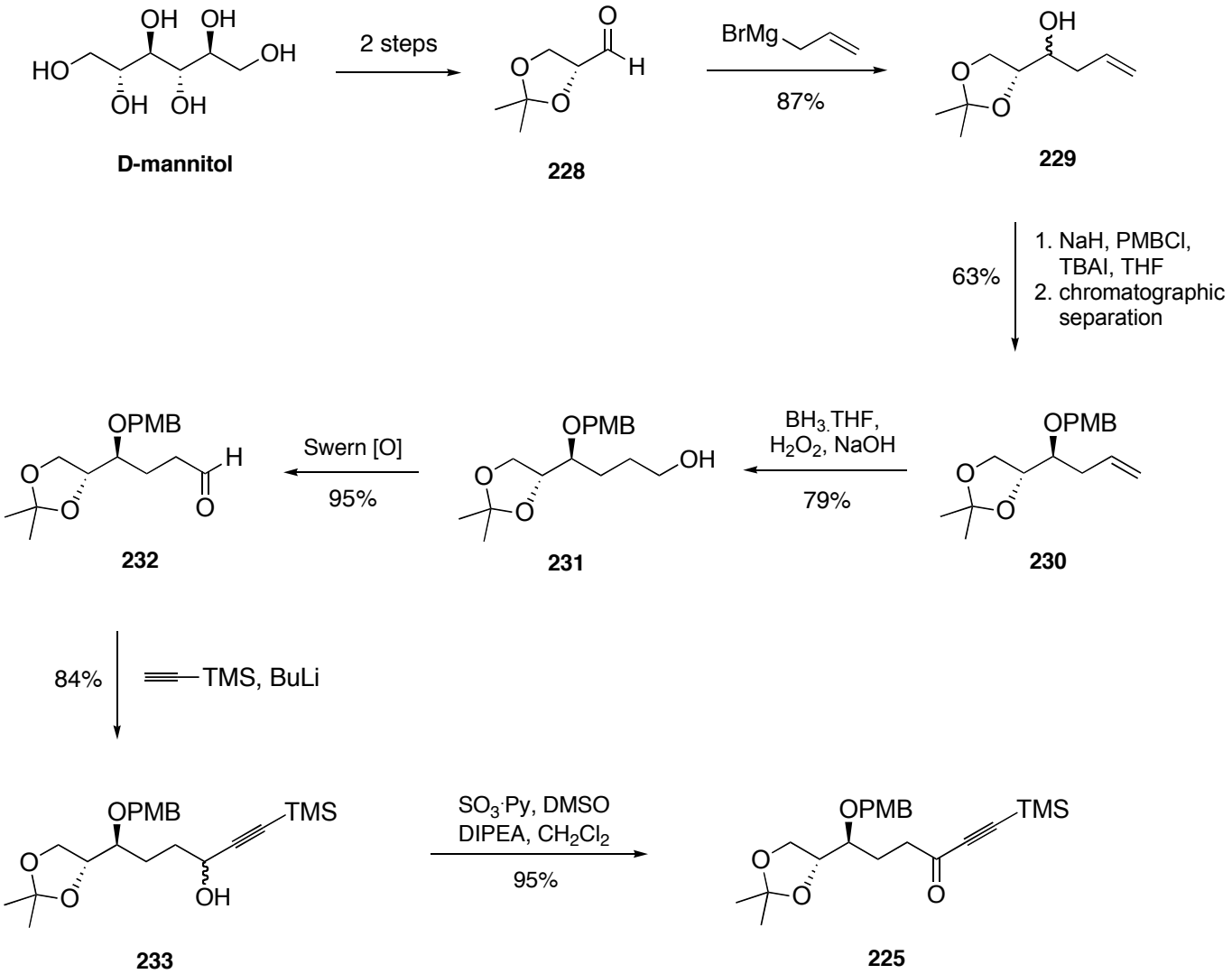


E. Abushanab *et al.*, *J. Org. Chem.*, **1988**, *53*, 2598

SYNTHESIS OF THE AB RING PRECURSOR

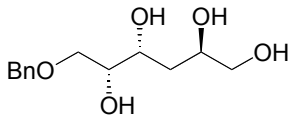


ALTERNATIVE ROUTE TO THE AB RING FROM D- MANNITOL



SYNTHESIS OF D RING

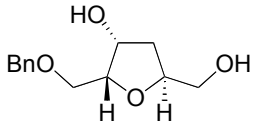
a)



238

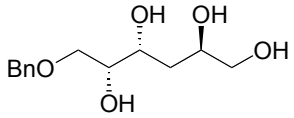
1. KHMDS, *1eq*.
2. Tris-Im, *1 eq*.
3. KHMDS, *2 eq*.

30%



239

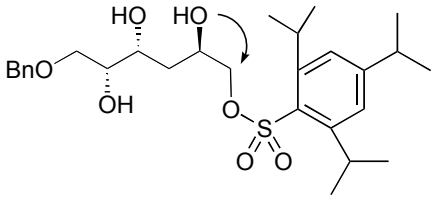
b)



from L-galactonolactone

TrisCl, DMEA
DMAP (cat.)

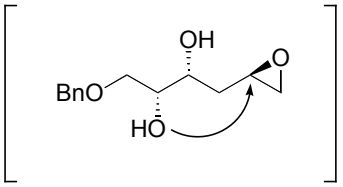
67%



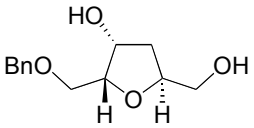
240

isolated

85% K_2CO_3, MeOH

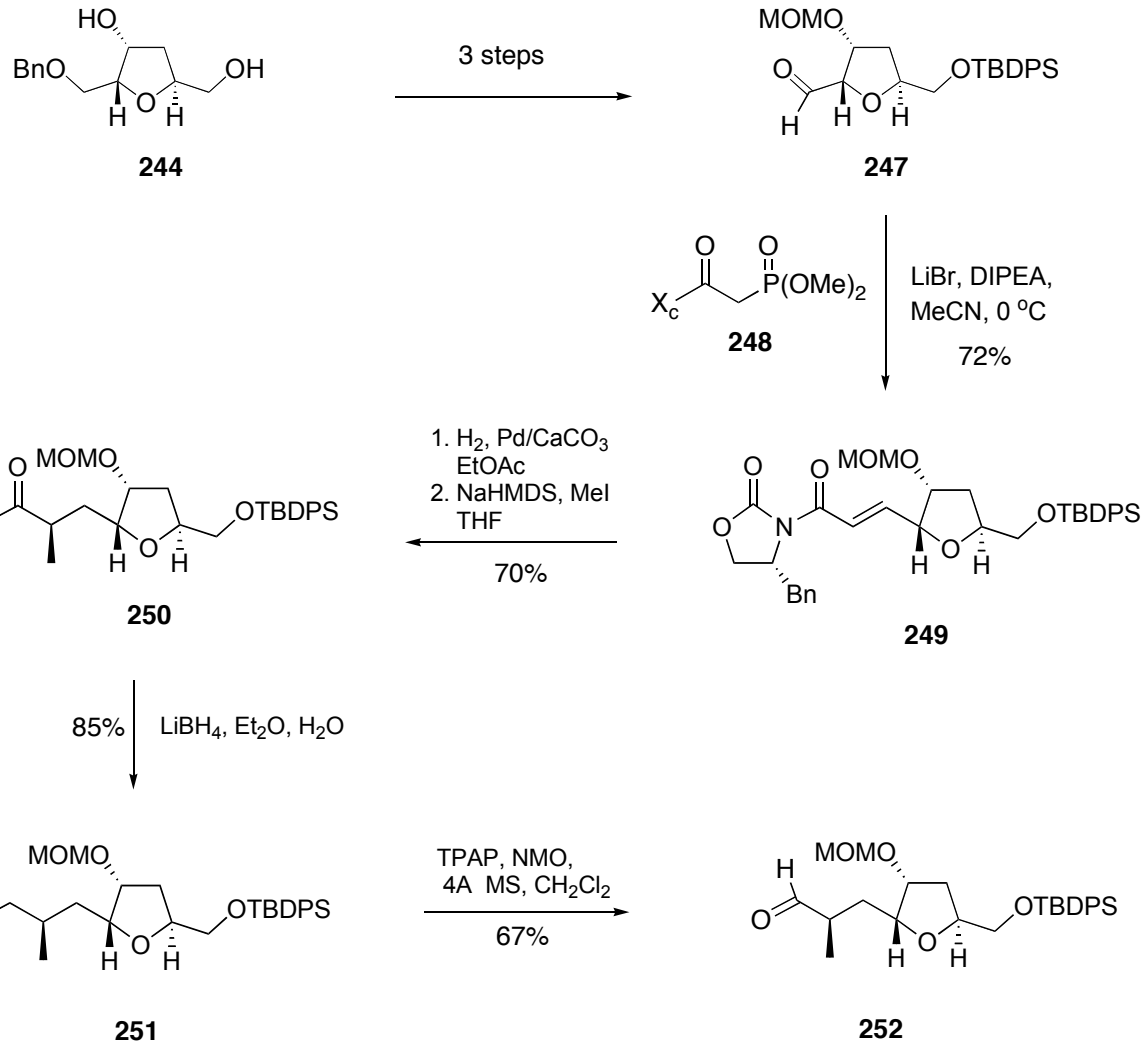


241

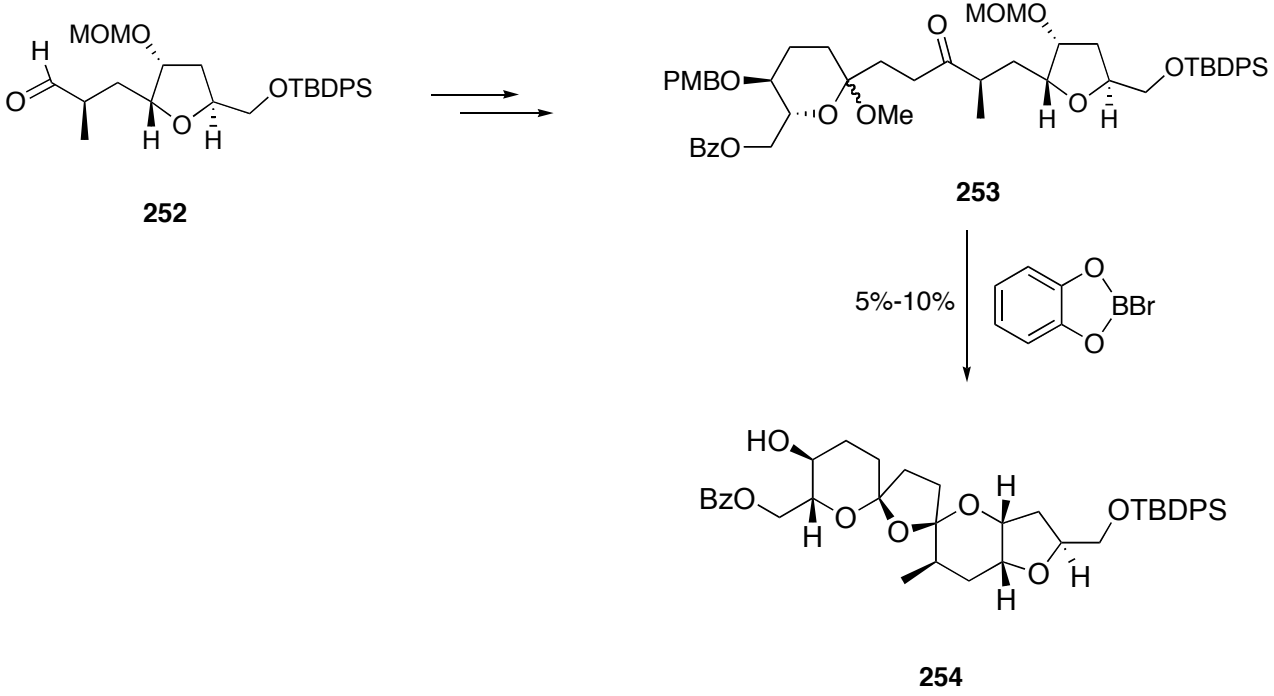


239

CD RING - FIRST VERSION

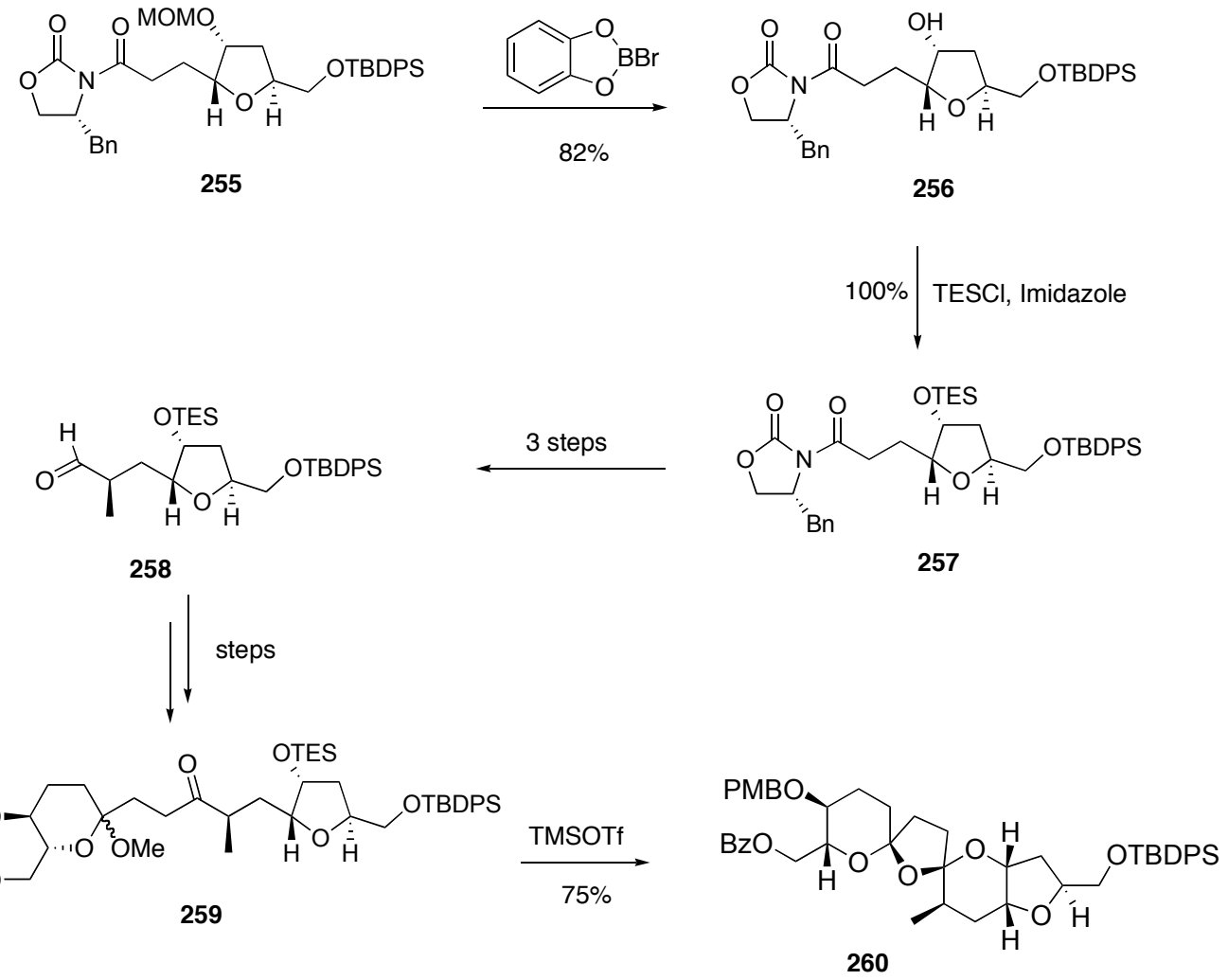


PROBLEM IN CYCLIZATION AND REMOVAL OF MOM GROUP

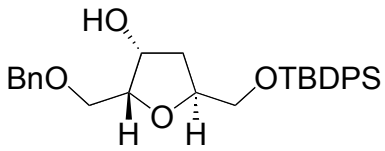


most material decomposed due to strong acidity

GOOD CYCLIZATION WITH MODIFIED CD RING



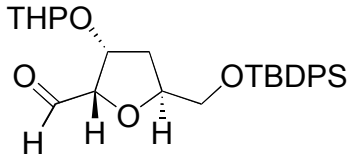
CD RING - FINAL VERSION



242

1. DHP, PPTS
2. $Pd/BaCO_3$, H_2, EtOH
3. Dess-Martin [O]

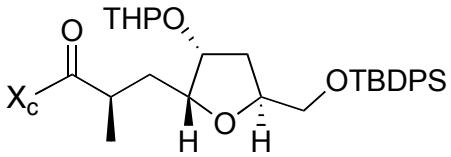
82%



263

54%

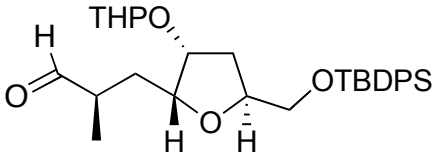
1. LiBr, **248**, DIPEA, MeCN,
2. H_2, $Pd/BaSO_4$, EtOAc
3. NaHMDS, MeI, THF



265

1. $LiBH_4$, Et_2O, H_2O
2. TPAP, NMO, 4A MS, CH_2Cl_2

77%



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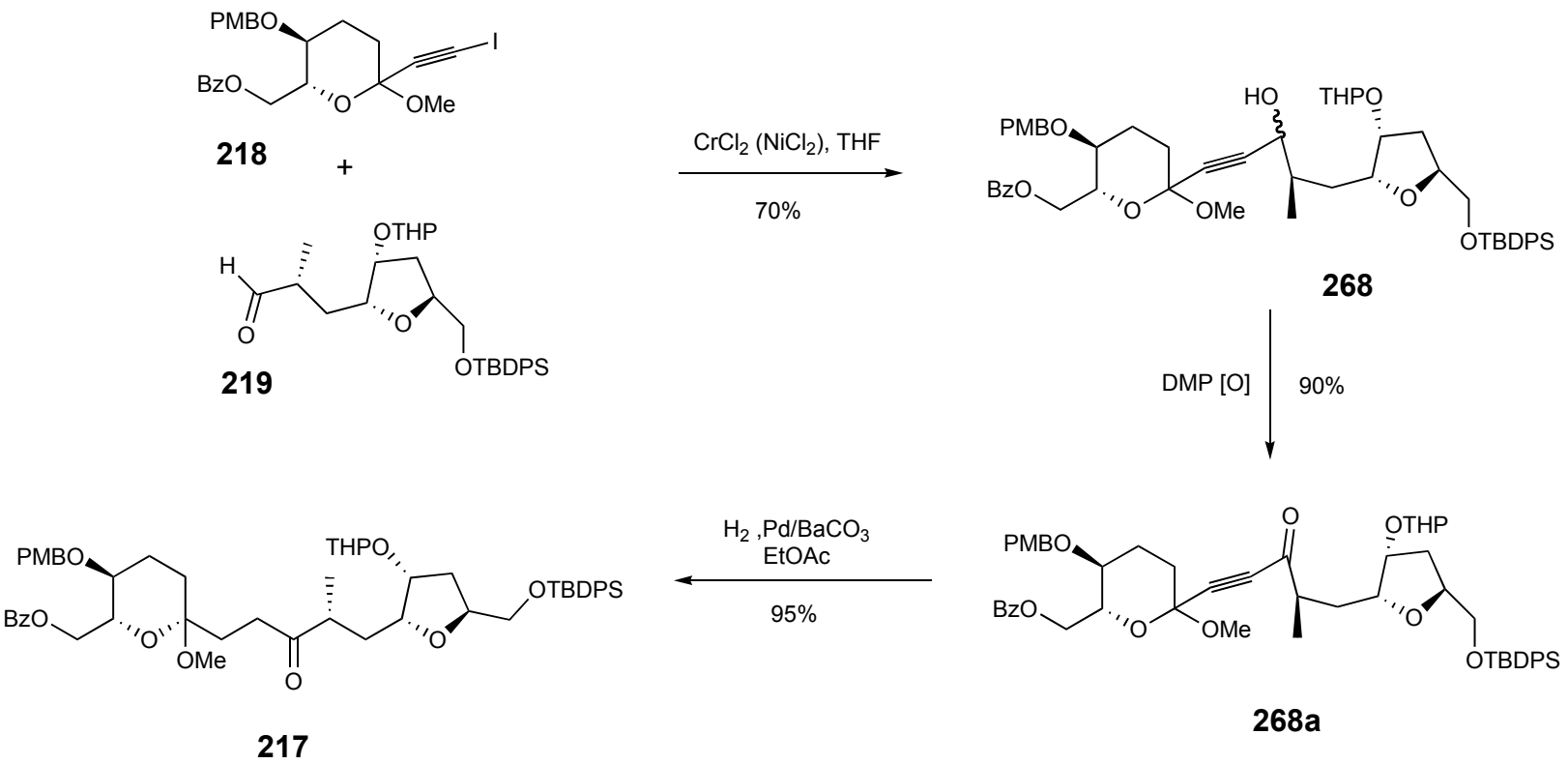
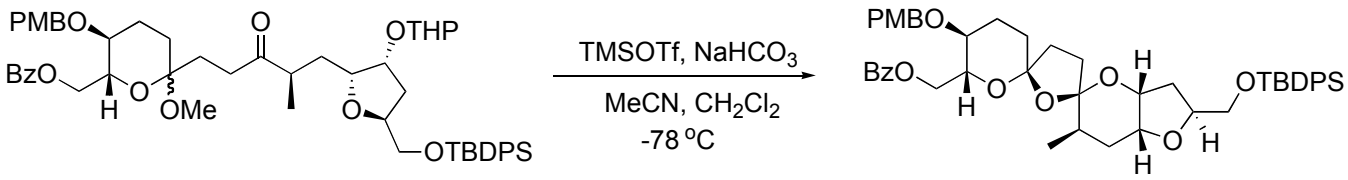
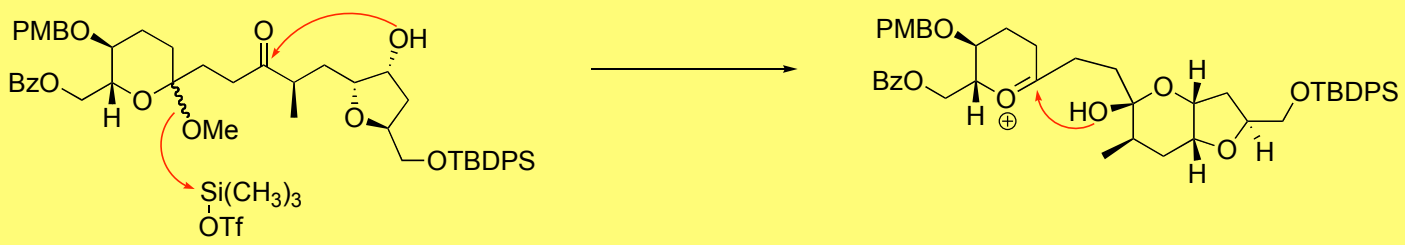
SYNTHESIS OF THE LINEAR PRECURSOR TO THE ABCD RING



CYCLIZATION CASCADE - FORMATION OF THE ABCD RING SYSTEM



Mechanism:



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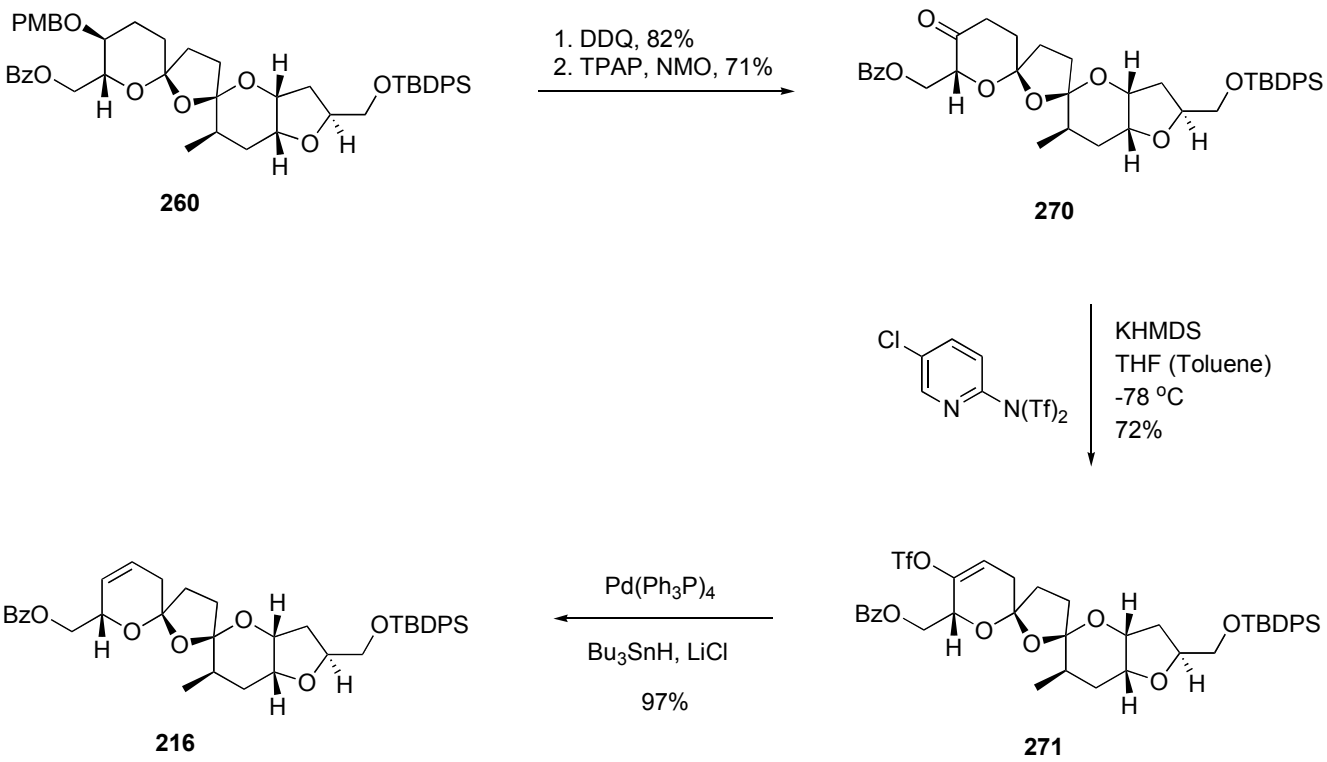
217b

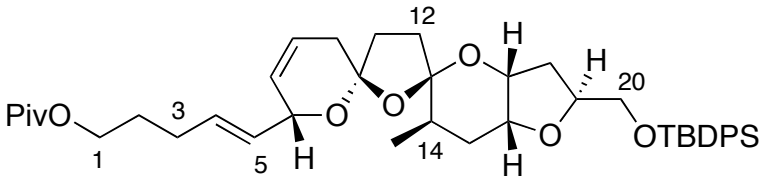
FORMATION OF THE A RING ALKENE



This route avoids the energy demanding E2 elimination process

Stille, J. K. *et al. J. Am. Chem. Soc*. **1986**, *108*, 3033.
Nicolaou, K. C. *et al. Angew. Chem. Int. Ed.* **2001**, *40*, 4068.

SECOND RETROSYNTHESIS FOR THE C1-C20 DOMAIN



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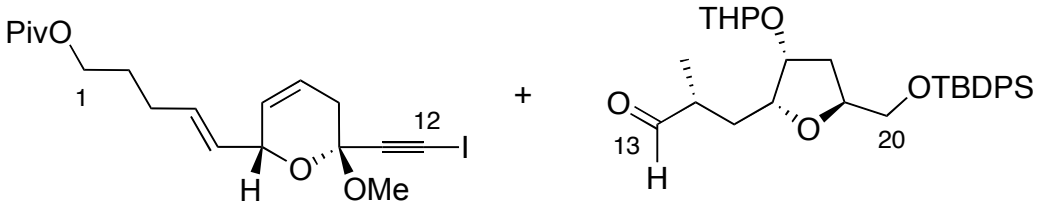

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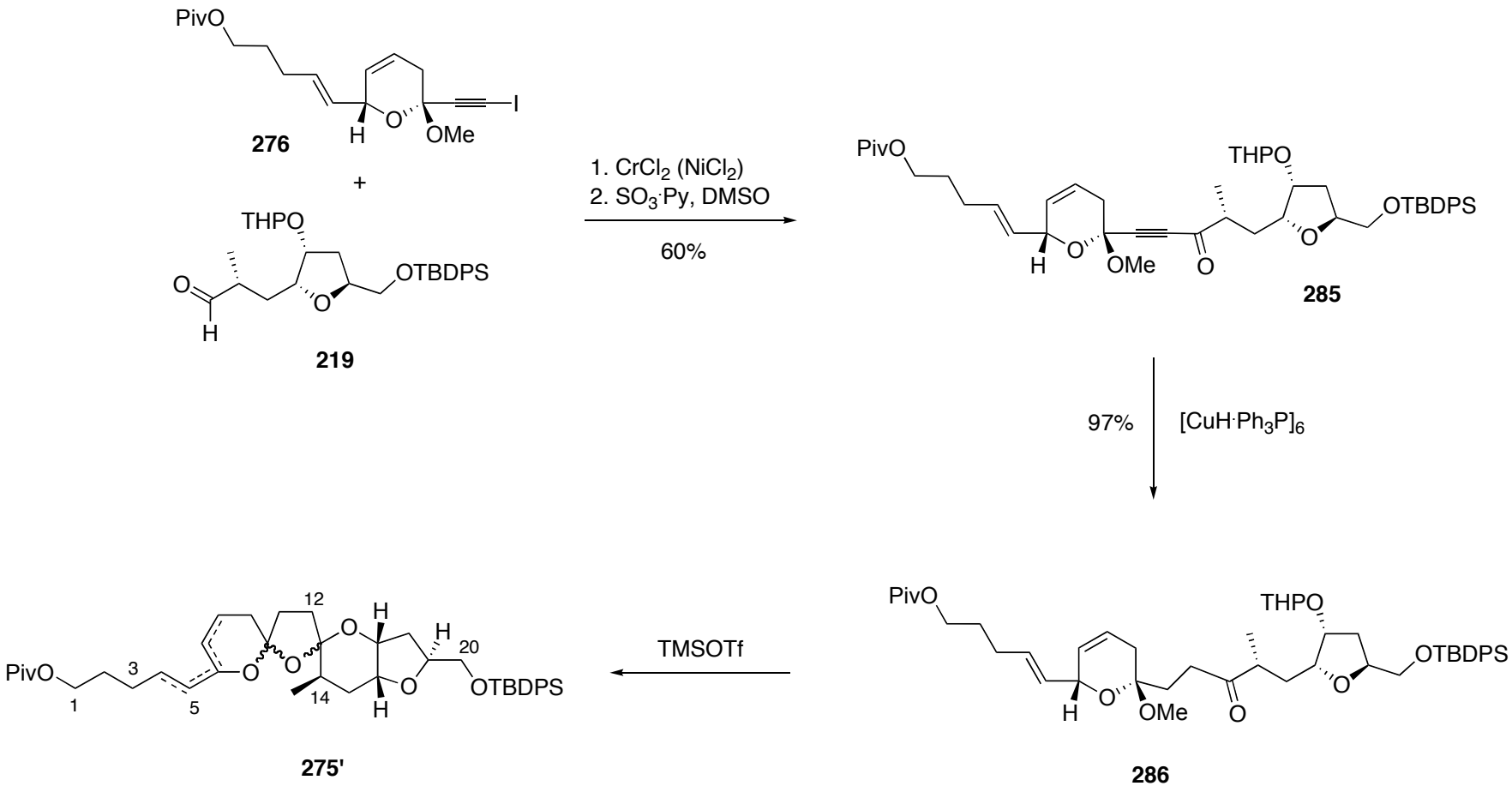
highly convergent but risky

ATTEMPTED CYCLIZATION WITH THE BIS-ALLYLIC MOIETY

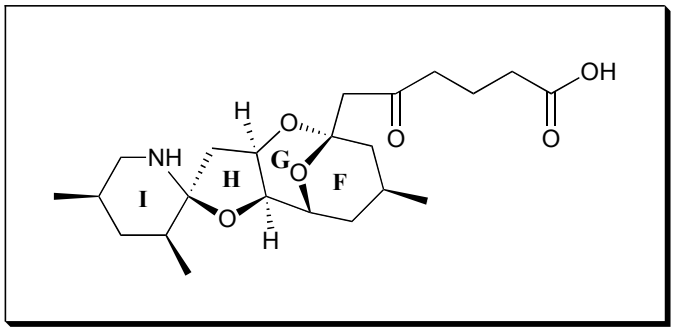


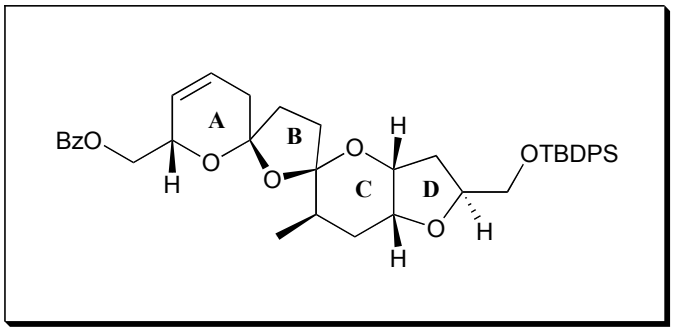
complicated mixture

Unpublished data

SUMMARY

1. Improved and scaled up the FGHI domain
2. Designed and synthesized a novel azaspiracid-based hapten for immunoassay studies
3. Correctly re-assigned the structure of the ABCD domain based on NMR and computed models
4. Developed efficient synthesis of the ABCD domain





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Thank you!