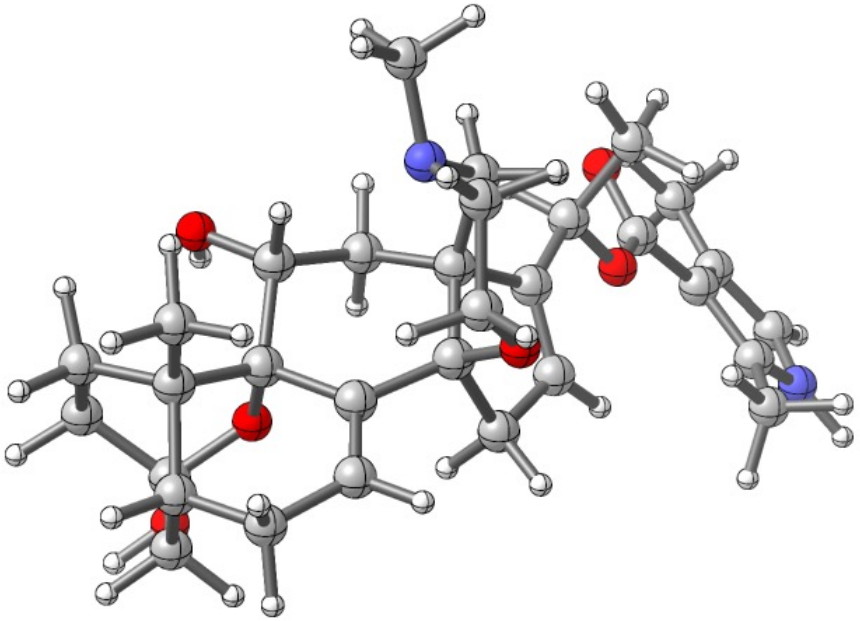
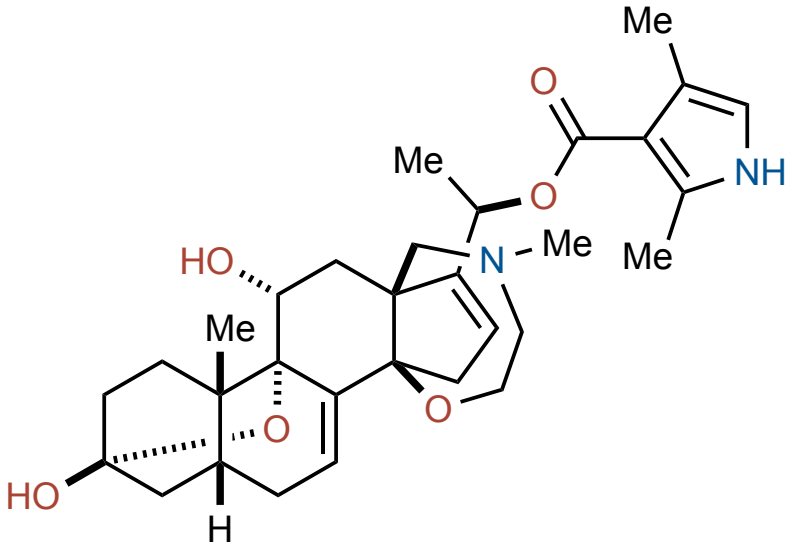
The Mysterious and Deadly Steroidal Alkaloid: BTX
Two Stories in the History of Total Synthesis





Roberto Serrano
Denmark Group Meeting
July 23rd, 2024

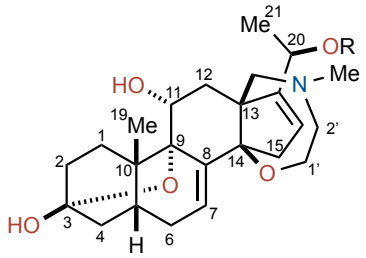
Overview

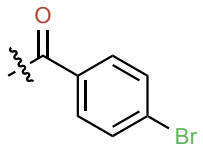
Part 1: Structural features and natural occurrence of different steroidal alkaloids

Part 2: Kishi's foundational studies

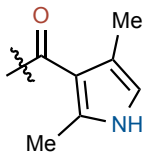
Part 3: Dubois' radical alternative

Part 1: Isolation and Characterization

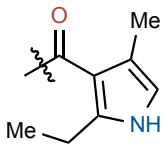




X_1



X_2



X_3

R = H, Batrachotoxinin A
R = X_1, Psuedobatrachotoxin
R = X_2, Batrachotoxin
R = X_3, Homobatrachotoxin

R = $COCHCHCH_3$, Batrachotoxinin A-20*R*-*cis*-crotonate
R = $COCH_2CH(OH)CH_2CH_3$, Batrachotoxinin A-20*R*-3'-hydroxypentanoate
R = Ac, Batrachotoxinin A-20*R*-acetate

- Batrachotoxin was first isolated in 1963 by F. Marki and B. Wiktop in the mountains of San Juan, Colombia.
- Kokoi frogs (*Phyllobates bicolor*) secrete the metabolite on their back as a defense mechanism against other hostile organisms.
- Witkop attained ~330 kokoi frogs and harvested the crude extract for characterization.
- Protection of the C_{20} hydroxyl with X_1, and crystallization from acetone gave rise to thin white needles suitable for X-ray diffraction.
- Partial syntheses from both Witkop and Imhof (ETH Zurich) further confirmed the nuclear magnetic resonance assignments.
- Testing of BTX revealed its potency with an LD_{50} of 2 μg/kg making it one of the deadliest sodium ion channel agonists ever discovered.

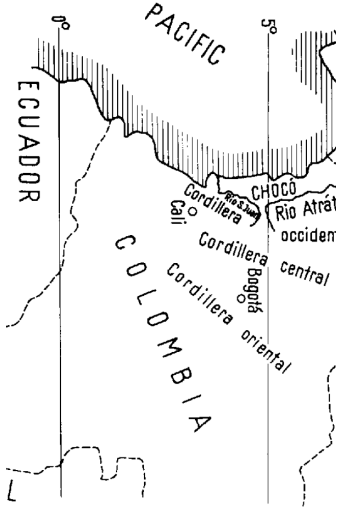
The 3 Main Players:

Phyllobates bicolor

Ifrita kowaldi

Choresine

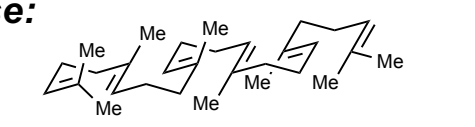
- Additionally, BTX has been isolated on the feathers of *Ifrita kowaldi* as well as in *Choresine* beetles.
- Kokoi frogs raised in captivity do not produce BTX and Choresine beetles may construct the alkaloid through plant phytosterols or a microbial symbiont.
- No biosynthesis available.



Isolation: Experientia. ***1963***, 19, 329–338; *J. Am. Chem. Soc.* ***1968***, 90, 1917–1918; *J. Am. Chem. Soc.* ***1969***, 91, 3931–3938.
Partial Synthesis: Helv. Chim. Acta. ***1972***, 55, 1151; *Helv. Chim. Acta.* ***1973***, 56, 139.
Biosynthetic Speculation: PNAS. ***2000***, 97, 12970-12975; *PNAS.* ***2004***, 101, 15857-15860; *ChemBioChem.* ***2019***, 20, 1231-1241.

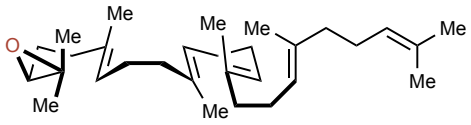
Part 1: General Biosynthetic Hypothesis

Cyclase:



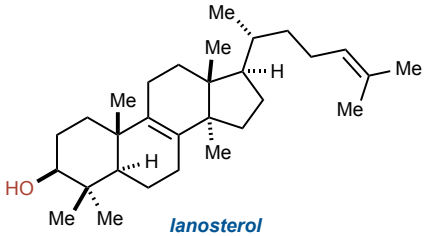
squalene

squalene epoxidase



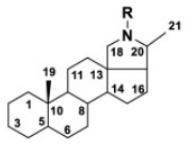
squalene oxide

oxidosqualene cyclase
(animals and fungi)

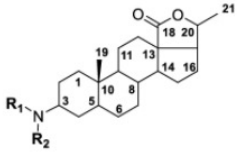


lanosterol
divergence via oxidation
and rearrangements

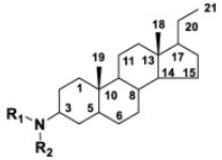
Oxidase and Rearrangements: Structural Variations



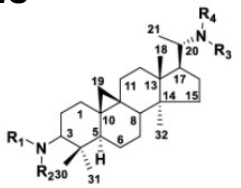
Conanine type



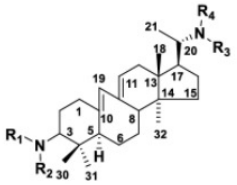
Paravallarine type



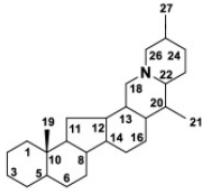
Pregnane type



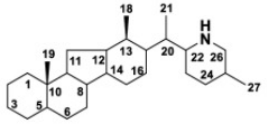
9β,19-Cyclo-14α-methylpregnane type



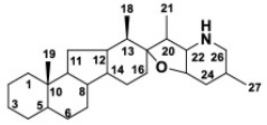
9(10→19) Abeo-14α-methylpregnane type



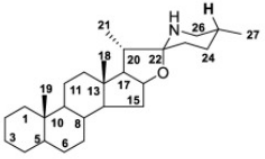
Cevanine type



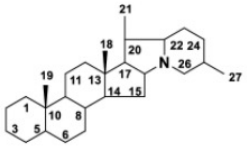
Veratramine type



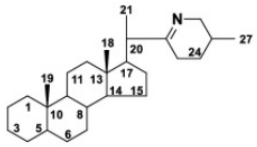
Jervine type



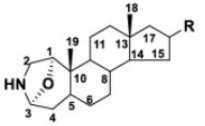
Spirosolane type



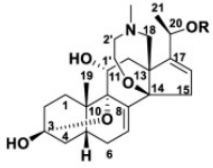
Solanidine type



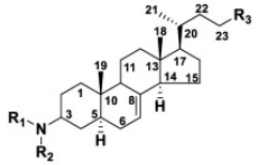
Verazine type



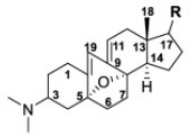
Samandarines



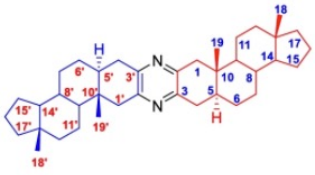
Batrachotoxins



Plakinamines



Cortistatins

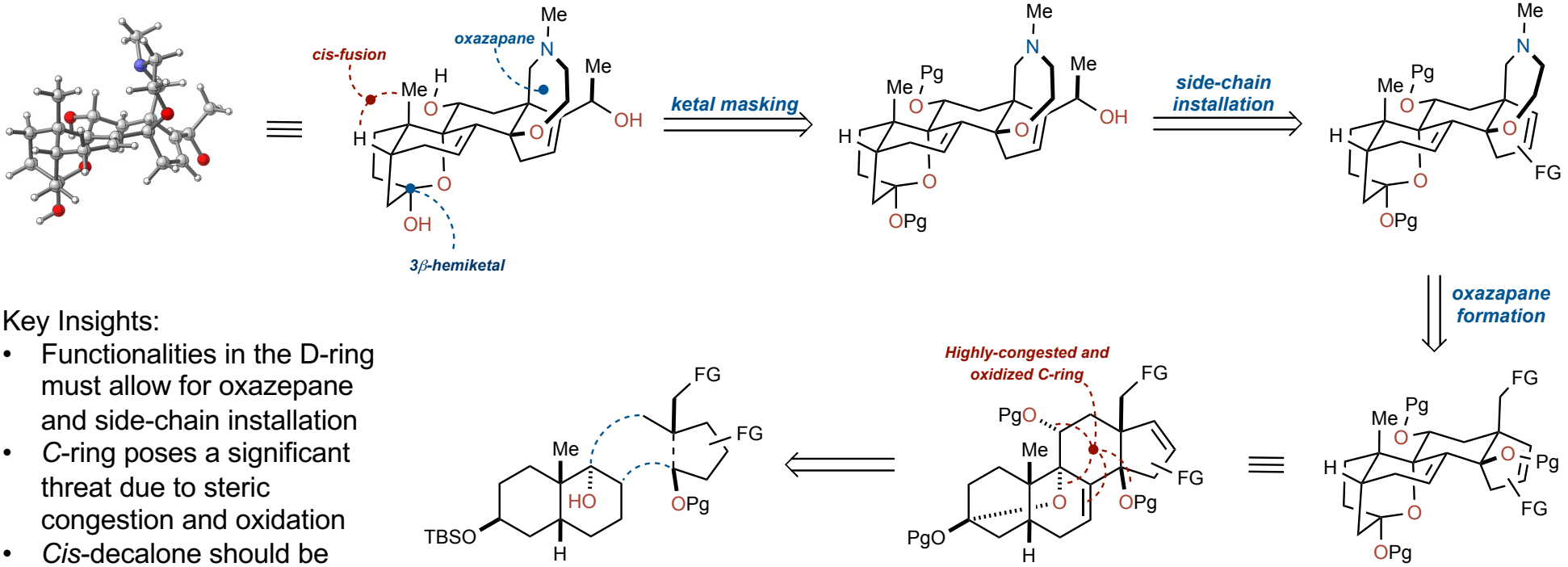


Cephalostatins and Ritterazines

Chem Rev. **2005**, 105, 4730–4756; *Isr. J. Chem.* **2011**, 51, 391–405; *Nat. Prod. Bioprospect.* **2022**, 12, 1–50.

Part 2: Kishi– Retrosynthetic Analysis

Structural Dissection and Retrosynthetic Analysis:



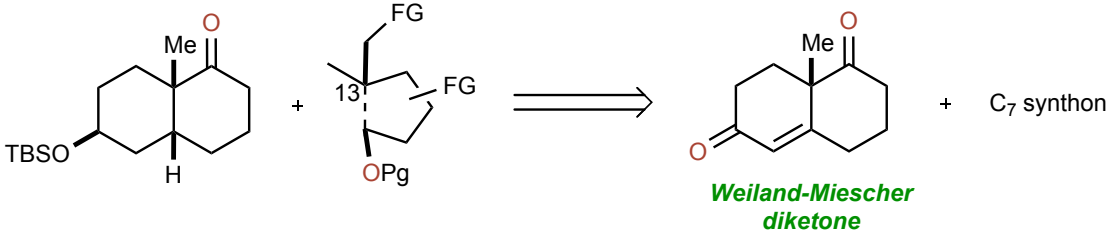
Key Insights:

- Functionalities in the D-ring must allow for oxazepane and side-chain installation
- *C*-ring poses a significant threat due to steric congestion and oxidation
- *Cis*-decalone should be assembled first and protected

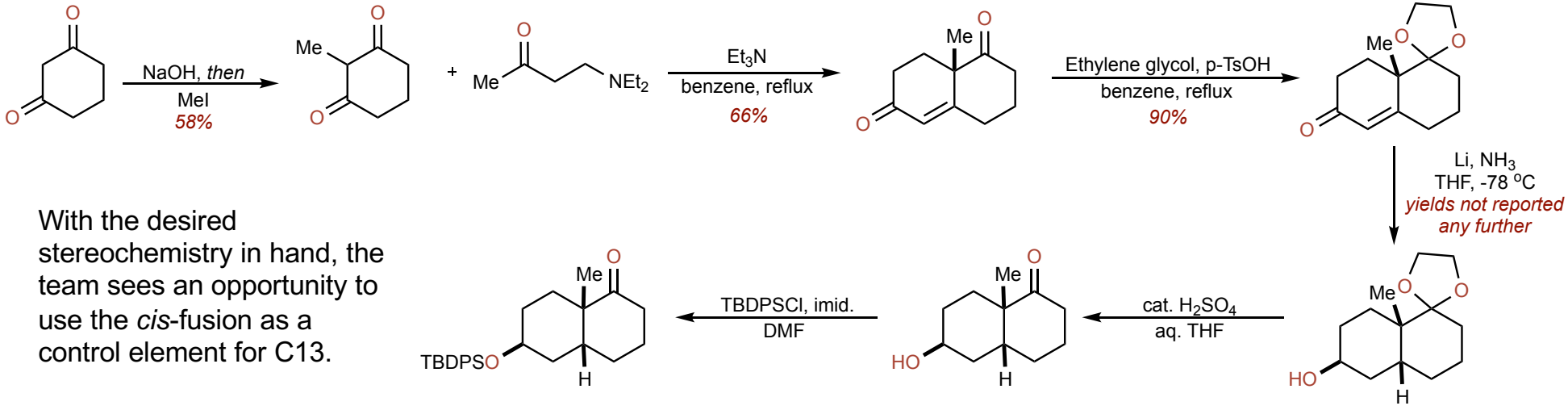
Part 2: Kishi– Model Studies

Final Retrosynthetic Traces:

Kishi's team identify the *cis*-decalone as a suitable starting point for programmed convergent coupling.



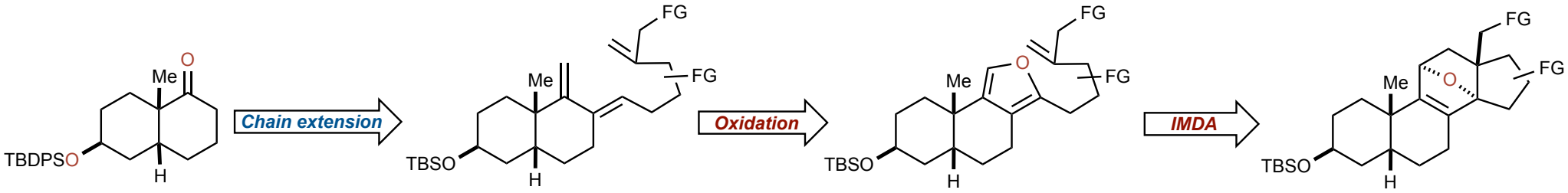
Commencement of the Synthesis:



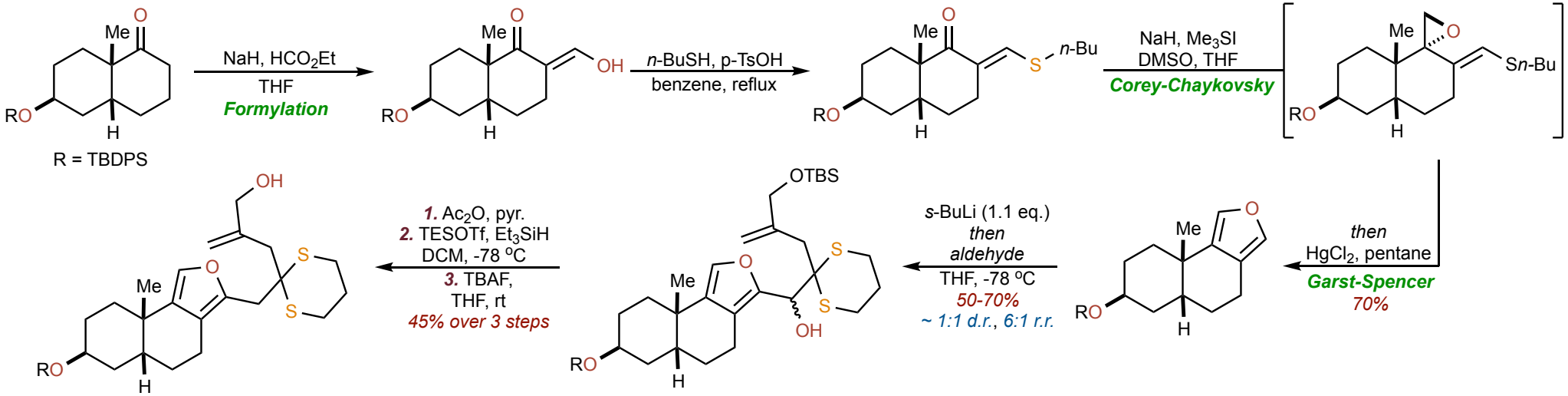
With the desired stereochemistry in hand, the team sees an opportunity to use the *cis*-fusion as a control element for C13.

Part 2: Kishi – The Intramolecular Diels-Alder

Formulation of the Furanyl-Intramolecular Diels-Alder:

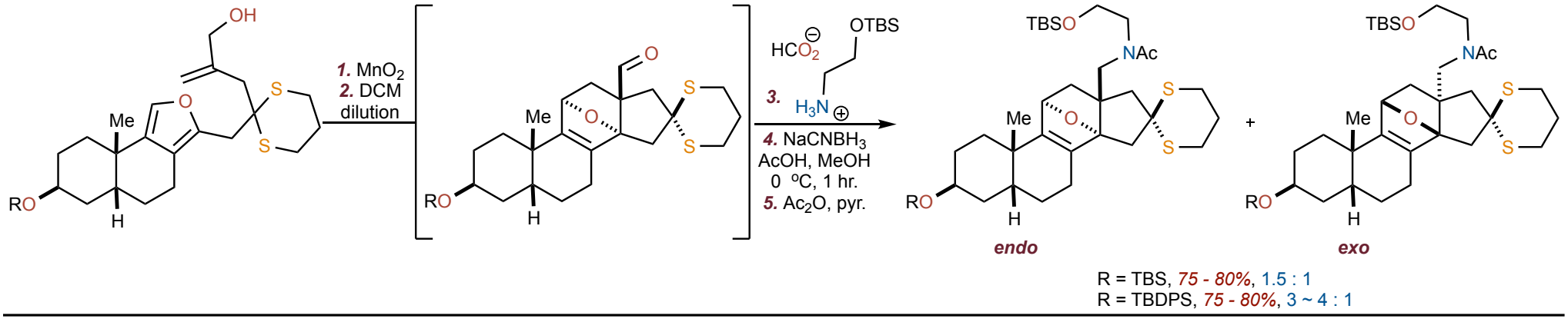


Synthesis of the critical intermediate:



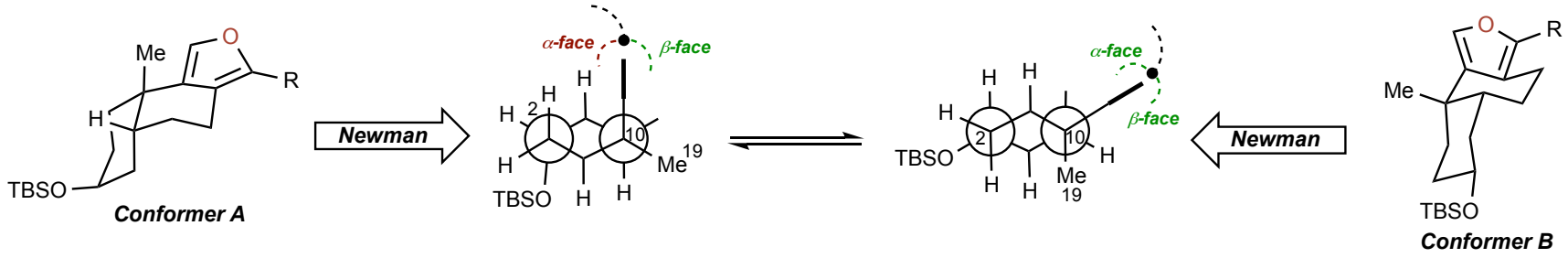
Part 2: Kishi – The Intramolecular Diels-Alder

Investigation of the IMDA:



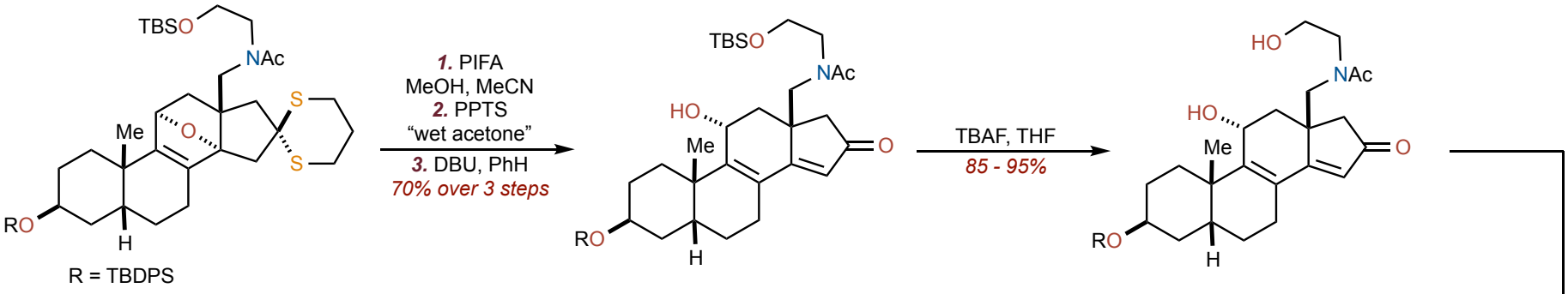
R = TBS, *75 - 80%*, 1.5 : 1
R = TBDPS, *75 - 80%*, 3 ~ 4 : 1

Stereochemical Analysis:

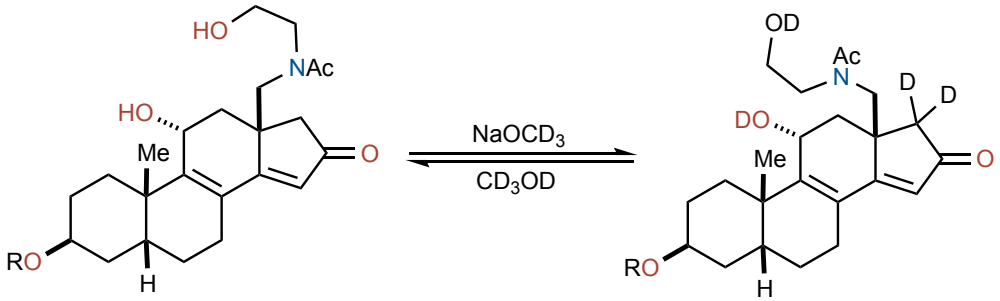


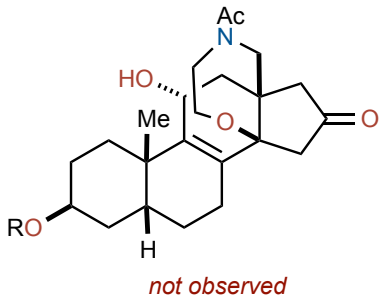
Part 2: Kishi – Oxazapane Formation

Intramolecular Oxa-Michael:



A Deeper Look:

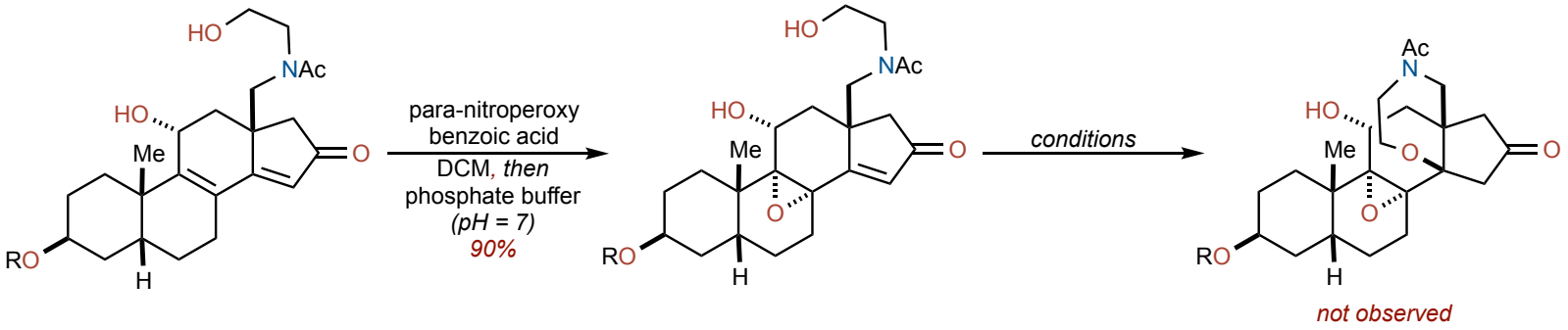




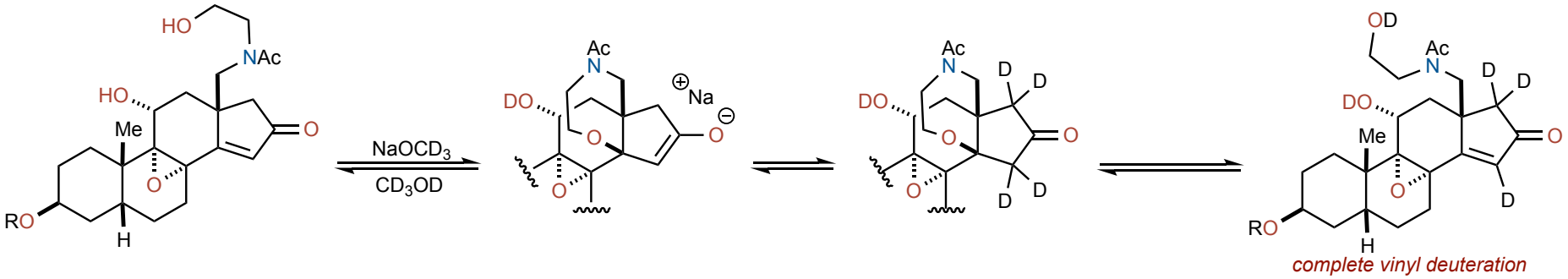
not observed

Part 2: Kishi – Oxazapane Formation

Epoxidation, then Intramolecular Oxa-Michael:

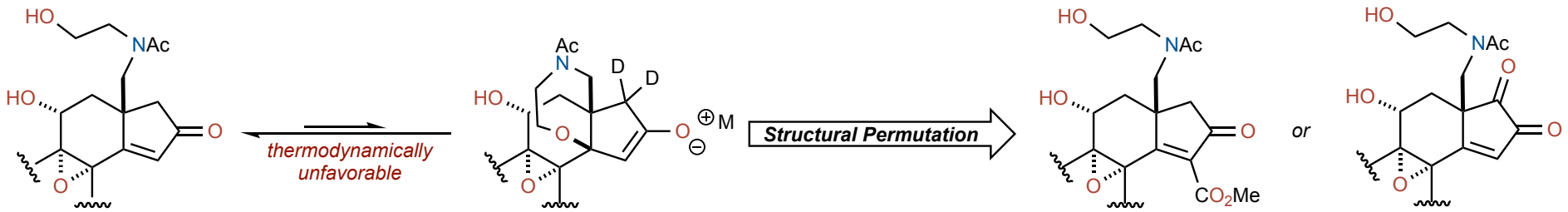


In-situ NMR:

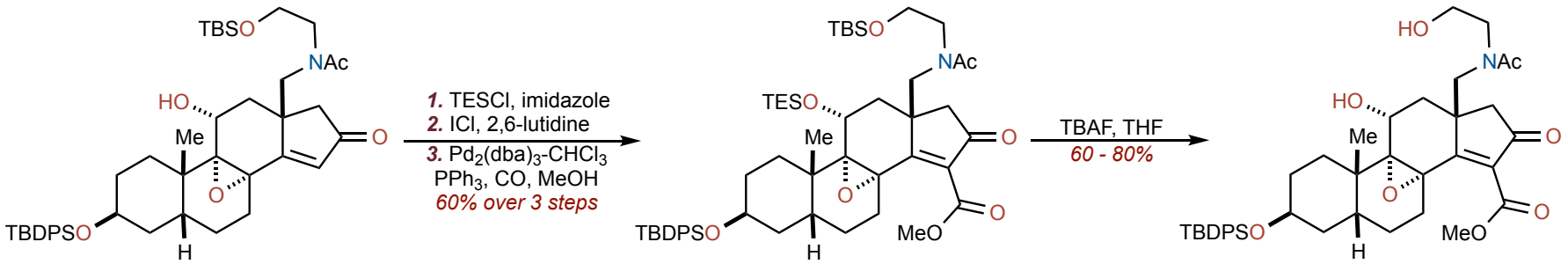


Part 2: Kishi – Oxazapane Formation

Improved Hypothesis: Structural Alteration of the Michael Acceptor

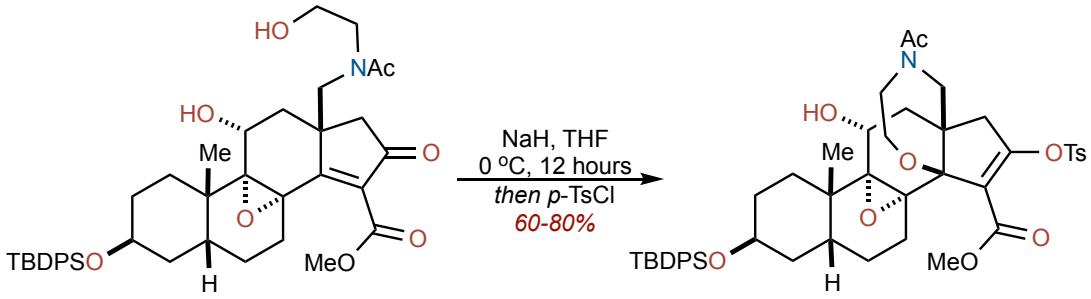


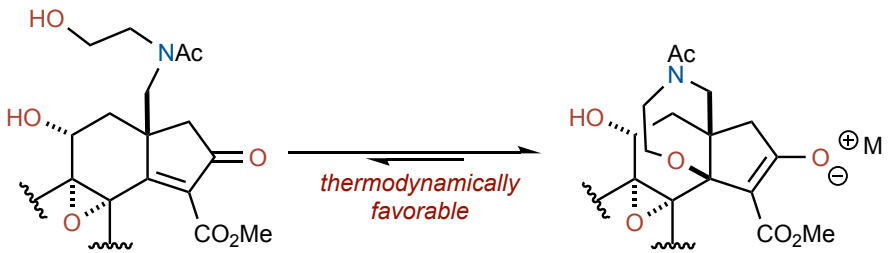
Synthesis of the Substrate:



Part 2: Kishi – Oxazapane Formation

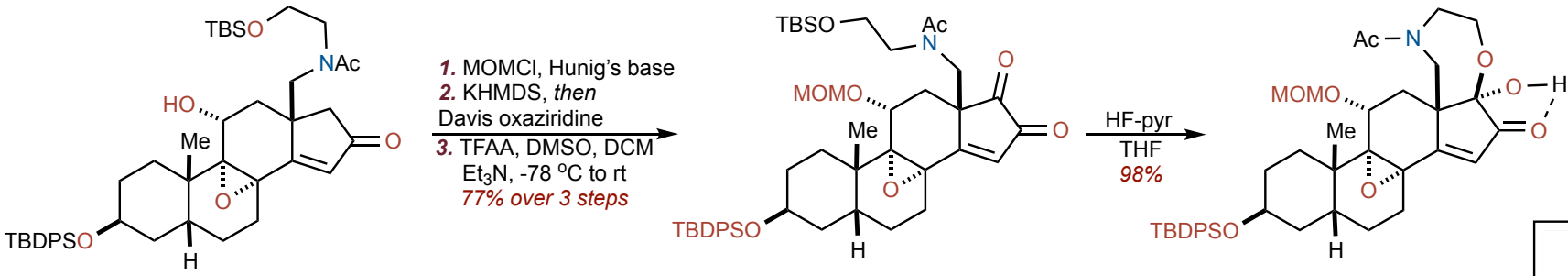
Cyclization Test:

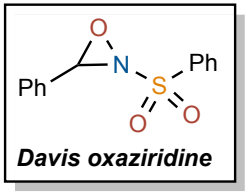




Although success was met, carbon connectivity is not optimal for further elaboration.

Probing the 2nd Permutation:

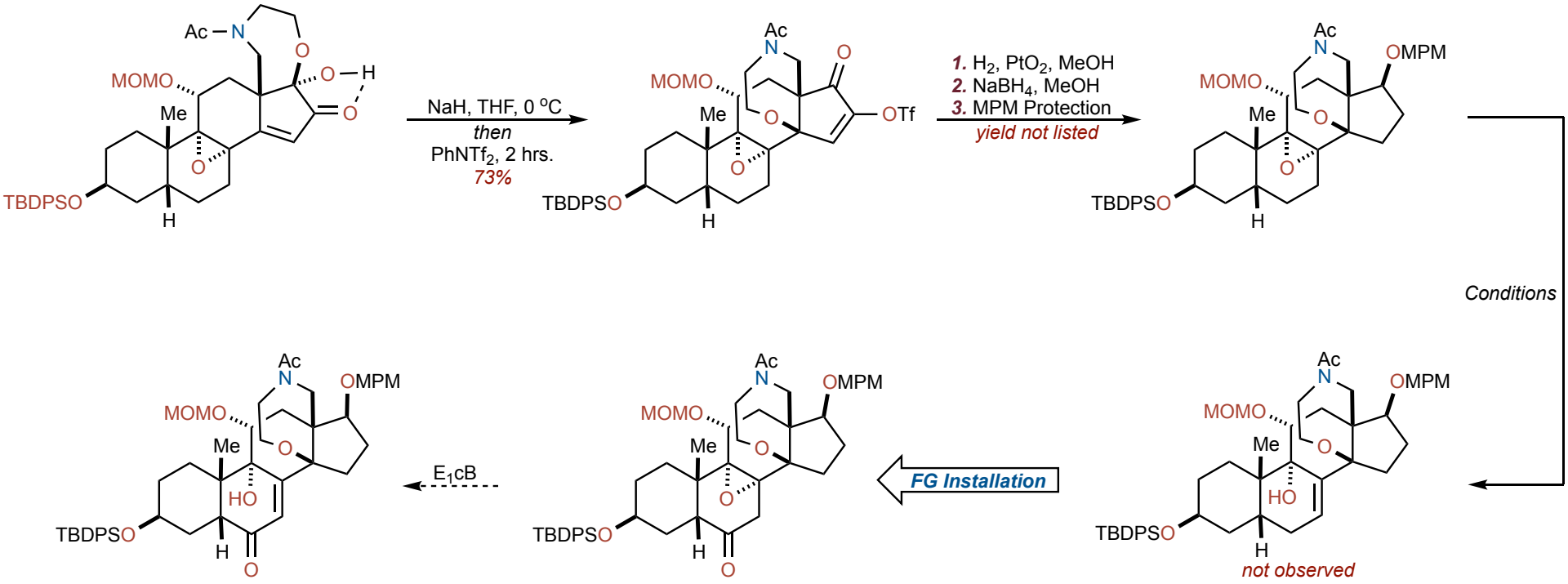




Davis oxaziridine

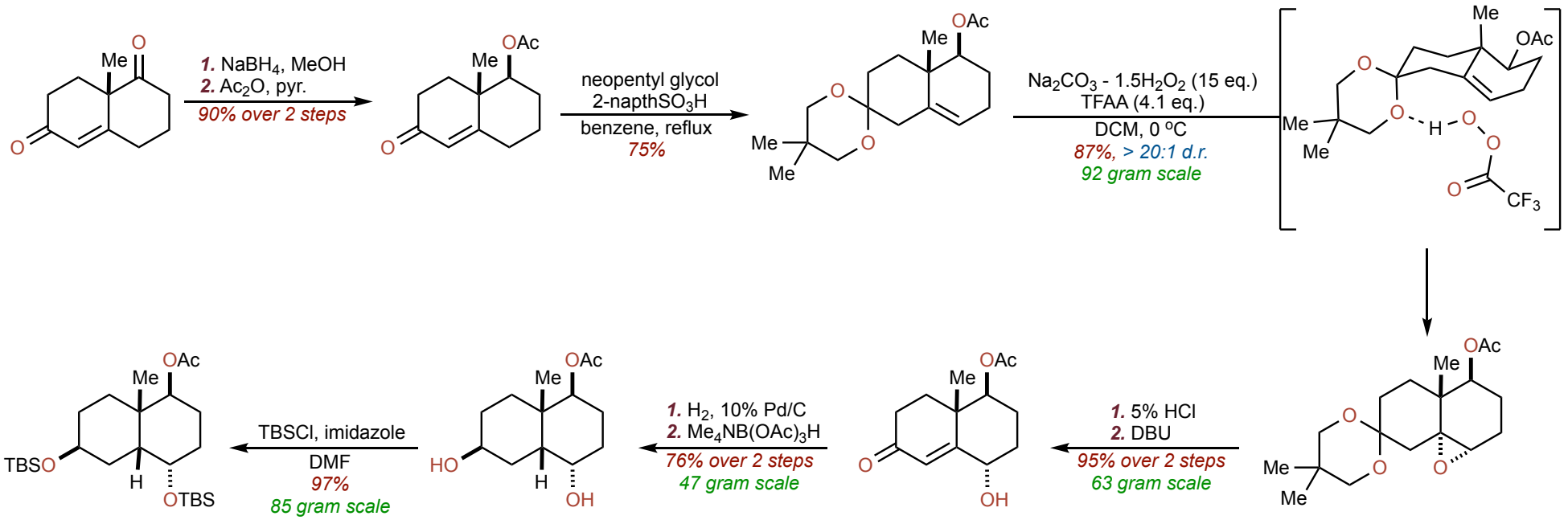
Part 2: Kishi – Oxazapane Formation

B-ring, C7-C8 Olefin:



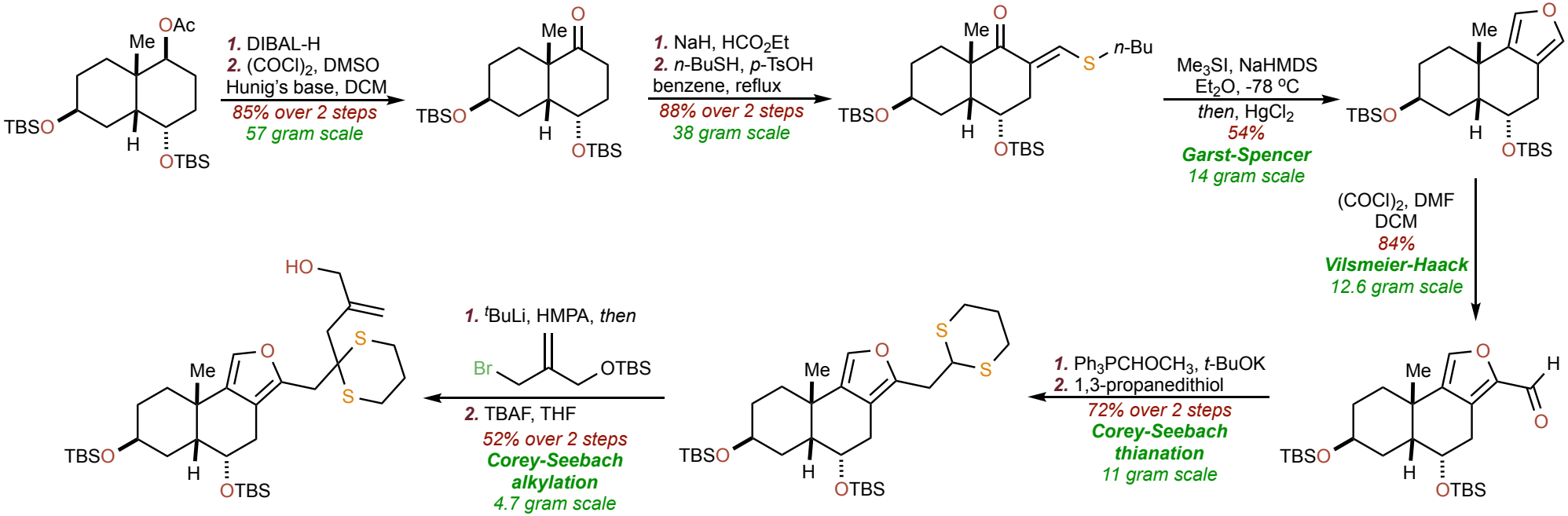
Part 2: Kishi – Oxidized *cis*-decalin

Redesigning the cis-decalin:



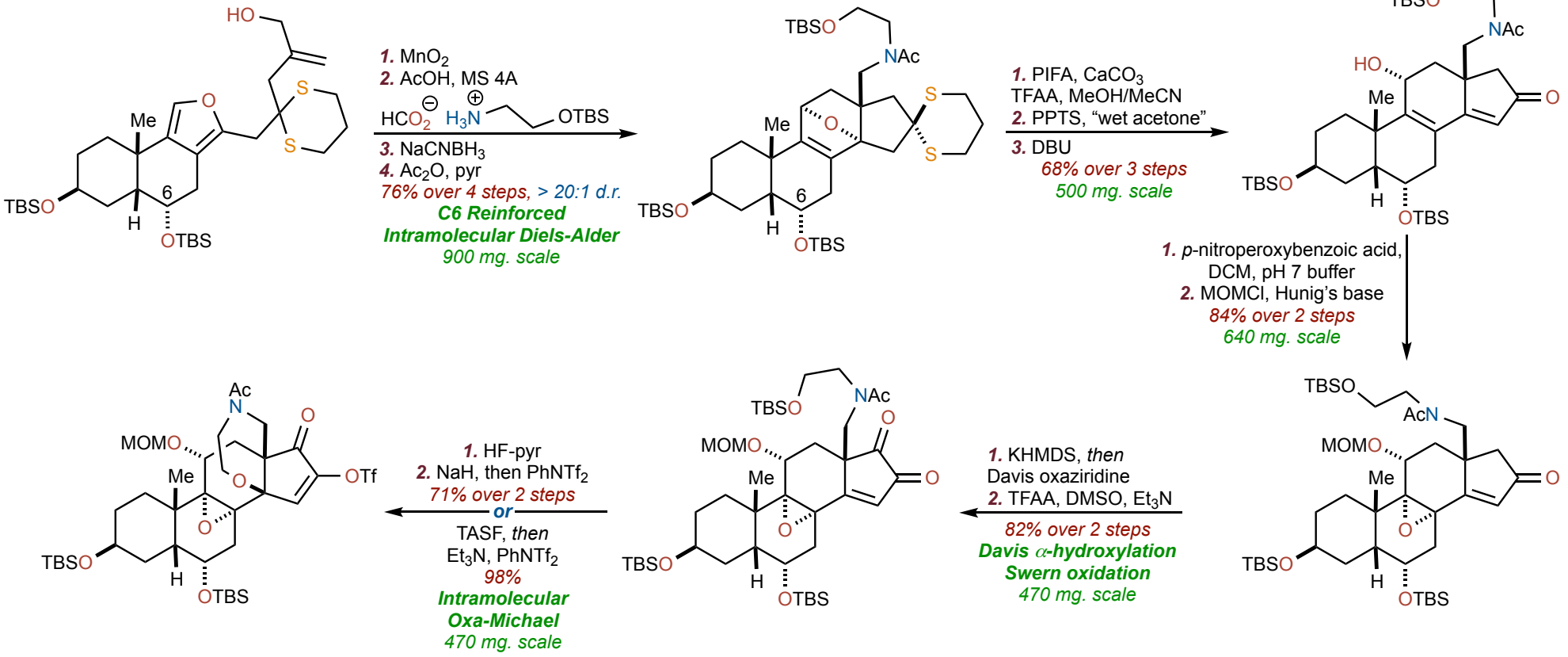
Part 2: Kishi – 2nd Generation Furan Synthesis

The Final Push: the oxidized tetracycle:



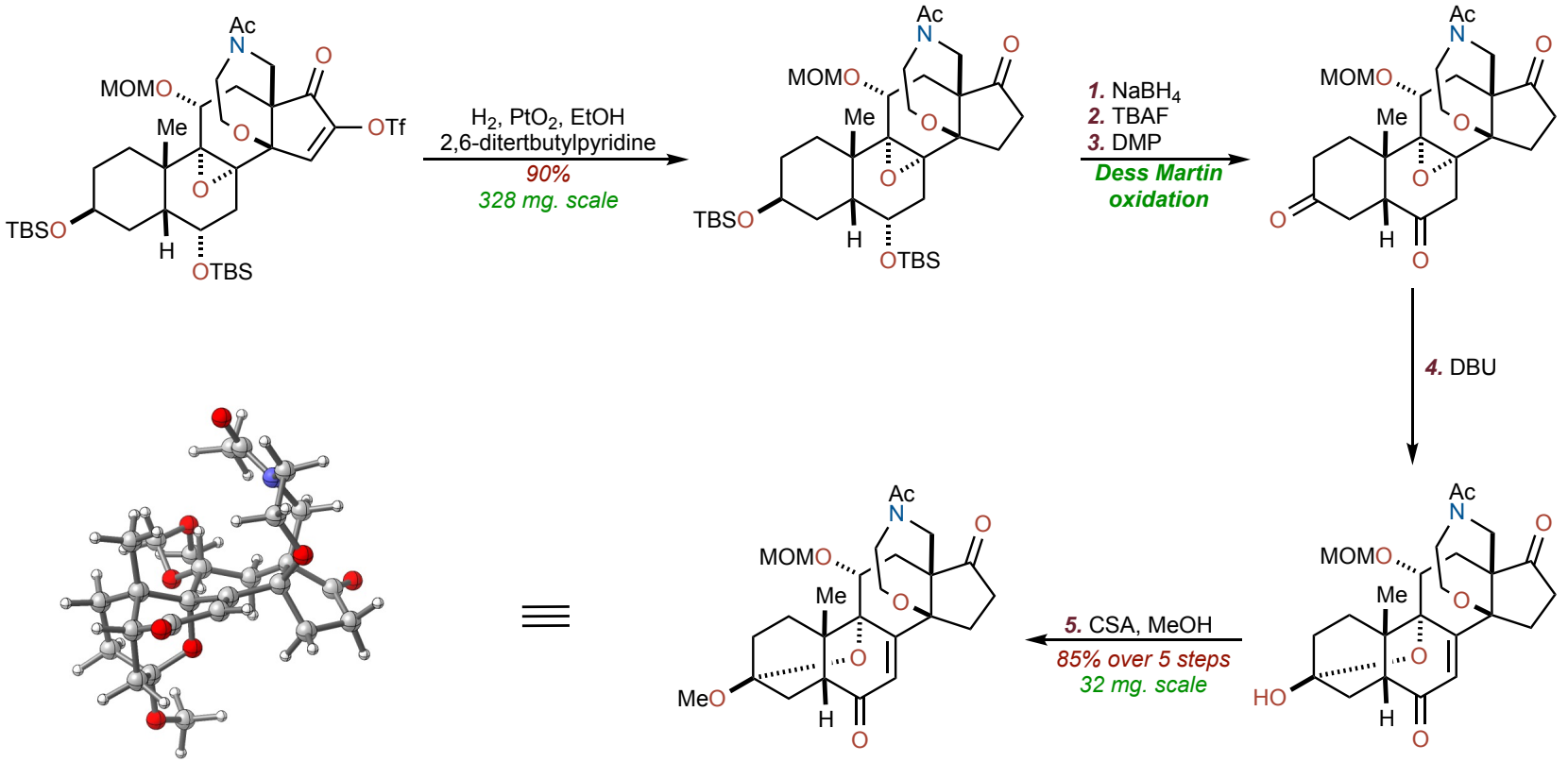
Part 2: Kishi – Reinforcements

C6-oxidized tetracycle synthesis:



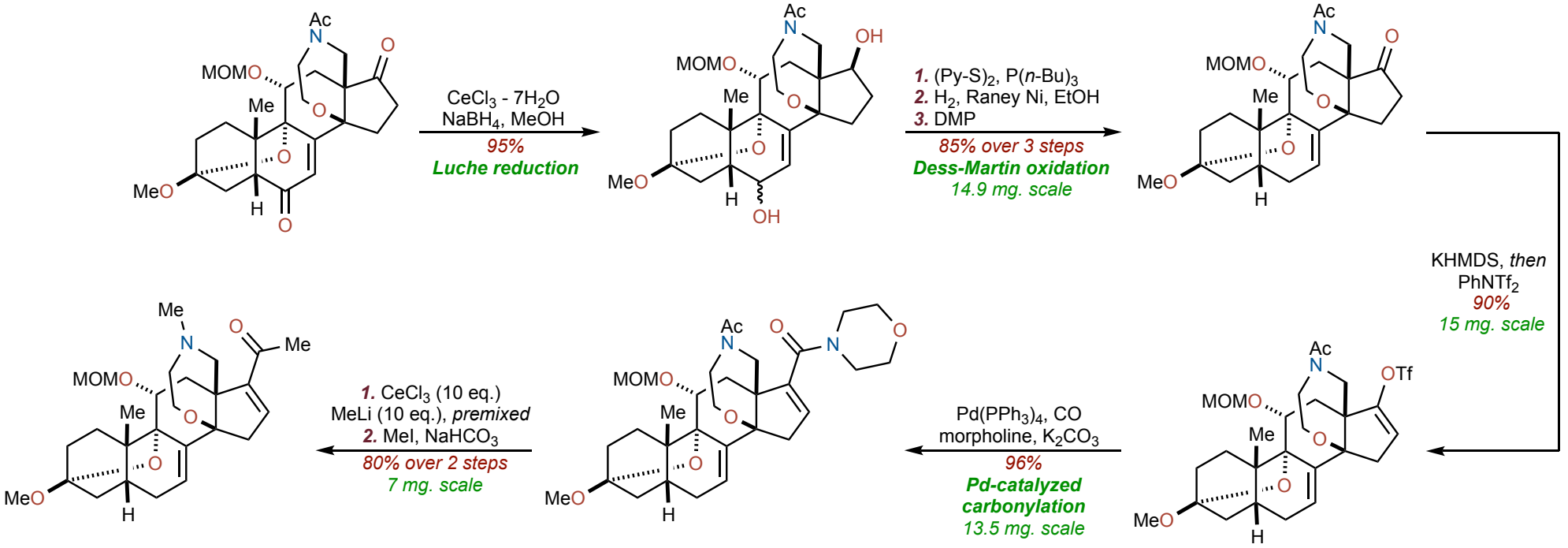
Part 2: Kishi – C7 - C8 Olefin

Redox Adjustments and testing the E1cB maneuver:



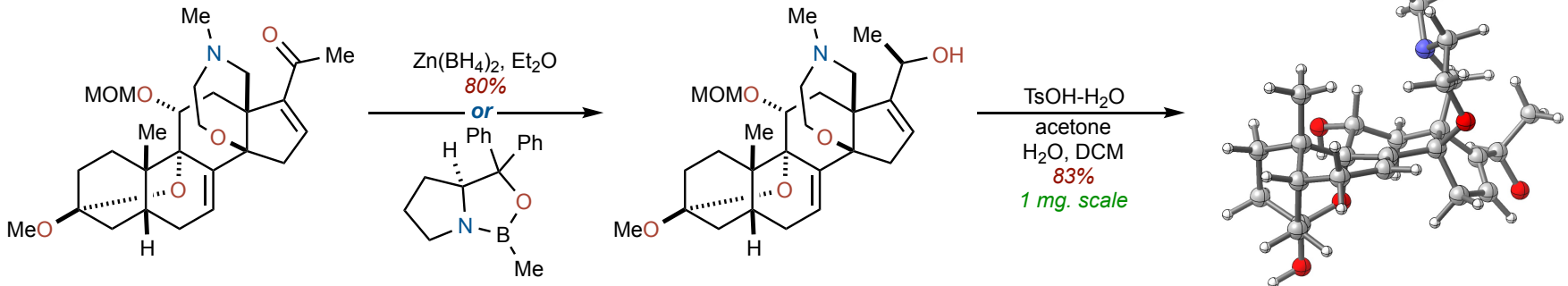
Part 2: Kishi – The Price of One Oxidation Site

Removal of the C6 Ketone and Side-Chain Installation:



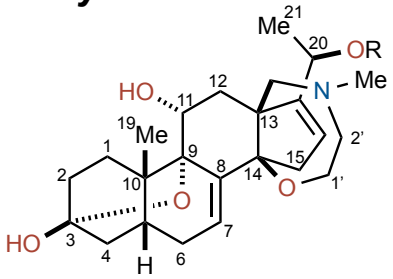
Part 2: Kishi – Final Operations and Reflections

Accessing BTX-A:



BTX-A
0.34% overall yield
49 steps LLS

Analysis:



49 steps LLS with
28 concession steps

Lessons Learned (Brief Summary):

1. *cis*-decalin is an optimal convergent building block, but controls stereochemistry at C13-C14.
2. Steroidal C-ring offers a nice point of convergency for coupling partners.
3. Oxazepane formation via oxa-Michael is difficult to the need for an irreversible process with high driving force.
4. C8-C9 epoxide is sterically hindered and inert to base-mediated isomerization processes. Best to establish C7-C8 olefin early in the synthesis.

Advancements:

New Garst-Spencer protocol, stereoselective furanyl-IMDA, oxa-Michael with α-keto enones, cerium-mediated ketone synthesis, and application of CBS-Itsuno resolution.

Overview

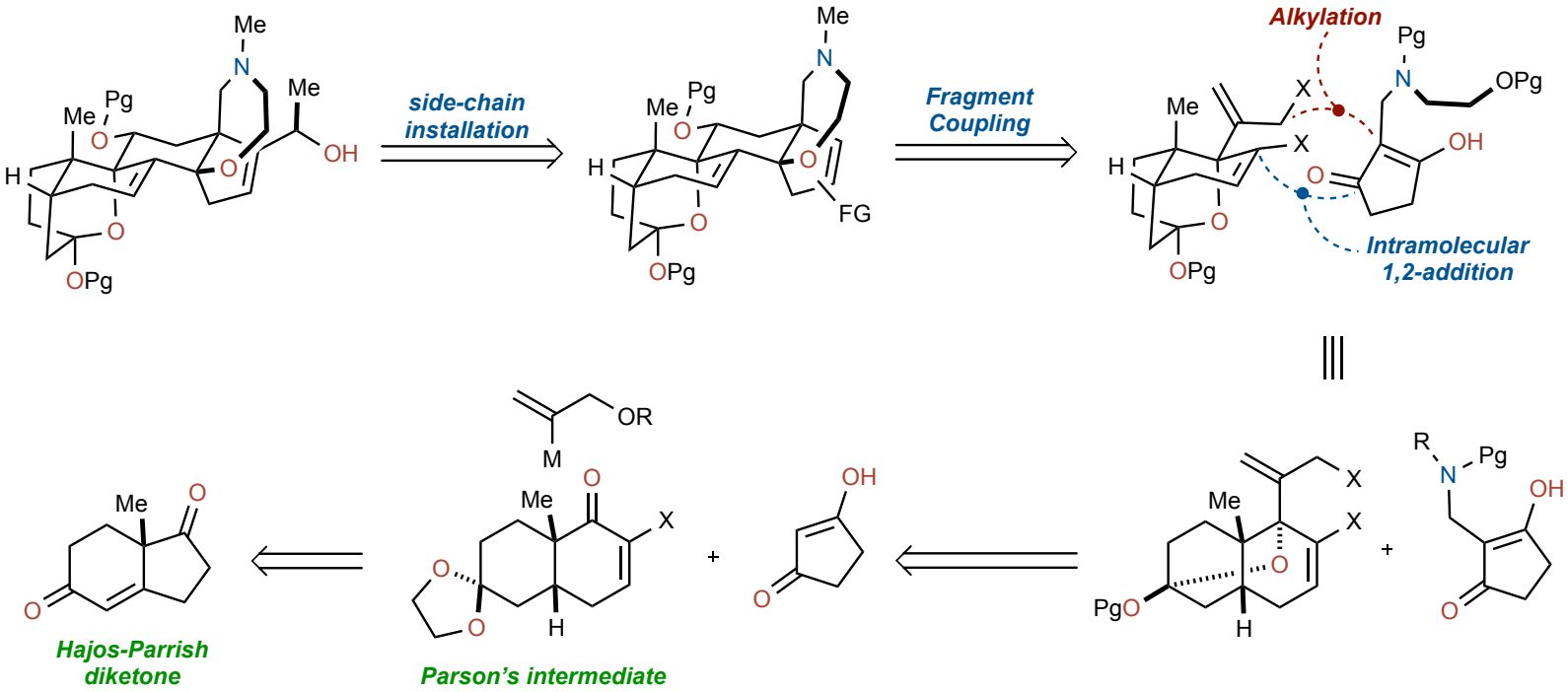
Part 1: Structural features and natural occurrence of different steroidal alkaloids

Part 2: Kishi's foundational studies

Part 3: Dubois' radical alternative

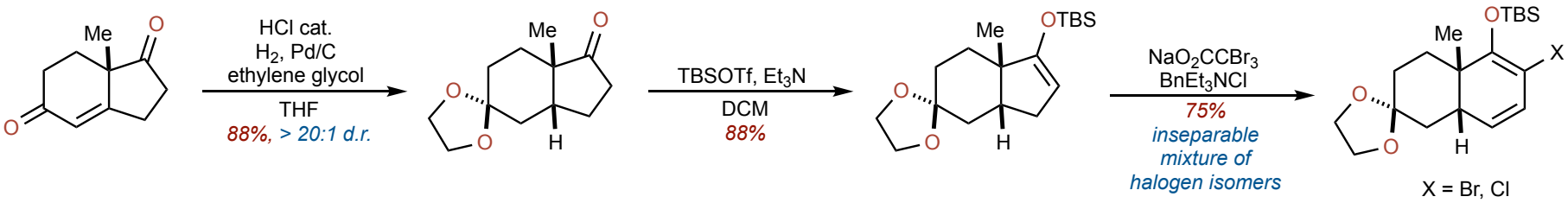
Part 3: Dubois – Retrosynthetic Analysis

Retrosynthetic Analysis:



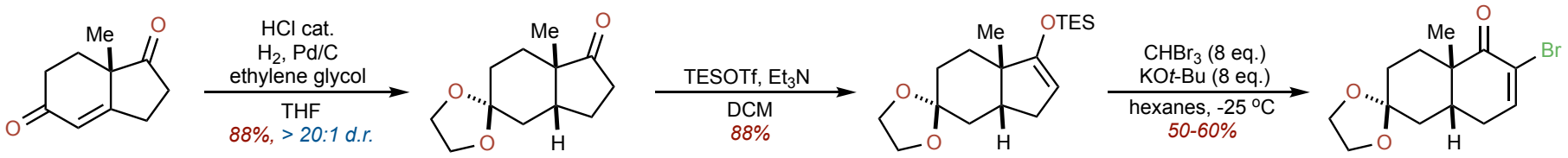
Part 3: Dubois – Decalin Synthesis

Building Block Synthesis:

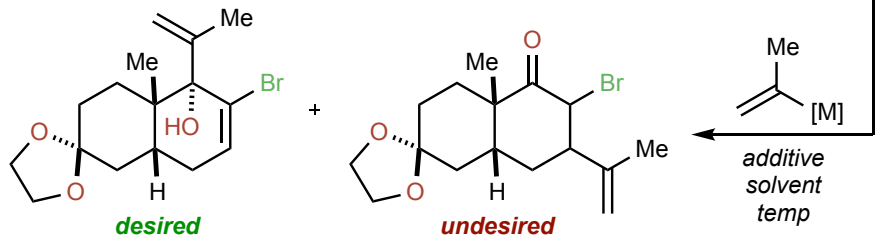


Part 3: Dubois – Decalin Synthesis

Building Block Synthesis:

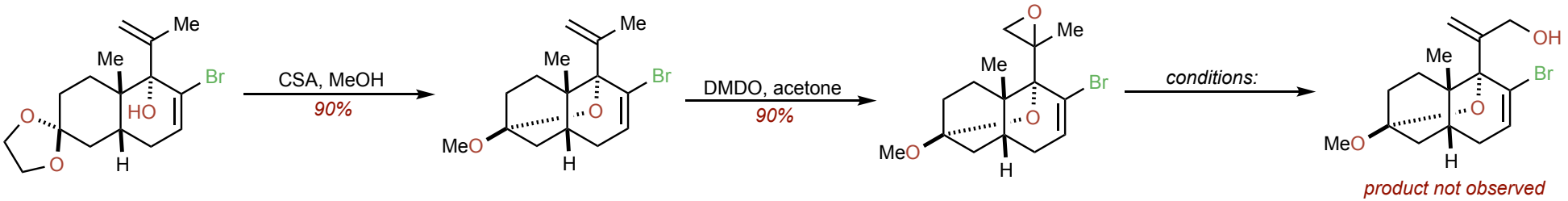


MgBr	-	THF	–78 °C to rt	-	:	-	-
MgBr	-	Et_2O	–78 °C to rt	-	:	-	-
MgBr	$LaCl_3$•2LiCl	THF	0 °C to rt	-	:	-	-
MgBr	$CeCl_3$•2LiCl	THF	–78 °C	1	:	1	35%
MgBr	$CeCl_3$	THF	–78 °C	0	:	1	-
Li	$CeCl_3$•2LiCl	THF	–78 °C to 0 °C	-	:	-	-
Li	-	THF	–78 °C	1	:	0	20%
Li	**LiBr**	**THF**	**–78 °C**	**1**	:	**0**	**31%**



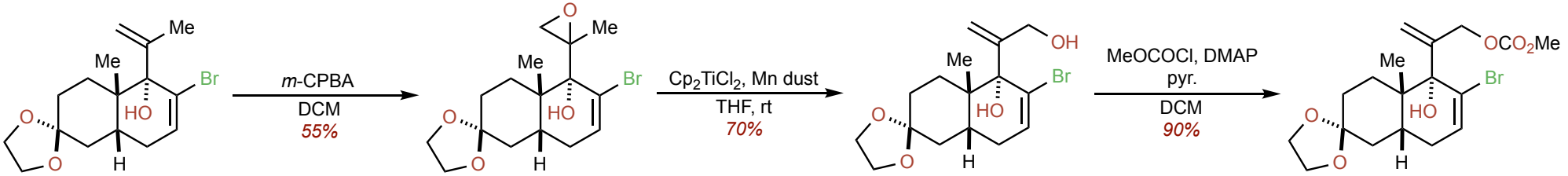
Part 3: Dubois – Allylic Electrophile Synthesis

Building Block Synthesis:

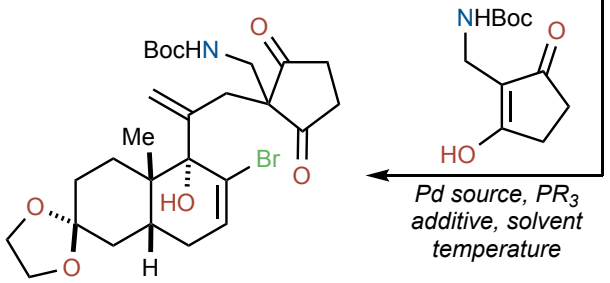


Part 3: Dubois – Allylic Electrophile Synthesis

Building Block Synthesis:

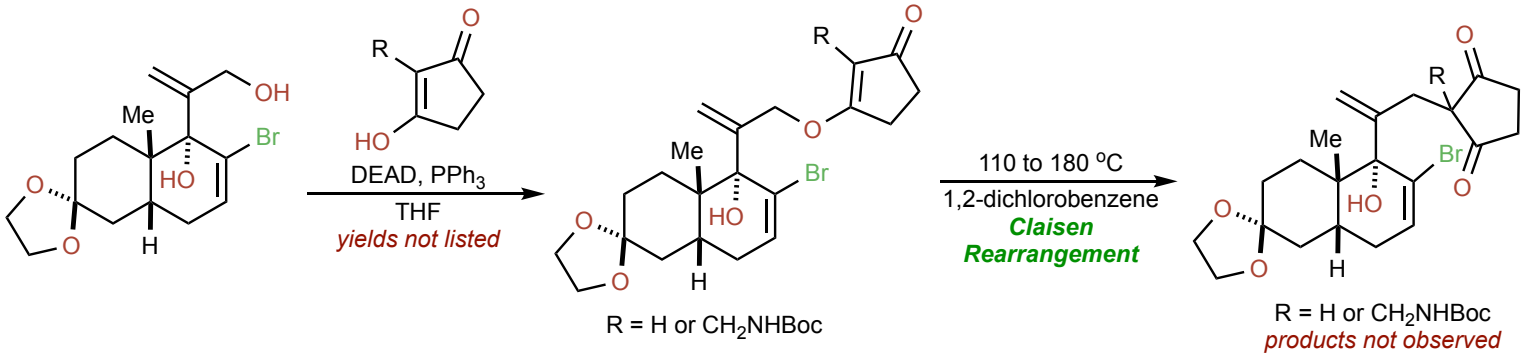


X	Pd Source	Phosphine	Additive	Solvent	Temp.	Yield
-OH	$Pd(OAc)_2$	PPh_3	Et_3B	THF	65 °C	N.R.
$-OCO_2Me$	$Pd_2(dba)_2 \cdot CHCl_3$	PPh_3	-	THF	65 °C	N.R.
$-OCO_2Me$	$Pd_2(dba)_2 \cdot CHCl_3$	Trost	-	THF	65 °C	N.R.
$-OCO_2Me$	$Pd_2(dba)_2 \cdot CHCl_3$	dppf	-	THF	65 °C	N.R.
$-OCO_2Me$	$Pd_2(dba)_2 \cdot CHCl_3$	Trost	-	dioxane	100 °C	N.R.
$-OCO_2Me$	$Pd_2(dba)_2 \cdot CHCl_3$	Trost	BSA/NaOAc	THF	65 °C	N.R.
-OAc	$Pd_2(dba)_2 \cdot CHCl_3$	Trost	BSA/NaOAc	THF	65 °C	N.R.
-OMs	$Pd_2(dba)_2 \cdot CHCl_3$	PPh_3	BSA/NaOAc	THF	65 °C	N.R.
-Br	$Pd_2(dba)_2 \cdot CHCl_3$	PPh_3	BSA/NaOAc	THF	65 °C	N.R.



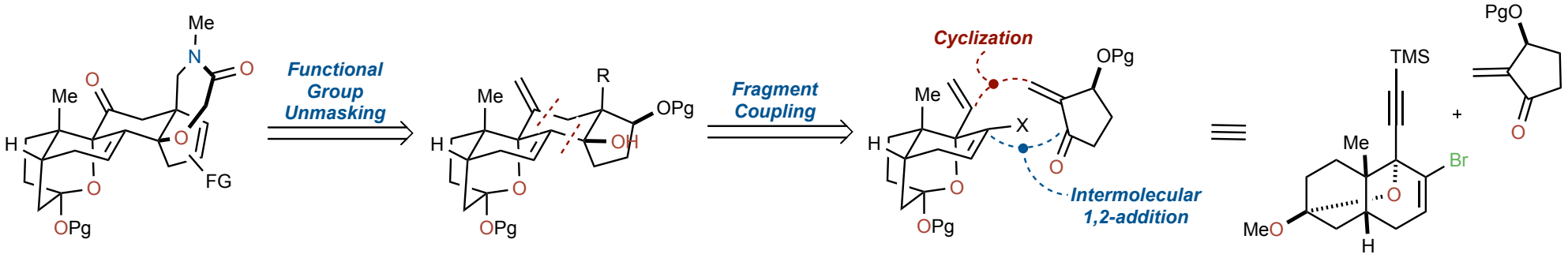
Part 3: Dubois – Desperate Measures

A last resort:



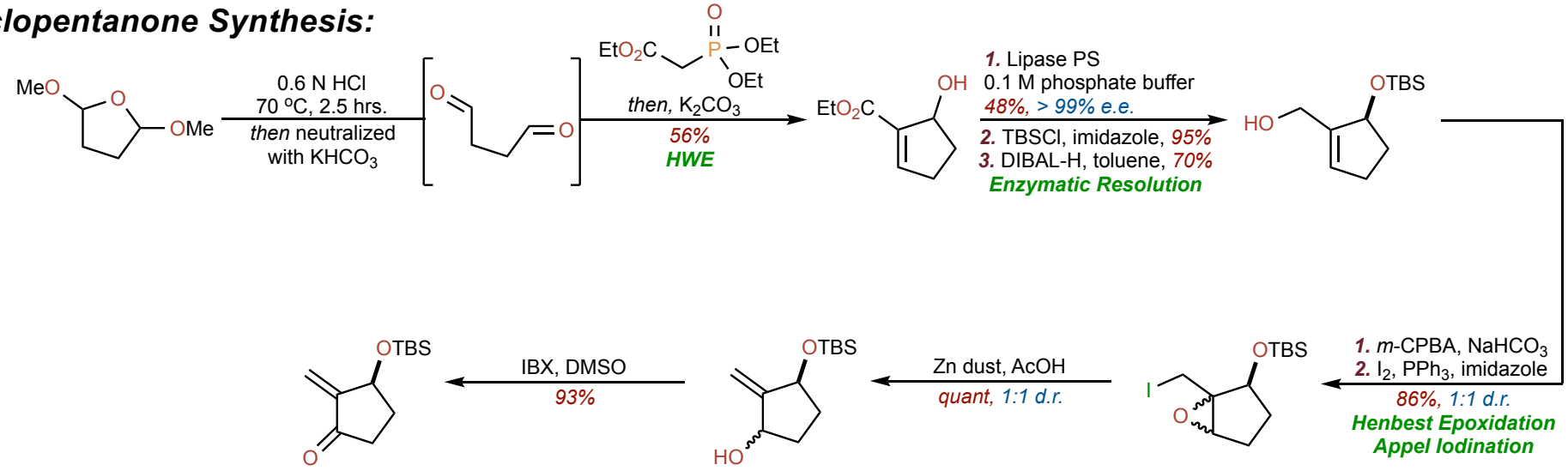
Electrophilic terminus despite being electronically activated is presumed to be too sterically encumbered for functionalization

2nd Generation Retrosynthesis:

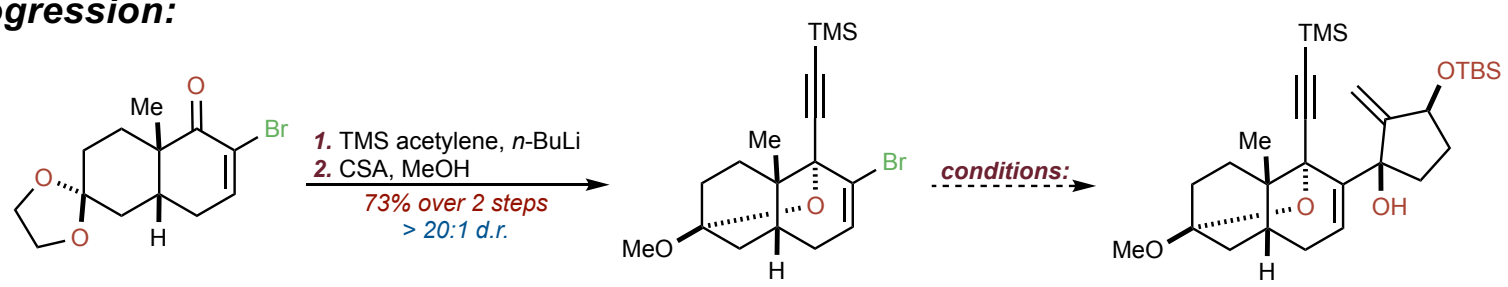


Part 3: Dubois – Building Block Synthesis

Cyclopentanone Synthesis:

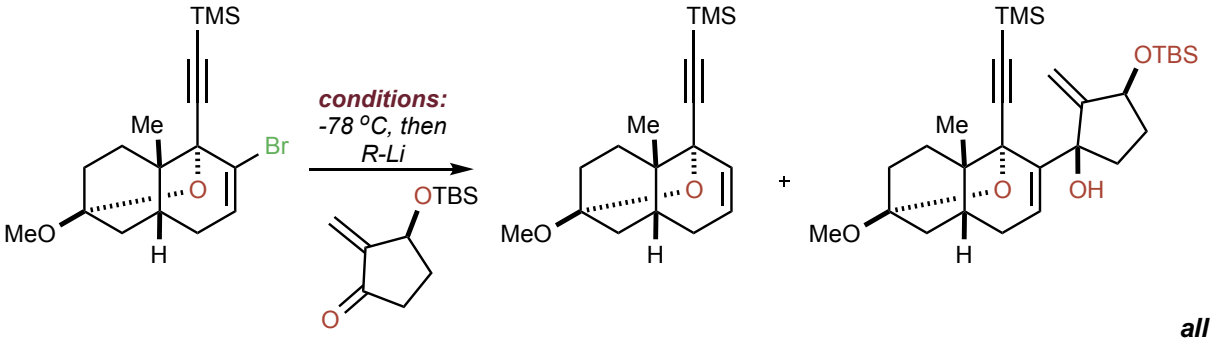


Decalin Progression:



Part 3: Dubois – Fragment Unification

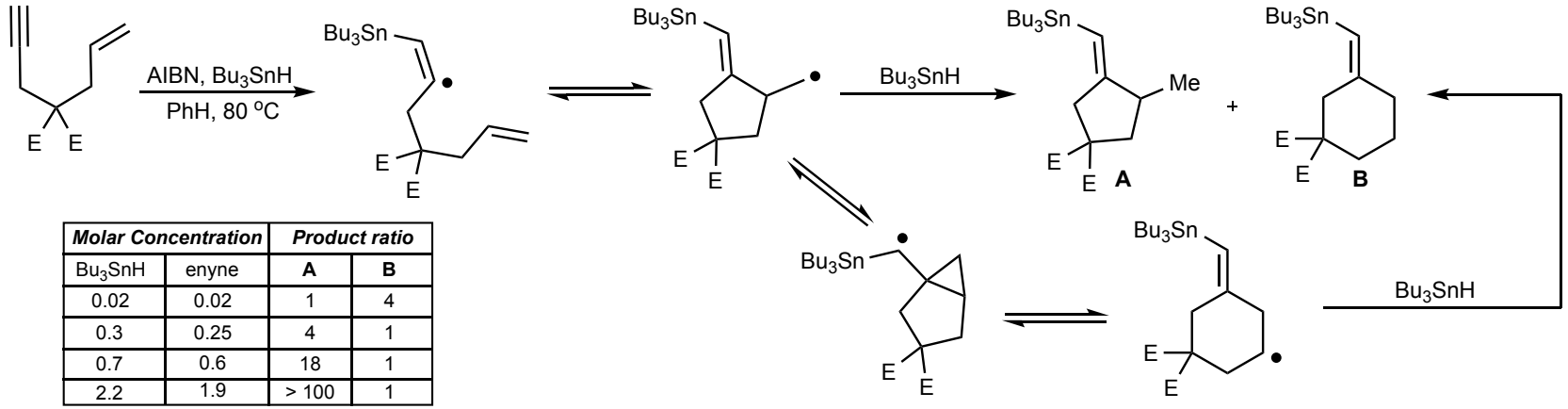
Investigation of 1,2-Addition:



-	THF	2 : 1	32%
-	Et_2O	1 : 0	0%
LiCl	THF	2 : 1	32%
LiBr	THF	2 : 1.5	40%
$MgBr•OEt_2$	THF	10 : 1	<5%
$ZnCl_2$	THF	1 : 0	0%
$ZnBr_2$	THF	5 : 1	<10%
$CeCl_3$	THF	5* : 2	<10%
$Yb(OTf)_3$	THF	1 : 0	0%
$CeCl_3•2LiCl$	THF	1.5* : 1	<10%
$LaCl_3•2LiCl$	THF	5* : 2	<10%
-	**THF**	**1 : 2.3**	**65%**

all entries are conducted with n-BuLi, except for the last one (t-BuLi).

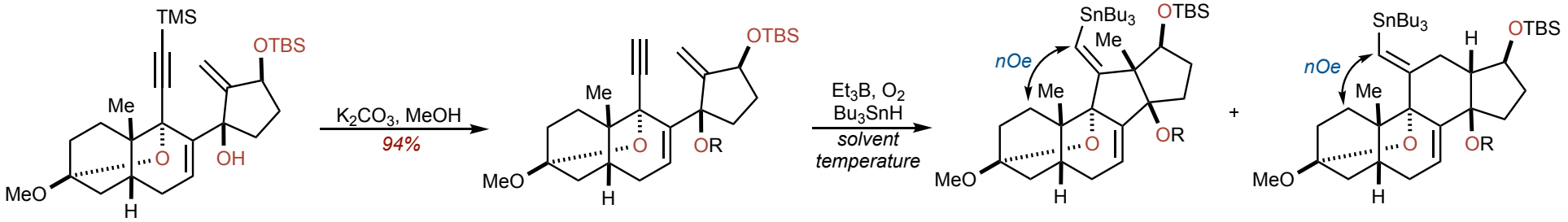
Unearthing Stork's Precedence:

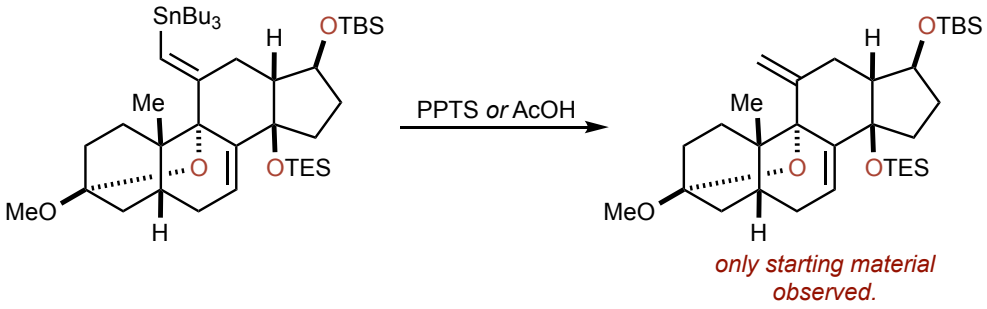


Molar Concentration		*Product ratio*	
Bu_3SnH	enyne	**A**	**B**
0.02	0.02	1	4
0.3	0.25	4	1
0.7	0.6	18	1
2.2	1.9	> 100	1

Part 3: Dubois – Radical Cyclization

Application of Stork's Precedent:



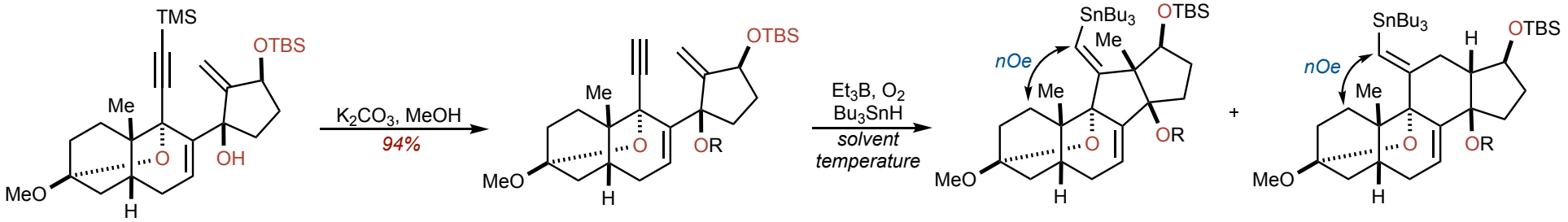


only starting material observed.

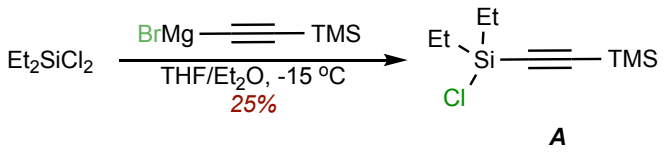
R	*Solvent*	*Temp (°C)*	*Ratio (A:B)*	
H	PhH	50	5	1
H	PhH	80	1	1
H	PhMe	110	1	1.2
H	xylenes	130	1	1.5
TMS	PhMe	110	1	3
TES	PhMe	110	1	9

Part 3: Dubois – Radical Cyclization

Application of Stork's Precedent:



Alkynyl Handle Synthesis:

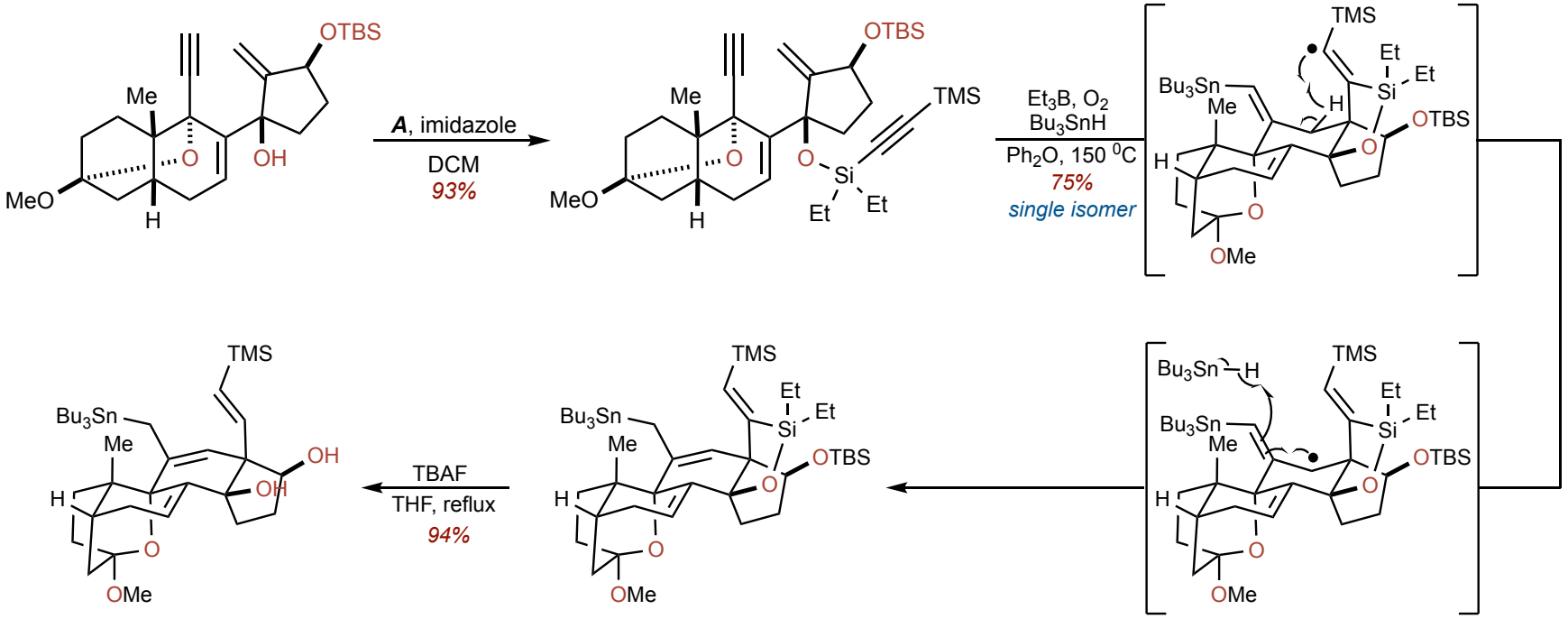


A

R	***Solvent***	***Temp (°C)***	***Ratio (A:B)***	
H	PhH	50	5	1
H	PhH	80	1	1
H	PhMe	110	1	1.2
H	xylenes	130	1	1.5
TMS	PhMe	110	1	3
TES	PhMe	110	1	9

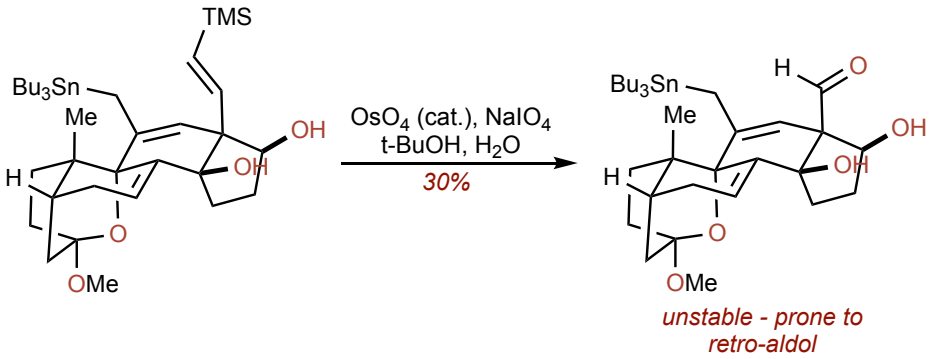
Part 3: Dubois – C-Ring Development

Implementation of the Alkynyl Handle:



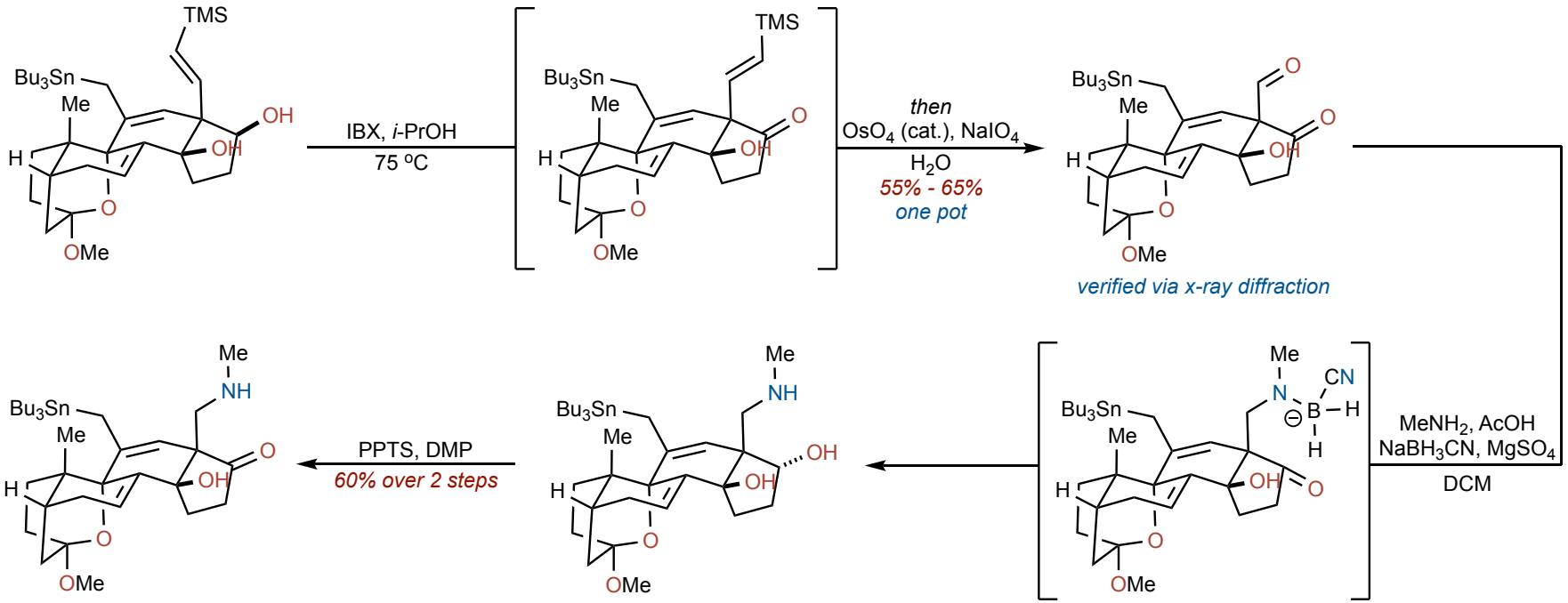
Part 3: Dubois – Redox Phase

Redox Choreography:



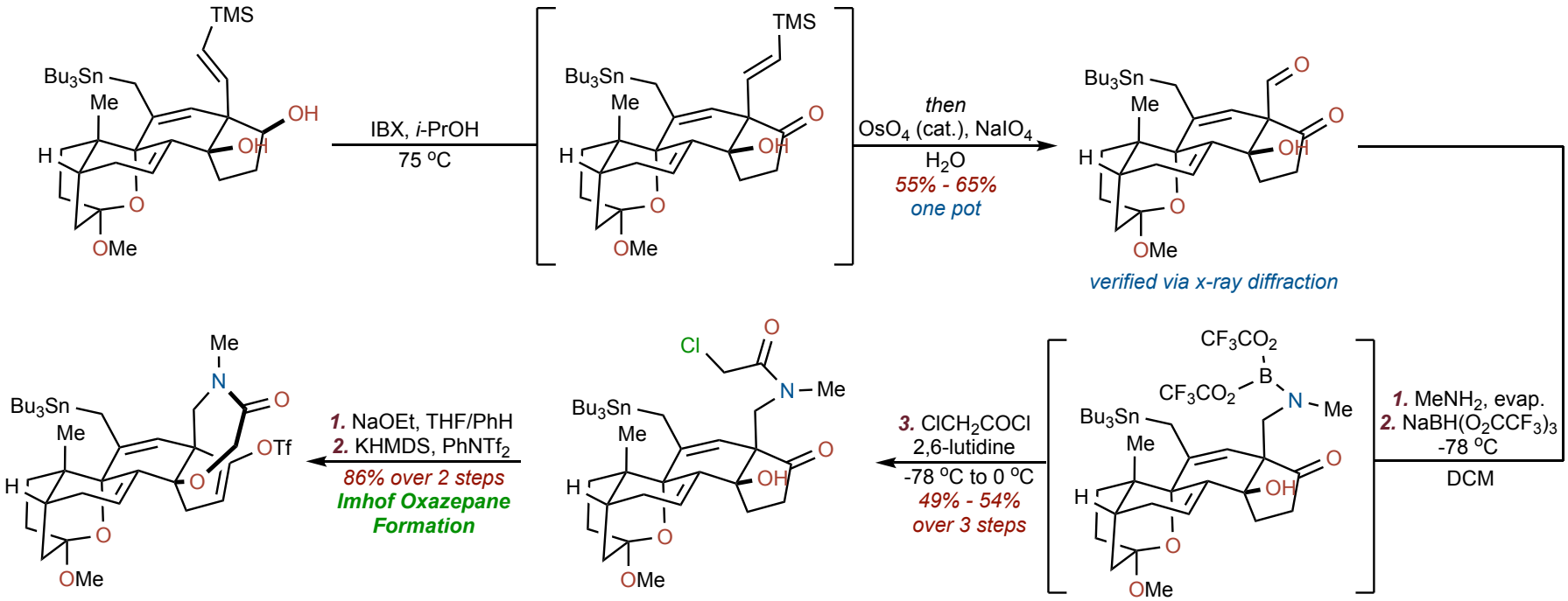
Part 3: Dubois – Redox Phase

Redox Choreography:



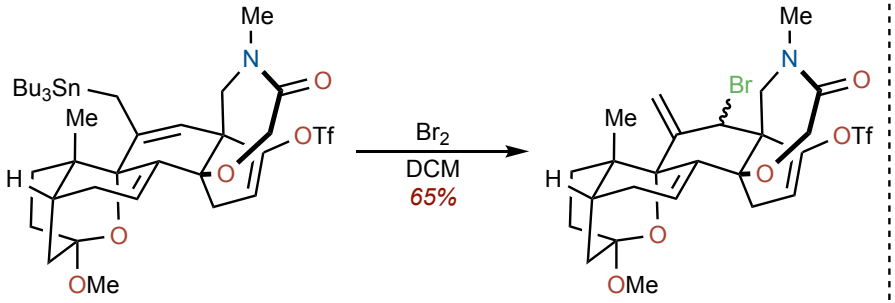
Part 3: Dubois – Redox Phase and Oxazepane Formation

Redox Choreography:

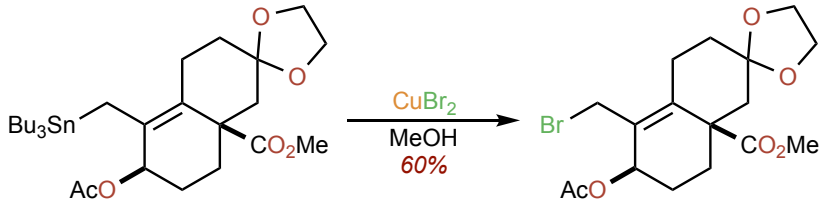


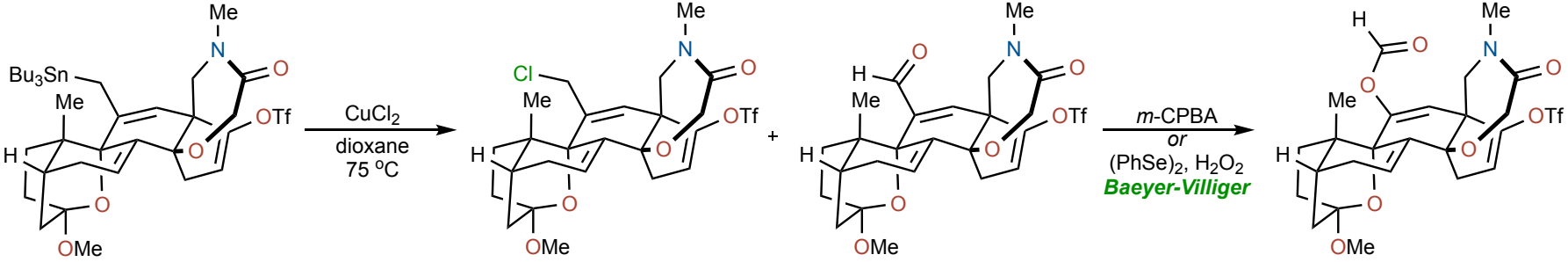
Part 3: Dubois – Destannylation

Oxidative Maneuvers:



Fuchs:





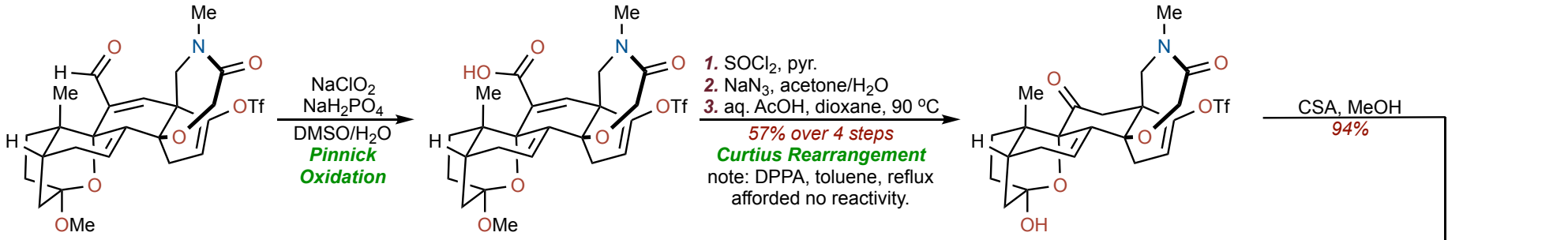
Modification	A	B
distilled dioxane	1.4	1
O_2 sparged dioxane	0	1

89% yield under optimized conditions

only recovered starting material was observed

Part 3: Dubois – Final Maneuvers

Demethylation and C11 Reduction:

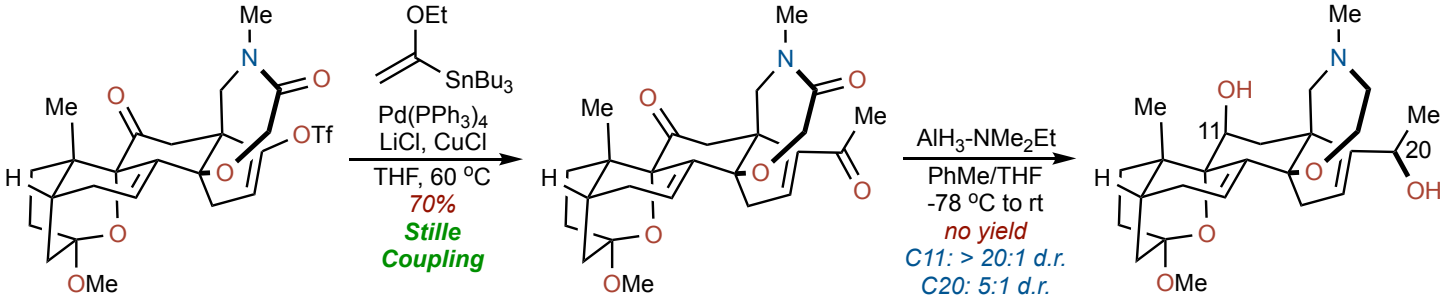


$NaBH_4$	MeOH	–78 to rt	1 : 0
$NaBH_4/CeCl_3$	MeOH	–78 to rt	1 : 0
$NH_3•BH_3$	THF	–20 to 60	1 : 0
$Zn(BH_4)_2$	THF	–78 to rt	no reaction
$LiBHEt_3$	THF	–78	decomp.
$LiBHs\text{-}Bu_3$	THF	–78 to rt	decomp.
(*S*)-Me-CBS	CH_2Cl_2	–20 to rt	1 : 0
SmI_2	THF	0 to rt	decomp.
SmI_2/1,4 cyclohexadiene	THF	–78 to 0	decomp.
Me_2AlCl/*i*-PrOH	PhMe	rt to 80	no reaction
$AlH_3•NMe_2Et$	PhMe	–78	decomp.

product not observed. undesired selectivity afforded in all cases.

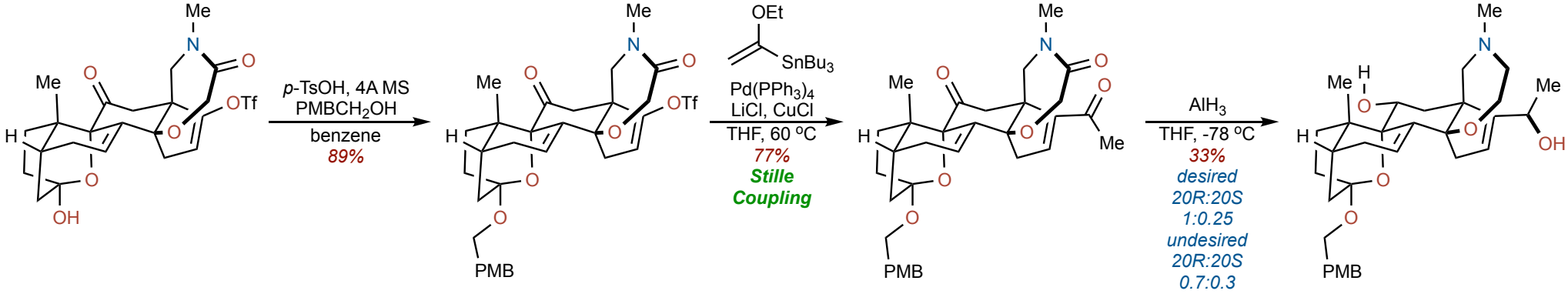
Part 3: Dubois – Final Maneuvers

Side-Chain Installation and Global Reduction:



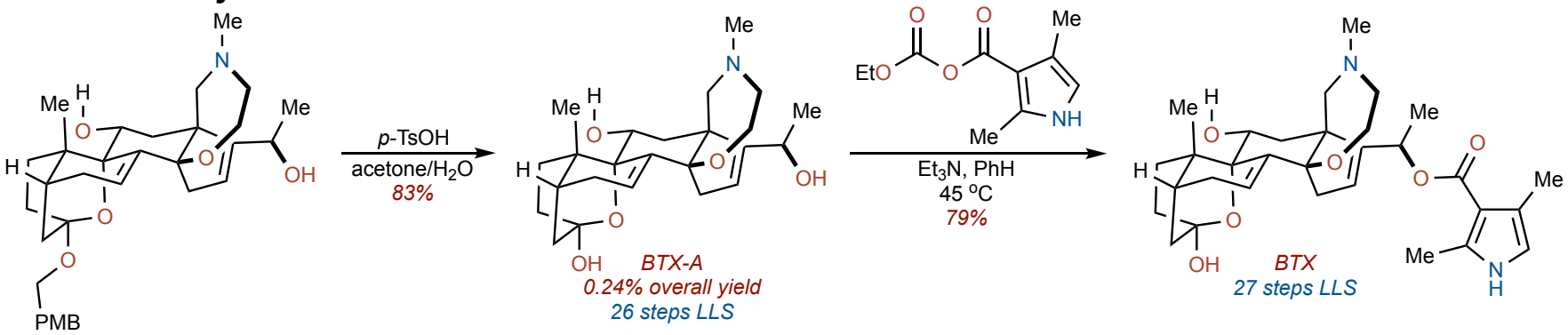
Despite chain extension, no C11 selectivity was observed and the diastereomeric mixture proved resistant to separation.

Installation of auxillary and reduction:

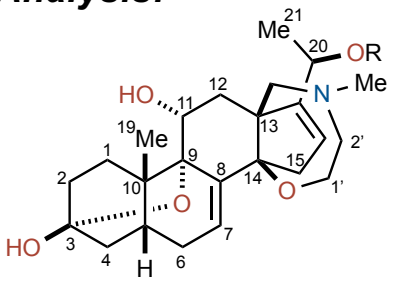


Part 3: Dubois – Final Maneuvers

Deprotection and Acylation:



Analysis:



26 LLS with
12 concession steps

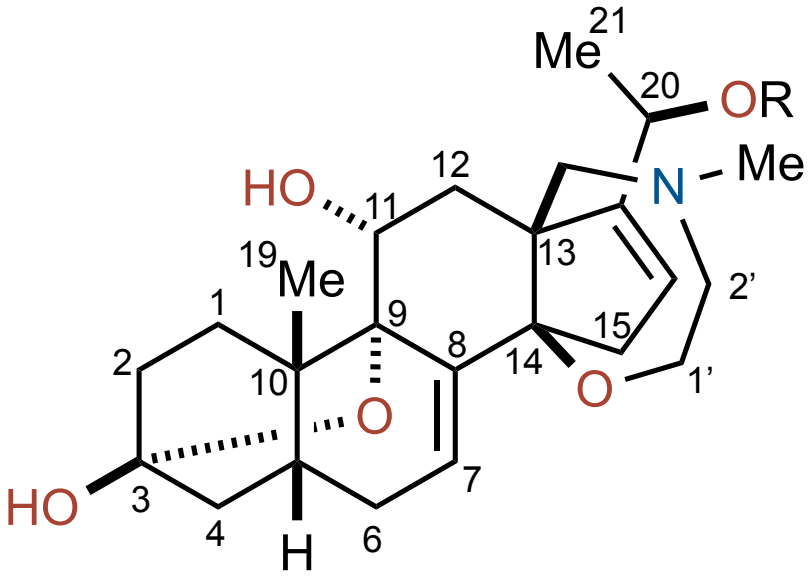
Lessons Learned (Brief Summary):

1. *Establishment of the C7-C8 olefin early in the synthesis dramatically improves efficiency.*
2. *Formation of the C12-C13 bond is difficult to steric constraints.*
3. *Direct construction of C8-C14 bond and the tertiary hydroxyl with correct stereochemistry dramatically decreases the need for redox maneuvers.*
4. *Demethylation via Curtius cost a price of 4 concession steps.*
5. *C11-ketone is highly hindered and difficult to reduce with the correct stereochemical outcome (substrate-structure dependence).*

Advancements:

Direct 1-2 addition of vinyl bromide to cyclopentone, engineered radical cyclization cascade, tandem reductive amination/acylation sequence, and stereoselective reduction for C20 with alane.

Comparison and Summary



Kishi:

- Intramolecular Diels-Alder provided the desired skeleton and C11 stereochemistry, but introduced unnecessary redox fluctuations via epoxidation, hydrogenolysis of a triflate, and introduction of a C6 ketone.
- Despite this, the route is high-yielding for a 49-step sequence.

Dubois:

- Highly convergent and direct approach between two optimized building block.
- Radical cyclization cascade established both the C11-C12 and angular C13 bonds rapidly.
- Unfortunately, 4 concession steps were introduced via demethylation, C3 protection with an auxiliary, and non-stereoselective reduction of C11.

Questions?

References

Kishi Synthesis:
***Tetrahedron Lett. 1994*, 35, 8333-8336.**
***Tetrahedron Lett. 1994*, 35, 8337-8340.**
***J. Am. Chem. Soc. 1998*, 120, 6627-6628.**

Dubois Synthesis:
***Science. 2016*, 354, 865-869.**
Matthew Logan Thesis:
https://stacks.stanford.edu/file/druid:ws244zb5741/thesis_logan-augmented.pdf

For more recent total syntheses regarding BTX see:
Tuoping Luo: *J. Am. Chem. Soc. 2020*, 142, 3675-3679.
https://doi.org/10.1021/jacs.9b12882
Masayuki Inoue: *Angew. Chem. Int. Ed. 2023*, 62, e202309688.
https://doi.org/10.1002/anie.202309688