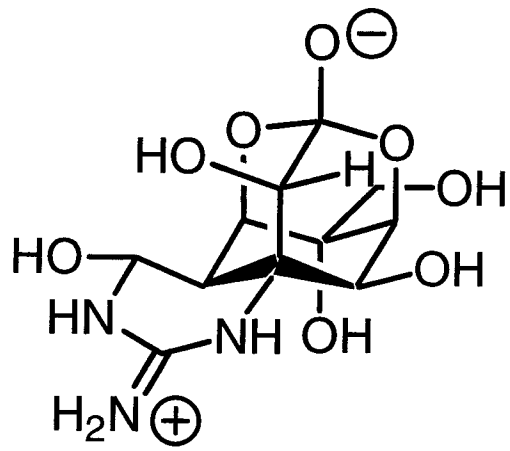
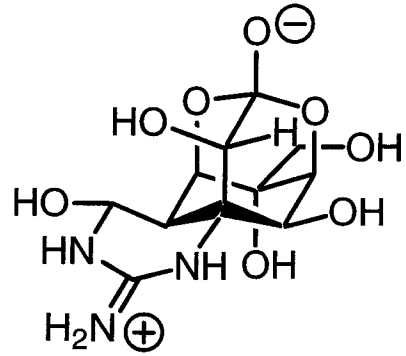
The Total Synthesis of Tetrodotoxin



John R. Heemstra Jr.
10/28/03

Tetrodotoxin: Background

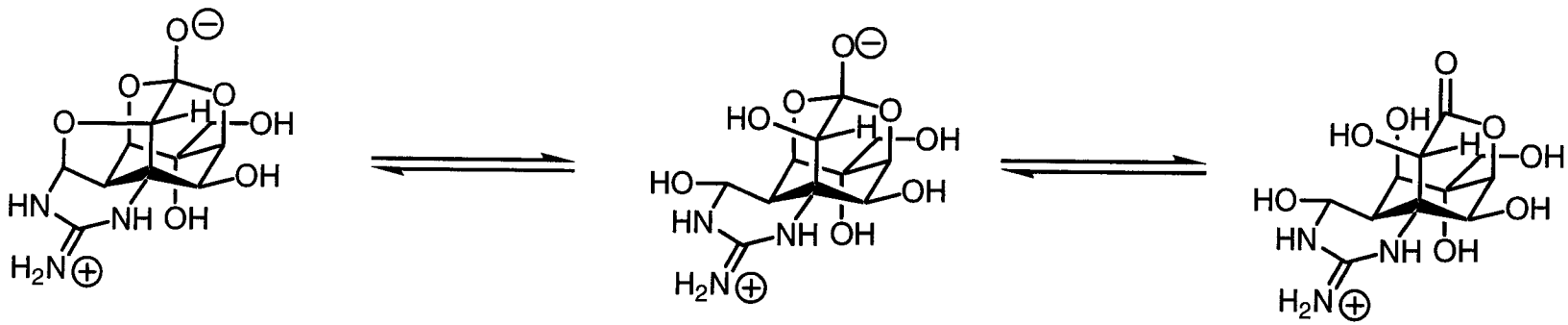


Certain varieties of puffer fish, especially the *tora fugu*, or tiger puffer (*S. rubripes*) and the closely related *ma fugu*, or common puffer (*S. porphyreus*), are highly prized as comestibles in Japan. The indulgence of the taste is fraught with some peril, since the livers and ovaries of the fish contain a powerful poison. The presence of this poison has been known through its effects since antiquity, but its labile nature and its extremely low concentration in its natural milieu made the isolation of the toxic principle extraordinarily difficult. Yokoo first succeeded in isolating the crystalline poisonous principle-now known as tetrodotoxin-on fourteen years ago (1950), and shortly thereafter Tsuda and Kawamura independently achieved its isolation in its pure state.

R. B. Woodward

Woodward, R. B. *Pure. Appl. Chem.* **1964**, *9*, 49-74

Tetrodotoxin: Physical Characteristics



4,9-anhydo-Tetrodotoxin **Tetrodotoxin**

- White crystalline solid
- Darkens above 220 °C, without melting
- Insoluble in all solvents except acids
- Weak base (pKa = 8.7)
- Exhibits no UV spectrum
- One of the most toxic compounds among poisons with low MW.
- Toxicity due to the blockage of sodium ion influx through sodium channel protiens.

Isolation Procedure

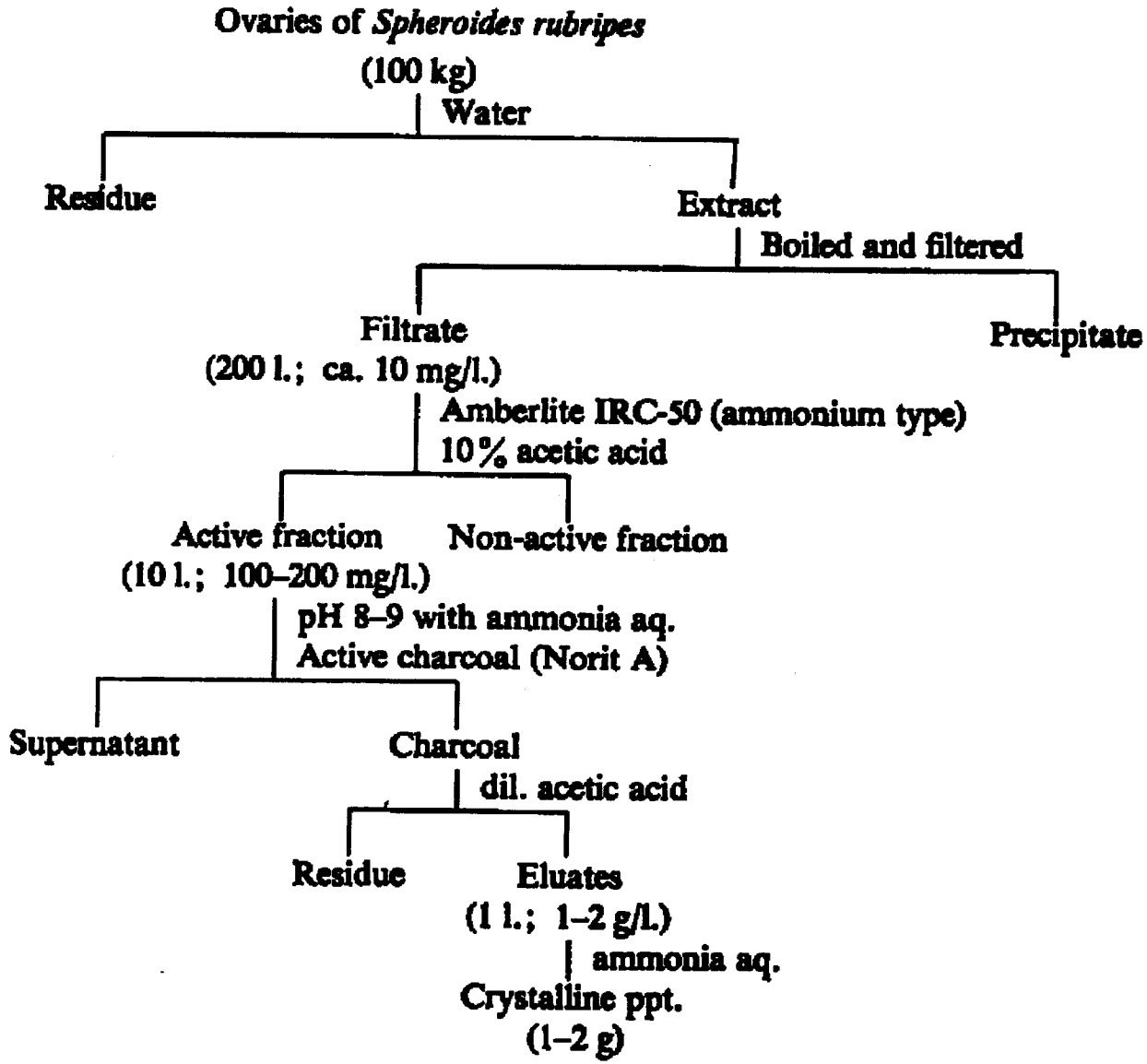
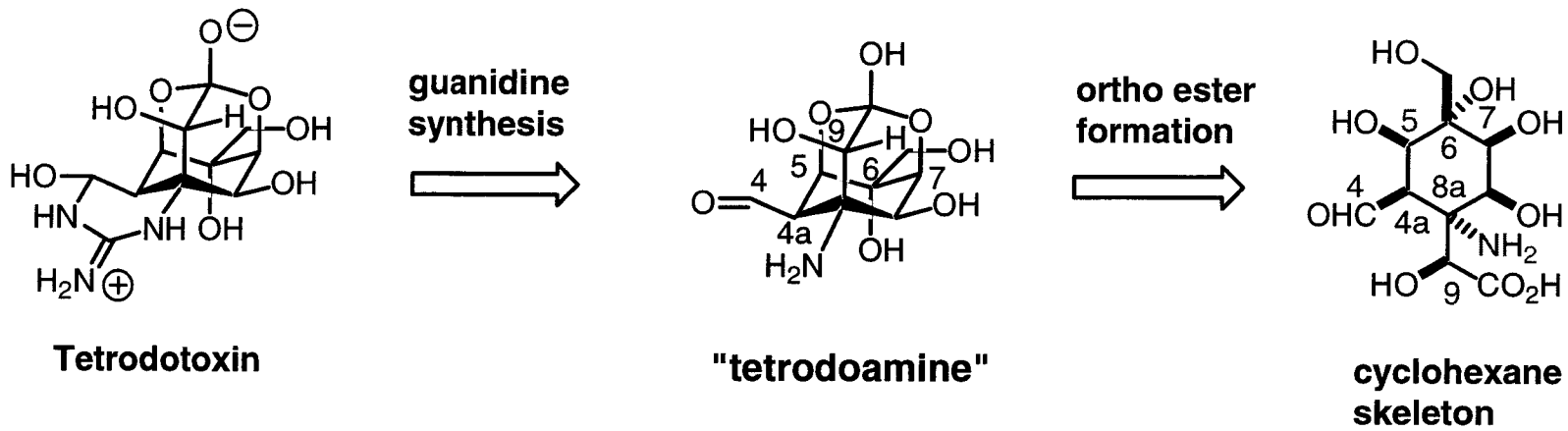


FIG. 1. Diagram of the isolation procedure.

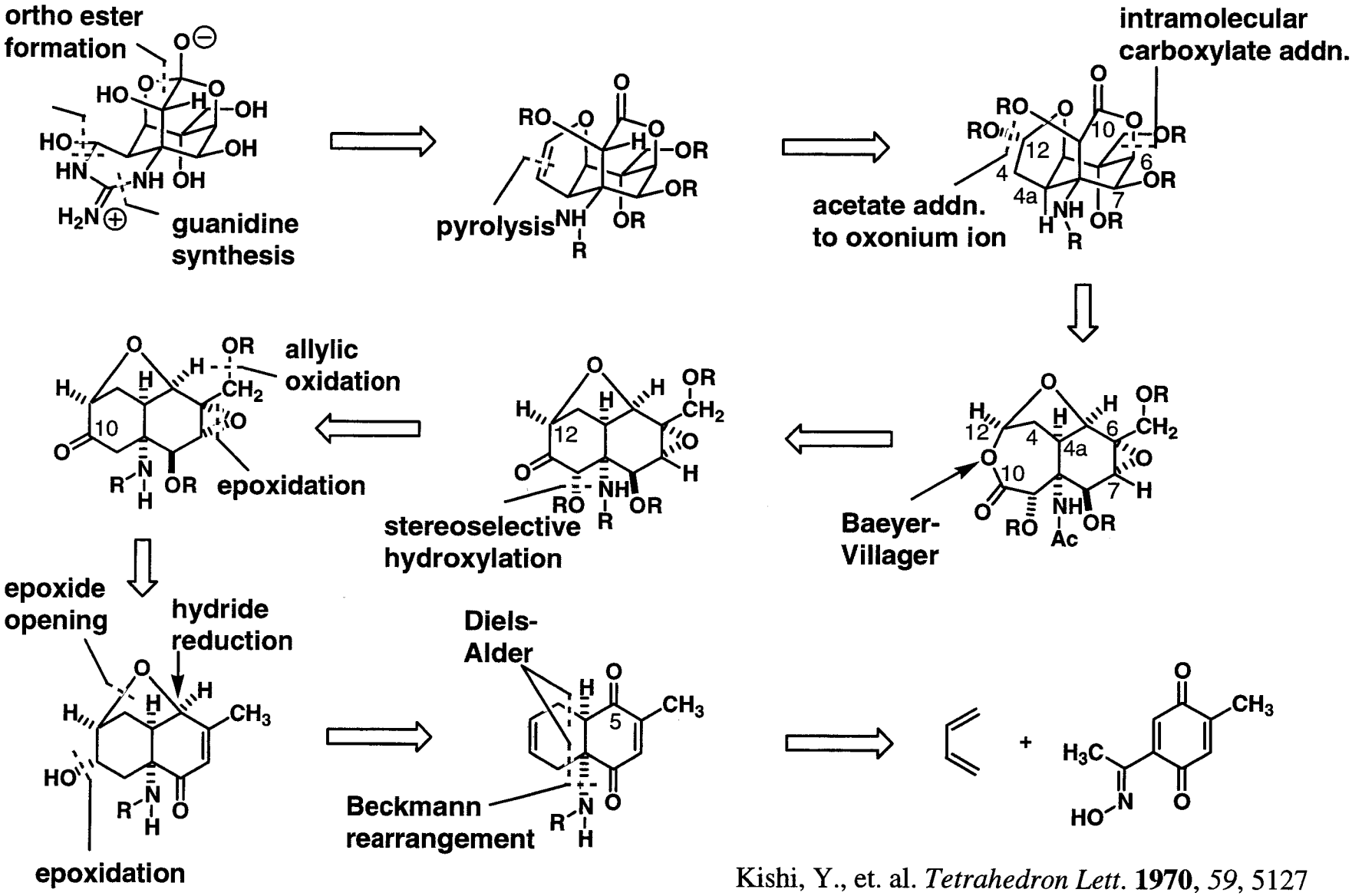
General Retrosynthetic Analysis



Key Synthetic Challenges:

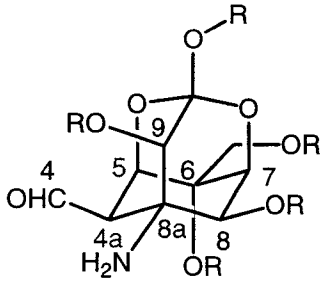
- Selection of acid labile protecting groups due to base senstivity of TTX
- Construction of the tetrasubstituted stereocenters C6 and C8a
- Introduction of the C8a amine
- Preventing epimerization at C9 and β-elimination of the hydroxyl at C5
- Determination of stereochemistry using coupling constants of vicinal hydrogens

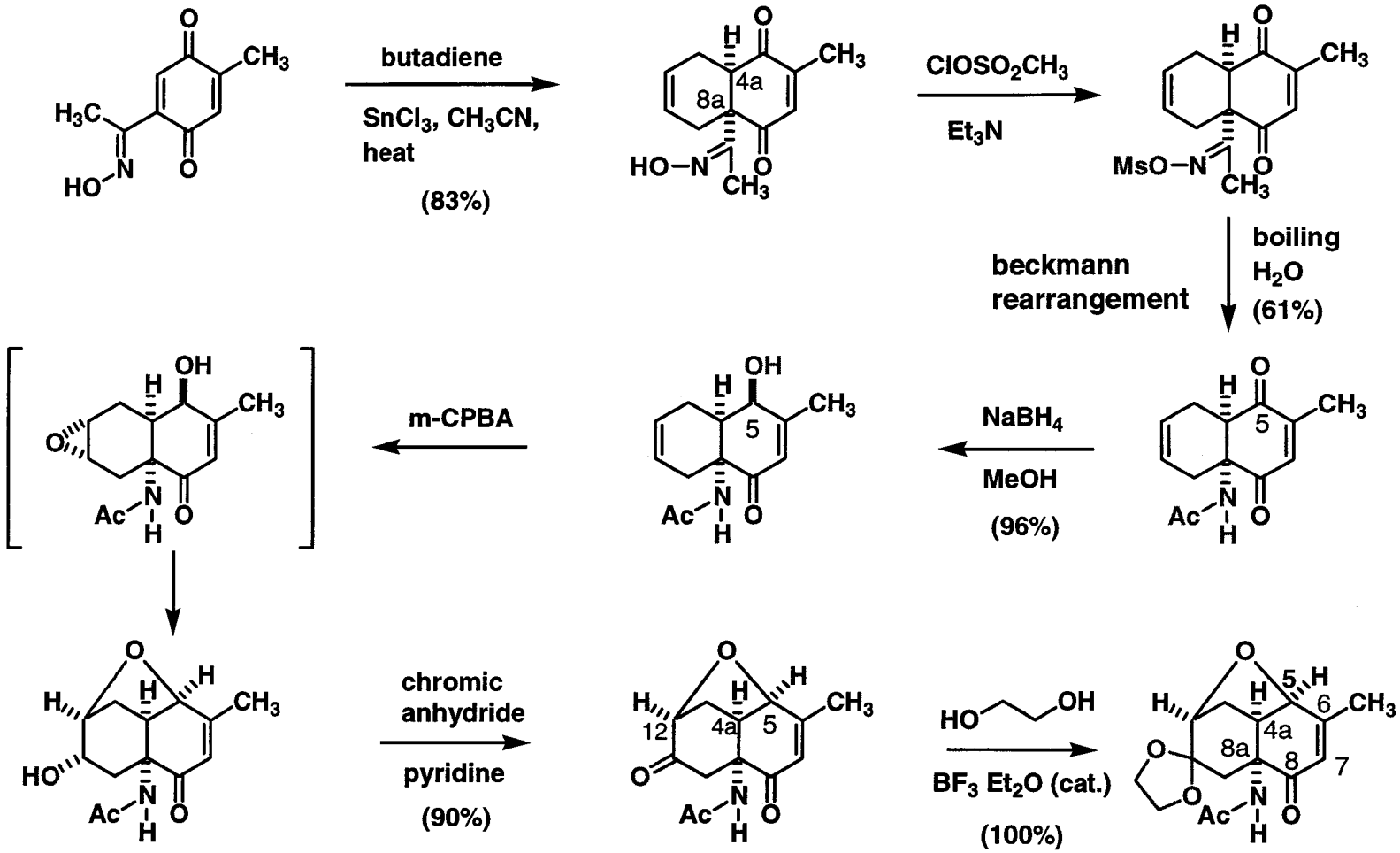
Kishi's Retrosynthetic Analysis - Racemic



Kishi, Y., et. al. *Tetrahedron Lett.* **1970**, *59*, 5127
Kishi, Y., et. al. *Tetrahedron Lett.* **1970**, *59*, 5129
Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9217
Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9219

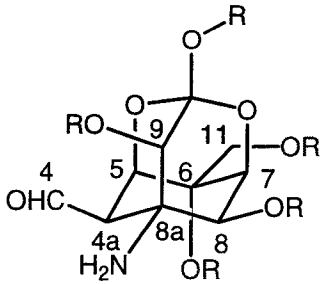
Synthesis of the Cyclohexane Skeleton: Diels-Alder and Beckmann Rearrangement

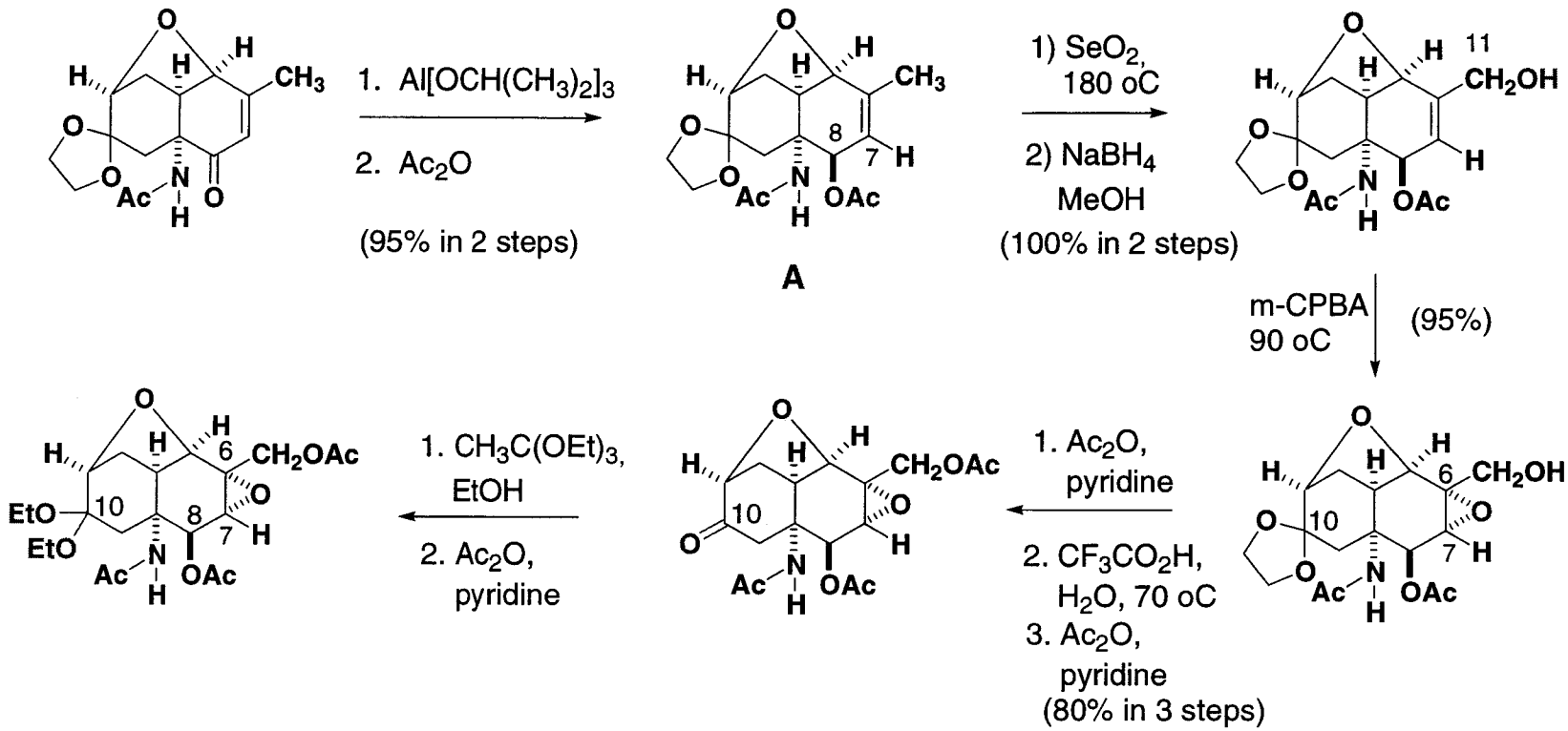




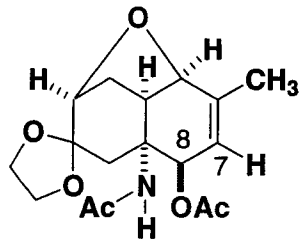
Kishi, Y., et. al. *Tetrahedron Lett.* **1970**, *59*, 5127
Kishi, Y., et. al. *Tetrahedron Lett.* **1970**, *59*, 5129

Synthesis of the Cyclohexane Skeleton: Installation of the C8 Stereocenter and Introduction of the C11 Alcohol

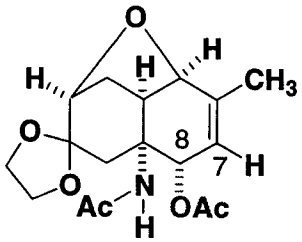




A



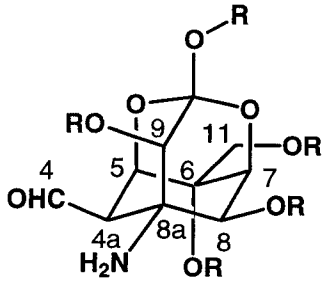
C(8)H and C(7)H diheral angle 90 J = 0 Hz

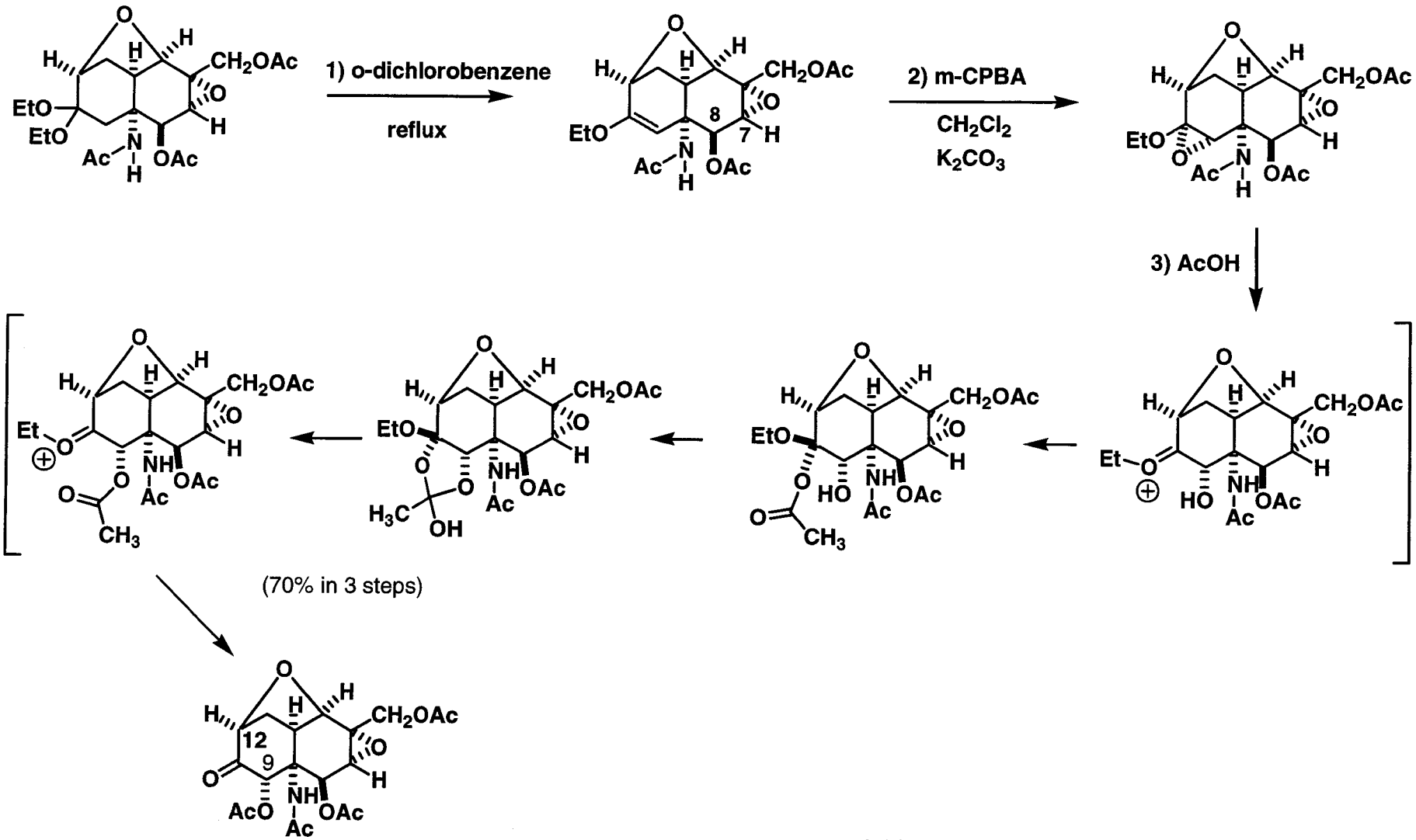


C(8)H and C(7)H diheral angle 30 J = 6 Hz

Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9217

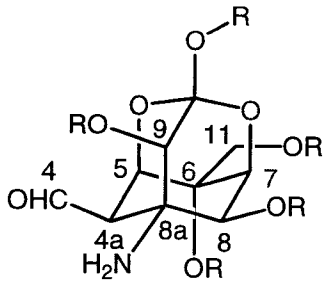
Synthesis of the Cyclohexane Skeleton: Introduction of the C9 Hydroxyl

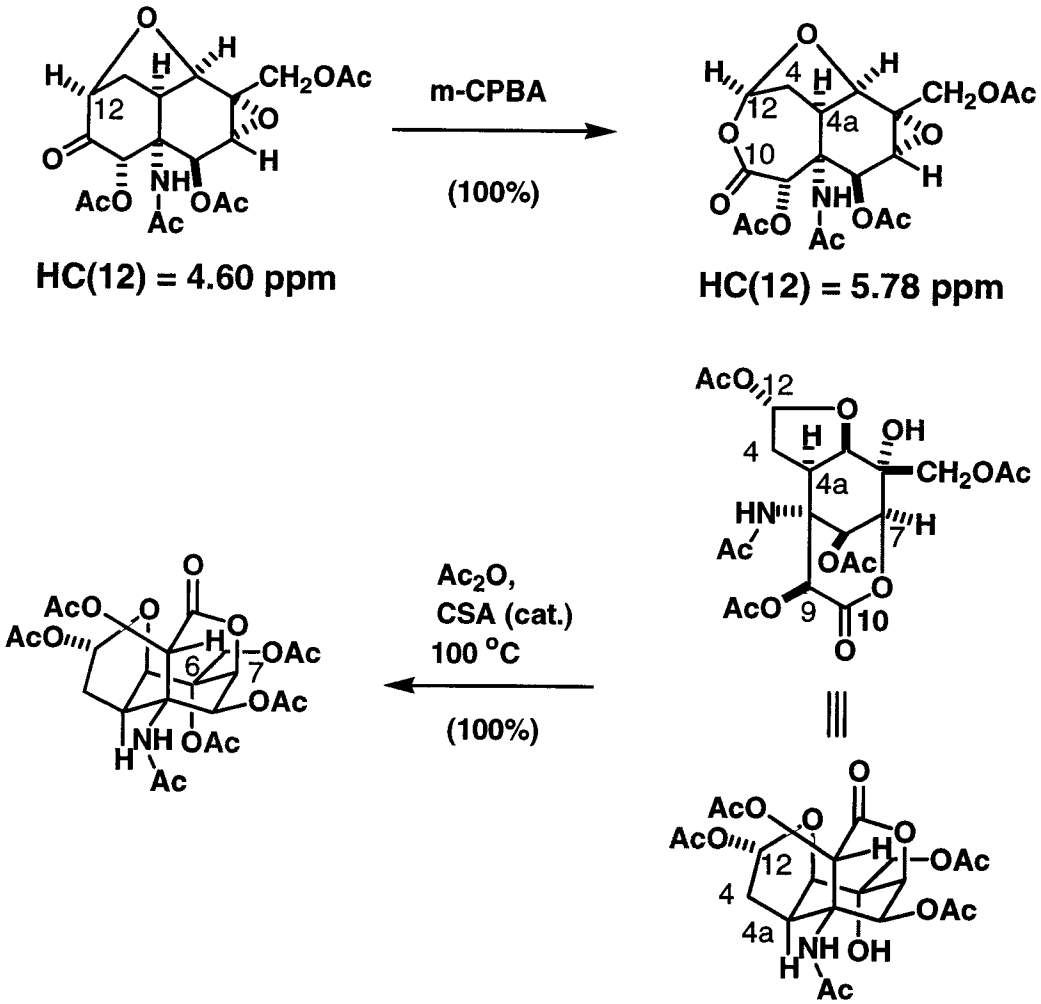




Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9217

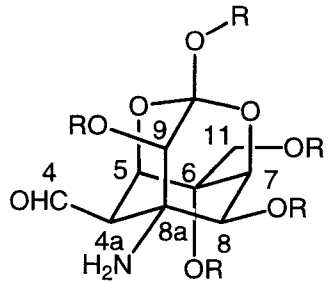
Stereoselective Synthesis of the Lactone Ring

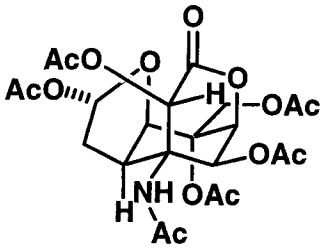




Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9217

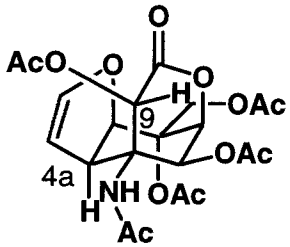
Synthesis of the Guanidine Moiety





290-300 °C

high vacuum



A

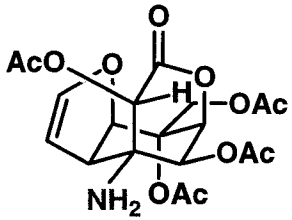
1) Et_3O BF_4

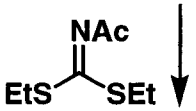
Na_2CO_3

then $AcOH/H_2O$

work-up

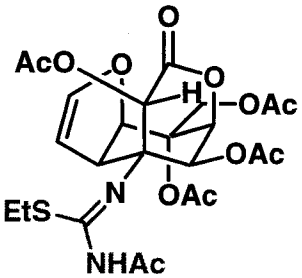
(92%)





120 oC

12 h

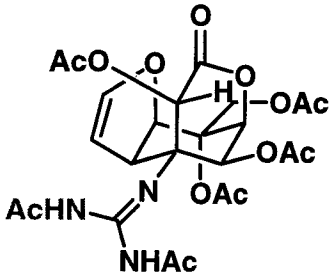


$AcNH_2$

150 oC

60 min

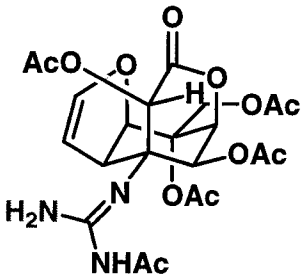
(20% 2 steps)



NH_3,

$MeOH/CH_2Cl_2$

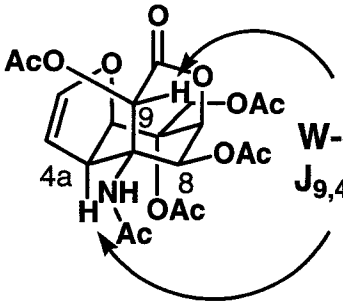
(100%)



+ $BF_3 \cdot OEt_2$

Determination of C9 configuration

A



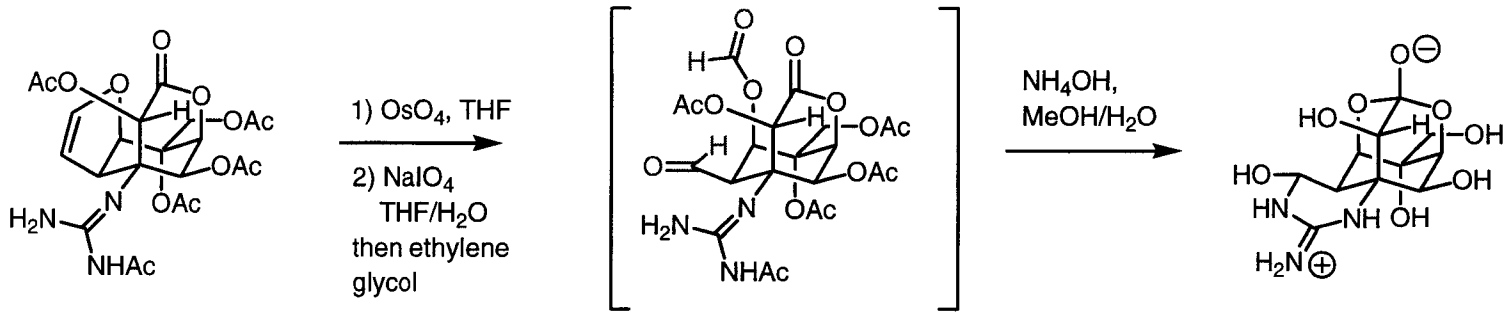
W-coupling

$J_{9,4a} = 1$ Hz

$J_{9,8} = 0$ Hz

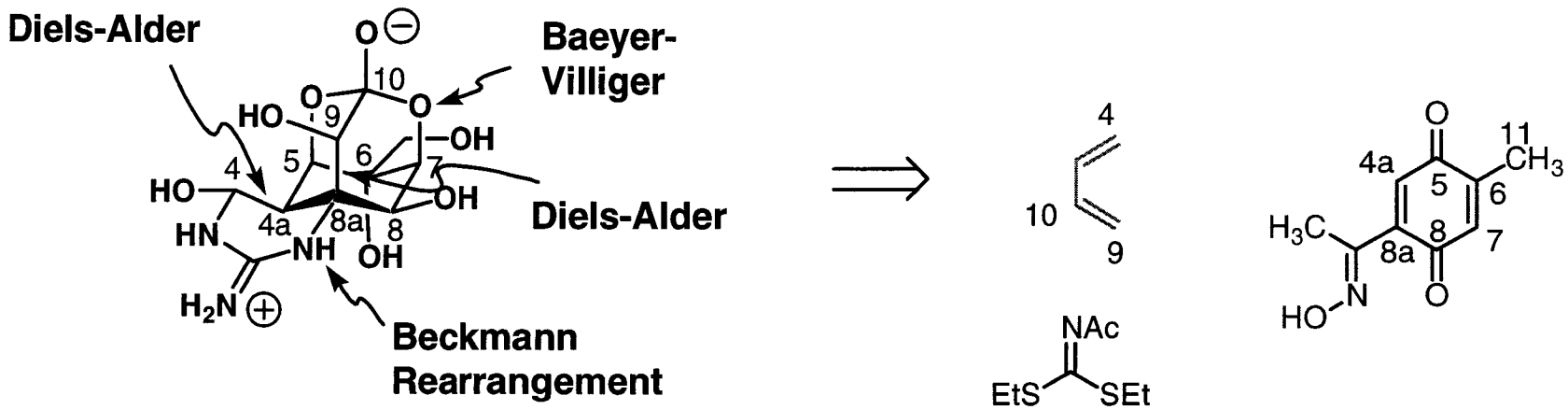
Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9219

Completion of the Synthesis of DL-Tetrodotoxin



Kishi, Y., et. al. *J. Am Chem. Soc.* **1972**, *94*, 9219

Summary of Kishi's Total Synthesis

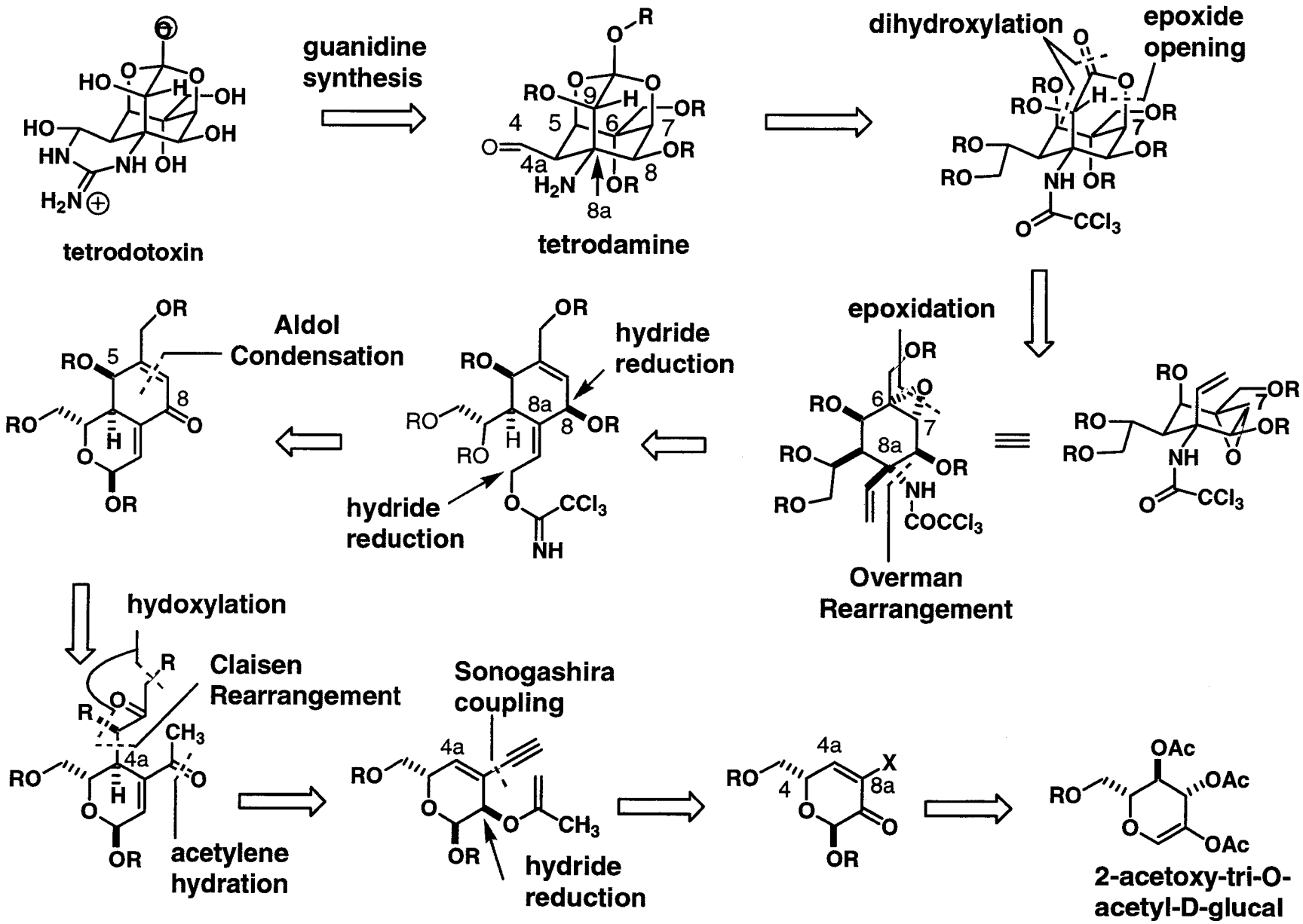


32 Total steps

C8a and C4a: diastereoselective Diels-Alder rxn.
C8 and C5: stereospecific, substrate controlled hydride reductions.
C6: stereospecific, substrate controlled epoxidation
C7: carboxylate attack onto epoxide
C9: stereospecific, substrate controlled epoxidation of enol ether

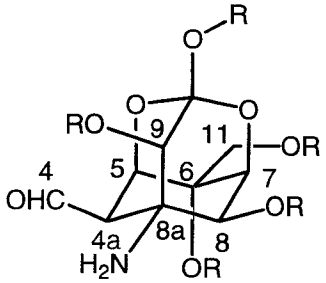
- Use of an unusual Diels-Alder dienophile containing an oxime.
- High degree of substrate control in creating stereocenters
- Development of a novel procedure to synthesize a guanidine moiety

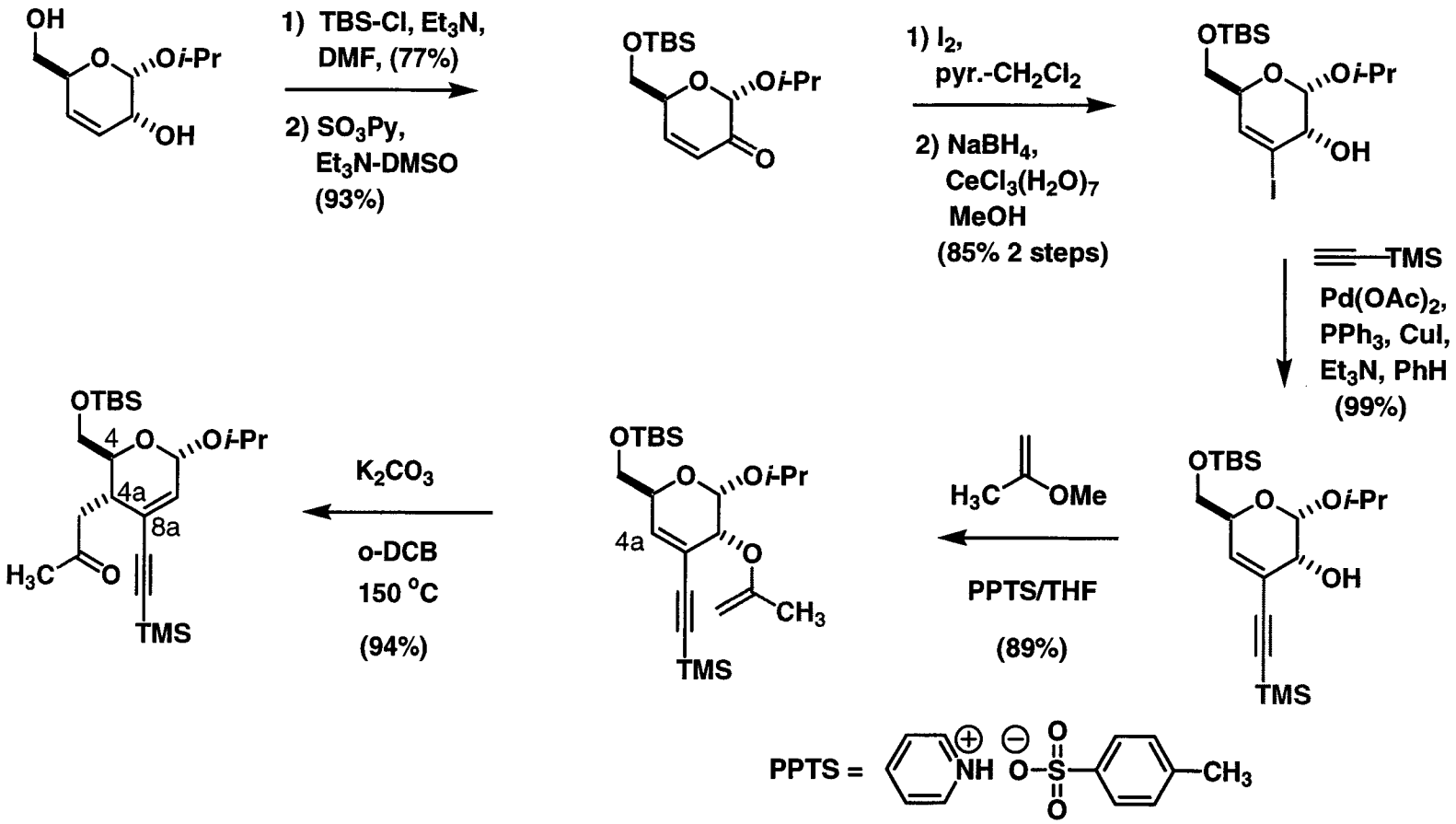
Isobe's Retrosynthetic Analysis - Asymmetric



Isobe, M., Ohyabu, T, Nishikawa, T. *J. Am Chem. Soc.* **2003**, *125*, 8798

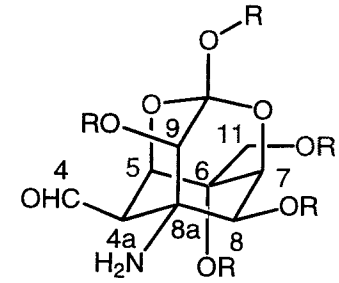
Synthesis of the Cyclohexane Skeleton: Sonogashira Coupling and Claisen Rearrangement

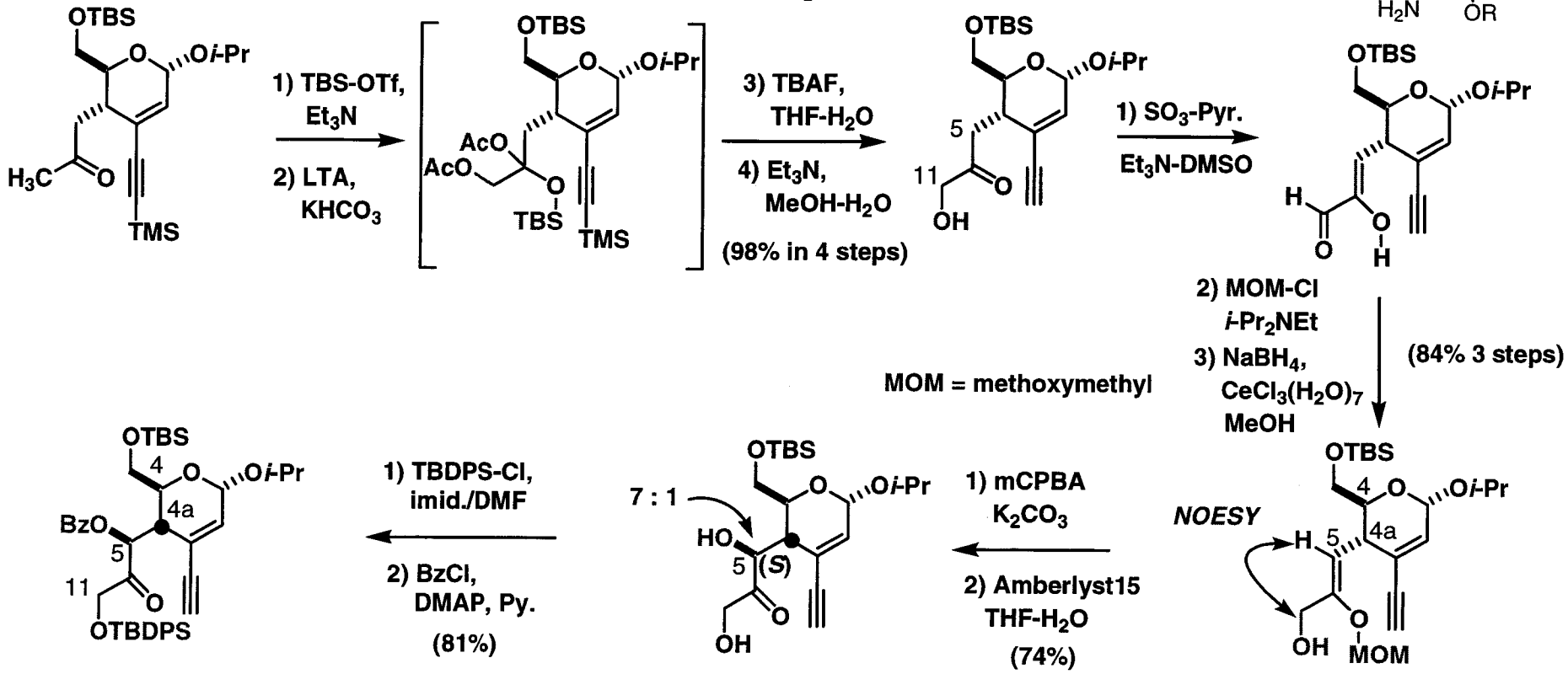




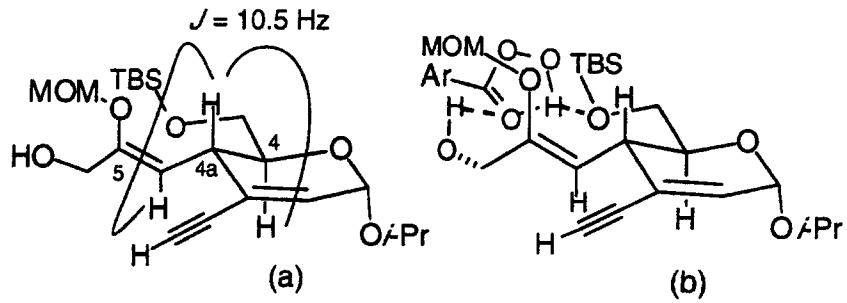
Isobe, M., Ohyabu, T, Nishikawa, T. *J. Am Chem. Soc.* **2003**, *125*, 8798

Synthesis of the Cyclohexane Skeleton: Introduction of the C5 and C11 Hydroxyl Groups



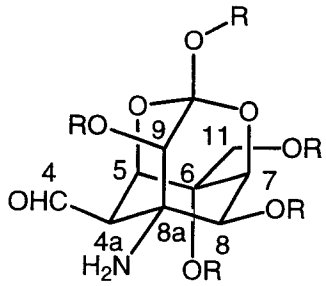


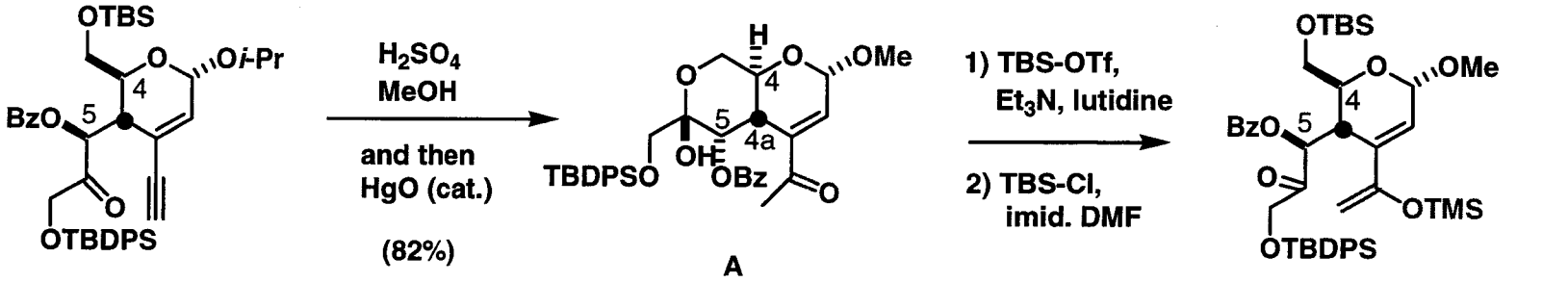
Conformation of A and possible Interactions with m-CPBA

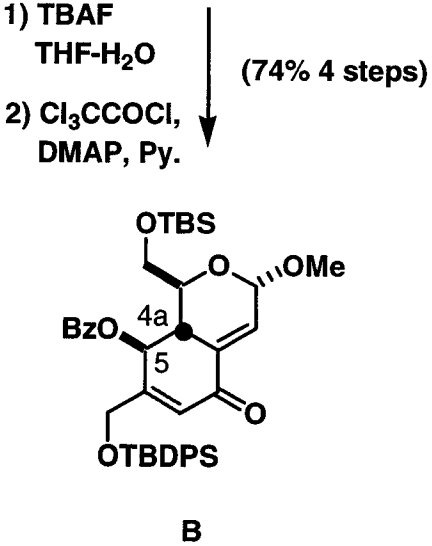


Isobe, M., Ohyabu, T, Nishikawa, T. *J. Am Chem. Soc.* **2003**, *125*, 8798

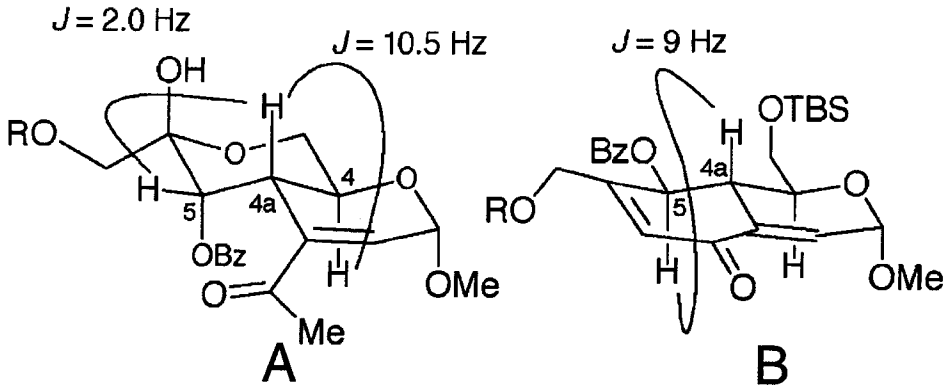
Synthesis of the Cyclohexane Skeleton: Acetylene Hydration and Intramolecular Aldol Condensation







Proof of the Configuration at the C-5 position



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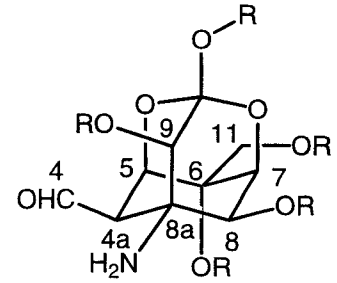
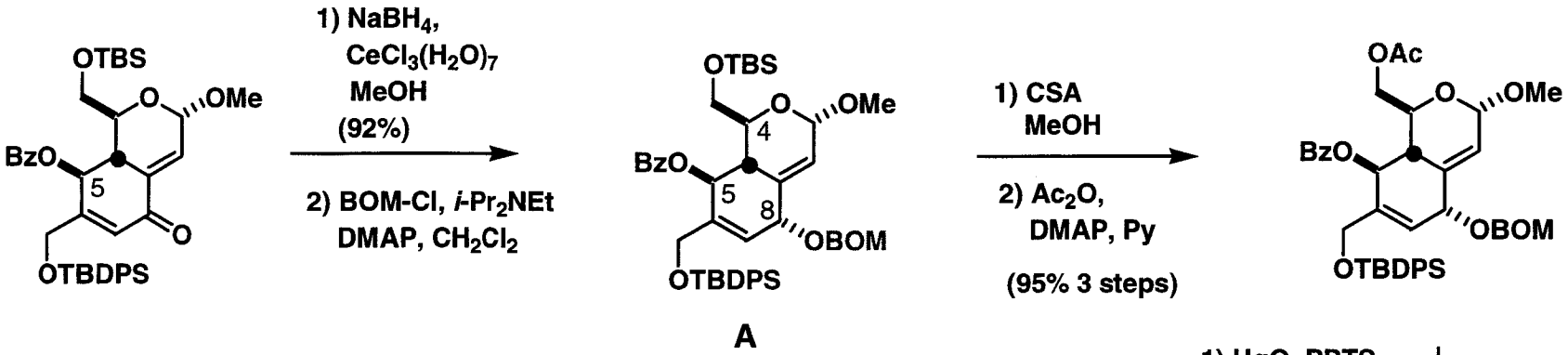
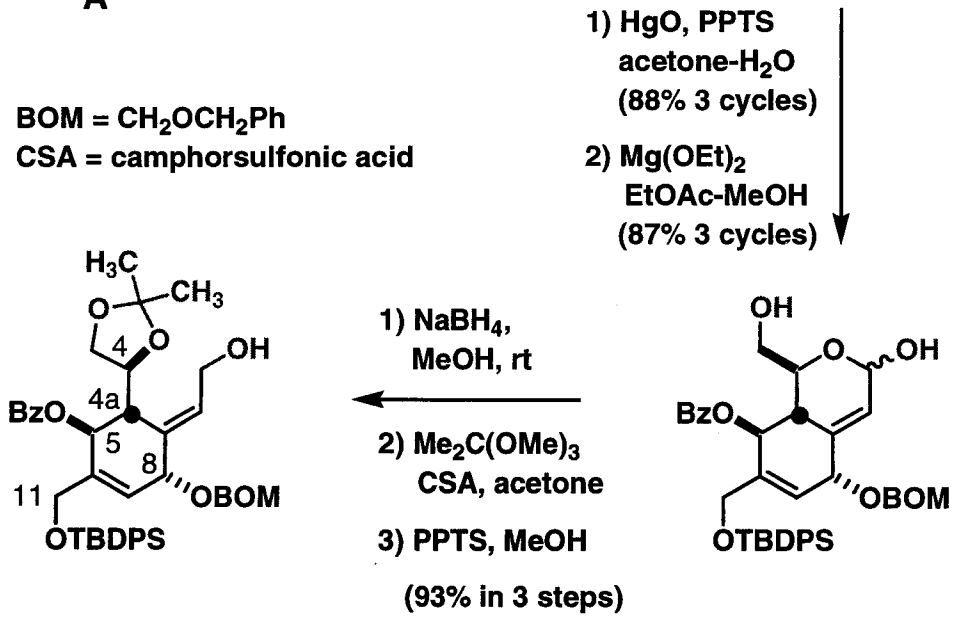
Synthesis of the Cyclohexane Skeleton: Exoolefin Synthesis

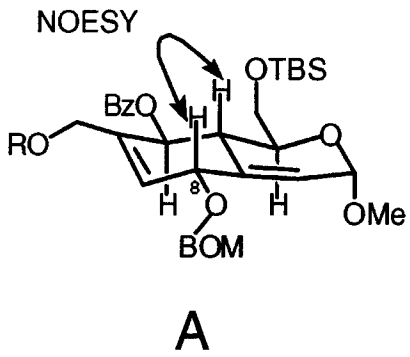




BOM = CH_2OCH_2Ph
CSA = camphorsulfonic acid

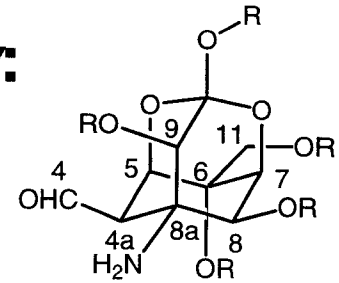


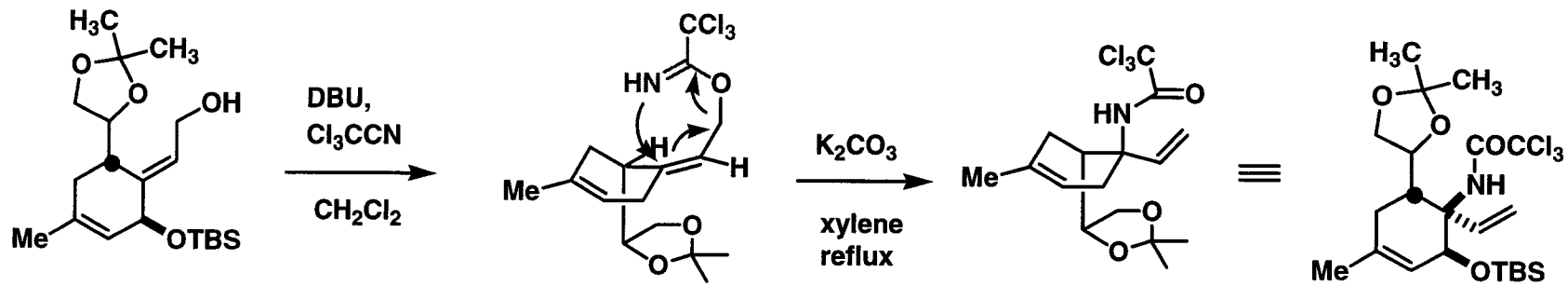
Proof of the Configuration at the C-8 position

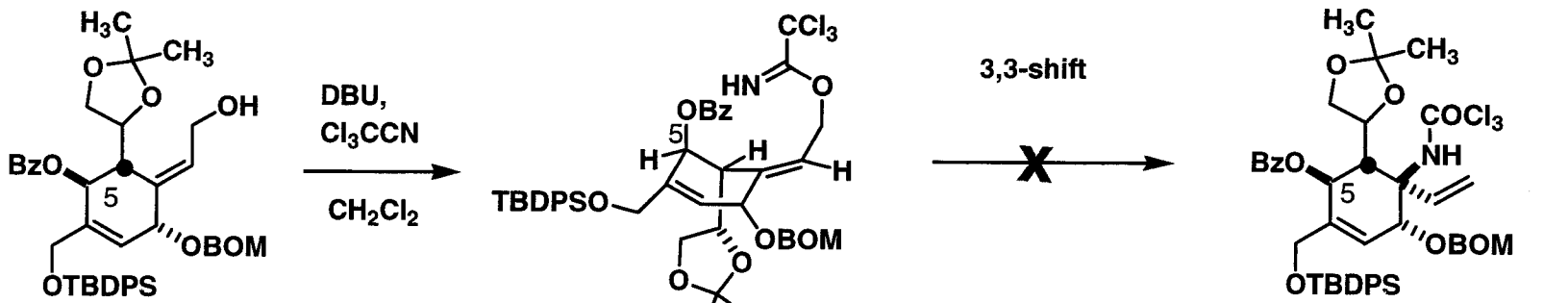


Isobe, M., Ohyabu, T, Nishikawa, T. *J. Am Chem. Soc.* **2003**, *125*, 8798

Introduction of the Nitrogen Functionality: Overman Rearrangement

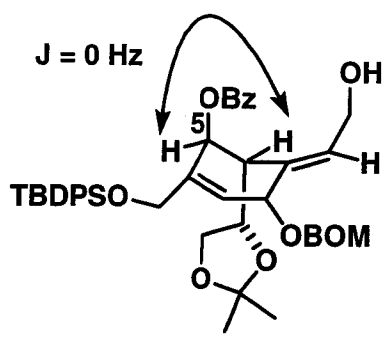


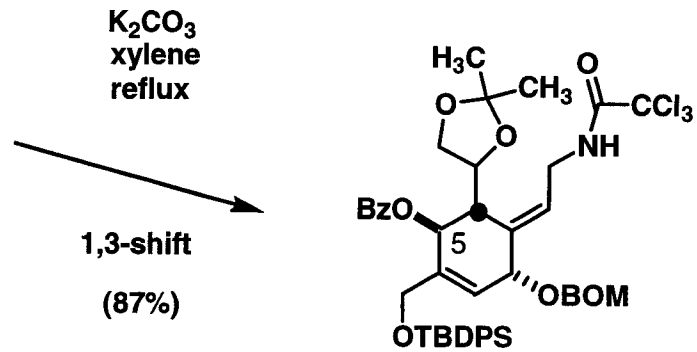




A

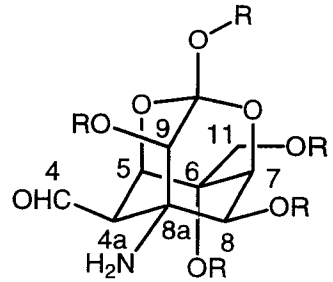
Proof of configuration Of A

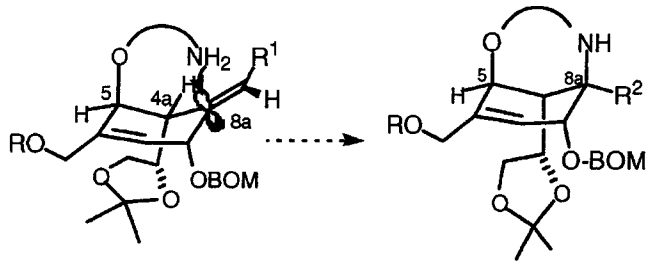


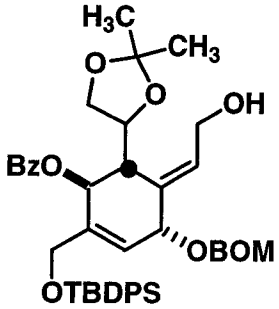


Isobe, M., Ohyabu, T, Nishikawa, T. *J. Am Chem. Soc.* **2003**, *125*, 8798

Introduction of the Nitrogen Functionality: Intramolecular Conjugate Addition

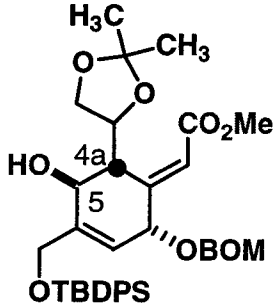






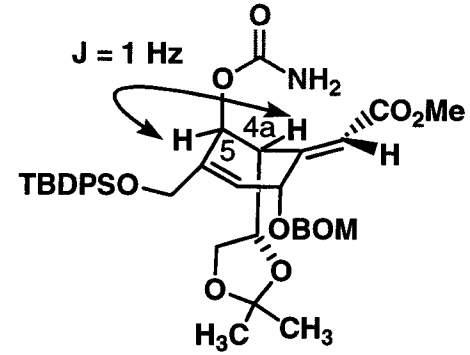
1) DIBAL-H
2) TEMPO, NCS
3) $NaClO_2$, NaH_2PO_4, $Me_2C{=}CHMe$, H_2O
4) TMS-CHN_2

(78% 4 steps)



1) $Cl_3CCONCO$, CH_2Cl_2
2) Et_3N, MeOH

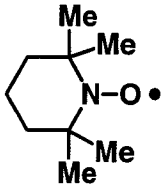
(99% 2 steps)

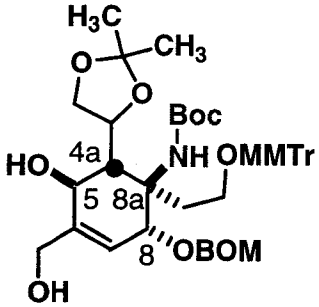


t-BuOK, THF
-78 oC to -15 oC

(90%)

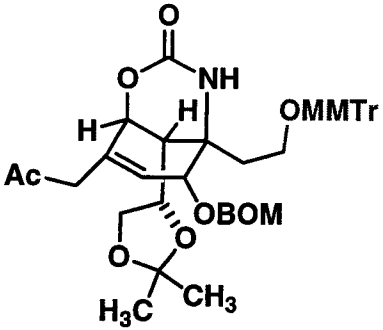
TEMPO =



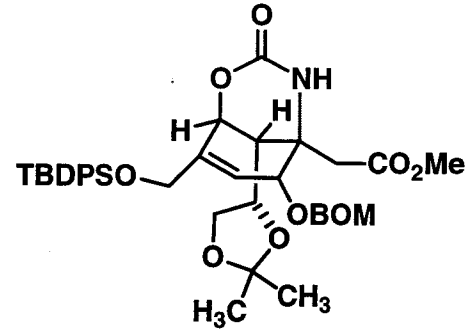


1) Boc_2O, Et_3N, DMAP, THF
2) LiOH, MeOH, $(CH_2Cl)_2$-H_2O

(84% 2 steps)



1) $LiBH_4$ THF
2) MMTr-Cl Pyr.



MMTr = p-methoxyphenyldiphenylmethyl
$C(C_6H_5)_2C_6H_4$-$_p$-OCH_3

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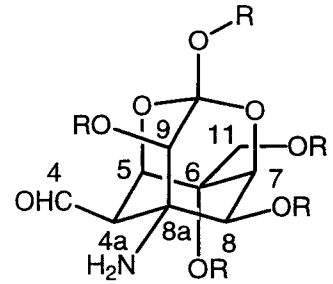
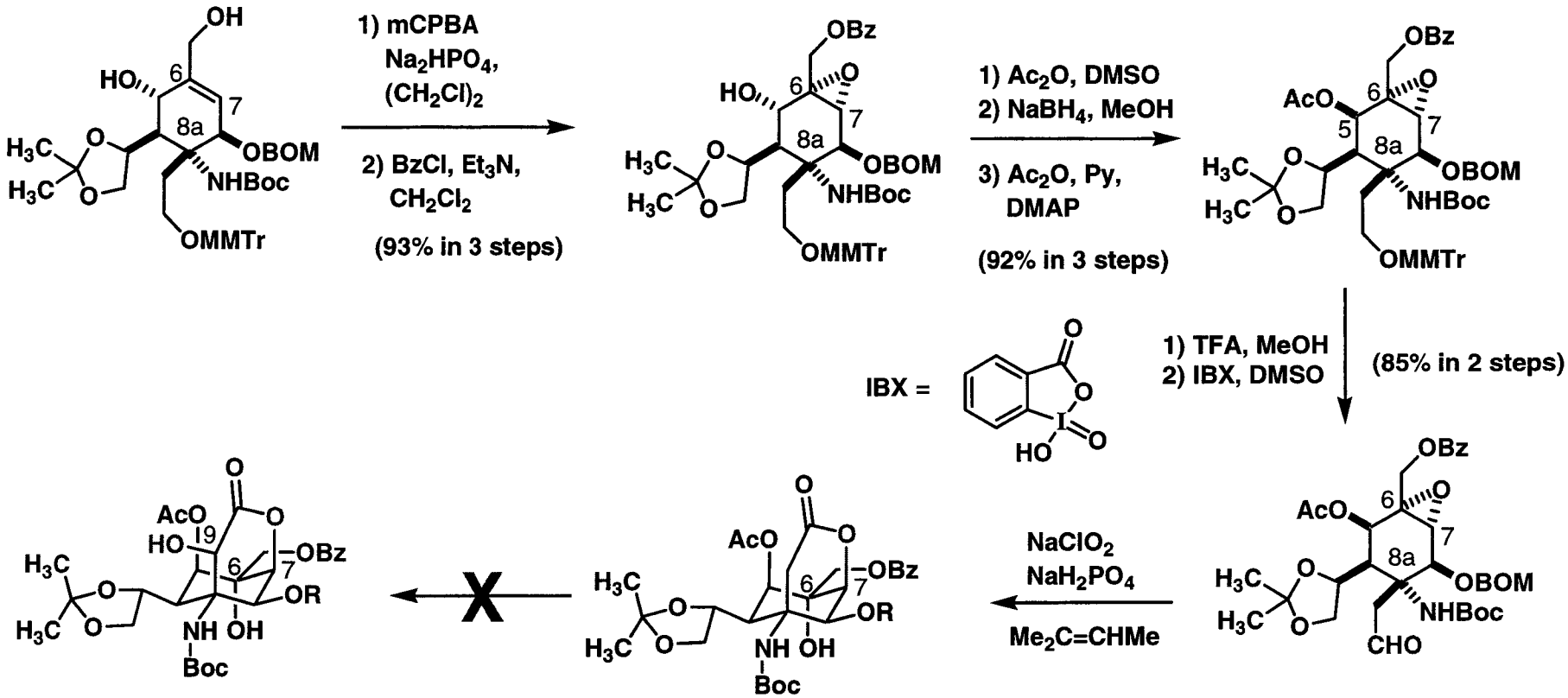
Stereoselective Synthesis of the Lactone Ring





All attempts at hydroxylation of C-9 failed

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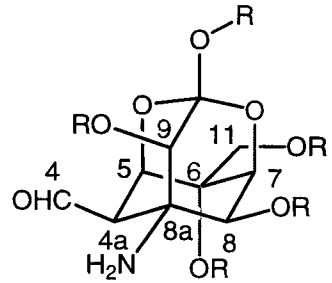
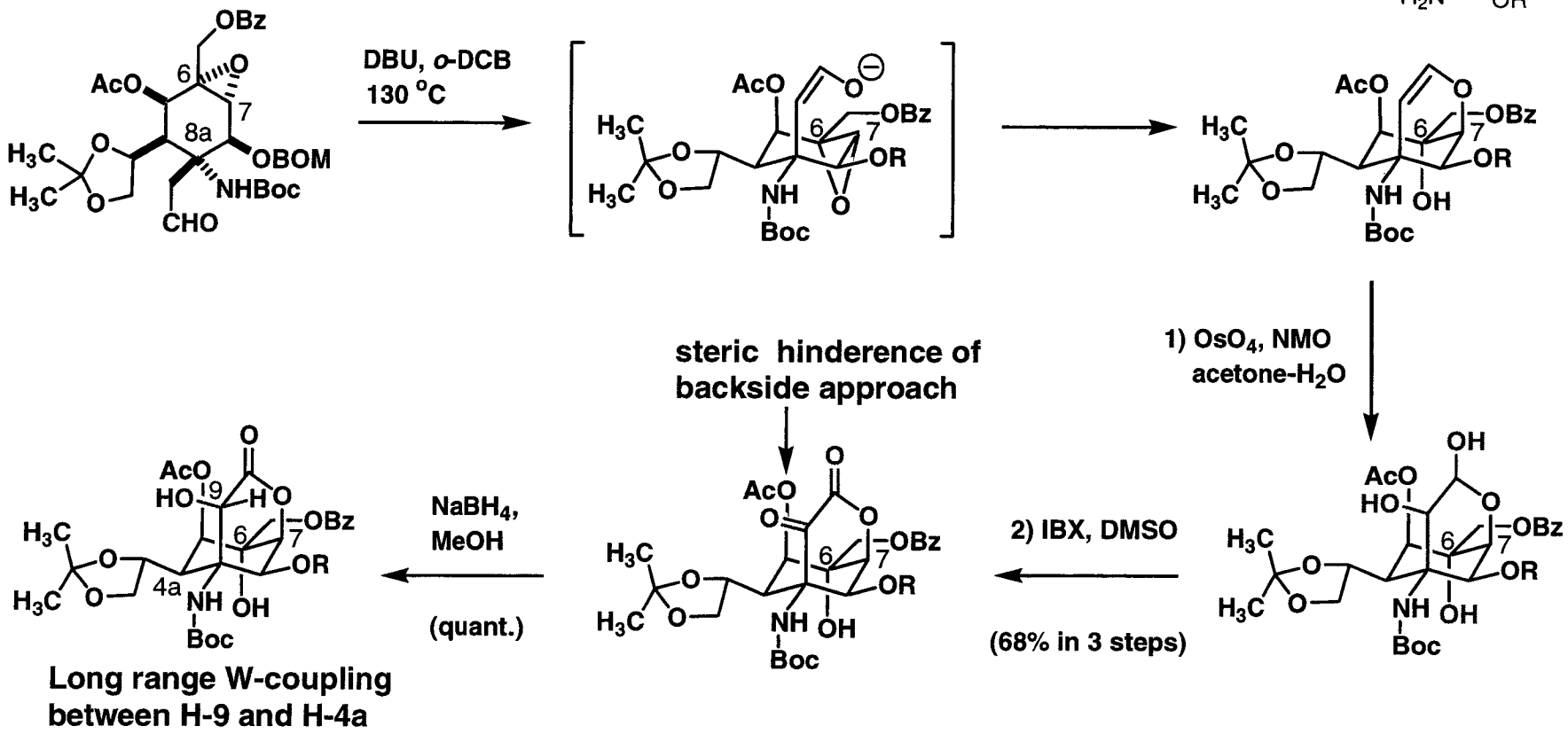
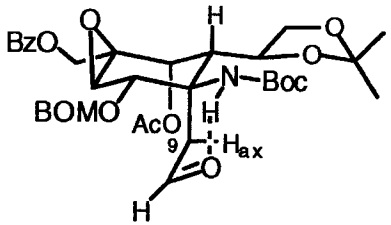
Stereoselective Synthesis of the Lactone Ring





Conformation of Enol precursor leading to Z-enolate



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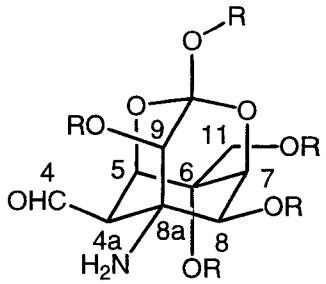
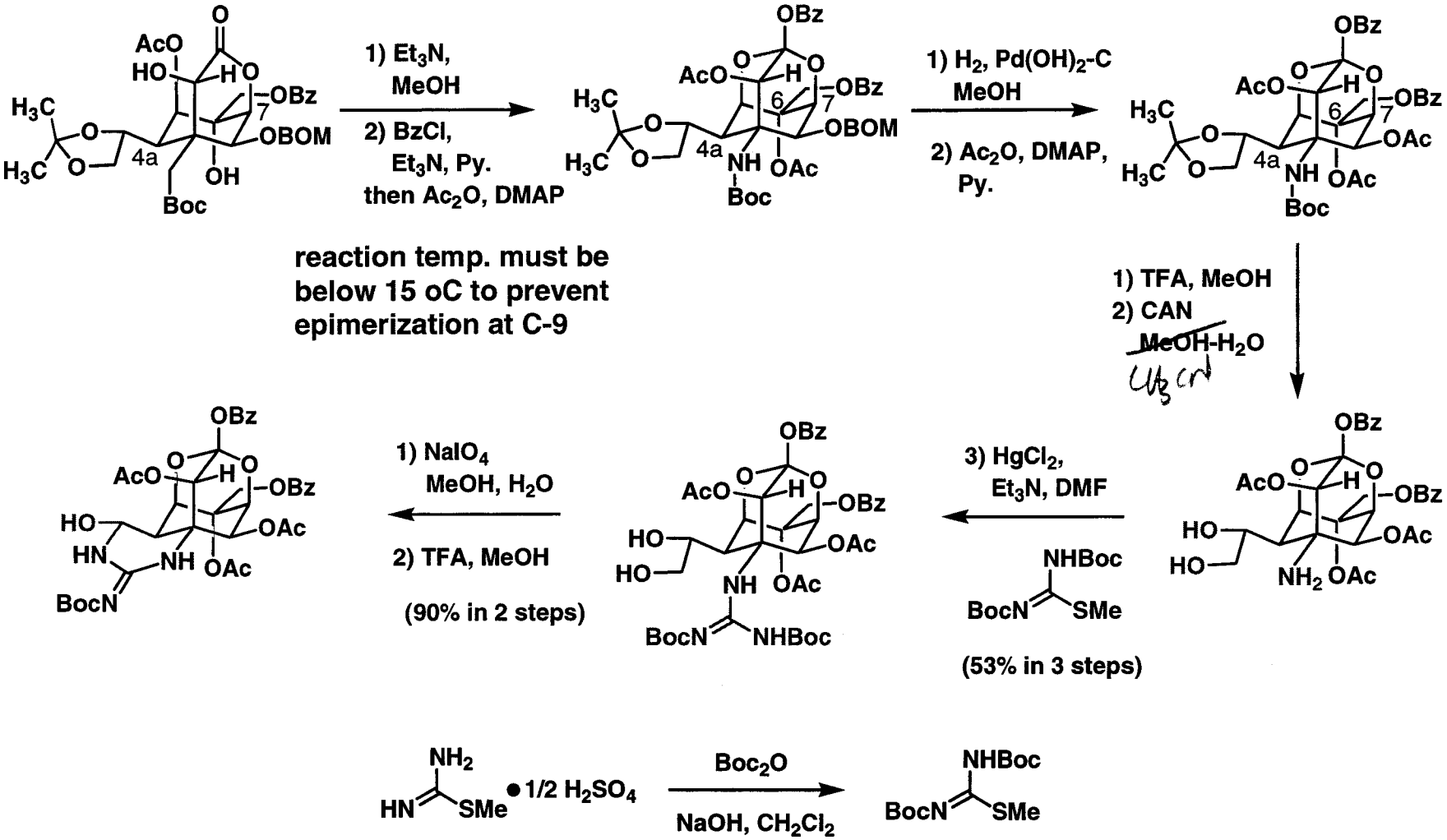
Introduction of the Guanidine Moiety





reaction temp. must be below 15 oC to prevent epimerization at C-9

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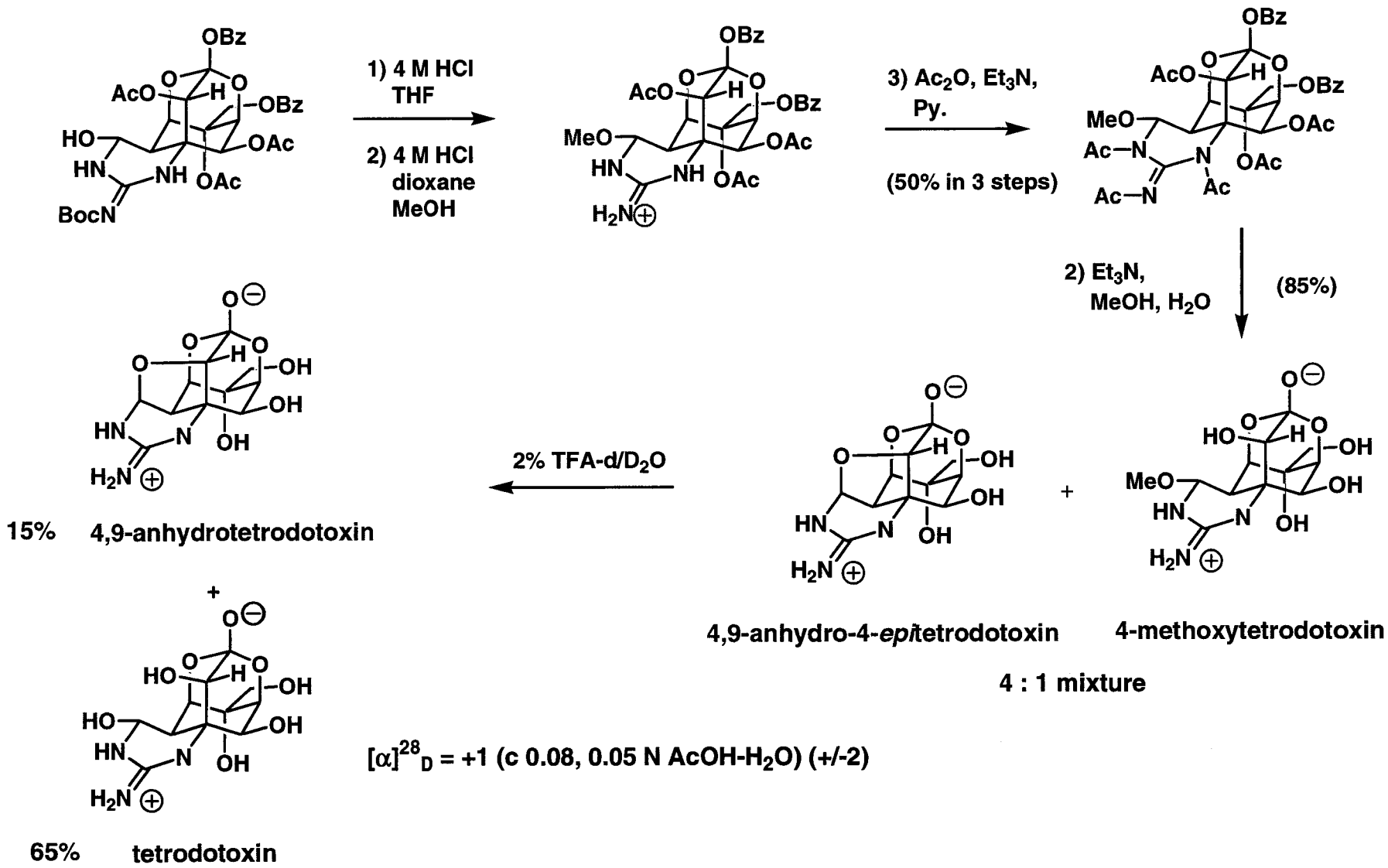
Completion of the Total Synthesis



$[\alpha]^{28}_{D}$ = +1 (c 0.08, 0.05 N AcOH-H_2O) (+/-2)

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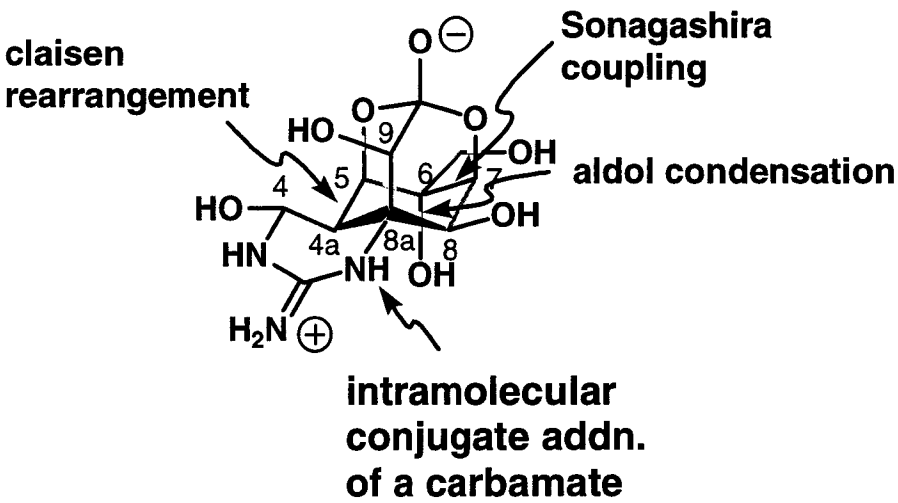
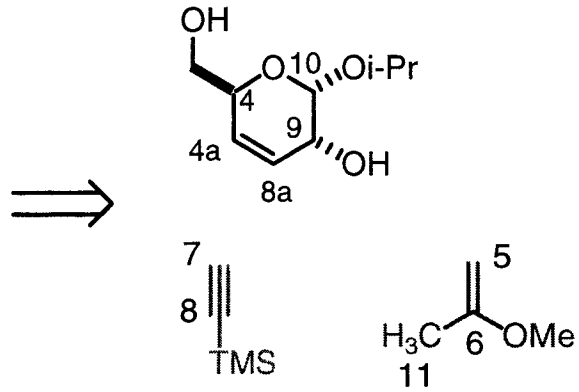
Summary of Isobe's Total Synthesis



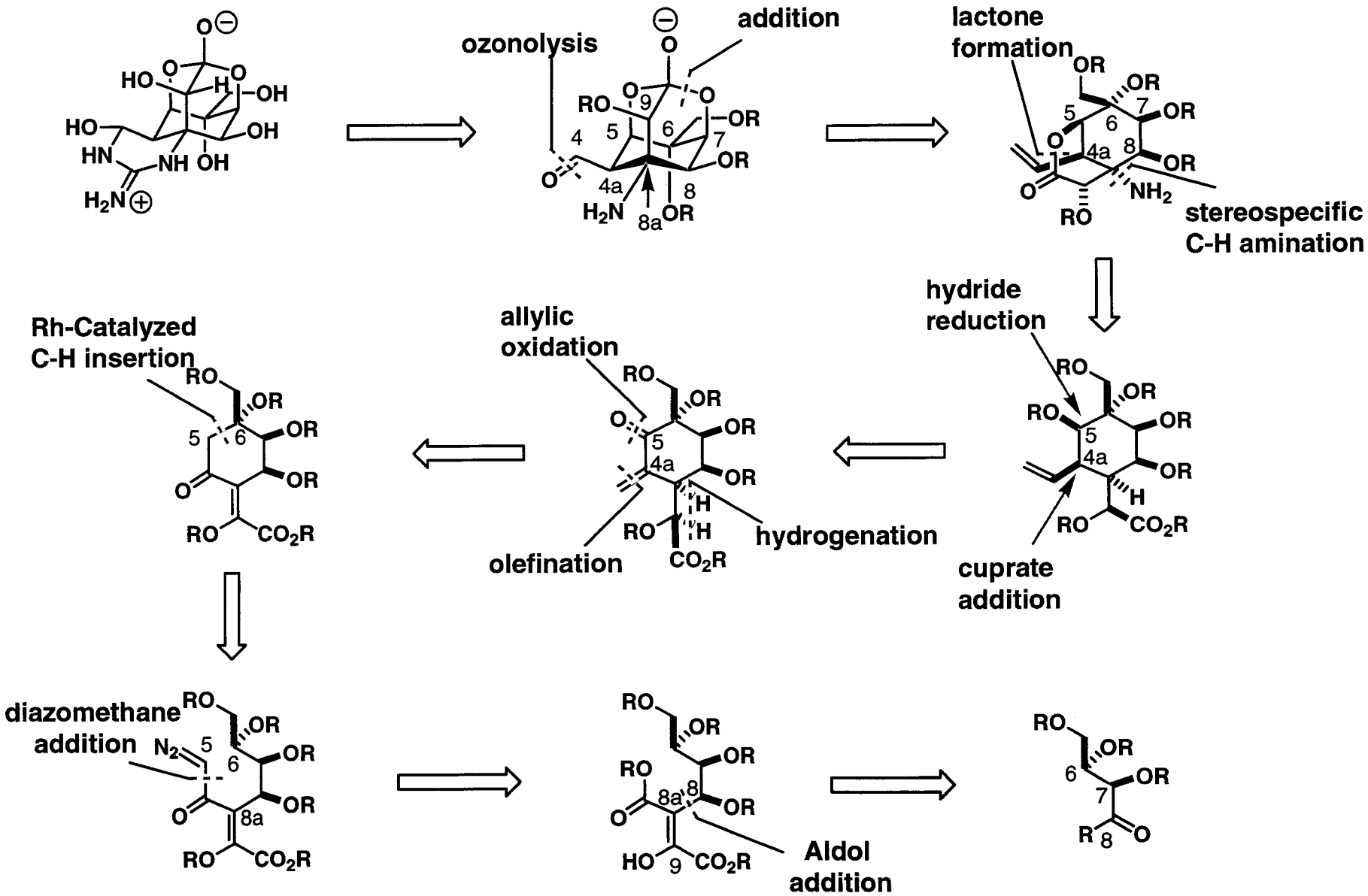


69 Total steps

C4a: Claisen rearrangement
C5: epoxidation of an enol ether and inversion
C6: stereospecific, substrate controlled epoxidation
C7: intramolecular enolate attack on epoxide
C8: Stereospecific, substrate controlled hydride reduction
C8a: intramolecular conjugate addition of a carbamate
C9: stereospecific, substrate controlled hydride reduction

- 25 steps involved in protection group manipulations.
- Required many steps to make Overman rearrangement precursor which was unsuccessful.

Du Bois' Retrosynthetic Analysis - Asymmetric



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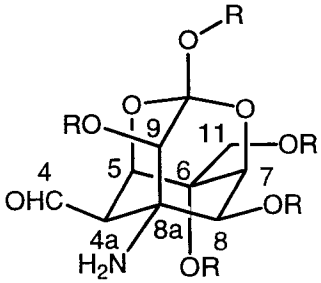
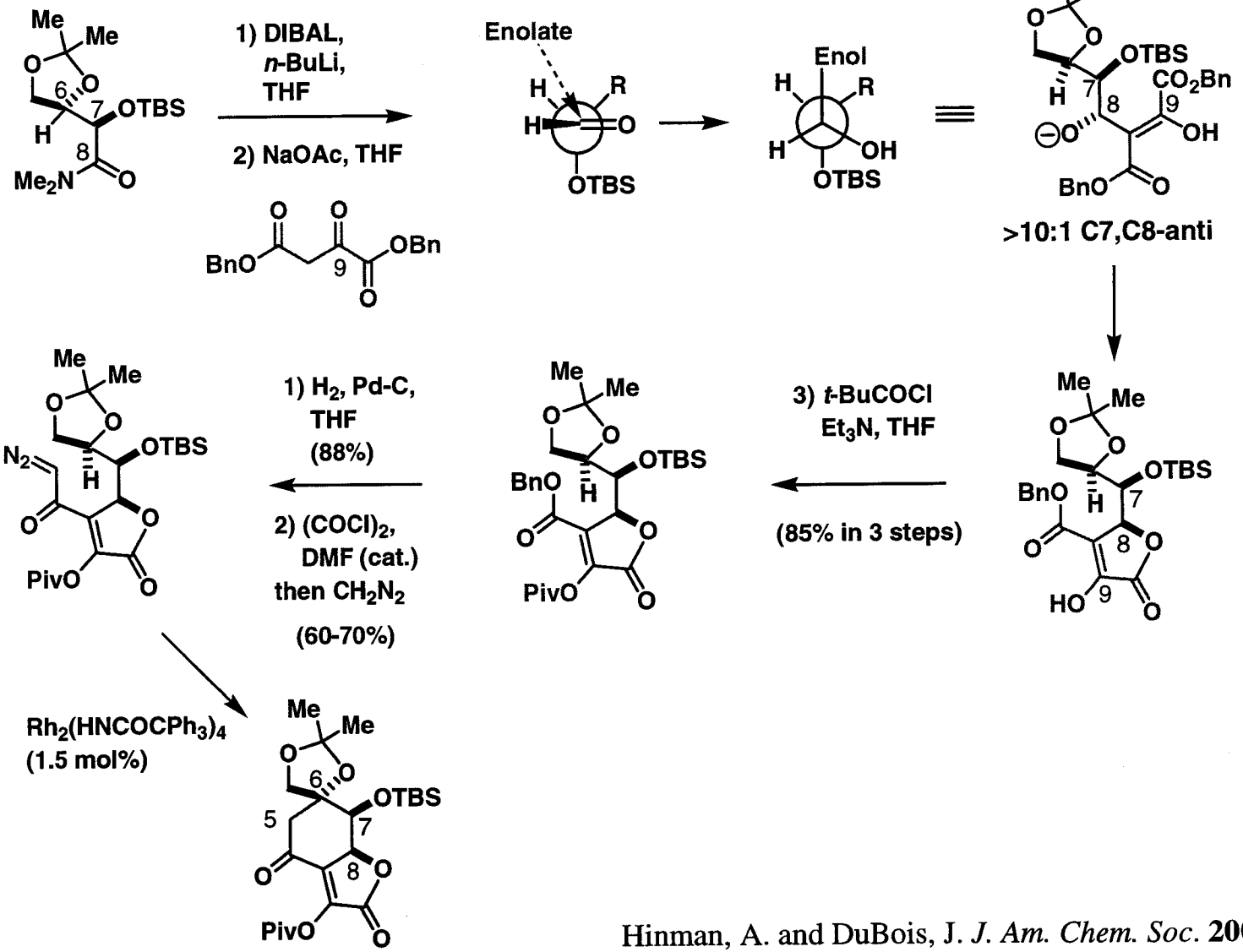
Synthesis of the Cyclohexane Skeleton: Aldol Addition and Rh-Catalyzed C-H Insertion





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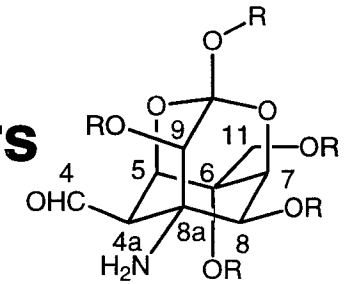
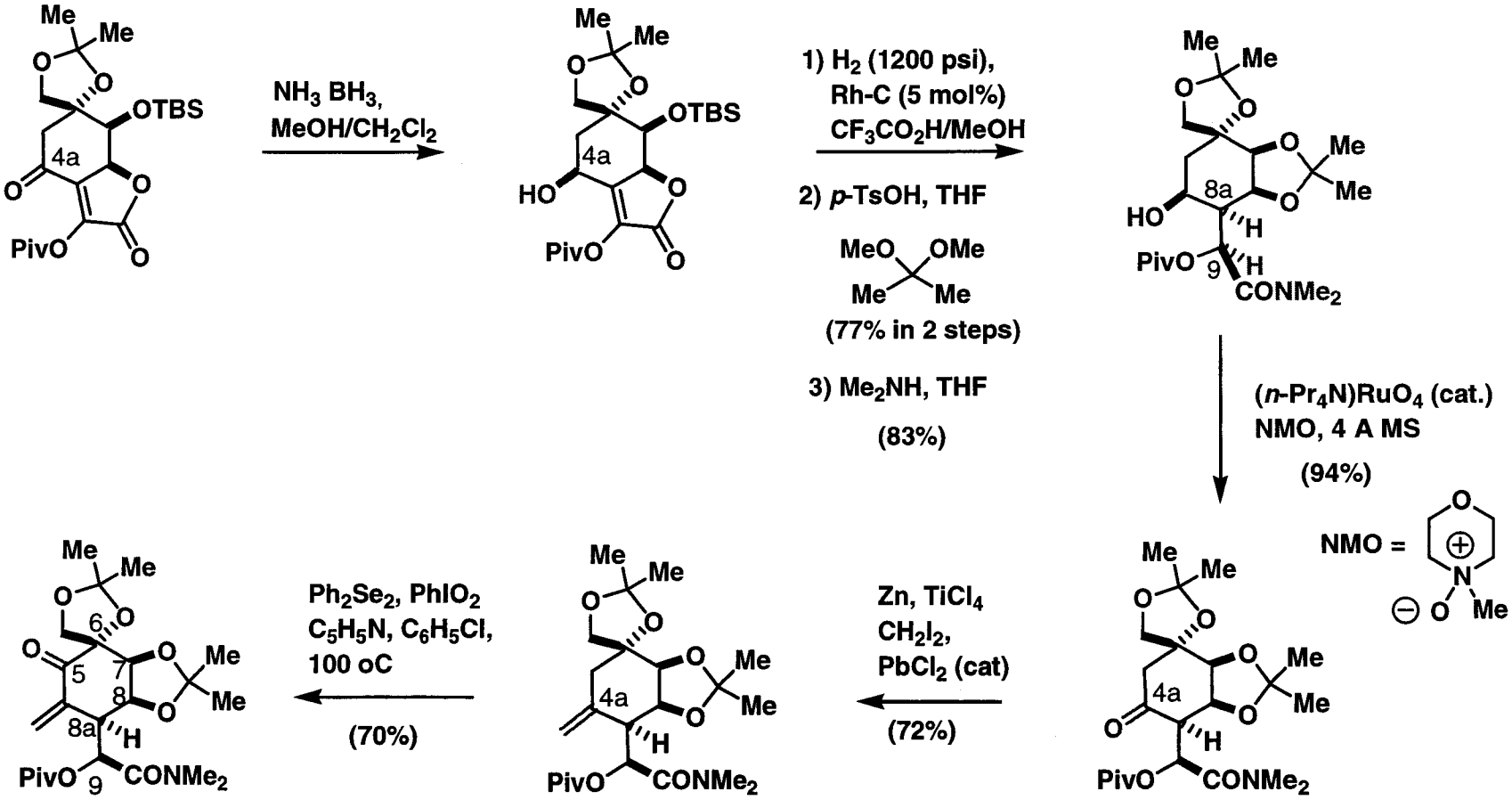
Synthesis of the Cyclohexane Skeleton: Installation of the C8a and C9 Stereocenters and Introduction of the C5 Alcohol





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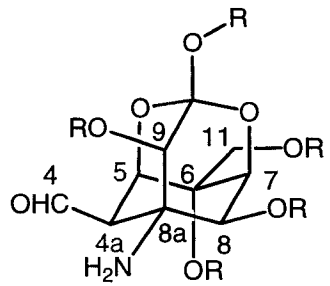
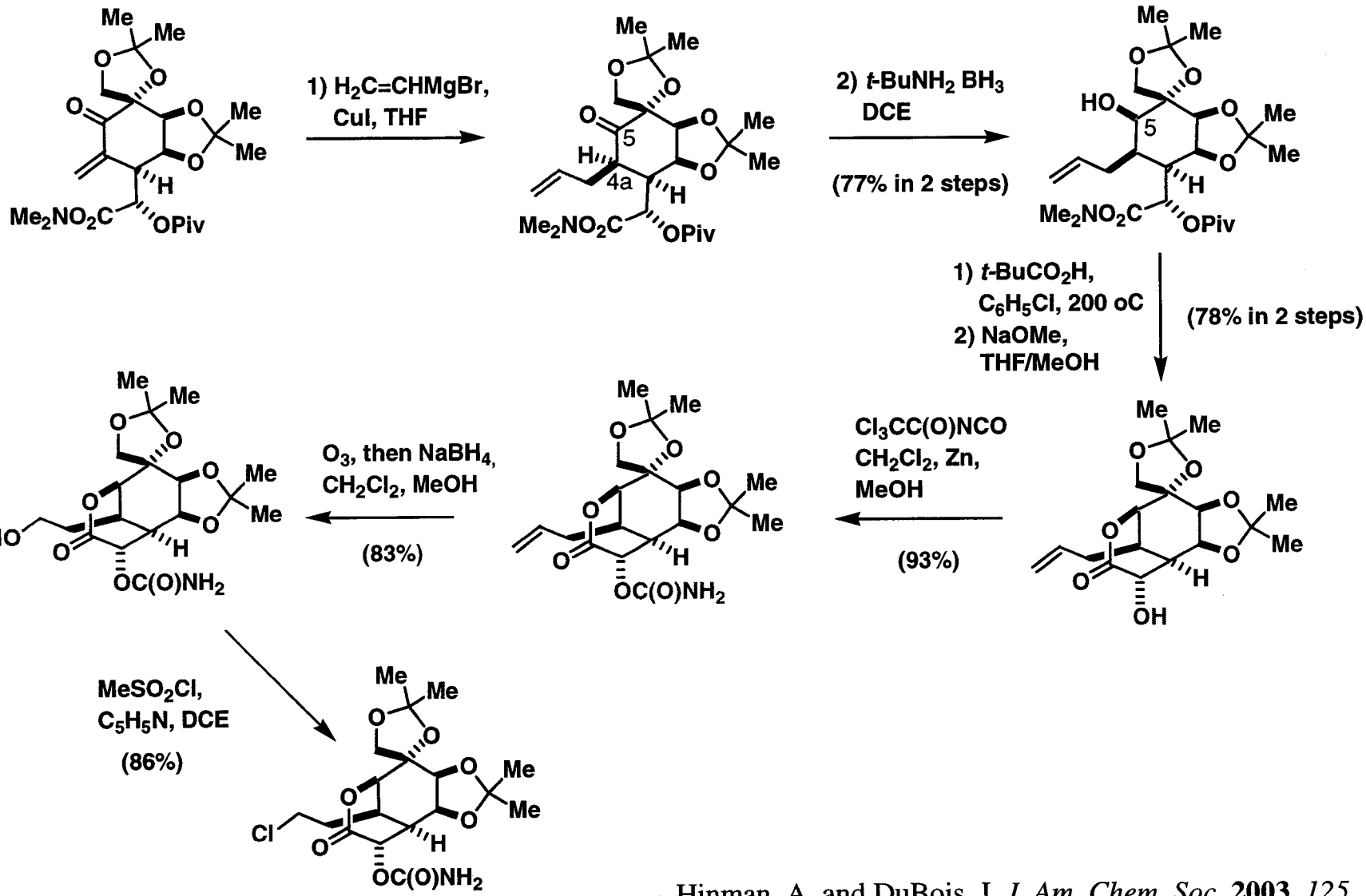
Completion of the Cyclohexane Skeleton And Synthesis of the Lactone Ring





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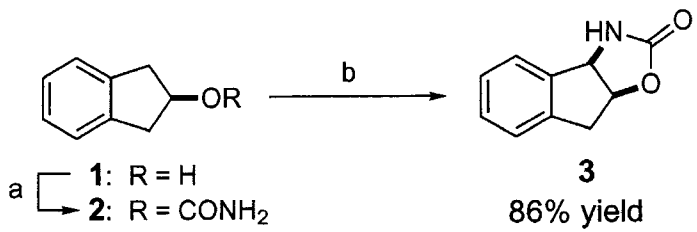
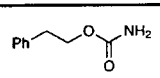
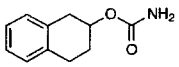
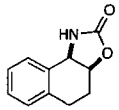
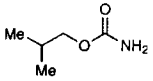
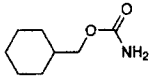
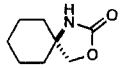
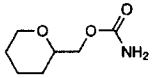
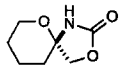
Rh-Catalyzed C-H Insertion Reaction for the Oxidative Conversion of Carbamates to Oxazolidinones



a. $CCl_3C(O)NCO$; MeOH, K_2CO_3
b. 5 mol% $[Rh_2(OAc)_4]$, $PhI(OAc)_2$, MgO, CH_2Cl_2, 40 °C, 12 hr

Entry	Substrate	Product	Catalyst[a]	Yield
1			B	74
2			B	77[c]
3			A	83
4			A	77
			B	79
5			A	82
			B	84

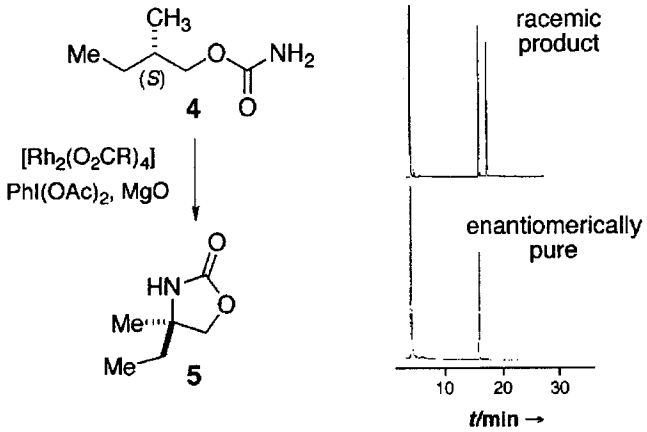
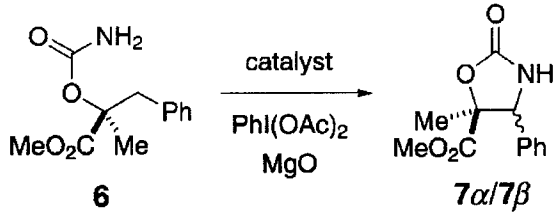


Figure 1. Cyclization of **4** into **5**. Gas chromatograms of the oxazolidinone **5** from racemic (top) and optically pure (bottom) carbamate **4**.

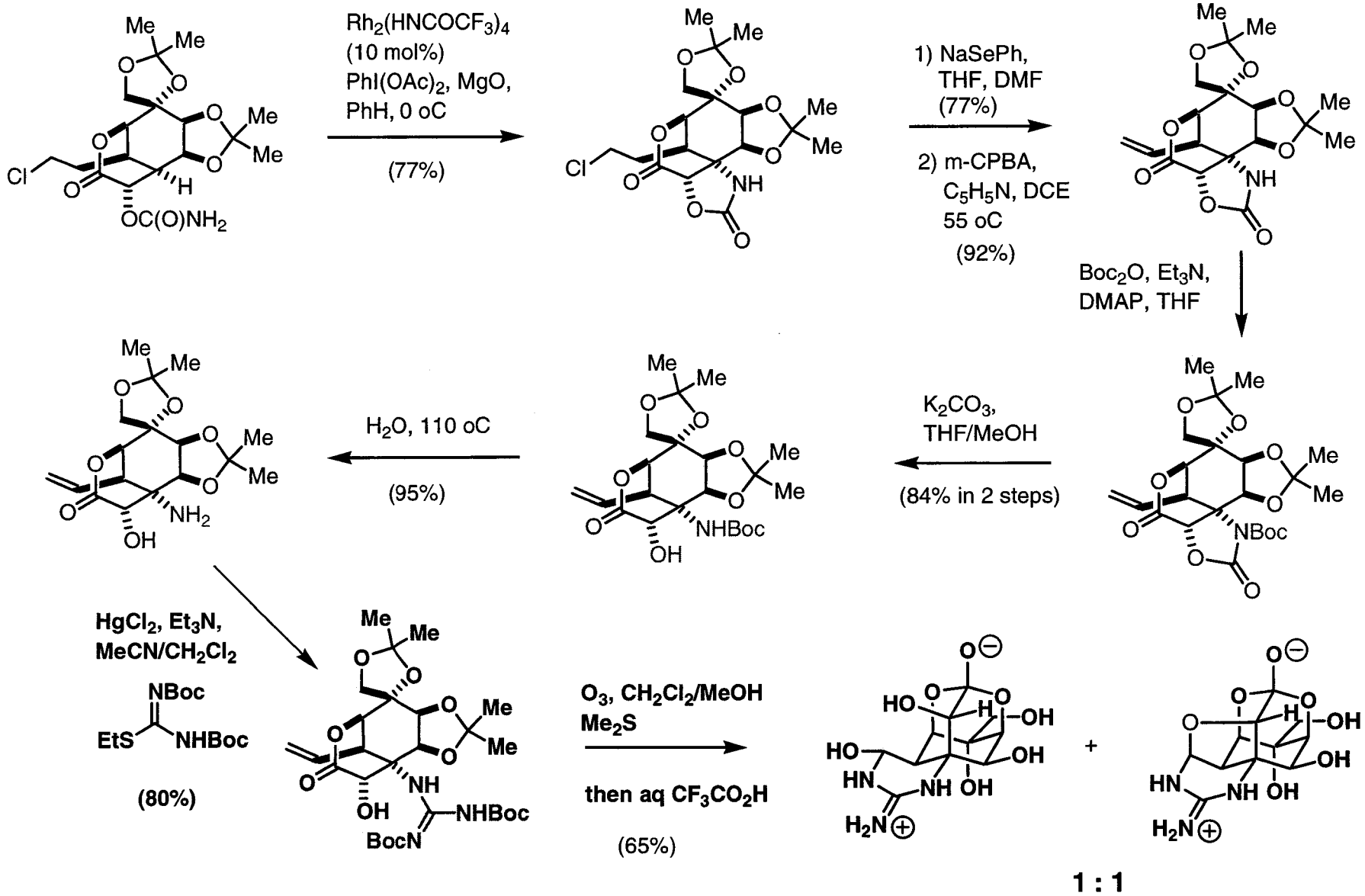
Reaction proceeds with retention of configuration, consistent with nitrene or nitrenoid intermediate.



	7α:7β
$[Rh_2(OAc)_4]$	2:1
$[Rh_2(tpa)_4]$	1:2.6

Rh catalyst is mediating the C-N insertion, a free carbamoylnitrene is not the active oxidant.

Completion of the Total Synthesis: Introduction of the Nitrogen and Guanidine Moiety



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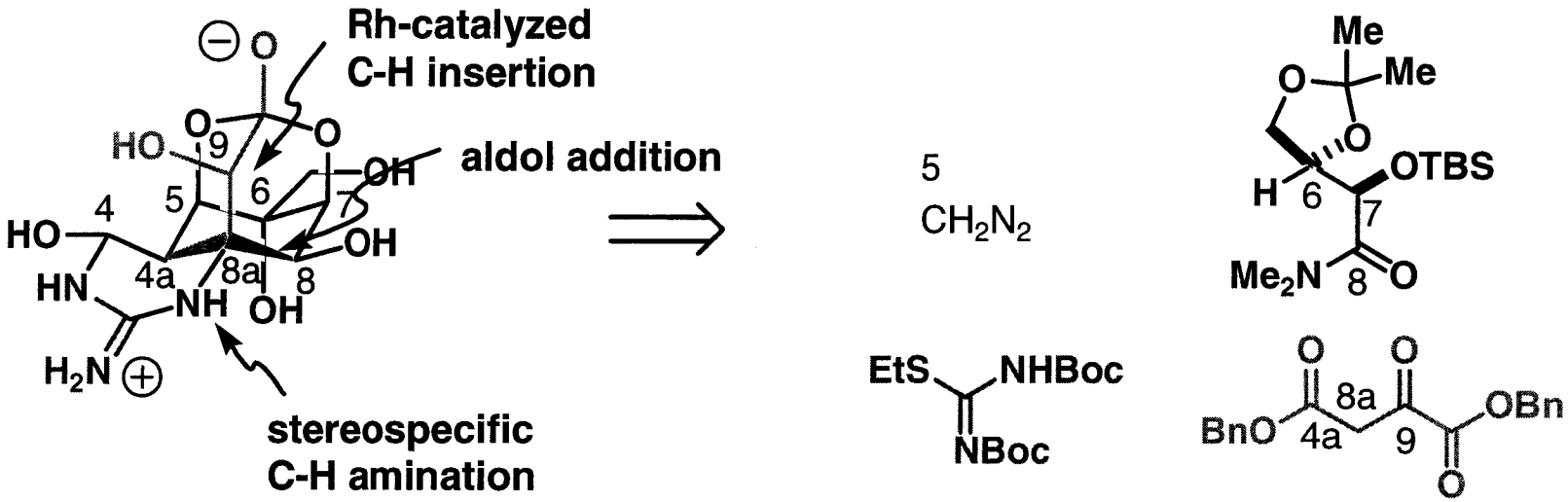
Summary of Du Bois' Total Synthesis



22 Total steps

C6 and C7: chiral starting material
C8: Diastereoselective aldol addition rxn.
C8a and C9: Stereoselective, substrate controlled hydrogenation
C4a: Stereoselective, substrate controlled protonation after conjugate addn.
C5: Stereoselective, subtrated controlled hydride reduction

- Utilized C-H functionalization for key steps, allowing for a short and concise synthesis
- Very few protecting group manipulation steps (5)

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