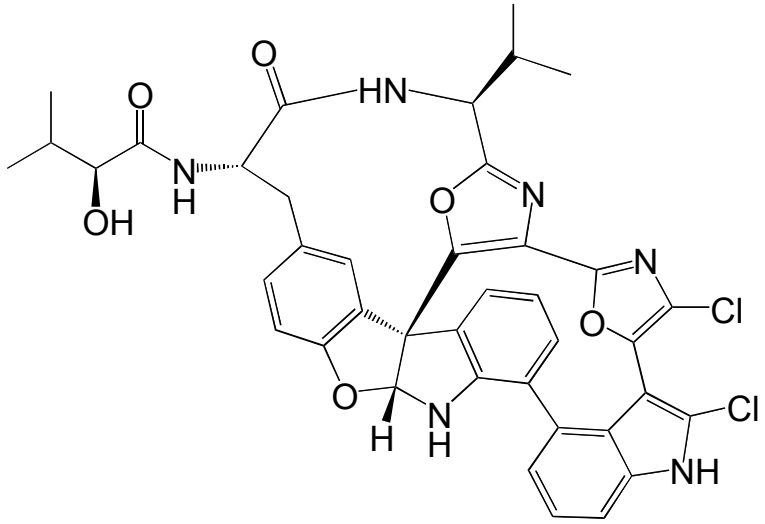
The Total Synthesis of Diazonamide A: Literature Highlights and Current Progress Toward the Asymmetric Formation of the C10 Quaternary Center



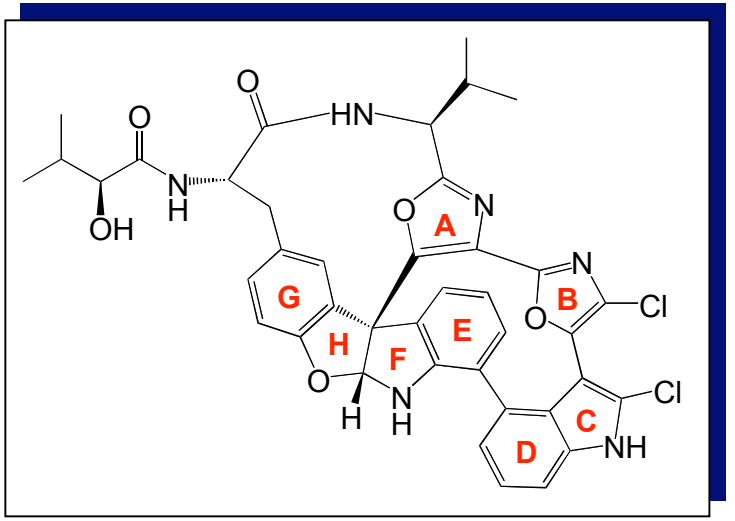
Erick B. Iezzi
University of Pittsburgh
Wipf Research Group

Presentation Outline

- Background, biochemical studies and structural assignments
- Total syntheses (Harran and Nicolaou)
- Partial syntheses and synthetic strategies
- Strategies of former Wipf group members
- Current strategy

Discovery and Biological Activity of Diazonamide A

- Isolated from the marine ascidian *Diazona Angulata* (originally misidentified as *Diazona chinensis*) in 1991 by William Fenical and co-workers at Scripps Institute of Oceanography[1,*]
- Novel macrocyclic peptide composed of three common amino acids: L-tyrosine, tryptophan and L-valine
- Demonstrated potent *in vitro* inhibition of HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell lines (IC_{50} values <15 ng/ml)[1,*]
- Synthetic Diazonamide A exhibited potent cytotoxic activity against ovarian carcinoma 1A9, breast carcinoma MCS-7 and taxol-resistant 1A9/PTX10 cell lines[2]
- Inhibitor of tubulin assembly (into microtubules), causing cells to accumulate at the G_2/M phase of the cell cycle

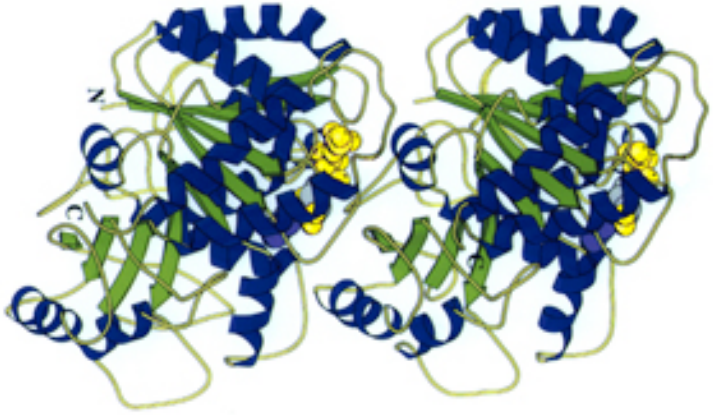


• *Diazonamide B also isolated (2.5x greater conc.), but much less active*

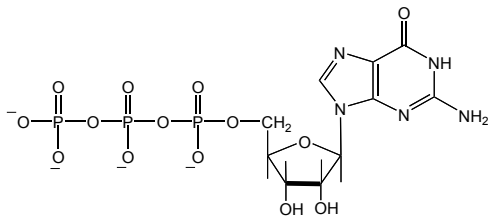
1. Fenical, et al. *J. Am. Chem. Soc.* **1991**, *113*, 2303.
2. Nicolaou, et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 3495.

Tubulin and the Assembly of Microtubules

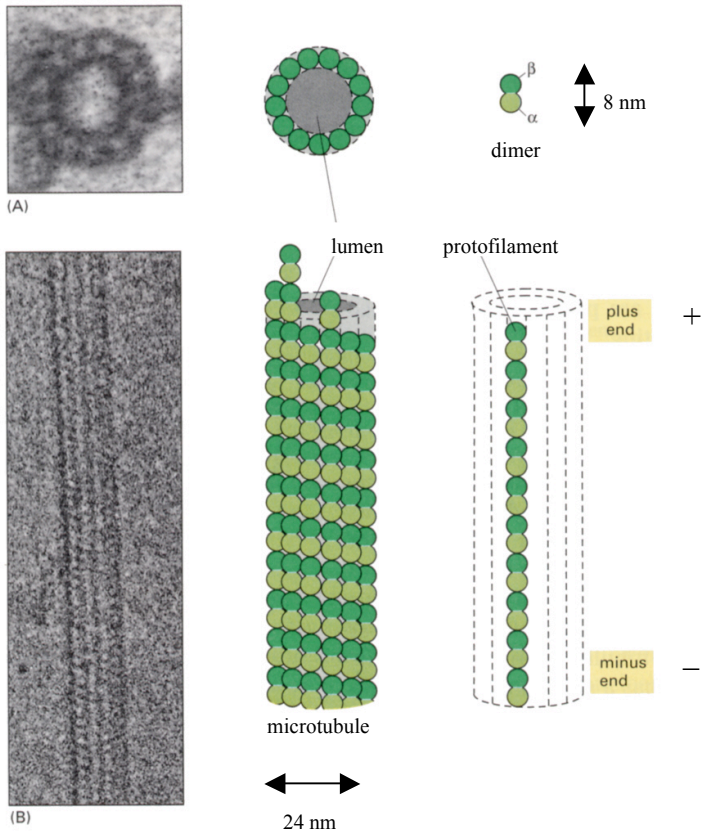
α,β-tubulin dimer



θ proteins are tightly bound by non-covalent bonds
θ bound guanosine triphosphate (GTP) nucleotide (yellow)



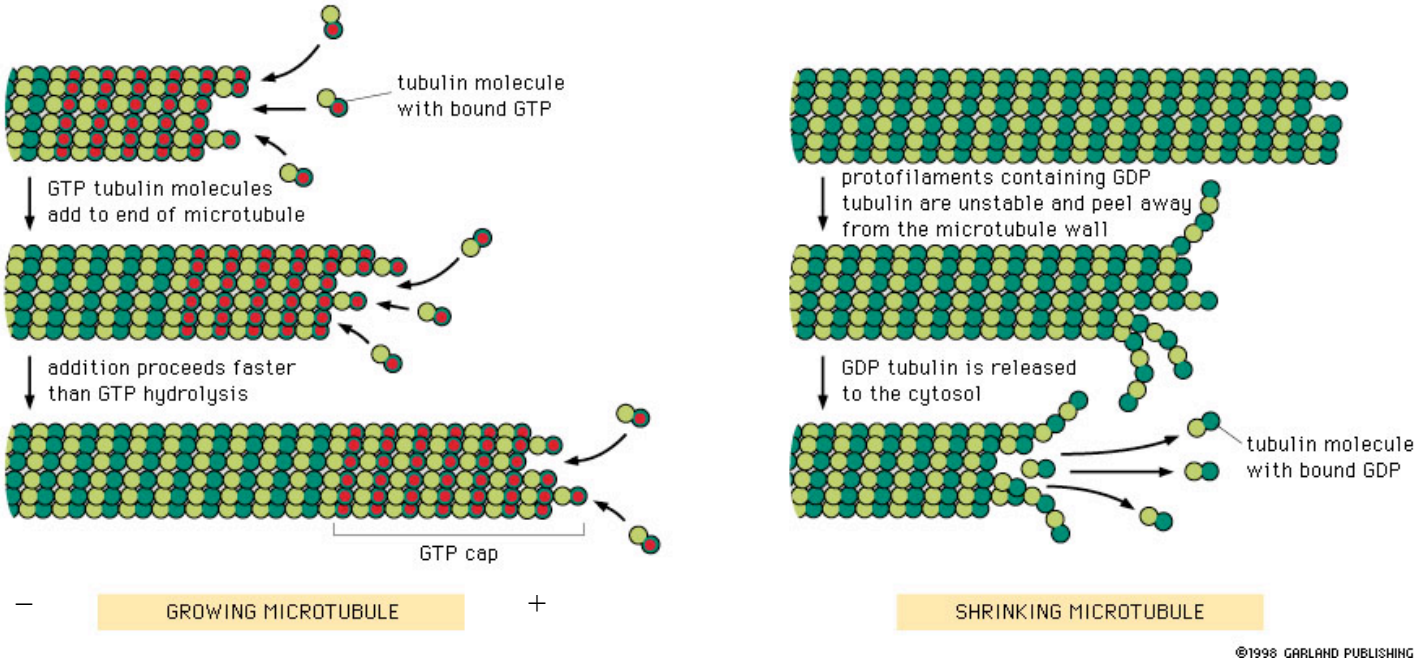
www.uib.no/aasland/ gensidene/tubulin.html



θ microtubules form the mitotic spindle during mitosis

www.tmd.ac.jp/artsci/ biol/textbook/cellmove.html

Microtubule Dynamics



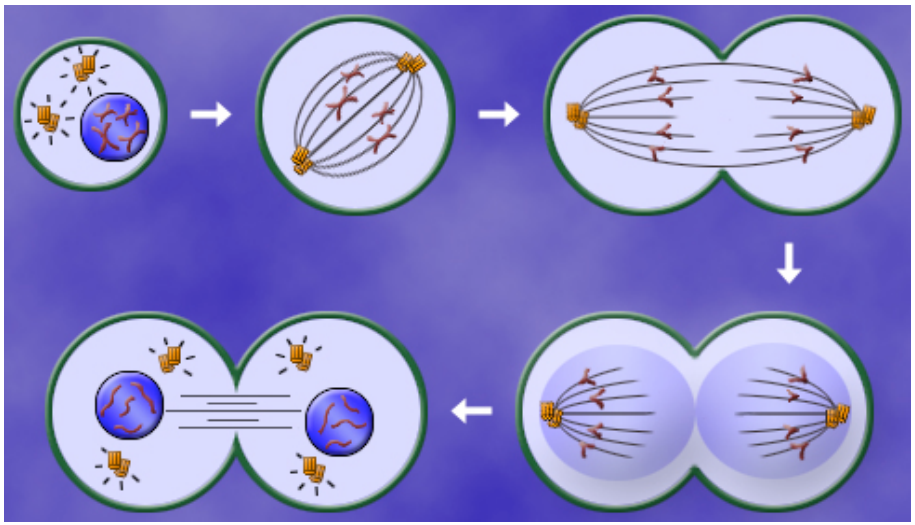
www.hykim.chungbuk.ac.kr/ lectures/cellbio/11/11.html

- stabilization of microtubules occurs when concentration of GTP is greater than GDP

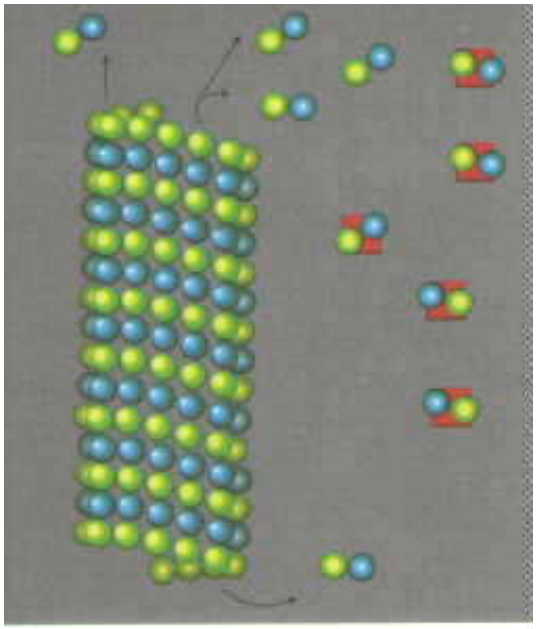
- depolymerization of endcapped microtubules containing GDP tubulin is ~100X faster than ones capped with GTP tubulin

- tubulin dimers can easily diffuse within the cytoplasm of the cell, whereas the polymer (microtubule) cannot

Mitosis and the Influence of Drugs on Microtubule Dynamics



www.swbic.org/products/clipart/images/mitosis.jpg



www.cellbio.utmb.edu/.../ microtubule_structure.html

Cell Cycle

- Interphase
  - G_1 phase - cell growth
  - S phase - DNA replication
  - G_2 phase - cell prepares to divide
- M-phase
  - mitosis - nuclear division
  - cytokinesis - cytoplasmic division

Mitosis

- Prophase
- Prometaphase
- Metaphase
- Anaphase
- Telophase

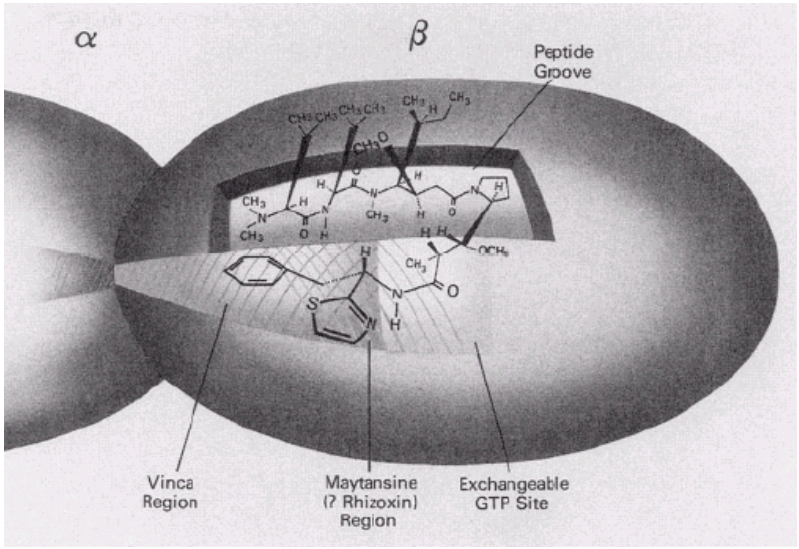
- Assembly and disassembly of microtubules are crucial for correct function of the mitotic spindle
- Tubulin dimers with bound drugs (red) cannot polymerize into microtubules

Diazonamide A: A Novel Inhibitor of Tubulin Assembly

Examples of microtubule-specific drugs:

θ Taxol, Discodermolide - bind to and stabilize microtubules during assembly
θ Colchicine, Colcemid and Nocodazole - bind to tubulin dimers and prevent assembly
θ Vinblastine, Vincristine and Dolastatin 10 - aggregate tubulin dimers which leads to depolymerization

Dolastatin 10 bound in subregion of vinca site



Bai, et al. *J. Bio. Chem.* **1990,** *265***,** 17141

* *Vinca domain* – binding site of vinca alkaloids

Biochemical properties of Diazonamide A

θ Potent inhibitor of tubulin assembly – equivalent to Dolastatin 10
θ Does not inhibit binding of Vinblastine, Dolastatin 10 or GTP exchange with tubulin
θ Does not stabilize Colchicine binding to tubulin

↓

Diazonamide A – inhibits assembly by: 1) binding to a unique site on the tubulin dimer or 2) binds to 'peptide site', but only when at end of growing tubes

Cruz-Monserrate, et al. *Mol. Pharmacol.* **2003**, *63*, 1273–1280

Studies of Cells Treated with Diazonamide A

I

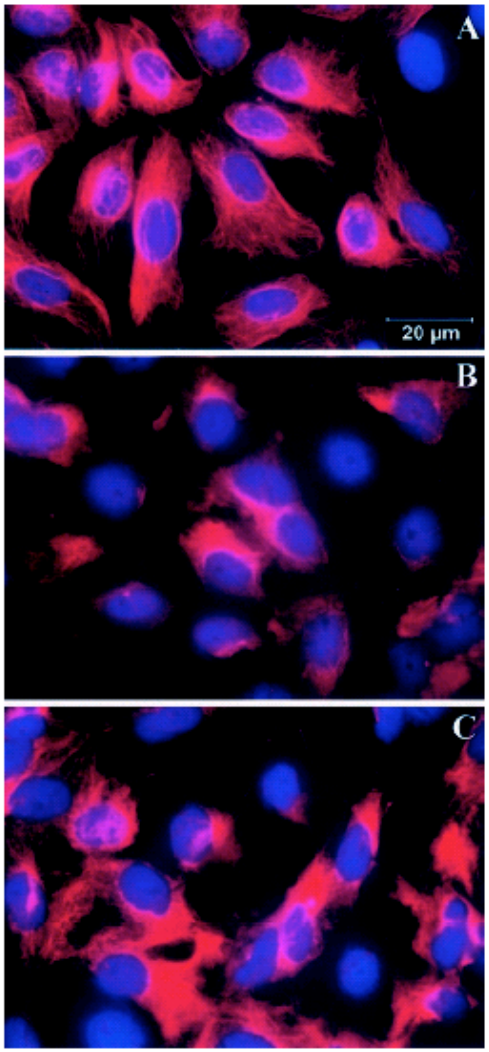


*figure shows DNA and tubulin florescense

II

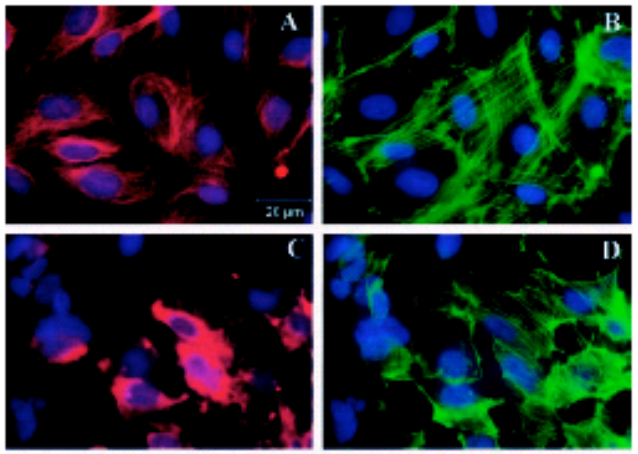


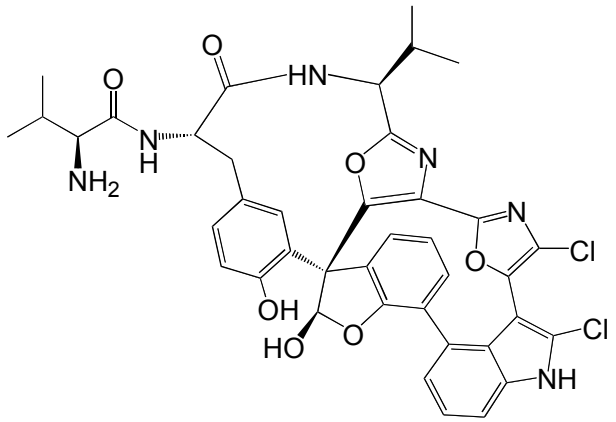
*figure shows (left) DNA and tubulin florescense, and (right) DNA and F-actin fluorescense

Figure I. PtK2 cells treated with IC_{50} concentration of Diazonamide A: **A**, no drug. **B**, 0.3 nM Diazonamide A for 16 h. **C**, 1.0 nM Diazonamide A for 16 h.

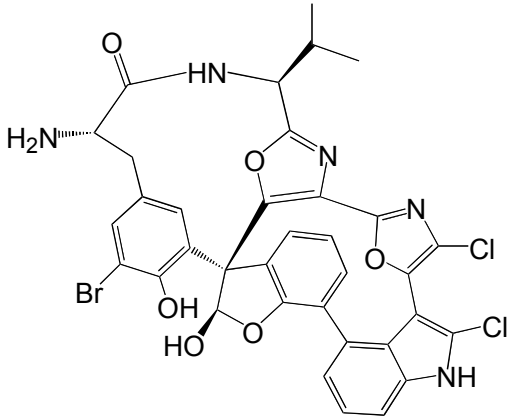
Figure II. PtK2 cells treated with 10 times IC_{50} concentration of Diazonamide A: **A** and **B**, no drug. **C** and **D**, 3 nM of Diazonamide A for 16 h.

Cruz-Monserrate, et al. *Mol. Pharmacol.* **2003**, *63*, 1273–1280

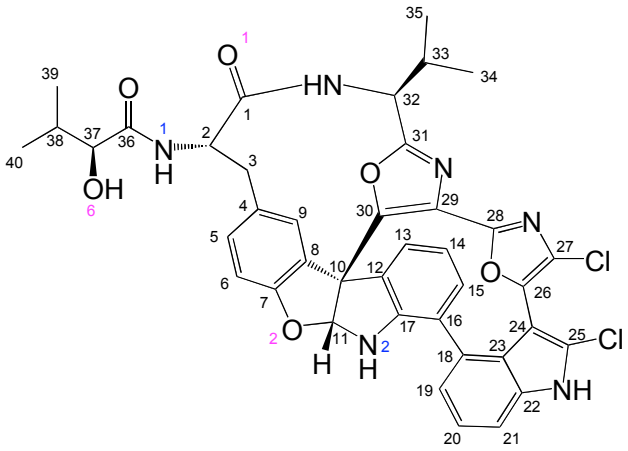
Structural Assignments of Diazonamide A and B



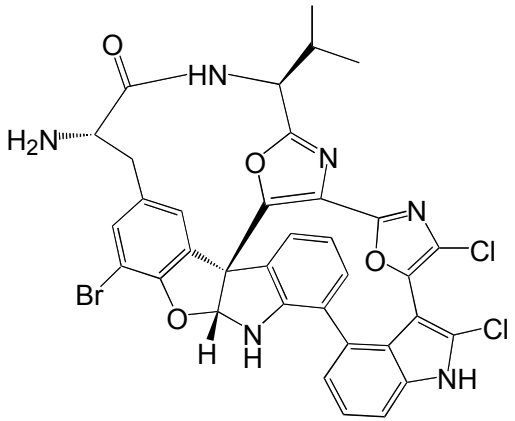
Nominal Diazonamide A (Fenical, 1991)



Nominal Diazonamide B (Fenical, 1991)

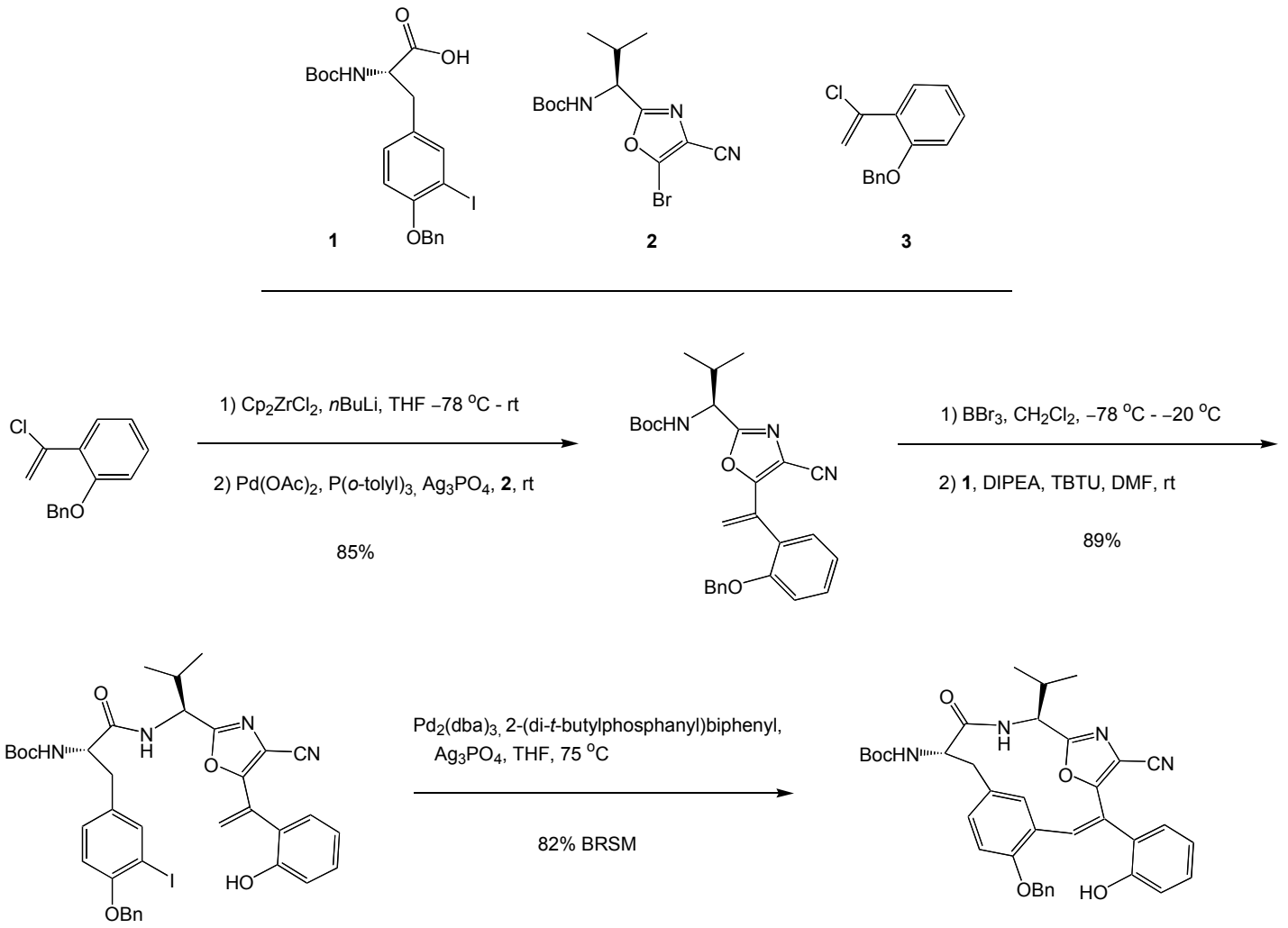


(–)-Diazonamide A (Harran, 2001)



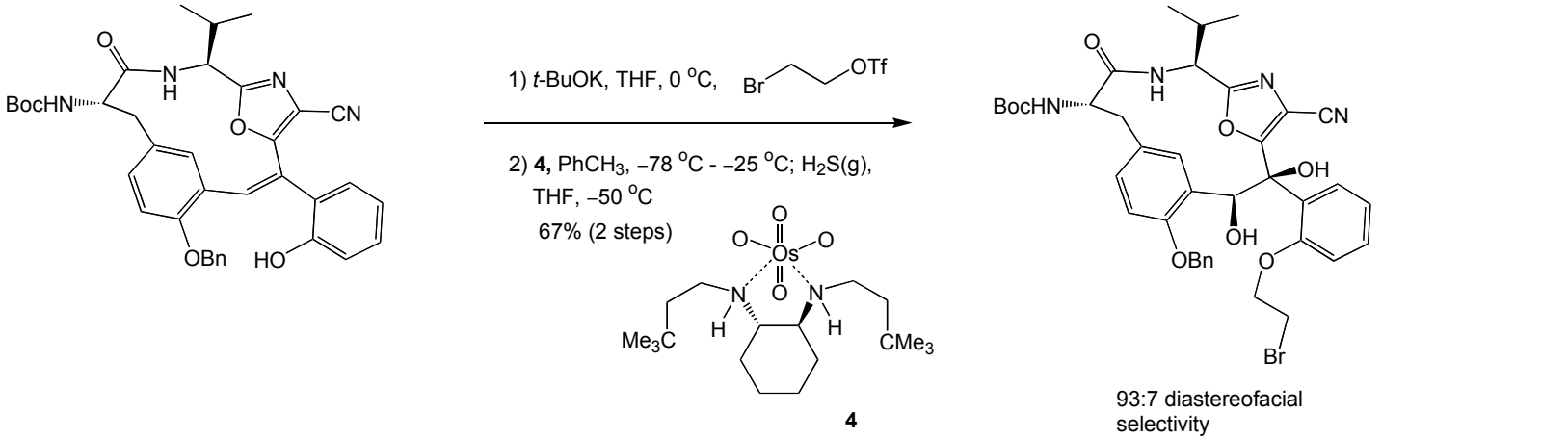
Diazonamide B (Harran, 2001)

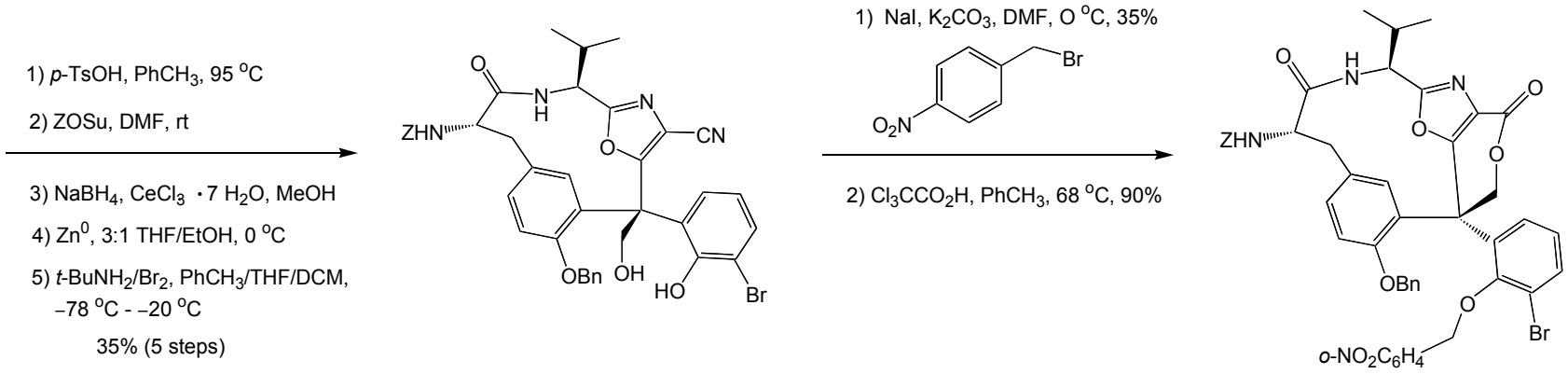
Harran's First Total Synthesis of Diazonamide A



Harran, et al. *Angew. Chem. Int. Ed.* **2001**, *40*, 4765–4769.

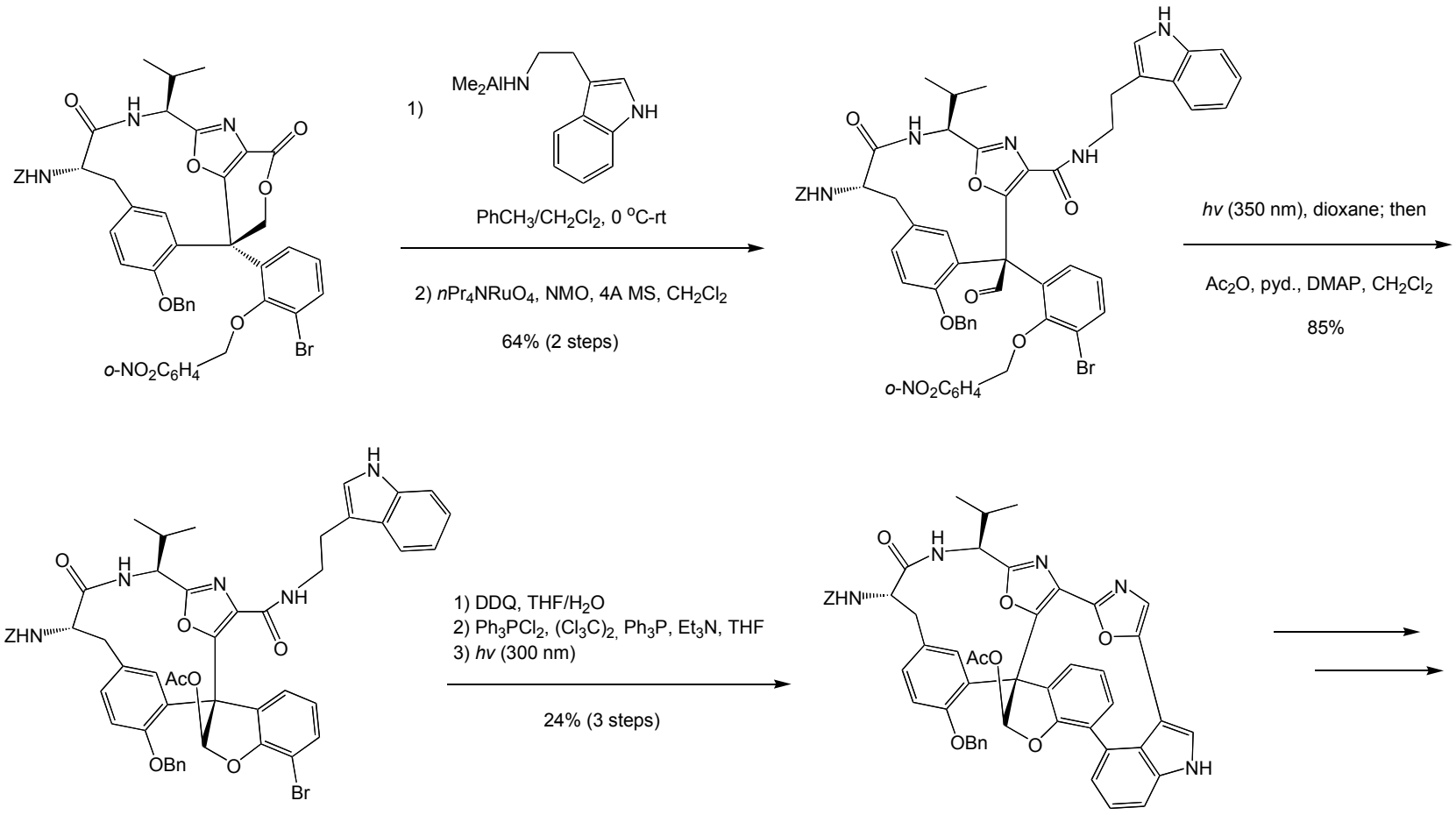
Harran's First Total Synthesis of Diazonamide A





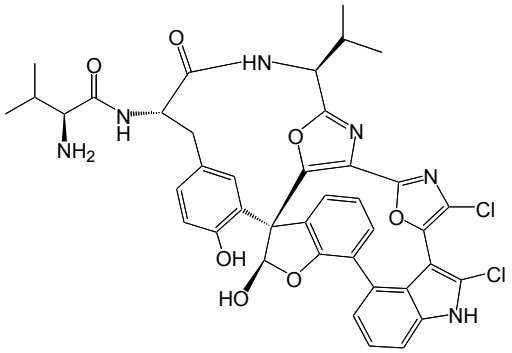
Harran, et al. *Angew. Chem. Int. Ed.* **2001**, *40*, 4765–4769.

Harran's First Total Synthesis of Diazonamide A



Harran, et al. *Angew. Chem. Int. Ed.* **2001**, *40*, 4765–4769.

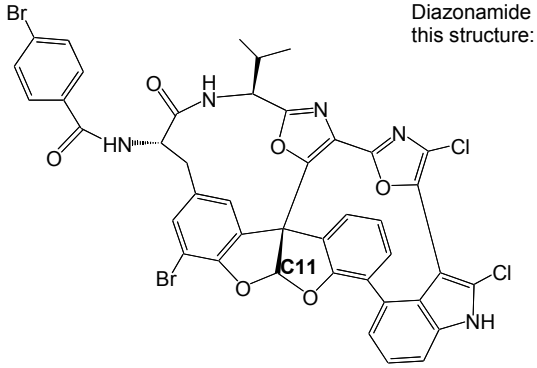
Harran's First Total Synthesis of Diazonamide A



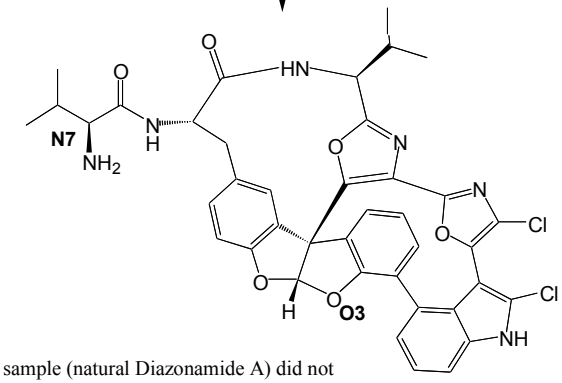
Initially proposed structure of Diazonamide A

- Harran's NMR data did not match that of isolated sample
  - C11 lactol not observed in natural product
- synthetic sample was unstable

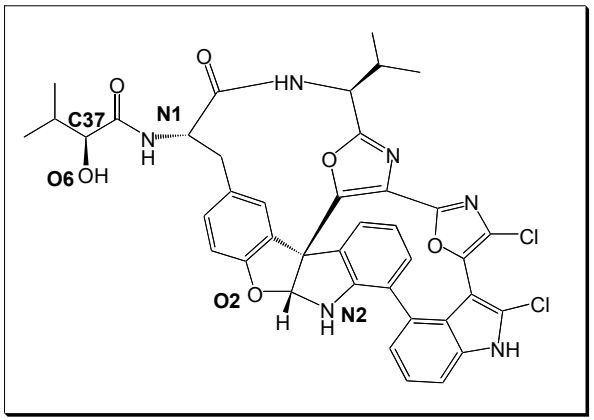
Fenical's X-ray strucuture of Diazonamide B was interpreted as this structure:



- Derivatization of Diazonamide B with p-Bromobenzoyl chloride and HRMS analysis were thought to eliminate water to form the acetal at C11

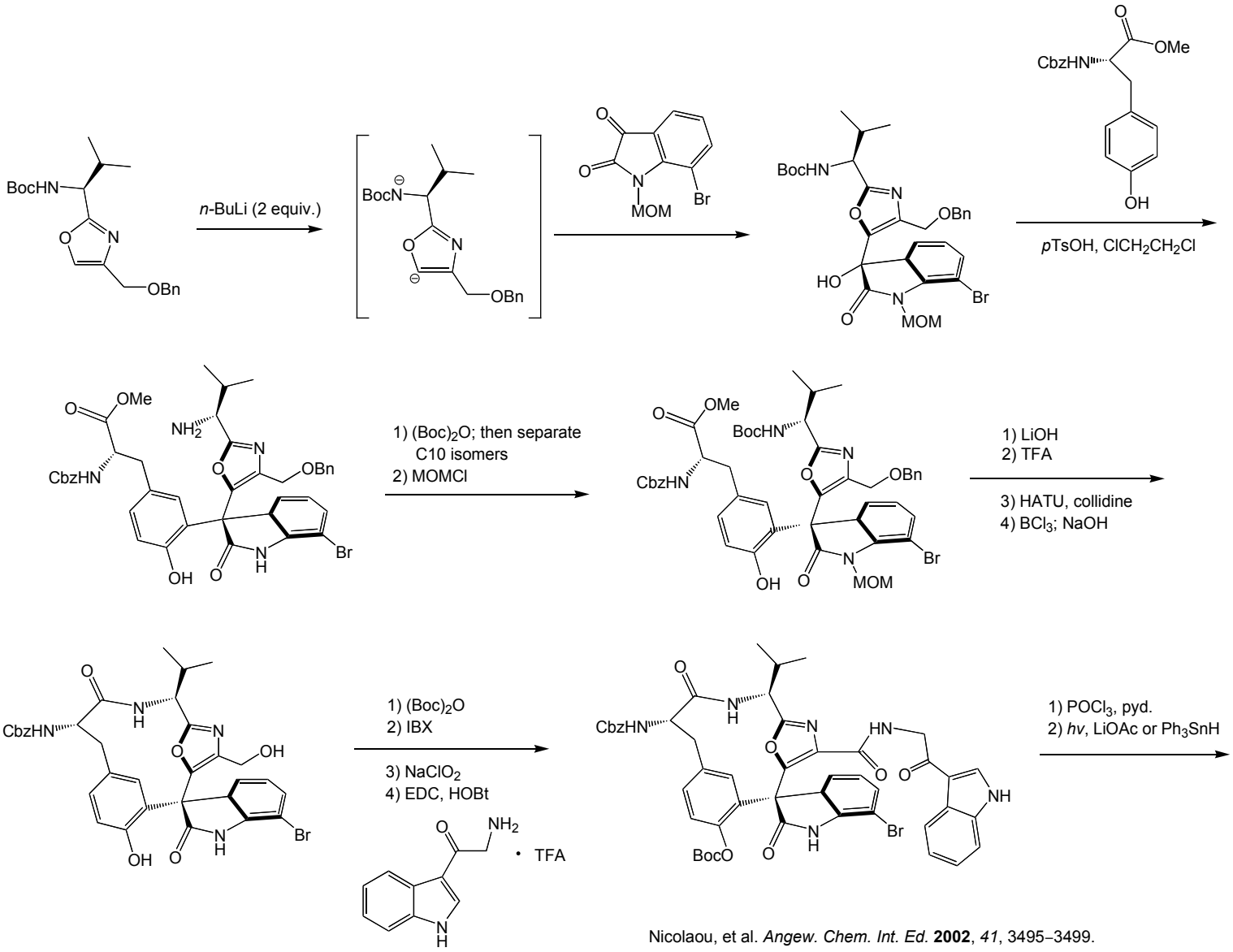


- Acid hydrolysis of a sample (natural Diazonamide A) did not yield valine
  - studies suggested that the amine at N7 should be an OH
- Mass of sample was off by 1Da, which meant that the O3 oxygen in the original crystal strucutre was an NH
- A derivative with (*S*)-hydroxy isovaleric acid yielded biological activity nearly identical to authentic Diazonamide A (>50 times more potent than amine derivative)



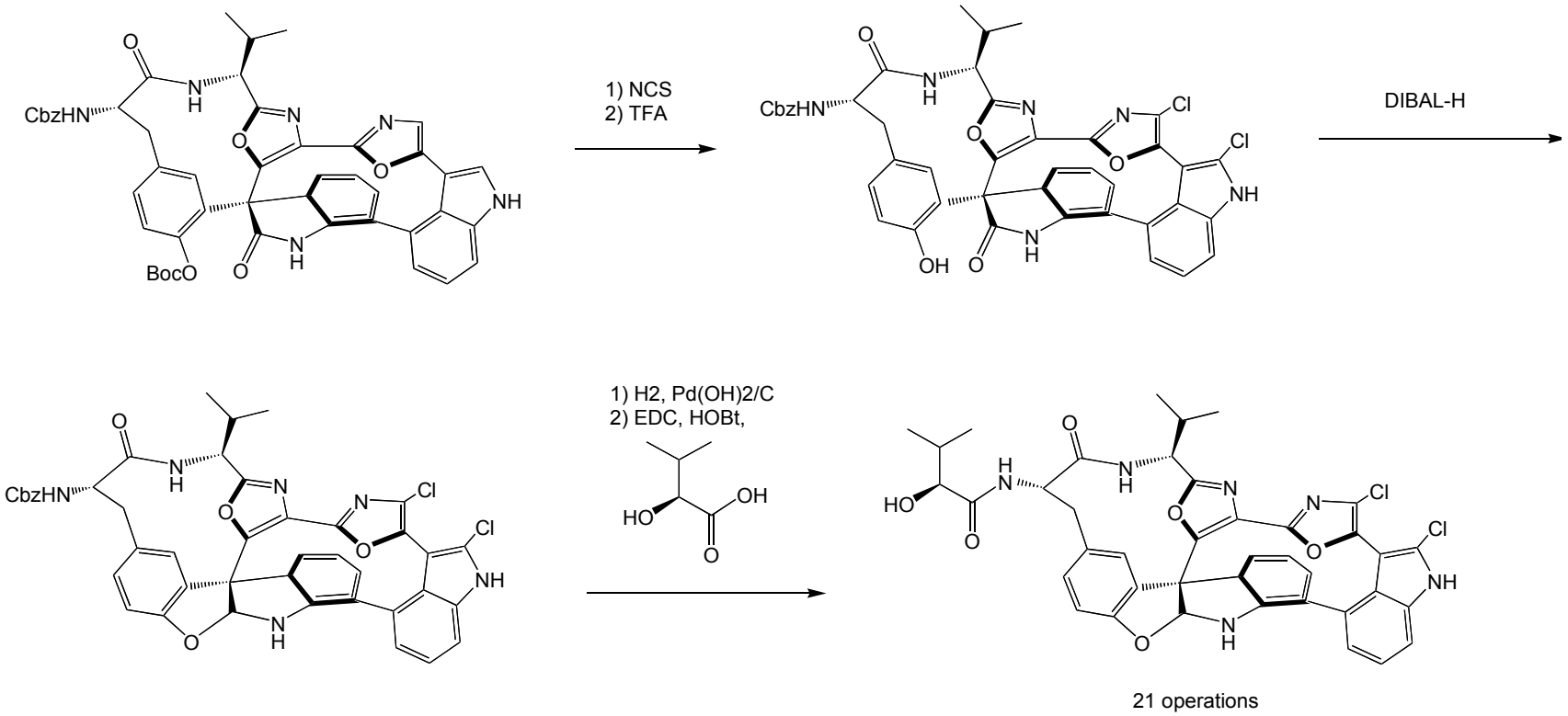
Revised structure of Diazonamide A
(Harran, et al. *Angew. Chem. Int. Ed.* **2001**, *40*, 4770)

Nicolaou's First Total Synthesis of Diazonamide A



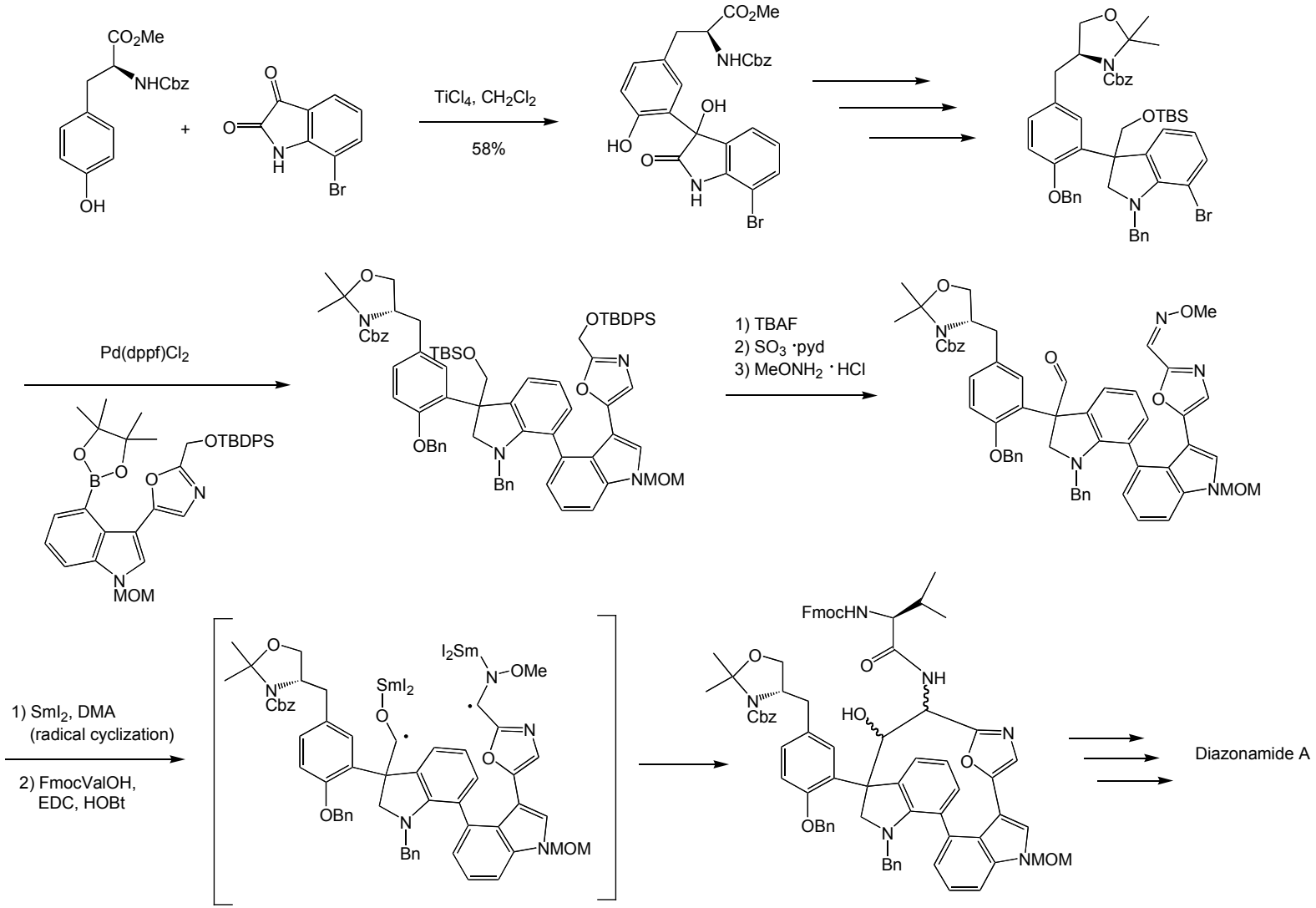
Nicolaou, et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 3495–3499.

Nicolaou's First Total Synthesis of Diazonamide A



Nicolaou, et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 3495–3499.

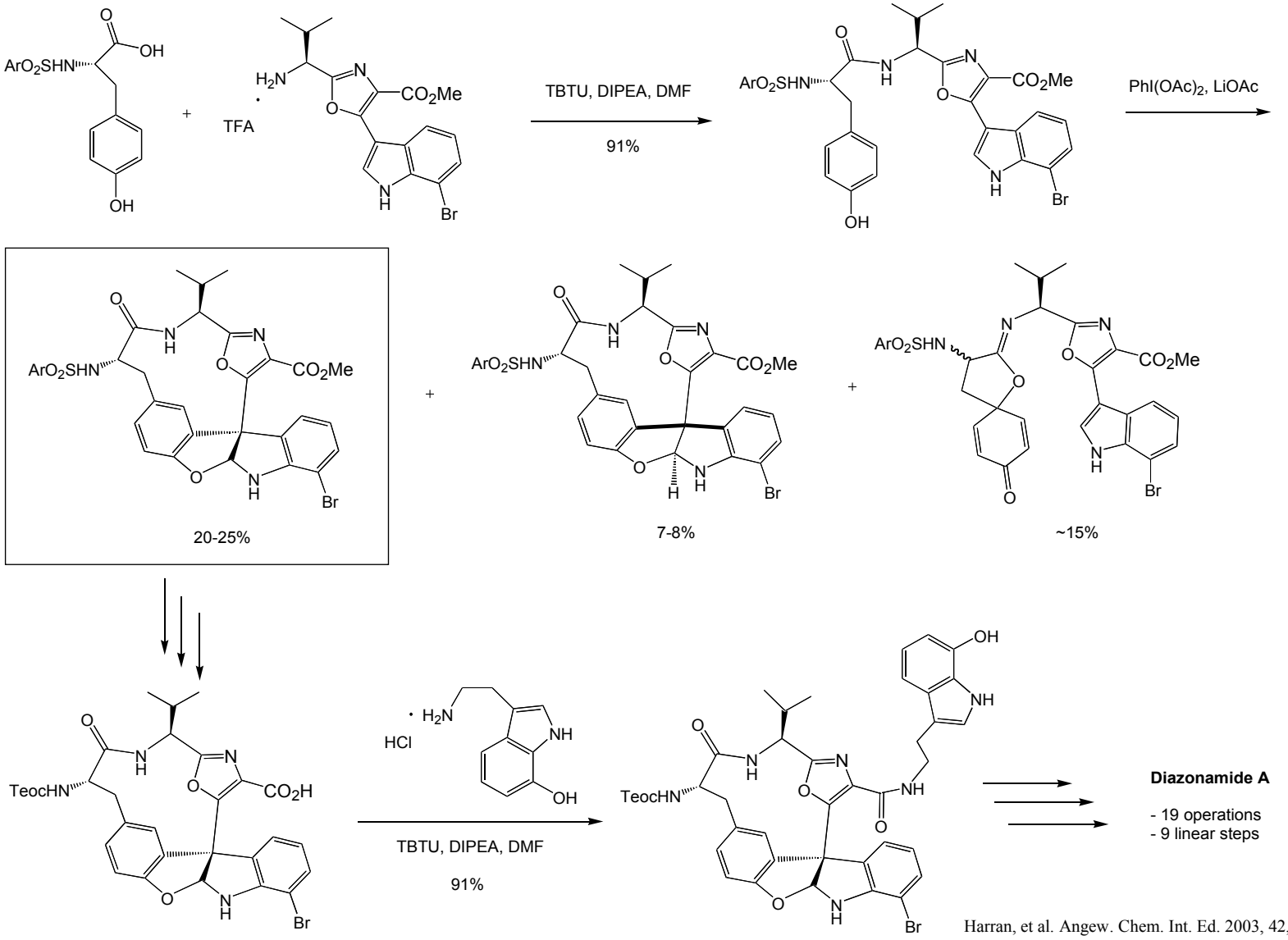
Nicolaou's Second Total Synthesis of Diazonamide A



Nicolaou, et al. *Angew. Chem. Int. Ed.* **2002**, *42*, 1753–1758.

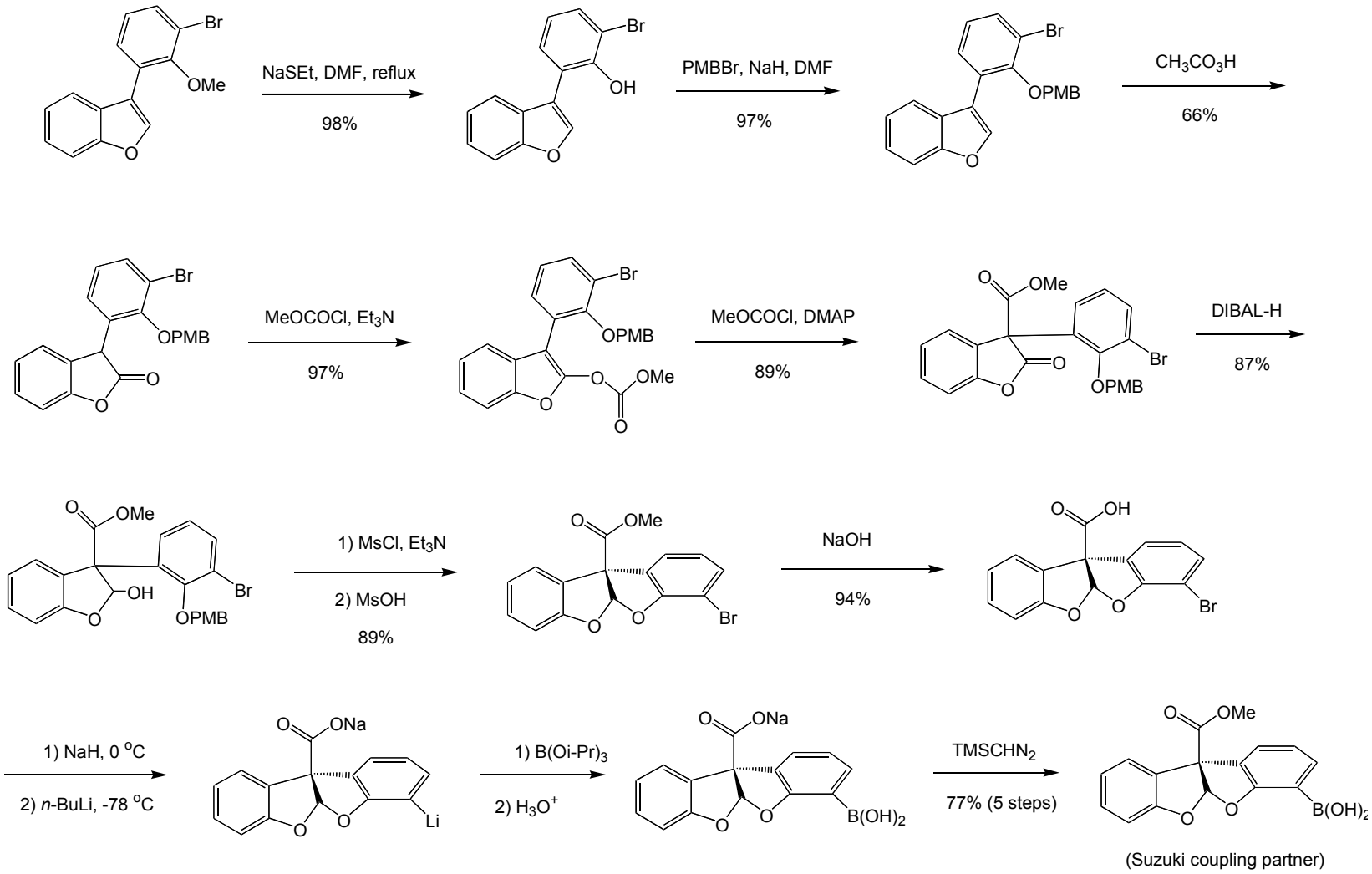
(34 total synthetic operations)

Harran's Second Total Synthesis of Diazonamide A



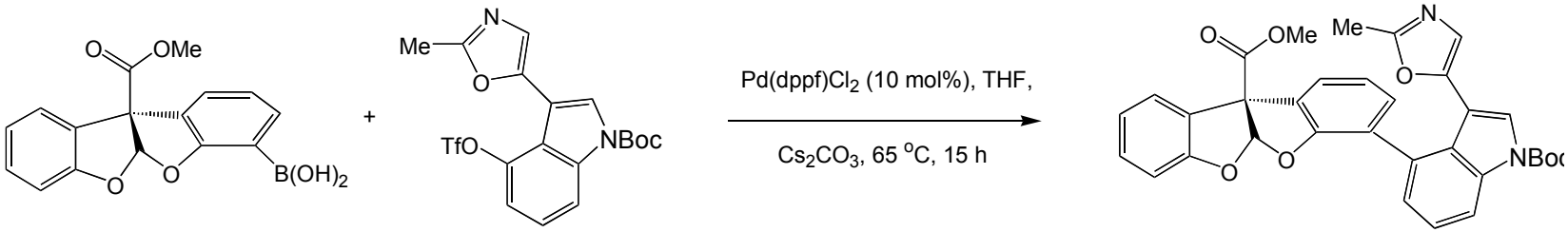
Harran, et al. Angew. Chem. Int. Ed. 2003, 42, 4961–4966

Imino-Dieckmann Cyclization Strategy (Vedejs, et al.)



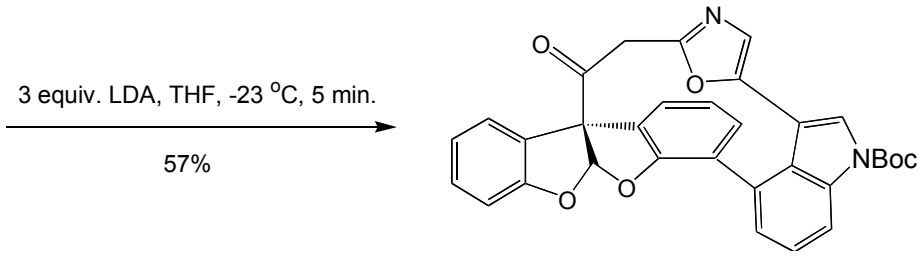
Vedejs, et al. *Org. Lett.* **2001**, *3*, 2451–2454

Imino-Dieckmann Cyclization Strategy (Vedejs, et al.)



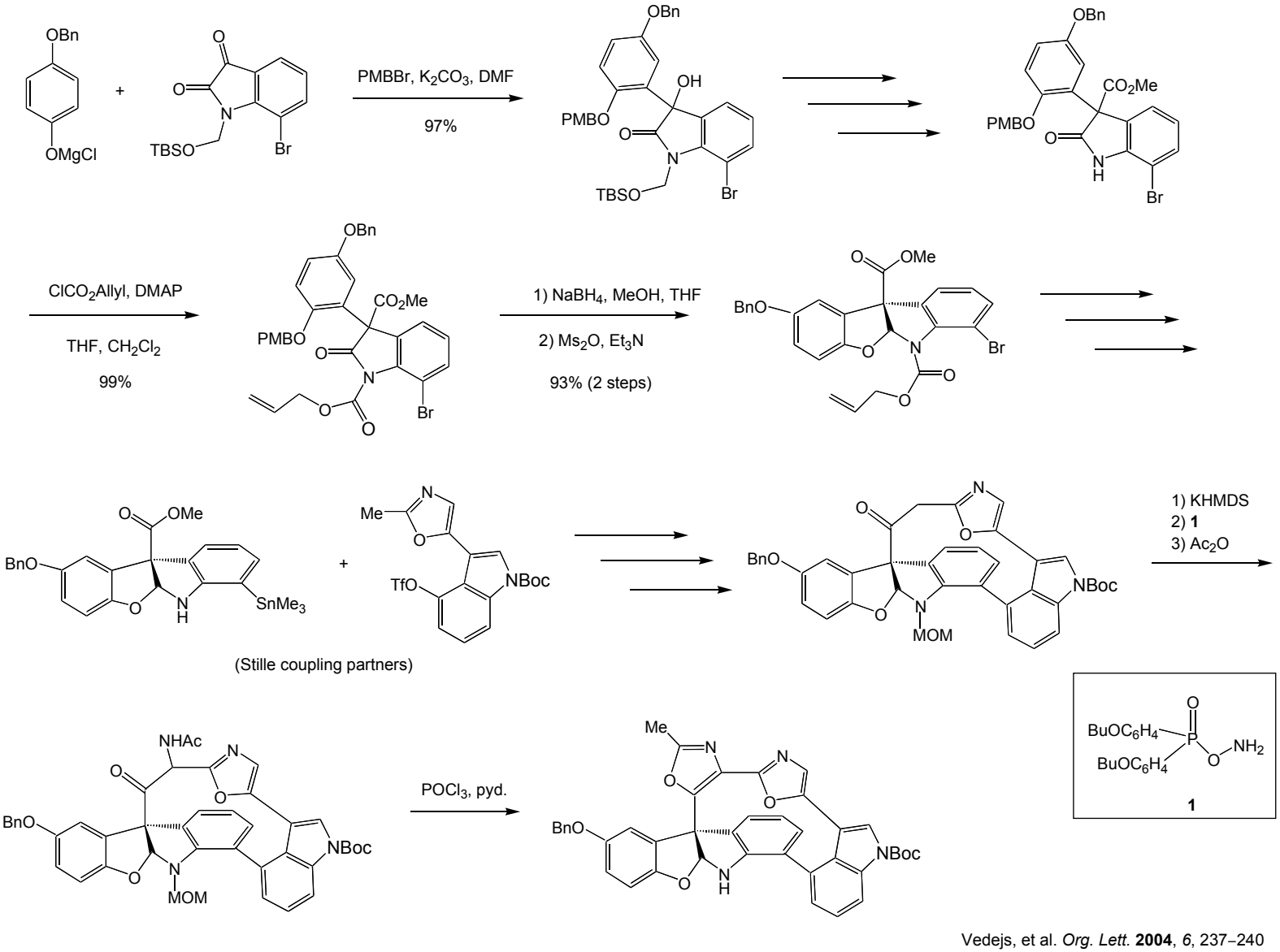
(Suzuki coupling partners)

(atropisomers)



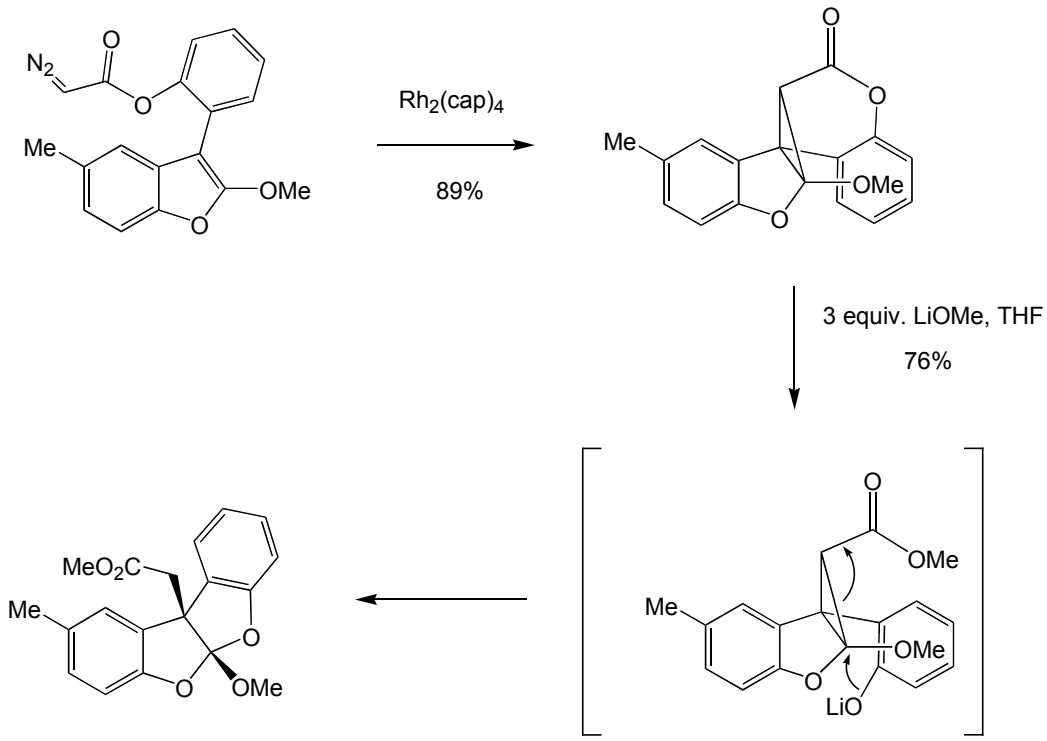
Vedejs, et al. *Org. Lett.* **2001**, *3*, 2451–2454

Imino-Dieckmann Strategy for Revised Structure



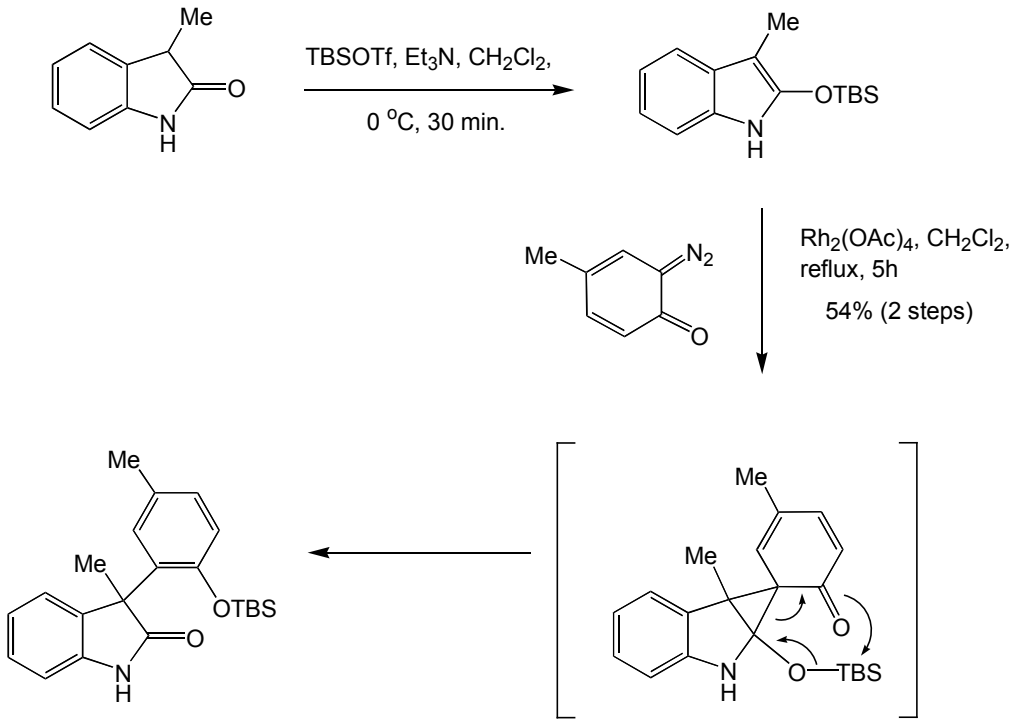
Vedejs, et al. *Org. Lett.* **2004**, *6*, 237–240

Cyclopropanation / Ring-Opening Strategy (Wood, et al.)



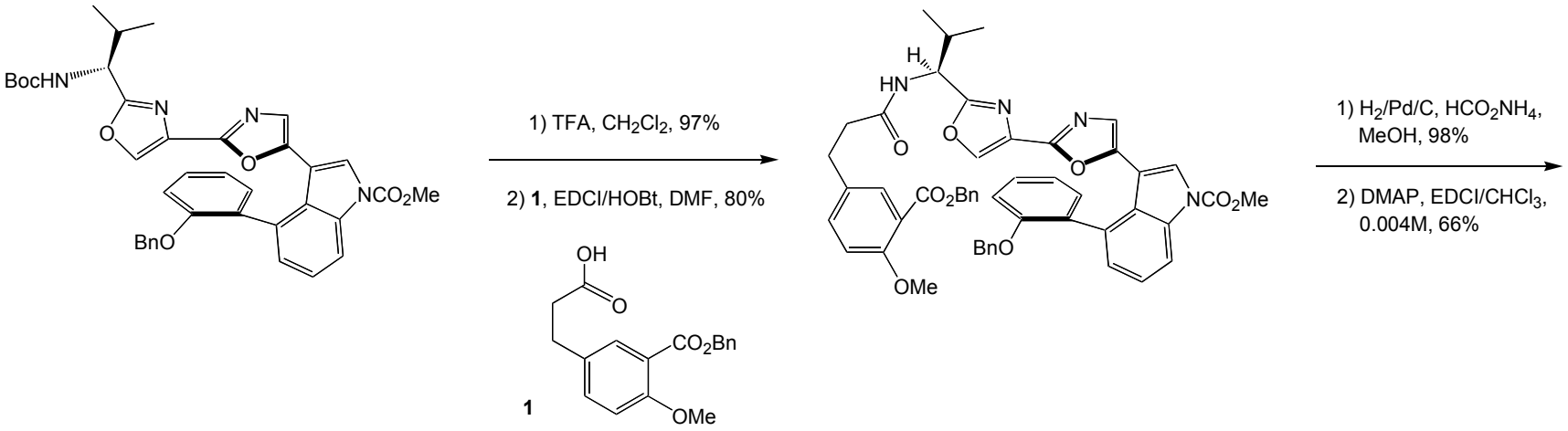
Wood, et al. *Org. Lett.* **2000**, *2*, 3521–3523

Wood's Strategy for Revised Structure



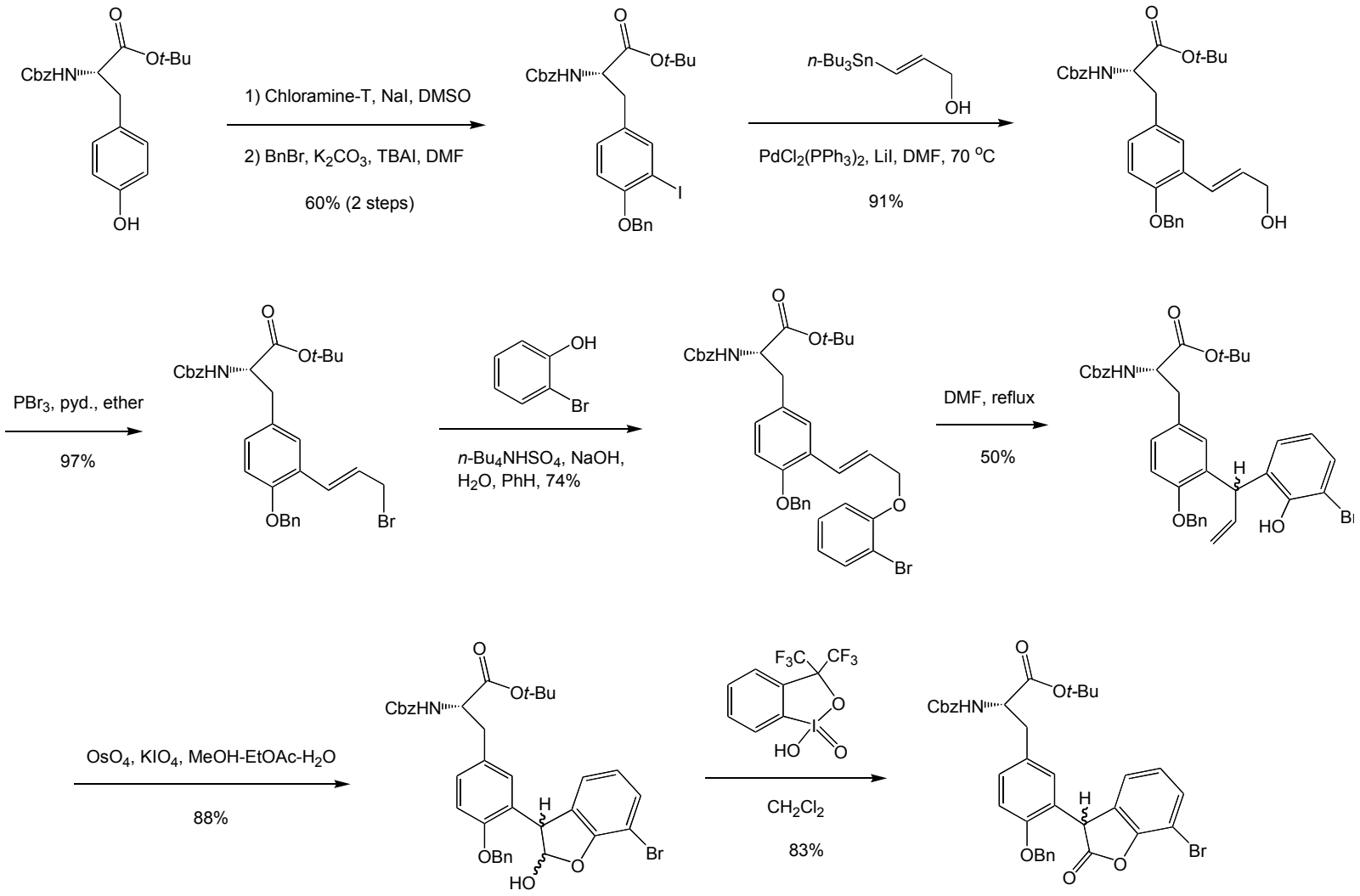
Wood, et al. *Tetrahedron Lett.* **2003**, *44*, 4919–4921

Photo-Fries Rearrangement Strategy (Magnus, et al.)



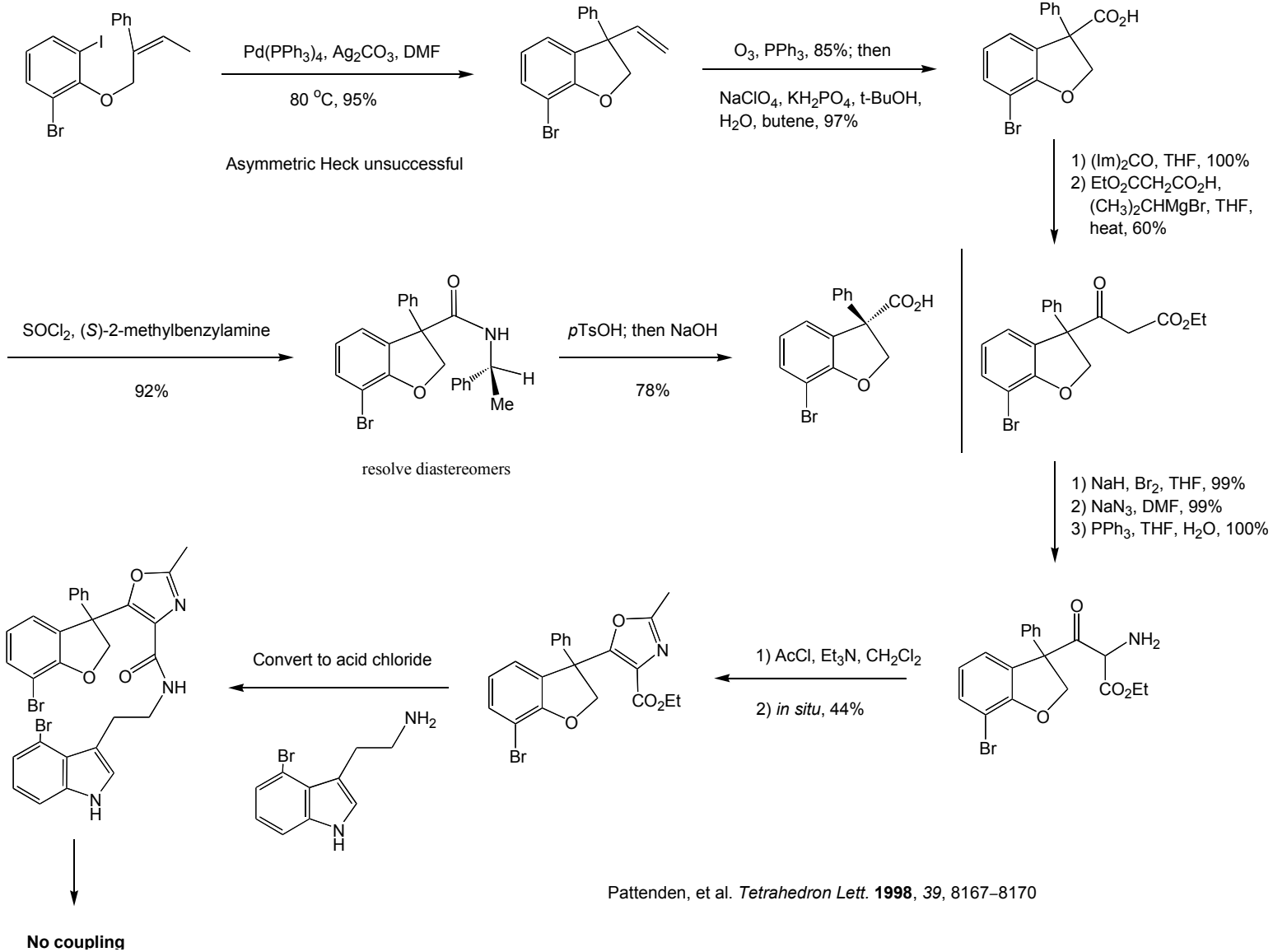
Magnus, et al. *Tetrahedron Lett.* **2001**, *42*, 7193–7196

Claisen Rearrangement Strategy (Moody, et al.)



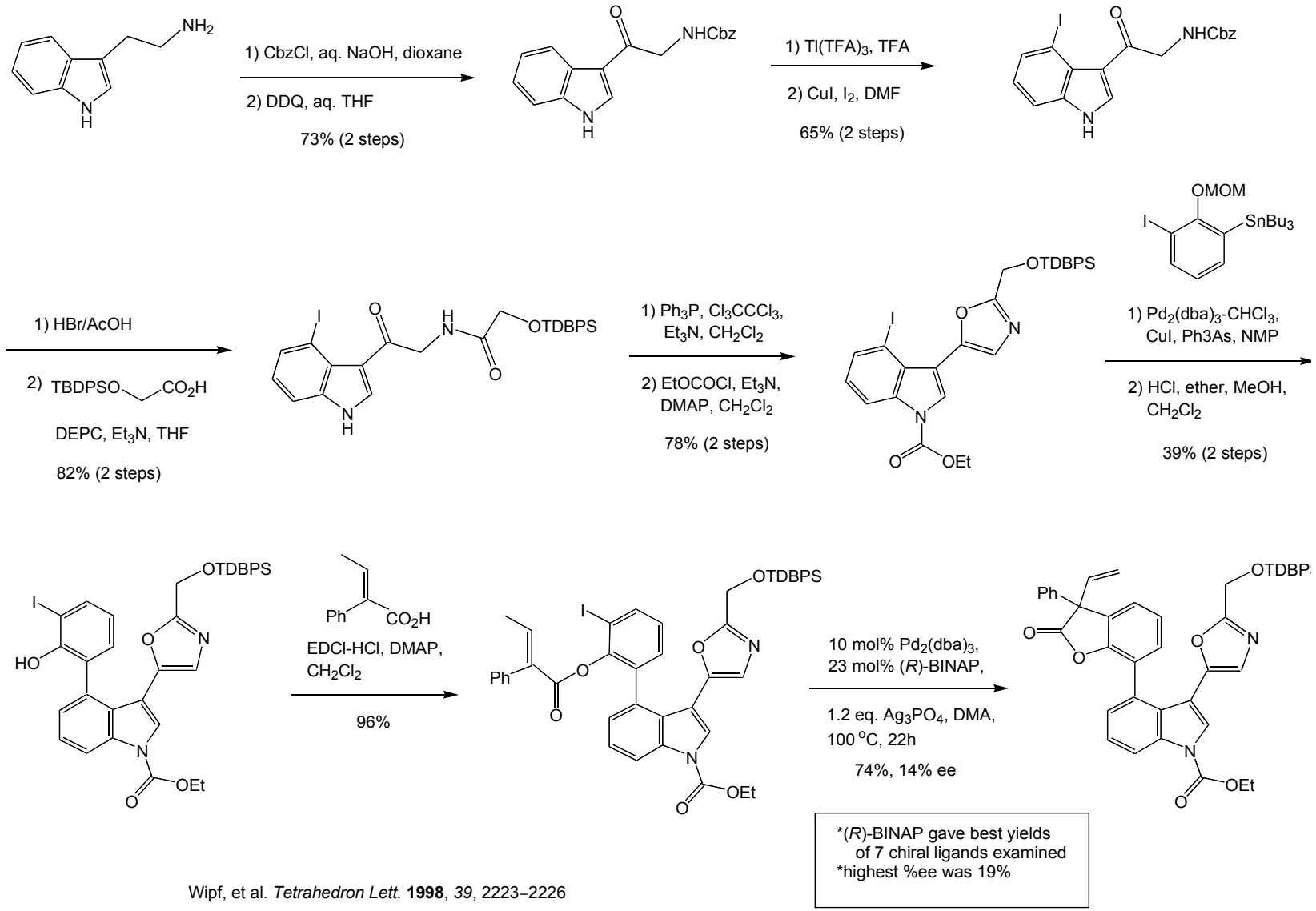
Moody, et al. *Tetrahedron Lett.* **2000**, *41*, 6893–6896

Heck and Ullmann Coupling Strategy (Pattenden, et al.)



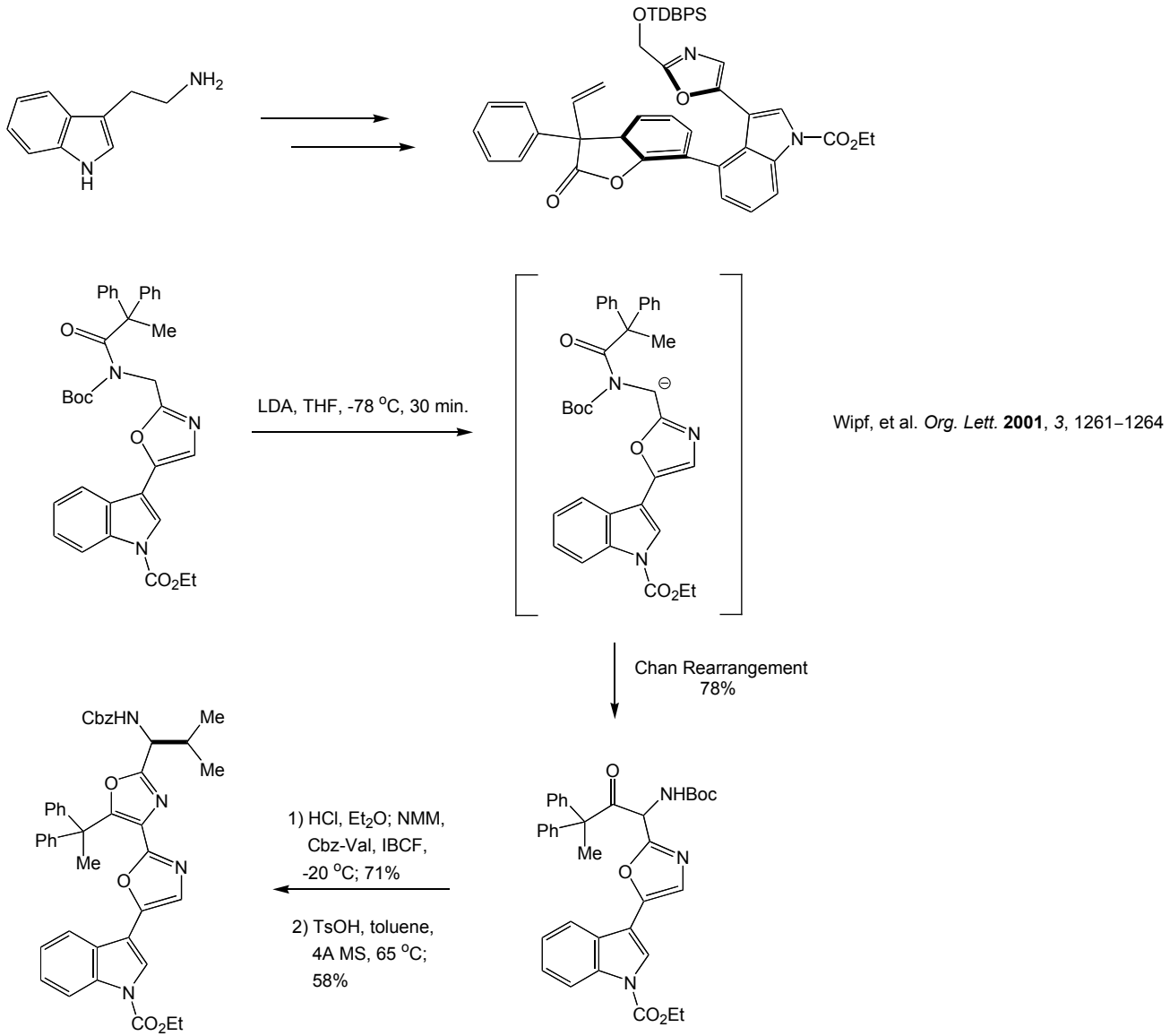
Pattenden, et al. *Tetrahedron Lett.* **1998**, *39*, 8167–8170

Strategies of Former Wipf Group Members (Fumiaki's)



Wipf, et al. *Tetrahedron Lett.* **1998**, *39*, 2223–2226

Strategies of Former Wipf Group Members (Joey's)



Wipf, et al. *Org. Lett.* **2001**, *3*, 1261–1264

The End

Questions?