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

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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
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.

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).

Synthetic Diazonamide A exhibited potent cytotoxic activity against ovarian carcinoma 1A9, breast carcinoma MCS-7 and taxol-resistant 1A9/PTX10 cell lines.

Inhibitor of tubulin assembly (into microtubules), causing cells to accumulate at the G$_2$/M phase of the cell cycle.

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Tubulin and the Assembly of Microtubules

α,β-tubulin dimer

- Proteins are tightly bound by non-covalent bonds
- Bound guanosine triphosphate (GTP) nucleotide (yellow)

Microtubules form the mitotic spindle during mitosis
Microtubule Dynamics

- 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

Cell Cycle
- **Interphase**
  - G₁ phase - cell growth
  - S phase - DNA replication
  - G₂ 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:

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

Biochemical properties of Diazonamide A

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

* Vinca domain – binding site of vinca alkaloids


Studies of Cells Treated with Diazonamide A

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.

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

1) Cp$_2$ZrCl$_2$, nBuLi, THF, $-78$ °C - rt
2) Pd(OAc)$_2$, P(o-tolyl)$_3$, Ag$_3$PO$_4$, 2, rt
85%

1) BBr$_3$, CH$_2$Cl$_2$, $-78$ °C - $-20$ °C
2) 1, DIPEA, TBTU, DMF, rt
89%

Harran’s First Total Synthesis of Diazonamide A

Harran’s First Total Synthesis of Diazonamide A

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

Revised structure of Diazonamide A

- 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 structure 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)

Fenical’s X-ray structure 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
Nicolaou’s First Total Synthesis of Diazonamide A

1) (Boc)$_2$O; then separate C10 isomers
2) MOMCl

1) LiOH
2) TFA
3) HATU, collidine
4) BCl$_3$, NaOH

1) (Boc)$_2$O
2) IBX
3) NaClO$_2$
4) EDC, HOBt

1) POCl$_3$, pyd.
2) hv, LiOAc or Ph$_3$SnH

Nicolaou’s First Total Synthesis of Diazonamide A

Nicolaou’s Second Total Synthesis of Diazonamide A


(34 total synthetic operations)
Harran’s Second Total Synthesis of Diazonamide A


Diazonamide A

- 19 operations
- 9 linear steps

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

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

(Suzuki coupling partners)

Pd(dppf)Cl₂ (10 mol%), THF,
Cs₂CO₃, 65 °C, 15 h

(atropisomers)

3 equiv. LDA, THF, -23 °C, 5 min.

57%

Imino-Dieckmann Strategy for Revised Structure

1) NaBH₄, MeOH, THF

(Stille coupling partners)

1) KHMDS
2) 1
3) Ac₂O

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

Wood’s Strategy for Revised Structure

\[ \text{Me} \text{H} \text{N} \text{O} \text{Rh}_2(\text{OAc})_4, \text{CH}_2\text{Cl}_2, \text{reflux, 5h} \]

54% (2 steps)

\[ \text{Me} \text{H} \text{N} \text{OTBS} \]

\[ \text{Me} \text{N}_2 \text{O} \text{Me} \text{N} \text{H} \text{O} \text{Me} \text{TBS} \]

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

1) TFA, CH₂Cl₂, 97%
2) 1, EDCI/HOBt, DMF, 80%

1) H₂/Pd/C, HCO₂NH₄, MeOH, 98%
2) DMAP, EDCI/CHCl₃, 0.004M, 66%

Claisen Rearrangement Strategy (Moody, et al.)

1) Chloramine-T, NaI, DMSO
2) BnBr, K$_2$CO$_3$, TBAI, DMF
60% (2 steps)

PdCl$_2$(PPh$_3$)$_2$, LiI, DMF, 70 ºC
91%

n-Bu$_3$Sn, pyd., ether
97%

n-Bu$_4$NHSO$_4$, NaOH, H$_2$O, PhH, 74%

DMF, reflux
50%

OsO$_4$, KIO$_4$, MeOH-ETOAc-H$_2$O
88%

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

Asymmetric Heck unsuccessful

SOCl₂, (S)-2-methylbenzylamine

Resolve diastereomers

Convert to acid chloride

No coupling

Strategies of Former Wipf Group Members (Fumiaki’s)

1) CbzCl, aq. NaOH, dioxane
2) DDQ, aq. THF
73% (2 steps)

1) Ti(TFA)3, TFA
2) Cul, I2, DMF
65% (2 steps)

1) HBr/AcOH
2) TBDPSO-CONHCO2H
82% (2 steps)

1) Pd2(dba)3-CHCl3, CuI, Ph3As, NMP
2) HCl, ether, MeOH, CH2Cl2
39% (2 steps)

*Highest %ee was 19%
Strategies of Former Wipf Group Members (Joey’s)

1) LDA, THF, -78 °C, 30 min.

2) TsOH, toluene, 4A MS, 65 °C; 58% 


Chem Rearrangement 78%

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The End

Questions?