Syntheses of the Kinamycin Family

Insights into natural diazo containing benzo[b]fluorenes

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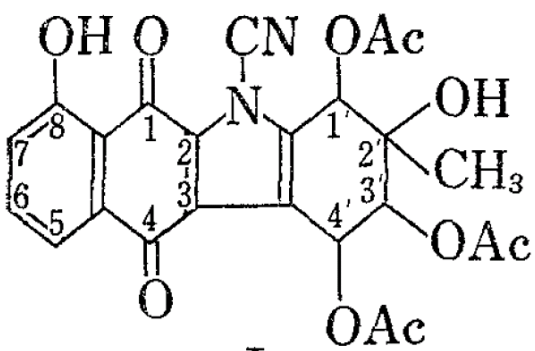
SED Group
Mar 16 2010

An Overview

- Isolation and characterization of the kinamycins, diazofluorene-containing natural products.
- Structural Reassignments
- Biological profile
- Total syntheses : 3 enantioselective and 1 racemic
- Efforts towards the synthesis of their dimer analogues

Meet the Kinamycins

- First isolated in 1970 by Omura et. al. from Streptomyces murayamaensis.
- Kinamycin family displays anti-tumor effects and is a potent Gram-positive antibiotic
- Assigned based on derivatization studies, IR, NMR (^{1}H and ^{13}C), X-Ray
- Initially assigned as an N-nitrile (cyanamide)
- Cyanamide group postulated based on X-ray data on a
  d ation 60 cm^{-1}
- " d frc



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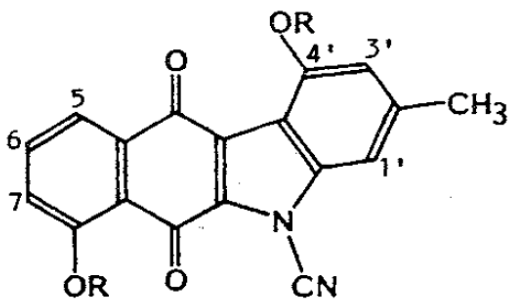
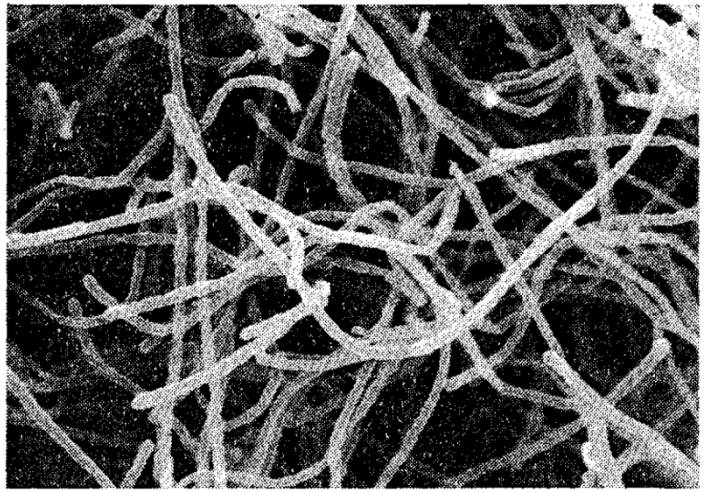
Kinamycin C

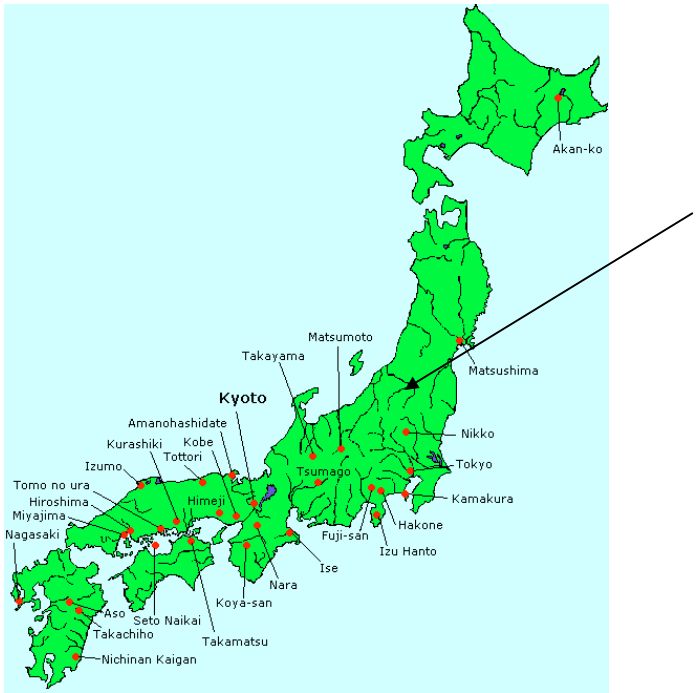


"Prekinamycin"

Omura et. al., J. Antibiot., **1970**, 23, 315
Omura et. al., Chem. Pharm. Bull., **1973**,
Gould et. al., J. Antibiot., **1989**, 42, 189

Isolation





Streptomyces murayamaensis, isolated from soil samples near Murayama in Japan

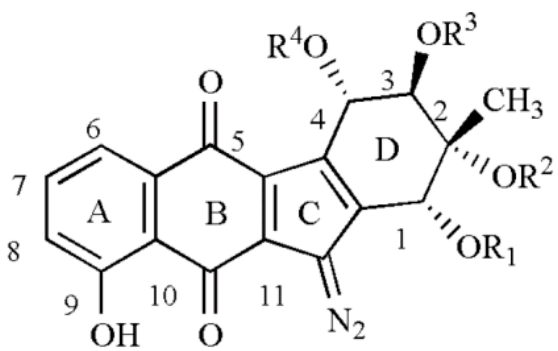
An actinomycete, largest class of antibiotic producing bacteria

Spore former.

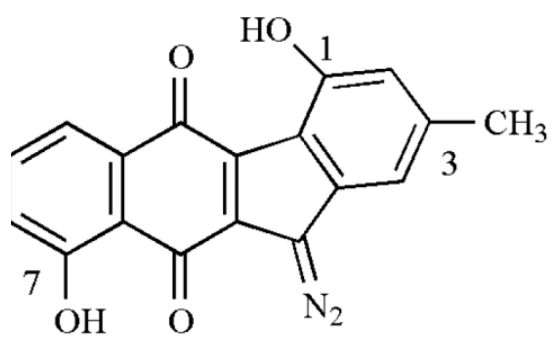
Omura et. al., J. Antibiot., **1970**, 23, 315
Omura et. al., Chem. Pharm. Bull., **1973**, 21
Gould et. al., J. Antibiot., **1989**, 42, 189
Image from www.japannet.de/japan/japan3

Now with Improved Spectral Data

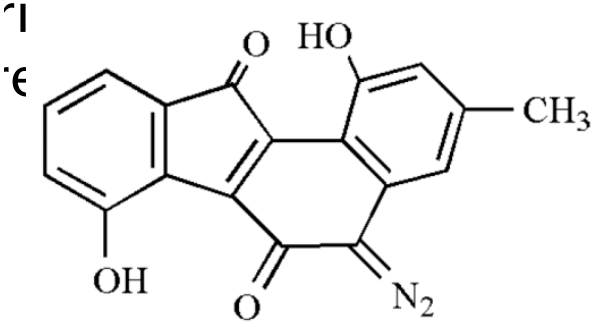
- Gould and Dmitrienko independently reassigned structure.
- Anomalous shift of nitrile carbon at 78 ppm (expected at ~110 ppm)
- IR absorbance of synthetic relatives did not match the nitrile signal.
- Echavarren synthesized presumed prekinamycin, inconsistent with proposed structure (was later renamed isoprekinamycin)



1 Kinamycin A (R^1= H, R^2= R^3= R^4= Ac)
2 Kinamycin B (R^1= R^3= R^4 = H, R^2= Ac)
3 Kinamycin C (R^1= R^3= R^4 = Ac, R^2= Ac)
4 Kinamycin D (R^1= R^3= Ac, R^2= R^4 = H)
5 Kinamycin E (R^1= Ac, R^2= R^3 = R^4 = H)
6 Kinamycin F (R^1= R^2= R^3 = R^4 = H)



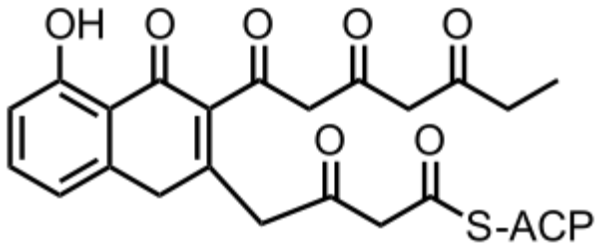
Prekinamycin (**9**)



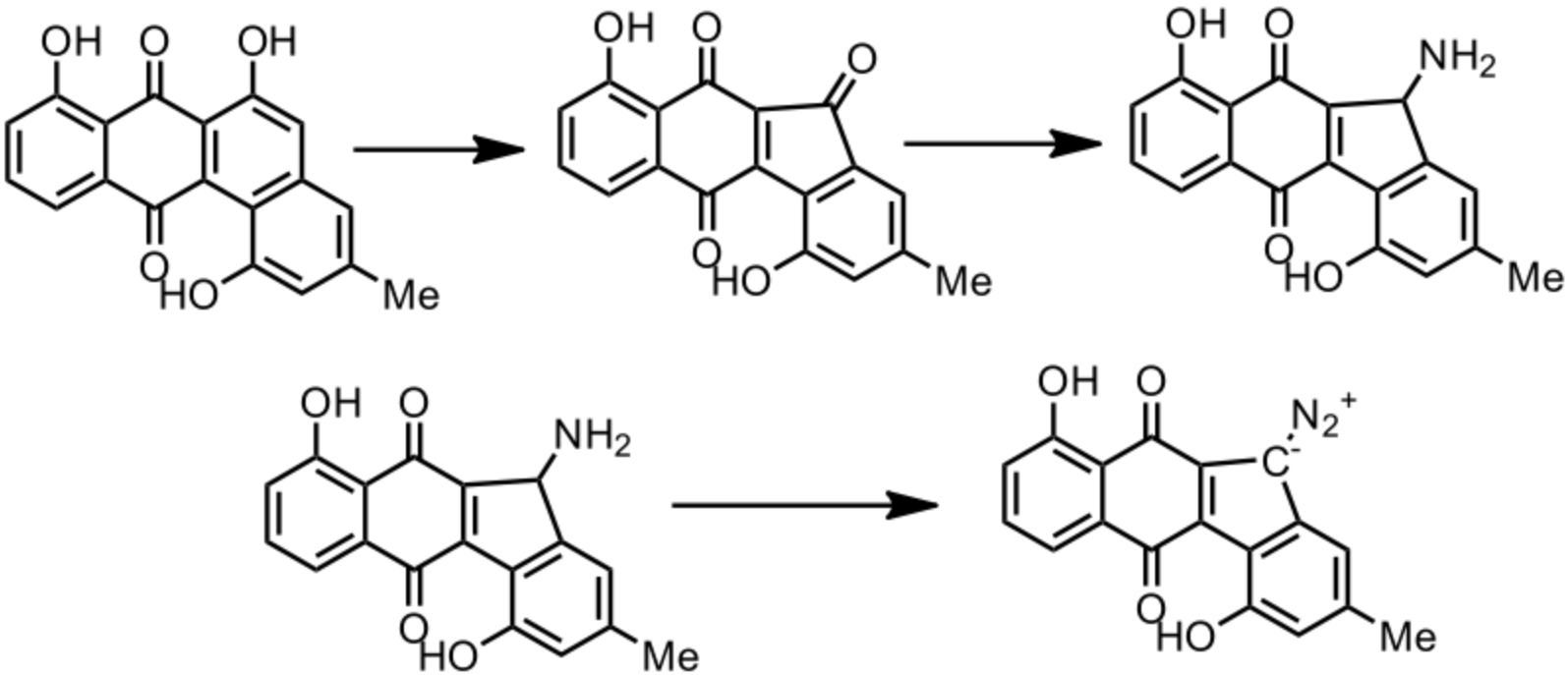
Isoprekinamycin (**10**)

Gould et. al., JACS, **1994**, 116, 2207
Dmitrienko et. al., JACS, **1994**, 116, 2209
Echavarren et. al. Tet. Lett., **1993**, 34,

Hypothesized Biosynthesis



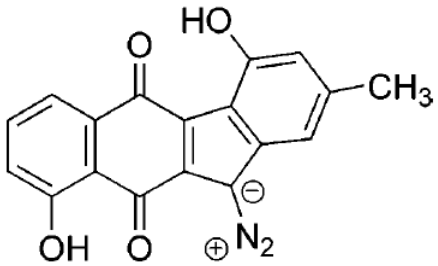
7,9,12-decaketide cyclization precursor

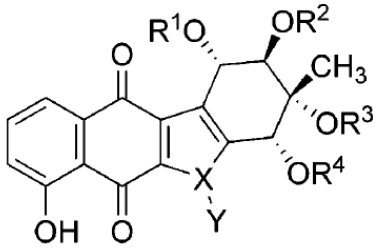


Annotated from the KEGG database

http://www.genome.jp/kegg-bin/show_pathway?rn01057+R06673

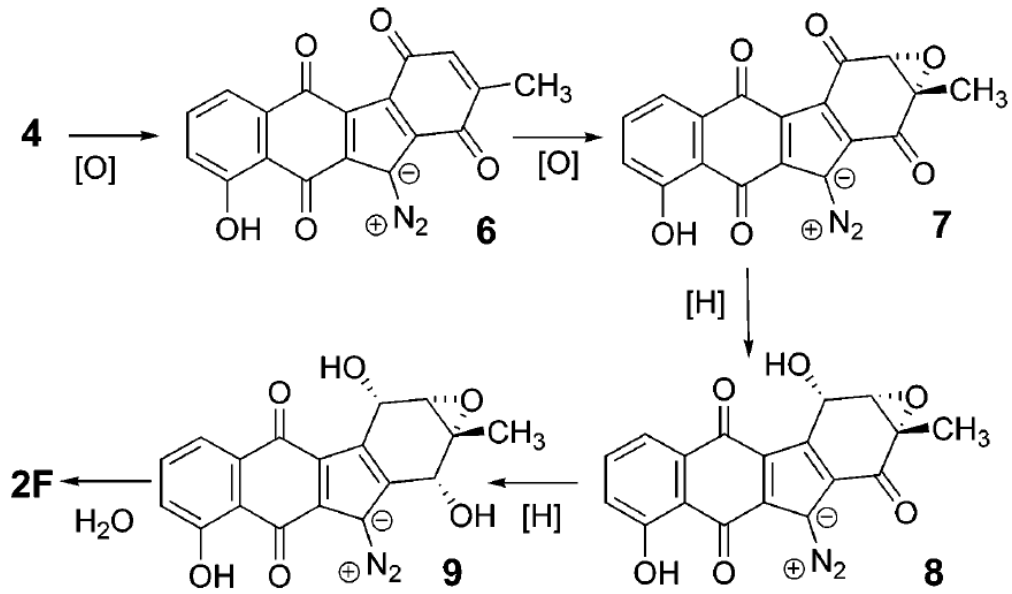
Hypothesized Biosynthesis





1 X = N; Y = CN
2 X = C⊖ ; Y = N_2⊕

	R^1	R^2	R^3	R^4
A:	H	Ac	Ac	Ac
C:	Ac	Ac	H	Ac
F:	H	H	H	H
J:	Ac	Ac	Ac	Ac



4, 8, O-acyl derivatives of 9 have been isolated.

Fairly extensive elaboration after diazotization.

Dmitrienko et. al., Org. Lett., **2008**, 10, 381

An Overview

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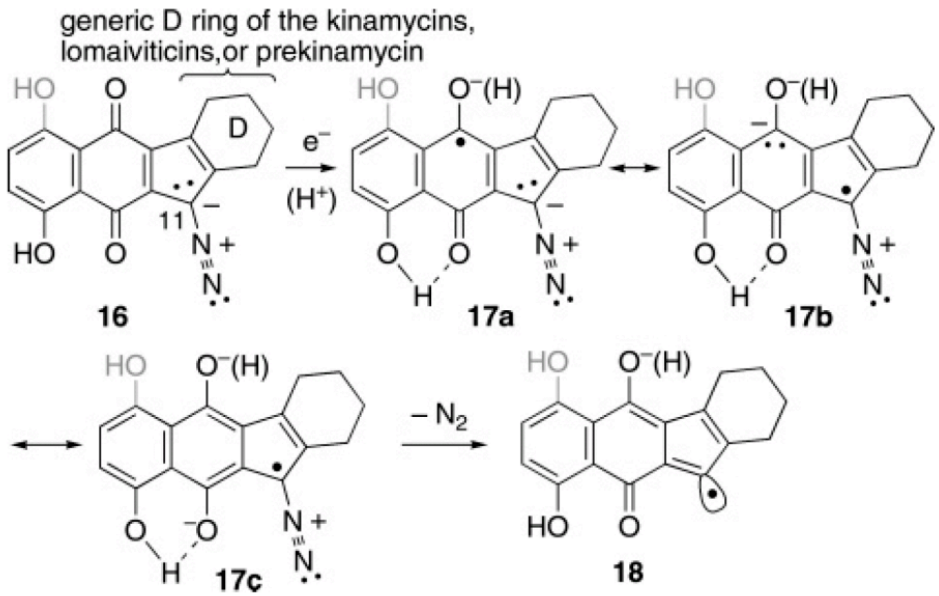
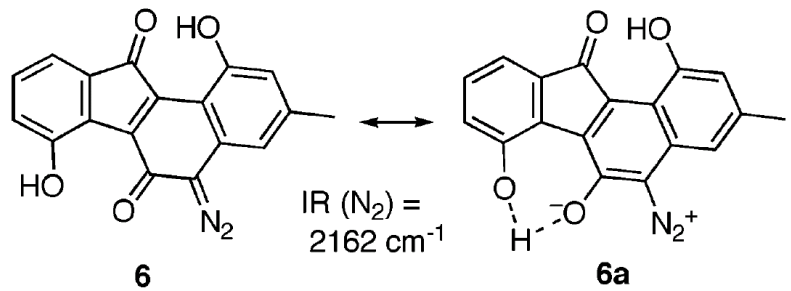
Structure and Biological Activity

- Weak binding to DNA (K_{app} = 90 μM)
- Unique activity against cancer cell line assay (IC_{50} < 1μM)
- Produces radicals under physiological conditions which cause DNA nicks.



Feldman et. al., JACS, **2006**, 128, 12562
Arya D. P., Top Heterocycl. Chem., **2006**, 2, 129
152
Hasinoff et. al. , Free Radical Bio. Med., **2007**,

Dmitrienko's Work



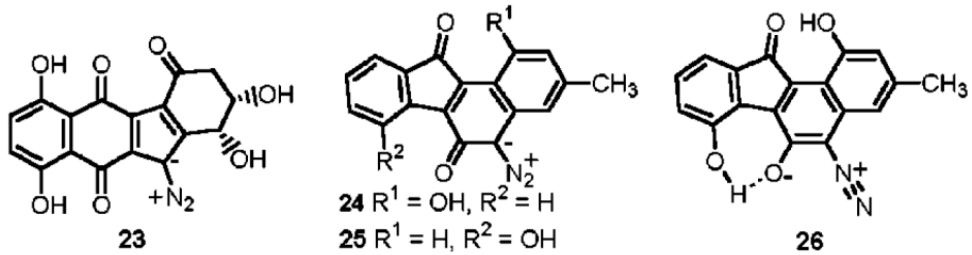


Table 1. Calculated C−N_2 Frequencies and N−N Bond Lengths

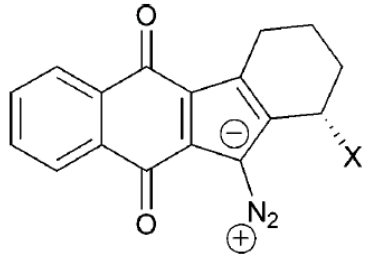
compound	calcd ν (cm^{-1})	calcd N–N (Å)
9-diazofluorene	1906	1.133
2,1-naphthoquinodiazide	2056	1.111
4	2087	1.108
24	2101	1.107
25	2125	1.105
2	2139	1.103
kinamycin B (**1**)	2188	1.099
23	2212	1.097
Ph-N≡N^+ Cl^-	2212	1.100

H-bonding proposed as method to increase diazonium character in isoprekinamycin.

Computational studies show significant diazonium character

Dmitrienko et al., JACS, **2002**, 124, 18
Feldman et. al., JACS, **2006**, 128, 1256

Conformational Analyses



X (equatorial) =	H	OAc	F	OH
ν_{N-N} (cm^{-1})	2104	2123	2158	2173
r_{N-N} (Å)	1.107	1.105	1.102	1.100

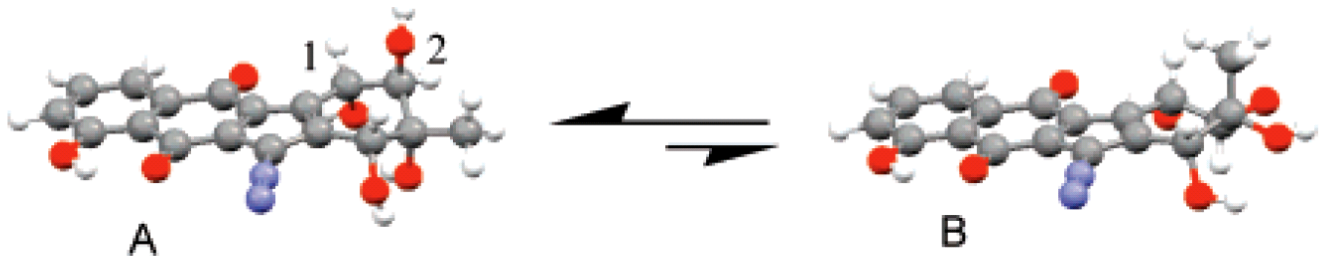


Figure 2. Calculated energy minimum conformations of kinamycin F.

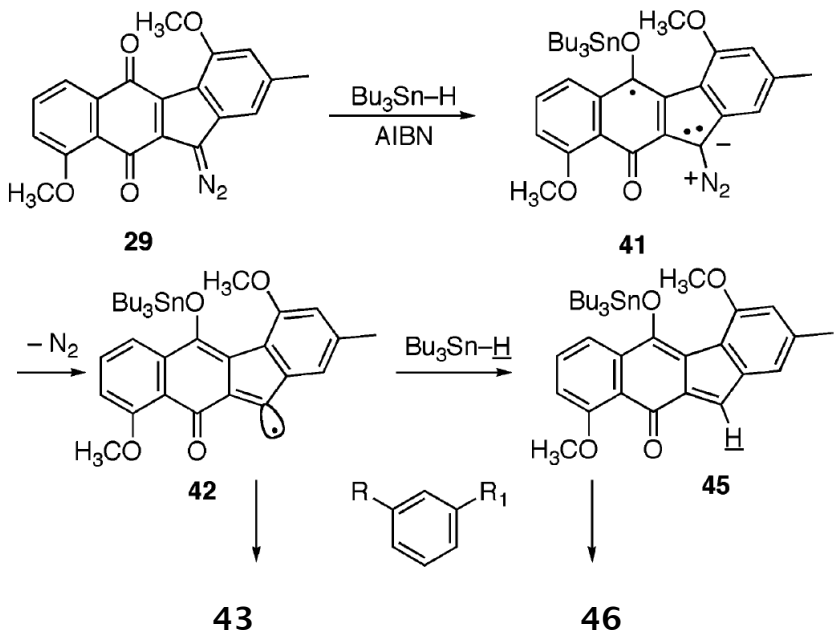
- Studies on Kinamycin derivatives by Dmitrienko.
- Favorable dipole interaction of C–N bond of the diazo group and the C–O ring bond postulated as possible reason for this conformational preference.
- Other kinamycins prefer conformation B
- Dmitrienko proposes that kinamycins get deacetylated to kinamycin F in vivo before displaying cytotixic activity based on the superior activity of kinamycin F

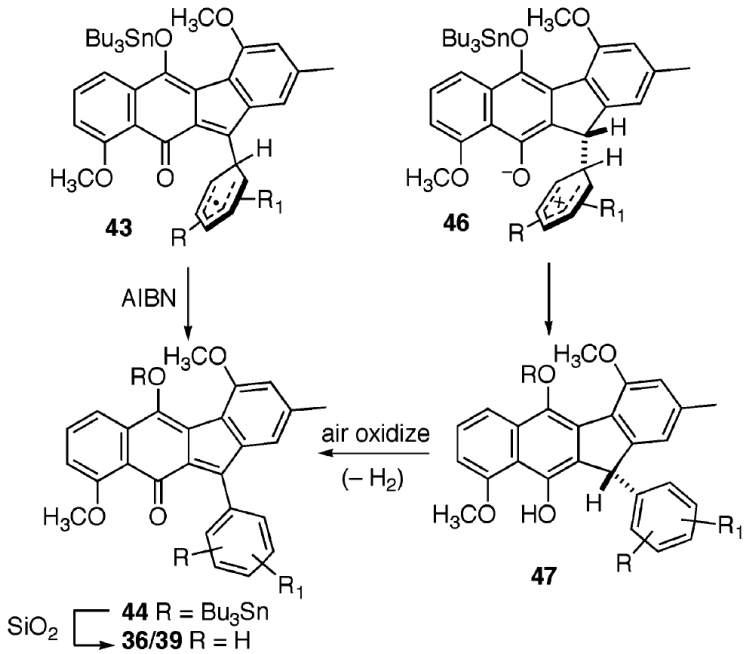
Dmitrienko et. al., Org. Lett., **2008**, 10,

Feldman's Mechanistic studies

Mechanism of Action of Prekinamycin

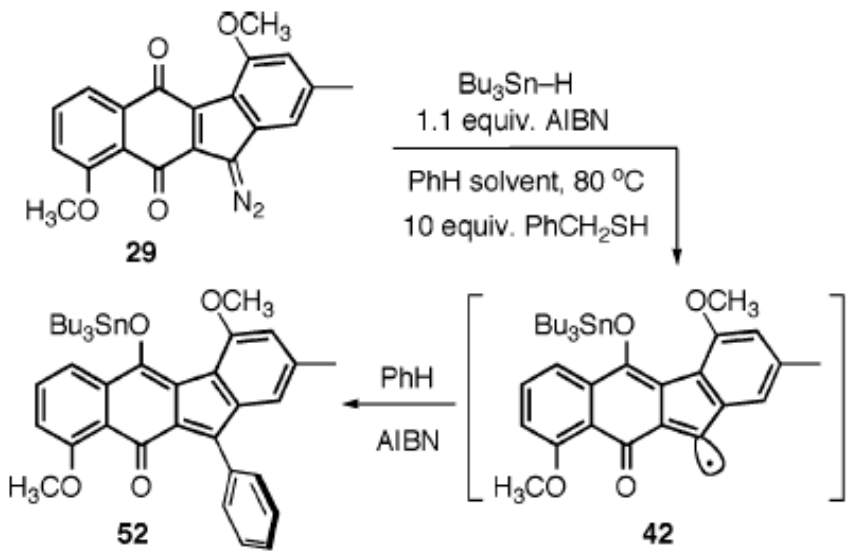
Scheme 5. Mechanistic Proposal for the Formation of Arene Adducts **36/39** from Diazoparaquinone **29**

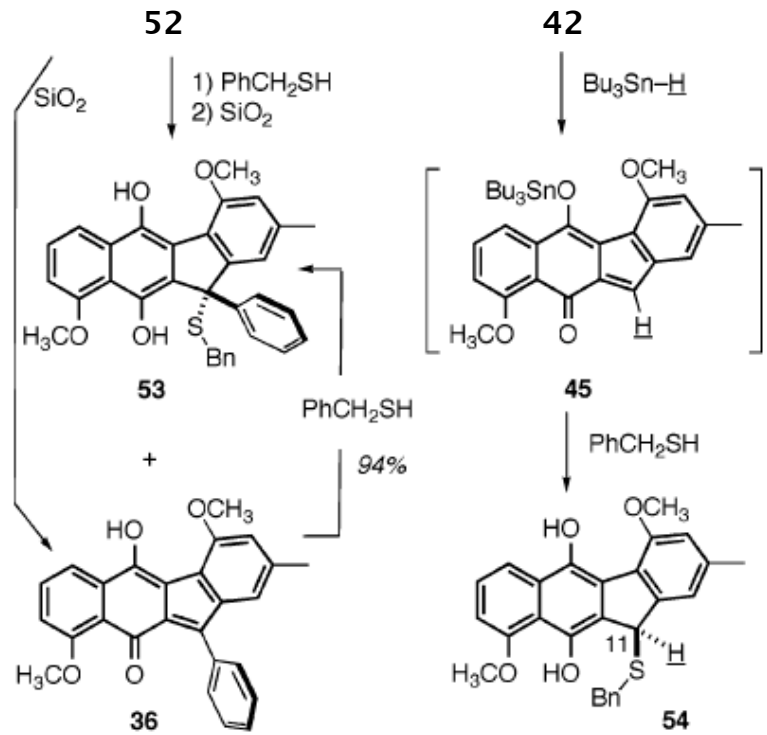




Feldman et. al., JACS, **2006**, 128, 125

Feldman's Mechanistic Studies





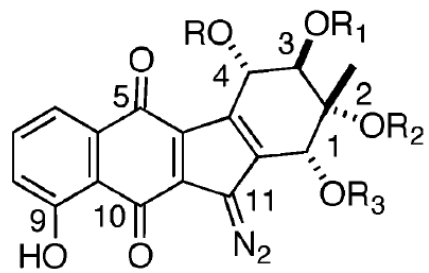
equiv. $Bu_3Sn–H$	total yield	**53** (%)	**36** (%)	**54** (%)
1.1	*83%*	*22%*	*15%*	*46%*
12	*90%*	*23%*	*10%*	*57%*

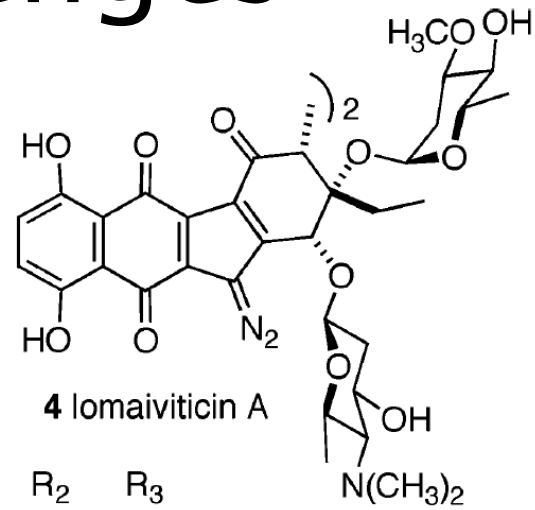
control: no $Bu_3Sn–H$ / AIBN, no reaction

Feldman et. al., JACS, **2006**, 128, 125

Synthetic Challenges

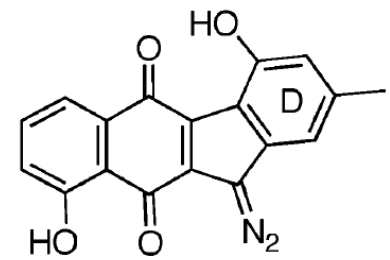
- 6-6-5-6 ring system
- Fluorene core
- Sensitive Diazo group
- Highly functionalized D ring.
- Tetrasubstituted quinone system
- Rich Ac substitution pattern on D ring.
- Dimer analog lomaiviticin A also has glycosylation pattern





4 lomaiviticin A

	R	R_1	R_2	R_3
1a kinamycin A	H	Ac	Ac	Ac
1b kinamycin B	H	H	Ac	H
1c kinamycin C	Ac	Ac	H	Ac
1d kinamycin D	H	Ac	H	Ac
1e kinamycin E	H	H	H	Ac
1f kinamycin F	H	H	H	H
1g kinamycin G	Ac	Ac	COiPr	Ac
1h kinamycin H	Ac	Ac	H	COiPr
1i kinamycin I	Ac	COiPr	H	COiPr
1j kinamycin J	Ac	Ac	Ac	Ac



5 prekinamycin

Feldman et. al., JACS, **2006**, 128, 125

An Overview

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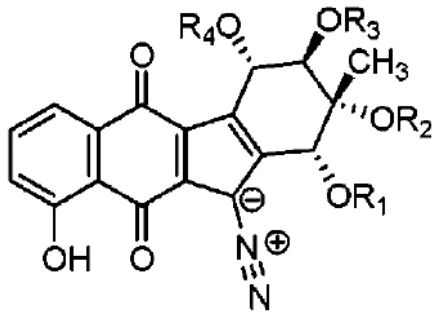
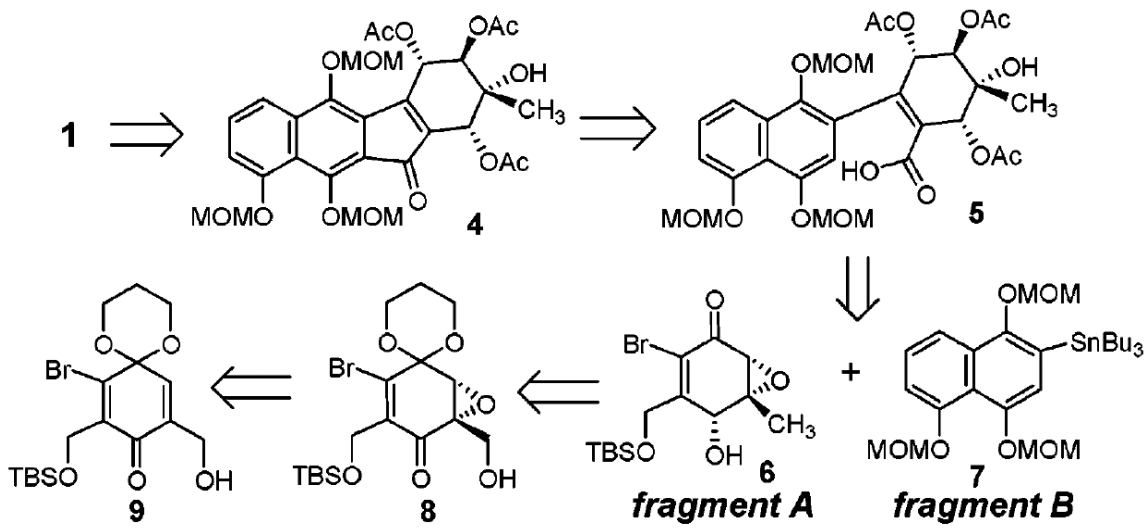
- Total syntheses : 3 enantioselective and 1 racemic
  - Porco
  - Nicolaou
  - Herzon
  - Ishikawa
- Efforts towards the synthesis of their dimer analogues and conclusions

First Total Synthesis

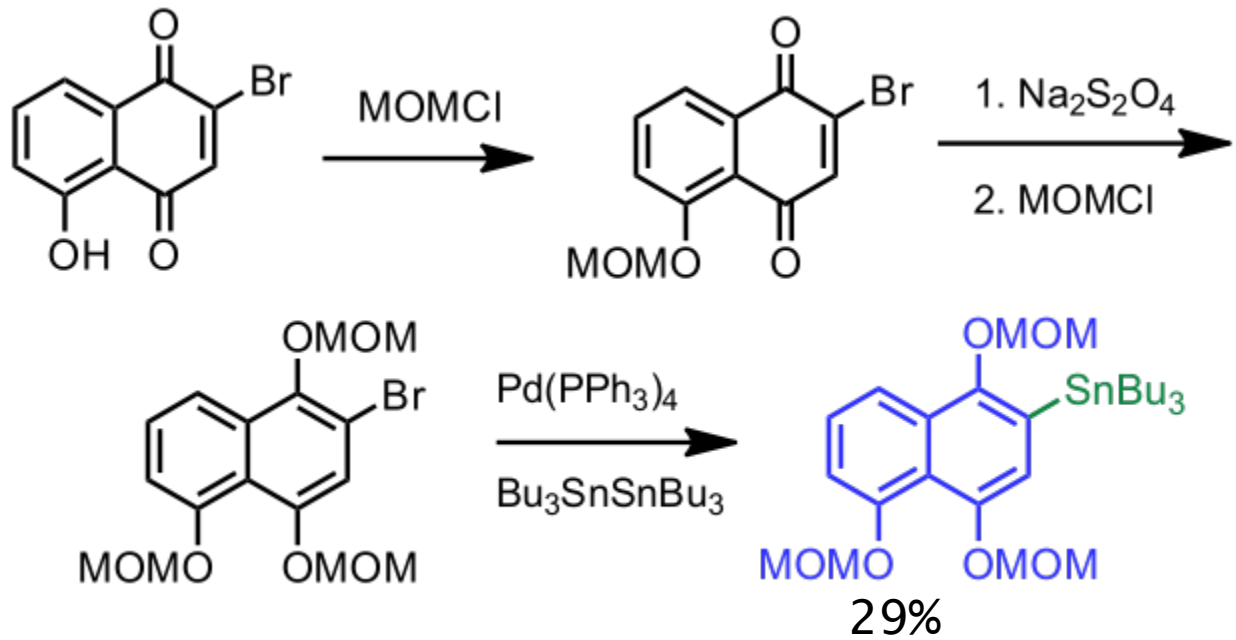


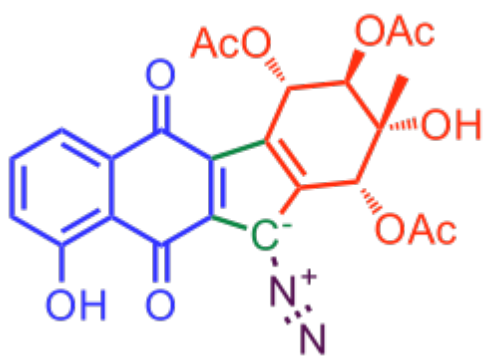
kinamycin A R_1= H R_2 = Ac R_3 =Ac R_4=Ac
kinamycin B R_1= H R_2 = Ac R_3 =H R_4=H
kinamycin C R_1= Ac R_2 = H R_3 =Ac R_4=Ac **1**
kinamycin D R_1= Ac R_2 = H R_3 =Ac R_4=H



Porco et. al., JACS, **2006**, 128, 14790

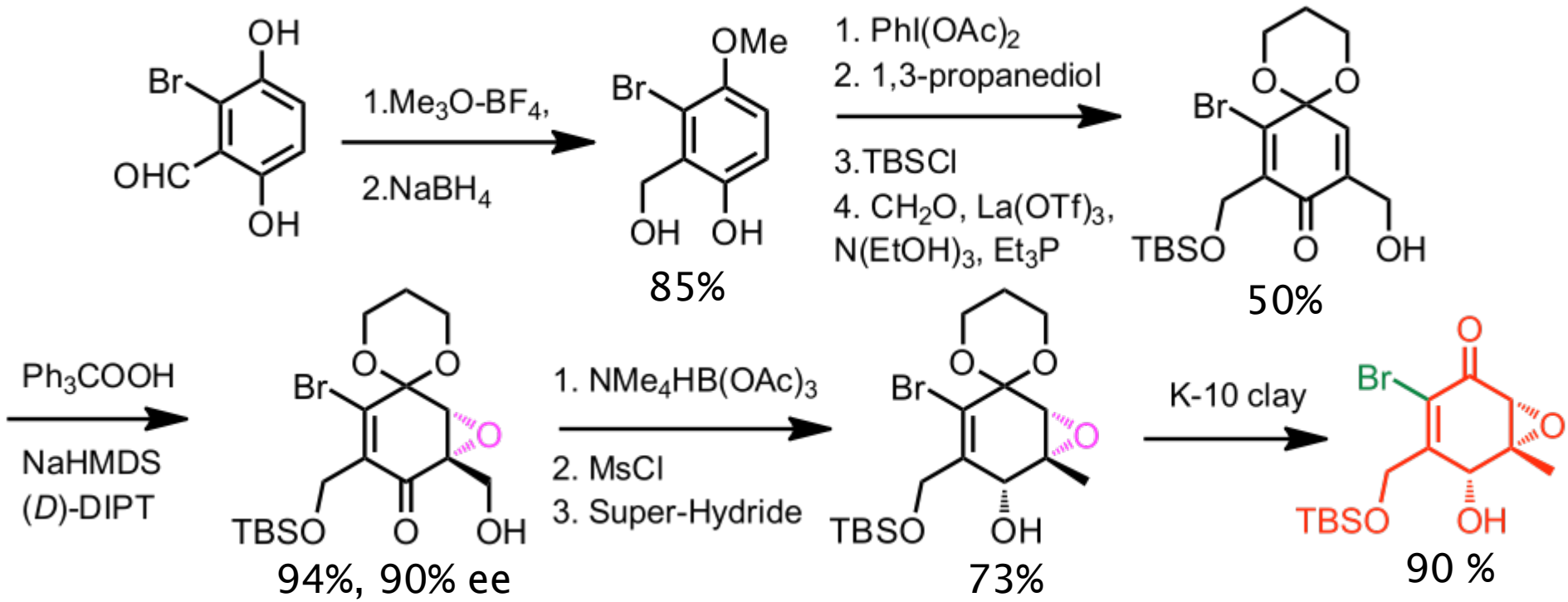
Synthesis of Fragment B



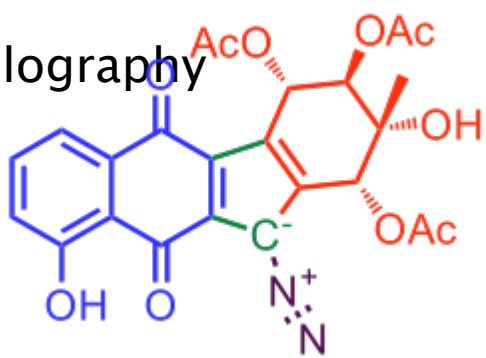


Porco et. al., JACS, **2006**, 128, 14790

Synthesis of Fragment A

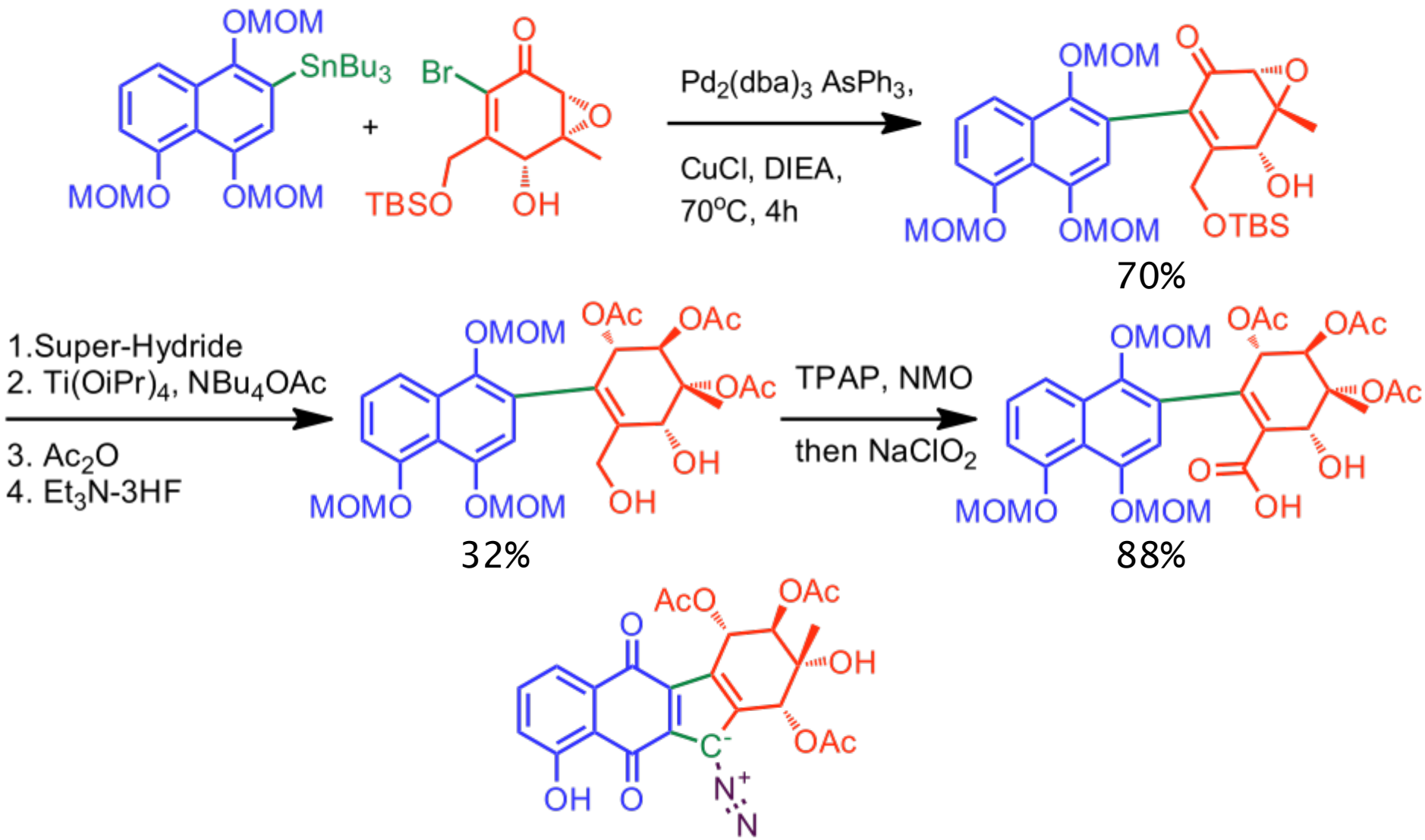


Absolute stereochemistry
confirmed by X-Ray crystallography



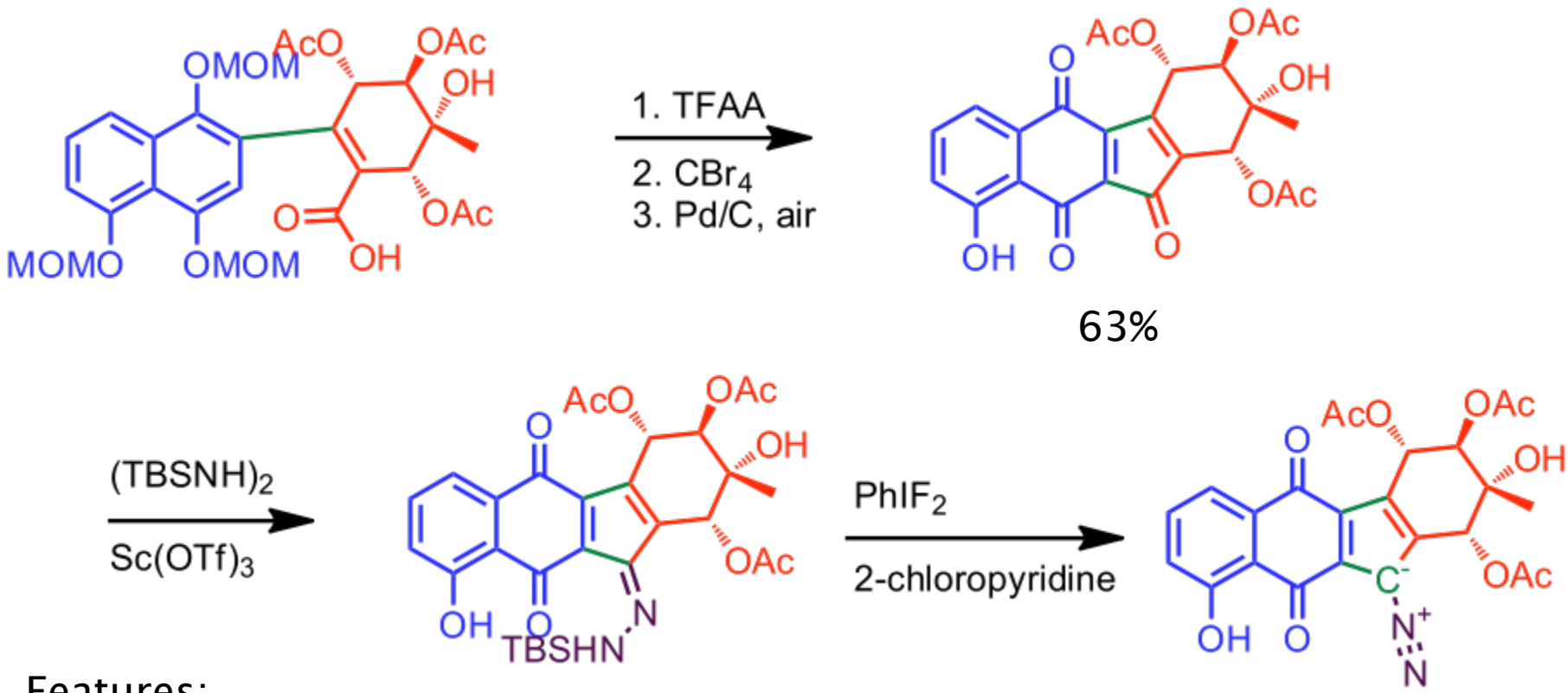
Porco et. al., JACS, **2006**, 128, 14790

Combining the Fragments



Porco et. al., JACS, **2006**, 128, 14790

Last Touches



Features:

- Late Stage Diazo-introduction
- Oxidation of silylhydrazine
- Pd-based coupling, followed by Friedel-Crafts cyclization
- Enantioselective epoxidation as chirality source
- Selective acylation of secondary hydroxyls
- 1.1% overall yield, 23 step longest linear sequence

Porco et. al., JACS, **2006**, 128, 14790

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  - Ishikawa
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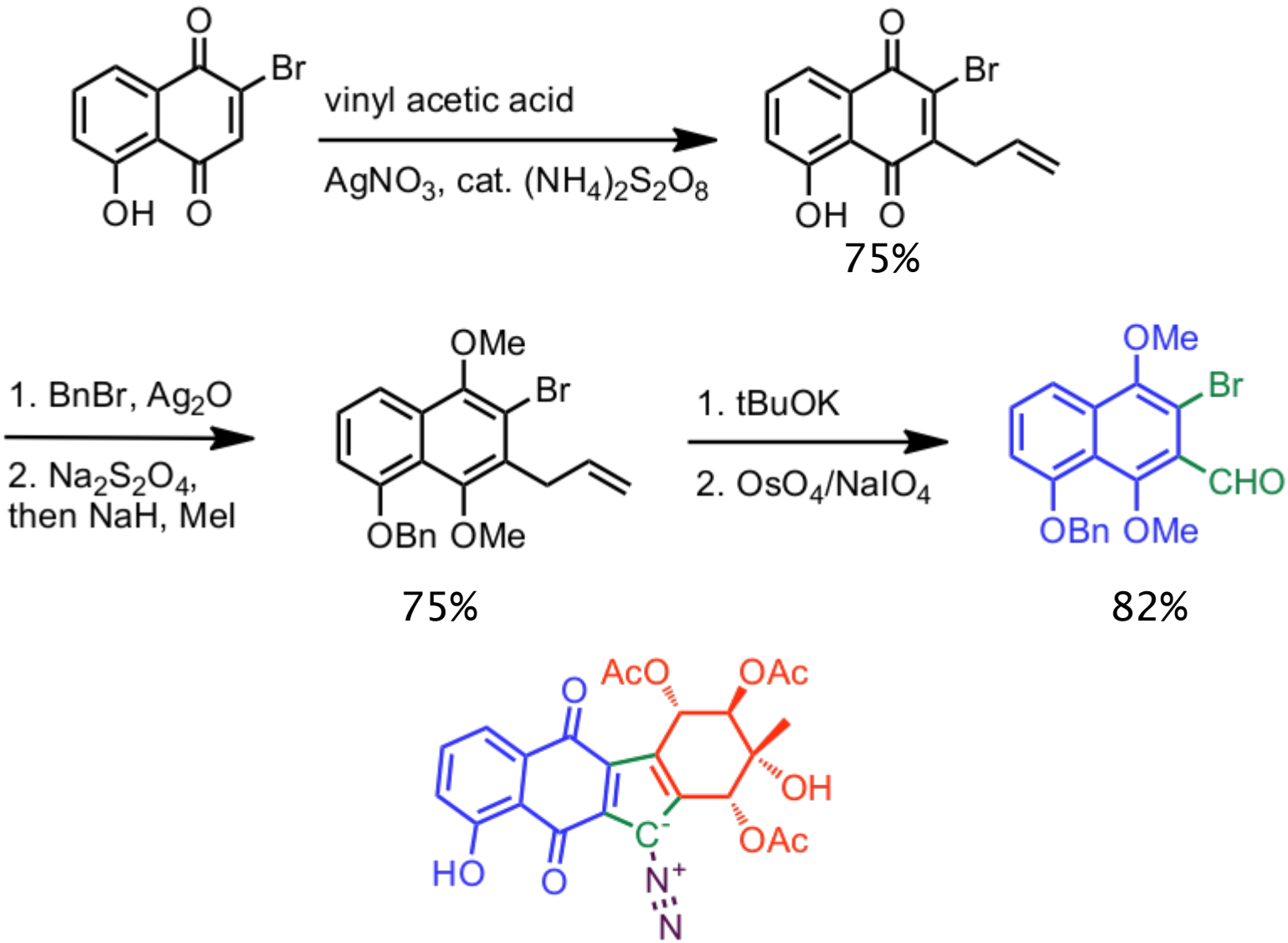
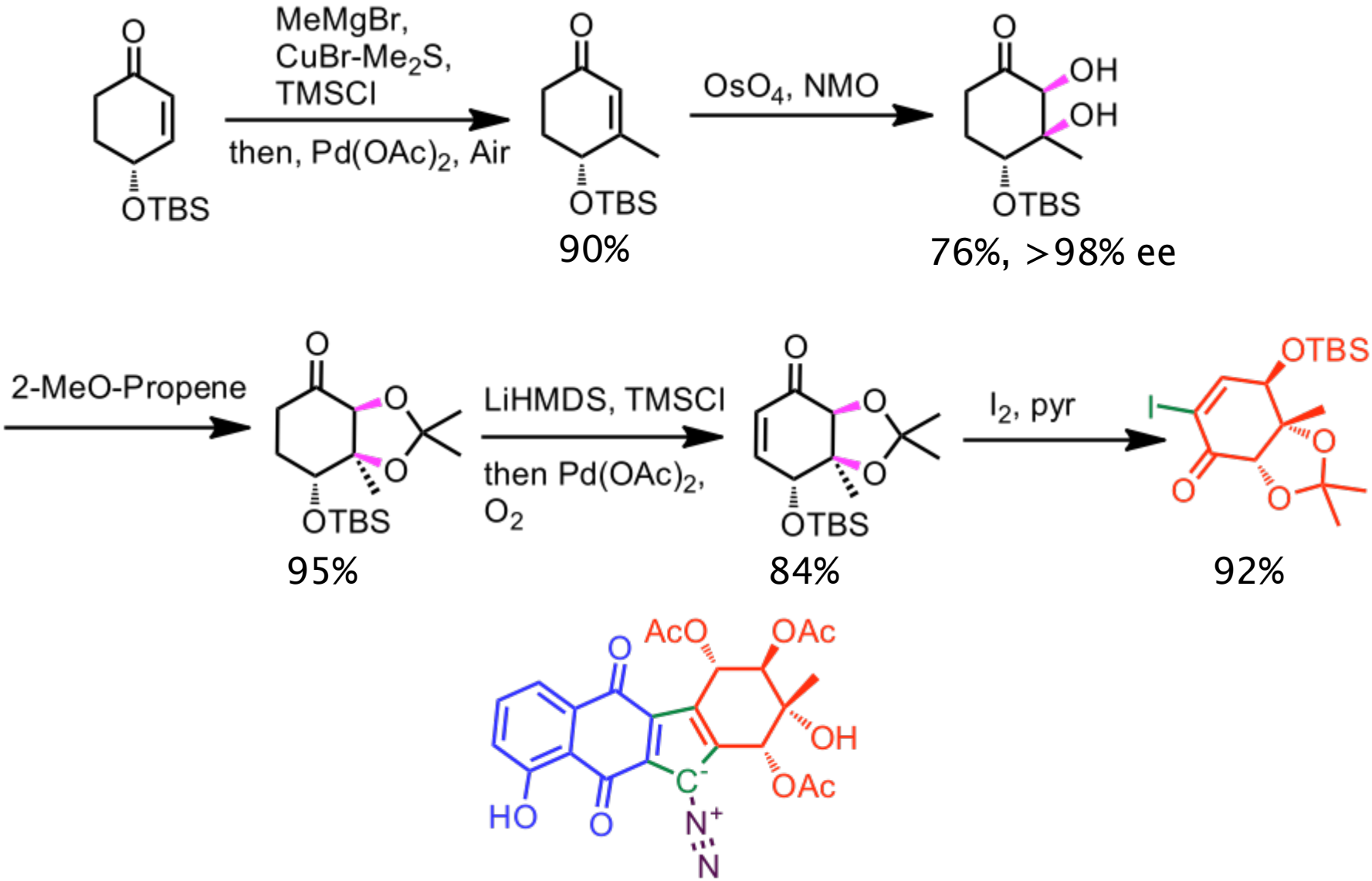
Nicolaou Synthesis, Part A



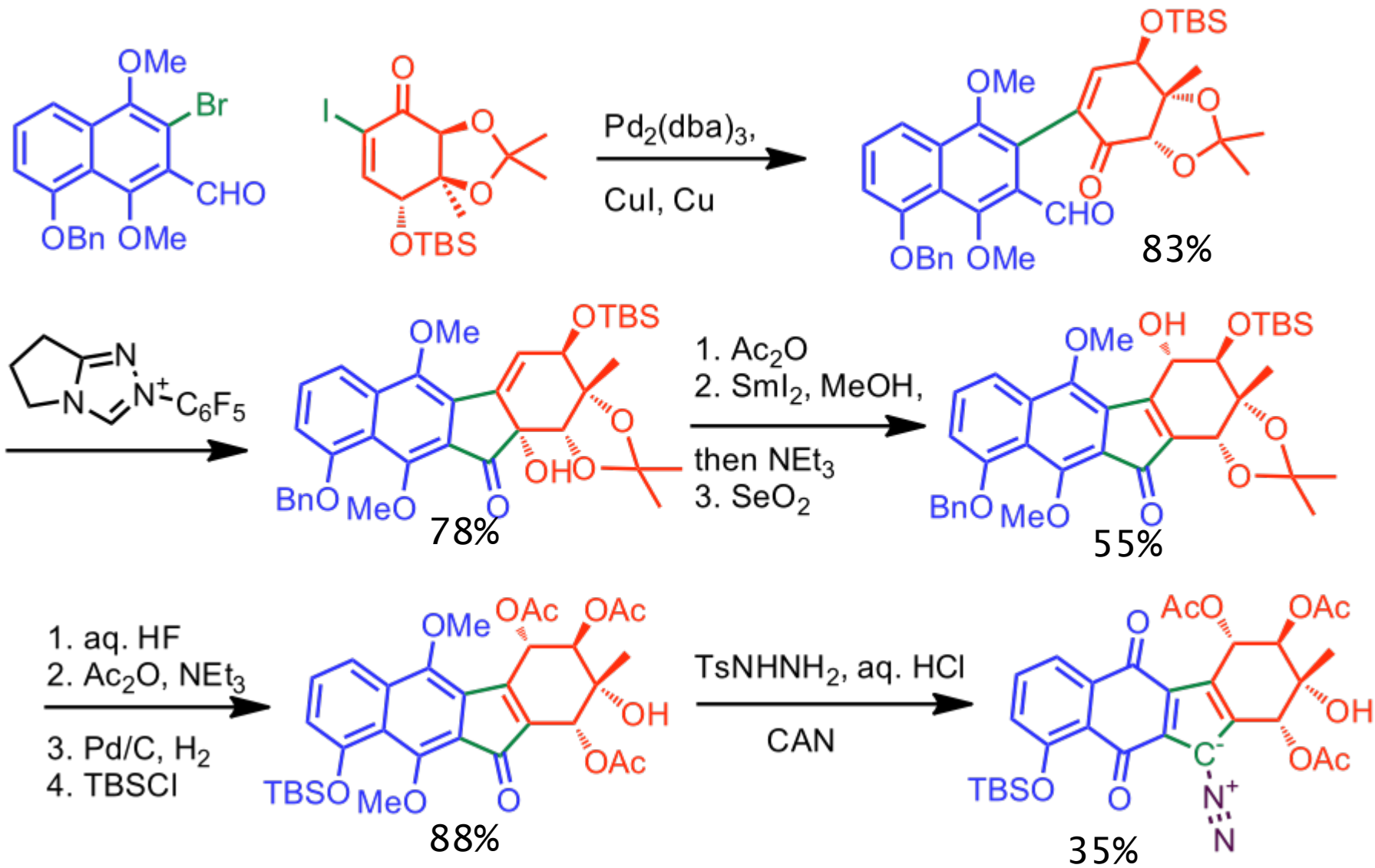
Nicolaou et. al., JACS, **2007**, 129, 103

Nicolaou Synthesis Part B



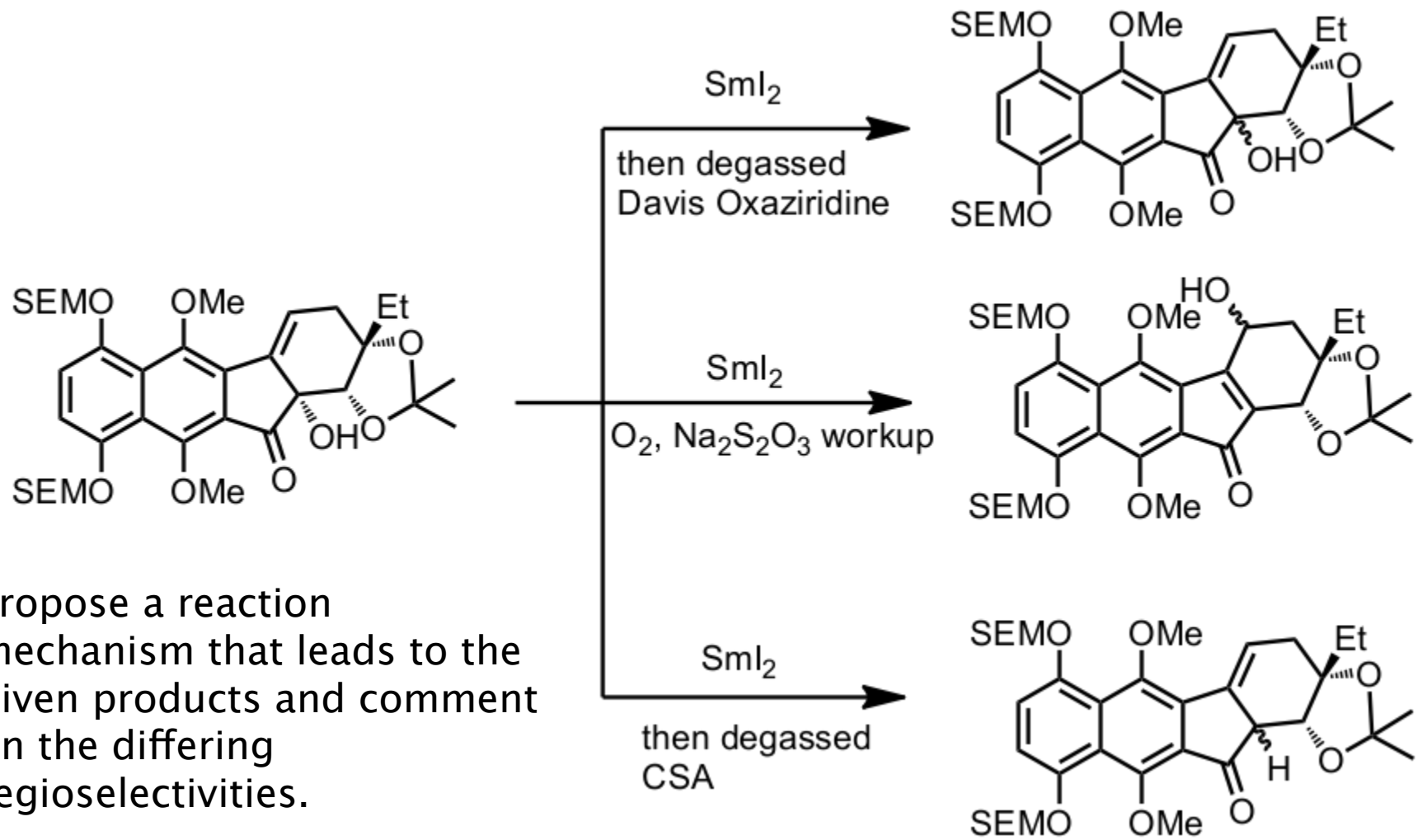
Nicolaou et. al., JACS, **2007**, 129, 103

Joining the Halves



Nicolaou et. al., JACS, **2007**, 129, 103

Group Problem



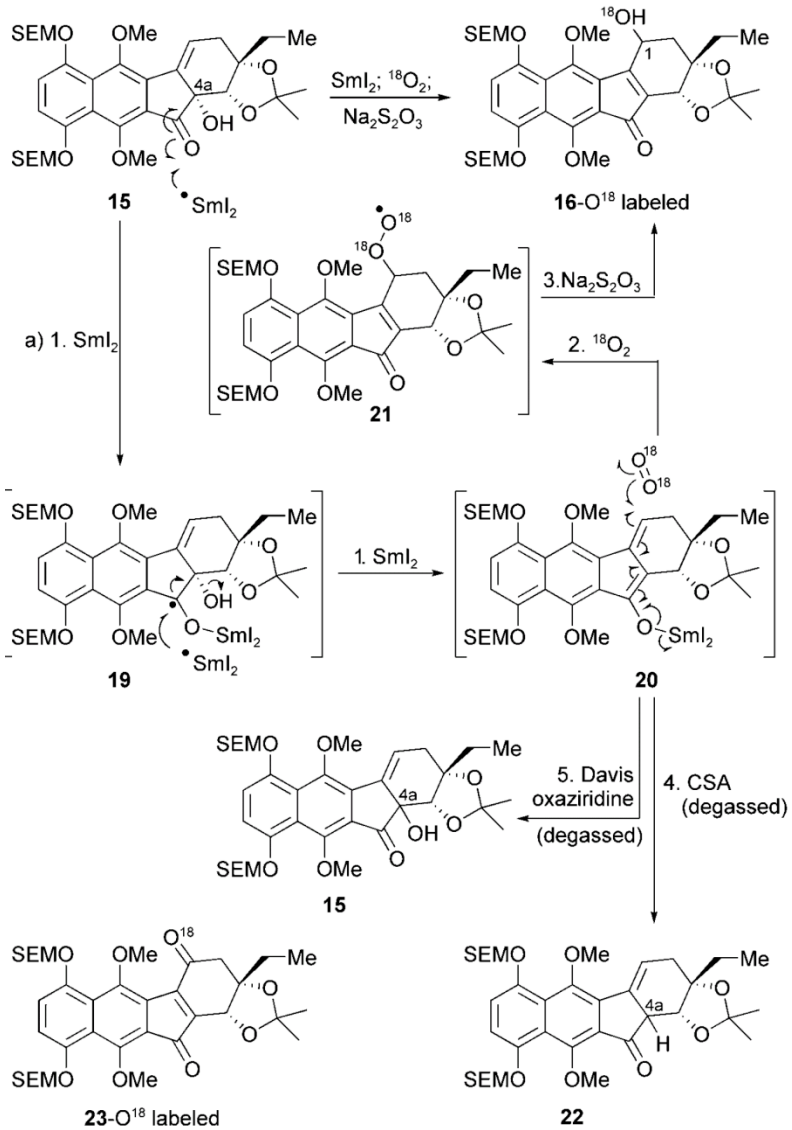
Propose a reaction mechanism that leads to the given products and comment on the differing regioselectivities.

Nicolaou et. al., ACIE, **2009**, 48, 5860

Group Answer

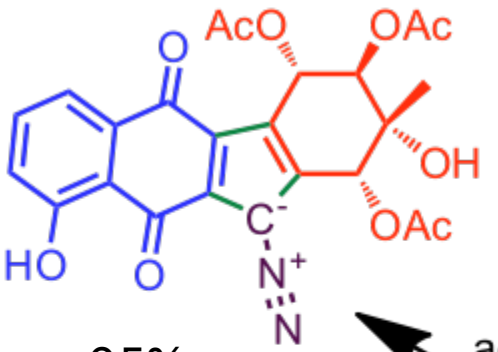
Labeling studies to determine
the fate of the proposed samarium enolate

Peroxide species 21 detected by NMR and MS



Nicolaou et. al., ACIE, **2009**, 48, 5860

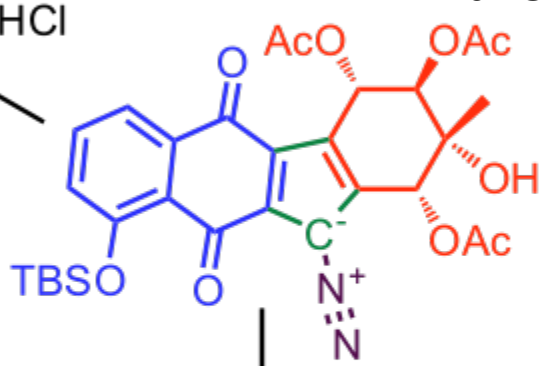
The Family Tree



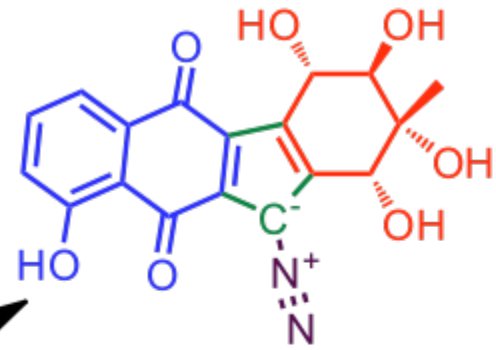
95%
Kinamycin C

aq. HCl

5.1% yield from juglone



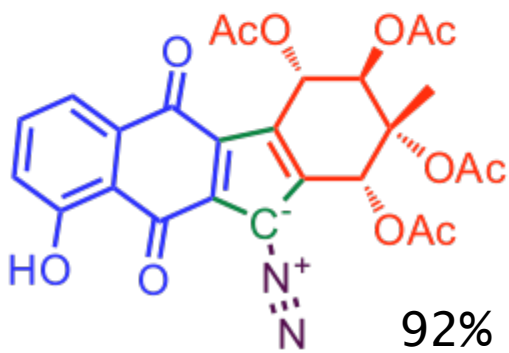
aq. LiOH



80%
Kinamycin F

Features:
- Modular
- Hydrazine oxidation
- Benzoin-type condensation and Ullmann coupling conditions to synthesize the fluorene core

Ac_2O, NEt_3
then aq. HCl



92%

Kinamycin J

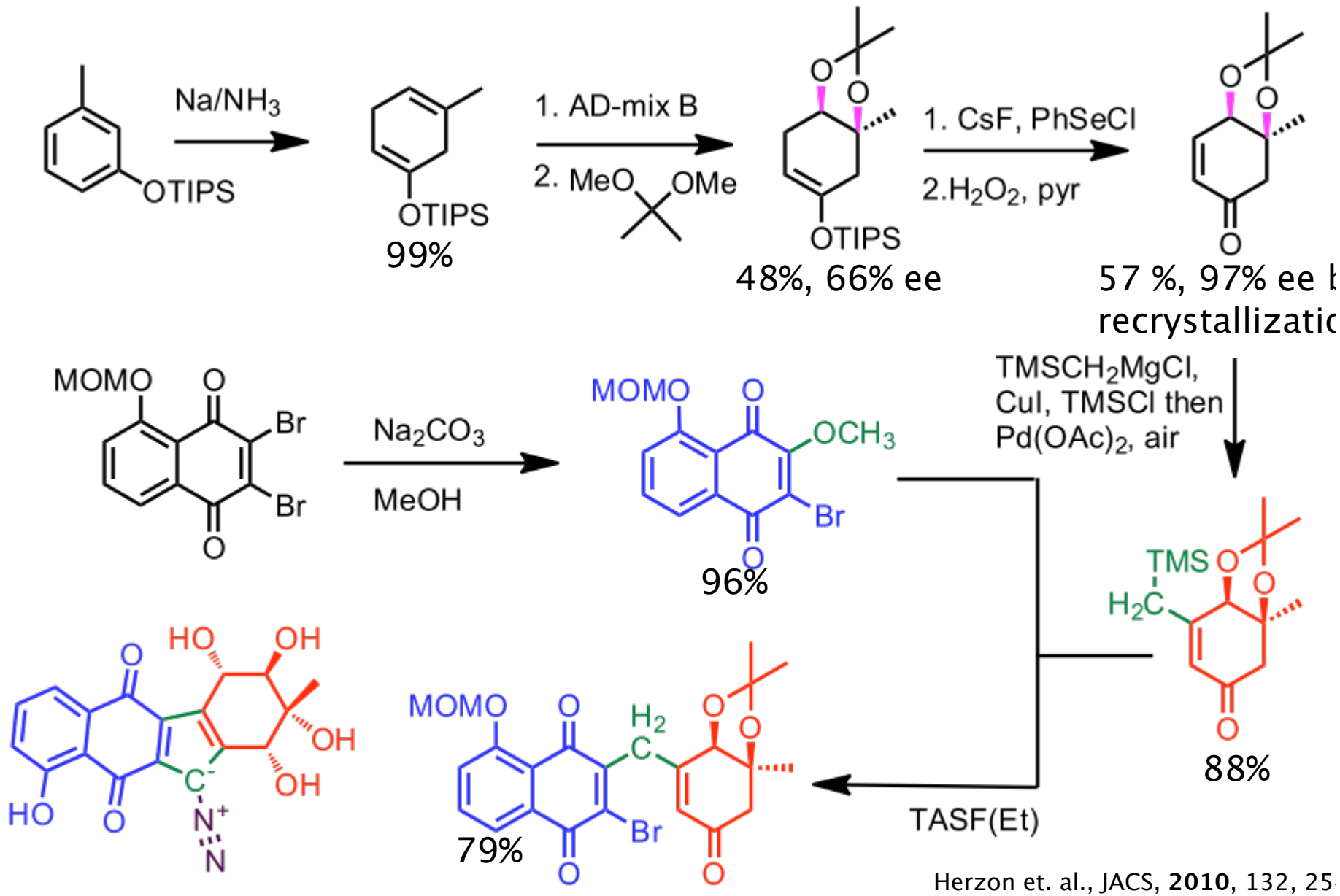
-Late stage diazo introduction
-Enantioselective dihydroxylation to set stereochemistry
- 17 step longest linear sequence to shown late-stage product.

Nicolaou et. al., JACS, **2007**, 129, 103

An Overview

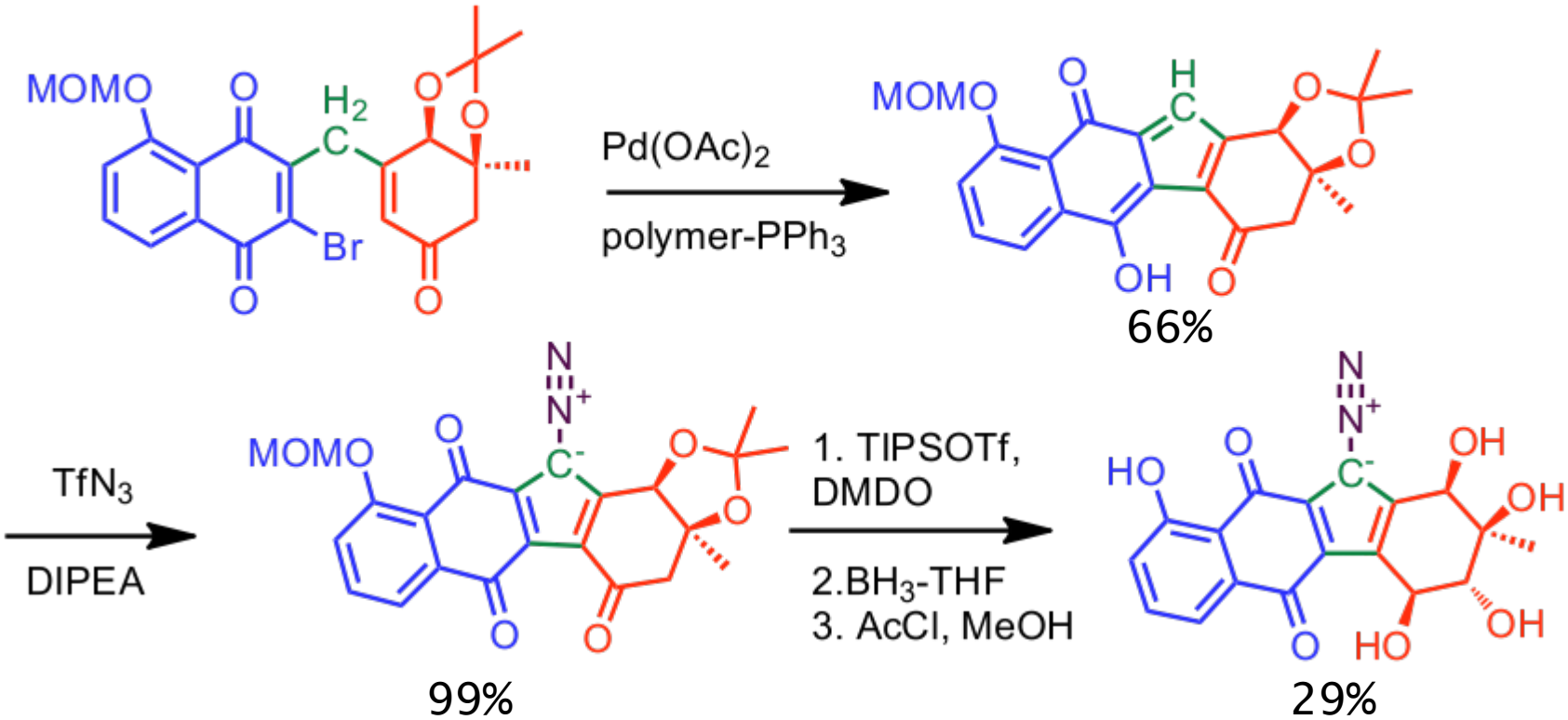
- Isolation and characterization of the Kinamycins, diazofluorene-containing natural products.
- Structural Reassignments
- Biological profile
- Total syntheses : 3 enantioselective and 1 racemic
  - Porco
  - Nicolaou
  - Herzon
  - Ishikawa
- Efforts towards the synthesis of their dimer analogues and conclusions

The Herzon Synthesis



Herzon et. al., JACS, **2010**, 132, 25

Kinamycin F



Features:
- Single step diazo introduction
- Late-stage construction of the D-ring.
- Michael-reaction and Heck-type coupling to sew the pieces.

- Enantioselective dihydroxylation to set stereochemistry (poor yield, poor ee)
- No worries about acylation pattern
- 3.6% yield, 12 step longest linear

Herzon et. al., JACS, 2010, 132, 25

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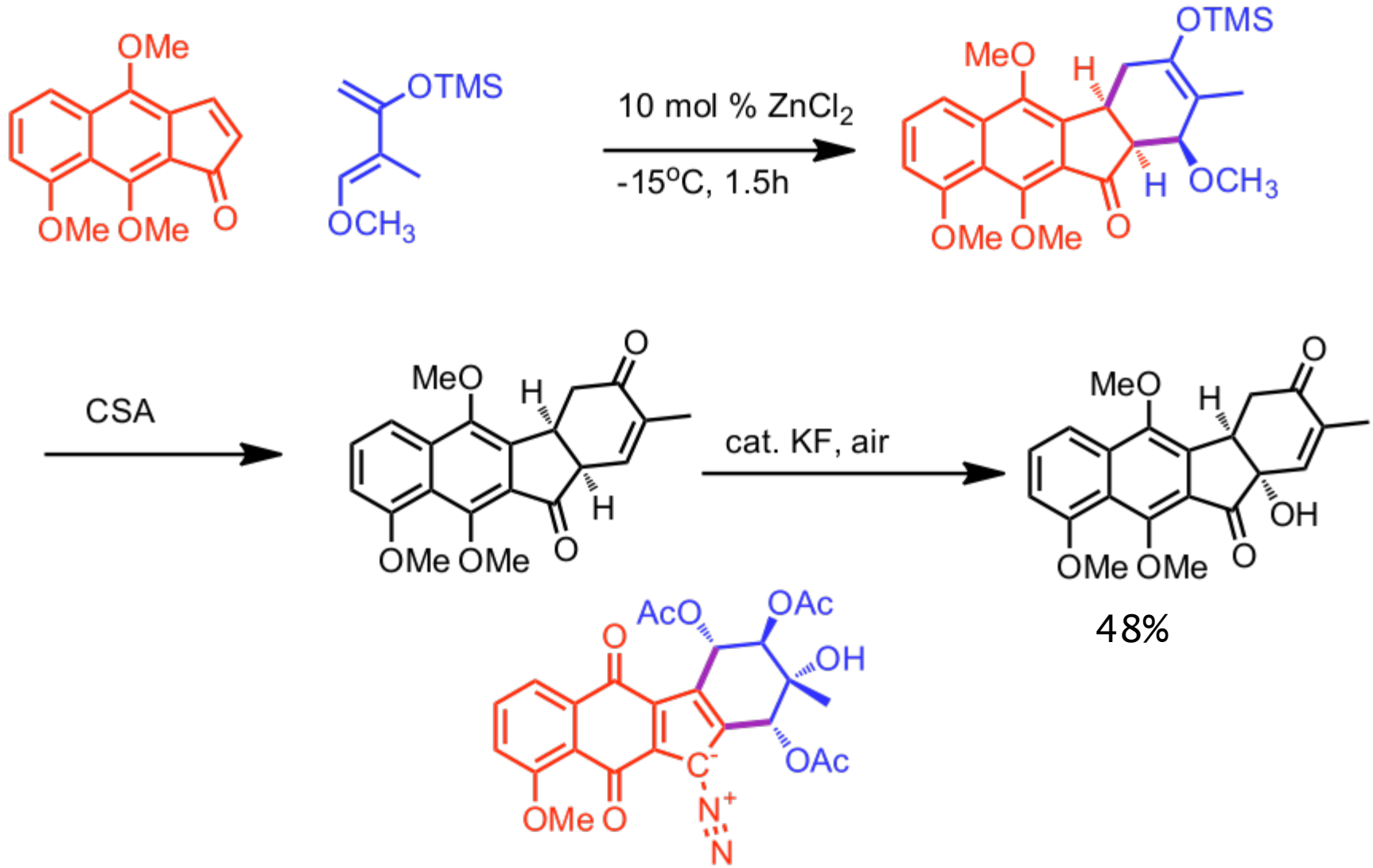
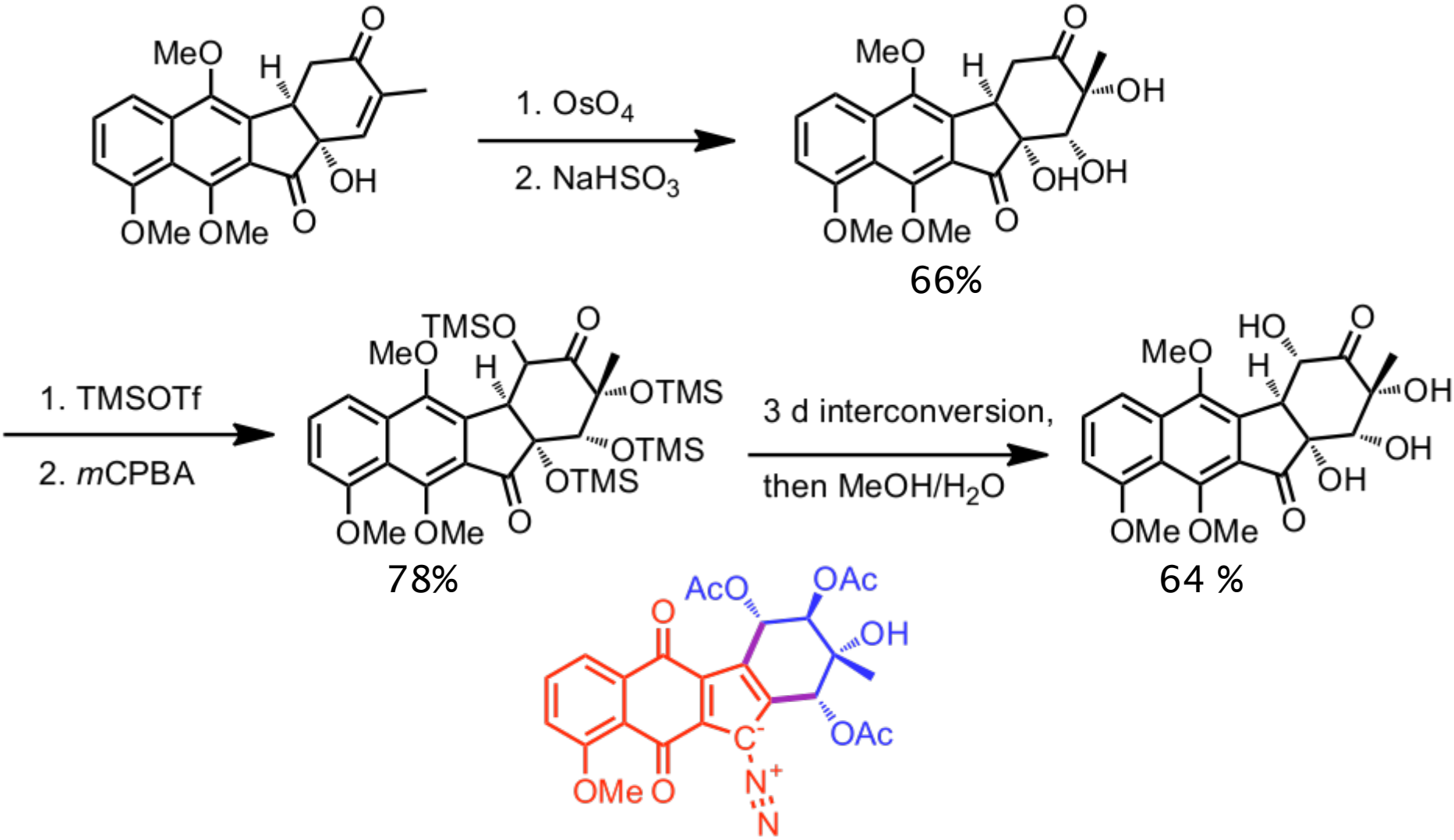
An Alternative Approach



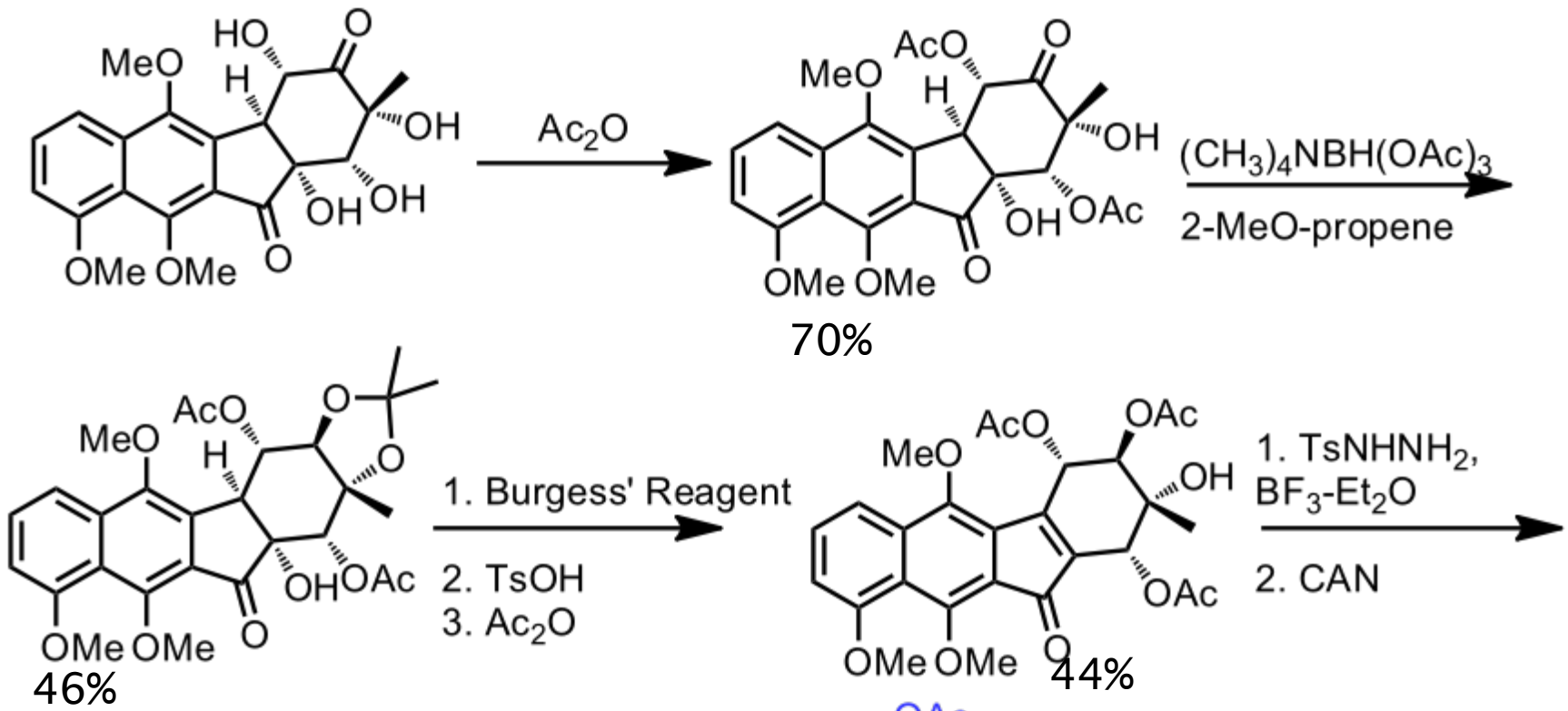
Ishikawa et. al., Helv. Chim. Acta, **2002**, 85,

Ring Elaboration

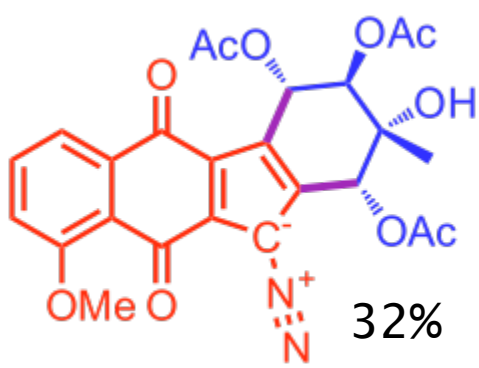


Ishikawa et. al., Tetrahedron, **2007**, 63, 5189

The Finish Line



Features:
Zn-catalyzed Diels-Alder
Racemic, although chiral DA catalysts known
Ring elaboration after DA



(±) O-methyl Kinamycin

Late Stage Diazo-introduction
Hydrazine Oxidation
Selective Acylation
0.7% yield, 16 steps from Diels-Alder precursors

Ishikawa et. al., Tetrahedron, **2007**, 63, 518

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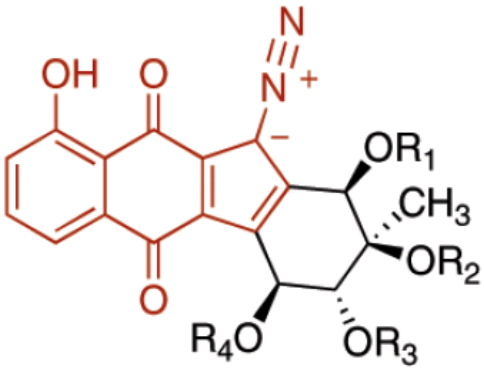
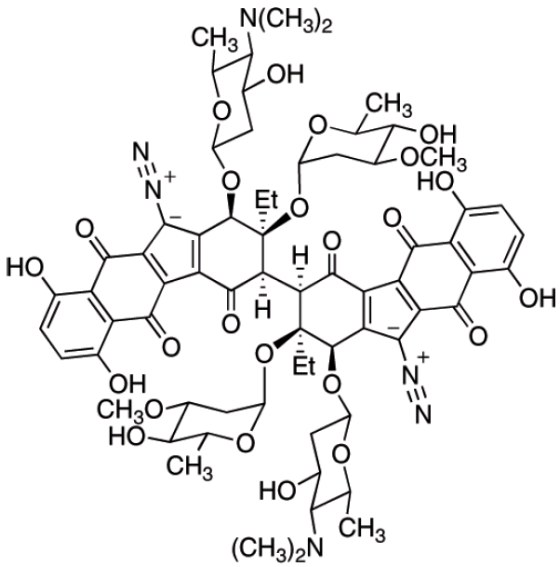
Whither Next?



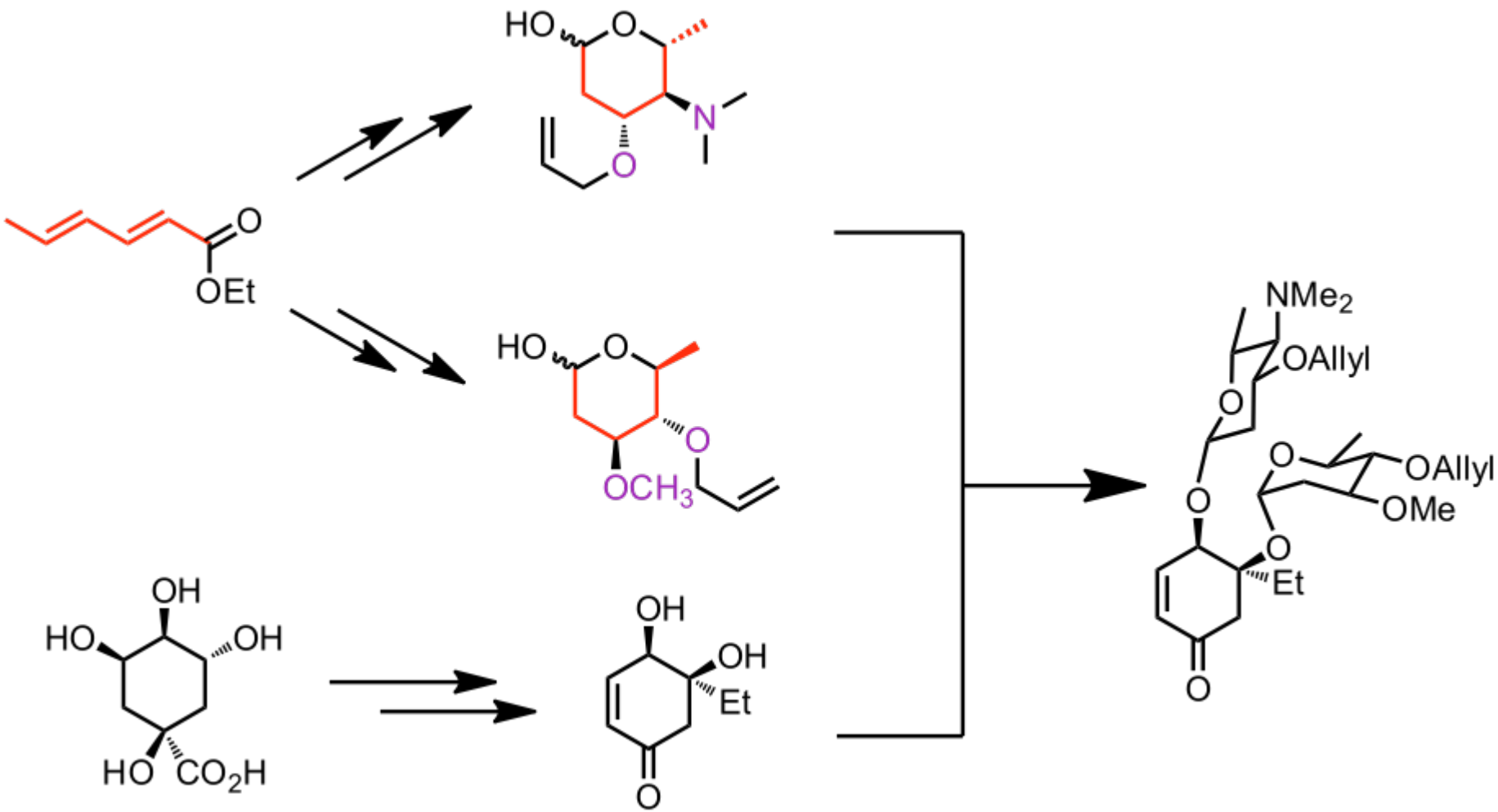
Kinamycin family backbone



Dimer of kinamycin-like precursor

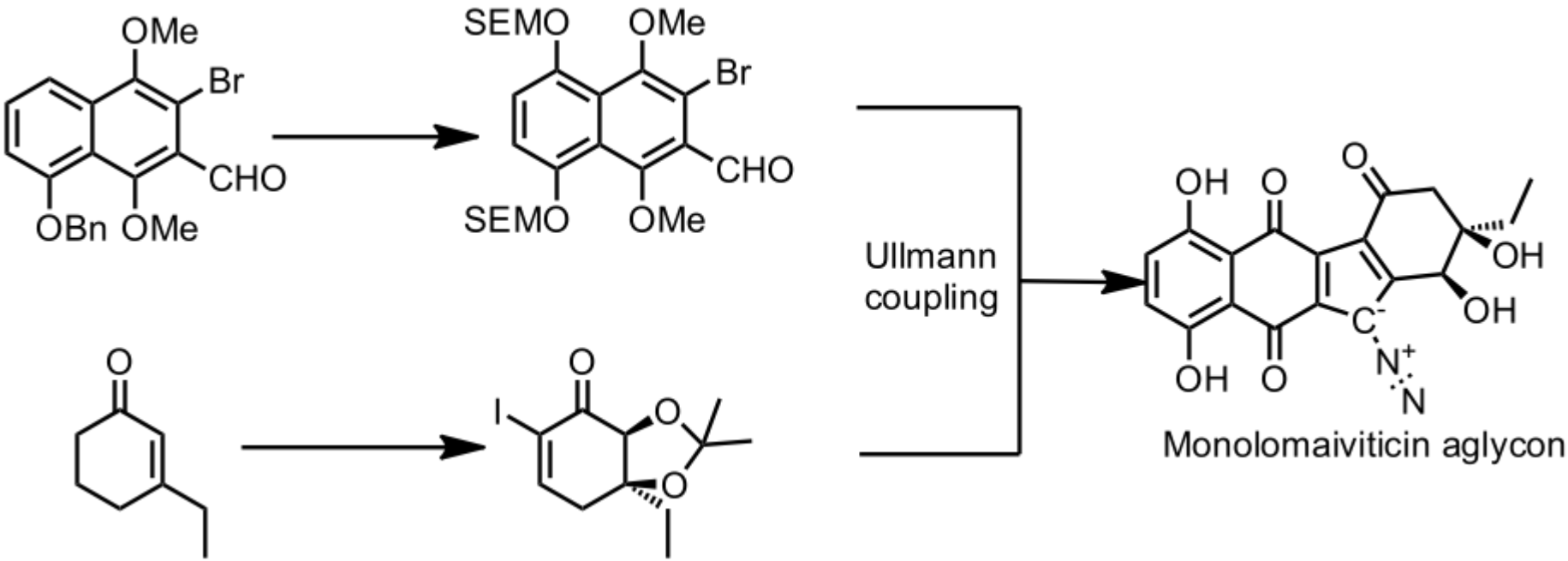
Herzon et. al., Org. Lett., **2009**, 11,

Herzon's Efforts



Herzon et. al., Org. Lett., **2009**, 11,

Nicolaou's Efforts



Nicolaou et. al., ACIE, **2009**, 48, 5860

To Be Solved for Lomaiviticin A

- Correct glycon monomer
- Efficient coupling reaction to make the dimer
- Derivative assays for activity.
- Biological profile : Mode of action, reason for superior activity, biosynthetic origins
- Relationship to the kinamycins both evolutionarily and enzymatically.

Conclusions

- Four different syntheses:
  - Porco : Stille Coupling, Friedel-Crafts reaction, epoxidation
  - Nicolaou : Ullmann Coupling, Benzoin-like condensation, desymmetrization of 4-OTBS-cyclohexanone
  - Herzon : Michael Reaction, Heck-type coupling, dihydroxylation
  - Kumamoto: Diels-Alder, racemic

  - Two efforts towards lomaiviticin A, dimer of a kinamycin-like backbone

  - Radical generation from diazo moiety indicated in bioactivity, derivative studies might help identify key structural features required for optimization.