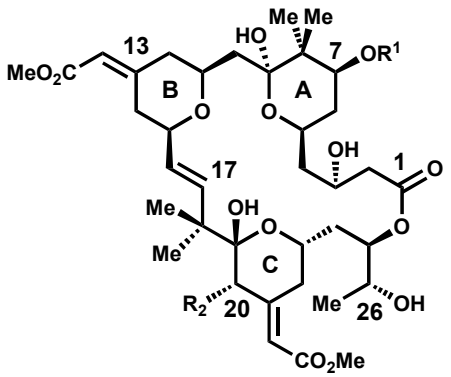
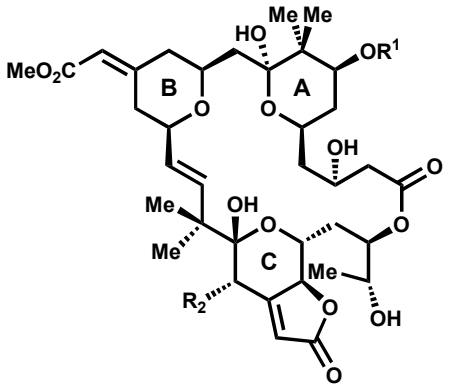
Bryostatins

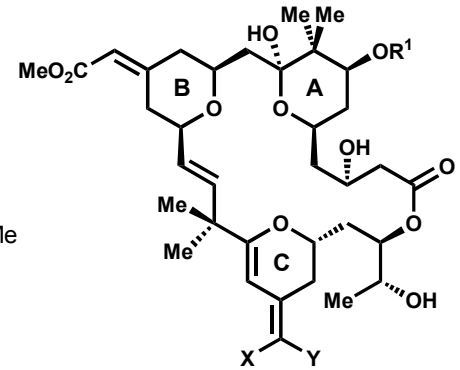
Benjamin Huffman





Bryostatin 1: R_1 = Ac, R_2 = $O_2C(CH)_4(CH_2)_2Me$
Bryostatin 2: R_1 = H, R_2 = $O_2C(CH)_4(CH_2)_2Me$
Bryostatin 4: R_1 = $COCH_2CHMe_2$, R_2 = $O_2C(CH_2)_2Me$
Bryostatin 5: R_1 = $COCH_2CHMe_2$, R_2 = OAc
Bryostatin 6: R_1 = $CO(CH_2)_2Me$, R_2 = OAc
Bryostatin 7: R_1 = Ac, R_2 = OAc
Bryostatin 8: R_1 = $CO(CH_2)_2Me$, R_2 = $O_2C(CH_2)_2Me$
Bryostatin 9: R_1 = Ac, R_2 = $O_2C(CH_2)_2Me$
Bryostatin 10: R_1 = Piv, R_2 = H
Bryostatin 11: R_1 = Ac, R_2 = H
Bryostatin 12: R_1 = $CO(CH_2)_2Me$, R_2 = $O_2C(CH)_4(CH_2)_2Me$
Bryostatin 13: R_1 = $CO(CH_2)_2Me$, R_2 = H
Bryostatin 14: R_1 = Piv, R_2 = OH
Bryostatin 15: R_1 = Ac, R_2 = $O_2C(CH)_4CH(OH)Et$

Bryostatin 3: R_1 = Ac, R_2 = $O_2C(CH)_4(CH_2)_2Me$



Bryostatin 16: R_1 = Piv, X = H, Y = CO_2Me
Bryostatin 17: R_1 = Piv, X = CO_2Me, Y = H
Bryostatin 18,19, 20 not yet characterized

Structural Features

- 20 membered macrolactone core
- 3 densely functionalized pyran rings
- ~ 11 stereocenters
- C_8 geminal methyls
- C_{16} - C_{17} trans olefin
- Differ in substitution at C_7 and C_{20}
- bryostatin 3 possess C_{22} oxygen; butenolide
- bryostatin 16 and 17 contain a dihydropyran C ring

History

- **1968: Jack Rudloe** sends samples of *Bugula neritina* to NCI
- **1976:** bryostatin 1 identified as active component in extracts
- **1982: George Pettit** reports crystal structure of bryostatin 1
- **1990: Masamune** reports the total synthesis of bryostatin 7
- **1998: Evans** reports the total synthesis of bryostatin 2
- **2000: Yamamura** reports the total synthesis of bryostatin 3
- **2008: Trost** reports the total synthesis of bryostatin 16
- **2011: Wender** reports the total synthesis of bryostatin 9
- **2011: Keck** reports the total synthesis of bryostatin 1
- **2011: Krische** reports the total synthesis of bryostatin 7

Shenvi Lab Group Meeting
04/10/2017

Bryostatins

Benjamin Huffman

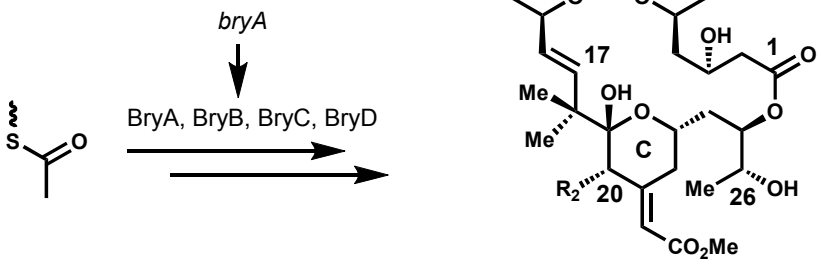
Bugula neritina

- Colonial Animal
- Individuals in a colony: Zooids
- Grow up to 15 cm in length
- Hermaphroditic
- Each zooid produces a single embryo
- Plot twist: bryostatins are actually produced from an uncultured symbiotic bacterium, *Endobugula sertula*
- believed that bryostatins protect developing larvae from predators
- 18g bryostatin 1 isolated from 10,000 gallons of wet animal ~ 14 tons or 12,700 Kg

Biosynthesis

1996: Kerr
- incubates *B. neritina* with radio labeled precursors
- Acetate, glycerol and S-adenosylmethionine (SAM)

2007: Haygood
- *E. sertula* consistently associated with *B. neritina*
- *E. sertula* vertically passed to next generation
- *E. sertula* eliminated with antibiotics
- *B. neritina* larvae devoid of bryostatin
- Entire PKS gene, *bryA*, identified and cloned
- Efforts to transfer biosynthetic machinery into other organisms are ongoing



Biology

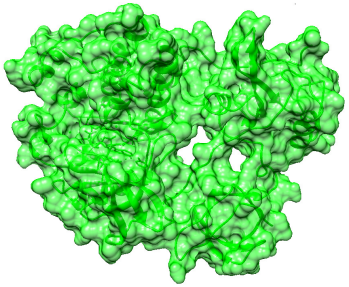
Anticancer:
- 43 separate phase I and phase II:
- Melanoma, myeloma, acute myeloid leukaemia, colorectal, renal, prostate, head and neck, cervix, ovarian, breast, peritoneal, stomach, oesophagus, anus, and lung cancer

Anti HIV:
- Reactivates HIV-1 from latency (PKC dependent)

Cognition:
- "Appropriate doses" improves rats' performance in spatial water maze
- Rescues induced depressive behavior

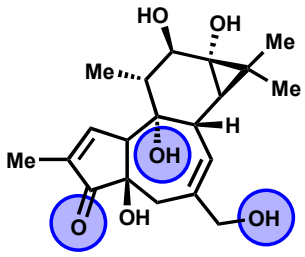
No Phase III advancement:
- Dose-limiting toxicity: severe myalgias
- Lack of efficacy

Biochemistry
- bryostatins target Protein Kinase C
- Protein Kinase C (PKC)
  - Serine/threonine specific kinases
  - 8 PKC isoforms
  - Regulate: proliferation, differentiation, motility, adhesion, and aptosis
- bryostatins bind to the C_1 domain of PKCs
- Triggers conformational change
- PKC becomes more lipophilic and translocates to membranes
- Membrane bound PKC phosphorylates target proteins

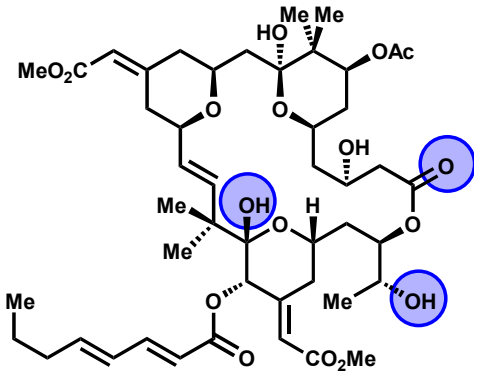


phorbol → Tumor promotion

bryostatin 1 → Tumor supression



phorbol



bryostatin 1

Isolation: JNP, 1991, 1265; **Biosynthesis:** TL, 1996, 8305; Chem. Biol. 2004, 1543; **Biology:** Curr. Med. Chem., 2012, 2652

Bryostatin 1,3 and 7

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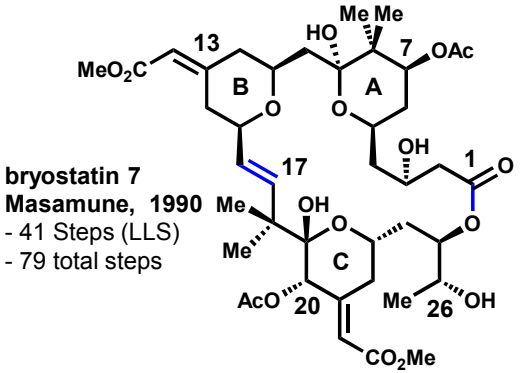
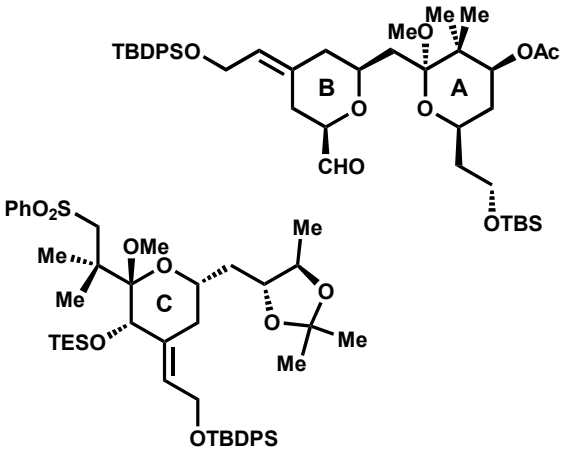
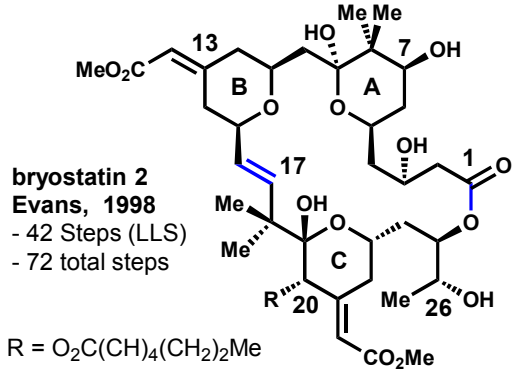


bryostatin 7
Masamune, 1990
- 41 Steps (LLS)
- 79 total steps

Julia Olefination / Yamaguchi Esterification

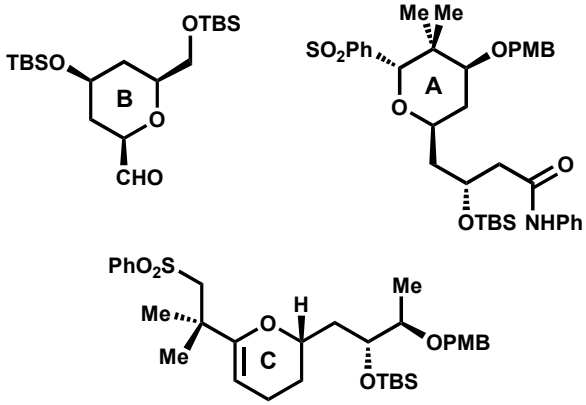


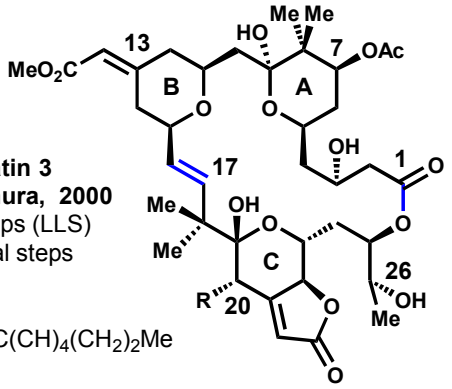


bryostatin 2
Evans, 1998
- 42 Steps (LLS)
- 72 total steps

R = $O_2C(CH)_4(CH_2)_2Me$

Julia Olefination / Yamaguchi Esterification

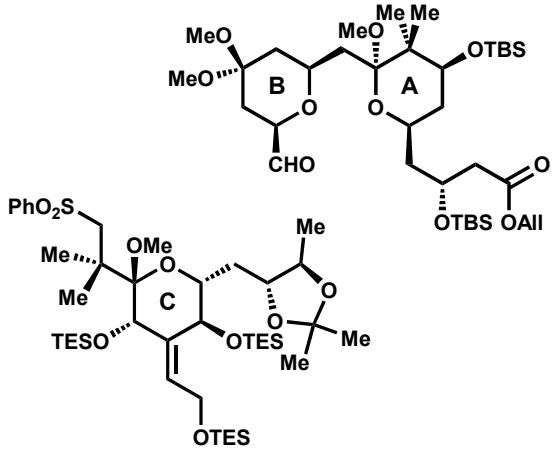




bryostatin 3
Yamamura, 2000
- 43 Steps (LLS)
- 88 total steps

R = $O_2C(CH)_4(CH_2)_2Me$

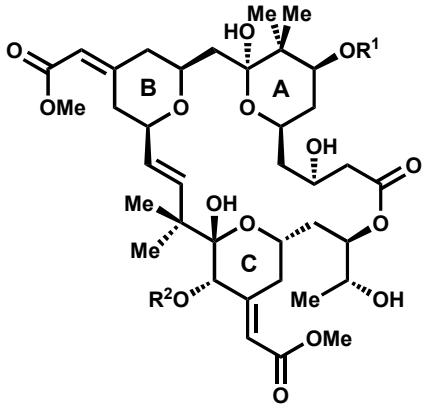
Julia Olefination / Yamaguchi Esterification



Masamune: JACS, 1990, 7407; **Evans**, JACS, 1999 ,7540; **Yamamura:** ACIE, 2000, 2290

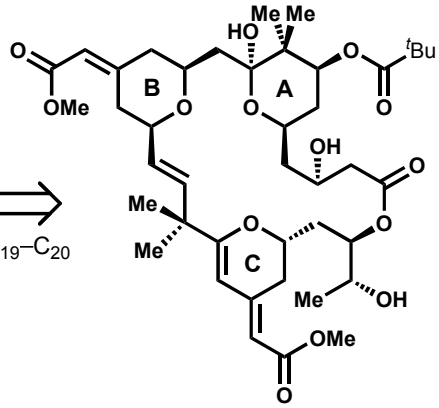
Bryostatin 16: Retrosynthesis

Benjamin Huffman



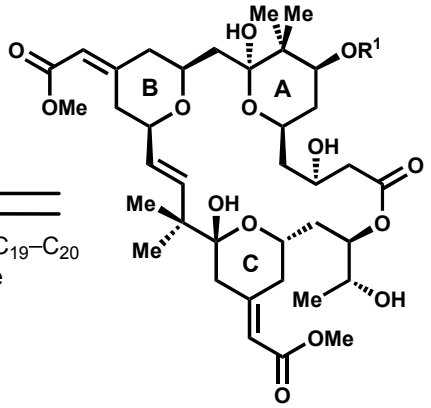
bryostatin 1,2,4 - 9, 12,14,15

Oxidation of C_{19}–C_{20} alkene

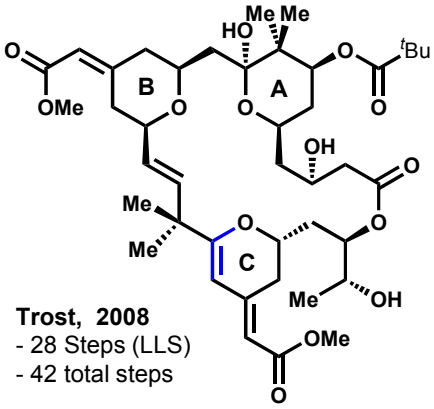


bryostatin 16

Hydration of C_{19}–C_{20} alkene



bryostatin 10, 11, 13, 18

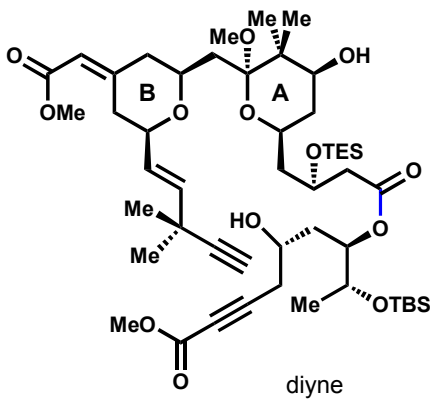


Trost, 2008
- 28 Steps (LLS)
- 42 total steps

bryostatin 16

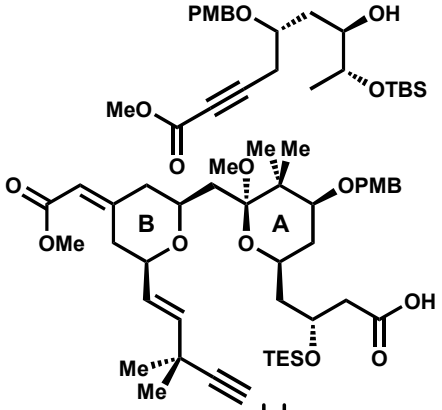
Palladium-catalyzed alkyne-alkyne coupling

Au-catalyzed 6-*endo*-*dig* cyclization

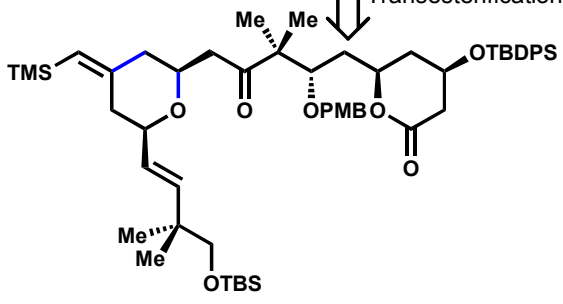


diyne

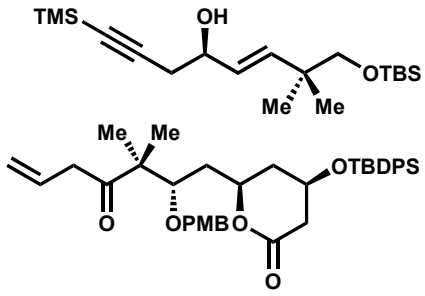
Yamaguchi Esterification

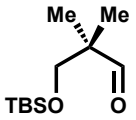


Transesterification



Ru-catalyzed tandem alkene/alkyne coupling/Michael addition

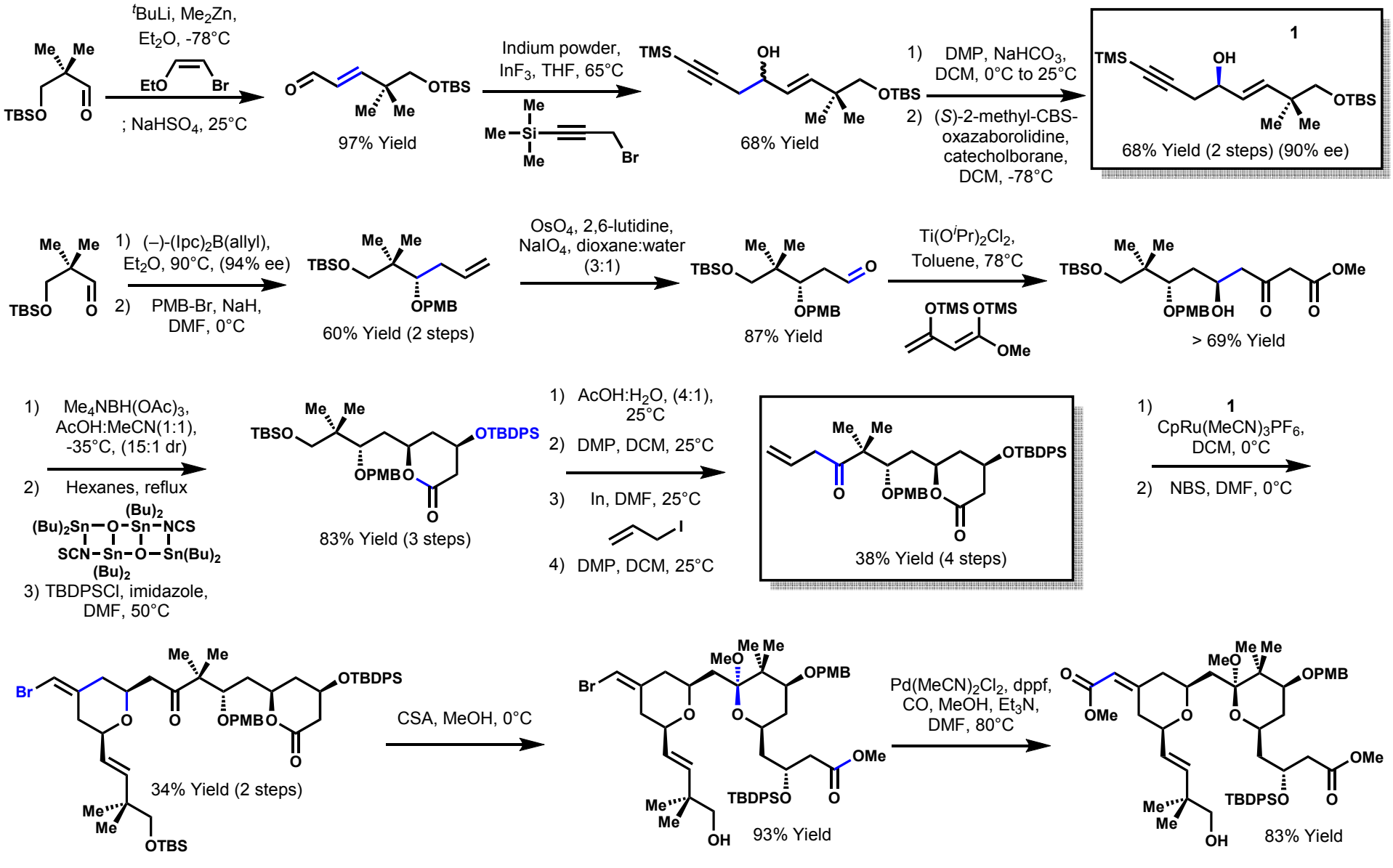




Nature, 2008, 485

Bryostatin 16: Northern Fragment

Benjamin Huffman



Bryostatin 16

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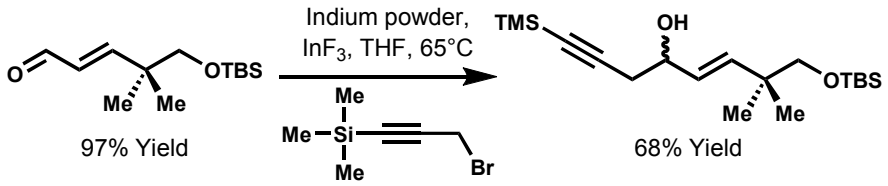

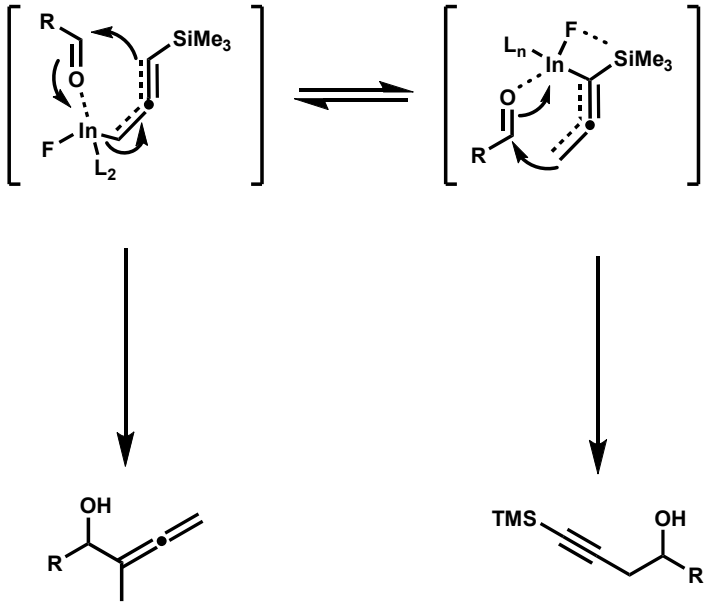

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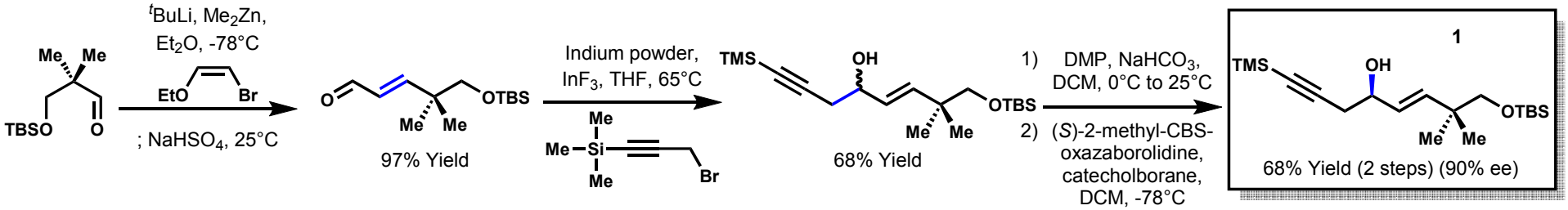

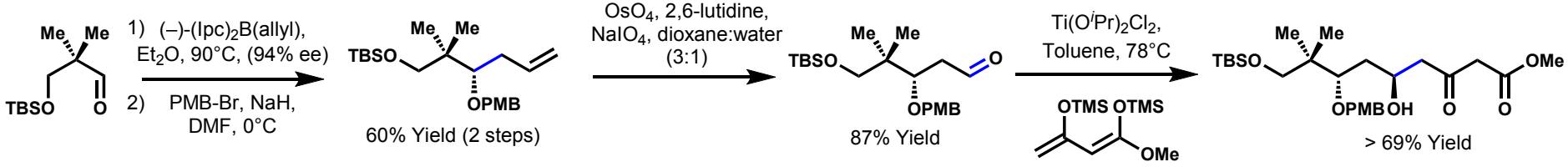

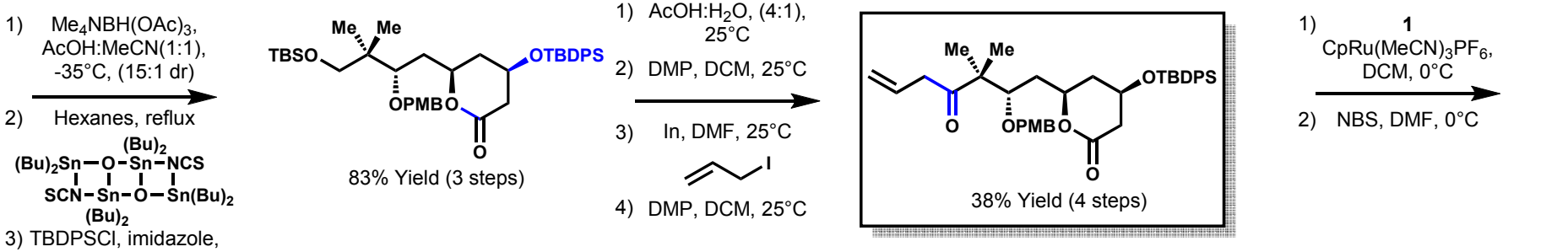

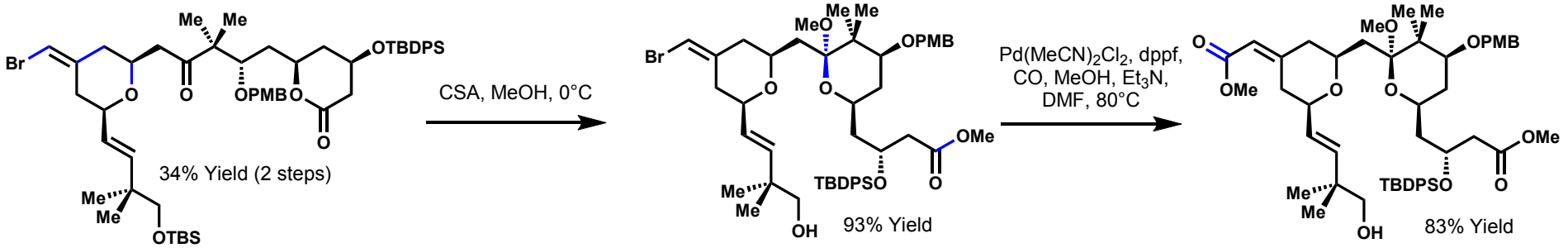

Bryostatin 16

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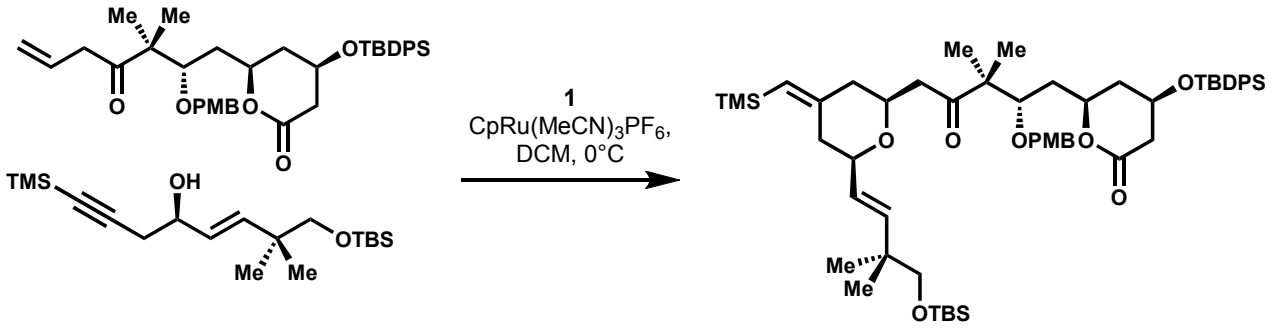
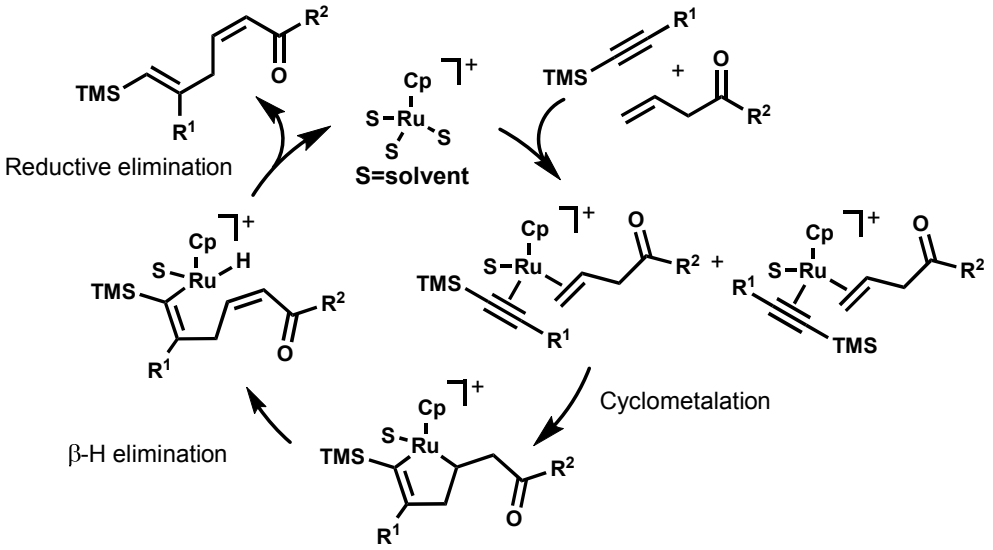
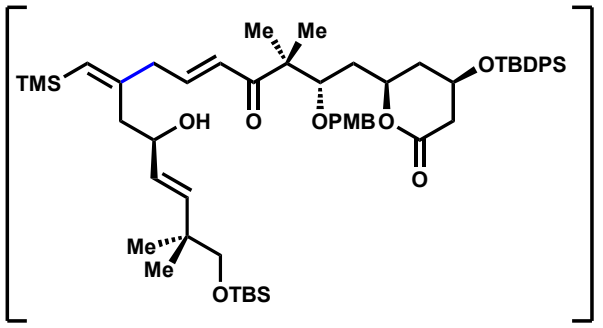


Ene-Yne Coupling

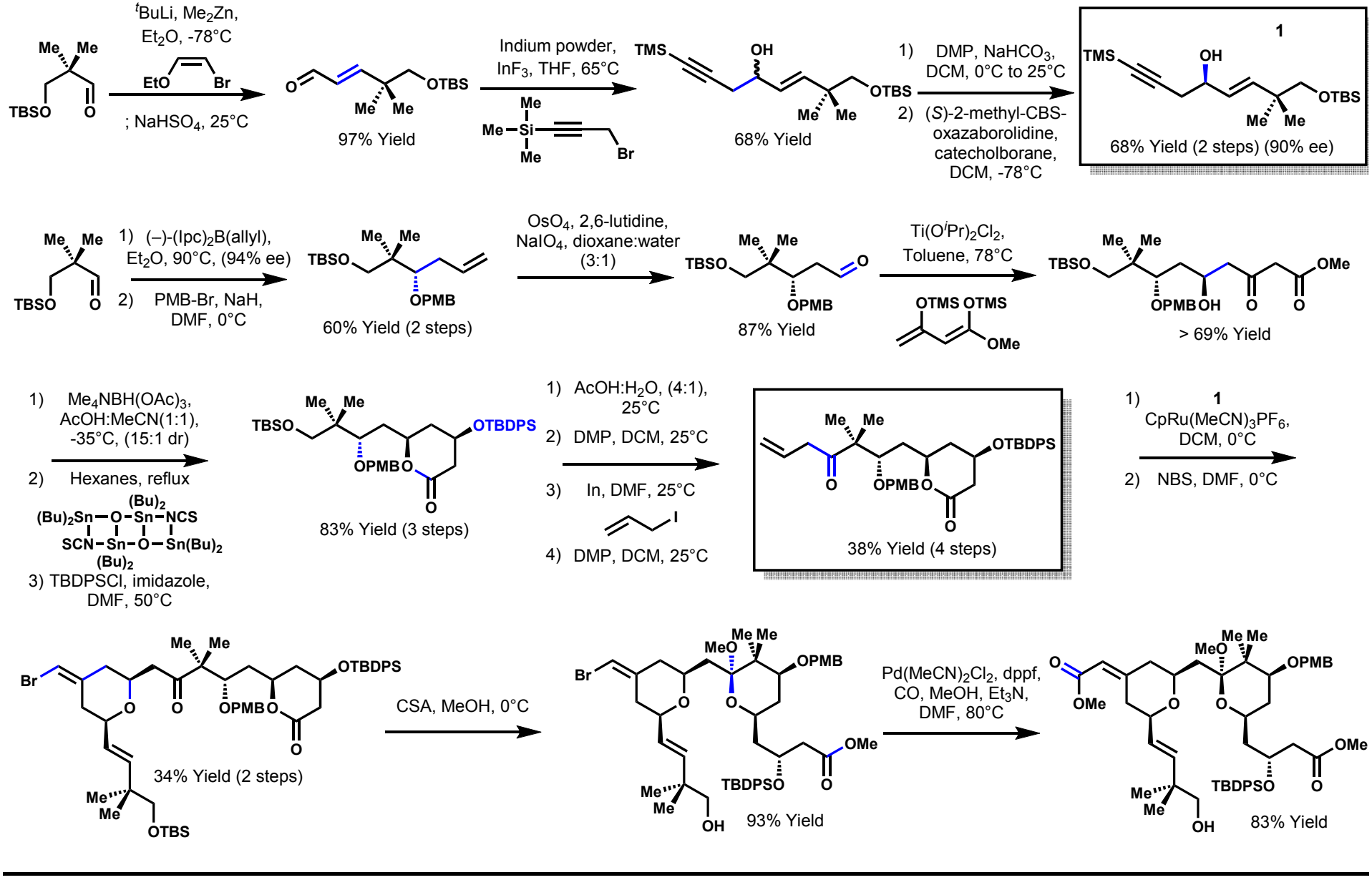




Shenvi Lab Group Meeting
04/10/2017

Bryostatin 16: Northern Fragment

Benjamin Huffman



Shenvi Lab Group Meeting
04/10/2017

Bryostatin 16: Southern Fragment

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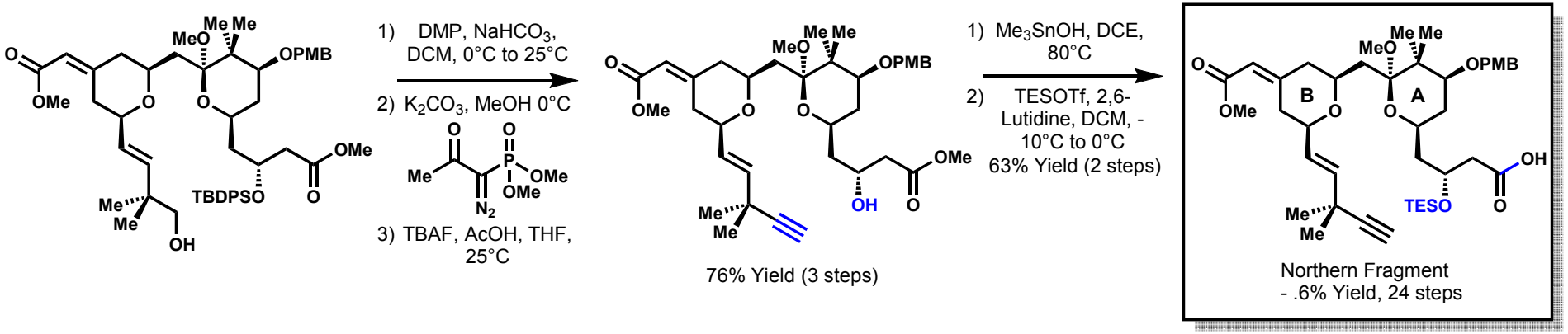
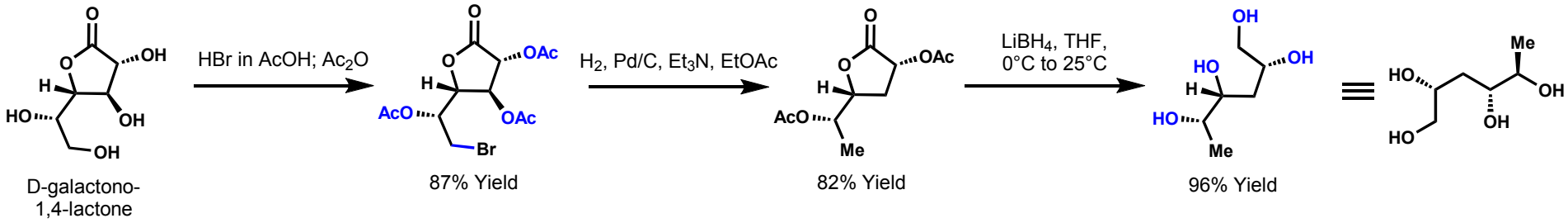
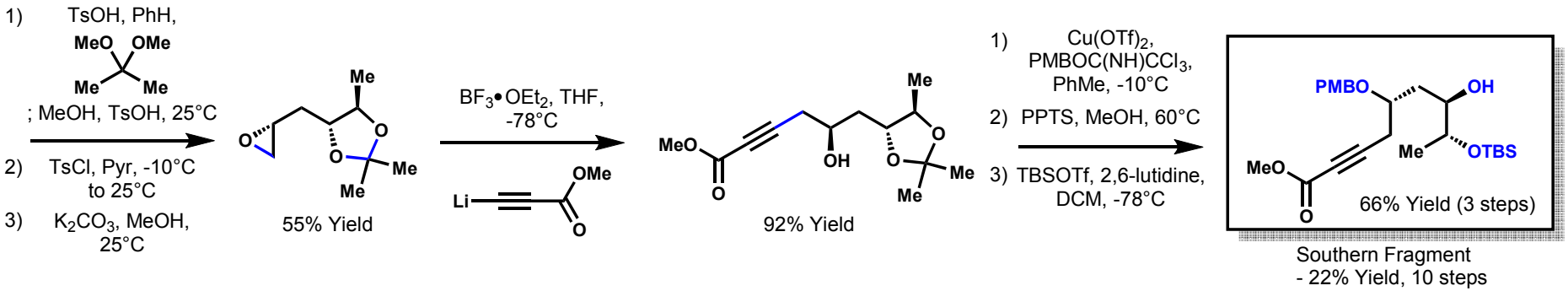


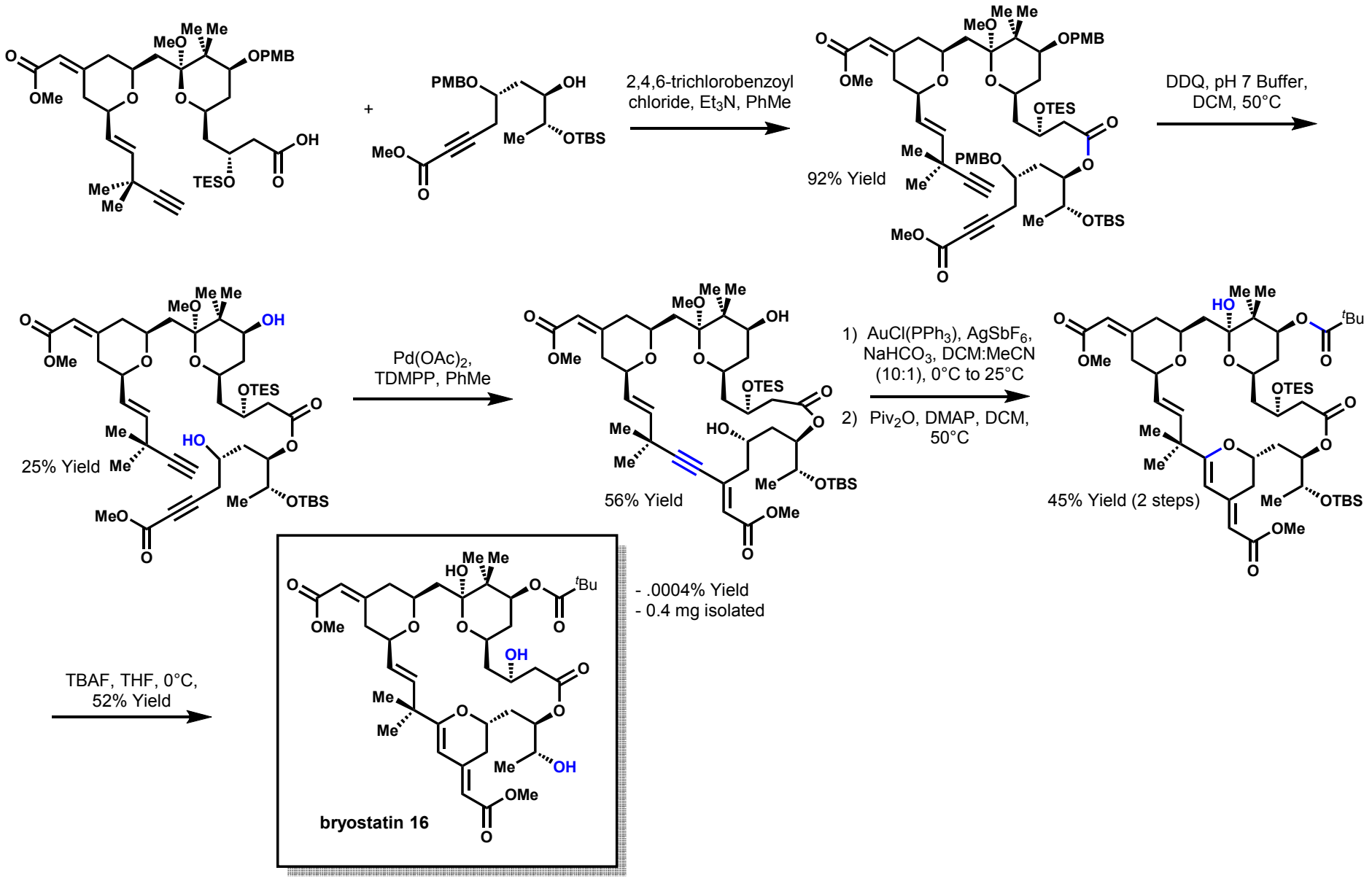
Synthesis of Southern Fragment





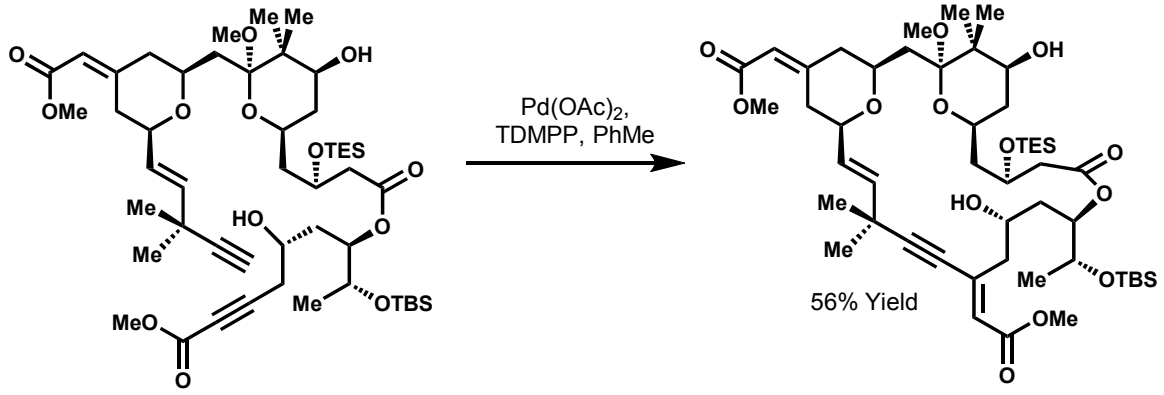
Bryostatin 16

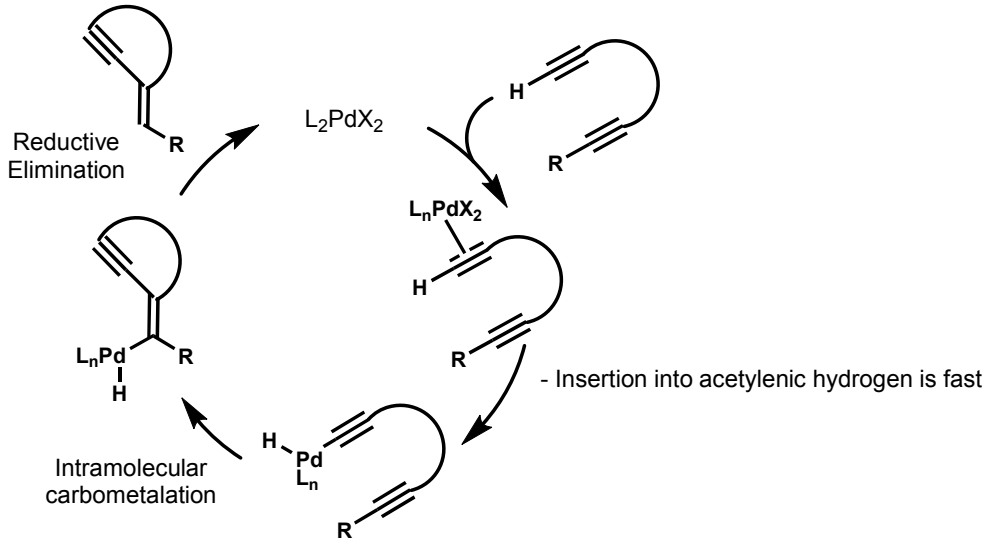
Benjamin Huffman



Bryostatin 16

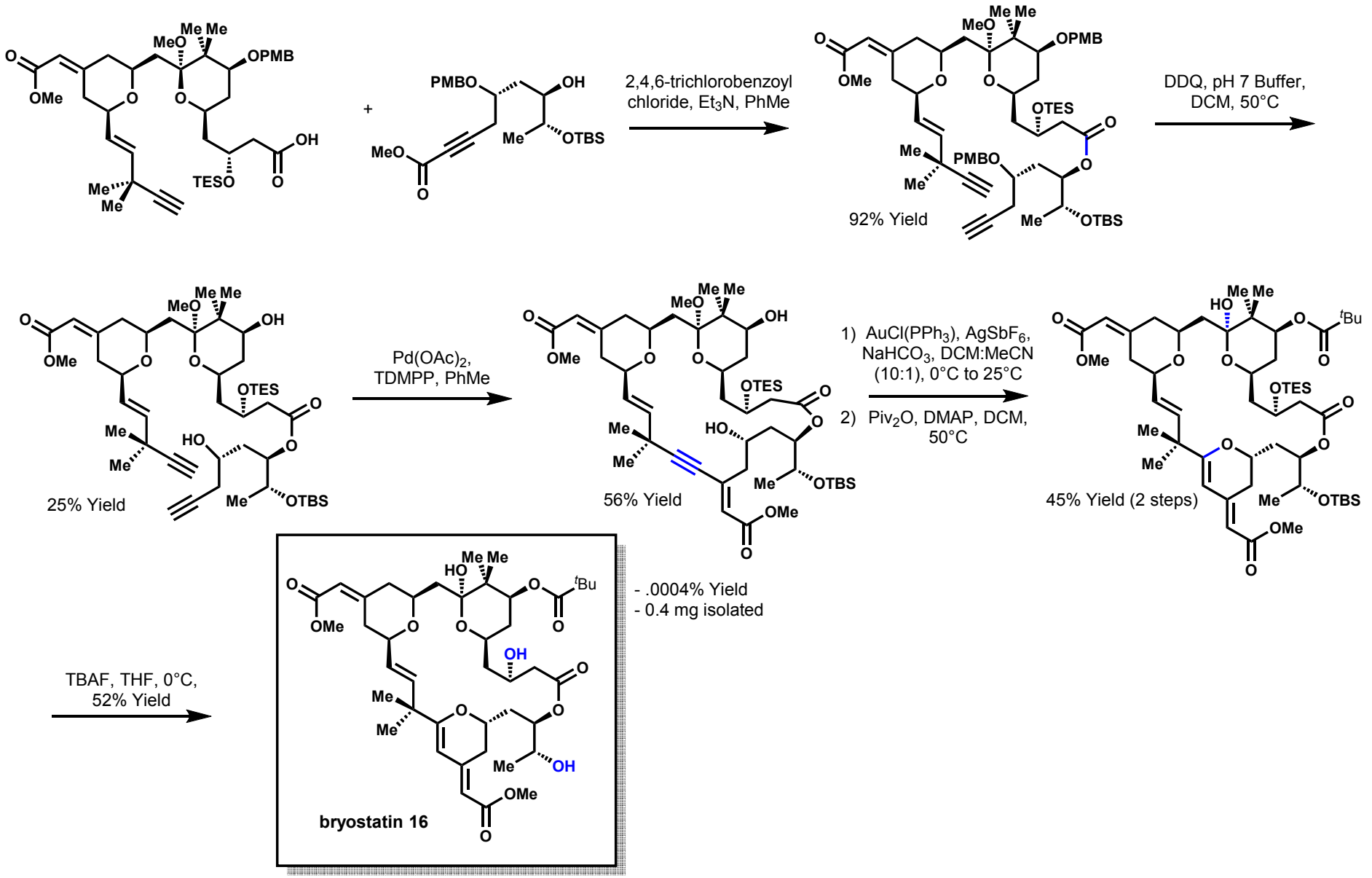
Benjamin Huffman





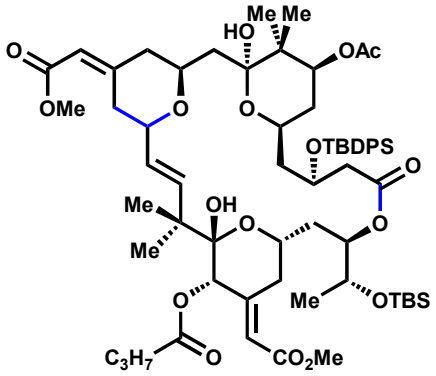
Bryostatin 16

Benjamin Huffman



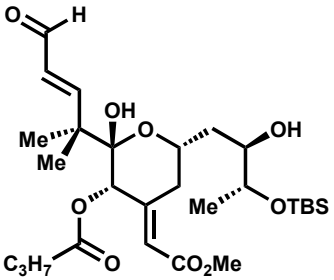
Bryostatin 9: Retrosynthesis

Benjamin Huffman

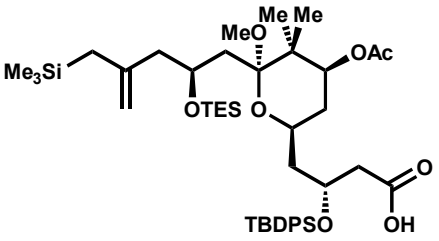


Prins-driven Macrocyclization

Yamaguchi Esterification



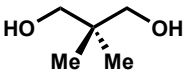
Southern Fragment



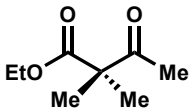
Northern Fragment

Wender, 2011
- 25 Steps (LLS)
- 43 total steps

19 steps



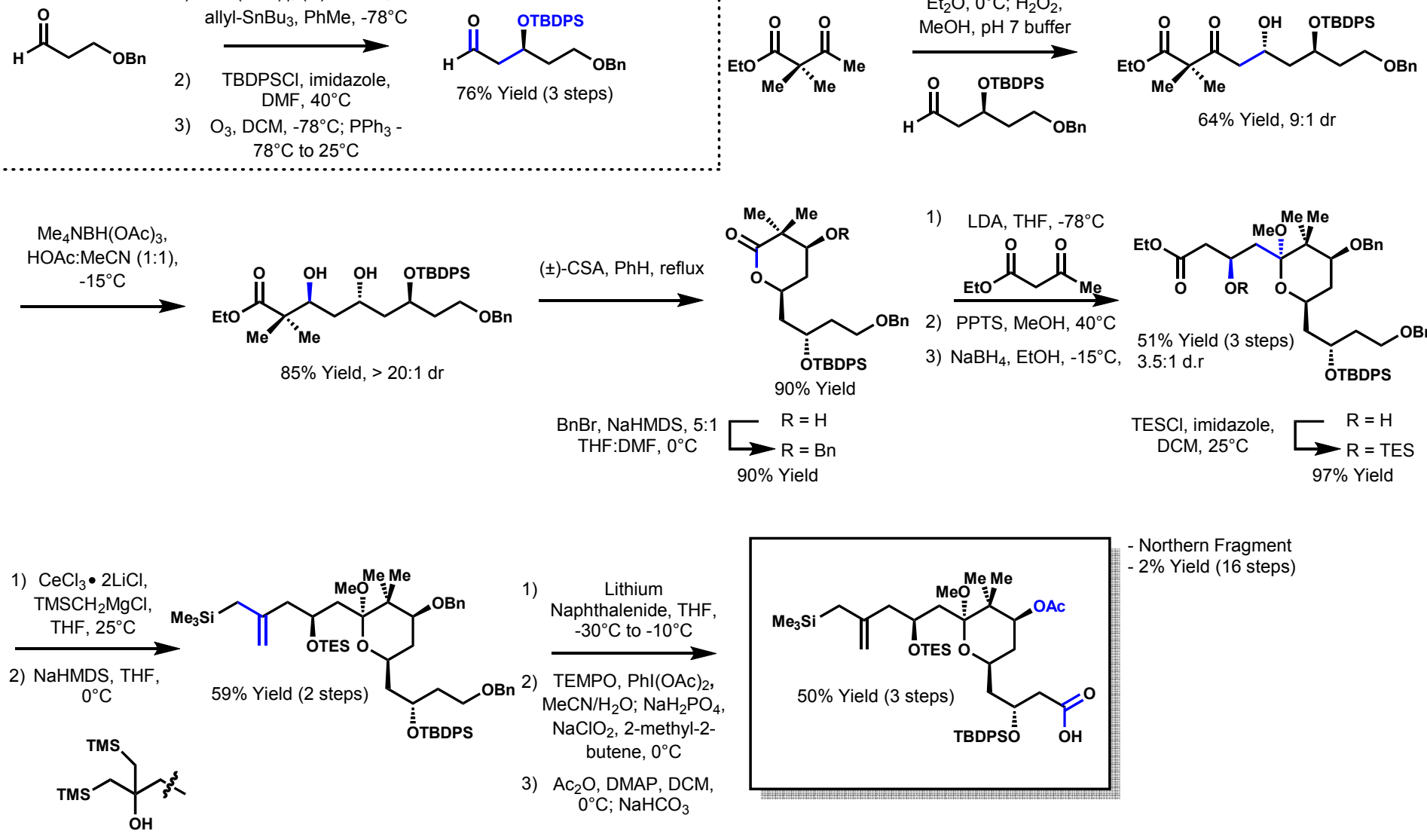
16 steps



Bryostatin 9: Northern Fragment

Benjamin Huffman

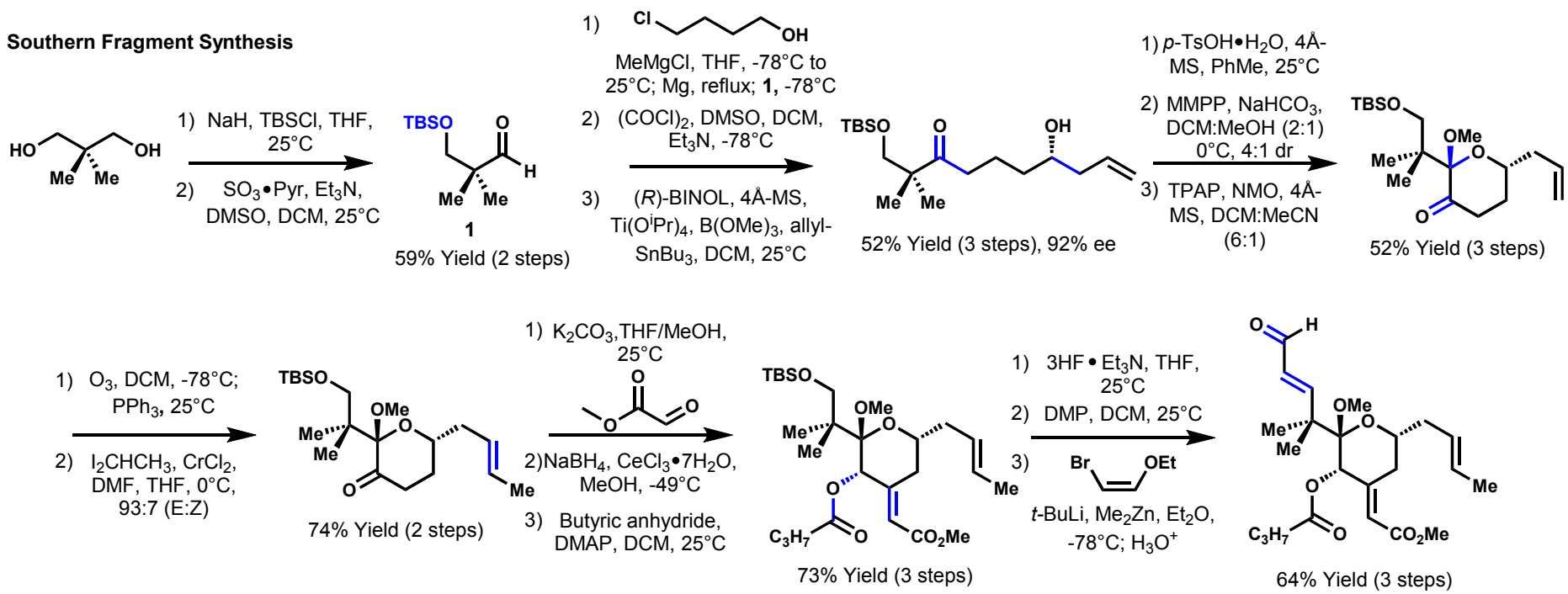
Northern Fragment Synthesis



Bryostatin 9: Southern Fragment

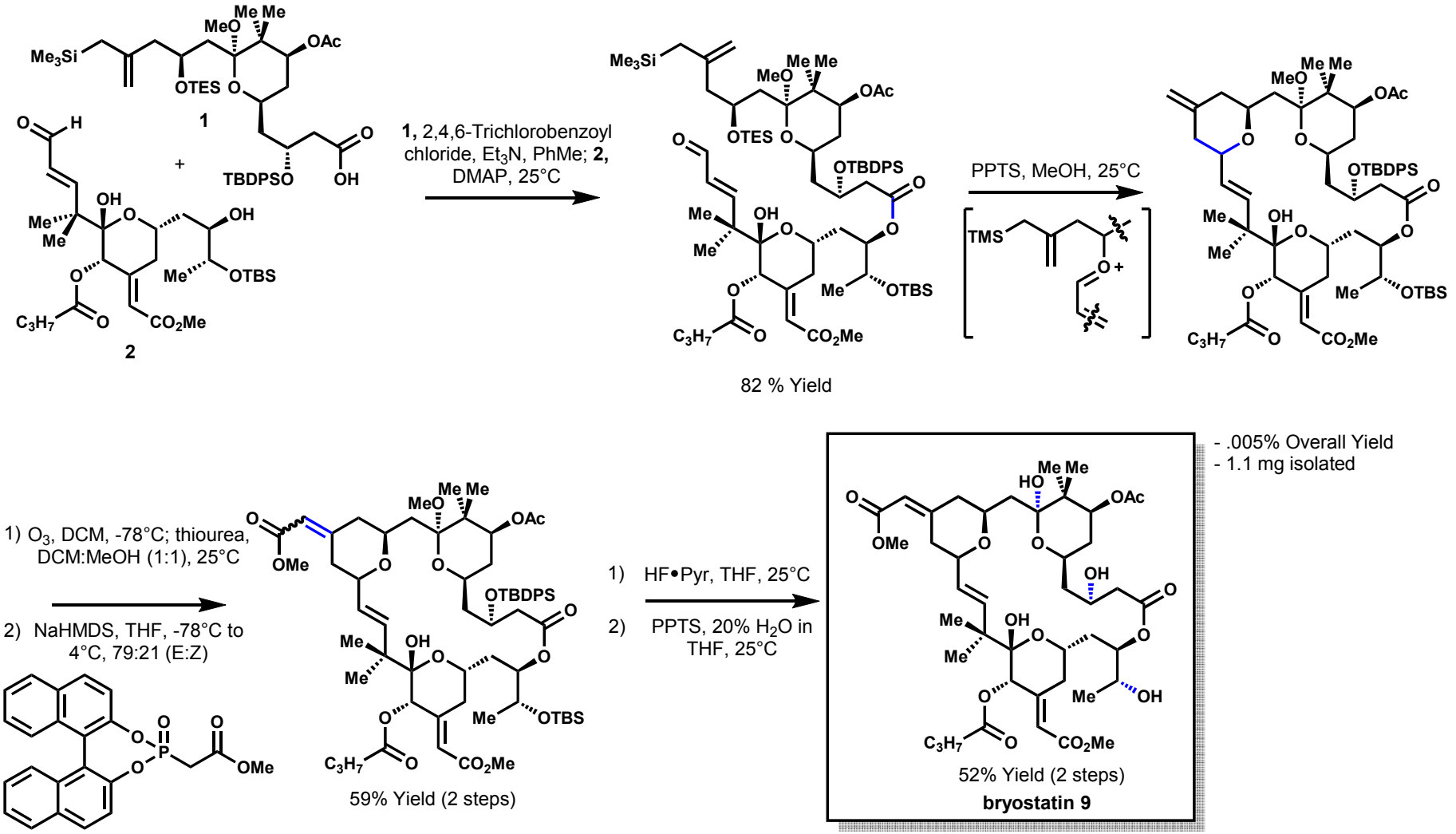
Benjamin Huffman

Southern Fragment Synthesis



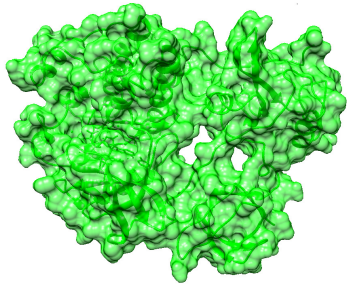
Bryostatin 9

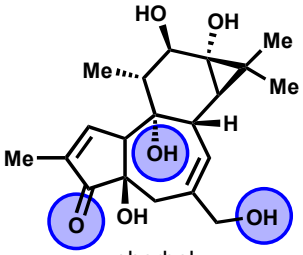
Benjamin Huffman



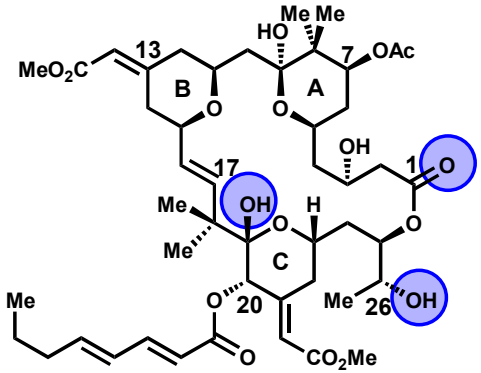
Notable Wender Bryologs

Benjamin Huffman





phorbol

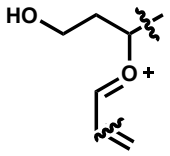


bryostatin 1 K_i = 1.35 nM

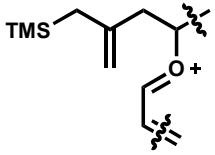
- Wender develops pharmacophoric model
- C_1, C_{19}, C_{26} oxygens spatially overlap with phorbol
- "Recognition domain" hypothesized

A ring construction

Acetalization

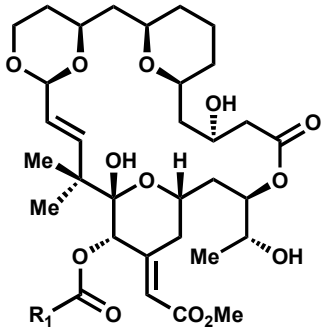


Prins



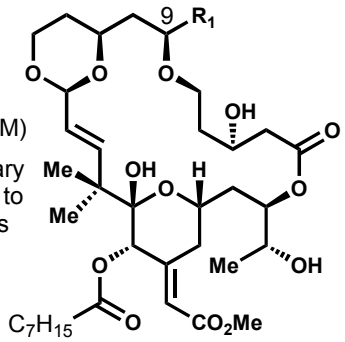
R_1 = Me (K_i = 232 nM)
R_1 = C_7H_{15} (K_i = 3.4 nM)
R_1 = $C_{13}H_{27}$ (K_i = 1.5 nM)
R_1 = Ph (K_i = 7 nM)

- Simplified A and B rings do not lose affinity for PKC

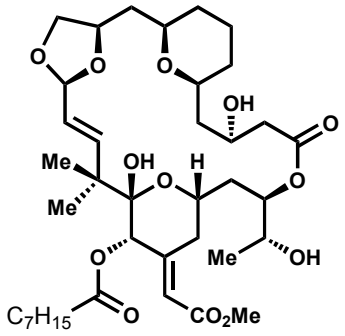


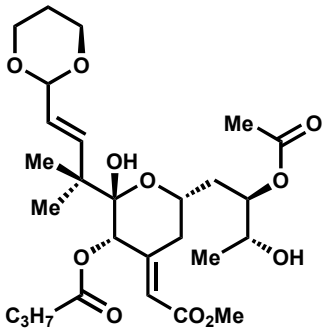
R_1 = H (K_i = 8 nM)
R_1 = *t*-Bu (K_i = 6.5 nM)
R_1 = Ph (K_i = 2.3 nM)
R_1 = Ph (p-Br) (K_i = 1.9 nM)

- Intact A ring not necessary
- C_9 region could be used to modify pharmacokinetics /dynamics

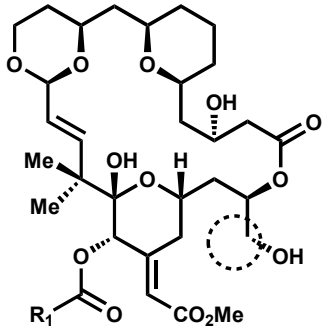


- K_i = 5.4 nM
- 5 - membered B ring retains single digit nM affinity
- Selectively translocates 4 of 8 PKC isozymes





- No significant bindining
- Recognition domain not sufficient by itself
- Macrocycle necessary

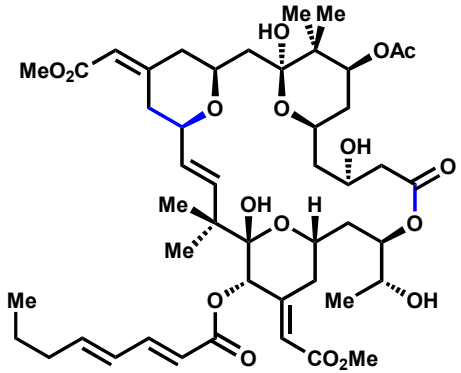


- "Picolog"
- K_i = 0.35 nM
- 100 fold Greater potency than bryostatin 1 in 24 of 35 cancer cell lines

PNAS, 1998, 6624; Org. Lett., 2006, 5299; JACS, 2008, 6658; Curr. Med. Chem., 2012, 2652

Bryostatin 1: Retrosynthesis

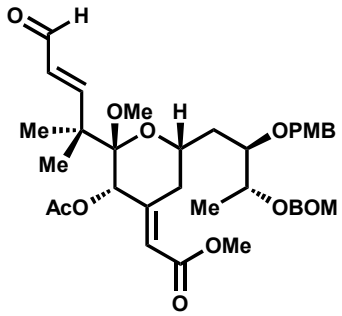
Benjamin Huffman

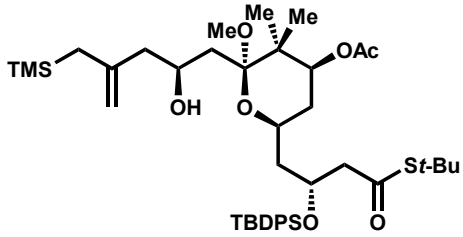


bryostatin 1

Prins-driven Macrocyclization

Yamaguchi Esterification

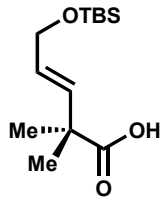


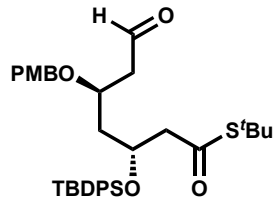


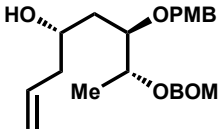
Keck, 2011
- 31 Steps (LLS)
- 58 total steps

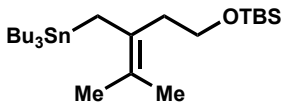
12 steps

20 steps



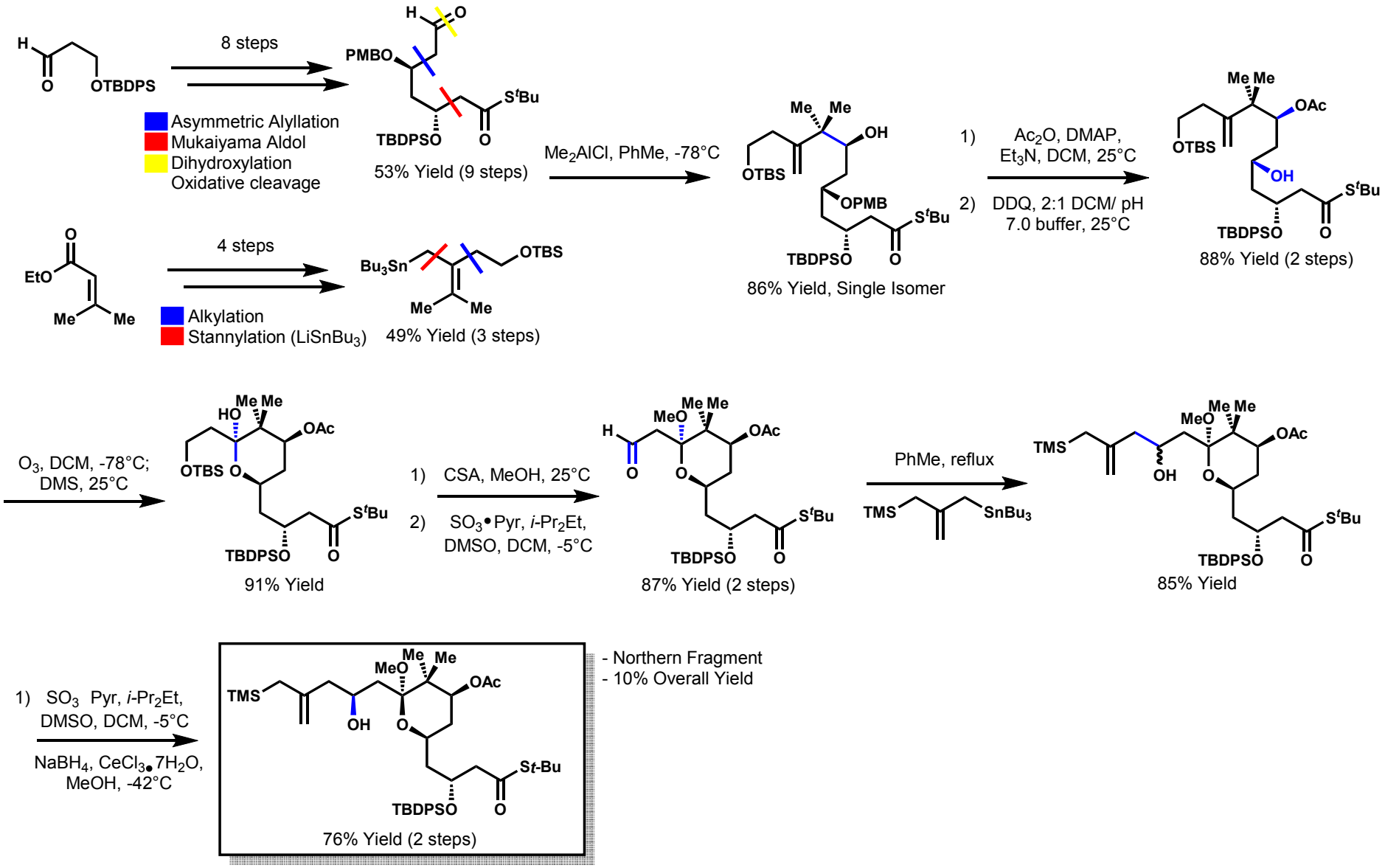






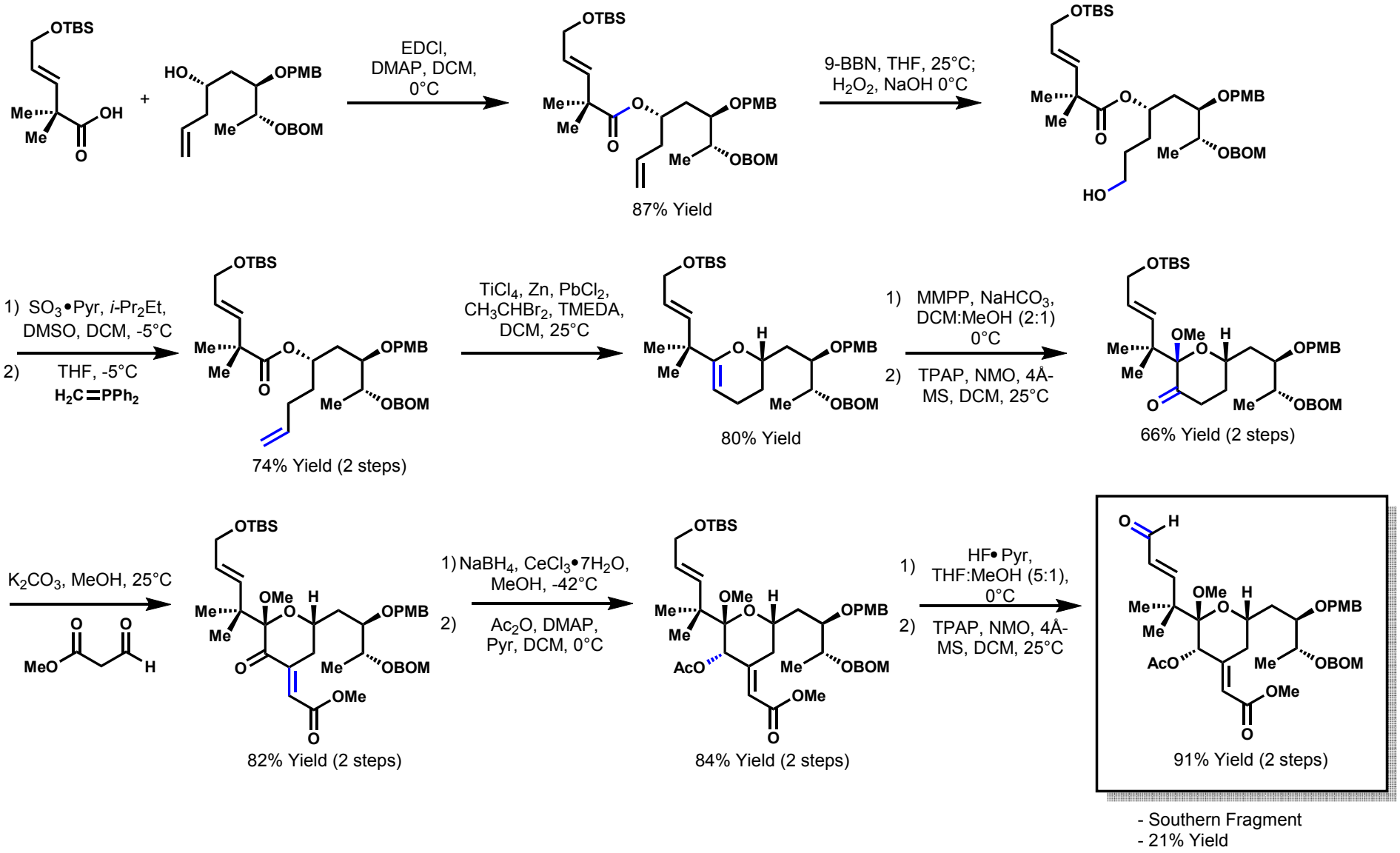
Bryostatin 1: Northern Fragment

Benjamin Huffman



Bryostatin 1: Southern Fragment

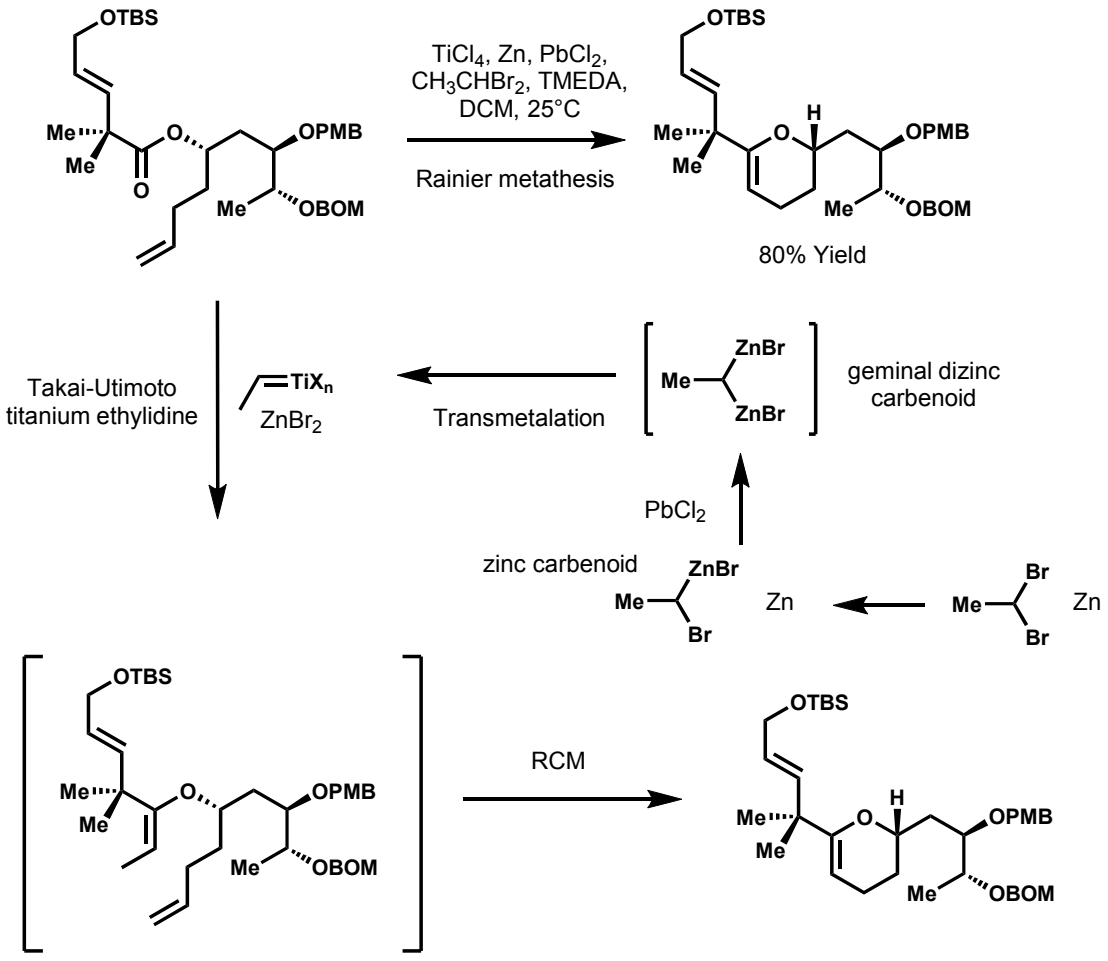
Benjamin Huffman



- Southern Fragment
- 21% Yield

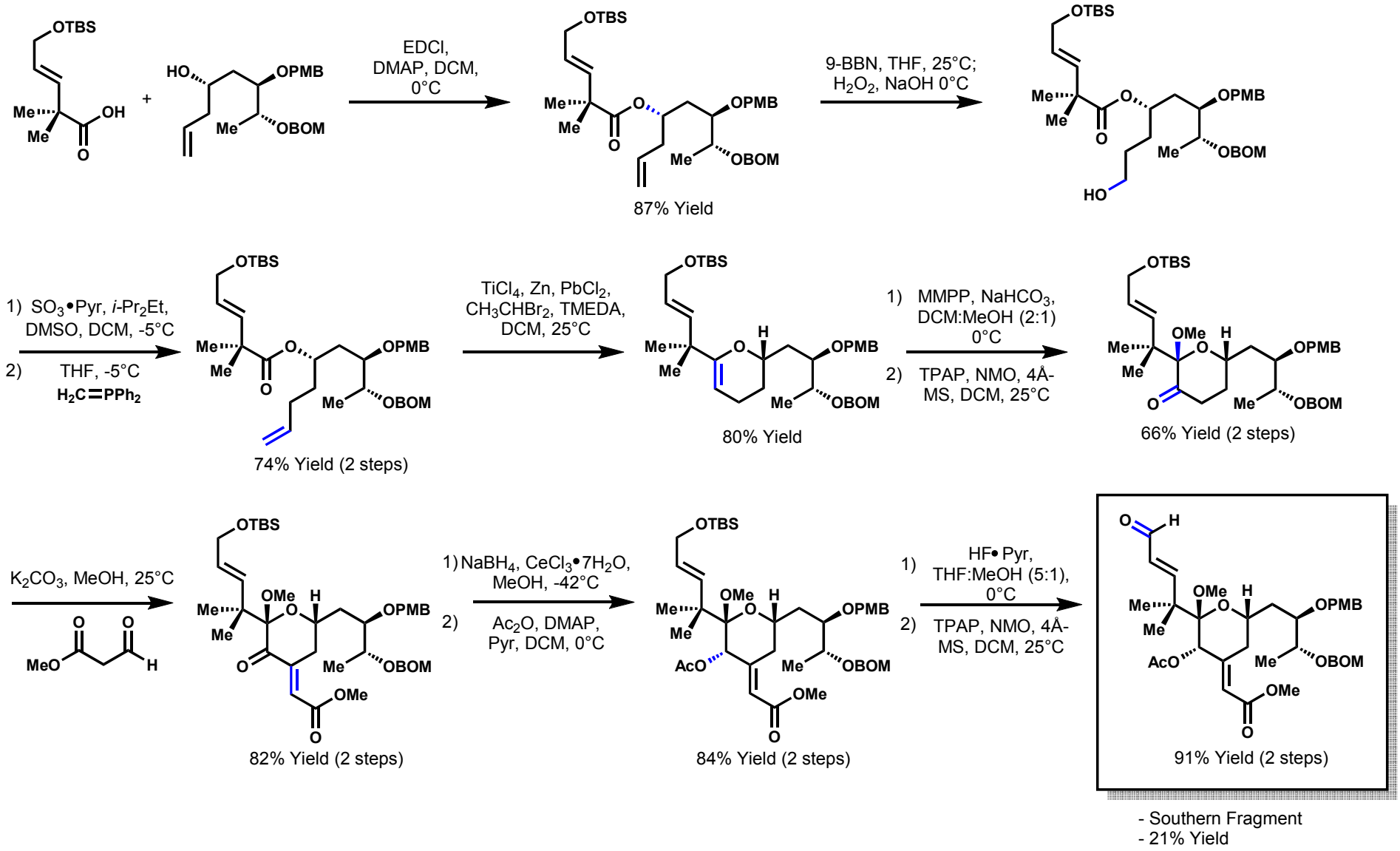
Bryostatin 1

Benjamin Huffman



Bryostatin 1: Southern Fragment

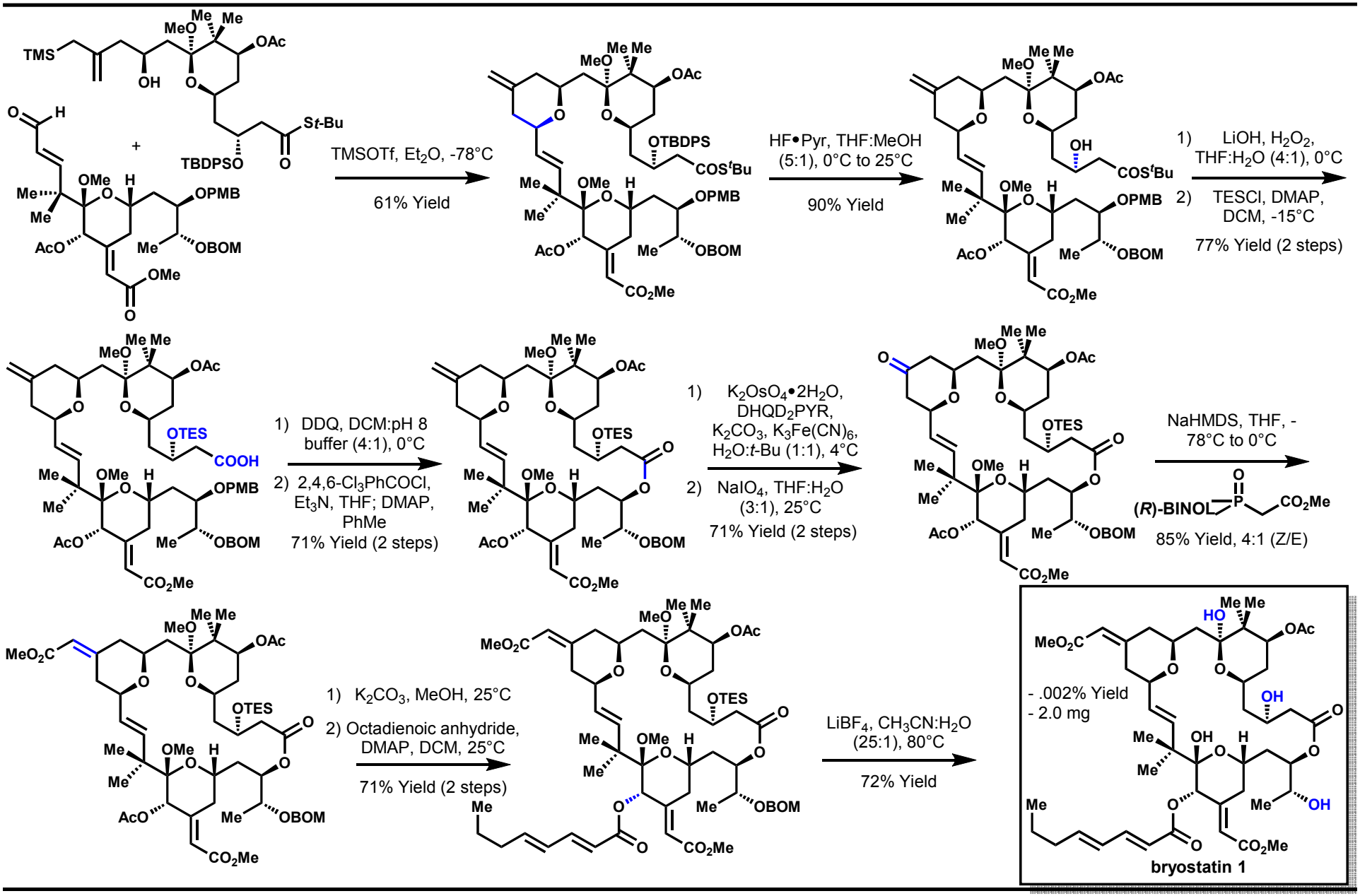
Benjamin Huffman



- Southern Fragment
- 21% Yield

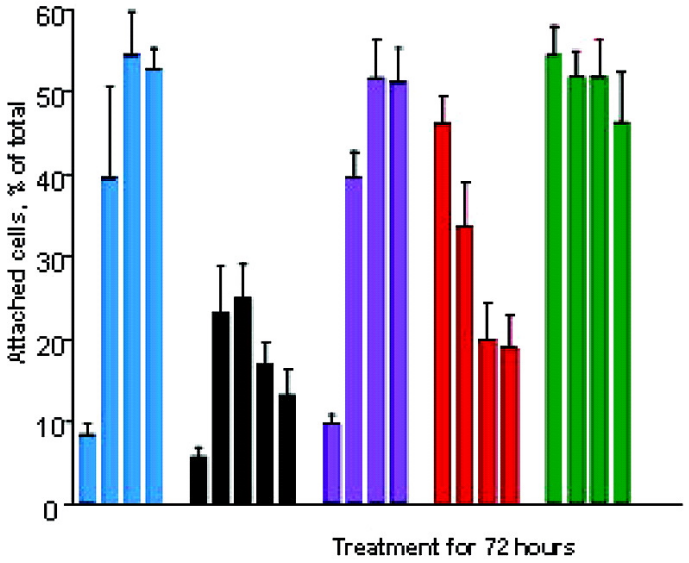
Bryostatin 1

Benjamin Huffman



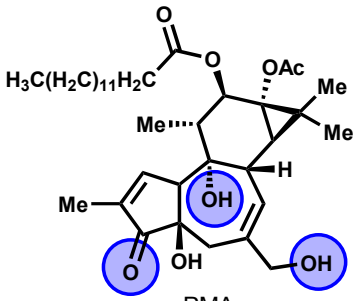
Notable Keck Bryologs

Benjamin Huffman

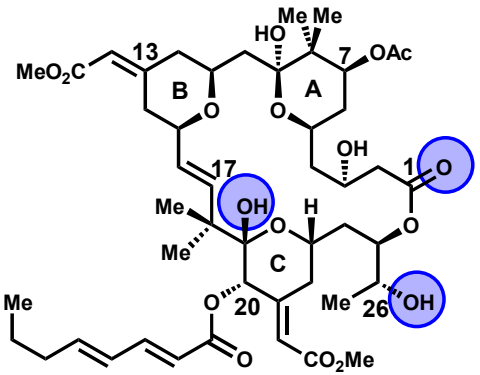


- PMA, a tumor promoter, increases attachment of U-937 leukemia cells in this assay

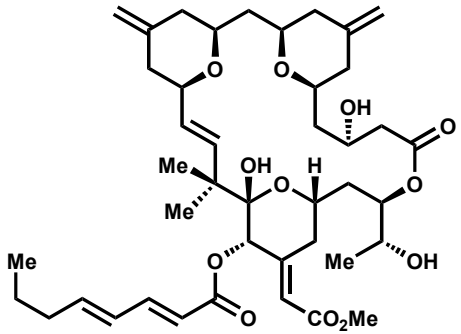
- Simple modification of A and B ring leads to tumor-promoting PMA activity



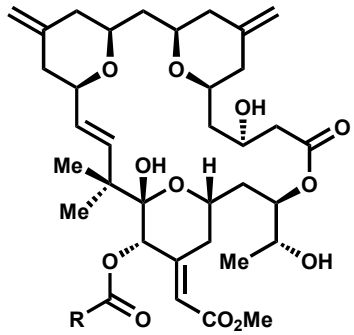
PMA



bryostatin 1



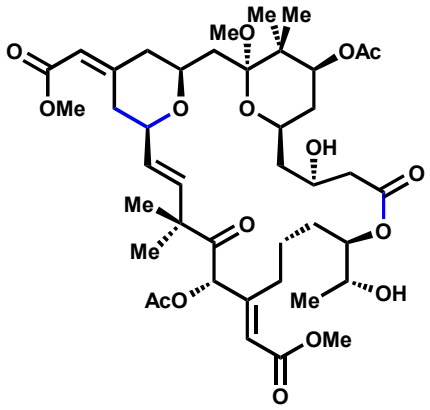
13, K_i = 0.70 nM
Merle 23



R_1 = Ph (K_i = 0.70 nM)
R_1 = C_7H_{15} (K_i = 1.05 nM)

Bryostatin 7: Retrosynthesis

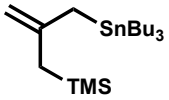
Benjamin Huffman

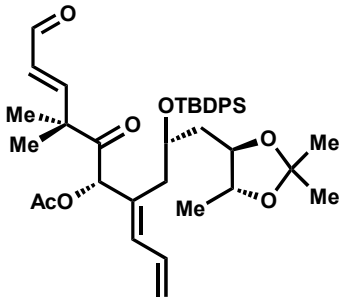


bryostatin 7

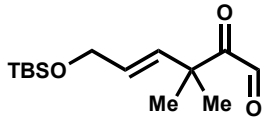
Prins-driven Macrocyclization

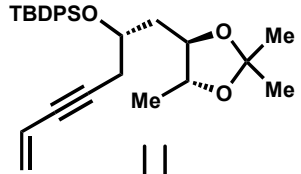
Yamaguchi Esterification

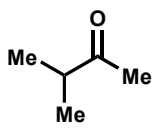


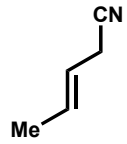


Hydrogenative coupling

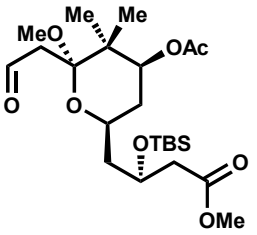


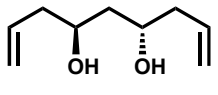


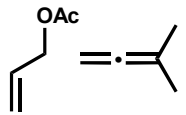


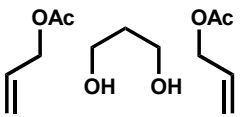


4 Hydrogenative couplings









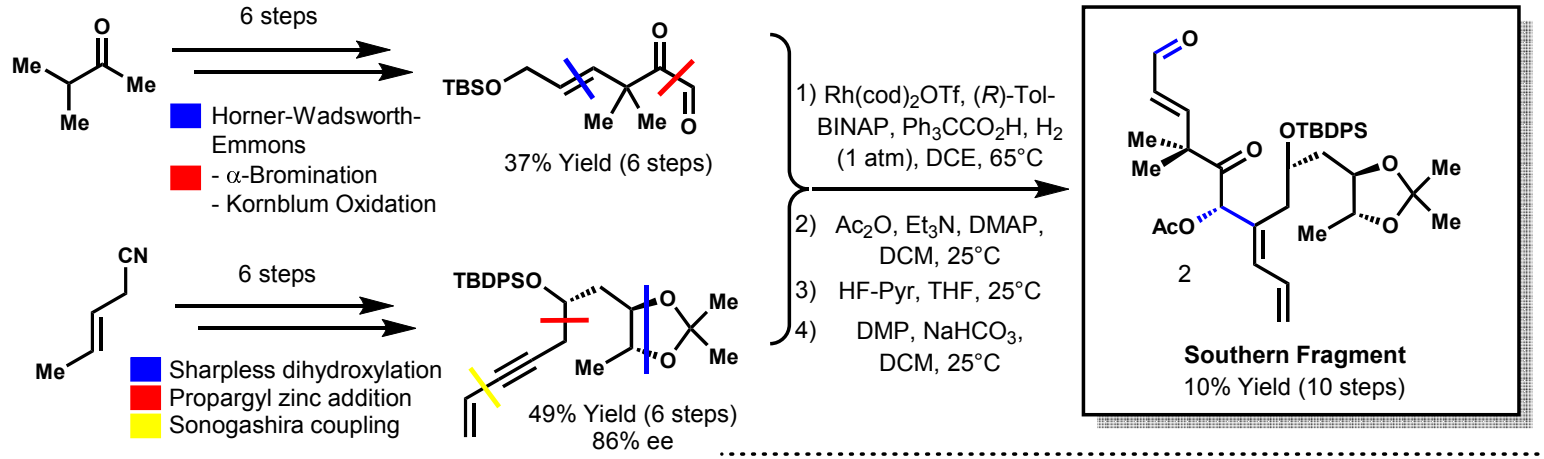
Krische, 2011
- 20 Steps (LLS)
- 36 total steps

JACS, 2011, 13876

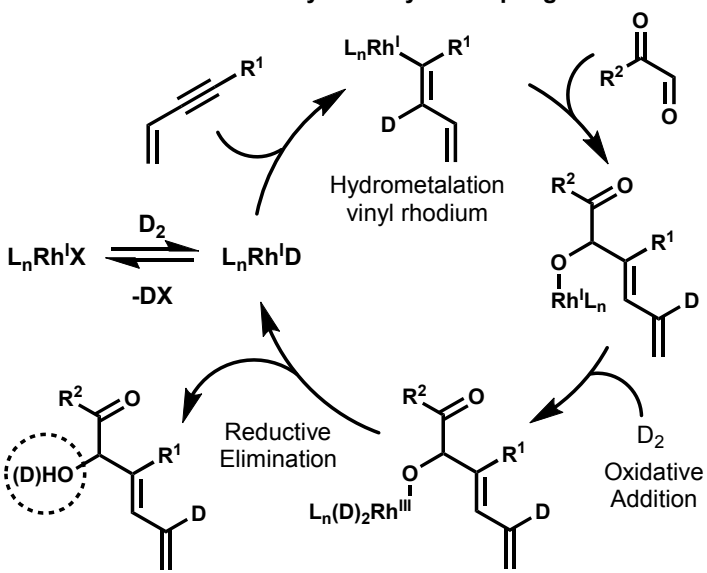
Bryostatin 7: Southern Fragment

Benjamin Huffman

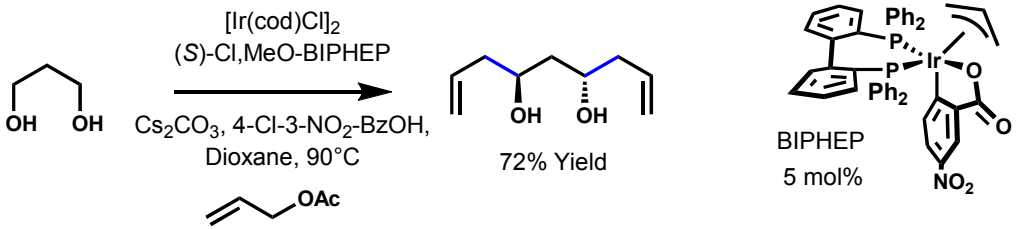
Synthesis of Southern Fragment



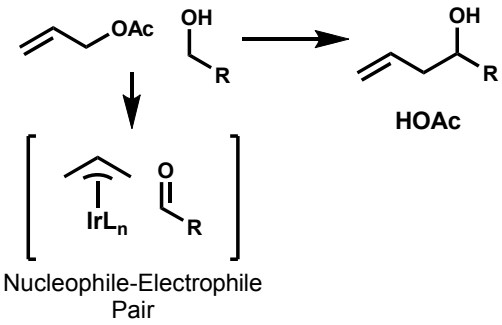
Glyoxal Enyne Coupling



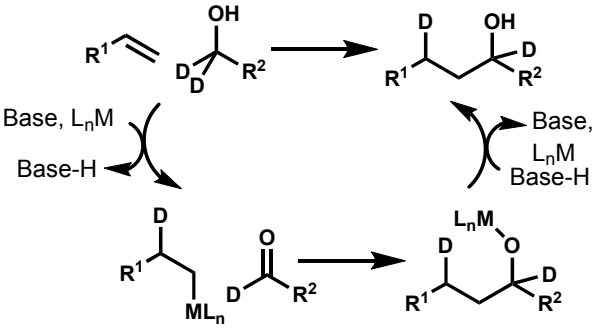
Synthesis of Northern Fragment



C-Allylation Via Transfer Hydrogenation

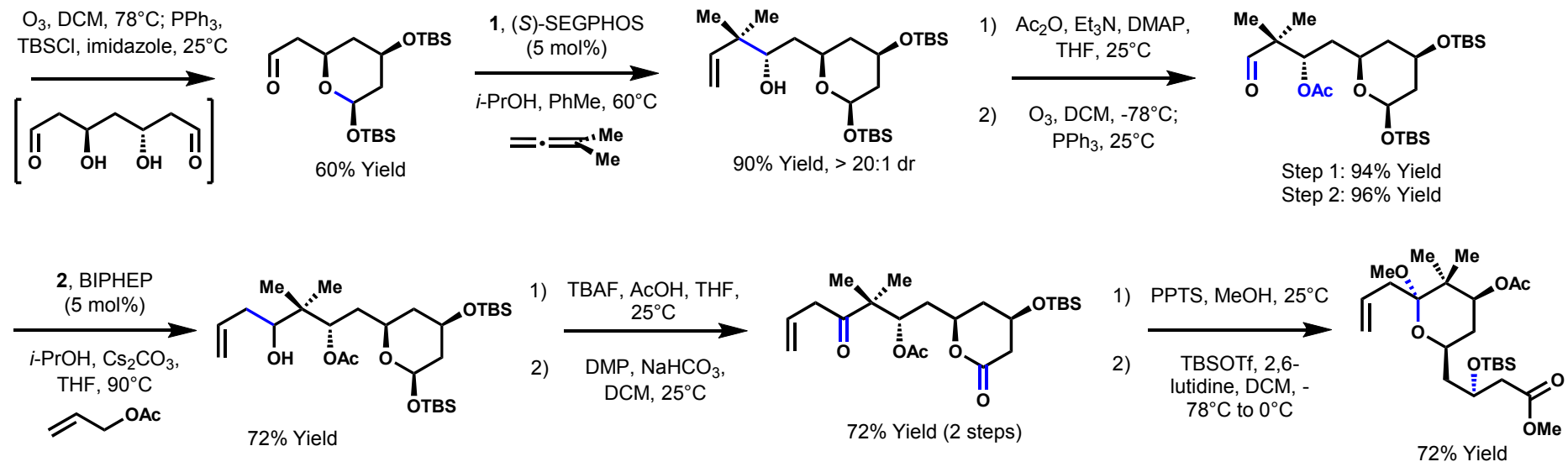


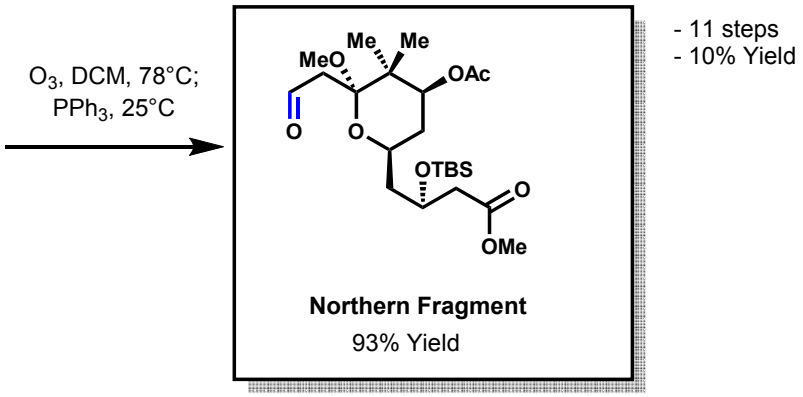
Hydrogen Auto-Transfer



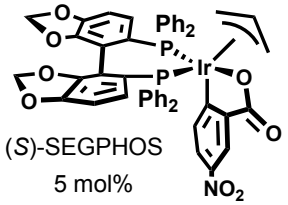
Bryostatin 7: Northern Fragment

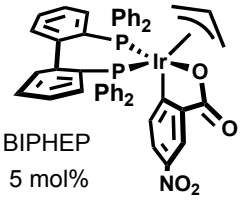
Benjamin Huffman





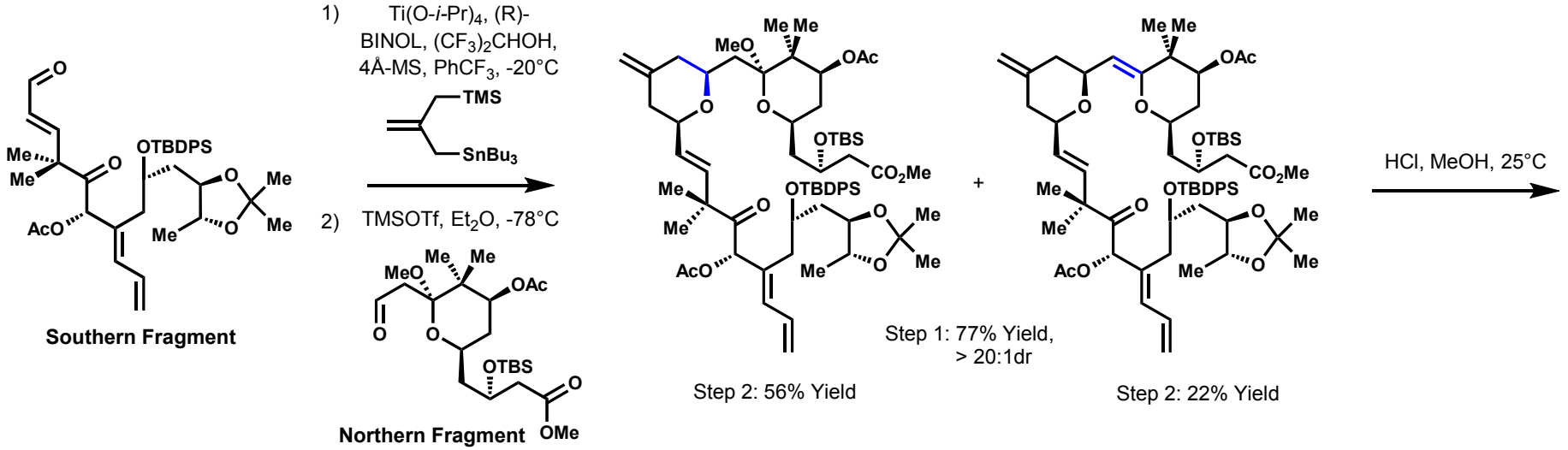
- 11 steps
- 10% Yield

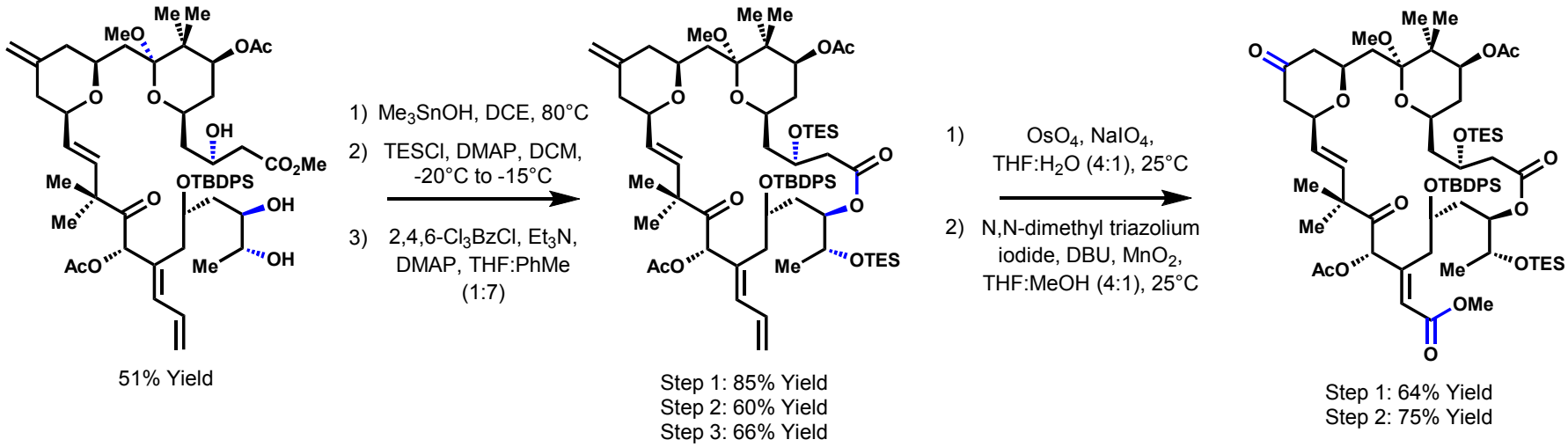




Bryostatin 7

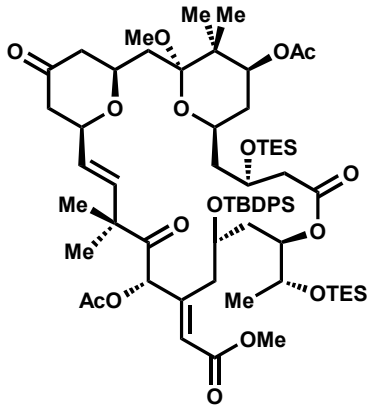
Benjamin Huffman





Bryostatin 7

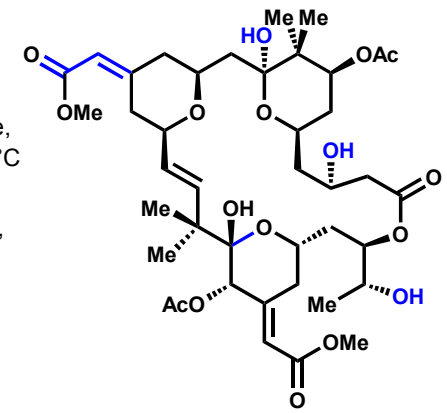
Benjamin Huffman



Step 1: 64% Yield
Step 2: 75% Yield

1) [(*R*)-BINOL]POCH$_2$CO$_2$Me, NaHMDS, THF, -78°C to 0°C

2) HF-Pyr, THF:H$_2$O (100:1), 35°C



Bryostatin 7

Step 1: 93% Yield, 6:1dr
Step 2: 60% Yield

- .00004 % Yield
- 1.7 mg