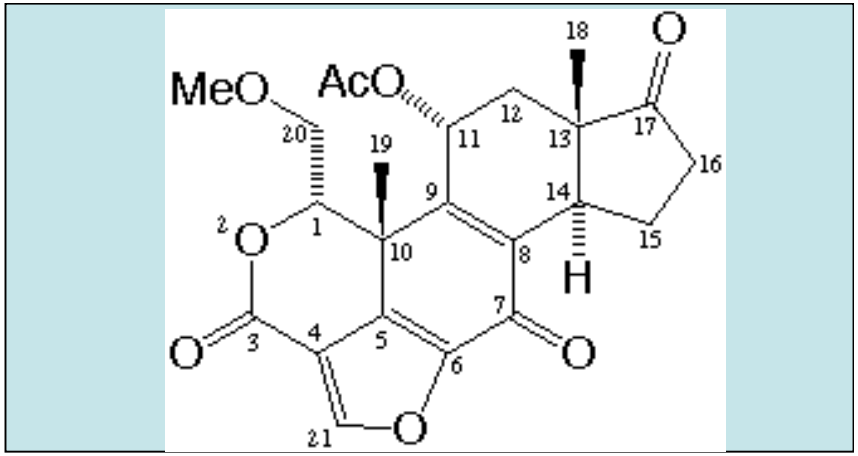
Research Topic Seminar

Dr. Claire Coleman

The Chemistry and Biology of Wortmannin



- Off white to pale yellow solid
- Hygroscopic/Light sensitive
- Small molecule natural product first isolated from culture filtrates of ***Penicillium Wortmanni*** (1957)
- Later found to be a metabolite of a variety of *Penicillium* and *Myrothecium* species
- Structure by chemical degradation and spectroscopic analysis 1972
- X-ray analysis 1972
- Clinically used as an immunosuppressive and anti-inflammatory
- Commercially available AG Scientific 25 mg $ 330

Wortmannin and simple analogs were found to be potent anti-inflammatory agents 1970's

Exhibited a high degree of toxicity-determined unsuitable for clinical development

20 years later Wortmannin was found to be a potent and selective inhibitor of PI 3-Kinase (IC_{50} = 4 nm)

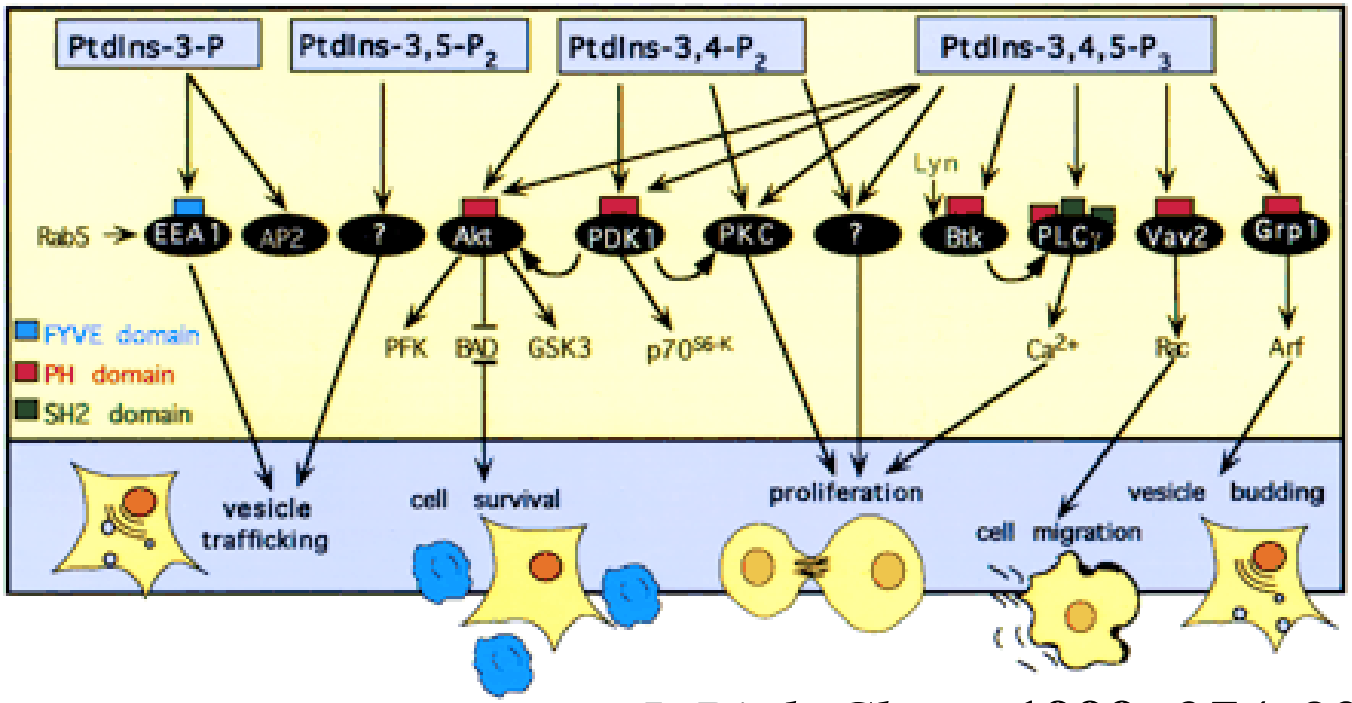
Phosphatidylinositol-3-kinase (PI-3-Kinase)

Important enzyme for intracellular signalling

Primary enzymatic activity of the PI-3-Kinases is the phosphorylation of inositol lipids at the 3 position

Different members of the PI-3-Kinase family generate different lipid products (lipid secondary messengers)

Vesicle trafficking
Cell survival
Proliferation
Cell migration
Vesicle budding



J. Biol. Chem. **1999**, *274*, 8347

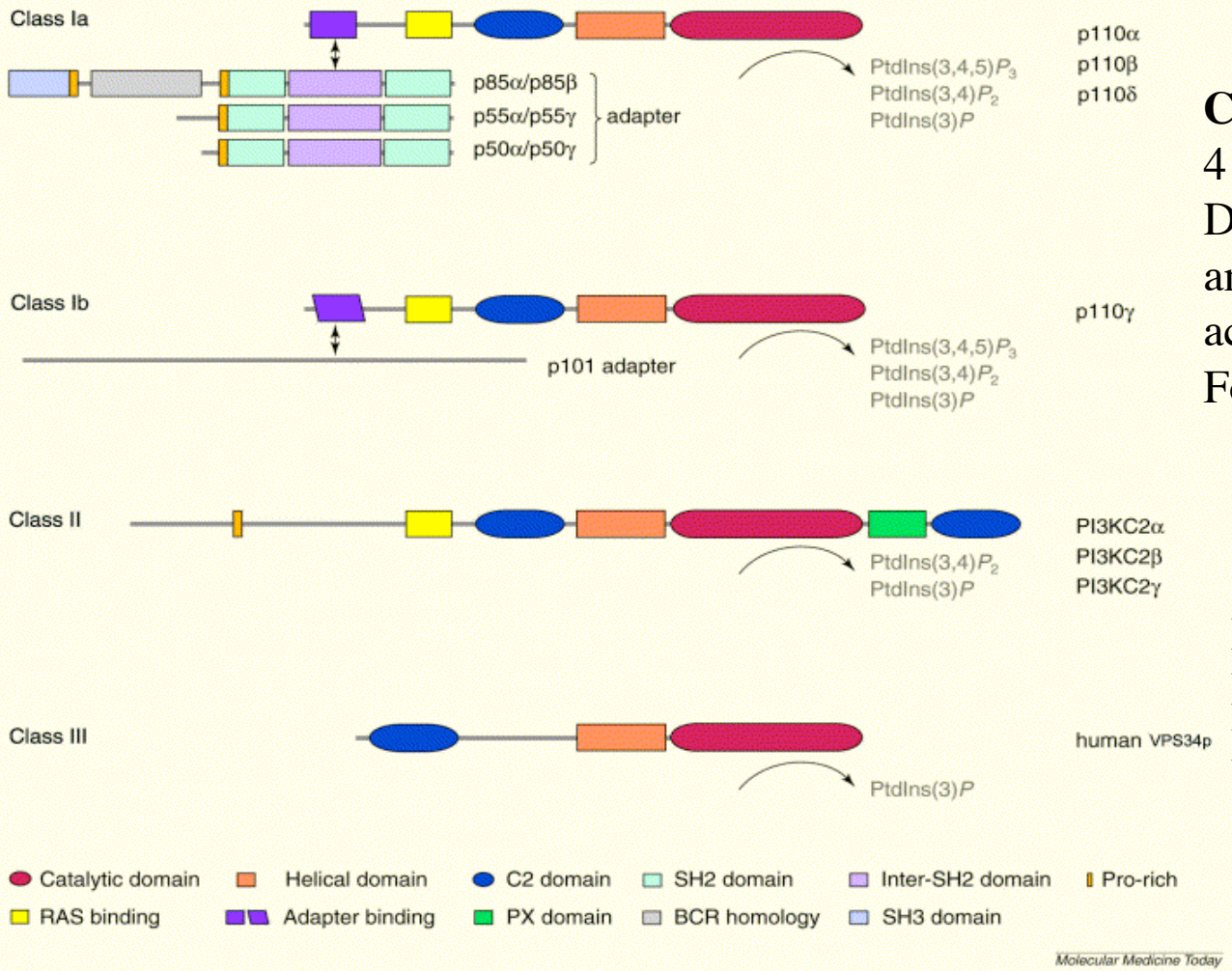
Signaling through PI 3-K lipid products and their targets. The lipid products of PI 3-K are indicated at the *top* of the figure, and the cellular processes affected by these lipids are indicated at the *bottom*. The *black ovals* indicate the direct targets of each lipid, and the *small boxes* indicate the protein domains that directly bind to them.

PI3-Kinase was initially purified and cloned as a heterodimeric complex consisting of

110 kDa catalytic subunit now called p110
85 kDa regulatory/adaptor subunit p85

9 mammalian PI-3-kinases have been identified

Divided into 3 classes based on sequence homology and substrate preference *in vitro*



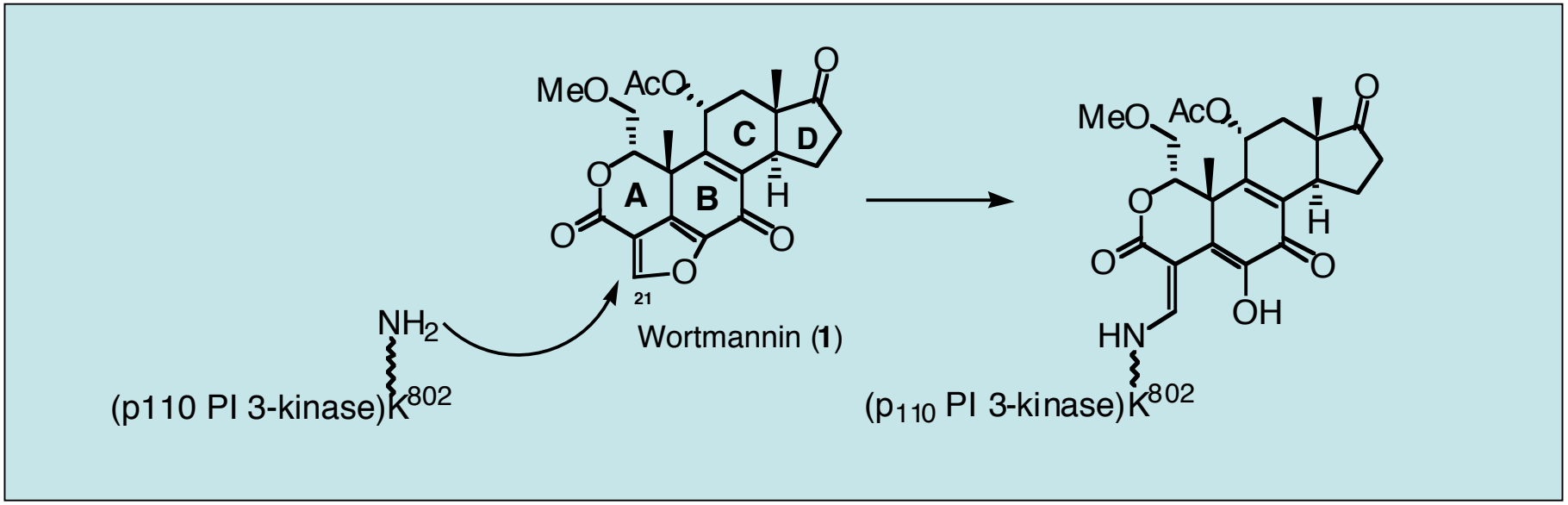
Class I
4 class I enzymes
Divided into 2 subclasses (Ia and Ib) based on mechanism of activation
Found in cytoplasm

Class II
3 members
Least understood
Larger than class I/III
Membrane associated

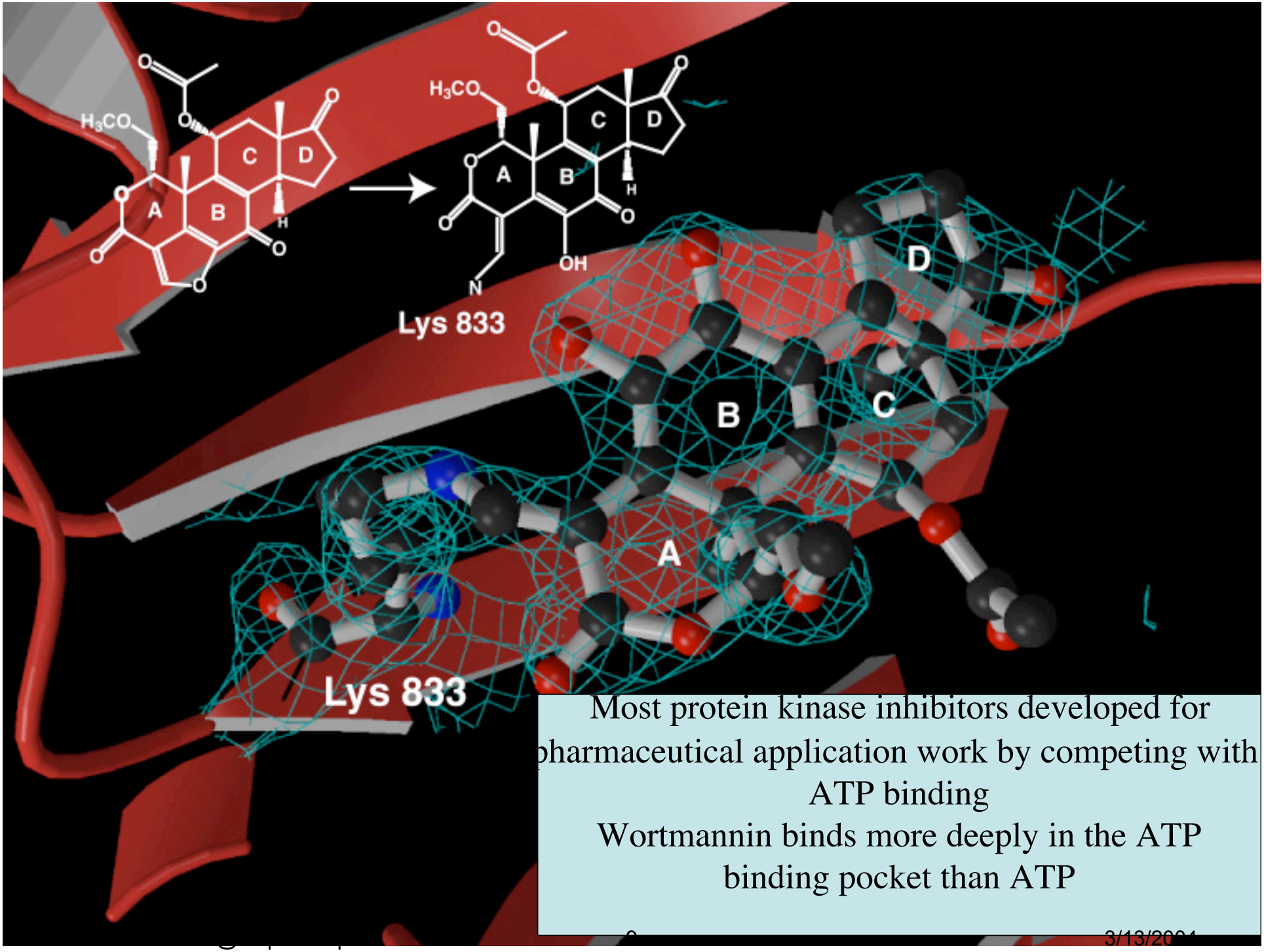
Class III
Traffic of proteins through the lysosome

Wortmannin-an **irreversible inhibitor** of PI 3-Kinase

Nucleophilic attack at the electrophilic C-21 position of the furan ring by Lys^{802} of p110 PI 3-Kinases



The Lysine residue resides in the ATP binding site of the p110 catalytic subunit--crucial role in the phosphotransfer reaction



Most protein kinase inhibitors developed for pharmaceutical application work by competing with ATP binding

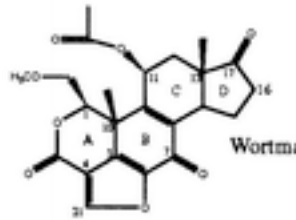
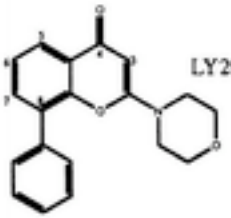
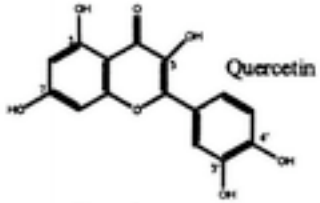
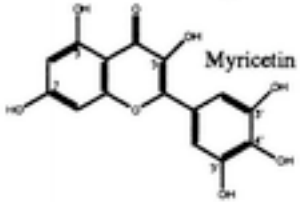
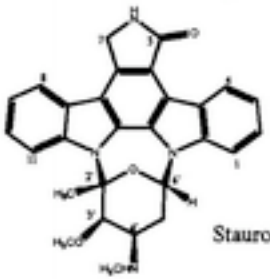
Wortmannin binds more deeply in the ATP binding pocket than ATP

Regulation of cell proliferation and the signaling pathways within cells that control it is important

If drugs can be developed to interfere with signal transductions then cancer cells may be made nonproliferating and new cancer therapies may emerge

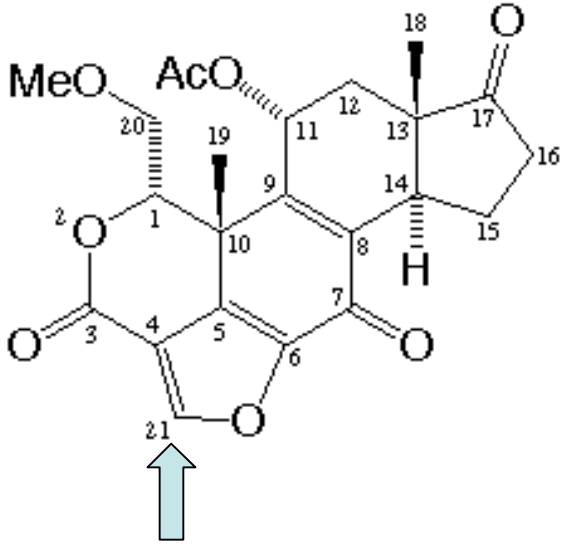
Inhibitors of PI 3-kinase (an enzyme that functions within signal transduction pathways) may be useful for new cancer and tumour therapies

PI 3-kinase Inhibitors other than Wortmannin

	Reported IC_{50}	K_d for PI3K γ
 Wortmannin	4.2 nM (Class IA PI3K)	-
 LY294002	1.4 μM (Bovine brain PI3K)	0.21 μM±0.04 μM
 Quercetin	3.8 μM (Bovine brain PI3K)	0.28 μM±0.04 μM
 Myricetin	1.8 μM (Class IA PI3K)	0.17 μM±0.04 μM
 Staurosporine	9 μM* (Class IA PI3K)	0.29 μM±0.06 μM

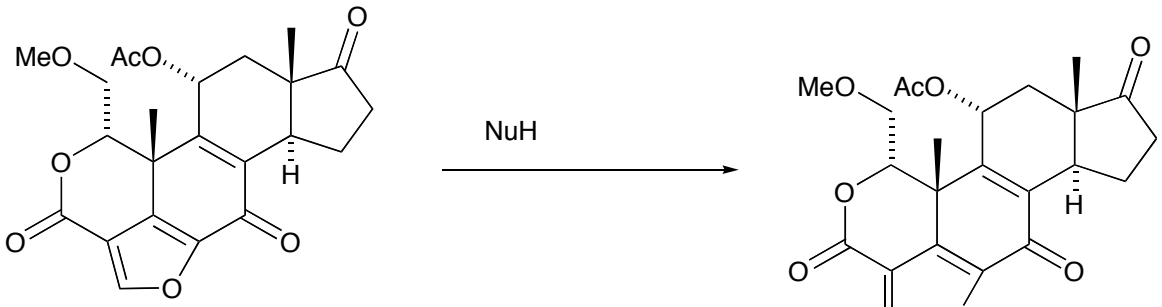
IC_{50} is 500 fold higher than wortmannin but is widely used in cell biology as it is much more stable in solution

The **furan ring** in Wortmannin is **essential** for biological activity



Chemists at Eli Lilly performed SAR on Wortmannin analogs to probe the structural requirements necessary for
PI 3-Kinase inhibition

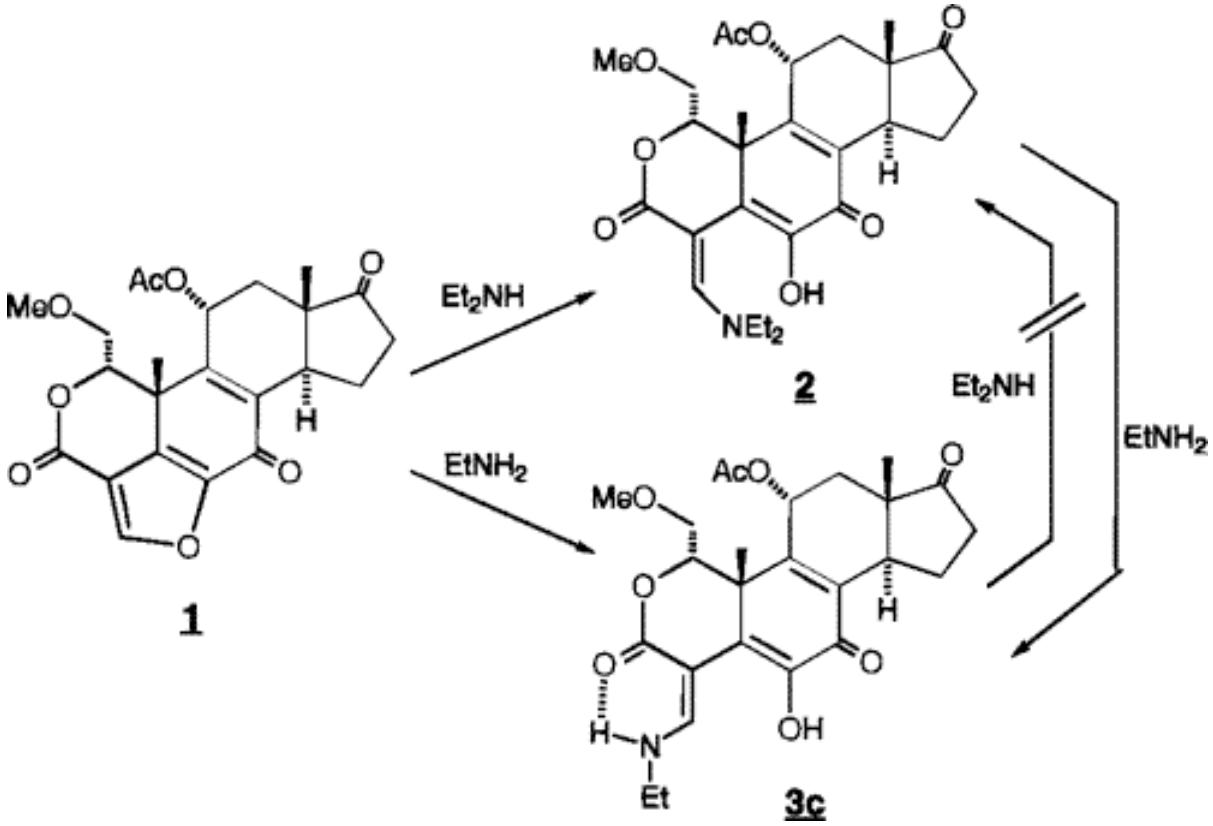
Made electronic/steric changes that influenced the elctrophilicity of the C-21 centre



Wortmannin (1)

	Nu	PI 3-K IC_{50} (nM)
⇒	Et_2N	80
	NH_2	>>500
	MeNH	>>500
	EtNH	>>500
	n-PrNH	>>500
⇒	*n*-BuS	52

1 and 6 retain activity but greatly reduced

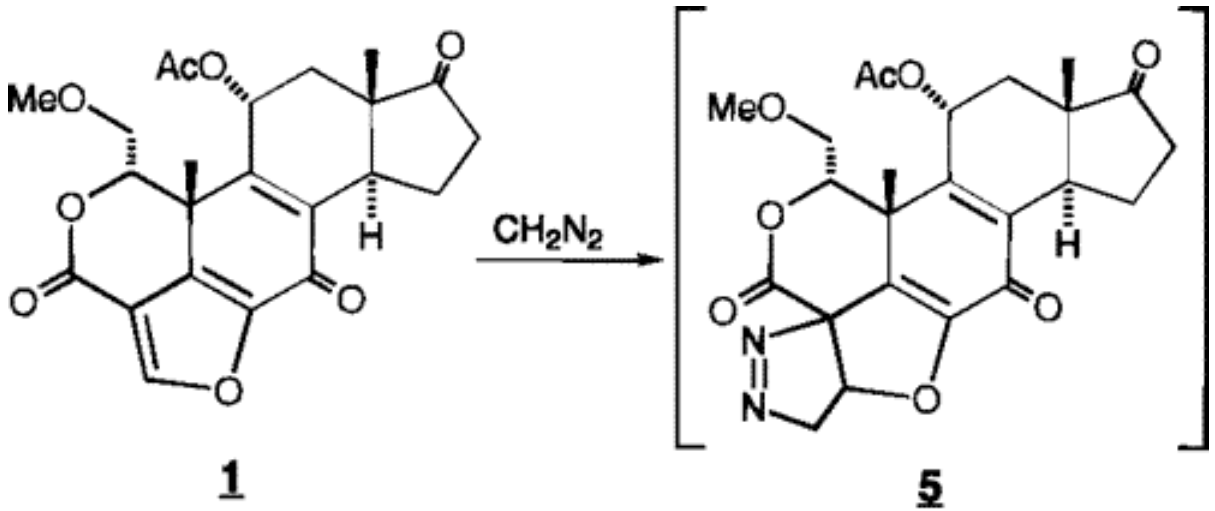


Primary (*Z*) vs secondary amine (*E*) adducts

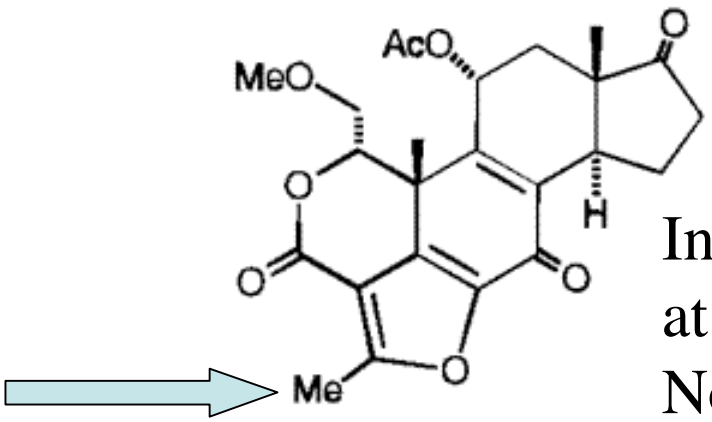
Difference in reactivity suggested to be difference in orientation of the enamine in relation to the lactone carbonyl

E configuration mimics wortmannin in ability to accept a nucleophile

Z--steric problems



1 **5**



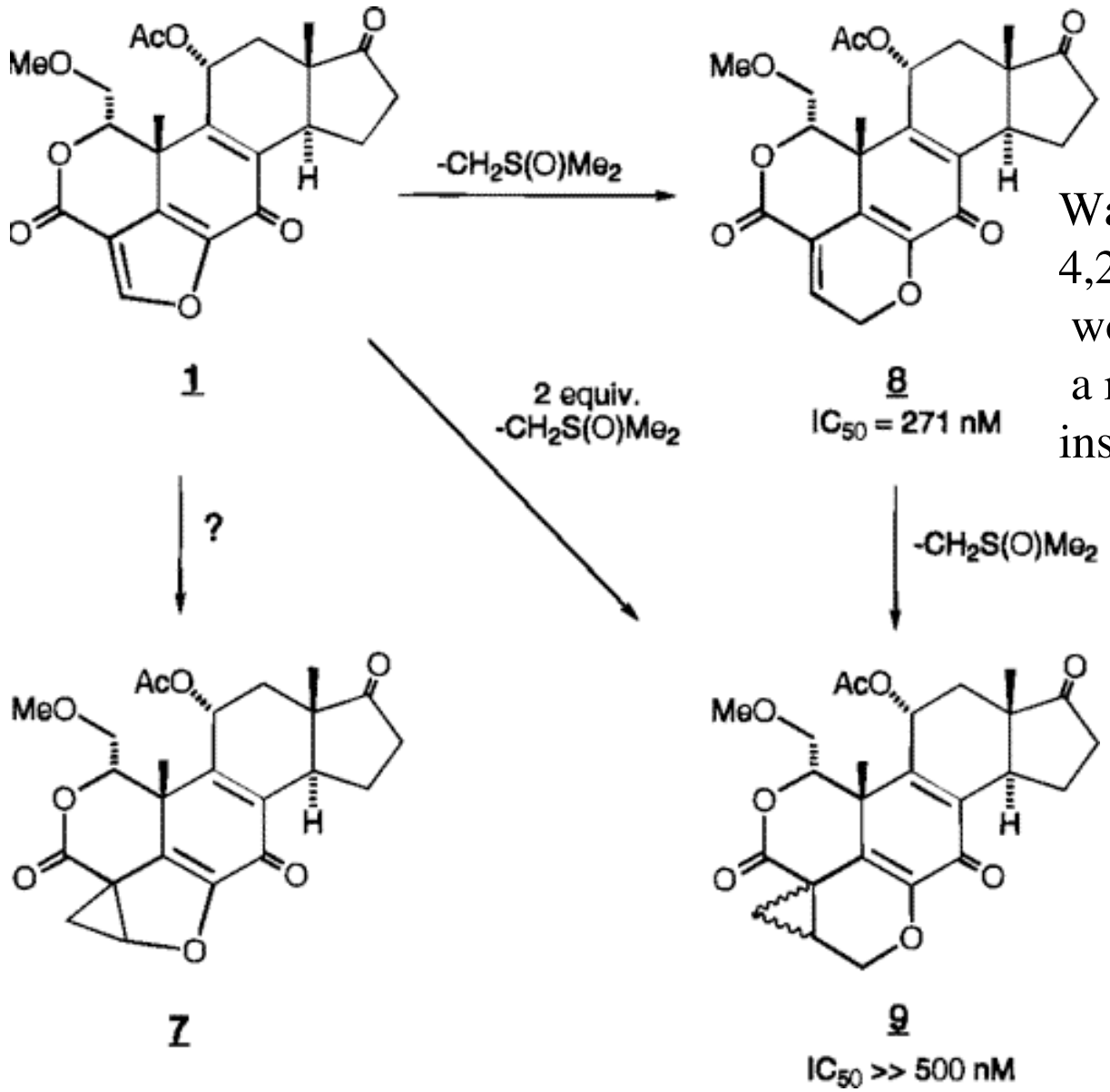
6

IC_{50} >> 500 nM

Introduction of methyl group at C-21

No inhibition up to 500 nM

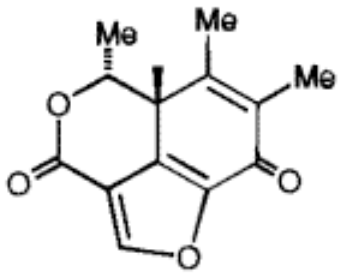
No reaction with diethylamine



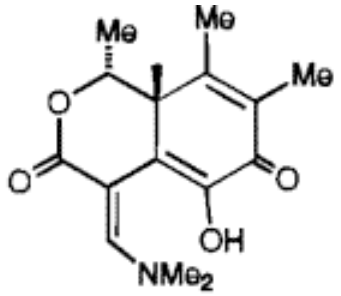
Wanted to prepare 4,21-cyclopropyl wortmannin but isolated a ring expansion product instead

Reacts with xs ylide to give a the desired product as a mixture of diasterisomers

When the furan is replaced by a pyran ring
it has only moderate activity--pyran is not planar/thermodynamics



Me_2NH



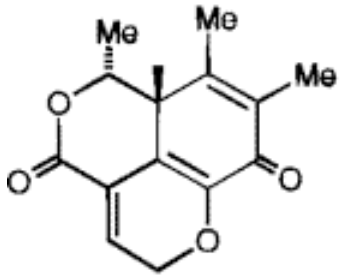
ΔH_f = -27.1 Kcal / mole

MODEL 1

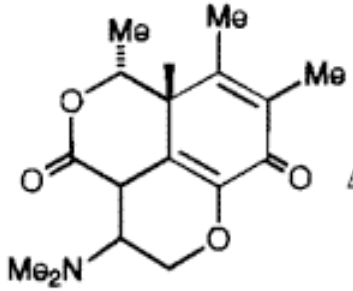
H_f = -88.9 Kcal / mole

MODEL 2

H_f = -116 Kcal / mole



Me_2NH



ΔH_f = -9 Kcal / mole

MODEL 8

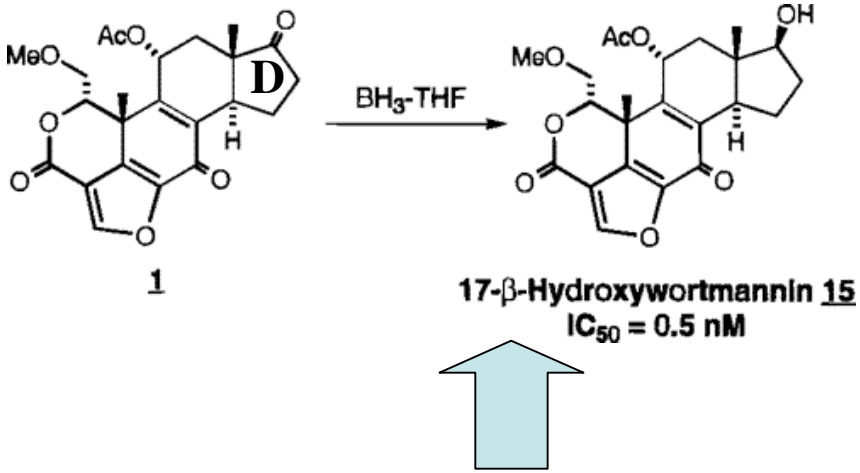
H_f = -108 Kcal / mole

MODEL 10

H_f = -117 Kcal / mole

D ring modifications

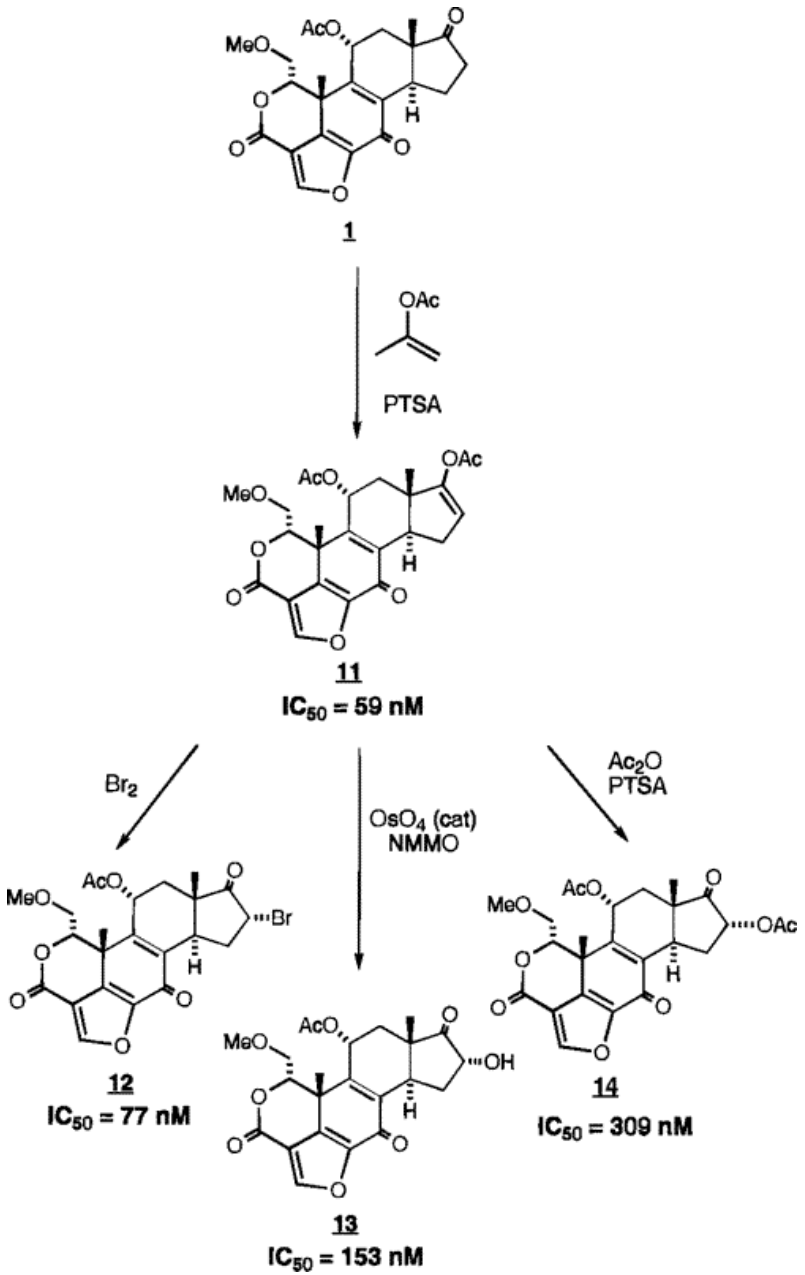
Remote from furan ring
Noticeable effects on inhibitor activity
D ring is an important recognition element



1

17-β-Hydroxywortmannin 15
IC_{50} = 0.5 nM

10 fold increase in activity



1

11
IC_{50} = 59 nM

12
IC_{50} = 77 nM

13
IC_{50} = 153 nM

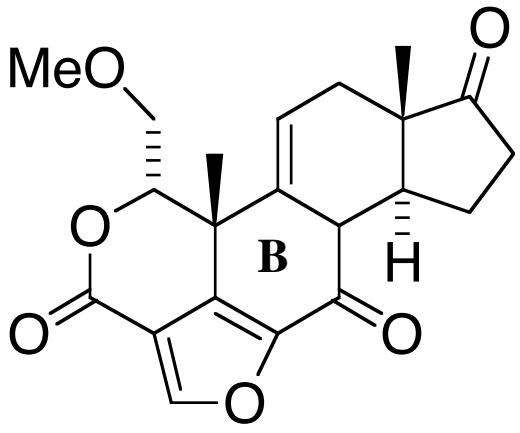
14
IC_{50} = 309 nM

B ring modifications

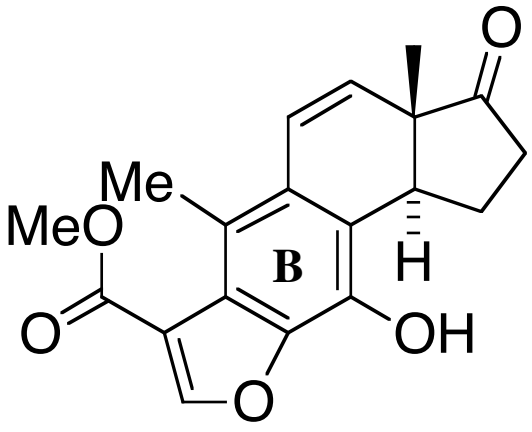
Have been limited

Deconjugated derivative remained active

Aromatized derivative lost activity

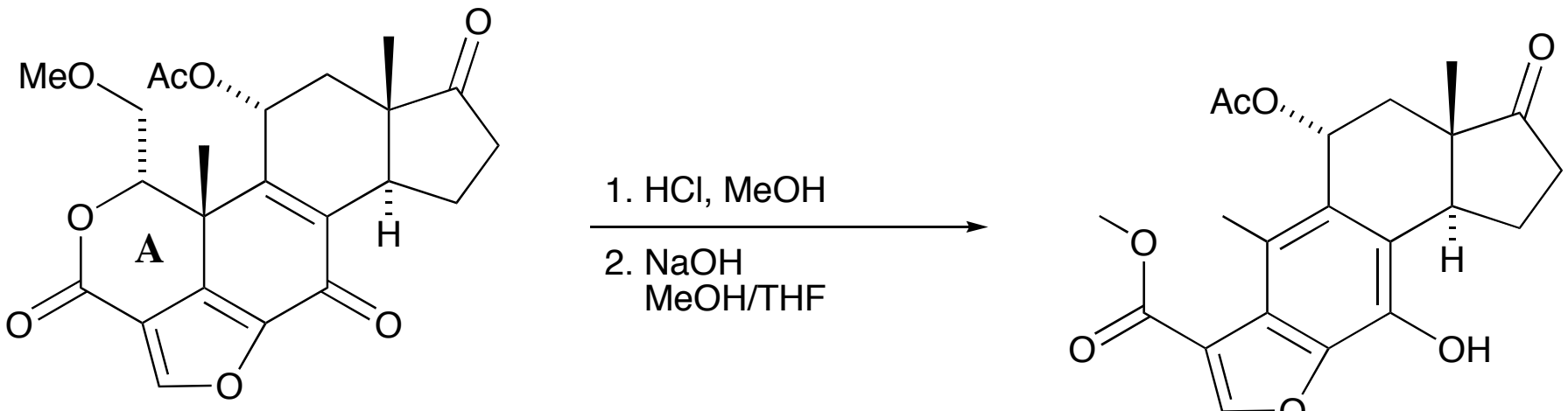


IC_{50} = 54 nM



IC_{50} = 4600 nM

A ring modifications



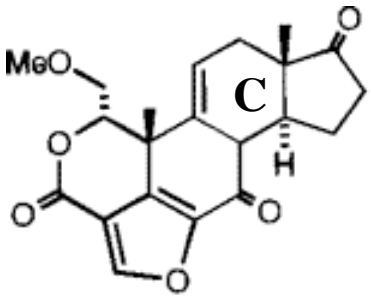
Wortmannin (1)

Bioorg. Med. Chem. Lett. **1995**, *5*, 1183

1000 fold loss of activity and no toxicity

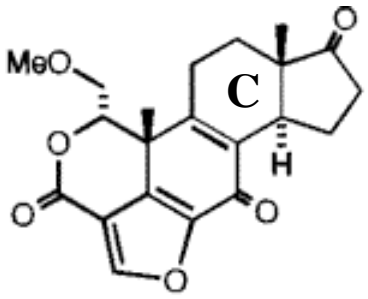
C ring modifications

Removal of C-11 acetoxy group



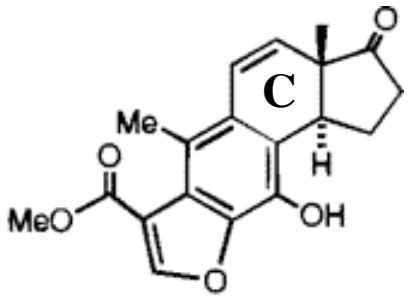
16

54 nM



17

17 nM



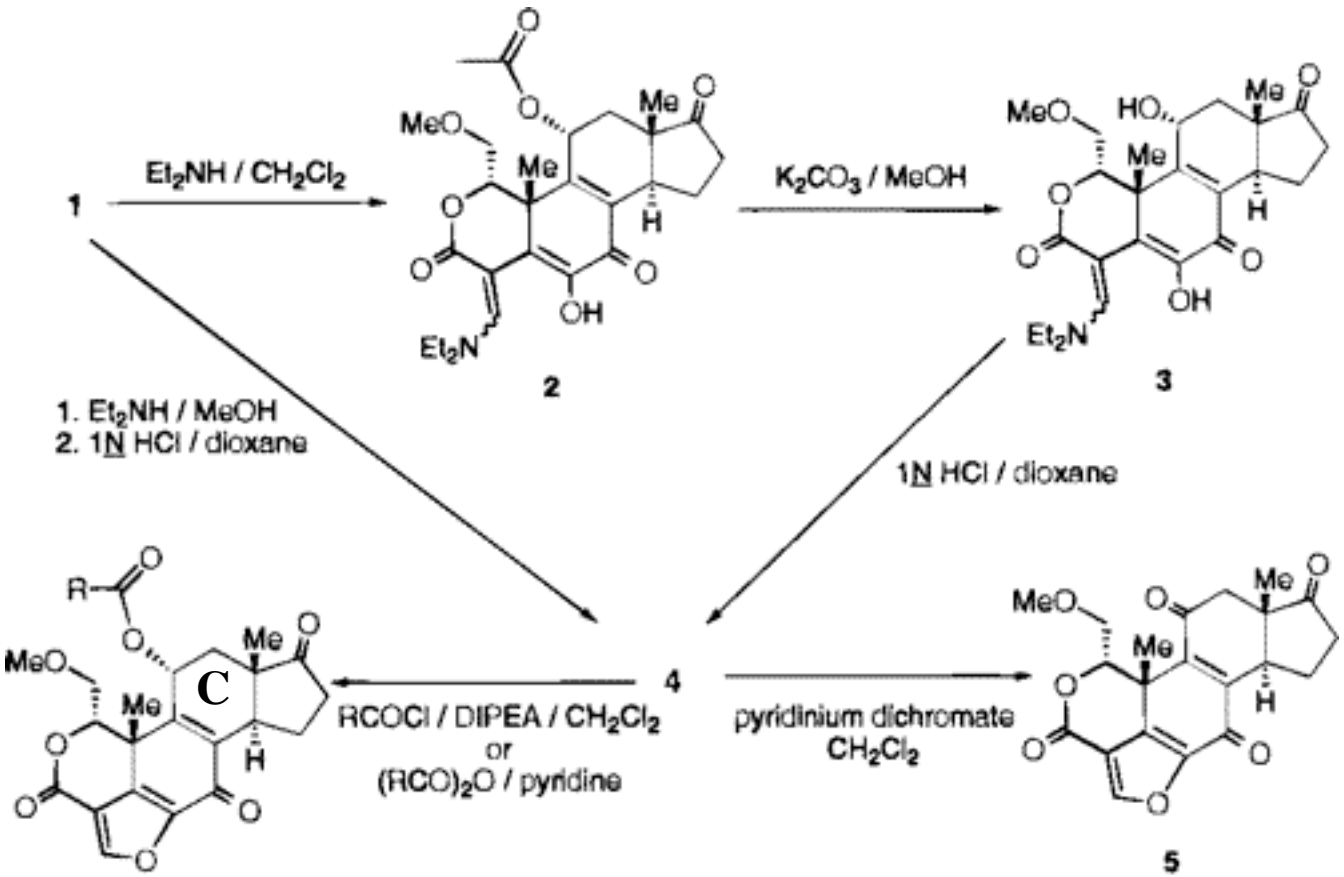
18

4600 nM

J. Med. Chem. **1996**, *39*,1106

Anti-tumour activity
(Wortmannin 4.2 nM)

Inhibitory activity increases with increasing lipophilic character



6, R = Et
9, R = Pr
10, R = Bu
11, R = $Ph(CH_2)$
12, R = CH_2=CH
13, R = CH_2Cl
14, R = $(CH_3)_2CHCH_2$

J. Med. Chem. **1996**, *39*, 5021-5024

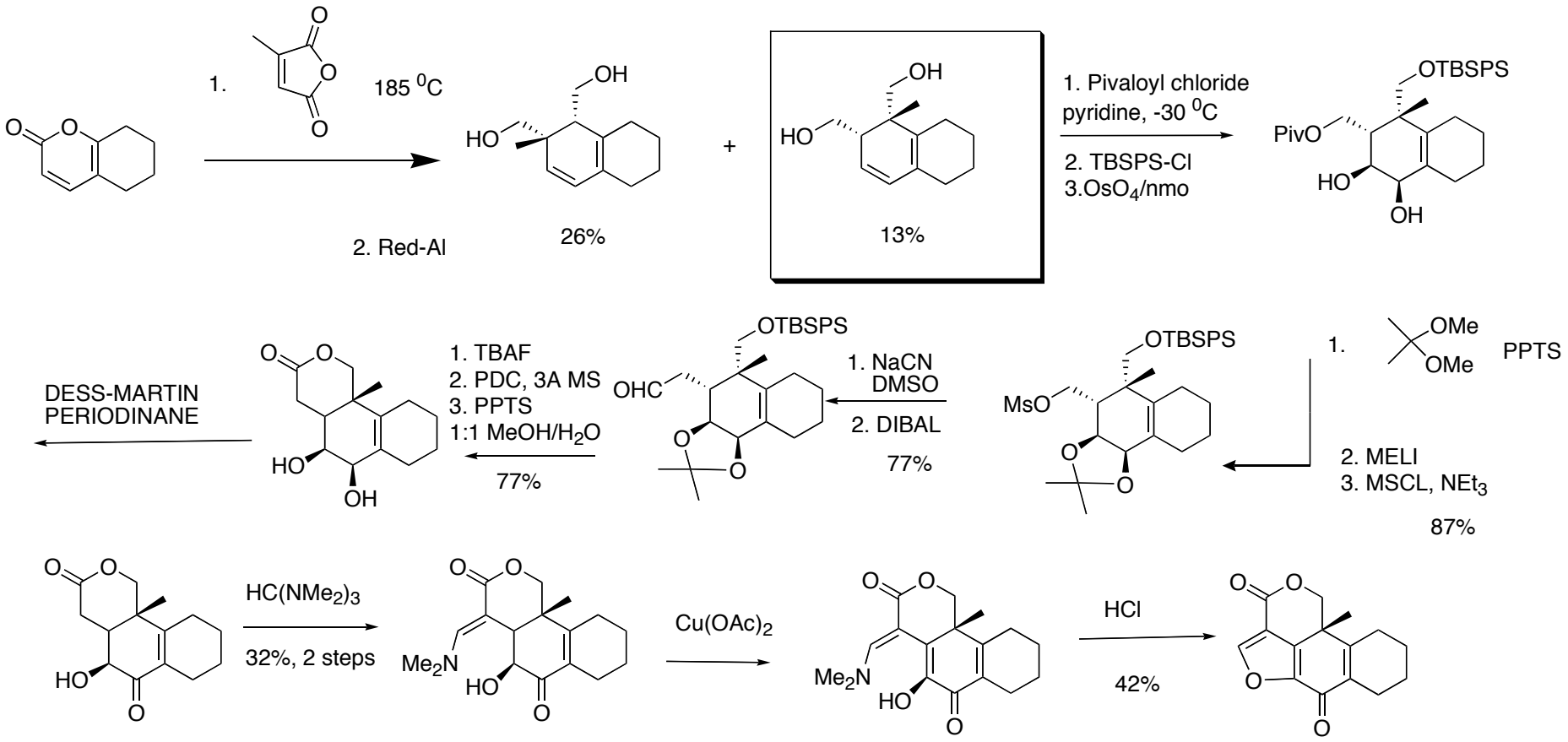
Wortmannin beyond anti-proliferation studies

- Radiation sensitizer (but may sensitize normal cells over tumour cells) *Rad. Res.* **2001**, *155*, 826.
- Alzheimers disease-alters effects on the metabolism of the Alzheimers amyloid precursor protein (APP) *J. Neurochem*, **1999**, *73*, 2316.
- Treatment of type I osteporosis-ability to inhibit osteoclasts from resorbing bone *Pharm. Exp. Ther.* **1995**, *277*, 543.

Synthetic Efforts towards Wortmannin

First synthesis of the furancyclohexadiene lactone subunit

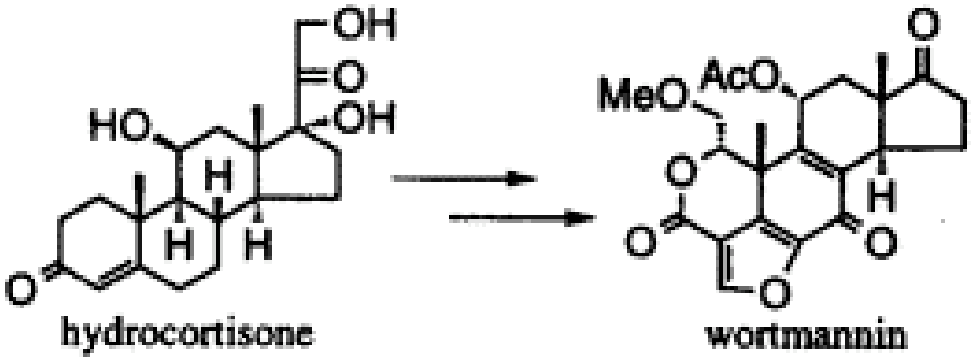
1992



J. Org. Chem. **1992**, *57*, 4888

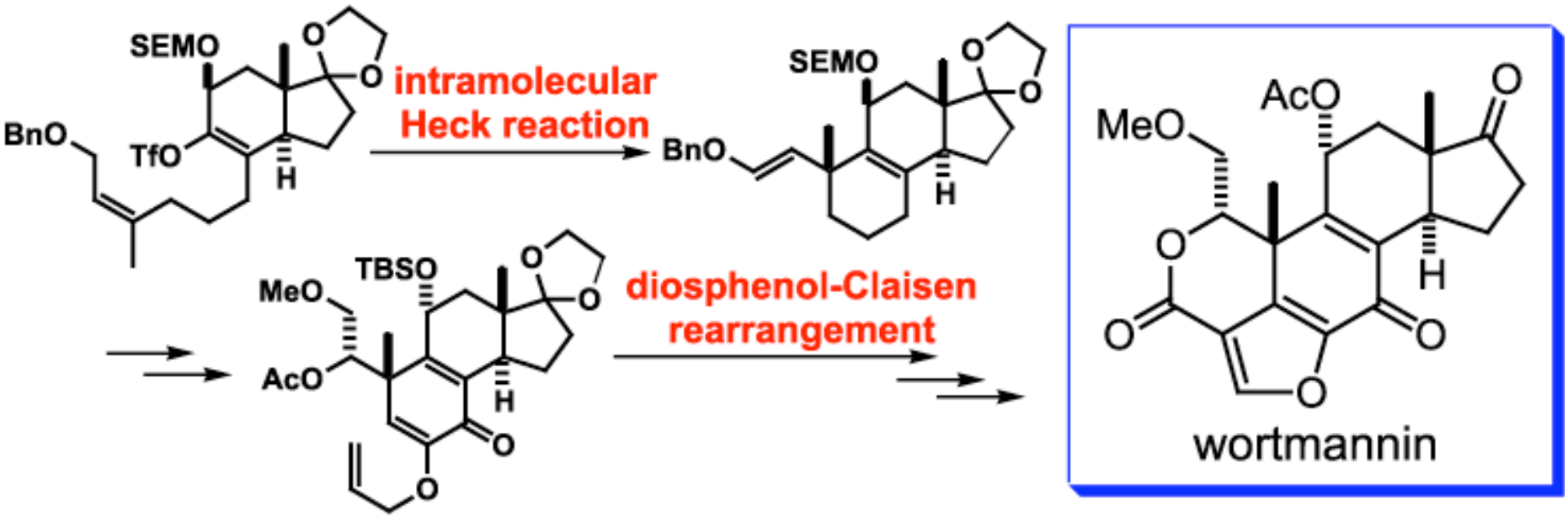
1996 First Chemical Synthesis

Starting from commercially available hydrocortisone

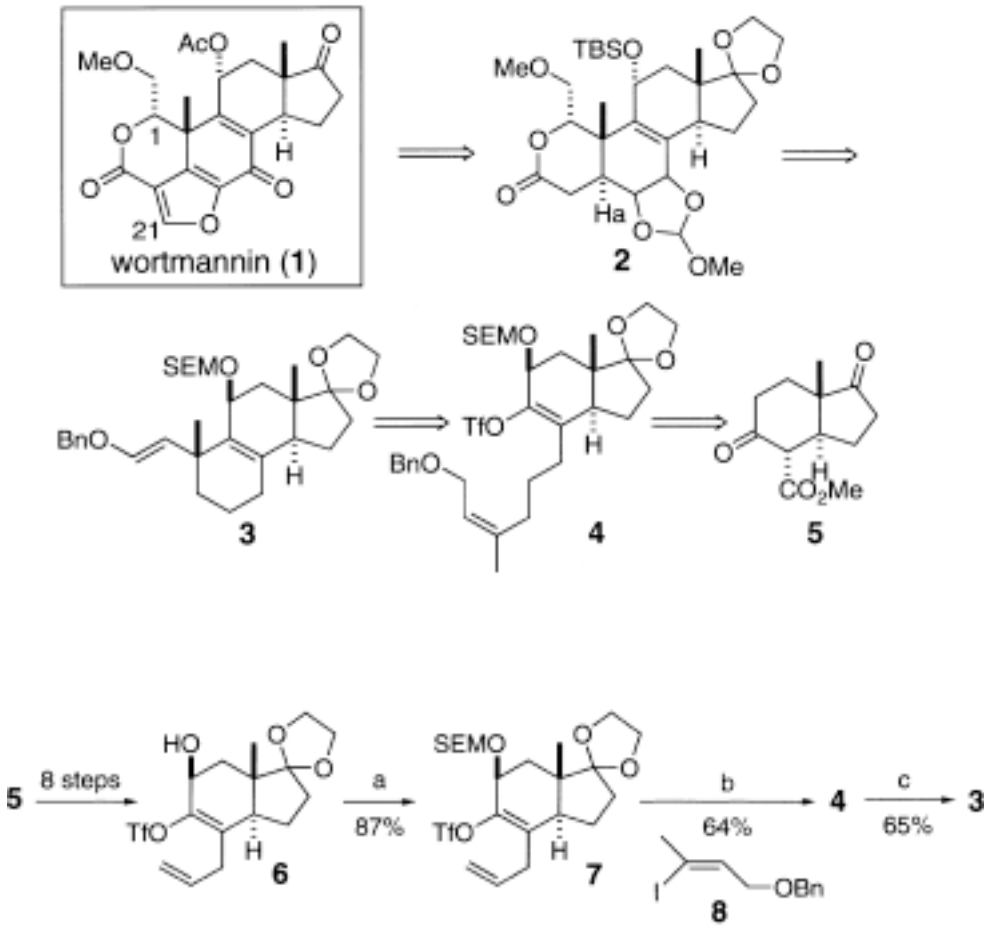


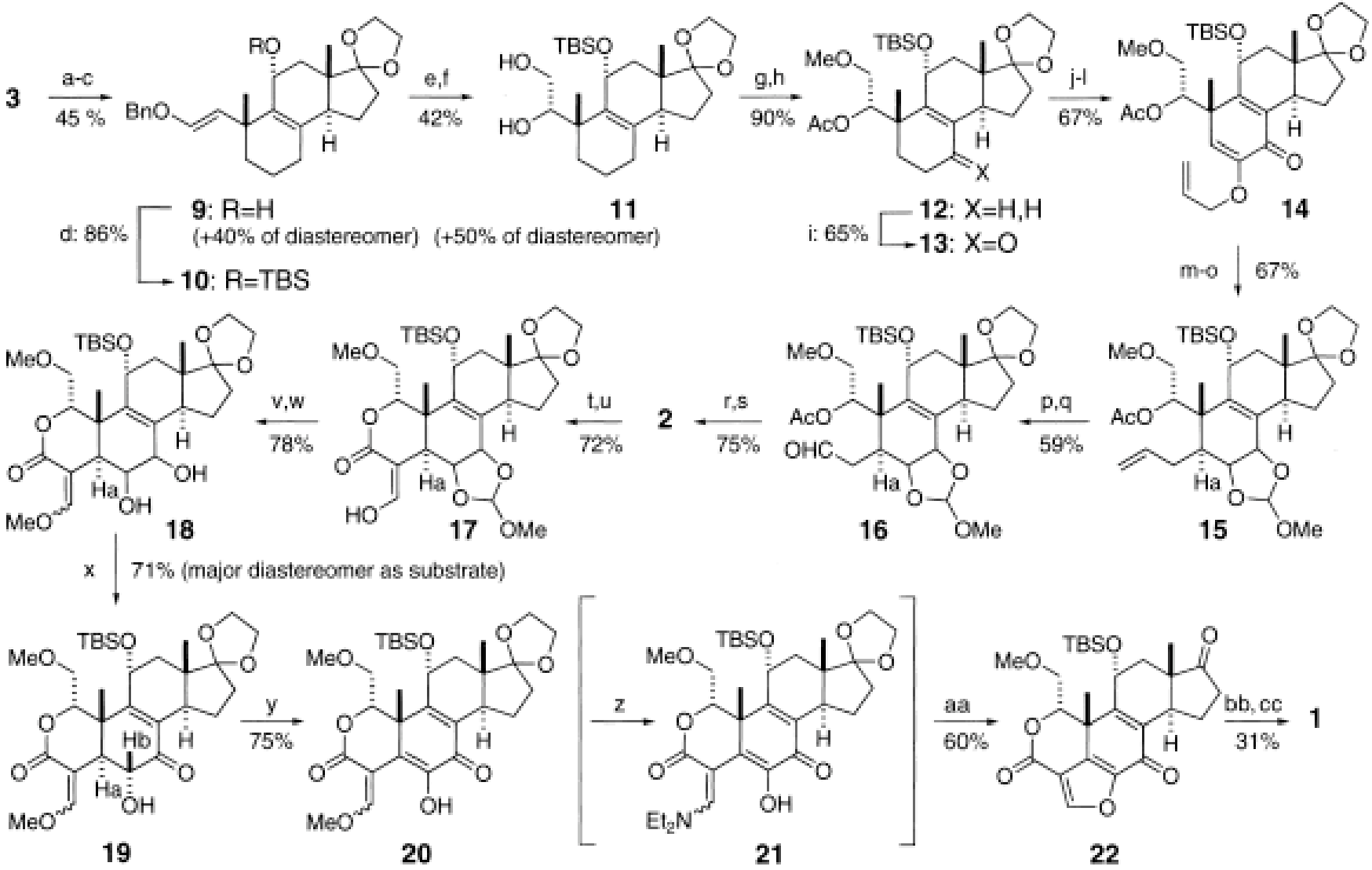
Lengthy 35 step synthesis, 0.01% overall yield

Total Synthesis of (±)–Wortmannin



T. Mizutani, S. Honzawa, S. Tosaki, M. Shibasaki, *Angew. Chem. Int. Ed.*, **2002**, *41*, 4680-4682


MeO
AcO
O
1
H
O
O
21
O
wortmannin (1)
TBSO
Ha
OMe
2
SEMO
BnO
3
TfO
4
CO2Me
5
HO
6
a
87%
7
b
64%
8
I
OBn
c
65%
5
8 steps


3
a-c
45 %
BnO
RO
H
e,f
42%
HO
HO
TBSO
11
g,h
90%
MeO
AcO
TBSO
X
12: X=H,H
13: X=O
i: 65%
j-l
67%
MeO
AcO
TBSO
14
m-o
67%
9: R=H
(+40% of diastereomer)
d: 86%
10: R=TBS
(+50% of diastereomer)
MeO
AcO
OHC
TBSO
16
p,q
59%
r,s
75%
2
t,u
72%
v,w
78%
MeO
HO
TBSO
17
OMe
MeO
OH
OH
TBSO
18
MeO
AcO
TBSO
15
OMe
x
71% (major diastereomer as substrate)
MeO
Hb
Ha
OH
TBSO
19
y
75%
MeO
OH
TBSO
20
z
MeO
Et2N
OH
TBSO
21
aa
60%
MeO
TBSO
22
bb, cc
31%
1