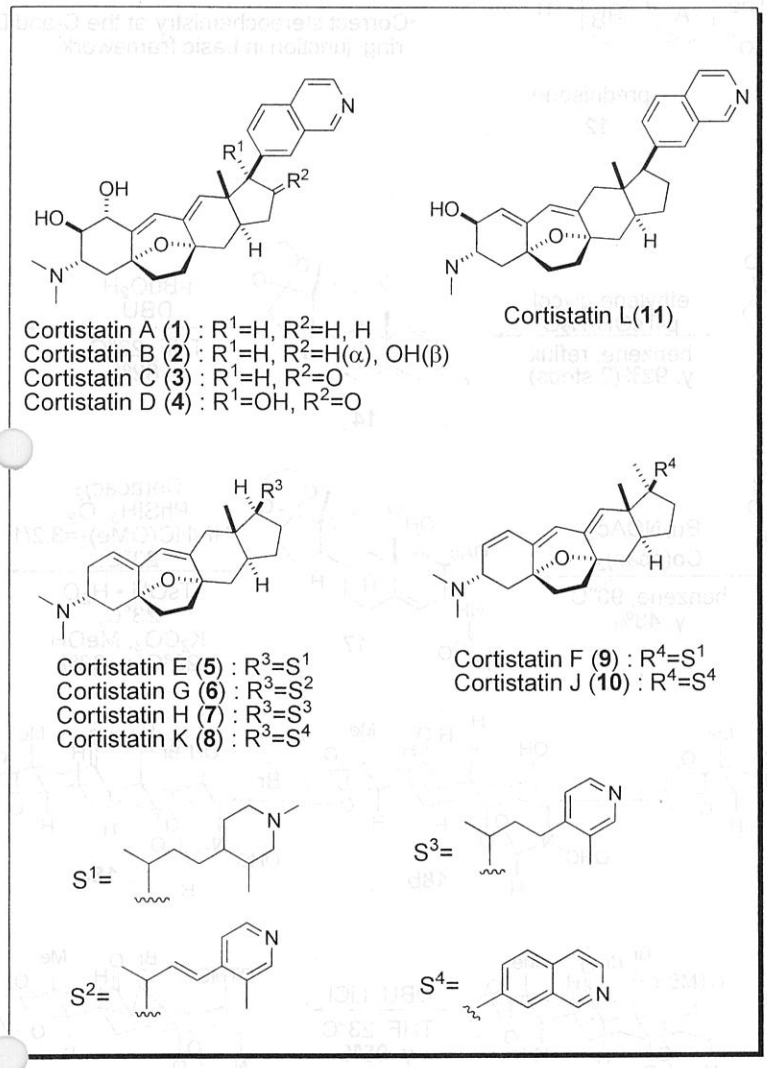
Cortistatins
Total Synthesis and Their Structure-Activity Relationship

Matsuzawa Akinobu(B4)



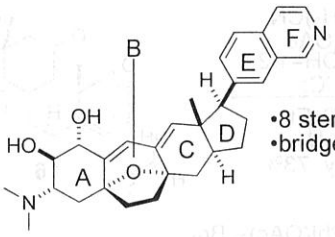
Isolation
M. Kobayashi et al. *JACS*, **2006**, *128*, 3148

Semisynthesis
P. S. Baran et al. *JACS*, **2008**, *130*, 7241

Total synthesis
M. D. Shair et al. *JACS*, **2008**, *130*, 16864
K. C. Nicolaou et al. *Angew. Chem. Int. Ed.* **2008**, *47*, 7310

Formal total synthesis
M. Hirama et al. *Tetrahedron. Lett.* **2009**, *50*, 3277
R. Sarpong et al. *Tetrahedron* In press

Other studies for the total synthesis
S. J. Danishefsky et al. *Tetrahedron. Lett.* **2008**, *49*, 6610
E. J. Corey et al. *Org. Lett.* **2008**, *10*, 5247
P. Magnus et al. *Org. Lett.* **2009**, *11*, 3938
B. W. Gung et al. *Chem. Eur. J.* **2010**, *16*, 639



•8 stereogenic centers
•bridged seven-membered B-ring

Cortistatin A (**1**)

Table 1. Selective Growth Inhibition of Cortistatins against HUVECs[a]

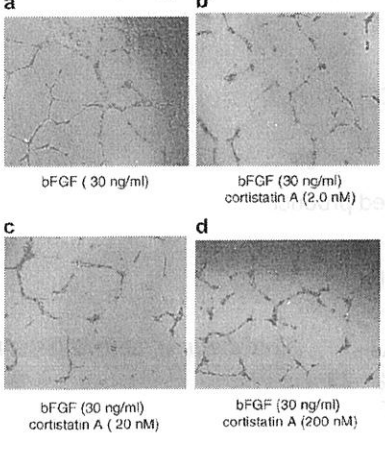
	1		2		3		4	
cell line	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.
HUVECs	0.0018	1	1.1	1	0.019	1	0.15	1
KB3−1	7.0	3900	120	110	150	7900	55	460
Neuro2A	6.0	3300	160	150	180	9500	>300	nd
K562	7.0	3900	200	180	>300	nd	>300	nd
NHDF	6.0	3300	>300	nd	>300	nd	>300	nd

[a] IC_{50} = μM; nd = not determined; S.I. = selective index: IC_{50} against testing cells/IC_{50} against HUVECs.

Cortistatins are first isolated from marine sponge *Corticium simplex* in 2006, as a selective inhibitor of the proliferation of HUVECs(human umbilical vein endothelial cells).
The most potent member of the familiy (IC_{50}=1.8nM), cortistatin A (**1**) demonstrated a selectivity index of more than 3000 against HUVECs in comparison with normal human dermal fibroblast(NHDF) and several other tumor cells(KB3-1, K562, and Neuro2A).

After that, cortistatin was found to inhibit not only proliferation, but also migration of HUVECs, thereby inhibited tubular formation.
What outstanding is that cortistatins are not cytotoxic but cytostatic, probably through reversible binding to the target protein.

Cortistatins are thought to be promising drug candidates or leads for the diseases related to angiogenesis.
These biological properties coupled with its unprecedented molecular architecture made cortistatin A a target for chemial synthesis. As a result of extensive studies, 5 groups have succeeded in its synthesis so far.

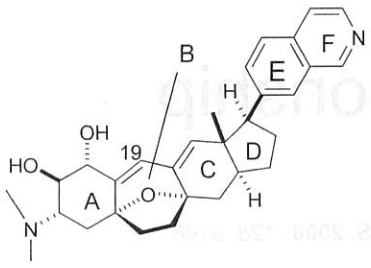


Contents

1. Semisynthesis by Baran

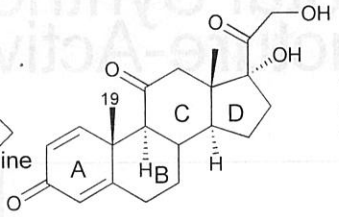
P. S. Baran et al. *JACS*, **2008**, *130*, 7241

Retrosynthesis



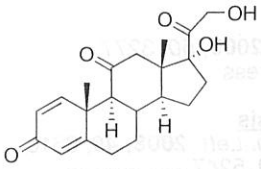
Cortistatin A (**1**)

1. A-ring functionalizaion
2. B-ring expansion
3. Oxidation at C19
4. coupling with isoquinoline
5. funcrtional group manipulation



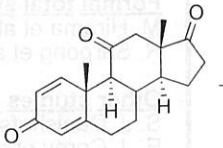
prednisone
12

- Prednisone is commercially available at $1.20/g and possesses 70% of carbon atoms.
- Correct stereochemistry at the C-and D-ring junction in basic framework.



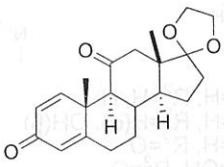
prednisone
12

BH_3•THF
THF, 0 to 23°C;
$NaIO_4$
acetone/H_2O=1/1
0 to 23°C



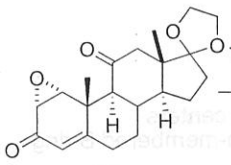
13

ethylene glycol
p-TsOH•H_2O
benzene, reflux
y. 92%(2 steps)



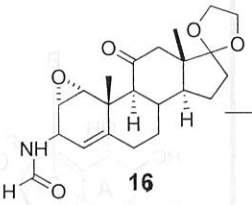
14

t-BuO_2H
DBU
THF, 23°C
y. 82%



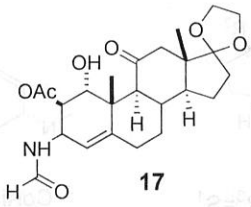
15

$Na(BH_3)CN$
NH_4OAc
THF/MeOH=1/2.4
23°C;
HCO_2Et
Et_3N
54°C, y. 73%



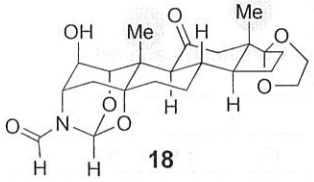
16

Bu_4NOAc
$Co(acac)_2$
benzene, 90°C
y. 48%



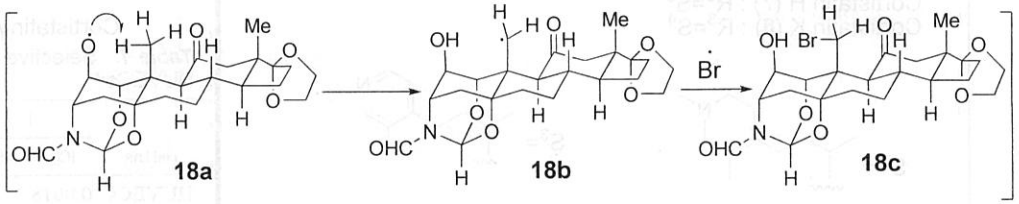
17

$Co(acac)_2$
$PhSiH_3$, O_2
THF/$HC(OMe)_3$=3.2/1
23°C;
TsOH • H_2O
23°C;
K_2CO_3, MeOH
23°C, y. 63%

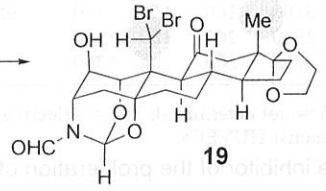


18

$PhI(OAc)_2$, Br_2
CH_2Cl_2
75W sunlamp
-30°C

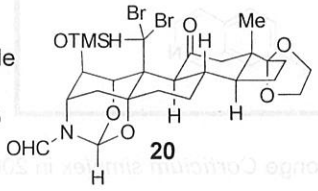


18a **18b** **18c**



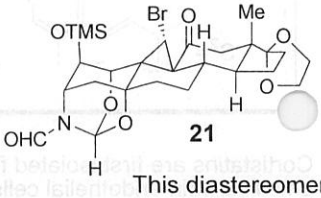
19

TMSCl, Imidazole
CH_2Cl_2, 0°C
y. 57% (2steps)



20

DBU, LiCl
THF, 23°C
y. 85%



21

This diastereomer only

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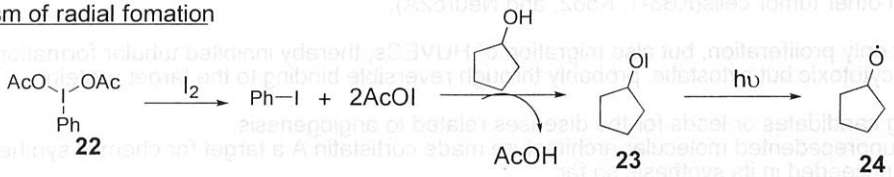
mechanism of radial fomation

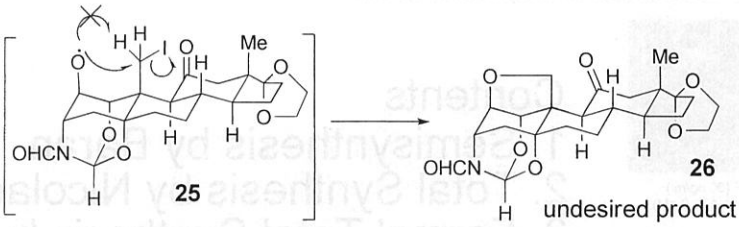


22 **23** **24**

Tetrahedron Lett. **1994**, *35*, 1003

in the case of I_2

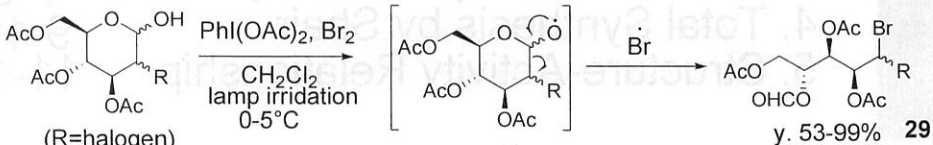
18 $PhI(OAc)_2$, I_2



25 **26**

undesired product

a precedent

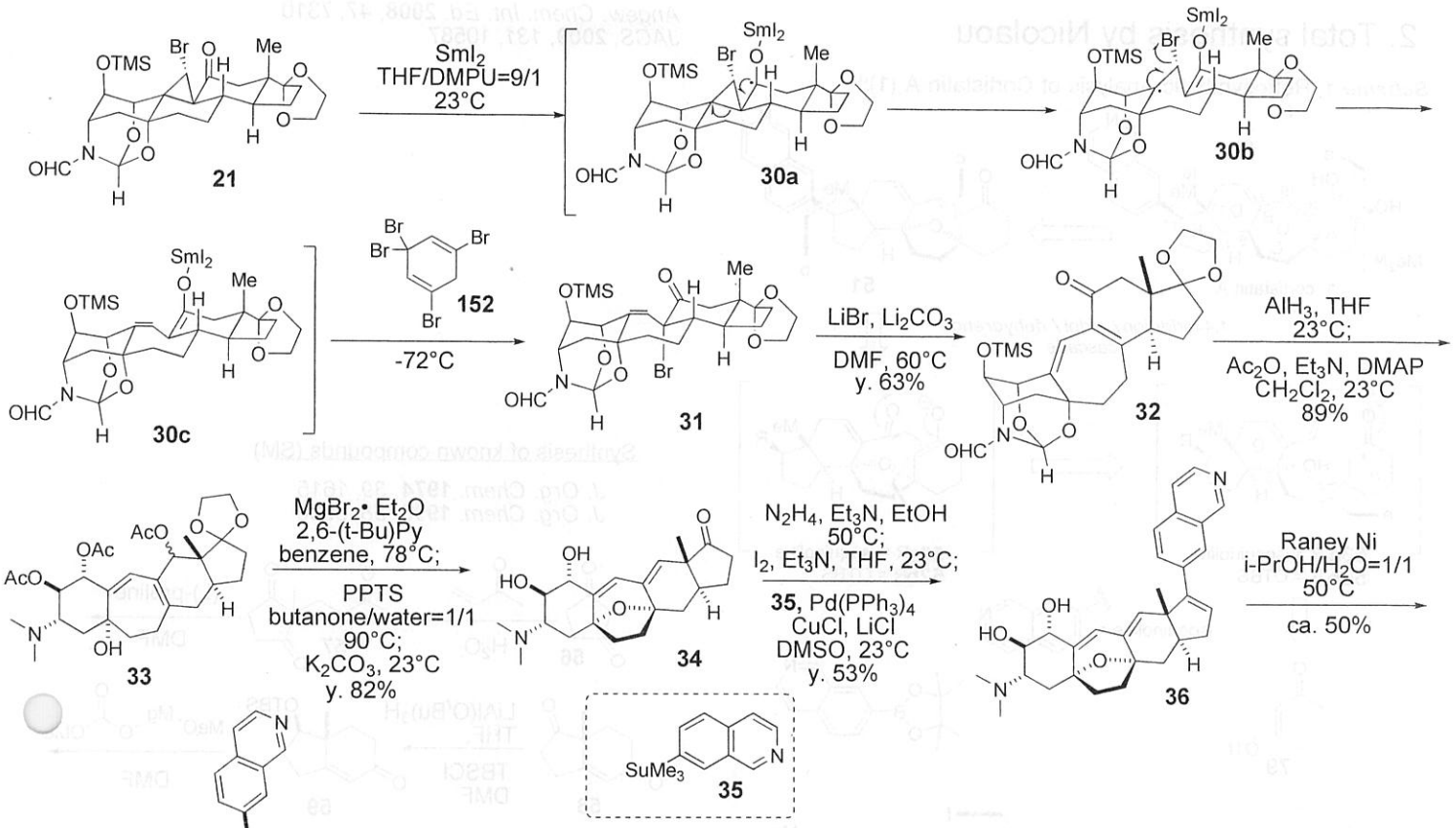


(R=halogen)
27 **28** **29**

$PhI(OAc)_2$, Br_2
CH_2Cl_2
lamp irridation
0-5°C

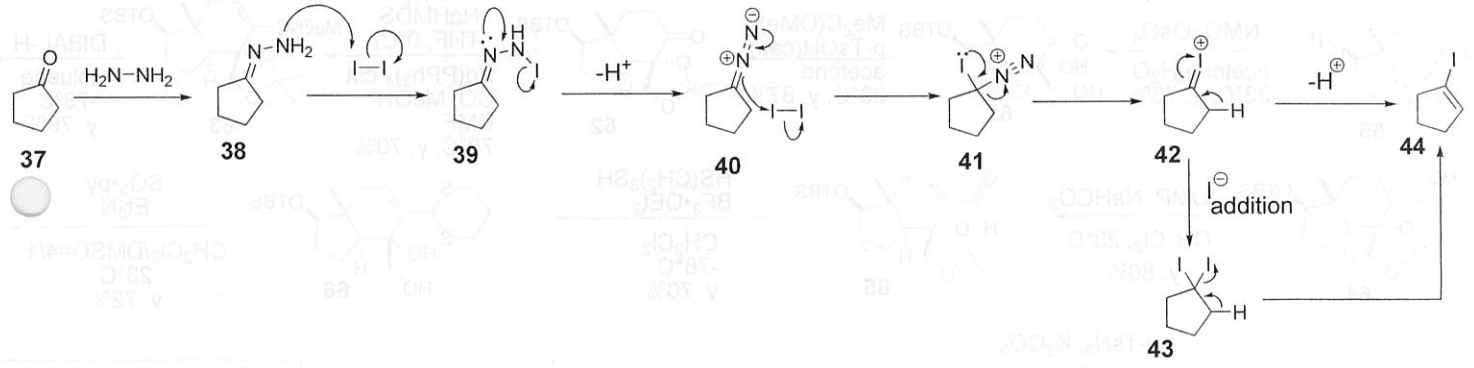
y. 53-99%
1:1-3:1 dr

Tetrahedron. Lett. **2003**, *44*, 6347

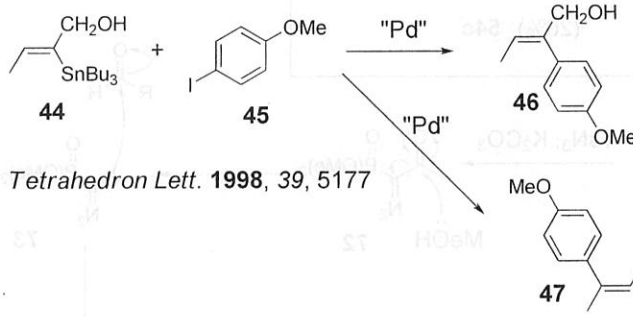


Generation of vinyl iodide from ketone

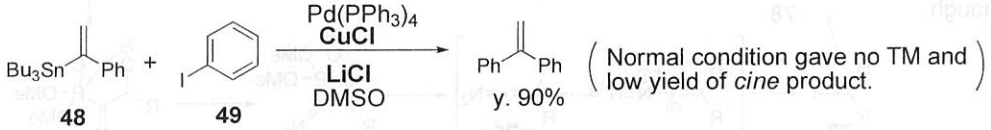
J. Chem. Soc. **1962**, 470



Stille coupling with strericaly hindered 1-substituted vinylstannanes

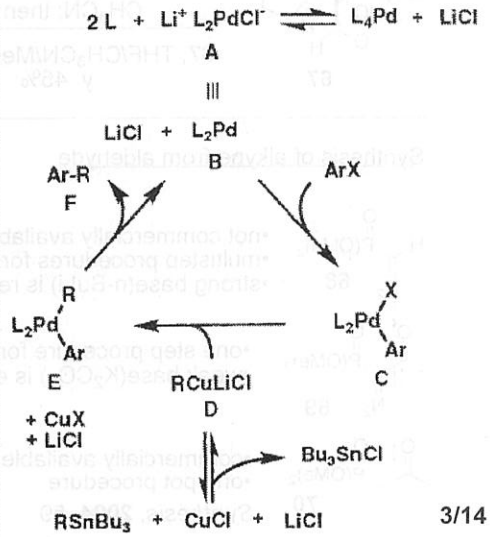


Tetrahedron Lett. **1998**, *39*, 5177



JACS, **1999**, *121*, 7600

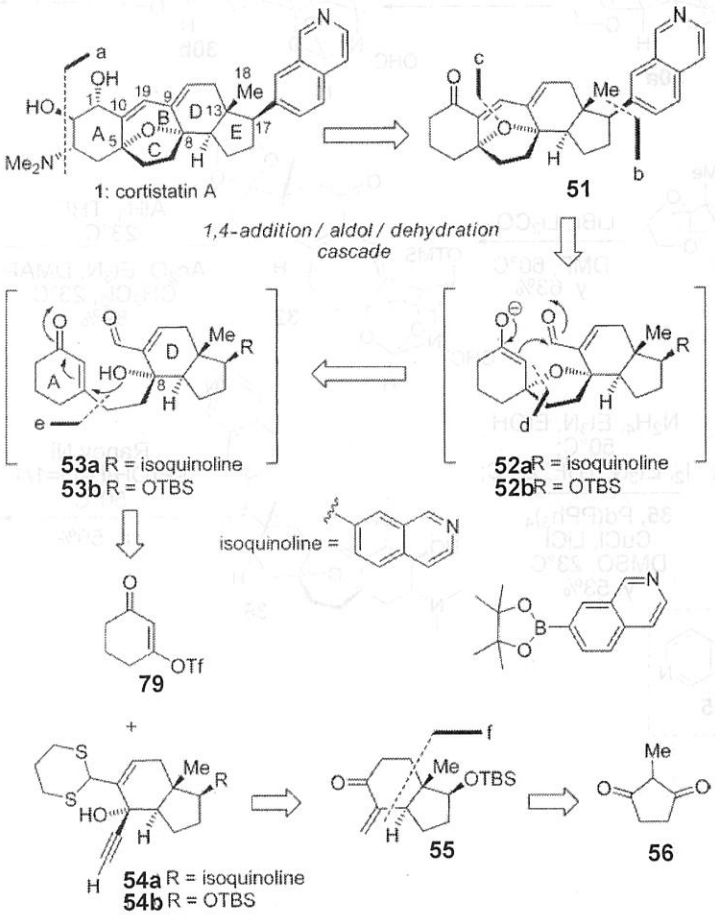
Proposed Catalytic Cycle



2. Total synthesis by Nicolaou

Angew. Chem. Int. Ed. **2008**, *47*, 7310
JACS, **2009**, *131*, 10587

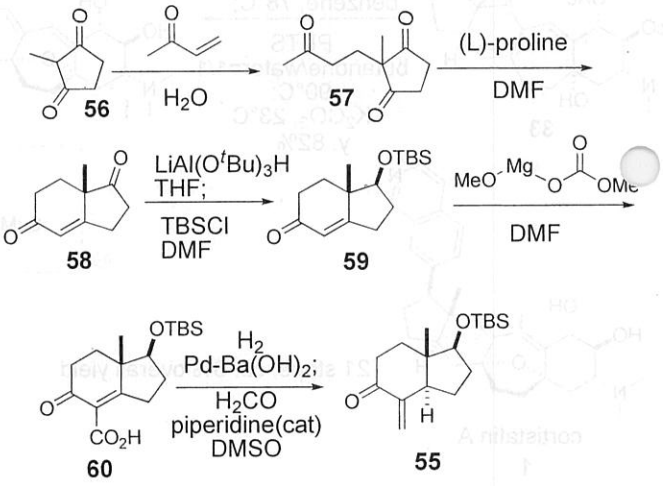
Scheme 1. Retrosynthetic Analysis of Cortistatin A (**1**)[a]

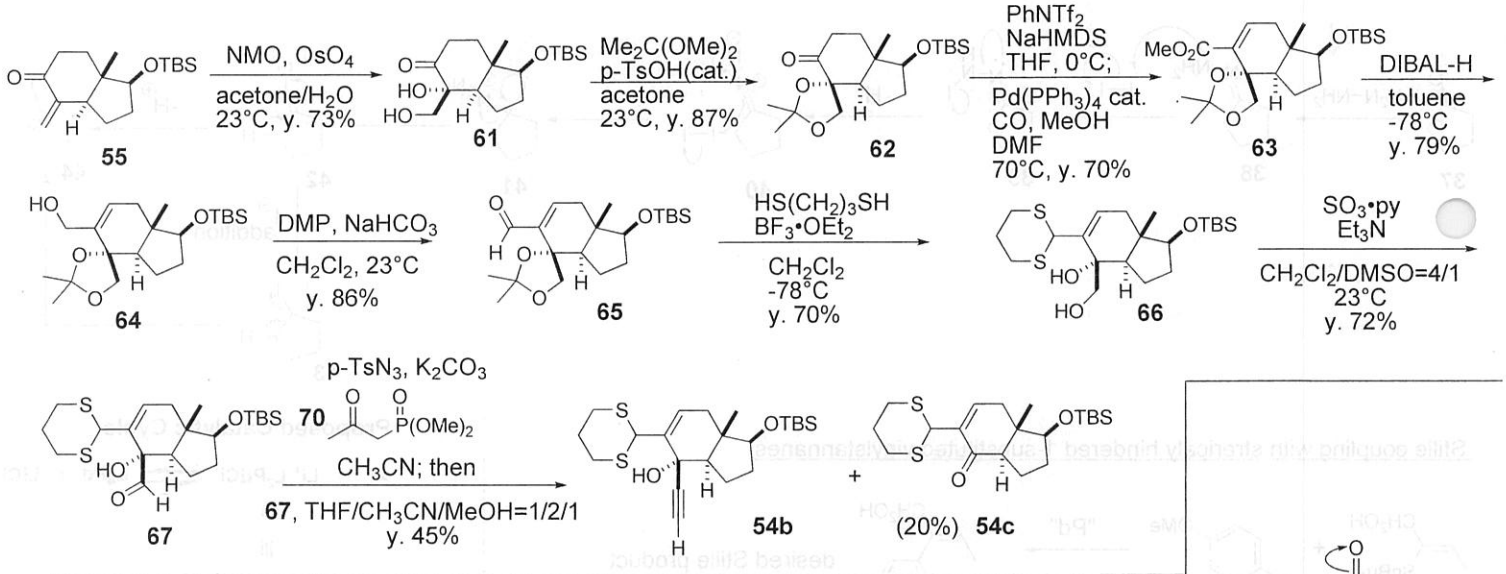


[a] Operations: (a) epoxide opening; (b) Suzuki–Miyaura coupling; (c) aldol condensation; (d) 1,4-addition; (e) Sonogashira coupling; (f) Hajos–Parrish ketone construction.

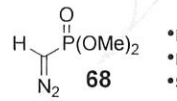
Synthesis of known compounds (SM)

J. Org. Chem. **1974**, *39*, 1615
J. Org. Chem. **1993**, *58*, 3938

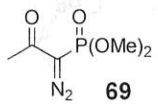




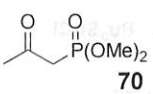
Synthesis of alkyne from aldehyde



- not commercially available
- multistep procedures for preparation
- strong base(n-BuLi) is required

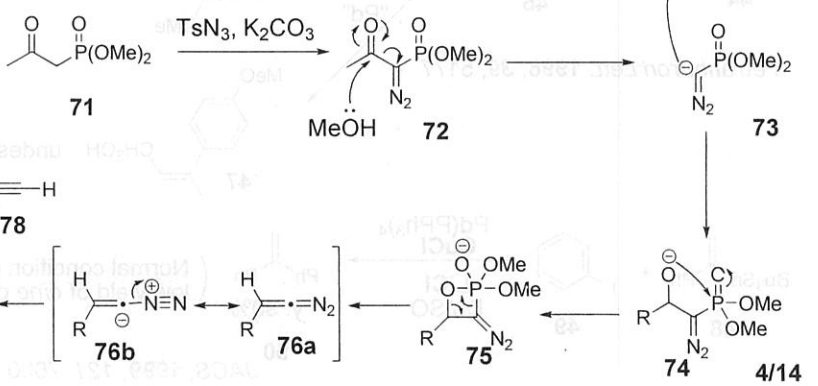


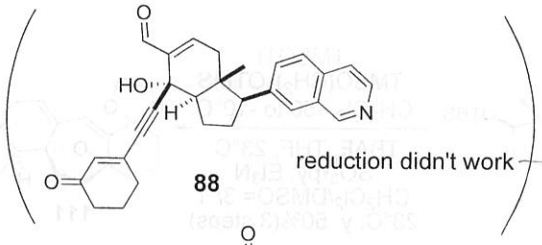
- one step procedure for preparation
- weak base(K_2CO_3) is enough

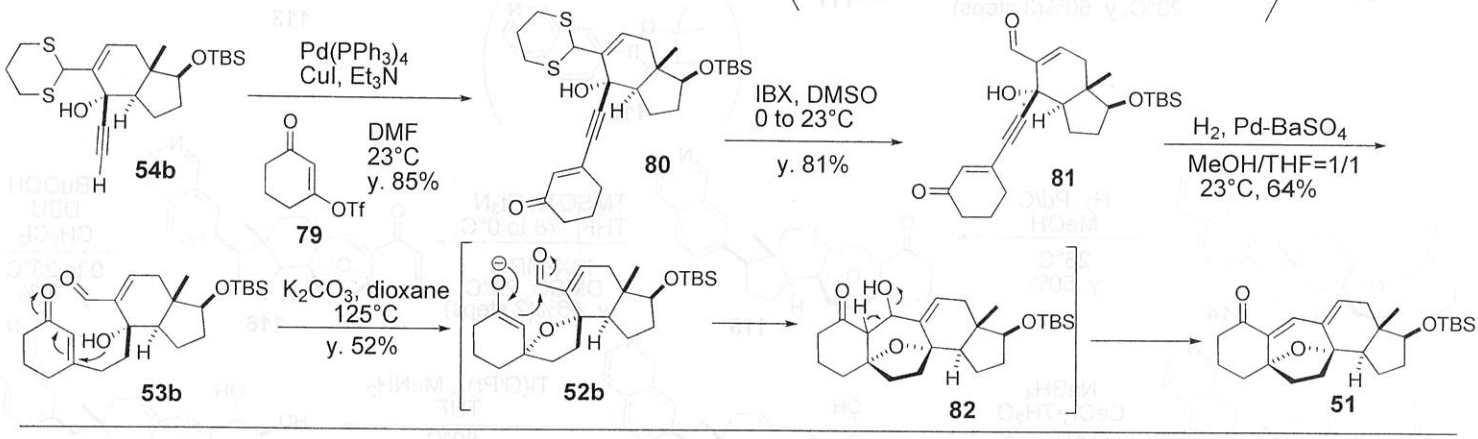


- commercially available
- one pot procedure

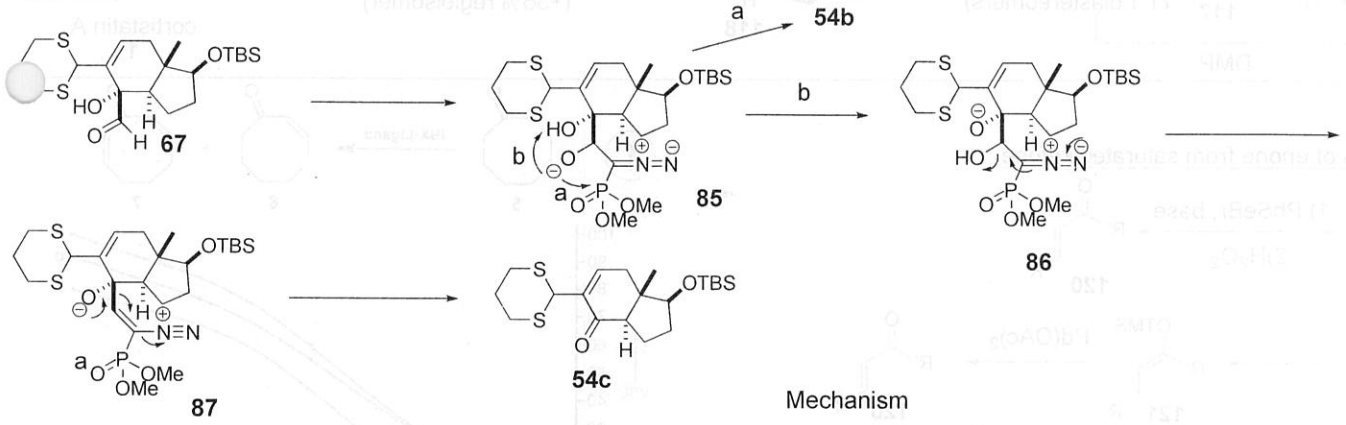
Synthesis, **2004**, 59



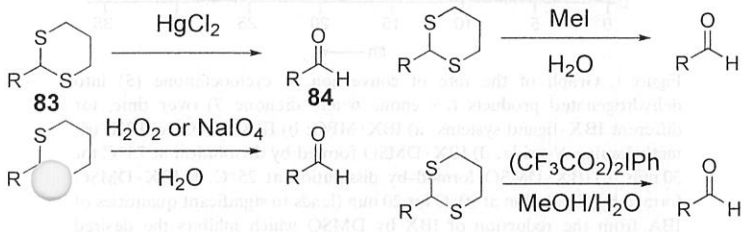




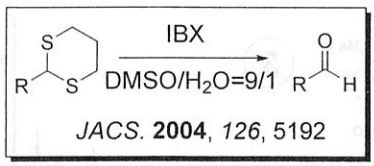
67→54c



Dethioacetalizaion

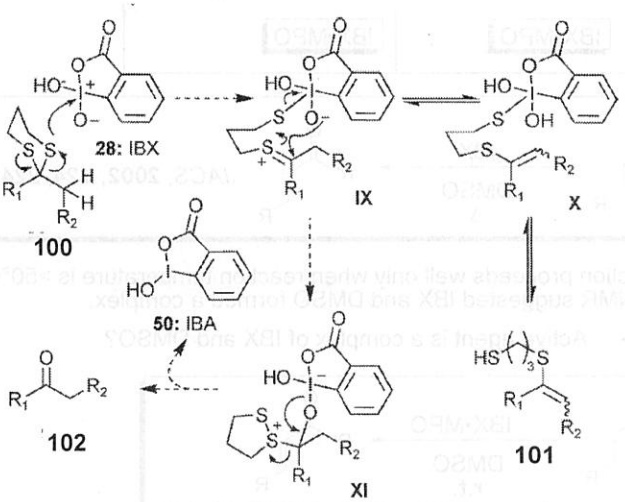


Tetrahedron Lett. **1989**, *30*, 287



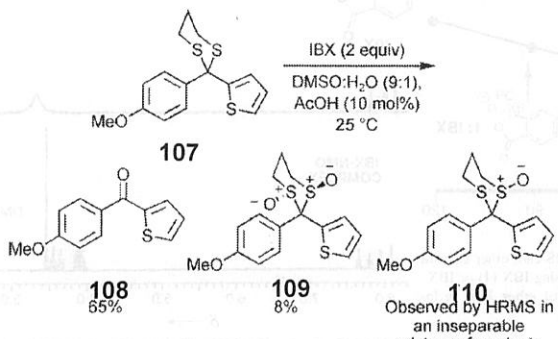
JACS. **2004**, *126*, 5192

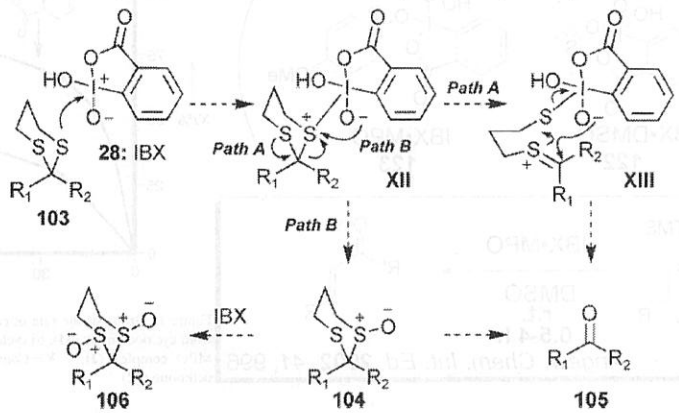
Mechanism

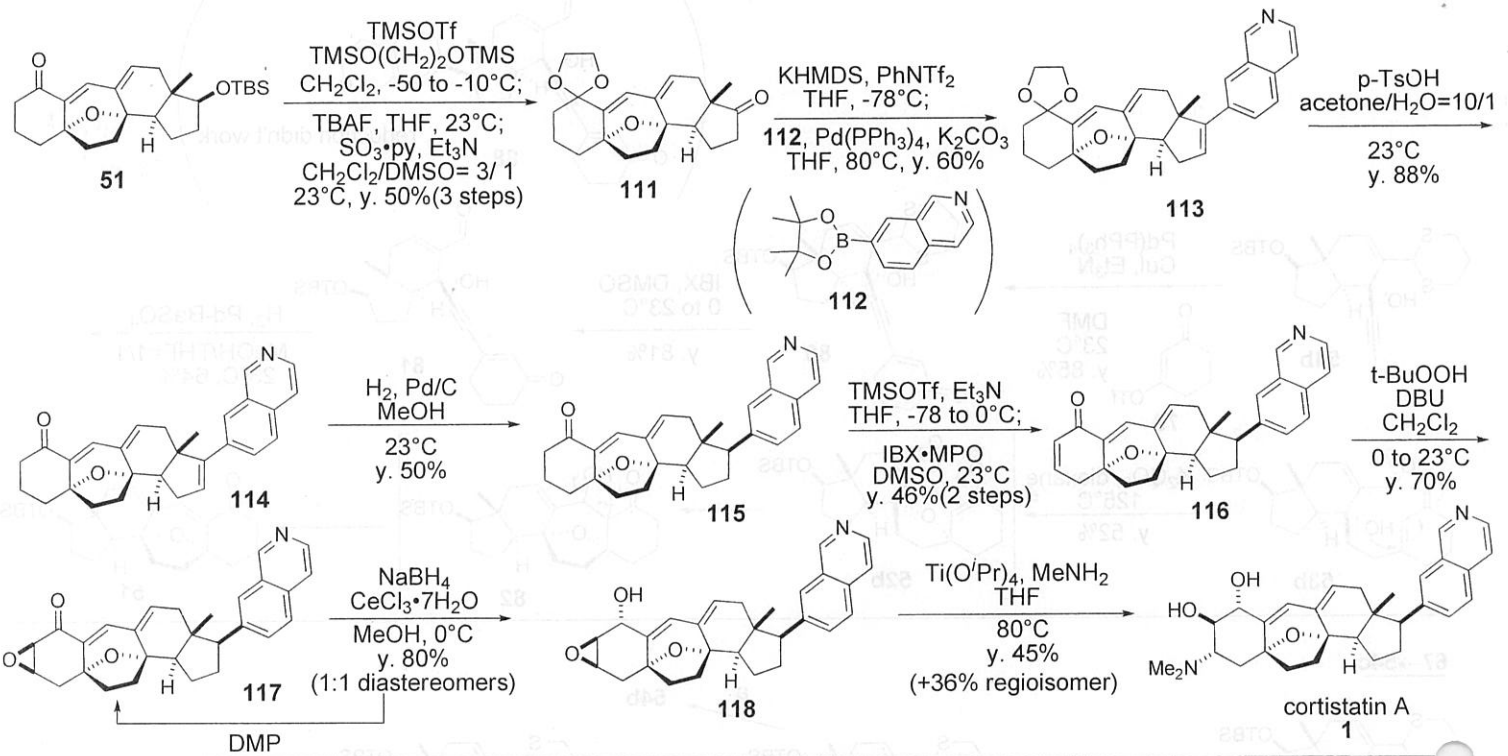


reaction mechanism of sterically hindered substrates

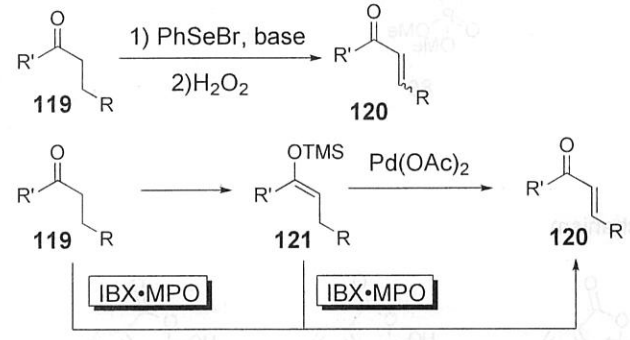
reactions of sterically hindered substrates (lacking α-protons)

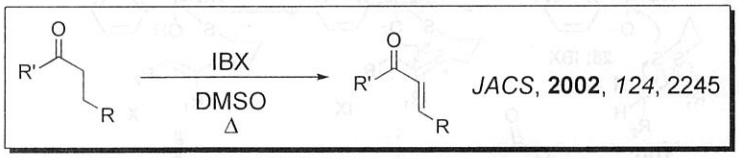






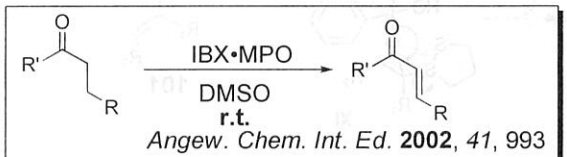
Synthesis of enone from saturated ketone

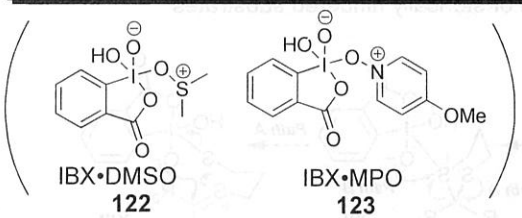


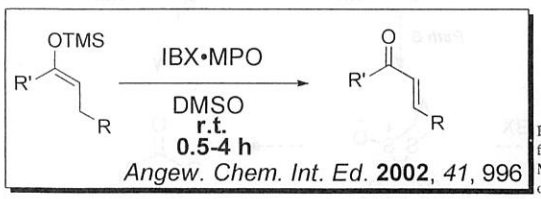


•Reaction proceeds well only when reaction temperature is >50°C
•1H-NMR suggested IBX and DMSO formed a complex.

⟶ Active agent is a complex of IBX and DMSO?







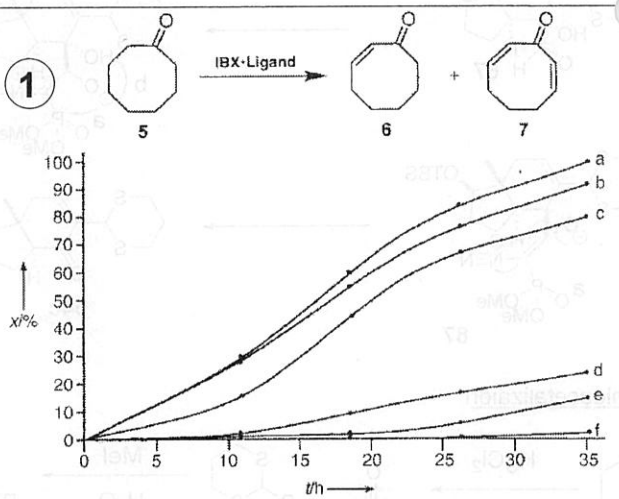


Figure 1. Graph of the rate of conversion of cyclooctanone (5) into dehydrogenated products (*cis* enone 6 and dienone 7) over time, for different IBX·ligand systems: a) IBX·MPO; b) IBX·NMO; c) IBX·trimethylamine-*N*-oxide; d) IBX·DMSO formed by dissolution at 75 °C for 30 min; e) IBX·DMSO formed by dissolution at 25 °C; f) IBX·DMSO formed by dissolution at 90 °C for 20 min (leads to significant quantities of IBA from the reduction of IBX by DMSO which inhibits the desired reaction[2]). Reagents and conditions: IBX/ligand 1:1 (2.2 equiv), [D6]DMSO, 25 °C, conversion monitored by means of ^{1}H NMR spectroscopy. x = Total dehydrogenation.

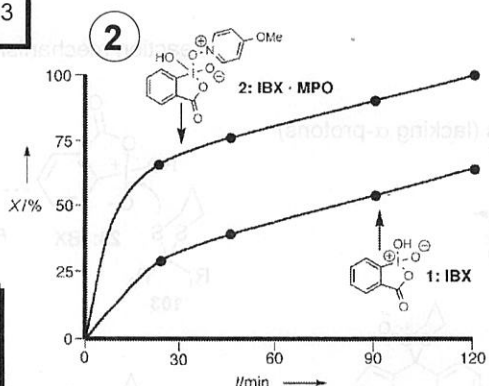


Figure 1. Graph of the rate of conversion of the TMS enol ether derived from cyclooctanone (31), to cyclooctenone (32) by using IBX (1) or IBX·MPO complex (2).[29] X = Conversion of TMS enol ether into cyclooctenone (32).

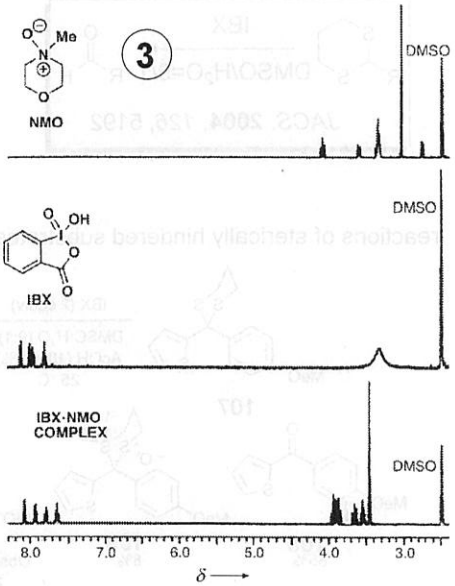
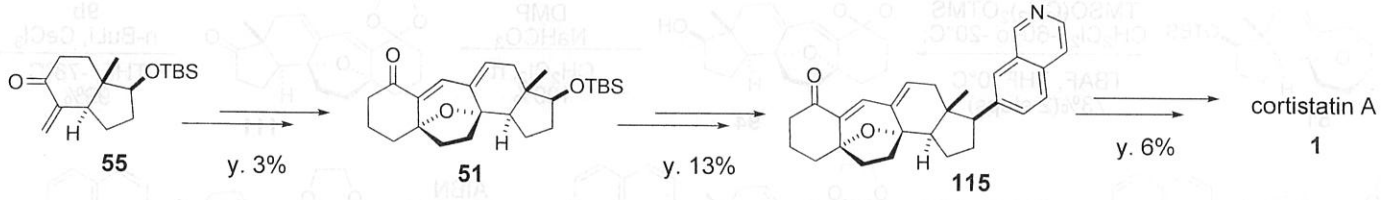


Figure 2. ^{1}H NMR spectra (500 MHz, [D6]DMSO) of NMO (top), IBX (center), and the observed NMO·IBX complex (bottom).

Althouth total synthesis was accomplished, overall yield was only 0.03%•••

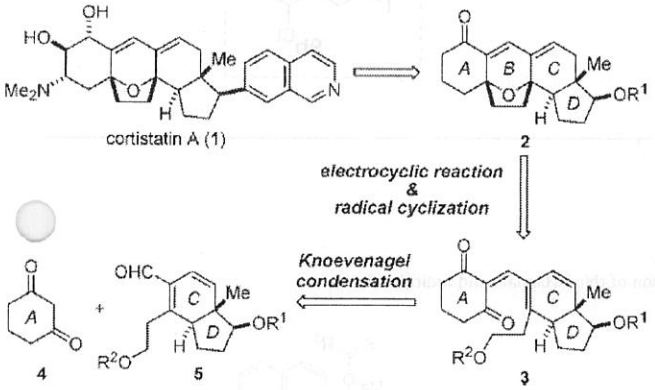


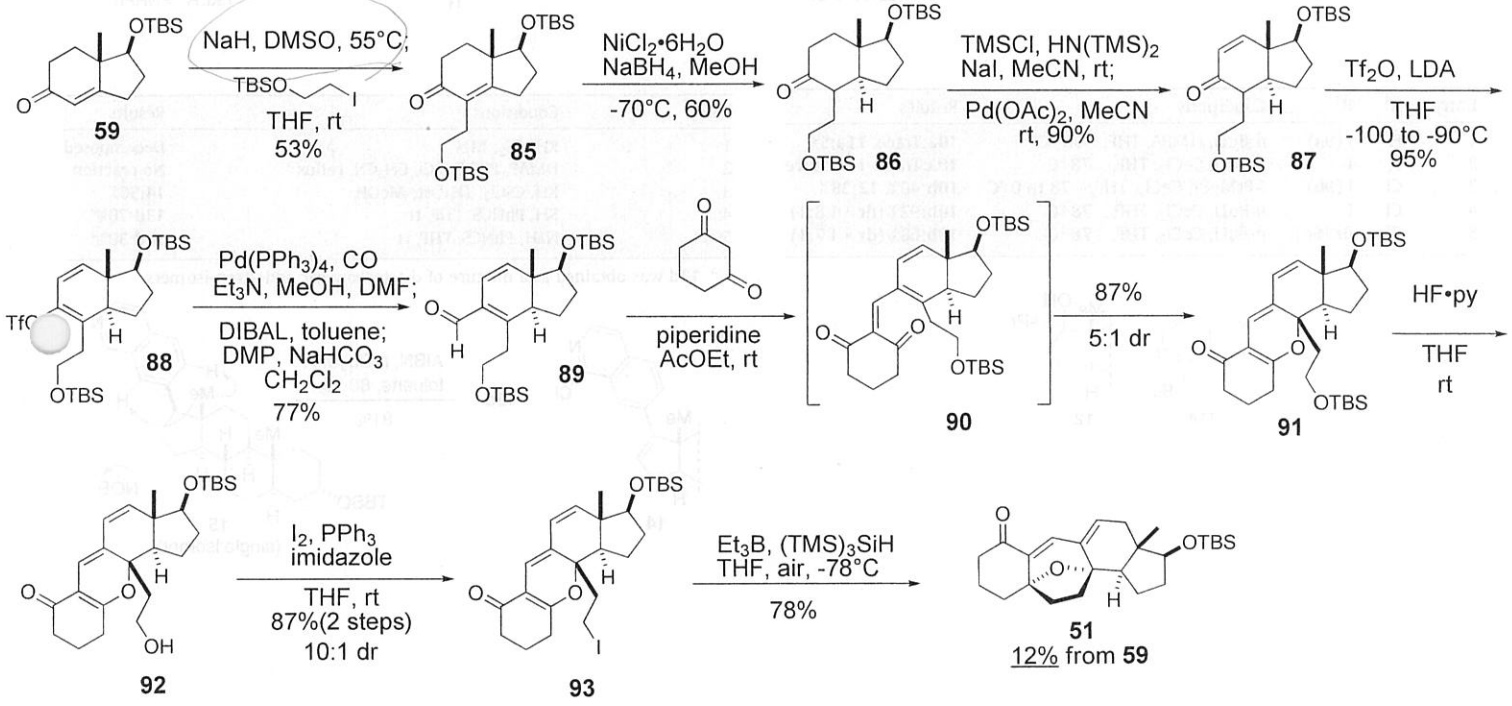
Improved synthesis of intermediate **51** and **115** was reported by Himura et al.

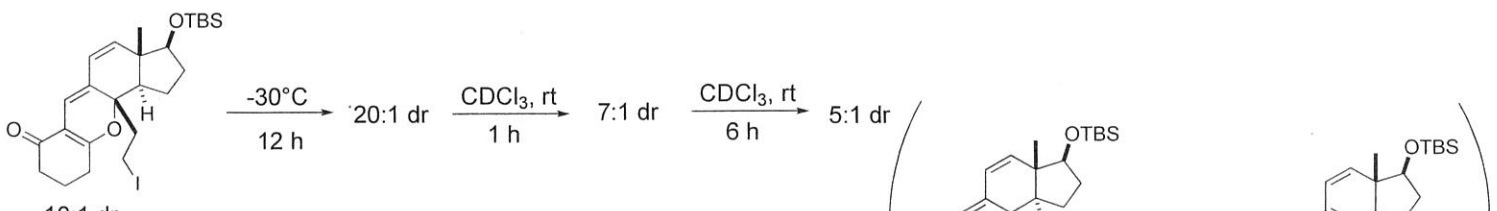
3. Formal total synthesis by Hirama

Org. Lett. **2008**, *10*, 3413
Tetrahedron Lett. **2009**, *50*, 3277

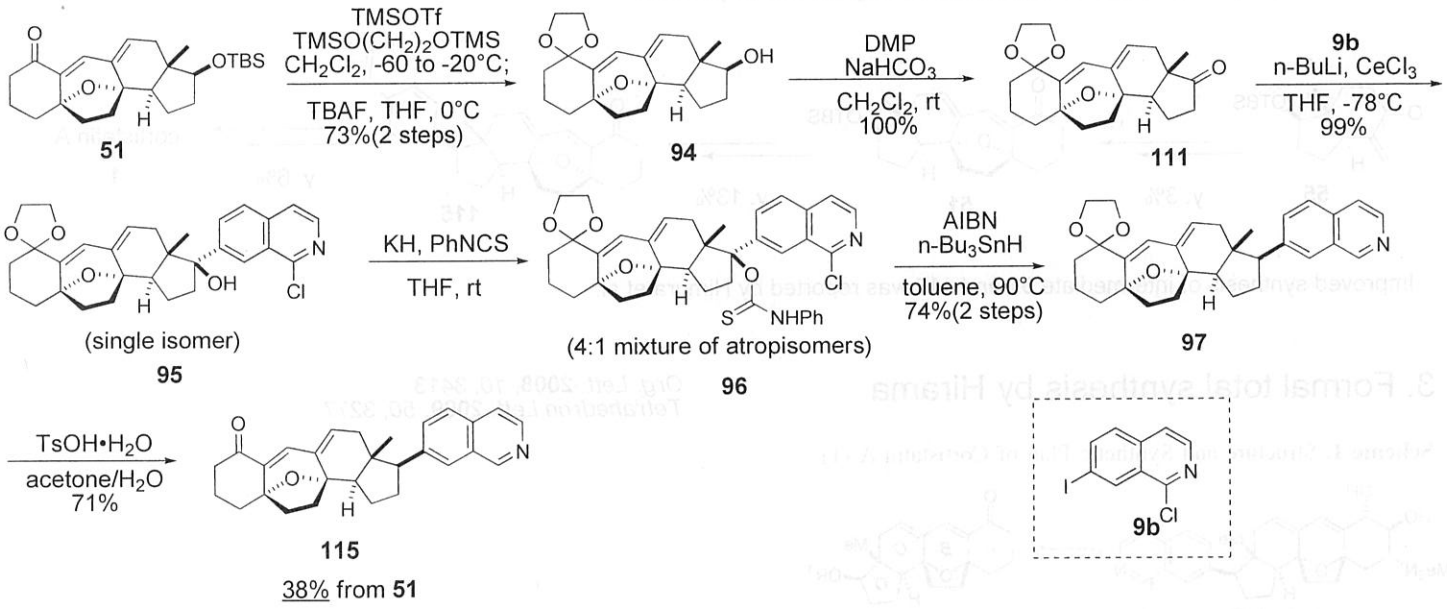
Scheme 1. Structure and Synthetic Plan of Cortistatin A (**1**)





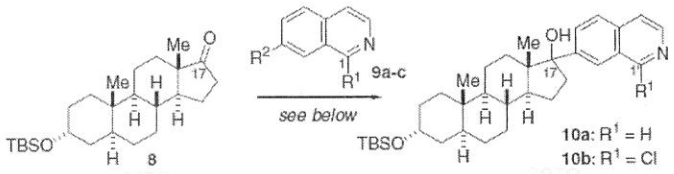


The dr of **93** changed spontaneously, which clealy indicated retrocyclization of **93** to corresponding dienone.



model study

Table 1
Nucleophilic addition of isoquinoline moiety



Entry	R^1	R^2	Conditions	Results
1	H	I (**9a**)	*n*-BuLi, HMPA, THF, −78 °C	**10a**:Trace, **11a**:5%
2	H	I	*n*-BuLi, $CeCl_3$, THF, −78 °C	**10a**:Trace, **11a**:Trace
3	Cl	I (**9b**)	*i*-PrMgBr, $CeCl_3$, THF, −78 to 0 °C	**10b**:40%, **12**:38%
4	Cl	I	*n*-BuLi, $CeCl_3$, THF, −78 °C	**10b**:92% (dr = 1.8:1)
5	Cl	Br (**9c**)	*n*-BuLi, $CeCl_3$, THF, −78 °C	**10b**:68% (dr = 1.7:1)

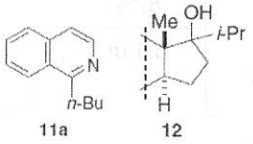
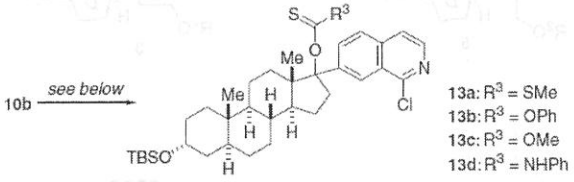
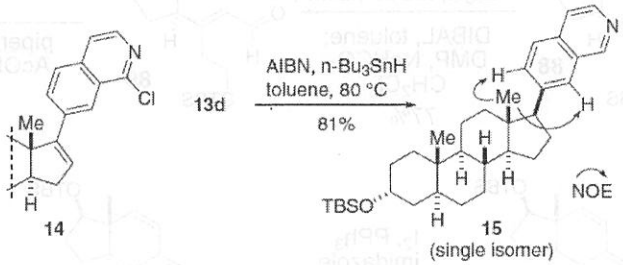


Table 2
Formation of thiocarbamate and radical reduction



Entry	Conditions	Results
1	KH, CS_2; MeI	Decomposed
2	DMAP, PhOC(S)Cl, CH_3CN, reflux	No reaction
3	KH, $CSCl_2$, THF, rt; MeOH	**14**:50%
4	KH, PhNCS, THF, rt	**13d**:70%[a]
5	NaH, PhNCS, THF, rt	**13d**:30%[a]

[a] **13d** was obtained as a mixture of diastereomeric and atrop-isomers.



4. Total synthesis by shair

Retrosynthesis

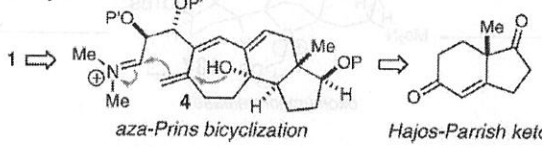
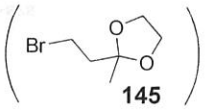
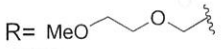
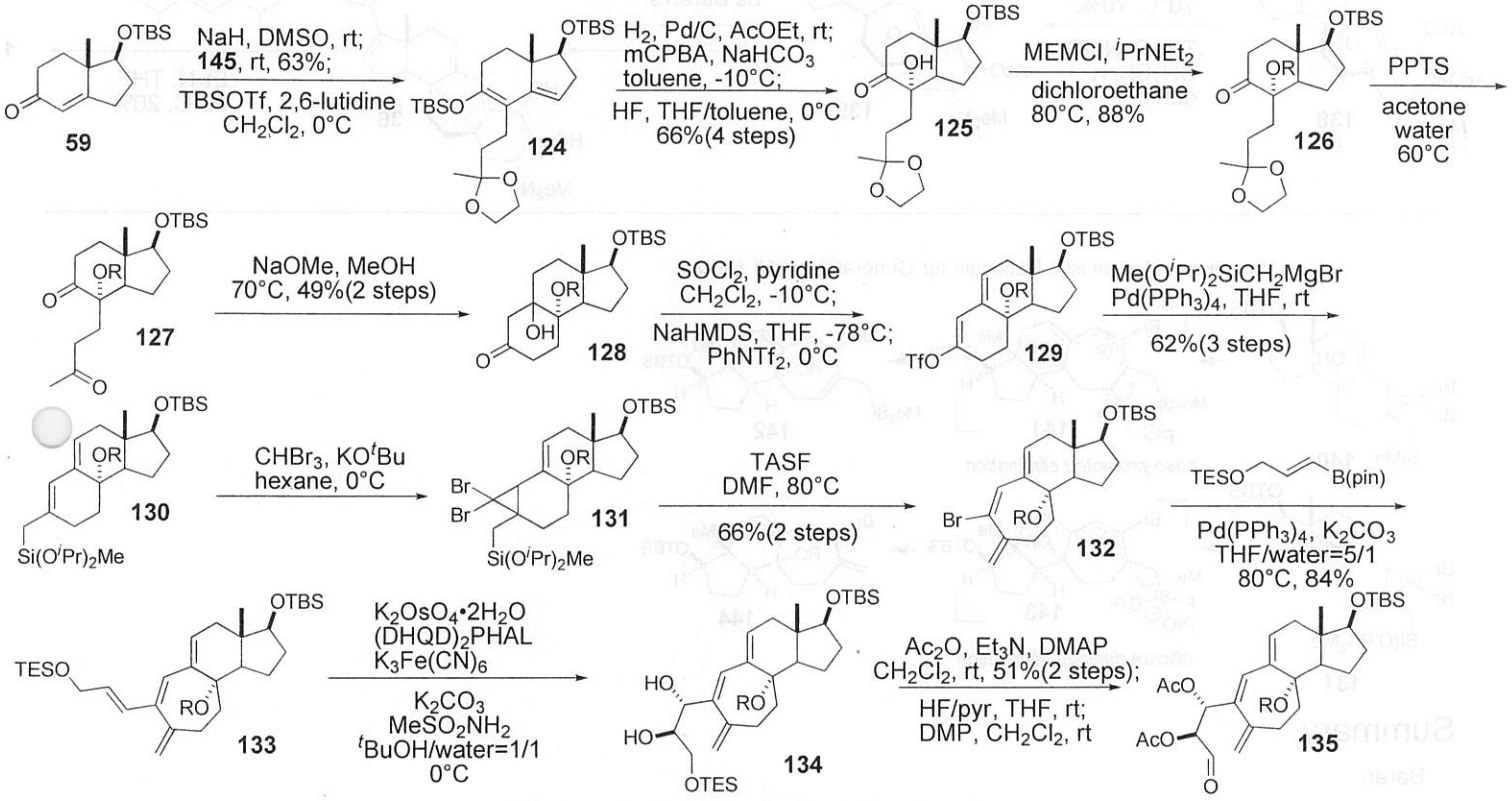


Figure 2. A key step of the cortistatin A synthesis is an aza-Prins/transannular etherification reaction. Enantiomerically enriched Hajos–Parrish ketone is the starting material.







Regioselectivity of asymmetric dihydroxylation

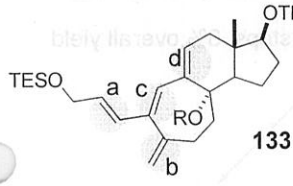


Table 27. The Selective Mono-dihydroxylation of Highly Conjugated Systems[117,†]

Entry	Substrate	Products	Ratio	% ee	% yield
1			6 : 1		60
2	51	52	4 : 1	92	68
3	53		4 : 1	98	87

† The AD reactions were performed under standard conditions[23a] using $(DHQD)_2PHAL$ and 0.2–1.0 mol % of OsO_4.

ligand interaction ??

In general, dihydroxylation maintains maximum degree of conjugation.

⇩

a, b, d>c

Table I. Ligand Effects on the Reactivity Hierarchy as a Function of Olefin Substitution Pattern[a]

OsO_4 alone	OsO_4 + Quinuclidine	OsO_4 + PHAL(DHQD)$_2$
14 ± 10% (29)	1200 ± 5% (16)	8800 ± 5% (23)
2.5 ± 10% (5.1)	320 ± 5% (4.8)	4100 ± 5% (11)
1.5 ± 10% (3)	210 ± 5% (3.2)	1400 ± 5% (3.7)
0.58 ± 10% (1.2)	100 ± 5% (1.5)	690 ± 5% (1.8)
0.58 ± 10% (1.2)	73 ± 5% (1.1)	560 ± 5% (1.5)
0.48 ± 10% (1)	66 ± 5% (1)	380 ± 5% (1)

[a] Rates in M^{-1} min^{-1}. Relative rates in parentheses. It is important to note that the relative rates only apply within a single column. The absolute rates can, of course, be compared throughout the table.

JACS, **1993**, *115*, 7047

trisubstituted, trans-disubstituted

V

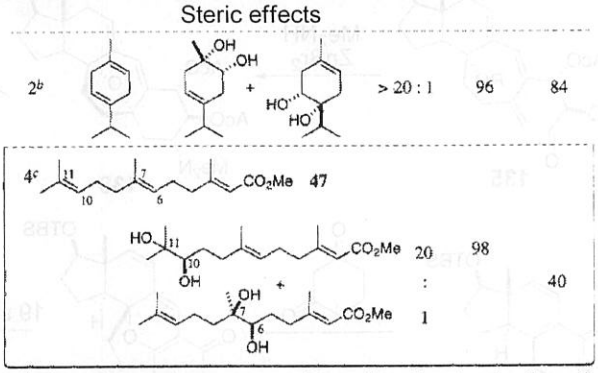
V

cis-disubstituted, 1,1-disubstituted
monosubstituted, tetrasubstituted

⇩

a, c, d>b

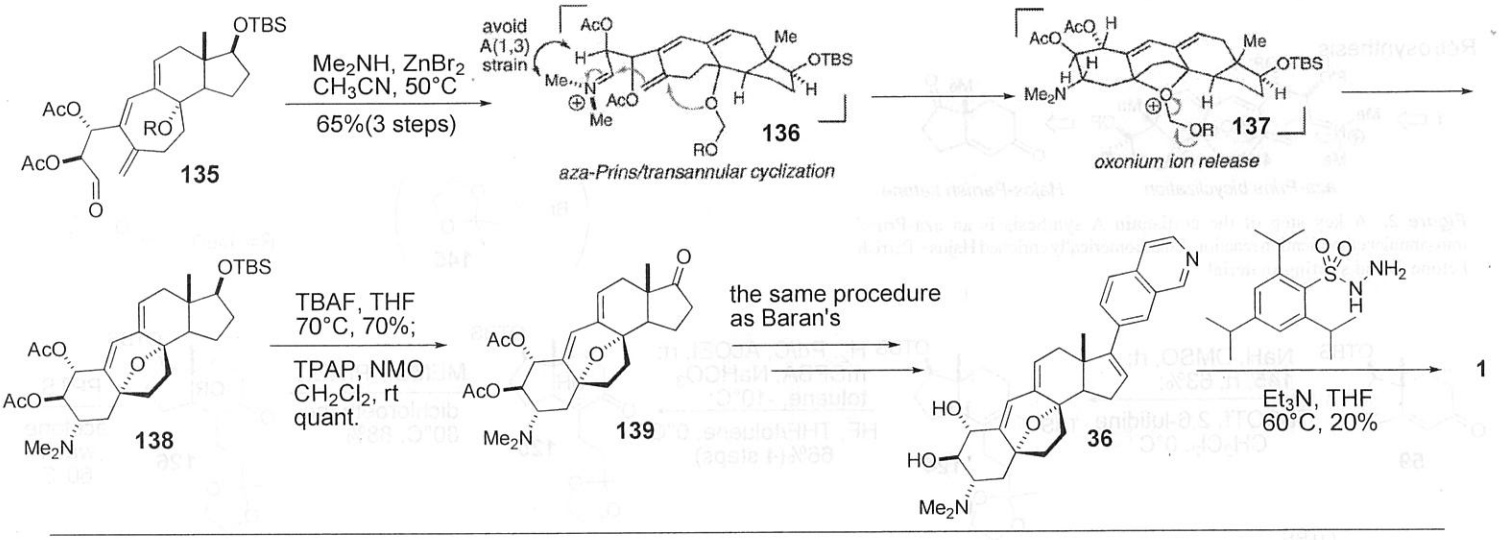
Steric effects



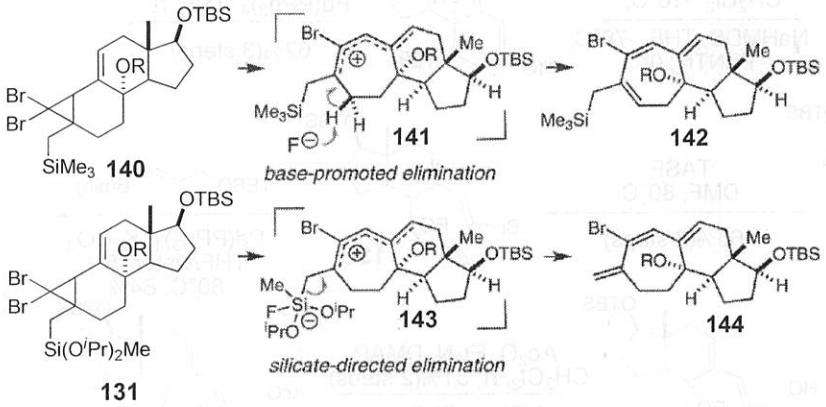
⇨ a>d

† The AD reactions were performed under standard conditions[23a] using $(DHQD)_2PHAL$ and 0.2–1.0 mol % of OsO_4. [a] % ee of the major product. [b] See ref 117. [c] See ref 121.

Chem. Rev. **1994**, *94*, 2483

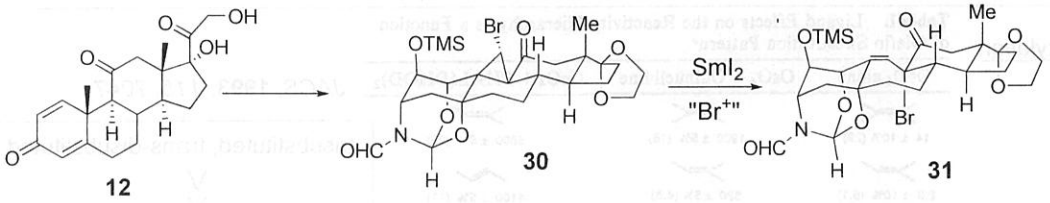


Scheme 3. Mechanistic Rationale for Generation of 12 and 20



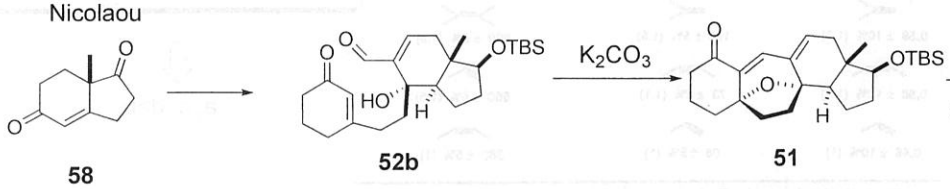
Summary

Baran

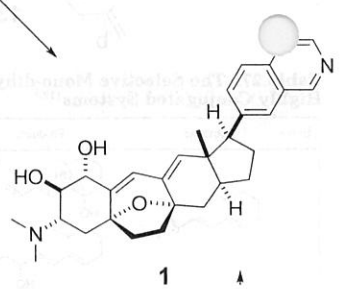


21 steps, 3% overall yield

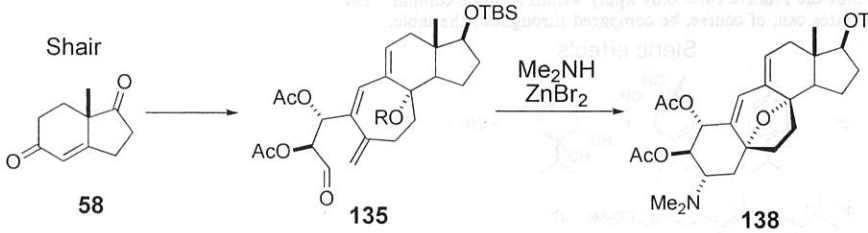
Nicolaou



24 steps(from **55**)
0.03% overall yield



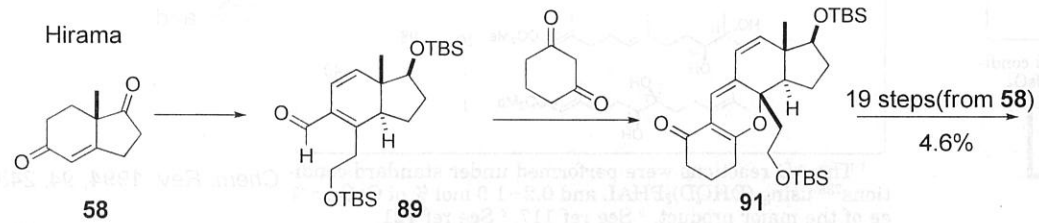
Shair



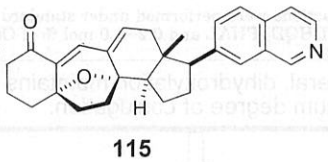
25 steps, 0.14% overall yield(from **59**)

5 steps
6%

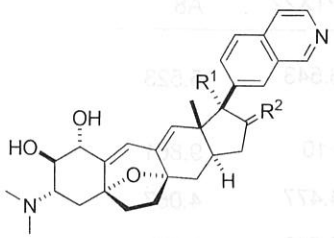
Hirama



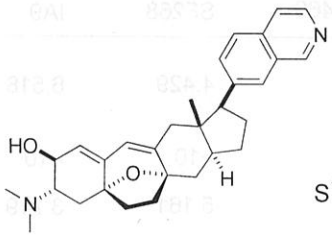
19 steps(from **58**)
4.6%



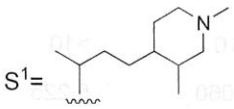
5. Structure-Activity Relationship

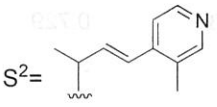


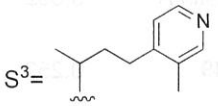
Cortistatin A (**1**) : R^1=H, R^2=H, H
Cortistatin B (**2**) : R^1=H, R^2=H(α), OH(β)
Cortistatin C (**3**) : R^1=H, R^2=O
Cortistatin D (**4**) : R^1=OH, R^2=O

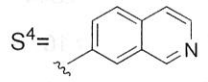


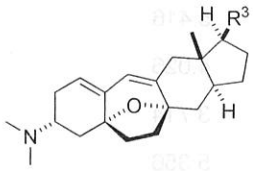
Cortistatin L(**11**)



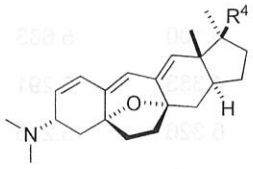








Cortistatin E (**5**) : R^3=S^1
Cortistatin G (**6**) : R^3=S^2
Cortistatin H (**7**) : R^3=S^3
Cortistatin K (**8**) : R^3=S^4



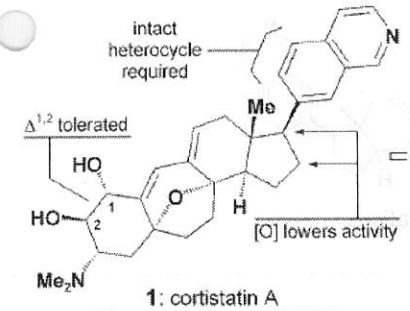
Cortistatin F (**9**) : R^4=S^1
Cortistatin J (**10**) : R^4=S^4

Table 1. Growth inhibition of cortistatins against HUVECs and various type of cell lines

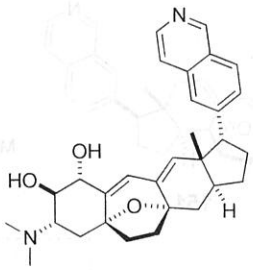
Cell line	A (1)		B (2)		C (3)		D (4)		E (5)	
	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
HUVEC	0.0018	1	1.1	1	0.019	1	0.15	1	0.45	1
KB3-1	7.0	3900	120	110	150	7900	55	460	2.5	6
Neuro2A	6.0	3300	160	150	180	9500	>300	n.d.	1.9	4
K562	7.0	3900	200	180	>300	n.d.	>300	n.d.	2.8	6
NHDF	6.0	3300	>300	n.d.	>300	n.d.	>300	n.d.	1.9	4

G (6)		H (7)		K (8)		F (9)		J (10)		L (11)	
IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
0.80	1	0.35	1	0.04	1	1.9	1	0.008	1	0.023	1
8.9	11	2.3	7	10.2	250	10.8	6	9.1	1100	14	610
4.0	5	2.2	6	3.0	80	4.0	2	3.3	410	2.8	120
3.8	5	2.7	8	3.9	100	4.0	2	3.3	410	4.3	190
2.9	4	2.7	8	2.5	60	4.1	2	2.4	300	2.4	100

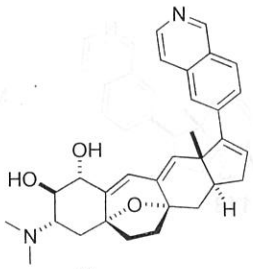
Kobayashi et al. *Bioorg. Med. Chem.* **2007**, *15*, 6758



1: cortistatin A
• 1.8 nM inhibition of HUVECs
• most potent member of cortistatin family



17-*epi*-cotristatin A
146



Δ^{16}-cortistatin A
147

Table 2: Selective growth inhibition of cortistatins against HUVECs.

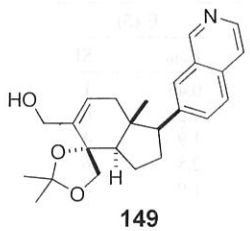
Substrate	IC_{50} [nM]
cortistatin A (1)	2.43[a], 1.8[b]
Δ^{16}-cortistatin A (2)	3.88
17-*epi*-cortistatin A (4)	> 1000
6d–g, 7a–f, 8e, 9a, 9d–e[c]	> 1000

[a] IC_{50} of synthetic cortistatin A tested by Pfizer Inc. [b] IC_{50} of natural cortistatin A tested by Kobayashi group.[4a] [c] The TBS groups were removed prior to testing. The results of **6e** and **7e** are from Ref. [4f].

Baran et al. *Angew. Chem. Int. Ed.* **2009**, *48*, 4328

compound	HUVEC	MCF-7	NCI-H460	SF268	IA9	PTX22	A8
synthetic cortistatin A	0.002	>10	7.786	4.429	6.518	6.543	5.523
149	0.253	>10	>10	>10	>10	>10	9.861
150	4.542	5.060	5.225	5.161	3.719	4.477	4.057
65	0.544	0.532	0.729	0.524	0.519	0.512	0.578
51	>10	-	-	-	-	-	-
111	>10	-	-	-	-	-	-
113	0.486	6.76	4.323	5.100	5.683	5.806	5.416
114	0.039	5.504	5.504	4.383	5.291	4.999	4.026
115	0.007	7.893	4.693	6.326	5.236	4.905	3.714
118	0.076	7.860	5.498	4.375	6.020	6.057	5.356
151	0.034	7.803	5.589	3.799	7.303	7.638	5.963
Taxol	0.005	0.007	0.006	0.006	0.005	0.058	0.052

GI_{50}(growth inhibition of 50%), μM



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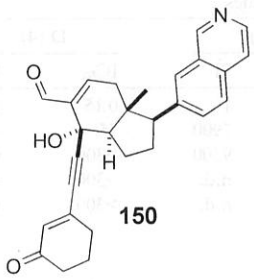

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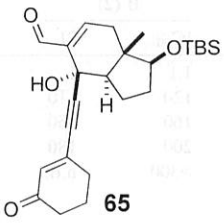

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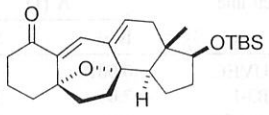

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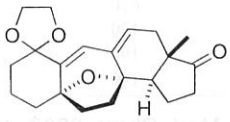

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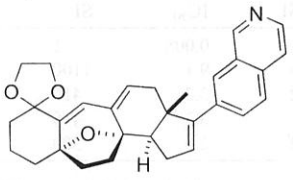

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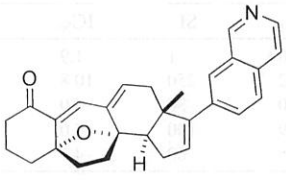

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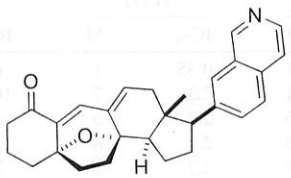

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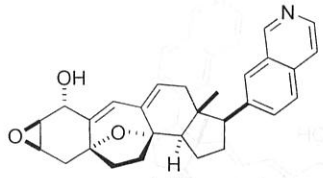

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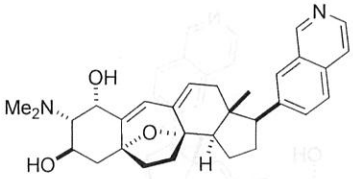

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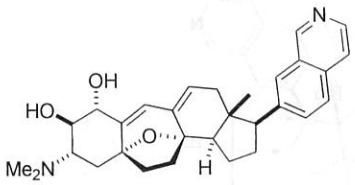
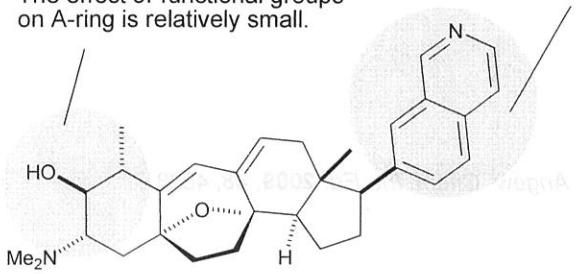
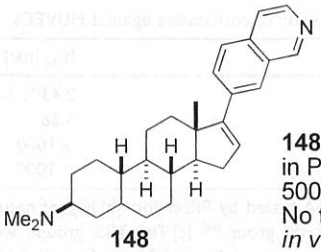


cortistatin A

Isoquinoline moiety is essential for the inhibition of cell prolification. This moiety is also neccesary for the selectivity.

The effect of functional groups on A-ring is relatively small.





148

IC_{50}=0.017 μM

148 inhibited retinal vessel formation in P6(6 days post birth) mice after 500 pmol of injection.
No toxity was observed at 50 μM *in vitro*.

Corey et al. *JACS*, **2009**, *131*, 9014

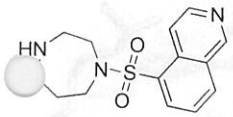
Cortistatin A was tested in high-throuphput binding assay against a panel of 402 kinases.

Nicolaou et al. *Angew, Chem, Int, Ed.* **2009**, *48*, 8952

Table 1: Kinase affinity of synthetic cortistatin A.

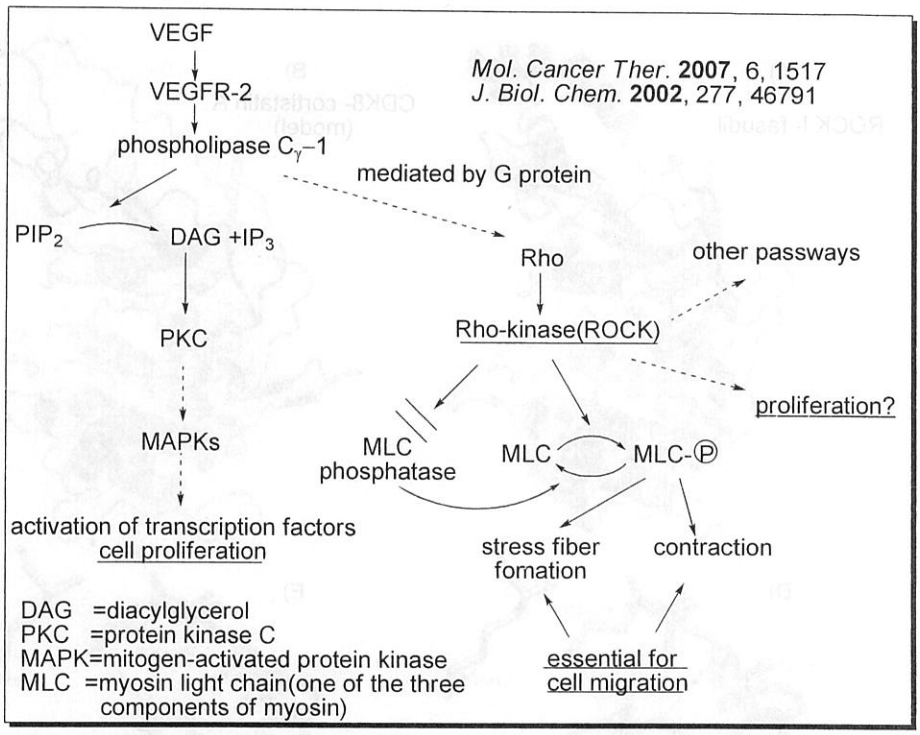
Kinase	POC (10 μM)[a]	K_d [nM][b]
ROCK II	0	220±7
CDK11	0.1	10±2
CDK8	0.95	17±2
LTK	2.9	ND
ALK	4.4	ND
PIM2	4.4	ND
PKACα	8.7	3500±212
PKACβ	13	ND
MET	18	ND
PRKG2	21	ND
RIOK2	21	ND
ROCK I	21	250±35
CLK4	26	ND
ROS1	26	ND
CIT	28	ND
JNK1	29	ND

[a] Kinases with POC < 35 are shown. [b] Average of two determinations ± SD; ND = not determined. POC=percent of control



ROCK I K_d=42nM
ROCK II K_d=34nM
CDK8 K_d>40000nM
CDK11 K_d>40000nM

fasudil (inhibitor of ROCK)



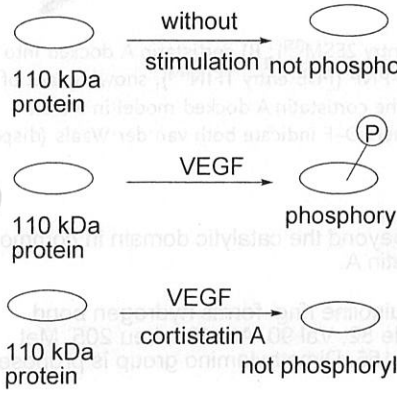
Mol. Cancer Ther. **2007**, 6, 1517
J. Biol. Chem. **2002**, 277, 46791

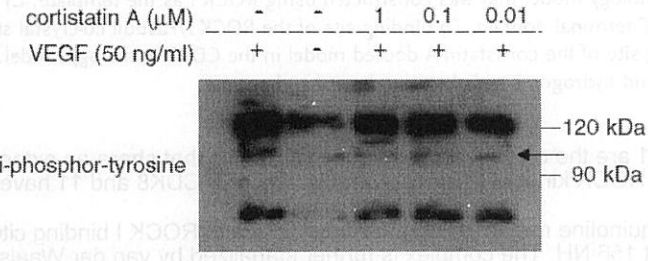
ROCK is a target protein of cortistatins?

Kobayashi et al. reported in 2007 that cortistatin A inhibited cell proliferation **without inhibition of MAPKs**

→ Another passway is the target.

They also reported that phosphorylation of **110 kDa protein** was reduced remarkably by the treatmet with cortistatin A.





ROCK phosphorylates **110 kDa subunit of MLC phosphatase** to promote cell migration.

→ ROCK seems to be a reasonable target.

(Further investigation is neccesary to confirm the conclusion.)

Because the function of CDK family is not known very well, it's difficult to ascetain whether the effects of corstatin A are consistent with inhibition of CDK8/11.

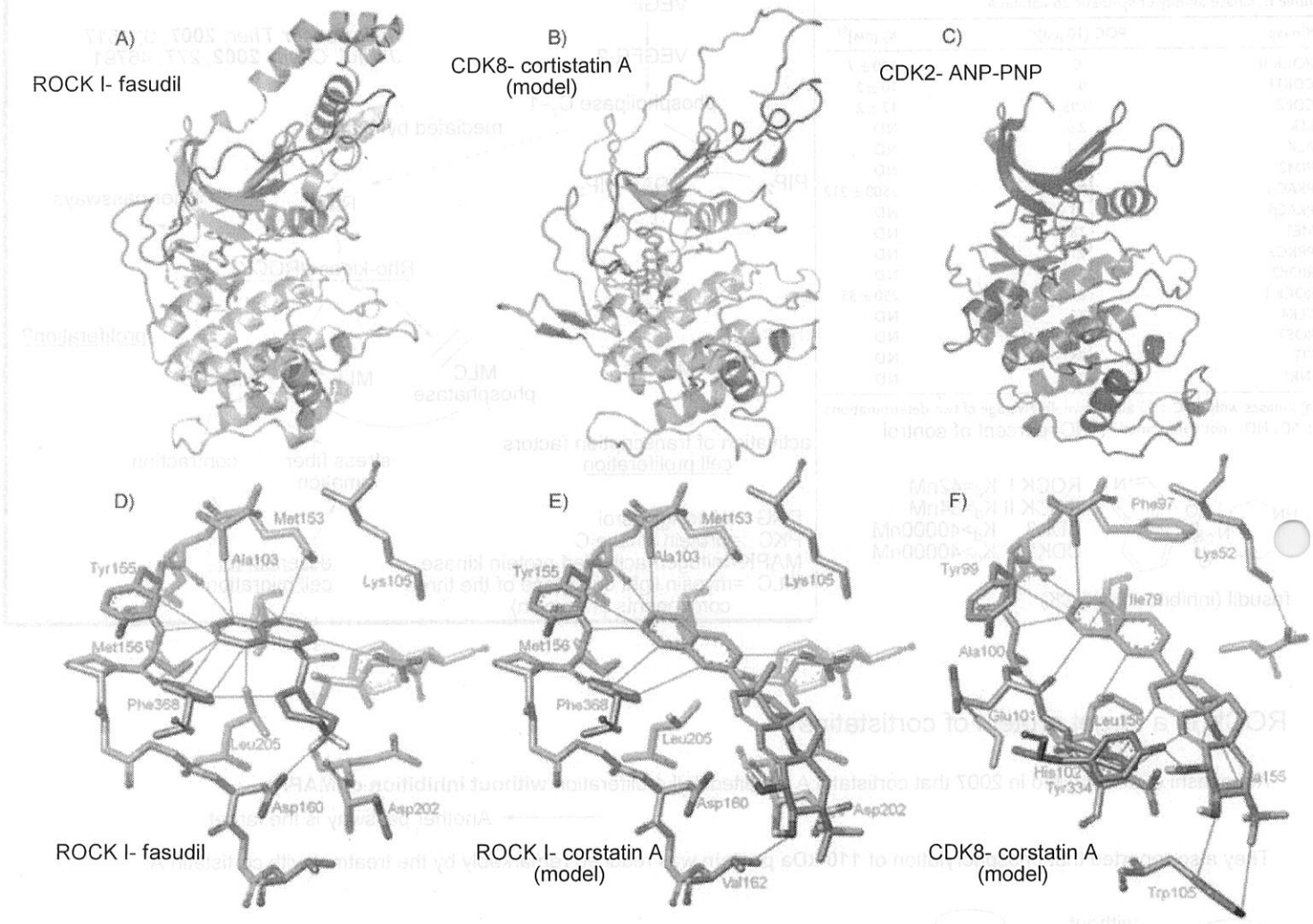
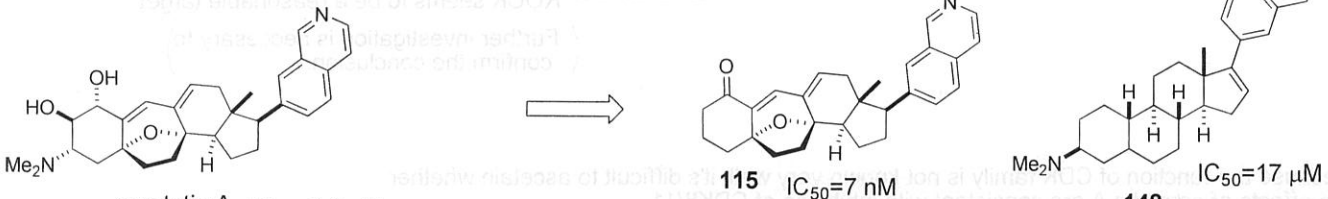


Figure 2. Structures and modeling of ROCK I, CDK8, and CDK2: A) ROCK I in complex with fasudil (PDB entry 2ESM[19]); B) cortistatin A docked into a CDK8 homology model that was constructed using ROCK I as the template; C) CDK2 in complex with ANP-PNP (PDB entry 1FIN[20]), showing lack of an extended C-terminal domain; D) binding site of the ROCK I/Fasudil co-crystal structure; E) binding site of the cortistatin A docked model in ROCK I; F) binding site of the cortistatin A docked model in the CDK8 homology model. Thin solid blue lines in panels D–F indicate both van der Waals (dispersion) contacts and hydrogen bonds between ligand and protein.

CDK8/11 are the only menbers of the CDK family that share an extended C-terminal domain beyond the catalytic domain in common with the ROCK kinases. This is probably why only CDK8 and 11 have high affinity with cortistatin A.

The isoquinoline ring of cortistatin A interacts with ROCK I binding cite. Nitrogen atom of isoquinoline ring forms hydrogen bond with Met 156 NH. The complex is further stabalized by van dar Waals contacts with **Phe 368**,Ile 82, Val 90, Ala 103, Leu 205, Met 153 as well as a CH•••O hydrogen bond with Asp 216 and an N•••CH hydrogen bond with Tyr 155. Dimethylamino group is proposed to form a salt bridge with Asp 202.

Future View



corstatin A IC_{50}=1.8 nM

115 IC_{50}=7 nM

148 IC_{50}=17 μM

Although the synthesis of corstatin A was accomplished, only tiny amount of cortistatin A was obtained so far. Further investigation might be difficult for this reason.

These less complex molecules will probably be a target for further investigation and lead compound for a new drug.