The Chemistry and Biology of Epothilones

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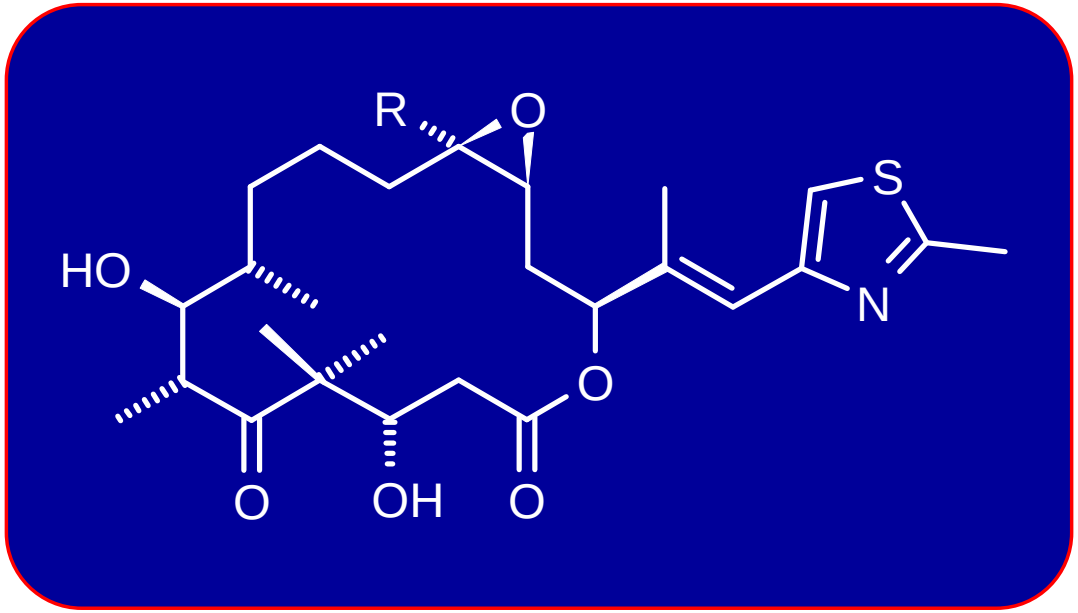
ORGANIC CHEMISTRY (IASOC)

Ischia, Sept. 16 - 21

Epothilones - 16-Membered Macrolides from Gliding Bacteria

ETH



R = H: Epothilone A

R = CH_3: Epothilone B

From myxobacterium *Sorangium cellulosum Soce90:*

- **Reichenbach et *al*., 1993, 1996**
- **Bollag et *al*., 1995**

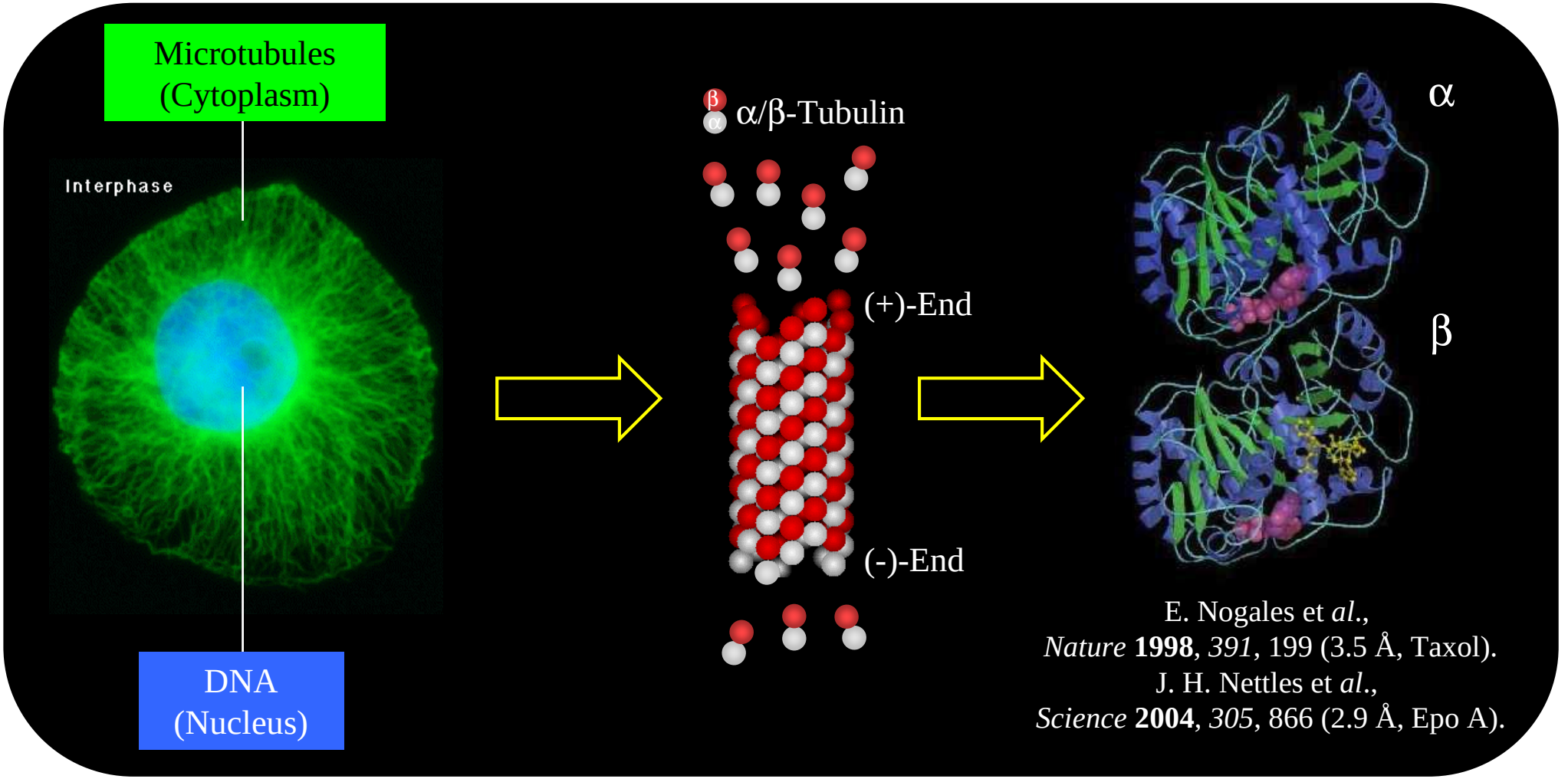
Cytoskeleton and Microtubule Structure

ETH

Microtubules and the **Cytoskeleton**

Microtubule Structure and **Dynamic Instability**

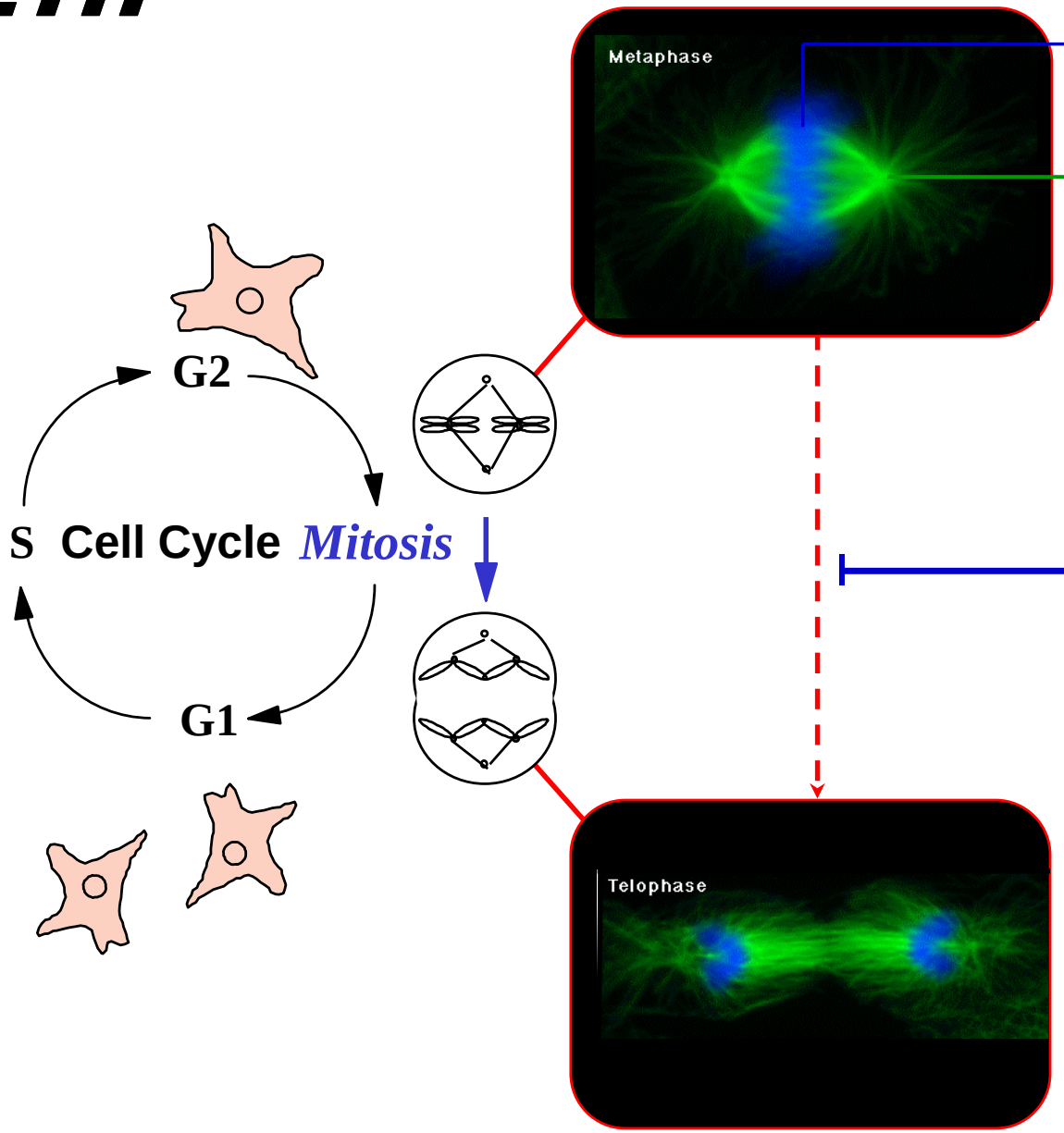
Tubulin Structure



E. Nogales et *al.*,
Nature **1998**, *391*, 199 (3.5 Å, Taxol).
J. H. Nettles et *al.*,
Science **2004**, *305*, 866 (2.9 Å, Epo A).

Taxol and Epothilones - G2/M Arrest and Induction of Apoptosis

ETH



- Inhibition of microtubule dynamics
- Cell cycle arrest at mitosis and induction of apoptosis (nM)
- Inhibition of human cancer cell growth (nM/sub-nM IC_{50}s)

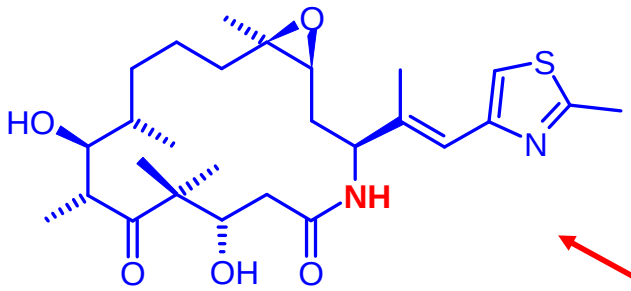
Epothilones - Growth Inhibition of Human Cancer Cell Lines

ETH

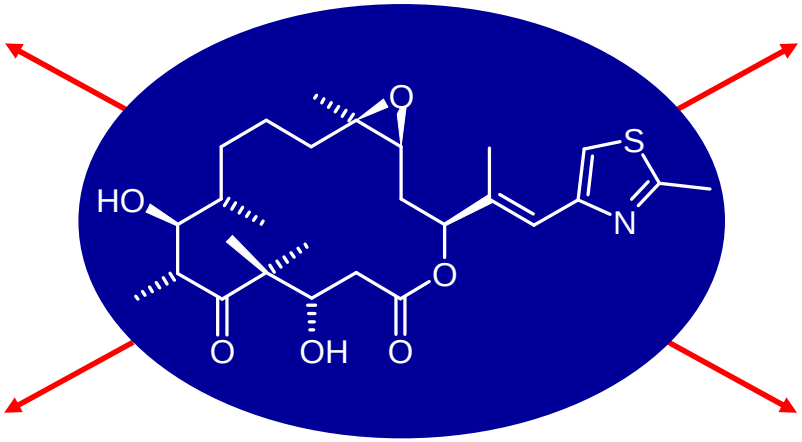
Cell Line		IC_{50} [nM]		
		Epothilone B	Epothilone A	Taxol
A-549	(Lung)	0.23	2.67	3.19
Du145	(Prostate)	0.31	4.86	2.79
HCT-116	(Colon)	0.32	2.51	1.66
MCF-7	(Breast)	0.18	1.49	1.80
MCF-7/ADR	(Breast, MDR)	2.92	27.5	9105
KB-31	(Epidermoid)	0.19	2.10	2.31
KB-8511	(Epidermoid, MDR)	0.19	1.90	533

Epothilone-Derived Agents – Compounds in Clinical Development

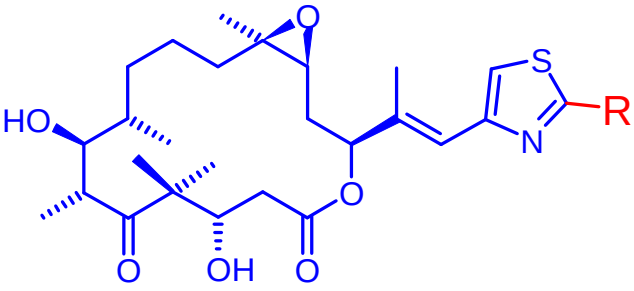
ETH



BMS-247550
(ixabepilone)
BMS, Phase I/II/III

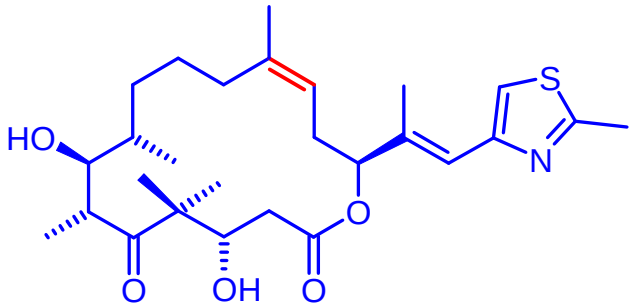


EPO906 (= Epo B)
(**patupilone**)
Novartis, Phase II/III



R = CH_2NH_2: BMS-310705
BMS, Phase I

R = SH_3: ABJ879
BMS, Phase I



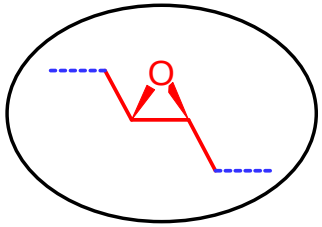
Epo D (KOS-862):
Roche/Kosan, Phase II

***Trans*-9,10-DidehydroEpo D**
(KOS-1584)
Roche/Kosan, Phase I

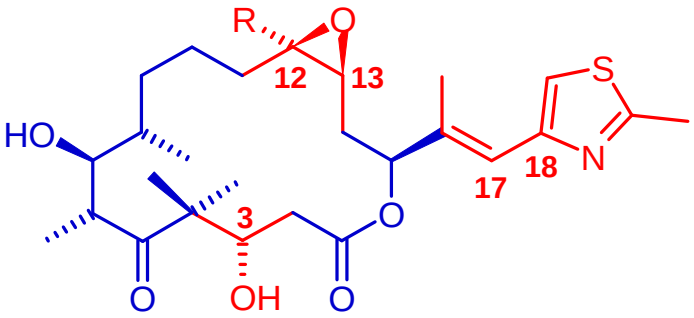
ZK-EPO (unknown structure)
Schering AG, Phase I/II

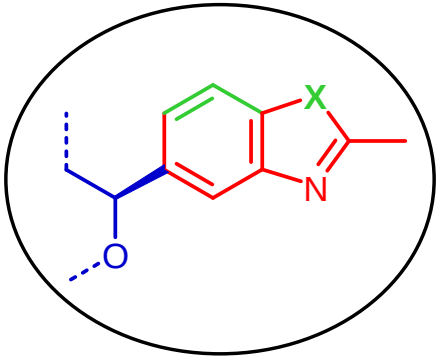
Evolving New Scaffolds for Microtubule Stabilization - Structural Entry Points

ETH

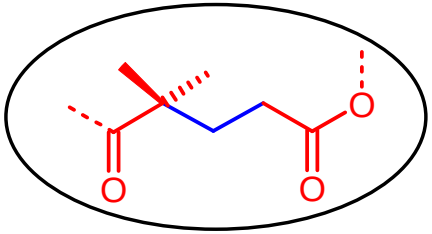


Epoxide geometry
(cis → trans**)**



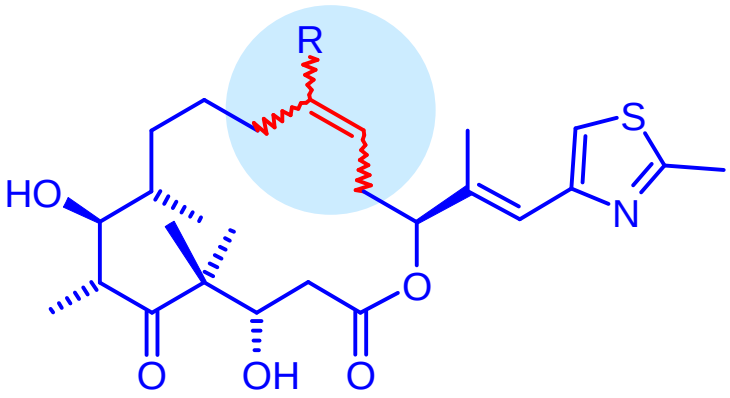


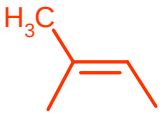
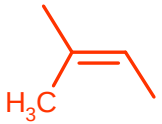
Side chain rigidification



3-Deoxy derivates

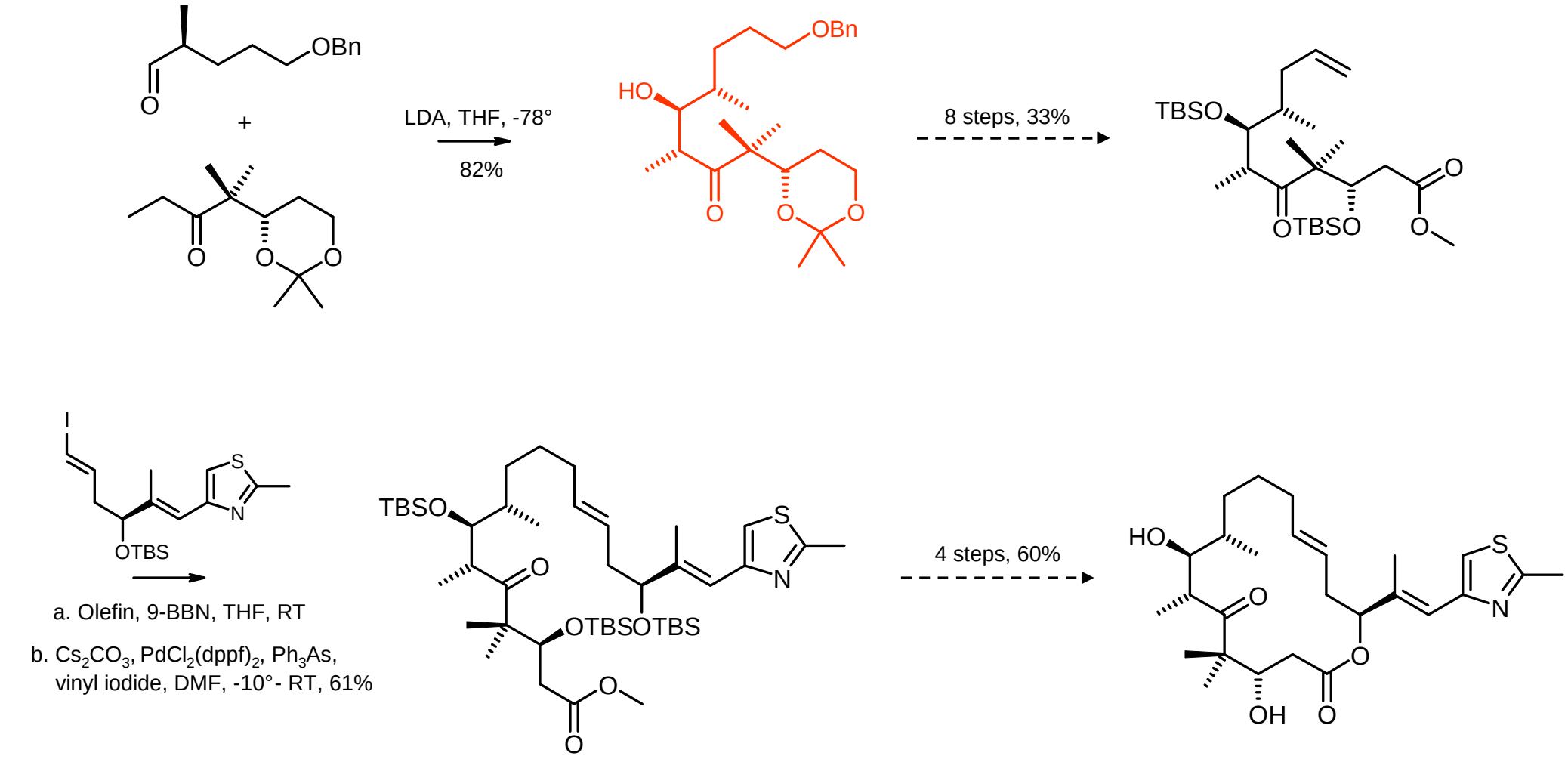
Deoxyepothilone SAR - Cis *vs* Trans *Olefins*



R	%Tubulin Polymerization	IC_{50} KB-31 [nM]	IC_{50} KB-8511 [nM]
	50	25	9.9
	48	52	23
	93	2.70	1.44
	38	44	34

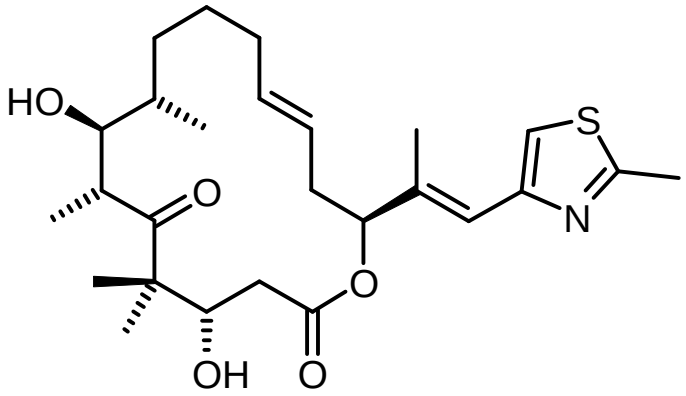
For first studies on *trans*-deoxyepothilones *cf*.: S. J. Danishefsky *et al*., *Angew. Chem. Int. Ed*. **1997**, *36*, 757.

Total Synthesis of trans-*Epothilone A – trans-Deoxy-Epothilone A*

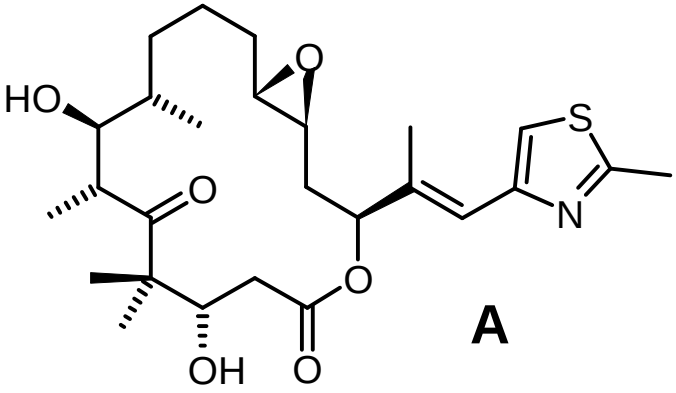


K.-H. Altmann, G. Bold, G. Caravatti, D. Denni, A. Flörsheimer, A. Schmidt, G. Rihs, M. Wartmann, *Helv. Chim. Acta* **2002**, *85*, 4086-4110.

trans-*Epothilone A - Epoxidation Selectivity*

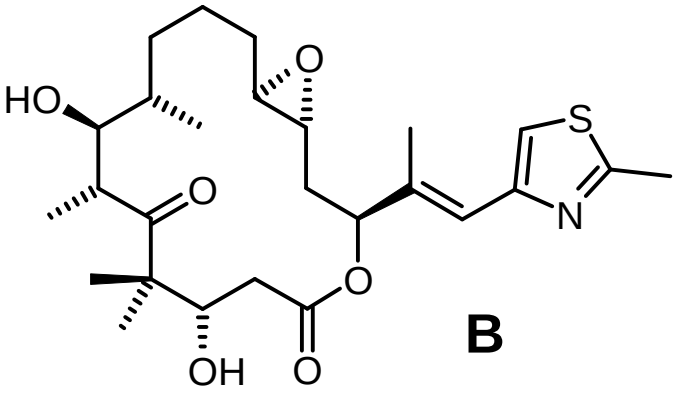


Oxidant →

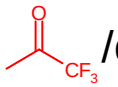
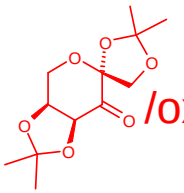


A

+



B

Oxidant	**A : B**	Yield (**A** + **B**)
 /oxone	1 : 1	28%
MCPBA	1 : 1	N.D.
 /oxone	8 : 1	27% [54%*]

*Based on recovered starting material

K.-H. Altmann, G. Bold, G. Caravatti, D. Denni, A. Flörsheimer, A. Schmidt, G. Rihs, M. Wartmann *Helv. Chim. Acta* **2002**, *85*, 4086-4110.

Epothilone SAR - Assessment of Biological Activity

ETH

- **Induction of tubulin polymerization**
  - Polymerization of MAP-rich porcine tubulin at pH 6.8 and 2 µM compound concentration.
  - Percent polymerization relative to the effect of 25 µM Epothilone B.

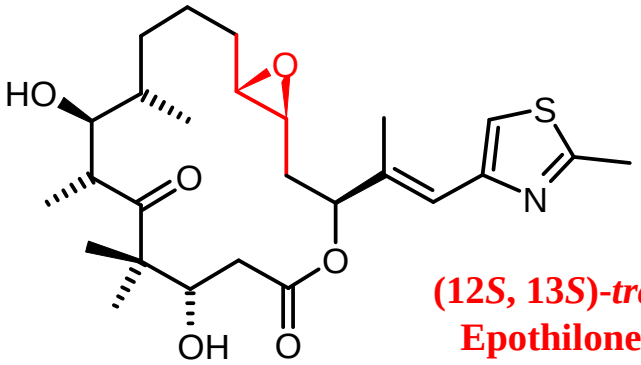
  → "%Tubulin Polymerization"

- **Cytotoxicity against KB-31 and KB-8511 (P-gp-overexpressing) cells**
  - Growth inhibition after 72h continuous exposure.

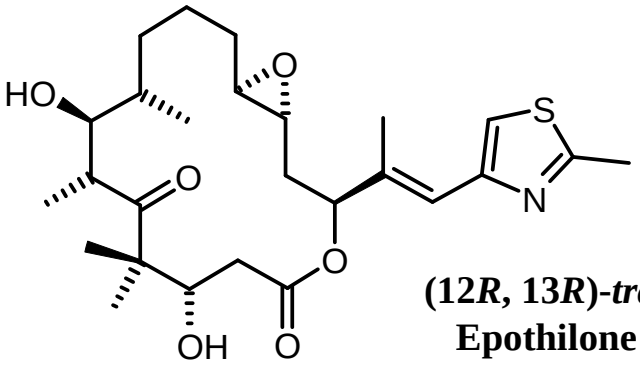
  → "IC50 KB-31 (KB-8511)"

Trans-*Epothilones A* - In vitro *Profile*

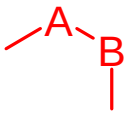
ETH



(12*S*, 13*S*)-*trans*-Epothilone A



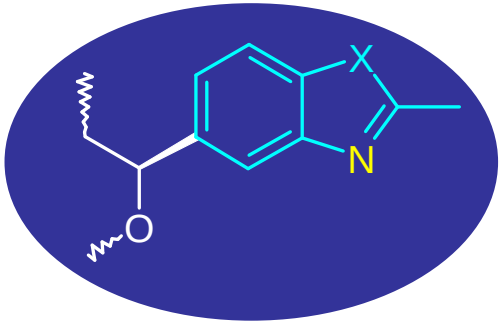
(12*R*, 13*R*)-*trans*-Epothilone A

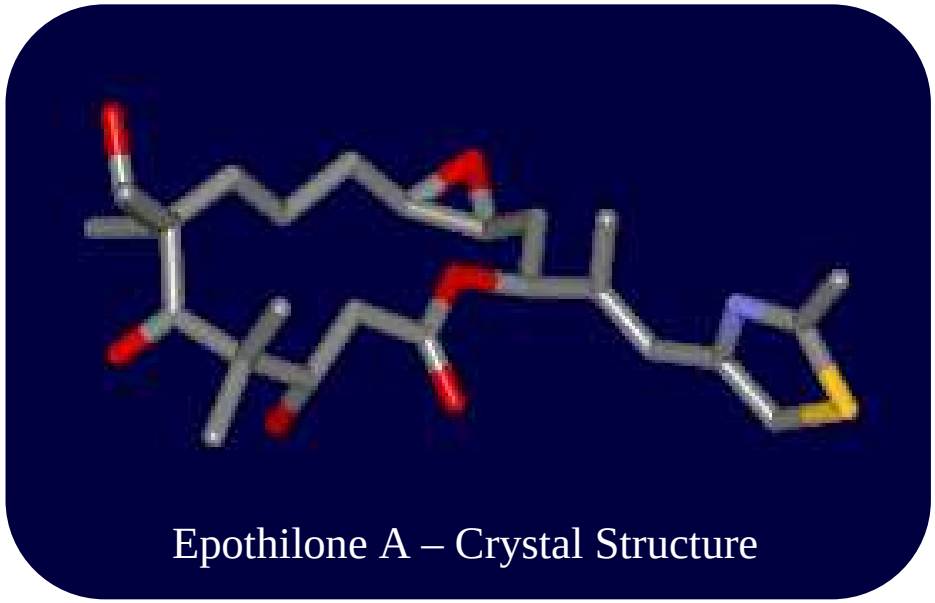
		EC_{50} Tubulin Polym. [μM]*	Growth inhibition (IC_{50} [nM]) KB-31	KB-8511	PC3-M	HCT-116
	(Epo A)	2.64	0.19	0.19	0.53	0.32
	(Epo B)	4.95	2.00	1.79	4.30	2.15
	(12*S*, 13*S*-*trans*-Epo A)	3.86	1.00	0.87	2.15	1.75
	(12*R*, 13*R*-*trans*-Epo A)	>50	523	305	N.D.	N.D.

*Concentration required to induce 50% tubulin polymerization

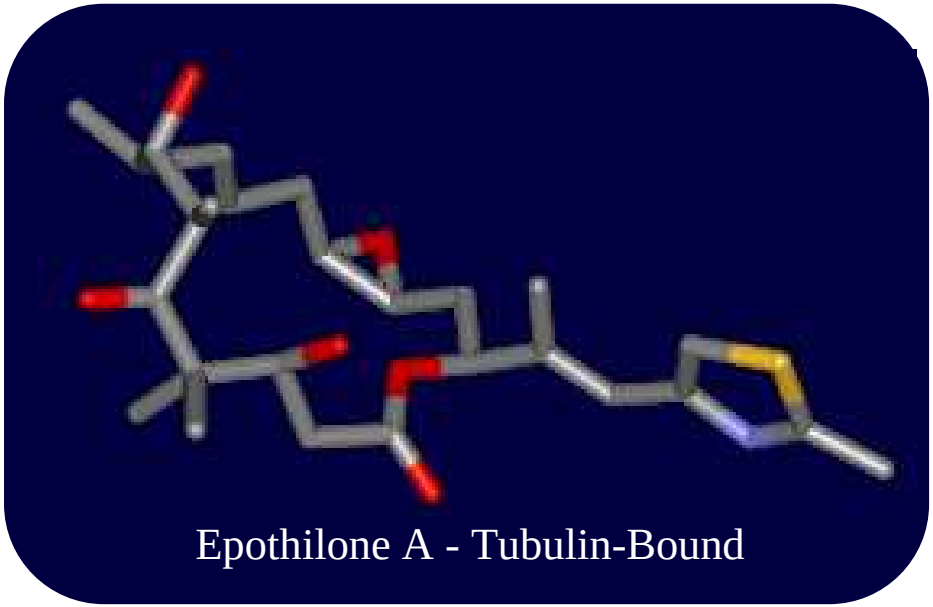
Epothilone A - Bioactive Conformation

ETH





Epothilone A – Crystal Structure



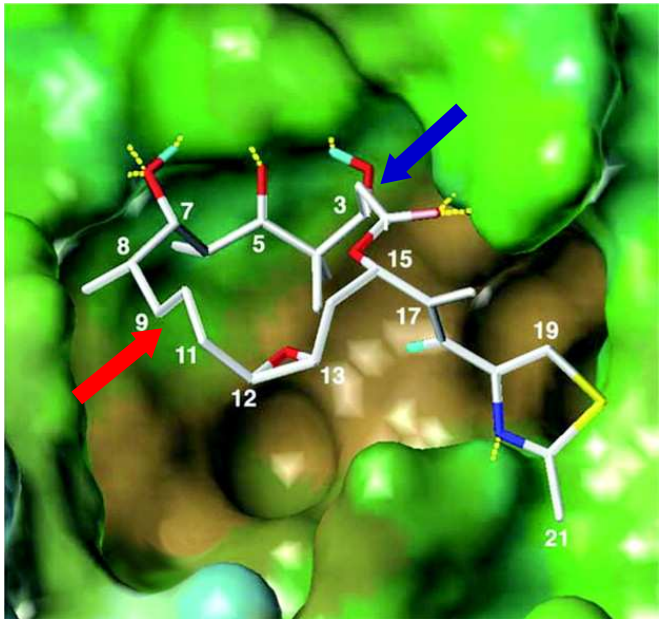
Epothilone A - Tubulin-Bound

T. Carlomagno, M. J. J. Blommers, J. Meiler, W. Jahnke, T. Schupp, F. Petersen, D. Schinzer, K.-H. Altmann, C. Griesinger
Angew. Chem. **2003**, *115*, 2615 - 2619.

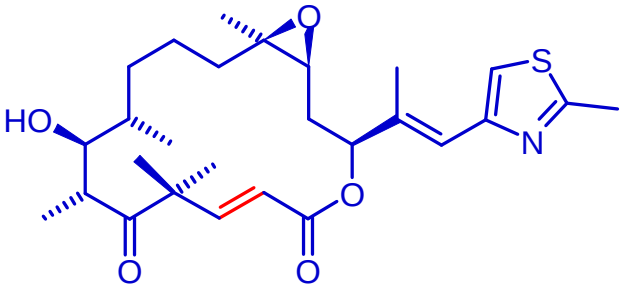
Epothilone A – Putative Bioactive Conformations

ETH

Electron crystallography & NMR analysis



Nettles *et al*.,
Science **2004**, *305*, 866-869.

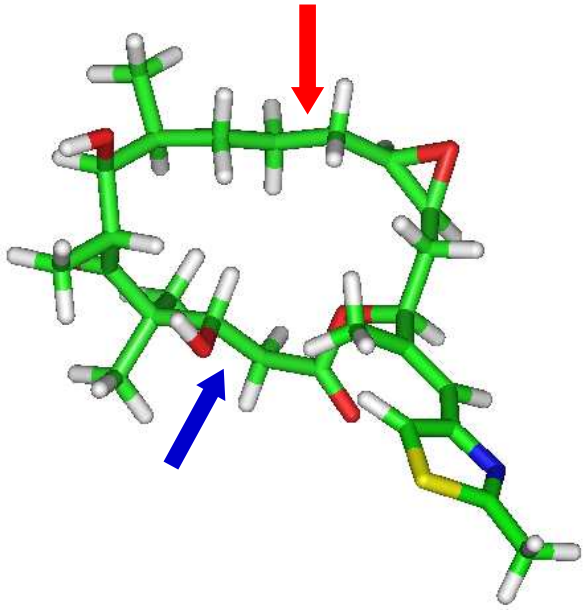


79% Tubulin polymerization

IC_{50} (KB-31): 1.81 nM

A. Flörsheimer, M. Wartmann, K.-H. Altmann, unpublished.
Cf. also: A. Regueiro-Ren et *al*., *Org. Lett.* **2002**, *4*, 3815-3818.

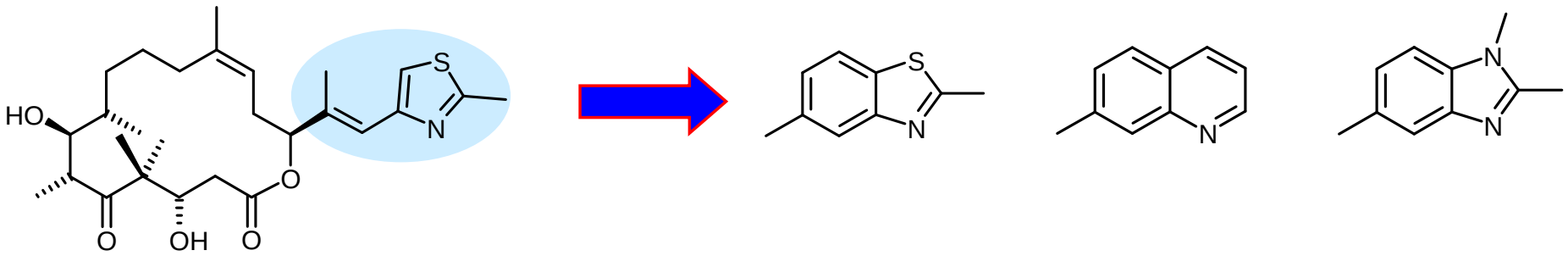
Liquid state NMR



Carlomagno *et al*.,
Angew. Chem. **2003**, *115*, 2615-2619.

Epothilone D Analogs with Constrained Side Chains

ETH

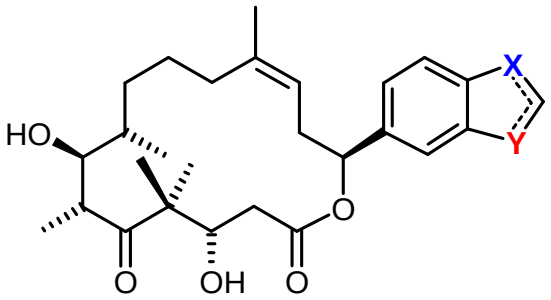


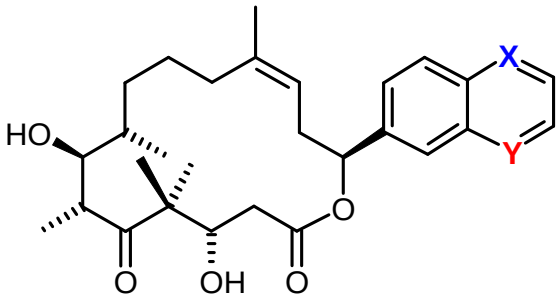
	Growth inhibition (IC_{50} [nM])			
KB-31 (Epidermoid):	**2.70**	**0.61**	**0.59**	**0.21**
A549 (Lung):	**4.62**	**1.00**	**0.90**	**0.20**
HCT-116 (Colon):	**4.48**	**1.22**	**1.13**	**0.41**
Activity increase:	**N.A.**	**4.2 x**	**2.9 x**	**14.4 x**

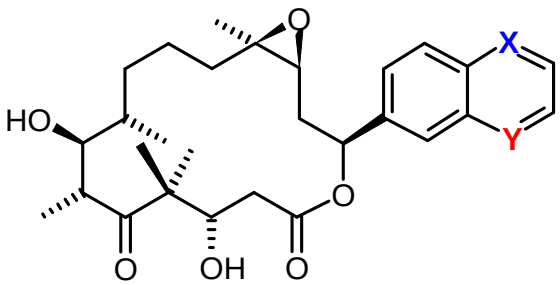
K.-H. Altmann, G. Bold, G. Caravatti, A. Flörsheimer, V. Guagnano, M. Wartmann, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2765-2768.

Epothilone Analogs with Constrained Side Chains – How important is the Position of the Nitrogen ?

ETH



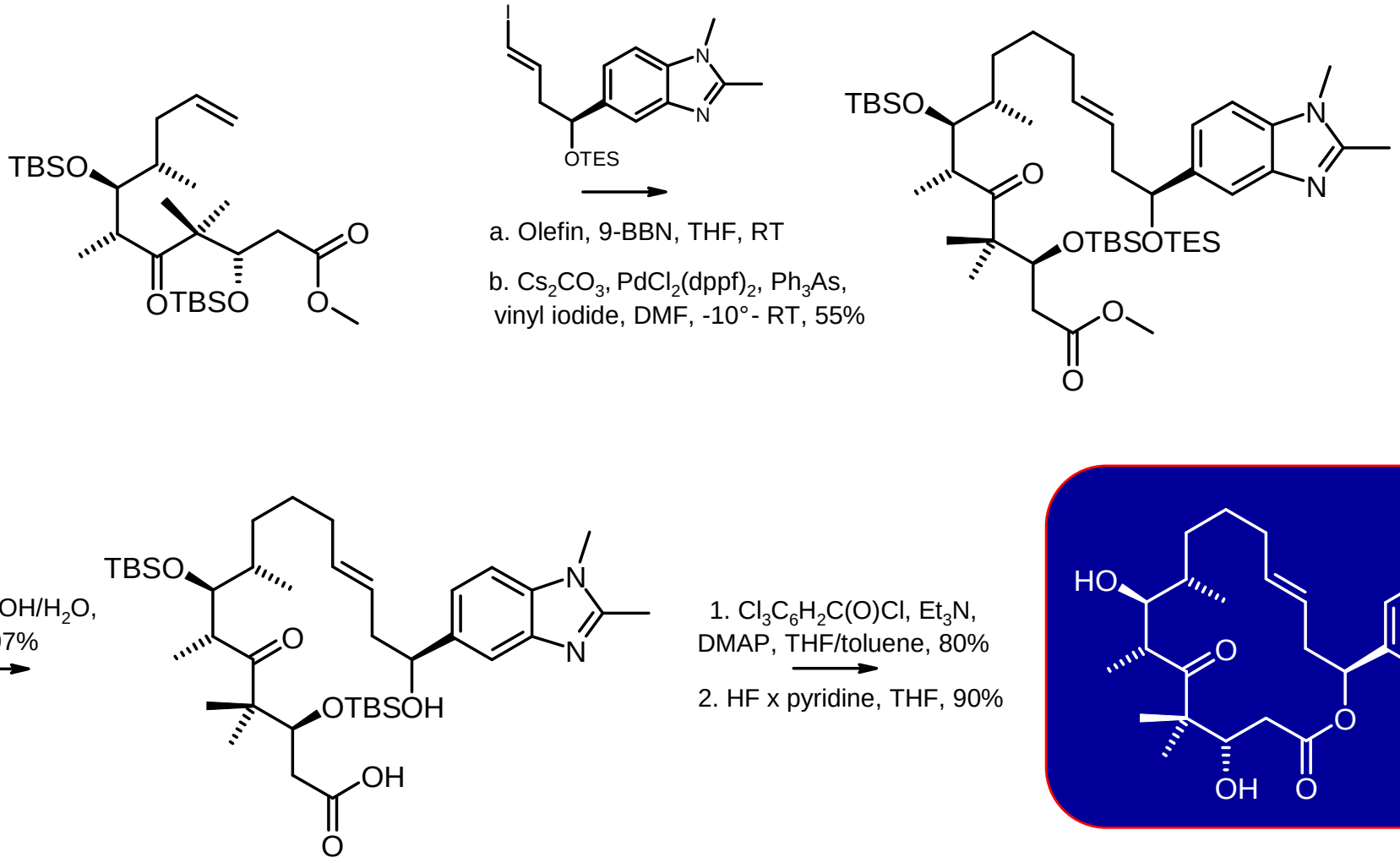




	EC_{50} Tubulinpol. [µM]	IC_{50} [nM] HCT-116	IC_{50} [nM] A549
X= S, Y = N	**1.02**	**1.22**	**1.00**
X = N, Y = S	**1.17**	**63.3**	**68.7**
X= CH, Y = N	**3.9**	**0.82**	**1.21**
X = N, Y = CH	**4.9**	**112**	**134**
X= CH, Y = N	**4.3**	**0.46**	**0.59**
X = N, Y = CH	**3.2**	**0.49**	**0.74**

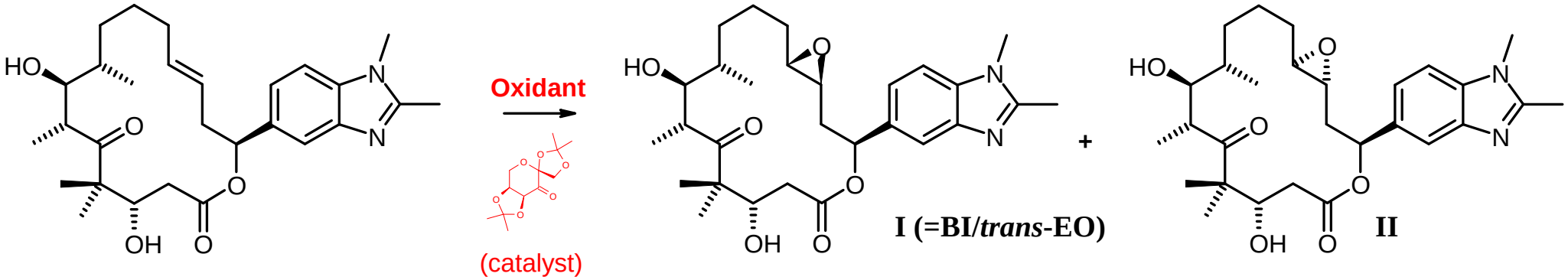
G. Bold, S. Wojeik, G. Caravatti, R. Lindauer, C. Stierlin, J. Gertsch, M. Wartmann, K.-H. Altmann, *ChemMedChem* **2006**, 1, 37-40.

Combining Backbone and Side Chain Modifications - Chemistry



F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann, *ChemBioChem* **2006**, *7*, 54-57.

Total Synthesis of Side-chain-modified trans-*Epothilone A*

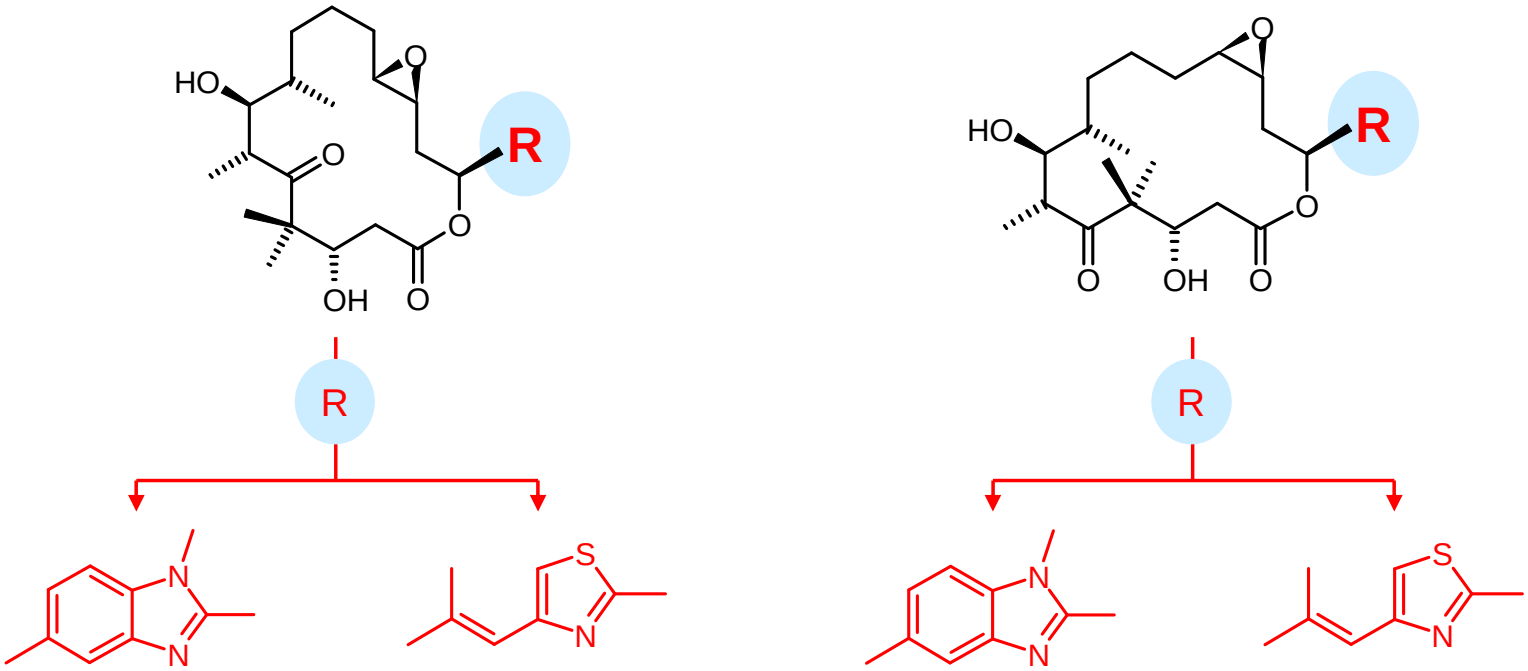


Oxidant	Conversion (HPLC %)	Ratio **I/II**	Yield (**I+II;** %)
$MeReO_3$, no catalyst	100	1/1	68
m-CPBA, no catalyst	50	1/1	30
Oxone®, 0.3 eq catalyst	50	8/1	25
Oxone®, 0.6 eq catalyst	90	8/1	68
***Oxone*®, 0.8 eq catalyst**	**100**	**8/1**	**70**

F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann, *ChemBioChem* **2006**, *7*, 54-57.

Benzimidazole-based Analogs of Epothilone A – Antiproliferative Activity

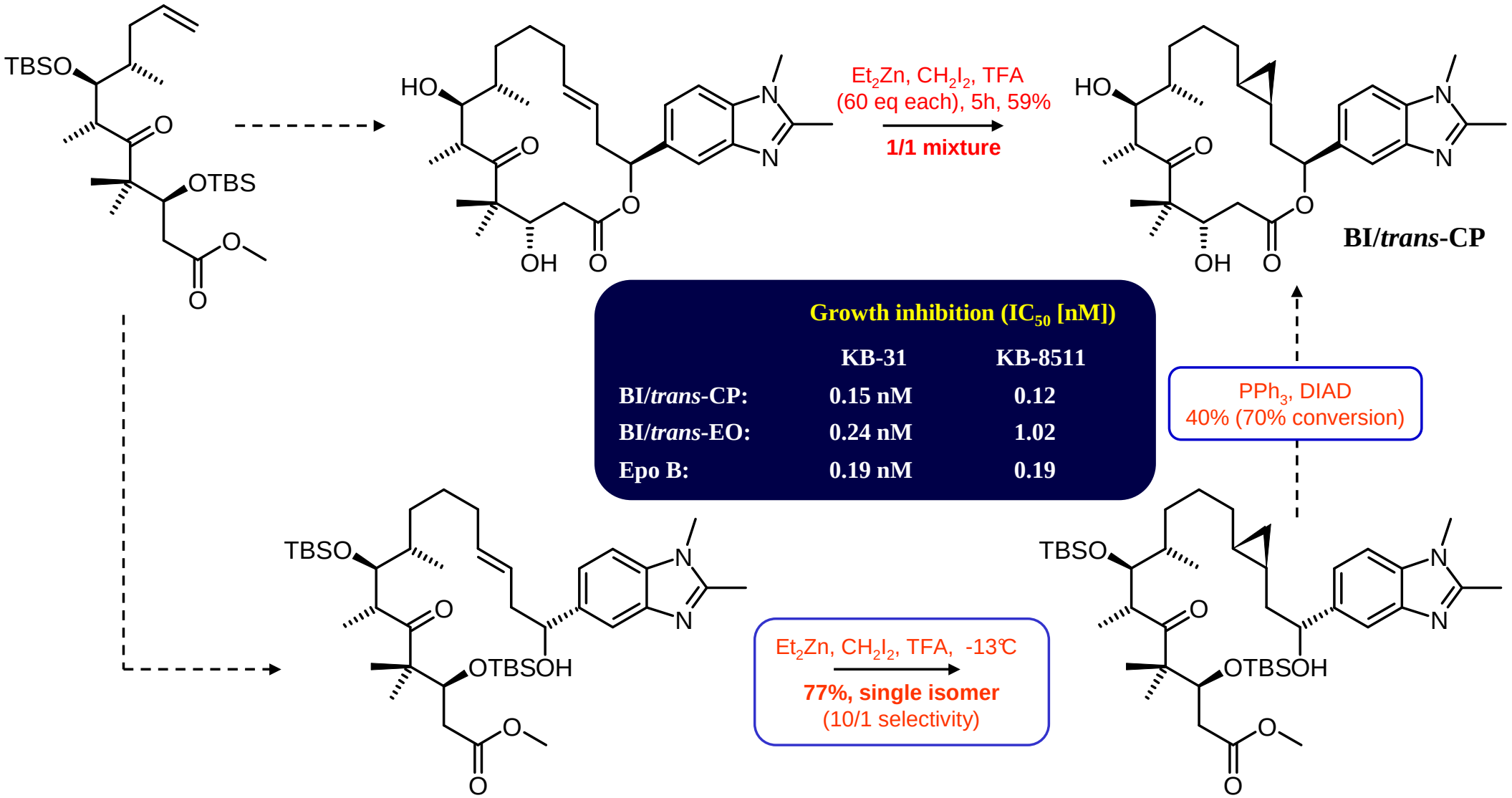
ETH



%Tubulin Polymerization.:	**70**	**85**	**73**	**65**
IC_{50} KB-31 [nM]:	**0.21**	**1.01**	**0.59**	**2.15**
IC_{50} KB-8511 [nM]:	**1.02**	**0.86**	**6.62**	**1.91**

F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann, *ChemBioChem* **2006**, *7*, 54-57.

Epoxide Replacement - Cyclopropane-based Analogs

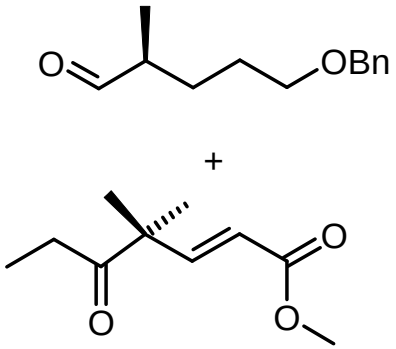


	Growth inhibition (IC_{50} [nM])	
	KB-31	KB-8511
BI/*trans*-CP:	0.15 nM	0.12
BI/*trans*-EO:	0.24 nM	1.02
Epo B:	0.19 nM	0.19

F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann, *Synlett* **2006**, 1384-1388.

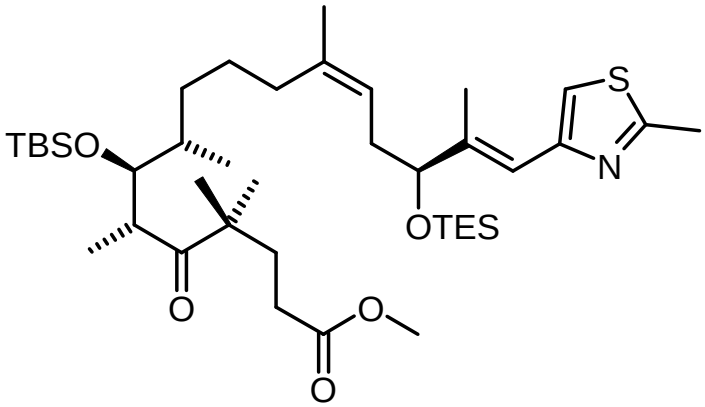
3-Deoxyepothilone B – Synthesis and Antiproliferative Activity

ETH



5 steps, 17%

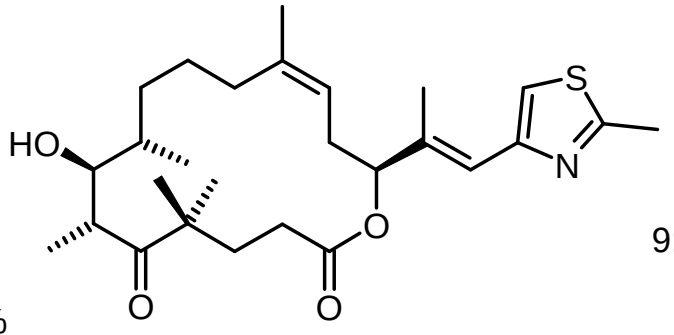
(Aldol selectivity *ca*. 2/1)



1. LiOH, i-PrOH/H_2O, 50°, 98%.

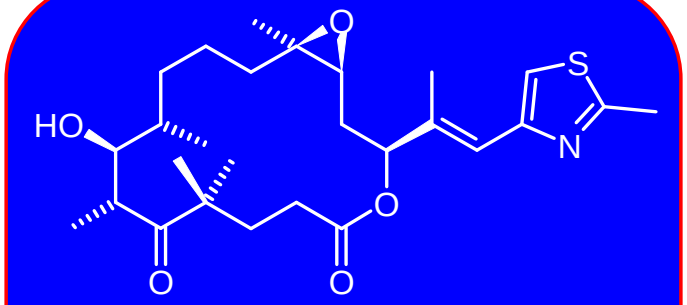
2. Macrolactonization, 93% (2/1 mixture of epimers at

3. HFxpyridine, THF, 80% (2/1 mixture)



$MeReO_3$, H_2O_2, CH_2Cl_2, 0°, 72%

9:1 (Epoxide isomers)



IC_{50} (KB-31): 5.12 nM

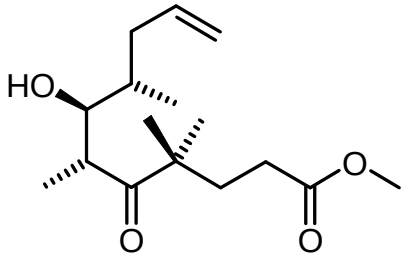
IC_{50} (KB-8511): 2.18 nM

Ca. **20-fold** activity loss *vs.* Epothilone B

F. Cachoux, et *al*., *Angew. Chem. Int. Ed.* **2005**, *44*, 7469-7473.

Combining Macrocycle and Side Chain Modifications – Towards new Scaffolds for Microtubule Inhibition

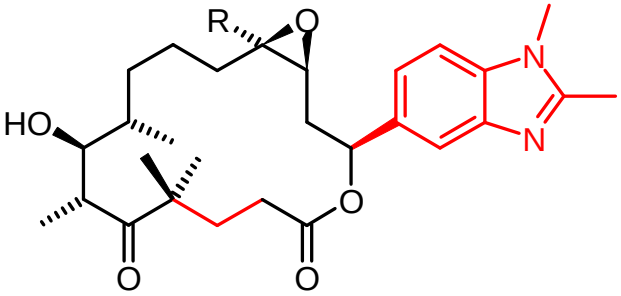
ETH

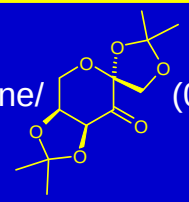


$MeReO_3$, H_2O_2, CH_2Cl_2, 0°

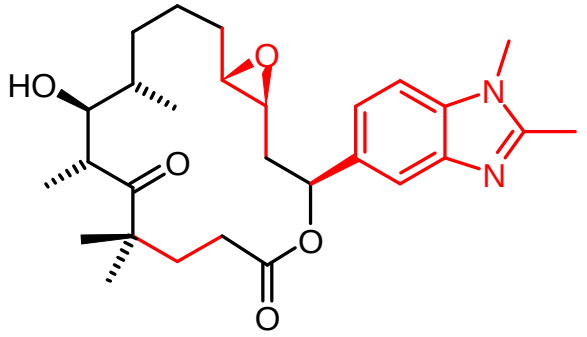
R = H: 2/1: 45%

R = CH_3: 10/1: 64%



Oxone/  (0.8 eq)

0 ℃, 3h, 65% (86%),
single isomer



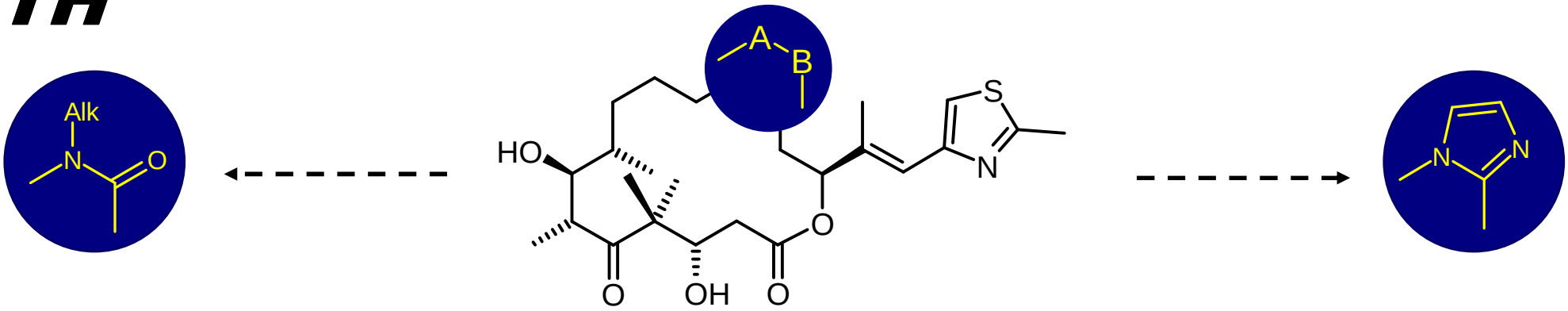
BI/*trans*-A

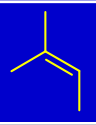
Growth inhibition (IC_{50} [nM])

	KB-31	KB-8511
BI/*cis*-A:	**7.40**	**37.6**
BI/*cis*-B:	**0.58**	**1.89**
BI/*trans*-A:	**3.16**	**7.60**
Epo A:	**2.00**	**1.79**

F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann *Angew. Chem. Int. Ed.* **2005**, *44*, 7469-7473.

N-Alkyl Amides and Imidazoles as Deoxyepothilone Mimetics

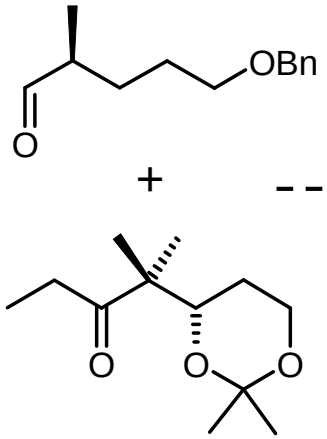


A–B	%Tubulin Polymerization	IC_{50} KB-31 [nM]	IC_{50} KB-8511 [nM]
	85	0.19	0.19
	93	2.70	1.44
	73	2.00	1.90
	50	25.0	9.90

For early work on deoxyepothilones *cf.*: K. C. Nicolaou et *al*., *Nature* **1997**, 387, 268; S. J. Danishefsky et *al*., *Angew. Chem. Int. Ed*. **1997**, *36*, 757.

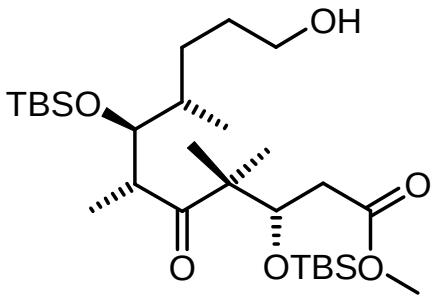
C12/C13-Amides and Other 12-Aza-Epothilones

ETH

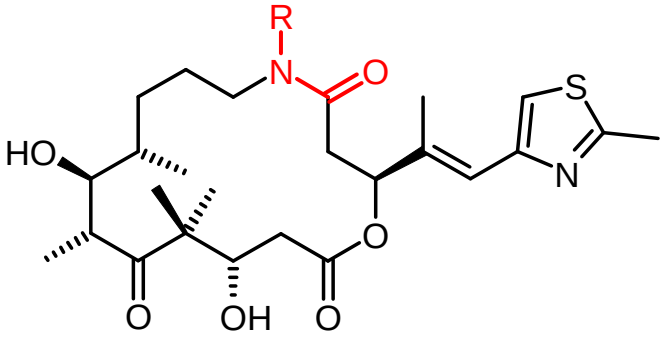


+

8 steps
32%

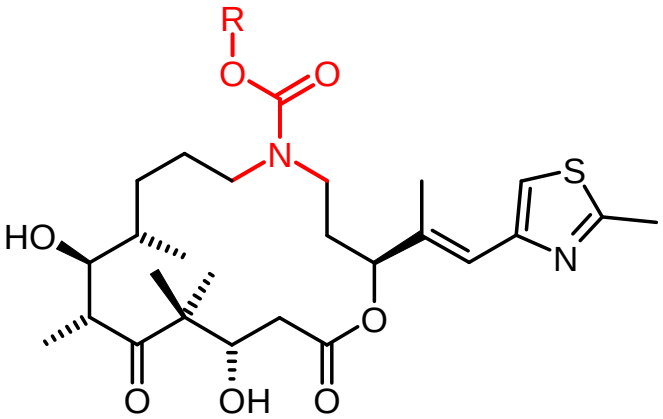


6 steps
~ 10%



%Tubulin Polymerization < 10

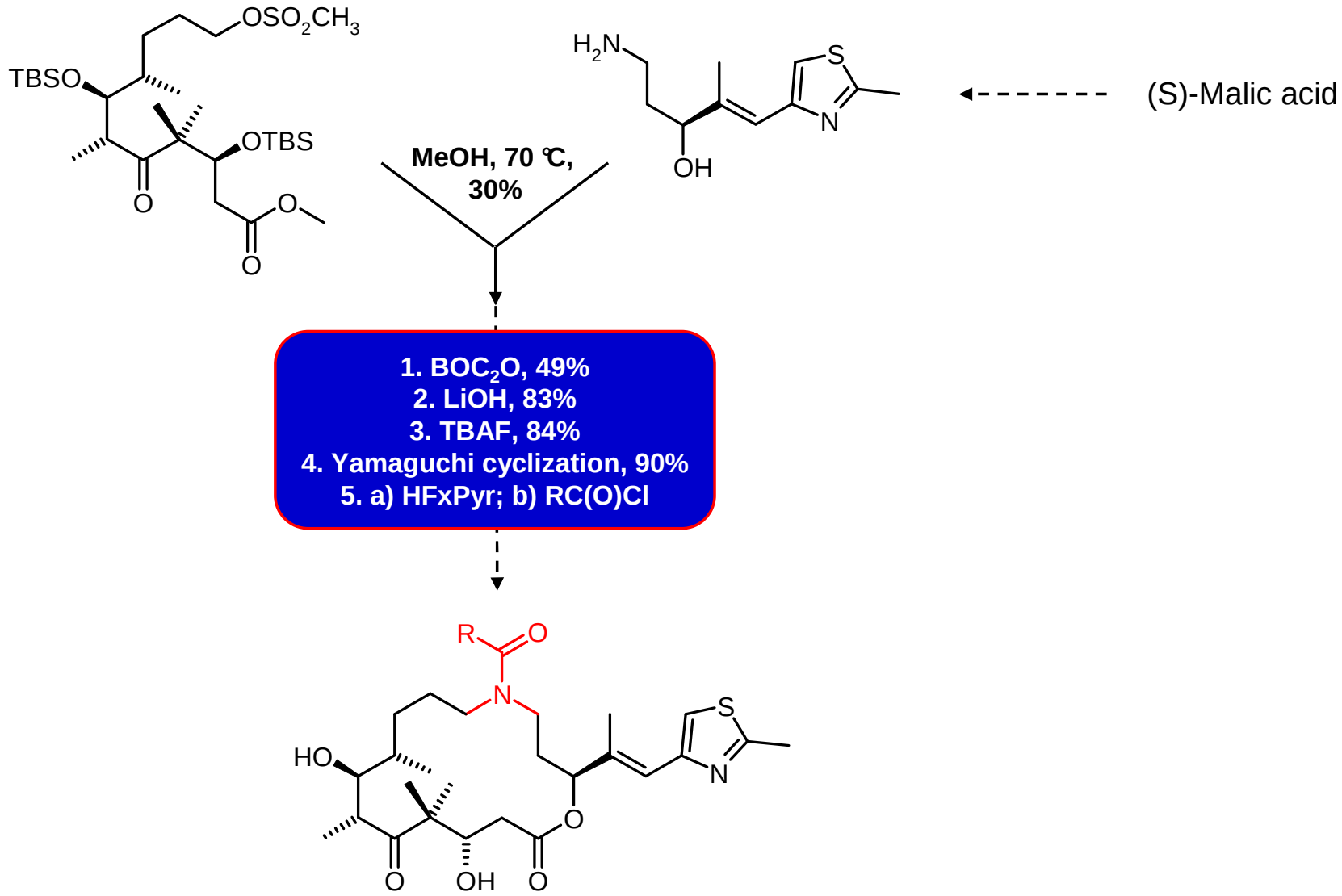
IC_{50} KB-31 > 1000 nM



IC_{50}s (KB-31)

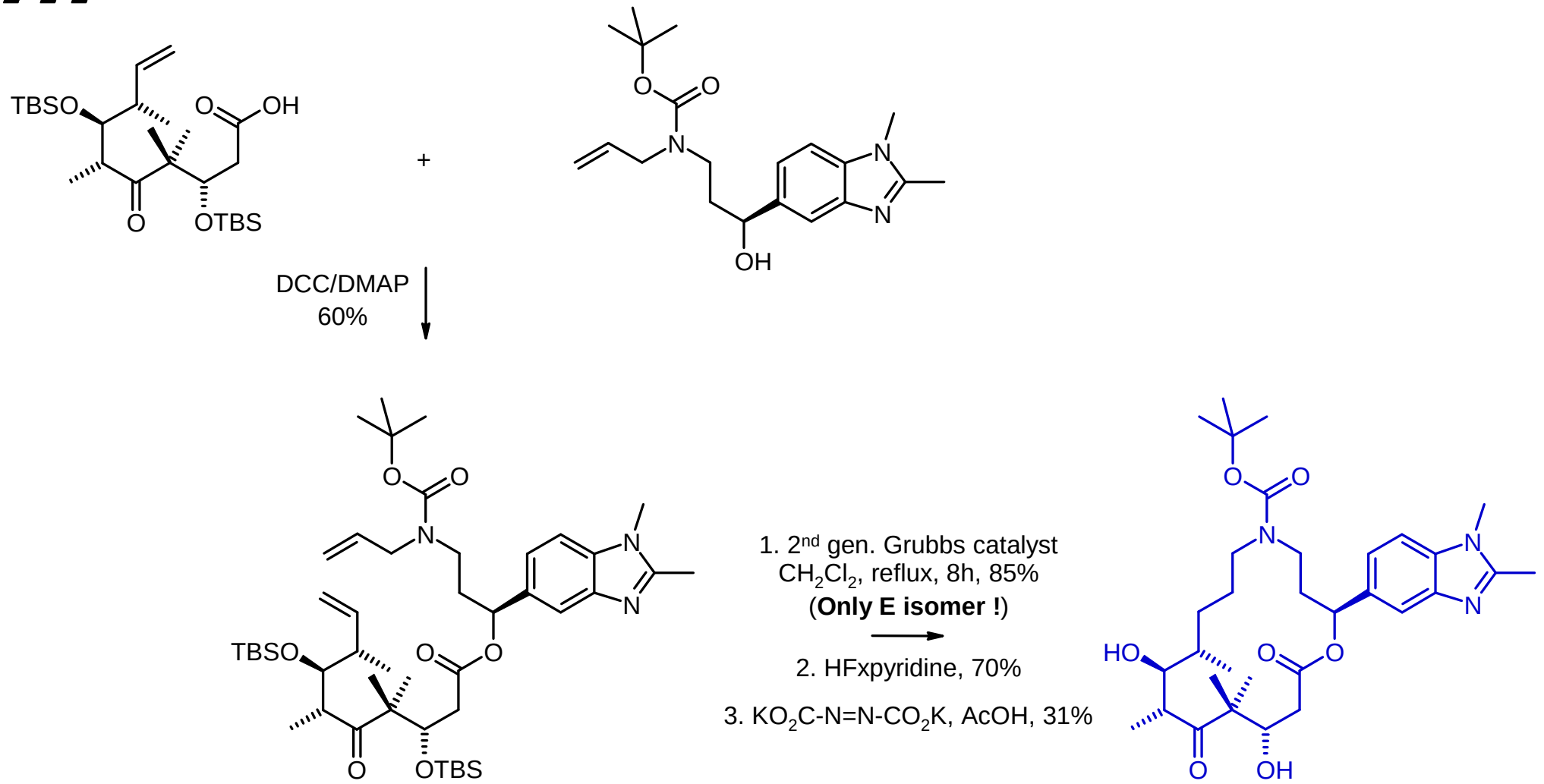
R = O-*tert.*-C_4H_9 :	31 nM
R = OC_2H_5 :	85 nM
R = OCH_2Ph :	297 nM
R = *tert.*-C_4H_9 :	206 nM
Epo A:	1.9 nM

Carbon Replacement in the Macrocycle - 12-Aza-Epothilones



12-Aza-Epothilones – A New Class of Potent Microtubule-Stabilizers

ETH



F. Feyen, J. Gertsch, M. Wartmann, K.-H. Altmann, *Angew. Chem. Int. Ed.* **2006**, *46*, in press.

EC_{50} (Tubulin): 3.9 µM (Epo A: 4.6 µM)

IC_{50} (KB-31): 0.33 nM (Epo A: 1.9 nM)