Progress Toward the Total Synthesis of Tubulysin D and its Analogues

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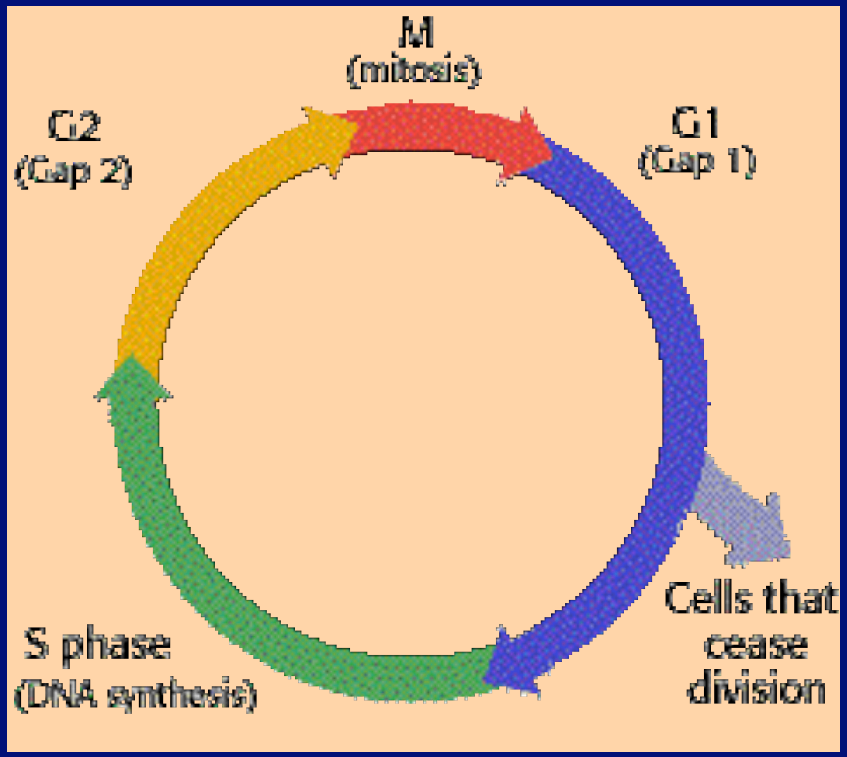
A General Look at the Cell Cycle

- Cell cycle is divided into four main parts
  - G_1 or Gap 1 phase
  - S or Synthesis phase
  - G_2 or Gap 2 period
  - M or Mitosis phase
- Cell cycle contains two major control points
  - G_1/S is the point at which cells commit to replicate genetic material, enter quiescence (G_0), or terminally differentiate and die
  - G_2/M is the point at which cells commit to division
  - Tubulin inhibitors typically cause treated cells to accumulate at the G_2/M checkpoint



http://www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells2.html

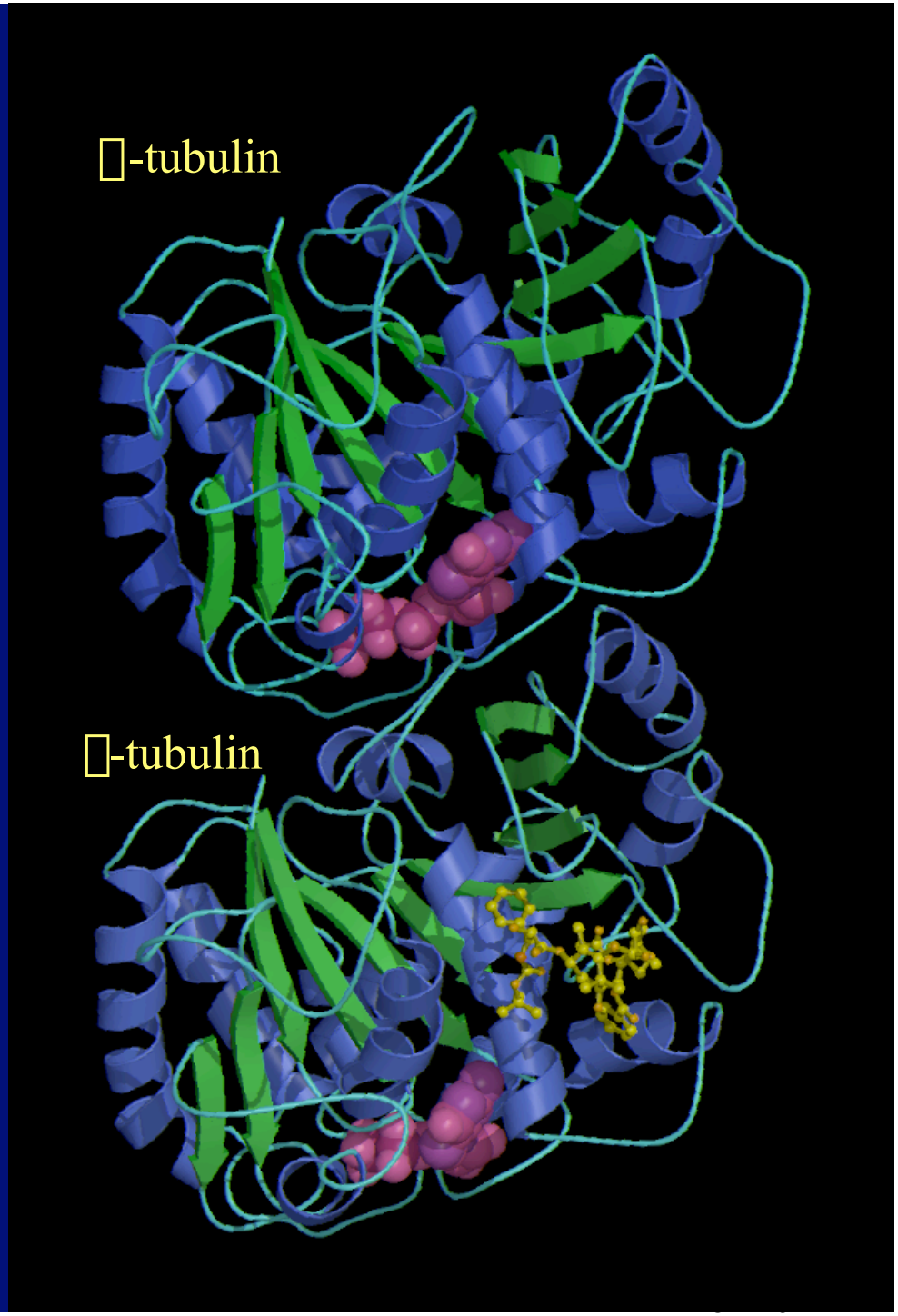
A Brief Introduction to Tubulin

- Tubulin exists as a heterodimeric structure of the □ and □ tubulin proteins.
  - tubulin proteins are highly conserved structures in eukaryotes
  - the □ and □-subunits are of similar secondary and tertiary structure
  - each subunit is ca. 55 kD in mass
  - the heterodimeric structure is tightly bound together and dissociates only under denaturing conditions

- Each tubulin monomer is capable of binding a molecule of GTP (guanosine triphosphate)
  - □-tubulin binds GTP and retains it in the heterodimer
  - □-tubulin binds a molecule of GTP and hydrolyses it to GDP during or shortly following the incorporation of the heterodimer into a protofilament

Structure of the □□-Tubulin Dimer from Electron Crystallography

- Purple subunits represent bound GTP (□-tubulin), and GDP (□-tubulin) each bound within a Rossman fold.
- Subtle differences between intradimeric and interdimeric binding contacts remain somewhat ambiguous.

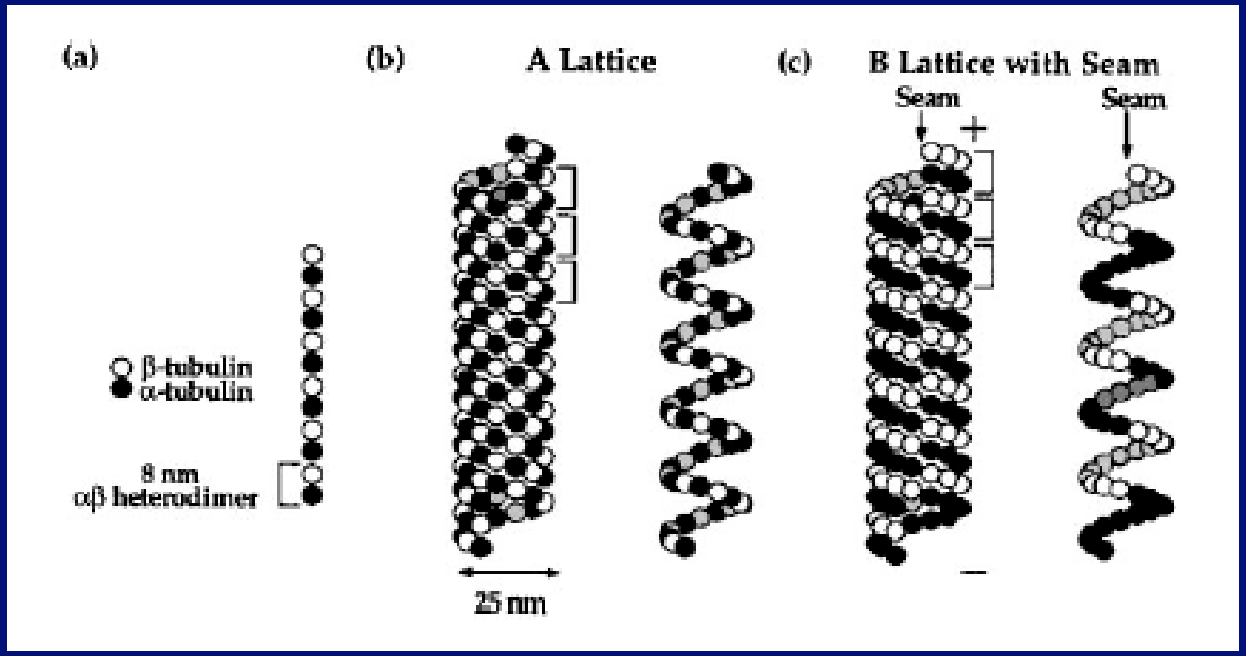


Nature **1998**, *391*, 199-203.

Introduction to Microtubules

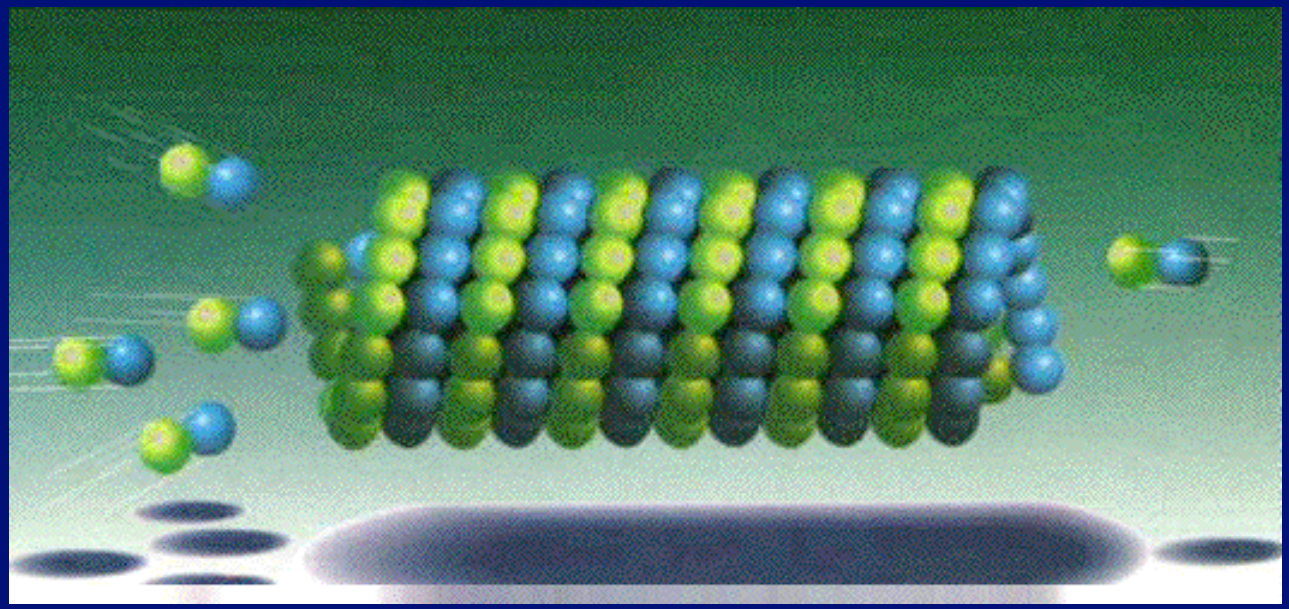
- Microtubules exist as helical entities consisting protofilaments
  - microtubules are “3-step” helices consisting of 13 protofilaments each
  - standard microtubules are 25 nm in diameter and vary in length depending on envionmental stimuli (dynamic instability)
  - protofilaments are polar entities consisting of a “+” (at □-tubulin) and “-” (at □-tubulin)
    - growth of the microtubule occurs at the “+” end
    - degredation of the microtubule occurs at “-” end

The Basics of Tubulin Polymerization



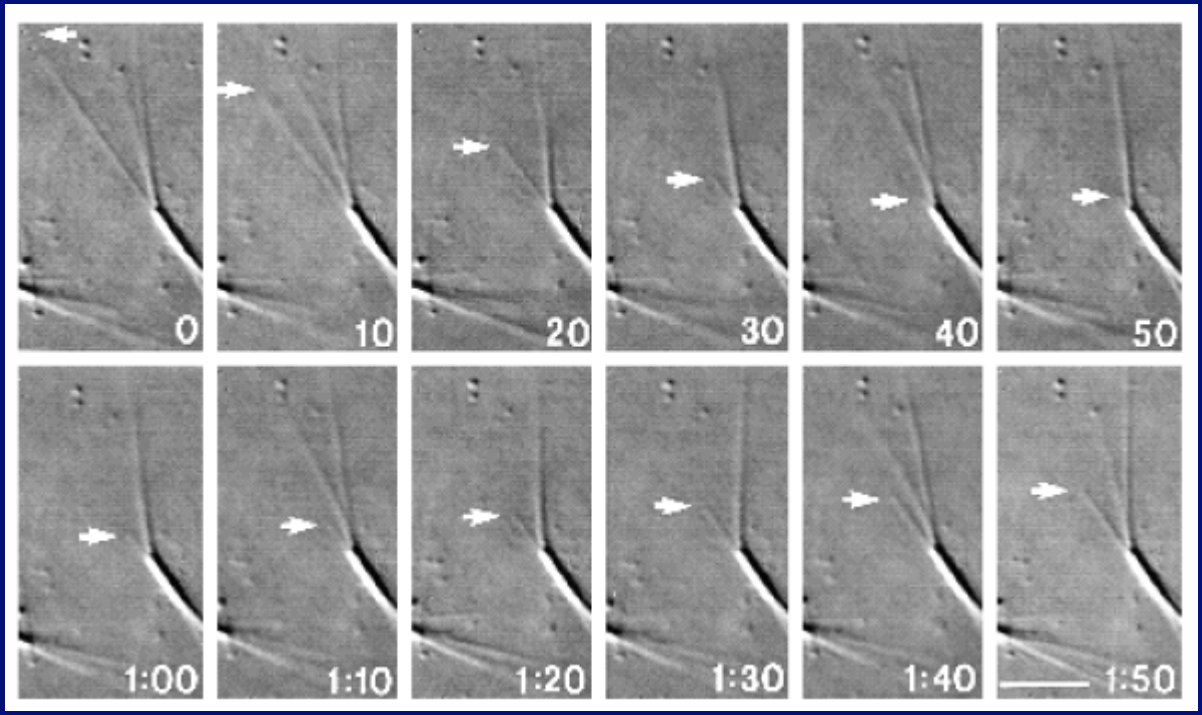
Annu. Rev. Cell Dev. Biol. **1997**, *13*, 83-117.

Dynamic Instability



http://www.ch.ic.ac.uk/local/projects/a_abowath/Tubulin.html

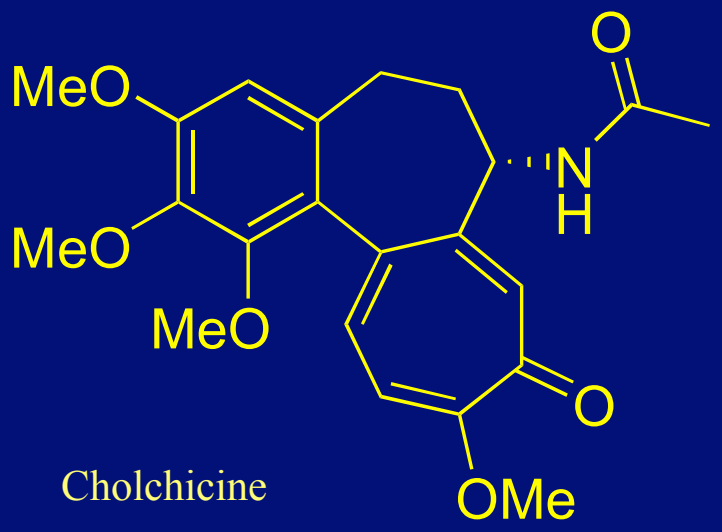
Microtubule Decomposition and Growth in Real Time



*J. Cell Sci.***1992**, *103*, 965-976.

A Survey of Tubulin Inhibitors – Colchicine Domain

- Alkaloid produced by the meadow saffron (*Colchicum autumnale)* .
- Inhibits microtubule-dependant cellular processes by strongly binding to □-tubulin, thus interfering with polymerization of tubulin protomers.
- Arrests mitotic cycle in plants and animals at metaphase.
- Commonly used in the treatment of Gout.
- The agent is not selectively toxic to cancer cell lines.

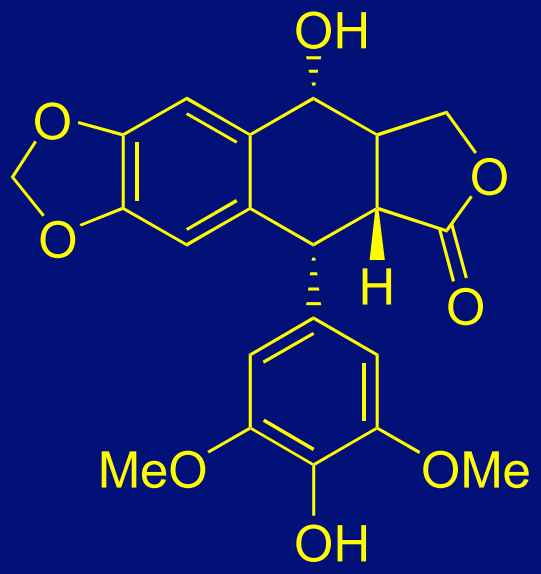


Cholchicine

(a) *Pharmac. Ther.,* **1991**, *51*, 377. (b) *Alkaloids* **1992**, *41*, 125. (c) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Colchicine Domain

- Obtained as an extract from the plant. *Podophyllum peltatum*
- Binding to tubulin is more rapid and reversible than colchicine derivitaves.
- Behaves as a competitive inhibitor of colchicine binding to tubulin.
- Inhibits tubulin dependent GTP hydrolysis (*vide supra*) thus suppressing the dynamic instability properties of microtubules.

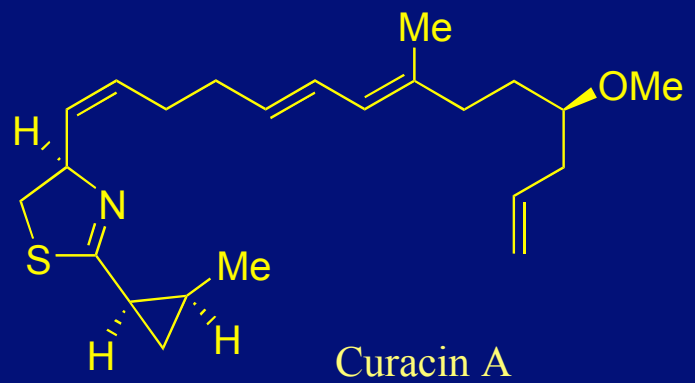


Podophillotoxin

(a) *Pharmac. Ther.*, **1993**, *59*, 163. (b) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Colchicine Domain

- Isolated from the marine cyanobacterium *Lynbya majuscula.*
- Inhibitor of mitosis, tubulin polymerization, and a competative inhibitor to colchicine binding.
- Interaction with tubulin characterized by rapid binding, slow dissociation, and induction of GTP hydrolysis uncoupled from normal tubulin assembly.
- Although an inhibitor of normal tubulin polymerization, treatment with curacin A often results in the formation of abnormal tubulin polymers.

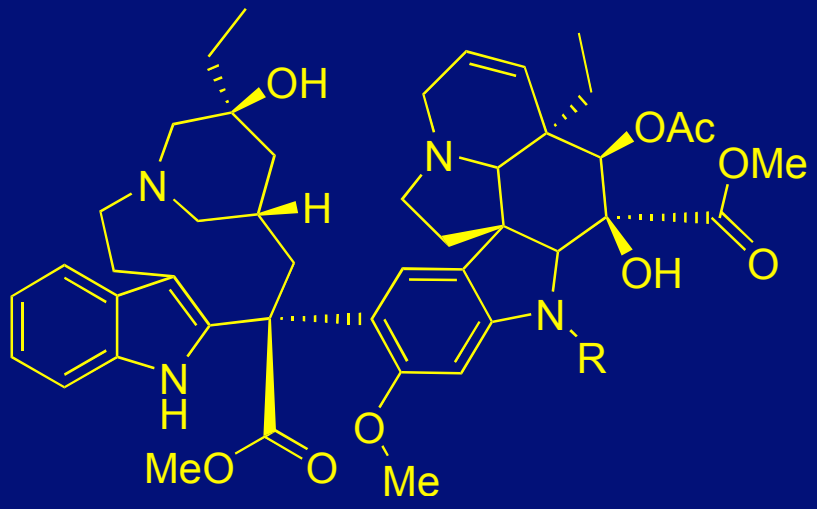


Curacin A

(a) *J. Org. Chem.* **1994**, *59*, 1243. (b) *Tetrahedron Lett.* **1995**, *34*, 110. (c) *Med. Res. Rev.* **1996**, *16*, 207-231. (d) *J. Org. Chem.* **1996**, *61*, 6556-6562. (e) *J. Med. Chem.* **2002**, *45*, 1901-1917.

A Survey of Tubulin Inhibitors – Vinca Site

- Isolated From the Madagascan periwinkle *Catharanus roseus* (formerly *Vinca rosea*).
- Inhibits of microtubule polymerization through binding to the vinca binding site on □- tubulin.
- Characteristically aberrant tubulin polymerization reactions result following *in vivo* treatment with *Vinca* alkaloids.
- The alkaloids strongly inhibit tubulin-dependent GTP hydrolysis, and weakly inhibits the binding of GTP and GDP at the exchangeable nucleotide site.
- Widely used in combination chemotherapy, often in conjunction with DNA damaging agents like bleomycin.



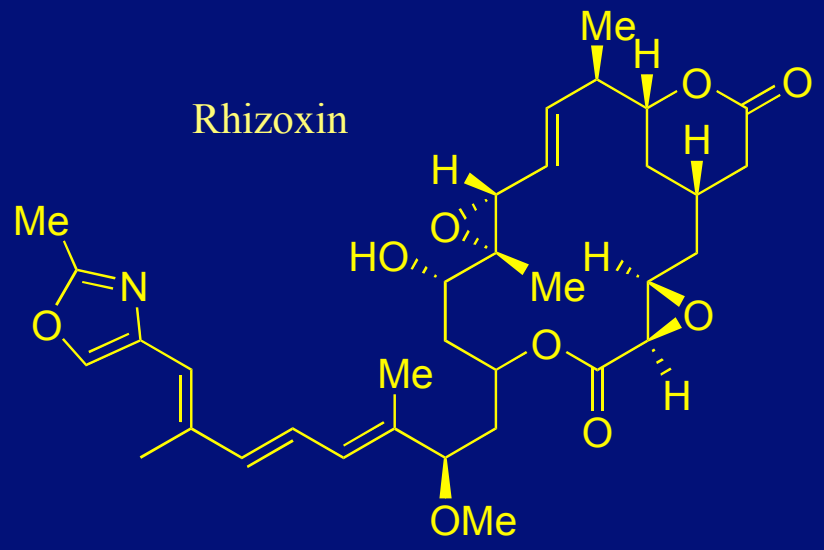
Vinblastine R = CH_3
Vincristine R = CHO

(a) *Pharmac. Ther.*, **1991**, *51*, 257. (b) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Vinca Site

- Isolated from fermentation cultures of the fungus *Rhizopus chinensis*.
- Reversibly binds to tubulin at 37 °C
- Agent demonstrates plant, fungal, and cellular toxicity.
- Under clinical evaluation for treatment in human cancers.
- Binding of radiolabled rhizoxane was competitively inhibited by vinblastine (K_i = 3 □M).
- Noncompetitive inhibition of Rhizoxin was observed with phomopsin A.

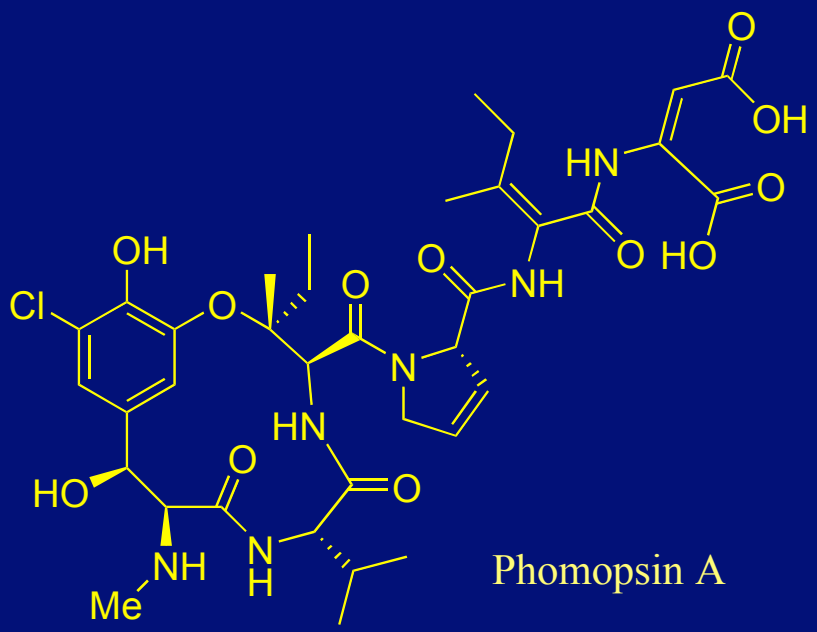
Rhizoxin



(a) *J. Antibiot.* **1984**, *37*, 354. (b) *Cancer Res.* **1992**, *52*, 2894. (c) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Vinca Domain

- A toxic secretion from the fungus *Phomopsis leptostomiformis.*
- Strong inhibitor of tubulin polymerization.
- The agent behaves as a noncompetitive inhibitor in the binding of [^{3}H] vincristine to tubulin (K_i = 2.8 □M), and as a competative inhibitor to dolostatin 10 / tubulin binding.
- Inhibits tubulin-dependent GTP hydrolysis, nucleotide exchange, and formation of the cys12 – cys 201/211 cross-link.
- Phomopsin strongly stabilizes tubulin conformation.



Phomopsin A

(a) *J. Chem. Soc. Chem. Commun.* **1983**, 1259. (b) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Vinca Domain

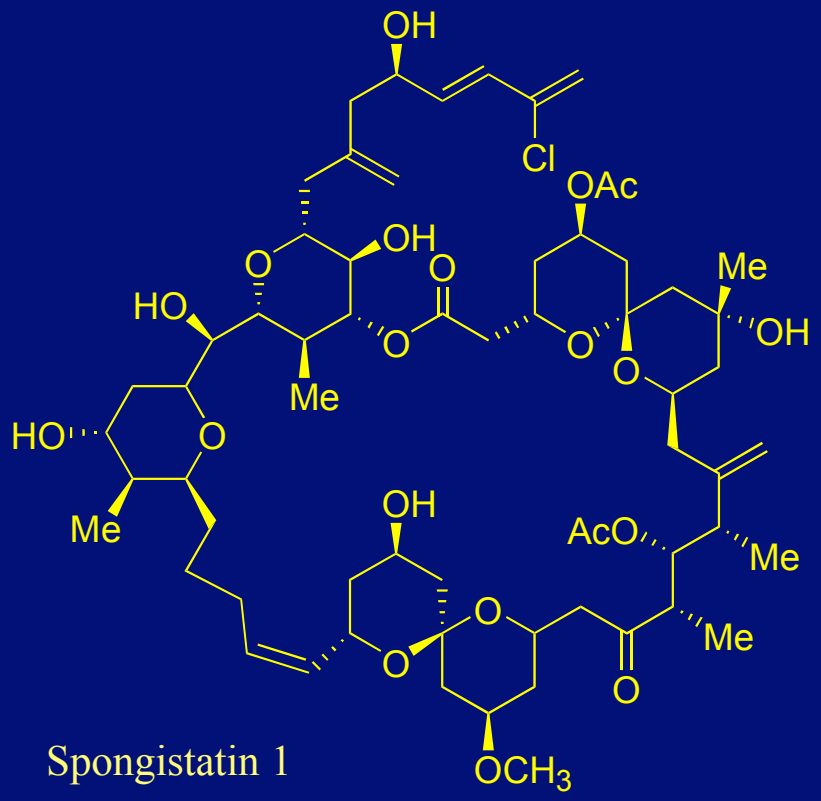
- An unusual peptide isolated from the shell-less mollusk *Dolabella auricularia*
- Agent causes mitotic arrest *in vivo*, with visible degredation of intracellular microtubules.
- The agent behaves as a noncompetitive inhibitor in the binding of [^{3}H] vincristine to tubulin (K_i = 1.4 □M), and as a competative inhibitor to radio labled phomopsin A– tubulin binding.
- Inhibits tubulin-dependent GTP hydrolysis, nucleotide exchange, and formation of the cys12 – cys 201/211 cross-link.

Dolostatin 10

(a) *Tetrahedron*, **1993**, 9151-9179. (b) *Med. Res. Rev.* **1996**, *16*, 207-231.

A Survey of Tubulin Inhibitors – Vinca Domain

- Isolated from the marine sponge *Spirastrella spinispirulifera* and *Hyrtios altum*..
- Highly toxic to a variety of human cancer lines in culture. (IC_{50} = 20 pM for L1210 murine leukemia cells).
- Noncompetitively inhibits the binding of both dolostatin 10 and vinblastine to tubulin.
- The agent is a strong inhibitor of GDP nucleotide exchange.

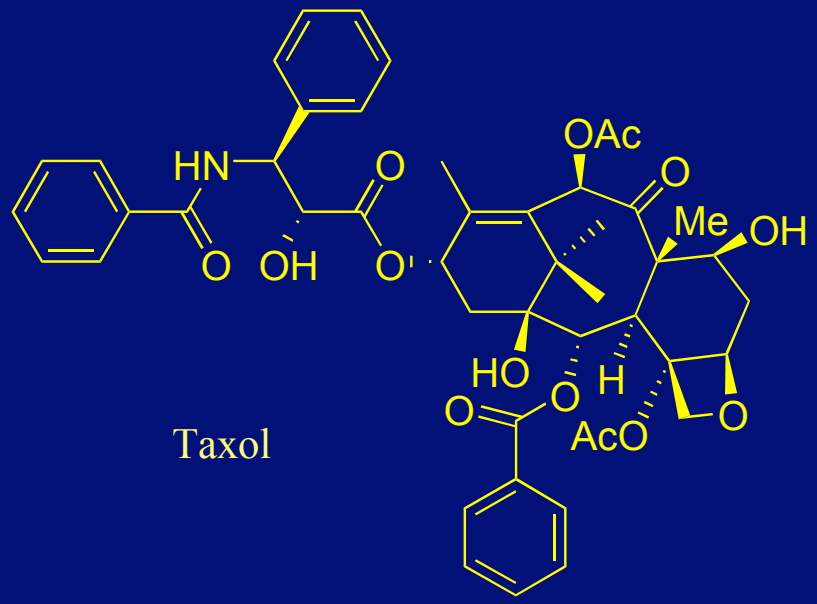


Spongistatin 1

(a) *J. Org. Chem.* **1993**, *58*, 1302. (b) *Tetrahedron Lett.* **1993**, *34*, 2795. (c) *Mol. Pharmacol.* **1993**, *44*, 757. (d) *Med. Res. Rev.* **1996**, *16*, 207-231. (e) *Angew. Chem. Int. Ed.* **1998**, *37*, 187-192. (f) *Angew. Chem. Int. Ed.* **1998**, *37*, 192-196.

Microtubule Stabilizing Agents – Taxol

- Originally isolated from the plant *Taxus brevifolia as a cytotoxic agent.*
- The agent was the first to derive its mechanism of action from the *stabilization* of microtubules rather than by the inhibition of their synthesis.
- Binding of Taxol occurs only with the tubulin polymer, and in a roughly 1:1 stoichiometry.
- Taxol binds to the polymer in a reversible fashion with a K_D = 1 □M.



Taxol

(a) *J. Am. Chem. Soc.,* **1971**, *93*, 2325. (b) *Nature*, **1979**, *277*, 665-667. (c) *J. Biol. Chem.* **1988**, *263,* 1342-1346. (d) *J. Med. Chem.* **1993**, *36*, 1918-1922. (e) *Med. Res. Rev.* **1996**, *16*, 207-231. (f) Khalil, M.-W. M. *Dissertation* **1999,** Technical University of Braunschweig.

Microtubule Stabilizing Agents – Epothilones

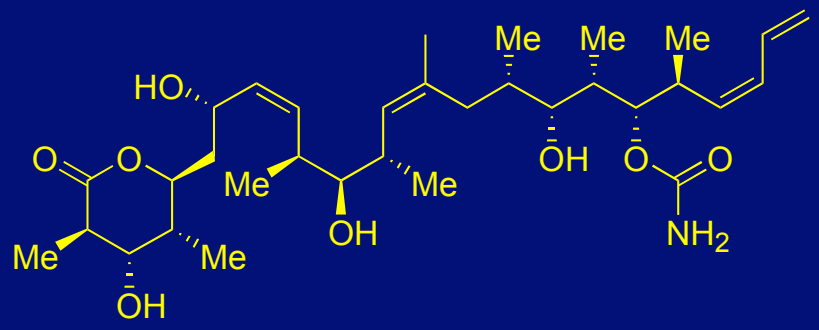
- Isolated from cultures of the myxobacterium *Sorangium cellulosum*.
- Epothilones were demonstrated to induce tubulin polymerization *in vitro*, in the absence of added GTP, and in a concentration dependent manner.
- The epothilones inhibited the binding of radiolabled paclitaxel to microtubules.

Epothilone A R = H
Epothilone B R = Me

(a) Höfle, G.; Bedorf, N.; Gerth, K.; Reichenbach, G. H. **1993**, (GBF) DE-4138042; *Chem. Abstr.* **1993**, *120*, 52841. (b) *Cancer Res.* **1995**, *55*, 2325-2333. (c) *Angew. Chem. Int. Ed.* **1996**, *35*, 1567-1569. (d) *J. Antibiot.* **1996**, *49*, 560-563. (e) *Med. Res. Rev.* **1996**, *16*, 207-231.

Microtubule Stabilizing Agents – Discodermolide

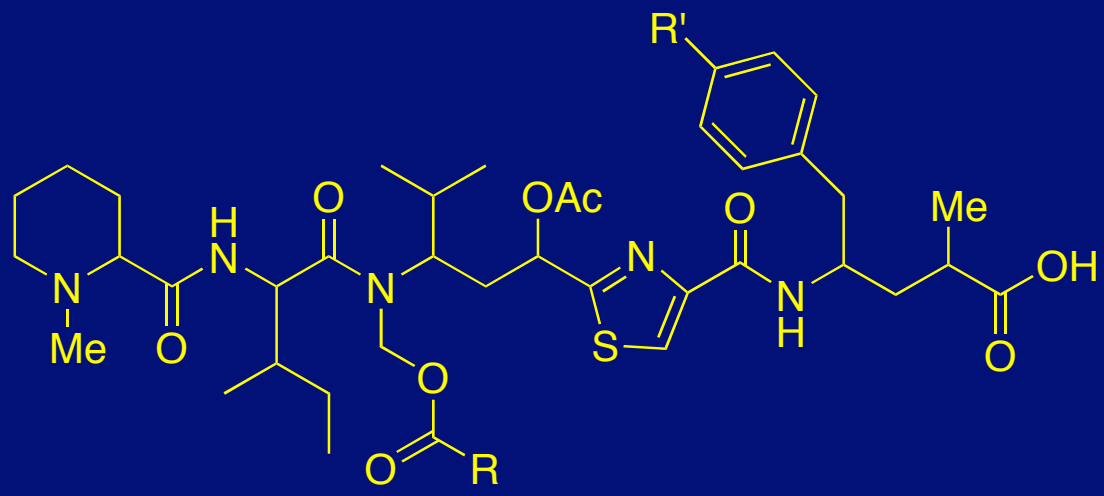
- Isolated from the marine sponge *Discodermia dissoluta.*
- Arrests the cell cycle of treated cells in G_2/M phase.
- The agent was more potent in microtubule stabilization than Taxol under a variety of reaction conditions.
- Discodermolide stabilized microtubules were morphologically distinct (shorter) from those resulting from their Taxol stabilized counterparts.



Discodermolide

(a) *J. Org. Chem.* **1990**, *55*, 4912-4915. (b) *J. Org. Chem.* **1991**, *56*, 1346. (c) *Chem. Biol.* **1994**, *1*, 67-71. (d) *Med. Res. Rev.* **1996**, *16*, 207-231. (e) *Biochemistry*, **1996**, *35*, 243-250.

Tubulysins: Potent Antimitotic Agents from *Archangium gephyra,* and *Angiococcus disciformis*



Tubulysin A	R = $CH_2CH(CH_3)_2$	R' = OH
Tubulysin B	R = $CH(CH_3)_2$	R' = OH
Tubulysin C	R = CH_2CH_3	R' = OH
Tubulysin D	R = $CH_2CH(CH_3)_2$	R' = H
Tubulysin E	R = CH_2CH_3)	R' = H
Tubulysin F	R = CH_2CH_3	R' = H

Sesse, F.; Steinmetz, J.; Heil, J.; Höfle, G. *J. Antibiot.* **2000**, *53*, 879-885.

Cytotoxicities of Various Selected Tubulin Inhibitors

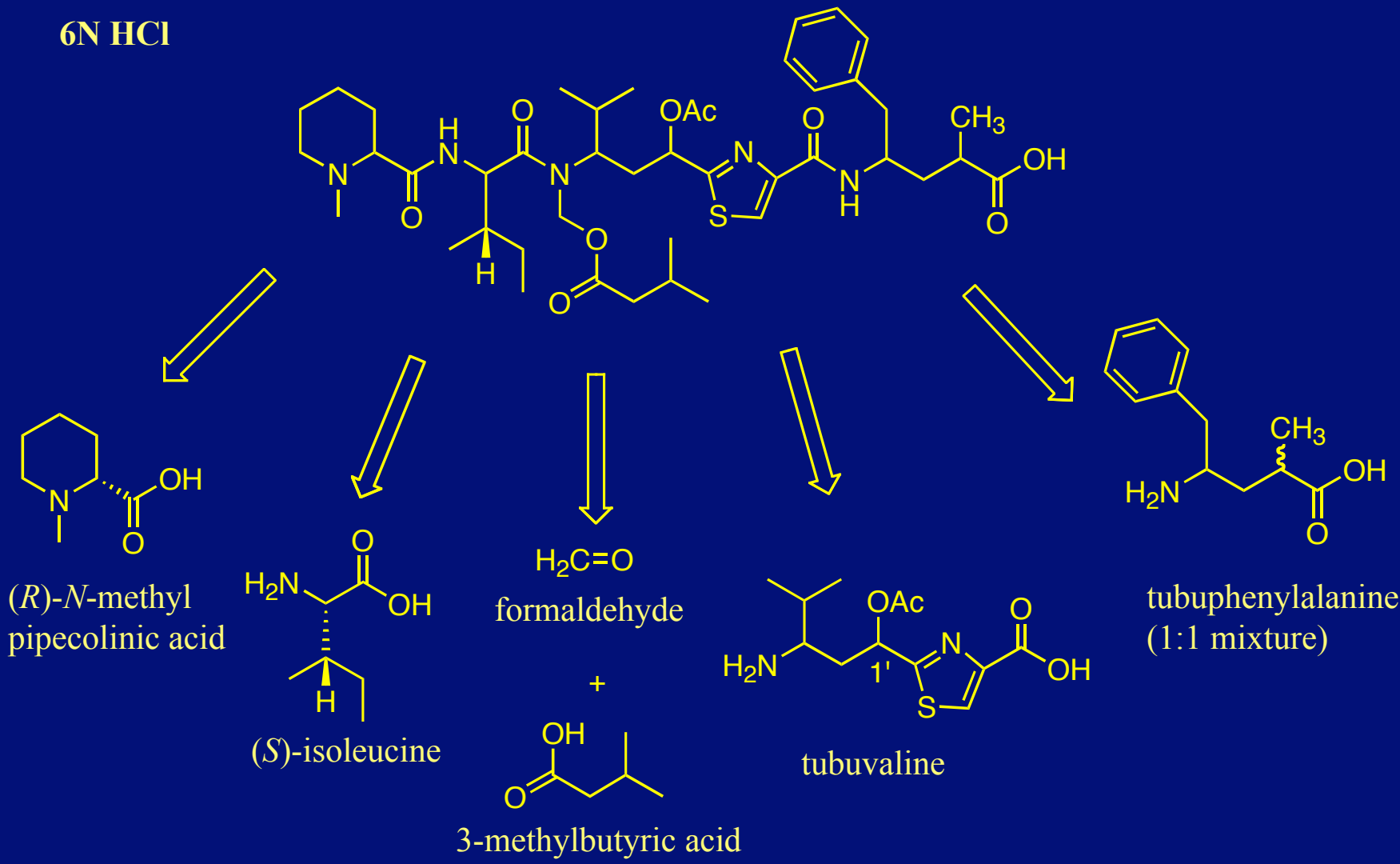
Cytotoxic activity of tubulin inhibitors for sensitive and resistant cell lines (IC_{50} ng/mL)

Compound	Cell Line			
	L929[a]	K562[b]	KB–3.1[c]	KB–VI[d]
Taxol	80	10	10	150
Vinblastine	15	6	7	120
Epothilone B	0.7	0.3	0.6	0.3
Dolostatin 10	0.1	0.1	0.2	1.2
Tubulysin A	0.2	0.07	0.2	0.4
Tubulysin B	0.4	0.2	0.3	1.0
Tubulysin D	0.03	0.02	0.2	0.08
Tubulysin E	0.1	0.05	0.03	0.1

[a] Fibroblast cell line from connective tissue of a mouse'
[b] Human myelogenous leukemia cell line.
[c] Human cervix carcinoma cell line.
[d] Multidrug-resistant KB clone.

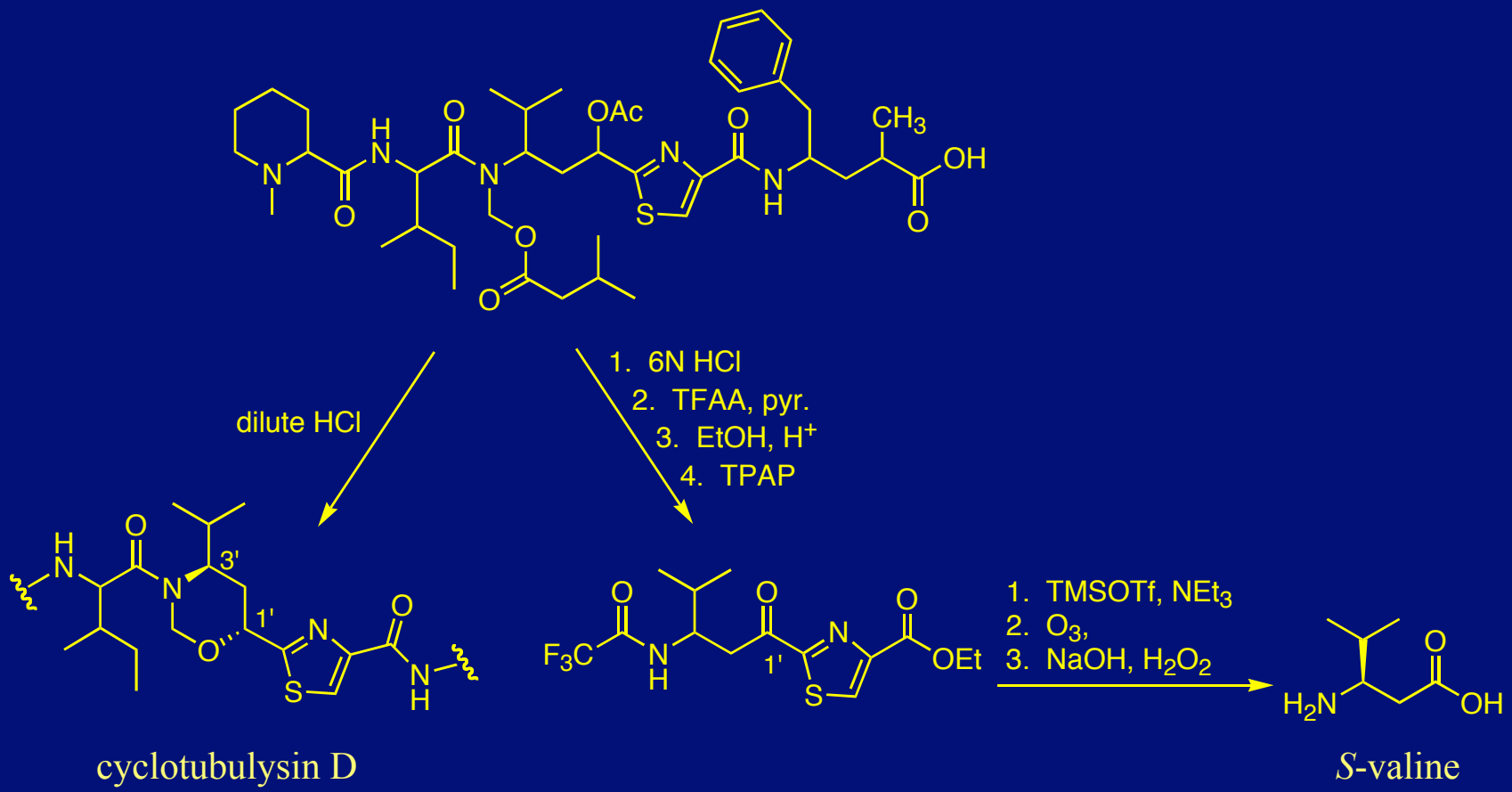
Pure Appl. Chem. **2003**, *75*, 167-178.

Degradation Studies: Total Acidic Hydrolysis of Tubulysin D



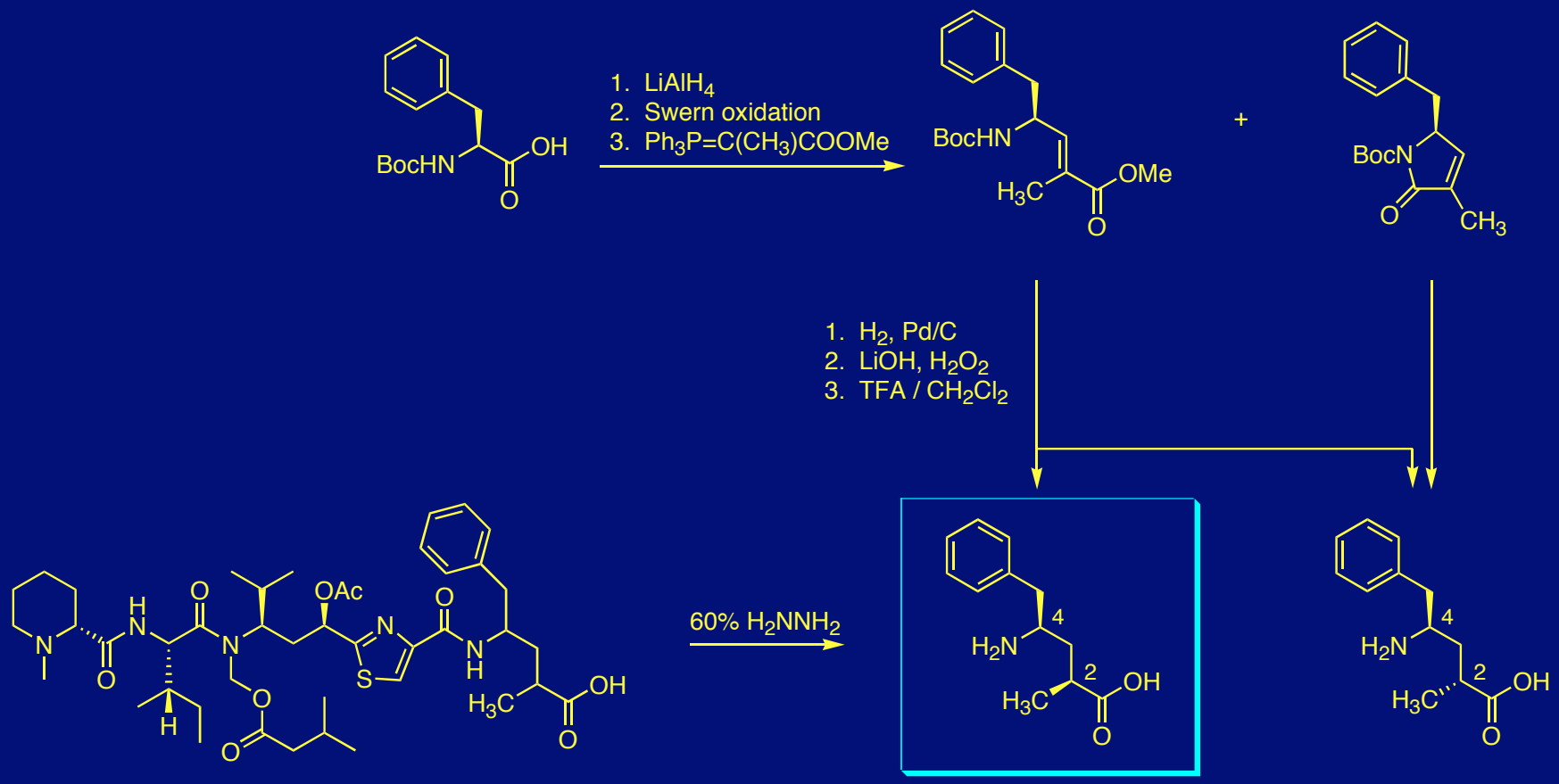
Pure Appl. Chem. **2003**, *75*, 167-178

Determination of the Absolute Stereochemistry of the Tubuvaline Subunit



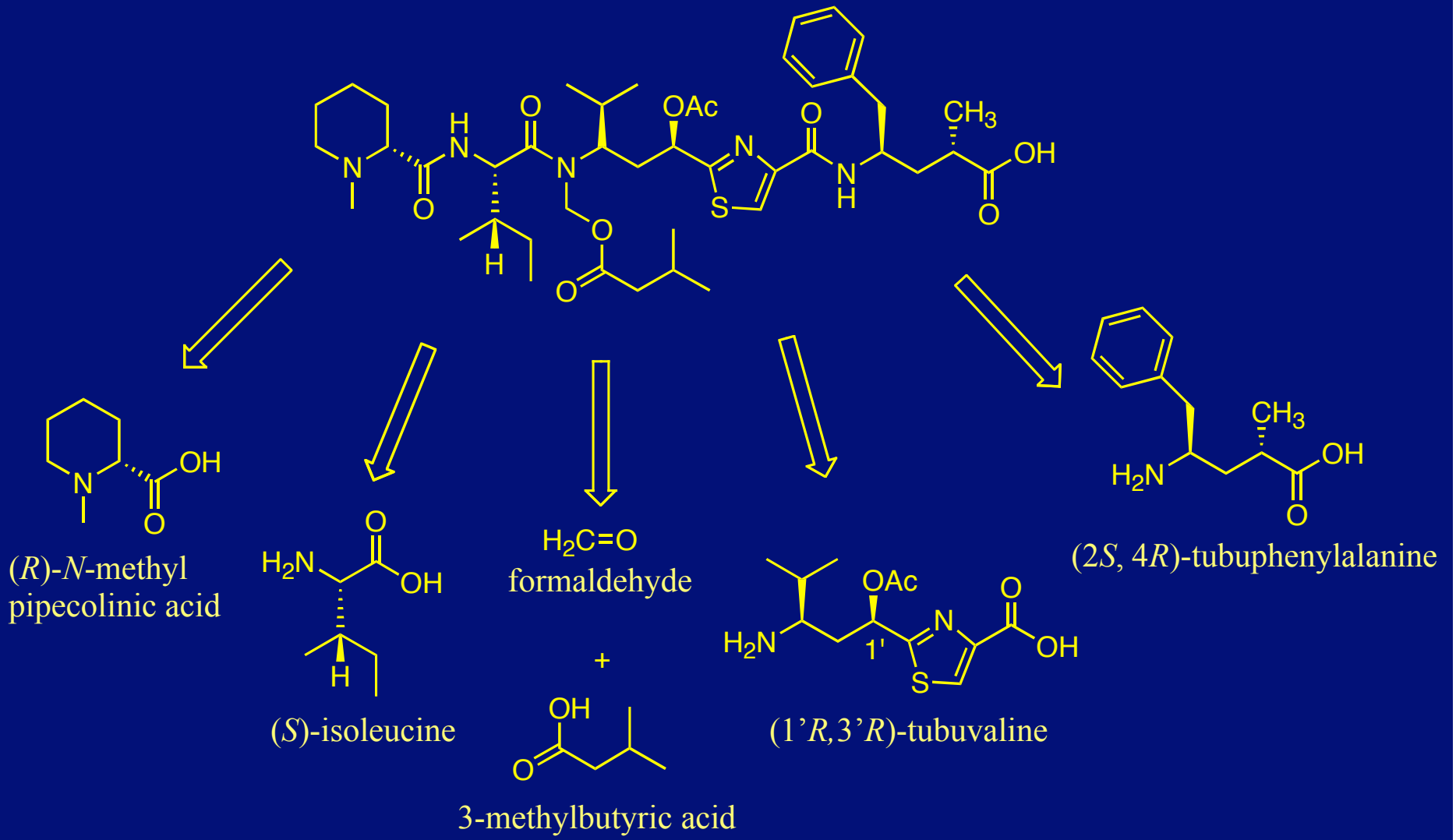
Pure Appl. Chem. **2003**, *75*, 167-178

Determination of the Absolute Stereochemistry of the Tubuphenylalanine Subunit



Pure Appl. Chem. **2003**, *75*, 167-178

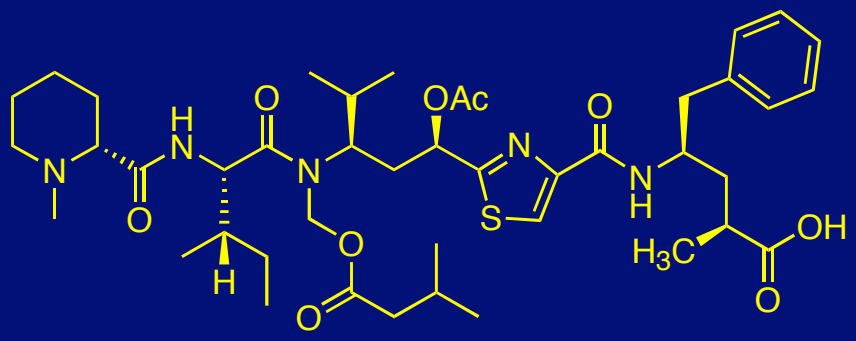
The Absolute Stereochemistry of the Tubulysins



Pure Appl. Chem. **2003**, *75*, 167-178

Dolostatin 10 and Tubulysin D: An Interesting Comparison

Dolostatin 10



Tubulysin D

(a) *Pure Appl. Chem.* **2003**, *75*, 167-178. (b) *J. Nat. Prod.* **2001**, *64*, 907-910.