Development of Taxol® as an Anticancer Drug

Symposium on "Natural Products in Anticancer Lead Discovery" in honor of Richard G. Powell (Sponsored by the *Journal of Natural Products*)

American Society of Pharmacognosy Annual Meeting

Copper Mountain, Colorado, July 27, 2015

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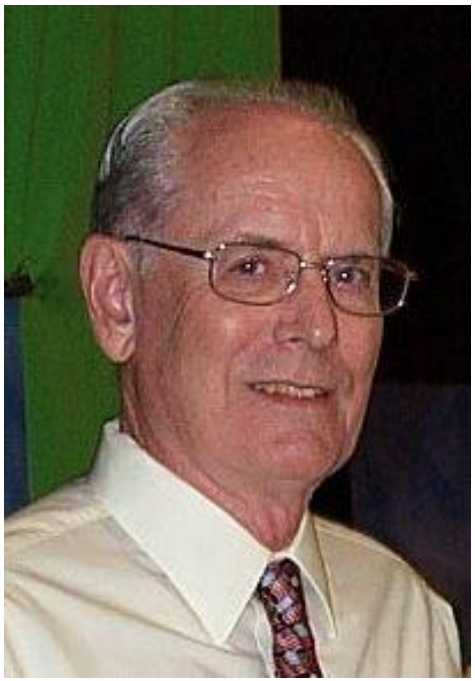

Richard G. Powell

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Outline

- History of the Development of Taxol
- The Chemistry of Taxol
- The Tubulin-Binding Structure of Taxol
- Acknowledgments

Taxol Discovery 1963-1971

1962 First collection of *Taxus brevifolia* by USDA botanists. Detection of activity in the bark of *Taxus brevifolia* Junod, *Life* , **1992**, *68*, 71

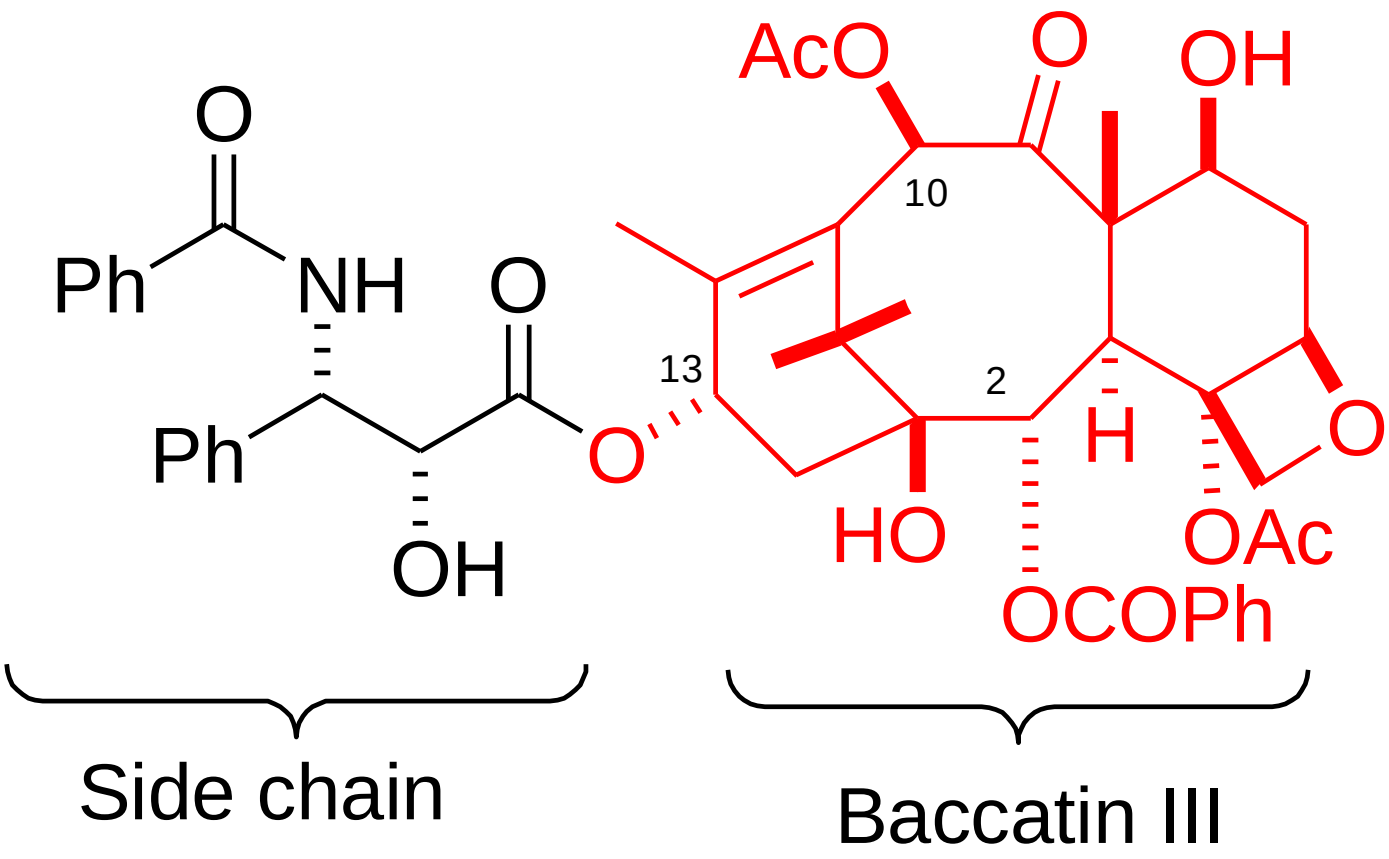
1963 Confirmed KB activity

1964 Recollection of bark and assignment to Dr. Monroe Wall, RTI

1969 Taxol first isolated in pure form (0.01% yield from bark)

1971 Isolation and structure first reported: Wall & Wani, *J. Am. Chem. Soc.*, **1971**, *93*, 2325.

Taxol Structure



Taxol Structure

Taxol Discovery 1963-1971

1971 Initial reaction to the discovery of taxol:
Underwhelming enthusiasm!!

Because of three problems:

A. The supply problem (0.02% from *T. brevifolia* bark)
B. The solubility problem (not water-soluble)
C. Its *in vivo* activity was mainly in mouse leukemia models, and it was not considered any better than many other leads.

Taxol Preclinical Development 1971-1977

1971-74 Isolation of additional quantities of taxol for *in vivo* testing.

1974 Discovery of taxol's activity in several new assays, including human tumor xenografts in nude mice. Good *in vivo* activity against B16 mouse melanoma

1975 B16 activity confirmed. Increased interest

1977 Decision by the National Cancer Institute to begin preclinical development of taxol

1977 Strong activity observed against human solid tumors, the MX-1 mammary and CX-1 colon xenograft models

Taxol Development 1977-1989

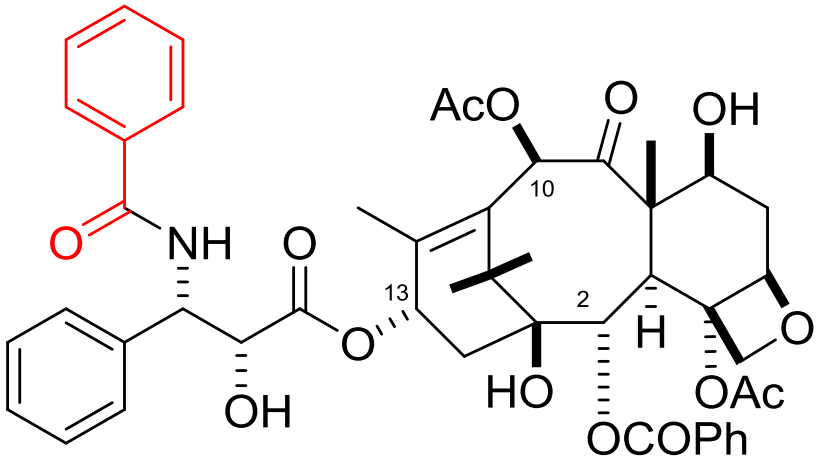
1978 Recognized as a mitotic spindle poison (Johnson et al.)

1977-83 Preclinical development; formulation, toxicology, etc.

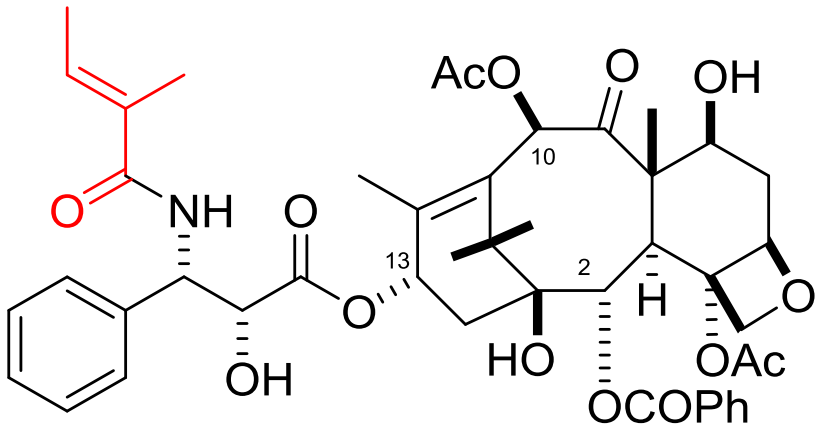
1979 Discovery of its mechanism of action by Susan Horwitz

1979 Discovery of cephalomannine by Powell et al.

Taxol Development 1977-1989



Taxol



Cephalomannine

- Has an N-tigloyl group instead of the N-benzoyl group of taxol
- Isolated from a plant initially identified as *Cephalotaxus mannii*, but later re-identified as *Taxus wallichiana* Zucc.

Powell, R. G.; Miller, R. W.; Smith, C. R., Jr. Cephalomannine; a New Antitumour Alkaloid from *Cephalotaxus mannii. J. Chem. Soc. Chem. Commun.* **1979,** *102-104.*
Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. Antileukemic Alkaloids from *Taxus wallichiana Zucc. J. Org. Chem.* **1981,** *46,* 1469-1474.
Miller, R. W.; Powell, R. G.; Smith, J. Cephalomannine and its use in Treating Leukemic Tumors. US Patent 4,206,221, June 3, 1980.

Taxol Development 1977-1989

1882 Approved for INDA filing with FDA

1984 Phase I clinical trials. Allergic reactions almost halt trials: *J. Clin. Oncol.* **1990**, 1263.

1985 Phase II clinical trials initiated

1989 First published report of clinical activity of taxol in ovarian cancer: McGuire et al. *Ann. Intern. Med.* **1989**, *111*, 273

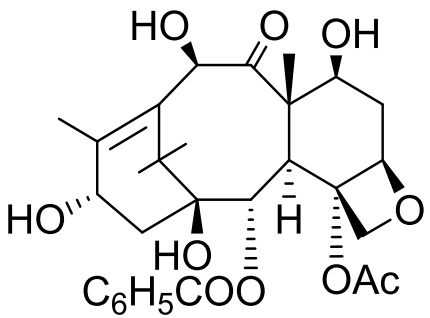
1989 Bristol-Myers Squibb (BMS) selected as Cooperative Research and Development Agreement (CRADA) partner.

1989 Taxol supply crisis: the available supplies were inadequate for the demand.

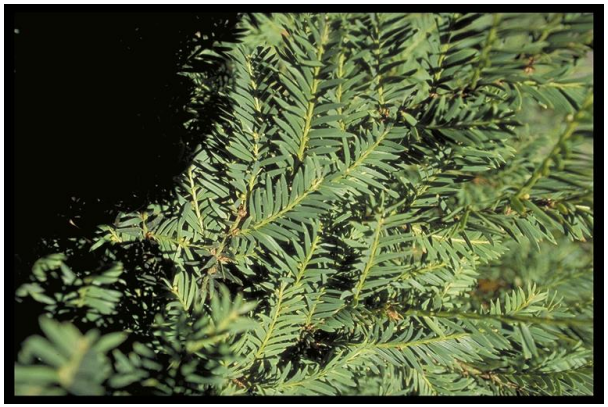
The Taxol Supply Crisis

- Yield from bark 1 g per 14 Kg dried bark, equivalent to 1.5 trees per 1 g
- Patient requirement 500 mg/patient/course x 4 courses = 2 g/patient
- 12,000 patients/year with ovarian cancer = 24 Kg = 36,000 trees
- *T. brevifolia* occurs primarily in the old growth forests of the Pacific Northwest
- These forests are the home of the spotted owl, an endangered species
- There was thus a conflict between environmentalists and patient advocates

A Solution to the Supply Crisis: Taxol Semisynthesis from 10-DAB

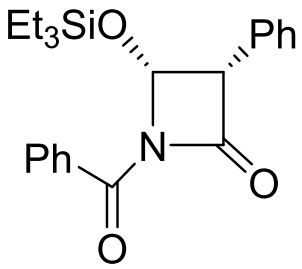


10-Deacetylbaccatin III



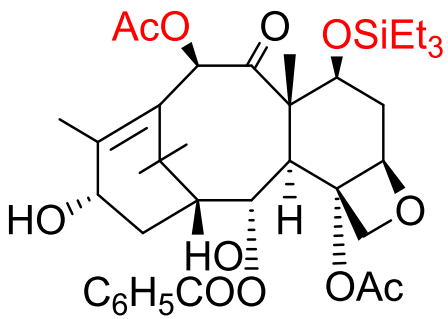
Taxus baccata needles

1. Ac_2O, pyridine
2. Et_3SiCl, pyridine



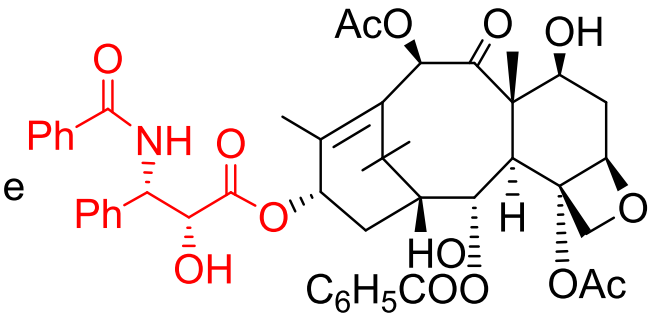
β-lactam

+



Baccatin III

1. nBuLi
2. HF/pyridine



Taxol (paclitaxel)

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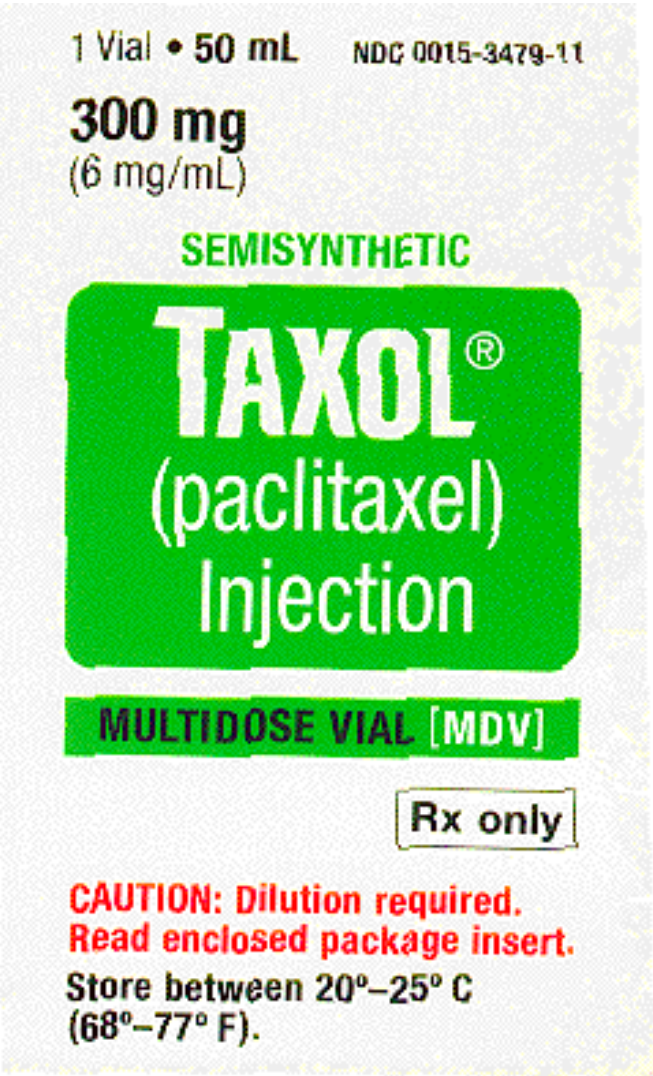
Taxol Development 1991 - present

1991	Activity observed against metastatic breast cancer
1992	FDA approval for treatment of refractory ovarian cancer
1994	FDA approval for use for treatment of refractory breast cancer
1994-	Phase III clinical trials, combination therapy
2000	Taxol sales over $1 billion/year
2009	Sales of Taxol & other taxanes over $3 billion in 2009.[1]

[1]Nat. Rev. Drug. Discovery **2010,** *9*, 677



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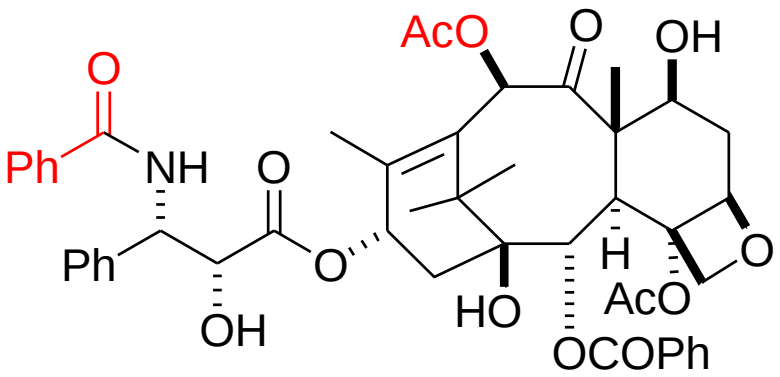
From taxol to Taxol™

1993 BMS trademarks the name Taxol for its formulation of taxol in Cremophor. The name paclitaxel is approved for generic use.

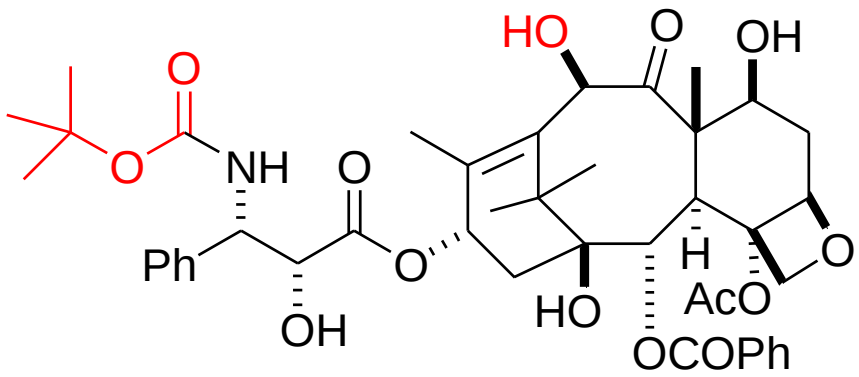
Why did they do this? Taxol had become a well-known drug because of the publicity over the supply crisis and also because of its sometimes dramatic effects. It was one of the few drugs whose names were known to the general public.

How did they do this? The name taxol had been in general scientific use for 20 years!!

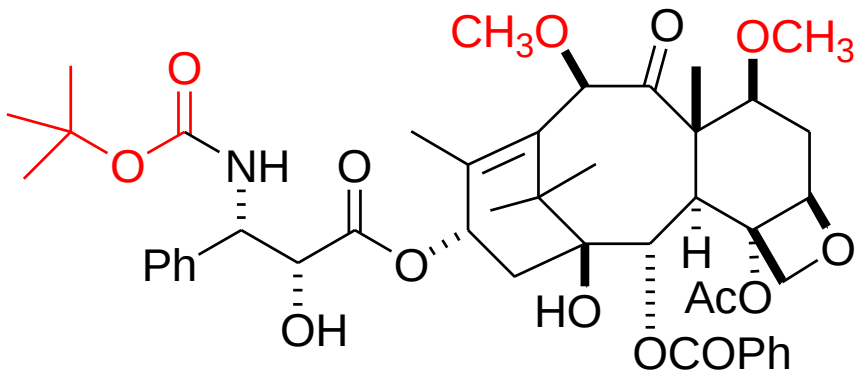
Taxanes in Clinical Use (2014)



Paclitaxel (Taxol)
Bristol-Myers Squibb, 1972



Docetaxel (Taxotere)
Aventis, 1996



Cabazitaxel (Jeftana)
Sanofi-Aventis, 2010

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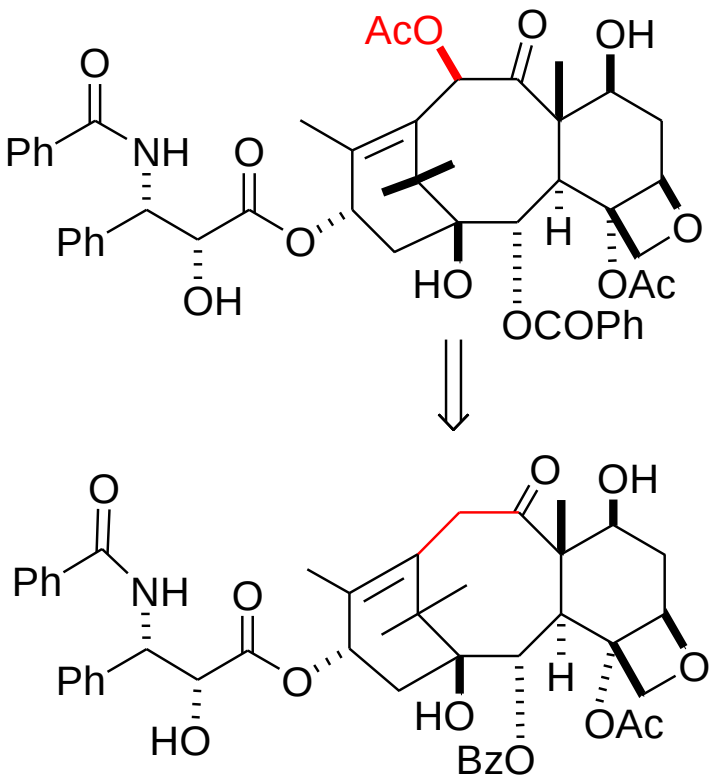
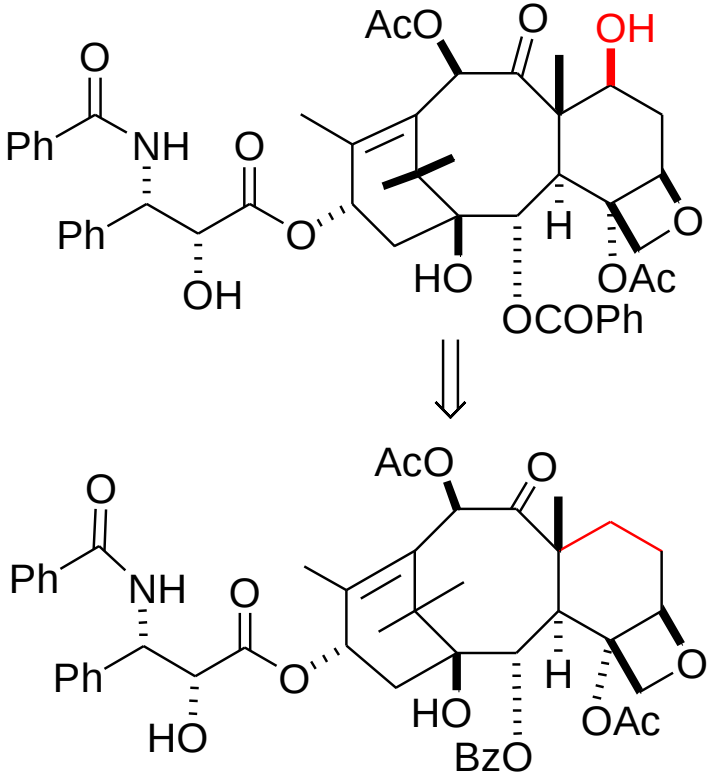
Deletion of some functional groups gives bioactive products



IC_{50}/IC_{50}(taxol) = 1.75 (HCT 116)
Chaudhary et al. Tetrahedron Lett., **1993**, 34, 4921-4924.



IC_{50}/IC_{50}(taxol) = 1.0 (HCT 116)
Chaudhary et al. J. Org. Chem., **1993**, 58, 3978-3979.

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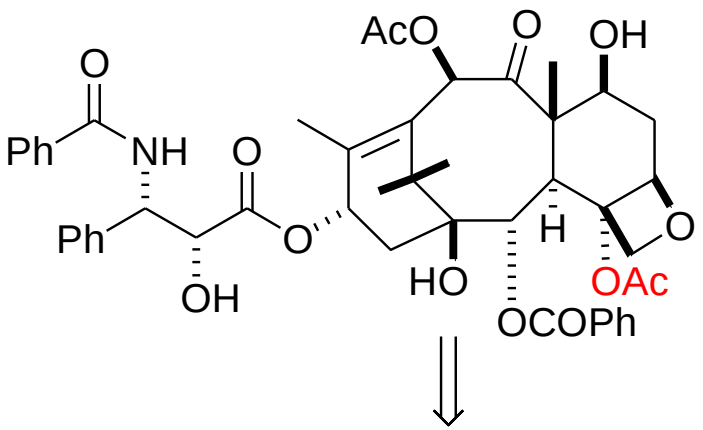
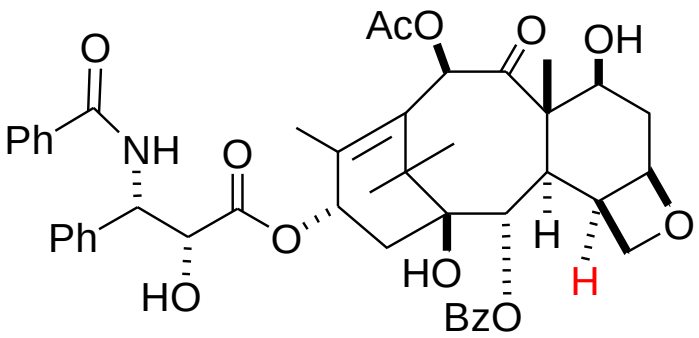
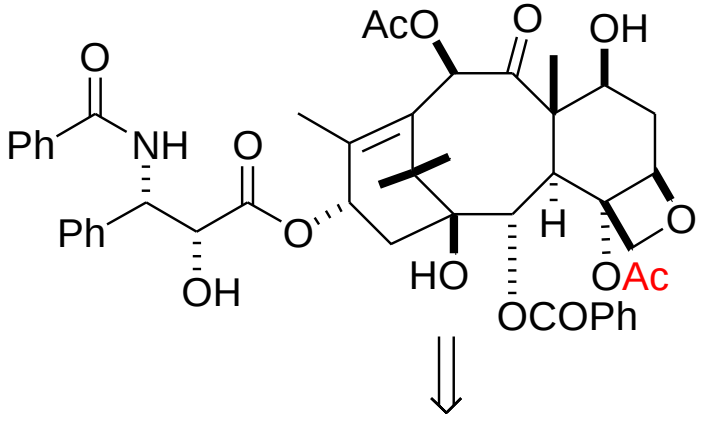
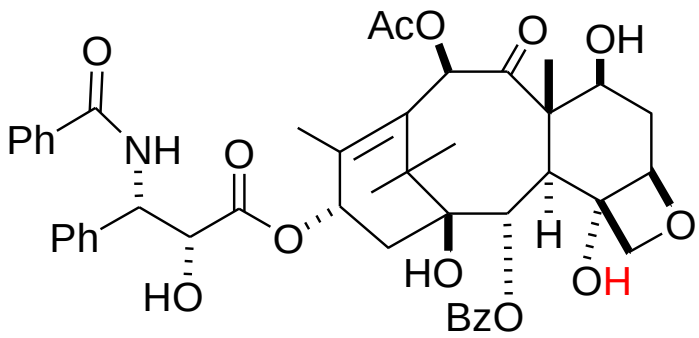
Deletion of others does not!





IC_{50}/IC_{50}(taxol) = 23 (CA46)
Chordia et al. *Tet. Lett.* **1994**, 6843





IC_{50}/IC_{50}(taxol) = >33 (CA46)
Neidigh et al. *Tet. Lett.* **1994**, 6839

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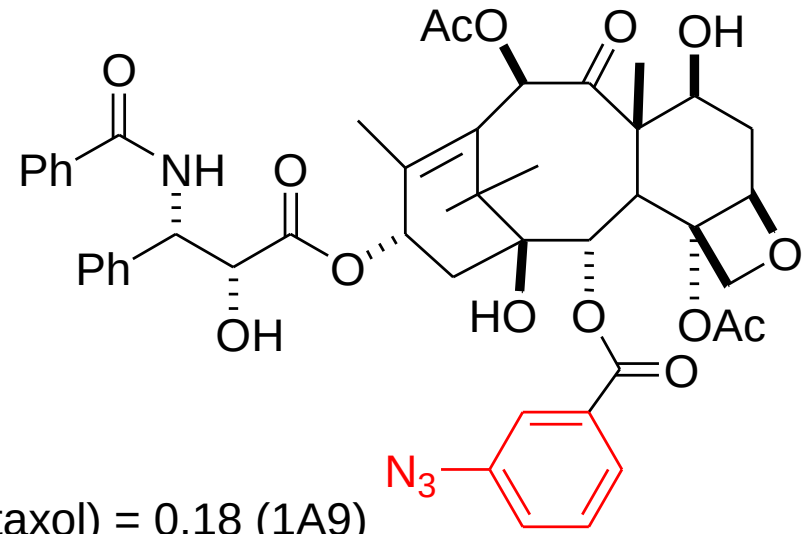
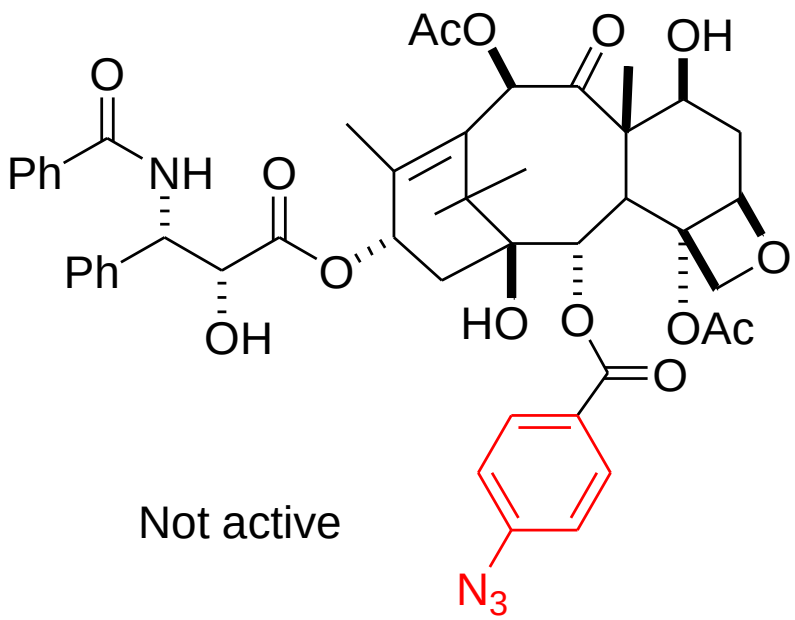
A Change of Substitution on the C2 Benzoate has a Major Effect on Activity



IC_{50}/IC_{50}(taxol) = 0.18 (1A9)



Not active

A. G. Chaudhary et al. *J. Am. Chem. Soc.* **1994**, *116*, 4097-4098.

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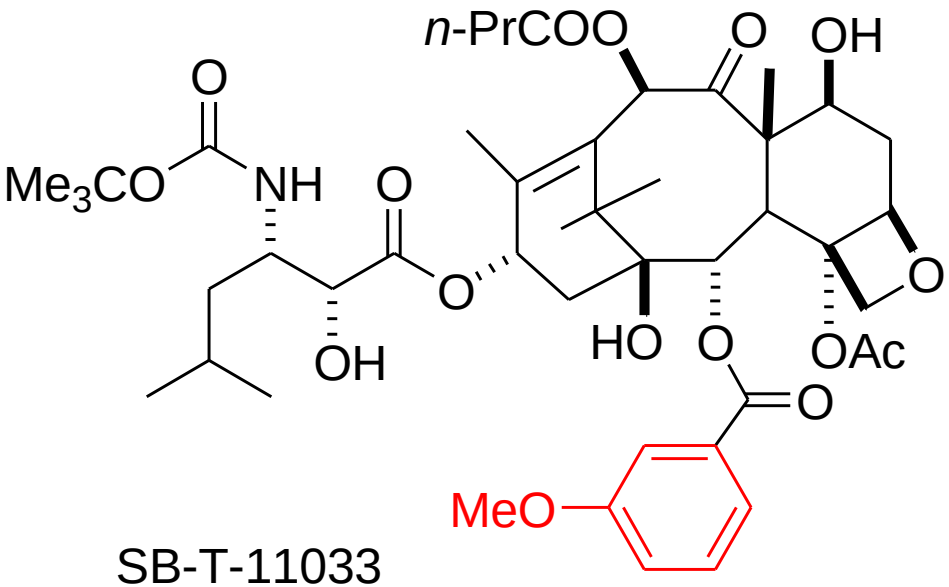
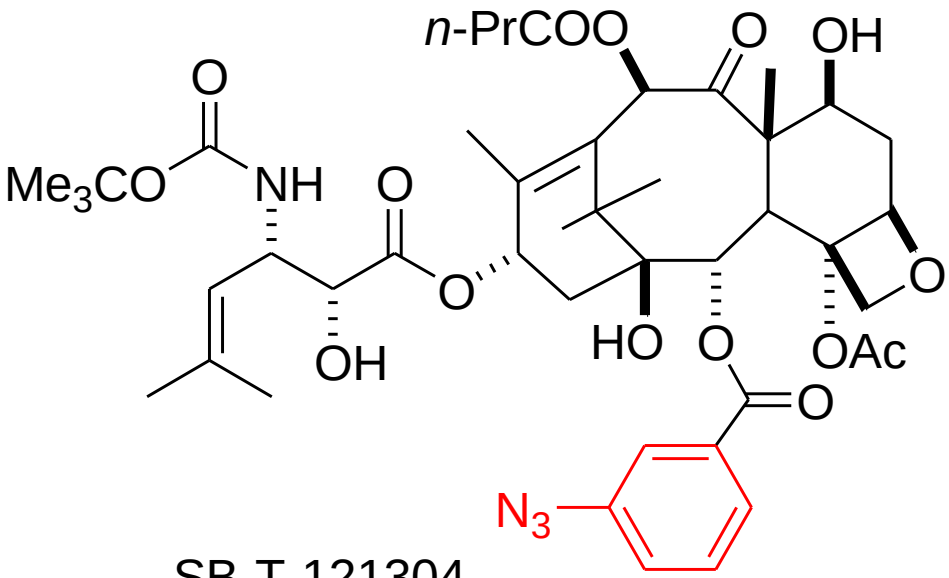
Which has Led to the Development of Clinical Candidates



SB-T-11033



SB-T-121304

Ojima et al. *Bioorg. Med. Chem. Lett.* **1999**, 3423

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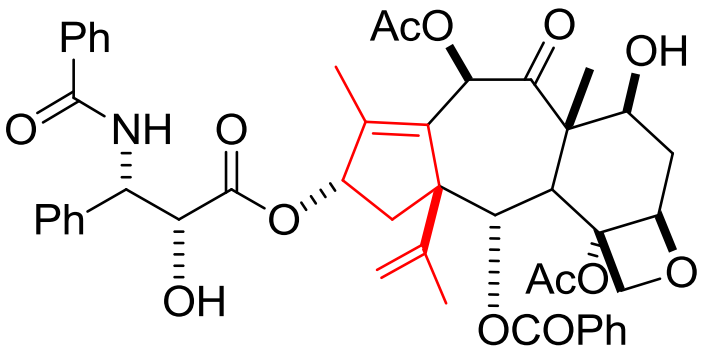
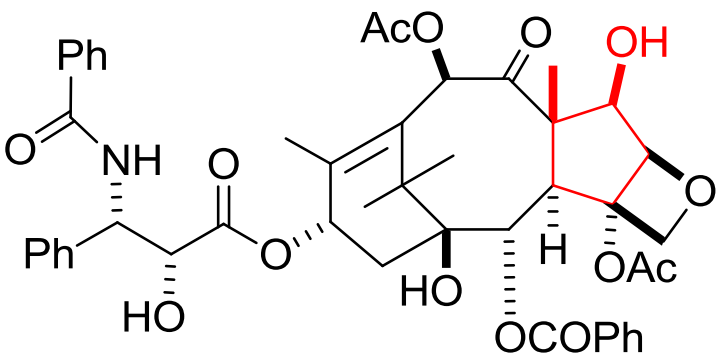
Contraction of the A and C Rings Reduces Activity



IC_{50}/IC_{50}(taxol) = >800 (A2780)
K_a/K_a(taxol) = <250 (association constant for MT binding)

Samaranayake et al. J. Org. Chem., **1991**, 56, 5114-5119.



IC_{50}/IC_{50}(taxol) = 9.6 (HCT116)
Liang et al. *Tet. Lett.* **1995**, 7795

Chaudhary et al. J. Org. Chem., **1993**, 58, 3978-3979.

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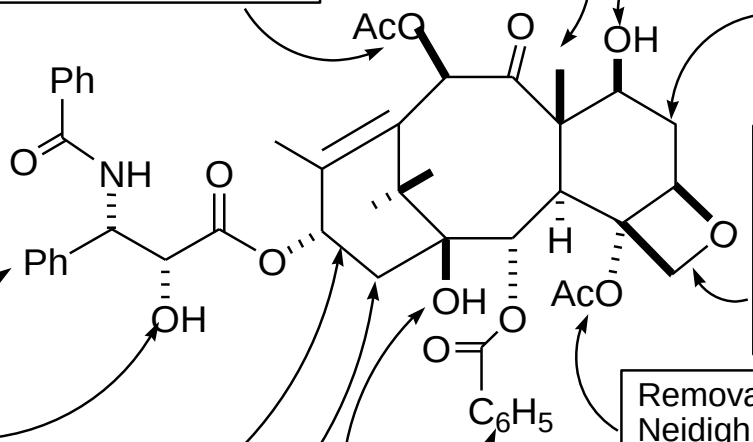
Taxol SAR



N-acyl group required; some acyl analogs have improved activity

Acetyl or acetoxy group may be removed without significant lossof activity; some acyl analogs have improved activity Chaudhary and Kingston, Tetrahedron Lett. 1993, 34, 4921-4924.

Reduction improves activity slightly

May be esterified, epimerized or removed without significant loss of activity. Some derivatives have improved activity Chaudhary et al. J. Org. Chem. 1993, 58, 3798-3799.

Contracted C-ring inactive Liang, X.; Kingston, D. G. I., Tetrahedron Lett. 1995, 36, 7795-7798.

Group may be changed to alkenyl or substituted phenyl. Some groups give improved activity.

Oxetane or cyclopropane ring required for activity; substitution of S for O reduces activity. Gunatilaka et al. J. Org. Chem. 1999, 64, 2694-2703.

Free 2'-hydroxyl group, or a hydrolysable ester thereof, required

Removal of acetate reduces activity. Neidigh et al., Tetrahedron Lett. 1994, 35, 6839-6842. Replacement by other groups can increase activity

Contracted A-ring inactive

Acyloxy group essential; certain alkenyl and substituted aromatic groups give improved activity Kingston et al., J. Med. Chem. 1998, 41, 3715

14β-hydroxyl group or 14β,1-carbonate analogs retain activity

Removal of hydroxyl group reduces activity Kingston et al. J. Org. Chem. 1999, 64, 1814-1822.

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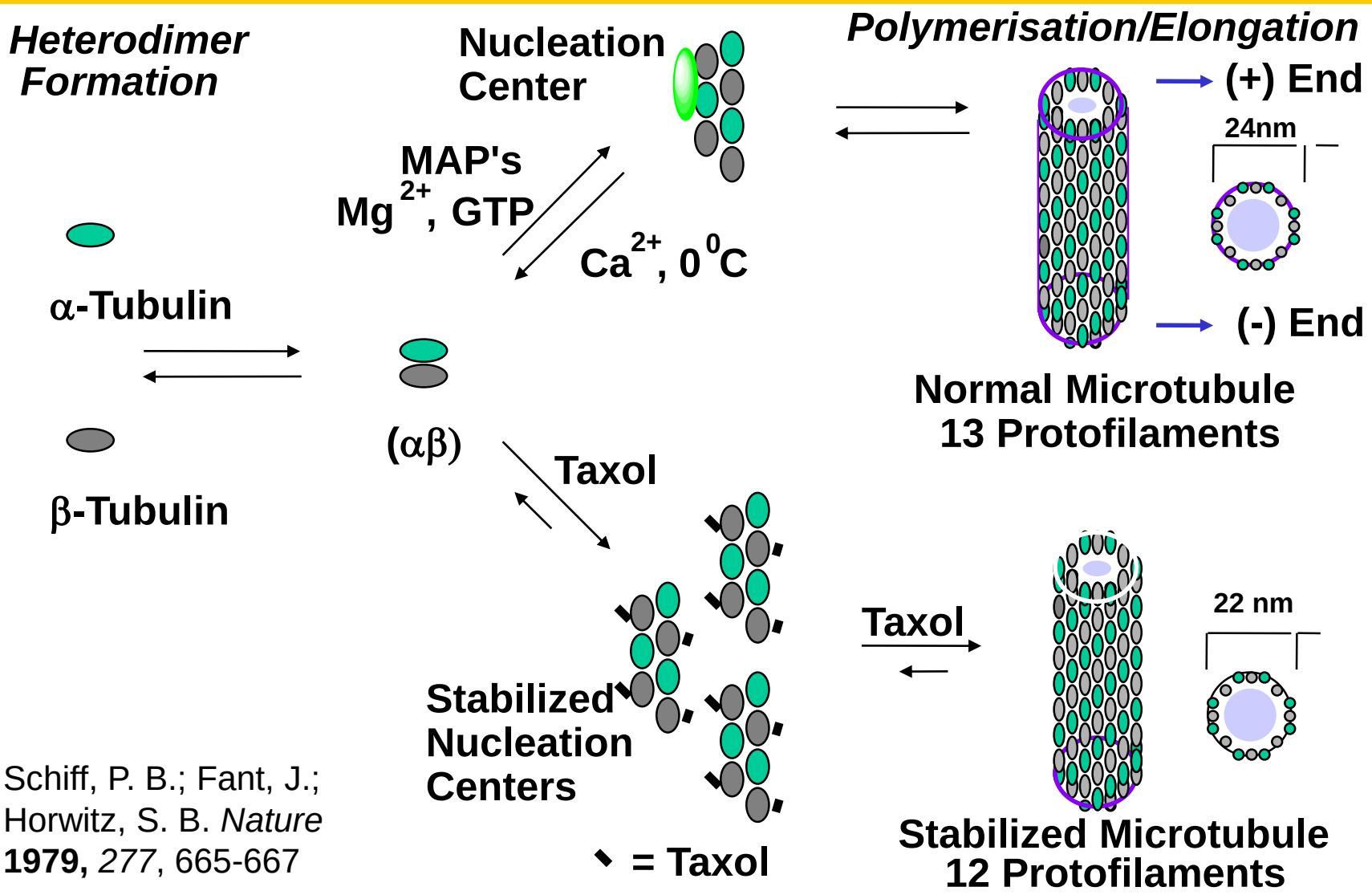
Taxol Disrupts this Equilibrium by Binding to the Microtubule and Stabilizing it



Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979,** *277*, 665-667

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The Binding Location of Taxol on the αβ-Tubulin Dimer is Known

Photoaffinity labeling of tubulin by taxol

Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. 3'-(*p*-Azidobenzamido)taxol Photolabels the N-Terminal 31 Amino Acids of β-Tubulin. *J. Biol. Chem.* **1994,** *269*, 3132-3134.

Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G. I.; Horwitz, S. B. Characterization of the Taxol-Binding Site on the Microtubule: 2-(*m*-Azidobenzoyl)taxol Photolabels a Peptide (amino acids 217-231) of β-tubulin. *J. Biol. Chem.* **1995,** *270,* 20235-20238.

Electron crystallography of taxol-stabilized zinc induced tubulin sheets at 3.7 Å

Nogales, E.; Wolf, S. G.; Downing, K. H. Structure of the αβ Tubulin Dimer by Electron Crystallography. *Nature* **1998**, *391*, 199-203.

Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. The Binding Conformation of Taxol in β–Tubulin: A Model Based on Electron Crystallographic Density. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5312-5316.

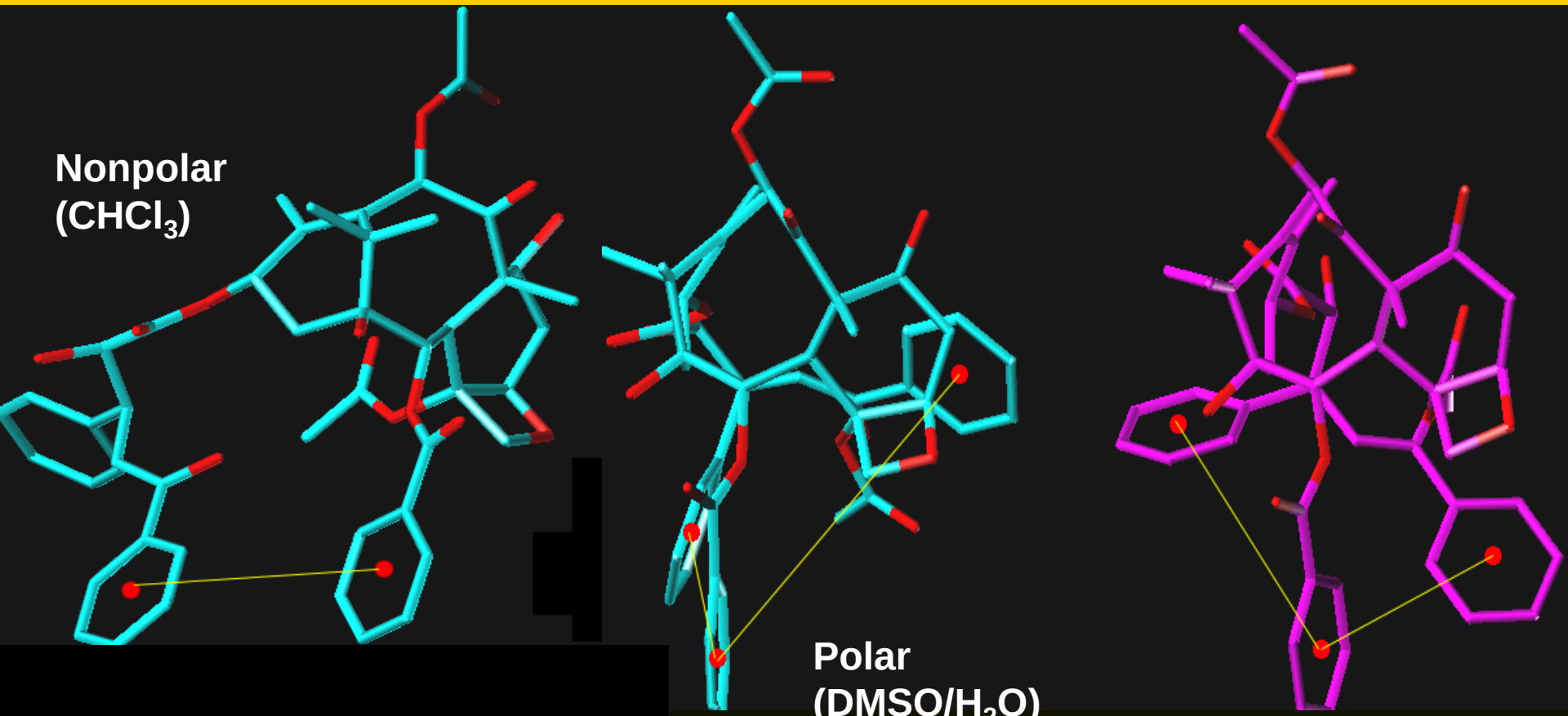
But the Tubulin-Binding Conformation of Taxol is Not

- The resolution of the electron crystallographic structure of the taxol-tubulin complex is too low to show the conformation of taxol
- A knowledge of the binding conformation(s) could lead to the design of improved analogs
- It could also lead to the design of simpler analogs which retain Taxol's tubulin-binding activity.

Five Tubulin-Binding Conformations Have Been Proposed for Taxol

- A “non-polar” conformation was proposed based on NMR studies in $CHCl_3$
- A “polar” conformation was proposed based on NMR studies in $DMSO/H_2O$
- A “T-Taxol” conformation was proposed based on NAMFIS NMR studies and molecular modeling
- A “REDOR-taxol” (also known as NY-PTX) was proposed by Ojima based in part on our REDOR NMR studies described below.
- 1JFF is an electron crystallographically refined structure

Proposed Tubulin-Binding Conformations



“Non-polar” conformer
Guénard, Guéritte-Voegelein 1993
Scott, Swindell et al 1993
Cachau, Gussio et al 1994

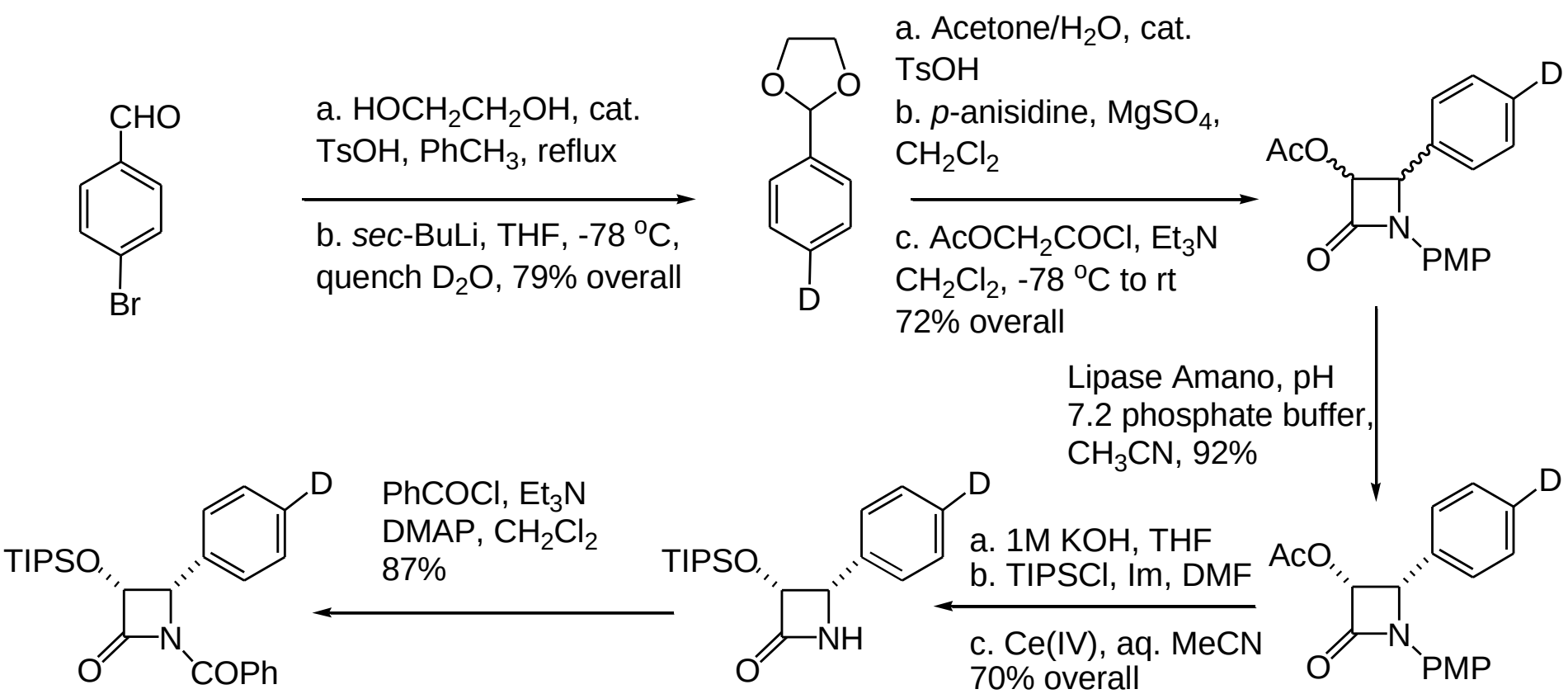
“Polar” conformer
Vander Velde, Georg et al 1993
Paloma, Nicolaou et al 1994
Ojima et al 1997

T-shaped conformer
Snyder et al.

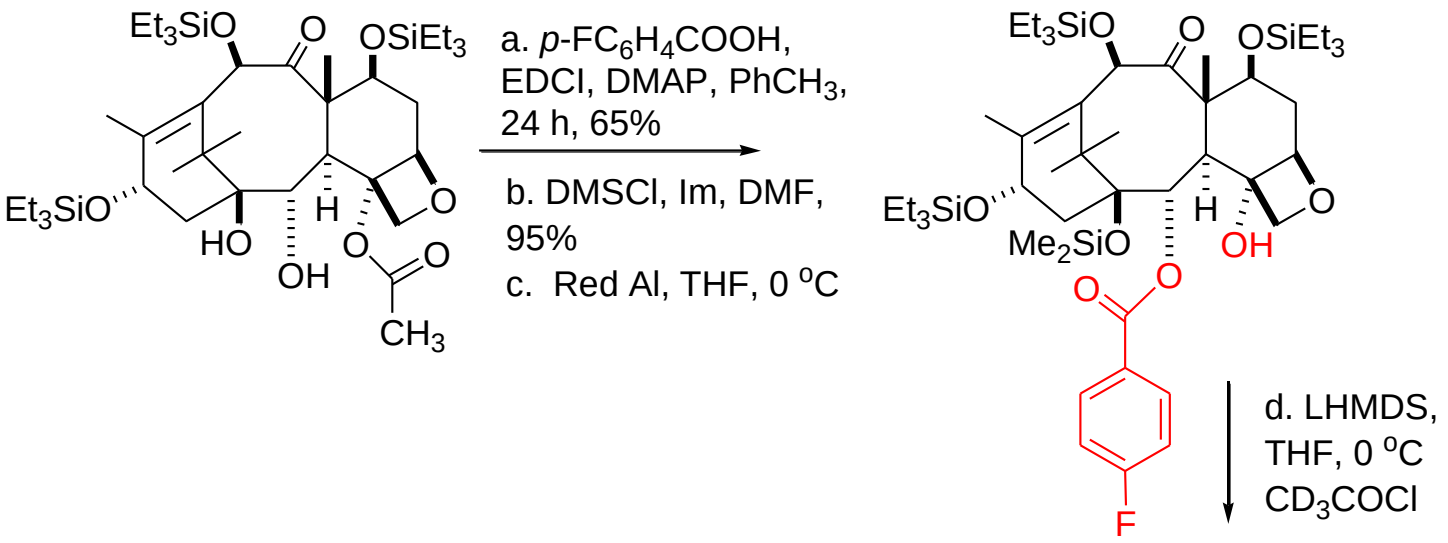
REDOR NMR Spectroscopy Can Distinguish Between These Conformations

- Solution-state NMR in the absence of tubulin does not give direct information on the tubulin-bound conformation, and it is not possible in the presence of tubulin, because addition of taxol to tubulin causes polymerization to microtubules
- REDOR NMR (Rotational-Echo, Double Resonance NMR) is a spectroscopic technique for solids spinning at the magic angle, so it can be used for ligand-bound microtubules
- It provides a direct measurement of heteronuclear dipolar coupling between pairs of labeled nuclei, and distances of up to 12 Å can be determined with 0.5 Å accuracy
- The method requires the synthesis of labeled ligands

Synthesis of Labeled Taxols for REDOR NMR



Synthesis of Labeled Taxols for REDOR NMR



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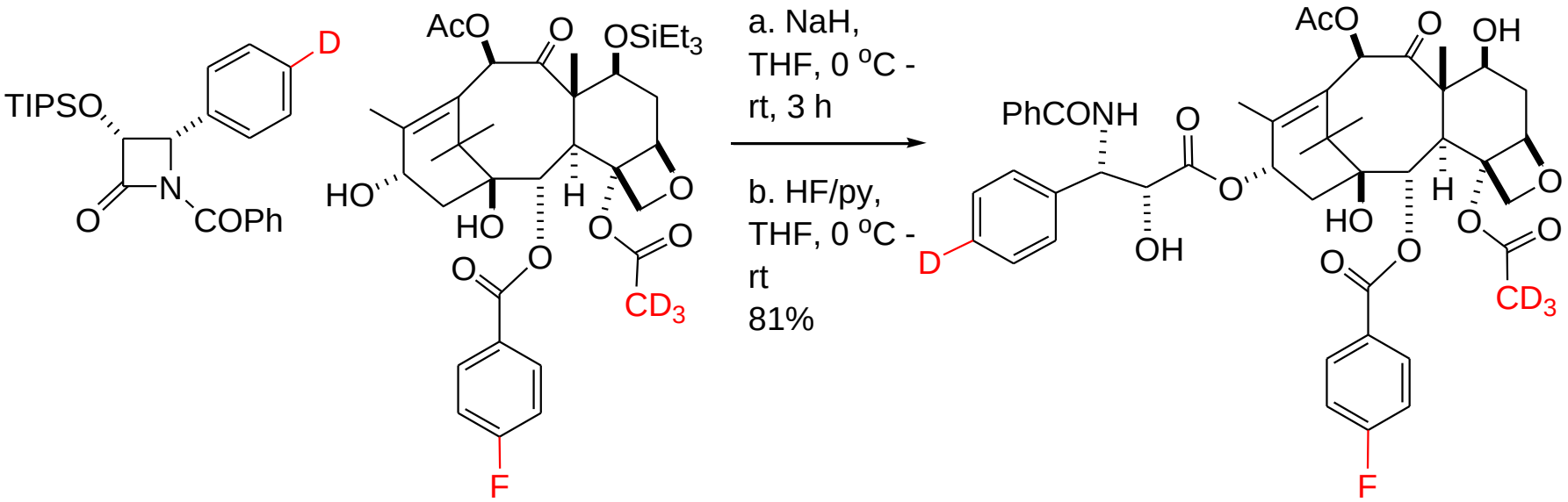
Synthesis of Labeled Taxols for REDOR NMR



Holton-Ojima β-lactam synthesis

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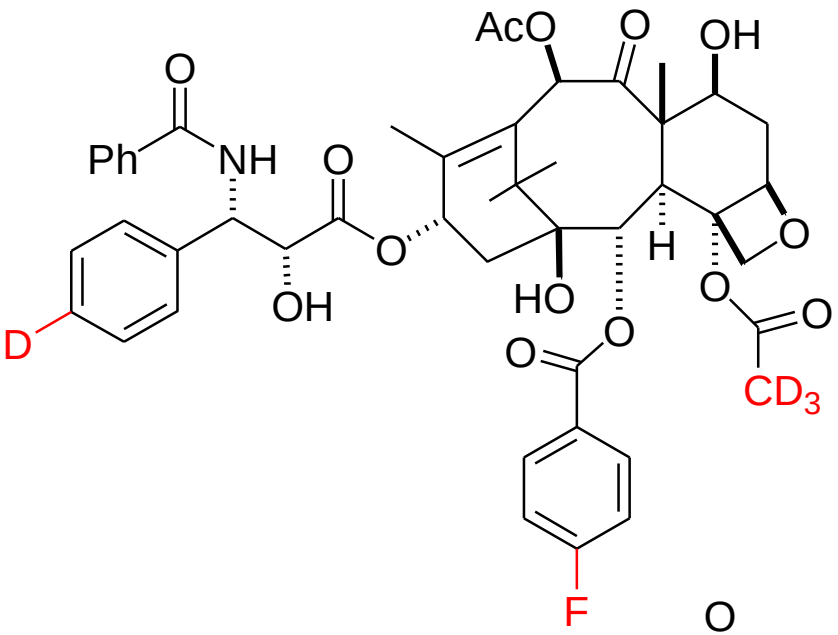
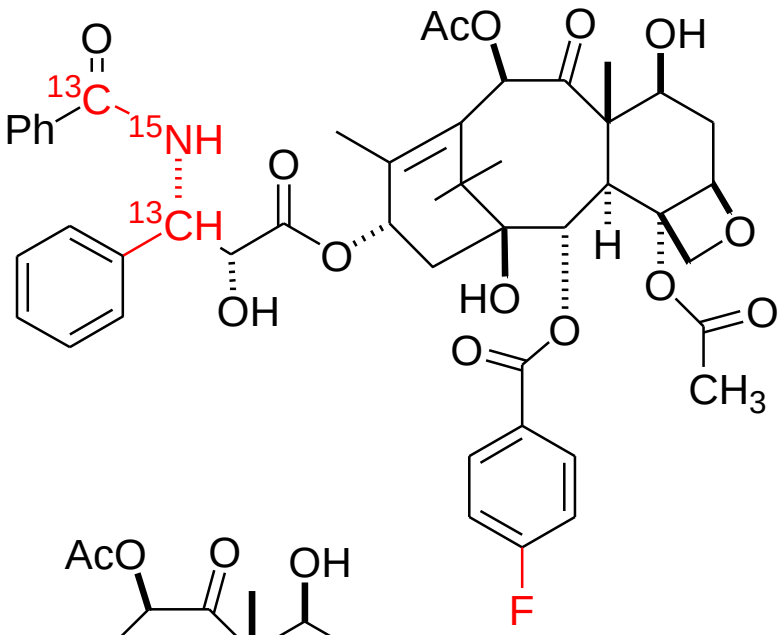
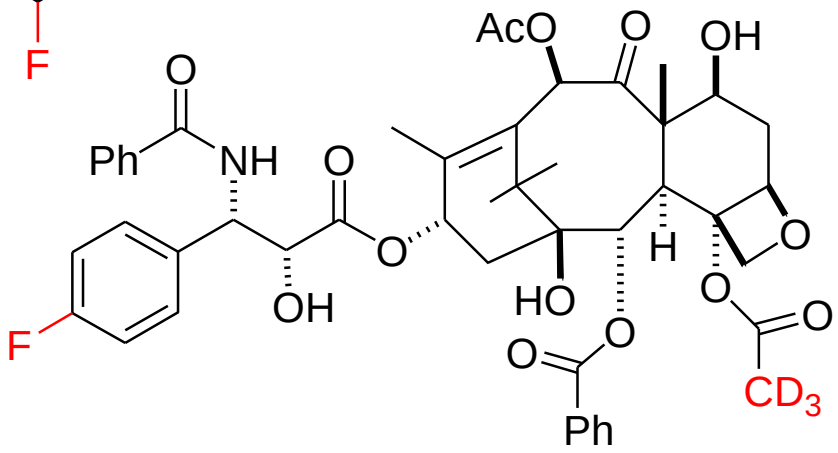
Labeled Taxols Prepared for REDOR NMR







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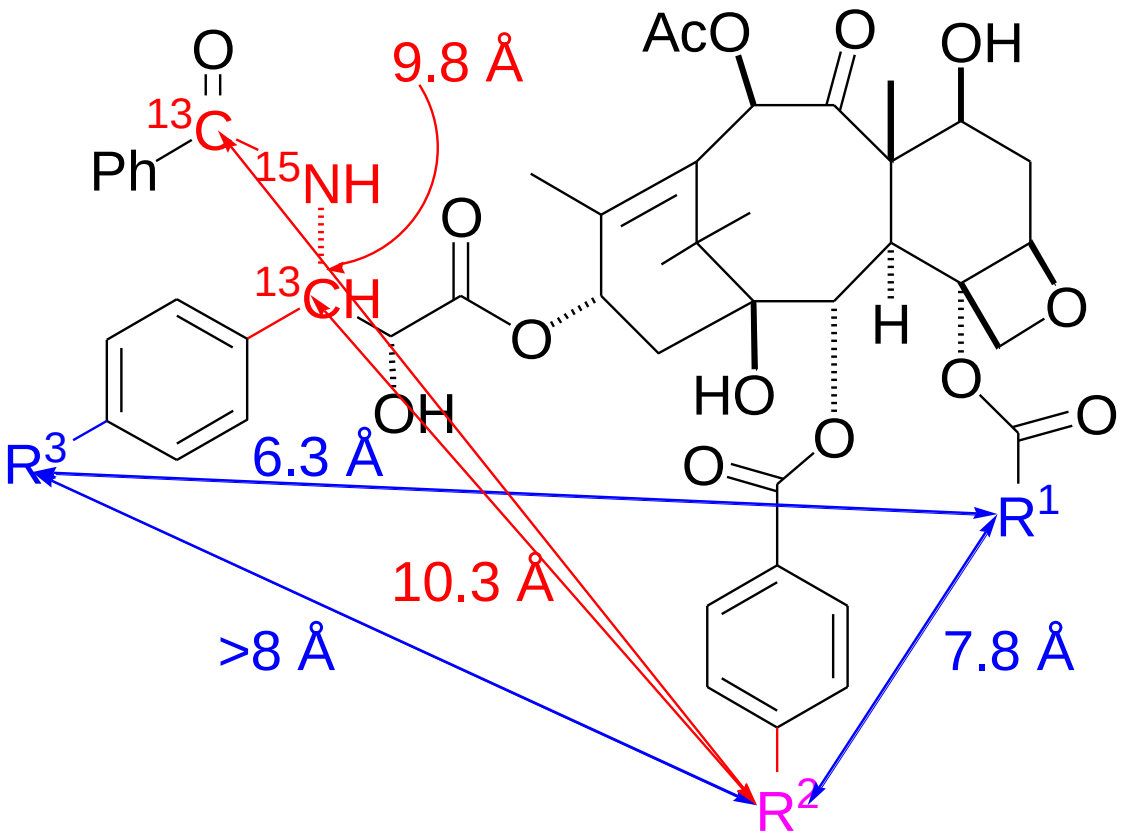
Three Separate REDOR NMR Experiments Gave Five Internuclear Distances



Li et al. *Biochemistry* 2000, 39, 281-291
Paik et al. *J. Am. Chem. Soc.* 2007, 129, 361-370

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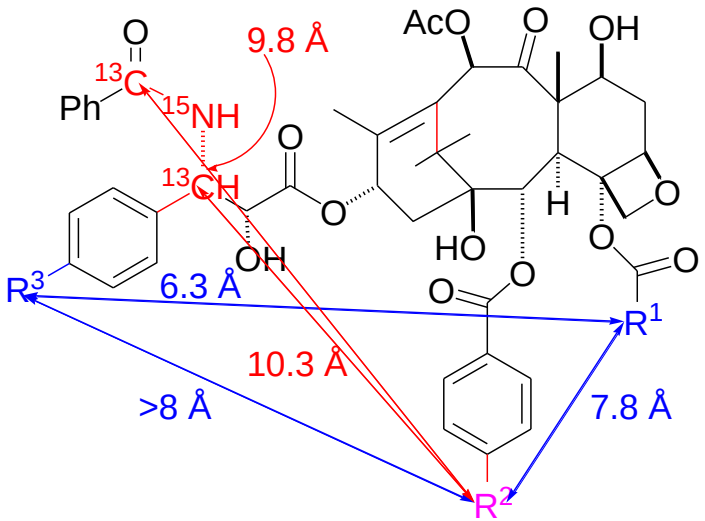
REDOR NMR Confirms T-Taxol Conformation



Calculated distances and experimental data from REDOR NMR

- **Green: Agrees with experiment within experimental error (0.7 A)**
- **Red: Does not agree with experiment**

Distances (A)	Polar	Non-polar	NY-PTX	1JFF	**T-Taxol**	Expt
R^1-R^2	7.9	8.0	7.5	6.5	**7.9**	**7.8**
R^1-R^3	5.9	7.2	8.5	7.3	**6.6**	**6.3**
R^2-R^3	4.6	12.5	14.4	11.6	**12.2**	**>8**
R^2-CH	9.6	8.5	10.1	9.3	**9.8**	**10.3**
R^2-C	10.4	6.2	10.3	8.1	**9.1**	**9.8**

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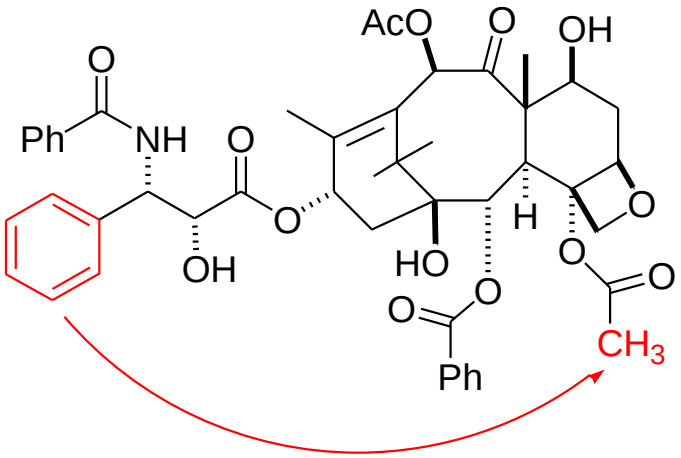
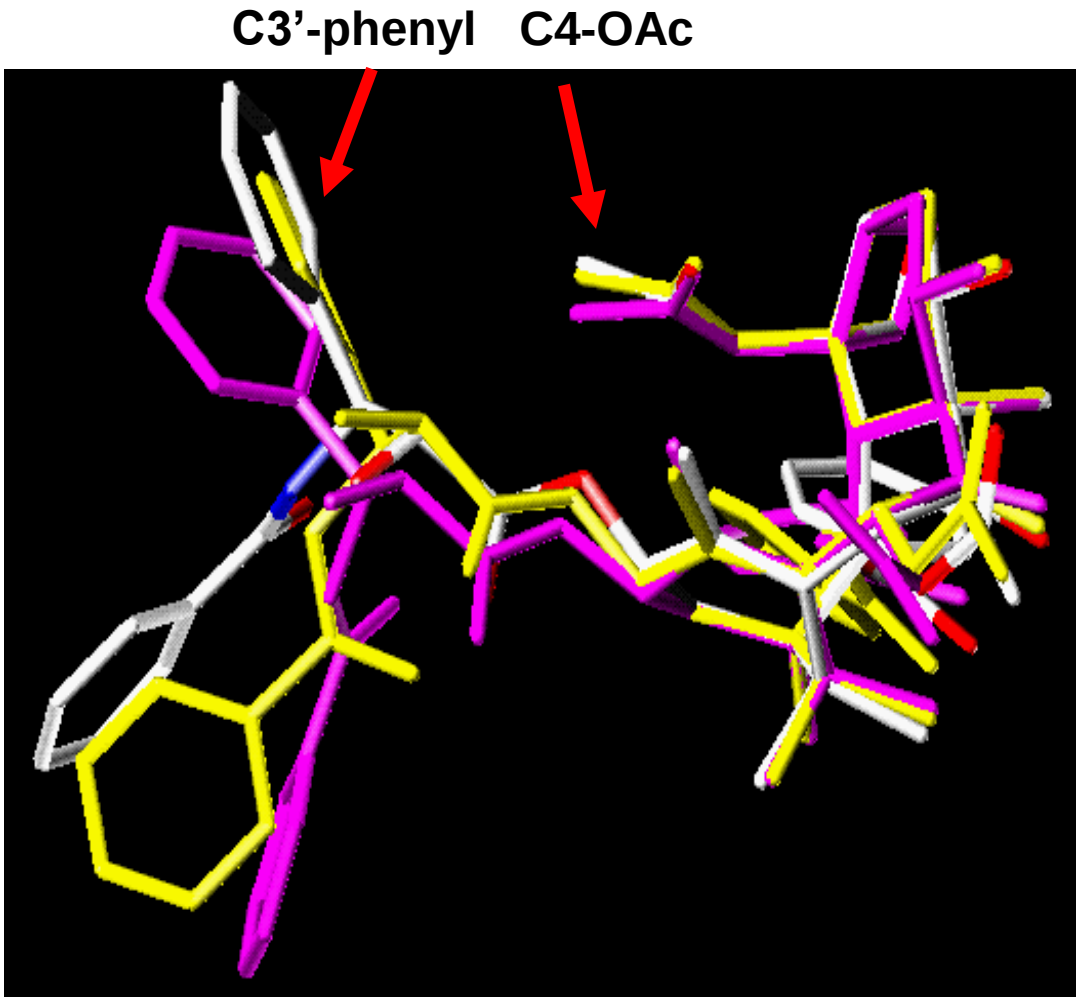
An Objective Test of the T-Taxol Binding Conformation Can be Devised





- The T-taxol conformation can be tested experimentally by the synthesis of bridged taxols which link the side chain and the C-4 acetate

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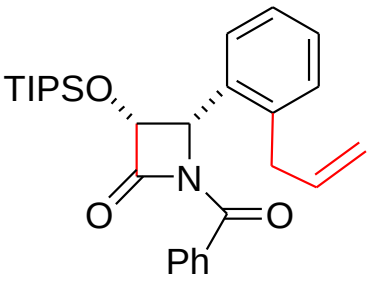
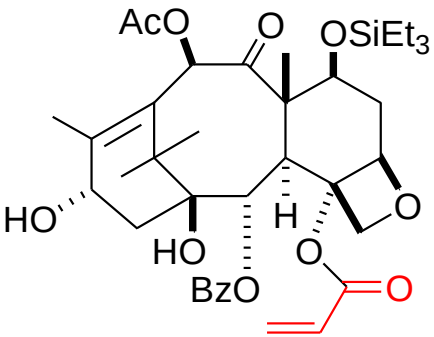
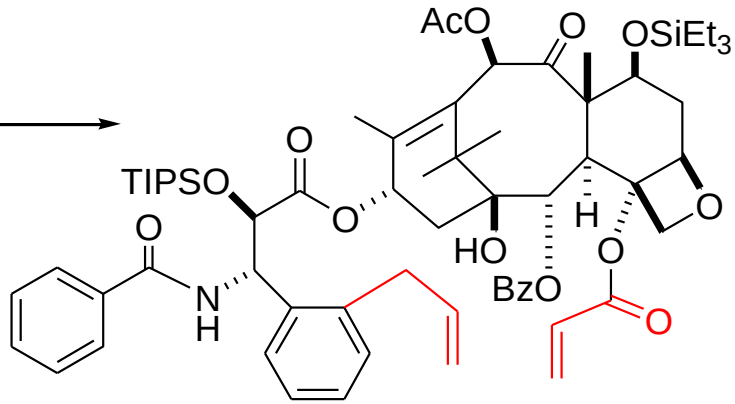
…and Indicated that the Synthesis of *Ortho* Bridged Analogs Should Yield Better Analogs

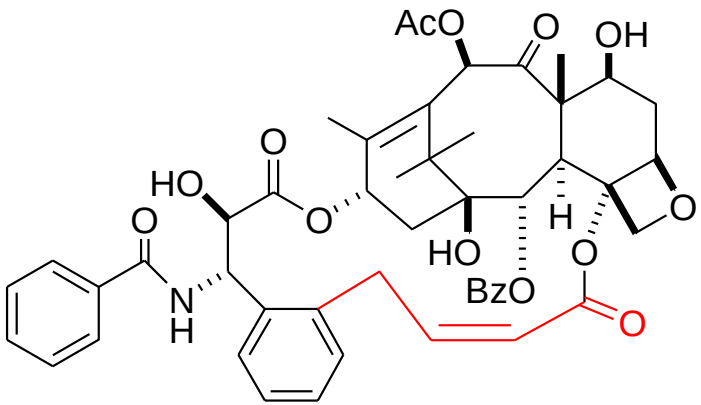






The β-lactam and baccatin III derivatives were prepared by modifications of known procedures

1. Grubbs' catalyst
2. HF/py



Britaxel-5
5-Atom Bridge (Phenyl-C4)

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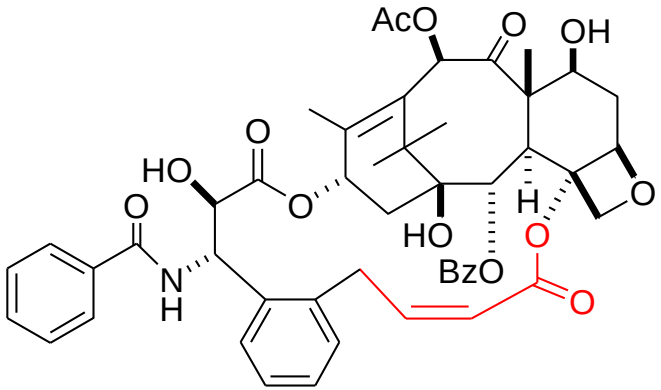
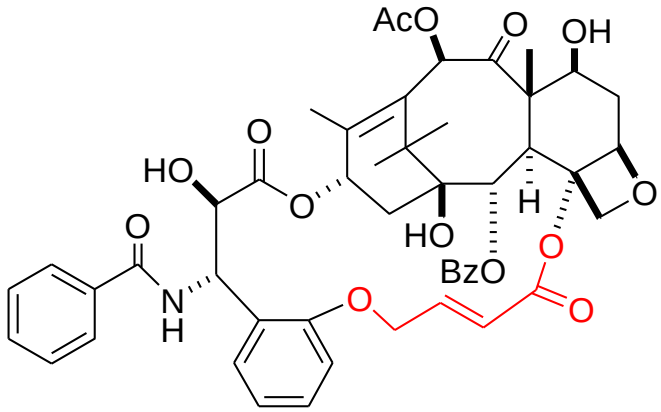
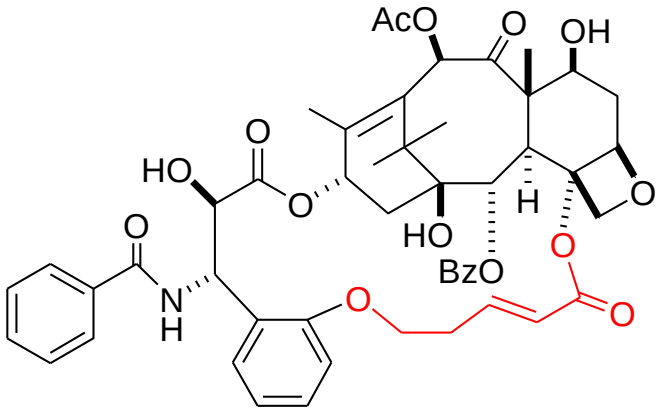
Several *Ortho*-C3'-Phenyl-C4 Bridged Analogs Were Prepared with Different Linker Lengths



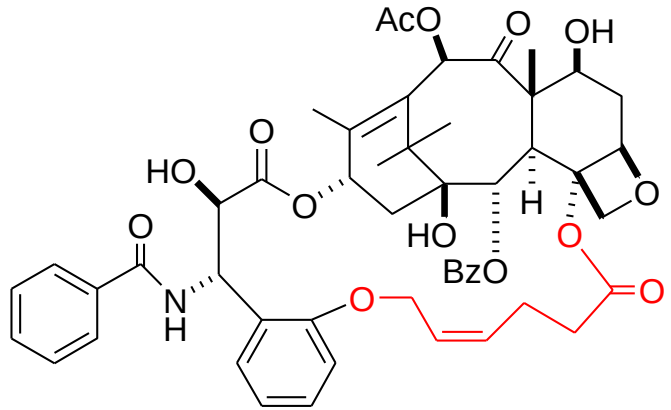
Z-Britaxel-5
Dihydrobritaxel-5



E-Britaxel-6



E-Britaxel-7



Z-Britaxel-8

All compounds were prepared by olefin metathesis as described for the *meta* bridged analog

T. Ganesh et al. Proc. Natl. Acad. Sci USA, **2004**, *101*, 10006-10011

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And Britaxel-5 Proved to be the Winner!

Compound	IC_{50}, A2780 (nM)	IC_{50}, PC3 (nM)	IC_{50}, Tb polymerizn. μM	Critical Tb conc., μM	Inhibition of binding of F-Taxol
Taxol	**6.64 ± 3.4**	**3.3 ± 0.30**	**0.42 ± 0.26**	**1.8 ± 0.30**	**26%**
***Z*-Britaxel-5**	**0.30 ± 0.22**	**2.4 ± 0.05**	**0.26 ± 0.16**	**0.53 ± 0.07**	**72%**
Dihydrobritaxel-5	**0.54 ± 0.3**	**2.4 ± 0.65**	**0.21 ± 0.01**	**0.35 ± 0.06**	**79%**
***E*-Britaxel-6**	**14.5 ± 0.7**	**15**	**0.31 ± 0.13**	**0.37 ± 0.20**	**ND**
***E*-Britaxel-7**	**20.7**	**16**	**0.67**	**ND**	**ND**
Dihydrobritaxel-8	**980**	**51**	**0.76**	**ND**	**ND**

Ganesh, T.; Guza, R. C.; Bane, S.; Ravindra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10006-10011.

Britaxel-5 is Even Better in Tubulin-Resistant Cell Lines

	1A9 (nM)	1A9-PTX10 (nM)	Relative resistance
Taxol	4.8	157	32.7
O-Britaxel-6	7.6	126	16.5
O-Britaxel-7	17.1	157	9.2
Britaxel-5	0.072	0.13	1.8
O-Britaxel-8	30.9	196	6.3
Iso-Britaxel-6	7.6	157	20.6
H_2-O-Britaxel-6	19.8	35.9	1.8
Dihydro-britaxel-5	0.083	1.03	12.4

Ganesh, T.; Yang, C.; Norris, A.; Glass, T. E.; Bane, S.; Ravindra, R.; Banerjee, A.; Metaferia, B.; Thomas, S. L.; Giannakakou, P.; Alcaraz, A. A.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *J. Med. Chem.* **2007,** *50*, 713-725.

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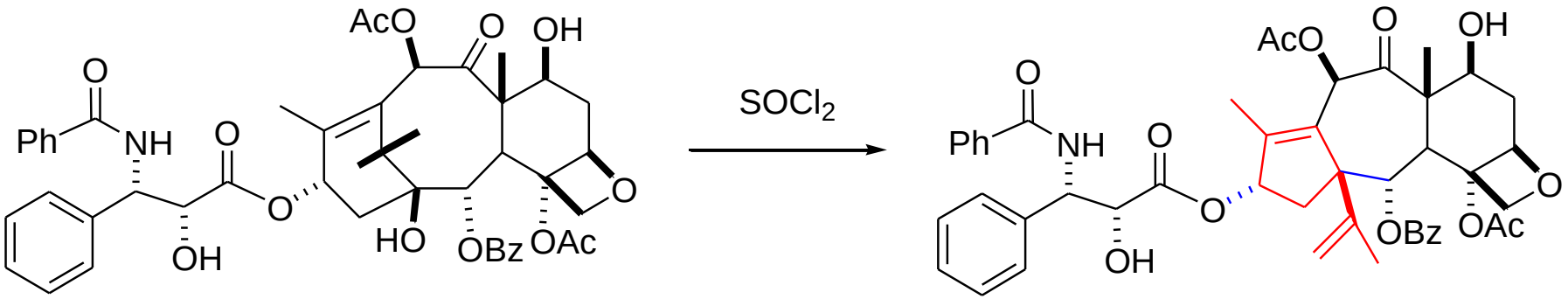
Can an Inactive Analog be Made Active by Bridging to the T-Taxol Conformation?



A-nor-Taxol

Samaranayake et al. J. Org. Chem. **1991**, 56, 5114-5119

- **A-*nor*-Taxol retains much of paclitaxel's tubulin-assembly promotion activity, but is NOT cytotoxic.**
- **Can we improve the cytotoxicity of A-*nor*-Taxol by making the bridged analog?**

Compound	IC_{50}, A2780 (nM)	IC_{50}, Tb polym (μM)
Taxol	2.1	1.5
A-*nor*-Taxol	>20,000	4.5

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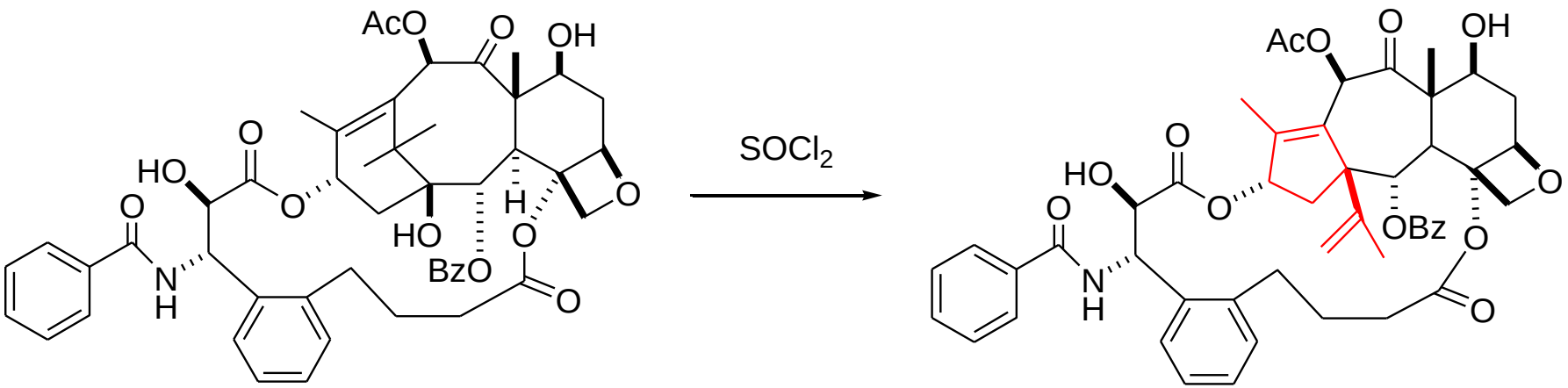
Yes it Can!



A-*nor*-Dihydrobritaxel-5

Compound	IC_{50}, A2780 (nM)	IC_{50}, Tb polym (μM)
Taxol	2.1	1.5
A-*nor*-Taxol	>20,000	4.5
A-*nor*-Dihydrobritaxel-5	77	0.78

Tang et al. Org. Lett. **2006**, 8, 3983-3986.

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Personal Motivation for Loving Natural Products

“O God, I am thinking Thy thoughts after Thee”

Johannes Kepler (1571-1630)
Discoverer of the Laws of Planetary Motion

Cited by C. Hummel, “The Galileo Connection”, InterVarsity Press: Downers Grove, IL, 1986.

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- Professor Susan Bane and her students, SUNY Binghamton (tubulin assays)
- Professor Jake Schaefer and his students, Washington University (REDOR NMR studies)

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