My 60-year love affair with natural products

Norman R. Farnsworth Lecture

March 26, 2021

College of Pharmacy

University of Illinois at Chicago

David G. I. Kingston
Department of Chemistry
Virginia Tech

Virginia Tech

Norman R. Farnsworth (1930-2011)

Norman Farnsworth was a towering figure in pharmacognosy from the 1960's to the present. Almost single-handedly, he established the Department of Pharmacognosy and Pharmacology (as it then was) at the University of Illinois, Chicago, as the preeminent place for pharmacognosy research in the USA. Although I never had the privilege of working with him, I regarded him as a scientific model, and I sought to emulate him in my research. He was also very generous to me, and wrote a letter of support for my tenure at Virginia Tech in the early 1970's and also, I believe, for my promotion to Full Professor a few years later. More recently I appreciated his wisdom and common sense as we met during the Annual ASP meetings to discuss the question of future Fellows of the ASP. He was also strongly committed to the National Center for Complementary and Alternative Medicine (NCCAM), and Dr. Josephine Briggs, the Director of NCCAM, paid a well-deserved tribute to him at a recent meeting of the NCCAM Advisory Council on October 15th. I am writing this from Ouro Preto, Brazil, at the 3rd Brazilian Conference on Natural Products, and both Lars Bohlin and I paid tribute to Norman in our lectures. — David Kingston, Virginia Tech

Virginia Tech

Cambridge

Queens' College

King's College

Queens' College

Punting on the Cam

Cambridge

Undergraduate studies 1957-1960
Graduate studies 1960-1963

University Chemical Laboratories

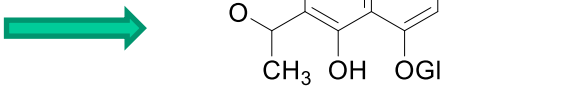
Lord Todd, Nobel Laureate

Donald Cameron

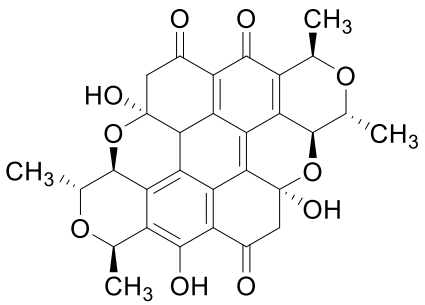
Virginia Tech

Ph.D. Research: Aphid Pigments 1960-1963

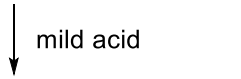
Black bean aphid
Aphis fabae

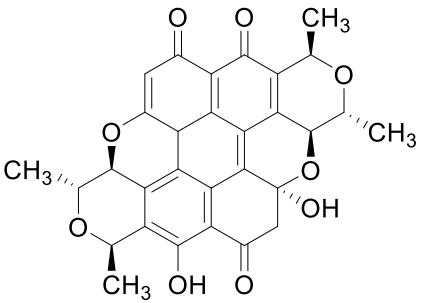


Protoaphin-fb
(Colorless)

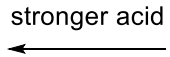


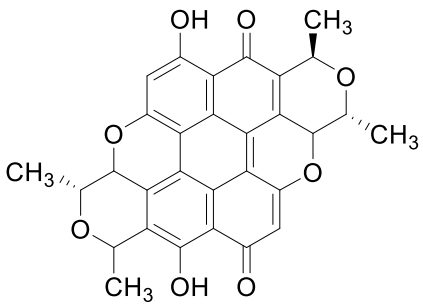
Xanthoaphin-fb
(Yellow)





Chrysoaphin-fb
(Orange)



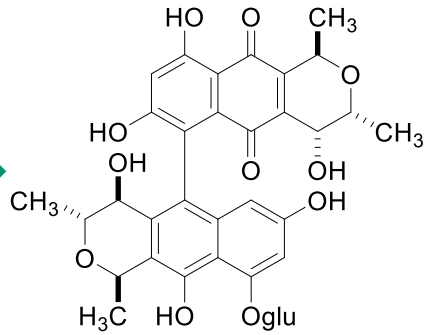


Erythroaphin-fb
(Red)

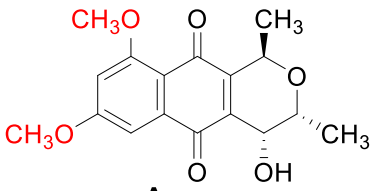
Virginia Tech

Ph.D. Research: Aphid Pigments 1960-1963

Tuberolachnus salignus
Giant willow aphid

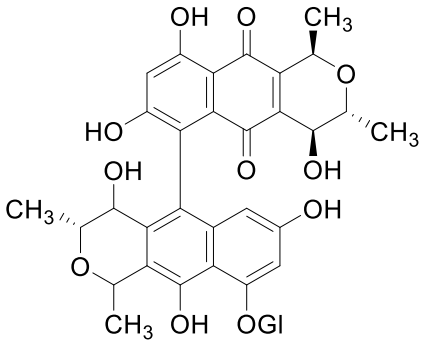


Protoaphin-sl
(from the willow aphid)



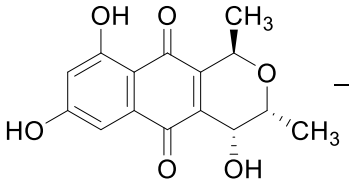
A

Me_2SO_4, base

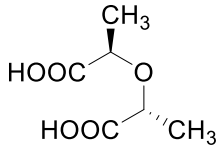


Protoaphin-fb
(Colorless)

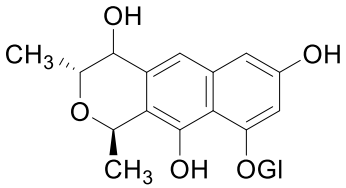
dithionite



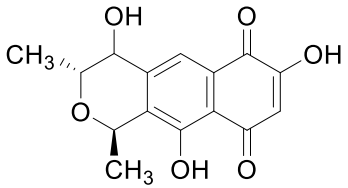
CrO_3



(+)-dilactic acid



Fremy's salt



Cameron, D. W.; Cromartie, R. I. T.; Kingston, D. G. I.; Todd, L. Colouring Matters of the Aphididae. Part XVII. The Structure and Absolute Stereochemistry of the Protoaphins. *J. Chem. Soc.* ***1964,*** 51-61.

Virginia Tech

Ph.D. Research: Aphid Pigments 1960-1963

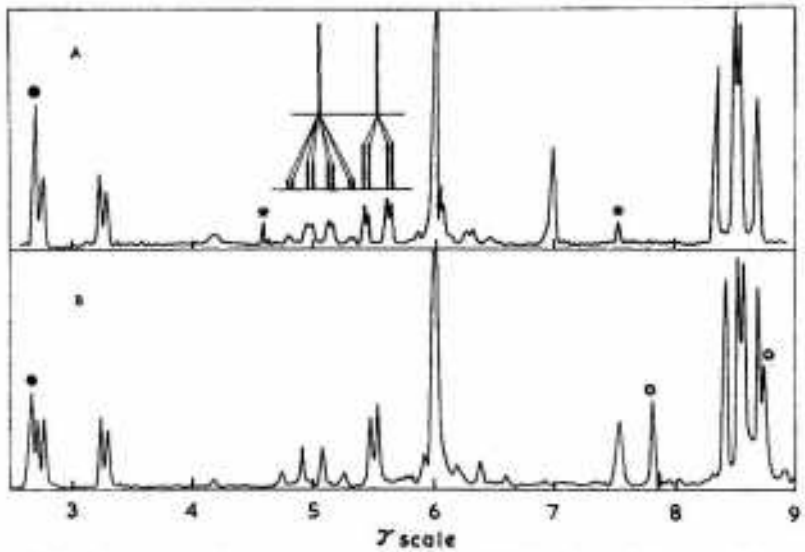
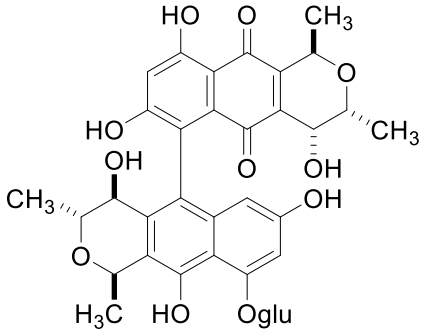
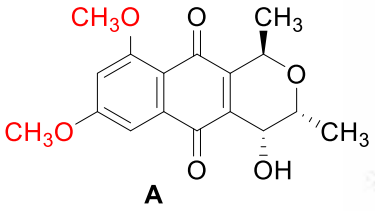


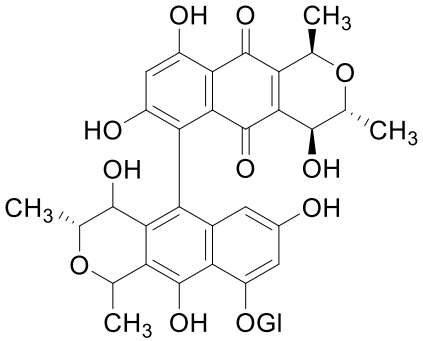
FIG. 2. Nuclear magnetic resonance spectra at 40 Mc./sec. of the naphthaquinone dimethyl ethers derived (A) from protoaphin-*fb*, (B) from protoaphin-*sl*, between 2-5 and 9 on the τ scale. ○ resonances believed to be caused by impurity; * spinning side-band; ● resonance of residual $CHCl_3$ in $CDCl_3$.



Protoaphin-sl
(from the willow aphid)

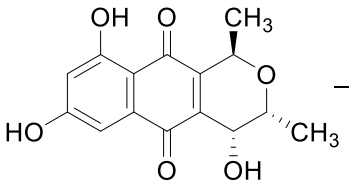


A

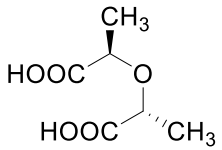


Protoaphin-fb
(Colorless)

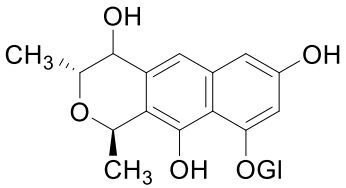
dithionite



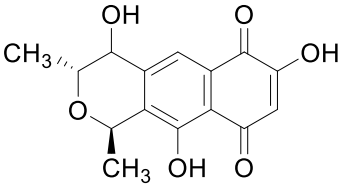
CrO_3



(+)-dilactic acid



Fremy's salt



Postdoctoral Research: MIT 1964-66

Worked on the enzyme formylglycinamide ribonucleotide amidotransferase, an enzyme of purine biosynthesis, under John M. Buchanan, Department of Biology, MIT

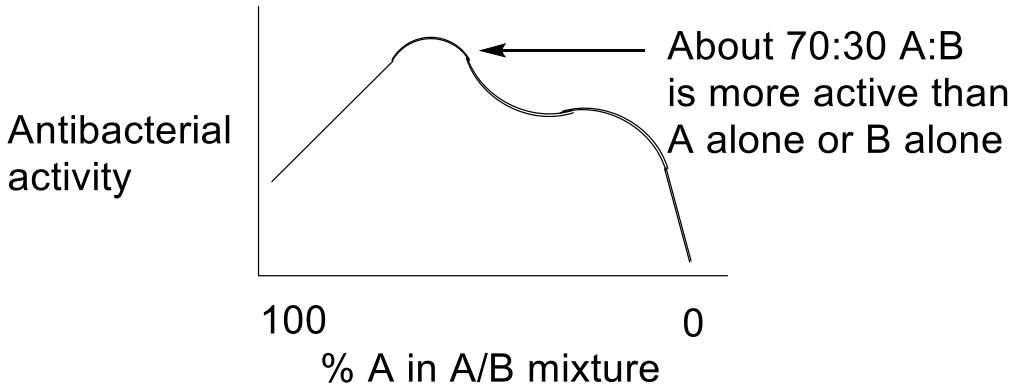
Also worked on getting to know this young lady, who later became Mrs. Kingston

Postdoctoral Research Cambridge: 1964-66
Ostreogrycins/Virginiamycins

Streptomyces ostreogriseus

↓

Ostreogrycins A and B
AKA
Streptogramins
Pristinamycins
Virginiamycins



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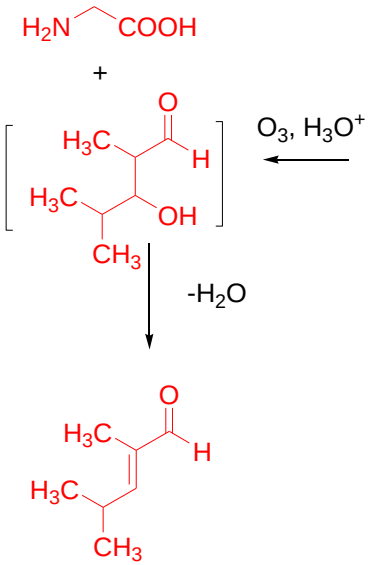
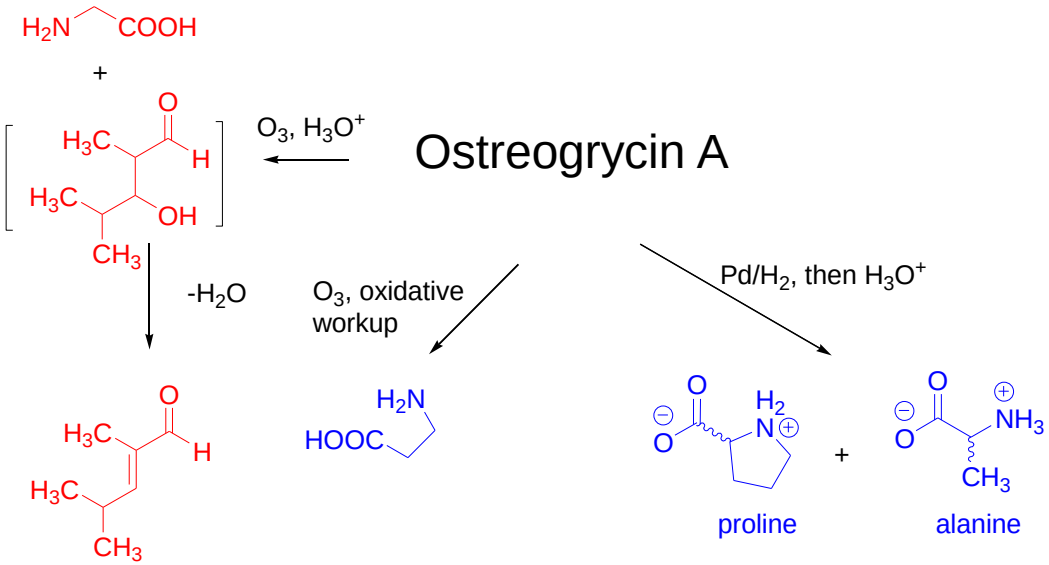
Postdoctoral Research Cambridge: 1964-66
Ostreogrycins/Virginiamycins



Ostreogrycin A

Postdoctoral Research Cambridge: 1964-66
Ostreogrycins/Virginiamycins



Postdoctoral Research Cambridge: 1964-66
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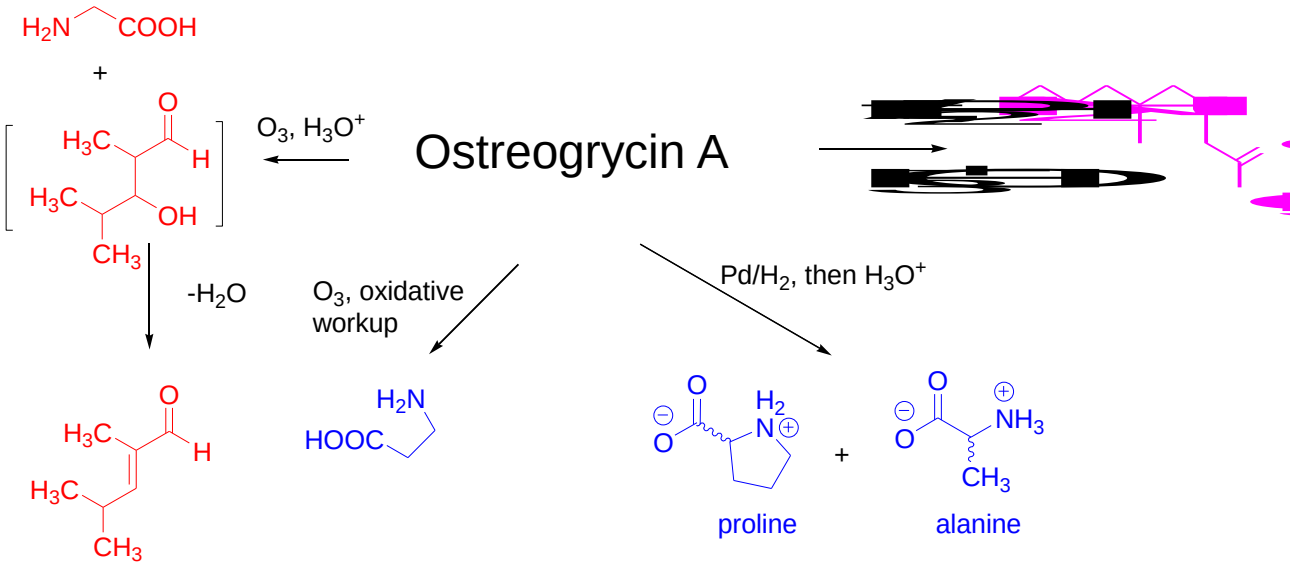

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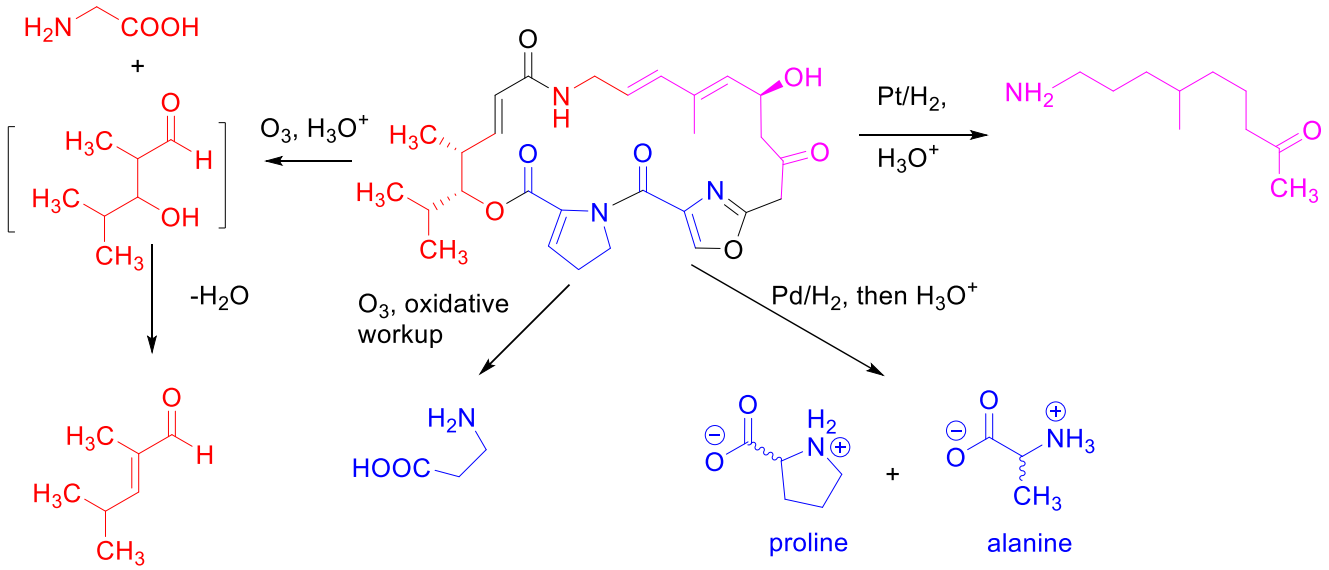

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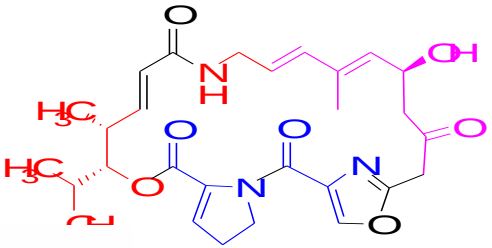
Ostreogrycins/Virginiamycins



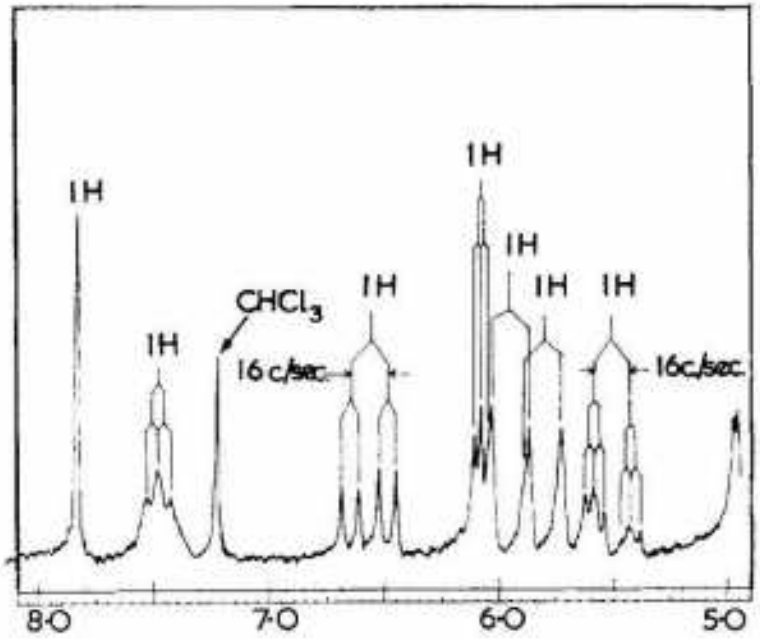


FIGURE 2 100 Mc./sec. n.m.r. spectrum of ostreogrycin A (δ = 5—8 p.p.m.)

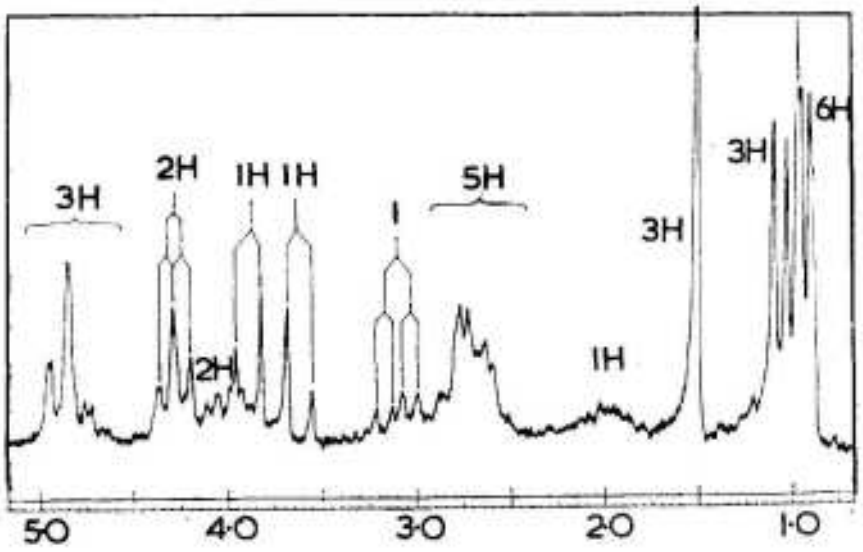


FIGURE 1 100 mc./sec. n.m.r. spectrum of ostreogrycin A (δ = 0·5—5 p.p.m.)

Kingston, D. G. I.; Todd, L.; Williams, D. H. Antibiotics of the Ostreogrycin Complex. Part III. The Structure of Ostreogrycin A. Evidence based on Nuclear Magnetic Double Resonance Experiments and High-resolution Mass Spectrometry. *J. Chem. Soc. C* ***1966, 1669-1676.***

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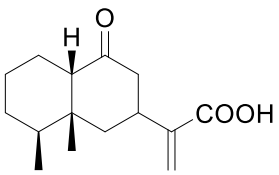
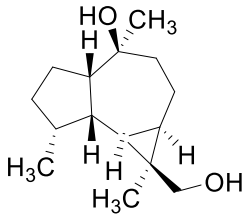
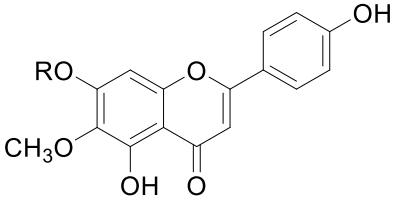
SUNY Albany: Work on Toxic Natural Products



flourensic acid



fluorensadiol



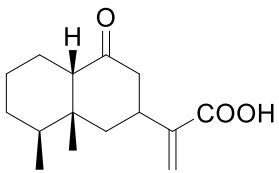
hispidulin (R = H)
cirsimaritin (R = CH_3)

Flourensia cernua

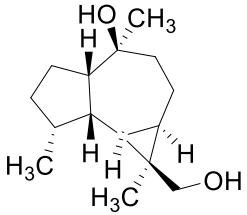
Rao, M. M., D. G. I. Kingston, et al. (1970). "Flavonoids from *Flourensia cernua*." *Phytochemistry* ***9: 227-228.***
Kingston, D. G. I., M. M. Rao, et al. (1971). "Isolation and Structure Determination of Flourensic Acid, A New Sesquiterpene of the Eremophilane Type." Tetrahedron Lett. **20:: 1613-1616.**

Virginia Tech

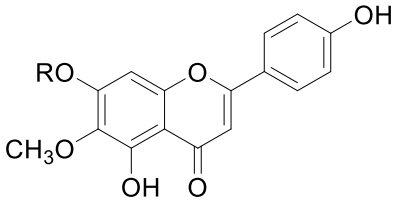
SUNY Albany: Work on Toxic Natural Products



flourensic acid



fluorensadiol



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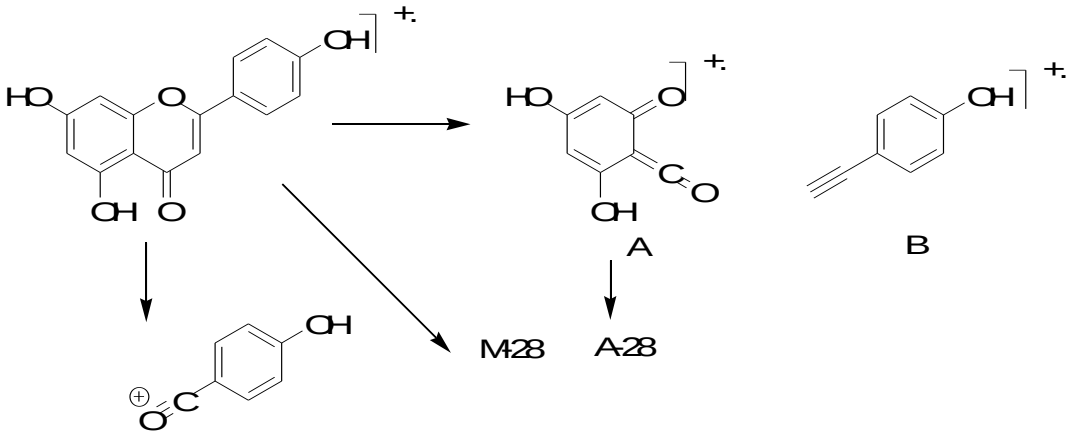
and Mass Spectrometry



Kingston, D. G. I. (1971). "Mass Spectrometry of Organic Compounds - VI. Mass Spectra of Flavonoid Compounds." Tetrahedron **27:** 2691-2700.

Virginia Tech

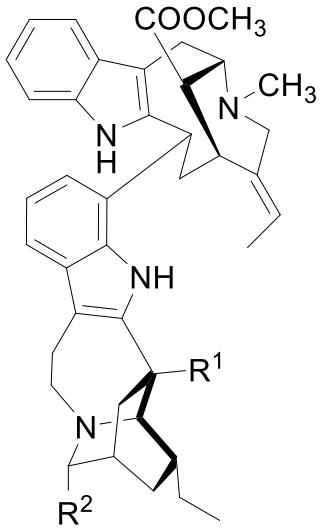
SUNY Albany: Beginnings of Anticancer Work

- My interest was inspired in part by a talk at the Gordon Conference on Natural Products by the late Morris Kupchan, who described the then novel approach of bioactivity-guided fractionation
- Jonathan Hartwell (1906 -1991) of the Natural Products Branch of the NCI was very supportive, and supplied active extracts from the NCI collection

"Dr. Hartwell was personally responsible for the initiation and early development of the research programs for natural products at the National Cancer Institute (NCI) and gave impetus to the creation of a systematic search for plants and marine animals with anticancer activity, an ongoing project for over 30 years"

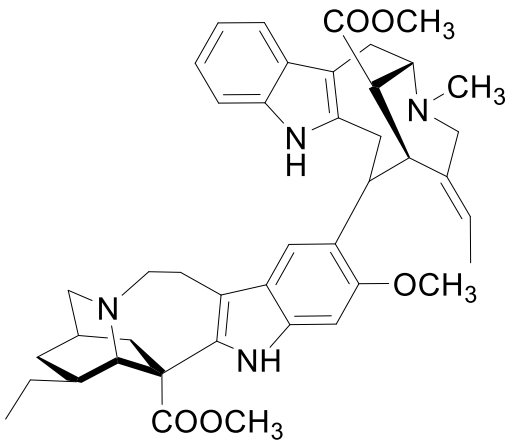
G. R. Pettit J. Nat. Prod. **1995**, *58*, 359-364

Virginia Tech: Anticancer Natural Products

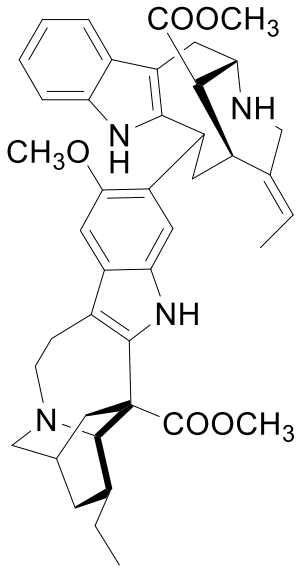


Tabernamine $R^1 = R^2 = H$

19-(2-oxopropyl)conodurine $R^1 = COOCH_3$, $R^2 = COCH_3$



Gabunamine



13'-Perivoacangine

Natural products with low micromolar ED_{50} values

Semisynthetic compound with a high nanomolar ED_{50} value

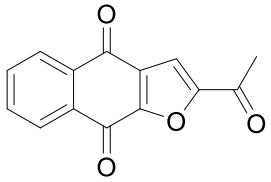
Kingston, D. G. I.; Gerhart, B. B.; Ionescu, F.; Mangino, M. M.; Sami, S. N. Plant Anticancer Agents V: New Bisindole Alkaloids from *Tabernaemontana johnstonii Stem Bark. J. Pharm. Sci.* ***1978, 67:, 249-251.***

Kingston, D. G. I., Plant Anticancer Agents VII: Structural Effects on Cytotoxicity of Bis-indole Alkaloids of Voacamine Type. *J. Pharm. Sci.* **1978,** 67:: 272-274.

Virginia Tech

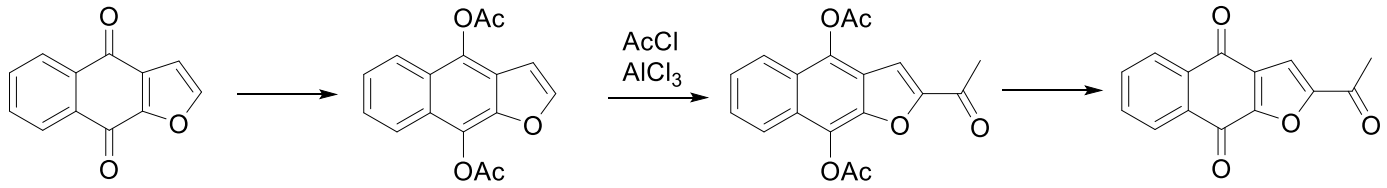
Virginia Tech: Napabucasin

2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-dione was isolated from *Tabebuia cassinoides.* It had only modest cytotoxic activity, with an ED_{50} value of 4.2 μM against the KB cell line.



2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-dione

Its structure was confirmed by synthesis from naphtho[2,3-b]furan-4,9-dione by reductive acetylation, Friedel-Crafts acylation, and hydrolysis followed by aerial oxidation.



naphtho[2,3-*b*]furan-4,9-dione

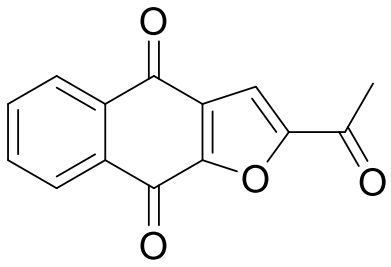
It was evaluated for *in vivo* activity in the P-388 leukemia mouse model, but it did not show any significant activity at the doses tested.

This appeared to be the end of the story, and the results were published: Rao, M. M.; Kingston, D. G. I., Plant Anticancer Agents. XII. Isolation and Structure Elucidation of New Cytotoxic Quinones from *Tabebuia cassinoides J. Nat. Prod.* **1982**, *45,* 600-604

Virginia Tech

Virginia Tech: Napabucasin

- Thirty-three years later, in 2015, a team at Boston Biomedical Inc. (now Sumitomo Dainippon Pharma Oncology, Inc.) reported that their experimental drug BBI608 had significant activity in suppressing cancer relapse and metastasis by inhibiting cancer stemness.
- Li et al. Suppression of cancer relapse and metastasis by inhibiting cancer stemness. Proc Natl Acad Sci U S A **2015**, 112, 1839-44.
- BBI608 was later identified as 2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-dione, and named napabucasin



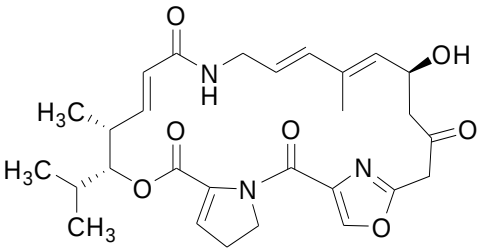
napabucasin

- Napabucasin has been shown to be activated by NAD(P)H:quinone oxidoreductase-1 (NQO1), leading to redox cycling and the generation of reactive oxygen species (ROS). This in turn causes numerous cellular changes, including a reduction in STAT3 phosphorylation, and leads to cell death. Froeling et al.. Clin Cancer Res **2019**, 25, 7162-7174.
- It is currently in Phase III clinical trials for colorectal cancer and various Phase I/II combination trials.
- Assuming a successful outcome of the clinical trials, napabucasin can be added to the long list of natural products as anticancer agents.

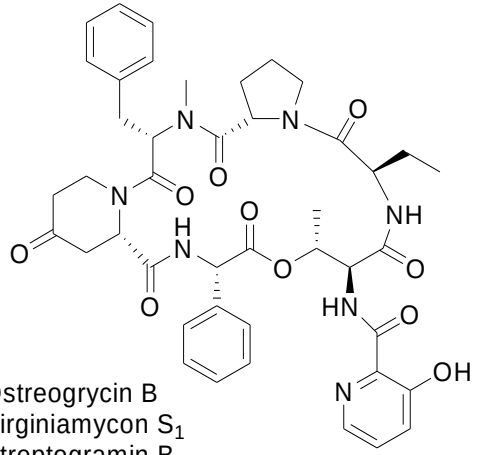
Virginia Tech

Biosynthesis of Ostreogrycins/Virginiamycins

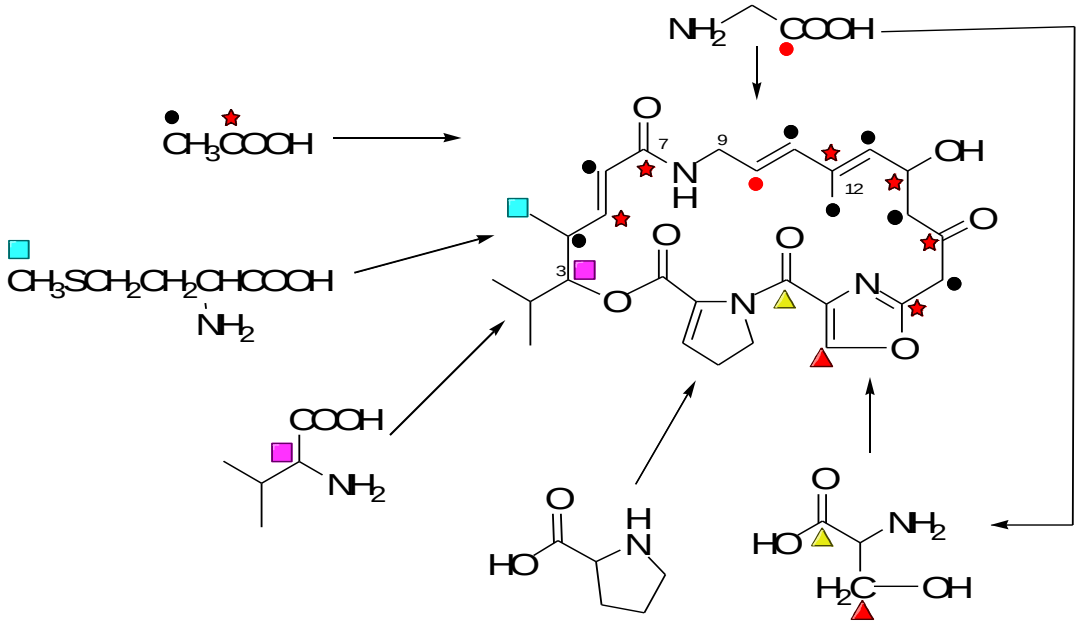
Streptomyces ostreogriseus



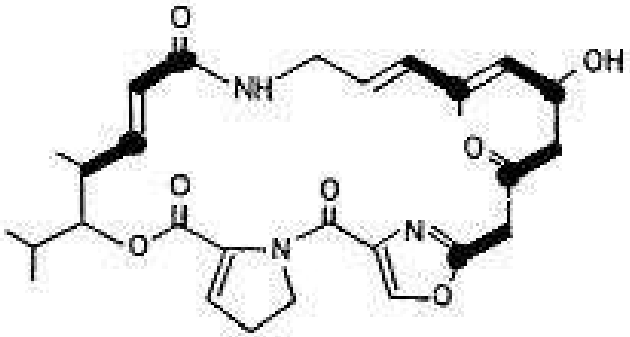
Ostreogrycin A
Virginiamycon M_1
Streptogramin A



Ostreogrycin B
Virginiamycon S_1
Streptogramin B

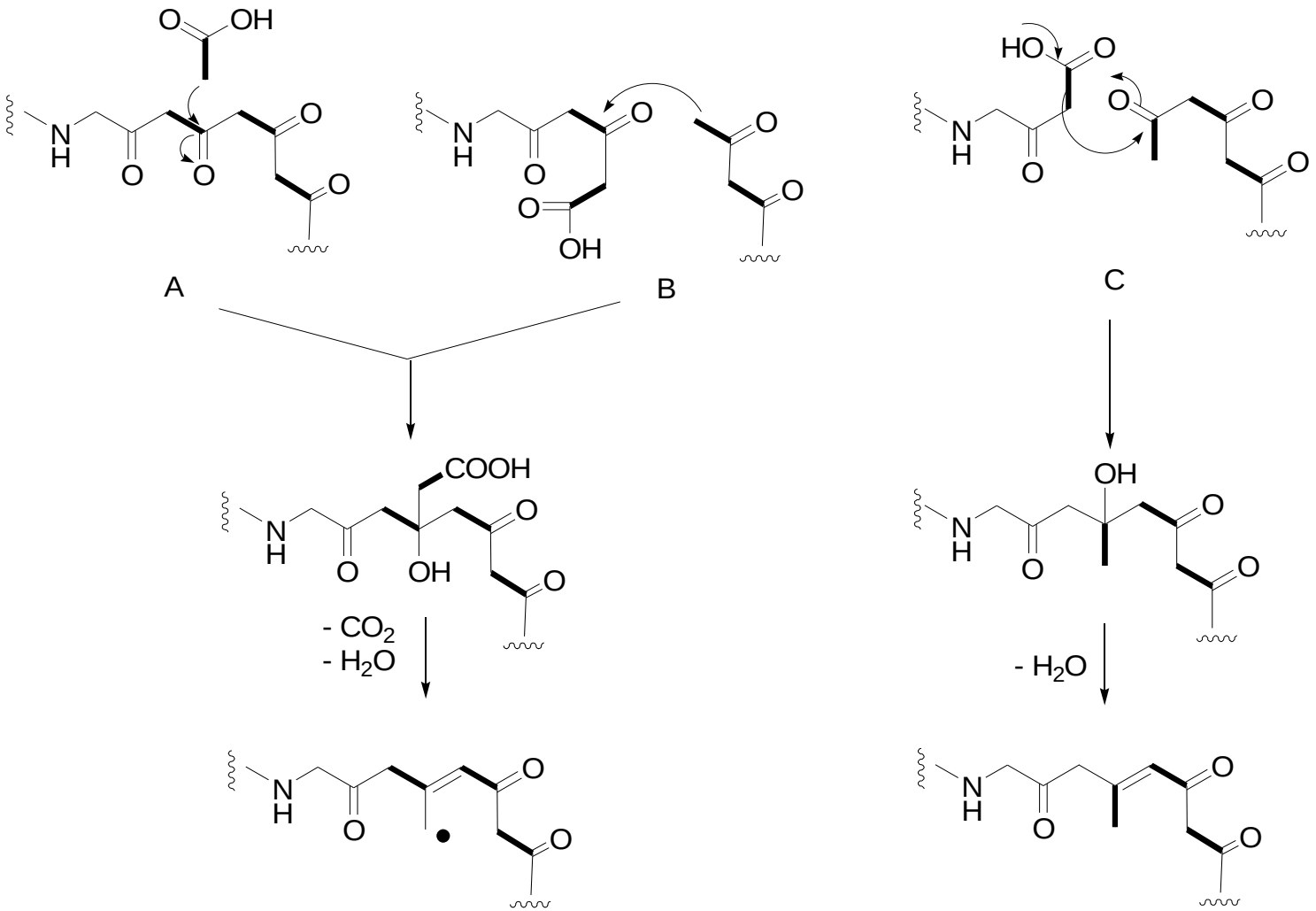


Biosynthesis of Virginiamycin M_1 from 1,2-^{13}C acetate

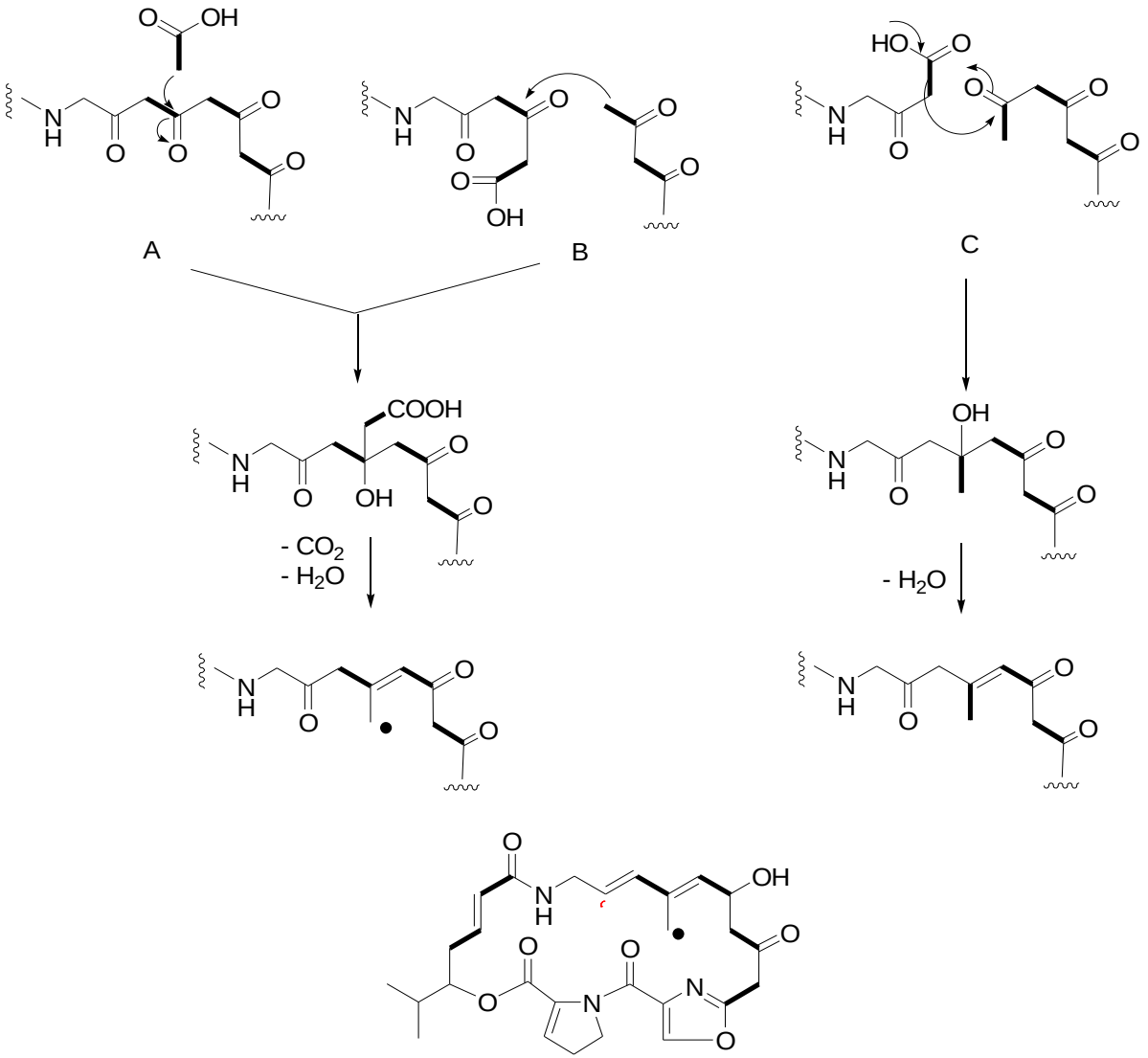


Biosynthesis of Ostreogrycins/Virginiamycins

How is the C-12 methyl group incorporated? It is not derived from S-adenosyl methionine, but it could be formed by three different pathways, A-C. The use of double labeled acetate provided a way to distinguish them.



Biosynthesis of Ostreogrycins/Virginiamycins



The result fits either pathway A or B but not pathway C Analysis of serine incorporation (serine to acetate by a roundabout path) suggests that the C-12 methyl group is incorporated later, and supports pathway A

Kingston, D. G. I.; Kolpak, M. X.; LeFevre, J. W.; Borup-Grochtmann, I. Biosynthesis of Anitbiotics of the Virginiamycin Family. 3. Biosynthesis of Virginiamycin M1 *J. Am. Chem. Soc.* ***1983 105:, 5106-5110***

Fecal Mutagens

- W. R. Bruce (Toronto) identified a highly potent ether-extractable mutagen in some samples of human feces in 1977
- This finding was confirmed by Tracy Wilkins at Virginia Tech in 1979
- The mutagen was only available in milligram quantities, but had a unique UV spectrum typical of pentaenes. It was also unstable
- Could this be a possible cause of colon cancer?

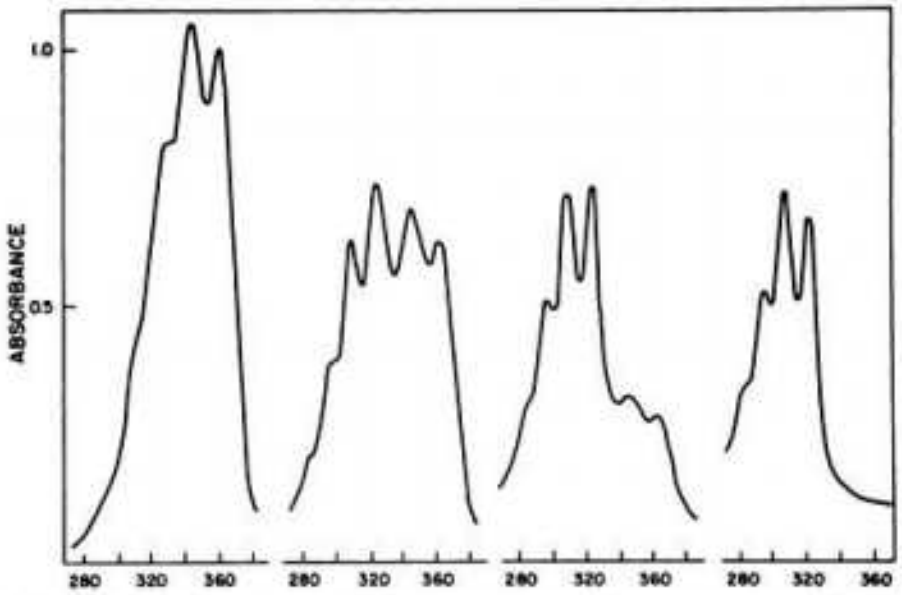
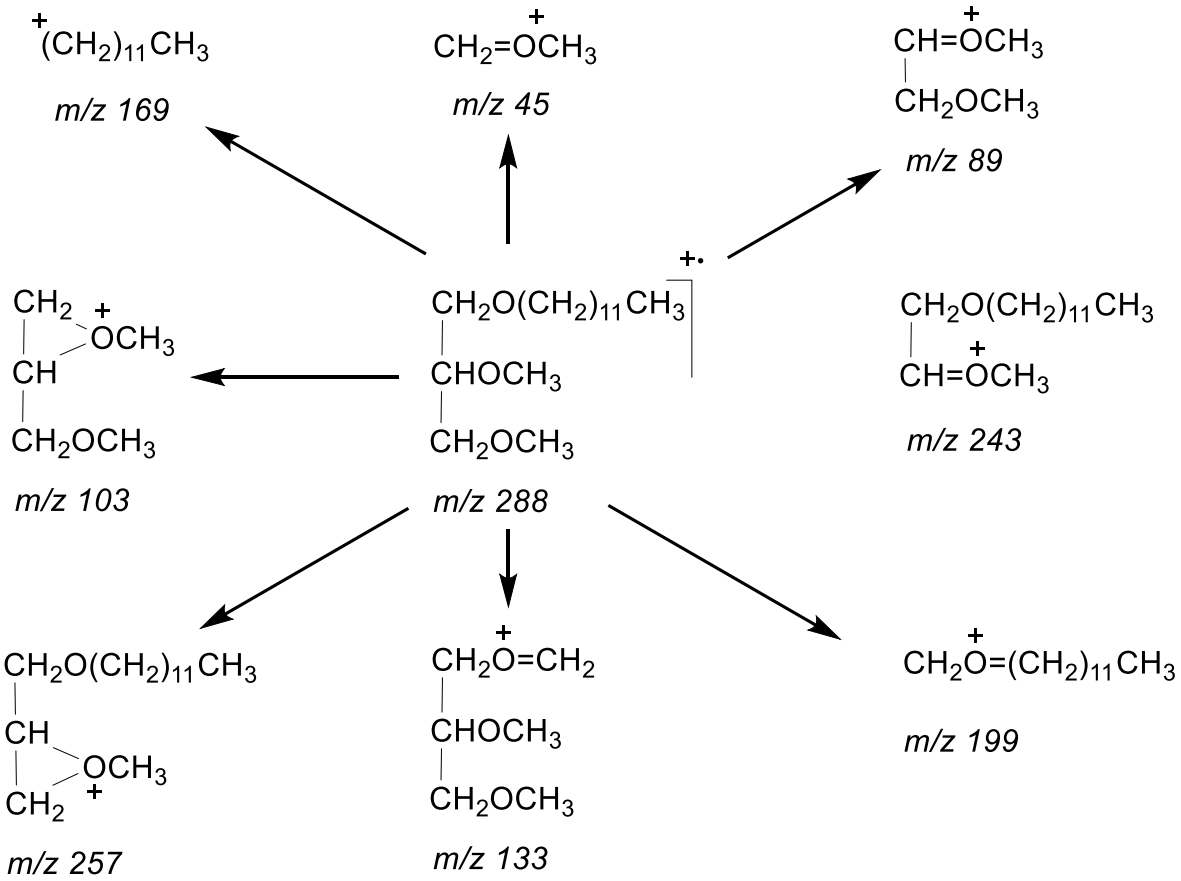


FIG. 1. The shift in the ultraviolet absorption spectrum of purified mutagen upon oxidation. Potassium superoxide (100 mg) was added to mutagen diluted in methylene chloride (2 ml). This oxidation from the "parent" state (*left*) to the oxidized state (*right*) took 3 hr.

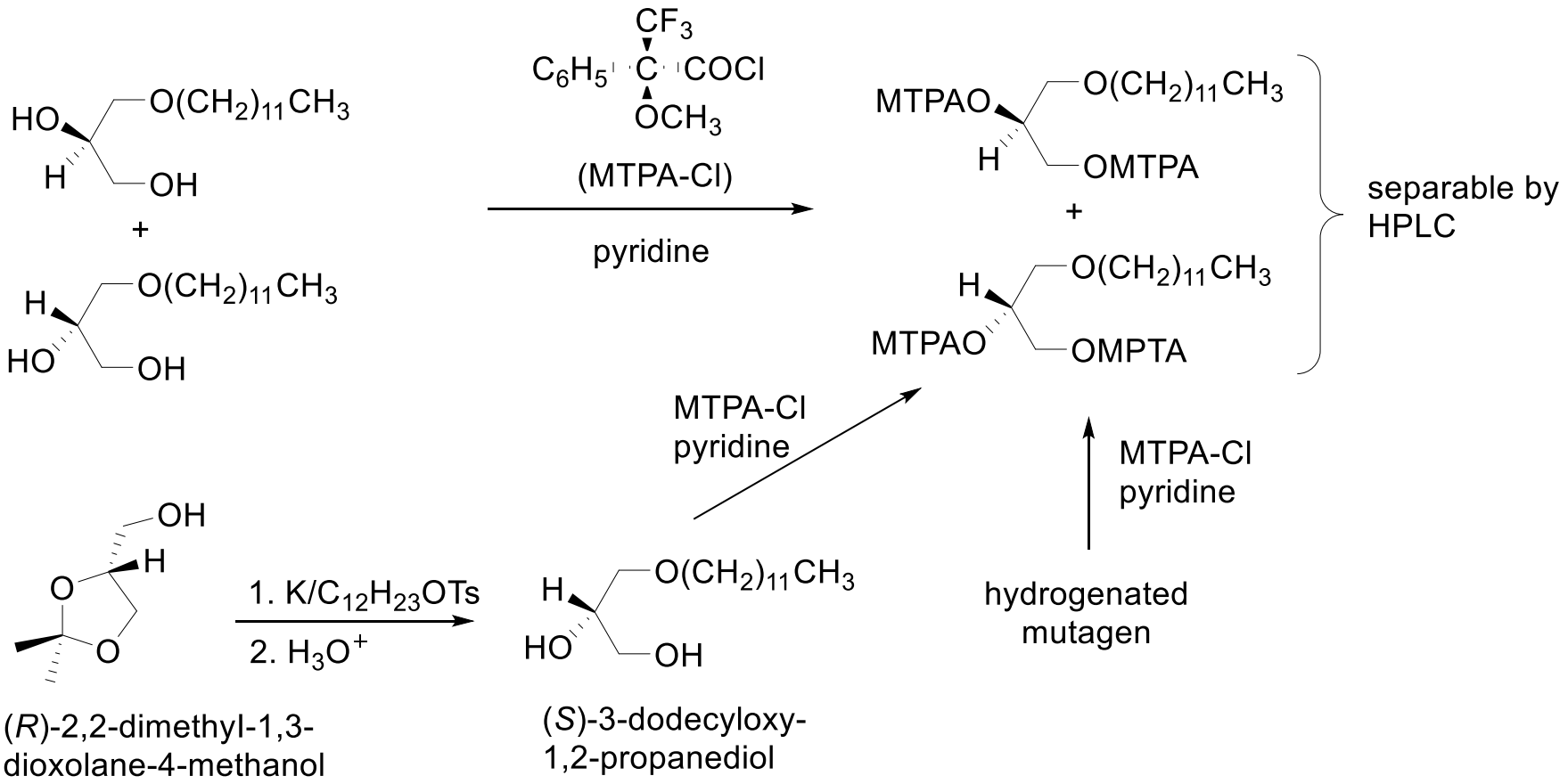
Fecal Mutagens

- The structure was solved by hydrogenation and methylation followed by mass spectrometry of the resulting dimethyl ether
- Comparison with the mass spectroscopic data of synthetic model compounds established the structure of the hydrogenated compound as the dimethyl ether of 3-dodecyloxy-1,2-propanediol



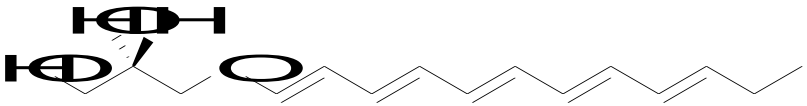
Fecal Mutagens

The absolute configuration of the 2-hydroxyl group was established by comparison of the HPLC retention times of the chiral (+)–α–methoxy-α-(trifluoromethyl)–α–phenylacyl (MTPA) esters of synthetic model compounds with that of the hydrogenated natural product



Fecal Mutagens

This led to to the following structure for fecapentaene-12, consistent with its ^{1}H NMR spectrum



Fecapentaene-12

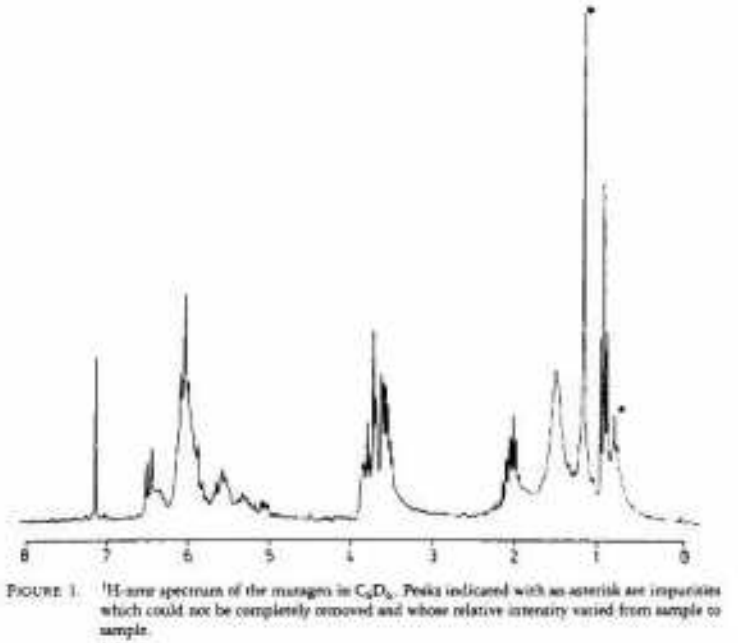


FIGURE 1. ^{1}H-nmr spectrum of the mutagen in C_6D_6. Peaks indicated with an asterisk are impurities which could not be completely removed and whose relative intensity varied from sample to sample.

So are the fecapentaenes significant contributors to colon cancer?

Hirai, N., D. G. I. Kingston, et al. (1982). "Structure Elucidation of a Potent Mutagen from Human Feces." J. Am. Chem. Soc. **104:: 6149-6150.**

Hirai, N., D. G. I. Kingston, et al. (1985). "Isolation and Structure Elucidation of Fecapentaenes-12, Potent Mutagens from Human Feces." J. Nat. Prod. **48:: 622-630.**

Fecal Mutagens

This led to to the following structure for fecapentaene-12, consistent with its ^{1}H NMR spectrum

Fecapentaene-12

So are the fecapentaenes significant contributors to colon cancer?

Probably not. A study by John Weisburger et al. treated both newborn mice and adult rats with fecapentaene-12. After 21 months the mice treated as newborns had developed various neoplasms, but the rats treated as adults did not display any neoplasia associated with the mutagen. The authors conclude “Fecapentaene may exert its effect in bacteria and in newborn mice through the generation of hydroxy radicals. However, adult rodent and human colon may have adequate biochemical defense mechanisms against low level, even continuous exposures to chemicals like FP-12, and thus be at low risk of neoplasia.”

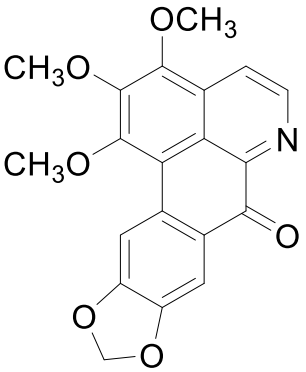
Weisburger, J. H.; Jones, R. C.; Wang, C.-X.; Backlund, J.-Y. C.; Williams, G. M. et al. Carcinogenicity Tests of Fecapentaene-12 in Mice and Rats. *Cancer Lett.* ***1990, 49, 89-98.***

Fecal Mutagens

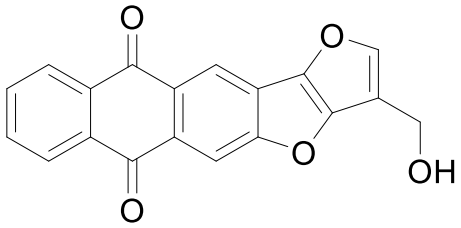
To Norm Farnsworth's disappointment the ASP Annual Meeting at the conclusion of my year as ASP President was an international joint meeting in Bonn, and he was not able to roast me as he wished.

Virginia Tech: Anticancer Natural Products

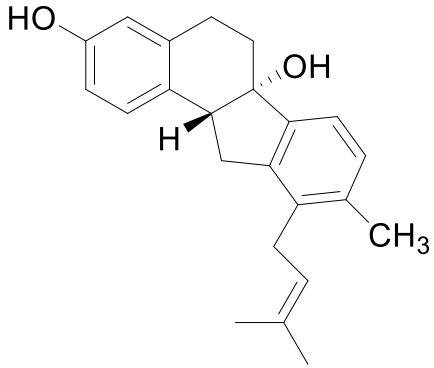
Compounds with activity against topoisomerase I or II



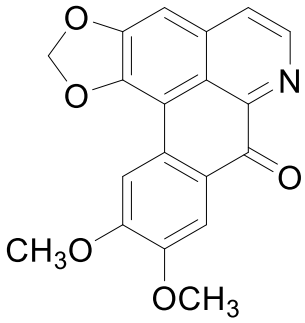
Xylopia aethiopica



Crescentia cujete



Erythrina burana



Dicentrinone
Inhibitor of
Topoisomerase 1

Gunatilaka, A. A. L.; Kingston, D. G. I.; Johnson, R. K. Mechanism-based Isolation and Structures of Some Anticancer Active Natural Products. Pure Appl. Chem. **1994,** 66, 2219-2222.

Zhou, B.-N.; Johnson, R. K.; Mattern, M. R.; Wang, X.; Hecht, S. M. et al. Isolation and Biochemical Characterization of a New Topoisomerase I Inhibitor from *Ocotea leucoxylum*. *J. Nat. Prod.* ***2000, 63, 217-221.***

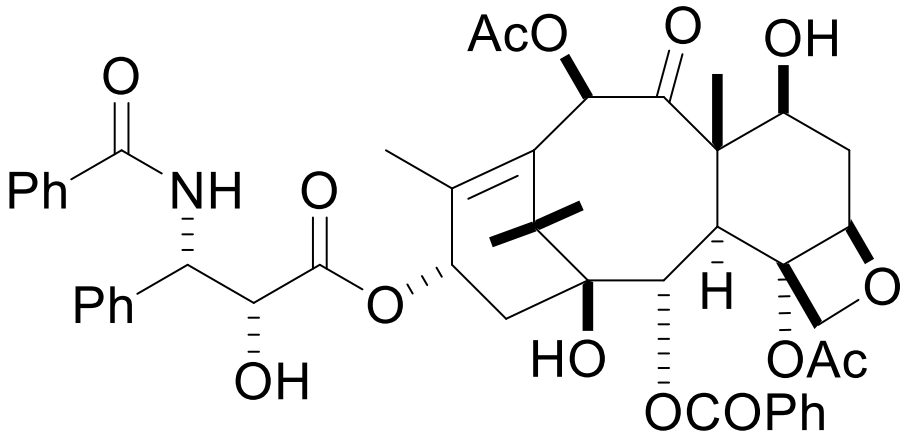
Virginia Tech

Taxol

Taxol was isolated by Monroe Wall and Mansukh Wani and its structure published in 1971

Mansukh Wani
1925 - April 11, 2020

Monroe Eliot Wall
(1916 – 2002)



Taxol

Initial reaction to the discovery of taxol: Underwhelming enthusiasm!!
Because of three problems:

A. The supply problem. *T. brevifolia* is not usually a large tree, and taxol was obtained in only 0.02% from its thin bark.

B. The solubility problem. Taxol is not water-soluble.

C. The activity problem. Its *in vivo* activity was mainly in mouse leukemia models, and it was not considered any better than many other leads.

D. The structure problem. Taxol has a very complex structure, and it was clearly not going to be accessible in high yield by total synthesis.

T. brevifolia in the Oregon State University Research Forest

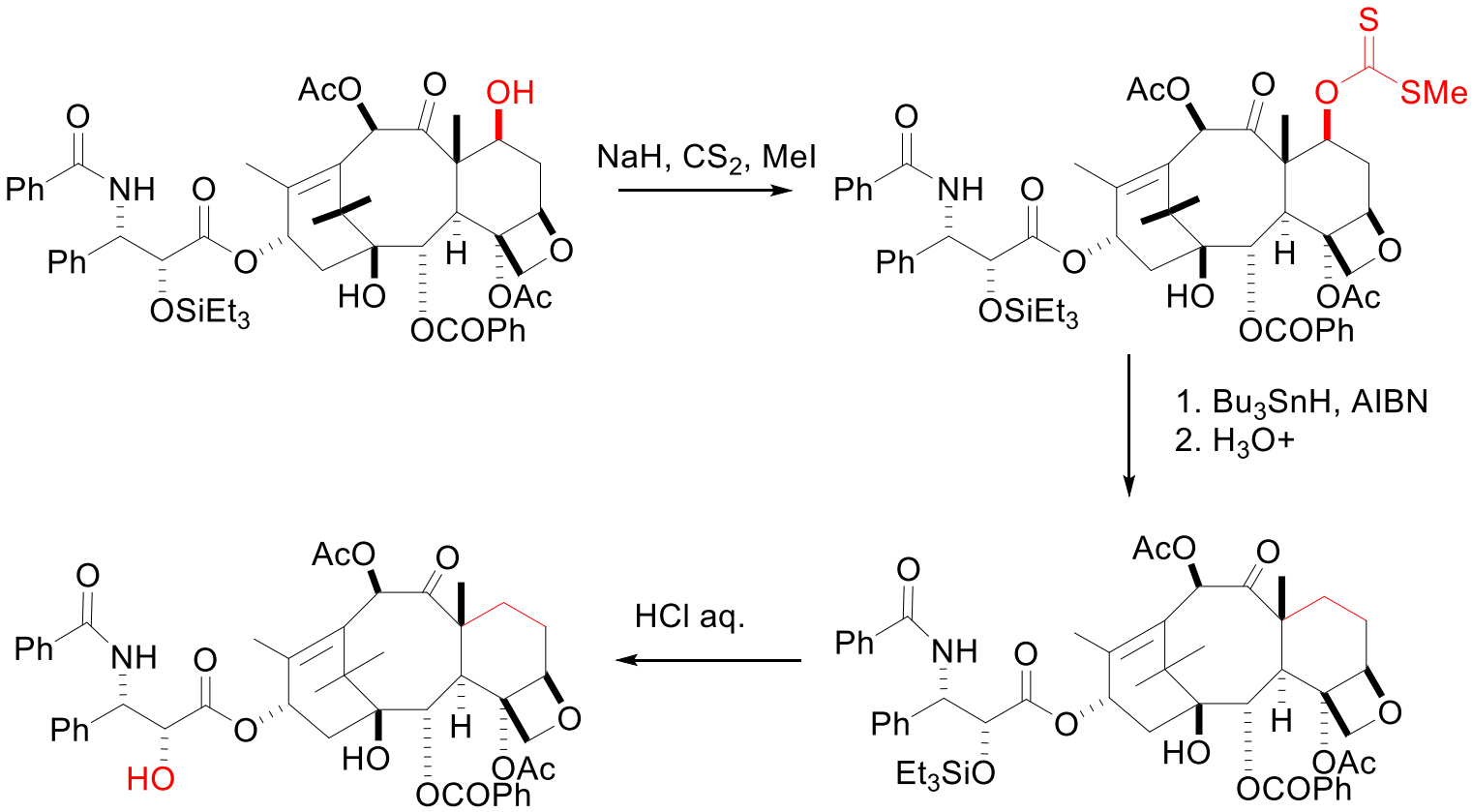
Taxol

- I began work on taxol in 1979, in a collaboration with Bob Holton
- My work was greatly helped by support from Matt Suffness, who arranged for me to receive crude taxol samples from PolySciences Inc., the contractor for taxol supplies for preclinical studies
- Matt was a major advocate for the development of taxol, and it is likely that taxol would not have been developed as a drug without his and Monroe Wall's advocacy

Matt Suffness
1943 - 1995

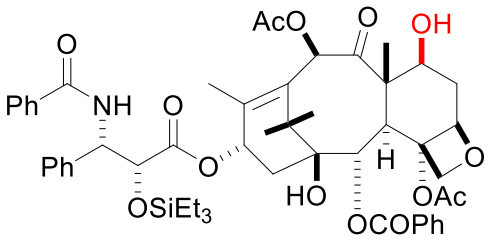
Taxol

Initial studies were aimed at uncovering taxol's SAR
It was found that deletion of some functional groups did not significantly affect taxol's bioactivity in cell culture

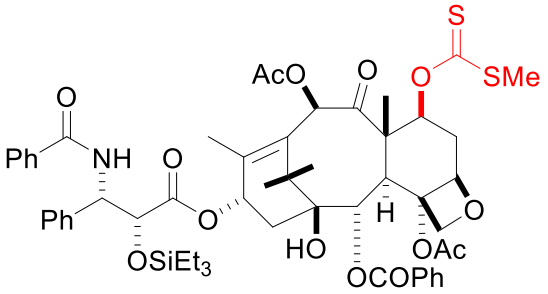


IC_{50}/IC_{50}(taxol) = 1.0 (HCT 116)
Chaudhary et al. J. Org. Chem., **1993**, *58*, 3978-3979.

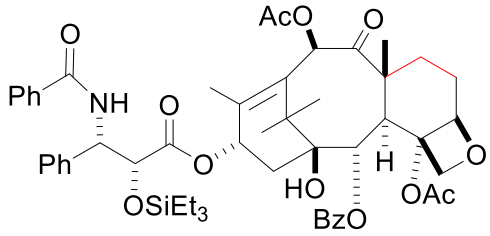
Taxol



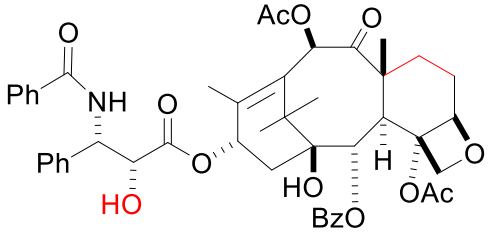
NaH, CS_2, MeI



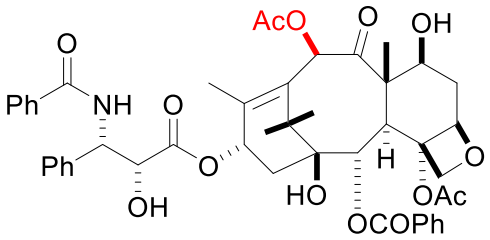
1. Bu_3SnH, AIBN
2. H_3O^+

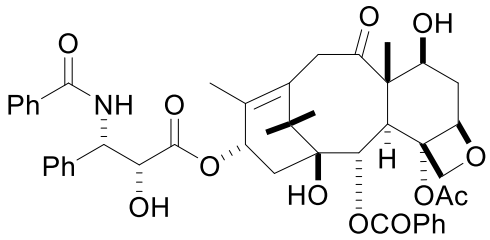


HCl aq.



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

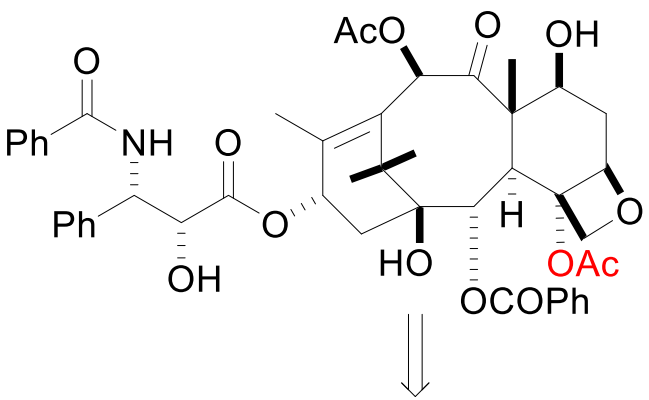


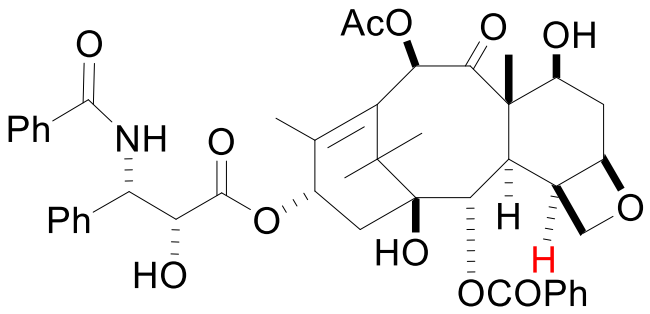


IC_{50}/IC_{50}(taxol) = 1.75 (HCT 116)
Chaudhary et al. Tetrahedron Lett., **1993**, 34, 4921-4924.

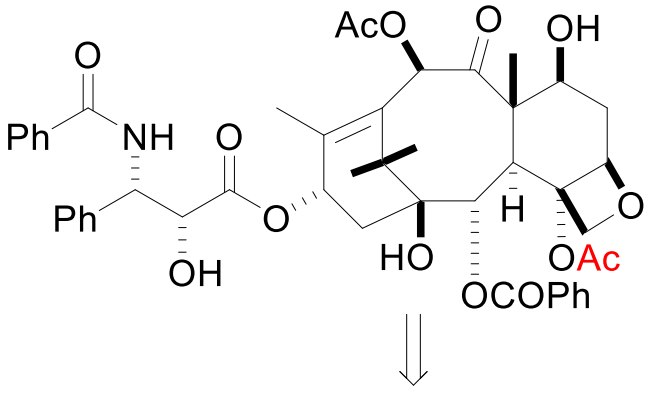
Taxol

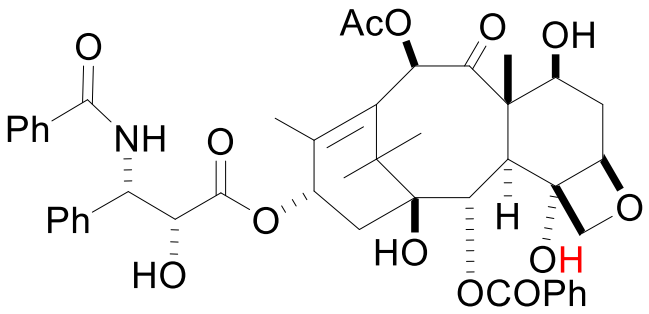
But deletion of other functional groups DID significantly affect taxol's bioactivity in cell culture





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



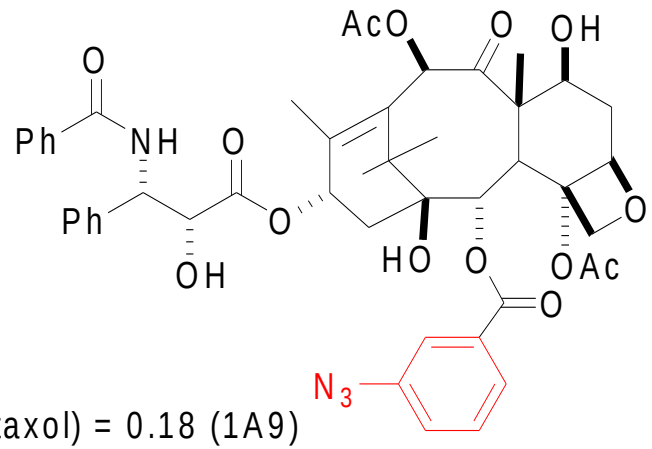


IC_{50}/IC_{50}(taxol) = >33 (CA46)
Neidigh et al. *Tet. Lett.* **1994**, 6839

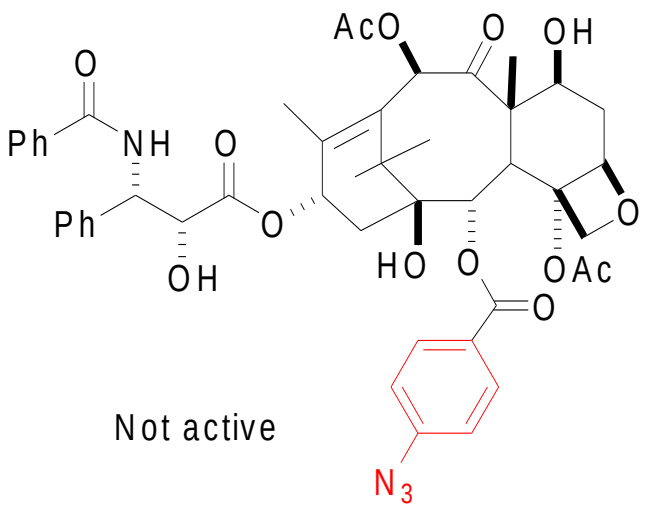
Virginia Tech

Taxol

In some cases a change of functional group could have either a positive or negative effect on taxol's bioactivity



IC_{50}/IC_{50}(taxol) = 0.18 (1A9)
Over fivefold more potent

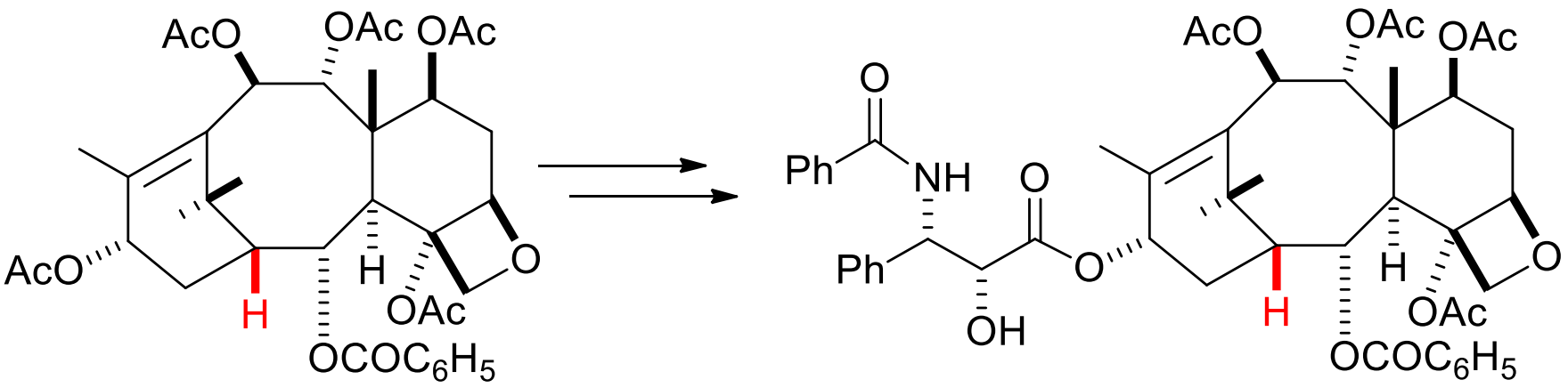


Not active

Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L. et al. Unexpectedly Facile Hydrolysis of the 2-Benzoate Group of Taxol and Synthesis of Analogs with Increased Activities. *J. Am. Chem. Soc.* ***1994, 116, 4097-4098.***

Taxol

Is the C1-hydroxyl group necessary for activity?

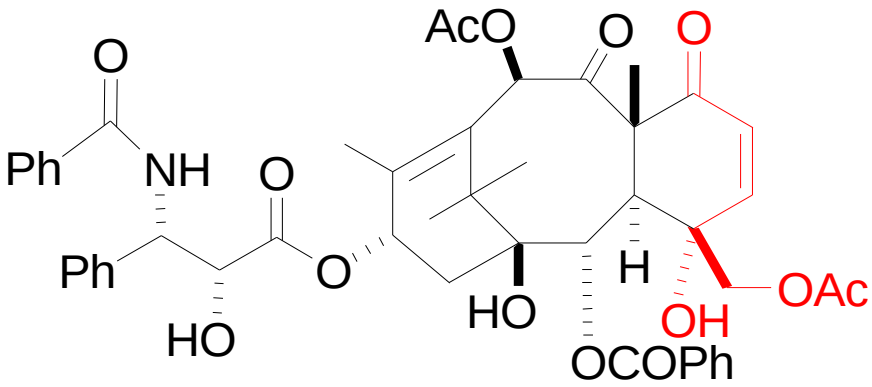


Removal of the C-1 hydroxyl group results in a small but significant loss in both tubulin-assembly activity and cytotoxicity compared with 7,9-diacetyl-9(R)-dihydropaclitaxel.

Kingston, D. G. I.; Chordia, M. D.; Jagtap, P. G.; Liang, J.; Shen, Y.-C. et al. Synthesis and Biological Evaluation of 1-Deoxypaclitaxel Analogues. J. Org. Chem. 1999, 64, 1814-1822.

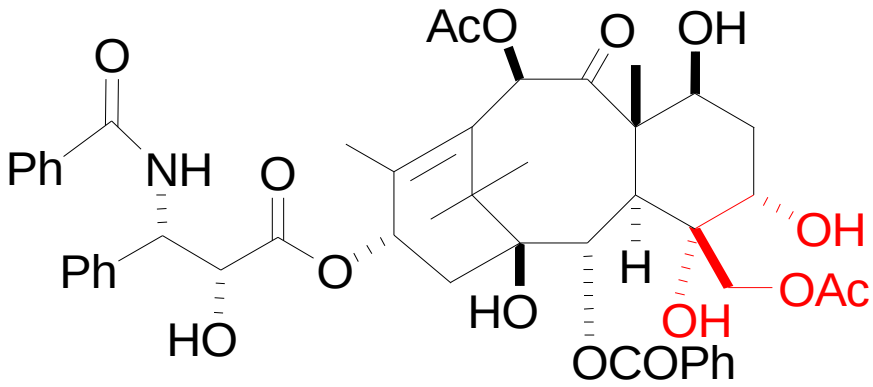
Taxol

Early studies indicated that the opening of the oxetane ring led to loss of activity



Inactive

Magri et al. J. Org. Chem., **1986,** 51, 797-802.



Inactive

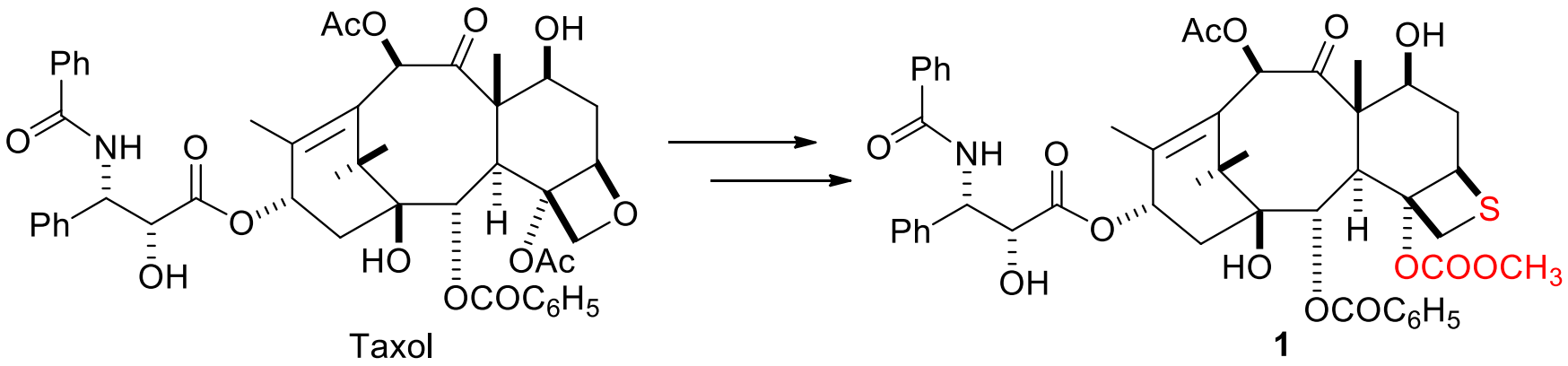
Samaranayake et al. J. Org. Chem., **1991**, 56, 5114-5119.

Was this due to an intrinsic requirement for the oxetane ring, or was it due to the conformational lock provided by the ring? What would be the effect of replacing the oxygen with sulfur?

Virginia Tech

Taxol

Comparison of biological activities of compound **1** with those of taxol.

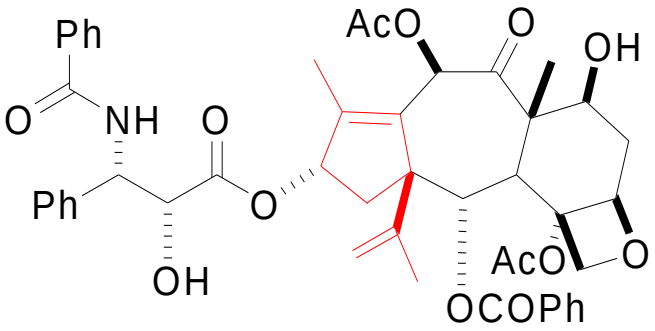


Compound	Tubulin polymer (EC_{50}, μM, ± SD)	Effects on human cancer cell growth IC_{50} (nM)	
		CA46 Burkitt lymphoma	PC3 Prostate carcinoma
Taxol	1.5 ± 0.2	5	4
1	13 ± 0.7	>1000	>2500

Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M. H.; Kingston, D. G. I.; Sackett, D. L. et al. Synthesis and Biological Evaluation of Novel Paclitaxel (Taxol) D-Ring Modified Analogues. J. Org. Chem. 1999, 64, 2694-2703.

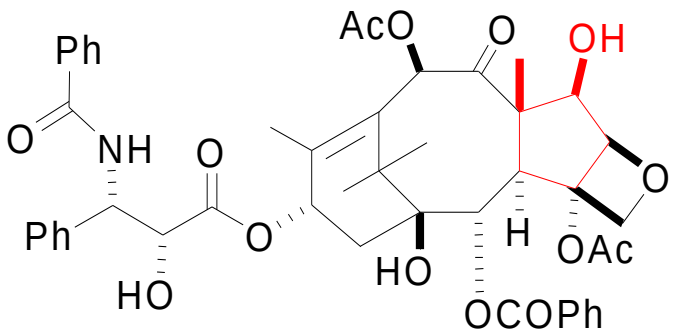
Taxol

Ring contraction of either the A or the C rings causes activity loss



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

Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. Modified Taxols. 5. Reaction of Taxol with Electrophilic Reagents, and Preparation of a Rearranged Taxol Derivative with Tubulin Assembly Activity. *J. Org. Chem.* ***1991, 56, 5114-5119.***

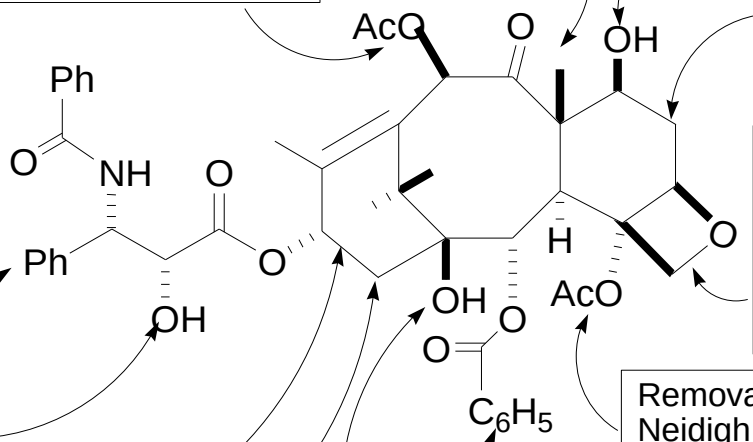


IC_{50}/IC_{50}(taxol) = 9.6 (HCT116)

Liang, X.; Kingston, D. G. I.; Long, B. H.; Fairchild, C. A.; Johnston, K. A. Synthesis, Structure Elucidation, and Biological Evaluation of C-Norpaclitaxel. *Tetrahedron Lett.* ***1995, 36, 7795-7798.***

Virginia Tech

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

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.

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

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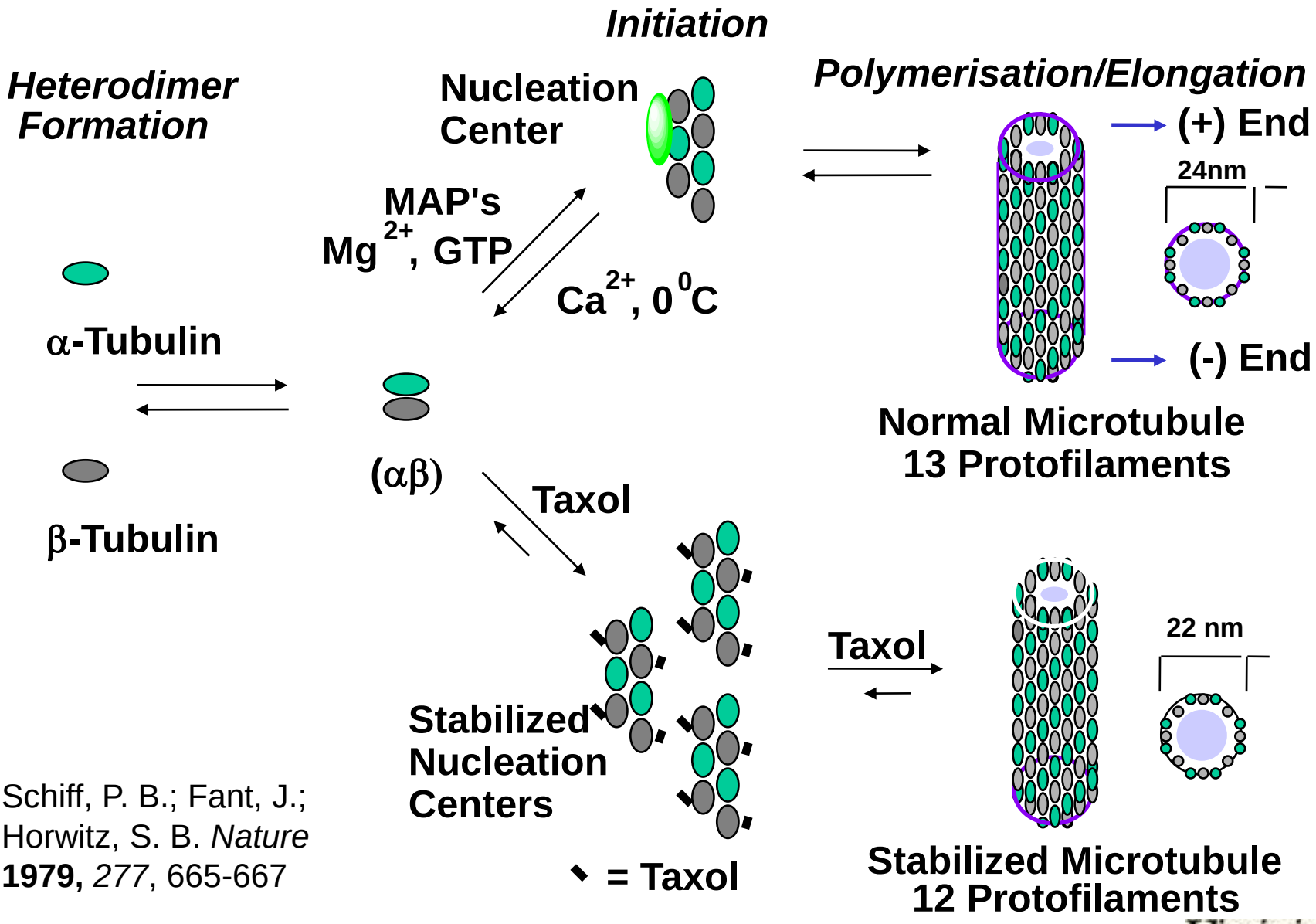
Taxol's Mechanism of Action



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

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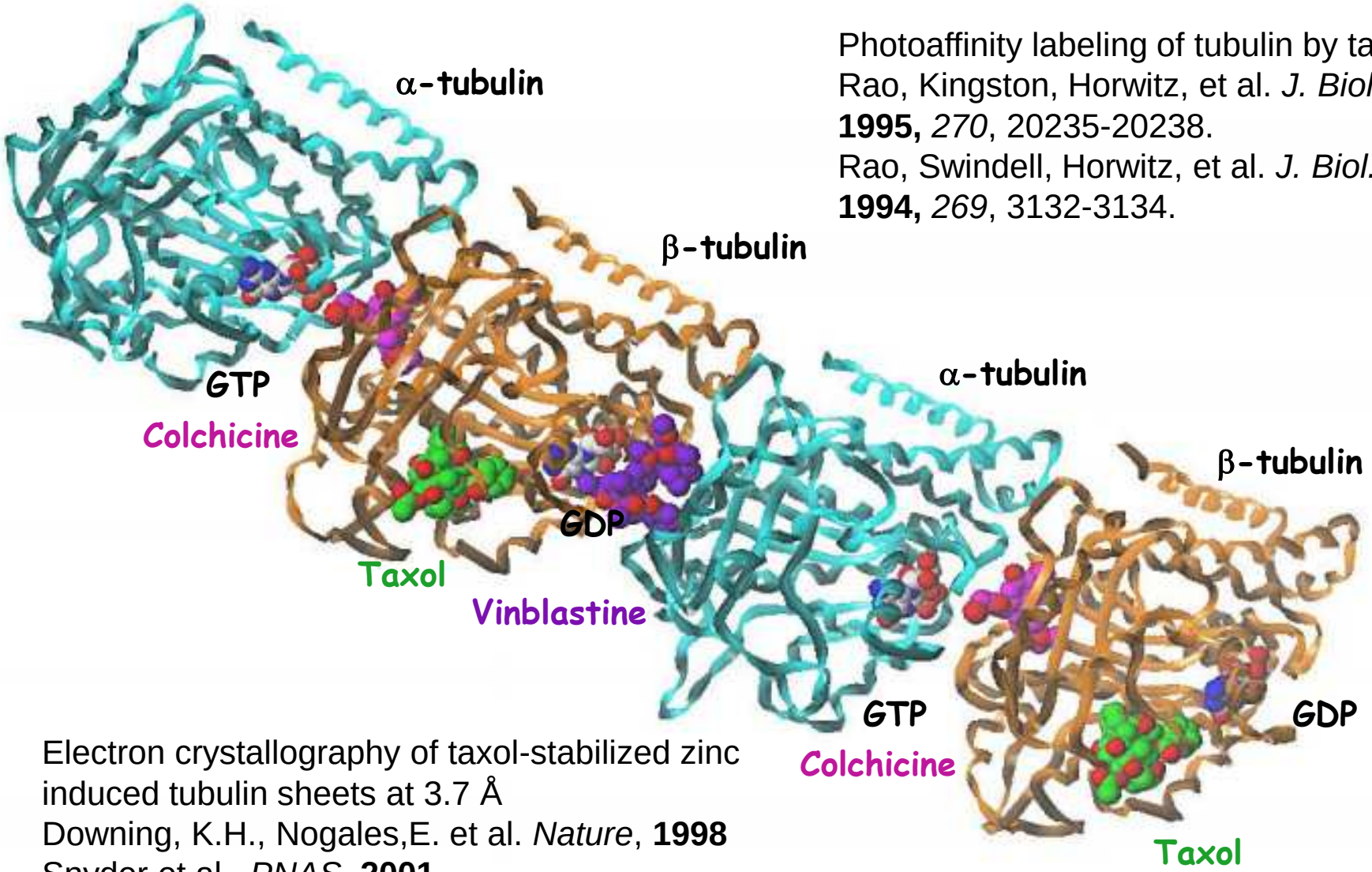
Taxol's Microtubule Binding



Photoaffinity labeling of tubulin by taxol
Rao, Kingston, Horwitz, et al. *J. Biol. Chem.* **1995,** *270*, 20235-20238.
Rao, Swindell, Horwitz, et al. *J. Biol. Chem.* **1994,** *269*, 3132-3134.

Electron crystallography of taxol-stabilized zinc induced tubulin sheets at 3.7 Å
Downing, K.H., Nogales,E. et al. *Nature*, **1998**
Snyder et al., *PNAS*, **2001**

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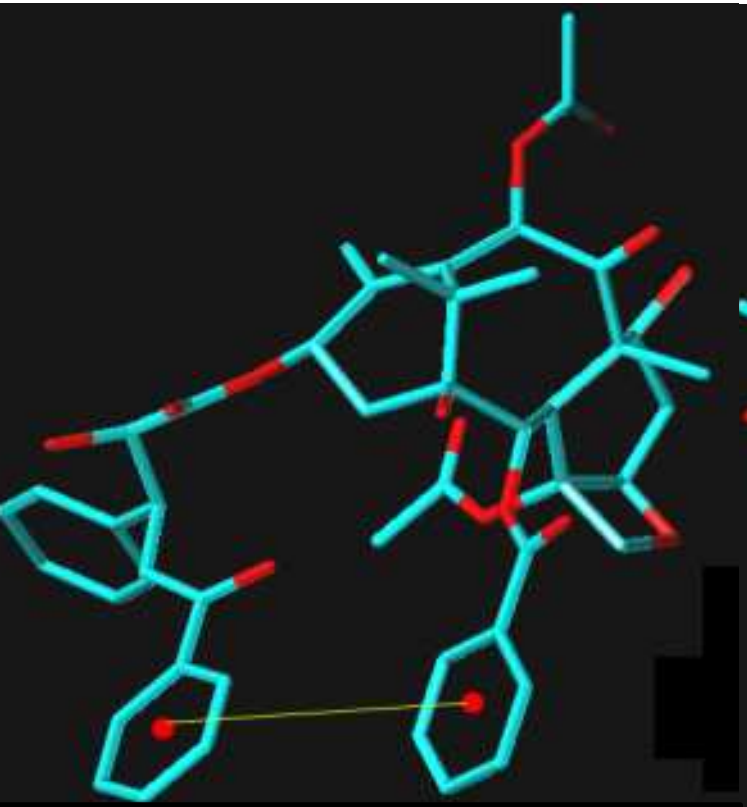
Taxol's Microtubule Binding

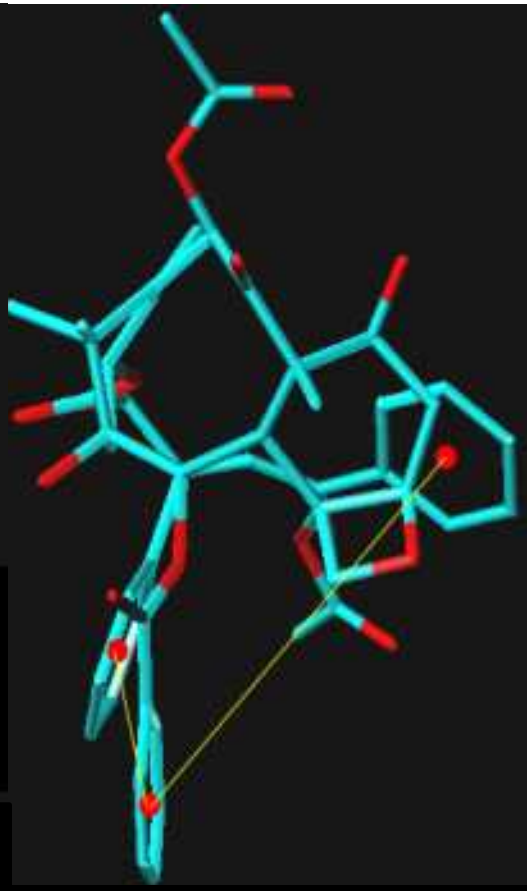
- The resolution of the electron crystallographic structure of the taxol-tubulin complex is too low to show the conformation of taxol
- A knowledge of the binding conformation(s) could lead to the design of improved analogs
- It could also lead to the design of simpler analogs which retain Taxol's tubulin-binding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

Taxol's Microtubule Binding

- 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
- 1JFF is an electron crystallographically refined structure

Taxol's Microtubule Binding





Non-polar
($CHCl_3$)

Polar
($DMSO/H_2O$)

"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

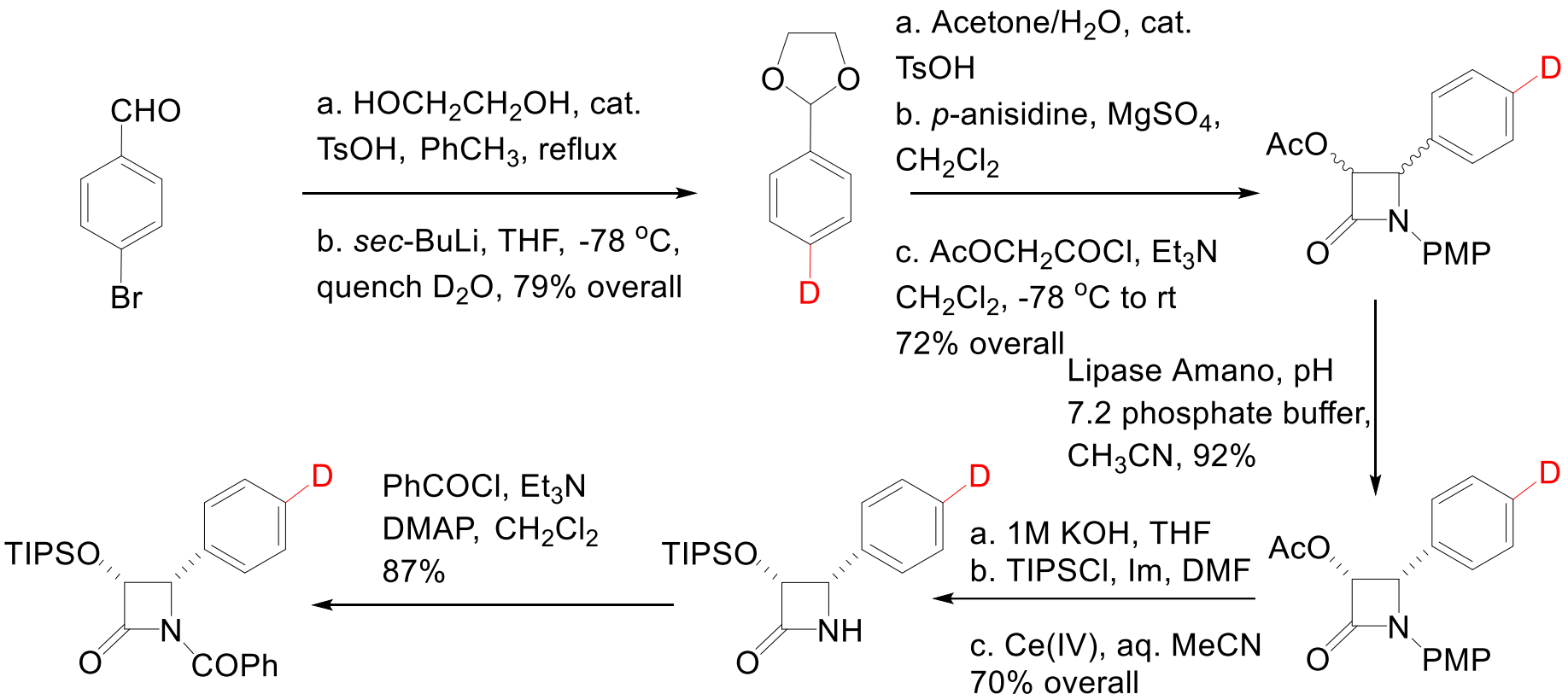
T-shaped conformer
Snyder et al.

Taxol's Microtubule Binding

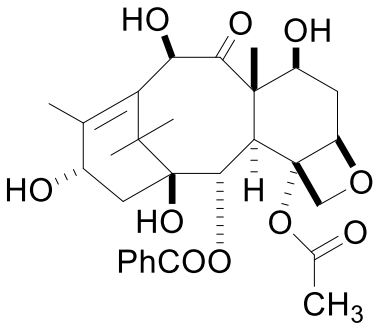
How can the proposed binding pose be dfistinguished?

- Solution-state NMR in the absence of tubulin does not give direct information on the tubulin-bound conformation
- Solution-state NMR is not possible in the presence of tubulin, because addition of taxol to tubulin causes polymerization to microtubules
- So a solid-state NMR method is needed
- 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 was developed by Jake Schaefer at Washington University, St. Louis.
- 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

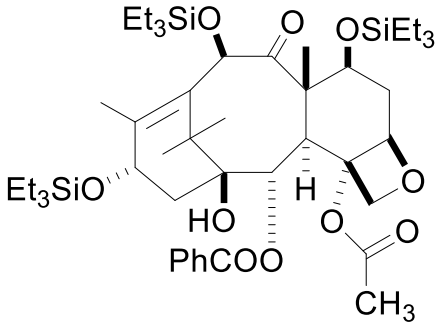
Taxol's Microtubule Binding



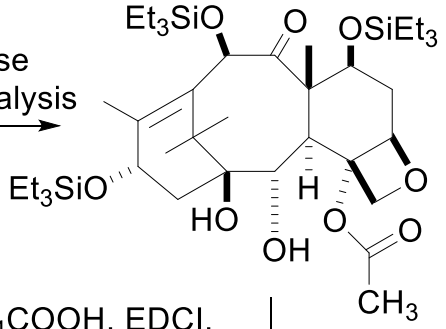
Taxol's Microtubule Binding



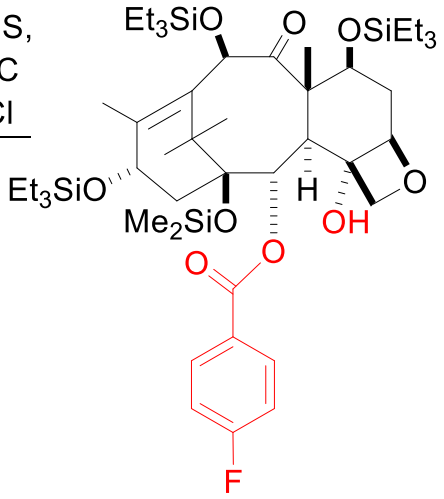
10-deacetylbaccatin III



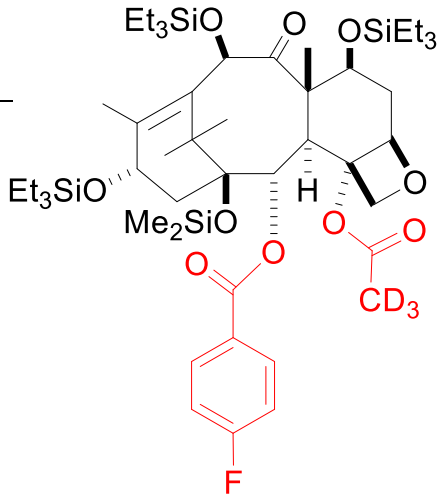
NaOH, phase transfer catalysis



a. *p*-FC_6H_4COOH, EDCI, DMAP, $PhCH_3$, 24 h, 65%
b. DMSCl, Im, DMF, 95%
c. Red Al, THF, 0 °C

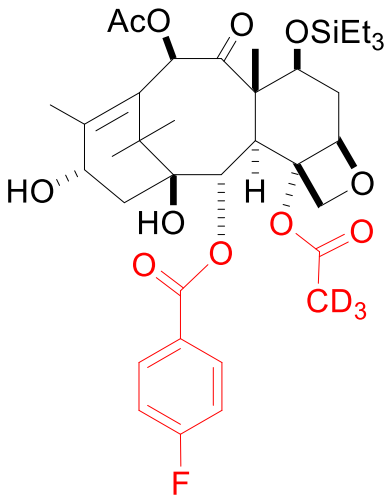


d. LHMDS, THF, 0 °C
CD_3COCl

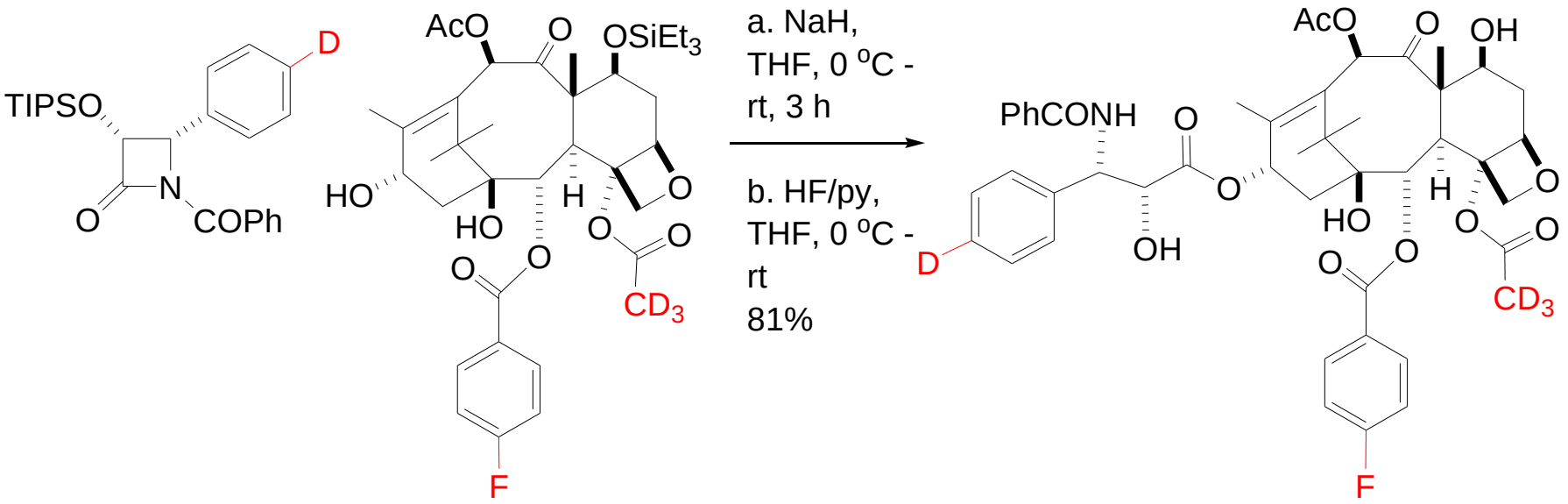


e. HF/Py,THF, 0 °- rt

f. Ac_2O, THF, $CeCl_3$
g. TESCl, Im, DMF, 94%



Taxol's Microtubule Binding



Holton-Ojima β-lactam synthesis

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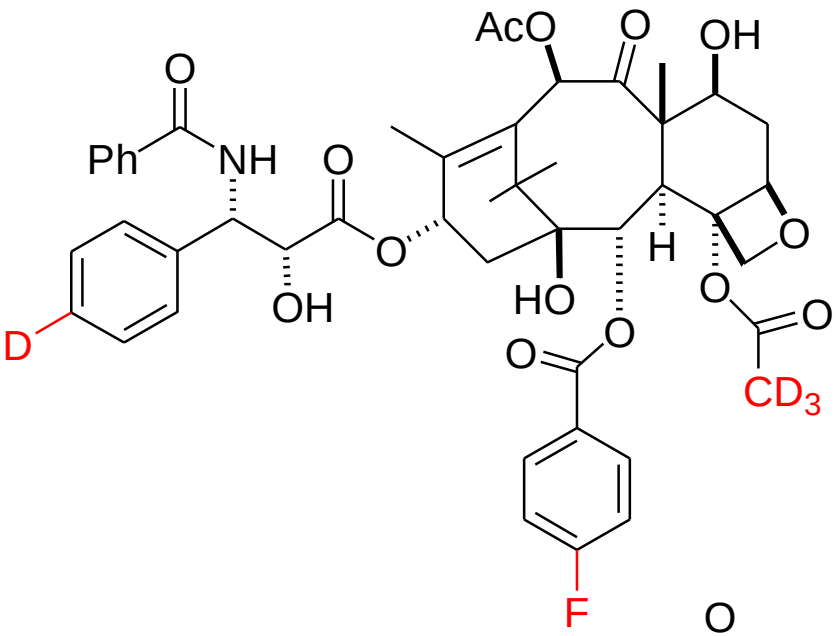
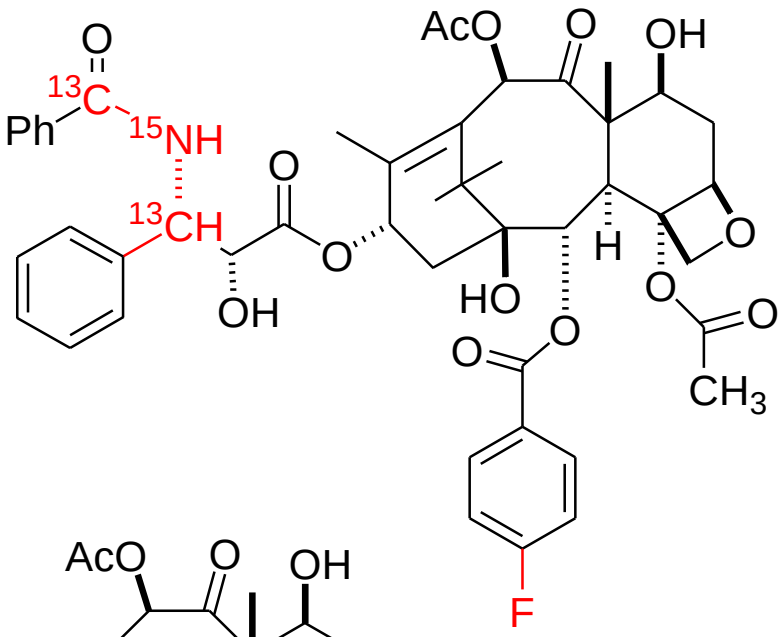
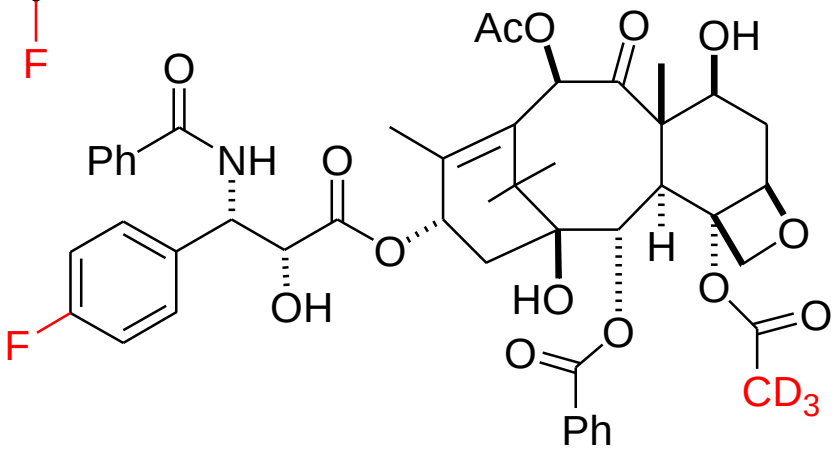
Taxol's Microtubule Binding







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REDOR Experiment

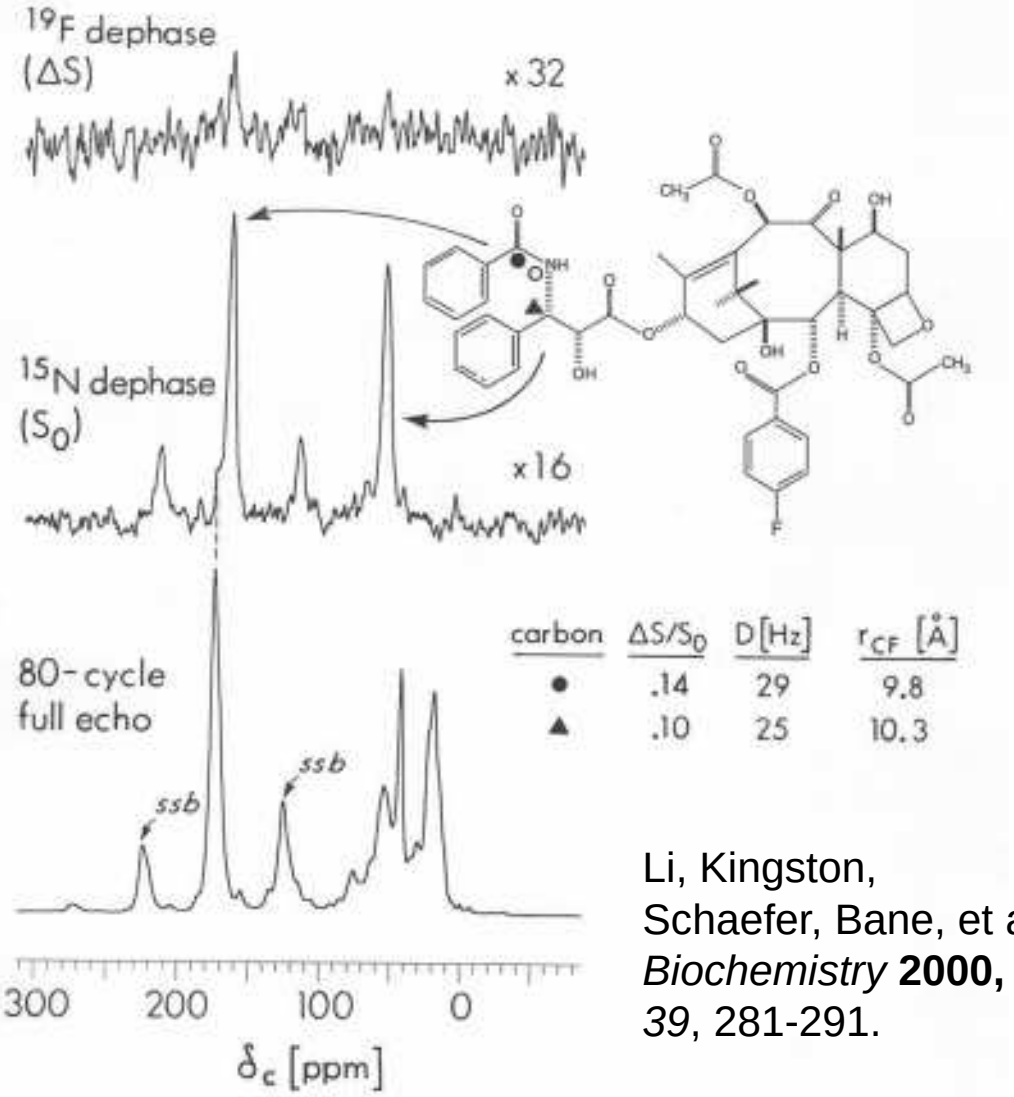
125-MHz $^{13}C(^{15}N$ or $^{19}F)$ Double REDOR Spectra of Labeled Taxol-Microtubule Complex.

Upper: Difference spectrum from ^{19}F dephasing (ΔS). This $^{13}C(^{19}F)$ spectrum gives peaks whose intensities relative to those in the middle spectrum are dependent on internuclear distances. The distance is proportional to the inverse sixth power of the relative intensity.

Middle: $^{13}C(^{15}N$) REDOR difference spectrum; background-free spectrum from the two ^{13}C labels.

Lower spectrum: Full-echo spectrum after 80 cycles.

8,000,000 scans (3 months)



Li, Kingston, Schaefer, Bane, et al. *Biochemistry* **2000,** *39*, 281-291.

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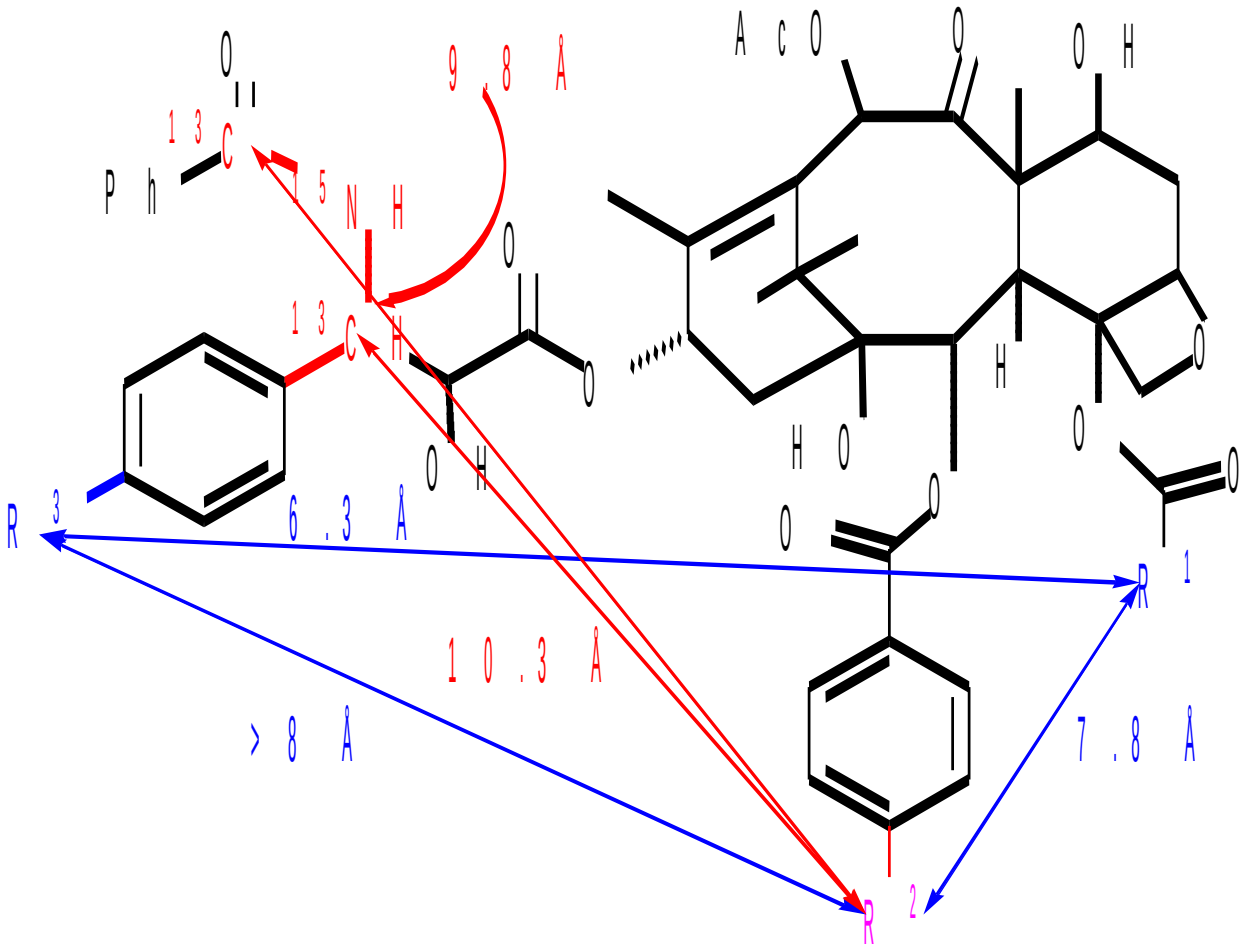
Combined REDOR Experiments



Li, Y.-K.; Poliks, B.; Cegelski, L.; Poliks, M.; Cryczynski, Z. et al. The Conformation of Microtubule-Bound Paclitaxel Determined by Fluorescence Spectroscopy and REDOR NMR. *Biochemistry* ***2000, 39, 281-291.***

Paik, Y.; Yang, C.; Metaferia, B.; Tang, S.; Bane, S. et al. Rotational-Echo Double-Resonance NMR Distance Measurements for the Tubulin-Bound Paclitaxel Conformation. *J. Am. Chem. Soc.* ***2007, 129, 361-370.***

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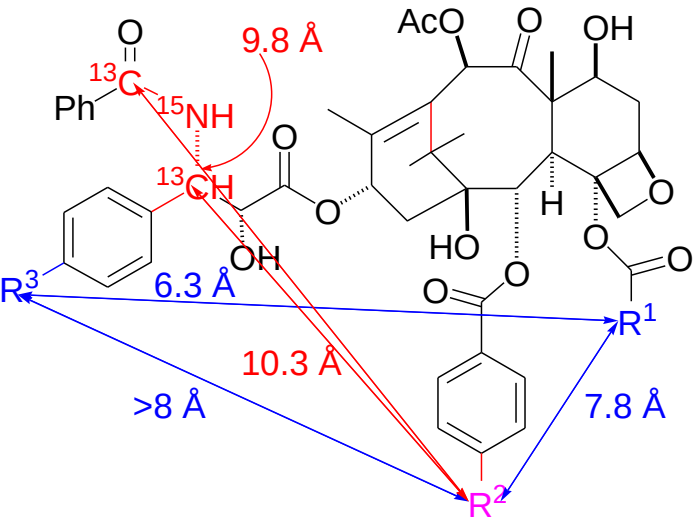
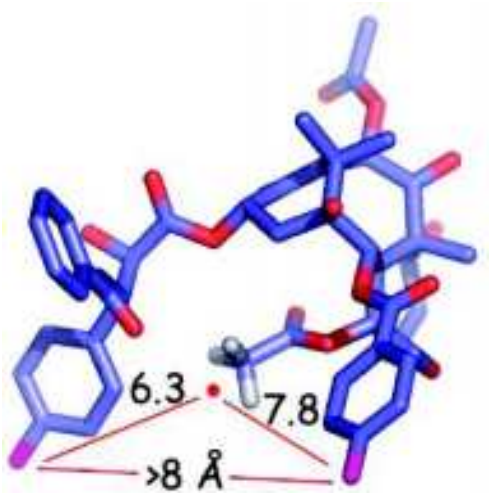
Combined REDOR Experiments





Calculated and experimental REDOR NMR distances

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

Distances (A)	Polar	Non-polar	**T-Taxol**	Expt
R^1-R^2	7.9	8.0	**7.9**	**7.8**
R^1-R^3	5.9	7.2	**6.6**	**6.3**
R^2-R^3	4.6	12.5	**12.2**	**>8**
R^2-CH	9.6	8.5	**9.8**	**10.3**
R^2-C	10.4	6.2	**9.1**	**9.8**

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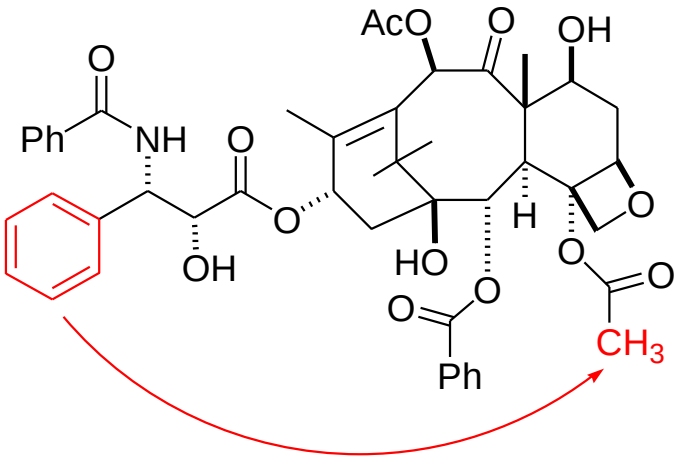
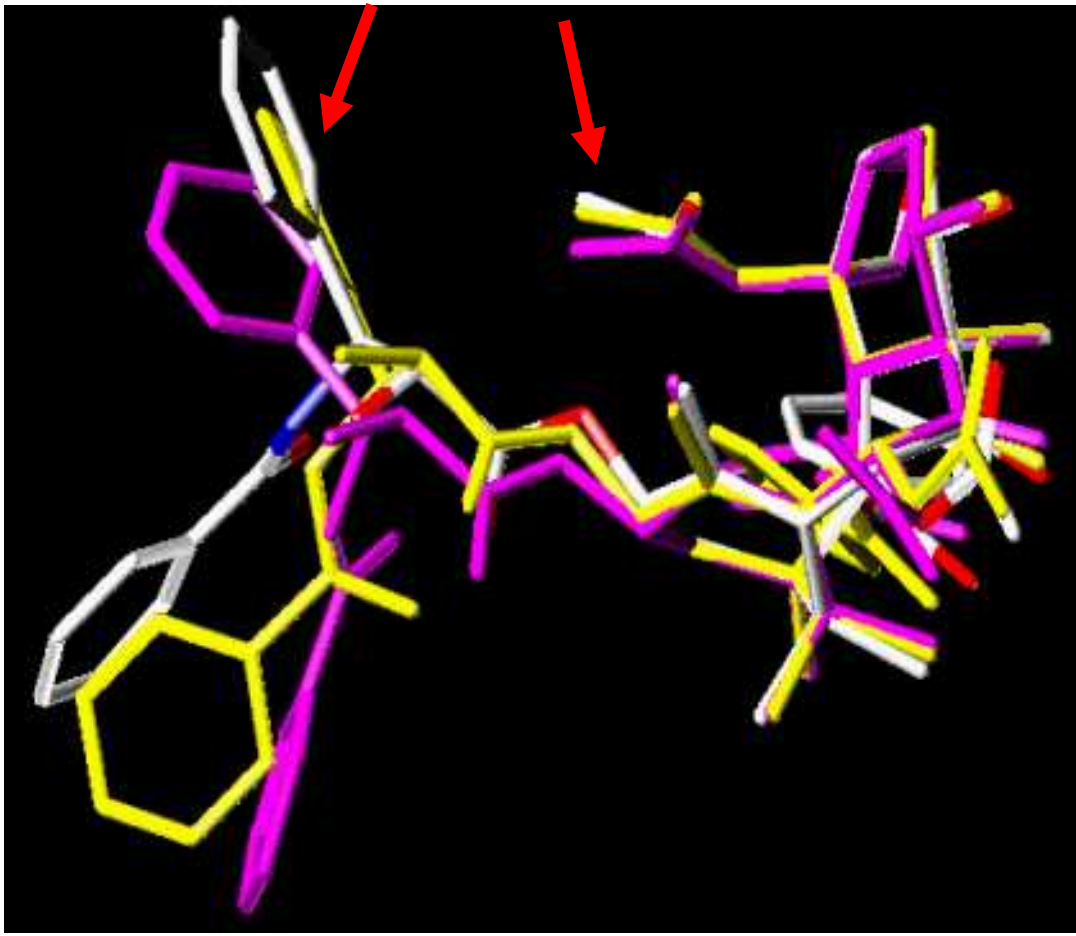
Synthesis of a T-Taxol Analog





- 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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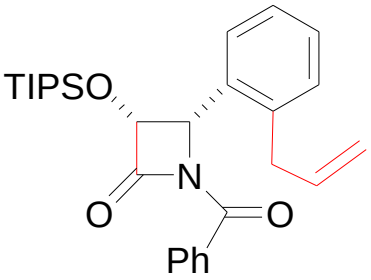
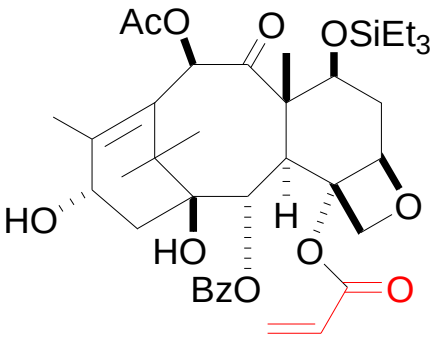
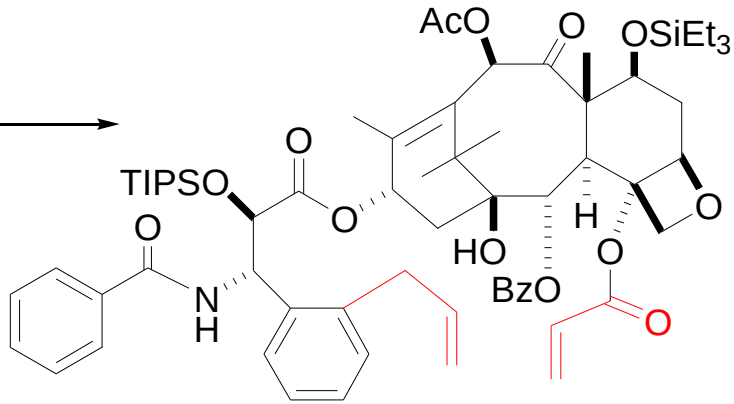
Synthesis of a T-Taxol Analog

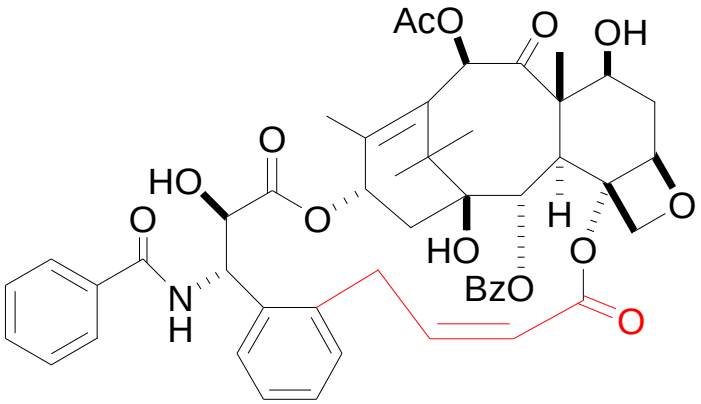






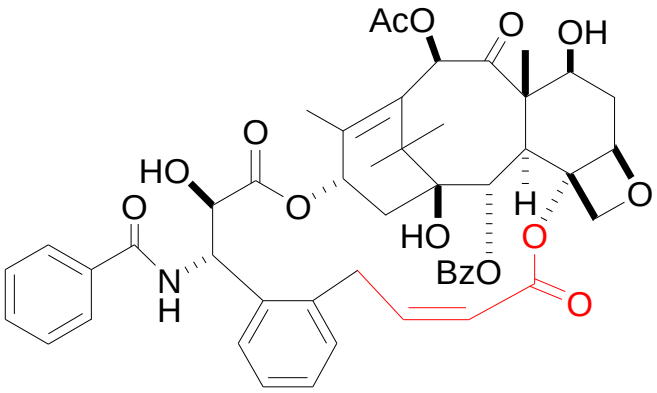
The β-lactam and baccatin III derivatives were prepared by modifications of known procedures

1. Grubbs' catalyst
2. HF/py

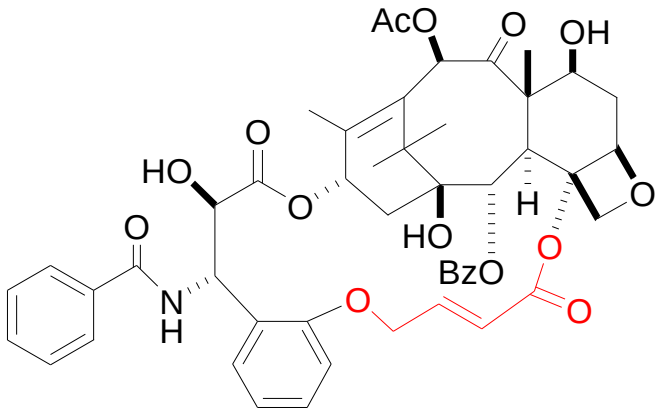


Britaxel-5
5-Atom Bridge (Phenyl-C4)

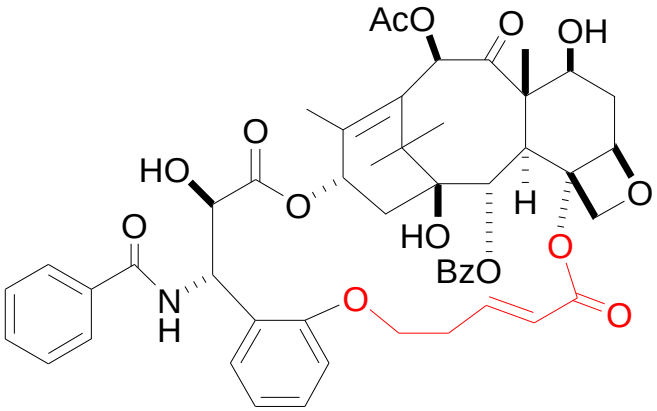
Synthesis of T-Taxol Analogs



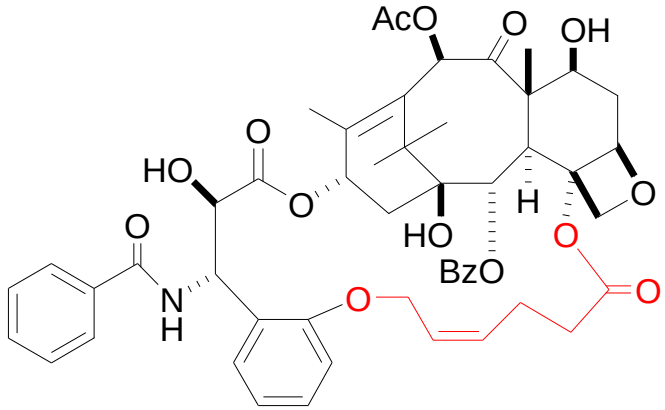
Z-Britaxel-5
Dihydrobritaxel-5



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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Biological Evaluation of T-Taxol Analogs

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**

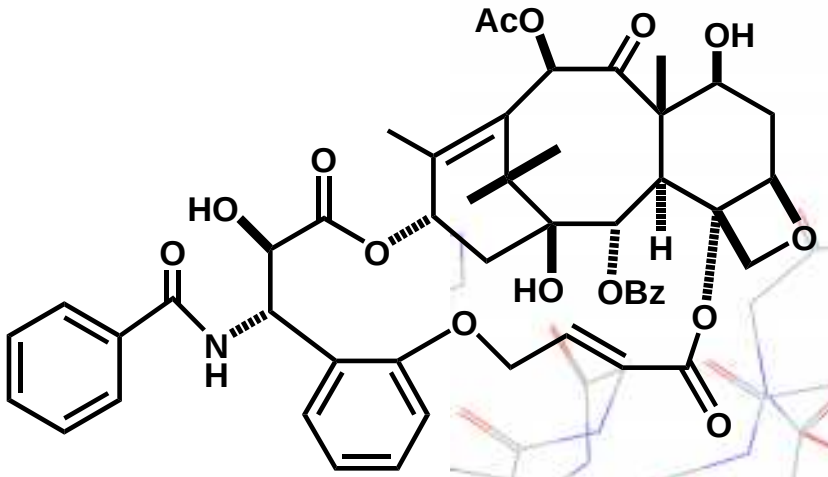
Ganesh, T.; Guza, R. C.; Bane, S.; Ravindra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10006-10011.

Biological Evaluation of T-Taxol Analogs

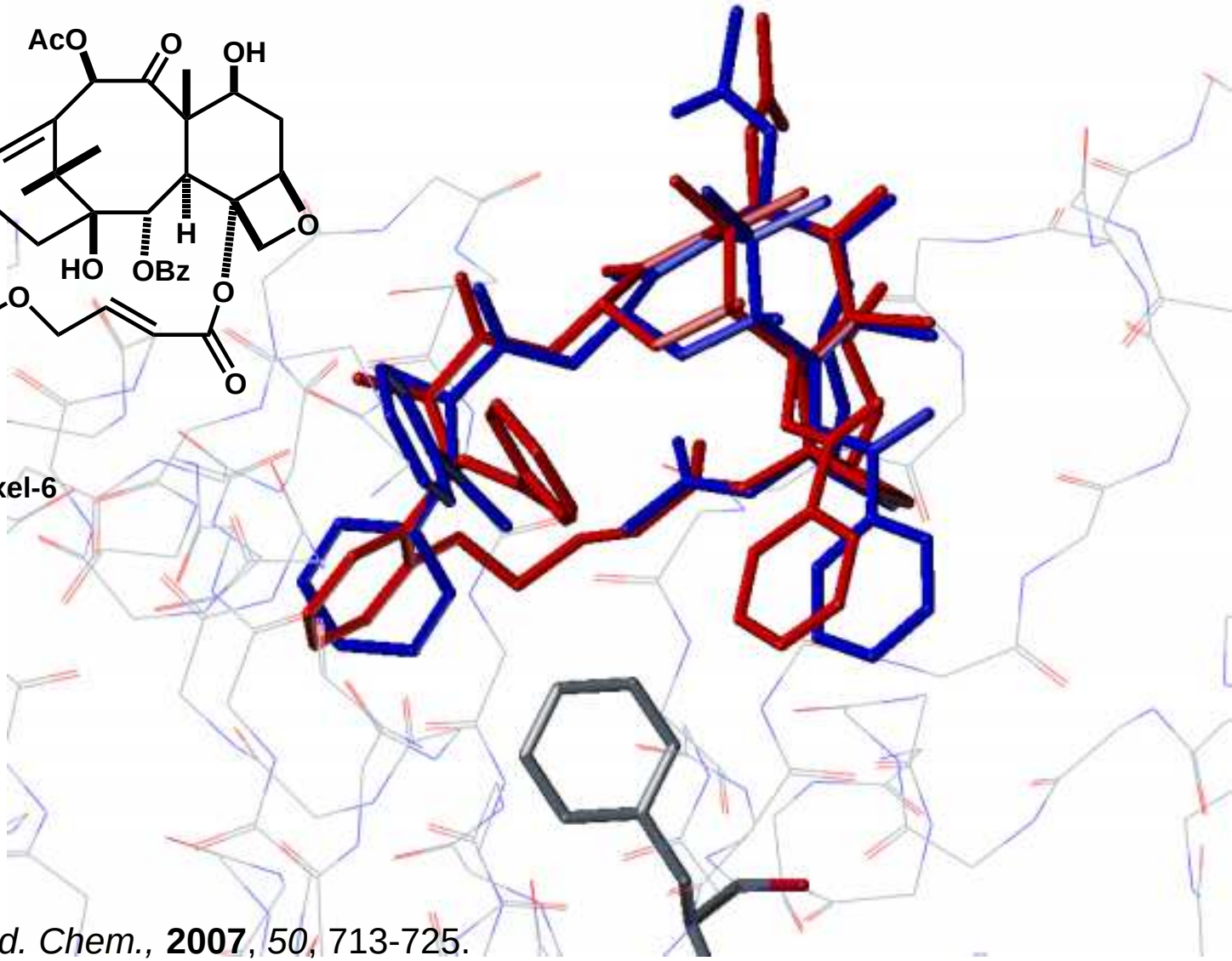
	1A9 (nM)	1A9-PTX10 (nM)	Relative resistance
Taxol	4.8	157	32.7
O-Britaxel-6	7.6	126	16.5
O-Britaxel-7	17.1	157	9.2
Britaxel-5	0.072	0.13	1.8
O-Britaxel-8	30.9	196	6.3
Iso-Britaxel-6	7.6	157	20.6
H_2-O-Britaxel-6	19.8	35.9	1.8
Dihydro-britaxel-5	0.083	1.03	12.4

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.

Comparison of Taxol and T-Taxol Conformations



***E*-Britaxel-6**



Ganesh et al. *J. Med. Chem.,* **2007**, *50*, 713-725.

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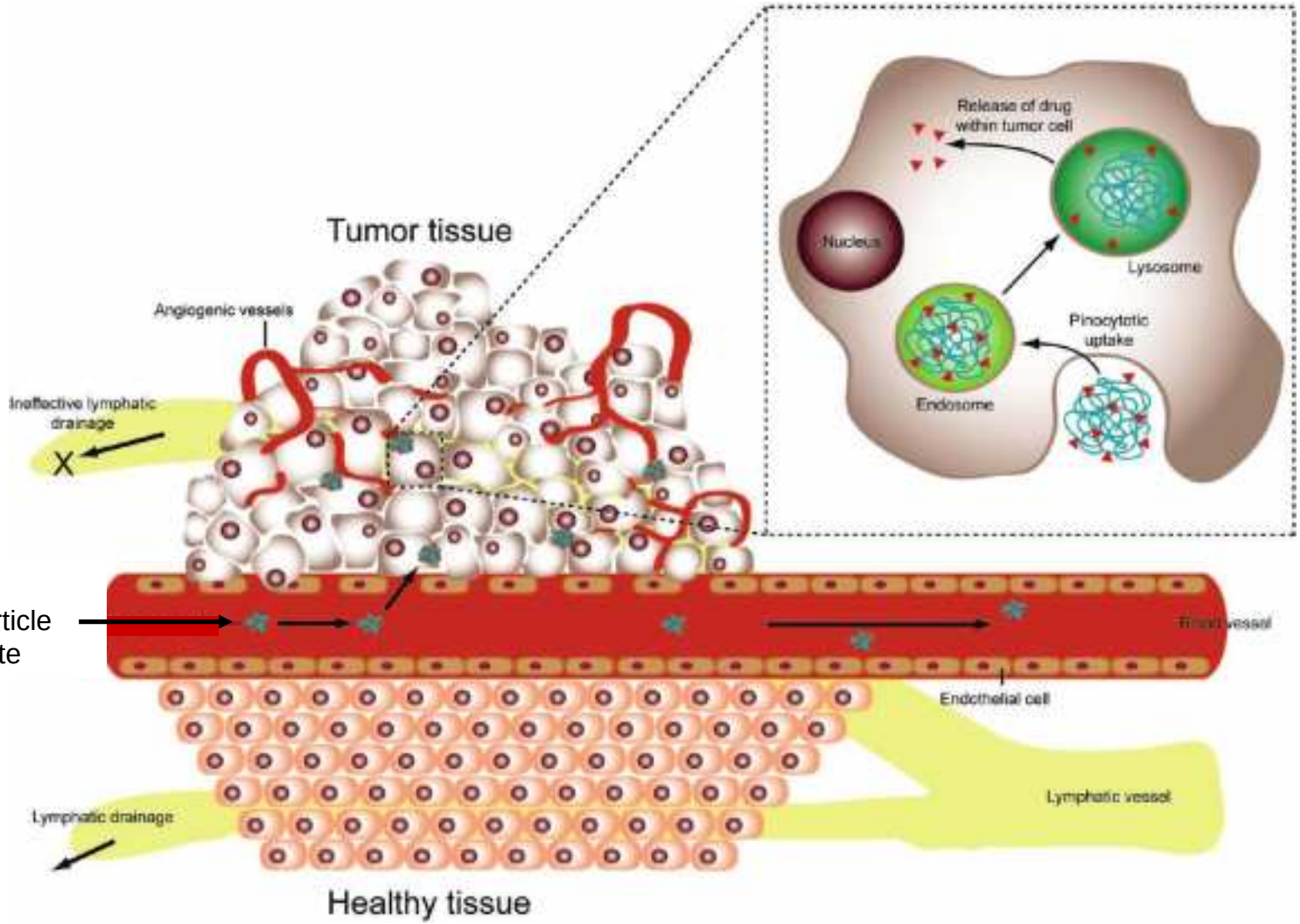
Nanoparticle Drug Delivery of Taxol



Gold nanoparticle conjugate

Adapted from Fox, M. E.; Szoka, F. C.; Fréchet, J. M. J., *Accounts of Chemical Research 2009, 42, 1141-1151.*

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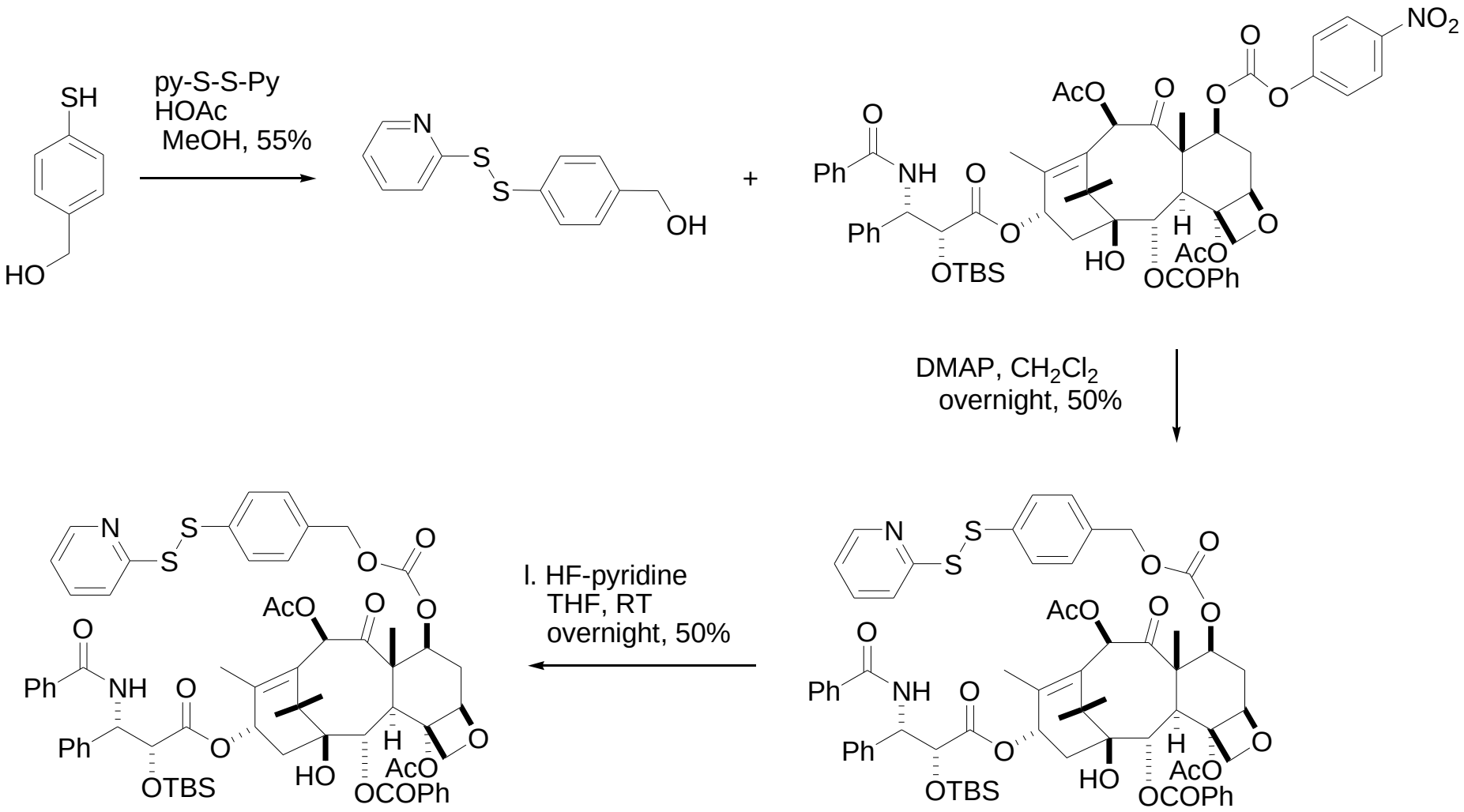
Nanoparticle Drug Delivery of Taxol

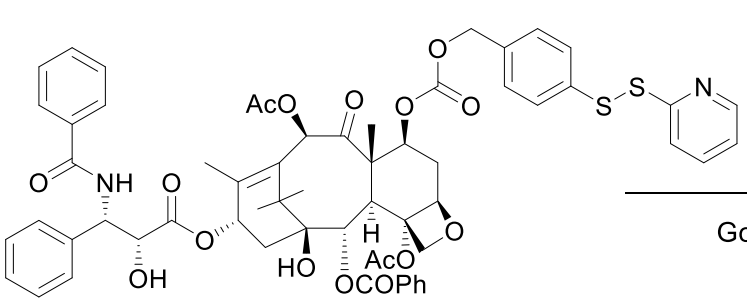
Colloidal gold nanoparticles passively accumulate in solid tumors but not in normal tissue because they cannot pass through normal vasculature. In CYT-21625 they are decorated with three different agents

- PEG-THIOL: Shields the nanoparticle drug from immune responses by the reticuloendothelial system
- Tumor Necrosis Factor (TNFα): This is both a tumor targeting ligand and a vascular disrupting agent (it is used clinically in treating cancer by the isolated limb perfusion technique)
- Paclitaxel Prodrug: Therapeutic to Treat TNF-Insensitive Tumor Cells; released in the cell

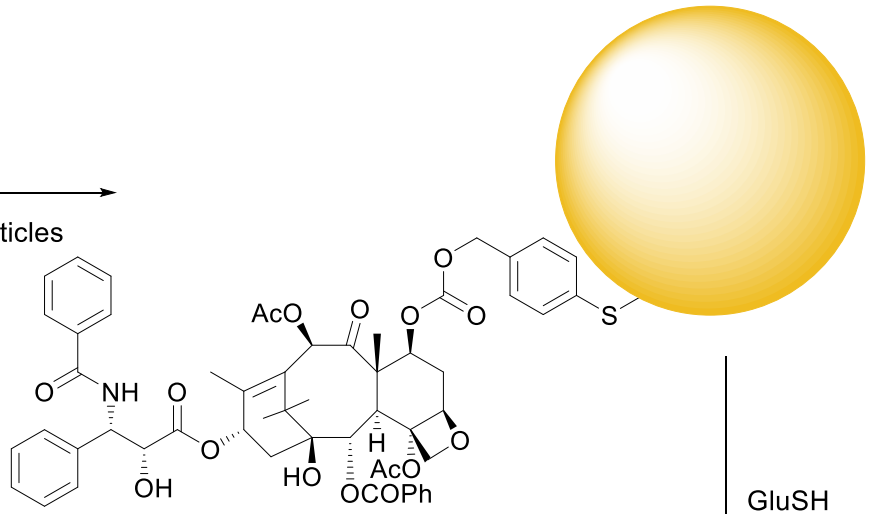
Nanoparticle Drug Delivery of Taxol. Synthesis



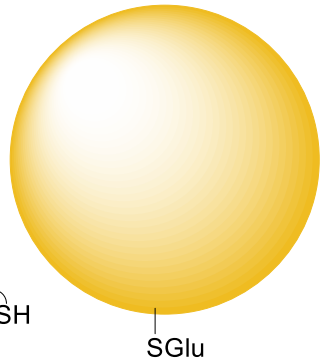
Nanoparticle Drug Delivery of Taxol. Drug Delivery



Gold nanoparticles

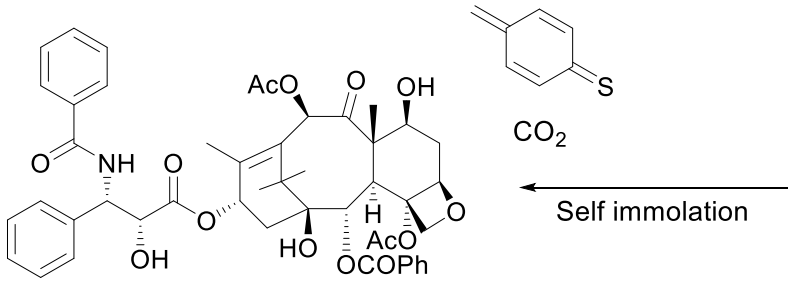


GluSH

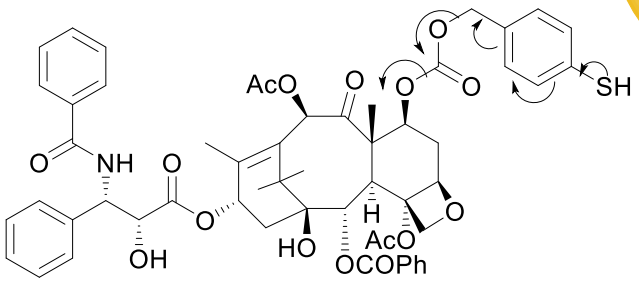


The tumor environment is a reducing one, with levels of glutathione (GluSH) approximately 100-fold higher than in the blood.

This reducing environment cleaves the gold-sulfur bond, and the linker then undergoes self-immolation to generate free paclitaxel in the tumor.



Self immolation

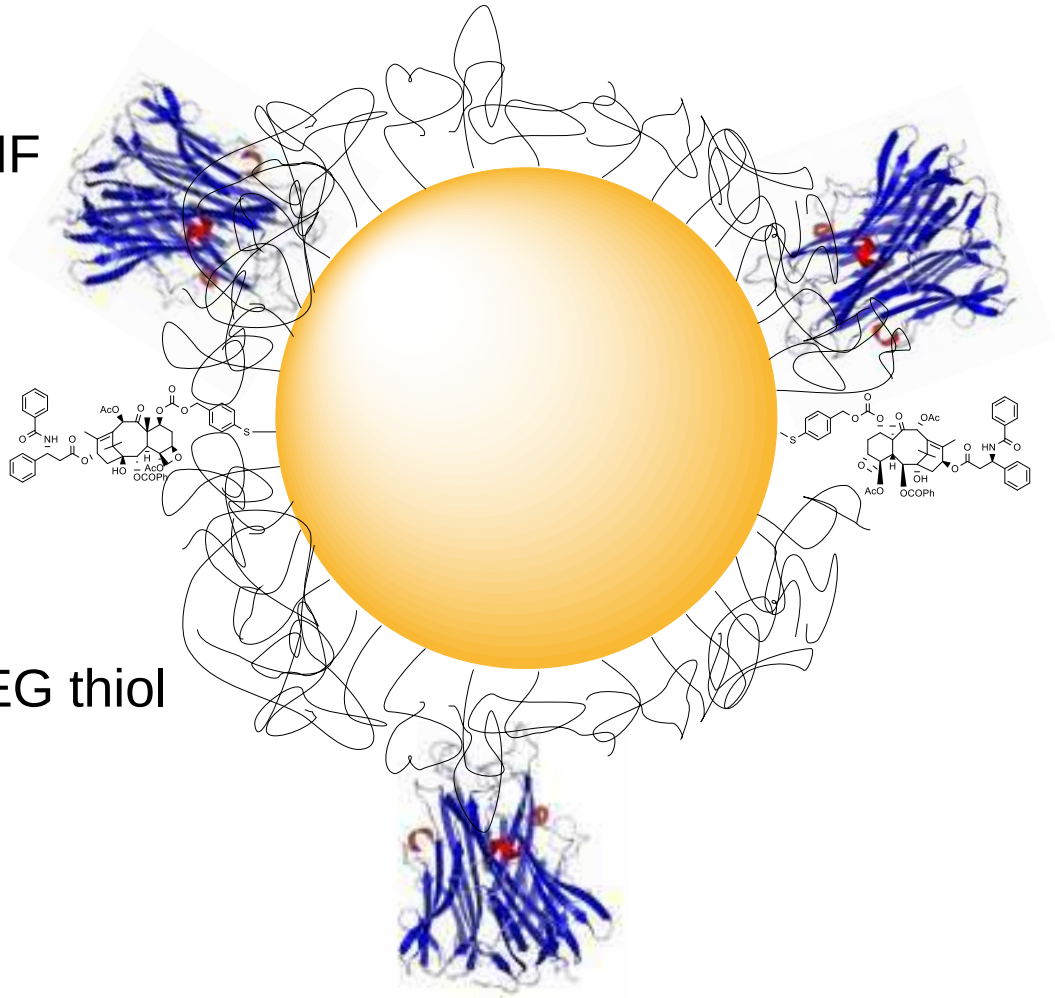


Nanoparticle Drug Delivery of Taxol

TNF

Paclitaxel
linked to gold

PEG thiol



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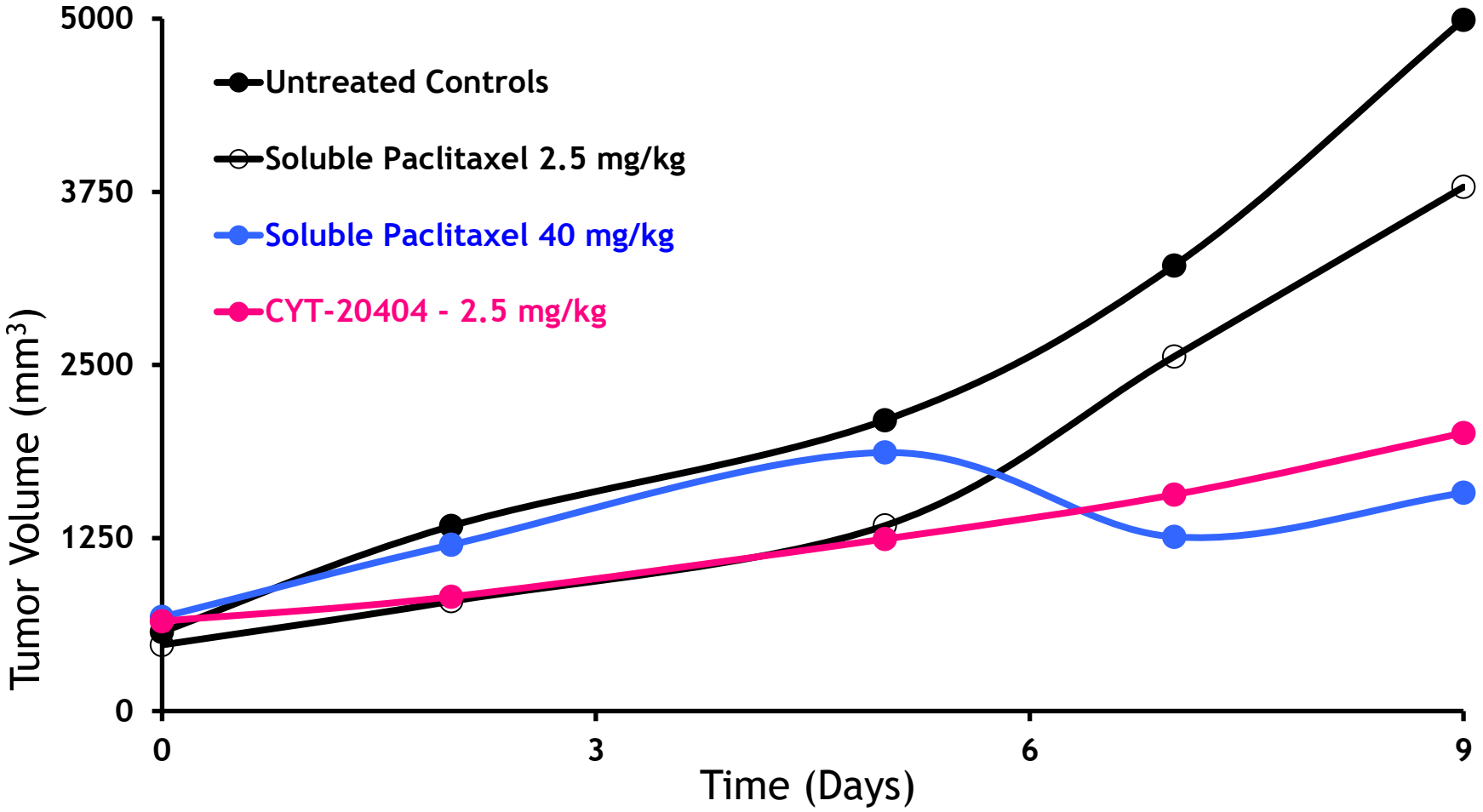
Nanoparticle Drug Delivery of Taxol. Animal Study



Anti-tumor efficacy of paclitaxel and the CYT-20000 series against B16/F10 tumors in C57BL/6 mice

Data from CytImmune Inc., Rockville, MD

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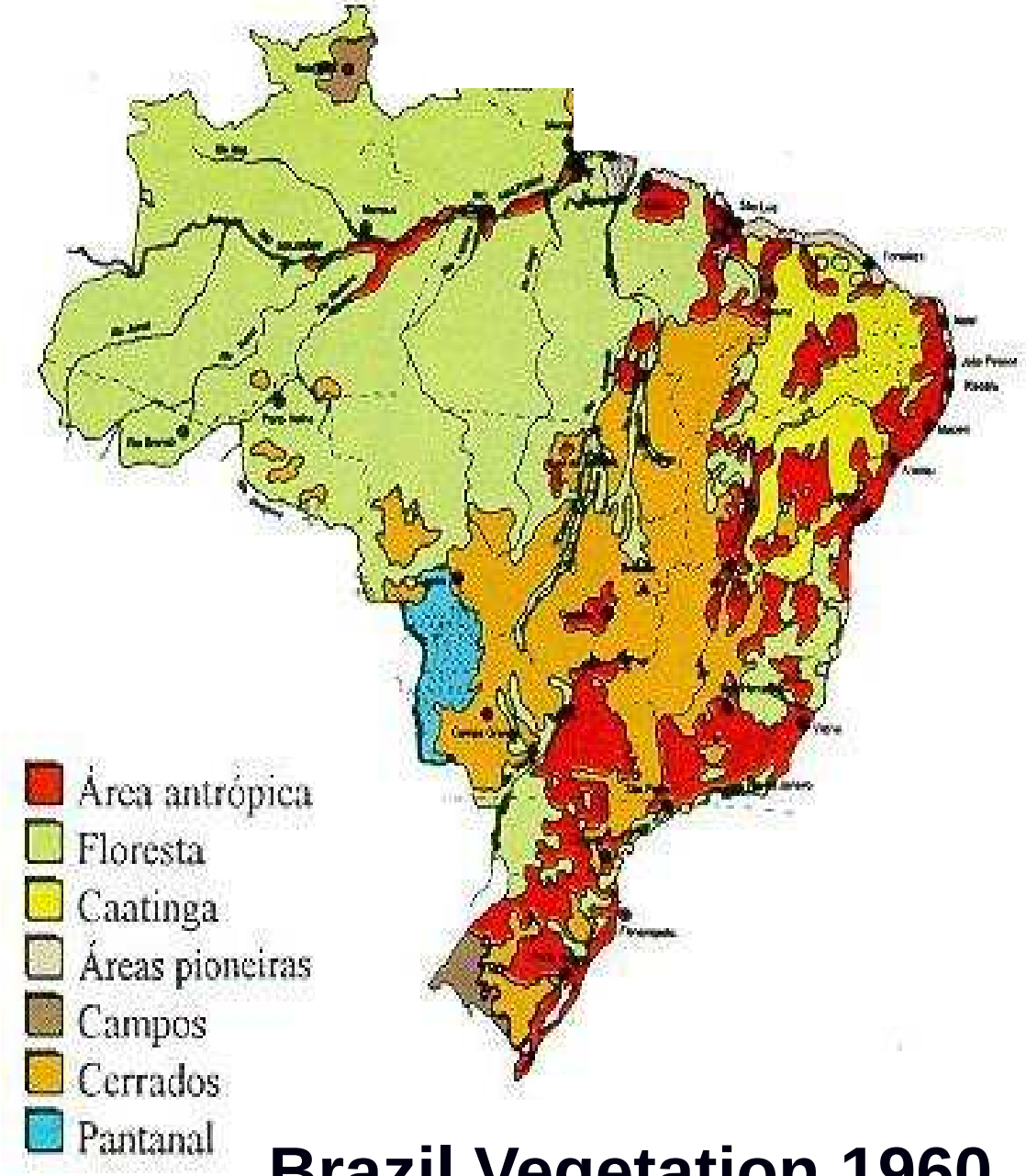
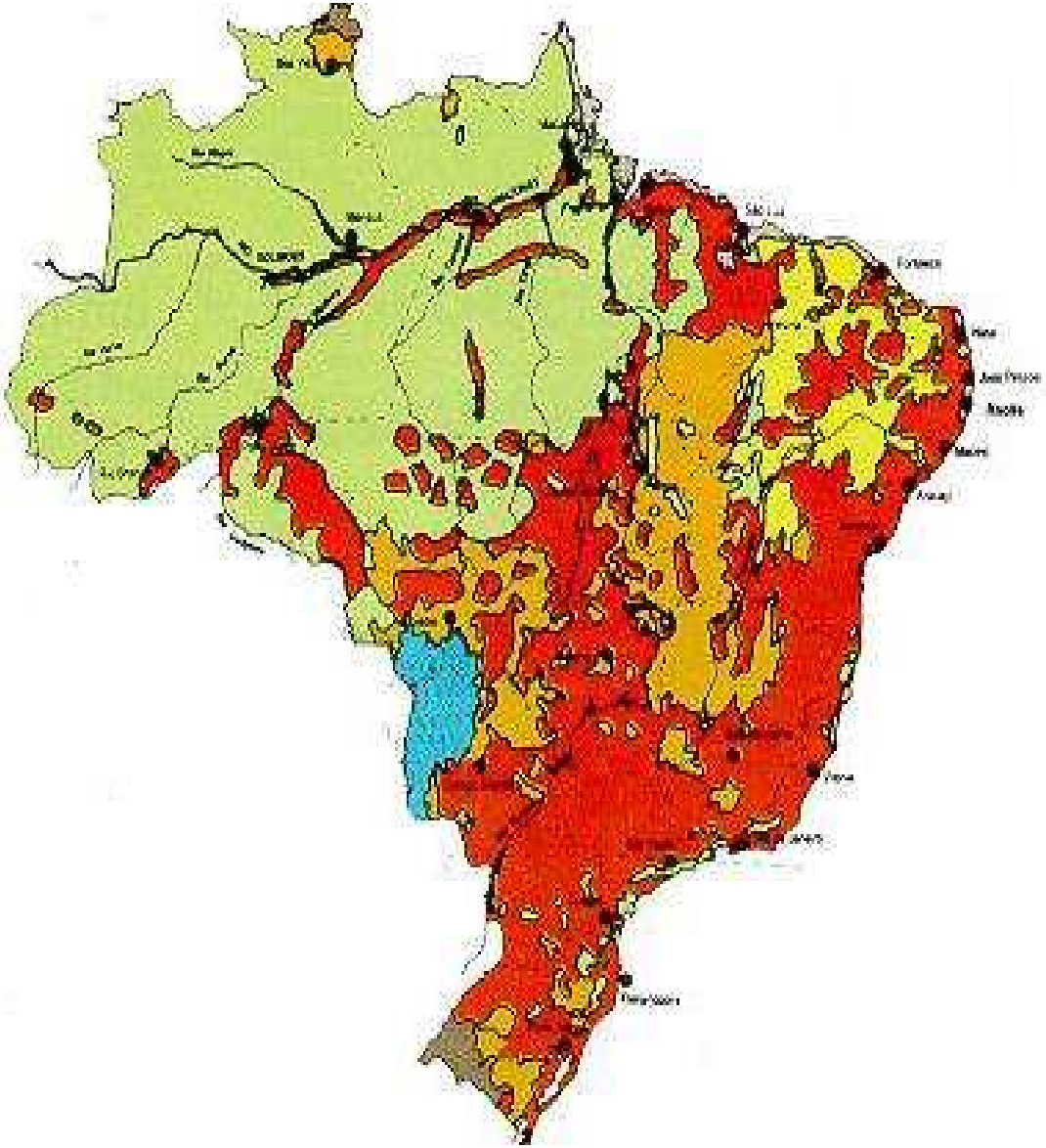
Drug Discovery and Biodiversity Conservation

Drug Discovery and Biodiversity Conservation



Brazil Vegetation 1960

Drug Discovery and Biodiversity Conservation



Brazil Vegetation 1988

Drug Discovery and Biodiversity Conservation

- Before 1991 most scientists collected biomass without regard to the Intellectual Property rights of the host country.
- This practice was declared to be illegal by the 1992 Rio Convention on Biodiversity (CBD).
- The basic objectives of the CBD are to promote sustainable use of biodiversity as well as conservation and benefit sharing; three objectives which are interrelated.
- This Convention has had both beneficial and detrimental effects on natural products-based drug discovery.

Drug Discovery and Biodiversity Conservation

- **Beneficial**
  - It provides a firm legal foundation for the work.
  - It is the right thing to do
- **Detrimental**
  - It created unrealistic expectations of huge royalties in some countries, which turned to disillusion when these royalties were not immediately forthcoming.
  - The process of obtaining the necessary agreements and permits on “mutually agreed terms” can be very difficult and time-consuming.
  - This difficulty is especially acute when tribal peoples are involved.
  - Some countries do not have the legal framework in place to allow biodiversity prospecting

Drug Discovery and Biodiversity Conservation: the ICBG Program

- In response to the need to promote ethical natural products discovery and with the active support of Norm Farnsworth and many others, the US established the International Cooperative Biodiversity Group (ICBG) program in 1991.
- This was unique in two ways. In the first place, its funding came from multiple sources within the US government, with major contributions from NIH, NSF, and initially USAID.
- Secondly, it required close cooperation between a diverse group of partners, including those with expertise in natural products, botany, and conservation, as well as host country and industrial participants.
- I was fortunate to receive funding for an ICBG program based in Suriname.

Drug Discovery and Biodiversity Conservation

- Who has the authority to allow plant collecting in a country like Suriname?

The government?

Or the tribal people?

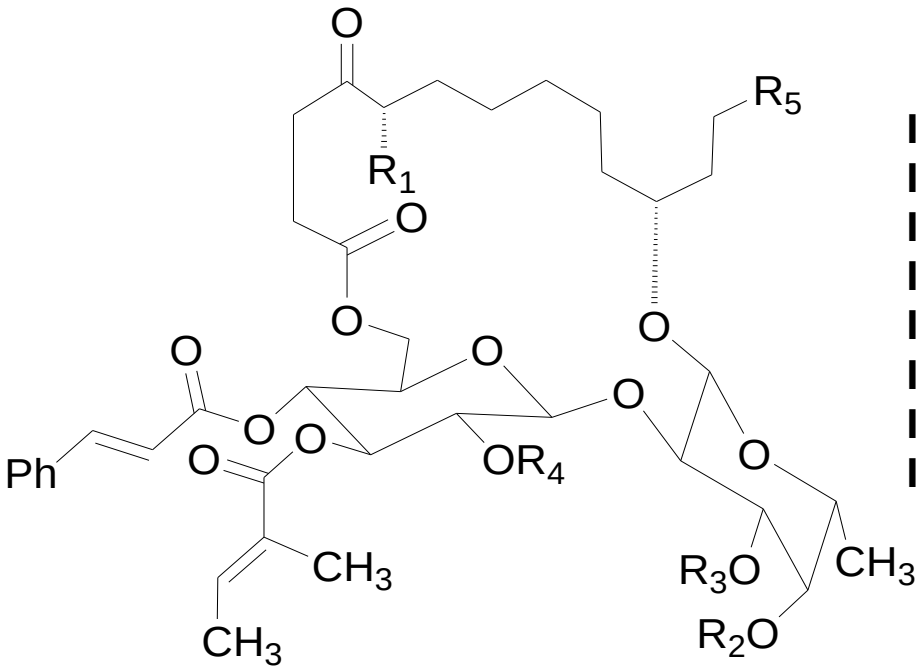
- In reality, both groups must give their approval.
- This complicates the collection process.

Drug Discovery and Biodiversity Conservation in Suriname

Drug Discovery and Biodiversity Conservation in Suriname

Antiproliferative activity against A2780 ovarian cancer cells

Ipomoeassin	**A**	**B**	**C**	**D**	**E**	**E-Ac**	**C-Ac**	**F**
IC_{50} (μM)	**0.5**	**0.4**	**2.9**	**0.035**	**3.3**	**15.8**	**19.1**	**0.036**



Ipo A $R_1 = R_3 = R_4 = R_5 = H$; $R_2 = Ac$
Ipo B $R_1 = R_2 = R_3 = R_4 = R_5 = H$
Ipo C $R_1 = OH$; $R_2 = Ac$; $R_3 = R_4 = R_5 = H$
Ipo D $R_1 = OAc$; $R_2 = Ac$; $R_3 = R_4 = R_5 = H$
Ipo E $R_1 = OAc$; $R_2 = R_3 = R_4 = R_5 = H$
Ipo E-Ac $R_1 = R_5 = H$; $R_2 = R_3 = R_4 = Ac$
Ipo C-Ac $R_1 = OAc$; $R_2 = R_3 = R_4 = Ac$; $R_5 = H$
Ipo F $R_1 = R_3 = R_4 = H$; $R_2 = Ac$; $R_5 = C_2H_5$

The eight similar compounds showed wide variations in activity, indicating that their activity is not due to a general detergent or similar effect

Cao, S.; Kingston, D. G. I., et al. *J. Nat. Prod.*, 2005, *68*, 487

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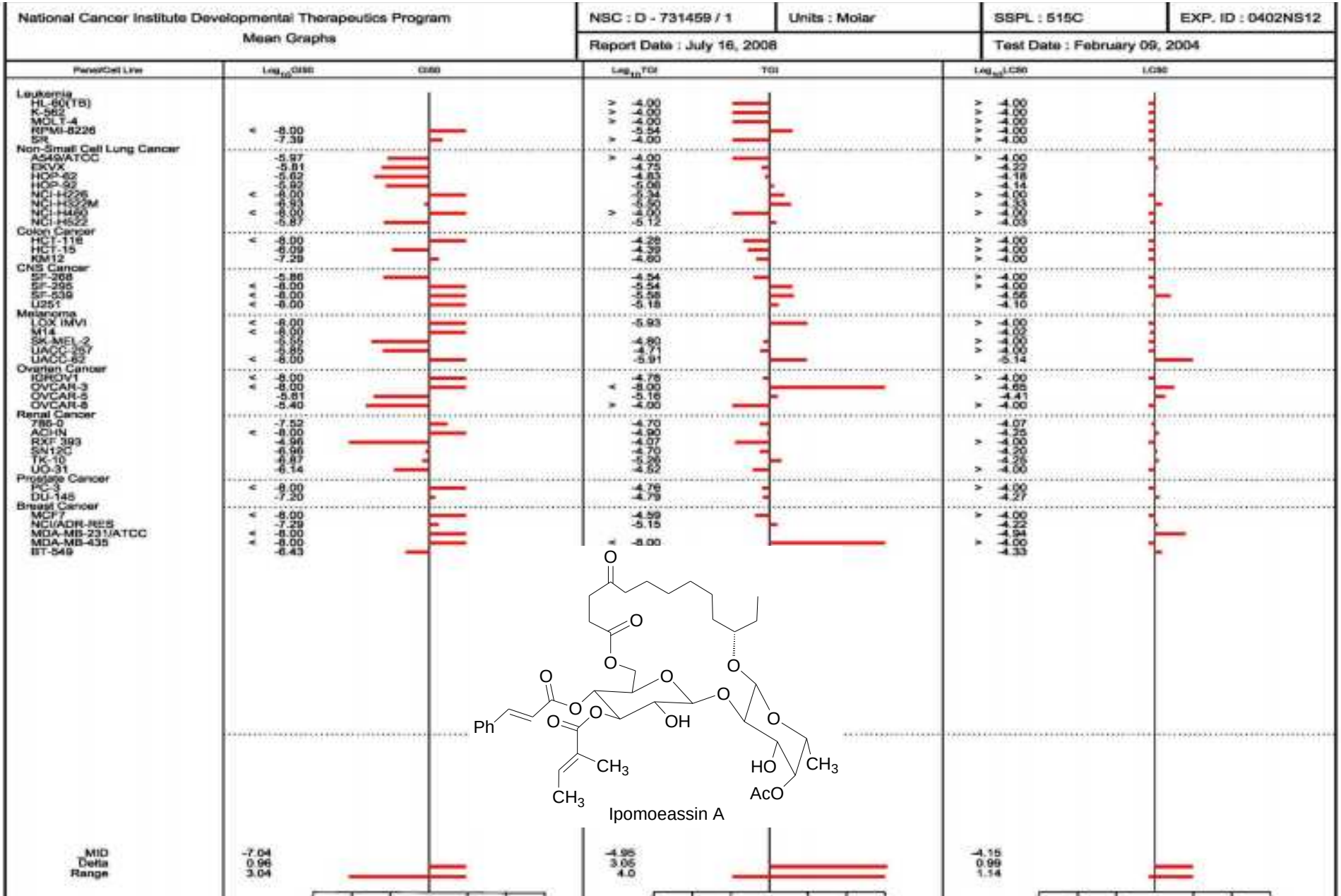
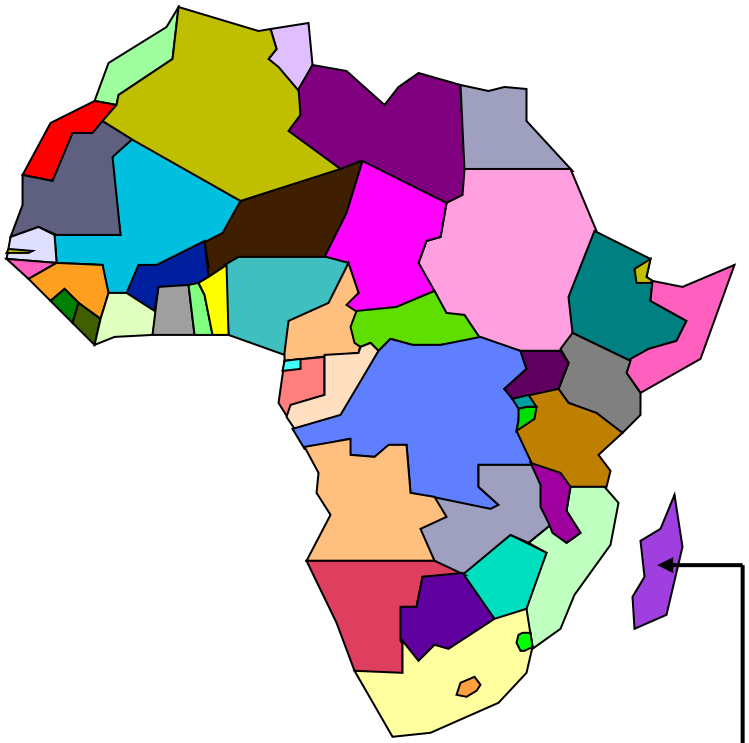
Drug Discovery and Biodiversity Conservation in Suriname



Drug Discovery and Biodiversity Conservation in Madagascar

- Madagascar is a prime source of biodiversity.
- Madagascar is one of the highest priority "Biodiversity Hotspots"
- Home to 25% of the plant species in the African region
- Approximately 80% of the plant species are endemic
- Great ecosystem diversity
- Work is urgent, since much of the country has been deforested



The Realities of Biodiversity Conservation

The Realities of Biodiversity Conservation

The Rewards of Biodiversity Conservation

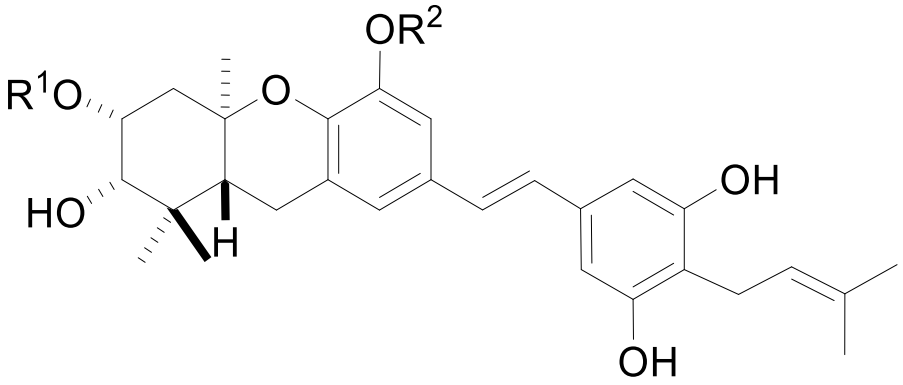
The Rewards of Biodiversity Conservation

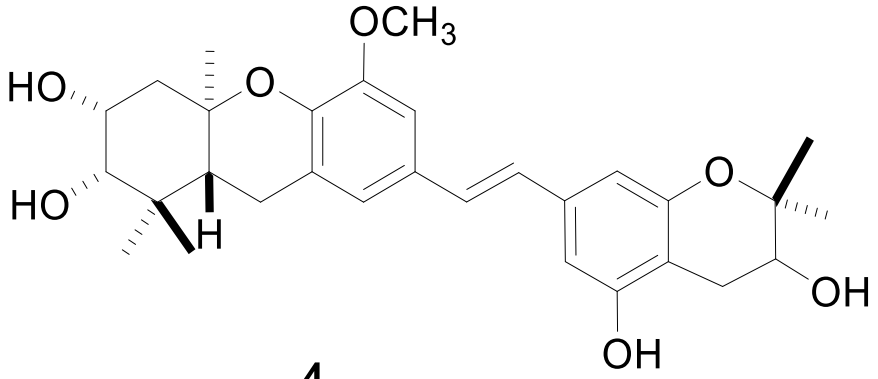
The Rewards of Biodiversity Conservation

The Rewards of Biodiversity Conservation

The Rewards of Biodiversity Conservation

Drug Discovery and Biodiversity Conservation in Madagascar





4

1 R^1 = OH, R^2 = CH_3 (Schweinfurthin E)

2 R^1 = H, R^2 = CH_3

3 R^1 = R^2 = H

5 R^1 =OH, R^2 = H

Compound	**1**	**2**	**3**	**4**	**5**
IC_{50} (μM, A2780)	0.26	5.0	0.39	4.5	0.13

- All compounds are potent cytotoxic agents
- Similar compounds were also isolated by John Beutler et al. at the NCI
- Development has been hampered by a lack of mechanistic understanding
- Work at NCI is overcoming this problem, and the compounds are being moved towards preclinical development

Yoder, B.; Cao, S.; Kingston, D. G. I. et al. *J. Nat. Prod.,* 2007, *70*, 342

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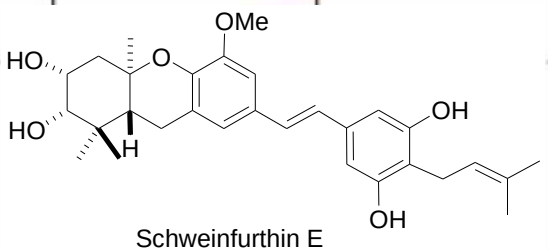
Drug Discovery and Biodiversity Conservation in Madagascar

National Cancer Institute Developmental Therapeutics Program
Mean Graphs

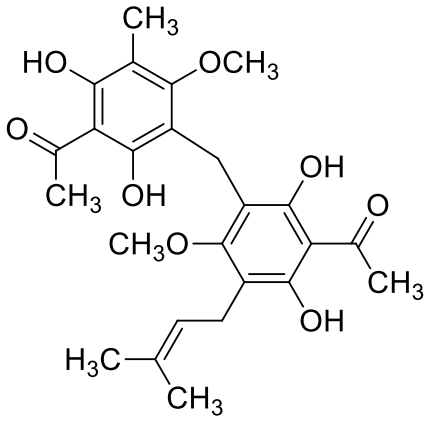
NSC : D - 732916 / 1 | Units : Molar | SSPL : 515C | EXP. ID : 0406RS17
Report Date : July 16, 2008 | Test Date : June 26, 2004

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-4.96		-4.44		> -4.00	
HL-60(TB)	-5.32		-4.72		> -4.00	
K-562	-5.32		> -4.00		> -4.00	
MOLT-4	-5.51		-4.98		> -4.00	
RPMI-8226	-5.48		-4.72		> -4.00	
SR	-5.35		-4.59		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-5.34		-4.61		> -4.00	
EKVX	-5.29		-4.18		> -4.00	
HOP-62	-7.86		-4.93		-4.42	
HOP-92	-4.90		-4.37		> -4.00	
NCI-H226	-5.18		-4.47		> -4.00	
NCI-H23	-5.35		-4.70		-4.29	
NCI-H322M	-5.98		-5.31		-4.51	
NCI-H460	< -8.00		-4.81		> -4.00	
NCI-H522	-5.31		-4.83		-4.09	
Colon Cancer						
COLO 205	-5.23		> -4.00		> -4.00	
HCT-116	-6.50		-5.43		-4.70	
HCT-15	-5.92		-4.80		-4.14	
HT29			> -4.00		> -4.00	
KM12	-6.33		-4.79		-4.12	
SW-620	-6.30		-4.86		> -4.00	
CNS Cancer						
SF-268	-5.49		-4.61		> -4.00	
SF-295	< -8.00		-7.02		-5.01	
SF-539	< -8.00		-6.18		-4.39	
SNB-19	-5.09		-4.01		> -4.00	
SNB-75	-4.86		-4.11		> -4.00	
U251	-7.38		-4.89		-4.22	
Melanoma						
LOX IMVI	-5.68		-4.84		-4.26	
M14	-6.76		-5.60		-5.00	
SK-MEL-2	-5.00		-4.58		-4.17	
SK-MEL-28	-4.98		> -4.00		> -4.00	
SK-MEL-5	-6.75		-5.79		-5.15	
UACC-257	-4.89		-4.57		-4.23	
UACC-62	-6.31		-5.46		-4.83	
Ovarian Cancer						
IGROV1	-5.09		-4.63		-4.22	
OVCAR-3	-5.62		-4.77		-4.24	
OVCAR-4	-4.67		> -4.00		> -4.00	
OVCAR-5	-4.86		-4.37		> -4.00	
OVCAR-8	-4.78		-4.34		> -4.00	
SK-OV-3	-4.77		> -4.00		> -4.00	
Renal Cancer						
A498	< -8.00		-6.60		-4.38	
ACHN	-5.86		-4.82		-4.28	
CAKI-1	-7.56		-5.61		-4.59	
RXF 393	-6.16		-4.55		> -4.00	
SN12C	-4.95		-4.51		-4.06	
TK-10	-6.59		-5.35		-4.42	
Prostate Cancer						
PC-3	-5.19		-4.60		-4.07	
DU-145	-5.29		-4.41		> -4.00	
Breast Cancer						
MCF7	-6.81		-5.28		-4.08	
NCI/ADR-RES	-4.95		> -4.00		> -4.00	
MDA-MB-231/ATCC	-5.70		-4.84		-4.23	
HS 578T	< -8.00		-5.48		> -4.00	
MDA-MB-435	-5.50		-4.16		> -4.00	
BT-549	-5.39		-4.66		-4.18	
T-47D	-4.84		> -4.00		> -4.00	
MID	-5.83				-4.19	
Delta	2.17				0.96	



Schweinfurthin E

Drug Discovery and Biodiversity Conservation in Madagascar



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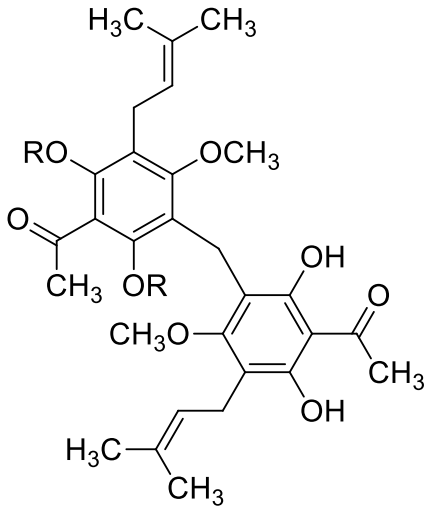

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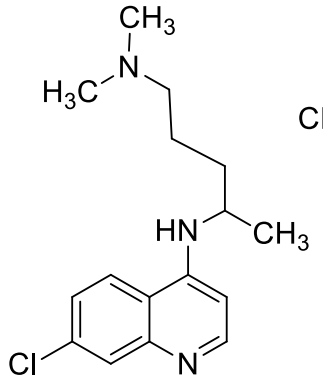
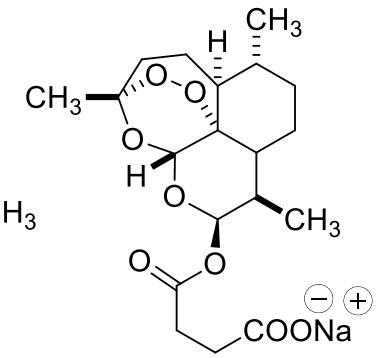


Chloroquine



Sodium artesunate

	Cytostatic activity, IC_{50} (μM)	Cytocidal activity, LD_{50} (μM)		Gametocytocidal activity, IC_{50} (μM)
Compound	Dd2	HB3	Dd2	NF54
1	0.75 ± 0.30	14.6 ± 0.7	6.7 ± 0.2	NA
2	0.14 ± 0.04	0.81 ± 0.05	0.80 ± 0.02	3.6 ± 0.2
Chloroquine	NT	0.10 ± 0.01	15.3 ± 0.9	NT
Artesunate	NT	NT	NT	2.3

Harinantenaina, Kingston, Cassera, Roepe, 2012

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The Rewards of Biodiversity Conservation. The Montagne de Francais is a New Protected Area

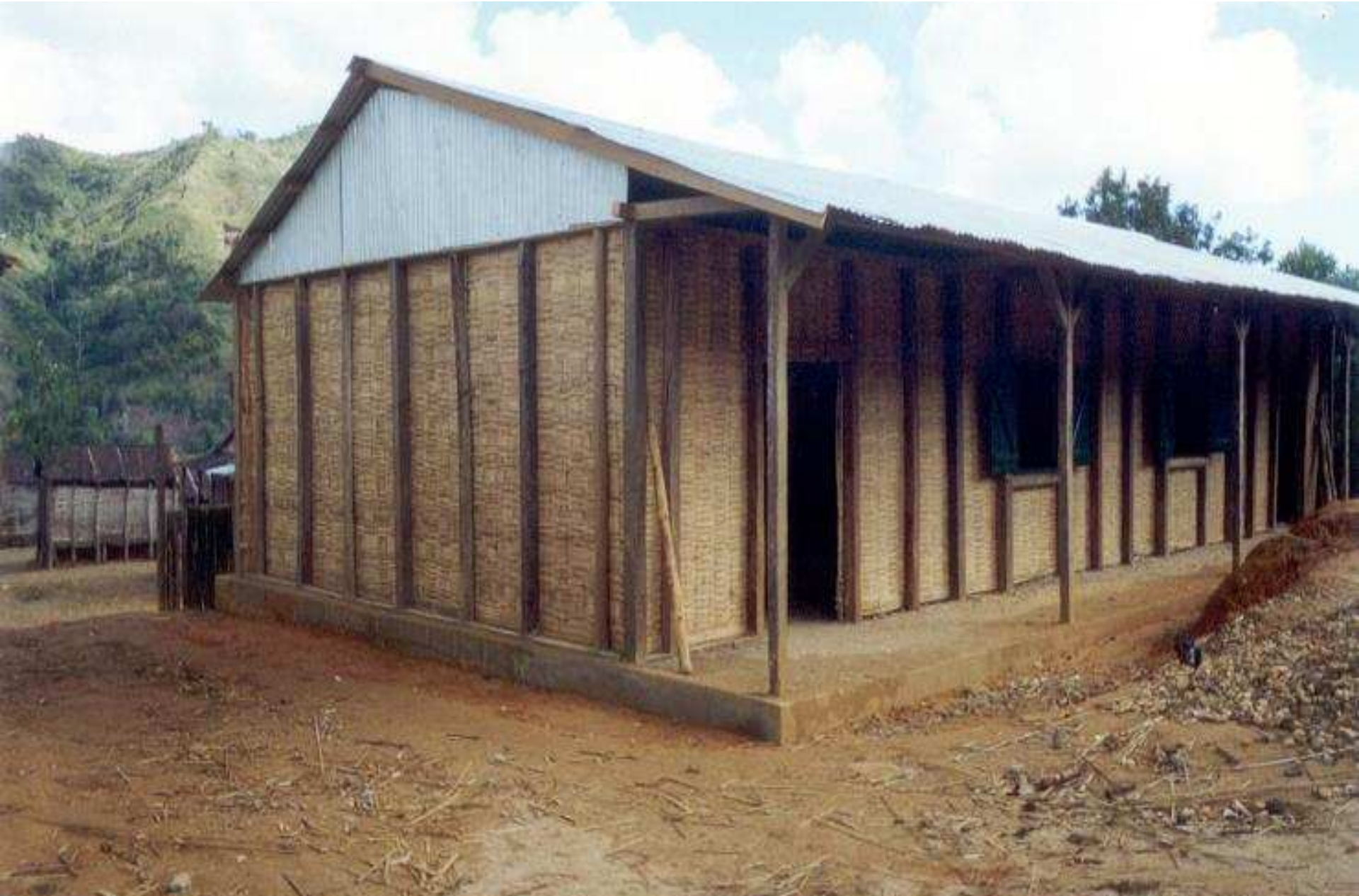
General view of Montagne de Francais Forest, Diego, N. Madagascar

The Rewards of Biodiversity Conservation. The Montagne de Francais is a New Protected Area

The Rewards of Biodiversity Conservation. The Montagne de Francais is a New Protected Area

The Rewards of Biodiversity Conservation. The Montagne de Francais is a New Protected Area

The Rewards of Biodiversity Conservation. New Infrastructure Projects Completed



The Rewards of Biodiversity Conservation. New Infrastructure Projects Completed

The Rewards of Biodiversity Conservation. New Infrastructure Projects Completed

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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Urban areas
MILES
0 25 50 75 100
Albers equal area projection
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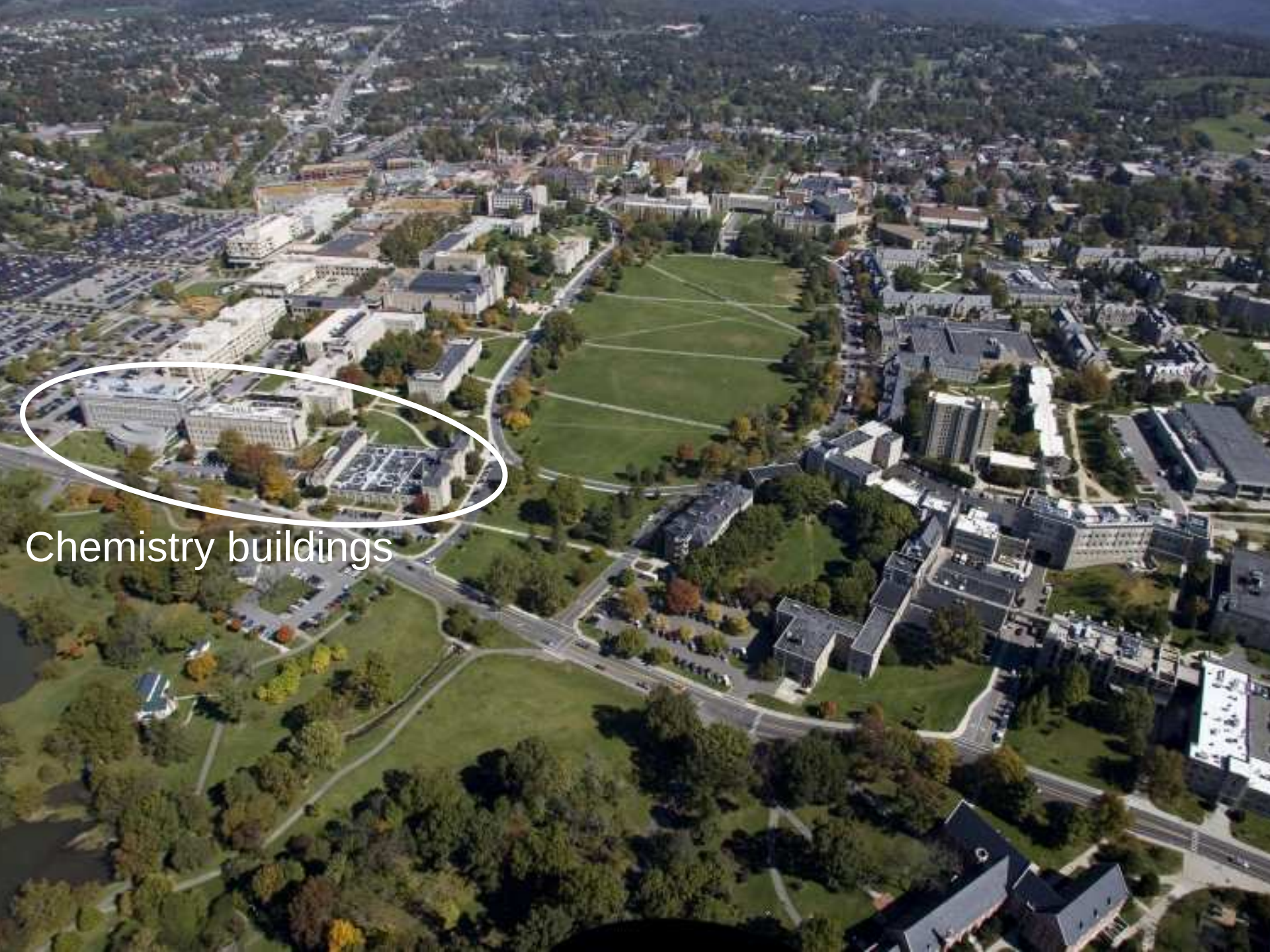
Chemistry buildings


TECHNIC INST