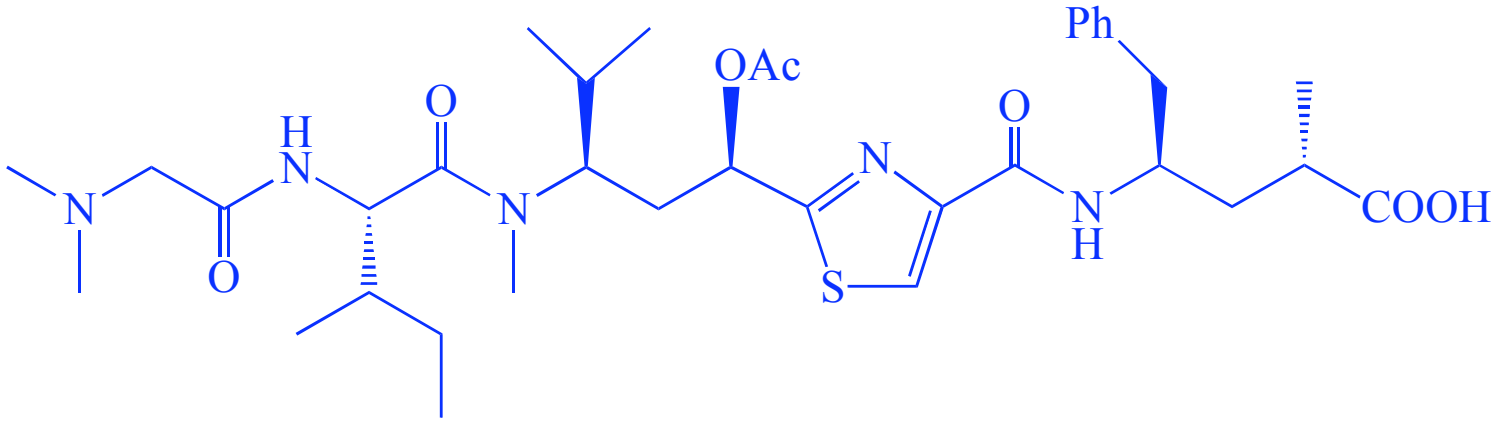
Part I. Progress toward the Total Synthesis of Tubulysin Analogs

Zhiyong Wang

Research Topic Presentation

September 16, 2006

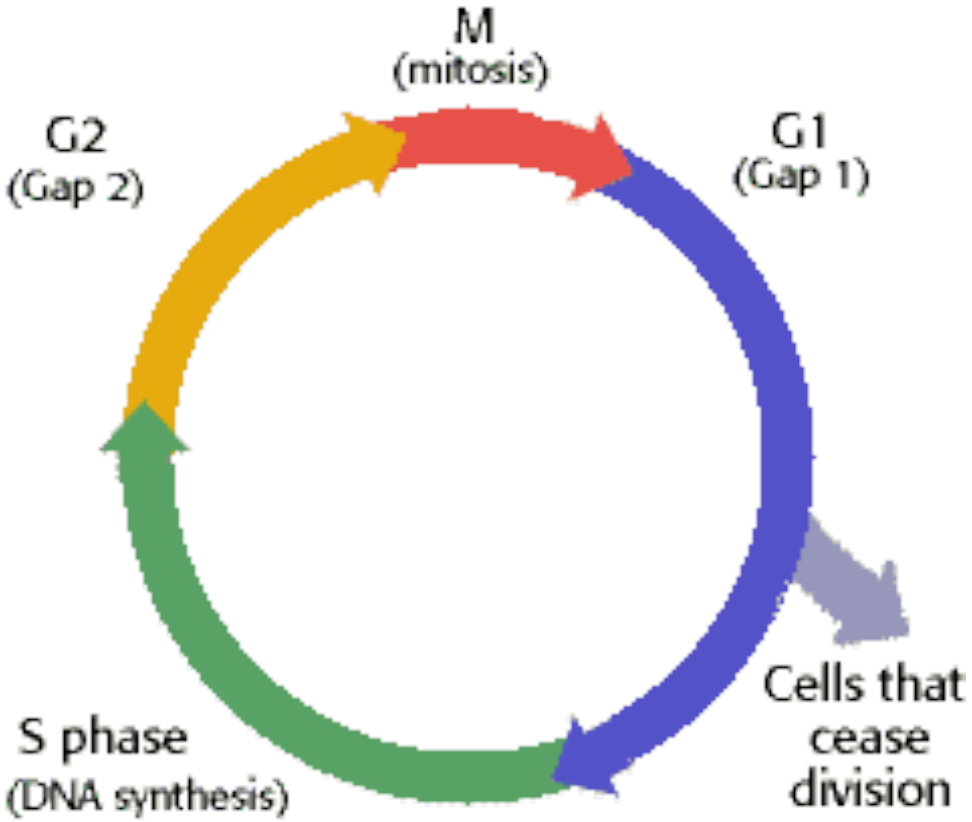


A General Look at the Cell Cycle

➢Four Stage of a Cell Cycle
G1-S-G2-M

➢Two Major Control Points for a Cell Cycle

– G1/S is the point at which cells commit to replicate generic material, enter quiescence (G0), or terminally differentiate and die.
– G2/M is the point at which cells commit to division.
– Tubulin inhibitors typically cause the treated cells to accumulate at the G2/M check point.



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

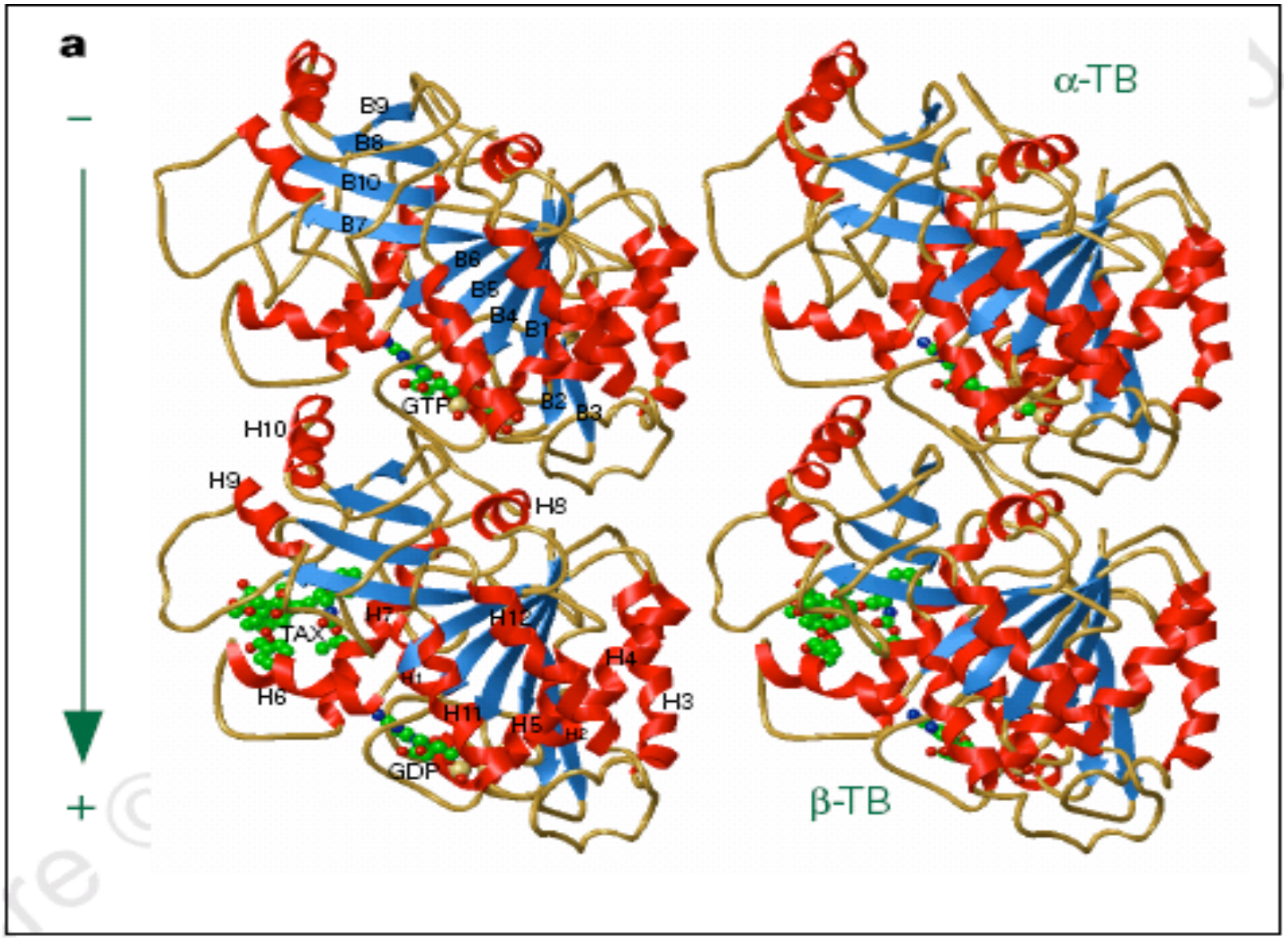
Microtubules and Tubulin

- Microtubules: cytoskeletal elements that are essential for intracellular transport and cell division in all eukaryotes.

- Tubulin is the structural subunit of microtubules, and it exists as a heterodimeric structure of the α and β tubulin proteins.
  - The α and β subunits are of similar secondary and tertiary structure.
  - Each subunit is ca. 55 kD in mass.
  - The heterodimeric structure is tightly bound together and dissociate only under denaturing conditions.

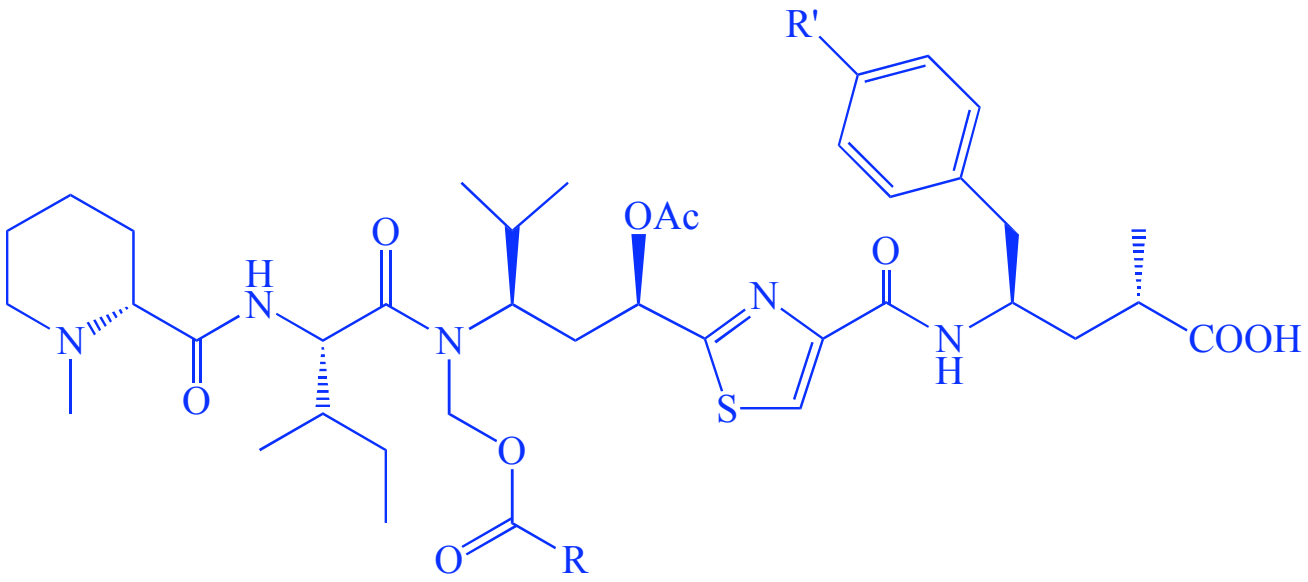
- Each tubulin monomer is capable of binding to a molecule of GTP.
  - α-Tubulin binds to GTP and retains it in the heterodimer.
  - β-Tubulin binds to GTP and hydrolyzes it to GDP during or shortly following the incorporation of the heterodimer into a protofilament.

Structure of the αβ Tubulin Dimer



Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199-203.

Tubulysins: Potent Antimitotic Agents from *Archangium Gephyra* and *Angiococcus Disciformis*



Tubulysin A: R = *iso*-butyl, R' = OH
Tubulysin B: R = *n*-propyl, R' = OH
Tubulysin C: R = ethyl, R' = OH
Tubulysin D: R= *iso*-butyl, R' = H
Tubulysin E: R = *n*-propyl, R' = H
Tubulysin F: R= ethyl, R' = H

Sasse, F.; Steinmetz, H.; Heil, J.; Hofle, G.; Reichenbach, H. *J. Antibiot.* **2000**, *53*, 879.

Depletion of Microtubules by Tubulysins

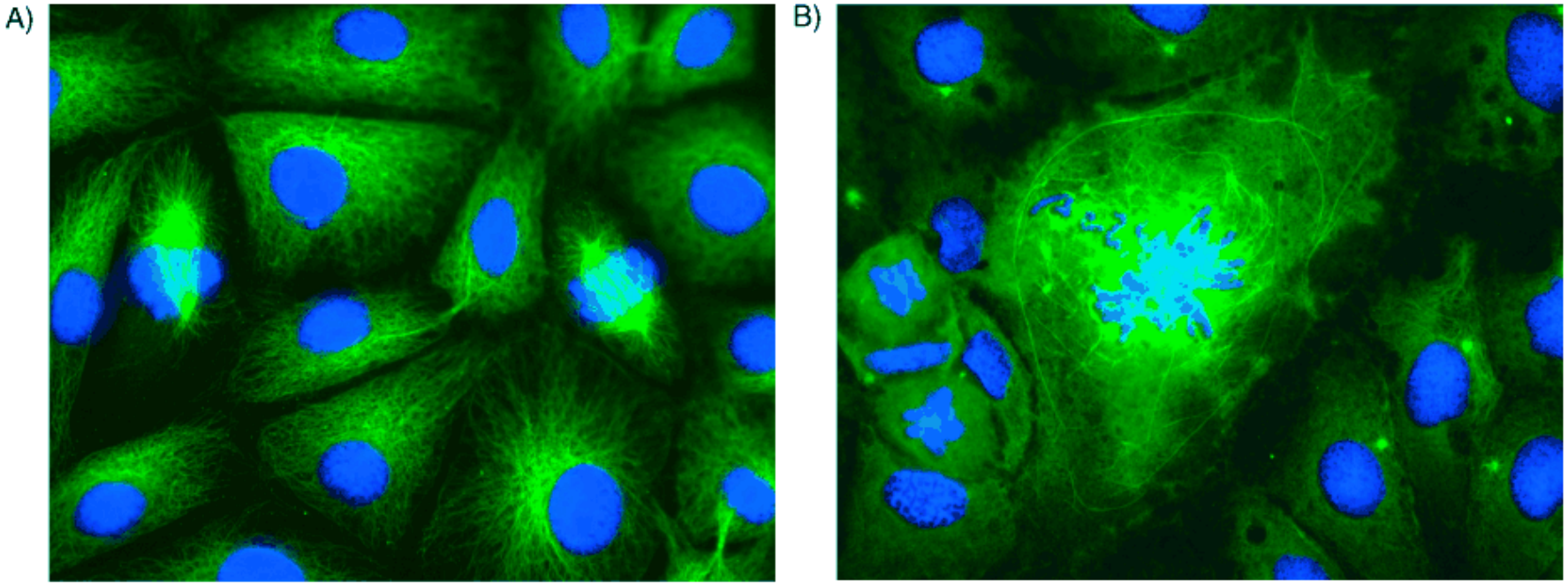


Figure 1. Tubulysin induces a depletion of microtubules. PtK2 potoroo kidney cells were cultured in the absence and presence of tubulysin A (59 nM) for 4 h. A) The control sample shows the normal microtubular network (green) in interphase cells and two mitotic bipolar spindles. Chromosomes and nuclei are stained blue. B) In tubulysin treated cells the microtubule network is less dense and the centrosomes become visible. The mitotic cell in the middle shows metaphase chromosomes, small tuberose microtubular structures, and few longer microtubules that do not form a mitotic spindle.

Khalil, M. W. et al. *ChemBioChem* **2006**, *7*, 678-683.

Influence on the Cell Cycle

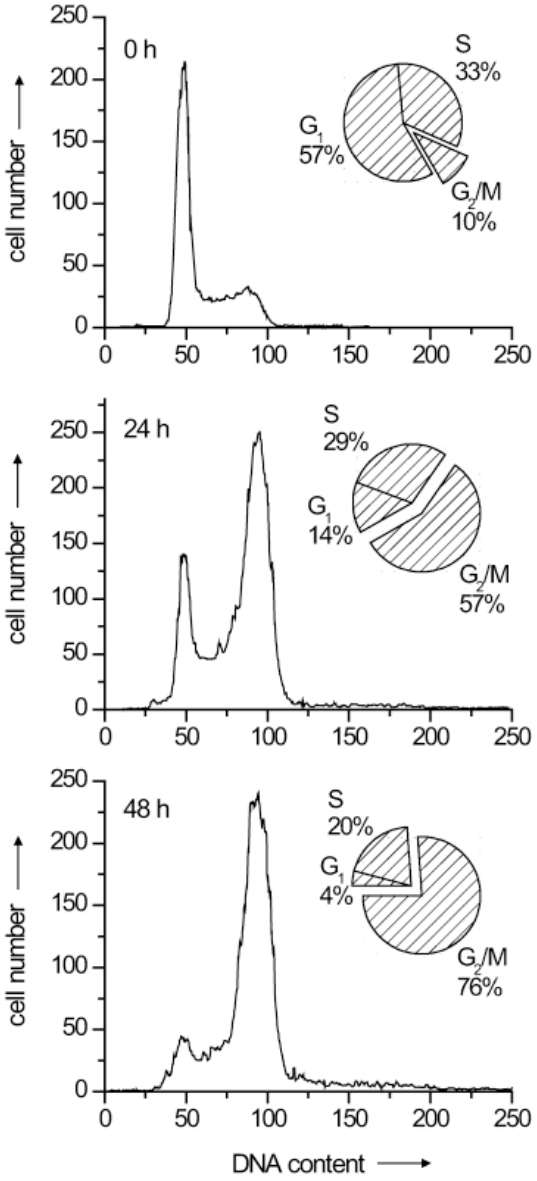


Figure 2. The influence of tubulysin on the cell cycle of L-929 cells. Without treatment the cells showed a high peak in the G1-phase. In the presence of tubulysin A (30 nM) the cells accumulated in the G2/M-phase, where 76 % were found after 2 days of incubation.

Khalil, M. W. et al. *ChemBioChem* **2006**, *7*, 678-683.

Inhibition of Tubulin Polymerization

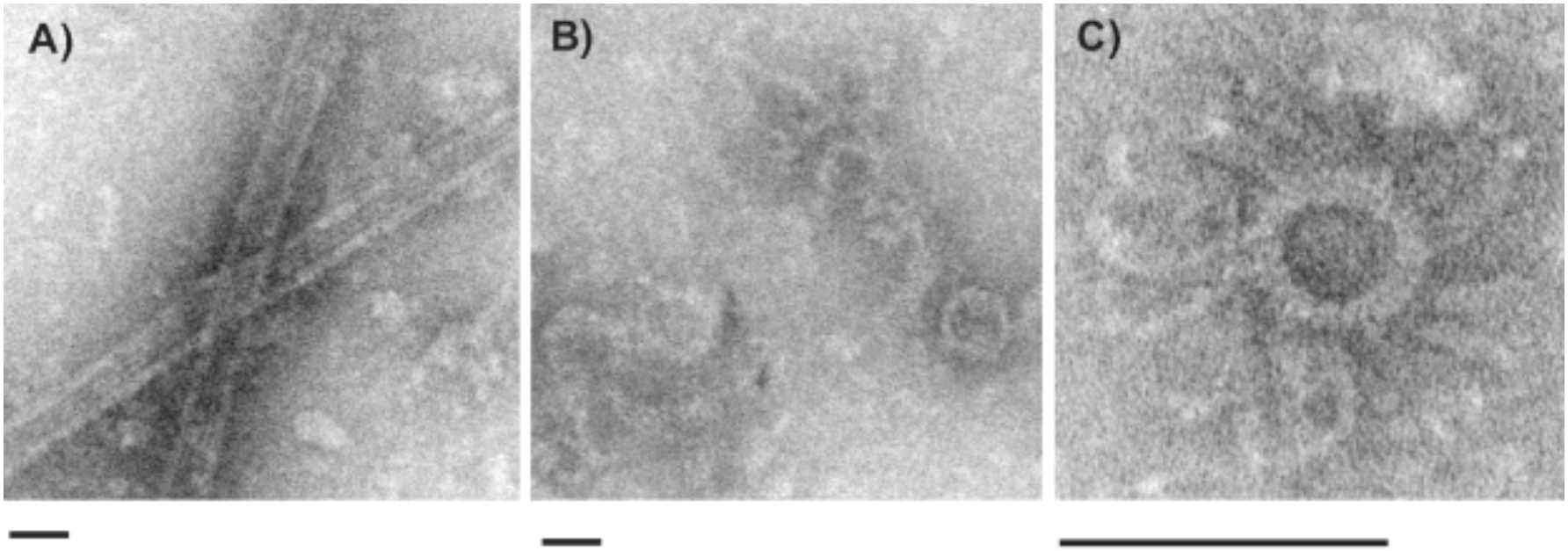


Figure 3. Oligomeric tubulin aggregates induced by tubulysin. Tubulysin was added to microtubules prepared from tubulin with MAPs at a concentration of 12 μM. A) Microtubules in the absence of tubulysins; B) rings and C) pinwheel structure in the presence of tubulysin A (2 μM). Scale bar is 50 nm for A), B), and 100 nm for C).

Khalil, M. W. et al. *ChemBioChem* **2006**, *7*, 678-683.

A Summary of Biological Effects

- Tubulysins act as antimitotic agents by inducing apoptosis.

- Tubulysins inhibits tubulin polymerization, as well as depolymerize formed microtubles. The effect is in contrast to paclitaxel and epothilones, which enhance tubulin polymerization.

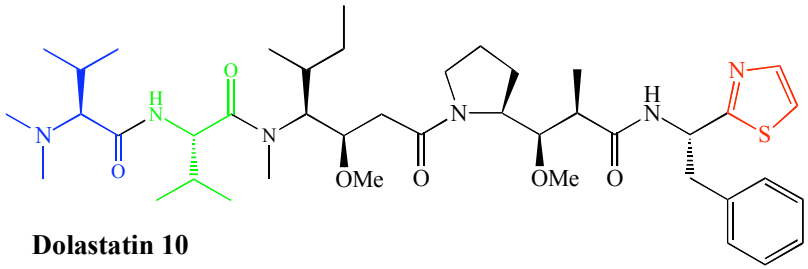
- Tubulysins bind to the peptide binding site of tubulin, which together with vinca site comprises the vinca domain of β tubulin.

- Tubulysins induce the formation of oligomeric tubulin aggregates at substoichiometric concentrations.

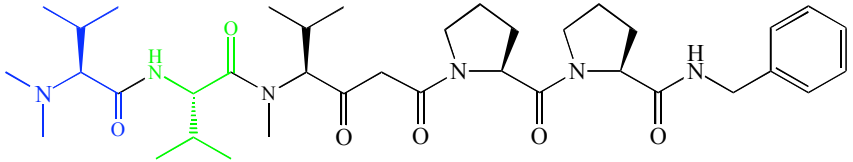
- Tubulysins are highly cytotoxic, and efforts to develop them into anticancer drugs are undergoing.

Khalil, M. W. et al. *ChemBioChem* **2006**, *7*, 678-683.

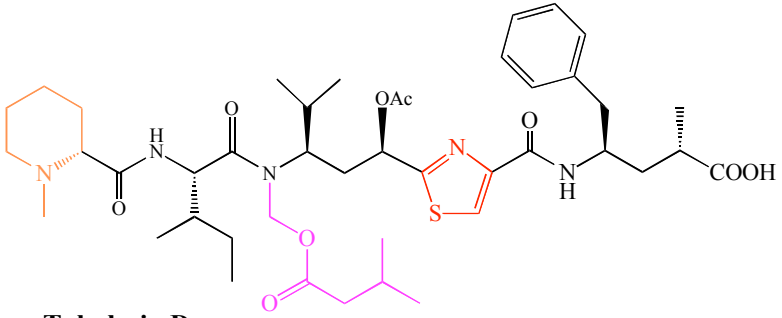
An Interesting Structural Comparison



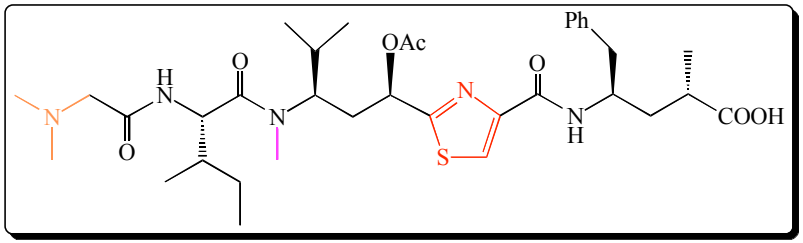
Dolastatin 10



LU103973

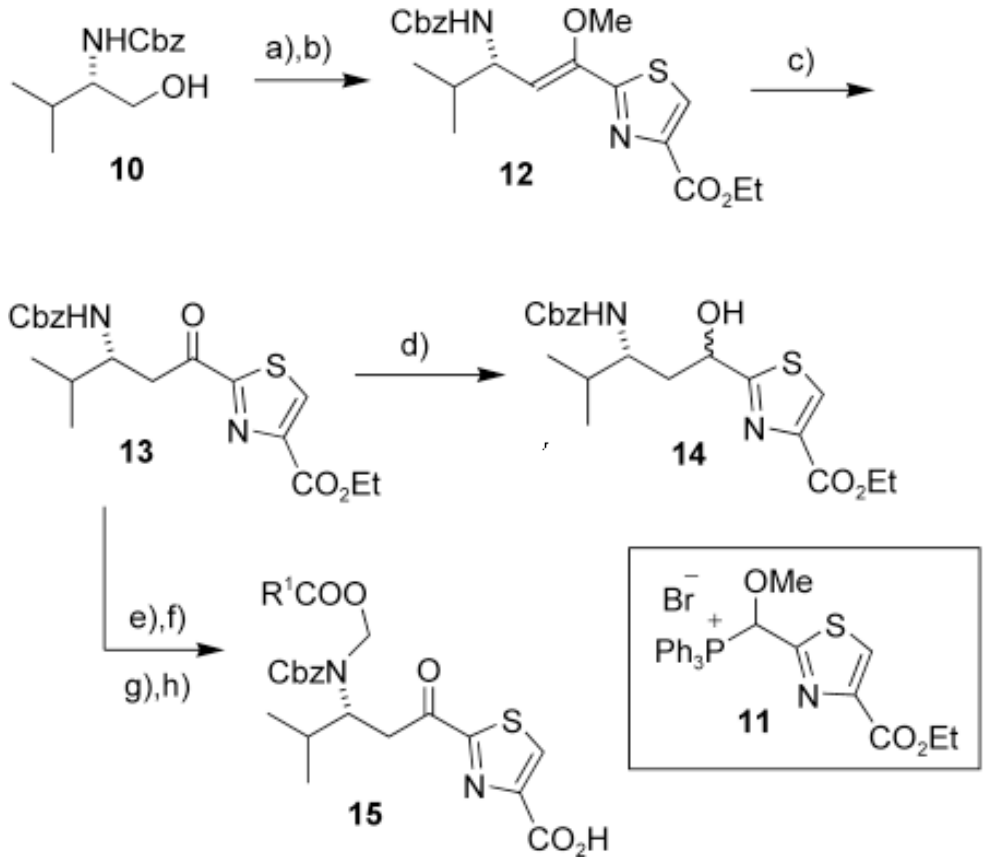


Tubulysin D



Hofle, G. et al. *Pure Appl. Chem.* **2003**, *75*, 167-178.
Jordan, M. A. et al. *Biochemistry* **1998**, *37*, 17571-17578.

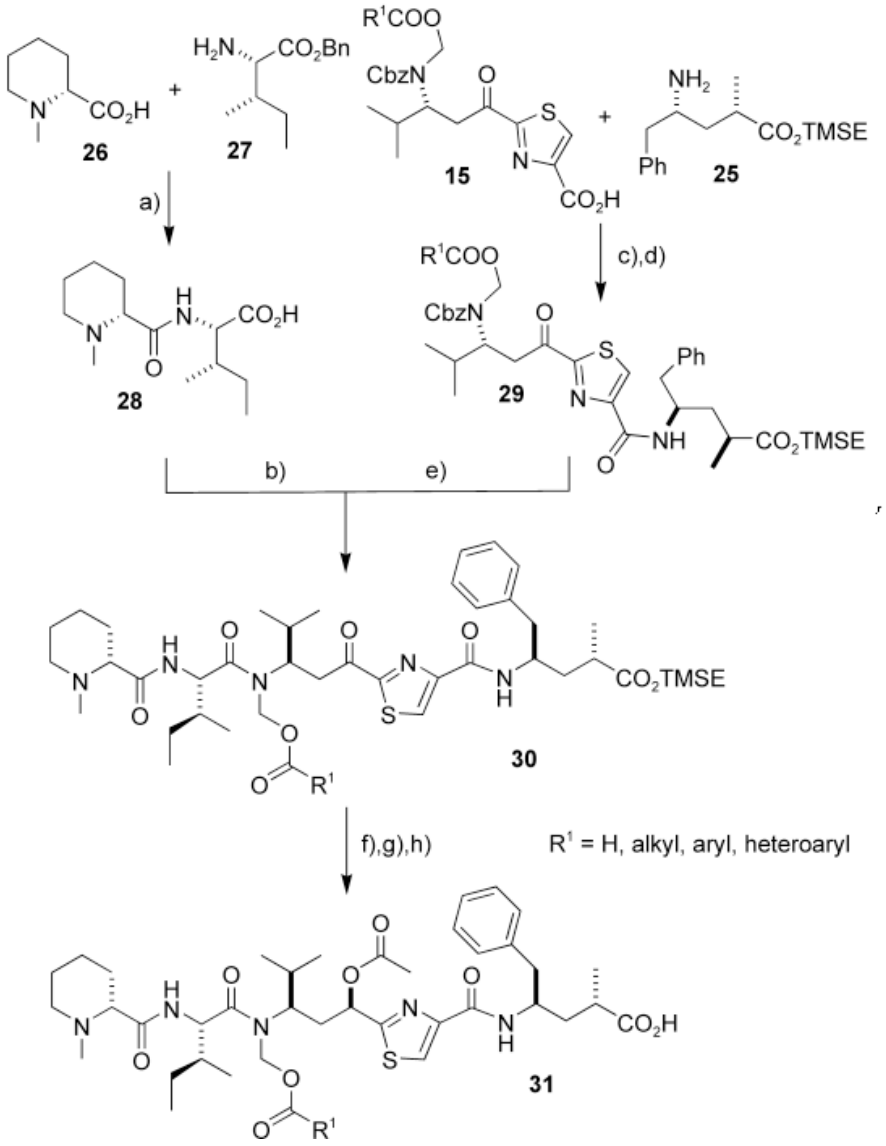
Previous Synthetic Studies on Tubulysins- GBF



Scheme 1. Synthesis of the Tuv fragment by Höfle et al.: a) Swern oxidation; b) DBU, **11**; c) THF, HCl (35 %); d) EtOH, $NaBH_4$; e) NaOH; f) TMSEtOH, DCC; g) NaH, $R^1CO_2CH_2Cl$; h) TBAF. Cbz=benzyloxycarbonyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DCC = *N*,*N* -dicyclohexylcarbodiimide, TBAF=tetrabutylammonium fluoride, TMSEtOH = 2-(trimethylsilyl)ethanol.

Höfle, G.; Leibold, T.; Steinmetz, H. (GBF), DE 10008089, **2001**.

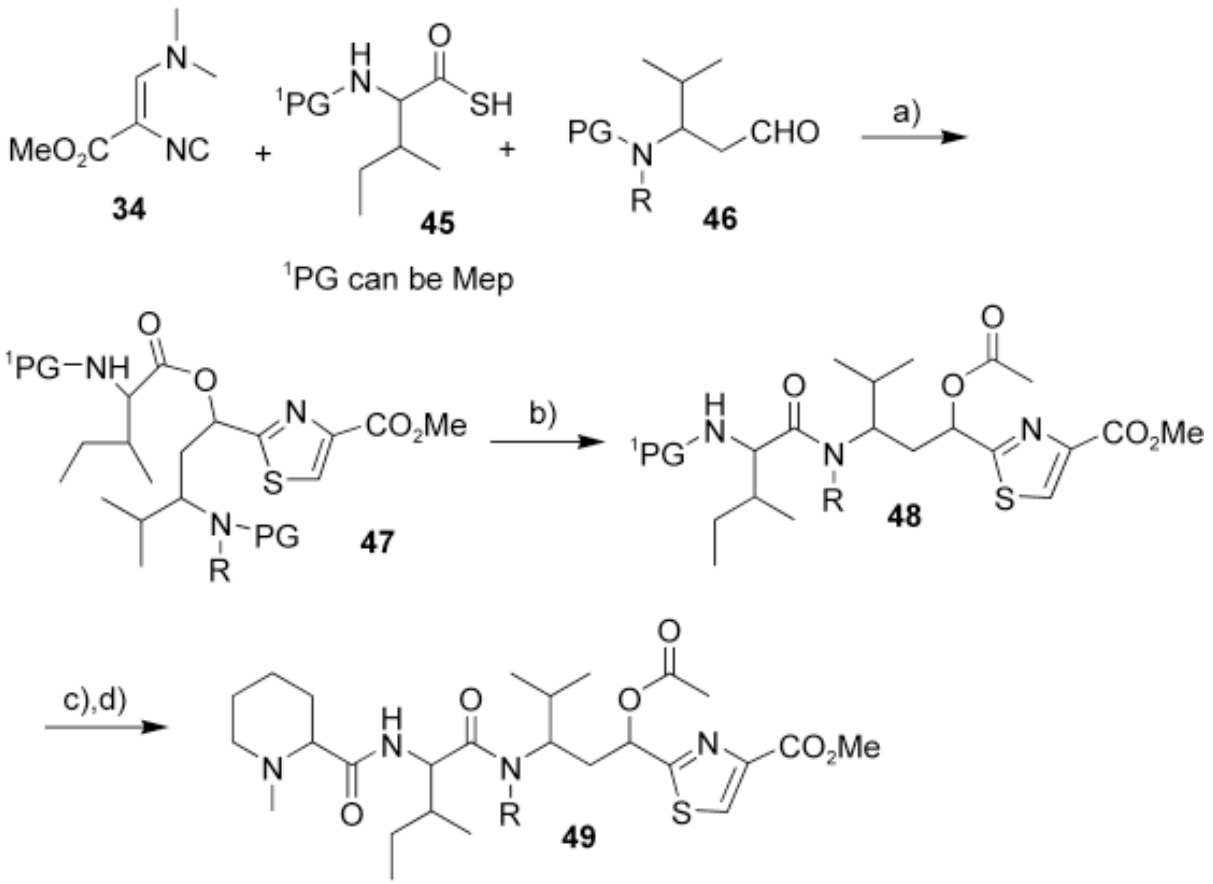
GBF Synthesis- Continued



Scheme 2. Completion of the synthesis of tubulysins by Höfle et al.: a) diethyl cyanophosphonate, Et_3N; b) pentafluorophenol trifluoroacetate; c) pentafluorophenol, DCC; d) Et_3N; e) Et_3N, Pd/C, H_2; f) $NaBH_4$; g) Ac_2O; h) TBAF. TMSE=2-(trimethylsilyl)ethyl.

Höfle, G.; Leibold, T.; Steinmetz, H. (GBF), DE 10008089, **2001**.

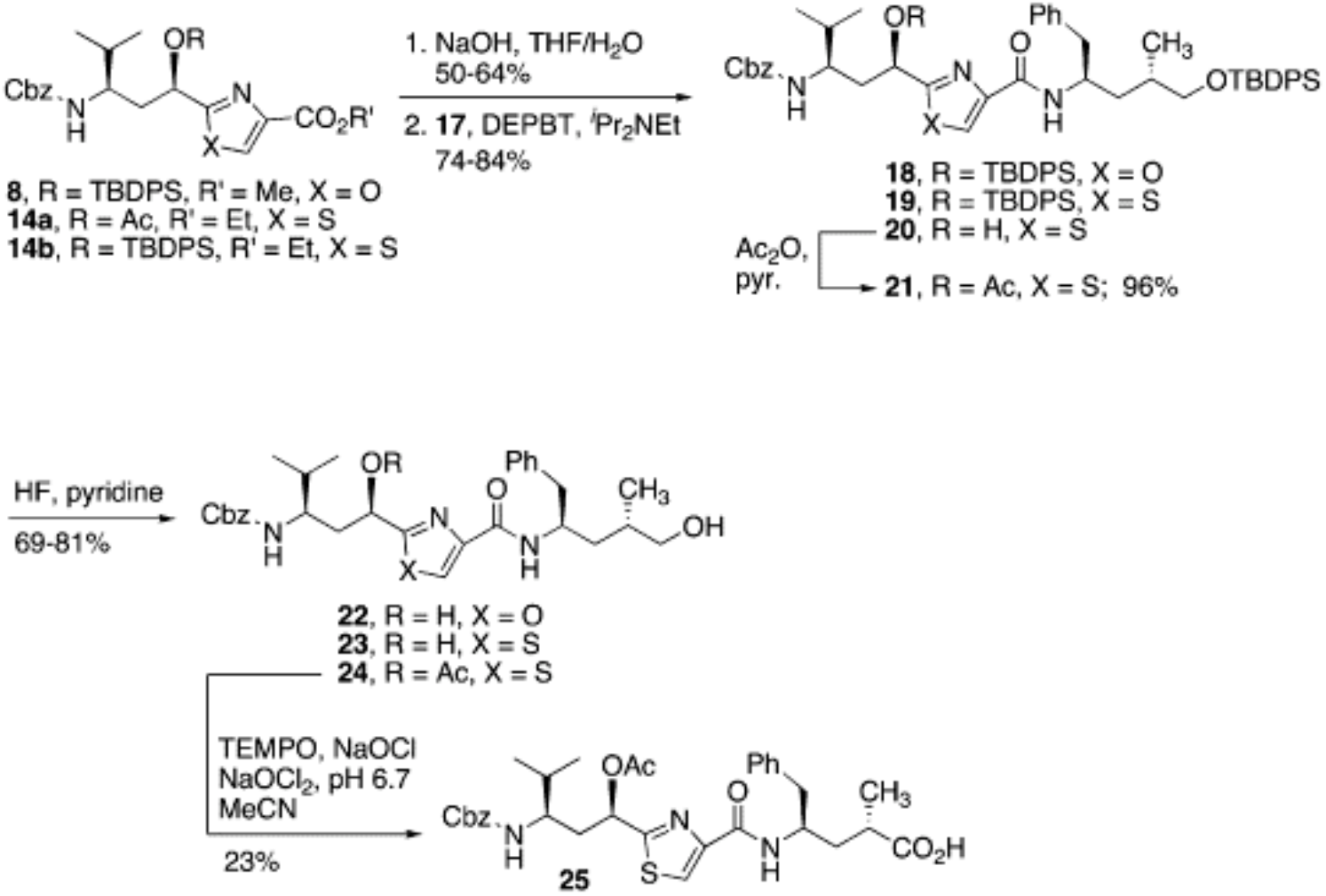
Previous Synthetic Studies on Tubulysins- Morphochem



Scheme 3. Alternative partial synthesis (Morphochem) of the tubulysin structure: a) $BF_3 \cdot OEt_2$, THF; b) Cleavage of PG and isomerization; c) Cleavage of PG1; d) Coupling with **26**.

Dömling, A.; Henkel, B.; Beck, B. (Morphochem), WO 2004005269, **2004**.
Dömling, A. et al. WO 2004005327, **2004**.

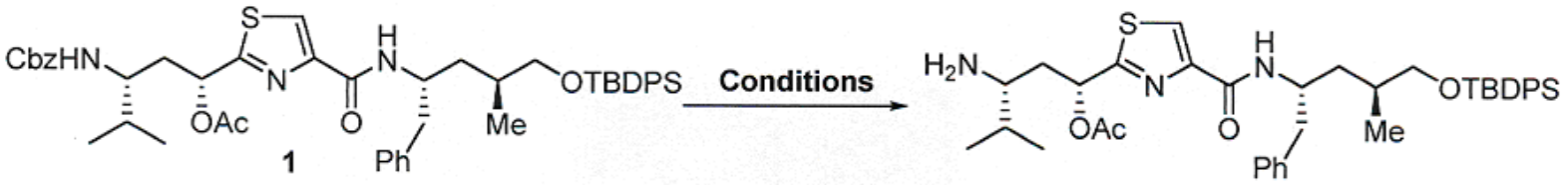
Previous Segment Synthesis- Wipf



Wipf, P.; Takada, T.; Rishel, M. J. *Org. Lett.* **2004**, *6*, 4057-4060.

A Dead End

Table 1. Attempted Cbz-removal from an advanced tubulysin intermediate.



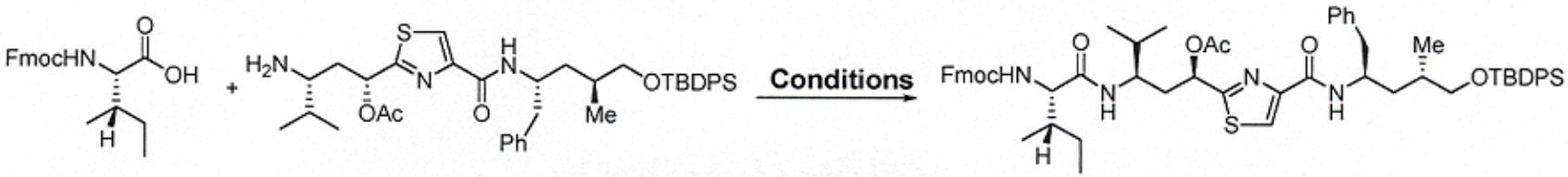
Catalyst	Equivalents	Time (h)	Temp. (°C)	H_2 Pressure (psi)	Solvent	Product (%)	SM (%)
10% Pd/C	0.35	14	24	500	MeOH	–	91
10% Pd/C	0.70	48	24	1000	MeOH	–	92
20% $Pd(OH)_2/C$	0.10	12	24	15	MeOH	–	94
20% $Pd(OH)_2/C$	0.20	12	24	100	MeOH	–	93
Pd-black	0.60	20	24	100	$EtOH/Et_3N$	30	64
Pd-black	1.2	16	24	500	$EtOH/Et_3N$	–	83
Pd-black	1.2	12	24	15	$EtOH/Et_3N$	8	84
Pd-black	2.5	96	24	160	$EtOH/Et_3N$	12	77
Raney nickel	0.20	12	24	15	MeOH	–	–
$Pd(OAc)_2$ $/Et_3SiH$	0.20	12	24	–	CH_2Cl_2/Et_3N	16	67
$Pd(OAc)_2$ $/Et_3SiH$	0.20	14	60*	–	CH_2Cl_2/Et_3N	–	70

*This reaction was performed in a sealed tube.

Rishel, M. *unpublished results*.

…And A Difficult Coupling

Table 2. Attempted coupling of an advanced tubulysin intermediate with Fmoc-Ile-OH



Coupling Reagent	Solvent	Time	Result
DEPBT	DMF	14 h	Recovered Amine (68%)
HATU/HOAt	DMF	36 h	Decomposition
Preformed OSu ester	Dioxane-H_2O	16 h	Recovered Amine (73%)
$(Fmoc\text{-}Ile)_2O$	9:1 DCE-DMF	2 h	Recovered Amine (81%)
Fmoc-Ile-Cl	DCM	16 h	Product (14%)*
BEP	DCM	16 h	Recovered Amine (83%)

* Isolated material may have been a product isomer

Rishel, M. *unpublished results*.