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Paclitaxel (taxol®), a structurally complex diterpenoid, has been developed as a potent drug.against a variety of cancers including breast cancer, ovarian cancer, Kaposi's sarcoma and advanced lung cancer. It possesses unique mechanism of action by binding microtubule and disrupting the microtubule network during cell division. However, the exact paclitaxel binding site on tubulin still remains unclear at the molecular level, and it is essential to understand the interactions of paclitaxel with tubulin and to identify the paclitaxel-tubulin binding interactions on the tubulin peptide in order to design and synthesize more potent analogs of paclitaxel.

The photoaffinity labeling approach is a very powerful method to identify the paclitaxel binding site and to study the interactions with tubulin. By attaching various photoaffinity labels to the paclitaxel molecule, the photolabeled analogues can be used as probes to map the paclitaxel binding site on tubulin.

Based on the proposal, I have successfully designed and prepared some of the photoaffinity analogs modified at C10, C7. The radioactive analogs modified at C7 were also completed. Biological evaluation of these analogs indicated that they possess good microtubule assembly activity and useful for probing paclitaxel binding site studies.

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Yanbin Liu 7-27-00

PI - Signature Date

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Introduction

Paclitaxel (taxol®) (**1**, Figure 1), a structurally complex diterpenoid, has been developed as a potent anticancer drug. It was first isolated in 1971[1] as a cytotoxic agent from the bark of the Pacific yew (*taxus brevifolia*). Its unique antimitotic mechanism of action has made it one of the most effective antitumor agents against a variety of cancers including breast cancer. In December 1992, paclitaxel, marketed by Bristol-Myers Squibb, was first approved by the FDA for the treatment of ovarian cancer. It was also approved by the FDA for treating breast cancer (April 1994), Kaposi's sarcoma (an AIDS-related cancer) (August 1997), and most recently, advanced lung cancer (June 1998.).

Although paclitaxel possesses strong antitumor activity by disruption of the microtubule network during cell division,[2] the exact paclitaxel binding site on tubulin still remains unclear at the molecular level. Therefore, it is essential to better understand the interactions of paclitaxel with tubulin and to identify the paclitaxel-tubulin binding interactions on the tubulin peptide in order to design and to synthesize more potent analogs of paclitaxel for cancer therapy.

The photoaffinity labeling approach is a very powerful method to identify the paclitaxel binding site and to study the interactions with tubulin. By attaching various photoaffinity labels to the paclitaxel molecule, the photolabeled analogs can be used as probes to map the paclitaxel binding site on tubulin. Based on the proposal, we have designed and synthesized some of the proposed photoaffinity analogs of paclitaxel.

Body of Report

Synthesis of C10 Photoaffinity Analogs of Paclitaxel

As stated in the original proposal, the C10 photoaffinity analogs were prepared as shown in scheme 1. The 10-acetyl group in paclitaxel was selectively removed under hydrogen peroxide condition[3] providing 10-deacetylpaclitaxel (10-DAT, **2**) in 86% yield. Protection of the 2' and 7 hydroxyl group with chloroacetyl groups was carried out in DMF in the presence of 2.5 equivalent of chloroacetyl anhydride and 4-dimethylaminopyridine (DMAP). The 2', 7 hydroxyl protected paclitaxel derivative (**3**) was obtained in greater than 60% yield. The C-10 hydroxyl group of **3** was then acylated with photolabeled acids (4-azidobenzoic acid, 3-azido-5-nitrobenzoic acid or 3-dimethylaminobenzoic acid) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and DMAP.[4] The resulting crude intermediates (**4**) were treated with thiourea to remove the protecting group at 2' and 7. This afforded the desired paclitaxel photolabels, **5**, **6** and **7** in variable yields. Among these three photolabels, compound **7** is a fluorescent analog.

Synthesis of Radiolabeled Pacltaxel Analogs with Photolabels at C10

Since above approach resulted in low yields, an alternative route using Lewis acid was attempted based on the chemistry used for acylation of 10-deacetylbaccatin III (**8**, scheme 2). The most reactive of the four alcohols of **8** is normally the 7-hydroxyl. However, selective acylation of the less reactive C10 hydroxyl in the presence of free C7 hydroxyl was achieved using catalytic cerium chloride and excess anhydride as the acylating reagents. Excellent yields were obtained.[5,6] Although its application to paclitaxel derivative was not known, we envisioned it was a potentially useful method to acylate the C10 hydroxyl of 10-DAT (**2**). Selective acylation of 10 over 7 hydroxyl would permit sequential radiolabeling at C7 with minimal protecting group manipulation.

a) Preparation of Acid Anhydrides

Since only acid anhydrides were allowed to use in the Lewis acid catalyzed chemistry, we prepared the target acid anhydrides as shown in schemes 3, 4 and 5. 4-Azidobenzoic acid anhydride (**10**) was prepared in one step from commercially available acid **9**. Treatment of **9** with 0.5 equivalents mesyl chloride and triethylamine (TEA) at -15 °C afforded the desired anhydride **10** in 68% yield (Scheme 3).

The noncommercially available 3-azidobenzoic acid **12** was prepared from 3-aminobenzoic acid **11** upon treatment with $NaNO_2$ followed by NaN_3 (Scheme 4). The corresponding anhydride **13** was obtained in 40% using the same chemistry as shown in scheme 3.

Preparation of anhydride **17** required conversion of acid **15** to acid chloride **16** using thionyl chloride as shown in scheme 5. Following the same chemistry as in scheme 4, aryl azide **15** was synthesized from commercially available **14** in 85% yield. Treatment of acid chloride **16** with the sodium salt of acid **15** afforded desired anhydride **17** in 58% yield. The use of mesyl chloride to prepare the corresponding anhydride as shown in scheme 3 and 4 was attempted and but failed to produce the desired product.

b) Synthesis of C10 Photolabels

Protection of the 2' hydroxyl of paclitaxel was necessary in order to block the hydrolysis of the side chain and the possible acylation of 2' hydroxyl in subsequent reactions. Treatment of paclitaxel with *tert*-butyldimethylsilyl chloride in the presence of DMAP afforded 2'-TBS-paclitaxel **18** in excellent yield (Scheme 6).[7] The C10 acetyl group was removed selectively using hydrazine monohydrate in 95% ethanol, providing 10-deacetylpaclitaxel derivative **19** in 87% yield. Selective

acylation of the C10 hydroxyl over the C7 hydroxyl of **19** was carried out in THF using the acid anhydrides prepared above (Scheme 3, 4 and 5) in the presence of a catalytic amount of cerium chloride. Good yields were obtained for compounds **20** and **21**. In the case of compound **22**, the lower yield was most likely due to the unstable 3-azido-5-nitrophenyl moiety. Removal of TBS protecting groups from **20** and **22** yielded the C10 photolabeled paclitaxel derivatives **5** and **6**, respectively (deprotection of **21** was not yet attempted).

Synthesis of C7 Radioactive Paclitaxel Analogs with C10 photolabels

Radiosynthesis of **21**, **22** and **23** modified at C7 was carried out using chemistry previously developed. Before conducting radiosynthesis using tritiated acetate, a cold run was carried out for each of the C10 photolabels (**21**, **22** and **23**) using nonradioactive acetate. In both cases, the acylations were carried out utilizing EDC, DMAP and sodium acetate (or tritiated sodium acetate) in CH_2Cl_2 at room temperature for 20 h (Scheme 7). The TBS protecting group at 2' was removed using acidic ethanol and the final products were isolated in good yields with sufficient radioactivity for microtubule binding studies. The cold runs gave higher yields than hot runs. For compound **25**, lower yields were obtained in both the cold run and hot run due to the light sensitive nature of the 3-azido-5-nitrophenyl moiety. The cold products were evaluated for their ability to promote tubulin assembly.

Synthesis of C2 Photoaffinity Analogs of Paclitaxel with a radioactive moiety at C7

Because the C2 photoaffinity analogs were shown to possess an excellent ability to promote tubulin assembly[7] and therefore were selected for use in labeling studies. In our paclitaxel binding studies, the radioactive form of these C2 analogs were required. Therefore, the C7 tritiated acetate derivatives of these C2 analogs were considered to be good target. Utilizing chemistry developed in our group, the 2-*O*-(3-azidobenzoyl)paclitaxel derivative **29** was synthesized as shown in scheme 8. Protection of the 2' and 7 hydroxyl group proceeded cleanly to afford compound **26**. The C2 benzoyl group was selectively removed by treating **26** with potassium *tert*-butoxide and water. Acylation of **27** with 3-azidobenzoic acid in the presence of DCC and DMAP afforded **28**, which was deprotected with pyridinium hydrogen fluoride to provide the C2 photolabeled paclitaxel analog **29**.

Radiosynthesis of **31** (Scheme 9) followed the same chemistry as shown in scheme 7. The more reactive 2' hydroxyl of **29** was protected with silyl group TBS in excellent yield using TBSCl in the presence of DMAP (Scheme 9). The tritiated sodium acetate was then utilized to acylate the C7 hydroxyl of **30**. Removal of the silyl group with HCl in ethanol afforded the desired radioactive C2 photoaffinity labeled compound in 43% overall yield with a specific radioactiviy 0.22 mCi / mMole.

Synthesis of C3' Photoaffinity Analogs of Paclitaxel

The C3' photolabeled pacllitaxel analog (**32**, scheme 10) constructed by replacing 3' phenyl group of paclitaxel with a 4-azidophenyl group was one of the proposed targets in the proposal. This could be achieved through the coupling of a β-lactam (**33**) with baccatin III (**34**) as outlined in scheme 10. The enantiomerically pure β-lactam (**33**) could be obtained using a [2 + 2] cycloaddition of the imine (**35**) and chiral auxiliary attached glycolate **36**. This glycolate was easily prepared according to well developed chemistry in our group.[8]

The synthesis of the C3' analog started with commercially available 4-aminobenzylalcohol **37**. Replacement of the amino group with a azido group provided **38** in excellent yield (Scheme 11). Pyridinium chlorochromate (PCC) oxidation of alcohol **38** to aldehyde **39** proceeded cleanly in 92% yield. Although in prior analogous reactions, generation of the *N*-TMS imine proceeded smoothly using lithium hexamethyldisilylazide. The *p*-azido imine was found to be unstable and promptly decomposed into a tarry residue. Attempts to isolate the imine using standard protocols also failed.

Direct use of the crude imine did not participate in the chiral ester enolate - imine cyclization. A numbers of attempts were made to prepare the imine **35** but all failed. Interestingly, with other aromatic aldehydes these complications were not seen.[8]

The instability of the silylimine of *p*-azidobenzaldehyde suggested the use of another imine. Therefore, generation of the *p*-methoxyphenyl (PMP) imine from *p*-azidobenzaldehyde (**39**) and anisidine (**40**) was carried out in ethanol. Imine **41** was obtained repeatedly in high yield as clean yellow needles (Scheme 12). The Bose - Staudinger reaction between imine **41** and *O*-TIPS glycoyl chloride proceeded smoothly overnight, providing β-lactam **42** in 80% yield. The PMP protecting group was removed under standard conditions to provide racemic **43**. After benzylation of **43** with benzyl chloride, the intermediate was coupled with 7 hydroxyl protected baccatin III. Finally, the 2' silyl group was removed by hydrogen fluoride to provide C3' photoaffinity analog **32**. Unfortunately. it was an inseparable diasteromers. Interestingly, when tested in tubulin assembly assay, its ability to promote tubulin assembly was found to be comparable to that of paclitaxel.

We would like to have an enantiomerically pure form of **32**. Therefore, our work toward this goal continues.

Biological Evaluation of Photoaffinity Analogs

All synthesized photoaffinity paclitaxel analogs were tested for their ability to promote tubulin assembly as shown in table 1.

Compounds **6** and **29** demonstrated strong microtubule assembly activity which was higher than paclitaxel. Other analogs also possessed reasonable potency. All analog would be good candidates for microtubule binding studies. The study of paclitaxel microtubule binding is underway using these photoaffinity analogs.

Key Research Accomplishments

- Synthesis and characterization of C10 photoaffinity paclitaxel analogs including a fluorescent labeled analog were completed.

  10-*O*-(*p*-azidobenzoyl)-10-deacetylpaclitaxel
  10-*O*-(3-azido-5-nitrobenzoyl-10-deacetylpaclitaxel
  10-*O*-(*m*-dimethylaminobenzoyl)-10-deacetylpaclitaxel

- A general chemistry for the synthesis of C10 paclitaxel analog using Lewis Acid was developed.

- C7 radioactive analogs with photolabels at C10 and C2 were completed.

  7-*O*-([$^3H_3$]-acetyl)-10-*O*-(*p*-azidobenzoyl)-10-deacetylpaclitaxel.
  7-*O*-([$^3H_3$]-acetyl)-10-*O*-(*m*-azidobenzoyl)-10-deacetylpaclitaxel
  7-*O*-([$^3H_3$]-acetyl)-10-*O*-(4-azido-5-nitrobenzoyl)-10-deacetylpaclitaxel
  2-O-(*m*-azidobenzoyl)-7-*O*-([$^3H_3$]-acetyl)-2-*O*-debenzoylpaclitaxel

- C7 nonradioactive acetate analogs were also prepared for the purposes of biological evaluation and characterization

  7-*O*-acetyl-10-*O*-(*p*-azidobenzoyl)-10-deacetylpaclitaxel.
  7-*O*-acetyl-10-*O*-(*m*-azidobenzoyl)-10-deacetylpaclitaxel
  7-*O*-acetyl-10-*O*-(4-azido-5-nitrobenzoyl)-10-deacetylpaclitaxel

- Preliminary study of preparation of C3' photoaffinity analog of paclitaxel was conducted and a mixture of diastereomers of C3' analog was obtained and evaluated.

- Biological evaluation of these prepared photoaffinity analogs were completed as well.

Reportable Outcomes

Flaherty, P. T.; Liu, Y.; Georg, G. I.; Himes, R. H. "Synthesis and Biological Evaluation of 10-Photoaffinity, 7-Radiolabeled Paclitaxel" *Abstract of Papers*, 219th National Meeting of the American Chemical Society, San Francisco, CA, March 26-30, 2000; MEDI 77.

Conclusions

Study of paclitaxel binding sites on microtubules at molecular level is of importance to design and develop the second generation paclitaxel derivatives with improved potency and reduced undesired properties. This requires further understand the exact interactions between paclitaxel and microtubules including the binding peptides, environments, types of binding and the conformational changes of the tubulin.

Photoaffinity analogs of ligands are powerful tools in the effort to identify ligand binding sites and domains in proteins by covalently attaching to the binding site upon UV irradiation. UV irradiation of aryl azides generates an aryl nitrene that covalently binds to peptides residues in close proximity.[9] Practical requirements of photoaffinity labels of paclitaxel include good affinity for the target, efficiency of photo conversion, sufficient biological activity and readily preparation. These are the basis for the proposed targets in the proposal.

As outlined in the proposal, I have planned to conduct two major areas of research: 1) synthesis and characterization of photoaffinity labels and 2) synthesis and characterization of radioactive paclitaxel analogs.

The first area of my project included design and synthesis of photoaffinity analogs of paclitaxel modified at C7, C10 and C3'. Previous structure-activity relationships studies documented that these positions were tolerated well with modifications without loss biological activity. With initial targeting at C10, I have successfully prepared the C10 aryl azides photoaffinity analogs and a fluorescent analog utilizing two chemical approaches. These analogs have been fully characterized. Biological evaluation of these analogs demonstrated that they displayed good ability to promote microtubule assembly, and therefore, are good candidates for probing paclitaxel binding site.

Fluorescence spectroscopy is a powerful tool for studying ligand-protein interactions due to its high sensitivity to the environments of the fluorophore. Information obtained from fluorescence studies could include the interchromophore distances of bound species and the flexibility of the bound molecule. The synthesized fluorescent C10 analog **7** in this study will also be utilized to explore paclitaxel binding environment on mcrotubules.

The second area was the synthesis and characterization of radioactive photoaffinity analogs with modifications at different positions of paclitaxel such as C7 and C10. In this study, an efficient methodology to prepare C7 tritiated acetate analogs has been utilized. This general method allowed me to synthesize and characterize several novel radiolabeled photoaffinity analogs (**23**, **24**, **25** and **31**). Their ability to promote microtubule assembly was found to be comparable with paclitaxel. In addition, these radioactive analogs possess sufficient specific activity to allow for the identification of labeled peptides.

In collaboration with Dr. Himes in the Department of Biochemistry at the University of Kansas, these prepared novel photoaffinity analogs will be utilized to map paclitaxel binding site.

Synthesis of C3' photoaffinity analog encountered some difficulties although a mixture of diastereomers was obtained.

In the future, different chemistry will be pursued to prepare the C3' analog. In addition to these aryl azides, small photoaffinity labels such as alkoxycarbonylazide and 2-diazo-3,3,3-trifluoropropionyl derivative attaching at C10, C7 and C3' of paclitaxel will be prepared and evaluated. These derivatives will be prepared following our previously established protocols.

In conclusion, studies outlined in annual research summary have provided a number of novel photoaffinity analogs that displayed sufficient biological activity for probing the paclitaxel-microtubule binding site and interactions.

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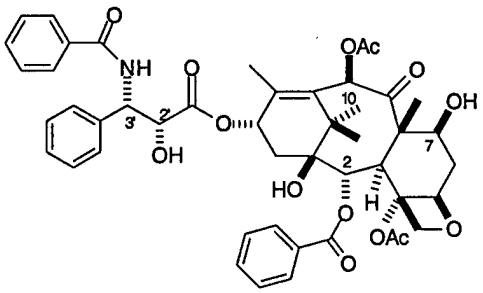
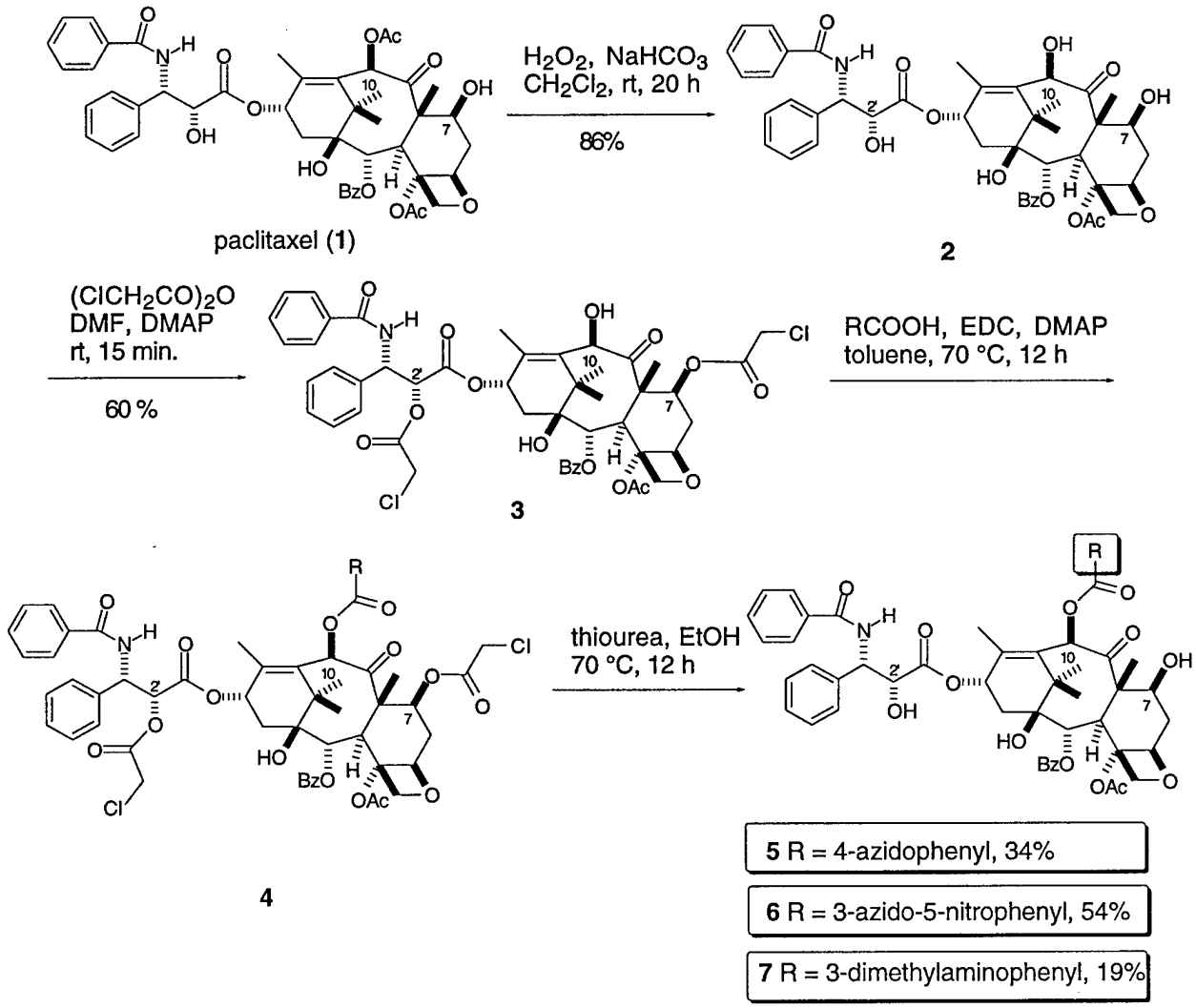
Addendum A

Figure 1. Structure of paclitaxel.

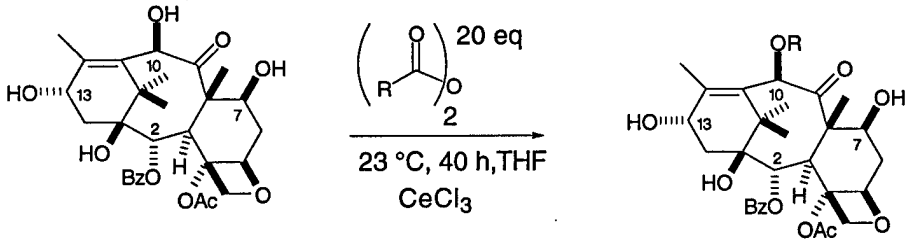


paclitaxel (**1**)

Scheme 1. Synthesis of C10 Photolabels.



Scheme 2. Lewis acid catalyzed acylation of C10 hydroxyl of 10-deacetyl baccatin III.



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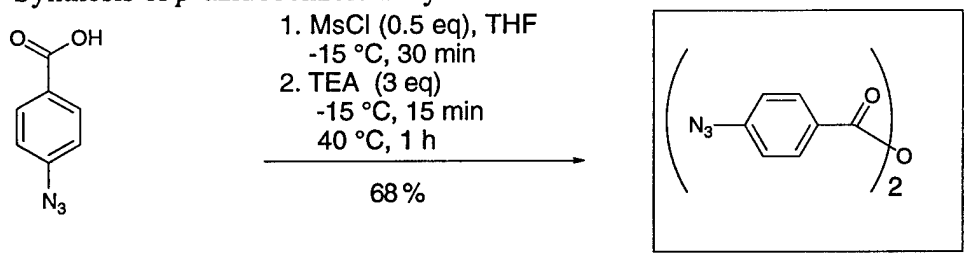
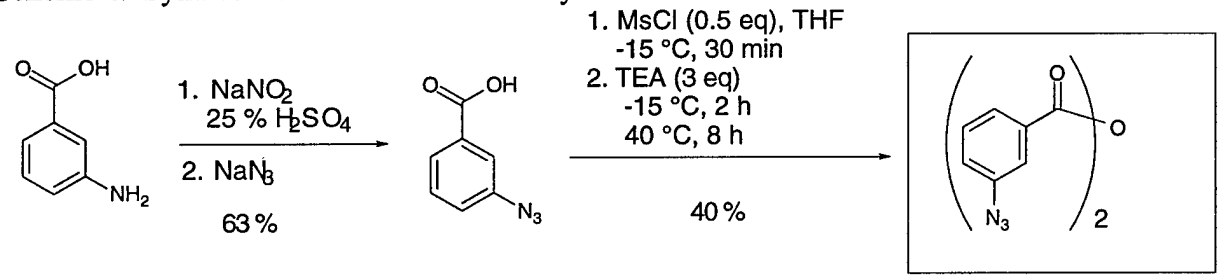
Scheme 3. Synthesis of *p*-azidobenzoic anhydride.



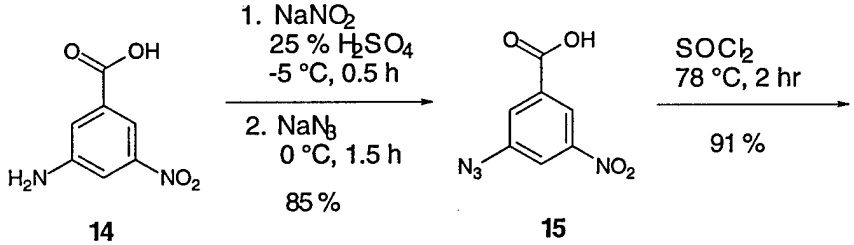
9 **10**

Scheme 4. Synthesis of *m*-azidobenzoic anhydride.

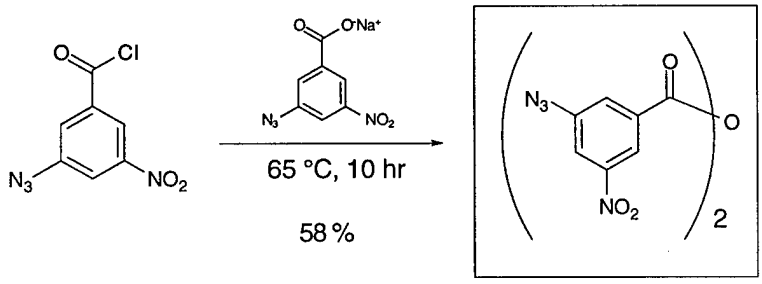


11 **12** **13**

Scheme 5. Synthesis of 3-azido-5-nitrobenzoic anhydride.

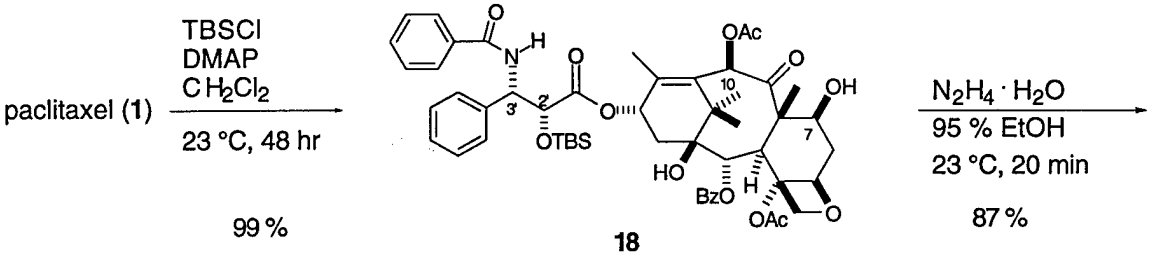


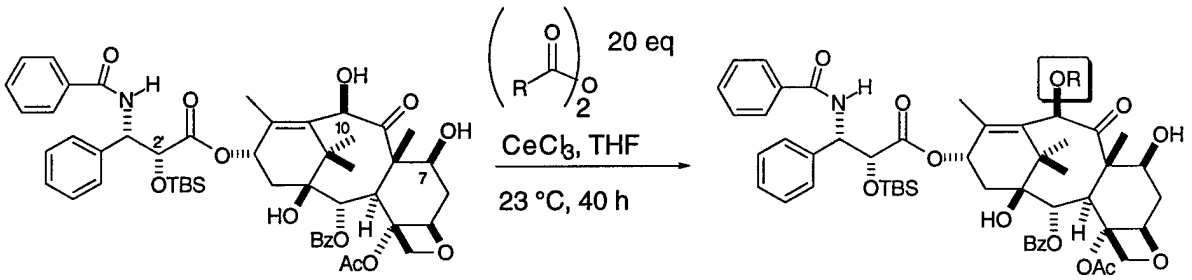
14 **15**



16 **17**

Scheme 6. Synthesis of C10 photolabels using Lewis acid catalyzed chemistry.





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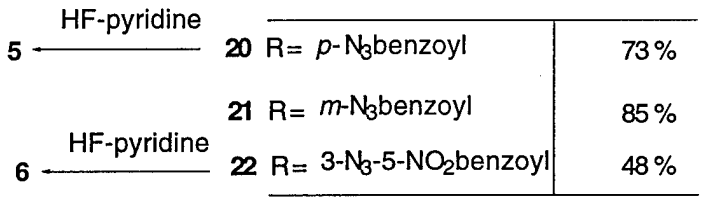
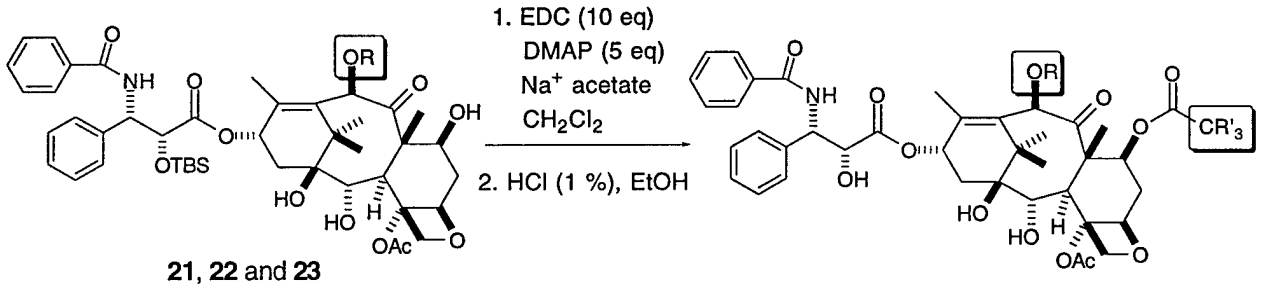


20 R= *p*-N_3benzoyl	73 %
21 R= *m*-N_3benzoyl	85 %
22 R= 3-N_3-5-NO_2benzoyl	48 %

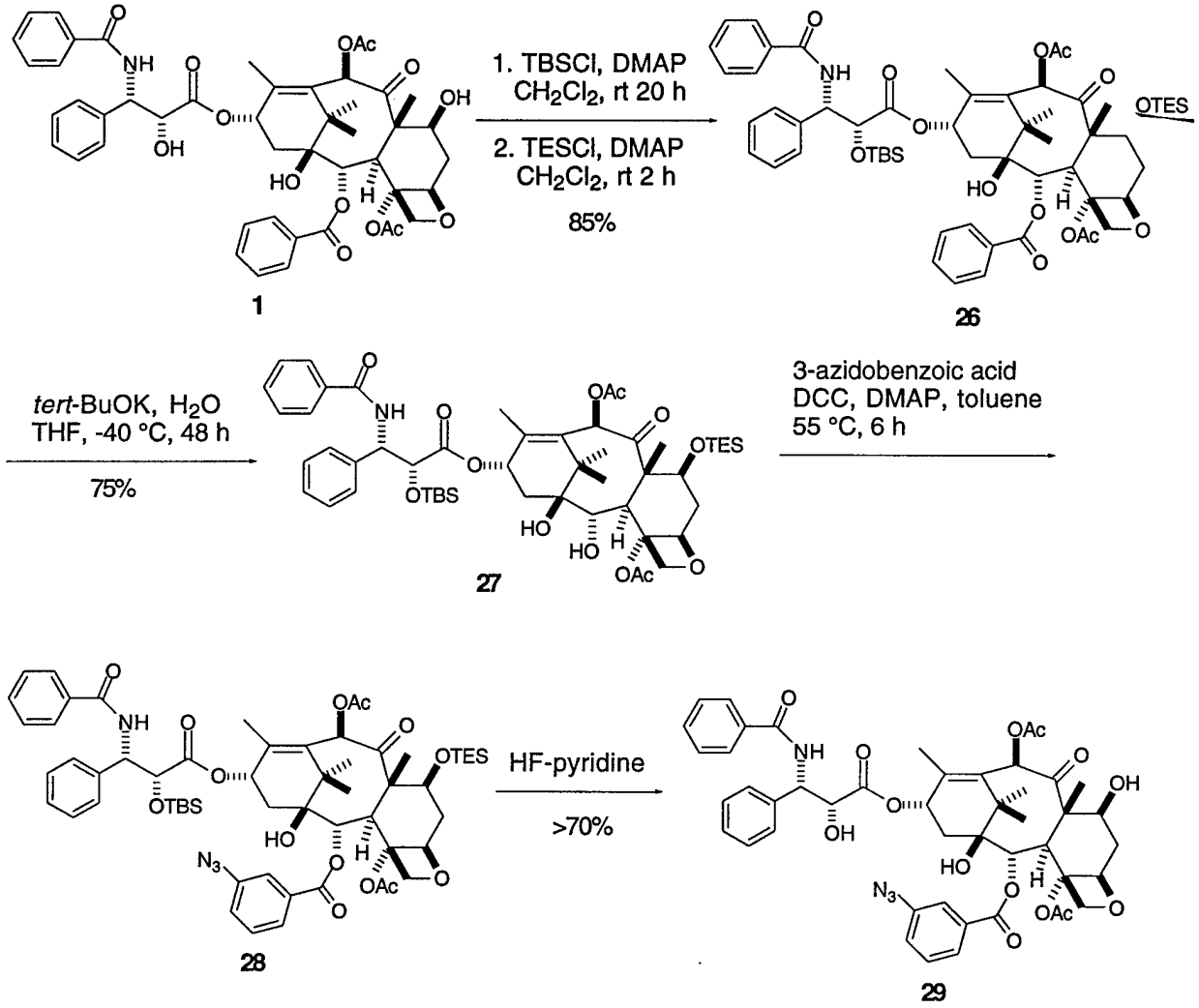
Scheme 7. Synthesis of C7 radioactive analogs with photoaffinity labels at C10.



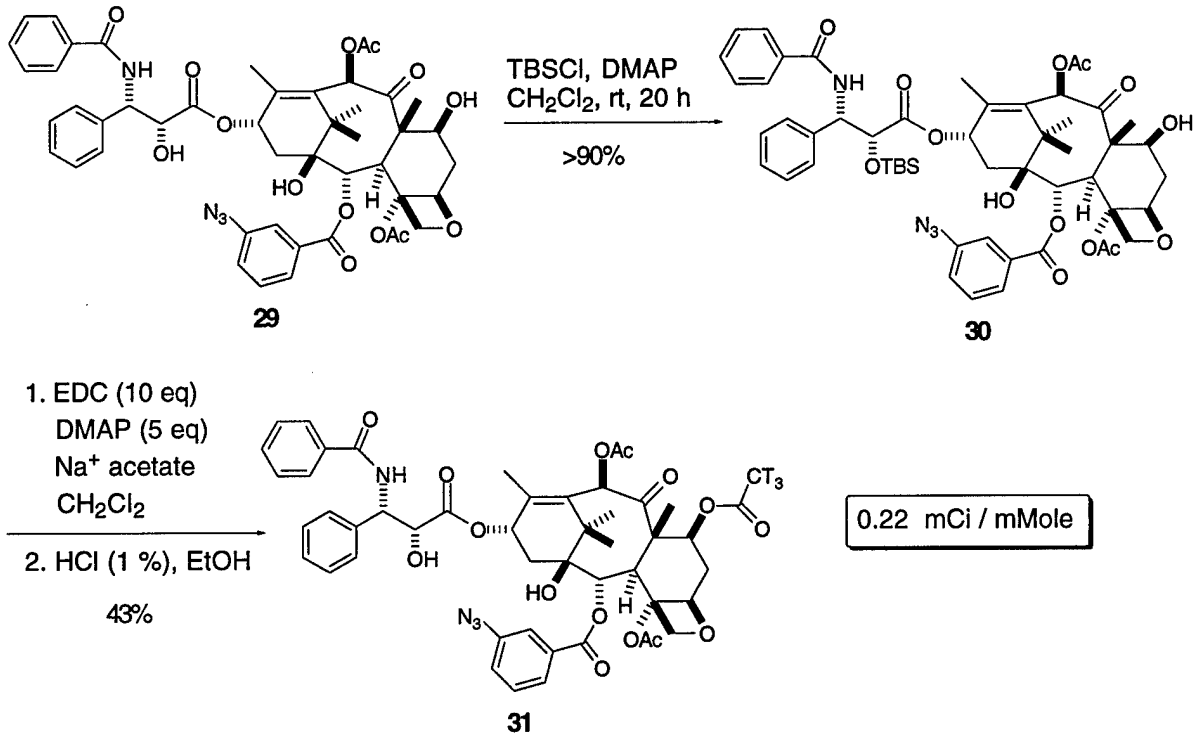
21, **22** and **23**

	cold, R' = H		radioactive, R' = ^3H
23 R= *p*-N_3benzoyl	73 %	43 %	0.28 mCi / mMole
24 R= *m*-N_3benzoyl	85 %	46 %	0.21 mCi /mMole
25 R= 3-N_3-5-NO_2benzoyl	48 %	22 %	0.73 mCi /mMole

Scheme 8. Synthesis of C2 photolabeled analog of paclitaxel.

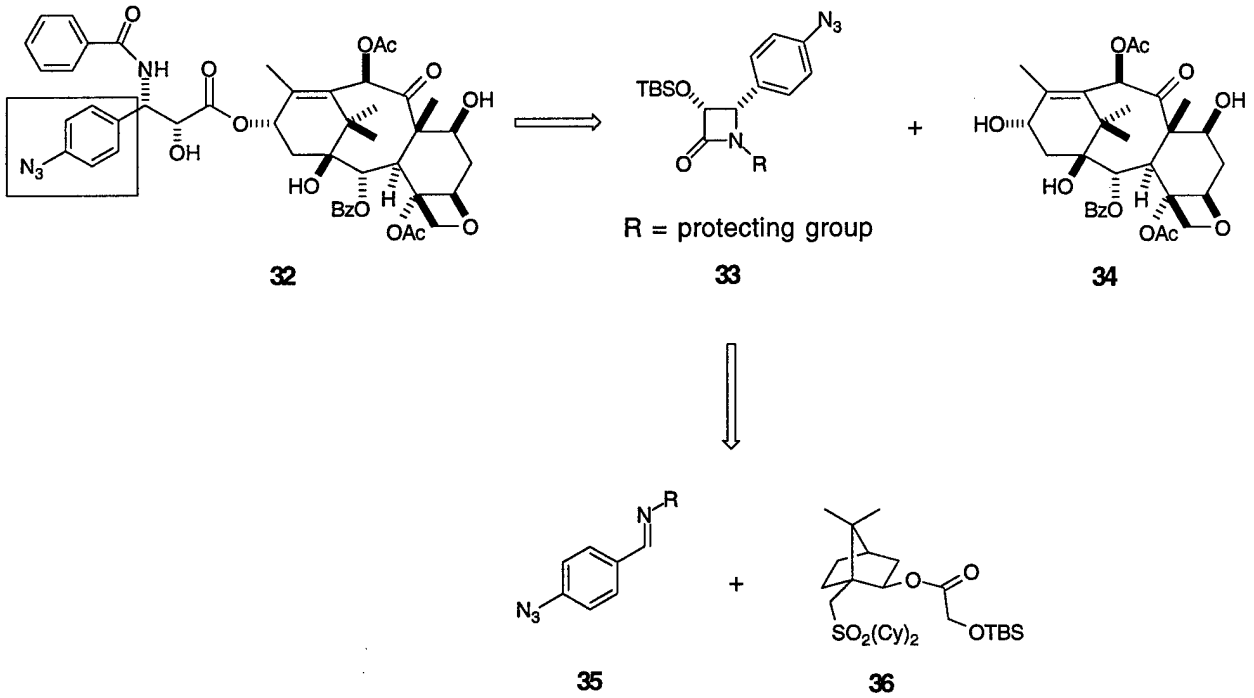


Scheme 9. Synthesis of C7 radioactive analog with a photolabel at C2 of paclitaxel.



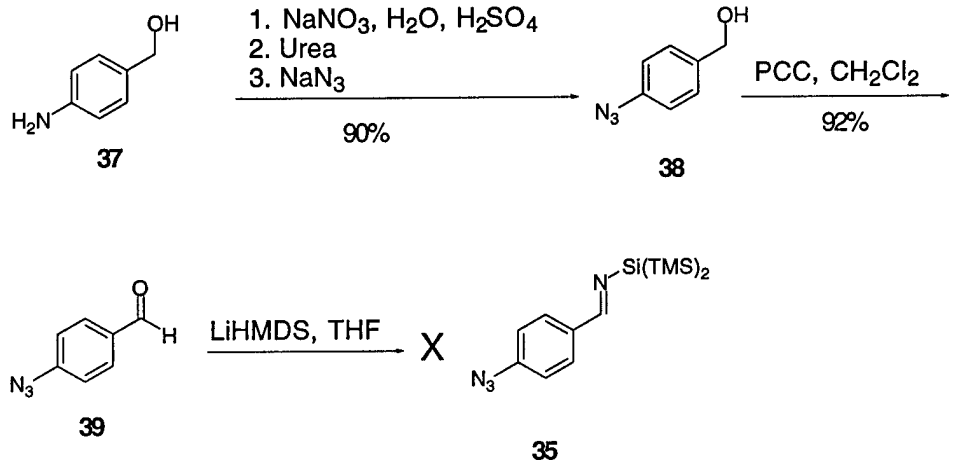
29 30 31

Scheme 10. Retrosynthetic analysis of C3' photoaffinity analog of paclitaxel.



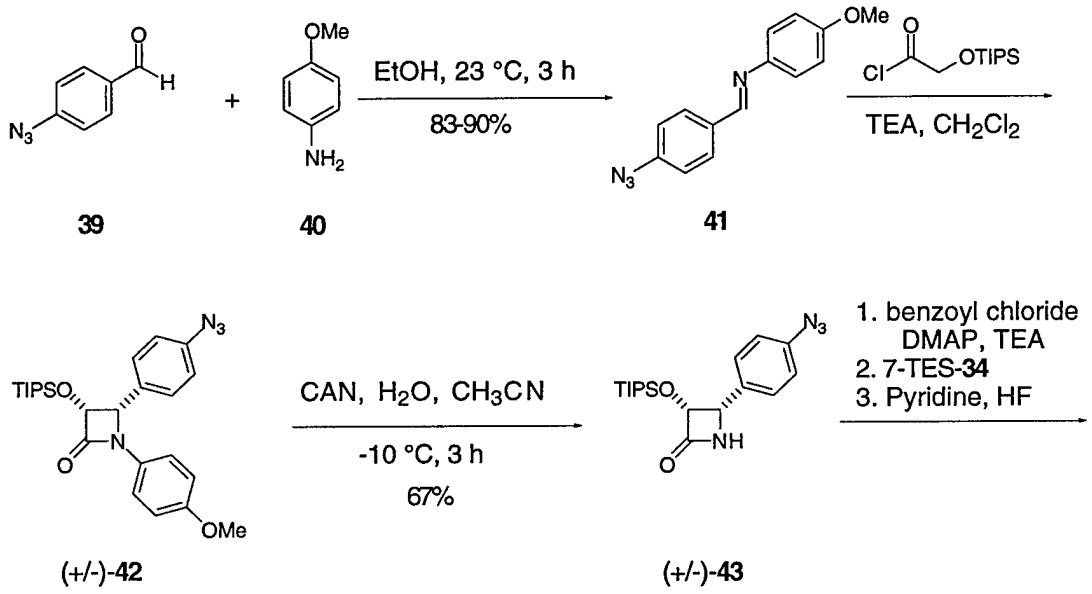
32 33 34 35 36

Scheme 11. Synthesis of azide imine.



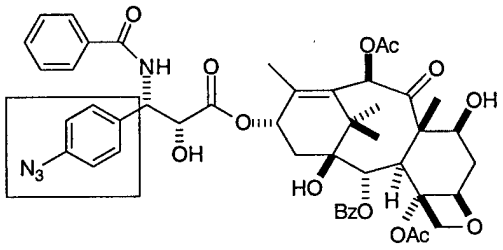
37 38 39 35

Scheme 12. Synthesis of C3' photoaffinity analog of paclitaxel.



39 **40** **41**

(+/-)-**42** (+/-)-**43**



32 (diastereomers)

Table 1. Biological Activity of Photoaffinity Labeled Analogs of Paclitaxel

Photoaffinity analogs	Microtubule assembly[a]
10-*O*-(*p*-azidobenzoyl)-10-deacetylpaclitaxel (**5**)	2.2
10-*O*-(3-azido-5-nitrobenzoyl-10-deacetylpaclitaxel (**6**)	0.8
10-*O*-(*m*-dimethylaminobenzoyl)-10-deacetylpaclitaxel (**7**)	1.6
7-*O*-acetyl-10-*O*-(*p*-azidobenzoyl)-10-deacetylpaclitaxel (**23**)	2.7
7-*O*-acetyl-10-*O*-(*m*-azidobenzoyl)-10-deacetylpaclitaxel (**24**)	2.0
7-*O*-acetyl-10-*O*-(4-azido-5-nitrobenzoyl)-10-deacetylpaclitaxel (**25**)	2.2
2-O-(*m*-azidobenzoyl)-2-*O*-debenzoylpaclitaxel (**29**)	0.4
3'-(*p*-azidobenzoyl)-3'-dephenylpaclitaxel (**32**)	2.0

a: numbers are given in ED_{50} ratio of analog over paclitaxel

Acronyms and Symbol Definition

MTs:	Microtubules
FDA:	Food & Drug Administration of the United States of America
UV:	Ultraviolet spectroscopy
EDC:	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
DMAP:	4-Dimethylaminopyridine
TBS:	*tert*-Butyldimethylsilyl
HF:	Hydrogen fluoride
H_2O_2:	Hydrogen peroxide
Ac:	Acetyl
Ar:	Aryl
Bz:	Benzoyl
Ph:	Phenyl
TES:	Triethylsilyl
Bn:	Benzyl
Bu:	Butyl
DCC:	1,3-Dicyclohexylcarbodiimide
THF:	Tetrahydrofuran
TMS:	Trimethylsilyl
Me:	Methyl
BuLi:	Butyl lithium

SYNTHESIS AND BIOLOGICAL EVALUATION OF 10-PHOTOAFFINITY, 7-RADIOLABELED PACLITAXEL ANALOGS.

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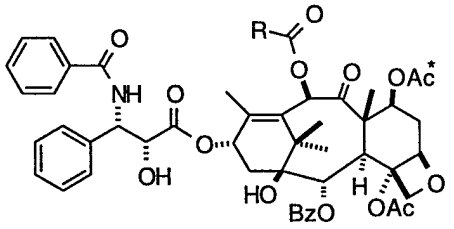


Background

•Paclitaxel (Taxol®) was isolated from the bark of *Taxus brevifolia* using bioassay directed isolation in 1964-1967 and exhibited potent anticancer activity against B16 melanoma cell lines.[1]

•Paclitaxel has been approved by the FDA for the treatment of ovarian cancer, breast cancer, and Karposi's sarcoma.

•Paclitaxel binds to tubulin dimers, induces polymerization, stabilizes microtubules, and shifts the dynamic equilibrium of tubulin toward the polymerized form.[1-3] See Tubulin Assembly Dynamics (page 2). This halts rapidly dividing cells at the *G2* to *M* transistion.

•The interaction of paclitaxel with tubulin requires further characterization because:

1) Specific interactions of paclitaxel with microtubulin require better characterization to assist the design of new and better analogues.

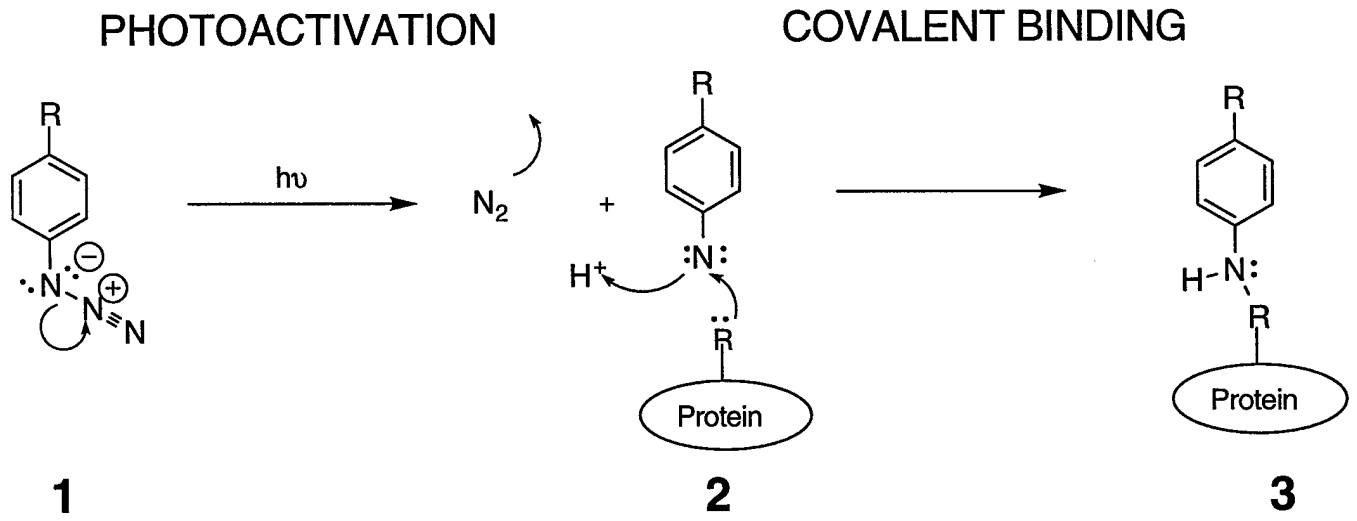
2) Paclitaxel binding to the α-tubulin subunit occurs, but this interaction has not been carefully characterized due to the low specific activity of prior paclitaxel radioanalogues.[2,4]

4) Paclitaxel and α,β-tubulin dimers have been analyzed by electron cyrstallography,[5] but the resolution is 3.5 Å. Detailed characterization of interactions require sharper resolution.

Tubulin Assembly Dynamics

Photoaffinity Labels

- Photoaffinity labels probe ligand-protein interactions by covalently attaching to the binding site upon UV irradiation. UV irradiation of aryl azides generates an aryl nitrene that covalently binds to Lewis bases in close proximity.[6]



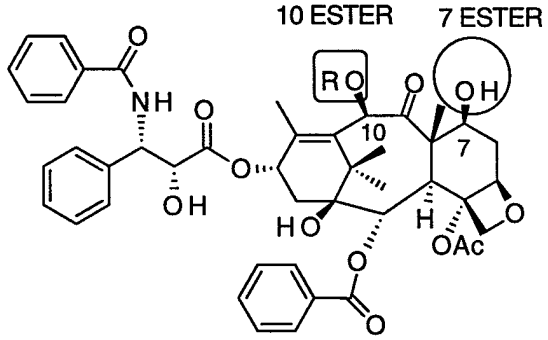
- The practical requirements of photoaffinity labels are:

1) good affinity for the target
2) efficiency of photoconversion
3) ability to be radiolabeled

General Approach / Specific Targets

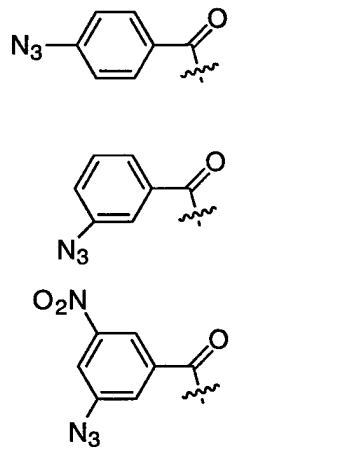
PHOTOAFFINITYLABEL

RADIOLABEL



Derivatives

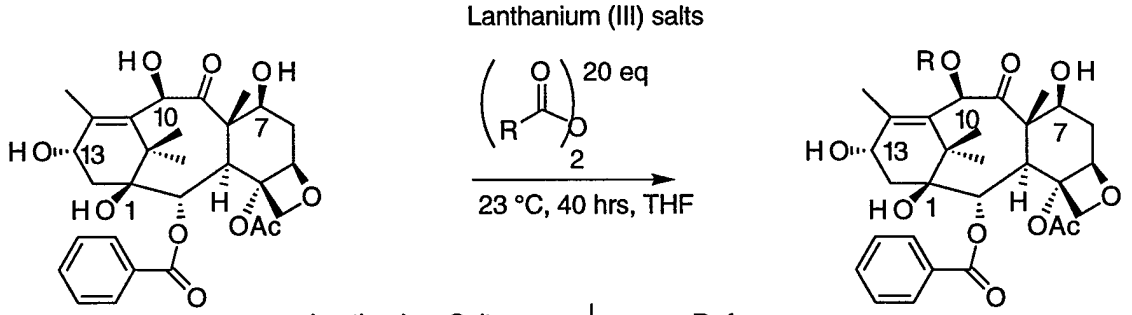
R=



- 7-cetates taxoids photolabeled elsewhere were effective paclitaxel probes.
- Prior work had identified the 10-esters as equipotent with paclitaxel.
- The 10-(3-azido-5-nitrobenzoyl)-paclitaxel derivative was more potent than paclitaxel.
  See Pannel 10.
- The 10-(3-azidobenzoyl)-paclitaxel derivative was not accessable *via* 7-*O*-TES-2'-*O*-
  TBS-paclitaxel using prior conditions (EDCI).
- Efficient synthesis with minimal protecting group manipulation is desired.

Lanthanide-Mediated Selective Acylation at 10

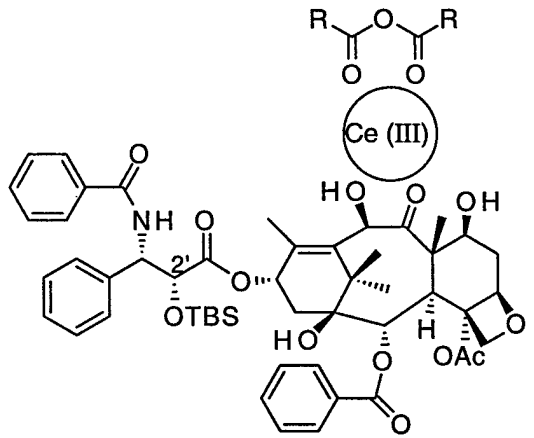
Previous Studies:



Lanthanium Salt	Reference
$CeCl_3$	Holton[8]
$CeCl_3$ $Yb(NO_3)_3$ $Yb(OTf)_3$ $Ln(OTf)_3$	Scheeren[9]

- There is prior literature precidence[7] for selective monoacylation of diols using lanthanide salts, specifically $Ce_2(SO_4)_3$.
- The most reactive of the four alcohols of 10-deacetyl baccatin III is normally the 7-hydroxyl.
- Both Holton[8] and Scheeren[9] identified only 10-acylation using catalytic $CeCl_3$ and excess anyhdride as the acylating reagent.
- Selective acylation of 10 over 7 should permit sequential photolabeling at 10 then radiolabeling at 7 with minimal protecting group manipulation.

Lanthanide-Mediated Selective Acylation at 10



UTILITY:

- Protection of the 2'-hydroxyl:
  - Protection of the sidechain from hydrolysis.
  - Blocking acylation of the 2'-hydroxyl.

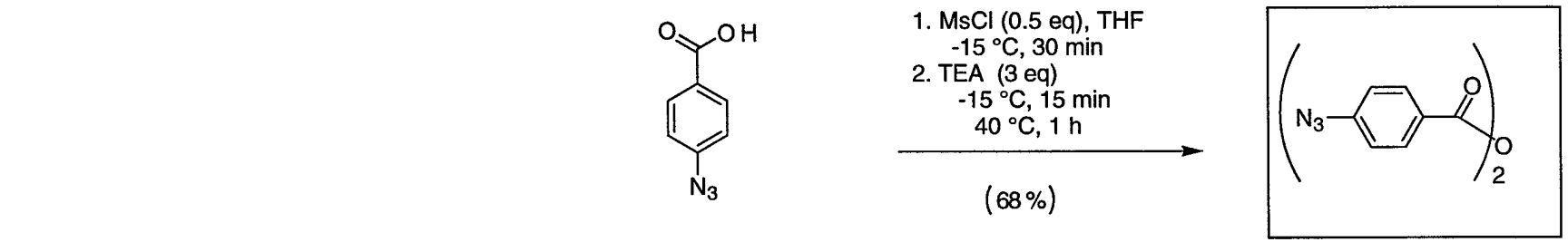
- The first attempt at acylating 2'-*O*-TBS-10-deacetyl-paclitaxel using acetic anhydride
  gave selective 10 acylation very cleanly.

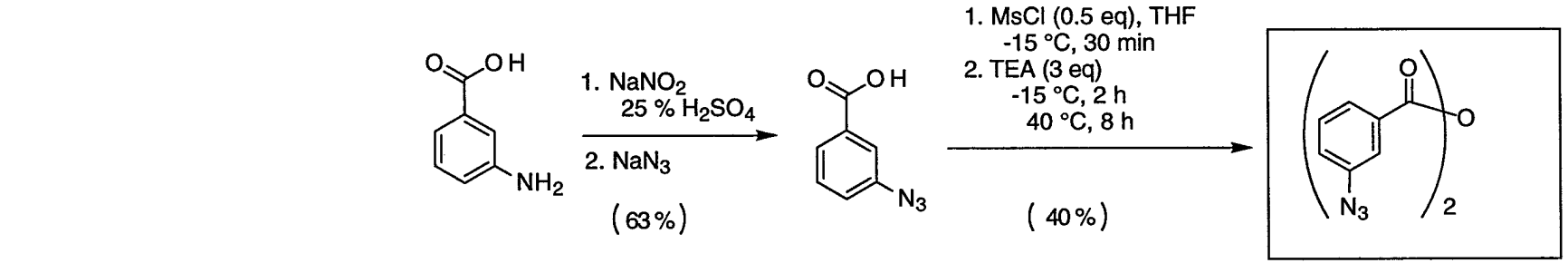
COORDINATION:

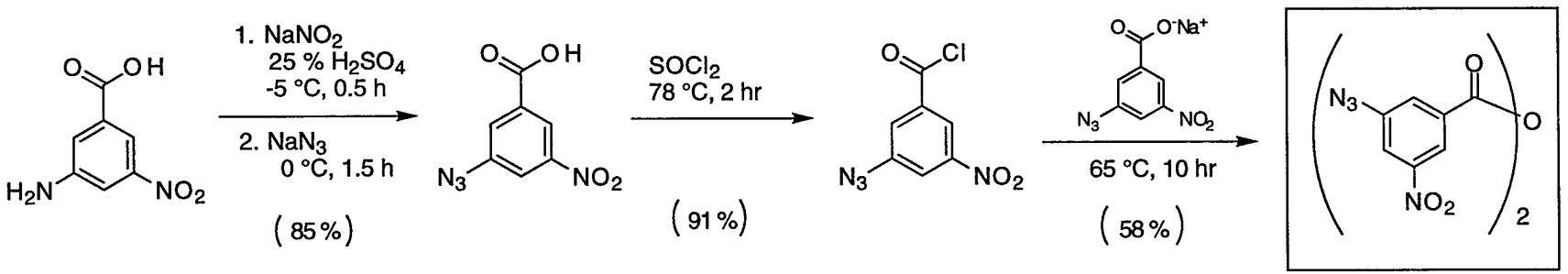
- Flexible 1,3 diacyl systems provide the best acylation selectivity.
  - Use of acetyl chloride gave acylation at both 10 and 7.
  - Use of DMAP gave acylation at both 10 and 7.
  - Cyclic anhydrides (ex: succinic anhydride) were unreactive.

- ^{1}H NMR of the 1:1 complex of 2'-*O*-TBS-10-deacetyl-paclitaxel to $CeCl_3$ (d_8-THF) showed coordination of Ce(III)[10] with both the 7- and 10- hydroxyls.

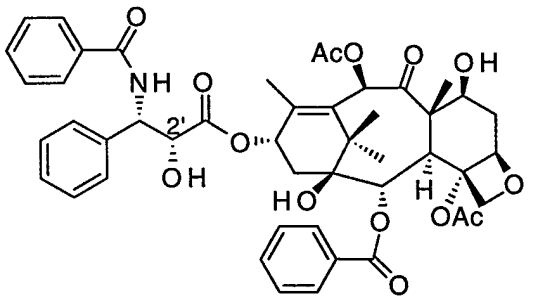
Syntheses of Required Anhydrides







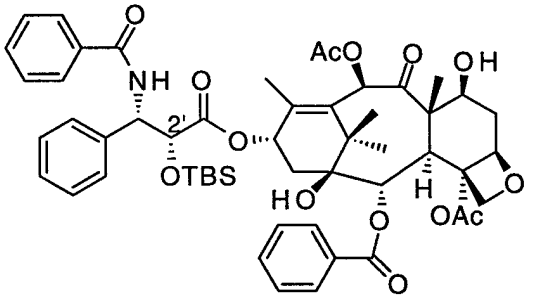
Taxane Chemistry



TBSCl
DMAP
CH_2Cl_2

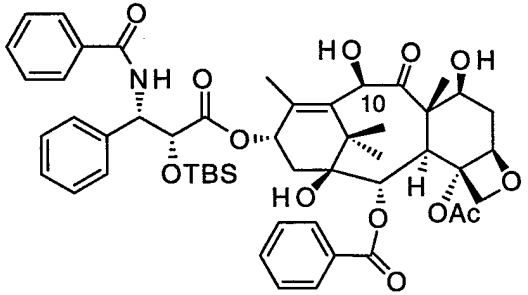
23 °C, 48 hr

(99 %)



$N_2H_4 \cdot H_2O$
95 % EtOH
23 °C, 20 min

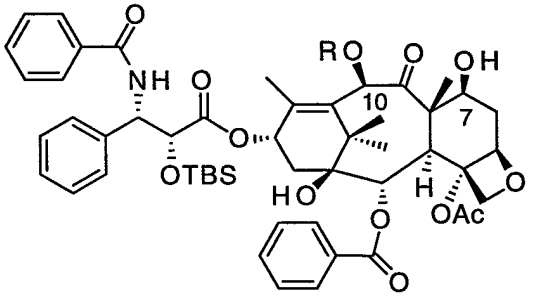
(97 %)



20 eq

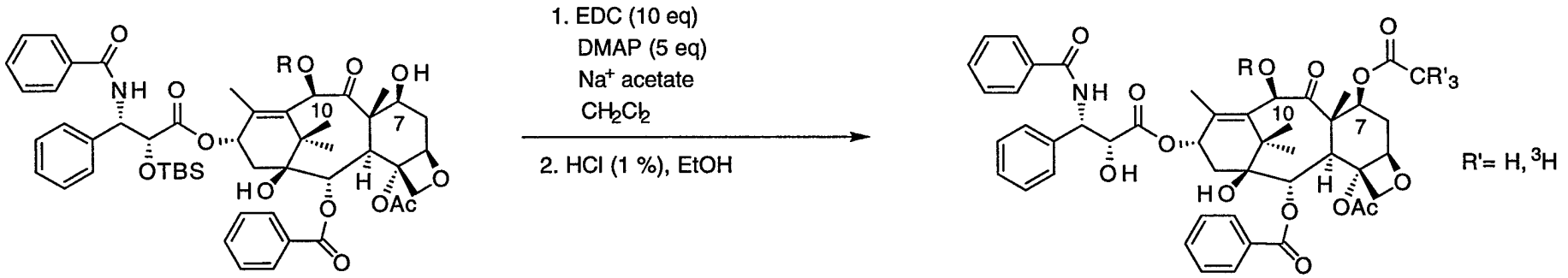
$CeCl_3$, THF

23 °C, 40 hrs



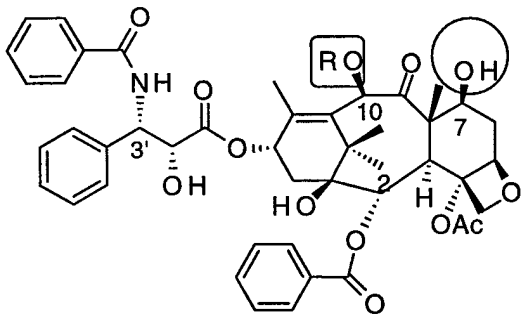
R= *p*-N_3benzoyl	73 %
R= *m*-N_3benzoyl	85 %
R= 3-N_3-5-NO_2benzoyl	48 %

Radiosyntheses



	cold	radioactive	
R= *p*-N_3benzoyl	73 %	43 %	0.28 mCi/μMole
R= *m*-N_3benzoyl	85 %	46 %	0.21 mCi/μMole
R= 3-N_3-5-NO_2benzoyl	48 %	22 %	0.73 mCi/μMole

Summary



PHOTOAFFINITYLABEL

RADIOLABEL

	Derivative	ED_{50}/ED_{50} (TAXOL)	Radioactivity µCi/µMole
7-OH	10-*p*-azidobenzoyl	2.2	
	10-*m*-azidobenzoyl		
	10-3-azido-5-nitrobenzoyl	0.8	
7-OAc	10-*p*-azidobenzoyl-7-acetyl	2.7	250
	10-*m*-azidobenzoyl-7-acetyl		150
	10-3-azido-5-nitrobenzoyl-7-acetyl	2.2	100

- All compouds were prepared using $CeCl_3$-mediated selective acylation at 10.
- 10-Derivatives previously inaccessable *via* 2'-*O*-TBS-7-*O*-TES-paclitaxel were prepared in high yeild.
- All of the 10-benzoyl-7-acetate derivatives displayed good activity in the microtubulin assembly assay.
- High specific activity of radioactive paclitaxel derivatives were obtained.
- The findings of pannel 6 suggest that other 1,3-diacyl acylating sources could find use in preparing other 10-paclitaxel analogs.

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Yanbin Liu predoctoral fellowship

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