Stony Brook University

Synthesis of SB-T-12301 Analogues and Tumor-Targeting Taxane-Based Drug Conjugates using Biotin as Tumor-Targeting Module

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Changwei Wang

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Abstract of the Thesis

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Cancer is one of the most serious diseases of the world, leading to one of eight deaths. Although the medical advancements have contributed greatly to the decline of other diseases, treatments for cancer have remained only modestly effective. Traditional chemotherapeutics such as Taxol and Taxotere generally lack specificity, and repeated administration often leads to multi-drug resistance (MDR). To increase the efficacy and specificity of drugs, both the synthesis and evaluation of lead compounds and the development of drug delivery system are equally important.

SB-T-1214 and three novel taxanes, SB-T-12301 and two of its analogues, were synthesized by the standard Ojima-Holton coupling protocol using the corresponding β–lactam and 7,10-dimethylated-10-DAB III and C-2 *meta*-methyl or fluorobenzoyl 7,10-dimethylated-10-DAB III. The β-lactam was obtained *via* two well-established synthetic routes: Staudinger [2+2] cycloaddition followed by enzymatic resolution protocol and the chiral ester enolate-imine cyclocondensation.

In addition, tumor-targeting taxane-based drug conjugate was synthesized by using biotin as the tumor-targeting module and a self-immolative disulfide linker, which can be cleaved in cancer cells due to high concentration of glutathione. Biotin plays a critical role in many biological processes. It is greedily taken up by tumor cells through a process of receptor-mediated endocytosis (RME) to meet its need of fast growth and propagation. The receptors of biotin are highly overexpressed on the surface of tumor cells and making them a good target for tumor-targeting drug design.

Dedicated to all who helped me out in Chemistry

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List of Abbreviations

ABC- ATP-binding cassette
Abl- Abelson proto-oncogene
Ac- acetyl
AcOH- acetic acid
Arg- arginine
atm- atmosphere
ATP- adenosine triphosphate
BMS – Bristol-Myers Squibb
Bn- benzyl
Boc- tert-butoxycarbonyl
BOM- benzyloxymethyl acetal
b.p.- boiling point
BQ - benzoylquinine
Bz- benzoyl
C- carbon
CAM- ceric ammonium molybdate
CAN- cerium(IV) ammonium nitrate
CML- chronic myelogenous leukemia
CRPC- castration-resistant prostate cancer
CSC– cancer stem cell
d- doublet
10-DAB III- 10-deacetyl baccatin III
dd- doublet of doublet
DCM- dichloromethane
DHA- docosahexaenoic acid
DIC- *N,N*-diisopropylcarbodiimide
DIPA- diisiopropylamine
DIPEA- *N, N*-Ddiisopropylethylamine
DMAP- 4-*N,N′*-dimethylaminopyridine
DMF- *N,N′*-dimethylformamide
DMSO- dimethyl sulfoxide
ee- enantiomeric excess
EGFR- epidermal growth factor receptor
EMEA- European Medicines Agency
EPA- eicosapentaenoic acid
eq- equivalent
Et- ethyl
et al.- and others
EtOAc- ethyl acetate
EtOH- ethanol
FA- folic acid
FBS- Fetal Bovine Serum
FIA- flow injection analysis

FDA- Food and Drug Administration
g- gram
GC- gas chromatography
GDP- guanosine 5'-diphosphate
GIST- gastrointestinal stromal tumors
Gly- glycine
gp60- glycoprotein 60
GSH- glutathione
GTP- guanosine 5'-triphosphate
HEX- hexanes
HL- Hodgkin lymphoma
HOSu- *N*-hydroxysuccinimide
HPLC- high performance liquid chromatography
hr/h – hour
HSA- human serum albumin
HSN- *N*-hydroxysuccinimide
Hz- hertz
IC50 - concentration for 50 % inhibition
iPr- isopropyl
IR- infrared spectroscopy
J- coupling constant
Kg- kilogram
L- liter
LDA- lithium diisopropylamide
LiHMDS- lithium bis(trimethylsilyl)amide
LNA- linolenic acid
m- multiplet
M- molar or molarity
mAb- monoclonal antibody
MAPs- microtubule associated proteins
MDR- multi-drug resistance
Me- methyl
MEM- β-methoxyethoxymethyl ether
MeOH- methanol
Mg- milligram
MHz- megahertz
Min- minute
mL- milliliter
mM- millimolar
mmol- millimole
mol- mole
m.p.- melting point
MRP- Multidrug Resistance-Associated Proteins
MS-mass spectrometry
n-BuLi- n-butyllithium

NCI- National Cancer Institute
nM- nanomolar
NSCLC- non-small cell lung cancer
NMR- nuclear magnetic resonance
o/n- overnight
PAMAM- polyamido amine
PBS- phosphate buffered saline
Pgp- P-glycoprotein
Ph- phenyl
PMP- *p*-methoxyphenyl
ppm- parts per million
p-TsOH- p-toluene sulfonic acid
PUFA- Polyunsaturated fatty acids
Py- pyridine
q- quartet
RME- receptor-mediated endocytosis
RPMI- Rosewell Park Memorial Institute medium
rt- room temperature
s- singlet
sALCL- systemic anaplastic large cell lymphoma
SAR- structure-activity relationship
SCID- Severe Combined Immunodeficiency
SPARC- secreted protein acidic and rich in cysteine
t- triplet
t-Bu- tert-butyl
TEA- triethylamine
Tert- tertiary
TES- triethylsilyl
THF- tetrahydrofuran
TIPS- triisopropylsilyl
TLC- thin layer chromatography
TMO- trimethyloxonium tetrafluoroborate
TMS- trimethylsilyl
Troc- 2,2,2-trichlorethoxycarbonyl chloride
TTM- tumor-targeting module
U.S.- United States
UV- ultraviolet-visible
WCA- Whitesells chiral auxilliary
Wt- weight
[α]- specific optical rotation
β-LSM- β-Lactam Synthon Method
δ- chemical shift
μl- microliter

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Chapter 1

Synthesis of β-Lactam and SB-T-1214

Chapter Contents

§1. 1 Introduction

§1.1.1 Cancer and treatment options

Cancer is one of the most serious worldwide diseases, leading to one of eight deaths. Despite advances made in medicinal chemistry, the number of global cancer patients has doubled over the last 3 decades. The rapid growth of populations will greatly increase the cancer burden. By 2030, 27 million incident cases of cancer are expected with annual deaths around 17 million, and 75 million people are anticipated to be living with cancer.[1] According to a study in 2009, cancer ranked 2nd among the leading causes of death in the United States, 1 in 2 men and 1 in 3 women in North America will develop some form of cancer in their lives.[2] It seems that cancer will outrank heart diseases as the No. 1 cause of death in the near future. Although the medical advancements have contributed greatly to the decline of other diseases, treatments for cancer have remained only modestly effective.

However, the greatest impact of cancer was felt among developing countries. These countries frequently have limited healthcare resources; cancer treatment facilities and life-saving therapies are not widely available for economic reasons. The rapid increase in the cancer burden represents a major crisis for public health systems worldwide. Finding sufficient treatment resources to treat cancers and provide palliative, supportive and terminal care for the large number of cancer patients are the most imperative task for most of the developing and developed countries.[1]

Many tumor risk factors, both external and internal, have been identified, such as smoking, alcohol consumption, radiation damage, obesity, dietary factors, occupational exposures, chronic infection, genetic mutations and immune deficiencies.[1] To some extent the risk of cancer could be reduced by changing lifestyle, but early detection and choice of the best option of treatment are the keys to improve the quality of life and increase the success rate of curing cancer.

There are several options for treating cancer such as surgery, radiation therapy, chemotherapy, and immunotherapy.[3] Typically, treatment depends on the type and stage of cancer progression as well as cancer patients' physical conditions, and in most cases treatments usually include combination of two or more of the options above. These treatment options may not provide a cure for cancer, but definitely could alleviate symptoms, extend life span, and improve the quality of life. Surgery is removing tumors by invasive techniques, the first choice if there is no metathesis. To prevent recurrence, it is important to remove all traces of cancer cells of the neighboring tissues. Radiation therapy is destroying cancer cells by high-energy X-rays, usually used for treating solid tumors and leukemia. It can be performed *via* both external beam radiotherapy and internal brachytherapy. The problem of this method is a substantial amount of the normal cells may also be destroyed simultaneously. Immunotherapy is fighting cancer cells by improving the patient's immune system. Vaccines, interferons and other cytokines, and bone marrow transplantation are often used to induce corresponding immune response. Chemotherapy is still the most widely used to kill cancer cells based on the assumption that chemical agents are capable of differentiating cancer cells from normal ones.[4] However, its low level of specificity often results in systemic toxicity and severe side effects such as irreversible damage to major organs, depression of the immune system.

§1.1.2 Tubulin-stabilizing agents and the mechanism of paclitaxel

Microtubules are a component of the cytoskeleton, found throughout the cytoplasm, and they are critcal in numerous cellular processes including cell division. Microtubules are composed of α– and β–tubulin dimers, growing and shrinking very rapidly depending on the concentration of cellular tubulin and the rate of guanosine 5'-triphosphate (GTP) hydrolysis. Generally, GTP, Mg^{2+}, and microtubules-associated proteins (MAPS) can assist the process of polymerization. Thus tubulin is a major target for anticancer drugs.[5] There are two main classes of tubulin- targeting drugs: preventing assembly by depolymerizing microtubules and promoting assembly by stabilizing microtubules. The former includes colchicine and vinca-alkaloids. Taxoids belongs to the second, which can lock the cellular division in the G2/M phase leading to early mitotic exit (**Figure 1-1**). [6, 7]

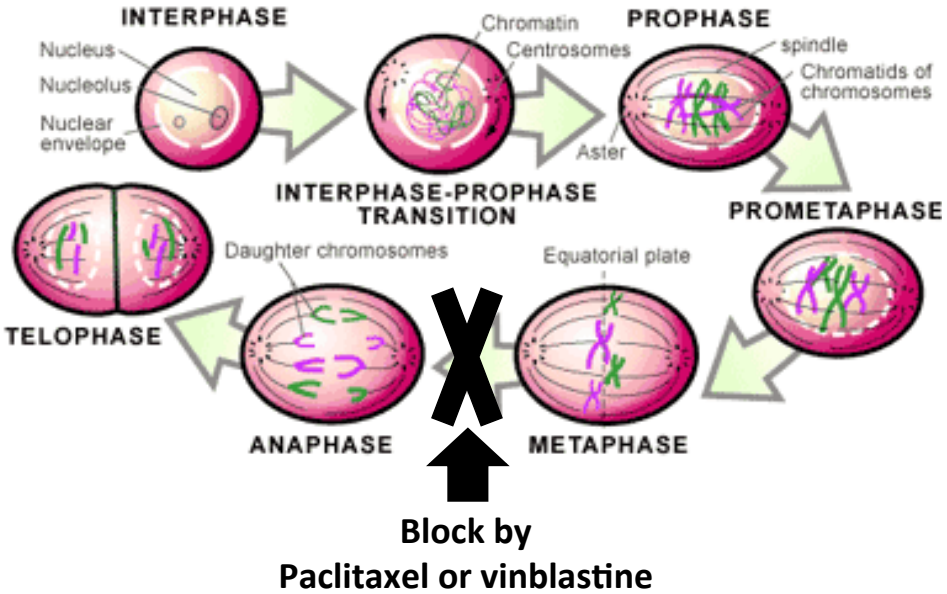


Figure 1-1: The function of anticancer agents on cell cycle (Adapted from (*6*))

Other tubulin stabilizing agents include epothilones, discodermolide, laulimalide and eleutherobin (**Figure 1-2**).[8] The epothilones are a new class of cancer drugs and they have better efficacy and milder adverse effects than taxoids in early trials, but the lactone structure makes them unstable and greatly reduced its usage. Although Bristol-Myers Squibb (BMS) once ambitiously wanted to replace taxoids, only ixabepilone was approved in October 2007 by United States FDA for the treatment of aggressive metastatic or locally advanced breast cancer no longer responding to currently available chemotherapies. However, in Novermber 2008, the European Medicines Agency (EMEA) refused authorization of its marketing in the Europe. There are cytotoxicity and supply problems with the other potential agents. It seems that paclitaxel are irreplaceable in the foreseeable future, particularly in developing countries.

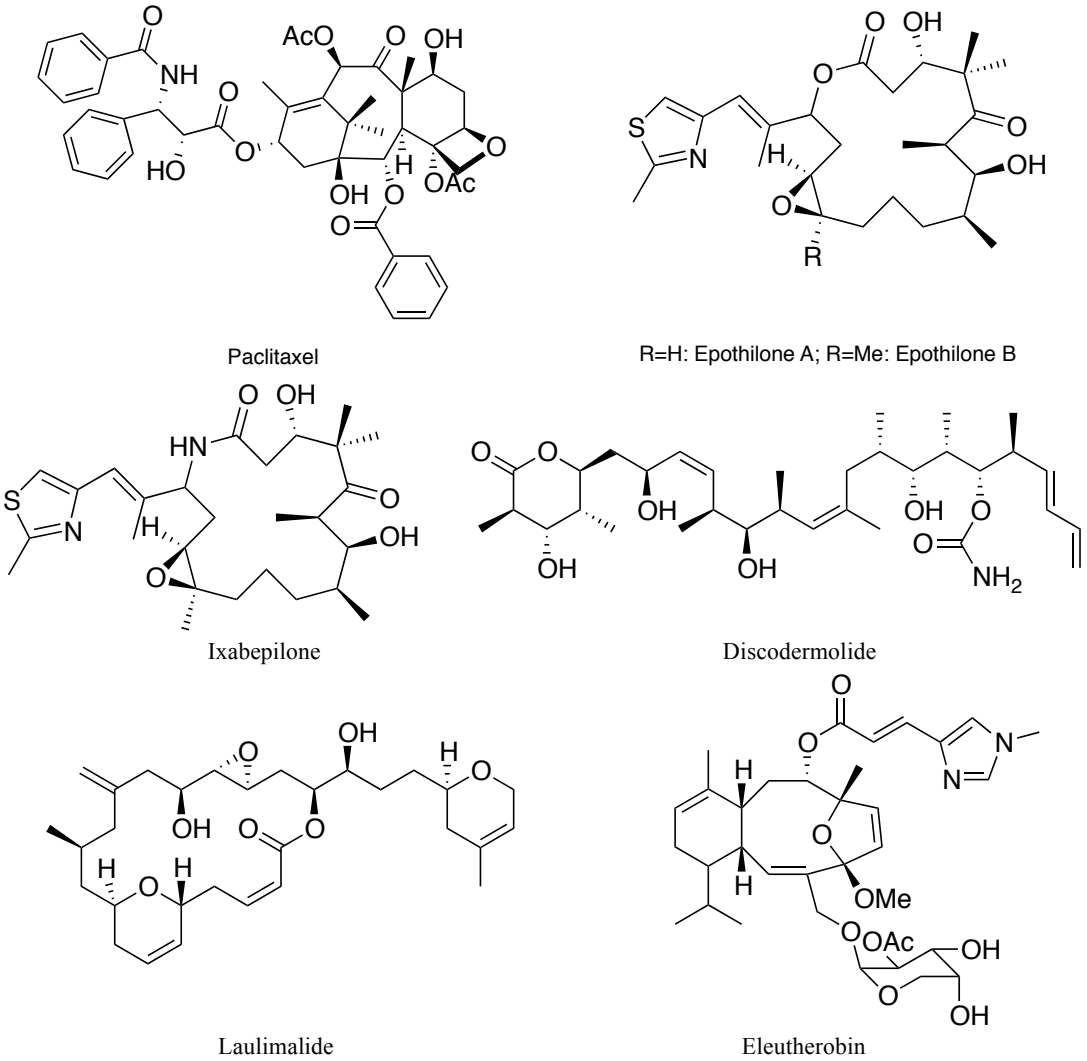


Figure 1-2: Microtubule-stabilizing anticancer agents (Adapted from ref. (*8*))

Horwitz *et al*. first reported the role of paclitaxel upon microtubule assembly.[5] It was found that paclitaxel could accelerate the rate and nucleation of microtubule formation. Moreover, the microtubule formed with paclitaxel is structurally different, containing 12 protofilaments with a diameter of 22 nm instead of the normal one (**Figure 1-3**).[5] The more tightly packed microtubules formed in the presence of paclitaxel can remain intact even in depolymerization conditions, either 4 ºC or 4 mM $CaCl_2$ solution.

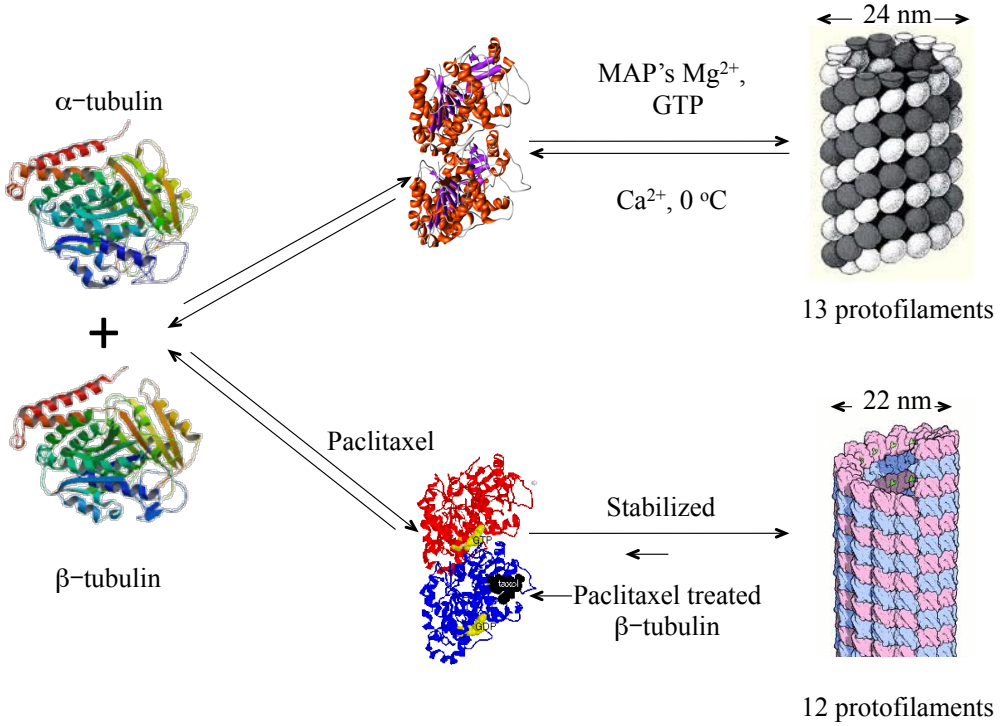


Figure 1-3: Difference of normal and paclitaxel-promoted microtubule polymerization (Adapted from ref. (*5*))

§1.1.3 Synthetic approaches to paclitaxel, docetaxel, and cabazitaxel

Since its discovery in the non-renewable bark of the *T. brevifolia*, paclitaxel was hardly commercialized due to its very limited supply in the early stage.[(9)] Total synthesis has been attempted and successfully made by the groups of Holton, Nicollaou, Danishefsky, Wender, Kuwajima, Mukaiyama, and Takahashi respectively. However, none of these could be industrialized due to high production cost and low yield until a breakthrough was achieved by semisynthesis.[(10)]

In 1981, 10-deacetylbaccatin III (**Figure 1-4**) was first isolated from the leaves of *T. baccata*, also known as European yew. Later it was found in other species and can be chemically transformed from various other kinds of taxoids. The reliable and sustainable supply of 10-deacetylbaccatin III guaranteed the commercialization of paclitaxel. [(9)]

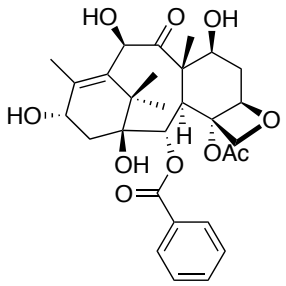
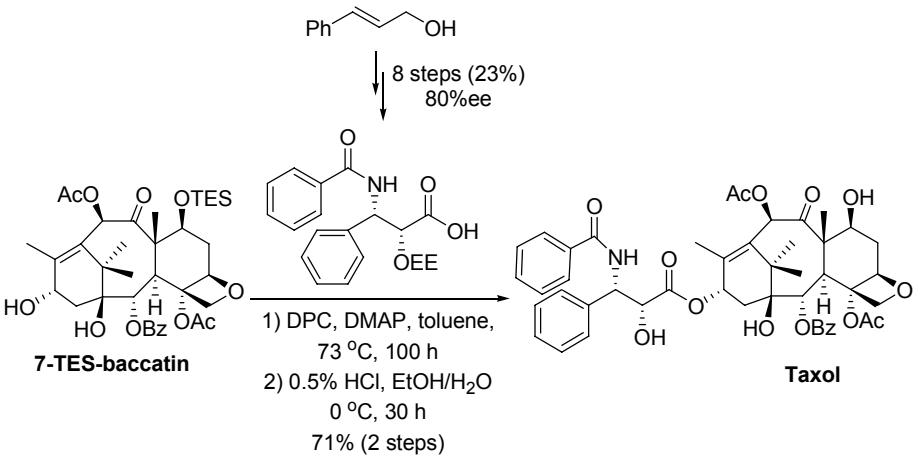


Figure 1-4: Structure of 10-deacetylbaccatin III

Several years later Greene and Potier published the first semi-synthetic route in 1988 (**Scheme 1-1**).[11]



Scheme 1-1: First semi-synthesis protocol published by Potier and Greene[11]

Later docetaxel was also synthesized from 10-DAB III, first developed and commercialized by Rhone-Poulenc Rover France, which is now a major medication owned by Sanofi, which also developed and commercialized cabazitaxel.[7]

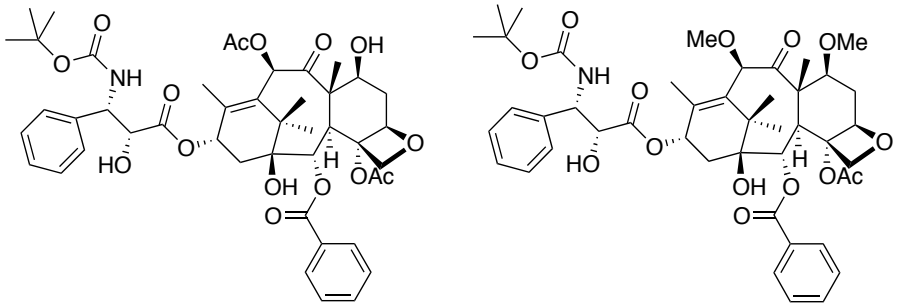
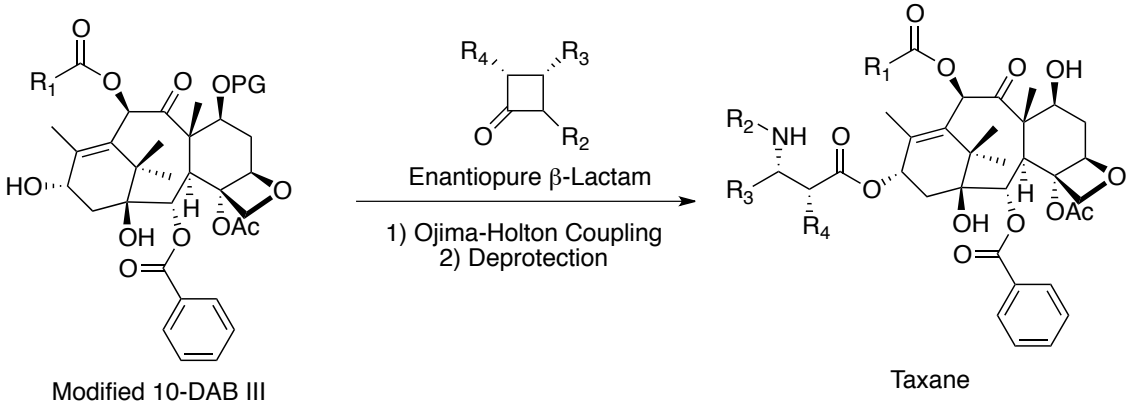


Figure 1-5: Structures of docetaxel and cabazitaxel

Following that, a lot of effort was put on the asymmetric synthesis of the side chain, (2*R*,3*S*) –*N*-benzoyl-3-phenylisoserine, the key component of semisynthesis. In 1990, Holton *et al.* published a β–lactam and 7-TES-baccatin III coupling protocol that could solve the problem of epimerization.[9] As this primary coupling protocol needs large excess of β–lactam and the reaction is also slow, Ojima *et al*. developed a more efficient approach to coupling the enantiopure β–lactam to the C-13 hydroxyl group of 7-TES-baccatin III, i.e., *β–lactam synthon method* (**Scheme 1-2**).[12]



Scheme 1-2: β–Lactam Synthon Method[13]

The development of *β–Lactam Synthon Method* was a major breakthrough of taxoids study and commercialization. First, under the condition of a strong base and low temperature, the reaction can be done in half an hour with a yield up to 93%, and only a slight excess of β–lactam side chain precursor is needed. Second, its C-3 hydroxyl group is capable of accommodating steric hindered protection group such as TIPS, which is critical for the synthesis of highly enantiopure β–lactam. This method provides a very efficient way for both the synthesis of new generation taxoids and SAR exploration.

§1.1.4 Multi-Drug Resistance (MDR) and Structure Activity Relationship (SAR)

Since 1990's, both Taxol® and Taxotere® (docetaxel) have become 2 of the most widely used anticancer chemotherapeutic agents.[14] Consistent administration has resulted in episodes of remission and reoccurrence rather than complete recovery and led to cases of multi-drug resistance (MDR). Although many factors such as drug target mutation, drug metabolism and sequestration can cause MDR, the overexpression of ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRP) of this family are playing an important role in MDR.[15] Overexpression of these proteins leads to the efflux of chemotherapeutic agents (**Figure 1-6**).[16] Up to now, P-gp is one of the best-studied models of all transmembrane polysubstrate efflux pumps that prevent chemotherapeutics from reaching the intracellular targets.[15]

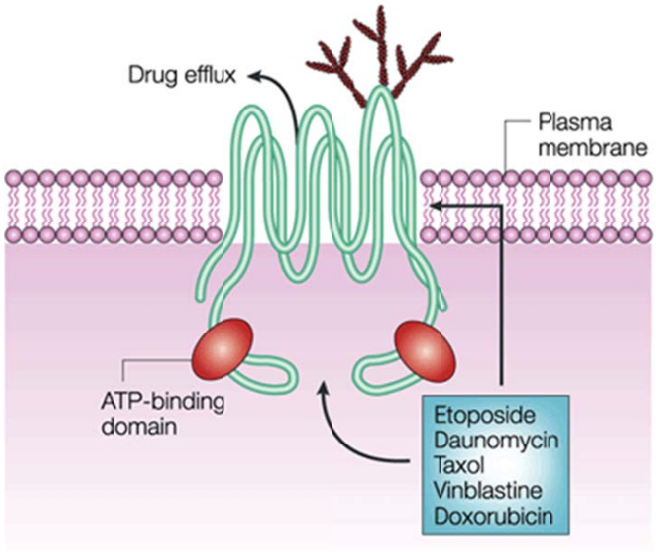


Figure 1-6: Efflux mechanism of resistance to hydrophobic anticancer drugs by Pgp[16]

Based on many years of extensive structure activity relationship (SARs) studies and biological cytotoxicity evaluation of taxoids (**Figure 1-7**), new taxoids have been designed, synthesized and biologically evaluated.[10]

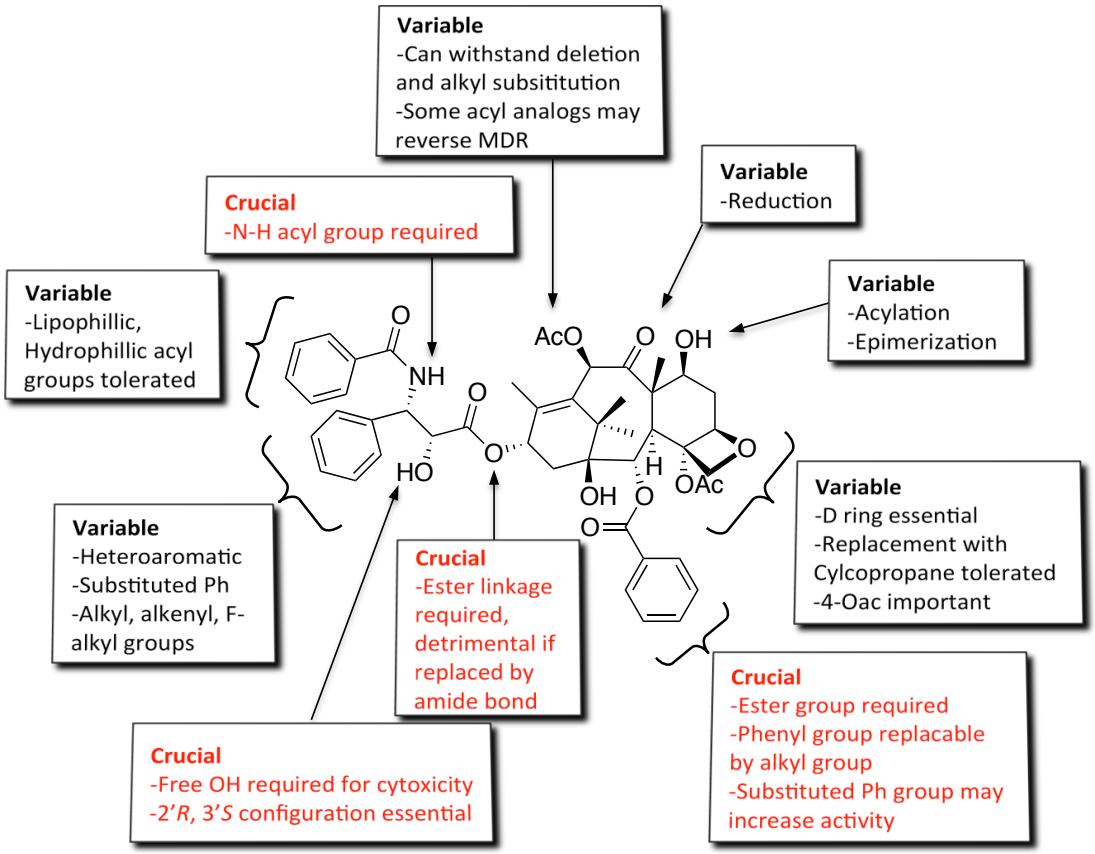


Figure 1-7: SAR study of paclitaxel[10, 13]

The side chain is essential to the activity of paclitaxel, whose efficacy will be lost if the ester is replaced by an amide bond.[17] It is crucially important to maintain the 2'*R* and 3'*S* configuration of the side chain that connected to the C-13 of paclitaxel's A ring.[18] The free hydroxyl group of C-2' is not only essential for activity due to its participation in a hydrogen bond with either the Arg 396 or Gly 370 protein of β-tubulin to ensure tight binding;[19] but also extremely important for designing pro-drugs because the functionality of taxoids could be masked by hydrolysable ester bond.[20] As C-3' acyl substitutes are variable, *tert*-butyl group has been chosen as an excellent substitute due to its favorable pharmacokinetic properties,[21] as proven in the difference between paclitaxel and docetaxel/cabazitaxel.

The D-ring, oxetane ring, is irreplaceable because it is a hydrogen-bond acceptor and also serves as a rigid "lock" on the taxoids skeleton. Modification of the C-2 and C-10 positions of B ring virtually could overcome the MDR effect in several cancer cell liners. It is also found that *meta* substitution of the benzoyl functionality can increase its biological activity.[10]

To P-gp, one of the best-known transmembrane polysubstrate efflux pumps, the recognition elements of paclitaxel were extensively studied (**Figure 1-8**).[22] P-gp shows stronger affinity towards Type II elements. A library of paclitaxel analogues has been synthesized by replacing these positions by other functional groups and many of which became unrecognizable to efflux pumps. Biological tests indicated that some of them have increased potency and capable of reversing the effects of MDR. [22]

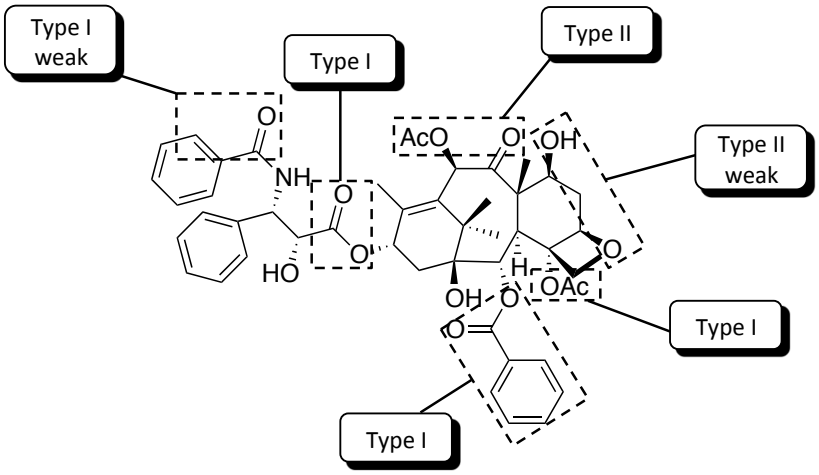


Figure 1-8: P-gp recognition elements of paclitaxel (Adapted from ref. (*22*))

Ojima group has made major contributions to the design and development of novel taxoids. SB-T-1212 and SB-T-1214, the second generation taxoids were synthesized and selected as lead compounds due to their effective 2 orders improvement in MDR cancer lines compared with paclitaxel and docetaxel (**Figure 1-9**).[23] Its remarkable efficacy and activity against cancer cells make it very promising to be another taxane that might be approved by FDA (**Table 1-1**).

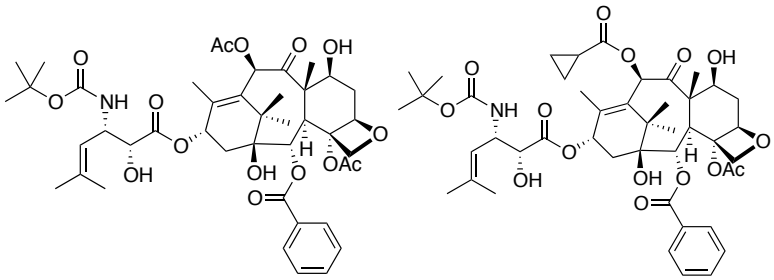


Figure 1-9: Structures of SB-T-1212 (left) and SB-T-1214 (right)

Table 1-1: IC_{50} results of SB-T-1212, and SB-T-1214 versus market Taxol® and Taxotere®[23, 24]

Taxane	A121 (ovarian)	A549 (NSCLC)	HT-29 (colon)	MCF (breast)	MCF7-R
Paclitaxel	6.1 nM	3.6 nM	3.2 nM	1.7 nM	300 nM
Docetaxel	1.2 nM	1.0 nM	1.2 nM	1.0 nM	235 nM
SB-T-1212	0.46 nM	0.27 nM	0.63 nM	0.55 nM	12 nM
SB-T-1214	0.26 nM	0.57 nM	0.36 nM	0.20 nM	2.1 nM

§1.1.5 Novel taxoids in advanced states

Besides ortataxel (IDN 5109), SB-T-1214 and other derivatives developed by Ojima's group, some other new generation taxoids such as larotaxel, tesetaxel (DJ-927), milataxel and so on are also in advanced state.[10]

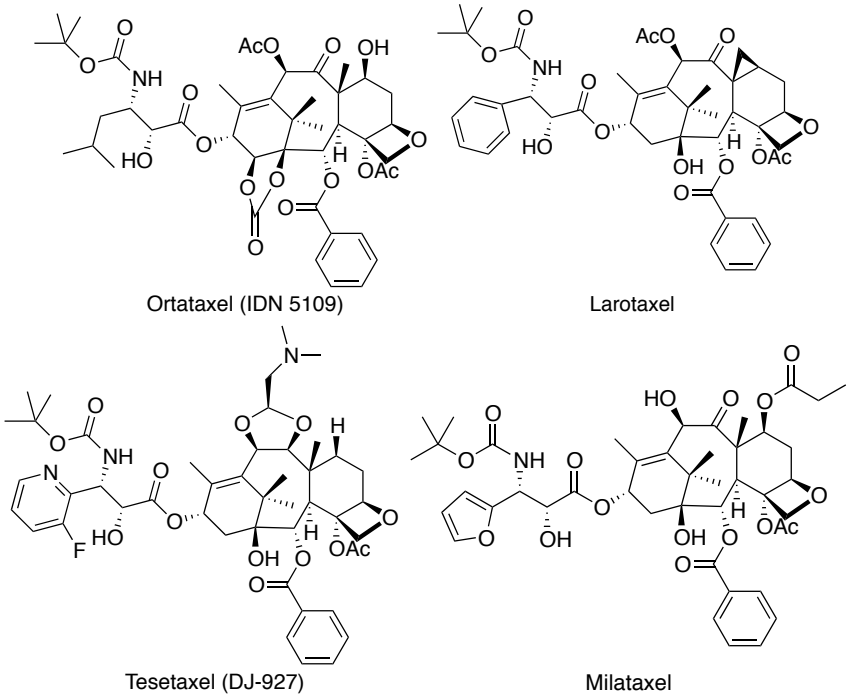


Figure 1-10: Structure of Ortataxel, Larotaxel, Tesetaxel, and Milataxel[10]

Ortataxel, is synthesized from 14β–hydroxybaccatin III, now under the development of Spectrum Pharmaceuticals. As a broad-spectrum modulator of ABC transmembrane transporters, it can overcome the MDR caused by P-gp efflux pumps. Although orally active, intravenous administration has been used for the initial studies. Phase I clinical trials of ortataxel showed good tolerability for both 3 weeks and weekly schedule while some side effects were observed in an early phase II trials, though generally well-tolerated. Its encouraging activity with manageable toxicity for patients with metastatic breast cancer was reported in a recent study.[10]

Tesetaxel was originally developed by Daiichi-Sankyo and is now licensed to Genta. It is orally active, low affinity to P-gp, showed that its oral administration provided well-controlled drug exposure, minimal side effects and disease stabilization in patients with advanced solid tumors in the initial phase I studies. Manageable toxicity and encouraging activity were observed during the phase II studies with advanced gastric cancer and colorectal cancer, but didn't demonstrate better efficacy over existing therapies. Renewed clinical evaluations proved that it is promising, particularly for combined therapy.[10]

Both larotaxel and milataxel are analogues of docetaxel. Larotaxel was developed by Sanofi and currently in the stage of Phase III clinical trial. Its low affinity towards P-gp permits it capable of crossing the blood-brain-barrier effectively and combination with cisplatin showed superior efficacy in phase II clinical studies. Milataxel was developed by Taxolog and currently under clinical evaluation. It has exhibited broad-spectrum anticancer efficacy in preclinical studies. Although it failed to show efficacy in a phase II clinical trial study, it demonstrated lasting responses for platinum-refractory non-small cell lung cancer (NSCLC), even previously treated by other taxanes.[10]

§1.2 Result and discussion

§1.2.1 Synthesis of β–Lactam

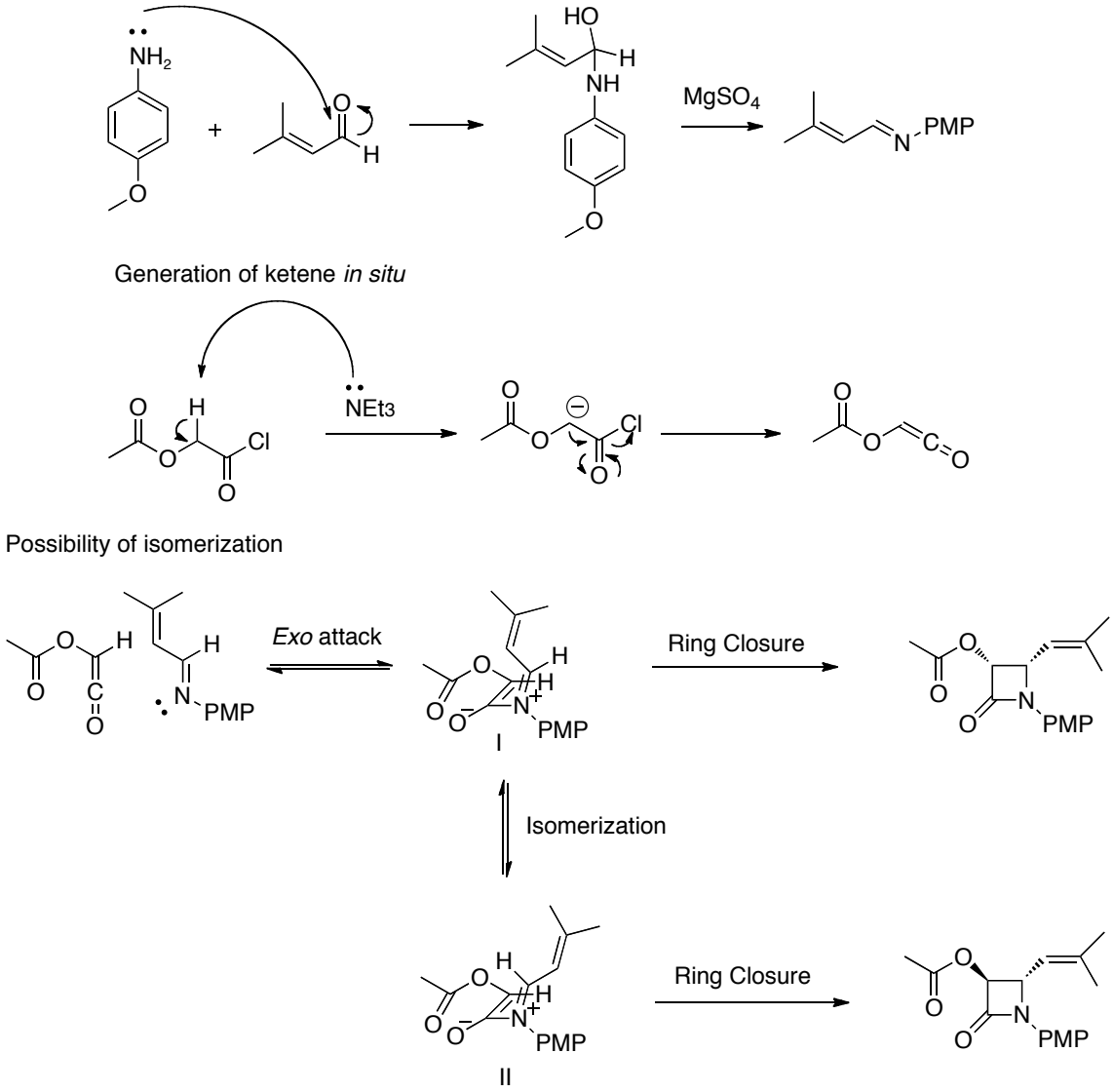
Although the first synthesis of β–lactam was credited to Hermann Staudinger through a reaction of the Schiff base of aniline and benzaldehyde with diphenylketene in a [2+2] cycloaddition early in 1907,[25] it didn't attract much attention until the first X-ray image of penicillin indicated how important the β–lactam structure was to the activity. Because β–lactam is the key structure of several most important antibiotics families such as penam, cephem, carbapenemes, and monobactams, efforts have been dedicated to the method study of β–lactam since the first total synthesis of penicillin V in 1957.[26]

In Ojima's group, enantiopure β–lactam was extensively studied and synthesized by two effcient methods: Staudinger [2+2] cycloaddition succeeded by enzymatic resolution and chiral ester enolate-imine cyclocondensation.[26, 27] The Staudinger [2+2] cycloaddition produced a racemic mixture, and the (-) enantiomer of which was resolved by the hydrolysis of the acetyl group removing the alcohol byproduct.[26] The chiral ester enolate-imine cyclocondensation was prepared utilizing the Whitesell's chiral auxiliary (WCA), which underwent the addition of an enolate to the imine, followed by cyclization to afford the β–lactam ring.[27, 28] Both methods can give the desired enantiomer, which was used to

synthesize the side chain precursor of SB-T-1214, SB-T-12301 and its analogues.

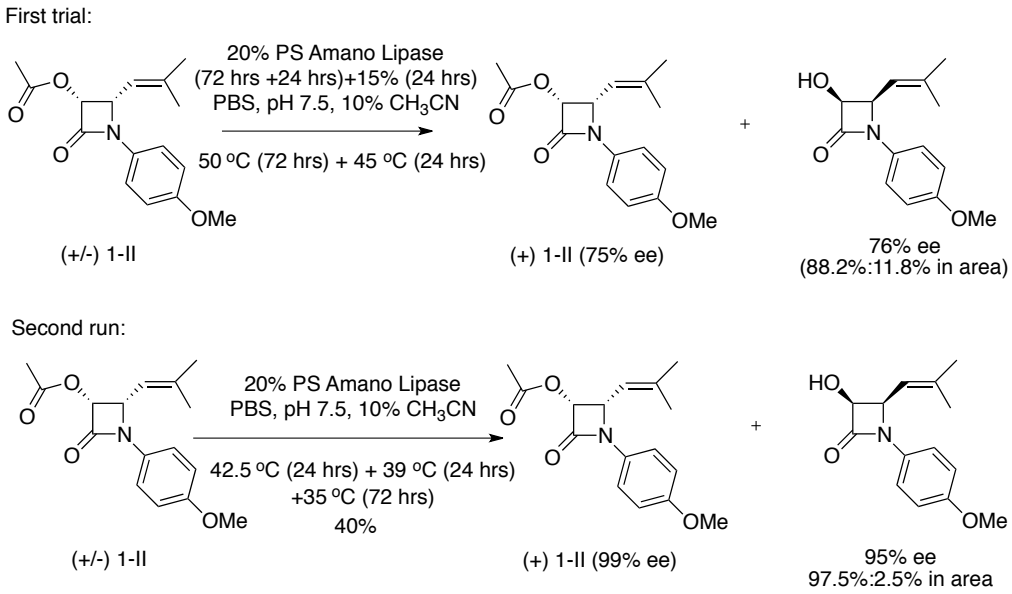
§1.2.1.1 Staudinger [2+2] Cycloaddition followed by Enzymatic Resolution

N-(4-methoxyphenyl)-3-methylbut-2-enaldimine (**1-I**) was prepared from *p*-anisidine and 3-methylbut-2-enal. Ketenes were generated in the reaction by eliminating hydrogen chloride from acetoxyacetyl chloride with a base. Imine attacked ketene as a nucleophile to give β-lactam through subsequent conrotatory ring closure.[29] The stereoselectivity was determined by the competition of the direct ring closure of the zwitterionic intermediate I and the isomerization of the imine moiety to the other zwitterionic intermediate II. Here the isomerization was controlled by running the reaction at low temperature and through slow addition of acetoxyacetyl chloride to prevent the formation of *trans*-(+/-)-β-lactam (**Scheme 1-3**).



Scheme 1-3: Mechanism of Staudinger [2+2] Cycloaddition

The enzymatic resolution with PS Amano Lipase selectively hydrolyzed the acetyl group of the (-) enantiomer of **1-II** (**Scheme 1-4**).[30] The activity of the lipase was variable and highly dependent on its batch, source, storage and reaction conditions and so on. Therefore, it is necessary that constant temperature and pH conditions be used to avoid denaturation.



Scheme 1-4: Enzymatic resolution

This reaction has been performed twice. For the first trial, it was monitored by ^{1}H NMR every 24 hrs. Unfortunately, after 3 days the conversion was no more than 15%. Instead of switching the enzyme, 15% of different source of Amano Lipase was added and the temperature was lowered to 45 ^{o}C. A day later, the conversion reached 50%. After quenched the reaction, the ee value was only around 75%. For the second run, the temperature was set to 42.5^{o}C. After 24 hours the ^{1}H NMR indicated that around 50% conversion was accomplished. To get products of higher ee value, the temperature was lowered to 39 ^{o}C after a day and then it was lowered again to 35 ^{o}C. **(+) 1-II** was obtained with an ee value of 99% (**Figure 1-11**).

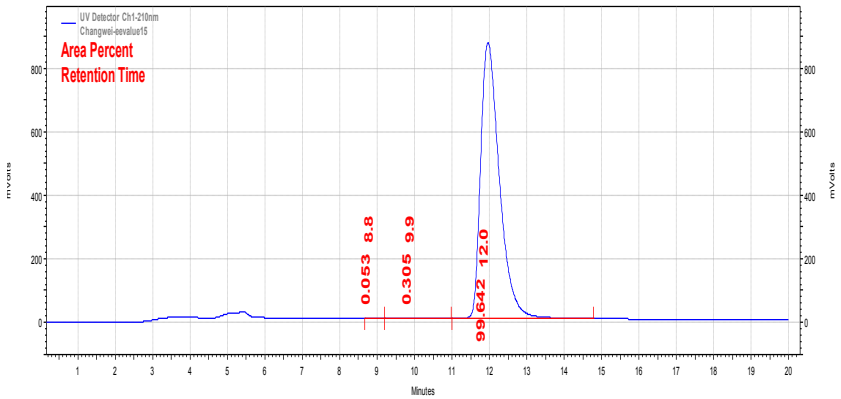
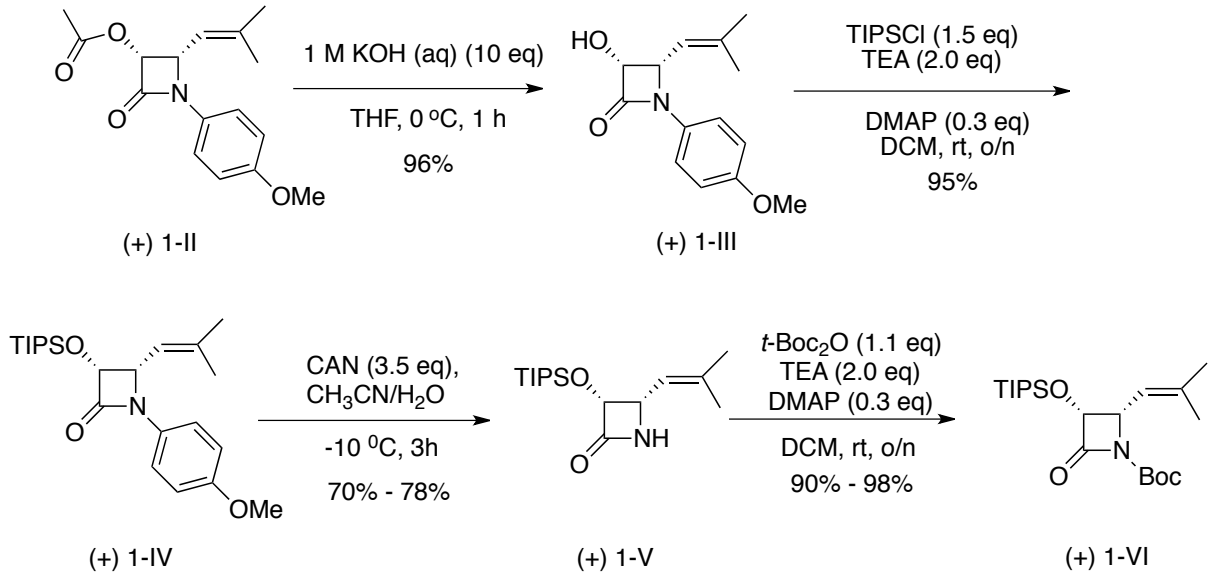


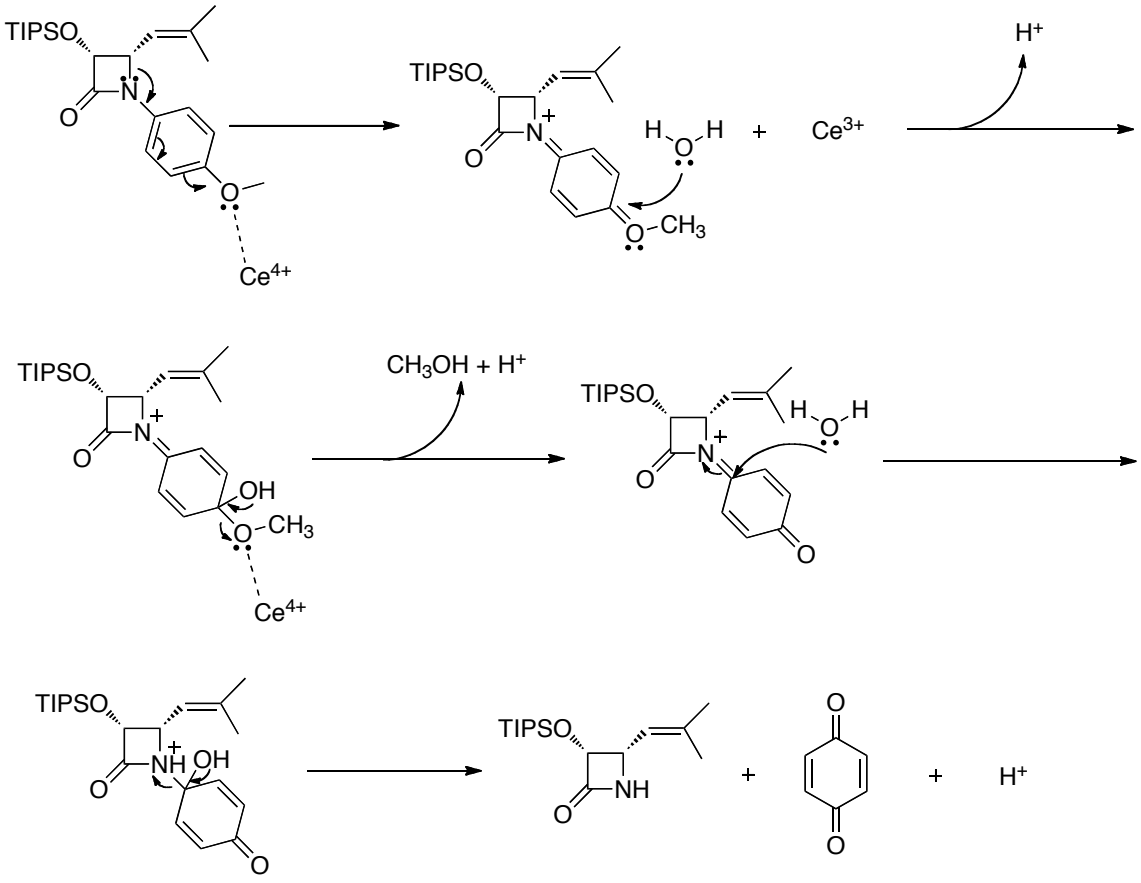
Figure 1-11: HPLC result of hydrolysis test (retention time: 12.0; 99% ee)

(+) 1- II underwent base-catalyzed hydrolysis in excellent yield. The alcohol was protected with TIPS. After cerium ammonium nitrate (CAN) deprotection, the amide was protected with Boc to yield the desired enantiopure **(+) 1-VI** (**Scheme 1-5**).[12, 31]



Scheme 1-5: Base hydrolysis, TIPS protection, CAN-deprotection, and *t*-Boc protection

In CAN deprotection, it involves an electron transfer from the PMP ether to CAN in which two Ce^{4+} ions are each reduced to Ce^{3+}. The first electron is removed from the aromatic phenyl ring to afford a resonance stabilized cationic radical. Water acts as a nucleophile and attacks the resonance-stabilized cation resulting in the hydrolysis of methanol. A second equivalent of Ce^{4+} produces the iminium cation, which can be hydrolyzed by another equivalent of water. Finally the reaction produces the deprotected amide, quinone, and methanol as the primary products (**Scheme 1-6**). [12, 31]



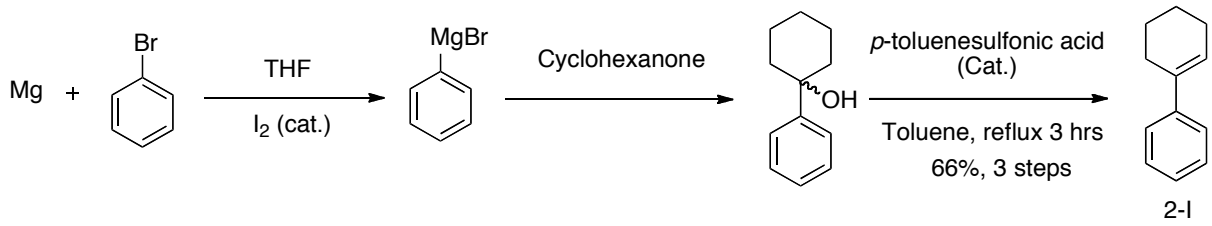
Scheme 1-6: Mechanism of CAN-deprotection

§1.2.1.2 Chiral ester enolate-imine cyclocondensation

Another efficient approach to obtain β–lactam is *via* chiral ester enolate-imine cyclocondensation. [27, 28] It was revisited, starting from the preparation of WCA.

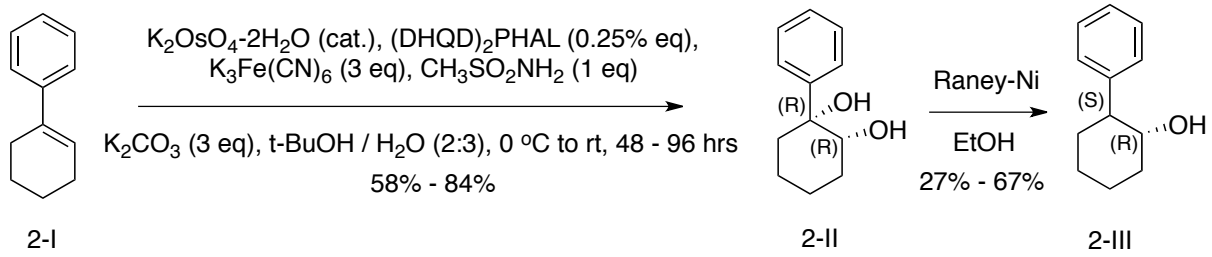
§1.2.1.2.1 Synthesis of Whitesell's chiral auxiliary

After the preparation of Grignard reagent, phenylmagnesium bromide reacted with cyclohexanone to afford the desired racemic 1-phenyl-1-cyclohexanol. The *p*-TsOH acted as a catalyst to afford the **2-I** as an elimination product *via* dehydration (**Scheme 1-7**).[32]



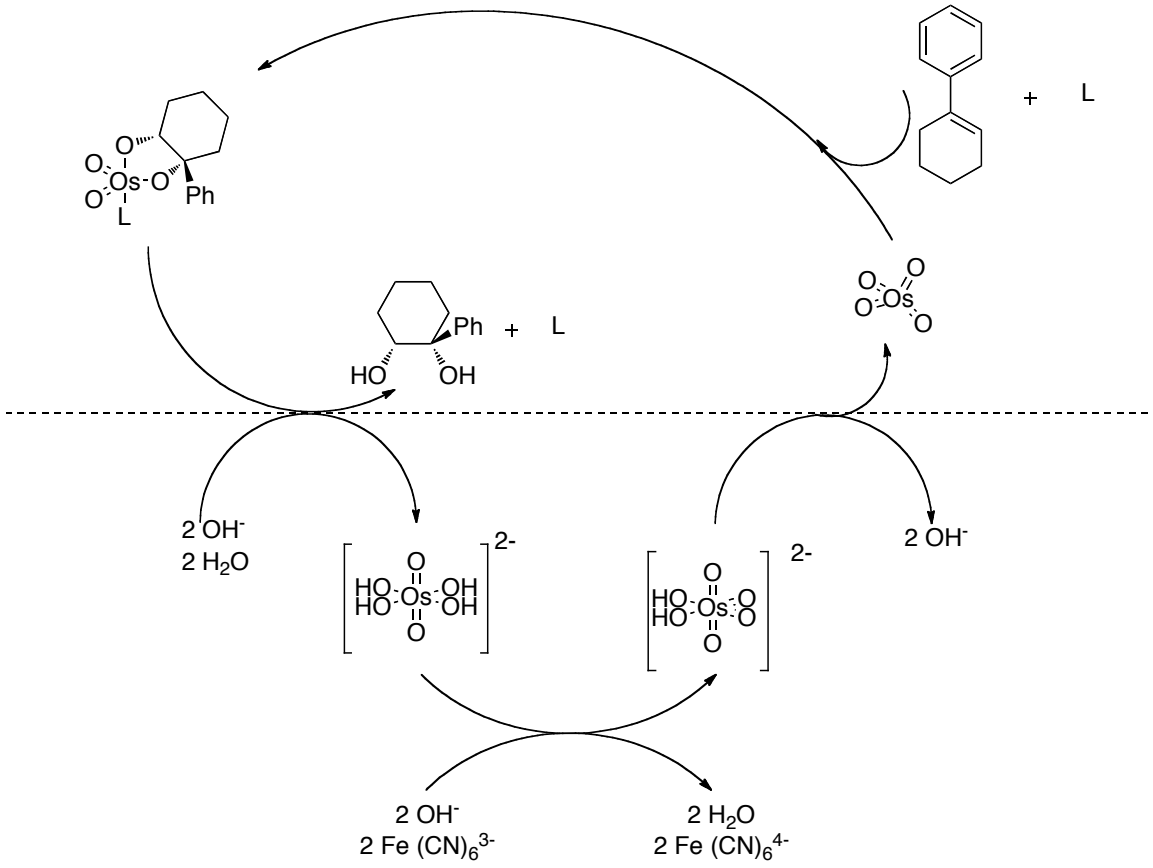
Scheme 1-7: Synthesis of 1-phenyl-1-cyclohexene (2-I)

To obtain the chiral auxiliary, **2-I** was advanced through an asymmetric dihydroxylation with the catalysis of osmium tetraoxide–ligand complex. The presence of methanesulfonamide, which could be removed by washing with 2M KOH, permitted the reaction to be run at 0 ^{o}C, leading to better regioselectivity (**Scheme 1-8**).[33]



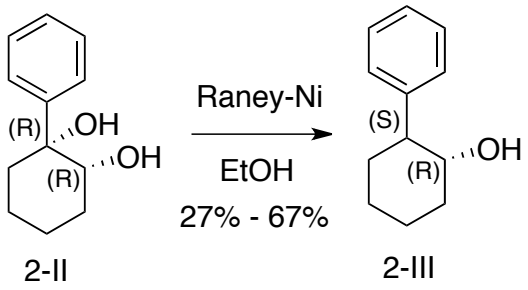
Scheme 1-8: Synthesis of (+)-(1*R*, 2*S*)-1-phenylcyclohexane-*cis*-1,2-diol

The mechanism of Sharpless asymmetric dihydroxylation is shown in the following **Scheme 1-9**.



Scheme 1-9: Mechanism of Sharpless asymmetric dihydroxylation

Compound **2-III** was obtained through dehydration of **2-II** using Raney nickel (**Scheme 1-10**).[34] The W-2 Raney nickel was prepared as slurry in wet ethanol under nitrogen atmosphere. The enantiomeric purity of the product was over 98% ee (**Figure 1-12**).



Scheme 1-10: Raney Nickel catalyzed dehydration

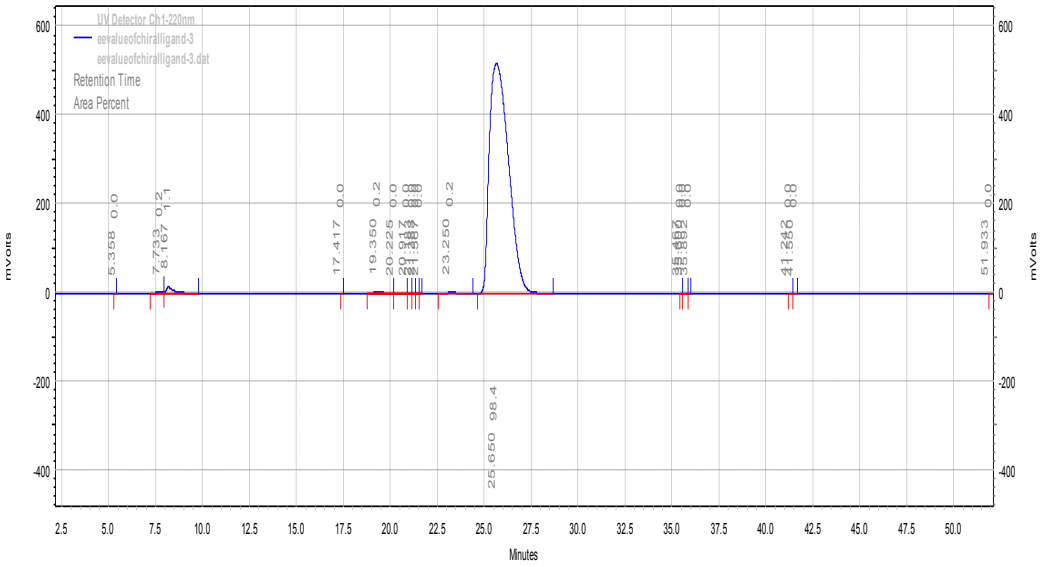
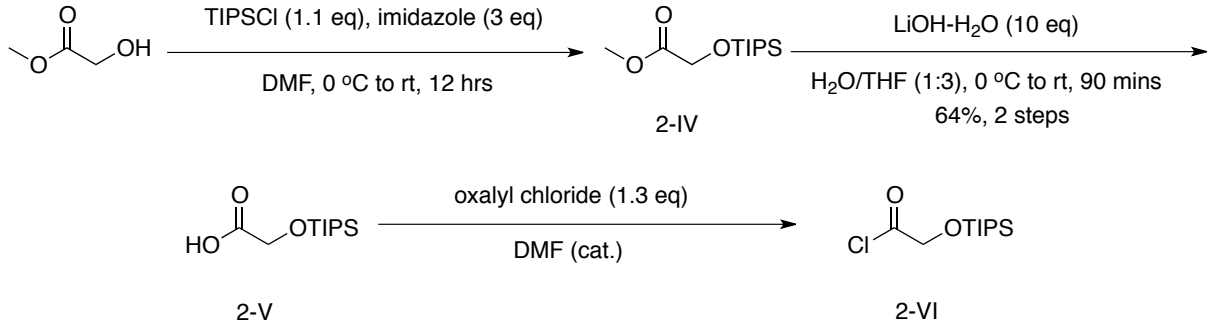


Figure 1-12: Enantiomeric purity (98% ee) of the chiral ligand

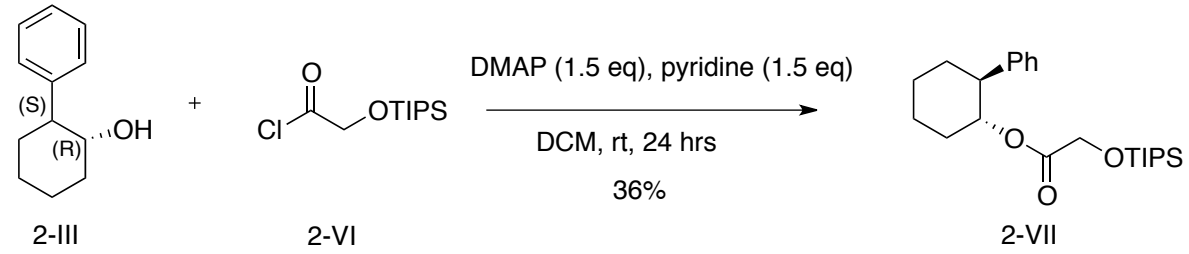
§1.2.1.2.3 Synthesis of chiral ester

TIPS protection of methyl glycolate was carried out at 0 ^{o}C, then followed by base hydrolysis, which selectively cleaved the ester while leaving silyl groups unaffected. TIPS-oxyacetyl chloride was generated by reacting TIPS-oxyacetic acid with oxalyl chloride in the presence of catalytic DMF (**Scheme 1-11**).[35]



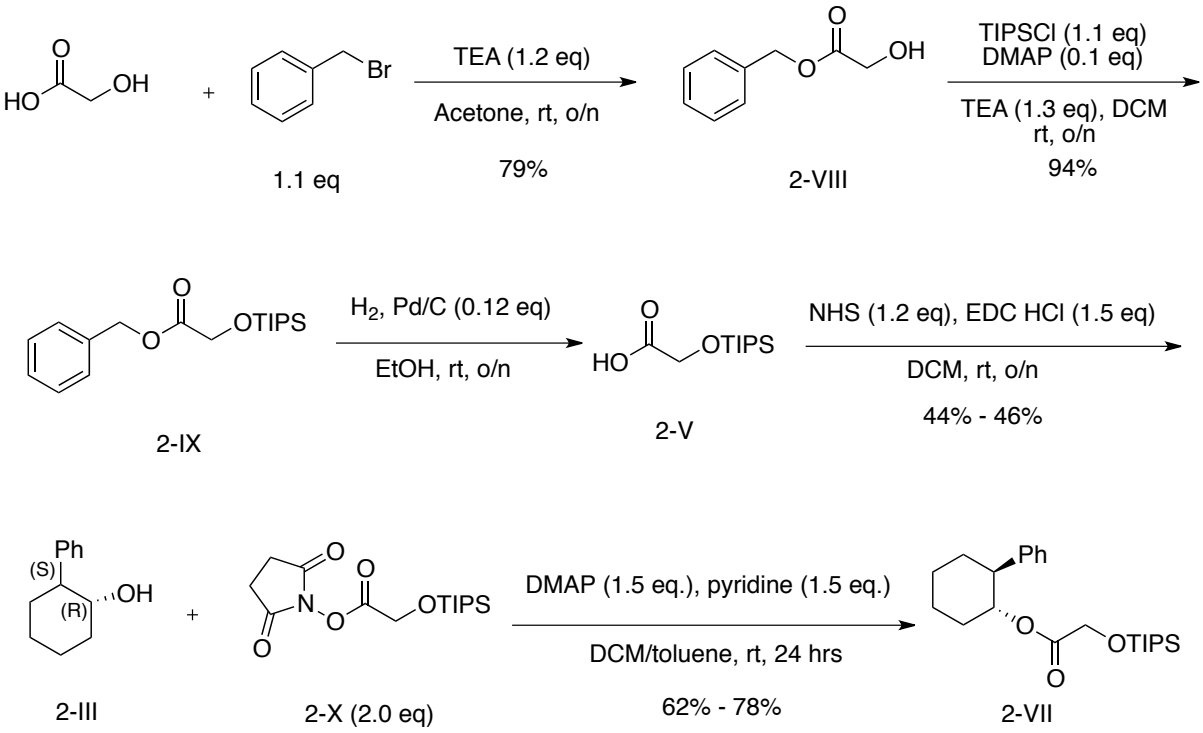
Scheme 1-11: TIPS protection, base hydrolysis, and oxalyl chloride activation

In the subsequent reaction, the chiral auxiliary **2-III** was coupled to **2-VI** in the presence of DMAP and pyridine (**Scheme 1-12**). The yield was low due to the use of column chromatography twice. The purification of this compound was found to be difficult because of its low polarity and interference with impurities.



Scheme 1-12: Synthesis of chiral ester

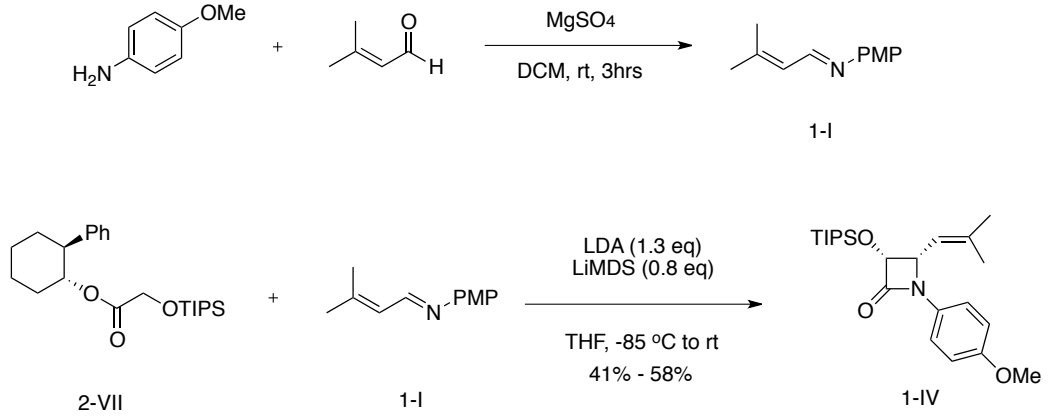
To increase the yield of chiral ester, the synthesis was improved *via* HSN activation (**Scheme 1-13**).[36] In this method, methyl glycolate was replaced by benzyl glycolate, which was obtained by reacting glycolic acid with benzyl bromide. TIPS protection and subsequent hydrogenolysis afforded intermediate **2-V**, which was activated by HSN to increase the yield of chiral ester.[36]



Scheme 1-13: Improved method for synthesizing chiral ester[36]

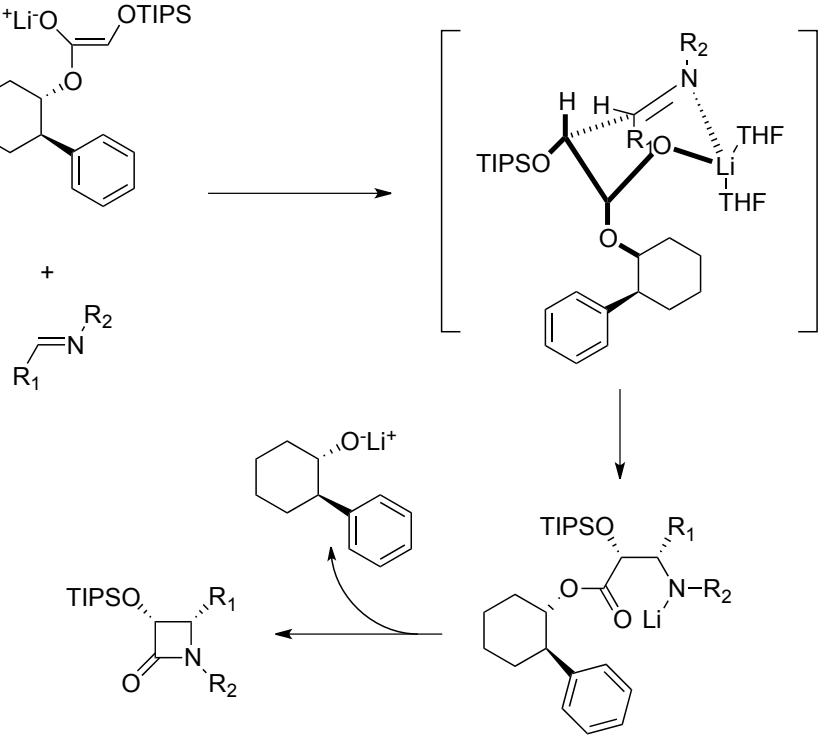
§1.2.1.2.4 Chiral ester enolate-imine cyclocondensation

After obtaining the key intermediate **2-VII**, β–lactam was synthesized efficiently with a decent yield, up to 58% (**Scheme 1-14**). Theoretically, most of the chiral auxiliary **2-III** could be recovered.



Scheme 1-14: Chiral Ester Enolate-imine Cyclocondensation

When the temperature was low enough, the double bond of (*E*)-enolate of **2-VII** attacked the *trans*-imine from the least hindered side and formed the β–amino ester intermediate, which underwent cyclization upon warming to room temperature and afforded *cis*-β–lactam. The WCA was also regenerated (**Scheme 1-15**).[12]

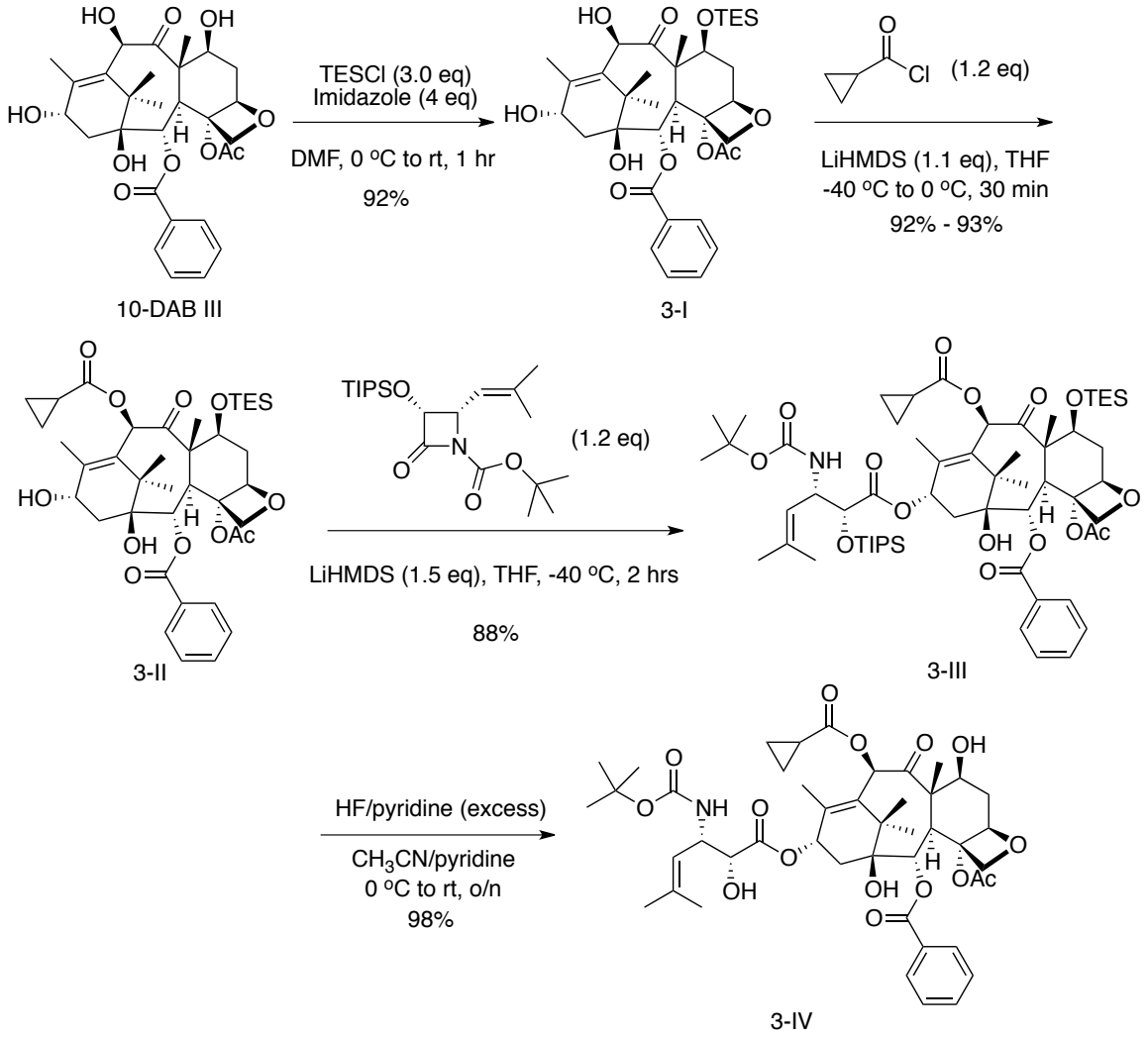


Scheme 1-15: Mechanism of enolate-imine cyclocondensation[12]

In the following steps, the highly enantiopure **1-IV** was modified in the same way as shown in **Scheme 1-5**. The final β–lactam was derived with decent yield.

§1.2.2 Synthesis of SB-T-1214

Out of all four free alcohol functionalities at C-1, C-7, C-10 and C-13 of 10-deacetylbaccatin III, the hydroxyl proton at C-7 was the most active one and was protected first. Then the hydroxyl of C-10 coupled to cyclopropanecarboxylic acid chloride. The reaction took place at -40 ^{o}C because the addition-elimination reaction of the alkoxide to the acyl chloride was exothermic. It was followed by Ojima-Holton coupling with β–lactam at C-13. C-1 is deeply buried inside, making it the least active one due to steric effect. In the process of Ojima-Holton coupling, lithium bis(trimethylsilyl)amide (LiHMDS), a non-nucleophilic base, reacted with the C-13 hydroxyl group of **3-II** and afforded a lithium-10-alkoxy salt, which acted as a nucleophile and attacked the carbonyl of β–lactam to open the ring. The deprotection of silyl groups afforded **3-IV**. (**Scheme 1-16**).[21]



Scheme 1-16: Synthesis of SB-T-1214 with Ojima-Holton Coupling[21]

§1.2.3 Conclusion and discussion

Two β–lactam synthesis methods and the synthesis of preparing SB-T-1214 were carried out. Despite of the successes, problems still exist in these standard protocols for preparing β–lactam. The space of further improvement might still exist.

The problems of the Staudinger reaction followed by enzymatic resolution are the unpredictable characteristics of enzymes; the activities of enzymes are different for different batches and require different reaction conditions. The enzymatic resolution was a heterogenous reaction; therefore reaction monitoring is not consistent with the reaction progress. However, the biggest problem of enzymatic resolution is the atom economy. Only half of the resolved material would be useful for taxoids synthesis, while the undesired enantiomer would be wasted. The other way of β–lactam synthesis, chiral ester enolate-imine cyclocondensation, involved the problems of preparing and regenerating WCA. Although it could theoretically be recovered, it is often messy, and a half of the material lost in the reaction and recovering processes. [37]

To synthesize β–lactam more efficiently, studies have been reported by replacing the chiral ligand like WCA with asymmetric catalyst like benzoylquinine (BQ) to save the troubles of making and recycling. If a new protocol of synthesizing β–lactam can be established by employing a suitable asymmetric catalyst, it might be superior to both of the well-established routes.[37]

§1.3 Experimental section

§1.3.1 General Methods and Materials:

^{1}H NMR was taken on a Varian 300, 400 or 500 MHz NMR spectrometer of the chemistry department. The melting points were carried out on a "Uni-melt" capillary melting point apparatus from Arthur H. Thomas Company, Inc. TLC analysis was accomplished on Merck DC-alufolien with Kieselgel 60F-254 and visualized by UV light and stained with sulfuric acid-EtOH, or 10 % Vanillin-EtOH with 1% sulfuric acid. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). Chiral HPLC analysis for the determination of enantiomeric excess was performed using a Shimazu HPLC, using a DAICEL-CHIRACEL OD chiral column (25 x 0.46 cm i.d.), utilizing hexane/2-propanol (85% : 15%) as the solvent system containing a flow rate of 0.6 ml / min.

The chemicals were purchased from Sigma Aldrich Company, Fischer Company or Acros Organic Company. 10-DAB III was donated by Indena, SpA, Italy. Dichloromethane and methanol were dried before use by distillation over calcium hydride under nitrogen. Ether and THF were dried before use by distillation over sodium-benzophenone kept under nitrogen. Dry DMF was purchased from EMD chemical company, and used without further purification. Reaction flasks were dried in 100 ^{o}C ovens and allowed to cool to room temperature in a desiccator over "*Drierite*" (calcium sulfate) and assembled under an inert nitrogen gas atmosphere.

§1.3.2 Synthetic procedure of β–lactam

N-(4-Methoxyphenyl)-3-methyl-2-butenaldimine [(+/-) 1-I][38]

p-Anisidine (7.495 g, 61 mmol) was dissolved in CH_2Cl_2 with anhydrous $MgSO_4$. To this solution, 3-methylbut-2-enal (5.6 mL, 74 mmol) was added dropwise under inert conditions. The reaction mixture was stirred at room temperature and monitored *via* TLC. After 3 hours, the solvent was evaporated and concentrated *in vacuo* to afford **1-I** as red oil, which was used immediately in the subsequent step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.96 (s, 3 H), 2.01 (s, 3 H), 3.81 (s, 3 H), 6.20 (d, J = 9.3 Hz, 1 H), 6.87 (d, J = 8.7 Hz, 2 H), 7.11 (d, J = 8.7 Hz, 2 H), 8.37 (d, J = 9.3 Hz, 1 H). All data are consistent with literature values. [38]

(+/-)-1-(4-Methoxyphenyl)-3-acetoxyl-4-(2-methylprop-1-enyl)azetidin-2-one [(+/-) 1-II][29]

An aliquot of **1-I** was dissolved in CH_2Cl_2, the solution was cooled to -78 ^{o}C and maintained at the temperature for 30 minutes. Then to this solution 1.5 eq of TEA (13.9 mL, 99.8 mmol) was added dropwise, followed by 1.2 eq. of acetoxyacetyl chloride (8.0 mL, 74.5 mmol). The resulting solution was stirred vigorously at -78 ^{o}C for 3 hours and then allowed to warm to room temperature overnight. The reaction was monitored *via* TLC and upon completion it was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The organic layers were combined and washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*, resulted in a dark red crude oil, which was purified by normal phase column chromatography (HEX: EtOAc = 2:1) to afford the product as a pale yellow solid. Recrystallization from hexanes yielded **(+/-) 1-II** (8.53g, 29.5 mmol, 49%) as a white crystal: m. p. 106–108 ^{o}C; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.58 (s, 3 H), 1.80 (s, 3 H), 1.83 (s, 3 H), 2.13 (s, 3 H), 3.79 (s, 3 H), 4.96 (dd, J_1 = 14.1 Hz, J_2 = 4.8 Hz, 1 H), 5.13 (d, J = 10.8 Hz, 1 H), 5.81 (d, J = 5.1 Hz, 1 H), 6.85 (d, J = 9.0 Hz, 1 H), 7.31 (d, J = 9.0 Hz, 1 H). All data are consistent with literature values. [29]

(+)-1-(4-Methoxyphenyl)-3-acetoxyl-4-(2-methylprop-1-enyl)azetidin-2-one [(+) 1-II] [39]

An aliquot of **(+/-) 1-II** (4.003g, 13.85 mmol) was dissolved in 500 mL of 0.2 M sodium phosphate buffer (pH 7.5) with 10% volume acetonitrile and heated to 42.5 ^{o}C. To this solution PS-Amino Lipase (0.8 g, 20%) was added and the mixture was stirred vigorously with a mechanical stirrer. The solution changed in color from yellow to red. The reaction was monitored *via* TLC and ^{1}H-NMR for 50% conversion of its C-3 and C-4 hydrogens. After 24 hrs the NMR indicated that around 50% conversion was almost accomplished. To get products of higher ee value, the temperature was lowered to 39 ^{o}C after a day and then was lowered again to 35 ^{o}C. When the last sample indicated that the ee value reached 99%, the remaining lipase was vacuum filtered and extracted with ethyl acetate (3 x 200 mL). The combined organic layers was washed with brine, dried over anhydrous $MgSO_4$ and concentrate *in vacuo*. The desired **(+) 1-II** (1.602 g, 5.54 mmol, 40% yield with >99% ee) was obtained as a white solid by performing normal phase column chromatography (HEX: EtOAc = 3:1). ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.57 (s, 3 H), 1.79 (s, 3 H), 1.82 (s, 3 H), 2.12 (s, 3 H), 3.78 (s, 3 H), 4.95 (dd, J_1 = 14.1 Hz, J_2 = 4.8 Hz,1 H), 5.11 (d, J = 10.8 Hz, 1 H), 5.80 (d, J = 5.1 Hz, 1 H), 6.84 (d, J = 9.0 Hz, 1 H), 7.30 (d, J = 9.0 Hz, 1 H). All data are consistent with literature values. [39]

(3*R*,4*S*)-1-(4-Methoxyphenyl)-3-hydroxy-4-(2-methoxyprop-1-enyl)azetidin-2-one [(+) 1-III] [12]

An aliquot of **(+) 1-II** (3.207 g, 11.1 mmol) was dissolved in 180 mL THF, cooled to 0°C. To this solution was added previously chilled 1M aqueous KOH (100 mL) in 100 mL THF. The mixture was stirred and monitored *via* TLC. Upon completion it was quenched with saturated aqueous NH_4Cl (100 mL), extract with CH_2Cl_2 (3 x 100 mL), washed with brine, dried over anhydrous $MgSO_4$, concentrated *in vacuo* and washed with hot hexanes to afford **(+) 1-III** (2.645 g, 10.7 mmol, 96%) as white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.79 (s, 3 H), 1.82 (s, 3 H), 2.12 (s, 3H), 3.78 (s, 3 H), 4.96 (dd, J_1 = 14.1 Hz, J_2 = 4.8 Hz, 1 H), 5.29 (d, J_1 = 10.8 Hz, J_2 = 6.9 Hz, 1 H), 5.80 (d, J = 5.1 Hz, 1 H), 6.84 (d, J = 9.0 Hz, 1 H), 7.30 (d, J = 9.0 Hz, 1 H). All data are consistent with literature values. [12]

(3*R*,4*S*)-1-*p*-Methoxyphenyl-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one [(+) 1-IV] [12]

An aliquot of **(+) 1-III** (1.076 g, 4.35 mmol) and DMAP (159 mg, 1.30 mmol) were dissolved in CH_2Cl_2 (20.5 mL). The mixture was cooled to 0 °C under inert condition. To this solution was added triethylamine (1.8 mL, 12.8 mmol) dropwise and stirred for 10 minutes, followed by the dropwise addition of chlorotriisopropylsilane (1.3 mL, 6.53 mmol). This mixture was stirred and allowed to warm up to room temperature. The reaction was monitored *via* TLC and upon completion it was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined the organic layers was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 95:5) to afford **(+) 1-IV** (1.675 g, 4.15 mmol, 95%) as a white crystalline with the strong scent of sand. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.05 (m, 21 H), 1.79 (s, 3 H), 1.84 (s, 3 H), 3.77 (s, 3 H), 4.78 (dd, J_1 = 14.7 Hz, J_2 = 4.8 Hz, 1 H), 5.05 (d, J = 4.8 Hz, 1 H), 5.31 (d, J = 9.9 Hz, 1 H), 6.82 (d, J = 9.0 Hz, 1 H), 7.30 (d, J = 8.7 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 12.14, 17.85, 26.33, 55.67, 57.79, 114.48, 118.57, 120.40, 131.75, 139.36, 156.23, 165.82. All data are consistent with literature values. [12]

(3*R*,4*S*)-3-Triisopropylsilyloxy-4-(2-methylpropen-2-yl)azetidin-2-one [(+) 1-V] [12]

An aliquot of **(+) 1-IV** (1.107 g, 2.74 mmol) was dissolved in acetonitrile (61.5 mL, 1:1 of each) and cooled to -10 °C. After 10 minutes, to this solution was added CAN (5.260 g, 9.59 mmol) dissolved in water (10 mL), dropwise. The reaction was monitored *via* TLC and upon completion it was quenched with saturated aqueous $NaHSO_3$ (20 mL). The resulting solution was extracted with aliquots of CH_2Cl_2 (3 x 130 mL). The combined organic layers was washed with distilled water (130 mL), sodium bicarbonate (2 x130 mL), and brine (2 x 150 mL), then dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by flash column chromatography on silica gel with increasing amounts of ethyl acetate in hexanes (HEX: EtOAc = 100:0- 85:15) to afford a pale yellow solid. After remove the color with a small amount of activated carbon, obtained compound **(+) 1-V** as a white solid (632 mg, 2.12 mmol, 77%): ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.03 (m, 21 H), 1.68 (s, 3 H), 1.76 (s, 3 H), 4.43 (dd, J_1 = 9.6 Hz, J_2 = 4.8 Hz, 1 H), 4.98 (m, 1 H), 5.29 (d, J = 9.6 Hz, 1 H), 5.85 (s, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 12.09, 17.83, 26.21, 53.70, 79.57, 121.62, 138.18, 170.17. All data are consistent with literature values. [12]

1-(*tert*-Butoxycarbonyl)-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one [(+)1-VI] [12]

An aliquot of **(+) 1-V** (506 mg, 1.70 mmol) and DMAP (53 mg, 0.43 mmol) were dissolved in CH_2Cl_2 (8.0 mL). To this solution, triethylamine (0.50 mL, 3.40 mmol) was added dropwise. While stirring, di-*tert*-butyl dicarbonate (409 mg, 1.87 mmol) in CH_2Cl_2 (8.0 mL) was added dropwise. Then the mixture was stirred for 24 hrs at room temperature and monitored *via* TLC. Upon completion the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers was washed with brine (30 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was performed by normal phase column chromatography on silica gel (HEX: EtOAc = 95:5) to obtain **1-VI** (658 mg, 1.65 mmol, 98%) as pale yellow oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.04 (m, 21 H), 1.48 (s, 9 H), 1.76 (s, 3 H), 1.79 (s, 3 H), 4.75 (dd, J_1 = 9.6 Hz, J_2 = 4.8 Hz, 1 H), 4.96 (d, J = 5.7 Hz, 1 H), 5.28 (d, J = 5.7 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 12.03, 17.74, 26.30, 28.26, 57.04, 83.16, 118.63, 139.86, 148.42, 166.63. All data are consistent with literature values. [12]

1-Phenyl-1-cyclohexanol and 1-phenyl-1-cyclohexene [2-I] [32]

Magnesium turnings (8.037 g, 329 mmol) was dried *via* oil bath (110 ^{o}C) *in vacuo*, then transferred to a 1000 mL three-neck round bottom flask that equipped with a condenser under nitrogen atmosphere. To this flask, THF (220 mL) was added and followed by a catalytic amount of I_2 (2.118 g, 16.7 mmol). To this mixture was added bromobenzene (42.3 mL, 402 mmol) dropwise at room temperature (exothermic) over 2 hrs by using a syringe pump. After the completion of adding bromobenzene, the mixture was heated to 65 ^{o}C and maintained for 1 hour until all of the magnesium turnings reacted. After it was cooled down to room temperature, cyclohexanone (23.0 mL, 222 mmol) was added dropwise over 5 hours by using a syringe pump. The reaction was allowed to stirred overnight and monitored *via* TLC. Upon completion, the reaction was quenched very slowly with saturated aqueous NH_4Cl (200 mL) and extracted with ethyl acetate (3 x 200 mL). The organic layers were combined and washed with brine (200 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to yield a yellow crude oil, which was dissolved in toluene (200 mL) in a 500 mL round bottom flask quipped with a Dean-Stark trap with 50 mL of toluene previously charged in the 60 mL solvent trap. To this solution was added a catalytic amount of *p*-toluene sulfonic acid and the solution was refluxed for 3 hours by oil bath (165 ^{o}C). The resulting solution was concentrated *in vacuo* to afford a brown crude oil, which was transferred to a 50 mL round bottom flask and distilled in Kugelrohr at 120 ^{o}C under reduced pressure 0.05 mmHg to afford **2-I** (26.4 mL, 147 mmol, 66%) as a clear oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.70 (m, 2 H), 1.80 (m, 2 H), 2.26 (m, 2 H), 2.44 (m, 2 H), 6.17 (m, 1 H), 7.28 (tt, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1 H), 7.36 (tt, J_1 = 8.1 Hz, J_2 = 2.1 Hz, 2 H), 7.42 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 2 H). All data are consistent with literature values. [32]

(+)-(1*R*,2*S*)-1-Phenylcyclohexane-*cis*-1,2-diol [2-II] [33]

To a 250 mL round bottom flask was added potassium ferrocyanide (31.367 g, 95.2 mmol), potassium carbonate (13.342 g, 96.5 mmol), and methanesulfonamide (3.160 g, 33.4 mmol), *tert*-butanol (32.5 mL) and distilled water (50.0 mL). This mixture was stirred vigorously and cooled down to 0 ^{o}C by ice bath. Then to this mixture was added potassium osmate dehydrate (35 mg + 41 mg) and $(DHQD)_2$PHAL ligand (644 mg, 0.813 mmol), and was allowed to

stirred for an additional 20 minutes. To this mixture was added 1-phenylcyclohexene (4.97 g, 31.4 mmol) dropwise, and allowed to warm to room temperature and stirred for 48 hours. This reaction mixture visibly changed from a dark orange to a light yellow color as potassium ferrocyanide was reduced. The reaction was monitored *via* TLC and upon completion ethyl acetate (25 mL) was added and allowed to stirred for 15 minutes. Then the solid potassium ferrocyanide was removed by filtering and the resulting liquid was diluted with an additional aliquot of ethyl acetate (100 mL). The resulting organic layer was washed with 2N KOH (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification was achieved by normal phase chromatography on silica gel to afford **2-II** (5.87 g, 26.5 mmol, 84%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.39 (m, 1 H), 1.52 (m, 2 H), 1.69 (s, 3 H), 1.87 (m, 3 H), 3.98 (dd, J_1 = 15.9 Hz, J_2 = 6.0 Hz, 1 H), 7.28 (tt, J_1 = 7.8 Hz, J_2 = 2.4 Hz, 1 H), 7.38 (tt, J_1 = 8.1 Hz, J_2 = 2.1 Hz, 2 H), 7.50 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 2 H). All data are consistent with literature values. [33]

(-)-*trans*-2-Phenyl-cyclohexanol [2-III] [33]

An aliquot of **2-II** (2.007 g, 10.4 mmol) was dissolved with absolute ethanol (20 mL) in a 250 mL round bottom flask and purged with N_2. To this solution was added Raney-Nickel 2800 catalyst. This mixture was allowed to heated to 105 ^{o}C while stirring and refluxing. The reaction was monitored *via* TLC and upon completion, it was cooled down to room temperature and filtered through celite. The resulting filtrate was concentrated *in vacuo*, and redissolved in ethyl acetate (30 mL). The organic layer was washed with brine (2 x 30 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification was done by normal phase chromatography on silica gel to afford the product as a white solid. Recrystallization from hexanes afforded **2-III** (1.222 g, 6.93 mmol, 67% in yield with >99% ee) as white crystal: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.40-1.43 (m, 2 H), 1.54 (m, 3 H), 1.77-1.84 (m, 1 H), 1.85 -1.88 (m, 2 H), 2.15 (m, 1 H), 2.44-2.52 (m, 1 H), 3.69 (m, 1 H), 7.28 -7.34 (m, 3 H), 7.37 (m, 2 H). All data are consistent with literature values. [33]

Triisopropylsiloxymethyl glycolate [2-IV][3]

To a 10 mL round bottom flask was added methyl glycolate (443 mg, 4.05 mmol), imidazole (805 mg, 12.2 mmol), and DMF (1.9 mL). This mixture was allowed to stirred and cooled down to 0 ^{o}C. Then to the mixture was added triisopropylsilyl chloride (0.9 mL, 4.2 mmol) dropwise. This mixture was allowed to stirred for 18 hours and monitored *via* TLC. Upon completion it was quenched with saturated aqueous NH_4Cl (5 mL). The resulting mixture was dissolved in ethyl acetate (30 mL) and was washed with saturated aqueous NH_4Cl solution (2 x 25 mL) and brine (25 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield **2-IV** (1.243 g, 4.77 mmol) as slightly yellow oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.04-1.07 (m, 21 H), 3.74 (s, 3 H), 4.32 (s, 2 H). All data are consistent with literature values. [3]

Triisopropylsiloxyacetic acid [2-V] [3]

To a 500 mL round bottom flask was added **2-IV** (3.624 g, 13.91 mmol) and THF (150 mL). It was cooled down to 0 ^{o}C and then to this solution was added aqueous 2M LiOH solution (68.7 mL, 152 mmol) dropwise with a syringe pump. The resulting solution was allowed to stirred for 2 hours at room temperature and monitored *via* TLC. Then it was diluted with 150 mL of water, removed the THF by rotary evaporation. The resulting aqueous solution was

extracted with diethyl ether (2 x 100 mL). Then diethyl ether (50 mL) was added and the pH was adjusted to 2.0 with 1N HCl. The aqueous solution was extracted with diethyl ether (2 x 150 mL) and dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to afford **2-V** (8.84 mmol, 2.056 g, 64%) as a clear oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.04 -1.06 (m, 21 H), 4.29 (s, 2 H). All data are consistent with literature values. [3]

Triisopropylsiloxyacetyl chloride [2-VI] [3]

To a 250 ml round bottom flask was added **2-V** (1.836 g, 7.90 mmol) and dichloromethane (80 mL), then to this solution was added oxalyl chloride (0.88 mL, 10.28 mmol) followed by 3 drops of DMF. The reaction was allowed to stirred under constant nitrogen flow for 24 hours and monitored *via* TLC. Upon completion, concentrated the mixture to afford **2-VI** as a slightly yellow oil, which is pure enough for the subsequent step without further purification.

(1*R*,2*S*)-(-)-2-Phenylcyclohexyl triisopropylsilyloxyacetate [2-VII][27]

To a 100 mL round bottom flask was added WCA (1.074 g, 6.07 mmol) and DMAP (670 mg, 5.47 mmol), followed by CH_2Cl_2 (9.0 mL) and pyridine (0.45 mL, 7.56 mmol). The mixture was allowed to stirred under nitrogen atmosphere. To this solution was added **2-VI** (1.775 g, 7.1 mmol) and the resulting mixture was allowed to stirred for 24 hours. The reaction was monitored *via* TLC and upon completion the reaction was quenched with saturated aqueous sodium bicarbonate (40 mL), extracted with ethyl acetate (300 mL) and washed with aqueous saturated sodium bicarbonate (300 mL), aqueous saturated cupric sulfate (300 mL) and brine (300 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to afford a crude oil. Purification was done by normal phase column chromatography on silica gel (HEX: EtOAc = 1% - 2%) to afford **2-VII** (0.842 g, 2.2 mmol, 36%) as a clear oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 0.93 -1.09 (m, 21 H), 1.34 - 1.55 (m, 4 H), 1.84 -1.99 (m, 3 H), 2.13 (d, J = 21.9 Hz, 1 H), 2.70 (t, J = 20.1 Hz, 1 H), 3.96 (d, J = 16.5 Hz, 1 H), 4.13 (d, J = 16.8 Hz, 1 H), 5.11 (t, J = 21.0 Hz, 1 H), 7.21 (d, J = 3.6 Hz, 3 H), 7.26 (t, J = 24.3 Hz, 3 H). All data are consistent with literature values. [27]

(3*R*,4*S*)-1-*p*-Methoxyphenyl-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one [(+) 1-IV] [27]

To a solution of THF and 1.3 eq of DIPA (0.50 mL, 3.514 mmol) that was previously cooled to -15 ^{o}C was added 1.3 eq of 2.5 M n-BuLi dropwise (1.41 mL, 3.514 mmol). This mixture was allowed to stirred for 1 hour. The reaction vessel was further cooled to -78 ^{o}C with dry ice–acetone bath for an additional hour. To this reaction mixture was added **2-VII** (1100 mg, 2.703 mmol) dissolved in THF dropwise over 2 hours with a syringe pump. Over the following 2 hours the solution was added 1.5 eq of **1-I** dissolved in THF, and the reaction was allowed to stirred for an additional 2 hours. To this reaction vessel was added 1.05 M LiHMDS in THF (2.1 mL, 2.21 mmol), and the mixture was allowed to warm to room temperature and stirred overnight. The reaction was monitored *via* TLC and quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford a crude oil, which was purified *via* flash column chromatography on silica gel. Recrystallization from hexanes to yield **(+) 1-IV** (633 mg, 1.568 mmol, 58%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.05 (m, 21 H), 1.79 (s, 3 H), 1.84 (s, 3 H), 3.77 (s, 3 H), 4.78 (dd, J = 14.7 Hz, J_2 = 6.8 Hz, 1 H), 5.05 (d, J = 4.8 Hz, 1 H), 5.31 (d, J = 9.9 Hz, 1 H), 6.82 (d, J = 9.0 Hz, 1 H), 7.30 (d, J = 8.7 Hz, 1 H). All

data are consistent with literature values. [27]

Benzyl glycolate [2-VIII][13, 36]
An aliquot of glycolic acid (3.387 g, 44.5 mmol) was dissolved in acetone, which was previously dried with $MgSO_4$. To this solution was added 1.2 eq of TEA (7.4 mL, 53.4mmol) dropwise and allowed to stirred for 10 minutes, then to this mixture was added 1.1 eq of benzyl bromide (5.8 mL, 49.0 mmol) dropwise and allowed to stirred for overnight. The reaction was monitored *via* TLC. Upon completion, it was quenched with saturated aqueous NH_4Cl, extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* afford **2-VIII** (5.875 g, 35.4 mmol, 79%) as a pale yellow oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 2.61 – 2.63 (m, 1 H), 4.20 (d, J = 5.7 Hz, 2 H), 5.23 (s, 2 H), 7.35 – 7.39 (m, 5 H). All data are consistent with literature values. [13, 36]

Triisopropylsiloxybenzylglycolate [2-IX] [36]
An aliquot of **2-VIII** (5.843 g, 35.2 mmol) was dissolved with CH_2Cl_2. To this solution was added 0.1 eq of DMAP (430 mg, 3.52 mmol) and 1.3 eq of TEA (6.4 mL, 45.8 mmol) and allowed to stirred for a while, then to this mixture was added 1.1 eq of triisopropylsilyl chloride (8.3 mL, 38.7 mmol) dropwise and allowed to stirred for 5 hours. The reaction was monitored *via* TLC. Upon completion, it was quenched with saturated aqueous NH_4Cl, diluted with water, extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to afford **2-IX** (10.672 g, 33.1 mmol, 94%): ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.04 – 1.11 (m, 21 H), 4.36 (s, 2 H), 5.19 (s, 2 H), 7.35 – 7.37 (m, 5 H). All data are consistent with literature values. [36]

Triisopropylsiloxyacetic acid [1-V] [3, 36]
An aliquot of **2-IX** (5.560 g, 17.2 mmol) was dissolved in ethanol. To this solution was added 0.12 eq of Pd/C under N_2 atmosphere, then the aspirate was purged by H_2. The reaction was monitored *via* TLC. Upon completion, it was concentrated *in vacuo* afford **1-V** as a pale yellow oil and used for subsequent reaction without purification.

Triisopropylsiloxyacetatoxy-*N*-succinimide [2-X] [36]
An aliquot of **1-V** and 1.2 eq of *N*-hydroxysuccinimde (2.182 g, 18.9 mmol) were dissolved in CH_2Cl_2 (57 mL). To this solution was added 1.5 eq of EDC (4.544 g, 23.7 mmol) dropwise and allowed to stirred overnight. The reaction was monitored *via* TLC. Upon completion, it was quenched with saturated aqueous NH_4Cl, extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Recrystallization afforded **2-X** (2.412 g, 7.3 mmol, 46%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.06 -1.09 (m, 21 H), 2.85 (s, 4 H), 4.67 (s, 2 H). All data are consistent with literature values. [36]

(1*R*,2*S*)-(-)-2-Phenylcyclohexyl triisopropylsilyloxyacetate [2-VII] [36]
To a round bottom flask was added WCA (727 mg, 4.13 mmol) and 0.8 eq of DMAP (404 mg, 3.3 mmol), followed by toluene (18 mL), then purged with N_2 atmosphere. To this mixture was added 2 eq of **2-X** (2.922 g, 8.88 mmol). The mixture was allowed to stirred for 72 hours and monitored *via* TLC. Upon completion, the reaction was quenched with saturated aqueous sodium bicarbonate, extracted with ethyl acetate, washed with saturated aqueous sodium bicarbonate, brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*.

Purification was performed by normal phase column chromatography on silica gel to afford **2-VII** (1.046 g, 3.18 mmol, 78%) as a clear oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 0.93 -1.09 (m, 21 H), 1.34 - 1.55 (m, 4 H), 1.84 -1.99 (m, 3 H), 2.13 (d, *J* = 21.9 Hz, 1 H), 2.70 (t, *J* = 20.1 Hz, 1 H), 3.96 (d, *J* = 16.5 Hz, 1 H), 4.13 (d, *J* = 16.8 Hz, 1 H), 5.11 (t, *J* = 21.0 Hz, 1 H), 7.21 (d, *J* = 3.6 Hz, 3 H), 7.26 (t, *J* = 24.3 Hz, 3 H). All data are consistent with literature values. [36]

§1.3.3 Synthetic procedure for SB-T-1214

10-Deacetyl-7-triethylsilylbaccatin III [3-I][11]

An aliquot of 10-DAB III (170 mg, 0.321 mmol) and 4.0 eq of imidazole (98 mg, 1.41 mmol) were dissolved in 3.5 mL of DMF (~ 50 mg/ml) and was cooled to 0 ^{o}C under inert conditions. To this mixture chlorotriethylsilane (0.17 mL, 0.74 mmol) was added dropwise. The mixture was stirred and allowed to warm to room temperature while being monitored by TLC. Upon completion the reaction was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate (3 x 10 mL). Washed the combined organic layers with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrate *in vacuo*. Purification was done *via* normal phase column chromatography on silica gel (HEX: EtOAc = 7:3) to yield **3-I** (193 mg, 0.295 mmol, 92%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.55 (m, 6 H), 0.93 (m, 9 H), 1.08 (s, 6 H), 1.73 (s, 3 H), 1.90 (m, 1 H), 2.08 (s, 3 H), 2.25 – 2.27 (m, 2 H), 2.28 (s, 3 H), 2.43 – 2.51 (m, 1 H), 3.95 (d, *J* = 6.9 Hz, 1 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.25 (s, 1 H), 4.31 (d, *J* = 8.4 Hz, 1 H), 4.38 – 4.43 (dd, J_1 = 10.8 Hz, J_2 = 8.4 Hz, 1 H), 4.85 – 4.87 (m, 1 H), 4.95 (d, *J* = 8.0 Hz, 1 H), 5.18 (s, 1 H), 5.59 (d, *J* = 6.8, 1 H), 7.47 (t, *J* = 7.6 Hz, 2 H), 7.60 (t, *J* = 7.6 Hz, 1 H), 8.09 (d, *J* = 7.2 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.2, 6.8, 10.0, 15.2, 19.5, 22.7, 26.7, 37.3, 38.6, 42.7, 47.0, 58.0, 68.0, 73.0, 74.7, 74.8, 78.8, 80.8, 84.3, 128.6, 129.4, 130.1, 133.6, 135.2, 141.8, 167.1, 170.8, 210.4. All data are consistent with literature values. [11]

10-Cyclopropanecarbonyl-10-deacetyl-7-triethylsilylbaccatin III [3-II][21]

An aliquot of **3-I** (745 mg, 1.130 mmol) was dissolved in THF (6.0 mL) and cooled to -40 ^{o}C under N_2 atmosphere. After 30 minutes at -40 ^{o}C, to the mixture 1.0 M LiHMDS in *tert*-butyl methyl ether (0.33 mL) was added dropwise and the resulting mixture was allowed to stirred for an additional 10 minutes, then to the mixture cyclopropanecarboxylic acid chloride (0.116 mL, 1.356 mmol) was added dropwise. The reaction was monitored *via* TLC. Upon completion, it was quenched with saturated aqueous NH_4Cl (2.0 mL). The resulting solution was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers was washed with brine (3 x 20 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was performed on normal phase column chromatography (HEX: EtOAc = 4:1) on silica gel to afford **3-II** (756 mg, 1.040 mmol, 93%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 0.56 (m, 6 H), 0.92 (m, 11 H), 1.05 (s, 3 H), 1.20 (s, 3 H), 1.68 (s, 3 H), 1.76 (m, 1 H), 1.97 (m, 1 H), 2.03 (m, 2 H), 2.19 (s, 3 H), 2.26 (s, 1 H), 2.28 (s, 3 H), 2.52 (m, 1 H), 3.88 (d, *J* = 7.1 Hz, 1 H), 4.13 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.47 (dd, *J* = 6.3, 10.5 Hz, 1 H), 4.85 (t, *J* = 8.0 Hz, 1 H), 4.95 (d, *J* = 9.0 Hz, 1 H), 5.64 (d, *J* = 6.8 Hz, 1 H), 6.46 (s, 1 H), 7.48 (t, *J* = 7.6 Hz, 2 H), 7.60 (t, *J* = 7.6 Hz, 1 H), 8.09 (t, *J* = 7.6 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.49, 6.98, 8.77, 8.90, 10.16, 13.24, 14.41, 15.13, 20.37, 21.26, 22.90, 27.06, 37.46, 38.46, 42.99, 47.49, 58.84, 60.62, 68.23, 72.56, 74.96, 75.76, 78.99, 81.11, 84.47, 128.80,

129.62, 130.32, 133.02, 133.83, 144.07, 167.34, 170.96, 173.40, 202.53. All data are consistent with literature values. [21]

2'-Triisopropylsilane-3'-dephenyl-10-(cyclopropanecarbonyl)-3'-(2-methyl-2-propenyl) docetaxel [3-III] [21]

An aliquot of **3-II** (198 mg, 0.271 mmol) and 1.2 eq of **(+) 1-VI** (132 mg, 0.324 mmol) were dissolved in 20 mL of THF and cooled to -40 ^{o}C. After 30 minutes, 1.0 M LiHMDS in *tert*-butyl methyl ether (0.42 mL) was added dropwise. The reaction was monitored *via* TLC. Upon completion it was quenched with saturated aqueous NH_4Cl (5 mL), extracted with CH_2Cl_2 (3 x 10 mL), the combined organic layers was washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification was done by performing normal phase column chromatography on silica gel (HEX: EtOAc = 1:1) to afford **3-III** (268 mg, 0.238 mmol, 88%) as a white solid: ^{1}H-NMR (300 MHz, $CDHCl_3$, ppm) 0.54 (m, 6 H), 0.91 (m, 11 H), 1.05 (s, 3 H), 1.19 (s, 3 H), 1.34 (s, 9 H), 1.62 (s, 3 H), 1.73 (m, 1 H), 1.75 (s, 3 H), 1.79 (s, 3 H), 1.92 (m, 1 H), 2.00 (m, 2 H), 2.26 (s, 1 H), 2.36 (s, 3 H), 2.45 (m, 1 H), 3.83 (d, *J* = 6.3 Hz, 1 H), 4.17 (d, *J* = 8.1 Hz, 1 H), 4.29 (d, *J* = 8.7 Hz, 1 H), 4.45 (d, *J* = 9.9 Hz, 1 H), 4.75 (t, *J* = 10.5 Hz, 1 H), 4.92 (d, *J* = 10.8 Hz, 1 H), 5.32 (d, *J* = 7.2 Hz, 1 H), 5.67 (d, *J* = 6.6 Hz, 1 H), 6.08 (d, *J* = 8.1 Hz, 1 H), 6.48 (s, 1 H), 7.43 (t, *J* = 15.6 Hz, 2 H), 7.58 (t, *J* = 14.4 Hz, 1 H), 8.09 (d, *J* = 7.2 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.52, 6.97, 8.85, 8.96, 10.30, 12.75, 13.17, 14.41, 14.53, 18.18, 18.25, 18.77, 21.26, 21.58, 22.78, 25.91, 26.51, 28.47, 35.76, 37.38, 43.46, 46.96, 58.54, 60.61, 72.12, 72.41, 75.01, 75.29, 75.49, 79.08, 81.29, 84.52, 122.38, 128.77, 129.67, 130.37, 133.60, 133.76, 141.09, 155.39, 167.08, 170.02, 171.38, 172.13, 173.29, 202.29. All data are consistent with literature values. [21]

SB-T-1214 (3'-Dephenyl-3'-(2-methyl-1-propenyl)-10-cyclopropanecarbonyldocetaxel) [3-IV] [21]

An aliquot of **3-III** (268 mg, 0.238 mmol) was dissolved in a 1:1 mixture of acetonitrile/pyridine (20 mL) and cooled to 0 ^{o}C under nitrogen atmosphere. To this mixture, excess HF (3.0 mL, 70% v/v in pyridine) was added dropwise. This mixture was allowed to stirred at room temperature overnight. The reaction was monitored *via* TLC. Upon completion, the reaction was quenched with 10% aqueous citric acid solution (2.0 mL) and neutralized with saturated aqueous $NaHCO_3$ (10 mL). The resulting solution was diluted and extracted with ethyl acetate (3 x 20 mL). The combined organic layer was washed with saturated aqueous cupric sulfate (2 x 30 mL) and then H_2O (30 mL), brine (3 x 30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done by normal phase column chromatography on silica gel (HEX: EtOAc = 1:1) to afford **SB-T-1214** (199mg, 0.233 mmol, yield 98%) as a white powder: m. p. 158 -160 ^{o}C (liter. 160 -161 ^{o}C); δ ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 0.97 (s, 2 H), 1.10 (m, 1 H), 1.15 (s, 3 H), 1.23 (s, 3 H), 1.35 (s, 9 H), 1.67 (s, 3 H), 1.74 – 1.79 (m, 10 H), 1.89 (s, 3 H), 2.03 (s, 3 H), 2.35 (s, 4 H), 2.53 (m, 1 H), 2.61 (d, *J* = 3.9, 1 H), 3.42 (d, *J* = 5.7 Hz, 1 H), 3.80 (d, *J* = 8.1 Hz, 1 H), 4.21 (m, 1 H), 4.29 (d, *J* = 8.1 Hz, 1 H), 4.41 (m, 1 H), 4.74 (t, *J* = 8.7 Hz, 1 H), 4.83 (m, 1 H), 4.94 (d, *J* = 8.1 Hz, 1 H), 5.30 (d, *J* = 8.4 Hz, 1 H), 5.65 (d, *J* = 7.2 Hz, 1 H), 6.14 (t, *J* = 13.5 Hz, 1 H), 6.30 (s, 1 H), 7.47 (t, *J* = 15.6 Hz, 2 H), 7.60 (t, *J* = 14.7 Hz, 1 H), 8.08 (d, *J* = 7.2 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 9.41, 9.66, 9.74, 13.25, 14.34, 14.42, 15.22, 18.80, 21.28, 22.18, 22.63, 25.95, 26.93, 28.45, 35.73, 35.82, 43.42, 45.86, 51.82, 58.82, 60.62, 72.46, 72.60, 73.98, 75.30, 75.66, 79.43, 80.21, 81.30, 84.69, 120.85, 128.87, 129.45, 130.38, 133.15, 133.91,

138.18, 142.90, 155.67, 167.19, 170.30, 175.37, 204.17. All data are consistent with literature values.[21]

Chapter 2

Synthesis of Tumor-Targeting Taxane-Based Drug Conjugates using Biotin as Tumor-Targeting Module

Chapter Contents

§2.1 Targeted chemotherapy

One of major problems of traditional chemotherapy is that most of the anticancer drugs lack of specificity.[4] Traditional chemotherapy is based on the assumption that the rate of cellular uptake is enhanced by the fast growth rate of cancer cells, making them more susceptible to these antineoplastic drugs. Unfortunately, both malignant and normal cells are often destroyed, resulting in various side effects such as hair loss, nausea and vomiting, diarrhea or constipation, anemia, and depression of the immune system.[4]

Although combined administration of efflux pump inhibitors of P-gp such as reversal agents could significantly reduce the efflux problems caused by MDR, this type of strategy has not advanced for treating patients. How to distribute the drugs to tumor selectively is the key to make drugs more powerful and effective.[40] To reduce these problems associated with chemotherapeutics, extensive effort has been dedicated to the development of drug delivery systems. Targeted cancer therapy is one of the possible solutions.

§2.1.1 Introduction to targeted chemotherapeutics

Targeted chemotherapy is aiming at specifically affecting cells, attack the cancer cells' inner working by interfering with cell growth signals, disrupting tumor angiogenesis, and inducing apoptosis of cancer cells. For example, Imatinib (Gleevec®) and Erlotinib hydrochloride (Tarceva®) (**Figure 2-1**), which were approved by the United States FDA in 2001 and 2004 respectively, are the drugs work against cancer cells by inhibiting certain tyrosine kinase enzymes, which are critical in a number of cell processes such as cellular differentiation and division.[41] Tyrosine kinases become one of the good targets of anticancer drugs because of their high overexpression.[42]

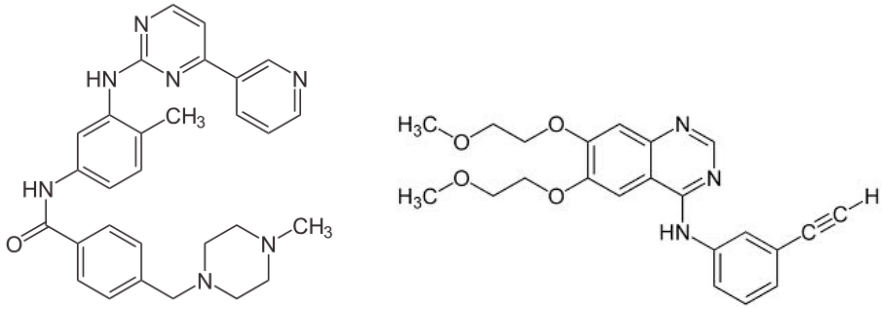


Figure 2-1: Structures of imatinib (left) and erlotinib (right)

Imatinib is marketed by Novartis, approved for treating chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs). As a 2-phenylaminopyrimidine derivative, imatinib is selective for the domain of Abelson proto-oncogene (abl) of tyrosine kinase enzymes. However, resistance has been frequently reported during recent years. Mutations of the kinase domain are able to reduce the affinity for drug binding due to conformational changes of the protein.[41]

Erlotinib, first commercialized by OSI Pharmaceuticals Inc., now has been approved for the treatment of non-small cell lung cancer and pancreatic cancer. It targets the tyrosine kinase of epidermal growth factor receptor (EGFR) by binding the ATP binding site reversibly.[41] To transmit signal, homodimers of EGFR molecules are necessary for

transphosphating on tyrosine residues with the help of ATP. The competitive inhibition of ATP receptor prevents the formation of phosphotyrosine in EGFR and calls off the initiation of signal cascade.[41] Iressa (geftinib) is the first anticancer drug of this type.

Seemingly effective and target specific as the compounds are, cellular resistances have been developed correspondingly (**Figure 2-2**).[42] The signal transduction pathways of human cells are fairly complex and there are always some rescue ways to transmit and amplify the messages. Unless a drug, or a combination therapy of drugs can eradicate all possible scenarios, resistance is almost inevitable.

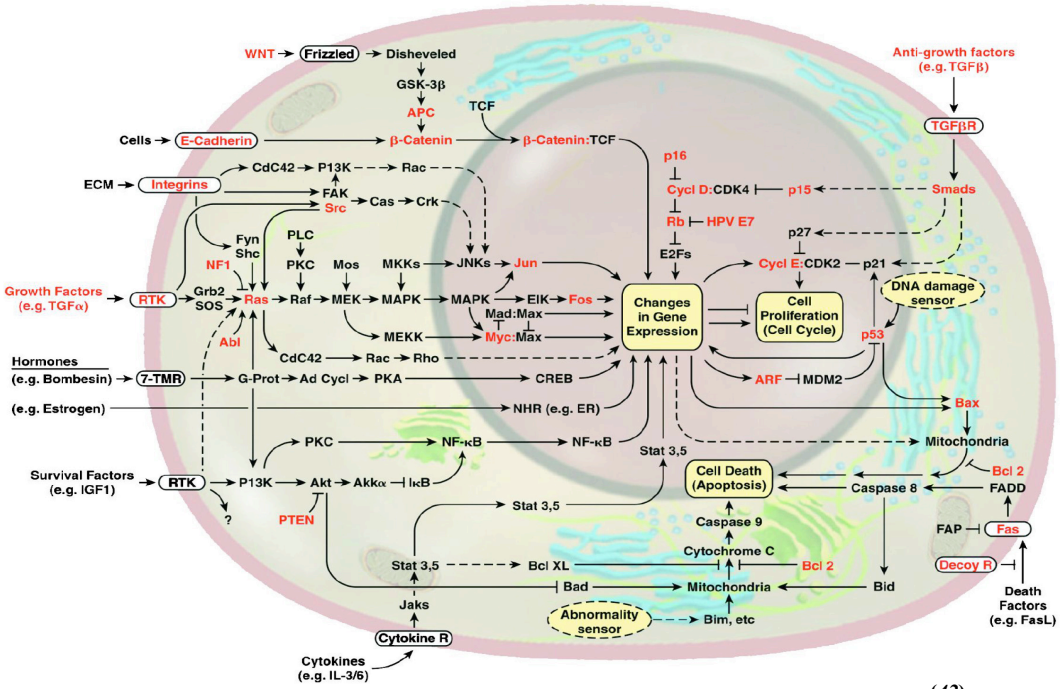


Figure 2-2: Brief summary of the cellular signal transduction[42]

Like imatinib and other ATP competitive inhibitors of tyrosine kinase, resistance rapidly occurs on the patients towards erlotinib, generally after 8-12 months of treatment.[41] More than half of the resistance is resulted from the mutation of ATP binding site by replacing a small polar threonine with a larger nonpolar methionine residue. Thus, the binding of erlotinib is prevented by steric hindrance.

It is clear that more effective drug delivery strategies must be explored to overcome these problems of resistance. Among which tumor-targeted drug delivery systems were extensively studied during recent years.

§2.1.2 Tumor-targeting drug delivery

Tumor-targeting drug conjugate, which consists of a Tumor Targeting Module (TTM) and a cytotoxic agent connected directly or through a suitable cleavable linker, is another way to specifically deliver chemotherapeutic agents. Taking advantages of the fundamental differences between cancer and normal cells that the tumor cells are more greedily requiring certain kinds of vitamins and nutrients to sustain their much faster limitless growth. Monoclonal antibodies, peptides, polyunsaturated fatty acids, and vitamins are often used as the tumor-targeting modules. In this way, the pro-drug complex can be kept inactive in the

bloodstream circulation and activated by chemical ligation after internalization (**Figure 2-3**).[4]

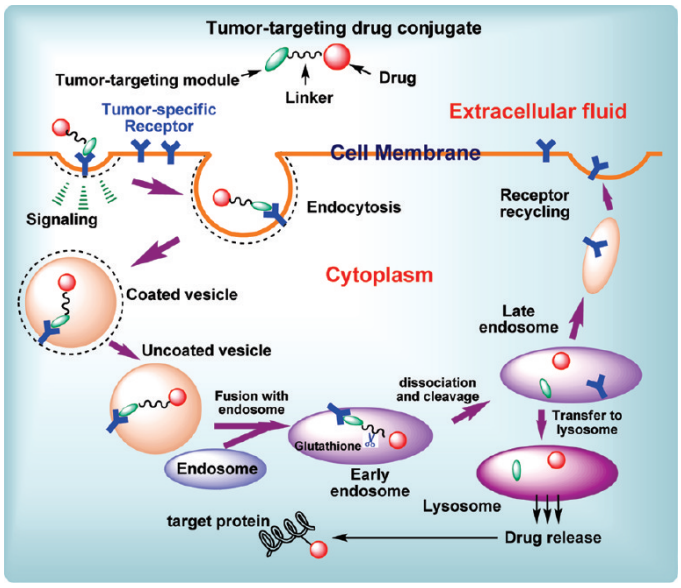


Figure 2-3: Receptor mediated endocytosis of TTM-linker-drug bioconjugate[4]

§2.1.2.1 Monoclonal antibody as tumor-targeting module

Because of the specificity towards antigens, monoclonal antibodies were used as targeting agents decades ago for drug design. For example, BR96-Doxorubicin was widely tested for clinical trials in 1990's;[43] HuP67.6-calicheamicin, Mab-MMAE conjugate have been approved by FDA in 2000 and 2011 respectively; and Herceptin-DM1 conjugate is being tested in phase III clinical trials (**Figure 2-4**).[44]

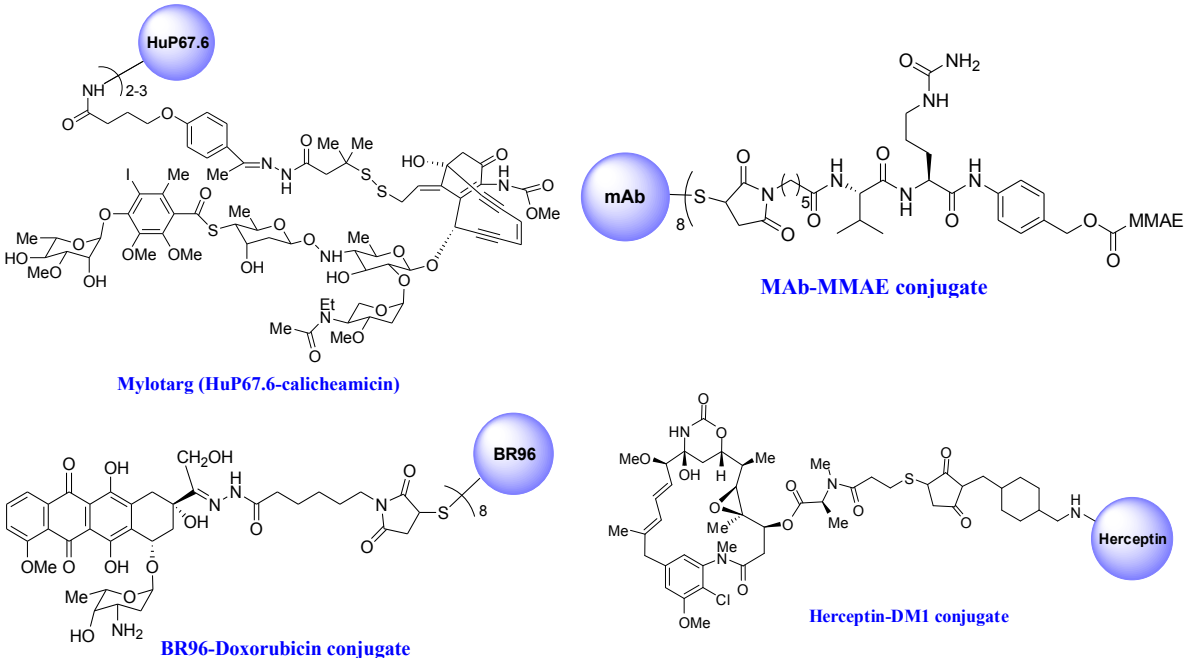


Figure 2-4: Examples of mAb-drug conjugate[44]

HuP67.6-calicheamicin, marketed by Wyeth as Mylotarg, is an antibody drug conjugate that was used for acute myelogenous leukemia before 2010. It was forced to be withdrawn from the market due to the fact that treatment with this drug increased death and lack of advantages over conventional chemotherapies.[45] Mab-MMAE conjugate, developed by Seattle Genetics with a trade name Adcetris, was granted accelerated approval for relapsed Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL). A year later it was also authorized by European Medicines Agency. The antibody is directed to the protein CD30 that is often expressed in classical HL and sALCL.[44]

§2.1.2.2 Peptides as tumor-targeting module

A number of peptides have their own receptors, which make them possibly be used as TTMs in drug design. For example, somatostatin, a hypothalamic peptide, and its analogs, which can inhibit the secretion of growth hormone, insulin and so on besides capable of playing a role of neural transmission, have their special receptors that can be used as targets for drug delivery (**Figure 2-5**). [46]

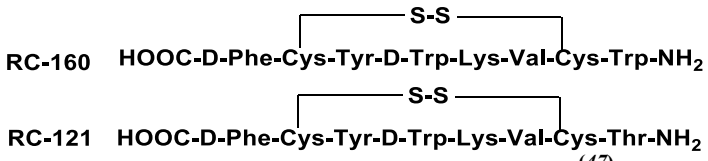


Figure 2-5: Somatostatin analogues[47]

Peptides have been used as TTM for taxane delivery for decades. Paclitaxel poliglumex (**Figure 2-6**) is one of the examples. It is an investigational conjugate by coupling paclitaxel with a biodegradable polyglutamate polymer. The activity of paclitaxel can be masked by the peptide, which can help paclitaxel to enter and accumulate in tumor cells by taking advantage of the porous abnormal blood vessels within tumor tissue. Once internalized, the paclitaxel can be released by biological metabolism. Currently, Paclitaxel poliglumex is under phase III trial for ovarian cancer, phase I/II for head and neck cancer.[48]

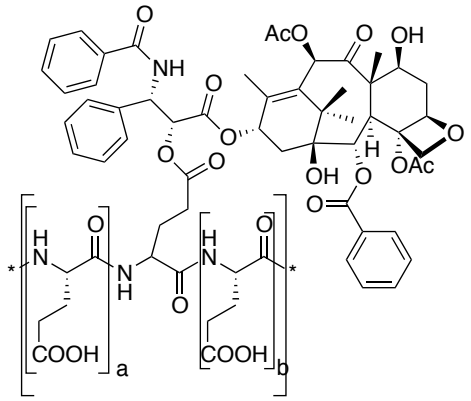


Figure 2-6: Paclitaxel poliglumex [49]

A recent study reported that after paclitaxel was conjugated to oligoarginine with a disulfide linker at C-2' or C-7. This known drug could overcome the efflux-based MDR of human ovarian carcinoma. The systemic cytotoxicity caused by this conjugate was no more than that of paclitaxel alone (**Figure 2-7**).[15]

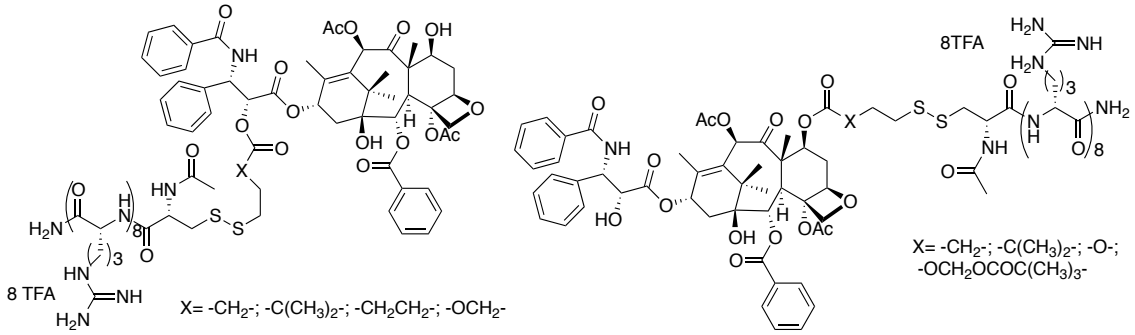


Figure 2-7: C-2 and C-7 conjugated paclitaxel polyarginine (Adapted from ref. (*15*))

§2.1.2.3 PUFA as tumor-targeting module

Some biologically derived acids such as Polyunsaturated fatty acid (PUFAs), hyaluronic acid and so on can be used as TTMs. PUFAs, particularly omega-3 fatty acids, are much easier to be taken by cancer cells as biochemical precursors and had been proved active against a number of cancer cell lines.[50-52] Linolenic acid (LNA) can be derived from vegetable oil and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are abundant in cold-water fish (**Figure 2-8**). They cannot be synthesized by human body but are beneficial for human health, vital for normal metabolism, and needed as the energy source and building material of cells.[53]

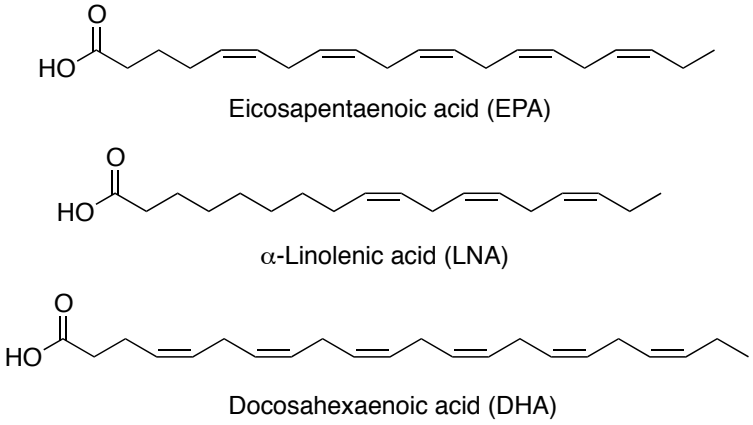


Figure 2-8: Structures of three omega-3 fatty acids

Omega-3 fatty acids are well known for its benefits for atherosclerosis, arthritis, neurological disorders, besides repressing some cancers. To make paclitaxel more water soluble and tumor-specific, it was conjugated to DHA. DHA-paclitaxel or Taxoprexin, which was developed by Protarga Inc., currently in phase III clinical trials, is not effective against MDR cancer cell lines.[54] To overcome the resistance problem, the Ojima group has designed and synthesized DHA-SB-T-1214 by replacing paclitaxel with SB-T-1214 (**Figure 2-9**). [54]

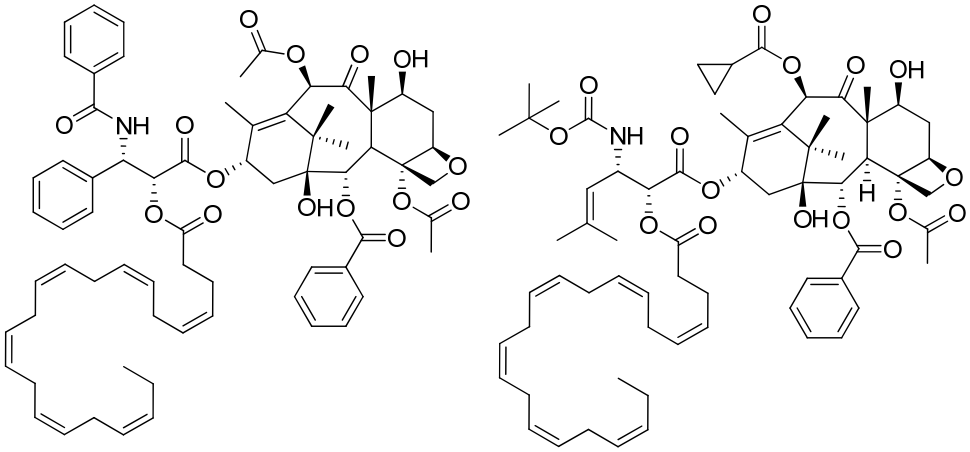


Figure 2-9: Structures of DHA-paclitaxel and DHA-SB-T-1214 conjugates[54]

Because DHA is readily taken up by cancer cell, the conjugates were shown with improved efficacy and reduced systemic toxicity. The stability of conjugates during circulation elongated the time of function and reduced unexpected side effects to normal tissues. In a series of systematic study, the improved efficacy was confirmed by the great changes of drug's pharmacokinetics and distribution.[55] There are two popular hypothesized mechanisms indicating how the conjugate is delivered to tumor.

One possibility is a process of gp-60 mediated transcytolysis from blood capillary to tumor interstitium (**Figure 2-10**).[55-57] The DHA moiety of the conjugate easily binds to human serum albumin (HSA), the most abundant protein of blood plasma. The hydrophobic drug conjugate is stabilized by HSA binding and the whole complex can be recognized by glycoprotein 60 (gp-60). Once the DHA conjugate is transported and accumulated in the tumor interstitium, it will be released from HSA with the function of secreted protein acidic and rich in cysteine (SPARC). The DHA conjugate is assumed to be absorbed on the surface of cancer cell and the free active drug is finally released. It is also assumed that the drug efficacy is to be enhanced by the involvement of DHA and its metabolite (**Figure 2-10**). [55-57]

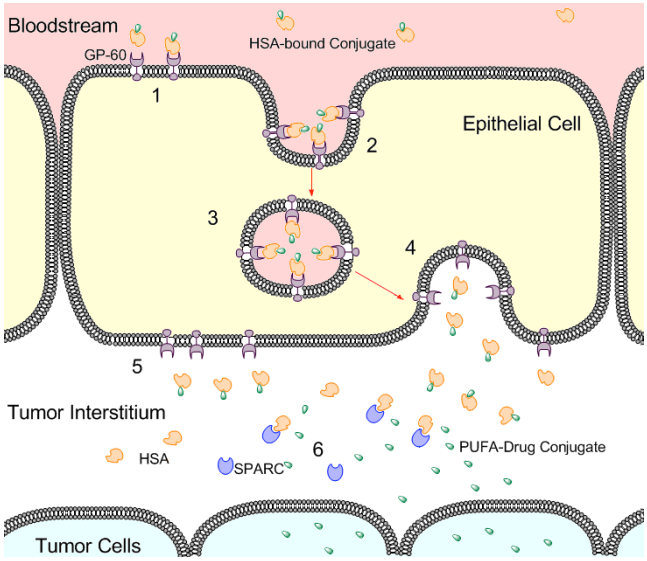


Figure 2-10: Gp60-mediated internalization of PUFA-drug conjugate[55]

Another hypothesized mechanism of DHA conjugate internalization and accumulation takes advantages of the abnormal blood vessels, which are developed through a process of angiogenesis induced by tumor.[58] These blood vessels have irregular shape, holes, pervascular detachment and vessel dilation. A possible scenario is the PUFA-drug conjugate can travel and fall through the holes of the tumor vessel, where it can be accumulated and internalized by the cancer cell. [58]

The efficacy of DHA-SB-T-1214 was evaluated and compared with taxoprexin by Ojima group, particularly tested against MDR cancer cell lines such as DLD-1 human colon tumor xenograph in mice. Although ineffective as shown by taxoprexin, complete regression was achieved by DHA-SB-T-1214 (**Figure 2-11**). [59]

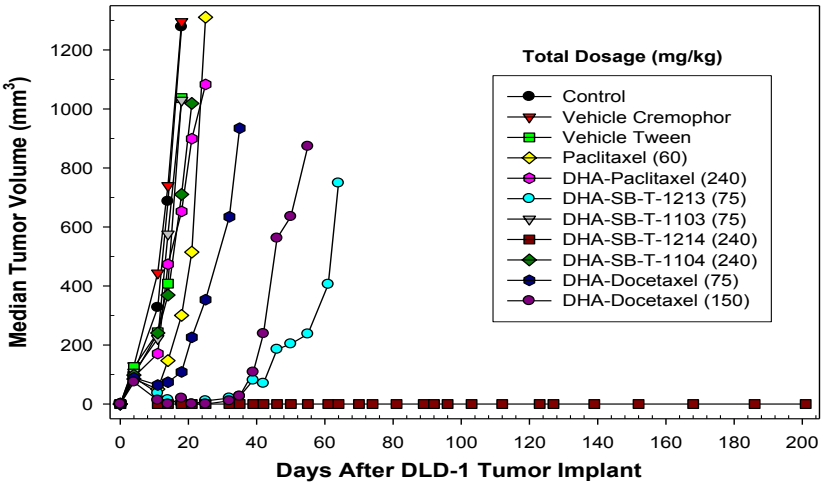


Figure 2-11: Evaluation of anticancer activity of DHA-SB-T-1214[59]

To improve the stability and ensure rapid release of drug after internalization, PUFA-SB-T-1214 conjugate was inserted a self-immolative disulfide linker. LNA-NHNH-Linker-Drug was designed, synthesized and evaluated (**Figure 2-12**).[4, 40] Among classes of linkers, disulfide linker has advantages. It is stable at physiological pH but could be cleaved in cancer cell because the concentration of glutathione is around 1000 times in cancer cells (millimolar range) compared to in blood plasma (micromolar range). The high level of glutathione enables the cancer cells capable of triggering the cleavage of disulfide bond and the release the drug in its original form (**Figure 2-12**). Thus the potential side effects could be greatly reduced and the drugs could be released in time to guarantee its effectiveness.[4]

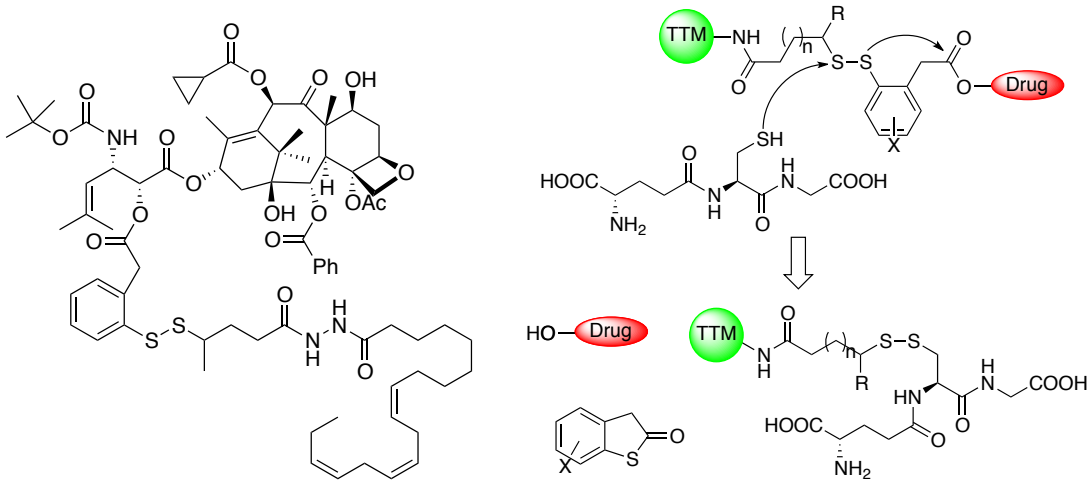


Figure 2-12: LNA-NHNH-Linker-Drug, glutathione-triggered self-immolative disulfide linker cleavage and drug release *(3, 4)*

The methyl group alpha to the disulfide bond might be able to stabilize the conjugate by preventing premature cleavage through steric hindrance. This drug-release mechanism was already validated by a time-dependent ^{19}F NMR experiment of model disulfide linked compound that was previously labeled by ^{19}F. *(40)*

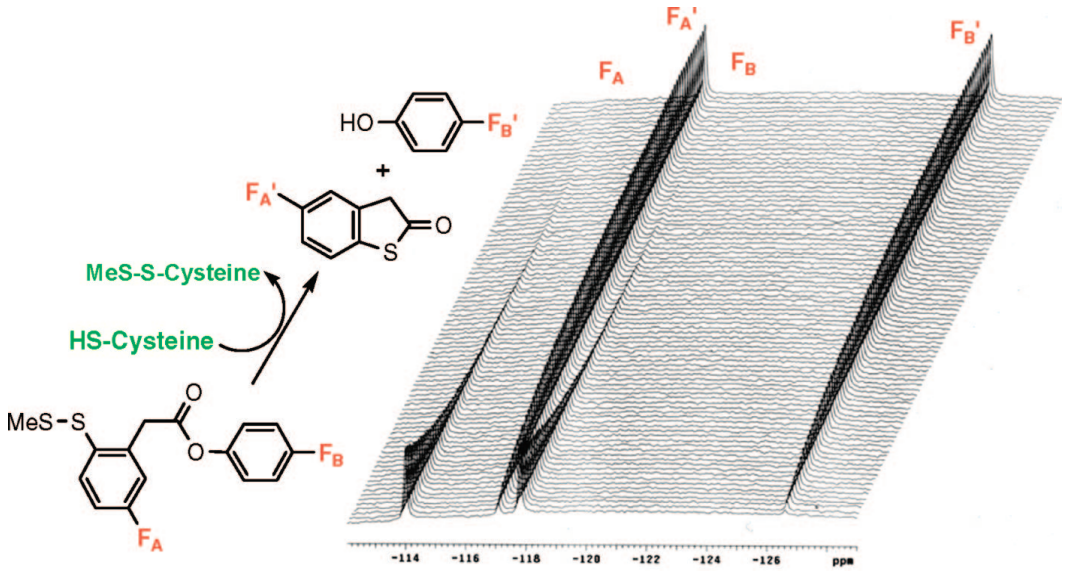


Figure 2-13: A model system showing ester bond cleavage by ^{19}F NMR *(40)*

§2.1.2.4 Vitamins as tumor-targeting module

Vitamins are essential to all living cells and tumor cells are particularly in need of that to maintain their fast growth and rapid proliferation. Therefore, their receptors are highly overexpressed on the cellular surface to ensure more vitamins can be absorbed. Among which folate and biotin receptors are particularly overexpressed in a number of cancer cell lines (**Table 2-1**).*(60)* These receptors are often used as favorable targets for biomarker as well as tumor-targeting drug delivery.

Table 2-1: Overexpressed folate and biotin receptors in various cancer cell lines (adapted from ref. (*60*))

Tumor	Mouse	Type	Folate	Biotin
O157	Balb/C	Bcell lymph	+/-	+/-
BW5147	AKR/J	Lymphoma	+/-	+/-
HCT-116	Balb/C-Nu	Colon	-	-
L1210	DBA/2	Leukemia	+/-	-
L1210FR	DBA/2	Leukemia	++	+++
Ov 2008	Balb/C-Nu	Ovarian	+++	++
ID-8	C57/B1	Ovarian	+++	++
Ovcar-3		Ovarian	+++	++
CT-26	Balb/C	Colon	+/-	+++
P815	DBA/2	Mastocytoma	+/-	+++
M109	Balb/C	Lung	+	+++
RENCA	Balb/C	Renal Cell	+	+++
RD995	C3H/HeJ	Renal Cell	+	+++
4T1	Balb/C	Breast	+	+++
JC	Balb/C	Breast	+	+++
MMT060562	Balb/C	Breast	+	+++

Folic acid (vitamin B_9), is very important for DNA synthesis and methylation, RNA synthesis as well as cell division and proliferation. Its receptors are highly expressed in ovarian, lung, kidney, endometrial, breast, brain and colon carcinomas and generally internalized *via* RME through proton-coupled folate transporters.[*(61)*] Moreover, folate-drug conjugates have high affinity of binding towards receptors, up to $K_d \sim 10^{-10}$ M. One of the most successful folate-drug conjugate designs is vintafolide, currently in phase III clinical trial for platinum resistant ovarian cancer and phase II for non-small cell lung cancer. The conjugate also exhibited synergy with doxorubicin conjugate. On 16 April 2012, the right of its development and commercialization was purchased by Merck from Endocyte with a price of up to $1 billion USD (**Figure 2-14**).[*(62)*]

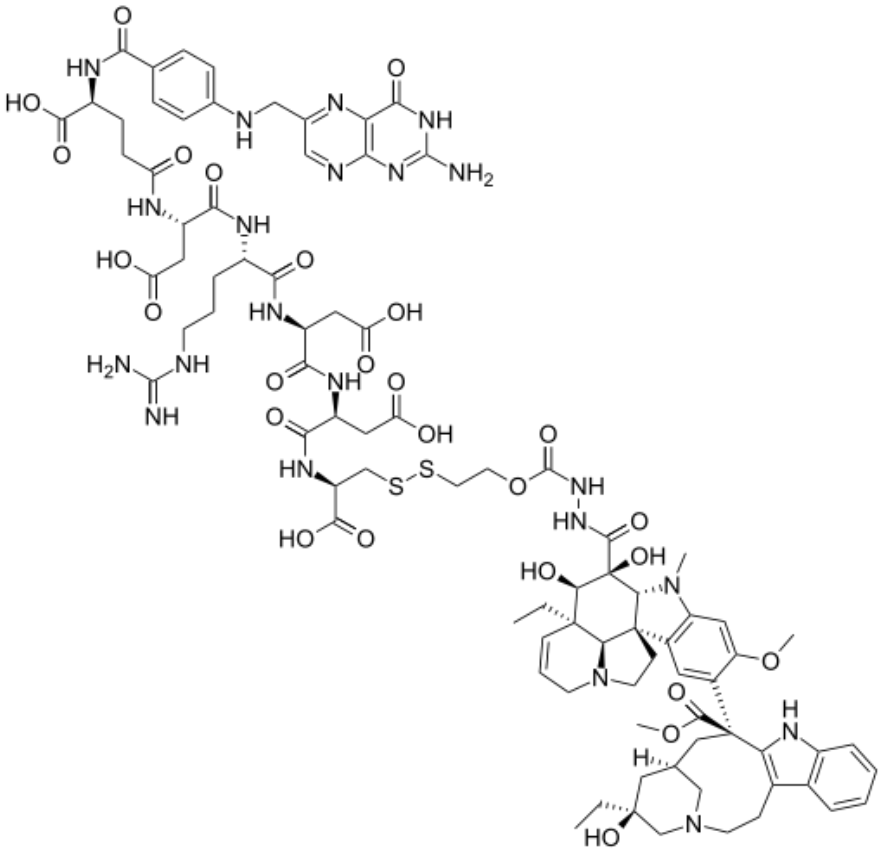


Figure 2-14: Structure of vintafolide[62]

Biotin also cannot be self-supplied by human. It must be obtained through intestinal absorption. Although similarly greedily needed by fast dividing cancer cells, the receptor of biotin was not systematically studied until recently. Actually they are even more overexpressed in a number of cancer cell lines and have been focused for tumor-targeting study.[60] The Ojima lab have designed and synthesized a series of conjugates based on biotin directed drug delivery system like biotin-PEG-Me-linker-SB-T-1214 conjugate, biotin-PEG-camptothecin drug conjugate, and the dual warheads delivery system (**Figure 2-15**).[24, 36]

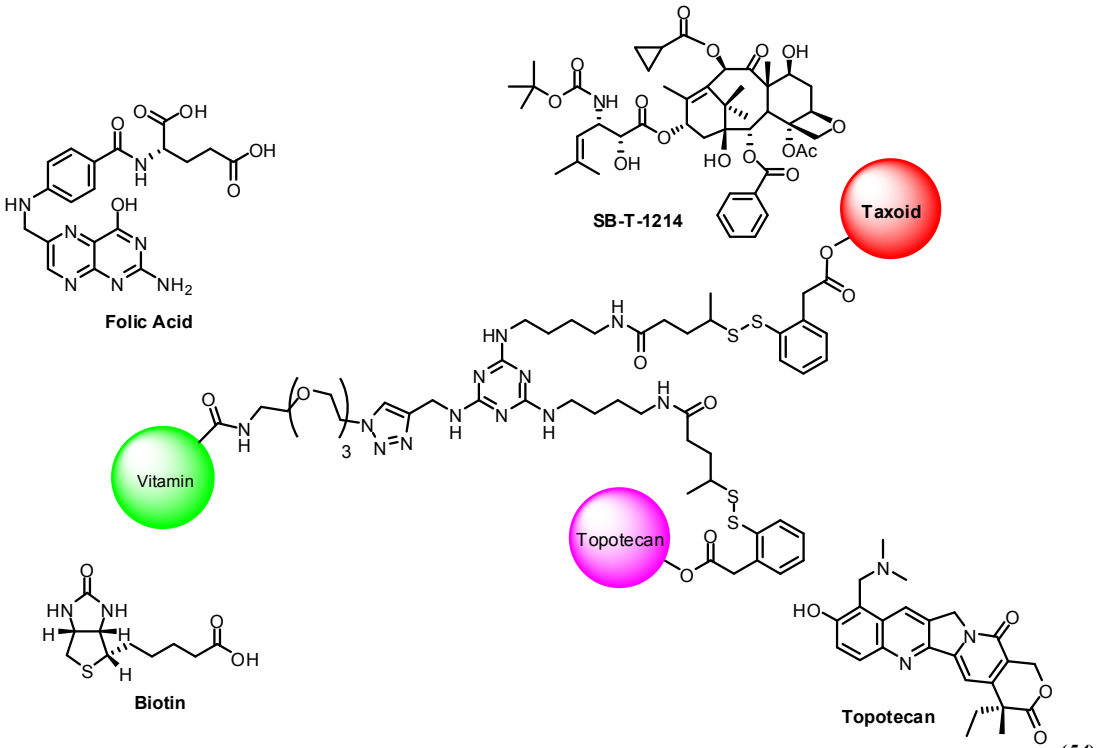


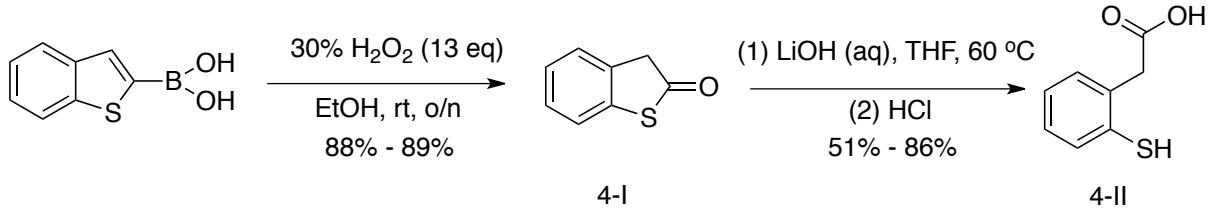
Figure 2-15: Structure of folate/biotin receptor directed dual-warhead conjugate[54]

In the long run, the conjugates with Polyamido amine (PAMAM) dendrimers, carbon nanotubes and so on are promising projects for the further developing of drug delivery system.[24, 63]

§2.2 Synthesis of biotin-methyl branched disulfide linker-SB-T-1214 conjugate

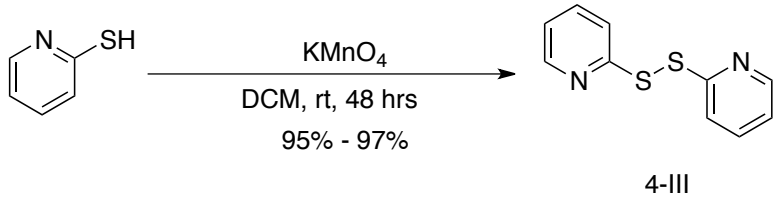
§2.2.1 Synthesis of Disulfide Linker

The synthesis of the disulfide linker was accomplished by synthesizing two fragments, upper and lower linker moieties. Oxidation of benzo-thiophen-2-ylboronic acid afforded the thiolactone, which was subsequently hydrolyzed with LiOH to open the ring to afford the upper moiety, **4-II** (**Scheme 2-1**). [64, 65] The compound was reserved under inert atmosphere and kept in refrigerator to prevent the formation of dimer.



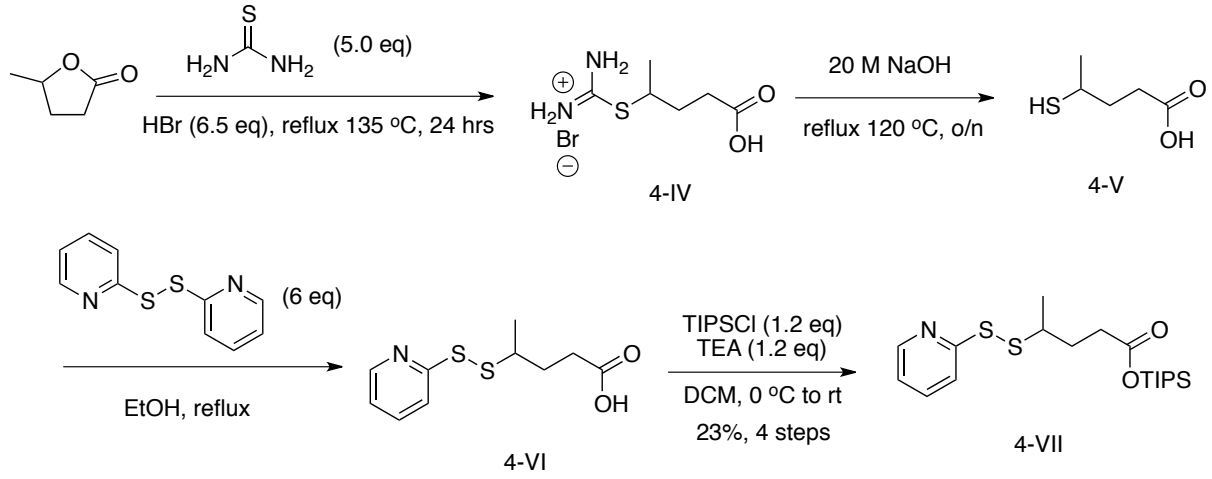
Scheme 2-1: Synthesis of (2-mercapto-phenyl) acetic acid[64, 65]

The synthesis of the lower moiety needs 4-III, which was obtained by oxidizing 2-pyridinethiol with approximately 3-4 equivalents of $KMnO_4$ in CH_2Cl_2, for the first disulfide bond exchange reaction (**Scheme 2-2**). [66, 67]



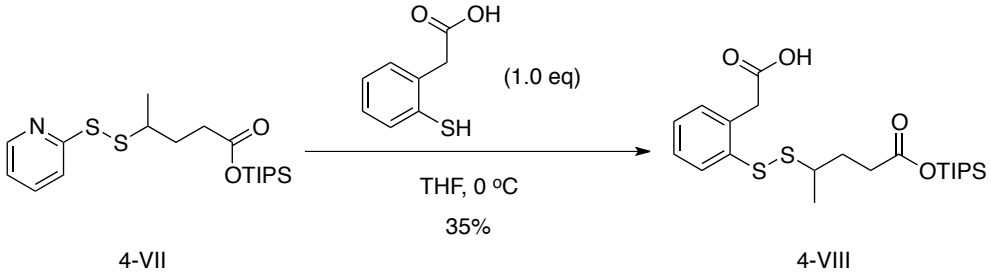
Scheme 2-2: Synthesis of 1,2-di(pyridine-2-yl)disulfane[66, 67]

The synthesis of the lower moiety initiated with a substitution reaction of γ-valerolactone. Here thiourea was used as a nucleophile to afford thiouronium bromide salt. These salts were then subsequently treated with aqueous NaOH to give the hydrolyzed product **4-V**.[68] The first disulfide exchange was performed with **4-III** and **4-V** under reflux condition. The 4-VI was protected with TIPS in the subsequent reaction to give the lower linker moiety (**Scheme 2-3**).[24]



Scheme 2-3: Synthesis of the lower linker moiety[24, 68]

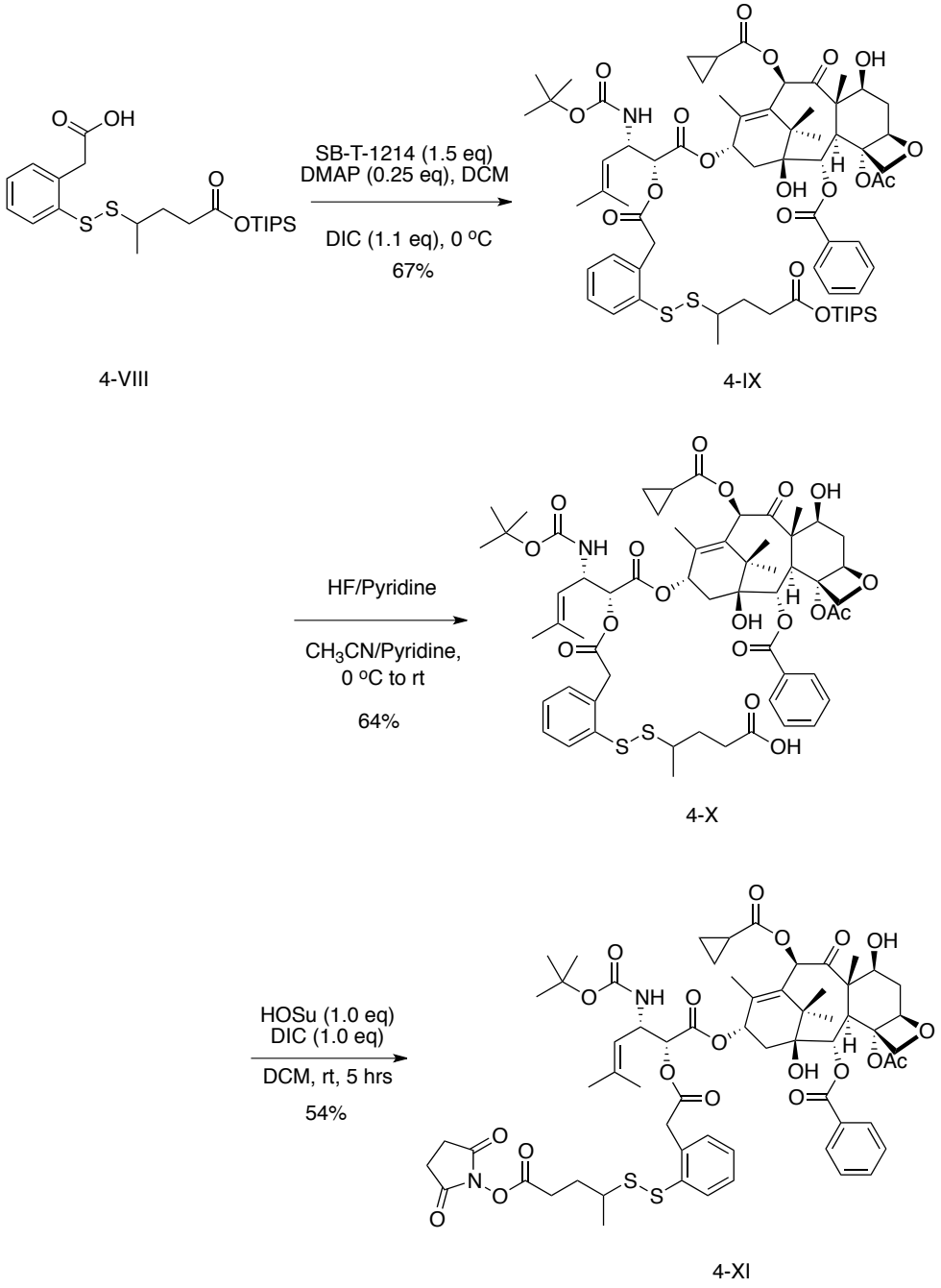
Finally the desired disulfide linker was afforded by the second disulfide exchange reaction of upper and lower linker moieties (**Scheme 2-4**). [4, 24]



Scheme 2-4: Synthesis of the final linker[4, 24]

§2.2.2 Synthesis of SB-T-1214-methyl branched disulfide-Linker-OSu[4, 24]

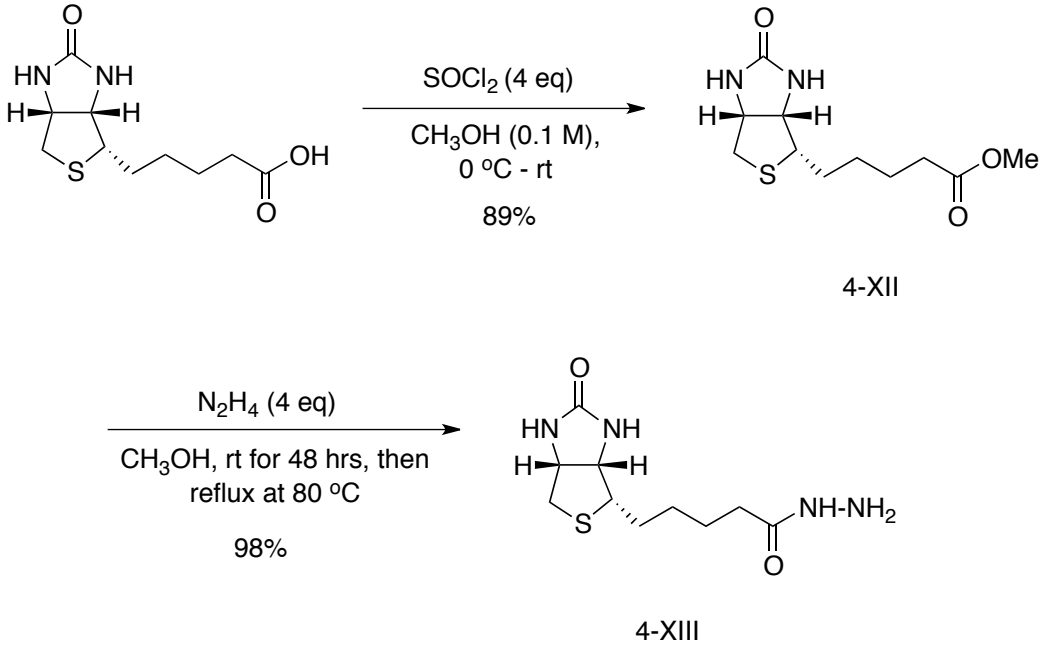
Due to the instability of TIPS group in the presence of carboxylic acid, the final linker was reacted immediately with SB-T-1214 by a diisopropyldiimide (DIC) coupling to afford TIPS protected drug-linker **4-IX**, in which the TIPS group was deprotected subsequently and activated by N-hydroxysuccinimide (HOSu) to afford the coupling-ready construct (**Scheme 2-5**).



Scheme 2-5: SB-T-1214- methyl branched disulfide-Linker-OSu[4, 24]

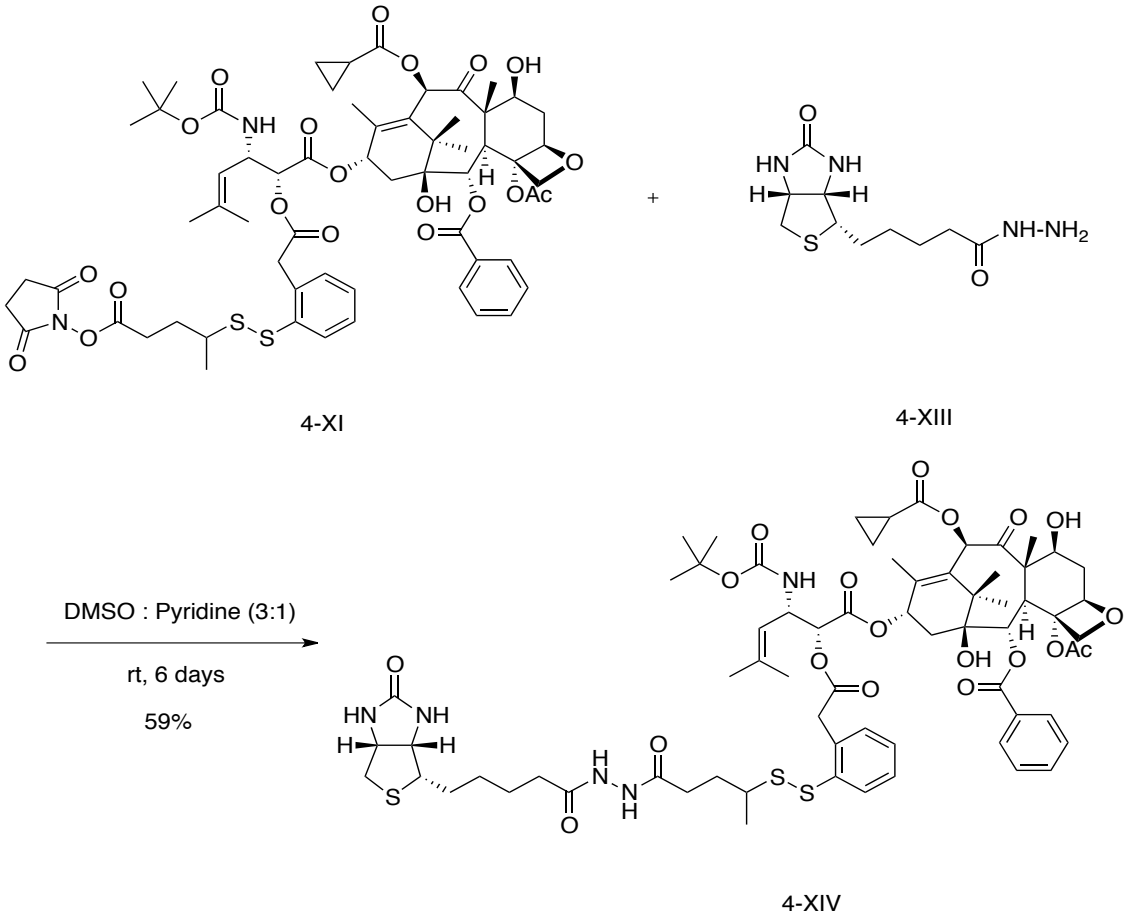
§2.2.3 Synthesis of Biotin-methyl branched disulfide linker-SB-T-1214 drug conjugate

To conjugate biotin with SB-T-1214-Me-linker- OSu (4-XI), the carboxylic acid moiety of biotin was converted to a methyl ester, to which was introduced an amine group by reacting with hydrazine in the subsequent step to afford biotin-hydrazide with a decent yield (Scheme 2-6).[4]



Scheme 2-6: Synthesis of biotin hydrazide[4]

Then **4-VIII** reacted with the previously HOSu-activated drug-linker to afford the final conjugate (**Scheme 2-7**).



Scheme 2-7: Synthesis of Biotin-methyl branched disulfide-linker-SB-T-1214 drug conjugate[4, 24]

§2.2.4 Biological evaluation

Biotin-Me-linker-SB-T-1214 drug conjugate has been tested and evaluated in various cancer cell lines by Edison Zuniga in the Ojima Lab.[24] According to the data presented in **Table 2-2**, it showed good efficacy against CT-26 and excellent activity in MX-1 after 48 and 72 hours incubation, indicating that both cancer cells not only can internalize the conjugate *via* receptor-mediated endocytosis (RME) successfully, but also can cleave the linker and release the active form of drug as anticipated. Generally, the result suggest that MX-1 cells overexpress biotin receptors on their surface more than they are expressed on CT-26 cells, therefore the cytotoxic activity of biotin-linker-SB-T-1214 is much higher in MX-1 compared to the activity observed CT-26 cells. [24]

Table 2-2: Biological activity of biotin-Me-linker-SB-T-1214 against two different cancer cell lines (Adapted from ref. (*24*))

Taxane	Time of Incubation with drug (hr)	IC_{50} (nM)	
		CT-26	MX-1
Paclitaxel[a]	48	>5000	0.54 ± 0.19
SB-T-1214[a]	48	0.41 ± 0.15	0.38 ± 0.17
biotin-linker-SB-T-1214[a]	48	16.4 ± 5.1	0.33 ± 0.04
biotin-linker-SB-T-1214[b]	72	4.92 ± 0.25	0.27 ± 0.03

[a] Cells were incubated for 48 hours at 37 °C with 5% CO_2 after administration of taxoid; [b] Cells were incubated for 72 hours at 37 °C with 5% CO_2 after administration of taxoid

- Cells were suspended in RPMI-1640 Medium 10 x (Sigma Aldrich, R1145) supplemented with tissue culture grade water, 0.3 g/L L-glutamine, 2.0 g/L sodium bicarbonate, 10% FBS, and 1% Penn Strep before administration of taxoid
- The optical density was determined using Acsent Multiskan optical density reader
- The reported values are a calculated averages of IC_{50} values determined from three individual experiments
- IC_{50} values were calculated using SigmaPlot v 10.0
- All IC_{50} values are report in nM scale unless otherwise noted

§2.3 Experimental procedure

§2.3.1 General Methods and Materials

^{1}H NMR was taken on a Varian 300, 400 or 500 MHz NMR spectrometer of the chemistry department. The melting points were carried out on a "Uni-melt" capillary melting point apparatus from Arthur H. Mass to charge values were measured by flow injection analysis on an Agilent Technologies LC/MSD VL. Thomas Company, Inc. TLC analysis was accomplished on Merck DC-alufolien with Kieselgel 60F-254 and visualized by UV light and stained with sulfuric acid-EtOH, 10 % Vanillin-EtOH with 1% sulfuric acid or DAKA. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM).

The chemicals were purchased from Sigma Aldrich Company, Fischer Company or Acros Organic Company. 10-DAB III was donated by Indena, SpA, Italy. Dichloromethane and methanol were dried before use by distillation over calcium hydride under nitrogen. DCM and THF were dried before use by distillation over sodium-benzophenone kept under nitrogen. Dry DMF was purchased from EMD chemical company, and used without further purification. Reaction flasks were dried in 100 ^oC ovens and allowed to cool to room temperature in a desiccator over "*Drierite*" (calcium sulfate) and assembled under an inert nitrogen gas atmosphere.

§2.3.2 Synthesis of Disulfide Linker

Benzothiophen-2-one [4-I][64, 65]

To a solution of thianaphthene-2-boronic acid (1.078 g, 6.06 mmol) in 12.0 mL of EtOH [~ 0.2 M], which was previously cooled to 0 ^{o}C for more than 10 minutes was added 30% H_2O_2 (8.0 mL) dropwise. The mixture was stirred and allowed to warm up to room temperature. The reaction was monitored *via* TLC. Upon completion, the mixture was diluted with water and extracted with aliquots of CH_2Cl_2 (3 x 15 mL). The combined organic layers was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was performed by normal phase column chromatography on silica gel (HEX: EtOAc = 3:1) to afford **4-I** (812 mg, 5.41 mmol, 89%) as a light yellow solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 3.99 (s, 2 H), 7.23-7.35 (m, 4 H). All data are consistent with literature values. [64, 65]

(2-Mercaptophenyl)acetic acid (4-II) [64, 65]

To a solution of **4-I** (0.812 g, 5.41 mmol) in 27.1 mL of THF [~ 0.2 M] being refluxed at 60 ^{o}C was added 6 eq of LiOH (1.375 g, 32.5 mmol) that previously dissolved in 28 mL of distilled water dropwise. The cloudy mixture was stirred overnight at 60 ^{o}C and monitored *via* TLC. Upon completion the resulting solution was cooled down to room temperature and diluted with a mixture of water (35 mL) and diethyl ether (20 mL). The mixture was chilled in an ice bath and its pH was then adjusted to 2 with 1 M HCl. The resulting organic layer was extracted with CH_2Cl_2 (3 x 20 mL), washed with brine (3 x 15 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Purification was performed by normal phase column chromatography on silica gel (HEX: EtOAc = 1:1) to afford **4-II** (0.789g, 4.67 mmol, 86%) as a yellow solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 3.50 (s, 1 H), 3.82 (s, 2 H), 7.17-7.26 (m, 3 H), 7.39-7.42 (m, 1 H). All data are consistent with literature values. [64, 65]

1,2-Di(pyridin-2-yl)disulfane (4-III)[66, 67]

An aliquot of 2-pyridinethiol (3.815 g, 34.3 mmol) was dissolved in 35 mL CH_2Cl_2 [0.1 M], then to this solution was added 3 eq of $KMnO_4$ (17.852 g, 113 mmol) and the reaction was stirred vigorously. Subsequently, the resulting solution was filtered over celite and concentrated *in vacuo* to afford **4-III** (3.657 g, 16.6 mmol) as a pale yellow crystal: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 7.09 – 7.13 (m, 2 H), 7.58 – 7.64 (m, 4 H), 8.46 (d, J = 4.6 Hz, 2 H). All data are consistent with literature values. [66, 67]

2-(5-Methoxy-5-oxooentan-2-yl)isothiouronium bromide (4-IV)[68]

To a mixture of γ–valerolactone (12.040 g, 120 mmol,) and 5.0 eq of thiourea (45.67 g, 600 mmol), 6.5 eq of aqueous 48 % HBr (88.5 mL, 780 mmol) was added dropwise. The solution was refluxed for 36 hours at 135 ^{o}C. Then the mixture was extracted with CH_2Cl_2 (3 x 50 mL). The resulting aqueous solution was used in the subsequent reaction without purification. [68]

4-Sulfhydryl-4-methylbutanoic acid (4-V) [68]

To the aqueous solution of **4-IV,** 20 M NaOH (48 mL) was added slowly to adjust pH to 13 and then refluxed at 120 ^{o}C overnight. Upon completion the resulting aqueous solution was cooled down to room temperature and then washed with CH_2Cl_2 (3 x 40 mL). The pH of the

aqueous solution was adjusted to 2 with 1 M HCl and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layers were subsequently washed with brine and H_2O, then dried with $MgSO_4$, and concentrated *in vacuo* to yield **4-V** (14.467 g, 108 mmol). [68]

4-(Pyridin-2-yl-disulfanyl)4-methylbutanoic acid (4-VI) [24]
To a solution of 1,2-di(pyridin-2-yl)disulfane (8.957 g, 40.7 mmol) dissolved in 203 mL EtOH [0.2 M], 0.17 eq of **4-V** (910 mg, 6.78 mmol) dissolved in 34 mL EtOH [0.2 M] was added slowly. The reaction was then refluxed under 100 ^oC and monitored *via* TLC. Upon completion, the resulting solution was evaporated, and concentrated *in vacuo* to yield the crude product **4-VI** as yellow-orange oil, which was used for the subsequent reaction without purification. [24]

Triisopropylsilyl 4-(pyridin-2-yldisulfanyl)-4-methyl butanoate (4-VII) [4, 24]
An aliquot of **4-VI** (1.711 g, 7.04 mmol) was dissolved in 70 mL CH_2Cl_2 [0.1 M], to this solution 1.2 eq of TEA (1.18 mL, 8.45 mmol) was added dropwise. After it was cooled down to 0 ^oC for 10 minutes, to this mixture 1.2 eq of chlorotriisopropylsilane (1.86 mL, 8.45 mmol) was added dropwise. It was stirred and allowed to warm to room temperature overnight. The reaction was monitored *via* TLC and upon completion, it was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done with normal phase column chromatography on silica gel (HEX: EtOAc = 5:1) to afford **4-VII** (703 mg, 1.76 mmol, 23% for 4 steps) as a light yellow oil, which was stored in freezer under nitrogen atmosphere: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.12 (d, 18 H), 1.35 (m, 6 H), 1.85 - 2.05 (m, 2 H), 2.56 – 2.59 (m, 2 H), 3.10 (m, 1 H), 7.12 (m, 1 H), 7.68 (t, J = 5.4 Hz, 1 H), 7.78 (d, J = 7.2 Hz, 1 H), 8.50 (m, 1 H). All data are consistent with literature values. [4, 24]

2-(4-Oxo-4-(triisopropylsilyloxy)-1-methylbutanyldisulfanylphenyl)acetic acid (4-VIII) [4, 24]

In a 50 mL round bottom flask an aliquot of **4-VII** (698 mg, 1.75 mmol) was dissolved in 17.5 mL of THF and cooled to 0 ^oC under nitrogen atmosphere. To this mixture was added 1 eq of **4-II** (296 mg, 1.76 mmol) dissolved in 17.6 mL of THF that previously cooled to 0 ^oC. The mixture was stirred at 0 ^oC for 3 hours and allowed to warm to room temperature. The reaction was monitored *via* TLC and upon completion, the solvent was rotary evaporated and purified by normal phase column chromatography (HEX: EtOAc = 4:1) on silica gel to afford **4-VIII** (279 mg, 0.610 mmol, 35%) as a pale yellow oil: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.10 (d, 18 H), 1.33 (m, 6 H), 1.82 – 2.03 (m, 2 H), 2.43 (t, 2 H), 2.95 (m, 1 H), 3.95 (s, 2 H), 7.24 (d, J = 6.3 Hz, 1 H), 7.29 – 7.39 (m, 2 H), 7.83 – 7.86 (d, J = 8.1 Hz, 1 H), 10.32 (s, 1 H, broad peak). All data are consistent with literature values. [4, 24]

§2.3.3 Synthesis of SB-T-1214-Me-Linker-OSu

SB-T-1214-Me-linker-TIPS ester conjugate (4-IX) [4, 24]
An aliquot of **4-VIII** (89 mg, 0.191 mmol) was dissolved in 1 mL of CH_2Cl_2. To this solution 1.0 eq of DIC (29 mg, 0.037 mL, 0.20 mmol) was added dropwise. After it was maintained under 0 ^oC for more than 10 minutes, to this mixture 1.5 eq of SB-T-1214 (244 mg, 0.29

mmol) and 0.25 eq of DMAP (6 mg, 0.049 mmol) previously dissolved in CH_2Cl_2 and cooled to 0 ^oC was added dropwise. The reaction was monitored *via* TLC and upon completion the reaction was quenched with saturated aqueous NH_4Cl (2 mL), extracted with ethyl acetate (3 x 15 mL). The combined organic layers eas washed with brine (2 x 20 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by normal phase column chromatography on silica gel to yield **4-IX** (166 mg, 0.128 mmol, 67 % yield). ^1H NMR (500 MHz, $CDCl_3$, ppm) δ 1.04 (m, 21 H), 1.07-1.10 (d, 4 H), 1.56 (s, 6 H), 1.66 (s, 3 H), 1.72 (s, 3 H), 1.86 – 1.90 (m, 4 H), 2.35 (s, 3 H), 2.37 (m, 1H), 2.42 (m, 2 H), 2.52 (m, 1 H), 2.59 (m, 1 H), 2.94 (m, 1 H), 3.79 (d, *J* = 7.1 Hz, 1 H), 3.92 -3.97 (m, 1 H), 4.08 – 4.13 (m, 1 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.42 (m, 1 H), 4.81 (d, 1 H), 4.92 – 4.97 (m, 3 H), 5.06 (d, *J* = 8.4 Hz, 1 H), 5.66 (d, *J* = 7.1 Hz, 1 H), 6.19 (t, 1 H), 6.28 (s, 1 H), 7.22 – 7.35 (m, 3 H), 7.47 (t, 2 H), 7.60 (t, 1 H), 7.79 (d, *J* = 7.8 Hz, 1 H), 8.11 (d, *J* = 7.4 Hz, 2 H). All data are consistent with literature values. [4, 24]

SB-T-1214-Me-linker-carboxylic acid conjugate (4-X) [4, 24]

An aliquot of **4-IX** (166 mg, 0.128 mmol) was dissolved in a 1:1 mixture of acetonitrile:pyridine (6.4 mL) and cooled to 0 °C under inert conditions. To this solution was added 70% HF/pyridine (1.3 mL) dropwise. The reaction was stirred at room temperature and monitored *via* TLC. Upon completion the reaction was quenched with 0.2 M citric acid (10 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layers eas washed with $CuSO_4$ (3 x 15 mL), brine (3 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done *via* column chromatography on silica gel using gradient eluent (HEX:EtOAc = 7:3 - 4:6) to afford **4-IX** (0.079 mmol, 89 mg, 65%) as a white solid: ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.952 – 0.971 (m, 2 H), 1.086 – 1.116 (m, 6 H), 1.202 – 1.252 (m, 9 H), 1.320 (s, 9H), 1.631 (s, 3 H), 1.681 – 1.698 (m, 6 H), 1.876 (s, 3 H), 2.005 (s, 4 H), 2.337 – 2.450 (m, 7 H), 2.451 – 2,528 (m, 1 H), 2.863 -2.952 (m, 1 H), 3.747 (d, *J*= 6.8 Hz, 1 H), 3.902 -3.953 (m, 1 H), 4.012 – 4.108 (m, 1 H), 4.141 (d, *J*= 8.4 Hz, 1 H), 4.256 (d, *J*= 8.4 Hz, 1 H), 4.365 -4.409 (m, 1 H), 4.925 – 4.938 (m, 4 H), 5.077 (d, 1 H), 5.630 (d, *J*= 7.0 Hz, 1 H), 6.166 (t, 1 H), 6.269 (s, 1 H), 7.181 – 7.320 (m, 3 H), 7.431 (t, 2 H), 7.540 (t, 1 H), 7.755 – 7.783 (m, 1 H), 8.054 (d, *J*= 7.4 Hz, 2 H), 9.322 (s, 1H, broad peak). All data are consistent with literature values. [4, 24]

SB-T-1214-Me-linker-OSu coupling-ready construct (4-XI) [4, 24]

An aliquot of **4-X** (88 mg, 0.078 mmol) was dissolved in CH_2Cl_2 (3.9 mL, ~ 0.02 M) and cooled to 0 °C with ice bath. To this solution was added 1.0 eq DIC (17 mg, 13 µl, 0.078 mmol), followed by the addition of HSN dissolved in DMF (3.9 mL) dropwise. The reaction was stirred at room temperature and monitored *via* TLC. Upon completion, the reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc (3 x 15 mL), washed with brine (3 x 15 mL), and concentrated *in vacuo*. Purification was done *via* normal phase column chromatography on silica gel to afford **4-IX** (53mg, 0.042 mmol, 54%): ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.952 – 0.971 (m, 2 H), 1.048 – 1.142 (m, 6 H), 1.238 – 1.317 (m, 9 H), 1.336 (s, 9H), 1.657 (s, 3 H), 1.705 – 1.760 (m, 6 H), 1.901 (s, 3 H), 2.041 - 2.054 (m, 4 H), 2.351 – 2.371 (m, 5 H), 2.503 – 2,564 (m, 1 H), 2.601 – 2.675 (m, 3 H), 2.823 (s, 4 H), 2.963 -3.025 (m, 1 H), 3.789 (d, *J* = 5.1 Hz, 1 H), 3.953 -3.994 (m, 1 H), 4.082 (d, 1 H), 4.165 (d, *J* = 8.4 Hz, 1 H), 4.288 (d, *J* = 8.4 Hz, 1 H), 4.410 -4.431 (m, 1 H), 4.785 (d, 1 H), 4.924 (t, 3 H), 5.083 (d, 1 H), 5.657 (d, *J* = 7.0 Hz, 1 H), 6.163 (t, 1 H), 6.278 (s, 1 H), 7.231 – 7.317 (m, 3

H), 7.451 (t, 2 H), 7.578 (t, 1 H), 7.784 (d, J = 7.6 Hz, 1 H), 8.101 (d, J = 7.4 Hz, 2 H); ^{13}C-NMR (Acetone-D_6, ppm) δ 0.43, 9.37, 10.60, 13.94, 15.35, 18.66, 18.99, 22.83, 23.50, 24.07, 26.41, 26.70, 27.49, 29.04, 29.33, 31.71, 37.21, 37.58, 39.51, 44.63, 46.85, 47.73, 50.79, 59.45, 72.80, 76.39, 76.51, 77.11, 79.18, 79.82, 82.18, 85.43, 121.88, 129.06, 129.57, 129.66, 131.22, 131.44, 131.53, 131.69, 132.50, 134.52, 134.67, 135.06, 135.11, 137.98, 138.62, 143.03, 156.47, 166.96, 169.70, 169.91, 170.91, 171.09, 171.50, 174.73, 204.32; FIA-MS MS(ESI) *m/z* calcd for $C_{62}H_{80}N_3O_{20}S_2$ $(M+NH_4)^+$ 1250.4, found 1250.4. All data are in agreement with literature values. [4, 24]

§2.3.4 Synthesis of Biotin-Methyl branched disulfide linker-SB-T-1214 drug conjugate

Biotin methyl ester (4-XII) [4]

An emulsion of biotin (274 mg, 1.12 mmol) in CH_3OH [0.1 M] was cooled to 0 ^oC. To the emulsion was added thionyl chloride (0.33 mL, 4.48 mmol) dropwise. The obtained clear mixture was stirred at room temperature monitored *via* TLC. Upon completion the mixture was evaporated and purified by normal phase column chromatography on silica gel to afford **4-XII** (257 mg, 0.99 mmol, 89%) as a white solid which was used in the next step without further purification: m.p. 165-166 ^oC (m.p. lit. 166 ^oC): ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 1.23-1.35 (m, 2 H), 1.36-1.65 (m, 4H), 2.27-2.31 (t, J = 7.6 Hz, 2H), 2.56 – 2.59 (d, J = 12.4 Hz, 1H), 2.79 - 2.84 (dd, J_1 = 12.4 Hz, J_2 = 5.2 Hz, 1H), 3.09 – 3.11 (m, 1H), 3.57 (s, 3H), 4.12 – 4.15 (m, 1H), 4.29 – 4.33 (m, 1H), 6.36 (s, 1H), 6.43 (s, 1H). All data are consistent with literature values. [4]

Biotin-hydrazide (4-XIII) [4]

To a solution of **4-XII** (157 mg, 0.61 mmol) dissolved in 3 mL of CH_3OH was added anhydrous hydrazine (0.085 mL, 3.05 mmol). The mixture was stirred at room temperature and monitored *via* TLC. Upon completion, the reaction solvent was evaporated and the resulting crude was diluted with water and washed with $CHCl_3$ (5 x 30 mL). The aqueous layer was collected and concentrated *in vacuo* to yield **4-XIII** (155 mg, quant. yield) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 1.23-1.35 (m, 2 H), 1.40-1.44 (m, 3 H), 1.45–1.65 (m, 1 H), 2.00 (t, J = 7.2 Hz, 2 H), 2.57 (d, J = 12.4 Hz, 1 H), 2.82 (dd, J_1 = 12.4 Hz, J_2 = 5.2 Hz, 1 H), 3.06-3.11 (m, 1 H), 4.11-4.14 (m, 3 H), 4.29-4.32 (m, 1 H), 6.35 (s, 1 H), 6.42 (s, 1 H), 8.91 (s, 1 H); ^{13}C-NMR (DMSO-d_6, ppm) δ 25.22, 28.00, 28.22, 33.22, 55.41, 59.18, 61.03, 162.70, 171.52. All data are consistent with literature values. [4]

Biotin-Me-linker-SB-T-1214 drug conjugate (4-XIV) [4, 24]

To a solution of **4-XI** (29 mg, 0.024 mmol) dissolved in 0.2 mL DMSO: pyridine (3:1, v/v) was added **4-XIII** (6.1 mg, 0.024 mmol) previously dissolved in 0.2 ml DMSO: pyridine (3:1, v/v) dropwise. The mixture was stirred at room temperature and monitored *via* TLC. Upon completion the reaction was diluted with water (1 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected and combined, washed with brine and dried over $MgSO_4$. Purification was done by normal phase column chromatography on silica gel to afford **4-XIV** (19 mg, 0.015 mmol, 59%), as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, ppm) δ 0.96-0.99 (t, J = 8.2 Hz, 2 H), 1.10-1.12 (t, J = 3.4 Hz, 2 H), 1.16 (s, 3 H), 1.23-1.26 (m, 5 H), 1.29 (d, J = 6.7 Hz, 1 H), 1.37 (s, 9 H), 1.41-1.67 (m, 6 H), 1.71-1.73 (m, 18 H), 1.86-1.89 (m, 2 H), 1.92 (s, 4 H), 2.20-2.23 (m, 5 H), 2.35-2.36 (m, 4 H), 2.49-2.52 (m, 1 H), 2.61 (s, 1

H), 2.71-2.73 (m, 1 H), 2.87-2.94 (m, 2 H), 3.10 (dd, J_1 = 11.2 Hz, J = 5.9 Hz, 1 H), 3.41-3.48 (m, 1 H), 3.80 (d, J = 7.0 Hz, 1 H), 3.97-4.01 (m, 1 H), 4.07-4.11 (m, 1 H), 4.16 (d, J = 7.5 Hz, 1 H), 4.28-4.32 (m, 2 H), 4.39- 4.40 (m, 1 H), 4.46-4.48 (m, 1 H), 4.95-4.98 (m, 3 H), 5.08-5.13 (m, 1 H), 5.67 (d, J = 7.0 Hz, 1 H), 5.84-5.86 (m, 1 H), 6.16 (m, 1 H), 6.33 (s, 1 H), 6.85 (s, 1 H), 7.23-7.32 (m, 2 H), 7.45-7.48 (m, 2 H), 7.59 (t, J = 7.5 Hz, 1 H), 7.78 (d, J = 8.0 Hz, 1 H), 8.09 (d, J = 8.0 Hz, 2 H), 8.55 (broad, 1 H), 9.45 (broad, 1 H); ^{13}C NMR ($CDCl_3$, ppm) δ 9.71, 13.00, 14.77, 18.53, 22.06, 22.52, 25.25, 25.80, 26.62, 27.91, 28.29, 31.10, 33.06, 35.53, 35.77, 38.81, 40.99, 43.23, 46.01, 46.40, 53.45, 58.38, 58.40, 60.33, 61.90, 71.85, 75.11, 75.52, 79.13, 81.11, 84.51, 128.01, 128.42, 128.67, 129.35, 130.16, 131.17, 132.64, 132.69, 133.66, 164.49, 166.91, 169.80, 170.66, 171.43, 172.07, 172.14, 174.84, 203.91. All data are in agreement with literature values. [4, 24]

Chapter 3

Synthesis of SB-T-12301 and its Analogues

Chapter Contents

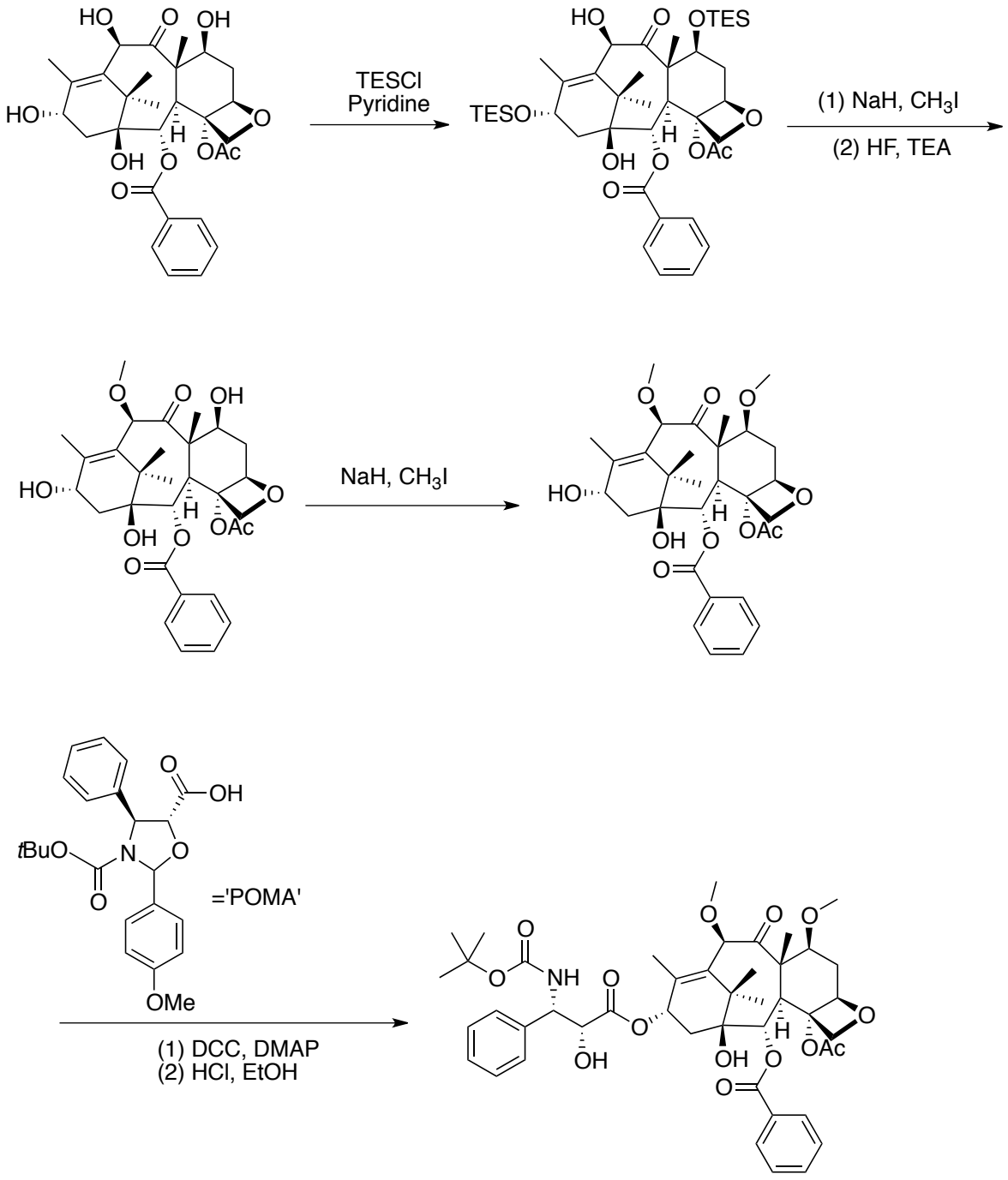
§3.1 Introduction

The functionalities of wide explored tumor-targeted drug conjugates are heavily relying on the efficacy of warhead. As consistent administration of certain drugs often leads to cases of MDR, chemotherapeutic drugs that can overcome MDR are being screened and tested extensively.[1] For marketed taxanes like paclitaxel and docetaxel, after having been used for two decades, recurrences are common and new agents that can overcome the failures are badly in need.[7, 23]

Cabazitaxel, developed by Sanofi-Aventis (now Sanofi), is the third taxane that has been approved by FDA on June 17, 2010. It is for the treatment of hormone-refractory prostate cancer. Prostate cancer is the most common malignancy in men and the third of mortality in the western countries. [7, 69] It is estimated that 240,890 new patients are diagnosed in the United States, and about 33, 720 of them die from this disease in 2011. Cabazitaxel is much more effective in treating castration-resistant prostate cancer (CRPC) than that of docetaxel because it is a poor substrate of P-gp, an ATP-dependent drug efflux pump.[23, 69]

§3.1.1 Review of cabazitaxel synthesis

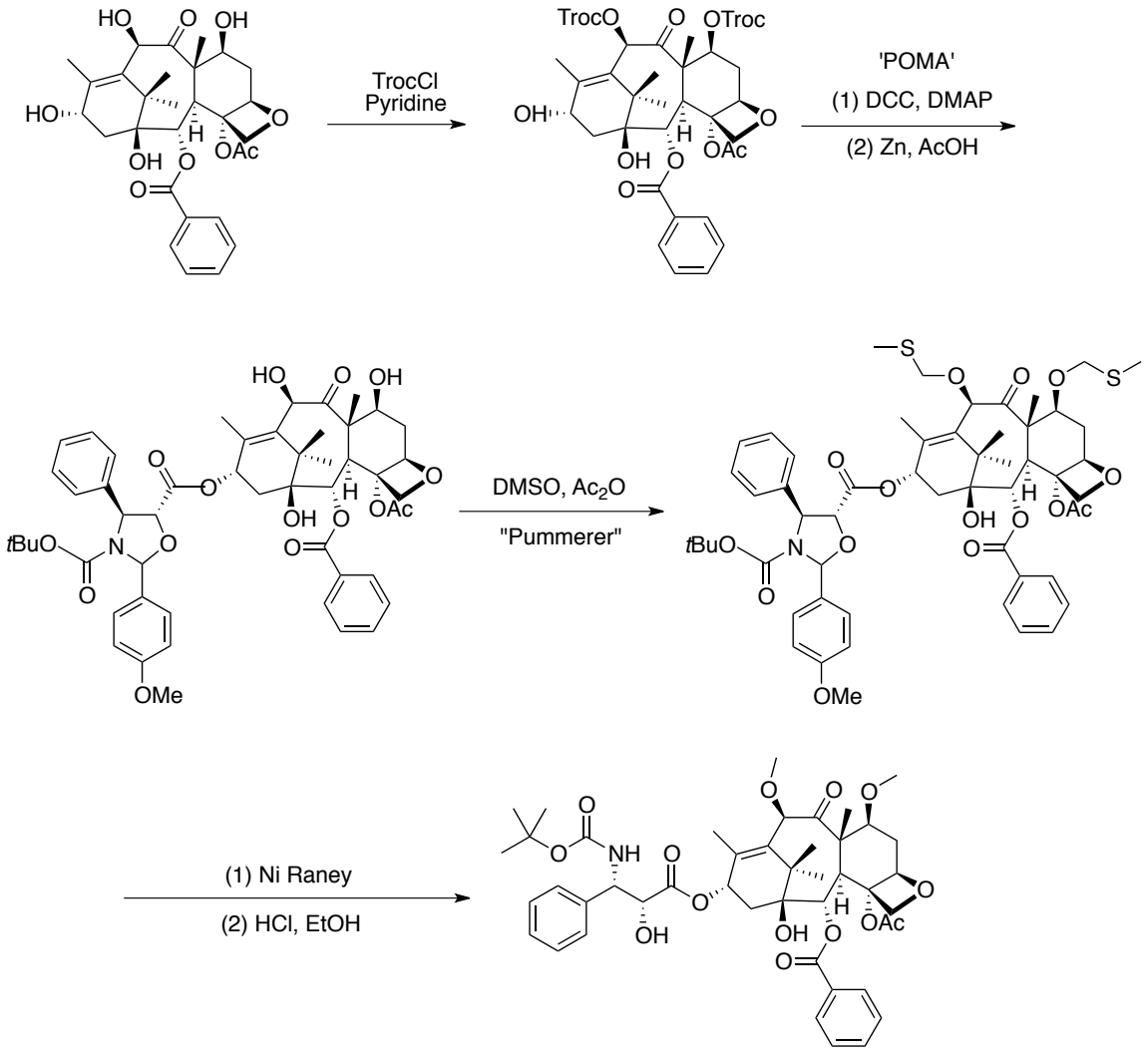
In spite of its importance, the reports of cabazitaxel synthesis are fairly limited. The first synthetic route was reported by Dr. Herve Bouchard *et al.* of Aventis Pharma in 2002.[70] 10-DAB III was used as the starting material. After protected the hydroxyl groups of C-7 and C-13 with TES, methyl group was introduced to the C-10 hydroxyl group in the presence of strong base NaH. Then TES deprotection and methylation of the C-7 hydroxyl group afforded the product by coupling with oxazolidine carboxylic acid side chain precursor and alcohol hydrolysis under acid condition (**Scheme 3-1**). [70] The overall yield of 6 steps was only 4.9%.



Scheme 3-1: The first synthetic route to Cabazitaxel[7]

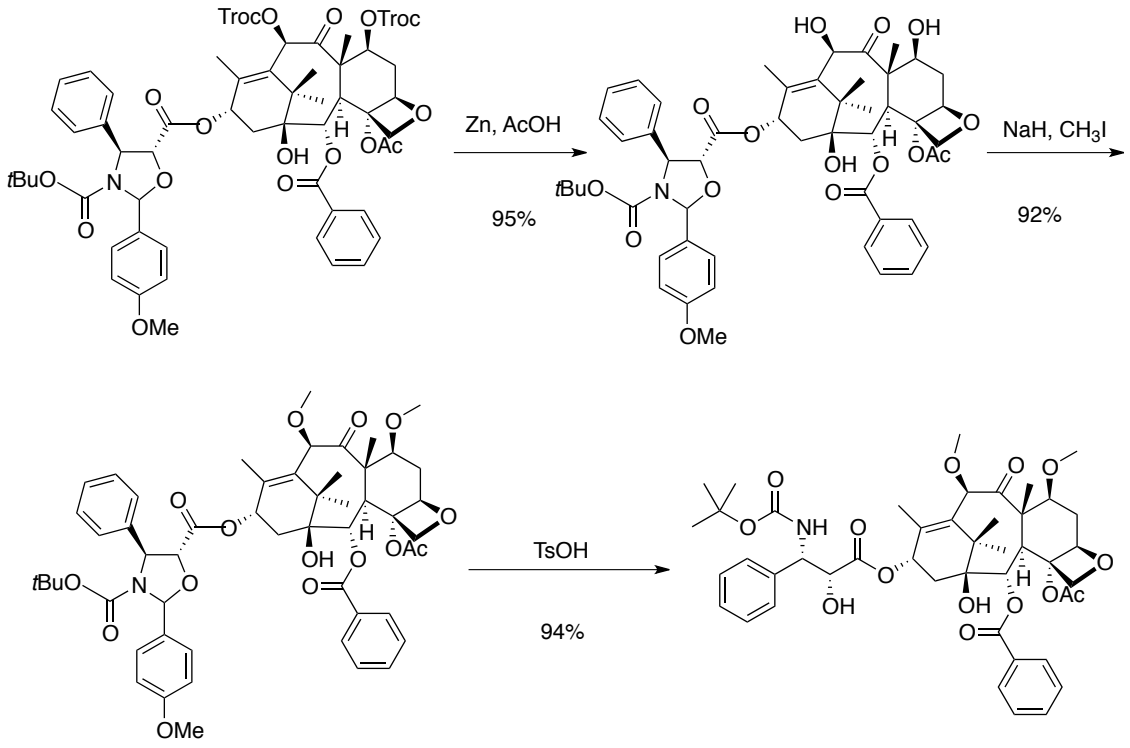
Later, the synthetic route was improved by protecting both C-7 and C-10 hydroxyl groups with Troc, succeeded by oxazolidine carboxylic acid coupling to introduce the side chain precursor. Troc deprotection followed by Pummerer rearrangement and Raney Nickel reduction to introduce methyl groups to the C-7 and C-10 hydroxyl groups, finally afforded the product by alcohol hydrolysis with a higher yield (**Scheme 3-2**).[7] The disadvantages of this method are high production cost due to the early introduction of chiral side chain

precursor, and average yields of Pummerer rearrangement and Raney Nickel reduction.[71]



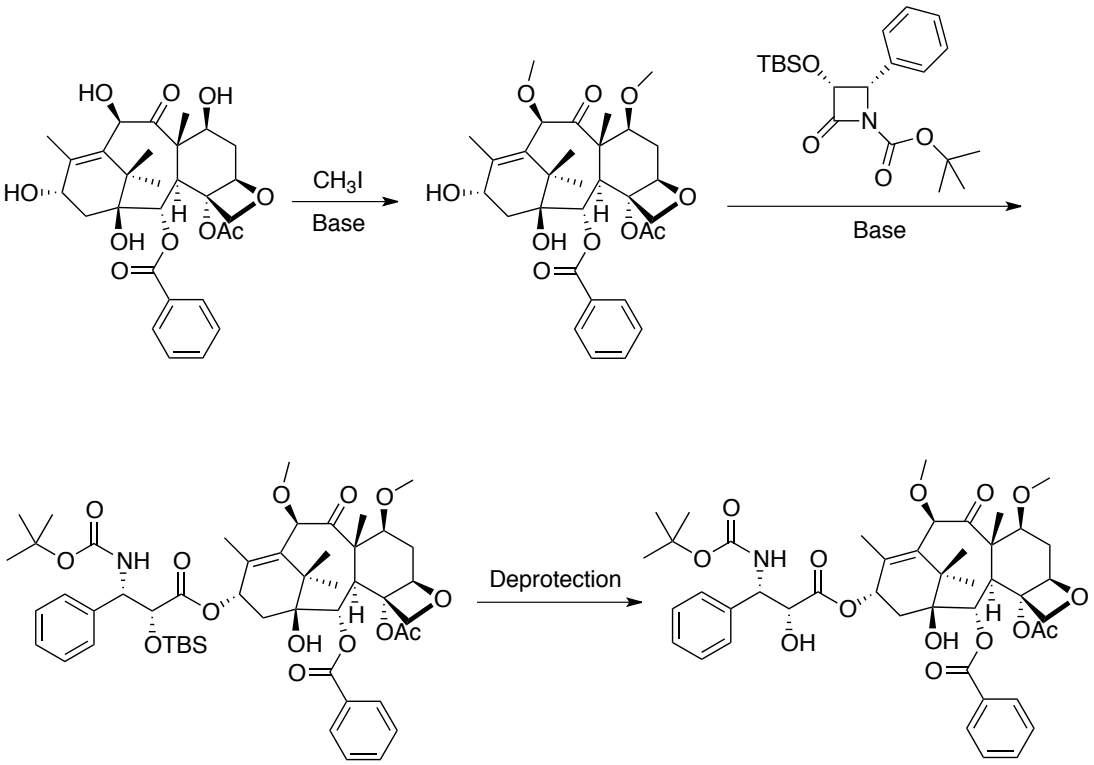
Scheme 3-2: Improved synthetic route to Cabazitaxel [7]

A similar patent was reported recently.[72] It started with the product of Oxazilidine carboxylic acid coupling, which is also an intermediate for docetaxel semi-synthesis. The Troc groups were deprotected in the presence of acetic acid and zinc powder, then the hydroxyls groups were methylated by CH_3I and NaH at position C-7 and C-10 positions simultaneously, finally yielding cabazitaxel after deprotection with TsOH (**Scheme 3-3**).[72] Although the yield for the 3 steps was 82%, the same problem is the higher production cost because of the early introduction of side chain precursor.



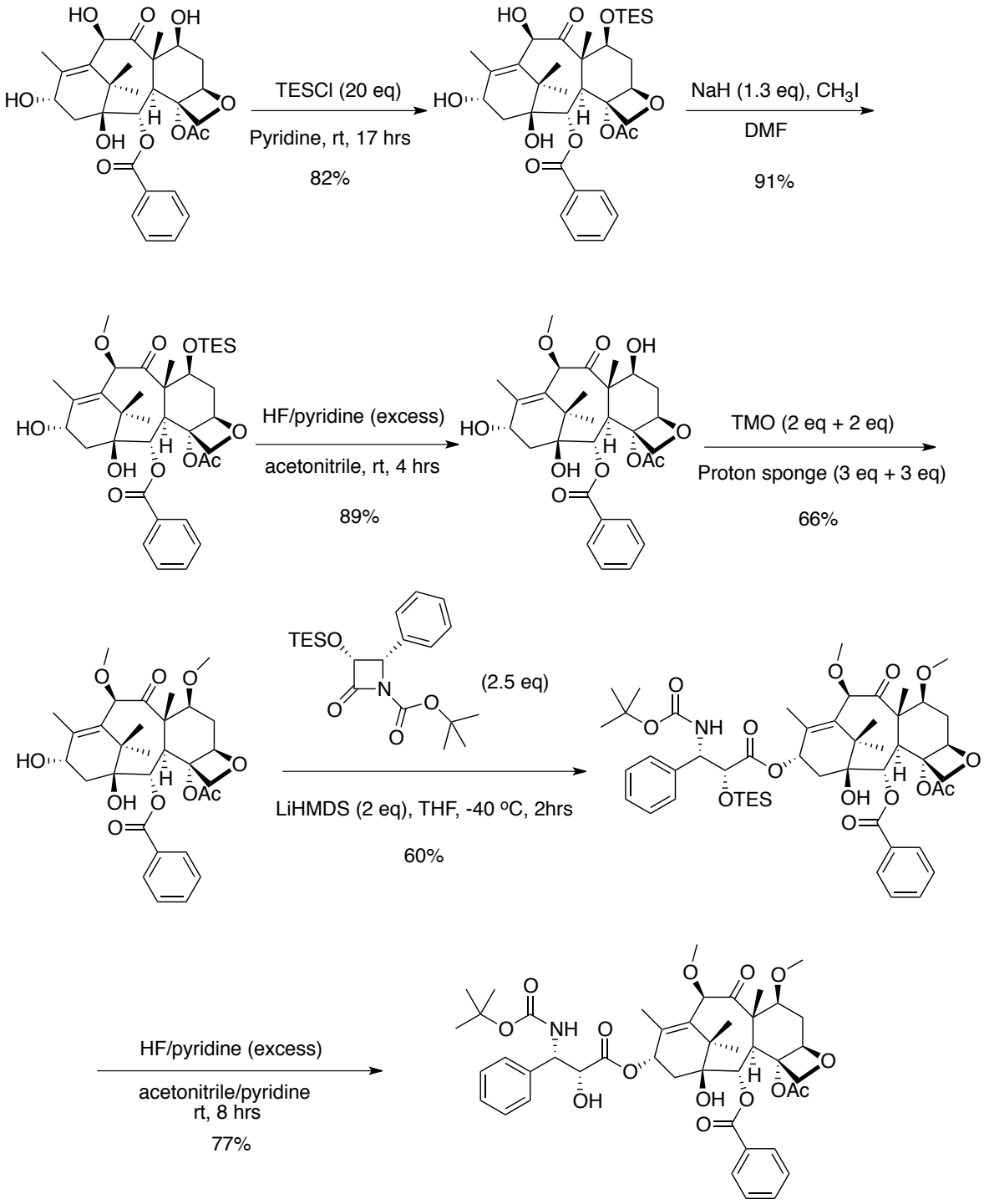
Scheme 3-3: Synthesis scheme of Zhang *et al*.[72]

In 2011, Sun *et al*. reported a synthesis route of cabazitaxel by Ojima-Holton coupling with an overall yield of 17.8% for 3 steps.[73] The yield of simultaneous methylation of both hydroxyl groups at C-7 and C-10 was only 24%. According to this patent, out of the methylation reagents that have been tried, CH_3I was better than both dimethyl sulfate and Meerwein's salt. Among all of the bases explored, LiHMDS was found to have the best efficacy. And the optimal reaction temperature was at -40 ^{o}C. The side chain precursor, (3*R*,4*S*)-β-lactam was prepared with the assistance of Oppolzer reagent (**Scheme 3-4**).[73]



Scheme 3-4: Synthesis scheme of Sun *et al*.[73]

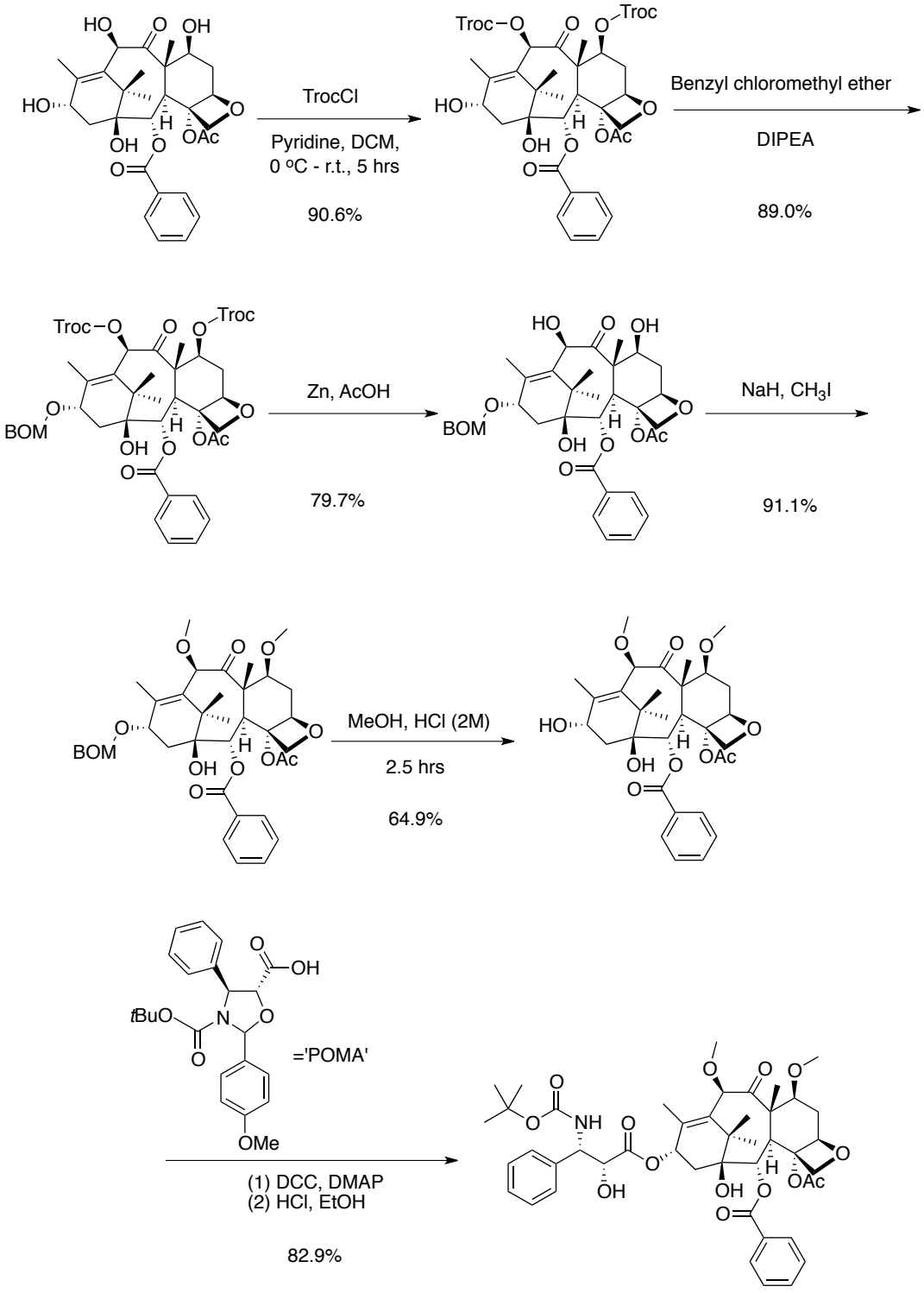
A following study indicated that the 7β-OH methylation of either 10-DAB III or 10-methyl-10-DAB III was inefficient under the presence of strong base, which often lead to epimerization through a retro-aldol reaction, making methylated 7α-OH isomer as the main product.[69] The switch to different methylating reagents such as CH_3I, $(CH_3O)_2SO_2$ and so on had little change on improving the yield. In a separate account, the yield was greatly improved by employing a mild base, proton sponge, and a more active methylating agent, trimethyloxonium tetrafluoroborate (TMO) (**Scheme 3-5)**.[69]



Scheme 3-5: Synthesis scheme of Zhang *et al*. [69]

A recent patent reported a new synthesis method that has improved the overall yield to 31.5% for 7 steps. The general strategy was that, after protection of C-7 and C-10 hydroxyl groups with Troc/TES/MEM, protect group such as BOM/TES/TMS is introduced to C-13 hydroxyl group. Then simultaneously deprotected and methylated the hydroxyl groups of C-7

and C-10, succeeded by deprotection to afford the key intermediate, 7,10-dimethyl-10-DAB III. The final product was obtained by standard oxazolidinecarboxylic acid coupling and alcohol hydrolysis (**Scheme 3-6**).[71]



Scheme 3-6: Synthesis scheme of Liu *et al.*[71]

Several other reported patents reported similar procedures.[74-76]

§3.1.2 SB-T-12301

Based on many years of extensive SAR studies and biological cytotoxicity evaluation of taxoids, SB-T-1214, a second generation taxane was synthesized, selected by the Ojima group as a lead compound due to its remarkable effect of 2 orders of improvement improvement against MDR cancer cell lines compared with paclitaxel and docetaxel.[23] As the side chain of SB-T-1214 is extremely important to maintain its function and the methylation of C-7 and C-10 hydroxyl groups are the key of making cabazitaxel superior to docetaxel, a compound that can combine both characteristics might be able to achieve a better effect (**Figure 3-1**).

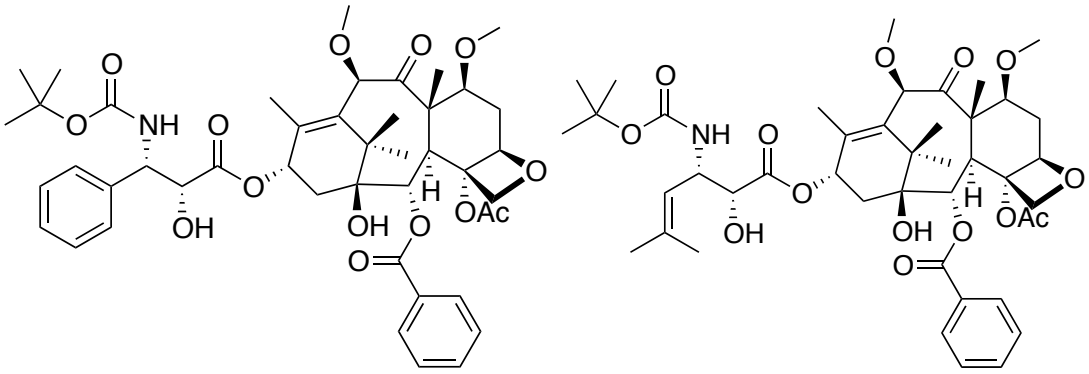
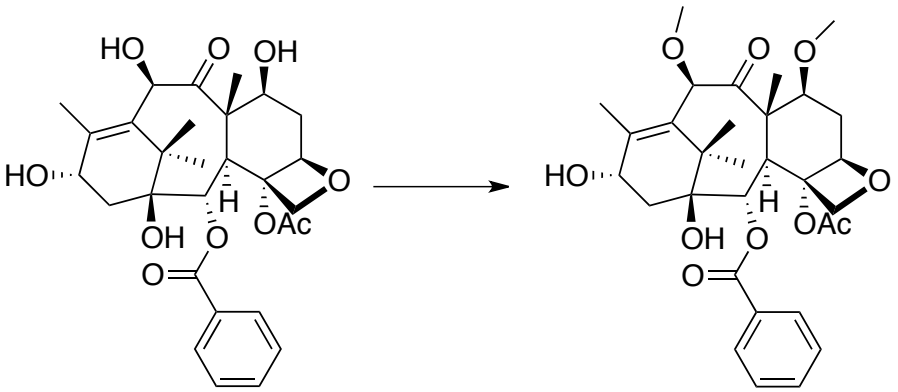


Figure 3-1: Structures of cabazitaxel and SB-T-12301

§3.2 Synthesis and biological evaluation of SB-T-12301

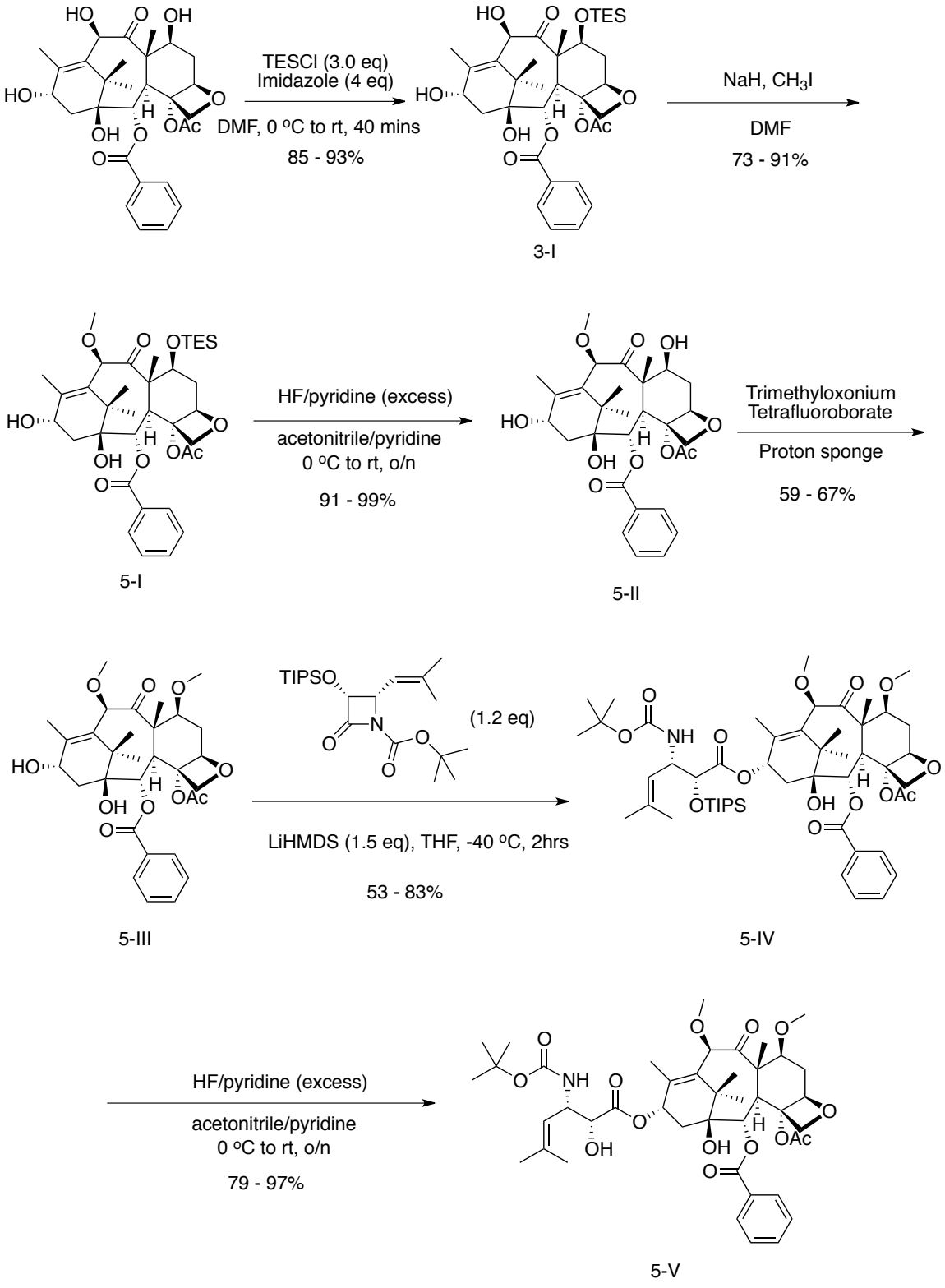
The key for synthesizing SB-T-12301 is to obtain 7,10-dimethyl-10-DAB III efficiently. There are two classes of methylation: simultaneous and stepwise. The simultaneous method was tried extensively but none was found practical in terms of yield **(Table 1)**. If the simultaneous methylation have been easy and efficient, there would be no intensive and laborious effort dedicated to improve the other stepwise synthetic routes. [7, 69]



Entry	Base (eq)	Methylating agent (eq)	Solvent	Temp. (^oC)	Time (hrs)	Conv.	Memo
1	NaH (3)	CH_3I (>20)	THF	~ -60	7	No	NA
2	NaH (5)	TMO (6)	DMF	0	5	Trace	NA
3	NaH (6)	TMO (4)	DMF	~ -45	6.5	No	NA
4	Proton Sponge (8)	TMO (5)	DMF	0	24	No	NA
5	Proton Sponge (7)	TMO (5)	DCM	0	19	Trace	NA
6	Proton Sponge (8)	TMO (6)	DCM	rt	96	Trace	Mixture
7	Proton Sponge (7)	TMO (5)	DCM	0	144	48%	Mixture
8	Proton Sponge (7)	TMO (5)	DCM	-10	336	28%	Mixture

Scheme 3-7: Trials of simultaneous methylation

Out of all stepwise methods of 7,10-dimethyl-10-DAB III synthesis, both the methods developed by Zhang *et al* and Liu *et al* are attractive due to their comparatively higher yields.[69,71] Although the overall yield of Zhang *et al*'s route was only 20.3%,[69] there should be a fairly handsome space to improve because several steps were not well run and the key intermediate, 10-methyl-10-DAB III can be recovered. And this synthesis route was tried successfully (**Scheme 3-8**). After obtaining the desired intermediate 7,10-dimethoxy-10-DAB III and β-lactam, the subsequent standard protocol of Ojima-Holton Coupling followed by deprotection afforded SB-T-12301 ((2'*R*, 3'*S*)-3'-dephenyl-3'-(2-methyl-1-propenyl) cabazitaxel, **Figure 3-2**). The first run of Ojima-Holton coupling was not well controlled. And there should be some space to improve the yield by preparing purer 7,10-dimethyl-10-DAB III (**5-III**).



Scheme 3-8: Synthesis of SB-T-12301

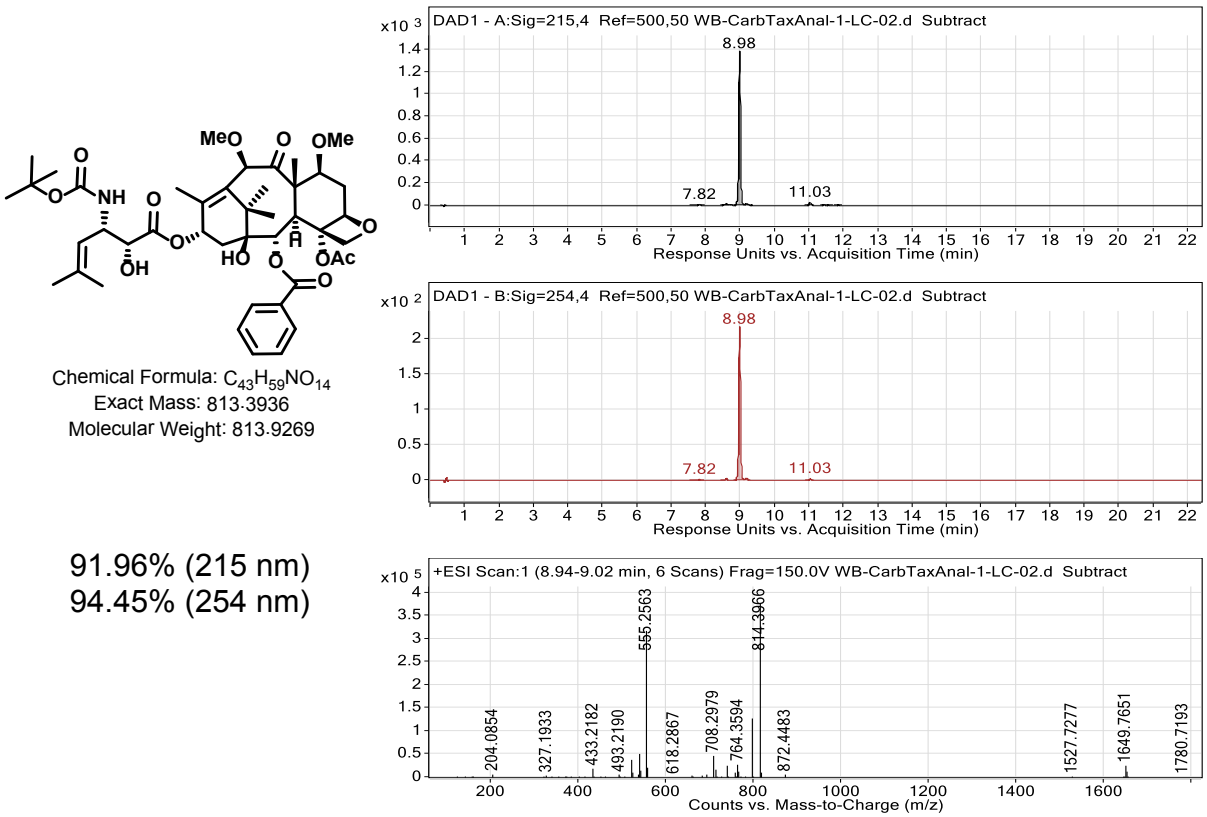


Figure 3-2: Chracterization of SB-T-12301

An important byproduct was identified during the process of methylating **5-II**, which was ignored at first because of its similar polarity with **5-III**. After TES protection of C-13 in the following route of synthesizing C-2 modified SB-T-12301 analogues, the TES protection byproduct was isolated and identified as shown in **Figure 3-3**.

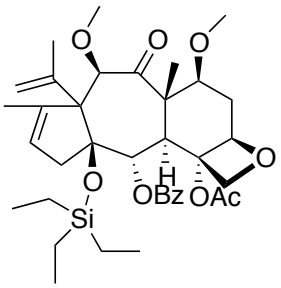
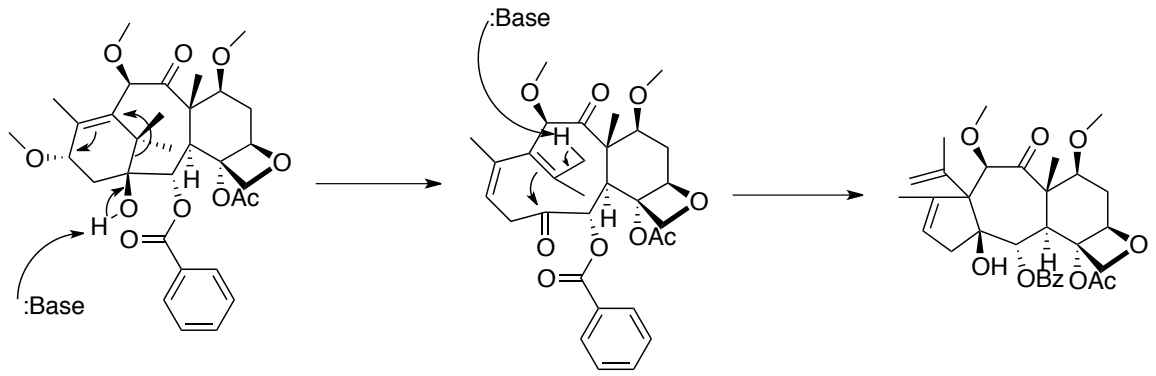


Figure 3-3: TMO methylation byproduct (5-III*) after C-13 protection

In spite of the reported mechanism by Samaranayake *et al.*,[77] in this case there was no acetyl chloride to make the hydroxyl group at C-1 a good leaving group. There was a base that can remove the hydrogen from C-1 hydroxyl group to initiate the rearrangement process depicted in **Scheme 3-9.** Based on the mechanism which has been reported,[77] acetylation of C-1 hydroxyl group followed by loss of this good leaving group yields an anion at C-1, succeeded by rearrangement *via* the migration of the C-11,C-15 bond. In the process a base deprotonated the allylic proton, pushing the double bond to attack the carbonyl group to

afford the final compound.



Scheme 3-9: Proposed mechanism for byproduct formation

§3.3 Synthesis of SB-T-12301 analogues: 2-Debenzoyl-2-*m*-methylbenzoyl-SB-T-12301 and 2-Debenzoyl-2-*m*-fluorobenzoyl-SB-T-12301

Studies have shown that the modification of C-2 benzoyl group at *meta* position with an electron rich substitute like methyl, methoxy, or fluoride could significantly increase the cytotoxicity against MDR cancer cell lines.[10] Cytotoxicity of benzoyl *meta* substituted SB-T-12301 analogues might be further enhanced, as it was shown in other *meta* modified taxanes, 2-3 orders of magnitude more potent than the parent molecules and similar IC values for both sensitive and MDR cancer cell lines.[3]

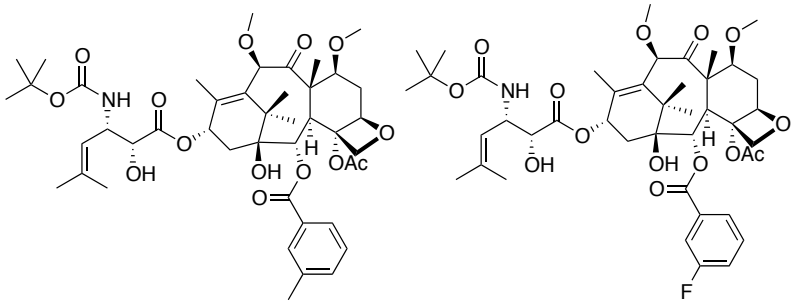
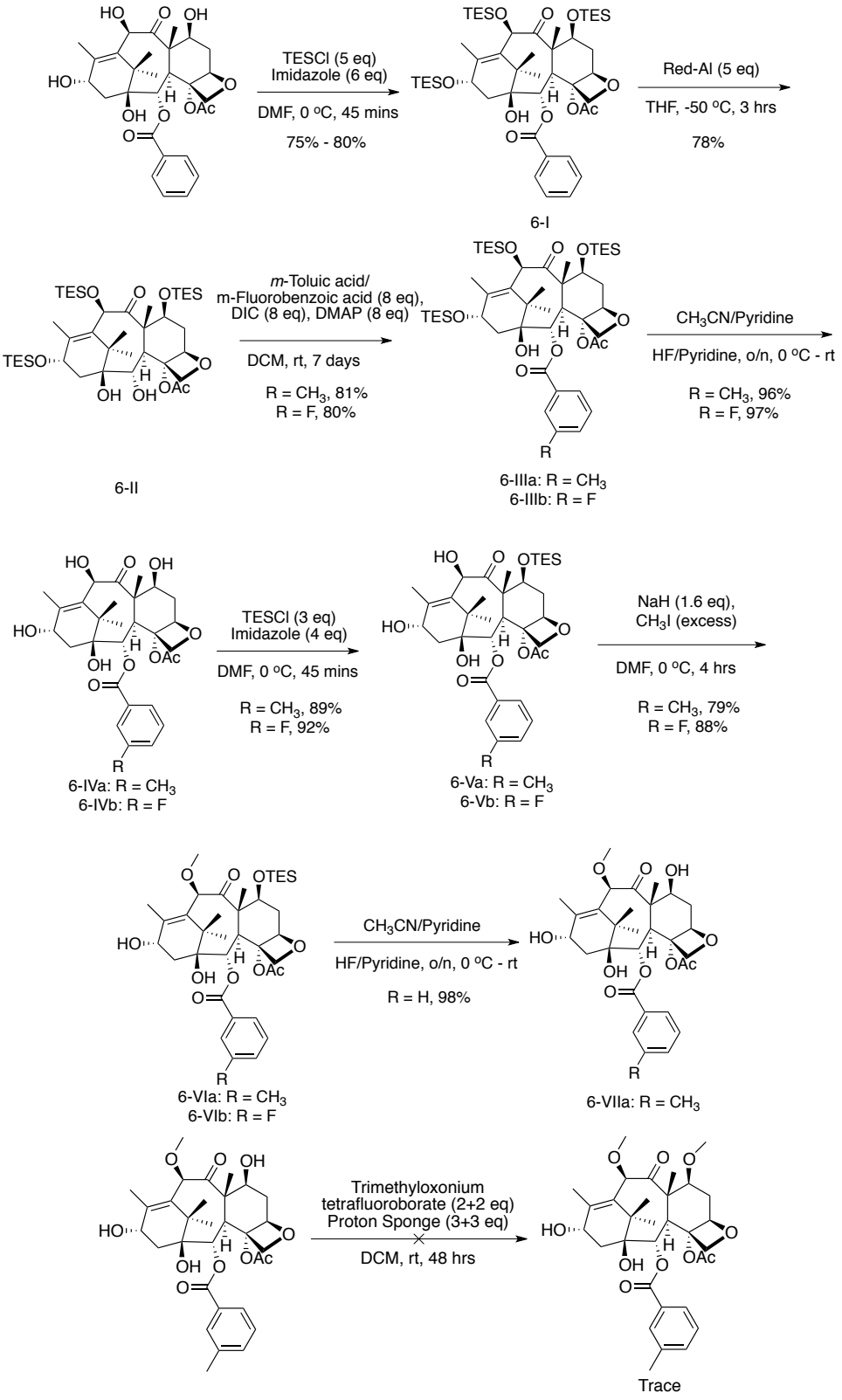


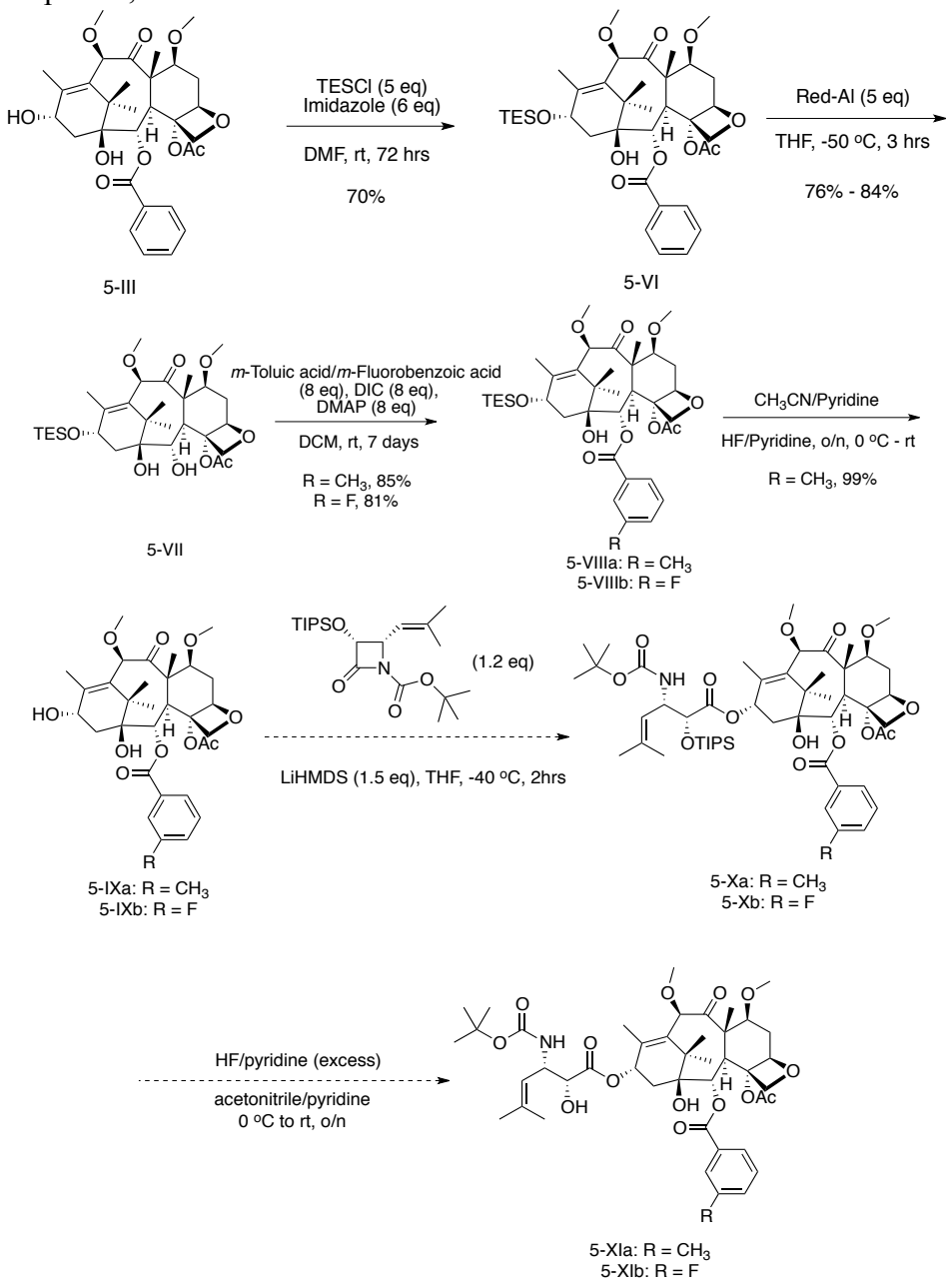
Figure 3-4: Structures of 2-Debenzoyl-2-*m*-methylbenzoyl-SB-T-12301 and 2-Debenzoyl-2-*m*-fluorobenzoyl-SB-T-12301

The initial synthetic route started from C-2 modification. TES protection of C-7, C-10, and C-13, followed by Red-Al cleaving of C-2 benzoate and coupling with toluic acid or *m*-fluorobenzoic acid, then deprotection afforded **6-IVa** / **6-IVb** for stepwise methylation. Unfortunately, the yield of 7-10 methylation was very low. And the desired **5-IXa** was mixed together with byproduct, which was very difficult to be removed from the desired product due to they have similar polarity (**Scheme 3-10**).



Scheme 3-10: First failed route to 2-Debenzoyl-2-*m*-methylbenzoyl-SB-T-12301 and 2-Debenzoyl-2-*m*-fluorobenzoyl-SB-T-12301

After the unexpected failure of the first synthetic route, an alternative route was carried out successfully. The first few steps were the same to that of synthesizing SB-T-12301 to obtain the key intermediate **5-III**. Then TES protection of C-13 hydroxyl group succeeded by Red-Al reaction cleaved C-2 benzoate and coupled with toluic acid / m-fluorobenzoic acid, deprotection afforded the key intermediates **5-IXa** and **5-IXb** with decent yields (**Scheme 3-11**). The coupling reaction between 5-VII and m-fluorobenzoic acid was exothermic. Ice water bath was needed to prevent the evaporation of solvent. Maybe this reaction was much faster than expected, but needs further confirmation.



Scheme 3-11: Second tried route to 2-Debenzoyl-2-*m*-methylbenzoyl-SB-T-12301 and 2-Debenzoyl-2-*m*-fluorobenzoyl-SB-T-12301

§3.4 Conclusion

Generally, the target compound has been successfully synthesized and its formation has been verified by spectroscopic methods and its cytotoxic activity against the MCF-7 cells has been evaluated preliminarily, about one order higher potency than that of paclitaxel. The improved activity might be due to a better penetration of the compound into the cell because in the new derivative the free hydroxyl group in paclitaxel has been methylated and also its acetate group has been replaced with a more lipophilic group. The alternative explanation could be better interaction of the new derivative with the target known as β-tubulin. And this hypothesis can be evaluated by running an *in vitro* target inhibition assay and comparing the inhibitory activity of new derivative and reference compound against the known target.

§3.5 Experimental section

§3.5.1 General Methods and Materials

^{1}H NMR was taken on a Varian 300, 400 or 500 MHz NMR spectrometer of the chemistry department. High-resolution mass spectrametric analyses were conducted with Agilent-TOF. The melting points were carried out on a "Uni-melt" capillary melting point apparatus from Arthur H. Thomas Company, Inc. Optical rotations were measured on Perkin-Elmer Model 241 polarimeter. TLC analysis was accomplished on Merck DC-alufolien with Kieselgel 60F-254 and visualized by UV light and stained with sulfuric acid-EtOH, 10 % Vanillin-EtOH with 1% sulfuric acid. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM).

The chemicals were purchased from Sigma Aldrich Company, Fischer Company or Acros Organic Company. 10-DAB III was donated by Indena, SpA, Italy. Dichloromethane and methanol were dried before use by distillation over calcium hydride under nitrogen. Ether and THF were dried before use by distillation over sodium-benzophenone kept under nitrogen. Dry DMF was purchased from EMD chemical company, and used without further purification. Reaction flasks were dried in 100 ^oC ovens and allowed to cool to room temperature in a desiccator over "*Drierite*" (calcium sulfate) and assembled under an inert nitrogen gas atmosphere.

§3.5.2 Synthetic procedure of SB-T-12301

10-Methyl-7-triethylsilyl-10-DAB III [5-I][(69)]

An aliquot of **3-I** (450 mg, 0.683 mmol) and 1.65 eq of NaH (~ 60%, 45 mg, 1.13 mmol) were dissolved in 7.0 mL of DMF (~ 50 mg/mL) and cooled to 0 ^oC for 10 minutes. To this mixture was added 20 eq of CH_3I (0.85 mL). The mixture was allowed to stirred for 2-4 hours at room temperature and the whole process was monitored *via* TLC. Upon completion, the reaction was quenched with saturated aqueous NH_4Cl (5 mL), extracted with ethyl acetate (3 x 30

mL). The combined organic layers was washed with brine (2 x 45 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done with normal phase chromatography on silica gel (HEX: EtOAc = 2:1) to afford **5-I** (418 mg, 0.622 mmol, 91%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.56 – 0.61 (m, 6 H), 0.93 – 0.98 (m, 9 H), 1.07 (s, 3 H), 1.17 (s, 3 H), 1.67 (s, 3 H), 1.85 - 1.91 (m, 1 H), 2.10 (s, 3 H), 2.23 – 2.26 (m, 2 H), 2.28 (s, 3 H), 2.43 – 2.54 (m, 1 H), 3.41 (s, 3 H), 3.88 (d, J = 6.9 Hz, 1 H), 4.14 (d, J = 8.1 Hz, 1 H), 4.25 (s, 1 H), 4.30 (d, J = 8.1 Hz, 1 H), 4.40 – 4.46 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H), 4.89 – 4.97 (m, 3 H), 5.60 (d, J = 7.2, 1 H), 7.46 (t, J = 7.2 Hz, 2 H), 7.59 (t, J = 7.2 Hz, 1 H), 8.10 (d, J = 7.5 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.63, 7.00, 10.06, 14.40, 15.39, 19.78, 21.26, 22.93, 27.06, 37.54, 38.57, 43.06, 47.54, 56.00, 58.45, 60.61, 68.22, 73.16, 75.06, 79.02, 81.22, 82.89, 84.54, 128.76, 129.68, 130.30, 133.75, 134.01, 143.25, 167.28, 171.02, 204.73. All data are in agreement with literature values. **(69)**

10-Methyl-10-DAB III [5-II] (69)

An aliquot of **5-I** (837 mg, 1.244 mmol) dissolved in 60 mL of acetonitrile / pyridine (1:1, v/v, 30 mL for each), to this solution HF in pyridine (70%, 8.4 mL) was added dropwise. The reaction was allowed to stirred for overnight under room temperature and monitored *via* TLC. Upon completion, the reaction was quenched with saturated aqueous $NaHCO_3$, extracted with ethyl acetate (3 x 100 mL). The combined organic layers was washed with brine (2 x 100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done with normal phase chromatography on silica gel to afford **5-II** (662 mg, 1.182 mmol, 95%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.08 (s, 3 H), 1.16 (s, 3 H), 1.68 (s, 3 H), 1.75 - 1.87 (m, 1 H), 2.09 (s, 3 H), 2.27 – 2.30 (m, 5 H), 2.54 – 2.63 (m, 1 H), 3.46 (s, 3 H), 3.95 (d, J = 7.2 Hz, 1 H), 4.14 (d, J = 5.2 Hz, 1 H), 4.26 – 4.33 (m, 2 H), 4.86 – 4.99 (m, 3 H), 5.63 (d, J = 7.2, 1 H), 7.48 (t, J = 7.2 Hz, 2 H), 7.61 (t, J = 7.5 Hz, 1 H), 8.10 (d, J = 8.4 Hz, 2 H); ^{13}C-NMR (CD_3OD, ppm) δ 10.42, 15.66, 21.16, 22.90, 27.28, 37.80, 40.67, 44.23, 56.44, 59.29, 68.33, 72.85, 76.49, 77.69, 79.49, 82.27, 84.63, 86.20, 129.78, 131.25, 131.78, 134.03, 134.61, 146.92, 167.90, 172.19, 209.26. All data are in agreement with literature values. **(69)**

7,10-Dimethyl-10-DAB III [5-III] (69)

An aliquot of **5-II** (165 mg, 0.295 mmol), 3 eq of proton sponge (190 mg, 0.885 mmol), 2 eq of trimethyloxonium tetrafluoroborate (88 mg, 0.590 mmol), and a certain amount of powdered $MgSO_4$ were suspended in 11 mL of CH_2Cl_2 under room temperature and purged with N_2 atmosphere. This mixture is allowed to stirred for 24 hours. The whole process was monitored *via* TLC. To push the reaction, more reagents, 3 eq of proton sponge (190 mg, 0.885 mmol) and 2 eq of trimethyloxonium tetrafluoroborate (88 mg, 0.590 mmol) were added again. Upon completion, the reaction was quenched with saturated aqueous NH_4Cl (20 mL), extracted with ethyl acetate (3 x 50 mL). The combined organic layers was washed with brine (2 x 60 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done with normal phase chromatography (HEX: EtOAc = 3:2 – 2:3) on silica gel to yield **5-III** (113 mg, 0.197 mmol, 67%) as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 1.07 (s, 3 H), 1.17 (s, 3 H), 1.71 (s, 3 H), 1.78 - 1.83 (m, 1 H), 2.11 (s, 3 H), 2.25 – 2.28 (m, 2 H), 2.29 (s, 3 H), 2.69 – 2.72 (m, 1 H), 3.31 (s, 3 H), 3.47 (s, 3 H), 3.88 – 3.95 (m, 2 H), 4.14 (d, J = 8.1 Hz, 1 H), 4.31 (d, J = 8.1 Hz, 1 H), 4.84 – 4.93 (m, 2 H), 5.01 (d, J = 9.6 Hz, 1 H), 5.59 (d, J = 7.2,

1 H), 7.45 (t, *J* = 8.4 Hz, 2 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 8.10 (d, *J* = 6.9 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 10.48, 15.53, 20.13, 22.96, 27.28, 32.30, 38.60, 43.04, 48.05, 57.16, 57.26, 57.52, 68.31, 74.78, 79.05, 81.11, 81.64, 83.37, 84.40, 128.80, 129.66, 130.30, 133.79, 134.86, 142.83, 167.24, 171.14, 205.69. All data are in agreement with literature values. **(69)**

1(15 → 11)-*abeo*Taxoid derivative byproduct [5-III*]
Obtained after C-13 TES protection as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 0.58 – 0.64 (m, 6 H), 0.97 – 1.01 (m, 9 H), 1.61 (s, 1 H), 1.63 (s, 3 H), 1.73 – 1.75 (m, 4 H), 1.77 - 1.84 (m, 4 H), 2.23 (s, 3 H), 2.33 – 2.38 (m, 1 H), 2.75 – 2.78 (m, 1 H), 3.32 (s, 3 H), 3.37 (s, 3 H), 3.49 (d, *J* = 7.6 Hz, 1 H), 4.04 – 4.08 (dd, J_1 = 9.6 Hz, J_2 = 7.2 Hz, 1 H), 4.20 (d, *J* = 8.0 Hz, 1 H), 4.26 (d, *J* = 8.0 Hz, 1 H), 4.49 (t, *J* = 6.4 Hz, 1 H), 4.67 (s, 1 H), 4.83 (s, 1 H), 4.89 (s, 1 H), 5.10 (d, *J* = 8.8 Hz, 1 H), 5.43 (d, *J* = 7.6, 1 H), 7.45 (t, *J* = 8.0 Hz, 2 H), 7.60 (t, *J* = 7.2 Hz, 1 H), 7.99 (d, *J* = 7.6 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 4.81, 6.78, 9.04, 11.72, 20.86, 21.70, 33.38, 42.93, 55.02, 56.81, 58.34, 63.30, 71.17, 74.46, 75.01, 79.18, 79.31, 81.36, 84.61, 112.72, 128.59, 129.62, 129.70, 133.45, 135.63, 145.50, 149.62, 165.15, 170.17, 206.20; HRMS (TOF) *m/z* calcd for $C_{37}H_{52}O_9Si$ $(M + H)^+$ 669.3453, found 669.3459.

3'-Dephenyl-3'-(2-methyl-1-propenyl)-2'-triisopropylsilyl cabazitaxel [5-IV]
An aliquot of **5-III** (92 mg, 0.161 mmol), and 1.5 eq of (+) **1-VI** (97 mg, 0.242 mmol) were dissolved in 2.0 mL of THF and cooled to -40 °C under inert conditions. To this mixture was added 1.2 eq of LiHMDS in 1.0 M THF (0.2 mL) dropwise. The mixture was stirred at -40 °C and monitored *via* TLC. Upon completion it was quenched with saturated aqueous NH_4Cl (3 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were combined, washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done *via* column chromatography on silica gel (HEX: EtOAc = 2:1) to yield **5-IV** (130 mg, 0.134 mmol, 83%) as a white soild: ^{1}H NMR (500 MHz, CDCl3) δ 1.115 (s, 3 H), 1.123 (s, 18 H), 1.190 (s, 3 H), 1.205 (s, 3 H), 1.342 (s, 9 H), 1.712 (s, 3 H), 1.797 (s, 3 H), 1.798 (s, 3 H), 1.952 (s, 3 H), 2.352 – 2.375 (m, 5 H), 2.668 – 2.729 (m, 1 H), 3.304 (s, 3 H), 3.446 (s, 3 H), 3.857 – 3.903 (m, 2 H), 4.195 (d, *J* = 8.4 Hz, 1 H), 4.305 (d, *J* = 8.4 Hz, 1 H), 4.438 (d, *J* = 2.7 Hz, 1 H), 4.774 – 4.834 (m, 3 H), 4.981 (d, *J* = 8.3 Hz, 1 H), 5.338 (d, *J* = 8.8 Hz, 1 H), 5.641 (d, *J* = 7.2 Hz, 1 H), 6.127 (t, *J* = 8.8 Hz, 1 H), 7.454 (t, *J* = 7.7 Hz, 2 H), 7.595 (t, *J* = 7.5 Hz, 1 H), 8.086 (d, *J* = 7.2 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 10.45, 12.57, 14.24, 14,61, 17.73, 18.02, 18.08, 18.61, 21.11, 22.64, 25.75, 26.49, 28.29, 31.99, 35.47, 43.33, 47.34, 52.10, 56.79, 57.15, 57.22, 60.44, 72.23, 74.89, 75.27, 78.99, 80.67, 81.59, 82.57, 84.25, 122.32, 128.58, 129.47, 130.16, 133.56, 134.87, 139.84, 166.85, 169.95, 205.00.

SB-T-12301 (3'-Dephenyl-3'-(2-methyl-1-propenyl)cabazitaxel) [5-V]
An aliquot of **5-IV** (128 mg, 0.132 mmol) was dissolved in a 1:1 mixture of acetonitrile:pyridine (7 mL) and cooled to 0 °C under inert conditions. To the mixture was added HF/pyridine (70%, 1.4 mL) dropwise. The reaction was stirred at room temperature and monitored *via* TLC. Upon completion it was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate (3 x 25 mL). The organic layers were combined, washed with $CuSO_4$, then brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done *via* column chromatography on silica gel (HEX: EtOAc = 1:1) to yield **5-V** (104 mg, 0.128 mmol, 97%) as a white solid, recrystallization to afford purer compound; m.p. 173 -175 ^oC; $[\alpha]_d$ = (+) 45.7 (methanol): ^{1}H NMR (300 MHz, CDCl3) δ 1.200 (s, 6 H), 1.364 (s, 9 H),

1.711 (s, 3 H), d1.763 (s, 6 H); 1.960 (s, 3 H), 2.285 – 2.373 (m, 5 H), 2.700 (m, 1 H), 3.296 (s, 3 H), 3.445 (s, 3 H), 3.814 – 3.895 (m, 2 H), 4.179 (d, J = 8.4 Hz, 1 H), 4.226 (dd, J_1 = 9.7 Hz, J_2 = 5.4 Hz, 1 H), 4.300 (d, J = 8.1 Hz, 1 H), 4.721 – 4.849 (m, 3 H), 4.982 (d, J = 8.4 Hz, 1 H), 5.305 (d, J = 8.4 Hz, 1 H), 5.627 (d, J = 7.2 Hz, 1 H), 6.163 (t, J = 9.0 Hz, 1 H), 7.460 (t, J = 7.8 Hz, 2 H), 7.597 (t, J = 7.5 Hz, 1 H), 8.073 (d, J = 7.2 Hz, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 10.57, 14.99, 18.83, 20.88, 22.70, 25.98, 26.94, 28.48, 32.34, 35.54, 43.52, 47.65, 51.70, 57.14, 57.27, 57.52, 72.63, 73.97, 74.84, 79.04, 80.17, 80.97, 81.93, 82.90, 84.34, 120.85, 128.81, 129.52, 130.34, 133.83, 135.73, 139.18, 167.13, 170.56, 205.18; HRMS (TOF) *m/z* calcd for $C_{43}H_{59}NO_{14}(M + H)^+$ 814.3936, found 814.3966.

§3.5.3 Synthetic procedure of 2-Debenzoyl-2-*m*-methylbenzoyl-SB-T-12301 and 2-Debenzoyl-2-*m*-fluorobenzoyl-SB-T-12301

7, 10-Dimethyl-13-triethylsilyl-10-DAB III [5-VI]

An aliquot of **5-III** (357 mg, 0.608 mmol) and 4.0 eq of imidazole (165 mg, 2.43 mmol) were dissolved in 7.0 mL of DMF (~ 50 mg/mL) and cooled to 0 °C under inert conditions. At 0 ^{o}C, an aliquot of chlorotriethylsilane (0.32 mL, 1.83 mmol) was added dropwise. The mixture was stirred and allowed to warm to room temperature. The reaction was monitored by TLC and upon completion, it was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by normal phase column chromatography on silica gel (HEX: EtOAc = 2:1) to yield **5-VI** (298 mg, 0.434 mmol, 70%) as a white solid: ^{1}H-NMR ($CDCl_3$) 0.67 – 0.69 (m, 6 H), 1.00 – 1.04 (m, 9 H), 1.14 (s, 3 H), 1.16 (s, 3 H), 1.69 (s, 3 H), 1.77 – 1.78 (m, 1 H), 2.06 (s, 3 H), 2.14 – 2.23 (m, 2 H), 2.29 (s, 3 H), 2.69 - 2.70 (m, 1 H), 3.31 (s, 3 H), 3.46 (s, 3 H), 3.84 (d, J = 6.8 Hz, 1 H), 3.90 – 3.95 (dd, J_1 = 10.8 Hz, J_2 = 6.4 Hz, 1 H), 4.14 (d, J = 8.4 Hz, 1 H), 4.29 (d, J = 8.4 Hz, 1 H), 4.82 (s, 1 H), 4.98 – 5.02 (m, 2 H), 5.59 (d, J = 8.4 Hz, 1 H), 7.47 (t, J = 8.4 Hz, 1 H), 7.32 - 7.41 (m, 2 H), 7.89 – 7.93 (m, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.07, 7.15, 10.58, 15.44, 21.29, 22.63, 27.00, 32.16, 40.08, 43.28, 47.74, 56.83, 57.30, 57.60, 68.76, 75.30, 77.15, 79.76, 81.03, 81.49, 83.35, 84.36, 128.78, 129.71, 130.26, 133.63, 133.75, 144.52, 167.26, 170.31, 205.92.

2-Debenzoyl-7, 10-dimethyl-13-triethylsilyl-10-DAB III [5-VII]

An aliquot of **5-VI** (298 mg, 0.434 mmol) was dissolved in 6.0 mL of THF (~ 50 mg/mL) and cooled to -50 ^{o}C under inert conditions. Then an aliquot of sodium bis(2-methoxyethyoxy) aluminum hydride, 65 wt% in toluene (0.22 mL, 2.32 mmol) was added to the solution dropwise. The reaction was monitored *via* TLC and upon completion, it was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers was washed with brine (2 x 30 mL), dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 3:2) to afford **5-VII** (244 mg, 0.365 mmol, 84 %) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) 0.63 – 0.69 (m, 6 H), 0.99 – 1.03 (m, 9 H), 1.06 (s, 3 H), 1.17 (s, 3 H), 1.66 (s, 3 H), 1.76 – 1.83 (m, 1 H), 1.89 – 1.95 (m, 1 H), 2.00 (s, 3 H), 2.07 – 2.11 (m, 1 H), 2.17 – 2.20 (m, 4 H), 2.45 (d, J = 5.6 Hz, 1 H), 2.67 - 2.74 (m, 1 H), 3.29 (s, 3 H), 3.43 (s, 3 H), 3.45 (d, J = 5.8 Hz, 1 H), 3.83 – 3.89 (m, 2 H), 4.57 – 4.64 (m, 2 H), 4.76 (s, 1 H), 4.98

– 5.03 (m, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.08, 7.17, 10.74, 15.42, 21.24, 22.66, 26.72, 32.30, 40.67, 42.79, 47.67, 56.86, 57.30, 57.56, 68.81, 74.88, 78.02, 79.03, 81.25, 82.70, 83.44, 84.01, 133.79, 144.23, 170.08, 206.48.

2-Debenzoyl-2-(3-methybenzoyl)-7,10-dimethyl-13-triethylsilyl-10-DAB III [5-VIIIa]
An aliquot of **5-VII** (50 mg, 0.086 mmol), 8 eq of DMAP (84 mg, 0.686 mmol), and 8 eq of 3-methylbenzoic acid (94 mg, 0.686 mmol) were dissolved in CH_2Cl_2 (1.0 mL) under inert conditions. An aliquot of DIC (0.11 mL, 0.686 mmol) was added to the mixture dropwise and allowed to stirred at room temperature for 7 days. The reaction was monitored *via* TLC and upon completion it was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate (3 x 10 mL). The combined organic layers was washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done using column chromatography on silica gel (HEX: EtOAc = 85:15) that afforded **5-VIIIa** (51 mg, 0.073 mmol, 85%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) 0.65 – 0.72 (m, 6 H), 1.01 – 1.05 (m, 9 H), 1.15 (s, 3 H), 1.17 (s, 3 H), 1.70 (s, 3 H), 1.74 – 1.81 (m, 1 H), 2.07 (s, 3 H), 2.12 – 2.26 (m, 2 H), 2.30 (s, 3 H), 2.41 (s, 3 H), 2.67 - 2.75 (m, 1 H), 3.31 (s, 3 H), 3.47 (s, 3 H), 3.84 (d, J = 6.4 Hz, 1 H), 3.91 – 3.95 (dd, J_1 = 10.8 Hz, J_2 = 6.4 Hz, 1 H), 4.15 (d, J = 8.4 Hz, 1 H), 4.30 (d, J = 8.4 Hz, 1 H), 4.83 (s, 1 H), 4.96 – 5.03 (m, 2 H), 5.57 (d, J = 6.4 Hz, 1 H), 7.34 – 7.42 (m, 2 H), 7.88 – 7.92 (m, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.06, 7.15, 10.59, 15.43, 21.30, 21.54, 22.57, 26.96, 32.14, 40.12, 43.24, 47.75, 56.80, 57.30, 57.59, 68.75, 75.17, 76.65, 79.82, 81.04, 81.50, 83.34, 84.34, 127.41, 128.67, 129.64, 130.94, 133.63, 134.47, 138.47, 144.50, 167.36, 170.26, 205.90.

4-Deacetyl-2-debenzoyl-2-(3-methylbenzoyl)-7,10-dimethyl-13-triethylsilyl-10-DAB III (5-VIIIa*)
^{1}H-NMR (400 MHz, $CDCl_3$, ppm) 0.76 – 0.85 (m, 6 H), 1.03 (s, 3 H), 1.07 – 1.11 (m, 9 H), 1.18 (s, 3 H), 1.59 (s, 3 H), 1.79 – 1.86 (m, 1 H), 2.06 (s, 3 H), 2.32 – 2.41 (m, 4 H), 2.59 – 2.68 (m, 2 H), 3.29 (s, 3 H), 3.46 (s, 3 H), 3.50 – 3.58 (m, 2 H), 4.70 (d, J = 9.3 Hz, 1 H), 4.83 (dd, J_1 = 9.8 Hz, J_2 = 3.4 Hz, 1 H), 4.87 (s, 1 H), 4.96 – 5.03 (m, 2 H), 5.51 (d, J = 5.4 Hz, 1 H), 7.31 – 7.39 (m, 2 H), 7.90 – 7.93 (m, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 4.87, 7.09, 10.22, 15.43, 18.00, 18.20, 21.46, 29.98, 32.08, 38.23, 42.30, 53.30, 57.03, 57.43, 57.72, 70.11, 73.30, 75.50, 79.50, 81.44, 84.12, 88.46, 127.60, 128.61, 129.43, 130.96, 134.46, 137.78, 138.49, 141.01, 167.28, 205.40.

2-Debenzoyl-2-(3-fluorobenzoyl)-7,10-dimethyl-13-triethylsilyl-10-DAB III [5-VIIIb]
An aliquot of **5-VII** (62 mg, 0.106 mmol), 8 eq of DMAP (104 mg, 0.851 mmol), and 8 eq of 3-fluorobenzoic acid (119 mg, 0.851 mmol) were dissolved in CH_2Cl_2 (1.2 mL) under inert conditions. An aliquot of DIC (0.14 mL, 0.851 mmol) was added dropwise to the mixture and allowed to stirred at room temperature for 7 days. The reaction was monitored *via* TLC and upon completion it was quenched with saturated NH_4Cl (2 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers was washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done using column chromatography on silica gel (HEX: EtOAc = 8:2) that afforded **5-VIIIb** (61 mg, 0.086 mmol, 81%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) 0.66 – 0.72 (m, 6 H), 1.01 – 1.05 (m, 9 H), 1.15 (s, 3 H), 1.17 (s, 3 H), 1.70 (s, 3 H), 1.75 – 1.81 (m, 1 H), 2.05 (s, 3 H), 2.07 – 2.23 (m, 2 H), 2.30 (s, 3 H), 2.68 - 2.75 (m, 1 H), 3.32 (s, 3 H), 3.47 (s, 3 H), 3.85 (d, J

= 7.2 Hz, 1 H), 3.91 – 3.95 (dd, J_1 = 10.8 Hz, J_2 = 6.0 Hz, 1 H), 4.15 (d, J = 8.0 Hz, 1 H), 4.30 (d, J = 8.0 Hz, 1 H), 4.83 (s, 1 H), 4.96 – 5.03 (m, 2 H), 5.57 (d, J = 7.2 Hz, 1 H), 7.29 – 7.33 (m, 1 H), 7.43 – 7.49 (m, 1 H), 7.77 (d, J = 6.8 Hz, 1 H), 7.88 (d, J = 6.0 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.06, 7.14, 10.56, 15.44, 21.26, 22.56, 26.97, 32.14, 40.04, 43.21, 47.72, 56.79, 57.30, 57.62, 68.69, 75.77, 76.50, 79.86, 80.99, 81.45, 83.33, 84.36, 117.10, 120.85, 126.05, 130.47, 131.89, 133.53, 144.59, 161.57, 164.03, 166.02, 170.27, 205.81.

2-Debenzoyl-2-(3-methylbenzoyl)-7, 10-dimethyl-10-DAB III [5-IXa]
An aliquot of **5-VIIIa** (51 mg, 0.073 mmol) was dissolved in a 1:1 mixture of acetonitrile:pyridine (3.6 mL) and cooled to 0 °C under inert conditions. To the mixture was added HF/pyridine (0.5 mL, 70%) dropwise. The reaction was stirred at room temperature and monitored *via* TLC. Upon completion it was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, washed with saturated aqueous $CuSO_4$, then brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by normal phase column chromatography on silica gel (HEX: EtOAc = 1:1 – 1:4) to yield **5-VIIIa** (43 mg, 0.072 mmol, 99%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) 1.08 (s, 3 H), 1.17 (s, 3 H), 1.71 (s, 3 H), 1.75 – 1.82 (m, 1 H), 2.12 (s, 3 H), 2.21 – 2.38 (m, 5 H), 2.43 (s, 3 H), 2.69 – 2.76 (m, 1 H), 3.32 (s, 3 H), 3.47 (s, 3 H), 3.89 – 3.95 (m, 2 H), 4.16 (d, J = 8.4 Hz, 1 H), 4.32 (d, J = 8.4 Hz, 1 H), 4.85 (s, 1 H), 4.89 (dd, J_1 = 9.0 Hz, J_2 = 2.7 Hz, 1 H), 5.02 (d, J = 8.0 Hz, 1 H), 5.57 (d, J = 6.4 Hz, 1 H), 7.34 – 7.42 (m, 2 H), 7.90 – 7.94 (m, 2 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 10.47, 14.41, 15.51, 20.12, 21.27, 21.59, 22.90, 27.26, 32.30, 38.60, 43.03, 48.05, 57.13, 57.25, 57.51, 60.61, 68.32, 74.61, 79.05, 81.12, 81.67, 83.37, 84.36, 127.43, 128.67, 129.55, 130.97, 134.52, 134.86, 138.50, 142.78, 167.36, 171.08, 205.67; HRMS (TOF) *m/z* calcd for $C_{32}H_{43}O_{10}$ $(M+H)^+$, 587.2851, found 587.2850.

7,10,13-Triethylsilyl-10-DAB III [6-I] (13)
An aliquot of 10-DAB III (539 mg, 0.989 mmol) and 5.0 eq of imidazole (344 mg, 4.95 mmol) were dissolved in 11 mL of DMF (~ 50 mg/mL) and cooled down to 0 °C in ice bath under inert conditions. To this mixture chlorotriethylsilane (1.14 mL, 4.95 mmol) was added dropwise. The mixture was stirred and allowed to warm to room temperature over 48 hours while being monitored by TLC. Upon completion the reaction was quenched with saturated NH_4Cl (10 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers was washed with brine (2 x 45 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done *via* normal phase column chromatography on silica gel (HEX: EtOAc = 9:1) to yield **6-I** (702 mg, 0.792 mmol, 80%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.55 -0.71 (m, 6 H), 0.96 – 1.02 (m, 9 H), 1.13 (s, 3 H), 1.19 (s, 3 H), 1.65 (s, 3 H), 1.86 – 1.92 (m, 1 H), 1.98 (s, 3 H), 2.05 – 2.28 (m, 5 H), 2.28 (s, 3 H), 2.42 – 2.52 (m, 1H), 3.85 (d, J = 7.2 Hz, 1H), 4.14 (d, J = 8.4 Hz, 1H), 4.28 (d, J = 8.4 Hz, 1H), 4.41 (dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, 1H), 4.93 – 4.96 (m, 2 H), 5.19 (s, 1H), 5.62 (d, J = 7.2 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 7.57 (t, J = 7.6 Hz, 1H), 8.08 (d, J = 1.2 Hz, 2H). All data are in agreement with literature values. [13]

2-Debenzoyl-7,10,13-tri(triethylsilyl)-10-DAB III [6-II] (13)
An aliquot of **6-I** (694 mg, 0.784 mmol) was dissolved in 14 mL of THF (~ 50 mg/mL) and cooled down to -50 °C in dry ice–acetone bath under inert conditions. An aliquot of sodium

bis(2-methoxyethyoxy) aluminum hydride, 65 wt% in toluene (0.38 mL, 2.32 mmol) was added to the solution dropwise. The reaction was monitored *via* TLC and upon completion, it was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers was washed with brine (2 x 45 mL), dried over anhydrous $MgSO_4$, and was concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 3:2) to afford **6-II** (479 mg, 0.612 mmol, 78 %) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.56 -0.67 (m, 18 H), 0.93 – 1.01 (m, 27 H), 1.08 (s, 3 H), 1.15 (s, 3 H), 1.61 (s, 3 H), 1.86 – 1.92 (m, 5 H), 2.01 – 2.07 (m, 1 H), 2.16 (s, 3 H), 2.35 (s, 1 H), 2.47 – 2.55 (m, 2 H), 3.45 (d, J = 7.8 Hz, 1 H), 3.86 (t, J = 6.4 Hz, 1 H), 4.34 (dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, 1 H), 4.54 (d, J = 9.2 Hz, 1 H), 4.61 (d, J = 9.2 Hz, 1 H), 4.93 – 4.95 (m, 2 H), 5.11 (s, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.06, 5.43, 6.18, 7.07, 7.12, 7.17, 10.84, 14.73, 20.83, 22.63, 26.25, 37.63, 40.70, 42.74, 47.07, 58.48, 68.61, 72.98, 75.16, 76.05, 78.23, 78.96, 82.34, 83.86, 136.19, 139.20, 169.86, 206.48. All data are in agreement with literature values. [(13)]

2-Debenzoyl-2-(3-methylbenzoyl)-7,10,13-tri(triethylsilyl)-10-DAB III [6-IIIa][(3)]
An aliquot of **6-II** (416 mg, 0.531 mmol), 8 eq of DMAP, and 8 eq of 3-methylbenzoic acid (578 mg, 4.25 mmol) were dissolved in CH_2Cl_2 (5.2 mL) under inert conditions. To the mixture an aliquot of DIC (0.66 mL, 4.25 mmol) was added dropwise and allowed to stirred at room temperature for 7 days. The reaction was monitored *via* TLC and upon completion it was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate (3 x 25 mL). The combined organic layers was washed with brine (2 x 30 mL), dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 9:1) to afford **6-IIIa** (386 mg, 0.428 mmol, 81%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.55 -0.70 (m, 18H), 0.94 – 1.04 (m, 27H), 1.13 (s, 3H), 1.19 (s, 3H), 1.65 (s, 3H), 1.85 – 1.92 (m, 1H), 1.98 (s, 3H), 2.07 – 2.13 (m, 1H), 2.20 – 2.26 (m, 1H), 2.28 (s, 3H), 2.40 (s, 3H), 2.48 – 2.56 (m, 1H), 3.85 (d, J = 6.8 Hz, 1H), 4.14 (d, J = 8.4 Hz, 1H), 4.28 (d, J = 8.4 Hz, 1H), 4.39 – 4.43 (dd, J_1 = 10.5 Hz, J_2 = 6.4 Hz, 1H), 4.90 – 4.96 (m, 2H), 5.19 (s, 1H), 5.59 (d, J = 7.2 Hz, 1H), 7.32 - 7.40 (m, 2H), 7.87 – 7.92 (m, 2H). All data are in agreement with literature values. [(3)]

2-Debenzoyl-2-(3-fluorobenzoyl)-7,10,13-triethylsilyl-10-DAB III [6-IIIb] [(78)]
An aliquot of **6-II** (0.261 mmol, 204 mg), 8 eq of DMAP, and 8 eq of *meta*-fluorobenzoic acid (292 mg, 2.09 mmol) were dissolved in CH_2Cl_2 (3.0 mL) under inert conditions. An aliquot of DIC (0.33 mL, 2.09 mmol) was added to the mixture dropwise and allowed to stirred at room temperature for 7 days. The reaction was monitored *via* TLC and upon completion it was quenched with saturated NH_4Cl (3.0 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layers was washed with brine (2 x 20 mL), dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 9:1) to afford **6-IIIb** (189 mg, 0.209 mmol, 80%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.56 – 0.71 (m, 18 H), 0.95 – 1.05 (m, 27 H), 1.14 (s, 3 H), 1.20 (s, 3 H), 1.66 (s, 3 H), 1.86 – 1.93 (m, 1 H), 1.99 (s, 3 H), 2.05 – 2.24 (m, 2 H), 2.29 (s, 3 H), 2.42 (s, 3 H), 2.50 – 2.57 (m, 1 H), 3.86 (d, J = 7.2 Hz, 1 H), 4.14 (d, J = 8.4 Hz, 1H), 4.28 (d, J = 8.4 Hz, 1 H), 4.42 (dd, J_1 = 10.6 Hz, J_2 = 6.6 Hz, 1 H), 4.91 – 4.97 (m, 2 H), 5.20 (s, 1 H), 5.59 (d, J = 7.2 Hz, 1 H), 7.30 - 7.32 (m, 1 H), 7.43 – 7.48 (m, 1 H); 7.78 (d, J = 8.8 Hz, 1 H), 7.88 (d, J = 6.8 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.09, 5.47,

6.20, 7.01, 7.12, 7.18, 10.65, 14.33, 14.80, 20.82, 22.56, 22.87, 26.54, 29.28, 31.81, 34.74, 37.50, 40.02, 43.20, 47.14, 58.44, 68.51, 72.88, 75.99, 76.21, 79.88, 81.02, 84.23, 116.98, 117.22, 120.66, 120.87, 126.08, 130.48, 132.12, 135.92, 139.71, 161.6, 164.03, 166.14, 170.13, 205.84. All data are in agreement with literature values. [78]

2-Debenzoyl-2-(3-methylbenzoyl)-10-DAB III [6-IVa] [3]

An aliquot of **6-IIIa** (386 mg, 0.428 mmol) was dissolved in a 1:1 mixture of acetonitrile:pyridine (15 mL) and cooled down to 0 °C under inert conditions. An aliquot of HF/pyridine (70%, 4.8 mL) was added dropwise and allowed to stirred at room temperature for overnight. The reaction was monitored by TLC and upon completion, it was quenched with 10 % citric acid (10 mL) and extracted with ethyl acetate (3 x 40 mL). The organic layers were combined and washed with saturated aqueous $CuSO_4$ and brine, dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 1:2) to yield **6-IVa** (229 mg, 0.410 mmol, 96%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 1.10 (s, 6H), 1.74 (s, 3H), 1.80 – 1.86 (m, 1H), 2.00 – 2.03 (m, 1H), 2.08 (s, 3H), 2.20 – 2.26 (m, 1H), 2.29 (s, 3H), 2.43 (s, 3H), 2.57 – 2.64 (m, 1H), 4.00 (d, J = 6.8 Hz, 1H), 4.16 (d, J = 8.4 Hz, 1H), 4.25 – 4.29 (dd, J_1 = 10.5 Hz, J_2 = 7.2 Hz, 1H), 4.33 (d, J = 8.4 Hz, 1H), 4.86 – 4.89 (m, 1H), 4.98 (d, J = 8.0 Hz, 1H), 5.25 (s, 1H), 5.61 (d, J = 7.2 Hz, 1H), 7.36 - 7.41 (m, 2H), 7.89 – 7.93 (m, 2H). All data are in agreement with literature values. [3]

2-Debenzoyl-2-(3-fluorobenzoyl)-10-DAB III [6-IVb] [78]

An aliquot of **6-IIIb** (188 mg, 0.208 mmol) was dissolved in a 1:1 mixture of acetonitrile:pyridine (7.4 mL) and cooled to 0 °C under inert conditions. To this solution an aliquot of HF/pyridine (2.3 mL) was added dropwise and allowed to stirred at room temperature for overnight. The reaction was monitored *via* TLC and upon completion, it was quenched with 10 % citric acid (20 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers was washed with saturated aqueous $CuSO_4$ and brine, dried over anhydrous $MgSO_4$, and concentrated in *vacuo*. Purification was done by flash column chromatography on silica gel (HEX: EtOAc = 1:3) to yield **6-IVb** (114 mg, 0.202 mmol, 97%) as a white solid: ^{1}H-NMR (400 MHz, CD_3OD, ppm) δ 1.08 (s, 3 H), 1.69 (s, 3 H), 1.78 – 1.85 (m, 1 H), 2.04 (s, 3 H), 2.20 – 2.24 (m, 1 H), 2.27 (s, 3 H), 2.32 – 2.37 (m, 1 H), 2.42 – 2.49 (m, 1 H), 3.98 (d, J = 7.2 Hz, 1 H), 4.1 - 4.22 (m, 2 H), 4.27 (dd, J_1 = 11.2 Hz, J_2 = 6.6 Hz, 1 H), 4.80 (t, J = 7.8 Hz, 1 H), 5.02 (d, J = 8.0 Hz, 1 H), 5.32 (s, 1 H), 5.59 (d, J = 7.2 Hz, 1 H), 7.37 - 7.41 (m, 1 H), 7.53 – 7.58 (m, 1 H); 7.80 (d, J = 9.6 Hz, 1 H), 7.94 (d, J = 8.0 Hz, 1 H); ^{13}C-NMR (CD_3OD, ppm) δ 10.48, 15.41, 20.96, 22.81, 27.35, 37.69, 40.83, 44.09, 59.04, 68.29, 72.93, 76.41, 77.15, 77.66, 79.54, 82.18, 86.11, 117.84, 127.13, 131.86, 135.89, 144.83, 172.09, 211.92. All data are in agreement with literature values. [78]

2-Debenzoyl-2-(3-methylbenzoyl)- 7-triethylsilyl-10-DAB III [6-Va] [3]

An aliquot of **6-IVa** (229 mg, 0.410 mmol) and 4.0 eq of imidazole (112 mg, 1.64 mmol) were dissolved in 4.5 mL of DMF (~ 50 mg/mL) and cooled to 0 °C under inert conditions. At 0 ^{o}C, an aliquot of chlorotriethylsilane (0.21 mL, 1.23 mmol) was added dropwise. The mixture was stirred and allowed to warm to room temperature. The reaction was monitored by TLC and upon completion, it was quenched with saturated NH_4Cl (2 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layer was washed with brine, dried over

anhydrous $MgSO_4$, and was concentrated *in vacuo*. Purification was done by normal phase column chromatography on silica gel (HEX: EtOAc = 3:1) to yield **6-Va** (245 mg, 0.364 mmol, 89 %) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.53 -0.57 (m, 6H), 0.92 – 0.95 (m, 9H), 1.08 (s, 6H), 1.73 (s, 3H), 1.87 – 1.94 (m, 1H), 2.01 – 2.03 (m, 1H), 2.08 (s, 3H), 2.25 – 2.27 (m, 1H), 2.29 (s, 3H), 2.42 (s, 3H), 2.44 – 2.51 (m, 1H), 3.94 (d, J = 7.2 Hz, 1H), 4.15 (d, J = 8.0 Hz, 1H), 4.25 (s, 1H), 4.31 (d, J = 8.4 Hz, 1H), 4.38 – 4.43 (dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, 1H), 4.85 – 4.88 (m, 1H), 4.95 (d, J = 8.0 Hz, 1H), 5.17 (s, 1H), 5.58 (d, J = 6.8 Hz, 1H), 7.33 - 7.42 (m, 2H), 7.89 – 7.93 (m, 2H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.37, 6.94, 10.13, 15.37, 19.71, 21.58, 22.79, 27.10, 37.47, 38.78, 42.91, 47.21, 58.15, 68.24, 73.18, 74.85, 79.02, 81.01, 84.45, 127.43, 128.68, 129.52, 130.95, 134.55, 135.46, 138.52, 141.87, 167.40, 170.96, 210.54. All data are in agreement with literature values. [3]

2-Debenzoyl-2-(3-fluorobenzoyl)-7-triethylsilyl-10-deacetylbaccatin III [6-Vb] [78]
An aliquot of **6-IVb** (114 mg, 0.202 mmol) and 4.0 eq of imidazole (55 mg, 0.808 mmol) were dissolved in 2.5 mL of DMF (~ 50 mg/mL) and cooled to 0 °C under inert conditions. At 0 ^{o}C, an aliquot of chlorotriethylsilane (0.11 mL, 0.606 mmol) was added dropwise. The mixture was stirred and allowed to warm to room temperature. The reaction was monitored by TLC and upon completion, it was quenched with saturated NH_4Cl (2 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers was washed with brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done by normal pahse column chromatography on silica gel (HEX: EtOAc = 2:1) to yield **6-Vb** (117 mg, 0.187 mmol, 92 %) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.49 – 0.64 (m, 6 H), 0.93 – 0.97 (m, 9 H), 1.09 (s, 6 H), 1.74 (s, 3 H), 1.88 – 1.95 (m, 1 H), 2.03 (d, J = 4.8 Hz, 1 H), 2.09 (s, 3 H), 2.26 (d, J = 8.0 Hz, 1 H), 2.30 (s, 3 H), 2.45 – 2.51 (m, 1 H), 3.96 (d, J = 7.2 Hz, 1 H), 4.16 (d, J = 8.4 Hz, 1H), 4.25 (s, 1 H), 4.31 (d, J = 8.4 Hz, 1 H), 4.42 (dd, J_1 = 10.4 Hz, J_2 = 6.4 Hz, 1 H), 4.86 – 4.91 (m, 1 H), 4.97 (d, J = 8.0 Hz, 1 H), 5.18 (s, 1 H), 5.58 (d, J = 7.2 Hz, 1 H), 7.30 - 7.34 (m, 1 H), 7.44 – 7.50 (m, 1 H); 7.79 (d, J = 8.1 Hz, 1 H), 7.90 (d, J = 7.8 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.39, 6.96, 10.14, 14.42, 15.41, 19.71, 22.81, 27.10, 37.46, 38.77, 42.88, 47.21, 58.16, 60.62, 68.18, 73.18, 74.89, 75.48, 79.10, 80.95, 84.49, 117.00, 117.23, 120.82, 121.03, 126.10, 130.47, 130.55, 131.83, 135.35, 142.00, 164.03, 166.08, 170.97, 210.47. All data are in agreement with literature values. [78]

2-Debenzoyl-2-(3-methylbenzoyl)-10-methyl-7-triethylsilyl-10-DAB III [6-VIa]
An aliquot of **6-Va** (240 mg, 0.357 mmol) and 1.6 eq of NaH (~60%, 24 mg, 0.572 mmol) were dissolved in 5.0 mL of DMF (~ 50 mg/mL) and cooled to 0 ^{o}C for 10 minutes. To this mixture was added 20 eq of CH_3I (0.45 mL, 7.14 mmol) dropwise. The mixture was allowed to stirred for 2-4 hours at this temperature and the whole process was monitored *via* TLC. Upon completion, the reaction was quenched with saturated aqueous NH_4Cl (3 mL), extracted with ethyl acetate (3 x 15 mL). The combined organic layers was washed with brine (2 x 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done with normal phase chromatography (HEX: EtOAc = 2:1) on silica gel to afford **6-VIa** (193 mg, 0.281 mmol, 79%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.52 -0.63 (m, 6H), 0.94 – 0.98 (m, 9H), 1.06 (s, 3H), 1.17 (s, 3H), 1.67 (s, 3H), 1.85 – 1.92 (m, 1H), 2.10 (s, 3H), 2.24 – 2.26 (m, 2H), 2.28 (s, 3H), 2.41 (s, 3H), 2.44 – 2.52 (m, 1H), 3.41 (s, 3H), 3.87 (d, J = 7.2 Hz, 1H), 4.13 (d, J = 8.4 Hz, 1H), 4.29 (d, J = 8.4 Hz, 1H), 4.40 – 4.45 (dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, 1H), 4.90 – 4.95 (m, 3H), 5.57 (d, J = 8.4 Hz, 1H), 7.32 - 7.41 (m, 2H), 7.89 – 7.93 (m, 2H);

^{13}C-NMR ($CDCl_3$, ppm) δ 5.64, 7.00, 10.09, 14.40, 15.39, 19.79, 21.26, 21.59, 22.88, 27.06, 37.55, 38.56, 43.07, 47.55, 56.00, 58.45, 60.62, 68.26, 73.19, 74.90, 79.05, 81.28, 82.90, 84.52, 127.44, 128.65, 129.59, 130.98, 134.07, 134.50, 138.48, 143.17, 167.41, 170.99, 204.71.

2-Debenzoyl-2-(3-fluorobenzoyl)-10-methyl-7-triethylsilyl-10-DAB III [6-VIb]

An aliquot of **6-Vb** (117 mg, 0.187 mmol) and 1.6 eq of NaH (~60%, 12 mg, 0.299 mmol) were dissolved in 2.4 mL of DMF (~ 50 mg/mL) and cooled to 0 ^{o}C for 10 minutes. To this mixture was added 20 eq of CH_3I (0.24 mL, 3.74 mmol) dropwise. The mixture was allowed to stirred for 2-4 hours at this temperature and the whole process was monitored *via* TLC. Upon completion, the reaction was quenched with saturated aqueous NH_4Cl (2 mL), extracted with ethyl acetate (3 x 10 mL). The combined organic layers was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification was done by normal phase chromatography (HEX: EtOAc = 3:2) on silica gel that afforded **6-VIb** (113 mg, 0.164 mmol, 88%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 0.53 – 0.65 (m, 6 H), 0.94 – 0.98 (m, 9 H), 1.07 (s, 3 H), 1.17 (s, 3 H), 1.66 (s, 3 H), 1.86 – 1.93 (m, 1 H), 2.10 (s, 3 H), 2.19 (broad s, 1 H), 2.22 – 2.29 (m, 5 H), 2.46 – 2.54 (m, 1 H), 3.42 (s, 3 H), 3.88 (d, J = 6.8 Hz, 1 H), 4.14 (d, J = 7.2 Hz, 1H), 4.29 (d, J = 8.4 Hz, 1 H), 4.43 (dd, J_1 = 10.4 Hz, J_2 = 6.8 Hz, 1 H), 4.91 – 4.98 (m, 3 H), 5.57 (d, J = 6.8 Hz, 1 H), 7.30 - 7.33 (m, 1 H), 7.43 – 7.48 (m, 1 H); 7.78 (d, J = 8.1 Hz, 1 H), 7.90 (d, J = 7.8 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, ppm) δ 5.43, 6.79, 9,87, 15.20, 19.56, 22.67, 26.85, 37.31, 38.32, 42.81, 47.33, 55.81, 58.22, 67.96, 72.95, 75.32, 78.90, 80.97, 82.67, 84.33, 116.78, 117.01, 120.54, 120.75, 125.92, 130.22, 130.30, 131.67, 131.74, 133.71, 143.12, 161.33, 163.80, 165.86, 170.78, 204.46; HRMS (TOF) *m/z* calcd for $C_{36}H_{55}FNO_{10}Si$ $(M + NH_4)^+$, 708.3574, found 708.3569.

2-Debenzoyl-2-(3-methylbenzoyl)- 10-methyl-10-deacetylbaccatin III [6-VIIa]

An aliquot of **6-VIa** (193 mg, 0.281 mmol) dissolved in 14 mL acetonitrile / pyridine (1:1), to this solution 2.0 mL of HF in pyridine (70%) was added dropwise. The reaction was allowed to stirred for overnight under room temperature and monitored *via* TLC. Upon completion it was quenched with 10 % citric acid (5 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers was washed with saturated aqueous $CuSO_4$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification was done using column chromatography on silica gel (HEX: EtOAc = 1:2) to yield **6-VIIa** (158 mg, 0.276 mmol, 98%) as a white solid: ^{1}H-NMR (400 MHz, $CDCl_3$, ppm) δ 1.08 (s, 3H), 1.16 (s, 3H), 1.68 (s, 3H), 1.76 – 1.83 (m, 1H), 2.09 (s, 3H), 2.25 – 2.35 (m, 2H), 2.28 (s, 3H), 2.42 (s, 3H), 2.56 – 2.64 (m, 1H), 3.46 (s, 3H), 3.94 (d, J = 7.2 Hz, 1H), 4.15 (d, J = 5.2 Hz, 1H), 4.29 (d, J = 8.4 Hz, 1H), 4.89 – 5.00 (m, 1H), 5.61 (d, J = 7.2 Hz, 1H), 7.34 - 7.42 (m, 2H), 7.89 – 7.93 (m, 2H); ^{13}C-NMR **(**$CDCl_3$, ppm) δ 9.82, 15.45, 20.18, 21.60, 22.80, 26.92, 37.40, 38.66, 42.92, 47.45, 57.05, 58.14, 68.21, 72.34, 74.85, 79.10, 81.21, 83.44, 84.46, 127.45, 128.71, 129.53, 130.98,134.34, 138.56, 143.27, 167.44, 170.88, 207.55; HRMS (TOF) *m/z* calcd for $C_{31}H_{44}NO_{10}$ $(M + NH_4)^+$, 590.296, found 590.2947.

§4.0 References

1. Boyle, P.; Levin, B., *World Cancer Report*. World Health Organization: **2008**.
2. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Thun, M. J., Cancer statistics, 2009. *CA Cancer J Clin* **2009,** *59*, 225-49.
3. Parasram, M. Synthesis of Next Generation Taxanes and Tumor-Targeting Taxane-Based Drug Conjugates using Linolenic Acid as Tumor-Targeting Module. Stony Brook University, **2011**.
4. Chen, S.; Zhao, X.; Chen, J.; Chen, J.; Kuznetsova, L.; Wong, S. S.; Ojima, I., Mechanism-based tumor-targeting drug delivery system. Validation of efficient vitamin receptor-mediated endocytosis and drug release. *Bioconjugate chem.* **2010,** *21*, 979-87.
5. Schiff, P. B.; Fant, J.; Horwitz, S. B., Promotion of microtubule assembly in vitro by taxol. *Nature* **1979,** *277*, 665-7.
6. Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Taxane Anticancer Agents: Basic Science and Current Status. In *American Chemical Society*, Washington D.C., **1995**.
7. Hervé, B., Cabazitaxel: Discovery and Development. In *BIT's 2nd international conference of Medichem*, **2011**.
8. Kingston, D. G., Tubulin-interactive natural products as anticancer agents. *J. Nat. Prod.* **2009,** *72*, 507-15.
9. Suffness, M., *Taxol: Science and Applications*. CRC Press: New York, **1995**.
10. Ojima, I.; Kamath, A.; Seitz, J. D., *Taxol, toxoids and related taxanes, Natural products in medicinal chemistry*. Wiley-VCH: **2013**.
11. Denis, J. N.; Greene, A. E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P., Highly efficient, practical approach to natural taxol. *J. Am. Chem. Soc.* **1988,** *110*, 5917-5919.
12. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T., New and efficient approaches to the semisynthesis of taxol and its C-13 side chain analogs by means of β- lactam synthon method. *Tetrahedron Lett.* **1992,** *48*, 6985-7012.
13. Sun, L. Design, Synthesis, Biological Evaluation and Molecular Modeling of Novel Taxane-Based Anticancer Agents and Paclitaxel Mimics. Stony Brook University, **2008**.
14. Rowinsky, E. K., The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu. Rev. Med.* **1997,** *48*, 353-74.
15. Wender, P. A.; Galliher, W. C.; Bhat, N. M.; Pillow, T. H.; Bieber, M. M.; Teng, N. N., Taxol-oligoarginine conjugates overcome drug resistance in-vitro in human ovarian carcinoma. *Gynecol. Oncol.* **2012,** *126*, 118-23.
16. Sorrentino, B. P., Gene therapy to protect haematopoietic cells from cytotoxic cancer drugs. *Nature Rev. Cancer* **2002,** *2*, 431-441.
17. Chen, S. F., V.; Vyas, D. M.; Doyle, T. W.; Long, B. H.; Fairchild, C. , Synthesis and Biological Evaluation of C-13 Amide-Linked Paclitaxel Analogs. *J. Org Chem.* **1996,** *61*, 2065- 2070.
18. Cabri, W. C., M.; Marcotullio, M. C.; Rosati, O. , A high yield semisynthetic approach to 2′ -epi-Taxol. *Teterahedron Lett.* **1996,** *37*, 4785-4786.
19. Guéritte-Voegelein, F. G. n., D.; Lavelle, F.; Le Goff, M. T.; Mangatal, L.; Potier, P. , Relationships between the structure of taxol analogs and their antimitotic activity. *J. Med. Chem.* **1991,** *34*, 992-998.
20. Ojima, I. L., S.; Wang, T. , Recent Advances in the Medicinal Chemistry of Taxoids

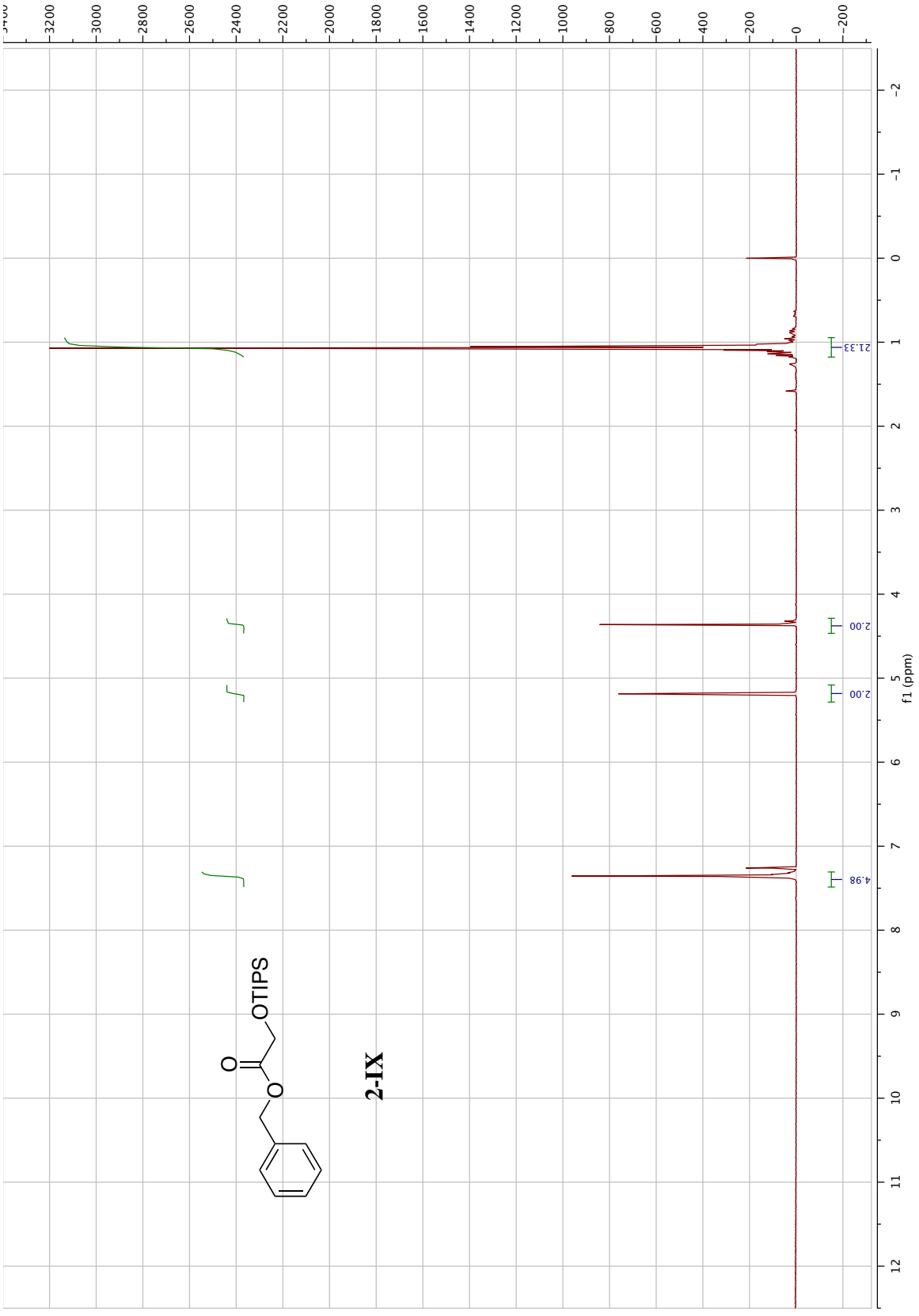
with Novel β-Amino Acid Side Chain. *Curr. Med. Chem.* **1999,** *6*, 927-953.
21. Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P. Y.; Vrignaud, P.; Bissery, M. C.; Veith, J. M.; Pera, P.; Bernacki, R. J., Syntheses and structure-activity relationships of the second-generation antitumor taxoids: exceptional activity against drug-resistant cancer cells. *J. Med. Chem.* **1996,** *39*, 3889-96.
22. Spletstoser, J. T. T., B. J.; Desino, K.; Rice, A. Datta, A.; Dutta, D.; Huff, J. K.; Himes, R. H.; Audus, K. L.; Seelig, A.; Georg, G. I. , Single-site Chemical Modification at C10 of the Baccatin Core of Palitaxel and Taxol C reduces P-glycoprotein interactions in bovine brain microvessel endothelial cells. *Bioorg. & Med. Chem. Lett.* **2006,** *16*, 495-498.
23. Ojima, I.; Das, M., Recent advances in the chemistry and biology of new generation taxoids. *J. Nat. Prod.* **2009,** *72*, 554-65.
24. Zuniga, E. S. Design, synthesis and biological evaluation of new-generation taxoid-based tumor-targeting drug conjugates. Stony Brook University, **2012**.
25. Staudinger, H.; Justus, L. A., Zur Kenntniss der Ketene. Diphenylketen. *Ann. Chem* **1907,** *356*, 51-123.
26. Singh, G. S., Beta-lactams in the new millennium. Part-I: monobactams and carbapenems. *Mini Rev Med Chem* **2004,** *4*, 69-92.
27. Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R., Efficient and Practical Asymmetric-Synthesis of the Taxol C-13 Side-Chain and Its Analogs Via Chiral 3-Hydroxy-4-Aryl-Beta-Lactams. *Abstr Pap Am Chem S* **1991,** *201*, 136-ORGN.
28. Ojima, I.; Kuduk, S. D.; Pera, P.; Veith, J. M.; Bernacki, R. J., Synthesis and structure-activity relationships of nonaromatic taxoids: effects of alkyl and alkenyl ester groups on cytotoxicity. *J. Med. Chem.* **1997,** *40*, 279-85.
29. Lin, S.; Geng, X.; Qu, C.; Tynebor, R.; Gallagher, D. J.; Pollina, E.; Rutter, J.; Ojima, I., Synthesis of highly potent second-generation taxoids through effective kinetic resolution coupling of racemic beta-lactams with baccatins. *Chirality* **2000,** *12*, 431-41.
30. Geney, R. S., L.; Pera, P.; Bernacki, R. J.; Xia, S.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I. , Use of the Tubulin Bound Paclitaxel Conformation for Structure-Based Rational Drug Design. *Chem. & Bio.* **2005,** *12*, 339-348.
31. Ojima, I. S., C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. D. , A Highly Efficient Route to Taxotere by the β. *Tetrahedron Lett.* **1993,** *34*, 4149-4152.
32. Berger, W. *Quarterly Report 1*; Chemistry Department, Stony Brook University: **2011**.
33. Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, B. K., Catalytic Asymmetric Dihydroxylation. *Chem. Rev.* **1994,** *94*, 2483-2547.
34. Schwartz, A.; Madan, P.; Whitesell, J. K.; Lawrence, R. M., Lipase-Catalyzed Kinetic Resolution of Alcohols via Chloroacetate Esters: (-)-(1R, 2S)-trans-2-phenyl-cyclohexanol and (+)-(1S, 2R)-trans-2-phenyl-cyclohexanol. *Org. Synth. Coll.* **1990,** *69*, 1-9.
35. Stayshich, R. M.; Meyer, T. Y., New Insights into Poly(lactic-co-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior. *J. Am. Chem. Soc.* **2010,** *132*, 10920-10934.
36. Seitz, J. D. The design, synthesis and biological evaluation of novel taxoid anticancer agents and their tumor-targeted drug conjugates. Stony Brook University, **2013**.
37. France, S.; Weatherwax, A.; Taggi, A. E.; Lectka, T., Advances in the catalytic, asymmetric synthesis of beta-lactams. *Acc. Chem. Res.* **2004,** *37*, 592-600.
38. Ojima, I.; Slater, J. C.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C. M.; Park,

Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J., Syntheses and structure-activity relationships of taxoids derived from 14 beta-hydroxy-10-deacetylbaccatin III. *J. Med. Chem.* **1997,** *40*, 267-78.
39. Brieva, R.; Crich, J. Z.; Sih, C. J., Chemoenzymic synthesis of the C-13 side chain of taxol: optically active 3-hydroxy-4-phenyl .beta.-lactam derivatives. *J. Org. Chem.* **1993,** *58*, 1068-1075.
40. Ojima, I., Guided Molecular Missiles for Tumor-Targeting Chemotherapy—Case Studies Using the Second-Generation Taxoids as Warheads. *Acc. Chem. Res.* **2008,** *41*, 108-119.
41. Tartarone, A.; Lazzari, C.; Lerose, R.; Conteduca, V.; Improta, G.; Zupa, A.; Bulotta, A.; Aieta, M.; Gregorc, V., Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. *Lung cancer* **2013**.
42. Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W. T.; Clarkson, B.; Kuriyan, J., Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **2000,** *289*, 1938-42.
43. Tolcher, A. W.; Sugarman, S.; Gelmon, K. A.; Cohen, R.; Saleh, M.; Isaacs, C.; Young, L.; Healey, D.; Onetto, N.; Slichenmyer, W., Randomized phase II study of BR96-doxorubicin conjugate in patients with metastatic breast cancer. *J. Clin. Oncol.* **1999,** *17*, 478-84.
44. Trail, P. A., Antibody Drug Conjugates as Cancer Therapeutics. *Antibodies* **2013,** *2*, 113-129.
45. Tallman, M. S.; McDonald, G. B.; DeLeve, L. D.; Baer, M. R.; Cook, M. N.; Graepel, G. J.; Kollmer, C., Incidence of sinusoidal obstruction syndrome following Mylotarg (gemtuzumab ozogamicin): a prospective observational study of 482 patients in routine clinical practice. *Int. J. Hematol.* **2013,** *97*, 456-64.
46. Kuriyama, I.; Miyazaki, A.; Tsuda, Y.; Yoshida, H.; Mizushina, Y., Inhibitory effect of novel somatostatin peptide analogues on human cancer cell growth based on the selective inhibition of DNA polymerase beta. *Bioorg. Med. Chem.* **2013,** *21*, 403-11.
47. Ojima, I. *Tumor-Targeted Cancer Chemotherapy*; Haikou City People's Hospital Haikou, Hainan, China, June 23, **2012**.
48. Jeyapalan, S.; Boxerman, J.; Donahue, J.; Goldman, M.; Kinsella, T.; Dipetrillo, T.; Evans, D.; Elinzano, H.; Constantinou, M.; Stopa, E.; Puthawala, Y.; Cielo, D.; Santaniello, A.; Oyelese, A.; Mantripragada, K.; Rosati, K.; Isdale, D.; Safran, H.; Study, A. B. U. O. G., Paclitaxel Poliglumex, Temozolomide, and Radiation for Newly Diagnosed High-grade Glioma: A Brown University Oncology Group Study. *Am. J. Clin. Oncol.* **2013**.
49. Li, C.; Price, J. E.; Milas, L.; Hunter, N. R.; Ke, S.; Yu, D. F.; Charnsangavej, C.; Wallace, S., Antitumor activity of poly(L-glutamic acid)-paclitaxel on syngeneic and xenografted tumors. *Clin. Cancer Res.* **1999,** *5*, 891-7.
50. Grammatikos, S. I.; Subbaiah, P. V.; Victor, T. A.; Miller, W. M., n-3 and n-6 fatty acid processing and growth effects in neoplastic and non-cancerous human mammary epithelial cell lines. *Br. J. Cancer* **1994,** *70*, 219-27.
51. Wigmore, S. J.; Ross, J. A.; Falconer, J. S.; Plester, C. E.; Tisdale, M. J.; Carter, D. C.; Fearon, K. C., The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition* **1996,** *12*, S27-30.
52. Hawkins, R. A.; Sangster, K.; Arends, M. J., Apoptotic death of pancreatic cancer cells induced by polyunsaturated fatty acids varies with double bond number and involves an

oxidative mechanism. *J. Pathol.* **1998,** *185*, 61-70.
53. Sauer, L. A., Dauchy, R.T., Blask, D.E. , Mechanism for the antitumor and anticachectic effects of n-3 fatty acids. *Cancer Res.* **2000,** *60*, 5289-5295.
54. Ojima, I.; Zuniga, E. S.; Berger, W. T.; Seitz, J. D., Tumor-targeting drug delivery of new-generation taxoids. *Future Med. Chem.* **2012,** *4*, 33-50.
55. Seitz, J. D. O., I. , Drug Conjugates with Polyunsaturated Fatty Acids. In Drug Delivery in Oncology: From Basic Research to Cancer Therapy. In Kratz, F. S., P.; Steinhagen, H., Eds., Ed. Wiley-VCH Verlag GmbH & co. KGaA.: **2010**; Vol. 3, pp 1323-1360.
56. Ibrahim, N. K.; Desai, N.; Legha, S.; Soon-Shiong, P.; Theriault, R. L.; Rivera, E.; Esmaeli, B.; Ring, S. E.; Bedikian, A.; Hortobagyi, G. N.; Ellerhorst, J. A., Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. *Clin. Cancer Res.* **2002,** *8*, 1038-44.
57. Desai, N.; Trieu, V.; Yao, Z.; Louie, L.; Ci, S.; Yang, A.; Tao, C.; De, T.; Beals, B.; Dykes, D.; Noker, P.; Yao, R.; Labao, E.; Hawkins, M.; Soon-Shiong, P., Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin. Cancer Res.* **2006,** *12*, 1317-24.
58. Rose, D. P.; Connolly, J. M., Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol. Ther.* **1999,** *83*, 217-44.
59. Kuznetsova, L.; Chen, J.; Sun, L.; Wu, X.; Pepe, A.; Veith, J. M.; Pera, P.; Bernacki, R. J.; Ojima, I., Syntheses and evaluation of novel fatty acid-second-generation taxoid conjugates as promising anticancer agents. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 974-7.
60. Russell-Jones, G.; McTavish, K.; McEwan, J.; Rice, J.; Nowotnik, D., Vitamin-mediated targeting as a potential mechanism to increase drug uptake by tumours. *J. Inorg. Biochem.* **2004,** *98*, 1625-33.
61. Xia, W.; Low, P. S., Folate-targeted therapies for cancer. *J. Med. Chem.* **2010,** *53*, 6811-24.
62. Lorusso, P. M.; Edelman, M. J.; Bever, S. L.; Forman, K. M.; Pilat, M.; Quinn, M. F.; Li, J.; Heath, E. I.; Malburg, L. M.; Klein, P. J.; Leamon, C. P.; Messmann, R. A.; Sausville, E. A., Phase I study of folate conjugate EC145 (Vintafolide) in patients with refractory solid tumors. *J. Clin. Oncol.* **2012,** *30*, 4011-6.
63. Chen, J.; Chen, S.; Zhao, X.; Kuznetsova, L. V.; Wong, S. S.; Ojima, I., Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. *J. Am. Chem. Soc.* **2008,** *130*, 16778-85.
64. Dickinson, R. P., Iddon, B. J. , Condensed thiophen ring systems. Part III. A new synthesis of benzo[b]thiophen-2(3H)-and-3(2H)-ones and some reactions of benzo-[b]thiophen-2(3H)-one with dimethyl sulphate in the presence of base *J. Chem. Soc.* **1970,** *14*, 1926-1928.
65. Bordwell, F. G., Fried, H.E. , Heterocyclic Aromatic Anions with 4n+2-Pi- Electrons. *J. Org. Chem.* **1991,** *56*, 4218-4223.
66. Samukov, V. V., A simple preparation of 3-(2-pyridyldithio)-propionic acid. *Synth. Commun.* **1998,** *28*, 3213 - 3217.
67. Shaabani, A.; Rahmati, A.; Naderi, S., A novel one-pot three-component reaction: synthesis of triheterocyclic 4H-pyrimido[2,1-b]benzazoles ring systems. *Bioorg. Med. Chem. Lett.* **2005,** *15*, 5553-7.

68. Widdison, W. C.; Wilhelm, S. D.; Cavanagh, E. E.; Whiteman, K. R.; Leece, B. A.; Kovtun, Y.; Goldmacher, V. S.; Xie, H.; Steeves, R. M.; Lutz, R. J.; Zhao, R.; Wang, L.; Blattler, W. A.; Chari, R. V., Semisynthetic maytansine analogues for the targeted treatment of cancer. *J. Med. Chem.* **2006,** *49*, 4392-408.
69. Zhang, G. N.; Fang, W. S., A new synthesis route of cabazitaxel. *J. Chin. Pharm. Sci.* **2012,** *21*, 472-476.
70. Bouchard, H.; Bourzat, J. D.; Commercon, A., 2002 US6372780, **2002**.
71. Liu, P.; Yuan, J. D. CN 102659721A, 2012.
72. Zhang, W. Z.; Zhang, A. P.; Wang, Q. Y.; Yin, Z. C.; Huang, W.; Cai, Z. X.; Tong, Z. B.; Gao, S. CN 102311410A, **2012**.
73. Sun, X. Q.; Chen, X.; Yang, H. T.; Miao, C. B. CN 102285947A, **2011**.
74. Yao, Q. X.; Chen, B.; Li, D. J.; Li, J. CN 102532065, **2012**.
75. Bai, J. S.; Che, D. H.; Chen, C. J. CN 102675256, **2012**.
76. Chen, B.; Yao, Q. X.; Li, J. CN 102336726, **2012**.
77. Samaranayake, G.; Margri, N. F.; Jitrangsri, C.; Kingston, G. I., Modified Taxols. 5. Reaction of Taxol with Electrophilic Reagents and Preperation of a Rearanged Taxol Derivative with Tubulin Assembly Activity. *J. Org. Chem.* **1991,** *56*, 5114-5119.
78. Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J., Synthesis and structure-activity relationships of new second-generation taxoids. *Bioorg. Med. Chem. Lett.* **1999,** *9*, 3423-8.

Appendix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2.00
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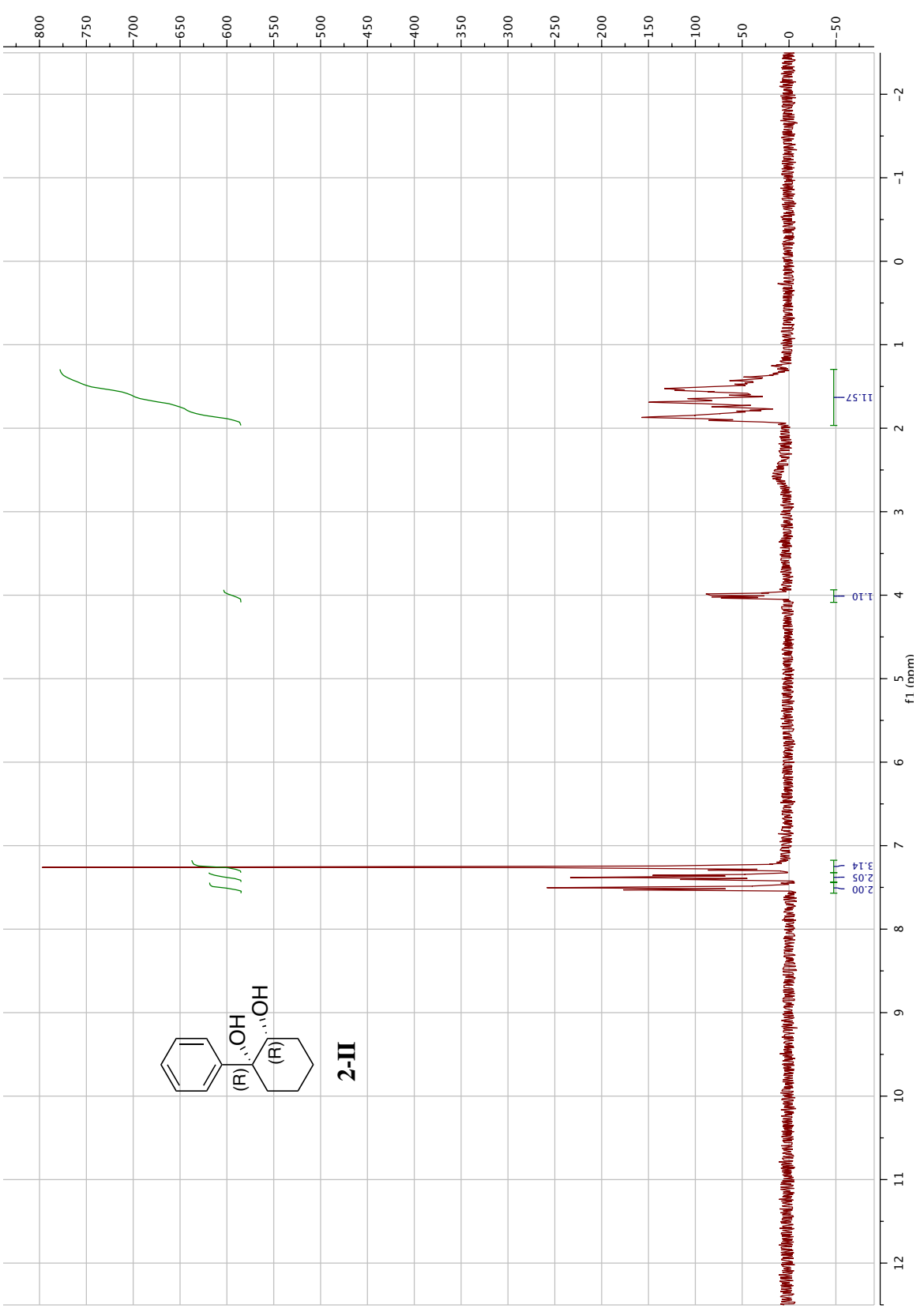
f1 (ppm)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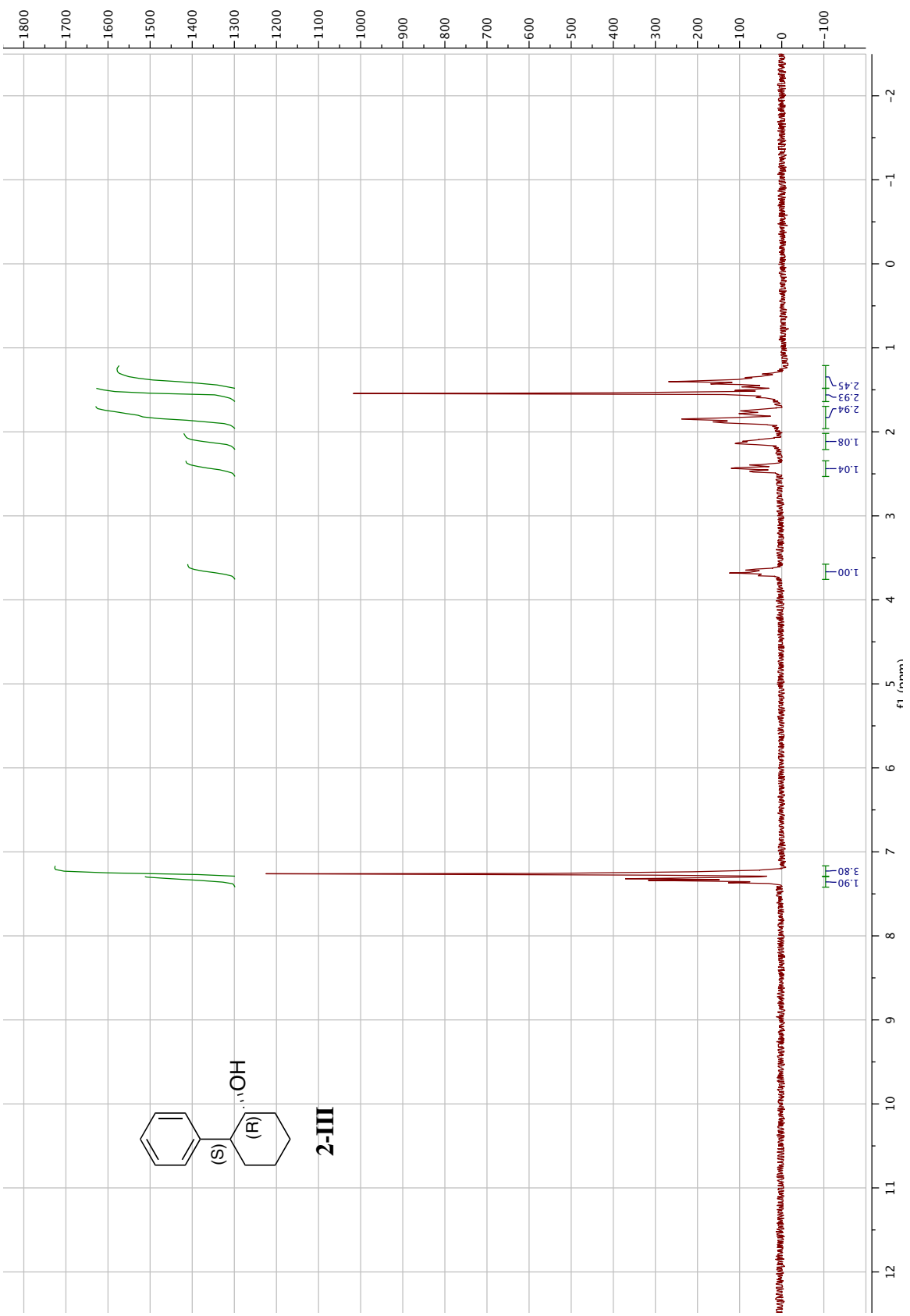
f1 (ppm)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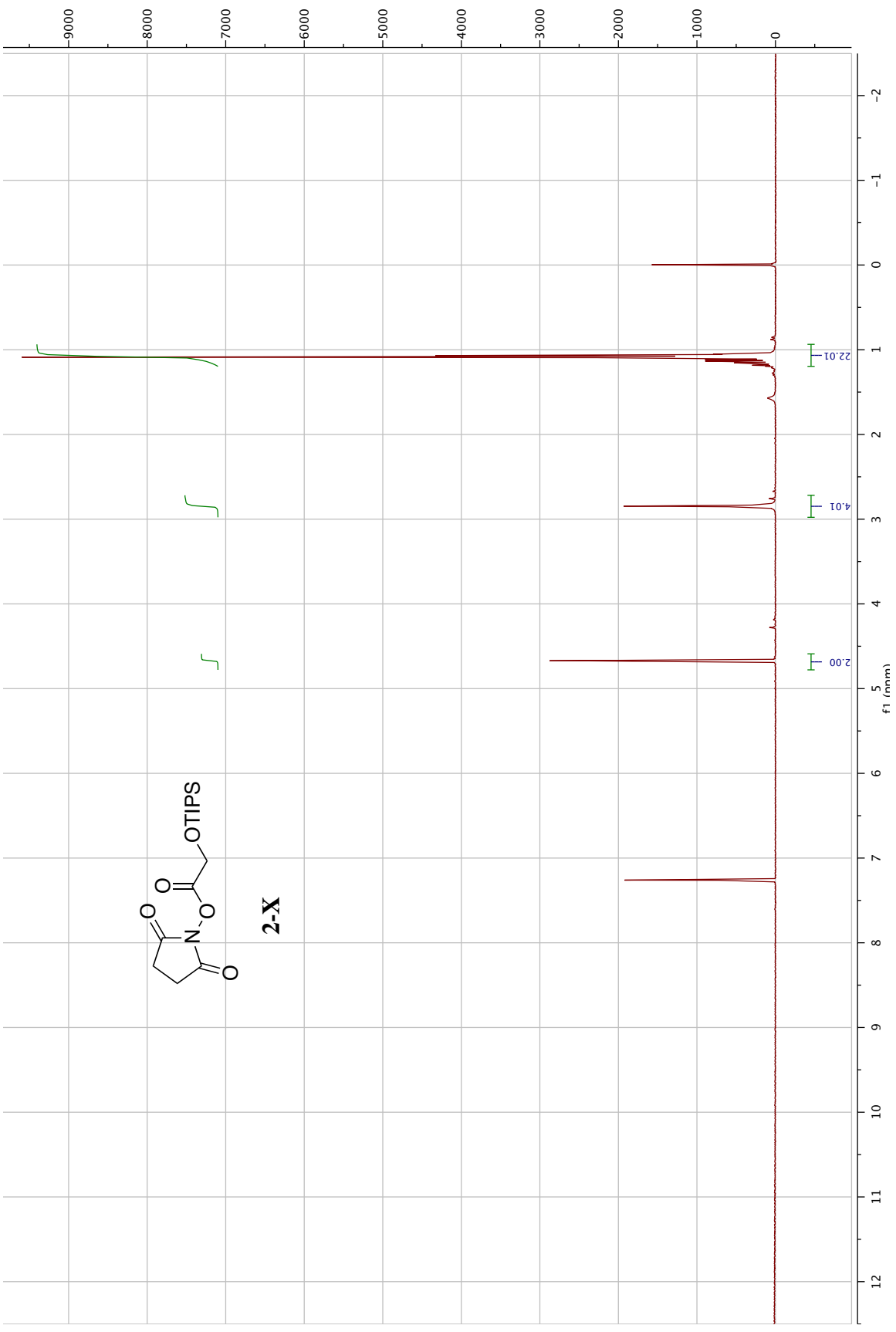
f1 (ppm)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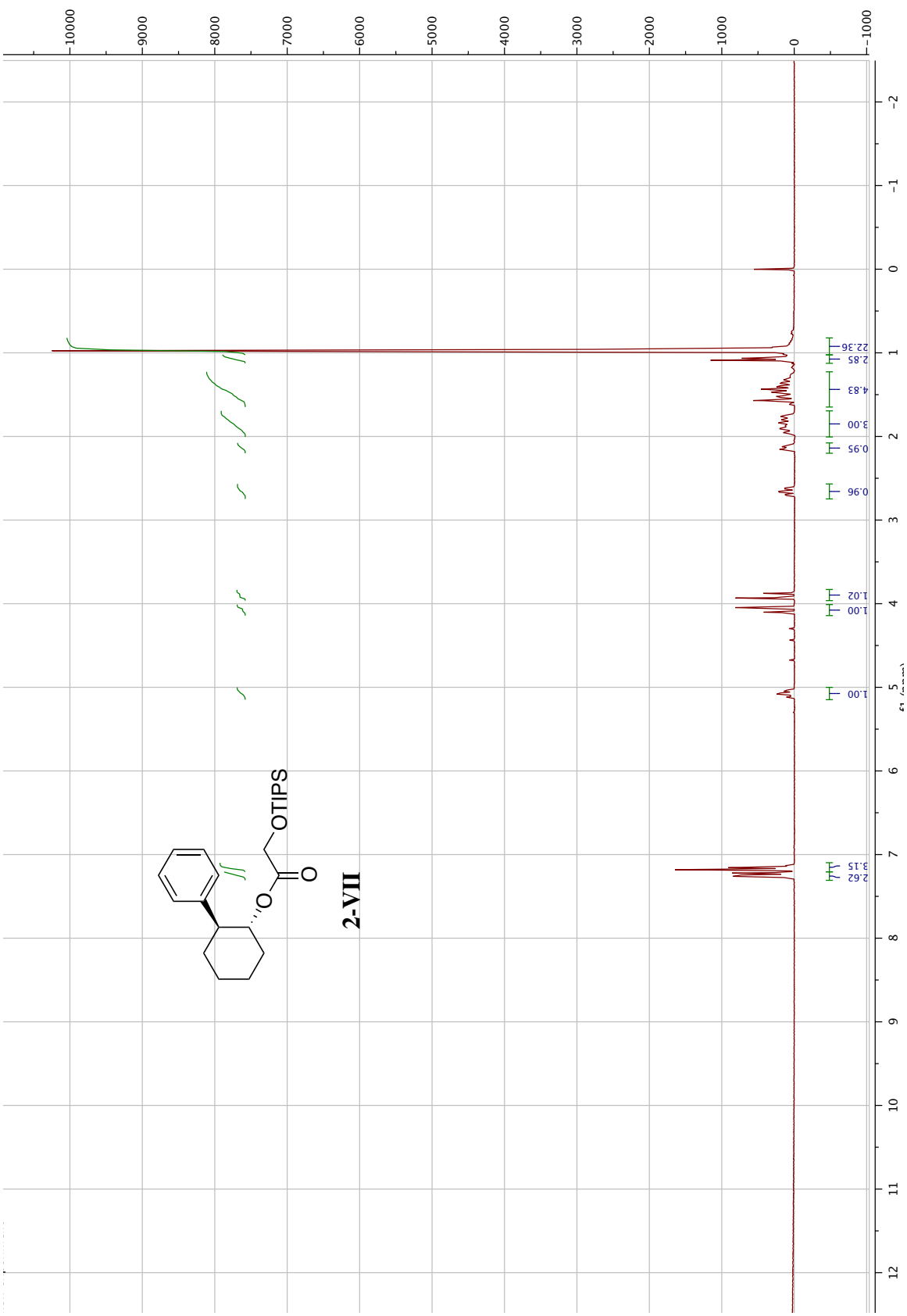
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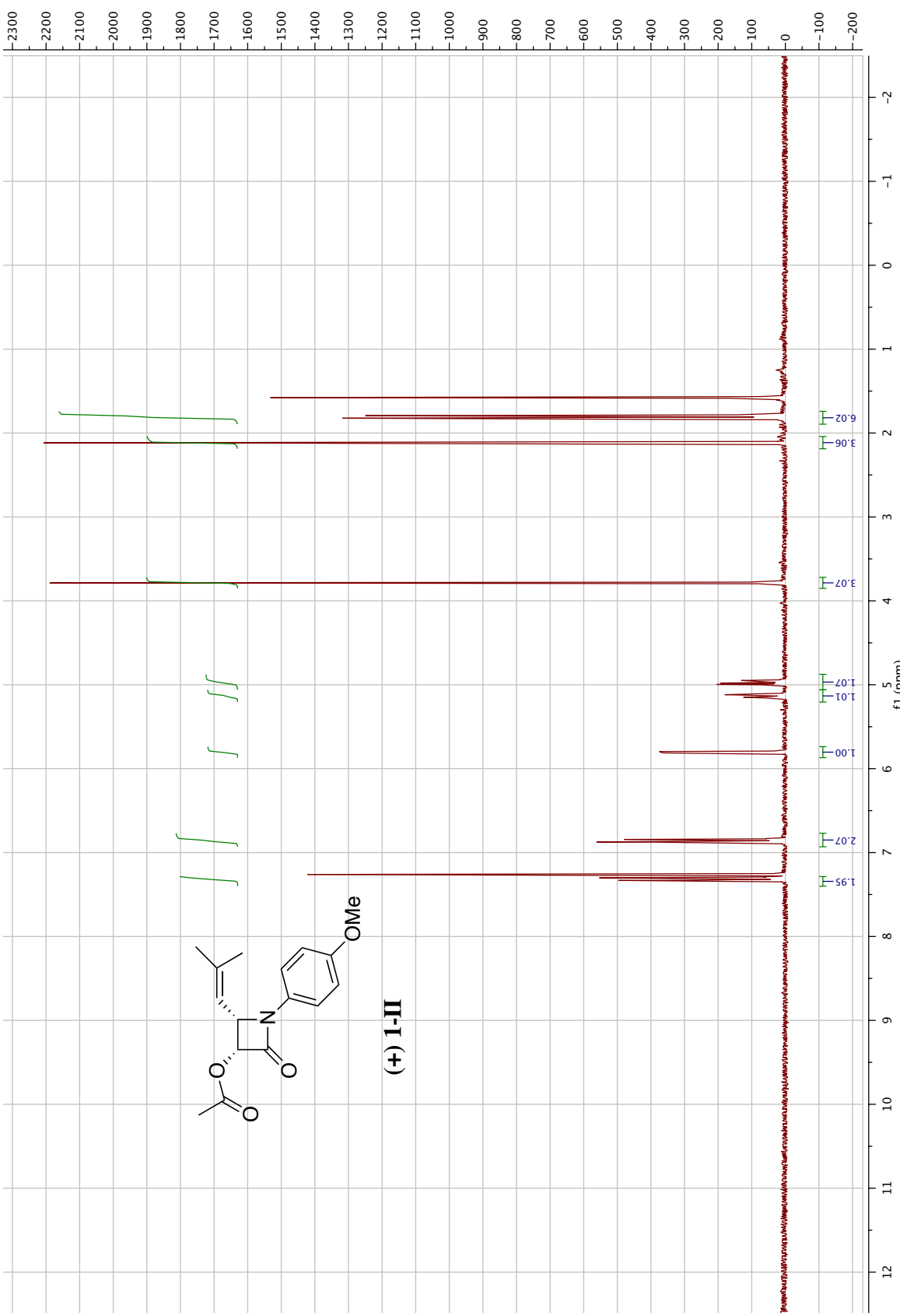
1.00
3.15
2.62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1.00
2.07
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f1 (ppm)

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7.317
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5.353
5.349
5.331
5.328
5.324
5.071
5.059
4.832
4.820
4.808
4.795
3.781
1.858
1.855
1.807
1.804
1.113
1.087
1.063
TIPSO
N
O
OMe
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ppm
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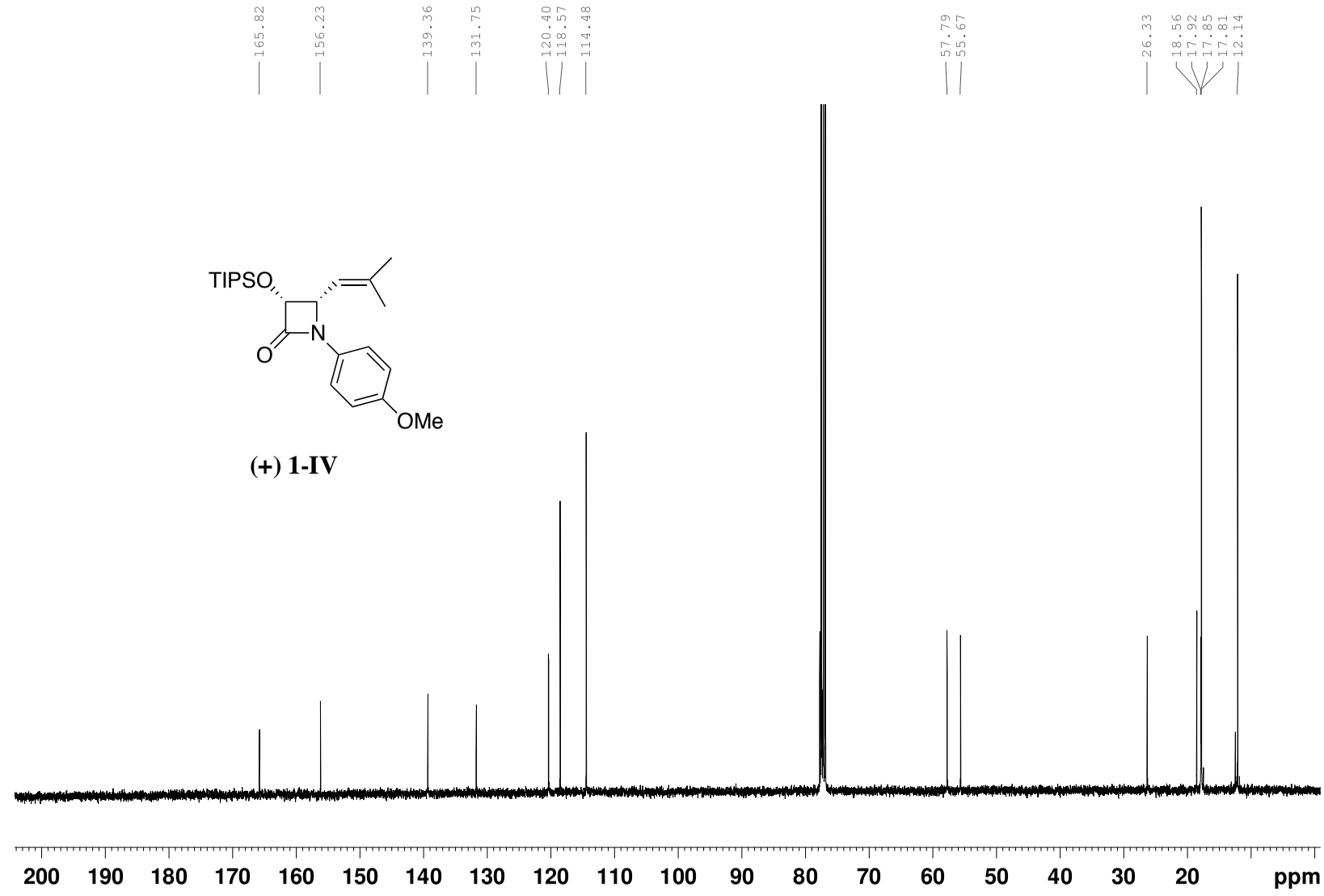
1.02
3.03
3.01
3.00
21.93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17.85
17.81
12.14
TIPSO
N
O
OMe
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

(+) 1-IV

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5.331
5.314
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5.308
5.007
5.002
4.995
4.990
4.466
4.454
4.442
4.431
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1.692
1.688
1.135
1.128
1.118
1.114
1.110
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1.096
1.085
1.069
1.059
1.043
1.036
1.032
TIPSO
NH
O
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1.00
0.97
1.02
21.20
3.06
3.07
ppm

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138.18
121.62
79.57
53.70
26.21
18.42
17.83
17.80
12.09
TIPSO
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O
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200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

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5.274
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4.969
4.784
4.769
4.759
4.745
1.800
1.798
1.775
1.772
1.494
1.075
1.060
1.050
1.034
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O
N
O
O
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7.0
6.5
6.0
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4.0
3.5
3.0
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1.5
1.0
0.5
ppm
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1.00
0.98
6.14
9.20
21.26

12.03
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17.74
18.47
26.30
28.26
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118.63
139.86
148.42
166.63
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O
N
O
O
(+) 1-VI
ppm
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190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

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1.890
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0.568
0.553
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0.495
OTES
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HO
O
O
O
O
H
3-I
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1.0
0.5
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1.01
1.03
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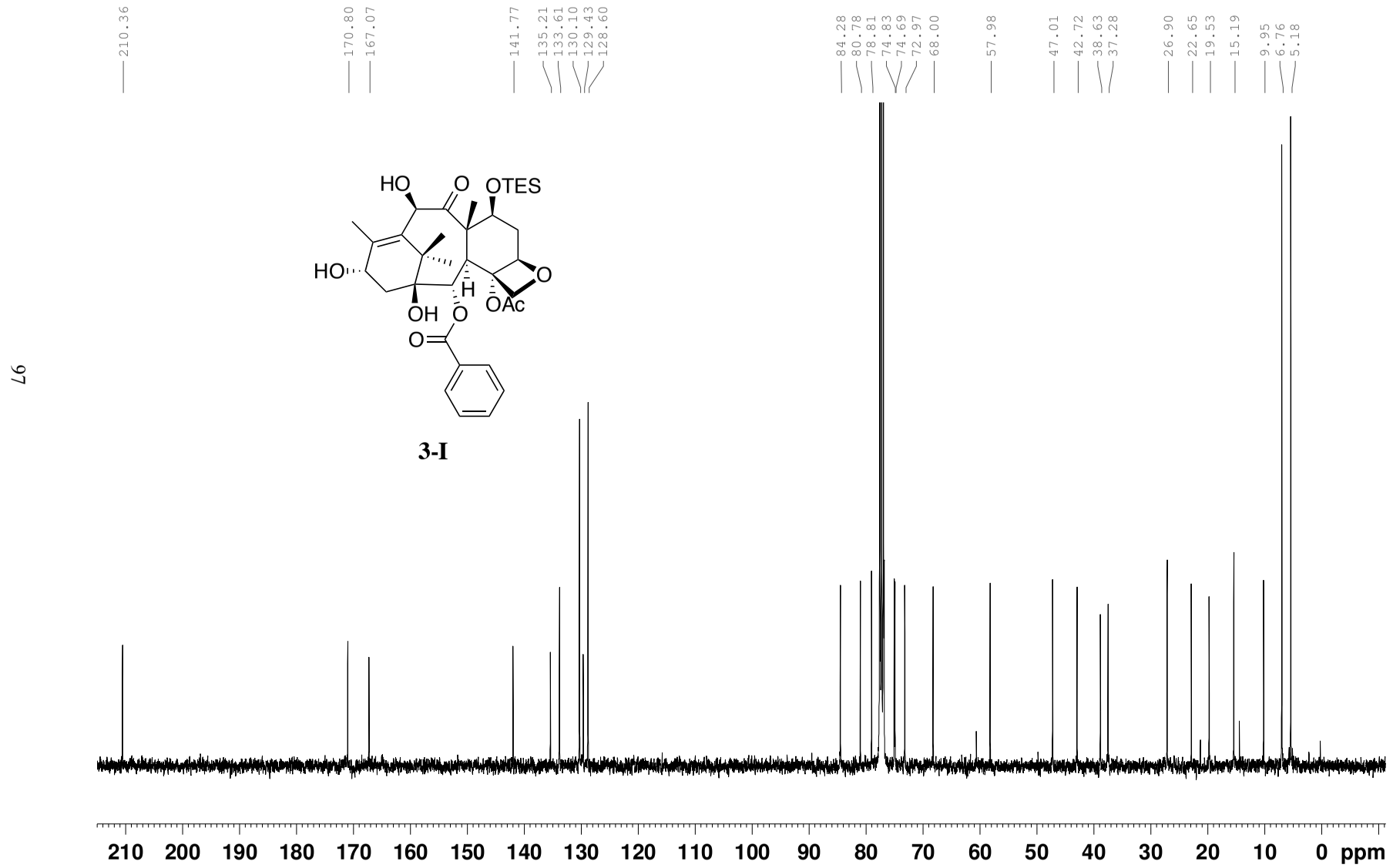
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15.19
9.95
6.76
5.18
HO
O
OTES
HO
O
H
OAc
OH
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

3-I

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4.119
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ppm
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84.47
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ppm

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4.450
4.443
4.435
4.318
4.297
4.214
4.193
3.859
3.841
2.361
2.015
1.801
1.799
1.760
1.758
1.693
1.650
1.346
1.281
1.264
1.246
1.239
1.199
1.189
1.145
1.133
1.120
1.111
1.100
0.940
0.919
0.900
0.597
0.589
0.578
0.569
0.558
0.549
0.538
0.528
OTES
OAc
OH
OTIPS
NH
3-III
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.99
1.01
2.00
1.00
1.00
1.02
1.01
1.07
2.00
2.02
1.06
1.01
1.01
1.05
5.03
2.90
1.18
8.03
2.98
3.25
8.90
2.00
4.05
20.40
11.37
5.97

8.85
8.96
10.30
12.75
13.17
14.41
14.53
18.18
18.25
18.77
21.26
21.58
22.78
25.91
26.51
28.47
35.76
37.38
43.46
46.96
58.54
60.61
72.12
72.41
75.01
75.29
75.49
79.08
81.29
84.52
122.38
128.77
129.67
130.37
133.60
133.76
141.09
155.39
167.08
170.02
171.38
172.13
173.29
202.29
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
OTES
OAc
OH
OTIPS
NH
3-III

8.049
8.045
7.561
7.543
7.447
7.427
7.408
6.255
6.133
6.129
5.631
5.613
4.925
4.905
4.707
4.694
4.369
4.268
4.247
4.175
4.169
4.158
4.148
4.127
3.774
3.757
2.515
2.506
2.340
2.333
2.307
1.995
1.853
1.850
1.734
1.722
1.719
1.716
1.637
1.625
1.520
1.306
1.227
1.217
1.209
1.191
1.112
1.096
1.085
0.974
0.968
0.956
0.948
0.936
3-IV
ppm
1.92
1.01
1.95
0.99
0.98
1.00
1.04
1.03
1.97
1.10
1.03
1.05
1.03
1.01
2.99
1.06
5.63
2.93
4.04
7.88
4.09
4.50
2.03

9.41
9.66
9.74
13.25
14.34
14.42
15.22
18.80
21.28
22.18
22.63
25.95
26.93
28.45
35.73
35.82
43.42
45.86
51.82
58.82
60.62
72.46
72.60
73.98
75.30
75.66
79.43
80.21
81.30
84.69
120.85
128.87
129.45
130.38
133.15
133.91
138.18
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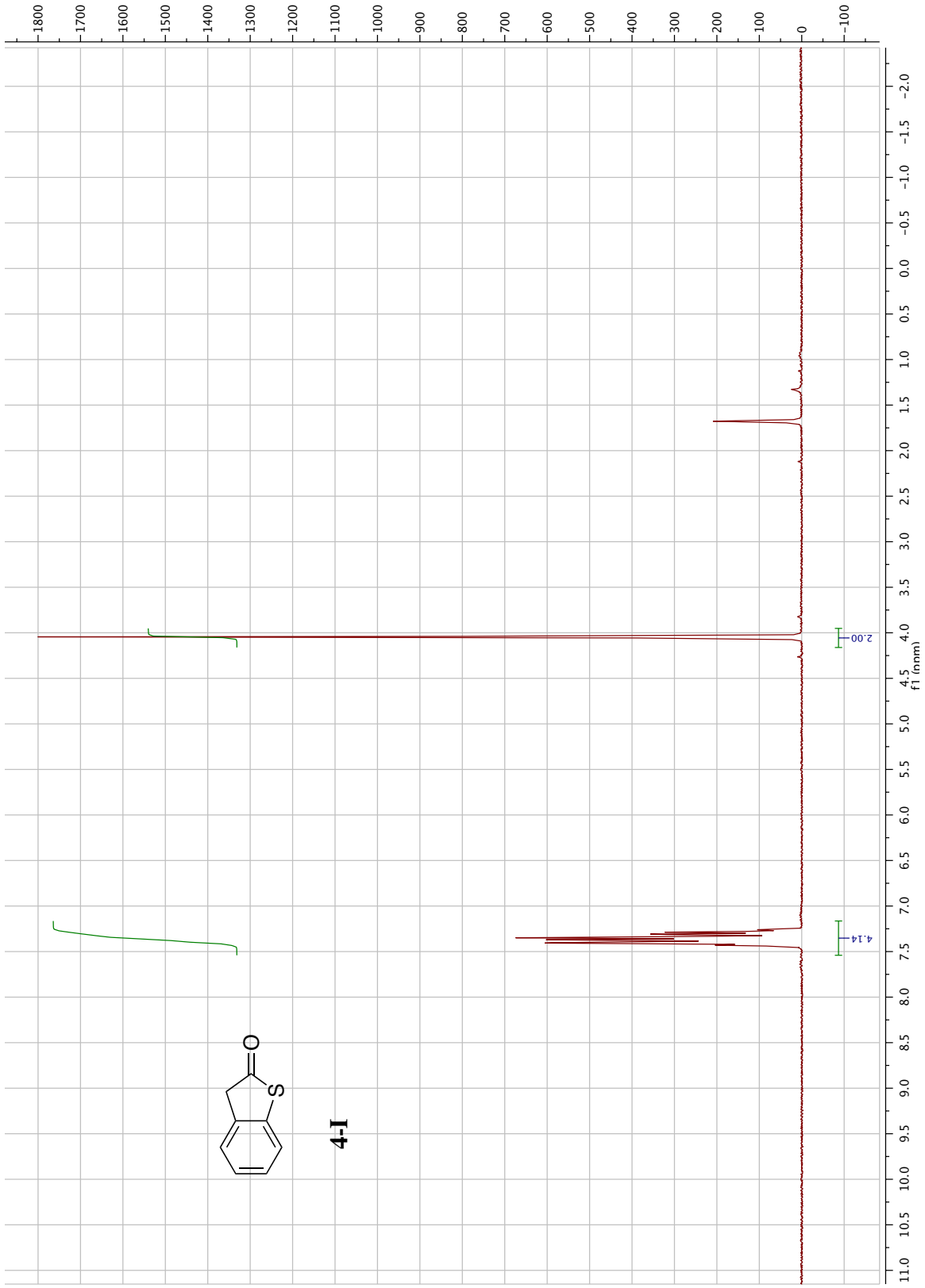
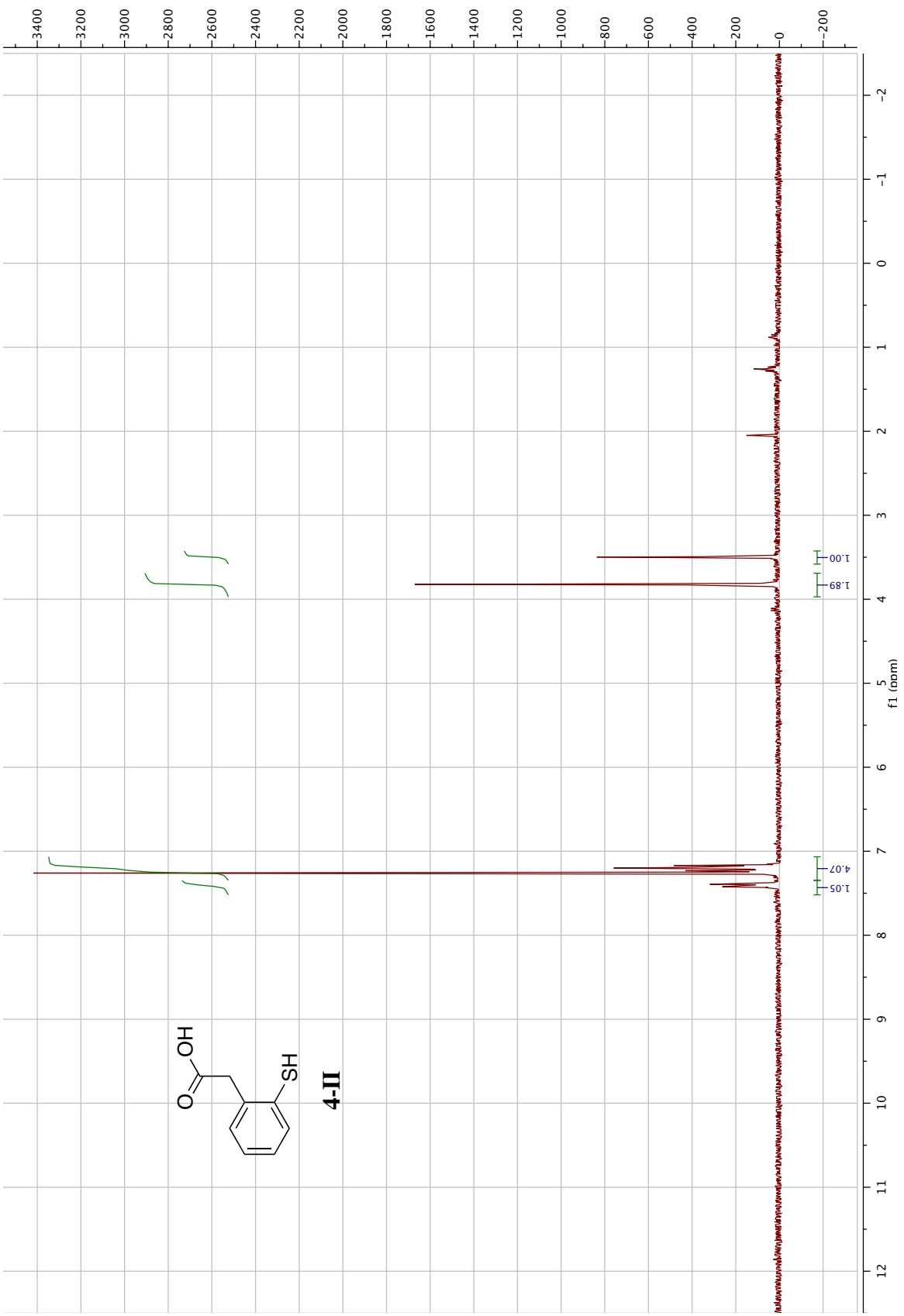
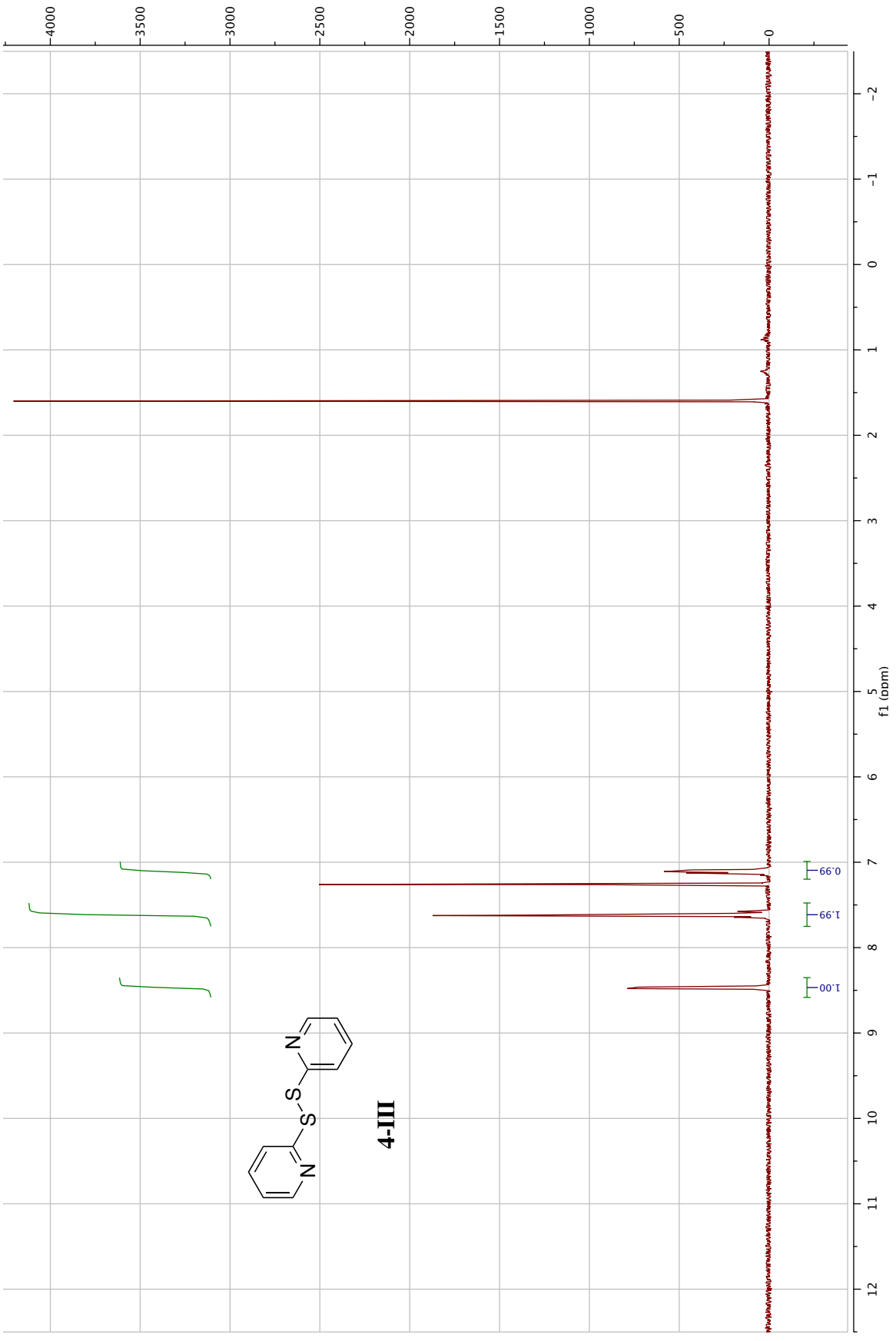
170.30
175.37
204.17
3-IV
OH
OAc
O
H
NH
HO
0 ppm



4-I



4-II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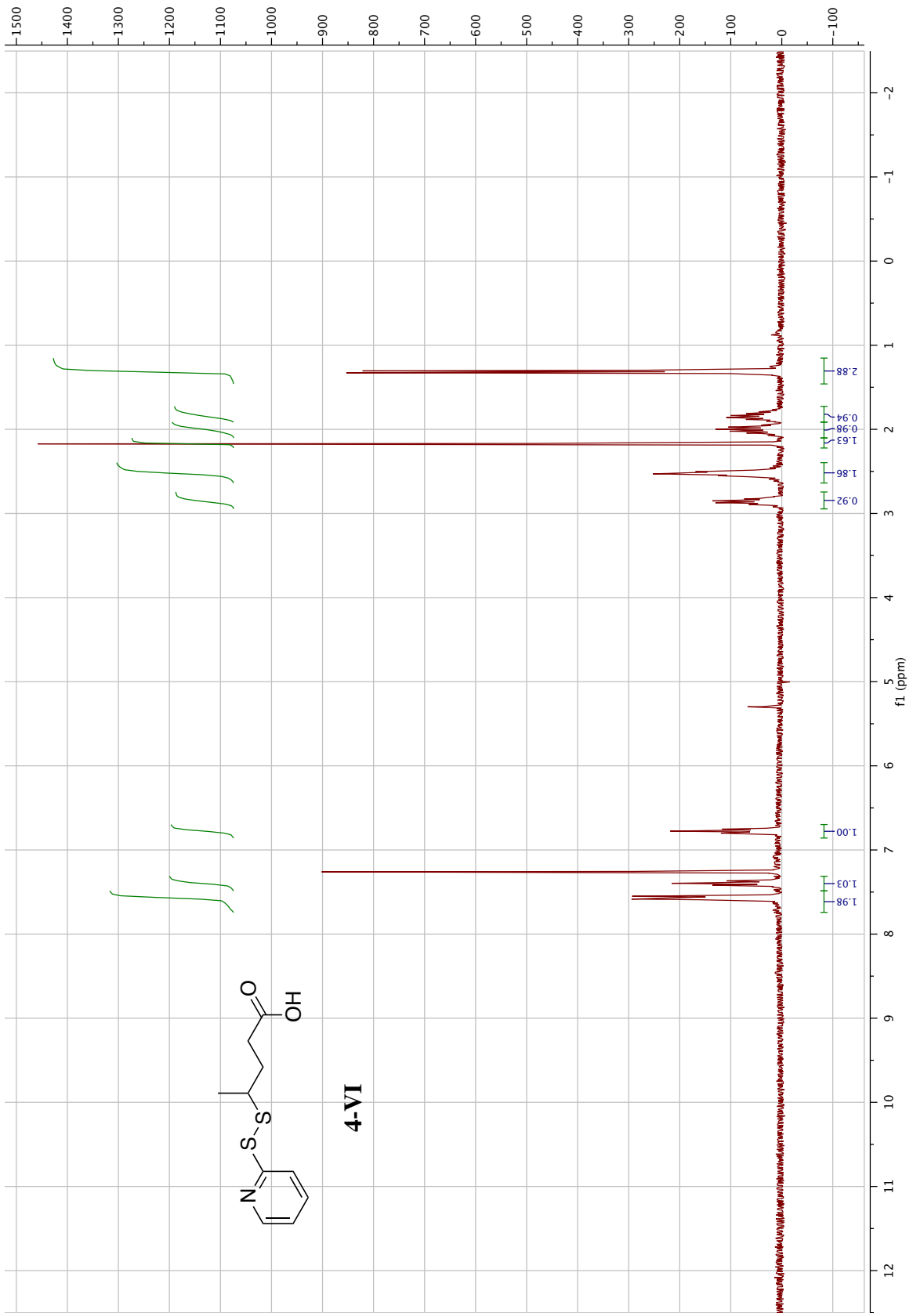
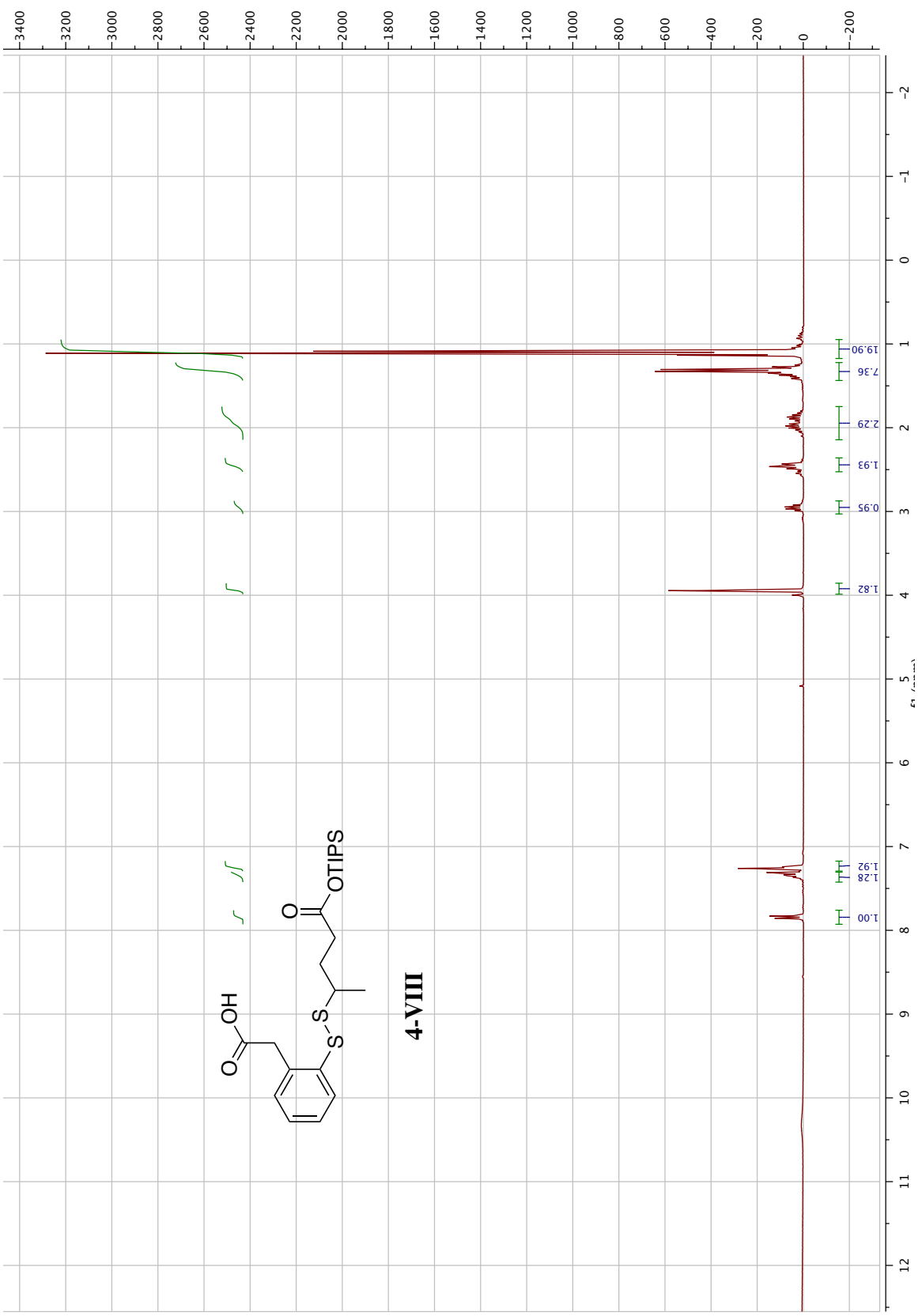
f1 (ppm)
0.99
1.99
1.00


4-VI
f1 (ppm)
2.88
0.94
0.98
1.63
1.86
0.92
1.00
1.03
1.98

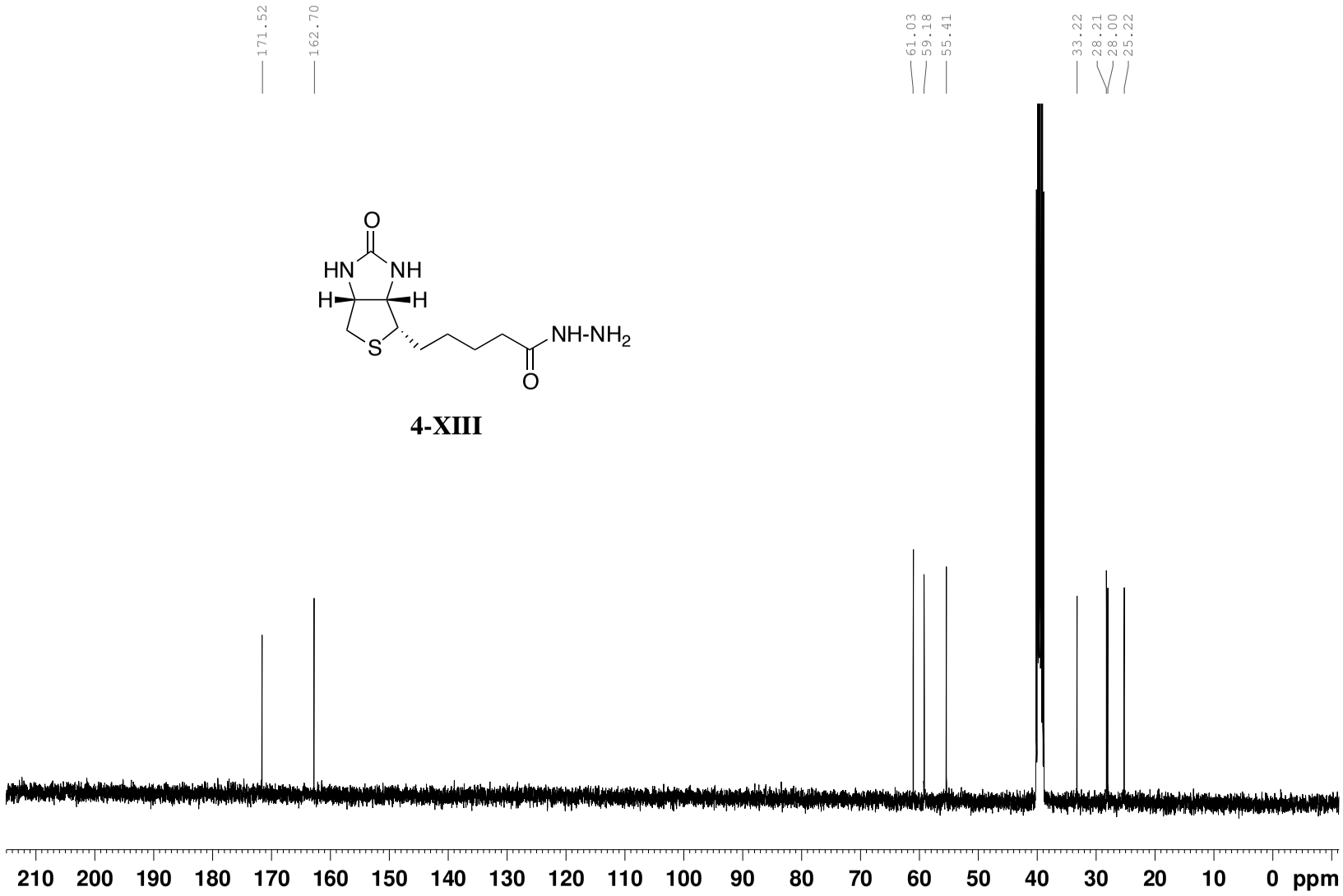

OTIPS
O
O
OH
S
S
4-VIII
19.90
7.36
2.29
1.93
0.95
1.82
1.92
1.28
1.00

8.137
8.119
7.860
7.841
7.656
7.557
7.537
7.518
7.374
7.361
7.357
6.448
5.726
5.708
5.006
4.985
4.965
4.185
4.080
4.047
3.966
3.888
3.871
2.883
2.875
2.741
2.456
2.437
2.064
2.058
2.053
2.047
2.042
1.970
1.762
1.752
1.666
1.385
1.349
1.343
1.332
1.326
1.240
1.220
1.079
1.072
1.063
1.058
1.047
1.000
0.988
0.973
0.968
0.956
0.947
4-XI
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.01
0.91
1.08
2.06
2.02
1.19
1.01
1.92
1.00
0.98
3.09
1.04
2.07
1.03
1.00
0.88
1.09
0.95
0.99
8.94
1.94
2.22
4.02
4.81
8.47
3.36
9.47
4.34
6.97
4.45
5.93

4-XI

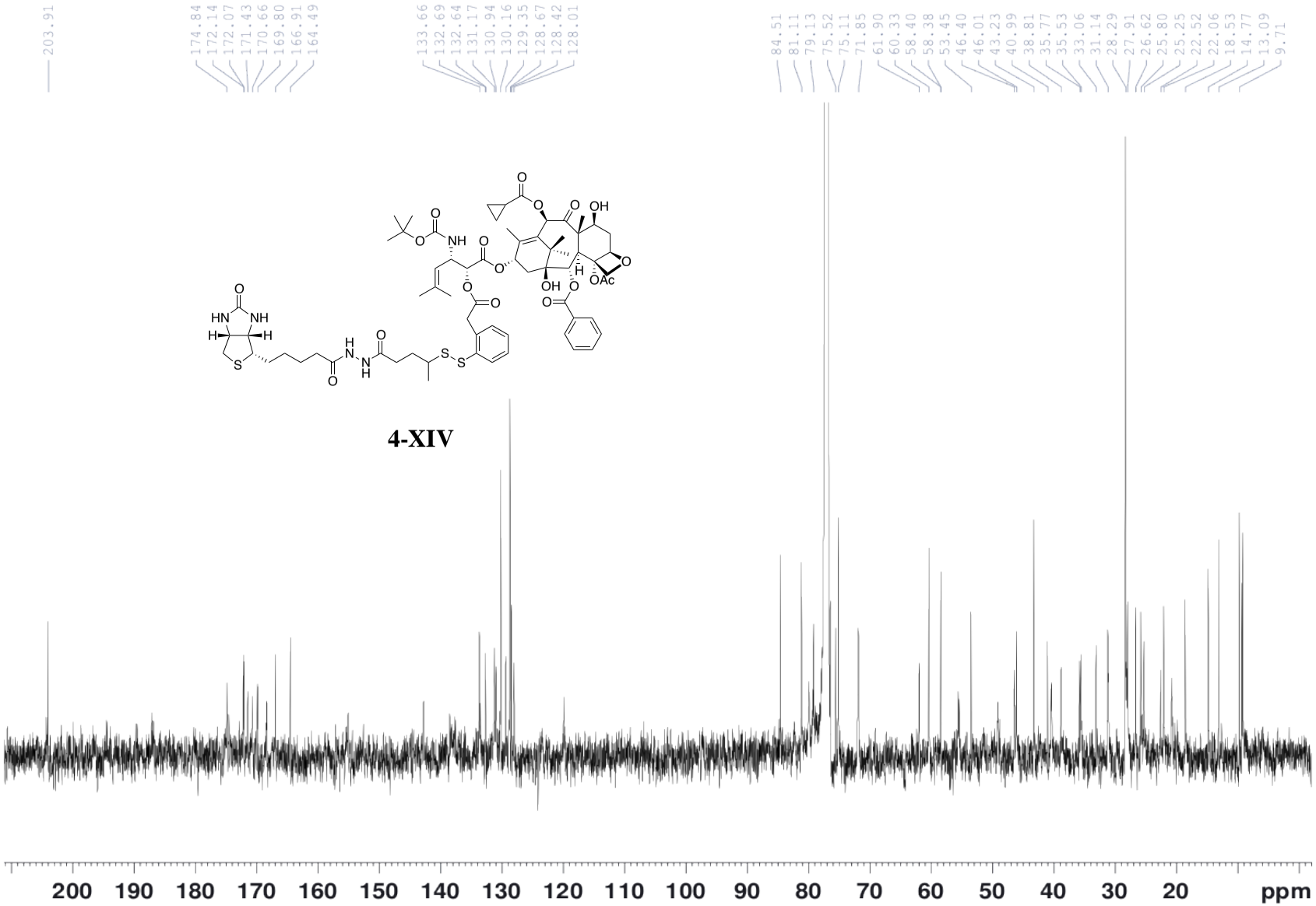
6.433
6.362
4.327
4.314
4.308
4.295
4.152
4.148
4.141
4.137
4.129
4.122
4.118
3.575
3.106
3.100
3.095
3.088
2.841
2.828
2.809
2.797
2.594
2.563
2.509
2.505
2.500
2.312
2.293
2.274
1.582
1.556
1.537
1.519
1.349
1.327
1.312
NH
HN
O
S
OMe
O
4-XII
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.05
3.30
1.08
1.05
1.00
1.00
0.95
2.04
2.93
1.19
2.20

8.912
6.418
6.350
4.317
4.304
4.298
4.285
4.140
4.136
4.129
4.124
4.117
4.110
4.105
3.113
3.098
3.092
3.087
3.081
3.065
2.840
2.827
2.809
2.796
2.589
2.558
2.022
2.004
1.985
1.634
1.626
1.616
1.611
1.601
1.592
1.586
1.576
1.561
1.543
1.536
1.518
1.501
1.481
1.472
1.459
1.450
1.437
1.424
1.402
1.337
1.320
1.303
1.298
1.281
1.264
1.242
1.233
NH-NH2
O
NH
HN
H
H
S
O
4-XIII
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.00
1.07
1.00
1.07
2.91
1.04
1.02
1.00
1.97
1.09
3.02
2.45

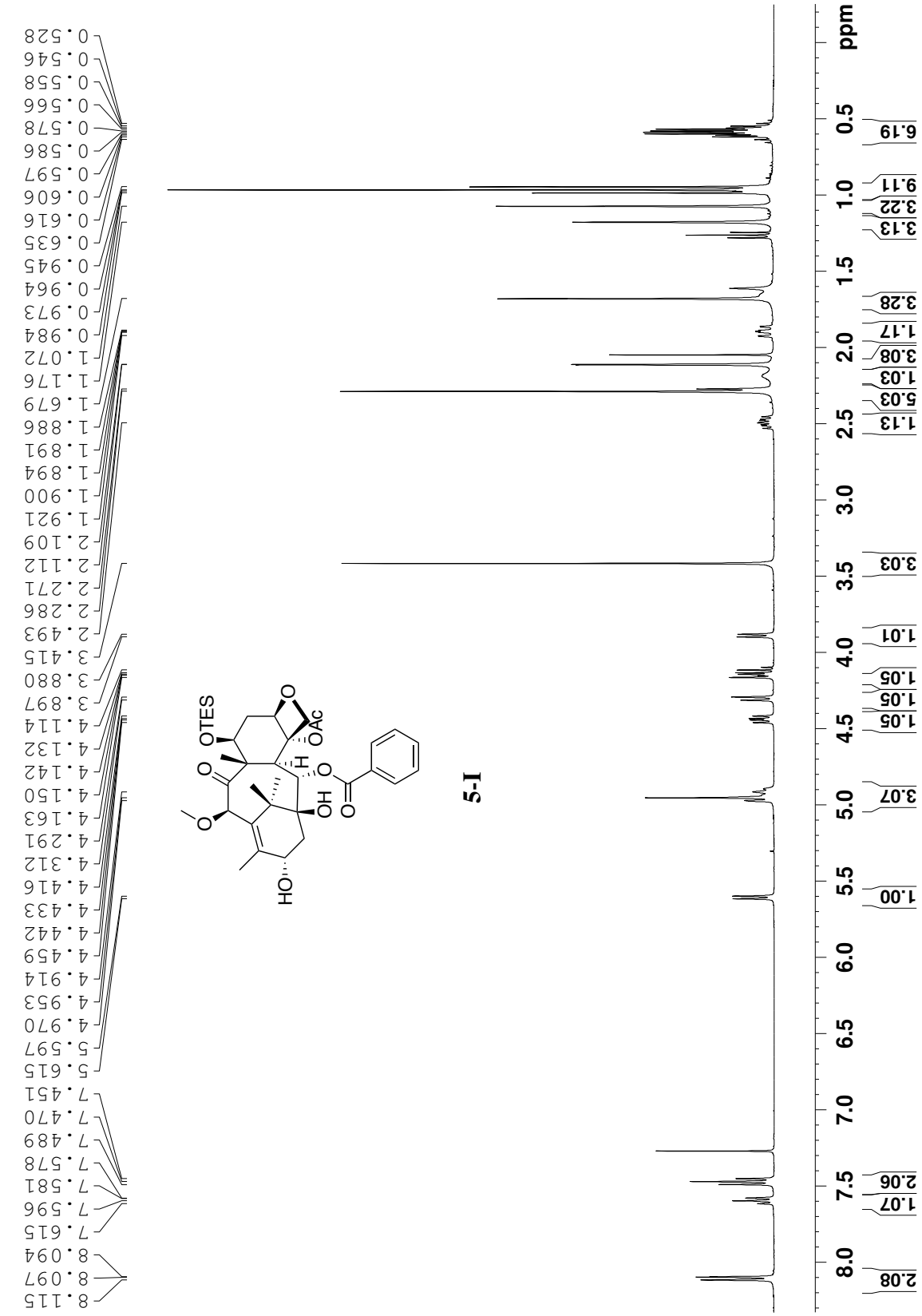

171.52
162.70
61.03
59.18
55.41
33.22
28.21
28.00
25.22
O
HN
NH
H
H
S
NH-NH2
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

4-XIII

4-XIV


203.91
174.84
172.14
172.07
171.43
170.66
169.80
166.91
164.49
133.66
132.69
132.64
131.17
130.94
130.16
129.35
128.67
128.42
128.01
84.51
81.11
79.13
75.52
75.11
71.85
61.90
60.33
58.40
58.38
53.45
46.40
46.01
43.23
40.99
38.81
35.77
35.53
33.06
31.14
28.29
27.91
26.62
25.80
25.25
22.52
22.06
18.53
14.77
13.09
9.71
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

4-XIV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1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
6.19
9.11
3.22
3.13
3.28
1.17
3.08
1.03
5.03
1.13
3.03
1.01
1.05
1.05
1.05
3.07
1.00
2.06
1.07
2.08
OTES
OAc
OH
HO
5-I

5.63
7.00
10.09
14.40
15.39
19.78
21.26
22.93
27.06
37.54
38.57
43.06
47.54
56.00
58.45
60.61
68.22
73.16
75.06
79.02
81.22
82.89
84.54
128.76
129.68
130.30
133.75
134.01
143.25
167.28
171.02
204.73
5-I
OTES
OAc
OH
HO
O
0 ppm

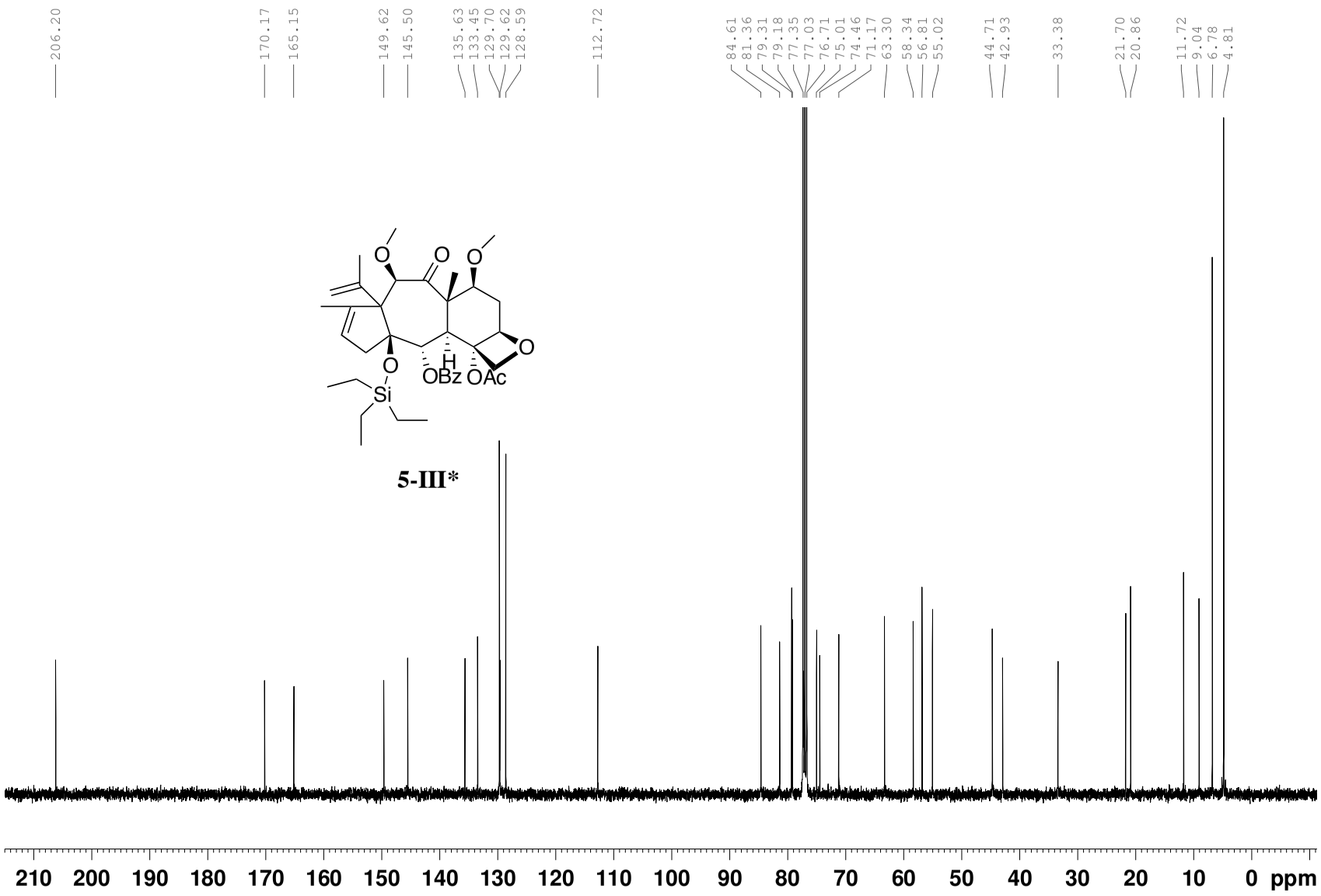
8.131
7.670
7.652
7.636
7.633
7.630
7.554
7.534
7.516
5.637
5.619
5.112
5.051
5.031
4.339
4.322
4.311
4.294
4.227
4.207
4.200
4.178
3.971
3.953
3.419
2.509
2.493
2.485
2.473
2.469
2.457
2.449
2.433
2.415
2.394
2.376
2.287
2.104
2.101
1.863
1.858
1.835
1.830
1.823
1.800
1.795
1.675
1.123
1.090
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.00
1.07
1.98
1.00
1.04
1.10
1.08
1.95
1.08
2.92
1.08
2.19
3.85
2.93
1.15
2.97
5.82
5-II

10.42
15.66
21.16
22.90
27.28
37.80
40.67
44.23
56.44
59.29
68.33
72.85
76.49
77.69
79.49
82.27
84.63
86.20
129.78
131.25
131.78
134.03
134.61
146.92
167.90
172.19
209.26
0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
OH
O
OAc
H
O
OH
O
O
HO
5-II

8.119
8.101
8.098
7.626
7.608
7.592
7.589
7.586
7.500
7.481
7.466
7.462
5.608
5.590
5.309
5.028
5.008
4.916
4.912
4.898
4.850
4.328
4.307
4.176
4.155
4.153
3.955
3.939
3.928
3.916
3.899
3.475
3.325
2.728
2.724
2.712
2.688
2.300
2.283
2.265
2.122
2.119
2.046
2.033
1.823
1.817
1.796
1.791
1.782
1.756
1.716
1.267
1.242
1.234
1.177
1.086
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.99
1.09
2.18
1.10
0.94
1.11
1.19
1.00
1.13
1.16
1.96
2.85
2.79
1.36
4.22
2.79
1.05
1.32
2.51
1.55
3.01
3.11
5-III

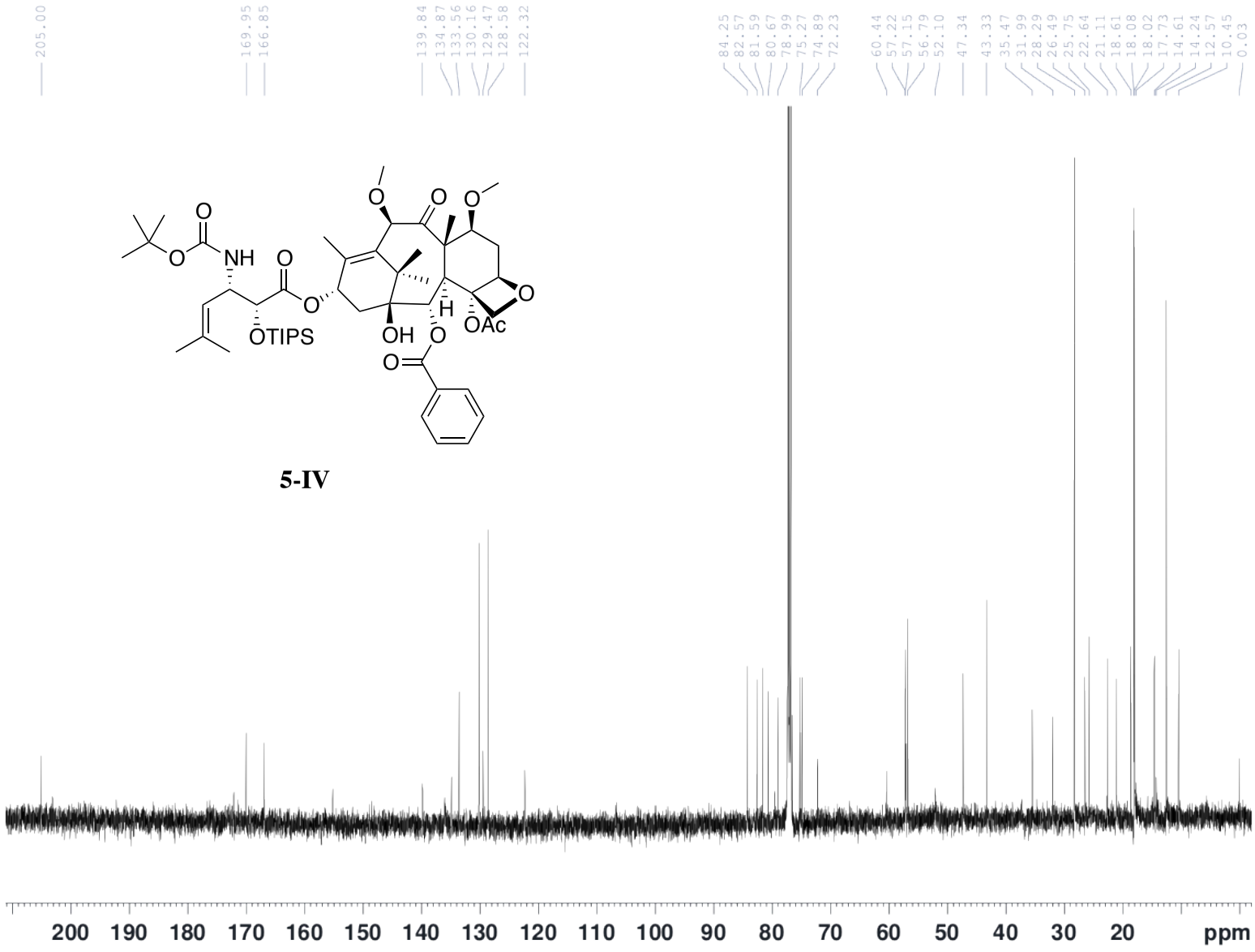
ppm
0
20
40
60
80
100
120
140
160
180
200
10.48
15.53
20.13
22.96
27.28
32.30
38.60
43.04
48.05
57.16
57.26
57.52
68.31
74.78
79.05
81.11
81.64
83.37
84.40
128.80
129.66
130.30
133.79
134.86
142.83
167.24
171.14
205.69
OAc
OH
HO
5-III

8.008
7.989
7.608
7.589
7.571
7.470
7.450
7.431
5.444
5.425
5.112
5.090
4.886
4.834
4.671
4.505
4.488
4.471
4.270
4.250
4.213
4.193
4.081
4.063
4.057
4.039
3.507
3.488
3.370
3.322
2.783
2.768
2.752
2.747
2.381
2.366
2.349
2.334
2.234
1.843
1.810
1.783
1.774
1.753
1.742
1.726
1.630
1.607
1.005
0.985
0.965
0.643
0.624
0.604
0.584
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.97
1.02
2.01
1.00
1.03
1.01
1.03
1.06
1.03
0.98
1.01
1.03
1.02
2.90
3.14
1.03
1.03
2.95
4.21
3.77
2.87
1.13
8.93
6.11
5-III*
OBz
OAc
H
O
Si


206.20
170.17
165.15
149.62
145.50
135.63
133.45
129.70
129.62
128.59
112.72
84.61
81.36
79.31
79.18
77.35
77.03
76.71
75.01
74.46
71.17
63.30
58.34
56.81
55.02
44.71
42.93
33.38
21.70
20.86
11.72
9.04
6.78
4.81
O
O
O
O
H
O
OBz
OAc
Si
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

5-III*

1.048
1.100
1.115
1.123
1.138
1.190
1.205
1.243
1.257
1.271
1.342
1.579
1.598
1.712
1.756
1.797
1.798
1.952
2.043
2.352
2.367
2.375
3.304
3.446
3.857
3.870
3.882
3.890
4.112
4.126
4.186
4.203
4.296
4.313
4.435
4.440
4.808
4.973
4.990
5.329
5.346
5.634
5.648
6.127
7.438
7.454
7.469
7.580
7.595
8.084
8.086
8.101
OAc
OH
OTIPS
NH
5-IV
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
19.75
5.71
1.38
8.47
1.57
2.97
6.64
2.82
0.67
4.60
1.01
2.86
2.88
1.96
0.47
0.96
0.97
0.97
2.91
1.05
1.03
1.00
1.01
1.00
1.95
1.91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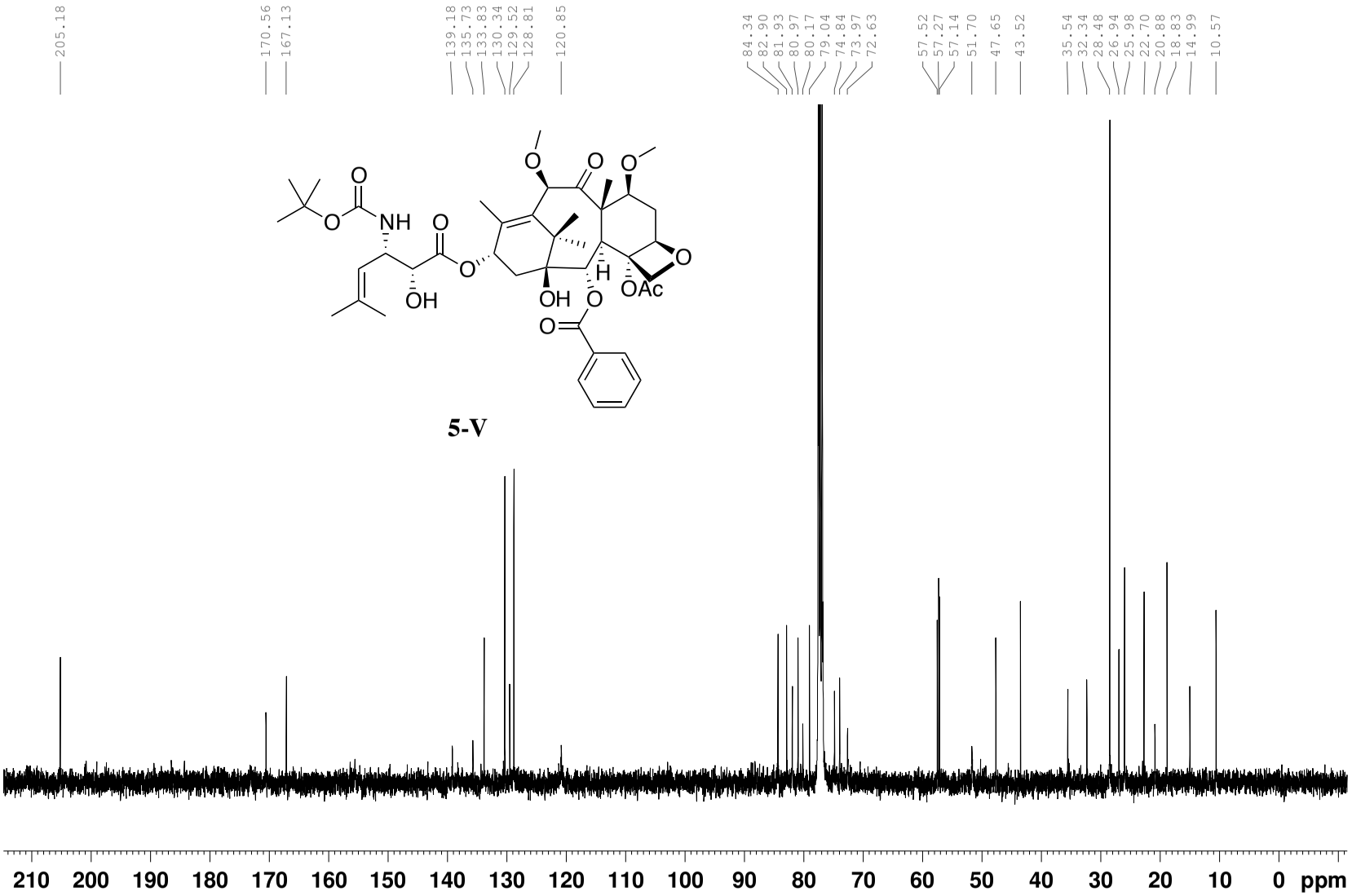
139.84
134.87
133.56
130.16
129.47
128.58
122.32
84.25
82.57
81.59
80.67
78.99
75.27
74.89
72.23
60.44
57.22
57.15
56.79
52.10
47.34
43.33
35.47
31.99
28.29
26.49
25.75
22.64
21.11
18.61
18.08
18.02
17.73
14.61
14.24
12.57
10.45
0.03
O
O
NH
O
O
OTIPS
O
O
O
H
OH
O
OAc
O
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

5-IV

8.084
8.065
7.603
7.584
7.566
7.469
7.449
7.430
6.179
6.158
6.136
5.628
5.611
5.318
5.296
4.985
4.962
4.946
4.924
4.806
4.778
4.772
4.756
4.750
4.294
4.273
4.232
4.225
4.215
4.208
4.176
4.155
3.883
3.867
3.857
3.840
3.830
3.812
3.602
3.584
3.436
3.286
2.688
2.344
2.308
2.286
1.954
1.791
1.755
1.702
1.355
1.192
1.125
1.109
0.946
0.926
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.05
1.03
2.06
1.00
1.00
1.02
2.06
2.00
1.13
1.01
0.99
2.09
1.05
2.93
2.96
0.99
5.17
2.95
1.01
6.99
2.94
9.20
5.94
2.14
0.99
5-V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167.13
139.18
135.73
133.83
130.34
129.52
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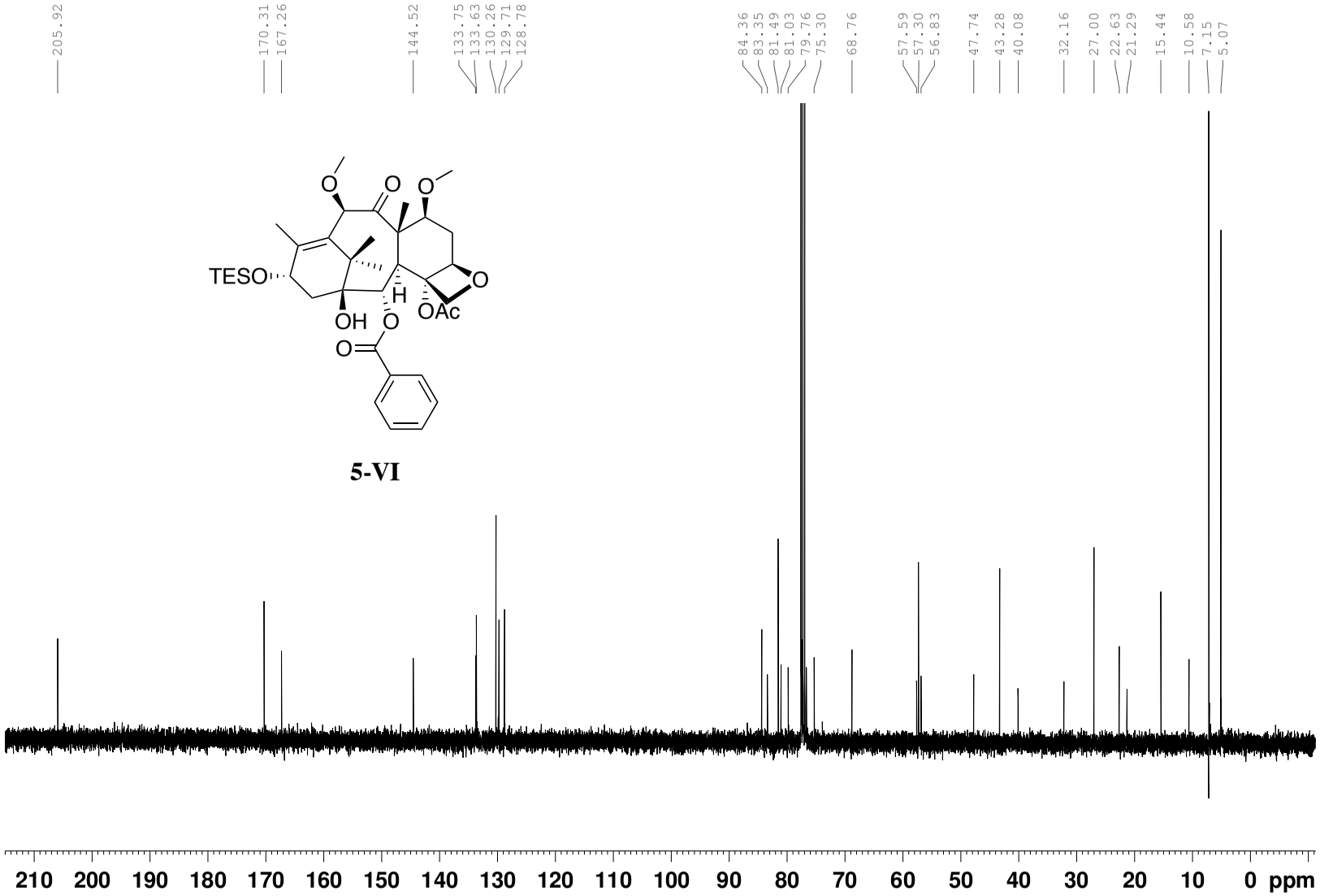
120.85
84.34
82.90
81.93
80.97
80.17
79.04
74.84
73.97
72.63
57.52
57.27
57.14
51.70
47.65
43.52
35.54
32.34
28.48
26.94
25.98
22.70
20.88
18.83
14.99
10.57
O
NH
OH
OAc
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

5-V

8.092
8.074
8.071
7.593
7.575
7.485
7.466
7.447
5.598
5.580
5.017
4.997
4.976
4.821
4.308
4.287
4.155
4.135
3.947
3.931
3.920
3.904
3.846
3.829
3.458
3.305
2.703
2.698
2.687
2.292
2.226
2.203
2.182
2.167
2.144
2.061
2.058
1.784
1.772
1.769
1.695
1.162
1.142
1.044
1.031
1.024
1.004
0.693
0.687
1.97
1.10
2.05
7.0
6.5
6.0
5.5
1.00
5.0
2.06
1.07
4.5
1.11
1.13
4.0
1.03
0.97
3.5
3.05
3.07
3.0
1.09
2.5
2.89
2.08
2.96
2.0
1.12
2.93
1.5
5.87
9.50
1.0
6.27
0.5
ppm
TESO
OAc
OH
5-VI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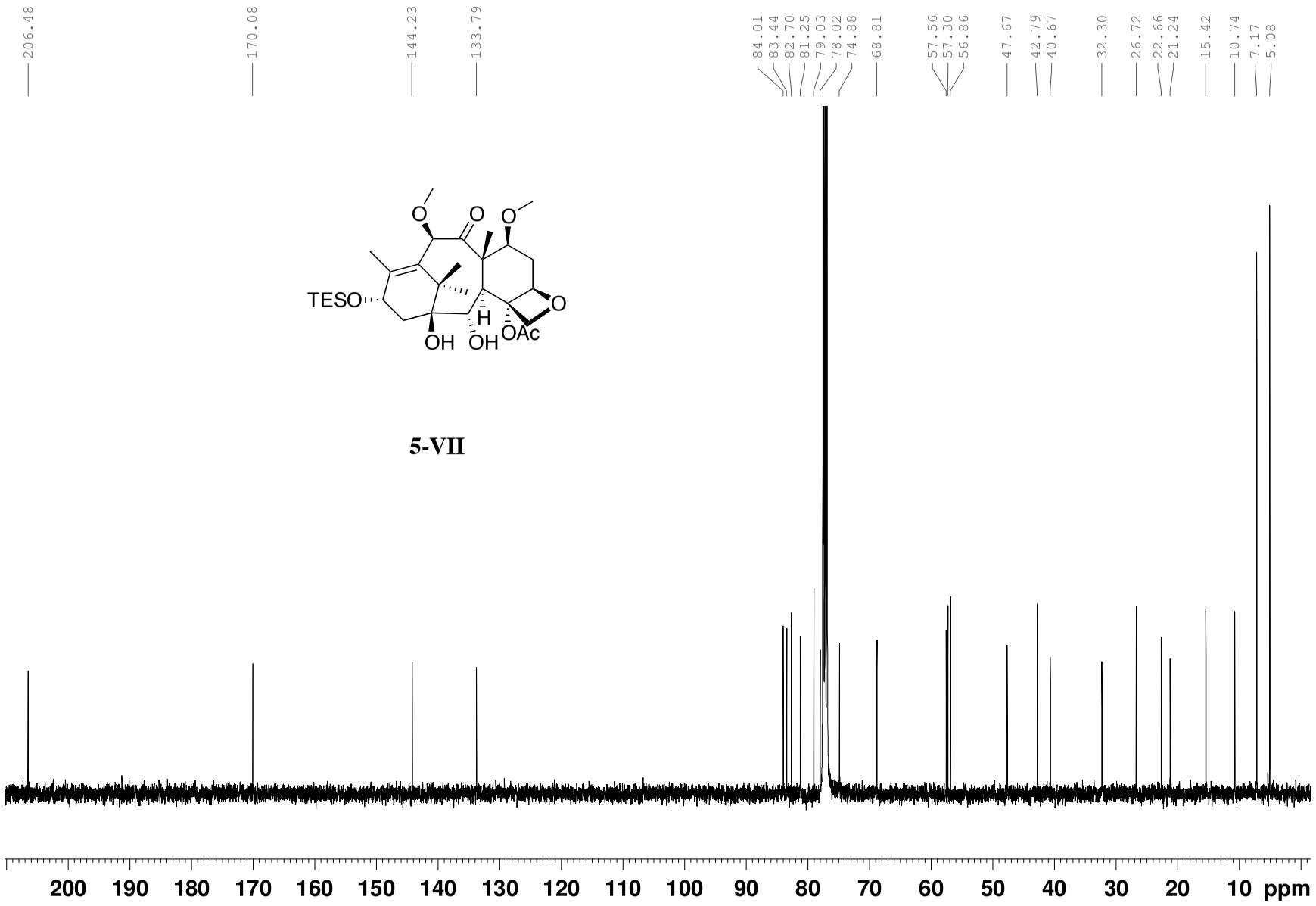
170.31
167.26
144.52
133.75
133.63
130.26
129.71
128.78
84.36
83.35
81.49
81.03
79.76
75.30
68.76
57.59
57.30
56.83
47.74
43.28
40.08
32.16
27.00
22.63
21.29
15.44
10.58
7.15
5.07
TESO
OH
OAc
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

5-VI

5-VII
TESO
OAc
OH
ppm
6.00
8.85
3.04
3.03
3.02
1.28
1.30
2.94
0.87
3.93
1.12
1.23
3.03
3.03
0.98
2.23
1.15
1.04
1.08
2.17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170.08
144.23
133.79
84.01
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82.70
81.25
79.03
78.02
74.88
68.81
57.56
57.30
56.86
47.67
42.79
40.67
32.30
26.72
22.66
21.24
15.42
10.74
7.17
5.08
TESO
OH
OH
H
OAc
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

5-VII

7.924
7.896
7.877
7.419
7.400
7.374
7.356
7.337
5.582
5.564
5.028
5.003
4.980
4.829
4.316
4.296
4.166
4.146
3.955
3.939
3.928
3.912
3.854
3.836
3.467
3.314
2.414
2.302
2.221
2.201
2.180
2.158
2.069
2.066
2.050
1.812
1.806
1.784
1.777
1.771
1.749
1.744
1.703
1.166
1.147
1.054
1.034
1.025
1.014
0.721
0.715
0.702
0.695
0.683
0.675
0.663
0.654
TESO
OAc
OH
5-VIIIa
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.17
2.31
1.00
2.01
1.03
1.00
0.99
1.00
0.99
1.04
3.04
3.05
1.00
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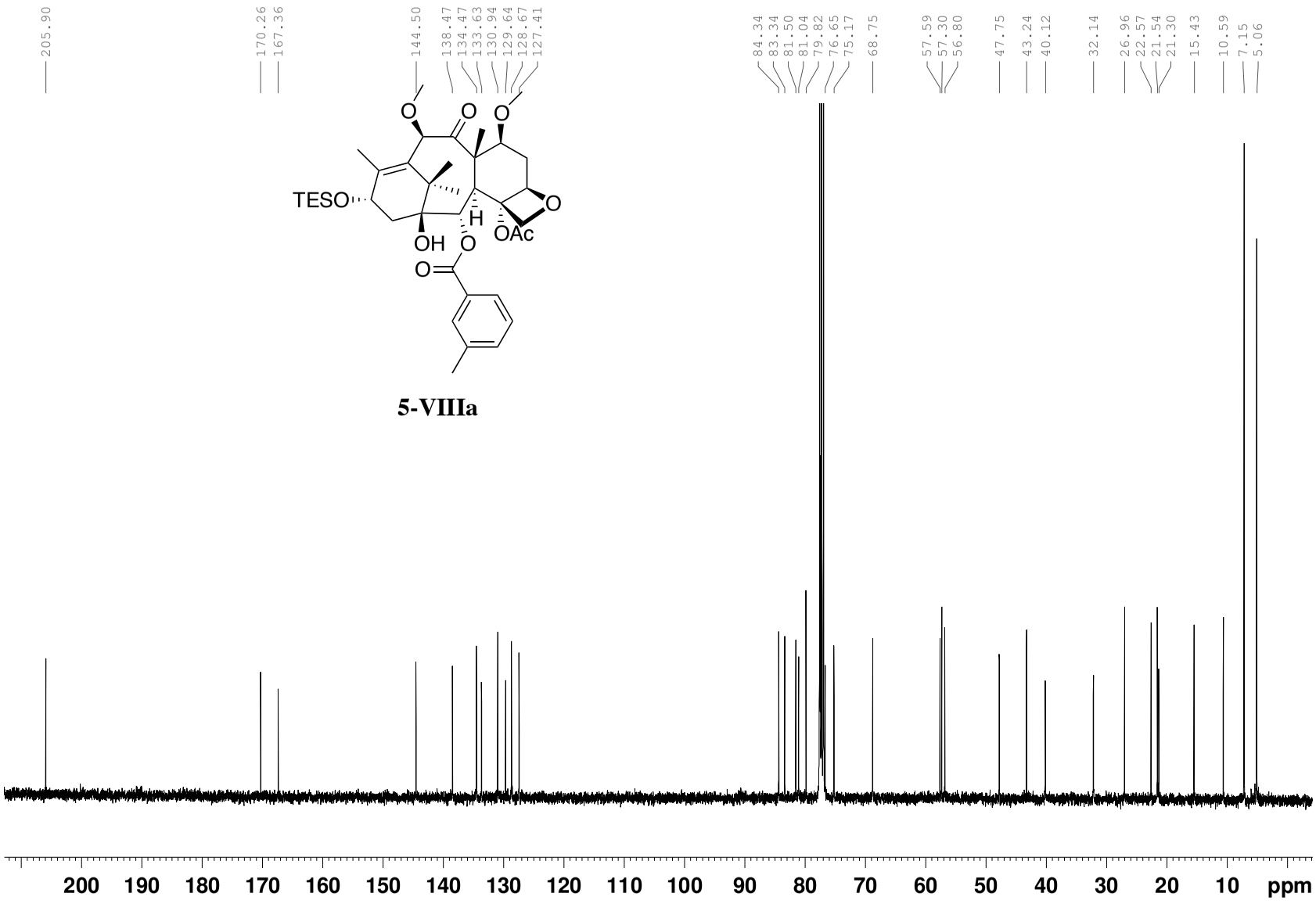
2.91
2.36
3.10
1.20
3.38
1.19
6.06
9.48
6.40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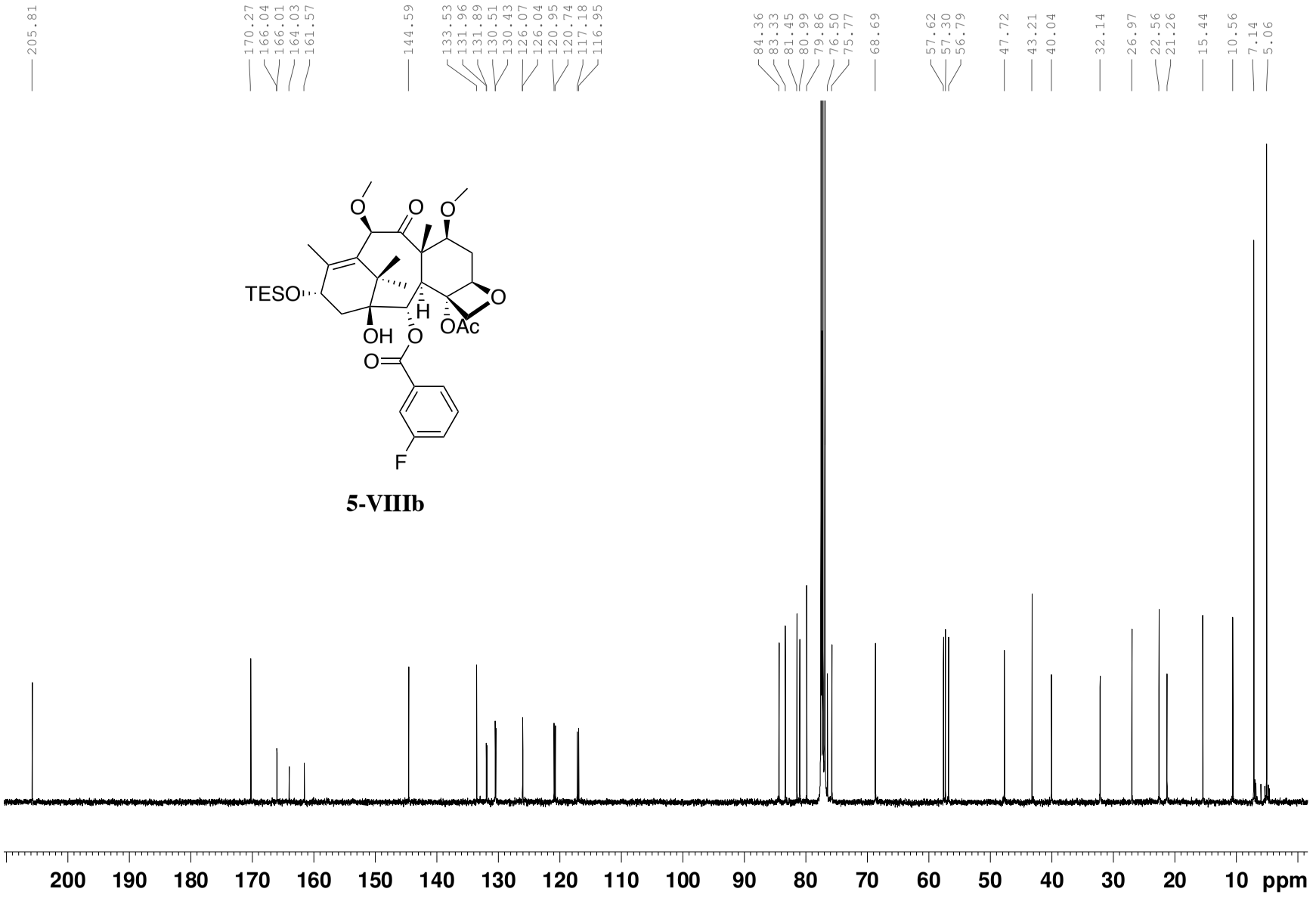
138.47
134.47
133.63
130.94
129.64
128.67
127.41
84.34
83.34
81.50
81.04
79.82
76.65
75.17
68.75
57.59
57.30
56.80
47.75
43.24
40.12
32.14
26.96
22.57
21.54
21.30
15.43
10.59
7.15
5.06
TESO
OH
OAc
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

5-VIIIa

7.892
7.873
7.793
7.787
7.783
7.770
7.766
7.760
7.468
7.454
7.448
7.434
7.312
7.307
5.574
5.556
5.033
5.012
5.008
4.981
4.828
4.308
4.287
4.155
4.134
3.953
3.937
3.926
3.910
3.857
3.839
3.467
3.316
2.304
2.191
2.170
2.149
2.070
2.068
1.780
1.775
1.704
1.515
1.166
1.150
1.054
1.034
1.014
0.723
0.718
0.704
0.698
0.684
0.677
0.665
0.657
TESO
OAc
OH
F
5-VIIIb
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.02
1.03
1.07
1.01
1.00
2.01
1.02
1.03
1.01
1.00
1.01
3.02
2.99
1.03
2.88
2.05
3.09
1.11
2.97
1.07
5.91
9.27
6.15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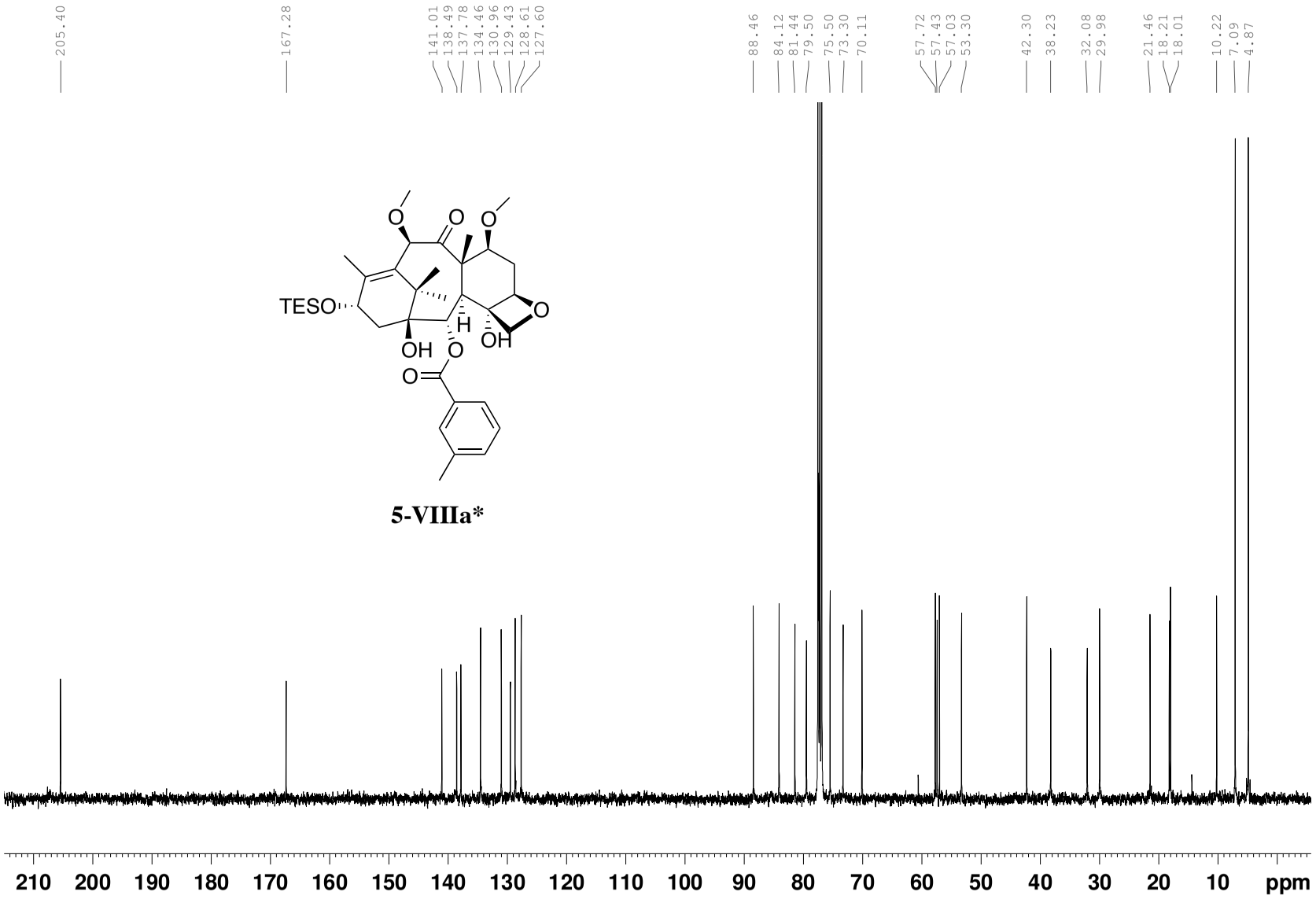
166.04
166.01
164.03
161.57
144.59
133.53
131.96
131.89
130.51
130.43
126.07
126.04
120.95
120.74
117.18
116.95
84.36
83.33
81.45
80.99
79.86
76.50
75.77
68.69
57.62
57.30
56.79
47.72
43.21
40.04
32.14
26.97
22.56
21.26
15.44
10.56
7.14
5.06
TESO
OH
H
OAc
F
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

5-VIIIb

7.931
7.918
7.899
7.387
7.367
7.343
7.324
7.305
5.525
5.511
4.870
4.847
4.838
4.822
4.814
4.711
4.688
4.300
4.280
4.235
4.215
3.571
3.556
3.533
3.518
3.503
3.459
3.295
2.645
2.641
2.632
2.627
2.593
2.587
2.412
2.389
2.369
2.367
2.058
1.832
1.825
1.818
1.591
1.183
1.113
1.094
1.074
1.026
0.848
0.828
0.818
0.810
0.798
0.790
0.778
0.771
0.757
TESO
OH
OH
O
O
O
O
O
O
5-VIIIa*
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.14
2.05
1.00
2.03
1.02
1.00
1.01
0.97
2.03
3.13
3.10
2.07
4.40
3.62
2.02
3.28
2.98
9.06
3.21
6.18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129.43
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88.46
84.12
81.44
79.50
75.50
73.30
70.11
57.72
57.43
57.03
53.30
42.30
38.23
32.08
29.98
21.46
18.21
18.01
10.22
7.09
4.87
TESO
OH
OH
H
O
O
O
O
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

5-VIIIa*

7.938
7.914
7.895
7.423
7.404
7.378
7.359
7.340
5.586
5.568
5.032
5.011
4.908
4.894
4.848
4.331
4.310
4.164
4.157
4.139
3.948
3.932
3.922
3.908
3.892
3.473
3.323
2.763
2.747
2.739
2.728
2.723
2.712
2.704
2.687
2.428
2.299
2.120
2.117
1.821
1.816
1.794
1.786
1.781
1.764
1.759
1.754
1.711
1.579
1.172
1.083
OAc
OH
HO
5-IXa
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.94
2.02
1.00
1.01
2.04
1.03
1.30
2.01
3.00
3.07
1.09
2.90
5.03
2.95
1.34
3.00
3.10
2.97

205.67
171.08
167.36
142.78
138.50
134.86
134.52
130.97
129.55
128.67
127.43
84.36
83.36
81.67
81.12
79.05
74.61
68.32
60.61
57.51
57.25
57.13
48.05
43.03
38.60
32.30
27.26
22.90
21.59
21.27
20.12
15.51
14.41
10.47
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
HO
OH
OAc
O
O
O
O
O
H

5-IXa

8.082
8.078
7.587
7.568
7.482
7.462
7.443
5.632
5.614
5.195
4.957
4.932
4.437
4.421
4.411
4.394
4.295
4.274
4.154
4.133
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3.846
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2.507
2.283
1.984
1.981
1.917
1.911
1.890
1.883
1.876
1.855
1.652
1.196
1.131
1.016
0.996
0.964
0.705
0.700
0.685
0.680
0.673
0.666
0.659
0.653
0.646
0.638
0.633
0.612
0.592
0.572
0.552
TESO
OTES
O
O
OAc
H
OH
O
O
TESO
6-I
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.22
1.32
2.36
1.28
1.32
2.29
1.44
1.23
1.25
1.24
1.67
2.77
1.56
3.12
3.00
2.86
3.03
24.00
17.05

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0.951
0.971
0.991
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1.154
1.610
1.651
1.856
1.866
1.888
1.912
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2.050
2.072
2.157
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2.486
2.504
2.510
2.521
2.528
2.545
3.443
3.460
3.840
3.856
3.873
4.324
4.341
4.351
4.367
4.535
4.558
4.604
4.627
4.927
4.950
5.111
TESO
O
OTES
TESO
OH
OH
H
OAc
O
6-II
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.00
2.00
0.99
1.00
1.01
0.99
0.99
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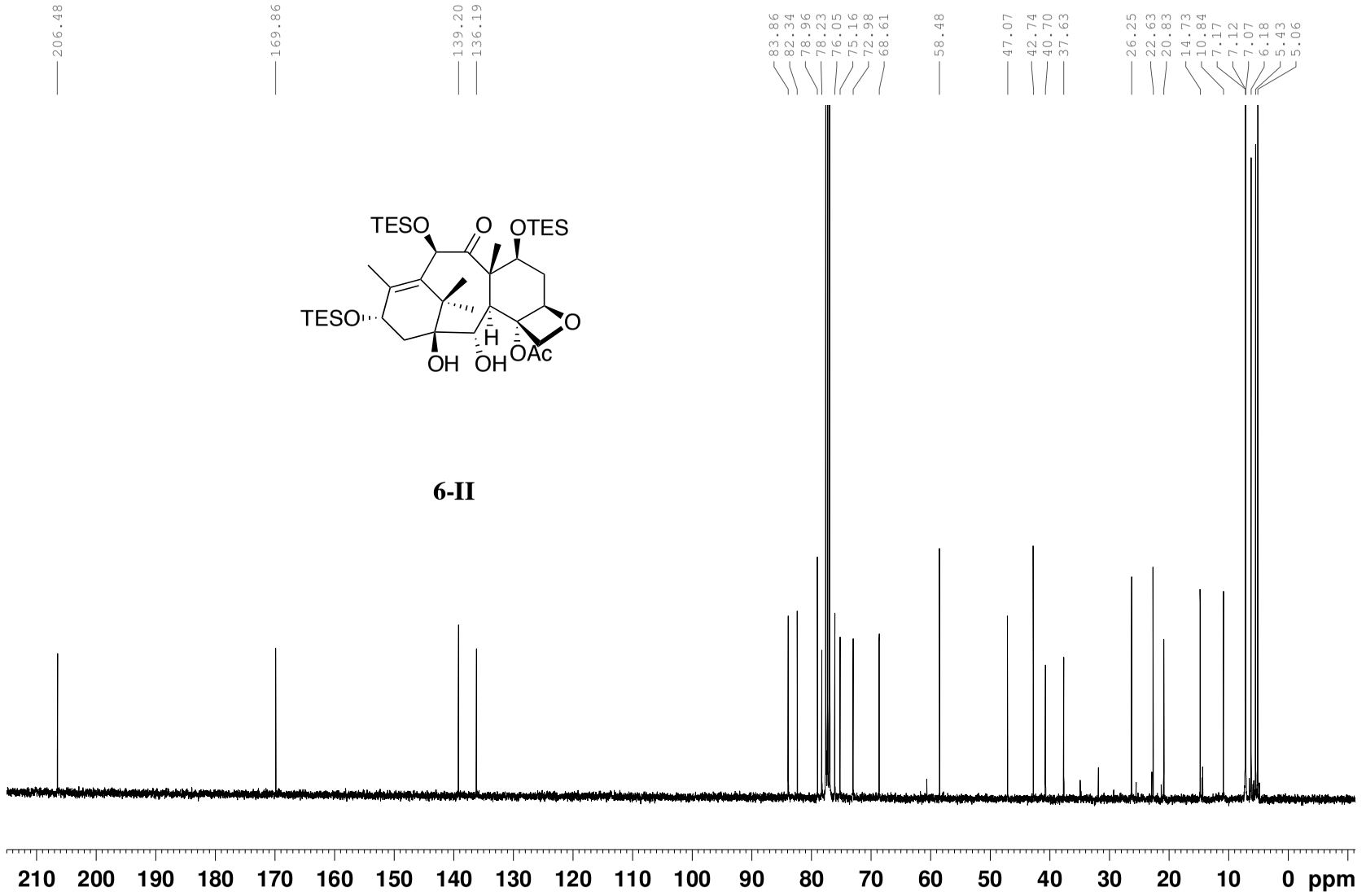
18.43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76.05
75.16
72.98
68.61
58.48
47.07
42.74
40.70
37.63
26.25
22.63
20.83
14.73
10.84
7.17
7.12
7.07
6.18
5.43
5.06
TESO
O
OTES
TESO
O
H
OH
OH
OAc
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

6-II

7.893
7.873
7.385
7.362
7.343
7.324
5.605
5.587
5.298
5.192
4.958
4.938
4.923
4.435
4.418
4.408
4.392
4.292
4.271
4.154
4.133
3.862
3.844
2.404
2.283
2.223
2.202
2.132
2.109
1.981
1.884
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1.189
1.126
1.037
1.017
0.996
0.983
0.976
0.963
0.944
0.704
0.699
0.685
0.679
0.672
0.665
0.658
0.652
0.645
0.637
0.632
0.626
0.611
0.592
0.572
0.552
TESO
OTES
O
OAc
H
O
OH
O
TESO
6-IIIa
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.10
2.18
1.04
1.02
2.07
1.07
1.03
1.02
1.00
1.03
2.94
2.92
1.05
1.00
2.92
1.03
3.00
2.97
2.99
25.16
17.30

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7.362
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4.317
4.294
4.285
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4.266
4.248
4.175
4.154
4.147
4.015
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2.618
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2.079
2.077
2.028
2.016
1.865
1.859
1.837
1.830
1.823
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1.594
1.427
ppm
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8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.97
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1.00
1.08
1.07
1.15
1.17
1.00
1.16
1.03
1.12
2.65
4.05
4.97
1.29
2.57
1.28
4.93
OH
O
HO
OH
OAc
H
O
O
OH
6-IVa

7.931
7.908
7.889
7.417
7.398
7.372
7.353
7.334
5.589
5.571
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5.167
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4.948
4.875
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2.251
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1.587
1.081
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0.935
0.915
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0.551
0.535
ppm
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.00
2.06
1.08
1.06
1.15
1.10
1.10
1.09
1.04
1.09
1.07
1.06
2.82
2.82
2.75
1.03
1.14
2.74
5.53
8.29
5.60
OTES
OAc
OH
HO
HO
6-Va

5.37
6.94
10.13
15.37
19.71
21.58
22.79
27.10
37.47
38.78
42.91
47.21
58.15
68.24
73.18
74.85
74.88
79.02
81.01
84.45
127.43
128.68
129.52
130.95
134.55
135.46
138.52
141.87
167.40
170.96
210.54
HO
O
OTES
OAc
H
OH
O
O
HO
6-Va
ppm
0
20
40
60
80
100
120
140
160
180
200

7.905
7.886
7.405
7.387
7.361
7.342
7.323
5.586
5.569
4.942
4.446
4.429
4.420
4.403
4.309
4.288
4.146
4.125
3.883
3.866
3.407
2.486
2.481
2.469
2.462
2.445
2.412
2.279
2.259
2.102
1.919
1.915
1.893
1.887
1.879
1.857
1.853
1.667
1.619
1.609
1.166
1.063
0.976
0.956
0.936
0.626
0.608
0.598
0.589
0.578
0.569
0.558
0.550
0.537
0.520
OTES
OAc
OH
HO
6-VIa
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.00
2.11
1.02
3.00
1.05
1.05
1.01
1.03
3.00
1.07
3.02
4.93
0.91
3.01
1.19
2.95
2.86
2.97
2.97
9.32
6.04

5.64
7.00
10.09
14.40
15.39
19.79
21.26
21.59
22.88
27.06
37.55
38.56
43.07
47.55
56.00
58.45
60.62
68.26
73.19
74.90
79.05
81.28
82.90
84.52
127.44
128.65
129.59
130.98
134.07
134.50
138.48
143.17
167.41
170.99
204.71
OTES
OAc
OH
HO
6-VIa
ppm
0
20
40
60
80
100
120
140
160
180
200

7.924
7.905
7.432
7.414
7.387
7.368
7.349
5.637
5.619
5.002
4.983
4.978
4.968
4.904
4.337
4.315
4.303
4.292
4.286
4.281
4.264
4.172
4.151
3.969
3.951
3.471
2.650
2.633
2.626
2.614
2.609
2.597
2.590
2.573
2.434
2.306
2.296
2.286
2.258
2.100
2.097
2.023
2.011
1.841
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1.813
1.807
1.799
1.777
1.772
1.692
1.590
1.370
1.348
1.174
1.092
ppm
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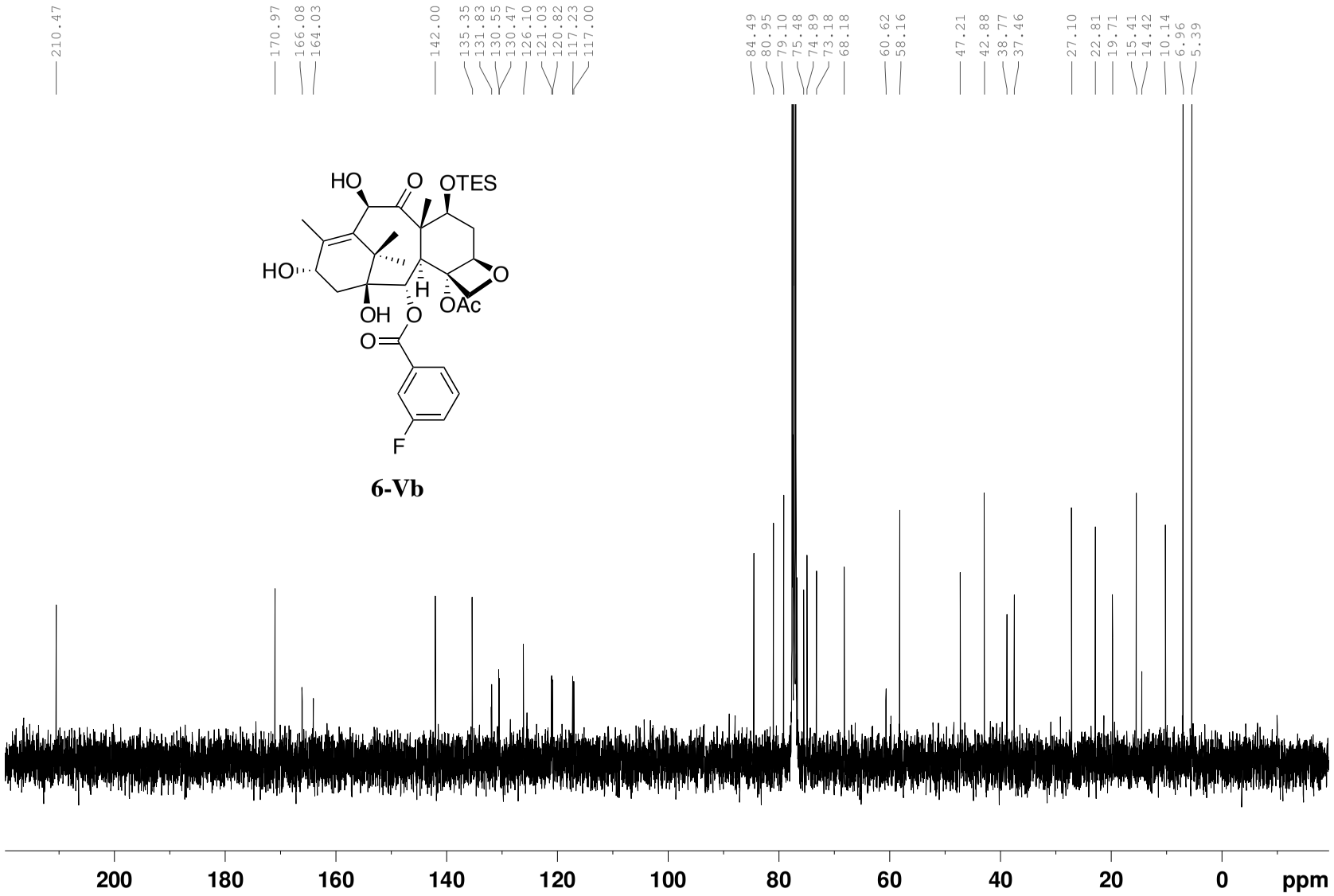
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.00
2.15
1.01
3.00
2.10
1.08
1.00
2.85
1.10
2.79
3.99
2.44
0.99
1.11
2.38
1.15
2.28
2.73
HO
O
OAc
H
O
OH
O
O
OH
O
6-VIIa

7.929
7.820
7.814
7.796
7.790
7.567
7.561
7.547
7.541
7.527
7.414
7.399
7.393
5.606
5.588
5.319
5.036
5.016
4.824
4.801
4.782
4.294
4.277
4.266
4.250
4.218
4.198
4.190
4.170
3.998
3.980
2.458
2.454
2.442
2.434
2.375
2.357
2.336
2.318
2.268
2.241
2.225
2.202
2.043
2.041
1.851
1.846
1.823
1.816
1.810
1.782
1.690
1.084
1.077
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
0.99
1.03
1.02
1.03
1.98
1.03
1.17
1.01
1.00
1.01
1.05
1.03
3.02
0.89
2.81
1.07
2.91
5.92
HO
OH
O
OAc
H
O
OH
O
F
6-IVb

10.48
15.41
20.96
22.81
27.35
37.69
40.83
44.09
59.04
68.29
72.93
76.41
77.14
77.66
79.54
82.18
86.11
117.60
117.84
121.35
127.13
131.78
131.86
135.89
144.83
172.09
211.92
0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
HO
O
OH
O
OAc
H
O
OH
O
F
6-IVb

7.913
7.893
7.814
7.809
7.791
7.787
7.482
7.476
7.462
7.456
7.442
7.320
7.314
5.589
5.572
5.183
5.178
4.981
4.961
4.888
4.873
4.437
4.420
4.410
4.394
4.323
4.303
4.254
4.249
4.169
4.149
3.970
3.953
2.494
2.297
2.271
2.251
2.094
2.034
2.022
1.919
1.914
1.742
1.092
0.966
0.946
0.926
0.619
0.600
0.586
0.581
0.566
0.562
0.546
0.526
0.508
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.05
1.05
1.07
1.12
1.08
1.06
1.09
1.10
1.09
1.05
1.07
1.03
1.11
1.15
5.02
2.81
1.08
1.10
2.83
5.68
9.01
6.00
HO
O
OTES
O
OAc
H
OH
O
O
F
HO
6-Vb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130.55
130.47
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121.03
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117.23
117.00
84.49
80.95
79.10
75.48
74.89
73.18
68.18
60.62
58.16
47.21
42.88
38.77
37.46
27.10
22.81
19.71
15.41
14.42
10.14
6.96
5.39
HO
O
OTES
HO
H
O
OAc
OH
O
O
F
200
180
160
140
120
100
80
60
40
20
0
ppm

6-Vb

7.909
7.906
7.903
7.889
7.886
7.883
7.810
7.806
7.803
7.786
7.782
7.780
7.463
7.450
7.444
7.308
7.306
7.301
7.299
5.584
5.566
4.977
4.952
4.456
4.439
4.429
4.413
4.303
4.283
4.150
4.132
3.897
3.879
3.415
2.288
2.274
2.256
2.112
2.110
1.676
1.647
1.171
1.073
0.985
0.965
0.945
0.617
0.607
0.598
0.587
0.579
0.567
0.559
0.549
0.546
OTES
OAc
OH
HO
F
6-VIb
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.01
1.03
1.06
1.01
3.03
1.03
1.05
1.10
1.04
3.02
1.04
4.96
1.00
3.03
1.08
3.02
2.99
3.01
9.28
6.22

5.43
6.79
9.87
15.20
19.56
22.67
26.85
37.31
38.32
42.81
47.33
55.81
58.22
67.96
72.95
75.32
78.90
80.97
82.67
84.33
116.78
117.01
120.54
120.75
125.92
130.22
130.30
131.67
131.74
133.71
143.12
161.33
163.80
165.86
170.78
204.46
OTES
OAc
OH
HO
O
F
6-VIb
0 ppm