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Design, synthesis and biological evaluation of novel taxane-based anticancer agents and their applications to tumor-targeting drug delivery systems

A Dissertation Presented

by

XIANRUI ZHAO

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Abstract of the Dissertation

Design, synthesis and biological evaluation of novel taxane-based anticancer agents and their applications to tumor-targeting drug delivery systems

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Paclitaxel (Taxol®) and docetaxel (Taxotere®) are currently two of the most important anticancer drugs. However, as others in cancer chemotherapy, they are ineffective against multidrug resistant (MDR) cancers and cause serious side effects. Based on structure-activity relationship study, a series of novel taxoids have been designed and synthesized utilizing the β-Lactam Synthon Method developed by Ojima's laboratory. Both *in vitro* and *in vivo* evaluations showed that these new generations of taxoids exhibited hundreds or thousands of times higher potency than that of paclitaxel, especially against MDR cell lines and xenografts.

One of the major parts in my research is Tumor-Targeting Drug Delivery System (TTDDS) containing tumor-targeting moiety (TTM), disulfide linker and taxoid, which are covalently connected as one conjugate. Undesired side effects are induced by destroying normal healthy cells (*e.g.*, bone marrow and hair) by paclitaxel and other anticancer drugs while malignant cells are killed. TTDDS may increase the drug efficiency and decrease side effects. With the help of TTM as the guide, the whole drug conjugate should be introduced into cancerous cells selectively without hurting normal cells. The function of the disulfide linker is to keep the whole conjugate stable during circulation in plasma, but to release the anticancer agents quickly and completely inside of tumor cell. The taxoid in its conjugate state is non-toxic during transportation, but its

potency is recovered once it is cleaved off from the whole conjugate and liberated at its original form inside the cancer cells. To achieve such goals, several disulfide-containing linker systems have been designed, synthesized, and screened with either model compounds or real taxoid-linker conjuages. Some key kinetic factors including substituent effect and acidity (pH) effect have been studied. Finally, a series of Biotin-Linker-Taxoid conjugates have been prepared and evaulated *in vitro*, in which biotin is serving as TTM. Different functional fluorescent molecules are also coupled to those conjugates, which facilitate the observation *in vitro* by confocal fluorescence microscopy. These cellular experiments have successfully provided the proof of concept, *i.e.*, internalization of the whole conjugate, disulfide bond cleavage by intracellular thiol source, fast intramolecular thiolactonization, the release of the free cytotoxic agent, and the binding of the free drug to its target protein.

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List of Symbols and Abbreviations

Å	angstrom
Ab	antibody
Ac	acetyl
AcOH	acetic acid
Anal	analysis
aq.	aqueous solution
atm	atmosphere
ATP	adenosine triphosphate
b	broad
bd	broad doublet
Bn	benzyl
bp	boiling point
bs	broad singlet
Boc	*tert*-butoxycarbonyl
t-Bu	*tert*-butyl
n-BuLi	*n*-butyllithium
Bz	benzoyl
calcd.	calculated value or theoretical value
CAN	ammonium cerium(IV) nitrate
d	doublet (in NMR spectrum) or day (in reaction time)
DAB	10-deacetylbaccatin III
DACA	4-*N,N*-Dimethylaminocinnamaldehyde
DCC	*N,N*'-dicyclohexylcarbodiimide
DCM	Dichloromethane or methylene dichloride
DCU	*N,N*'-dicyclohexylurea
dd	doublet of doublet
d.e. or de	diastereomeric excess
DHA	docosahexaenoic acid
DIBALH	diisobutylalluminum hydride
DIC	*N,N'*-diisopropylcarbodiimide
DICU	*N,N'*-diisopropylurea
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethylsulfoxide
DMSO-d^6	deuterium dimethylsulfoxide
DMTr	4,4'-dimethoxyltrityl
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
e.e. or ee	enantiomeric excess
EE	1-ethoxyethyl
eq or eq.	equivalent
ESI	Electron Spray Ionization
Et	ethyl
EtOAc	ethyl acetate

EVE	ethyl vinyl ether
F-C Reaction	Friedel-Crafts Reaction
FDA	Food and Drug Administration
FITC	Fluorescein isothiocyanate
g	gram
GSH	glutathione
GTP	guanosine 5'-triphosphate
h	hour
Hex	hexanes
HMC or 7-HMC	7-Hydroxy-4-methylcoumarin
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HOBT	1-Hydrobenzotriazole hydrate
HOSu	*N*-hydroxylsuccimide
HONp	4-Nitrophenol
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Hertz
IC_{50}	concentration for 50 % inhibition
*i*Pr	isopropyl
IR	infrared spectroscopy
J	coupling constant
kDa	kilodalton
kg	kilogram
KHMDS	potassium 1,1,1,3,3,3-hexamethyldisilazide
L	liter
LDA	lithium diisopropylamide
LiHMDS	lithium 1,1,1,3,3,3-hexamethyldisilazide
lit.	literature or literature value
m	multiplet
M	molar concentration
MAP	microtubule associated protein
MDR	multidrug resistance
MDS	methyldisulfanyl
Me	methyl
mg	milligram
MHz	megahertz
min	minute
mL	milliliter
mmol	millimole
mol	mole
mp or m.p.	melting point
MS	mass spectrometry
NaHMDS	sodium 1,1,1,3,3,3-hexamethyldisilazide
NCI	National Cancer Institute
nM	nanomolar
NMR	nuclear magnetic resonance

PG	protecting/protective/protection group
Pgp	P-glycoprotein
Ph	phenyl
PLAP	pig liver acetone powder
PMP	*para*-methoxyphenyl
ppm	parts per million
p-TSA	*p*-toluenesulfonic acid
Py	pyridine
q	quartet
Red-Al	bis(methoxyethoxy)aluminum hydride
r.t. or rt	room temperature
s	singlet (in NMR spectrum) or second (in reaction time)
SAR	structure-activity relationship
SM	starting material
t	triplet
TAP	tumor activated prodrug
TBDMS or TBS	*tert*-butyldimethylsilyl
TEA	triethylamine
tert	tertiary
TES	triethylsilyl
Tf	triflouromethanesulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	tri*iso*propylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
v	volume
v/v	volume ratio
wt	weight
[α]	specific optical rotation
δ	chemical shift
μM	micromolar

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1. Chen, Jingyi; Chen, Shuyi; **Zhao, Xianrui**; Kuznetsova, Larisa V.; Wong, Stanislaus S.; Ojima, Iwao. *J. Am. Chem. Soc.* **2008**, *130*, 16778-16785.
2. Ojima, Iwao; Chen, Jin; Sun, Liang; Borella, Christopher; Wang, Tao; Miller, Michael; Lin, Songnian; Geng, Xudong; Kuznetsova, Larisa; Qu, Chuanxing; Gallager, David; **Zhao, Xianrui**; Zanardi, Ilaria; Xia, Shujun; Horwitz, Susan; Clair, Jon Mallen-St; Guerriero, Jennifer; Bar-Sagi, Dafna; Veith, Jean; Pera, Paula; Bernacki, Ralph *J. Med. Chem.* **2008**, *51*, 3203-3221.
3. Chen, Jin; Jaracz, Stanislav; **Zhao, Xianrui**; Chen, Shuyi; Ojima, Iwao. *Expert Opin. Drug Deliv.* **2005**, *2*, 873-890.
4. **Zhao, Xianrui**; Li, Chongxi. Synthesis ester of nitrogen containing carboxylic acids with metronidazole. *Beijing Daxue Xuebao, Ziran Kexueban* **2002**, *38(2)*, 175-180.

Meetings and Presentations (selected):

1. **Zhao, Xianrui**; Chen, Shuyi; Chen, Jin; Chen, Jingyi; Iwao Ojima. Synthesis and application of fluorescence-labeled biotin-taxoid conjugate for the investigation into efficacious tumor-targeting drug delivery system. *234th ACS National Meeting*, Boston, MA, United States, 2007.
2. **Zhao, Xianrui**; Chen, Jin; Chen, Shuyi; Jaracz, Stanislav; Das, Manisha; Iwao Ojima. Novel disulfide linkers for tumor-targeting drug delivery. *234th ACS National Meeting*, Boston, MA, United States, 2007.
3. **Zhao, Xianrui**; Chen, Jin; Commandeur, Claude; Ojima, Iwao. New approaches to tumor-targeted chemotherapy: Development of "coupling-ready" taxoid-linker constructs. *229th ACS National Meeting*, San Diego, CA, United States, 2005.

Field of Study

Organic and medicinal chemistry

Chapter One
Paclitaxel and β-Lactam Synthon Method

§ 1.1. Cancer and Chemotherapy

Cancer refers to a group of diseases, in which cell growth is out of control and these abnormal cells tend to invade surrounding tissues or migrate to other body parts. Migration, which is also called metastasis, usually is lethal. Man has noticed cancer for thousands of years. For example, cancer was observed by ancient Egyptians and Greeks. However, it draws more and more attention nowadays in modern human society.[1]

First, cancer is accompanied by high mortality. According to the statistical data from 1996 to 2003, only 5% pancreatic cancer patients would be still alive 5 years after their first diagnoses.[2] The 5-year survival rate is 11% for liver and 16% for lung cancer patients. Cancer usually means lifetime is counting down. Secondly, new cases keep increasing every year although cancer is not contagious, and prevention seems complicated. It's well-known that smoking is related to higher risk for lung cancer, but the real pathology for most types of cancer is not yet fully understood. By estimation, 1.44 million new cancer patients occured in U.S. in 2008 (compared to 1.39 million in 2006), and 0.56 million will die of cancer (*i.e.*, 1500 people per day). Third, cancer may recur, and more seriously, cancerous cells may develop drug-resistance and survive. Moreover, cancer is complicated by other problems than death, such as medical insurance and cost ($ 219 billion in 2007), which may delay cancer treatment.[2] The American Cancer Society stated that cancer, surpassing heart disease, was the first leading cause of death in the U.S. for people under 85 years old in 1999.[3] Overall, cancer is a growing public health problem.

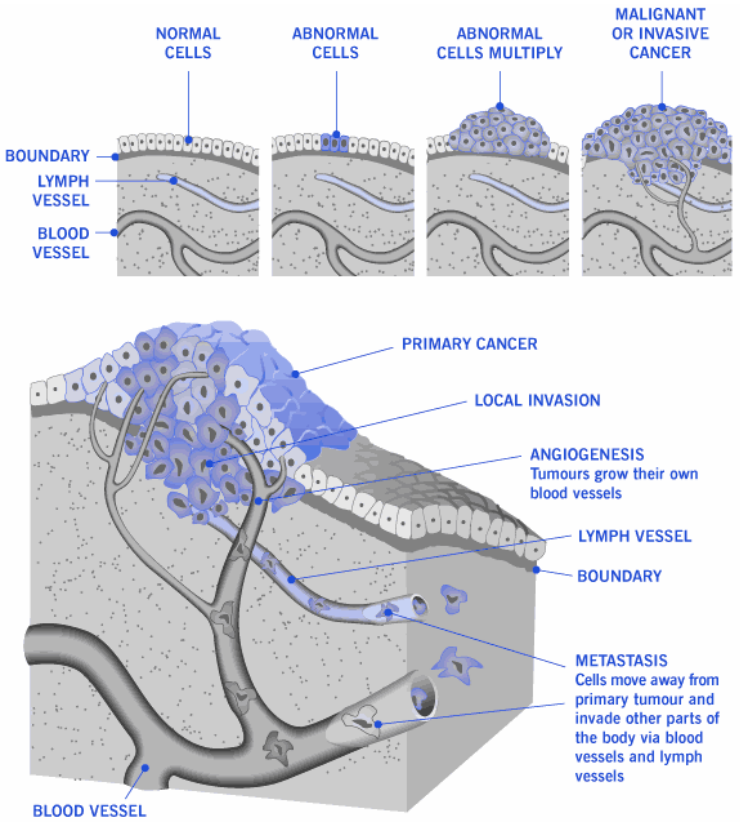


Figure 1-1. Formation of cancer [4]
(adapted from http://www.cancervic.org.au/cancer1/students/pics/what_is_cancer.gif)

Many factors may cause cancer, including external (chemicals, radiation, and life style) and internal (hormones, immune condition, and inherited mutations) ones. All of them are believed to damage DNA and cause gene mutations, which may produce abnormal proteins and cellular signal messengers. Under such stimulations, normal cells may become abnormal cells and form agglomerates, which could happen anywhere inside the body (Figure 1-1).[4,5] It should be noted that the proliferation of these abnormal cells is still under control and usually they are covered by a membrane. If the abnormal cells grow out of regulations, they will break out of the membrane and spread to invade surrounding tissues, including lymph and blood systems. This means the tumor cells turn into a malignancy. Later, new blood vessels will be formed inside the mass, *i.e.*, malignant tumor, to further facilitate the growth of tumor cells. The process of formation of blood vessels is named angiogenesis. At a certain stage, the tumor is not limited locally, and cancerous cells, after released from their matrix, may travel through blood circulation to other body parts. Due to special cellular signal mechanism, cancerous cells usually have much stronger ability to survive under unfriendly conditions. If those malignant cells continue to proliferate in the new body site, a new tumor is generated afar. This is called metastasis, which is fatal to mammals mainly because it may cause multiple system organ failure (MSOF), especially when vital organs are involved. Compared to the localized tumor, the metastasis tumor is usually responsible for death, and 5-year survival rate drops dramatically as shown in the statistical data,[2] for example,

that 91% of patients with melanoma of the skin would be still alive if there were no metastasis, but the rate decreases down to only 15% if metastasis occurs.

Based on its progress, cancer could be also divided into several different stages. One of the staging systems is named TNM method, which focuses on local primary tumor (T), regional lymph node infection within the same organ or tissue (N), and remote metastasis (M). Stages I to IV thus are assigned accordingly from primary tumor to advanced distant tumor.[2]

In the nomenclature, tumor or neoplasm refers to an agglomerate of uncontrolled cells, which could be either benign or malignant. Malignant neoplasm is invasive and metastatic, but not benign neoplasm. When cancer is discussed, it usually refers to the malignant tumor.

Currently, the treatments against cancer involve surgery, radiation, chemotherapy, gene therapy, and other biological methods. The choice on therapy depends on the location and status of the tumor and the physical condition of the patient. Different therapeutic methods may be combined together to achieve the best result. In recent years, chemotherapy develops quickly and greatly improves the quality of life of patients.

Chemotherapy involves the use of cytotoxic drugs to kill cancerous cells, shrink tumor size, stop the angiogenesis and metastasis, and ultimately eradicate cancer. Chemotherapy is very effective against tumors with high proliferation rate, such as acute myelogenous leukemia. These chemical drugs usually stop cell division or DNA/protein synthesis *via* various mechanisms, thus combination of different drugs in chemotherapy is normally adopted. One drawback in chemotherapy is the potential damage on healthy cells, especially those fast dividing normal cells, such as bone marrow, epithelial and hair cells. Common side effects are hair loss, nausea and vomiting, diarrhea or constipation, anemia and depression of immune system. To minimize those undesired effects, targeted drug delivery is conceived and currently under investigation. Multi-drug resistance (MDR) is another disadvantage for chemotherapy. After several cycles of chemotherapeutic treatments, the tumor cells develop resistance against a variety of drugs with distinct structures, and reduce or disable drug functions. Thus, new drugs are required to overcome MDR.

Most chemotherapeutic drugs can be categorized into DNA-targeting agents (*e.g.* cisplatin), antimetabolites, anthracyclines (*e.g.* doxorubicin), plant alkaloids (*e.g.* vinca alkaloids and taxanes), topoisomerase inhibitors (*e.g.* camptothecin and teniposide), antitumor antibiotics (*e.g.* dactinomycin), and most recently kinase inhibitors (*e.g.* erlotinib). Paclitaxel, docetaxel and other taxoids will be the focus in this dissertation (Figure 1-2).

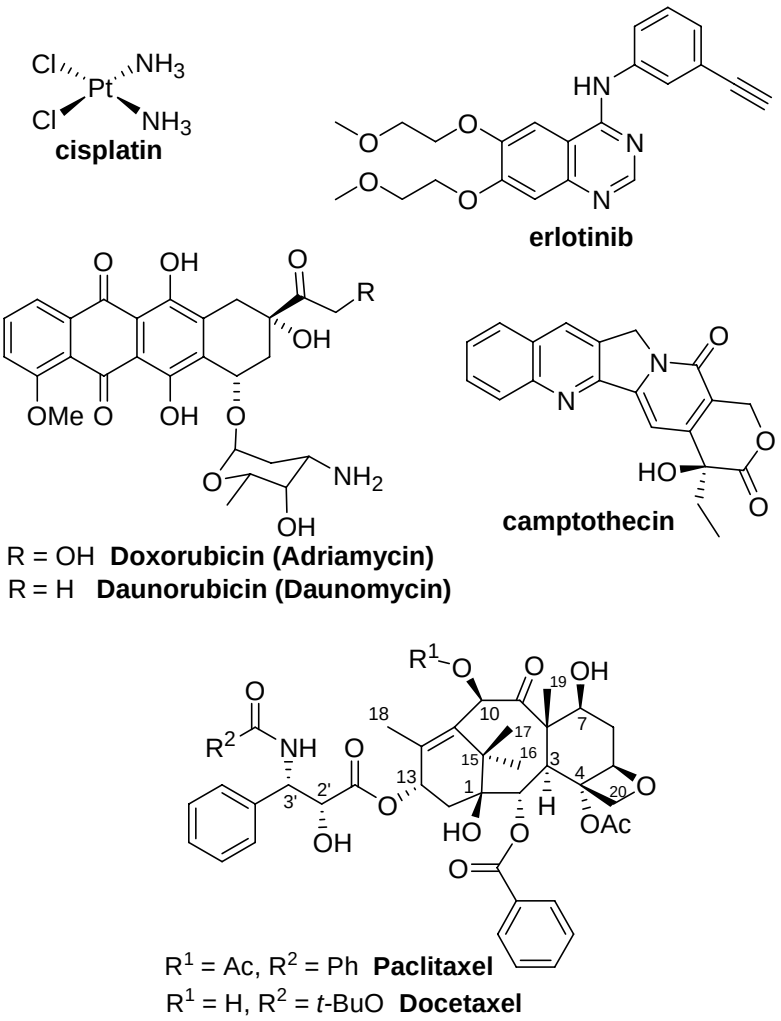


Figure 1-2. Some of anticancer drugs used in clinic

§ 1.2. Paclitaxel, Docetaxel and Taxoids

§ 1.2.1. History of paclitaxel

Paclitaxel (Taxol®, Figure 1-2a) is a leading drug in chemotherapy, and its story may be tracked back to 1950s, when the U. S. National Cancer Institute (NCI) initiated a program to find anticancer plants in 35,000 species.[6] In 1966, crude extracts from the bark of the pacific yew tree (*Taxus brevifolia*) was found to have activity against tumor and mouse leukemia. It was in 1971 that Wani and his group isolated and characterized the active component in this extract.[7] Due to its hydroxyl groups, the molecule was initially named taxol. Later on, paclitaxel showed extremely potent activity against several tumor cell lines. Recognizing paclitaxel's potential property, Bristol-Myers-Squibb (BMS) was authorized by NCI to develop this powerful anticancer candidate. After the U. S. Food and Drug Administration (FDA) approved for the treatment of refractory ovarian cancer, paclitaxel hit the market by BMS with its commercial name Taxol® in December 1992. Soon after, paclitaxel was approved for breast cancer in 1994, Kaposi's sarcoma in 1997, and lung cancer in 1998. Although paclitaxel has become a generic drug, some Phase II and III clinical trials for the treatment of other cancers, such as colon and prostate cancers, and combination with other therapies, are also in progress.

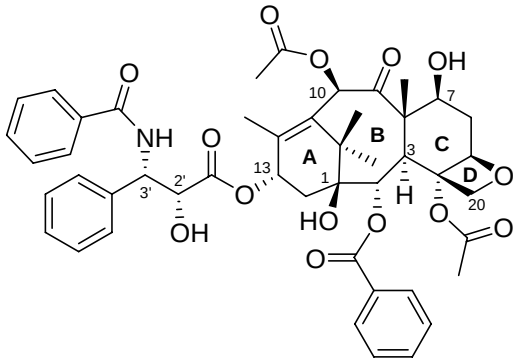


Figure 1-2a. Structure of paclitaxel (Taxol®)

Paclitaxel ($C_{47}H_{51}NO_{14}$, molecular weight 853.91) is a complicated diterpene containing a fused tetracyclic skeleton (*i.e.*, baccatin core) with a chiral *N*-benzoylphenylisoserine side chain attached to the C-13 hydroxy group *via* ester bond. It is one but famous representative in taxane family. The essential rigid ring system (namely A-D rings) and 11 chiral centers exhibit great attractions and challenges to both synthetic and medicinal chemists.

§ 1.2.2. Mechanism of inhibition in cell cycle

Horwitz and co-workers made landmark discovery in the function of paclitaxel around 1980.[8-10] It has turned out that inside the cell, paclitaxel promotes the polymerization of tubulins into microtubules, stabilizes the microtubules and prevents further depolymerization of microtubules. This, when discovered, was a totally new mechanism. At that time, it was well known that a series of natural spindle toxins, such as vinca alkaloids and podophyllotoxin, inhibit tubulin polymerization and assembly of microtubules. However, paclitaxel behaves uniquely in a contrary manner.

Microtubules exist in all eukaryotic cells with large quantities, and they are involved in the maintenance of cell motility and shape. More importantly, microtubules play a crucial role in the dynamic process of cell cycle as shown in Figure 1-3.[6]

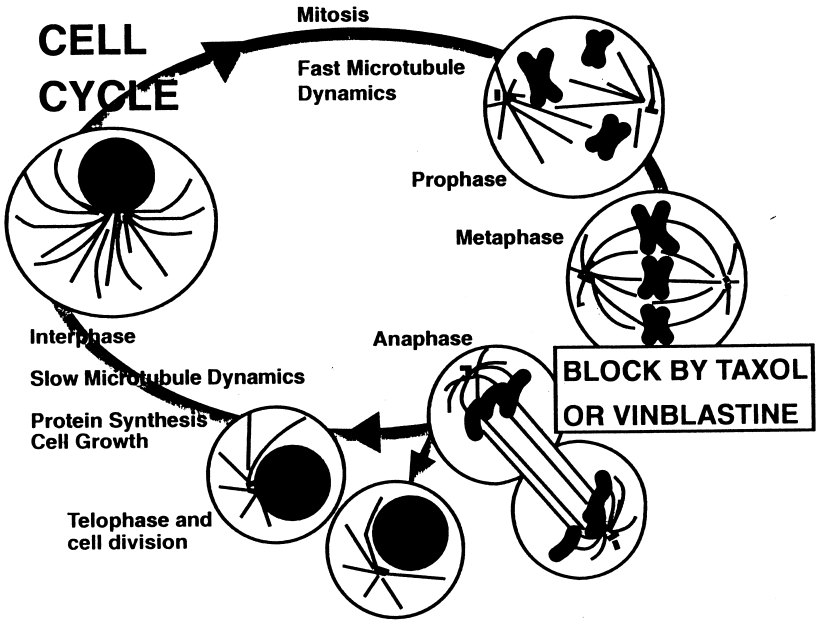


Figure 1-3. Cell division and block of mitosis [6]
(Picture was adapted from the cited reference.)

The cell division in eukaryote consists of two major phases based on cellular activity: *interphase* and *mitosis*. During *interphase*, cell grows larger because of the duplication of genetic materials and proteins. During *mitosis*, cells divide into two new daughter cells.

Mitosis is composed of several small phases. At the first stage, *prophase*, the centrosome splits into two daughter centrosomes, which will serve as the spindle poles later. After a "sun-burst" arrangement, spindles, which are formed by microtubules, start to radiate out from each centrosome to reach into the nucleus as the nuclear membrane breaks down. The spindles catch the sets of chromosomes and align them along the middle of the cell. Then the cell has reached the second stage called *metaphase*. It is during *anaphase* (third stage) that the sister chromatids of each chromosome split apart. And they will move towards the pole because of the shortening of the microtubules that they are attached. When the chromosomes nearly reach their respective poles, the cell enters the *telophase*. In this stage, the nuclear envelope reforms around each set of chromosomes and the cell membrane pinches off to form two new cells.

Clearly, assembly (elongation) and disassembly (shortening) of microtubules are indispensable during cell division. It is between *metaphase* and *anaphase* that microtubule-targeting agents, such as paclitaxel or vinblastine, disturb the regular cell cycle. As a result of this mitotic block, apoptosis or "programmed cell death" is eventually induced, and the cell will be destroyed.

Horwitz and her group[9,10] also recognized that paclitaxel promotes the rate, extent, and nucleation phase of tubulin polymerization and stabilizes the microtubules. Microtubules are primarily formed by the dimerization of two similar polypeptide subunits, α- and β-tubulin, each of which contains approximately 440 amino acid residues (about 50 kD). In the presence of Mg^{2+}, guanosine 5'-triphosphate (GTP), and microtubule-associated proteins (MAPs), the α- and β-tubulins form dumbbell-shaped heterodimers. If these heterodimers accumulate, microtubule rings will be formed. The sizes of microtubule rings can vary under different conditions. For example, the diameter is about 24 nm when 13 heterodimers form a microtubule ring naturally. Some drugs, such as colchicine, podophyllotoxin may bind to the tubulin heterodimers, but prevent their polymerization into microtubules. Contrarily, paclitaxel also binds to the *α,β*-tubulin heterodimer (more accurately, *β*-tubulin) with up to 1:1 ratio to promote the polymerization and stabilize the resulting microtubules, which contains only 12 photofilaments with a diameter of about 22 nm. This progress can happen even without Mg^{2+}, GTP and MAPs. The paclitaxel-microtubule complex is very stable under usual depolymerization conditions: low temperature (4 ^{o}C) or 4 mM $CaCl_2$ solution. Their results are illustrated in Figure 1-4.

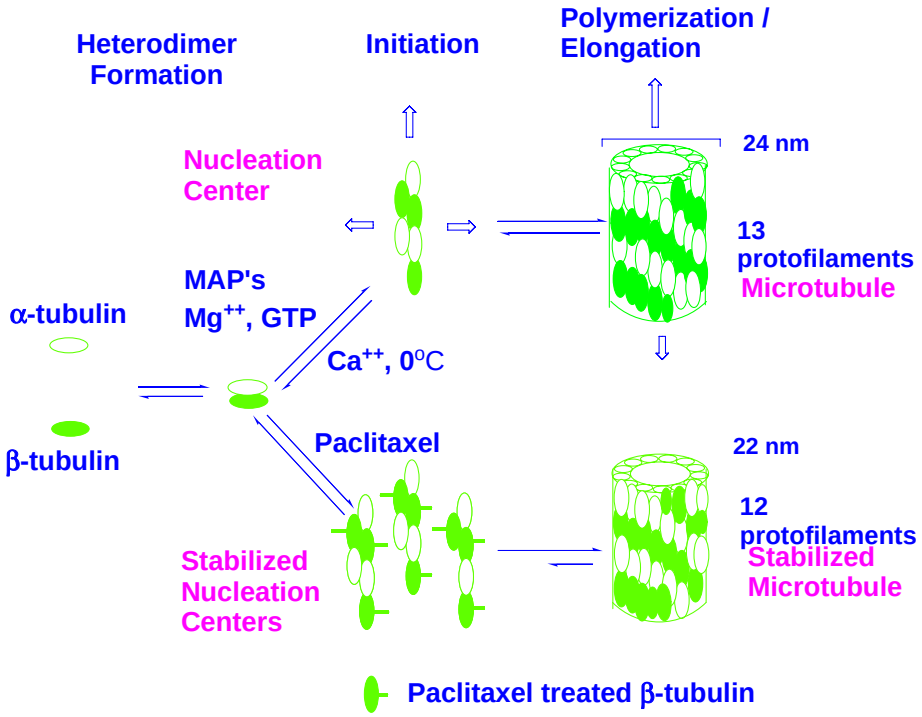


Figure 1-4. Formation of microtubule and the mechanism of action of paclitaxel[8-10]

It was found later that this unique mechanism is shared among some other natural products, which contain distinct structures from that of paclitaxel. Epothilones A and B[11] (from the myxobacterium, *Sorangium cellulosum*), eleutherobin[12] (from a soft coral of the *Eleutherobia* sp.), discodermolide[13] (from the marine sponge *Discodermia dissoluta*), sarcodictyin,[14] and laulimalide[15] all show comparable activities to paclitaxel in cytotoxicity and inhibition of microtubules disassembly in purified tubulin assembly assays.[12,16-18]

Recently when Wang and co-workers explored the cellular functions of paclitaxel, they suggested another mechanism, in which paclitaxel may also induce apoptosis *via* cellular signaling pathways.[19] When cells are treated with low concentrations of paclitaxel (10-100 nM), formation of multinuclei and damage of chromosomal DNA were observed. The latter was known to trigger p53-induced apoptosis (Figure 1-5).[20] Further investigation is still going on in Wang's group.

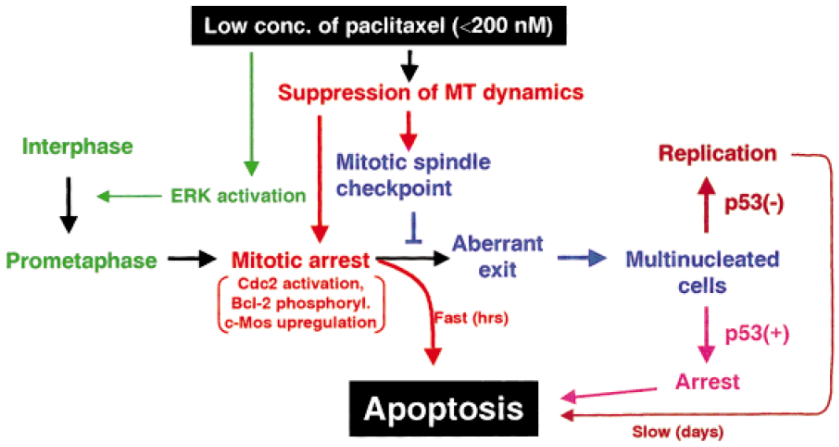


Figure 1-5. Another mechanism proposed by Wang et al (MT: microtubule)[19]

Because of its novel structure and unique mechanism(s) against tumor cells, paclitaxel exhibits powerful activities against many cancers that were not effectively treated by other existing drugs before paclitaxel was found. To cure patients and support on-going clinical trials, large quantity of paclitaxel was urgently needed.

§ 1.2.3. Synthesis of paclitaxel

Applications and clinical trials of paclitaxel suffered from the inadequate supply of paclitaxel. Initially, isolation from the extracts of the bark of the pacific yew tree was the only way. Although the yew tree is about 25 feet tall after fully grown, it takes decades for those slow-growing coniferous trees to develop in the American Pacific Northwest, and cutting off the bark will inevitably cause death of those trees and irreversibly destroy the current balance of ecosystem in the forest. The chemical extraction is a time-consuming and highly expensive process with low yields, which makes the scenario even worse. For instance, 10,000 pounds of bark will be necessary to obtain 1 pound of paclitaxel, which means sacrifice of approximately 3,000 yew trees. (It was estimated that thirty 100-year-old yew trees may provide 1 ounce paclitaxel, but the question is how many such 100-year-old trees exist.). As such 1 pound of paclitaxel is barely enough for the treatment of approximately 250 patients. Obviously, the natural source is not sufficient at all.[6] Consequently, alternative ways would have to be explored to achieve sustainable sources.

Chemical synthesis is definitely one of the most promising means for supplying paclitaxel. Although the structure of paclitaxel is very intricate, total synthesis has been accomplished by several research groups, including Holton,[21,22] Nicolaou,[23-27]

Danishefsky,[28] Wender,[29,30] Kuwajima,[31] and Mukaiyama.[32] These indeed are fabulous academic achievements because new synthetic routes and reagents were proposed and the syntheses were accomplished. However, none of those syntheses is applicable in mass production due to long multi-step synthesis and thus very low overall chemical yield, as well as the reaction conditions and reagents that are not suitable in scale-up production.

Fortunately, 10-deacetylbaccatin III (10-DAB III, Figure 1-6), a natural product isolated from the leaves of European yew (*Taxus baccata*), was identified in 1980.[6,33] Most strikingly, this compound contains the tetracyclic core of paclitaxel, but without the C-13 isoserine side chain and C-10 ester group. As the readily accessible starting material with relatively good yield from natural source (1 pound out of 1000 pound fresh leaves), it could be converted into paclitaxel within a few steps to complete the semi-synthesis.[34] 10-DAB not only guarantees the sustainable supply of paclitaxel, but also enables development of novel taxoids to achieve better potency and pharmacological property.

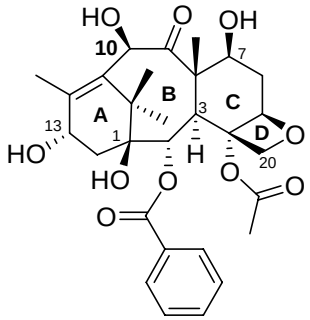
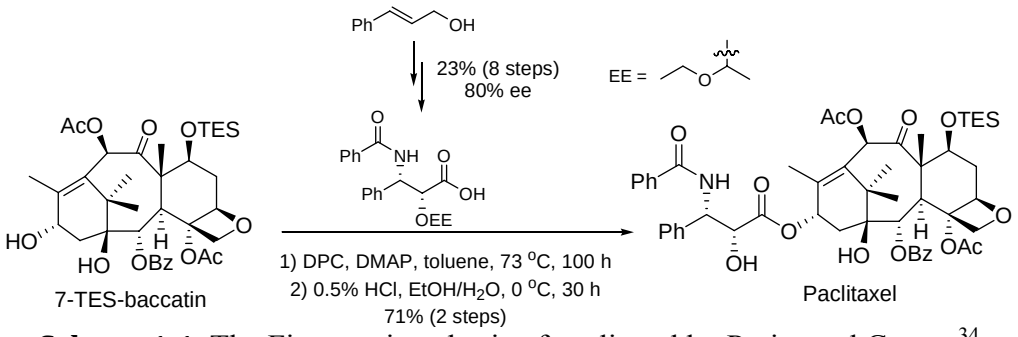


Figure 1-6. Structure of 10-Deacetylbaccatin III (10-DAB III)

In 1988, the first semi-synthesis of paclitaxel utilizing 10-DAB III was accomplished by Greene and Potier using a direct esterification of 7-TES-baccatin and (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine (side chain in paclitaxel) as shown in Scheme 1-1.[34] They also found that those four hydroxyl groups of 10-DAB have different reactivity towards acylation or silylation, which allows for the step-wise protection of the hydroxyl groups at C-7 and C-10. The isoserine acid, in which hydroxyl was protected by 1-ethoxylethyl (EE) was synthesized in 8 steps with a modest 80% enantiomeric excess (e.e.), from *cis*-cinnamyl alcohol, which was later purified to 98% e.e..[35,36] Due to the steric hindrance at the C-13 position, the installation of a protected side chain precursor was carried out under harsh conditions, which gave protected paclitaxel in 80% yield based on 50% conversion. However, significant epimerization occurred at the C-2' position in the side chain at high reaction temperature and long reaction time. The subsequent deprotection using diluted HCl afforded paclitaxel in 89% yield.

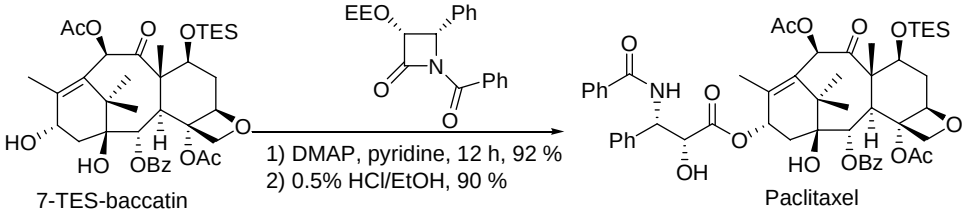


Scheme 1-1. The First semisynthesis of paclitaxel by Potier and Greene[34]

Following Potier's pioneering work, much effort was focused on the asymmetric syntheses of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine (side chain in paclitaxel) *via* various approaches,[37-39] such as Sharpless dihydroxylation,[40] Sharpless'[41] and Jacobsen's[42] asymmetric epoxidation, Chen's synthesis *via* enzymatic trans-esterification,[43] aldol reaction,[44] Sharpless asymmetric aminohydroxylation,[45] and Kobayashi's chiral Lewis acid catalyzed Mannich-type reaction.[46] But the epimerization under the forced coupling condition implied that the coupling method of side chain to the tetracyclic system was also needed to be further explored and improved.

Novel esterification strategies were also developed, which included Holton oxazinone coupling,[37,47] Ojima-Holton β-lactam coupling,[5,48,49] Commerçon oxazolidine - carboxylic acid coupling,[50] and Kingston oxazolinecarboxylic acid coupling[51]. Among all these coupling methods, the Ojima-Holton *β-Lactam Synthon Method* (β-LSM) is the most efficient and powerful route, thus frequently adopted in the total and semi-syntheses of paclitaxel.[21-23,28,30] Versatile lactams were also applied to obtain various non-natural taxoids, which greatly facilitated structure-activity relationship (SAR) studies.

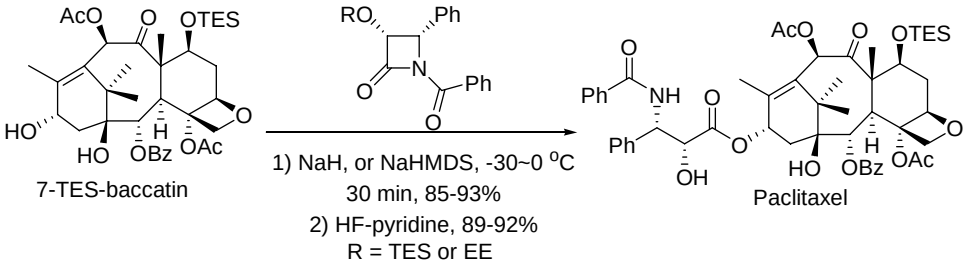
Holton tried direct coupling between (3*R*,4*S*)-*N*-benzoyl-3-*O*-(1'-ethoxylethoxyl)-4-phenylazetidin-2-one (β-lactam) and 7-TES-baccatin III with the help of 4-dimethylaminopyridine (DMAP), and the 7-TES-2'-*O*-EE-paclitaxel was obtained in 92% yield. After subsequent deprotection, paclitaxel was finally obtained in 90% yield (Scheme 1-2).[52] Holton's method showed high overall yield, and no epimerization happened in the process. However, this method also suffered from using a large excess of β-lactam (5 equivalents) and slow reaction rate, and it was limited to *N*-benzoyl protected β-lactam.



Scheme 1-2. Holton's protocol towards the semi-synthesis of paclitaxel

In early 1990s, Ojima and co-workers developed a more practical and efficient semi-synthesis of paclitaxel using β-LSM and protected 10-DAB III (Scheme 1-3).[5,53,54] The coupling reaction between chiral β-lactam with modified baccatin was carried out under strong basic condition, followed by deprotection to finish the synthesis in high

overall yield. After a series of bases, such as NaH, *n*-BuLi, LDA, LiHMDS, NaHMDS, and KHMDS, were screened, NaHMDS was found to be the best, and the temperature was also controlled (-30~0 ^{o}C) for the reaction process. Compared to Holton's protocol, Ojima's new approach had at least three advantages. Instead of huge amount of β-lactam (5 eq.) used in Holton's method, only slightly excess of β-lactam (1.1~1.5 eq.), which was usually not easy to obtain, was good enough to complete the reaction in excellent yield. The reaction time was reduced to within 30 min in Ojima's procedure, while 12 hours were necessary in Holton's method. Different protecting groups on the β-lactam in Ojima's tactic, as well as the deprotections, greatly extended the reaction potentials, and paved the way to prepare novel taxoids, which would eventually benefit the SAR study on paclitaxel. The optically pure β-lactam could be produced by either enolate-imine cyclocondensation in the presence of a chiral auxiliary or ketene-imine cycloaddition followed by enzymatic resolution. Both methods, which will be discussed later, achieved very high yield and excellent enatiomeric purity (*i.e.*, > 96% e.e.).[48,49,55]



Scheme 1-3. Ojima's approach[5,54]

Recently, based on the better understanding of paclitaxel biosynthesis and the help of genetic engineering in molecular biology, cell culture fermentation was employed as an industrial method to produce paclitaxel.[56,57]

§ 1.2.4. Structure-activity relationship (SAR) studies and docetaxel

Exploration of semi-synthesis of paclitaxel led to many taxoid analogs. For example, docetaxel (Taxotère®, Figure 1-7) was discovered by Potier and co-workers, and developed by Rhone-Poulenc Rorer (a French pharmaceutical company, now Sanofi-Aventis) in 1984.[58-60]

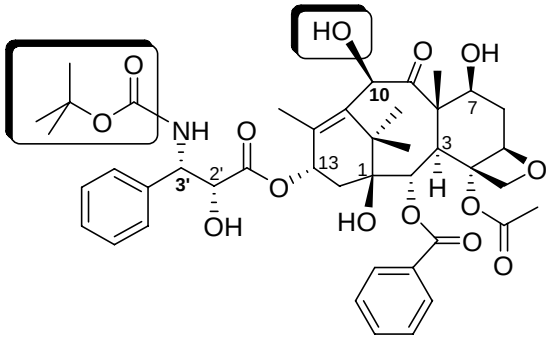


Figure 1-7. Structure of Docetaxel

Compared to paclitaxel, docetaxel has a C3'-*N*-tert-butoxycarbonyl (Boc) moiety and a free alcohol at C-10, which replace benzoyl and acetate moieties in paclitaxel, respectively. Apparently, docetaxel and paclitaxel are sharing the same mechanism, but the interesting property of docetaxel is its strong potency, *i.e.*, twice as high as paclitaxel in the inhibition of microtubule depolymerization. Docetaxel was also approved by the FDA for the treatment of breast cancer in 1996, and further clinical applications in various stages are going on worldwide.[6]

However, a number of undesirable side effects come with the applications of paclitaxel and docetaxel, including allergy, infection, hair loss, joint and muscle pain, diarrhea, and neuropathy. Meanwhile, the efficacy of taxoids suffers from multi-drug resistance (MDR).[61] Consequently, new taxoids with better pharmacological profiles are definitely needed. The practice of semi-synthesis greatly facilitates the development of new generation taxoids, and sustains the structure-activity relationship (SAR) study.[39,60,62,63] Besides modifications on those hydroxyl groups, the rigid tetracyclic ring system could also be opened under suitable conditions. Several comprehensive reviews have been published on SAR with paclitaxel,[6,39,62,64] and Figure 1-8 shows some of the key features.

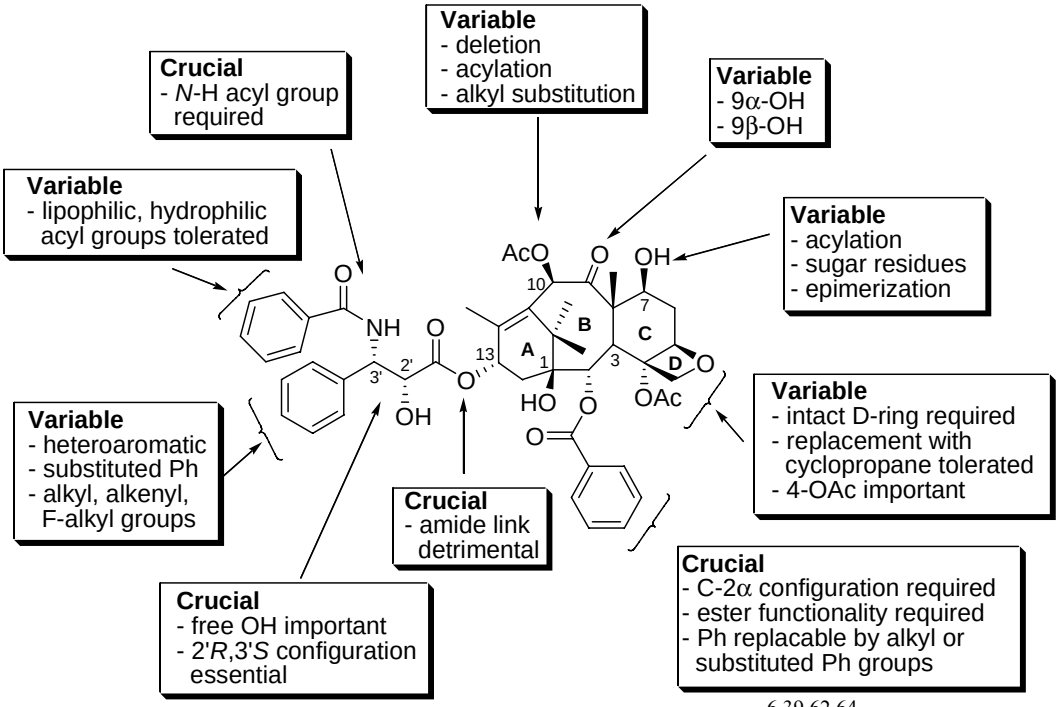


Figure 1-8. Summary of SAR for paclitaxel[6,39,62,64]

1) Baccatin structure

Various modifications have been done on the tetracyclic core, focusing mainly either ring size or peripheral oxygens.[65-67]

Shrinkages on A-ring[68] and C-ring[69] resulted considerable decrease in activity, while compounds after B-ring contraction still showed some cytotoxicity.[70] Converting the olefin within A-ring into epoxide[71] and reducing the olefin to saturated structure[72] were also done, respectively. But both operations showed less toxicity in cellular assay.

Taxoid derivatives with open-ring structures were also investigated. Ojima and Appendino prepared several A-seco analogs from the C-14β-hydroxy-10-DAB,[73,74] which were 15-20 times less cytotoxic than paclitaxel. C-*seco* taxoids, prepared through an oxidation-reduction protocol, were reported. Among them, **IDN5390** (Figure 1-9) has shown strong antiangiogenic and antimetastatic activities, although with slightly reduced cytotoxicity.[75-77] Further development is on the way. The intact D-ring, *i.e.*, oxetane, is responsible for the high level of activity, and modifications by ring-opening,[69,70,77] and formation of azetidine ring[78,79] or thietane,[80] caused loss of potency. Recently, compounds containing cyclopropane as D-ring resulted in a similar activity to that of paclitaxel.[81]

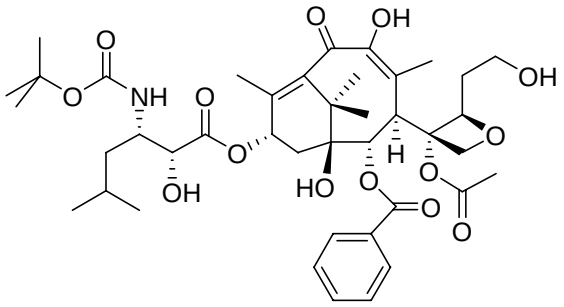


Figure 1-9. Structure of IDN 5390, a C-seco taxoid

In paclitaxel, there are 4 free hydroxyl groups and 3 ester groups, all of which are associated with chiral carbon centers. It is not surprising that extensive modifications have been done on those oxygen-containing functional moieties, such as epimerization, esterification or de-esterification, and replacement by other hetero-atoms.

C-2 benzoate is an interesting position, and was shown to be essential for potency. Epimerization or hydrolysis of the ester at C-2 led to a drastic reduction in activity.[82] Substitutions on the benzene ring were also a part of SAR study.[83] While *para*-substitution impaired the potency, *meta*-substitution derivatives showed greater cytotoxicity than paclitaxel (activity: F > Cl > OMe > N_3 > Me > $CH{=}CH_2$ > H). It has been found that *meta*- position was involved in the metabolism of paclitaxel.[84] Consequently, derivatives with slower metabolic rate at *meta*- position should demonstrate higher activity, such as methoxy or halogen.[85] Depending on the modeling, it was proposed that *para*- groups might disrupt the hydrophobic interactions between the C-2 benzoate residue and the phenyl group at C-3', which might be necessary for the active conformation of paclitaxel, and *meta*- groups might enhance such interactions.[86] Studies in Ojima's lab revealed that the activity could be improved if the phenyl moiety of the C-2 benzoate group would be replaced by an alkyl or alkenyl group.[87]

The C-4 position was found to be crucial to paclitaxel's activity. Hydrolysis of the C-4 ester to alcohol exhibited a significant decrease in cytotoxicity. Steric hindrance might play a role at C-4 ester, too. For example, if the acetyl group was replaced by a cyclopropanecarbonyl group, the new analog showed slightly better activity, but the potency dropped greatly when a benzoyloxy group was installed at C-4.[88,89] Transformations at C-7, C-9, and C-10 were tolerated. Extensive studies have been carried out on the C-7 hydroxyl moiety.[90-92] It has demonstrated that acylation,

epimerization, and even deoxygenation, did not significantly alter the *in vitro* activities. As shown before, C-10 was a hydroxyl in docetaxel, but an acetate in paclitaxel, and both were potent. In general, most of the analogs bearing ester or carbamate at C-10 possessed either retained or even better biological activities. Reduction of the C-9 ketone in paclitaxel to either an α- or β-hydroxyl group had little effect on the cytotoxicity,[93,94] while the formation of C-9, C-10 diol showed decrease in activity.[95-97]

To explore and overcome the multi-drug resistance (MDR) against paclitaxel, a series of second-generation taxoids were prepared by Ojima and co-workers. They found that the exceptional activity of these novel taxoids against the drug-resistant cell lines could be clearly attributed to the modification at the C-10 position,[98,99] although previous studies have concluded that modification of the C-10 position has little effect on the *in vitro* cytotoxicity.[100,101] As a result, the combination of C-10 and C-2 modifications in the 3rd-generation taxoids virtually conquered the MDR caused by P-glycoprotein in several cancer cell lines.[102]

Epimerization of hydroxyl at C-13 or an amide linkage at C-13 caused a complete loss in activity.[103,104]

2) Side chain at C-13

C-13 side chain in paclitaxel and docetaxel, *i.e.*, (2*R*,3*S*)-*N*-acyl-3-phenylisoserine, plays an extremely important role in cytotoxicity and antitumor activity. For example, loss of this moiety causes more than 1,000-fold reduction in the potency, and change of the stereochemistry also reduced activity up to 500-fold.[105,106]

It was known that C-2' hydroxyl was vital. Protection, acylation, deletion, or epimerization of the C-2' hydroxyl leads to substantial decreases in activity.[107] Recent study showed C-2' hydroxyl group may be involved in direct interactions between paclitaxel and β-tubulin, and a hydrogen bond (H-bond) between the C-2' hydroxyl group and either Arg 369 or His 229 of the β-tubulin backbone was formed, which well explained the previous experimental results.[108] The *in vivo* activity would be comparable to the parent compound if modification groups at C-2' could be removed (*e.g.*, hydrolysis of an ester, cleavage of the protecting group) inside the cell. This finding eventually gave birth to the design of "prodrug", *e.g.*, based on C-2'-acylation.[39,109-112]

Certain modifications at the C-3'-*N* position were tolerated while a free C3'-NH_2 significantly decreased the potency. Derivatives containing lipophilic or hydrophilic acyl groups, which replaced benzoyl, exhibited activity similar to paclitaxel.[113] Actually, a large number of analogs with a *tert*-butoxycarbonyl group, such as docetaxel, showed better activity than paclitaxel.[39,98,99,114] Certain analogs bearing *N*-cycloalkenoyl substituents also possessed improved biological activity.[115] But replacement of C-3'-*N*-benzoyl with *n*-alkanoyl groups was not successful.[38,98,113]

C-3'-phenyl group was carefully investigated as well.[38,39,62] Substitution of a number of heterocyclic aromatic groups at C-3' instead of the phenyl group resulted in comparable or better activity than paclitaxel.[113] 2nd-Generation taxoids developed in Ojima's lab, which possess C-3' alkyl, alkenyl, fluorinated alkyl, and epoxy moieties, exhibited extremely high potency, especially against MDR cancer cell lines (see *vide infra*).[39,62,98,99,114,116,117] A very recent paper is also available for more information.[118]

§ 1.3. *β*-Lactam Synthon Method (*β*-LSM)

§ 1.3.1. β-Lactam and taxoid

β-Lactam (azetidin-2-one) has been known to chemists for more than one hundred years, and it is a very important and typical structure in antibiotics and some other natural compounds, such as penam, cephem, and penem (Figure 1-10), which, together with synthetic analogs, are widely used in clinic as antibacterial drugs nowadays.[119-121] *β*-Lactam has drawn significant interest by synthetic chemists because of its unique structure.

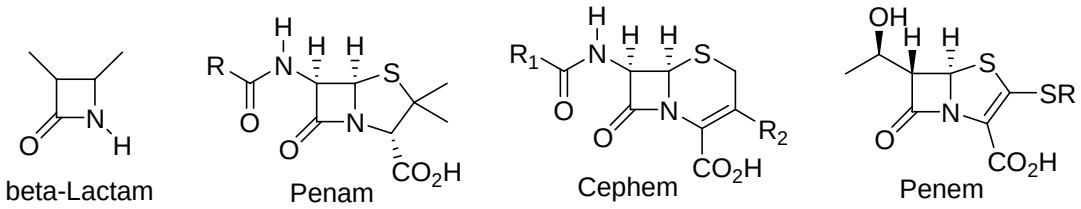
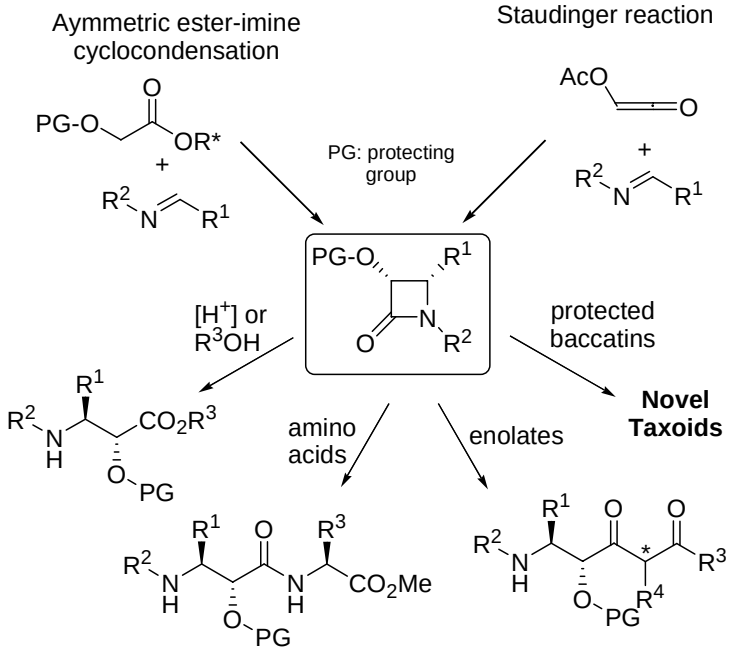


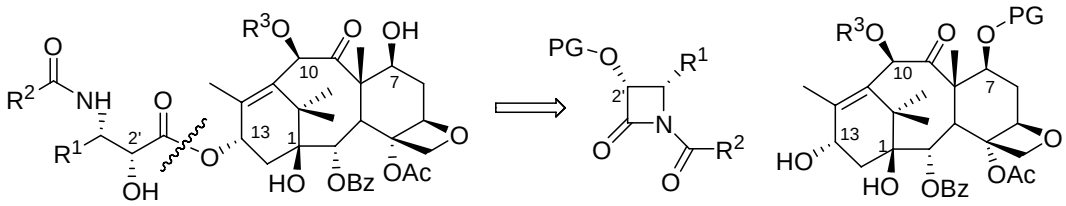
Figure 1-10. Representative structures of *β*-lactams

In the early 1980s, Ojima and co-workers started their pioneering work to explore applications of *β*-lactam to synthetic chemistry, which now is named as "*β-Lactam Synthon Method (β-LSM)*". By the 1990s, Ojima and other groups had elegantly demonstrated the enantiomerically pure β-lactams as essential intermediates and versatile building blocks towards the asymmetric synthesis of a variety of natural and unnatural amino acids, peptides, peptide mimetics, as well as taxoid anticancer agents.[122-124] This *β*-LSM is well documented.[39,53,54,62,124,125]



Scheme 1-4. Applications of *β*-Lactam Synthon Method (*β*-LSM)

Serving as the C-13 side chain precursor is one of the most important applications of β-LSM, which greatly boosted the semi-synthesis of paclitaxel and docetaxel, and more importantly, paved the road to numerous taxoids with novel structures.[5,21,23,28,30] This approach, which is now named as "Ojima-Holton β-lactam coupling", reacts a modified DAB with an enantiomerically pure (3*R*,4*S*)-*N*-benzoyl-β-lactam in the presence of base. Due to its high selectivity and yield as well as mild reaction conditions, this method has proven to be the most efficient and versatile route (Scheme 1-5).[37,39,60,105] Also, the supply of paclitaxel other than natural source, which is very scarce, has been secured *via* this route because scale-up is feasible.



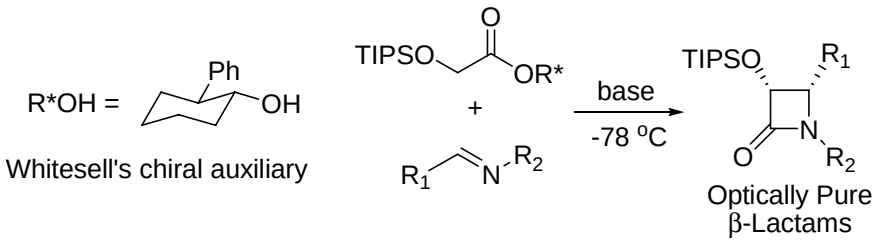
Scheme 1-5. Retro-synthesis of taxoids *via* β-LSM

β-Lactams could be prepared by various synthetic methods, including hydroxamate cyclization,[126] ester enolate-imine condensation,[127-130] chromium carbene-imine reaction,[131] isocyanate-alkene cycloaddition,[132] ketene-imine cycloaddition (*i.e.*, Staudinger reaction),[133,134] and some others.[135,136] We have adopted the following two methods to obtain chiral β-lactams, which are required for the synthesis of taxoids mentioned above. The first one is asymmetric ester-imine cyclocondensation, and the second one is a Staudinger reaction followed by enzymatic resolution. Both methods can achieve high overall yield and enantiomeric purity, and can be scaled up easily.

§ 1.3.2. Synthesis of β-lactam *via* cyclocondensation reaction

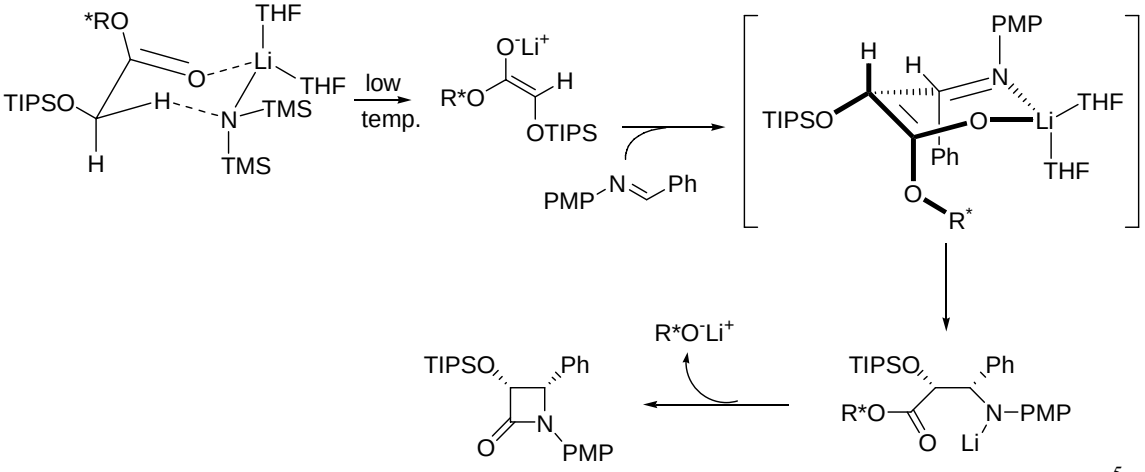
§ 1.3.2.1. Introduction

Due to the important chiral centers in the side chain of paclitaxel, docetaxel and other taxoids, enantiomerically pure β-lactams are highly demanded, because they serve as the precursors of the side chains as discussed before.[5,55] After investigations, our laboratory found that ester enolate-imine cyclocondensation reaction provides β-lactams efficiently. If a chiral ester is employed, β-lactam could be obtained with good yields and high enantiomeric purity.[5,48] The chiral auxiliary used for the ester moiety is (-)-*trans*-2-phenylcyclohexanol (Whitesell's chiral auxiliary).[137,138] It could be fully recycled with high e.e. after the cyclocondensation step, and thus could be used again (Scheme 1-6).



Scheme 1-6. Chiral auxiliary and cyclocondensation reaction towards β-lactam

The cyclocondensation reaction, in the presence of PMP-imine and TIPSO-ester with Whitesell's chiral auxiliary, selectively produces the *cis*-β-lactam with high optical purity. Several features should be noted (Scheme 1-7).[5] First of all, (*E*)-enolate is predominantly generated at low temperature. Next, the imine is added from the less hindered face to form the complex *via* 6-member-ring transition state, and consequently provides the β-amino ester intermediate, which could be isolated if the reaction would be stopped at low temperature. Finally, when warmed up to room temperature, the intermediate collapses and cyclization happens leading to the formation of the corresponding chiral β-lactam, and meanwhile the chiral auxiliary is released as its lithium salt.



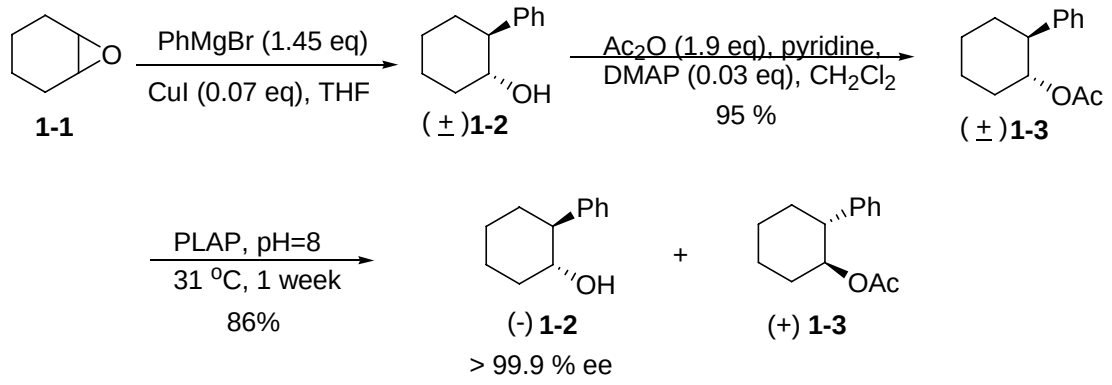
Scheme 1-7. A proposed mechanism of chiral ester enolate-imine cyclocondensation[5]

The diversity of this method comes from the variable substituents on the imine part. The phenyl moiety[49,55] could be replaced by substituted phenyl groups,[139] isobutenyl group,[98,102] as well as other groups[139] at the C-4 position on the β-lactam ring. Thus, various β-lactams with high enantiopurities were prepared in excellent yields.

§ 1.3.2.2. Results and Discussion

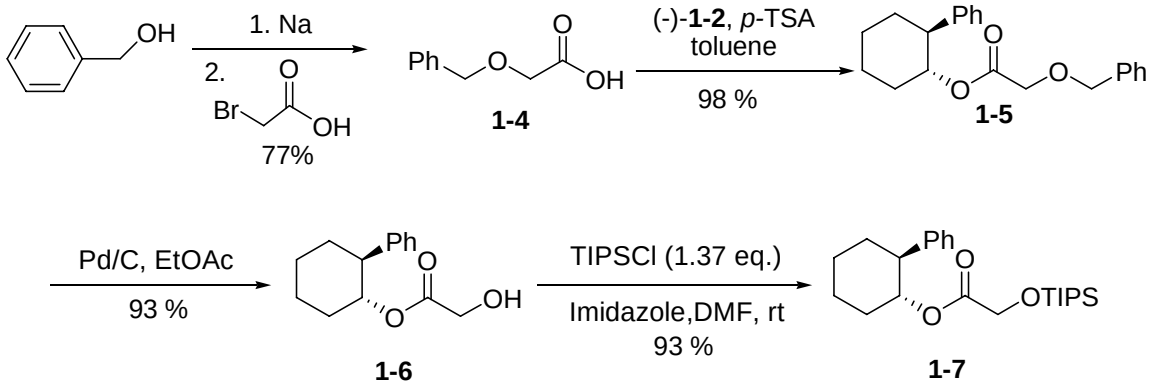
The synthesis of chiral β-lactam[5] through enolate-imine cyclocondensation started from cyclohexene oxide (**1-1**), which was converted into racemic acetate after epoxide opening and protection of alcohol. Stereo-selective attack of phenyl Grignard reagent on the epoxide provided the *trans*-2-phenylcyclohexanol (**1-2**) in the presence of catalytic amount of cuprous iodide. After acetylation, the racemic esters (**1-3**) were ready for the

enzymatic kinetic resolution step. Screening of a series of esterases showed that the powder from the acetone extract of pig liver (PLAP) gave satisfactory results.[140] After optimization, the chiral auxiliary with correct configuration, which was (-)-(1*R*,2*S*)-2-phenylcyclohexanol, was obtained in more than 93% theoretical yield and more than 99% e.e after one week (Scheme 1-8).



Scheme 1-8. Synthesis of Whitesell's chiral auxiliary{Whitesell, 1986 #141}

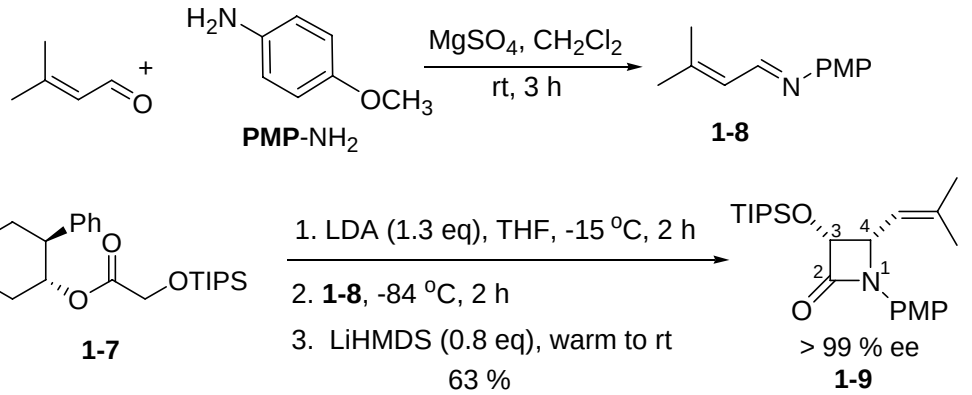
The chiral auxiliary (-)-**1-2** was coupled to benzyloxyacetic acid (**1-4**), which was prepared by sodium salt of benzyl alcohol and bromoacetic acid, to generate chiral ester **1-5**. Removal of benzyl moiety by hydrogenolysis followed by TIPS protection provided chiral TIPS-ester **1-7** in excellent overall yield (Scheme 1-9).



Scheme 1-9. Synthesis of chiral TIPS-ester{Ojima, 1992 #49}

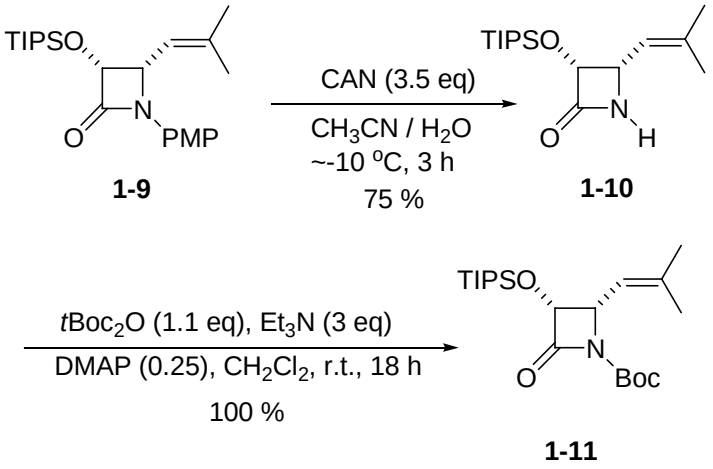
To synthesize the isobutenyl substituted β-lactam (**1-9**), 3-methyl-2-butenal was first converted to the corresponding imine with *p*-anisidine in the presence of dehydrating reagent. After chiral TIPS-ester enolate was produced from **1-7** with LDA in THF, the crude *para*-methoxyphenyl (PMP)-protected *iso*butenylimine **1-8** was added slowly at low temperature. The reaction mixture was allowed to warm up slowly to ambient temperature to give the desired chiral β-lactam (**1-9**). The cyclocondensation step was

found to be sensitive to moisture and temperature. The quality of LDA determines the final yield. If the cyclization is not complete, adding some LiHMDS would help (Scheme 1-10).



Scheme 1-10. Synthesis of 1-PMP-4-*iso*butenyl-*β*-lactam **1-9**{Ojima, 1992 #49}

Further modifications towards the final *β*-lactam were pretty straightforward. The PMP group was removed by oxidant, ceric ammonium nitrate (CAN), in aqueous acetonitrile *via* Single-Electron Transfer (SET) mechanism.[141] The resulting NH-free amide **1-10** was then protected by reacting with di-*tert*-butyldicarbonate anhydride to afford the desired (3*R*,4*S*)-4-*iso*butenyl-1-(*tert*-butoxycarbonyl)-3-(tri*iso*propylsilyloxy)azetidin-2-one (**1-11**) (Scheme 1-11) with excellent yield and high enantiomeric purity.[53]



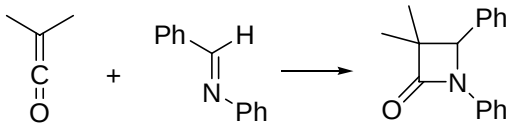
Scheme 1-11. Synthesis of C-4-isobutenyl-*β*-lactam **1-11**{Ojima, 1992 #49}

Overall, the required *β*-lactams were obtained in 11 steps by cyclocondensation in about 16% overall yield.

§ 1.3.3. Synthesis of β-lactam via cycloaddition (Staudinger) reaction

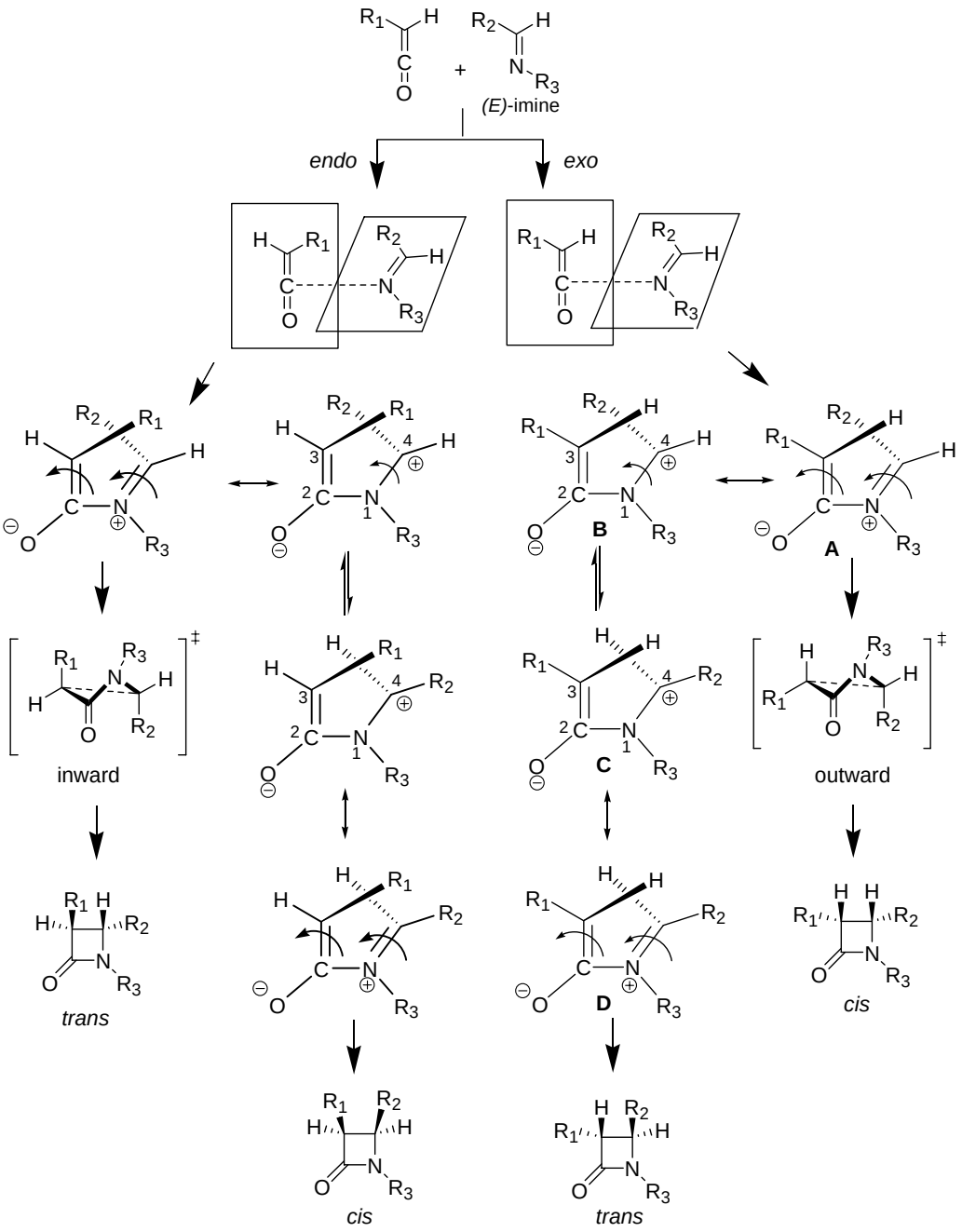
§ 1.3.3.1. Introduction

Staudinger reaction, *i.e.*, ketene-imine cycloaddition or [2+2] cycloaddition reaction, is another efficient way to prepare *β*-lactams.[136,142] Actually, the first *β*-lactam was synthesized by Staudinger and co-workers using this method around 1907 (Scheme 1-12).[133] In general, *cis*-*β*-lactams are the major product although two possible chiral centers (4 possible enantiomers) will be formed after the reaction. Unfortunately, the mechanism is not yet clearly understood even now,[135,143,144] but it does not impede Staudinger reaction becoming one of the most important routes for the synthesis of *β*-lactam.[134] It gives versatile products in high yields very quickly, and the reaction conditions are very mild.



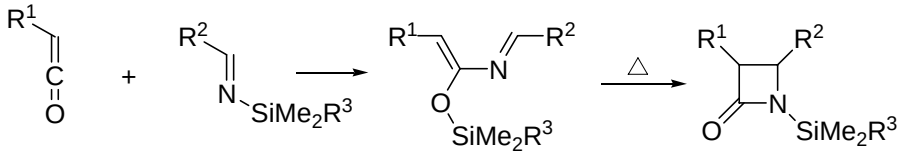
Scheme 1-12. An example of Staudinger's original experiments[133]

Basically, this reaction could be divided into two steps. The first step is considered as a nucleophilic addition, *i.e.*, the *sp*-hybridized carbon in ketene is attacked by the nitrogen in imine, which forms a zwitterionic intermediate. The second step is thermal conrotatory electrocyclization, which follows Woodward-Hoffman rule, to form a *β*-lactam ring. In the initial step, it is assumed that the attack usually happens at the less hindered site on the ketene. Thus, in general, imines with *E*- configuration will give *cis*-*β*-lactams, and *trans*-*β*-lactams are derived from *Z*-imines (Scheme 1-13). However, unexpected results were also observed: *E*-imines led to the formation of *trans*-*β*-lactams although the yield was low.[145] These unusual phenomena could be explained by the faster rotation of N1-C4 bond than the conrotatory cyclization as shown from A to D in Scheme 1-13.[146,147]



Scheme 1-13. Mechanism study on Staudinger reaction[135,144,147]

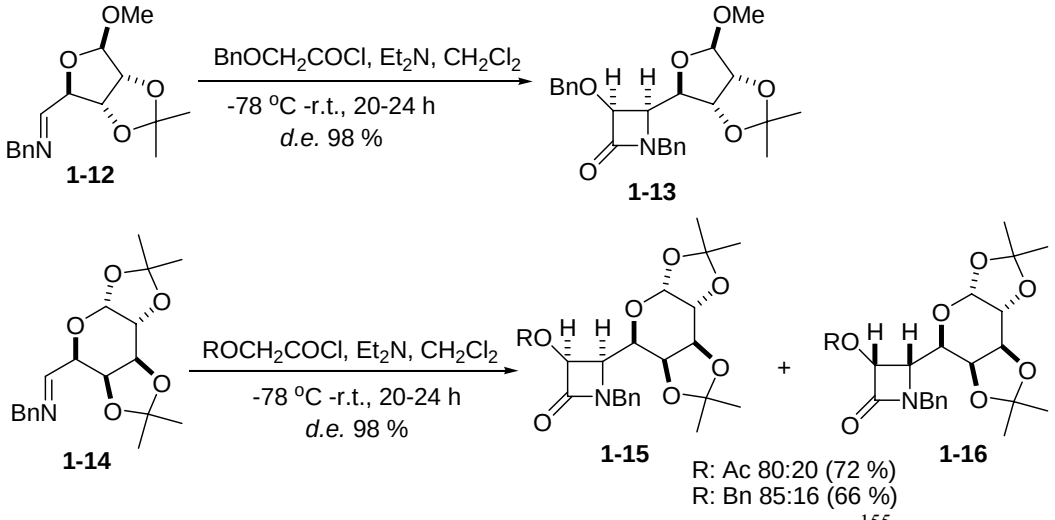
The zwitterionic intermediate was detected or even trapped by many different methods, such as adduct with SO_2[148,149] and EtOH[150], both of which were the solvents used in this type of reaction. An interesting but important result was reported by Pannunzio and co-workers, and the *O*-silyl intermediates were all identified unambiguously and were converted into the final β-lactams (Scheme 1-14).[151,152] All of these strongly supported the proposed mechanism in Scheme 1-13. Theoretical calculations were also tried with model structures.[153,154]



Scheme 1-14. Direct proof of the putative intermediate[151,152]

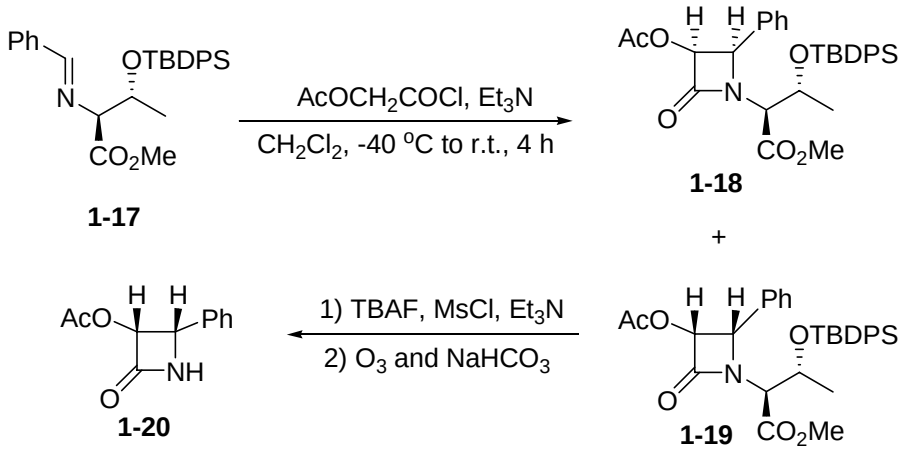
To avoid the direct use of a highly active and unstable ketene, surrogates such as acid chloride are applied to the above reaction in practice. The acid chloride, if there is at least one proton on the α-carbon, is converted into ketene *in situ* after elimination of HCl in the presence of a base at low temperature.

Our interest is focused on finding suitable *β*-lactams with correct chirality as the side chain of taxoids, which means a *cis*-*β*-lactams with (3*R*,4*S*) configuration and an oxygen atom at C-3. Consequently, asymmetric synthesis of *β*-lactam is required. There are several strategies to introduce chirality into *β*-lactam, including N1 induction, C3 or C4 induction, and a chiral catalyst. But asymmetric synthesis of chiral 3-hydroxy (alkoxy)-*β*-lactams was limited. Palomo and co-workers reported a successful synthesis by combination of C3- and C4-inductions (Scheme 1-15).[155] Compound **1-13** was achieved with high diastereomeric excess (*d.e.*). However, chiral imine **1-14** did not give high selectivity, which indicated that the diastereoselectivity depended on substrates.



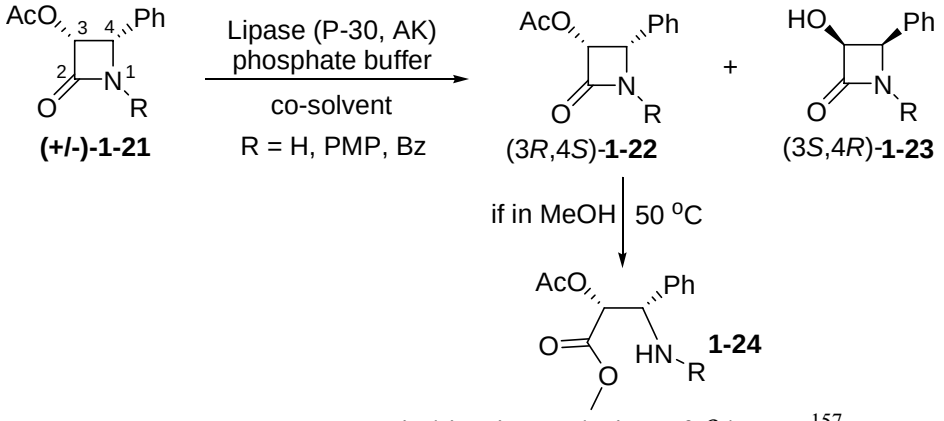
Scheme 1-15. Chiral imines in Staudinger reaction (1)[155]

The diastereoselectivity usually is poor when the chiral centers are connected to both N1 and C4. A relatively high diastereoselectivity is shown in Scheme 1-16. Imine **1-17** with two chiral centers and a bulky silyl group produced a mixture of **1-18** and **1-19** with 1 to 11.5 ratio. After further steps, the desired precursor for the side chain of paclitaxel was obtained from the major isomer **1-19**.[156]



Scheme 1-16. Chiral imines in Staudinger reaction (2)[156]

Enlightened by the enzymatic resolution in cyclocondensation method, we envisioned the promising application of esterase for kinetic resolution of two enantiomers. In 1993, Sih and co-workers reported such resolution of racemic *cis*-3-acetoxyl-4-phenyl-*β*-lactams (**1-21**) by commercially available lipases.[157] Five different lipases and two penicillinases were screened, and the e.e. values were measured by NMR with chiral shift reagents. In fact, some *Pseudomonas* lipases in phosphate buffer, such as P-30, AK and K-10, can selectively hydrolyze (*3S*,*4R*)-3-*O*-acetyl-4-phenyl-*β*-lactam into alcohol **1-23**, and (3*R*,4*S*)-*β*-lactam (**1-22**) remains, which is the correct precursor to taxoids. However, they also found that if the reaction was carried out in MeOH at elevated temperature, the *β*-lactam ring would be opened (**1-24**), which resulted in low yield. After optimizations, including co-solvent, buffer, temperature, and reaction time, they found that when PMP was used as the protecting group, P-30 lipase showed the best result (49% yield and >99.5% e.e.) in the presence of 10% CH_3CN in water around pH 7.5 at room temperature after 35 hours.

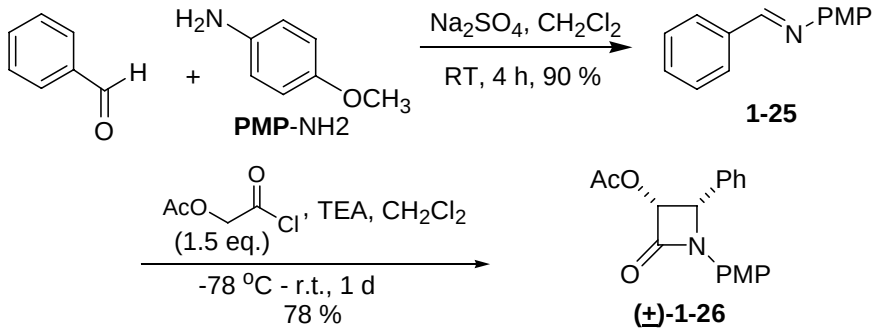


Scheme 1-17. Enzymatic kinetic resolution of *β*-lactam[157]

§ 1.3.3.2. Results and Discussion

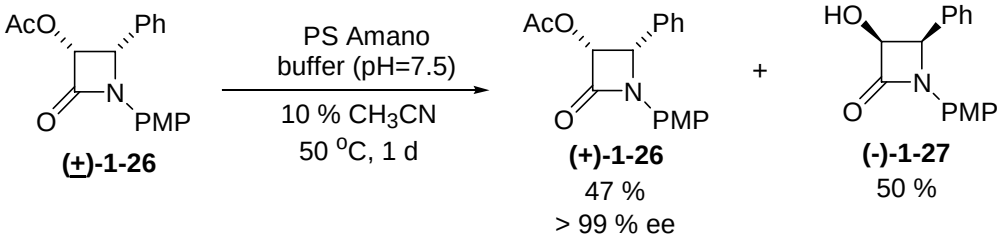
The synthesis of the necessary *β*-lactam for the side chain of paclitaxel started from benzaldehyde and *p*-anisidine (PMP-NH_2). The *para*-methoxyphenyl (PMP)

substituted imine (**1-25**) was obtained through condensation, which was subjected to the Staudinger reaction with acetoxyacetyl chloride and triethylamine. The yield of β-lactam **1-26** was good in this step, and the major by-product was acyclic amide (up to 8%). Control of temperature was important. The yield would be low if the reaction mixture was warmed up to room temperature too quickly. Again, addition of LiHMDS would help the cyclization (Scheme 1-18).



Scheme 1-18. Synthesis of racemic β-lactam **(±)-1-26**

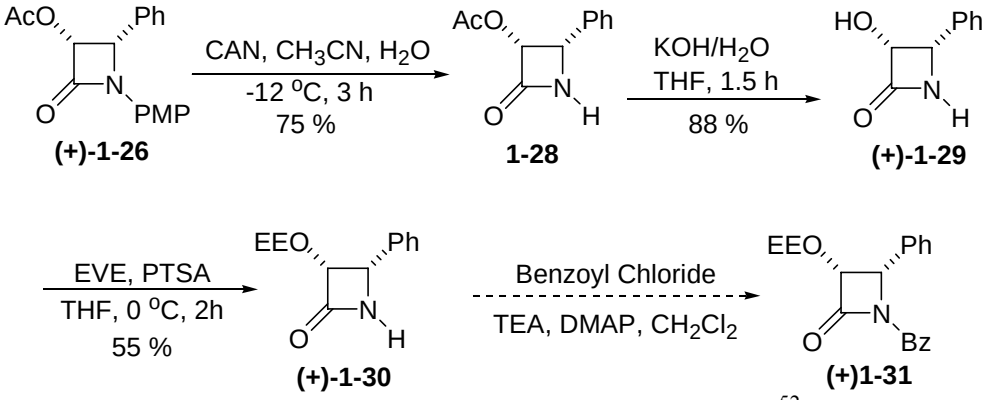
Enzymatic resolution was carried out under optimized conditions, and the reaction process was monitored by ^{1}H NMR. The commercial name of the lipase used here was "PS-Amano". Compared with the resolution of 2-acetoxyphenylcyclohexane in cyclocondensation method (7 days in average), it took less time in this case (usually within 2 days). In order to secure high e.e., the reaction must be overrun, which was responsible for the low yield. But in fact, the enzymatic hydrolysis of the mis-matched substrate is relatively slow, compared with that of the matched one. PBS buffer was used to control the acidity of the solution, but water was suspected to open the β-lactam ring at elevated temperature and with basic buffer, which might be a reason leading to low yield in this step. The intact enantiomer **1-26** and hydrolyzed product **1-27** were easily separable by chromatography. The e.e. of 3-acetoxy-β-lactam **(+)-1-26** was determined by chiral HPLC.[157] It is worth to note here that enzymatic resolution, which avoids using chiral ketenes or chiral imines, offers an economical method to obtain enantiomeric β-lactam. There is only a modicum loss in enzyme activity after the enzyme is recycled three times.[157]



Scheme 1-19. Enzymatic resolution of racemic β-lactam **(±)-1-26**[157]

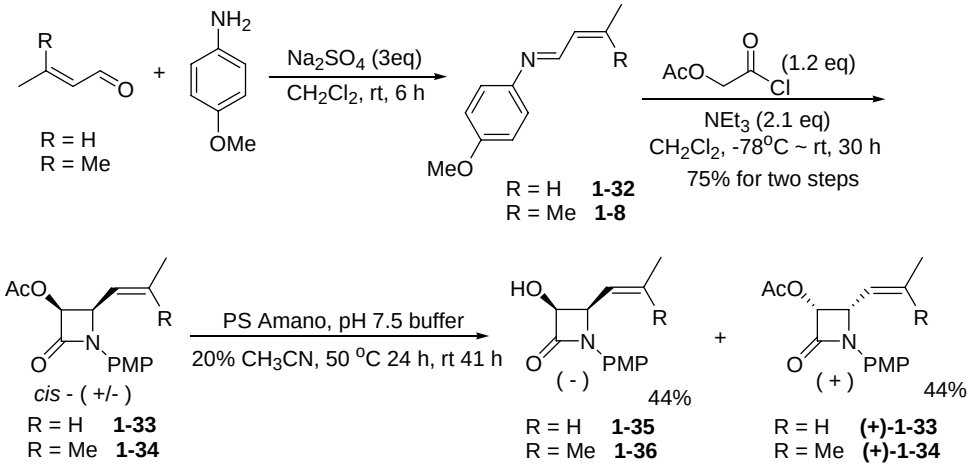
The removal of PMP *via* oxidative cleavage by ceric ammonium nitrate (CAN) and basic hydrolysis of acetate provided **1-29**, which then was protected with ethyl vinyl

ether (EVE) to give **1-30** (EE = 1-ethoxyethyl). Prior hydrolysis of acetate would cause oxidation of free alcohol when CAN is used. Since EE is not stable under CAN condition, the CAN reaction has to be done first. It should be noted that EE introduced another chiral center to β-lactam **1-30**, which showed up in ^{1}H NMR as two sets of peaks and the ratio was approximately 2 to 1 (Scheme 1-20).[52] Compared with cyclocondensation, where TIPS was used to protect the hydroxyl group, protection with EE, which is much smaller, may make the coupling easier between a modified baccatin and a β-lactam. The next step will be the protection of nitrogen with benzoyl group to give **1-31**, which is ready to be coupled with modified 10-DAB core. However, this process was not performed in this dissertation work.



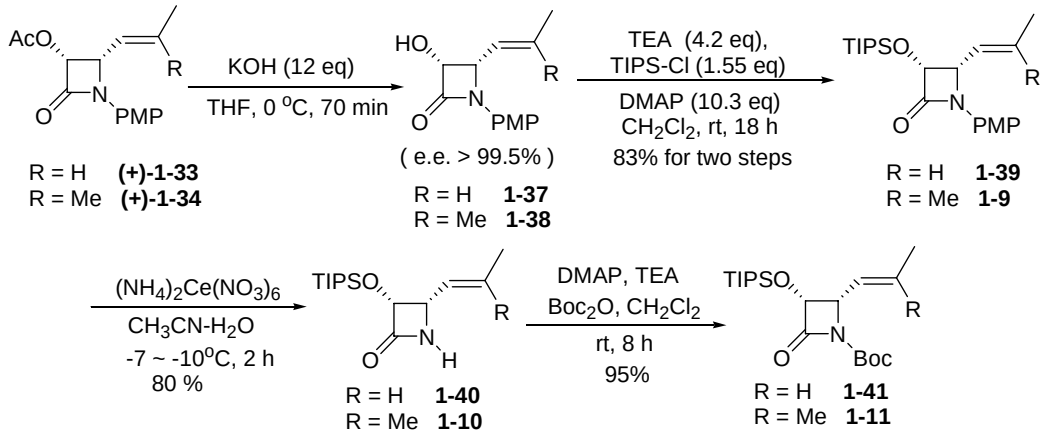
Scheme 1-20. Synthesis of β-lactam **(+)-1-31** [52]

Following the success of Staudinger cycloaddition, several β-lactams were also prepared, in which the phenyl group in **1-31** was replaced by alkenyl (such as *iso*butenyl and 1-propenyl) and the hydroxyl group was protected by TIPS (Scheme 1-21).



Scheme 1-21. Synthesis of β-lactams bearing alkenyl moieties

Thus, starting from α,β-unsaturated aldehydes, the racemic β-lactams were successfully prepared through Staudinger Reaction. The configuration of olefin was predominantly *trans*- when R was H (**1-33**). After the enzymatic resolution provided the desired enantiomers, similar chemical modifications were done as shown in Scheme 1-22. After basic hydrolysis, the high e.e. value was confirmed by chiral HPLC. When 2-butenyl was employed, the corresponding β-lactam was obtained in gram-scale quantities in 21% overall yield for 7 steps.



R = H **(+)-1-33**
R = Me **(+)-1-34**

R = H **1-37**
R = Me **1-38**

R = H **1-39**
R = Me **1-9**

R = H **1-40**
R = Me **1-10**

R = H **1-41**
R = Me **1-11**

Scheme 1-22. Future modifications on β-lactams[98]

Compared with the 4-phenyl counterpart, the 4-alkenyl-β-lactams possessed smaller polarity and thus had greater solubilities in organic solvents.[158]

These new β-lactams would serve as the side chain precursors for "second-generation" taxoids that have been developed by Ojima's lab.

§1.4. Summary

Paclitaxel (Taxol®) and docetaxel (Taxotère®) are two important examples of the taxane-based anticancer drugs in chemotherapy approved by FDA. Their unique mechanism of action, *i.e.*, binding to the *β*-tubulin subunits and stabilizing the microtubules, induces cell apoptosis. However, compared with the strong demands for treating various human cancers, the scarcity of the natural source of paclitaxel (bark of yew tree) strongly urged its chemical synthesis.

Among the semi-synthesis of paclitaxel, docetaxel and other analogs, the *β*-lactam synthon method (*β*-LSM) has proven to be the most efficient and versatile strategy, which was developed by Ojima and Holton. The enantiomerically pure *β*-lactams are coupled to a modified 10-DAB III, which comes from the extract of renewable plant leaves with relatively higher abundance in nature.

The required chiral *β*-lactams can be obtained by either asymmetric ester-imine cyclocondensation or the Staudinger reaction followed by enzymatic resolution, which are discussed in this chapter.

Because Ojima-Holton *β*-lactam coupling method can be used to obtain both natural and unnatural taxoids, it has greatly facilitated the structure-activity relationship (SAR) study. Ojima and co-workers have discovered a series of "second-generation" taxoids that exhibit extremely high biological activities against tumor cells, which will be discussed in next chapter.

§ 1.5. Experimental Section

General Methods: ^{1}H and ^{13}C NMR spectra were measured on a Varian 300 or 400 MHz NMR spectrometer. Melting points were measured on a "Uni-melt" capillary melting point apparatus from Arthur H. Thomas Company, Inc., and were not corrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. GC-MS analyses were performed on an Agilent 6890 Series GC system equipped with the HP-5HS capillary column, (50 m X 0.25 mm, 0.25 um) and with the Agilent 5973 network mass selective detector. LC-MS analyses were carried out on an Agilent 1100 Series Liquid Chromatograph Mass Spectrometer (Agilent Technologies, Palo Alto, CA). TLC analyses were performed on Merck DC-alufolien with Kieselgel 60F-254, and were visualized with one of the following methods: UV light, iodine chamber, 10% sulfuric acid in ethanol, 10% ceric sulfate and 15% sulfuric acid in water, and 10% phosphomolybdic acid in ethanol. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). Chemical purity was determined with a Waters HPLC assembly consisting of dual Waters 515 HPLC pumps, a PC workstation running Millennium 32, and a Waters 996 PDA detector, using a Curosil-B column from Phenomenex, employing CH_3CN/water as the solvent system with a flow rate of 1 mL/min, or the Agilent HPLC system. Chiral HPLC analysis for the determination of enantiomeric excess was carried out with a Waters HPLC assembly, comprising Waters M45 solvent delivery system, Waters Model 680 gradient controller, Water M440 detector (at 254 nm) equipped with a Spectra Physics Model SP4270 integrator. The system uses a Daicel-Chiral OD chiral column (25 x 0.46 cm *i.d.*), employing hexane/2-propanol (90/10 or 95/5) as the mobile phase with a flow rate of 1.0 ml/min.

Chemicals and Materials: Chemicals were purchased from Sigma-Aldrich or Fisher (including Acros) Company. 10-Deacetyl baccatin III (DAB) was donated by Indena, SpA, Italy. All solvents, right before use, were distilled under nitrogen or argon atmosphere unless otherwise mentioned. Dichloromethane (DCM) and methanol were dried over calcium hydride. Toluene and benzene were dried over sodium metal. Diethyl ether and THF were dried over sodium with benzophenone as the indicator. Toluene, THF, diethyl ether, and dichloromethane were also purified through PURE SOLVTM from Innovative technology Inc., and used without further purification. Anhydrous DMF was purchased from EMD, and used as it is. Glassware was dried in a 110 ^{o}C oven and allowed to cool to room temperature in a desiccator over "Drierite" (calcium sulfate).

Racemic *trans*-2-Phenylcyclohexanol [(±)-1-2]:[137]

A solution of phenylmagnesium bromide in THF (125 mL) was prepared from magnesium (3.54 g, 0.15 mol) and bromobenzene (15.6 mL, 0.15 mol) with reported conditions.[137] After cooling the Grignard solution to -30 ^{o}C, Cu_2I_2 (1.27 g, 6.6 mmol) was added. The resulting solution was stirred for approximately 10 min before a solution of cyclohexene oxide (**1-1**, 10.0 mL, 0.103 mol) in THF (25 mL) was added dropwise over 1 h. The reaction mixture was then allowed to warm to 0 ºC and stirred for an additional 2 h. The reaction was then quenched at 0 ºC by saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was washed with a saturated aqueous NH_4Cl solution until no color change was observed in the aqueous layer. The

combined aqueous layers were extracted with ether, and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Recrystallization of the crude product from hexane gave **1-2** (12.8 g, 73% yield) as a white solid: mp 57-58 °C [lit.[137] 57-58 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25-1.53 (m, 4 H), 1.62 (s, 1 H), 1.76 (m, 1 H), 1.84 (m, 2 H), 2.11 (m, 1 H), 2.42 (ddd, *J* = 16.6, 10.8, 5.4 Hz, 1 H), 3.64 (ddd, *J* = 16.6, 10.8, 5.4 Hz, 1 H), 7.17-7.35 (m, 5 H). All data are in agreement with literature values.[137]

Racemic *trans*-2-Phenylcyclohexyl acetate [(±)-(1-3)]:[137]

To a solution of 4-dimethylaminopyridine (DMAP, 0.295 g, 2.4 mmol) and racemic alcohol **1-2** (10.00 g, 57 mmol) in pyridine (10.0 mL, 124 mmol) and CH_2Cl_2 (16 mL) was added dropwise a solution of acetic anhydride(12.0 mL, 127 mmol) in CH_2Cl_2 (10 mL) in 2 h. The reaction mixture was then poured into a mixture of 6 *N* HCl (30 mL), ice (40 g) and ether (90 mL). The organic layer was washed with 2 *N* HCl (20 mL) and the combined aqueous layers were extracted with ether. The organic layer was then washed with saturated $NaHCO_3$ solution, followed by NaCl solution. After drying over anhydrous $MgSO_4$, the solution was filtrated and concentrated *in vacuo* to give **(±)-1-3** (11.64 g, 94% yield) as pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (m, 1 H), 1.41 (m, 1 H), 1.46 (m, 1 H), 1.56 (m, 1 H), 1.74 (s, 3 H), 1.78 (m, 1 H), 1.84 (m, 1 H), 1.93 (m, 1 H), 2.65 (ddd, *J* = 16.6, 11.0, 5.4 Hz, 1 H), 7.17-7.35 (m, 5 H). All data are in agreement with literature values.[137]

***trans*-(+)-2-Phenylcyclohexyl acetate [(+)-1-3] and *trans*-(-)-2-Phenylcyclohexanol [(-)-1-2]:** [137]

To aqueous KH_2PO_4/K_2HPO_4 buffer (0.50 M, pH = 8.0, 350 mL) was added racemic acetate **(±)-1-3** (12.09 g, 0.25 mol) in ether (60 mL) at 31 ºC. After stirring for 30 min, pig liver acetone powder (PLAP, 2.48 g) was added. The mixture was stirred for 8 days at 31 ºC, until ^{1}H NMR of the crude organic layer showed nearly 1:1 ratio of alcohol **(-)-1-2** and acetate **(+)-1-3**. The reaction was quenched by acidifying to pH 4 by HCl (2.0 M). To the resulting mixture was added ether (100 mL) and the mixture was stirred for 1 h. After PLAP settled down, the supernatant organic layer was separated. The aqueous layer was extracted with ether 3 times, and combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel (hexane/EtOAc = 8/1) to afford **(+)-1-3** (slightly yellow oil, 5.43 g, 93% yield) and alcohol **(-)-1-2** as a white solid. Recrystallization of the alcohol from hexane afforded of pure **(-)-1-2** (4.00 g, 85% yield) with mp: 62-63 ºC [lit.[137] 62-65 ºC]. All data are in agreement with literature values.[137]

Benzyloxyacetic acid (1-4): [159]

Sodium (6.40 g, 0.28 mol) was dissolved in benzyl alcohol (110 mL) at room temperature, and the mixture was kept at 150 ºC for 3 h for the reaction to complete. To the resulting solution, bromoacetic acid (17.82 g, 0.13 mol) in THF (25 mL) was added dropwise. The reaction mixture was stirred at 150 ºC for another 3 h and then cooled to room temperature, and then cold water was added slowly. The aqueous layer was carefully extracted by DCM to remove any remaining benzyl alcohol. The water layer was then acidified with 10% HCl to pH 1-2 and extracted with ether. The organic layer

was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The oily residue was distilled under reduced pressure (bp 138-143 ºC at 2.5 mm Hg; lit.[159] 140-150 ºC at 0.8 mm Hg) to afford **1-4** (16.51 g, 77% yield) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17 (s, 2 H), 4.67 (s, 2 H), 7.38 (m, 5 H). All data are in agreement with reported values.[159]

(1*R*,2*S*)-(-)-2-Phenylcyclohexyl benzyloxyacetate (1-5): [159]

A toluene (40 mL) solution of **1-2** (3.38 g ,19 mmol), benzyloxyacetic acid **1-4** (3.464 g, 21 mol) and a catalytic amount of *p*-toluenesulfonic acid (pTSA, 0.030 g, 1.7 mmol) was refluxed overnight. After toluene was evaporated *in vacuo*, the reaction mixture was diluted with ether and washed with saturated $NaHCO_3$ solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford **1-5** (6.05 g, 98% yield) as a white solid: mp 52-53 ºC [lit.[159] 52-53 ºC]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26-1.63 (m, 4 H), 1.76-1.99 (m, 3 H), 2.10-2.20 (m, 1 H), 2.70 (dt, J = 11.0, 4.1 Hz, 1 H), 3.73 (d, J = 16.5 Hz, 1 H), 3.84 (d, J = 16.5 Hz, 1 H), 4.25 (s, 1 H), 5.13 (td, J = 11.0, 4.1 Hz, 1 H), 7.16-7.39 (m, 5 H). All data are in agreement with reported values.[159]

(1*R*,2*S*)-(-)-2-Phenylcyclohexyl hydroxyacetate (1-6): [119]

A mixture of 10% palladium on carbon (1.770 g) and **1-5** (6.02 g, 18.5mmol) in THF (56 mL) was stirred at 45 ºC under H_2. After completion of the reaction, the mixture was filtered through celite and concentrated *in vacuo* to afford **1-6** (4.02 g, 93% yield) as a white solid: mp 59-60 ºC [lit.[159] 59-60 ºC]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.3-1.7 (m, 4 H), 1.8-2.0 (m, 3 H), 2.1-2.2 (m, 2 H), 2.6 (dt, J = 11.0, 4.1 Hz, 1 H), 3.7 (d, J = 17.0 Hz, 1 H), 3.9 (d, J = 17.0 Hz, 1 H), 5.1 (td, J = 11.0, 4.1 Hz, 1 H), 7.16-7.32 (m, 5 H). All data are in agreement with literature values.[119]

(1*R*, 2*S*)-(-)-2-Phenylcyclohexyl tri*iso*propylsiloxyacetate (1-7): [160]

To a solution of imidazole (2.722 g, 40 mmol) and **1-6** (3.70 g, 15.8 mmol) in DMF (10 mL) was added tri*iso*propylsilyl chloride (TIPS-Cl, 4.71 g, 24.4 mmol) at room temperature. The mixture was stirred under N_2 for 24 h, quenched by water, and extracted with ether. The organic layer was washed several times with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was distilled (bp: 206-209 ºC at 1.2 mm Hg; lit.[160] 171-176 ºC at 0.2 mm Hg) to afford **1-7** (5.73 g, 93% yield) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.9-1.2 (m, 21 H), 1.3-1.7 (m, 4 H), 1.8-2.0 (m, 3 H), 2.1-2.2 (m, 1 H), 2.7 (dt, J = 11.0, 4.2 Hz, 1 H), 3.9 (d, J = 16.5 Hz, 1 H), 4.1 (d, J = 16.5 Hz, 1 H), 5.1 (td, J = 11.0, 4.2 Hz, 1 H), 7.1-7.3 (m, 5 H). All data are in agreement with literature values.[160]

***N*-(4-Methoxyphenyl)-3-methyl-2-butenaldimine (1-8):** [98]

To a mixture of *p*-anisidine (9.70 g, 79 mmol; recrystallized from ether) and anhydrous Na_2SO_4 (35.0 g, 246 mmol) in DCM (50 mL) was added 3-methylbutyraldehyde (10.4 mL, 110 mmol) dropwise, and the mixture was stirred at room temperature for 6 h. After filtration and evaporation to remove the solvent, the brown residue was left under high vacuum (oil pump) to yield imine **1-8** as yellow and viscous oil, which was immediately used for the synthesis of β-lactam without further

purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (s, 3 H), 2.00 (s, 3 H), 3.8 (s, 3 H), 6.2 (d, *J* = 9.6 Hz, 1 H), 6.89 (d, *J* = 6.9 Hz, 2 H), 7.11 (d, *J* = 6.9 Hz, 2 H), 8.38 (d, *J* = 9.6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.0, 27.0, 55.7, 114.1, 122.1, 126.2, 146.1, 149.5, 157.3, 158.5. All data are in agreement with literature values.[98]

1-*p*-Methoxyphenyl-3-tri*iso*propylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-9): [161]

To a solution of di*iso*propylamine (0.346 ml, 2.5mmol) in THF (10 mL) was added *n*-butyllithium (2.5 M in hexanes, 1.00 ml, 2.5mmol) at -15 °C. After stirring for 1 h, the solution was cooled to -85 °C, and a solution of TIPS-ester **1-7** (0.740 g, 1.9 mmol) in THF (10mL) was slowly added by use of a cannula over a period of 1 h. After stirring for an additional 1.5 h, a solution of imine **1-8** (3.3 mmol) in THF (10 mL) was carefully added *via* cannula over a period of 2 h. The mixture was stirred overnight at -85°C. Then, LiHMDS (1.50 mmol) was added to the reaction mixture and stirred for another 2 h. The reaction was then quenched with a saturated aqueous NH_4Cl solution. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel (hexane/EtOAc = 20/1) to afford **1-9** (488 mg, 63% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00-1.16 (m, 21 H), 1.79 (s, 3 H), 1.84 (s, 3 H), 3.77 (s, 3 H), 4.80 (dd, *J* = 9.9, 4.8 Hz, 1 H), 5.05 (d, *J* = 5.1 Hz, 1 H), 5.32 (d, *J* = 9.9 Hz, 1 H), 6.84 (d, *J* = 9.0 Hz, 2 H), 7.33 (d, *J* = 9.0 Hz, 2 H). All data are in agreement with literature values.[161]

(3*R*,4*S*)-(+)-3-Tri*iso*propylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-10): [99]

To a solution of **1-9** (6.00 g, 15 mmol) in acetonitrile (300 mL) and water (40 mL) at -13 °C was slowly added a solution of ceric ammonium nitrate (CAN, 30 g, 52 mmol) in water (260 mL) over a period of 1.5 h. The mixture was stirred for 3 h at -10 °C before the reaction quenched by saturated Na_2SO_3 solution (300 mL). The aqueous layer was extracted with ether, and the combined organic layer was washed with water and saturated brine to afford **1-10** (3.5 g, 80% yield) after column chromatography on silica gel (hexane/EtOAc = 3/1): mp 84.5-86.0 °C [lit.[99] 85-86 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00-1.16 (m, 21 H), 1.69 (s, 3 H), 1.76 (s, 3 H), 4.44 (q, *J* = 9.6 Hz, 4.8 Hz, 1 H), 5.05 (q, *J* = 4.8 Hz, 2.1 Hz, 1 H), 5.32 (d, *J* = 9.3 Hz, 1 H), 5.78 (s, 1 H). All data are in agreement with literature values.[99]

(3*R*,4*S*)-(+)-1-(*tert*-Butyloxycarbonyl)-3-tri*iso*propylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-11): [99]

To a solution of **1-10** (3.00 g, 11 mmol), triethylamine (4.2 mL, 30 mmol) and dimethylaminopyridine (DMAP, 0.300 g, 2.4 mmol) in CH_2Cl_2 (30 mL) was added di-*tert*-butyl dicarbonate (2.42 g, 12 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at room temperature overnight and the reaction was quenched with saturated NH_4Cl solution. The reaction mixture was extracted by EtOAc (400 mL). The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified on a silica gel column (hexane/EtOAc = 4/1) to yield **1-11** (oil, 3.77 g, 95% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00-1.16 (m, 21 H), 1.48 (s, 9 H), 1.76 (s,

3 H), 1.79 (s, 3 H), 4.78 (q, J = 9.9, 5.7 Hz, 1 H), 4.96 (d, J = 5.7 Hz, 1 H), 5.32 (d, J = 9.8 Hz, 1 H). All data are in agreement with literature values.[99]

_N_-(4-Methoxyphenyl)benzaldimine (1-25): [5]

To a suspension of *p*-anisidine (10.0 g, 81.2 mmol; purified by ether extraction) and anhydrous $MgSO_4$ (19.5 g, 162 mmol) in DCM (150 mL) was added benzaldehyde (9.48 g, 89.3 mmol). The mixture was stirred at room temperature for 2 h and solid was filtered off. The filtrate was concentrated to give the crude imine, which was further purified by recrystallization from hexane and dichloromethane to give pure **1-25** (15 g, 90% yield) as white solid: mp 70-71 °C [lit.[5] 71-72 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 3 H), 6.93 (d, J = 8.8 Hz, 2 H), 7.23 (d, J = 8.8 Hz, 2 H), 7.46 (m, 3 H), 7.87 (m, 2 H), 8.48 (s, 1 H). All data are consistent with the reported values.[5]

General procedure for Staudinger Reaction ([2+2] Cycloaddition Reaction): [162]

To a solution of imine (10.0 mmol) and triethylamine (20.0 mmol) in dichloromethane (80 mL) at -78 °C was added dropwise a solution of acetoxyacetyl chloride (12.2 mmol) in DCM (80 mL). The reaction mixture was allowed to warm to room temperature over 18 h, and then diluted by DCM (100 mL). The organic layer was washed by water (100 mL) and saturated ammonium chloride solution twice (90 mL each), dried over sodium sulfate, and concentrated to provide a crude solid product. Although the color of the crude solid varied from yellow to dark brown, it was good enough to be used for the next enzymatic resolution step without further purification. The pure sample (white solid) was obtained after column chromatography on silica gel (hexane/EtOAc = 10/1).

***cis*-1-*p*-Methoxyphenyl-3-acetoxy-4-phenylazetidin-2-one (1-26):** [52]

Following the general procedure in Staudinger Reaction mentioned above, crude racemic **1-26** was obtained as yellow solid: mp 161-163 °C [lit.[52] 160-162 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68 (s, 3 H), 3.75 (s, 3 H), 5.34 (d, J = 4.8 Hz, 1 H), 5.93 (d, J = 5.1 Hz, 1 H), 6.81 (d, J = 9.0 Hz, 2 H), 7.3-7.4 (m, 7 H). All data are consistent with literature data.[52]

General procedure for the enzymatic enantioselective hydrolysis of *β*-lactam [162]

To a solution of racemic 3-acetoxy-*β*-lactam **1-26** (54 mmol) in sodium phosphate buffer (0.20 M, pH = 7.5, 1.2 L) containing 10-15% (V/V) acetonitrile (120-180 mL) was added PS-Amano lipase (8.00 g), and the mixture was vigorously stirred at 50 ^{o}C. After 24-48 h (monitored by ^{1}H NMR), the reaction was terminated by extraction of the mixture with ethyl acetate three times (500 mL each). After removal of organic solvent, the residue was purified on a silica gel chromatography (eexane/EtOAc = 3/1 to 1/1) to provide the ester and the alcohol.

(+)-*cis*-l-(*p*-Methoxyhenyl)-3-acetoxy-4-phenylazetidin-2-one ((+)-1-26): [162]

(+)-1-26 was isolated as white solid in 47% yield after column chromatography on silica gel (hexane/EtOAc = 3/1). Its ^{1}H NMR was the same as the racemic **1-26**.

***cis*-(-)-l-(*p*-Methoxyhenyl)-3-hydroxy-4-phenylazetidin-2-one ((-)-1-27):**

(-)-1-27 was obtained as white solid in 50% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (d, *J* = 9.3 Hz, 1 H), 3.76 (s, 3 H), 5.18 (q, *J* = 9.0, 4.8 Hz, 1 H), 5.28 (d, *J* = 5.4 Hz, 1 H), 6.80 (d, *J* = 9 Hz, 2 H), 7.2-7.4 (m, 7 H).

(3*R*,4*S*)-(-)-3-Acetoxy-4-phenylazetidin-2-one (1-28): [157]

To a solution of **(+)-1-26** (0.73 g, 2.3 mmol) in acetonitrile (80 mL) and water (5 mL) at -13 °C was slowly added a solution of ceric ammonium nitrate (4.504 g, 8.2 mmol) in water (28 mL) over 30 min. The mixture was stirred for 3.5 h at -10 °C and then the reaction was quenched with saturated $NaHSO_3$. The aqueous layer was extracted with ether (400 mL), and the combined organic layers were washed with water and brine. After drying over $MgSO_4$ and concentrating under reduced pressure, the crude product was purified on a silica gel column (hexanes/EtOAc = 3/1) yielding **1-28** (white solid, 0.35 g, 75 % yield): mp 148-151 °C [lit.[157] 151-153 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68 (s, 3 H), 5.04 (d, *J* = 4.8 Hz, 1 H), 5.89 (q, *J* = 4.8, 3.0 Hz, 1 H), 6.17 (m, 1 H), 7.4-7.2 (m, 5 H). All data are consistent with literature data.[157]

(3*R*,4*S*)-(+)-3-Hydroxy-4-phenylazetidin-2-one (1-29): [52]

To a solution of THF (14 mL) and 1 M KOH (18 mL) at 0 °C was added a solution of **1-28** (0.300 g, 1.50 mmol) in THF (20 mL). The solution was stirred at 0 °C for 1.5 h and 10 mL saturated sodium bicarbonate was added. The mixture was extracted with ethyl acetate and the combined organic layers were dried over sodium sulfate and concentrated to give **(+)-1-29** (0.210 g, 88 % yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (d, *J* = 9.0 Hz, 1 H, O<u>H</u>), 4.96 (d, *J* = 6.4 Hz, 1 H), 5.09-5.15 (m, 1 H), 6.16 (m, 1 H), 7.25-7.35 (m, 5 H). All data are in agreement with literature values.[52]

(3*R*,4*S*)-(+)-3-(l-Ethoxyethoxy)-4-phenyl-2-azetidinone (1-30): [5]

A mixture of **(+)-1-29** (0.200 g, 1.23 mmol), ethyl vinyl ether (0.30 mL, 3.1 mmol) and pTSA (0.010 mg, 0.060 mmol) in THF (2 mL) was stirred for 1.5 h at 0 ^oC. Then, the reaction mixture was diluted with ether, washed with saturated $NaHCO_3$, and extracted with dichloromethane. The combined extracts were dried over anhydrous $MgSO_4$ and concentrated. After purification on silica gel chromatography (hexane/EtOAc = 4/1), **1-30** was obtained (160 mg, 55% yield) as slightly yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (d, *J* = 5.4 Hz, 1.88 H), 1.08 (d, *J* = 5.7 Hz, 1.60 H), 1.15 (t, *J* = 4.2 Hz, 3.23 H), 3.20 (m, 0.67 H), .40 (m, 1 H), 3.70 (m, *J* = 7.2 Hz, 0.67 H), 4.50 (q, *J* = 5.4 Hz, 0.52 H), 4.72 (q, *J* = 5.7 Hz, 0.62 H), 4.87 (q, *J* = 11.4, 4.8 Hz, 1 H), 5.21-5.24 (m, 1 H), 6.16 (m, 1 H), 7.40-7.34 (m, 5 H). All data are in agreement with literature values.[5]

***cis*-1-Methoxyphenyl-3-acetoxy-4-propenylazetidin-2-one (1-33):** [98]

To a suspension of anhydrous Na_2SO_4 (7.1 g, 50 mmol) and *p*-anisidine (1.23 g, 10 mmol) in DCM (15 mL) was added but-2-enal (0.93 mL, 11 mmol). After stirring the mixture for 4 h at room temperature, the solvent was evaporated. The crude brown residue was checked by NMR and used for the Staudinger Reaction without further purification.

Following the protocol similar to **1-26**, the crude residue was dissolved in DCM (100 mL) and TEA (2.10mL, 15 mmol), and then acetoxyacetyl chloride (1.33 mL, 12 mmol) in

CH_2Cl_2 (50 mL) was added slowly at -78 °C. After purification on a silica gel column (hexane/EtOAc = 3/1), **1-33** was obtained (0.32 g, 10% yield) as slightly yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (dd, *J* = 6.6, 1.8 Hz, 3 H), 2.13 (s, 3 H), 3.79 (s, 3 H), 4.72 (dd, *J* = 8.1, 4.8 Hz, 1 H), 5.40 (m, 1 H), 5.80 (m, 1 H), 5.95 (m, 1 H), 6.85 (d, *J* = 9.3 Hz, 2 H), 7.36 (d, *J* = 9.3 Hz, 2 H).

***cis*-1-Methoxyphenyl-3-acetoxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-34):**[98]

Following the protocol similar to **1-26**, the title compound **1-34** (17.15 g, 75% yield) was obtained as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79 (s, 3 H), 1.82 (s, 3 H), 2.11 (s, 3 H), 3.79 (s, 3 H), 4.97 (dd, *J* = 9.0, 4.8 Hz, 1 H), 5.12 (m, *J* = 9.0 Hz, 1 H), 5.80 (d, *J* = 5.1 Hz, 1 H), 6.85 (d, *J* = 9.0 Hz, 2 H), 7.11 (d, *J* = 9.0 Hz, 2 H). All data are in agreement with literature values.[98]

(+)-*cis*-1-(*p*-Methoxyphenyl)-3-acetoxy-4-propenyl-azetidin-2-one ((+)-1-33) and **(-)-*cis*-l-(*p*-Methoxyhenyl)-3-hydroxy-4-propenyl-azetidin-2-one (1-35):** [98]

After enzymatic resolution of racemic **1-33** (578 mg) by using PS Amano (400 mg) at 50 °C after 35 h, **(+)-1-33** (263 mg, 45% yield) and **1-35** (227 mg, 46% yield) were isolated with column chromatography on silica gel (hexane/EtOAc = 3/1 to 1/1). **1-35** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80 (dd, *J* = 6.6, 1.8 Hz, 3 H), 2.58 (d, *J* = 8.1 Hz, 1 H), 3.79 (s, 3 H), 4.65 (m, 1 H), 5.00 (m, 1 H), 5.60 (m, 1 H), 5.95 (m, 1 H), 6.85 (d, *J* = 9.3 Hz, 2 H), 7.36 (d, *J* = 9.3 Hz, 2 H). All data are in agreement with literature values.[98]

(+)-*cis*-l-(*p*-Methoxyhenyl)-3-acetoxy-4-(2-methylpropen-2-yl)azetidin-2-one (**(+)-1-34**) and **(-)-*cis*-l-(*p*-methoxyhenyl)-3-hydroxy-4-(2-methylpropen-2-yl)azetidin-2-one** (**1-36**)**:** 44% and 50% yield, respectively. For **1-36**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (s, 6 H), 2.74 (d, *J* = 7.8 Hz, 1 H), 3.78 (s, 3 H), 4.89 (q, *J* =9.0, 4.8 Hz, 1 H), 5.02 (q, *J* = 7.8, 4.8 Hz, 1 H), 5.26 (d, *J* = 9 Hz, 1 H), 6.83 (d, *J* = 9 Hz, 2 H), 7.32 (d, *J* = 9 Hz, 2 H). All data are in agreement with literature values.[98]

(3*R*,4*S*)-l-(*p*-Methoxyhenyl)-3-hydroxy-4-propenylazetidin-2-one (1-37): [98]

To a solution of **1-33** (263 mg, 0.96 mmol) in THF (18 mL) was added 1.0 M KOH (10 mL, 10 mmol) at 0 °C. After the solution was stirred for 1 h, saturated NH_4Cl (20 mL) was added. The mixture was extracted with EtOAc (20 mL x 2) and the combined organic layers were dried over anhydrous $MgSO_4$, and concentrated. Purification of the residue on a silica gel column (hexane/EtOAc = 1/1) gave **1-37** (190 mg, 85% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80 (dd, *J* = 6.6, 1.8 Hz, 3 H), 2.58 (d, *J* = 8.1 Hz, 1 H), 3.79 (s, 3 H), 4.65 (m, 1 H), 5.00 (m, 1 H), 5.60 (m, 1 H), 5.95 (m, 1 H), 6.85 (d, *J* = 9.3 Hz, 2 H), 7.36 (d, *J* = 9.3 Hz, 2 H). All data are in agreement with literature values.[98]

(3*R*,4*S*)-l-(*p*-Methoxyphenyl)-3-hydroxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-38): [98]

To a solution of **1-34** (6.90 g, 24 mmol) in THF (160 mL) and water (20 mL) was added KOH (2.0 M, 144 mL, 288 mmol) at 0 °C. The solution was stirred for 70 min and saturated NH_4Cl solution (100 mL) was added. The mixture was extracted with EtOAc (100 mL x 3) and the combined organic layers were dried over anhydrous $MgSO_4$ and

concentrated to give **1-38** (5.86 g) as white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (s, 6 H), 3.78 (s, 3 H), 4.88 (q, *J* = 9.0, 5.1 Hz, 1 H), 5.02 (d, *J* = 5.4 Hz, 1 H), 5.26 (d, *J* = 9.0 Hz, 1 H), 6.84 (d, *J* = 9.0 Hz, 2 H), 7.33 (d, *J* = 9.0 Hz, 2 H). All data are in agreement with literature values.[98] Enantiomer excess value (e.e.) was more than 99.5% [measured on chiral OD column on Waters M45 HPLC pump, Waters 484 tunable absorbance detector, isopropanol:hexane (5: 95 v/v), 0.5 mL/min].

(3*R*,4*S*)-l-(*p*-Methoxyphenyl)-3-tri*iso*propylsiloxy-4-propenylazetidin-2-one (1-39): [98]

To a solution of **1-37** (190 mg, 0.81 mmol) and DMAP (30 mg, 0.24 mmol) in DCM (5.6 mL) and TEA (0.48 mL, 3.4 mmol) was added tri*iso*propylsilyl chloride (TIPS-Cl, 0.28 mL, 1.26 mmol). The reaction was monitored by TLC. After stirring for 36 h at room temperature, the reaction was quenched by saturated NH_4Cl solution. The aqueous layer was extracted by CH_2Cl_2, and extracts were dried over anhydrous $MgSO_4$. After concentration and separation on a silica gel column (hexane/EtOAc = 20/1), **1-39** was obtained (353 mg, containing some $TIPS_2O$) as a colorless crystals: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (m, 21 H), 1.78 (dd, *J* = 6.6, 1.8 Hz, 3 H), 3.79 (s, 3 H), 4.57 (m, 1 H), 5.00 (m, 1 H), 5.60 (m, 1 H), 5.90 (m, 1 H), 6.85 (d, *J* = 9.3 Hz, 2 H), 7.36 (d, *J* = 9.3 Hz, 2 H). All data are in agreement with literature values.[98]

(3*R*,4*S*)-3-Tri*iso*propylsilyloxy-4-propenylazetidin-2-one (1-40): [98]

To a solution of **1-39** (310 mg, 0.8 mmol) in acetonitrile (30 mL) at -13 °C was slowly added a solution of ammonium ceric nitrate (CAN, 1.53 g, 2.8 mmol) in water (10 mL) over a 30-min period. The mixture was stirred for 1 h at -10°C and the reaction was quenched with saturated Na_2SO_3 aqueous solution (50 mL). The aqueous layer was extracted with ether (400 mL), and the combined organic layers were washed with water and saturated aqueous NaCl solutions. Purification by column chromatography on silica gel (hexane/EtOAc = 4/1) afforded **1-40** (210 mg, 90% yield for two steps) as slightly yellow powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (m, 21 H), 1.74 (dd, *J* = 6.6, 1.8 Hz, 3 H), 4.16 (m, 1 H), 4.97 (m, 1 H), 5.54 (m, 1 H), 5.70 (m, 1 H), 6.14 (br, 1 H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.8, 17.60, 17.64, 58.0, 79.3, 127.9, 130.8, 169.7. All data are in agreement with literature values.[98,118]

(3*R*,4*S*)-1-(*tert*-Butyloxycarbonyl)-3-tri*iso*propylsiloxy-4-propenylazetidin-2-one (1-41): [98,118]

To a solution of **1-40** (210 mg, 0.74 mmol) and DMAP (22.6 mg, 0.19 mmol) in TEA (0.32 mL, 2.2 mmol) and CH_2Cl_2 (2 mL) was added di-*tert*-butyl dicarbonate (179 mg, 0.82 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at room temperature overnight and the reaction was quenched with saturated NH_4Cl solution. The aqueous layer was extracted with DCM (30 mL), and the DCM layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. After purification on a silica gel column (hexane/EtOAc = 20/1), **1-41** was obtained (260 mg, 92% yield) as colorless sticky oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (m, 21 H), 1.45 (s, 9 H), 1.76 (dd, *J* = 6.6, 1.8 Hz, 3 H), 4.45 (m, 1 H), 4.96 (m, 1 H), 5.53 (m, 1 H), 5.85 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.7, 17.45, 17.51, 17.9, 27.9, 61.0, 83.1, 124.8, 132.7, 148.2, 166.1. All data are in agreement with literature values.[98,118]

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Chapter Two
Syntheses of Novel Taxoids

§ 2.1. Second- and Third-Generation Taxoids

§ 2.1.1. Development of Second- and Third-Generation Taxoids

Due to their remarkable activities against various tumors, paclitaxel (Taxol®) and docetaxel (Taxotére®) are widely used in clinic as powerful anticancer drugs. However, two problems limit their efficiency: one is the multi-drug resistance (MDR),[163,164] and the other is the undesirable side effect. Consequently, novel taxane-based drugs with better pharmacological properties and activities are required. During the extensive structure-activity relationship (SAR) studies,[165-169] thousands of such molecules have been synthesized and evaluated. Among these studies, Ojima and co-workers have developed "second-generation" taxoids.[170-173] (Figure 2-1)

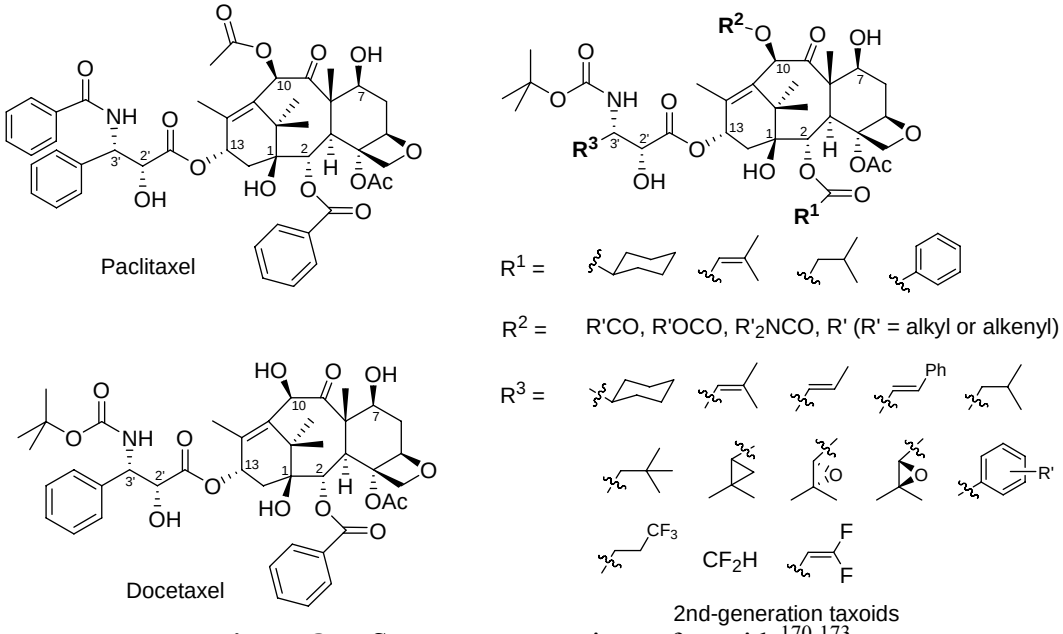


Figure 2-1. Structure comparison of taxoids[170-173]

As shown in Figure 2-1, there are some significant modifications in second-generation taxoids. A *tert*-butoxycarbonyl group at C-3'-*N* (docetaxel and second-generation taxoids) replaces the N-benzoyl group in paclitaxel; various alkyl or alkenyl groups are introduced to the C-3' position instead of the phenyl group in paclitaxel and docetaxel; modifications at the C-10 position are also important. Compared to paclitaxel and docetaxel, these second-generation taxoids showed 10 times higher potencies against drug-sensitive cancer cell lines, but 100-1000 times higher potencies against drug-resistant cell lines expressing MDR phenotypes (Table 2-1).[170,171,174]

In 1998, Kingston and co-workers published their study on the effect of the *meta* substitution of the C-2 benzoate on potency (Figure 2-2), which revealed that the cytotoxicity was significantly increased against the P-388 cell line by the introduction of *meta*-substitutents.[175]

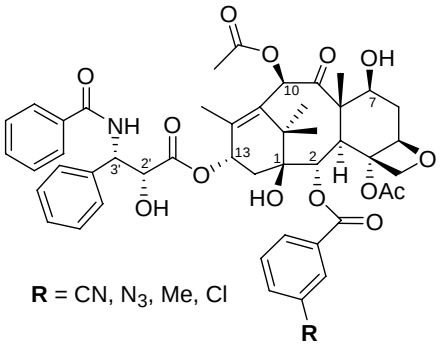


Figure 2-2. Kingston's *meta*-substituted paclitaxel analogs[175]

Inspired by Kingston's results, Ojima and co-workers designed and synthesized a new series of taxoids, which introduced a *meta*-substituted benzoate at C-2 into the second-generation taxoids. Besides their exceptional potency and cytotoxicity compared to paclitaxel or docetaxel, the most amazing character of these new taxoids was that they were almost equally potent against drug-resistant and drug-sensitive cell lines (*e.g.*, **SB-T-121303** exhibited virtually the same IC_{50} values for MCF-7 (0.36 nM) and MCF-7R (0.33 nM), which meant MDR was totally circumvented (Table 2-1). These taxoids are named "third-generation taxoids" (Figure 2-3).[174]

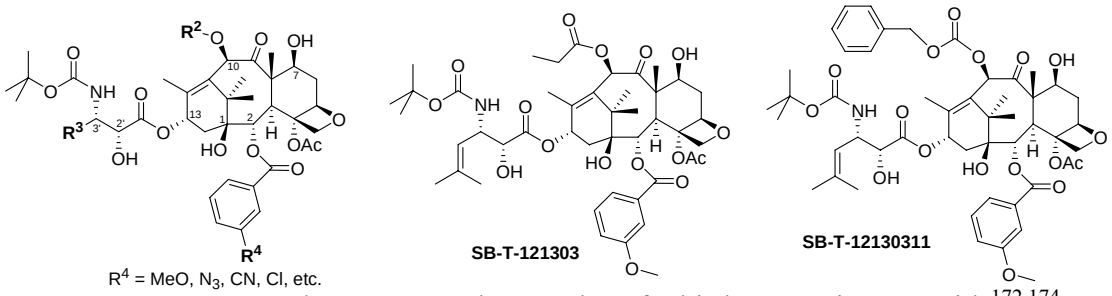


Figure 2-3. General structure and examples of "third-generation" taxoids[172,174]

The *in vitro* cytotoxicity data of these novel taxoids are showed in Table 2-1. **SB-T-1213**, **SB-T-1103** and **SB-T-1214** are representatives of second-generation taxoids, while **SB-T-12130211** and **SB-T-11033** belong to the third-generation taxoids (Figure 2-4). The screened human tumor cell lines included A121 (ovarian), A549 (NSCL), HT-29 (colon), MCF-7 (breast), LCC-WT (breast), MCF7-R (breast) and LCC-MDR (breast), and the latter two are multiple drug-resistant cancer cell lines.[172] Clearly, novel taxoids are very potent against MDR cell lines, for which paclitaxel and docetaxel failed. Thus, it is very clear from Table 2-1 that MDR has been overcome by these new members in taxane family. More experimental data are available in the literature.[170,172,174]

Both second- and third-generation taxoids promote microtubule polymerization as paclitaxel does, but the time they need is basically much shorter than paclitaxel, which implies that the new taxoids are much more effective than paclitaxel in acceleration of polymerization. Meanwhile, from the morphology, the characteristics of the resulting tubulins after treatment with these novel taxoids are much greater in number and much shorter in length than either normal ones promoted by GTP or those obtained after paclitaxel treatment.[174]

Table 2-1. Cytotoxicity (IC_{50}, nM)[a] of taxoids. [170,172,174]

Taxoids	*Cancer Cell Lines*						
	A121[b] (ovarian)	**A549[c] (NSCL)**	**HT-29[d] (colon)**	**MCF-7[e] (breast)**	**MCF7-R[f] (breast)**	**LCC6 -WT[g] (breast)**	**LCC6 -MDR[h] (breast)**
Paclitaxel	**6.3**	**3.6**	**3.6**	**1.7**	**299**		
Docetaxel	**1.2**	**1.0**	**1.2**	**1.0**	**235**		
SB-T-1103	**0.41**	**0.53**	**0.53**	**0.35**	**2.8**		
SB-T-1213	**0.12**	**0.29**	**0.31**	**0.18**	**2.2**		
SB-T-1214	**0.26**	**0.57**	**0.36**	**0.20**	**2.1**		
SB-T-121303		**0.05**		**0.36**	**0.33**	**1.0**	**0.9**
SB-T-11033		**0.046**		**0.36**	**0.43**	**0.9**	**0.8**
SB-T-12130311				**0.2**	**1.5**	**1.2**	**1.8**
SB-T-12130211						**1.2**	**4.1**

The cytotoxicity assays were carried out in Dr. Ralph Bernacki's lab in Roswell Park Cancer Institute. [a]The concentration of compound which inhibits 50% (IC_{50}, nM) of the growth of human tumor cell line after 72 h drug exposure; [b] A121: ovarian carcinoma; [c] A549: non-small-cell lung carcinoma; [d] HT-29: colon carcinoma; [e] MCF-7, breast carcinoma; [f] MCF7-R: multi-drug resistant human breast carcinoma; [g] LCC6-WT: human breast carcinoma cell line (Pgp-); [h] LCC6-MDR: mdr1 transduced cell line (Pgp+).

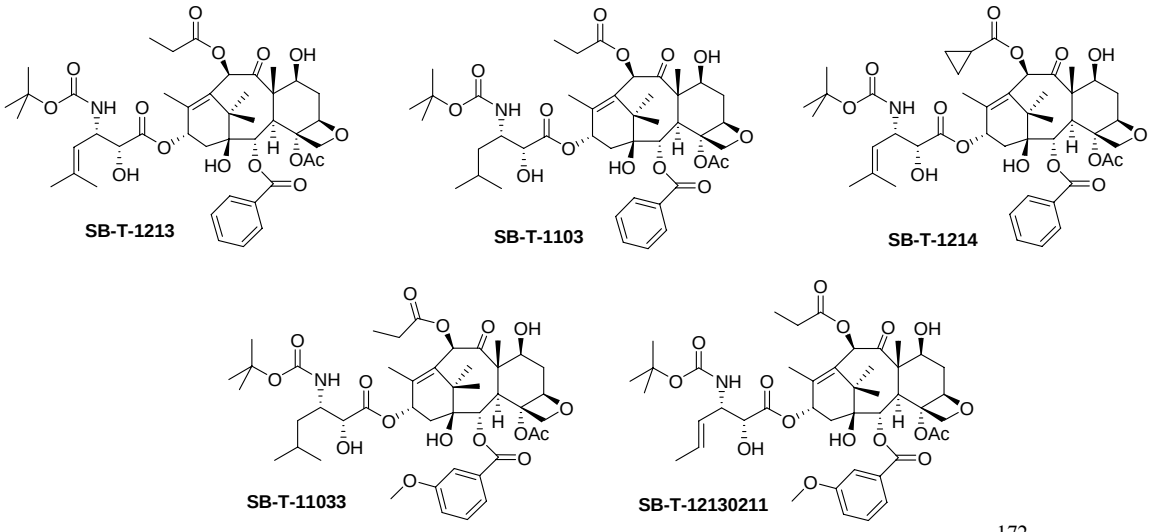


Figure 2-4. Examples of novel taxoids developed by Ojima's group[172]

Recently, **SB-T-1214** and **SB-T-121303** have also been evaluated against pancreatic cancer cell lines, *e.g.*, MiaPaca, BXPC3, CFPac and Panc 1, some of which are MDR cell lines.[174,176] The biological assays were carried out by Jon Mallen-St. Clair and Jennifer Curato in Dr Dafna Bar-Sagi's lab at the Department of Molecular Genetics

and Microbiology at Stony Brook University. Pancreatic cancer is one of the most fatal cancers, and its 5-year survival is only around 5%.[177] Even worse, MDR is commonly seen in pancreatic cancer cells, and conventional therapy (such as cisplatin and paclitaxel) showed little success. However, these novel taxoids showed particularly promising effectiveness (Table 2-2). Both **SB-T-1214** and **SB-T-121303** were able to strongly inhibit the growth of pancreatic cancer cells *in vitro*. Interestingly, these taxanes are not toxic to primary pancreatic ductal cells in the therapeutic concentrations.

Table 2-2. Cytotoxicities (IC_{50}, nM) of taxoids **SB-T-1214** and **SB-T-121303** against pancreatic cancer cell lines [174,176]

Taxoids	MiaPaca	CFPac	BXPC3	Panc 1
SB-T-1214	**0.92**	**0.83**	**1.04**	**3.68**
SB-T-121303	**0.68**	**0.89**	**3.03**	**22.6**

Further evaluation of **SB-T-1214** was performed against a xenograft model of pancreatic cancer. Thus, mice were inoculated with 1 million CFPAC pancreatic cancer cells that express high levels of MDR1 in each flank, and tumors were allowed to grow to a volume of approximately 100 mm^3, which was monitored and measured with calipers. Then, **SB-T-1214** (1 mg) solution was injected once in 3 days and 3 injections (q3d x 3) to the mice. As shown in Figure 2-5, the tumor volume was reduced, and the tumor was still under complete control after 8 weeks.[176]

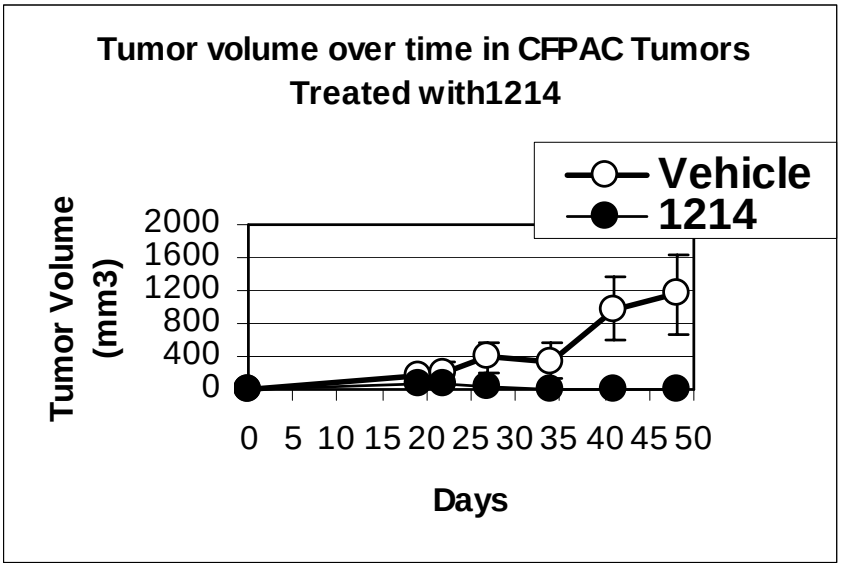


Figure 2-5. Tumor volume in CFPAC xenograft treated by **SB-T-1214** [176]

More *in vitro* and *in vivo* experiments as well as other biological results in detail are reported elsewhere.[172,174,178]

§ 2.1.2. Syntheses and Discussions

§ 2.1.2.1. Syntheses of second-generation taxoids SB-T-1213, SB-T-1103, and SB-T-1214

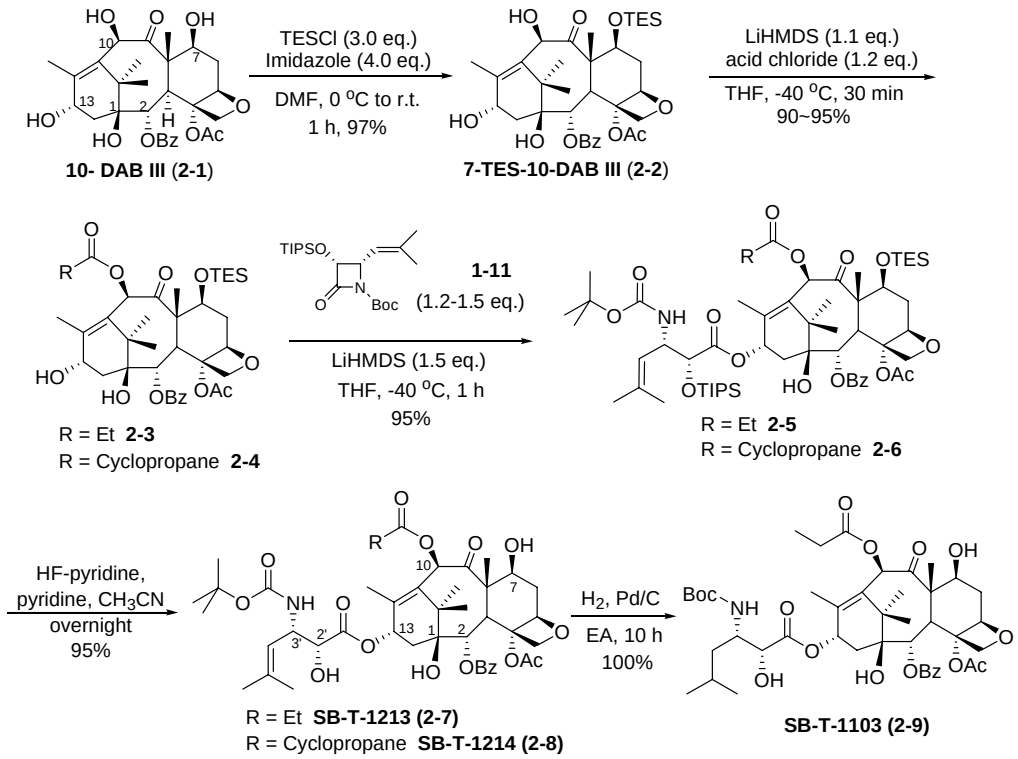
The syntheses of second- and/or third-generation taxoids started from the natural product 10-DAB III (**2-1**, Figure 1-6), which was introduced in Chapter 1. There are four hydroxyl groups in 10-DAB III, but their different chemical activities (order: C-7-OH > C-10-OH > C-13-OH > C-1-OH) enable selective modifications to distinguish them from each other.[179]

Thus, the C-7 hydroxyl group was converted to triethylsilyl (TES) ether by triethylsilyl chloride (TES-Cl, 3 eq.) and imidazole (4 eq.) in anhydrous dimethylformamide (DMF). Due to the high concentration and activity, the reaction usually gave excellent yield (> 95%) within 1 h at room temperature, and longer reaction time caused by-product formation, leading to lower yield.[170]

Next step was the modification at C-10 hydroxyl group (most active one among the remaining three alcohols), and different reagents, such as acid chloride, acid anhydride, free acid, or alkyl chloride, were applied depending on the target taxoids. For instance, cyclopropanecarbonyl chloride should be used for the synthesis of **SB-T-1214**. After 7-TES-10-DAB **2-2** was treated with lithium bis(trimethylsilyl)amide (LiHMDS) at -40 ^{o}C, addition of the corresponding acid chloride afforded 7-TES-10-cyclopropanecarbonyl-baccatin **2-3** in high yield.[170] Sometimes the coupling would not go smoothly due to the bulky acylation moiety, but another method should be considered which generates the C-10 ester after oxidation of C-13 alcohol.[174] It is also worth to mention that C-10 acylation can be done with the help of an Lewis acid when C-7 is a free hydroxyl group (*vide infra* in the synthesis of third-generation taxoid).[174]

The side chain at the C-13 hydroxyl position was installed through Ojima-Holton Coupling by using proper *β*-lactams (such as **1-11**), prepared in Chapter 1. The coupling reaction was carried out at low temperature and in the presence of LiHMDS to produce the protected taxoid in excellent (usually > 93%) yield. Finally, the silyl groups were removed globally by HF-pyridine to complete the semi-synthesis of second-generation taxoids, such as **SB-T-1213** and **SB-T-1214**. The overall yield for these 4 steps was around 85% (Scheme 2-1). According to the above protocol, **SB-T-1214** was prepared in a 3-gram scale.

The olefin moiety at the terminal of the side chain was reduced through hydrogenation over palladium on carbon to give **SB-T-1103**. During the reaction, the carbon-carbon double bond on the tetracyclic ring was not affected because of its steric hindrance.[170]



Scheme 2-1. Synthesis of second-generation taxoids[170]

§ 2.1.2.2. Crystal structure of SB-T-1214

For the research on taxoids, crystallographic studies play an important role by revealing the structural information of these complex molecules for both molecular structure determination and structure-activity relationship (SAR) investigation. The most important results include the crystal structures of docetaxel[180] and paclitaxel[181]. Some of other taxoid derivatives showing either comparable bioactivity, such as dibromo-7-*epi*-10-deacetylcephalomannine[182], or much less or even no anti-tumor activity, such as 2-carbamate taxol[183], 2-debenzoylpaclitaxel[184], 7-mesylpaclitaxel[185], and 10-deacetyl-7-*epi*taxol[186], have also been investigated by single-crystal X-ray analysis.

SB-T-1214 crystals suitable for X-ray analysis were obtained after slow evaporation of dichloromethane solution. The solvated crystal (later, it turned out to be **SB-T-1214**·$3CH_2Cl_2$) showed air sensitivity, and it readily collapsed from high quality colorless chunky plate into white powder, once taken out of the CH_2Cl_2-saturated atmosphere, presumably due to the loss of solvent molecules trapped in the crystal lattice. Therefore, the single-crystal sample had to be sealed in a capillary tube containing mother liquor for data collection. The structure elucidation was done by Dr Zhong Li in the laboratory of Dr Frank Fowler and Dr Joseph W. Lauher, Department of Chemistry, Stony Brook University.

In the crystal structure, **SB-T-1214** ($C_{45}H_{59}NO_{15}$) forms solvated crystals with a 1:3 ratio to dichloromethane, *i.e.*, the solvent molecule. All chiral centers were clearly assigned, which confirmed the validity of the β-Lactam Synthon Method. The taxoid

molecules stack in a $P2_12_12_1$ space group through the intermolecular hydrogen bonds, N3'—H3'---O5 and O1—H1---O4'.

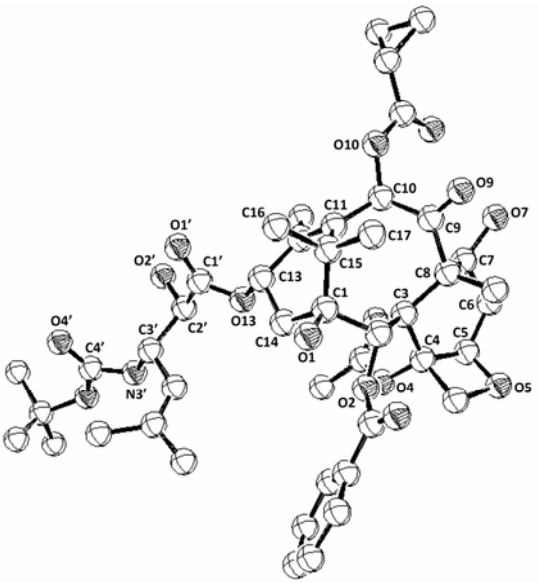


Figure 2-6. Crystal structure of **SB-T-1214**
(The atom-numbering scheme (important ones only) and 50% probability displacement ellipsoids are shown. The hydrogen atoms and disordered solvent molecules are not shown for clarity.)

The conformation of the core tetracyclic ring system in **SB-T-1214** is essentially identical to that of other taxoids, whose crystal structures were resolved. In addition, the C-13 side chain shows an extended conformation (Figure 2-6).[180] It should be noted that the specific geometries of the C-13 side chain in crystal are very similar to those of paclitaxel or docetaxel. Interestingly, the "hydrophobic collapse" conformation observed primarily in aqueous environment according to a series of NMR and modeling studies[181] is also found in this case, although the crystals were obtained from pure nonpolar environment. Such conformation is characterized by the presence of interactions between the C2-benzoyl and C4-acetyl groups of the core and C3'-isobutenyl group of the side chain. Similar conformation existing in a nonpolar environment has only been reported once in the crystal structure of 2-carbamate-taxol[183], which doesn't show comparable anti-tumor activity. Through the entire crystal lattice, hydrophobic channels form as the result of the combined hydrophobic-hydrophobic interactions due to the "hydrophobic collapse" conformation as well as the close contact between the C10-cyclopropylcarbonyl and N3'-*tert*-butoxycarbonyl groups of neighboring **SB-T-1214** molecules (Figure 2-7). The solvent molecules fill these cavities efficiently to stabilize the crystals in a disordered manner, with a ratio of 3 CH_2Cl_2 molecules per taxoid. However, since there are no obvious interactions between the solvent and solvate molecules, the trisolvate crystals show high air sensitivity.

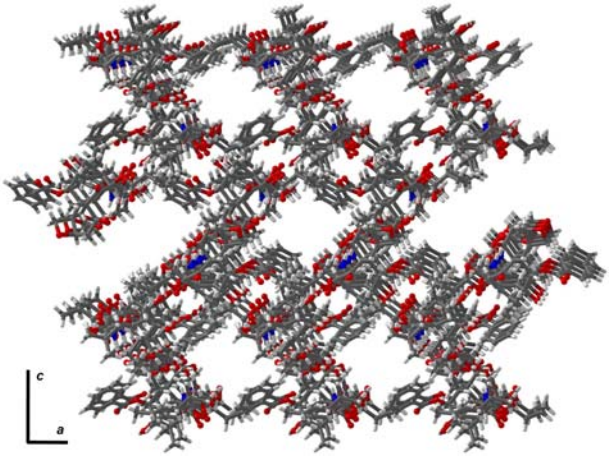


Figure 2-7. The solvent channel formed by the hydrophobic substituents of **SB-T-1214** (Disordered solvent molecules are not shown for clarity.)

The hydrogen-bonding property of the taxoid molecules is usually highly concerned due to the fact that it is directly related to the receptor binding mechanism. In the crystal structure of **SB-T-1214**, it is found that there are three pairs of intramolecular hydrogen bonds, O7—H7---O9, O7—H7---O10' and O2'—H2'---O1'. More importantly, there are two pairs of intermolecular hydrogen bonds, O1—H1---O4' and N3'—H3'---O5, which help the stacking of neighboring **SB-T-1214** molecules via a 2_1 axis along the *c* axis. These two intermolecular hydrogen bonds have been seen in neither of the crystal structures of paclitaxel and docetaxel.

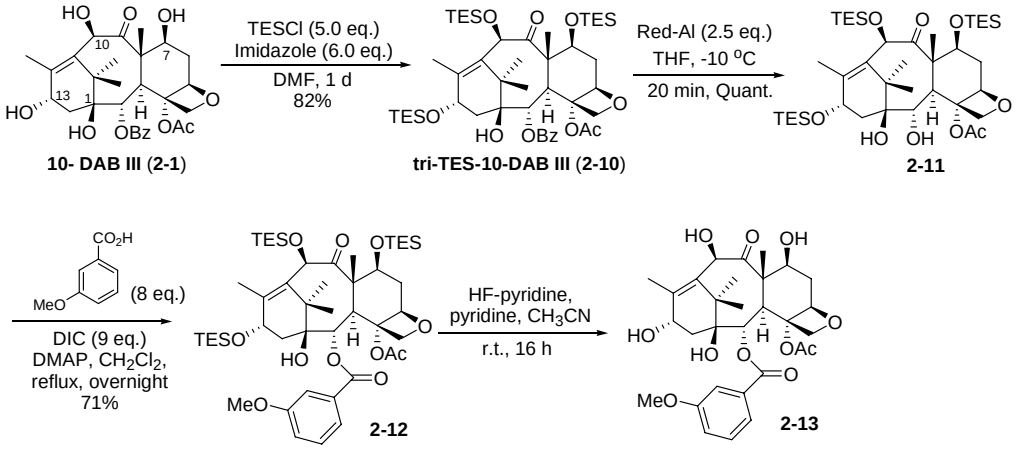
Although such a conformation adopted by **SB-T-1214** in crystalline state may not necessarily represent the effective one in the actual biological environment, it can still provide some useful information for the investigation of the receptor binding activities of this particular taxoid at the molecular level, as well as for the development of future taxoids.

§ 2.1.2.3. Synthesis of third-generation taxoids

Due to the modifications at the C-2 benzoate moiety, the preparation of third-generation taxoids contains more steps than those for second-generation taxoids. Several groups had studied the chemistry of the C-2 benzoate. Vyas and co-workers used Red-Al, *i.e.*, bis(methoxyethoxy)aluminum hydride, to cleave the benzoate through reduction of the ester,[187] while Holton[188] and Nicolaou[189] used a Grignard or a lithium reagent with baccatin-1,2-carbonate to re-install the benzoate. Other procedures were also reported including hydrolysis of taxoids by Triton B[175] or KOH[190] followed by re-acylation of the C-2 alcohol. Ojima and co-workers have successfully applied a modified Vyas's procedure for a tri-TES-baccatin to remove the C-2 benzoate[170], and the produced diol was reacted with *meta*-substituted benzoic acids in the presence of carbodiimide and DMAP to obtain the desired modified baccatin core.[172,174]

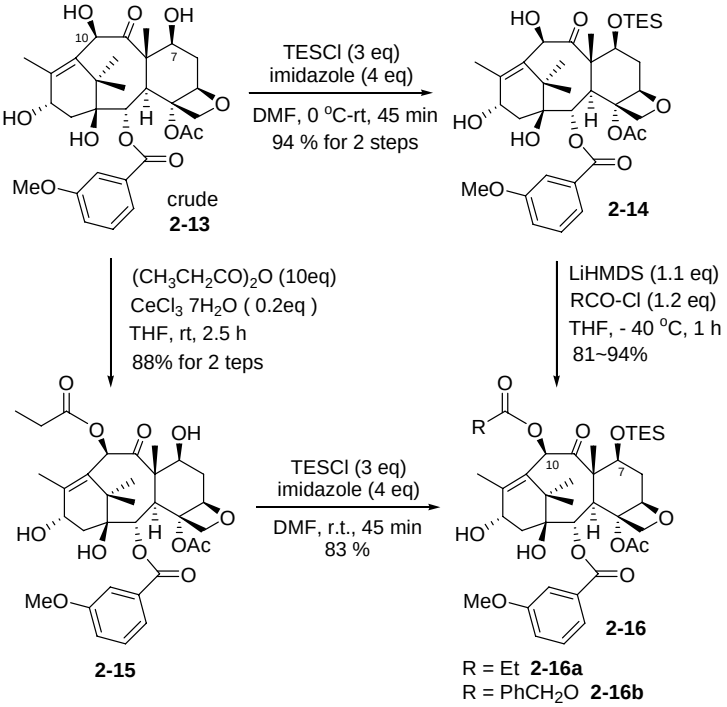
Scheme 2-2 shows the initial steps for the synthesis of those third-generation taxoids. After full protection of 10-DAB III with TES-Cl (5 eq.) and imidazole (6 eq.), C-2 benzoate was removed by Red-Al at low temperature to give 1,2-diol **2-11** in good

yield, which, after purification, was subsequently coupled to *m*-anisic acid at the C-2 position (Scheme 2-2). The three silyl groups were then removed to afford the crude tetraol **2-13**.[172,174]



Scheme 2-2. Modifications on C-2 benzoate [172,174]

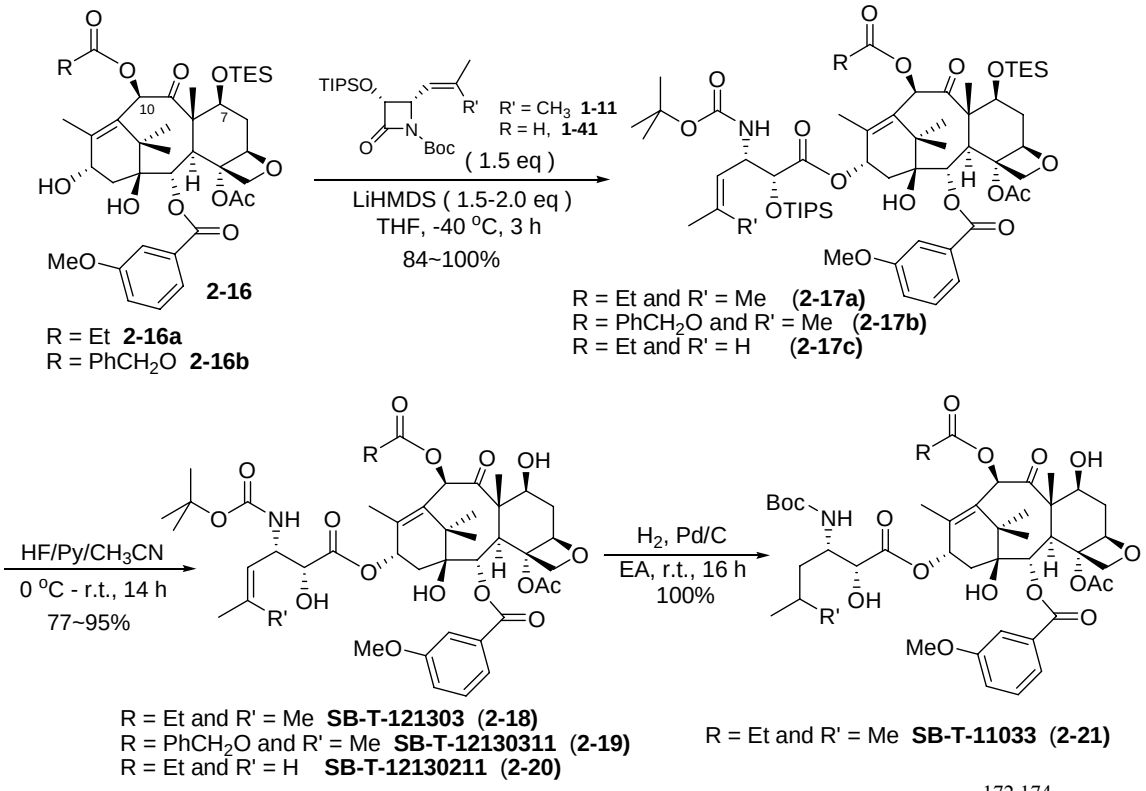
There were two different methods when **2-13** was in hand. The first choice was the same as that shown in Scheme 2-1, *i.e.*, C-7 protection prior to C-10 acylation (from **2-13** to **2-16** *via* **2-14** in Scheme 2-3). The second way was to acylate C-10 prior to protection at C-7 (from **2-13** to **2-16** *via* **2-15** in Scheme 2-3).[191]



Scheme 2-3. Two different protocols in the synthesis of third-generation taxoids[174]

Usually, the free hydroxyl group at C-7 is more reactive than that at C-10, but the latter would react first with anhydride in the presence of Lewis acid. Although the mechanism is not fully understood yet,[192] it is reasonable to assume that the coordination of oxygen of C-9 carbonyl to the metal center can activate the C-10 hydroxyl group because such activation and acceleration for the esterification of acyloin-type hydroxyl groups (even tertiary alcohols) in the presence of Lewis acid was observed previously[193].

Following the standard conditions,[172,174] the final third-generation taxoids were obtained after β-lactam coupling and deprotection of silyl groups. Syntheses of three of them are shown here, and **SB-T-121303** further was hydrogenated to give **SB-T-11033** bearing a saturated the side chain (Scheme 2-4).

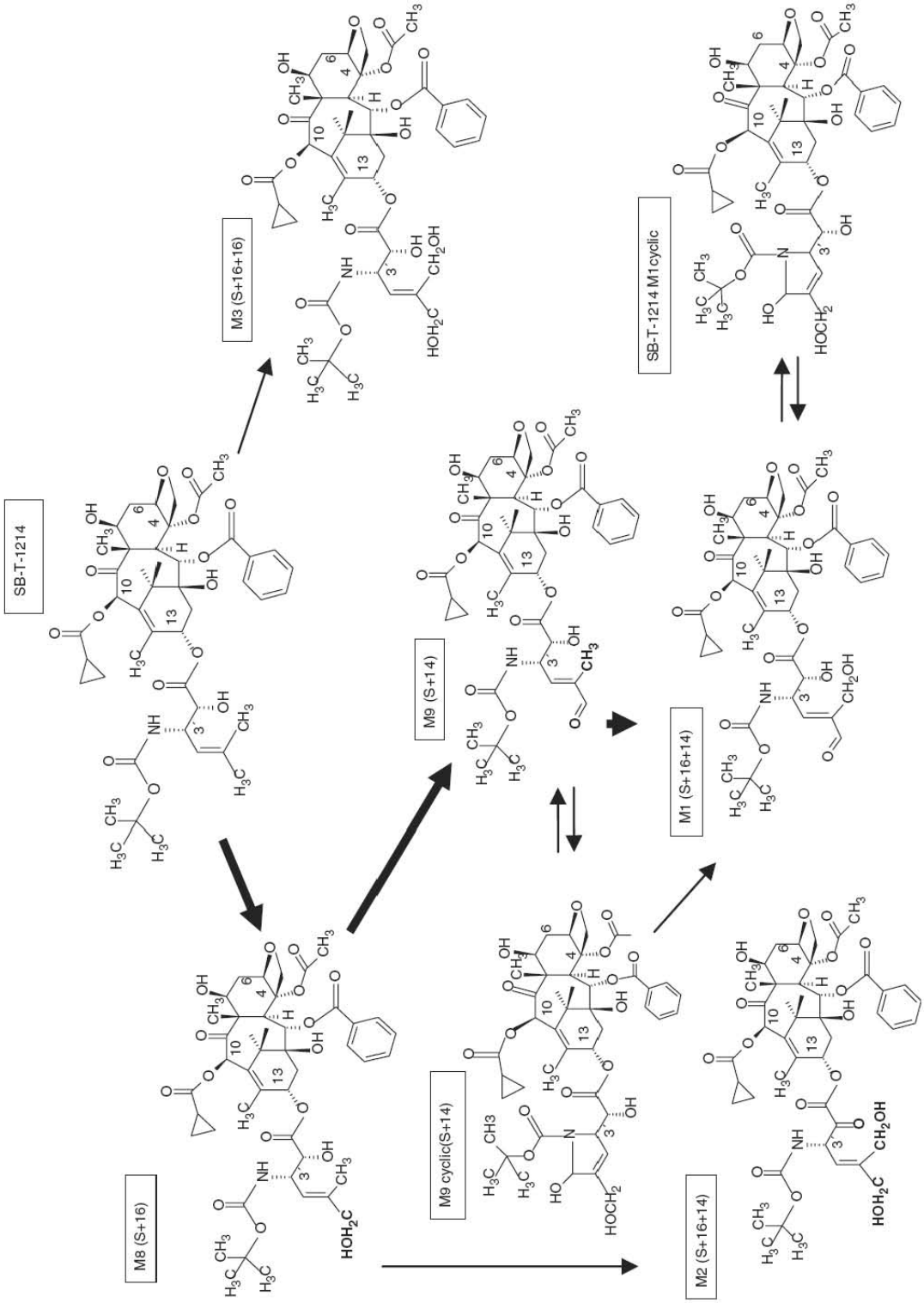


Scheme 2-4. Final steps in the preparation of third-generation taxoids[172,174]

§ 2.1.3. Metabolism of Second-Generation Taxoids

The CYP metabolism of some second-generation taxoids (such as **SB-T-1103**, **SB-T-1214** and **SB-T-1216**) was studied by Gut and co-workers.[194] These experiments were carried out with human, pig, minipig and rat liver microsomes, and the metabolites were purified by reverse-phase HPLC and analyzed by HPLC/MS/MS. Major pathways (big black arrows in Scheme 2-5) were disclosed, where **SB-T-1214** was taken as example. It was known that the *tert*-butyl group in the side chain of docetaxel was hydroxylated during metabolism. However, the active site switched to other moieties in second-generation taxoids. For instance, oxidative hydroxylation happened at the *iso*butenyl moiety in **SB-T-1214**, but not *tert*-butyl. After analyzing data in detail, they suggested that relatively slow metabolism of second-generation taxoids might contribute to their high antitumor activity.[194] In addition, they also confirmed that CYP3A played a

very important role in the metabolism of second-generation taxoids, while CYPs 1A2, 1B1, 2A6, 2C9 and 2E1 were totally inactive. Further study is underway.



Scheme 2-5. Metabolism of **SB-T-1214** in liver microsomes[194]

Based on the experiments mentioned above, Ojima designed several fluorine-containing second-generation taxoids, which took advantages of stable fluorine-carbon bond during metabolism caused by P450.[195] For example, if a difluorovinyl group replaces the *iso*butenyl moiety in **SB-T-1214**, then the new taxoid should have sturdy resistance towards metabolism, and consequently, these fluorinated taxoids should have even longer excretion time and should have much stronger tumor inhibition effect.

§2.2. Difluorovinyl-Containing Taxoids

§2.2.1. Fluorine in Organic and Medicinal Chemistry

As one of the smallest atoms, fluorine has been widely applied to chemistry, medicine, agriculture and material science. For example, chlorofluorocarbons (CFCs) are widely used in daily life as coolant in refrigerators worldwide. They are not flammable or corrosive, which replaced the previously used coolants ammonia and sulfur dioxide immediately in history. Although now CFCs are abandoned after their damage to the ozone layer was discovered, it changed daily life greatly. Teflon® and other perfluoronated material are stable in air and process very small surface tension, which enable them to be used as very good coatings on cooking utensils and industry, and even spaceship.[196] Many FDA-approved drugs contain fluorine(s), such as 5-fluorouracil (5-FU, anticancer), fluoxetine (Prozac®, antidepressant), atorvastatin (Lipitor®, cholesterol-lowering), and ciprofloxacin (Ciprobay®, antibacterial).[197] It was estimated that the ratio of the drugs that contains at least one fluorine atom is 20-25% among the pharmaceutical pipeline.[197,198]

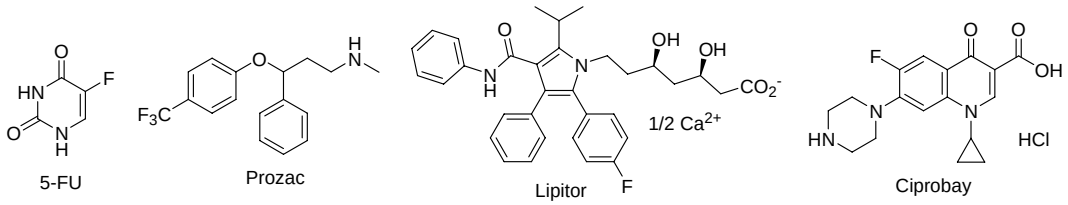


Figure 2-8. Some fluorinated drugs

In general, fluorine always shows up as mono-valent species. It can chemically take the place of hydrogen, hydroxyl (OH), amino (NH_2), methyl (CH_3) and many other groups. However, most fluorinated compounds are artificial, and such fluorinated compounds are rarely seen in nature while fluorine is holding the 13th position in the abundant element list of the Earth crust. This may be due to the high chemical activity of fluorine atom, which is usually considered as the strongest electron-negative element. It is so active that there is no free fluorine atom on the Earth.[196,197]

It has clearly shown that simple replacement of hydrogen by fluorine has profound effect on the property of the compound, for instance, uracil and 5-FU. The fluorine effects can be discussed mainly in terms of molecular structure and change in chemical and biological property. Also the stereoelectronic effect on molecular conformation is actively under investigation nowadays.

(1) Characteristics of C-F bond compared with C-H bond and molecular structure

Among the data shown in Table 2-3, C-F bond is a little bit longer than C-H bond mainly because fluorine atom is larger than hydrogen atom.[199] Actually, the shape and structure of the whole molecule do not change much after one or few fluorine atoms replacing hydrogen atoms.

Bond energy of C-F is higher than that of C-H, which means C-F is more stable than C-H. Significant changes happen with the dipole moment.[199] Not only much larger is the dipole moment of C-F than C-H, but also the direction of dipole moment is reversed.

Table 2-3. Comparison of C-F bond and C-H bond[199]

Bond	**C-H**	**C-F**	**C-Cl**	**C-O**
Bond length (Å)	1.08~1.10	1.26~1.40	1.79	1.42
Van der Waals (Å)	2.00 (CH_3)	2.25 (CF_3)		
Bond energy (KJ/mol)	397	439	340	380 (C-OH)
Dipole moment (Dy)	0.3	1.4		0.7

(2) Change in chemical and biological property

Introduction of fluorine may alter the physical, chemical and biological properties of the molecule. As discussed above, the stability of C-F bond and reversed dipole moment are crucial for those changes. Contrast to a C-H bond, a C-F bond is not easily oxidized, which increases stability of the fluorinated compounds in the metabolism. Thus, prolonged excretion time and enhanced effective time of the molecule are usually seen in drugs containing fluorine.[197]

The change in dipole moment causes profound effects on the molecule. It may change the basicity and acidity of the neighboring group through inductivity. More fluorine atoms usually imply stronger electron-withdrawing abilities.[200] This may change the compactness and density of the molecule, molecular cavity or molecular clusters, and modulate the interactions between molecules and their receptors. Eventually, the biological activity of the fluorinated molecule is altered.[198]

Moreover, trifluoromethyl and difluoromethyl groups have shown much higher lipophilic property than methyl group, which leads to increased membrane permeability.[201]

(3) Other benefits brought by fluorine

Introduction of fluorine may enhance the hydrophobic binding to protein. Some evidence implies the weak interactions between F (from C-F bond in substrate molecule) and carbon in carbonyl in the backbone of protein. This, as well as interaction between F and aromatic moiety on the side chain of aminoacid residue, strengthens the binding ability of fluorinated substrate. Consequently, fluorine(s) in bioactive molecules provide(s) a unique and valuable tool for *in vitro* and *in vivo* study by taking advantage of ^{19}F NMR.[202] Another advantage of *in vivo* ^{19}F NMR studies is that the living organism does not contain naturally occuring fluorinated organic compounds.[196]

Although fluorine chemistry has been developed for decades, it is still not easy to predict the real overall influence of fluorine substitution. Meanwhile, the H bond between hydrogen and fluorine (which is connected to a carbon) is yet another argument.

(4) Introduction of Fluorine to Organic Compound [203-205]

Several strategies have been explored to embed fluorine into organic compounds. The most direct way is the addition of F_2 to unsaturated double or triple bonds. The fluorine source could be XeF_2 as well. However, this method is not convenient to carry out due to extremely high reactivity of F_2 and elaborated reaction conditions. Addition of HF to alkene or alkyne is another method. Substitution reaction has been applied to the

synthesis of fluorinated compounds by using some fluoride, such as CsF, MnF_3, AgF_2, ClF_5, in place of for HF. In recent years, numerous N-F (such as *N*-fluorobenzenesulfonimide (NFSI)) and S-F (such as diethylaminosulfur trifluoride (DAST)) compounds have been developed, which provide versatile reaction conditions to install fluorine.

§2.2.2. Metabolism Inhibition by Difluorovinyl Taxoids

As discussed in **§2.1.1**, *iso*butenyl (dimethylvinyl) moiety is serving as an important moiety in some of second-generation taxoids, such as **SB-T-1213** and **SB-T-1214**. Although these novel taxoids exhibit very strong cytotoxicity, the methyl groups of the *iso*butenyl group may be metabolized into hydroxymethyl in liver microsomes mainly by cytochrome P450, which was mentioned in **§2.1.3**.[194] This may lead to undesirable bioavailability and low efficacy. In order to inhibit this major metabolism pathway, Ojima and co-workers designed and successfully synthesized several difluorovinyl-containing taxoids, such as **SB-T-12851** (Figure 2-9).

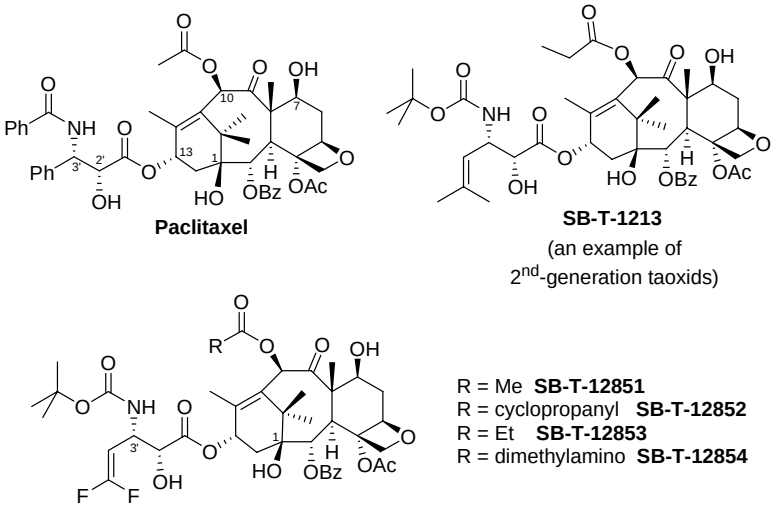


Figure 2-9. Several difluorovinyl-containing taxoids (adapted from Dr. Ojima's proposal)

The novel taxoids mentioned above have also shown very strong cytotoxicity, especially against drug-resistance cell lines. Usually 50-300 times higher potency than that of paclitaxel was observed, and data are shown in Table 2-4.[174] Other difluorovinyl-taxoids and their data were also reported.[174] Holding compatible activity to normal second-generation taxoids (i.e., **SB-T-1213**), these difluorovinyl taxoids are more promising regarding their pharmacokinetics although the metabolism study of these fluorinated taxoids is under investigation.

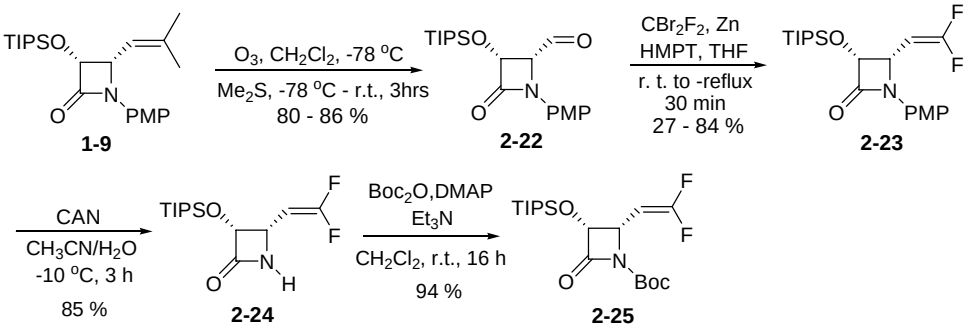
Table 2-4. *In vitro* cytotoxicity (IC_{50} nM) of C-3'-difluorovinyl-taxoids[174]

Taxoid	MCF7-S (breast)	MCF7-R (breast)	R/S
Paclitaxel	1.7	300	176
SB-T-1213	0.18	2.2	12
SB-T-12851	0.14	0.95	6.7
SB-T-12852	0.17	6.03	35
SB-T-12853	0.17	1.2	7.1
SB-T-12854	0.19	4.27	22

Besides the difluorovinyl taxoids, some other fluorinated taxoids containing difluoromethyl or trifluoromethyl moiety were also prepared. [206] Again, their potencies were extremely high.[206]

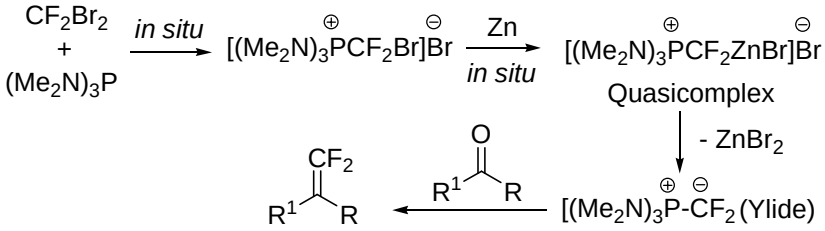
§2.2.3. Synthesis of Fluoro-β-Lactam and SB-T-12851

The synthesis of **SB-T-12851** required 3-difluorovinyl-β-lactam, which could be obtained from chiral material **1-9** after several transformations shown in Scheme 2-6. First, the olefin moiety in **1-9** was subjected to ozonolysis to provide aldehyde **2-22**, which was converted to fluorinated derivative **2-23** by CBr_2F_2, Zinc, and hexamethylphosphoroustriamide (HMPA) in THF.[207,208] After removal of the PMP group by cerium ammonium nitrate (CAN), the amide was protected by Boc_2O to complete the synthesis in excellent yield.



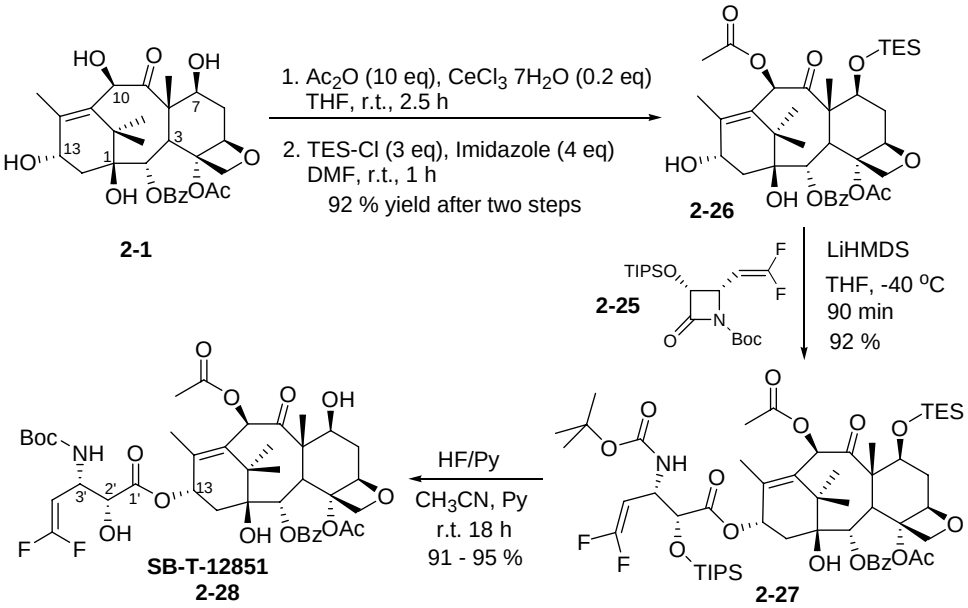
Scheme 2-6. Synthesis of chiral 3-difluorovinyl-β-lactam

The conversion of **2-22** to **2-23** was based on the formation of active species $[R_3PCF_2Br]^+Br^-$, which was generated by the reaction between HMPA and CBr_2F_2 *in situ*. A highly exothermic reaction occurred immediately due to the formation of the quasi-complex with zinc, which rapidly collapsed into ylide intermediate. The ylide then attacked the aldehyde to afford the desired fluorinated compound (Scheme 2-7).[209]



Scheme 2-7. Mechanism of 1,1-difluoroolefin formation[209]

With the chiral 3-difluorovinyl-β-lactam **2-25** in hand, the synthesis of **SB-T-12851** (**2-28**) began from baccatin core **2-1**, which was modified according to standard procedures described previously (§2.1.2).[170] Finally, **SB-T-12851** was obtained in 3-gram scale after 4 steps in 80% overall yield.



Scheme 2-8. Synthesis of **SB-T-12851**

§ 2.3. Conclusion

Paclitaxel and docetaxel are ineffective against multidrug-resistant (MDR) cancers. After extensive structure-activity relationship (SAR) studies on paclitaxel and docetaxel, highly active second- and third-generation taxoids have been successfully developed, in which the functional groups at C-2, C-10, C-3' and the C-3'N positions are modified. Compared to paclitaxel and docetaxel, most of these novel taxoids demonstrate much higher potency, especially against MDR tumor cell lines (including breast and pancreatic cancer cell lines as well as ovarian cancer cell lines bearing point mutations in the paclitaxel bingding site), in which hundreds to thousands of times greater cytotoxicity are observed. As a matter of fact, some third-generation taxoids showed equally potent against drug-sensitive and MDR cell lines. **SB-T-1213**, **SB-T-1214**, and **SB-T-121303** are three representatives among them. *In vitro* assays also showed that these new taxoids promote the polymerization of tubulins faster and stabilize the microtubules much more strongly than paclitaxel. *In vivo* studies of these novel taxoids were also done with SCID or nude mice bearing different MDR tumor xenografts, such as DLD-1 (colon) and CFPAC-1 (Pancreas). In these experiments, tumor sizes were under control and eventually the tumor agglomerate disappeared within 6-8 weeks.[174]

Metabolism studies on some second-generation taxoids, including **SB-T-1214** and **SB-T-1103**, led to the design of difluorovinyl taxoids, which exhibit similar biological activities as non-fluorinated taxoids but expected to be superior in pharmacokinetics.

Continuous efforts on the development of paclitaxel have resulted in highly potent taxane-based anticancer agents with much better biological activities. Together with the advances in drug-resistance mechanism study and tumor-targeting drug delivery strategy, these novel taxoids, as well as paclitaxel and docetaxel, should have broader applications to the future cancer chemotherapy.

§ 2.4. Experimental Section

General Methods: ^{1}H, ^{13}C and ^{19}F NMR spectra were measured on a Varian 300 or 400 MHz NMR spectrometer. The melting points were measured on a "Uni-melt" capillary melting point apparatus from Arthur H. Thomas Company, Inc., which were not corrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. GC-MS analyses were performed on an Agilent 6890 Series GC system equipped with the HP-5HS capillary column, (50 m X 0.25 mm, 0.25 um) and with the Agilent 5973 network mass selective detector. LC-MS analyses were carried out on an Agilent 1100 Series Liquid Chromatograph Mass Spectrometer (Agilent Technologies, Palo Alto, CA). TLC analyses were performed on Merck DC-alufolien with Kieselgel 60F-254, and were visualized with either of the following methods: UV light, iodine chamber, 10% sulfuric acid in ethanol, 10% ceric sulfate and 15% sulfuric acid in water, and 10% phosphomolybdic acid in ethanol. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). Chemical purity was determined with a Waters HPLC assembly consisting of dual Waters 515 HPLC pumps, a PC workstation running Millennium 32, and a Waters 996 PDA detector, using a Curosil-B column from Phenomenex, employing CH_3CN/water as the solvent system with a flow rate of 1 mL/min, or the Agilent HPLC system. Chiral HPLC analysis for the determination of enantiomeric excess was carried out with a Waters HPLC assembly, comprising Waters M45 solvent delivery system, Waters Model 680 gradient controller, Water M440 detector (at 254 nm) equipped with a Spectra Physics Model SP4270 integrator. The system uses a Daicel-Chiral OD chiral column (25 x 0.46 cm *i.d.*), employing hexane/2-propanol (90/10 or 95/5) as the mobile phase with a flow rate of 1.0 ml/min. IR spectra were measured on a Shimadzu FTIR-8400s spectrophotometer. High-resolution mass spectrametric analyses were conducted at the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

Chemicals and Materials: The chemicals were purchased from Sigma-Aldrich or Fischer (including Acros) Company. 10-Deacetyl baccatin III (DAB) was donated by Indena, SpA, Italy. All solvents, right before use, were distilled under nitrogen or argon atmosphere unless mentioned. Thus, dichloromethane (DCM) and methanol were dried over calcium hydride. Toluene and benzene were dried over sodium metal. Diethyl ether and tetrahydrofuran (THF) were dried over sodium with benzophenone as the indicator. Toluene, THF, diethyl ether, and DCM were also purified through PURE SOLVTM from Innovative technology Inc., and used without further purification. Anhydrous DMF was purchased from EMD, and used as it is. The glasses were dried in oven at 110 ^{o}C and allowed to cool to room temperature in a desiccator over "Drierite" (calcium sulfate) before use.

Different biological assays were carried out in Dr. Ralph Bernacki's lab, Roswell Park Cancer Institute, or. by Jon Mallen-St. Clair and Jennifer Curato in Dr Dafna Bar-Sagi's lab, Department of Molecular Genetics and Microbiology, Stony Brook University, or Dr. Gut[210] in National Institute of Public Health, Czech Republic, respectively, which have been mentioned above.

7-Triethylsilyl-10-deacetylbaccatin III (2-2):[170]

To a solution of 10-deacetylbaccatin III (**2-1**) (1.58 g, 2.91 mmol) and imidazole (790 mg, 11.6 mmol) in DMF (32 mL) was added chlorotriethylsilane (TES-Cl, 1.47 mL, 8.73 mmol) dropwise at 0 ^{o}C. The mixture was stirred for 2 h at room temperature and the reaction was quenched by saturated ammonium chloride (75 mL) and water (25 mL). The reaction mixture was extracted by EtOAc (100 mL x 5), and the organic layers were combined, washed with H_2O (50 mL x 3), brine (50 mL), dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified on a silica gel column using hexanes:EtOAc (1:1) as eluant to give **2-2** as a white solid (1.85 g, 97% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (m, 6 H), 0.94 (m, 9 H), 1.08 (s, 6 H), 1.59 (d, J = 2.5 Hz, 1H), 1.73 (s, 3H), 1.90 (dt, 1 H) (H_{6a}), 2.05 (d, J = 4.8 Hz, 1H), 2.08 (s, 3 H), 2.24 (s, 1 H), 2.28 (s, 3 H) (OAc), 2.48 (ddd, 1 H) (H_{6b}), 3.95 (d, J = 7.1 Hz, 1 H) (H_3), 4.16 (d, J = 8.3 Hz, 1 H) (H_{20a}), 4.25 (s, 1 H), 4.31 (d, J = 8.3 Hz, 1 H) (H_{20b}), 4.40 (dd, J = 6.4, 10.5 Hz, 1 H) (H_7), 4.85 (t, 1 H) (H_{13}), 4.95 (d, J = 8.0 Hz, 1 H) (H_5), 5.17 (s, 1 H) (H_{10}), 5.60 (d, J = 7.0 Hz, 1 H) (H_2), 7.47 (t, J = 7.5 Hz, 2 H), 7.60 (t, J = 7.5 Hz, 1 H), 8.10 (d, J = 7.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 75.0 MHz) δ 5.1, 6.7, 9.9, 15.1, 19.5, 22.6, 26.8, 37.2, 38.6, 42.7, 47.0, 57.9, 67.9, 72.9, 74.6, 74.8, 76.5, 78.8, 80.7, 84.2, 87.6, 128.6, 129.4, 130.0, 133.6, 135.1, 141.8, 167.0, 170.7, 210.3. All data are consistent with the reported values.[170]

7-Triethylsilyl-10-deacetyl-10-propanoylbaccatin III (2-3):[170]

To a solution of **2-2** (222 mg, 0.34 mmol) in THF (3 mL) was added 1.0 M LiHMDS (0.40 mL, 0.41 mmol) at -40 °C. After the mixture was stirred for 5 min, propanoyl chloride (0.030 mL, 0.41 mmol) was added dropwise. The mixture was then allowed to stir for 30 min at -40 °C. The reaction was quenched with saturated aqueous NH_4Cl solution, and extracted twice with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Purification was done by column chromatography on silica gel (hexanes/EtOAc = 3/1) to give 7-triethylsilyl-10-deacetyl-10-propanoylbaccatin III (**2-3**) as a white solid (237 mg, 98% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, J = 7.8 Hz, 6 H), 0.89 (t, J = 7.8 Hz, 9 H), 1.15 (s, 3 H), 1.26 (s, 3 H), 1.36 (s, 9 H), 1.68 (s, 3 H), 1.77 (s, 6 H), 1.90 (s, 4 H), 2.25 (s, 3 H), 2.36 (s, 3 H), 2.43 (m, 2 H), 3.82 (d, J = 7.0 Hz, 1 H), 4.20 (d, J = 8.5 Hz, 1 H), 4.31 (d, J = 8.5 Hz, 1 H), 4.43 (m, 1 H), 4.78 (m, 1 H), 4.96 (d, J = 8.1 Hz, 1 H), 5.32 (d, J = 8.3 Hz, 1 H), 5.67 (d, J = 7.0 Hz, 1 H), 6.17 (t, J = 8.6 Hz, 1 H), 6.51 (s, 1 H), 7.45 (m, 2 H), 7.59 (m, 1 H), 8.10 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.2, 6.7, 9.2, 9.9, 14.9, 20.1, 22.6, 26.7, 27.6, 37.2, 38.3, 42.7, 58.6, 67.8, 72.3, 74.7, 78.7, 80.0, 84.2, 128.5, 129.4, 130.0, 132.6, 133.5, 143.9, 167.8, 170.7, 174.5, 202.3. All data are consistent with the reported values.[170]

7-Triethylsilyl-10-deacetyl-10-cyclopropanecarbonylbaccatin III (2-4):[170]

Following the protocol for the synthesis of **2-3**, the title compound **2-4** was obtained (1.96 g, 90% yield) as white powder from **2-2** (1.85 g, 2.81 mmol), 1.0 M LiHMDS (3.09 mL, 3.09 mmol), and cyclopropanecarbonyl chloride (0.29 mL, 3.09 mmol) in THF (52 mL): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.90 (m, 9 H), 1.00

(s, 3 H), 1.16 (s, 3 H), 1.64 (s, 3 H), 1.73 (m. 1 H), 1.82 (m, 1 H) (H_{6a}), 2.14 (s, 3 H), 2.23 (m, 2 H), 2.24 (s, 3 H) (OAc), 2.49 (m, 1 H) (H_{6b}), 3.84 (d, J = 6.9 Hz, 1 H) (H_3), 4.11 (d, J = 8.0 Hz, 1 H) (H_{20a}), 4.26 (d, J = 8.2 Hz, 1 H) (H_{20b}), 4.45 (dd, J = 6.7, 10.3 Hz, 1 H) (H_5), 4.77 (t, J = 7.8 Hz, 1 H) (H_{13}), 4.92 (d, J = 8.6 Hz, 1 H) (H_5), 5.59 (d, J = 7.0 Hz, 1 H) (H_2), 6.43 (s, 1 H) (H_{10}), 7.43 (t, J = 7.4 Hz, 2 H), 7.56 (t, J = 7.6 Hz, 1 H), 8.05 (d, J = 7.3 Hz, 2 H). All data are consistent with the reported values.[170]

7-Triethylsilyl-2'-tri*iso*propylsilyl-3'-dephenyl-3'-(2-methylprop-1-enyl)-10-deacetyl-10-propanoyldocetaxel (2-5):[170]

To a solution of **2-3** (4.41.g, 6.16 mmol) and β-lactam **1-11** (3.68 g, 9.25 mmol) in THF (300 mL) was added LiHMDS (9.25 mL, 9.25 mmol) at -40 ^oC. The solution was stirred at -40 ^oC for 1 h and the reaction was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layers were combined and dried over anhydrous $MgSO_4$ and concentrated after filtration. The residue was purified on a silica gel column using Hexanes/EtOAc (8/1) as the eluant to afford **2-5** (6.53 g, 95% yield) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.91 (m, 9 H), 1.11 (m, 21 H), 1.17 (s, 3 H), 1.20 (s, 3 H), 1.22 (s, 3 H), 1.33 (s, 9 H), 1.69 (s, 3 H), 1.75 (s, 3 H), 1.79 (s, 3 H), 1.87 (m, 1 H) (H_{6a}), 2.01 (s, 3 H), 2.36 (s, 3 H) (OAc), 2.46 (m, 3 H), 3.84 (d, J = 7.0 Hz, 1 H) (H_3), 4.18 (d, J = 8.3 Hz, 1 H) (H_{20a}), 4.29 (d, J = 8.4 Hz, 1 H) (H_{20b}), 4.42 (d, J = 2.2 Hz, 1 H), 4.47 (dd, J = 10.4, 6.6 Hz, 1 H) (H_7), 4.80 (m, 2 H), 4.93 (d, J = 8.8 Hz, 1 H) (H_5), 5.32 (d, J = 8.2 Hz, 1 H), 5.68 (d, J = 7.0 Hz, 1 H) (H_2), 6.08 (t, J = 8.8 Hz, 1 H), 6.48 (s, 1 H) (H_{10}), 7.45 (t, J = 7.5 Hz, 2 H), 7.59 (t, J = 7.3 Hz, 1 H), 8.09 (d, J = 7.5 Hz, 2 H). All data are consistent with the reported values.[170]

7-Triethylsilyl-2'-tri*iso*propylsilyl-3'-dephenyl-3'-(2-methylprop-1-enyl)-10-cyclopropanecarbonyldocetaxel (2-6):[170]

Similar to that of **2-5**, compound **2-6** was obtained (2.24 g, 95% yield) as white solid from **2-4** (1.85 g, 2.55 mmol), **1-11** (1.59 g, 4.00 mmol) and LiHMDS (4 mmol) in THF (250 mL): ^1H NMR (300 MHz, $CDCl_3$): δ 0.55 (m, 6 H), 0.91 (m, 11 H, TES and cyclopropane (2)), 1.10 (m, 21 H, TIPS), 1.19 (s, 3 H), 1.24 (s, 3 H), 1.20-1.25 (m, 2 H, cyclopropane (2)), 1.34 (s, 9 H), 1.68 (s, 3 H), 1.74 (s, 3 H), 1.75 (s, 3 H), 1.7-1.8 (m, 2 H, C-14 (1) and cyclopropane (1)), 1.89 (m, 1 H, C-6 (1)), 2.00 (s, 3 H), 2.35 (s, 3 H), 2.41 (m, 3 H, C-14 (1), C-6 (1) and OH), 3.84 (d, J = 7.2 Hz, 1 H), 4.20 (d, J = 8.4 Hz, 1 H), 4.29 (d, J = 8.4 Hz, 1 H), 4.47 (m, 2 H), 4.77-4.87 (m, 2 H), 4.92 (d, J = 8.4 Hz, 1 H), 5.33 (d, J = 8.7 Hz, 1 H), 5.68 (d, J = 7.2 Hz, 1 H), 6.09 (t, J = 8.4 Hz, 1 H), 6.48 (s, 1 H), 7.46 (t, J = 7.5 Hz, 2 H), 7.60 (t, J = 7.2 Hz, 1 H), 8.10 (d, J = 7.2 Hz, 2 H). All data are consistent with the reported values.[170]

3'-Dephenyl-3'-(2-methyl-2-propenyl)-10-deacetyl-10-propanoyldocetaxel (2-7, SB-T-1213):[170]

To a solution of **2-5** (301 mg, 0.356 mmol) in CH_3CN (3 mL) and pyridine (3 mL) was added HF-pyridine (3 mL) at 0 ^oC, and the temperature was slowly warmed up to room temperature. After stirring overnight, the reaction was quenched with saturated aqueous $NaHCO_3$ solution and diluted with EtOAc, washed with saturated aqueous $CuSO_4$ solution, H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and

filtered. After the solvent was removed, residue was purified on a silica gel column using hexanes/EtOAc (1/1) as the eluant to afford **SB-T-1213** (**2-7)** (215 mg, 95 % yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (s, 3 H), 1.13-1.18 (m, 6 H), 1.28 (s, 9 H), 1.60 (s, 3 H), 1.69 (br s, 6 H), 1.72 (m, 1 H), 1.83 (s, 3 H), 2.29 (s, 3 H), 2.31 (s, 2 H), 2.44 (m, 3 H), 3.38 (br s, 1 H), 3.74 (d, *J* = 6.9 Hz, 1 H), 4.10 (d, *J* = 8.1 Hz, 1 H), 4.13 (br s, 1 H), 4.22 (d, *J* = 8.1 Hz, 1 H), 4.33 (dd, *J* = 10.1, 7.5 Hz, 1 H), 4.67 (m, 2 H), 4.88 (d, *J* = 9.3 Hz, 1 H), 5.23 (d, *J* = 8.4 Hz, 1 H), 5.59 (d, *J* = 6.9 Hz, 1 H), 6.06 (m, 1 H), 6.24 (s, 1 H), 7.37 (t, *J* = 6.9 Hz, 2 H), 7.51 (t, *J* = 7.2 Hz, 1 H), 8.01 (d, *J* = 7.2 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 9.5, 14.9, 18.5, 21.8, 22.3, 25.7, 26.6, 27.5, 28.2, 35.5, 43.1, 45.6, 51.6, 55.5, 58.5, 72.1, 72.3, 73.7, 75.0, 75.4, 76.4, 76.5, 77.0, 77.5, 79.1, 79.9, 81.0, 84.3, 120.6, 128.6, 129.2, 130.1, 132.9, 133.6, 137.8, 142.4, 155.4, 166.9, 170.1, 173.0, 174.6, 203.8. All data are consistent with the reported values.[170]

3'-Dephenyl-3'-(2-methyl-2-propenyl)-10-deacetyl-10-cyclopropanecarbonyl-docetaxel (2-8, SB-T-1214):[170]

Following the protocol for the synthesis of **SB-T-1213**, **SB-T-1214** was obtained (1.68 g, 99% yield) as white powder from **2-6** (2.24 g, 2.0 mmol): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98-1.05 (m, 2 H), 1.13-1.21 (m, 8 H, C-16, C-17 and cyclopropane (2)), 1.37 (s, 9 H), 1.42 (m, 1 H), 1.69-1.90 (m, 13 H, C-19, C-5' (6), cyclopropane (1), C-6 (1), C-14 (1) and OH), 2.03 (s, 3 H, C-18), 2.37 (br s, 4 H), 2.55 (m, 1 H), 2.63 (d, *J* = 3.9 Hz, 1 H), 3.83 (d, *J* = 7.2 Hz, 1 H), 4.21 (m, 2 H), 4.33 (d, *J* = 8.1 Hz, 1 H), 4.43 (m, 1 H), 4.76 (t, *J* = 6.0 Hz, 1 H), 4.85 (d, *J* = 8.4 Hz, 1 H), 4.97 (d, *J* = 9.3 Hz, 1 H), 5.33 (d, *J* = 8.4 Hz, 1 H), 5.68 (d, *J* = 7.2 Hz, 1 H), 6.19 (t, *J* = 7.8 Hz, 1 H), 6.32 (s, 1 H), 7.49 (t, *J* = 8.1 Hz, 2 H), 7.61 (t, *J* = 7.2 Hz, 1 H), 8.11 (d, *J* = 8.4 Hz, 2 H). All data are consistent with the reported values.[170] The purity of **SB-T-1214** was checked by HPLC: 98.0% purity was observed when Phenomenex C-18 column 10 μL (CH_3CN/H_2O = 60/40, 0.5 mL/min, retention time: 12.86 min) was used, and 98.4% purity was seen with Waters C-18 column (CH_3CN/H_2O = 60/40, 0.5 mL/min, retention time: 8.93 min).

3'-Dephenyl-3'-(2-methyl-2-propenyl)-10-deacetyl-10-pentanoyldocetaxel (2-9, SB-T-1103):[170]

To a flask with 10% Pd/C (1.38 g) was added a solution of **2-7** (4.12 g, 4.88 mmol) in 200 mL EtOAc with several drops of MeOH under hydrogen atmosphere. The mixture was stirred overnight and reaction mixture was filtered through celite and purified on a silica gel column using hexanes/EtOAc (1/1) as the eluant to afford **SB-T-1103** (4.14 g, 100% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (m, 6 H), 1.13 (s, 3 H), 1.22-1.27 (m, 6 H), 1.30 (s, 9 H), 1.63 (s, 3 H), 1.73 (s, 2 H), 1.82 (m, 1 H), 1.88 (s, 3 H), 2.36 (s, 3 H), 2.40 (s, 3 H), 2.46 (m, 1 H), 2.49 (m, 2 H), 3.25 (br s, 1 H), 3.79 (d, *J* = 6.9 Hz, 1 H), 4.09 (d, *J* = 8.1 Hz, 1 H), 4.16 (br s, 1 H), 4.27 (d, *J* = 8.1 Hz, 1 H), 4.38 (dd, *J* = 10.2, 6.6 Hz, 1 H), 4.57 (d, *J* = 9.6 Hz, 1 H), 4.94 (d, *J* = 8.1 Hz, 1 H), 5.64 (d, *J* = 7.2 Hz, 1 H), 6.13 (m, 1 H), 6.30 (s, 1 H), 7.43 (t, *J* = 8.1 Hz, 2 H), 7.56 (t, *J* = 7.2 Hz, 1 H), 8.08 (d, *J* = 8.1 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 9.5, 14.9, 21.8, 21.9, 22.5, 23.2, 24.6, 26.5, 27.5, 28.1, 29.6, 35.5, 41.2, 43.1, 45.6, 51.3, 58.5, 72.1, 72.6, 73.0, 75.1, 75.4, 76.4, 76.5, 77.0, 77.5, 79.1, 79.7, 81.0, 84.4, 128.6, 129.2, 130.1, 132.9, 133.6,

142.4, 155.5, 166.9, 169.9, 173.9, 174.6, 203.8. All data are consistent with the reported values.[170]

7,10,13-Tris(triethylsilyl)-10-deacetylbaccatin III (2-10):[172,211]

To a solution of 10-DAB III (900 mg, 1.65 mmol) and imidazole (675 mg, 9.91 mmol) in dry DMF (3 mL) was added chlorotriethylsilane (1.386 mL, 8.260 mmol) dropwise via syringe at room temperature. The mixture was stirred for 1 day at room temperature and diluted with EtOAc (150 mL). The reaction mixture was then washed with water (20 mL x 3), brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified on a silica gel column (hexanes:EtOAc = 10:1) to give **2-10** (1.20 g, 82% yield) as a white solid: mp 187-189 ^oC; $[\alpha]_D^{20}$ -38.8 (*c* 0.28, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.65 (m, 18 H), 0.99 (m, 27 H), 1.11 (s, 3 H), 1.18 (s, 3 H), 1.64 (s, 3 H), 1.87 (m, 1 H), 1.97 (s, 3 H), 2.08 (dd, *J* = 15.2, 8.4 Hz, 1 H), 2.21 (dd, *J* = 15.2, 8.4 Hz, 1 H), 2.28 (s, 3 H), 2.51 (m, 1 H), 3.84 (d, *J* = 6.8 Hz, 1 H), 4.13 (d, *J* = 8.4 Hz, 1 H), 4.27 (d, *J* = 8.4 Hz, 1 H), 4.40 (dd, *J* = 10.4, 6.4 Hz, 1 H), 4.92 (m, 2 H), 5.19 (s, 1 H), 5.61 (d, *J* = 7.2 Hz, 1 H), 7.44 (t, *J* = 7.6 Hz, 2 H), 7.57 (t, *J* = 7.6 Hz, 1 H), 8.08 (d, *J* = 7.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.7, 5.2, 5.9, 6.9, 10.4, 14.8, 20.5, 22.4, 26.3, 37.3, 19.8, 42.4, 46.8, 58.2, 68.3, 72.6, 75.4, 75.7, 76.6, 79.5, 80.7, 83.9, 128.5, 129.6, 130.0, 133.4, 135.7, 139.3, 167.1, 169.7, 205.6. All data are in agreement with literature values.[172]

7,10,13-Tris(triethylsilyl)-2-debenzoyl-10-deacetylbaccatin III (2-11):[172]

To a solution of **2-10** (870 mg, 0.98 mmol) in dry THF (45 mL) at -10 ^oC was added dropwise a solution of Red-Al in toluene (65% wt, 0.59 mL, 1.96 mmol), and the reaction mixture was stirred for 20 min at -10 ^oC. The reaction was quenched with aqueous saturated NH_4Cl solution (20 mL), and the aqueous layer was extracted with EtOAc (50 mL x 3). The combined extracts were then dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexanes:EtOAc = 8:1 followed by 3:1) to afford **2-11** (768 mg, 100% yield) as a white solid: mp 68-70 ^oC; $[\alpha]_D^{20}$ -35.6 (*c* 0.87, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.57 (m, 18 H), 0.94 (m, 27 H), 1.11 (s, 3 H), 1.55 (s, 3 H), 1.87 (m, 1 H), 1.88 (s, 3 H), 1.94 (m, 1 H), 2.00 (m, 1 H), 2.12 (s, 3 H), 2.47 (m, 1 H), 3.42 (d, *J* = 6.6 Hz, 1 H), 3.80 (d, *J* = 6.6 Hz, 1 H), 4.31 (dd, *J* = 10.5, 6.6 Hz, 1 H), 4.50 (d, *J* = 9.0 Hz, 1 H), 4.57 (d, *J* = 9.0 Hz, 1 H), 4.63 (s, 1 H), 4.89 (d, *J* = 8.4 Hz, 1 H), 4.91 (m, 1 H), 5.08 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.7, 5.1, 5.8, 6.7, 6.8, 10.5, 14.4, 20.5, 22.3, 37.3, 40.3, 42.5, 58.1, 65.0, 66.3, 72.6, 74.6, 75.7, 77.9, 78.5, 81.9, 83.7, 126.8, 127.4, 128.4, 135.9, 138.9, 169.6, 206.3. All data are in agreement with literature values.[172]

2-Debenzoyl-2-(3-methoxybenzoyl)-7,10,13-tris(triethylsilyl)-10-deacetyl-baccatin III (2-12):[172]

To a solution of **2-11** (400 mg, 0.56 mmol), *m*-anisic acid (660 mg, 4.36 mmol) and 4-dimethylaminopyridine (DMAP, 600 mg, 4.92 mmol) in CH_2Cl_2 (5 mL) was added di*iso*propylcarbodiimide (DIC, 0.76 mL, 4.92 mmol), and the mixture was refluxed overnight. The reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (10 mL), H_2O (10 mL) and brine (10 mL). The

organic lawyer was dried over anhydrous $MgSO_4$. After the solvent was removed under reduced pressure, the residue was purified on a silica gel column (hexanes:EtOAc = 20:1) to afford **2-12** (330 mg, 71% yield) as a white solid: mp 201-202 ^oC; $[\alpha]_D^{20}$ -40.6 (*c* 0.32, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (m, 18 H), 0.98 (m, 27 H), 1.11 (s, 3 H), 1.18 (s, 3 H), 1.57 (bs, 1 H), 1.62 (s, 3 H), 1.85 (m, 1 H), 1.96 (s, 3 H), 2.14 (m, 2 H), 2.26 (s, 3 H), 2.50 (m, 1 H), 3.83 (m, 4 H), 4.13 (d, *J* = 7.9 Hz, 1 H), 4.30 (d, *J* = 8.1 Hz, 1 H), 4.39 (dd, *J* = 10.4, 6.9 Hz, 1 H), 4.92 (m, 2 H), 5.17 (s, 1 H), 5.59 (d, *J* = 6.9 Hz, 1 H), 7.10 (m, 1 H), 7.35 (t, *J* = 7.9 Hz, 1 H), 7.61 (s, 1 H), 7.66 (d, *J* = 7.7 Hz, 2 H). All data are in agreement with literature values.[172]

2-Debenzoyl-2-(3-methoxybenzoyl)-10-deacetylbaccatin III (2-13):[172]

To a solution of **2-12** (330 mg, 0.255 mmol) in 8 mL of pyridine/acetonitrile (1:1) was added dropwise HF/pyridine (70:30, 2.0 mL) at 0 ^oC, and the mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (5.0 mL). The reaction mixture was then diluted with EtOAc (60 mL), washed with saturated aqueous $CuSO_4$ solution (10 mL x 3) and H_2O (10 mL), dried over anhydrous $MgSO_4$ and concentrated to afford **2-13** (229 mg) as a white solid, which was used for the next step directly without further purification.

2-Debenzoyl-2-(3-methoxybenzoyl)-7-triethylsilyl-10-deacetylbaccatin III (2-14): [172]

To a solution of **2-13** (141 mg crude, 0.245 mmol) and imidazole (66.82 mg, 0.982 mmol) in DMF (4.2 mL) was added TES-Cl (0.145 ml, 0.859 mmol), and the mixture was allowed to stir at room temperature for 1 h. After the reaction was quenched with saturated aqueous NH_4Cl, the aqueous solution was extracted with EtOAc. Then the organic layers were washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified by column chromatography on silica gel (hexanes/EtOAc = 5/1) to give **2-16 (**157 mg, 94 % yield) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (m, 6 H), 0.98 (m, 9 H), 1.11 (s, 3 H), 1.18 (s, 3 H), 1.57 (bs, 1 H), 1.62 (s, 3 H), 1.85 (m, 1 H), 1.96 (s, 3 H), 2.14 (m, 2 H), 2.26 (s, 3 H), 2.50 (m, 1 H), 3.83 (m, 4 H), 4.13 (d, *J* = 7.9 Hz, 1 H), 4.30 (d, *J* = 8.1 Hz, 1 H), 4.39 (dd, *J* = 10.4, 6.9 Hz, 1 H), 4.92 (m, 2 H), 5.17 (s, 1 H), 5.59 (d, *J* = 6.9 Hz, 1 H), 7.10 (m, 1 H), 7.35 (t, *J* = 7.9 Hz, 1 H), 7.61 (s, 1 H), 7.66 (d, *J* = 7.7 Hz, 2 H). All data are consistent with the reported values.[172]

2-Debenzoyl-2-(3-methoxybenzoyl)-10-deacetyl-10-propanoylbaccatin III (2-15):[172]

To a solution of **2-13** (229 mg crude, 0.39 mmol) and $CeCl_3 \cdot 7H_2O$ (14.2 mg, 0.038 mmol) in THF (5.4 mL) was added propanoic anhydride (0.48 mL, 2.00 mmol) dropwise *via* syringe at room temperature, and the mixture was stirred for 2 h at room temperature and the reaction was quenched with $NaHCO_3$ (20 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with water (15 mL x 3), brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified on a silica gel column using hexanes/EtOAc (3/1 followed by 1/1) as the eluant to give **2-15** (200 mg, 88% yield) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.1 (m, 5 H), 1.23 (t, *J* = 7.2 Hz, 4 H), 1.54 (m, 6 H), 1.66 (s, 3 H), 1.82 (m, 1 H), 2.05 (m, 4 H), 2.27 (m, 5 H), 2.52 (m, 4 H), 3.88 (d, *J* = 7.2 Hz, 4 H), 4.12 (d, *J* = 8.7 Hz, 1 H), 4.26 (d, *J* = 8.7 Hz, 1

H), 4.40 (m, 1 H), 4.85 (m, 1 H), 4.95 (d, *J* = 8.4 Hz, 1 H), 5.59 (d, *J* = 7.2 Hz, 1 H), 6.33 (s, 1 H), 7.10 (dd, *J* = 8.1, 2.2 Hz, 1 H), 7.36 (t, *J* = 8.1 Hz, 1 H), 7.60 (s, 1 H), 7.66 (d, *J* = 7.6 Hz, 1 H). All data are in agreement with literature values.[172]

2-Debenzoyl-2-(3-methoxybenzoyl)-7-triethylsilyl-10-deacetyl-10-propanoylbaccatin III (2-16a) from 2-14:[172]

To a solution of **2-14** (25 mg, 0.036 mmol) in dry THF (0.66 mL) was added 1.0 M LiHMDS in THF (0.040 mL, 0.040 mmol) dropwise *via* syringe at -40 ^{o}C. The mixture was stirred at -40 ^{o}C for 15 min, and then propanoyl chloride (0.0039 mL, 0.043 mmol) was added dropwise. After 45 min, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (10 mL x 3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified on a silica gel column to afford **2-16a** (22 mg, 81.5% yield) as a white solid: mp 105-107 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J* = 7.8 Hz, 6 H), 0.85 (t, *J* = 7.8 Hz, 9 H), 0.99 (s, 3 H), 1.20 (m, 7 H), 1.64 (s, 3 H), 1.83 (m, 1 H), 2.00 (s, 3 H), 2.16 (s, 3 H), 2.23 (m, 3 H), 2.40 (m, 3 H), 3.85 (m, 4 H), 4.09 (m, 2 H), 4.29 (d, *J* = 8.3 Hz, 1 H), 4.45 (dd, *J* = 10.2, 6.7 Hz, 1 H), 4.79 (t, *J* = 7.9 Hz, 1 H), 4.94 (d, *J* = 8.9 Hz, 1 H), 5.57 (d, *J* = 6.9 Hz, 1 H), 6.45 (s, 1 H), 7.09 (dd, *J* = 8.3, 2.2 Hz, 1 H), 7.33 (t, *J* = 8.1 Hz, 1 H), 7.60 (s, 1 H), 7.66 (d, *J* = 7.7 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5.3, 6.7, 9.2, 9.9, 14.2, 14.9, 20.1, 22.5, 26.8, 27.7, 37.2, 38.3, 42.7, 47.2, 55.3, 58.6, 60.4, 67.8, 72.3, 74.8, 75.6, 76.4, 78.7, 80.8, 84.2, 114.7, 119.9, 122.5, 129.6, 130.6, 132.6, 144.1, 159.6, 166.9, 170.6, 172.7, 202.4.

2-Debenzoyl-2-(3-methoxybenzoyl)-7-triethylsilyl-10-deacetyl-10-propanoylbaccatin III (2-16a) from 2-15:[172]

To a solution of **2-15** (251 mg, 0.40 mmol) and imidazole (107 mg, 1.60 mmol) in dry DMF (3.4 mL) was added TES-Cl (0.20 mL, 0.162 mmol) dropwise *via* syringe at room temperature. The mixture was stirred for 40 min, and then the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with EtOAc (15 mL x 3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified on a silica gel column using hexanes/EtOAc (3/1) to afford **2-16a** as a white solid (248 mg, 83% yield). ^{1}H and ^{13}C NMR data were identical to those mentioned above.

2-Debenzoyl-2-(3-methoxybenzoyl)-7-triethylsilyl 10-deacetyl-10-benzyloxy-carbonyl-baccatin III (2-16b):[172,174]

To a solution of **2-14** (25 mg, 0.036 mmol) in dry THF (0.66 mL) was added 1.0 M LiHMDS in THF (0.040 mL, 0.207 mmol) dropwise *via* syringe at -40 ^{o}C. The mixture was stirred at -40 ^{o}C for 15 min, and then Cbz-Cl (0.0064 mL, 0.043 mmol) was added dropwise. After 1.5h, the reaction was quenched with saturated aqueous NH_4Cl (10 mL), and extracted with DCM (15 mL x 3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified on a silica gel column to afford **2-16b** (28 mg, 94% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.59 (q, *J* = 7.8 Hz, 6 H), 0.91 (t, *J* = 7.8 Hz, 9 H), 1.03 (s, 3 H), 1.17 (s, 3 H),

1.25 (s, 1 H), 1.62 (m, 2 H), 1.68 (s, 3 H), 1.87 (m, 1 H), 2.08 (m, 1 H), 2.20 (s, 3 H), 2.27(m, 5H), 2.54 (m, 1 H), 3.85 (m, 4 H), 4.14 (d, J = 8.4 Hz, 1 H), 4.33 (d, J = 8.4 Hz, 1 H), 4.50 (dd, J = 6.6, 3.9 Hz, 1 H), 4.84 (m, 1 H), 4.97 (d, J = 8.7 Hz, 1 H), 5.20 (dd, J = 12, 10.5 Hz, 2 H), 5.61 (d, J = 6.9 Hz, 1 H), 6.30 (s, 1 H), 7.13 (dd, J = 8.1, 2.1 Hz, 1 H), 7.33-7.41 (m, 6 H), 7.64 (s, 1 H), 7.69 (d, J = 7.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.3, 6.8, 9.9, 15.1, 19.9, 22.7, 26.6, 37.2, 38.2, 42.6, 47.2, 55.4 (MeO), 58.5, 67.9, 69.9 ($PhCH_2O$), 72.3, 74.5, 76.5, 78.7, 79.2, 80.6, 84.2, 144.6, 120.0, 122.5, 128.3, 128.4, 128.5, 129.6, 130.6, 132.3, 135.2, 144.7, 154.3, 159.6, 166.9, 170.7, 201.5 (overall 38 different carbons were observed). All data are in agreement with literature values.[174]

3'-Dephenyl-3'-(2-methyl-1-propenyl)-2-debenzoyl-2-(3-methoxybenzoyl)-10-propanoyldocetaxel (2-18, SB-T-121303):[172]

To a solution of baccatin **2-16a** (245 mg, 0.33 mmol) and β-lactam **1-11** (192 mg, 0.50 mmol) in dry THF (18 mL) was added 1.0 M LiHMDS in THF (0.52 mL, 0.52 mmol) dropwise at -40 ^{o}C, and the solution was stirred at -40 ^{o}C for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (20 mL), and the aqueous layer was extracted with EtOAc (15 mL x 3). The combined extracts were then dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The residue was purified on a silica gel column using hexanes:EtOAc (8:1) as the eluant to afford the coupling product **2-17a** (381 mg, 100% yield) as white solid.

To a solution of **2-17a** (381 mg, 0.33 mmol) thus obtained in 16 mL of pyridine/acetonitrile (1:1) was added dropwise HF/pyridine (70:30, 4.0 mL) at 0 ^{o}C, and the mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (20 mL). The mixture was then diluted with EtOAc (90 mL), washed with saturated aqueous $CuSO_4$ solution (30 mL x 3) and NaCl (30 mL x 3), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column using hexanes:EtOAc (1:1) as the eluant to afford **SB-T-121303** (**2-18**, 267 mg, 92% yield**)** as a white solid: mp 130-132 ^{o}C; $[\alpha]_D^{20}$ -75.0 (*c* 0.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 3 H), 1.28 (m, 8 H), 1.33 (s, 9 H), 1.66 (m, 3 H), 1.74 (s, 3 H), 1.77 (s, 3 H), 1.89 (m, 5 H), 2.37 (m, 5 H), 2.52 (m, 4 H), 3.80 (d, J = 6.9 Hz, 1 H), 3.86 (s, 3 H), 4.12 (m, 2 H), 4.32 (d, J = 8.5 Hz, 1 H), 4.40 (dd, J = 10.6, 6.8 Hz, 1 H), 4.72 (m, 2 H), 4.96 (d, J = 8.3 Hz, 1 H), 5.30 (d, J = 7.6 Hz, 1 H), 5.64 (d, J = 7.0 Hz, 1 H), 6.16 (t, J = 8.6 Hz, 1 H), 6.30 (s, 1 H), 7.13 (d, J = 7.9 Hz, 1 H), 7.33 (t, J = 8.0 Hz, 1 H), 7.62 (s, 1 H), 7.68 (d, J = 7.6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 9.5, 14.9, 18.5, 21.8, 22.4, 25.7, 26.6, 27.5, 28.2, 35.5, 43.2, 45.6, 51.5, 55.3, 58.5, 72.2, 72.3, 73.7, 75.1, 75.4, 76.2, 79.1, 79.9, 81.1, 84.4, 114.6, 120.1, 120.6, 122.5, 129.6, 130.4, 132.9, 137.8, 142.5, 155.4, 159.6, 166.8, 170.0, 174.0, 174.6, 203.8. All data are in agreement with literature values.[172] The purity of **SB-T-121303** determined by HPLC analysis was 99%.

3'-Dephenyl-3'-(2-methyl-1-propenyl)-2-debenzoyl-2-(3-methoxybenzoyl)-10-benzyloxycarbonyldocetaxel (2-19, SB-T-12130311):[172]

To a solution of baccatin **2-16b** (21 mg, 0.0255 mmol) and β-lactam **1-11** (15.2 mg, 0.038 mmol) in dry THF (3 mL) was added 1.0 M LiHMDS in THF (0.038 mL,

0.038 mmol) dropwise at -40 °C, and the solution was stirred at -40 °C for 1.5 h. The reaction was quenched with saturated aqueous NH_4Cl solution (5 mL), and the aqueous layer was extracted with EtOAc (15 mL x 3). The combined extracts were then dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column to afford the coupling product **2-17b** (24.6 mg, 80% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.59 (q, *J* = 7.8 Hz, 6 H), 0.91 (t, *J* = 7.8 Hz, 9 H), 1.10 (s, 21 H, TIPS and Me), 1.18 (s, 3 H), 1.20 (S, 3 H), 1.25 (s, 1 H), 1.34 (s, 9 H), 1.62 (m, 2 H), 1.68 (s, 3 H), 1.72 (m, 1 H), 1.75 (s, 3 H), 1.76 (s, 3 H), 1.89 (m, 1 H), 2.02 (s, 3 H), 2.35 (m, 5 H), 2.27(m, 5H), 2.52 (m, 1 H), 3.80(d, *J* = 6.6 Hz, 1 H), 3.85 (s, 3 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.43 (m, 1 H), 4.48 (dd, *J* = 6.6, 3.9 Hz, 1 H), 4.81 (m, 2 H), 4.93 (d, *J* = 8.4 Hz, 1 H), 5.20 (dd, *J* = 12.3, 11.7 Hz, 2 H), 5.35 (d, *J* = 8.7 Hz, 1 H), 5.66 (d, *J* = 7.2 Hz, 1 H), 6.10 (t, *J* = 9.0 Hz, 1 H), 6.30 (s, 1 H), 7.13 (dd, *J* = 8.1, 2.1 Hz, 1 H), 7.33-7.42 (m, 6 H), 7.64 (s, 1 H), 7.69 (d, *J* = 7.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.3, 6.7, 10.0, 12.5, 14.4, 17.9, 18.0, 18.5, 21.1, 22.6, 25.7, 26.1, 28.2, 35.3, 37.1, 43.1, 46.6, 55.3 (MeO), 58.2, 69.9 ($PhCH_2O$), 71.9, 72.2, 74.9, 75.3, 76.5, 78.6, 78.7, 79.5, 81,1, 84.2, 114.6, 119.9, 122.1, 122.5, 128.3, 128.4, 128.5, 129.5, 130.6, 132.9, 135.1, 136.2, 141.7, 154.2, 155.1, 159.6, 166.7, 169.8, 171.9, 201.2 (overall 50 different carbons were observed.).

To a solution of **2-17b** (24 mg, 0.020 mmol) thus obtained in pyridine/acetonitrile (1:1, 1 mL) was added dropwise HF/pyridine (70:30, 0.25 mL) at 0 °C. After the mixture was stirred overnight at room temperature, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5.0 mL). The mixture was then diluted with EtOAc (50 mL), washed with saturated aqueous $CuSO_4$ solution (5 mL x 3) and saturated NaCl (10 mL x 3), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column to afford **SB-T-12130311** (**2-19**, 17.7 mg, 95% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3 H), 1.24 (s, 3 H), 1.34 (s, 9 H), 1.70 (s, 3 H), 1.72 (s, 1 H), 1.74 (s, 3 H), 1.77 (s, 3 H), 1.87 (m, 1 H), 1.92 (s, 3 H), 2.35 (s, 3 H), 2.38 (m, 2 H), 2.56 (m, 2 H), 3.39 (d, *J* = 6.4 Hz, 1 H), 3.79 (d, *J* = 7.2 Hz, 1 H), 3.87 (s, 3 H), 4.19 (m, 2 H), 4.35 (d, *J* = 8.4 Hz, 1 H), 4.40 (m, 1 H), 4.72 (m, 2 H), 4.97 (d, *J* = 8.4 Hz, 1 H), 5.24 (s, 2 H), 5.32 (d, *J* = 8.4 Hz, 1 H), 5.67 (d, *J* = 7.2 Hz, 1 H), 6.14 (s, 1 H), 6.17 (t, *J* = 8.8 Hz, 1 H), 7.13 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.39 (m, 6 H), 7.63 (m, 1 H), 7.69 (d, *J* = 8.0 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.5, 15.0, 18.5, 21.7, 22.4, 25.7, 26.4, 28.2, 29.7, 35.5, 35.6, 43.1, 45.6, 51.5, 55.3, 58.6, 70.6, 72.1, 72.3, 73.7, 75.1, 76.4, 78.4, 79.1, 79.9, 81.0, 84.4, 114.7, 120.1, 120.6, 122.5, 128.5, 128.6, 129.7, 130.4, 132.5, 134.7, 137.9, 143.5, 155.2, 155.4, 159.6, 166.8, 170.0, 173.0, 203.9. HRMS (FAB): *m/e* calcd for $C_{50}H_{63}NO_{17}\cdot H^+$: 950.4174, found: 950.4164 (Δ = -1.1 ppm). All data are in agreement with literature values.[174]

3'-Dephenyl-3'-(1-propenyl)-2-debenzoyl-2-(3-methoxybenzoyl)-10-propanoyldocetaxel (2-20, SB-T-12130211):[172]

To a solution of baccatin **2-16a** (22 mg, 0.030 mmol) and *β*-lactam **1-41** (17 mg, 0.044 mmol) in dry THF (1.8 mL) was added 1.0 M LiHMDS in THF (0.044 mL, 0.044 mmol) dropwise at -40 °C, and the solution was stirred at -40 °C for 1.5 h. The reaction was quenched with saturated aqueous NH_4Cl solution (5 mL), and the aqueous layer was extracted with EtOAc (10 mL x 3). The combined extracts were then dried over

anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column to afford **2-17c** (31 mg, 93% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J* = 7.8 Hz, 6 H), 0.85 (t, *J* = 7.8 Hz, 9 H), 0.99 (s, 3 H), 1.10 (s, 24 H, including TIPS and Me), 1.18 (s, 3 H), 1.20 (s, 3 H), 1.23 (s, 3 H), 1.31 (s, 9 H), 1.68 (s, 3 H), 1.72 (m, 6 H), 1.89 (m, 1 H), 2.00 (s, 3 H), 2.16-2.55 (m, 8 H), 3.85 (m, 4 H), 4.17 (d, *J* = 8.7 Hz, 1 H), 4.35 (d, *J* = 8.7 Hz, 1 H), 4.46 (m, 1 H), 4.51 (m, 1 H), 4.59 (m, 1 H), 4.94 (m, 2 H), 5.57 (dd, *J* = 12, 5.4 Hz, 1 H), 5.67 (m, 2 H), 6.13 (t, *J* = 8.7 Hz, 1 H), 6.48 (s, 1 H), 7.09 (dd, *J* = 8.3, 2.2 Hz, 1 H), 7.33 (t, *J* = 8.1 Hz, 1 H), 7.60 (s, 1 H), 7.66 (d, *J* = 7.7 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.3, 6.7, 9.2, 9.9, 12.5, 14.4, 17.7, 17.90, 17.95, 21.2, 22.7, 26.3, 27.6, 28.1, 35.3, 37.2, 43.2, 46.7, 55.3, 58.4, 71.2, 72.2, 74.8, 74.9, 78.7, 79.6, 81.1, 84.2, 114.4, 120.2, 122.6, 127.6, 128.6, 129.7, 130.4, 133.3, 140.7, 155.3, 159.6, 166.9, 169.8, 171.7, 172.7, 201.9 (There should be 46 different carbons, but only 44 showed up in the spectrum).

To a solution of **2-17c** (27 mg, 0.028 mmol) thus obtained in pyridine/acetonitrile (1:1, 1.1 mL) was added dropwise HF/pyridine (70:30, 0.3 mL) at 0 °C. After the mixture was stirred overnight at room temperature, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (5.0 mL). The mixture was then diluted with EtOAc (20 mL), washed with saturated aqueous $CuSO_4$ solution (10 mL x 3) and saturated NaCl (10 mL x 3), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column to afford **SB-T-12130211** (**2-20**, 15.8 mg, 77% yield) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (s, 3 H), 1.23 (t, *J* = 7.6 Hz, 3 H), 1.25 (s, 3 H), 1.33 (s, 9 H), 1.66 (s, 3 H), 1.72 (s, 1 H), 1.75 (d, *J* = 6.4 Hz, 3 H), 1.82 (m, 1 H), 1.88 (s, 3 H), 2.32 (m, 2 H), 2.38 (s, 3 H), 2.52 (m, 4 H), 3.26 (d, *J* = 6.4 Hz, 1 H), 3.81 (d, *J* = 7.2 Hz, 1 H), 3.89 (s, 3 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.31 (m, 1 H), 4.35 (d, *J* = 8.4 Hz, 1 H), 4.44 (m, 1 H), 4.58 (m, 1 H), 4.87 (d, *J* = 9.2 Hz, 1 H), 4.96 (d, *J* = 8.0 Hz, 1 H), 5.56 (dd, *J* = 14.4, 6.4 Hz, 1 H), 5.66 (d, *J* = 6.8 Hz, 1 H), 5.78 (m, 1 H), 6.22 (t, *J* = 8.8 Hz, 1 H), 6.31 (s, 1 H), 7.13 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.38 (t, *J* = 8.0 Hz, 1 H), 7.63 (m, 1 H), 7.70 (d, *J* = 8.0 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0, 9.6, 14.8, 17.8, 21.9, 22.5, 26.7, 27.6, 28.0, 29.7, 35.5, 35.6, 43.2, 45.7, 55.3, 58.6, 72.2, 72.3, 73.0, 75.1, 75.3, 76.5, 79.0, 80.0, 81.2, 84.5, 114.1, 120.7, 122.7, 127.3, 128.8, 129.8, 130.2, 133.3, 142.3, 155.3, 159.7, 167.1, 170.3, 173.1, 174.6, 203.6; HRMS (FAB): *m/e* calcd for $C_{44}H_{59}NO_{16}H^+$: 858.3912, found: 858.3880 (Δ = -3.7 ppm). All data are in agreement with literature values.[174]

SB-T-11033 (**2-21**):[172]

To a reaction flask with activated Pd/C (4 mg) was added a solution of **2-18** (25 mg, 0.029 mmol) in EtOAc (7 mL) with three drops of MeOH under hydrogen atmosphere. The mixture was stirred overnight and the reaction mixture was filtered through celite. The crude product was purified on a silica gel column using hexanes/EtOAc (1/1) as the eluant to afford **SB-T-11033** (**2-21**) as a white solid (25 mg, 100% yield): mp 132-134 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (m, 6 H), 1.13 (s, 3 H), 1.28 (m, 8 H), 1.66 (m, 6 H), 1.88 (s, 3H), 2.37 (m, 6 H), 2.52 (m, 4 H), 3.21 (bs, 1 H), 3.80 (d, *J* = 6.9 Hz, 1 H), 3.86 (s, 3 H), 4.12 (m, 2 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.40 (dd,

J = 10.5, 6.8 Hz, 1 H), 4.57 (d, *J* = 9.6 Hz, 1 H), 4.96 (d, *J* = 8.1 Hz, 1 H), 5.63 (d, *J* = 6.9 Hz, 1 H), 6.16 (t, *J* = 8.4 Hz, 1 H), 6.30 (s, 1 H), 7.13 (d, *J* = 7.8 Hz, 1 H), 7.33 (t, *J* = 8.1 Hz, 1 H), 7.62 (s, 1 H), 7.68 (d, *J* = 7.5 Hz, 1 H). All data are in agreement with literature values.[172]

1-(4-Methoxyphenyl)-4-oxo-3-triisopropylsilanyloxyazetidine-2-carbaldehyde (2-22):[212]

To a solution of **1-9** (206 mg, 0.5 mmol) in CH_2Cl_2 (17 mL) and MeOH (8 mL) was bubbled with nitrogen and oxygen for 5 min each at -78 ^oC. Then O_3 was bubbled into the solution until the color of the solution turned blue. Oxygen was bubbled into the solution until the blue color disappeared. After nitrogen was bubbled for another 30 min, Me_2S (0.20 mL, 25 mmol) was injected, and the mixture was slowly warmed up to room temperature. After 3 h, solvent was removed, and the resulting yellow oil was purified on an alumina column (Hexane/EtOAc = 15/1) to give **2-22** (155 mg, 85% yield) as white wax-like solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.04-1.17 (m, 21 H), 3.77 (s, 3 H), 4.44 (dd, *J* = 5.4, 4.2 Hz, 1 H), 5.28 (d, *J* = 5.4 Hz, 1 H), 6.84 (d, *J* = 6.9 Hz, 2 H), 7.25 (d, *J* = 6.9 Hz, 2 H), 9.75 (d, *J* = 4.2 Hz, 1 H). All data are in agreement with reported values.[212]

1-(4-Methoxyphenyl)-3-triisopropylsiloxy-4-(2,2-difluorovinyl)azetidin-2-one (2-23):[212]

To a solution of hexamethylphosphorous triamide (HMPT, 0.32 mL, 1.75 mmol) in THF (3 mL) at 0 °C was added dibromodifluoromethane (0.13 mL, 0.88 mmol). The white precipitate was transferred into a suspension of **2-22** (165 mg, 0.44 mmol) and Zn (142 mg, 2.2 mmol) in THF (6 mL) at 0 °C. The mixture was allowed to reflux for 2 h. The solution was concentrated and the residue was purified by silica gel column to yield **2-23** (100 mg, 56% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 1.08-1.15 (m, 21 H), 3.79 (s, 3 H), 4.54 (ddd, *J* = 16.5, 6.3, 1.5 Hz, 1 H), 4.83 (m, 1 H), 5.14 (d, *J* = 5.1 Hz, 1 H), 6.87 (d, *J* = 9.0 Hz, 2 H), 7.32 (d, *J* = 9.0 Hz, 2 H). All data are in agreement with reported values.[212]

3-Triisopropylsilanyloxy-4-(2,2-difluorovinyl)azetidin-2-one (2-24):[212]

To a solution of **2-23** (1.17 g, 2.85 mmol) in CH_3CN (106 mL) and water (40 mL) at -10 ^oC was added dropwise a solution of ammonium ceric nitrate (5.46 g, 9.96 mmol) in water (66 mL). The mixture was stirred for 3 h, and the reaction quenched by saturated Na_2SO_3 solution. The aqueous layer was extracted with EtOAc, and the combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated. The product mixture was purified on a silica gel column to afford **2-24** (807 mg, 92% yield) as yellowish oil: ^1H NMR (300 MHz, $CDCl_3$) δ 1.03-1.18 (m, 21 H), 4.44-4.59 (m, 2 H), 5.06 (dd, *J* = 2.4, 1.5 Hz, 1 H), 5.96 (br, 1 H). All data are in agreement with reported values.[212]

1-(*tert*-Butoxycarbonyl)-3-triisopropylsiloxy-4-(2,2-difluorovinyl)azetidin-2-one (2-25):[212]

To a solution of **2-24** (503 mg, 1.65 mmol), triethylamine (0.70 mL, 4.95 mmol), and DMAP (50 mg, 0.41 mmol) in CH_2Cl_2 (6 mL) was added Boc_2O (407 mg, 1.81 mmol) in CH_2Cl_2 (4 mL) at 0 ^oC. The mixture was stirred for 18 h and the reaction was

quenched by water. The reaction mixture was diluted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crude residue was purified by column chromatography on silica gel to afford **2-25** (644 mg, 96% yield) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04-1.17 (m, 21 H), 1.50 (s, 9 H), 4.50 (ddd, *J* = 23.7, 9.9, 1.5 Hz, 1 H), 4.75 (dddd, *J* = 9.9, 5.7, 2.1, 1.2 Hz, 1 H), 5.04 (1 H, d, *J* = 5.7 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ -85.8 (dd, *J* = 30.5, 24.3 Hz, 1 F), -81.3 (d, *J* = 30.5 Hz, 1 F). All data are in agreement with reported values except coupling constants in ^{19}F NMR.[212]
From Dr Larisa Kuznetsova's Dissertation [212]: ^{19}F NMR (282 MHz, $CDCl_3$) δ -81.20 (d, 1 F, J = 31.0 Hz), -85.83 (dd, 1 F, J = 5.6 Hz, J = 29.3 Hz).

7-Triethylsilylbaccatin III (2-26):[170]

To a solution of **2-1** (1.200 g, 2.2 mmol) and $CeCl_3 \cdot 7H_2O$ (82 mg, 0.22 mmol) in dry THF (65 mL) was added acetic anhydride (2.12 mL, 22 mmol) dropwise *via* syringe at room temperature. The mixture was stirred for 2 h at room temperature, and the reaction was quenched by $NaHCO_3$ (20 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with water (50 mL x 3), brine (50 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product (1.366 g) was used for the next step without further purification.

To a solution of 7-TES-baccatin III thus obtained and imidazole (600 mg, 8.8 mmol) in dry DMF (24 mL) was added TES-Cl (1.11 mL, 6.6 mmol) dropwise *via* syringe at room temperature, and the mixture was stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (80 mL x 3). The combined extracts were dried over anhydrous MgSO4 and concentrated *in vacuo*. The crude product was purified on a silica gel column using hexanes:EtOAc/ (2/1) to afford **2-26** (1.422 g, 92% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J* = 7.8 Hz, 6 H), 0.85 (t, *J* = 7.8 Hz, 9 H), 1.04 (s, 3 H), 1.20 (s, 3 H), 1.61 (s, 1 H), 1.68 (s, 3 H), 1.88 (m, 1 H), 2.00 (m, 1 H), 2.18 (s, 3 H), 2.20 (s, 3 H), 2.27 (m, 2 H), 2.29 (s, 3 H), 2.51 (m, 1 H), 3.88 (d, *J* = 6.9 Hz, 1 H), 4.14 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.49 (dd, *J* = 10.5, 6.9 Hz, 1 H), 4.84 (m, 1 H), 4.95 (d, *J* = 7.2 Hz, 1 H), 5.63 (d, *J* = 7.2 Hz, 1 H), 6.46 (s, 1 H), 7.47 (t, *J* = 8.1 Hz, 2 H), 7.50 (m, 1 H), 8.09 (d, *J* = 7.2 Hz, 1 H). All data are in agreement with literature values.[170]

SB-T-12851 (2-28):[212]

To a solution of **2-26** (951 mg, 1.36 mmol) and *β*-lactam **2-25** (772 mg, 1.90 mmol) in dry THF (160 mL) was added LiHMDS in THF (1.2 M, 1.71 mL, 2.04 mmol) dropwise at -40 ^{o}C, and the mixture was stirred at -40 ^{o}C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl solution (50 mL), and the aqueous layer was extracted with EtOAc (80 mL x 3). The combined extracts were then washed by water and brine and dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column using hexanes/EtOAc (5/1) as the eluant to afford the coupling product **2-27** (1.22 g, 93% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J* = 7.8 Hz, 6 H), 0.85 (t, *J* = 7.8 Hz, 9 H), 1.04 (s, 3 H), 1.11 (m, 21 H), 1.20 (s, 3 H), 1.29 (s, 9 H), 1.61 (s, 1 H), 1.67 (s, 3 H), 1.68 (br, 1 H), 1.88 (m, 1 H), 2.00 (s, 3 H), 2.16 (s, 3 H), 2.24 (m, 1 H), 2.35 (m, 1 H), 2.36 (s, 3 H), 2.50 (m, 1 H), 3.80 (d, *J* = 6.9

Hz, 1 H), 4.14 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.48 (m, 3 H), 4.90 (m, 3 H), 5.65 (d, *J* = 7.2 Hz, 1 H), 6.16 (t, *J* = 8.7 Hz, 1 H), 6.45 (s, 1 H), 7.47 (t, *J* = 8.1, 2 H), 7.50 (m, 1 H), 8.09 (d, *J* = 7.2 Hz, 1 H). All data are in agreement with reported values.[212]

To a solution of **2-27** (1.25 g, 1.16 mmol) in pyridine-acetonitrile (29 mL each) was added dropwise HF/pyridine (70/30, 15 mL) at 0 ^{o}C, and the mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The mixture was then diluted with EtOAc (300 mL), washed with saturated aqueous $CuSO_4$ solution (35 mL x 6) and NaCl (50 mL x 3), dried over anhydrous MgSO4 and concentrated *in vacuo*. The residue was purified on a silica gel column using hexanes/EtOAc (1/1) as the eluant to afford **SB-T-12851** (**2-28**, 938 mg, 95%) as a white solid: mp 157-159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (s, 3 H), 1.25 (m, 3 H), 1.30 (s, 9 H), 1.68 (s, 3 H), 1.75 (bs, 1 H), 1.88 (m, 4 H), 2.24 (s, 3 H), 2.33 (m, 2 H), 2.39 (s, 3 H), 2.49 (d, *J* = 3.6 Hz, 1 H), 2.55 (ddd, *J* = 14.8, 9.6, 6.4, Hz, 1 H), 3.52 (d, *J* = 5.6 Hz, 1 H), 3.81 (d, *J* = 7.2 Hz, 1 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.28 (d, *J* = 2.8 Hz, 1 H), 4.31 (d, *J* = 8.4 Hz, 1 H), 4.44 (m, 1 H), 4.58 (ddd, *J* = 24.8, 9.6, 1.2 Hz, 1 H, H-3'-vinyl), 4.87 (t, *J* = 8.8 Hz, 1 H), 4.96 (d, *J* = 9.6 Hz, 2 H), 5.66 (d, *J* = 7.2 Hz, 1 H), 6.24 (t, *J* = 8.8 Hz, 1 H), 6.30 (s, 1 H), 7.49 (t, *J* = 7.6 Hz, 2 H), 7.61 (t, *J* = 7.2 Hz, 1 H), 8.11 (d, *J* = 7.2 Hz, 2 H). All data are in agreement with reported values.[212]

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Chapter Three

Design, Synthesis, Evaluation and Development of Self-Immolative Disulfide-Containing Linkers for Efficient Intracellular Release of Anticancer Agents

§ 3.1. Introduction

§ 3.1.1. Tumor-Targeting Prodrug (TTP) and Drug Delivery System

Two major problems associated with current anticancer chemotherapy are the inefficacy against multidrug resistant (MDR) cancer and the lack of specificity between cancerous and normal cells. However, the efficacy against MDR cancer can be increased by developing novel drugs, such as second- and third-generation taxoids, which are discussed in Chapter Two. This chapter will focus on the tumor-targeting drug delivery system (TTDDS).

As shown in Figure 1-1 in Chapter One, cancerous cells originate from normal cells. Consequently, there are no significant differences between tumor cells and normal cells, thereby making cancer cells difficult to be destroyed without harming normal cells. The basic assumption in conventional cancer chemotherapy is that tumor cells are more likely to be killed than normal cells because tumor cells grow faster and therefore, they need higher leverls of vitamins, nutrients and enzymatic activity to support their faster proliferation. When tumor cells greedily obtain the necessary substance from the media, they uptake more anticancer agents than normal cells do. However, this premise cannot guarantee that normal cells are not damaged at all. Normal cells, such as hair and bone marrow cells, also have an elevated growth rate. The reality in clinic is that these anti-cancer agents, such as paclitaxel, cisplatin and doxorubicin, can kill tumor cells, as well as normal cells, which leads to numerous side effects. Thus, patients would be at risk if they are exposed to the high doses of cytotoxic agents that are required to eradicate the tumor completely.[213] In addition, some current anticancer agents in chemotherapy are inefficient against some solid tumors because these solid tumor cells grow slowly.[214]

One of the most promising strategies to solve the tumor-specificity problem is the development of tumor-targeting drug delivery systems (TTDDS).[215] TTDDS, or more a general term, tumor-targeting prodrug (TTP), should recognize the "small" differences between normal cells and cancer cells, and deliver the drug molecules to tumor site only. In general, TTDDS consists of tumor recognition moiety(ies) and cytotoxic warhead(s) with or without a suitable linker(s) to form the drug conjugate (prodrug). The conjugate itself should be systemically non-toxic, but should release the active cytotoxic agent after it is internalized into the cancer cell. This also requires that the linker or linkage must be stable during circulation in the blood stream.

The ideal recognition moiety or guiding molecule must show very high affinity to tumor cells but not to normal cells, and should also trigger the internalization (endocytosis) of the entire drug conjugate to enter the cancer cell (Figure 3-1). Advances in molecular cell biology have clearly illustrated that certain receptors, such as vitamin receptors, hormone receptors (*e.g.*, epidermal growth factor receptor (EGFR)), and saccharide receptors, are overexpressed on the surface of malignant cells. Consequently,

monoclonal antibodies (mAb),[216] polyunsaturated fatty acids,[217] vitamins (such as folic acid[218] and biotin[219]), hyaluronic acid,[220] aptamer,[221] oligopeptides,[222] and others[223] have been successfully serving as the tumor-targeting moiety.[213,215,224] Among them, one mAb-drug conjugate, Gemtuzumab-ozogamicin (Mylotarg®) was approved by FDA in 2000 for the treatment of acute myelogenous leukaemia.[225]

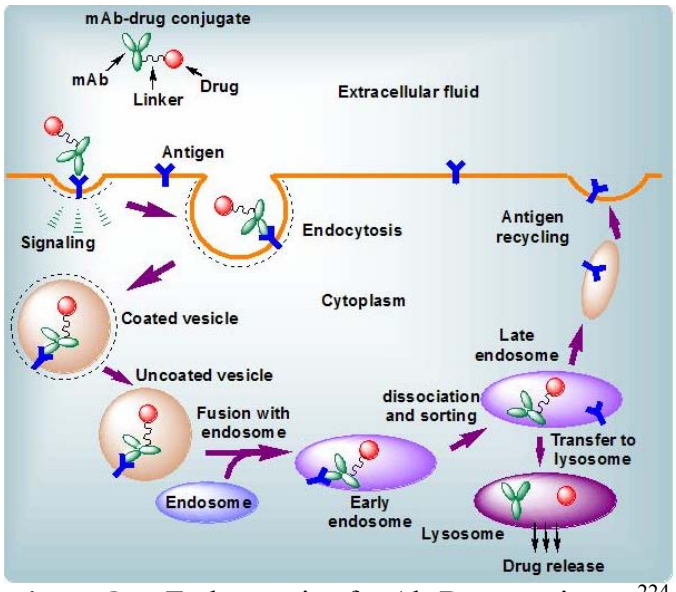


Figure 3-1. Endocytosis of mAb-Drug conjugate[224]

Regarding the linker or linkage part, acid-labile moieties, such as an ester, imine and hydrazone, are usually employed because of lower pH levels inside tumor cells.[213,226,227]. The loaded drug would be released from the conjugate by proteolysis or acid-catalyzed hydrolysis. To our interest, a disulfide linker was chosen due to its stability and activity.[213,216] Accordingly, a thiol-disulfide exchange will set the drug free.

§ 3.1.2. First-Generation Disulfide Linkers and Their Applications to "Taxoid-Monoclonal Antibody Conjugates"

Paclitaxel and docetaxel have been applied to TTDDS.[215] However, the cytotoxicity (*i.e.*, IC_{50}) of the drug for the effective immunoconjugates must be around $10^{-10\sim-11}$M or lower, based on the number of receptors on the surface of tumor cell (for example, EGFR is estimated to be 10^5 per cell).[213] Obviously, the IC_{50} of paclitaxel ($10^{-7\sim-9}$ M) or docetaxel ($10^{-8\sim-9}$ M) does not meet the requirement.[228,229] In addition, those conjugates are anticipated to be ineffective against MDR cancers.

The second- and third-generation taxoids (IC_{50}:$10^{-9\sim-11}$ M) developed in the Ojima lab exhibit extremely high potency even against MDR cell lines, as described in Chapter Two,[230,231] and they are active enough to be the ideal warhead for tumor-targeting drug conjugates. Ojima and co-workers designed and prepared mAb-taxoids conjugates by using disulfide linkers, shown in Figure 3-2.[216]

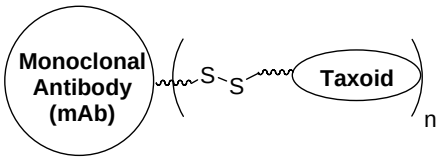


Figure 3-2. mAb-taxoid immunoconjugates

One of the second-generation taxoids, **SB-T-12162** was selected and connected to three mAb, ***KS61*** *(IgG2a)*, ***KS77*** *(IgG1)* and ***KS78*** *(IgG2a)*, which are known to target EGFR on tumor cell surface, as well as ***mN901***, which does not bind to EGFR. The results were quite impressive and promising. The immunoconjugates are cytotoxic at the 10^{-9} M level, specifically against A-431 cancer cells expressing EGFR, while non-binding immunoconjugate, ***mN901*-SB-T-12136**, exhibited no cytotoxicity. *In vivo* antitumor activities of two conjugates, ***KS61*-SB-T-12136** and ***KS77*-SB-T-12136**, were evaluated against human squamous cancer (A431) xenografts in SCID mice. Both of them showed remarkable antitumor activity, while at the same dose, free taxoid **SB-T-12136** at the same dose showed no therapeutic effect (Figure 3-3). Moreover, the immunoconjugates were totally non-toxic to the mice as demonstrated by the absence of body weight loss. These exciting results indicated excellent specificity of the immunoconjugates.[216]

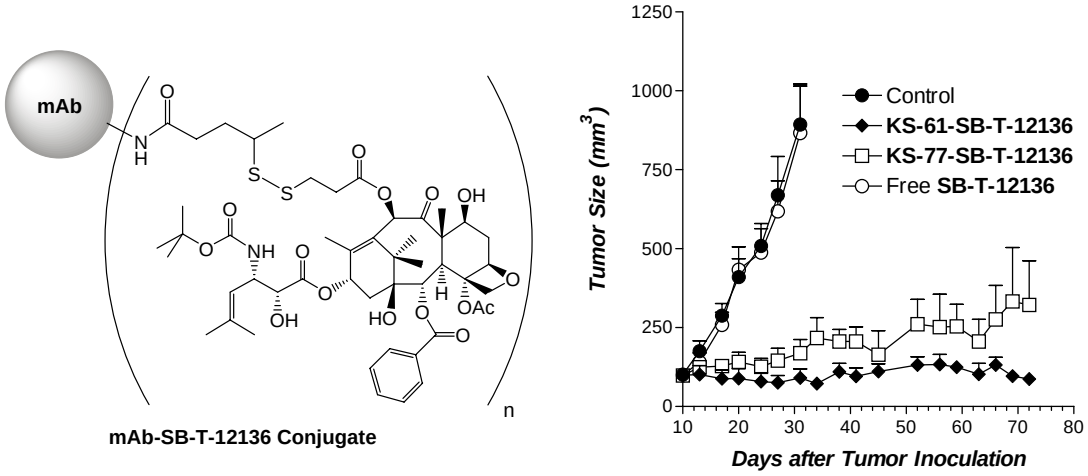
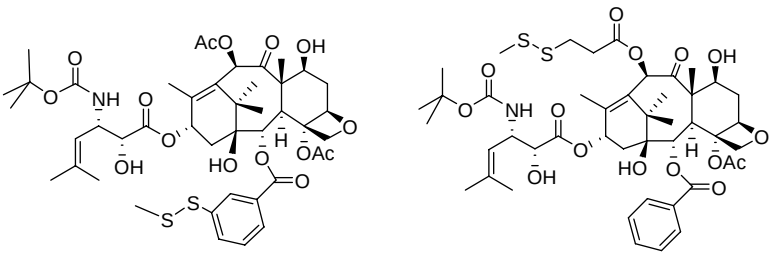


Figure 3-3. Antitumor activity of anti-EGFR mAb-taxoid conjugates against A-431 xenografts in SCID mice[216]

Some novel taxoids bearing a disulfide moiety at different positions were designed and prepared to search for the best potency (Figure 3-4). However, there is still a sulfhydryl moiety at the C-10 position of the taxoid after cleavage of disulfide linkage. Unfortunately, this residual sulfhydryl is responsible for 6~8 times less cytotoxic activity (**SB-T-12136** *vs* **SB-T-1213**), as shown in Table 3-1.[216,232] It is necessary to eliminate the sulfhydryl moiety to recover the original potency of the taxoid.



SB-T-121203 SB-T-12136

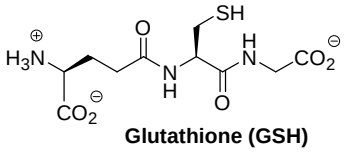
Figure 3-4. Taxoids with disulfide moiety

Table 3-1. *In vitro* cytotoxicity (IC_{50}, nM)[a] of taxoids

Drug	A-431[b]	A-549[c]
Paclitaxel	-	3.6
Taxotere	-	1.0
SB-T-1213	0.09	0.1
SB-T-121203	>3.0	>3.0
SB-T-12136	0.5	0.8

a.The concentration of compound which inhibits 50% of the growth of cancer cell line after 72 h drug exposure. b.Human epidermoid carcinoma. c.Non-small cell lung carcinoma.

It is worthy of note here that a disulfide bond is the bridge between mAb and taxoids, which takes advantage of different concentrations of glutathione (γ-glutamyl-cysteinyl-glycine, GSH, a natural tripeptide containing cysteine, Figure 3-5) in the blood stream and inside tumor cells. It was reported that GSH shows 1~2 μM in human plasma but around 4 mM in normal cells. In several different tumor tissues, the concentrations of GSH are 2 times higher, and 10-fold higher in drug-resistant human ovarian tumor tissue.[233] Based on the GSH concentration data and mAb-disulfide-taxoid experimental results, it is confirmed that the disulfide linkage is indeed stable during circulation in plasma because of low concentration of GSH, and that drug molecules are quickly released inside tumor cells due to the very high concentration of GSH. Thus, the disulfide linker is reliable, and worth to be further evaluated in other TTDDS.



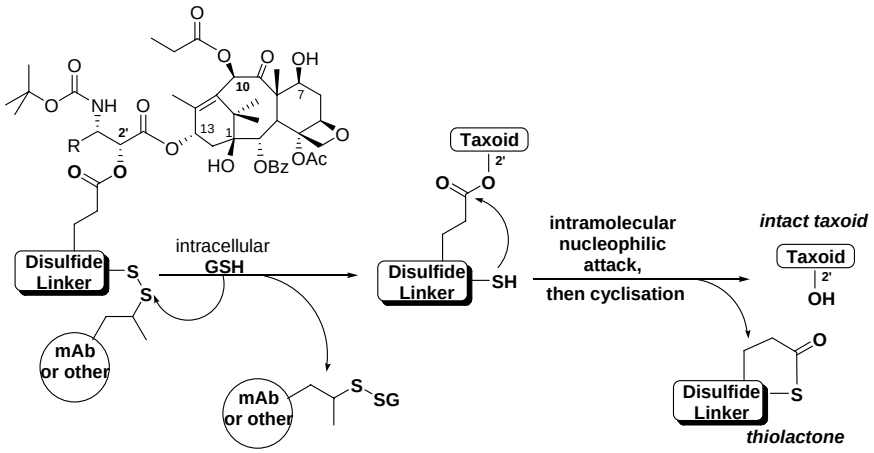
Glutathione (GSH)

Figure 3-5. Structure of glutathione (GSH)

§ 3.1.3. Self-Immolative Second-Generation Disulfide Linkers

§ 3.1.3.1. New hypothesis and design of second-generation linkers with benzothio-lactone systems

Ojima designed a series of novel disulfide linkers with new hypothesis shown in Scheme 3-1.



Scheme 3-1. New hypothesis (adapted from Ojima's proposal)

There are several key features in the new design. Compared to the previous single-step process to release the cytotoxic agent with an undesired sulfhydryl group, the new linker system undergoes a two-step process to liberate the anticancer warhead. After the reductive cleavage of the disulfide bond by intracellular glutathione (GSH), the intermediate, *i.e.*, taxoid-linker conjugate containing sulfhydryl (thiol), will cyclize intramolecularly, and release the active taxoid and thiolactone (Scheme 3-1). Because the taxoid is released in its original form, its potency should be completely recovered, in sharp contrast to the previous taxoid bearing the free sulfhydryl (6-8 times less cytotoxic). Noticeably, the linker in the new design is coupled to the C-2' hydroxyl position, which has two advantages. First, C-2' hydroxyl is very essential to the cytotoxicity of the taxoid (discussed in Chapter One). Consequently, an ester functional group at the C-2' hydroxyl position will substantially reduce the potency of the taxoid, assuring that the taxoid-linker conjugates have no or little systematic cytotoxicity during circulation inside human plasma (also because of low concentration of GSH in human plasma). The second advantage is that the C-2' hydroxyl moiety is the most reactive site in chemical reactions among the four free hydroxyls at the C-1, C-7, C-10 (if free), and C-2' positions of the taxoid. Thus, the installation of the linker moiety should be easier at the C-2' hydroxyl moiety than that of C-10, where was the position of linkage in the previous taxoid-mAb conjugate.

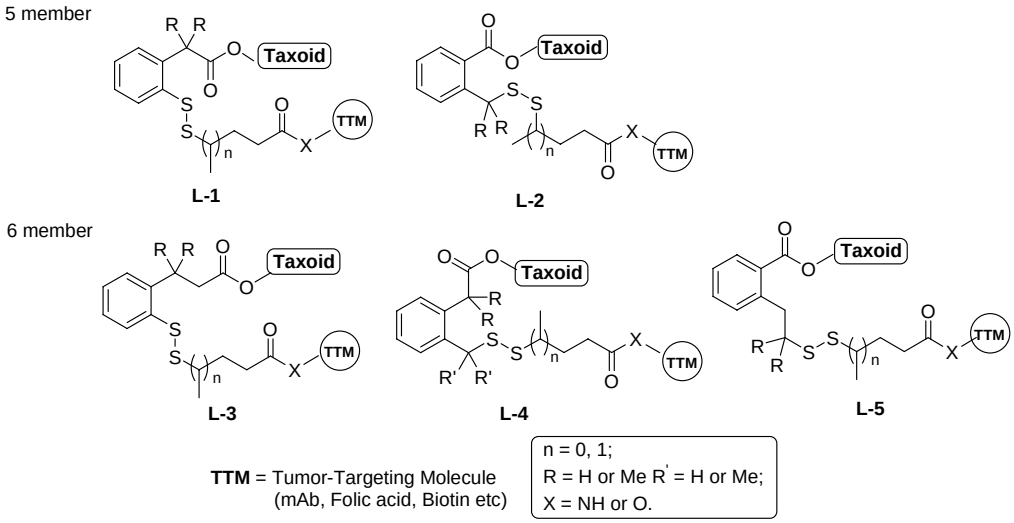


Figure 3-6. Design of new linkers (adapted from Ojima's proposal)

Based on the mechanism mentioned above, two series of disulfide linker systems were designed (Figure 3-6). Depending on the size of the thiolactone generated, one series is the 5-member ring system, and the other is the 6-member system. These second-generation linkers are composed of an asymmetrical disulfide moiety, which has an aromatic group at one end and an alkyl group at the other. The chemical difference between aryl and alkyl groups enables easy modifications on each part to tune the overall electronic and kinetic properties of the linkers, which are very crucial for the drug release. For example, electron-withdrawing or electron-donating substituents should show different electronic effects on the reactivity of the disulfide bond.

It is also worth to mention here that the introduction of substituents, such as mono- or di-methyl substitutions, at the carbon next to the sulfur atom (*e.g.*, L-1 when n = 1) will increase the steric hindrance to the disulfide bond. Thus, these methyl-branched linkers should have better stability in human plasma. Actually, long and branched aliphatic chains as part of the disulfide linker have drawn attention among research groups, *e.g.*, Chari[234], Hamann[235], and Ojima[216]. In Ojima's paper, a 4-mercaptopentanoic acid moiety was employed to connect two fragments: a monoclonal antibody and a taxoid.[216] Hamann[235] and Chari[234] chose a bulky alkyl chain, which was 4-mercapto-4-methylpentanoic acid.

Moreover, *gem*-dimethyl moieties on the carbon next to the carbonyl (ester) carbon (*e.g.*, L-3 when R = Me) is believed to help the cyclization process.[236,237]

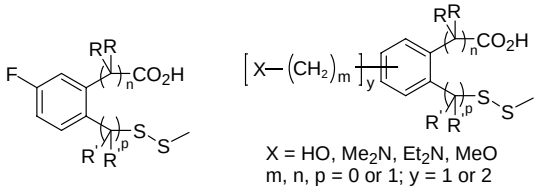


Figure 3-7. Fluorinated linkers and hydrophilic linkers

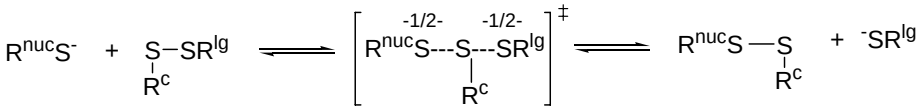
A fluorine atom was also introduced at the *para*-position to the disulfide moiety on the phenyl ring, and ^{19}F NMR helped to track the conversion quantitively. It is known

that taxoids are hydrophobic. In order to increase the water solubility of the taxoid-linker conjugate, linkers bearing hydrophilic groups on phenyl rings, such as hydroxyl, dialkylamino, hydroxymethyl, or dialkylaminomethyl groups, are also considered. For comparison, a methoxyl group was also prepared. (Figure 3-7)

Particularly in my research, linkers L-1 and L-3 and their derivatives have been studied.

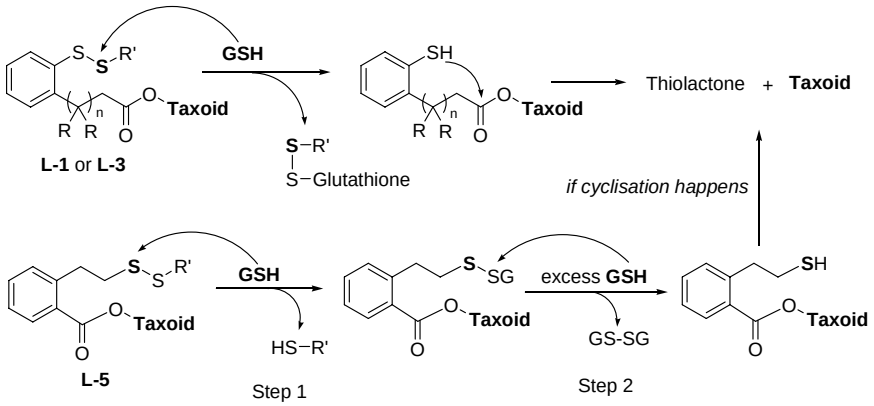
§ 3.1.3.2. Thiol-disulfide exchange reaction and cyclization step

The collapse of a disulfide into a thiol and another new disulfide is usually initiated by a free thiol, as shown in Scheme 3-2. This step is called a thiol-disulfide exchange reaction. This type of reaction is not only essential in disulfide cleavage and drug release in the designed linkers, but also important and widely used in the synthesis of asymmetric disulfide compounds. Mechanistic studies suggest that this process follows an S_N2 mechanism in which a thiolate anion attacks the disulfide bond along the S-S axis.[238] Ester, amide, acid, alcohol and amine functional groups have shown to be tolerated.[238]



Scheme 3-2. Mechanism of thiol-disulfide exchange reaction[238]

Because all of the new generation linkers are not symmetrical disulfides, two sets of products would be generated in theory, and obviously only one combination would result in the final thiolactone and taxoid. We also notice that in the transition state, the sulfur in the leaving group is holding a partial negative charge, which should be directly stabilized by the phenyl ring in L-1 and L-3. As a consequence after the exchange reaction, benzenethiol is the dominant product, which leads to the formation of thiolactone and free taxoid. In the other linkers, such as L-5, due to the high intracellular concentration of GSH, the exchange reaction will be controlled by Le Chatelier's Principle, *i.e.*, the desired thiol intermediate should also be produced although it might need two steps (Scheme 3-3).



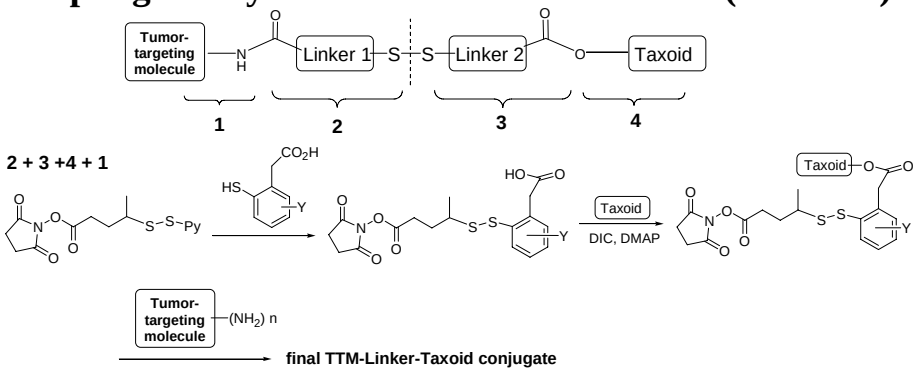
Scheme 3-3. Generation of desired intermediate (thiol or thiolate)

Clearly, different substituents on the phenyl ring will cause dramatic effects on this process, especially in L-1 and L-3. For example, an electron-withdrawing group should boost the exchange reaction, but might retard the cyclization due to a decreased nucleophilicity of the sulfur.

Solution acidity is another factor that may affect the reaction. For instance, slightly basic conditions would help to generate the thiolate to initiate the exchange reaction, and the resulting intermediate (still a thiolate) should be more feasible to undergo cyclization than a neutral thiol.

In addition, this process is sensitive to the steric hindrance in both the exchange step and the cyclization step.

§ 3.1.4. Coupling-Ready Warhead-Linker Construct (CRWLC)



Scheme 3-4. Strategy to assemble TTM-Linker-Taxoid conjugate

From a synthetic chemistry standpoint, a TTM-Linker-Taxoid conjugate is composed of four parts, *i.e.*, TTM, a part of linker 1 (one side of the disulfide linkage), a part of linker 2 (the other side of the disulfide linkage) and a taxoid. Many approaches may work. But, to keep the diversity at both warhead (taxoid) and TTM (mAb), the disulfide linkage was first prepared, as shown in Scheme 3-4. The new disulfide linker possesses a carboxylic acid terminus and an active ester terminus (*N*-hydroxysuccimide (HOSu) or *p*-nitrophenol (Np)). The carboxylic acid is coupled to a taxoid (anticancer drug) to afford the corresponding "coupling-ready" warhead-linker construct (CRWLC, details in Figure 3-8). Finally, the construct is attached to the TTM, such as mAb, fatty acid, and biotin, to complete the whole conjugate (Scheme 3-4).

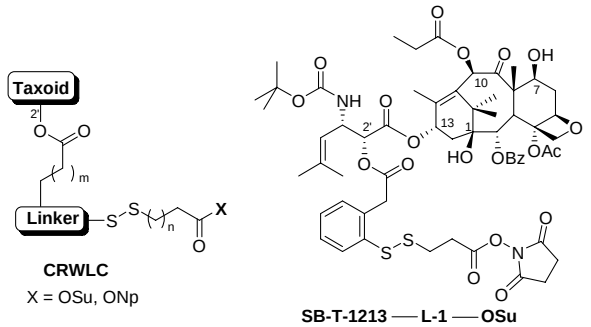


Figure 3-8. Coupling-ready warhead-linker constructs (CRWLC)

CRWLC is very useful and versatile because it contains all the necessary elements, and ready to be coupled with any TTM containing free amine(s). If a TTM does not hold such an amino group, *e.g.*, biotin (carboxylic acid), hydrazine or other alkyldiamine (*e.g.*, ethylene diamine) should be used as the tether to connect both parts. This also illustrates that the disulfide linkage, in principle, is universal and can be modified to accept any TTM.

So far, CRWLC has been successfully applied to lysine-containing oligopeptide (tripeptide), mAb,[239] and aptamer[239]. Among them, a tripeptide was designed to mimic mAb, and the optimized coupling condition between CRWLC and the tripeptide was also modified and applied to the synthesis of mAb-taxoid and aptamer-taxoid conjugates.[239]

§ 3.2. Results and Discussions

§ 3.2.1. Validation of thiolactone formation and drug release

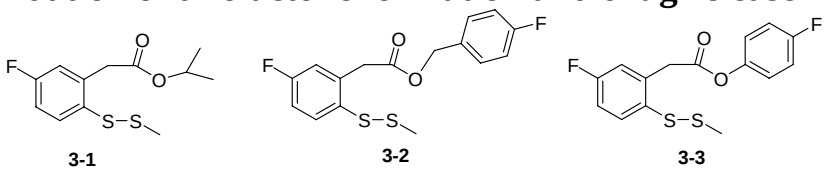
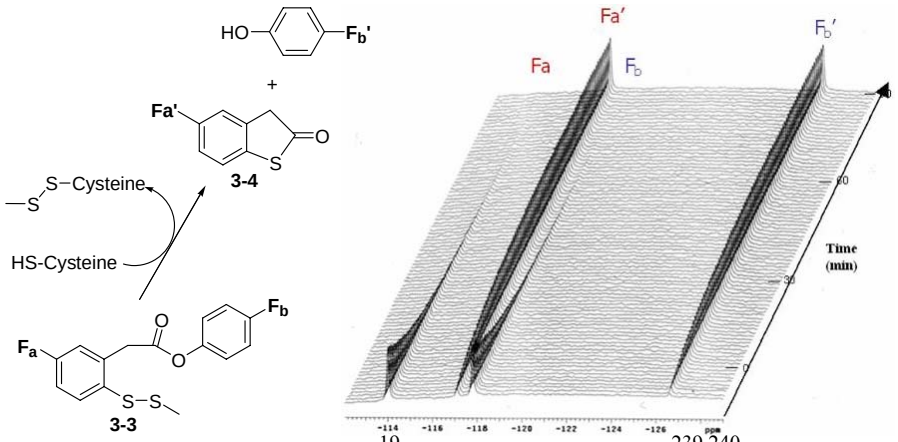


Figure 3-9. Some model compounds used for proof-of-concept

For a proof of concept, a series of model compounds with fluorine *para* to the disulfide moiety on the phenyl ring of the linker (L-1) were prepared, and the release of the fluorinated thiolactone in the presence of cysteine in PBS buffer was monitored by GC-MS, TLC, HPLC, ^{1}H NMR and ^{19}F NMR. However, there was no product from **3-1**. The reaction of **3-2** stopped after disulfide cleavage, but futher cyclization did not happen until the pH was adjusted to 10, which is far beyond physiological conditions (usually pH 7.4 and more acidic in cancer cells[226]).[239] Only **3-3** showed satisfying results even at room temperature (Scheme 3-6).[240] These results strongly implied that a good leaving group (phenol *vs* primary alcohol) is important to undergo the desired intramolecular cyclization step.

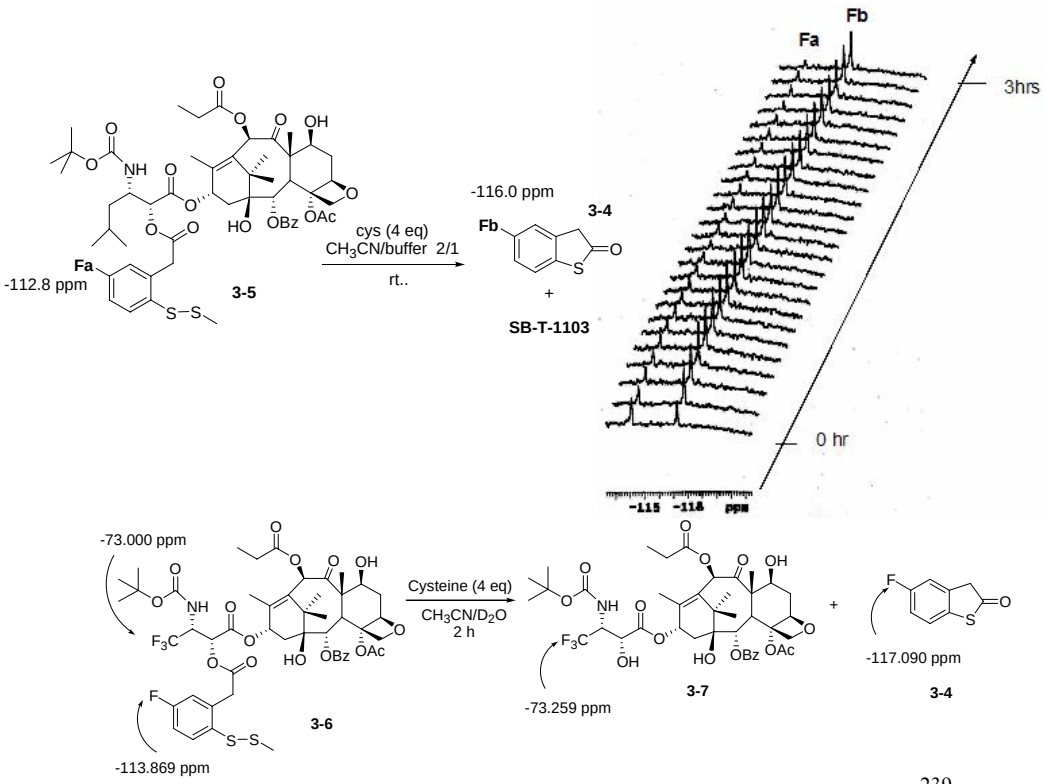


Scheme 3-6. ^{19}F NMR result of **3-3** [239,240]

Accordingly, two taxoid-linker conjugates were also prepared (Scheme 3-7). Fortunately, both of them were able to liberate free taxoids completely at physiological pH levels, monitored by TLC and ^{19}F NMR. Because the C-2' hydroxyl moiety in a taxoid is only a secondary alcohol and similar to isopropanol (see Compound **3-1**, which failed to produce free isopropanol), the neighboring groups in the taxoid, such as a carbonyl (ester) at C-1'and Boc protected amino at C-3', might play a role to push the cyclization step to completion.[239] The ester bond between the linker and the taxoid was relatively stable if there was no free thiol, *i.e.*, the hydrolysis of C-2' ester under physiological condition is very slow.

Different thiol sources were also employed, such as dithiothreitol, glutathione, and benzenethiol.[239] Cysteine and glutathione are preferred over the other two. Considering water solubility, glutathione is preferred over cysteine.

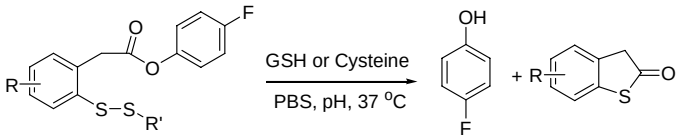
The experimental results clearly show that basic conditions help the release of phenol or a taxoid much more than acidic conditions.[239]



Scheme 3-7. Two taxoid-linker conjugates and their results[239]

Among the linker systems from L-1 to L-5, two of them, L-1 and L-3, have successfully demonstrated their ability to perform the reaction and release the corresponding thiolactone completely, and thus worth to be explored further. Linker **L-4** is not efficient because its reaction was sluggish and a high pH was needed to finish the whole process.[239] Other linkers are still under investigation.

After the validation of function and the potentials of the linkers mentioned above, a model reaction was chosen to investigate the kinetic properties of these novel linkers quickly and efficiently (Scheme 3-8). A taxoid molecule was replaced by *para*-fluorophenol although both molecules were known to be good leaving groups. Due to the cost and ease of chemistry, *para*-fluorophenol was a good choice. To mimic the real drug conjugate, a longer disulfide chain containing ester was employed instead of a methyl disulfide, which was too short.

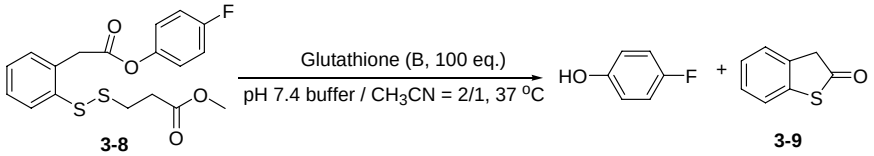


Scheme 3-8. Model reaction and condition

Thus, the rate constant, the substitution effect on the phenyl ring of the linker, the pH profile, as well as steric hindrance, were measured and evaluated by the model reaction described above with different linker models.

§ 3.2.2. Kinetic Study of Phenol/Taxoid Release Process

§ 3.2.2.1. Rate Constant Determination [239]



Scheme 3-9. Model reaction to measure the rate constant

The reaction used for rate constant measurement is shown in Scheme 3-9. To mimic biological conditions, the reaction was carried out in PBS buffer at pH 7.4 at 37 °C in the presence of 100 equivalents of glutathione. Due to the poor solubility of the linker model, a 2 to 1 ratio of buffer to acetonitrile co-solvent was used. The concentration of the starting material, *i.e.*, a linker model, was monitored by HPLC using naphthalene as the internal standard at different times (Figure 3-10).[239]

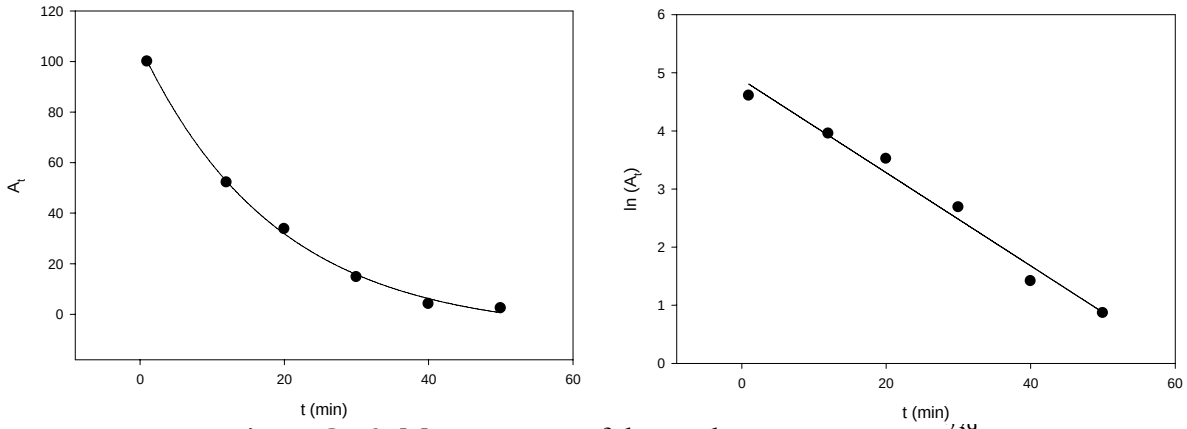
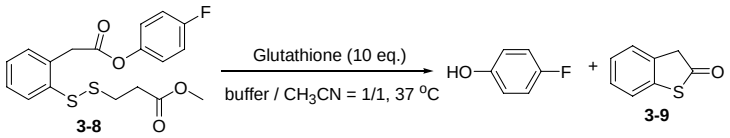


Figure 3-10. Measurement of drug-release rate constant[239]

According to 2nd-order rate laws associated with S_N2 reactions (rate = -d[A]/dt = k[A][B]), if B is in large excess, then rate = -d[A]/dt = q[A], in which q = k[B], and now the reaction should be a pseudo-first order reaction. Therefore, $\ln(A_t) = \ln(A_0)-qt$. After measurement and calculations, $q = 1.3\times10^{-3}$ sec^{-1}, half time $t_{1/2} = 8.9$ min (Figure 3-10).[239]

§ 3.2.2.2. pH dependence of the drug-release process [239]

It is well known that the physiological pH is 7.4, while tumor tissues are normally acidic.[227]. The experiments were performed at pH 5.0, 7.4, and 10.0 (Scheme 3-10). From the concentration curve of the product (benzothiolactone) determined by HPLC with naphthalene as the internal standard, the reaction went faster under basic conditions, but slower under acidic conditions, compared to the physiological conditions (Figure 3-11).[239]



Scheme 3-10. Model reaction in pH dependence measurement [239]

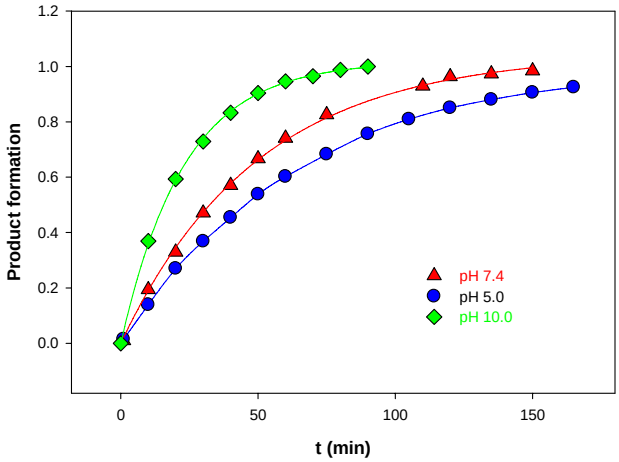
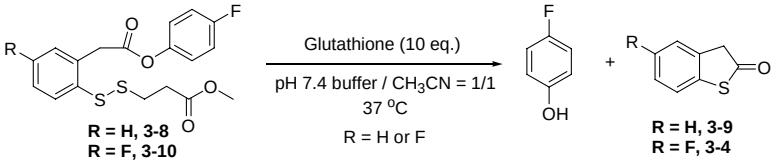


Figure 3-11. pH dependence plot

§ 3.2.2.3. Substituent effect [239]

Different substituents were introduced at the *para*- position to the disulfide moiety on the phenyl ring of the linker, such as F, OMe, NO_2, OH, NMe_2, etc, and some of them were evaluated for the substituent effect. (Scheme 3-11 and Figure 3-12) Compared to the fluorinated derivative, the unsubstituted derivative (hydrogen) underwent faster disulfide cleavage and thiolactonization.



Scheme 3-11. Model reaction to study substituent effect

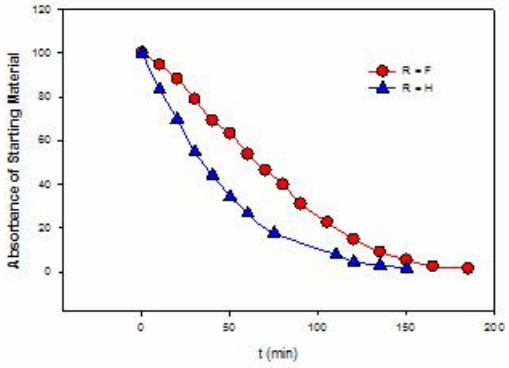


Figure 3-12. Plot of concentration of starting material *vs* time[239]

Another experiment was done by measuring the ratio of products, as shown in Figure 3-13.[241] Thus, a MeO-substituted derivative was mixed with equal moles of non-substituted derivative, and both were treated with one equivalent of cysteine. The products were separated by chromatography, which exhibited the ratio of 70 *vs* 30 favoring the MeO-product. While the same condition was applied to a mixture of fluorinated derivative and non-substituted derivative, the result was almost 50 *vs* 50. Consequently, the reaction rate followed the sequence: MeO > H ≈ F.

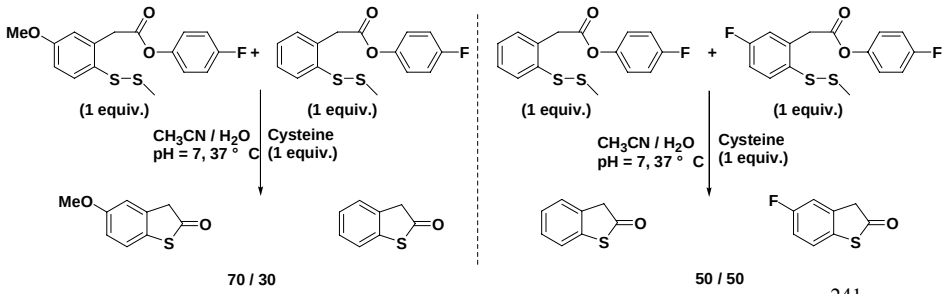


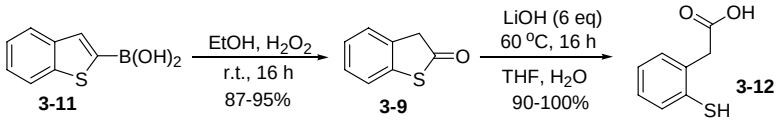
Figure 3-13. Ratios of thiolactones from different sources[241]

§ 3.3. Synthesis of Novel Linkers and Their Derivatives

§ 3.3.1. Second-Generation Linkers and Their Derivatives

§ 3.3.1.1. Synthesis of 3-methyl-3*H*-benzo[b]thiophen-2-ones (5-member benzothiolactones) and their hydrolysis

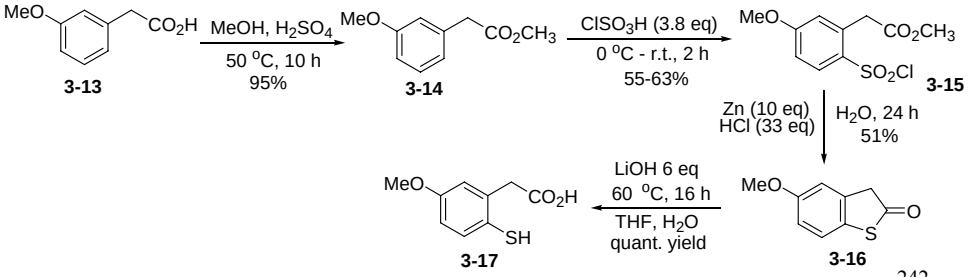
The L-1 linker was the first to successfully cyclize and release the corresponding thiolactone and fluorophenol. Its synthesis, starting from commercially available compound **3-11**, is shown in Scheme 3-12, while other methods are also studied.[239] The hydrogen peroxide reaction was done in a beaker for safety reason, and the hydrolysis must be carried out in inert atmosphere, *e.g.*, nitrogen, to avoid oxidation of free thiol.



Scheme 3-12. Synthesis of 5-member benzothiolactone and its acid

Compound **3-9** (slightly yellow chunks) and **3-12** (yellow powder or golden crystals) should be stored under inert atmosphere, and if available, the containers should be covered by foil to keep off light. During the storage, the color of **3-9** may change to red or even dark brown, but the sample is still good for the following reactions.

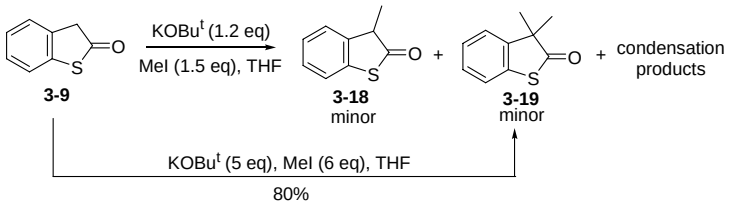
A few more steps would be required if there is a substituent on the phenyl ring. While the important *para*-fluorobenzothiolactone (**3-4**) was discussed by a previous group member (Dr. Ioana Ungureanu), the preparation of *para*-methoxyl derivative was achieved according to Scheme 3-13.



Scheme 3-13. Synthesis of *para*-methoxybenzolactone and its acid[242]

The commercially available material **3-13** was first converted to its methyl ester, followed by sulfonation, which installed the sulfur at the *para*-position to the methoxyl. Zinc reduction and acidic cyclization afforded the thiolactone **3-16**.[242] The desired mercaptophenylacetic acid was obtained through basic hydrolysis in good overall yield.

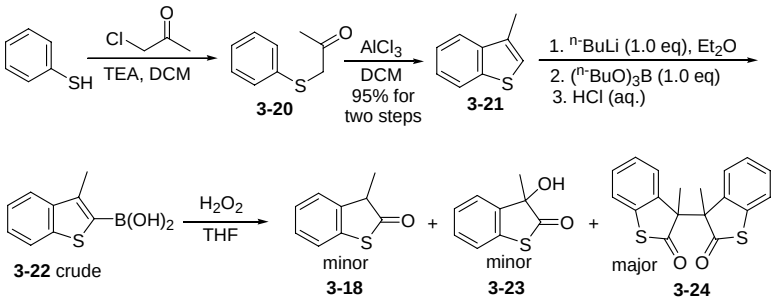
To furnish the mono-methyl substituent at the benzylic position in compound **3-9** was troublesome. Since **3-9** was readily available, direct methylation under basic condition was attempted. However, it turned out that the reaction was very messy. After stirring at room temperature for 2 h, at least five new spots showed up on TLC plate. Messy NMR spectrum suggested mono- and di-methyl derivatives, and *O*-methylation as well. Although the di-methylation underwent smoothly, the mono-methylation was not successful even after several optimizations, such as sequence of addition of reagents, slow addition, and temperature control (Scheme 3-14).



Scheme 3-14. Direct methylation at benzylic position

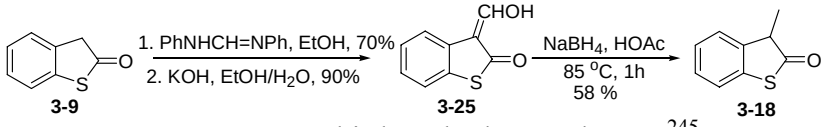
It was reported that the pKa of compound **3-9** was around 10.5,[243] and **3-18** would be even more acidic than **3-9**. Accordingly, the reaction might not stop at the mono-methylation step, and go quickly into side reactions.

Based on the former synthesis of **3-4**,[239] the following procedure was tested (Scheme 3-15). Benzenethiol underwent substitution and Friedel-Crafts acylation to provide **3-21**, which has a strong smell as that of naphthelene. Lithiation and boration should afford benzothiophene borate **3-22**. But two products showed up in the crude **3-21** (solid), and an attempted separation by chromatography failed. It was reported that a similar procedure produced a polymer of boric acid anhydride.[244] Consequently, the mixture was treated with H_2O_2, and amazingly, two different fractions were collected after column. Based on the ^{1}H NMR, only the first fraction contained the desired thiolactone **3-18**, but it was contaminated by an unknown (probably by-product **3-23**). The second fraction was the major product, which was recrystallized from chloroform. X-ray crystallography showed the correct structure was racemic 3,3'-Dimethyl-3*H*,3'*H*-[3,3']bi[benzo[b]thiophenyl]-2,2'-dione (**3-24)**, which was a dimer of **3-18**. But, the mechanism is not clear yet.



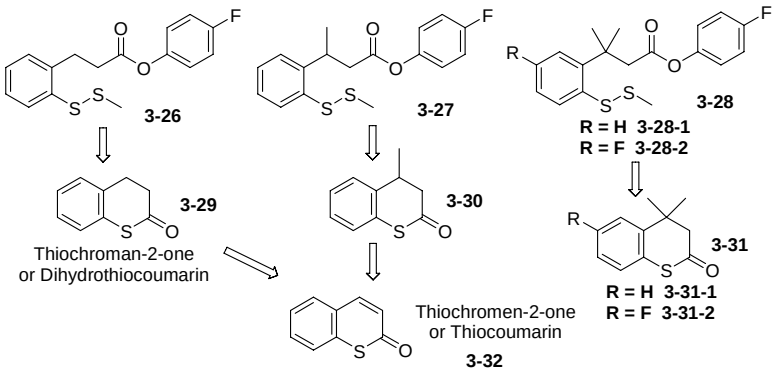
Scheme 3-15. Second route towards mono-methyl substance **3-18**

Finally, **3-18** was successfully obtained by the method shown in Scheme 3-16.[245] It should be noted that the reduction would not go without HOAc. Also the clean ^{1}H NMR confirmed the existence of **3-18** in a mixture with **3-23**.



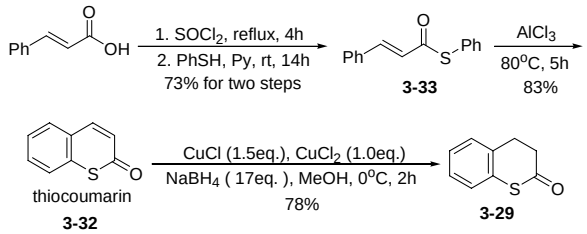
Scheme 3-16. Third method towards **3-18**[245]

§ 3.3.1.2. Synthesis of thiochroman-2-ones (6-member benzothiolactones) and their hydrolysis



Scheme 3-17. Retro-synthesis of 6-member thiolactones

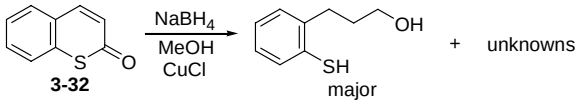
From Scheme 3-17, thiocoumarin **3-32** would most likely be the key intermediate to get the non- or mono-methyldihydrothiocoumarins **3-29** and **3-30** *via* reduction and Michael Addition, respectively. Actually, thiocoumarin **3-32** could be easily prepared from cinnamic acid in a large quantity in good yield and high purity, as shown in Scheme 3-18.



Scheme 3-18. Synthesis of thiocoumarin and its reduction

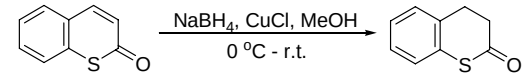
The reduction of an unsaturated aldehyde or ketone with sodium borohydride only often leads to the formation of an allylic alcohol through 1,2-reduction. In order to promote the desired 1,4-reduction selectively, copper salts were applied, which were reported by many research groups.[246]

Indeed, this protocol worked well, and the yield of the reduction was around 80 %. However, the results could not be repeated for unknown reasons. Although different conditions and hydride sources were employed, the major product was the primary alcohol (over-reduction product). Using a smaller amount of hydride resulted in an incomplete reaction, but the over-reduction product was still observed. (Scheme 3-19 and Table 3-2)



Scheme 3-19. Over-reduction product by sodium borohydride

Table 3-2. Results of the reduction of **3-32**

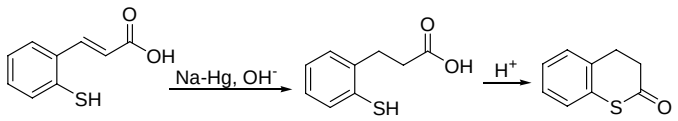


81 mg (0.5 mmol)

Entry	CuCl (eq.)	$NaBH_4$ (eq.)	Time (h)	SM:Prod:Byprod
1	1.5 (old)	15	3.5	unknown mix.
2	1.5 (fresh)	15	5	over-reduced
3	1.5 (old)	20	5	over-reduced
4	1.5 (fresh)	10	1	1: 1: 0.3
5	0.75 (fresh)	5	2	1: 1: 0.1

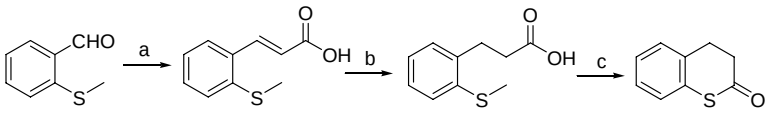
As a known compound, the synthesis of dihydrothiocoumarin has been explored by some groups. But, relatively few information was published.

In 1972, Cotterill and co-workers reported their synthesis.[247] The double bond was reduced by sodium amalgam and alkali, followed by cyclization catalyzed by an acid (Scheme 3-20).



Scheme 3-20. Synthesis of **3-29** [247]

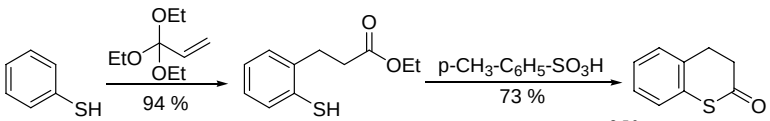
With a similar strategy but starting with 2-(methylthio)benzaldehyde, Christiaens *et al.* reported a three-step synthesis in 1981, as shown in Scheme 3-21.[248] However, tedious work was needed to obtain the starting material, *i.e.*, 2-mercaptobenzaldehyde, and it may polymerize during storage.[249]



a. malonic acid, pyridine; b. $CuSO_4$, N_2H_4, HOAc; c. SO_2Cl_2, H_3PO_2

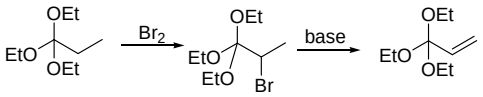
Scheme 3-21. Synthesis of **3-29** [248,249]

Another synthesis was reported by Rapoport and co-workers in 1982.[250] The benzenethiol was converted to (1,1-diethoxyallyl)phenylsulfane as an intermediate, followed by thermal rearrangement to ethyl 3-(2-mercaptophenyl)propanoate in one-pot reaction.



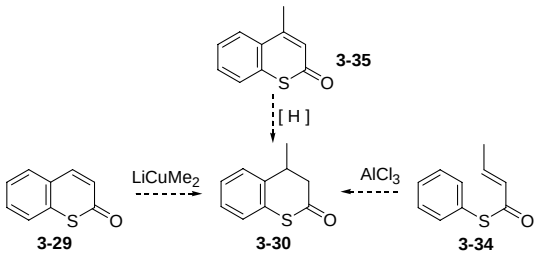
Scheme 3-30. Synthesis of **3-29** [250]

Ethyl orthoacrylate could be prepared from ethyl propionate after bromination and elimination, as shown in Scheme 3-31.[251] Currently, Rapoport's method is under investigation in my study.



Scheme 3-31. Synthesis of ethyl orthoacrylate [251]

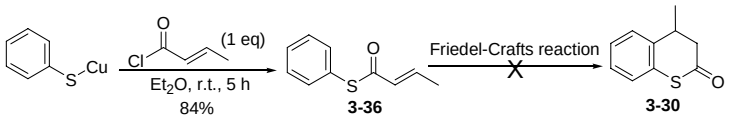
Mono-methylthiolactone **3-30** could be obtained by three approaches: direct Michael addition to **3-29**, Friedel-Crafts reaction (F-C reaction) of **3-34**, and reduction of **3-35**.



Scheme 3-32. Three approaches towards **3-30**

Based on a reported procedure where methyl cinnamate was used as the substrate,[252] the conversion of **3-29** to **3-30** was performed. However, the reaction was messy on TLC and NMR, and no desired **3-30** was isolated in two entries.

Compound **3-36** was prepared by the known procedure in moderate yield (Scheme 3-32).[253] It was subjected to different Lewis acids and solvents at various temperatures. However, none of them showed promising results. (Table 3-3)

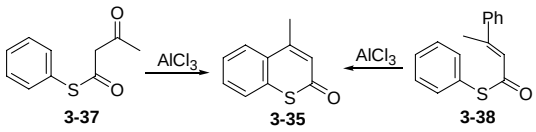


Scheme 3-32. Synthesis of **3-35** from **3-36**

Table 3-3. Results of Friedel-Crafts Reaction from **3-36** to **3-35**

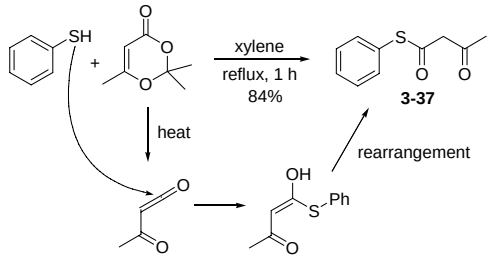
entry	amount	catalyst amount	solvent	temperature	reaction time	result
1	434 mg	$AlCl_3$ (1.5 eq)	DCM	r.t.	8 h	no reaction
2	413 mg	$AlCl_3$ (2.2 eq)	DCM	r.t.	27 h	no reaction
3	410 mg	$AlCl_3$ (2.2 eq)	DCM	50 ^oC	8 h	messy TLC
4	208 mg	$TiCl_4$ (2 eq)	DCM	r.t.	32 h	205 mg SM recovered
5	205 mg	$AlCl_3$ (4 eq)	CH_3NO_2	r.t.	24 h	major is SM
6	185 mg	$AlCl_3$ (4 eq)	CH_3NO_2	90 ^oC	24 h	messy

With the unsuccessful Michael Addition and F-C reaction in Scheme 3-32, I switched to explore the route from **3-35** to **3-30**. Indeed, Compound **3-35** was synthesized by two different methods (Scheme 3-33). Both were F-C type reactions.



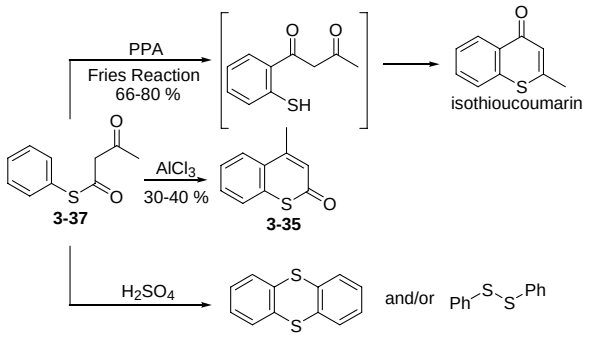
Scheme 3-33. Synthetic method towards **3-35**

Substrate **3-37** was prepared from 2,2,6-trimethyl-4H-1,3-dioxin-4-one and thiophenol cuprate according to the literature procedure shown in Scheme 3-34, in which an active ketene intermediate was involved.[254]



Scheme 3-34. Synthesis of **3-37** [254]

However, the conversion of **3-37** to **3-35** is puzzling. Based on a literature, the product depends on the reaction conditions. [255] If polyphosphoric acid is used as the catalyst, the isothiocoumarin is the major product. Fries rearrangement may happen prior to the F-C reaction. Only one research group claimed that when $AlCl_3$ serves as the catalyst, and the major product was **3-35** in 40-50% yield.[256] (Scheme **3-35**)

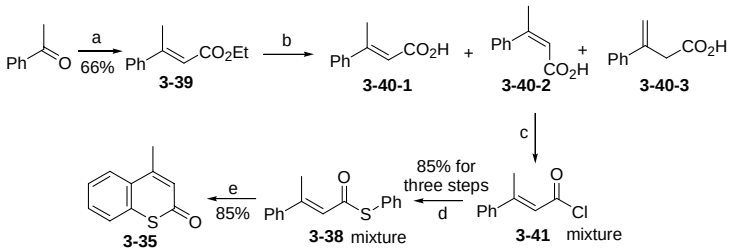


Scheme 3-35. F-C reaction of **3-37** at different conditions [256]

Following the reported procedure[256], desired **3-35** was successfully obtained from **3-37** in 31% yield after purification. Since there is no significant difference between **3-35** and isothiocoumarin in spectra, the structure of **3-35** was confirmed when compared with that from **3-38**.

Compound **3-35** was also prepared from acetophenone, as shown in Scheme 3-36. After a Horner-Wadsworth-Emmons reaction, the ester **3-39** was hydrolyzed to give an acid.[257] When the hydrolysis was carried out at room temperature for 2 days, no **3-40-2** was detected, and the ratio of **3-40-1** *vs* **3-40-3** was 1 *vs* 0.22, based on ^{1}H NMR. When the condition was 50 ^{o}C for 12 h, all three acids would be produced. Under such

conditions, the ratio of **3-40-1** *vs* **3-40-2** was 1 *vs* 1.2. In general, **3-40-3** could be isolated from the mixture of acids.



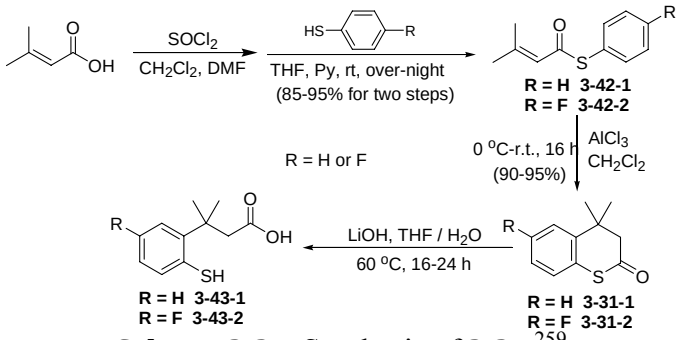
a. $(EtO)_2P(O)CH_2CO_2Et$ (1.2 eq), NaH (1.2 eq); b. KOH, THF, H_2O, r.t.;
c. $SOCl_2$ (3 eq), DCM, reflux, 6 h; d. PhSH (0.9 eq), Py (2 eq), Et_2O, r.t., 8 h;
e. $AlCl_3$ (5 eq), 80 ^oC, 5 h.

Scheme 3-36. Synthesis of **3-35** from **3-38** [257,258]

The chlorination of **3-40-2** with $SOCl_2$ did not change the product ratio estimated from NMR, and thioester **3-38** was generated smoothly in the presence of a base, followed by a F-C reaction leading to the formation of **3-35** in good yield.[258] The ^1H NMR of **3-35** thus obtained was identical to that obtained from **3-37**.

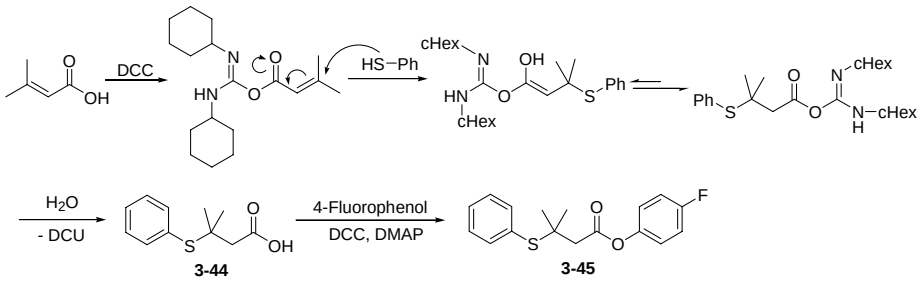
The reduction of **3-35** to **3-30** is currently under investigation.

The dimethyl substituted thiolactone **3-31** was also a known compound, and could be prepared in high yield (Scheme 3-37).



Scheme 3-37. Synthesis of **3-31** [259]

However, the thioester **3-42** could not be obtained using the usual DCC/DMAP coupling method because it gave a mixture of the desired product (**3-42**) and a by-product **3-44** (Scheme 3-38). The structure of the by-product was determined by ^1H NMR, and a plausible mechanism was proposed based on steric hindrance of the material. However, **3-44** could be coupled to 4-fluorophenol, and the resulting ester **3-45** could be applied for model reaction conditions to explore the stability of the ester bond since it can be argued that the release of the fluorophenol or a taxoid is caused by hydrolysis of the ester bond rather than the mechanism we have designed.

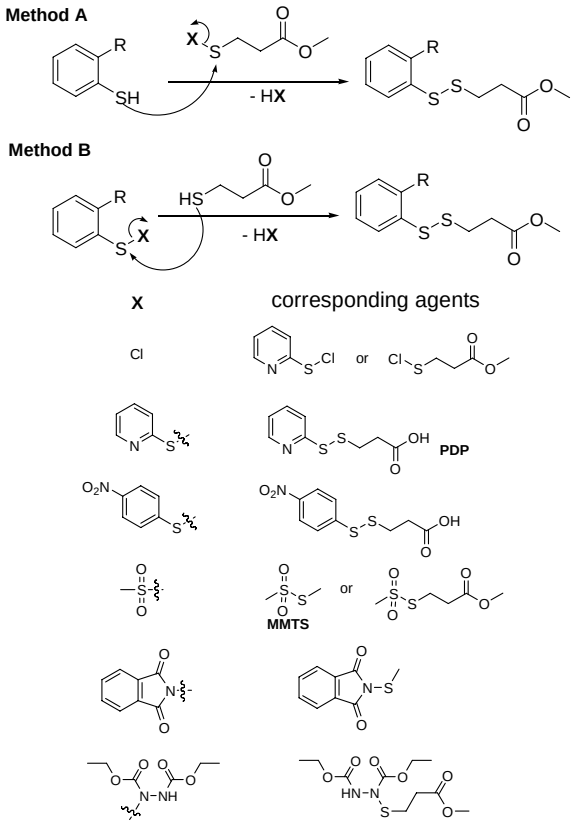


Scheme 3-38. By-product in the presence of DCC

The *gem*-dimethyl groups caused some trouble in the hydrolysis of **3-41**. It took a longer time to open the thiolactone, and during the acidic work-up, some **3-43** recyclized back to **3-41**. However, this implied that the *gem*-dimethyl substrate **3-43** and/or **3-28** definitely favors the cyclization process that meets our aim. Thus in the synthesis, the crude di-lithium salt of **3-43** should be applied in the next step, and indeed it worked.

§ 3.3.2. Thiol-Disulfide Exchange Reactions (1): Synthesis of 3-(Pyridin-2-yl-disulfanyl)-propionic Acid (PDP) and Its Derivatives

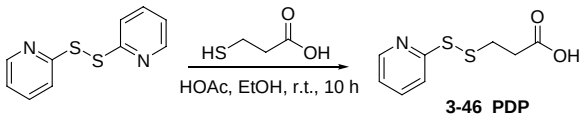
There are several ways to synthesize disulfide compounds, but Method A and B in Scheme 3-39 are the most commonly employed to prepare asymmetrical disulfide. The activation of one sulfur atom is necessary. If X is a good leaving group, the reaction would be useful and practical in the preparation of disulfides. Otherwise, the reaction would be controlled by concentrations of reagents, as discussed in § 3.1.3.2. It should be mentioned here that the disulfides were usually obtained by Method A, but the model reactions proceeded *via* Method B, where in GSH or cysteine took place of mercaptopropanoic acid as the thiol source.



Schem 3-39. Synthesis of Ar-Alkyl disulfide

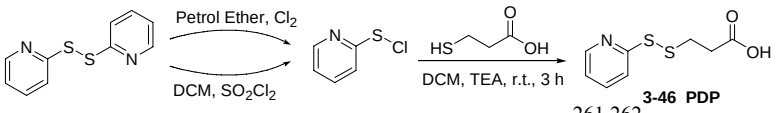
Preparation of disulfides containing the pyridin-2-yldisulfanyl moiety is discussed here. MMTS and methyldisulfanyl will be described in § 3.3.4 and miscellaneous disulfides in § 3.3.5.

Three different procedures have been reported in literature to synthesize 3-(pyridin-2-yl disulfanyl)propanoic acid (PDP).[260-263]



Scheme 3-40. Synthesis of PDP by Carlsson[260]

Jan Carlsson et al first reported the synthesis of PDP in 1978[260] in which dipyridyl disulfide was employed, and PDP was obtained as yellow oil in 40% yield after direct thiol-disulfide exchange reaction (Scheme 3-40). Under the same reaction conditions and separation conditions, Ghadiri and co-workers, in 1998, claimed the yield was improved up to 82.2%.[264] In their preparation, an alumina column was necessary ddssto purify PDP. But in my practice, pure PDP was obtained as white solid after an alumina column and a silica gel column.

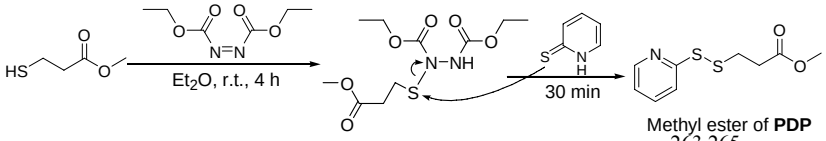


Scheme 3-41. Samukov's Method[261,262]

In 1985, Samukov *et al*. preactivated dipyridyl disulfide by chlorine gas, and the intermediate, 2-pyridylsulfenyl chloride, was reacted with mercaptopropanoic acid to provide PDP (Scheme 3-41).[261] Compared to Carlsson's method, chloride ion seemed to be a better leaving group. However, due to high activity of the intermediate and heterogeneous reaction conditions, the yield of this method varied at different entries. Moreover, chlorine is highly toxic, and extra protection should be made. Later in 1998, Samukov published another paper by using sulfuryl chloride (SO_2Cl_2) instead of chlorine.[262] The reaction condition was mild, and the yield was higher (77%) and reproducible.

The afore-mentioned two strategies were focused on activating the aromatic thiol to produce the asymmetric Ar-alkyl disulfides. Activation of alkyl thiol also worked, in which dialkyl azodicarboxylate (such as DEAD) was found to be useful.

Loccufier and Schacht reported the synthesis of methyl ester of PDP by using DEAD in 1988,[263] but the method was first developed by Mukaiyama and Takahashi[265] in 1968 to synthesize asymmetric disulfide (Scheme 3-42).

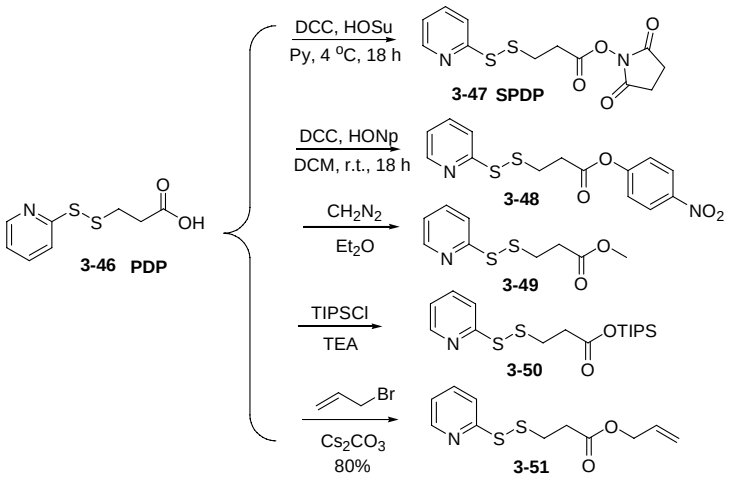


Scheme 3-42. DEAD in the synthesis of PDP[263,265]

This was a one-pot synthesis, and the intermediate adduct was not isolated. Although the yield was not given in their paper, the spectra data of PDP methyl ester were reported in detail,[51] which were taken as the standard to validate PDP formation.

At first, Carlsson's method was followed, but the formation of 3,3'-dithio-dipropionic acid was always a problem although in their paper they claimed that this side reaction could be surpressed by using two equivalents 2,2'-dipyridyl disulfide. The real yield in my experiments was around 50-60%. However, the price of 2,2'-dipyridyl disulfide is expensive, and a half of it is wasted in Carlsson's method. Thus, Samukov's approach was explored. So far, the best yield was 60-70% for two reasons: the formation of 3,3'-dithiodipropionic acid and the incompletion of the first activation step. Due to the atomic economy and ease in work-up, Samukov's approach was applied in a large scale (20 g) synthesis of PDP.

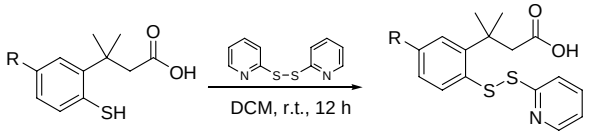
With PDP in hand, a series of its derivatives became quickly accessible (Scheme 3-43). Among them, 3-(pyridin-2-yldisulfanyl)propionic acid 2,5-dioxo-pyrrolidin-1-yl ester (SPDP, **3-47**), [260,263,266] methyl ester **3-49**[263] and TIPS ester **3-50** served as the key intermediates for other syntheses.



Scheme 3-43. Synthesis of PDP derivatives

Bifunctional SPDP (**3-47**), composed of an activated ester (ready for amide formation, *esp.* lysine) and a potential disulfide (ready for disulfide bond with cysteine), is widely used in protein research, *e.g.*, ligation, cross-coupling and conjugation.[260,267] However, from a small molecule synthetic chemistry standpoint, it would be better if amide formation is done prior to the disulfide exchange, because of the polarity and instability of activated HOSu ester. Based on experience, control of reaction temperature was very crucial because elevated temperature would cause β-elimination to form acrylate. [267] DCC was better than DIC or EDCI for this reaction.

Meanwhile, dipyridinyl disulfide was also applied to the synthesis of a methyl-branched linker, which will be discussed in § 3.3.6, and two model compounds used in the proof-of-concept reactions with 6-member linkers (Scheme 3-44).



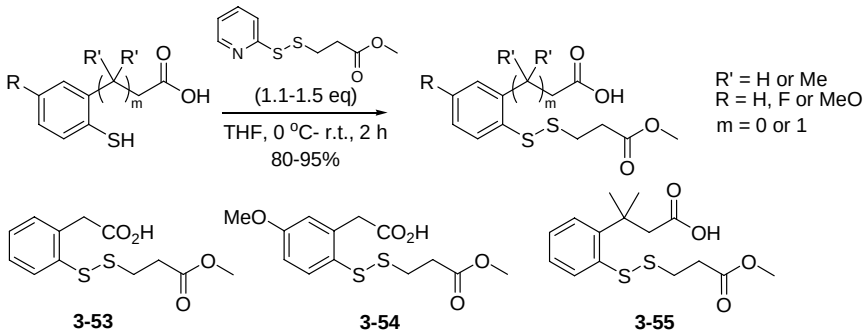
R = H 3-43-1
R = F 3-43-2

R = H 3-52-1
R = F 3-52-2

Scheme 3-44. Thiol-disulfide exchange reaction with dipyridinyl disulfide

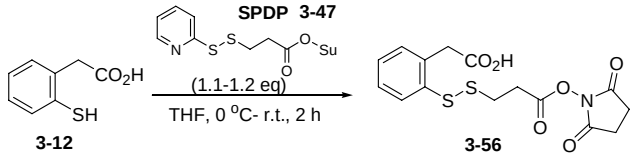
§ 3.3.3. Thiol-Disulfide Exchange Reactions (2): Applications of 3-(Pyridin-2-yl-disulfanyl)propionic Acid (PDP) Derivatives

Although the synthesis of PDP suffered from low yields, harsh conditions and tedious work-up protocols, the following reactions between its derivatives and aromatic thiols were very efficient and showed desired product in high yield and good purity (Scheme 3-45). Three compounds were prepared for the kinetic study.



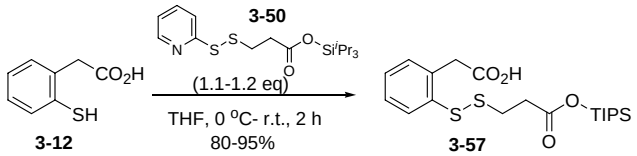
Scheme 3-45. Thiol-disulfide exchange reaction (aromatic thiol and PDP derivatives)

In Scheme 3-46, SPDP was employed in the reaction with acids containing a mercapto moiety. The reaction was very clean and efficient, but the products were difficult to be purified due to its high polarity. Consequently, crude products should be directly applied to the next step (formation of CRWLC).



Scheme 3-46. SPDP in thiol-disulfide exchange reaction

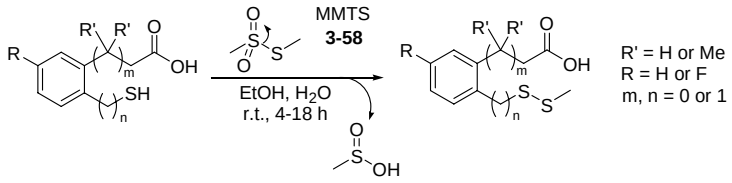
Because **3-56** proved problematic in its preparation and future reactions, **3-50** and **3-57** (Scheme 3-47) were developed to replace SPDP and **3-56**. Surprisingly, the reaction worked very smoothly and cleanly in high yield with easy purification. The tri*iso*-propylsily ester was reported unstable,[268] but **3-50** and **3-57** could be stored in a freezer for up to two months without affecting the next steps.



Scheme 3-47. Thiol-disulfide exchange reaction by using **3-50**

§ 3.3.4. Thiol-Disulfide Exchange Reactions (3): *S*-Methyl methanethiosulfonate (MMTS) and methyldisulfanyl derivatives

S-Methyl methanethiosulfonate (MMTS, **3-58**)[269] was successfully employed in the synthesis of methyldisulfanyl (CH_3-S-S-) compounds (Scheme 3-48). Although MMTS has a special odor, this reaction usually is clean, mild and gives high yield. The by-product, the dimer of the thiol (starting material), could be suppressed by adding thiol slowly into MMTS solution at low temperature. Interestingly, the reaction went smoothly even without a base.[234] However, according experimental results, it was better to use a base, *i.e.*, either organic (triethylamine) or inorganic base (sodium bicarbonate).



Scheme 3-48. Application of MMTS to thiol-disulfide exchange reaction

The following compounds (Figure 3-14) except **3-60** were prepared from the corresponding mercapto-acids according to Scheme 3-38.

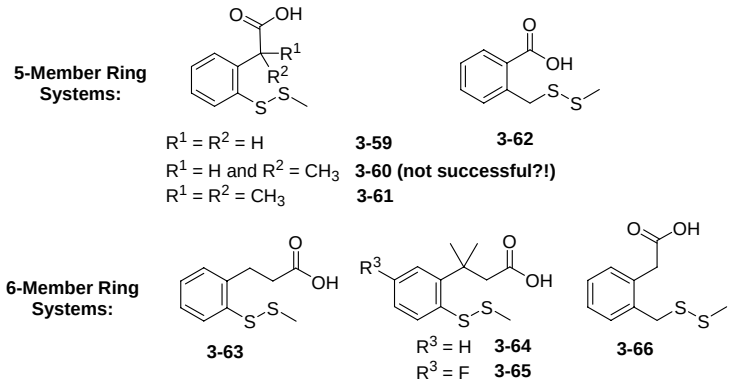
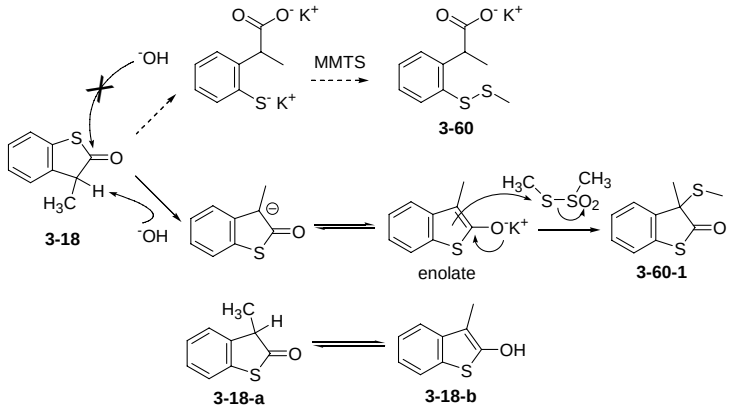


Figure 3-14. Some methyldisulfanyl compounds prepared *via* MMTS

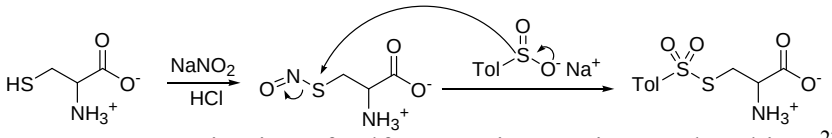
However, the hydrolysis of **3-18** with KOH followed by MMTS treatment did not provide **3-60**, but, interestingly, **3-60-1** in 60% yield, which was confirmed by LCMS and NMR. This may be attributed to the stability of the enolate of the intermediate caused by deprotonation at the benzylic position, rather than nucleophilic attack at the carbonyl (Scheme 3-49). Also it was purposed that the equilibrium between **3-18-a** and **3-18-b** (although not detectable in NMR) was shifted in the presence of base since benzylic protons in this type of compounds were found to be very acidic.[243] In addition, the feasible generation of the enolate may also be one of the reasons why mono-methylation with **3-9** was not successful.



Scheme 3-49. Putative mechanism of formation of **3-60-1**

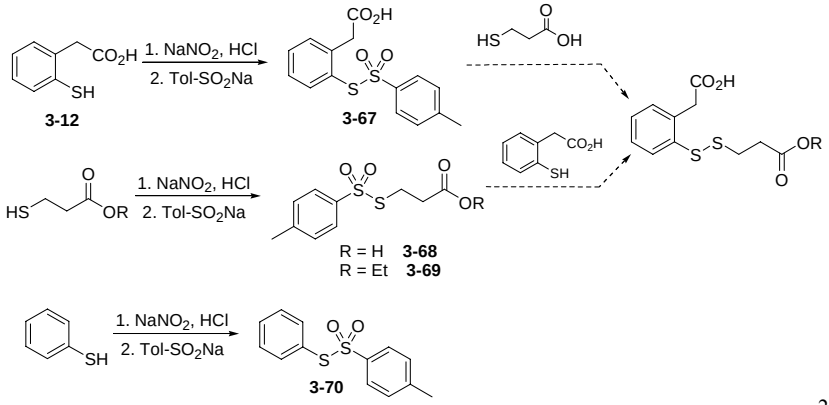
To obtain **3-60**, the acidic hydrolysis of **3-18**, or the alkylation of the methyl ester of **3-12**, should be investigated.

Usually, MMTS is prepared by oxidation of dimethyl disulfide because of its symmetry. Asymmetric MMTS-type compounds would not be easy to prepare.[270] However, cysteine or glutathione could form the MMTS-type derivatives with treatments of $NaNO_2$ and sodium *p*-toluenesulfinic acid ($CH_3C_6H_4SO_2Na•H_2O$), as shown in Scheme 3-50.[271]



Scheme 3-50. Activation of sulfur atom in cysteine or glutathione[271]

Initiated by this result, the novel formation of a disulfide linker through the activation of an aromatic sulfur followed by the attack of an alkyl thiol was elucidated (Scheme 3-51). Thus, **3-12** could be converted to **3-67** by a reported procedure.[271]

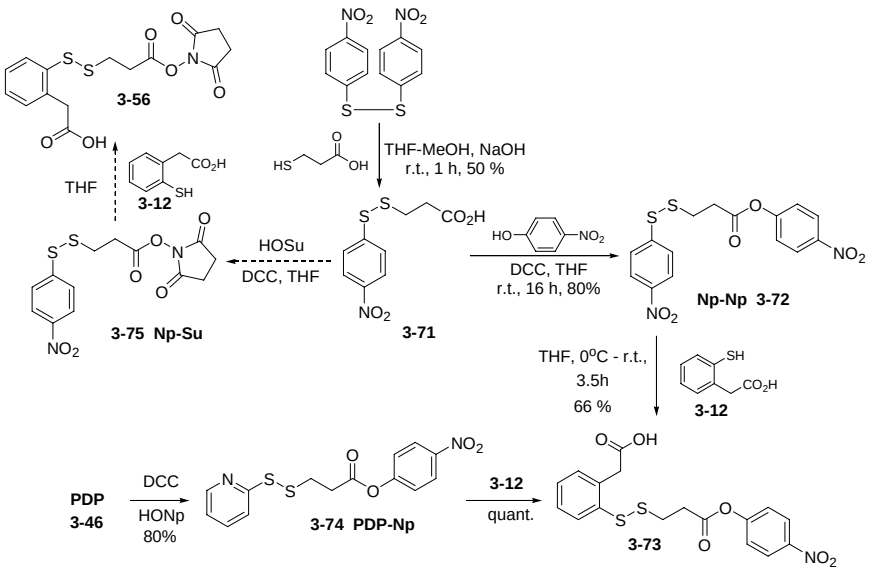


Scheme 3-51. New design towards the synthesis of disulfide linkers [271-274]

Since **3-67** is a literature unknown compound, **3-70** and **3-68** were prepared to verify this procedure. **3-70** is a known compound and obtained by a different method.[274] **3-68** is also unknown, but its structure (alkyl thiol) is similar to cysteine, and its ethyl ester **3-69** is a known substance[273]. Finally, **3-67** (confirmed by LCMS and NMR), **3-68** and **3-69** were successfully prepared *via* Marr's method[271], which was believed reliable.

The advantage of this strategy is diversity. The thiol-disulfide exchange reaction could be considered as the final step to assemble the target molecule. Although not tried in my research yet, it has been reported in literature.[272] As a complementary method to the previous one (activation at alkyl sulfur), this new route (activation at aromatic sulfur) is worth to be further explored.

§ 3.3.5. Miscellaneous Disulfides

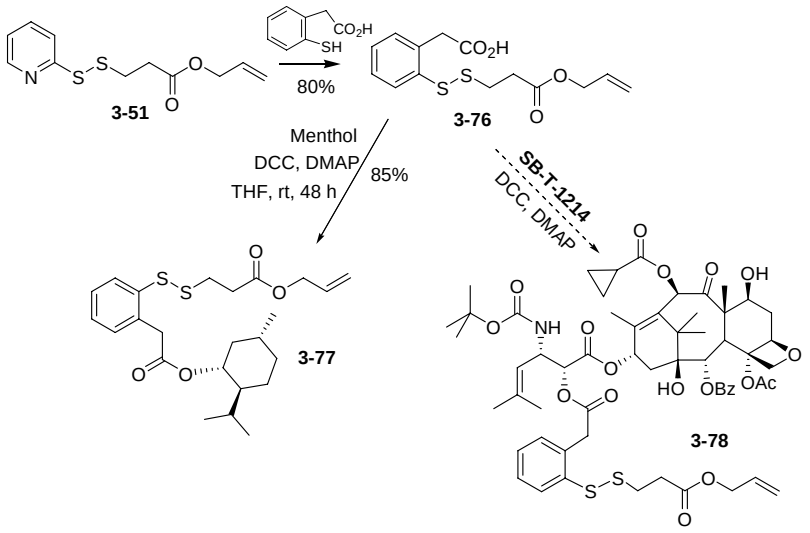


Scheme 3-52. 4-Nitrobenzenethiol in the formation of disulfide

As well known, 4-nitrophenol (HONp) and 4-nitrobenzenethiol are good leaving groups, which could play an important role in disulfide preparation. Thus, **3-71**[275] was coupled to HONp, and later led to **3-73** through thiol-disulfide exchange reaction (Scheme 3-52). However, **3-73** could also be obtained from **3-74** in high yield, which was derived from PDP **3-46**. From experimental results, the reaction of **3-12** towards **3-73** did not reach completion when only one equivalent of **3-72** was present, while it showed full conversion with an equal amount of **3-74**. Obviously, **3-72** was not as active as **3-74**.

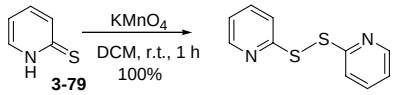
The HONp ester has at least three advantages over the HOSu ester. First, the HONp ester is more active than the HOSu ester in amide formation reaction. For example, secondary amines react faster with the former, but much slower with the latter. Second, HONp esters are usually less polar than HOSu esters. Third, although most HONp esters are colorless or slightly yellow, free HONp shows a strong bright yellow color, which is intensive and sensitive enough to be utilized to monitor the progress of the reaction. However, the HONp ester is known to decompose in aqueous solution while the HOSu ester is more resistant against water, thus the HOSu ester could be stored for long periods of time without decay. While some HONp esters are liquid, most HOSu esters are nice crystal or solid.

To explore the coupling conditions of a disulfide linker to a taxoid at the C-2' hydroxyl position, *i.e.*, ester bond formation, menthol was taken as a mimic of a taxoid, both of which are bulky secondary alcohols (Scheme 3-53). In addition, an allyl ester was introduced. It was reported that an allyl ester was removed by $Pd(PPh_3)_4$ in the presence of a disulfide bond,[276] and $Pd(PPh_3)_4$ should be tolerated by other functional groups in the taxoid-linker conjugate. However, the removal of the allyl group from **3-77** did not go under the reported conditions.



Scheme 3-53. Mimic reaction towards the coupling step with a taxoid

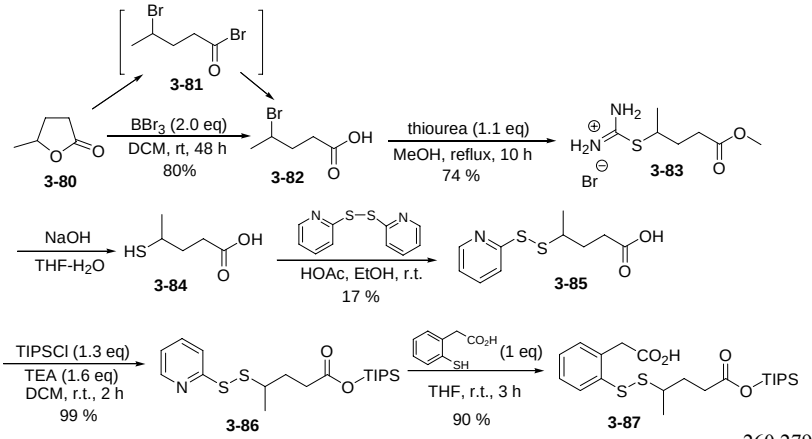
Also it is valuable to mention that thiopyridinone, a co-product after the thiol-disulfide exchange reaction using dipyridinyl disulfide, could be converted to dipyridinyl disulfide, which is expensive (Scheme 3-54).[277] Oxidation with SO_2Cl_2 is not efficient in my practice[278]. However, oxidation with $KMnO_4$ goes completely and smoothly without generation of the over-oxidized product.[277]



Scheme 3-54. Recycle of dipyridinyl disulfide from thiopyridinone[277]

§ 3.3.6. Methyl-Branched Disulfide Linkers

As discussed above, the purpose to install methyl substitution at the alkyl chain is aiming at steric hindrance, which may stabilize the disulfide linkers. Consequently, mono-methyl-branched alkyl chain and corresponding disulfide linkers were prepared.[279,280] The route shown in Scheme 3-55 resulted in racemic compounds, but an enantiomeric pure isomer was also reported.[234]

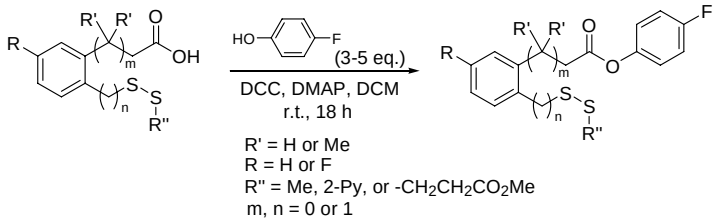


Scheme 3-55. Synthesis of methyl-branched disulfide linkers [260,279,280]

Therefore, starting from γ-valerolactone (**3-80**) and borane tribromide (BBr_3), 4-bromopentanoic acid **3-82** was obtained in good yield *via* the intermediate **3-81**.[279] After reaction with thiourea in methanol under reflux condition, sulfur was embedded into the molecule and the corresponding the methyl ester was formed.[280] After basic hydrolysis, crude **3-84** was directly subjected to the next step to yield **3-85**, which could be stored safely. Following previous procedures, **3-87** was obtained in very high yield after two steps. (Scheme 3-55) The applications of such branched linkers are easily envisioned.

§ 3.3.7. Synthesis of Linker Esters with 4-Fluorophenol

The synthesis of the 4-fluorophenol ester with a linker was straightforward, which was usually done in the presence of 3 to 4 equivalents of phenol together with DCC and DMAP (Scheme 3-56).



Scheme 3-56. Synthesis of 4-fluorophenol ester

Figure 3-15 lists all 4-fluorophenol esters I prepared. The purities of some of them were determined by HPLC for the kinetic study on release of 4-flurophenol.

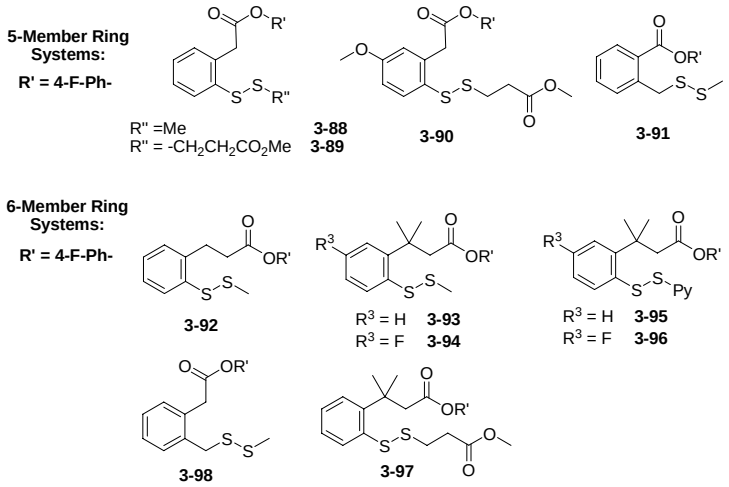
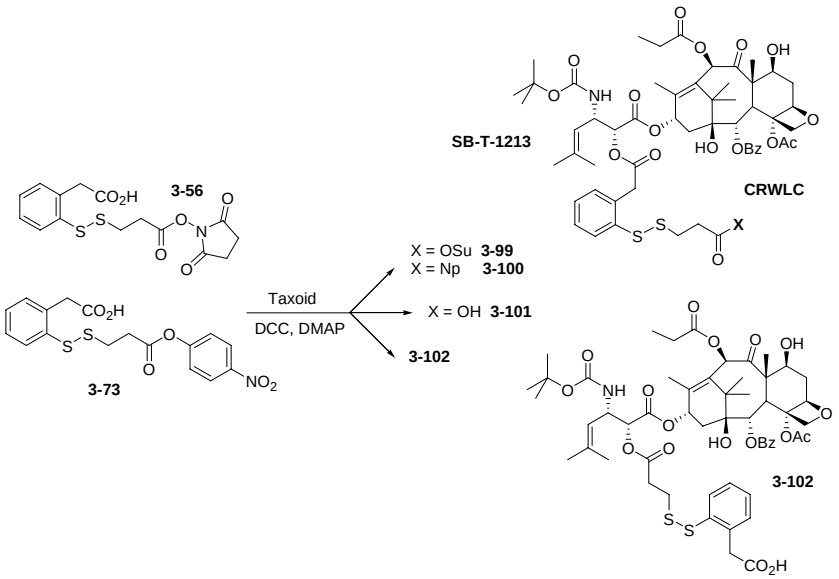


Figure 3-15. Structures of 4-fluorophenol esters

§ 3.3.8. Coupling-Ready Warhead-Linker Construct (CRWLC)

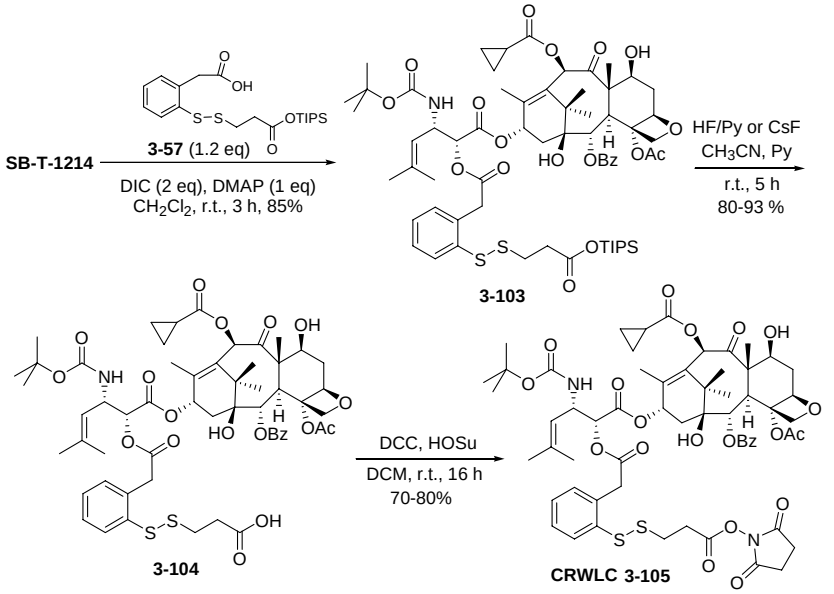
The Coupling-Ready Warhead-Linker Constructs (CRWLC) were prepared from direct reactions between **3-56** and taxoids in the presence of DCC (or DIC) and DMAP. (Scheme 3-47) The coupling did not proceed without DMAP. However, the reaction sometimes was not clean even though two different activated esters were applied, *i.e.*, **3-56** and **3-73**. Sometimes the loss of the OSu or ONp moiety was observed in NMR and

LCMS, such as **3-101**, which led to the confusing question: Was it **3-101** or indeed **3-102**? It was suspected that DMAP might partially activate OSu or ONp (as it actually does in amide formation), thus, the taxoid then might be coupled to the undesired terminal, *e.g.*, **3-102**. Consequently, the purification was troublesome.



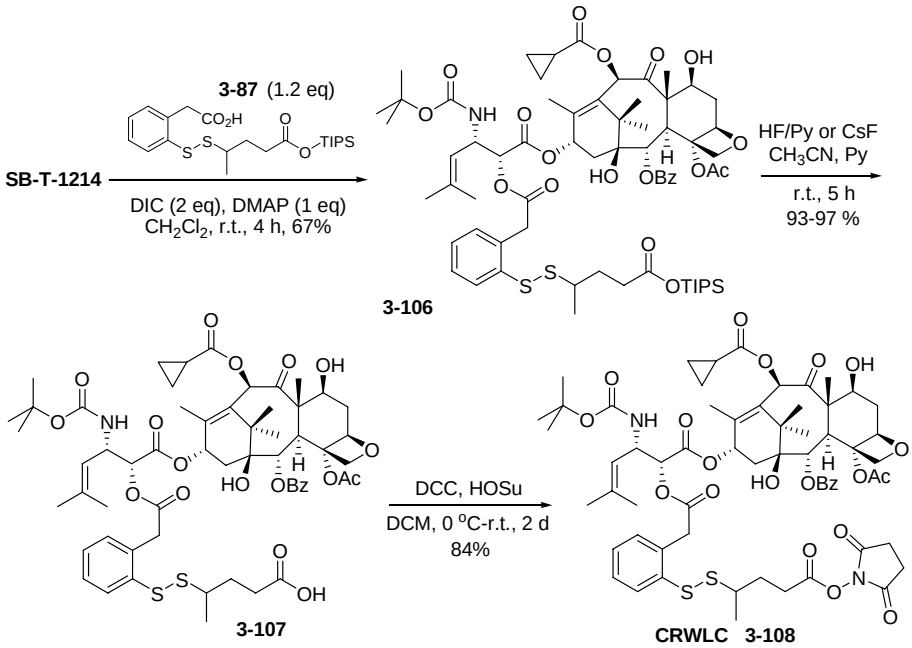
Schem 3-57. Direct coupling between activated ester and taxoid

Introduction and development of **3-57** solved the problem. Although the new route has more steps, the quality of the final CRWLC was guaranteed. (Scheme 3-58) Eventually, both methods showed same analytical results of the final desired CRWLC compounds.



Scheme 3-58. New route towards CRWLC by **3-57**

Moreover, CRWLC containing methyl-branched disulfide linker was also synthesized successfully by similar method (Scheme 3-59).



Scheme 3-59. CRWLC containing methyl-branched linker

CRWLC **3-99**, **3-105** and **3-108** have been successfully applied to the TTDDS, where polyunsaturated fatty acids and aptamers serve as TTMs, respectively.[239] Experimentally, **3-105** and **3-108** were connected to biotin (TTM), which will be discussed in the next Chapter in this dissertation. More examples have been reported in a recent publication.[219]

§ 3.4. Summary and Conclusion

Tumor-targeting prodrug (TAP) and tumor-targeting drug delivery systems (TTDDS) have been emerging and developing rapidly in recent years. These two concepts have been elaborated in this Chapter. The essentials are defining a specific tumor-targeting moiety (TTM) and a stable but efficient linker component.

Based on a new hypothesis, second-generation disulfide-containing linkers have been designed, synthesized, and evaluated in model reactions. While the first-generation disulfide linkers were not able to release the original taxoid from the taxoid-linker conjugate and resulting in compromised cytotoxicity, the novel self-immolative linkers, because of their distinguished structures and different connecting position on the taxoid, can indeed liberate the intact taxoid from the conjugate and thus the potency of taxoid is fully restored. The model reactions were monitored carefully by ^{1}H NMR, ^{19}F NMR, TLC, HPLC, and GC-MS. A series of delicate experiments provided the proof of concept, *i.e.*, smooth cleavage of a disulfide bond by intracellular thiol source, fast intramolecular thiolactonization, and the rapid release of free cytotoxic agent. In addition, these bifunctional disulfide linker systems are versatile and can be easily modified to adapt various drug molecules and TTMs.

With the success in getting proof of concept, some important kinetic factors involved in this drug-release process, such as reaction rate, susbstituent and pH effect, were investigated after suitable and meaningful substrates were synthesized.[239,281] Although the study is not completely finished, the current results have already shed some light on the design of better bifunctional linker candidates for future use.

Meanwhile, the "coupling-ready warhead-linker construct" (CRWLC) has been conceived as an extension of the new disulfide linkers and TTDDS. The CRWLC can serve in TTDDS as both crucial synthetic building blocks and a key cytotoxic segment. While the synthesis of a taxoid-containing CRWLC is established after several attempts, some taxoid-containing CRWLCs have been successfully applied to a variety of TTMs, including polyunsaturated fatty acid (PUFA), folic acid, monoclonal antibody (mAb), aptamer and biotin.[219,239] The biotin-mediated TTDDS will be discussed in the next Chapter in this dissertation. It is hopeful that these new CRWLC-containing disulfide linkages and taxoids will provide fruitful utilities in the battle against cancer in the near future.

§ 3.5. Experimental Section

General Methods: ^{1}H, ^{13}C and ^{19}F NMR spectra were measured on a Varian 300 or 400 MHz NMR spectrometer. The melting points were measured on a "Uni-melt" capillary melting point apparatus from Arthur H. Thomas Company, Inc., which were not corrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. GC-MS analyses were performed on an Agilent 6890 Series GC system equipped with the HP-5HS capillary column, (50 m X 0.25 mm, 0.25 um) and with the Agilent 5973 network mass selective detector. LC-MS analyses were carried out on an Agilent 1100 Series Liquid Chromatograph Mass Spectrometer (Agilent Technologies, Palo Alto, CA). TLC analyses were performed on Merck DC-alufolien with Kieselgel 60F-254, and were visualized with either of the following methods: UV light, iodine chamber, 10% sulfuric acid in ethanol, 10% ceric sulfate and 15% sulfuric acid in water, and 10% phosphomolybdic acid in ethanol. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). Chemical purity was determined with a Waters HPLC assembly consisting of dual Waters 515 HPLC pumps, a PC workstation running Millennium 32, and a Waters 996 PDA detector, using a Curosil-B column from Phenomenex, employing CH_3CN/water as the solvent system with a flow rate of 1 mL/min, or the Agilent HPLC system. Chiral HPLC analysis for the determination of enantiomeric excess was carried out with a Waters HPLC assembly, comprising Waters M45 solvent delivery system, Waters Model 680 gradient controller, Water M440 detector (at 254 nm) equipped with a Spectra Physics Model SP4270 integrator. The system uses a Daicel-Chiral OD chiral column (25 x 0.46 cm *i.d.*), employing hexane/2-propanol (90/10 or 95/5) as the mobile phase with a flow rate of 1.0 ml/min. IR spectra were measured on a Shimadzu FTIR-8400s spectrophotometer. High-resolution mass spectrametric analyses were conducted at the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

Chemicals and Materials: The chemicals were purchased from Sigma-Aldrich or Fischer (including Acros) Company. 10-Deacetyl baccatin III (DAB) was donated by Indena, SpA, Italy. All solvents, right before use, were distilled under nitrogen or argon atmosphere unless mentioned. Thus, dichloromethane (DCM) and methanol were dried over calcium hydride. Toluene and benzene were dried over sodium metal. Diethyl ether and tetrahydrofuran (THF) were dried over sodium with benzophenone as the indicator. Toluene, THF, diethyl ether, and DCM were also purified through PURE SOLVTM from Innovative technology Inc., and used without further purification. Anhydrous DMF was purchased from EMD, and used as it is. The glasses were dried in oven at 110 ^{o}C and allowed to cool to room temperature in a desiccator over "Drierite" (calcium sulfate) before use.

3H-Benzo[b]thiophen-2-one (3-9):[31]

To a solution of thianaphthene-2-boronic acid (3.09 g, 17 mmol) in EtOH (30 mL) was added hydrogen peroxide (30%, 5.6 mL) dropwise. The color was changed from pink to red. After stirring for 8 h, the solution was carefully evaporated in vacuum. Then the crude residue was dissolved in saturated NaCl solution, and extracted by $CHCl_3$ three times. The organic layers wwere combined and dried over $MgSO_4$. After removal of the

solvent and purification on a silica gel column (hexane/EtOAc = 20/1), pure **3-9** was obtained (2.25 g, 86.5% yield) as slightly yellow solid: mp 45-46 ˚C [lit.[31] 43.5-44.0 ˚C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (s, 2 H), 7.2-7.4 (m, 4 H).

2-Mercaptophenylacetic acid (3-12):

To a solution of **3-9** (310 mg, 2.0 mmol) in THF (10 mL) and H_2O (2 mL) was added LiOH hydrate (508 mg, 12 mmol) in H_2O (8 mL). Then, the whole mixture was kept at 60 ˚C for 16 h. After cooled down, the solution was diluted using H_2O (5 mL) and diethyl ether (10 mL). After separation, the aqueous layer was adjusted to pH 2 by HCl (2 mol/L), followed by diethyl ether extraction. The organic layer was separated, washed with saturated NaCl, and dried over Na_2SO_4. After the solvent was removed in vacuum, the residue was purified on a silica gel column (hexane/EtOAc = 10/1) to give pure **3-12** (302 mg, 90% yield) as golden solid or yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (s, 1 H, S-H), 3.83 (s, 2 H), 7.18-7.29 (m, 3 H), 7.41 (m, 1 H), 10.10 (b, 1 H).

Sometimes, 2,2'-(2,2'-disulfanediylbis(2,1-phenylene))diacetic acid (dimer of **3-12**) was observed as by-product in the reaction where **3-12** was involved: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (s, 4 H), 7.17-7.32 (m, 6 H), 7.43 (d, J = 7.8 Hz, 2 H), 10.60 (br, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.03, 128.29, 129.49, 131.22, 134.60, 136.33, 177.69.

Methyl 2-(3-methoxyphenyl)acetate (3-14):[242]

To a solution of 2-(3-methoxyphenyl)acetic acid (**3-13**, 2.60 g, 15.7 mmol) in MeOH (20 mL) was added conc. H_2SO_4 (4.0 mL) at room temperature. After stirring for 23 h, the solvent was evaporated, and the residue was diluted by K_2CO_3 and Et_2O while pH was 1. Ether layer was washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. After removal of ether, desired compound **3-14** was obtained (2.68 g, 95% yield) as colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (s, 2 H), 3.69 (s, 3 H), 3.80 (s, 3 H), 6.81-6.87 (m, 3 H), 7.24 (t, J = 6.0 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.1, 51.9, 55.1, 112.6, 114.9, 121.5, 129.4, 135.3, 159.7, 171.8. All data were in agreement with literature values.[242]

Methyl 2-(2-(chlorosulfonyl)-5-methoxyphenyl)acetate (3-15): [242]

To a flask containing **3-14** (2.22 g, 12.3 mmol) was added sulfurochloridic acid (3.2 mL, 46.7 mmol) at 0 ˚C. After 30 min at 0 ˚C and 1h at room temperature, the reaction was quenched by ice-water (50 mL), and the solution was extracted by chloroform (40 mL x 3). The organic layer was washed by water and brine till pH became 6. After removal of solvent, the desired product was obtained (2.15 g, 63% yield) as white waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (s, 3 H), 3.91 (s, 3 H), 4.13 (s, 2 H), 6.9-7.0 (m, 2 H), 8.05 (dd, J = 8.1, 1.2 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.5, 52.4, 55.9, 112.8, 119.4, 131.9, 135.0, 135.9, 164.6, 170.2. All data were in agreement with literature values.[242]

5-Methoxybenzo[b]thiophen-2(3H)-one (3-16):[242]

To a suspension of zinc (2.0 g, 30 mmol) in water (5.5 mL) was slowly added **3-15** (1.5 g, 5.4 mmol) in three portions. After stirring at room temperature for 2 h, the mixture was heated to 60 ˚C for 1 h. After the reaction mixture was cooled down, zinc

(0.30 g) was added. Then, a solution of hydrochloric acid (13.5 mL) in water (3.5 mL) was added to the mixture at 0-10 ˚C. After stirring for 18 h at room temperature, zinc (1.3 g) was added and the mixture was refluxed for 2 h. The aqueous layer was extracted by ethyl ether (50 mL x 3). The organic layer was washed by saturated brine to pH 6, and dried over sodium sulfate. After purification on a silica gel column (hexane/EtOAc = 20/1), the desired product was isolated (494 mg, 50% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3 H), 3.93 (s, 2 H), 6.8-6.9 (m, 2 H), 8.05 (m, 1 H). All data were in agreement with literature values.[242]

2-(2-Mercapto-5-methoxyphenyl)acetic acid (3-17):

To a solution of **3-16** (176 mg, 1.0 mmol) in THF (3 mL) was added LiOH hydrate (252 mg, 6 mmol) in H_2O (3 mL) at 60 ˚C. After stirring for 16 h and cooling down, the solution was diluted with H_2O (2 mL) and diethyl ether (80 mL). The pH was adjusted to 2 by HCl (2 mol/L). The organic layer was separated, washed with saturated NaCl, and dried over Na_2SO_4. After the solvent was removed in vacuum, the crude product was obtained (192 mg, quant. yield) as white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30 (s, 1 H, SH), 3.80 (s, 3 H), 3.86 (s, 2 H), 6.76 (dd, *J* = 8.4, 2.7 Hz, 1 H), 6.84 (d, *J* = 2.7 Hz, 1 H), 7.40 (d, *J* = 8.4 Hz, 1 H). The crude sample was used for the next step without further purification.

1-(Phenylthio)propan-2-one (**3-20**)**:**

To a solution of thiophenol (0.50 mL, 5 mmol) and triethylamine (TEA) in DCM was added chloroacetone (0.48 mL, 5.5 mmol) at 0 ^{o}C. White precipitations appeared immediately. After stirring at room temperature for 3 h, the mixture was diluted by ether, and the organic layer was washed by citric acid, sodium bicarbonate, and brine. Removal of the solvents gave crude **3-20** (790 mg, 95% yield) as colorless oil, which was used in the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (s, 3 H), 3.66 (s, 2 H), 7.2-7.4 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.94, 44.64, 126.86, 129.13, 129.49, 134.65, 203.49. All data were in agreement with literature values.[282]

3-Methylbenzo[b]thiophene (**3-21**)**:**

To a suspension of $AlCl_3$ (1.33 g, 10 mmol) in DCM was injected **3-21** (790 mg, 4.8 mmol) at 0 ^{o}C. After stirred for 5 h at room temperature, the reaction was quenched by ice-water. The aqueous layer was extracted by ether, which was washed by diluted HCl and brine. After removal of solvent and purification on a silica gel column (hexane/EtOAc = 40/1), the desired **3-21** (472 mg, 67% yield) was obtained as colorless oil [bp 72-74 ^{o}C at 2 mm Hg; data from Aldrich catalog 2005-2006, pp 1580]: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.51 (d, *J* = 0.9 Hz, 3 H), 7.13 (q, *J* = 0.9 Hz, 1 H), 7.39-7.49 (m, 2 H), 7.78 (m, 1 H), 7.92 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.83, 121.41, 121.64, 122.70, 123.76, 124.02, 132.05, 139.60, 140.22. All data were in agreement with literature values.[283]

3,3'-Dimethyl-3H,3'H-[3,3']bi[benzo[b]thiophenyl]-2,2'-dione (3-24), 3-Hydroxy-3-methyl-3H-benzo[b]thiophen-2-one (3-23), and **3-Methyl-3*H*-benzo[b]thiophen-2-one (3-18)**

To a solution of **3-21** (469 mg, 3.2 mmol) in ether (6 mL) was added *n*-butyllithium (1.6 M in hexane, 2.18 mL, 3.5 mmol) at 0 ^{o}C. The brown solution became milky after 10 min. After stirring at room temperature for 2 h, tributyl borate (1.02 mL, 3.8 mmol) in ether (3 mL) was injected to the solution at 0 ^{o}C. Brown color disappeared quickly, and the whole solution became yellowish and finally cloudy after stirring for 2 h at room temperature. The reaction was quenched by adding HCl (6 M, 4mL) and water (4 mL) at 0 ^{o}C. After another 2 h at room temperature, the solution was extracted by ether, which was washed by water and brine, and dried by $MgSO_4$. White solid (**2-22**, 520 mg, crude) was obtained after column chromatography over silica gel (hexane/EtOAc = 5/1 to 2/1). NMR of the crude product was messy and two spots showed up in TLC with long tails.

The above mixture was subjected into H_2O_2 (35 %, 1.0 mL, 12 mmol) and ethanol (5 mL). After overnight, the solution was diluted with water (20 mL), wherein the pH was round 4. The aqueous layer was extracted by ether (40 mL x 3), and the organic layers were washed by brine and dried over $MgSO_4$. Separation by silica gel column gave two components.

Component 1: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, *J* = 7.5 Hz, 3 H), 1.70 (s, 2 H), 3.80 (q, *J* = 7.2 Hz, 1 H), 7.0-7.15 (m, 2 H), 7.2-7.4 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.10, 18.80, 51.96, 62.51, 122.13, 122.85, 124.36, 125.62, 125.77, 126.17, 128.25, 128.73, 133.74, 135.24, 137.67, 138.07, 206.60, 207.09. It seemed there were two compounds based on NMR analysis. One of them seemed to be **3-18**,[284] and other one seemed to be **3-23** (reported in literature but with incomplete data[244]). But further purification or analysis has not been done.

Component 2: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66 (s, 3 H), 6.62-6.25 (d, *J* = 7.8 Hz, 1 H), 7.10 (m, 1 H), 7.23-7.33 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.55, 62.96, 122.89, 125.44, 125.71, 129.09, 135.78, 137.66, 205.60. Some crystals were obtained in chloroform after slow evaporation. The X-ray crystallography of the crystal showed that this product was **3-24**. The crystallography data will be reported in the future.

3-(Hydroxymethylene)benzo[b]thiophen-2(3H)-one (**3-25**):[245]

N,*N*'-Diphenylformimidamide (HC(=NPh)NHPh, 1.14 g, 5.7 mmol) and benzo[b]-thiophen-2(3H)-one (847 mg, 5.65 mmol) were dissolved in absolute EtOH. The yellow solution was refluxed for 2 h, during which yellow precipitation appeared. After filtration, the yellow cake was washed by cold EtOH and dried over $MgSO_4$. Evaporation of the solvent gave 3-((phenylimino)methyl)benzo[b]thiophen-2(3H)-one (990 mg, 70% yield) as yellow powder

The yellow powder was treated with KOH (1.14 g, 21 mmol) in H_2O (20 mL) and EtOH (12 mL) at 100 ^{o}C for 2 h, which gave a clear yellow solution. The basic solution was acidified by aq. HCl. The crude was obtained after filtration. After recrystallization from ethyl acetate and hexane, there were still two spots showed up on TLC plate. The crude **3-25** (627 mg) was used for the next step without further purification, but the data of this compound were available in literature.[245]

3-Methylbenzo[b]thiophen-2(3H)-one (**3-18**)**:**[284]

3-(Hydroxymethylene)benzo[b]thiophen-2(3H)-one (**3-25**, 400 mg) was dissolved in HOAc (5 mL), and heated to 85 ^{o}C. Then, $NaBH_4$ (590 mg) was added in two portions.

After 90 min, the reaction was quenched with water, and extracted by ethyl ether. The pure **3-18** (214 mg, 58% yield) was obtained after column chromatography on silica gel (hexane/EtOAc = 20/1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, *J* = 7.8 Hz, 3 H), 3.80 (q, *J* = 7.8 Hz, 1 H), 7.2-7.4 (m, 4 H). All data were in agreement with literature values.[284]

_S_-Phenyl thiocinnamate (3-33):[258,259]

To a solution of cinnamic acid (1.48 g, 10 mmol) in CH_2Cl_2 (30 mL) was added $SOCl_2$ (1.2 mL, 15 mmol) dropwise at room temperature, and the solution was reflux for 4 h. After concentration *in vcuo*, the residue was dissolved in toluene (15 mL). To this solution was added a solution of thiophenol (1.35 mL, 12 mmol) and pyridine (1.4 mL, 17 mmol) in toluene (5 mL). The reaction mixture was left overnight at room temperature and after washed by water, diluted HCl and saturated NaCl solution, the organic layer was removed *in vacuo*. Recrystallization of the residue from EtOAc and hexane gave **3-33** (1.74 g, 73% yield) as white solid: mp 91-92 ^{o}C [lit.[46] 92-93 ^{o}C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.8 (d, *J* = 16 Hz, 1 H), 7.4-7.5 (m, 10 H), 7.6 (d, *J* = 16 Hz, 1 H). All data are in agreement with literature values.[258,259]

Thiocoumarin (3-32):[258]

Compound **3-33** (1.00 g, 4.2 mmol) was mixed with anhydrous $AlCl_3$ (2.83 g, 21 mmol) and the mixture was kept 80 ^{o}C for 5 h. TLC was used to monitor the disappearance of the thiocinnamate. The reaction was quenched by an aqueous solution of HCl and ice. The aqueous layer was extracted by CH_2Cl_2. After drying over anhydrous $MgSO_4$, the solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (hexane/EtOAc = 5/1) to give **3-32** (557 mg, 83% yield) as a white or pink solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.5 (d, *J* = 12 Hz, 1 H), 7.4-7.6 (m, 4 H), 7.7 (d, *J* = 12 Hz, 1 H). All data are in agreement with literature values.[258]

3, 4-Dihydrothiocoumarin (3-29):[246]

Reduction of thiocoumarin **3-32** (82 mg, 0.5 mmol) in MeOH (10 mL) was performed using $NaBH_4$ (460 mg, 85 mmol) and Cu_2Cl_2 (149 mg, 0.75 mmol) with magnetic stirring at 0 ^{o}C. After 2 h, the reaction was quenched by 5% HCl solution and the aqueous layer was extracted by ether (60 mL x 3). After evaporation of ether, purification on silica gel column (hexane/EtOAc = 30/1) gave **3-29** (46 mg, 78% yield based on 70% conversion) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.8 (m, 2 H), 3.0 (m, 2 H), 7.1-7.3 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.6, 39.4, 126.90, 126.92, 127.4, 128.6, 131.8, 134.0, 200.4. All data are in agreement with literature values.[250]

Meanwhile, **3-32** (23 mg) was recycled after column purification.

_S_-Phenyl 3, 3-dimethylthioacrylate (3-42-1):[259]

To a solution of 3,3-dimethylacrylic acid (1.65 g, 16.5 mmol) and 2 drops DMF in CH_2Cl_2 (50 mL) was added $SOCl_2$ (7.3 mL, 100 mmol) dropwise at room temperature and the solution was reflux for 4 h. After evaporation *in vacuo*, the residue was dissolved in THF (50 mL), and to this solution was added a solution of thiophenol (1.54 mL, 15 mmol) and pyridine (2.83 mL, 35 mmol) in THF. The reaction was left overnight at room temperature, and the solvent was evaporated to dryness. The residue was dissolved

in Et_2O, washed by water, diluted HCl and saturated NaCl solution. Removal of the organic layer *in vacuo* gave **3-42** (2.77 g, 96% yield) as brown oil, which was pure based on TLC anaylsis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.9 (s, 3 H), 2.15 (s, 3 H), 6.0 (m, 1 H), 7.4 (m, 5 H). All data are in agreement with literature values.[259] The crude was used for the next step without further purification.

3-Methylbut-2-enethioic acid *S*-(4-fluorophenyl) ester (3-42-2): 85% yield; sticky oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.92 (s, 3 H), 2.15 (s, 3 H), 6.06 (m, 1 H), 7.05 (t, *J* = 8.7 Hz, 2 H), 7.54 (dd, *J* = 7.2 Hz, 5.4 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 27.4, 116.2 (d, *J* = 21 Hz), 121.9, 136.7 (d, *J* = 8 Hz), 156.2, 161.7, 165.0, 187.2, 212.0; ^{19}F NMR (282 MHz, $CDCl_3$) δ -112.0.

4, 4-Dimethyl-2-oxo-3,4-dihydro-2H-1-benzothiopyran (3-31-1):[259]

To a suspension solution of $AlCl_3$ (1.40 g, 10 mmol) in CH_2Cl_2 (10 mL) was added **3-42-1** (1.344 g, 7 mmol) in CH_2Cl_2 (7 mL) at 0 ^{o}C. The mixture was stirred and left overnight at room temperature. The reaction was quenched by an aqueous solution of HCl and ice. The aqueous layer was extracted by CH_2Cl_2. After dried over anhydrous $MgSO_4$, the solvent was removed *in vacuo* to afford **3-31-1** (1.275 g, 96% yield) as oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 6 H), 2.68 (s, 2 H), 7.2-7.4 (m, 4 H). All data are in agreement with literature values.[259]

6-Fluoro-4,4-dimethylthiochroman-2-one (3-31-2): 95% yield; solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 6 H), 2.68 (s, 2 H), 6.94 (m, 2 H), 7.43 (dd, *J* = 9.6, 5.7 Hz, 4 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -115.5.

3-(2-Mercaptophenyl)-3-methylbutanoic acid (3-43-1):

To a solution of **3-31-1** (1.09 g, 5.7 mmol) in THF (25 mL) and H_2O (5 mL) was added a suspension solution of LiOH (1.66 g, 36 mmol) in H_2O (5 mL) at room temperature. After the solution was kept at 60 ^{o}C for 16 h, diluted citric acid solution was used to adjust the pH to 2-3. The aqueous layer was extracted by Et_2O. After removal of the solvent *in vacuo*, a mixture of **3-43-1** and **3-31-1** was obtained, which was used directly in the next step without further purification.

3-(2-Mercapto-5-fluorophenyl)-3-methylbutanoic acid (3-43-2):

A Mixture of **3-43-2** and **3-31-2** was obtained in the same manner and used in the next step directly: ^{1}H NMR for **3-43-2** (300 MHz, $CDCl_3$) δ 1.55 (s, 6 H), 3.08 (s, 2 H), 3.70 (s, 1 H), 6.80 (m, 1 H), 6.96 (m, 1 H), 7.30 (m, 1 H); ^{19}F NMR of **3-43-2** (282 MHz, $CDCl_3$) δ -118.

3-(Pyridin-2-yldisulfanyl)propionic acid (PDP, **3-46**, Carlsson's method**)**: [260]

To a solution of dipyridine disulfide (17.66 g, 80 mmol) in EtOH (100 mL) and HOAc (1.90 mL, 40 mmol) was added $HSCH_2CH_2CO_2H$ (4.24 g, 40 mmol) dropwise at 0^{o}C. After stirring for 12 h and evaporation of the yellow solution in vacuum, chromatography was done by using neutral Alumina (DCM-MeOH-HOAc), and then by silica gel column (hexane/EtOAc = 3/1) to give **3-46** (7.19 g, 81% yield) as white powder or chunks: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.79 (t, *J* = 6.9 Hz, 2 H), 3.06 (t, *J* = 6.9 Hz, 2

H), 7.14 (m, 1 H), 7.66 (m, 1 H), 8.48 (dd, J = 3.3, 1.2 Hz, 1 H), 10.4 (br, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.8, 34.1, 120.5, 121.2, 137.4, 149.4, 159.2, 176.1. All data were in agreement with literature values.[264]

3-(Pyridin-2-yldisulfanyl)propanoic acid (**PDP**, **3-46**, Samukov's Method)[262]

To a solution of dipyridyl disulfide (200 mg, 0.91 mmol) in DCM (1.5 mL) was added sulfuryl chloride (75 μL, 0.94 mmol) in DCM (0.3 mL) dropwise at 0 ^{o}C. After stirring for 40 min at room temperature, the yellow solution was chilled to 0 ^{o}C, and 3-mecaptopropionic acid (0.15 mL, 1.7 mmol) and triethylamine (0.23 mL, 1.7 mmol) in DCM (1.0 mL) was added. After 30 min at room temperature, the solvent was evaporated, and the residue was purified on a silica gel column (hexane/EtOAc = 3/1) to give the desired PDP (191 mg, 57% based on 85% conversion) as white solid. Dipyridyl disulfide (30 mg) was recovered.

In another entry, the first step was stirred at 0 ^{o}C for 1.5 h, and the final yield was 70%.

3-(Pyridin-2-yldisulfanyl)propionic acid 2,5-dioxo-pyrrolidin-1-yl ester (3-47, SPDP):[260,263,266]

To a solution of **3-46** (205 mg, 1 mmol) and HOSu (115 mg, 1 mmol) in pyridine (1.5 mL) was added DCC (227 mg, 1.1 mmol) at 0 ^{o}C. After stirring for 2 h, the solution was kept in refrigerator for 20 h. The urea was removed by filtration with CH_2Cl_2. After column chromatography on silica gel (hexane/EtOAc = 2/1), pure **SPDP** was obtained (105 mg, 36% yield) as waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.84 (s, 4 H), 3.0-3.1 (m, 4 H), 7.20 (m, 1 H), 7.67 (m, 2 H), 8.50 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5, 30.8, 32.7, 119.9, 121.0, 137.3, 149.7, 159.0, 166.9, 168.9. All data were in agreement with literature values. [260,263,266] The yield of this reaction varied from 20% to 76%.

It is worthy to note here that DCU came out of silica gel column when hexane/EtOAc (4/1 or 3/1) was used as the eluant, *i.e.*, DCU came out earlier than SPDP.

Tri*iso*propylsilyl 3-(pyridin-2-yldisulfanyl)propanoate (3-50):

To a solution of PDP (**3-46**, 562 mg, 2.6 mmol) and TEA (0.60 mL, 4.2 mmol) in CH_2Cl_2 (10 mL) was added TIPSCl (0.75 mL, 3.4 mmol) dropwise at 0 ^{o}C. After stirring at room temperature for 1 h, the reaction mxiture was diluted by ether and washed by $NaHCO_3$ (aq, s) and brine. After purification by column chromatography on silica gel (hexane/EtOAc = 15/1), the desired product was obtained (900 mg, 93% yield) as colorless sticky oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (d, J = 7.2 Hz, 18 H), 1.29 (m, 3 H), 2.80 (t, J = 7.2 Hz, 2 H), 3.04 (t, J = 7.2 Hz, 2 H), 7.09 (m, 1 H), 7.66 (m, 2 H), 8.45 (d, J = 8.4 Hz, 1 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 12.07, 17.93, 34.04, 35.51, 119.94, 120.92, 137.26, 149.84, 160.13, 171.61.

The main purpose of running chromatography here was to remove TIPS-Cl, TIPS-O-TIPS and TIPS-OH. Silica gel column could be skipped if the crude is pure enough.

Allyl 3-(pyridin-2-yldisulfanyl)propanoate (3-51):[285]

To a solution of **PDP** (54 mg, 0.25 mmol) in MeOH (2.2 mL) was added Cs_2CO_3 (42 mg, 0.12 mmol). After refluxing for 2h, the solvent was evaporated, and redissolved in DMF (2.0 mL). Then, allyl bromide (54 μL, 0.62 mmol) was added. The solution was

stirred for 18h. After purification by column chromatography on silica gel (hexane/EtOAc = 6/1), the desired product was obtained (51 mg, 80% yield) as oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.78 (t, *J* = 7.2 Hz, 2 H), 3.05 (t, *J* = 7.2 Hz, 2 H), 4.59 (d, *J* = 5.6 Hz, 2 H), 5.22 (d, *J* = 8.4 Hz, 1 H), 5.29 (d, *J* = 17.2 Hz, , 1 H), 5.90 (m, 1 H), 7.08 (m, 1 H), 7.65 (m, 2 H), 8.45 (d, *J* = 4.8 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.5, 33.9, 65.6, 118.7, 119.9, 120.9, 132.0, 137.2, 149.8, 159.9, 171.3.

3-Methyl-3-(2-(pyridin-2-yldisulfanyl)phenyl)butanoic acid (3-52-1):

To a solution of **3-43-1** and **3-31-1** (0.331 g) in THF-H_2O was added 2,2'-dipyridyl disulfide (0.363 g). After 12 h at room temperature, the reaction was quenched by diluted HCl to pH 1-2, followed by extraction with EtOAc. **3-52-1** was separated (57 mg, 12% yield) as white solid by column chromatography on silica gel (DCM/MeOH = 20/1): mp 122-125^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67 (s, 6 H), 3.32 (s, 2 H), 7.11 (m, 1 H), 7.17 (m, 2 H), 7.38 (m, 1 H), 7.5-7.6 (m, 2 H), 7.80 (m, 1 H), 8.44 (d, *J* = 4.5 Hz, 1 H), 105 (broad, 1 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 29.7, 38.6, 44.8, 120.5, 121.3, 127.1, 127.5, 127.6, 128.8, 134.4, 137.4, 144.9, 149.6, 158.7, 175.7.

3-[5-Fluoro-2-(pyridin-2-yldisulfanyl)phenyl]-3-methylbutanoic acid (3-52-2): 40% yield; white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 6 H), 3.32 (s, 2 H), 6.84 (m, 1 H), 7.17 (m, 2 H), 7.35 (m, 1 H), 7.60 (m, 2 H), 8.46 (d, *J* = 3.6 Hz, 1 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.

2-(3-Methoxy-3-oxopropyldisulfanyl)phenylacetic acid (3-53):

To a solution of methyl 3-(pyridin-2-yldisulfanyl)propanoate **3-49** (103 mg, 0.451 mmol) in THF (5.0 mL) was added **3-12** (74.4 mg, 0.442 mmol) in THF (2.5 mL) dropwise at 0 ^{o}C. After stirring for 2-3 h at room temperature, the solvent was removed in vacuum. After purification by column chromatography on silica gel (hexane/EtOAc = 4/1), **3-53** was obtained (65 mg, 51% yield) as sticky oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.71 (t, *J* = 6.9 Hz, 2 H), 2.92 (t, *J* = 6.9 Hz, 2 H), 3.66 (s, 3 H), 3.89 (s, 2 H), 7.25 (m, 2 H), 7.29 (m, 1 H), 7.74 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.98, 33.58, 38.90, 51.87, 127.97, 128.41, 130.54, 131.01, 133.58, 136.69, 172.13, 176.80.

5-Methoxy-2-((3-methoxy-3-oxopropyl)disulfanyl)phenylacetic acid (3-54):

To a solution of methyl 3-(pyridin-2-yldisulfanyl)propanoate **3-49** (173 mg, 0.75 mmol) in THF (3.0 mL) was added **3-17** (143 mg, 0.7 mmol) in THF (1.5 mL) at 0 ^{o}C dropwise. The solution was kept 0 ^{o}C for 30 min and room temperature for 1-2 h. After removal of THF, the residue was purified on a silica gel column (hexane/EtOAc = 4/1), and the desired product was obtained (113 mg, 50% yield) as beige solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.73 (t, *J* = 7.2 Hz, 2 H), 2.89 (t, *J* = 7.2 Hz, 2 H), 3.67 (s, 3 H), 3.80 (s, 3 H), 3.91 (s, 2 H), 6.8-6.9 (m, 2 H), 7.60 (m, 1 H), 9.0 (vb, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.7, 33.9, 39.5, 52.0, 55.5, 114.1, 117.0, 127.5, 135.2, 137.2, 160.4, 172.5, 176.9.

2,5-Dioxopyrrolidin-1-yl 3-(2-hydroxycarbonylmethylphenyldisulfanyl)propanoate (3-56):

To a solution of SPDP **3-47** (312 mg, 1.0 mmol) in THF (10 mL) was added **3-12** (168 mg, 1.0 mmol) in THF (6 mL) dropwise at 0 ^{o}C. After stirring for 2 h, the solvent was removed in vacuum. After purification by column chromatography on silica gel (hexane/EtOAc = 1/1 with 0.5% aq. HCl), **3-56** was obtained (270 mg, 75% yield) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (s, 4 H, OSu), 2.90 (m, 4 H, -CH_2-CH_2-), 3.92 (s, 2 H), 7.18-7.35 (m, 3 H), 7.85 (d, *J* = 7.8 Hz, 1 H), 8.6 (b, 1 H).

2-((3-Oxo-3-(tri*iso*propylsiloxy)propyl)disulfanyl)phenylacetic acid (3-57):

To a solution of **3-50** (900 mg, 2.4 mmol) in THF (10 mL) was added **3-12** (407 mg, 2.4 mmol) in THF (6 mL) dropwise at 0 ^{o}C. After stirring for 2-3 h, the solvent was removed in vacuum. After purification by column chromatography on silica gel (hexane/EtOAc = 5/1), desired product was obtained (868 mg, 84% yield) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, *J* = 7.2 Hz, 18 H), 1.31 (m, 3 H), 2.76 (t, *J* = 6.9 Hz, 2 H), 2.92 (t, *J* = 6.9 Hz, 2 H), 3.90 (s, 2 H), 7.28 (m, 3 H), 7.77 (d, *J* = 8.4 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.05, 17.93, 33.78, 35.53, 39.09, 128.23, 128.62, 131.06, 131.18, 134.00, 137.00, 171.85, 176.55.

2-(Methyldisulfanyl)phenylacetic acid (**3-59**):

To a solution of 2-(2'-mercaptophenyl)acetic acid **3-12** (contributed from **Shuyi Chen**, 196 mg, 1.17 mmol) in ethanol-water (2 mL-1 mL) was added MMTS (0.12 mL, 1.2 mmol) in ethanol (2.0 mL) at 0 ^{o}C. After stirring overnight, the reaction was quenched with water and sodium carbonate, and extracted by DCM. Then the aqueous layer was acidified by HCl to pH 2, and extracted by ether. The extract was washed by water and brine. The residue, after evaporation, was purified by silica gel column (hexane/EtOAc = 8/1) to give **3-59** (164 mg, 65% yield) as waxy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (s, 3 H), 3.90 (s, 2 H), 7.2 (m, 2 H), 7.30 (m, 1 H), 7.76 (d, *J* = 6.0 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.64, 38.83, 127.94, 128.38, 130.58, 131.00, 133.69, 136.68, 176.90.

Synthesis towards 3-60 (Hydrolysis of **3-18** followed by treatment with **MMTS**):

To a suspension of 3-methylbenzo[b]thiophen-2(3H)-one (**3-18**, 110 mg, 0.67 mmol) in H_2O (1.5 mL) and 1,4-dioxane (0.5 mL) was added KOH (1.4 mmol in 0.7 mL H_2O) at 0 ^{o}C. After 10 min at room temperature, the color of the solution changed to green, then became blue after around 30 min. Finally, the color turned purple after no late than 1 h. The hydrolysis was quenched after 2.5 h at room temperature by adding MMTS solution. Thus, MMTS (0.076 mL, 0.80 mmol) in H_2O (0.5 mL) and 1,4-dioxane (1.5 mL) was added at 0 ^{o}C. After stirring at room temperature overnight, the bright blue solution was extracted by ethyl ether. The aqueous layer was acidified to pH 2 by aq. HCl, and was extracted by ethyl ether. The organic layer was washed by water and brine, dried over $MgSO_4$, and concentrated. However, no desired product **3-60** was found. Meanwhile, pink solid was separated after column chromatography on silica gel (hexane/EtOAc = 10/1), which may be responsible for the color change. Its structure needs to be elucidated in the future.

3-Methyl-3-(methylthio)benzo[b]thiophen-2(3*H*)-one (**3-60-1**):

3-60-1 was obtained (84 mg, 60% yield) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.71 (s, 3 H, CCH_3), 1.96 (s, 3 H, SCH_3), 7.2-7.4 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.74 (SCH_3), 22.89 (CCH_3), 60.30, 123.21, 125.45, 127.11, 129.48, 134.24, 138.82, 204.17; MS (ESI) 233.0 (M+Na), 163.0 (M-SCH_3).

2-Methyl-2-(2-methyldisulfanylphenyl)propanoic acid (**3-61**):

To a solution of 3,3-dimethylbenzo[b]thiophen-2(3H)-one **3-19** (contributed from **Shuyi Chen**, 207 mg, 1.16 mmol) in H_2O (1.4 mL) and 1,4-dioxane (0.4 mL) was added KOH (2.4 mmol in 1.2 mL H_2O) at 0 ^oC. The heterogeneous solution was stirred for 5 h at 60 ^oC. MMTS (0.14 mL, 1.4 mmol) in H_2O (0.5 mL) and 1,4-dioxane (2.5 mL) was added at 0 ^oC. After stirred at room temperature overnight, the solution was acidified to pH 2 by aq. HCl, and extracted by ethyl ether. The product was contaminated by MMTS even after column chromatography on silica gel column (hexane/EtOAc = 15/1). Based on NMR analysis, the yield was calculated as 83% after two steps: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 6 H), 2.44 (s, 3 H), 7.2-7.4 (m, 3 H), 7.90 (d, J = 7.5 Hz, 1 H), 10.2 (br, 1 H).

2-(Methyldisulfanylmethyl)benzoic acid (**3-62**):

To a solution of 2-(mercaptomethyl)benzoic acid (contributed from **Shuyi Chen**, 170 mg, 1 mmol) in ethanol-water (4 mL-1 mL) was added MMTS (0.12 mL, 1.2 mmol). After stirring overnight, the reaction was quenched with water, and extracted by ether. The extract was washed by water and brine. The residue, after evaporation, was purified by silica gel column (hexane/EtOAc = 10/1) to give **3-62** (187 mg, 87% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3 H), 4.38 (s, 2 H), 7.40 (m, 2 H), 7.52 (m, 1 H), 8.11 (m, 1 H).

3-(2-Methyldisulfanylphenyl)propionic acid (3-63):

Hydrolysis of **3-29**. To a solution of **3-29** (76 mg, 0.46 mmol) in THF (1.5 mL) and water (1.5 mL) was added LiOH monohydrate (116.8 mg, 2.8 mmol), and the mixture was kept at 60 ^oC overnight. The reaction mixture was acidified by 10% citric acid (aq.) to pH~2, and extracted by ether. Evaporation of ether gave a solid (messy ^{1}H NMR), which was used without further purification.

Reaction with MMTS. The above solid was slowly added to a MMTS (31.5 mg, 0.024 mL, 0.25 mmol) solution in DCM (1.0 mL) and TEA (0.07 ml, 0.51 mmol) at 0 ^oC. After stirring overnight at room temperature, extraction was done by ether at pH 2. After purification on a silica gel column (hexane/EtOAc = 10/1), 32 mg solid was obtained, but ^{1}H NMR indicated that this solid was a mixture of **3-63** and dimer of 3-(2'-mercaptophenyl)propanoic acid with a molar ratio of 1 to 0.6. Based on the molar ratio, 16.4 mg **3-63** was obtained.

3-Methyl-3-(2-methyldisulfanylphenyl)-butanoic acid (3-64):

Methyl methanethiosulfonate (MMTS, 213 mg, 1.7mmol) was added to a solution of a mixture of **3-43-1** and **3-31-1** (3:1 molar ratio; 500 mg) in EtOH (8 mL) and H_2O (3 mL). The solution was stirred overnight at room temperature. Final pH of the solution was about 2. After neutralization with Na_2CO_3, the solution was acidified to pH 2 again. The crude product was extracted by Et_2O. After purification by column chromatography

on silica gel (hexane/EtOAc =10/1), **3-64** was obtained (300 mg, 47% for two steps based on 485 mg **3-31-1** used in the previous hydrolysis reaction) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) for **3-64** δ 1.58 (s, 6 H), 2.43 (s, 3 H, S-SCH_3), 3.14 (s, 2 H), 7.1-7.4 (m, 3 H), 7.94 (d, *J* = 7.8 Hz, 1 H), 10.4 (broad, 1 H, -COOH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 22.6, 29.4, 38.4, 44.5, 126.9, 127.2, 127.4, 130.5, 135.4, 145.7, 176.6.

3-(5-Fluoro-2-methyldisulfanylphenyl)-3-methylbutyric acid (3-65): 76% yield for two steps from mixture of **3-43-2** and **3-31-2**; pale yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 6 H), 2.43 (s, 3 H), 3.09 (s, 2 H), 6.84 (m, 1 H), 7.30 (m, 1 H), 7.72 (m, 1 H), 10.4 (broad, 1 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.

2-(Methyldisulfanylmethyl)phenylacetic acid (**3-66**):

The starting material, *i.e.*, 2-(mercaptomethyl)phenylacetic acid, was supplied (728 mg, 4.0 mmol) by **Shuyi Chen**. Following the same procedure for the preparation of **3-62**, **3-66** was obtained (690 mg, 76% yield) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3 H), 3.82 (s, 2 H), 4.00 (s, 2 H), 7.2-7.4 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.87, 38.27, 40.39, 127.57, 127.99, 131.08, 131.10, 132.51, 135.53, 176.49.

2-(4-Methylbenzenesulfonylthio)phenylacetic acid (3-67):[271]

To a solution of 2-mecaptophenylacetic acid (**3-12**, 168 mg, 1.0 mmol) in MeOH (2 mL) and HCl (2.0 mol/L, 1.0 mL) was added sodium nitrite ($NaNO_2$, 69 mg, 1 mmol) in water (0.5 mL) at 0 ^{o}C. Brown color was formed immediately, and the solution was stirred at 5 ^{o}C for 1 h and sodium *p*-toluenesulfinic acid salt ($CH_3C_6H_4SO_2Na•H_2O$, 356 mg, 2 mmol) in water (2 mL) was added. After 90 min, the solution was diluted and adjusted to pH 1 by water and HCl. The crude product was extracted by DCM and purified on a silica gel column (1% MeOH in DCM) to give **3-67** (200 mg, 60% yield) as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3 H), 3.58 (s, 2 H), 7.19 (d, *J* = 8.4 Hz, 2 H), 7.20 (m, 1 H), 7.28-7.35 (m, 2 H), 7.40 (d, *J* = 8.4 Hz, 2 H), 7.41 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.66, 39.07, 127.55, 128.18, 128.43, 129.54, 131.38, 131.99, 138.41, 139.46, 140.10, 145.05, 176.42; IR (liquid film in DCM, cm^{-1}) 1326, 1145; MS (ESI) 323 (M+H).

3-(4-Methylbenzenesulfonylthio)propanoic acid (3-68): [271]

To a solution of 3-mecaptopropanoic acid (0.087 mL, 1 mmol) in HCl (2.0 mol/L, 1.0 mL) was added sodium nitrite ($NaNO_2$, 69 mg, 1 mmol) in water (0.5 mL) at 0 ^{o}C. Red color was formed immediately, and the solution was stirred at 5 ^{o}C for 40 min and sodium *p*-toluenesulfinic acid salt ($CH_3C_6H_4SO_2Na•H_2O$, 356 mg, 2 mmol) was added. After 90 min, the solution was diluted and adjusted to pH 1 by water and HCl. The crude product was extracted by DCM and purified on silica gel column (1% MeOH in DCM) to give **3-68** (184 mg, 71% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3 H), 2.76 (t, *J* = 6.9 Hz, 2 H), 3.15 (t, *J* = 6.9 Hz, 2 H), 7.33 (d, *J* = 7.8 Hz, 2 H), 7.77 (d, *J* = 8.4 Hz, 2 H), 9.60 (br, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.57, 29.97, 33.73, 126.94, 129.93, 141.25, 145.12, 176.75.

Ethyl 3-(4-methylbenzenesulfonylthio)propanoate **3-69** was reported.[273]

***S*-Phenyl 4-methylbenzenethiosulfonate (3-70)**:[271]

To a solution of benzenethiol (0.10 mL, 110 mg, 1 mmol) in MeOH (2 mL) and HCl (2.0 mol/L, 1 mL) was added sodium nitrite ($NaNO_2$, 69 mg, 1 mmol) in water (0.5 mL) at 0 ^oC. Red color was formed immediately, and the solution was stirred for 10 min and sodium *p*-toluenesulfinic acid salt ($CH_3C_6H_4SO_2Na•H_2O$, 360 mg, 2 mmol) was added. After 4 h, the solution was diluted and adjusted to pH 1 by water and HCl. The crude product was extracted by ether and purified by silica gel column (hexane/EA = 40/1) to give **3-70** as an oil: ^1H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3 H), 7.20 (d, *J* = 8.4 Hz, 2 H), 7.31 (m, 4 H), 7.45 (d, *J* = 8.4 Hz, 2 H), 7.46 (m, 1 H). All data are in accordance with the literature value.[274]

3-(4-Nitrophenyldisulfanyl)propionic acid (3-71): [275]

To a solution of di-4,4'-nitrophenyl disulfide (0.750 g, 2.4 mmol) in THF (40 mL) and MeOH (12.5 mL) was added 3-mercaptopropanoic acid (172 mg, 1.6 mmol) in THF (10 mL) by syringe pump over 30 min at room temperature. After 5min, 2 drops of NaOH solution (10 mol/L, aq) were added, which caused the color of the mixture changing into dark red immediately. The mixture was stirred for one additional hour. Then pH was adjusted to 1 by HCl (2 mol/L, aq), followed by extraction with ether. The organic layer was dried over MgSO4 and evaporated in vacuum. The crude yellow solid was purified by silica gel column (hexane/EtOAc = 6/1) to give pure **3-71** (94 mg, 23% yield) as a yellow solid: ^1H NMR (300 MHz, CDCl3) δ 2.79 (t, *J* = 6.9 Hz, 2 H), 3.01 (t, *J* = 6.9 Hz, 2 H), 7.67 (d, *J* = 9.0 Hz, 2 H), 8.19 (d, *J* = 9.0 Hz, 2 H), 10.7 (br, 1 H).

3-(4-Nitrophenyldisulfanyl)propanoic acid 4-nitrophenyl ester (3-72):

To a DCM (0.7 mL) solution of *p*-nitrophenol (HONp, 42.8 mg, 0.31 mmol) and **3-71** (76 mg, 0.29 mmol) was added DCC (66.4 mg, 0.32 mmol) at 0^oC. After stirring overnight, the mixture was filtrated, followed by purification on a silica gel column to afford **3-72** (101 mg, 90% yield) as a yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 3.05 (t, *J* = 6.9 Hz, 2 H), 3.13 (t, *J* = 6.9 Hz, 2 H), 7.28 (d, *J* = 9.0 Hz, 2 H), 7.67 (d, *J* = 9.0 Hz, 2 H), 8.19 (d, *J* = 9.0 Hz, 2 H), 8.28 (d, *J* = 9.0 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.9, 33.8, 122.2, 124.2, 125.2, 126.1, 145.5, 145.8, 146.5, 154.9, 168.9.

3-(2-Hydroxycarbonylmethylphenyldisulfanyl)propanoic acid 4-nitrophenyl ester (3-73):

From **3-72**: To a solution of **3-72** (38 mg, 0.10 mmol) in THF (0.5 mL) was added **3-12** (16.8 mg, 0.10 mmol) in THF (0.2 mL) dropwise. After stirring for 3.5 h, the solvent was removed in vacuum. **3-73** (26 mg, 66% yield) was obtained after purification on silica gel column (hexane/EtOAc = 3/1) as yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 3.03 (m, 4 H), 3.90 (s, 2 H), 7.2-7.4 (m, 5 H), 7.77 (d, *J* = 7.2 Hz, 1 H), 8.24 (d, *J* = 9.0 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.6, 33.9, 38.9, 122.4, 125.2, 128.2, 128.6, 130.7, 131.2, 133.7, 136.4, 145.4, 155.1, 169.3, 176.6. Meanwhile unreacted **3-72** (about 10 mg) was recovered.

4-Nitrophenyl 3-(pyridin-2-yldisulfanyl)propanoate (3-74):

To a DCM (1.5 mL) solution of *p*-nitrophenol (HONp, 86 mg, 0.63 mmol) and PDP **3-46** (162 mg, 0.75 mmol) was added DCC (195 mg, 0.94 mmol) at 0^oC. After stirring overnight at room temperature, the mixture was filtrated, and the liquid was

evaporated, followed by silica gel column purification (pure DCM as the only eluant) to afford **3-74** (189 mg, 89% yield, purity 95%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06-3.17 (m, 4 H), 7.12 (m, 1 H), 7. 27 (d, *J* = 7.2 Hz, 2 H), 7.65 (m, 2 H), 8.26 (d, *J* = 7.2 Hz, 2 H), 8.47 (d, *J* = 4.8 Hz, 1 H).

2-(3-Allyloxy-3-oxopropyl)disulfanylphenylacetic acid (3-76):

The reaction was carried out in the same manner as that described for the synthesis of **3-53**. The desired product **3-76** (80% yield) was obtained after silica gel column (hexane/EtOAc = 5/1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (t, *J* = 7.2 Hz, 2 H), 2.96 (t, *J* = 7.2 Hz, 2 H), 3.89 (s, 2 H), 4.59 (d, *J* = 5.6 Hz, 2 H), 5.22 (d, *J* = 8.4 Hz, 1 H), 5.29 (d, *J* = 17.2 Hz, , 1 H), 5.90 (m, 1 H), 7.2-7.3 (m, 3 H), 7.77 (d, *J* = 7.2 Hz, 1 H), 9.0 (vb, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.9, 33.7, 38.9, 65.5, 118.5, 127.9, 128.4, 130.5, 131.0, 131.8, 133.6, 136.7, 171.3, 176.8.

Menthol 2-(3-allyloxy-3-oxopropyl)disulfanylphenylacetate (3-77):

To a solution of **3-76** (27 mg, 0.086 mmol) and menthol (16.2 mg, 0.104 mmol) in THF (0.2 mL) was added DCC (19.6 mg, 0.095 mmol) and DMAP (2.0 mg, 0.017 mmol). After stirring for 16 h, the solution was filtered and the solvent was evaporated. The residue was purified by silica gel column (hexane/EtOAc = 20/1) to give the desired product **3-77** (33 mg, 85% yield) as colorless sticky oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72 (d, *J* = 3.6 Hz, 3 H), 0.86 (m, 6 H), 1.0 (m, 2 H), 1.4 (m, 3 H), 1.67 (m, 2 H), 1.80 (m, 1 H), 2.00 (m, 1 H), 2.72 (t, *J* = 7.2 Hz, 2 H), 2.96 (t, *J* = 7.2 Hz, 2 H), 3.82 (m, 2 H), 4.59 (m, 2 H), 4.70 (m, 1 H), 5.23 (d, *J* = 8.4 Hz, 1 H), 5.29 (d, *J* = 17.2 Hz, , 1 H), 5.90 (m, 1 H), 7.2-7.3 (m, 3 H), 7.77 (d, *J* = 7.2 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.3, 20.7, 22.0, 23.4, 26.1, 31.3, 33.0, 33.7, 34.2, 39.6, 40.7, 46.9, 65.4, 74.9, 118.4, 127.7, 128.0, 130.0, 130.8, 131.9, 134.5, 136.5, 170.4, 171.2.

Dipyridinyl disulfide (from **3-79)**:[277]

To a yellow solution of **3-79** (111 mg, 1 mmol) in DCM (12 mL) was added $KMnO_4$ (490 mg, 3.1 mmol). The reaction was finished within 1 h which was monitored by TLC. After filtration and evaporation, the crude solid was pure enough to do further reactions. Its ^{1}H NMR was very clean and matched the commercial reagent very well.

4-Bromopentanoyl bromide (3-81):

This was the intermediate detected when **3-80** was converted to **3-82**. ^{1}H NMR for **3-81** (300 MHz, $CDCl_3$) δ 1.74 (d, *J* = 4.8 Hz, 3 H), 2.08 (m, 1 H), 2.22 (m, 1 H), 3.27 (m, 2 H), 4.13 (m, 1 H).

4-Bromopentanoic acid (3-82):[279]

To a solution of γ-valerolactone (**3-80**, 1.0 mL, 10.6 mmol) in DCM (10 mL) was added BBr_3 (1.0 mol/L in DCM, 20 mL, 20 mmol) at 0 ^{o}C. After stirring for 16 h, the solution was quenched by ice-water, and the pH was changed to 2 by $NaHCO_3$. The aqueous layer was extracted by DCM (100 mL x 3). The organic layers were combined and washed by saturated NaCl till pH 5: After evaporation, desired product was obtained (1.75 g, crude, 80% yield) together with SM (4:1 ratio): ^{1}H NMR (300 MHz, $CDCl_3$) δ

1.74 (d, J = 4.8 Hz, 3 H), 2.14 (m, 2 H), 2.60 (m, 2 H), 4.15 (m, 1 H), 11.27 (vb, 1 H). Literature showed that the mp of this compound is 23.5 ^{o}C.[279]

2-(5-Methoxy-5-oxopentan-2-yl)isothiouronium bromide (3-83):[280]

To a solution of **3-82** (41 mg, 0.23 mmol) in methanol (1.0 mL) was added thiourea (20 mg, 0.27 mmol). After refluxing for 20 h, desired product **3-83** (43.5 mg, 74% yield) was obtained as brown solid after recystallization from MeOH-DCM: ^{1}H NMR (400 MHz, CD_3OD) δ 1.42 (d, J = 6.0 Hz, 3 H), 2.0 (m, 2 H), 2.55 (ddd, J = 7.2, 7.2, 2.0 Hz, 2 H), 3.70 (s, 3 H), 3.77 (m, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 20.7, 31.6, 32.7, 42.5, 52.6, 172.2, 175.2.

4-Mercaptopentanoic acid (**3-84**):

2-(5-Methoxy-5-oxopentan-2-yl)isothiouronium bromide (**3-83**, 2.70 g, 10 mmol) was dissolved in 8.0 M KOH (22 mL), and the solution was kept stirring at 60 ^{o}C for 16 h. After cooling down, the basic solution was neutralized by aqueous HCl to pH 1, and extracted by ethyl ether. The extract was washed by water and brine several times and was dried over $MgSO_4$. Evaporation of organic layer gave **3-84** (778 mg, 58% yield) as slightly yellow but stench oil in good purity: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (d, J = 6.9 Hz, 3 H), 1.45 (d, J = 7.2 Hz, 1 H, S<u>H</u>), 1.70-1.82 (m, 1 H), 1.91-2.03 (m, 1 H), 2.44-2.62 (m, 2 H), 2.96 (m, 1 H), 10.3 (br, 1 H). All data were in agreement with literature.[234]

Some **3-80** was also found after the reaction, which implied cyclization reaction happened as side reaction, in which thiourea served as a good leaving group. This could also have happen when **3-84** was converted into **3-85** (by elimination of PySSH).

4-(Pyridin-2-yldisulfanyl)pentanoic acid (**3-85**):[234]

To a solution of dipyridyl disulfide (3.10 g, 14 mmol) in HOAc (0.15 mL) and MeOH (15 mL) was added **3-84** (778 mg, 5.8 mmol). After stirring overnight, the solvent was evaporated. The crude was purified on a neutral alumina column then silica gel column to give **3-85** (243 mg, in 17% yield) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, J = 6.6 Hz, 3 H), 1.8-2.1 (m, 2 H), 2.54 (t, J = 8.1 Hz, 2 H), 3.04 (m, 1 H), 7.08 (m, 1 H), 7.63 (m, 1 H), 7.72 (m, 1 H), 8.46 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.50, 30.50, 31.29, 46.07, 120.08, 120.72, 137.05, 149.35, 160.58, 178.02. All data were in agreement with literature.[234]

4-(Pyridin-2-yldisulfanyl)-pentanoic acid, Tri*iso*propylsilyl ester (3-86):

In a similar manner to **3-50**, **3-86** was obtained (160 mg, 99% yield) as colorless oil after purification by a silica gel column (hexane/EtOAc = 20/1) from the reaction mixture of **3-85** (97 mg, 0.4 mmol), TIPSCl (1.3 eq) and TEA (1.6 eq): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, J = 7.2 Hz, 18 H), 1.20 (q, J = 7.2 Hz, 3 H), 1.32 (d, J = 6.8 Hz, 3 H), 1.90 (m, 2 H), 2.49 (m, 2 H), 3.00 (hexad, J = 6.8 Hz, 1 H), 7.00 (m, 1 H), 7.64 (dt, J = 8.0, 2.0 Hz, 1 H), 7.68 (d, J = 8.4 Hz, 1 H), 8.38 (d, J = 4.8 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.98, 17.87, 20.60, 31.26, 33.33, 46.29, 119.88, 120.65, 137.03, 149.45, 160.90, 173.05.

4-(2-Hydroxylmethlylcarbonymethyl-phenyldisulfanyl)pentanoic acid, Tri*iso*propylsilyl ester (3-87):

In a similar manner to **3-57**, **3-87** was obtained (83 mg, 90% yield) as colorless oil after purification by a silica gel column (hexane/EtOAc = 5/1) from the reaction mixture of **3-86** (80 mg, 0.2 mmol) and **3-12** (1.0 eq) in THF: ^{1}H NMR (300 MHz, CDCl3) δ 1.05 (d, *J* = 7.2 Hz, 18 H), 1.25 (m, 6 H), 1.80 (m, 1 H), 2.00 (m, 1 H), 2.40 (m, 2 H), 2.89 (hexad, *J* =6.6 Hz, 1 H, S-C<u>H</u>), 3.88 (dd, *J* =23.7 Hz, 2 H), 7.20 (m, 2 H), 7.30 (m, 1 H), 7.80 (d, *J* =7.5 Hz, 1 H), 10.0 (br, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.01, 17.91, 20.52, 31.13, 33.22, 39.10, 46.07, 127.62, 128.39, 130.23, 130.94, 133.31, 137.90, 173.45, 176.54.

4-Fluorophenyl 2-(methyldisulfanyl)phenylacetate (**3-88**):

Following the same procedure as that for the preparation of **3-98**, **3-88** was obtained with 94% yield as colorless sticky oil. HPLC showed 98.5% purity (C18 column, CH_3CN:H_2O = 70:30, 1.0 mL/min).

3-Methyl-3-(2-methyldisulfanylphenyl)butanoic acid 4-fluorophenyl ester (3-93):

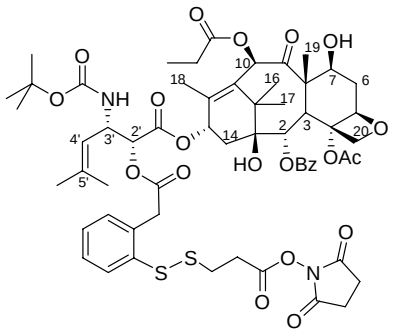
To a solution of **3-64**, (100 mg, 0.4 mmol, 1.0 eq), 4-fluorophenol (54.3 mg, 0.48 mmol, 1.2 eq) and DMAP (24.4 mg, 0.2 eq) in CH_2Cl_2 was added diisopropyl carbodiimide (DIC,76 microliter,.5-2.0 eq) at 0^{o}C. The solution was left overnight with stirring. After filtration to remove the urea, the crude compound was purified on a silica gel column (hexane:EtOAc = 10/1) to give **3-93** (123 mg, 62% yield) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (s, 6 H), 2.52 (s, 3 H), 3.44 (s, 2 H), 6.70 (m, 2 H), 7.00 (t, *J* = 9.0 Hz, 2 H), 7.2-7.3 (m, 2 H), 7.36 (dd, *J* = 7.8 Hz, 1.5 Hz, 1 H), 7.96 (dd, *J* = 7.8 Hz, 1.2 Hz, 1 H); ^{19}F NMR (282 MHz, $CDCl_3$): δ -119.2.

3-Methyl-3-[2-(pyridin-2-yldisulfanyl)phenyl]butyric acid 4-fluoro-phenyl ester (3-95): 66% yield; oil; ^{1}H NMR (300 MHz, $CDCl_3$,) δ 1.74 (s, 6 H), 3.43 (s, 2 H), 6.60 (m, 2 H), 6.93 (m, 2 H), 7.07 (m, 1 H), 7.20 (m, 2 H), 7.42 (m, 2 H), 7.59 (d, *J* = 7.8 Hz, 1 H), 7.77 (m, 1 H), 8.46 (d, *J* = 3.6 Hz, 1 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -119.1.

4-Fluorophenyl 2-(methyldisulfanyl)methylphenylacetate (**3-98**):

To a solution of **3-66** (114 mg, 0.5 mmol), 4-fluorophenol (168 mg, 1.5 mmol) and DMAP (122 mg, 1 mmol) in DCM was added DCC (124 mg, 0.6 mmol) at 0 ^{o}C. After stirring overnight, the mixture was filtered and solvent was removed. The residue was purified on silica gel column (hexane:EtOAc = 10/1) to give **3-98** (142 mg, 88% yield) as colorless sticky oil: HPLC showed 98.3% purity (C18 column, CH_3CN:H_2O = 70:30, 1.0 mL/min).

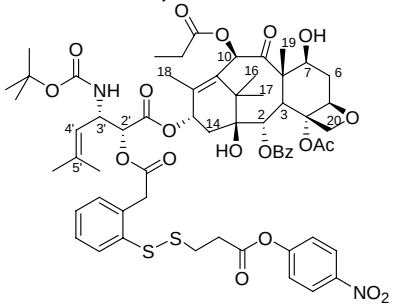
SB-T-1213-Linker-OSu (CRTLC 3-99):



To a DCM (7 mL) solution of **3-56** (40 mg, 0.1 mmol), DMAP (12 mg, 0.1 mmol) and **SB-T-1213** (89 mg, 0.1 mmol) and was added 1,3-diisopropylcarbodiimide (DIC, 0.044 mL, 0.11 mmol) at 0°C. After stirring for 3 h, the solvent was removed in vacuum. After purification on silica gel column (hexane/EtOAc = 1/1 to 1/2), **3-99** was obtained (65 mg, 55% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3 H) (H17/16/10), 1.23 (s, 3 H) (H10/16/17), 1.25 (s, 3 H) (H16/17/10), 1.35 (s, 9 H) (Boc), 1.67 (s, 3 H) (H19), 1.76 (s, 6 H) (isobutenyl), 1.85 (m, 1 H) (H6a), 1.90 (s, 3 H) (H18), 1.90 (s, 1 H) (OH), 2.36 (s, 3 H) (OAc), 2.39 (s, 1 H) (OH), 2.53 (m, 3 H) (H6b, H14), 2.84 (s, 4 H) (OSu), 2.87 (d, *J* =6 Hz, 2 H) (CH_2-CO_2Su), 2.94 (d, *J* =6 Hz, 2 H) (S-CH_2), 3.82 (d, *J* = 7.2 Hz, 1 H) (H3), 4.17 (d, *J* = 8.7 Hz, 1 H) (H20a), 4.18 (s, 2 H) (Ph-CH_2-CO_2), 4.31 (d, *J* = 8.7 Hz, 1 H) (H20b), 4.43 (dd, *J* = 10.6, 6.6 Hz, 1 H) (H7), 4.9-5.0 (m, 4 H) (H3', H4'isoButenyl, H5, H2'), 5.32 (s, 1 H) (NH), 5.67 (d, *J* = 7.2 Hz, 1 H) (H2), 6.17 (t, *J* = 8.7 Hz, 1 H) (H13), 6.31 (s, 1 H) (H10), 7.36 (m, 3 H) (Ph linker), 7.47 (t, *J* = 7.5 Hz, 2 H) (Bz), 7.51 (t, *J* = 7.3 Hz, 1 H) (Bz), 7.80 (d, *J* = 7.2 Hz, 1 H) (Ph linker), 8.09 (d, *J* = 7.4 Hz, 2 H) (Bz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 9.5, 14.7, 18.5, 22.1, 22.4, 25.6, 25.7, 26.6, 27.5, 28.2, 32.6, 33.3, 35.5, 35.7, 43.2, 45.6, 49.0, 58.5, 71.8, 72.1, 74.8, 75.2, 75.4, 76.4, 79.2, 79.9, 81.0, 84.4, 119.9, 128.3, 128.6, 129.0, 129.3, 130.1, 130.8, 131.0, 131.9, 132.5, 133.6, 136.4, 137.9, 143.2, 154.9, 166.4, 167.0, 168.1, 169.0, 169.6, 171.0, 174.6, 204.0 (There should be 53 different carbons, but only 52 showed up in my spectrum.); HRMS (FAB): *m/e* calcd. for $C_{59}H_{72}N_2O_{20}S_2H^+$: 1193.4198; Found: 1193.4183 (Δ = -1.3 ppm).

A by-product (about 36 mg), which lost OSu at the active ester terminus and had almost the same R_f as **SB-T-1213**, was also isolated.

SB-T-1213-Linker-ONp (CRTLC 3-100):



To a solution of **SB-T-1213** (25 mg, 0.030 mmol), DMAP (3.6 mg, 0.030 mmol) and 3-73 (13 mg, 0.033 mmol) in DCM (1.0 mL) was added DIC (7.0 μL, 0.060 mmol) at 0°C. The solution was stirred for 3 h. After filtration and concentration of liquid, the

crude was purified on silica gel column to give **3-100** (10 mg, 22% yield) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3 H) (H17/16/10), 1.23 (s, 3 H) (H10/16/17), 1.25 (s, 3 H) (H16/17/10), 1.35 (s, 9 H) (Boc), 1.67 (s, 3 H) (H19), 1.76 (s, 6 H) (*iso*butenyl), 1.85 (m, 1 H) (H6a), 1.90 (s, 3 H) (H18), 1.90 (s, 1 H) (OH), 2.36 (s, 3 H) (OAc), 2.39 (s, 1 H) (OH), 2.53 (m, 3 H) (H6b, H14), 2.87 (d, *J* =6 Hz, 2 H) (CH_2-CO_2Su in linker), 3.00 (d, *J* = 6.0 Hz, 2 H) (S-CH_2 in linker), 3.82 (d, *J* = 7.2 Hz, 1 H) (H3), 4.17 (d, *J* = 8.7 Hz, 1 H) (H20a), 4.18 (s, 2 H) (Ph-CH_2-CO_2 in linker), 4.31 (d, *J* = 8.7 Hz, 1 H) (H20b), 4.43 (dd, *J* = 10.6, 6.6 Hz, 1 H) (H7), 4.75 (d, *J* = 9.3 Hz, 1 H) (H2'), 4.9-5.0 (m, 3 H) (H3', H4'isoButenyl, H5), 5.32 (d, *J* = 7.2 Hz, 1 H) (NH), 5.67 (d, *J* = 7.2 Hz, 1 H) (H2), 6.17 (t, *J* = 8.7 Hz, 1 H) (H13), 6.31 (s, 1 H) (H10), 7.32 (d, *J* = 9.0 Hz, 2 H) (Nitrophenyl at linker), 7.36 (m, 3 H) (Ph at linker), 7.47 (t, *J* = 7.5 Hz, 2 H) (Bz), 7.51 (t, *J* = 7.3 Hz, 1 H) (Bz), 7.80 (d, *J* = 7.2 Hz, 1 H) (Ph linker), 8.09 (d, *J* = 7.4 Hz, 2 H) (Bz), 8.25 (d, *J* = 9.0 Hz, 2 H) (Nitrophenyl at linker).

A by-product (about 6 mg), which lost Np at the active ester terminus, was also isolated.

SB-T-1214-Linker-OTIPS (3-103):

To a solution of **SB-T-1214** (42 mg, 0.049 mmol), DMAP (1.2 mg, 0.01 mmol) and **3-57** (22 mg, 0.051 mmol) in DCM (2.5 mL) was added DIC (9.2 μL, 0.059 mmol), and the solution was stirred for 16 h at room temperature before the solvent was evaporated. The crude was purified on a silica gel column (hexane/EtOAc = 3/1) to give desired product **3-103** (85 mg, 85 % yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.9-1.05 (m, 20 H, cyclopropyl(2) and TIPS(18)), 1.13-1.21 (m, 2 H, cyclopropyl), 1.15 (s, 3H, C17), 1.26 (s, 3H, C16), 1.29 (m, 3 H, TIPS), 1.35 (s, 9 H, Boc), 1.62 (m, 2 H, OH and C6(1)), 1.65 (s, 3 H, C5'), 1.67 (s, 3 H, C5'), 1.72 (m, 1 H, cyclopropyl), 1.75 (s, 3 H, C19), 1.92 (s, 3 H, C18), 2.24 (m, 2 H, C6(1) and C14(1)), 2.32 (s, 3 H, Ac), 2.52 (m, 1 H, C14(1)), 2.75 (t, *J* = 6.6 Hz, 2 H, linker), 2.95 (t, *J* = 6.6 Hz, 2 H, linker), 3.80 (d, *J* = 6.9 Hz, 1 H, C3), 3.94 (d, *J* = 16.5 Hz, 1 H, linker(1)), 4.07 (d, *J* = 16.5 Hz, 1 H, linker(1)), 4.16 (d, *J* = 8.4 Hz, 1 H, C20(1)), 4.28 (d, *J* = 8.4 Hz, 1 H, C20(1)), 4.42 (dd, *J* = 10.5, 6.9 Hz, 1 H, C7), 4.75-4.97 (m, 4 H, C2', C3', C4' and C5), 5.06 (d, *J* = 8.7 Hz, 1 H, NH), , 5.66 (d, *J* = 6.9 Hz, 1 H, C2), 6.18 (t, *J* = 8.1 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 7.30 (m, 3 H, linker), 7.43 (t, *J* = 7.8 Hz, 2 H, Bz), 7.60 (t, *J* = 7.2 Hz, 1 H, Bz), 7.75 (d, *J* = 7.2 Hz, 1 H, linker), 8.06 (d, *J* = 8.4 Hz, 2 H, Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 1.22, 9.32, 9.53, 9.75, 13.21, 13.90, 15.00, 18.75, 19.32, 22.42, 22.63, 23.69, 25.80, 25.94, 26.91, 28.44, 29.90, 30.86, 32.84, 33.54, 35.67, 35.95, 43.40, 45.84, 49.20, 58.71, 64.56, 72.05, 72.37, 75.05, 75.45, 75.66, 76.62, 79.51, 80.07, 81.22, 84.70, 120.15, 128.57, 128.85, 129.20, 129.51, 130.38, 131.03, 131.27, 132.12, 132.76, 133.82, 136.70, 138.16, 143.58, 155.13, 166.58, 167.21, 168.39, 169.20, 169.86, 171.19, 175.30, 204.30.

SB-T-1214-Linker-OH (3-104):

To a solution of **3-103** (50 mg, 0.040 mmol) in 2 mL of pyridine/acetonitrile (1/1) was added dropwise HF/pyridine (70/30, 0.5 mL) at 0 °C, and the mixture was stirred for 17 h at room temperature. The mixture was then diluted with ethyl acetate (30 mL), washed with aqueous saturated copper sulfate solution (30 mL x 3) and water (30 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude was purified on a silica gel column (hexane/EtOAc = 1/1) to afford desired compound **3-104**

(39 mg, 89% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 3 H), 1.14 (s, 3 H), 1.25 (s, 4 H), 1.34 (s, 9 H), 1.66 (s, 3 H), 1.71 (s, 1 H), 1.75 (s, 6 H), 1.82 (s, 1 H), 1.86(m, 1 H), 1.91 (s, 3 H), 2.31 (s, 1 H), 2.33 (s, 1 H), 2.37 (s, 3 H), 2.60 (m, 2 H), 2.87 (d, *J* = 6 Hz, 2 H), 2.97 (d, *J* = 6 Hz, 2 H), 3.80 (d, *J* = 7.2 Hz, 1 H), 4.17 (s, 2 H), 4.19 (d, *J* = 8.7 Hz, 1 H), , 4.30 (d, *J* = 8.7 Hz, 1 H), 4.43 (dd, *J* = 10.6, 6.6 Hz, 1 H), 4.9-5.0 (m, 4 H), 5.19 (s, 1 H), 5.66 (d, *J* = 7.2 Hz, 1 H), 6.17 (t, *J* = 8.7 Hz, 1 H), 6.29 (s, 1 H), 7.34 (m, 3 H), 7.47 (t, *J* = 7.5 Hz, 2 H), 7.60 (t, *J* = 7.3 Hz, 1 H), 7.80 (d, *J* = 7.2 Hz, 1 H), 8.10 (d, *J* = 7.4 Hz, 2 H).

SB-T-1214-Linker-OSu (3-105):

To a solution of Taxoid-Linker-OH (98 mg, 0.088 mmol) and HOSu (50.8 mg, 0.44 mmol) in THF (0.88 mL) was added DCC (21.8 mg, 0.106 mmol) at 0 ^{o}C. After stirring at 0 ^{o}C for 1.5 h, the reaction flask was kept at 4 ^{o}C in the refrigerator overnight. The reaction mixture was filtered through celite, and the white solid was washed by EtOAc. The organic solutions were combined, washed by water and brine, and dried over $MgSO_4$. After evaporation of the solvents, the crude (145 mg) was purified on a silica gel column (hexane/EtOAc = 1/2 or 1-2% MeOH/DCM) to give desired product (32 mg, 62% yield based on 50% conversion) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.985 (m, 2 H), 1.141 (m, 4 H), 1.25 (br s, 4 H), 1.33 (s, 9 H), 1.65 (s, 3 H), 1.69-1.93 (m, 9 H), 1.8-1.9 (m, 1 H), 1.90 (s, 3 H), 2.35 (m, 4 H), 2.52 (m, 1 H), 2.64 (d, *J* = 4.0 Hz, 1 H), 2.83 (s, 4 H, OSu), 3.02 (m, 4 H, linker), 3.79 (d, *J* = 7.2 Hz, 1 H), 4.00 (dd, *J* = 16.4 Hz, 2 H, linker), 4.17 (d, *J* = 8.1 Hz, 1 H), 4.29 (d, *J* = 8.1 Hz, 1 H), 4.42 (m, 1 H), 4.82 (d, *J* = 8.4 Hz, 1 H), 4.95 (m, 3 H), 5.09 (d, *J* = 8.4 Hz, 1 H), 5.66 (d, *J* = 6.8 Hz, 1 H), 6.17 (t, *J* = 8.4 Hz, 1 H), 6.27 (s, 1 H), 7.28-7.38 (m, 3 H, linker), 7.47 (t, *J* = 8.0 Hz, 2 H), 7.59 (t, *J* = 7.6 Hz, 1 H), 7.77 (d, *J* = 7.6 Hz, 1 H, linker), 8.11 (d, *J* = 7.6 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.28, 9.47, 9.72, 13.17, 14.95, 18.66, 22.37, 22.57, 23.66, 25.75, 25.89, 26.85, 28.39, 29.85, 31.15, 32.40, 35.65, 38.89, 43.35, 45.83, 49.20, 58.62, 68.12, 72.03, 72.24, 75.14, 75.39, 75.63, 76.55, 79.44, 80.00, 81.13, 84.66, 120.12, 128.54, 128.79, 128.81, 129.47, 130.33, 130.82, 131.39, 132.59, 133.74, 133.95, 136.09, 137.95, 143.57, 155.07, 167.14, 168.23, 169.12, 169.79, 170.25, 175.18, 204.26.

The unreacted Taxoid-Linker-OH (50 mg) was recycled after column (hexane/EtOAc = 1/4).

SB-T-1214-MeLinker-OTIPS (3-106):

To a solution of MeLinker-OTIPS (83 mg, 0.18 mmol), SB-T-1214 (154 mg, 0.17 mmol) and DMAP (22 mg, 0.16 mmol) in DCM (9.0 mL) was added DIC (0.056 mL, 0.36 mmol) at 0 ^{o}C. After stirring at r.t. for 4 h, no precipitation was observed. TLC analysis showed that the reaction was not finished yet because SB-T-1214 was still left, but two new spots containing taxoid were also observed on the TLC plate. Thus, the reaction was stopped and the reaction mixture was concentrated. The residue was purified on a silica gel column (hexane/EtOAc = 1/1) to afford the desired product (147 mg, 67% yield) as white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, *J* = 7.2 Hz, 20 H, TIPS and Cyclopropane), 1.14 (m, 5 H, TIPS and Cyclopropane), 1.16-1.28 (m, 11 H), 1.34 (s, 9 H), 1.65 (s, 3 H), 1.70 (s, 3 H), 1.71 (s, 3 H), 1.74-1.86 (m, 4 H, cyclopropane, OH, C14 (1), and C6 (1)), 1.89 (s, 3 H), 2.2-2.6 (m, 8 H, OAc, linker (2), OH, C14 (1), and C6 (1)), 2.95 (hex, 1 H, S-C<u>H</u>), 3.79 (d, *J* = 7.2 Hz, 1 H), 3.91-3.98 (dd, *J* = 16.2, 4.2

Hz, 1 H, linker), 4.07-4.12 (dd, *J* = 16.2, 4.2 Hz, 1 H, linker), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.42 (m, 1 H), 4.80 (m, 1 H), 4.85-5.00 (m, 3 H), 5.07 (m, 1 H), 5.66 (d, *J* = 6.9 Hz, 1 H), 6.19 (t, *J* = 9 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 7.2-7.3 (m, 3 H, linker), 7.47 (t, *J* = 7.5 Hz, 2 H), 7.60 (m, 1 H), 7.80 (m, 1 H, linker), 8,12 (d, *J* = 7.5 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.07, 9.28, 9.50, 11.83, 12.9, 14.156, 14.76, 17.73, 18.43, 20.45, 20.50, 22.20, 22.36, 24.89, 25.57, 25.64, 26.65, 28.16, 30.93, 32.92, 32.96, 33.87, 35.38, 35.48, 38.73, 43.13, 45.56, 45.97, 49.11, 58.43, 60.35, 71.77, 72.10, 74.92, 75.21, 75.42, 76.35, 79.23, 79.76, 80.92, 84.46, 119.98, 127.64, 128.26, 128.57, 129.27, 130.08, 130.12, 130.81, 132.40, 133.1, 133.53, 137.35, 137.59, 143.45, 154.87, 166.93, 168.01, 169.57, 170.07, 171.10, 172.93, 175.04, 204.06.

However, the unreacted taxoid came out together with DICU from the silica gel column. Thus, the conversion was not quantified.

SB-T-1214-MeLinker-OH (3-107):

by HF/Py: To a solution of Taxoid-MeLinker-OTIPS (70 mg, 0.054 mmol) in Py (1.2 mL) and CH_3CN (1.2 mL) was added HF/Py (70/30, 0.70 mL) at 0 ^oC. After stirring at room temperature for 18 h, the reaction was quenched with citric acid (5% aq.) and EtOAc. The organic layer was washed with $CuSO_4$, water and NaCl, and dried over $MgSO_4$. The crude residue was purified on a silica gel column (DCM/MeOH = 100/3) to give the desired product (60 mg, 97% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (m, 2 H), 1.06-1.28 (m, 13 H), 1.34 (s, 9 H), 1.65 (s, 3 H), 1.70 (s, 3 H), 1.71 (s, 3 H), 1.74-1.86 (m, 4 H), 1.89 (s, 3 H), 2.2-2.6 (m, 8 H), 3.0 (m, 1 H, S-C<u>H</u>), 3.77 (d, *J* = 6.9 Hz, 1 H), 4.00 (dd, *J* = 16.5 Hz, 2 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.40 (m, 1 H), 4.8-5.0 (m, 4 H), 5.10 (m, 1 H), 5.65 (d, *J* = 6.9 Hz, 1 H), 6.19 (m, 1 H, C13), 6.276 and 6.283 (s, 1 H, C10), 7.2-7.3 (m, 3 H), 7.45 (t, *J* = 7.5 Hz, 2 H), 7.59 (m, 1 H), 7.80 (m, 1 H), 8,08 (d, *J* = 7.5 Hz, 2 H); MS (ESI) for $C_{58}H_{73}NO_{18}S_2H^+$ *calcd* 1136.4, found 1136.4.

Three abnormal proton peaks should be noted in the above ^{1}H NMR. The peak of proton at the C-13 position was not triplet. C-10 proton showed two peaks at 6.28 ppm. The peak of methine (S-CH) moiety in the linker, which should be hexad, was irregular. All of these should be attributed to the introduction of the racemic linker, which resulted in two distereomers.

by CsF[286]: To a solution of crude Taxoid-MeLinker-OTIPS containg some urea (104 mg, 0.080 mmol) in ACN (3 mL) and MeOH (2 mL) was added CsF (24.4 mg, 0.16 mmol) at 0 ^oC, which resulted in a pink-color solution after stirring for 15 min. After 1 h at r.t., the reaction was almost done based on TLC analysis. The reaction mixture was diluted with DCM (5 mL), followed by addition of citric acid (3% aq.), and the pink color disappeared quickly. The reaction mixture was stirred at r.t. for another 1 h, and DCM was used in extraction. The organic layers were combined, washed by water and brine, and dried over $MgSO_4$. After evaporation of solvents, the crude residue (95 mg) was purified on a silica gel column (hexane/EtOAc = 2.5/1 to remove the urea, followed by 2.5-5% MeOH in DCM to flush out the prod). The desired product was obtained (84 mg, 93% yield) as white solid. The NMR was identical to the above one.

SB-T-1214-MeLinker-OSu (CRWLC 3-108):

To a solution of Taxoid-MeLinker-OH (60 mg, 0.052 mmol) and HOSu (30.4 mg, 0.264 mmol) in Py (0.2 mL) and THF (0.1 mL) was added DIC (0.0165 mL, 0.106 mmol) at 0 ^{o}C. After stirring at r.t. overnight, the reaction was not finished yet based on TLC analysis. The reaction mixture was kept at 4 ^{o}C for 1 d to complete the conversion. After evaporation of the solvents, the crude residue was purified on a silica gel column (hexane/EtOAc = 2/1 to 1/1) to give the desired product (54 mg, 84% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (m, 2 H), 1.06-1.28 (m, 13 H), 1.34 (s, 9 H), 1.65 (s, 3 H), 1.70 (s, 3 H), 1.71 (s, 3 H), 1.74-1.86 (m, 4 H), 1.89 (s, 3 H), 2.2-2.6 (m, 6 H), 2.66 (t, *J* = 7.2 Hz, 2 H, C<u>H</u>$_2$-CO_2Su), 2.83 (s, 4 H, OSu), 3.0 (m, 1 H, S-C<u>H</u>), 3.80 (d, *J* = 6.9 Hz, 1 H), 4.00 (dd, *J* = 16.2 Hz, 1 H, linker (1)), 4.08 (dd, *J* = 16.2 Hz, 1 H linker (1)), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.40 (m, 1 H, C7), 4.80 (m, 1 H), 4.95 (m, 3 H), 5.10 (m, 1 H), 5.65 (d, *J* = 7.8 Hz, 1 H), 6.20 (t, *J* = 7.8 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 7.2-7.3 (m, 3 H), 7.47 (t, *J* = 7.5 Hz, 2 H), 7.60 (m, 1 H), 7.80 (d, *J* = 7.5 Hz, 1 H, linker), 8,08 (d, *J* = 7.5 Hz, 2 H).

In another experiment, EDC (2 eq.) was used as the coupling reagent together with HOSu (5 eq.) in THF (concentration of taxoid was 0.1 mmol/mL). But only about 60% conversion was achieved at r.t. after 36 h. The results implied that high concentration in this reaction might be preferred.

§ 3.6. List of References

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Chapter Four

Synthesis and Evaluation of a Biotin-Mediated Tumor-Targeting Drug Delivery System Containing Novel Disulfide Linker and Fluorescent Probe

The purpose of this project is to develop novel tumor-targeting drug delivery system containing taxoid as the warhead, biotin as the tumor-targeting moiety, and disulfide linker as the linkage. With the synergistic functions of the three parts, this system should transfer the cytotoxic agent selectively into cancerous cells, but not normal cells. Introduction of proper fluorescent probes to this system should facilitate the observation of the *in vitro* processes through confocal fluorescence microscopy.

§ 4.1. Tumor-Targeting Drug Delivery

Destruction of normal healthy cells by chemotherapeutic drugs/agents leads to serious undesirable side effects. This fact necessitates improvements of old drugs, new drug research, review of existing therapeutic theory, and development of novel concepts. Tumor-Activated Prodrug (TAP)[287] and Tumor-Targeting Drug Delivery[288] are two of emerging ideas, both of which take advantage of the difference between tumor cells and normal cells, and deliver the therapeutic agents only to tumor tissue or cells. As a consequence, the malignant cells are destroyed, but normal healthy cells are not affected. In general, these two new strategies should increase drug efficiency and reduce side effects.

To achieve this tumor-targeting delivery, a "smart" drug conjugate (also prodrug) that can differentiate tumor cells from normal cells is required. Usually, this smart drug is composed of three parts: tumor-targeting moiety (TTM), suitable linkage, and cytotoxic agent. Each of them has different functions, but they work synergistically. TTM, after recognizing tumor cell specifically, should guide the whole conjugate entering into cancerous cell, where the drug is released from the linker to kill the malignant cell through various mechanisms. The responsibilities of proper linker are to keep the whole conjugate stable during transportation in plasma, and to liberate the drug quickly and efficiently inside cell.

In previous chapters, 2nd-generation taxoids (warhead) and novel disulfide linkers have been designed and evaluated. This chapter will focus on TTM.

The ideal TTM (guide molecule) must show very high affinity to tumor cells, and this could be achieved *via* particular receptor for TTM or other biomarkers on the surface of tumor cells. Studies in cancer biology have already indicated that certain receptors or biomarkers are overexpressed on the malignant cell surface, such as EGFR and vitamin receptors.[287,288] This is reasonable that tumor cell needs much more such receptors than normal cell to get more necessary materials to support their rapid division and growth. The number of receptors may vary.

Indeed, the interactions between TTM and its receptor will cause the internalization of the whole conjugate through endocytosis (Figure 4-1).[288]

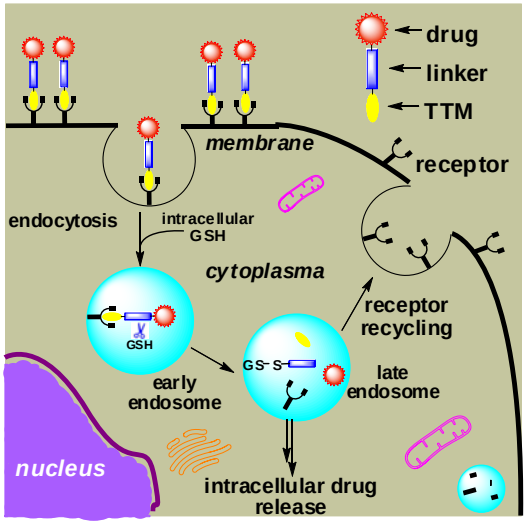


Figure 4-1. Receptor-Mediated Endocytosis[288]

As shown in Figure 4-1, a drug conjugate specifically binds to tumor cell surface through TTM and the receptor, which generates the signal that induces internalization. After cell membrane sinks, coated vesicle forms, followed by formation of early endosome *via* fusion of coated vesicle and intracellular substances, including glutathione (GSH), which may reduce the disulfide linker and trigger the drug release from the conjugate (discussed in Chapter Three). Finally, a cytotoxic agent is released to cytoplasm in the late endosome or lysosome stage. Meanwhile, the receptors are recycled after it is separated from TTM.

In my research, biotin was chosen as TTM because its receptors are overexpressed in various types of cancer cell lines, and the number of the receptors is much greater than normal cell.[289]

§ 4.2. Biotin-Mediated Tumor-Targeting Drug Delivery System

§ 4.2.1 Biotin and Its Functions

Biotin (Figure 4-2), also named vitamin H, is an essential vitamin for all organisms. It is found in liver, kidney, pancreas, and milk. It was first isolated from egg yolk in 1936, and its structure was elucidated in 1942, which was confirmed by X-ray crystallography in 1966. Since 1943, a variety of methods have been developed towards the total synthesis of biotin.[290]

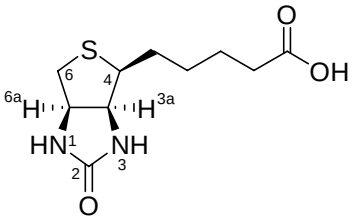


Figure 4-2. Structure of biotin

Biotin plays an important role in many biological processes, mainly carboxylation, decarboxylation, and transcarboxylation. The putative mechanism is shown in Figure 4-3.[291] Biotin is the active cofactor of pyruvate carboxylase, and pyruvate is converted to oxaloacetate, which participates in many metabolic reactions (Figure 4-4).

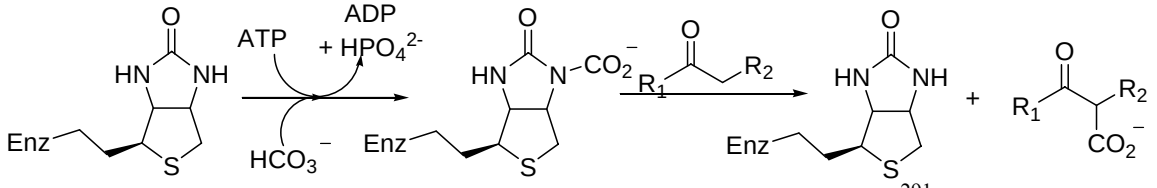


Figure 4-3. Mechanism of biotin as coenzyme factor[291]

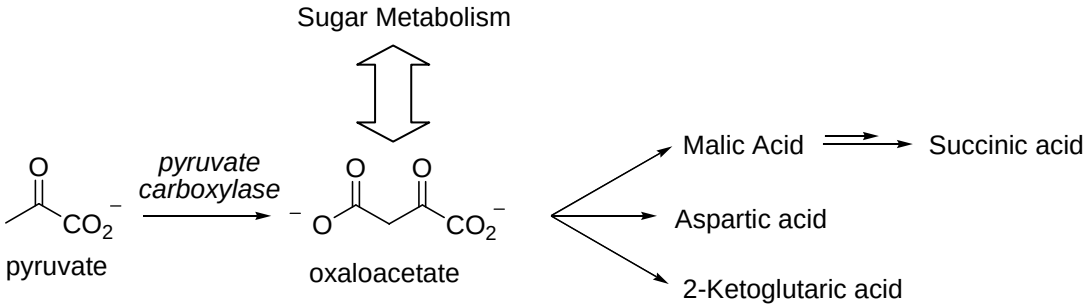


Figure 4-4. Importance of oxaloacetate

Human body can't generate biotin, but can acquire it by two different exterior ways: one is diet and the other is colon bacteria that can synthesize biotin. Inside human body, intestine and colon are the organs that absorb biotin. It has been found that both the sodium-dependent multivitamin transporter 1 (SMVT1) and monocarboxylate transporter 1 are responsible to transfer biotin across the membrane. Yet, the structures of these two carriers or receptors are still unknown.[292,293]

Biotin is also important for cell growth.[294] The effect of biotin on SV3T3 cell growth is 5-10 times faster than control in the presence of normal calf serum.[295] When

exogenous biotin was added to the media lacking biotin wherein the reduced rate of HeLa cell growth was observed, the growth rate was enhanced.[296] Moreover, the concentration of biotin in cancer tissues was found higher than normal.[297] Obviously, tumor cells need more biotin because of rapid growth. Thus, the receptors for biotin may be overexpressed on the tumor cell surface, which has been confirmed by Russell-Jones.[289] Fifteen tumor cell lines were screened for the study of the folate or vitamin B_{12} receptor, and most of them were found to overexpress the biotin receptors as well. Therefore, any biotin conjugate should be attractive to such tumor cells, and biotin-drug conjugates are promising candidates for cancer chemotherapy.[298]

§ 4.2.2 Biotin Receptor and Receptor-Mediated Endocytosis

Compared to the transporters of many other micronutrients, the structures of biotin transporters are poorly known. However, it is clear now in normal concentration, biotin is transported into the cell by certain transporters or receptors (*i.e.*, receptor-mediated endocytosis is the main mechanism), and passive diffusion becomes predominant at much higher concentrations.[293]

Sodium-dependent multivitamin transporter 1 (SMVT1), a protein composing of 634 amino acid residues and 12 putative trans-membrane domains, is now considered as one of the biotin transporters. [292] It was found SMVT1 can transport biotin to HeLa cells. In fact, it can transport not only biotin, but also lipoic acid and panthothenic acid according to experimental observations. [292] Obviously, the common moiety within these three acids is the valeric acid fragment. However, still some experiments argue against SMVT1.[292]

It is generally believed that TTM (as well as the conjugate) will be detached from the receptor in lysosome. However, Choi *et al.* thought vitamins might not enter the lysosome, but would stay in endosome because of its biofunctions.[298] If the TTM is a monoclonal antibody or a hormone, it eventually transmits a signal to the tumor cell. This signal may cause harmful damage to the cancer cell if it remains for a longer period of time, and the cancer cell itself tries to eliminate this signal quickly. Therefore, the whole conjugate will be rapidly transferred into lysosome, wherein the whole conjugate is destroyed totally. On the contrary, vitamins are nutrition, and tumor cell will consume them. That is why vitamin conjugates stay longer time and still function very well inside the tumor cell, as observed by Leamon and Low on folate.[299]

§ 4.2.3 Biotin-Drug Conjugate

From the discussion mentioned above, biotin-anticancer drug conjugates, yet not fully developed,[300] would have prospective advantages in cancer chemotherapy. The biotin can serve well as a guide molecule to satisfy the tumor-specific delivery duty, and the potent cytotoxic drug can be released inside of the tumor cell if appropriate linker is chosen.

Paclitaxel and other taxoids have been extensively studied in our group, and highly potent 2nd-generation taxoids were developed,[301] which can be conjugated to biotin to form an inactive pro-drug. In this project, **SB-T-1214** was chosen.

Also, novel disulfide linkers designed by Ojima[302] have shown attractive property and diversity for drug delivery systems by taking advantage of glutathione, a tripeptide that exists at higher concentration in tumor cells.[303]

§ 4.3. Research Plan and Application of Fluorescent Probes

The aim of the research is to validate Biotin-Linker-Taxoid conjugate(s) as promising tumor-targeting chemotherapeutic agent. In order to obtain unambiguous experimental results, the following three points need to be confirmed. First, biotin is indeed a good TTM, and able to induce internalization of the whole conjugate. Second, the disulfide linker works well in cancer cells as it does in the model reaction discussed in Chapter Three, *i.e.*, intracellular GSH reduces the disulfide linkage, and intramolecular cyclisation happens to release anticancer agent. Third, liberation of free intact taxoid should be detected in cancer cells. To doubly confirm the drug release, binding of taxoid to its cellular target, *i.e.*, microtubules, should be utilized.

Introducing proper fluorescent molecules as probes should help to monitor the proposed processes in cancer cells. Consequently, in order to monitor the biological processes of the internalization, cleavage of the linker and release of the original taxoid, at least one final compound containing fluorophore is required for each step.

If a simple system, *i.e.*, single TTM and single cytotoxic agent, should work well, the more complicated systems, such as single TTM with multiple identical cytotoxic agents (e.g., taxoid only) or single TTM with multiple different cytotoxic agents (e.g., taxoid and cisplatin) or multiple TTMs with multiple cytotoxic agents, should be explored to increase the therapeutic efficiency.

§ 4.3.1. Biotin-Linker-Taxoid Conjugate (single TTM-single cytotoxic agent)

The molecule in Figure 4-5 was designed to see if biotin would be efficient enough to take the whole conjugate into the tumor cell through its interactions with its receptors over-expressed on the surface of the tumor cell. Once the internalization happens, the fluorophore, *e.g.*, fluoroscein isothiocynate (FITC) or rhodamine, should light up the whole cell. Considering structures of biotin and common fluorophore, a hydrazine linkage was considered appropriate.

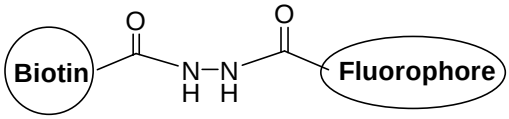


Figure 4-5. Target Compound A for the first step

For the second step, a biotin-linker-fluorophore was designed as the target compound (Figure 4-6). Here a pro-fluorophore (fluorogenic probe) [304,305] ought to be employed. In its conjugated status, this pro-fluorophore does not emit fluorescence. However, when it is detached from the conjugate and becomes free, the fluorescence will be regenerated, which can be detected by either fluorometer or microscopy. Obviously, the pro-fluorophore also serves as a substitute of the cytotoxic agent.

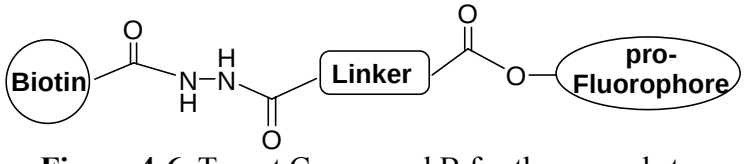


Figure 4-6. Target Compound B for the second step

To envision the third step, the conjugate in Figure 4-7 should be useful. Actually, many fluorescent taxoids have been synthesized and applied to cellular assays, which can be applied to our plan.[306] A fluorescent moiety, which can shine up all the time during the progress, ought to be installed in the taxoid. Nicolaou[307] and Lai[308] coupled either FITC or rhodamine to paclitaxel at the C-7 hydroxyl group with *β*-alanine or caproic acid. A small spacer between the fluorophore and the taxoid moiety was necessary in their synthesis. Kingston and Bane also prepared some fluorescent paclitaxel derivatives with much weaker fluorescence.[309]

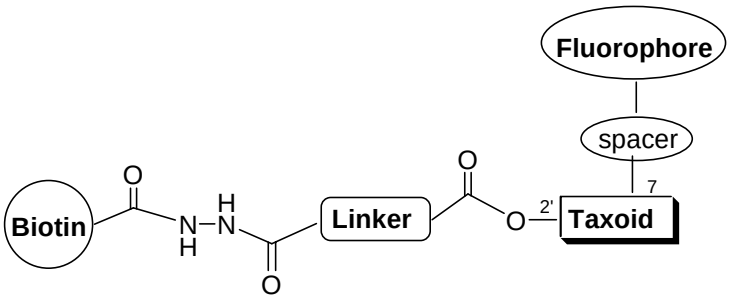


Figure 4-7. Target Compound C for the third step

§ 4.3.2. Single TTM and Multiple Cytotoxic Agents

The following model (Figure 4-8) structures were also designed to take advantages of synergistic effects of two different drugs. Either a tri-substituted phenyl ring or a lysine serves as the splitter. It should be noted that Drug 2 should have a different mechanism of action from that of taxoid, and should not counteract to taxoid.

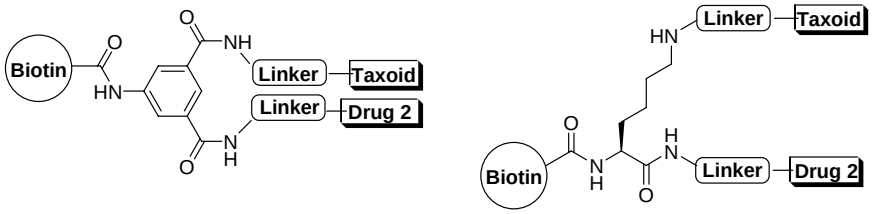


Figure 4-8. Single TTM with multiple therapeutic agents (adapted from Ojima's proposal)

§ 4.3.3. SWNT-Drug Conjugate (Multiple TTM and Multiple Cytotoxic Agents)

To accommodate multiple TTMs and multiple drug molecules, a suitable multi-valent carrier, such as dendrimers,[310,311] polymers,[312,313] and carbon nanotube (CNT), is needed.

After proper modifications, single-walled carbon nanotube (SWNT) has been used to transport a wide range of molecules across the membranes into living cells, such as DNA fragments, proteins, and drug molecules.[314] Meanwhile, several experiments successfully showed that functionalized SWNTs were biocompatible.[315] Consequently, SWNT should be a safe and promising drug carrier to fight against human diseases, which is envisioned by Ojima and others.[316]

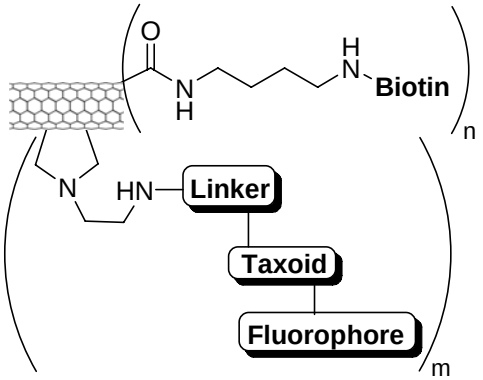


Figure 4-9. SWNT as the carrier of multi-TTM-multi-Drug conjugates[316]

Figure 4-9 shows the structure of a biotin-functionalized SWNT-drug conjugate containing a cleavable disulfide linker. It should be noted that the biotins are installed at both ends as well as some parts of the side wall of the SWNT, which may help the internalization of the carbon nanotube conjugate. In addition, cytotoxic agents are connected to the side wall of SWNT. The modified SWNT shown above should be able to deliver massive drug molecules into the tumor cell, and thus destroy the tumor cell more efficiently. As discussed before, three different SWNT conjugates would be prepared to validate the function of each component.

This is only a system containing multiple identical cytotoxic agents, and in the future, systems with multiple different cytotoxic agents (including cytotoxic proteins and DNA or RNA fragments) should definitely be of interest.

§ 4.3.4. Fluorescent Probes

To date, confocal fluorescent microscopy has demonstrated as one of the most valuable techniques in the biological sciences, drug discovery and study of delivery system.[317-319] A wide variety of fluorescent molecules has been successfully attached to proteins or drugs of interest (Figure 4-10). Due to high quantum yield and respective excitation-emission spectrum, fluorescein (or rhodamine), BODIPY (boron-dipyrrin)[320,321], and cyanines (*e.g.*, ICG, Cy5.5)[322] have been widely used as the most useful small molecule fluorescent probes. Meanwhile, macromolecular fluorescent substances, such as Green Fluorescent Protein (GFP)[323], are also well developed. Now, with the help of fluorescence and spectroscopic methods, even quantification of drug release is achievable, and assays could be done both *in vitro* and *in vivo*.[324]

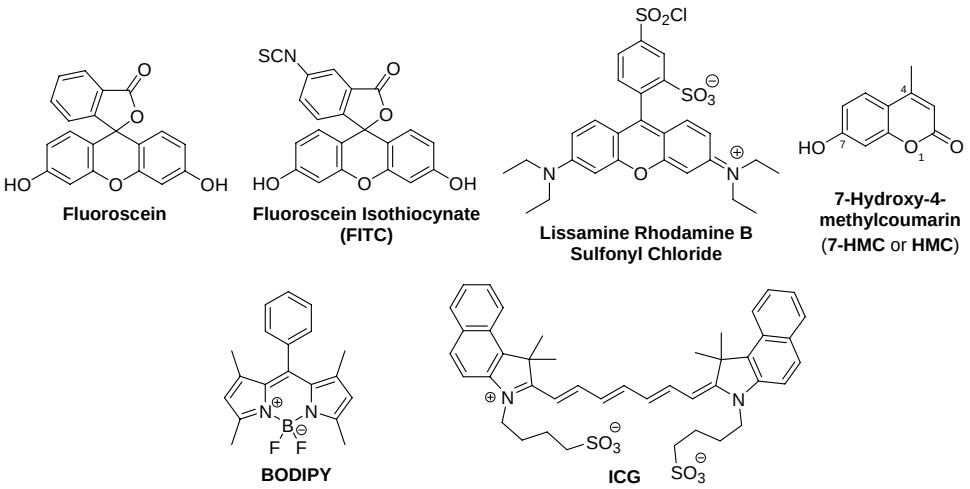


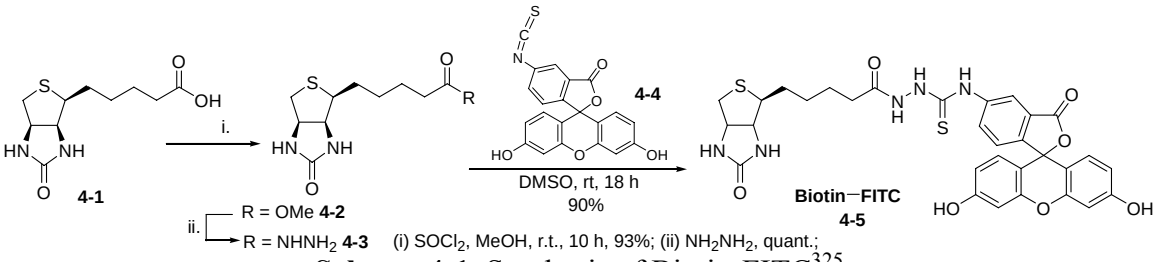
Figure 4-10. Some fluorescent molecules

§ 4.4. Results and Discussion

§ 4.4.1. Synthesis

§ 4.4.1.1. Biotin-Fluorophore (A)

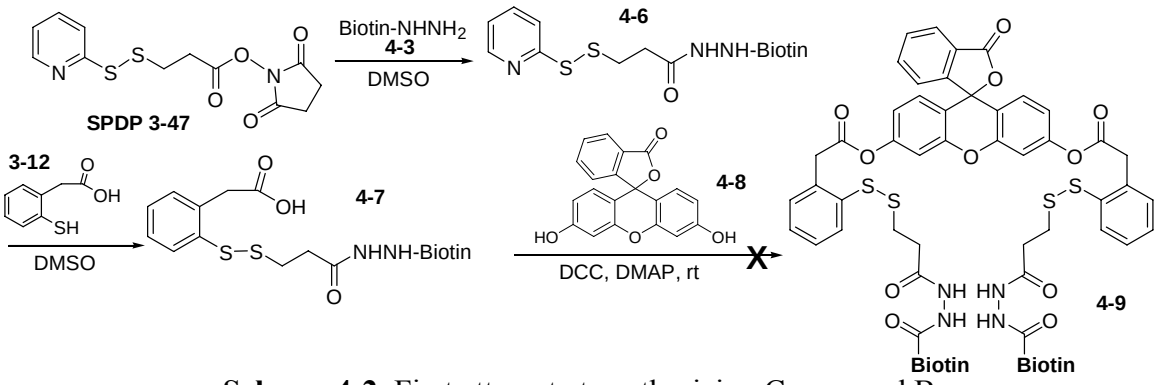
Biotin hydrazide (**4-2**), obtained from biotin (**4-1**) *via* methyl ester with excellent yield,[325] was directly reacted with FITC (**4-4**) to provid the desired Biotin-FITC (**4-5**).



Scheme 4-1. Synthesis of Biotin-FITC[325]

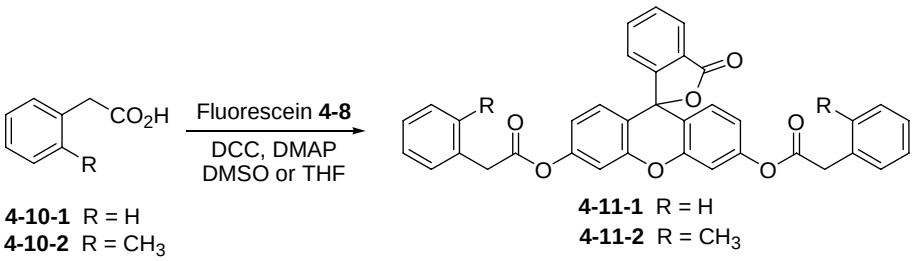
§ 4.4.1.2. Biotin-Linker-pro-Fluorophore (B)

It is known that when and only when both hydroxyl groups are converted to ester, fluorescein (**4-8**) becomes non-fluorescent. If any of the two hydroxyl groups is in the free phenol form, it is still fluorescent. Consequently, **4-9** (Scheme 4-2) was designed as a probe for the test on the function of a disulfide linker in cancer cells.



Scheme 4-2. First attempt at synthesizing Compound B

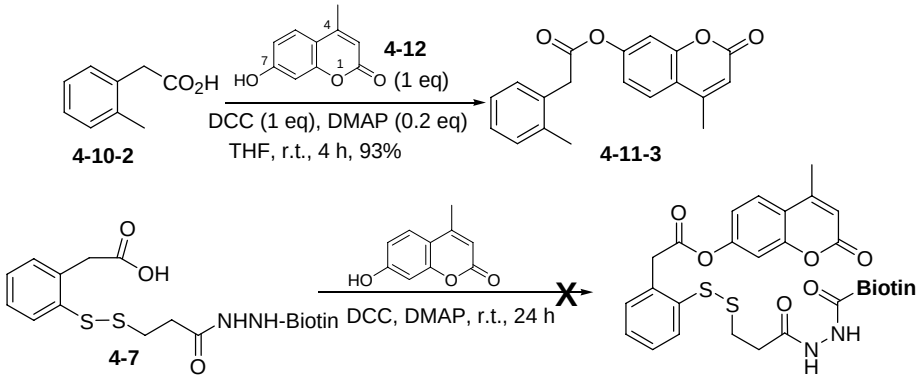
The biotin-SSPy (**4-6**), prepared from biotin-$NHNH_2$ (**4-3**) and SPDP (**3-47**), underwent thiol-disulfide exchange reaction smoothly with **3-12** to give Biotin-Linker-COOH (**4-7**). The conversion was very high (monitored by TLC), but the yield (70%) was relatively low, which was due to the very high polarity of the product that had to be precipitated out by ether. However, the next coupling step did not provide the desired product **4-9** at all.



Scheme 4-3. A model study for the final coupling step in Scheme 4-2

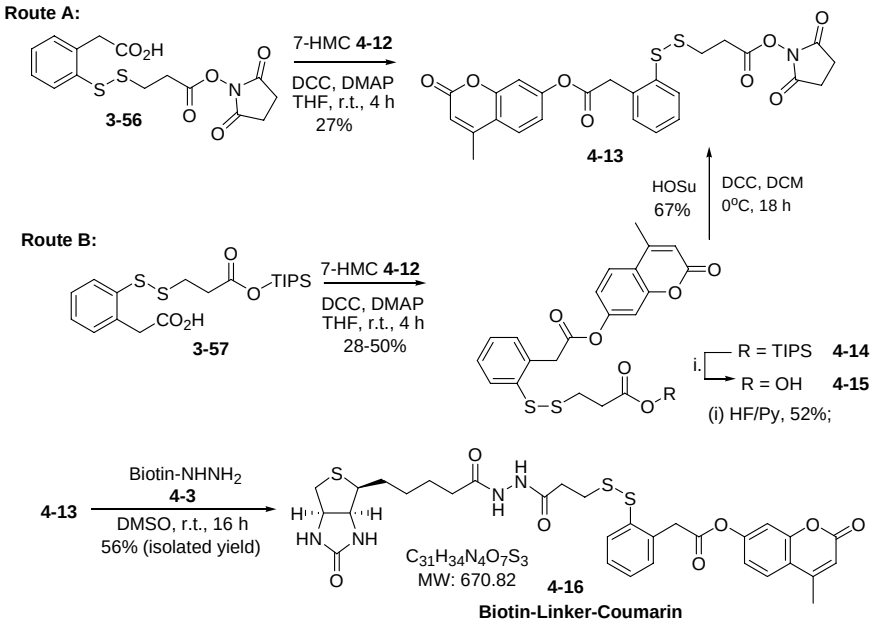
Actually, before the final coupling step in Scheme 4-2 was performed, some model compounds were prepared to explore the reaction conditions for the di-ester of fluorescein (Scheme 4-3). The ratio of **4-10** : **4-8** : DCC was ranging from 10 : 1 : 10 to 5 : 1 : 5. The reaction time was ranging from 12 h up to 2 days. However, none of those reactions showed completion. Regarding solvent, results in THF (80% yield) was much better than those in DMSO (41% yield), but **4-7** did not dissolve in THF. Other coupling reagents and methods, such as BOP-Cl, were also tried, but did not afford any interesting findings.

Then, 7-hydroxy-4-methylcoumarin (7-HMC or HMC, **4-12**), although its fluorescence is much less intensive compared to fluorescein, was considered to replace fluorescein in Compound B.[326] The model study gave exciting yield and purity. Unfortunately, the final coupling of Biotin-Linker-CO_2H (**4-7**) with HMC (**4-12**) did not proceed well. (Scheme 4-4)



Scheme 4-4. A model reaction with HMC
and an attempted final coupling of **4-7** with HMC

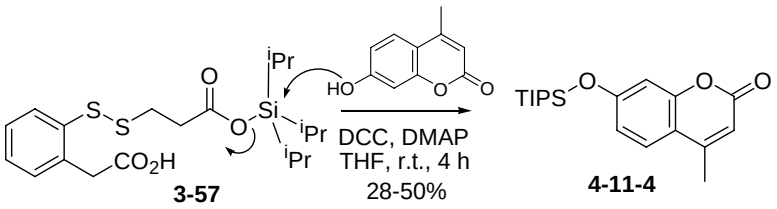
These results implied that the coupling reaction with fluorescein or HMC could proceed, but the biotin derivative **4-7** was not a good substrate, maybe due to its very high polarity and relatively large size or possible intramolecular interactions. Thus, we thought that the coupling of a fluorophore (here it was HMC) should be done prior to the installation of biotin moiety. As a consequence, a different protocol in Scheme 4-5 was explored.



Scheme 4-5. New routes towards B

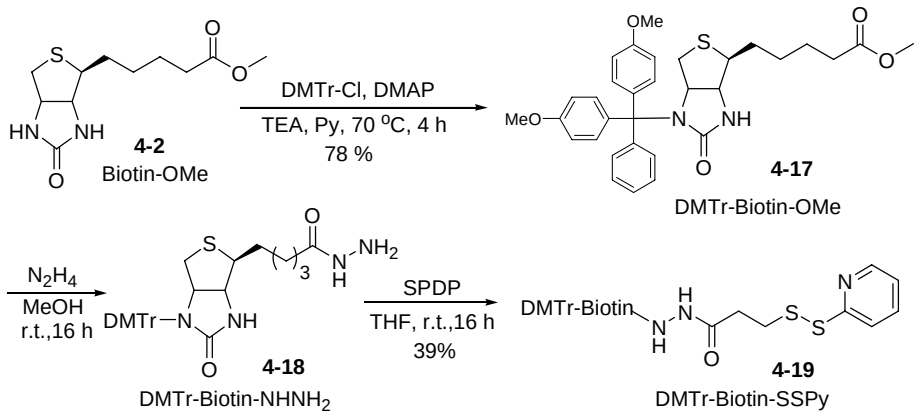
In the presence of DCC and DMAP, **3-56** was converted to coumarin ester **4-13**, which could also be prepared from **3-56** as shown as Route B in Scheme 4-5. Final amide formation completed the synthesis of Biotin-Linker-Coumarin **4-16**, which was applied to the cellular assay to check the function of linker.

Interestingly, TIPS ether of HMC was isolated when **3-57** was converted to **4-14**, and maybe it was one of the reasons why the yield was moderate (Scheme 4-6).



Scheme 4-6. By-product in the synthesis of **4-14** from **3-57**

Because all biotin derivatives described above had poor solubility in usual organic solvents, several DMTr-protected biotin derivatives[327,328] were prepared, which were known to greatly improve the solubility. Some of the reactions are summarized in Scheme 4-7.

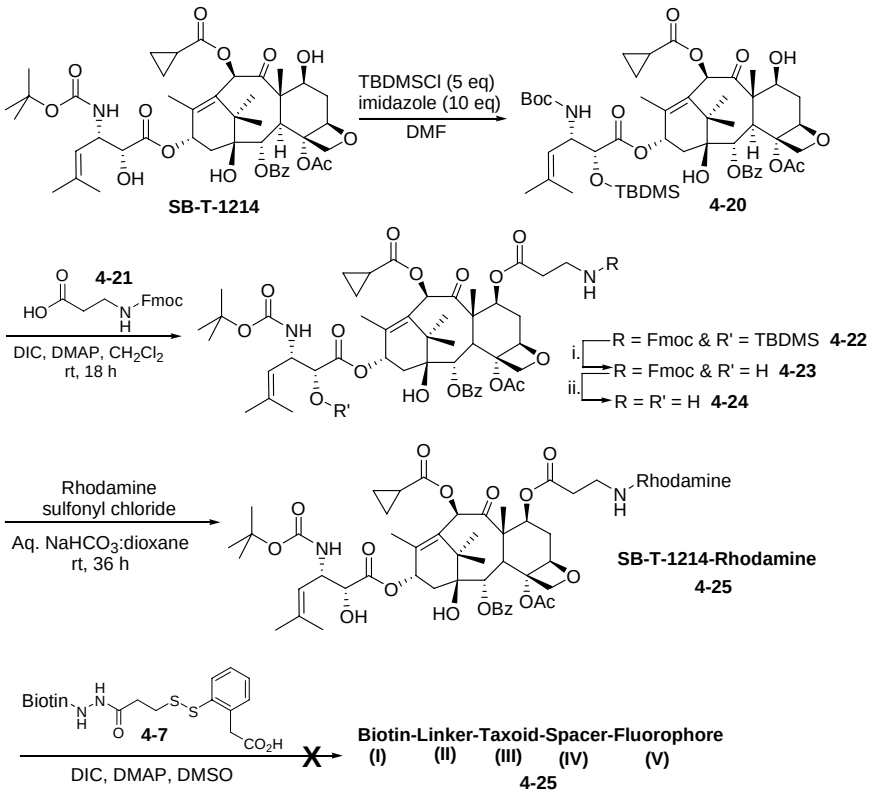


Scheme 4-7. Synthesis of DMTr-Biotin derivatives

4,4'-Dimethoxytrityl (DMTr) chloride was used to protect one of the amide bonds (*i.e.*, at N-6) in the biotin ring. Those DMTr-biotins, such as DMTr-biotin-$NHNH_2$, dissolved very well in either DCM or THF, and the previous troubles in using DMSO as the only solvent were thus circumvented[329,330]. Unfortunately, DMTr group is very sensitive to acid. For instance, trace HCl in commercial $CDCl_3$ is strong enough to remove DMTr, which gave a messy NMR spectrum. I also noticed that at room temperature, both silica gel and acetic acid cleaved DMTr partially. Further modifications on **4-19** were not performed.

§ 4.4.1.3. Biotin-Linker-Taxoid-Fluorophore (C)

Initially, a taxoid-rhodamine complex was designed to meet the requirement because paclitaxel-rhodamine using amino acid as the linkage had been reported[307,308]. Due to the existence of Boc and alkene functional groups in the side chain of **SB-T-1214**, the choices of spacers between the taxoid and the fluorophore weree limited to Fmoc-protected amino acid, which led to the synthesis route shown in Scheme 4-8.[331]



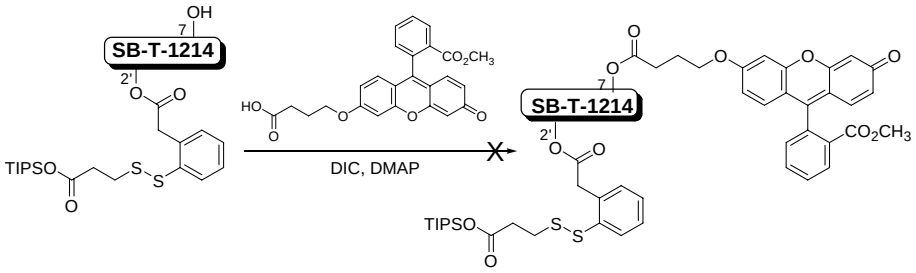
(i). HF/Py, CH_3CN, Py, 0°C-rt, 18 h; (ii). Piperidine, CH_2Cl_2, r.t., 2 h.

Scheme 4-8. Taxoid-Rhodamine complex[308,331]

However, the final coupling, for some reason, did not work, and we noticed that similar failures happened in Scheme 4-2 and Scheme 4-4 as well. On the basis of the successful coupling reaction without a biotin hydrazide moiety (see **4-34** in Scheme 4-13), it strongly suggested that biotin moiety or its hydrazide might disturb the coupling reaction. Another problem regarding this route was that the conversion of **4-24** to **4-25** was less than 80% by NMR although different conditions were tried, and preparative HPLC was applied since the flash chromatography separation was not sufficint.

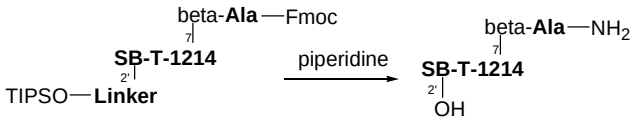
After unsuccessful trials, the structure of the whole conjugate (**4-25**) was carefully examined. Based on the experience in preparations of the second compound, it seemed that the assembling sequence of the five moieties was very critical. Some other synthetic strategies towards **4-25** were also conceived and explored.

The unsuccessful "**(I + II) + [(III + IV) + V**" sequence was described above. Because Linker-Taxoid complex was extensively explored in the synthesis of the Coupling-Ready Warhead-Linker Constructs (CRWLC), the combinations of "**I + [(II + III) + (IV + V)]**", "**[I + (II + III)] + IV + V**", and "**I + [(II + III) + IV + V]**" were tried. However, one of the key steps, esterification of Linker-Taxoid (or Biotin-Linker-Taxoid) with a spacer (or a spacer-fluorophore) did not proceed (Scheme 4-9). This indicated that the prior combination of **II** and **III** was not wise.



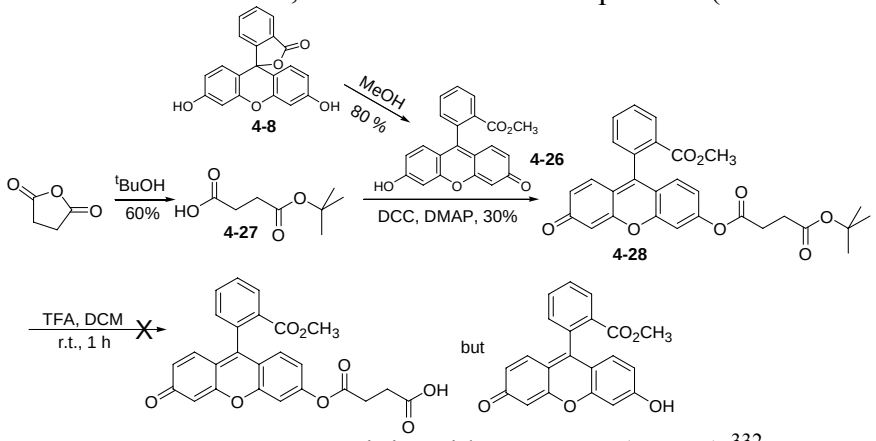
Scheme 4-9. Esterification of Taxoid-Linker at C-7

Next, the "**I + [[II + (III + IV)] + V]**" sequence was tried. However, even at a low concentration, piperidine, which was used in the standard procedure to remove the Fmoc in **IV** to generate free amine for the coupling with **V**, also destroyed the ester bond at 2'-position in the taxoid, which was the linkage between **II** and **III** (Scheme 4-10).



Scheme 4-10. Linkage damage between **II** and **III** by piperidine

Then, succinic acid was chosen to serve as **IV** to avoid the trouble caused by amino acid. Moreover, a fluorescein methyl ester **4-26**[332] was chosen from the fluorescent compound pool. However, the deprotection of *tert*-Butyl ester **4-28**, which was prepared from **4-26** and **4-27**, resulted in total decomposition (Scheme 4-11).



Scheme 4-11. Succinic acid as Part **IV** (spacer) [332]

To avoid the instability of phenolic ester, a hydroxyl-carboxylic acid[332,333] was finally employed as **IV** (spacer), and it was connected to **V** through ether bond and to **III** (taxoid) *via* ester bond to complete the synthesis of **4-25** (Figure 4-11).

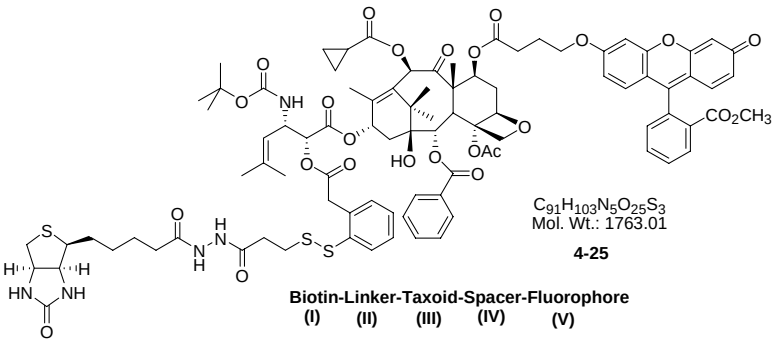
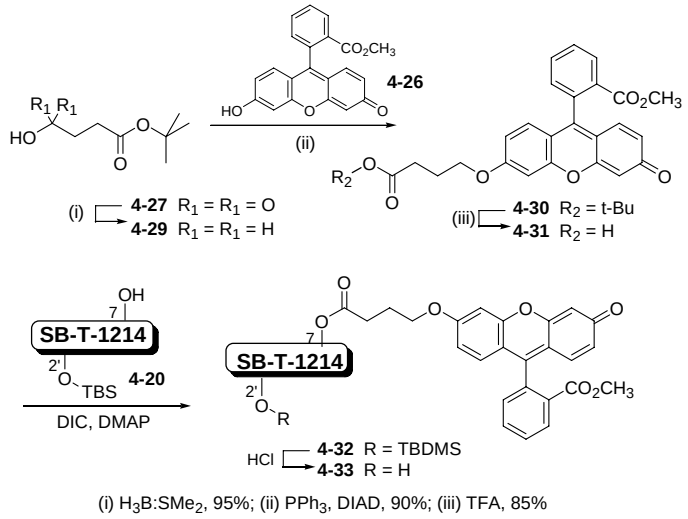


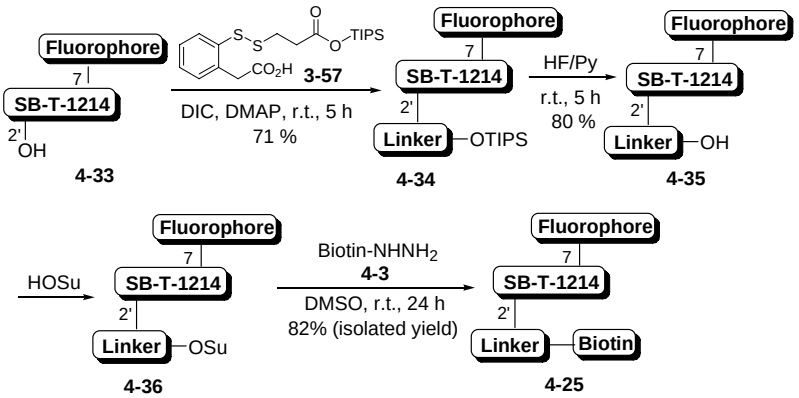
Figure 4-11. Final Biotin-Linker-Taxoid-Fluorophore with new spacer and fluorophore

The successful route turned out to be "**I** + [**II** + [**III** + (**IV** + **V**)]]". Started from Mistunobu reaction, alcohol **4-29** (obtained from **4-27**) was converted to **4-30** wherein ether bond was formed. Then, *tert*-Butyl group in **4-30** was removed, followed by coupling with protected taxoid **4-20** to give **4-32**, which was ready to be connected to linker moiety after silyl protection was removed (Scheme 4-12).



Scheme 4-12. Assemble of Part **III**, **IV** and **V**

To my delight, further steps were done smoothly. The silyl-protected linker moiety **3-57** was reacted at the C-2' hydroxyl moiety of "**III**+**IV**+**V**" piece to afford **4-34**. After removal of TIPS, the resulting carboxylic acid was activated by HOSu. The final amide formation completed the whole synthesis of desired compound **4-25** for *in vitro* assays (Scheme 4-13).



Scheme 4-13. Final steps towards Compound C

It is worth to mention here that after the fluorophore was coupled, it should be better to treat all following compounds and to run reactions in dark.

Same as CRWLC, **4-36** could be applied to all kinds of tumor-targeting moieties (TTM). As it has the strong fluorophore on it, **4-36** should be an indispensable intermediate for the synthesis of various fluorescent probes.

§ 4.4.1.4. Biotin-Linker-Taxoid conjugates without fluorophore

Usually, the cytotoxicities of fluorescent taxoids are compromised because of the existence of the fluorophore moiety at the C-7 position in the taxoid. For example, compared to that of paclitaxel, fluorescent paclitaxel **4-37** showed about 100 times less potency against Hela cervical carcinoma (HeLa), while **4-38** showed 10 times less, based on a XTT assay.[307,308] In another study, paclitaxel, **4-39** and **4-40** were tested against H-460 human non-small cell lung cancer (NSCLC) cells by MTT assay.[307,308] IC_{50} of paclitaxel fell in nanomolar range, but **4-39** and **4-40** exhibited only micromolar IC_{50}.[307,308]

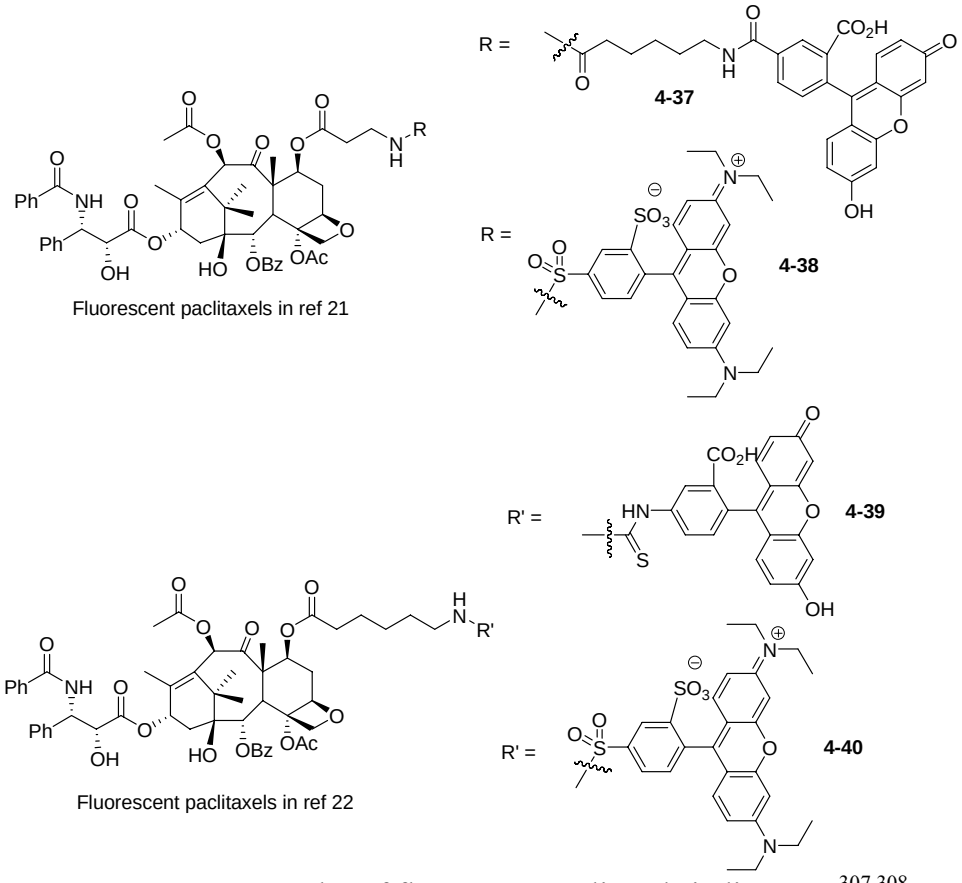


Figure 4-12. Examples of fluorescent paclitaxels in literature[307,308]

These results clearly imply that the cytotoxicity of Biotin-Linker-Taxoid-Fluorophore conjugate does not represent the real potency of Biotin-Linker-Taxoid. To measure the real potency, the conjugates without fluorophore moieties, *i.e.*, Biotin-Linker-Taxoid, are needed. Moreover, the scope of novel self-immolative disulfide-containing linkers, which have been discussed in former chapter, should be further explored. Consequently, two biotin-taxoid conjugates containing different linkers were designed (Figure 4-13).

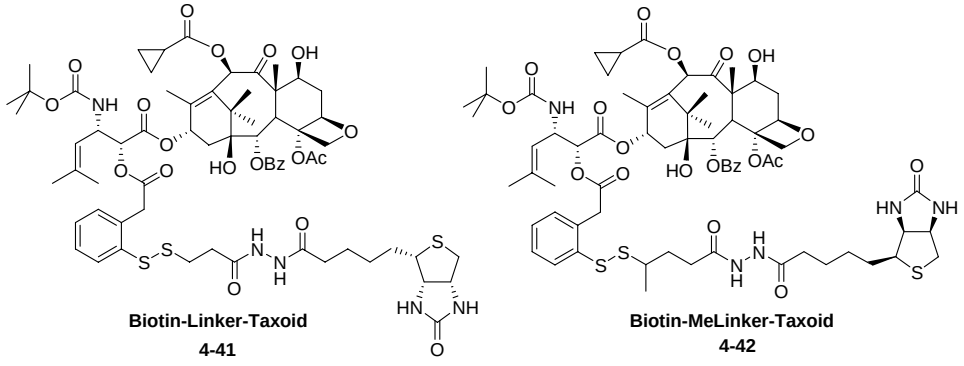


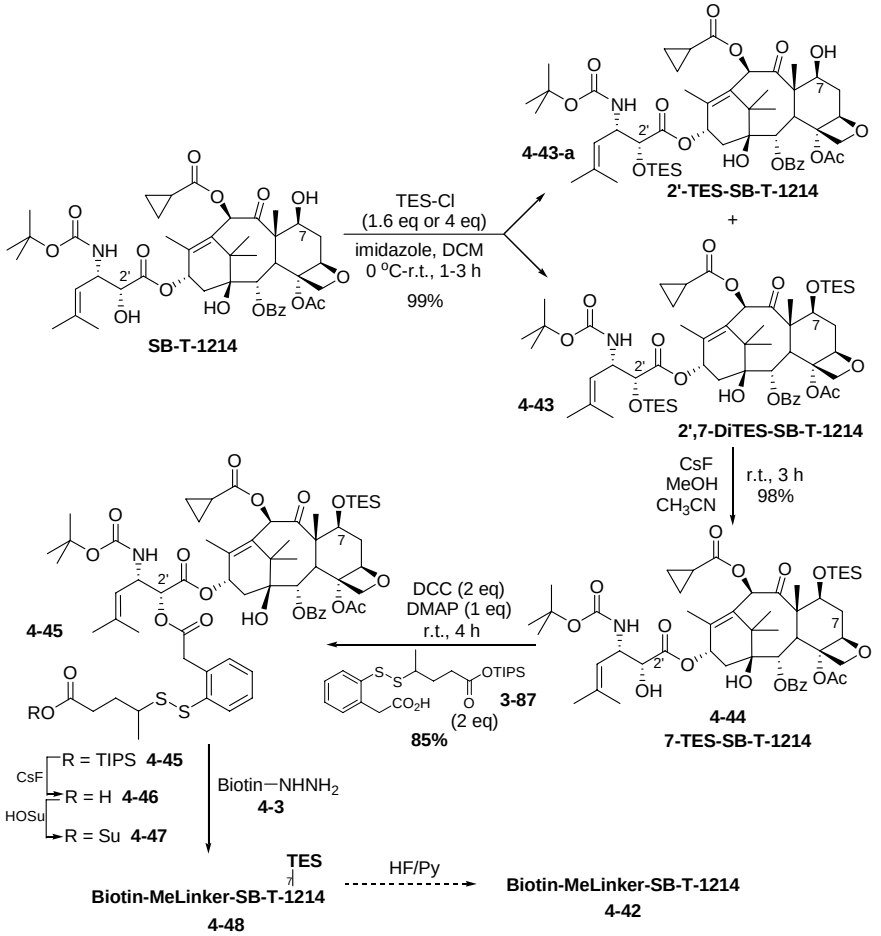
Figure 4-13. Biotin-Disulfide Linker-Taxoid conjugates without fluorophore

The preparations of these two conjugate were very straightforward by reacting biotin-hydrazide with CRWLC **3-105** or **3-108**, which were discussed in Chapter Three.

However, the synthesis of **3-108** was not satisfactory because the coupling reaction between the linker moiety and **SB-T-1214** suffered from low conversion, low yield (65%) and formation of by-product (presumably C-7 ester). To improve the reactions, a new route was purposed by blocking the hydroxyl at the C-7 position on the taxoid (Scheme 4-14).

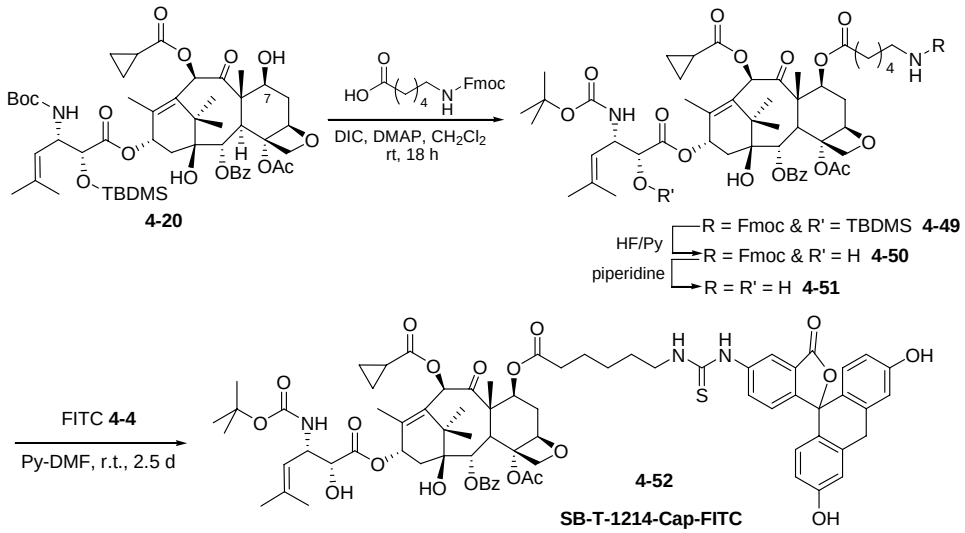
Because the hydroxyl group at the C-2' position on **SB-T-1214** is more active in chemical reactions than that at the C-7 position, direct protection of C-7 hydroxyl by a silyl group is not applicable with a free C-2' hydroxyl. If **SB-T-1214** was treated with limited amount of TES-Cl, e.g., 1.6 equivalents, the major product was 2'-TES-**SB-T-1214** (**4-43-a**). But when 4 eq. TES-Cl was used, 2',7-DiTES-**SB-T-1214** (**4-43**) was obtained almost quantitatively. After selective removal of TES at C-2' hydroxyl under mild condition, the resulting **4-44** was ready to be coupled to the methyl-branched linker **3-87**.

The coupling reaction by using 2 equivalents **3-87** resulted in 85% yield, which was much better than the previous 65% yield. Next, the TIPS group was removed by CsF, followed by the activation of carboxylic acid with HOSu in the presence of DCC. Amide formation by using Biotin-NNH_2 gave **4-48** successfully. The future step would be the deprotection of the hydroxyl at C-7 position to complete the synthesis (Scheme 4-14).



Scheme 4-14. 7-TES-**SB-T-1214** and its application in the synthesis of **4-42**

Moreover, to further explore the effect of fluorophore moiety on cytotoxicity and study the behavior of free taxoid without biotin (TTM) and linker *in vitro*, a **SB-T-1214**-FITC conjugate containing a longer spacer at the C-7 hydroxyl position on taxoid was designed and synthesized (Scheme 4-15). Caproic acid was chosen as the spacer based on the literature.



Scheme 4-15. Synthesis of a SB-T-1214-Cap-FITC conjugate

§ 4.4.1.5. Fluorescent Linker-Taxoid conjugate with SWNT [316]

As discussed above, one of other applications with **4-36** could be in combination with nano-technology. The coupling reactions, when SWNT was used, were carried out by Dr. Jingyi Chen and Dr. Shuyi Chen. Consequently, three SWNT conjugates were successfully prepared and evaluated by a variety of cellular assays, and the results have been reported recently.[316]

§ 4.4.2. *In vitro* Assay of Biotin-Linker-Taxoid-Fluorophore in L1210FR cell line

With fluorescent probes in hand, the *in vitro* assays were performed on L1210FR cell line, which was reported to over-express biotin receptors.[289]

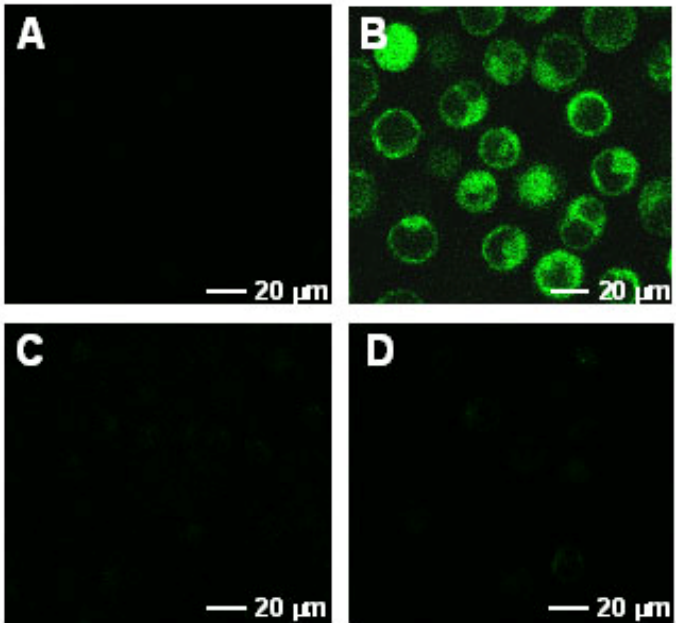


Figure 4-14. Endocytosis of Biotin-FITC **4-5** with L1210FR cells
(Confocal fluorescent images of L1210FR cells after incubation with 100 nM Biotin-FITC (**4-5**) at different conditions: (A) Blank control; (B) at 37 ^{o}C for 3 h; (C) at 4 ^{o}C for 3 h; (D) at 37 ^{o}C pretreated with excess biotin (2 mM) for 3 h.)

When L1210FR cells were treated with Biotin-FITC (**4-5**) at 37 ^{o}C for 3 h, clear and strong fluorescence inside the cells was captured by confocal fluorescence microscopy (Figure 4-14-B). Time-dependence assay also showed that the intensity of fluorescence kept increasing when time went by. The results indicated that Biotin-FITC indeed entered into the cells. Inhibition test was done at 4 ^{o}C for 3 h. It is known that receptor-mediated endocytosis, which is an ATP-dependent process, is inhibited at this temperature. As shown in Figure 4-14-C, only very weak fluorescence was observed. Competition binding against biotin receptor was also examined. Thus, the cells were first incubated in 2 mM free biotin solution at 37 ^{o}C for 2 h, followed by addition of Biotin-FITC **4-5**. Vitually no fluorescence was observed, as showed in Figure 4-14-D, which strongly suggested that internalization of Biotin-FITC occurred through receptor-mediated endocytosis.

When Biotin-Linker-Coumarin (**4-16**) was used, glutathione ethyl ester (GSH-OEt) was introduced extracellularly in order to observe the result quickly.

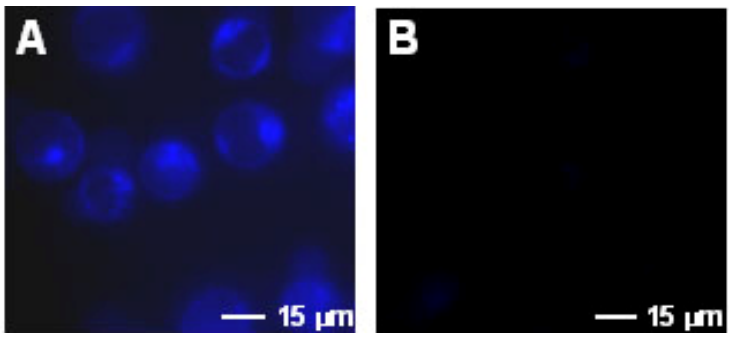


Figure 4-15. *in vitro* results from pro-fluorophore (**4-16**)

Figure 4-15-A is the epifluorescence image of L1210FR cells that were initially incubated with Biotin-Linker-Coumarin (**4-16**), which was not fluorescent, followed by treatment with GSH-OEt to cleave the disulfide bond triggering the release of free HMC, showing fluorescent blue and activate the dye. Figure 4-15-B is the epifluorescent image of L1210FR cells just after incubation with Biotin-Linker-Coumarin (**4-16**), which shows only very weak fluorescence.

Finally, L1210FR cells were treated with fluorescent taxoids. Confocal fluorescence image A in Figure 4-16 was obtained after cells were first incubated with Biotin-Linker-Taxoid-Fluorophore (**4-25**), followed by treatment with GSH-OEt to release fluorescent taxoid **4-33**. The fluorescent-labeled microtubule networks in the cells were clearly visualized. Image B shows the result after cells were incubated with fluorescent taxoid **4-33**. This was a control experiment to confirm that released taxoid did bind to microtubule network. If the cells were only treated with **4-25**, the result is shown in image C.

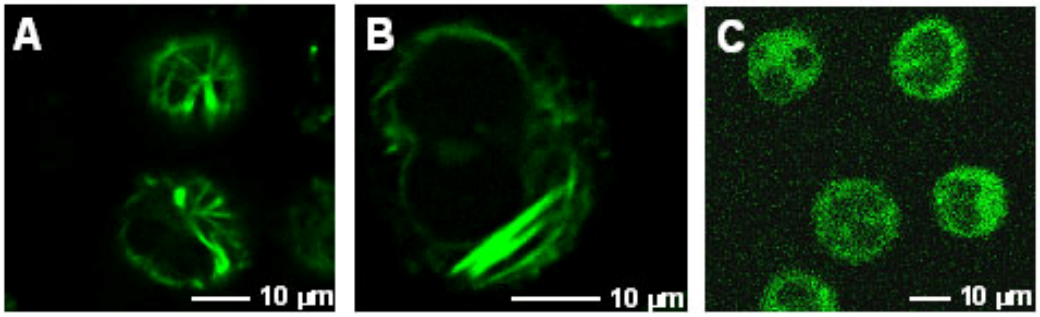


Figure 4-16. L1210FR cells treated with Biotin-Linker-Taxoid conjugate **4-25** and a fluorescent taxoid **4-33**

These confocal fluorescent images clearly demonstrate that the designed mechanism has worked successfully, which includes drug conjugate internalization by tumor-targeting moiety (biotin), disulfide linker cleavage by intracellular thiols (GSH), free intact drug (taxoid) release and its binding to the target protein, microtubules. The purposed tumor-targeting drug delivery was verified.

Moreover, the cytotoxicity of these taxoid conjugates against L1210FR cells were also examined by MTT assay (Table 4-1). Amazingly, Biotin-Linker-Taxoid conjugate (**4-41**) showed the same or slightly better potency than **SB-T-1214**, which also validated that taxoid was indeed released in its original form.

Table 4-1. *In vitro* cytotoxicity of some taxoids or conjugates against L1210FR

	Taxol	**SB-T-1214**	Biotin-Linker-**SB-T-1214** (**4-41**)	**SB-T-1214**-Fluorescein (**4-33**)
IC_{50} (nM)*	122	8.90	8.80	87.6

*: The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure.

Furthermore, the cytotoxicities of Biotin-Linker-**SB-T-1214** (**4-41**) against tumor cells and normal cells were also measured. L1210 (leukemia) and WI38 (non-cancerous human embryonic fibroblast cells) are two types of cell lines that are lack of biotin receptor on their surface, compared with L1210FR cells that overexpress biotin receptor on their surface. Shown in Table 4-2, the cytotoxicity of **4-41** against L1210 cells was 522 nM, which was 60 times less compared with that against L1210FR cells. This, again, indicates clearly that the biotin receptor plays an important role to transfer the biotin-drug conjugate into the cells. Those cells were treated with **4-41** for 72 hours before the MTT assay was carried out, and the drug may be completely released from the conjugate in such long incubation time, i.e., 72 hours in our experiment. The data also showed that the biotin-drug conjugate **4-41** seemed not toxic against the cells with limited number of biotin receptors on their surface, whatever the cells are malignant (L1210) or not (WI38).

Table 4-2. *In vitro* cytotoxicity of Biotin-Linker-Taxoids on different cell lines

Cell lines	L1210FR	L1210	WI38
IC_{50} (nM) *	8.8	522	570

*: The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure.

§ 4.5. Summary

A tumor-targeting drug delivery system (TTDDS), composed of a tumor-targeting moiety (TTM), a disulfide linker and a taxoid, was successfully designed and developed, in which biotin served as the TTM. With the help of TTM as the guide, the whole drug conjugate should be introduced into cancerous cells selectively without hurting normal cells. The function of the disulfide linker is to keep the whole conjuage stable during circulation in blood, but to release the anticancer agents completely and quickly inside the tumor cell. The taxoid in its conjugate state is non-toxic during transportation, but its potency is recovered once it is cleaved off from the whole conjugate and liberated at its original form inside the cancer cells.

A series of biotin-linker-taxoid conjugates were designed and synthesized, and the cellular experiments successfully provided the proof of concept, *i.e.*, internalization of the whole conjugate, disulfide bond cleavage by intracellular thiol source, fast intramolecular thiolactonization, the release of the free cytotoxic agent and the binding of the free cytotoxic agent to the intracellular target protein. Different functional fluorescent molecules were also coupled to those conjugates, which facilitated the observation *in vitro* by confocal fluorescence microscopy.

In addition, similar idea was concieved, in which single-walled carbon nanotube (SWNT) served as the drug carrier. After proper chemical modifications, SWNT was able to successfully deliver massive therapeutic agents into tumor cells, and the whole process was unambiguously validated in the experiments *in vitro* by means of confocal fluorescence microscopy.

§ 4.6. Experimental section

General Methods: NMR spectra were measured on a Varian 300 or Varian 400 NMR spectrometer. TLC was performed on Merck DC-aluminfolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). Solvents were purified either by distillation after dried with respective drying agents according to standard protocols or by PureSolv^{TM} (Innovative Technology, Inc) under N_2. De-gassed solvents were applied when necessary. Analytical HPLC was performed on Shimadzu L-2010A with CH_3CN and H_2O as the mobile phase. The columns for HPLC were Waters Nova-Pak® (C18, 3.9 x 150 mm) and Phenomenex® (Curosil–B, 5μ, 250 x 4.60 mm). IR was measured on Shimadzu FTIR-8400S. High-resolution mass spectrometry (HRMS) analyses were conducted at the Mass Spectrometry Laboratory of the University of Illinois at Urbana-Champaign.

Chemicals and reagents, if not specified, were used as received from commercial sources. Dark room, aluminum foil, and inert nitrogen atmosphere was applied when necessary. The staining agent on TLC for biotin derivatives was 4-*N, N*-Dimethylamino-cinnamaldehyde (DACA) solution prepared according to literature.[334]

The *in vitro* cellular assays were carried out by Dr. Jingyi Chen and Dr. Shuyi Chen.

Biotin hydrazide (4-3):[325]

To a suspension of biotin (**4-1**, 300 mg, 1.23 mmol) in MeOH (3 mL) was added $SOCl_2$ (0.30 mL, 4.0 mmol), and the solution was stirred overnight at room temperature, which formed a clear solution. After evaporation of the solvent at reduced pressure, the crude biotin methyl ester **4-2** was obtained (296 mg, 93% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.4-1.7 (m, 6 H), 2.34 (t, J = 7.2 Hz, 2 H), 2.76 (d, J = 13.2 Hz, 1 H), 2.91 (dd, J_1 = 13.2, 4.8 Hz, 1 H), 3.17 (m, 1 H), 3.67 (s, 3 H), 4.32 (m, 1 H), 4.52 (m, 1 H), 5.08 (s, 1 H), 5.37 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.5, 28.1, 28.4, 33.6, 40.0, 51.6, 55.4, 62.2, 63.8, 163.9, 174.3. All data were in agreement with literature values.[325]

Biotin methyl ester was dispersed in MeOH (2.5 mL), and hydrazine (0.30 mL, 10 mmol) was added. After stirring for 16 h, the solution was condensed and diluted by water. The aqueous layer was washed by chloroform three times to remove the unreacted biotin methyl ester, and water was removed to give biotin hydrazide **4-3** (296 mg, 99%) as white solid: ^{1}H NMR (400 MHz, D_2O) δ 1.40 (m, 2 H), 1.5-1.7 (m, 4 H), 2.3 (t, J = 7.2 Hz, 2 H), 2.8 (d, J = 13.2 Hz, 1 H), 3.0 (dd, J = 13.2, 4.8 Hz, 1 H), 3.4 (m, 1 H), 4.4 (m, 1 H), 4.6 (m, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 25.0, 27.8, 28.0, 33.6, 39.9, 55.5, 60.5, 62.3, 165.6, 175.8. All data were in agreement with literature values. [325]

Biotin-FITC (4-5):

To a solution of biotin hydrazide **4-3** (52 mg, 0.2 mmol) in dimethylsulfoxide (DMSO, 1 mL) was added fluorescein isothiocynate (FITC, **4-4**, 130 mg, 0.3 mmol). The yellow solution was stirred for 36 h at room temperature. DMSO was removed and the residue was purified by column chromatograph on silica gel (2-5% MeOH in DCM) to give the desired product (120 mg, 93% yield) as a yellow or brown solid: ^{1}H NMR (300 MHz, CD_3OD) δ 1.4-1.8 (m, 6 H), 2.35 (t, J = 7.5 Hz, 2 H), 2.68 (d, J = 12.9 Hz, 1 H), 2.90 (dd, J = 12.9, 4.8 Hz, 1 H), 3.20 (m, 1 H), 4.29 (m, 1 H), 4.47 (m, 1 H), 6.57 (m, 2

H), 6.67 (m, 2 H), 6.76 (m, 2 H), 7.15 (d, J = 8.4 Hz, 1 H), 7.87 (dd, J = 8.4, 1.8 Hz, 1 H), 8.13 (d, J = 1.8 Hz, 1 H); ^{13}C NMR (75 MHz, CD_3OD) δ 26.3, 29.6, 29.9, 34.7, 41.2, 57.1, 61.7, 63.4, 103.7, 111.7, 114.0, 121.8, 125.6, 128.8, 130.5, 133.6, 142.4, 150.3, 154.4, 161.7, 166.2, 171.0, 175.8, 184.2; HRMS (ESI) $C_{31}H_{29}N_5O_7S_2H^+$ *calc*. 648.1581, found 648.1587 (Δ = -0.9 ppm); the HPLC purity was 93.1% by Waters Nova-Pak® C-18 column (CH_3CN/H_2O = 20/80, 1 mL/min, UV 254 nm detector, retention time 3.30 min), and was 90.3% by Phenomenex C-18 column (CH_3CN/H_2O = 20/80, 1 mL/min, UV 254 nm detector, retention time 5.67 min).

***N'*-Biotinyl-3-(pyridin-2-yldisulfanyl)propanehydrazide (4-6):**

To a solution of SPDP (**3-47**, 75 mg, 0.24 mmol) in DMSO (1 mL) was added biotin hydrazide (**4-3**, 51.6 mg, 0.2 mmol). After stirring for 16 to 24 h, DMSO was removed and desired product **4-6** was obtained (82 mg, 90% yield) as white powder after washing by ether and methanol (no chromatography was necessary): ^{1}H NMR (300 MHz, DMSO-d^6) δ 1.2-1.6 (m, 6 H), 2.10 (t, J = 7.2 Hz, 2 H), 2.5-2.6 (m, 3 H), 2.80 (dd, J = 12.3, 5.1 Hz, 1 H), 3.0 (m, 3 H), 4.14 (m, 1 H), 4.28 (m, 1 H), 6.34 (s, 1 H), 6.39 (s, 1 H), 7.24 (m, 1 H), 7.78-7.83 (m, 2 H), 8.45 (m, 1 H), 9.73 (s, 1 H), 9.83 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 25.06, 28.05, 28.11, 32.61, 32.95, 33.78, 55.44, 59.25, 61.09, 119.31, 121.27, 137.94, 149.65, 159.06, 162.77, 168.90, 171.04.

2-(3-Biotinylhydrazinyl-3-oxopropyl)disulfanylphenylacetic acid (4-7):

To a solution of **4-6** (82 mg, 0.18 mmol) in DMSO (6 mL) was added **3-12** (30.3 mg, 0.18 mmol) at room temperature. After stirring for 4 h, DMSO was removed and the desired product was isolated as a white powder after washed by ether. ^{1}H NMR (300 MHz, CD_3OD): δ 1.3-1.6 (m, 6 H), 2.25 (t, J = 6.9 Hz, 2 H), 2.6-2.7 (m, 3 H), 2.9-3.0 (m, 3 H), 3.20 (m, 1 H), 3.84 (s, 2 H), 4.29 (m, 1 H), 4.46 (m, 1 H), 7.25-7.33 (m, 3 H), 7.78 (d, J = 7.8 Hz, 1 H).

Fluorescein bis(phenylacetate) (4-11-1):

To a solution of phenylacetic acid (**4-10-1**, 136.2 mg, 1 mmol), fluorescein (**4-8**, 33.2 mg, 0.1 mmol) and DMAP (24.4 mg, 0.2 mmol) in THF (1 mL) was added DCC (206 mg, 1 mmol) at 0 ^{o}C. After stirring for 40 h at room temperature, DCU was filtered off. After purification by a silica gel column (hexane/EtOAc = 6/1), the desired product was obtained (40 mg, 70% yield) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 4 H), 6.78 (m, 4 H), 7.05 (m, 2 H), 7.13 (m, 1 H), 7.3-7.4 (m, 10 H), 7.6-7.7 (m, 2 H), 8.0 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.34, 81.56, 110.25, 116.48, 117.61, 123.98, 125.21, 126.01, 127.50, 128.79, 128.90, 129.26, 130.02, 133.00, 135.26, 151.49, 152.07, 152.93, 169.10, 169.41. Mono-substituted derivative was not successfully separated from DCU.

Under the same conditions by using DMSO as the solvent, the yield for **4-11-1** was 44%. When the amount of acid was reduced to 5 equivalents regarding to fluorescein, the isolated yield for **4-11-1** in THF was 21%; the mono- was 36%; and the unreacted fluorescein was 43%.

Fluorescein bis(2-*o*-tolylacetate) (4-11-2):

To a solution of *o*-tolylacetic acid (**4-10-2**, 120 mg, 0.8 mmol) and TEA (0.23 mL, 1.6 mmol) in THF (1 mL) was added bis(2-oxooxazolidin-3-yl)phosphinic chloride (BOP-Cl, 203.7 mg, 0.8 mmol) at 0 ^{o}C. After stirring for 18 h, the yellow milky solution was diluted by EtOAc, which was washed by $NaHCO_3$ (aq, s) and brine. After purification on a silica gel column (hexane/EtOAc = 6/1), the desired product **4-11-2** was obtained (48 mg, 80% yield) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (s, 6 H), 3.88 (s, 4 H), 6.80 (m, 4 H), 7.05 (m, 2), 7.1-7.4 (m, 9 H), 7.6-7.7 (m, 2 H), 8.0 (m, 1 H).

4-Methyl-2-oxo-2H-chromen-7-yl 2-*o*-tolylacetate (4-11-3):

To a solution of *o*-tolylacetic acid (150 mg, 1 mmol), **4-12** (177 mg, 1 mmol) and DMAP (24 mg, 0.2 mmol) in THF (3 mL) was added DCC (206 mg, 1 mmol) at 0 ^{o}C. After stirring for 2 h at room temperature, DCU was filtered off, and the crude was purified by silica gel column (DCM/MeOH = 100/1) to give the desired product **4-11-3** (287 mg, 93% yield) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3 H), 2.44 (s, 3 H), 3.94 (s, 2 H), 6.25 (q, *J* = 1.5 Hz, 1 H), 7.06 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.11 (d, *J* = 2.1 Hz, 1 H), 7.2-7.4 (m, 4 H), 7.63 (d, *J* = 8.7 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.64, 19.65, 39.17, 110.22, 114.44, 117.81, 117.94, 125.43, 126.37, 127.85, 130.23, 130.58, 131.72, 136.88, 151.97, 153.11, 154.08, 160.36, 169.27.

4-Methyl-2-oxo-2H-chromen-7-yl
2-(2,5-dioxopyrrolidin-1-yloxycarbonylethyldisulfanyl)phenylacetate (4-13):

*from **4-15***: To a solution of **4-15** (40 mg, 0.093 mmol) and *N*-hydroxysuccinimide (HOSu, 11 mg, 0.093 mmol) in DCM (0.25 mL) and pyridine (0.25 mL) was added DCC (20 mg, 0.098 mmol) at 0 ^{o}C. After stirring for 18 h, the solvent was evaporated, and the residue was further purified on a silica gel column (hexane/EtOAc = 1/2 to 1/3) to give the desired product (33 mg, 67% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (m, 3 H), 2.81-2.85 (s and s, 4 H), 3.02 (m, 4 H), 4.0-4.2 (s and s, 2 H), 6.25 (m, 1 H), 7.04-7.12 (m, 2 H), 7.20-7.33 (m, 3 H), 7.56 (d, *J* = 8.4 Hz, 1 H), 7.80 (m, 1H).

*from **3-56***: To a solution of **3-56** (112 mg, 0.30 mmol), **4-12** (53.3 mg, 0.30 mmol) and DMAP (4 mg, 0.03 mmol) in THF (1.0 mL) was added DCC (64 mg, 0.31 mmol) at 0 ^{o}C. After stirring 4 h at room temperature, the solution was evaporated, and the crude mixture was separated on a silica gel column (hexane/EtOAc = 1/2), which gave the desired product (42 mg, 27% yield) as white solid. NMR was identical with that from **4-15**. Di-HMC ester was also observed as a by-product after separation on a silica gel column along with DCU in this procedure.

4-Methyl-2-oxo-2H-chromen-7-yl
2-(tri*iso*propylsiloxycarbonylethyldisulfanyl)phenylacetate (4-14):

To a solution of **3-57** (113 mg, 0.26 mmol), 4-dimethylaminopyridine (DMAP, 16 mg, 0.13 mmol) and 7-hydroxy-4-methylcoumarin (**HMC**, **4-12** 142 mg, 0.78 mmol) in THF (2 mL) was added dicyclohexylcarbodiimide (DCC, 56 mg, 0.26 mmol) at 0 ^{o}C. After stirring for 3 h at room temperature, the precipitation was filtered off, and the desired product was obtained (43 mg, 28% yield) after silica gel column purification (hexane/EtOAc = 8/1). Rotamers were observed in NMR by using either $CDCl_3$ or CD_3OD or DMSO-d^6 at different temperatures: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (m, 18 H), 1.31 (m, 3H), 2.40-2.42 (m, 3 H), 2.77 (t, *J* = 7.2 Hz, 1.6 H), 2.96 (t, *J* = 6.8 Hz,

1.6 H), 3.04 (m, 0.8 H), 3.90 (s, 0.4 H), 4.14 (s, 1.6 H), 6.25 (m, 1 H), 7.04-7.12 (m, 2 H), 7.20-7.33 (m, 3 H), 7.56 (m, 1 H), 7.80 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.02, 17.90, 18.85 (CH_3), 33.84, 35.43, 39.53, 110.50, 114.69, 118.03, 118.16, 125.50 (110.5-125.5 coumarin), 128.54, 128.76, 131.31, 131.33, 133.87, 136.75 (128.6-136.8 linker), 152.01, 153.28, 154.30, 160.58, 168.97, 171.51. Some other peaks may belong to the rotamer: 17.85 (TIPS CH_3), 32.88, 34.02, 40.93 (linker, Ar-CH_2), 114.75, 118.12, 125.55, 127.99, 128.29, 134.87, 136.58, 153.01, 169.67, 170.72.

4-Methyl-7-(tri*iso*propylsiloxy)-2H-chromen-2-one (4-11-4):

The title compound was isolated by column chromatography on silica gel (hexane/EtOAc = 10/1) when **3-57** was converted into **4-14**: ^{1}H NMR for **4-11-4** (300 MHz, $CDCl_3$) δ 1.10 (m, 18 H), 1.26 (m, 3H), 2.38 (d, J = 0.9 Hz, 3 H), 6.12 (q, J = 1.2 Hz, 1 H), 6.82 (m, 2 H), 7.43 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.80, 17.99, 18.81, 107.71, 112.23, 114.19, 117.21, 125.60, 152.66, 155.20, 159.75, 161.51.

3-[2-(4-Methyl-2-oxo-2H-chromen-7-yloxycarbonylmethyl)phenyldisulfanyl]propanoic acid (4-15):

To a solution of **4-14** (105 mg, 0.18 mmol) in pyridine (1.6 mL) and acetonitrile (1.6 mL) was added HF/Py (70% weight, 0.80 mL) at 0 ^{o}C. After stirring for 5 h, the solution was diluted by EtOAc and washed by water, $CuSO_4$ (aq, s) and brine. After purification by silica gel column (hexane/EA = 1/1.5), the desired product was obtained (40 mg, 57% yield) as white solid. Rotamers were observed in NMR by using $CDCl_3$: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (m, 3 H), 2.77 (t, J = 7.2 Hz, 1.4 H), 2.95 (t, J = 7.2 Hz, 1.4 H), 3.04 (m, 1.2 H), 3.90 (s, 0.7 H), 4.14 (s, 1.3 H), 6.26 (m, 1 H), 7.04-7.12 (m, 2 H), 7.20-7.33 (m, 3 H), 7.56 (d, J = 8.4 Hz, 1 H), 7.80 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.86 (HMC CH_3), 32.84, 33.69, 39.51 (linker, Ph-CH_2), 110.51, 114.64, 118.10, 118.19, 125.56, 128.56, 128.83, 131.04, 131.42, 133.71, 136.53, 152.21, 153.27, 154.25, 160.80, 169.03, 176.88. Some other peaks may belong to the rotamer: 32.89, 34.06, 39.10, 110.54, 114.72, 118.06, 125.55, 128.36, 128.71, 130.78, 131.34, 133.91, 136.69, 152.16, 153.00, 160.73, 169.75, 176.52.

4-Methyl-2-oxo-2H-chromen-7-yl 2-(2-((3-(2-biotinylhydrazinyl)-3-oxopropyl)disulfanyl) phenyl)acetate (4-16):

To a solution of **4-13** (75 mg, 0.14 mmol) in DMSO (1 ml) was added **4-3** (33 mg, 0.13 mmol). After stirring for 16 h at room temperature, the solution was subjected to silica gel column directly (DCM:MeOH = 10:1) to give the title compound **4-16** (48 mg, 56% yield) as white powder: ^{1}H NMR (300 MHz, CD_3OD) δ 1.4-1.8 (m, 6 H, biotin side chain), 2.25 (t, J = 7.2 Hz, 2 H, biotin), 2.48 (m, 3 H, HMC), 2.65-2.73 (m, 3 H, biotin (1) and linker (2)), 2.90 (m, 1 H, biotin), 3.0 (m, 2 H, linker), 3.20 (m, 1 H, biotin), 3.86 and 4.21 (s, 2 H, integrations were 0.78 and 1.24, respectively, linker), 4.27(m, 1 H, biotin), 4.45 (m, 1 H, biotin), 6.32 (m, 1 H, HMC), 7.15 (m, 2 H, linker), 7.2-7.4 (m, 3 H, linker (1) and HMC (2)), 7.78 (m, 2 H, linker (1) and HMC (1)); ^{13}C NMR (100 MHz, DMSO-d^6) δ 18.16 (HMC), 25.02, 28.01, 28.08 (25.1-28.1 biotin), 32.68, 32.92 (linker two carbons), 33.41 (biotin), 38.57 (linker, Ar-CH_2), 39.86 (biotin), 55.39, 59.19, 61.04 (55.4-61.0 biotin), 109.85, 113.85, 117.69, 118.19, 126.58 (109.8-126.6 HMC), 127.59, 128.67, 130.15, 131.63, 133.78, 136.15 (127.6-136.2 linker), 152.76, 152.90, 153.55, 159.58 (152.8-159.6 HMC), 162.71 (biotin), 168.82 (amide), 169.64 (amide), 170.90 (ester).

Some other peaks may belong to the rotamer: 32.57, 37.83, 109.97, 113.81, 117.62, 118.30, 126.46, 127.88, 128.14, 130.69, 136.29, 152.68, 152.92, 153.50, 159.57, 162.67, 168.19, 169.64, 171.00; HRMS (ESI) $C_{31}H_{34}N_4O_7S_3H^+$ *calc.* 671.1662, found 671.1674 (Δ = 1.8 ppm); the HPLC purity was 94.8% by Waters Nova-Pak® C-18 column (CH_3CN/H_2O = 70/30, 0.5 mL/min, UV 254 nm detector, retention time 2.91 min), and was 95.3% by Phenomenex C-18 column (CH_3CN/H_2O = 70/30, 0.5 mL/min, UV 254 nm detector, retention time 6.71 min).

1-(4,4'-Dimethoxytrityl)biotin methyl ester (DMTr-Biotin-OMe, 4-17): [327,330]

To a suspension of Biotin-OMe (**4-2**, 51.6 mg, 0.2 mmol), DMAP (6 mg, 0.05 mmol) and DMTr-Cl (203.3 mg, 0.6 mmol) in pyridine (1 mL) was injected TEA (0.030 mL, 0.2 mmol) at room temperature. After stirring at 70 ^oC for 4 h, the reaction was quenched by MeOH (5 mL) at room temperature. Evaporation of the solvent gave a mixture, which was purified on a silica gel column (DCM:MeOH = 100:1) to give **4-17** (87 mg, 78% yield) as yellow powder: ^1H NMR (300 MHz, $CDCl_3$*) δ 1.4-1.7 (m, 6 H), 2.26 (m, 1 H), 2.31 (t, *J* = 7.5 Hz, 2 H), 2.46 (dd, *J* = 13.2, 1.8 Hz, 1 H), 3.17 (m, 1 H), 3.67 (s, 3 H), 3.80 (s, 6 H), 4.36 (m, 2 H), 4.56 (br, 1 H), 6.81 (m, 4 H), 7.16 (m, 4 H), 7.30 (m, 5 H). All data were in accord with reported values.[327,330]

* It's very important to mention that $CDCl_3$ must be free of acids before using. This did not show in the above literature.

1-(4,4'-Dimethoxytrityl)biotin hydrazide (DMTr-Biotin-NHNH2, 4-18):

To a solution of **4-17** (56 mg, 0.1 mmol) in MeOH (0.25 mL) was added anhydrous hydrazine (0.030 mL, 0.9 mmol). After stirring at room temperature for 20 h, the solvent was evaporated in vacuum, and the desired product was obtained (53.2 mg, 95% yield) as white powder: ^1H NMR (300 MHz, $CDCl_3$*) δ 1.4-1.7 (m, 6 H), 1.96 (t, *J* = 7.5 Hz, 2 H), 2.25 (dd, *J* = 13.2, 5.4 Hz, 1 H), 2.46 (dd, *J* = 12.9, 1.8 Hz, 1 H), 3.07 (m, 1 H), 3.6-3.9 (br, 2 H), 3.79 (s, 6 H), 4.2-4.4 (m, 2 H), 5.56 (br, 1 H), 6.81 (m, 4 H), 7.16 (m, 5 H, including CO-N<u>H</u>-NH_2), 7.30 (m, 5 H).

* It's very important to mention that $CDCl_3$ must be free of acids before using.

***N'*-[1-(4,4'-Dimethoxytrityl)biotinyl]-3-(pyridin-2-yldisulfanyl)propanehydrazide (4-19):**

To a solution of **4-18** (53.2 mg, 0.095 mmol) in THF (0.5 mL) was added SPDP (**3-47**, 38.5 mg, 0.123 mmol). After stirring for 16 h at room temperature, the crude was directly purified by silica gel column (5% MeOH in DCM) to give the desired product (28 mg, 39% yield).

2'-*tert*-Butyldimethylsilyl-SB-T-1214 (SB-T-1214-2'-TBDMS, 4-20): [331]

To a flask containing **SB-T-1214** (343 mg, 0.40 mmol), TBDMSCl (302 mg, 2.0 mmol) and imidazole (273 mg, 4.0 mmol) was added dry DMF (0.54 mL). After stirring at room temperature for 18 h (some other group members suggested that 2 to 4 h was enough), the reaction mixture was diluted with EtOAc (90 mL), which was washed by water and brine. After evaporation of the solvent and purification on a silica gel column (hexane/EtOAc = 3/1), the desired product **4-20** was obtained (330 mg, 85% yield) as white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.080 (s, 3 H), 0.12 (s, 3 H), 0.98-1.05 (m, 2

H, cyclopropyl), 1.13-1.21 (m, 2 H, cyclopropyl), 1.15 (s, 3H, C17), 1.26 (s, 3H, C16), 1.35 (s, 9 H, Boc), 1.37 (s, 9 H), 1.66 (s, 3 H, C19), 1.74-1.79 (m, 8 H, C5', cyclopropyl, and OH), 1.86 (m, 1 H, C6(1)), 1.87 (s, 3 H, C18), 2.2-2.4 (m, 2 H, C6(1) and OH), 2.41 (s, 3 H, OAc), 2.55 (m, 1 H, C14(1)), 2.58 (d, J = 3.9 Hz, 1 H, C14(1)), 3.81 (d, J = 7.2 Hz, 1 H, C3), 4.17 (d, J = 8.1 Hz, 1 H, C20(1)), 4.23 (d, J = 3.6 Hz, 1 H, C2'), 4.30 (d, J = 8.1 Hz, 1 H, C20(1)), 4.40 (m, 1 H, C7), 4.78 (m, 2 H, C3' and C4'), 4.95 (d, J = 7.8 Hz, 1 H, C5), 5.21 (d, J = 8.4 Hz, 1 H, NH), 5.65 (d, J = 7.2 Hz, 1 H, C2), 6.19 (t, J = 7.2, 1 H, C13), 6.28 (s, 1 H, C10), 7.49 (t, J = 8.1 Hz, 2 H), 7.61 (t, J = 7.2, 1 H), 8.11 (d, J = 8.4 Hz, 2 H). The purity was more than 99% in HPLC analysis. Another procedure with higher temperature and shorter reaction time was reported recently.[335]

2'-*tert*-Butyldimethylsilyl-7-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propano-yl]-SB-T-1214 (SB-T-1214-2'-TBDMS-7-β-Ala-Fmoc, 4-22): [308,336,337]

See the references for details.

2'-*tert*-Butyldimethylsilyl-7-(3-aminopropanoyl)-SB-T-1214 (SB-T-1214-2'-TBDMS-7-β-Ala-NH_2, 4-23): [308,336,337]

See the references for details.

SB-T-1214-2'-OH-7-β-Ala-Rhodamine (**4-25**): [308,336,337]

See the references for details.

Methyl 2-(3-hydroxy-6-oxo-6H-xanthen-9-yl)benzoate (Fluorescein-OMe, 4-26): [332]

To a solution of fluorescein (**3-8**, 1.04 g, 3 mmol) in dry MeOH was added H_2SO_4 (0.80 mL, 15 mmol). After refluxing for 14 h, the solvent was removed, and the residue was diluted with EtOAc. The organic layer was washed by $NaHCO_3$ (aq, s), water and brine. After evaporation of the solvent, the title compound **4-26** was obtained (648 mg, 62% yield) as dark red powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.58 (s, 3 H), 6.80 (dd, J = 9.3, 2.1 Hz, 2 H), 6.89 (d, J = 2.1 Hz, 2 H), 6.96 (d, J = 9.0 Hz, 2 H), 7.30 (dd, J = 7.5, 1.2 Hz, 1 H), 7.63-7.75 (m, 2 H), 8.22 (dd, J = 7.5, 1.2 Hz, 1 H), 11.50 (br, 1 H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 52.40, 102.87, 115.26, 121.14, 129.32, 130.43, 130.48, 130.76, 131.09, 133.20, 133.56, 157.10, 165.11, 172.52 (One carbon less may be due to overlapping, possibly at 158 ppm). All data were in agreement with literature values.[332]

Mono-*tert*-butyl succinate (4-27): [333]

To a flask containing succinic anhydride (3.00 g, 30 mmol), HOSu (1.00 g, 9 mmol) and DMAP (0.35 g, 3 mmol) was added ^{t}-BuOH (8.50 mL, 90 mmol), toluene (17.5 mL) and TEA (1.26 mL, 9 mmol). After stirring at 80°C for 12 h, the resulting brown solution was diluted with EtOAc, and washed by 10% citric acid and brine. After dried over $MgSO_4$, the solvent was removed. The title compound **4-27** was obtained (2.58 g, 49% yield) as white solid after recrystallization from EtOAc-hexanes: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 2.51-2.54 (m, 2 H), 2.60-2.63 (m, 2H), 11.5 (br, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.17, 29.36, 30.26, 81.18, 171.56, 178.82. All data are in agreement with literature values.[333]

***tert*-Butyl 9-(2-methoxycarbonylphenyl)-6-oxo-6H-xanthen-3-yl succinate (4-28):**

To a solution of fluorescein methyl ester (**4-26**, 100 mg, 0.29 mmol), *tert*-butyl 4-hydroxybutanoate (**4-27**, 400 mg, 2.3 mmol) and DMAP (77 mg, 0.63 mmol) in THF (4 mL) was added DCC (474 mg, 2.3 mmol). After stirring at room temperature for 21 h, the solution was filtered and concentrated. The crude mixture was purified on a silica gel column (hexane/EtOAc = 1/1.5) to give the desired product **4-28** (44 mg, 30% yield) as yellow powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9 H), 2.65 (m, 2 H), 2.84 (m, 2 H), 3.64 (s, 3 H), 6.43 (dd, *J* = 1.8, 0.6 Hz, 1 H), 6.52 (ddd, *J* = 9.9, 2.1, 0.4 Hz, 1 H), 6.84 (d, *J* = 9.6 Hz, 1 H), 6.93 (m, 2 H), 7.27-7.30 (m, 2 H), 7.67 (td, *J* = 7.5, 1.2 Hz, 1 H), 7.74 (td, *J* = 7.5, 1.2 Hz, 1 H), 8.22 (dd, *J* = 7.5, 1.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.22, 29.69, 30.29, 52.61, 81.35, 106.33, 110.32, 118.35, 119.03, 119.70, 128.64, 129.99, 130.40, 130.44, 130.78, 130.87, 131.40, 132.99, 134.45, 149.09, 152.91, 154.18, 158.75, 165.64, 170.48, 171.26, 186.05.

***tert*-Butyl 4-hydroxybutanoate (4-29):** [333]

The title compound was prepared according to literature: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9 H), 1.80 (m, 2 H), 2.31 (t, *J* = 6.9 Hz, 2 H), 2.50 (s, 1 H, OH), 3.62 (t, J =6.3 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.78, 27.98, 32.31, 61.96, 80.43, 173.41. All data are in accordance with literature values.[333]

Methyl 2-[3-(4-*tert*-butoxy-4-oxobutoxy)-6-oxo-6H-xanthen-9-yl]benzoate (4-30): [332]

To a solution of **4-26** (293 mg, 0.85 mmol), **4-29** (400 mg, 2.5 mmol) and Ph_3P (656 mg, 2.5 mmol) in THF and CH_3CN was added diisopropylazodicarboxylate (**DIAD**, 0.33 mL, 1.7 mmol). After stirring at room temperature for 3 h, the solvent was removed. After column purification on silica gel (DCM/MeOH = 100/1), the desired product **4-30** was obtained (372 mg, ~90% yield, calc. based on NMR) as yellow powder, which was slightly contaminated by Ph_3PO: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12 (m, 2 H), 2.59 (t, *J* = 6.9 Hz, 2 H), 3.62 (s, 3 H), 4.24 (t, *J* = 6.0 Hz, 2 H), 6.4-6.8 (br, 1 H), 6.88 (m, 2 H), 6.96 (s, 1 H), 7.06 (dd, *J* = 9.0, 6.0 Hz, 2 H), 7.16 (m, 1 H), 7.31 (dd, *J* = 7.5, 1.2 Hz, 1 H), 7.63-7.75 (m, 2 H), 8.30 (dd, *J* = 7.5, 1.2 Hz, 1 H). Ph_3PO was totally removed after treatment with TFA to remove *tert*-Butyl ester in the preparation of **4-33**.

4-[9-(2-Methoxycarbonylphenyl)-6-oxo-6H-xanthen-3-yloxy]butanoic acid (4-31):

The title compound was obtained by treating **4-30** with TFA/DCM (100 mg / 2 mL) for 2 h. After evaporation, the crude product was used without further purification.

SB-T-1214-2'-TBDMS-7-Fluorescein (**4-32**): [336,337]

See the references for details.

SB-T-1214-2'-OH-7-Fluorescein (**4-33**): [336,337]

See the references for details.

7-Fluorophore-SB-T-1214-2'-Linker-OTIPS (4-34):

To a solution of **4-33** (84 mg, 0.066 mmol), **3-57** (34 mg, 0.079 mmol) and DMAP (8 mg, 0.066 mmol) in DCM was added DIC (0.020 mL, 0.132 mmol) at 0 ^{o}C. After stirring for 4 h at room temperature, the reaction mixture was concentrated. The residue was purified on a silica gel column (hexane/EtOAc = 1/2 to 1/4), and the desired

product was obtained (65 mg, 71% yield) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98-1.05 (m, 2 H, cyclopropyl), 1.07 (d, *J* = 6.9 Hz, 18 H, TIPS), 1.13-1.21 (m, 2 H, cyclopropyl), 1.15 (s, 3H, C17), 1.26 (s, 3H, C16), 1.29 (m, 3 H, TIPS), 1.35 (s, 9 H, Boc), 1.62 (m, 2 H, OH and C6(1)), 1.65 (s, 3 H, C5'), 1.67 (s, 3 H, C5'), 1.72 (m, 1 H, cyclopropyl), 1.75 (s, 3 H, C19), 1.92 (s, 3 H, C18), 2.12 (m, 2 H, tether), 2.24 (m, 2 H, C6(1) and C14(1)), 2.32 (s, 3 H, Ac), 2.41 (m, 2 H, tether), 2.52 (m, 1 H, C14(1)), 2.72 (t, *J* = 6.6 Hz, 2 H, linker), 2.91 (t, *J* = 6.6 Hz, 2 H, linker), 3.58 (d, *J* = 0.9 Hz, 3 H, OCH_3), 3.87 (m, 2 H, C3 and linker(1)), 4.02-4.14 (m, 4 H, C20(1), linker(1) and tether (2)), 4.26 (d, *J* = 8.4 Hz, 1 H, C20(1)), 4.7-4.9 (m, 4 H, C2', C3', C4' and C5), 5.04 (d, *J* = 8.4 Hz, 1 H, NH), 5.55 (dd, *J* = 10.5, 6.9 Hz, 1 H, C7), 5.63 (d, *J* = 6.9 Hz, 1 H, C2), 6.11 (t, *J* = 7.2 Hz, 1 H, C13), 6.25 (s, 1 H, C10), 6.41 (s, 1 H), 6.47 (d, *J* = 9.9 Hz, 1 H), 6.68 (dd, *J* = 9.0, 2.1 Hz, 1 H), 6.80 (m, 2 H), 6.92 (t, *J* = 2.1 Hz, 1 H), 7.27 (m, 4 H, fluorophore(1) and linker(3)), 7.43 (t, *J* = 7.8 Hz, 2 H, taxoid), 7.54-7.73 (m, 4 H, taxoid(1), fluorophore(2) and linker(1)), 8.06 (d, *J* = 8.4 Hz, 2 H, taxoid), 8.19 (dd, *J* = 8.1 Hz, 1.5 Hz, 1 H, fluorophore). Some of the product (~15 mg), contaminated by **4-33**, was also collected during the column purification (2% MeOH in DCM as the eluant).

7-Fluorophore-SB-T-1214-2'-Linker-OH (4-35):

To a solution of **4-34** (65mg, 0.039 mmol) in pyridine and CH_3CN was added HF/Py (70% wt, 0.50 mL) at 0 ^oC. After stirred for 5 h at room temperature, the solution was diluted with EtOAc, and washed thoroughly by $CuSO_4$ (aq, s) several times, followed by water and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column (hexane/EtOAc = 1/4 to 1/8) to give the desired product (43 mg, 80% yield) as yellow powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98-1.05 (m, 2 H, cyclopropyl), 1.13-1.21 (m, 8 H, cyclopropyl, C17 and C16), 1.35 (s, 9 H, Boc), 1.62 (m, 2 H, OH and C6(1)), 1.72-1.78 (m, 10 H, cyclopropyl(1), C5'(6) and C19), 1.97 (s, 3 H, C18), 2.12 (m, 2 H, tether), 2.3-2.6 (m, 8 H, Ac(3), C6(1), C14(2) and tether(2)), 2.68 (t, *J* = 6.9 Hz, 2 H, linker), 2.95 (m, 2 H, linker), 3.62 (d, *J* = 0.9 Hz, 3 H, OCH_3), 3.94 (m, 2 H, C3 and linker(1)), 4.02-4.25 (m, 4 H, C20(1), linker(1) and tether (2)), 4.33 (d, *J* = 8.7 Hz, 1 H, C20(1)), 4.90-4.97 (m, 4 H, C2', C3', C4' and C5), 5.12 (d, *J* = 8.4 Hz, 1 H, NH), 5.58 (dd, *J* = 10.5, 6.9 Hz, 1 H, C7), 5.69 (d, *J* = 6.9 Hz, 1 H, C2), 6.17 (t, *J* = 7.2 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 6.58-6.62 (m, 2 H), 6.74 (dd, *J* = 9.0, 2.1 Hz, 1 H), 6.88 (m, 2 H), 7.03 (t, *J* = 1.8 Hz, 1 H), 7.27 (m, 4 H, fluorophore(1) and linker(3)), 7.43 (t, *J* = 7.8 Hz, 2 H, taxoid), 7.54-7.73 (m, 4 H, taxoid(1), fluorophore(2) and linker(1)), 8.06 (d, *J* = 8.4 Hz, 2 H, taxoid), 8.19 (dd, *J* = 8.1 Hz, 1.5 Hz, 1 H, fluorophore); MS (ESI) $C_{81}H_{87}NO_{24}S_2H^+$ *calc.* 1522.5, found 1522.4 (M+H), and $C_{81}H_{87}NO_{24}S_2Na^+$ *calc.* 1544.5, found 1544.4. For some reason, the proton for CO_2H was not observed in NMR analysis.

7-Fluorescein-SB-T-1214-2'-Linker-OSu (4-36):

To a solution of **4-35** (43 mg, 0.028 mmol) and HOSu (16 mg, 0.14 mmol) in THF (0.3 mL) was added DCC (14 mg, 0.068 mmol). After stirring for 36 h at room temperature, the reaction mixture was concentrated. The residue was purified by silica gel column (DCM/MeOH = 100/3) to give the desired product (48 mg, >100% yield) as yellow powder, together with some urea: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98-1.05 (m, 2 H, cyclopropyl), 1.13-1.21 (m, 8 H, cyclopropyl, C17 and C16), 1.34 (s, 9 H, Boc), 1.62

(m, 2 H, OH and C6(1)), 1.72-1.78 (m, 10 H, cyclopropyl(1), C5'(6) and C19), 1.97 (s, 3 H, C18), 2.12 (m, 2 H, tether), 2.3-2.6 (m, 8 H, Ac(3), C6(1), C14(2) and tether(2)), 2.83 (s, 4 H, OSu), 3.02 (m, 4 H, linker), 3.62 (d, J = 0.9 Hz, 3 H, OCH_3), 3.94 (m, 2 H, C3 and linker(1)), 4.02-4.25 (m, 4 H, C20(1), linker(1) and tether (2)), 4.33 (d, J = 8.7 Hz, 1 H, C20(1)), 4.90-4.97 (m, 4 H, C2', C3', C4' and C5), 5.12 (d, J = 8.4 Hz, 1 H, NH), 5.57 (dd, J = 10.5, 6.9 Hz, 1 H, C7), 5.67 (d, J = 6.9 Hz, 1 H, C2), 6.17 (t, J = 7.2 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 6.45 (d, J = 2.1 Hz, 1 H), 6.52 (dd, J = 9.6, 1.8 Hz, 1 H), 6.72 (dd, J = 9.0, 2.4 Hz, 1 H), 6.85 (m, 2 H), 6.96 (t, J = 2.4 Hz, 1 H), 7.27 (m, 4 H, fluorophore(1) and linker(3)), 7.47 (t, J = 7.8 Hz, 2 H, taxoid), 7.54-7.73 (m, 4 H, taxoid(1), fluorophore(2) and linker(1)), 8.09 (d, J = 8.4 Hz, 2 H, taxoid), 8.24 (dd, J = 8.1, 1.5 Hz, 1 H, fluorophore); MS (ESI) $C_{85}H_{90}N_2O_{26}S_2H^+$ *cal.* 1619.5, found 1619.5, and $C_{85}H_{90}N_2O_{26}S_2Na^+$ *cal.* 1641.5, found 1641.5.

Biotin-Linker-Taxoid-Fluorophore (**Compound C**, **4-25** in Scheme 4-13):

To a solution of **4-36** (45 mg, 0.022 mmol) in DMSO (0.2 mL) was added Biotin-$NHNH_2$ (**4-3**, 5.4 mg, 0.020 mmol) at room temperature. After stirring for 24 h, the solution was loaded onto silica gel column directly. The desired product **4-25** was obtained (24 mg, 82%) as yellow powder after column chromatography (DCM/MeOH = 100/6), and the unreacted **4-36** was recovered as well: ^{1}H NMR for **4-25** (300 MHz, $CDCl_3$) δ 0.80-1.10 (m, 4 H, cyclopropyl(2) and biotin(2)), 1.13-1.41 (m, 21 H, biotin (4), cyclopropyl(2), C17(3), C16(3) and Boc(9)), 1.5-1.8 (m, 12 H, OH, cyclopropyl(1), C6(1), C5'(6) and C19(3)), 1.95 (s, 3 H, C18), 2.1-2.74 (m, 15 H, tether(2 at 2.10 ppm), biotin(2 at 2.26 ppm), Ac(3), C6(1), C14(2), tether(2), biotin (1 at 2.60 ppm) and linker(2)), 2.85 (m, 1 H, biotin), 2.95 (m, 2 H, linker), 3.10 (m, 1 H, biotin), 3.62 (d, J = 1.2 Hz, 3 H, OCH_3), 3.94-4.20 (m, 6 H, C3(1), linker(2), C20(1) and tether (2)), 4.33 (m, 2 H, C20(1) and biotin(1)), 4.47 (m, 1 H, biotin), 4.90-5.2 (m, 5 H, C2', C3', C4', C5 and NH from taxoid), 5.59 (dd, J = 10.5, 6.9 Hz, 1 H, C7), 5.68 (d, J = 6.9 Hz, 1 H, C2), 5.83 (s, 1 H, urea NH from biotin), 6.16 (t, J = 7.2 Hz, 1 H, C13), 6.28 (s, 1 H, C10), 6.54 (d, J = 1.5 Hz, 1 H, f-phore), 6.62 (dd, J = 9.6, 1.8 Hz, 1 H, f-phore), 6.66 (br, 1 H, urea NH from biotin), 6.75 (dd, J = 9.0, 2.4 Hz, 1 H, f-phore), 6.88 (m, 2 H, f-phore), 7.02 (t, J = 2.1 Hz, 1 H, f-phore), 7.27-7.35 (m, 4 H, fluorophore(1) and linker(3)), 7.43 (t, J = 7.8 Hz, 2 H, taxoid), 7.54-7.73 (m, 4 H, taxoid(1), fluorophore(2) and linker(1)), 8.06 (d, J = 8.4 Hz, 2 H, taxoid), 8.19 (dd, J = 8.1, 1.5 Hz, 1 H, fluorophore), 9.13 (br, 1 H, CONH-N from biotin), 9.31 (br, 1 H, CONH-N from biotin); ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.98, 11.05, 13.05, 14.68, 18.73, 21.58, 22.60, 23.88, 25.28, 25.62, 25.95, 26.45, 27.96, 28.08, 28.45, 30.48, 33.16, 33.44, 33.45, 35.63, 38.90, 40.62, 43.45, 47.04, 49.21, 52.61, 55.66, 56.19, 60.57, 62.08, 68.25, 71.71, 71.99, 74.76, 75.14, 75.23, 76.49, 78.82, 80.09, 80.92, 84.07, 100.92, 105.64, 114.70, 114.89, 117.43, 119.94, 128.20, 128.81, 129.12, 129.47, 129.92, 130.33, 130.45, 130.68, 131.31, 132.57, 132.87, 133.61, 133.80, 134.70, 136.81, 138.32, 141.72, 152.16, 154.83, 155.38, 159.50, 164.19, 164.80, 165.71, 166.92, 168.64, 169.89, 170.05, 170.64, 172.03, 172.15, 172.81, 173.05, 185.82, 202.61 (Four carbons are missing in the double bond range); Mass (ESI) $C_{91}H_{103}N_5O_{25}S_3H^+$ *cal.* 1762.6, found 1762.4 (M+H), and $C_{91}H_{103}N_5O_{25}S_3Na^+$ *cal.* 1784.6, found 1785.2 (M+1+Na, isotope peak of M+Na, which showed higher abundance than the original M+Na peak).

SB-T-1214-Linker-Biotin (**4-41**):

To a solution of **CRWLC 3-105** (32 mg, 0.030 mmol) in DMSO was added biotin-$NHNH_2$ (**4-3**, 7.0 mg, 0.027 mmol) at r.t., and the solution was stirred at r.t. for 2 d. The crude solution was purified on a silica gel column (MeOH/DCM = 7/100) to give **4-41** (25 mg, 81% yield) as white powder: HRMS (ESI) $C_{66}H_{85}N_5O_{19}S_3H^+$ *calc* 1348.5079, found 1348.5087 (Δ = 0.8 ppm).

2',7-DiTES-SB-T-1214 (**4-43**) and **2'-TES-SB-T-1214** (**4-43a**):

To a solution of **SB-T-1214** (78 mg, 0.092 mmol) and imidazole (31.3 mg, 0.46 mmol) in DCM (2 mL) was added TES-Cl (0.025 mL, 0.15 mmol, 1.6 eq.) at 0 ^oC. After the reaction mixture was stirred at 0 ^oC for 30 min, two taxoid-containing spots showed up on TLC plate, and no SM left. The reaction was quenched with water, and diluted with DCM. After separation, the DCM layer was washed by water and brine, and dried over $MgSO_4$. DCM was removed, and the residue was purified on a silica gel column to give **4-43** (hexane/EtOAc = 4/1) and **4-43a** (hexane/EtOAc = 2/1).

4-43 was obtained (16 mg, 16% yield) as white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.5-0.7 (m, 12 H), 0.85-1.05 (m, 21 H), 1.20 (s, 3 H), 1.22 (m, 1 H), 1.23 (s, 3 H), 1.35 (s, 9 H), 1.68 (s, 3 H), 1.70 (s, 1 H), 1.72 (m, 1 H), 1.75 (s, 3 H), 1.77 (s, 3 H), 1.88 (m, 1 H), 2.00 (s, 3 H), 2.2-2.5 (m, 6 H containing a singlet at 2.39 ppm for 3 H), 3.84 (d, *J* = 6.9 Hz, 1 H), 4.19 (d, *J* = 8.4 Hz, 1 H), 4.26 (d, *J* = 3.6 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.46 (m, 1 H), 4.72 (m, 1 H), 4.84 (d, *J* = 9.6 Hz, 1 H), 4.93 (d, *J* = 9.3 Hz, 1 H), 5.28 (d, *J* = 8.7 Hz, 1 H), 5.68 (d, *J* = 7.2 Hz, 1 H), 6.13 (t, *J* = 7.8 Hz, 1 H), 6.48 (s, 1 H), 7.46 (t, *J* = 8.1 Hz, 2 H), 7.60 (m, 1 H), 8.10 (d, *J* = 8.4 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.79, 5.49, 6.89, 6.94, 8.80, 8.92, 10.28, 13.16, 14.33, 18.79, 21.59, 22.78, 25.92, 26.55, 28.44, 35.60, 37.37, 43.45, 46.91, 51.87, 58.52, 71.65, 72.36, 74.94, 75.29, 79.14, 79.60, 81.19, 84.49, 122.00, 128.75, 129.60, 130.34, 133.61, 133.73, 136.72, 140.97, 155.25, 167.14, 170.13, 172.01, 173.20, 202.23.

4-43a was obtained (67 mg, 75% yield) as white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (m, 6 H), 0.92-1.02 (m, 11 H), 1.14 (m, 2 H), 1.15 (s, 3 H), 1.25 (s, 3 H), 1.34 (s, 9 H), 1.66 (s, 3 H), 1.74 (s, 3 H), 1.77 (s, 3 H), 1.81 (m, 2 H), 1.87 (m, 1 H), 1.88 (s, 3 H), 2.2-2.6 (m, 7 H containing a singlet at 2.37 ppm for 3 H), 3.82 (d, *J* = 6.9 Hz, 1 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.25 (d, *J* = 3.6 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.42 (m, 1 H), 4.72 (m, 1 H), 4.85 (d, *J* = 9.6 Hz, 1 H), 4.95 (d, *J* = 9.3 Hz, 1 H), 5.24 (d, *J* = 8.7 Hz, 1 H), 5.65 (d, *J* = 7.2 Hz, 1 H), 6.15 (t, *J* = 7.8 Hz, 1 H), 6.28 (s, 1 H), 7.45 (t, *J* = 8.1 Hz, 2 H), 7.59 (m, 1 H), 8.09 (d, *J* = 8.4 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.79, 5.49, 6.89, 6.94, 8.80, 8.92, 10.28, 13.16, 14.33, 18.79, 21.59, 22.78, 25.92, 26.55, 28.44, 35.60, 37.37, 43.45, 46.91, 51.87, 58.52, 71.65, 72.36, 74.94, 75.29, 79.14, 79.60, 81.19, 84.49, 122.00, 128.75, 129.60, 130.34, 133.61, 133.73, 136.72, 140.97, 155.25, 167.14, 170.13, 172.01, 173.02, 202.23.

In another experiment, TES-Cl (0.071 mL, 0.43 mmol, 4 eq.) was added into a solution of SB-T-1214 (90 mg, 0.106 mmol) and imidazole (43 mg, 0.64 mmol) in DCM (2 mL) at 0 ^oC. After the reaction mixture was stirred at r.t. for 3 h, the reaction was quenched with $NaHCO_3$ (aq., s) and diluted with EtOAc. After separation, the organic layer was washed by citic acid (5% aq.) and brine to pH 7, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by column chromatography on silica gel (hexane/EtOAc = 8/1) to give **4-43** (94 mg, 99% yield based on 84% conversion) as

white powder. Its ^{1}H NMR was identical to the one reported above. In addition, the unreacted SB-T-1214 was cycled after column.

7-TES-SB-T-1214 (**4-44**):

To a 2',7-diTES-**SB-T-1214** (94 mg, 0.087 mmol) solution in CH_3CN (2.4 mL) and MeOH (1.2 mL) was added CsF (39.6 mg, 0.26 mmol) at 0 ^oC. After the reaction solution was stirred at r.t. for 3 h, three spots showed up on the TLC plate, which indicated over-reaction. The reaction solution was diluted with EtOAc, washed with citric acid (3% aq.) and brine, and dried over $MgSO_4$. After removal of the solvents, the three components mentioned above were separated on a silica gel column.

The desired product **4-44**, *i.e.*, the first component flushed out of column around hexane/EtOAc = 2.2/1, was obtained (83 mg, 98% yield) as white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.92-1.02 (m, 11 H), 1.10-1.25 (m, 2 H), 1.18 (s, 3 H), 1.23 (s, 3 H), 1.34 (s, 9 H), 1.68 (s, 3 H), 1.75 (m, 8 H), 1.87 (m, 1 H), 2.00 (s, 3 H), 2.32 (br s, 5 H), 2.49 (m, 1 H), 3.42 (d, *J* = 6.0 Hz, 1 H, 2'-OH), 3.81 (d, *J* = 7.2 Hz, 1 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.20 (m, 1 H), 4.28 (d, *J* = 8.4 Hz, 1 H), 4.43 (m, 1 H), 4.73 (m, 1 H), 4.84 (d, *J* = 8.8 Hz, 1 H), 4.92 (d, *J* = 9.2 Hz, 1 H), 5.30 (d, *J* = 8.4 Hz, 1 H), 5.66 (d, *J* = 6.8 Hz, 1 H), 6.11 (t, *J* = 8.8 Hz, 1 H), 6.46 (s, 1 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.58 (t, *J* = 7.2 Hz, 1 H), 8.08 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.23, 6.71, 8.54, 8.66, 9.98, 12.91, 14.35, 18.53, 21.02, 22.37, 25.67, 26.42, 28.18, 35.38, 37.18, 43.20, 46.74, 51.54, 58.44, 60.34, 72.21, 73.71, 74.78, 74.85, 78.77, 79.85, 81.09, 84.17, 120.64, 128.54, 129.26, 130.08, 133.55, 133.76, 137.86, 140.14, 155.35, 166.90, 170.06, 172.90, 201.94 (two carbons were missing in the C-C/C-Si single bond area).

The second component came out of the column around hexane/EtOAc = 1.8/1, which gave a by-product 7-TES-10-Cp-13-OH (10 mg, 16% yield) as white powder. Its ^{1}H NMR was identical to that of Compound **2-4**.

When hexane/EtOAc = 1/1 was used, the third component was obtained (3 mg) as film/solid, but its NMR spectrum was very messy.

7-TES-SB-T-1214-MeLinker-OTIPS (**4-45**):

To a solution of 7-TES-**SB-T-1214** (60 mg, 0.06 mmol), MeLinker-OTIPS (**3-87**, 56 mg, 0.12 mmol) and DMAP (7.4 mg, 0.06 mmol) in DCM (1.5 mL) was added DCC (25 mg, 0.12 mmol) at 0 ^oC. After stirring at 0 ^oC for 25 min and r.t. for 3.5 h, the reaction mixture was filtered through celite and the filtrate was concentrated. The residue was purified through a silica gel column (hexane/EtOAc = 5/1) to give the desired product (75 mg, 89% yield) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.92-1.02 (m, 11 H), 1.05 (m, 23 H, TIPS and cyclopropane (2)), 1.18 (s, 3 H), 1.23 (s, 3 H), 1.29 (d, *J* = 6.8 Hz, 3 H, linker C<u>H</u>$_3$), 1.34 (s, 9 H), 1.67 (s, 3 H), 1.69 (s, 3 H), 1.71 (s, 3 H), 1.75 (m, 1 H, cyclopropane), 1.86 (m, 4 H, C-14 (1), C-6 (1) and linker (2)), 2.00 (s, 3 H), 2.25-2.6 (m, 8 H, linker (2), OAc (3), C-14 (1), C-6 (1) and OH), 2.94 (m, 1 H, S-C<u>H</u>), 3.82 (d, *J* = 6.8 Hz, 1 H), 3.95 (dd, *J* = 16.0, 4.8 Hz, 1 H), 4.10 (dd, *J* = 16.0, 4.8 Hz, 1 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.45 (m, 1 H), 4.83 (m, 1 H), 4.94 (m, 3 H), 5.07 (d, *J* = 7.6 Hz, 1 H), 5.69 (d, *J* = 6.8 Hz, 1 H), 6.15 (t, *J* = 8.8 Hz, 1 H), 6.46 (s, 1 H), 7.2-7.3 (m, 3 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.58 (t, *J* = 7.2 Hz, 1 H), 7.79 (d, *J* = 7.6 Hz, 1 H), 8.08 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.42, 6.93, 8.69, 8.80, 10.25, 12.04, 13.09, 14.33, 17.84, 17.93, 18.65, 20.65, 20.70, 21.57, 22.58,

25.84, 26.58, 28.37, 31.13, 33.12, 33.17, 35.44, 37.33, 38.93, 43.42, 46.16, 46.89, 49.19, 58.49, 72.00, 72.28, 74.88, 75.07, 75.26, 79.13, 79.95, 81.12, 84.43, 120.20, 127.86, 128.44, 128.74, 129.54, 130.22, 130.33, 131.06, 133.32, 133.42, 133.68, 137.49, 137.81, 141.13, 155.08, 167.18, 168.23, 169.68, 170.19, 172.90, 173.14, 202.35.

7-TES-SB-T-1214-MeLinker-OH (**4-46**):

To a solution of -7-TES-**SB-T-1214**-MeLinker-OTIPS (69 mg, 0.049 mmol) in CH_3CN (3 mL) and MeOH (2 mL) was added CsF (15.2 mg, 0.10 mmol) at 0 ^oC. After stirring at 0 ^oC for 20 min and at r.t. for 1 h, the reaction was finished based on the TLC analysis. No pink color was observed. The reaction was diluted with DCM and quenched with citric acid (2% aq.). After extraction and separation, the organic layer was washed with water and brine, and dried over $MgSO_4$. The solvents were removed, and the crude (65 mg) was purified on a silica gel column to give the desired product (54 mg, 88% yield) as white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.92-1.02 (m, 11 H), 1.05 (m, 2, cyclopropane (2)), 1.18 (s, 3 H), 1.23 (s, 3 H), 1.29 (d, *J* = 6.8 Hz, 3 H, linker C<u>H</u>$_3$), 1.34 (s, 9 H), 1.67 (s, 3 H), 1.69 (s, 3 H), 1.71 (s, 3 H), 1.75 (m, 1 H, cyclopropane), 1.86 (m, 4 H, C-14 (1), C-6 (1) and linker (2)), 2.00 (s, 3 H), 2.25-2.6 (m, 8 H, linker (2), OAc (3), C-14 (1), C-6 (1) and OH), 2.94 (m, 1 H, S-C<u>H</u>), 3.82 (d, *J* = 6.8 Hz, 1 H), 3.95 (d, *J* = 16.0 Hz, 1 H), 4.10 (d, *J* = 16.0 Hz, 1 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.45 (m, 1 H), 4.83 (m, 1 H), 4.94 (m, 3 H), 5.07 (d, *J* = 7.6 Hz, 1 H), 5.69 (d, *J* = 6.8 Hz, 1 H), 6.15 (t, *J* = 8.8 Hz, 1 H), 6.46 (s, 1 H), 7.2-7.3 (m, 3 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.58 (t, *J* = 7.2 Hz, 1 H), 7.79 (d, *J* = 7.6 Hz, 1 H), 8.08 (d, *J* = 8.0 Hz, 2 H). The proton of the carboxylic acid did not show up in NMR, and the sample also contained some DMF.

7-TES-SB-T-1214-MeLinker-OSu (**4-47**):

To a solution of 7-TES-**SB-T-1214**-MeLinker-OH (50 mg, 0.04 mmol) and HOSu (23 mg, 0.20 mmol) in THF (0.5 mL) was added DCC (16.5 mg, 0.08 mmol) at 0 ^oC, and the reaction mixture was stirred at r.t. overnight. After evaporation of the solvent, the crude residue was purified on a silica gel column (hexane/EtOAc = 2/1 to 1/1) to afford the desired product (42 mg, 72% yield) as white powder: ^1H NMR (400 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.92-1.02 (m, 11 H), 1.05 (m, 2, cyclopropane (2)), 1.18 (s, 3 H), 1.23 (s, 3 H), 1.29 (d, *J* = 6.8 Hz, 3 H, linker C<u>H</u>$_3$), 1.34 (s, 9 H), 1.67 (s, 3 H), 1.69 (s, 3 H), 1.71 (s, 3 H), 1.75 (m, 1 H, cyclopropane), 1.86 (m, 4 H, C-14 (1), C-6 (1) and linker (2)), 2.00 (s, 3 H), 2.25-2.6 (m, 6 H, OAc (3), C-14 (1), C-6 (1) and OH), 2.67 (t, *J* = 7.6 Hz, 2 H, linker (2)), 2.82 (s, 4 H, OSu), 2.94 (m, 1 H, S-C<u>H</u>), 3.82 (d, *J* = 6.8 Hz, 1 H), 3.95 (d, *J* = 16.0, 1 H), 4.10 (d, *J* = 16.0 Hz, 1 H), 4.16 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.45 (m, 1 H), 4.83 (m, 1 H), 4.94 (m, 3 H), 5.07 (d, *J* = 7.6 Hz, 1 H), 5.69 (d, *J* = 6.8 Hz, 1 H), 6.15 (t, *J* = 8.8 Hz, 1 H), 6.46 (s, 1 H), 7.2-7.3 (m, 3 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.58 (t, *J* = 7.2 Hz, 1 H), 7.79 (d, *J* = 7.6 Hz, 1 H), 8.08 (d, *J* = 8.0 Hz, 2 H).

7-TES-SB-T-1214-MeLinker-Biotin (**4-48**):

To a solution of 7-TES-**SB-T-1214**-MeLinker-OSu (40 mg, 0.030 mmol) in DMF (0.6 mL) was added Biotin-$NHNH_2$ (7.1 mg, 0.027 mmol), and the reaction mixture was stirred at r.t. for 36 h. The solvent was removed, and the residue was purified on a silica gel column to give the desired product (33 mg, 75% yield, but containing some HOSu) as

white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (m, 6 H), 0.92-1.02 (m, 11 H), 1.18 (s, 3 H), 1.2-1.3 (m, 8 H, cyclopropane (2), C-16/17 (3), linker CH_3 (3)), 1.35 (br s, 11 H, Boc and biotin (2)), 1.6-1.84 (m, 17 H, biotin (4), cyclopropane (1), C-6/C-14 (1), C-5' (6), C-19 (3) and linker (2)), 2.0 (s, 3 H, C-18), 2.1-2.4 (m, 10 H, biotin (2) at 2.28 ppm), OAc (3), C-6 and C-14 (2), OH, linker (2)), 2.48 (m, 1 H, taxoid), *2.60 (s, 1 H, free HOSu)*, 2.72 (d, *J* = 12.8 Hz, 1 H, biotin), 2.89 (m, 2 H, biotin (1) and linker S-CH), 3.10 (m, 1 H, biotin), 3.80 (d, *J*= 6.8 Hz, 1 H, C-3), 3.90-4.10 (d, *J* = 16.0 Hz, 1 H, linker), 4.10-4.20 (d, *J* = 16.0 Hz, 1 H, linker), 4.16 (d, *J* =8.0 Hz,1 H, C-20 (1)), 4.29 (m, 2 H, C-20(1) and biotin (1)), 4.47 (m, 2 H, biotin (1) and taxoid (1)), 4.80-5.05 (m, 4 H, taxoid), 5.10 (m, 1 H, taxoid), 5.68 (d, *J* = 6.8 Hz, 1 H, C-2), 6.02 (d, *J* = 16.8 Hz, 1 H, urea NH from biotin), 6.15 (m, 1 H, C-13), 6.45 (s, 1 H, C-10), 7.05 (br s, 1 H, urea NH from biotin), 7.2-7.3 (m, 3 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.56 (t, *J* = 7.2 Hz, 1 H), 7.77 (d, *J* = 7.6 Hz, 1 H), 8.09 (d, *J* = 7.2 Hz, 2 H, taxoid), 8.81 (br s, 1 H, CONH-N from biotin), 9.65 (br s, 1 H, CONH-N from biotin); ^{13}C NMR (100 MHz, $CDCl_3$): δ 5.45, 6.95, 8.74, 8.85, 10.29, 13.15, 14.31, 18.70, 20.88, 21.65, 22.69, 25.40, 25.95, 26.64, 28.18, 28.47, 31.36, 33.27, 35.56, 37.34, 38.93, 40.66, 41.17, 43.48, 46.46, 46.89, 49.24, 55.83, 58.48, 60.49, 62.08, 72.31, 74.88, 75.24, 75.37, 79.11, 79.16, 79.95, 81.14, 84.43, 120.21, 128.00, 128.13, 128.51, 128.76, 129.62, 130.31, 130.76, 131.25, 133.57, 133.69, 137.63, 140.93, 141.00, 155.21, 164.83, 167.08, 168.47, 169.91, 170.53, 171.22, 171.90, 173.02, 202.24.

SB-T-1214-MeLinker-Biotin (**4-42**):

SB-T-1214-MeLinker-OSu (**3-108**, 50 mg, 0.040 mmol) was mixed with Biotin-$NHNH_2$ (11.5 mg, 0.044 mmol) in DMSO (0.50 mL) and DCM (0.3 mL), and the reaction solution was stirred at r.t. for 36 h. After evaporation of the solvents, the crude residue was purified on a silica gel column (5-6% MeOH in DCM) to give the desired product (36 mg, 65% yield) as white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92-1.02 (m, 2 H), 1.18 (s, 3 H), 1.2-1.3 (m, 8 H, cyclopropane (2), C-16/17 (3), linker CH_3 (3)), 1.35 (br s, 11 H, Boc and biotin (2)), 1.6-1.84 (m, 18 H, biotin (4), cyclopropane (1), C-6/C-14 (1), C-5' (6), C-19 (3), OH and linker (2)), 2.0 (s, 3 H, C-18), 2.1-2.4 (m, 10 H, biotin (2) at 2.28 ppm), OAc (3), C-6 and C-14 (2), OH, linker (2)), 2.50 (m, 1 H, taxoid), *2.60 (s, 1 H, free HOSu)*, 2.73 (d, *J* = 12.8 Hz, 1 H, biotin), 2.86 (m, 2 H, biotin (1) and linker S-CH), 3.10 (m, 1 H, biotin), 3.79 (d, *J*= 6.8 Hz, 1 H, C-3), 3.90-4.10 (d, *J* = 16.0 Hz, 1 H, linker), 4.10-4.20 (d, *J* = 16.0 Hz, 1 H, linker), 4.16 (d, *J* =8.0 Hz,1 H, C-20 (1)), 4.25-4.50 (m, 4 H, C-20 (1), biotin (2) and taxoid (1)), 4.80-5.15 (m, 5 H, taxoid), 5.66 (d, *J* = 6.8 Hz, 1 H, C-2), 6.11 (m, 2 H, C-13 and urea NH from biotin), 6.32 (s, 1 H, C-10), 6.95 (br s, 1 H, urea NH from biotin), 7.2-7.3 (m, 3 H), 7.45 (t, *J* =7.6 Hz, 2 H), 7.56 (t, *J* = 7.2 Hz, 1 H), 7.77 (d, *J* = 7.6 Hz, 1 H), 8.09 (d, *J* = 7.2 Hz, 2 H, taxoid), 8.86 (br s, 1 H, CONH-N from biotin), 9.59 (br s, 1 H, CONH-N from biotin).

2'-TBS-SB-T-1214-7-Caproyl-Fmoc (**4-49**):

To a solution of 2'-TBS-**SB-T-1214** (**4-20**, 76 mg, 0.078 mmol), Fmoc-Cap-OH (prepared from caproic acid and Fmoc-OSu, 124 mg, 0.35 mmol) and DMAP (9.5 mg, 0.078 mmol) in DCM (2 mL) was added DCC (72 mg, 0.35 mmol) at 0 ^{o}C. After stirring at room temperature for 18 h, the reaction mixture was filtered through celite, and the white solid was washed with DCM (5 mL). The filtrate was combined and evaporated, and the crude residue was purified on a silica gel column (hexane/EtOAc = 2.5/1) to give

the desired product (108 mg, 90% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 3 H), 0.11 (s, 3 H), 0.85-0.99 (m, 11 H, containing 9 H as a singlet at 0.94 ppm), 1.10 (m, 2 H), 1.17 (s, 3 H), 1.23 (s, 3 H), 1.35 (m, 11 H, containing 9 H as a singlet at 1.35 ppm and caproyl (2)), 1.42-1.62 (m, 4 H, caproyl (4)), 1.65-1.72 (m, 2 H), 1.74 (s, 3 H), 1.77 (s, 3 H), 1.80 (s, 3 H), 1.94 (m 4 H, containing 3 H as a singlet at 1.94 ppm), 2.28 (m, 3 H), 2.41 (m, 4 H, containing 3 H as a singlet at 2.40 ppm), 2.58 (td, *J* = 14.3, 9.4 Hz, 1 H), 3.19 (q, *J* = 6.1 Hz, 2 H, N-C<u>H</u>$_2$ in Fmoc), 3.97 (d, *J* = 6.9 Hz, 1 H), 4.16-4.26 (m, 2 H, C-20 (1) and CH in Fmoc), 4.24 (d, *J* = 3.3 Hz, 1 H), 4.32 (d, *J* = 8.4 Hz, 1 H, C-20 (1)), 4.38 (d, *J* = 6.9 Hz, 2 H, C<u>H</u>$_2$ in Fmoc), 4.79 (m, 3 H, taxoid (3) and Fmoc-N<u>H</u>), 4.95 (d, *J* = 7.9 Hz, 1 H), 5.24 (d, *J* = 8.4 Hz, 1 H), 5.59 (dd, *J* = 10.6, 7.0 Hz, 1 H, C-7), 5.68 (d, *J* = 7.0 Hz, 1 H), 6.14 (t, *J* = 8.7 Hz, 1 H), 6.32 (s, 1 H), 7.30 (t, *J* = 7.4 Hz, 2 H), 7.39 (t, *J* = 7.1 Hz, 2 H), 7.47 (t, *J* = 7.6 Hz, 2 H), 7.5-7.6 (m, 3H), 7.75 (d, *J* = 7.4 Hz, 2 H), 8.10 (d, *J* = 7.2 Hz, 2 H).

SB-T-1214-7-Caproyl-Fmoc (**4-50**):

To a solution of 2'-TBS-**SB-T-1214**-7-Caproyl-Fmoc (108 mg, 0.083 mmol) in Py (1.7 mL) and CH_3CN (2.3 mL) was added HF/Py (70/30, 1.0 mL) at 0 ^{o}C. After stirring at room temperature for 18 h, the reaction was quenched with $NaHCO_3$ and EtOAc. The organic layer was washed with $CuSO_4$, water and NaCl, and dried over $MgSO_4$. The crude residue (white solid, 94 mg) was pure based on the TLC analysis (hexane/EtOAc = 1/1 as the developing solvent system), and thus was used in the next step without further purification.

SB-T-1214-7-Cap-OH (**4-51**):

SB-T-1214-7-Cap-Fmoc (93 mg, 0.078 mmol) was dissolved in DCM (0.80 mL), and piperidine (0.2 mL) was added at 0 ^{o}C. After stirring for 5min at 0 ^{o}C and 30 min at r.t., TLC analysis showed no more SM left in the reaction, and the reaction mixture was concentrated. The residue was purified on a silica gel column (7% MeOH-DCM together with 0.5% TEA) to give the desired product (70 mg, 93% yield) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85-1.1 (m, 4 H), 1.17 (s, 3 H), 1.21 (s, 3 H), 1.28 (m, 2 H, caproyl), 1.35 (s, 9 H), 1.40-1.58 (m, 4 H, caproyl (4)), 1.69 (m, 1 H), 1.74 (br s, 6 H), 1.79 (s, 3 H), 1.83 (m, 1 H), 1.95 (s, 3 H), 2.1-2.4 (m, 8 H, containing 3 H as a singlet at 2.34 ppm), 2.55 (m, 1 H), 3.5-3.8 (v br, 3 H, NH_2 and OH), 3.93 (d, *J* = 6.6 Hz, 1 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.21 (d, *J* = 3.0 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.74 (dt, *J* = 8.7, 2.7 Hz, 1 H), 4.88 (d, *J* = 8.7 Hz, 1 H), 4.93 (d, *J* = 8.1 Hz, 1 H), 5.30 (d, *J* = 8.4 Hz, 1 H), 5.54 (dd, *J* = 10.5, 7.2 Hz, 1 H), 5.66 (d, *J* = 6.9 Hz, 1 H), 6.11 (t, *J* = 8.6 Hz, 1 H), 6.30 (s, 1 H), 7.46 (t, *J* = 7.6 Hz, 2 H), 7.60 (t, *J* = 7.4 Hz, 1 H), 8.08 (d, *J* = 7.2 Hz, 2 H). Two protons (N-CH_2 in caproyl) were missing, which might be overlapping with TEA peak at 2.63 ppm.

SB-T-1214-7-Cap-FITC (**4-52**):

To a solution of **SB-T-1214**-7-Cap-OH (70 mg, 0.072 mmol) in Py (0.5 mL) and DMF (1.0 mL) was added FITC (42 mg, 0.108 mmol). After stirring at r.t. for 1.5 d, the reaction was not completed based on TLC analysis. Second portion of FITC (42 mg, 0.108 mmol) was added. After stirring for 1 d (total 2.5 d), SM was still shown up on the TLC plate. The reaction mixture was concentrated, and the brown residue was purified

twice by column chromatography on silica gel (first column condition was hexane/EtOAc = 2/1 to 1/4, but the sample (58 mg) was not clean on the TLC plate, and consequently, hexane/EtOAc = 1/1 with 2% HOAc was used for the second column) to give the desired product (26 mg, 26% yield) as yellow solid.

Cell culture and fluorescent imaging assay

L1210FR cell line was received as a gift from Dr. Gregory Russell-Jones [289] (Access Pharmaceuticals Australia Pty Ltd., Targeted Delivery, Unit 5, 15-17 Gibbes St, Chatswood, NSW, Sydney 2067, Australia). The fluorescent images were obtained on Zeiss LSM 510 confocal microscope.

Cell Culture: L1210FR cells were grown in RPMI-1640 cell culture medium (Invitrogen) in the absence of folate receptor (FR) supplemented with 10% fetal bovine serum (FBS). Prior to incubation, the cells were collected by centrifugation at 1000 rpm for 6 min and resuspended in RPMI medium without FR at a cell density of 5×10^5 cells/mL.

Incubation of Cells with the Conjugates (Biotin-FITC, or Fluorescein-Taxoid-Linker-Biotin): The cell suspension mentioned above (1 mL) was added to microtube. The conjugates (10 μL) in DMSO were added to the microtube at a final concentration of 100 nM and incubated at 37 ^{o}C for 3 h. After incubation, the cells were washed with PBS and collected by centrifugation twice, and resuspended in 100 μL PBS for imaging.

Low Temperature Incubation of Cells with the Conjugates: The incubation of L1210FR with the conjugates was carried out in the cold room at ~4 ^{o}C. The isolation and washing of the cells were carried out as described above.

Blocking the Receptors on L1210FR cells with Excess Biotin: Before incubation with the conjugates, the cells were treated with 2 mM of biotin at final concentration for 1 h.

Release of the Coumarin or Taxoid in L1210FR cells: The conjugates (10 μL) in DMSO were added to 1 mL of cells in the microtube at a final concentration of 1 μM for Coumarin-Linker-Biotin (**4-16**) and 20 μM for Fluorescein-Taxoid-Linker-Biotin (**4-25**), respectively. After incubation at 37 ^{o}C for 3 h, the cells were washed twice by PBS to remove excess conjugates and resuspend in the medium. Glutathione ethyl ester (10 μL) was then added to the suspension at a final concentration of 2 mM and incubated for another 2 h. The excess glutathione ethyl ester was removed by washing twice with PBS and the cells were resuspended in 100 μL PBS before imaging.

Confocal Fluorescence Microscopy Imaging of the Treated Cells: All the confocal images were taken immediately after the incubation and washing steps. The cell suspension (100 μL) was transferred to the bottom-glass dish using micropipette and imaged by a Zeiss LSM 510 confocal fluorescence microscope.

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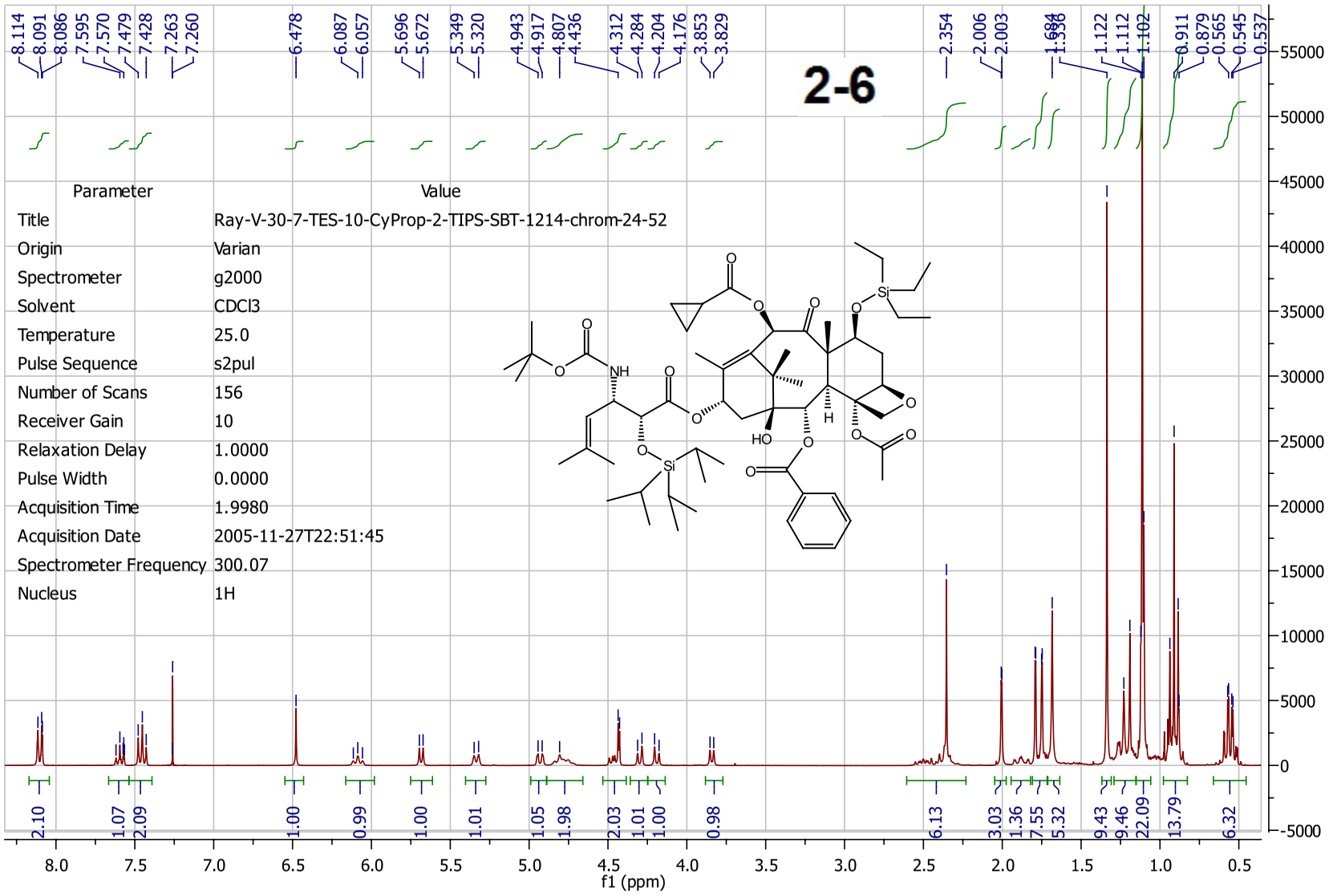
Appendix 1. NMR Spectra

Appendix 2. Crystal data of SB-T-1214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6.057
5.696
5.672
5.349
5.320
4.943
4.917
4.807
4.436
4.312
4.284
4.204
4.176
3.853
3.829
2.354
2.006
2.003
1.684
1.338
1.122
1.112
1.102
0.911
0.879
0.565
0.545
0.537
Parameter Value
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Spectrometer g2000
Solvent CDCl3
Temperature 25.0
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Number of Scans 156
Receiver Gain 10
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.9980
Acquisition Date 2005-11-27T22:51:45
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Nucleus 1H
2.10
1.07
2.09
1.00
0.99
1.00
1.01
1.05
1.98
2.03
1.01
1.00
0.98
6.13
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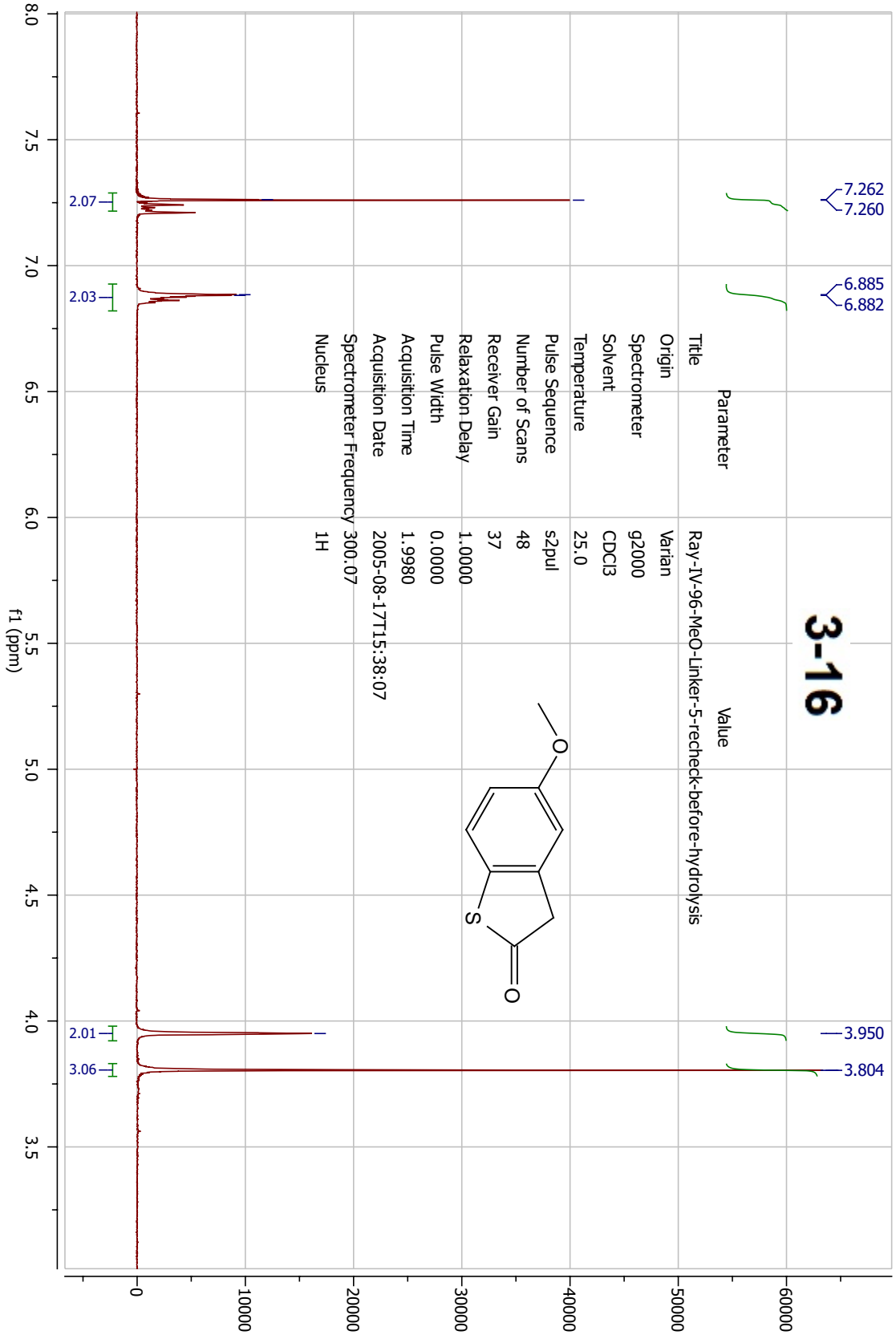
5.32
9.43
9.46
22.09
13.79
6.32
f1 (ppm)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Value
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Spectrometer
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Solvent
CDCl3
Temperature
25.0
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s2pul
Number of Scans
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Receiver Gain
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Relaxation Delay
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Pulse Width
0.0000
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Acquisition Date
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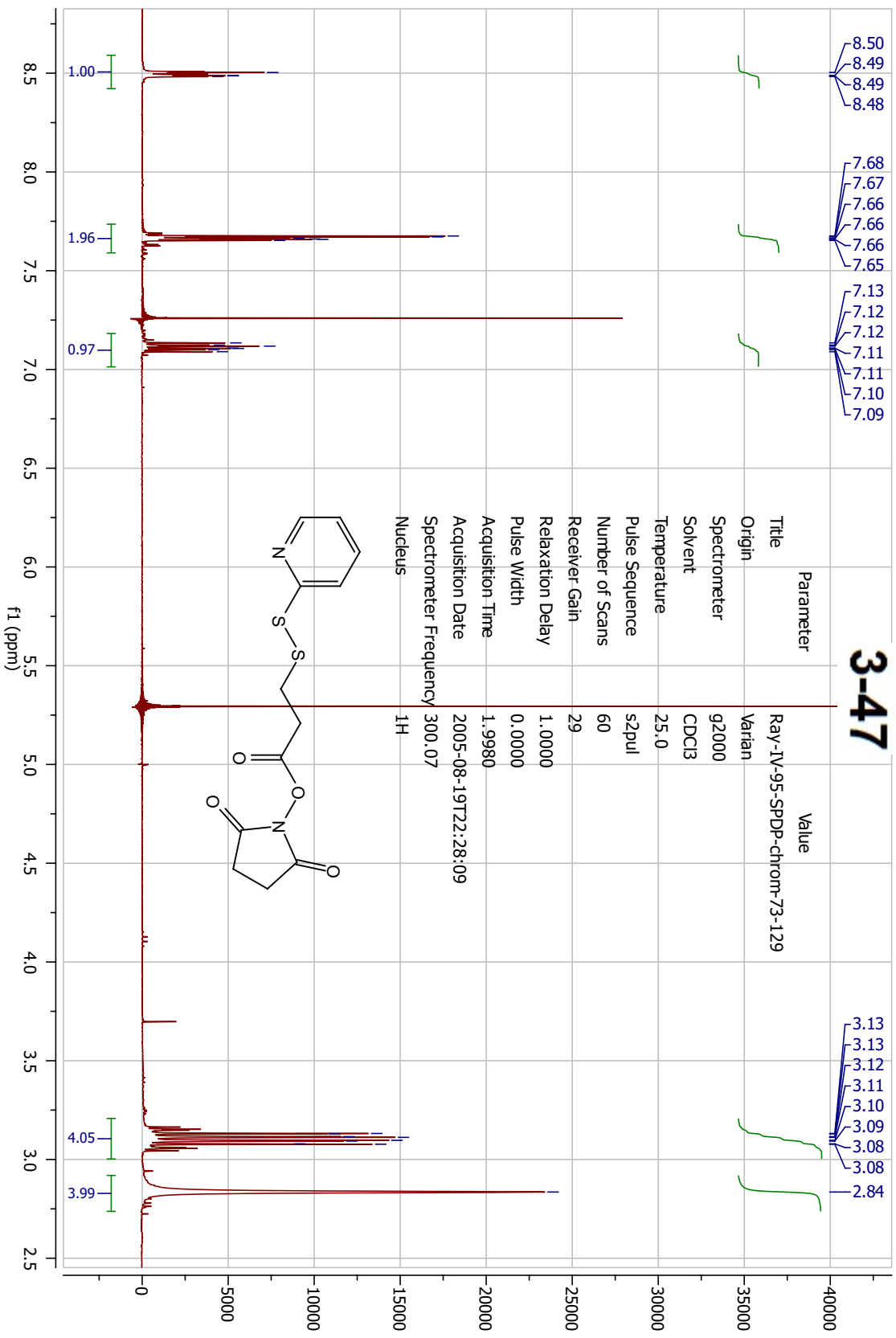
2.01
3.06
O
S
O
f1 (ppm)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Solvent
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25.0
Pulse Sequence
s2pul
Number of Scans
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29
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Pulse Width
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7.65
7.13
7.12
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7.11
7.11
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7.09
3.13
3.13
3.12
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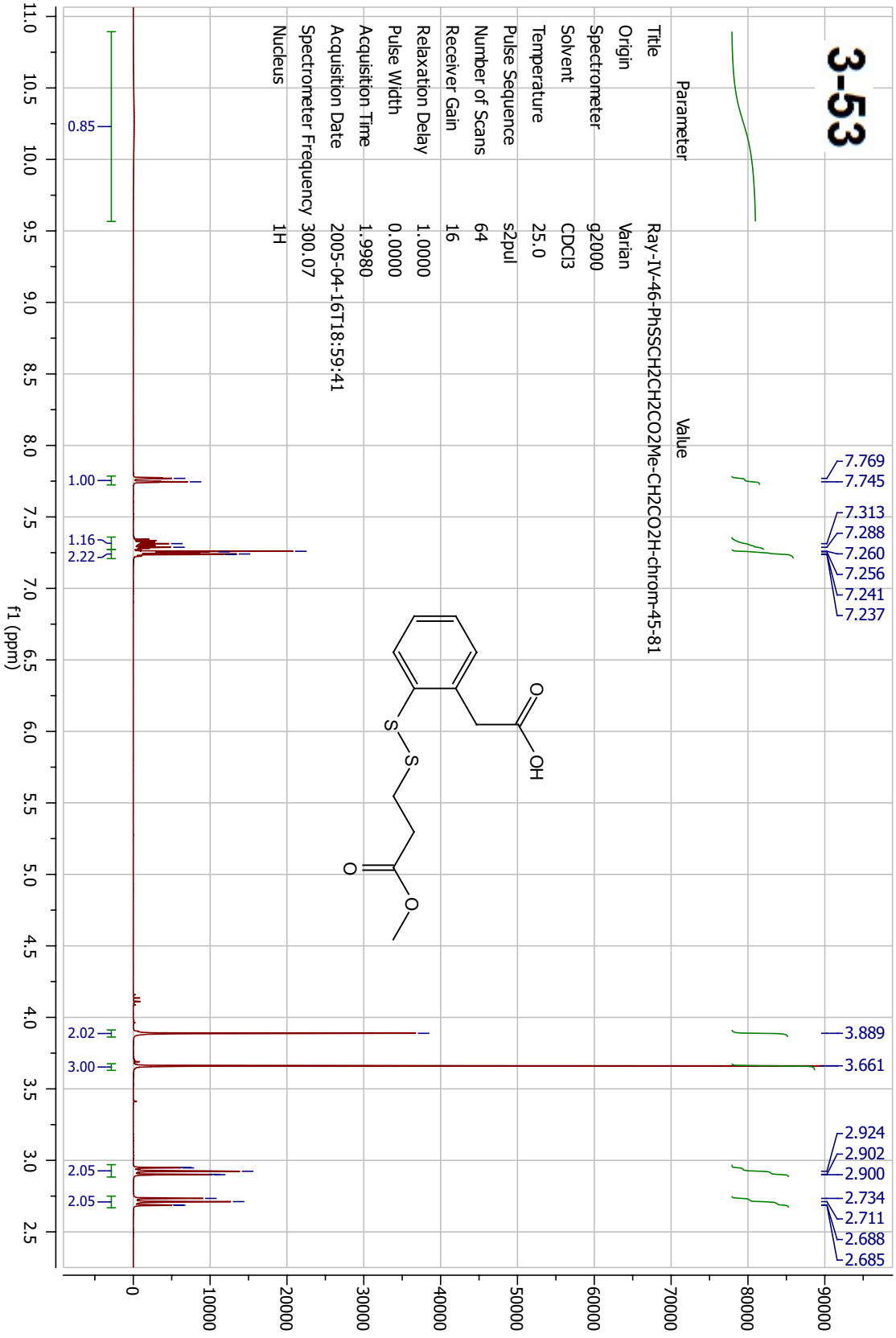
f1 (ppm)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Varian
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Solvent
CDCl3
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s2pul
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Pulse Width
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Acquisition Date
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7.256
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7.237
3.889
3.661
2.924
2.902
2.900
2.734
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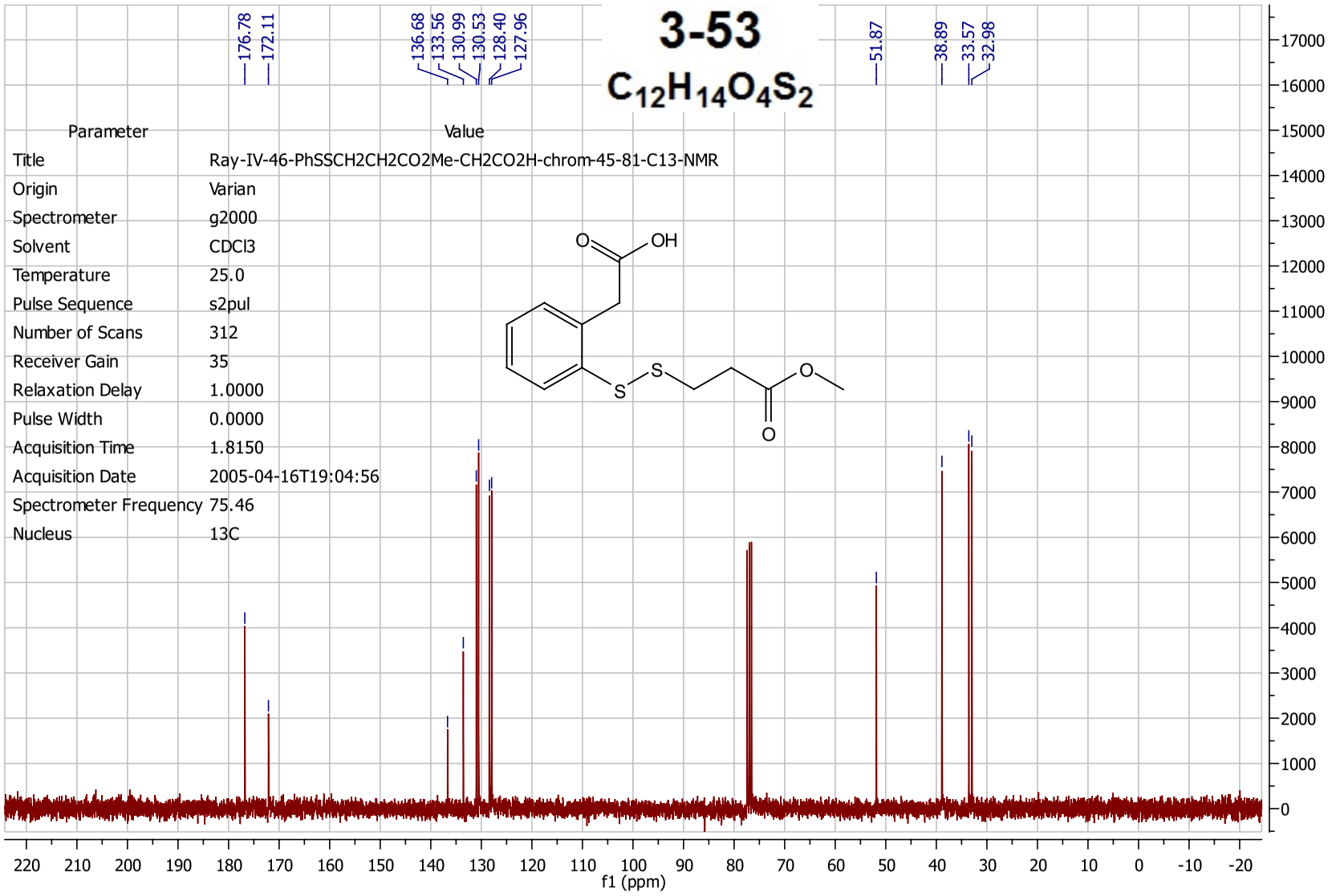
2.05
f1 (ppm)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38.89
33.57
32.98
Parameter
Value
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Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
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s2pul
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312
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35
Relaxation Delay
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0.0000
Acquisition Time
1.8150
Acquisition Date
2005-04-16T19:04:56
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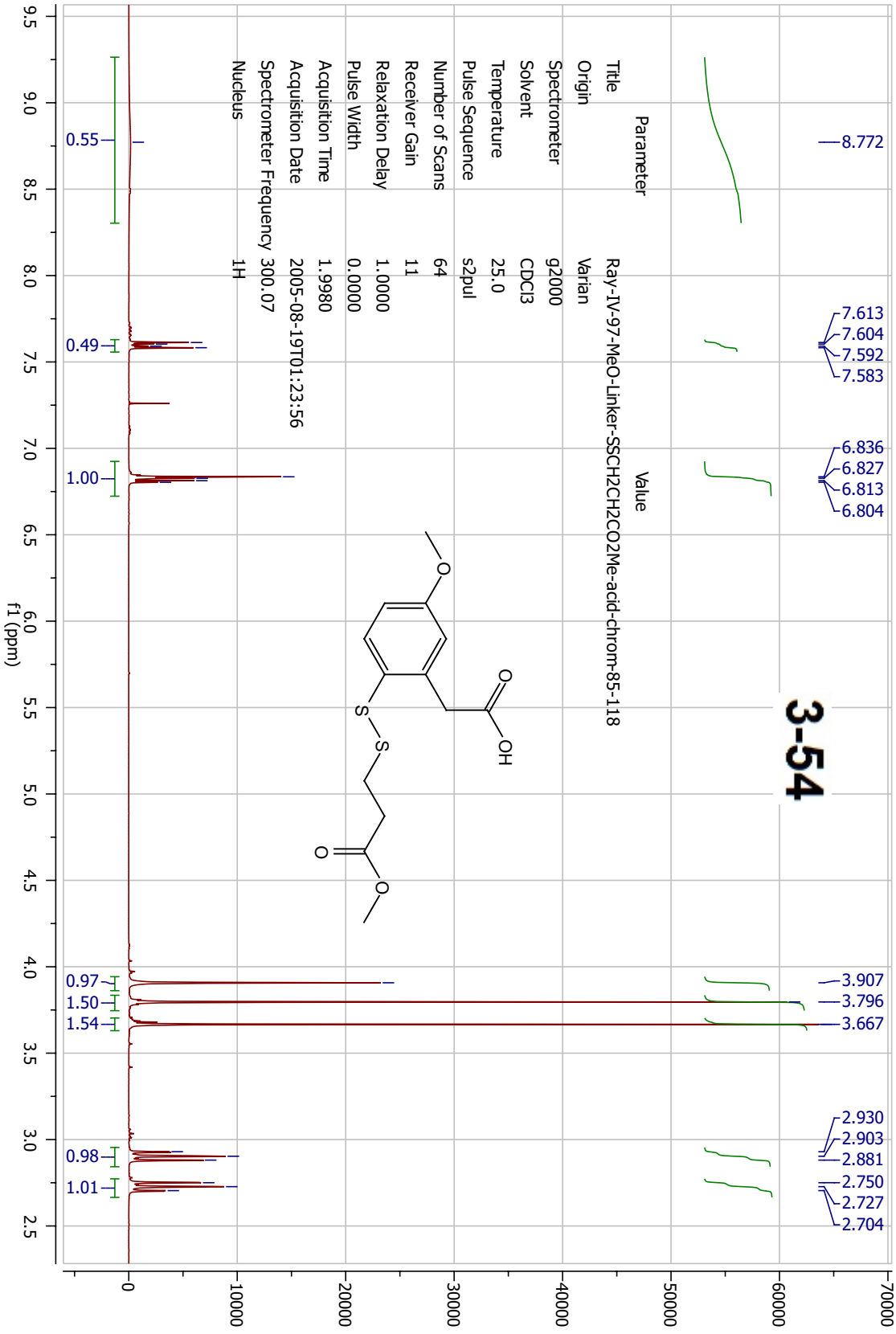
Nucleus
13C
O
OH
S
S
O
O
f1 (ppm)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3.796
3.667
2.930
2.903
2.881
2.750
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2.704
0.55
0.49
1.00
0.97
1.50
1.54
0.98
1.01
Parameter
Value
Title
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Varian
Spectrometer
g2000
Solvent
CDCl3
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25.0
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s2pul
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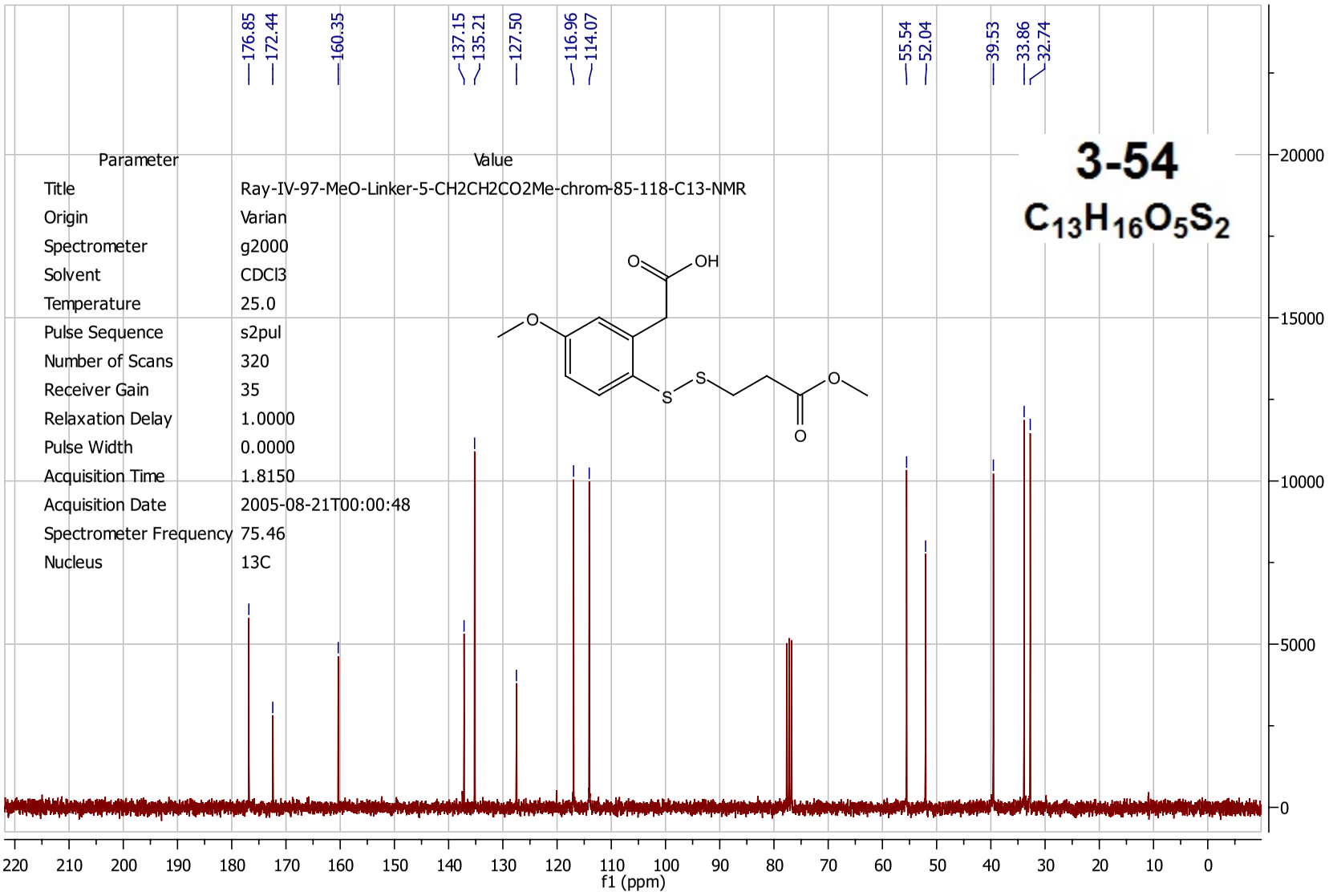
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52.04
39.53
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32.74
Parameter
Value
Title
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Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
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O
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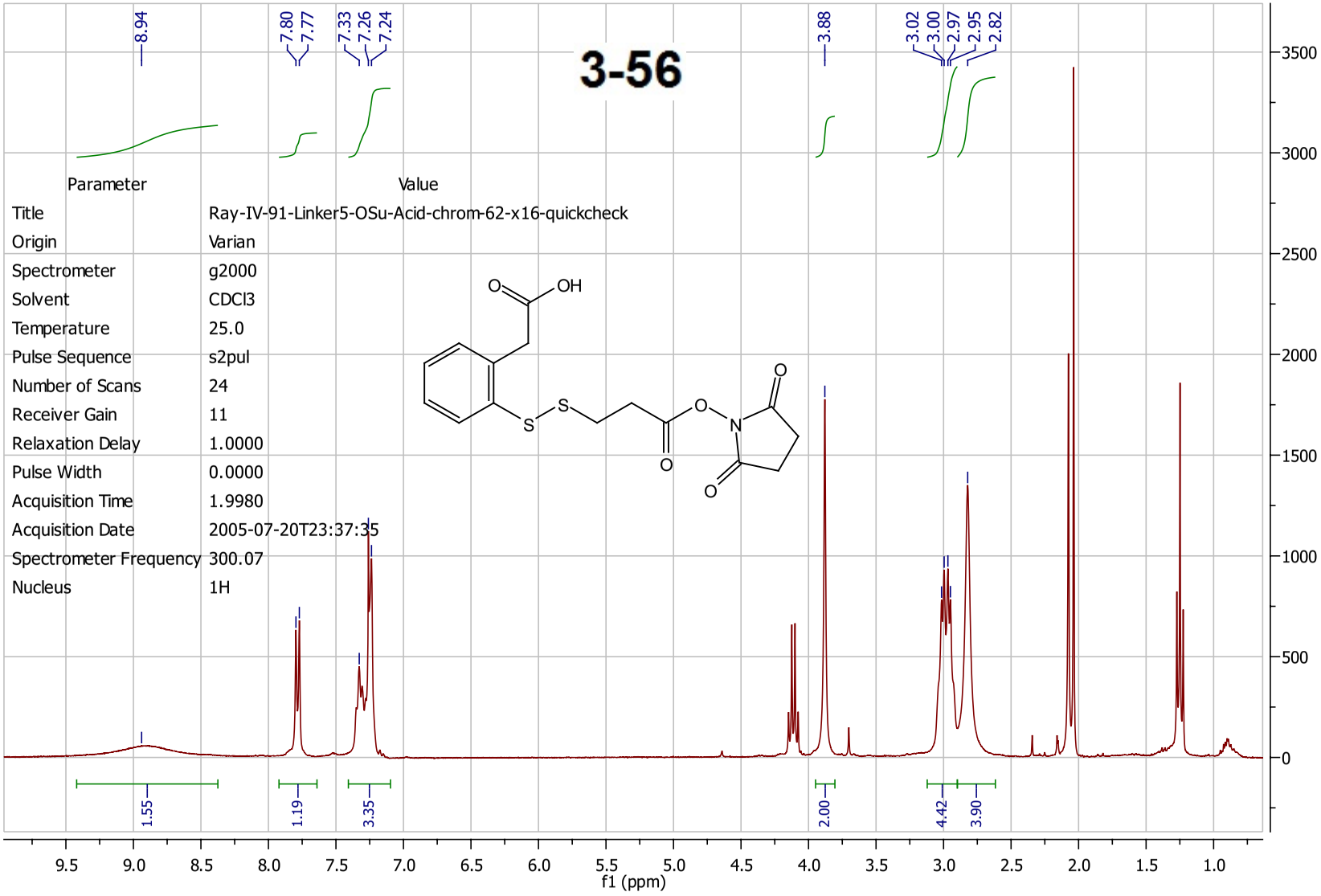
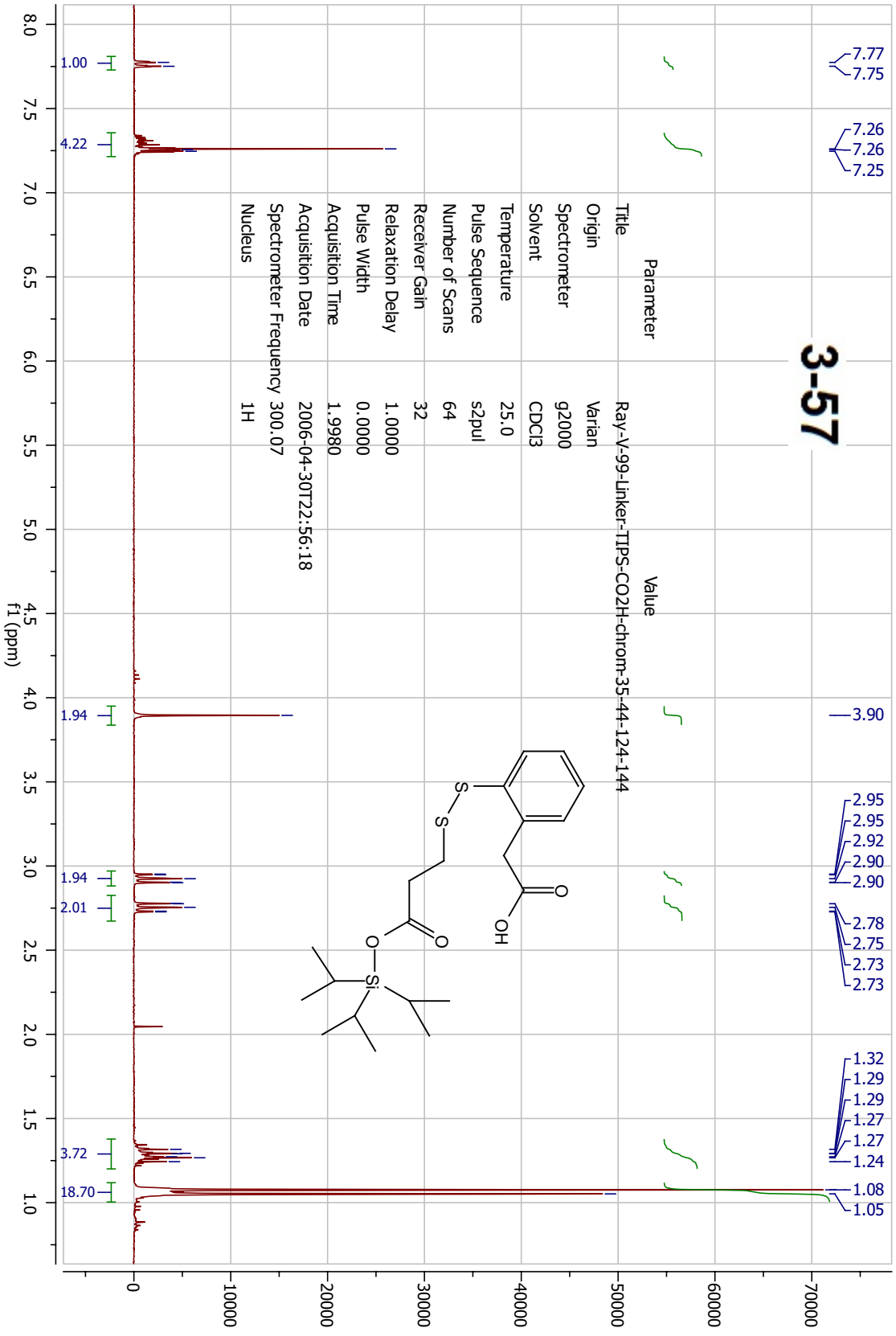
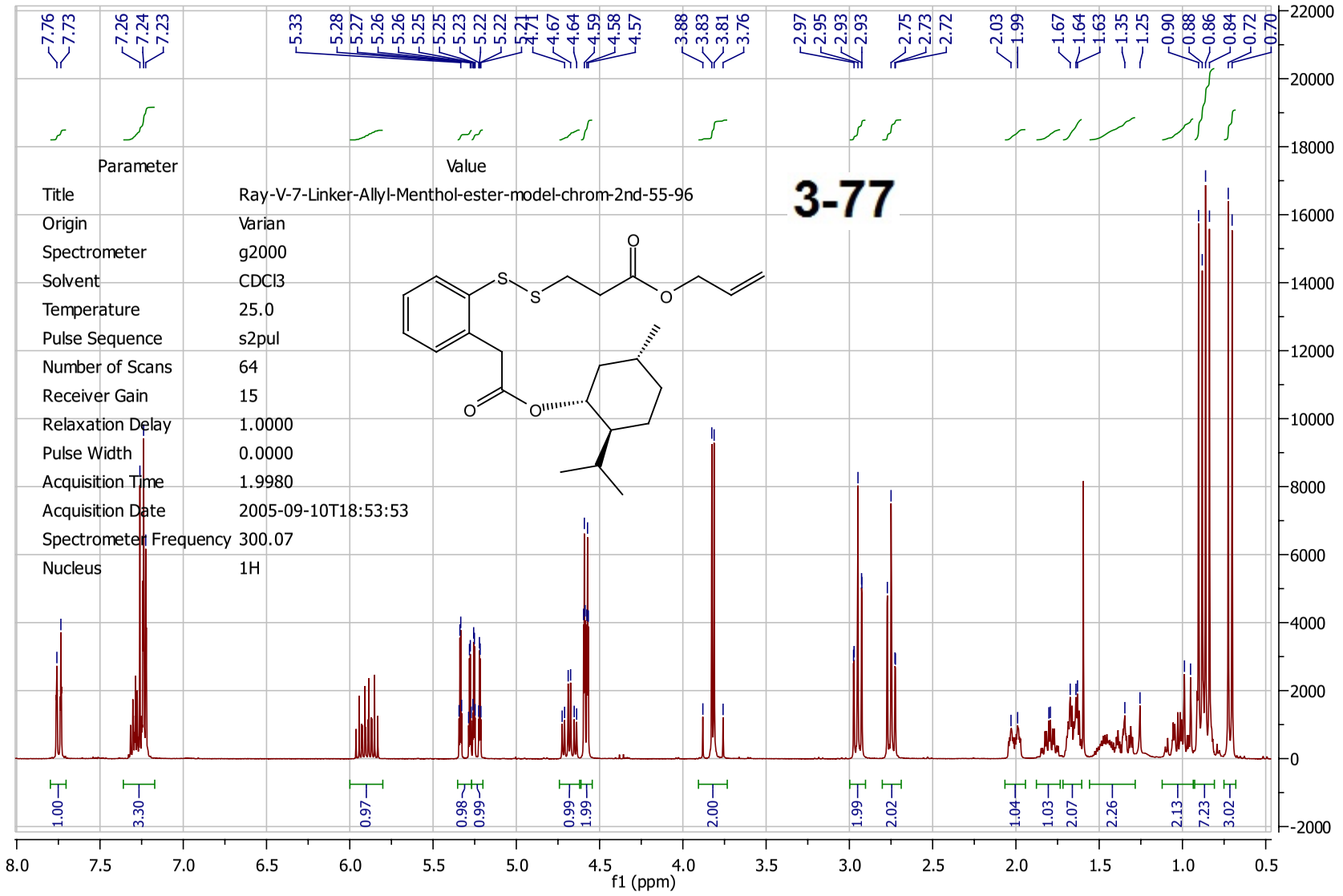
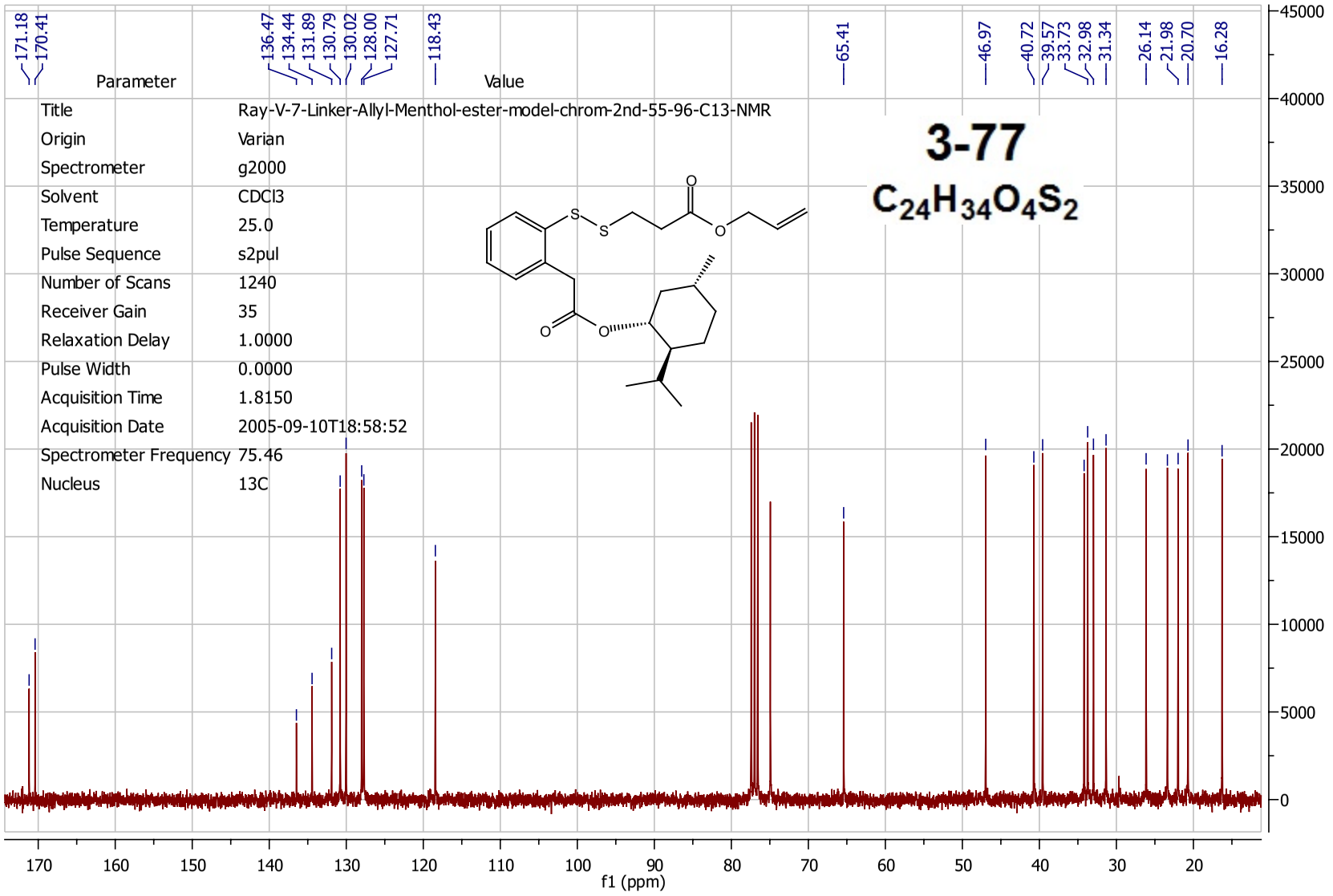
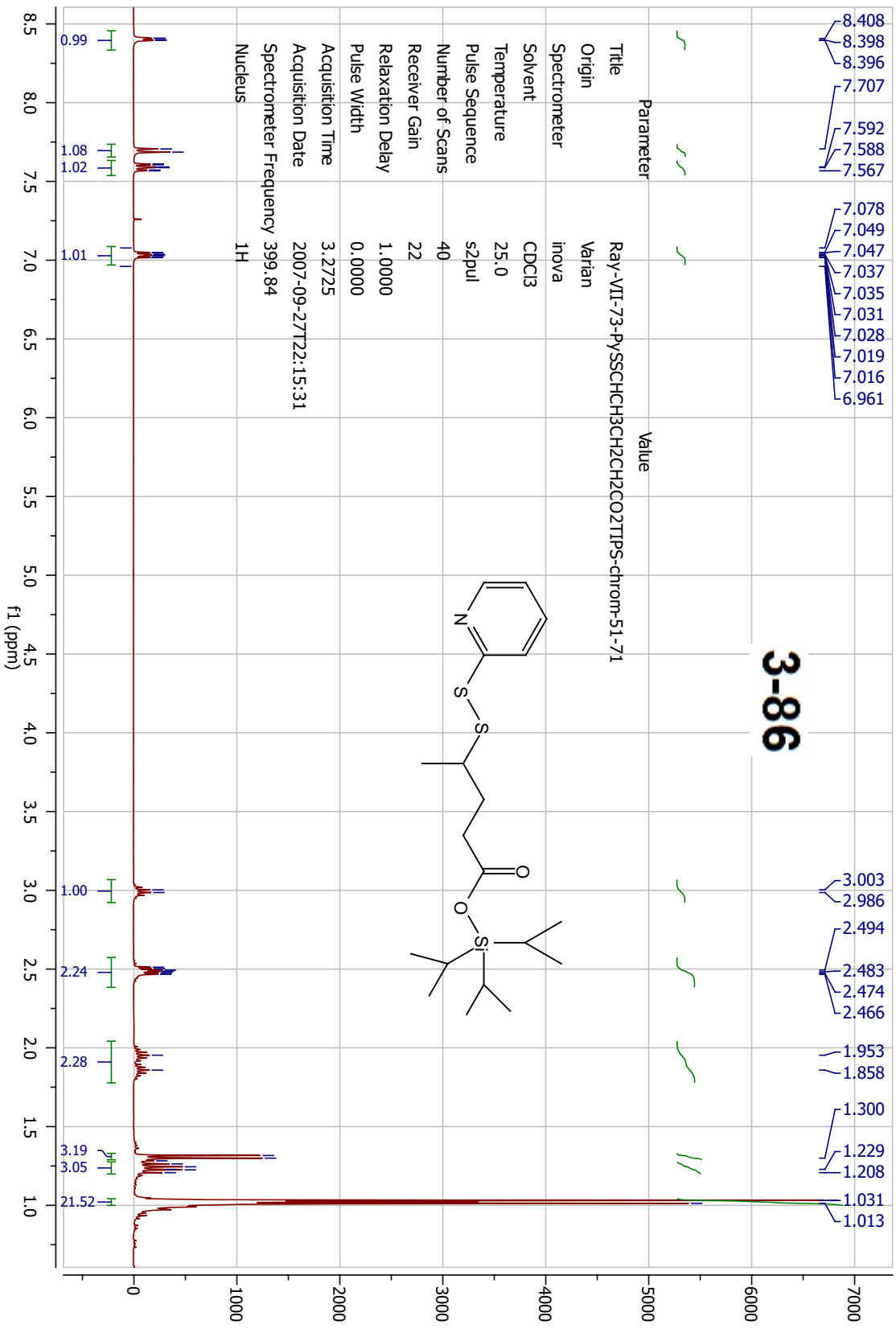
10000
5000
0
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

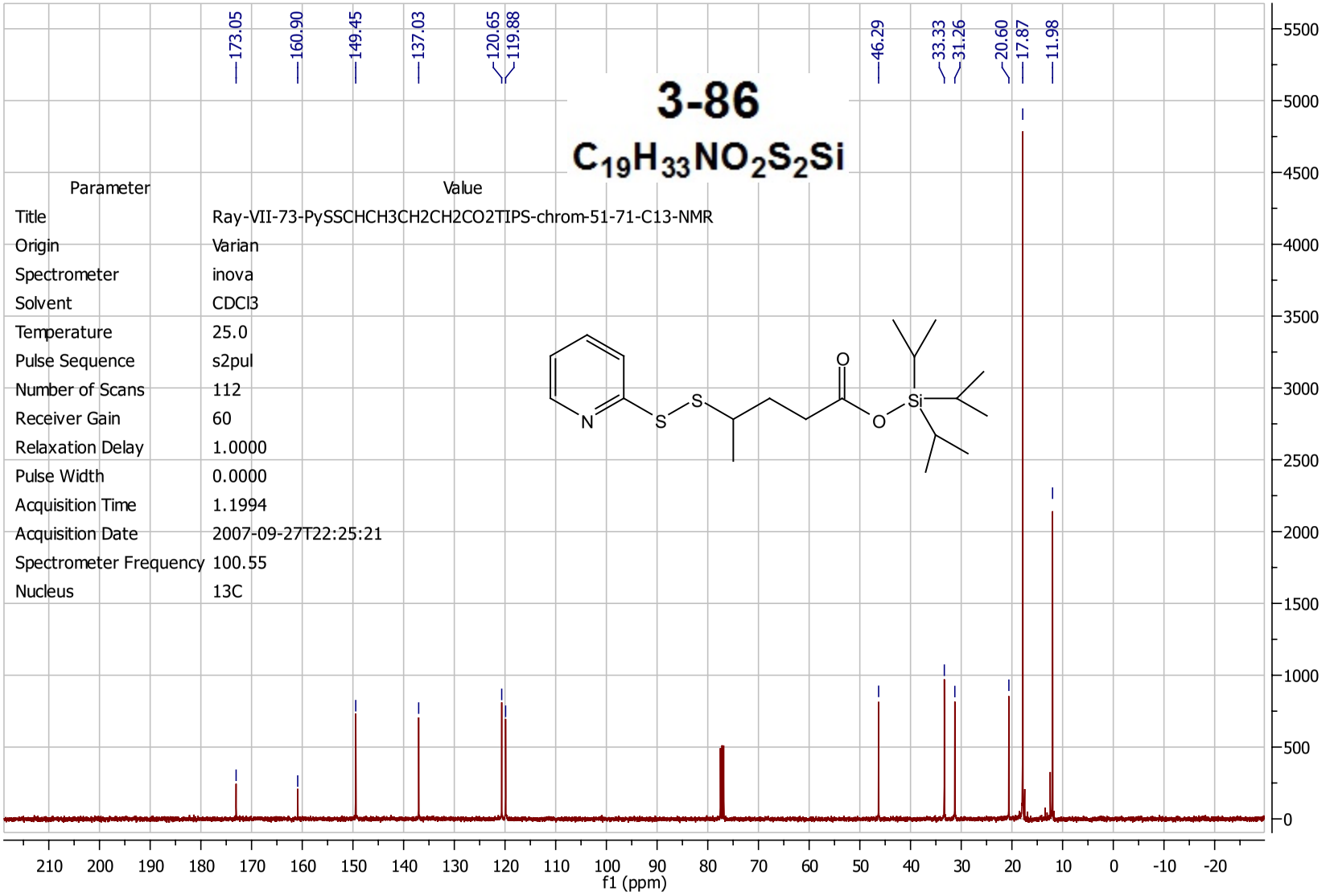

3-56
8.94
7.80
7.77
7.33
7.26
7.24
3.88
3.02
3.00
2.97
2.95
2.82
Parameter
Value
Title
Ray-IV-91-Linker5-OSu-Acid-chrom-62-x16-quickcheck
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
24
Receiver Gain
11
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2005-07-20T23:37:35
Spectrometer Frequency
300.07
Nucleus
1H
O
OH
S
S
O
O
N
O
O
1.55
1.19
3.35
2.00
4.42
3.90
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
f1 (ppm)
3500
3000
2500
2000
1500
1000
500
0

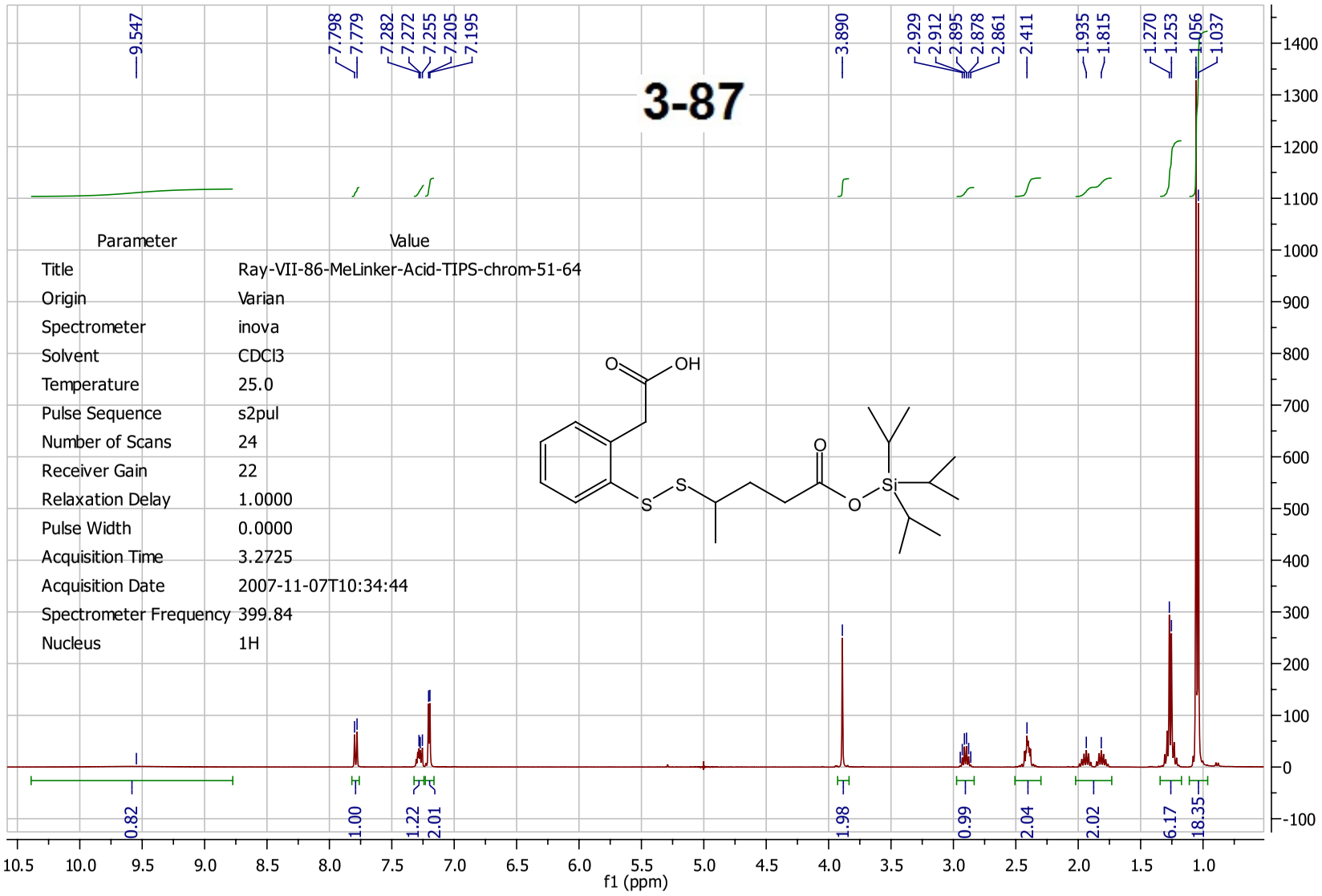

3-57
Parameter
Value
Title
Ray-V-99-Linker-TIPS-CO2H-chrom-35-44-124-144
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
64
Receiver Gain
32
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-04-30T22:56:18
Spectrometer Frequency
300.07
Nucleus
1H
7.77
7.75
7.26
7.26
7.25
3.90
2.95
2.95
2.92
2.90
2.90
2.78
2.75
2.73
2.73
1.32
1.29
1.29
1.27
1.27
1.24
1.08
1.05
1.00
4.22
1.94
1.94
2.01
3.72
18.70
f1 (ppm)

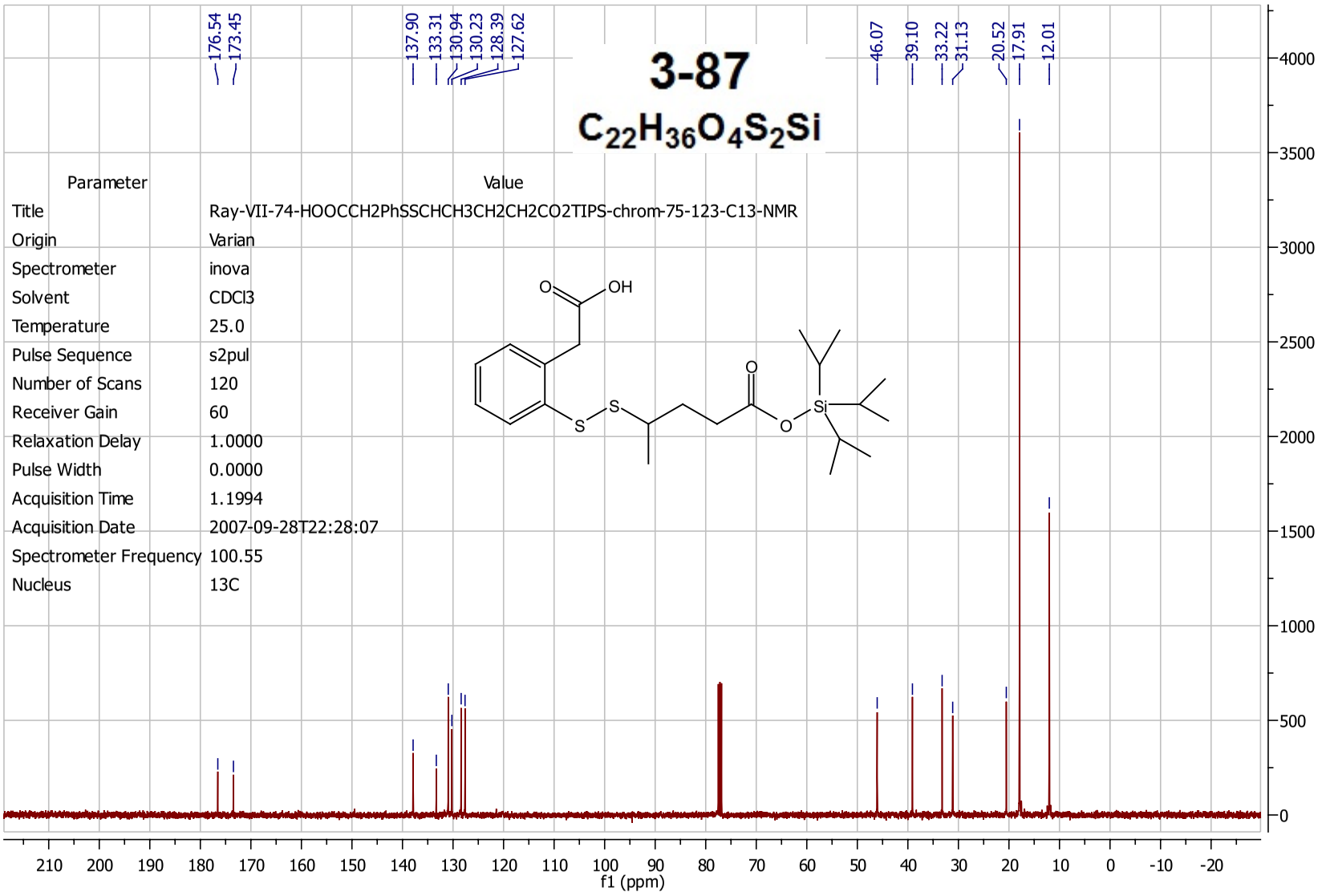

7.76
7.73
7.26
7.24
7.23
5.33
5.28
5.27
5.26
5.26
5.25
5.25
5.23
5.22
5.22
5.21
4.71
4.67
4.64
4.59
4.58
4.57
3.88
3.83
3.81
3.76
2.97
2.95
2.93
2.93
2.75
2.73
2.72
2.03
1.99
1.67
1.64
1.63
1.35
1.25
0.90
0.88
0.86
0.84
0.72
0.70
Parameter
Value
Title
Ray-V-7-Linker-Allyl-Menthol-ester-model-chrom-2nd-55-96
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
64
Receiver Gain
15
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2005-09-10T18:53:53
Spectrometer Frequency
300.07
Nucleus
1H
3-77
S
S
O
O
O
1.00
3.30
0.97
0.98
0.99
0.99
1.99
2.00
1.99
2.02
1.04
1.03
2.07
2.26
2.13
7.23
3.02
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
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
f1 (ppm)


171.18
170.41
136.47
134.44
131.89
130.79
130.02
128.00
127.71
118.43
65.41
46.97
40.72
39.57
33.73
32.98
31.34
26.14
21.98
20.70
16.28
Parameter
Value
Title
Ray-V-7-Linker-Allyl-Menthol-ester-model-chrom-2nd-55-96-C13-NMR
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
1240
Receiver Gain
35
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.8150
Acquisition Date
2005-09-10T18:58:52
Spectrometer Frequency
75.46
Nucleus
13C
3-77
C24H34O4S2
S
S
O
O
O
O
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


3-86
Parameter
Value
Title
Ray-VII-73-PySSCHCH3CH2CH2CO2TIPS-chrom-51-71
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
40
Receiver Gain
22
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2007-09-27T22:15:31
Spectrometer Frequency
399.84
Nucleus
1H
8.408
8.398
8.396
7.707
7.592
7.588
7.567
7.078
7.049
7.047
7.037
7.035
7.031
7.028
7.019
7.016
6.961
3.003
2.986
2.494
2.483
2.474
2.466
1.953
1.858
1.300
1.229
1.208
1.031
1.013
0.99
1.08
1.02
1.01
1.00
2.24
2.28
3.19
3.05
21.52
f1 (ppm)

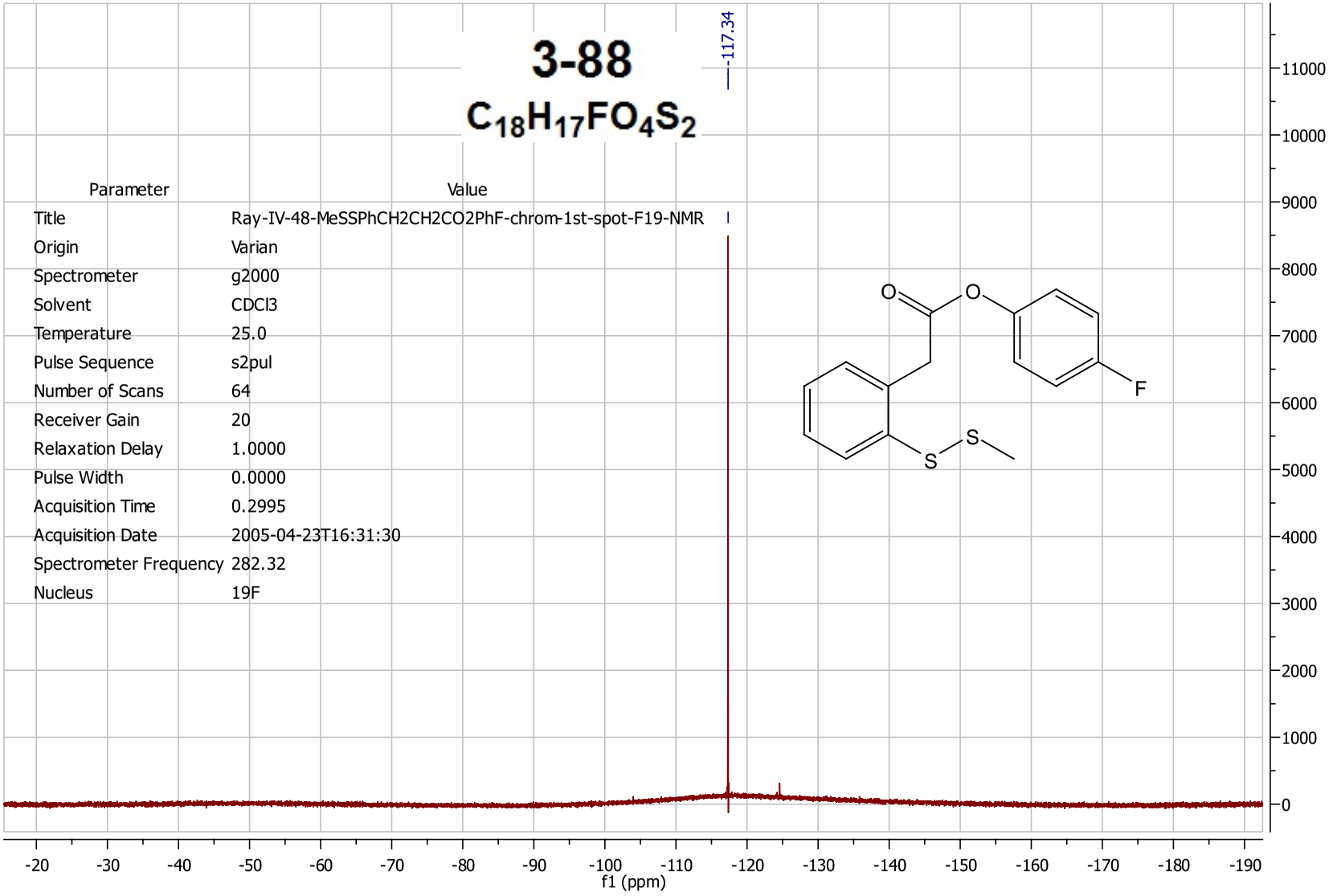

173.05
160.90
149.45
137.03
120.65
119.88
46.29
33.33
31.26
20.60
17.87
11.98
3-86
C19H33NO2S2Si
Parameter Value
Title Ray-VII-73-PySSCHCH3CH2CH2CO2TIPS-chrom-51-71-C13-NMR
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 112
Receiver Gain 60
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.1994
Acquisition Date 2007-09-27T22:25:21
Spectrometer Frequency 100.55
Nucleus 13C
N
S
S
O
O
Si
f1 (ppm)


3-87
9.547
7.798
7.779
7.282
7.272
7.255
7.205
7.195
3.890
2.929
2.912
2.895
2.878
2.861
2.411
1.935
1.815
1.270
1.253
1.056
1.037
Parameter Value
Title Ray-VII-86-MeLinker-Acid-TIPS-chrom-51-64
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 24
Receiver Gain 22
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2007-11-07T10:34:44
Spectrometer Frequency 399.84
Nucleus 1H
O
OH
S
S
O
O
Si
0.82
1.00
1.22
2.01
1.98
0.99
2.04
2.02
6.17
18.35
f1 (ppm)

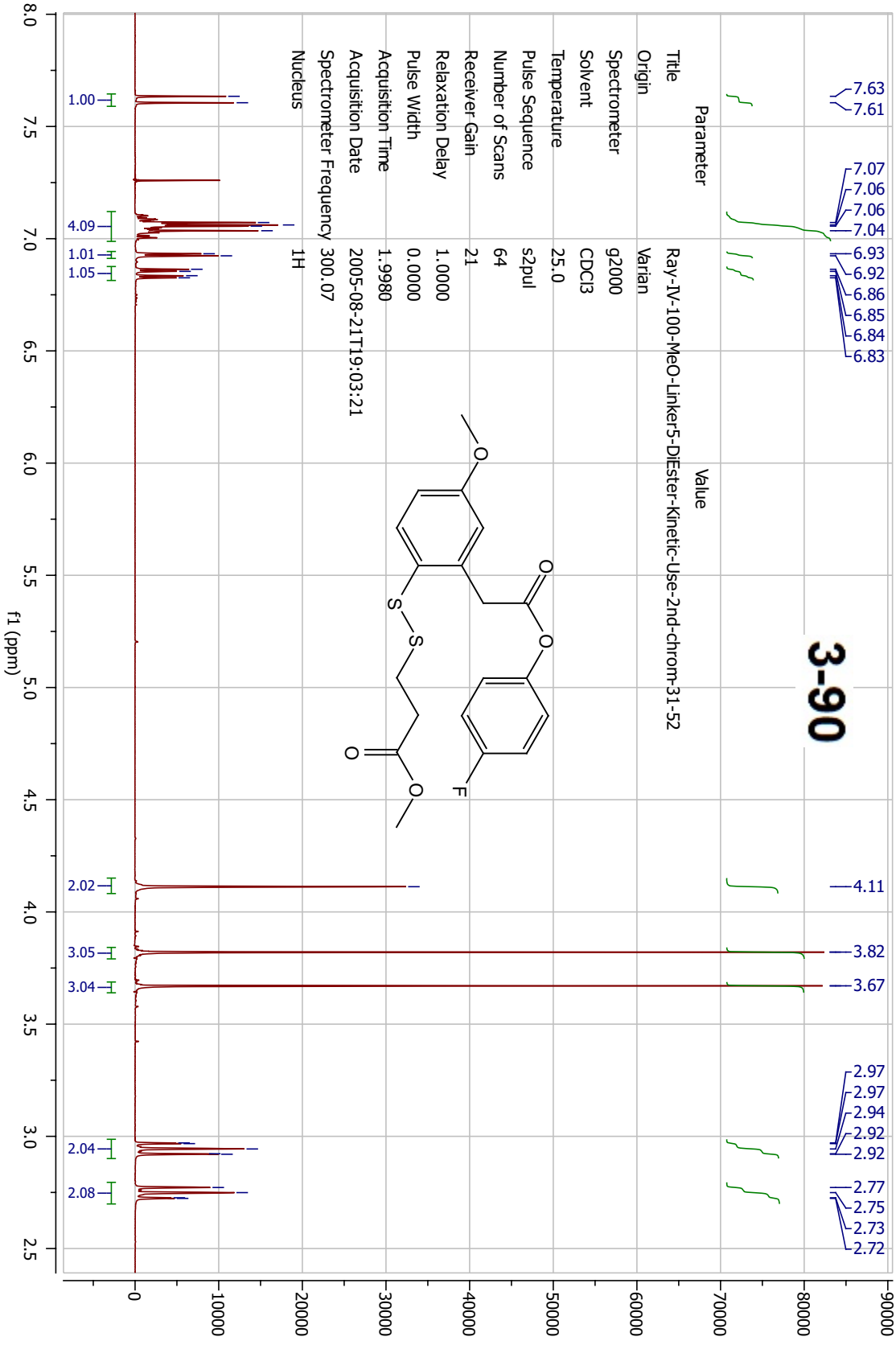

176.54
173.45
137.90
133.31
130.94
130.23
128.39
127.62
46.07
39.10
33.22
31.13
20.52
17.91
12.01
3-87
C22H36O4S2Si
Parameter
Value
Title
Ray-VII-74-HOOCCH2PhSSCHCH3CH2CH2CO2TIPS-chrom-75-123-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-09-28T22:28:07
Spectrometer Frequency
100.55
Nucleus
13C
O
OH
S
S
O
O
Si
4000
3500
3000
2500
2000
1500
1000
500
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

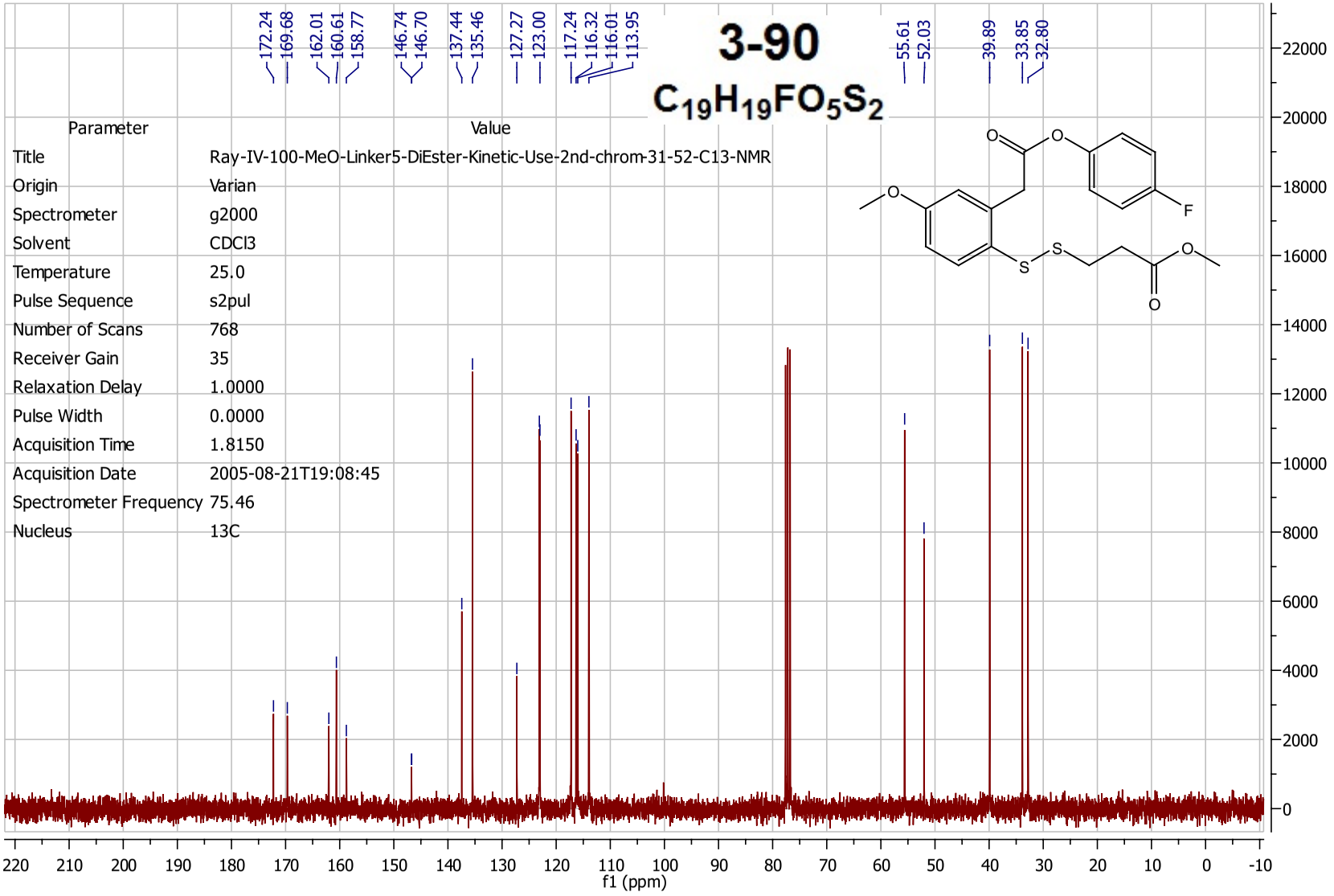
3-88

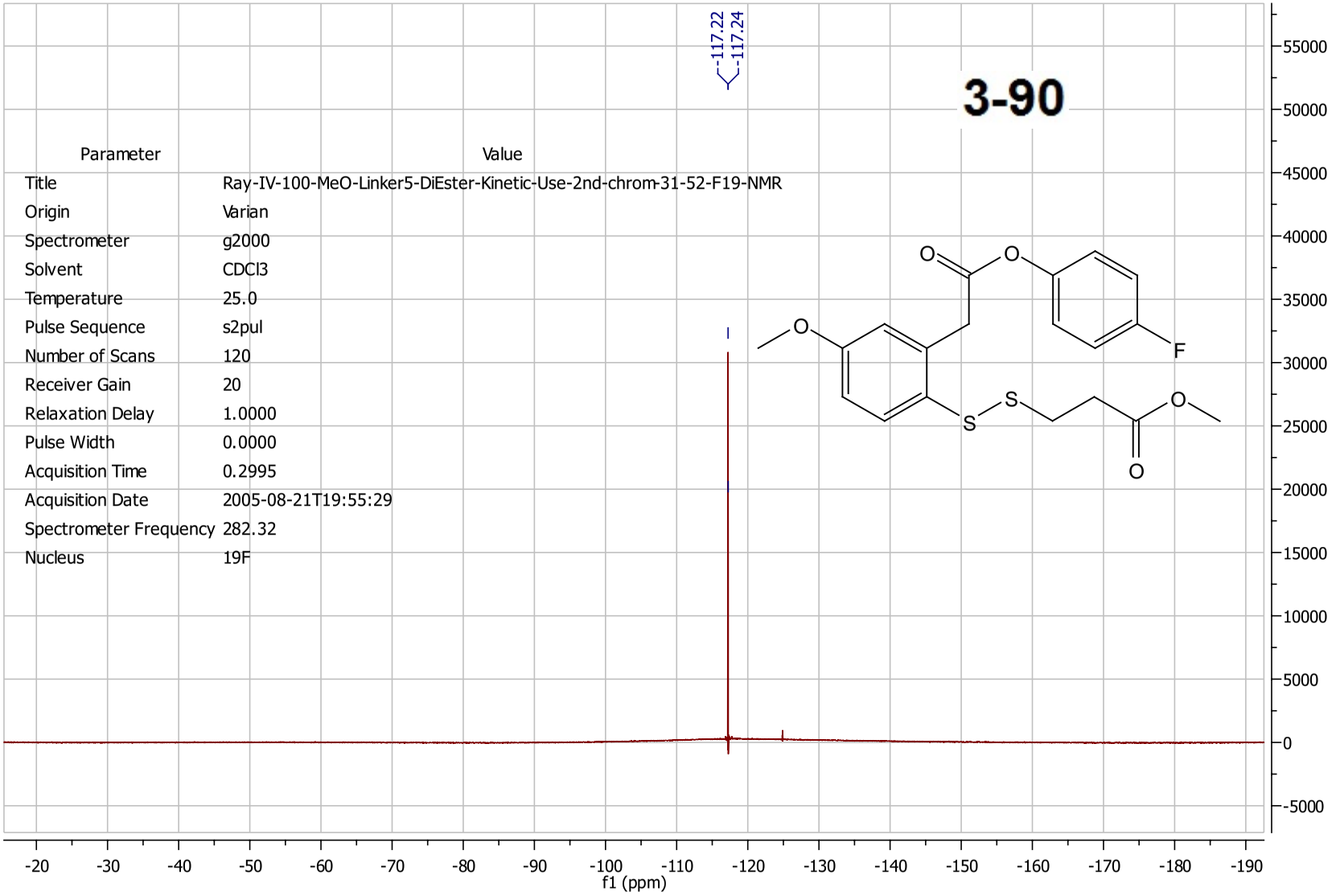
$C_{18}H_{17}FO_4S_2$

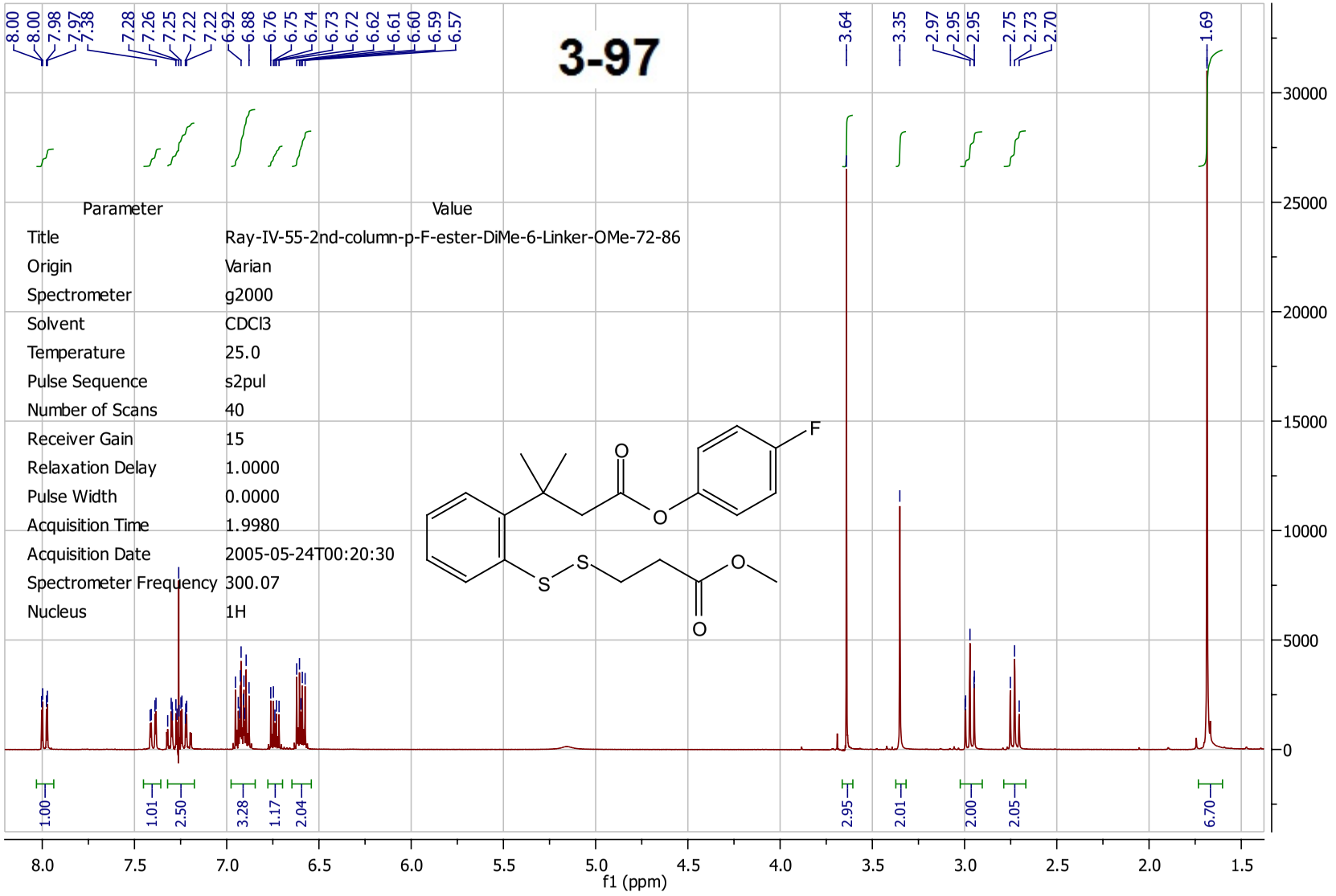


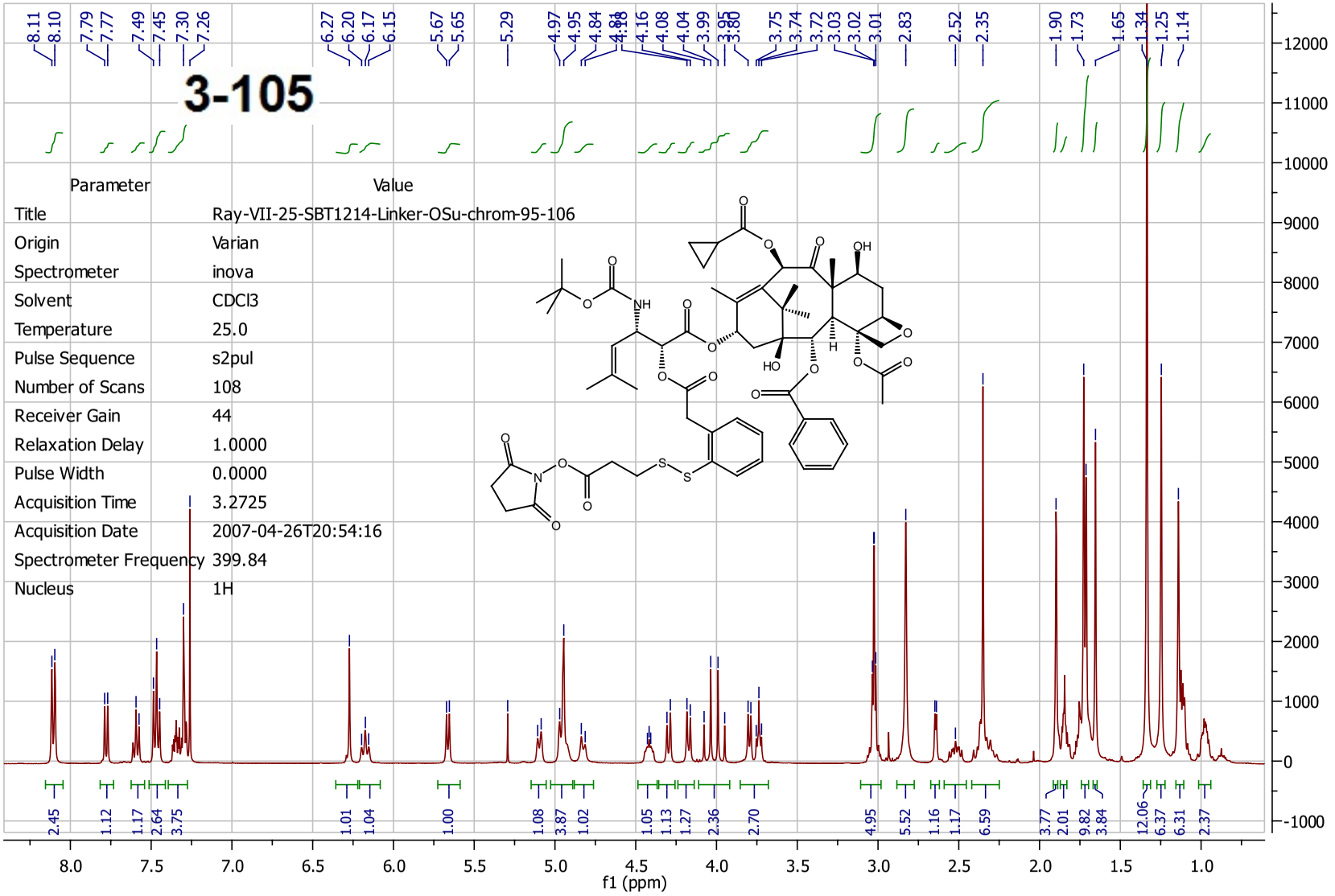
Parameter	Value
Title	Ray-IV-48-MeSSPhCH2CH2CO2PhF-chrom-1st-spot-F19-NMR
Origin	Varian
Spectrometer	g2000
Solvent	CDCl3
Temperature	25.0
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	20
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	0.2995
Acquisition Date	2005-04-23T16:31:30
Spectrometer Frequency	282.32
Nucleus	19F

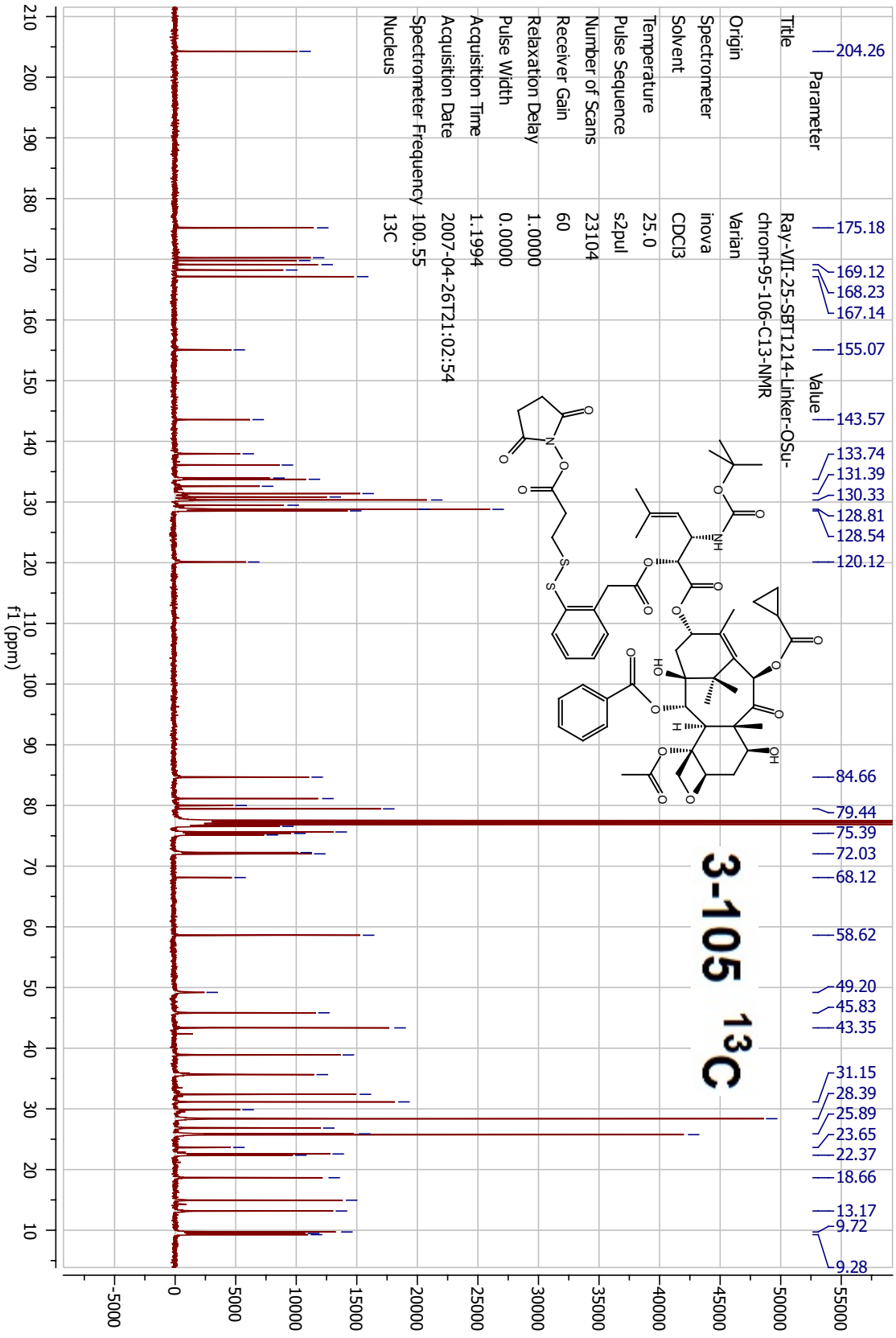

3-90
Parameter
Value
Title
Ray-IV-100-MeO-Linker5-DiEster-Kinetic-Use-2nd-chrom-31-52
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
64
Receiver Gain
21
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2005-08-21T19:03:21
Spectrometer Frequency
300.07
Nucleus
1H
7.63
7.61
7.07
7.06
7.06
7.04
6.93
6.92
6.86
6.85
6.84
6.83
4.11
3.82
3.67
2.97
2.97
2.94
2.92
2.92
2.77
2.75
2.73
2.72
1.00
4.09
1.01
1.05
2.02
3.05
3.04
2.04
2.08
f1 (ppm)

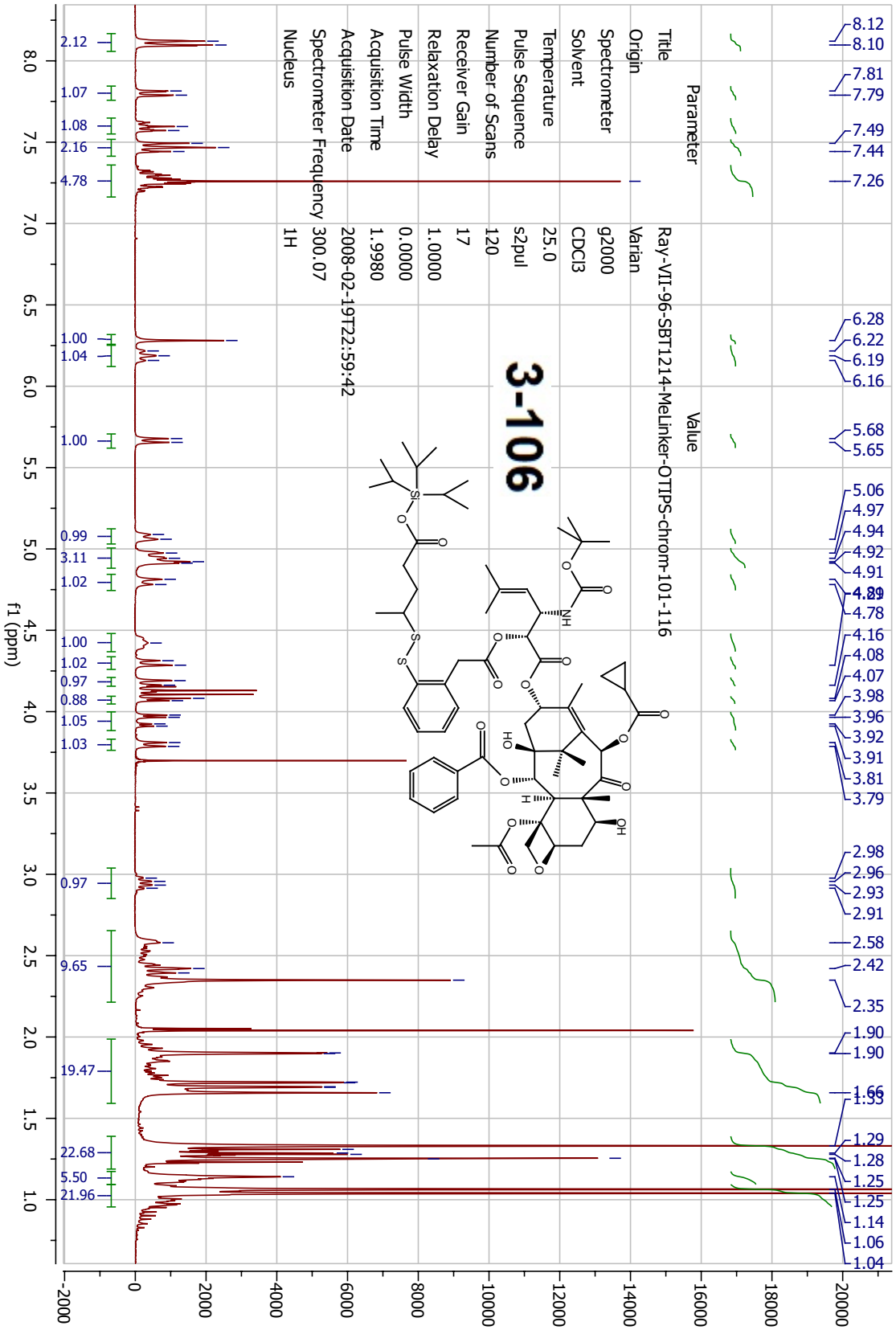

172.24
169.68
162.01
160.61
158.77
146.74
146.70
137.44
135.46
127.27
123.00
117.24
116.32
116.01
113.95
55.61
52.03
39.89
33.85
32.80
3-90
C19H19FO5S2
Parameter
Value
Title
Ray-IV-100-MeO-Linker5-DiEster-Kinetic-Use-2nd-chrom-31-52-C13-NMR
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
768
Receiver Gain
35
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.8150
Acquisition Date
2005-08-21T19:08:45
Spectrometer Frequency
75.46
Nucleus
13C
f1 (ppm)

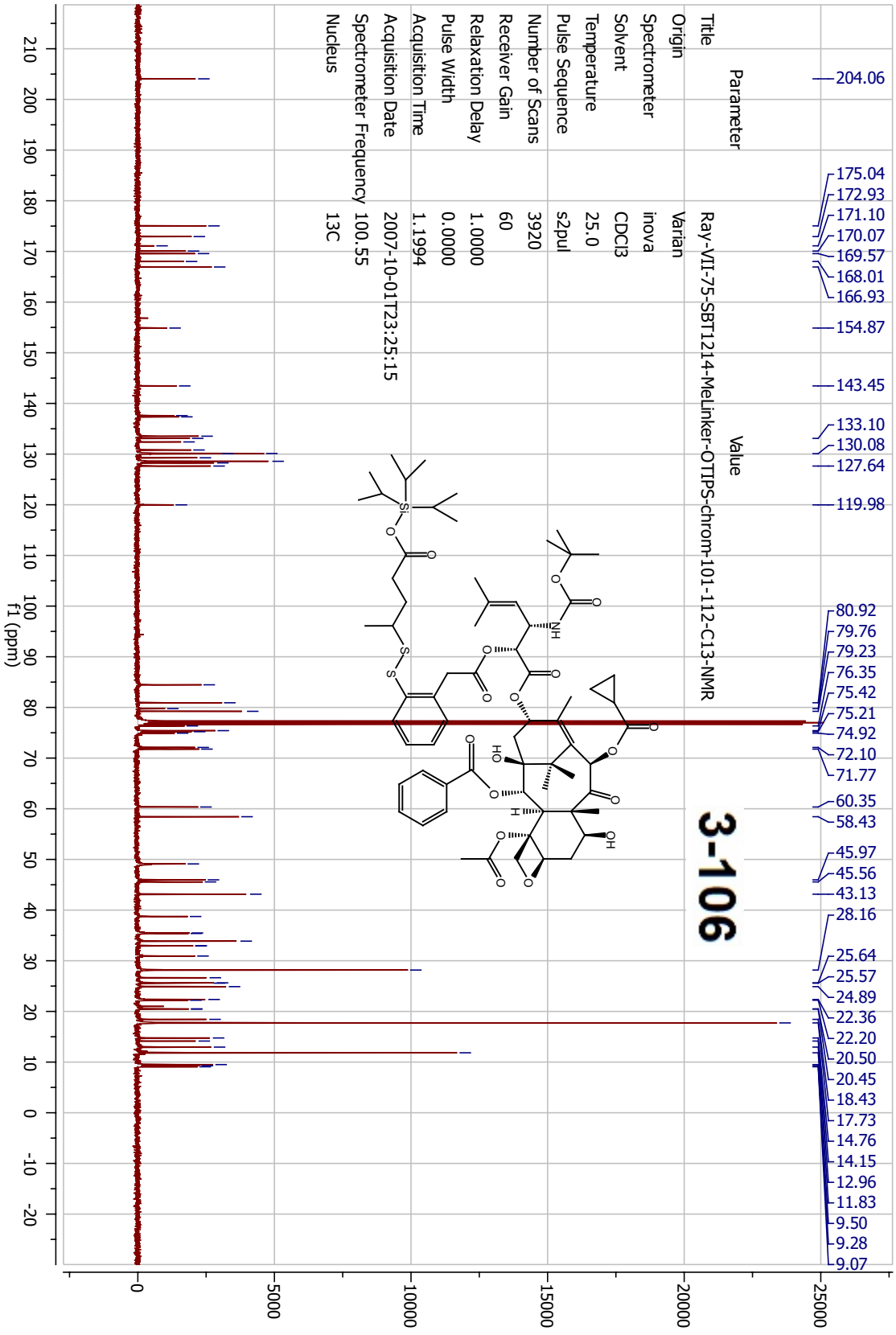

-117.22
-117.24
3-90
Parameter Value
Title Ray-IV-100-MeO-Linker5-DiEster-Kinetic-Use-2nd-chrom-31-52-F19-NMR
Origin Varian
Spectrometer g2000
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 120
Receiver Gain 20
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 0.2995
Acquisition Date 2005-08-21T19:55:29
Spectrometer Frequency 282.32
Nucleus 19F
O
O
O
F
S
S
O
O
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
-20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190
f1 (ppm)

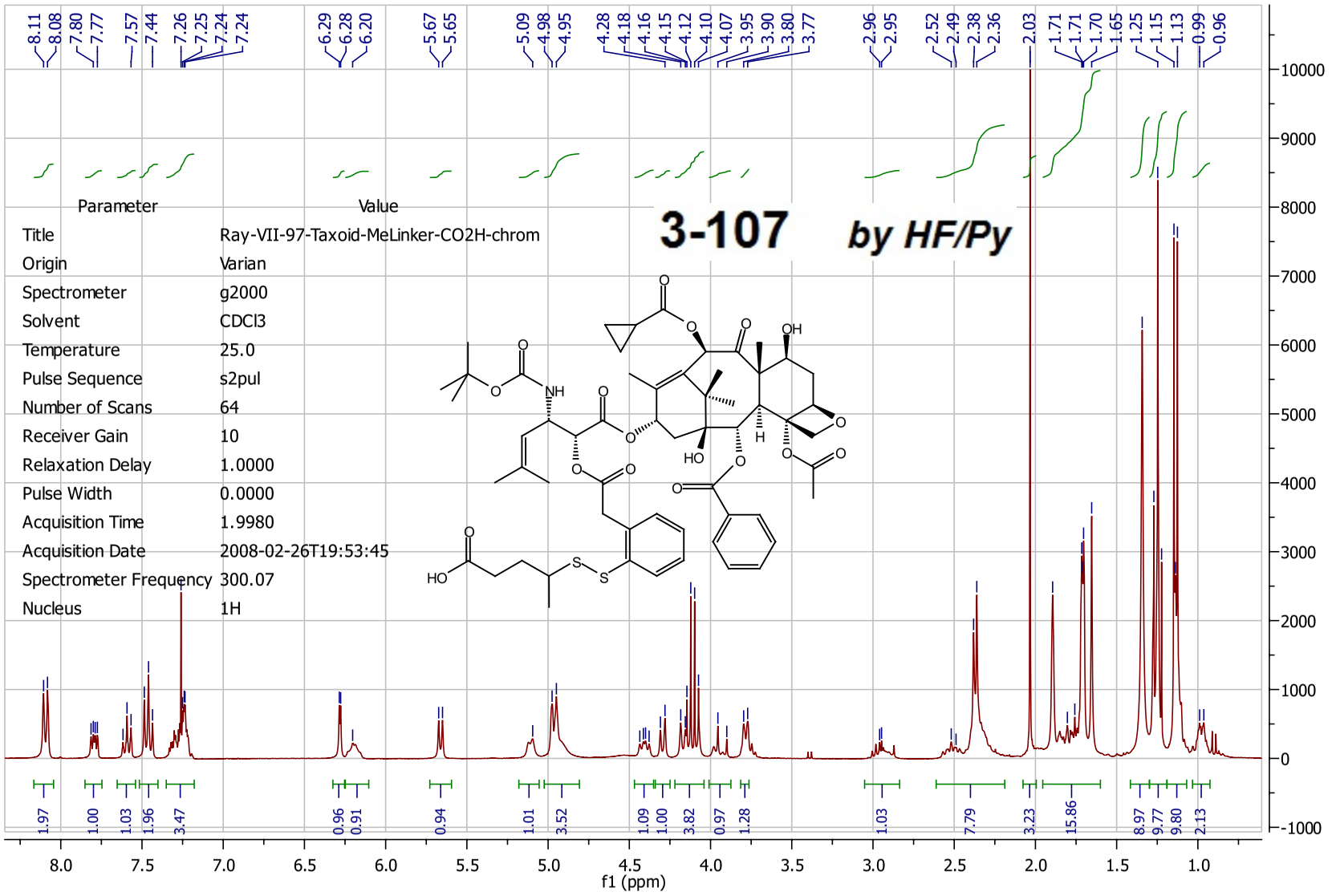

3-97
8.00
8.00
7.98
7.97
7.38
7.28
7.26
7.25
7.22
7.22
6.92
6.88
6.76
6.75
6.74
6.73
6.72
6.62
6.61
6.60
6.59
6.57
3.64
3.35
2.97
2.95
2.95
2.75
2.73
2.70
1.69
Parameter
Value
Title
Ray-IV-55-2nd-column-p-F-ester-DiMe-6-Linker-OMe-72-86
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
40
Receiver Gain
15
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2005-05-24T00:20:30
Spectrometer Frequency
300.07
Nucleus
1H
F
O
O
S
S
O
O
30000
25000
20000
15000
10000
5000
0
1.00
1.01
2.50
3.28
1.17
2.04
2.95
2.01
2.00
2.05
6.70
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
f1 (ppm)

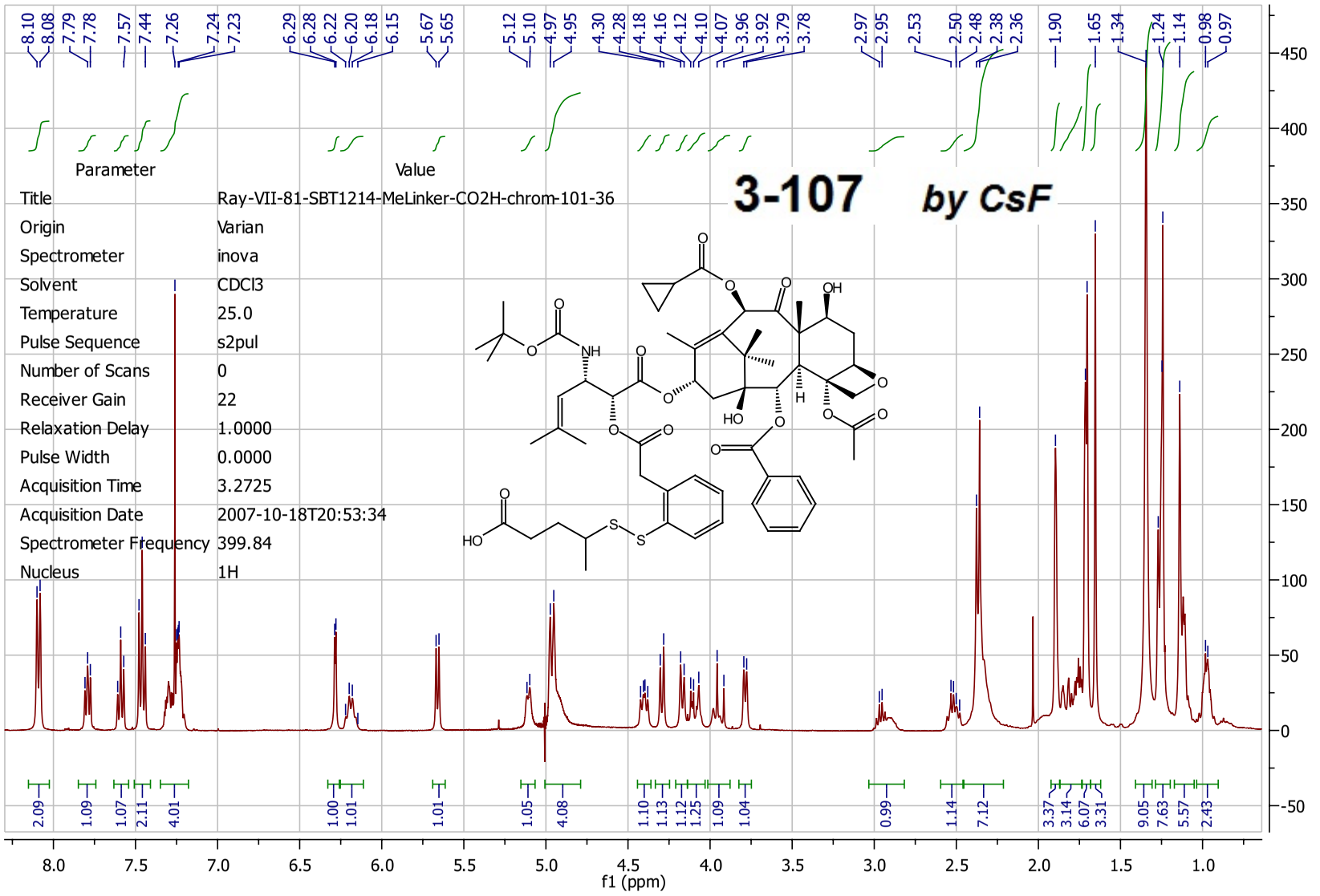

3-105
Parameter Value
Title Ray-VII-25-SBT1214-Linker-OSu-chrom-95-106
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 108
Receiver Gain 44
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2007-04-26T20:54:16
Spectrometer Frequency 399.84
Nucleus 1H
f1 (ppm)

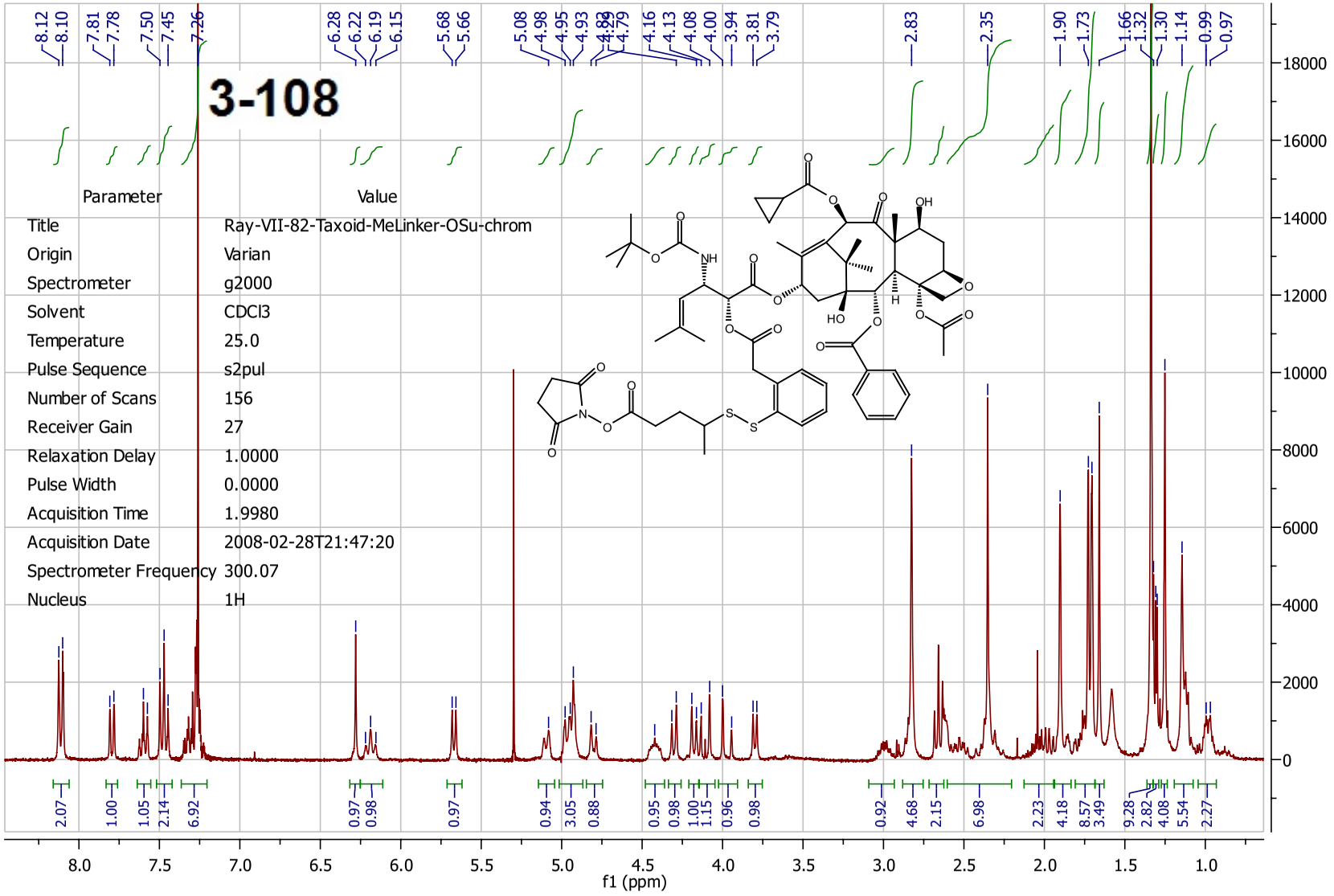

Parameter
Value
Title
Ray-VII-25-SBT1214-Linker-OSu-chrom-95-106-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
23104
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-04-26T21:02:54
Spectrometer Frequency
100.55
Nucleus
13C
3-105 13C
204.26
175.18
169.12
168.23
167.14
155.07
143.57
133.74
131.39
130.33
128.81
128.54
120.12
84.66
79.44
75.39
72.03
68.12
58.62
49.20
45.83
43.35
31.15
28.39
25.89
23.65
22.37
18.66
13.17
9.72
9.28
f1 (ppm)

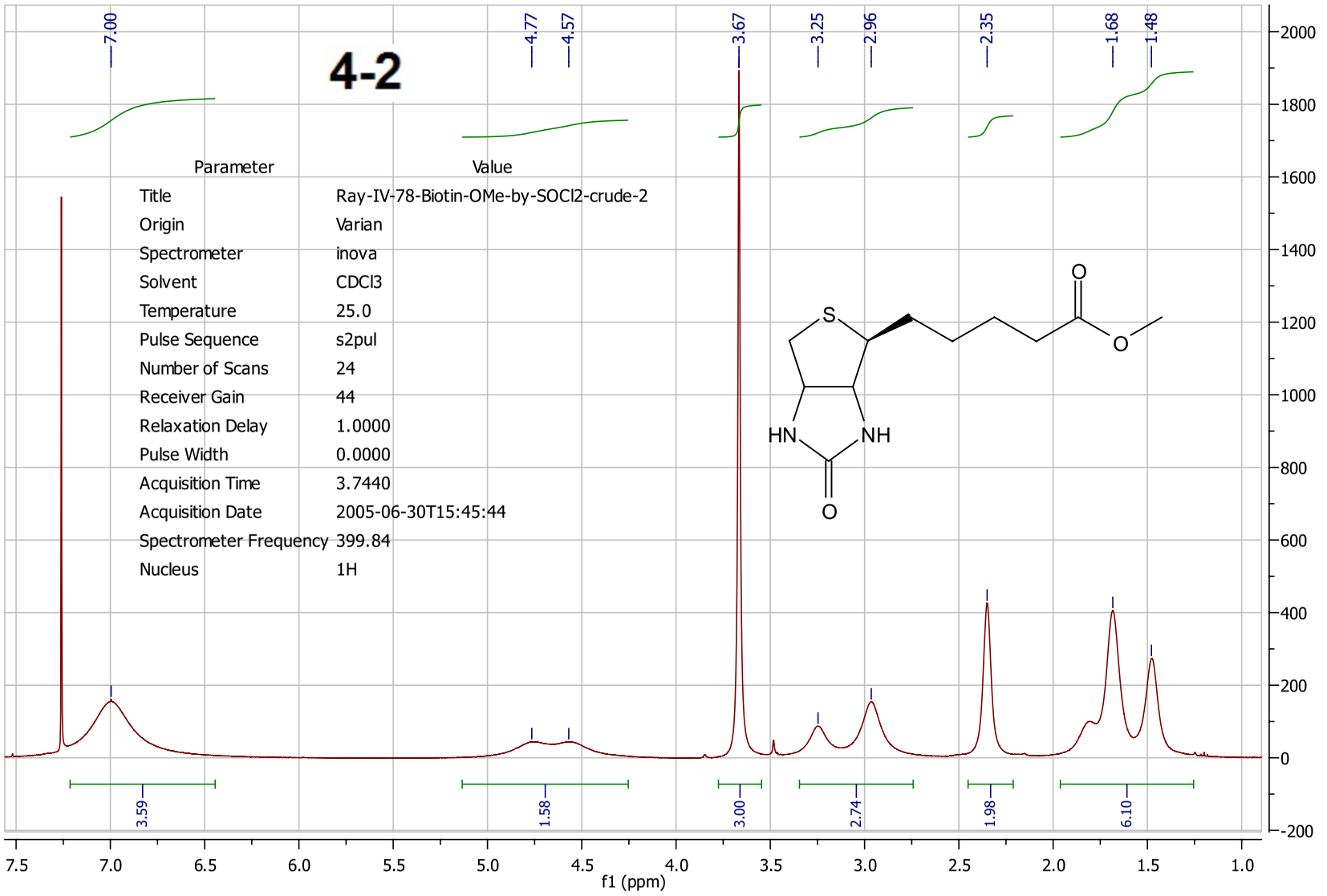

3-106
Parameter
Value
Title
Ray-VII-96-SBT1214-MeLinker-OTIPS-chrom-101-116
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
17
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2008-02-19T22:59:42
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

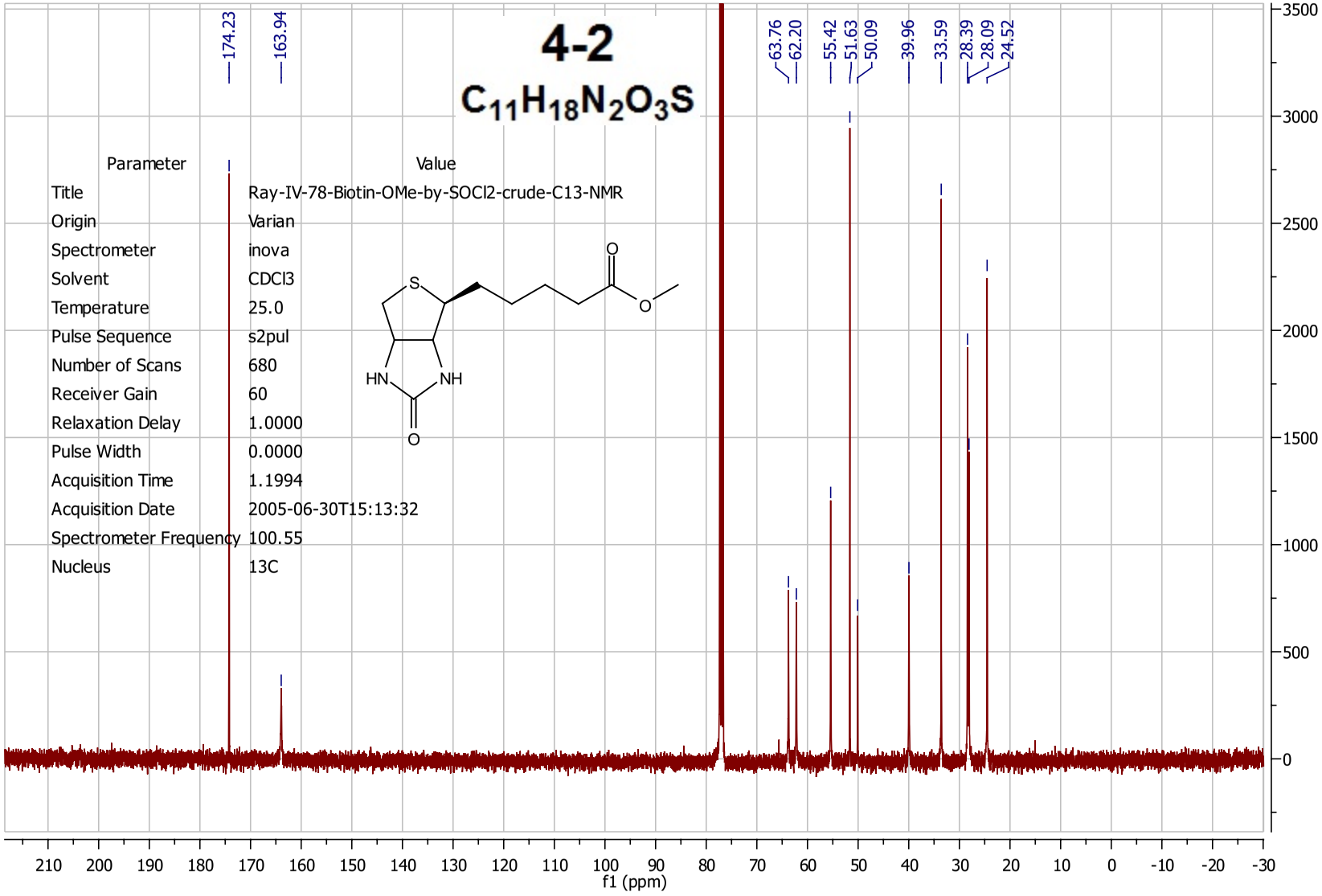

Parameter
Value
Title
Ray-VII-75-SBT1214-MeLinker-OTIPS-chrom-101-112-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
3920
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-10-01T23:25:15
Spectrometer Frequency
100.55
Nucleus
13C
3-106
204.06
175.04
172.93
171.10
170.07
169.57
168.01
166.93
154.87
143.45
133.10
130.08
127.64
119.98
80.92
79.76
79.23
76.35
75.42
75.21
74.92
72.10
71.77
60.35
58.43
45.97
45.56
43.13
28.16
25.64
25.57
24.89
22.36
22.20
20.50
20.45
18.43
17.73
14.76
14.15
12.96
11.83
9.50
9.28
9.07
f1 (ppm)

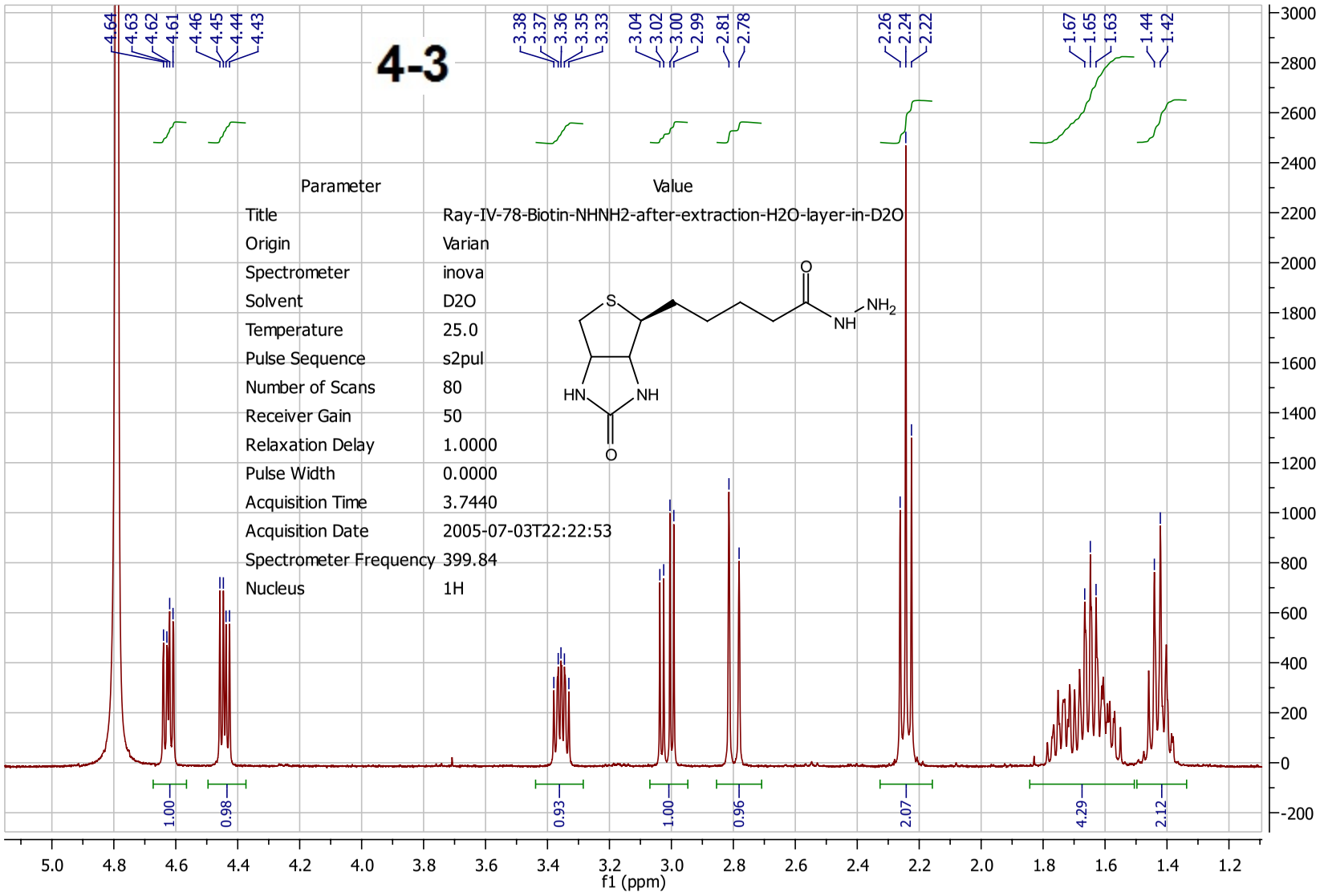

8.11 8.08 7.80 7.77 7.57 7.44 7.26 7.25 7.24 7.24 6.29 6.28 6.20 5.67 5.65 5.09 4.98 4.95 4.28 4.18 4.16 4.15 4.12 4.10 4.07 3.95 3.90 3.80 3.77 2.96 2.95 2.52 2.49 2.38 2.36 2.03 1.71 1.71 1.70 1.65 1.25 1.15 1.13 0.99 0.96
Parameter
Value
Title Ray-VII-97-Taxoid-MeLinker-CO2H-chrom
Origin Varian
Spectrometer g2000
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 64
Receiver Gain 10
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.9980
Acquisition Date 2008-02-26T19:53:45
Spectrometer Frequency 300.07
Nucleus 1H
3-107
by HF/Py
1.97 1.00 1.03 1.96 3.47 0.96 0.91 0.94 1.01 3.52 1.09 1.00 3.82 0.97 1.28 1.03 7.79 3.23 15.86 8.97 9.77 9.80 2.13
f1 (ppm)


8.10 8.08 7.79 7.78 7.57 7.44 7.26 7.24 7.23 6.29 6.28 6.22 6.20 6.18 6.15 5.67 5.65 5.12 5.10 4.97 4.95 4.30 4.28 4.18 4.16 4.12 4.10 4.07 3.96 3.92 3.79 3.78 2.97 2.95 2.53 2.50 2.48 2.38 2.36 1.90 1.65 1.34 1.24 1.14 0.98 0.97
Parameter
Value
Title Ray-VII-81-SBT1214-MeLinker-CO2H-chrom-101-36
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 0
Receiver Gain 22
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2007-10-18T20:53:34
Spectrometer Frequency 399.84
Nucleus 1H
3-107 by CsF
2.09 1.09 1.07 2.11 4.01 1.00 1.01 1.01 1.05 4.08 1.10 1.13 1.12 1.25 1.09 1.04 0.99 1.14 7.12 3.37 3.14 6.07 3.31 9.05 7.63 5.57 2.43
f1 (ppm)


3-108
Parameter Value
Title Ray-VII-82-Taxoid-MeLinker-OSu-chrom
Origin Varian
Spectrometer g2000
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 156
Receiver Gain 27
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.9980
Acquisition Date 2008-02-28T21:47:20
Spectrometer Frequency 300.07
Nucleus 1H
f1 (ppm)

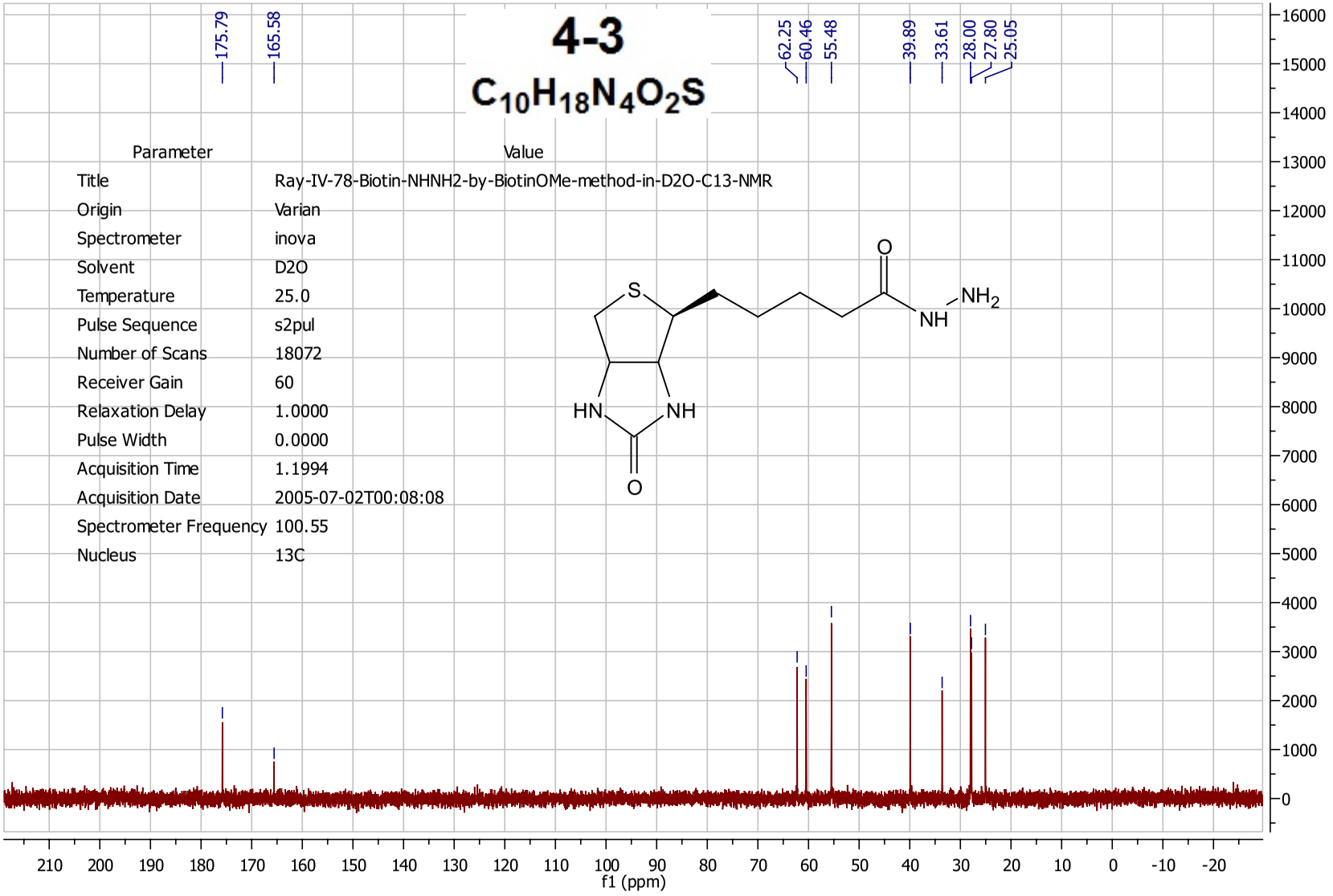

4-2
7.00
4.77
4.57
3.67
3.25
2.96
2.35
1.68
1.48
Parameter Value
Title Ray-IV-78-Biotin-OMe-by-SOCl2-crude-2
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 24
Receiver Gain 44
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.7440
Acquisition Date 2005-06-30T15:45:44
Spectrometer Frequency 399.84
Nucleus 1H
S
O
O
HN
NH
O
3.59
1.58
3.00
2.74
1.98
6.10
f1 (ppm)


4-2
$C_{11}H_{18}N_2O_3S$
174.23
163.94
63.76
62.20
55.42
51.63
50.09
39.96
33.59
28.39
28.09
24.52
Parameter
Value
Title
Ray-IV-78-Biotin-OMe-by-SOCl2-crude-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
680
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2005-06-30T15:13:32
Spectrometer Frequency
100.55
Nucleus
13C
S
O
O
HN
NH
O
3500
3000
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

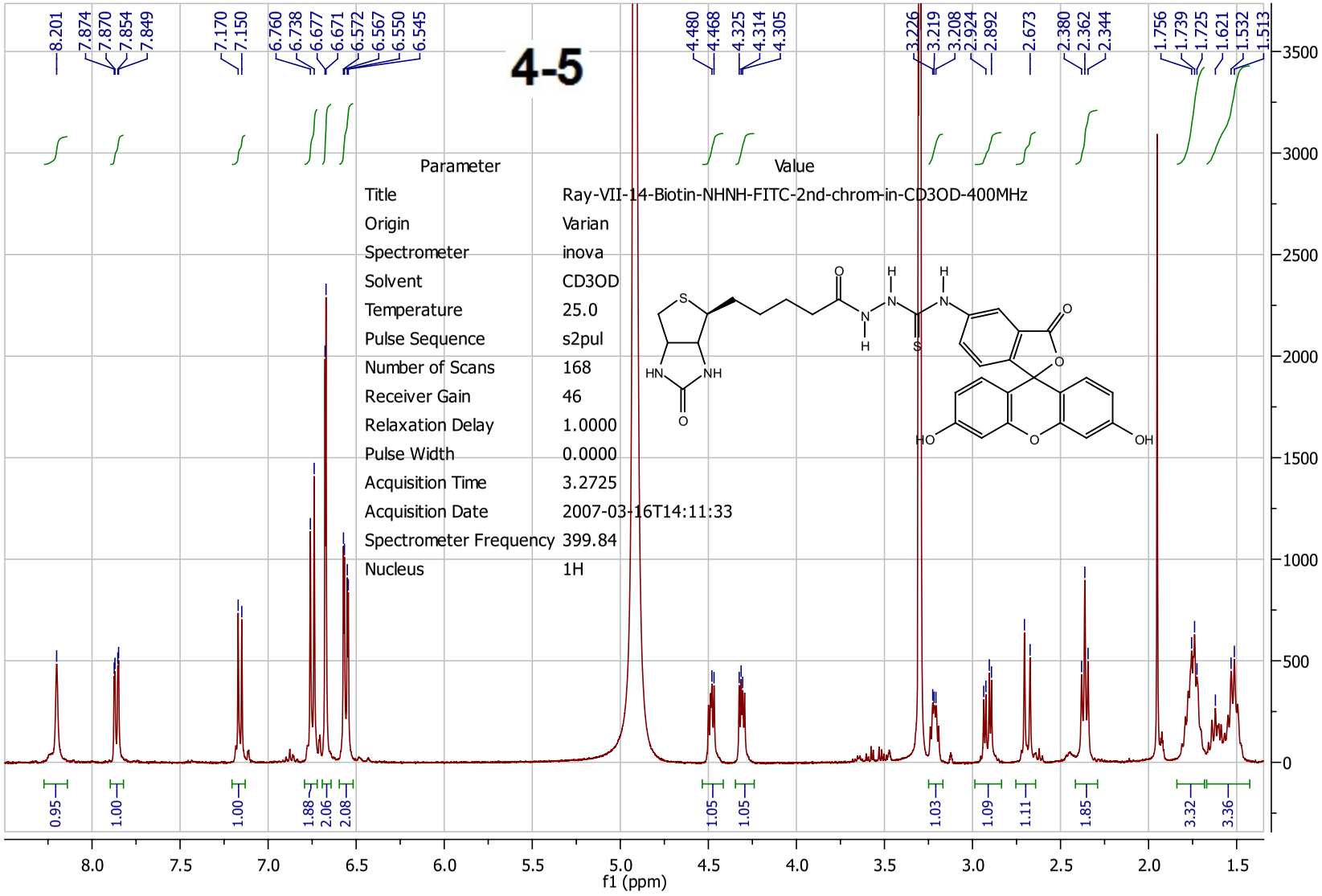

4-3
4.64
4.63
4.62
4.61
4.46
4.45
4.44
4.43
3.38
3.37
3.36
3.35
3.33
3.04
3.02
3.00
2.99
2.81
2.78
2.26
2.24
2.22
1.67
1.65
1.63
1.44
1.42
Parameter
Value
Title
Ray-IV-78-Biotin-NHNH2-after-extraction-H2O-layer-in-D2O
Origin
Varian
Spectrometer
inova
Solvent
D2O
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
80
Receiver Gain
50
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.7440
Acquisition Date
2005-07-03T22:22:53
Spectrometer Frequency
399.84
Nucleus
1H
1.00
0.98
0.93
1.00
0.96
2.07
4.29
2.12
f1 (ppm)

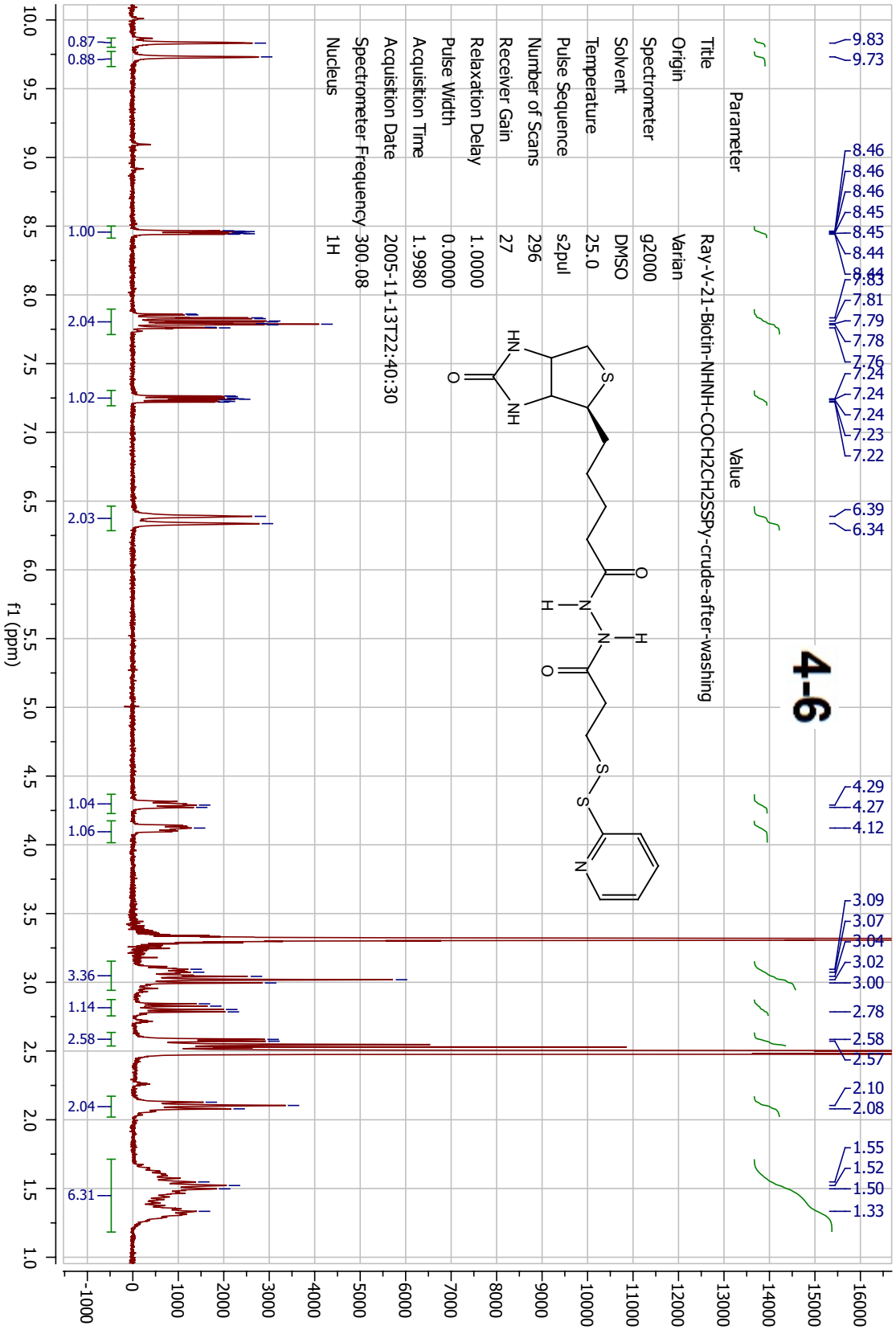
4-3

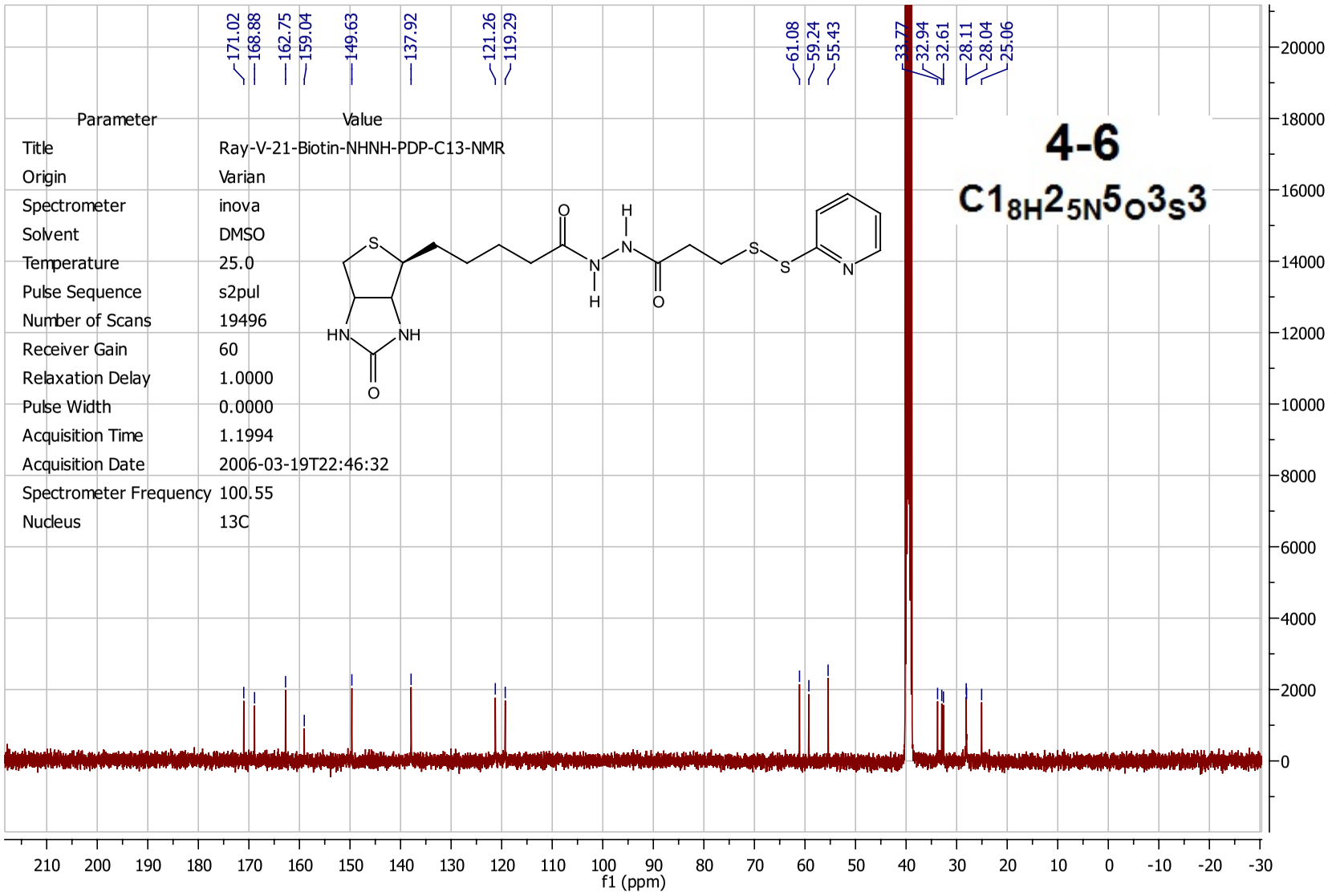
$C_{10}H_{18}N_4O_2S$

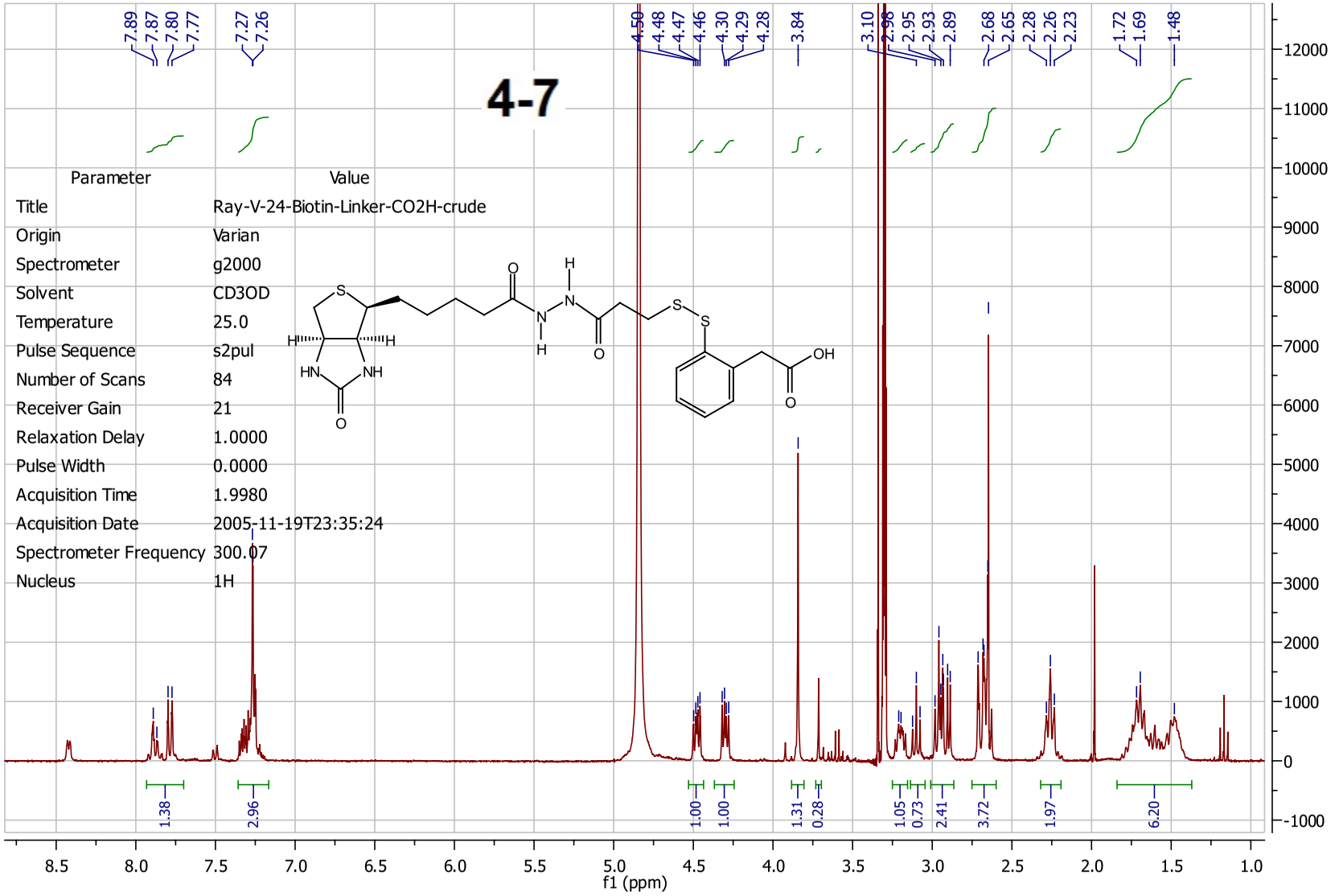


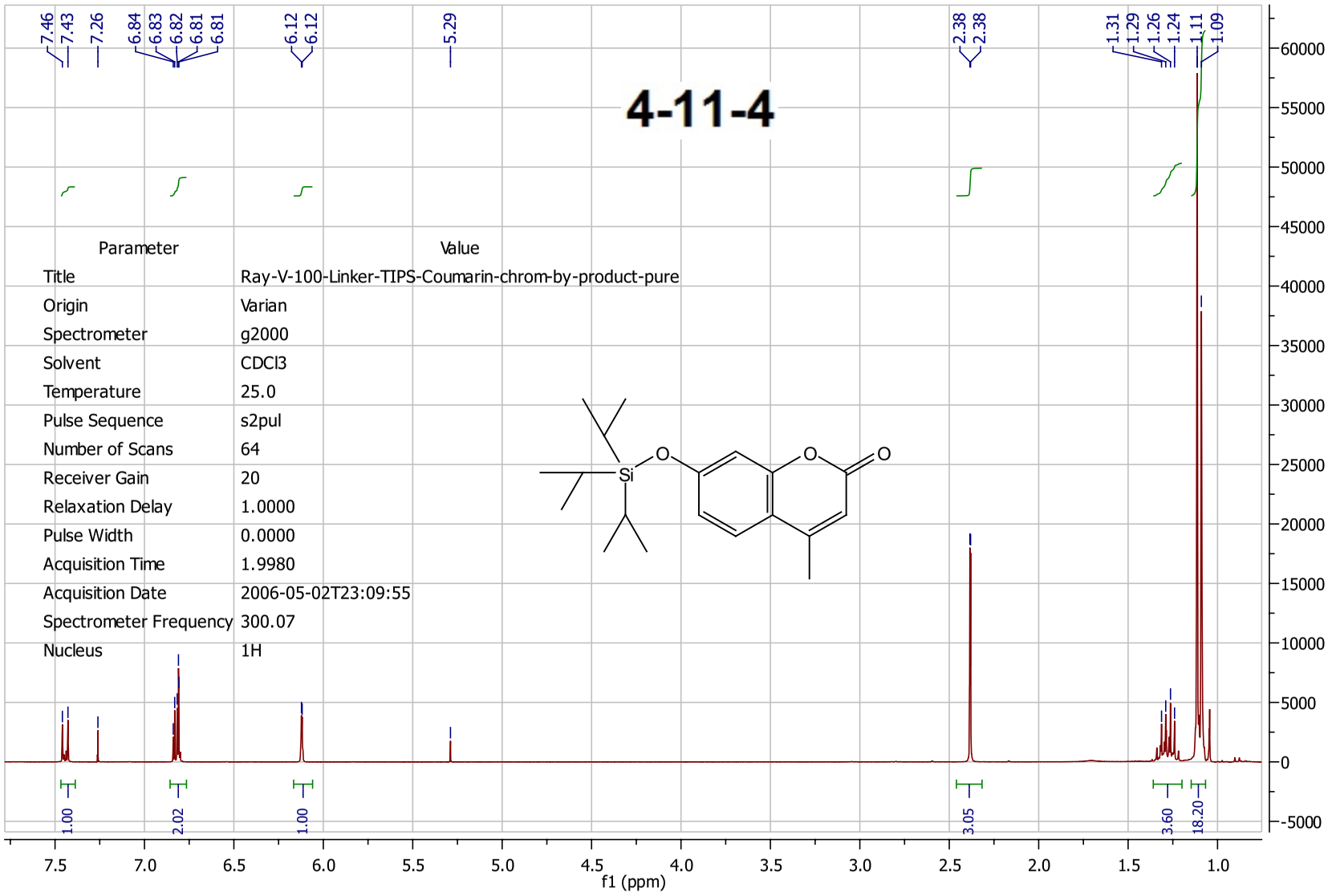
Parameter	Value
Title	Ray-IV-78-Biotin-NHNH2-by-BiotinOMe-method-in-D2O-C13-NMR
Origin	Varian
Spectrometer	inova
Solvent	D2O
Temperature	25.0
Pulse Sequence	s2pul
Number of Scans	18072
Receiver Gain	60
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	1.1994
Acquisition Date	2005-07-02T00:08:08
Spectrometer Frequency	100.55
Nucleus	13C

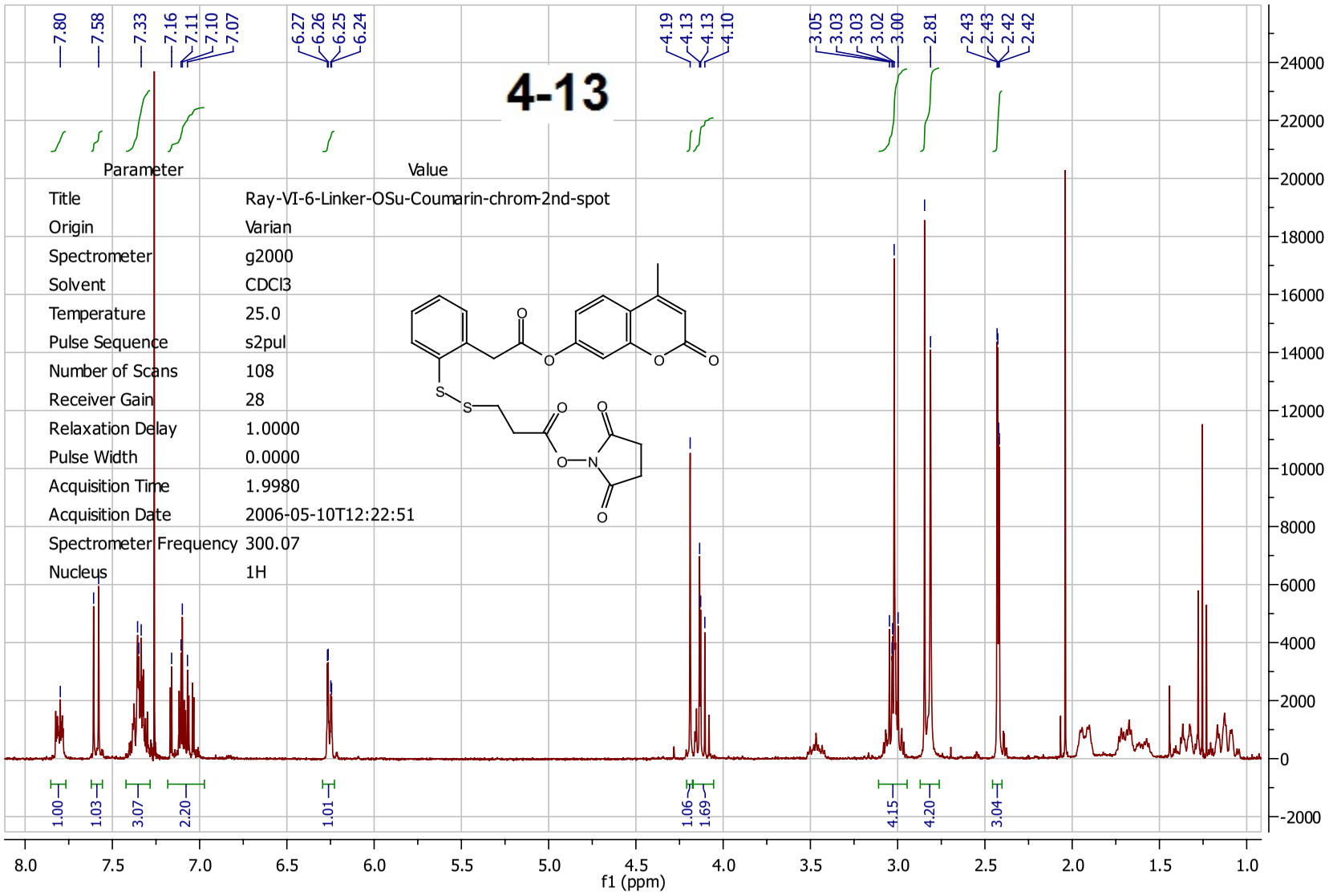

4-5
8.201
7.874
7.870
7.854
7.849
7.170
7.150
6.760
6.738
6.677
6.671
6.572
6.567
6.550
6.545
4.480
4.468
4.325
4.314
4.305
3.226
3.219
3.208
2.924
2.892
2.673
2.380
2.362
2.344
1.756
1.739
1.725
1.621
1.532
1.513
Parameter
Value
Title
Ray-VII-14-Biotin-NHNH-FITC-2nd-chrom-in-CD3OD-400MHz
Origin
Varian
Spectrometer
inova
Solvent
CD3OD
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
168
Receiver Gain
46
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2007-03-16T14:11:33
Spectrometer Frequency
399.84
Nucleus
1H
0.95
1.00
1.00
1.88
2.06
2.08
1.05
1.05
1.03
1.09
1.11
1.85
3.32
3.36
f1 (ppm)

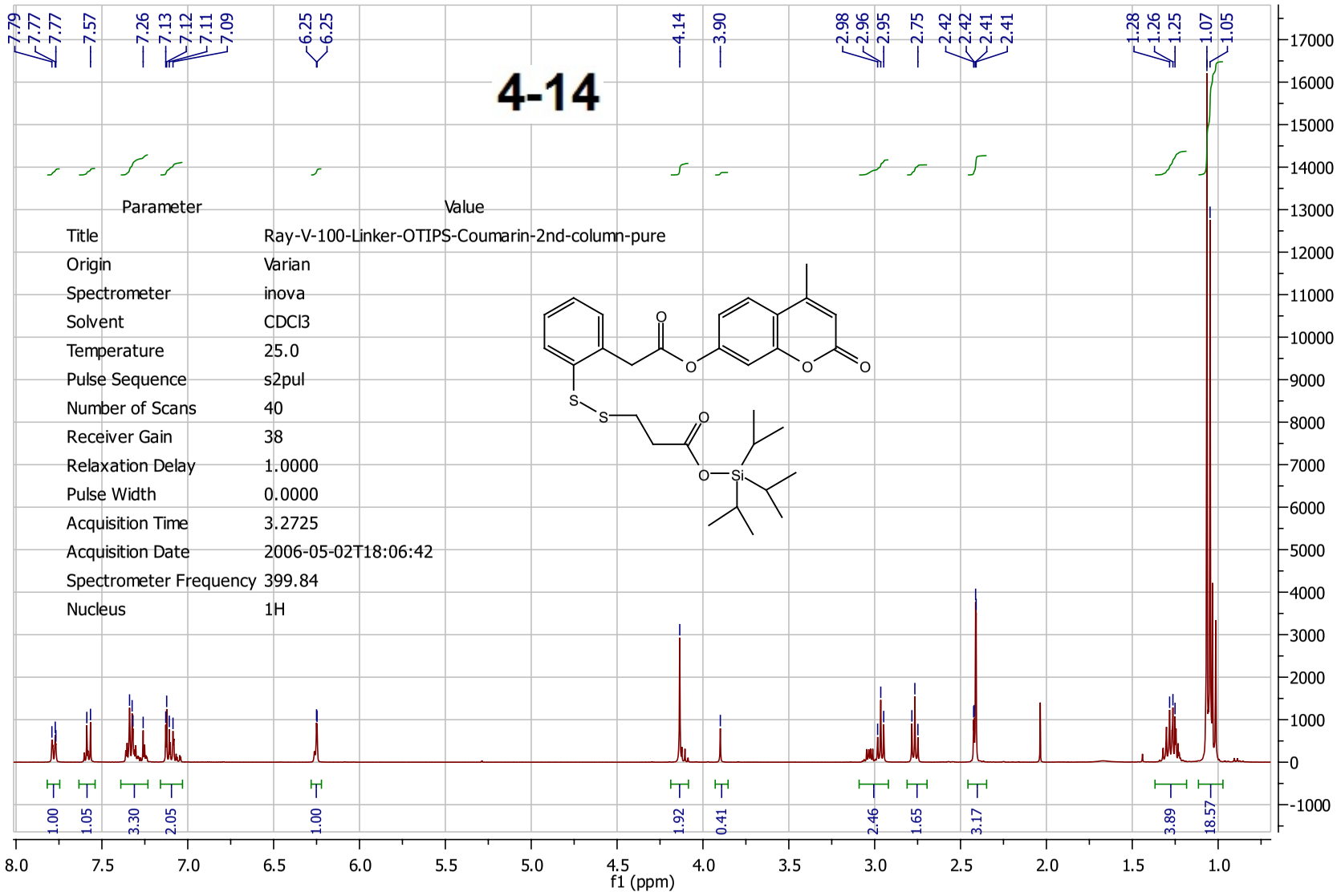

4-6
Parameter
Value
Title
Ray-V-21-Biotin-NHNH-COCH2CH2SSPy-crude-after-washing
Origin
Varian
Spectrometer
g2000
Solvent
DMSO
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
296
Receiver Gain
27
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2005-11-13T22:40:30
Spectrometer Frequency
300.08
Nucleus
1H
f1 (ppm)

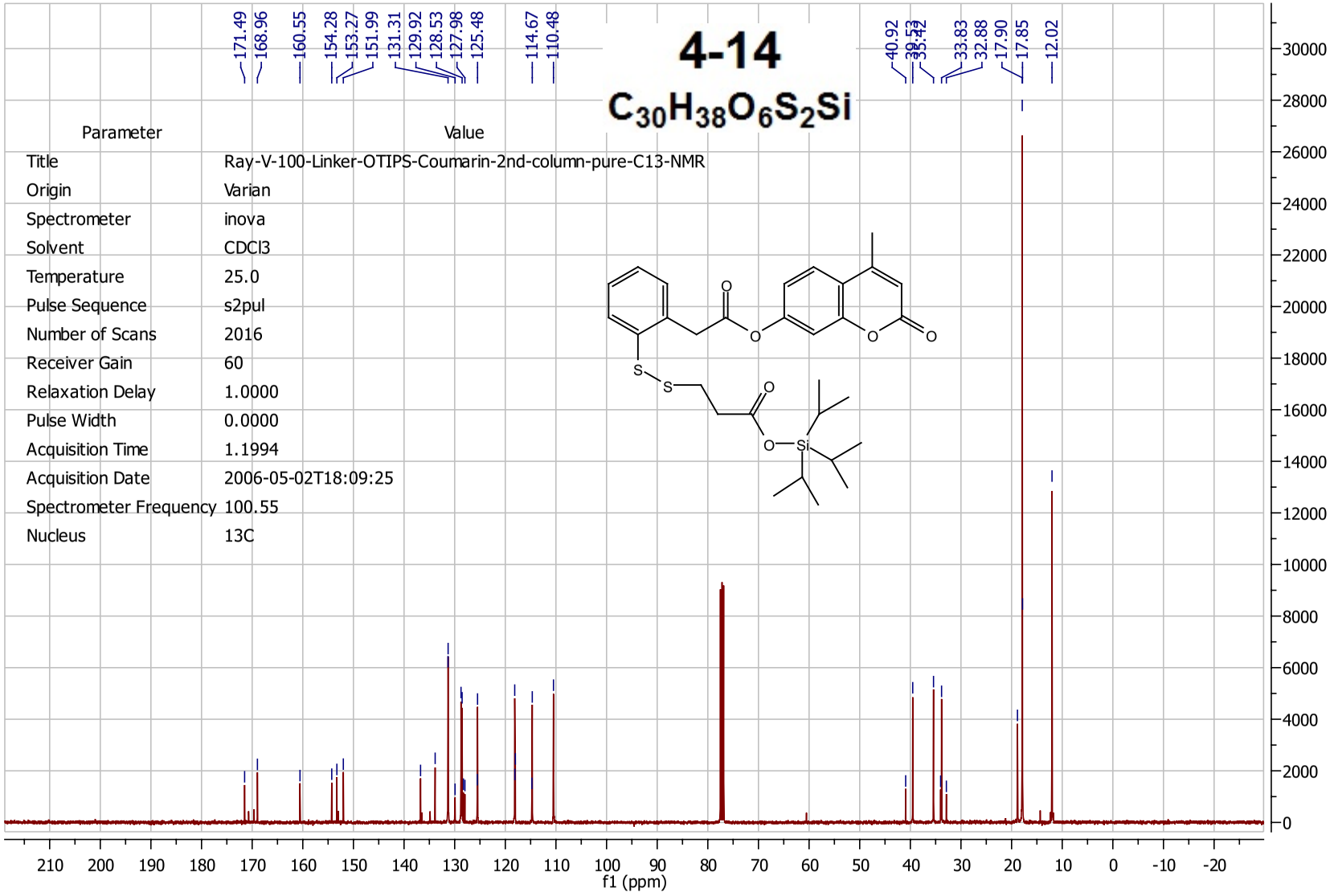

171.02
168.88
162.75
159.04
149.63
137.92
121.26
119.29
61.08
59.24
55.43
33.77
32.94
32.61
28.11
28.04
25.06
Parameter Value
Title Ray-V-21-Biotin-NHNH-PDP-C13-NMR
Origin Varian
Spectrometer inova
Solvent DMSO
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 19496
Receiver Gain 60
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.1994
Acquisition Date 2006-03-19T22:46:32
Spectrometer Frequency 100.55
Nucleus 13C
4-6
C18H25N5O3S3
f1 (ppm)

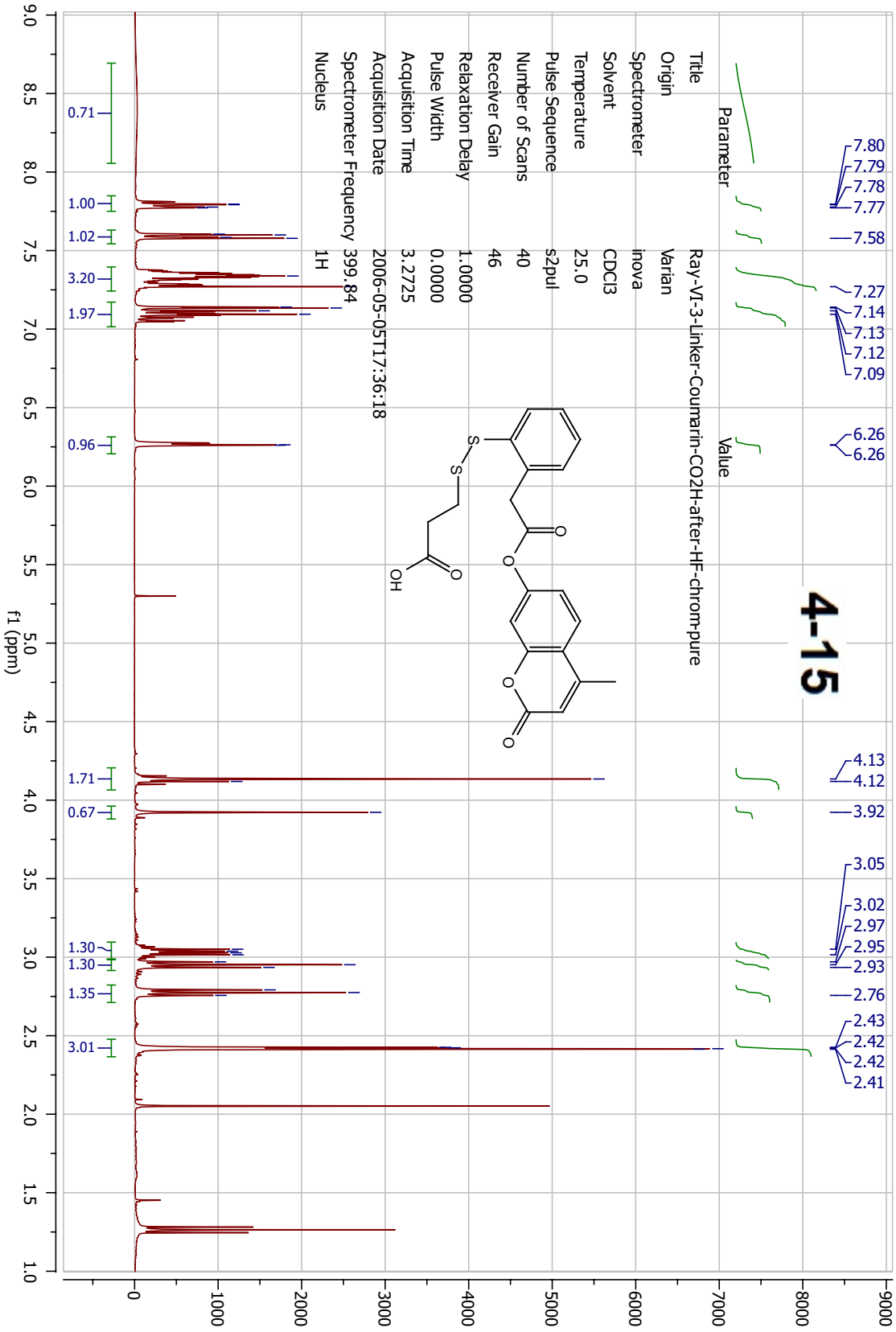

4-7
7.89
7.87
7.80
7.77
7.27
7.26
4.50
4.48
4.47
4.46
4.30
4.29
4.28
3.84
3.10
2.98
2.95
2.93
2.89
2.68
2.65
2.28
2.26
2.23
1.72
1.69
1.48
Parameter Value
Title Ray-V-24-Biotin-Linker-CO2H-crude
Origin Varian
Spectrometer g2000
Solvent CD3OD
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 84
Receiver Gain 21
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.9980
Acquisition Date 2005-11-19T23:35:24
Spectrometer Frequency 300.07
Nucleus 1H
1.38
2.96
1.00
1.00
1.31
0.28
1.05
0.73
2.41
3.72
1.97
6.20
f1 (ppm)

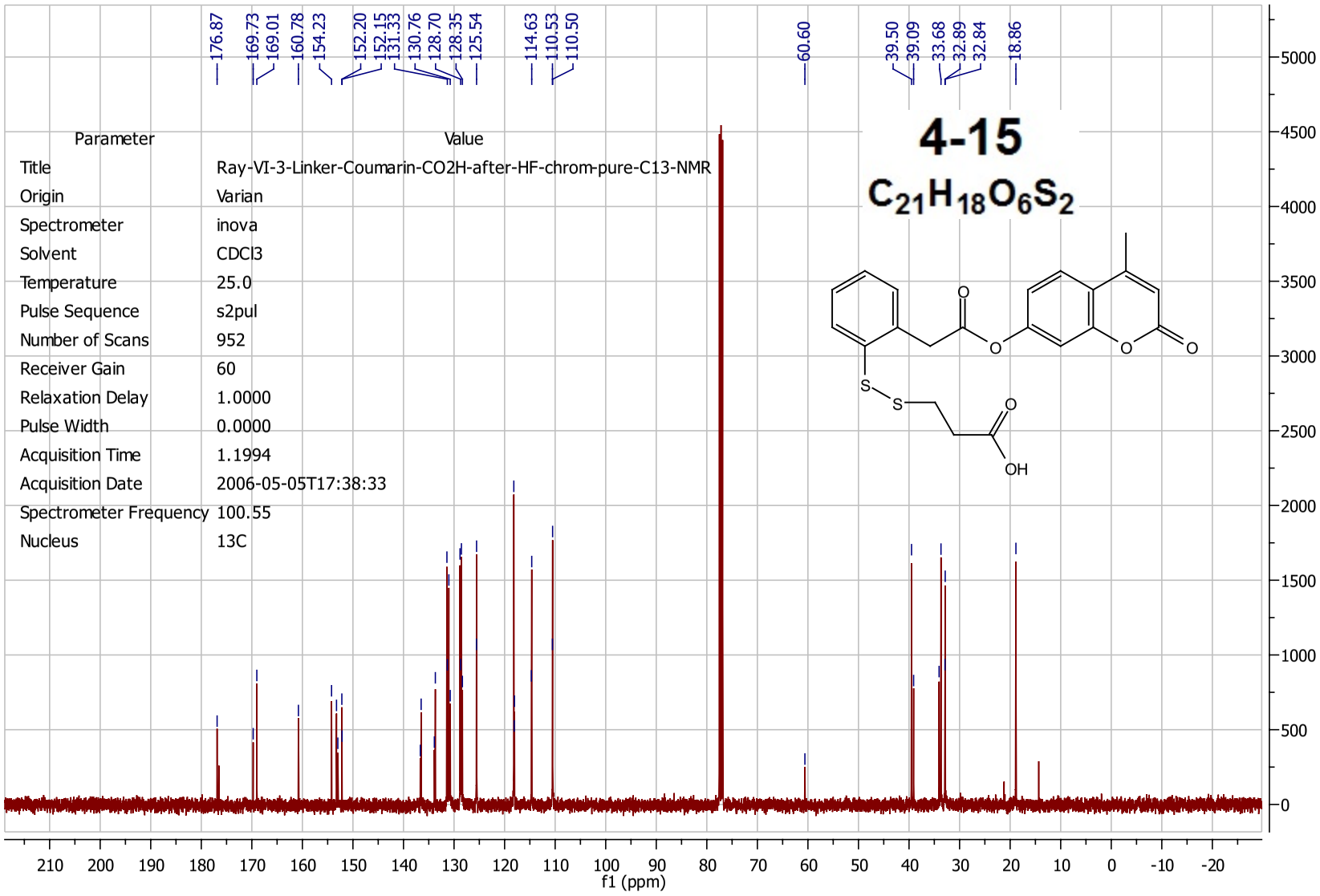

4-11-4
7.46
7.43
7.26
6.84
6.83
6.82
6.81
6.81
6.12
6.12
5.29
2.38
2.38
1.31
1.29
1.26
1.24
1.11
1.09
Parameter
Value
Title
Ray-V-100-Linker-TIPS-Coumarin-chrom-by-product-pure
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
64
Receiver Gain
20
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-05-02T23:09:55
Spectrometer Frequency
300.07
Nucleus
1H
Si
O
O
O
1.00
2.02
1.00
3.05
3.60
18.20
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
f1 (ppm)
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000

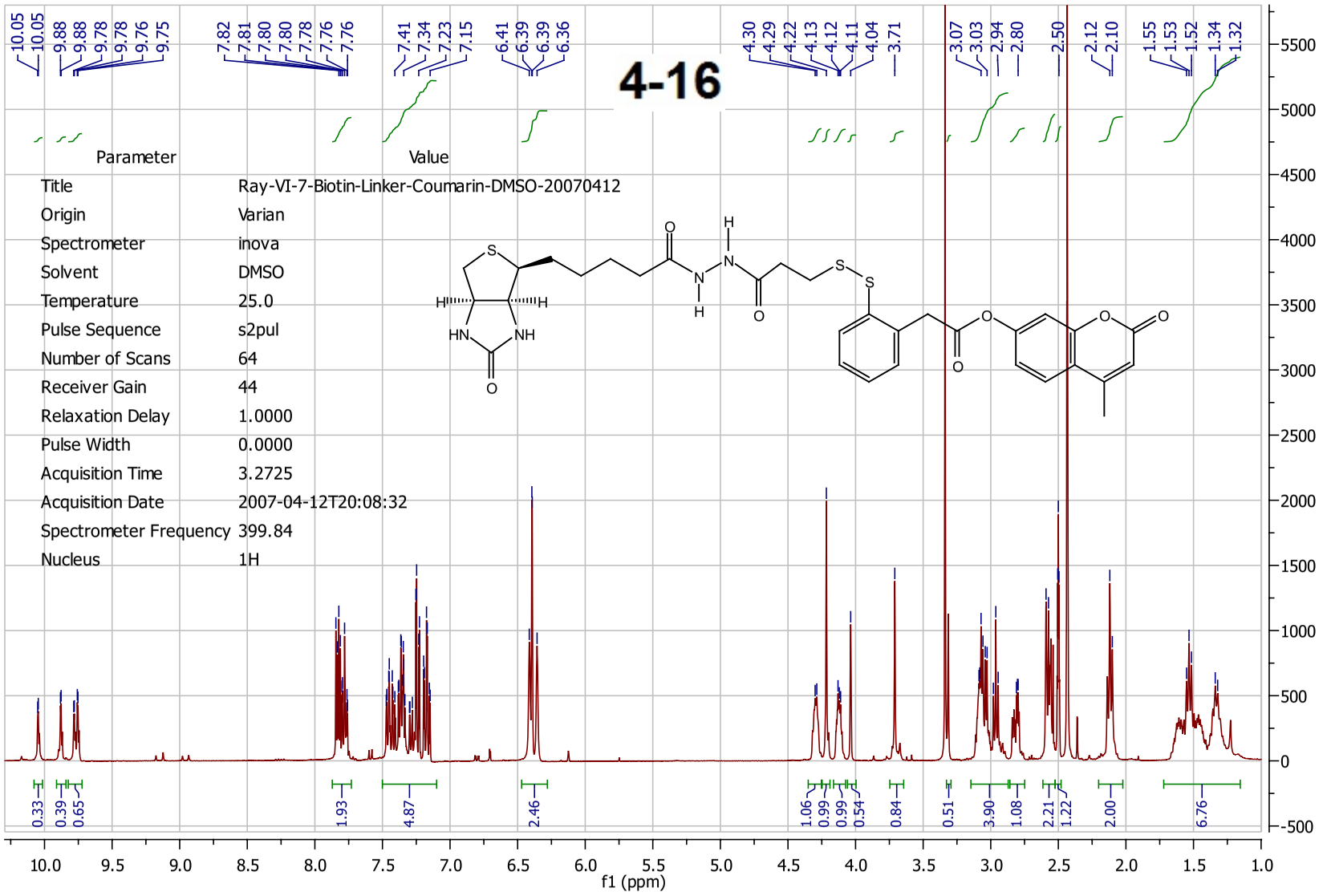

4-13
Parameter
Value
Title
Ray-VI-6-Linker-OSu-Coumarin-chrom-2nd-spot
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
108
Receiver Gain
28
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-05-10T12:22:51
Spectrometer Frequency
300.07
Nucleus
1H
7.80
7.58
7.33
7.16
7.11
7.10
7.07
6.27
6.26
6.25
6.24
4.19
4.13
4.13
4.10
3.05
3.03
3.03
3.02
3.00
2.81
2.43
2.43
2.42
2.42
1.00
1.03
3.07
2.20
1.01
1.06
1.69
4.15
4.20
3.04
f1 (ppm)

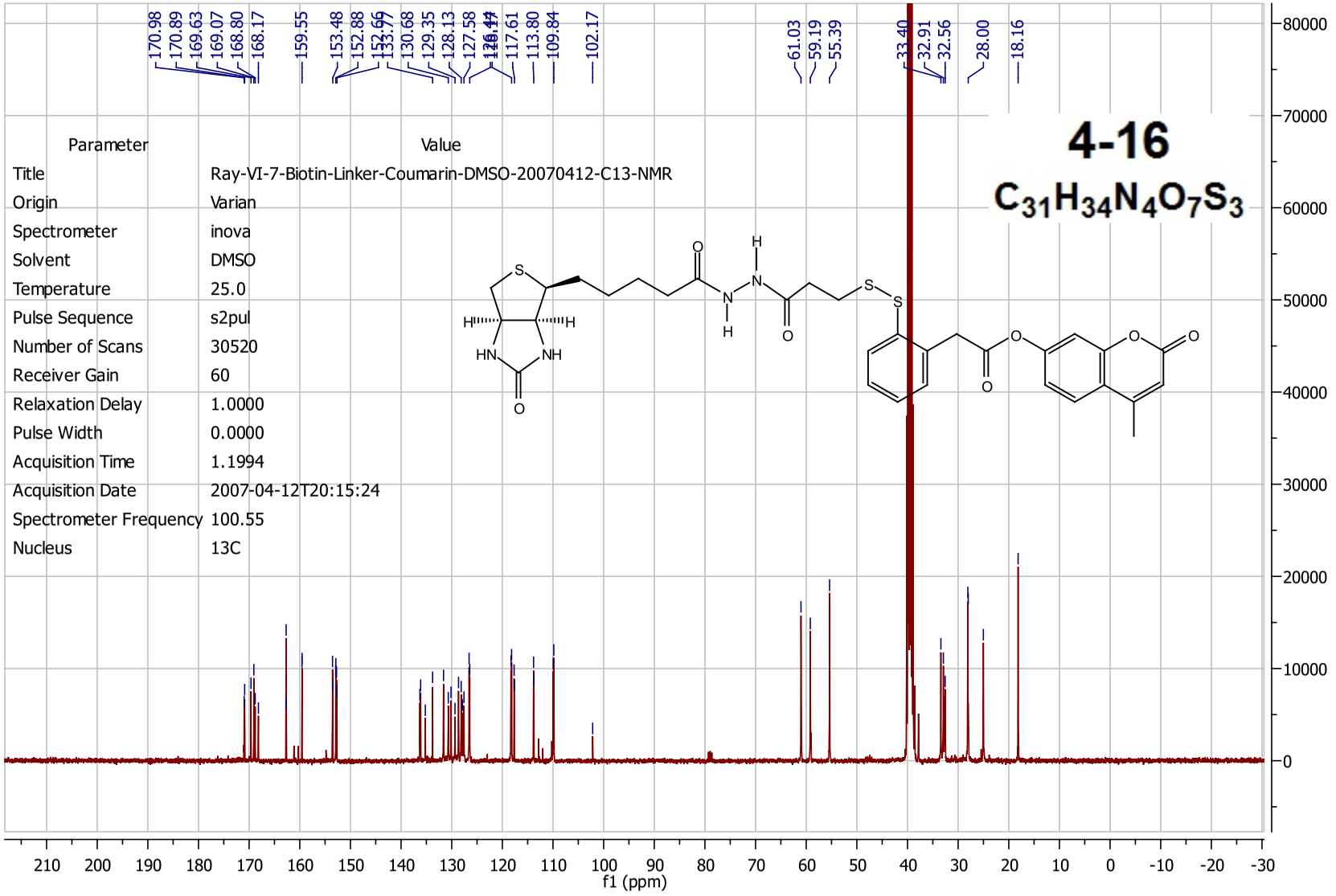

4-14
7.79
7.77
7.77
7.57
7.26
7.13
7.12
7.11
7.09
6.25
6.25
4.14
3.90
2.98
2.96
2.95
2.75
2.42
2.42
2.41
2.41
1.28
1.26
1.25
1.07
1.05
Parameter Value
Title Ray-V-100-Linker-OTIPS-Coumarin-2nd-column-pure
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 40
Receiver Gain 38
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2006-05-02T18:06:42
Spectrometer Frequency 399.84
Nucleus 1H
1.00
1.05
3.30
2.05
1.00
1.92
0.41
2.46
1.65
3.17
3.89
18.57
f1 (ppm)

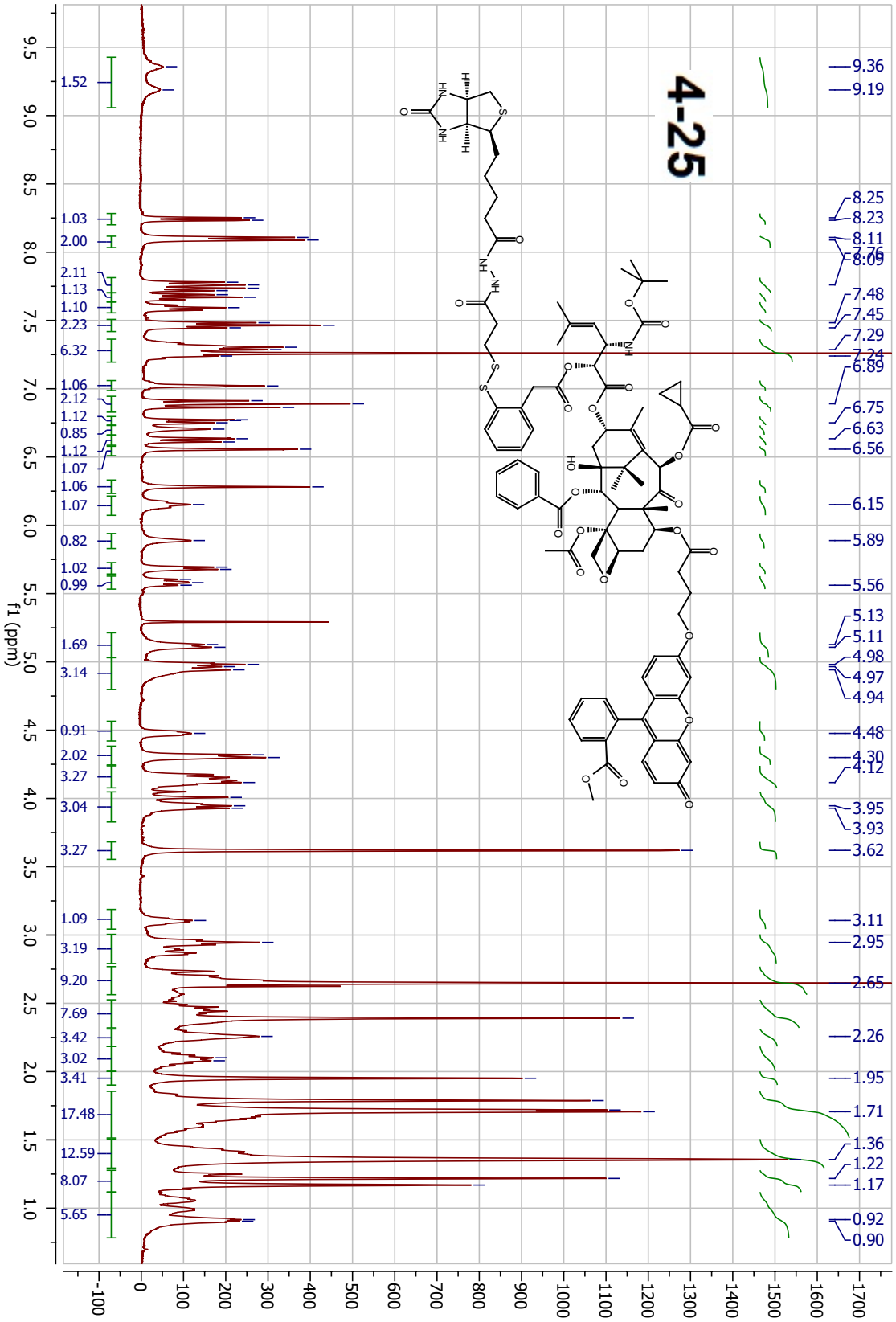

4-14
$C_{30}H_{38}O_6S_2Si$
171.49
168.96
160.55
154.28
153.27
151.99
131.31
129.92
128.53
127.98
125.48
114.67
110.48
40.92
39.53
33.83
32.88
17.90
17.85
12.02
Parameter
Value
Title
Ray-V-100-Linker-OTIPS-Coumarin-2nd-column-pure-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
2016
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2006-05-02T18:09:25
Spectrometer Frequency
100.55
Nucleus
13C
f1 (ppm)

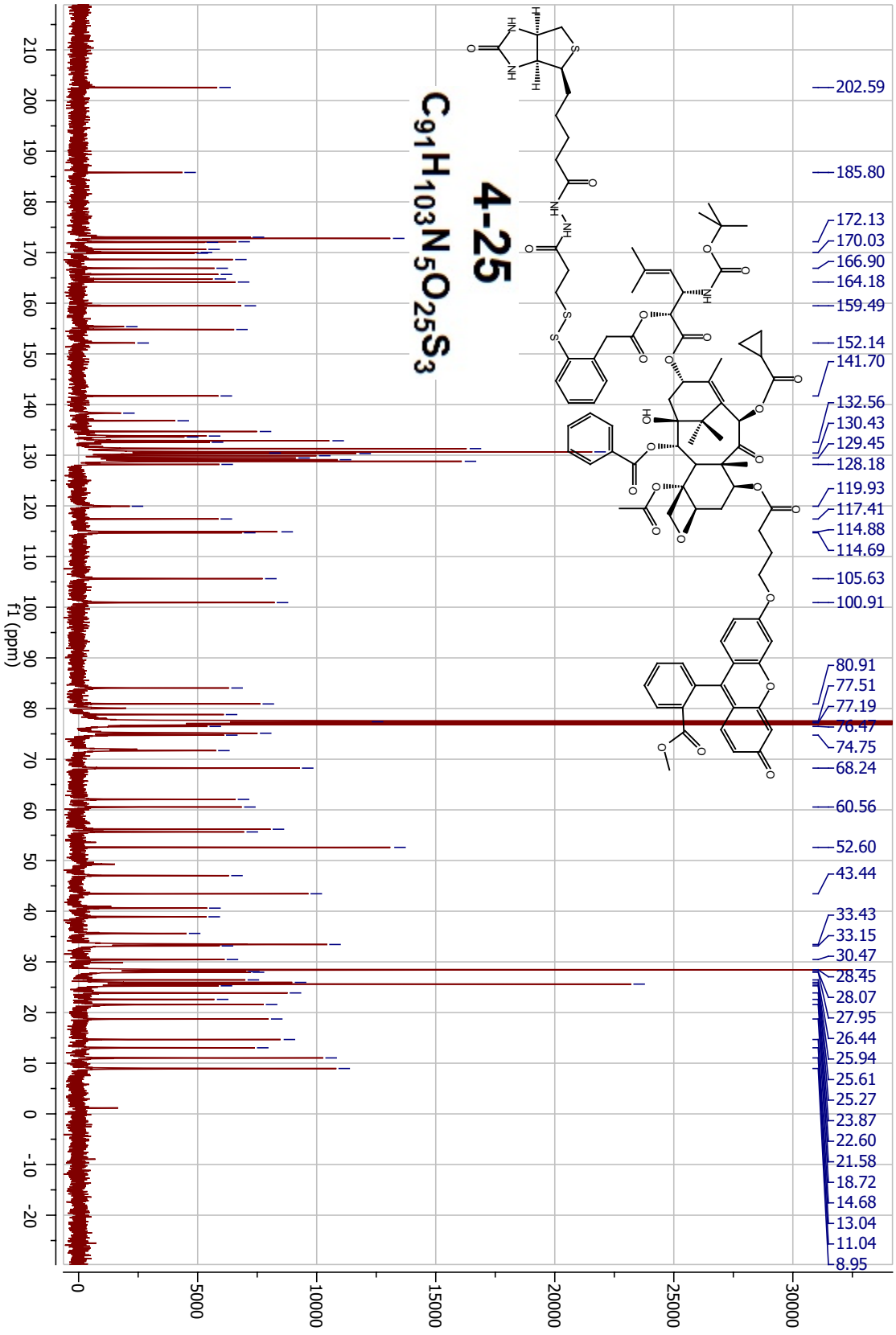

4-15
Parameter
Value
Title
Ray-VI-3-Linker-Coumarin-CO2H-after-HF-chrom-pure
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
40
Receiver Gain
46
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2006-05-05T17:36:18
Spectrometer Frequency
399.84
Nucleus
1H
7.80
7.79
7.78
7.77
7.58
7.27
7.14
7.13
7.12
7.09
6.26
6.26
4.13
4.12
3.92
3.05
3.02
2.97
2.95
2.93
2.76
2.43
2.42
2.42
2.41
0.71
1.00
1.02
3.20
1.97
0.96
1.71
0.67
1.30
1.30
1.35
3.01
f1 (ppm)

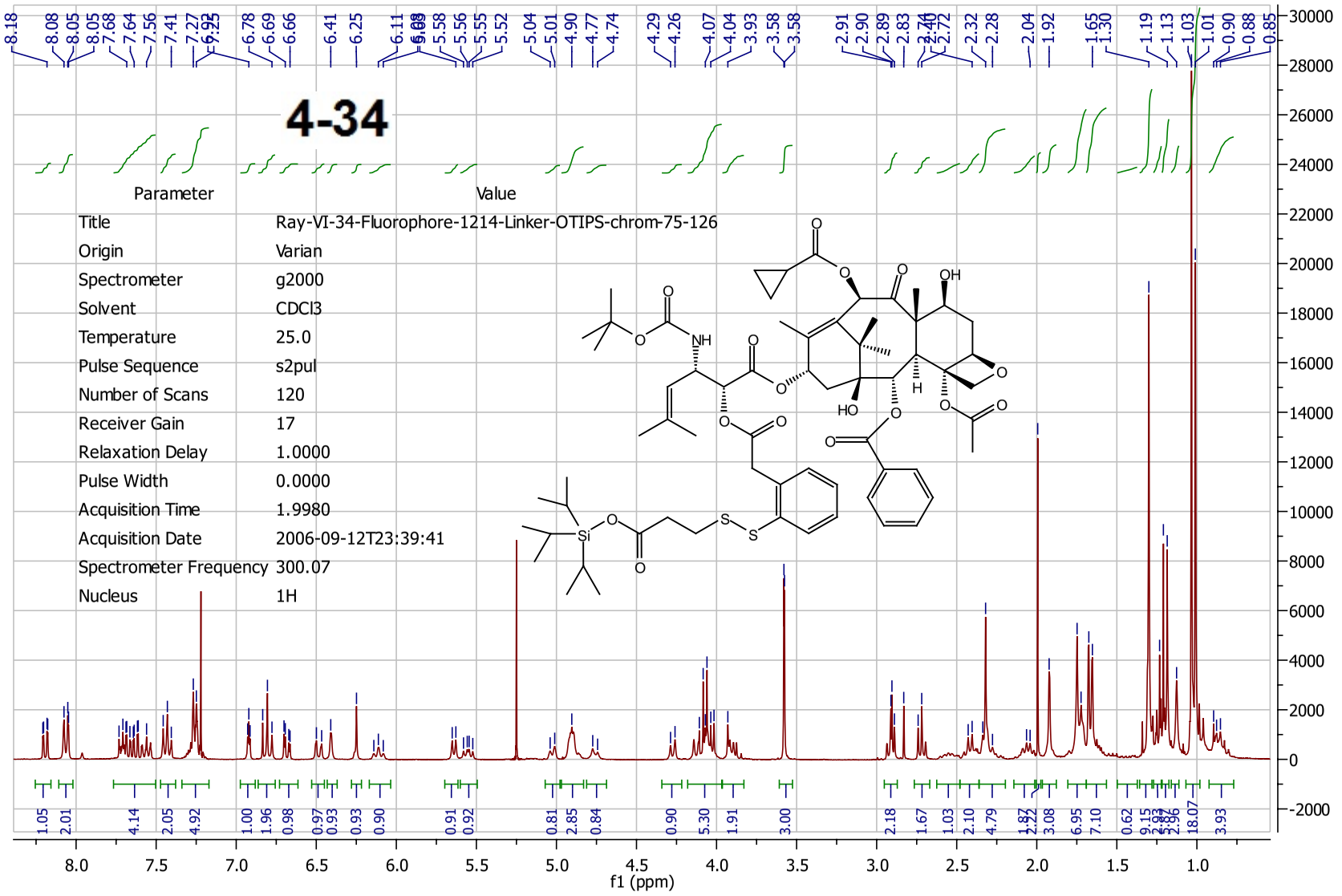

176.87
169.73
169.01
160.78
154.23
152.20
152.15
131.33
130.76
128.70
128.35
125.54
114.63
110.53
110.50
60.60
39.50
39.09
33.68
32.89
32.84
18.86
Parameter Value
Title Ray-VI-3-Linker-Coumarin-CO2H-after-HF-chrom-pure-C13-NMR
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 952
Receiver Gain 60
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.1994
Acquisition Date 2006-05-05T17:38:33
Spectrometer Frequency 100.55
Nucleus 13C
4-15
C21H18O6S2
O
O
O
O
S
S
O
OH
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

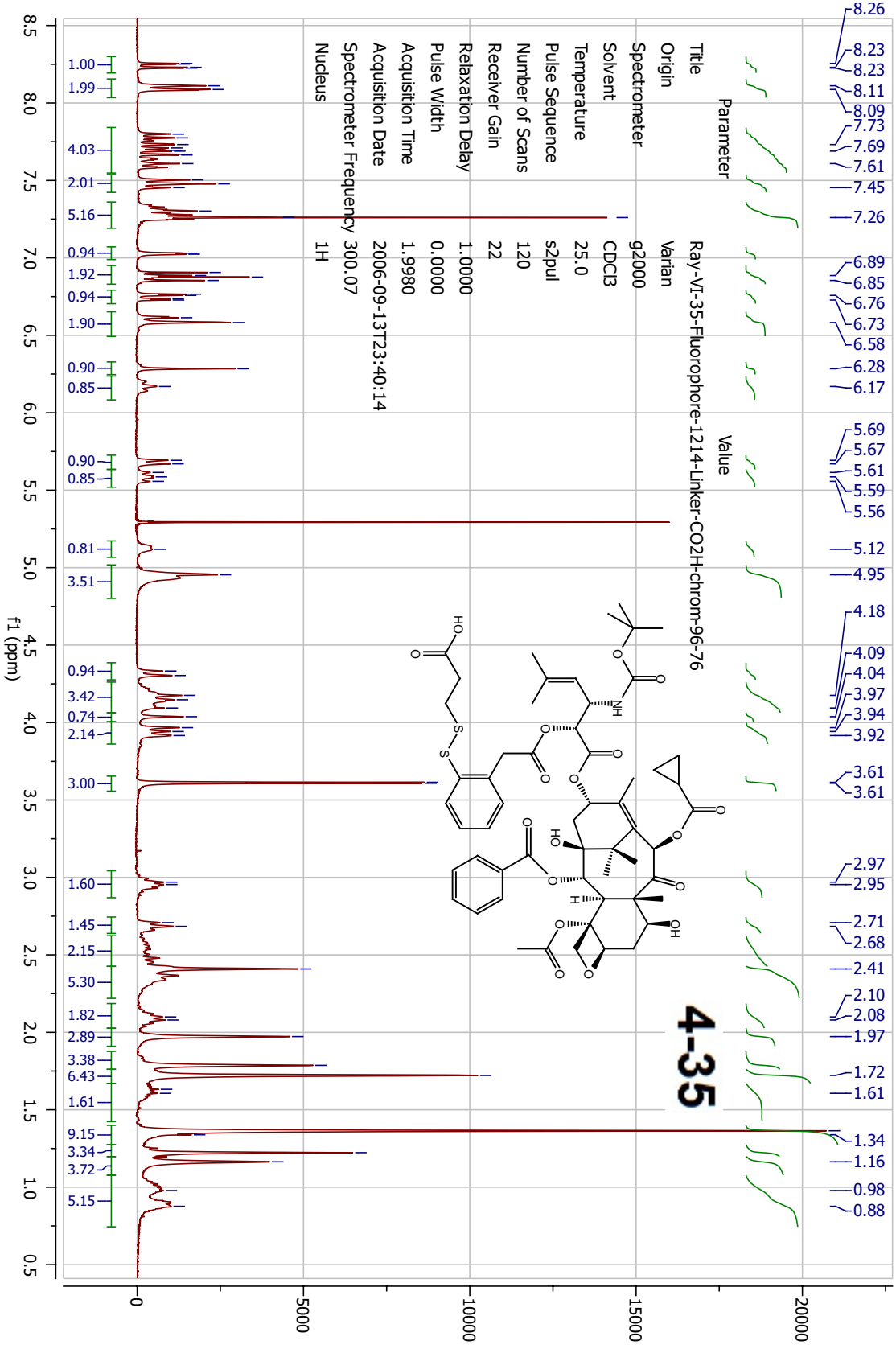

4-16
Parameter Value
Title Ray-VI-7-Biotin-Linker-Coumarin-DMSO-20070412
Origin Varian
Spectrometer inova
Solvent DMSO
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 64
Receiver Gain 44
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2007-04-12T20:08:32
Spectrometer Frequency 399.84
Nucleus 1H
f1 (ppm)

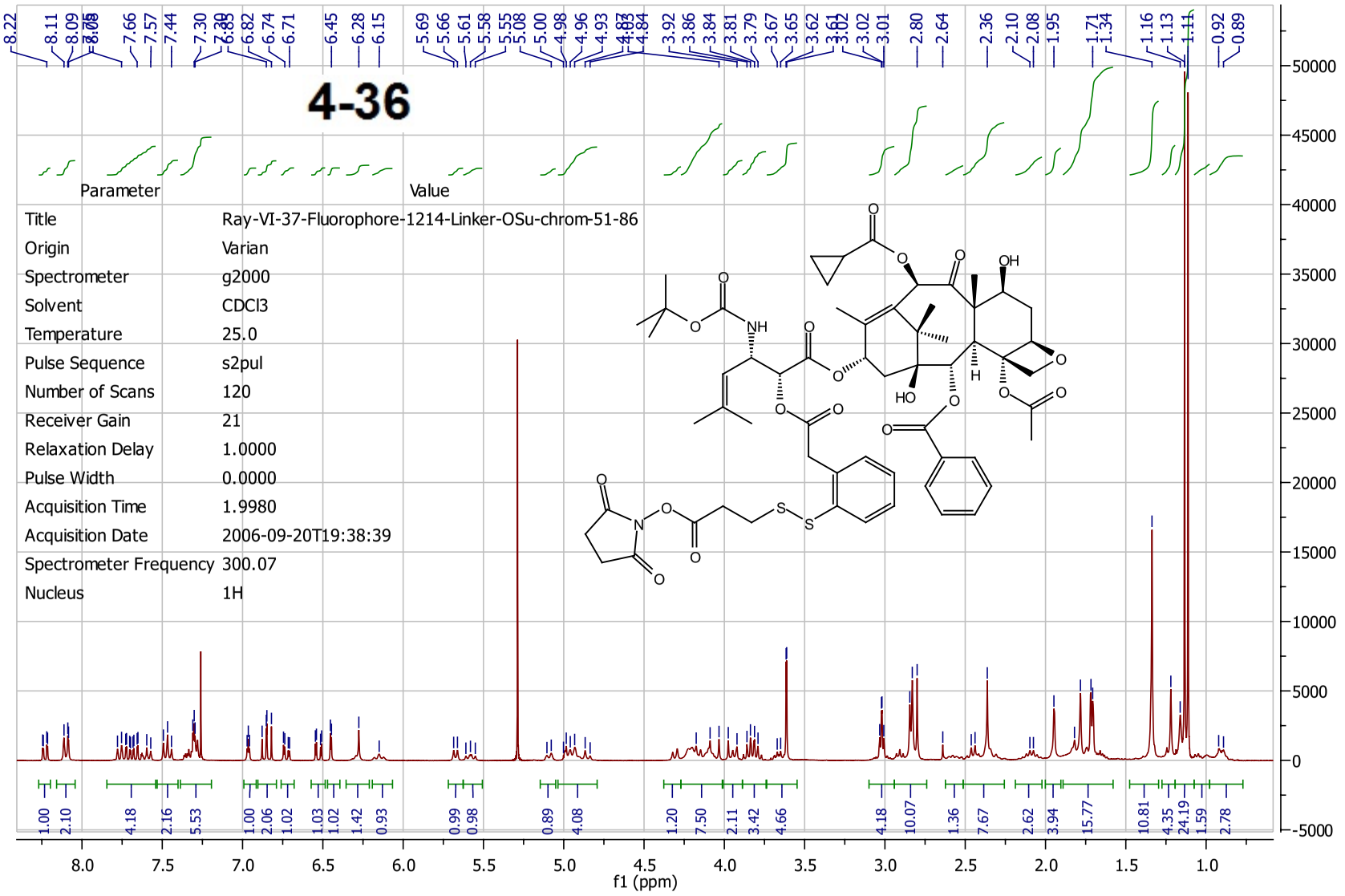

4-16
$C_{31}H_{34}N_4O_7S_3$
Parameter Value
Title Ray-VI-7-Biotin-Linker-Coumarin-DMSO-20070412-C13-NMR
Origin Varian
Spectrometer inova
Solvent DMSO
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 30520
Receiver Gain 60
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.1994
Acquisition Date 2007-04-12T20:15:24
Spectrometer Frequency 100.55
Nucleus 13C
170.98 170.89 169.63 169.07 168.80 168.17 159.55 153.48 152.88 152.66 133.77 130.68 129.35 128.13 127.58 117.61 113.80 109.84 102.17 61.03 59.19 55.39 33.40 32.91 32.56 28.00 18.16
f1 (ppm)

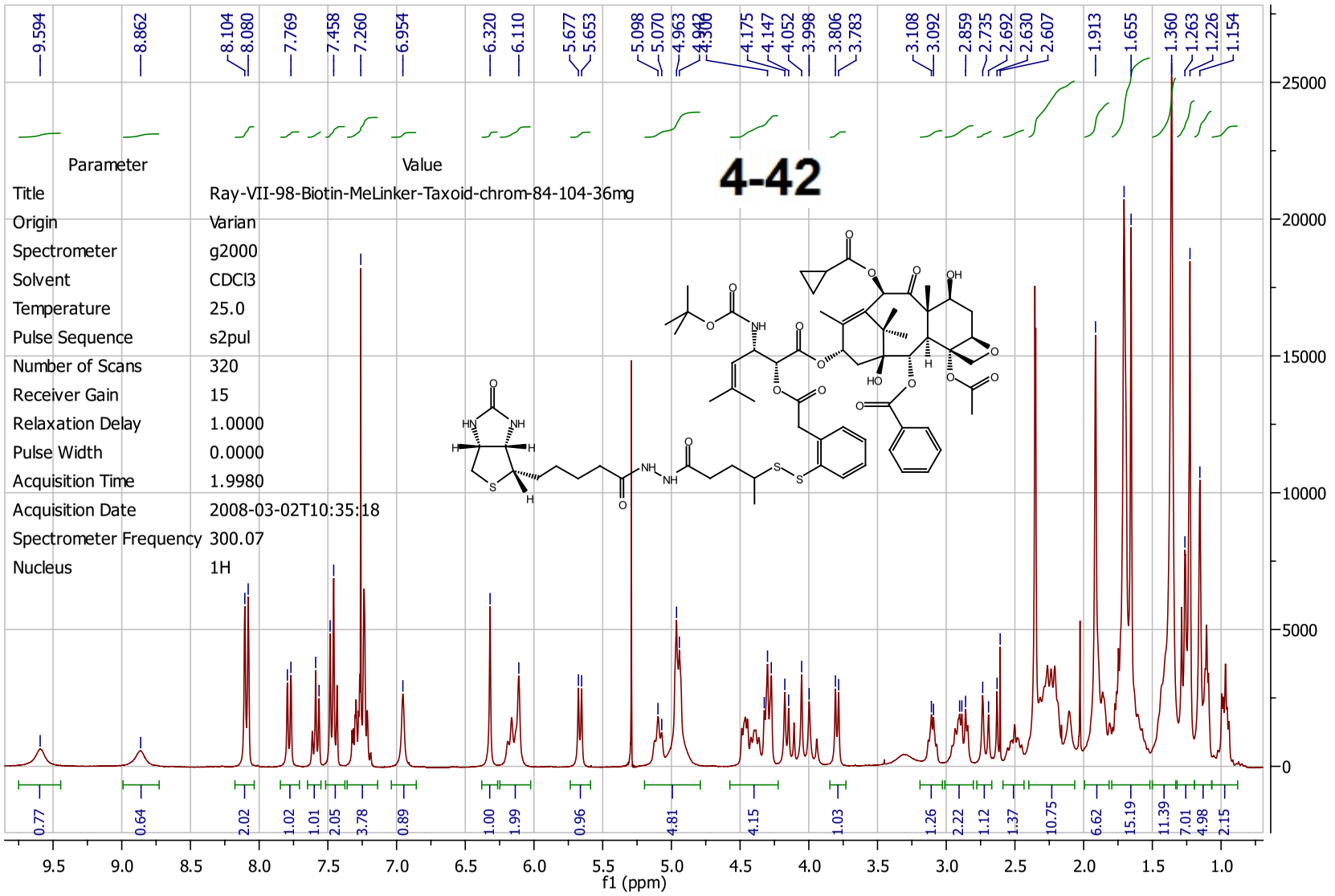

4-25
f1 (ppm)

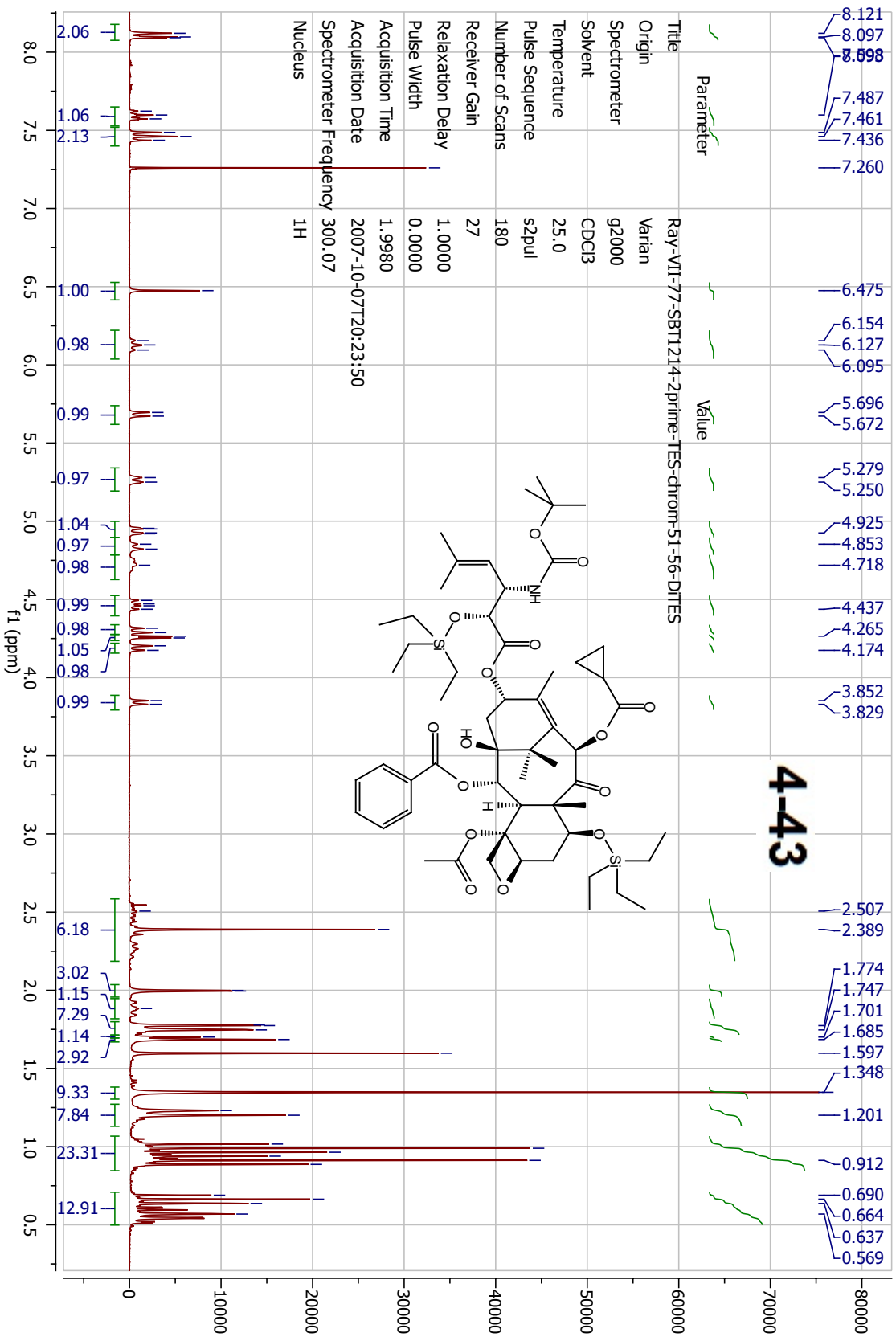

4-25
$C_{91}H_{103}N_5O_{25}S_3$
f1 (ppm)
202.59
185.80
172.13
170.03
166.90
164.18
159.49
152.14
141.70
132.56
130.43
129.45
128.18
119.93
117.41
114.88
114.69
105.63
100.91
80.91
77.51
77.19
76.47
74.75
68.24
60.56
52.60
43.44
33.43
33.15
30.47
28.45
28.07
27.95
26.44
25.94
25.61
25.27
23.87
22.60
21.58
18.72
14.68
13.04
11.04
8.95

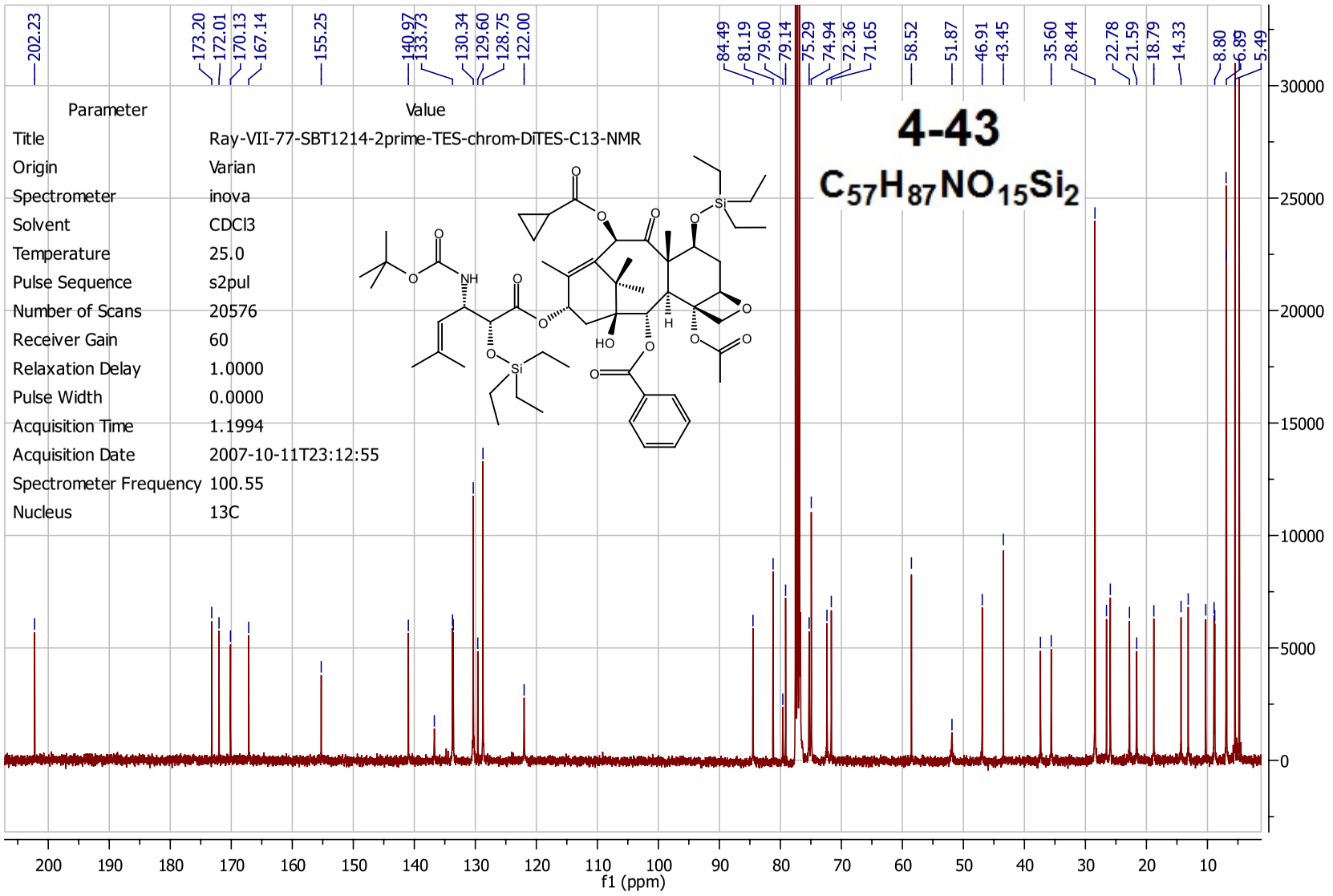

4-34
Parameter
Value
Title
Ray-VI-34-Fluorophore-1214-Linker-OTIPS-chrom-75-126
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
17
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-09-12T23:39:41
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

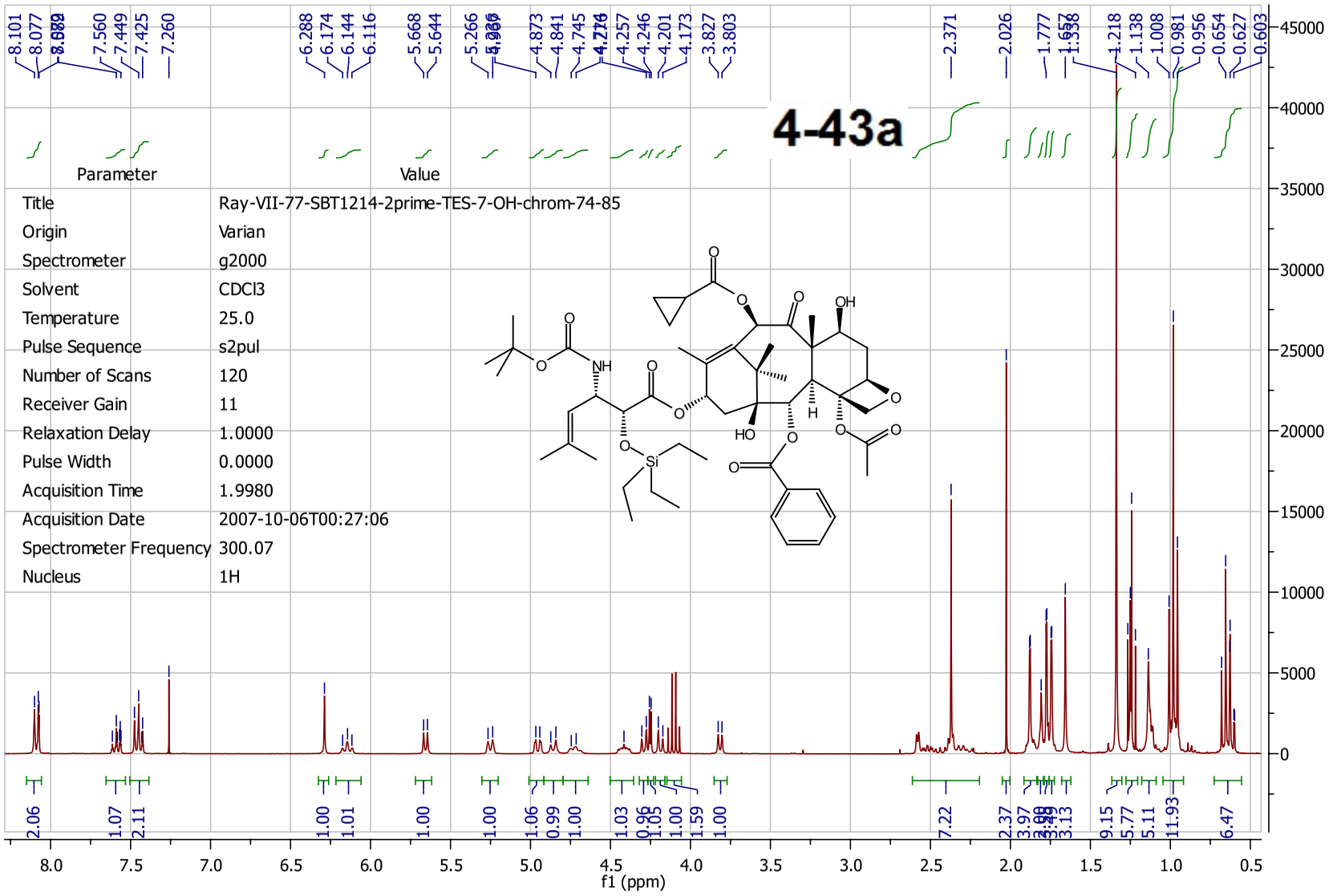

Parameter
Value
Title
Ray-VI-35-Fluorophore-1214-Linker-CO2H-chrom-96-76
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
22
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-09-13T23:40:14
Spectrometer Frequency
300.07
Nucleus
1H
4-35
f1 (ppm)

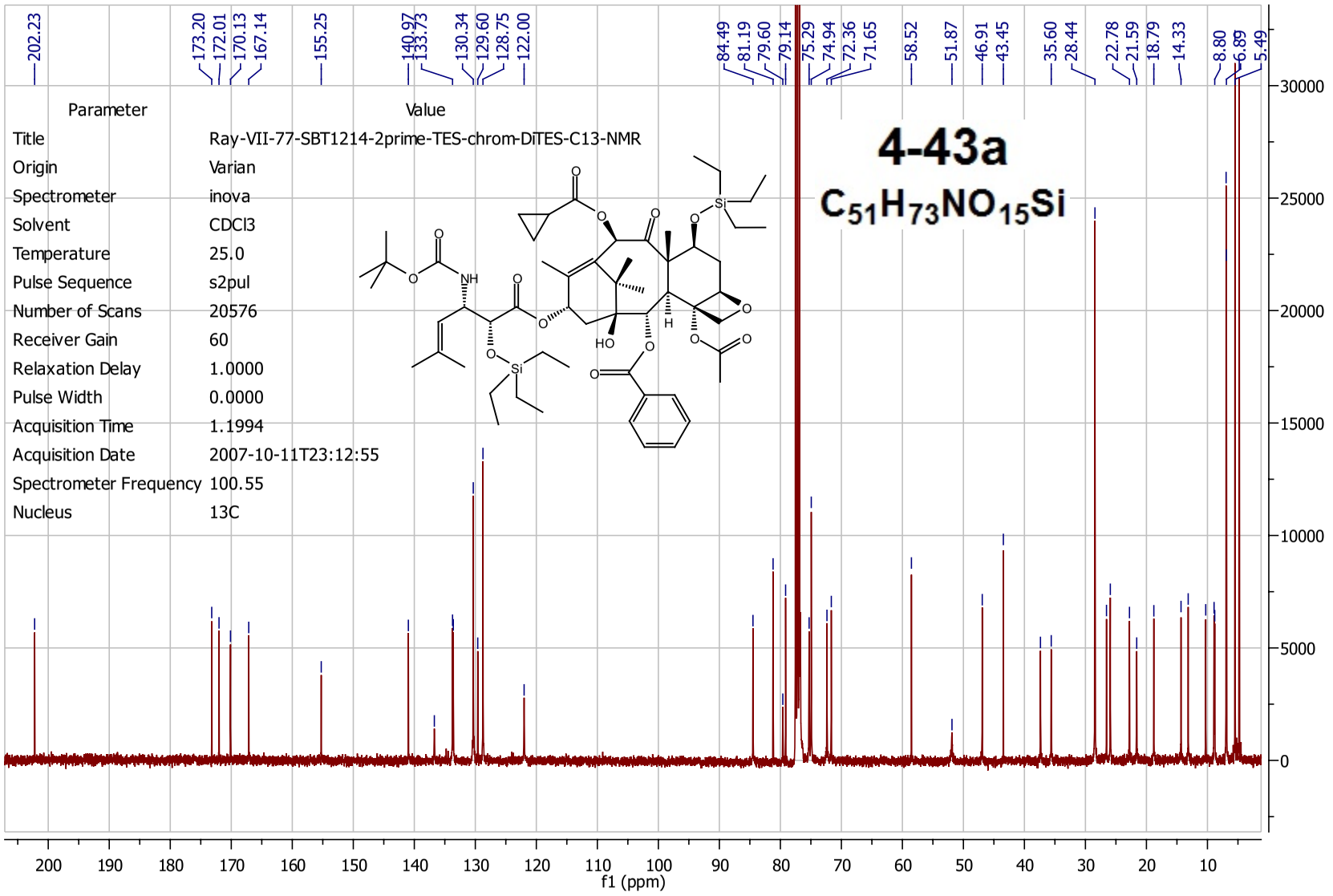

4-36
Parameter
Value
Title
Ray-VI-37-Fluorophore-1214-Linker-OSu-chrom-51-86
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
21
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2006-09-20T19:38:39
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

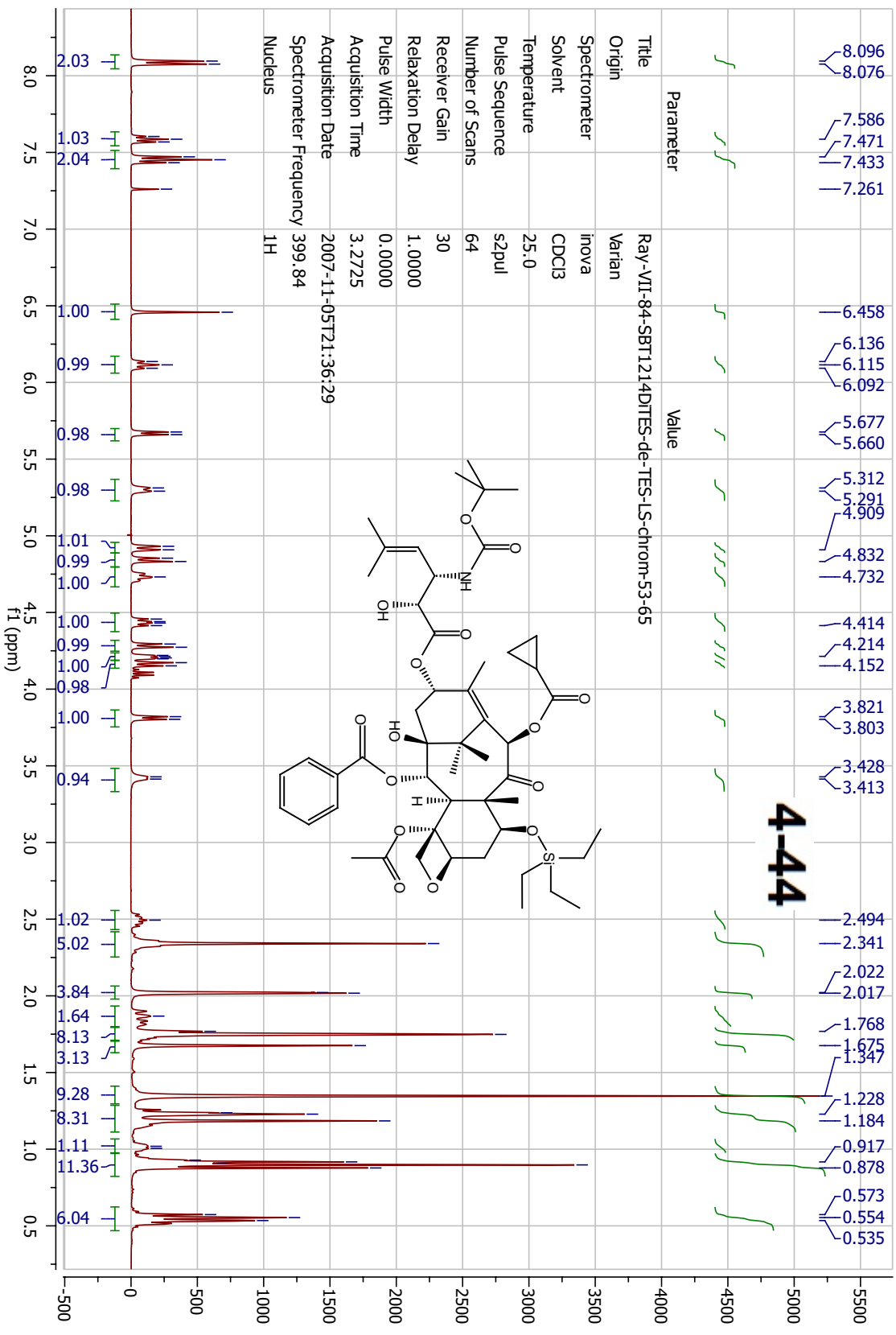

4-42
Parameter
Value
Title
Ray-VII-98-Biotin-MeLinker-Taxoid-chrom-84-104-36mg
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
320
Receiver Gain
15
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2008-03-02T10:35:18
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

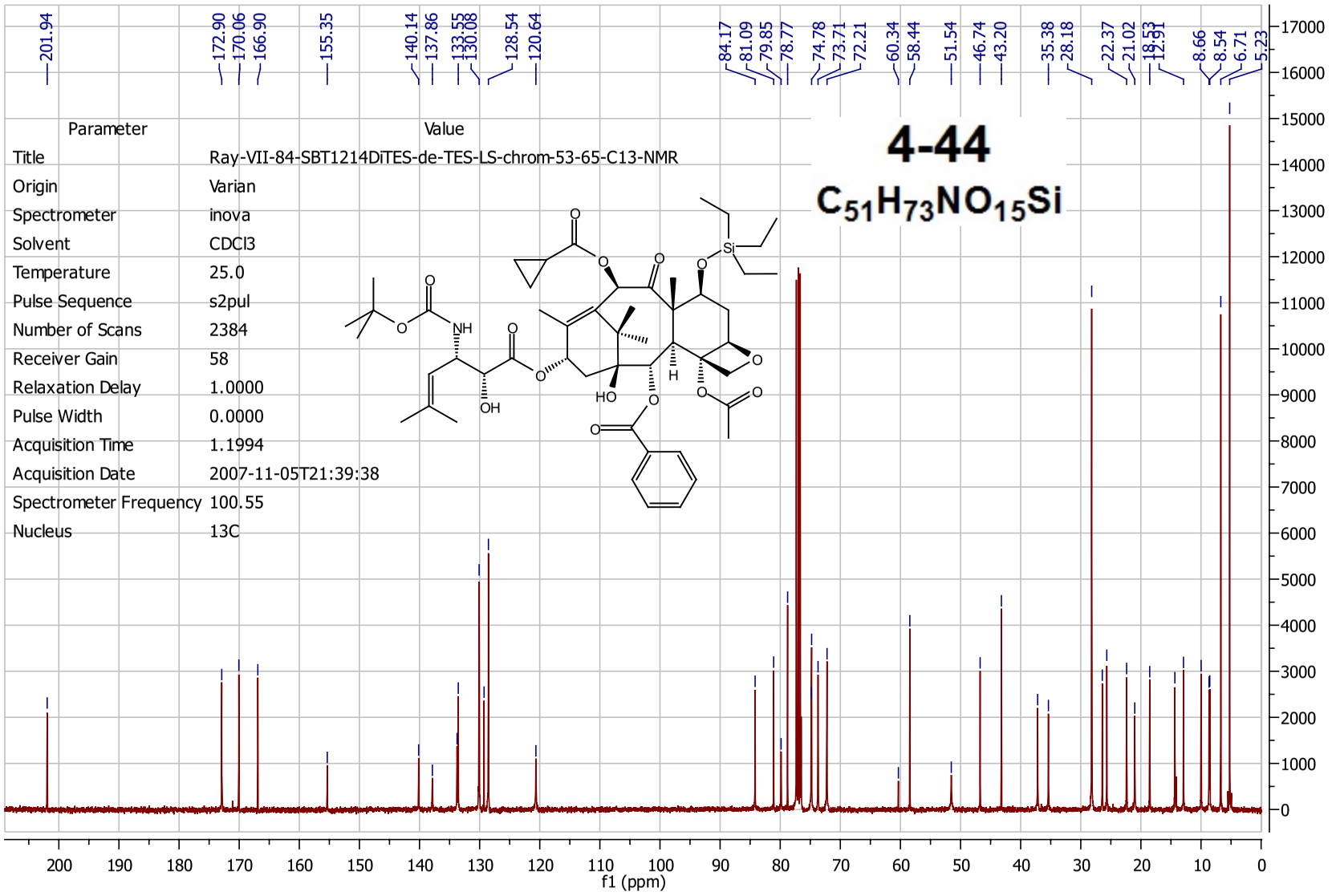

4-43
Parameter
Value
Title
Ray-VII-77-SBT1214-2prime-TES-chrom-51-56-DITES
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
180
Receiver Gain
27
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2007-10-07T20:23:50
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

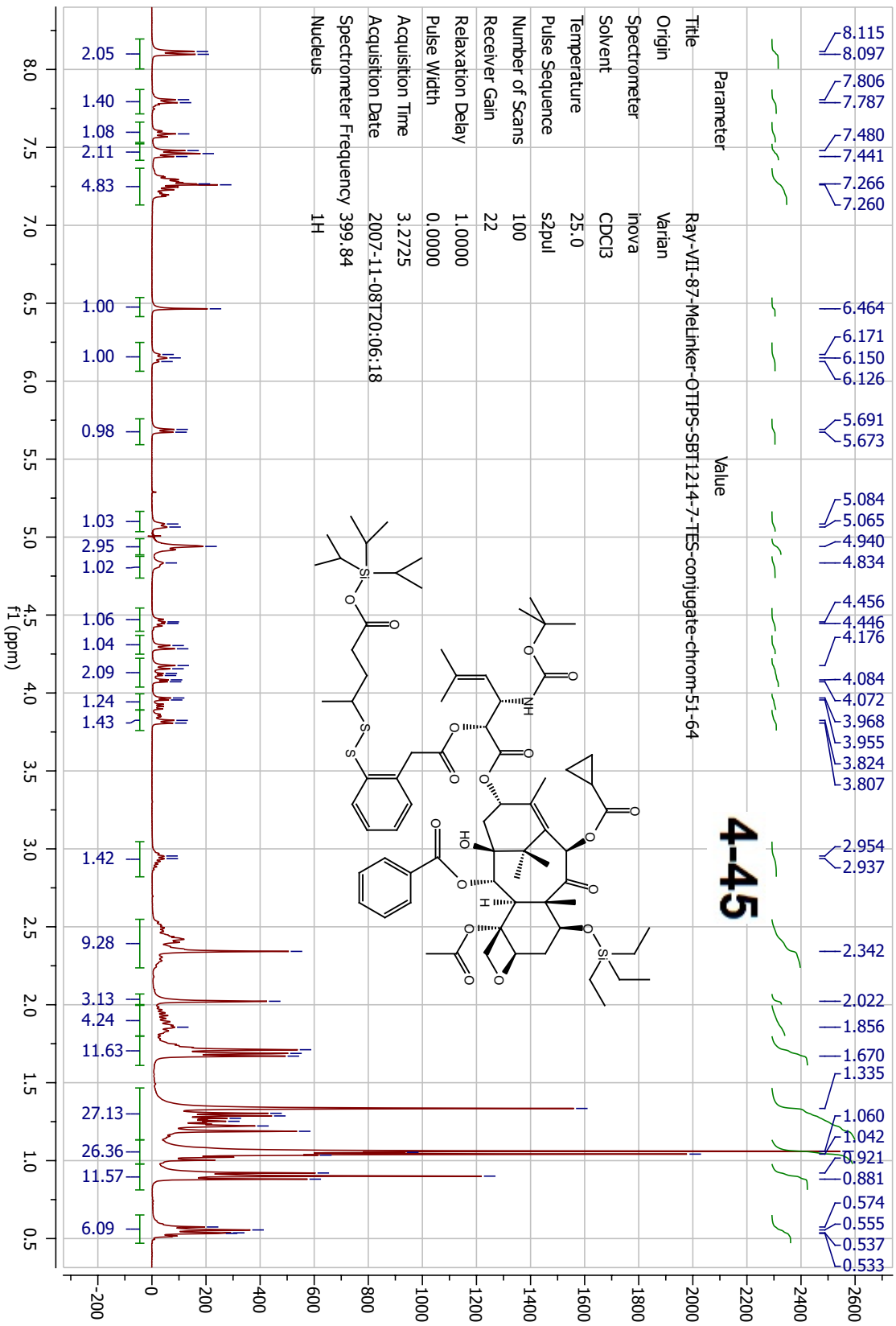

202.23
173.20
172.01
170.13
167.14
155.25
140.97
133.73
130.34
129.60
128.75
122.00
84.49
81.19
79.60
79.14
75.29
74.94
72.36
71.65
58.52
51.87
46.91
43.45
35.60
28.44
22.78
21.59
18.79
14.33
8.80
6.89
5.49
Parameter
Value
Title
Ray-VII-77-SBT1214-2prime-TES-chrom-DiTES-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
20576
Receiver Gain
60
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-10-11T23:12:55
Spectrometer Frequency
100.55
Nucleus
13C
4-43
C57H87NO15Si2
30000
25000
20000
15000
10000
5000
0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

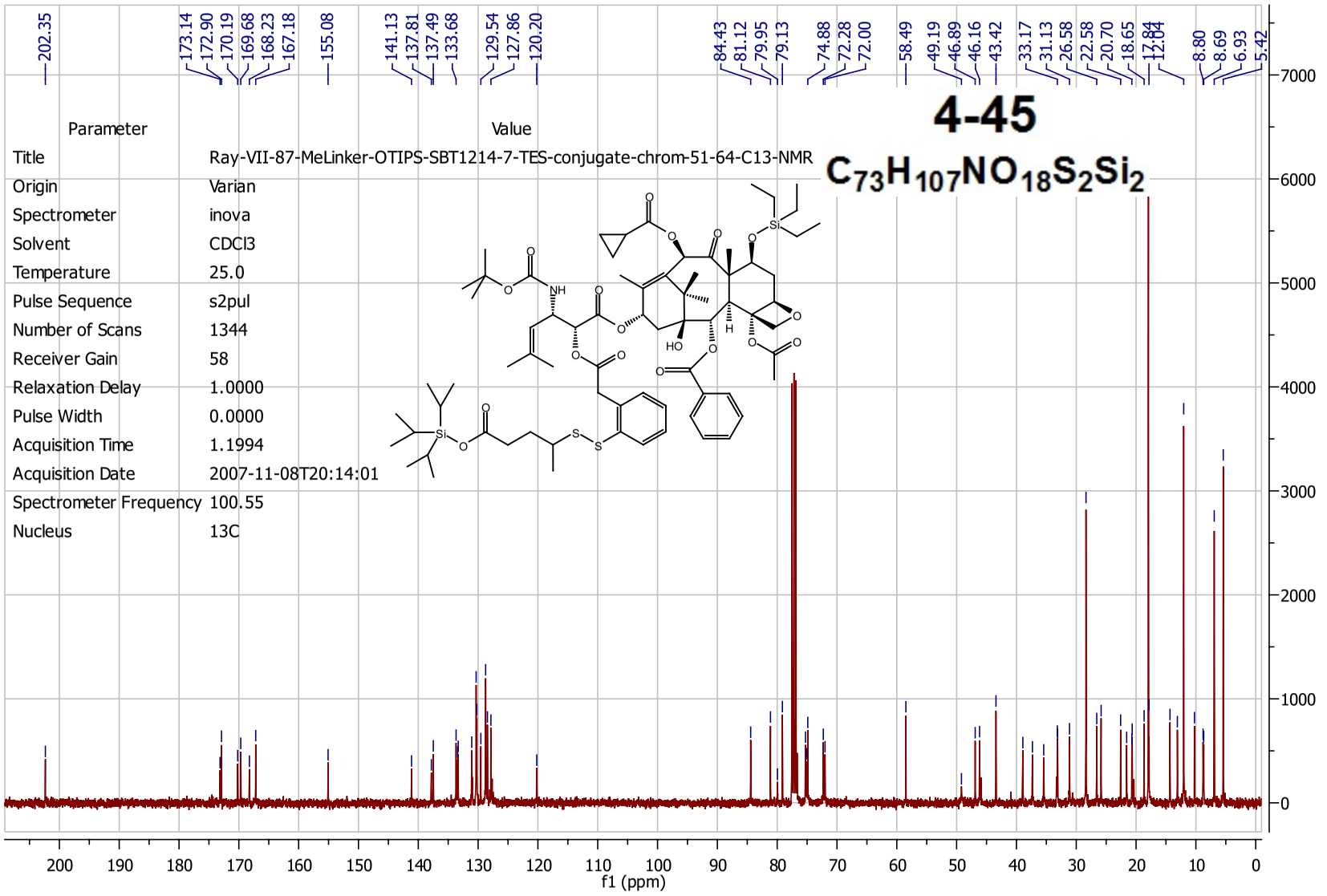

4-43a
Parameter
Value
Title
Ray-VII-77-SBT1214-2prime-TES-7-OH-chrom-74-85
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
11
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2007-10-06T00:27:06
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)

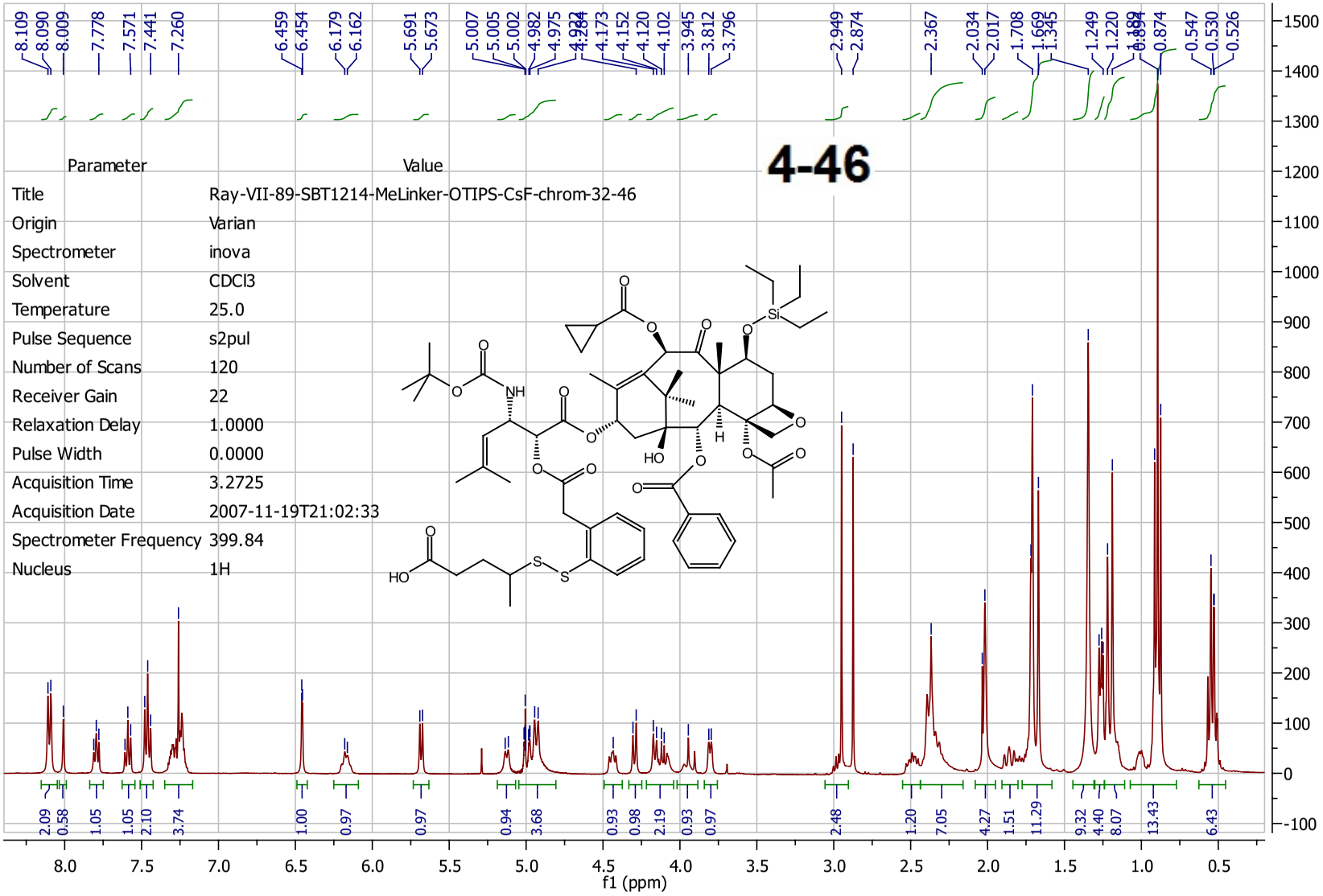

202.23
173.20
172.01
170.13
167.14
155.25
140.97
133.73
130.34
129.60
128.75
122.00
84.49
81.19
79.60
79.14
75.29
74.94
72.36
71.65
58.52
51.87
46.91
43.45
35.60
28.44
22.78
21.59
18.79
14.33
8.80
6.89
5.49
Parameter Value
Title Ray-VII-77-SBT1214-2prime-TES-chrom-DiTES-C13-NMR
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 20576
Receiver Gain 60
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 1.1994
Acquisition Date 2007-10-11T23:12:55
Spectrometer Frequency 100.55
Nucleus 13C
4-43a
C51H73NO15Si
30000
25000
20000
15000
10000
5000
0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

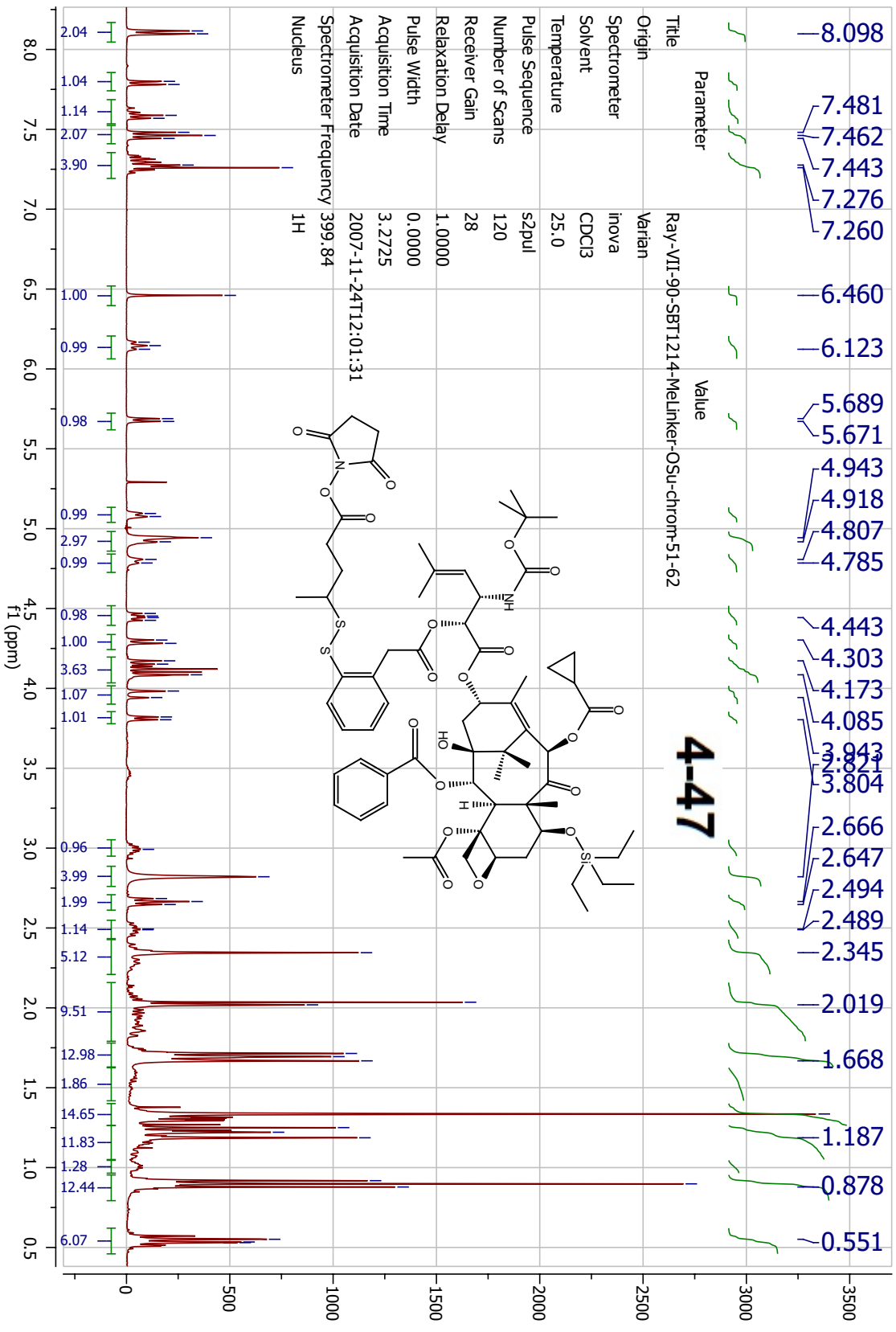

4-44
Parameter
Value
Title
Ray-VII-84-SBT1214DiTES-de-TES-LS-chrom-53-65
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
64
Receiver Gain
30
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2007-11-05T21:36:29
Spectrometer Frequency
399.84
Nucleus
1H
f1 (ppm)

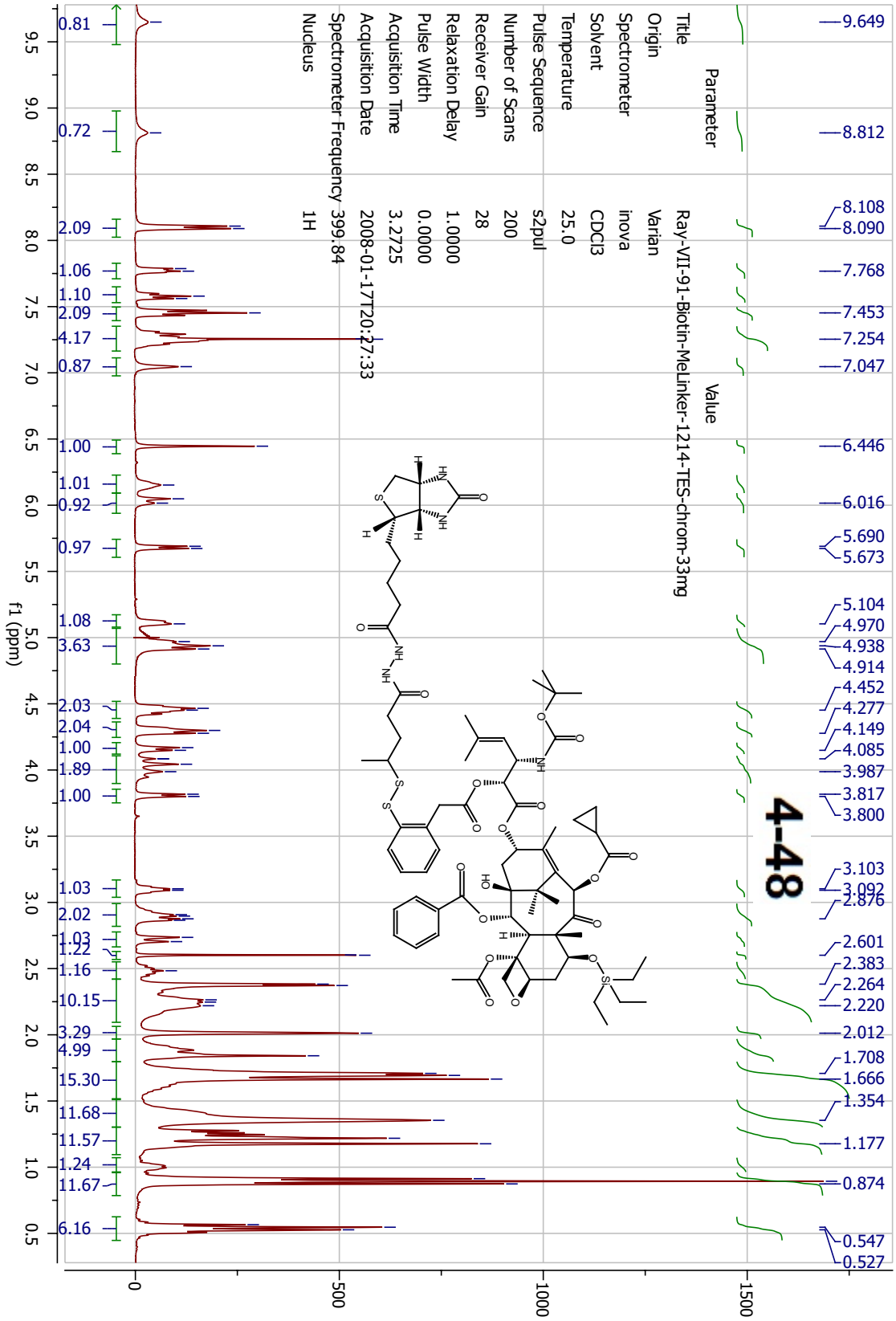

201.94
172.90
170.06
166.90
155.35
140.14
137.86
133.55
130.08
128.54
120.64
84.17
81.09
79.85
78.77
74.78
73.71
72.21
60.34
58.44
51.54
46.74
43.20
35.38
28.18
22.37
21.02
18.53
12.91
8.66
8.54
6.71
5.23
Parameter
Value
Title
Ray-VII-84-SBT1214DiTES-de-TES-LS-chrom-53-65-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
2384
Receiver Gain
58
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-11-05T21:39:38
Spectrometer Frequency
100.55
Nucleus
13C
4-44
C51H73NO15Si
f1 (ppm)

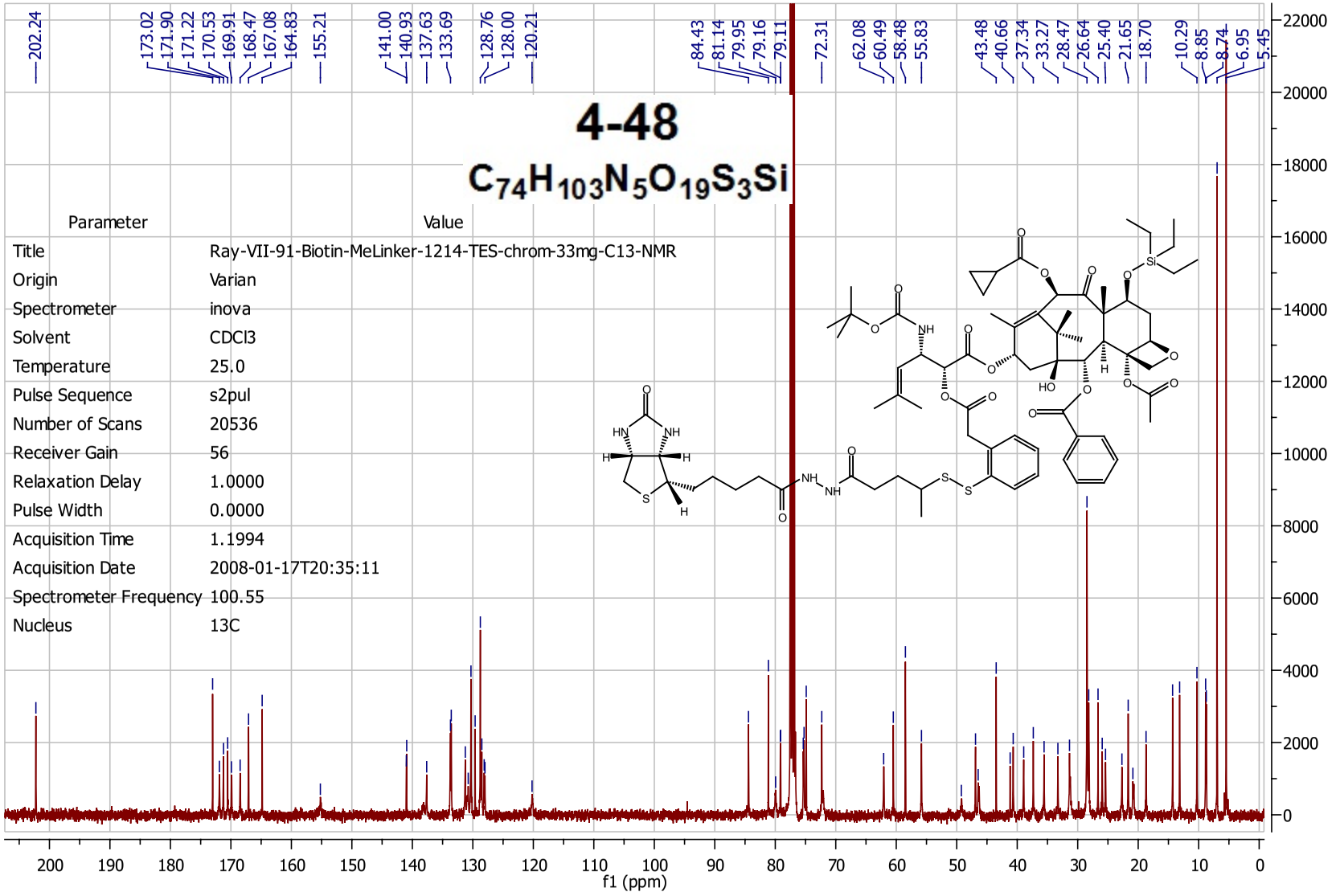

4-45
Parameter
Value
Title
Ray-VII-87-MeLinker-OTIPS-SBT1214-7-TES-conjugate-chrom-51-64
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
100
Receiver Gain
22
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2007-11-08T20:06:18
Spectrometer Frequency
399.84
Nucleus
1H
f1 (ppm)

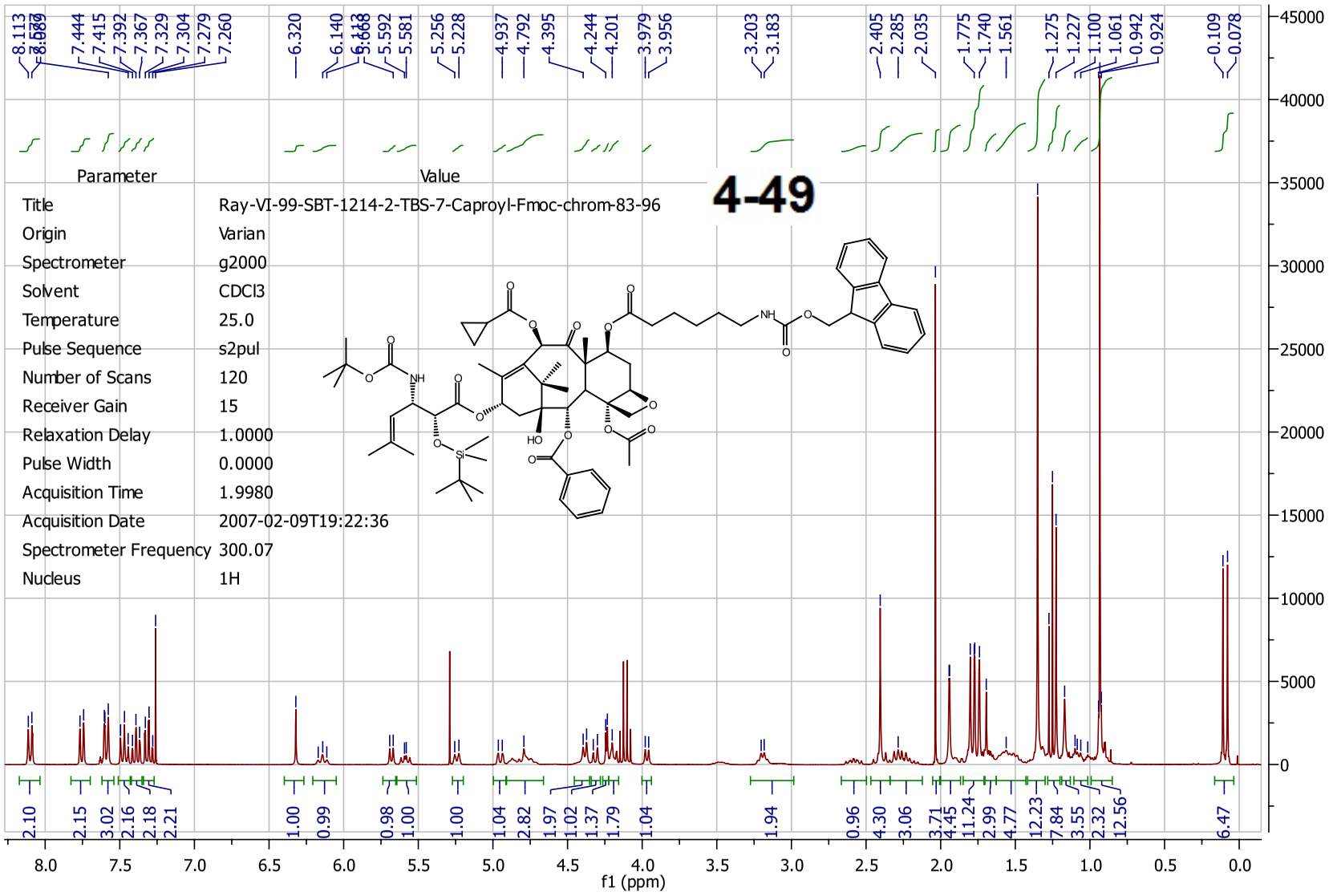

202.35
173.14
172.90
170.19
169.68
168.23
167.18
155.08
141.13
137.81
137.49
133.68
129.54
127.86
120.20
84.43
81.12
79.95
79.13
74.88
72.28
72.00
58.49
49.19
46.89
46.16
43.42
33.17
31.13
26.58
22.58
20.70
18.65
17.84
12.04
8.80
8.69
6.93
5.42
4-45
C73H107NO18S2Si2
Parameter
Value
Title
Ray-VII-87-MeLinker-OTIPS-SBT1214-7-TES-conjugate-chrom-51-64-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
1344
Receiver Gain
58
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2007-11-08T20:14:01
Spectrometer Frequency
100.55
Nucleus
13C
f1 (ppm)

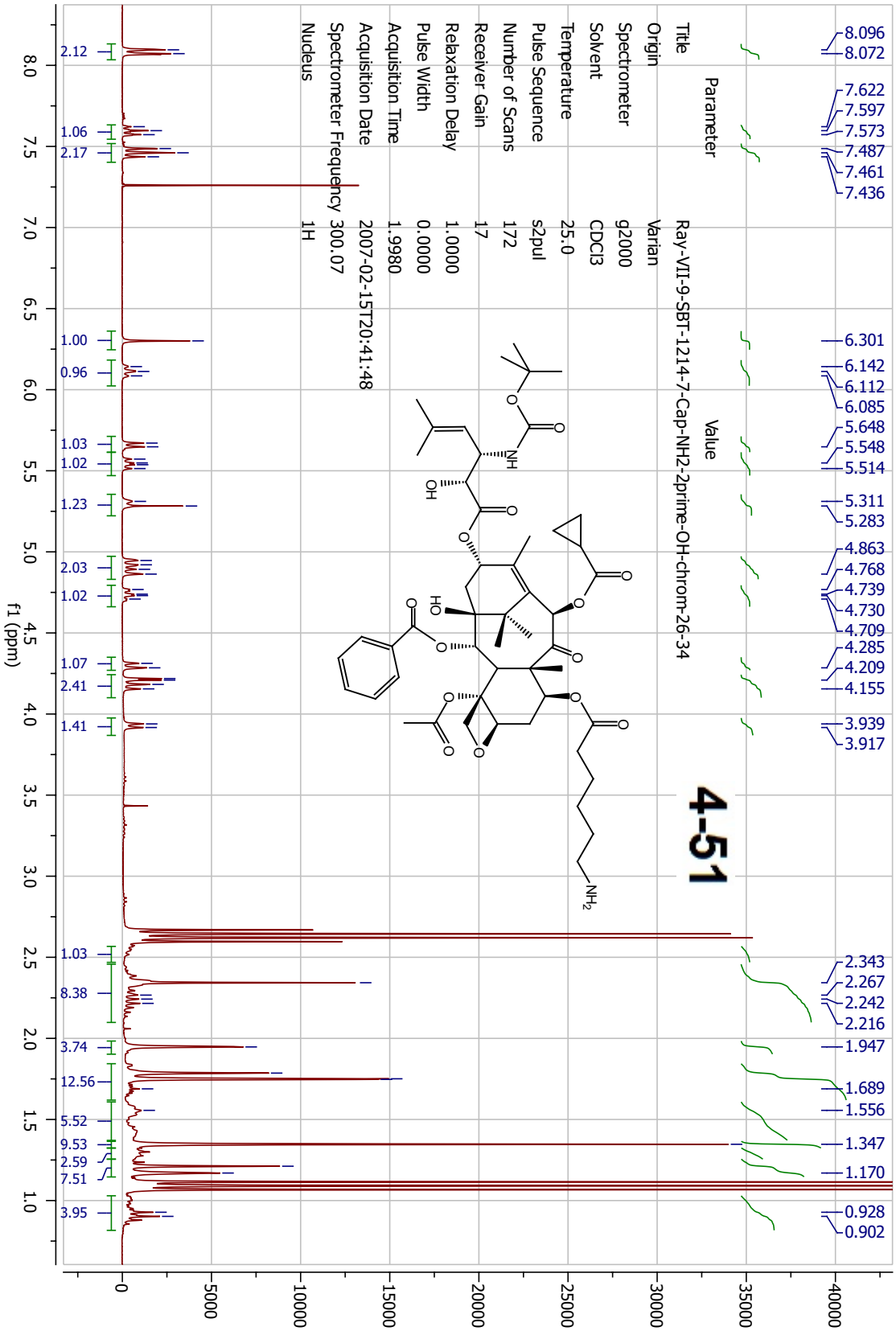

4-46
Parameter Value
Title Ray-VII-89-SBT1214-MeLinker-OTIPS-CsF-chrom-32-46
Origin Varian
Spectrometer inova
Solvent CDCl3
Temperature 25.0
Pulse Sequence s2pul
Number of Scans 120
Receiver Gain 22
Relaxation Delay 1.0000
Pulse Width 0.0000
Acquisition Time 3.2725
Acquisition Date 2007-11-19T21:02:33
Spectrometer Frequency 399.84
Nucleus 1H
f1 (ppm)


4-47
Parameter
Value
Title
Ray-VII-90-SBT1214-MeLinker-OSu-chrom-51-62
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
28
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2007-11-24T12:01:31
Spectrometer Frequency
399.84
Nucleus
1H
8.098
7.481
7.462
7.443
7.276
7.260
6.460
6.123
5.689
5.671
4.943
4.918
4.807
4.785
4.443
4.303
4.173
4.085
3.943
3.821
3.804
2.666
2.647
2.494
2.489
2.345
2.019
1.668
1.187
0.878
0.551
f1 (ppm)


4-48
Parameter
Value
Title
Ray-VII-91-Biotin-MeLinker-1214-TES-chrom-33mg
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
200
Receiver Gain
28
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
3.2725
Acquisition Date
2008-01-17T20:27:33
Spectrometer Frequency 399.84
Nucleus
1H
f1 (ppm)


202.24
173.02
171.90
171.22
170.53
169.91
168.47
167.08
164.83
155.21
141.00
140.93
137.63
133.69
128.76
128.00
120.21
84.43
81.14
79.95
79.16
79.11
72.31
62.08
60.49
58.48
55.83
43.48
40.66
37.34
33.27
28.47
26.64
25.40
21.65
18.70
10.29
8.85
8.74
6.95
5.45
4-48
C74H103N5O19S3Si
Parameter
Value
Title
Ray-VII-91-Biotin-MeLinker-1214-TES-chrom-33mg-C13-NMR
Origin
Varian
Spectrometer
inova
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
20536
Receiver Gain
56
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.1994
Acquisition Date
2008-01-17T20:35:11
Spectrometer Frequency
100.55
Nucleus
13C
f1 (ppm)


4-49
Parameter
Value
Title
Ray-VI-99-SBT-1214-2-TBS-7-Caproyl-Fmoc-chrom-83-96
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
120
Receiver Gain
15
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2007-02-09T19:22:36
Spectrometer Frequency
300.07
Nucleus
1H
f1 (ppm)


Parameter
Value
Title
Ray-VII-9-SBT-1214-7-Cap-NH2-2prime-OH-chrom-26-34
Origin
Varian
Spectrometer
g2000
Solvent
CDCl3
Temperature
25.0
Pulse Sequence
s2pul
Number of Scans
172
Receiver Gain
17
Relaxation Delay
1.0000
Pulse Width
0.0000
Acquisition Time
1.9980
Acquisition Date
2007-02-15T20:41:48
Spectrometer Frequency
300.07
Nucleus
1H
4-51
f1 (ppm)

checkCIF/PLATON report

No syntax errors found. **CIF dictionary** **Interpreting this report**

Datablock: I

```
Bond precision:  C-C = 0.0077 A          Wavelength=0.71073

Cell:            a=12.7345(7)     b=18.5715(10)     c=22.8765(13)
                 alpha=90         beta=90           gamma=90
Temperature:     273 K

                 Calculated                  Reported
Volume           5410.3(5)                   5410.3(5)
Space group      P 21 21 21                  P 21 21 21
Hall group       P 2ac 2ab                   ?
Moiety formula   C45 H59 N O15, 3(C H2 Cl2)  C45 H59 N O15, 3(C H2 Cl2)
Sum formula      C48 H65 Cl6 N O15           C48 H65 Cl6 N O15
Mr               1108.71                     1108.75
Dx,g cm-3        1.361                       1.361
Z                4                           4
Mu (mm-1)        0.382                       0.382
F000             2328.0                      2328.0
F000'            2332.65
h,k,lmax         17,24,30                    15,24,28
Nref             7470[ 13633]                12050
Tmin,Tmax        0.909,0.926
Tmin'            0.909

Correction method= Not given

Data completeness= 1.61/0.88       Theta(max)= 28.450

R(reflections)= 0.0777( 5159)      wR2(reflections)= 0.2517( 12050)

S = 0.921                  Npar= 621
```

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level B

PLAT201_ALERT_2_B Isotropic non-H Atoms in Main Residue(s) 3

Author Response: The disordered t-butyl group was left isotropic for getting best refinement.

```
PLAT241_ALERT_2_B Check High      Ueq as Compared to Neighbors for        C28
```

Author Response: The highly disorder solvent molecules in close range cause this misbehavior on the concerned objectives.

```
PLAT413_ALERT_2_B Short Inter XH3 .. XHn     H39C   ..  H42A    ..       1.96 Ang.
```

Author Response: The disordered t-butyl group was left isotropic for getting best refinement.

Alert level C

```
RFACR01_ALERT_3_C  The value of the weighted R factor is > 0.25
           Weighted R factor given   0.252
SHFSU01_ALERT_2_C  The absolute value of parameter shift to su ratio > 0.05
           Absolute value of the parameter shift to su ratio given   0.092
           Additional refinement cycles may be required.
PLAT026_ALERT_3_C Ratio Observed / Unique Reflections too Low ....         43 Perc.
PLAT080_ALERT_2_C Maximum Shift/Error ..........................       0.09
PLAT202_ALERT_3_C Isotropic non-H Atoms in Anion/Solvent .........         4
PLAT220_ALERT_2_C Large Non-Solvent    C     Ueq(max)/Ueq(min) ...       3.12 Ratio
```

Author Response: .See above

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PLAT222_ALERT_3_C Large Non-Solvent    H     Ueq(max)/Ueq(min) ...       3.80 Ratio
PLAT230_ALERT_2_C Hirshfeld Test Diff for    O5     --  C5      ..       6.18 su
PLAT230_ALERT_2_C Hirshfeld Test Diff for    C31    --  C32     ..       5.05 su
PLAT242_ALERT_2_C Check Low       Ueq as Compared to Neighbors for        C26
PLAT242_ALERT_2_C Check Low       Ueq as Compared to Neighbors for        C34
PLAT340_ALERT_3_C Low Bond Precision on C-C Bonds (x 1000) Ang ...         8
PLAT412_ALERT_2_C Short Intra XH3 .. XHn     H13    ..  H16C    ..       1.83 Ang.
PLAT194_ALERT_1_C Missing _cell_measurement_reflns_used datum ....         ?
PLAT195_ALERT_1_C Missing _cell_measurement_theta_max   datum ....         ?
PLAT196_ALERT_1_C Missing _cell_measurement_theta_min   datum ....         ?
PLAT234_ALERT_4_C Large Hirshfeld Difference C27    --  C28     ..       0.20 Ang.
```

Author Response: .See above

```
PLAT243_ALERT_4_C High  'Solvent' Ueq as Compared to Neighbors for        C42
PLAT244_ALERT_4_C Low   'Solvent' Ueq as Compared to Neighbors for        C41
PLAT244_ALERT_4_C Low   'Solvent' Ueq as Compared to Neighbors for        C43
PLAT950_ALERT_1_C Reported and Calculated Hmax Values Differ by ..        2
PLAT952_ALERT_1_C Reported and Calculated Lmax Values Differ by ..        2
```

Alert level G

```
REFLT03_ALERT_1_G ALERT: Expected hkl max differ from CIF values
          From the CIF: _diffrn_reflns_theta_max          28.45
          From the CIF: _reflns_number_total             12050
          From the CIF: _diffrn_reflns_limit_ max hkl   14.   23.   28.
          From the CIF: _diffrn_reflns_limit_ min hkl  -15.  -24.  -15.
          TEST1: Expected hkl limits for theta max
          Calculated maximum hkl   17.   24.   30.
          Calculated minimum hkl  -17.  -24.  -30.
```

```
REFLT03_ALERT_4_G Please check that the estimate of the number of Friedel pairs is
           correct. If it is not, please give the correct count in the
           _publ_section_exptl_refinement section of the submitted CIF.
          From the CIF: _diffrn_reflns_theta_max          28.45
          From the CIF: _reflns_number_total             12050
          Count of symmetry unique reflns         7470
          Completeness (_total/calc)            161.31%
          TEST3: Check Friedels for noncentro structure
          Estimate of Friedel pairs measured      4580
          Fraction of Friedel pairs measured     0.613
          Are heavy atom types Z>Si present        yes
PLAT301_ALERT_3_G Note Main Residue  Disorder .....................      5.00 Perc.
PLAT199_ALERT_1_G Check the Reported _cell_measurement_temperature       273 K
PLAT200_ALERT_1_G Check the Reported _diffrn_ambient_temperature .      273 K
PLAT720_ALERT_4_G Number of Unusual/Non-Standard Labels ..........        5
PLAT779_ALERT_4_G Suspect or Irrelevant (Bond) Angle in CIF ......     29.50 Deg.
             C40' -C37  -C40     1.555   1.555   1.555
PLAT779_ALERT_4_G Suspect or Irrelevant (Bond) Angle in CIF ......     34.90 Deg.
             C39' -C37  -C39     1.555   1.555   1.555
PLAT779_ALERT_4_G Suspect or Irrelevant (Bond) Angle in CIF ......     30.30 Deg.
             C38' -C37  -C38     1.555   1.555   1.555
PLAT791_ALERT_4_G The Model has Chirality at C1      (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C2      (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C2'     (Verify) ....        R
PLAT791_ALERT_4_G The Model has Chirality at C3      (Verify) ....        R
PLAT791_ALERT_4_G The Model has Chirality at C3'     (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C4      (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C5      (Verify) ....        R
PLAT791_ALERT_4_G The Model has Chirality at C7      (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C8      (Verify) ....        S
PLAT791_ALERT_4_G The Model has Chirality at C10     (Verify) ....        R
PLAT791_ALERT_4_G The Model has Chirality at C13     (Verify) ....        S
```

```
  0 ALERT level A = In general: serious problem
  3 ALERT level B = Potentially serious problem
 22 ALERT level C = Check and explain
 20 ALERT level G = General alerts; check

  8 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
 11 ALERT type 2 Indicator that the structure model may be wrong or deficient
  6 ALERT type 3 Indicator that the structure quality may be low
 20 ALERT type 4 Improvement, methodology, query or suggestion
  0 ALERT type 5 Informative message, check
```

Publication of your CIF in IUCr journals

A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

PLATON version of 09/04/2009; check.def file version of 08/04/2009

Datablock I - ellipsoid plot