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Synthesis of New-Generation Taxoid and Taxoid-Based Tumor-Targeting Drug Conjugates

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Abstract of the Thesis

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Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells, and it is the leading cause of death around the world. Despite of great advance made in the medical research, there is no universal treatment for cancer. Chemotherapy is advantageous and widely used because it is not invasive, and there are many different chemotherapeutic agents to different cancers. Chemotherapy relies on the premise that rapid dividing cells are more likely to be attacked by cytotoxic agents.

Paclitaxel (Taxol) is a mitotic inhibitor used in cancer chemotherapy and is one of the most effective antitumor drugs available for various cancers, such as ovarian cancer, breast cancer, and AIDS-related Kaposi's sarcoma. Its analogues docetaxel (Taxotere®) and cabazitaxel (Jevtana®) are also chemotherapeutic agents for breast cancer and prostate cancer respectively. Taxoids can be accessed through modified 10-DAB III intermediate coupled with enantiopure *β*-

lactam via Ojima-Holton coupling to afford the taxoids. Using this coupling method, new generation of taxoids have been synthetized effectively.

Lack of tumor specificity is the main challenges which cause undesired side effects of chemotherapy. In last few decades, various drug delivery protocol and systems have been explored. In general, tumor-targeting drug-delivery system consists of a cytotoxic agent and a tumor-targeting moiety which can be linked directly or bridged by a suitable linker to form a conjugate. The conjugate should be systemically non-toxic, stable in circulation, and delivered to tumor cells specifically. As the tumor-targeting moiety (TTM) is recognized by receptors of the tumor cells, the conjugate can be internalized into the cells and cleaved to regenerate the active cytotoxic agent. Ojima group has constructed many tumor-targeting drug-delivery systems; one of the examples is using biotin (vitamin B) as tumor targeting moiety, new-generation taxoid (SB-T-1214) as cytotoxic agent, and they are bound together by methyl-branch disulfide linker. The syntheses of these drug conjugates will be discussed.

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List of Abbreviations

°C	Degrees Celsius
10-DAB III	10-Deacetylbaccatin III
ABC	ATP-binding cassette
Ac	Acetyl
ADC	Antibody-drug conjugate
AIDS	Acquired immune deficiency syndrome
Arg	Arginine
Asp	Asparagine
ATP	Adenosine triphosphate
Az	Azide
BOC	*tert*-Butyl carbonate
Bu	Butyl
BuLi	Butyl lithium
CAN	Ceric ammonium nitrate
CML	chronic myeloid leukemia
CN	nitrile
CRPC	castration-resistant prostate cancer
d	Day
DCM	dichloromethane
DDS	Drug delivery system
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
EDC·HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	Enantiomeric excess
EGFR	Epidermal growth factor receptor
eq	equivalent
Et	Ethyl
EtOH	Ethanol
FA	Folic acid
FDA	Food and drug administration
FITC	Fluorescein isothiocyanate
Fmoc	Fluorenylmethyloxycarbonyl
g	Gram
G_2/M	Gap 2/ mitosis

GIST	malignant gastrointestinal stromal tumor
Glu	Glutamic acid
Gly	Glycine
GSH	Glutathione
GTP	Guanosine-5'-triphosphate
h	Hours
HPLC	High-performance liquid chromatography
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
IPA	Isopropanol
k	Kilo
L	Liter
LDA	Lithium diisopropylamide
Leu	Leucine
LiHMDS	Lithium bis(trimethylsilyl)amide and lithium hexamethyldisilazide
Lys	Lysine
m	Meter
m.p.	Melting point
mAbs	Monoclonal antibodies
MDR	Multidrug resistance
Me	Methyl
mg	Milligram
MHz	Megahertz
min	Minutes
mL	Milliliter
mmol	Millimole
MsCl	Methanesulfonyl chloride
NCI	National Cancer Institute
NHS, HOSu	*N*-Hydroxysuccinimide
NMR	Nuclear magnetic resonance
NSCLC	non-small cell lung cancer
OC	Ovarian cancer
PBS	Phosphate buffer solution
PEG	Polyethylene glycol
PG	Protecting group
Pgp	P-glycoprotein
Ph	Phenyl
Phe	Phenylalanine
PMA	Phosphomolybdic acid
PMP	p-Methoxyphenyl
Pr	Propyl

py	Pyridine
r.t.	Room temperature
RME	Receptor-mediated endocytosis
RNA	Ribonucleic acid
s	Seconds
SAR	Structure-activity relationship
SB-T	Stony Brook taxoid
SET	Single electron transfer
TEA	triethylamine
tert	tertiary
TES	Triethylsilyl
TESCl	Triethylsilyl chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TIPSCl	Triisopropylsilyl chloride
TLC	Thin layer chromatography
TPPO	Triphenyphosphineoxide
TTM	Tumor-targeting module
WCA	Whitesell Chiral Auxiliary

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Chapter 1

Synthesis of New-Generation Taxoid Antitumor Agents

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§1.1 Introduction

§1.1.1 Cancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, the disease can invade vital organs, resulting in death. Cancer begins with a mutation in the genetic code, which results in cellular changes. It makes the cells to undergo uncontrolled abnormal cell division, and form a malignant tissue. As the disease progresses, the cancer could spread through the body via circulatory and lymphatic systems, a process known as metastasis.[1] Cancer was the leading cause of death in the developed countries and second leading cause of death in the developing countries.[2] According to the statistics of the American Cancer Society, a total of 1.6 million new cancer cases and half a million cancer deaths are projected to occur in the United States in 2013.[3] Since the risk of being diagnosed with cancer increase with age, most cases occur in adult who are middle age or older.

Despite of great advance made in the medical research, there is no universal treatment for cancer. There are many established treatments which may be used individually or in combination. Cancer is currently treated by a combination of surgery, radiation therapy, immunotherapy and chemotherapy. Surgery is an invasive treatment, and surgeons use operative instruments to physically remove the malignant tissue from patients. Radiation therapy: high-energy beams, such as X-ray or gamma rays, were used to kill the malignant tissue or shrink the tumor. For example, gamma rays damage DNA, causing the tumor cells to die. Both surgery and radiation therapy are local treatments. On the other hand, chemotherapy and immunotherapy are systemic. Thus, they can be used to treat cancer after it has metastasized or spread. Immunotherapy is a treatment of stimulating patients' own immune system to work harder or smarter to kill cancer cells.[4] Chemotherapy is the used of specific chemical agents to kill cancer cells.

Chemotherapy is advantageous and widely used because it is not invasive, and there are many different chemotherapeutic agents that work to control different cancers. Chemotherapy relies on the premise that rapid dividing cells are more likely to be affected by cytotoxic agents that act on the processes involved in cell division. Common mechanisms of actions for these chemotherapeutic agents are to disrupt the process of mitosis (e.g. paclitaxel and vincristine), inhibit DNA replication and transcription (e.g. camptothecin) **(Figure 1-1)**.[5,6]

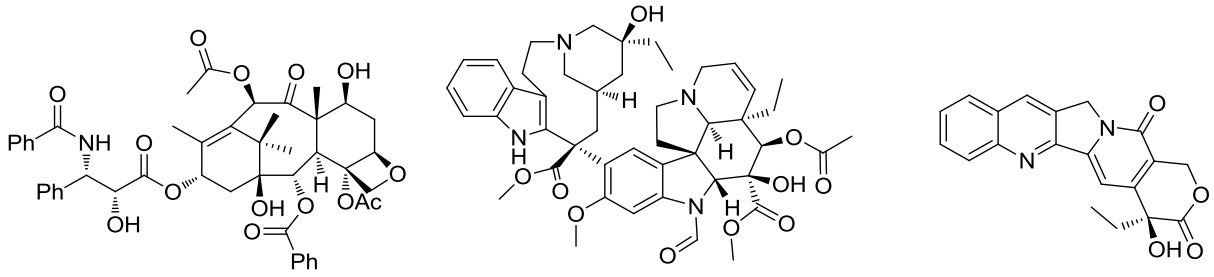


Figure 1-1: Examples of chemotherapeutic drugs.

§1.1.2 Anticancer drug: Paclitaxel and its Developments

Paclitaxel (Taxol®), isolated from the bark of the Pacific yew (*Taxus brevifolia*), is a mitotic inhibitor used in cancer chemotherapy and is one of the most widely used antitumor drugs available for various cancer through its unique anti-mitotic mechanism of action.[7-9] Paclitaxel has been approved by the FDA for the treatment of advanced ovarian cancer (1992), breast cancer (1994), and AIDS-related Kaposi's sarcoma (1997), and nonsmall lung cancer (2006).

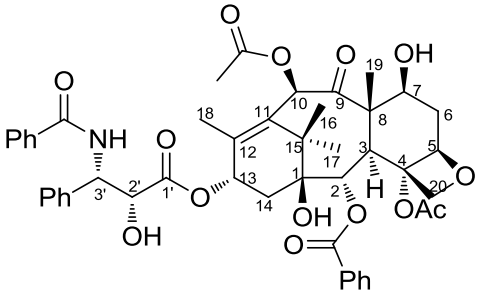


Figure 1-2: Structure of Paclitaxel.

Paclitaxel promotes and stabilizes microtubule assembly, which means it inhibits the disassembly of the mitotic spindle, stops chromosome segregation and suspends mitosis in the G_2/M phase in cell cycle, ultimately resulting in cell death **(Figure 1-3)**.[10,11] The mechanism of action is by binding to tubulin heterodimers which stabilize microtubules formation and thus disrupt the dynamic process of cell division.[8] The formation and dissociation of microtubules are

highly dynamic, but once Taxol binds to them, they become extremely stable and static, making cell division impossible, which induces apoptosis.[8]

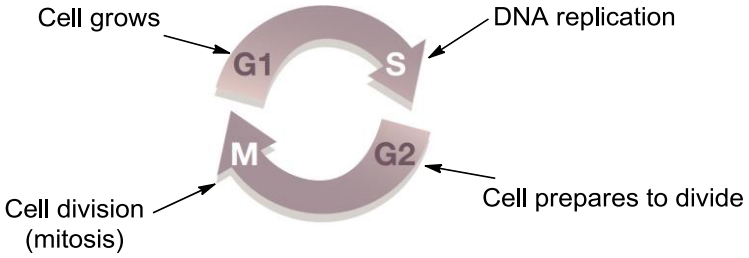


Figure 1-3: Cell cycle diagram.[10,11]

Docetaxel (Taxotere®) **(Figure 1-4)**, a semi-synthetic analog of paclitaxel, was discovered by Potier *et. al*. and developed by the French pharmaceutical company Rhone-Poulenc Rorer (currently Sanofi-Aventis).[12,13] This analog of paclitaxel has an *N-tert*-butoxycarbonyl moiety on C-3' position and a hydroxyl group at C-10 position. Docetaxel has the same mechanism of action, and is as potent as paclitaxel against B16 melanoma, colon adenocarcinoma 38, and pancreatic ductal adenocarcinoma 03.[14] The FDA approved docetaxel for the treatment of breast cancer, prostate cancer and non-small cell lung cancer.[15-17]

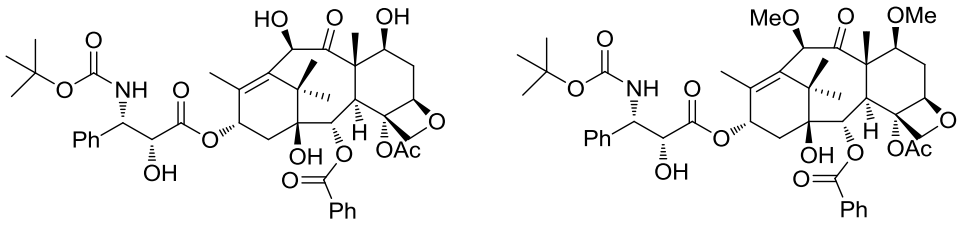


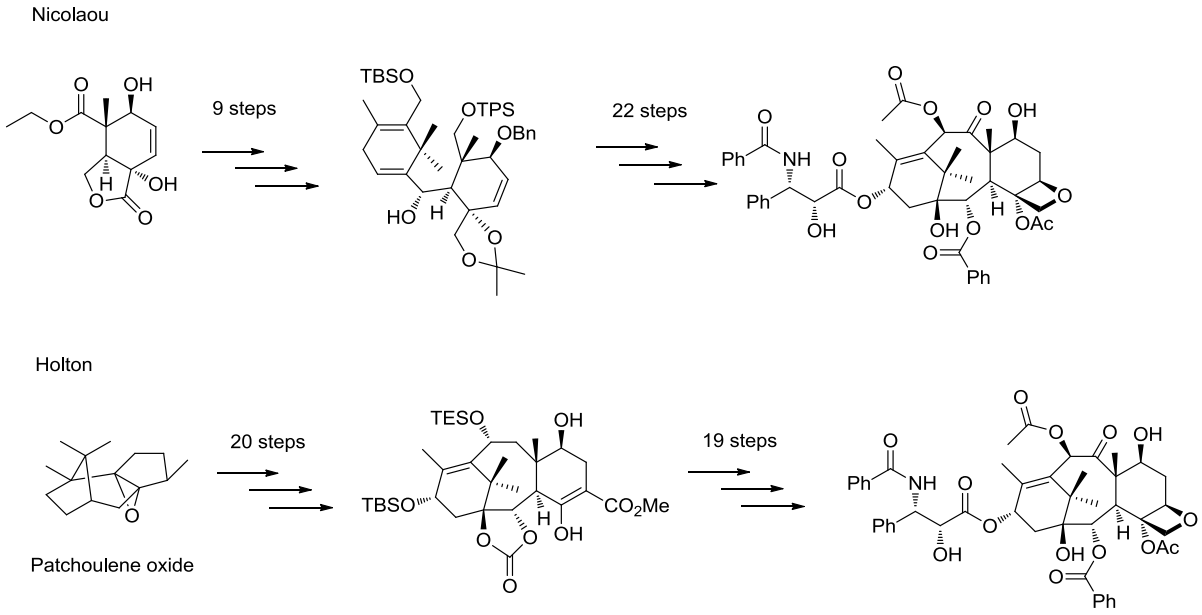
Figure 1-4: Docetaxel(Taxotere®) and cabazitaxel (Jevtana®).

Cabazitaxel (Jevtana®) **(Figure 1-4)** is another taxane compound for cancer treatment and approved by FDA in 2010.[18] It is an analog of docetaxel, and has modification on C-7 and C-10 of baccatin core. Cabazitaxel is a second-line treatment for patients with advanced castration-resistant prostate cancer (CRPC) who are treated with docetaxel first but showed no significant improvement.[19,20]

§1.1.3 Synthesis of Paclitaxel

Paclitaxel was a widely used chemotherapeutic agent for various cancers, but the source was really limited. The initial supply of paclitaxel was extraction from the bark of Pacific yew (*Taxus brevifolia*). It is a slowly growing tree and primarily localized in the coastal areas. The isolation procession is difficult and the isolated yield was low; 12 kg air-dry barks could only isolated 0.5 g of paclitaxel, 0.004% isolated yield.[9] The limited source and supply tempted chemists to search useful synthetic route to paclitaxel.

The complicated structure and 11 stereocenters made paclitaxel an extremely difficult molecule to construct. In 1994, two research teams Nicolaou's and Holton's group simultaneously published two different total syntheses of Taxol **(Scheme 1-1)**.[21,22,23] Nicolaou's group reported a synthetic route with three pre-assembled synthons, including *β*-lactam. Holton's group published a total synthesis of Taxol. It was a linear synthetic route starting from a bicyclic epoxy alcohol fragment, patchoulene oxide, and needed more than 35 steps to construct Taxol and the total yield was 4-5 %. Both synthetic routes are not acceptable for factory production.



Scheme 1-1: Total syntheses of Taxol

In late 1980's Potier isolated a semisythetic precursor 10-deacetylbaccatin III (10-DAB III) from the leaves of European Yew (*Taxus baccata*).[24,25] 10-Deacetylbaccatin III (10-DAB III) has the same polycyclic core structure and correct stereochemistry as Paclitaxel. In addition, the leaves of European Yew are renewable source and isolation yield of 10-DAB III is higher than that of Paclitaxel, obtaining 10-DAB III in yield of 1 gram per kilogram of fresh leaves.[24] Thus, 10-DAB III is a good access point to Paclitaxel through a semi-synthetic process.

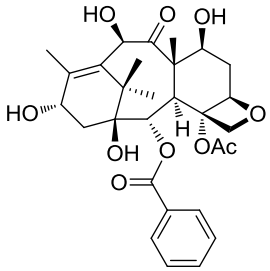
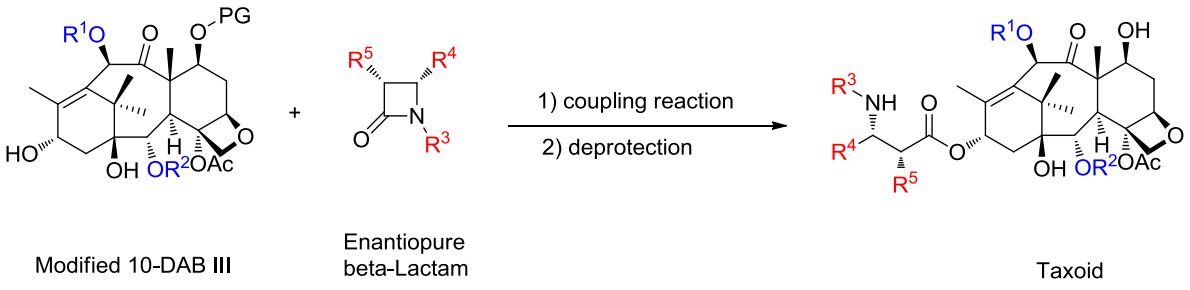


Figure 1-5: 10-deacetylbaccatin III.

In 1988, research groups led by Greene and Potier published the first semi-synthesis of paclitaxel using modified 10-DAB III, but it required high temperature (73 ^{o}C) and long reaction time (100 h).[24] In 1991, a research group led by Ojima found the β-lactam ring, the core structure of many antibiotics, could be used in the semisynthesis of Taxol without emperization.[26] Taxoids can be accessed through modified 10-DAB III intermediate coupled with enantiopure β-lactam via the Ojiam-Holton coupling.[26,27]



Scheme 1-2: General scheme for the Ojima-Holton coupling protocol.

§1.1.4 Multidrug Resistance

Paclitaxel and docetaxel are useful anticancer drugs in chemotherapy; however, clinical treatment of both taxane agents has showed undesired side effects and multidrug resistance (MDR). The principle of multidrug resistance is cancer cells develop resistance to drugs. In clinical study, reduction in effectiveness of chemotherapeutic agents after patients treated with chemotherapy. The mechanism of MDR includes decreased drug permeability to the cells, increased drug efflux from cancer cells, insensitivity to drug-induced apoptosis, and altered drug-targeted sites.[28-30]

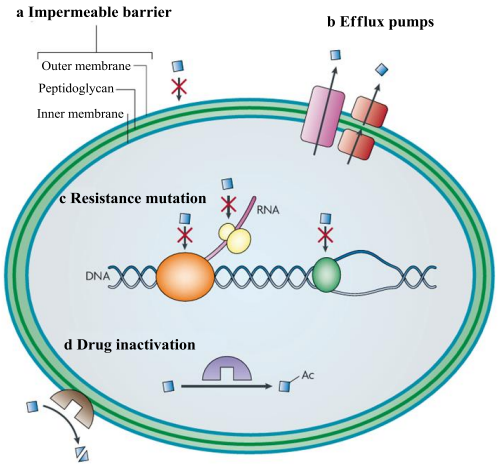


Figure 1-6: Proposed mechanisms of multidrug resistance (MDR).[28]

Classical MDR was resulted from overexpression of ATP-binding cassette (ABC) transporter which accelerates efflux of anticancer drug.[31] ABC transporters are transmembrane proteins which carries out various substances, and P-glycoprotein (Pgp) and multidrug resistance-associated proteins (MRP) are two molecular pumps from the ABC family that have been related with MDR.[28,30] Taxane clinical treatment encountered MDR caused by overexpression of Pgp. The overexpressed proteins make the efflux of chemotherapeutic agents resulting in a loss of drug efficiency (**Figure 1-7**). Thus, new drugs have been developed with enhanced efficiency against MDR cell lines.

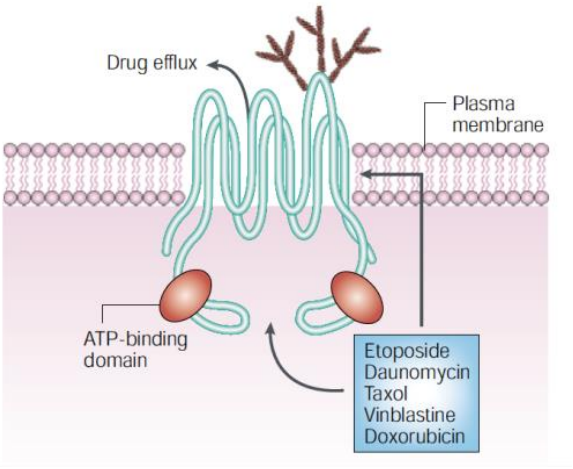


Figure 1-7: Efflux of chemotherapeutic agents.[32]

§1.1.5 Structure Activity Relationships of Taxoids

Using this Ojima-Holton coupling method, new generation of taxoids have been synthetized.[33-35] Accordingly, extensive Structure–Activity Relationship (SAR) studies have been conducted on the taxane family. These finding are summarized in **Figure 1-6**.

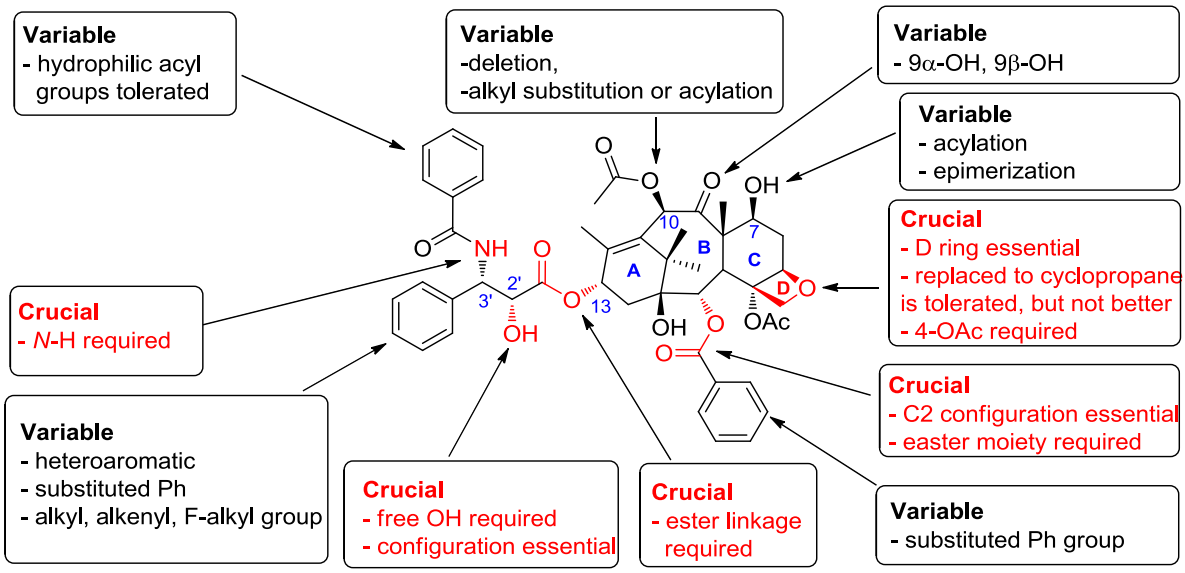


Figure 1-8: Summary of SAR studies of paclitaxel.[36,37]

SAR studies have shown that the C-13 side chain is an essential component for the cytotoxicity and antitumor activity of paclitaxel.[38] The stereochemistry of the ester group at C-13 position is essential and replacement with amide bond results in loss of activity.[39] The stereochemistry of C-2' and C-3' are important for activity; free hydroxyl group on C-2' is essential to participate in hydrogen bonding with the Arg 359 of *β*-tubulin backbone.[40] The C-3' and C-3'-N positions are tolerable to other functionality.[41] The substitution of C-3' position has been studied systematically, second-generation taxoids with C-3' alkyl, alkenyl, or fluorinated alkyl groups have been developed by our laboratory and were found to be potent, especially analogs with isobutenyl moiety.[27,35,42-45]

A, B, C and D rings are essential for optimal cytotoxicity.[46,47] Modification of the C-7 and C-10 positions is well-tolerated. Certain modifications of C-10 position showed similar potency to paclitaxel against cancer cell line (MCF-7, NCI/ADR-RES), but less interaction with Pgp.[48] Taxoid with dimethylcarbamoyl substitution on C-10 showed better cytotoxicity than Taxol in HCT 116 human colon carcinoma cell line.[49] The substitution of benzoyl group on C-2 position has shown improvement of anticancer activity.[50,51] The *meta* substitution at C-2 benzoyl group showed good activity (2-3 order of magnitude higher potency) than paclitaxel and docetaxel against drug-sensitive cancer cell lines (LCC6-MDR, MCF7, MCF7-R).[52,53] Ojima et al. reported that the second-generation toxoids with modification at C-10, C-3' showed better cytotoxicity than paclitaxel against human ovarian (A 121), colon (HT-29), and breast (MCF-7) cancer cell lines and effective inhibition of Pgp binding.[35]

§1.1.6 *β*-Lactam Synthon Method

The synthesis of taxoid **(Scheme 1-2)** could be approached via Ojima-Holton coupling, and it is required the synthesis of enantiopure *β*-lactam moiety.[54] Using different substituents on the *β*-lactam, different taxoids with modifications on C'3 position or *N*-position can be synthesized. There are two common synthetic routes to these enantiopure *β*-lactams. The first synthetic route is Staudinger [2+2] cycloaddition. This reaction gave the racemic *β*-lactam, and the desired enantiomer was obtained by enzymatic resolution. The second synthetic route is chiral ester enolate-imine cyclocondensation. Using both of these methods, the *β*-lactam was obtained with excellent enantiomeric purity.

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

The Staudinger [2+2] ketene-imine cycloaddition reaction has been considered a useful method for the synthesis of *β*-lactams. The mechanism of the cycloaddition includes the imine acting as a nucleophile to attack the ketene, which generates a zwitterionic intermediate that undergoes con-rotatory ring closure to yield the 4-membered *β*-lactam, shown in **Figure 1-9**. If the a substituted ketene and imine are used, the reaction produces two stereocenters in *β*-lactam, and cis and trans isomers can be obtained in any ratio, depending on the substituted group of imine and ketene and the reaction conditions.[55] In general, the use of (*E*)-imines results in *cis-β*-lactam and (*Z*)-imines results in *trans-β*-lactams. The imine prefers *exo* and con-rotatory ring closure to form the *cis-β*-lactam if the substituent group of the ketene is electron donating. The nonpolar solvent are prefer cis-*β*-lactam formation because the intermediate is not stable and speed up the ring closure. In polar solvent, the zwitterionic intermediate is stable, so isomerization of the double bond is prior to ring closure. [56-58]

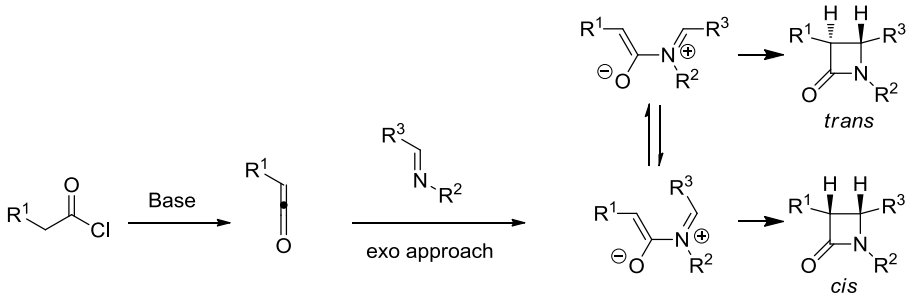


Figure 1-9: Mechanism of Staudinger [2+2] ketene imine cycloaddition.[59]

§1.1.6.2 Chiral Ester Enolate- Imine Cyclocondensation

The chiral ester and imine react via enolate-imine cyclocondensation to afford enantiopure *β*-lactam. The (*E*)-lithium enolate is formed dominantly in the presence of LDA at low temperature. The (*E*)-lithium enolate reacts with imine to form chair-like transition state to give *β*-amino ester intermediate that undergoes cyclization to form the enantiopure *β*-lactam **(Figure 1-10)**.[34]

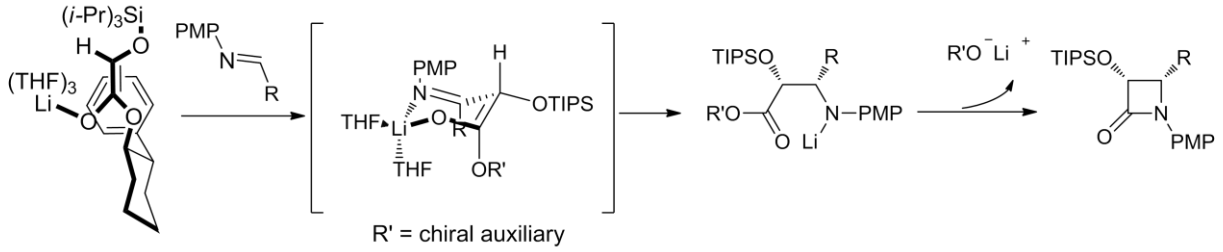


Figure 1-10: Transition state of chiral ester enolate-imine cyclocondensation.

§ 1.2 Result and Discussion

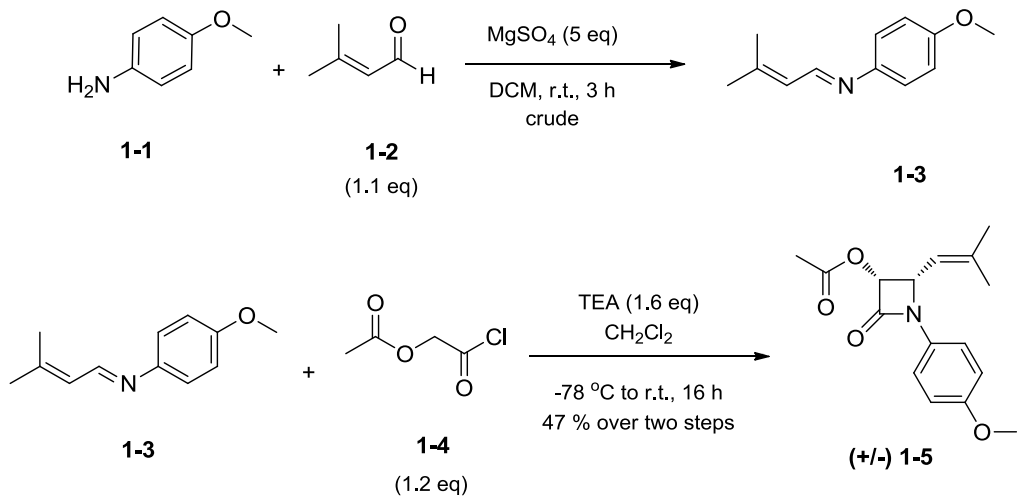
§ 1.2.1 Synthesis of *β*-Lactam via Enzymatic Resolution

The Staudinger [2+2] ketene-imine cycloaddition results in the production of racemic *β*-lactam,[60] so it is followed by enzymatic kinetic resolution to give the enantiopure product.[61]

The imine was formed from 3-methyl-2-butenal treated with *p*-anisidine **1-1** via aldehyde-amine condensation in the presence of $MgSO_4$. The free amine acted as a nucleophile to attack aldehyde, and elimination of water was followed. The presence of water could hydrolyze the product, therefore excess $MgSO_4$ was used as drying reagents to prevent the revere reaction. The imine was sensitive to air and light, so the reaction was performed in the dark and under nitrogen.

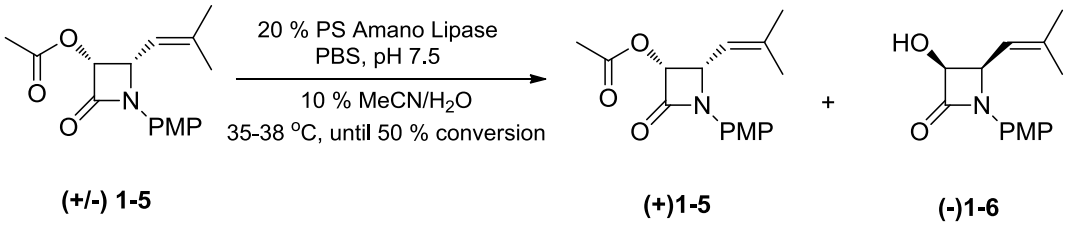
The acyl chloride was treated with triethylamine to form the ketene, and coupled with imine to form the racemic *β*-lactam. However, as the reaction was not symmetry-restricted, the

reaction temperature should remain very low to favor *cis*-isomer. The product was purified via column chromatography and recrystallization to afford white solid **(+/-)1-5 (Scheme 1-3)**.[55]



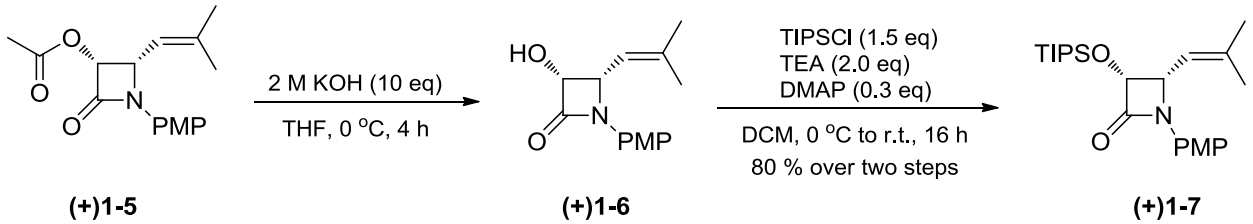
Scheme 1-3: Formation of *β*-lactam via Staudinger [2+2] cycloaddition,

The enzymatic resolution with PS Amino Lipase selectively hydrolyzed the acetyl group of the (-) enantiomer of compound **(+/-)1-5 (Scheme1-4).** The reactivity of the enzyme highly depended on the environment; therefore, the pH value of the reaction and temperature should remain constant. After 50% conversion observed by NMR, reaction was quenched and the enantiomeric purity was confirmed by chiral HPLC in order to obtain product with high *ee.* value.



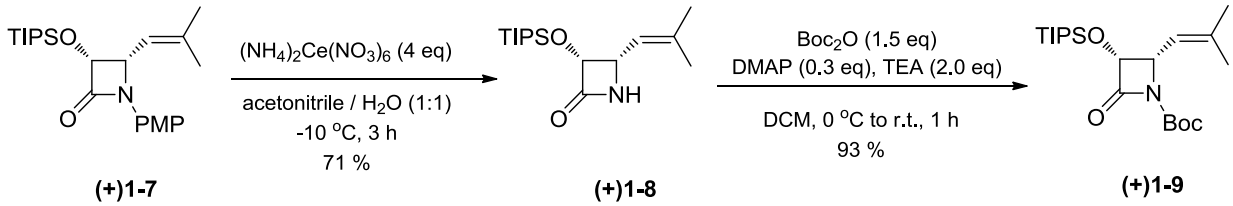
Scheme 1-4: Enzymatic resolution via PS Amano Lipase to afford enantiomer **(+)1-5.**

The ester group of **(+)1-5** was hydrolyzed to the alcohol by KOH. The free alcohol was then protected by chlorotriisopropylsilane using triethylamine to afford TIPS- protected *β*-lactam (+)**1-7 (Scheme 1-5)**.



Scheme 1-5: Formation of TIPS-protection in *β*-lactam.

The PMP-group of *β*-lactam was deprotected by using cerium ammonium nitrate (CAN). The two cerium Ce (IV) atom were both reduced to Ce (III) through electron transfer.[34] After depotection, the free lactam was protected using Boc anhydride. The DMAP served as a nucleophilic catalyst to activate the *t*-Boc group, forming an intermediate salt with electron-donating group at *para* position for stabilization. The free amine underwent nuclecophilic substituent reaction to afford protected *β*-lactam **1-9 (Scheme 1-6)**.

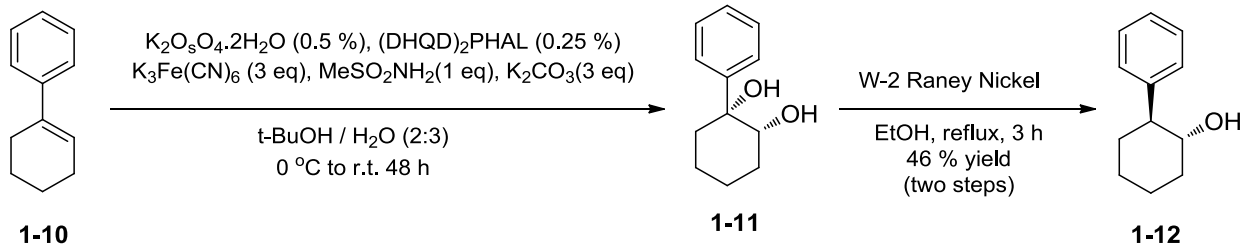


Scheme 1-6: Formation of Boc-protected *β*-lactam.[35]

§1.2.2 Synthesis of *β*-Lactam via Enolate-Imine Cyclocondensation

In the methodology of chiral ester-enolate-imine cyclocondensation, Whitesell's chiral auxiliary (WCA) is coupled to triisopropsilyoxyacetyl chloride to obtain the chiral ester. This ester undergoes a cyclocondensation with an imine to afford the desired *β*-lactam.

In order to introduce the chiral center, 1-phenylcyclohexene **1-10** was treated with osmium-catalyzed Sharpless asymmetric dihydroxylation (AD) to obtain chiral diol **1-11**. Reductive dehydroxylation of **1-11** was carried out to afford compound **1-12**, using Raney nickel, a compound used as heterogeneous catalyst for hydrogenation. The reduction only specifically dehydroxylated on benzylic position because the phenyl group helped to stabilize the radical or cation transition state. When setting up this reaction, the mechanical stirring equipment was required, and the Raney nickel is extremely pyrophoric when dry, so it had to be submerged in liquid in all times.[62,63]

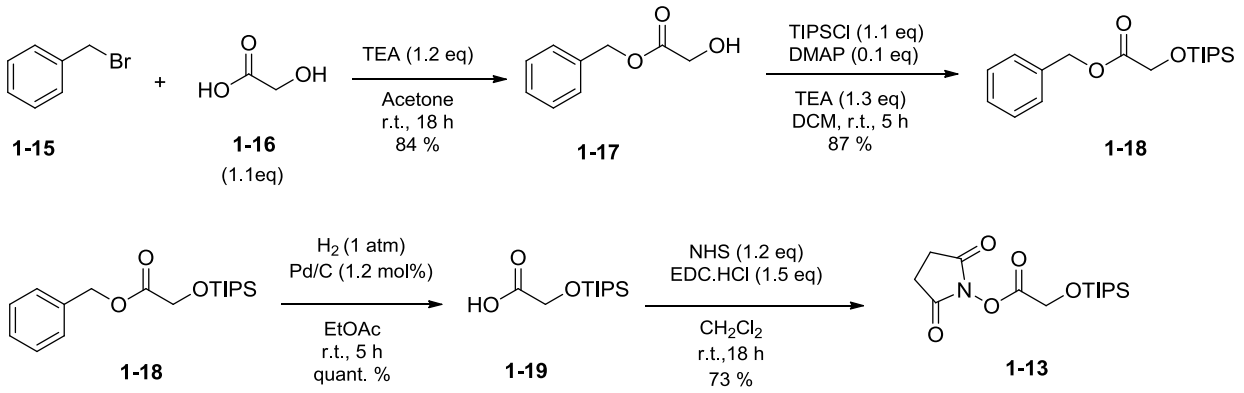


Scheme 1-7: Synthesis of Whitesell chiral auxiliary (WCA).

The purity of the chiral ester is one of the key for success of enolate-imine cyclocondensation. If the chiral ester is not absolutely pure, the reaction will not proceed, and material will be lost. The 2-(triisopropylsilyloxy) acetic acid could be activated to acid chloride or succinimidyl ester for the coupling reaction. The acetic chloride **1-14** is an oil; on the other hand, the succinimidyl ester **1-13** is a solid. The succinimidyl ester was chosen because it could be recrystallization. Thus, the purity of the chiral ester was improved, leading to more reproducible results in the cyclization reaction.

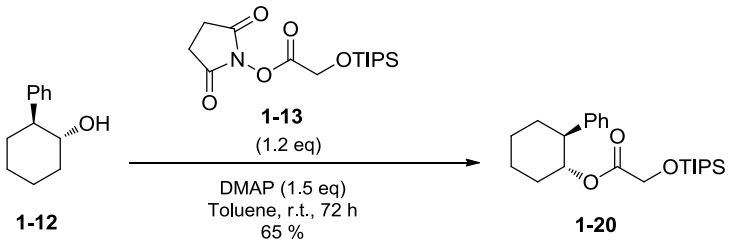
Figure 1-11: Structure of activated forms of TIPS acetic acid.

The approach to the chiral ester started with commercial available glycolic acid undergoing selective benzyl protection of the acid, followed by TIPS protection on the hydroxyl group to afford the benzyl TIPS protected product **1-18**. The compound **1-18** underwent hydrogenolysis to remove the benzyl group and NHS activation to afford the activated ester via EDC coupling. The compound **1-13** was a white solid and purified via recrystallization from hexane.



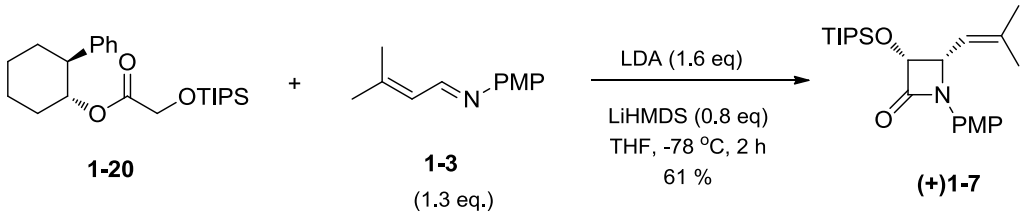
Scheme 1-8: Synthesis of activatived ester of compound **1-13.**

Chiral Auxiliary **1-12** coupled with activated ester **1-13**, in the presence of DMAP and toluene as solvent to afford chiral ester **1-20**. Toluene is used as solvent because NHS is not very soluble, and much of the NHS can be filtered off. Because of the purity of **1-20** greatly affects the success of the cyclocondensation, only very pure fractions were collected in column chromatography. Therefore, the isolated yield was low. The impurities was



Scheme 1-9: Chiral ester formation.

The chiral ester **1-20** reacted with imine **1-3** via chiral ester enolate-imine cyclocondensation to afford *β*-lactam **1-7**. This reaction is extremelysensitive to the purity of the materials and the presence of water. When assessing the purity of the starting material **1-20**, the ^{1}H NMR spectrum of this compound should be very clean without other undesired peaks, with special attention paid to the upfield 1-1.3 ppm region. The quality of the base is important, as lithium diisopropylamide (LDA) is a strong base and sensitive to moisture. Using a new bottle of LDA or making it fresh from n-butyllithium and 1,2-diisoproylamine helps to ensure success in this reaction. Also, as THF was a water-miscible organic solvent, it must be freshly distilled from benzophenone and sodium to remove any moisture.



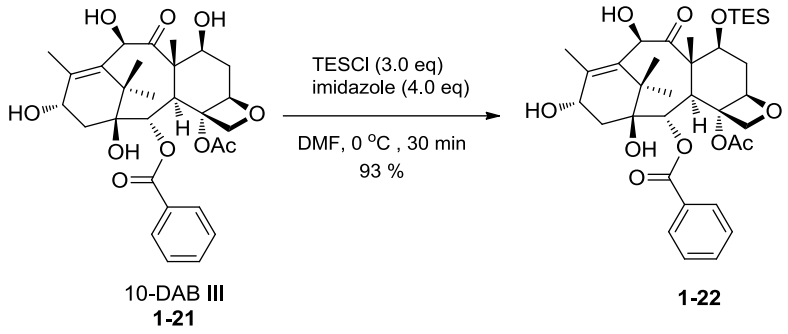
Scheme 1-10: Chiral ester enolate-imine condensation to afford *β*-lactam.

The compound **1-7** underwent PMP-deprotection by using cerium ammonium nitrate (CAN), and Boc protection to afford the *β*-lactam **1-9** through the same synthetic route shown in **Scheme 1-6**.

§1.2.3 Synthesis of SB-T-1214

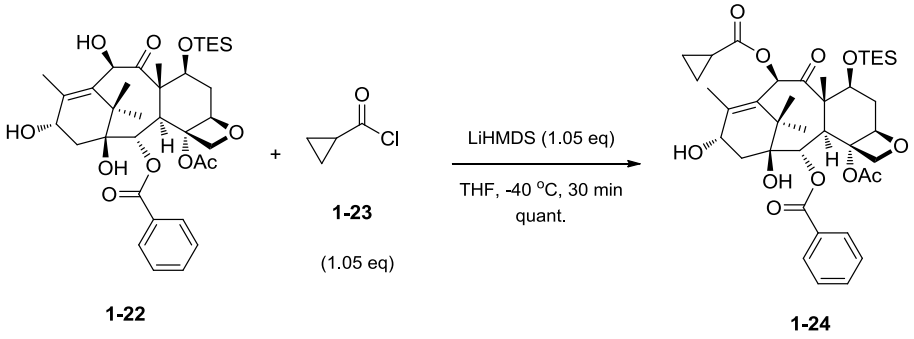
The 10-deacetylbaccatin III contains four free hydroxyl groups at carbons 1, 7, 10, and 13. In the final structure of SB-T-1214, the C-10 is coupled with cyclopropanecarbonyl chloride, and the C-13 underwent the Ojima-Holton coupling. However, the hydroxyl group at C-7 was very active, so it needed to be protected in order to do the following reaction. The reactivity of hydroxyl groups in 10-deacetylbaccatin III in order of decreasing activity is C-7, C-10, and C-13. C-1 is very sterically hindered and cannot react with most electrophiles.[35]

In 10-deacetylbaccatin III, C-7 hydroxyl group was treated with triethylsilyl chloride in the presence of imidazole to form TES-protected ether. The reaction was very fast, so the reaction temperature should maintained at 0 ^{o}C until the reaction completed. Even though the hydroxyl group at C-7 is more reactive, the other hydroxyl groups might also react. Therefore, the reaction progress was monitored via TLC carefully and it was stopped when no starting material remained.



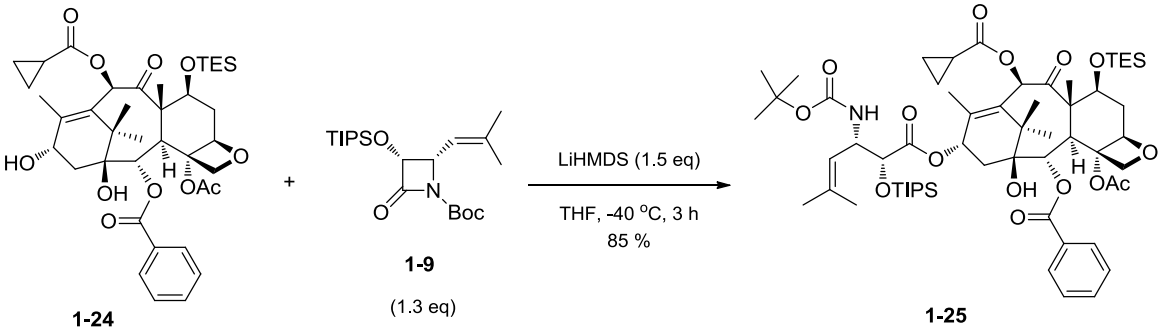
Scheme 1-11: TES-protection of the hydroxyl group at C-7 on 10-DAB III.

In the functionalization of the hydroxyl group at C-10, lithium bis(trimethylsilyl)amide is a non-nucleophilic base used to deprotonate the C-10 hydroxyl group to form an alkoxide, which underwent nucleophilic attack to cyclopropanecarbonyl chloride to afford the C-10 functionalized baccatin as white solid.



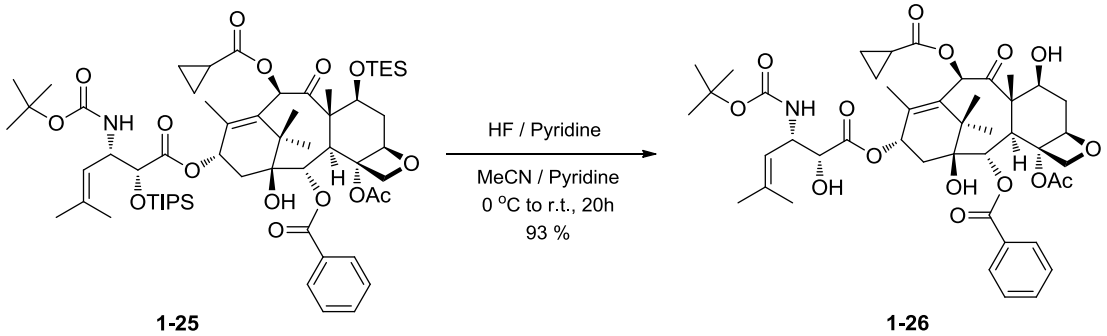
Scheme 1-12: Functionalization of the hydroxyl group at C-10.

The β-lactam was coupled to the C-13 position of compound **1-23** through the Ojima-Holton coupling reaction. LiHMDS acted as a base to deprotonate the alcohol proton on C-13 position, and the lithium-C13-alkoxide underwent nucleophilic attack to the carbonyl carbon on β-lactam to open the ring and maintained its stereocenters.



Scheme 1-13: Ojima-Holton coupling reaction.

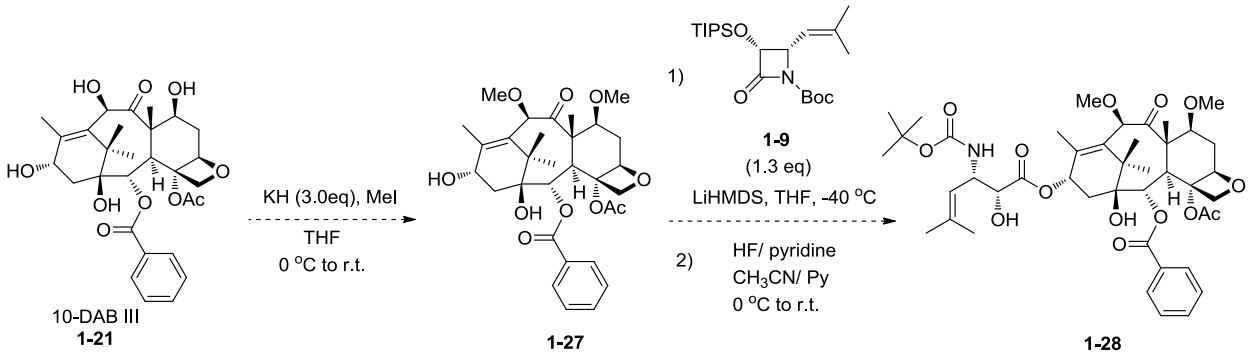
The last step in the synthetic route was the removal of the silyl protection groups at C-2' and C-7 position using hydrofluoric acid in pyridine. The conjugate base fluoride anion attacked the silyl protection group, resulting in a very stable silicon-fluorine bond. The reaction was exothermic, so the reaction temperature was kept at 0 ^{o}C and allowed to warm to room temperature and stirred for 20 hours.



Scheme 1-14: Silyl deprotection of C-2' and C-7 position to afford SB-T-1214.

§1.2.4 Synthesis of SB-T-12301

Cabazitaxel is an analog of docetaxel that has dimethoxyl groups on C-7 and C-10 positions in 10-deacetylbaccatin III skeleton. The methoxyl groups on C-7 and C-10 positions combined with the isobutenyl group on the side chain might give a potent taxoid. The proposed synthetic route of this analog is shown at **Scheme 1-15**. The compound **1-21** (10-DAB III) underwent dimethylation to afford compound **1-27,**[64] which could be coupled with *β*-lactam **1-9** and silyl group deprotection to afford the SB-T-12301 **1-28.**

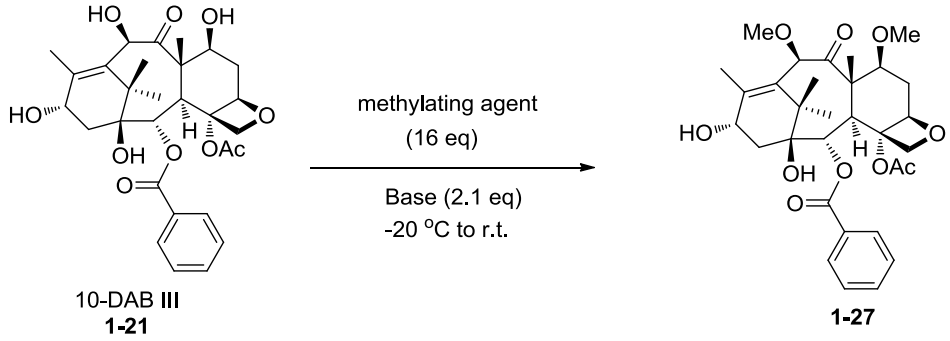


Scheme 1-15: Proposed synthetic route of SB-T-12301.

Base on SAR, the reactivity of hydroxyl group on baccatin core are followed by C-7, C-10, C-13 and C-1. Thus, dimethylation the C-7 and C-10 positions at the same might work. However, the direct dimethylation of 10-DAB-II was found to be problematic.

The methylation of C-7 and C-10 position at the same time did not obtain the desired product. We followed the reference from Didier's US patent in 1999, many methylating agents, and bases were discussed.[64] The product was successfully obtained in many different reagents. Initially we chose dimethyl sulfate and sodium hydride as our reagents for dimethylation; however, there was no reaction at all. We screened different methylating agents (dimethyl sulfate, methyl iodide, and methyl triflate), bases (sodium hydride and potassium hydride) and solvents (THF and DMF), but none of the conditions gave good outcome. They resulted in no desired product, decomposition or 10-DAB III recovered.

Table 1-1: Dimethylation of 10-deacetylbaccatin III



10-DAB III
1-21

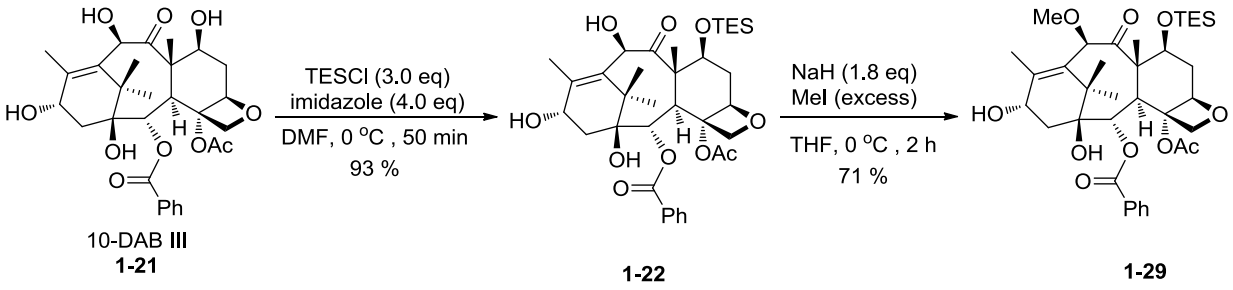
1-27

Entry	Methylating Agent	Base	Solvent	Temp.[a]	Time	Result
1	Dimethyl sulfate	NaH[b]	THF	-20°C to r.t.	6 h	SM recovered[c]
2	Methyl iodide	NaH	THF	-20°C to r.t.	9 h	No desired product[d]
3	Methyl iodide	KH	THF	-20°C to r.t.	9 h	No desired product[d]
4	Methyl triflate	NaH	THF	-20°C to r.t.	3h	Decomposed[c]
5	Methyl triflate	NaH	THF	-20°C	25 min	SM recovered[e]
6	Methyl iodide	NaH	DMF	-20 °C to 0 °C	9 h	SM recovered[e]

[a] Temperature maintained at -20 °C for 2 h, 0 °C for 2 h, and move to r.t. gradually
[b] sodium hydride (3.0 eq)
[c] 10-DAB III recovered
[d] The reaction did go, but the isolated product was not desired compound confirmed by proton NMR
[e] The mixture became sticky at 0 °C, and remained the same as warmed to r.t.

In addition, if stronger base was applied, a retro-aldol reaction might occur at C-7 position of 10-DAB III that could change the chirality of the compound. Therefore, we decided to protect the C-7 position first, methylate the C-10 position and methylate the C-7 position with less basic reagent.[65]

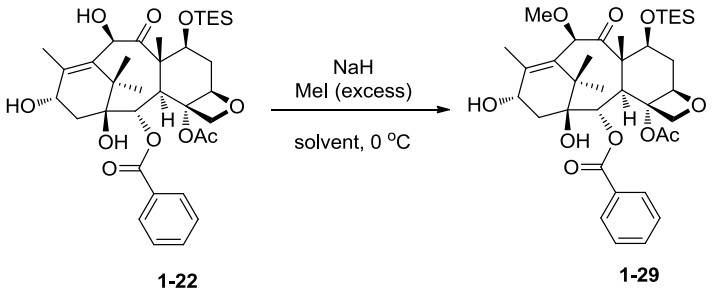
10-DAB III was treated with TES-protection using imidazole as a base. Methylation on C-10 position was used sodium hydride as base and methyl iodide as methylating agent to afford the desired product **1-29**.



Scheme 1-16: Synthesis of 10-methoxyl-7-(triethylsilyl)-10-Deacetylbaccatin III.

Optimization for C-10 methylation is shown in Table **1-2**. When less base was applied, the reaction did not progress to completion, and a greater excess of base was used, it resulted in lower yield due to formation of the dimethylated compound. It was found that using 1.8 equivalents of base and THF as solvent gave a better result. The reaction in THF was faster than which in DMF because THF is less polar compared to DMF. The epoxide is more active to undergo the methylation.

Table 1-2: Optimization for methylated condition of **1-29**



Entry	Base	Solvent	Time	Result
1	NaH (1.3 eq)	DMF	8 h	Mixture of 1-22 & 1-29
2	NaH (2.0 eq)	DMF	8 h	58% isolated yield [a]
3	NaH (2.2 eq)	DMF	7 h	51% isolated yield
4	NaH (2.0 eq)	THF	5 h	62% isolated yield
5	NaH (1.8 eq)	THF	2 h	71% isolated yield

[a] In column chromatography, starting material was observed on TLC.

Compound **1-29** underwent TES deprotection to afford compound **1-30** which had free hydroxyl group on C-7 position. The chosen base for C-7 positon is crucial because the retro-aldol reaction should be avoided. Proton sponge **(Figure 1-12)** acts as a highly selective non-nucleophilic base because of its sterically hindered structure, making it a weak nucleophile. Thus, proton sponge was applied as base in C-7 methylation and trimethyloxonium tetrafluoroborate as methylating agent.[65]

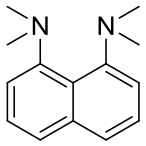
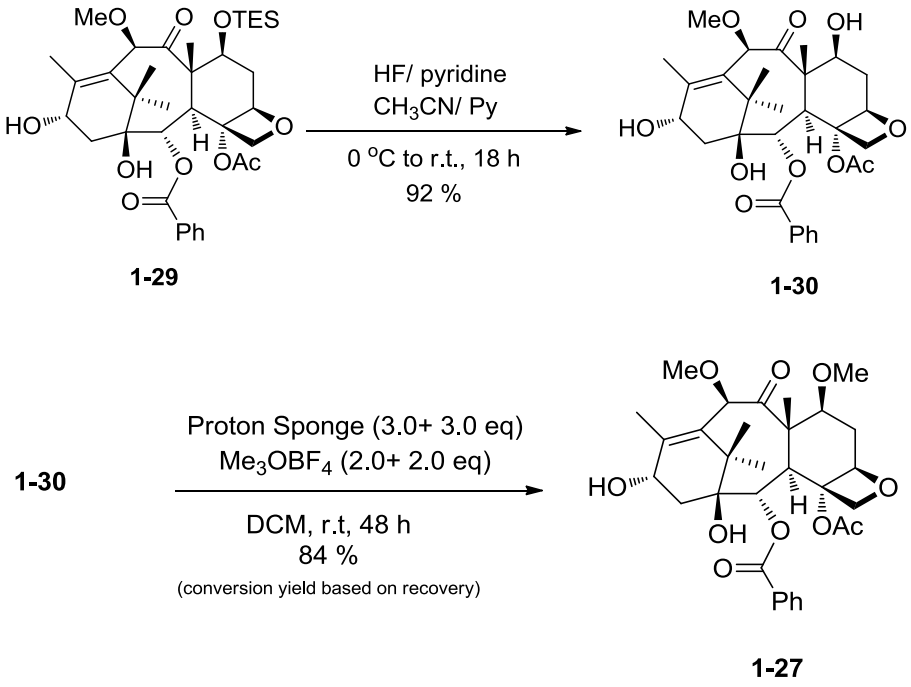


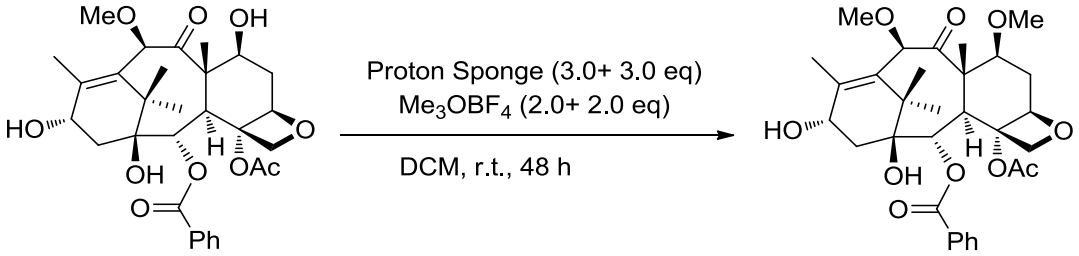
Figure 1-12: Structure of Proton sponge



Scheme 1-17: Synthesis of dimethylated 10-DAB III.

The solubility of the compound **1-30** and **1-27** were both poor in many solvents, such as dichloromethane (DCM), and THF. In order to dissolve all of the starting material, a large amount of solvent was used, but the reaction progress was slow and lots of starting material remained. The amount of the solvent was reduced, making the mixture more concentrated, and resulted in higher yield. However, the reaction still did not go to completion, and starting material **1-30** was recovered (34 %). The solubility of the product and starting material made completion of this reaction difficult.

Table 1-3: Optimization for methylated condition of compound **1-27**

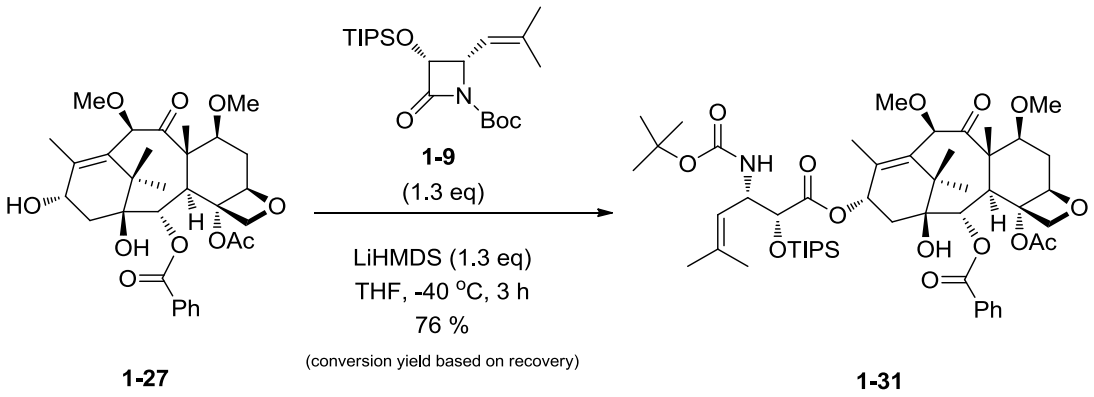


1-30 **1-27**

Entry	Solvent	Concentration (M)	Isolated Yield (%)
1	DCM	0.013[a]	12
2	DCM	0.028[b]	49
3	DCM	0.052[b]	56

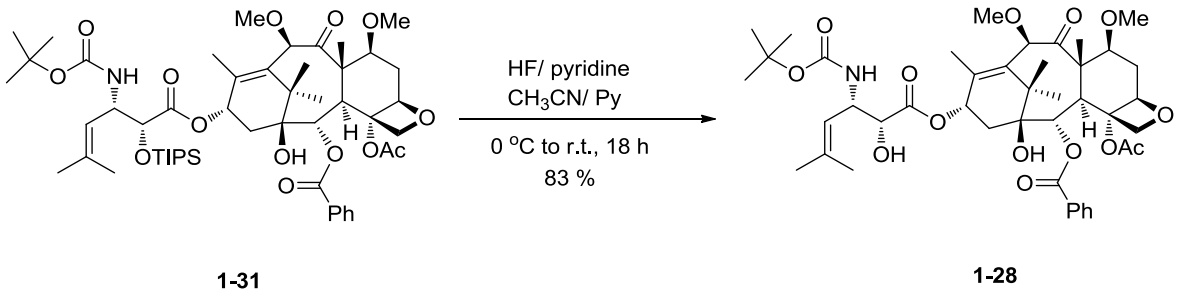
[a] All compound **1-30** was dissolved in solvent, homogeneous.
[b] Suspension condition.

The compound **1-27** was coupled with *β*-lactam **1-9** through the Ojima-Holton coupling to afford the product **1-31**. The yield of this step was low because the reaction did not go to completion after three hours. The starting material **1-27** was recovered (27 %). The reaction could be improved by longer reaction time, more concentrated solution or higher reaction temperature (no higher than 0 ^{o}C).



Scheme 1-18: Ojima-Holton *β*-lactam coupling of SB-T-12301.

The compound **1-31** underwent the deproteciton of the silyl group on the side chain by using hydrogen fluoride in pyridine to afford the final product **1-28,** SB-T-12301.



Scheme 1-19: TIP deprotection of SB-T-12301.

§1.3 Conclusion

The enantionpure β-lactam is a key intermediate of taxoid synthesis and it was successfully synthesized using two synthetic routes. One was Staudinger [2+2] cycloaddition followed by enzymatic resolution, and the other was through a chiral ester enolate-imine cyclocondensation. For the Staudinger [2+2] cycloaddition method, the enzymatic resolution was the key in the synthetic route to produce the enantiopure product, but it took long time for enzyme to hydrolyze the undesired enantiomer at lower temperature (35- 38 ^{o}C). The reaction progress was faster at higher temperature, but at the higher temperature, the selectivity of the enzyme was reduced, and could result in lower yield. Most importantly, this route wasted half of the β-lactam because it produced a racemic mixture. On the other hand, the chiral ester enolate-imine cyclocondensation gave β-lactam with excellent enantiomeric purity (98%). The reaction condition was very critical for many factors, such as purity of chiral ester, quality of base and solvent. This reaction might fail if any of the factors was not controlled well. However, it gave highly enantioenriched β-lactam and the chiral auxiliary could be recycled after the reaction. It was a better method to afford the desired β-lactam than the enzymatic resolution protocol.

Many different taxoids could be synthesized from modified 10-DAB III coupling with different β-lactam structures via the Ojima-Holton coupling reaction. The structure of baccatin skeleton was fixed, but modifications could be made at the C-2, C-7 or, C-10 positions. The β-lactam could be modified on N-position and its adjacent position, to give different analogues on the side chain of C-13. Therefore, the docetaxel, cabazitaxel, and their analogues could be obtained by the Ojima-Holton coupling reaction.

§1.4 Experimental Section

General information

All chemical were purchased from VWR International, Fisher Scientifi and Sigma-Aldrich. All reactions were carried out under nitrogen in dried glassware which were dried in a 110 ^{o}C oven and allowed to cool to room temperature in a desiccator over "Drierite" (calcium sulfate) and assembled under nitrogen gas. Dichloromethane was distilled prior to use under nitrogen from calcium hydride. Tetrahydrofuran was also freshly distilled from sodium metal and benzophenone. Toluene was also distilled immediately prior to use under nitrogen from calcium hydride. Anhydrous dimethylformamide (DMF) was used commercially available from EMD chemical company, and used without further purification. Reactions were monitored by thin layer chromatography (TLC) using E. Merck 60F254 precoated silica gel plates and alumina plates, depending on the compounds. Flash chromatography was performed with the indicated solvents using Fisher silica gel (particle size 170-400 Mesh). Yields refer to chromatographically or spectroscopically pure compounds. ^{1}H, and ^{13}C data were obtained using either 300 MHz Varian Gemni 2300 spectrometer, the 400 MHz Varian INOVA 400 spectrometer or the 500 MHz Varian INOVA 500 (125 MHz ^{13}C) in deuterated solvent. Chemical shifts (δ) are reported in ppm and standardized with solvent as internal standard based on literature reported values.[66] Melting points were measured on Thomas Hoover Capillary melting point apparatus and are uncorrected. Optical rotations were measured on Perkin-Elmer Model 241 polarimeter.

N-(4-Methoxyphenyl)-3-methyl-2-butenaldimine (1-3)[27]

To a solution of *p*-anisidine (400 mg, 3.25 mmol) and anhydrous Mg_2SO_4 (1.96 g, 16.3 mmol) in dichloromethane (8 mL), and 3-methyl-2-butenal (273 mg, 3.25 mmol) was added. The mixture was stirred at room temperature and monitored by TLC. After three hour, the reaction mixture was filtered and concentrated *in vacou* to afford compound **1-3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3 H), 2.17 (s, 3 H), 3.97 (s, 3 H), 6.37 (td, *J* = 9.6, 2.7 Hz, 1 H), 7.03–7.07 (m, 2 H), 7.26-7.30 (m, 2 H), 8.54 (d, *J* = 9.6 Hz, 1 H). All data are consistent with the reported values.[27]

(+/-)-1-(4-Methoxyphenyl)-3-acetoxyl-4-(2-methyl-2-propenyl)azetidin-2-one (1-5)[67]

To a solution of compound **1-3** (5.37 g, 0.028 mol) in dichloromethane (50 mL) was placed in inert atmosphere and cooled to -78 ^{o}C for 30 min. The mixture was added TEA (2.87 g, 0.045 mol) and followed by slow addition of acetoxyacetyl chloride 1-4 (4.66 g, 0.034 mol). The reaction mixture was stirred at -78 ^{o}C over 2 hours, and was warmed to room temperature gradually. The reaction was monitored by TLC. After completion (18 h), it was quenched with satureated NH_4Cl (30 mL), extracted with DCM (40 mL x 3) and washed with brine. The organic extract was dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography and trituration with hexanes to afford racemic product, compound **(+/-)1-5** (3.83 g, 47 % over two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79 (s, 3 H), 1.82 (s, 3 H), 2.11 (s, 3 H), 3.78 (s, 3 H), 4.97 (dd, *J* = 9.3, 4.8 Hz, 1 H), 5.13 (td, *J* = 9.3, 1.5 Hz, 1 H), 5..80 (d, *J* = 5.1 Hz, 1 H), 6.84–6.87 (m, 2 H), 7.29–7.33 (m, 2 H). All data are consistent with the reported values.[67]

Enzymatic resolution of compound 1-5[61]

To a mixture of (+/–) compound **1-5** (4.48, 0.016 mol) in phosphate buffered saline (pH 7.5) (560 mL) with 10 volume % acetonitrile/water (28 mL/ 28 mL) and heated to 30-38 ^{o}C. The reaction mixture was added 20 weight % PS-Amano Lipase (0.90 g). The reaction was stirred vigorously and monitored by ^{1}H NMR, looking for 50 % conversion of the acetate moiety to hydroxyl moiety. Upon 50 % conversion, the remaining Lipase was removed via vacuum filtration and extracted with DCM. The organic extract was washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **(+)1-5** and compound **(-)1-6**. The enantiopurity of (+)1-5 was confirmed *via* chiral HPLC analysis on normal phase with chiral OD-H column using hexanes: isopropanol (85:15, 0.6 mL/min). The enantiomeric excess value was 98 %.

(3*R*, 4*S*)-1-(4-Methoxyphenyl)-3-hydroxy-4-(2-methyl-2-propenyl)azetidin-2-one ((+)1-6)[68]

To solution of compound **(+)1-5** (2.01 g, 6.95 mmol) in THF (138 mL) was cooled to 0 ^{o}C. The reaction mixture was added 2 M KOH and monitored via TLC. After 4 hours, the reaction mixture was quenched with saturated NH_4Cl (50 mL) and extracted with DCM (70 mL). The combined organic layers were washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou* to afford compound **(+)1-6** (1.74 g, crude) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (s, 6 H), 3.78 (s, 3 H), 4.89 (dd, *J* = 6.8, 5.1 Hz, 1 H), 5.02 (d, *J* = 5.4 Hz, 1 H), 5.26 (td, *J* = 8.7, 1.5 Hz, 1 H), 6.83–6.86 (m, 2 H), 7.30–7.33 (m, 2 H). All data are consistent with the reported values.[68]

1-*p*-Methoxyphenyl-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-7)

Method A (Enantiopure *β*-Lactam via Enzymatic Resolution): To a solution of compound **(+/-)1-6** (1.74 g, 7.02 mmol), TEA (1.42 g, 14.0 mmol) and DMAP (257 mg, 2.11 mmol) in CH_2Cl_2 (77 mL) was under inert condition and cooled to 0 ^{o}C. The mixture was added chlorotriisopropylsilane (2.25 mL, 10.5 mmol), producing a yellow solution. The mixture was allowed to warm to room temperature and the reaction was monitored via TLC. After 18 hours, the reaction was quenched with saturated NH_4Cl (80 mL) and extracted with ethyl ether (80 mL x 3). The combined organic layer was washed brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The product was purified via flash silica gel column chromatography to yield the compound **1-7** (2.26 g, 80 % over two steps) as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02–1.10 (m, 21 H), 1.80 (s, 3 H), 1.85 (s, 3 H), 3.78 (s, 3 H), 4.81 (dd, *J* = 4.8, 7.1 Hz, 1 H), 5.04 (d, *J* = 6 Hz, 1 H), 5.33 (m, 1H), 6.82–6.86 (m, 2 H), 7.29–7.33 (m, 2 H). All data are consistent with the reported values.[68]

Methaod B (chiral ester enolate- imine cyclocondensation): To a solution of dry THF (1.6 mL) placed under inert atmosphere and cooled to -78 ^{o}C. The reaction mixture was added 2.0 M LDA (0.41 mL, 0.819 mmol) dropwise and stirred at -78 ^{o}C for 30 min. The reaction mixture was added compound **1-20** (200 mg, 0.512 mmol) dissolved in THF (2 mL) and stirred over 1 hour.

The mixture was added compound **1-3** (126 mg, 0.666 mmol) in THF (1 mL) over a period of 3 hour. After 2 hours, the reaction was added 1.0 M LiHMDS in THF (0.41 mL, 0.410 mmol). The reaction was allowed to warm to room temperature for 20 min. The reaction was quenched with saturated NH_4Cl (5 mL), extracted with ethyl acetate (5 mL x 3), washed with brine, dried with $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-7** (126 mg, 61 %) as light yellow solid: mp 120–125 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 1.03–1.10 (m, 21 H), 1.80 (s, 3 H), 1.85 (s, 3 H), 3.77 (s, 3 H), 4.81 (dd, *J* = 4.8, 7.1 Hz, 1 H), 5.05 (d, *J* = 6 Hz, 1 H), 5.34 (m, 1H), 6.82–6.85 (m, 2 H), 7.29–7.33 (m, 2 H). All data are consistent with the reported values.[27]

3-Triisopropylsilyoxy-4-(2-methyl-2-propenyl)azetidin-2-one (1-8)[27]

To a solution of compound **1-7** (400 mg, 0.991 mmol) in acetonitrile (17 mL) at -10 ^oC, an aqueous solution of cerium ammonium nitrate (2.17 g, 3.95 mmol) in water (17 mL) was added dropwise via additional funnel. The reaction mixture was stirred at -10 ^oC and monitored by TLC. After completion (3 h), the reaction was quenched with saturated Na_2SO_4 (10 mL), extracted with DCM (15 mL x 3), washed with H_2O, and brine. The organic extract was dried with $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-8** (210 mg, 71 %) as white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 1.04–1.12 (m, 21 H), 1.68 (d, *J* = 1.2 Hz, 3 H), 1.76 (d, *J* = 1.2 Hz, 3 H), 4.44 (dd, *J* = 7.5, 4.7 Hz, 1 H), 5.00 (dd, *J* = 4.7, 2.2 Hz, 1 H), 5.32 (dt, *J* = 9.6, 1.4 Hz, 1 H). All data are consistent with the reported values.[27]

1-(*tert*-Butoxycarbonyl)-3-triisopropylsilyoxy-4-(2-methyl-2-propenyl)azetidin-2-one (1-9)[27]

To a solution of compound **1-8** (605 mg, 2.03 mmol), DMAP (68 mg, 0.61 mmol) and triethylamine (411 mg, 4.07 mmol) in dichloromethane (11.5 mL) was cooled to 0 ^oC under inert atmosphere. The reaction mixture was added di-*tert*-butyl dicarbonate (666 mg, 3.05 mmol) dissolved in dichloromethane dropwise, and stirred over 1 hour at room temperature. After completion, the reaction was quenched with saturated NH_4Cl (7 mL) and extracted with ethyl

acetate (15 mL x 3). The organic extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacou* to afford crude oil. The product was purified via silica gel column chromatography to get compound **1-9** (750 mg, 93 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01–1.10 (m, 21 H), 1.47 (s, 9 H), 1.75 (s, 3 H), 1.77 (s, 3 H), 1.47 (s, 9 H), 4.74 (dd, *J* = 7.8, 5.8 Hz, 1 H), 4.95 (d, *J* = 5.7 Hz, 1 H), 5.27 (d, *J* = 12.1 Hz, 1 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.8, 17.5, 18.2, 26.0, 28.0, 56.8, 77.2, 82.9, 118.4, 139.6, 148.2, 166.4. All data are consistent with the reported values.[27]

(+)-(1*R*,2*R*)-1-Phenylcyclohexane-cis-1,2-diol (1-11)[62]

To a vessel containing $K_3Fe(CN)_6$ (43.8 g, 0.133 mol), anhydrous K_2CO_3 (18.4 g, 0.133 mol) and methanesulfonamide (4.20 g, 0.044 mol) was added *tert*-butanol and distilled H_2O (40 mL/60 mL), the reaction mixture was cooled to 0 ^{o}C, and $K_2OsO_4 \cdot 2H_2O$ (8.2 mg, 0.022 mmol) and (DHQD)PHAL (86.2 mg, 0.111 mmol) was added. After vigorously stirred 20 min, 1-phenylcyclohexene (7.00 g, 0.044 mol) was added dropwise. The reaction mixture was allowed to equilibrate to room temperature and stirred for two days. The appearance of the slurry gradually changed from a mixture containing red granules (ferricyanide) to yellow flakes, which were presumably a salt of iron (II). After completion (48 h), the product was exacted with ethyl acetate (50 mL x 3), filtered through medium-fritted glass and washed with ethyl acetate. The organic extract was washed vigorously with 2 M potassium hydroxide (40 mL x 2) to remove methanesulfonamide, dried over anhydrous $MgSO_4$ and concentrated *in vacou* to afford the crude diol **1-11** (8.41 g, quant. yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (m, 1 H), 1.64–1.76 (m, 3 H), 1.80–1.92 (m, 3 H), 2.59 (d, *J* = 1.8 Hz, 1 H), 7.27 (m, 1 H), 7.35–7.41 (m, 2 H), 7.50–7.53 (m, 2 H). All data are consistent with the reported values.[62]

(-)-(1*R*,2*S*)-*trans*-2-Phenyl-1-cyclohexanol (1-12)[63]

To a solution of diol **1-11** (8.00 g, 0.045 mol), added to slurry of W-2 Raney nickel in wet ethanol (70 % v/v) under inert atmosphere. The reaction mixture was heated to 100 ^{o}C for three hours and monitored by TLC. After completion, the reaction mixture was cooled to 40–50 ^{o}C, filtered through a layer of celite and washed with ethanol. The filtrate was concentrated *in vacou*

to remove most ethanol. The two-phase mixture was diluted with brine (10 mL) and extracted with ethyl acetate (50 mL x 3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacou* to obtain a white solid. The crude product was purified via recrystallization in pentane to afford compound **1-12** (3.62 g, 46 % over two steps) as a colorless crystal: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31–1.54 (m, 4 H), 1.76 (m, 1 H), 1.84–1.88 (m, 2 H), 2.11 (m, 1 H), 2.39–2.46 (m, 1 H), 3.65 (m, 1 H), 7.22–7.26 (m, 3 H), 7.33 (m, 2 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 25.0, 26.0, 33.3, 34.4, 53.2, 74.4, 126.8, 127.9, 128.8, 143.2. All data are consistent with the reported values.[63]

2-(Triisopropylsilyloxy) acetic acid succinimidyl ester (1-13)

To a solution of compound **1-19** (350mg,1.51 mmol), EDC·HCl (433 mg, 2.56 mmol) in dichloromethane (8 mL), and a solution of *N*-hydroxysuccinimide (208 mg, 1.81 mmol) in dichloromethane (4 mL). The reaction mixture was stir at room temperature and monitored via TLC. Upon completion (18 h), the reaction mixture was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo.* The crude product was purified by recrystallization with hexanes to give compound **1-13** (360 mg, 73 %) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (d, J = 6.2 Hz, 18 H), 1.11–1.18 (m, 3 H), 2.84 (s, 4 H), 4.67 (s, 2 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.8, 17.7, 25.6, 60.0, 167.1, 168.8.

Benzyl 2-hydroxyacetate (1-17)[69]

To a solution of glycolic acid **1-16** (3.00 g, 39.4 mmol) in acetone (37 mL), and triethylamine (4.38 g, 43.0 mmol) was added slowly and the reaction mixture was stir at room temperature for 10 min. The benzyl bromide **1-15** (6.13 g, 35.8 mmol) was added dropwise within 20 min. The reaction was monitored via TLC plate. After completion (18 h), the mixture was added with water (15mL), and extracted with DCM (40 x 3 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give product **1-17** (5.73 g, 84 %), as light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20 (s, 2 H), 5.23 (s, 2 H), 7.35–7.40 (m, 5 H). All data are in agreement with literature values.[69]

Benzyl 2-(triisopropylsiloxy)acetate (1-18)[70]

To a solution of compound **1-17** (4.23 g, 25.4 mmol) in dichloromethane (55 mL), was added 4-dimethylaminopyridine (311 mg, 2.54 mmol) and triethylamine (3.45 g, 33.1 mmol). The chlorotriisopropylsilane (5.40 g, 28.0 mmol) was then added dropwise. The reaction was monitored via TLC plate. After completion (5 h), the reaction mixture was quenched by NH_4Cl (10 mL) and diluted with water (50 mL). The mixture was extracted with DCM (50 mL x 3). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude was purified by silica gel column chromatography to afford compound **1-18** (6.70 g, 82 %) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06–1.14 (m, 21 H), 4.36 (s, 2 H), 5.19 (s, 2 H), 7.32–7.37 (m, 5 H), ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.9, 17.8, 62.0, 66.4, 128.4, 135.6, 171.4. All data are consistent with the reported values.[70]

2-(Triisopropylsilyloxy) acetic acid (1-19)[70]

To a solution of compound **1-18** (509 mg, 1.55 mmol) and 10 wt % palladium on carbon (20 mg, 1.2 mol %) in ethyl acetate (7 mL). The flask was vacuumed and purged with nitrogen gas three times. After that the flask was aspirated and purged with hydrogen gas three times, kept filled with hydrogen gas, and was allowed to stir at room temperature. The reaction was monitored by TLC plate. Upon completion (5 h), the reaction mixture was filtered with layer of celite to remove the catalyst. The filtrate was concentrated *in vacuo* to give crude compound **1-19** (357 mg, quant. yield) as light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04–1.12 (m, 21 H), 4.30 (s, 2 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.7, 17.7, 61.7. All data are consistent with the reported values.[70]

(1*R*, 2*S*)-*trans*-2-Phenylcyclohexyl-1-triisopropylsiloxyacetate (1-20)[34]

To a solution of compound **1-12** (500 mg, 2.84 mmol) and DMAP (520 mg, 4.26 mmol) in toluene (8.3 mL) under inert conditions was added compound **1-13** (1.12 g, 3.41 mmol). The reaction was stirred at room temperature for 72 hours. Upon completion, the solvent was evaporated and washed with brine, extracted with ethyl acetate. The combined organic layer was dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude product was further purified

by column chromatography on silica gel to afford compound 1-20 (720 mg, 65 %) as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00–1.02 (m, 21 H), 1.27–1.62 (m, 4 H), 1.75–2.00 (m, 3 H), 2.14 (m, 1 H), 2.62–2.71 (dt, *J* = 13.5, 3.9 Hz, 1 H), 3.91 (d, *J* = 16.5 Hz, 1 H), 4.08 (d, *J* = 16.5 Hz, 1 H), 5.05–5.13 (dt, *J* = 3.9, 6.5 Hz, 1 H), 7.13–7.19 (m, 3 H), 7.22–7.27 (m, 2 H). All data are consistent with the reported values.[34]

7-Triethylsilyl-10-deacetylbaccatin III (1-22)[35]

To solution of 10-deacetylbaccatin III **1-21** (500 mg, 0.918 mmol) and imidazole (250 mg, 3.67 mmol) in DMF (9.2 mL) and cooled to 0 ^{o}C. The reaction mixture was added TESCl (415 mg, 2.75 mmol) dropwise, and remained at low temperature. The reaction was monitored by TLC plate. After completion (30 min), the reaction mixture was quenched with saturated NH_4Cl (10 mL) and extracted with ethyl acetate (20 mL x 3). The organic extract was washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-22** (562 mg, 93 %) as white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.49–0.62(m, 6 H), 0.93 (t, *J* = 8.0 Hz, 9 H), 1.08 (s, 6 H), 1.73 (s, 3 H), 1.90 (m, 1 H), 2.01 (d, *J* = 4.7 Hz, 1 H), 2.08 (d, *J* = 1.1 Hz, 3H), 2.25–2.29 (m, 5 H), 2.48 (m, 1 H), 3.95 (d, *J* = 6.9 Hz, 1 H), 4.16 (d, *J* = 8.5 Hz, 1 H), 4.26 (d, *J* = 2.1 Hz, 1 H), 4.31 (d, *J* = 8.3 Hz, 1 H), 4.41 (dd, *J* = 10.7, 6.7 Hz, 1 H), 4.87 (m, 1 H), 4.95 (d, *J* = 7.8 Hz, 1 H), 5.17 (d, *J* = 2.1 Hz, 1 H), 5.60 (d, *J* = 7.0 Hz, 1 H), 7.47 (t, *J* = 7.8 Hz, 2 H), 7.59 (t, *J* = 7.5 Hz, 1 H), 8.10 (d, *J* = 7.2 Hz, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 5.1, 6.7, 9.9, 15.2, 19.5, 22.6, 26.9, 37.2, 38.6, 42.7, 46.9, 58.0, 72.9, 74.7, 74.8, 78.8, 80.7, 84.2, 128.6, 129.4, 130.1, 133.6, 135.2, 141.7, 167.0, 170.8, 210.3. All data are consistent with the reported values.[35]

10-Cyclopropylcarbonyl-7-(triethylsilyl)-10-deacetylbaccatin III (1-24)[35]

To a solution of compound **1-22** (446 mg, 0.677 mmol) in THF (5 mL) and cooled to -40 ^{o}C. The reaction mixture was added 1M LiHMDS in THF (0.71 mL, 0.71 mmol) dropwise and cyclopropanecarbonyl chloride **1-23** (74.3 mg, 0.711 mmol). The temperature of the reaction was maintained at -40 ^{o}C and the reaction was monitored by TLC plate. After completion (30 min), the reaction mixture was quenched with saturated NH_4Cl (5 mL) and extracted with ethyl acetate

(15 mL x 3). The organic extract was washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-24** (492 mg, quant. yield) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.53–0.60 (m, 6 H), 0.90–0.94 (m, 10 H), 1.00–1.04 (m, 4 H), 1.15–1.28 (m, 4 H), 1.68 (s, 3 H), 1.76 (m, 1 H), 1.85 (m, 1 H), 2.01 (m, 1 H), 2.20 (s, 3 H), 2.26–2.28 (m, 5 H), 2.52 (m, 1 H), 3.88 (d, *J* = 7.0 Hz, 1 H), 4.15 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.2 Hz, 1 H), 4.48 (dd, *J* = 8.6, 6.7 Hz, 1 H), 4.84 (m, 1 H), 4.96 (d, *J* = 8.0 Hz, 1 H), 5.64 (d, *J* = 7.0 Hz, 1 H), 6.46 (s, 1 H), 7.48 (t, *J* = 15.4 Hz, 2 H), 7.60 (t, *J* = 14.8 Hz, 1 H), 8.11 (d, *J* = 8.5 Hz, 2 H). All data are consistent with the reported values.[35]

2'-Triisoproylsily-3'-dephenyl-10-(cyclopropylcarbonyl)-3'-(2-methyl-2-propenyl)–docetaxel (1-25)[35]

To a solution of compound **1-24** (498 mg, 0.685 mmol) in anhydrous THF (10 mL) and cooled to -40 ^{o}C. The mixture was added 1.0 M LiHMDS in THF (0.89 mL, 0.891 mmol) and a solution of compound **1-9** (350 mg, 0.880 mmol) in THF (6 mL). The temperature of the reaction was maintained at -40 ^{o}C and the reaction was monitored by TLC. After completion (3 h), the reaction was quenched with saturated NH_4Cl (10 mL) and extracted with ethyl acetate (20 mL x 3). The combined organic layer was washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-25** (654 mg, 85%) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.52–0.59 (m, 6 H), 0.89–0.93 (m, 10 H), 1.10–1.11 (m, 21 H), 1.15–1.19 (m, 4 H), 1.25–1.29 (m, 4 H), 1.34 (s, 9 H), 1.68 (s, 3 H), 1.72–1.75 (m, 5 H), 1.79 (s, 3 H), 1.88 (m, 1 H), 2.01 (s, 3 H), 2.35 (s, 3 H), 2.45 (m, 2 H), 2.51 (m, 1 H), 3.84 (d, *J* = 7.0 Hz, 1 H), 4.19 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.43 (d, *J* = 2.8 Hz, 1 H), 4.46 (m, 1 H), 4.75–4.81 (m, 2 H), 4.93 (d, *J* = 8.2 Hz, 1 H), 5.33 (d, *J* = 8.6 Hz, 1 H), 5.68 (d, *J* = 7.1 Hz, 1 H), 6.08 (t, *J* = 17.6 Hz, 1 H), 6.48 (s, 1 H), 7.46 (t, *J* = 15.5 Hz, 2 H), 7.60 (t, *J* = 14.8 Hz, 1 H), 8.10 (d, *J* = 7.3 Hz, 2 H). All data are consistent with the reported values.[35]

3'-Dephenyl-10-(cyclopropylcarbonyl)-3'-(2-methyl-2-propenyl)docetaxel [SB-T-1214] (1-26)[35]

To a solution of compound **1-25** (505 mg, 0.499 mmol.) in acetonitrile (8 mL) and pyridine (8 mL) and cooled to -0 ^{o}C. The reaction mixture was added 1M HF/pyridine (5 mL) dropwise at 0 ^{o}C, and allowed to warm to room temperature gradually. The reaction was monitored by TLC plate. After completion (20 h), the reaction was quenched with saturated $NaHCO_3$ (10 mL) and extracted with ethyl acetate (15 mL x 3). The aqueous phase was treated with solid potassium hydroxide to adjust the pH value to 7-8 and extracted with ethyl acetate (15 mL x 3). The first and second organic phases were combined together and washed with saturated copper sulfate, brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-26** (357mg, 93%) as white solid: mp 178–179 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13–1.16 (m,5 H), 1.35 (s, 9 H), 1.67 (s, 3 H), 1.77 (m, 8 H), 1.83–1.92 (m, 4 H), 2.04 (s, 1 H), 2.35–2.38 (m, 5 H), 2.50–2.58(m, 2 H), 3.39 (s, 1 H), 3.81 (d, *J* = 6.8 Hz, 1 H), 4.19 (m, 2 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.42 (m, 1 H), 4.74–4.80(m, 2 H), 4.96 (d, *J* = 8.2 Hz, 1 H), 5.32 (d, *J* = 7.6 Hz, 1 H), 5.67 (d, *J* = 7.2 Hz, 1 H), 6.17 (t, *J* = 17.0 Hz, 1 H), 6.30 (s, 1 H), 7.47 (t, *J* = 15.4 Hz, 2 H), 7.61 (t, *J* = 14.8 Hz, 1 H), 8.10 (d, *J* = 7.3 Hz, 2 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 9.2, 9.4, 9.5, 13.0,, 14.2, 15.0, 18.6, 22.0, 22.4, 25.7, 26.7, 28.2, 35.5, 35.6, 43.2, 45.6, 51.6, 58.6, 60.4, 72.2, 72.4, 79.2, 80.0, 81.0, 84.5, 120.6, 128.6, 129.2, 130.1, 132.9, 133.7, 137.9, 142.7, 155.5, 166.9, 170.1, 175.1, 203.9. All data are consistent with the reported values.[35]

10-Methoxyl-7-(methoxyl)-10-deacetylbaccatin III (1-27)[64,65]

A mixture of compound **1-30** (82.3 mg, 0.147 mmol), 1,8-Bis(dimethylamino) naphthalene (proton sponge) (94.7 mg, 0.442 mmol), trimethyloxonium tetrafluoroborate (43.6 mg, 0.295mmol) and molecular sieve (4 Å, 83mg) in (1.3 mL) was stirred for 24 hours at room temperature, and another portion of proton sponge (94.7 mg) and trimethyloxonium tetrafluoroborate (43.6 mg) was added. The mixture was stirred at room temperature for another 24 hours. The mixture was extracted with ethyl acetate (5 mL x 3). The combined organic layers were washed brine, dried over anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-27** (46.9 mg, 84%) as

white solid, and the compound **1-30** (28 mg, 34%) was recovered: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (s, 3 H), 1.09 (s, 3 H), 1.56 (s, 1 H), 1.71 (s, 3 H), 1.78 (m, 1 H), 2.02 (m, 1 H), 2.11 (d, *J* = 1.3 Hz, 3 H), 2.25–2.29 (m, 4 H), 2.72 (m, 1 H), 3.32 (s, 3 H), 3.47 (s, 3 H), 3.89–3.95 (m, 2 H), 4.15 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.3 Hz, 1 H), 4.84 (s, 1 H), 4.89 (m, 1 H), 5.00 (d, *J* = 8.7 Hz, 1 H), 5.59 (d, *J* = 8.8 Hz, 1 H), 7.47 (d, *J* = 7.7 Hz, 2 H), 7.60 (tt, *J* = 9.1, 1.5 Hz, 1 H), 8.09–8.11 (m,2 H). All data are consistent with the reported values.[64,65]

3'-Dephenyl-3'-(2-methyl-2-propenyl)cabazitaxel [SB-T-12301] (1-28)

To a solution of compound **1-31** (17.2 mg, 0.018 mmol) in acetonitrile (0.5 mL) and pyridine (0.5 mL) and cooled to -0 ^{o}C. The reaction mixture was added 1 M HF/pyridine (0.2 mL) dropwise at 0 ^{o}C, and allowed to warm to room temperature gradually. The reaction was monitored by TLC plate. After completion (18 h), the reaction was dilute with water (3 mL) and quenched with saturated $NaHCO_3$ (10 mL) and extracted with ethyl acetate (15 mL x 3). The combined organic layers were washed with saturated copper sulfate, brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou.* The product was purified using silica gel column chromatography to afford compound **1-28** (12mg, 83%) as white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21 (s, 6 H), 1.37 (s, 9 H), 1.48 (s, 1 H), 1.63 (s, 1 H), 1.71 (s, 3H), 1.77 (s, 6 H), 1.81 (m, 1 H), 2.00 (s, 3 H), 2.29 (m, 1 H), 2.36 (s, 3 H), 2.40 (m, 1 H), 2.70 (m, 1 H), 3.30 (s, 3 H), 3.42 (d, *J* = 6.8 Hz, 1 H), 3.45 (s, 3 H), 3.83 (d, *J* = 6.9 Hz, 1 H), 3.87 (dd, *J* = 10.7, 6.6 Hz, 1 H), 4.17 (d, *J* = 8.5 Hz, 1 H), 4.23 (dd, *J* = 6.7, 2.8 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1H), 4.76 (m, 1 H), 4.81 (m, 2 H), 4.98 (d, *J* = 8.4, 1 H), 5.30 (d, *J* = 9.7 Hz, 1 H), 5.63 (d, *J* = 7.0 Hz, 1 H), 6.17 (t, *J* = 8.6 Hz, 1 H), 7.47 (t, *J* = 7.8, 2 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 8.09 (d, *J* = 7.3 Hz, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 10.3, 11.4, 14.1, 14.8, 18.6, 20.6, 22.5, 22.7, 25.3, 25.8, 26.2, 26.7, 27.9, 28.2, 29.7, 31.6, 31.9, 32.1, 34.6, 35.3, 43.3, 47.4, 51.5, 56.9, 57.0, 57.3, 72.4, 73.7, 74.6, 78.8, 79.9, 80.7, 81.7, 82.6, 84.1, 120.6, 128.6, 129.3, 130.1, 133.6, 135.4, 139.0, 155.4, 166.9, 170.3, 205.0.

10-Methoxyl-7-(triethylsilyl)-10-deacetylbaccatin III (1-29)[65]

A solution of compound **1-22** (150 mg, 0.228 mmol) and 60 % sodium hydride (16.4 mg, 0.410 mmol) in THF (6.6 mL) at 0 ^{o}C, and methyl iodide (0.94 mL) was added. The reaction mixture

was stirred at 0 ^{o}C and monitored via TLC. After completion (2 h), the mixture was quenched by saturated NH_4Cl (3 mL), and extracted with ethyl acetate (10 mL x 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified using silica gel column chromatography to afford compound **1-29** (108 mg, 71%) as white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 053–0.62 (m, 6 H), 1.00 (t, *J* = 8.0 Hz, 9 H), 1.07 (s, 3 H), 1.18 (s, 3 H), 1.58 (s, 1 H), 1.68 (s, 3 H), 1.86–1.91 (m, 1 H), 2.02 (m, 1 H), 2.11 (d, *J* = 1.2 Hz, 3 H), 2.27 (m, 4 H), 2.46–2.52 (m, 1 H), 3.41 (s, 3 H), 3.88 (d, *J* = 7.0 Hz, 1 H), 4.15 (d, *J* = 8.3 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.43 (dd, *J* = 10.5, 6.7 Hz, 1 H), 4.90–5.00 (m, 3 H), 5.60 (d, *J* = 7.0 Hz, 1 H), 7.47 (t, *J* = 7.8 Hz, 2 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 8.08–8.11 (m, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ5.4, 6.8, 9.9, 14.1,15.2, 19.5, 22.6, 22.7, 25.3, 26.8, 31.6, 34.6, 37.3, 38.3, 42.8, 47.3, 55.8, 58.2, 68.0, 72.9, 74.8, 78.8, 81.0, 82.7, 84.3, 128.5, 129.4, 130.1, 133.5, 142.9, 167.1, 170.8, 204.4. All data are consistent with the reported values.[65]

10-Methoxyl-10-deacetylbaccatin III (1-30)[65]

To a solution of compound **1-29** (102 mg, 0.152 mmol) in acetonitrile (1.4 mL) and pyridine (1 mL) and cooled to -0 ^{o}C. The reaction mixture was added 1 M HF/pyridine (0.7 mL) dropwise at 0 ^{o}C, and allowed to warm to room temperature gradually. The reaction was monitored by TLC plate. After completion (18 h), the reaction was dilute with water (3 mL) and quenched with saturated $NaHCO_3$ (10 mL) and extracted with ethyl acetate (15 mL x 3). The combined organic layers were washed with saturated copper sulfate, brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou* to afford compound **1-30** (78 mg, 92%) as white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.08 (s, 3H), 1.17 (s, 3H), 1.39 (d, *J* = 8.8 Hz, 1 H), 1.57 (s, 1 H), 1.68 (s, 3 H), 1.77–1.82 (m, 1 H), 2.03 (d, *J* = 4.9 Hz, 1 H), 2.88 (d, *J* = 0.9 Hz, 3 H), 2.29 (m, 5 H), 2.57–2.63 (m, 1 H), 3.46 (s, 3 H), 3.95 (d, *J* = 7.1 Hz, 1 H), 4.15 (d, *J* = 8.4 Hz, 1 H), 4.26–4.32 (m, 2 H), 4.91 (m, 1 H), 4.97 (m, 2 H), 5.64 (d, *J* = 7.2 Hz, 1 H), 7.48 (t, *J* = 7.8 Hz, 2 H), 7.61 (t, *J* = 7.4 Hz, 1 H), 8.11 (d, *J* = 7.2 Hz, 2 H). All data are consistent with the reported values.[65]

2'-Triisoproylsily-3'-dephenyl-3'-(2-methyl-2-propenyl)cabazitaxel (1-31)

To a solution of compound **1-27** (22.5 mg, 0.039 mmol) in anhydrous THF (0.5 mL) was cooled to -40 ^{o}C. The mixture was added 1.0 M LiHMDS in THF (0.06 mL, 0.059 mmol) and a solution of **1-9** (20 mg, 0.050 mmol) in THF (0.5 mL). The temperature of the reaction was maintained at -40 ^{o}C and the reaction was monitored by TLC plate. After 3 hours, the reaction was quenched with saturated NH_4Cl (2 mL) and extracted with ethyl acetate (10 mL x 3). The combined organic layer was washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **1-31** (21 mg, 76 %) as white solid and starting material **1-27** (6.1 mg, 27 %) was recovered: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.06–1.14(m, 21 H), 1.19 (s, 3 H), 1.21 (s, 3 H), 1.34 (s, 9 H), 1.42 (m, 1 H), 1.59 (s, 1 H), 1,71 (m, 4 H), 1.76 (s, 3 H), 1.80 (m, 4 H), 1.95 (s, 3 H), 2.36 (m, 4 H), 2.69 (m, 1 H), 3.30 (s, 3 H), 3.45 (s, 3 H), 3.86–3.90 (m, 2 H), 4.19 (d, *J* = 8.5 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.44 (d, *J* = 2.7 Hz, 1 H), 4.80 (m, 3 H), 4.98 (d, *J* = 8.3 Hz, 1 H), 5.33 (d, *J* = 8.8 Hz, 1 H), 5.64 (d, *J* = 7.1 Hz, 1 H), 6.13 (t, *J* = 8.8 Hz, 1 H), 7.45 (t, *J* = 7.8 Hz, 2 H), 7.60 (t, *J* = 7.4 Hz, 1 H), 8.09 (d, *J* = 7.3 Hz, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 10.4, 12.5, 14.1, 14.6, 18.0, 18.6, 21.1, 22.6, 25.3, 25.7, 26.4, 28.2, 30.0, 31.6, 31.9, 35.4, 43.3, 47.3, 52.1, 56.7, 57.1, 57.2, 72.2, 74.8, 75.2, 76.5, 78.9, 80.6, 81.6, 82.5, 84.2, 122.3, 128.5, 129.4, 130.1, 133.5, 134.8, 139.8, 155.2, 166.8, 169.9, 172.0, 205.0.

§1.5 Reference

(1) Evan, G. I.; Vousden, K. H.: Proliferation, cell cycle and apoptosis in cancer. *Nature* **2001**, *411*, 342-8.

(2) Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D.: Global Cancer Statistics. *Ca-Cancer J Clin* **2011**, *61*, 69-90.

(3) Siegel, R.; Naishadham, D.; Jemal, A.: Cancer statistics, 2013. *Ca-Cancer J Clin* **2013**, *63*, 11-30.

(4) Blattman, J. N.; Greenberg, P. D.: Cancer immunotherapy: a treatment for the masses. *Science* **2004**, *305*, 200-5.

(5) Manfredi, J. J.; Horwitz, S. B.: Taxol - an Antimitotic Agent with a New Mechanism of Action. *Pharmacol Therapeut* **1984**, *25*, 83-125.

(6) Ulukan, H.; Swaan, P. W.: Camptothecins: a review of their chemotherapeutic potential. *Drugs* **2002**, *62*, 2039-57.

(7) Nicolaou, K. C.; Guy, R. K.; Potier, P.: Taxoids: new weapons against cancer. *Scientific American* **1996**, *274*, 94-8.

(8) Schiff, P. B.; Fant, J.; Horwitz, S. B.: Promotion of microtubule assembly in vitro by taxol. *Nature* **1979**, *277*, 665-7.

(9) Wall, M. E.; Wani, M. C.: Camptothecin and taxol: discovery to clinic--thirteenth Bruce F. Cain Memorial Award Lecture. *Cancer research* **1995**, *55*, 753-60.

(10) Kastan, M. B.; Bartek, J.: Cell-cycle checkpoints and cancer. *Nature* **2004**, *432*, 316-23.

(11) Vermeulen, K.; Van Bockstaele, D. R.; Berneman, Z. N.: The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell proliferation* **2003**, *36*, 131-49.

(12) Guenard, D.; Guerittevoegelein, F.; Potier, P.: Taxol and Taxotere - Discovery, Chemistry, and Structure-Activity-Relationships. *Accounts Chem Res* **1993**, *26*, 160-167.

(13) Guerittevoegelein, F.; Guenard, D.; Mangatal, L.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C.: Structure of a Synthetic Taxol Precursor - N-Tert-Butoxycarbonyl-10-Deacetyl-N-Debenzoyltaxol. *Acta Crystallogr C* **1990**, *46*, 781-784.

(14) Bissery, M. C.; Guenard, D.; Guerittevoegelein, F.; Lavelle, F.: Experimental Antitumor-Activity of Taxotere (Rp-56976, Nsc-628503), a Taxol Analog. *Cancer research* **1991**, *51*, 4845-4852.

(15) Lyseng-Williamson, K. A.; Fenton, C.: Docetaxel: a review of its use in metastatic breast cancer. *Drugs* **2005**, *65*, 2513-31.

(16) Belani, C. P.; Eckardt, J.: Development of docetaxel in advanced non-small-cell lung cancer. *Lung cancer* **2004**, *46 Suppl 2*, S3-11.

(17) Dagher, R.; Li, N.; Abraham, S.; Rahman, A.; Sridhara, R.; Pazdur, R.: Approval summary: Docetaxel in combination with prednisone for the treatment of androgen-independent hormone-refractory prostate cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2004**, *10*, 8147-51.

(18) Galsky, M. D.; Dritselis, A.; Kirkpatrick, P.; Oh, W. K.: Cabazitaxel. *Nature reviews. Drug discovery* **2010**, *9*, 677-8.

(19) Paller, C. J.; Antonarakis, E. S.: Cabazitaxel: a novel second-line treatment for metastatic castration-resistant prostate cancer. *Drug Des Dev Ther* **2011**, *5*, 117-124.

(20) Villanueva, C.; Bazan, F.; Kim, S.; Demarchi, M.; Chaigneau, L.; Thiery-Vuillemin, A.; Nguyen, T.; Cals, L.; Dobi, E.; Pivot, X.: Cabazitaxel A Novel Microtubule Inhibitor. *Drugs* **2011**, *71*, 1251-1258.

(21) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J.: Total Synthesis of Taxol. *Nature* **1994**, *367*, 630-634.

(22) Holton, R. A.; Kim, H. B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S. C.; Nadizadeh, H.; Suzuki, Y.; Tao, C. L.; Vu, P.; Tang, S. H.; Zhang, P. S.; Murthi, K. K.; Gentile, L. N.; Liu, J. H.: First Total Synthesis of Taxol .2. Completion of the C-Ring and D-Ring. *J Am Chem Soc* **1994**, *116*, 1599-1600.

(23) Holton, R. A.; Somoza, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S. C.; Nadizadeh, H.; Suzuki, Y.; Tao, C. L.; Vu, P.; Tang, S. H.; Zhang, P. S.; Murthi, K. K.; Gentile, L. N.; Liu, J. H.: First Total Synthesis of Taxol .1. Functionalization of the B-Ring. *J Am Chem Soc* **1994**, *116*, 1597-1598.

(24) Denis, J. N.; Greene, A. E.; Guenard, D.; Guerittevoegelein, F.; Mangatal, L.; Potier, P.: A Highly Efficient, Practical Approach to Natural Taxol. *J Am Chem Soc* **1988**, *110*, 5917-5919.

(25) Guerittevoegelein, F.; Senilh, V.; David, B.; Guenard, D.; Potier, P.: Chemical Studies of 10-Deacetyl Baccatin .3. Hemisynthesis of Taxol Derivatives. *Tetrahedron* **1986**, *42*, 4451-4460.

(26) Ojima, I.; Habus, I.; Zhao, M. Z.; Georg, G. I.; Jayasinghe, L. R.: Efficient and Practical Asymmetric-Synthesis of the Taxol C-13 Side-Chain, N-Benzoyl-(2r,3s)-3-Phenylisoserine, and Its Analogs Via Chiral 3-Hydroxy-4-Aryl-Beta-Lactams through Chiral Ester Enolate-Imine Cyclocondensation. *J Org Chem* **1991**, *56*, 1681-1683.

(27) Ojima, I.; Slater, J. C.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C. M.; Park, Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J.: Syntheses and structure-activity relationships of taxoids derived from 14 beta-hydroxy-10-deacetylbaccatin III. *J Med Chem* **1997**, *40*, 267-278.

(28) Allen, H. K.; Donato, J.; Wang, H. H.; Cloud-Hansen, K. A.; Davies, J.; Handelsman, J.: Call of the wild: antibiotic resistance genes in natural environments. *Nature reviews. Microbiology* **2010**, *8*, 251-9.

(29) Dean, M.; Fojo, T.; Bates, S.: Tumour stem cells and drug resistance. *Nature reviews. Cancer* **2005**, *5*, 275-84.

(30) Gottesman, M. M.: Mechanisms of cancer drug resistance. *Annual review of medicine* **2002**, *53*, 615-27.

(31) Croop, J. M.: Evolutionary relationships among ABC transporters. *Methods in enzymology* **1998**, *292*, 101-16.

(32) Sorrentino, B. P.: Gene therapy to protect haematopoietic cells from cytotoxic cancer drugs. *Nature reviews. Cancer* **2002**, *2*, 431-41.

(33) Ojima, I.: Recent Advances in the Beta-Lactam Synthon Method. *Accounts Chem Res* **1995**, *28*, 383-389.

(34) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T.: New and Efficient Approaches to the Semisynthesis of Taxol and Its C-13 Side-Chain Analogs by Means of Beta-Lactam Synthon Method. *Tetrahedron* **1992**, *48*, 6985-7012.

(35) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P. Y.; Vrignaud, P.; Bissery, M. C.; Veith, J. M.; Pera, P.; Bernacki, R. J.: Syntheses and structure-activity

relationships of the second-generation antitumor taxoids: Exceptional activity against drug-resistant cancer cells. *J Med Chem* **1996**, *39*, 3889-3896.

(36) Kingston, D. G. I.: Recent advances in the chemistry of taxol. *J Nat Prod* **2000**, *63*, 726-734.

(37) Geney, R.; Ungureanu l, M.; Li, D.; Ojima, I.: Overcoming multidrug resistance in taxane chemotherapy. *Clinical chemistry and laboratory medicine : CCLM / FESCC* **2002**, *40*, 918-25.

(38) Kingston, D. G.; Jagtap, P. G.; Yuan, H.; Samala, L.: The chemistry of taxol and related taxoids. *Fortschritte der Chemie organischer Naturstoffe = Progress in the chemistry of organic natural products. Progres dans la chimie des substances organiques naturelles* **2002**, *84*, 53-225.

(39) Chen, S. H.; Farina, V.; Vyas, D. M.; Doyle, T. W.: Synthesis and biological evaluation of C-13 amide-linked paclitaxel (Taxol) analogs. *J Org Chem* **1996**, *61*, 2065-2070.

(40) Geney, R.; Sun, L.; Pera, P.; Bernacki, R. J.; Xia, S. J.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I.: Use of the tubulin bound paclitaxel conformation for structure-based rational drug design. *Chem Biol* **2005**, *12*, 339-348.

(41) Guerittevoegelein, F.; Guenard, D.; Lavelle, F.; Legoff, M. T.; Mangatal, L.; Potier, P.: Relationships between the Structure of Taxol Analogs and Their Antimitotic Activity. *J Med Chem* **1991**, *34*, 992-998.

(42) Georg, G. I.; Harriman, G. C. B.; Hepperle, M.; Clowers, J. S.; VanderVelde, D. G.; Himes, R. H.: Synthesis, conformational analysis, and biological evaluation of heteroaromatic taxanes. *J Org Chem* **1996**, *61*, 2664-2676.

(43) Ojima, I.; Duclos, O.; Zucco, M.; Bissery, M. C.; Combeau, C.; Vrignaud, P.; Riou, J. F.; Lavelle, F.: Synthesis and Structure-Activity-Relationships of New Antitumor Taxoids - Effects of Cyclohexyl Substitution at the C-3' and/or C-2 of Taxotere (Docetaxel). *J Med Chem* **1994**, *37*, 2602-2608.

(44) Ojima, I.; Lin, S. N.: Efficient asymmetric syntheses of beta-lactams bearing a cyclopropane or an epoxide moiety and their application to the syntheses of novel isoserines and taxoids. *J Org Chem* **1998**, *63*, 224-225.

(45) Ojima, I.; Lin, S. N.; Wang, T.: Recent advances in the medicinal chemistry of taxoids with novel beta-amino acid side chains. *Curr Med Chem* **1999**, *6*, 927-954.

(46) Kingston, D. G. I.: The Chemistry of Taxol. *Pharmacol Therapeut* **1991**, *52*, 1-34.

(47) Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A.: The Chemistry of Taxol, a Clinically Useful Anticancer Agent. *J Nat Prod* **1990**, *53*, 1-12.

(48) Spletstoser, J. T.; Turunen, B. J.; Desino, K.; Rice, A.; Datta, A.; Dutta, D.; Huff, J. K.; Himes, R. H.; Audus, K. L.; Seelig, A.; Georg, G. I.: Single-site chemical modification at C10 of the baccatin III core of paclitaxel and Taxol C reduces P-glycoprotein interactions in bovine brain microvessel endothelial cells. *Bioorg Med Chem Lett* **2006**, *16*, 495-498.

(49) Kant, J.; Okeeffe, W. S.; Chen, S. H.; Farina, V.; Fairchild, C.; Johnston, K.; Kadow, J. F.; Long, B. H.; Vyas, D.: A Chemoselective Approach to Functionalize the C-10 Position of 10-Deacetylbaccatin-Iii - Synthesis and Biological Properties of Novel C-10 Taxol(R) Analogs. *Tetrahedron Lett* **1994**, *35*, 5543-5546.

(50) Ojima, I.: Tumor-targeting drug delivery of chemotherapeutic agents. *Pure Appl Chem* **2011**, *83*, 1685-1698.

(51) Ojima, I.; Zuniga, E. S.; Berger, W. T.; Seitz, J. D.: Tumor-targeting drug delivery of new-generation taxoids. *Future medicinal chemistry* **2012**, *4*, 33-50.

(52) Kingston, D. G. I.; Chaudhary, A. G.; Chordia, M. D.; Gharpure, M.; Gunatilaka, A. A. L.; Higgs, P. I.; Rimoldi, J. M.; Samala, L.; Jagtap, P. G.; Giannakakou, P.; Jiang, Y. Q.; Lin, C. M.; Hamel, E.; Long, B. H.; Fairchild, C. R.; Johnston, K. A.: Synthesis and biological evaluation of 2-acyl analogues of paclitaxel (Taxol). *J Med Chem* **1998**, *41*, 3715-3726.

(53) Ojima, I.; Wang, T.; Miller, M. L.; Lin, S. N.; Borella, C. P.; Geng, X. D.; Pera, P.; Bernacki, R. J.: Synthesis and structure-activity relationships of new second-generation taxoids. *Bioorg Med Chem Lett* **1999**, *9*, 3423-3428.

(54) Holten, K. B.; Onusko, E. M.: Appropriate prescribing of oral beta-lactam antibiotics. *Am Fam Physician* **2000**, *62*, 611-620.

(55) Cossio, F. P.; Arrieta, A.; Sierra, M. A.: The mechanism of the ketene-imine (staudinger) reaction in its centennial: still an unsolved problem? *Acc Chem Res* **2008**, *41*, 925-36.

(56) Cossio, F. P.; Ugalde, J. M.; Lopez, X.; Lecea, B.; Palomo, C.: A Semiempirical Theoretical-Study on the Formation of Beta-Lactams from Ketenes and Imines. *J Am Chem Soc* **1993**, *115*, 995-1004.

(57) Lopez, R.; Sordo, T. L.; Sordo, J. A.; Gonzalez, J.: Torquoelectronic Effect in the Control of the Stereoselectivity of Ketene Imine Cycloaddition Reactions. *J Org Chem* **1993**, *58*, 7036-7037.

(58) Cossio, F. P.; Arrieta, A.; Lecea, B.; Ugalde, J. M.: Chiral Control in the Staudinger Reaction between Ketenes and Imines - a Theoretical Scf-Mo Study on Asymmetric Torquoselectivity. *J Am Chem Soc* **1994**, *116*, 2085-2093.

(59) Das, M.: Design, Synthesis and Biological Evaluation of Novel Tumor-targeting Taxane-based Drug Delivery Systems. Stony Brook University, 2010.

(60) Staudinger, H.: Announcements from the chemical instiute at Strasbourg University in Alsace, France - Ketene. *Liebigs Ann Chem* **1907**, *356*, 51-123.

(61) Brieva, R.; Crich, J. Z.; Sih, C. J.: Chemoenzymatic Synthesis of the C-13 Side-Chain of Taxol - Optically-Active 3-Hydroxy-4-Phenyl Beta-Lactam Derivatives. *J Org Chem* **1993**, *58*, 1068-1075.

(62) Kolb, H. C.; Vannieuwenhze, M. S.; Sharpless, K. B.: Catalytic Asymmetric Dihydroxylation. *Chem Rev* **1994**, *94*, 2483-2547.

(63) Gonzalez, J.; Aurigemma, C.; Truesdale, L.: Synthesis of (+)-(1S,2R)- and (-)-(1R,2S)-trans-2-phenylcyclohexanol via Sharpess Asymmetric Dihydroxylation. . *Organic Syntheses* **2002**, *79*, 93.

(64) Didier, E. L., P.; Oddon, G.; Pauze, D.; Riguet, D.: Process for preparing derivatives of the taxoid family. Patent, U. S., Ed., Oct 5, 1999; Vol. 5,962,705.

(65) Zhang, G.; Fang, W.: A new synthesis route of cabazitaxel. *Journal of Chinese Pharmaceutical Sciences* **2012**, *21*, 471–476.

(66) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A.: NMR chemical shifts of common laboratory solvents as trace impurities. *J Org Chem* **1997**, *62*, 7512-7515.

(67) Lin, S. N.; Geng, X. D.; Qu, C. X.; Tynebor, R.; Gallagher, D. J.; Pollina, E.; Rutter, J.; Ojima, I.: Synthesis of highly potent second-generation taxoids through effective kinetic resolution coupling of racemic beta-lactams with baccatins. *Chirality* **2000**, *12*, 431-441.

(68) Kuznetsova, L.; Ungureanu, I. M.; Pepe, A.; Zanardi, I.; Wu, X. Y.; Ojima, I.: Trifluoromethyl- and difluoromethyl-beta-lactams as useful building blocks for the synthesis of fluorinated amino acids, dipeptides, and fluoro-taxoids. *Journal of fluorine chemistry* **2004**, *125*, 487-500.

(69) Barrish, J. C.; Lee, H. L.; Mitt, T.; Pizzolato, G.; Baggiolini, E. G.; Uskokovic, M. R.: Total Synthesis of Pseudomonic Acid-C. *J Org Chem* **1988**, *53*, 4282-4295.

(70) Yu, H. W.; Ballard, C. E.; Boyle, P. D.; Wang, B. H.: An inexpensive carbohydrate derivative used as a chiral auxiliary in the synthesis of alpha-hydroxy carboxylic acids. *Tetrahedron* **2002**, *58*, 7663-7679.

Chapter 2

Synthesis of Taxoid-Based Tumor-Targeting Drug Conjugates

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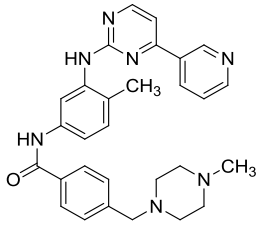
§2.1 Introduction

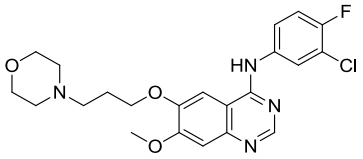
Except MDR leads to drug ineffectiveness (Chapter 1), lack of tumor specificity was another challenge for chemotherapy which would result in severe side effects. The premise of chemotherapy is that rapidly proliferating cancer cells would be killed by the cytotoxic agents. However, the cytotoxic agents have little or no specificity which means that the chemotherapeutic agent could harm some normal cells which also proliferated fast, such as hair cells and immune cells, resulting in severe side effects, such as hair loss, anemia, damage to kidney and bone marrow. Therefore, targeted cancer therapy and tumor specific drug delivery system (DDS) have been developed to solve the problem.

§2.1.1 Targeted Cancer Therapy

Targeted cancer therapy is using drugs which specially target cancer cells to disrupt their growth, and result in apoptosis.[1] These drugs only target to cancer cells because they recognize some biomarkers of cancer cells which normal cells do not have any. For instance, imatinib mesylate (Gleevec®)[2] was approved by FDA for the treatment of chronic myeloid leukemia (CML) in 2001, and malignant gastrointestinal stromal tumors (GISTs).[3] Imatinib mesylate is a tyrosine kinase inhibitor, which inhibit the Bcr-Abl tyrosine kinase that overexpressed in CML cells.[4] Since this enzyme only exists in cancer cells and not in the normal healthy cells, imatinib mesylate only affects the growth of cancer cells and leading to apoptosis. Furthermore, gefitinib (Iressa®) is an inhibitor of epidermal growth factor receptor (EGFR), and shows good response in treatment of non-small cell lung cancer (NSCLC). It has been proven that mutant EGFR in NSCLCs would activate anti-apoptotic pathways and induce proliferation.[5,6] Thus, gefitnib targets to ECFR, inhibits its kinase activity, and stops cell proliferation. Both Gleevec and Iressa are good examples for targeted cancer therapy.



Imatinib Mesylate (Gleevec®)



Gefitinib (Iressa®)

Figure 2-1: Targeted cancer therapeutic agents.

§2.1.2 Tumor-Targeting Drug Delivery System

Whereas targeted cancer therapy, the development of new and effective tumor-targeting drug delivery system is urgently necessary. Thus, chemotherapeutic agents only target to cancer cells and reduce the side effects. In last few decades, various drug delivery protocol and systems have been explored.[7] In general, tumor-targeting drug-delivery system consists of a cytotoxic agent and a tumor-targeting moiety which can be combined directly or be bridged by a suitable linker to form a conjugate. The conjugate, regarded as "prodrug", should be systemically non-toxic, stable in circulation, and delivered to tumor cells specifically.[8] As the tumor-targeting moiety (TTM) is recognized by receptors of the tumor cells, the conjugate can be internalized into the cells and cleaved to regenerate the active cytotoxic agent.

Cancer cells overexpress many tumor-specific receptors, which could be used as biomarker to deliver chemotherapeutic agents into cancer cells. It could be introduced to target the cancer cells and delivered the cytotoxic agent specifically. In other words, these drug conjugates identify and utilize the innate morphological and physiological difference between the normal and cancer cells.[9] For instance, tumor cells overexpress particular receptors to increase the absorption of nutrients and vitamins for fast proliferation. These TTMs could be targeted to delivery chemotherapeutic agents into cancer cells through receptor-mediated endocytosis (RME), such as monoclonal antibodie,[10,11] polyunsaturated fatty acid,[12,13] folic acid[14] and vitamin.[15] Those tumor-targeting molecules should have high tumor specificity and high affinity for tumor-specific receptor, and could be linked to cytotoxic drug by a linker.

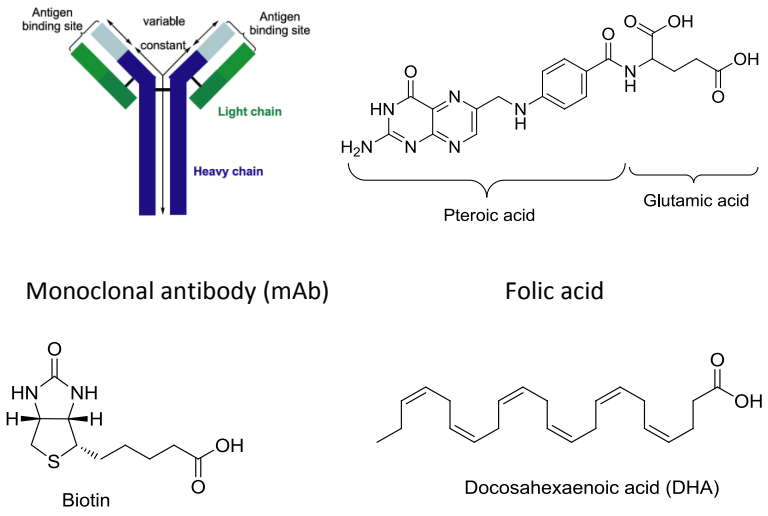


Figure 2-2: Example of tumor-targeting molecules.[15]

§2.1.3 Cleavable Linker: Disulfide Linker

The suitable linker of for tumor-targeted drug-delivered system should be able to connect drug to TTM efficiently. The drug conjugate "prodrug" must be stable and non-toxic in the circulation, but can be cleaved effectively by certain intracellular condition, such as acidic condition or the presence of some specific substances. For instance, hydrazone linker is cleaved under acidic condition (pH 4.5 – 6.5) to release the cytotoxic drug.[16,17] The hydrazone linker are relatively stable at neutral environment (pH 7.3 – 7.5), but it undergoes hydrolysis in lysosome (~pH 5.0) and release the drug in active form. It was used in gemtuzumab ozogamicin (Mylotarg®), a prodrug conjugate with antibody and cytotoxic drug. However, it was proven that prematul cleavage of hydrazone under physiological pH, suggesting the instability of the linker in circulation.[17] Thus, peptide linker is better alternative for lysosomal hydrolysis. Monopeptide Lys,[18] and dipepride Phe-Lys[19] have been applied in DDS, and both of them are much more stable than hydrazone linker. For example, immunoconjugate of doxorubicin (Adriamycin®) and TTM monoclonal antibody contained peptide linker **(Figure 2-3)**.[18]

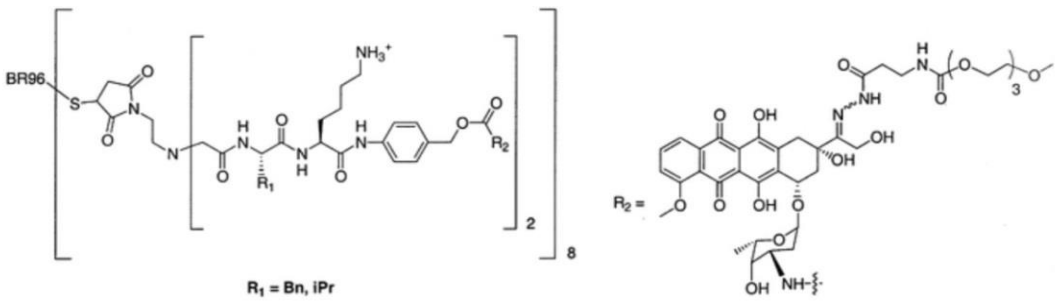


Figure 2-3: Antibody conjugates of doxorubicin prepared with branched peptide linkers.[19]

Disulfide linker is another good option for tumor-targeting drug delivery system, and it is cleavable inside the tumor cells via disulfide exchange with an intracellular thiol substance, such as glutathione (GSH).[20] It has been proved that the concentration of glutathione in tumor cells is much higher (> 100times) than it in blood plasma.[15,21-23] Thus, it is stable in circulation and no premature cleavage.

The disulfide linker is combined with tumor-target moiety and cytotoxic agent. Once, the conjugate is internalized by the targeted cell, the GSH level inside cancer cell is high enough to trigger the self-immolative thiolactonization. The thiol group undergoes intramolecular nucleophile substitution to form a five-member thiolactone and releases the free active cytotoxic agents **(Figure 2-4)**.[15]

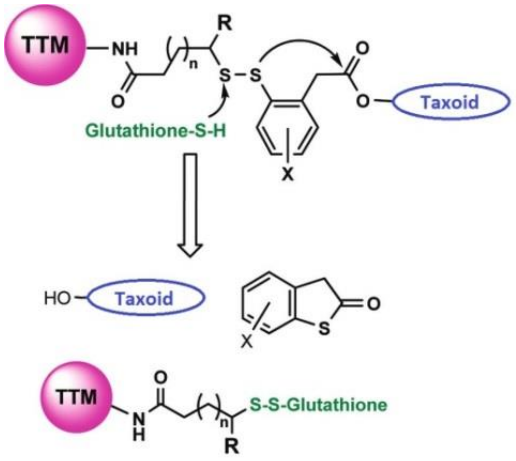


Figure 2-4: Second-generation self-immolative disulfide linkers.[15]

§2.1.4 Receptor-Mediated Endocytosis

Vitamin-linker-drug conjugate could be internalized into cancer cells through receptor-mediated endocytosis (RME) **(Figure 2-5)**.[8,15] As the vitamin moiety binds to the receptor on the surface of targeted cells, it initiates a signal which induces the membrane to form a membrane invagination, and leads to a coated vesicle. The vesicle fuses with endosome to form the early endosome, and the prodrug is released from the receptor and is the receptors are recycled to the cell surface. In endosome, the linkage of the conjugates is cleaved by reducing substance, low pH or endogenous enzymes to release the active cytotoxic agent, and it binds to its targeted protein.

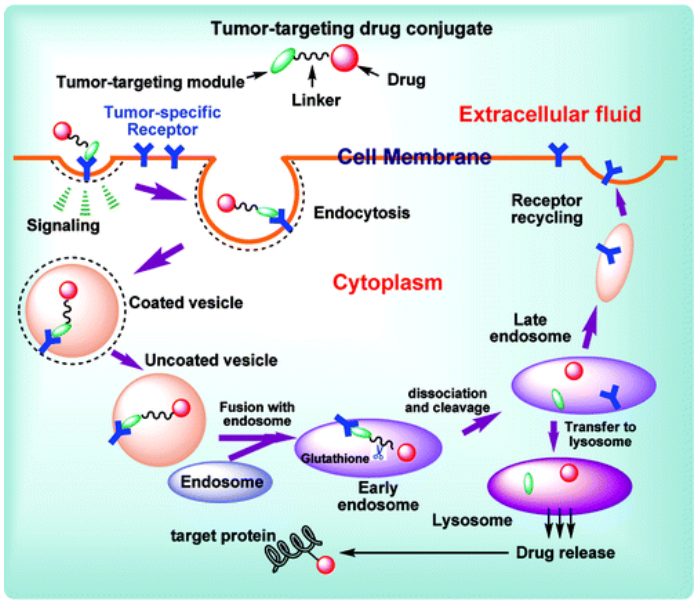


Figure 2-5: Illustration of receptor-mediated endocytosis (RME).[15]

Ojima group has constructed many tumor-targeting drug-delivery systems; one of the examples is using biotin (vitamin B) as tumor targeting moiety, new-generation taxoid (SB-T-1214) as cytotoxic agent, and they are bound together by methyl-branch disulfide linker **(Figure 2-6)**.[15]

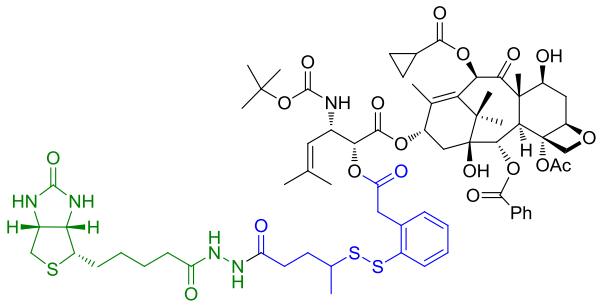


Figure 2-6: Biotin-Me-linker-taxoid conjugate.

§2.2 Results and Discussions: Tumor-Targeting Drug Conjugates

In general, the tumor-targeting drug delivery system contains three parts. First, tumor targeting modules could be antibody or vitamin. Second, cleavable linker could be peptide linker or disulfide linker. Third, cytotoxic agent could be taxoid, such as SB-T-1214.

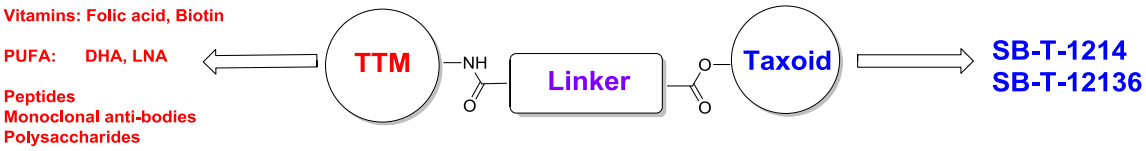
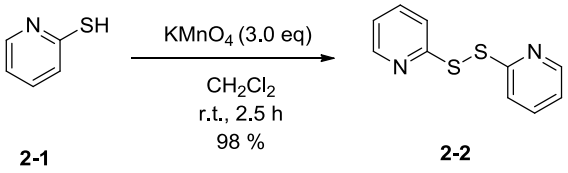


Figure 2-7: Tumor-targeting drug conjugate.

§2.2.1 Synthesis of Disulfide Linker Intermediate

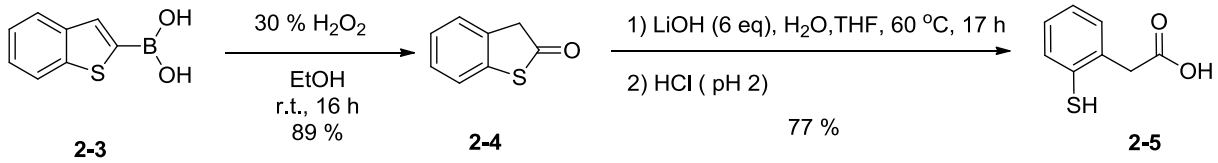
The synthesis of the methyl-branched disulfide linker started with two key intermediate syntheses. These intermediates were required for the two following thiol-disulfide exchange reactions.

The first intermediate was obtained from pyridine-2-thiol **2-1** underwent oxidation of sulfhydryl group to afford **2-2**. The reaction was completed in two and a half hours and afforded the desired compound **2-2** in excellent yield.



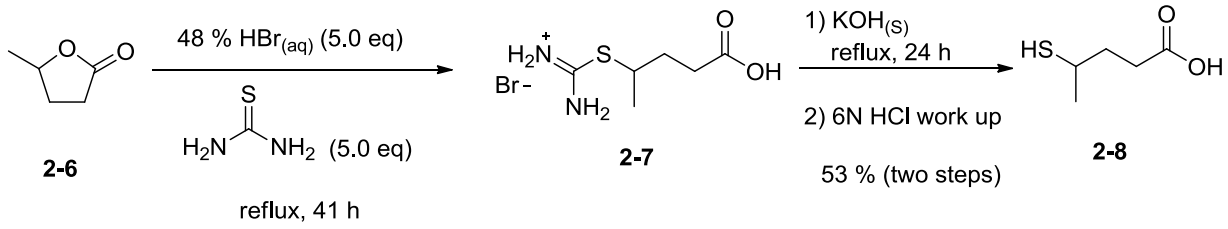
Scheme 2-1: Synthesis of 2,2'-Dithiodipyridine.

The second intermediate, compound **2-5** was prepared from the oxidation of 2-benzothienylboronic acid **2-3** in the presence of hydrogen peroxide to afford desired thiolactone **2-4**. The thiolactone **2-4** was treated with lithium hydroxide to produce sulfhydryphenylacetic acid **2-5**. It was base-mediated ring opening of the thiolactone followed by acidic work-up via addition of 1 N HCl to yield the free sulfhydryl group and the free carboxylic acid. The compound **2-5** should be store under nitrogen and at low temperature in case of dimerization.



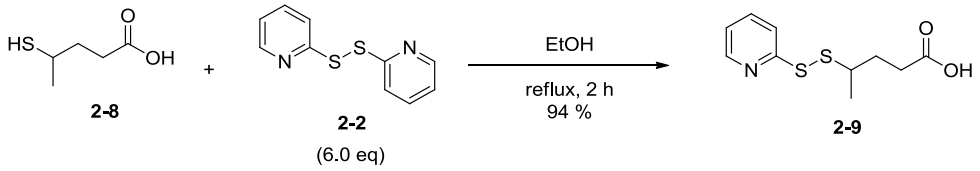
Scheme 2-2: Sulfhydryphenylacetic acid synthesis.

Starting the drug-linker synthesis, γ-valerolactone **2-6** is stirred under reflux condition with HBr, activating the lactone for nucleophilic attack by thiourea, which was added after reflux condition established. Then, the salt **2-7** was hydrolyzed under extremely harsh condition, potassium hydroxide, to afford color-less oil compound **2-8**. It's been observed that the ration of HBr to thiourea should be one to one, and the five equivalent of each gave higher yield in comparison to other amounts. Increasing the amount of both reagents might increase the overall yield, but recovery of unreacted starting materials could become a problem. Furthermore, instead of intermolecular nucleophilic addition, intramolecular ring closure might occur by carboxylate anion. Thus, any lactone or excess thiourea were removed from aqueous layer including **2-7**.



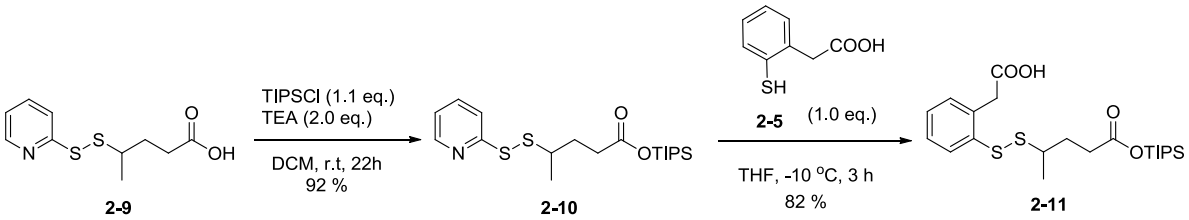
Scheme 2-3: Synthesis of 4-sufhydrylpentanoic acid.

The 4-sufhydrylpentanoic acid **2-8** was treated with 2,2'-Dithiodipyridine **2-2** in reflux condition to carry out the first thiol-disulfide exchange reaction. The slow addition of **2-8** to disulfide compound **2-2** ensured minimal dimer formation and formation of desired product **2-9** in excellent yield.



Scheme 2-4: Thiol disulfide exchange.

After the first thiol-disulfide exchange, TIPS protection of the desired carboxylic acid was followed to afford **2-10** which was stable under nitrogen and stored in the refrigerator. However, the second thiol-disulfide exchange is problematic. The reaction was done under slightly acidic condition, but it has been experimentally observed that as the reaction time goes longer, the TIPS moiety might be cleaved. The reaction rate is slowed because of steric hindrance generated by the methyl group. Therefore, the reaction should be stopped after the initial signs of TIPS deprotection. The compound **2-11** was put under nitrogen gas and low temperature in case of silyl deprotection. Therefore, it should be coupled with taxoid, SB-T-1214, right away.

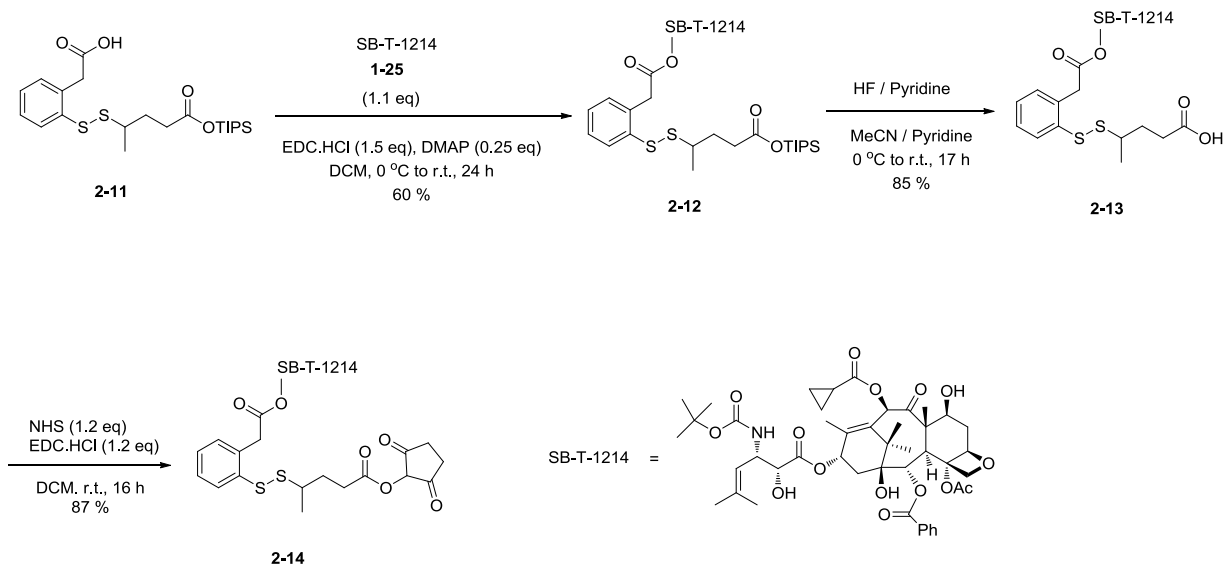


Scheme 2-5: Final steps towards synthesis of methyl-branched disulfide linker.

§2.2.2 Synthesis of Coupling-Ready Drug-Linker Construct

The disulfide linker **2-11** was coupled with SB-T-1214 via EDC coupling to afford compound **2-12**. This reaction was carried out at low temperature (0 ^{o}C) and was allowed to warm to room temperature for longer time if the starting material did not consume yet. The yield of this reaction was low because some of the starting material (SB-T-1214) was recovered and some other product was collected in the column chromatography. Based on proton NMR, it was desired product without TIPS protecting group.

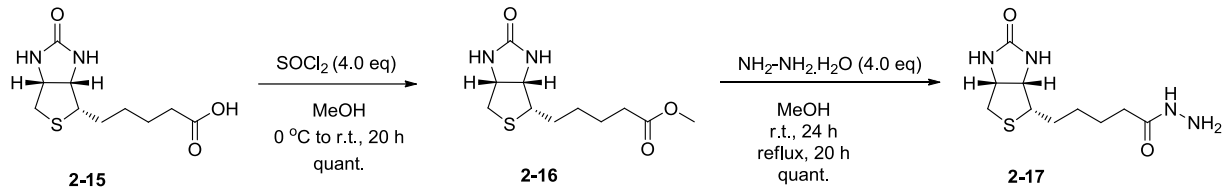
The compound **2-12** underwent silyl-group deprotection by HF in pyridine to afford the carboxylic acid **2-13.** Activation of carboxylic acid in the presence of NHS and EDC·HCl gave the coupling-ready warhead-linker construct **2-14**. It could be coupled to any kind of tumor-targeting moiety or other construct intermediate with free amine.



Scheme 2-6: Synthesis of coupling-ready drug-linker construct.

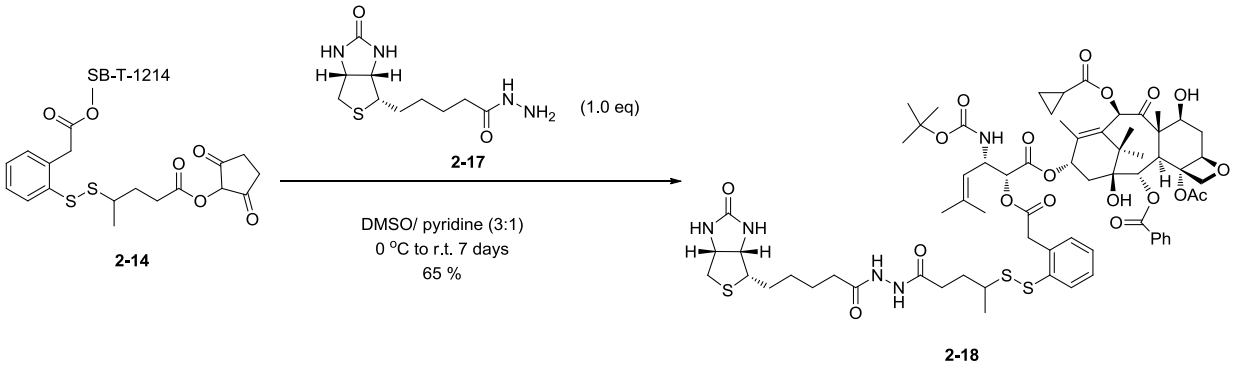
§2.2.3 Synthesis of Biotin-Linker-Taxoid Drug Conjugate

The TTM biotin conjugate started from commercially available biotin, which was treated with thionyl chloride and methanol to give biotin methyl ester **2-16**. Conversion of biotin methyl ester was treated with hydrazine in methanol under reflux condition to afford biotin-hydrazide conjugate **2-17**.[24]



Scheme 2-7: Synthesis of biotin-hydrazide conjugate.

The biotin-linker-drug conjugate was obtained by coupling **2-14** and **2-17** in the present of DMSO and pyridine at room temperature for seven days. The activity of hydrazide was not fast as normal amine for coupling reaction, so the rate of the reaction was slow which took long time for completion.

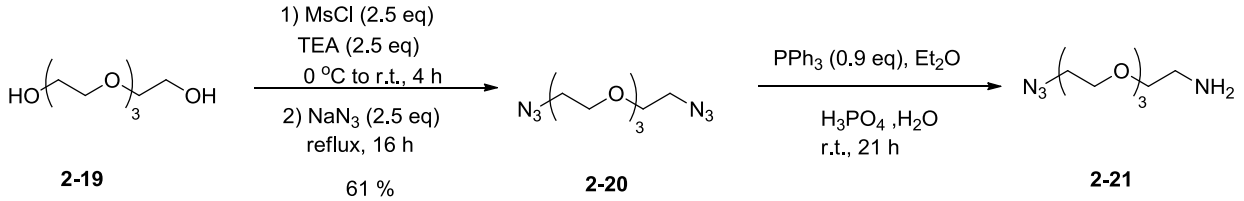


Scheme 2-8: Synthesis of biotin-Me-linker-SB-T-1214 drug conjugate.

§2.2.4 Synthesis of Folate- Linker-Taxoid Drug Conjugate

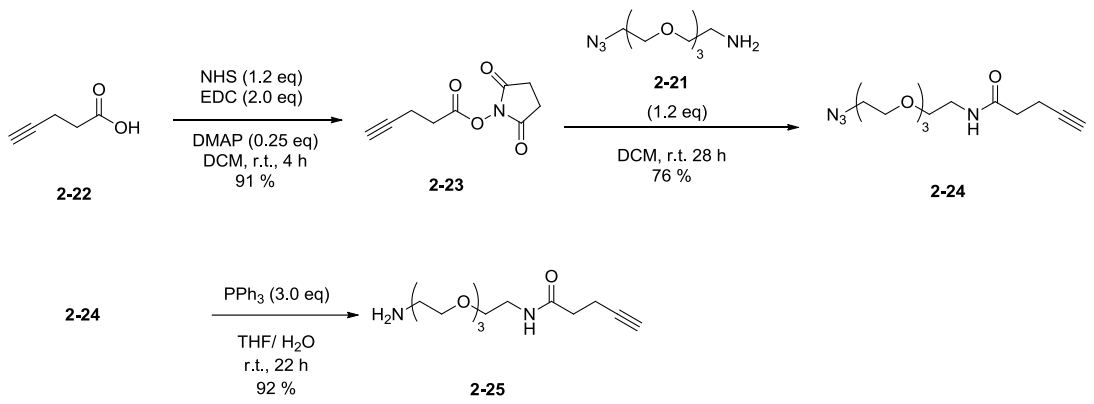
Folic acid was introduced as tumor-targeted moiety in tumor-targeting delivery. The drug-linker-folate conjugate contained two parts, one was alkyne-linker-drug conjugate, the other was peptide-folate-azide conjugate, two of they could be coupled together via click chemistry reaction.

To start the synthesis of PEG-alkyne conjugate, the polyethylene glycol (PEG) conjugates was introduced. Tetraethylene glycol was treated with methanesulfonyl chloride and sodium azide to form the diazido compound **2-20**. The diazido compound was carefully mono-reduced to form desired amino azide compound **2-21**. The idea of mono-reduction was to use the different solubilities of diazide and amine in organic solvent. The mixture contained organic layer and aqueous layer. Once one of the diazide was reduced to amine, it would be carried to the dilute aqueous layer (phosphoric acid), preventing overreduction. Therefore, the mono-reduced amino azide was favored than diamino compound.[25]



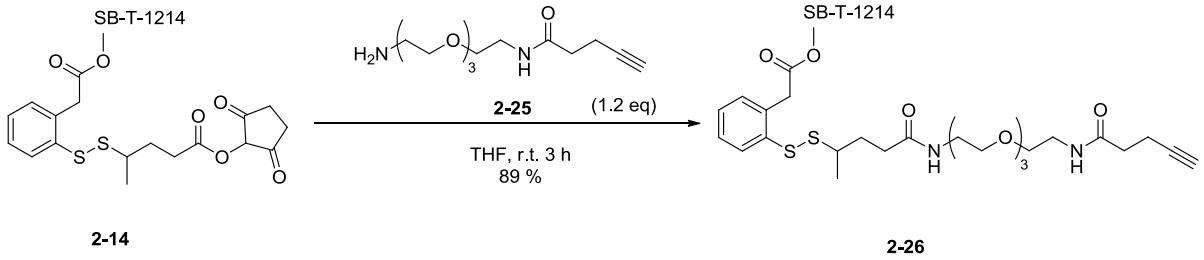
Scheme 2-9: Synthesis of asymmetric polyethylene glycol conjugate.

Activation of 4-pentynoic acid to activated ester via EDC coupling with *N*-hydroxysuccinimide (NHS) to afford succinimidyl ester **2-23**, which coupled with **2-21** to afford azido alkyne intermediate **2-24**. The compound **2-24** was reduced to amine to afford the amino-alkyne compound **2-25** and ready to couple with coupling-ready construct **2-14**.



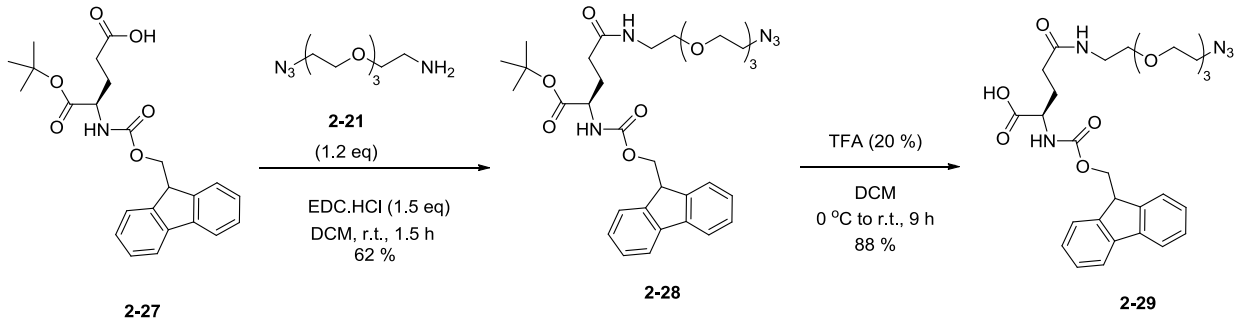
Scheme 2-10: Synthesis of PEG-alkyne conjugate.

The amino-alkyne compound **2-25** was coupled with coupling-ready drug-linker construct to form the desired alkyne-linker-drug conjugate **2-26**, which would be coupled with peptide-folic acid-azide conjugates via click chemistry.



Scheme 2-11: Synthesis of alkyne-linker-drug conjugate.

The synthesis of peptide-folic acid-azide conjugates started with commercial available Fmoc-Glu-OtBu which coupled with asymmetric azido amino **2-21** to introduce the PEG chain. Later, TFA deprotection of *t*-Boc group on the amino acid conjugates to form the Fmoc-Glu (PEG)-COOH conjugates **2-29.**

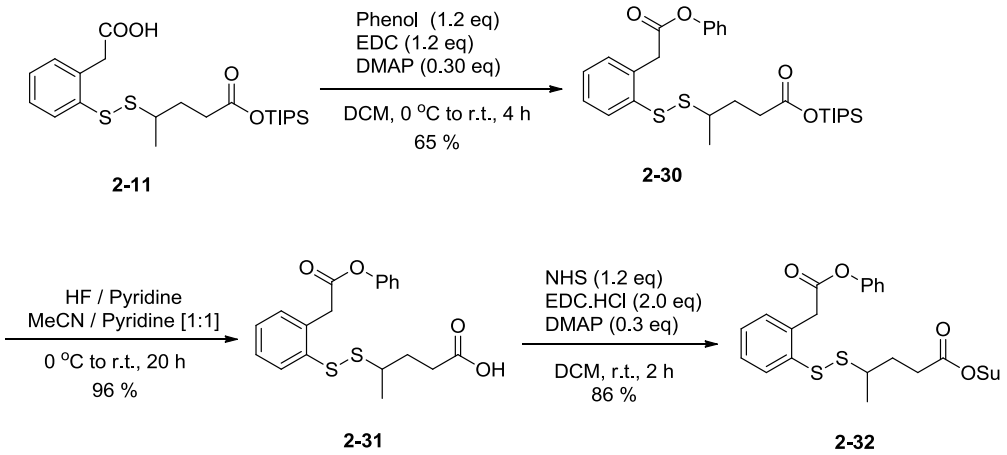


Scheme 2-12: Synthesis of Fmoc-Glu-PEG-azide conjugate.

§2.2.5 Synthesis of Drug-Conjugates bearing One Dummy Molecule

To further evaluate the activity of the cytotoxic agent, the dummy molecule was introduced for comparison in biological tests. With the structure of coupling-ready drug-linker construct, the drug was replaced by dummy molecule.

For linker intermediate compound **2-11**, instead of coupling with drug, phenol was introduced as dummy molecule for comparison the biological evaluation, such as IC_{50} in cell line. As the same synthetic route with drug-linker conjugate, the phenyl-linker conjugates was prepared via EDC coupling with disulfide linker intermediate **2-11** to give product **2-30**. The compound **2-31** underwent deprotection of silyl group by HF in pyridine, and NHS activation to afford the coupling-ready dummy-linker construct, compound **2-32**.



Scheme 2-13: Synthesis of dummy-molecule conjugate.

§ 2.3 Conclusions

Vitamin-drug conjugates have potency in tumor-targeting drug delivery system. Once the coupling-ready drug-linker construct was synthesized, it could couple to different tumor targeting moieties. Biotin and folic acid are both good options for tumor-targeting modules. They are not endogenously produced in mammals. Fast proliferate cancer cells eagerly uptake nutrients through external supplies. Thus, biotin and folic acid are great options for tumor-targeting drug conjugates. The synthesis of biotin moiety for TTM was straightforward. Commercially available biotin was converted to biotin hydrazide conjugate in two steps and coupled with coupling-ready drug-linker construct to yield the biotin-linker-drug conjugate. For folate conjugate, it involved more steps to construct. The solubility of folic acid was poor, so PEG chains and amino acids were introduced in the construct to increase its solubility.

The Ojima group has conducted second-generation taxoids and methyl-branched disulfide linker in tumor-targeting drug delivery system. For instance, SB-T-1214 drug-linker construct coupled with biotin moiety to form biotin-linker-taxoid drug conjugate. It is ready for further biological evaluation. In conclusion, vitamins as tumor-targeting modules in tumor-targeting drug conjugates should be explored for further study.

§2.4 Experimental Section

General information

All chemical were purchased from VWR International, Fisher Scientifi and Sigma-Aldrich. All reactions were carried out under nitrogen in dried glassware which were dried in a 110 ^{o}C oven and allowed to cool to room temperature in a desiccator over "Drierite" (calcium sulfate) and assembled under nitrogen gas. Dichloromethane was distilled prior to use under nitrogen from calcium hydride. Tetrahydrofuran was also freshly distilled from sodium metal and benzophenone. Toluene was also distilled immediately prior to use under nitrogen from calcium hydride. Anhydrous dimethylformamide (DMF) was used commercially available from EMD chemical company, and used without further purification. Reactions were monitored by thin layer chromatography (TLC) using E. Merck 60F254 precoated silica gel plates and alumina plate depending on the compounds. Flash chromatography was performed with the indicated solvents using Fisher silica gel (particle size 170-400 Mesh). Yields refer to chromatographically or spectroscopically pure compounds. ^{1}H, and ^{13}C data were obtained using either 300 MHz Varian Gemni 2300 spectrometer, the 400 MHz Varian INOVA 400 spectrometer or the 500 MHz Varian INOVA 500 (125 MHz 13C) in deuterated solvent. Chemical shifts (δ) are reported in ppm and standardized with solvent as internal standard based on literature reported values. Melting points were measured on Thomas Hoover Capillary melting point apparatus and are uncorrected. Optical rotations were measured on Perkin-Elmer Model 241 polarimeter.

2,2'-Dithiodipyridine (2-2)[26]

A solution of pyridine-2-thiol (1.00 g, 9.01 mmol) in 90 mL DCM, and $KMnO_4$ (4.27 g, 27.0 mmol) was added slowly over a period of fifteen minutes. The reaction mixture was stirred vigorously and monitored by TLC. Upon completion (2.5 h), the solution was filtered over celite and concentrated *in vacou*. The residue crude was purified via silica gel column chromatography to afford compound **2-2** (0.97 g, 98 %) as dark purple solid: ^{1}H NMR (400 HMz, $CDCl_3$) δ 7.09 (m, 2 H), 7.59 (m, 4 H), 8.44 (m, 2 H); ^{13}C NMR (400 HMz, $CDCl_3$) δ 119.6, 121.1, 137.3, 149.5, 158.8. All data are consistent with the reported values.[26]

3*H*-Benzo[*b*]thiophen-2-one (2-4)[27,28]

A solution of the 2-Benzothienylboronic acid (2.00 g, 11.2 mmol) in EtOH (40 mL) was added 30 % hydrogen peroxide (16 mL) dropwise. The reaction mixture was stirred at room temperature for 16 hours. Upon completion, the reaction mixture was diluted with water and extracted with dichloromethane (50 mL x 3). The combined organic layers was dried over $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **2-4** (1.50 g, 89 %) as brown solid: ^{1}H NMR (400 HMz, $CDCl_3$) δ 3.93 (s, 2 H), 7.20 (m, 1 H), 7.29 (m, 3 H); ^{13}C NMR (400 HMz, $CDCl_3$) δ 47.2, 122.9, 124.7, 126.0, 128.2, 132.1, 136.9, 202.8. All data are consistent with the reported values.[27,28]

2-Sulfhydryphenyl acetic acid (2-5)[15]

A solution of compound **2-4** (1.40 g, 9.32 mmol) in THF (20 mL) and heated to 60 ^{o}C under inert condition. The reaction mixture was added a solution of LiOH (2.35 g, 55.9 mmol) in water (20 mL) dropwise. The mixture was stirred at 60 ^{o}C for 17 hours. Upon completion, the reaction mixture was cooled down to room temperature and diluted with water. The pH value of the mixture was adjusted to pH 2 using 1 M HCl. The acidified solution was extract with ethyl ether (20 mL x 3). The combined organic layer was dried with $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **2-5** (1.20 g, 77 %) as dark purple solid: ^{1}H NMR (400 HMz, $CDCl_3$) δ 3.49 (s, 1 H), 3.82 (s, 2 H), 7.19 (m, 2 H), 7.24 (m, 1 H), 7.41 (m, 1 H); ^{13}C NMR (400 HMz, $CDCl_3$) δ 40.0, 126.9, 128.2, 130.7, 131.0, 132.4, 133.3, 177.1. All data are consistent with the reported values.[15]

4-Sulfhydrypentanoic acid (2-8)[29]

A solution of γ-valerolactone (4.20 g, 42.0 mmol) and 48 % HBr (23 mL, 210 mmol) was heated to 100 ^{o}C, and followed by slow addition of thiourea (16.0 g, 210 mmol). The reaction mixture was stirred under reflux condition for 42 hours. Then, the mixture was allowed to cool down to

room temperature and extracted with DCM (30 mL x 3) to remove excess thiourea. The pH value of the aqueous phase was adjusted to 13-14 using KOH. The aqueous phase was stirred vigorously under reflux condition for 24 hours. Upon completion, the reaction mixture was allowed to cool to room temperature and diluted with water. The pH value of the mixture was adjusted to 2 using 1N HCl, and extract with DCM (30 mL x 3). The combined organic layer was washed with brine, dried with $MgSO_4$ and concentrated *in vacou*. The product was purified using silica gel column chromatography to afford compound **2-8** (3.00 g, 53 %) as light-yellow oil: ^{1}H NMR (500 HMz, $CDCl_3$) δ 1.37 (d, *J* = 6.8 Hz, 3 H), 1.45 (d, *J* = 7.1 Hz, 1 H), 1.76 (m, 1 H), 2.00 (m, 1 H), 2.47–2.59 (m, 2 H), 2.95 (m, 1 H); ^{13}C NMR (500 HMz, $CDCl_3$) δ 25.6, 31.8, 34.9, 35.4, 179.7. All data are consistent with the reported values.[29]

4-(Pyridin-2-yldisulfanyl)pentanoic acid (2-9)[29]

A solution of compound **2-2** (5.89 g, 26.7 mmol) in EtOH(132 mL) under inert condition was added a solution of compound **2-8** (600 mg, 4.46 mmol) in EtOH (22.3 mL) slowly. The reaction mixture was stirred at reflux condition and monitored via TLC. Upon completion (2 h), the solvent was evaporated and the residual was purified via silica gel column chromatography to afford compound **2-9** (1.01 g, 94 %): ^{1}H NMR (500 HMz, $CDCl_3$) δ 1.34 (d, *J* = 6.8 Hz, 3 H), 1.91 (m, 1 H), 2.00 (m, 1 H), 2.54 (m, 2 H), 3.03 (m, 1 H), 7.10 (dt, *J* = 1.6, 13.0 Hz, 1 H), 7.65 (dt, *J* = 1.8, 17.4 Hz, 1 H), 7.73(d, *J* = 8.1 Hz,1 H), 8.46 (d, *J* = 4.3 Hz, 1 H); ^{13}C NMR (500 HMz, $CDCl_3$) δ 20.5, 30.5, 31.3, 46.1, 120.2, 120.8, 137.2, 149.2, 160.5, 177.9. All data are consistent with the reported values.[29,30]

Triisopropylsilyl 4-(pyridine-2-yldisufany)pentanoate (2-10)[30]

A solution of compound **2-9** (1.01 g, 4.17 mmol) and TEA (1.16 mL, 8.34 mmol) in DCM (23 mL) was cooled to 0 ^{o}C under inert condition and TIPSCl (0.88 g, 4.59 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature gradually and stirred for 22 hour. After completion, the reaction was quenched with saturated NH_4Cl (5 mL) and extracted with DCM (20 mL x 3). The combined organic extract was washed with brine, dried

with $MgSO_4$ and concentrated *in vacou*. The product was purified via silica gel column chromatography to afford compound **2-10** (1.53 g, 92 %) as light-yellow oil: ^{1}H NMR (500 HMz, $CDCl_3$) δ1.04–1.05 (m, 21 H), 1.33(d, *J* = 6.8 Hz, 3 H), 1.87 (m, 1 H), 1.98 (m, 1 H), 2.51 (m, 2 H), 3.02 (q, *J* = 6.8 Hz, 1 H), 7.06 (m, 1 H), 7.62 (m, 1 H),7.72 (m, 1 H), 8.43(m,1 H); ^{13}C NMR (500 HMz, $CDCl_3$) δ11.8, 17.7, 20.5, 31.1, 33.2, 46.2, 119.8, 120.5, 136.9, 149.3, 160.8, 172.9. All data are consistent with the reported values.[30]

2(2-5-Oxo-5-(triisopropylsilyloxy)pentan-2-yldisulfanylphenyl) acetic acid (2-11)[30]

A solution of compound **2-10** (300 mg, 0.751 mmol) in THF (7.5 mL) and cool to -10 ^{o}C under inert condition. The reaction mixture was slowly added a solution of compound **2-5** (126 mg, 0.751 mmol) in THF (7.5 mL). The reaction mixture was stirred at -10 ^{o}C for 3 hours. Upon completion, the solvent was evaporated and the residual was purified via silica gel column chromatography to afford compound **2-11** (280 mg, 82 %) as light-yellow oil: ^{1}H NMR (500 HMz, $CDCl_3$): δ 1.01–1.05 (m, 18 H), 1.24–1.29 (m, 6 H), 1.80 (m, 1 H), 1.97 (m, 1 H), 2.41 (m, 2 H), 2.89 (q, *J* = 6.8 Hz, 1 H), 3.89 (d, *J* = 4.2 Hz, 2 H), 7.21 (m, 2 H), 7.29 (m, 1 H), 7.79 (d, *J* = 7.7 Hz, 1 H); ^{13}C NMR (500 HMz, $CDCl_3$): δ 11.8, 17.7, 20.2, 31.0, 33.1, 38.9, 45.8, 127.5, 128.3, 130.1, 130.8, 133.1, 137.8, 173.5, 176.0. All data are consistent with the reported values.[30]

Drug-linker-TIPS (2-12)[31]

A solution of compound **2-11** (100 mg, 0.219 mmol), SB-T-1214 **1-26** (206 mg, 0.241 mmol) and DMAP (6.69 mg, 0.055 mmol) in DCM (4.4 mL), and cooled to 0 ^{o}C under inert condition. The reaction mixture was added EDC (62.9 mg, 0.328 mmol). The reaction mixture was stirred at 0 ^{o}C first and warm to room temperature gradually and it was monitored via TLC. Upon completion (24 h), the mixture was washed with water and extract with DCM (5 mL x 3). The combined organic extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacou*. The product was purified via silica gel column chromatography to afford compound **2-12** (171 mg, 60 %) as white solid: ^{1}H NMR (500 HMz, $CDCl_3$, ppm) δ 1.01–1.05 (m, 18 H), 1.08 (m, 2 H), 1.15 (m, 3 H), 1.26 (m, 8 H), 1.36 (s, 6 H), 1.66 (s, 2 H), 1.72 (s, 2 H), 1.78 (m, 3 H), 1.90 (m,

2 H), 2.04 (s, 3 H), 2.38 (m, 4 H), 2.54 (m, 1 H), 3.78 (s, 1 H), 4.13 (s, 2 H), 4.18 (m, 1 H), 4.31 (m, 1 H), 4.41 (m, 1 H), 4.98 (m, 2 H), 5.14 (m, 1 H), 5.68 (d, J = 7 Hz, 1 H), 6.22 (m, 1 H), 6.30 (d, *J* = 7 Hz, 1 H), 7.30 (m, 1 H), 7.60 (t, *J* = 5 Hz, 1 H), 7.80 (t, *J* = 11 Hz, 1 H), 8.10 (d, *J* = 6.4 Hz, 1 H). All data are consistent with the reported values.[31]

Drug-linker-COOH (2-13)[31]

A solution of compound **2-12** (147 mg, 0.114 mmol) in acetonitrile (5 mL) and pyridine (5 mL) and cooled to 0 ^{o}C under inert condition. The reaction mixture was added HF/pyridine(1.5 mL) dropwise. The mixture was allowed to warm to room temperature and monitored via TLC. Upon completion (24 h), the reaction mixture was quenched with 0.2 M citric acid (3 mL) and extracted with ethyl acetate (10 mL x 3). The combined organic phase was washed with $CuSO_4$, brine, dried over $MgSO_4$ and concentrated *in vacou*. The product was purified via silica gel column chromatography to afford compound **2-13** (110 mg, 85%): 1H NMR (500 MHz, CDCl3, ppm) δ 1.00 (m, 2 H), 1.08 (m, 2 H), 1.15 (m, 3 H), 1.26 (m, 9 H), 1.36 (s, 6 H), 1.66 (s, 2 H), 1.72 (s, 2 H), 1.78 (m, 3 H), 1.90 (m, 2 H), 2.04 (s, 3 H), 2.38 (m, 4 H), 2.54 (m, 1 H), 3.78 (s, 1 H), 4.13 (s, 2 H), 4.18 (m, 1 H), 4.31 (m, 1 H), 4.41 (m, 1 H), 4.98 (m, 2 H), 5.14 (m, 1 H), 5.68 (d, *J* = 7 Hz, 1 H), 6.22 (m, 1 H), 6.30 (d, *J* = 7 Hz, 1 H), 7.30 (m, 1 H), 7.59 (t, *J* = 5 Hz, 1 H), 7.80 (t, *J* = 11 Hz, 1 H), 8.10 (d, *J* = 6.4 Hz, 1 H). All data are consistent with the reported values.[31]

Coupling-ready drug-linker construct (2-14)[31]

A solution of compound **1-13** (110 mg, 0.097 mmol) and EDC (22.3 mg, 0.116mmol) in THF (4.84 mL) was added NHS (13.4mg, 0.116 mmol). The reaction mixture was stirred at room temperature and monitored via TLC. Upon completion (18 h), the mixture was washed with brine, dried over $MgSO_4$ and concentrated *in vacou*. The product was purified via silica gel column chromatography to afford compound **2-14** (104 mg, 87 %) as white solid: ^{1}H NMR (400 HMz, $CDCl_3$) δ 0.93–1.00 (m, 3 H), 1.11–1.14 (m, 5 H), 1.23–1.27 (m, 5 H), 1.31 (m, 3H), 1.34 (s, 9H), 1.66 (m, 5H), 1.71 (s, 3H), 1.73 (s, 3H), 1.90 (s, 3H), 1.98 (q, *J* = 7.2 Hz, 1H), 2.04 (s,

3H), 2.29–2.39 (m, 5 H), 2.53 (m, 1H), 2.66 (t, *J* = 7.5 Hz, 2 H), 2.82 (s, 4H), 3.00 (m, 1H), 3.80 (d, *J* = 7.0 Hz, 1 H), 3.97 (m, 1 H), 4.07 (m, 1 H), 4.10 (d, *J* = 7.2 Hz, 1 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.42 (q, *J* = 5.8 Hz, 1 H), 4.80 (m, 1 H), 4.92–4.97 (m, 3 H), 5.09 (m, 1 H), 5.67 (d, *J* = 7.1 Hz, 1 H), 6.18 (t, *J* = 8.9 Hz, 1 H), 6.28 (s, 1 H), 7.26–7.33 (m, 3 H), 7.47 (t, *J* = 7.7 Hz, 2 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 7.79 (d, *J* = 7.5 Hz, 1 H), 8.11 (d, *J* = 7.3 Hz, 1 H); ^{13}C NMR (400 HMz, $CDCl_3$) δ 9.5, 13.0, 14.8, 18.5, 20.4, 21.0, 22.4, 25.6, 25.7, 26.7, 28.2. 43.2, 45.6, 58.5, 60.4, 75.2, 75.4, 81.0, 84.5, 128.0, 128.4, 128.6, 129.3, 130.2, 130.6, 130.8, 131.0, 133.6, 137.2, 137.7, 143.5, 167.0, 168.0, 169.0, 169.6, 170.1, 171.1, 175.1, 177.9, 204.1. All data are consistent with the reported values.[31]

Biotin methyl ester (2-16)[15,24]

An emulsion of biotin (100 mg, 0.409 mmol) in CH_3OH (4.1 mL) was cooled to 0 °C. To the emulsion was added thionyl chloride (195 mg, 1.64 mmol) dropwise. As the addition was complete, the reaction solution became clear, and the mixture was stirred at room temperature and monitored via TLC. After 20 h, the reaction solvent was evaporated, and the product was purified via flash silica gel column chromatography to afford compound **2-16** (104 mg, quant. yield) as white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 1.44 (quin, *J* = 6.5 Hz, 2 H), 1.53–1.76 (m, 4H), 2.34 (t, *J* = 7.4 Hz, 2 H), 2.70 (d, *J* = 12.8 Hz, 1H), 2.92 (dd, *J* = 5.0, 12.8 Hz, 1H), 3.20 (quin, *J* = 4.8 Hz, 1 H), 3.65 (s, 3 H), 4.30 (m, 1 H), 4.49 (m, 1 H); ^{13}C NMR (400 MHz, CD_3OD) δ 24.0, 27.6, 27.8, 32.7, 39.2, 50.2, 55.1, 59.8, 61.5, 164.3,174.2. All data are consistent with the reported values.[15,24]

Biotin hydrazide (2-17)[15,24]

To a solution of **2-16** (104 mg, 0.403 mmol) in CH_3OH (4.1 mL) was added anhydrous hydrazine (80.6 mg, 1.61 mmol). The mixture was stirred at room temperature for 24 hour and reflux for another 16 hours. After completion, the reaction solvent was evaporated. The resulting crude was diluted with water and washed with $CHCl_3$ (20 mL x 5). The aqueous layer was collected and concentrated *in vacuo* to yield compound **2-16** (110 mg, quant. yield), as a white solid: ^{1}H NMR (400 MHz, H_2D) δ 1.41 (qui, *J* = 6.0 Hz, 2 H), 1.55–1.77 (m, 4 H), 2.24 (t, *J* = 5.9 Hz, 2 H), 2.79 (d, *J* = 10.4 Hz, 1 H), 3.01 (dd, *J* = 5.0, 13 Hz, 1 H), 3.45 (m, 1 H), 4.44 (m, 1

H), 4.61 (m,1 H); ^{13}C NMR (400 MHz, H_2D) δ 24.8, 27.6, 33.4, 55.3, 60.2, 62.0, 165.4, 175.6. All data are consistent with the reported values.[15,24]

Biotin-Me-linker-SB-T-1214 drug conjugate (2-18)[15]

To a solution of 11 (140 mg, 0.1 mmol) dissolved in DMSO [0.2 M] was added **13** (30 mg, 0.1 mmol) dissolved in DMSO [0.2 M], dropwise. The mixture was stirred at room temperature and the reaction was monitored via TLC. After 8 h, the reaction was diluted with water resulting in a white precipitate. The precipitate was collected and purified using flash column chromatography on silica gel (10% CH_3OH in CH_2Cl_2) to yield **5-18** (10 mg, 56 % yield), as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83–0.87 (m, 2 H), 1.09–1.13 (m, 9 H), 1.23–1.34 (m, 6 H), 1.64–1.69 (m, 21 H), 1.89 (s, 4 H), 2.02 (s, 3 H), 2.14–2.45 (m, 10 H), 2.64 (m, 3 H), 2.95 (m, 3 H), 3.61 (t, *J* = 8.5 Hz, 1 H), 3.77 (d, *J* = 7.2 Hz, 1 H), 3.97 (br s, 2 H), 4.13 (d, *J* = 8.8 Hz, 1 H), 4.26 (d, *J* = 8.8 Hz, 1 H), 4.35–4.39 (m, 1 H), 4.92–5.12 (m, 4 H), 5.64 (d, *J* = 8.5 Hz, 1 H), 6.14 (m, 1 H), 6.30 (s, 1 H), 7.42–7.49 (m, 2 H), 7.56–7.58 (m, 1 H), 7.77 (m, 1 H), 8.07 (d, *J* = 7.2 Hz, 2 H), 8.93 (br s, 1 H), 9.34 (s, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 1.21, 9.38, 9.50, 9.9, 13.3, 13.7, 15.0, 18.7, 22.2, 22.7, 25.3, 26.0, 26.8, 27.9, 28.5, 28.6, 29.9, 33.2, 33.5, 35.7, 36.0, 38.9, 40.2, 43.4, 46.2, 55.5, 58.6, 61.4, 62.8, 72.0, 75.2, 75.7, 79.3, 81.3, 84.6, 128.1, 128.8, 129.5, 130.3, 130.5, 131.4, 132.9, 133.4, 133.8, 137.0, 142.8, 164.4, 167.1, 168.6, 170.0, 170.8, 172.2, 175.0, 204.0. All data are consistent with the reported values.[15]

1,11-Diazido-3,6,9-trioxaundecane (2-20)[25]

A solution of tetraethylene glycol (6.00 g, 30.9 mmol) and methanesulfonyl chloride (6.00 mL, 77.2 mmol) in THF (60 mL) was cooled to 0 ^{o}C via ice bath. The mixture was added a solution of Et_3N (10.8 mL, 77.2 mmol) in THF (6 mL) to form a yellow-white precipitate. After one hour, the ice bath was removed, and the mixture was stirred for another 3.5 hours. Addition of H_2O dissolved the solid, forming two liquid phases, and pH value of the mixture was adjusted to pH 8 via addition of saturated $NaHCO_3$ in a cold water bath. The mixture was added followed by NaN_3 (5.02 g, 77.2 mmol), and reflux for 24 hours. The aqueous layer was extracted Et_2O (20 mL x 5), and each Et_2O layer was backwashed with the same 15-mL aliquot of saturated NaCl. The organic layers were combined, dried over magnesium sulfate, filtered, and concentrated *in vacou*

to afford compound **2-20** (4.59g , 60.8 %) as light yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.38 (t, *J* = 5.1 Hz, 4 H), 3.67 (m, 12 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 50.6, 70.0, 70.7. All data are consistent with the reported values.[25]

1-Amino-11-azido-3,6,9-trioxaundecane (2-21)[25]

A solution of Diazide compound **2-20** (3.46 g, 14.1 mmol) 0.65 M aqueous H_3PO_4 (33 mL) was stirred at room temperature while Ph_3P (3.23 g, 12.3 mmol) in Et_2O (24.3 mL) was added dropwise over 30 min followed by 3 mL of ether to rinse the addition funnel. After stirring under nitrogen gas for 24 h, the aqueous layer was washed with Et_2O (30 mL x 3), and 2.6 g of KOH was added. After traces of ether had evaporated, the mixture was cooled to 4 °C for 16 h, and Ph_3PO was removed by filtration. Addition of 14 g of KOH to the aqueous solution, and it extracted with CH_2Cl_2 (20 mLx 7), dried over $MgSO_4$ and concentrated *in vacou*. The product was purified via flash aluminium oxide column chromatography compound **2-21** (3.15 g, 75%) as clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ1.84 (br s, 2 H), 2.81 (t, *J* = 5.0 Hz, 2 H), 3.33 (t, *J* = 5.1 Hz, 2 H), 3.46 (t, *J* = 5.2 Hz, 2 H), 3.57–3.62 (m, 10 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 41.6, 50.5, 69.9, 70.1, 70.5, 73.1. All data are consistent with the reported values.[25]

4-Pentynoic acid succinimidyl ester (2-23)[32]

A solution of 4-pentynoic acid (43 mg, 0.438 mmol), EDC·HCl (168 mg, 0.877 mmol) and DMAP (13.4 mg, 0.110 mmol) in dichloromethane (1 mL) was stirred at room temperature for 10 minutes, and a solution of NHS (115 mg, 0.526 mmol) in DCM (1 mL) was added. The reaction was monitored via TLC plate. After 4 hours, the mixture was washed with water (5 mL x 2). The combined organic layer was washed with brine, dried with $MgSO_4$ and concentrated *in vacou*. The product was purified via silica gel column chromatography to afford compound **2-23** (78 mg, 91 %) as white solid: ^{1}H NMR (500 HMz, $CDCl_3$) δ 2.04 (t, *J* = 2.7 Hz, 1 H), 2.62 (m, 2 H), 2.84 (br s, 4 H), 2.88 (m, 2 H); ^{13}C NMR (500 HMz, $CDCl_3$) δ 14.1, 25.5, 30.3, 70.0, 80.8, 167.0, 168.9. All data are consistent with the reported values.[32]

***N*-(11-Azido-3,6,9-trioxaundecyl)pent-4-ynamide (2-24)**

A solution of compound **2-23** (100 mg, 0.512 mmol) and compound **2-21** (183 mg, 0. 615 mmol) in dichloromethane (3 mL) was stirred at room temperature and monitored via TLC plate. After 28 hours, the solvent was evaporated, and the residual was purified by column chromatography to give compound **2-24** (116 mg, 76%) as clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (t, *J* = 2.6 Hz, 1 H), 2.41 (t, *J* = 7.3 Hz, 2 H), 2.38 (m, 2 H), 3.39 (t, *J* = 5.01 Hz, 2 H), 3.47 (m, 2H), 3.57 (t, *J* = 5.0 Hz, 2 H), 3.62–3.66 (m, 10 H), 6.18 (br s, 1 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.8, 35.2, 39.2, 50.6, 69.2, 69.7, 70.0, 70.1, 70.5, 70.6, 82.9, 170.9.

***N*-(11-Amino-3,6,9-trioxaundecyl)pent-4-ynamide (2-25)**

A solution of compound **2-24** (50 mg, 0.168 mmol) in THF (1.3 mL) was added a solution of triphenylphosphine (132 mg, 0.503 mmol) in water (0.37 mL). The mixture was stirred at room temperature and monitored via TLC. After 22 hours, the solvent was evaporated and the residual was extracted with benzene (5 mL x3) and collect the aqueous layers. The product was concentrated *in vacou* to afford the compound **2-25** (42 mg, 92 %) as clear oil: 1H NMR (500 MHz, $CDCl_3$) δ 1.91 (br s, 2 H), 2.00 (t, *J* = 2.6 Hz, 1 H), 2.41 (t, *J* = 7.3 Hz, 2H), 2.52 (m, 2 H), 2.86 (t, *J* = 5.2 Hz, 2 H), 3.45 (m, 2 H), 3.51–3.56 (m, 4 H), 3.61–3.64 (m, 8 H), 6.96 (br s, 1 H); 13C NMR (500 MHz, $CDCl_3$) δ 14.9, 35.2, 39.2, 41.6, 69.1, 70.0, 70.1, 70.2, 70.5, 70.6, 73.2, 83.2, 171.0.

PEG-Azide-Me-linker-SB-T-1214 drug conjugate (2-26)

A solution of compound **2-14** (20 mg, 0.016 mmol) and amine-PEG-azide **2-25** (9.0 mg, 0.033 mmol) in dichloromethane (1 mL) was stirred at room temperature and the reaction was monitored via TLC. After 3 hours, the mixture was washed with water. The organic phase was collected, dried over $MgSO_4$ and concentrated *in vacou* to give the compound **2-26** (20 mg, 89 %) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (s, 3 H), 1.25 (s, 3 H), 1.29(d, *J* = 6.8 Hz, 3

H), 1.34 (s, 9 H), 1.66 (s, 3 H), 1.71 (s, 3 H), 1.73 (s, 3 H), 1.86–1.91 (m, 6 H), 2.03 (t, *J* = 2.6 Hz, 1H), 2.05 (t, *J* = 2.7 Hz, 1H), 2.12 (m, 2 H), 2.39 (m, 4 H), 2.52 (m, 3 H), 2.62 (m, 2 H), 2.68 (m, 1 H), 2.84 (s, 3H), 2.89 (m, 3 H), 3.39 (m, 2 H), 3.44–3.49 (m, 2 H), 3.51–3.58 (m, 4 H), 3.64 (br s, 8 H), 3.73 (m, 1 H), 3.80(d, *J* = 3.7 Hz, 1 H), 3.99 (s, 1 H), 4.08 (d, *J* = 1.0 Hz, 1 H), 4.18 (d, *J* = 4.2, 1 H), 4.30 (d, *J* = 4.2, 1 H), 4.40 (m, 1 H), 4.95–4.98(m, 4 H), 5.10 (br s, 1 H), 5.68 (d, *J* = 7.2 Hz, 1 H), 6.01 (br s, 1 H), 6.19 (t, *J* = 8.7 Hz, 1 H), 6.29 (m, 2 H), 7.31–7.33 (m, 3 H), 7.47 (t, *J* = 7.2, 2 H), 7.60 (t, *J* = 7.4 Hz, 1 H), 7.80 (m, 1 H), 8.11 (d, *J* = 3.9 Hz, 1 H).

N_γ-((((Azidoethoxy)ethoxy)ethoxy)ethyl)-*α*-(*tert*butyl)-N_α-fmoc-*L*-glutamin (2-28)

To a solution of Fmoc-Glu-OtBu (250 mg, 0.588 mmol), EDC·HCl (169 mg, 0.883 mmol) and azide-PEG-amine **2-24** (154 mg, 0.706 mmol) in DCM [0.058M]. The reaction mixture was stirred at room temperature for one and a half hour. Upon completion, the mixture was concentrated *in vacou*, and the product was purified using silica gel column chromatography to afford compound **2-28** (228 mg, 62 %), as yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47(s, 9 H), 1.96 (m, 1 H), 2.23 (m, 2 H), 3.36 (t, *J* = 5.0 Hz, 2 H), 3.45 (t, *J* = 5.1 Hz, 2 H), 3.55 (t, *J* = 5.3 Hz, 2 H), 3.63–3.65 (m, 10 H), 4.22 (m, 2 H), 4.39 (quin, *J* = 5.1 Hz, 2 H), 5.65 (d, *J* = 8.0 Hz, 1 H), 6.25 (br s, 1 H), 7.32 (tt, *J* = 7.4, 1.2 Hz, 2 H), 7.40 (t, *J* = 7.5 Hz, 2 H), 7.61 (m, 2 H), 7.77 (d, *J* = 7.3 Hz, 2 H). All data are consistent with the reported values.[33]

N_γ-((((Azidoethoxy)ethoxy)ethoxy)ethyl)-*α*-(*tert*butyl)-*L*-glutamin (2-29)

A solution of compound **2-28** (327 mg, 0.523 mmol) in DCM [0.03M] was cooled to 0 ^{o}C, and TFA (3.27 mL, 0.04 mol) was added. The reaction mixture was stirred at 0 ^{o}C for two hours and the reaction temperature move to room temperature gradually and stirred for another 7 hours. Upon completion, the reaction mixture was washed with water (15 mL x 4) and concentrated *in vacou* to afford the compound **2-29** (263 mg, 88 %), as white-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.15 (m, 2 H), 2.47 (m, 2 H), 3.37 (t, *J* = 4.9 Hz, 2 H), 3.47 (m, 2 H), 3.57 (t, *J* = 4.5 Hz, 2 H), 3.63–3.65 (m, 10 H), 4.21(t, *J* = 7.0 Hz, 1 H), 4.37 (quin, *J* = 8.68 Hz, 3 H), 6.01 (d, *J* = 6.4 Hz, 1 H), 6.60 (br s, 1 H), 7.31 (t, *J* = 7.4 Hz, 2 H), 7.40 (t, *J* = 7.4 Hz, 2 H), 7.59 (t, *J* = 5.8

Hz, 2 H), 7.76 (d, *J* = 7.6 Hz, 2 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.6, 28.7, 31.5, 32.2, 39.6, 47.0, 50.6, 53.3, 67.0, 69.3, 69.9, 70.1, 70.4, 119.9, 125.1, 127.1, 127.7, 141.2, 143.7, 143.9, 156.2, 173.4, 173.5. All data are consistent with the reported values.[33]

Phenol-Me-linker-TIPS (2-30)[31]

A solution of compound **2-11** (370 mg, 0.810 mmol), EDC·HCl (186 mg, 0.972 mmol), and DMAP (30mg, 0.243 mmol) in DCM [0.1 M] was under inert condition at 0 ^{o}C, and a solution of phenol (91.5 mg, 0.972 mmol) in 4 mL DCM was added into the flask. The temperature of reaction mixture was warm to room temperature gradually and it was monitored via TLC. After 4 hours, the reaction was washed with brine. The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue crude was purified via silica gel column chromatography to afford the compound **2-30** (280 mg, 65%), as light-yellow oil: ^{1}H NMR (400 HMz, $CDCl_3$) δ 1.06–1.09 (m, 18H), 1.24–1.28(m, 3H), 1.31 (d, *J* = 6.8 Hz, 3H), 1.86 (m, 1H), 1.96 (m, 1H), 2.36–2.49 (m, 2H), 2.96 (sext, *J* = 6.8 Hz, 1H), 4.12 (s, 2H), 7.11 (m, 2H), 7.21 (tt, *J* = 7.4, 1.1 Hz, 1H), 7.26 (m, 1H), 7.29–7.38 (m, 4H), 7.82 (m, 1H); ^{13}C NMR (400 HMz, $CDCl_3$) δ11.8, 17.8, 20.5, 31.0, 33.0, 39.4, 46.1, 121.4, 125.8, 127.6, 128.2, 129.3, 130.2, 130.8, 133.3, 137.6, 150.8, 173.0. All data are consistent with the reported values.[31]

Phenol-Me-linker-CO_2H (2-31)[31]

A solution of compound **2-30** (274 mg, 0.514 mmol) in a 1:1 mixture of CH_3CN:py [0.05 M] was cooled to 0 ^{o}C and HF:py (2.7 mL) was added. The mixture was warmed to room temperature gradually and the reaction was monitored via TLC. After 20 h, the reaction was quenched with 0.2 M citric acid and extracted with EA (2 x 20 mL). The organic layer was washed with aqueous $CuSO_4$ (2 x 20 mL), brine (3 x 20 mL), and dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified using silica gel column chromatography to yield compound **2-31** (188 mg, 96% yield), as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, *J* = 6.8 Hz, 3H), 1.86 (sext, J = 1.86 Hz, 1H), 1.97 (sex, *J* = 7.5 Hz, 1H), 2.42 (m, 2H), 2.95 (q, *J* = 6.7 Hz, 1H), 4.12 (s, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.19–7.28 (m, 2H), 7.30–7.38 (m, 4H), 7.81 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 20.4, 30.3, 31.1, 39.4, 45.8, 121.4,

125.8, 127.8, 128.3, 129.3, 130.4, 130.9, 133.5, 137.5, 150.7, 169.4, 178.7. All data are consistent with the reported values.[31]

Phenol-Me-linker-OSu (2-32)[31]

A solution of compound **2-31** (176 mg, 0.467 mmol), EDC·HCl (179 mg, 0.934 mmol), and DMAP (17 mg, 0.14 mmol) in DCM [0.1 M] was added a solution of NHS (64.5 mg, 0.560 mmol) in 2 mL DCM. The mixture was stirred at room temperature and the reaction was monitored via TLC. After 2 h, the mixture was washed with water (2 x 15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified using silica gel column chromatography to yield compound **2-32** (189 mg, 86% yield), as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (d, *J* = 6.8, 3H), 1.96 (m, 1H), 2.06 (m, 1H), 2.66 (t, *J* = 7.5 Hz, 2 H), 2.82 (br s, 4 H), 3.00 (sex, *J* = 6.2 Hz, 1 H), 4.13 (s, 2 H), 7.11 (m, 2 H), 7.21 (tt, *J* = 7.4, 2.3 Hz, 1 H), 7.25–7.28 (m, 1H), 7.31–7.38 (m, 4 H), 7.81 (m, 1 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 20.4, 25.6, 28.3, 30.3, 39.4, 45.6, 121.5, 125.8,127.9, 128.4, 129.4, 130.5, 130.9, 133.6, 168.1, 169.0, 169.4. All data are consistent with the reported values.[31]

§2.5 References

(1) Sawyers, C.: Targeted cancer therapy. *Nature* **2004**, *432*, 294-297.

(2) Dagher, R.; Cohen, M.; Williams, G.; Rothmann, M.; Gobburu, J.; Robbie, G.; Rahman, A.; Chen, G.; Staten, A.; Griebel, D.; Pazdur, R.: Approval summary: Imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. *Clin Cancer Res* **2002**, *8*, 3034-3038.

(3) Demetri, G. D.; von Mehren, M.; Blanke, C. D.; Van den Abbeele, A. D.; Eisenberg, B.; Roberts, P. J.; Heinrich, M. C.; Tuveson, D. A.; Singer, S.; Janicek, M.; Fletcher, J. A.; Silverman, S. G.; Silberman, S. L.; Capdeville, R.; Kiese, B.; Peng, B.; Dimitrijevic, S.; Druker, B. J.; Corless, C.; Fletcher, C. D.; Joensuu, H.: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *The New England journal of medicine* **2002**, *347*, 472-80.

(4) Druker, B. J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G. M.; Fanning, S.; Zimmermann, J.; Lydon, N. B.: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* **1996**, *2*, 561-566.

(5) Cohen, M. H.; Williams, G. A.; Sridhara, R.; Chen, G.; McGuinn, W. D.; Morse, D.; Abraham, S.; Rahman, A.; Liang, C. Y.; Lostritto, R.; Baird, A.; Pazdur, R.: United States Food and Drug Administration drug approval summary: Gefitinib (ZD1839; Iressa) tablets. *Clin Cancer Res* **2004**, *10*, 1212-1218.

(6) Sordella, R.; Bell, D. W.; Haber, D. A.; Settleman, J.: Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* **2004**, *305*, 1163-1167.

(7) Jaracz, S.; Chen, J.; Kuznetsova, L. V.; Ojima, L.: Recent advances in tumor-targeting anticancer drug conjugates. *Bioorgan Med Chem* **2005**, *13*, 5043-5054.

(8) Ojima, I.: Guided molecular missiles for tumor-targeting chemotherapy-case studies using the second-generation taxolds as warheads. *Accounts Chem Res* **2008**, *41*, 108-119.

(9) Ojima, I.; Zuniga, E. S.; Berger, W. T.; Seitz, J. D.: Tumor-targeting drug delivery of new-generation taxoids. *Future medicinal chemistry* **2012**, *4*, 33-50.

(10) Chari, R. V. J.: Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. *Advanced drug delivery reviews* **1998**, *31*, 89-104.

(11) Ojima, I.; Geng, X. D.; Wu, X. Y.; Qu, C. X.; Borella, C. P.; Xie, H. S.; Wilhelm, S. D.; Leece, B. A.; Bartle, L. M.; Goldmacher, V. S.; Chari, R. V. J.: Tumor-specific novel taxoid-monoclonal antibody conjugates. *J Med Chem* **2002**, *45*, 5620-5623.

(12) Bradley, M. O.; Webb, N. L.; Anthony, F. H.; Devanesan, P.; Witman, P. A.; Hemamalini, S.; Chander, M. C.; Baker, S. D.; He, L. F.; Horwitz, S. B.; Swindell, C. S.: Tumor targeting by covalent conjugation of a natural fatty acid to paclitaxel. *Clin Cancer Res* **2001**, *7*, 3229-3238.

(13) Kuznetsova, L.; Chen, J.; Sun, L.; Wu, X. Y.; Pepe, A.; Veith, J. A.; Pera, P.; Bernacki, R. J.; Ojima, I.: Syntheses and evaluation of novel fatty acid-second-generation taxoid conjugates as promising anticancer agents. *Bioorg Med Chem Lett* **2006**, *16*, 974-977.

(14) Lee, J. W.; Lu, J. Y.; Low, P. S.; Fuchs, P. L.: Synthesis and evaluation of taxol-folic acid conjugates as targeted antineoplastics. *Bioorgan Med Chem* **2002**, *10*, 2397-2414.

(15) Chen, S. Y.; Zhao, X. R.; Chen, J. Y.; Chen, J.; Kuznetsova, L.; Wong, S. S.; Ojima, I.: Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated Endocytosis and Drug Release. *Bioconjugate Chem* **2010**, *21*, 979-987.

(16) Hamann, P. R.; Hinman, L. M.; Beyer, C. F.; Lindh, D.; Upeslacis, J.; Flowers, D. A.; Bernstein, I.: An anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Choice of linker. *Bioconjugate Chem* **2002**, *13*, 40-46.

(17) Kaneko, T.; Willner, D.; Monkovic, I.; Knipe, J. O.; Braslawsky, G. R.; Greenfield, R. S.; Vyas, D. M.: New Hydrazone Derivatives of Adriamycin and Their Immunoconjugates - a Correlation between Acid Stability and Cytotoxicity. *Bioconjugate Chem* **1991**, *2*, 133-141.

(18) King, H. D.; Dubowchik, G. M.; Mastalerz, H.; Willner, D.; Hofstead, S. J.; Firestone, R. A.; Lasch, S. J.; Trail, P. A.: Monoclonal antibody conjugates of doxorubicin prepared with branched peptide linkers: Inhibition of aggregation by methoxytriethyleneglycol chains. *J Med Chem* **2002**, *45*, 4336-4343.

(19) Dubowchik, G. M.; Firestone, R. A.; Padilla, L.; Willner, D.; Hofstead, S. J.; Mosure, K.; Knipe, J. O.; Lasch, S. J.; Trail, P. A.: Cathepsin B-labile dipeptide linkers for lysosomal release of doxorubicin from internalizing immunoconjugates: Model studies of

enzymatic drug release and antigen-specific in vitro anticancer activity. *Bioconjugate Chem* **2002**, *13*, 855-869.

(20) Chari, R. V. J.: Targeted cancer therapy: Conferring specificity to cytotoxic drugs. *Accounts Chem Res* **2008**, *41*, 98-107.

(21) Zheng, Z. B.; Zhu, G. Z.; Tak, H.; Joseph, E.; Eiseman, J. L.; Creighton, D. J.: N-(2-hydroxypropyl)methaerylamide copolymers of a glutathione (GSH)-activated glyoxalase I inhibitor and DNA alkylating agent: Synthesis, reaction kinetics with GSH, and in vitro antitumor activities. *Bioconjugate Chem* **2005**, *16*, 598-607.

(22) Kosower, N. S.; Kosower, E. M.: The glutathione status of cells. *International review of cytology* **1978**, *54*, 109-60.

(23) Britten, R. A.; Green, J. A.; Warenius, H. M.: Cellular glutathione (GSH) and glutathione S-transferase (GST) activity in human ovarian tumor biopsies following exposure to alkylating agents. *International journal of radiation oncology, biology, physics* **1992**, *24*, 527-31.

(24) Corey, E. J.; Mehrotra, M. M.: A Simple and Enantioselective Synthesis of (+)-Biotin. *Tetrahedron Lett* **1988**, *29*, 57-60.

(25) Schwabacher, A. W.; Lane, J. W.; Schiesher, M. W.; Leigh, K. M.; Johnson, C. W.: Desymmetrization reactions: Efficient preparation of unsymmetrically substituted linker molecules. *J Org Chem* **1998**, *63*, 1727-1729.

(26) Shaabani, A.; Tavasoli-Rad, F.; Lee, D. G.: Potassium permanganate oxidation of organic compounds. *Synthetic Commun* **2005**, *35*, 571-580.

(27) Dickinso.Rp; Iddon, B.: Condensed thiophen ring systems. Part III. A new synthesis of benzo[b]thiophen-2(3H)-and-3(2H)-ones and some reactions of benzo-[b]thiophen-2(3H)-one with dimethyl sulphate in the presence of base *J Chem Soc C* **1970**, 1926-28.

(28) Bordwell, F. G.; Fried, H. E.: Heterocyclic Aromatic Anions with 4n+2-Pi-Electrons. *J Org Chem* **1991**, *56*, 4218-4223.

(29) Widdison, W. C.; Wilhelm, S. D.; Cavanagh, E. E.; Whiteman, K. R.; Leece, B. A.; Kovtun, Y.; Goldmacher, V. S.; Xie, H. S.; Steeves, R. M.; Lutz, R. J.; Zhao, R.; Wang, L. T.; Blattler, W. A.; Chari, R. V. J.: Semisynthetic maytansine analogues for the targeted treatment of cancer. *J Med Chem* **2006**, *49*, 4392-4408.

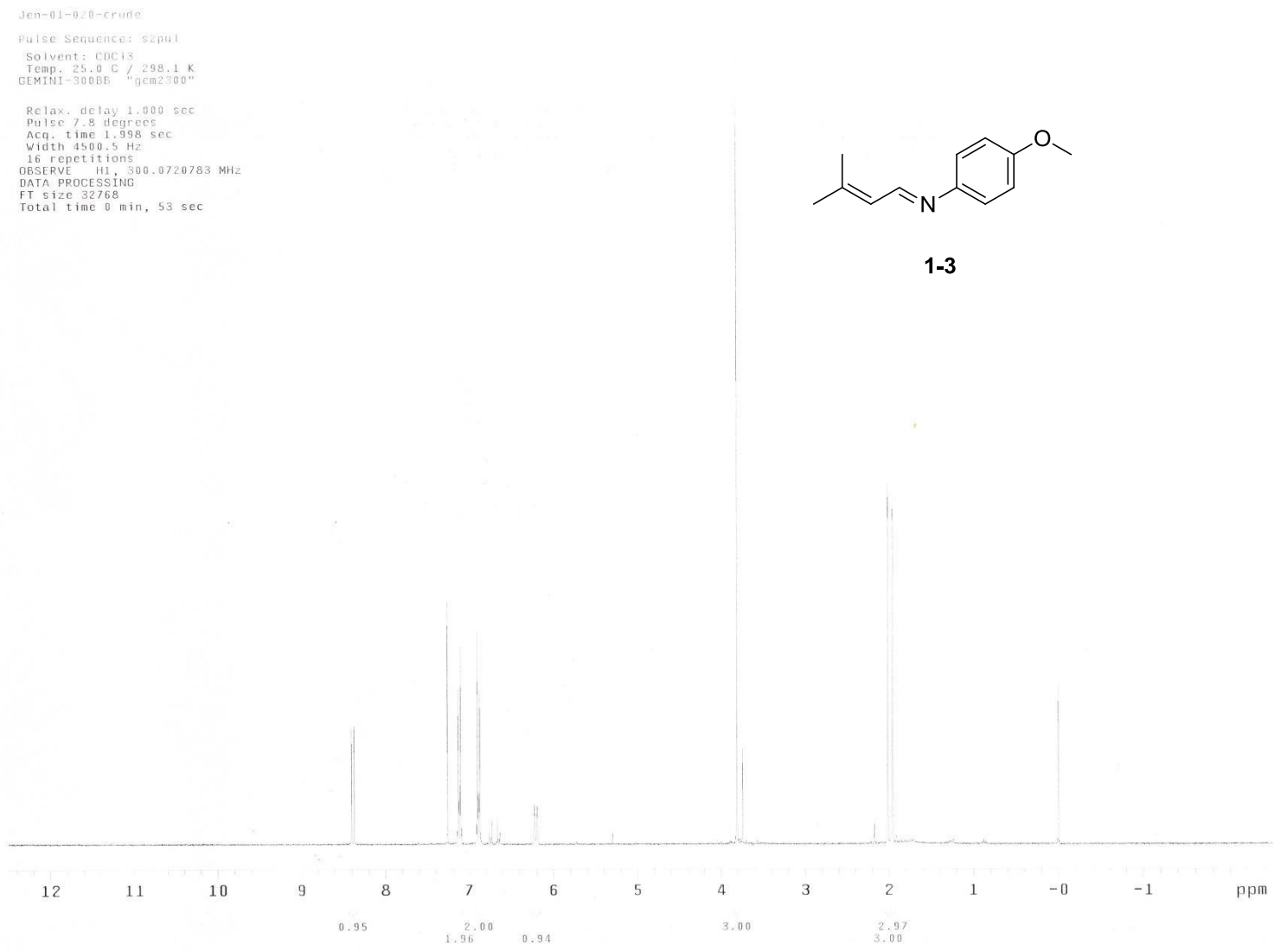
(30) Banerjee, P. S.; Zuniga, E. S.; Ojima, I.; Carrico, I. S.: Targeted and armed oncolytic adenovirus via chemoselective modification. *Bioorg Med Chem Lett* **2011**, *21*, 4985-4988.

(31) Zuniga, E. S.: Design, synthesis and biological evaluation of new-generation taxoid-based tumor-targeting drug conjugates. Stony Brook University, 2012.

(32) Galibert, M.; Dumy, P.; Boturyn, D.: One-Pot Approach to Well-Defined Biomolecular Assemblies by Orthogonal Chemoselective Ligations. *Angewandte Chemie-International Edition* **2009**, *48*, 2576-2579.

(33) Willibald, J.; Harder, J.; Sparrer, K.; Conzelmann, K. K.; Carell, T.: Click-modified anandamide siRNA enables delivery and gene silencing in neuronal and immune cells. *J Am Chem Soc* **2012**, *134*, 12330-3.

Appendix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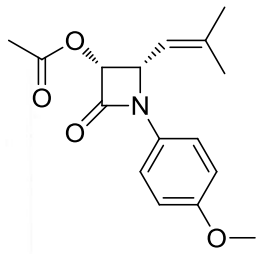

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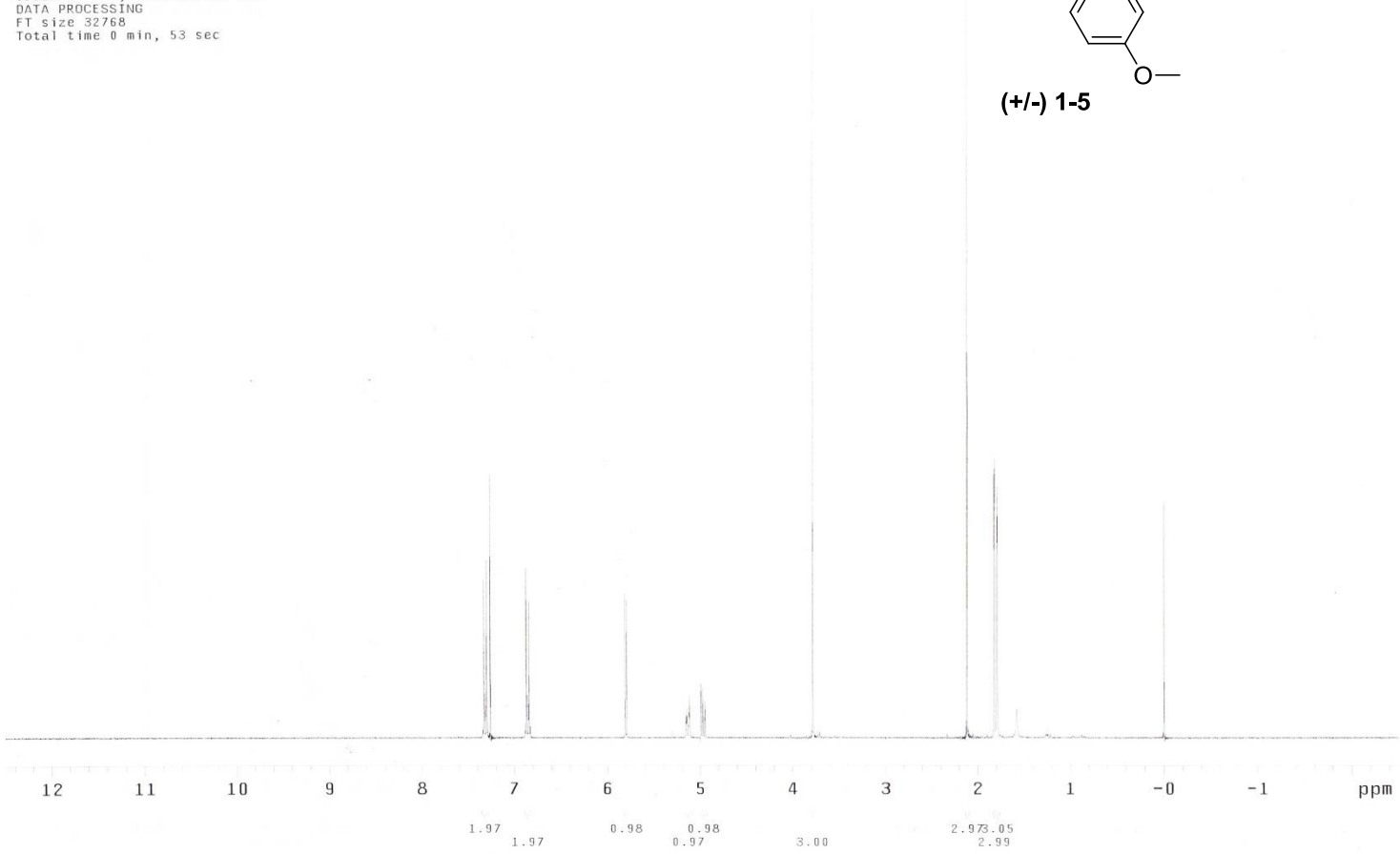

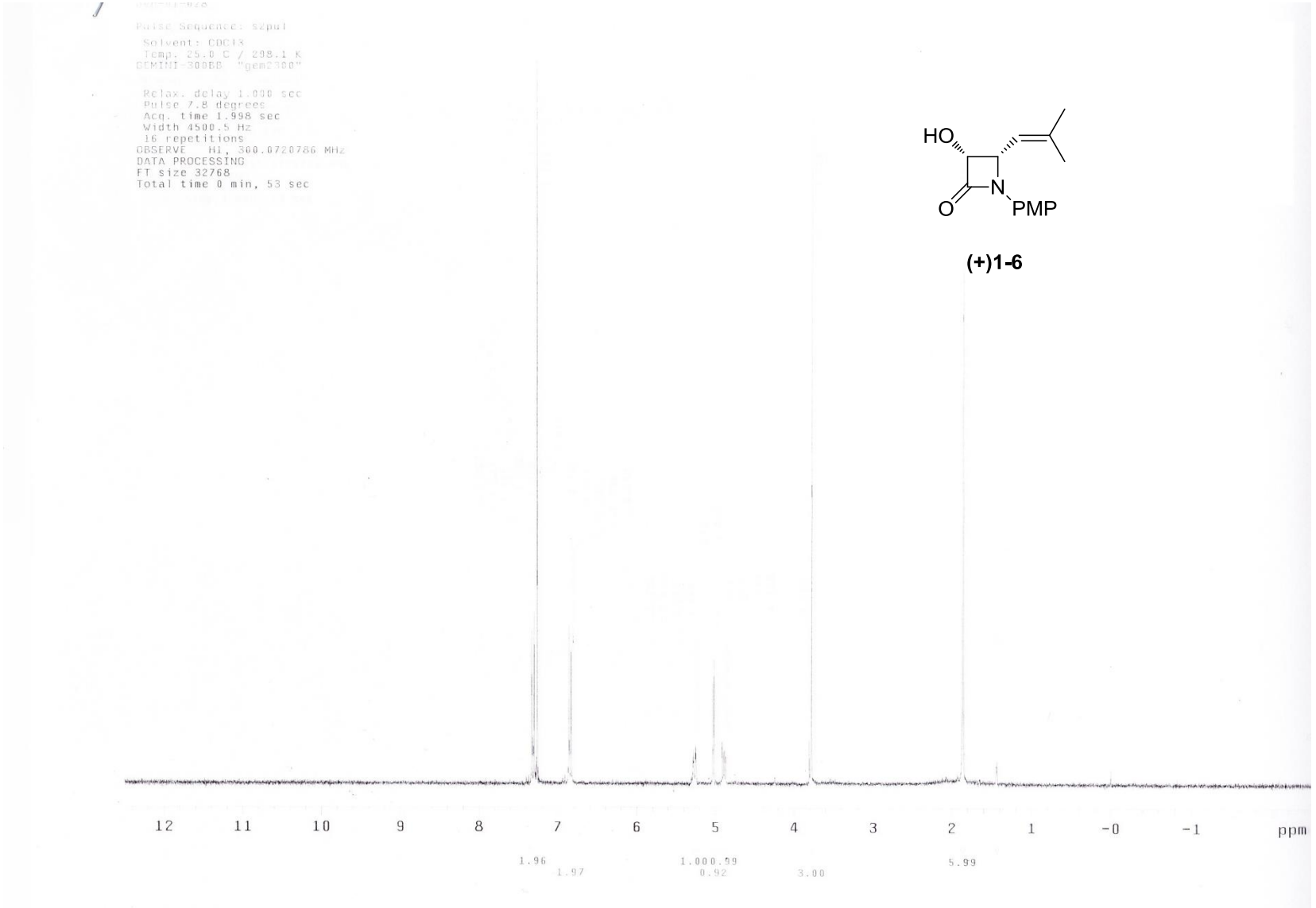
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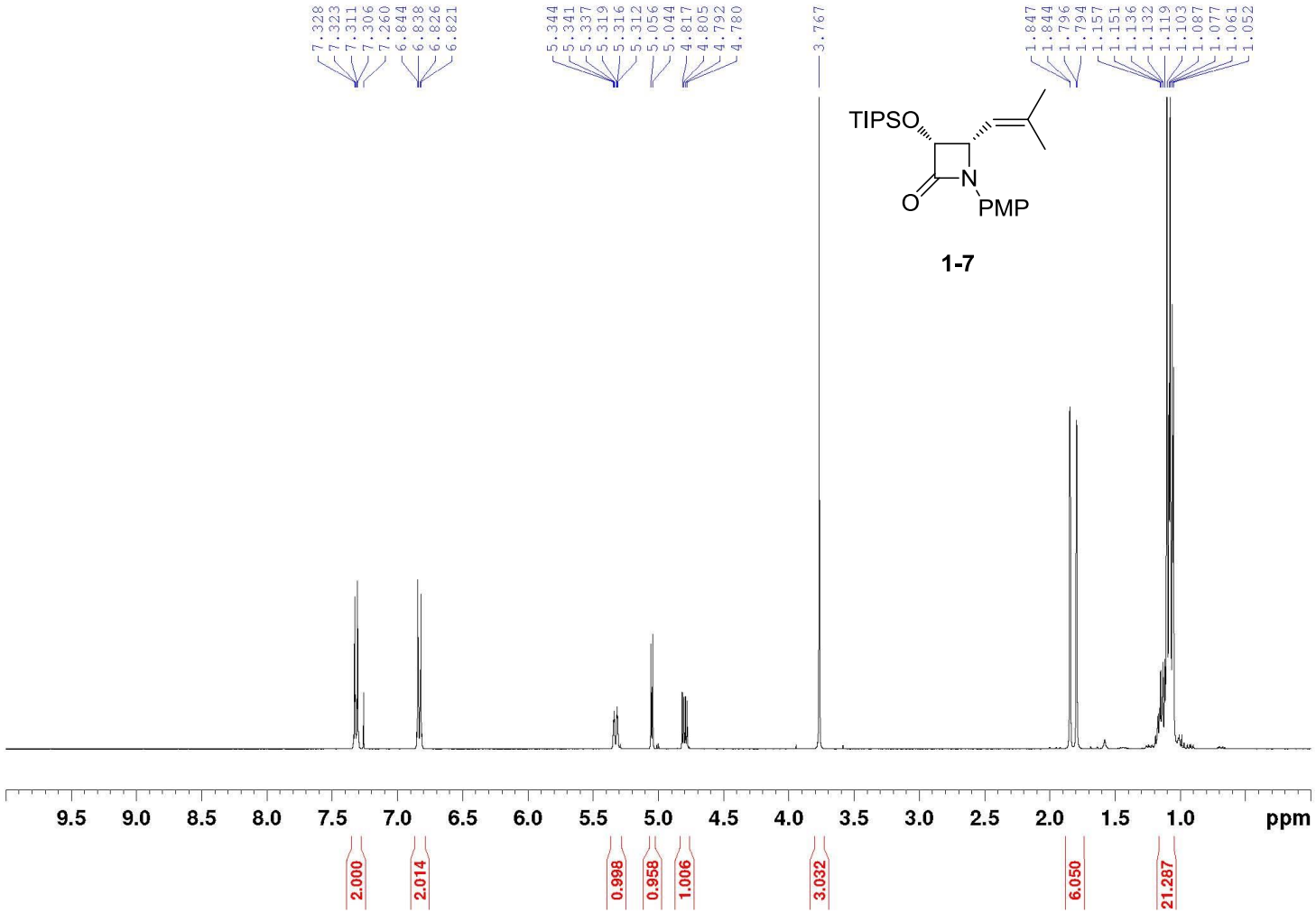
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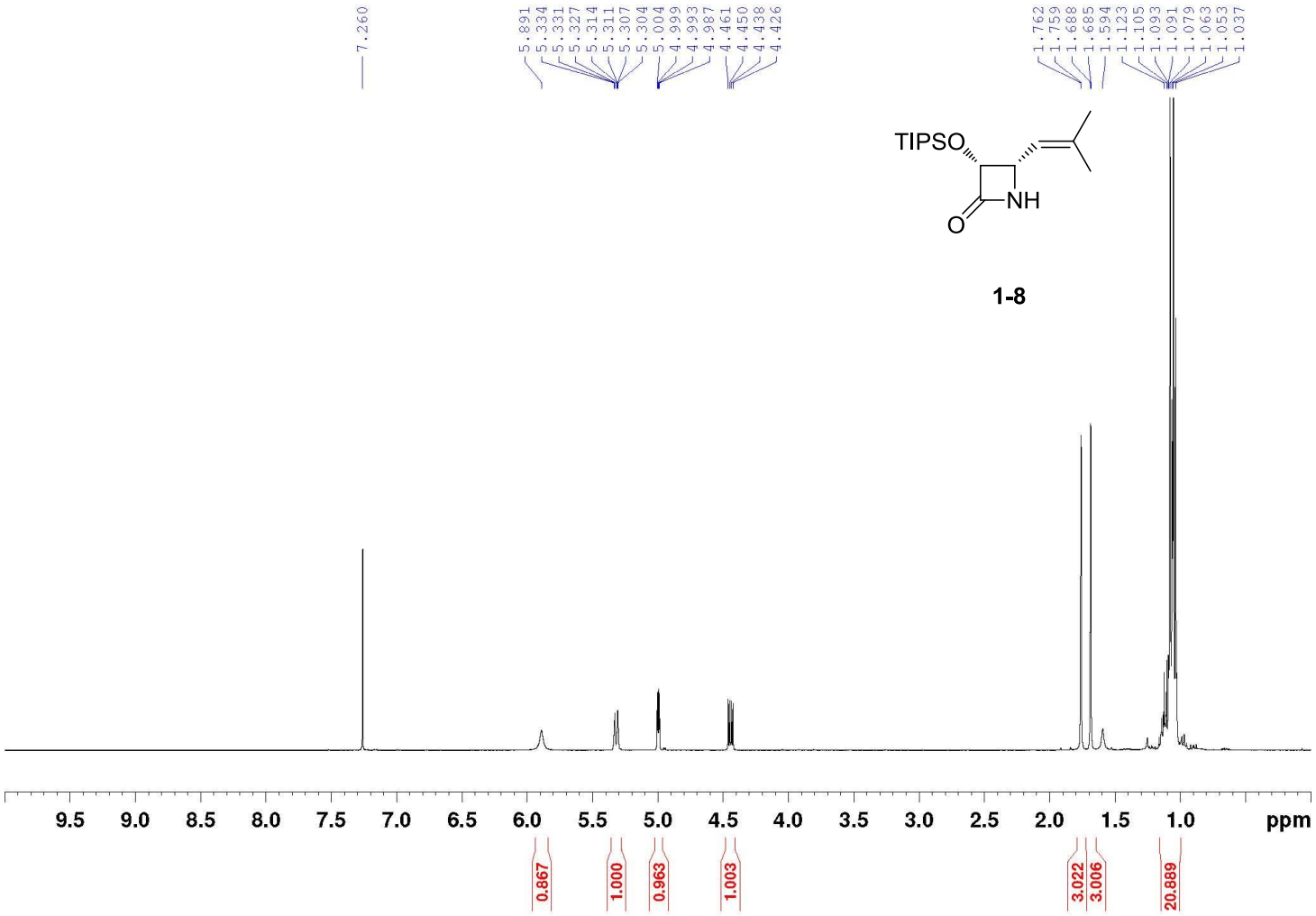
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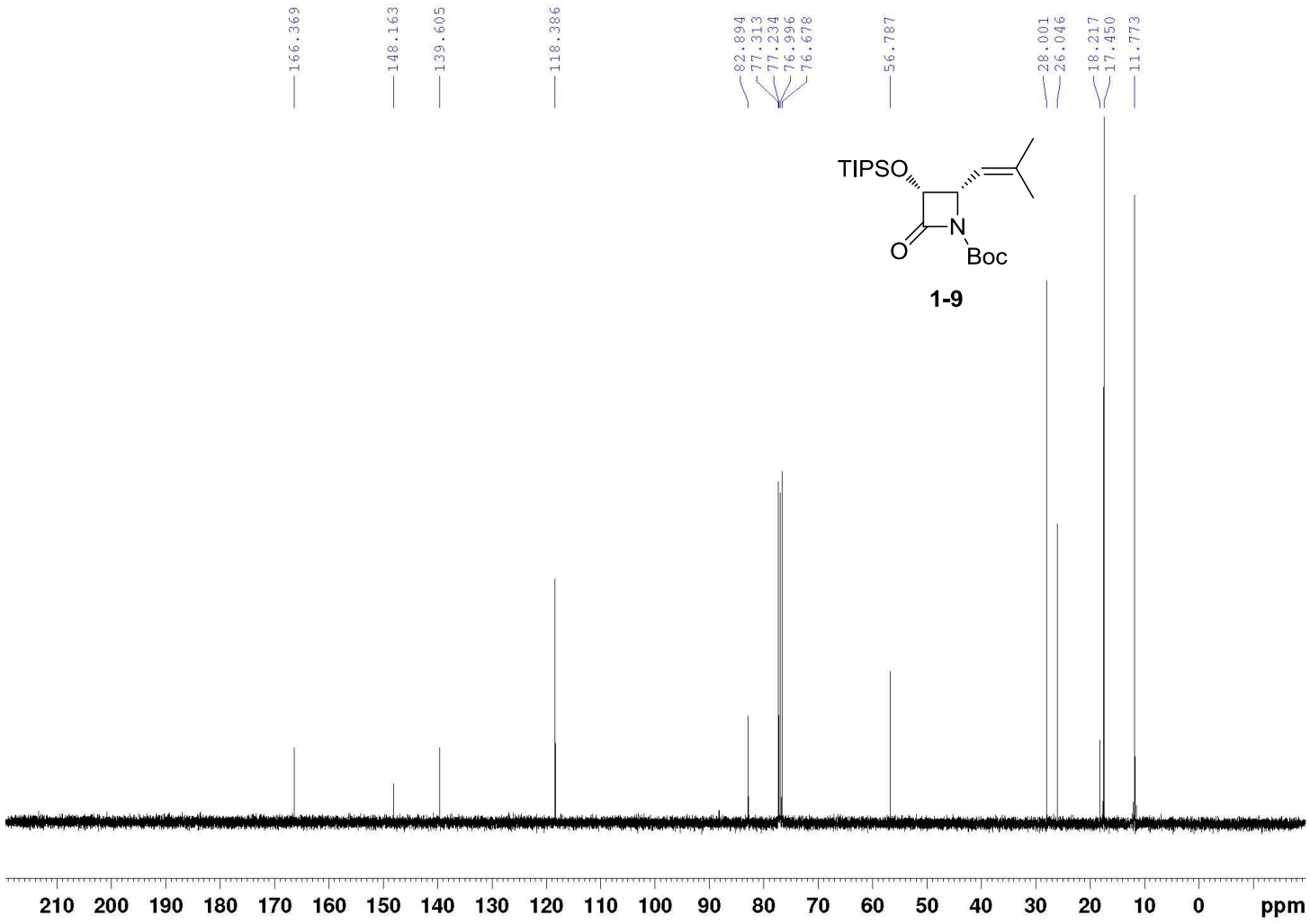
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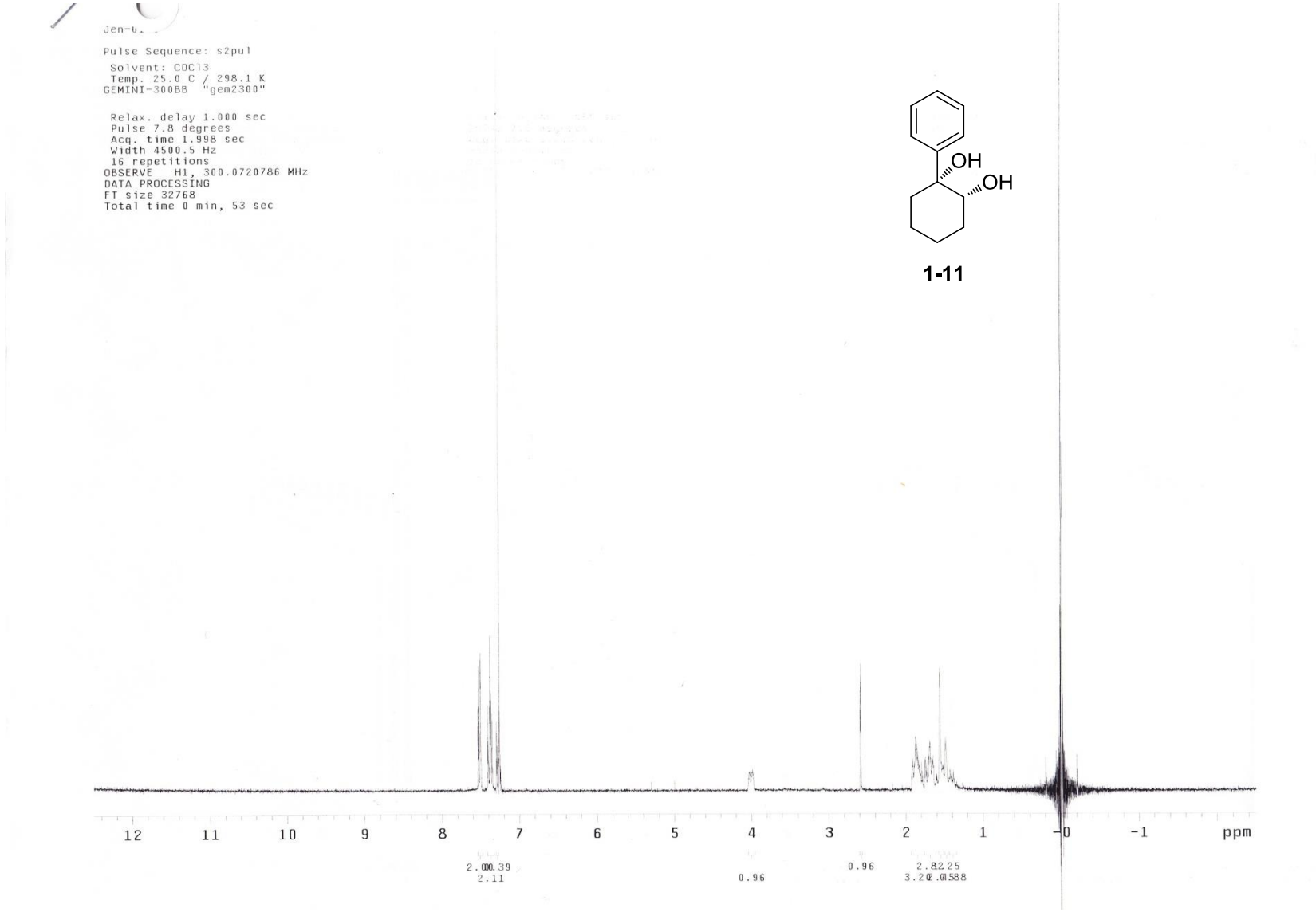
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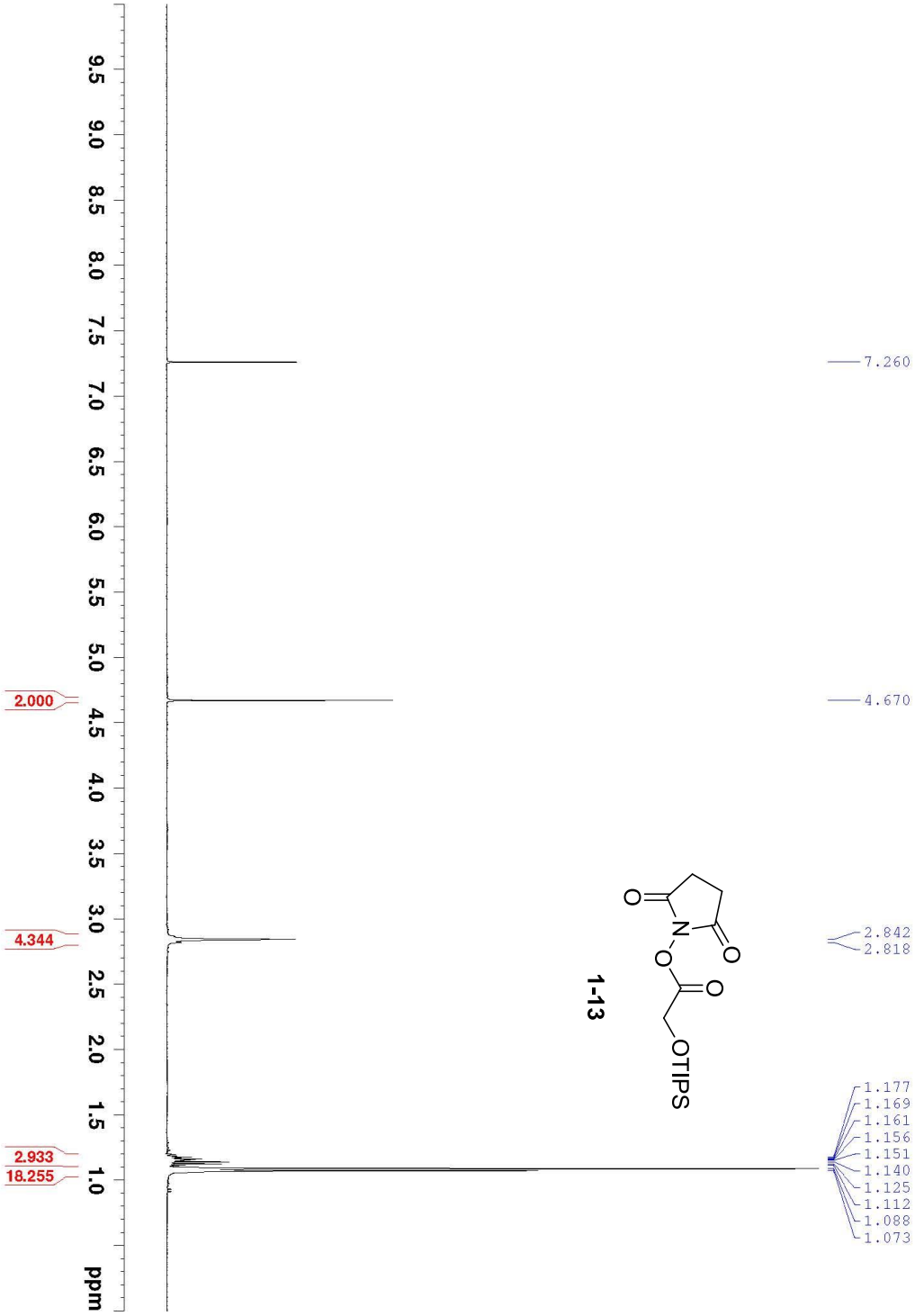
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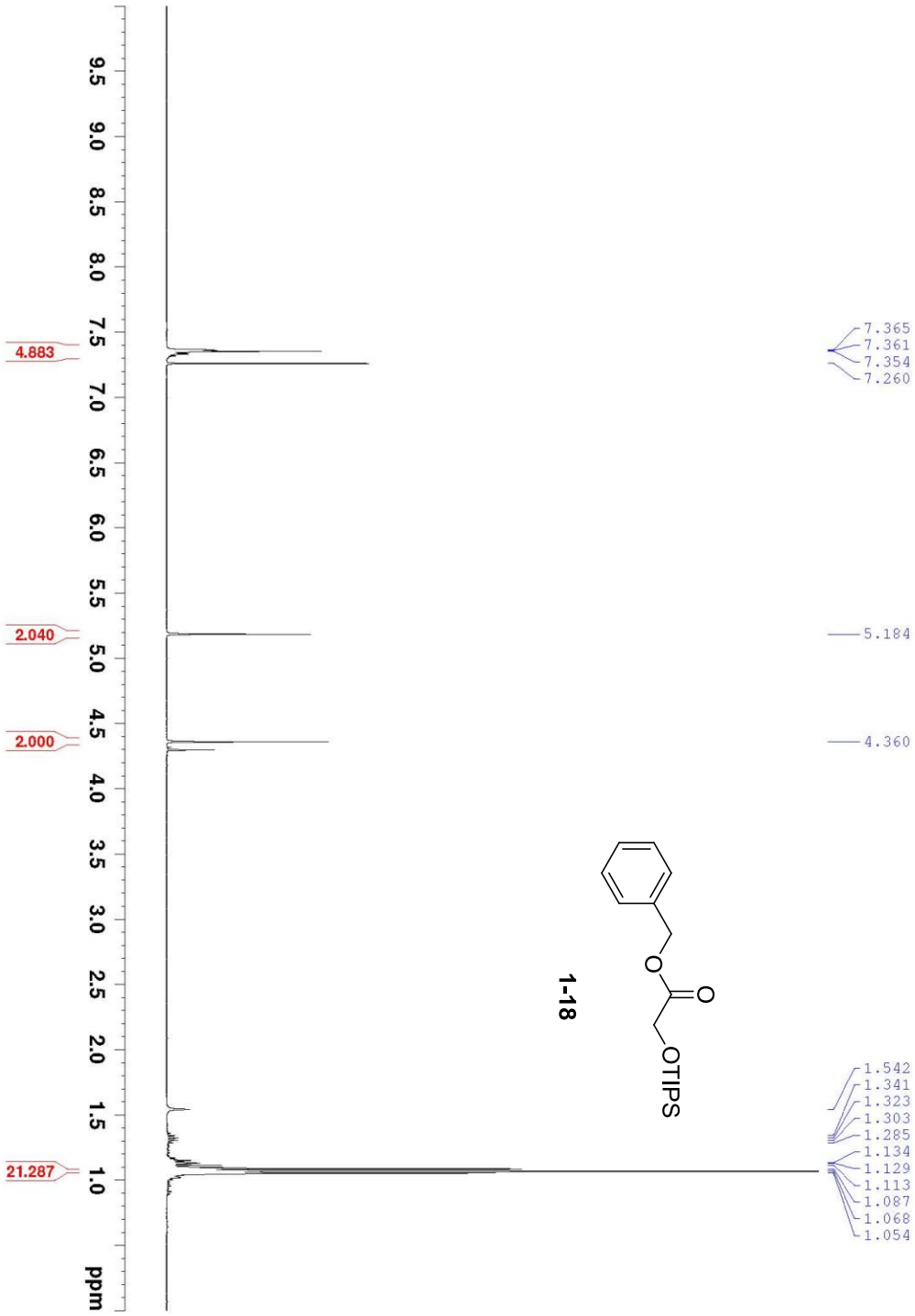
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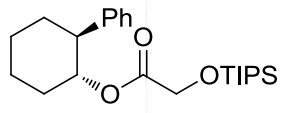
O
OTIPS
1-18

Jen-01-011

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
GEMINI-300BB "gem2300"

Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0720778 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 53 sec



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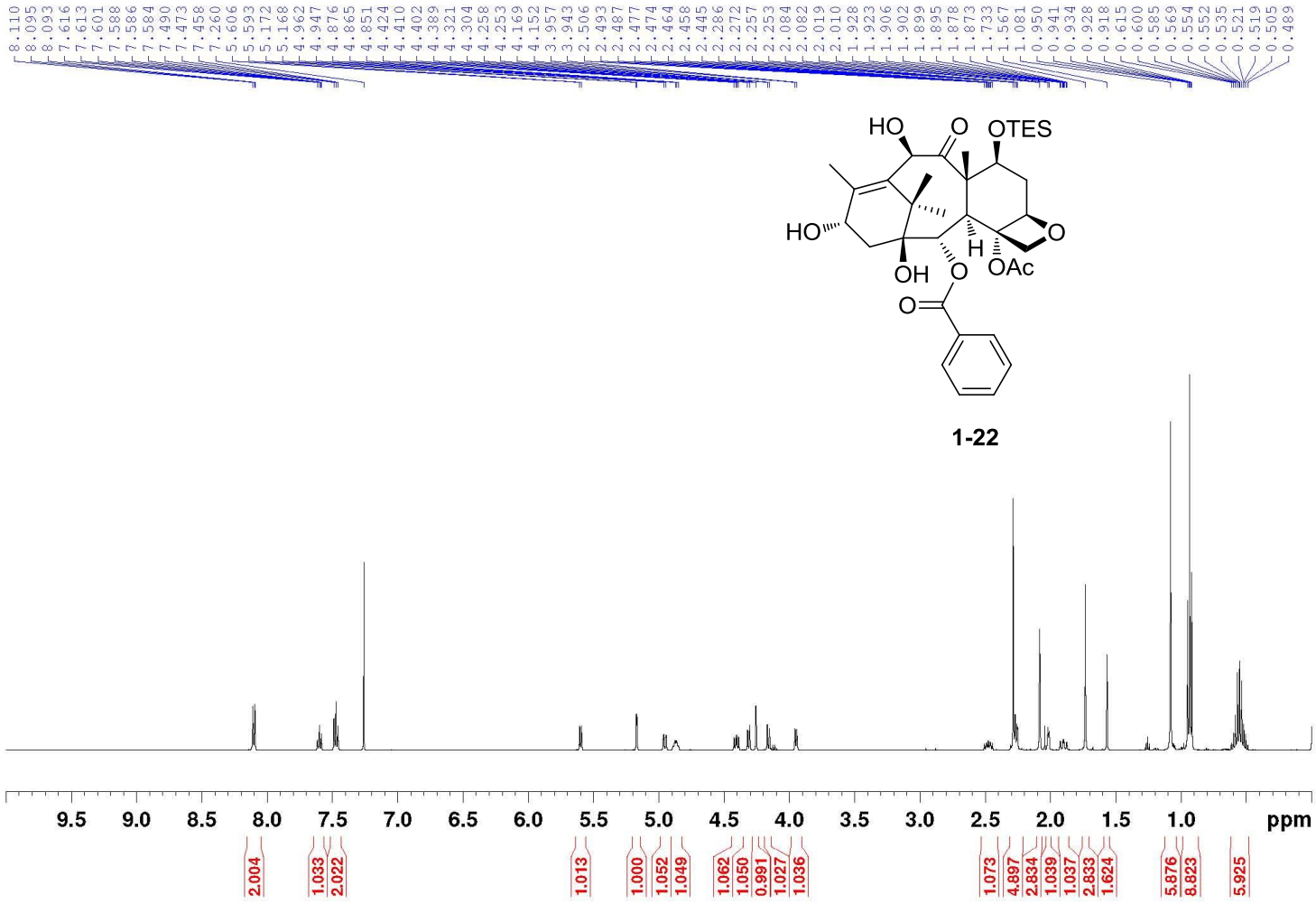
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7.260
5.606
5.593
5.172
5.168
4.962
4.947
4.876
4.865
4.851
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4.410
4.402
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4.321
4.304
4.258
4.253
4.169
4.152
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2.493
2.487
2.477
2.474
2.464
2.458
2.445
2.286
2.272
2.257
2.253
2.084
2.082
2.019
2.010
1.928
1.923
1.906
1.902
1.899
1.895
1.878
1.873
1.733
1.567
1.081
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0.934
0.928
0.918
0.615
0.600
0.585
0.569
0.554
0.552
0.535
0.521
0.519
0.505
0.489
HO
O
OTES
HO
H
O
OH
O
OAc
O
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
ppm
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2.022
1.013
1.000
1.052
1.049
1.062
1.050
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8.823
5.925

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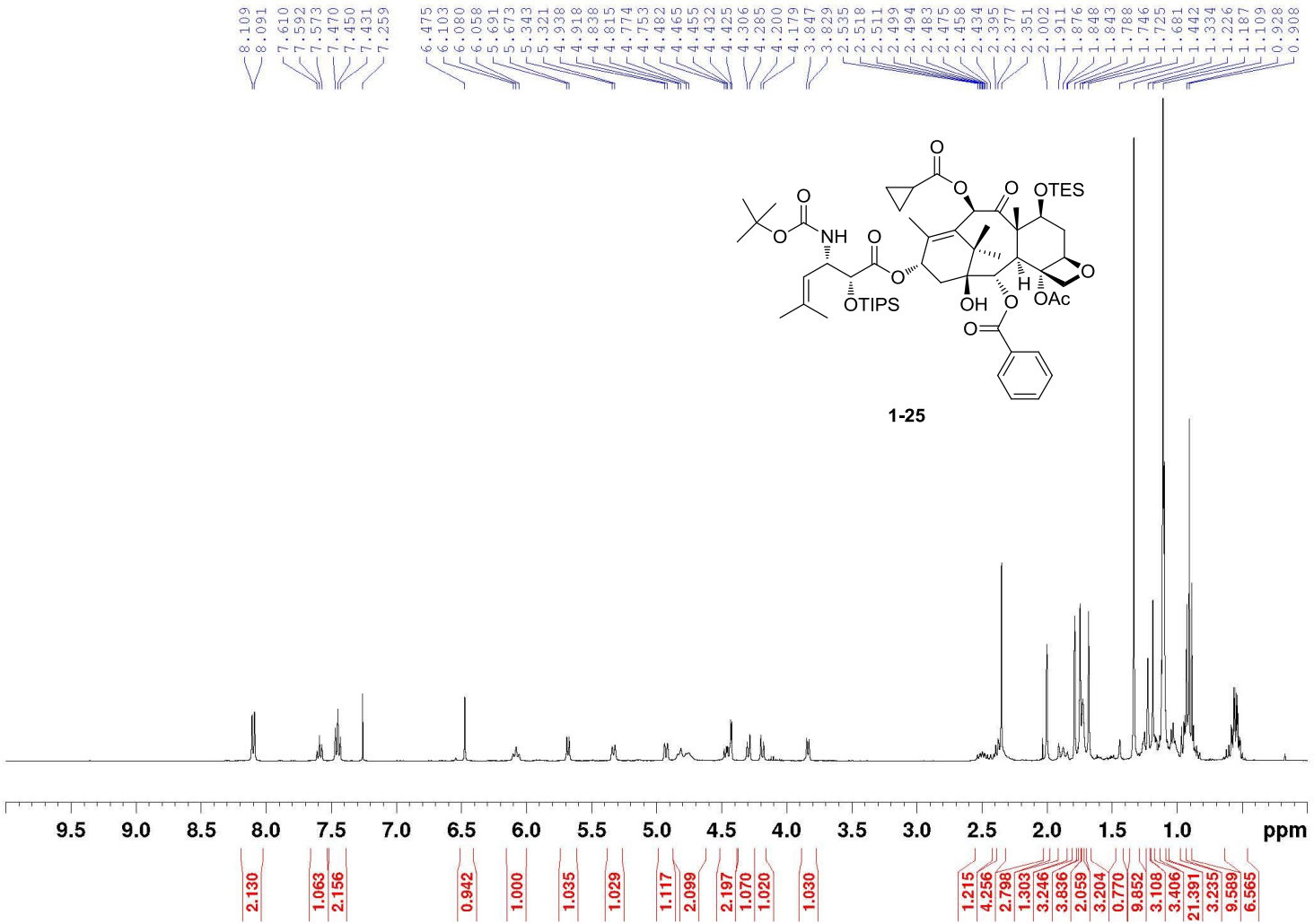
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5.691
5.673
5.343
5.321
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4.918
4.838
4.815
4.774
4.753
4.482
4.465
4.455
4.432
4.425
4.306
4.285
4.200
4.179
3.847
3.829
2.535
2.518
2.511
2.499
2.494
2.483
2.475
2.458
2.434
2.395
2.377
2.351
2.002
1.911
1.876
1.848
1.843
1.788
1.746
1.725
1.681
1.442
1.334
1.226
1.187
1.109
0.928
0.908
O
O
O
O
OTES
O
NH
O
O
H
O
OTIPS
OH
O
OAc
O
1-25
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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1.035
1.029
1.117
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2.197
1.070
1.020
1.030
1.215
4.256
2.798
1.303
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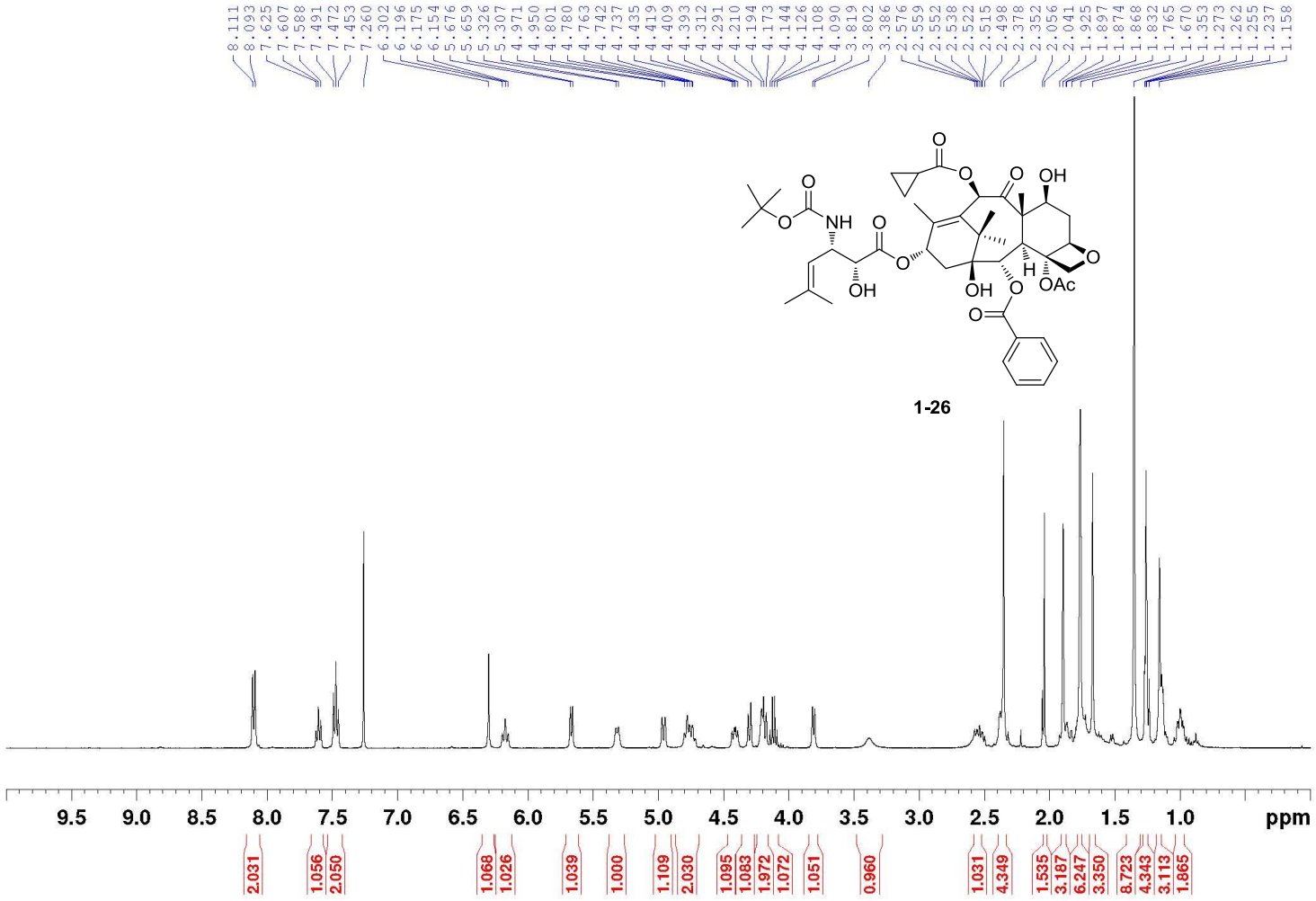
9.589
6.565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6.154
5.676
5.659
5.326
5.307
4.971
4.950
4.801
4.780
4.763
4.742
4.737
4.435
4.419
4.409
4.393
4.312
4.291
4.210
4.194
4.173
4.144
4.126
4.108
4.090
3.819
3.802
3.386
2.576
2.559
2.552
2.538
2.522
2.515
2.498
2.378
2.352
2.056
2.041
1.925
1.897
1.874
1.868
1.832
1.765
1.670
1.353
1.273
1.262
1.255
1.237
1.158
1-26
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
ppm
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2.050
1.068
1.026
1.039
1.000
1.109
2.030
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1.083
1.972
1.072
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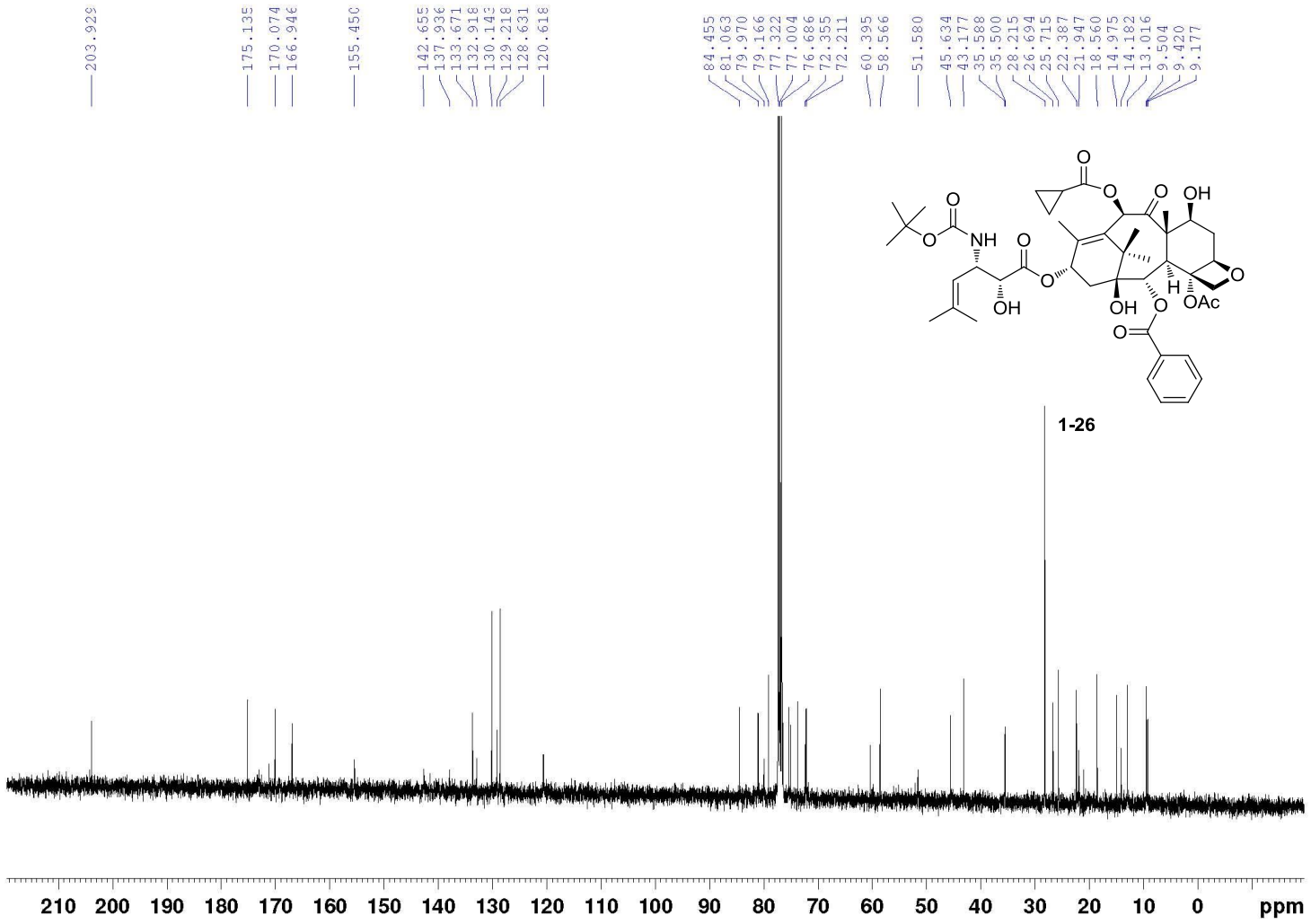
3.113
1.865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137.936
133.671
132.918
130.143
129.218
128.631
120.618
84.455
81.063
79.970
79.166
77.322
77.004
76.686
72.355
72.211
60.395
58.566
51.580
45.634
43.177
35.588
35.500
28.215
26.694
25.715
22.387
21.947
18.560
14.975
14.182
13.016
9.504
9.420
9.177
1-26
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

8.1074
8.0931
8.0906
7.6131
7.5983
7.5833
7.4875
7.4716
7.4565
7.2600
5.5956
5.5815
5.0175
5.0006
4.9013
4.8906
4.8400
4.3166
4.3002
4.1611
4.1440
3.9397
3.9269
3.9180
3.9048
3.8900
3.8747
3.4647
3.3154
2.7478
2.7348
2.7284
2.7193
2.7158
2.7064
2.7003
2.6871
2.2912
2.2827
2.2688
2.2547
2.1115
2.1096
1.7055
1.5558
1.2523
1.1662
1.0745
MeO
O
OMe
HO
H
O
OH
O
OAc
O
1-27
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1.0396
1.7850
1.0000
1.0765
1.0874
0.9543
1.1733
1.0176
1.8745
3.1982
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1.3207
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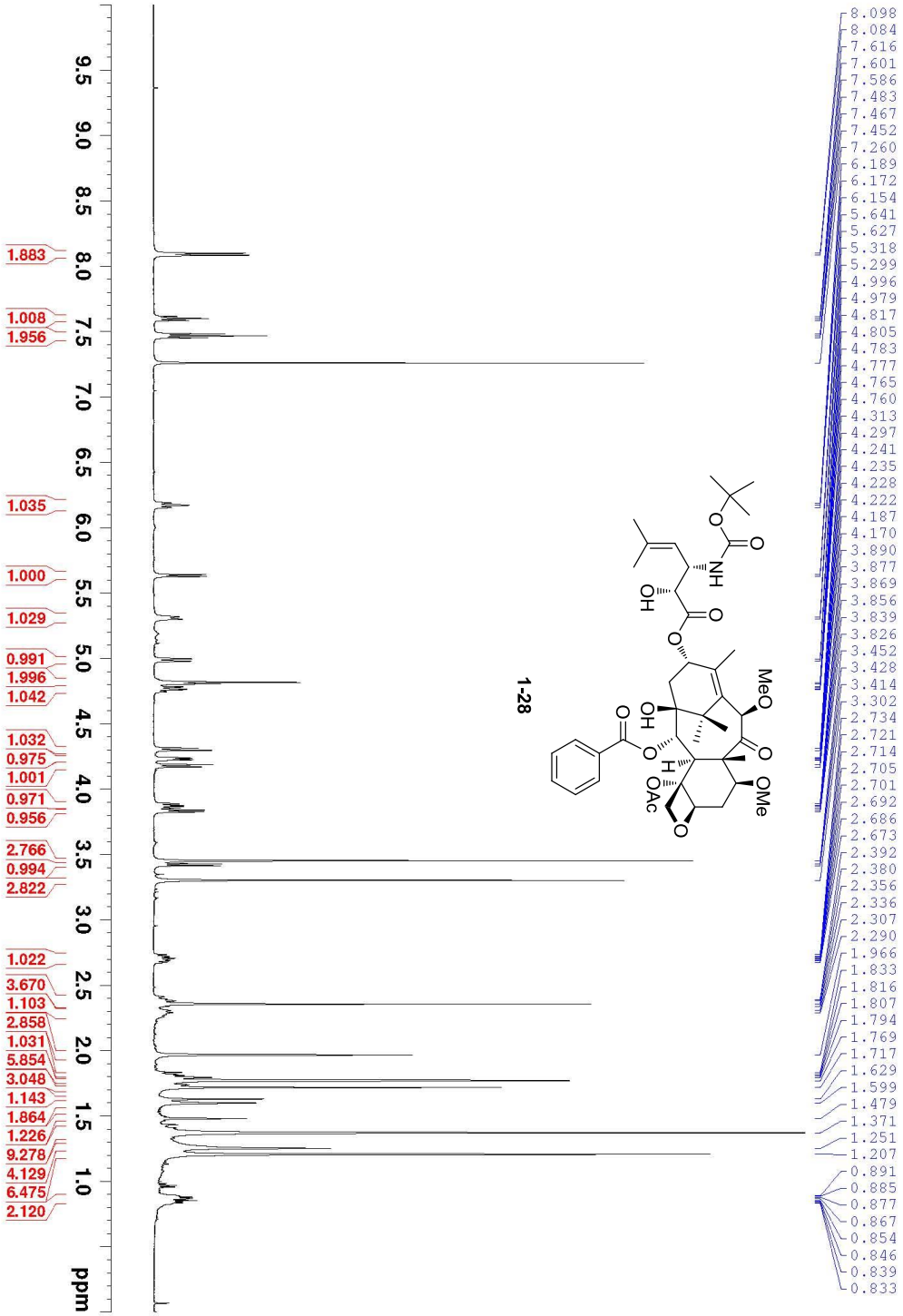
4
6
8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OAc
OH
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6.172
6.154
5.641
5.627
5.318
5.299
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4.979
4.817
4.805
4.783
4.777
4.765
4.760
4.313
4.297
4.241
4.235
4.228
4.222
4.187
4.170
3.890
3.877
3.869
3.856
3.839
3.826
3.452
3.428
3.414
3.302
2.734
2.721
2.714
2.705
2.701
2.692
2.686
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2.392
2.380
2.356
2.336
2.307
2.290
1.966
1.833
1.816
1.807
1.794
1.769
1.717
1.629
1.599
1.479
1.371
1.251
1.207
0.891
0.885
0.877
0.867
0.854
0.846
0.839
0.833
1.883
1.008
1.956
1.035
1.000
1.029
0.991
1.996
1.042
1.032
0.975
1.001
0.971
0.956
2.766
0.994
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1.103
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1.143
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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
ppm

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133.606
130.106
129.252
128.579
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84.091
82.644
81.664
80.723
79.920
78.786
77.252
76.999
76.744
74.574
73.720
72.393
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57.041
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51.483
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35.266
34.643
32.086
31.911
31.571
29.685
28.233
27.930
26.680
26.180
25.754
25.256
22.679
22.469
20.615
18.602
14.758
14.113
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OMe
NH
OH
OAc
H
1-28
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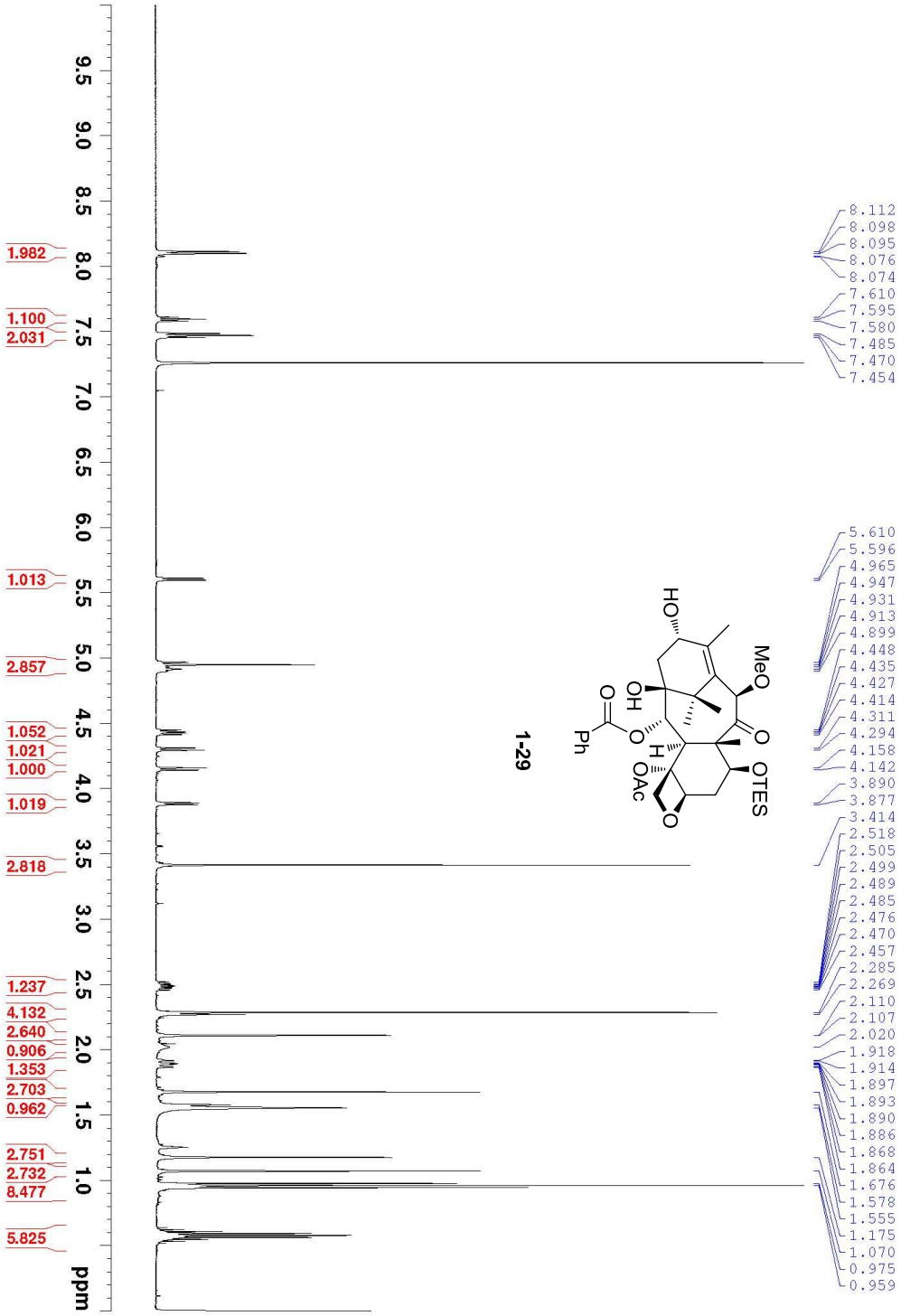
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MeO
OH
O
Ph
O
H
OAc
O
OTES
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8.076
8.074
7.610
7.595
7.580
7.485
7.470
7.454
5.610
5.596
4.965
4.947
4.931
4.913
4.899
4.448
4.435
4.427
4.414
4.311
4.294
4.158
4.142
3.890
3.877
3.414
2.518
2.505
2.499
2.489
2.485
2.476
2.470
2.457
2.285
2.269
2.110
2.107
2.020
1.918
1.914
1.897
1.893
1.890
1.886
1.868
1.864
1.676
1.578
1.555
1.175
1.070
0.975
0.959
1.982
1.100
2.031
1.013
2.857
1.052
1.021
1.000
1.019
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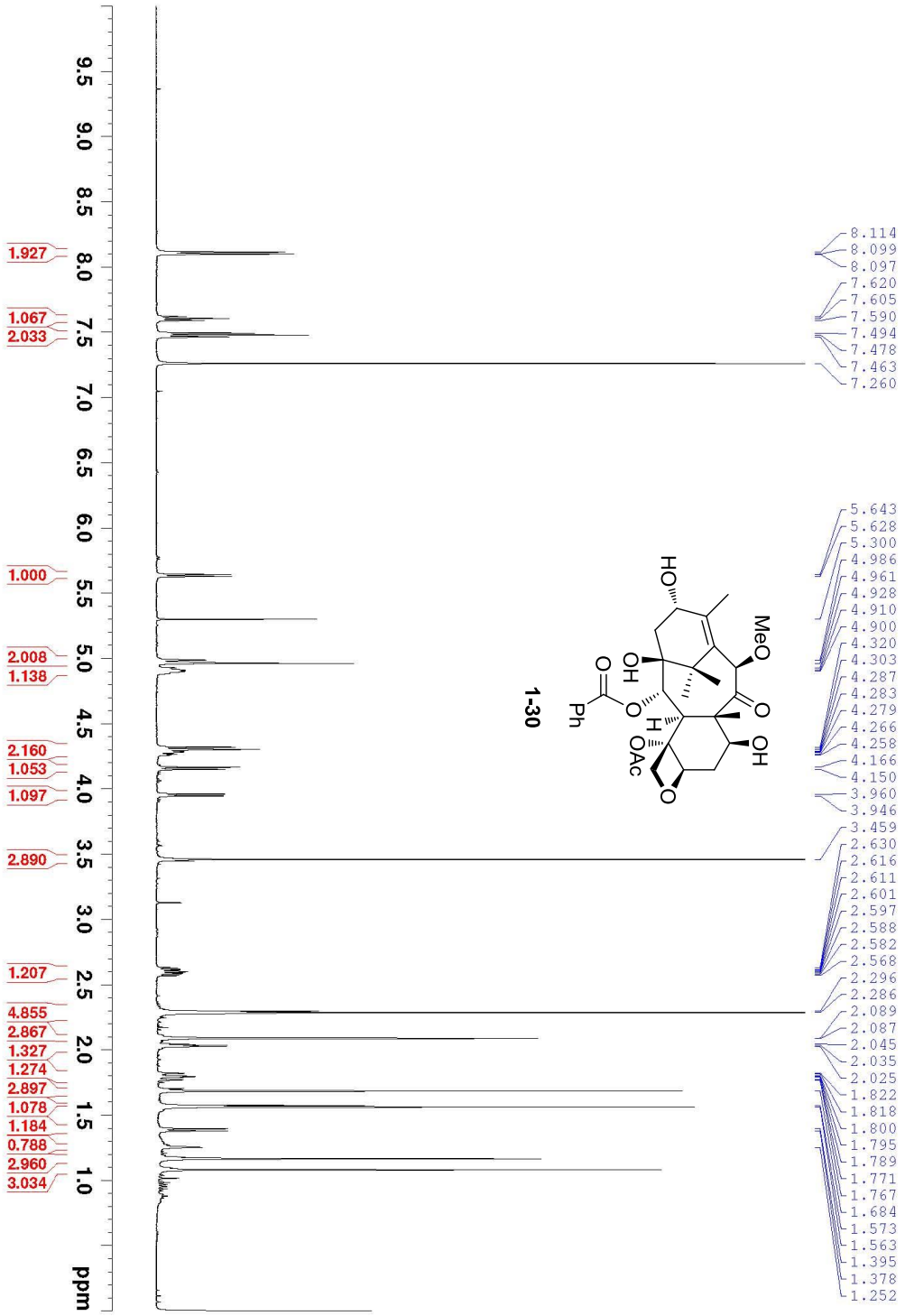
ppm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OH
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Ph
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H
OAc
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7.590
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7.478
7.463
7.260
5.643
5.628
5.300
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4.961
4.928
4.910
4.900
4.320
4.303
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4.279
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4.258
4.166
4.150
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2.582
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2.089
2.087
2.045
2.035
2.025
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1.800
1.795
1.789
1.771
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1.684
1.573
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1.395
1.378
1.252
1.927
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1.000
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1.138
2.160
1.053
1.097
2.890
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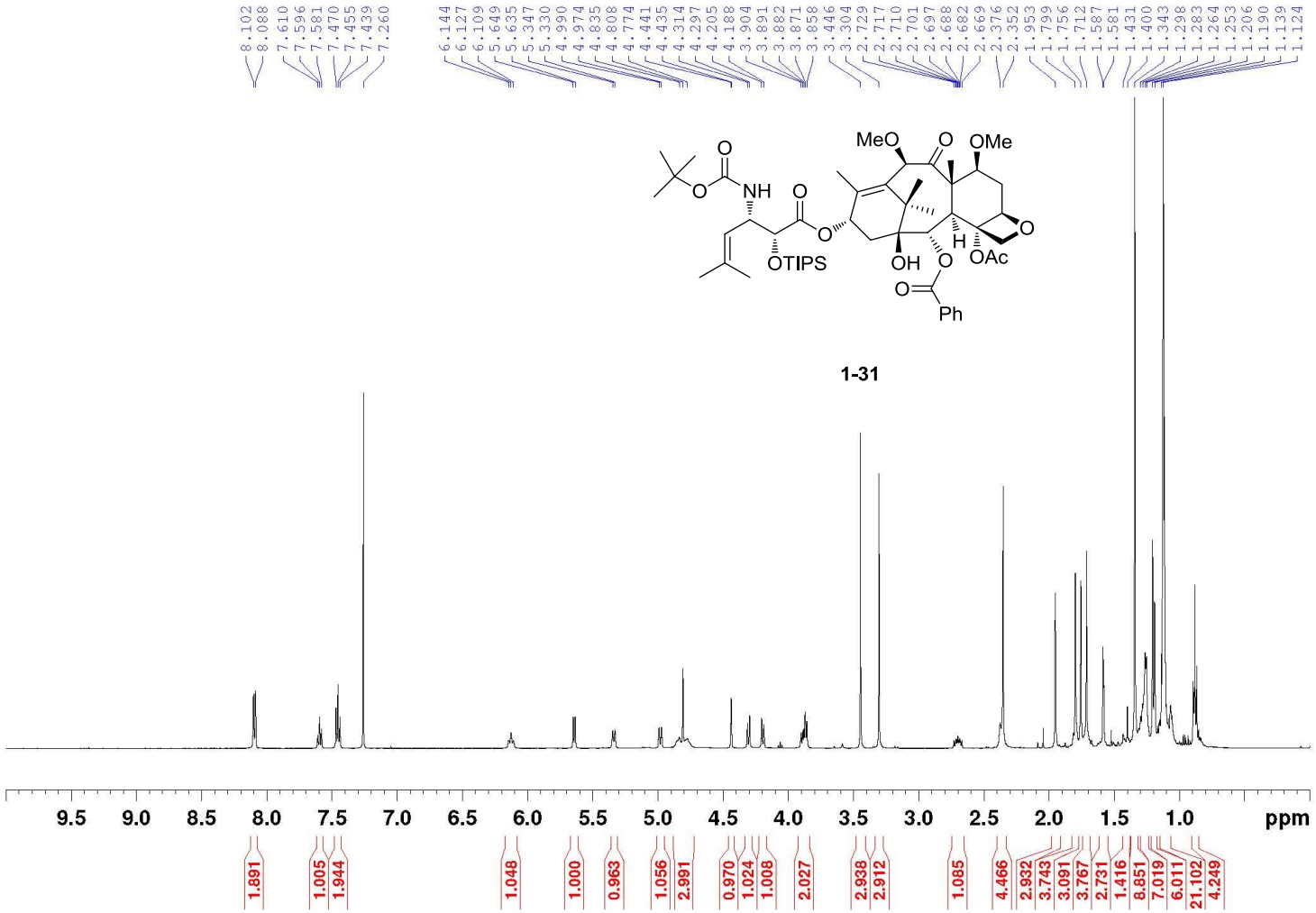
ppm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5.635
5.347
5.330
4.990
4.974
4.835
4.808
4.774
4.441
4.435
4.314
4.297
4.205
4.188
3.904
3.891
3.882
3.871
3.858
3.446
3.304
2.729
2.717
2.710
2.701
2.697
2.688
2.682
2.669
2.376
2.352
1.953
1.799
1.756
1.712
1.587
1.581
1.431
1.400
1.343
1.298
1.283
1.264
1.253
1.206
1.190
1.139
1.124
MeO
O
OMe
NH
O
O
O
O
H
OTIPS
OH
O
OAc
O
Ph
1-31
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7.0
6.5
6.0
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5.0
4.5
4.0
3.5
3.0
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2.0
1.5
1.0
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1.000
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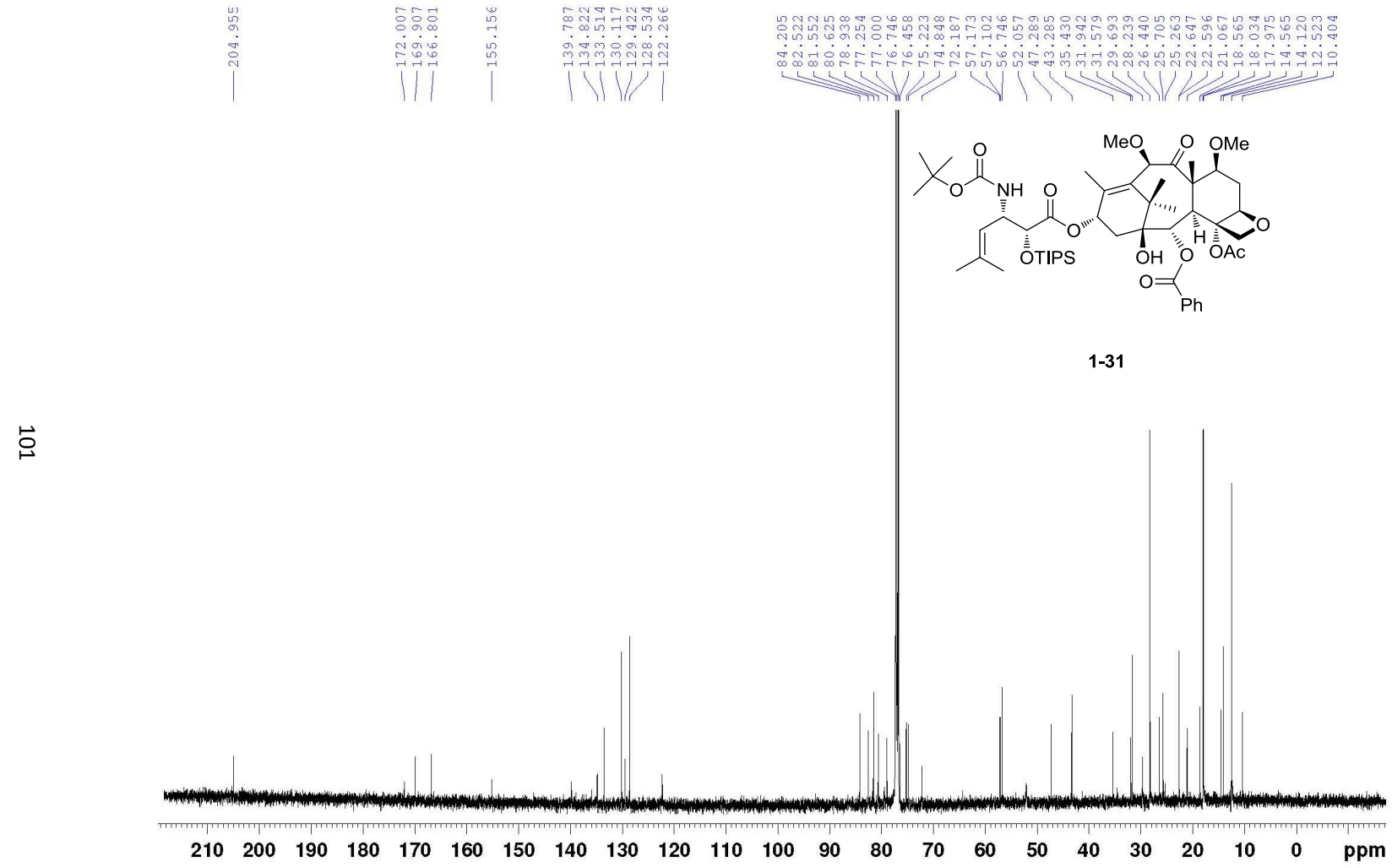
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166.801
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139.787
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129.422
128.534
122.266
84.205
82.522
81.552
80.625
78.938
77.254
77.000
76.746
76.458
75.223
74.848
72.187
57.173
57.102
56.746
52.057
47.289
43.285
35.430
31.942
31.579
29.693
28.239
26.440
25.705
25.263
22.647
22.596
21.067
18.565
18.034
17.975
14.565
14.120
12.523
10.404
MeO
O
OMe
O
NH
O
O
OTIPS
H
OAc
OH
O
O
Ph
1-31
210
200
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100
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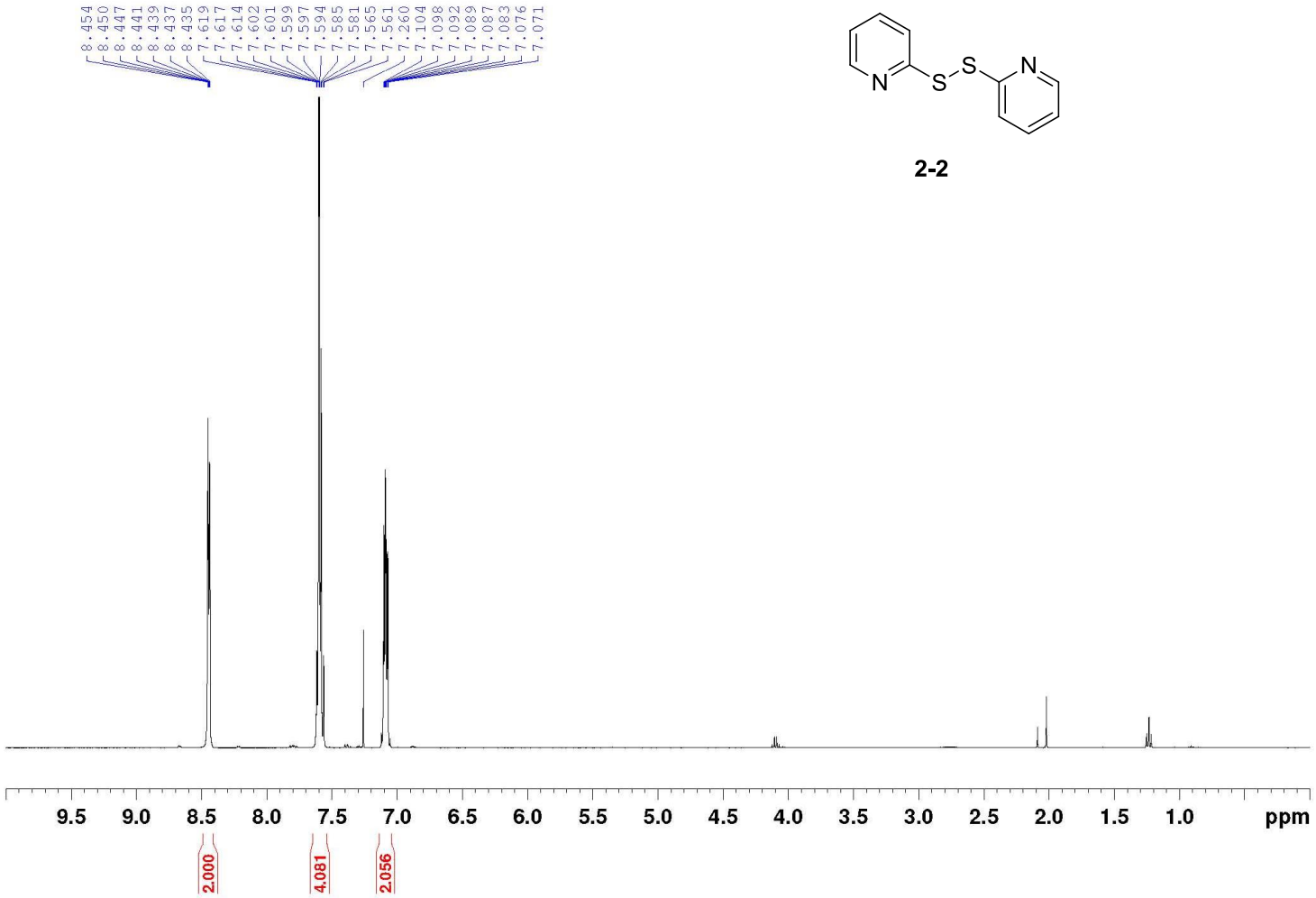
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7.601
7.599
7.597
7.594
7.585
7.581
7.565
7.561
7.260
7.104
7.098
7.092
7.089
7.087
7.083
7.076
7.071
N
S
S
N
2-2
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.5
3.0
2.5
2.0
1.5
1.0
ppm
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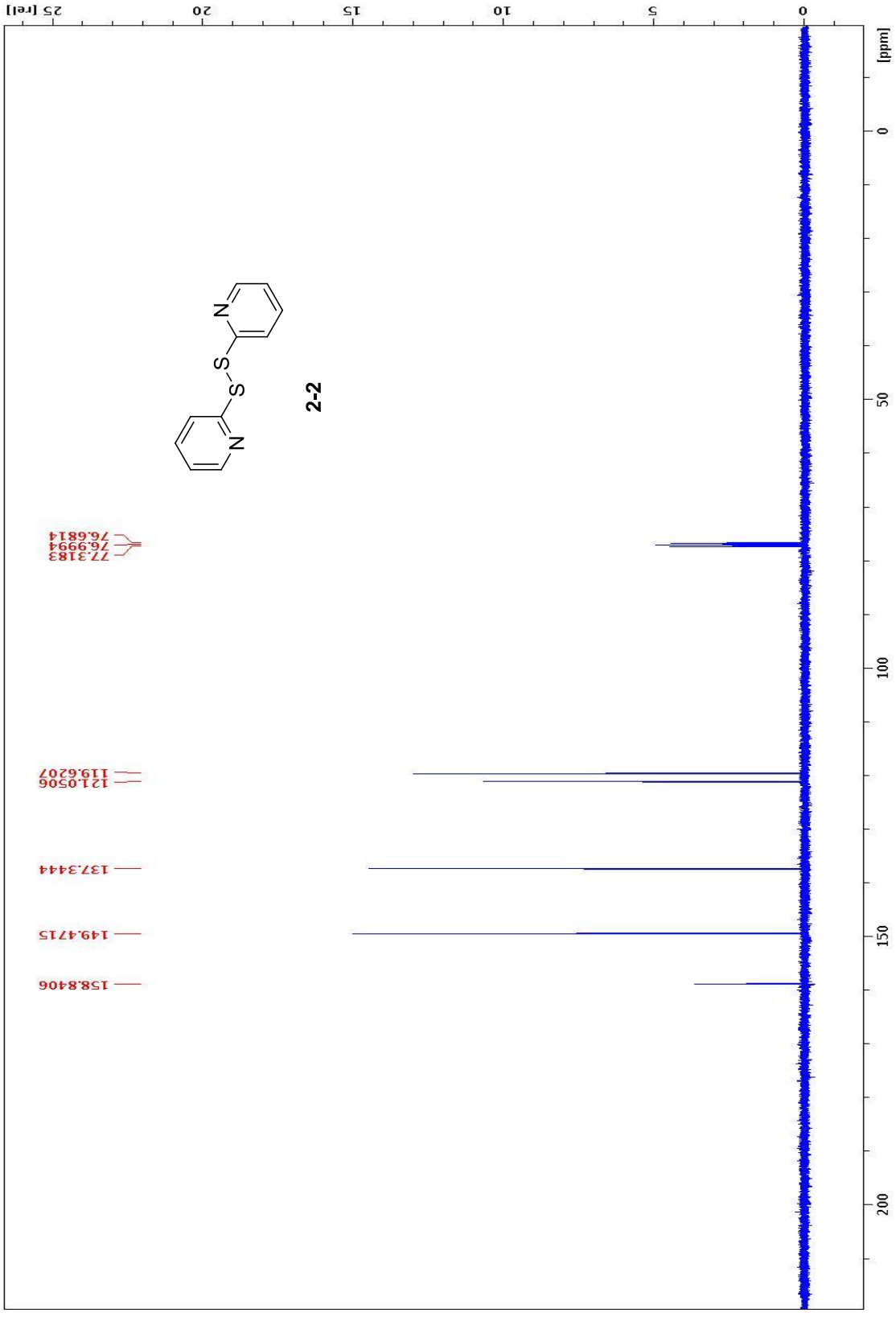
2.056


2-2
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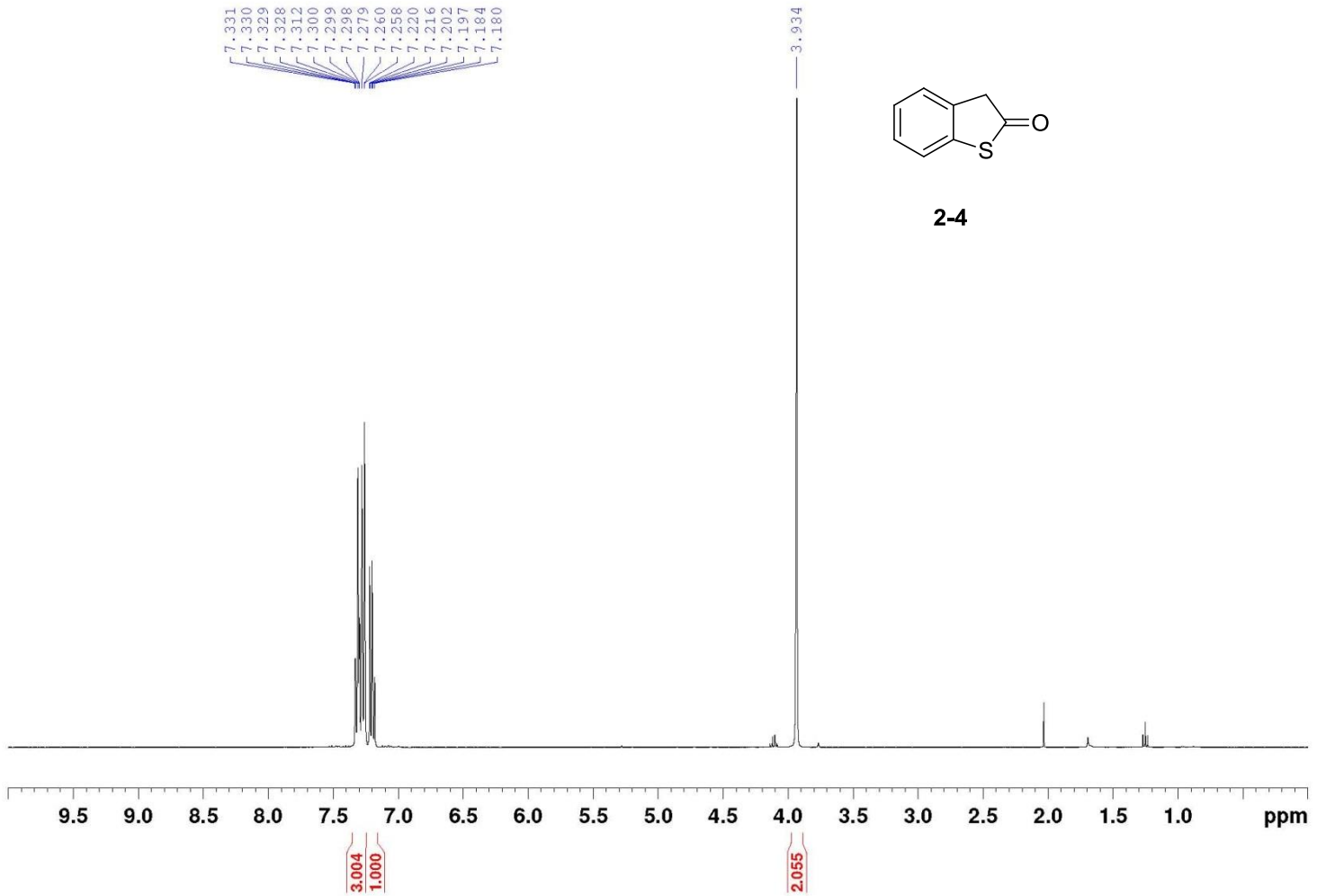
76.9994
76.6814
[ppm]
[rel]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7.328
7.312
7.300
7.299
7.298
7.279
7.260
7.258
7.220
7.216
7.202
7.197
7.184
7.180
3.934
O
S
2-4
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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1.000
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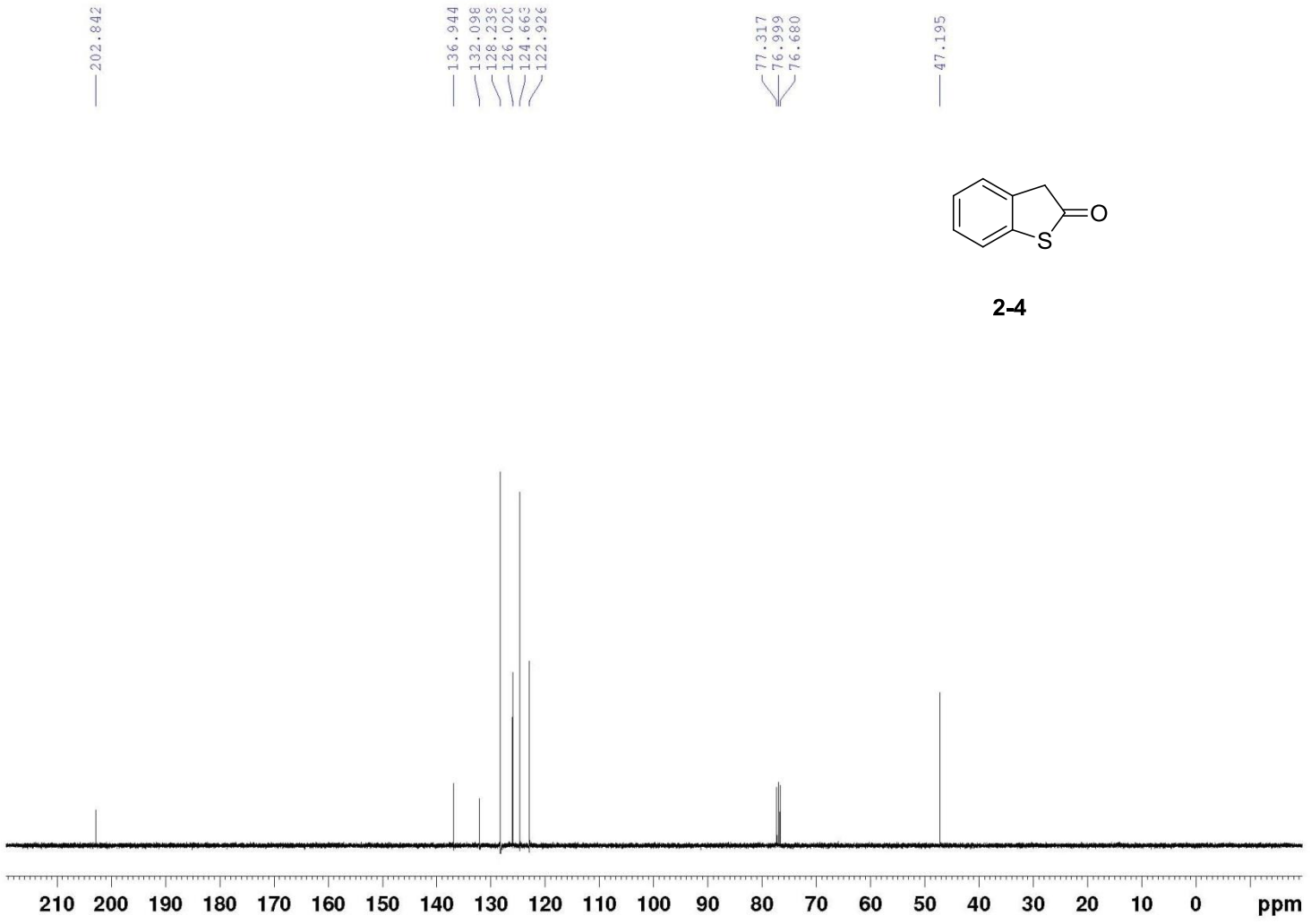
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76.680
47.195
O
S
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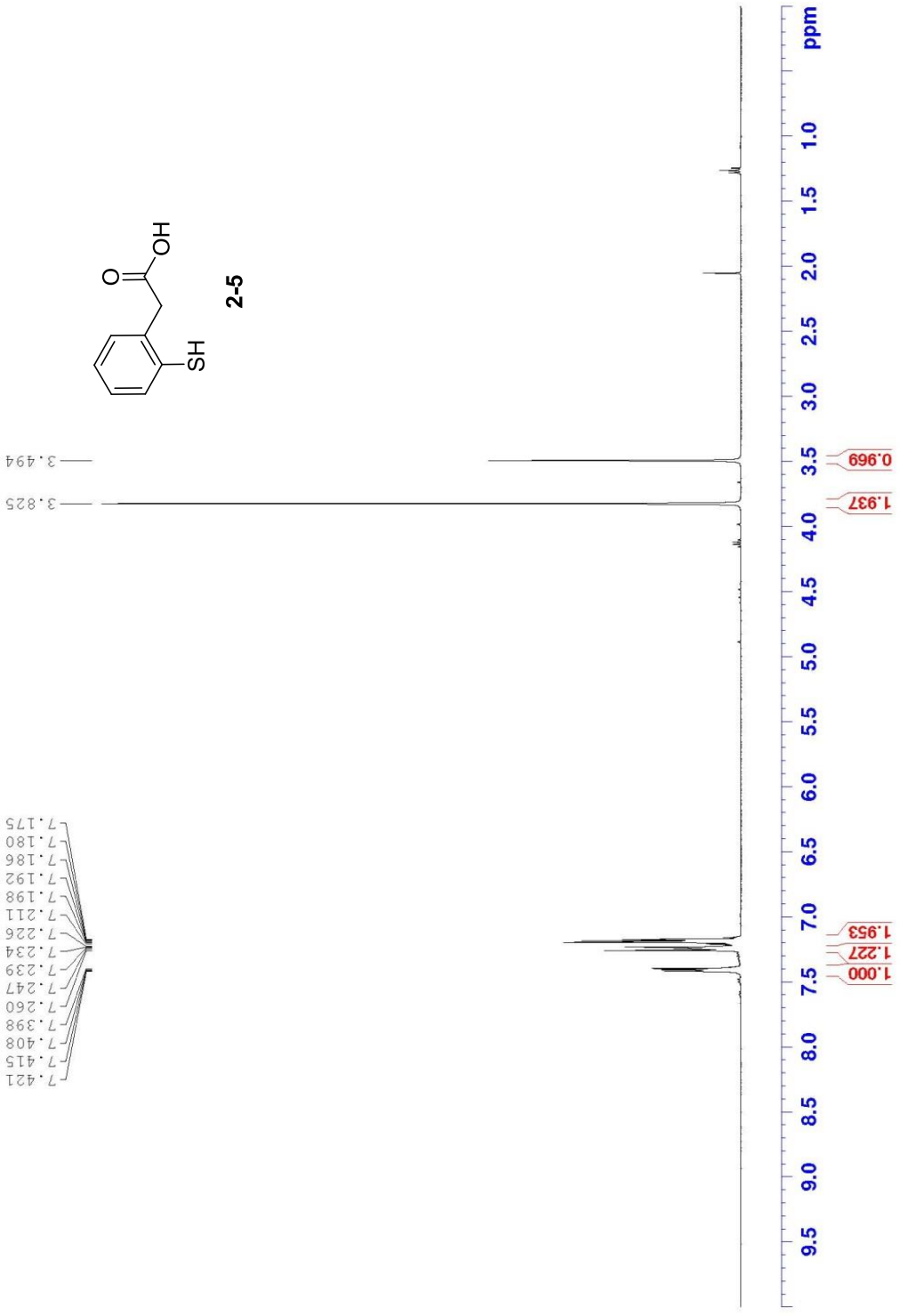
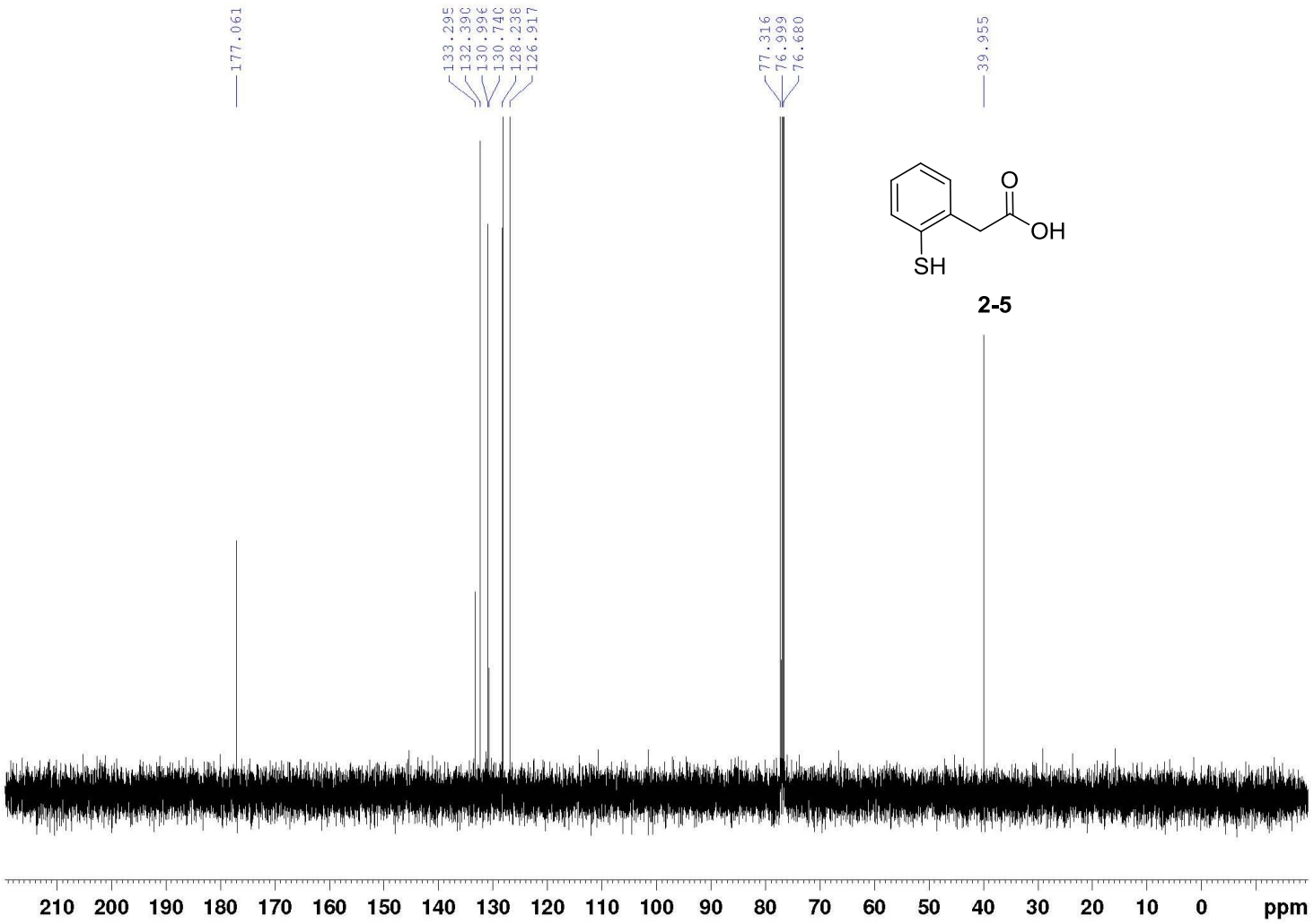
2-4


2-5
OH
O
SH
3.494
3.825
7.421
7.415
7.408
7.398
7.260
7.247
7.239
7.234
7.226
7.211
7.198
7.192
7.186
7.180
7.175
0.969
1.937
1.953
1.227
1.000
ppm


177.061
133.295
132.390
130.996
130.746
128.238
126.917
77.316
76.999
76.680
39.955
O
OH
SH
2-5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

11.153
7.260
5.292
2.981
2.970
2.967
2.964
2.957
2.954
2.950
2.943
2.940
2.937
2.926
2.591
2.579
2.574
2.561
2.558
2.546
2.541
2.534
2.528
2.520
2.517
2.503
2.493
2.487
2.484
2.470
2.004
1.994
1.990
1.987
1.980
1.976
1.973
1.965
1.962
1.959
1.952
1.948
1.945
HS
O
OH
2-8
11
10
9
8
7
6
5
4
3
2
1
ppm
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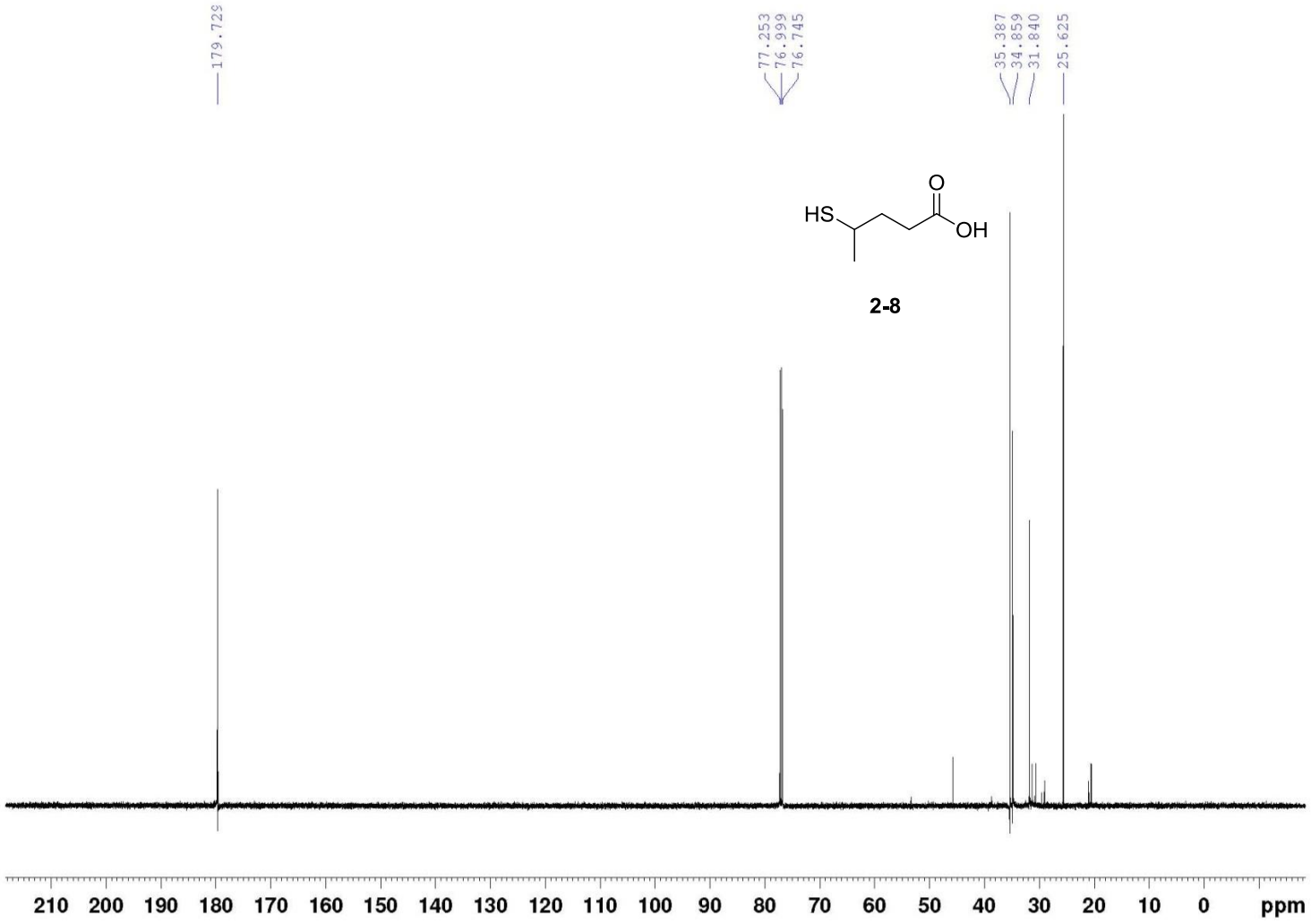
1.000
2.460
1.216
1.041
0.959
3.146
0.696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76.999
76.745
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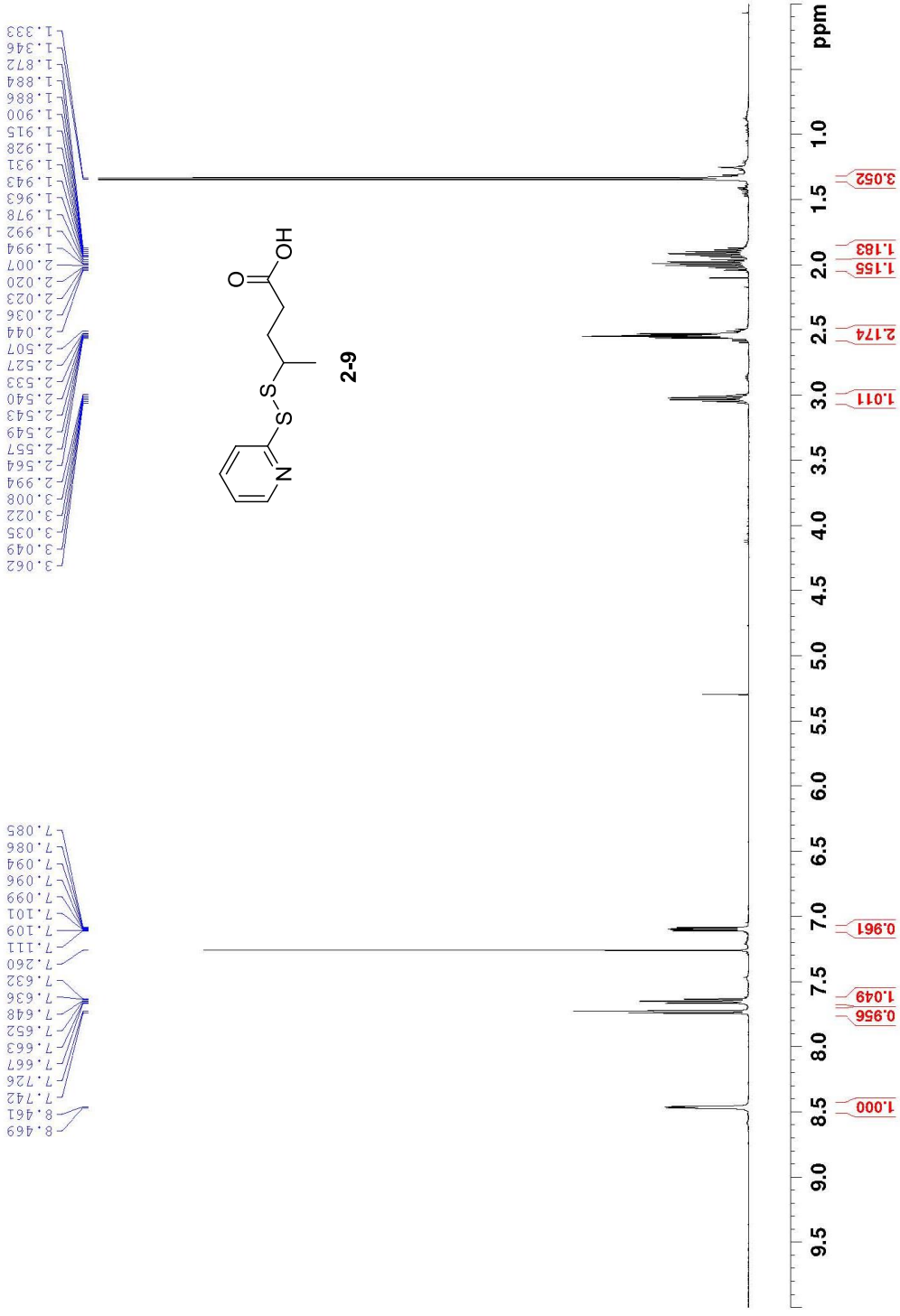
31.840
25.625
HS
O
OH
2-8
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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1.346
1.872
1.884
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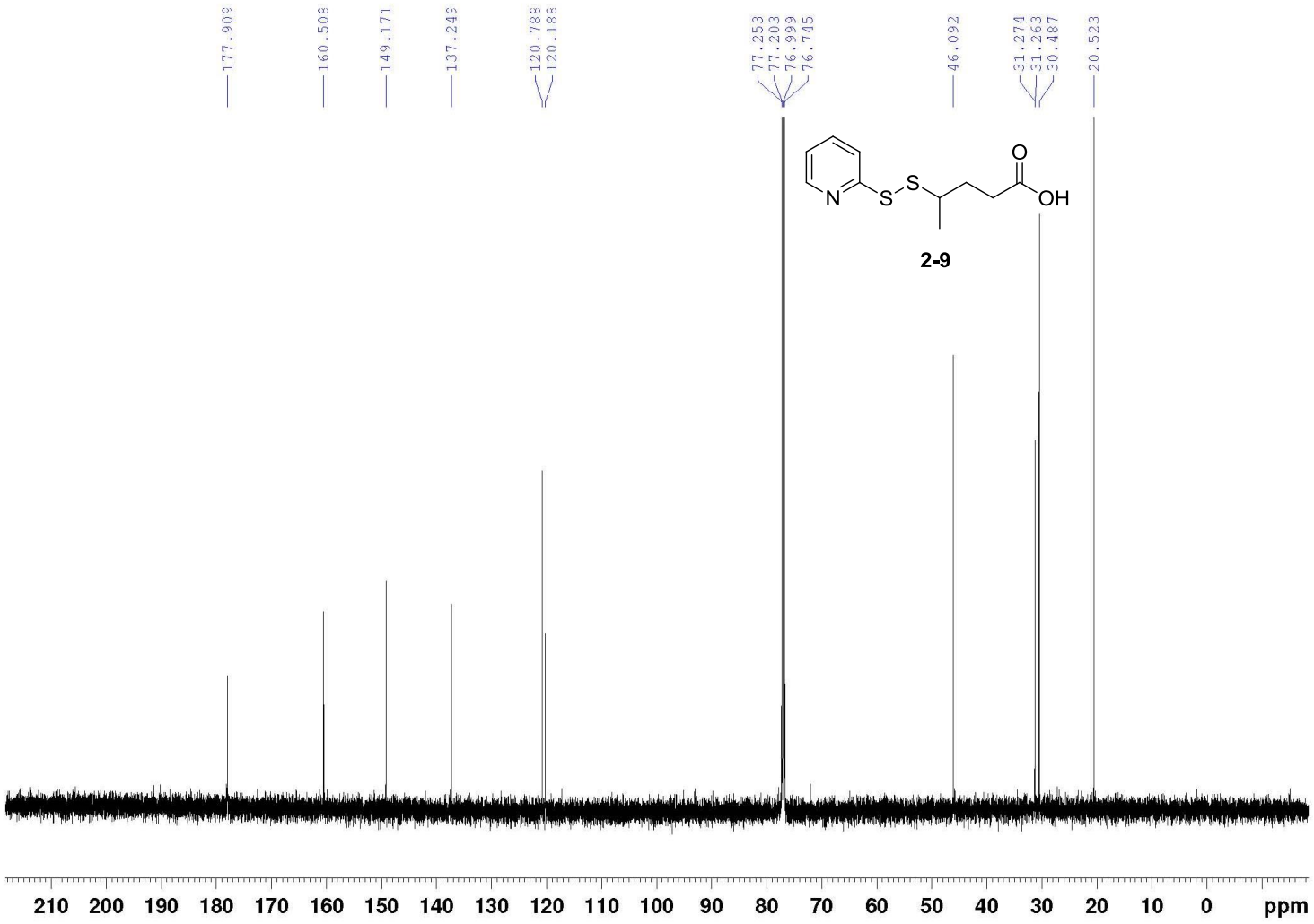
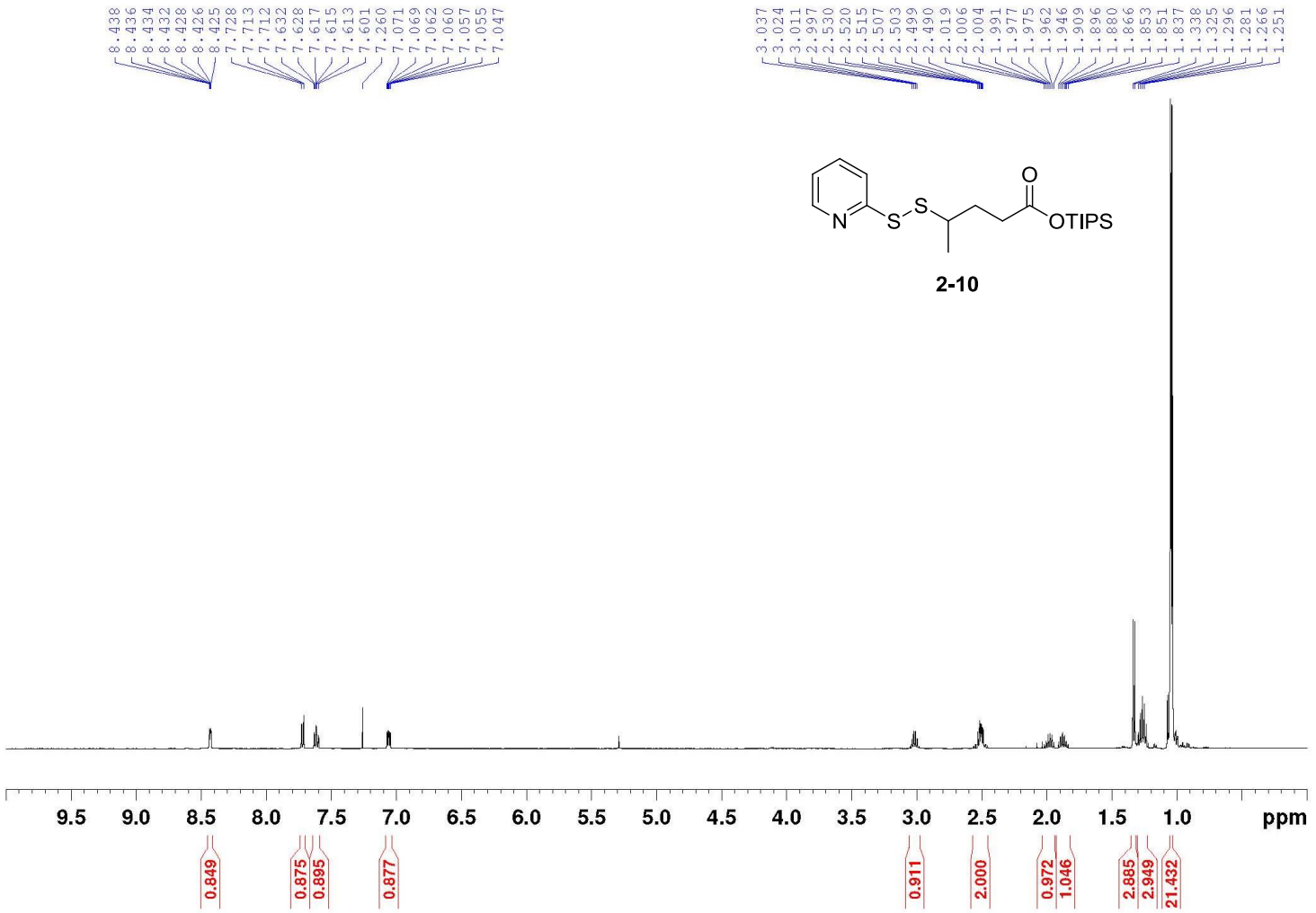
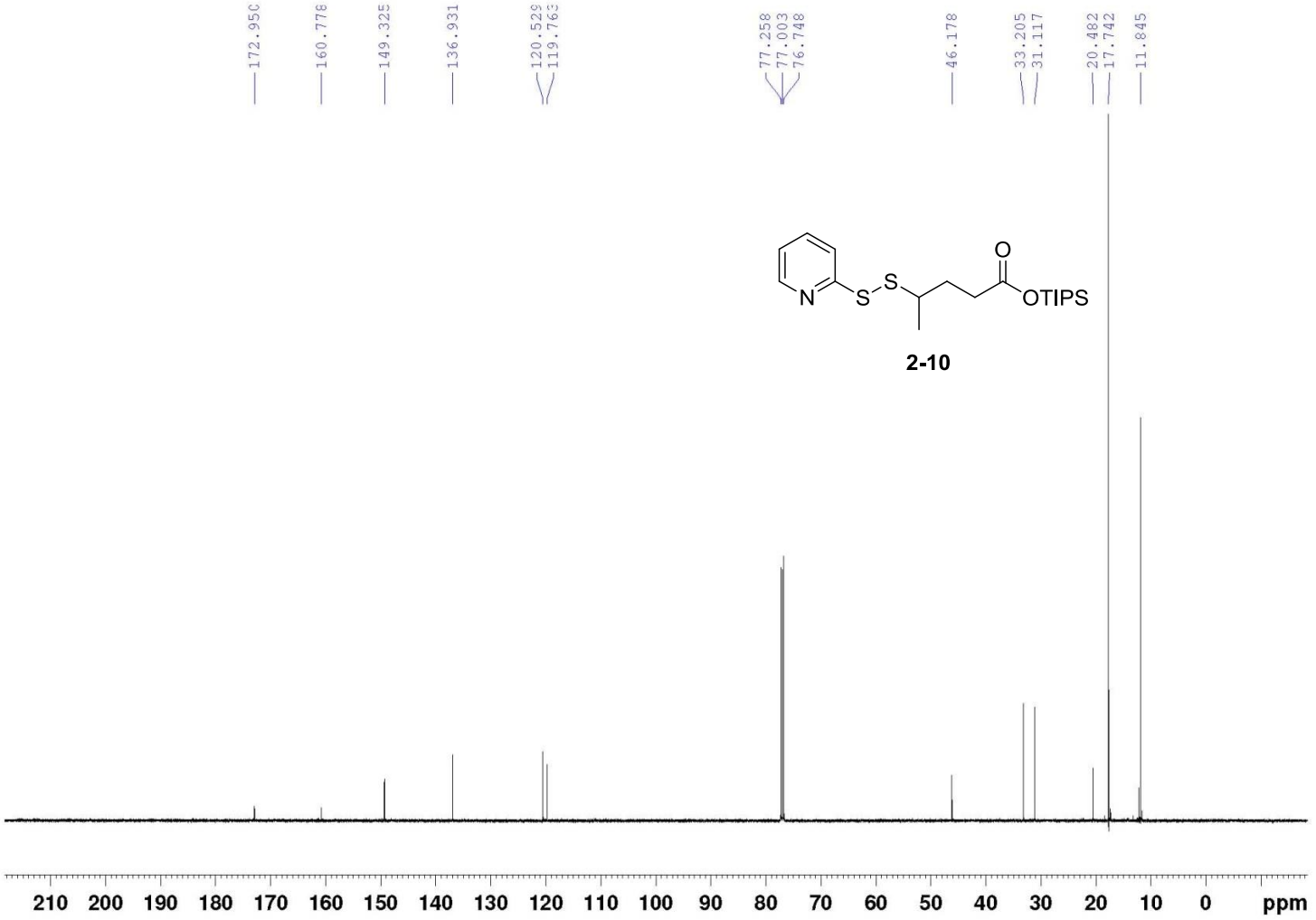
1.900
1.915
1.928
1.931
1.943
1.963
1.978
1.992
1.994
2.007
2.020
2.023
2.036
2.044
2.507
2.527
2.533
2.540
2.543
2.549
2.557
2.564
2.994
3.008
3.022
3.035
3.049
3.062
7.085
7.086
7.094
7.096
7.099
7.101
7.109
7.111
7.260
7.632
7.636
7.648
7.652
7.663
7.667
7.726
7.742
8.461
8.469
O
OH
S
S
N
2-9
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
3.052
1.183
1.155
2.174
1.011
0.961
1.049
0.956
1.000


177.909
160.508
149.171
137.249
120.788
120.188
77.253
77.203
76.999
76.745
46.092
31.274
31.263
30.487
20.523
N
S
S
O
OH
2-9
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


8.438
8.436
8.434
8.432
8.428
8.426
8.425
7.728
7.713
7.712
7.632
7.628
7.617
7.615
7.613
7.601
7.260
7.071
7.069
7.062
7.060
7.057
7.055
7.047
3.037
3.024
3.011
2.997
2.530
2.520
2.515
2.507
2.503
2.499
2.490
2.019
2.006
2.004
1.991
1.977
1.975
1.962
1.946
1.909
1.896
1.880
1.866
1.853
1.851
1.837
1.338
1.325
1.296
1.281
1.266
1.251
N
S
S
O
OTIPS
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
ppm
0.849
0.875
0.895
0.877
0.911
2.000
0.972
1.046
2.885
2.949
21.432

2-10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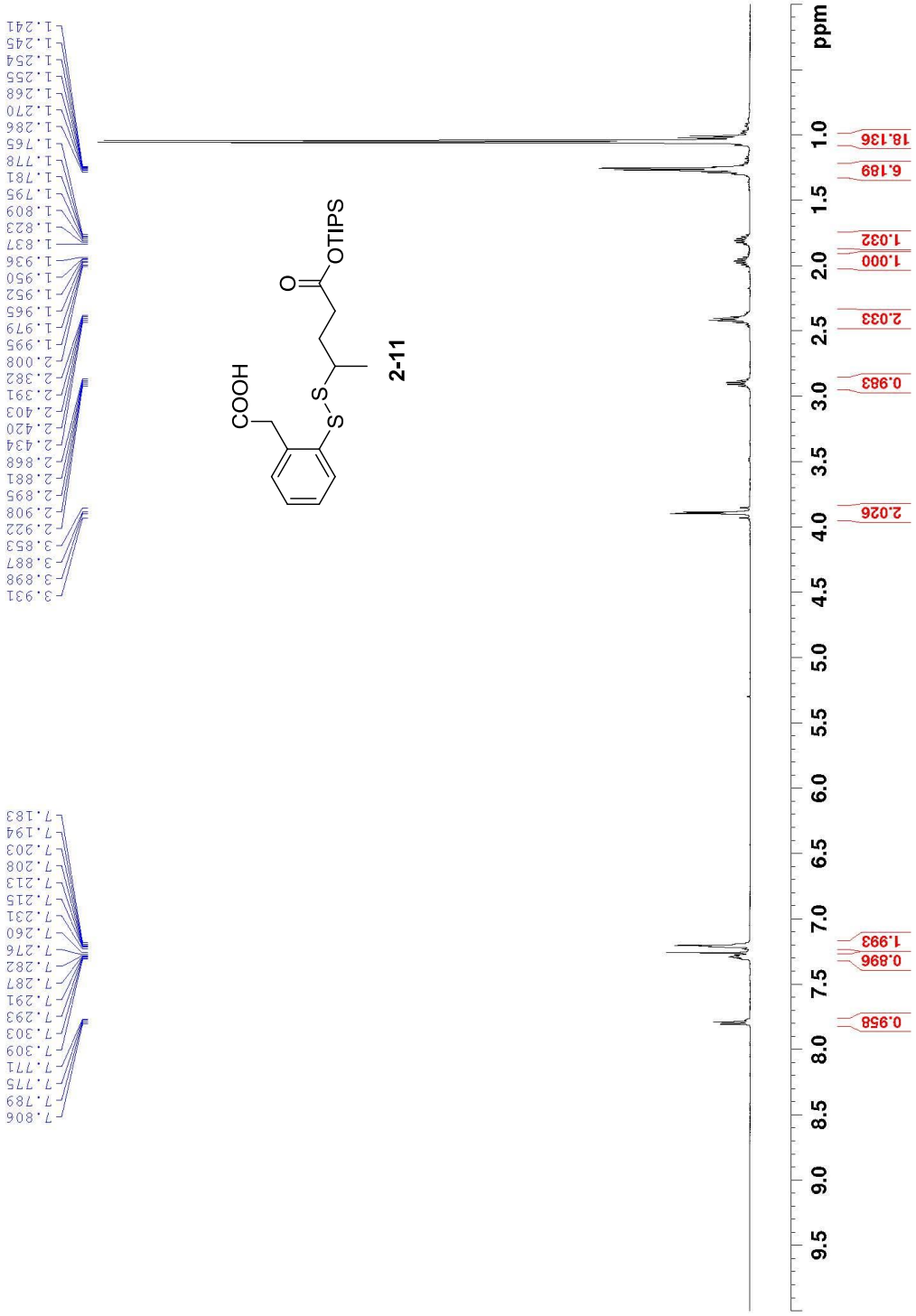
160.778
149.325
136.931
120.529
119.763
77.258
77.003
76.748
46.178
33.205
31.117
20.482
17.742
11.845
N
S
S
O
OTIPS
2-10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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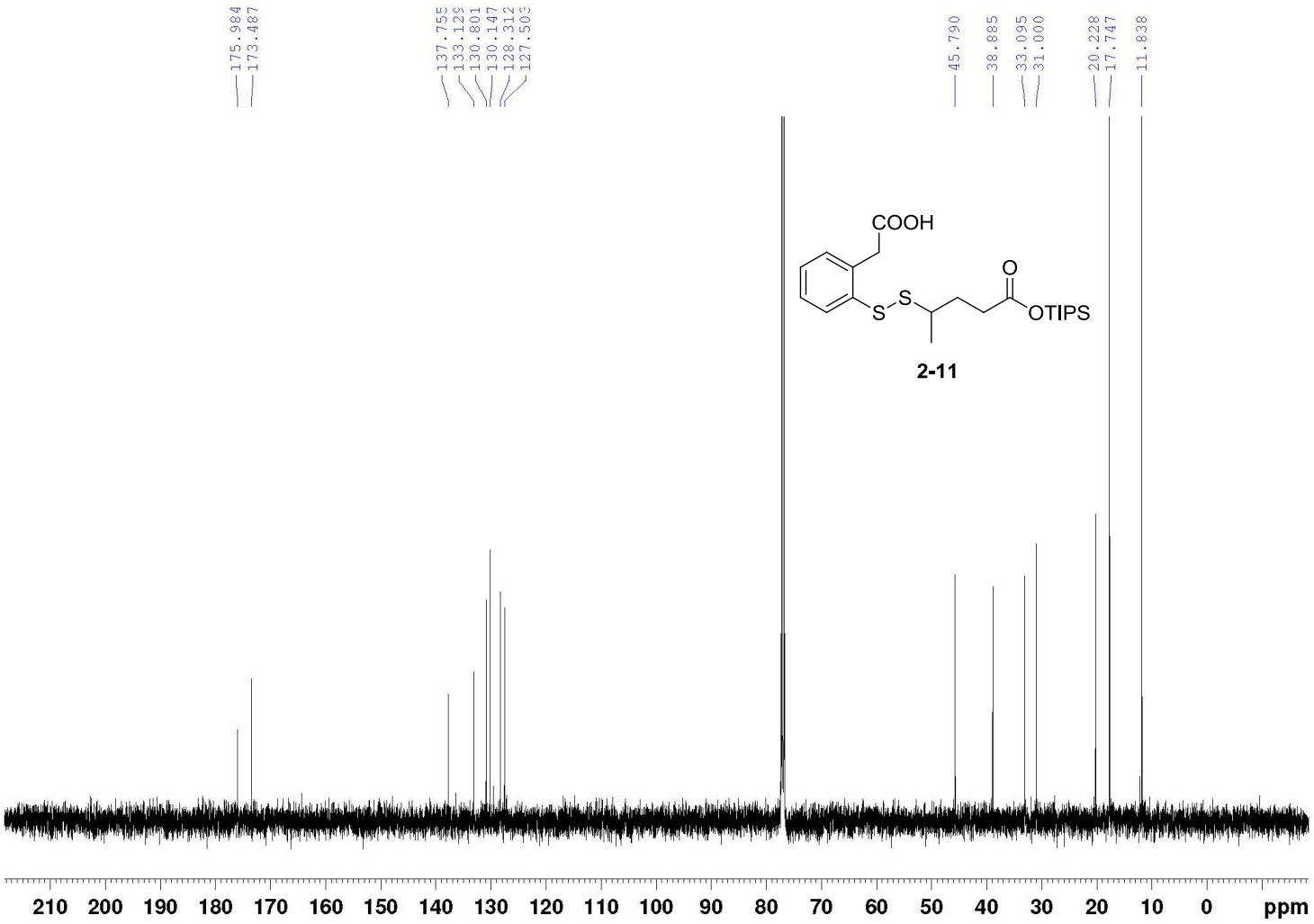
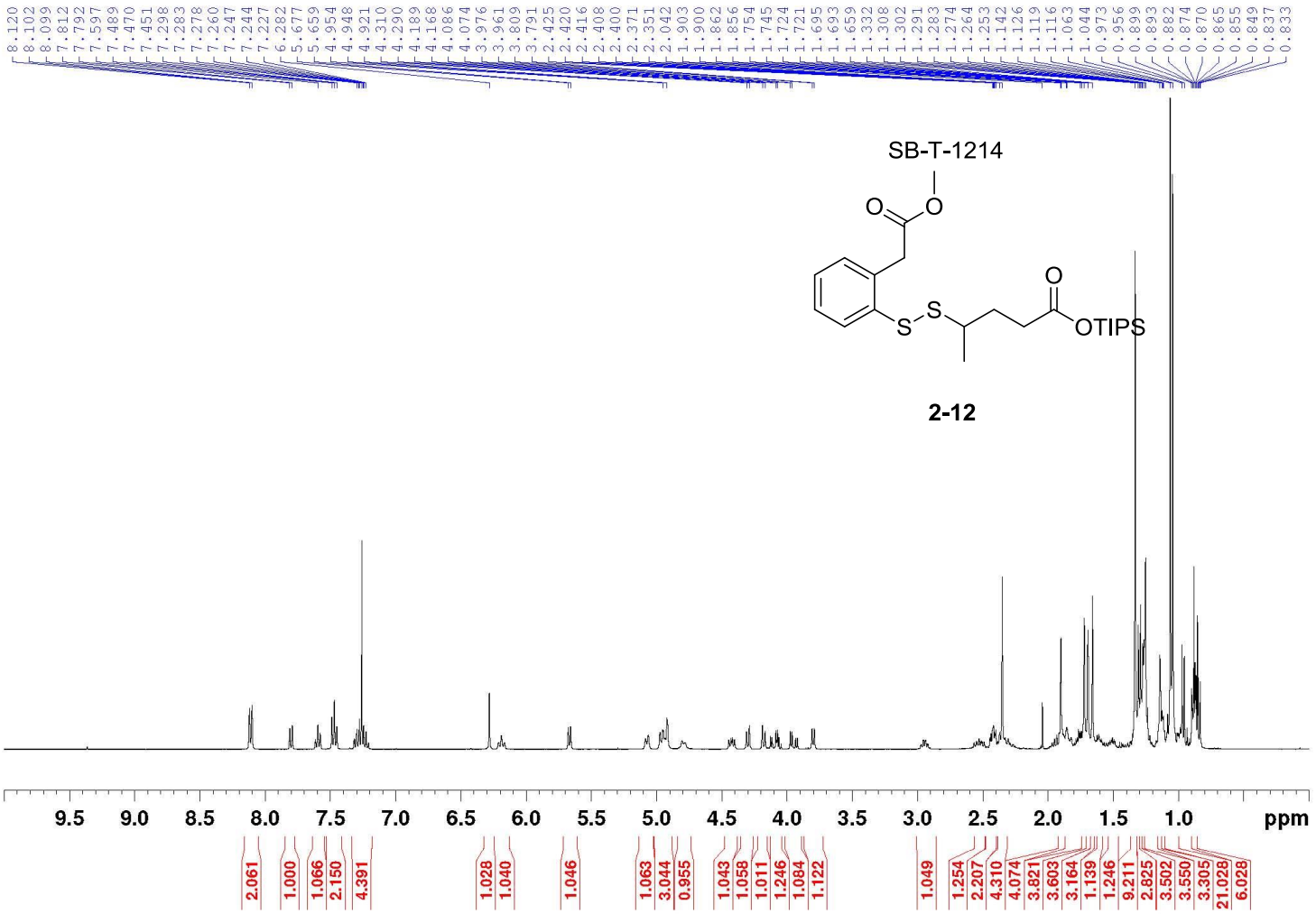
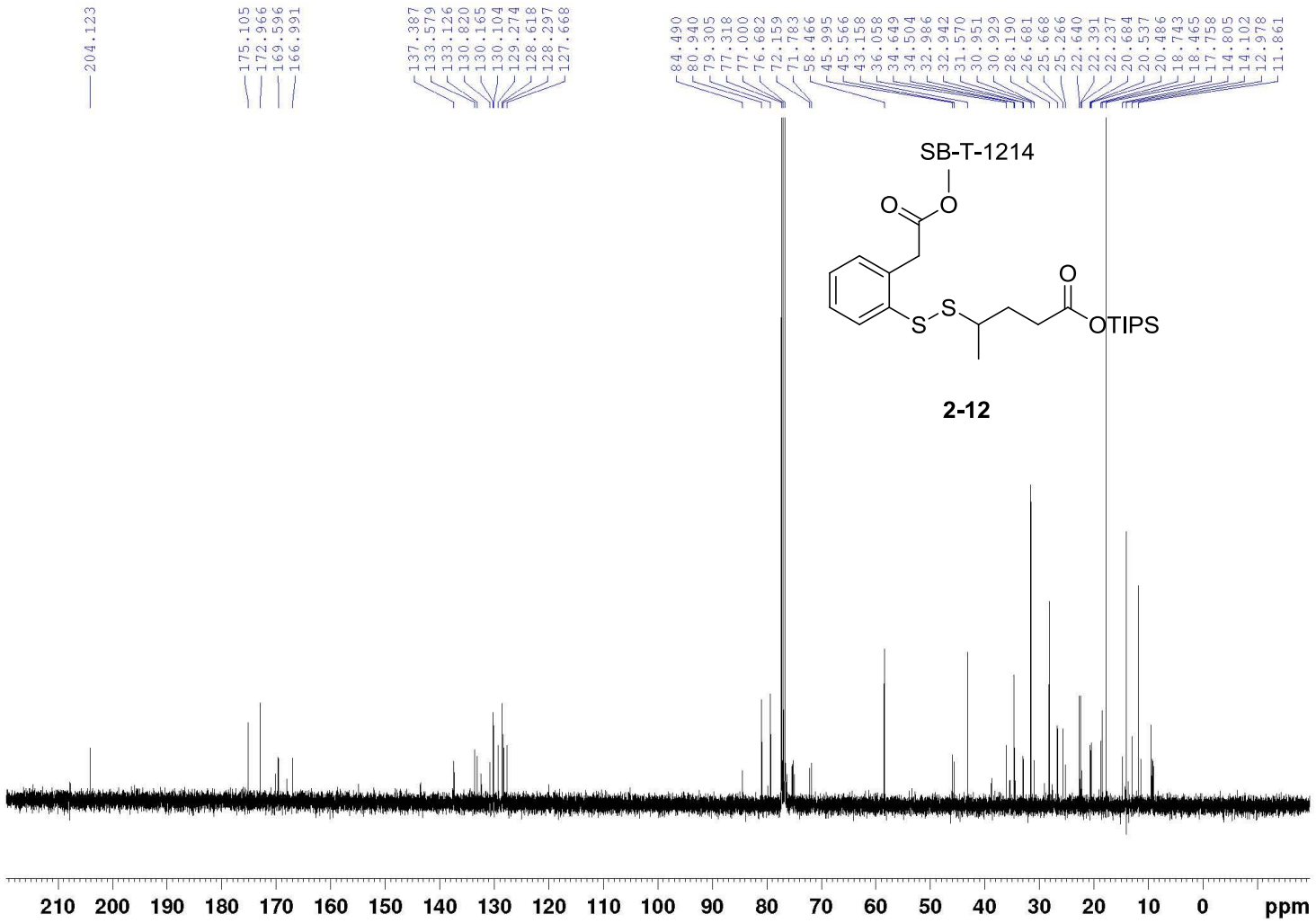
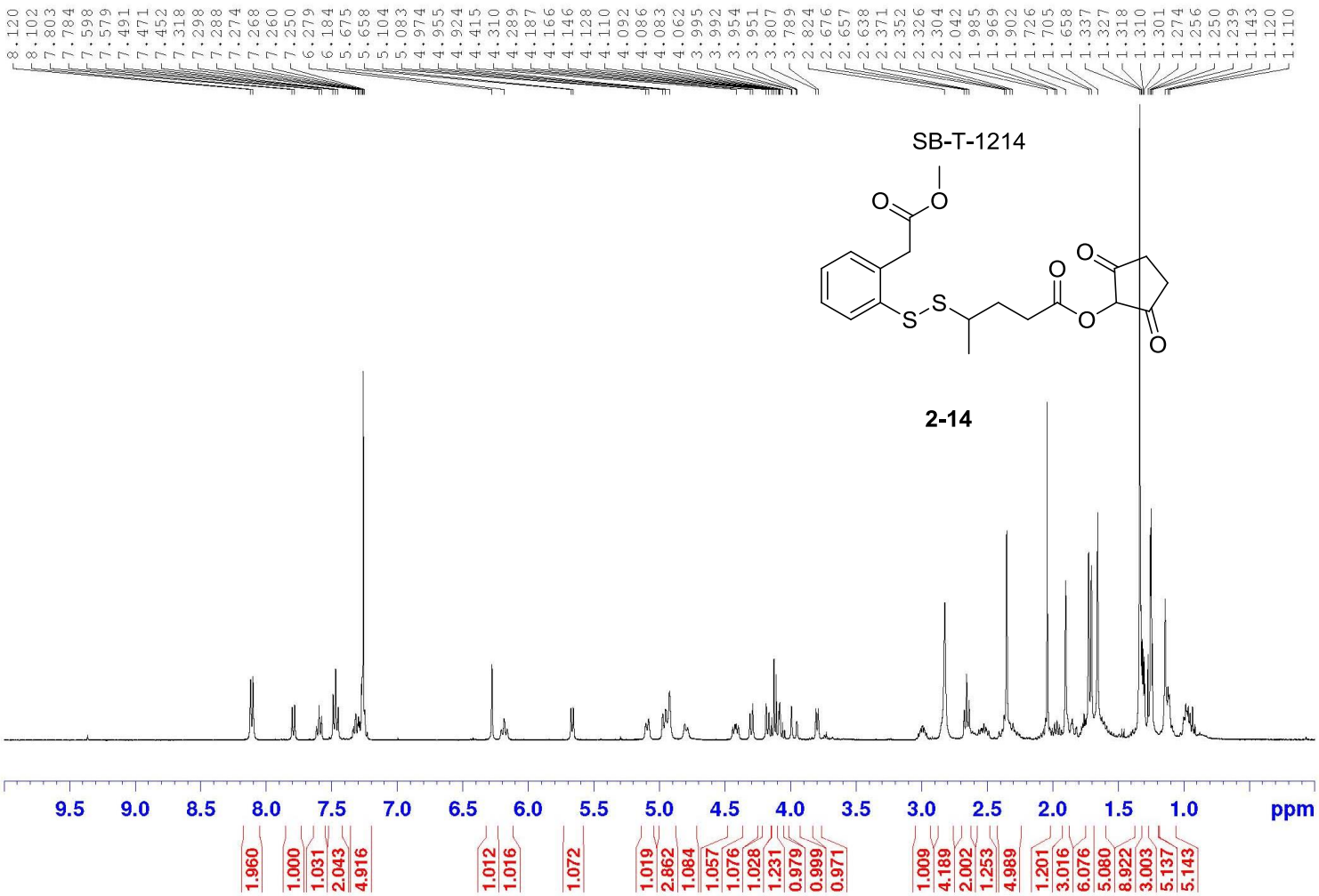
1.245
1.254
1.255
1.268
1.270
1.286
1.765
1.778
1.781
1.795
1.809
1.823
1.837
1.936
1.950
1.952
1.965
1.979
1.995
2.008
2.382
2.391
2.403
2.420
2.434
2.868
2.881
2.895
2.908
2.922
3.853
3.887
3.898
3.931
7.183
7.194
7.203
7.208
7.213
7.215
7.231
7.260
7.276
7.282
7.287
7.291
7.293
7.303
7.309
7.771
7.775
7.789
7.806
COOH
S
S
O
OTIPS
2-11
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
18.136
6.189
1.032
1.000
2.033
0.983
2.026
1.993
0.896
0.958


175.984
173.487
137.755
133.128
130.801
130.147
128.312
127.503
45.790
38.885
33.095
31.000
20.228
17.747
11.838
COOH
O
S
S
OTIPS
2-11
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


SB-T-1214
O
O
O
S
S
OTIPS
2-12
ppm


204.123
175.105
172.966
169.596
166.991
137.387
133.579
133.126
130.820
130.165
130.104
129.274
128.618
128.297
127.668
84.490
80.940
79.305
77.318
77.000
76.682
72.159
71.783
58.466
45.995
45.566
43.158
36.058
34.649
34.504
32.986
32.942
31.570
30.951
30.929
28.190
26.681
25.668
25.266
22.640
22.391
22.237
20.684
20.537
20.486
18.743
18.465
17.758
14.805
14.102
12.978
11.861
SB-T-1214
O
O
O
S
S
OTIPS
2-12
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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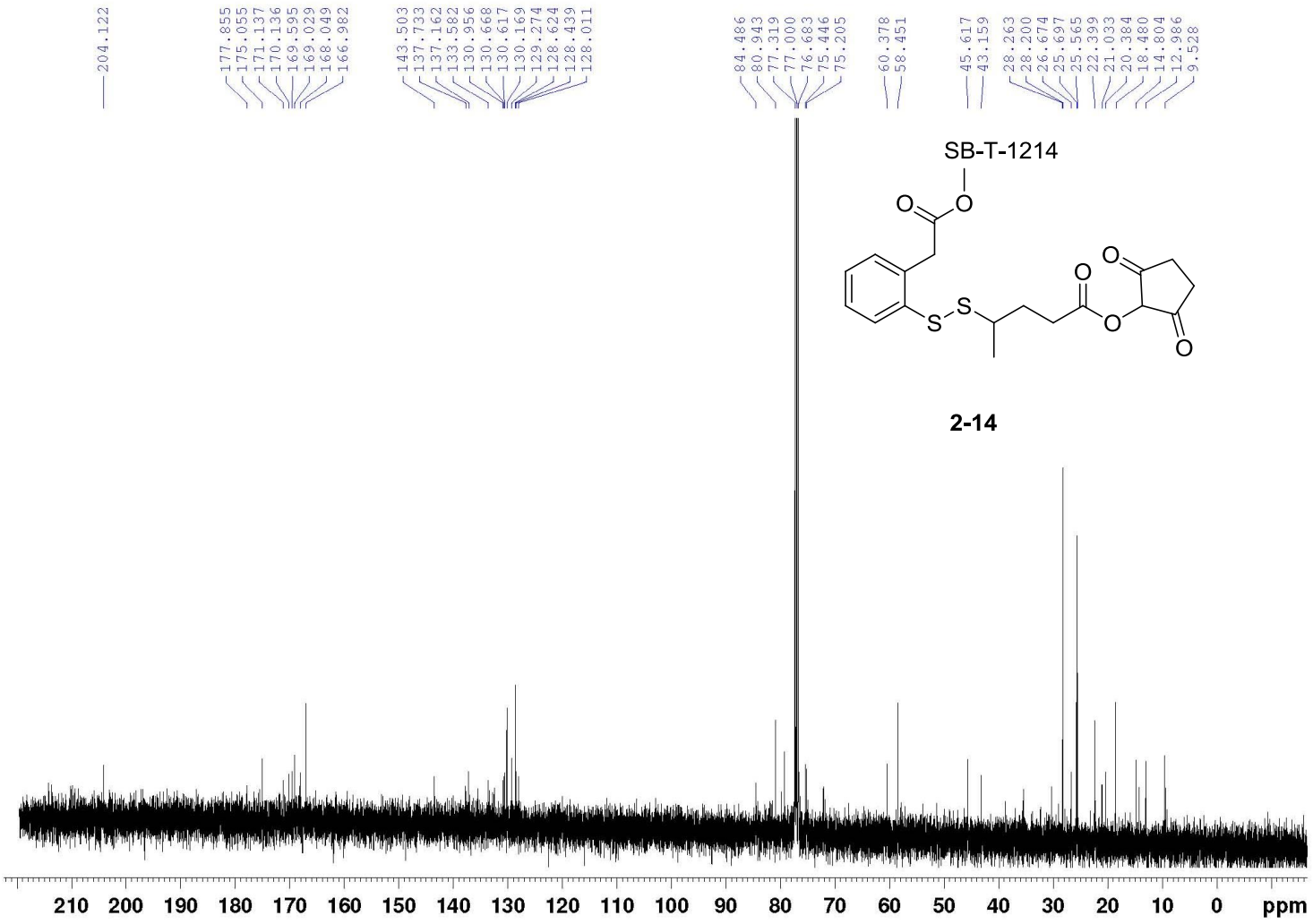
8.102
7.803
7.784
7.598
7.579
7.491
7.471
7.452
7.318
7.298
7.288
7.274
7.268
7.260
7.250
6.279
6.184
5.675
5.658
5.104
5.083
4.974
4.955
4.924
4.415
4.310
4.289
4.187
4.166
4.146
4.128
4.110
4.092
4.086
4.083
4.062
3.995
3.992
3.954
3.951
3.807
3.789
2.824
2.676
2.657
2.638
2.371
2.352
2.326
2.304
2.042
1.985
1.969
1.902
1.726
1.705
1.658
1.337
1.327
1.318
1.310
1.301
1.274
1.256
1.250
1.239
1.143
1.120
1.110
SB-T-1214
2-14
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
ppm
1.960
1.000
1.031
2.043
4.916
1.012
1.016
1.072
1.019
2.862
1.084
1.057
1.076
1.028
1.231
0.979
0.999
0.971
1.009
4.189
2.002
1.253
4.989
1.201
3.016
6.076
5.080
8.922
3.003
5.137
5.143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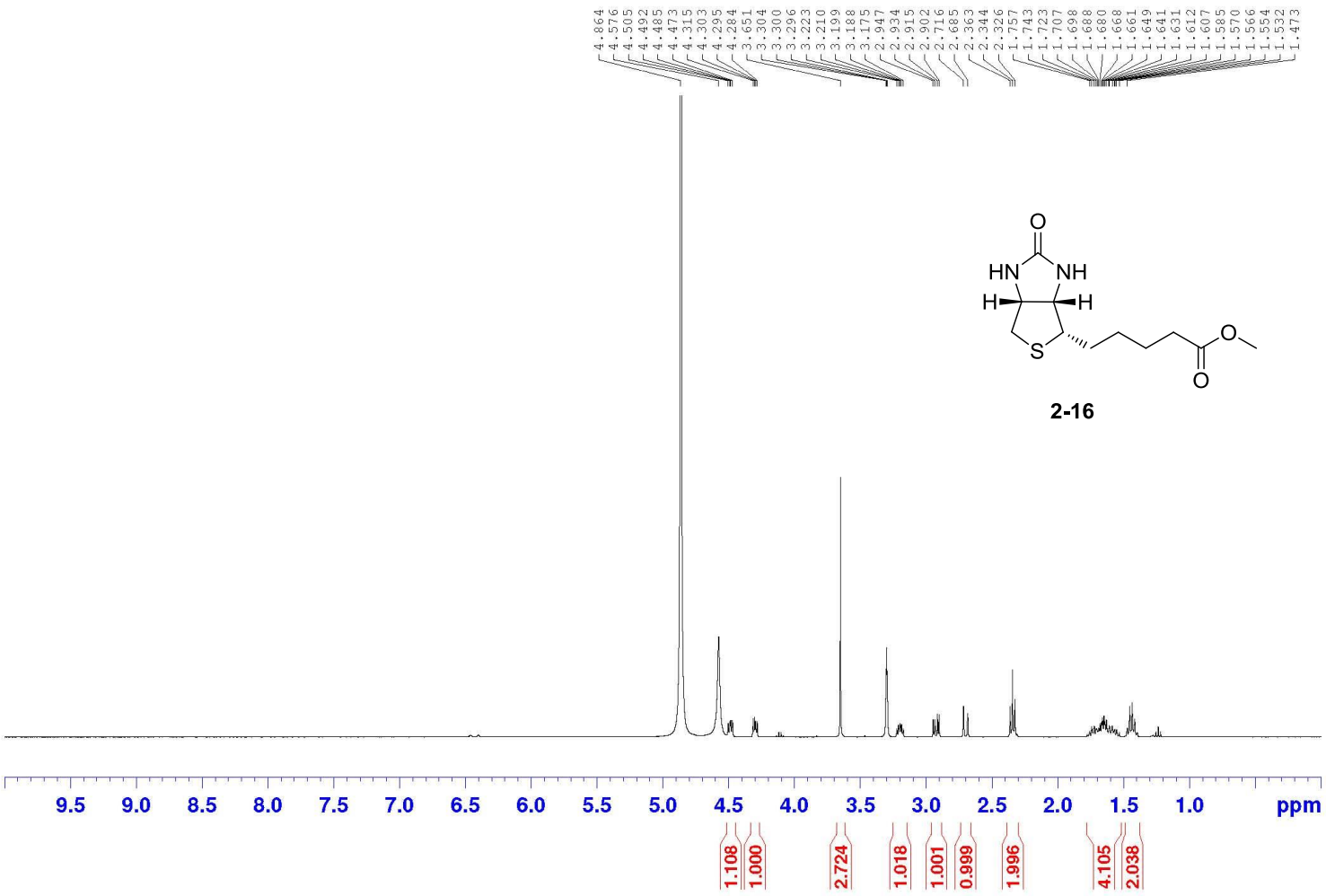
175.055
171.137
170.136
169.595
169.029
168.049
166.982
143.503
137.733
137.162
133.582
130.956
130.668
130.617
130.169
129.274
128.624
128.439
128.011
84.486
80.943
77.319
77.000
76.683
75.446
75.205
60.378
58.451
45.617
43.159
28.263
28.200
26.674
25.697
25.565
22.399
21.033
20.384
18.480
14.804
12.986
9.528
SB-T-1214
O
O
O
O
S
S
O
O
O
2-14
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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4.505
4.492
4.485
4.473
4.315
4.303
4.295
4.284
3.651
3.304
3.300
3.296
3.223
3.210
3.199
3.188
3.175
2.947
2.934
2.915
2.902
2.716
2.685
2.363
2.344
2.326
1.757
1.743
1.723
1.707
1.698
1.688
1.680
1.668
1.661
1.649
1.641
1.631
1.612
1.607
1.585
1.570
1.566
1.554
1.532
1.473
O
HN
NH
H
H
S
O
O
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
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1.000
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0.999
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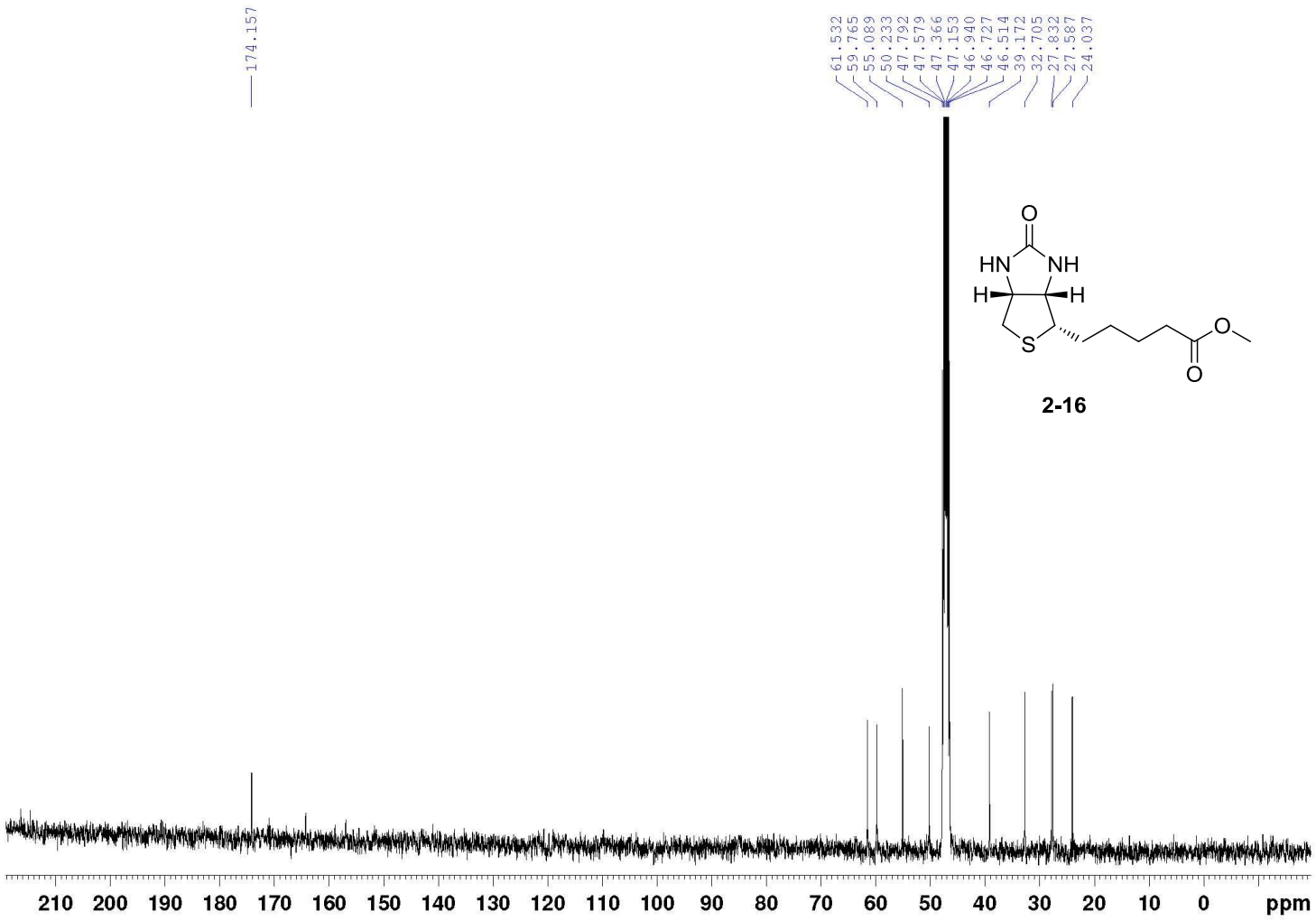
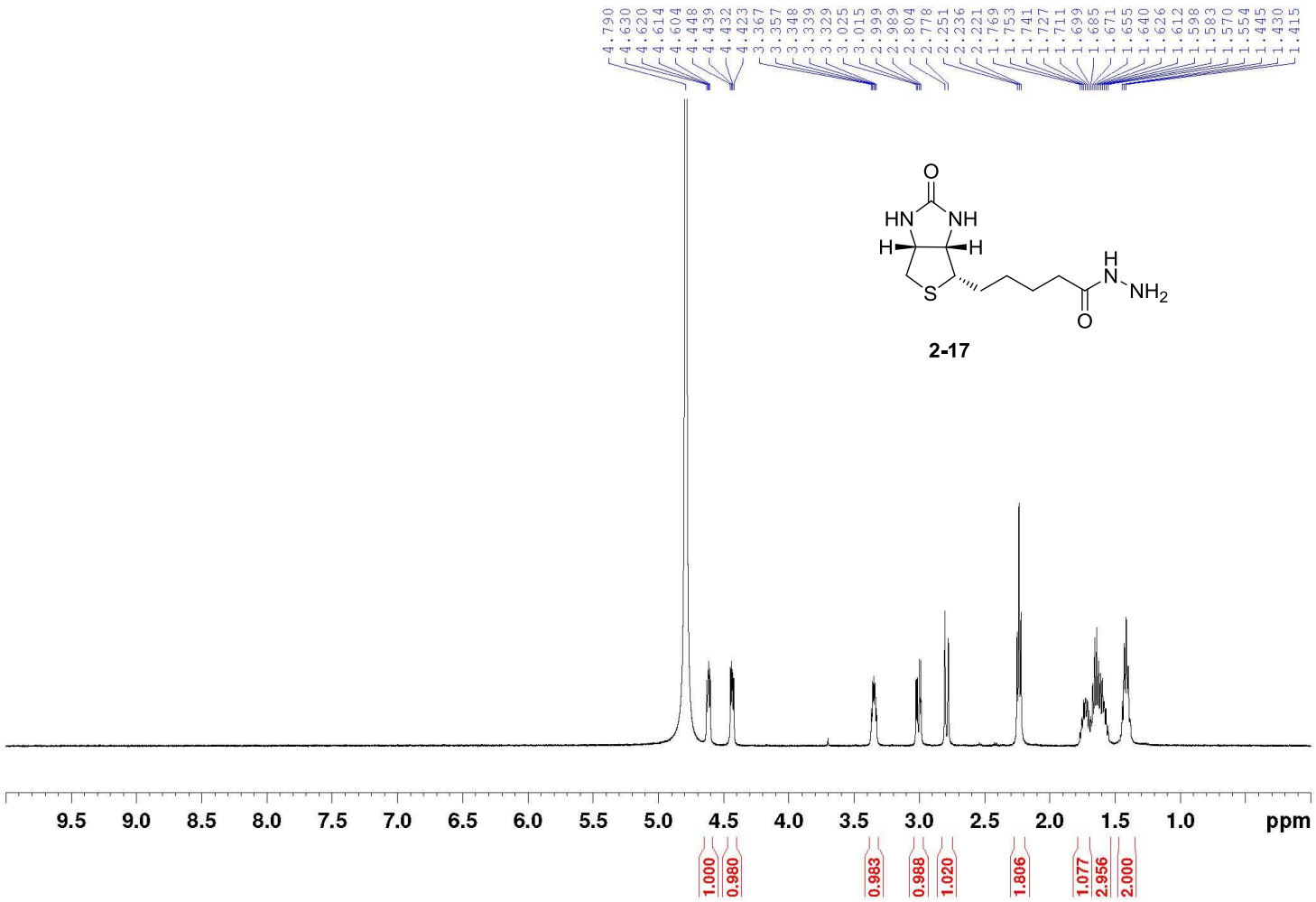
4.105
2.038

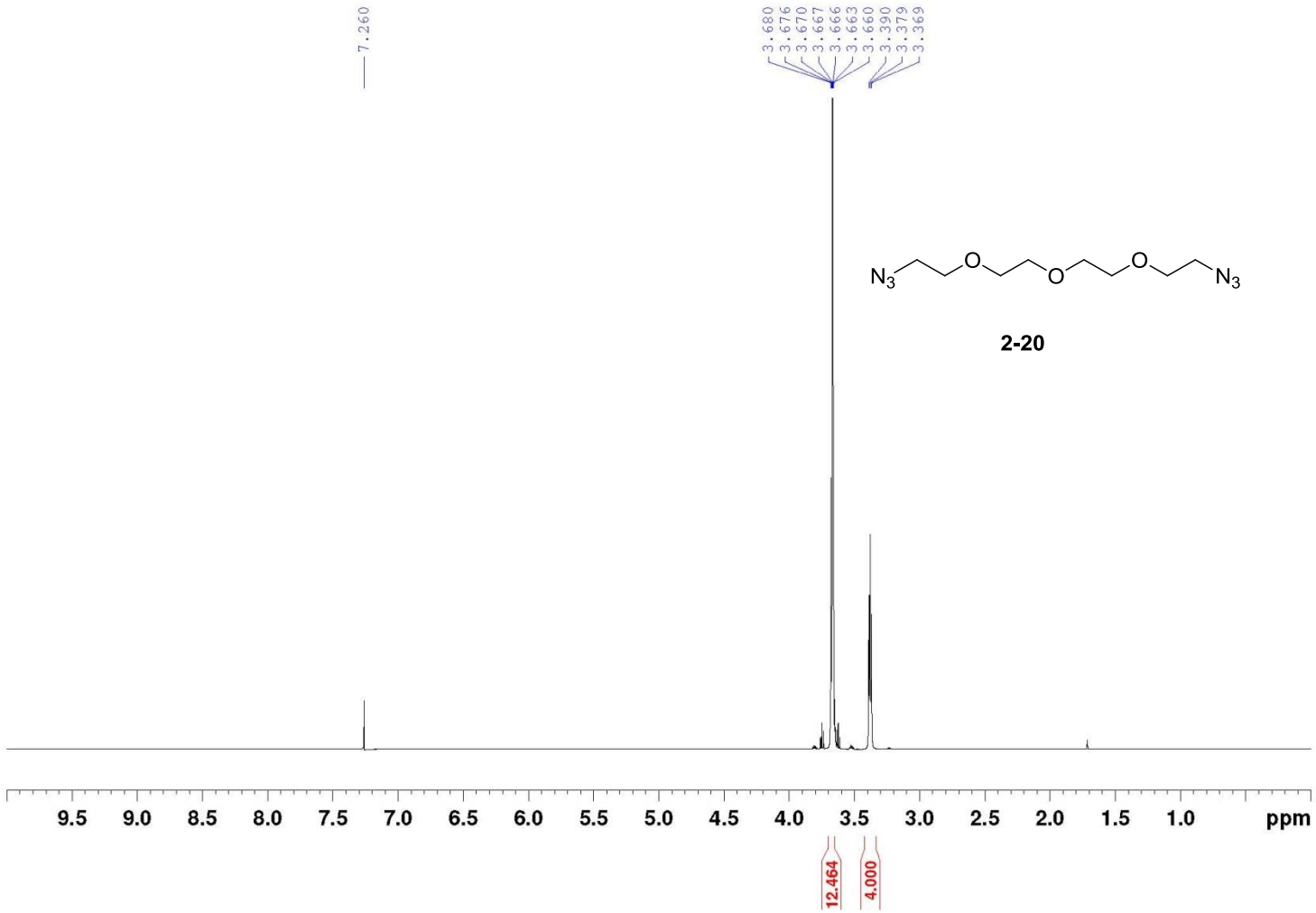
2-16


174.157
61.532
59.765
55.089
50.233
47.792
47.579
47.366
47.153
46.940
46.727
46.514
39.172
32.705
27.832
27.587
24.037
O
HN
NH
H
H
S
O
O
2-16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


4.790
4.630
4.620
4.614
4.604
4.448
4.439
4.432
4.423
3.367
3.357
3.348
3.339
3.329
3.025
3.015
2.999
2.989
2.804
2.778
2.251
2.236
2.221
1.769
1.753
1.741
1.727
1.711
1.699
1.685
1.671
1.655
1.640
1.626
1.612
1.598
1.583
1.570
1.554
1.445
1.430
1.415
HN
NH
H
H
S
O
O
H
N
NH2
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
ppm
1.000
0.980
0.983
0.988
1.020
1.806
1.077
2.956
2.000

2-17

175.622
165.395
62.035
60.243
55.258
39.654
33.356
27.563
24.817
O
HN
NH
H
H
S
H
N
NH2
O
2-17
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


7.260
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3.670
3.667
3.666
3.663
3.660
3.390
3.379
3.369
N3
O
O
O
N3
2-20
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
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1.5
1.0
ppm
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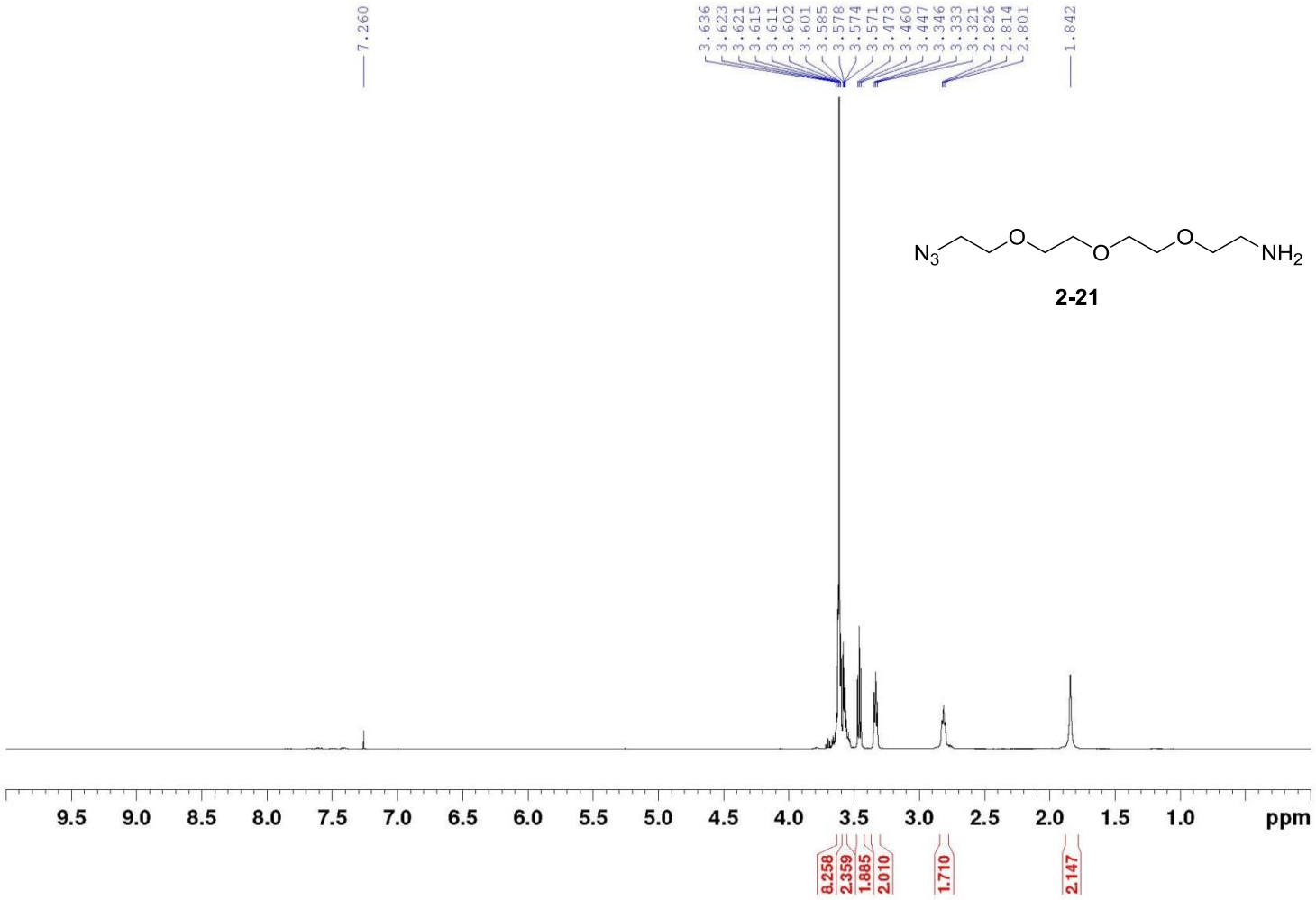
4.000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3.621
3.615
3.611
3.602
3.601
3.585
3.578
3.574
3.571
3.473
3.460
3.447
3.346
3.333
3.321
2.826
2.814
2.801
1.642
N3
O
O
O
NH2
2-21
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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2.0
1.5
1.0
ppm
8.258
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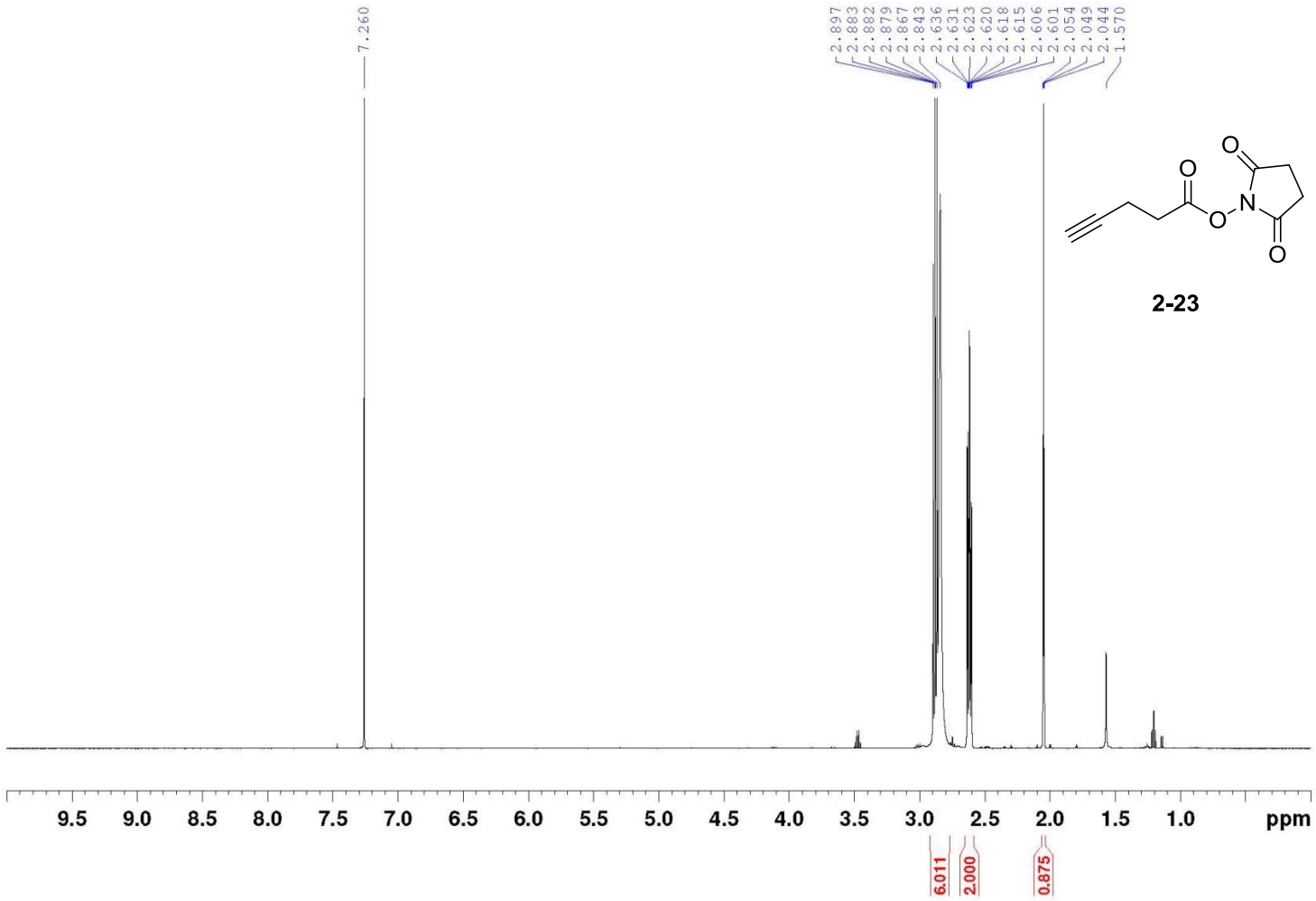
2.010
1.710
2.147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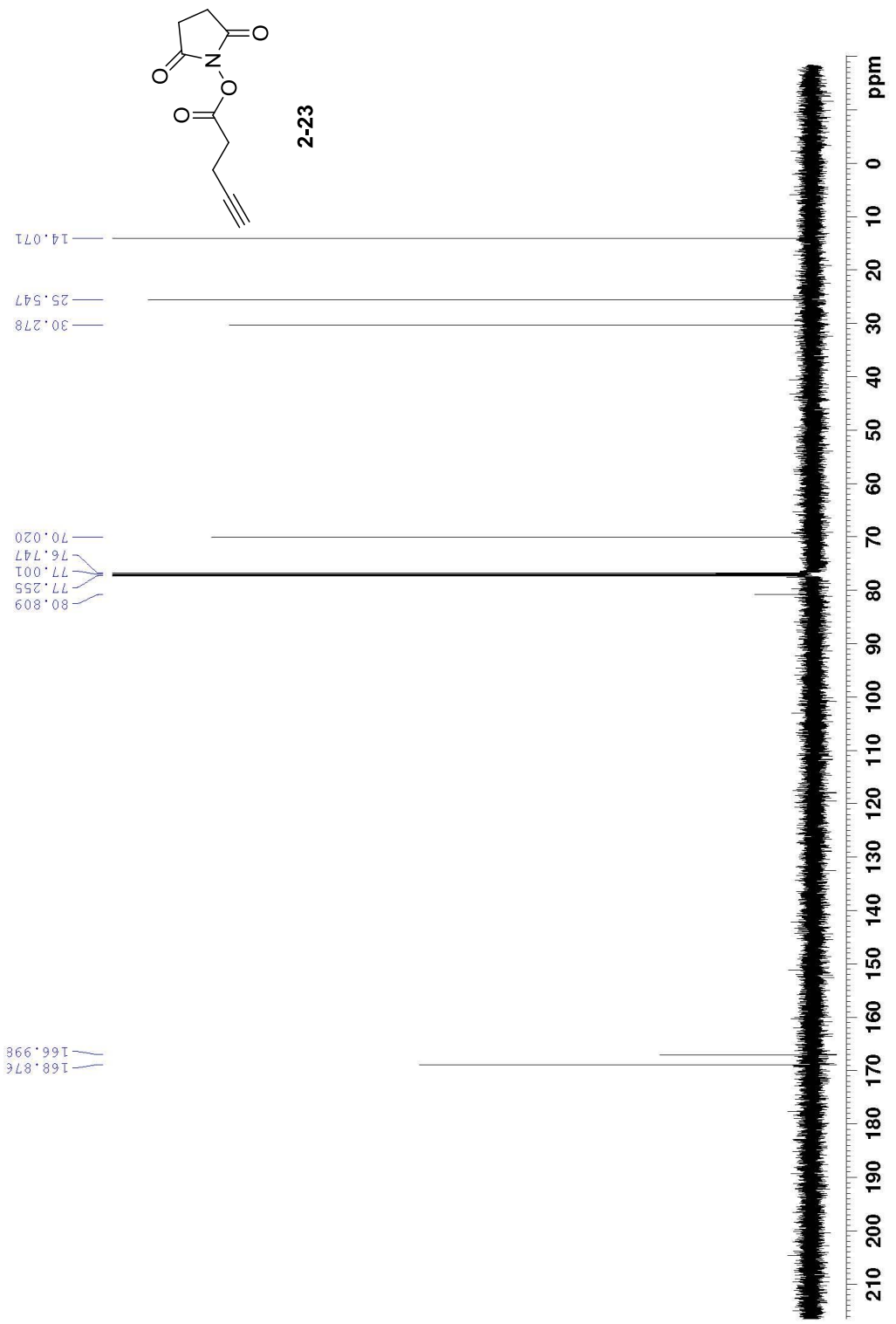
2.636
2.631
2.623
2.620
2.618
2.615
2.606
2.601
2.054
2.049
2.044
1.570
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
6.011
2.000
0.875

2-23


2-23
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30.278
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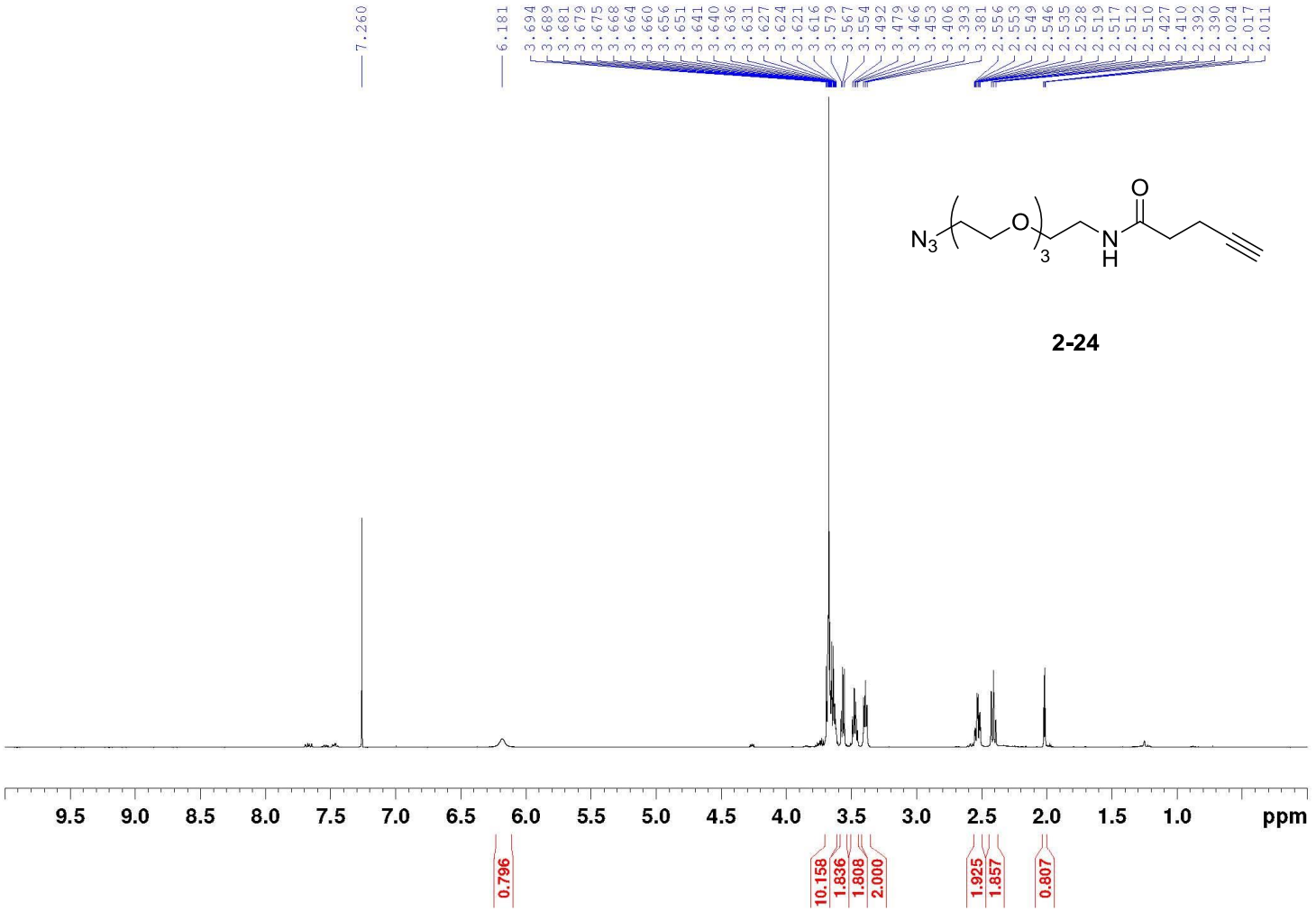
76.747
77.001
77.255
80.809
168.876
166.998
ppm


7.260
6.181
3.694
3.689
3.681
3.679
3.675
3.668
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3.660
3.656
3.651
3.641
3.640
3.636
3.631
3.627
3.624
3.621
3.616
3.579
3.567
3.554
3.492
3.479
3.466
3.453
3.406
3.393
3.381
2.556
2.553
2.549
2.546
2.535
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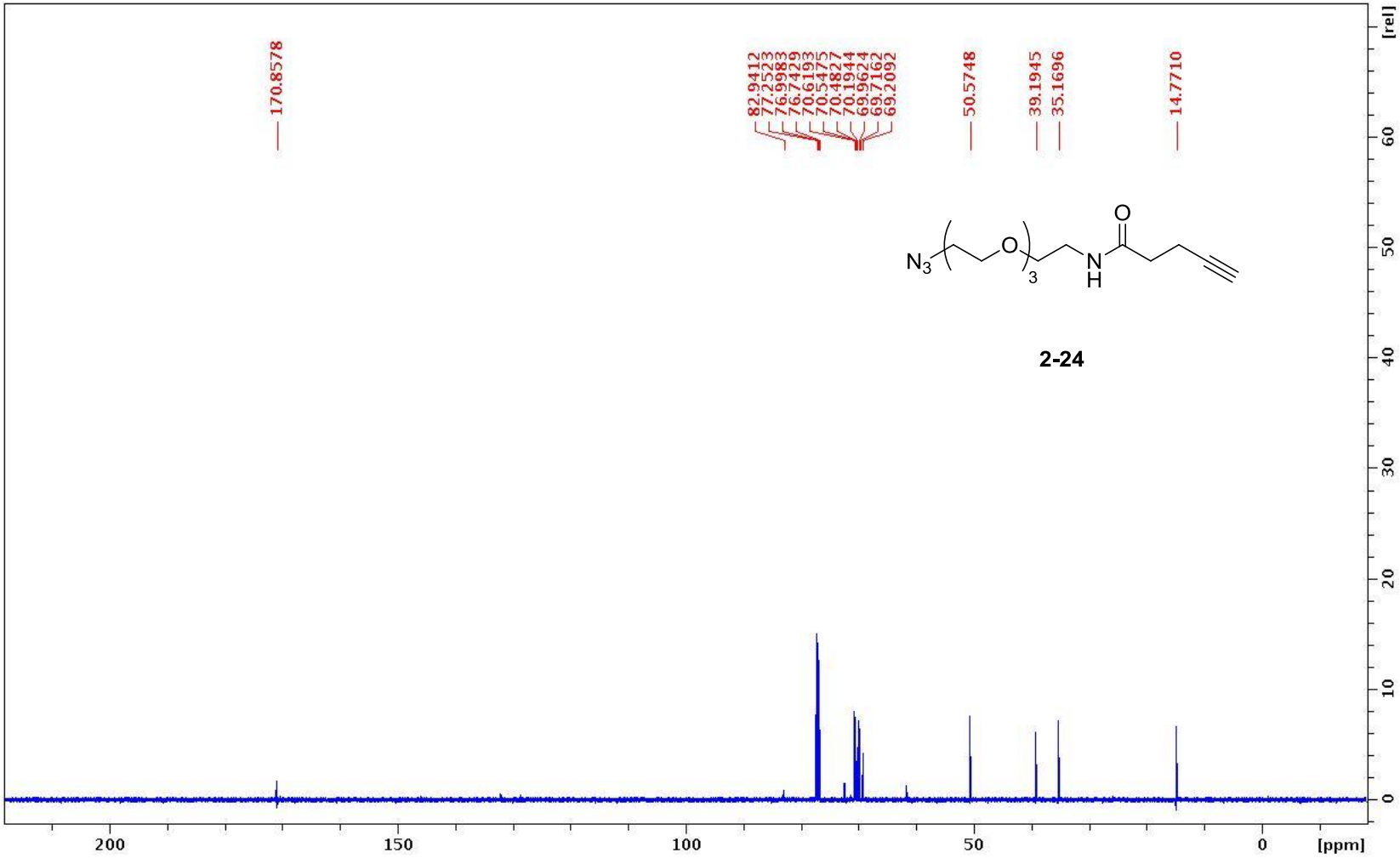
2.519
2.517
2.512
2.510
2.427
2.410
2.392
2.390
2.024
2.017
2.011
N3
O
3
N
H
O
2-24
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.796
10.158
1.836
1.808
2.000
1.925
1.857
0.807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82.9412
77.2523
76.9983
76.7429
70.6193
70.5475
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70.1944
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69.7162
69.2092
50.5748
39.1945
35.1696
14.7710
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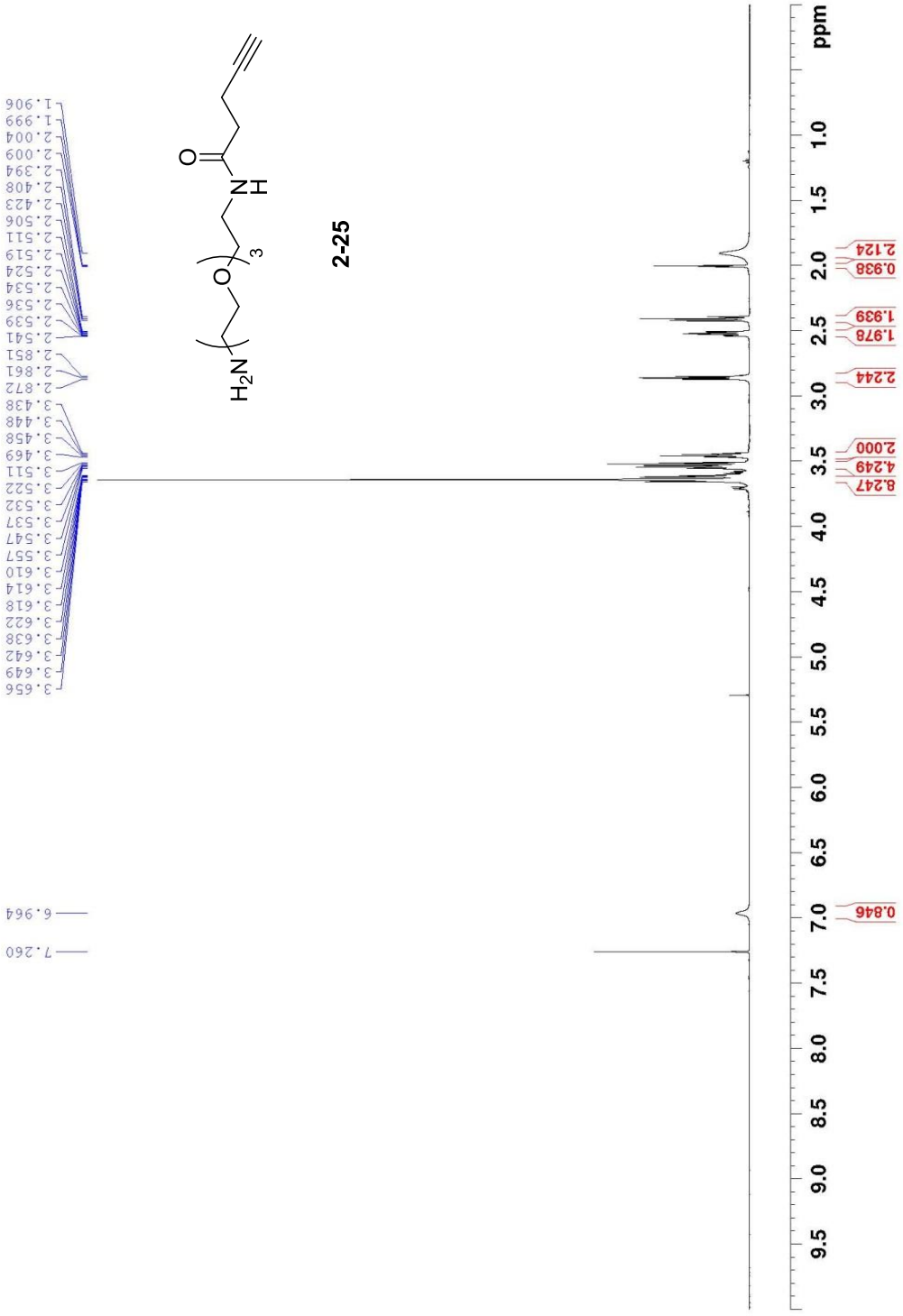
[ppm]
200
150
100
50
0
N3
O
3
N
H
O

2-24


2-25
ppm
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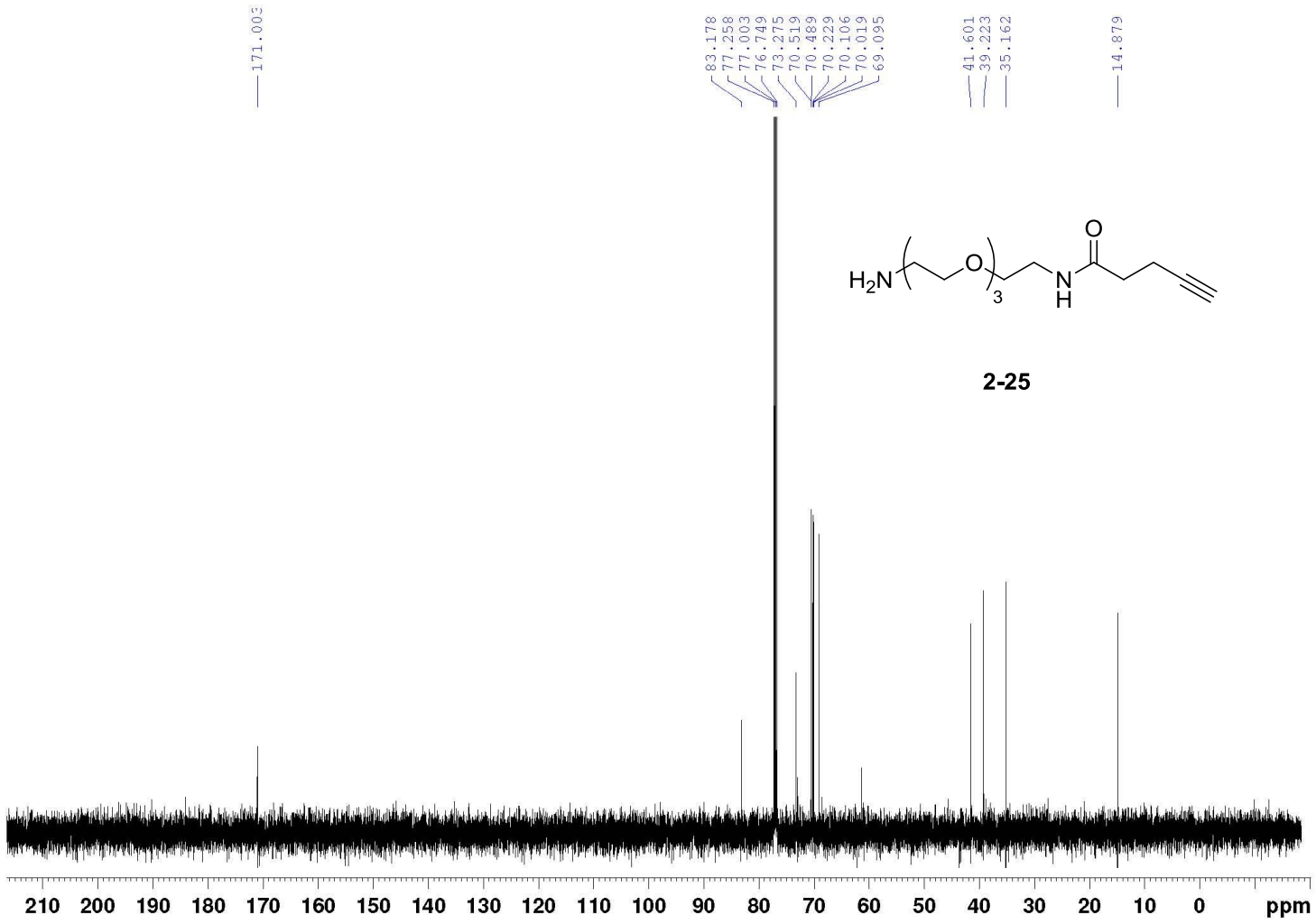
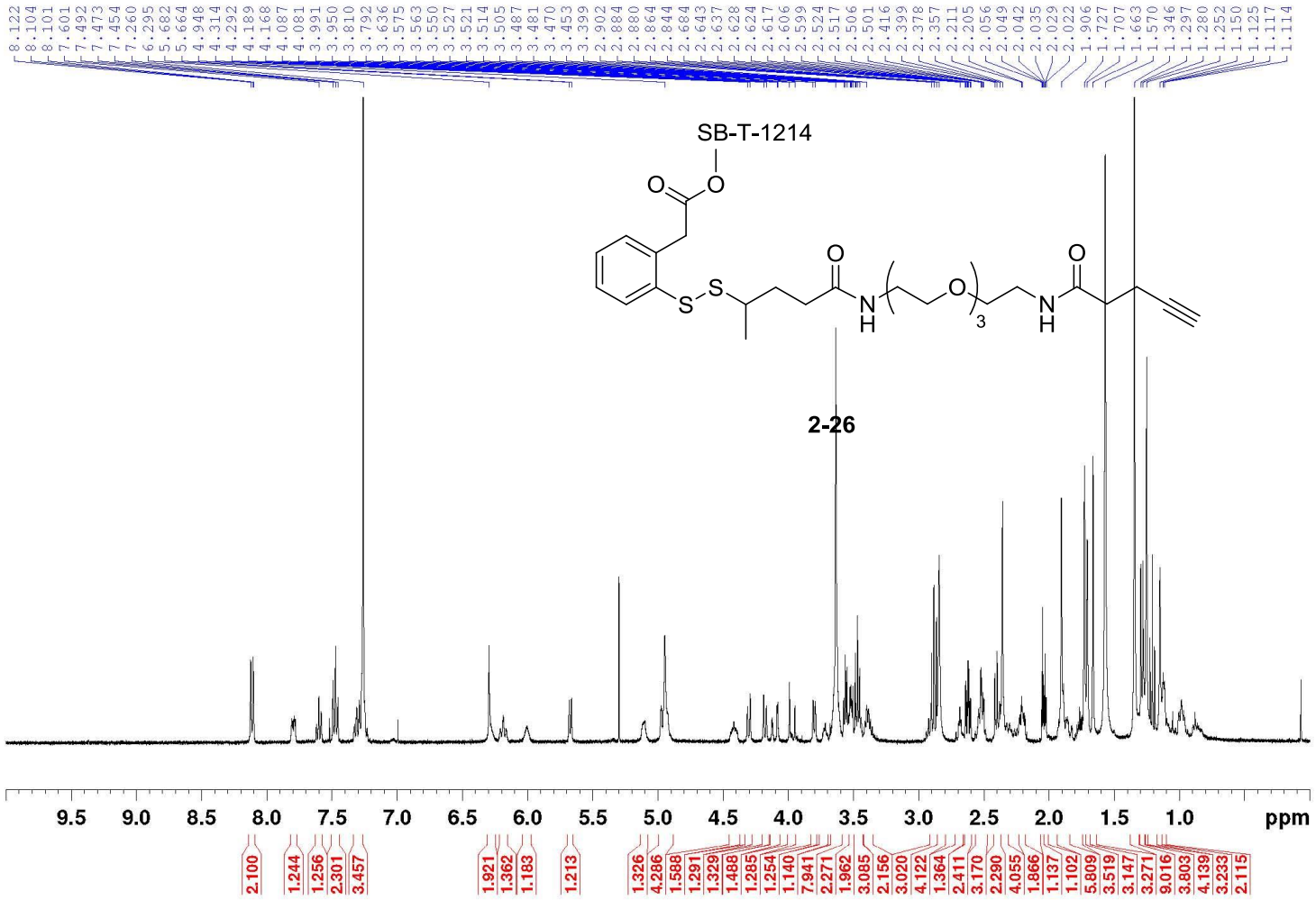
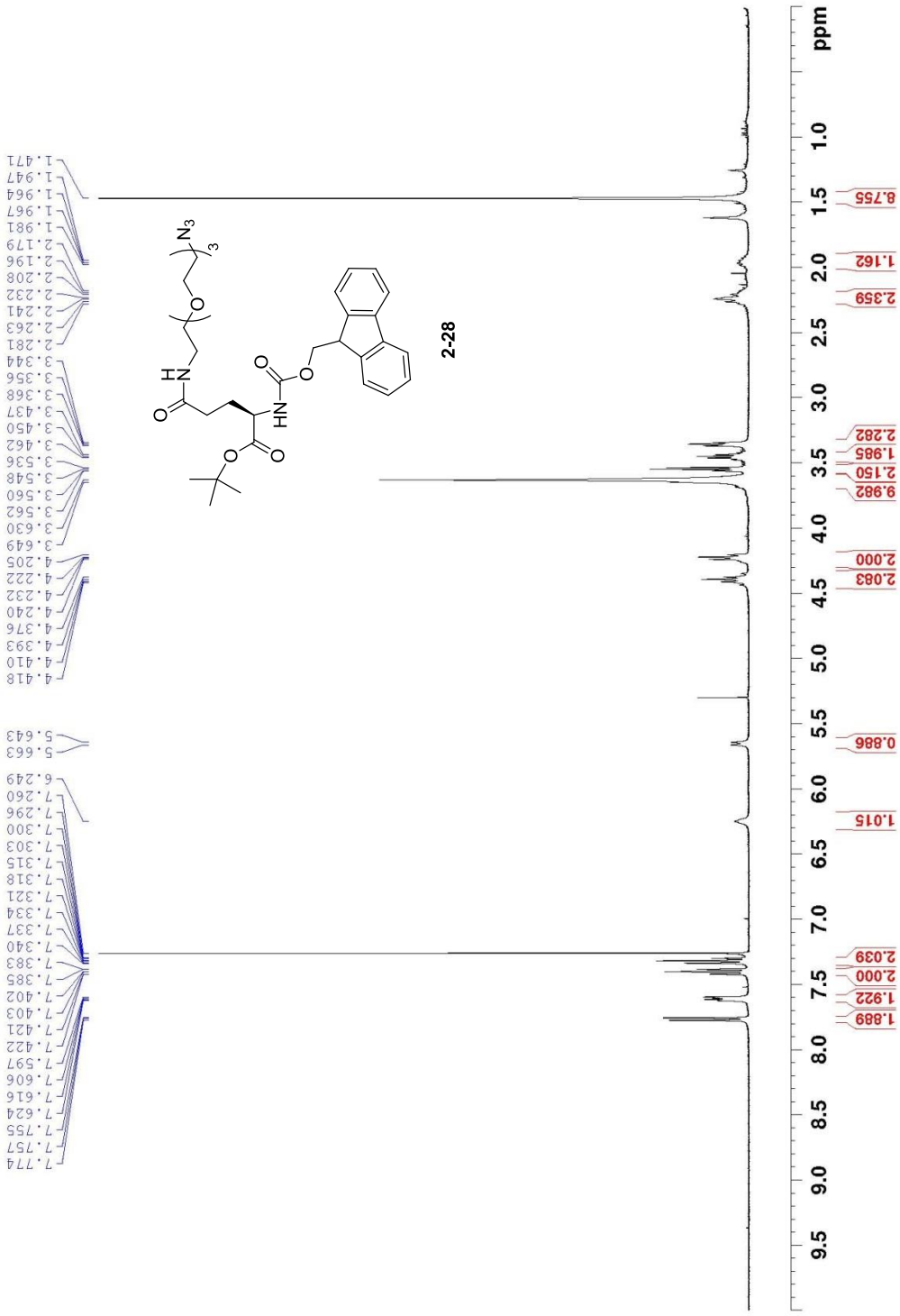
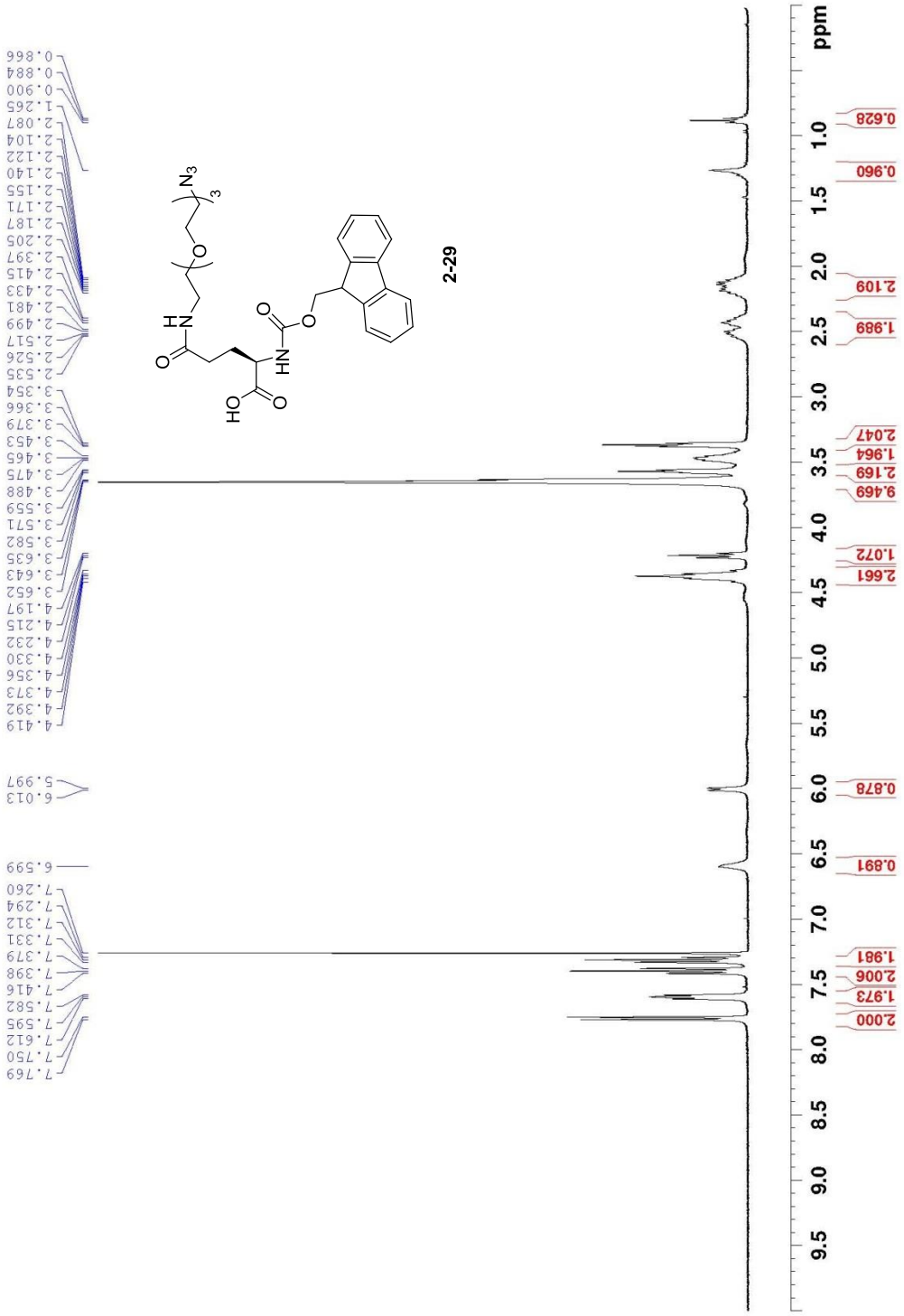
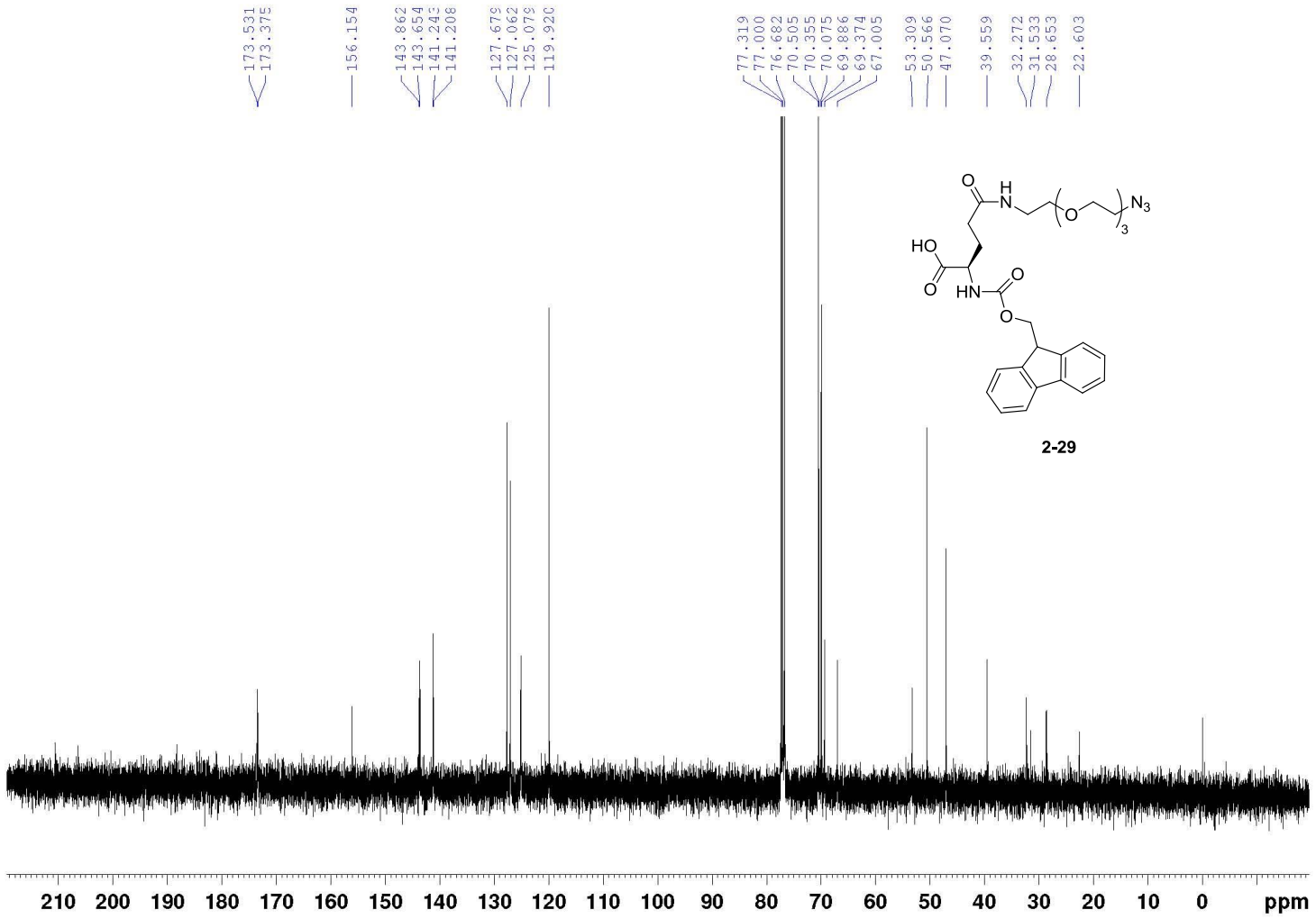
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.846
8.247
4.249
2.000
2.244
1.978
1.839
0.938
2.124
7.260
6.964
3.656
3.649
3.642
3.638
3.622
3.618
3.614
3.610
3.557
3.547
3.537
3.532
3.522
3.511
3.469
3.458
3.448
3.438
2.872
2.861
2.851
2.541
2.539
2.536
2.534
2.524
2.519
2.511
2.506
2.423
2.408
2.394
2.009
2.004
1.999
1.906


171.003
83.178
77.258
77.003
76.749
73.275
70.519
70.489
70.229
70.106
70.019
69.095
41.601
39.223
35.162
14.879
H2N
O
3
N
H
O
2-25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


SB-T-1214
2-26
ppm


2-28


2-29
ppm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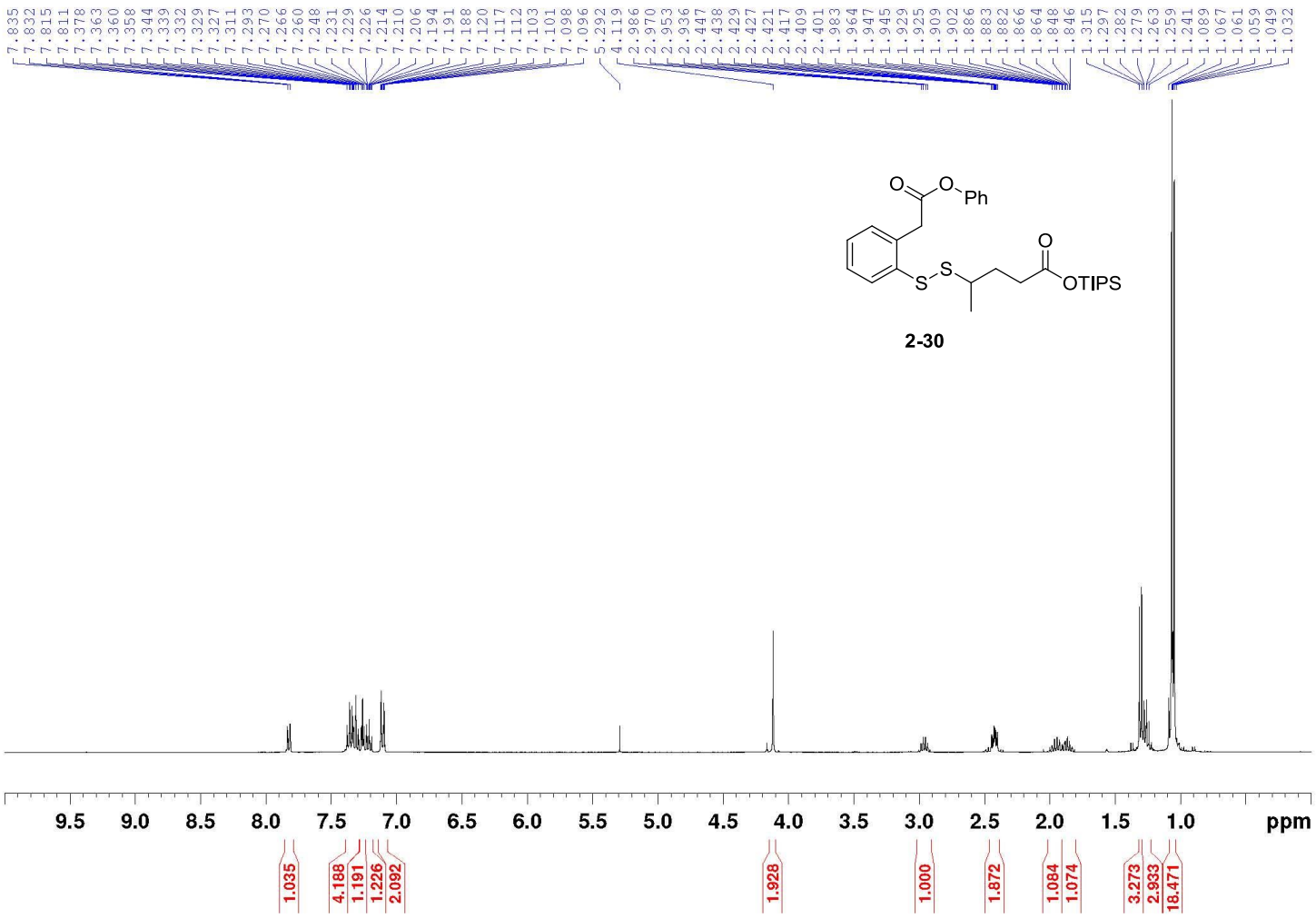
173.375
156.154
143.862
143.654
141.243
141.208
127.679
127.062
125.079
119.920
77.319
77.000
76.682
70.505
70.355
70.075
69.886
69.374
67.005
53.309
50.566
47.070
39.559
32.272
31.533
28.653
22.603
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

2-29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7.815
7.811
7.378
7.363
7.360
7.358
7.344
7.339
7.332
7.329
7.327
7.311
7.293
7.270
7.266
7.260
7.248
7.231
7.229
7.226
7.214
7.210
7.206
7.194
7.191
7.188
7.120
7.117
7.112
7.103
7.101
7.098
7.096
5.292
4.119
2.986
2.970
2.953
2.936
2.447
2.438
2.429
2.427
2.421
2.417
2.409
2.401
1.983
1.964
1.947
1.945
1.929
1.925
1.909
1.902
1.886
1.883
1.882
1.866
1.864
1.848
1.846
1.315
1.297
1.282
1.279
1.263
1.259
1.241
1.089
1.067
1.061
1.059
1.049
1.032
O
O
Ph
O
S
S
OTIPS
2-30
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
ppm
1.035
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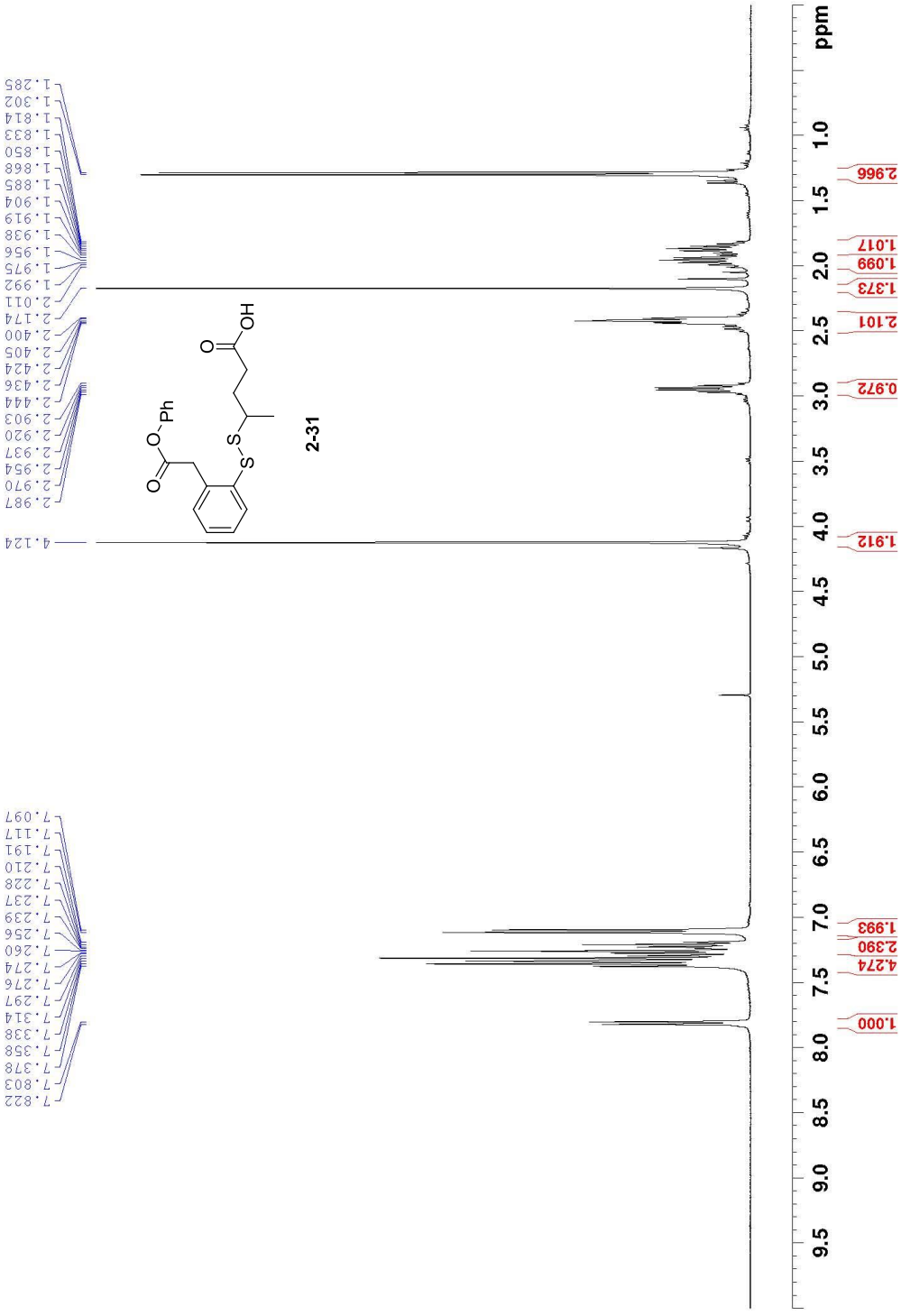
1.191
1.226
2.092
1.928
1.000
1.872
1.084
1.074
3.273
2.933
18.471


2-31
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7.0
6.5
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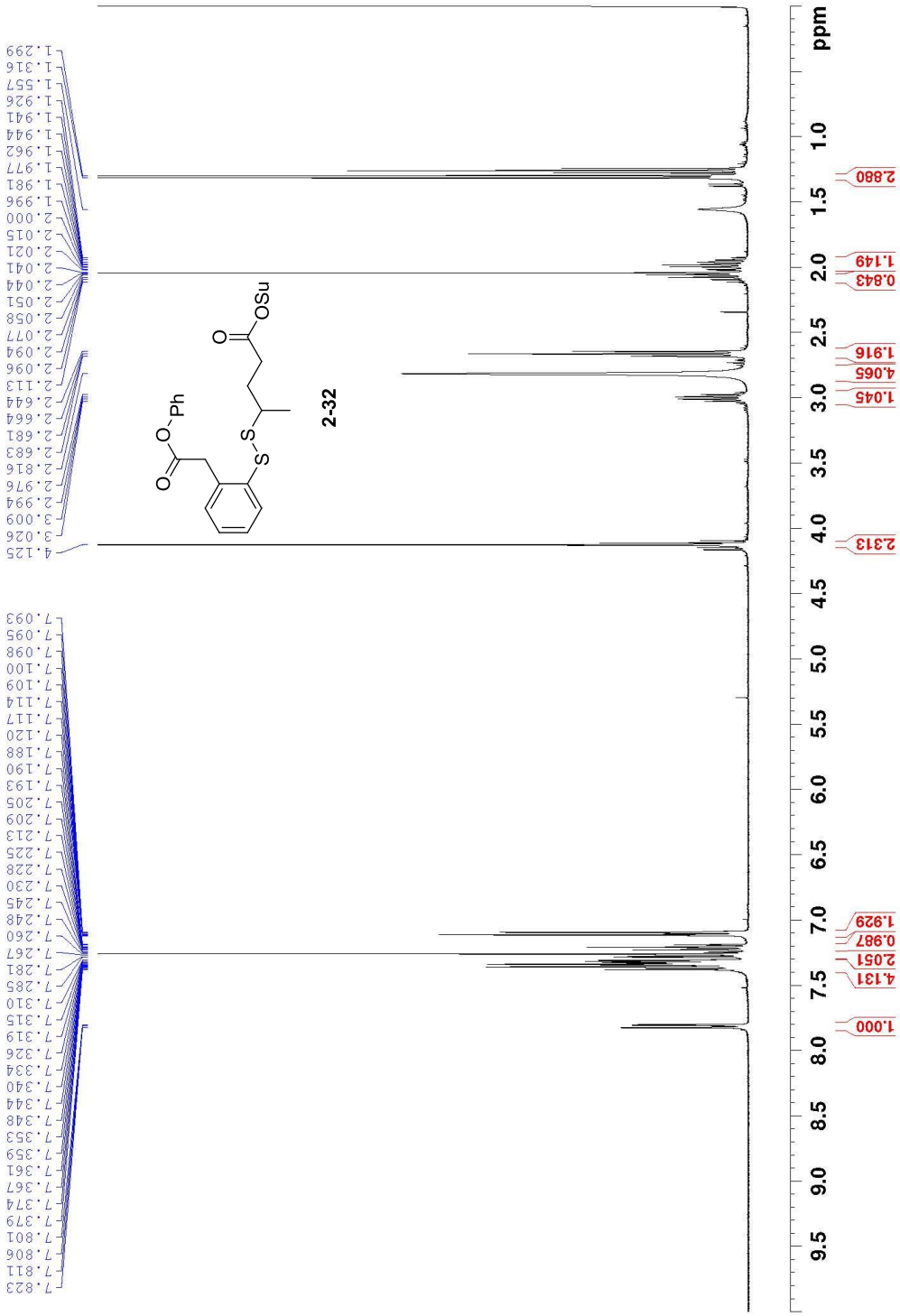
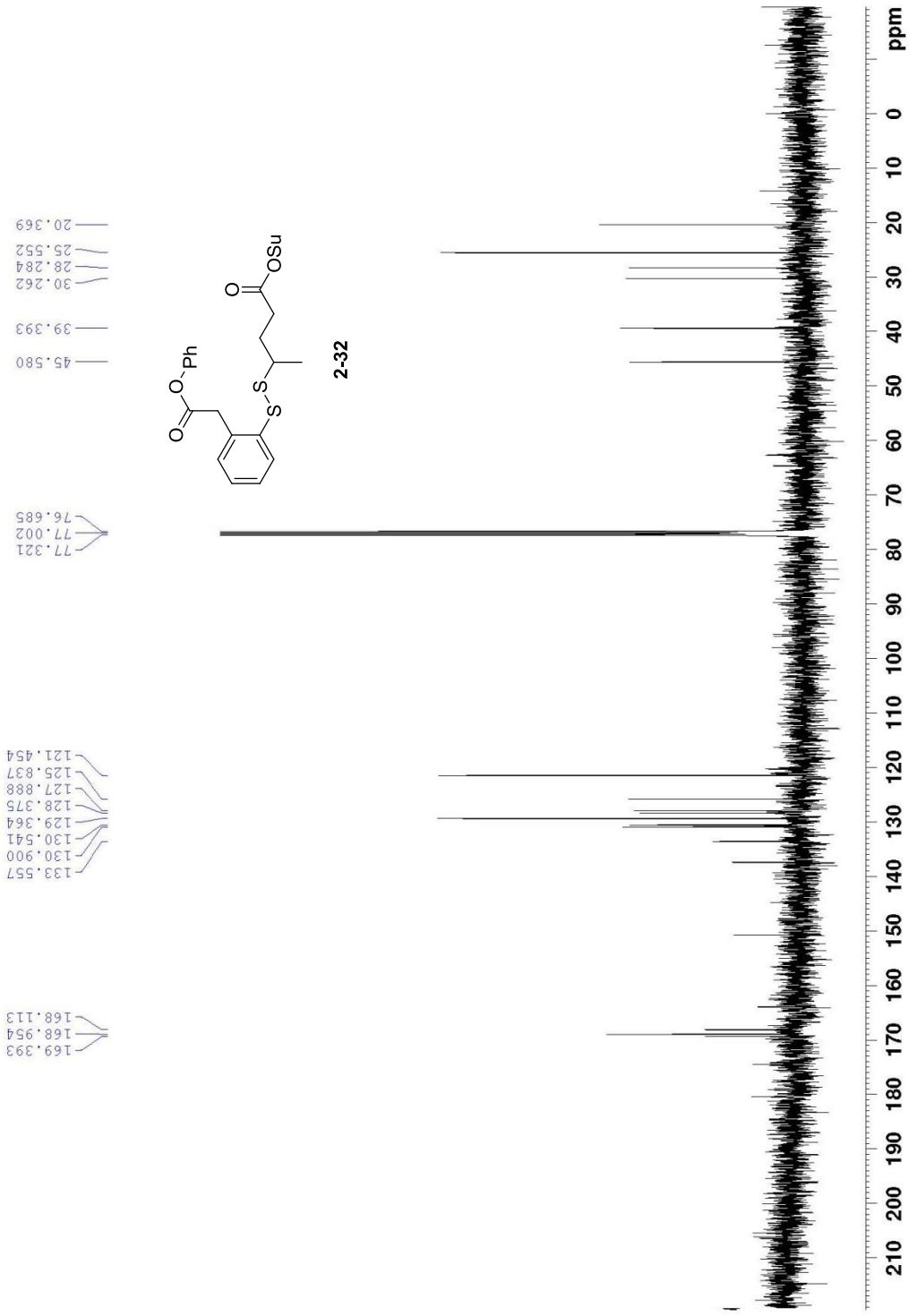
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
2.966
1.017
1.099
1.373
2.101
0.972
1.912
1.993
2.390
4.274
1.000
4.124
2.987
2.970
2.954
2.937
2.920
2.903
2.444
2.436
2.424
2.405
2.400
2.174
2.011
1.992
1.975
1.956
1.938
1.919
1.904
1.885
1.868
1.850
1.833
1.814
1.302
1.285
7.822
7.803
7.378
7.358
7.338
7.314
7.297
7.276
7.274
7.260
7.256
7.239
7.237
7.228
7.210
7.191
7.117
7.097


ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
2.880
1.149
0.843
1.916
4.065
1.045
2.313
1.929
0.987
2.051
4.131
1.000
O
OSu
O
Ph
O
S
S
2-32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28.284
30.262
39.393
45.580
76.685
77.002
77.321
121.454
125.837
127.888
128.375
129.364
130.541
130.900
133.557
168.113
168.954
169.393
O
OSu
O
O
Ph
S
S
2-32
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210