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Synthesis and biological evaluation of histone deacetylase inhibitor largazole and analogs

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Synthesis and Biological Evaluation of Histone Deacetylase

Inhibitor Largazole and Analogs

by

Pravin R. Bhansali

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Medicinal Chemistry

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An Abstract of

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Epigenetics, along with genetic mutations, are major etiological factors in carcinogenesis. Epigenetics are heritable changes in gene expression that take place without modifying gene sequence. Histone acetylation modulated by two protein families, histone acetyltransferase (HAT) and histone deacetylase (HDAC) are the most extensively studied of these epigenetic processes. The fine balance of acetylation-deacetylation of histones in normal cells is disturbed in cancer cells. HDACs are a class of enzymes responsible for removal of acetyl groups from substrate proteins.

Recently isolated natural HDAC inhibitor largazole is very potent and selective in its activity for cancer cells. A molecule requires three structural elements to inhibit HDAC:

1) a zinc-binding moiety, 2) a hydrophobic surface recognition group which occupies the lipophilic region of the enzyme and 3) a linker which interacts with hydrophobic channel. The zinc-binding unit and surface recognition group are the potential targets for modification to achieve variations in activity and selectivity profiles of HDAC inhibitors.

We have successfully synthesized largazole and several largazole analogs with modified surface recognition groups and zinc-binding moieties to modulate potency and selectivity. Substitution of L-valine of largazole by hydrophobic amino acids such as D-naphthylalanine, L-naphthylalanine and L-allylglycine generated analogs with an altered hydrophobic core. Replacement of the thiol group of largazole with moieties containing multiple heteroatoms and hydroxamic acid gave analogs with modifications in the Zn^{2+} binding moiety.

In our synthetic strategy, a stereoselective aldol condensation reaction set the stereochemistry at C-17 of the molecule. Synthesis of (*R*)-α-methylcysteine as well as its enantiomer (*S*)-α-methylcysteine provided access to the synthesis of largazole and its C-7 epimer for structure activity relationship (SAR) studies. Determination of the biological properties of these molecules has provided valuable SAR information that can direct the design and development of additional largazole analogs.

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I am lucky to have caring family members. A special thanks to my soul mate, Meenakshi for her love, faith and unconditional support. I cannot forget my son, Tanishk who always took care of mine. In the end, I thank the almighty for his guidance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF SPECTRA

LIST OF ABBREVIATIONS

AAPS	American Association of Pharmaceutical Scientists
ACS	American Chemical society
AML	Acute myeloid leukemia
AMP	Adenosine 5'-monophosphate
Boc	*tert*-butyloxycarbonyl
brsm	Based on recovered starting material
$CDCl_3$	Deuterated chloroform
CDI	carbonyl diimidazole
CTCL	Cutaneous T-cell lymphoma
D_2O	Deuterated water
DIBAL-H	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine (Hunig's base)
DMAP	Dimethylaminopyridine
DME	Dimethoxyethane
DMF	Dimthylformamide
DMSO	Dimethyl sulphoxide
DMSO-d_6	Deuterated dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EGFR	Epidermal growth factor receptor
FK 228	Depsipeptide Romidepsin
Fmoc	9*H*-Fluoren-9-ylmethoxycarbonyl
HA	Hydroxamic acid
HAT	Histoneacetyltransferase
HATU	O-(7-Azabenzotriazole-1-yl)-N, N,N'N'-tetramethyluronium hexafluorophosphate
HDAC	Histonedeacetylase
HDACi	Histonedeacetylase inhibitor
HDACis	Histonedeacetylase inhibitors
HDACs	Histone deacetylases
HDLP	histone deacetylase like protein
HER	Human epidermal growth factor receptor
HOAt	1-Hydroxy-7-azabenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectra
HSP-90	Heat shock protein 90

Hunig's base	Diisopropylethylamine, DIPEA
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium
NAD^+	Nicotinamide adenine dinucleotide
NMR	nuclear magnetic resonance
PKA	Protein kinase
RNA	Ribonucleic acid
RT	Room temperature
SAHA	Suberoylanilide hydroxamic acid
SAR	Structure activity relationship
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SM	Starting Material
S_N1	First oder nucleophilic substitution
S_N2	Second order nucleophilic substitution
TBAB	tetrabutylamonnium bromide
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFMK	Trifluoromethylketone
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
TRAIL R1	Tumor necrosis factor related apoptosis inducing ligand receptor 1
TRAIL R2	Tumor necrosis factor related apoptosis inducing ligand receptor 2
TSA	Trichostatin A
USFDA	United State Food and Drug Administration
UT	The University of Toledo

Chapter 1

1 Significance

A major challenge in cancer chemotherapy is the development of drugs which selectively kill cancer cells without affecting normal healthy cells. This is required to increase potency as well as to reduce toxic side effects. A cancer is a group of cells that undergo rapid multiplication due to disruption of the control mechanisms that regulate cell division. In most respects they are like normal cells and therefore, selectively targeting a drug molecule to cancer cells remains a challenging task. Natural products are a main source of lead molecules in the design and development of anticancer agents.[1] Many natural product based drugs such as vincristine, vinblastin, paclitaxel, docetaxel, etopside, teniposide, topotecan, irinotecan, actinomycin D, and doxorubicin with different mechanism of action have been used in cancer treatment (Figure 1). The blockbuster drug paclitaxel is an anti-mitotic agent and has limitations in clinical use due to development of multiple drug resistance. Actinomycin D is an antibiotic which inhibits RNA polymerase, while doxorubicin is a DNA intercalater. A common problem associated with all anticancer agents is lack of selectivity.

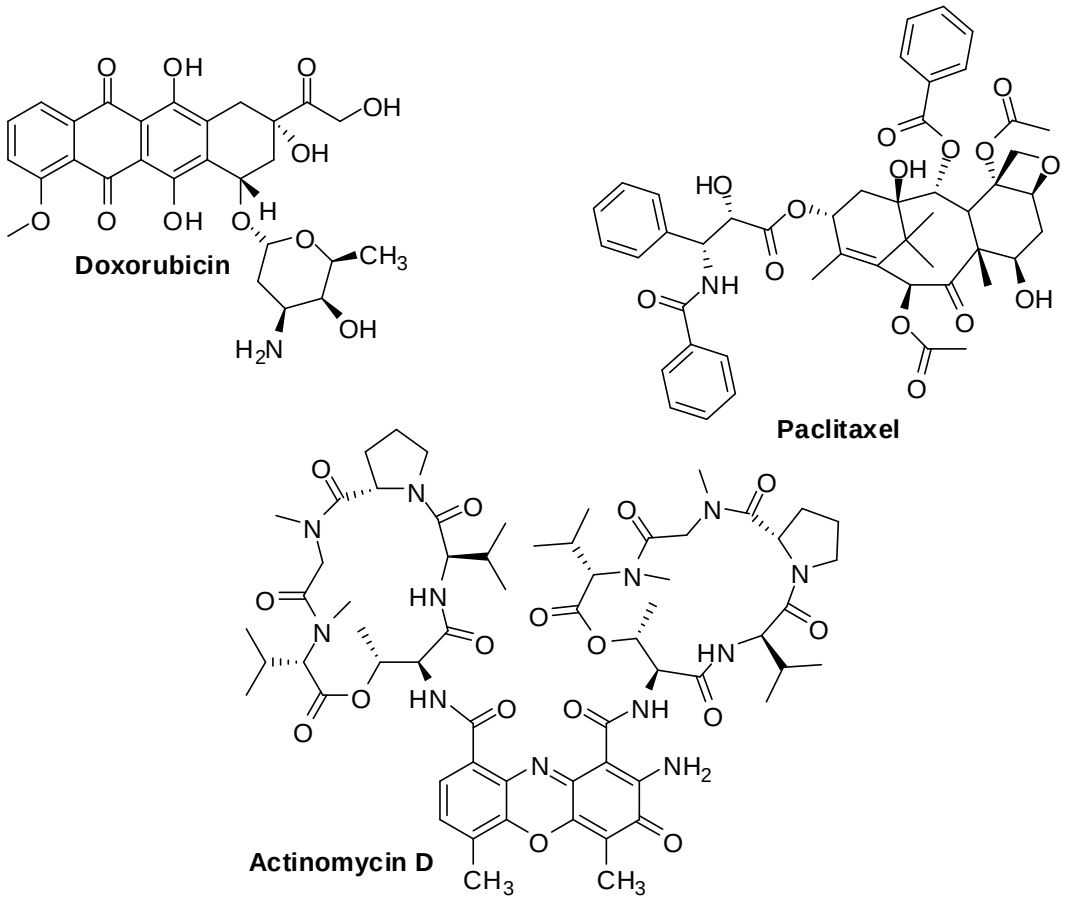


Figure 1. Natural product anticancer drugs.

Recently, a new natural product largazole (Figure 2), isolated from a marine cynobacterium of the genus *Symploca*, was reported to possess surprisingly selective growth inhibitory activity on highly invasive transformed human mammary epithelial cells (MDA-MB-231) and transformed fibroblastic osteosarcoma (USOS) cells when compared to the corresponding normal cell lines NMuMG and NIH3T3, respectively (Table 1).[2]

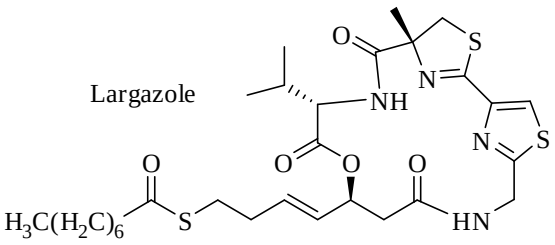


Figure 2. Structure of largazole

Table 1: Growth-inhibitory activity (GI_{50}) of natural product drugs[2]

	MDA-MB-231	**NMuMG**	**USOS**	**NIH3T3**
Largazole	7.7 nM	122 nM	55 nM	480 nM
Paclitaxel	7.0 nM	5.9 nM	12 nM	6.4 nM
Actinomycin D	0.5 nM	0.3 nM	0.8 nM	0.4 nM
Doxorubicin	310 nM	63 nM	220 nM	47 nM

Subsequent mechanistic studies showed that largazole is an inhibitor of histone deacetylase (HDAC), an enzyme that plays a key role in cell division.[3] Histone deacetylases (HDACs) are a class of enzymes responsible for removal of acetyl groups from substrate proteins, while the reverse reaction i.e. transfer of acetyl group from acetyl coenzyme Q to histone is catalyzed by histone acetyl transferase (HAT)[4] (Figure 3). The normal cell's finely balanced status of acetylation and deacetylation is modified in tumorogenesis.[5] The deacetylated protein, positively charged at physiological pH, interacts with the negatively charged DNA core to form transcriptionally inactive condensed forms of genes. These epigenetic changes can result in silencing of tumor suppressor genes leading to tumorogenesis. Inhibition of HDAC activates the

inappropriately silenced genes[6] and is thus an attractive target for anticancer drug development.

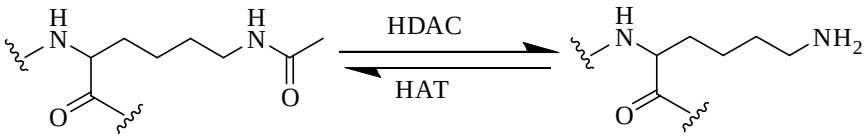


Figure 3. Acetylation and deacetylation processes.

1.1 Role of epigenetics in cancer

Apart from genetic mutations, epigenetics are a major player in carcinogenesis. Epigenetics are heritable changes which take place without modifying gene sequence.[6] Chromatin is made up of many units of nucleosome which consists of 146 base pairs of DNA wrapped around 8 histone molecules, 2 each of H_2A, H_2B, H_3, and H_4. Histones, upon deacetylation, acquire positive charges at physiological pH and tightly interact with the negatively charged DNA. In contrast, acetylated histones release DNA which is transcriptionally active.[4, 6] In cancer, aberrant epigenetic silencing of several genes including tumor suppressor genes is a common occurrence.[7] Aberrant epigenetic silencing occurs as a result of abnormal promoter region CpG island DNA methylation in concert with changes in covalent modification of histone proteins.[7g] Enzymatic modifications such as acetylation, methylation and phosphorylation of the lysine and arginine residues of the N-terminus of histones regulate the access to DNA by transcriptional factors thereby regulating gene expression.[8] Histone acetylation modulated by the two protein families histone acetyltransferase and histone deacetylase is the most extensively studied of these processes.[9] Epigenetic changes, unlike DNA

sequence changes, are reversible and thus targeting epigenetic gene regulation as an anticancer strategy has attracted the attention of the scientific community.

HDAC proteins are a family of 18 enzymes and are classified into four classes based on their size, cellular localization, number of catalytic active sites and homology to yeast HDAC proteins:[10]

Class I: HDAC1, -2, -3, and -8 resides in nucleus

Class IIa: HDAC4, -5, -7, and -9 shuttles between nucleus and cytoplasm

Class IIb: HDAC6 and -10 shuttles between nucleus and cytoplasm

Class III: Sirtuin proteins, consists of 7 types of proteins

Class IV: HDAC11 shuttles between nucleus and cytoplasm

Zn^{2+} is predominantly required for activity of class I, II and IV HDACs. Class III HDACs do not require Zn^{2+} but require NAD^{+} for their catalytic activity. HDAC henceforth in following discussion refers to class I, -II and –IV HDACs only as HDAC inhibitors (HDACis) inhibit all classes of HDAC other than class III. Association of class III Sirtuin proteins in offsetting age-related diseases such as type II diabetes, obesity and neurodegenerative diseases is known.[9b] Although their full characterizations remains to be accomplished, these HDAC isoforms have generally noticeable gene expression patterns and may also differ in cellular localization and function.[10] HDAC proteins are involved in basic cellular events and disease states including cell growth, differentiation and cancer formation.[11] Distinctively over-expression of class I and class II HDAC proteins have been noticed in some cancers including ovarian (HDAC1-3), gastric (HDAC2) and lung cancers (HDAC1 and -3) among others.[11] It has been proposed that

there is a possible correlation between HDAC8 and acute myeloid leukemia (AML). Over-expression of HDAC6 was induced in some breast cancer cells.[11] Inhibition of HDAC3 and -8 has been shown to be essential for inhibition of MCF-7 and KYO-1 cancer cell growth.[12] Targeting a single isoform/class can increase selectivity as well as decrease toxicity.

All of the HDAC proteins except HDAC6 exist in multi-protein complexes with other regulatory proteins and isolation of individual isoforms becomes difficult, which deters the development of isoform selective HDAC inhibitors.[13] Class I enzymes consist of around 500 amino acids while class II are made of about 1000 amino acids.[14] Class IIa enzymes consist of a 600 amino acid residue extension at the N-terminus and they play a role in regulatory and functional properties.[15] HDAC1 and -2 are highly related enzymes with an overall sequence identity of about 82%. Phosporylation status of HDAC1 and -2 affects their activity. Hyperphosphorylation increases deacetylase activity while hypophoshorylation has the opposite effect.[16] HDAC3 has 34% overall sequence identity with HDAC8.[16] HDAC4 and -5 are largely similar to each other (with overall sequence identity 70%), and HDAC7 in turn is related to HDAC4 and -5 with ~ 58 and ~ 57% overall similarity, respectively.[16] HDAC4, and to a lesser extent HDAC8 and -9, seem to be expressed more in tumor tissues than in normal tissues.[16] HDAC5, -7 and -9 are expressed in heart tissues and they can be correlated with their role in the development of cardiac muscle tissue.[16] Increased levels of HDAC5 mRNA in depressive patients have been reported.[17] HDAC6 has two independent catalytic domains- one at C-terminal and the other at the N-terminal of the protein. C-terminal catalytic domain maintains its

activity even upon trimming of the N-terminal catalytic domain. Tubulin deacetylation activity is present in the C-terminal domain.[17] HDAC6 deacetylates tubulin and heat shock proteins apart from histones and is highly expressed in tumor cells.[16-17] It has been suggested that HDAC6 inhibitors are potential anticancer agents in the treatment of multiple myeloma[18] and they are also useful in inflammation, Huntington's disease and various neurodegenerative disorders.[17, 19] Studies have shown that HDACs deacetylate many non-histone proteins as well. More than 1700 non-histone proteins have been detected as substrates for HDACs.[13] These non-histone proteins play a regulatory role in processes such as cell migration, cell proliferation and cell death.[20]

X-ray crystallography and SAR studies of various HDAC inhibitors have shown that three key structural elements are required for a molecule to be effective as a HDAC inhibitor (Figure 4):[21]

i) a metal binding domain which chelates with the Zn^{2+} cation present at the active site
ii) a linker, which occupies the hydrophobic channel
iii) a surface recognition group, which interacts with hydrophobic residues on the rim of the active site

Most HDAC inhibitors, including those in clinical trials, are reversible inhibitors, although irreversible inhibitors are known.[17] Irreversible inhibitors with a keto-epoxide covalently interacts with nucleophilic residues such as histidine, aspartate, and tyrosine of the active site.[17] The variety of functional groups used as zinc binding moieties include thiol, epoxy ketone, carboxylic acid, boronic acid, sulfoxide, sulfonamide,

phosphonamidate, phosphonate, phosphinate, trifluoroketone, hydroxamic acid, 2-amino benzamide, oxime amide, and α-thioamide.[7e, 17]

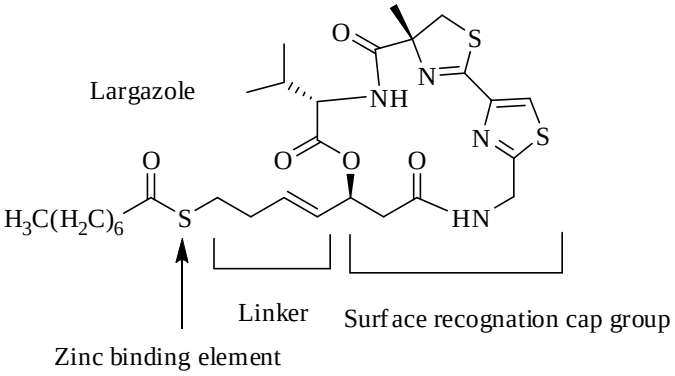


Figure 4. HDACi binding elements.

The selective activity of an HDAC inhibitor on a particular type of cancer cells may be attributed to variations in the composition of HDAC proteins. HDAC inhibitors have become a major area of research in the development of new drugs for the treatment of cancer. The HDAC inhibitors suberoylanilide hydroxamic acid (SAHA) (Figure 5), and depsipeptide FK 228 (Figure 6) were approved by the US FDA in October 2006 and November 2009, respectively, for the treatment of cutaneous T-Cell lymphoma (CTCL).[13]

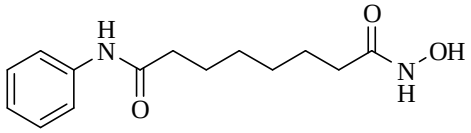


Figure 5. Structure of SAHA.

A number of other HDAC inhibitors are in various phases of clinical trials (Table 2). SAHA inhibits all isoforms of HDAC (thus called a pan-HDAC inhibitor) to varying degrees. The small cap group of SAHA interacts with the highly conserved region of the enzyme rim and is unable to make contact with the variable region which is further away from the opening of the channel of HDAC. The inability to interact with the variable region is responsible for its non-specificity.[22]

Table 2. A list of HDACis in clinical trials[7b, 13, 23]

Compound	**Chemical class**	**Status**	**Indication**
Butyrate	Short chain Fatty acid	Phase II	Non-small cell lung cancer
Valproic acid	Carboxylic acid	Phase I /II	Non-small cell lung cancer
Pivanex/ AN 9	Ester	Phase I/II	Chronic Lymphocytic Leukemia
Phenylbutyrare	Ester	Phase II	Progressive or Recurrent Brain Tumors
Belinostat	Hydroxamate	Phase II	Refractory Peripheral T-Cell Lymphoma
Panobinostat	Hydroxamate	Phase II Phase II/III	Refractory Clear Cell Renal Carcinoma, Metastatic Thyroid Cancer, Refractory Colorectal Cancer Chronic Myeloid Leukemia
CUDC-101	Hydroxamate. Inhibits human epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2) and histone deacetylase (HDAC)	Phase I	Advanced Solid Tumors
JNJ-26481585	Hydroxamate	Phase I	Advanced Solid Malignancies and Lymphoma
CHR-3996	Hydroxamate	Phase I	Advanced Solid Tumors
AR-42	Hydroxamate	Phase I	Advanced or Relapsed Multiple Myeloma, Chronic Lymphocytic Leukemia, or

			Lymphoma
SB939	Hydroxamate	Phase II	Recurrent or Metastatic Prostate Cancer, Locally Advanced or Metastatic Solid Tumors
PCI-24781	Hydroxamate	Phase II	Follicular Lymphoma

Table 2 Continued from page 9

ITF2357/ Givinostat	Hydroxamate	Phase II	Refractory/Relapsed Lymphocytic Leukemia
4SC-201/ Resminostat	Hydroxamate	Phase II	Relapsed or Refractory Hodgkin's Lymphoma
N-Acetyl Dinaline (CI-994)/ Tacedinaline	Aminobenzamide	Phase II	Advanced Myeloma
MGCD0103	Aminobenzamide	Phase II	Refractory Chronic Lymphocytic Leukemia
MS-275/ Entinostat	Aminobenzamide	Phase II Phase I	Metastatic Melanoma Advanced Solid Tumors or Lymphoma
CG200745	Aminobenzamide	Phase II	Solid Tumors
4SC-202	2-Aminophenylcarbamoyl	Phase I	Advanced Hematologic Malignancies

Due to its highly selective activity on selected cancer cell lines and a strong unprecedented picomolar inhibitory bias for class I HDACs (HDAC1, -2 and -3) over class II HDAC6,[3] largazole provides a new attractive lead molecule in the development of class and /isoform selective HDAC inhibitors. Interestingly, largazole shares a common 3-hydroxy-7-mercapto-4-heptenoic acid side chain with related natural product HDAC inhibitors such as FK228 **2**, FR901375 **3**, and spiruchostatins **4** (Figure 6). All of these agents are prodrugs which upon activation release the free thiol group necessary for HDAC inhibitory activity. The thioester group present in largazole is hydrolyzed by cellular esterases and/or lipases to release the free thiol function which constitutes the

domain that chelates Zn^{2+}.[3b] In compounds **2**, **3** and **4** the masked thiol is present as a disulfide bridge with a cysteine residue in the depsipeptide ring and is reduced to free thiol by glutathione mediated cellular reduction.[24]

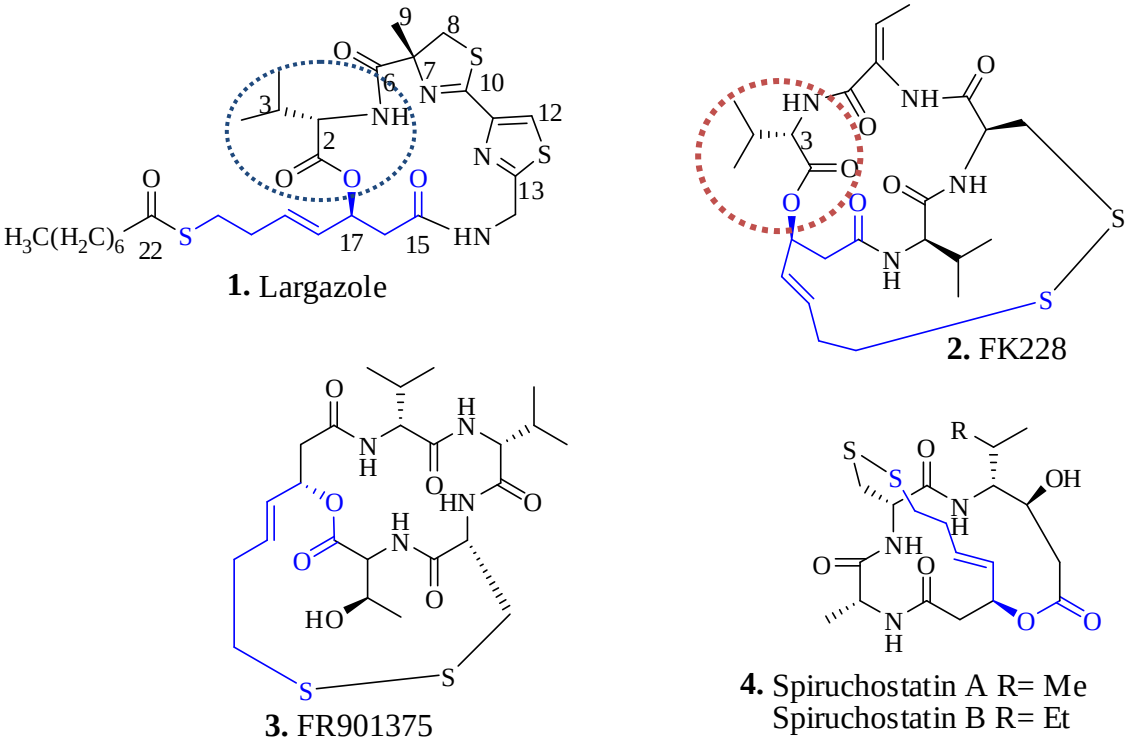


Figure 6. Largazole and related natural products.

Total synthesis of largazole and several analogs along with some preliminary structure-activity relationship (SAR) studies has been published.[3, 25] SAR studies (Figure 7) have shown that variations in the length of the side-chain (**A**, **B**, **C**) resulted in decreased activity.[25c, d] Diminished activity resulted upon replacement of the side chain sulphur atom with oxygen (**D**) or a methylene moiety (**E**).[25a] α-Aminoanilide group as a zinc binding agent is known to produce HDAC1 and -2 selective activity[26] while α-mercaptoacetamide compounds[27] showed preferential binding to HDAC6 over HDAC1, -

2, -8 and -10. However, thiol replacement with α-aminoanilide (**F**, **G**) or α-mercaptoacetamide (**H**, **I**) did not produce noticeable selectivity.[25g] The C-2 epimer **J**, non-natural largazole enantiomer **K**, analog **L** with proline substituted for valine all had reduced anticancer activity.[25g, 28] On the other hand, substitution of the thiazole-thiazoline moiety with an oxazole-oxazoline moiety (**M**) and cysteine substitution for α-methyl cysteine (**N**) gave equi-potent analogs.[25g] Replacement of thiazole ring with pyridine (**O**) resulted in a 3-4 fold enhancement in HDAC inhibitory activity. In fact, the pyridine analog **O** is the most potent HDAC inhibitor known.[25g] The amide analog formed by substitution of ester oxygen atom at C-17 with an NH moiety (**P**) led to reduced activity. This was attributed to a conformational change in the cap group of the molecule rather than to a change in hydrogen bonding ability as it occupies the hydrophobic region of the active site.[25h]

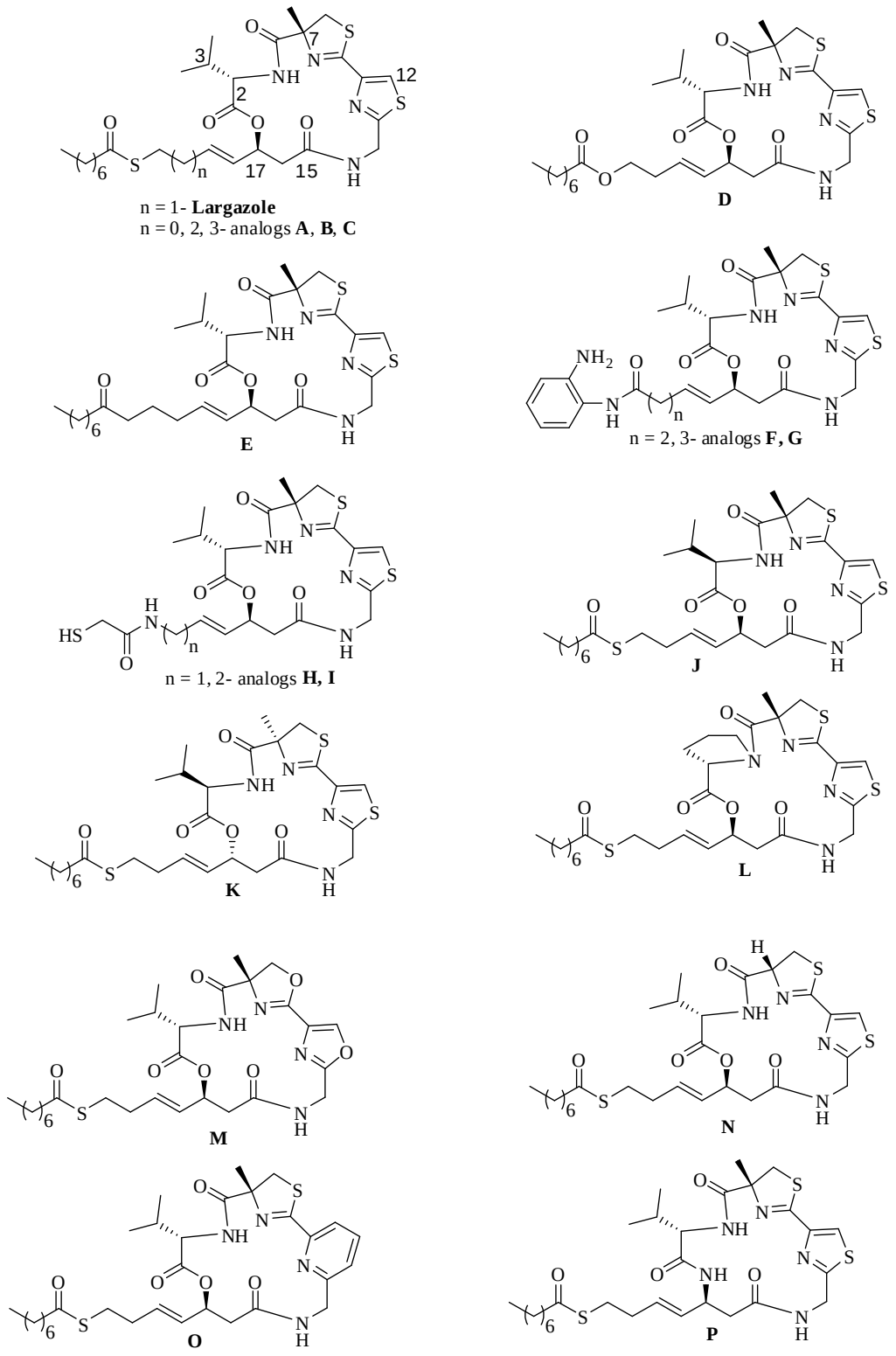


Figure 7. Largazole analogs synthesized in other laboratories.

1.2 Crystal structure and mechanistic pathways

Crystal structure information can dispense valuable directions for the design and development of isoform and/ class-selective HDAC inhibitors. Currently, crystal structures of HDAC2, -4, -7 and -8 are known.[13, 15, 17] It is assumed that the acetyl group of the lysine residue is held in the binding site by multiple hydrogen bonds during deacetylation by HDAC.[22] A general catalytic mechanism for HDAC based on computational studies with histone deacetylase like protein (HDLP) by Vanommeslaeghe is shown in Figure 8.[29] The Lewis acid-catalyzed carbonyl group activation by Zn^{2+} facilitates the nucleophilic attack by a H_2O molecule to generate a tetrahedral intermediate, which is stabilized by Tyr^{297} and Zn^{2+}. The stabilization of the intermediate transition state is mediated by Tyr^{297} in HDLP, Tyr^{303} in HDAC1, Tyr^{304} in HDAC2, Tyr^{306} in HDAC8, His^{976} and H_2O in HDAC4 and His^{709} in HDAC7. The tetrahedral intermediate breaks down to yield deacetylated lysine protein and acetate ion.[22]

Class IIa HDAC enzymes have low activity as a result of low transition state stabilization due to the absence of Tyr^{306}.[15] A 320 amino acid residue structural homology between class I and –II HDACs at the catalytic site is known.[17] Class IIb enzymes are characterized by two catalytic domains.[15] Structures of HDAC4, -7 and -8 consist of two potassium ions near the catalytic site.[15] The four conserved zinc-binding ligands in class IIa HDACs are His^{665}, Cys^{667}, His^{678}, and Cys^{751} which are missing in other HDACs.[15] Lack of transition state stabilizer Tyr^{306} in HDAC4, and -7 (which is present in class I HDACs) is responsible for their reduced enzymatic activity.[15] In HDAC4, Zn^{2+} ion is

loosely bound to the protein.[15] In addition to inhibiting the enzyme, HDAC4 inhibitors also hinder the role of class I HDAC3 as a promoter of HDAC4-

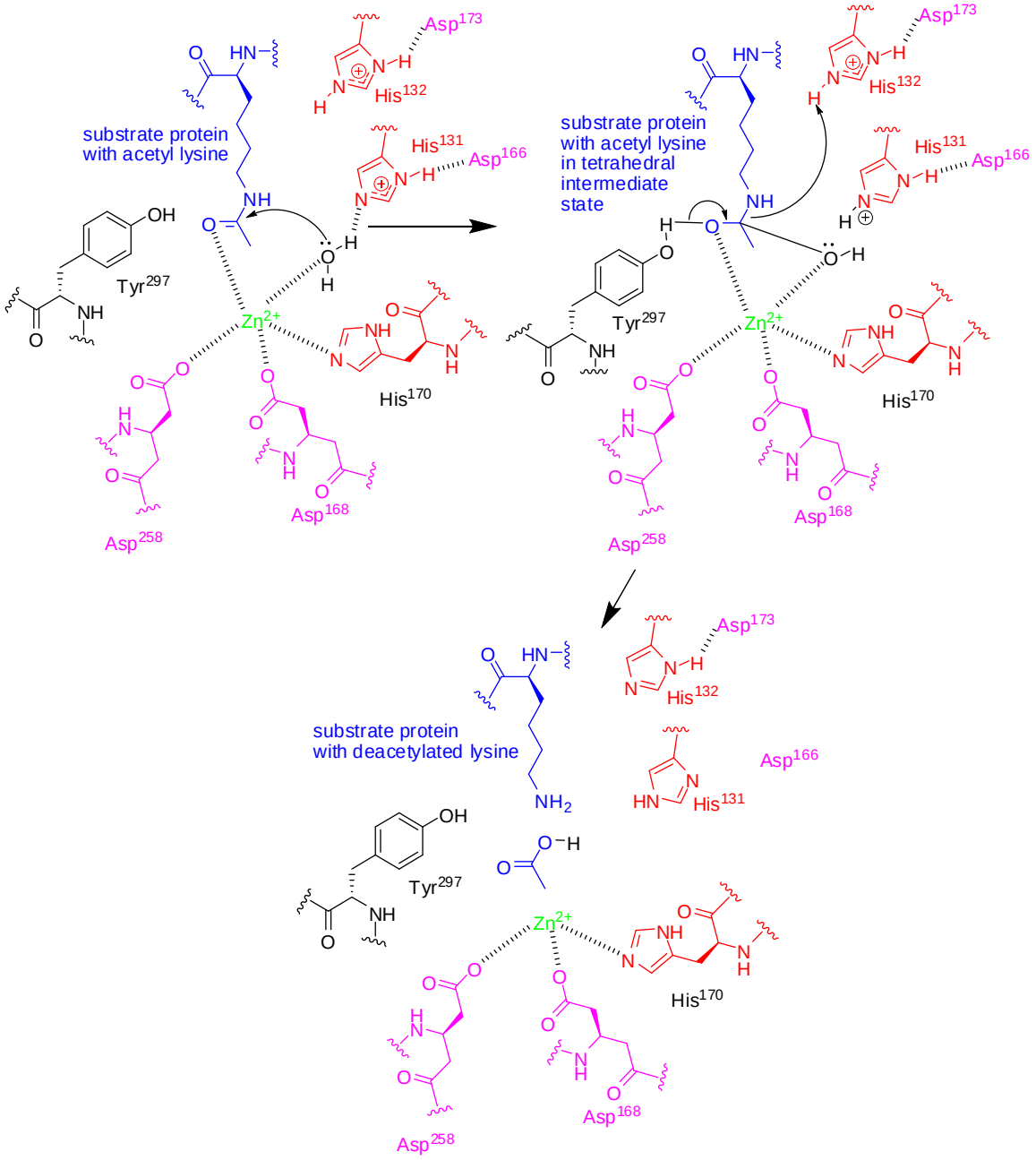


Figure 8. Proposed mechanism of action of Zn^{2+}- dependent HDACs. (Adapted from *Nat. Prod. Rep.*, **2009**, *26*, 1293–1320).

regulated genes.[15] In a X-ray crystallographic study with HDAC4, it has been observed that the trifluoromethyl ketone (**TFMK**) (Figure 9), upon hydration binds to HDAC4 in a bidentate fashion as compared to the hydroxamate (**HA**) which interacts in a monodentate manner. Unlike **HA**, **TFMK** fills the gap near Pro^{800} and this leads to higher potency of **TFMK** over **HA** (Figure 10).[15] As this pocket is larger in HDAC1, -2 and -3 enzymes, **TFMK** type compounds typically display 10-100 fold greater inhibition of class II HDACs than class I HDACs.[15] Class IIa HDACs have a longer binding pocket near His^{976} due to the outward positioning of His^{976} side chain.[15]

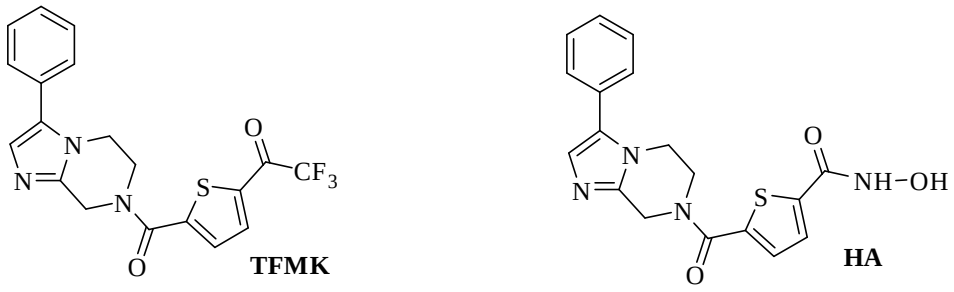


Figure 9. HDACis used in binding studies with HDAC4.

HDAC7 has a major role in cardiovascular development and disorders.[30] Inhibitor binding studies with HDAC7 by X-ray crystallography (Figure 11) revealed that benzamide inhibitors (**MS-275**, **CI-994**, **MGCD-0103**), cyclic tetrapeptides (apicidin) and short chain fatty acids (butyrate, and valproic acid) did not show binding abilities.[31] Binding of hydroxamate HDAC inhibitors (SAHA, TSA) to the active site Zn^{2+} of HDAC7 takes place in monodentate manner with participation of hydroxyl oxygen only.[31] The carbonyl oxygen of hydroxamate is directed away from the zinc ion and is connected to a water molecule through hydrogen bonding. Amino acids present at the periphery of the pocket show conformational changes to the inhibitor upon its binding.[31]

HDAC7 alone has low intrinsic catalytic activity while it is activated in the presence of HDAC3.[31]

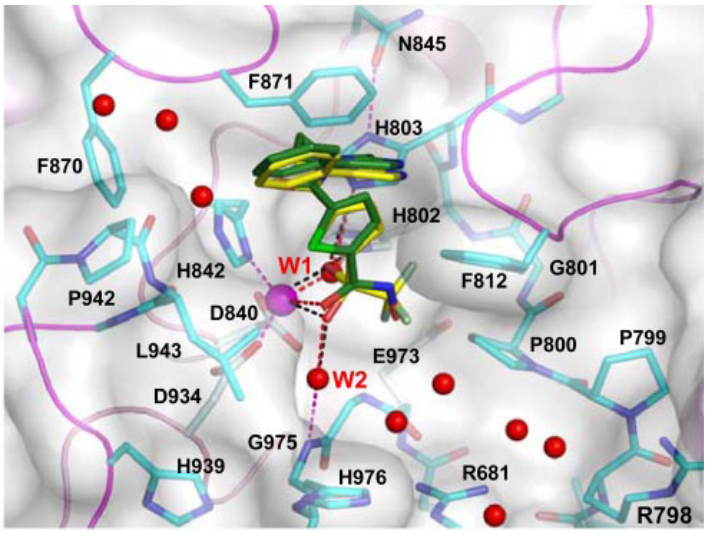


Figure 10. Interaction of TFMK (yellow carbons) and HA (green carbons) with HDAC4 catalytic domain. Reprinted from *Journal of Biological Chemistry* **2008**, *283*(39), 26694-26704.

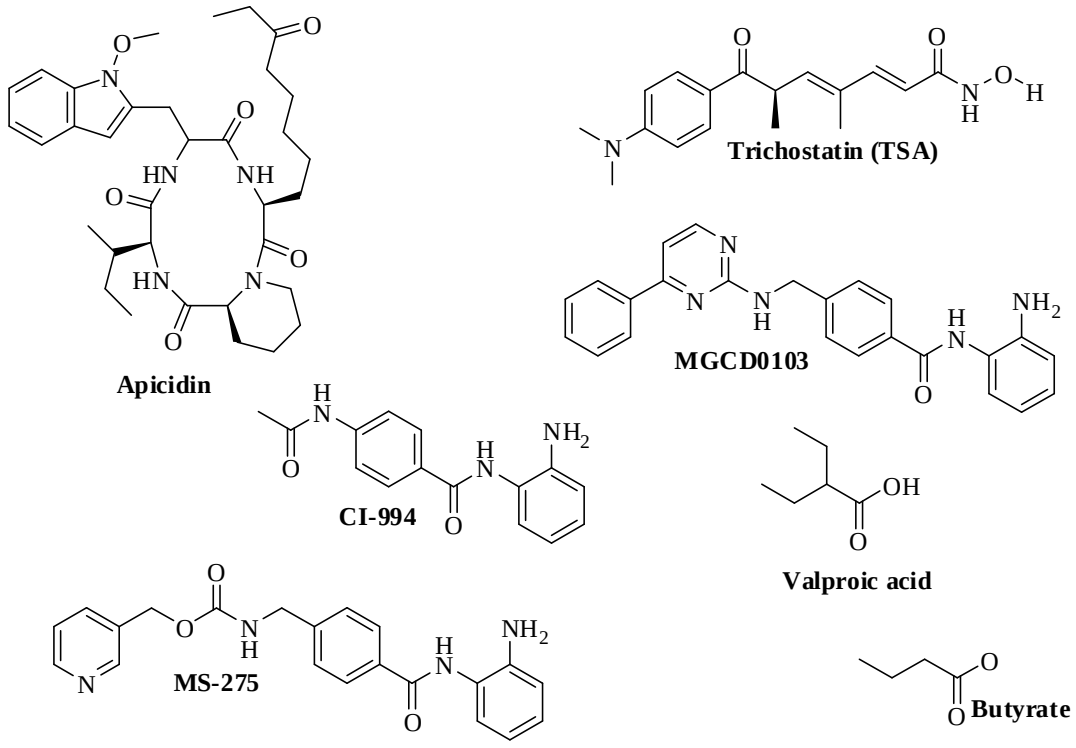


Figure 11. HDACis used in binding studies with HDAC7.

HDAC8 consists of 377 amino acids and is closely associated with phylogenetic border between class I and class II HDACs.[32] HDAC8 activity is decreased upon cyclic AMP-dependent protein kinase (PKA) phosphorylation of ser[39] which is responsible for the hyperacetylation of histone H3 and H4. Serine is positioned at the surface of HDAC8, approximately 20 Å from the opening to the active site and about 13 Å from the opening to the second binding site (Figure 12).[32] A possible role of HDAC8 in acute myeloid leukemia (AML) has been indicated.[32] Inversion, a chromosomal translocation, often takes place in AML and produces a protein product which causes abnormal transcription repression. HDACis reverse the repression produced by protein product association with HDAC8.[32] HDAC8 consists of a wider active site pocket with broad surface opening.[32] The hydrophobic wall of the tunnel is lined with Phe[152], Phe[208], His[180], Gly[151], Met[274] and Tyr[306]. These are preserved across the class I enzymes except that met[274] is replaced with Leu in the other family members.[32]

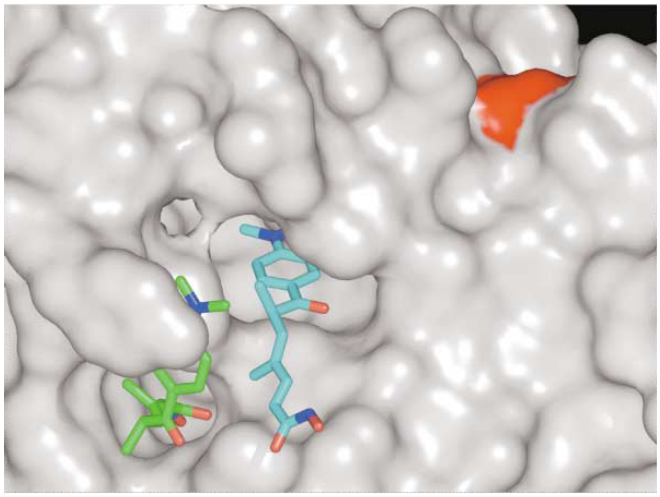


Figure12. Serine phosphorylation site (red) in relation to the active site of HDAC8 where it shows interaction with two molecules of TSA. Reprinted with permission from *Structure* **2004**, 12, 1325–1334.

In a HDACis binding study with HDAC8, it has been shown that hydroxamate inhibitors bind in bidentate fashion. Somoza et al[33] observed that HDAC8 binding site is very flexible and can accommodate ligands with different structural motifs (Figure 13, 14) .

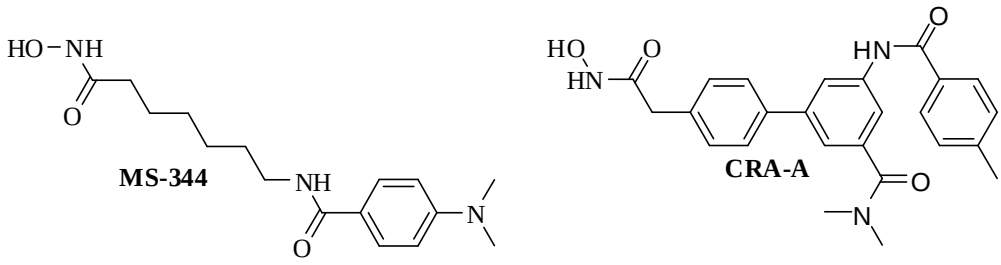


Figure 13. Some of the HDACis used in binding studies with HDAC8.

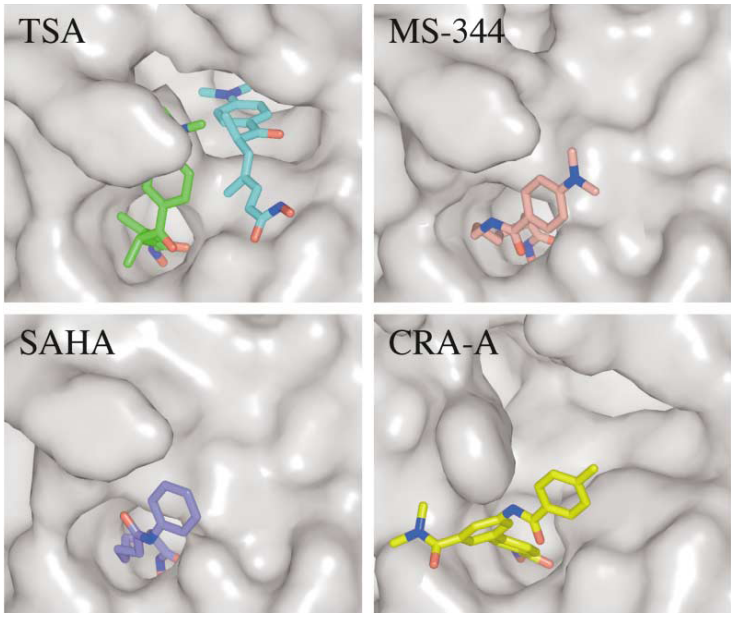
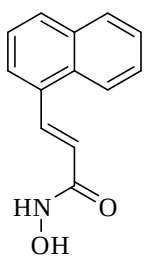
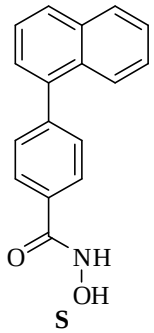


Figure 14. Interaction of HDACis with flexible catalytic pockets of HDAC8. TSA and CRA-A occupy two binding pockets while SAHA and MS-344 engage only one. Reprinted with permission from *Structure* **2004**, 12, 1325–1334.

This information was used by Ulrich et al[34] in the rational design of linkerless selective HDAC8 inhibitors (Figure 15: compound **R**, **S**).





	R	**S**
HDAC1 IC_{50} (μM)	> 100.0	>100.0
HDAC6 IC_{50} (μM)	82.0	55.0
HDAC8 IC_{50} (μM)	0.7	0.3

Figure 15. Linkerless selective HDAC8 inhibitors.

It has been observed that proliferation of human lung, cervical and colon cancer cell lines were inhibited by knockdown of HDAC8 by RNA interference studies.[35] HDAC1 and -3 have 43 % sequence identity with HDAC8.[35] In general, HDAC8 exists as a dimer of two molecules interacting head to head and each monomer binds with one Zn^{2+} and two K^+ ions.[35] The presence of K^+ in the active site can affect the catalytic mechanism of the enzyme's deacetylation process.[35] K^+ can stabilize the transition state oxyanion and/ or the negatively charged acetate product. Also, the presence of K^+ ion may deliver the conformation required for catalytic activity.[35] Effective zinc binding of the large zinc interacting group, a 2-aminobenzanilide of **MS-275** (Figure 16), is hindered because of the presence of Trp^{141} at the bottom of the active site and leads to lack of inhibition of HDAC8 by this compound as compared to HDAC1 and -3 which have Leu at 141 position (Figure 17).[35]

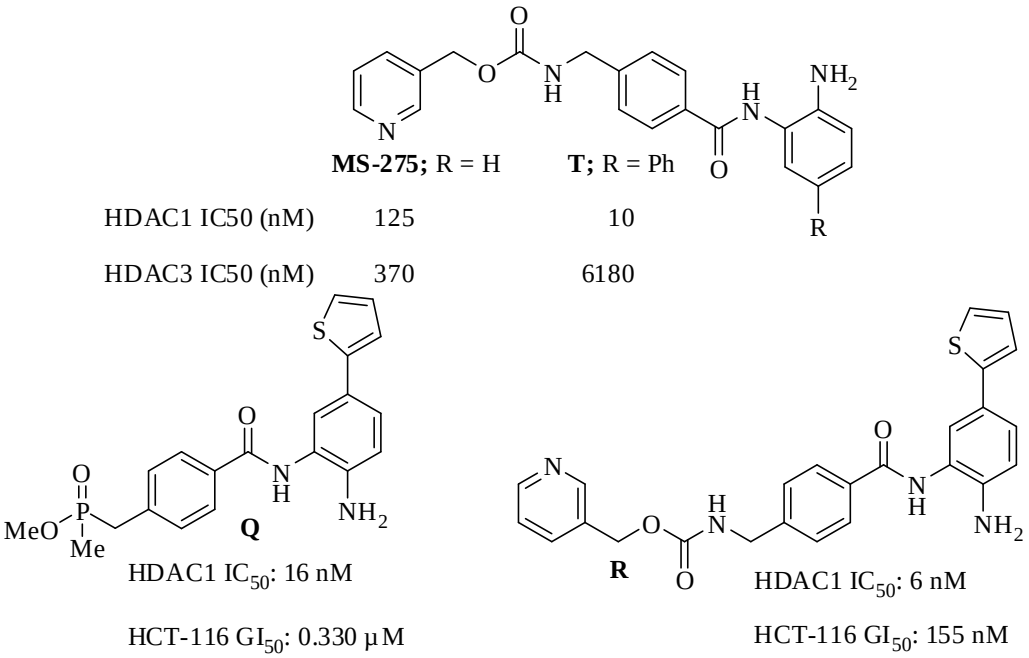


Figure 16. Representative examples of selective HDAC1 and -2 inhibitors.

The presence of an aryl moiety para to amino group in aminobenzamide HDACis increases selectivity toward HDAC1 and -2 (Figure 16). This group is accommodated by HDAC1 and -2 and not by other HDAC isoforms. This structural information was utilized by Miller et al of Merck research laboratories to develop several classes of selective HDAC1 and -2 inhibitors (Figure 16: compound **Q**, **R**).[26]

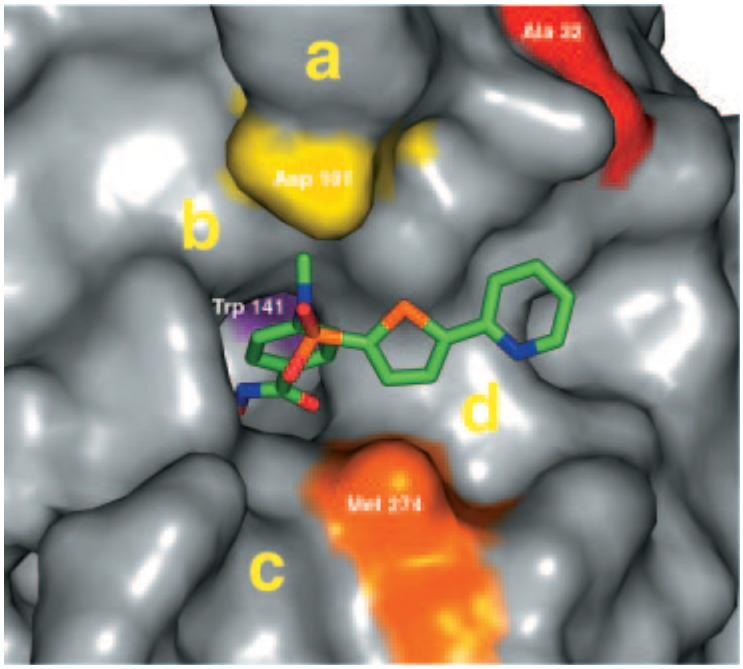


Figure 17. Zinc binding site of HDAC8 showing the presence of Trp^{141} at the bottom of the site. Reprinted with permission from *Proc. Natl. Acad. Sci. U. S. A.* **2004,** *101* (42), 15064-15069. "Copyright 2004 National Academy of Sciences, U.S.A."

α-Mercaptoacetamide compounds showed preferential binding to HDAC6 over HDAC1, -2, -8 and -10. These compounds showed better neuroprotection.[27] As HDAC1 is required for neuronal cell survival, selectivity of α-mercaptoacetamide compounds for HDAC6 over HDAC1 might be responsible for observed neuroprotection.[27]

It has been suggested that HDACis arrest cell cycle at both the G1/S and G2/M phases.[36] As HDAC acts on histone as well as multiple non-histone proteins, many mechanistic pathways have been suggested to include all possible events.[4] Acetylated histones were the earliest substrates identified for HDACs. But a large number of other nuclear and cytoplasmic proteins regulating different biological processes, whose activities are modified through acetylation/ deacetylation, have been identified. Thus, this

posttranslational modification involving acetylation is emerging as a major mechanism of regulating protein and cell function.[36]

Proliferation arrest and TRAIL (Tumor Necrosis Factor Related Apoptosis Inducing Ligand) mediated apoptosis are other outcomes of HDAC inhibition. HDACis induce p21 as well as TRAIL expression and thus cause tumor-selective death signaling in acute myeloid leukemia (AML) cells and the blasts of individuals with AML. RNA interference studies have shown that induction of p21, TRAIL and differentiation are separate activities of HDACis.[4] It has been observed that HDACis which act on HDAC1 and -2 cause TRAIL sensitization. These TRAIL proteins activate TRAIL receptors TRAIL-R1 and TRAIL-R2 which commence activation of intrinsic and extrinsic death signaling cascades ultimately resulting in apoptosis.[17, 37] It has been suggested that HDAC inhibitors are responsible for an increase in reactive oxygen species (ROS) in cancer cells preferentially over normal cells.[4] Reversible inhibition by HDAC inhibitors provide time for the normal cells to recover from the effect of the inhibitor, and thus survive in contrast to cancer cells. This could be another reason for selective inhibition of growth of cancer cells over normal cells.[20] Other proposed mechanisms include transformed cell cycle arrest, terminal cell differentiation, cell death by induction of intrinsic apoptotic pathway, activation of extrinsic apoptotic pathway, mitotic failure, autophasic cell death, polyploidy and senescence.[20]

The effect of HDACis is not limited to cancer cells; they have also shown promising effects in a range of other disease states such as neurological disorders, arthritis,

inflammation, infectious diseases (such as HIV infections, malaria, fungal infections), sickle cell anemia and cardiovascular diseases.[38] Toxicities associated with clinically tested HDAC inhibitors include fatigue, vomiting, hypokalemia, diarrhea, and thrombocytopenia.[5] Cardiac irregularities are also reported with many HDAC inhibitors.[5] HDAC isoform and/or class-selective inhibitors are likely to reduce side-effects and to have increased potency.

1.3 Innovation (Drug Design and Rationale)

In order to develop largazole analogs as potent isoform / class selective anticancer agents, it was decided to:

1. Synthesize several largazole analogs of varying structures to determine the structural elements required for improved activity with low toxicity. The designed analogs incorporated changes in the surface recognition group as well as in the zinc binding domain of largazole.
2. Determine HDAC inhibitory activity and HDAC isoform selectivity of these synthesized analogs. This will also help to understand the role of individual HDAC isoforms in certain disease conditions.

Less sequence homology between isoforms at the surface near the opening to the binding pocket may be exploited to design isoform selective inhibitors. The area of the HDAC enzyme bordering the binding pocket consists of many grooves which could be varyingly occupied by multiple cap groups to impact selectivity as well as potency.[17] modifications of the zinc binding group and cap group has been extensively used to modulate selectivity of HDACi.[11, 17] In order to increase isoform and/ or class selectivity for HDAC inhibitory activity, we propose to synthesize a number of largazole analogs **T1- T7** (Figure 18) with modifications in the zinc binding group and cap group and to evaluate their HDAC inhibitory activity and selectivity profile. These compounds may help to define the role and possible pharmacological significance of isoform/class specific HDAC inhibitors in cancer treatment.[21, 39]

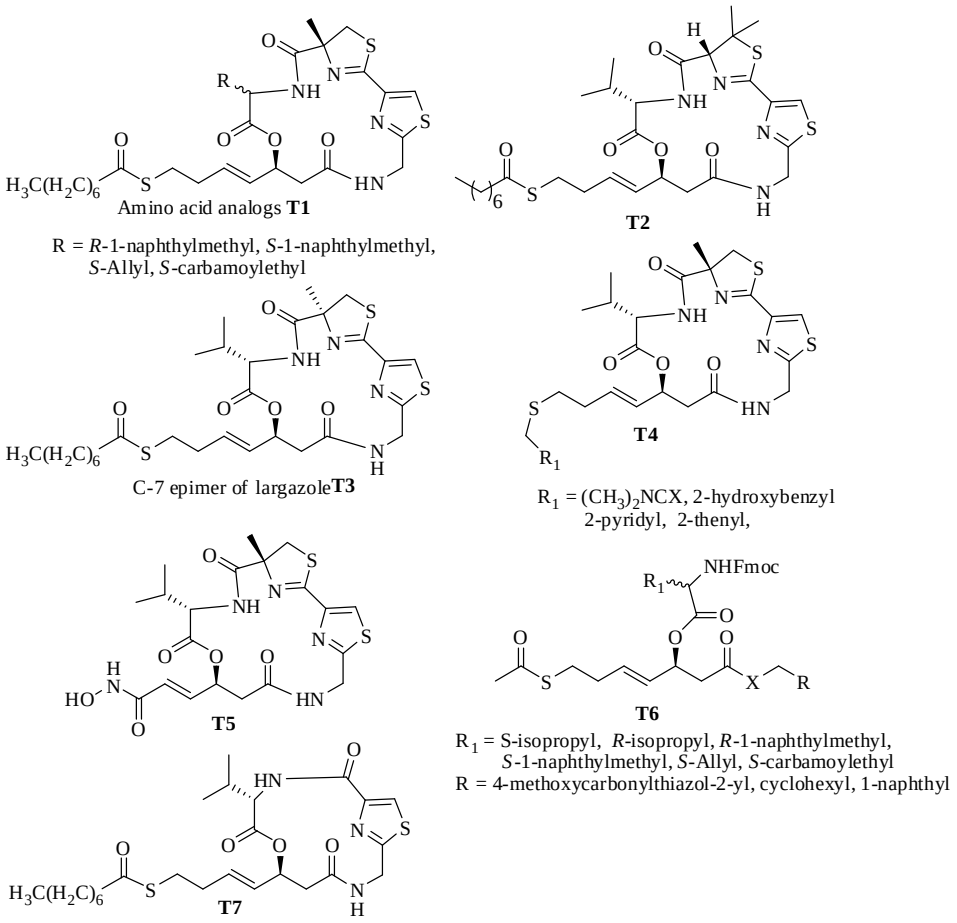


Figure 18. HDAC inhibitors designed for synthesis

It has been shown that the replacement of L-valine (circled in structure of FK228 and largazole in Figure 5) with L-naphthylalanine and L-phenylalanine in FK228 resulted in enhancement of antitumor activity.[25l, 40] Interaction of the hydrophobic side chain of naphthylalanine with the hydrophobic elements of HDAC active site and changes in hydrogen bonding interactions are likely to be responsible for this enhanced activity profile. In order to determine the effect of such structural change in largazole, we designed several amino acid analogs of **T1** series bearing hydrophobic as well as hydrophilic side chains such as *R*-1-naphthylmethyl, *S*-1-naphthylmethyl, *S*-Allyl, *S*-carbamoylethyl.

Analog **T2** with a substituted thiazoline ring is designed to determine the effect of increasing the steric bulk at C-8 on activity and selectivity. Also it will determine if (*R*)-α-methyl cysteine HCl which is expensive and not readily available can be replaced with inexpensive and commercially available L-penicillamine.

Analog **T3** is the C-7 epimer of largazole for structure activity relationship studies. Changing configuration at C-7 changes the shape of the depsipeptide ring and will provide additional information about the SAR requirements of largazole.

Analogs **T4** are designed such that sulphur and a second heteroatom present in the side-chain are 2/3 atoms away from each other can form a cyclic 5/6 membered transition state with Zn^{2+} of HDAC enzyme (Figure 19).[41] As Zn (II) is a soft metal ion which can show different coordination numbers such as tetra-, penta and hexa-coordination,[42] binding of two heteroatoms to the Zn^{2+} cation can increase its affinity for the enzyme and may lead to enhancements in activity.

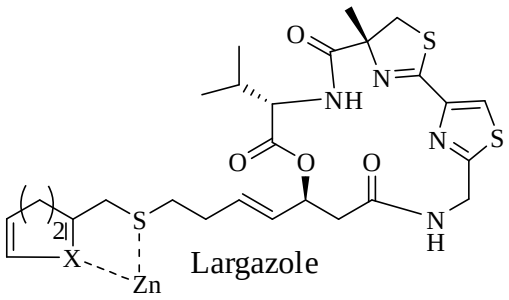


Figure 19. Proposed binding of T4 series analogs with HDAC active site.

The clinically approved HDAC inhibitor SAHA has a hydroxamic acid moiety as the zinc binding group. Although hydroxamic acid is rapidly hydrolyzed to the inactive carboxylic acid and is toxic, it is a widely studied zinc binding group because it is one of the most potent zinc binding agents and also it can form hydrogen bonds with various amino acids present in the enzyme.[22] Considering one order magnitude higher binding aptitude of hydroxamic acid over thiol,[42] we propose to replace the thiol group of largazole with a hydroxamic acid moiety to generate analog **T5**.

Molecules shown in Figure 19 are highly potent and selective HDAC6 inhibitors.[18b] Unique structural features of HDAC6 include a C-terminal zinc finger moiety and two catalytic domains. HDAC6 is also responsible for deacetylation of non-histone proteins such as α-tubulin and HSP-90. It is a much larger protein which does not complex with other isoforms and is widely present in the body.[17] It has been reported that HDAC6 inhibitors are Y-shaped molecules.[22] Molecules shown in Figure 20 are highly potent and selective HDAC6 inhibitors.[18b] Target molecules **T6** are designed to meet these structural requirements. It has been suggested that Boc cap group present in these molecules has

some favorable interactions with the HDAC6 enzyme.[17] Retaining the Fmoc group in the designed analogs **T6** will mimic steric hydrophobic bulk of Boc group to preserve its complimentary interaction with the HDAC6 surface.[17] They will be synthesized and tested against HDACs for HDAC6 isoform selectivity. HDAC6 inhibitors can be used in the treatment of multiple myeloma and neurodegenerative Huntington's disease.[17-19]

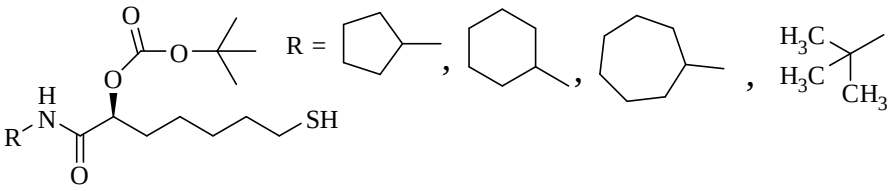


Figure 20. Selective HDAC6 inhibitors.

Analog **T7** is a smaller ring version of largazole designed to explore if the thiazoline ring is required for activity.

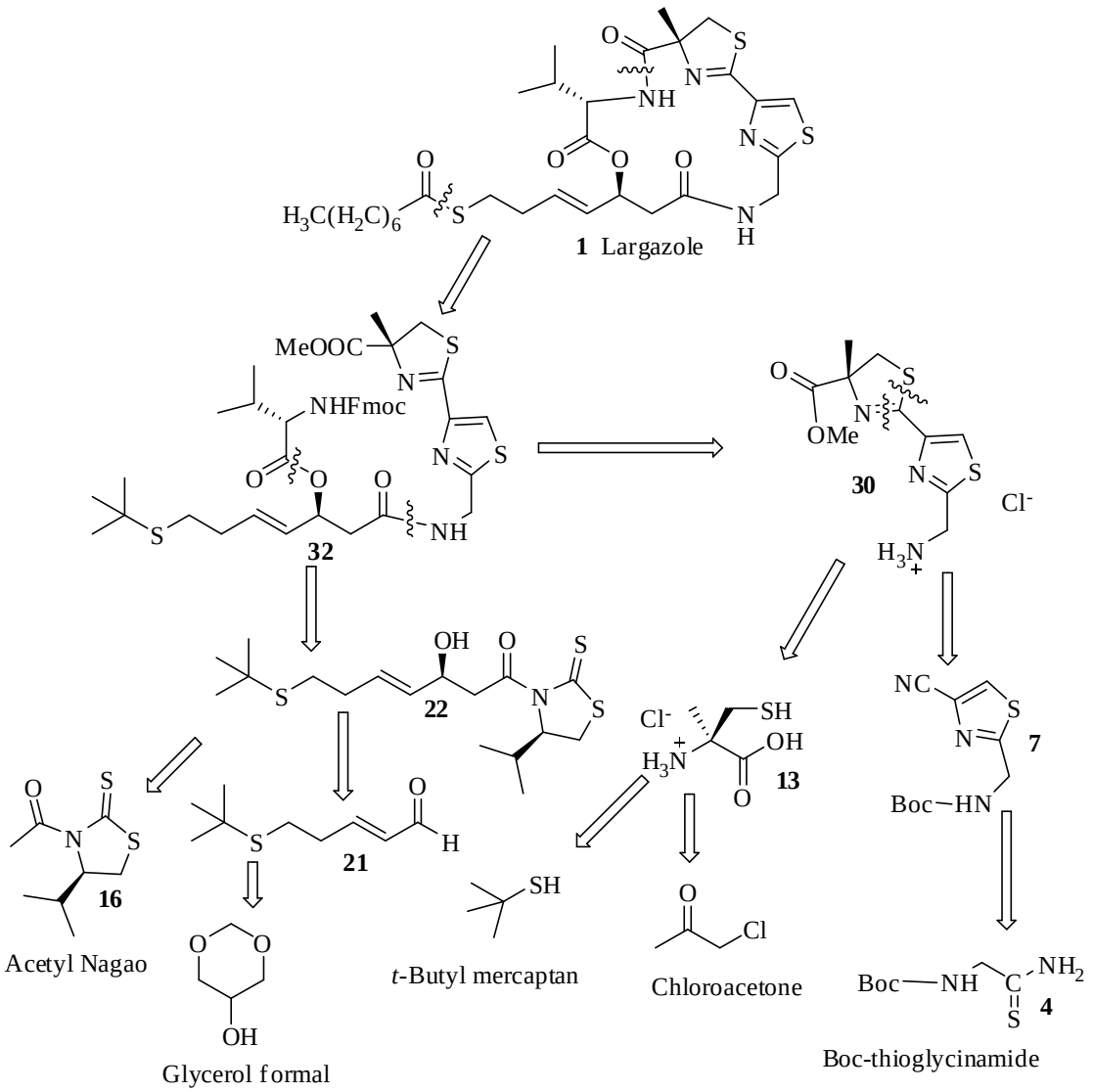
The synthesized compounds will be tested to determine GI_{50} values by antiproliferative assay with cancer cell lines, HDAC inhibitory activity, effect on HDAC6 and HDAC isoform selectivity. Most of the biological testing would be carried out in the laboratory of Dr. Robert A. Casero, Professor of Oncology, the Sidney Kimmel Comprehensive Cancer Center, the Johns Hopkins University, School of Medicine, Baltimore.

Chapter 2

2 Results and Discussions

2.1 Synthesis of largazole

The convergent synthetic approach adopted for synthesis of largazole is shown in retrosynthetic analysis (Scheme 1). It involves macrolactamization and side chain thioesterification of the intermediate **32** which could be derived by acyl transfer from fragment **22** to **30** followed by coupling with Fmoc-valine. Intermediate **30** can be synthesized from the thiazole nitrile **7** and (*R*)-α-methylcysteine hydrochloride **13**. Synthesis of **13** would also generate its enantiomer useful for making the C-7 epimer of largazole analogs. Fragment **22** is the product of acetate aldol reaction of the aldehyde **21** using acetyl Nagao **16** as the chiral auxiliary.[43]

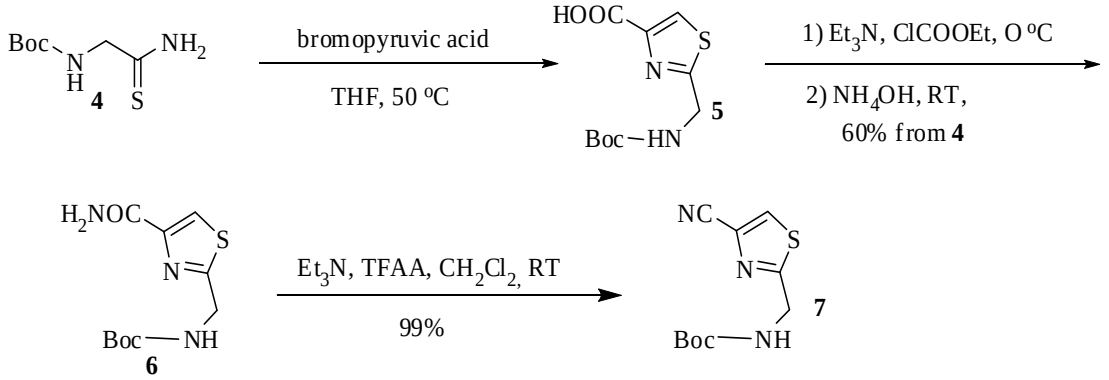


Scheme 1. Retrosynthetic analysis of largazole

2.1.1 Synthesis of nitrile 7

The synthesis of thiazole derivative **7** is shown in Scheme 2. Thiazole amide **6** was made in 60% overall yield from commercially available Boc-thioglycinamide and bromopyruvic acid in a one-pot reaction in which the initially formed carboxylic acid, after removal of water, was activated and treated with ammonia. This one pot protocol was found to be more efficient and gave the product in higher yield and in a much shorter

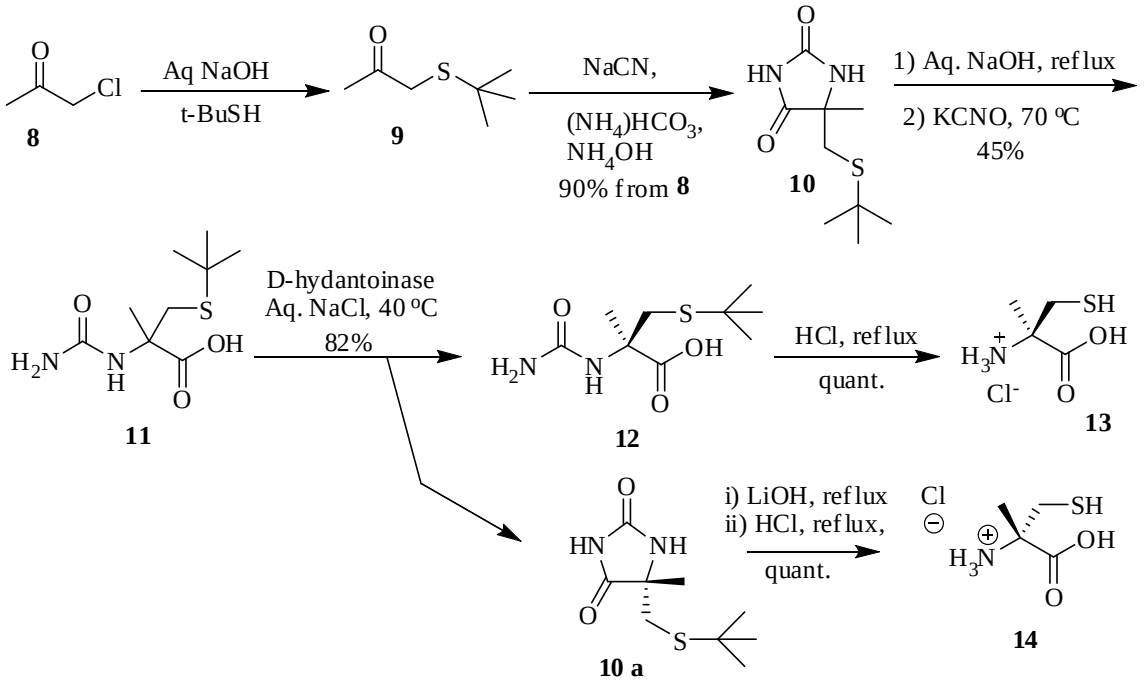
period of time than the previously reported methods.[25a] Amide **6** was converted to the nitrile **7** using standard conditions in 99% yield.[25a] We improved our own protocol by performing all reactions from Boc-thioglycinamide **4** to nitrile **7** in one pot with improved yield of 65% as compared to previous 59% overall yield. It also saved time and solvents required in the tedious purification of amide **6**.



Scheme 2. Synthesis of fragment **7**

2.1.2 Synthesis of (*R*)- and (*S*)-α-methylcysteine

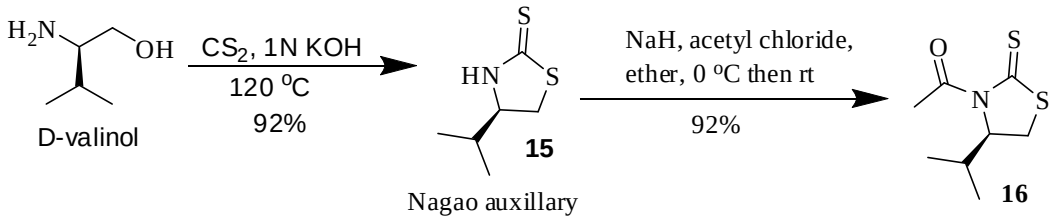
The synthesis of (*R*)-α-methylcysteine HCl **13** and its (S)-enantiomer **14** was achieved via the enzymatic resolution of racemic precursor **11** (Scheme 3).[44] Reaction of chloroacetone and *t*-butyl mercaptan under basic conditions gave **9**, which was subjected to Bucherer-Berg conditions to produce hydantoin **10**. This one-pot protocol gave 90% yield of **10** after two steps. Hydantoin **10** was converted to **11** in one-pot by hydrolysis with aqueous NaOH followed by KCNO treatment. Resolution of **11** with D-hydantoinase afforded **12** and the hydantoin enanantiomer **10a** which were converted to **13** and **14,** respectively, in quantitative yields.[44]



Scheme 3. Synthesis of (*R*)- and (*S*)-α-methylcysteine

2.1.3 Synthesis of acetyl Nagao auxillary (16)

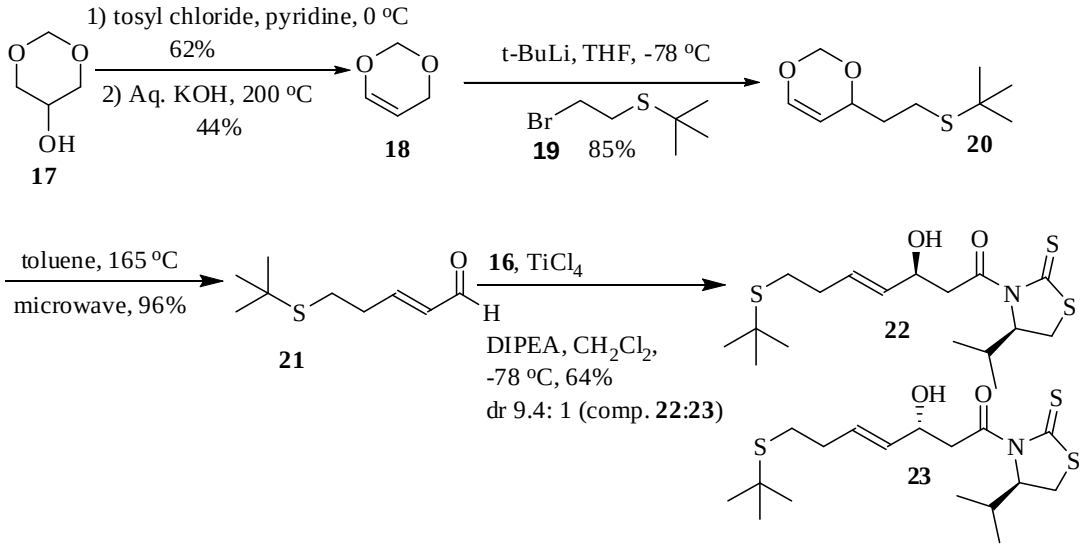
Nagao Auxillary was synthesized by refluxing D-valinol with CS_2 in 1 N KOH (Scheme 4).[45] Acetyl Nagao **16** was synthesized by reacting Nagao auxillary with NaH and acetyl chloride in 92% yield.



Scheme 4. Synthesis of acetyl Nagao auxillary (**16**)

2.1.4 Synthesis of the *t*-butyl protected β-hydroxyamide 22

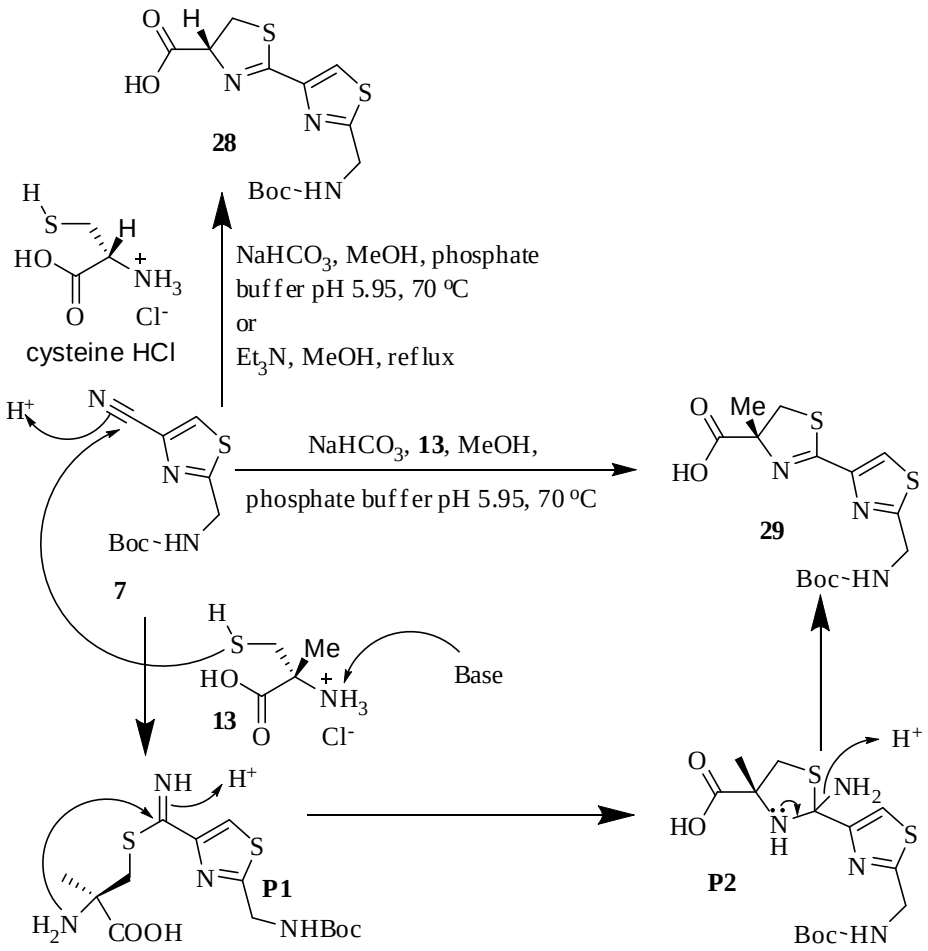
Turning our attention to the synthesis of **22** (Scheme 5), commercially available glycerol formal **17** was converted to dioxene **18** in two steps. Glycerol formal was converted to the tosylate which was heated at 200 ^{o}C in the presence of KOH to generate the dioxene **18**. Alkylation of **18** with 2-[(2-bromoethyl)sulfanyl]-2-methylpropane (**19**) via the lithium derivative gave **20**. Compound **20** underwent retro Diels-Alder reaction to give the aldehyde **21**[46] which was used in acetate aldol reaction to furnish the alcohol **22** in 81% diastereomeric excess. The two diastereomers were purified by flash column chromatography on silica gel in ethyl acetate: hexanes (10-30%). The *S*-configuration of the newly created chiral center of molecule **22** was established by modified Mosher ester analysis.[47]



Scheme 5. Synthesis of the *t*-butyl protected β-hydroxycarboxamide **22**

2.1.5 Attempts for synthesis of carboxylic acid 29

The thiazole nitrile **7** was reacted with (*R*)-α-methyl cysteine HCl **13** to synthesize the thiazole-thiazoline derivative **29** as shown in Scheme 6 (vide infra).[25a, 25c] Compound **7** consists of cyanide which acts as an electrophile and reacts with the nucleophilic thiol to give intermediate **P1**. Another nucleophilic attack by amine leads to formation of intermediate **P2**. Lone pair electron assistance for displacement of ammonia leads to the formation of product **29**. This reaction was initially performed using L-cysteine HCl instead of (*R*)-α-methyl cysteine HCl **13** in a model reaction to obtain product **28**. As the reaction sequence requires conversion of the carboxylic acid **29** to its methyl ester, we attempted another model reaction (not shown) using the methyl ester of L-cysteine to see if the formation of the ester can be accomplished in one step. However, when ethyl or methyl ester of cysteine HCl was used under similar conditions, reaction was very sluggish even after 3 days of refluxing conditions with Et_3N in MeOH or stirring at 70^o C with $NaHCO_3$ in MeOH and phosphate buffer pH 5.95 for 3 days.

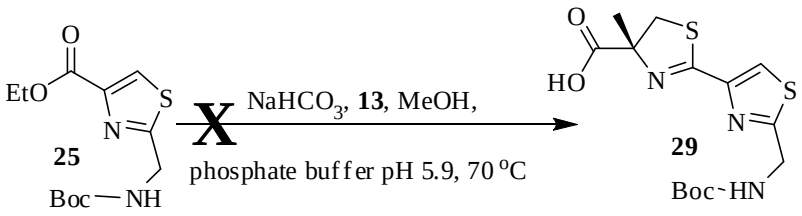


Scheme 6. Mechanism for conversion of nitrile **7** to thiazole thiazoline derivative **29**

A number of alternative methods were attempted by different routes before settling to the synthesis of **29** from compound **7** with $NaHCO_3$, MeOH and phosphate buffer pH 5.95 as in Scheme 6. Following paragraphs discuss each of the failed attempts.

Attempt 1

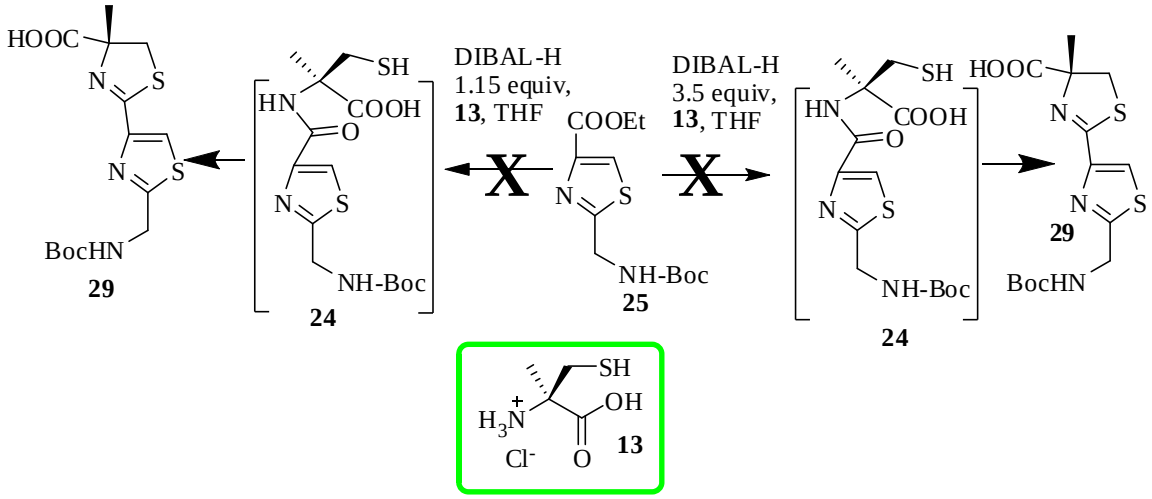
We initially used the more easily obtained ester **25** as the electrophile in expectation to obtain the same product **29**. Unfortunately reaction of ester **25** with compound **13** under similar conditions did not produce **29** (Scheme 7).



Scheme 7. Failed attempt 1 for synthesis of **29**

Attempt 2

Conversion of esters to amides by DIBAL-H is known.[48] If compound **25** is converted to intermediate **24** by DIBAL-H in the presence of **13**, the intermediate **24** may undergo cyclization to produce compound **29** (Scheme 8). However when the ester **25** and **13** were treated with DIBAL-H (1.15 equiv), there was no disappearance of starting material. Increasing the DIBAL-H equivalence to 3.5 did not change reaction outcome, most probably due to the highly sterically hindered environment of the ester group or due to the presence of hetero atoms in compound **25** which can chelate with DIBAL-H.

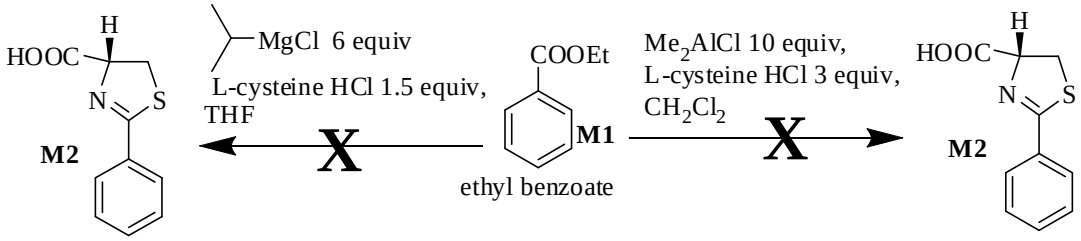


Scheme 8. Failed attempt 2 for synthesis of **29**

Subsequent attempts were made using model reactions.

Attempt 3

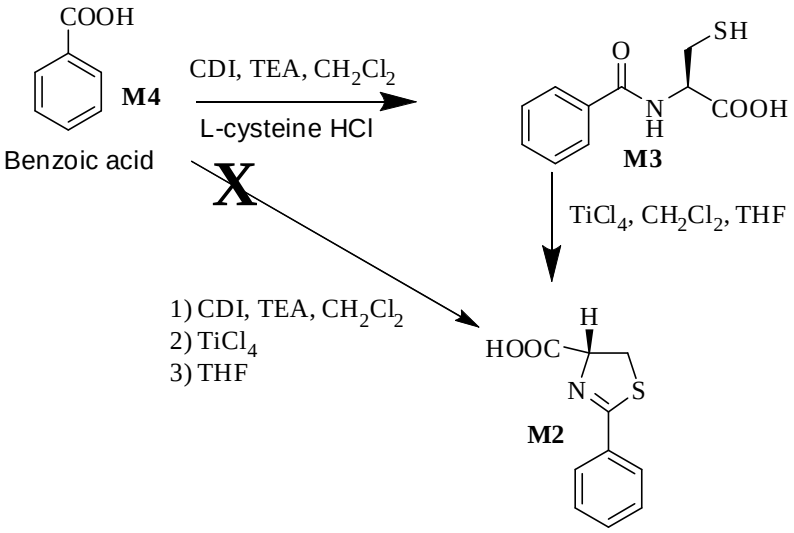
Formation of amides from esters and amines using isopropyl magnesium chloride as Lewis acid is known.[49] However, reaction of ethyl benzoate **M1** and L- cysteine HCl in the presence of different Lewis acids including isopropyl magnesium chloride failed to give product **M2** (Scheme 9). Increasing the amount of Lewis acid had no effect on the outcome of the reaction.



Scheme 9. Failed Attempt 3 (model reaction 1)

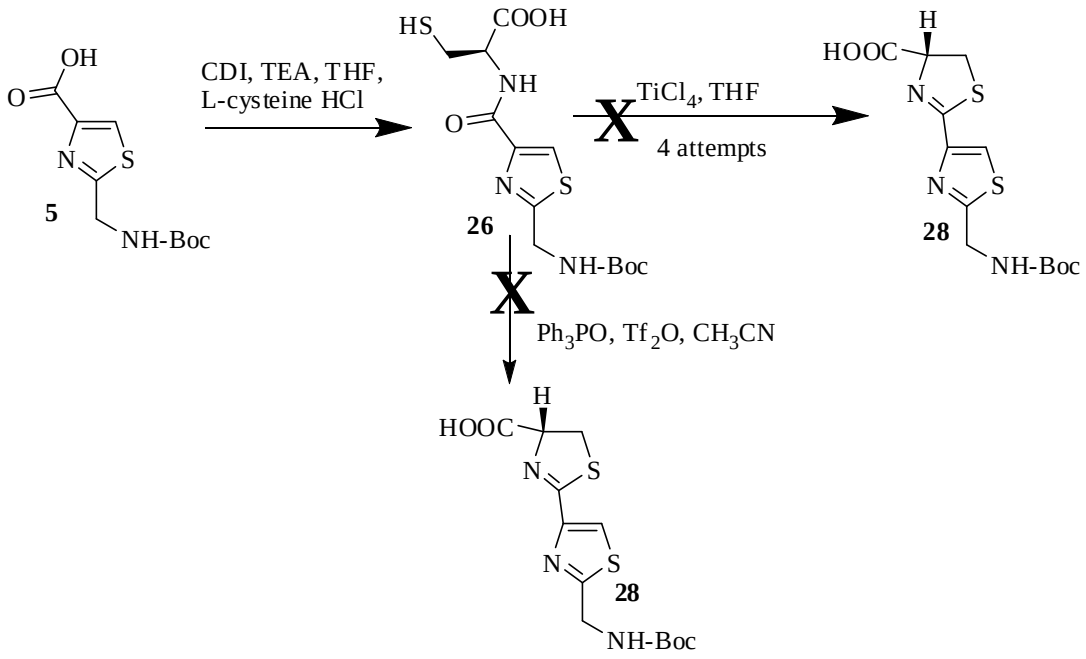
Attempt 4

In the next model reactions, amide coupling of benzoic acid **M4** with L-cysteine HCl in the presence of CDI and triethylamine yielded compound **M3** (Scheme 10). Although **M3** was effectively cyclized by $TiCl_4$ to **M2**, one pot-direct conversion of **M4** to **M2** was not successful.



Scheme 10. Reaction of benzoic acid with L-cysteine HCl

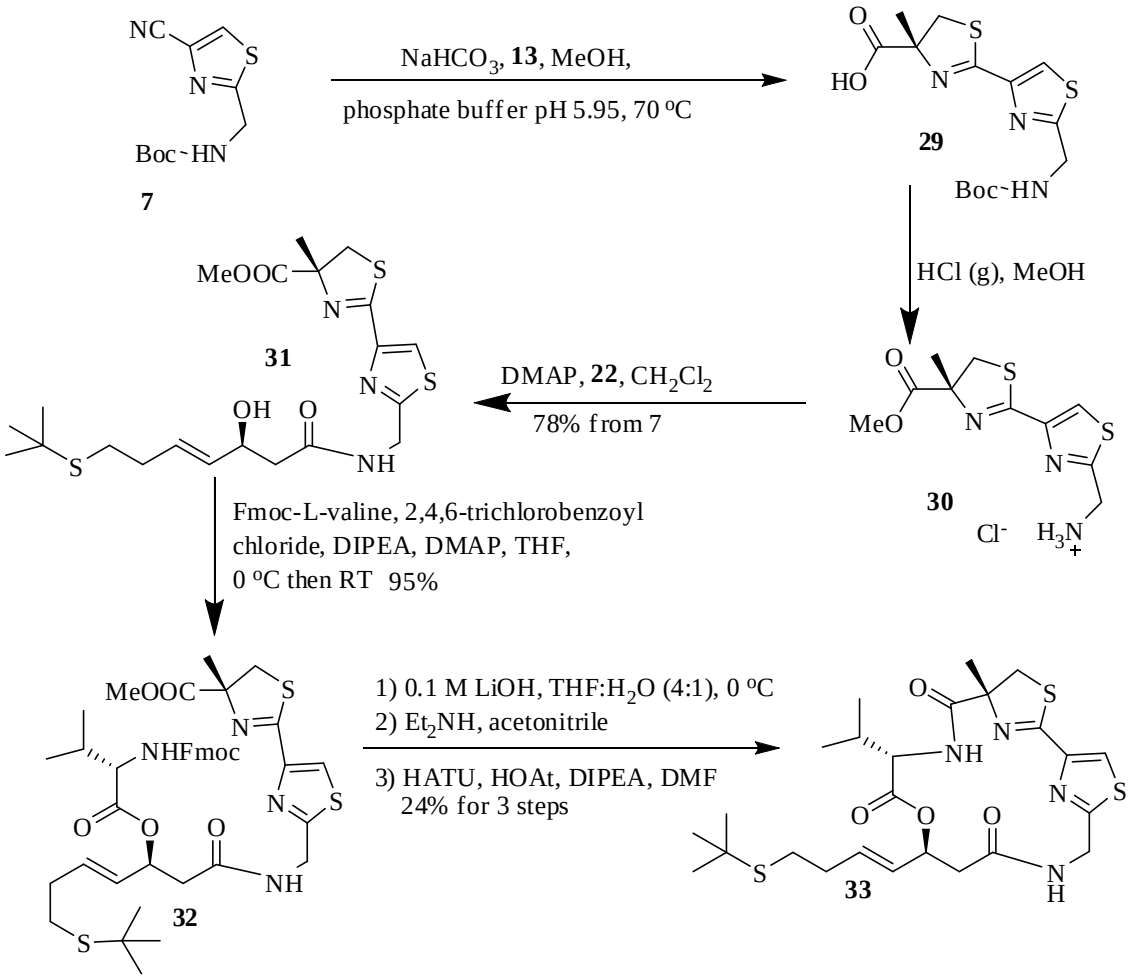
The reaction was repeated with compound **5** in place of benzoic acid. Compound **5** was converted to amide **26** by reacting it with L-cysteine HCl, CDI and triethylamine (Scheme 11). However, $TiCl_4$ mediated cyclization of intermediates **26** did not produce **28**. Attempted cyclization by an alternative reported method[50] with Ph_3PO and Tf_2O was also unsuccessful.



Scheme 11. Failed attempt 5 (model reaction 2)

2.1.6 Synthesis of cyclic core 33

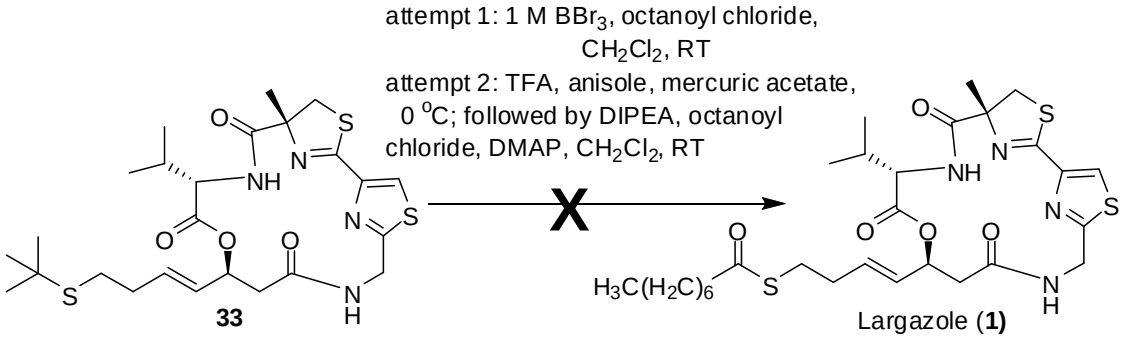
With the necessary building blocks in hand for largazole synthesis, the assembly of cyclic core **33** was undertaken as shown in Scheme 12. The nitrile **7** was condensed with (*R*)-α-methylcysteine HCl **13** to obtain the thiazole-thiazoline carboxylic acid **29**.[25a, 25c] The crude product **29,** after simultaneous removal of Boc group and esterification of the free carboxylic acid under acidic conditions in MeOH, gave **30.** Acyl group was transferred[51] from **22** to **30** to obtain alcohol **31** in the presence of DMAP in dichloromethane. The formation of **29** from nitrile **7** and the one pot conversion of **29** to alcohol **31** were carried out efficiently with 78% yield for 3 steps. Yamaguchi esterification[3a, 52] was used to couple Fmoc-L-valine to **31** to afford the acyclic precursor **32** in 95% yield. After saponification and Fmoc group removal, macrocyclization with HOAt, HATU, and Hunig's base yielded the cyclized product **33** in 24% overall yield over the 3 steps.



Scheme 12. Synthesis of cyclic core **33**

2.1.7 Failed attempts for conversion of cyclic core 33 to largazole

Having synthesized the advanced intermediate **33**, what remained to be done to prepare largazole was to remove the *t*-butyl protecting group and to convert the resulting thiol to the octanoate ester. Unfortunately, all efforts to remove the *t*-butyl group as illustrated in Scheme 13 (with 1 M BBr_3;[53] trifluoroacetic acid, anisole, mercuric acetate[54]) and esterification with octanoyl chloride to form largazole failed. With BBr_3, mostly starting material was recovered. Treatment with TFA/anisole/mercuric acetate indicated the formation of a polar product (TLC) which was subjected to thioesterification. However no largazole, largazole thiol or starting material was recovered.

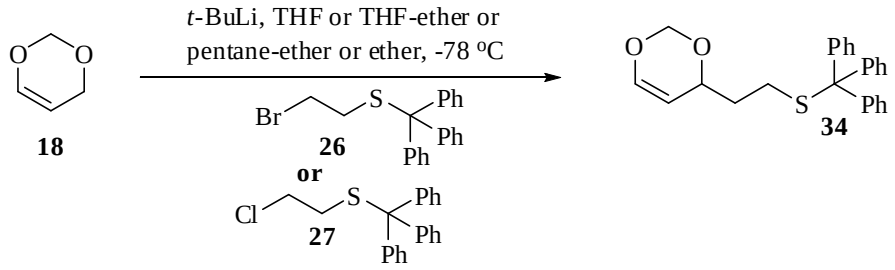


Scheme 13. Attempted conversion of **33** to largazole

2.1.8 Alternative Strategy to largazole with trityl as thiol protecting group

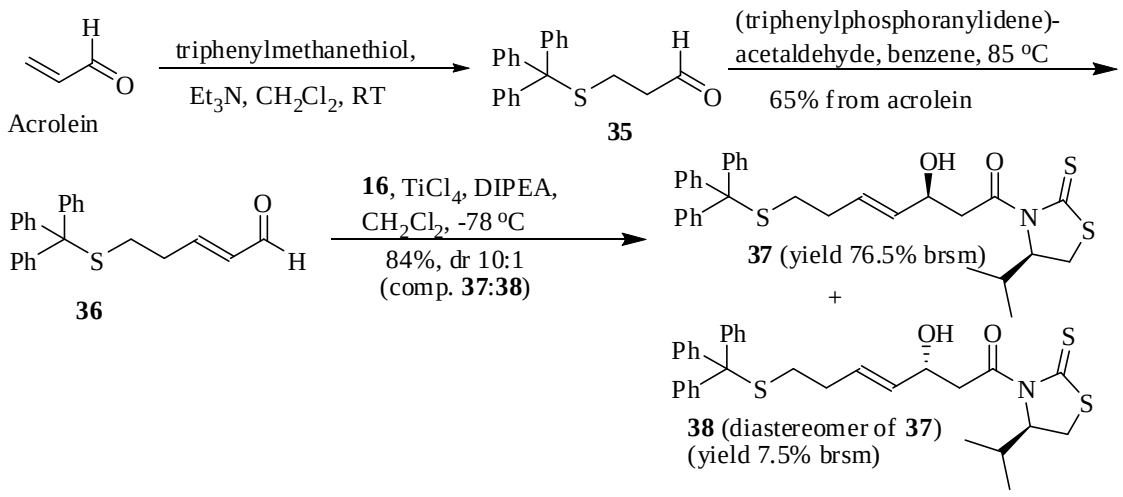
After unsuccessful attempts to remove *t*-butyl protecting group and to convert the resulting thiol to largazole in the final step, it was decided to change the protecting group from *t*-butyl to trityl group. For this purpose, synthesis of compound **34** was approached in a way similar to the synthesis of **20** (Scheme 14). Dioxene **18** was reacted with *t*-butyl lithium and reaction of the lithiated compound with **26** did not give product **34**. Changing the order of addition i. e. slow addition of a solution of lithiated compound in THF to a

pre-cooled solution of **26** in THF at -78 ^{o}C did not help either. Attempts using the chloro compound **27** instead of the bromo compound **26** or changing reaction solvents too failed to yield **34**. After these unproductive efforts, it was decided to follow Janda's protocol[55] for synthesis of aldehyde **36** (Scheme 15).



Scheme 14. Attempts to synthesis of compound **34**

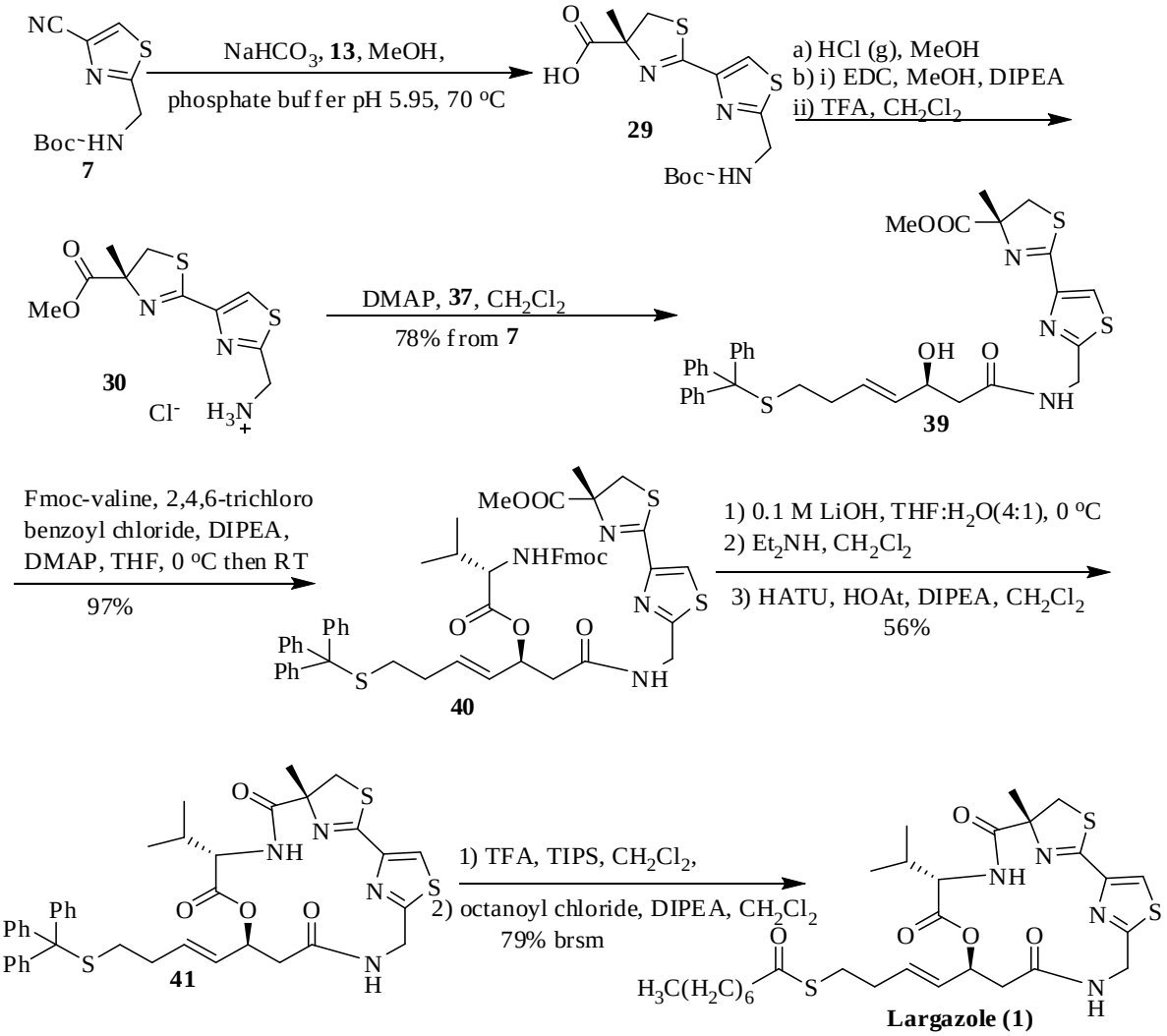
Synthesis of alcohol **37** (Scheme 15) started with conjugate addition of triphenylmethanethiol to acrolein to give **35** which was used in a Wittig reaction with commercially available (triphenylphosphoranylidene)-acetaldehyde to yield aldehyde **36**.[55] This one-pot protocol gave 65% overall yield for the two steps. Acetate aldol condensation of the aldehyde **36** with acetyl Nagao auxiliary (**16**) was used to synthesize alcohol **37** in 82% diastereomeric excess.[43] The two diastereomers were purified by flash column chromatography on silica gel in dichloromethane: hexanes (25-90%). The *S*-configuration of the newly created chiral center of molecule **37** was established by modified Mosher ester analysis.[47]



Scheme 15. Synthesis of the β-hydroxycarboxamide **37**

With the necessary building blocks in hand, the assembly of largazole was undertaken as shown in Scheme 16. The nitrile **7** was condensed with (*R*)-α-methylcysteine HCl **13** to obtain the thiazole-thiazoline carboxylic acid **29**.[25a] After simultaneous removal of Boc group and esterification of the free carboxylic acid under acidic conditions in MeOH, acyl group was transferred[51] from **37** to **30** to obtain alcohol **39**. The formation of **29** from nitrile **7** and the one pot conversion of **29** to alcohol **39** proved very efficient with 78% yield for 3 steps. Yamaguchi esterification[3a, 52] was used to couple Fmoc-valine to alcohol **39** to afford the acyclic precursor **40**. After saponification and Fmoc group removal, macrocyclization with HOAt, HATU, and Hunig's base yielded the cyclized product **41** in 56% overall yield over the 3 steps. Deprotection of trityl group gave largazole thiol which was esterified with octanoyl chloride using Hunig's base to give largazole in 79% yield (based on recovered largazole thiol in esterification step) over two steps.

Final scheme for largazole synthesis



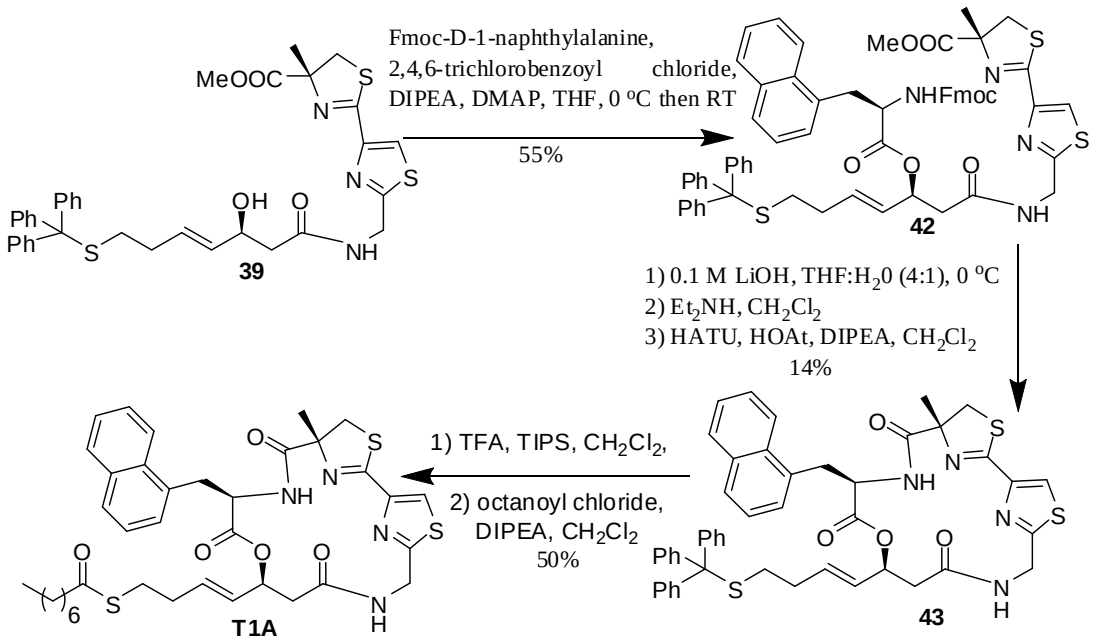
Scheme 16. Synthesis of largazole

2.2 Synthesis of analogs of T1 series

The three analogs of **T1** series were synthesized in a similar manner to largazole except using Fmoc-L-allylglycine, Fmoc-D-1-naphthylalanine and Fmoc-L-1-naphthylalanine instead of Fmoc-L-valine in Yamaguchi condensation (conversion of compound **39** to compound **40** for largazole).

Synthesis of analog T1A

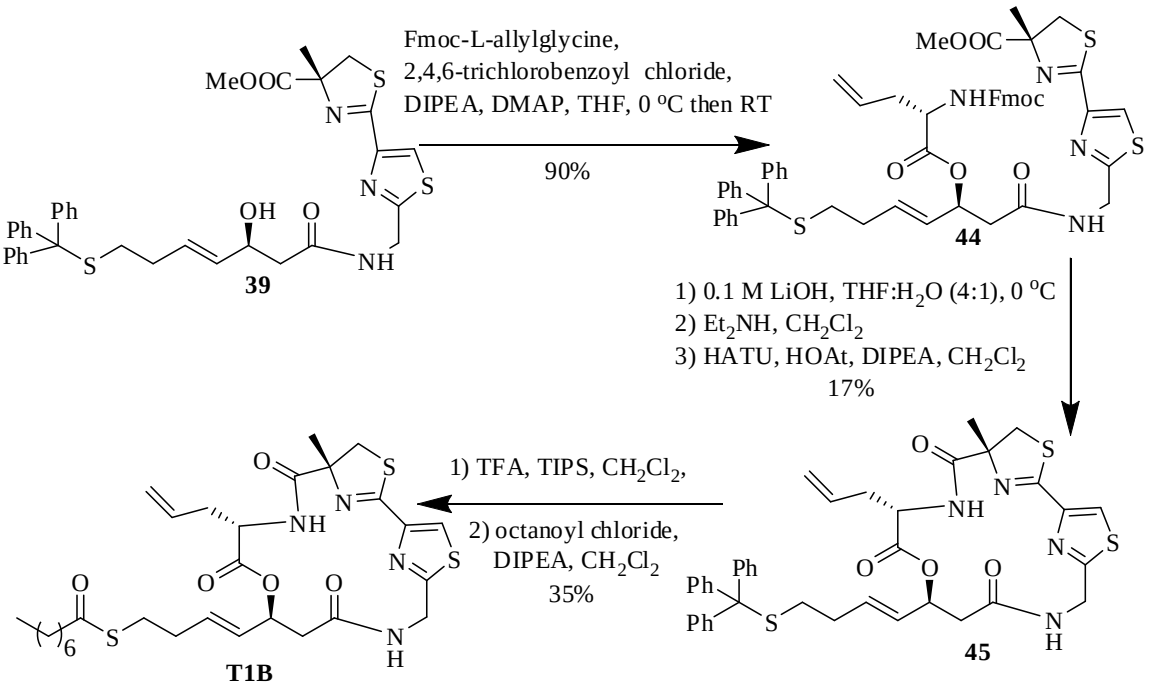
Synthesis of analog **T1A** as shown in Scheme 17 started with the synthesis of compound **42** by Yamaguchi coupling of Fmoc-D-1-naphthylalanine with alcohol **39**. Cyclization of compound **42** after hydrolysis of methyl ester and removal of Fmoc group gave cyclic core **43** in low yield of 14%. Gratifyingly, the final transformation of thioesterification after trityl group deprotection was more successful to yield the analog **T1A** in 50% overall yield for two steps.



Scheme 17. Synthesis of analog **T1A**

Synthesis of analog T1B

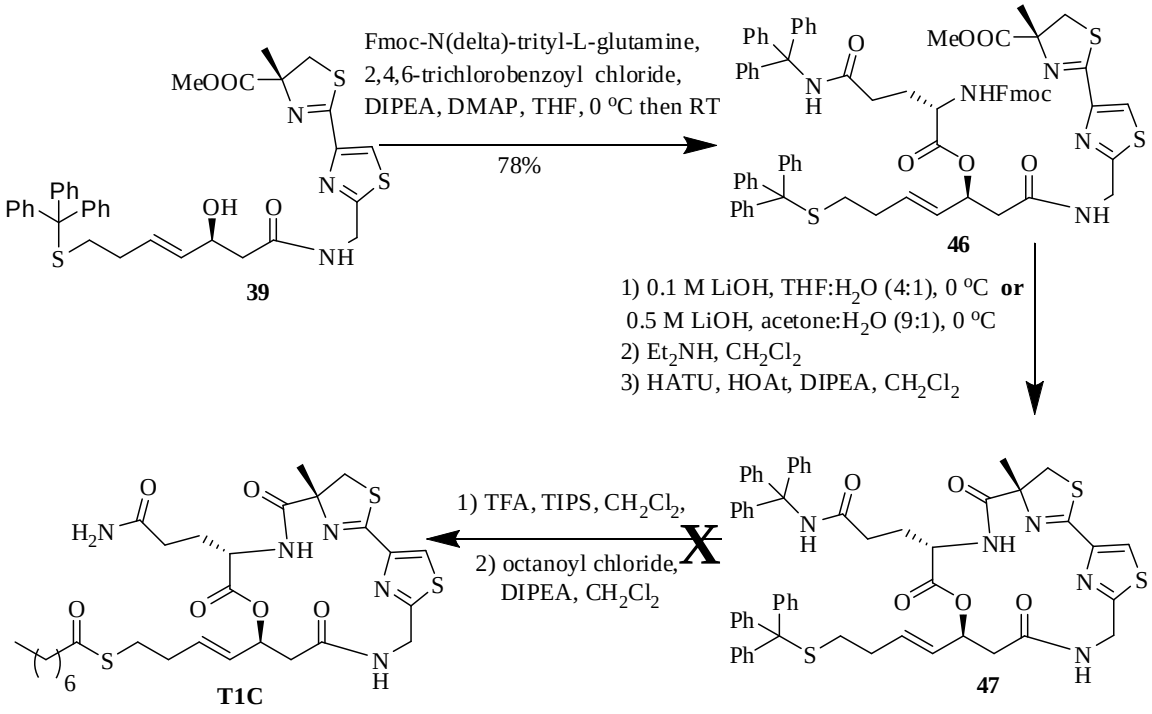
Synthesis of analog **T1B** is shown in Scheme 18. Yamaguchi coupling of alcohol **39** with Fmoc-L-allylglycine produced compound **44**. Macrocyclization of it after removal of protecting groups gave cyclic core **45** which was converted to final analog **T1B** as before in 35% overall yield for the two steps.



Scheme 18. Synthesis of analog **T1B**

Unsuccessful attempt in the synthesis of analog T1C

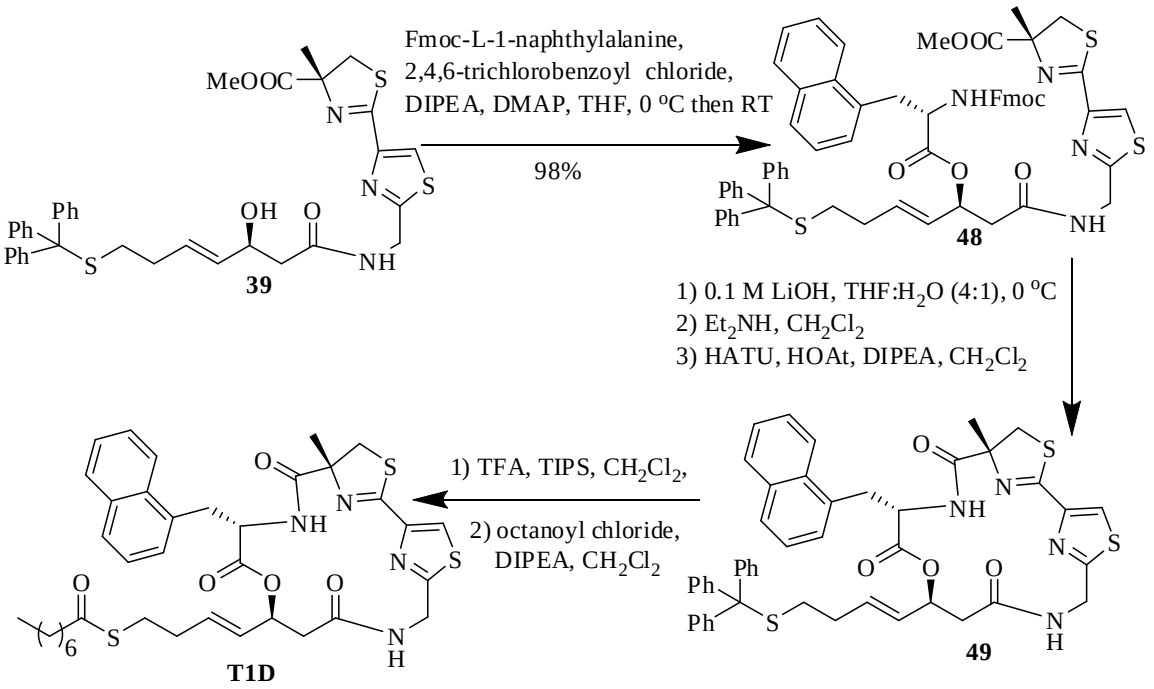
Attempted synthesis of analog **T1C** as shown in Scheme 19 started with the synthesis of compound **46** by Yamaguchi coupling of Fmoc-N^{δ}-trityl-L-glutamine with alcohol **39**. Cyclization of compound **46** after removal of protecting groups gave cyclic core **47** in very low yield. Despite several attempts of purifications, it was not possible to obtain cyclic core **47** in pure form. Therefore, it was decided to carry out next two transformations without further purification. However, thioesterification after trityl group deprotection was unsuccessful and no product of analog **T1C** was obtained.



Scheme 19. Attempted of Synthesis of analog **T1C**

Synthesis of analog T1D

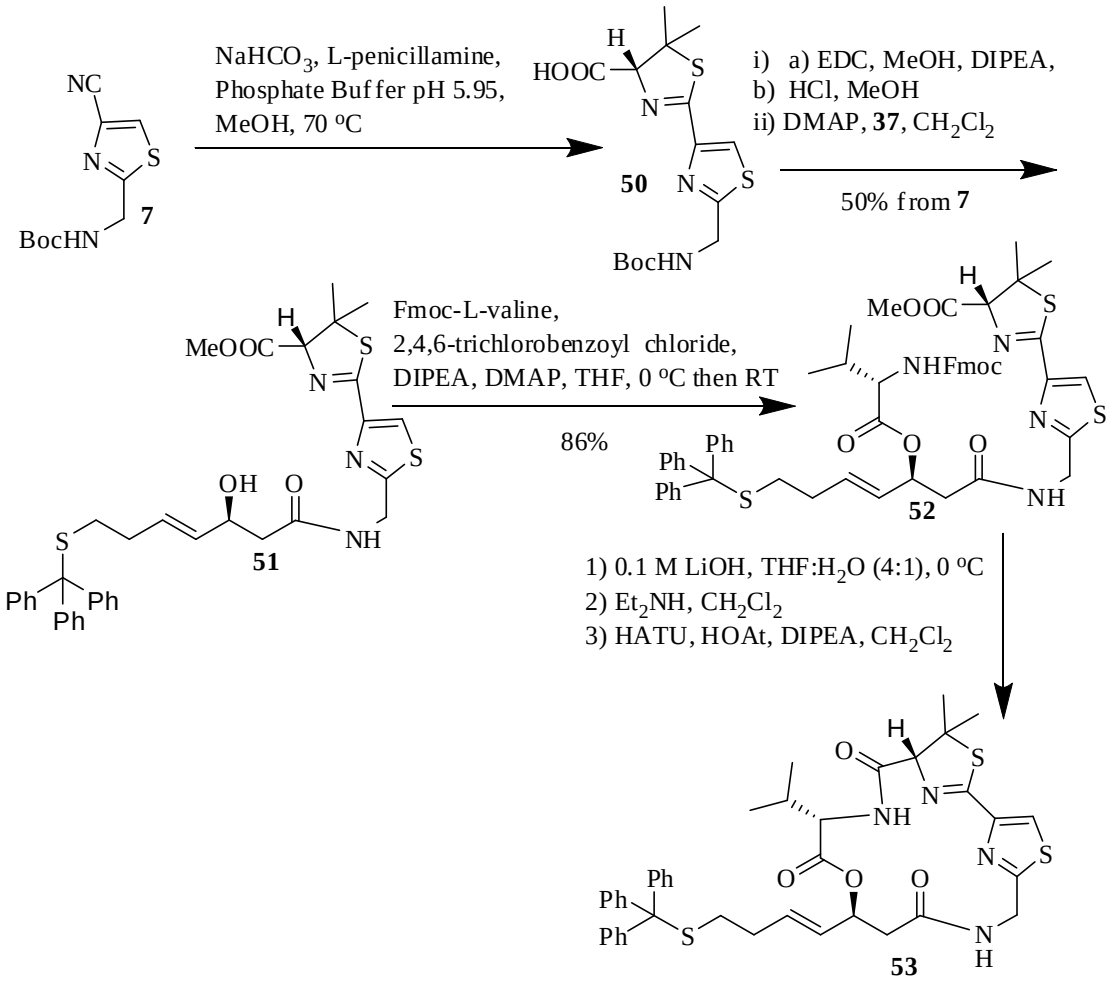
Attempted synthesis of analog **T1D** is shown in Scheme 20. Yamaguchi coupling of alcohol **39** with Fmoc-L-1-naphthylalanine produced compound **48**. Macrocyclization of it following removal of protecting groups gave cyclic core **49** in very low yield. Despite several attempts, it was not possible to obtain pure product of cyclic core **49**. Therefore the last two transformations were carried out without further purification. Thioesterification upon trityl deprotection followed by reverse phase HPLC purification on C18 column gave small amount of the partially purified product **T1D**. The synthesis needs to be repeated to generate sufficient material for biological evaluations.



Scheme 20. Attempt for synthesis of analog **T1D**

2.3 Unsuccessful attempt in the synthesis of analog T2

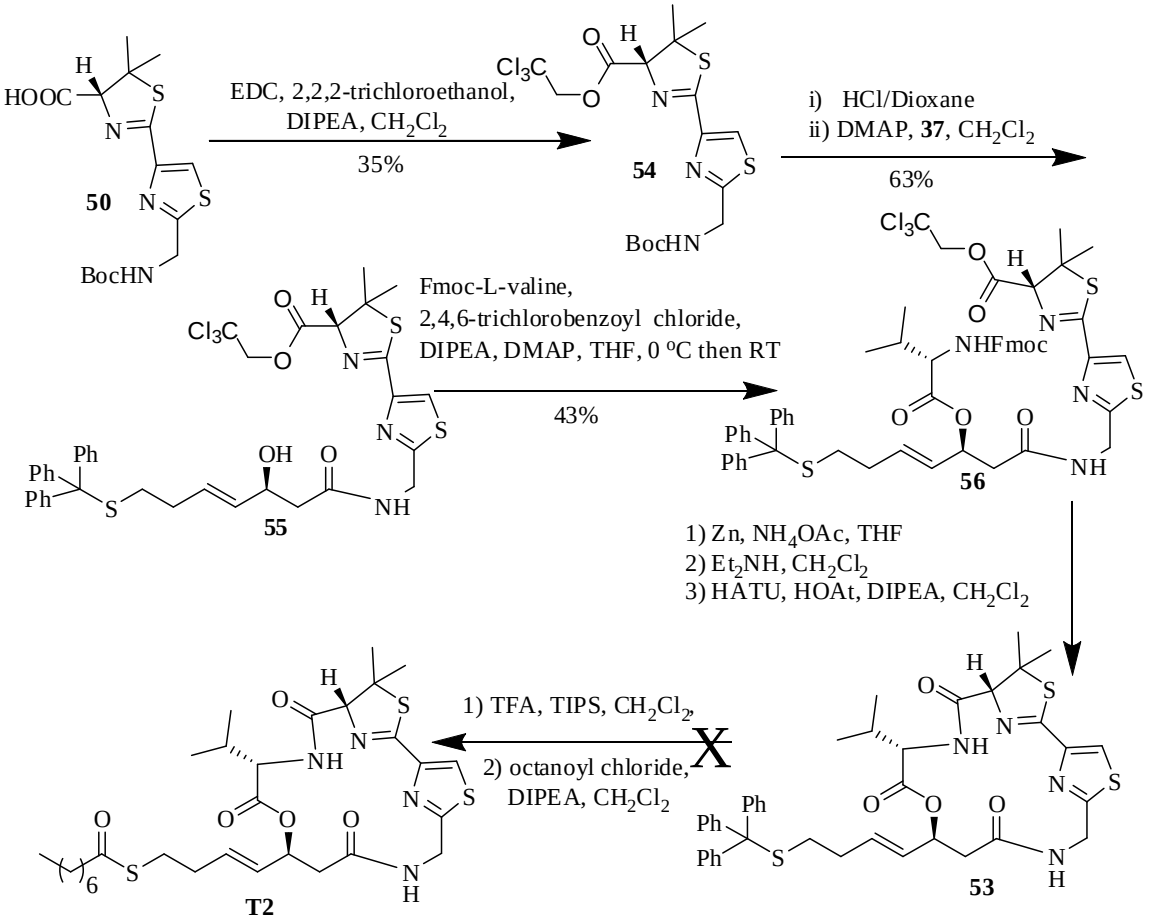
The assembly of analog **T2** was undertaken as shown in Scheme 21. The nitrile **7** was condensed with commercially available L-penicillamine to obtain the thiazole-thiazoline carboxylic acid **50**.[25a] After simultaneous removal of Boc group and esterification of the free carboxylic acid under acidic conditions in MeOH, acyl group was transferred[51] from **37** to obtain **51**. The formation of **50** from nitrile **7** and the one pot conversion of **50** to alcohol **51** proved very efficient with 50% yield for the 3 steps. Yamaguchi esterification[3a, 52] was used to couple Fmoc-valine to alcohol **51** to afford the acyclic precursor **52**. After saponification and Fmoc group removal, macrocyclization with HOAt, HATU, and Hunig's base yielded the cyclized product **53** in low yield over the 3 steps. As before, despite several attempts, pure product of cyclic core **53** could not be obtained. Incomplete saponification of **52** by LiOH in THF:H_2O was responsible for low yield of **53**.



Scheme 21. Synthesis cyclic core **53** for analog **T2**

As above mentioned route produced insufficient amount of cyclic core **53**, an alternative route to compound **53** was attempted. It started with esterification of carboxylic acid **50** with 2,2,2-trichloroethanol in presence of EDC and Hunig's base to get **54** as shown in Scheme 22. The Boc group of ester **54** was removed with acid and acyl group of **37** was transferred to obtain alcohol **55**. The ester **56** was produced by Yamaguchi condensation of alcohol **55** with Fmoc-L-valine. The trichloroethyl ester group of **56** was cleaved with Zn and NH_4OAc in THF.[43] Usual Fmoc group removal followed by cyclization produced only 2 mg of impure cyclized core **53** (from 146 mg of compound **56**). The impure

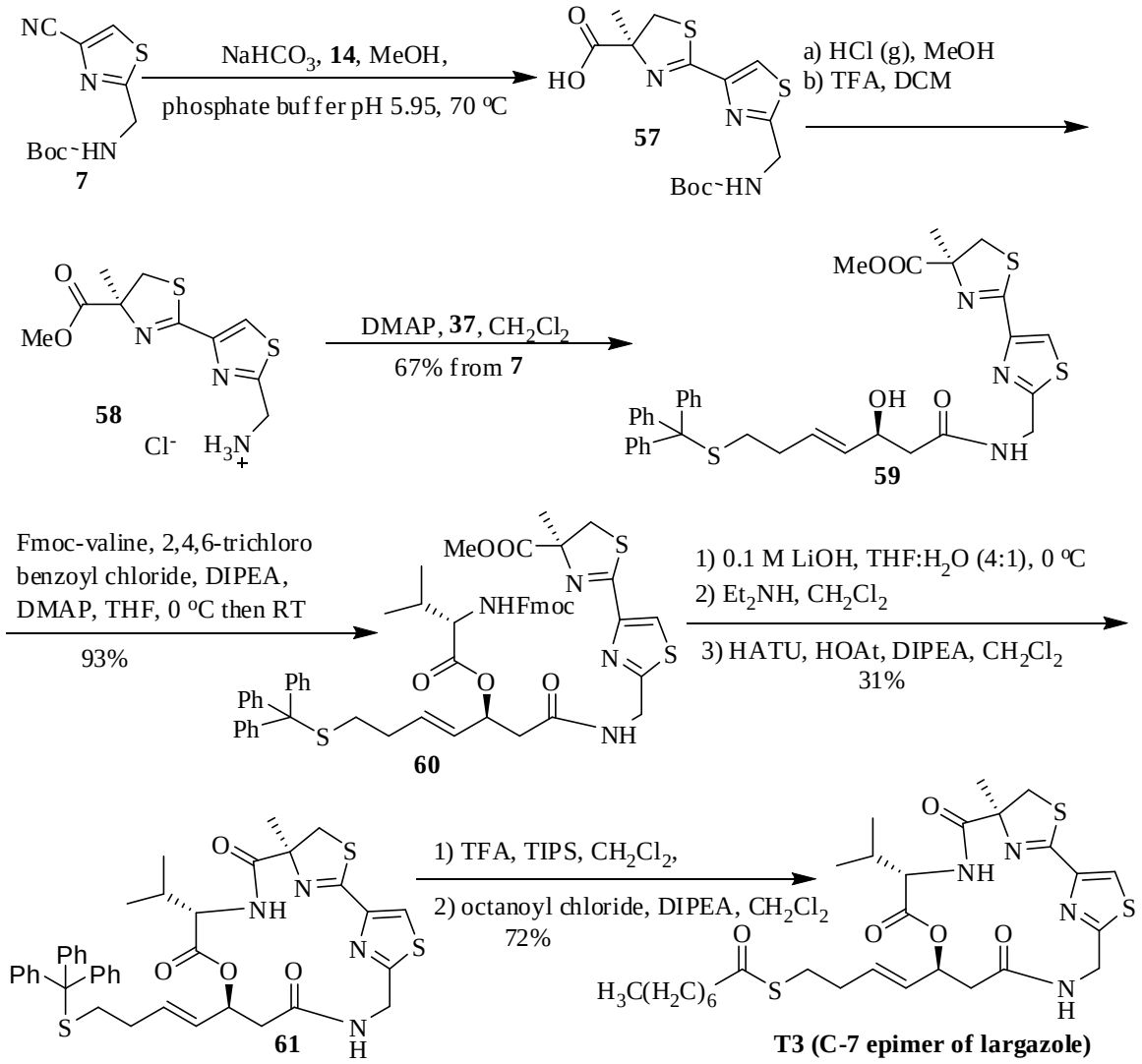
product, upon subjection to removal of trityl group and thioesterification did not produce analog **T2**.



Scheme 22. Alternative route for analog **T2**

2.4 Synthesis of analog T3

Synthesis of target molecule **T3** followed the same route as for largazole synthesis using (*S*)-α-methylcysteine HCl (**14**) instead of (*R*)-α-methylcysteine HCl to generate C-7 epimer of largazole (Scheme 23). The nitrile **7** was condensed with (*S*)-α-methylcysteine to obtain the thiazole-thiazoline carboxylic acid **57**.[25a] After simultaneous removal of Boc group and esterification of the free carboxylic acid under acidic conditions in MeOH, acyl group was transferred[51] from **37** to compound **58** to obtain alcohol **59**. The formation of carboxylic acid **57** from nitrile **7** and the one pot conversion of **57** to alcohol **59** proved very efficient with 67% yield for 3 steps. Yamaguchi esterification[3a, 52] was used to couple Fmoc-valine to alcohol **59** to afford the acyclic precursor **60** in 93% yield. After saponification and Fmoc group removal, macrocyclization with HOAt, HATU, and Hunig's base yielded the cyclized product **61** in 31% yield over the 3 steps. Thioesterification with octanoyl chloride of cyclized core **61** upon removal of trityl group produced analog **T3** in 72% overall yield for two steps.

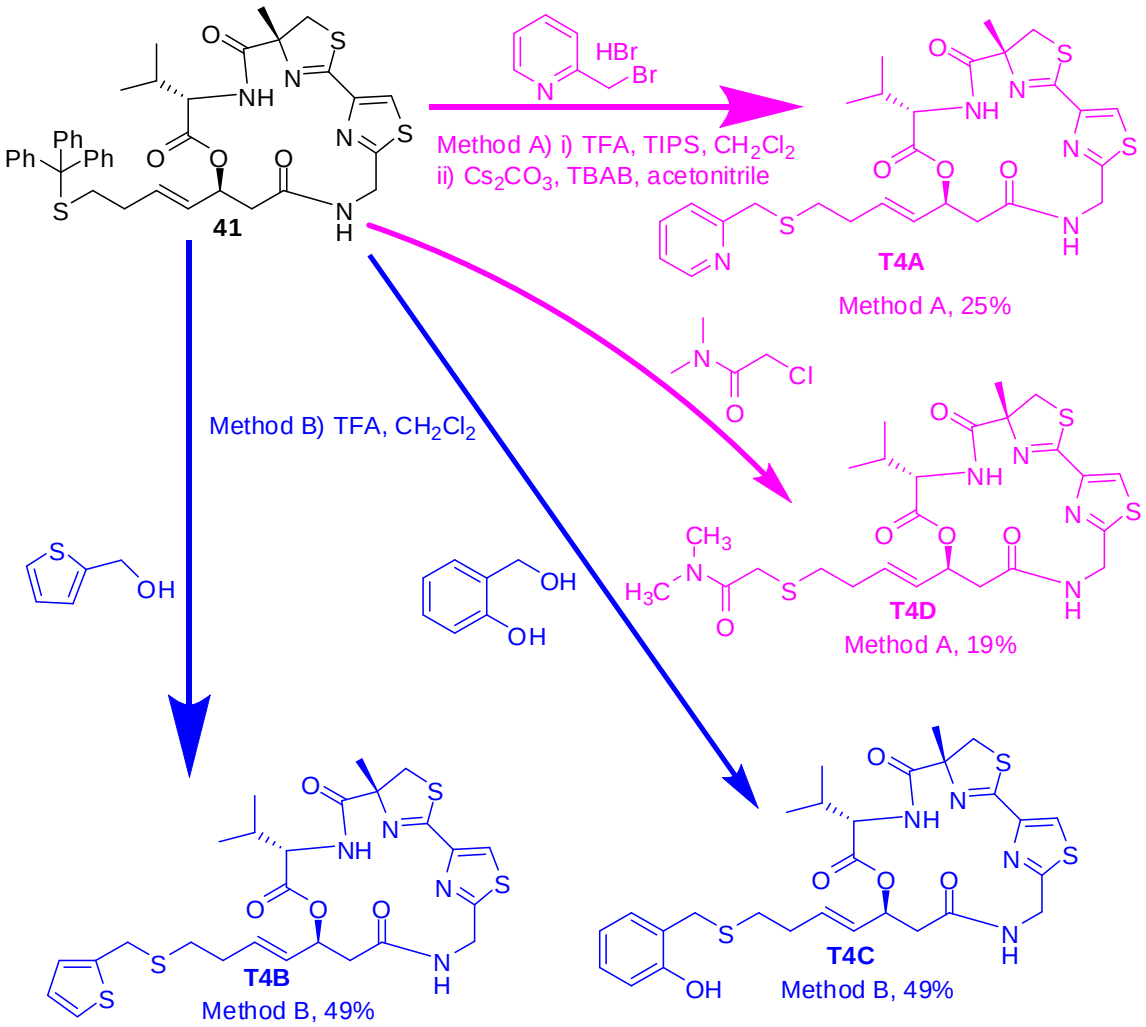


Scheme 23. Synthesis of analog **T3** (C-7 epimer of largazole)

2.5 Synthesis of analogs of T4 series

Scheme 24 shows the general method used for the synthesis of largazole analogs **T4**, which incorporate modifications in the zinc binding motif. These analogs are designed such that sulphur and a second heteroatom in the side chain are 2-3 atoms apart and are thus capable of interacting with Zn^{2+} via 5/6 membered cyclic transition states (Figure 19).

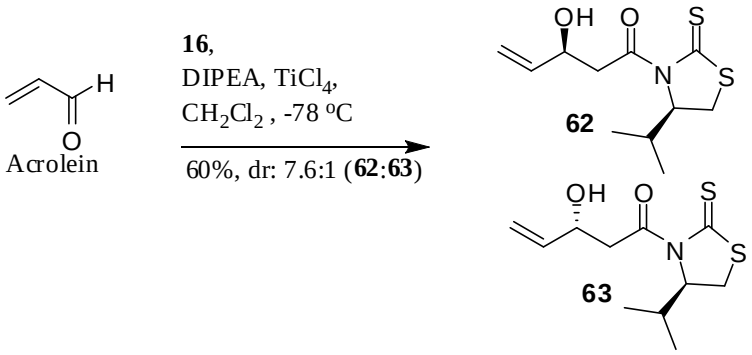
The analogs **T4A**, **T4B**, **T4C and T4D** were synthesized from the advanced intermediate **41** after conversion to free thiol, by nucleophilic substitution of the corresponding precursors by largazole thiol by either method A (basic)[56] or method B (acidic)[57] as shown in Scheme 24. Method A comprises of removal of trityl group by TFA to give thiol which reacted with alkyl halide precursor in the presence of $CsCO_3$ and tetrabutylammonium bromide (TBAB) to give S_N2 displacement product. This two pot protocol gave an overall yield of 25%, and 19% of analogs **T4A and T4D**, respectively. Analogs **T4B** and **T4C** were synthesized by method B. This one pot protocol consisted of removal of trityl group of compound **41** by TFA followed by reaction of the resulting thiol with corresponding alcohol precursor in the same pot to give the S_N1 substituted product. This method gave 49% overall yield for both analogs **T4B** and **T4C**.



Scheme 24. Synthesis of analogs of type **T4** with modified metal-binding domain

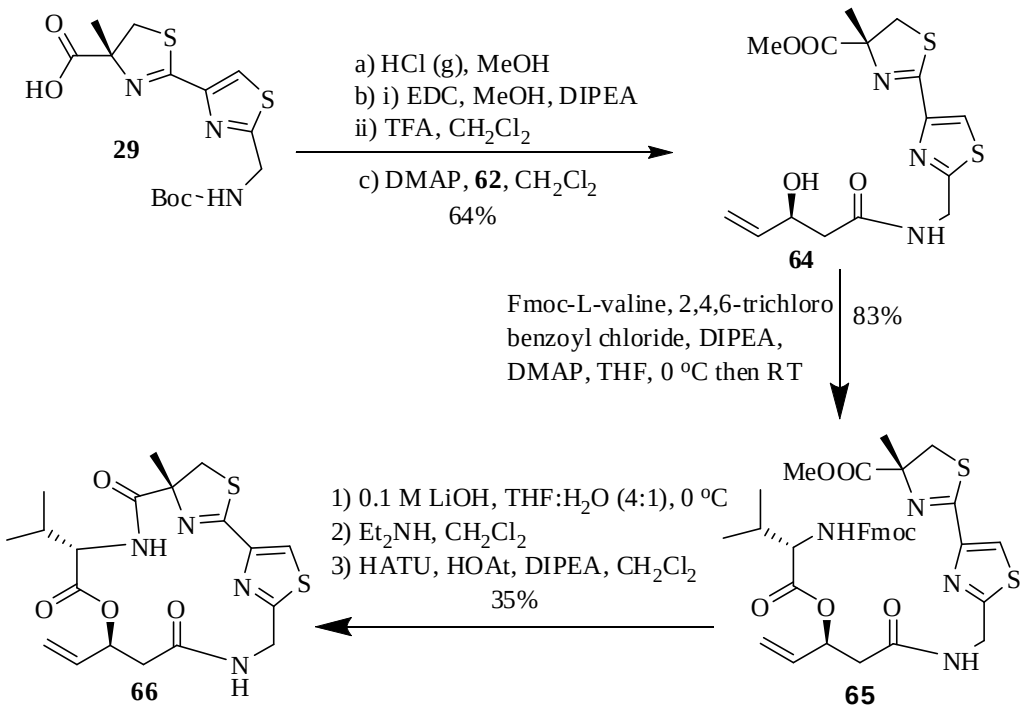
2.6 Synthesis of analog T5

Synthesis of target molecule **T5** began with synthesis of alcohol **62** by aldol reaction of acrolein with acetyl Nagao auxillary (**16**) (Scheme 25). The two diastereomers were separated by flash column chromatography on silica gel in ethyl acetate and hexanes (20-90%) to get **62** in 77% diastereomeric excess. The absolute stereochemistry of newly generated chiral center of **62** was determined by modified Mosher ester analysis.[47]



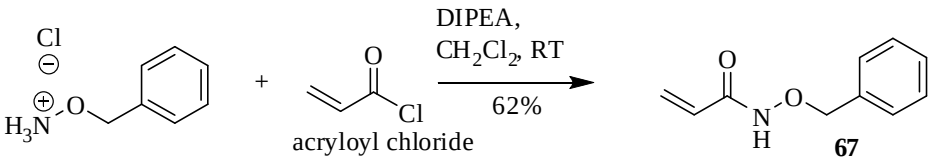
Scheme 25. Synthesis of alcohol **62** for the synthesis of cyclic core of **T5**

Alcohol **62** was used to synthesize the cyclic core **66** as in the synthesis of largazole (Scheme 26). The assembly of cyclic core **66** began with simultaneous removal of Boc group and esterification of the free carboxylic acid of **29** under acidic conditions in MeOH and acyl group transfer[51] from **62** to obtain alcohol **64** in one pot with 64% yield for 2 steps. Yamaguchi esterification[3a, 52] was used to couple Fmoc-L-valine with alcohol **64** to afford the acyclic precursor **65** in 83% yield. After saponification and Fmoc group removal, macrocyclization with HOAt, HATU and Hunig's base yielded the cyclized product **66** in 35% yield over the 3 steps.



Scheme 26. Synthesis of cyclic core **66**

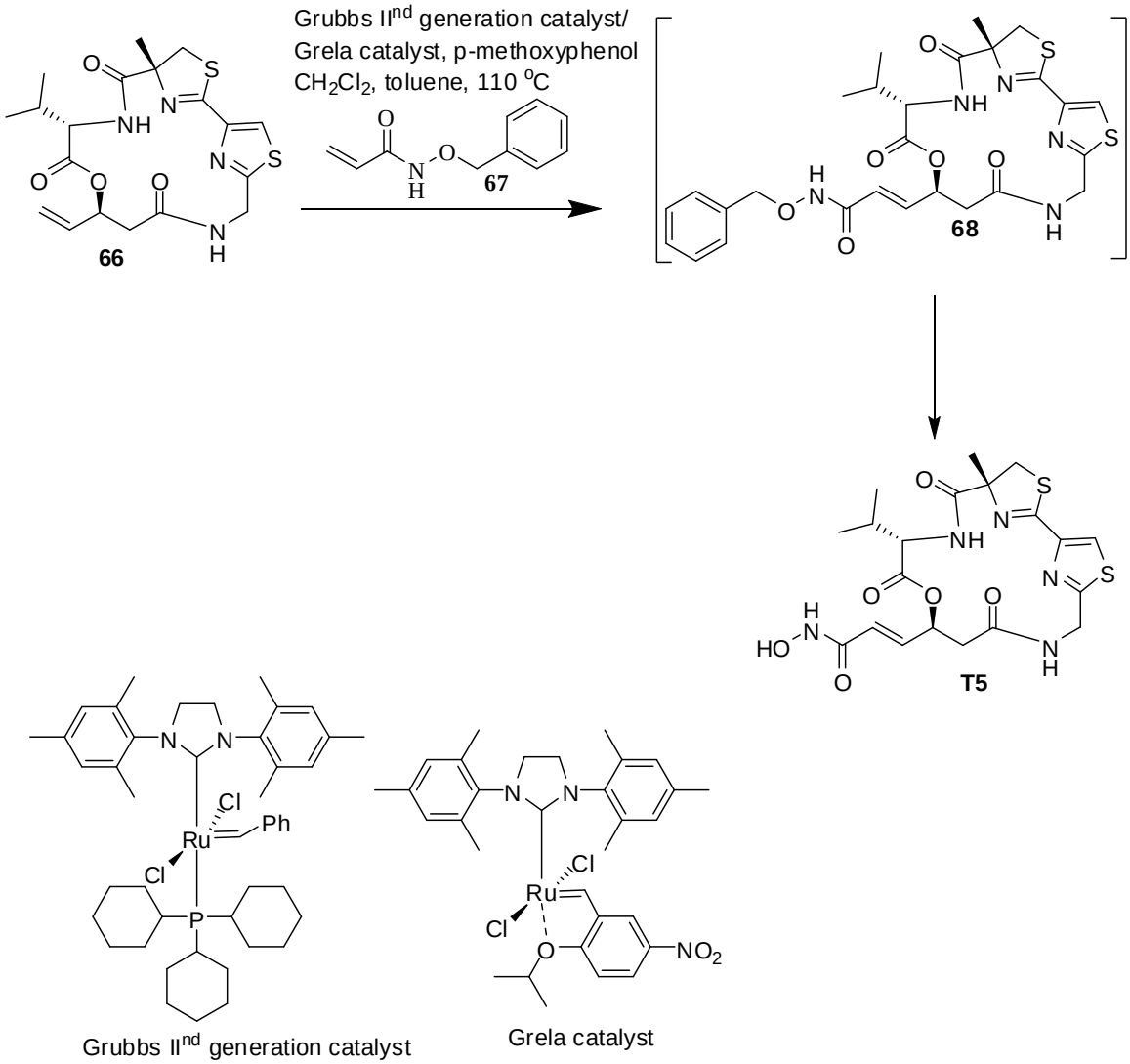
Synthetic pathway to analog **T5** side chain is depicted in Scheme 27. Reaction of acryloyl chloride with o-benzylhydroxylamine HCl in the presence of Hunig's base gave product **67** in 62% yield.



Scheme 27. Synthetic pathway for side chain of target molecule **T5**

The cyclic core **66** was subjected to olefin metathesis with compound **67** using Grubbs IInd generation or Nitro-Grela catalyst to afford precursor **68** of target molecule **T5** (Scheme 28). Gratifyingly, olefin metathesis was accompanied by debenzylation and the

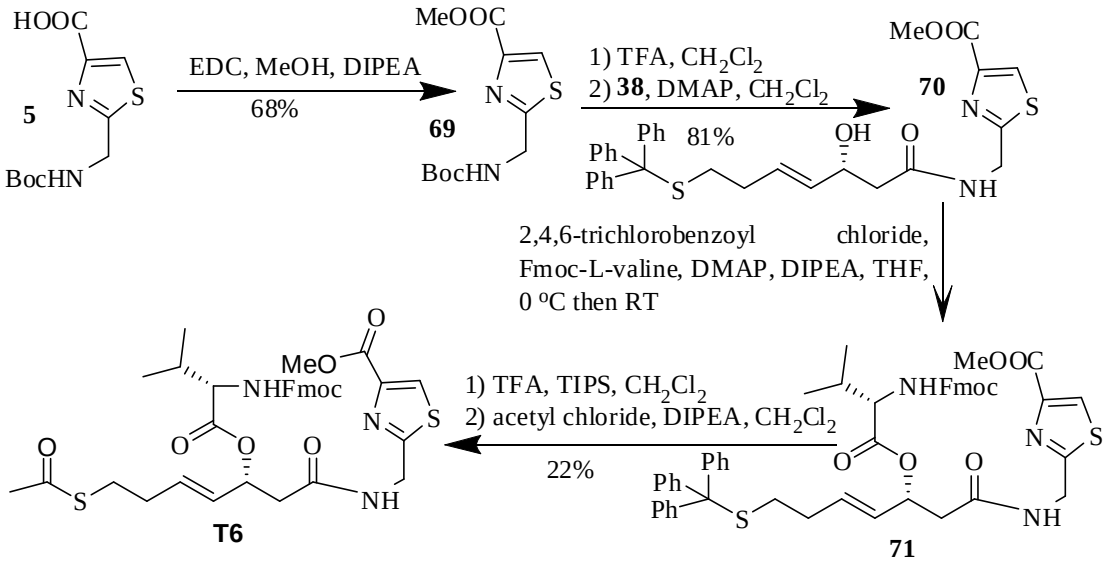
product **T5** was isolated by reverse phase HPLC purification of the crude product on a C18 column.



Scheme 28. Synthesis of analog **T5**

2.7 Synthesis of analogs of T6 series

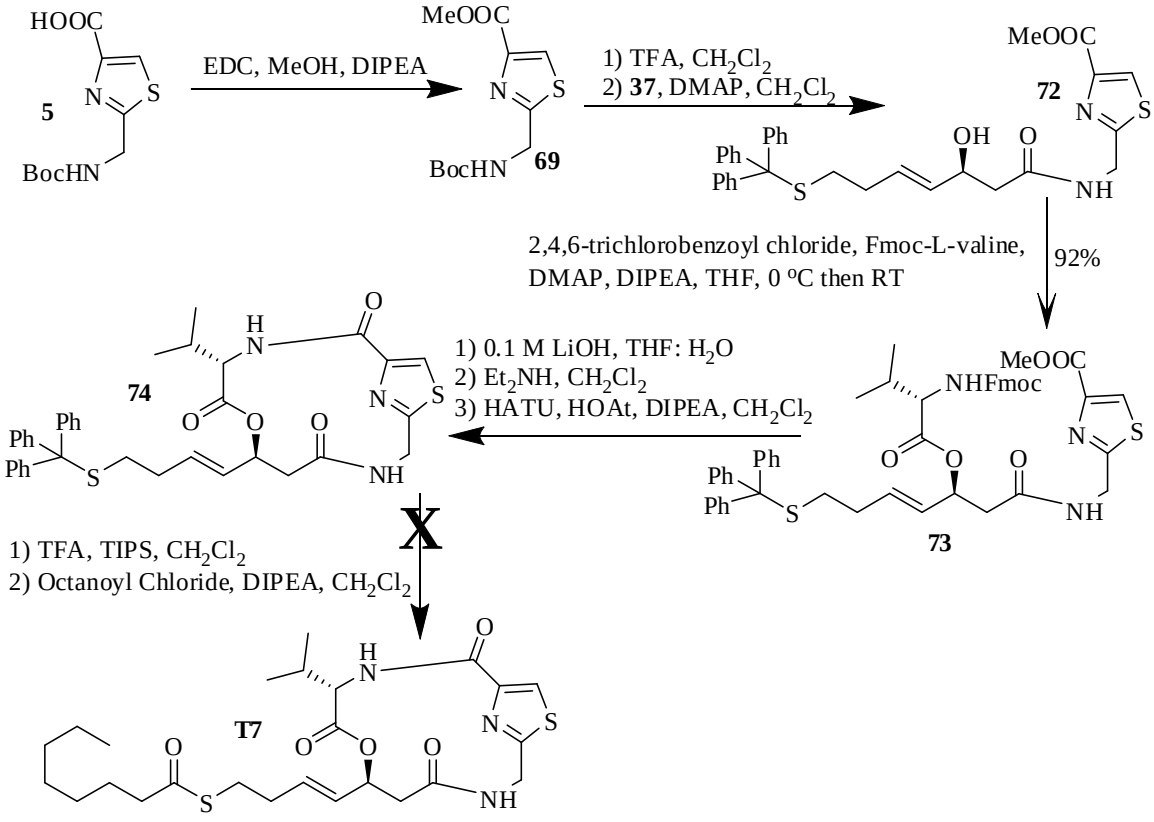
Synthesis of analog **T6A** as shown in Scheme 29 started with esterification of carboxylic acid **5** to get compound **69**. Removal of Boc group of compound **69** by TFA in CH_2Cl_2 and acyl group transfer[51] from **38** gave the alcohol **70** in 81% yield. Yamaguchi esterification[3a, 52] was used to couple Fmoc-L-valine to alcohol **70** to afford the precursor **71**. Compound **71** was taken to the next step without further purification. After removal of trityl group of compound **71** and thioesterification with acetyl chloride, it yielded analog **T6** in 22% yield over the 2 steps.



Scheme 29. Synthesis of analog **T6**

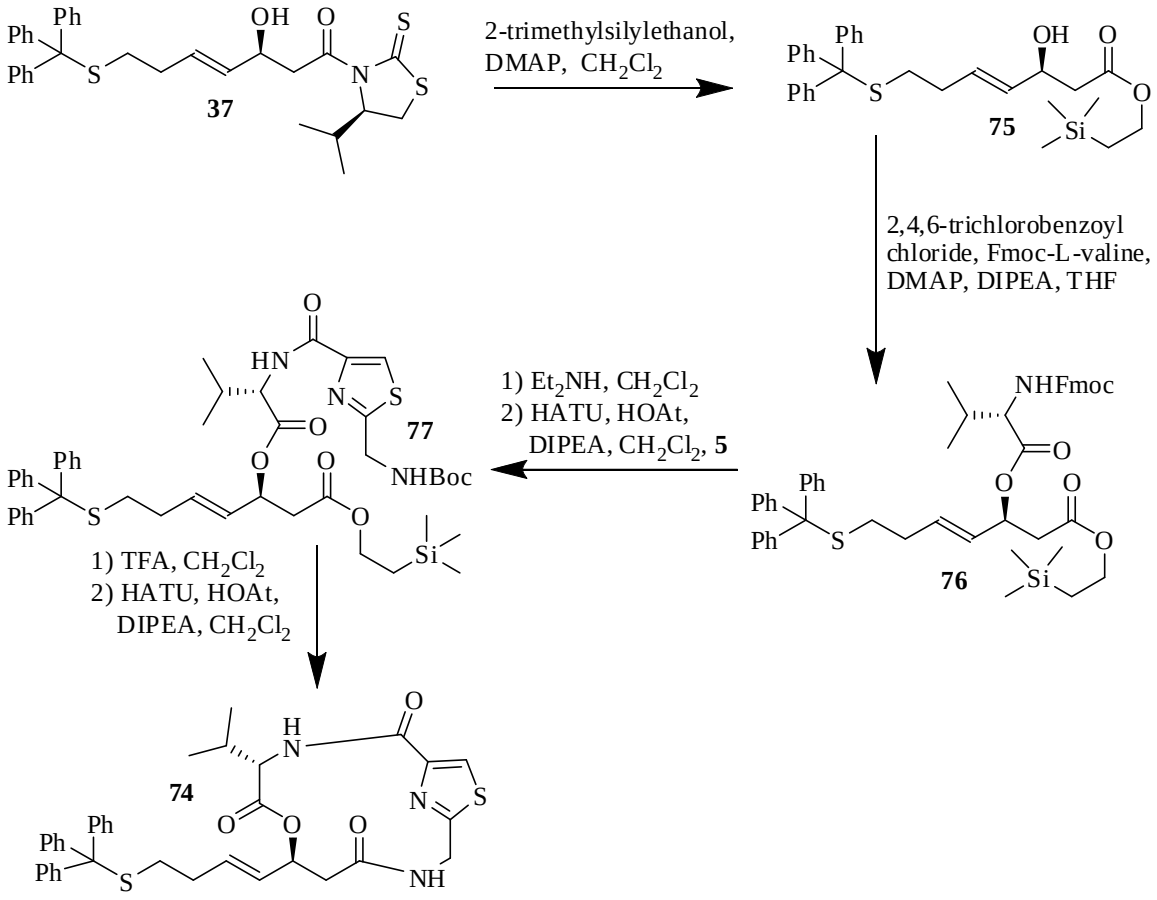
2.8 Attempts to synthesize 13-membered ring analog T7

Scheme 30 depicts attempts to synthesis of analog **T7**. Removal of Boc group of compound **69** with TFA, and transfer of acyl group from **37** with DMAP as acyl transfer agent gave alcohol **72**. Fmoc-L-valine coupling of alcohol **72** by Yamaguchi's protocol yielded compound **73** in 92% yield. Macrocyclization after removal of protecting groups yielded partially purified 13-membered cyclic core **74**. As each purification step resulted in loss of material, it was subjected to next set of reactions without further purification. Usual protocol of trityl group removal with TFA and thioesterification with octanoic acid did not yield analog **T7**. As this approach gave low yields of the cyclic core **74** an alternative route to its synthesis was undertaken.



Scheme 30. Attempt to the synthesis of 13 membered ring analog **T7**

Acyl transfer from alcohol **37** to 2-trimethylsilylethanol yielded compound **75** which upon coupling with Fmoc-L-valine gave compound **76** (Scheme 31). This was coupled with carboxylic acid **5** to get precursor **77** for cyclic core synthesis. Although cyclization after deprotection yielded compound **74**, the amount obtained after purification was low. Every purification step led to loss of material. Instability of cyclic core **74** on silica gel could be the reason for low yields obtained with its purification. Insufficient amount of cyclic core material **74** obtained deterred attempts to convert it to analog **T7**.



Scheme 31. Alternative route of synthesis to cyclic core **74**

2.9 Biological Studies

2.9.1 Results

The Biological studies were carried out in the laboratory of Dr. Robert A. Casero, Professor of Oncology, the Sidney Kimmel Comprehensive Cancer Center, the Johns Hopkins University, School of Medicine, Baltimore. The antiproliferative activity of analogs **T1A**, **T1B**, **T3**, **T4A**, **T4B, T4C** and **T6** were evaluated in the HCT116 colon adenocarcinoma cell line by a standard 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reduction assay (Promega) using largazole as the control as previously reported.[58] Figure 21 show the results of the MTS assay after 96 h of treatment with 10 nM, 100 nM, 1 µM and 5 µM concentrations of the compounds. Largazole inhibited the growth of HCT116 cells with a GI_{50} of ~ 30 nM whereas the three side chain analogs **T4A**, **T4B** and **T4C** showed activity only at higher concentrations. Of the three, the pyridine analog **T4A** demonstrated the greatest effect on growth inhibition with a GI_{50} of 1.0 µM. Of the analogs incorporating changes in the depsipeptide ring, the C-2 L-allyl substituted analog **T1B** showed a GI_{50} value of 10 nM. The C-7 epimer of largazole **T3** is less active than largazole with a GI_{50} value of 5 µM.

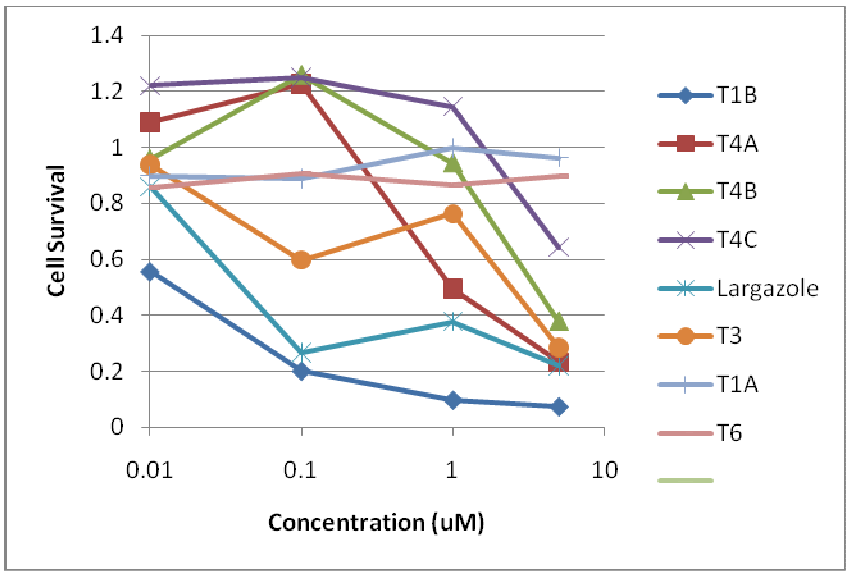


Figure 21. Growth inhibitory effects of largazole and analogs on HCT116 colon carcinoma cells.

To evaluate the effects of analogs **T1B**, **T3**, **T4A**, **T4B,** and **T4C** on HDAC activity, the downstream effects of HDAC inhibition on global histone H3 acetylation was evaluated by Western blot analysis after 24 hours of cellular exposure to 10 nM, 100 nM, and 1 μM of each compound. Although no increase in global acetylation was observed in treated cells exposed to analogs **T3**, **T4A**, **T4B** and **T4C** at 100 nM, analog **T1B** showed prominent increase in global acetylation at 100 nM. The effect of analog **T1B** on global histone H3 acetylation is quite promising and is comparable to that of largazole at 100 nM. Analogs **T4A** and **T4B** showed a significant increase in global acetylation at 1μM, though the effect is much lower than what was observed with largazole.

In order to ascertain the effect of analogs **T4A**, **T4B** and **T4C** on HDAC6 activity, the levels of α-tubulin acetylation after exposure to 10 nM, 100 nM and 1 μM were studied using Western blot analysis of whole cell lysates from cells treated for 24 hours. No

changes in α-tubulin acetylation were observed by exposure to any of the analogs, including largazole. This conforms to earlier findings that HDAC6 is not a target for largazole and **T4** series analogs.[10]

2.9.2 Discussions

Largazole inhibited the growth of HCT116 cells with a GI_{50} of ~30 nM. The three side chain analogs **T4A**, **T4B** and **T4C** were less active, the pyridine analog **T4A** being the most active of the three with a GI_{50} of ~1.0 μM. The other two analogs were active at higher concentrations. This is consistent with significant increase of global H3 acetylation observed with largazole at 1 μM concentration and modest induction of global H3 acetylation observed for analogs **T4A** and **T4B**. There was no change in acetylated α-tubulin levels with any of the compounds indicating they have no effect on HDAC6. Thioethers bind weakly to Zn^{2+}.[59] These largazole analogs were designed to see whether the introduction of a second hetero atom would lead to stronger binding to the metal ion and also isoform/class selectivity. However, it is not possible to suggest if the diminished biological activity observed is due to poor affinity of thioether group for Zn^{2+} ion or incompatibility of the modified metal binding moiety with the HDAC active site. Despite the high sequence similarity within the active site, presence of discrete binding cavities in the vicinity of the metal ion may be taken advantage of to achieve isoform selectivity as exemplified by HDACis with substituted benzamide metal-binding domains which display class I selectivity.[26d, 41] The exploration of alternative metal-binding domains is one of the ways forward to the development of such isoform and class-selective HDACis.

Analog **T1B** with allyl group replacement of isopropyl group of valine was more potent than largazole. Substitution of the isopropyl group of valine at C-2 with other

hydrophobic and hydrophilic groups may lead to molecules with improved activity and selectivity.

Determination of HDAC isoform/class selectivity of all synthesized analogs is in progress.

2.10 Future Directions

The HDAC isoforms or subsets of isoforms pertinent to antitumor therapy are not clearly understood. In addition, which HDAC substrates have the most critical roles in a particular type of tumors is not known.[36] Although trial and error and serendipity have played an important role in the development of HDAC inhibitors due to lack of information about individual HDAC isoforms, rational drug design has led to a number of clinically important HDACis.[36] Some success has been achieved in the development of isoform and/class selective HDACis based on binding studies of molecules to HDAC proteins by X-ray crystallography. Apart from extending the synthetic efforts to generate additional analogs of current series, HDAC4 selective compounds (analogs **T8A** and **T8B**), compounds (analogs **T10**, **T11**,) with multiple sites of activity and HDAC6 selective analogs (**T12)** as shown in Figure 22 are designed to increase the therapeutic efficacy of these molecules.

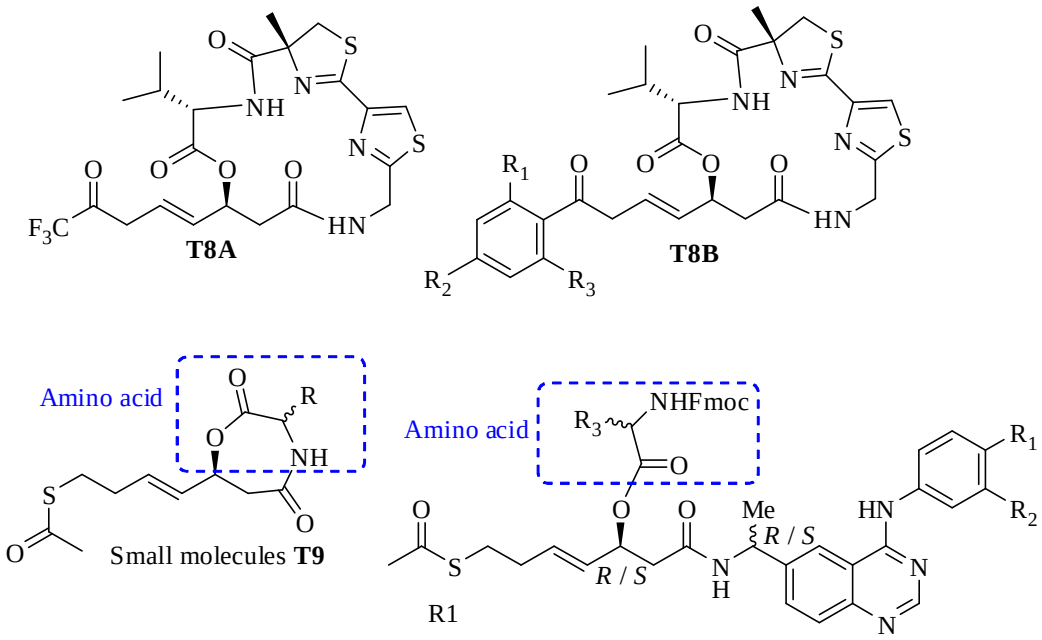
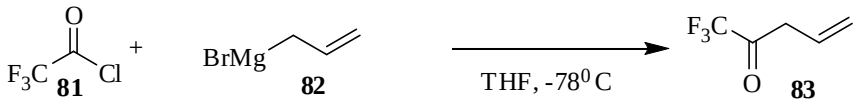


Figure 22. Additional target molecules designed for future directions.

Target molecules of T8 series

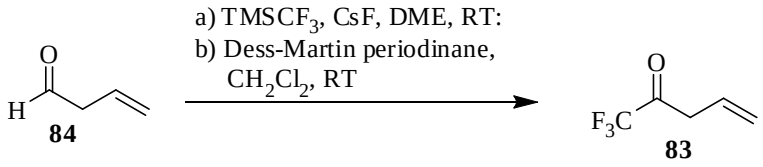
In target molecule **T8**, the thiol group of largazole is replaced with a trifluoromethyl ketone moiety as HDACi with trifluoromethyl ketone functionality typically display a 10-100 fold greater inhibition of class II than class I HDACs.[15] It has been shown that HDAC4 (class II) inhibitors produce cellular sensitization to ionizing radiations.[39] Although the trifluoromethyl group fills the gap near Pro^{800}, it is far away from His^{976} which lies at the bottom of the biding pocket of HDAC4. Analogs of type **T8B** are designed to see if substituted phenyl ketone fills this gap and leads to additional HDAC4 selectivity. Electron withdrawing groups such as $-NO_2$, -CN, $-SO_2NH_2$, -COOR on phenyl ring serve three purposes: 1) they increase electropositivity of ketone; 2) they can form hydrogen bonds with His^{976}, Pro^{800} and other amino acids; 3) they fill the pocket around His^{976} and Pro^{800}. Additionally, presence of sterically hindered groups at 2,6-positions decreases chances of stabilization of hydrated ketone by Tyr^{306} in class I HDAC and thus increases their selectivity for class II HDAC.

Proposed synthesis of target molecule **T8A** can begin with Grignard reaction between **81** and **82** to give **83** as shown in Scheme 32. If Grignard reaction is complicated by formation of tertiary alcohol, Weinreb amide can be used instead of acid chloride to make **83**. An alternative route to **83** is depicted in scheme 33.[60] The cyclic core **66** (scheme 26) can be subjected to olefin metathesis with **83** to afford the target molecule **T8A**.



Scheme 32: Synthesis of side chain fragments **83** of target molecule **T8A**

Target molecule **T8B** can be synthesized in a similar manner.

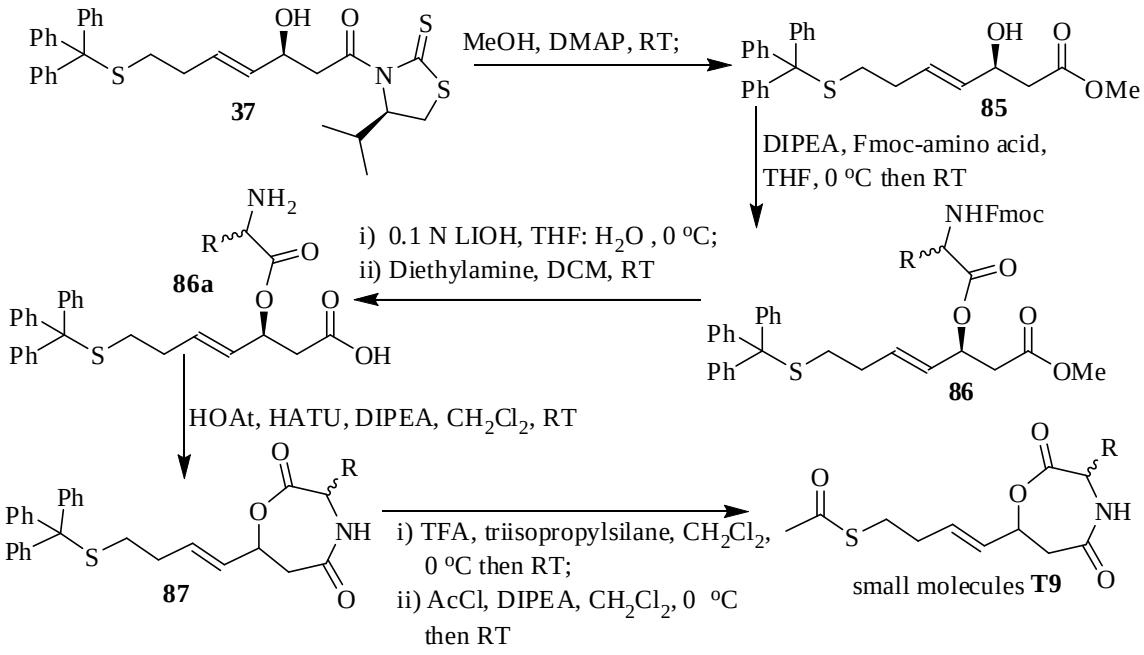


Scheme 33. Alternative route to compound **83**

Target molecules of T9 series:

Small molecules generally are preferred as drug candidates as their large-scale synthesis for clinical use is more viable commercially. Seven-membered ring compounds **T9** are designed as small molecule analogs of largazole. Several analogs of **T9** can be generated using different amino acids. Also, stereochemical requirements can be deduced by generating analogs using both D- and L-amino acids.

Synthesis of target molecule **T9** is shown in Scheme 34. Acyl group transfer from **37** to methanol gave **85** which can undergo Yamaguchi esterification with various Fmoc amino acids to afford **86**. After saponification and Fmoc group removal, it can be subjected to lactamization under specified conditions to give the cyclic intermediate **87**. Removal of trityl group of **87** followed by acetylation will yield analogs of **T9** series.



Scheme 34. Synthesis of small ring analogs **T9**

Target molecules T10, T11 series

Involvement of human epidermal growth factor receptor (HER) family in tumorogenesis is known. The four structurally linked tyrosine kinase receptor proteins of HER family are the epidermal growth factor receptor (EGFR): (HER1), HER2, HER3 and HER4.[61] EGFR/HER2 inhibitor lapatinib and EGFR inhibitors erlotinib and gefitinib are FDA approved drugs used against solid tumors. As these drugs suffer from limitations due to development of drug resistance, Curis Inc. combined features of HDACi and EGFR/HER2 inhibitors in CUDC-101 (Figure 23), which is in phase I clinical trials for advanced head and neck, gastric, breast, liver and non-small cell lung cancer tumors.[61] Based on the success of CUDC-101,[61] the proposed molecules **T10, T11** are designed to have multiple sites of activity in a single molecule. The molecules combine HDAC,

EGFR, and HER2 inhibitory activity. In addition, analogs **T11** are designed to include two heteroatoms two atoms away from each other. The binding of these two heteroatoms to Zn^{2+} of HDAC enzymes would form a stable 5 membered transition state which may potentiate the activity of these analogs.

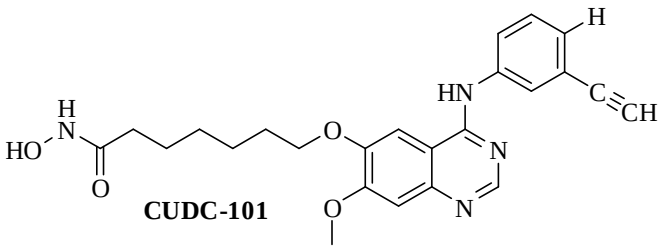


Figure 23. Structure of phase I molecule CUDC-101.

Target molecules T12 series

HDAC inhibitors with phenylisoxazole as cap group are reported to have HDAC6 selectivity.[62] Few of these compounds have been shown to be 10-fold more potent than SAHA.[62] Analogs **T12** are designed to consist of phenylisoxazole along with Fmoc as cap groups and are Y shape molecules which retain their HDAC6 selectivity features.

Chapter 3

3 Experimental Sections

3.1 Experimental Chemistry

General:

THF was refluxed with Na and benzophenone and freshly distilled prior to use. NMR spectra were recorded on Varian INOVA 600 MHz and Varian VXRS 400 MHz instruments and calibrated using residual undeuterated solvent as internal reference ($CDCl_3$: ^1H NMR at δ 7.26, ^{13}C NMR at δ 77.23; D_2O: ^1H NMR at δ 4.8; DMSO-d_6: ^1H NMR at δ 2.5, ^{13}C NMR at δ 39.51). Optical rotations were recorded on an AUTOPOL III 589/546 polarimeter. High-resolution mass spectra (HRMS) were recorded on a Micromass LCT Electrospray mass spectrometer at the Central Instrument Facility, the Wayne State University, Detroit, Michigan and on a Micromass Q-Tof II mass spectrometer at Mass Spectrometry and Proteomics Facility, the Ohio State University, Columbus, Ohio. Crude products were purified by flash column chromatography on silica gel (32-63 μ) purchased from Dynamic Adsorbents Inc. and by preparative thin layer chromatography on 1000 μ Uniplates purchased from Analtech Inc. using commercial solvents as specified. Microwave experiments were performed using Biotage Initiator. Combiflash Companion by Teledyne ISCO Inc. was used for flash chromatographic

separations. HPLC analyses were performed on a Waters 1525 Binary Pump HPLC system with Waters 2487 Dual Wavelength Absorbance Detector on a Symmetry C18 column (reverse phase, 5 □, 4.6 mm x 150 mm) using a liner gradient of 10-100% H_2O:MeOH over 15-20 min; flow rate of 1 mL/min and UV detection at 254 nm. Luna 5 µ C_{18} (2) 100A (size 250 X 10 mm 5 micron) column by Phenomenex Inc. was used for HPLC separations. D-Hydantoinase, recombinant, immobilized from *E. Coli* (catalog no: 53765; CAS: [9030-74-4]) was purchased from Fluka Chemie AG.

***tert*-Butyl (4-carbamoylthiazol-2-yl)methylcarbamate (6).**

A mixture of Boc-thioglycinamide **4** (0.95 g, 5 mmol, 1 equiv) and bromopyruvic acid (0.835 g, 5 mmol, 1 equiv) in dry THF (20 mL) was stirred at 50 ^{o}C under nitrogen for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was dried under reduced pressure by repeated azeotropic removal of moisture with toluene. The crude carboxylic acid residue was dissolved in dry THF (50 mL) and triethylamine (1.3 g, 12.94 mmol, 2.6 equiv) and ethyl chloroformate (0.681 g, 6.24 mmol, 1.25 equiv) were added at 0 ^{o}C. The reaction mixture was stirred for 30 minutes at the same temperature. Ammonium hydroxide (~ 1.1 g, 31.76 mmol, 5 equiv) was added and the reaction mixture was stirred at room temperature for 2 h. It was concentrated *in vacuo* and purified by flash column chromatography on silica gel in ethyl acetate/hexanes (33-100%) to yield **6** (0.768 g, 60% over two steps from Boc-thioglycinamide); mp 153-154 ^{o}C (lit.[25c] mp 153 ^{o}C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.13 (br s, 1H), 5.94 (br s, 1H), 5.34 (br s, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 163.1, 155.8, 149.3, 124.8, 80.8, 42.5, 28.5.

***tert*-Butyl-(4-cyanothiazol-2-yl)methylcarbamate (7).**

To a solution of amide **6** (0.204 g, 0.797 mmol, 1 equiv) in dichloromethane (20 mL) at 0 ^{o}C was added triethylamine (0.174 g, 1.725 mmol, 2.16 equiv) followed by dropwise addition of trifluoroacetic anhydride (0.181 g, 0.863 mmol, 1.08 equiv). The reaction mixture was stirred at room temperature for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-50%) to obtain the nitrile **7** (0.189 g, 99%). mp 84-85 ^{o}C (lit.[25c] mp 84 ^{o}C). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95 (s, 1H), 5.3 (s, 1H), 4.62 (d, J = 6 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 155.8, 131.1, 126.6, 114, 81, 42.5, 28.5.

5-(*tert*-Butylthiomethyl)-5-methylimidazolidine-2,4-dione (10).

To a 5% aqueous solution of NaOH (96 mL, 120 mmol, 1.2 equiv) was added *t*-butyl mercaptan (9.02 g, 100 mmol, 1 equiv) at 0 ^{o}C. After 30 minutes, chloroacetone (9.25 g, 100 mmol, 1 equiv) was added at 0 ^{o}C and the mixture was stirred at room temperature for 3 h. The two yellow layers of the reaction mixture became homogenous after addition of NaCN (5.88 g, 120 mmol, 1.2 equiv), $(NH_4)HCO_3$ (27.7 g, 350 mmol, 3.5 equiv), and ammonium hydroxide solution (14.8 M, 31 ml, 459 mmol, 4.59 equiv). After stirring at 60 ^{o}C overnight, the reaction mixture was cooled and the pH was adjusted to 7-7.6 with HCl. The precipitated product was filtered and dried *in vacuo*. It was recrystallized from 50% ethanol to get hydantoin **10** (19.44 g, 90%); mp 208-210 ^{o}C (lit.[63] mp 208-210 ^{o}C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (s, 1H), 7.89 (s, 1H), 2.75 (s, 2H), 1.31 (s, 3H), 1.22 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.2, 156.2, 62.1, 41.8, 35.1, 30.7, 23.6.

3-(*tert*-Butylthio)-2-methyl-2-ureidopropanoic acid (11).

A solution of hydantoin **10** (2.37 g, 10.97 mmol, 1 equiv) in aqueous NaOH (10% w/v, 35 mL, 87.5 mmol, 7.97 equiv) was refluxed at 120 ^{o}C for 60 h. After the completion of the reaction (TLC, 10% MeOH: CH_2Cl_2) the pH of the reaction mixture was adjusted to 8 with HCl when a thick precipitate was formed. The suspension was heated to 70 ^{o}C and a solution of KCNO (3.1 g, 38.27 mmol, 3.49 equiv) in water (15 mL) was added drop wise over 40 minutes along the sides of the flask. The reaction mixture was stirred at 70 ^{o}C for 7 h. After cooling, the pH of the reaction mixture was adjusted to 2 with HCl. The product was filtered off, washed with water and dried to get compound **11** (1.15 g, 45%). The crude product was taken to the next step without further purification.

(*R*)-3-(*tert*-Butylthio)-2-methyl-2-ureidopropanoic acid (12).

To an aq NaCl solution (3.2%, 7 mL) was added compound **11** (1.35 g, 5.77 mmol, 1 equiv) and pH of the mixture was adjusted to 6.5 with aq. NaOH (10%) solution. Immobilized hydantoinase (0.45 g, 23.9 U) and magnesium sulphate monohydrate (8.0 mg) were added to it and the reaction mixture was stirred at 40 ^{o}C for 2 days while maintaining the pH at 6.5 using 10% aq. H_2SO_4. The reaction mixture was cooled and the precipitate of the enzyme and compound **10a** was filtered off. The precipitate was washed with water and extracted with ethyl acetate. The organic layer was washed with water and concentrated to get **10a** (0.553 g, 81.9%). The filtrate was washed with ethyl acetate (15 mL) twice. The pH of the aqueous layer was adjusted to 3 using HCl and the precipitate of compound **12** was filtered off. It was washed with water and dried to get compound **12** (0.510 g, 81.9%).

(*R*)-α-Methylcysteine HCl (13).

A solution of compound **12** (1.5 g, 6.41 mmol, 1 equiv) in HCl (15 mL) was refluxed under nitrogen for 3 days. The reaction mixture was azeotropped with isopropanol three times. It was stirred with toluene at 60 ^{o}C for 1 hour and cooled. The crystals were filtered, washed with toluene and dried *in vacuo* to get compound **13** (1.1 g, quantitative). $[\alpha]_D^{20}$ + 7.88 (*c* 0.33, H_2O) (lit.[64] $[\alpha]_D$ + 8.13 ((*c* 1.58, H_2O)). ^{1}H NMR (400 MHz, D_2O): δ 3.16 (d, *J* = 15.2 Hz, 1H), 2.86 (d, *J* = 15.2 Hz, 1H), 1.57 (s, 3H). ^{13}C NMR (100 MHz, H_2O): δ 173.2, 61.6, 30.3, 21.3.

(*R*)-4-isopropylthiazolidine-2-thione (15).[45]

A mixture of D-valinol (4 g, 38.83 mmol, 1 equiv), aqueous 1 N potassium hydroxide (200 mL, 200 mmol, 5.15 equiv) and CS_2 (15.14 g, 199 mmol, 5.13 equiv) was refluxed at 105 ^{o}C overnight. The reaction mixture was cooled to room temperature and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulphate and evaporated *in vacuo*. The crude product was purified by flash column chromatography on silica gel in ethyl acetate: hexanes (5-20%) to obtain **15** (5.9 g, 92 %). $[\alpha]_D^{20}$ + 34.0 (*c* 1.0, $CHCl_3$). (lit.[65] $[\alpha]_D^{20}$ + 37 (c 1.0, $CHCl_3$)). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (br s, 1H), 4.03 (dd, *J* = 8.4, 15.6 Hz, 1H), 3.52 (dd, *J* = 7.8, 10.8 Hz, 1H), 3.34 (dd, *J* = 9.0, 11.4 Hz, 1H), 1.93-1.99 (m, 1H), 1.04 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.3, 70.2, 36.2, 32.3, 19.0, 18.5.

(*R*)-1-(4-isopropyl-2-thioxothiazolidin-3-yl)ethanone (16).

To a mixture of NaH (1.15g of 60% dispersion in oil, 28.65 mmol, 2.25 equiv) and Nagao auxillary (**15)** (2.05 g, 12.7 mmol, 1 equiv) was added dry ether (100 mL) at 0 ^{o}C. The reaction mixture was stirred for 10 minute at 0 ^{o}C and acetyl chloride (1.1 g, 14.01 mmol, 1.1 equiv) was added very slowly at 0 ^{o}C. It was stirred for 10 minutes at 0 ^{o}C and then for 1 h at room temperature. The reaction mixture was treated with 1M HCl and extracted with ethyl acetate (3 times). The organic extract was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude reaction product was purified by flash column chromatography on silica gel in ethyl acetate: hexanes (5%) to get compound **16** (2.378 g, 92%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.13-5.16 (m, 1H), 3.50 (dd, *J* = 7.8, 11.4 Hz, 1H), 3.02 (dd, *J* = 1.2, 11.4 Hz, 1H), 2.77 (s, 3H), 2.33-2.38 (m, 1H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 203.4, 170.9, 71.4, 30.9, 30.6, 27.2, 19.3, 18.0.

1,3-Dioxene (18).[66]

To a solution of glycerol formal (available as approx. 60: 40 isomeric mixture of of 5-hydroxy-1,3-dioxane: 4-hydroxymethyl-1,3-dioxolane) (26 g, 0.25 mol, 1 equiv) and toluenesulfonyl chloride (50 g, 0.2625 mol, 1.05 equiv) in dichloromethane (50 mL) was added anhydrous pyridine (25.5 mL, 0.316 mol, 1.26 equiv) through a dropping funnel at 0 °C over 2 h. 4-(Dimethylamino)pyridine (5.0 mg, 0.041 mmol,) was added and the reaction mixture was stirred for 16 h at room temperature. It was partitioned between H_2O and dichloromethane and the aqueous phase was extracted with dichloromethane. The combined organic extract was dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was crystallized from Et_2O and hexanes to give 5-

tosyloxy-1,3-dioxane (27.34 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 4.74-4.82 (m, 2H), 4.44-4.48 (m, 1H), 3.99 (d, *J* = 3.2 Hz, 1H), 3.96 (d, *J* = 3.6 Hz, 1H), 3.78 (dd, *J* = 6.0, 12.0 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.5, 133.6, 130.2, 130.1, 128.2, 128.0, 93.7, 70.8, 68.8, 21.9.

To a homogeneous solution of KOH (9.8 g, 0.175 mol, 25 equiv) and triethylene glycol (60 mL) at 50 °C was added 5-tosyloxy-1,3- dioxane (18 g, 0.07 mol, 1 equiv) in one portion. The resulting mixture was heated slowly to 200 °C under stirring, and H_2O and dioxene (**18)** formed were condensed in a Dean-Stark trap equipped with a dry ice condenser (-78 °C). *n*-Decane (100 mL) was added to the condensate, the phases were separated and the aqueous phase was extracted with *n*-decane. The combined organic extract was dried over sodium sulphate and the crude product was distilled to get **18** (2.32 g, 44%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.56 (dt, *J* = 2.4, 6.6 Hz, 1H), 5.06 (s, 2H), 4.92 (dt, *J* = 2.4, 6.6 Hz, 1H), 4.25 (t, *J* = 1.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.8, 103.0, 90.7, 63.8.

6-(2-(*tert*-Butylthio)ethyl)-6*H*-1,3-diox-4-ene (20).[46]

To a solution of dioxene **18** (0.516 g, 6 mmol, 1 equiv) in THF (8 mL) was added *t*-BuLi in pentane (1.6 M, 4 ml, 6.4 mmol, 1.07 equiv) with syringe pump over 25 minutes to give a light yellow solution. A precooled solution of 2-[(2-bromoethyl)sulfanyl]-2-methylpropane (**19**) (1.18 g, 6 mmol, 1 equiv) in THF (8 mL) at -78 ^{o}C was canulated slowly to the reaction mixture. After stirring for 4 hours at -78 ^{o}C it was allowed to warm to rt with stirring overnight. The reaction mixture was quenched with water and extracted with ether. The organic layer was washed with brine, dried over anhydride sodium

sulphate and concentrated *in vacuo*. It was partially purified on silica gel in ethyl acetate: hexanes (0-2%) to afford **20** (1.03 g, ~ 85%). This was taken to the next step without further purification.

5-(*tert*-Butylthio)pent-2-*E*-enal (21).[46]

Partially purified compound **20** (0.248 g, 0.0012 mmol) in anhydrous toluene (1.8 ml) was heated in a sealed vial in a microwave synthesizer at 165 ^{o}C for 110 min. The reaction mixture was concentrated and purified on silica gel in ethyl acetate: hexanes (1-2%) to give **21** (0.211 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.52 (d, 7.6, 1H), 6.87 (dt, *J* = 6.8, 15.6 Hz, 1H), 6.16 (dd, *J* = 8.0, 16.0 Hz, 1H), 2.7 (t, *J* = 6.4 Hz, 2H), 2.61 (q, *J* = 6.8 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.0, 156.3, 133.8, 42.7, 32.9, 31.1, 26.6. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_9H_{17}OS$, 173.1000; found, 173.0999.

7-(*tert*-Butylthio)-3*S*-hydroxy-1-(4*R*-isopropyl-2-thioxothiazolidin-3-yl)hept-4*E*-en-1-one (22).[43]

To a stirred solution of acetyl Nagao chiral auxiliary **16** (0.318 g, 1.57 mmol, 1 equiv) in dichloromethane (15 mL) at 0 ^{o}C was added $TiCl_4$ (0.327 g, 1.723 mmol, 1.1 equiv). After stirring for 5 minutes, the reaction mixture was cooled to –78 ^{o}C and Hunig's base (0.222 g, 1.723 mmol, 1.1 equiv) was added. The reaction mixture was stirred for 2 h at the same temperature and the aldehyde **21** (0.27 g, 1.57 mmol, 1.0 equiv) in dichloromethane (4 mL) was added dropwise. The reaction mixture was stirred for 1 h at -78 ^{o}C. It was removed from cooling bath, treated with water (15 mL), and diluted with dichloromethane (50 mL). The layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic layer was washed with saturated NaCl (20

mL) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel in ethyl acetate/hexanes (5-33%) to give the major isomer **22** as a thick yellow oil (0.216 g, 58% brsm), and the diastereomer **23** (0.023 g, 6%) with recovery of acetyl Nagao chiral auxiliary (**16**) (0.115 g). Data for major isomer **22**: $[\alpha]_D^{20}$ - 277.6 (*c* 0.135, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73-5.80 (m, 1H), 5.59 (dd, *J* = 6.0, 15.2 Hz, 1 H), 5.15 (t, *J* = 7.6 Hz, 1H), 4.63 (m, 1H), 3.61 (dd, *J* = 2.8, 17.6 Hz, 1H), 3.52 (dd, *J* = 8.0, 11.6 Hz, 1H), 3.30 (dd, *J* = 9.2, 17.6 Hz, 1H), 3.03 (d, *J* = 11.6 Hz, 1H), 2.85 (s, 1H), 2.57 (t, *J* = 7.6 Hz, 2H), 2.27-2.39 (m, 3H), 1.30 (s, 9H), 1.05 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 7.2 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$) δ 203.1, 172.7, 131.9, 130.7, 71.6, 68.7, 45.5, 42.23, 32.7, 31.2, 31.0, 30.8, 28.0, 19.29, 18.0. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{17}H_{29}NO_2S_3Na$, 398.1258; found, 398.1245.

(*R*)-2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazol-4-yl)-4,5-dihydrothiazole-4-carboxylic acid (29).[25a, 25c]

To a well stirred mixture of the nitrile **7** (0.096 g, 0.4 mmol, 1 equiv) and $NaHCO_3$ (0.232 g, 2.76 mmol, 5.6 equiv) in methanol (5 mL) was added (*R*)-α-methylcysteine hydrochloride **13** (0.084 g, 0.491 mmol, 1.23 equiv) followed by phosphate buffer pH 5.95 (2.5 mL). The reaction mixture was degassed with nitrogen before stirring it under nitrogen at 70 ^{o}C for 1 h. It was acidified with 1 M HCl and extracted with ethyl acetate (15 mL) three times. The combined organic extract was washed with saturated NaCl solution, dried over anhydrous sodium sulphate and concentrated to obtain the carboxylic acid **29** (0.137 g) which was used in the next step without further purification.

(*R*)-Methyl 2-(2-(((*S*,*E*)-7-(*tert*-butylthio)-3-hydroxyhept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (31).

A solution of carboxylic acid **29** (0.121 g, 0.34 mmol, 1 equiv) in anhydrous methanol (5 mL) was bubbled with HCl gas for 5 minutes. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo* to give compound **30** which was azeotropped using toluene before taking it to the next step. A mixture of above obtained compound **30** and DMAP (0.108 g, 0.886 mmol, 2.61 equiv) in dichloromethane (2 mL) was stirred for 5 minutes and a solution of aldol product **22** (0.124, 0.34 mmol, 1 equiv) in dichloromethane (1 mL) was added. The reaction mixture was stirred for 2 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-100%) to afford the alcohol **31** (0.140 g, 78% over three steps from nitrile **7**). $[\alpha]_D^{20}$ – 15 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.32 (s, 1H), 5.66-5.72 (m, 1H), 5.52 (dd, *J* = 6.4, 15.2 Hz, 1H), 4.66-4.72 (m, 2H), 4.48 (s, 1H), 3.83 (d, *J* = 11.2 Hz, 1H), 3.76 (s, 3H), 3.24 (d, *J* =11.2 Hz, 1H), 2.52 (t, *J* = 7.6 Hz, 2H), 2.39-2.64 (m, 2H), 2.24 (q, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.1, 168.2, 162.9, 148.3, 132.3, 130.6, 122.4, 84.6, 69.2, 53.1, 43.0, 42.2, 41.6, 40.9, 32.6, 31.1, 27.85, 24.1.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-8-((*E*)-4-(*tert*-butylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (32)

To a solution of Fmoc-L-valine (0.013 g, 0.0383 mmol, 1.55 equiv) in THF (1 mL) at 0 ^oC were added Hunig's base (0.007 g, 0.0575 mmol, 2.32 equiv) and 2,4,6-trichlorobenzoyl chloride (0.0125 g, 0.0512 mmol, 2.07 equiv). The reaction mixture was

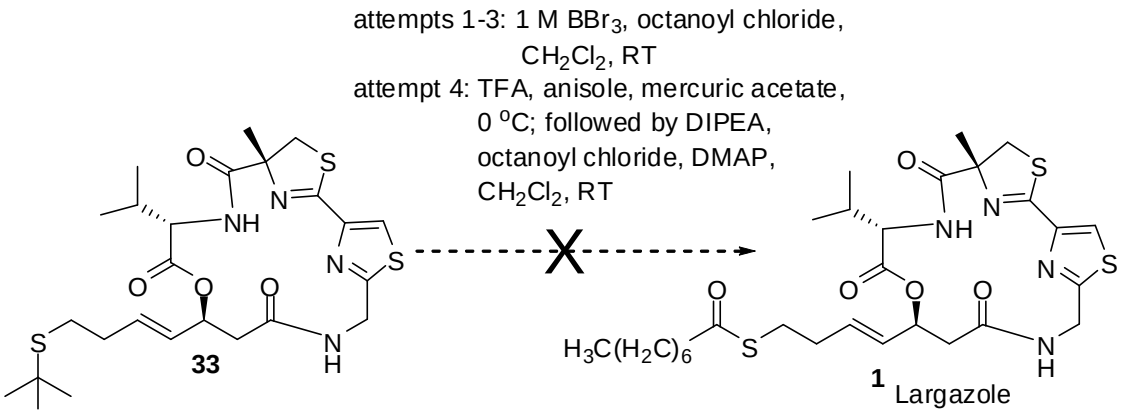
stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **31** (0.012, 0.247 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-100%) yielded the acyclic precursor **32** (0.019 g, 95%). $[\alpha]_D^{20}$ - 42 (*c* 0.095, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.39 (dt, *J* = 2.0, 7.6 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 6.86 (t, *J* = 6.0 Hz, 1H), 5.82-5.90 (m, 1H), 5.65-5.70 (m, 1H), 5.56 (dd, *J* = 7.6, 15.6 Hz, 1H) 5.27 (d, *J* = 8.0 Hz, 1H), 4.69-4.79 (m, 2H), 4.32-4.41 (m, 2H), 4.19 (t, *J* = 6.8 Hz, 1H), 4.05-4.13 (m, 1H), 3.85 (d, *J* = 11.2 Hz, 1H), 3.78 (s, 3H), 3.24 (d, *J* = 11.6 Hz, 1H), 2.63 (d, *J* = 5.6 Hz, 2H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.29 (q, *J* = 7.2 Hz, 2H), 2.06-2.13 (m, 1H), 1.76 (s, 1H), 1.62 (s, 3H), 1.29 (s, 9H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 171.5, 169.2, 168.6, 162.9, 156.6, 148.5, 143.9, 143.9, 141.5, 141.5, 134.5, 127.9, 127.5, 127.3, 127.3, 125.3, 125.2, 122.3, 120.2, 120.2, 84.7, 72.5, 67.3, 59.7, 53.1, 47.3, 42.3, 41.7, 41.3, 32.7, 31.2, 31.1, 27.7, 24.2, 19.3, 18.2.

Cyclic core 33

To a stirred solution of **32** (0.029 g, 0.0351 mmol, 1 equiv) in THF/H_2O (4:1, 1.1 mL) at 0 ^oC was added 0.1 M LiOH (0.35 mL, 0.035 mmol, 1.0 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 6 h it was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The product was purified by prep TLC to give the carboxylic acid. The carboxylic acid was dissolved in acetonitrile (3 mL) and treated with diethylamine (0.150 g, 1.46 mmol, 41.59 equiv). After stirring at room temperature for 3 h, it was concentrated *in vacuo* to dryness to afford the free amino derivative. After drying azeotropically with toluene, it was treated with HATU (0.03 g, 0.0789 mmol, 2.25 equiv), HOAt (0.010 g, 0.0735 mmol, 2.09 equiv), dimethylformamide (30 mL, ~ 1 mM), and Hunig's base (0.023 g, 0.173 mmol, 4.91 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-80%) to yield the cyclic core **33** (0.0048 g, 24% over 3 steps from **27**). ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.17 (d, *J* = 9.6 Hz, 1H), 6.5 (dd, *J* = 3.0, 9.6 Hz, 1H), 5.87-5.92 (m, 1H), 5.68 (dt, *J* = 2.4, 7.2 Hz, 1H), 5.52 (dd, *J* = 7.2, 15.6 Hz, 1H), 5.29 (dd, *J* = 9.6, 17.4 Hz, 2H), 4.60 (dd, *J* = 3.0, 9.6 Hz, 1H), 4.26 (dd, *J* = 3.0, 17.4 Hz, 1H), 4.03 (d, *J* = 11.4 Hz, 1H), 3.28 (d, *J* = 11.4 Hz, 1H), 2.87 (dd, *J* = 4.2, 16.2 Hz, 1H), 2.71 (dd, *J* = 3.0, 16.2 Hz, 1H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.29-2.34 (m, 2H), 2.08-2.14 (m, 1H), 1.86 (s, 3H), 1.30 (s, 9H), 0.69 (d, *J* = 7.2 Hz, 3H), 0.50 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ173.3, 169.7, 169.1, 168.2, 147.6, 133.9,

127.8, 124.4, 84.6, 72.3, 58.0, 43.5, 41.3, 40.8, 38.8, 34.4, 32.8, 31.2, 27.7, 24.4, 19.1, 16.8.

Attempted conversion of 33 to largazole:



Attempt 1:[53] To a stirred solution of **33** (0.0032 g, 0.00579 mmol, 1 equiv) in dichloromethane (1 mL) were added octanoyl chloride (0.0019 g, 0.01166 mmol, 2 equiv) and BBr_3 in dichloromethane (1 M, 7 µL, 0.007 mmol, 1.21 equiv). The mixture was stirred overnight at room temperature. After TLC analysis, another portion of octanoyl chloride (0.0019 g, 0.01166 mmol, 2 equiv) was added and the mixture was stirred for another 5 h. Concentration of the reaction mixture *in vacuo* and purification by silica gel chromatography in ethyl acetate: hexanes gave the starting material **33** (0.002 g) and no largazole was isolated.

Attempt 2: To a stirred solution of **33** (0.002 g, 0.00362 mmol, 1 equiv) in dichloromethane (0.5 mL) were added octanoyl chloride (0.01435 g, 0.0876 mmol, 24 equiv) and BBr_3 in dichloromethane (1 M, 14 µL, 0.014 mmol, 3.87 equiv). The reaction

mixture was stirred for 2 days at room temperature. TLC analysis (4% MeOH: DCM) showed a new spot of low Rf (0.05), but chromatographic separation on silica gel in methanol: dichloromethane (4%) did not give any free thiol, largazole or the starting material.

Attempt 3: To a stirred solution of **33** (0.004 g, 0.00724 mmol, 1 equiv) in dichloromethane (0.5 mL) were added octanoyl chloride (0.0048 g, 0.0285 mmol, 3.93 equiv) and BBr_3 in dichloromethane (1 M, 14 µL, 0.014 mmol, 1.94 equiv). The reaction mixture was stirred at room temperature for 6 h. After monitoring by TLC more BBr_3 solution (2 equiv) and octanoyl chloride (8 equiv) were added and the reaction mixture was stirred overnight. Purification by column chromatography resulted in recovery of staring material (0.0025 g) but no thiol or largazole was isolated.

Attempt 4:[54] Trifluoroacetic acid (0.148 g, 1.30 mmol, 288 equiv) was added to the thioether **33** (0.0025 g, 0.0045 mmol, 1 equiv) at 0 ^{o}C. To the stirred reaction mixture were added anisole (0.001 g, 0.009 mmol, 2 equiv) and mercuric acetate (0.0015 g, 0.0047 mmol, 1.04 equiv) and the mixture was stirred for 30 min. at room temperature. After the disappearance of the starting material (TLC) in favor of a more polar compound, reaction mixture was concentrated *in vacuo*. The residue was dissolved in acetonitrile (1 mL), and H_2S gas was bubbled through it to precipitate mercury as HgS. The mixture was filtered and concentrated *in vacuo*.

The residue was dissolved in dry dichloromethane (0.5 mL) and treated with Hunig's base (0.0059 g, 0.046 mmol, 10.23 equiv) and octanoyl chloride (0.0057 g, 0.035 mmol, 7.77 equiv). The reaction mixture was stirred at rt for 4 h. More Hunig's base (0.0045 g, 0.035 mmol, 7.77 equiv) and DMAP (0.001 g, 0.0082 mmol, 1.82 equiv) were added and the mixture was stirred at room temperature. It failed to give any starting material or product.

(2*E*)-5-[(Triphenylmethyl)thio]-2-pentenal (36).[55]

To a solution of triphenylmethanethiol (6.91g , 25 mmol, 2.09 equiv) in dichloromethane (100 mL) were added acrolein (1.965 g, 35 mmol, 2.9 equiv) and triethylamine (3.56 g, 35 mmol, 2.9 equiv). The reaction mixture was stirred for 1 h at room temperature and was concentrated to give the aldehyde **35** as a white solid, which was used in the next step without purification. A solution of the aldehyde **35** obtained above and (triphenylphosphoranylidene)acetaldehyde (3.64 g, 11.96 mmol, 1 equiv) in dry benzene (150 mL) was refluxed for 8 h. The reaction mixture was concentrated and purified by flash chromatography on silica gel in dichloromethane/hexanes (20-25%) to afford aldehyde **36** (5.83 g, 65% over the two steps); mp 140-141 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.43 (d, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 2.4, 7.6 Hz, 6H), 7.29 (dt, *J* = 2.0, 6.8 Hz, 6H), 7.22 (dt, *J* = 2.4, 7.2 Hz, 3H), 6.60-6.67 (m, 1H), 5.95-6.01 (dd, *J* = 8.0, 15.6 Hz, 1H), 2.29-2.37 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194, 156, 144.7, 133.8, 129.7, 128.2, 127, 67.2, 31.9, 30.2.

3*S*-Hydroxy-1-(4*R*-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4*E*-en-1-one (37).

To a stirred solution of acetyl Nagao chiral auxiliary **16** (1.493 g, 7.355 mmol, 1 equiv) in dichloromethane (60 mL) at 0 ^{o}C was added $TiCl_4$ (1.72 g, 9.05 mmol, 1.23equiv). After stirring for 5 minutes, the reaction mixture was cooled to –78 ^{o}C and Hunig's base (1.872 g, 9.2 mmol, 1.25 equiv) was added. The reaction mixture was stirred for 2 h at the same temperature and the aldehyde **36** (2.6 g, 7.263 mmol, 0.987 equiv) in dichloromethane (8 mL) was added dropwise. The reaction mixture was stirred for 1 h at -78 ^{o}C. It was removed from cooling bath, treated with water (15 mL), and diluted with dichloromethane (50 mL). The layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic layer was washed with saturated NaCl (40 mL) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel in dichloromethane/hexanes (25-90%) to give the major isomer **37** as a thick yellow oil (1.963 g, 76.5% brsm), and the diastereomer **38** (0.193 g, 7.5%) with recovery of acetyl Nagao chiral auxiliary (**16**) (0.513 g). Data for major isomer **37**: $[\alpha]_D^{20}$ - 149 (*c* 3.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.2 Hz, 6H), 7.28 (t, *J* = 7.2 Hz, 6H), 7.21 (t, *J* = 7.2 Hz, 3H), 5.61-5.55 (m, 1H), 5.46 (dd, *J* = 6.0, 15.2 Hz, 1H), 5.12 (t, *J* = 6.8 Hz, 1H), 4.57 (t, *J* = 5.8 Hz, 1H), 3.56 (dd, *J* = 2.8, 17.6 Hz, 1H), 3.47 (dd, *J* = 7.6, 11.6 Hz, 1H), 3.28 (dd, *J* = 8.8, 17.6 Hz, 1H), 2.99 (d, *J* = 11.6 Hz, 1H), 2.82 (s, 1H), 2.37-2.32 (m, 1H), 2.21 (t, *J* = 7.2 Hz, 2H), 2.09 (q, *J* = 7.2 Hz, 2H), 1.05 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.1, 172.7, 145, 132, 130.2, 129.7, 128, 126.8, 71.6, 68.6, 66.7, 45.4, 31.6, 31.6, 31, 30.8, 19.3, 18.0.

(*R*)-Methyl 2-(2-((3*S*-hydroxy-7-(tritylthio)hept-4*E*-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (39).

A solution of carboxylic acid **29** (0.136 g, 0.38 mmol, 1 equiv) in anhydrous methanol (5 mL) was bubbled with HCl gas for 5 minutes. The reaction mixture was stirred overnight and concentrated *in vacuo* to give compound **30** which was azeotropped with toluene before taking it to the next step. A mixture of above obtained compound **30** and DMAP (0.121 g, 0.992 mmol, 2.61 equiv) in dichloromethane (2 mL) was stirred for 5 minutes and a solution of aldol product **37** (0.214, 0.38 mmol, 1 equiv) in dichloromethane (1 mL) was added. The reaction mixture was stirred for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-100%) to afford the alcohol **39** (0.191 g, 78% over 3 steps a-c). $[\alpha]_D^{20}$ - 11 (*c* 3.55, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 6H), 7.26 (t, *J* = 7.6 Hz, 6H), 7.19 (t, *J* = 7.6 Hz, 3H), 7.07 (t, *J* = 6.0 Hz, 1H), 5.50-5.58 (m, 1H), 5.37-5.43 (dd, *J* = 6.0, 15.2 Hz, 1H), 4.63-4.74 (m, 2H), 4.43 (m, 1H), 3.86 (d, *J* = 11.6 Hz, 1H), 3.78 (s, 3H), 3.48 (s, 1H), 3.25 (d, *J* = 11.6 Hz, 1H), 2.34-2.46 (m, 2H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.05 (q, *J* = 7.2, Hz, 2H), 1.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.0, 168.1, 162.9, 148.4, 144.9, 132.4, 130.3, 129.7, 128, 126.8, 122.4, 84.6, 69.2, 66.7, 53.1, 42.9, 41.6, 40.9, 31.6, 31.4, 24.1. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{36}H_{38}N_3O_4S_3$, 672.2019; found, 672.2024.

(4*R*)-Methyl 2-(2-((8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)-but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (40).

To a solution of Fmoc-L-valine (0.09 g, 0.266 mmol, 1 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.45 g, 0.345 mmol, 1.29 equiv) and 2,4,6-trichlorobenzoyl chloride (0.78 g, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **39** (0.07 g, 0.104 mmol, 0.39 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-100%) yielded the acyclic precursor **40** (0.100 g, 97%). $[\alpha]_D^{20}$ -12 (*c* 6.83, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.76 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.41-7.37 (m, 8 H), 7.30 (t, *J* = 7.2 Hz, 2H), 7.26-7.29 (m, 6 H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.74 (t, *J* = 8.4 Hz, 1H), 5.69-5.65 (m, 1H), 5.61 (dd, *J* = 6.0, 13.2 Hz, 1H), 5.42 (dd, *J* = 7.8, 15.0 Hz, 1H), 5.21 (d, *J* = 7.8 Hz, 1H), 4.7 (d, *J* = 6.0 Hz, 2H), 4.38 (dd, *J* = 7.2, 10.8 Hz, 1H), 4.33 (dd, *J* = 6.6, 10.8 Hz, 1H), 4.19 (t, *J* = 6.6 Hz, 1H), 4.05 (dd, *J* = 6.0, 8.4 Hz, 1H), 3.85 (d, *J* = 10.8 Hz, 1H), 3.78 (s, 3H), 3.24 (d, *J* = 10.4 Hz, 1H), 2.58 (d, *J* = 6.0 Hz, 2H), 2.2-2.12 (m, 2H), 2.07- 2.01 (m, 2H) 1.62 (s, 3H), 0.9 (d, *J* = 7.2 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 169.2, 168.6, 163, 156.6, 148.5, 144.9, 143.6, 141.5, 134.2, 129.7, 128.03, 127.9, 127.7, 127.3, 126.8, 125.2, 122.3, 120.15, 84.7, 72.42, 67.2, 66.8, 59.6, 53.1, 47.3, 41.7, 41.6, 41.3, 31.5, 31.3, 31, 29.9, 24.1, 19.2, 18.1. HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{56}H_{57}N_4O_7S_3$, 993.3397; found, 993.3389.

Cyclic core 41.[3a]

To a stirred solution of **40** (0.133 g, 0.134 mmol, 1 equiv) in THF/H_2O (4:1, 4 mL) at 0 ^oC was added 0.1 M LiOH (1.4 mL, 0.14 mmol, 1.045 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 1 h, it was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated and purified by prep TLC in ethyl acetate to give the carboxylic acid which was carried to the next step. The carboxylic acid was dissolved in dichloromethane (13 mL) and treated with diethylamine (0.462 g, 6.32 mmol, 47.16 equiv). After stirring at room temperature for 3 h, it was concentrated to dryness to afford the free amino derivative. After drying azeotropically with toluene, it was treated with HATU (0.105 g, 0.276 mmol, 2.06 equiv), HOAt (0.038 g, 0.279 mmol, 2.08 equiv), dichloromethane (130 mL, ~ 1 mM), and Hunig's base (0.074 g, 0.575 mmol, 4.29 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-60%) to yield the cyclic core **41** (0.056 g, 57% over three steps from **2**). $[\alpha]_D^{20}$ + 2.5 (*c* 0.95, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 6H), 7.26 (t, *J* = 8 Hz, 6H), 7.20-7.15 (m, 4H), 6.49 (dd, *J* = 2.8, 9.2 Hz, 1H), 5.68-5.71 (m, 1H), 5.60 (m, 1H), 5.38 (dd, *J* = 6.8, 15.6 Hz, 1H), 5.19 (dd, *J* = 8.8, 17.6 Hz, 1H), 4.55 (dd, *J* = 3.2, 9.6 Hz, 1H), 4.11 (dd, *J* = 3.2, 17.6 Hz, 1H), 4.01 (d, *J* = 11.2Hz, 1H), 3.26 (d, *J* = 11.6 Hz, 1H), 2.77 (dd, *J* = 9.6, 16.4 Hz, 1H), 2.64 (dd, *J* = 3.2, 16 Hz, 1H), 2.15-2.19 (m, 2H), 1.98-2.06 (m, 2H), 1.82 (s, 3H), 0.67 (d, *J* = 6.8 Hz, 3H), 0.50 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 173.7, 169.4, 168.9, 168.1, 164.6, 147.6, 144.9, 133.3, 129.7, 128.1, 126.8, 124.3, 84.6, 72.0, 66.8, 58.0, 43.5, 41.2, 40.8, 34.2, 31.5, 31.4, 29.9, 24.4, 19.1, 16.9.

Largazole (1).

To a solution of **41** (0.033 g, 0.045 mmol, 1 equiv) in dichloromethane (5 mL) at 0 ^{o}C was added triisopropylsilane (0.013 g, 0.083 mmol, 1.85 equiv) followed by trifluoroacetic acid (0.307 g, 2.69 mmol, 59.85 equiv). After stirring for 3 h at room temperature, the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate to obtain the largazole thiol (0.022 g). To a stirred solution of largazole thiol in dichloromethane (7 mL) at 0 ^{o}C, were added, Hunig's base (0.045 g, 0.35 mmol, 7.7 equiv) and octanoyl chloride (0.044 g, 0.27 mmol, 6 equiv). Catalytic DMAP (1.0 mg) in dichloromethane (0.1 mL) was added to the reaction mixture. After stirring for 4 h at room temperature, reaction was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by preparative thin layer chromatography on silica gel in ethyl acetate gave largazole (0.010 g, 79%, based on recovered largazole thiol 0.012 g). $[\alpha]_D^{20}$ + 19.5 (*c* 0.2, $CHCl_3$) (lit.[3a] $[\alpha]_D^{26.6}$ +38.9 (*c* 0.027, MeOH)). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.15 (d, *J* = 9.6 Hz, 1H), 6.41 (dd, *J* = 2.4, 9.0 Hz, 1H), 5.79-5.84 (m, 1H), 5.66 (m, 1H), 5.50 (dd, *J* = 6.6, 15.6 Hz, 1H), 5.29 (dd, *J* = 9.6, 18.0 Hz, 1H), 4.61 (dd, *J* = 3.0, 9.0 Hz, 1H), 4.27 (dd, *J* = 3.0, 17.4 Hz, 1H), 4.04 (d, *J* = 11.4 Hz, 1H), 3.28 (d, 11.4 Hz, 1H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.85 (dd, *J* = 10.8, 16.2 Hz, 1H), 2.53 (t, *J* = 7.8 Hz, 2H), 2.31 (q, *J* = 7.2, Hz, 2H), 2.10-2.12 (m, 1H), 1.87 (s, 3H), 1.62-1.67 (m, 2H), 1.26-1.31 (m, 8H), 0.87 (t, *J* = 7.2 Hz, 3H), 0.68 (d, *J* = 7.2 Hz, 3H), 0.5 (d, 6.6 Hz, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 199.6, 174.7, 169.6, 169.1, 168.1, 164.7, 147.7, 133.0, 128.6, 124.4, 84.7, 72.3, 57.9, 44.4, 43.6, 41.3, 40.7, 34.4, 32.5, 31.8, 29.1, 28.1, 25.9, 24.4, 22.8, 19.1, 16.8, 14.3. HRMS- ESI (*m/z*): $[M + Na]^+$ calcd for $C_{29}H_{42}N_4O_5S_3Na$, 645.2211 found, 645.2215. % purity: 95.31% (HPLC)

(*R*)-Methyl 2-(2-((5*R*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (42).

To a solution of Fmoc-D-1-naphthylalanine (0.130 g, 0.297 mmol, 3.06 equiv) in THF (1 mL) at 0 ^oC were added Hunig's base (0.045 g, 0.345 mmol, 3.55 equiv) and 2,4,6-trichlorobenzoyl chloride (0.078 g, 0.32 mmol, 3.3 equiv). The reaction mixture was stirred at 0 ^oC for 1 h. When TLC indicated formation of the anhydride, alcohol **39** (0.065 g, 0.097, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^oC. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-100%) yielded the acyclic precursor **42** (0.058 g, 55%). $[\alpha]_D^{20}$ - 1.38 (*c* 0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, *J* = 8.0 Hz, 1H), 7.80-7.85 (m, 2H), 7.75- 7.77 (m, 3H), 7.44-7.51(m, 4H), 7.34-7.41 (m, 9H), 7.23-7.29 (m, 9H), 7.15-7.19 (t, *J* = 7.2 Hz, 3H), 5.94 (t, *J* = 5.6 Hz, 1H), 5.55-5.62 (m, 1H), 5.39-5.46 (m, 2H), -5.32 (dd, *J* = 7.2, 15.6 Hz, 1H), 4.73 (q, *J* = 7.2 Hz, 1H), 4.52 (dd, *J* = 6.0, 16.0 Hz, 1H), 4.42 (dd, *J* = 5.6, 16.0 Hz, 1H), 4.35 (dd, *J* = 7.2, 10.4 Hz, 1H), 4.26 (dd, *J* = 7.2, 10.4 Hz, 1H), 4.13 (t, *J* = 7.2 Hz, 1H), 3.87 (d, *J* = 11.2 Hz, 1H), 3.79 (s, 3H), 3.49 (d, *J* = 8.8 Hz, 1H), 3.24 (d, *J* = 11.2 Hz, 1H), 2.28 (dd, *J* = 6.0, 14.4 Hz, 1H), 2.15 (t, *J* = 7.2 Hz, 2H), 2.00-2.09 (m, 2H), 1.63 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$) δ 173.8, 170.9, 168.9, 167.9, 162.9, 155.8,

148.5, 144.9, 143.9, 143.8, 141.4, 133.9, 133.9, 132.5, 132.1, 129.7, 129.1, 128.2, 128.1, 127.9, 127.8, 127.3, 127.3, 127.2, 126.9, 126.8, 126.3, 125.6, 125.3, 125.2, 123.6, 122.1, 120.2, 84.7, 72.7, 67.3, 66.8, 55.0, 53.2, 47.2, 41.7, 41.3, 41.1, 36.2, 31.5, 31.3, 24.2.

Cyclic core 43.

To a stirred solution of **42** (0.058 g, 0.0532 mmol, 1 equiv) in THF/H_2O (4:1, 1.6 mL) at 0 ^oC was added 0.1 M LiOH (0.53 mL, 0.053 mmol, 1.00 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 1 h, it was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The residue was purified by prep TLC in ethyl acetate to give the carboxylic acid. The carboxylic acid was dissolved in dichloromethane (13 mL) and treated with diethylamine (0.1917 g, 2.70 mmol, 50.75 equiv). After stirring at room temperature for 3 h, it was concentrated *in vacuo* to dryness to afford the free amino derivative. After drying azeotropically with toluene, it was treated with HATU (0.041 g, 0.108 mmol, 2.02 equiv), HOAt (0.015 g, 0.110 mmol, 2.07 equiv), dichloromethane (55 mL, ~ 1 mM), and Hunig's base (0.030 g, 0.23 mmol, 4.32 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-60%) to yield the cyclic core **43** (0.0057 g, 14%). $[\alpha]_D^{20}$ + 39.3 (*c* 0.112, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H) 7.68 (d, *J* = 9.0 Hz, 1H), 7.67 (s, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 6H), 7.32 (d, *J* = 7.2 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 6H), 7.19 (t, *J* = 7.2 Hz, 3H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 6.6 Hz, 1H), 6.39 (s, 1H), 5.68 (t, *J* = 9.6 Hz, 1H), 5.61-5.66 (m, 1H), 5.21 (dd, *J* = 8.4, 15.6 Hz, 1H),

5.05 (dd, *J* = 8.4, 17.4 Hz, 1H), 4.83 (m, 1H), 4.20-4.24 (m, 2H), 3.68 (dd, *J* = 5.4, 13.8 Hz, 1H), 3.19-3.22 (m, 2H), 2.81 (dd, *J* = 9.8, 16.8 Hz, 1H), 2.55 (d, *J* = 16.2 Hz, 1H), 2.05-2.21 (m, 3H), 2.00-2.03 (m, 1H), 1.72 (s, 3H), 1.21 (dd, *J* = 6.6, 16.2 Hz, 1H), 0.92-1.02 (m, 2H). ^{13}C (150 MHz, $CDCl_3$) δ 174.2, 169.6, 167.9, 167.4, 162.9, 147.8, 145.0, 134.8, 134.0, 132.5, 132.1, 129.8, 128.8, 128.3, 128.2, 128.1, 126.9, 126.7, 125.9, 124.9, 124.5, 124.3, 84.9, 72.8, 66.9, 55.7, 41.8, 41.5, 40.3, 37.1, 31.6, 31.2, 26.4. HRMS- ESI (*m/z*): $[M + H]^+$ calcd for $C_{48}H_{45}N_4O_4S_3$, 837.2603; found, 837.2599.

Analog T1A.

To a solution of **43** (0.005 g, 0.006 mmol, 1 equiv) in dichloromethane (1 mL) at 0 ^oC was added triisopropylsilane (0.0023 g, 0.0147 mmol, 2.44 equiv) followed by trifluoroacetic acid (0.046 g, 0.404 mmol, 67.25 equiv). After stirring for 3 h at room temperature, the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate to obtain the thiol. To a stirred solution of the thiol in dichloromethane (1 mL) at 0 ^oC were added Hunig's base (0.006 g, 0.046 mmol, 7.7 equiv) and octanoyl chloride (0.006 g, 0.035 mmol, 5.83 equiv). After stirring for 4 h at room temperature, the reaction was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by preparative thin layer chromatography on silica gel in ethyl acetate and final purification by HPLC on C_{18} column in methanol: water (20-100%) gave analog **T1A** (0.0022 g, 50%). $[\alpha]_D^{20}$ + 45.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.40 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.68 (s, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 6.6 Hz, 1H), 7.26-7.29 (m, 1H), 7.15 (d, *J* = 7.2 Hz, 1H), 6.39-6.41 (m,

1H), 5.73-5.78 (m, 2H), 5.35 (dd, *J* = 8.4, 15.6 Hz, 1H), 5.07 (dd, *J* = 7.8, 16.8 Hz, 1H), 4.86 (q, *J* = 7.8 Hz, 1H), 4.25 (dd, *J* = 4.8, 16.8 Hz, 1H), 4.21 (d, *J* = 11.4 Hz, 1H), 3.72 (dd, *J* = 4.8, 13.8 Hz, 1H), 3.25 (dd, *J* = 9.6, 13.8 Hz, 1H), 3.21 (d, *J* = 11.4 Hz, 1H), 2.83-2.88 (m, 3H), 2.59 (d, *J* = 16.8 Hz, 1H), 2.51 (t, *J* = 7.2 Hz, 2H), 2.22-2.31 (m, 2H), 1.74 (s, 3H), 1.60-1.65 (m, 2H) 1.25 (m, 11H), 0.87 (t, *J* = 7.2 Hz, 3H). ^{13}C (150 MHz, $CDCl_3$) δ 199.5, 174.2, 169.6, 167.9, 167.4, 162.9, 147.9, 134.2, 134.0, 132.5, 132.2, 128.9, 128.7, 128.3, 128.2, 126.7, 125.9, 124.9, 124.4, 124.3, 114.2, 85.0, 72.8, 55.7, 44.4, 41.8, 41.5, 40.4, 37.1, 32.4, 31.8, 29.9, 29.1, 28.0, 26.4, 25.8, 22.8, 14.2. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{32}H_{34}N_4O_5S_3Na$, 743.2372; found, 743.2372.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-5-allyl-1-(9*H*-fluoren-9-yl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (44).

To a solution of Fmoc-L-allylglycine (0.046 g, 0.137 mmol, 2 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.030 g, 0.230 mmol, 3.38 equiv) and 2,4,6-trichlorobenzoyl chloride (0.047 g, 0.192 mmol, 2.83 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **39** (0.046 g, 0.068 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-100%) yielded the acyclic precursor **44** (0.061 g, 90%). $[\alpha]_D^{20}$ - 12.53 (*c* 1.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 1H), 7.37-7.40 (m, 8H), 7.25-7.31 (m, 3H), 7.20 (t, *J* = 6.8 Hz, 3H), 6.84 (t, *J* = 6.0 Hz, 1H), 5.57-5.68 (m, 3H), 5.42 (dd, *J* = 7.2, 15.2, Hz, 1H), 5.32 (d, *J* = 7.6 Hz, 1H), 5.06-5.10

(m, 2H), 4.68 (d, *J* = 6.0 Hz, 2H), 4.31-4.38 (m, 2H), 4.26 (q, *J* = 6.4 Hz, 1H), 4.19 (t, *J* = 7.2 Hz, 1H), 3.85 (d, *J* = 11.2 Hz, 1H), 3.78 (s, 3H), 3.24 (d, *J* = 11.6 Hz, 1H), 2.58 (d, *J* = 6.4 Hz, 2H), 2.49-2.54 (m, 1H), 2.38-2.44 (m, 1H), 2.19 (t, *J* = 6.8 Hz, 2H), 2.05 (q, *J* = 7.2 Hz, 2H), 1.85 (s, 1H), 1.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 171.0, 169.2, 168.5, 163.0, 156.1, 148.5, 144.9, 143.9, 143.8, 141.5, 141.4, 134.0, 132.0, 129.7, 128.1, 127.9, 127.6, 127.3, 126.8, 125.3, 125.2, 122.3, 120.2, 119.8, 84.7, 72.7, 67.3, 66.8, 53.6, 53.1, 47.2, 41.7, 41.6, 41.2, 36.3, 31.5, 31.3, 24.1. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{56}H_{54}N_4O_7S_3Na$, 1013.3052; found, 1013.3058.

Cyclic core 45.

To a stirred solution of **44** (0.061 g, 0.0615 mmol, 1 equiv) in THF/H_2O (4:1, 2 mL) at 0 ^oC was added 0.1 M LiOH (0.71 mL, 0.071 mmol, 1.15 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 1 h, it was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated *in vacuo*. The reaction mixture was purified by preparative TLC in ethyl acetate: hexanes (70%) to give the carboxylic acid (0.043 g, 0.044 mmol, 1 equiv) which was carried to the next step. During purification, starting material **44** (0.01 g) was recovered. The carboxylic acid was dissolved in dichloromethane (4.5 mL) and treated with diethylamine (0.156 g, 2.14 mmol, 48.4 equiv). After stirring at room temperature for 3 h it was concentrated to dryness to afford the free amino derivative. After drying azeotropically with toluene it was treated with HATU (0.041 g, 0.108 mmol, 2.45 equiv), HOAt (0.015 g, 0.110 mmol, 2.29 equiv), dichloromethane (60 mL, ~ 1 mM), and Hunig's base (0.030 g, 0.23 mmol, 4.79 equiv) and the mixture was stirred for 30 h at room temperature. The reaction

mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-60%) to yield the cyclic core **45** (5.5 mg, 17%). $[\alpha]_D^{20}$ +20.0 (*c* 0.28, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 6H), 7.27 (t, *J* = 8.4 Hz, 6H), 7.19 (t, *J* = 8.4 Hz, 3H), 6.42 (dd, *J* = 3.6, 9.0 Hz, 1H), 5.64-5.70 (m, 2H), 5.36 (dd, *J* = 6.6, 15.0 Hz, 1H), 5.20 (dd, *J* = 9.6, 17.4 Hz, 1H), 4.69 (d, *J* = 16.8 Hz, 1H), 4.62-4.65 (m, 1H), 4.35 (d, *J* = 10.2 Hz, 1H), 4.16 (dd, *J* = 3.6, 18.0 Hz, 1H), 4.07 (d, *J* = 11.4 Hz, 1H), 4.21 (d, *J* = 11.4 Hz, 1H), 2.81 (dd, *J* = 10.2, 16.8 Hz, 1H), 2.63 (dd, *J* = 3.0, 16.8 Hz, 1H), 2.47-2.50 (m, 1H), 2.31-2.36 (m, 1H), 2.15-2.20 (m, 1H), 1.99-2.08 (m, 2H), 1.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 169.5, 169.1, 167.8, 164.4, 147.9, 145.0, 133.7, 131.5, 129.8, 128.1, 128.0, 126.9, 119.5, 84.5, 72.5, 52.7, 42.7, 41.3, 40.6, 38.0, 31.6, 31.4, 24.8. HRMS-ESI (*m/z*):: $[M + Na]^+$ calcd for $C_{40}H_{40}N_4O_4S_3Na$, 759.2109; found, 759.2039.

Analog T1B.

To a solution of **45** (0.0055 g, 0.0075 mmol, 1 equiv) in dichloromethane (1 mL) at 0 ^oC was added triisopropylsilane (0.0023 g, 0.0147 mmol, 1.96 equiv) followed by trifluoroacetic acid (0.057 g, 0.498 mmol, 66.78 equiv). After stirring for 3 h at room temperature the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate to obtain the thiol. To a stirred solution of the thiol in dichloromethane (1 mL) at 0 ^oC were added Hunig's base (0.006 g, 0.046 mmol, 7.7 equiv) and octanoyl chloride (0.006 g, 0.035 mmol, 5.83 equiv). After stirring for 4 h at room temperature reaction was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by preparative thin layer

chromatography on silica gel in ethyl acetate as solvent gave analog **T1B** (0.0016 mg, 35%). $[\alpha]_D^{20}$ + 25.56 (*c* 0.08, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 6.36 (dd, *J* = 3.0, 9.0 Hz, 1H), 4.79-4.83 (m, 1H), 5.73 (t, *J* = 8.4 Hz, 1H), 5.50 (dd, *J* = 7.2, 15.6 Hz, 1H) 5.26-5.32 (m, 3H), 4.67-4.72 (m 2H), 4.35 (d, *J* = 16.2 Hz, 1H), 4.27 (dd, *J* = 3.6, 18.0 Hz, 1H), 4.10 (d, *J* = 11.4 Hz, 1H), 3.22 (d, *J* = 11.4 Hz, 1H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.87 (dd, *J* = 10.8, 16.8 Hz, 1H), 2.68 (dd, *J* = 2.4, 16.2 Hz, 1H), 2.53 (t, *J* = 7.2 Hz, 3H), 2.34-2.39 (m, 1H), 2.31 (q, *J* = 7.2 Hz, 2H), 1.85 (s, 3H), 1.63-1.67 (m, 2H), 1.25-1.32 (m, 13H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.7, 173.7, 169.5, 169.1, 167.8, 164.4, 147.9, 133.2, 131.5, 128.5, 124.3, 121.8, 119.5, 84.5, 72.6, 52.8, 44.4, 42.7, 41.3, 38.0, 32.5, 31.8, 29.9, 29.1, 28.1, 25.9, 24.8, 22.8, 14.29. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{29}H_{40}N_4O_5S_3Na$, 643.2059; found, 643.2046.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-3,6,10-trioxo-5-(3-oxo-3-(tritylamino)propyl)-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (46).

To a solution of Fmoc-L-Gln(Trt)-OH (0.096 g, 0.157 mmol, 2.00 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.028 g, 0.219 mmol, 2.78 equiv) and 2,4,6-trichlorobenzoyl chloride (0.047 g, 0.192 mmol, 2.43 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **39** (0.053 g, 0.079 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-60%) yielded the acyclic precursor **46** (0.079, 78%). $[\alpha]_D^{20}$ - 11.22 (*c* 4.07, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (s, 1H), 7.73 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 7.6 Hz, 2H), 7.37 (d, *J* = 7.6 Hz, 7H), 7.17- 7.28 (m, 27 H) 7.11 (t, *J* = 6.0 Hz, 1H), 6.86 (s, 1H), 5.76 (d, *J* = 7.2 Hz, 1H), 5.57-5.64 (m, 1H), 5.50 (q, *J* = 5.6 Hz, 1H), 5.40 (dd, *J* = 7.2, 15.2 Hz, 1H), 4.60 (dd, *J* = 6.4, 16.0 Hz, 1H), 4.47 (dd, *J* = 6.0, 16.0 Hz, 1H), 4.3-4.40 (m, 2H), 4.17 (t, *J* = 6.8, 1H), 3.82 (d, *J* = 11.2 Hz, 1H), 3.76 (s, 3H), 3.22 (d, *J* = 11.2 Hz, 1H), 2.42-2.52 (m, 2H), 2.26-2.34 (m, 2H), 2.14-2.21 (m, 2H), 2.00-2.08 (m,2H), 1.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 171.0, 169.4, 168.9, 163.0, 156.5, 148.3, 144.9, 144.6, 143.9, 143.8, 141.4, 133.4, 129.7, 128.8, 128.1, 128.0, 127.9, 127.6, 127.3, 127.2, 126.8, 125.3, 122.1, 120.184.7, 72.7, 70.8, 67.1, 66.8, 54.1, 53.1, 47.3, 41.6, 41.4, 41.2, 33.0, 31.4, 31.3, 27.2, 24.1. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{36}H_{37}N_3O_4S_3Na$, 1286.4206; found, 1286.4214.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (48).

To a solution of Fmoc-L-1-naphthylalanine (0.052 g, 0.119 mmol, 1.95 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.028 g, 0.219 mmol, 3.59 equiv) and 2,4,6-trichlorobenzoyl chloride (0.031 g, 0.128 mmol, 2.09 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **39** (0.041 g, 0.061 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-60%) yielded the acyclic precursor **48** (0.065 g, 98%). $[\alpha]_D^{20}$ - 9.05 (*c* 3.25, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.80 (m, 2H), 7.73 (dd, *J* = 2.4, 7.2 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.42-7.50 (m, 4H), 7.36-7.39 (m, 8H), 7.24-7.29 (m, 10H), 7.18 (t, *J* = 7.2 Hz, 4H), 6.61 (t, *J* = 6.0 Hz, 1H), 5.39-5.43 (m, 2H), 5.30-5.35 (m, 1H), 5.11 (dd, *J* = 7.2, 15.6 Hz, 1H), 4.53-4.60 (m, 2H), 4.52 (dd, *J* = 6.0, 16.2 Hz, 1H), 4.28-4.35 (m, 2H), 4.09-4.11 (m, 1H), 3.82 (d, *J* = 11.4 Hz, 1H), 3.76 (s, 3H), 3.40-3.49 (m, 2H), 3.20 (d, *J* = 10.8 Hz, 1H), 2.41 (dd, *J* = 4.2, 14.4 Hz, 1H), 3.30 (dd, *J* = 6.6, 14.4 Hz 1H), 2.13 (t, *J* = 7.8 Hz, 2H) 1.97 (q, *J* = 7.2 Hz, 2H), 1.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 171.2, 169.2, 168.8, 163.0, 156.1, 148.5, 145.0, 143.8, 143.8, 141.5, 134.0, 133.6, 132.1, 132.1, 84.7, 72.7, 71.6, 67.3, 66.8, 60.6, 55.1, 53.1, 47.2, 41.6, 41.5, 41.2, 35.5, 31.5, 31.3, 24.1, 21.3, 20.6, 14.4. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{64}H_{58}N_4O_7S_3Na$: 1113.3365; found: 1113.3384.

(*R*)-2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylic acid (50).

To a well stirred mixture of the nitrile **7** (0.048 g, 0.2 mmol, 1 equiv) and $NaHCO_3$ (0.120 g, 1.43 mmol, 7.14 equiv) in methanol (2.5 mL) was added L-penicillamine (0.04 g, 0.268, 1.234 equiv) followed by phosphate buffer pH 5.95 (1.25 mL). The reaction mixture was degassed with nitrogen before stirring it under nitrogen at 70 ^oC for 1 h. It was acidified with 1 M HCl and extracted with ethyl acetate (15 mL) three times. The combined organic extract was washed with saturated NaCl solution, dried over anhydrous sodium sulphate and concentrated to obtain the carboxylic acid **50** (0.07 g) which was used in the next step without further purification.

(*R*)-Methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (51).

A solution of carboxylic acid **50** (0.07 g, 0.189 mmol, 1 equiv) in anhydrous methanol (5 mL) was bubbled with HCl gas for 5 minutes. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo* and was azeotropped using toluene before taking it to the next step. A mixture of above obtained compound and DMAP (0.06 g, 0.492 mmol, 2.60 equiv) in dichloromethane (2 mL) was stirred for 5 minutes, and a solution of aldol product **37** (0.106, 0.189 mmol, 1 equiv) in dichloromethane (1 mL) was added. The reaction mixture was stirred for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-100%) to afford the alcohol **51**. $[\alpha]_D^{20}$ - 5.28 (*c* 3.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 6H), 7.26 (t, *J* = 8.0 Hz, 6H), 7.19 (t, *J* = 7.2 Hz, 3H), 7.11 (q, *J* = 6.0 Hz, 1H), 5.50-5.57 (m, 1H), 5.39 (dd, *J* = 6.4, 15.6 Hz, 1H), 4.83 (s, 1H), 4.44-4.72 (m, 2H),

4.42 (s, 1H), 3.79 (s, 3H), 3.53 (s, 1H), 2.43 (dd, *J* = 3.6, 15.2 Hz, 1H), 2.38 (dd, *J* = 5.2, 13.2 Hz, 1H), 2.18 (t, *J* = 7.6 Hz, 2H), 2.03-2.08 (m, 2H), 1.75 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 170.0, 168.1, 164.8, 148.8, 145.0, 132.4, 130.2, 129.7, 128.0, 126.8, 122.4, 86.0, 69.2, 66.7, 60.7, 60.6, 42.8, 40.9, 31.6, 31.4, 28.6, 26.1, 21.2, 14.4.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (52).

To a solution of Fmoc-L-valine (0.080 g, 0.236 mmol, 2.48 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.040 g, 0.310 mmol, 3.26 equiv) and 2,4,6-trichlorobenzoyl chloride (0.069 g, 0.281 mmol, 2.96 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **51** (0.065 g, 0.095 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-60%) yielded the acyclic precursor **52**. $[\alpha]_D^{20}$ - 14.94 (*c* 0.435, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.76 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.35-7.41 (m, 8H), 7.25-7.35 (m, 8H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.77 (q, *J* = 8.4 Hz, 1H), 5.59-5.70 (m, 2H), 5.42 (dd, *J* = 7.6, 15.6 Hz, 1H), 5.24 (d, *J* = 8.4 Hz, 1H), 4.83 (s, 1H), 4.63-4.72 (m, 2H), 4.31-4.39 (m, 2H), 4.19 (t, *J* = 6.8 Hz, 1h), 4.06 (t, *J* = 8.4 Hz, 1H), 3.80 (s, 3 H), 2.57 (d, *J* = 6.0 Hz, 2H), 2.12-2.20 (m, 2H), 1.99-2.08 (m, 3H), 1.75 (s, 3H), 1.43 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 0.84 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.2, 168.7, 164.9, 156.5, 148.7, 144.9, 144.9, 143.9, 143.8, 141.4, 141.4, 134.1, 129.7, 128.0,

127.9, 127.7, 127.3, 127.2, 126.8, 125.2, 125.2, 120.1, 86.0, 72.4, 67.2, 66.7, 60.6, 60.5, 59.5, 52.4, 47.3, 41.5, 41.2, 31.4, 31.2, 31.0, 28.6, 26.8, 19.2, 18.0.

(*R*)-2,2,2-Trichloroethyl 2-(2-((*tert*-butoxycarbonylamino)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (54).

To a solution of carboxylic acid **50** (0.410 g, 1.105 mmol, 1 equiv), EDC.HCl (0.380 g, 1.98 mmol, 1.79 equiv) and DMAP (0.146 g, 1.2 mmol, 1.09 equiv) in dichloromethane (2 mL) were added Hunig's base (0.519 g, 4.02 mmol, 3.63 equiv) and trichloroethanol (1.55 g, 10 mmol, 9.05 equiv). The reaction mixture was stirred overnight at room temperature. The reaction mixture is partitioned between water and dichloromethane. The organic layer was washed with 10% HCl, 5% $NaHCO_3$, and brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude reaction product was purified by flash column chromatography on silica gel in ethyl acetate: hexanes (9-25%) to get pure ester **54** (0.195, 35%). $[\alpha]_D^{20}$ - 5.77 (*c* 0.26, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95 (s, 1H), 5.23 (s, 1H), 4.98 (s, 1H), 4.92 (d, *J* = 12.0 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 6.6 Hz, 2H), 1.83 (s, 3H), 1.52 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 168.1, 165.7, 155.9, 148.9, 122.1, 94.6, 85.8, 80.6, 74.9, 60.6, 42.5, 28.6, 28.3, 26.4.

(*R*)-2,2,2-Trichloroethyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (55).

To a solution of ester **54** (0.111 g, 0.221 mmol, 1 equiv) in dichloromethane (3 mL) was added 4N HCl in 1,4-dioxane (4.5 mL) over 5 minutes. The reaction mixture was stirred for 1 h and concentrated *in vacuo*. The residue was azeotropped with toluene before taking it to the next step. A mixture of above obtained compound and DMAP (0.07 g, 0.574 mmol, 2.60 equiv) in dichloromethane (2 mL) was stirred for 5 minutes and a solution of aldol product **37** (0.124, 0.221 mmol, 1 equiv) in dichloromethane (1 mL) was added. The reaction mixture was stirred for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-100%) to afford the alcohol **55** (0.111 g, 63%). $[\alpha]_D^{20}$ - 5.6 (*c* 1.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9 (s, 1H), 7.37 (d, *J* = 7.6 Hz, 6H), 7.25 (t, *J* = 7.2 Hz, 6H), 7.18 (t, *J* = 6.8 Hz, 3H), 5.49-5.55 (m, 1H), 5.39 (dd, *J* = 6.0, 15.6 Hz, 1H), 5.00 (s, 1H), 4.92 (d, *J* = 12.0 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.30-4.70 (m, 2H), 4.41 (s, 1H), 3.58 (s, 1H), 2.35-2.44 (m, 2H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.00-2.07 (m, 1H), 1.82 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 168.4, 168.1, 165.8, 148.7, 145.1, 132.6, 130.3, 129.8, 128.1, 126.9, 122.6, 94.6, 85.7, 74.9, 69.3, 66.8, 60.8, 42.95, 41.0, 31.7, 31.5, 28.3, 26.4.

(*R*)-2,2,2-Trichloroethyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (56).

To a solution of Fmoc-L-valine (0.146 g, 0.431 mmol, 1.98 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.074 g, 0.575 mmol, 2.64 equiv) and 2,4,6-trichlorobenzoyl chloride (0.125 g, 0.512 mmol, 2.39 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **55** (0.175 g, 0.218 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-60%) yielded the acyclic precursor **56** (0.106 g, 43%). $[\alpha]_D^{20}$ - 7.14 (*c* 0.28, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.75 (d, *J* =10.8 Hz, 2H), 7.57 (d, *J* = 10.8 Hz, 2H), 7.37-7.39 (m, 8H), 7.26-7.32 (m, 8H), 7.20 (t, *J* = 11.4 Hz, 3H), 6.85 (q, *J* = 9.0 Hz, 1H), 5.60-5.69 (m, 2H), 5.42 (dd, *J* = 10.8, 15.2 Hz, 1H), 5.30 (d, *J* = 12.0 Hz, 1H), 4.95 (s, 1H), 4.92 (d, *J* = 18.0 Hz, 1H), 4.77 (d, *J* = 18.6 Hz, 1H), 4.79 (d, *J* = 8.4 Hz, 1H), 4.31-4.41 (m, 2H), 4.19 (t, *J* = 10.8 Hz, 1H), 4.06-4.12 (m, 1H), 2.58 (d, *J* = 9.0 Hz, 2H), 2.06-2.21 (m, 2H), 2.00-2.08 (m, 1H), 1.82 (s, 3H), 1.51 (s, 3H), 0.90 (d, *J* = 10.2 Hz, 3H), 0.85 (d, *J* =10.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 168.8, 168.1, 165.6, 158.2, 156.6, 148.7, 145.0, 144.0, 143.9, 141.52, 134.3, 129.8, 128.1, 128.0, 127.8, 127.4, 126.9, 125.3, 125.3, 122.4, 120.2, 94.6, 85.8, 74.9, 72.5, 67.3, 66.9, 59.7, 47.4, 41.7, 41.4, 31.5, 31.4, 29.9, 28.3, 26.4, 21.3, 19.3, 18.1.

(*S*)-2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylic acid (57).

To a well stirred mixture of the nitrile **7** (0.096 g, 0.4 mmol, 1 equiv) and $NaHCO_3$ (0.232 g, 2.76 mmol, 5.6 equiv) in methanol (5 mL) was added (*s*)-α-methylcysteine hydrochloride **14** (0.084 g, 0.491, 1.23 equiv) followed by phosphate buffer pH 5.95 (2.5 mL). The reaction mixture was degassed with nitrogen before stirring it under nitrogen at 70 ^oC for 1 h. It was acidified with 1 M HCl and extracted with ethyl acetate (15 mL) three times. The combined organic extract was washed with saturated NaCl solution, dried over anhydrous sodium sulphate and concentrated to obtain the carboxylic acid **57** (0.112g) which was used in the next step without further purification.

(*S*)-Methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (59).

A solution of carboxylic acid **57** (0.056 g, 0.157 mmol, 1 equiv) in anhydrous methanol (5 mL) was bubbled with HCl gas for 5 minutes. The reaction mixture was stirred overnight and concentrated *in vacuo* to give compound **58** which was azeotropped with toluene before taking it to the next step. A mixture of above obtained compound **58** and DMAP (0.05 g, 0.410 mmol, 2.61 equiv) in dichloromethane (2 mL) was stirred for 5 minutes and was added a solution of aldol product **37** (0.088, 0.157 mmol, 1 equiv) in dichloromethane (1 mL). The reaction mixture was stirred for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (20-100%) to afford the alcohol **59** (0.07 g, 67% from nitrile **7** over 3 steps). $[\alpha]_D^{20}$ - 0.77 (*c* 0.65, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.37 (d, *J* = 7.2, Hz, 6H), 7.26 (t, *J* = 6.6 Hz, 6H), 7.19 (t, *J* = 7.2 Hz, 3H), 6.89 (t, *J* = 6.0 Hz, 1H), 5.52-5.56 (m, 1H), 5.40

(dd, *J* = 6.6, 15.0 Hz, 1H), 4.71 (m, 2H), 4.43 (m, 1H), 3.86 (d, *J* = 11.4 Hz, 1H), 3.78 (s, 3H), 3.26 (d, *J* = 11.4 Hz, 1H), 2.44 (dd, *J* = 3.0, 15.6 Hz, 1H), 2.38 (dd, *J* = 8.4, 15.0 Hz, 1H), 2.18 (t, 7.8 Hz, 2H), 2.05 (q, *J* = 7.2 Hz, 2H), 1.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.0, 167.9, 162.9, 148.5, 145.0, 132.4, 130.5, 129.7, 128.1, 126.8, 122.4, 84.7, 69.4, 66.8, 53.2, 42.9, 41.7, 41.0, 31.6, 31.5, 24.2. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{36}H_{37}N_3O_4S_3Na$, 694.1844; found, 694.1851.

(*S*)-Methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (60).

To a solution of Fmoc-L-valine (0.071 g, 0.209 mmol, 2 equiv) in THF (1 mL) at 0 ^oC were added Hunig's base (0.040 g, 0.31 mmol, 2.98 equiv) and 2,4,6-trichlorobenzoyl chloride (0.062 g, 0.255 mmol, 2.45 equiv). The reaction mixture was stirred at 0 ^oC for 1 h. When TLC indicated formation of the anhydride, alcohol **59** (0.07 g, 0.104 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^oC. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (20-100%) yielded the acyclic precursor **60** (0.095 g, 93%). $[\alpha]_D^{20}$ - 4.18 (*c* 2.75, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.38 (d, *J* = 7.2 Hz, 8H), 7.25-7.30 (m, 8H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.91 (t, *J* = 5.6 Hz, 1H), 5.59-5.71 (m, 2H), 5.39-5.46 (m, 1H), 5.30 (d, *J* = 8.4 Hz, 1H), 4.69 (d, *J* = 6.0 Hz, 2H), 4.30-4.40 (m, 3H), 4.19 (q, *J* = 7.2 Hz, 1H), 4.07 (dd, *J* = 3.6, 6.0 Hz, 1H), 3.83 (d, *J* = 11.2 Hz, 1H), 3.77 (s, 3H), 3.24 (d, *J* = 11.6, 1H), 2.57 (d, *J* = 5.6 Hz, 2H), 2.14-2.20 (m, 2H), 2.00-2.07 (m, 2H), 1.62 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H). ^{13}C

NMR (100 MHz, $CDCl_3$): δ 173.8, 169.3, 168.6, 163.1, 156.6, 148.3, 144.9, 144.0, 143.9, 143.8, 141.4, 141.4, 134.2, 129.7, 127.9, 127.7, 127.3, 126.8, 125.2, 125.2, 84.6, 72.4, 67.2, 66.8, 59.6, 53.1, 47.3, 47.3, 41.6, 41.6, 41.2, 31.5, 31.3, 31.0, 24.1, 19.2, 18.1. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{36}H_{37}N_3O_4S_3Na$, 1015.3209; found, 1015.3203.

Cyclic core 61.

To a stirred solution of **60** (0.095 mg, 0.096 mmol, 1 equiv) in THF/H_2O (4:1, 3 mL) at 0 ^oC was added 0.1 M LiOH (0.96 mL, 0.096 mmol, 1.0 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 1 h it was acidified with 1 M HCl solution and was extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated and purified by preparative TLC in ethyl acetate to give the carboxylic acid (0.052 g, 0.053 mmol, 1 equiv) which was carried to the next step. Also recovered starting ester **60** (0.039 g) during purification. The carboxylic acid was dissolved in dichloromethane (4 mL) and treated with diethylamine (0.213 g, 2.92 mmol, 55.05 equiv). After stirring at room temperature for 3 h it was concentrated to dryness to afford the free amino derivative. After drying azeotropically with toluene it was treated with HATU (0.041 g, 0.108 mmol, 2.04 equiv), HOAt (0.015 g, 0.110 mmol, 2.08 equiv), dichloromethane (60 mL, ~ 1 mM), and Hunig's base (0.030 g, 0.230 mmol, 4.33 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-60%) to yield the cyclic core **61** (0.013 g, 31%). $[\alpha]_D^{20}$ - 17.08 (*c* 0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 1H), 7.49 (d, *J* = 10.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 6H), 7.29 (t, *J* = 6.8 Hz, 6H), 7.23 (t, *J* = 7.2 Hz, 3H), 6.72 (t, *J* = 6.0 Hz, 1H), 5.56-5.57 (m, 1H), 5.14-5.20 (m, 1H),

5.03 (dd, *J* = 6.0, 15.6 Hz, 1H), 4.79 (dd, *J* = 7.2, 15.6 Hz, 1H), 4.57 (dd, *J* = 6.8, 14.4 Hz, 1H), 4.17 (d, *J* = 11.6 Hz, 1H), 3.94 (dd, *J* = 5.2, 15.6 Hz, 1H), 3.27 (d, *J* = 11.6 Hz, 1H), 2.64 (dd, *J* = 4.0, 10.4 Hz, 1H), 2.51 (dd, *J* = 4.0, 10.8 Hz, 1H), 2.07-2.25 (m, 3H), 1.80 (s, 3H), 1.52-1.58 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 169.3, 167.5, 163.0, 148.6, 144.7, 130.9, 129.8, 129.7, 128.2, 128.1, 127.1, 121.8, 85.2, 71.5, 67.2, 58.3, 42.9, 42.3, 32.7, 31.1, 31.1, 26.6, 19.3, 18.2. HRMS (ESI): *m/z*: $[M + Na]^+$ calcd for $C_{40}H_{42}N_4O_4S_3Na$: 761.2266; found: 761.2266.

Analog T3 (C-7 epimer of largazole).

To a solution of **61** (0.013 g, 0.0176 mmol, 1 equiv) in dichloromethane (2.5 mL) at 0 ^oC was added triisopropylsilane (0.007 g, 0.044 mmol, 2.5 equiv) followed by trifluoroacetic acid (0.1535 g, 1.35 mmol, 76.5 equiv). After stirring for 3 h at room temperature the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by methanol: ethyl acetate (1%) to obtain the thiol. To a stirred solution of the thiol in dichloromethane (1 mL) at 0 ^oC were added Hunig's base (0.012 g, 0.092 mmol, 5.23 equiv) and octanoyl chloride (0.0114 g, 0.0699 mmol, 3.97 equiv). After stirring for 4 h at room temperature, reaction was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by preparative thin layer chromatography on silica gel in ethyl acetate: hexanes (30-60%) gave analog **T3** (0.008 g, 72%). $[\alpha]_D^{20}$ - 28.57 (*c* 0.175, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (s, 1H), 7.37 (d, *J* = 10.2 Hz, 1H), 6.65 (t, *J* = 6.0Hz, 1H), 5.62 (q, *J* = 4.2 Hz, 1H), 5.16-5.24 (m, 2H), 4.85 (dd, *J* = 7.2, 15.6 Hz, 1H), 4.62 (dd, *J* = 6.6, 10.2 Hz, 1H), 4.56 (dd, *J* = 4.8,

15.6 Hz, 1H), 4.18 (d, *J* = 11.4 Hz, 1H), 3.31 (d, *J* = 11.4 Hz, 1H), 2.51-2.65 (m, 4H), 2.51 (t, *J* = 7.8 Hz, 2H), 2.14-2.20 (m, 1H), 1.96 (q, *J* = 7.2 Hz, 2H), 1.84 (s, 3H), 1.62-1.65 (m, 8H), 1.25-1.29 (m, 10 H), 1.03 (d, *J* = 7.2 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 199.6, 174.2, 169.2, 169.1, 167.7, 163.9, 148.4, 130.6, 127.0, 122.7, 84.9, 71.4, 58.4, 44.4, 42.4, 42.2, 40.3, 32.9, 32.3, 31.8, 29.9, 29.1, 27.8, 26.4, 25.9, 22.8, 19.2, 18.3, 14.3. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{29}H_{42}N_4O_5S_3Na$, 645.2215; found, 645.2197.

Analog T4A.

To a solution of **41** (0.016 g, 0.0217 mmol, 1 equiv) in dichloromethane (2.5 mL) at 0 ^oC was added triisopropylsilane (0.007 g, 0.044 mmol, 2.03 equiv) followed by trifluoroacetic acid (0.1535 g, 1.35 mmol, 62.05 equiv). After stirring for 3 h at room temperature the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate to obtain the largazole thiol. To a stirred mixture of largazole thiol, 2-bromomethylpyridine HCl (0.012 g, 0.0472 mmol, 2.17 equiv), tetrabutylammonium bromide (0.007 g, 0.0217 mmol, 1 equiv), and cesium carbonate (0.033 g, 0.101 mmol, 4.66 equiv), was added acetonitrile (1.0 mL), and stirred for 20 h at room temperature. The reaction mixture was concentrated *in vacuo* and purified by preparative thin layer chromatography in methanol: dichloromethane (2.5%) to obtain analog **T4A** (0.002 g, 25%). $[\alpha]_D^{20}$ + 13.64 (*c* 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, CDCl3): δ 8.52 (d, *J* = 4 Hz, 1H), 7.75 (s, 1H), 7.66 (dt, *J* = 1.6, 7.6 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.16 (m, 2H), 6.53 (dd, *J* = 3.2, 9.6 Hz, 1H), 5.82-5.89 (m, 1H), 5.63-5.67 (m, 1H), 5.48 (dd, *J* = 6.8, 15.6 Hz, 1H), 5.28 (dd, *J* = 9.6, 17.6 Hz, 1H), 4.59 (dd, *J*

= 3.2, 9.2 Hz, 1H), 4.26 (dd, *J* = 3.2, 17.6 Hz), 4.03 (d, *J* = 11.2 Hz, 1H), 3.82 (s, 2H), 3.27 (d, *J* = 11.2 Hz, 1H), 2.85 (dd, *J* = 10.4, 16.4 Hz, 1H), 2.69 (dd, *J* = 3.2, 16.4 Hz, 1H), 2.53 (t, *J* = 7.6 Hz, 2H), 2.30 (q, *J* = 6.8 Hz, 2H), 2.05-2.12 (m, 1H), 1.85 (s, 3H), 0.69 (d, *J* = 6.8 Hz, 3H), 0.51 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, CDCl3) δ 173.8, 169.6, 169.1, 168.2, 164.8, 159.0, 149.4, 147.7, 137.0, 133.3, 128.2, 124.3, 123.3, 122.2, 84.7, 72.2, 58.0, 43.5, 41.3, 40.7, 38.3, 34.3, 32.2, 31.1, 24.5, 19.1, 16.9. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{27}H_{33}N_5O_4S_3Na$, 610.1592; found, 610.1581.

Analog T4B.

To a solution of **41** (0.016 g, 0.0217 mmol, 1 equiv) in dichloromethane (2.5 mL) at 0 ^oC was added triisopropylsilane (0.007 g, 0.044 mmol, 2.03 equiv) followed by trifluoroacetic acid (0.1535 g, 1.35 mmol, 62.05 equiv). After stirring for 3 h at room temperature was added the 2-thiophene methanol (0.0126 g, 0.110 mmol, 5.06 equiv) and the reaction mixture was stirred for 50 minutes at room temperature. It was concentrated and purified by column chromatography on silica gel in ethyl acetate: hexanes (20-70%) which was repurified by prep TLC in methanol: dichloromethane (2.5%) to obtain analog **T4B** (0.0063 g, 49%). $[\alpha]_D^{20}$ + 13.92 (*c* 0.395, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.21 (t, *J* = 3.0 Hz, 1H), 7.17 (d, *J* = 9.6 Hz, 1H), 6.90-6.92 (m, 2H), 6.49 (dd, *J* = 3.0, 9.0 Hz, 1H), 5.84-5.89 (m, 1H), 5.64-5.68 (m, 1H), 5.49 (dd, *J* = 7.2, 15.6 Hz, 1H), 5.27 (dd, *J* = 9.0, 16.8 Hz, 1H), 4.60 (dd, *J* = 3.6, 9.6 Hz, 1H), 4.26 (dd, *J* = 3.0, 17.4 Hz, 1H), 4.03 (d, *J* = 11.4 Hz, 1H), 3.90 (s, 3H), 3.27 (d, *J* = 11.4 Hz, 1H), 2.84 (dd, *J* = 10.2, 16.2 Hz, 1H), 2.69 (dd, *J* = 3.6, 16.8 Hz, 1H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.30 (q, *J* = 7.2 Hz, 2H), 2.08-2.11 (m, 1H), 1.84 (s, 3H), 0.69 (d, *J* = 7.2 Hz, 3H), 0.50 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.8, 169.6, 169.1, 168.1, 164.8, 147.7, 142.2,

133.3, 128.2, 126.9, 126.3, 125.1, 124.4, 84.6, 72.2, 58.0, 43.5, 41.3, 40.7, 34.3, 32.1, 31.0, 30.8, 24.4, 19.1, 16.8. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{26}H_{32}N_4O_4S_4Na$, 615.1204; found, 615.1184. % Purity: 95.7% (HPLC).

Analog T4C.

To a solution of **41** (0.0078 g, 0.0106 mmol, 1 equiv) in dichloromethane (1.25 mL) at 0 ^oC was added triisopropylsilane (0.00464 g, 0.029 mmol, 2.77 equiv) followed by trifluoroacetic acid (0.092 g, 0.808 mmol, 76.22 equiv). After stirring for 3 h at room temperature was added the 2-hydroxybenzyl alcohol (0.004 g, 0.0323 mmol, 3.04 equiv) and it was stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* and purified by column chromatography on silica gel in ethyl acetate: hexanes (20-100%) followed by preparative TLC in ethyl acetate: hexanes (75%) to obtain analog **T4C** (0.0031 g, 49%). $[\alpha]_D^{20}$ + 17.86 (*c* 0.14, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.79 (s, 1H), 7.46 (s, 1H), 7.20 (d, *J* = 9.6 Hz, 1H), 7.14 (dd, *J* = 1.8, 7.2 Hz, 1H), 7.08 (dt, *J* = 1.8, 7.8 Hz, 1H), 6.82 (dt, *J* = 1.2, 7.8 Hz, 1H), 6.73 (dd, *J* = 1.2, 8.4 Hz), 6.70 (dd, *J* = 3.6, 8.4 Hz, 1H), 5.82-5.87 (m, 1H), 5.68-5.71 (m, 1H), 5.62 (dd, *J* = 6.0, 15.6 Hz, 1H), 5.19 (dd, *J* = 9.0, 17.4 Hz, 1H), 4.60 (dd, *J* = 3.6, 9.0 Hz, 1H), 4.20 (dd, *J* = 3.0, 17.4 Hz), 4.06 (d, *J* = 11.4 Hz, 1H), 3.80 (dd, *J* = 12.6, 27.6 Hz, 2H), 3.31 (d, *J* = 11.4 Hz, 1H), 2.88 (dd, *J* = 9.0, 16.2 Hz, 1H), 2.70 (dd, *J* = 3.0, 16.2 Hz, 1H), 2.58-2.62 (m, 1H), 2.46-2.51 (m, 1H), 2.28-2.33 (m, 2H), 2.06-2.12 (m, 1H), 1.85 (s, 3H), 0.71 (d, *J* = 7.2 Hz, 3H), 0.54 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, CDCl3) δ 173.6, 170.0, 168.9, 168.6, 165.8, 155.3, 147.4, 132.4, 130.9, 129.2, 128.9, 124.5, 123.6, 120.5, 116.7, 84.4, 71.7, 58.1, 43.6, 41.3, 40.9, 34.1, 32.2, 32.2, 31.1, 24.3, 19.1, 17.0. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{28}H_{34}N_4O_5S_3Na$, 625.1589; found, 625.1562.

Analog T4D.

To a solution of **41** (0.0067 g, 0.0091 mmol, 1 equiv) in dichloromethane (1.25 mL) at 0 ^{o}C was added triisopropylsilane (0.004 g, 0.025 mmol, 2.69 equiv) followed by trifluoroacetic acid (0.077 g, 0.675 mmol, 74.22 equiv). After stirring for 3 h at room temperature the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate to obtain the largazole thiol. To a stirred mixture of largazole thiol, N,N-dimethyl-2-chloroacetamide (0.0142 g, 0.116 mmol, 12.77 equiv), tetrabutylammonium bromide (0.005 g, 0.0155 mmol, 1.7 equiv), and cesium carbonate (0.025 g, 0.0767 mmol, 8.42 equiv), was added acetonitrile (0.4 mL), and stirred for 20 h at room temperature. The reaction mixture was concentrated *in vacuo* and the crude mixture was purified by preparative TLC in methanol:dichloromethane (2.5 %) followed by another preparative TLC in methanol:ethyl acetate (10%, 4 parts) and hexane (1 part) to obtain analog T4D (0.001 g, 19%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.15 (d, *J* = 9.6 Hz, 1H), 6.47 (dd, *J* = 3.0, 9.0 Hz, 1H), 5.85-5.90 (m, 1H), 5.64-5.67 (m, 1H), 5.52 (dd, *J* = 6.6, 15.6 Hz, 1H), 5.27 (dd, *J* = 9.0, 17.4 Hz, 1H), 4.58 (dd, *J* = 3.6, 9.6 Hz, 1H), 4.26 (dd, *J* = 3.6, 18.0 Hz, 1H), 4.02 (d, *J* = 11.4 Hz, 1H), 3.26 (d, *J* = 10.8 Hz, 1H), 3.05 (s, 3H), 2.95 (s, 3H), 2.84 (dd, *J* = 10.2, 16.2 Hz, 2.66-2.70 (m, 3H), 2.36 (q, *J* = 7.2 Hz, 2H), 2.07-2.10 (m, 1H), 1.85 (s, 3H),0.68 (d, *J* = 7.2 Hz, 3H), 0.50 (d, *J* =7.2 Hz, 3H).

(S)-3-Hydroxy-1-((R)-4-isopropyl-2-thioxothiazolidin-3-yl)pent-4-en-1-one (62).

To a stirred solution of acetyl Nagao chiral auxiliary (**16)** (0.625 g, 3.079 mmol, 1 equiv) in dichloromethane (25 mL) at 0 ^{o}C was added $TiCl_4$ (0.645 g, 3.39 mmol, 1.10 equiv). After stirring for 5 minutes, the reaction mixture was cooled to –78 ^{o}C and Hunig's base (0.438 g, 3.39 mmol, 1.10 equiv) was added. The reaction mixture was stirred for 2 h at the same temperature. Acrolein (0.176 g, 3.146 mmol, 1.02 equiv) was added dropwise and the reaction mixture was stirred for 2.5 h at -78 ^{o}C. It was removed from cooling bath, treated with water (15 mL), and diluted with dichloromethane (50 mL). The layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic layer was washed with saturated NaCl (40 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel in dichloromethane/hexanes (25-90%) to give the major isomer **62** as a thick yellow oil (0.425 g, 53%), and the diastereomer **63** (0.053 g, 7.0%). Data for major isomer **62**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.91-5.96 (m, 1H), 5.34 (dt, *J* = 1.2, 16.2 Hz, 1H), 5.15-5.19 (m, 2H), 4.66-4.68 (m, 1H), 3.67 (dd, *J* = 3.0, 18.0 Hz, 1H), 3.53 (dd, *J* = 7.8, 11.4 Hz, 1H), 3.31 (dd, *J* = 9.0, 17.4 Hz, 1H), 3.04 (dd, *J* = 1.2, 12.0 Hz, 1H), 2.87 (d, *J* = 4.8 Hz, 1H), 2.34-2.40 (m, 1H), 1.07 (d, *J* = 6.6 Hz, 3H), 0.99 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.2, 172.5, 138.9, 115.4, 71.5, 68.9, 45.2, 30.9, 30.8, 19.2, 17.9.

(*R*)-Methyl 2-(2-(((*S*)-3-hydroxypent-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (64).

A solution of carboxylic acid **29** (0.047 g, 0.131 mmol, 1 equiv) in anhydrous methanol (5 mL) was bubbled with HCl gas for 5 minutes. The reaction mixture was stirred overnight and concentrated *in vacuo* which was azeotropped with toluene before taking it to the next step. A mixture of above obtained compound and DMAP (0.042 g, 0.344

mmol, 2.63 equiv) in dichloromethane (2 mL) was stirred for 5 minutes, and a solution of aldol product **62** (0.035, 0.135 mmol, 1.03 equiv) in dichloromethane (1 mL) was added. The reaction mixture was stirred for 1 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (25-100%) to afford the alcohol **64** (0.031 g, 64%). $[\alpha]_D^{20}$ - 23.04 (*c* 1.15, $CHCl_3$) (lit.[3a] $[\alpha]_D^{26.3}$ – 17.3 (*c* 1.00, $CHCl_3$)). ^{1}H NMR (600 MHz, CDCl3): δ 7.95 (s, 1H), 6.76 (s, 1H), 5.87-5.92 (m, 1H), 5.33 (d, *J* = 16.8 Hz, 1H), 5.16 (d, *J* = 10.2 Hz, 1H), 4.74-4.82 (m, 2H), 4.57 (s, 1H), 3.89 (d, *J* = 11.4 Hz, 1H), 3.81 (s, 3H), 3.29 (d, *J* = 11.4 Hz, 1H), 2.55 (dd, *J* = 3.0, 15.6 Hz, 1H), 2.45 (dd, *J* = 8.4, 15.0 Hz, 1H), 1.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.1, 168.0, 162.9, 148.4, 139.2, 122.6, 115.6, 84.7, 69.6, 53.2, 42.7, 41.7, 40.9, 24.2.

(*R*)-Methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-vinyl-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (65).

To a solution of Fmoc-L-valine (0.110 g, 0.325 mmol, 2.26 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.056 g, 0.431 mmol, 2.99 equiv) and 2,4,6-trichlorobenzoyl chloride (0.094 g, 0.384 mmol, 2.67 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **64** (0.053 g, 0.144 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (25-100%) yielded the acyclic precursor **65** (0.082, 83%). $[\alpha]_D^{20}$ - 2.07 (*c* 4.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.73 (d, *J* = 7.6 Hz, 2H), 7.55 (d, *J* = 6.8 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 2H), 7.19 (t, *J* = 6.0 Hz, 1H), 5.81-5.89 (m, 1H), 5.69 (q, *J* =

6.0 Hz, 1H), 5.45 (d, *J* = 8.4 Hz, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.22 (d, *J* = 10.8 Hz, 1H), 4.64-4.75 (m, 2H), 4.19-4.40 (m, 2H), 4.17 (t, *J* = 7.2 Hz, 1H), 4.11 (dd, *J* = 6.0, 8.0 Hz, 1H), 3.82 (d, *J* = 11.6 Hz, 1H), 3.75 (s, 3H), 3.22 (d, *J* = 11.6 Hz, 1H), 2.62 (d, *J* = 6.0 Hz, 2H), 2.03-2.11 (m, 1H), 1.6 (s, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 171.4, 169.2, 168.6, 162.9, 156.6, 148.3, 143.8, 143.8, 141.4, 134.4, 127.8, 127.2, 125.2, 122.3, 120.1, 118.6, 84.6, 72.5, 67.1, 59.7, 53.0, 47.2, 41.5, 41.2, 30.9, 24.0, 19.1, 18.0, 14.3.

Cyclic core 66.

To a stirred solution of **65** (0.0805 g, 0.117 mmol, 1 equiv) in THF/H_2O (4:1, 4 mL) at 0 ^{o}C was added 0.1 M LiOH (1.2 mL, 0.12 mmol, 1.03 equiv) dropwise over a period of 15 minutes. After being stirred at 0 ^{o}C for 1 h, it was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated to give the carboxylic acid which was carried to the next step. The carboxylic acid was dissolved in dichloromethane (10 mL) and treated with diethylamine (0.426 g, 5.84 mmol, 49.88 equiv). After stirring at room temperature for 3 h, it was concentrated *in vacuo* to dryness to afford the free amino derivative. After drying azeotropically with toluene, it was treated with HATU (0.089 g, 0.234 mmol, 2.00 equiv), HOAt (0.032 g, 0.235 mmol, 2.00 equiv), dichloromethane (90 mL, ~ 1 mM), and Hunig's base (0.061 g, 0.472 mmol, 4.034 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated to dryness and was purified by flash chromatography on silica gel in ethyl

acetate/hexanes (10-70%) to yield the cyclic core **66** (0.018 g, 35%). $[\alpha]_D^{20}$ + 36.82 (*c* 0.44, $CHCl_3$) (lit.[3a] $[\alpha]_D^{29.7}$ + 22.8 (*c* 0.33, MeOH)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.18 (d, *J* = 9.2 Hz, 1H), 6.47 (dd, *J* = 2.4, 9.2 Hz, 1H), 5.66-5.71 (m,1H), 5.24-5.38 (m, 3H), 4.62 (dd, *J* = 3.2, 9.2 Hz, 1H), 4.26 (dd, *J* = 3.2, 17.6 Hz, 1H), 4.07 (d, *J* = 11.2 Hz, 1H), 3.27 (d, *J* = 11.2 Hz, 1H), 2.86 (dd, *J* = 10.4, 16.4 Hz, 1H), 2.72 (dd, *J* = 2.8, 16.4 Hz, 1H), 2.06-2.14 (m, 1H), 1.86 (s, 3H), 0.69 (d, *J* = 6.8 Hz, 3H), .51 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 169.5, 169.1, 168.1, 164.8, 147.6, 134.9, 124.5, 118.1, 84.6, 72.5, 57.9, 43.5, 41.3, 40.3, 34.4, 24.4, 19.1, 16.8.

N-(benzyloxy)acrylamide (67).

To a solution of o-benzyl hydroxylamine HCl (0.176 g, 1.1 mmol, 1.1 equiv) in dichloromethane (2 mL) was added Hunig's base (0.284 g, 2.2 mmol, 2.2 equiv). The reaction mixture was cooled to 0 ^{o}C and was added acryloyl chloride (0.905 g, 1 mmol, 1 equiv) at 0 ^{o}C and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography on silica gel in ethyl acetate: hexanes (10-20%) to get compound **67** (0.11 g, 62%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.3(s, 1H), 7.35-7.39 (m, 5H), 6.16 (dd, *J* = 1.8, 17.4 Hz, 1H), 6.03 (dd, *J* = 10.2, 16.8 Hz, 1H), 5.65 (dd, *J* = 1.8, 10.2 Hz, 1H), 4.83 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0, 135.3, 129.3, 128.7, 128.6, 127.6, 127.4, 78.3.

Analog T5.

A reaction mixture of cyclic core **66** (0.030 g, .0687 mmol, 1 equiv), compound **67** (0.015 g, 0.0847 mmol, 1.23 equiv), Grubb's second generation catalyst (0.025 g, 0.0294

mmol, 0.428 equiv) and p-methoxyphenol (0.009 g, 0.0726 mmol, 1.06 equiv) in dichloromethane (1.5 mL) was refluxed at 40 ^{o}C for 36 hours. As TLC analysis indicated formation of a small amount of a new compound, additional Grubb's catalyst (0.007 g, 0.008 mmol, 0.12 equiv) in toluene (1.2 mL) was added and the mixture was stirred at 110 ^{o}C for 7 hours. Following TLC analysis, another portion of compound **67** (0.015 g, 0.0847 mmol, 1.23 equiv) in toluene (0.5 mL) was added and the mixture was stirred at 110 ^{o}C for additional 7 hours. The reaction mixture was cooled, treated with DMSO (100 µl) and stirred overnight at room temperature. The reaction mixture was concentrated in *vacuo* and purified by repeated flash chromatography on silica gel in ethyl acetate:hexanes (33-100%), and methanol:ethyl acetate (2%), followed by purification by successive preparative TLC in methanol:dichloromethane (4%), and acetone:hexane (10%) and reverse phase HPLC on C18 column in water: methanol (10-100%) to obtain **T5** (0.0005 g, 1.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ7.74 (s, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 6.0 Hz, 1H), 5.53 (q, *J* = 6.8 Hz, 1H), 5.26 (dd, *J* = 8.8, 16.8 Hz, 1H), 4.65 (dd, *J* = 6.6, 8.8 Hz, 1H), 4.25 (dd, *J* = 3.2, 17.2 Hz, 1H), 4.03 (d, *J* = 11.6 Hz, 1H), 3.49 (d, *J* = 4.8 Hz, 2H), 3.31-3.35 (m, 2H), 3.11 (dd, *J* = 5.2, 16.8 Hz, 1H), 2.05-2.15 (m, 1H), 1.86 (m, 3H), 0.74 (d, *J* = 2.0 Hz, 3H), 0.73 (d, *J* = 2.0 Hz, 3H).

Methyl 2-((*tert*-butoxycarbonylamino)methyl)thiazole-4-carboxylate (69).

To a solution of carboxylic acid **5** (0.050 g, 0.195 mmol, 1 equiv), EDC.HCl (0.075 g, 0.39 mmol, 2 equiv) in MeOH (2 mL) was added with Hunig's base (0.104 g, 0.8 mmol, 4 equiv). The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*. The residue was partitioned between water and ethyl acetate. The aqueous layer was extracted three times with ethyl acetate and the combined organic

extract was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude reaction mixture was purified by flash column chromatography on silica gel in ethyl acetate: hexanes (20-50%) to obtain **69** (0.036 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 5.38 (s, 1H), 4.62 (d, *J* = 6.0 Hz, 2H), 3.92 (s, 3H), 1.44 (s, 9H). ^{13}C (100 MHz, $CDCl_3$): δ 170.4, 161.9, 155.8, 146.6, 128.3, 80.7, 52.7, 42.5, 28.5.

(*R*,*E*)-Methyl 2-((3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazole-4-carboxylate (70).

Ester **69** (0.086 g, 0.316 mmol, 1 equiv) was treated with dichloromethane: trifluoroacetic acid (2:1, 1.8 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature and concentrated *in vacuo* which was azeotropped with toluene before taking it to the next step. A mixture of above obtained compound and DMAP (0.039 g, 0.320 mmol, 1.01 equiv) in dichloromethane (2 mL) was treated with Hunig's base (0.082 g, 0.636 mmol, 2.01 equiv) and stirred for 5 minutes. To the reaction mixture was added a solution of aldol diastereomer **38** (0.178, 0.316 mmol, 1 equiv) in dichloromethane (1 mL). The reaction mixture was stirred for 2 h, concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (25-80%) to afford the alcohol **70** (0.147 g, 81% over two steps from ester **69**). $[\alpha]_D^{20}$ + 4.78 (*c* 3.35, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.36 (d, *J* = 7.2 Hz, 6H), 7.25 (t, *J* = 7.2 Hz, 6H), 7.17 (t, *J* = 7.2 Hz, 3H), 5.50-5.54 (m, 1H), 5.38 (dd, *J* = 6.0, 15.0 Hz, 1H), 4.64-4.73 (m, 2H), 4.41 (br s, 1H), 3.89 (s, 3H), 2.43 (dd, *J* = 3.0, 15.0, 2.37 (dd, *J* = 9.0, 15.0 Hz, 1H), 2.16 (t, *J* = 7.8 Hz, 2H), 2.03 (q, *J* = 7.2 Hz, 2H). ^{13}C (150 MHz, $CDCl_3$): δ 172.3, 168.8, 161.8, 146.2, 144.9, 132.4, 130.2, 129.7, 128.7, 128.0, 126.8, 69.2, 66.7, 52.7, 42.9, 41.0, 31.5, 31.4. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{32}H_{32}N_2O_4S_2Na$, 595.1701; found, 595.1719.

Methyl 2-((5*S*,8*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (71).

To a solution of Fmoc-L-valine (0.018 g, 0.053 mmol, 2.0 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.0104 g, 0.08 mmol, 3.07 equiv) and 2,4,6-trichlorobenzoyl chloride (0.0156 g, 0.064 mmol, 2.44 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **70** (0.015 g, 0.0262 mmol, 1 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (33-800%) yielded partially pure **71**. This was taken to the next step without further purification.

Methyl 2-((5*S*,8*R*)-8-((*E*)-4-(acetylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (analog T6).

To a solution of **71** (0.035 g, 0.04 mmol, 1 equiv) in dichloromethane (5 mL) at 0 ^{o}C was added triisopropylsilane (0.013 g, 0.083 mmol, 2.08 equiv) followed by trifluoroacetic acid (0.307 g, 2.7 mmol, 67.5 equiv). After stirring for 3 h at room temperature, the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by methanol: ethyl acetate (2%) to obtain the thiol. To a stirred solution of the thiol in dichloromethane (2 mL) at 0 ^{o}C were added Hunig's base (0.0334 g, 0.2588 mmol, 6.47 equiv) and acetyl chloride (0.0132 g, 0.168 mmol, 4.2 equiv). After stirring for 4 h at room temperature, reaction was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by flash column chromatography

on silica gel in ethyl acetate: hexanes (40-100%) followed by preparative thin layer chromatography purification in ethyl acetate: hexanes (75%) gave analog **T6** (0.006 g, 22%). $[\alpha]_D^{20}$ + 10.71 (*c* 0.28, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.30 (dt, *J* = 4.2, 7.2 Hz, 2H), 6.61 (t, *J* = 6.0 Hz, 1H), 5.73-5.76 (m, 1H), 5.66 (dd, *J* = 4.8, 12.0 Hz, 1H), 5.51 (dd, *J* = 6.6, 15.6 Hz, 1H), 3.36 (d, *J* = 9.0, 1H), 4.76 (dd, *J* = 6.0, 16.2 Hz, 1H), 4.69 (dd, *J* = 6.0, 16.2 Hz, 1H), 4.39 (dd, *J* = 7.2, 10.2 Hz, 1H), 4.33 (dd, *J* = 6.6, 10.2 Hz, 1H), 4.19-4.23 (m, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 2.61 (dd, *J* = 7.8, 14.4 Hz, 1H), 2.55 (dd, *J* = 4.8, 15.0 Hz, 1H), 2.25-2.28 (m, 5H), 2.23-2.11 (m, 2H), 1.42 (d, *J* = 6.0 Hz, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H). ^{13}C (150 MHz, $CDCl_3$): δ 195.9, 171.1, 171.0, 169.2, 168.1, 161.8, 156.6, 156.6, 146.6, 144.0, 143.9, 141.5, 133.0, 128.6, 128.4, 127.9, 127.2, 125.3, 120.2, 72.4, 67.2, 59.3, 52.7, 47.3, 41.6, 41.2, 32.3, 31.2, 30.8, 28.3, 19.2, 17.7, 17.6. HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{35}H_{39}N_3O_8S_2Na$, 716.2076; found, 716.2056.

(*S*, *E*)-Methyl 2-((3-hydroxy-7-(tritylthio)hept-4enamido)methyl)thiazole-4-carboxylate (72).

Compound **69** (0.036 g, 0.132 mmol, 1 equiv) was treated with TFA: DCM (1:5) (2 mL). The reaction mixture was stirred for 2 h at room temperature. It was concentrated *in vacuo* and azeotropped many times with toluene. The residue was treated with Hunig's base in MeOH, stirred for 1 h at room temperature and concentrated *in vacuo*. To a mixture of the residue and DMAP (0.042 g, 0.344 mmol, 2.6 equiv) in dichloromethane (1mL), was added aldol product **37** (0.074 g, 0.131 mmol, 1 equiv) in dichloromethane (1 mL). The reaction mixture was stirred for 2 hours at room temperature. The reaction

mixture was concentrated *in vacuo* and purified by flash column chromatography on silica gel in ethyl acetate: hexanes (33-100%) to get **72** (0.07 g, 92%). $[\alpha]_D^{20}$ - 4.85 (*c* 0.7, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.37 (dd, *J* = 1.8, 9.0 Hz, 6H), 7.25 (t, *J* = 9.0 Hz, 6H), 7.18 (t, *J* = 7.2 Hz, 3H), 5.50-5.54 (m, 1H), 5.39 (dd, *J* = 6.6, 15.6 Hz, 1H), 4.64-4.73 (m, 2H), 4.42 (s, 1H), 3.89 (s, 3H), 3.54 (s, 1H), 2.43 (dd, *J* = 3.0, 15.0 Hz, 1H), 2.37 (dd, *J* = 9.0, 15.6 Hz, 1H), 2.17 (t, *J* = 7.8 Hz, 2H), 2.00-2.05 (m, 2H). ^{13}C (100 MHz, $CDCl_3$): δ 172.2, 168.7, 161.7, 146.2, 144.9, 132.4, 130.3, 129.7, 128.6, 128.0, 126.8, 69.2, 66.7, 52.7, 42.9, 41.0, 31.4.

Methyl 2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (73).

To a solution of Fmoc-L-valine (0.107 g, 0.316 mmol, 1 equiv) in THF (1 mL) at 0 ^oC were added Hunig's base (0.059 g, 0.46 mmol, 1.46 equiv) and 2,4,6-trichlorobenzoyl chloride (0.094 g, 0.38 mmol, 1.21 equiv). The reaction mixture was stirred at 0 ^oC for 1 h. When TLC indicated formation of the anhydride, alcohol **72** (0.09 g, 0.157 mmol, 0.5 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^oC. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification of the residue on silica gel in ethyl acetate/hexanes (20-80%) yielded the acyclic precursor **73** (0.13 g, 92%). $[\alpha]_D^{20}$ - 8.74 (*c* 1.75, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.02 (s, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7-37-7.40 (m, 8H), 7.25-7.31 (m, 8H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.91 (t, *J* = 6.0 Hz, 1H), 5.6-5.7 (m, 2H), 5.41 (dd, *J* = 7.6, 15.6 Hz, 1 H), 5.31 (d, *J* = 8.4 Hz, 1H), 4.64-4.77 (m, 2H), 4.28-4.39 (m, 2H), 4.17 (t, *J* = 6.8 Hz, 1H), 4.06 (t, *J* = 6.8 Hz, 1H), 3.89 (s, 3H), 2.58 (d, *J* = 5.6 Hz,

2H), 2.13-2.19 (m, 2H), 1.99-2.07 (m, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 171.4, 169.7, 169.2, 161.8, 156.6, 146.4, 145.0, 143.9, 143.9, 141.5, 141.4, 134.2, 129.7, 128.6, 128.1, 127.9, 127.6, 127.3, 127.3, 126.8, 125.2, 125.2, 120.2, 120.2, 72.4, 67.3, 66.8, 59.6, 526, 47.3, 41.5, 41.4, 31.5, 31.3, 31.0, 19.2, 18.1.

Synthesis of cyclic core 74.

To a stirred solution of **73** (0.130 mg, 0.153 mmol, 1 equiv) in THF/H_2O (4:1, 5 mL) at 0 ^oC was added 0.1 M LiOH (1.6 mL, 0.16 mmol, 1.046 equiv) dropwise over a period of 15 minutes. After stirring at 0 ^oC for 4.5 h, the reaction mixture was acidified with 1 M HCl solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, concentrated and purified by preparative TLC in ethyl acetate to give the carboxylic acid.

The carboxylic acid (0.097g, 0.118 mmol, 1 equiv) was dissolved in dichloromethane (10 mL) and treated with diethylamine (0.831 g, 11.38 mmol, 96.44 equiv). After stirring at room temperature for 3 h, it was concentrated *in vacuo* to dryness to afford the free amino derivative.

After drying azeotropically with toluene it was treated with HATU (0.084 g, 0.221 mmol, 2.0 equiv), HOAt (0.03 g, 0.221 mmol, 2.0 equiv), dichloromethane (110 mL, ~ 1 mM), and Hunig's base (0.067 g, 0.517 mmol, 4.38 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated *in vacuo* to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-100%) to yield the partially purified cyclic core **74** which was repurified by preparative TLC in ethyl acetate to obtain still partially pure cyclic core **74** (~ 0.012 g). This partially purified cyclic core was used in the next step without further purification.

Attempt to synthesize analog T7.

To a solution of **74** (0.012 g, 0.0188 mmol, 1 equiv) in dichloromethane (2.5 mL) at 0 ^{o}C was added triisopropylsilane (0.013 g, 0.083 mmol, 1.85 equiv) followed by trifluoroacetic acid (0.153 g, 1.35 mmol, 71.8 equiv). After stirring for 3 h at room temperature the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel in ethyl acetate/hexanes (20%) to first remove impurity followed by ethyl acetate and methanol: ethyl acetate (20%) to obtain the thiol. To a stirred solution of thiol in dichloromethane (1 mL) at 0 ^{o}C were added Hunig's base (0.012 g, 0. 08 mmol, 4.28 equiv) and octanoyl chloride (0.0095 g, 0.058 mmol, 3.1 equiv). After stirring for 2.5 h at room temperature, reaction mixture was quenched with methanol and the mixture was concentrated *in vacuo*. Purification of the crude product by flash column chromatography on silica gel in ethyl acetate: hexanes (10-100%) did not yield product.

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-hydroxy-7-(tritylthio)hept-4-enoate (75).

To a mixture of compound **37** (0.163 g, 0.29 mmol, 1 equiv) and DMAP (0.004 g, 0.033 mmol, 0.1 equiv) in dichloromethane (2 mL) was added 2-(trimethylsilyl)ethanol (0.043 g, 0.35 mmol, 1.2 equiv) and the mixture was stirred overnight at room temperature. It was concentrated *in vacuo* and purified by flash chromatography on silica gel in ethyl acetate/hexanes (3-10%) to afford the alcohol **75** (0.05 g, 33%). $[\alpha]_D^{20}$ - 1.27 (*c* 0.315, $CHCl_3$) (lit.[3b] $[\alpha]_D^{24}$ - 1.1 (*c* = 2, CHCl3)). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.39 (d, *J* = 7.2 Hz, 6H), 7.26 (t, *J* = 7.8 Hz, 6H), 7.19 (dt, *J* = 1.2, 7.2 Hz, 3H), 5.54-5.58 (m, 1H), 5.40 (dd, *J* = 6.6, 15.6 Hz, 1H), 4.42 (s, 1H), 4.17 (t, *J* = 9.0 Hz, 2H), 2.88 (s, 1H), 2.40-2.49 (m, 1H), 2.19 (t, *J* = 7.2 Hz, 2H), 2.03-2.08 (m, 2H), 0.97 (t, *J* = 9.0 Hz, 2H), 0.02 (s,

9H). ^{13}C (100 MHz, $CDCl_3$): δ 172.7, 145.0, 132.1, 130.3, 129.7, 128.0, 126.8, 68.8, 63.3, 41.7, 31.6, 31.5, 17.5, -1.3.

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-7-(tritlthio)hept-4-enoate (76).

To a solution of Fmoc-L-valine (0.05 g, 0.147mmol, 1 equiv) in THF (1 mL) at 0 ^{o}C were added Hunig's base (0.026 g, 0.196 mmol, 1.33 equiv) and 2,4,6-trichlorobenzoyl chloride (0.041 g, 0.166 mmol, 1.13 equiv). The reaction mixture was stirred at 0 ^{o}C for 1 h. When TLC indicated formation of the anhydride, alcohol **75** (0.05 g, 0.096 mmol, 0.66 equiv) in THF (1 mL) was added to the reaction mixture at 0 ^{o}C. It was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and flash chromatography purification on silica gel in ethyl acetate/hexanes (5-10%) yielded the compound **76** (0.077 g, 99%). $[\alpha]_D^{20}$ - 14.0 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.61 (dd, *J* = 3.0, 6.8 Hz, 2H), 7.40-7.42 (m, 8H), 7.26-7.35 (m, 8H), 7.21 (t, *J* = 7.2 Hz, 3H), 5.62-5.72 (m, 2H), 5.38 (dd, *J* = 7.6, 15.2 Hz, 1H), 5.31 (d, *J* = 8.8 Hz, 1H), 4.34-4.44 (m, 2H), 4.29 (dd, *J* = 4.4, 9.2 Hz, 1H), 4.26 (t, *J* = 7.2 Hz, 1H), 4.16 (t, *J* = 8.8, 2H), 2.69 (dd, *J* = 8.0, 15.6 Hz, 1H), 2.56 (dd, *J* = 5.6, 16.0 Hz, 1H), 2.11-2.21 (m, 3H), 2.05 (q, *J* = 6.8 Hz, 2H), 0.89-0.99 (m, 5H), 0.81 (d, *J* = 7.2 Hz, 3H), 0.03 (s, 9H). ^{13}C (100 MHz, $CDCl_3$): δ = 171.1, 169.8, 156.3, 145.0, 144.1, 144.0, 141.5, 134.2, 129.7, 128.0, 127.9, 127.2, 126.8, 125.3, 120.2, 120.1, 72.0, 67.2, 66.8, 63.3, 58.9, 47.4, 39.9, 31.6, 31.5, 31.3, 19.2, 17.5, 17.5, -1.3.

(*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(2-((*tert*-butoxycarbonylamino)methyl)thiazole-4-carboxamido)-3-methylbutanoyloxy)-7-tritylthio)hept-4-enoate (77)

The compound **76** (0.078g, 0.098 mmol, 1 equiv) was dissolved in dichloromethane (4.5 mL) and treated with diethylamine (0.355 g, 4.86 mmol, 49.6 equiv). After stirring at room temperature overnight, it was concentrated to dryness to afford the free amino derivative.

The free amine derivative along with HATU (0.074 g, 0.195 mmol, 2 equiv), HOAt (0.027 g, 0.195 mmol, 2 equiv), compound **5** (0.038 g, 0.147 mmol, 1.52 equiv) were dissolved in dichloromethane (4 mL). To the above reaction mixture was added Hunig's base (0.051 g, 0.39 mmol, 4 equiv) and the reaction mixture was stirred overnight at room temperature. It was concentrated *in vacuo* and purified by flash column chromatography on silica gel in ethyl acetate: hexanes (10-20%) to get **77** (0.064 g, 77%). $[\alpha]_D^{20}$ - 1.5 (*c* 0.4, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.72 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 1.8, 7.2 Hz, 6H), 7.28 (dt, *J* = 1.8, 7.2 Hz, 6H), 7.21 (dt, *J* = 1.2, 7.2 Hz, 3H), 5.67-5.71 (m, 1H), 5.63 (dd, *J* = 5.4, 7.8 Hz, 1H), 5.37 (dd, *J* = 7.8, 15.6 Hz, 1H), 4.67 (dd, *J* = 4.8, 9.0 Hz, 1H), 4.54-5.64 (m, 2H), 4.13 (m, 2H), 2.68 (dd, *J* = 7.8, 15.6 Hz, 1H), 2.54 (dd, *J* = 5.4, 15.6 Hz, 1H), 2.20-2.23 (m, 1H), 2.14-2.18 (m, 2H), 2.04 (q, *J* = 7.2 Hz, 2H), 1.47 (s, 9H), 0.94 (d, *J* = 7.2 Hz, 3H), 0.87 (d, *J* = 7.2 Hz, 3H), 0.00 (s, 9H). ^{13}C (100 MHz, $CDCl_3$): δ = 170.8, 169.8, 169.4, 160.9, 155.8, 149.6, 145.0, 134.2, 129.7, 128.0, 127.9, 126.8, 124.1, 80.6, 72.0, 66.8, 63.3, 56.9, 42.5, 39.9, 31.8, 31.5, 31.2, 28.5, 19.3, 17.8, 17.4, -1.3.

Cyclic core 74.

To a solution of compound **77** (0.06 g, 0.07 mmol, 1 equiv) in dichloromethane was added trifluoroacetic acid (1.075 g, 9.42 mmol, 134.6 equiv) very slowly. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*.

After drying azeotropically with toluene the residue was treated with HATU (0.054 g, 0.142 mmol, 2.0 equiv), HOAt (0.02 g, 0.147 mmol, 2.0 equiv), dichloromethane (70 mL, ~ 1 mM), and Hunig's base (0.060 g, 0.46 mmol, 6.57 equiv) and the mixture was stirred for 30 h at room temperature. The reaction mixture was concentrated *in vacuo* to dryness and was purified by flash chromatography on silica gel in ethyl acetate/hexanes (10-100%) to yield the partially pure cyclic core **74** (~ 0.002 g).

3.2 Experimental Biology

Cytoproliferation assay:

Cell proliferation was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reduction assay with the CellTiter 96 One solution MTS assay as described by the manufacturer (Promega, Madison, WI). Briefly, cells ($3x10^4$ cells/well) were seeded in quadruplicate in 96- well plates and allowed to attach overnight. Media was replaced with 100 uL of fresh medium containing the appropriate concentration of compounds **T1B**, **T3**, **T4A**, **T4B**, **T4C**, or largazole. Following incubation at 37 °C, 5% CO_2, 20 µL of CellTiter 96 One Solution was added per well and incubated 1.5 h at 37 °C and absorbance measured at 490 nm.

Evaluation of global histone acetylation levels:

HCT116 cells were treated for 24 hours with 10 nM, 100 nM, or 1 uM or DMSO. Cell lysates were extracted using RIPA buffer and equal amounts (30 ug/Lane) were loaded onto an SDS-PAGE gel. Standard Western blotting protocols were used using the Invitrogen NuPAGE western blotting system. Primary antibodies used were AcH3 (Millipore), α-tubulin (Sigma), and β-actin (Sigma). Dye-conjugated secondary antibodies from Li-Cor Biosciences were used for detection and scanned using the Odyssey Infrared Detection System (LI-COR Biosciences, Lincoln, NE).

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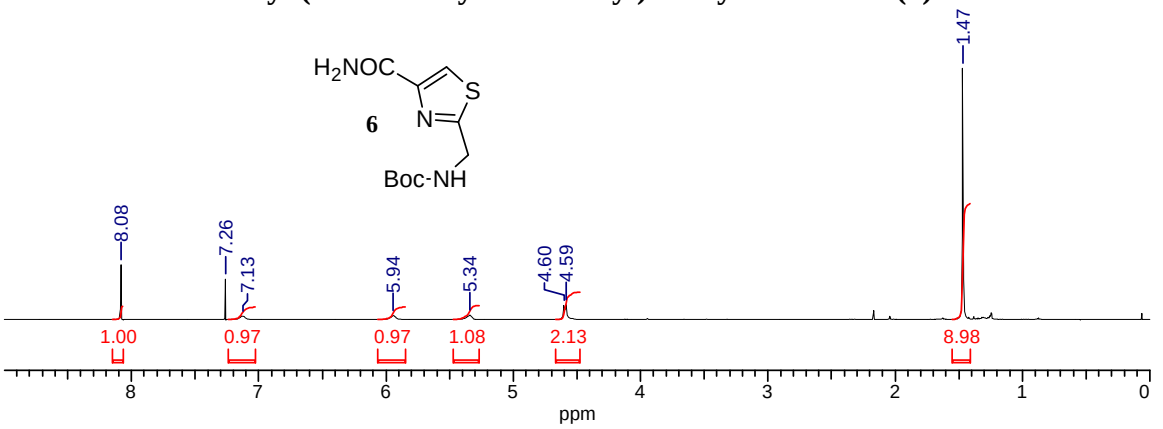
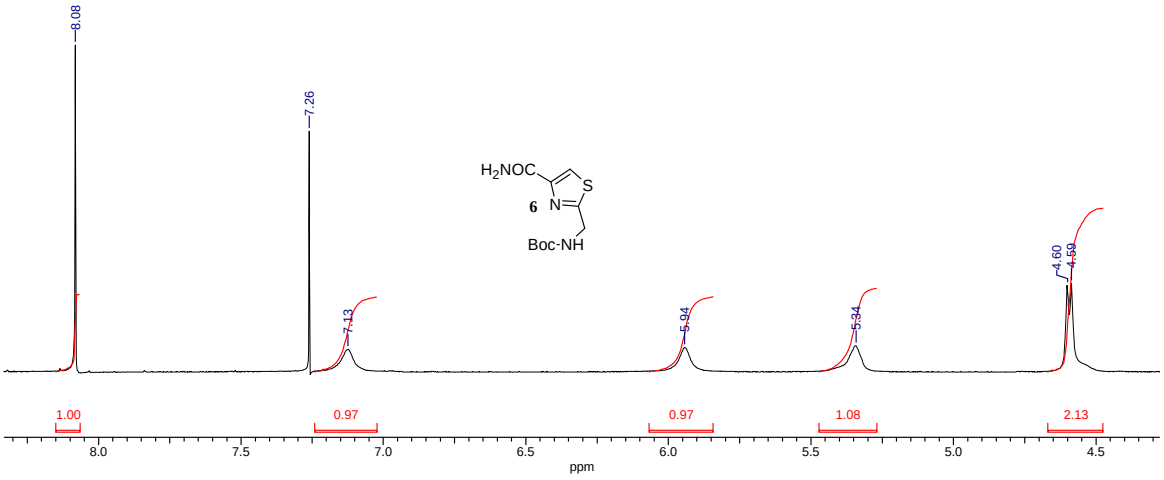
Appendix

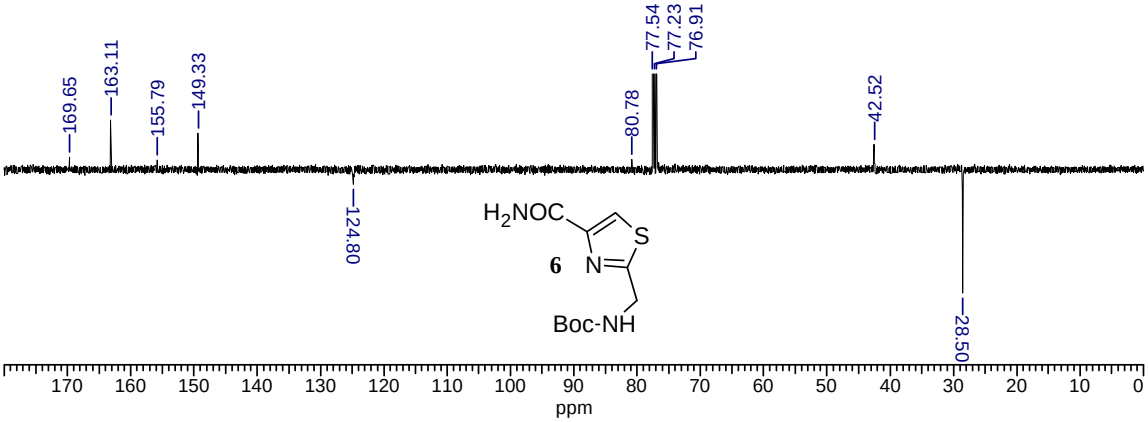
^{1}H and ^{13}C NMR Spectra

^{1}H NMR of *tert*-butyl (4-carbamoylthiazol-2-yl)methylcarbamate (6)

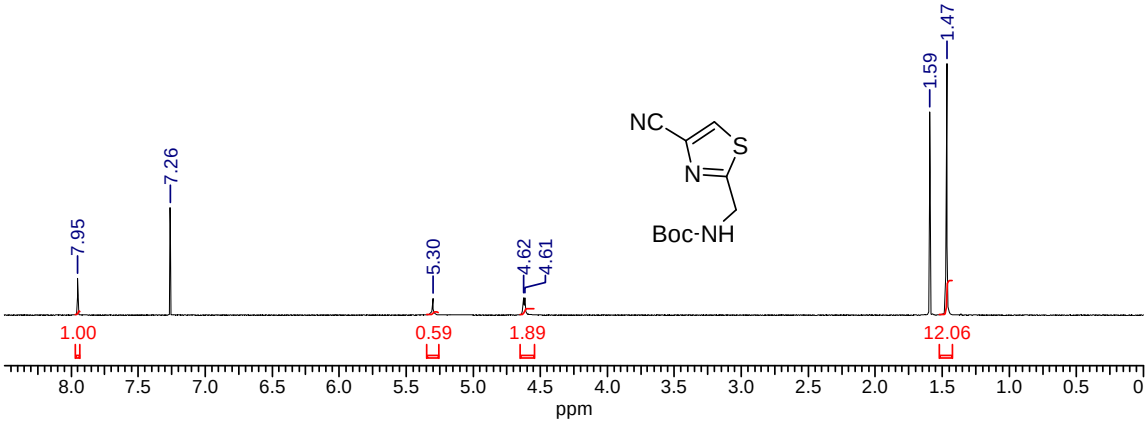


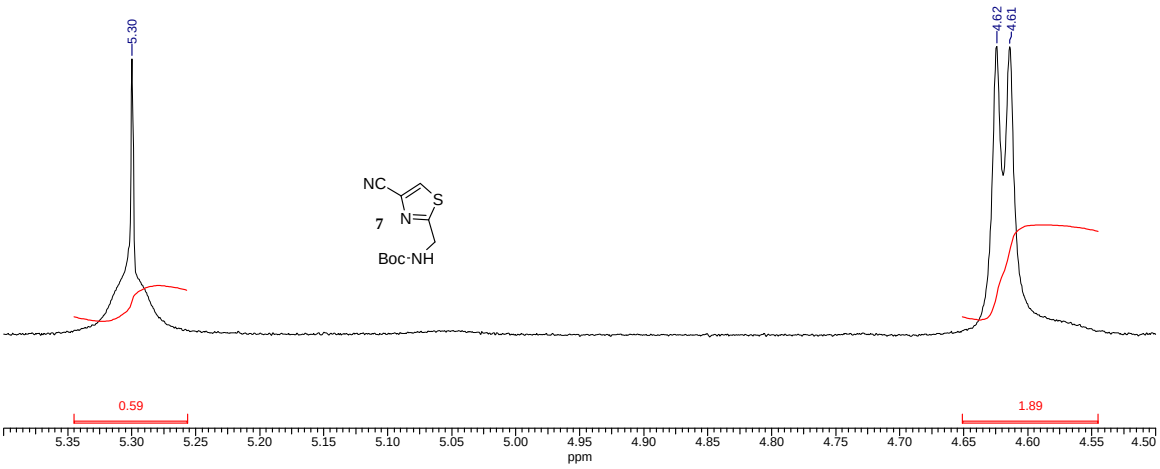


^{13}C NMR of *tert*-butyl (4-carbamoylthiazol-2-yl)methylcarbamate (6)

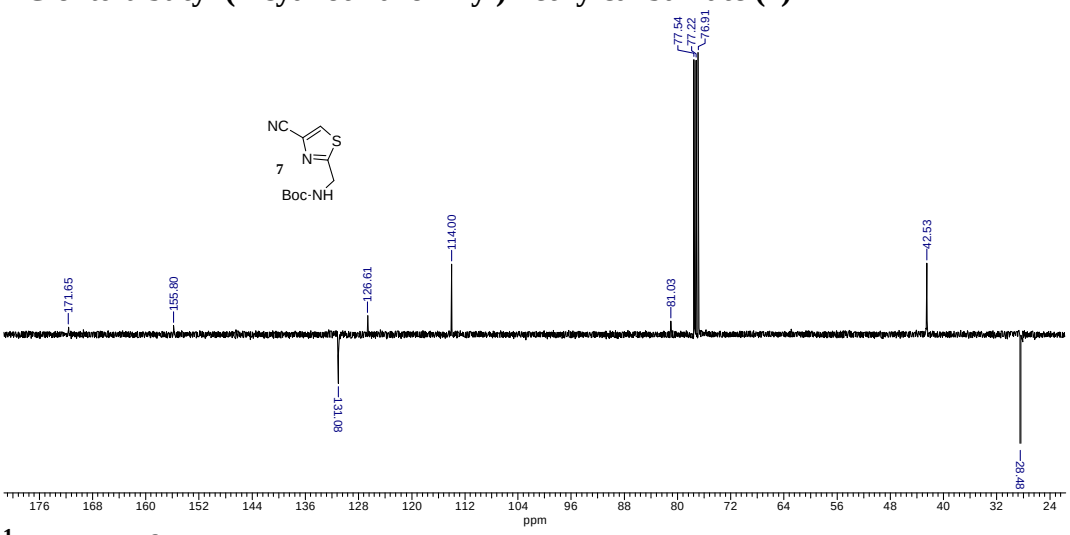


^{1}H NMR of *tert*-butyl (4-cyanothiazol-2-yl)methylcarbamate (7)

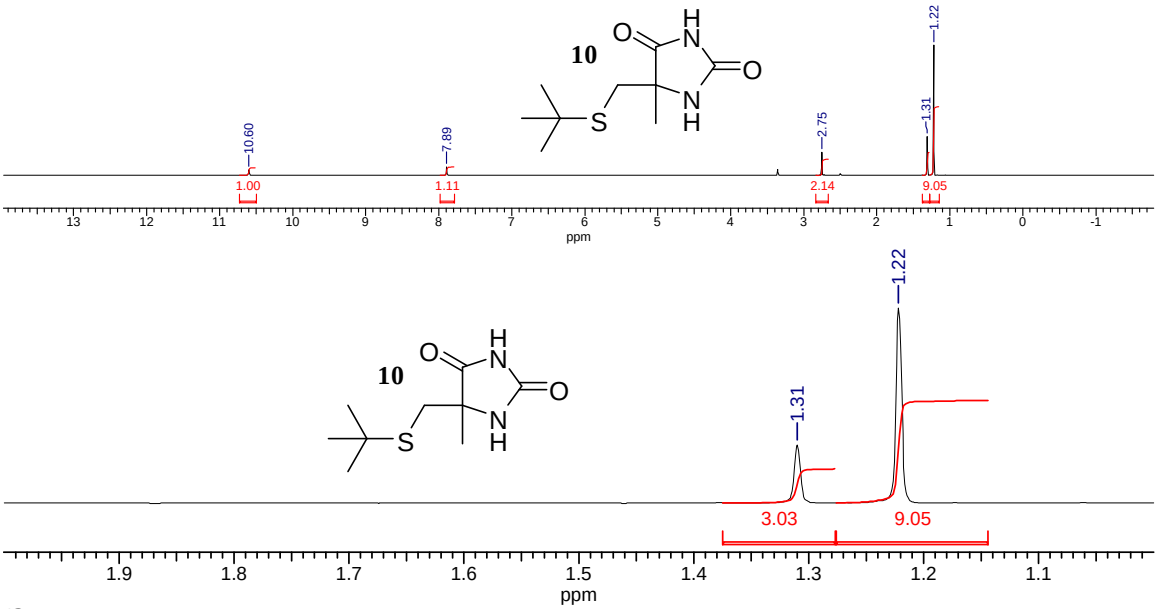




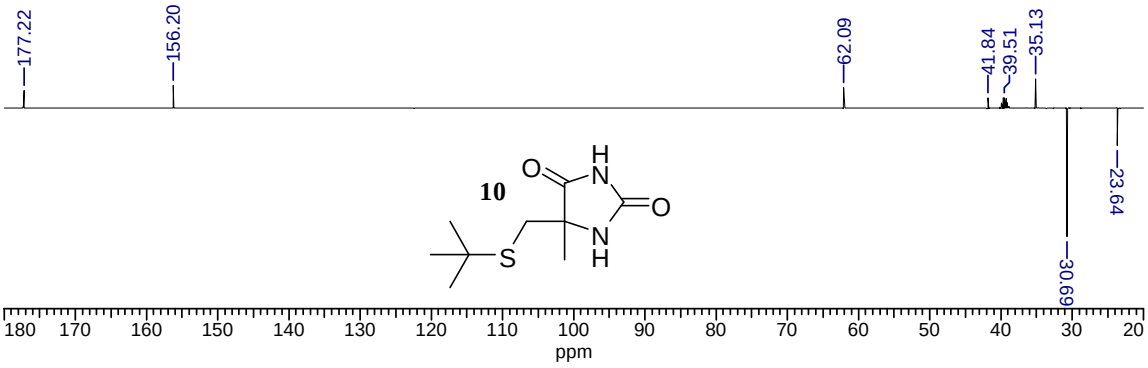
^{13}C of *tert*-butyl (4-cyanothiazol-2-yl)methylcarbamate (7)



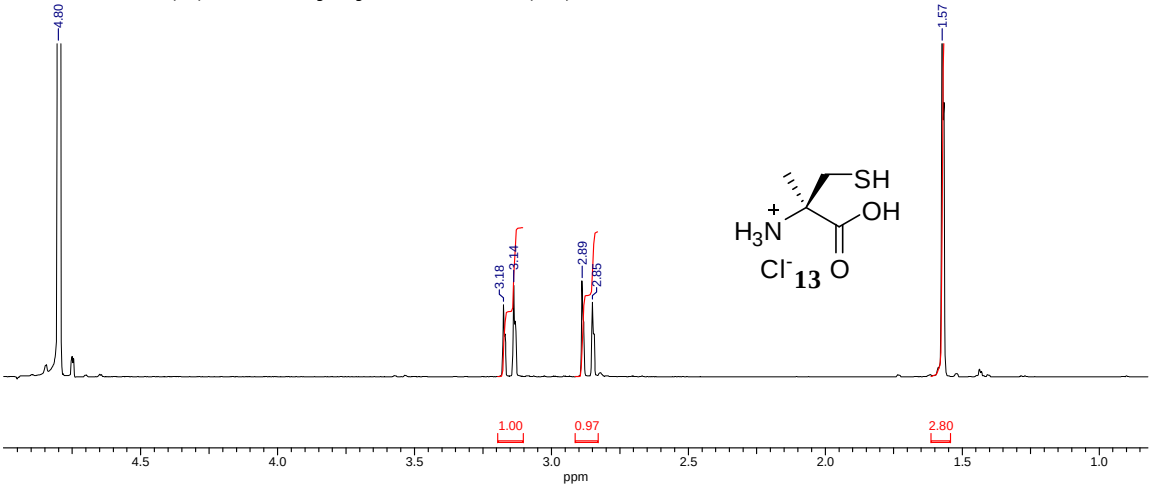
^{1}H NMR of 5-(*tert*-butylthiomethyl)-5-methylimidazolidine-2,4-dione (10)



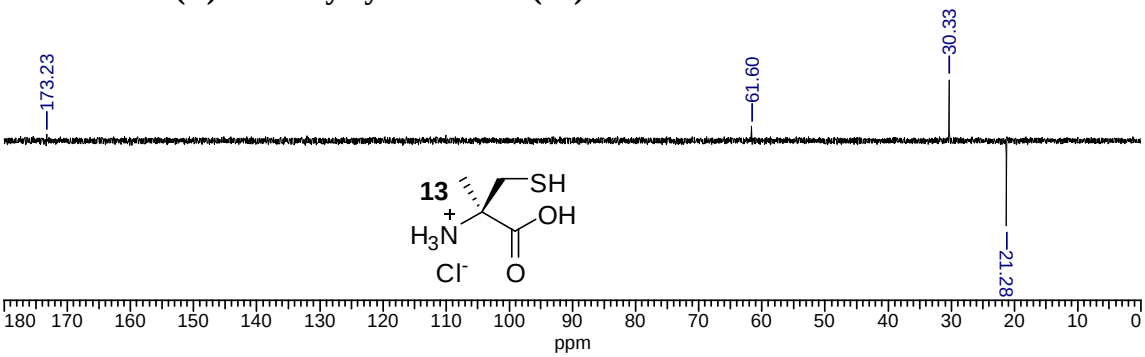
^{13}C NMR of 5-(*tert*-butylthiomethyl)-5-methylimidazolidine-2,4-dione (10)



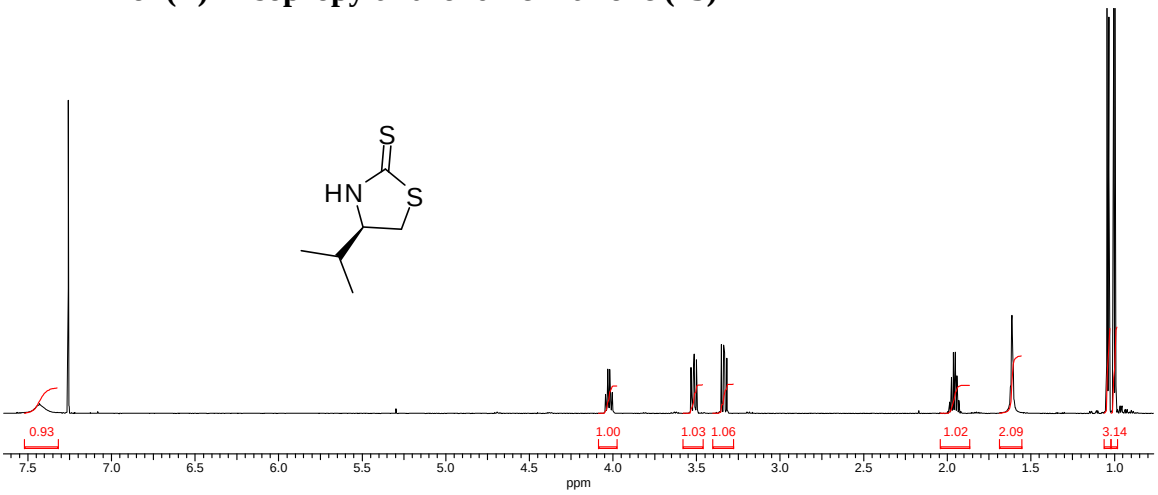
^{1}H NMR of (*R*)-α-methylcysteine HCl (13)



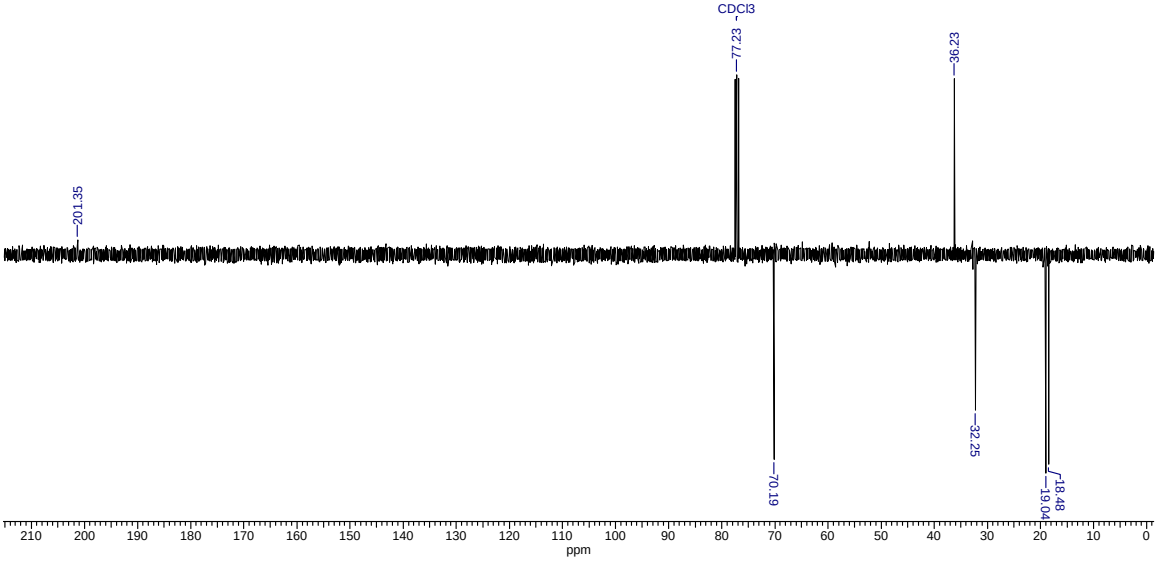
^{13}C NMR of (*R*)-α-methylcysteine HCl (13)



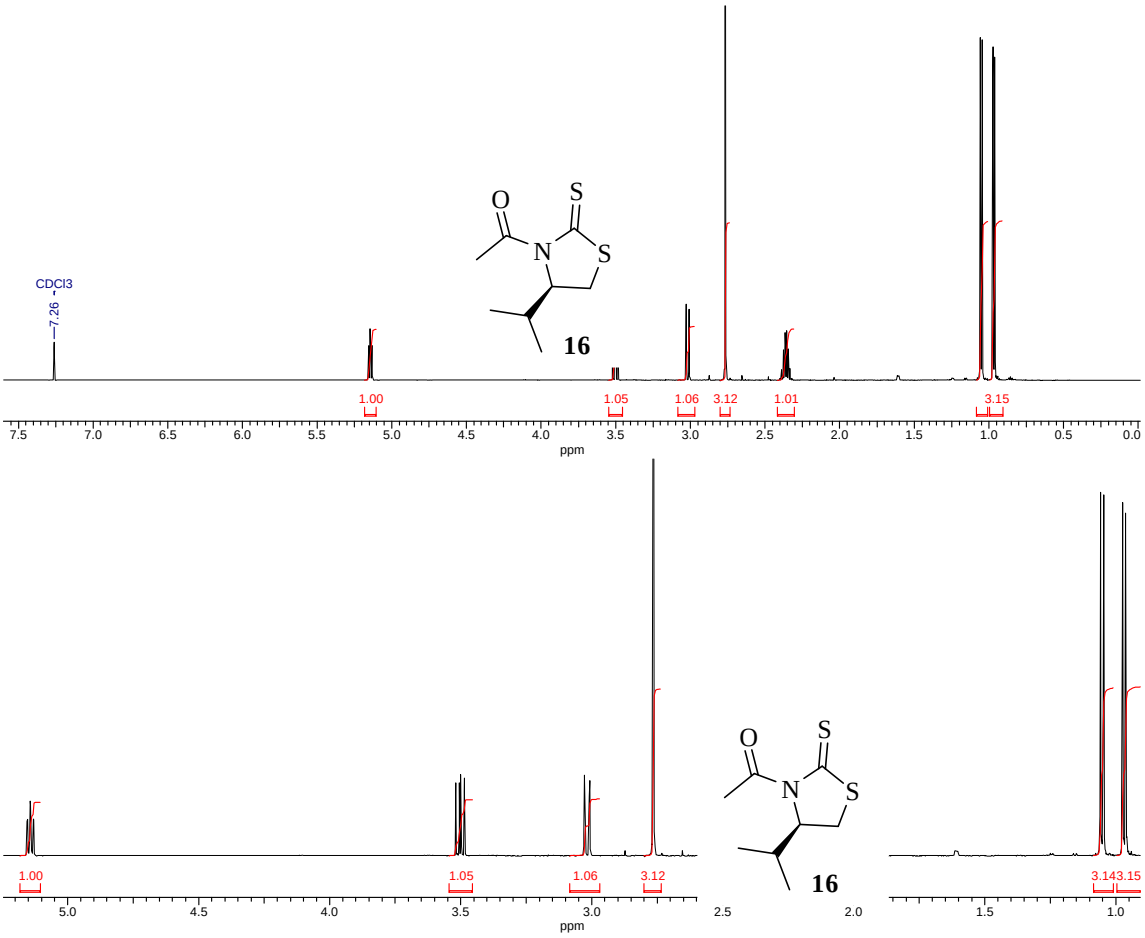
^{1}H NMR of (*R*)-4-isopropylthiazolidine-2-thione (15)



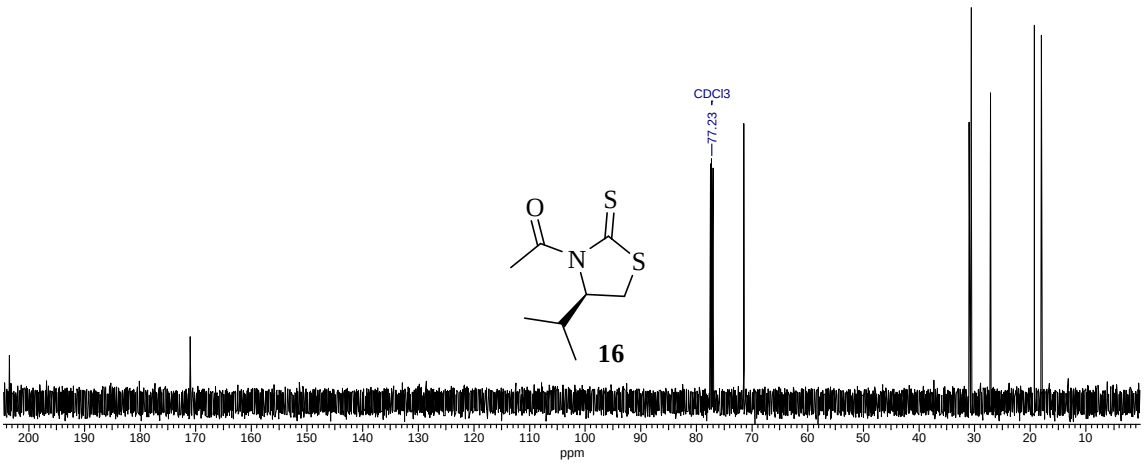
^{13}C NMR of (*R*)-4-isopropylthiazolidine-2-thione (15)



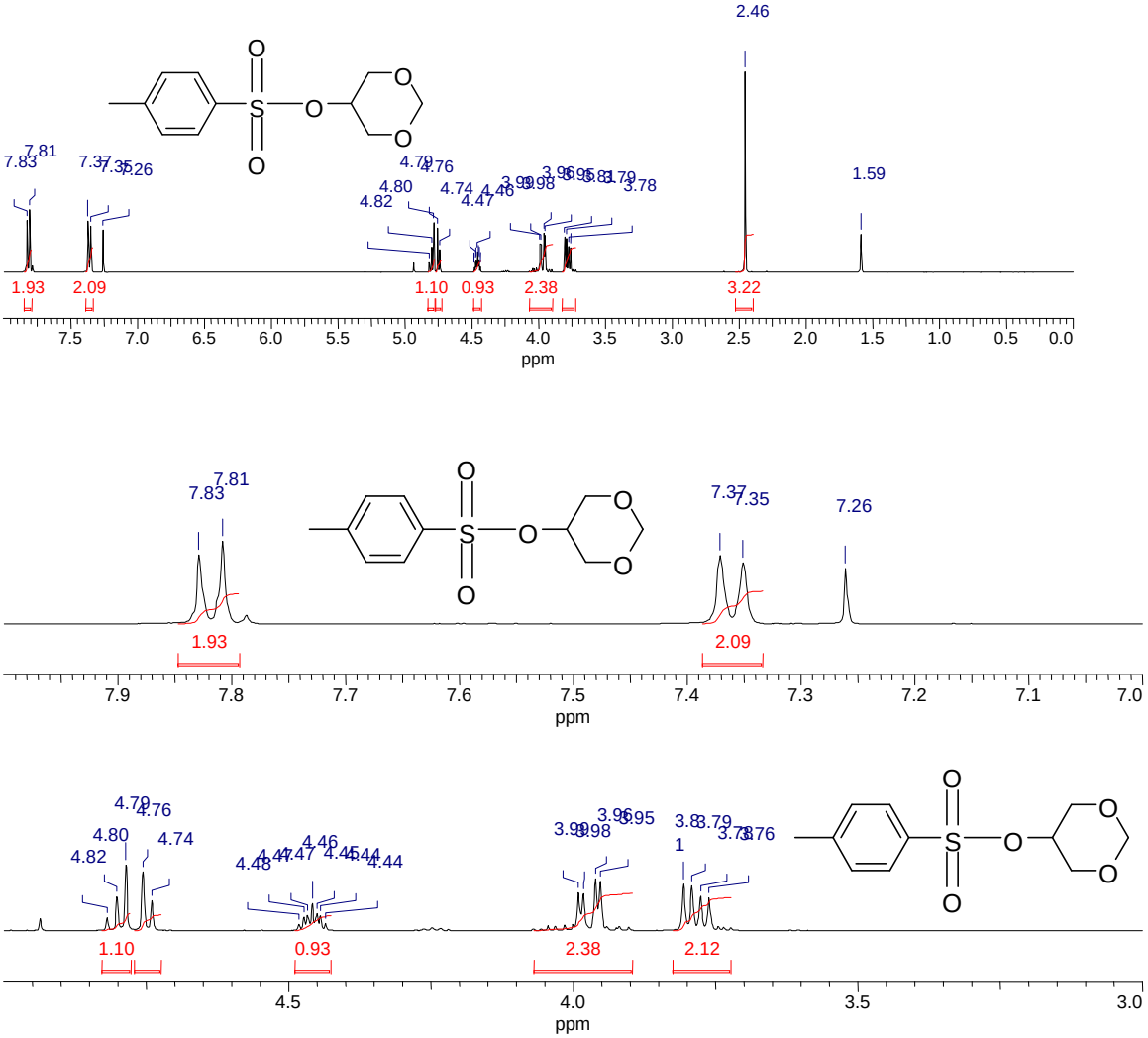
^{1}H NMR of acetyl Nagao (16)



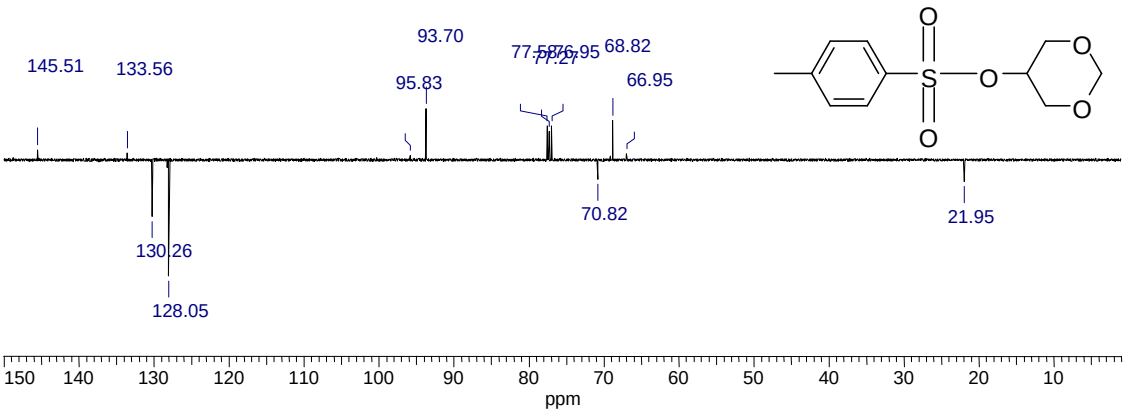
^{13}C NMR of acetyl Nagao (16)



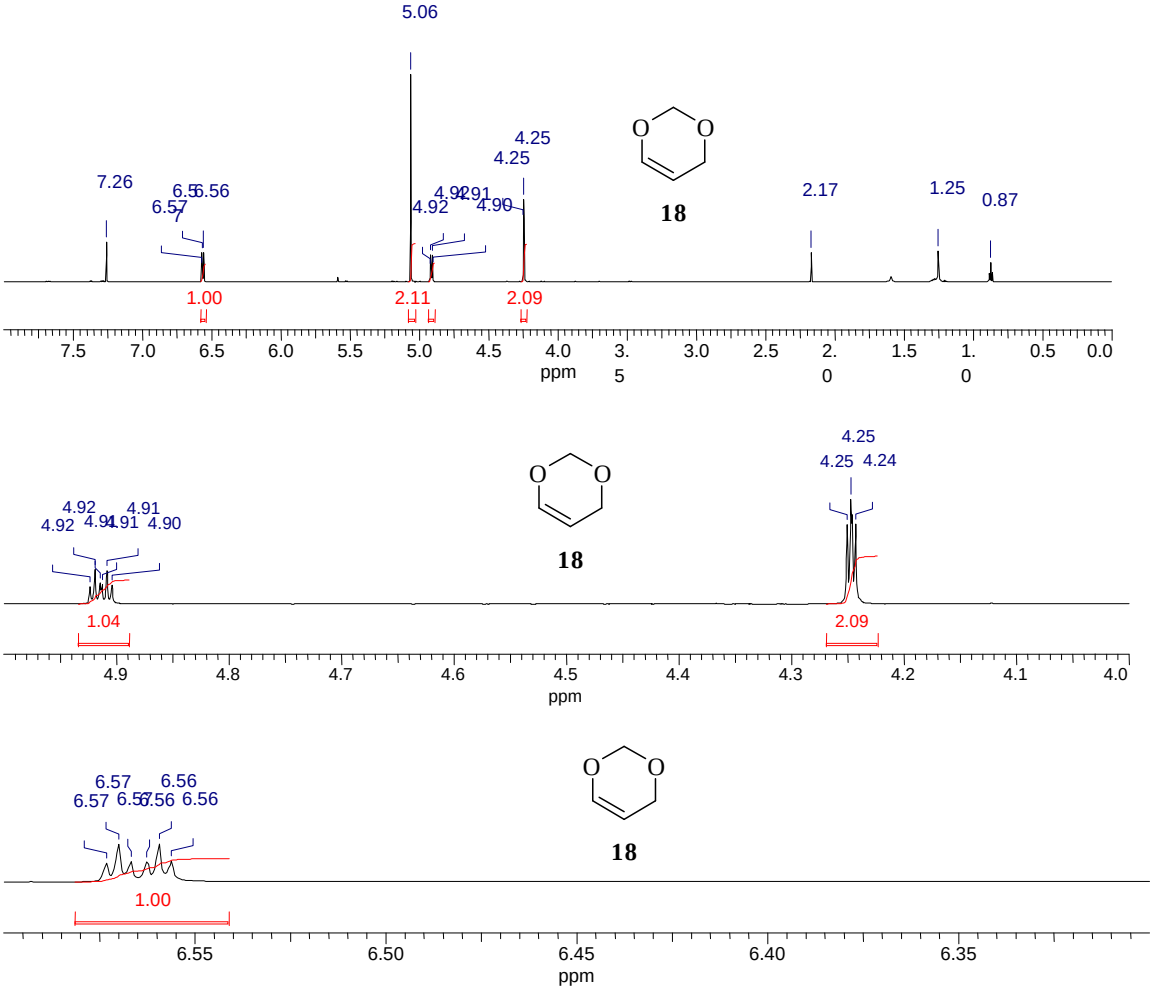
^{1}H NMR of 5-tosyloxy-1,3-dioxolane



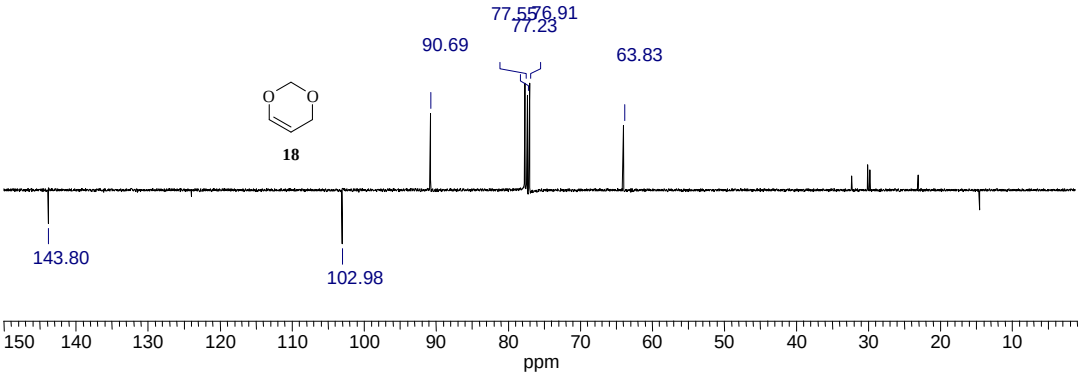
^{13}C NMR of 5-tosyloxy-1,3-dioxolane



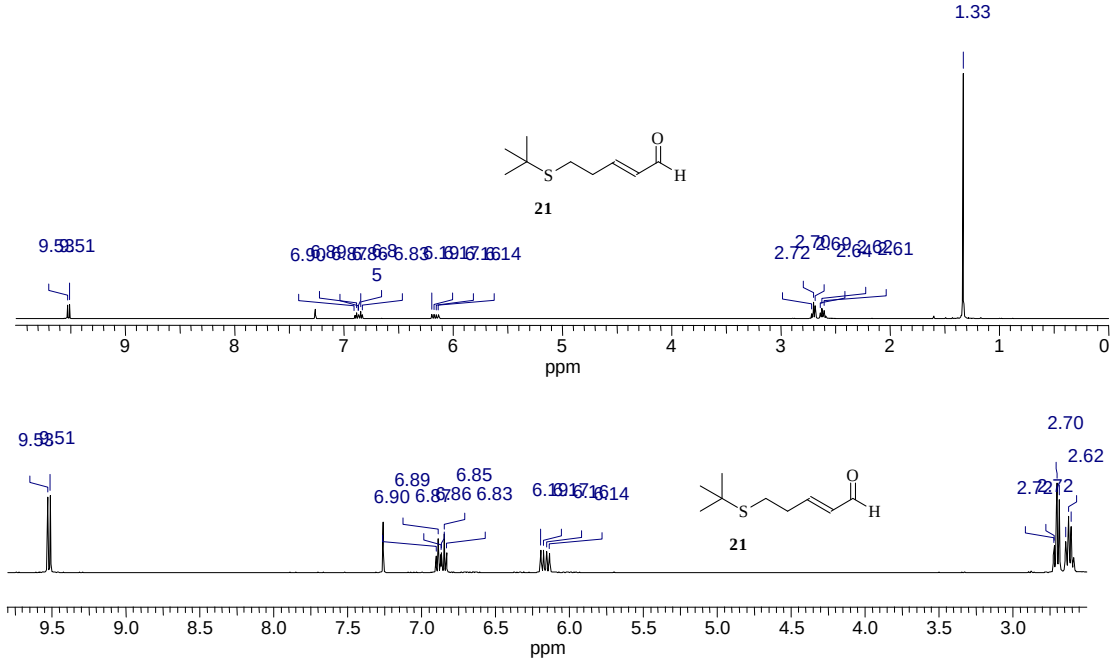
^{1}H NMR of 1,3-dioxene (18)



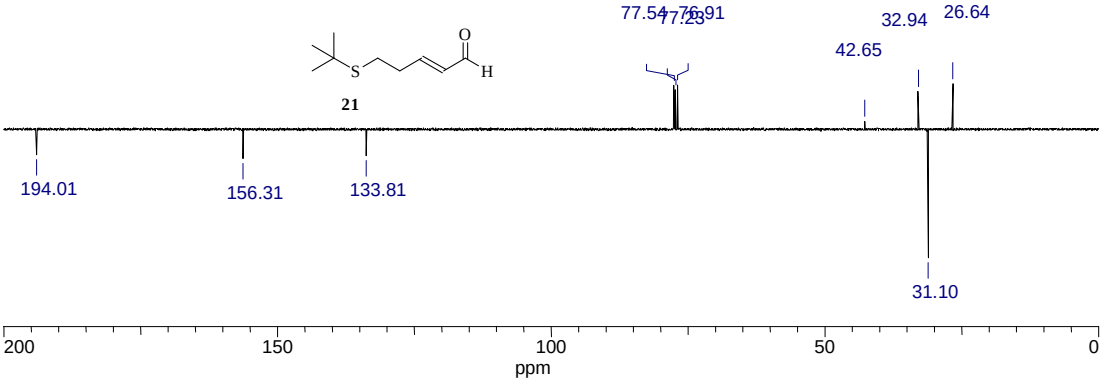
^{13}C NMR of 1,3-dioxene (18)



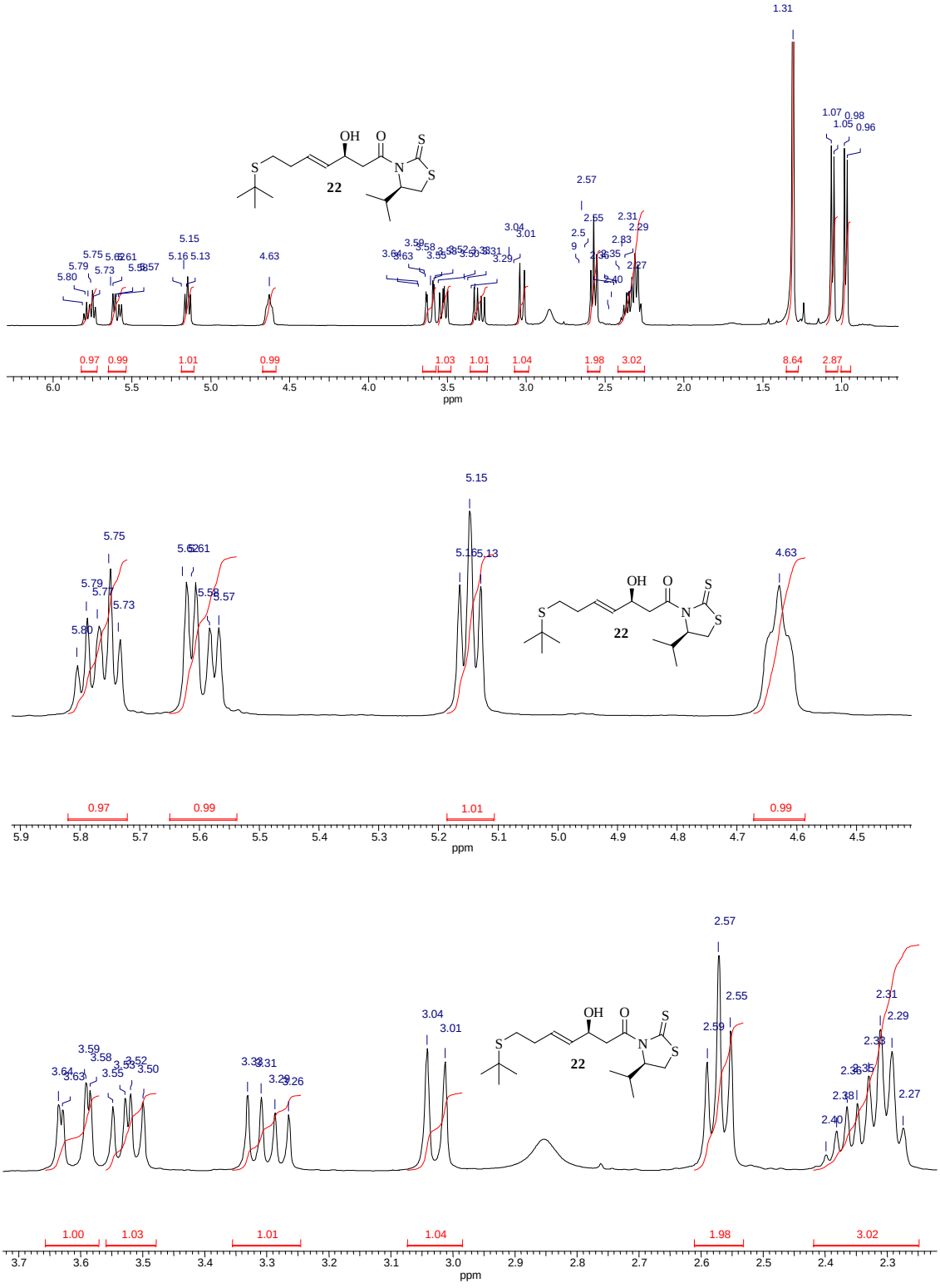
^{1}H NMR of 5-(*tert*-butylthio)pent-2*E*-enal (21)

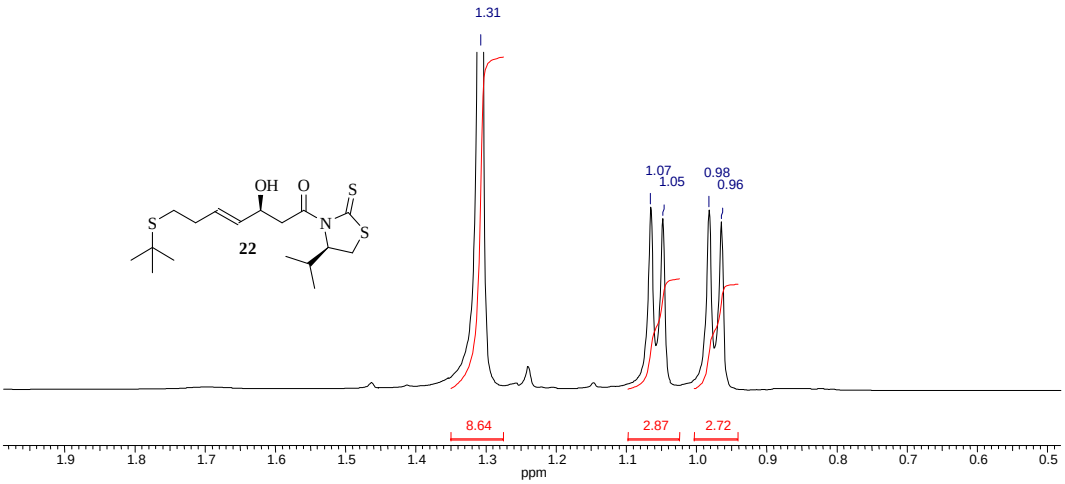


^{13}C NMR of 5-(*tert*-butylthio)pent-2*E*-enal (21)

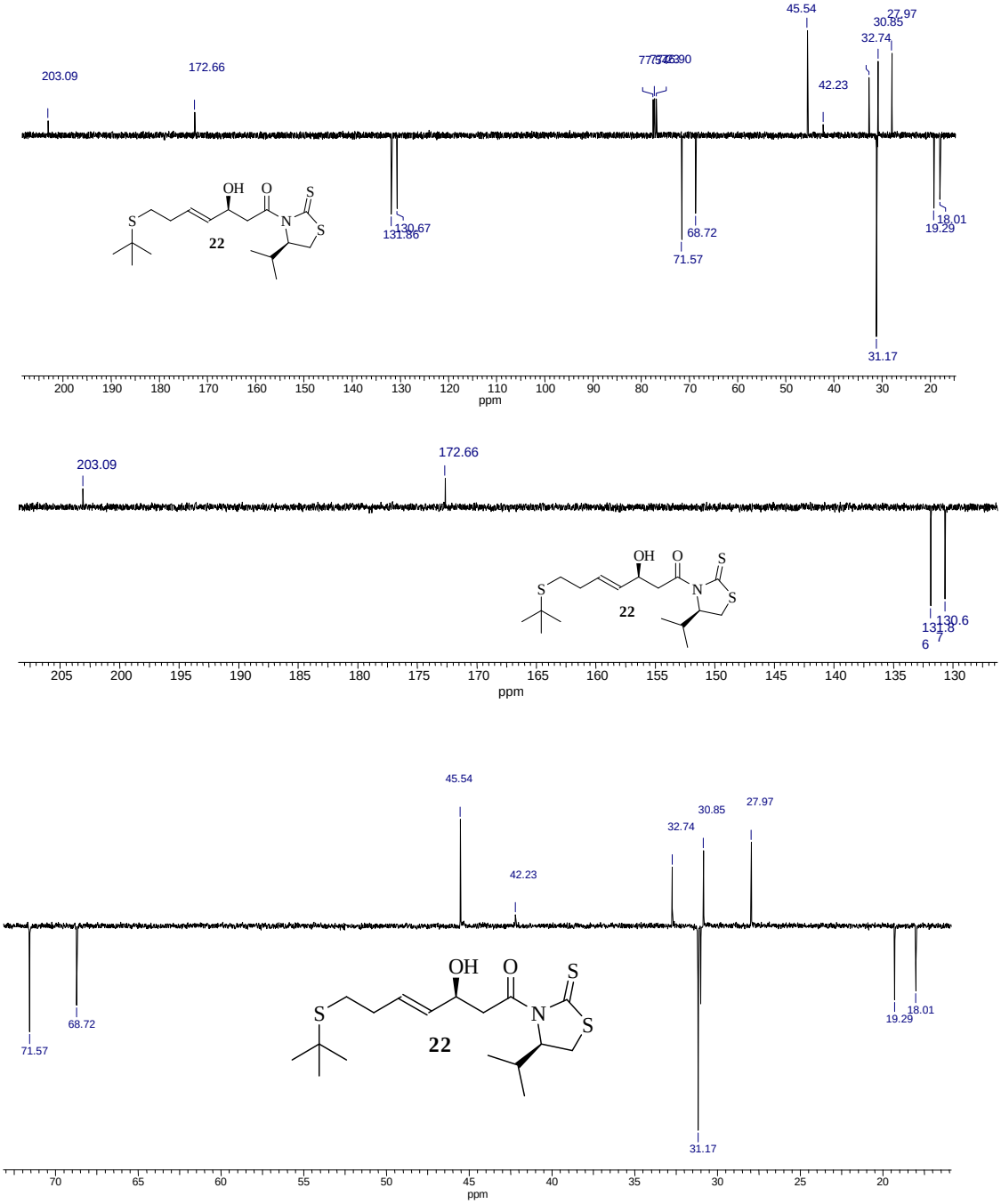


^{1}H NMR of 7-(*tert*-butylthio)-3*S*-hydroxy-1-(4*R*-isopropyl-2-thioxothiazolidin-3-yl)hept-4*E*-en-1-one (22)

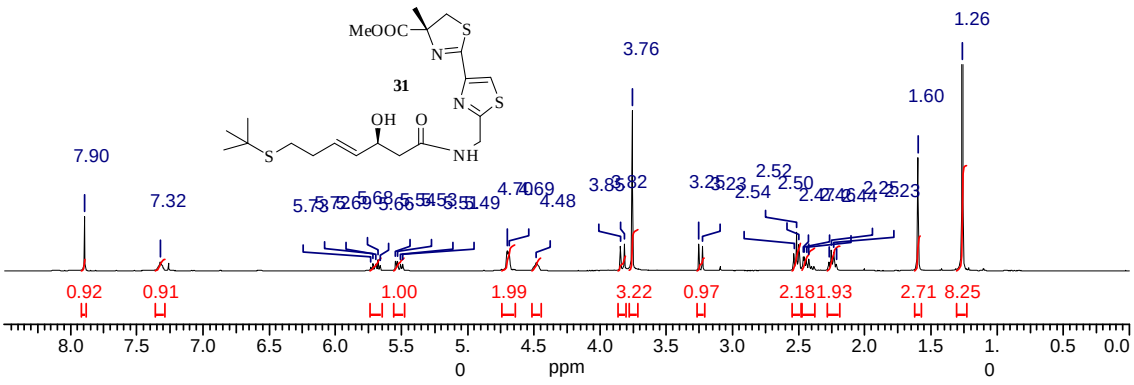


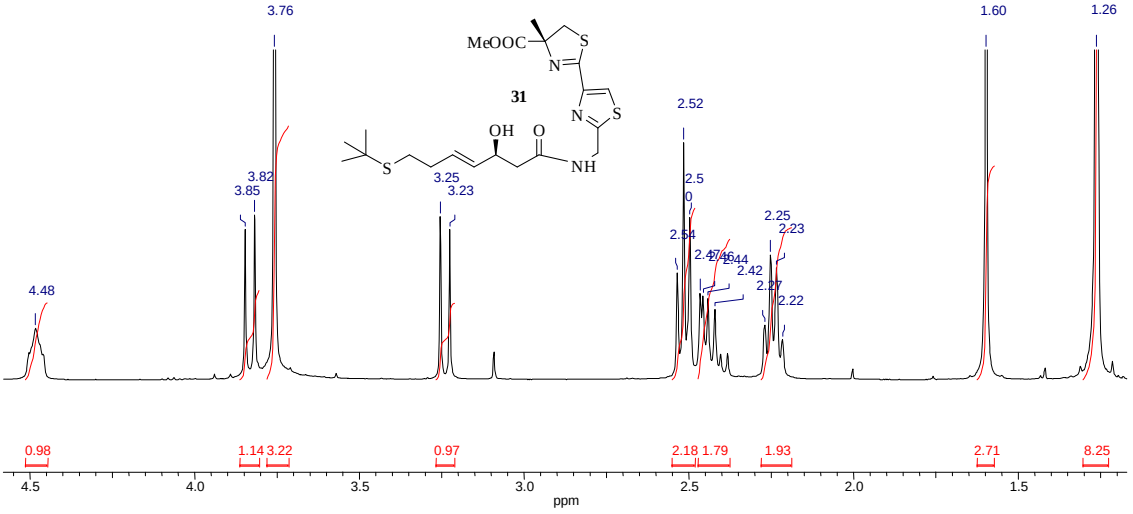

1.31
1.07
1.05
0.98
0.96
OH
O
S
S
N
S
22
8.64
2.87
2.72
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
ppm

^{13}C NMR of 7-(*tert*-butylthio)-3*S*-hydroxy-1-(4*R*-isopropyl-2-thioxothiazolidin-3-yl)hept-4*E*-en-1-one (22)

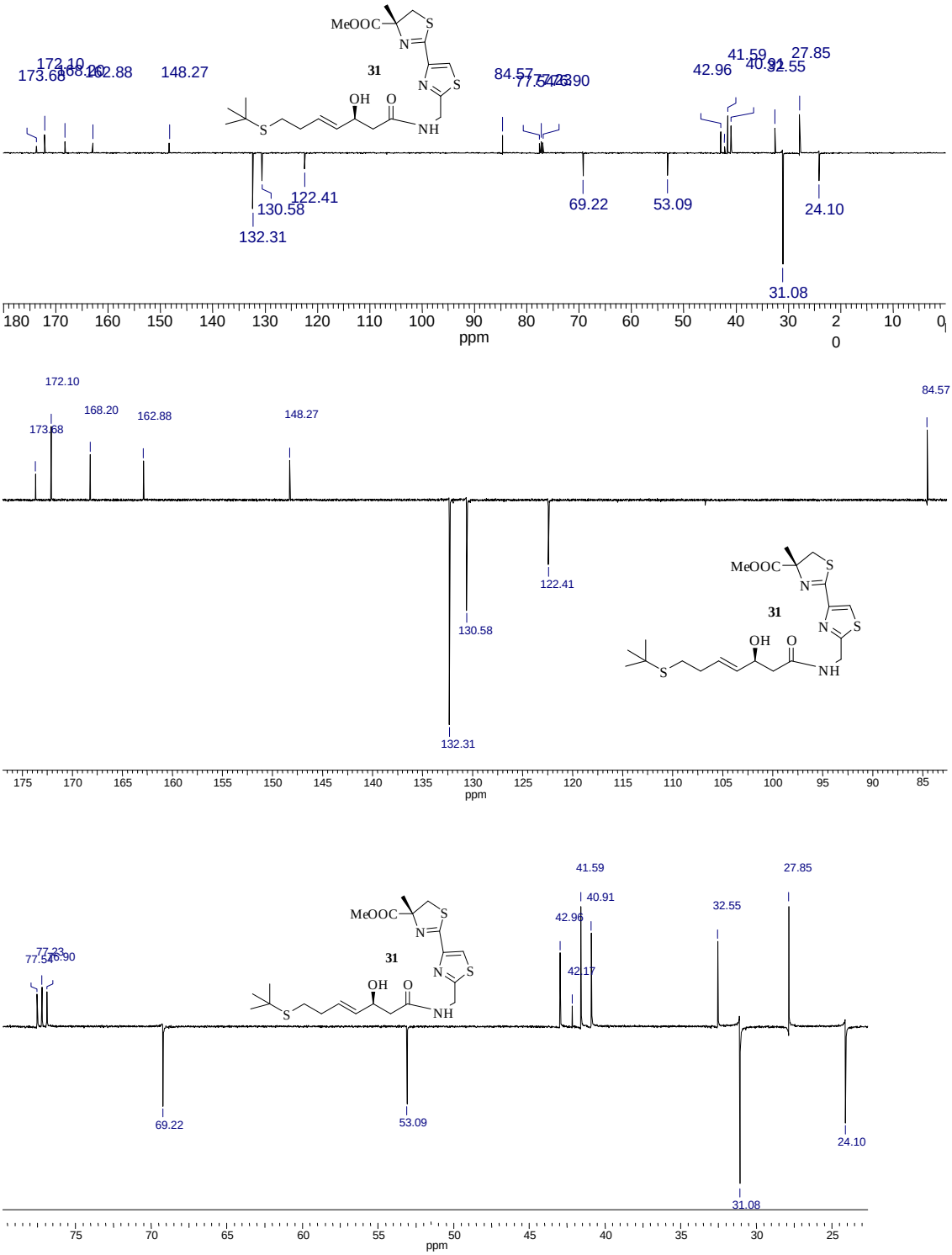


^{1}H NMR of (*R*)-methyl 2-(2-(((*S*,*E*)-7-(*tert*-butylthio)-3-hydroxyhept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (31)

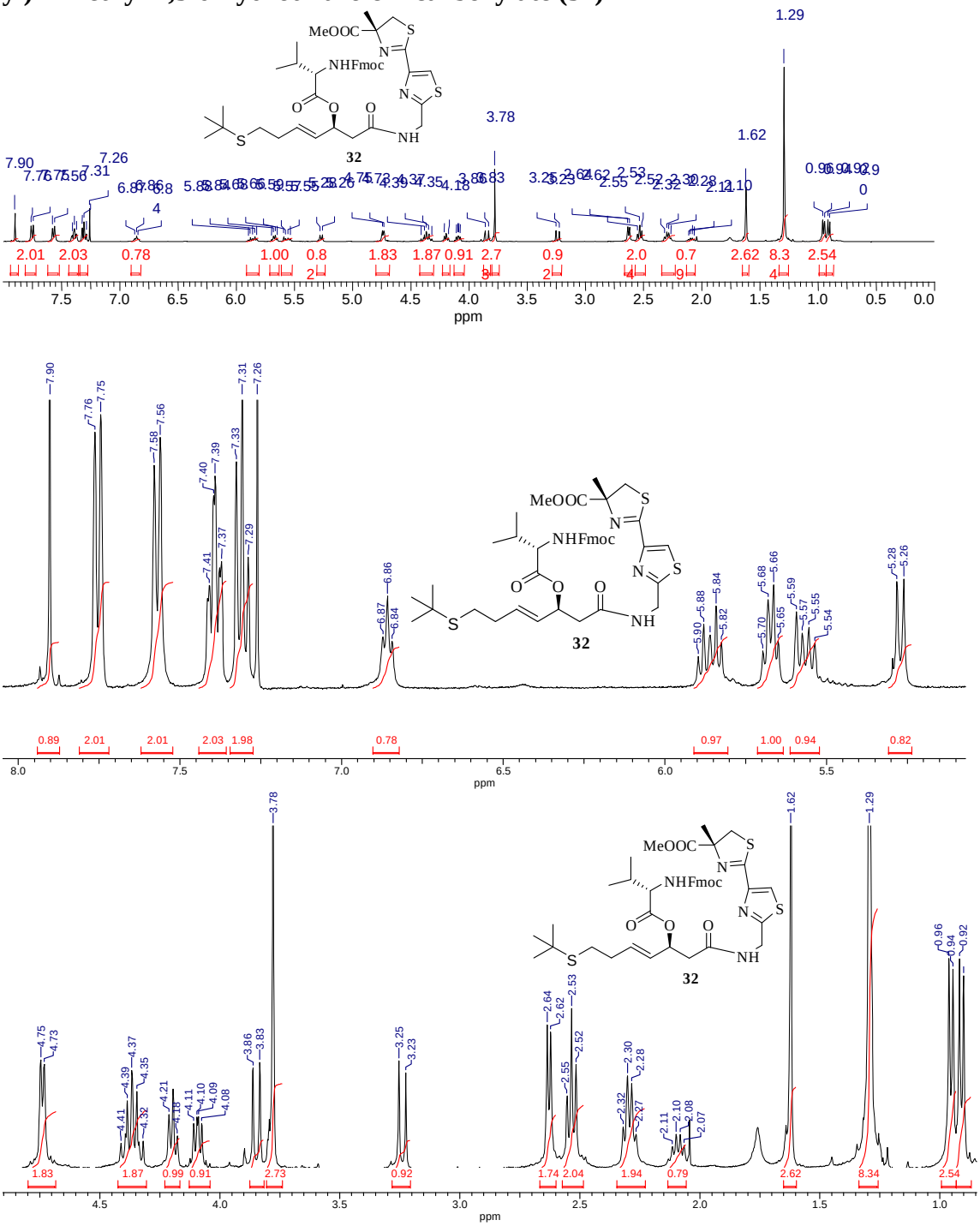




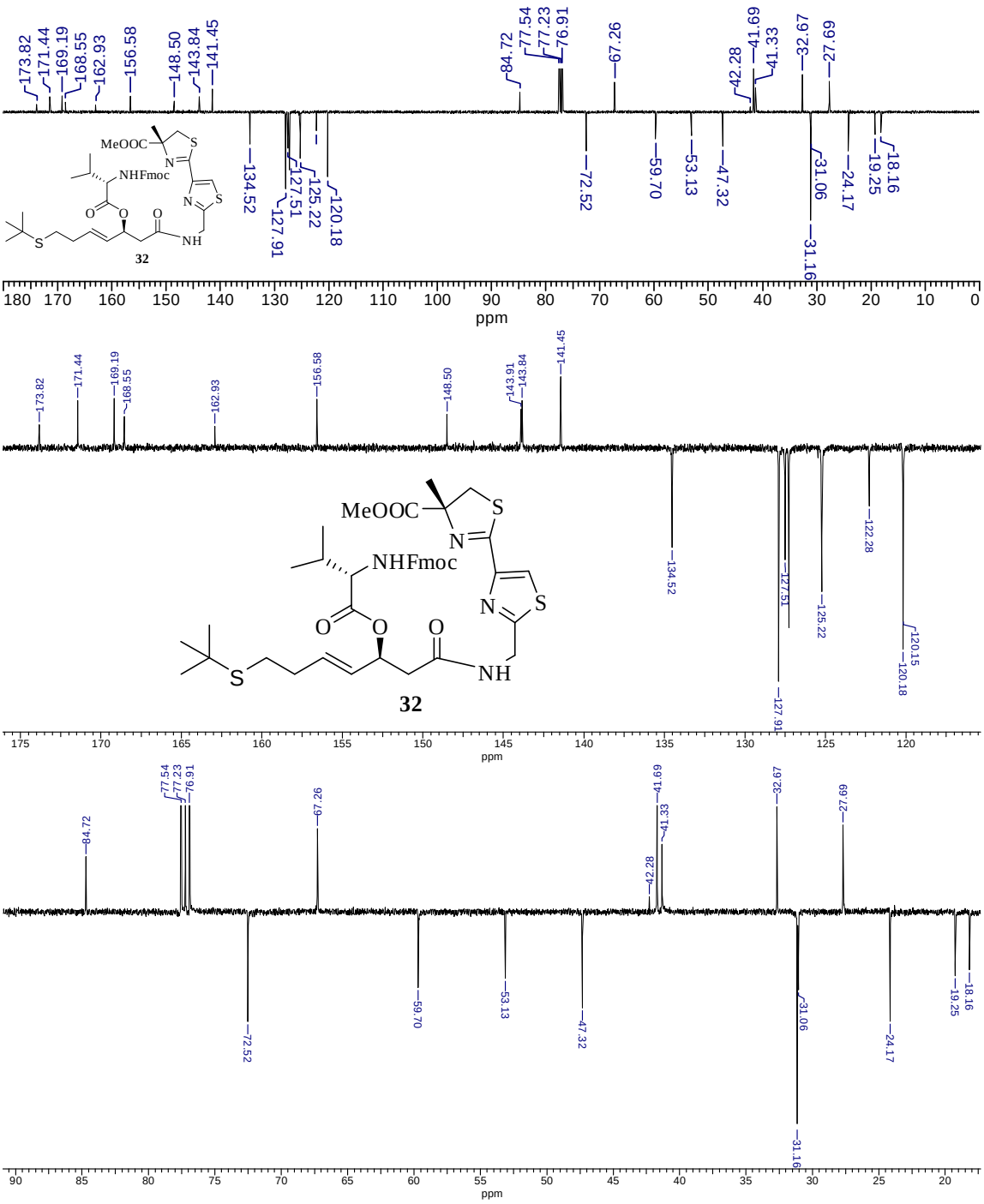
^{13}C NMR of (*R*)-methyl 2-(2-(((*S*,*E*)-7-(*tert*-butylthio)-3-hydroxyhept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (31)



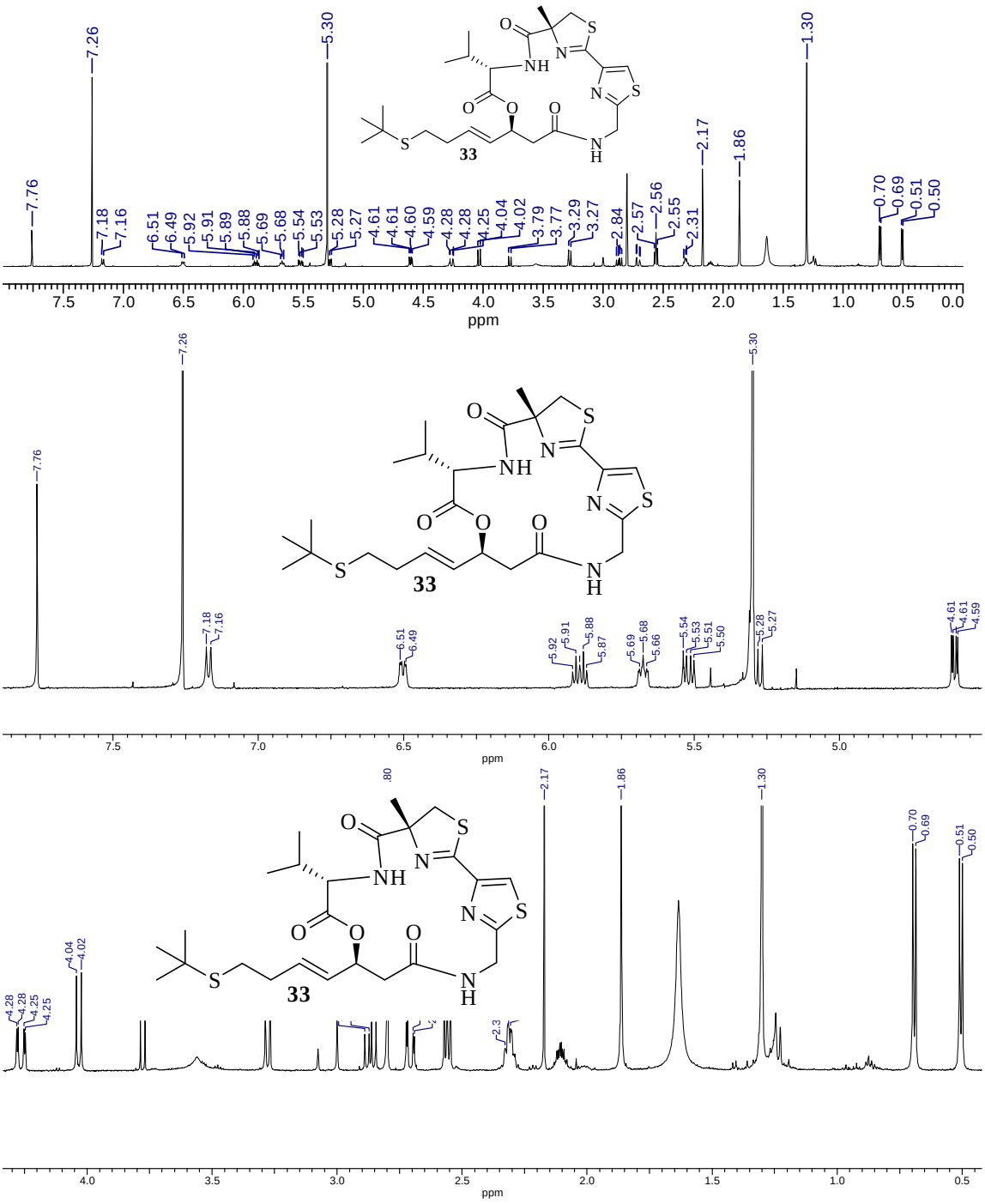
^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-8-((*E*)-4-(*tert*-butylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (32)



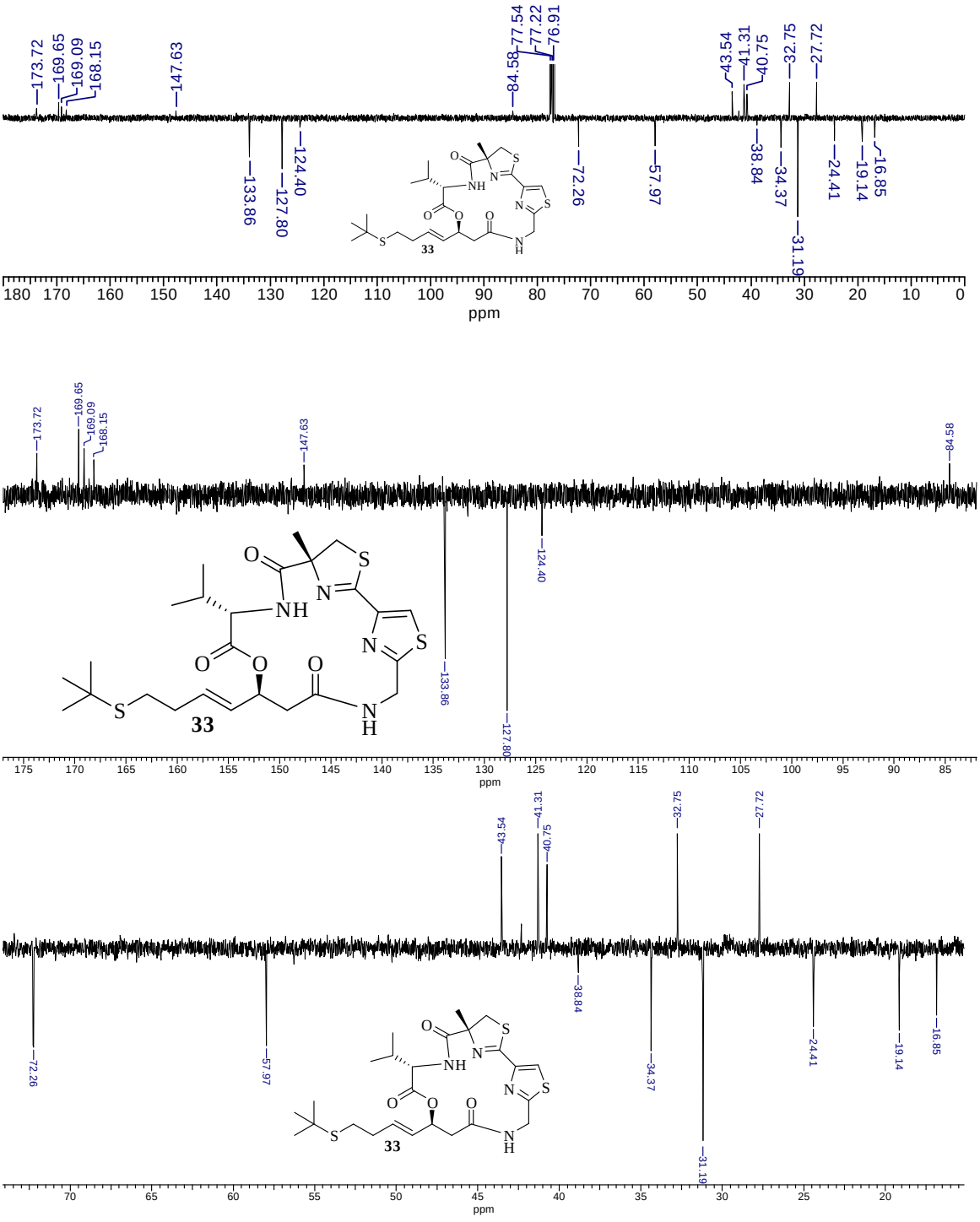
^{13}C NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-8-((*E*)-4-(*tert*-butylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (32)



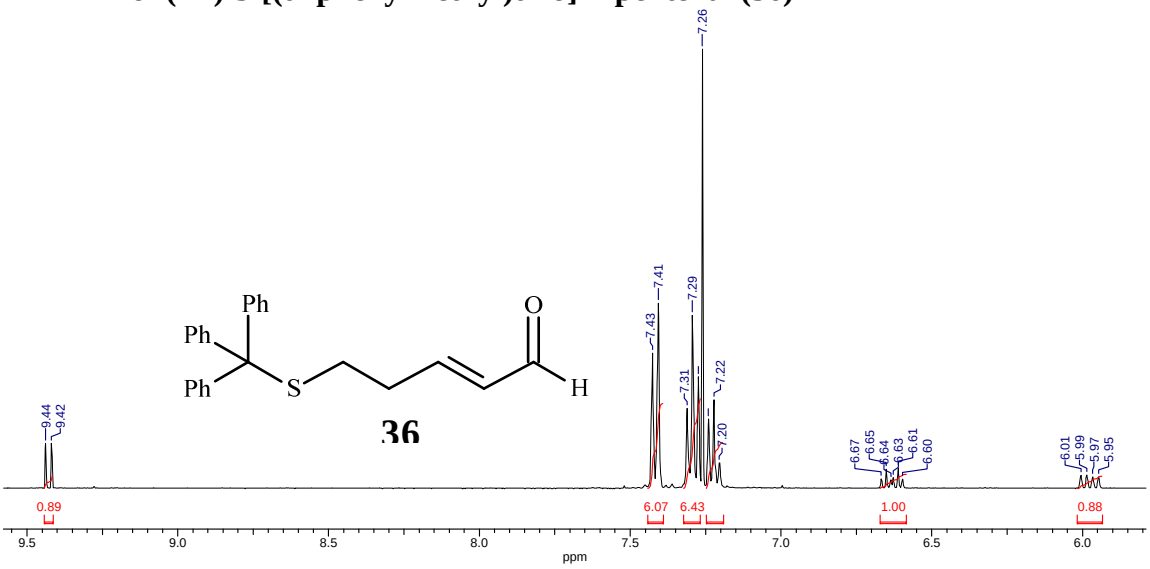
^{1}H NMR of cyclized product 33

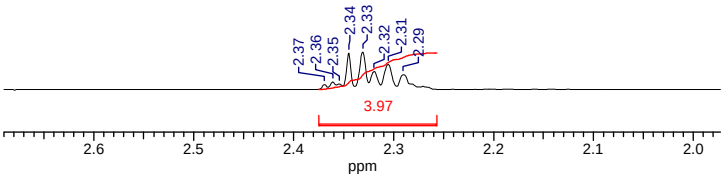


^{13}C NMR of cyclized product 33

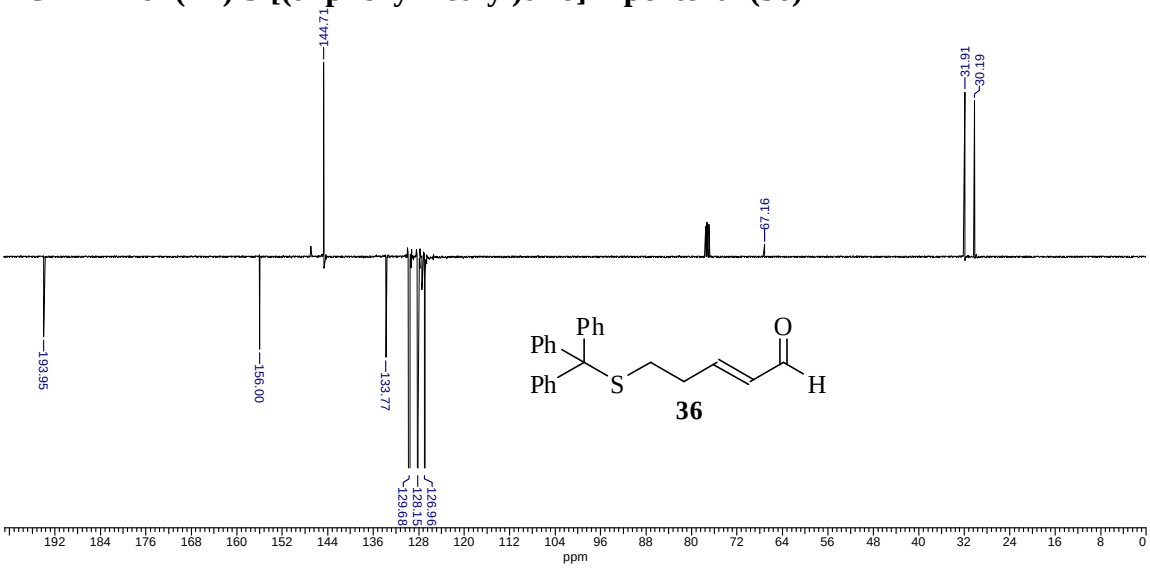


^{1}H NMR of (2*E*)-5-[(triphenylmethyl)thio]-2-pentenal (36)

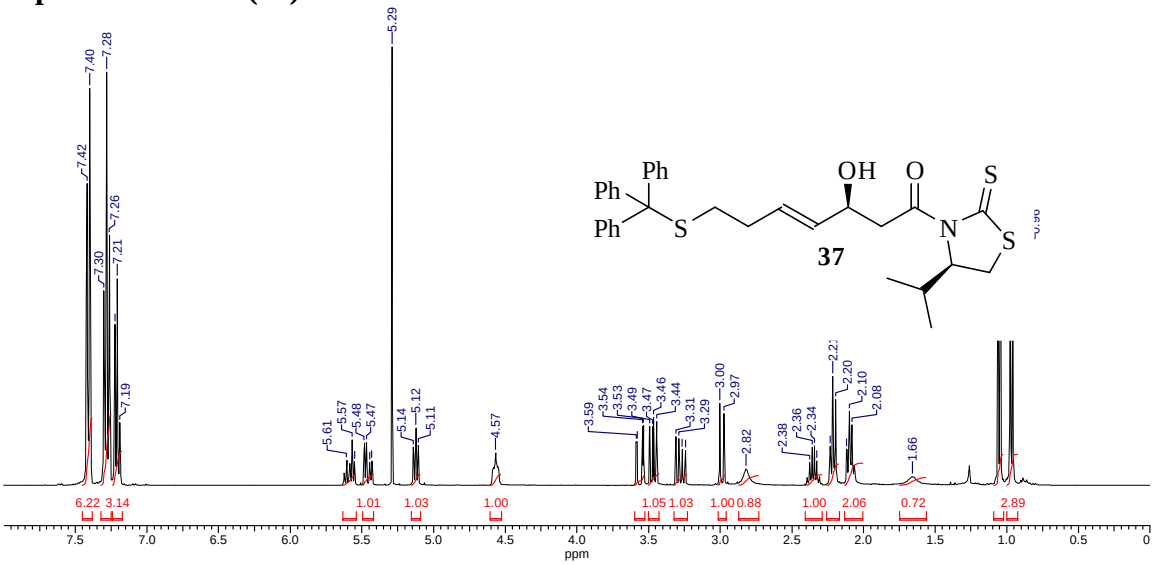


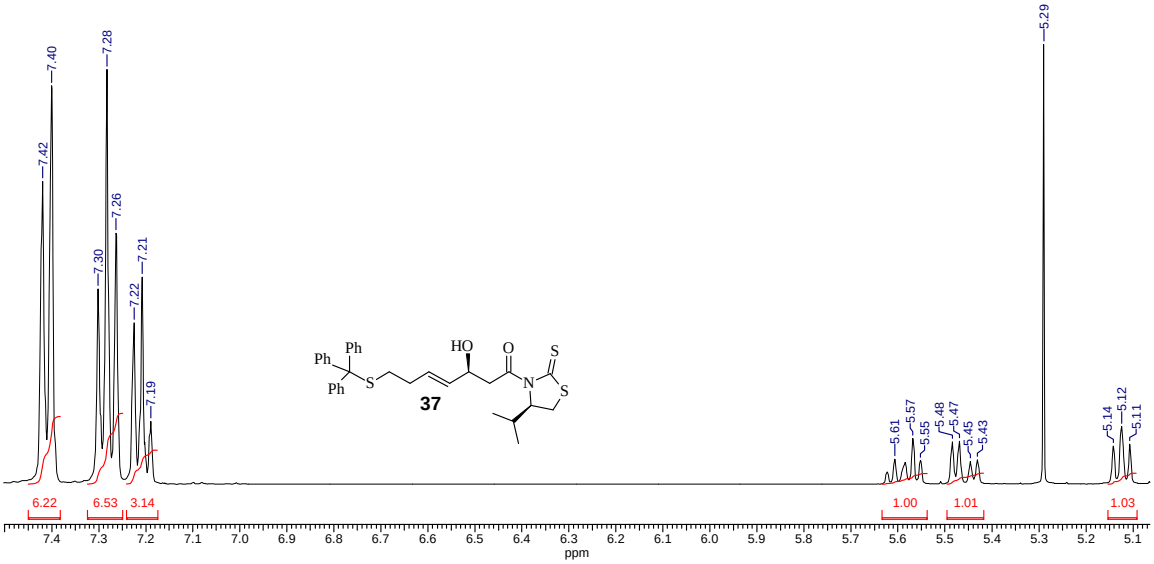


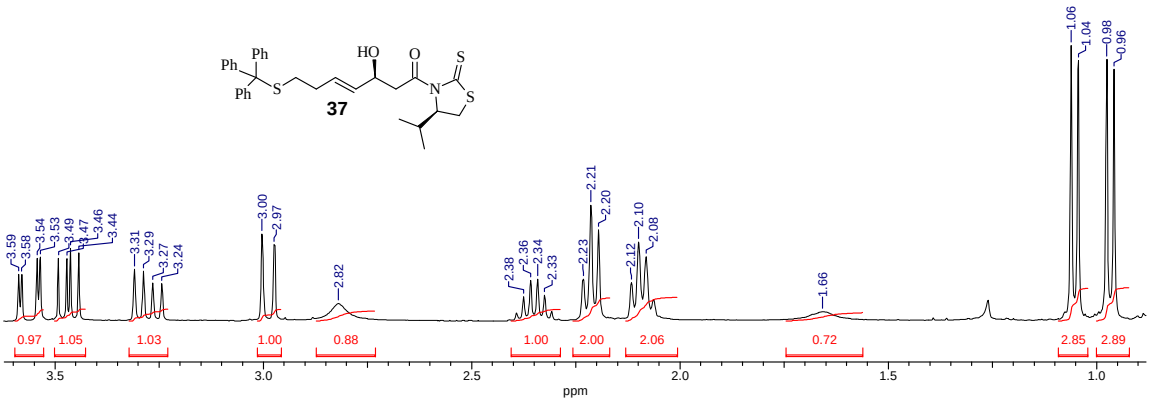
^{13}C NMR of (2*E*)-5-[(triphenylmethyl)thio]-2-pentenal (36)



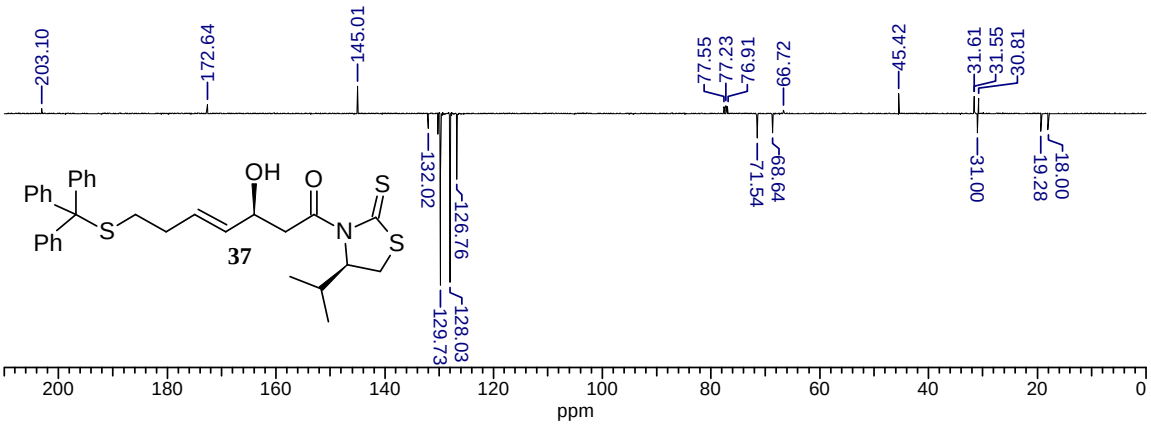
^{1}H NMR of 3*S*-hydroxy-1-(4*R*-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4*E*-en-1-one (37)



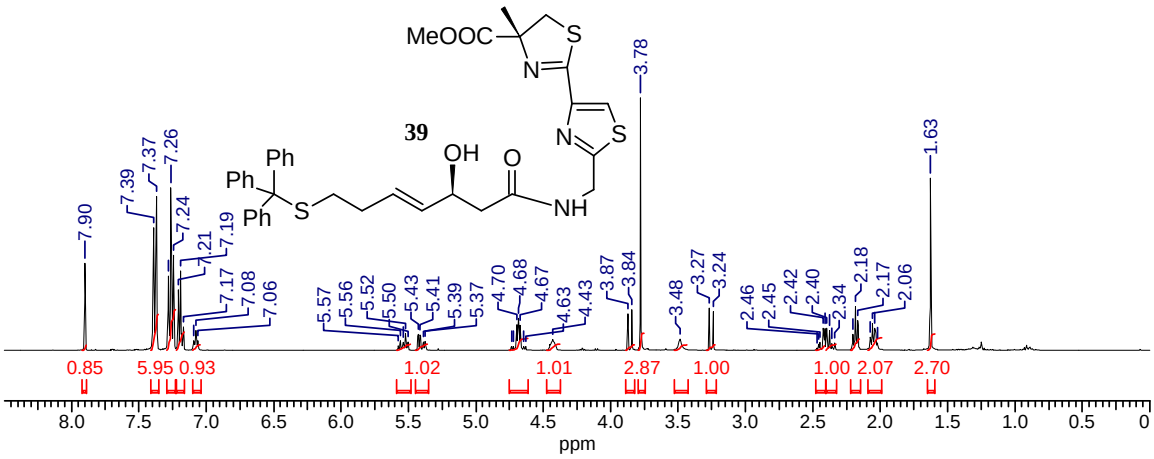


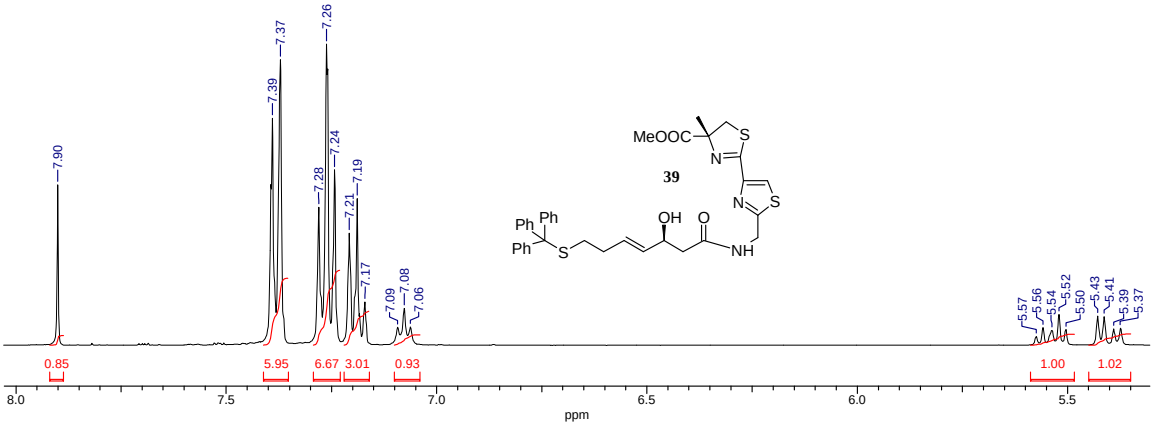


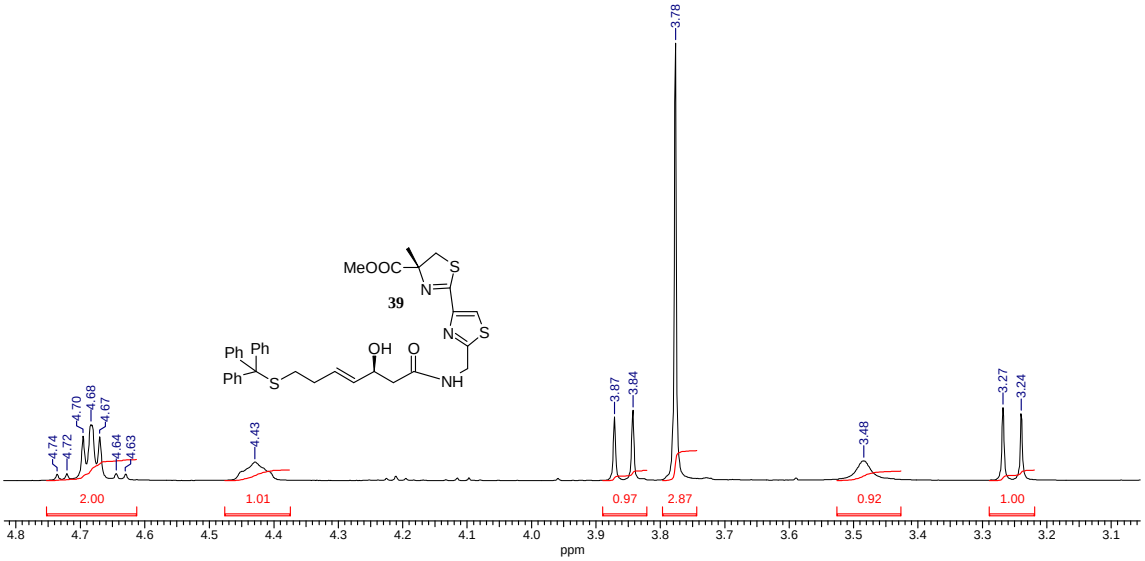
^{13}C NMR of 3*S*-hydroxy-1-(4*R*-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4*E*-en-1-one (37)

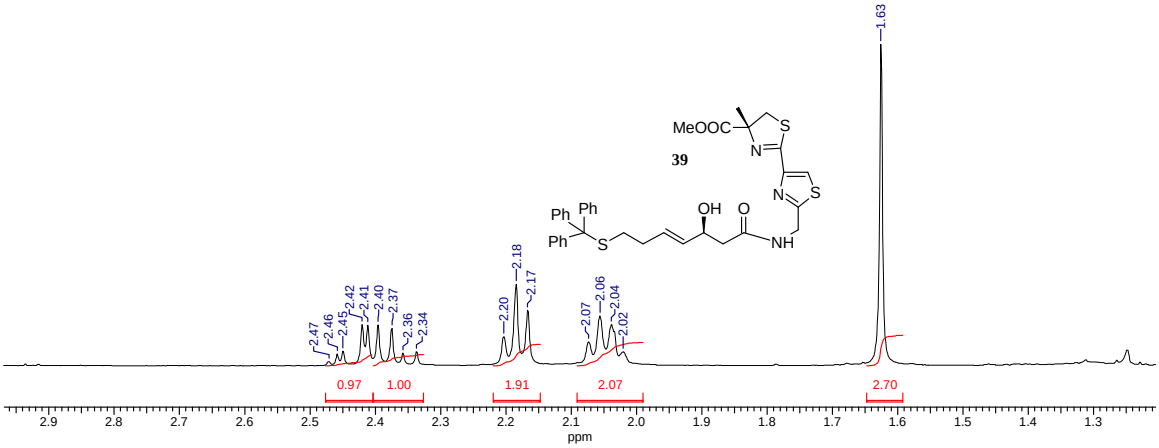


^{1}H NMR of (*R*)-methyl 2-(2-((3*S*-hydroxy-7-(tritylthio)hept-4*E*-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (39)

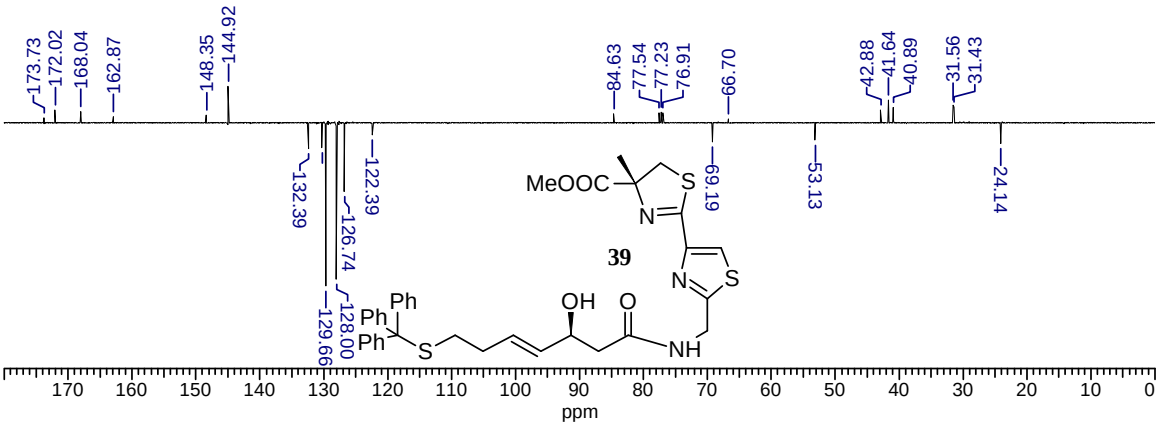


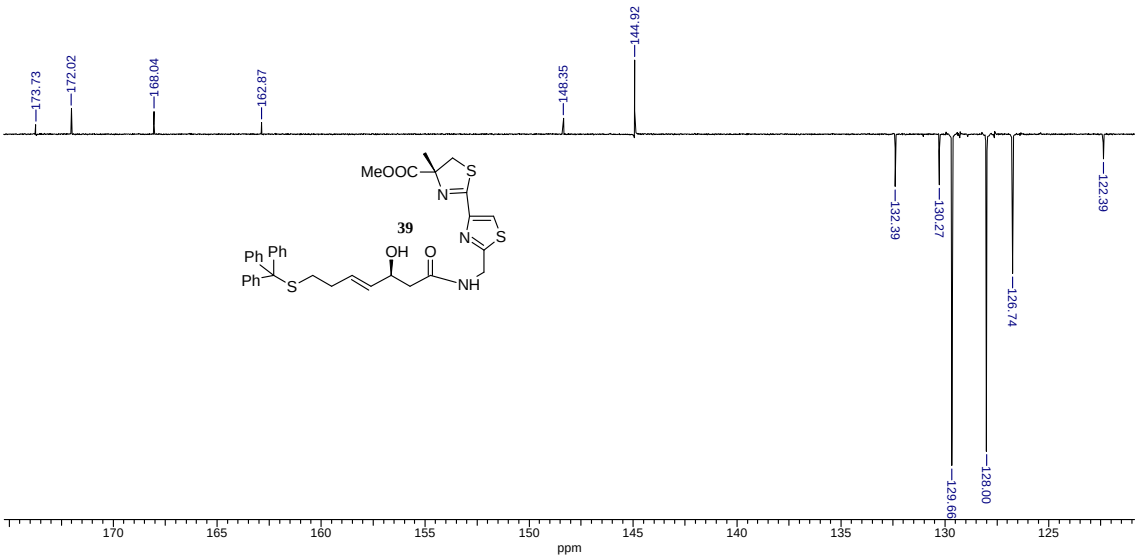


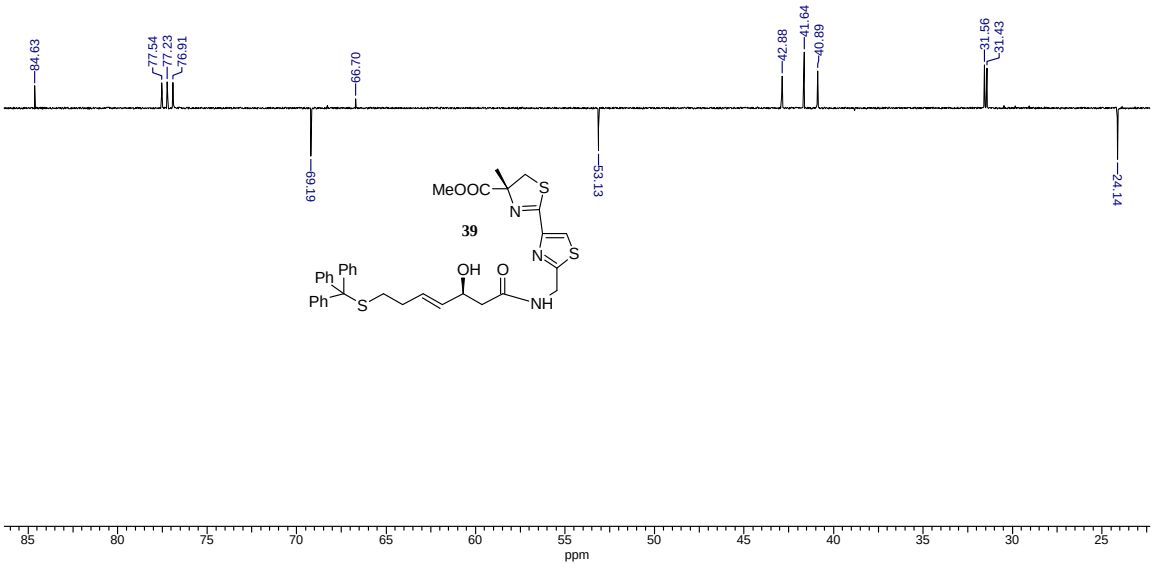




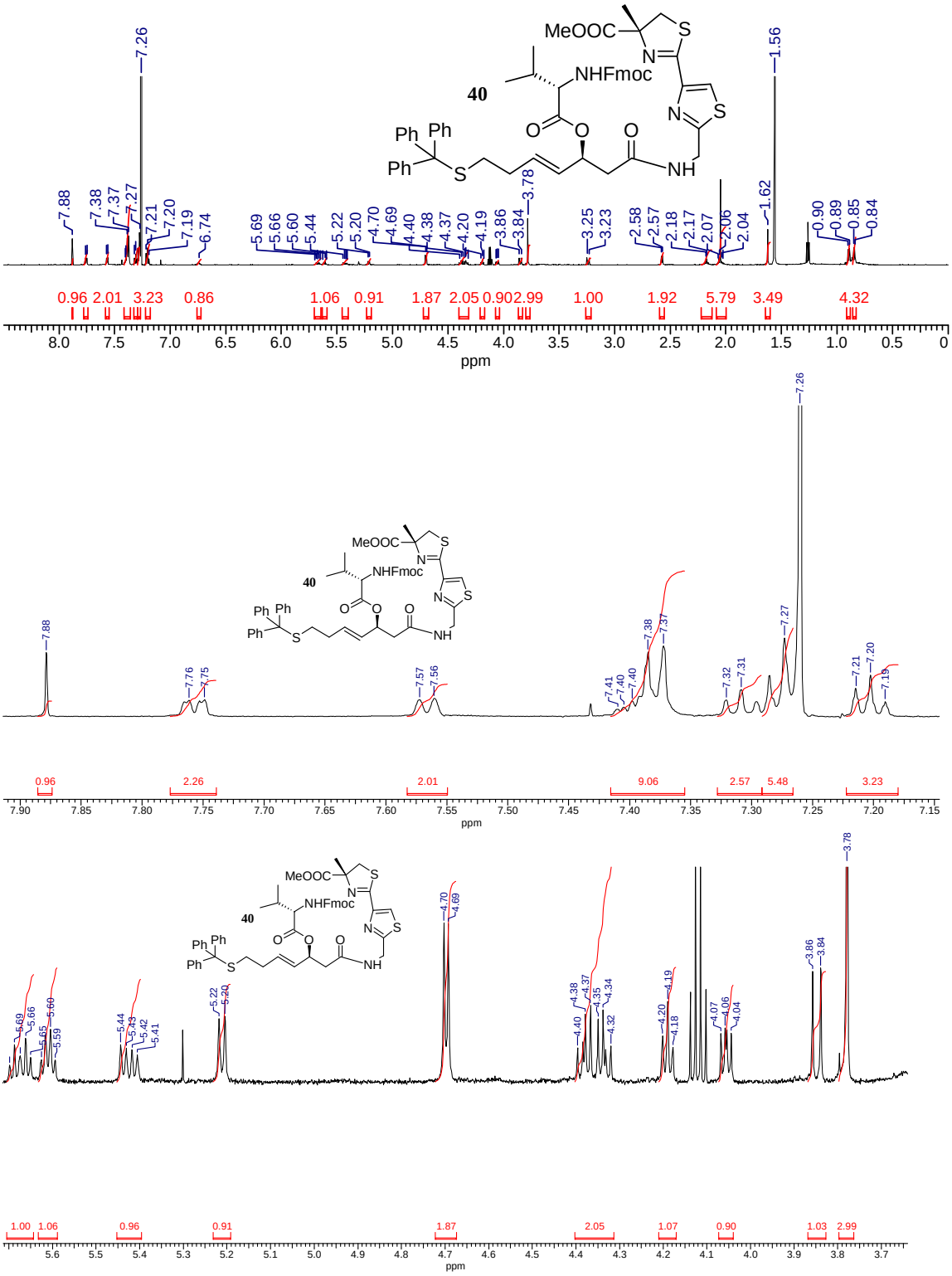
^{13}C NMR of (*R*)-methyl 2-(2-((3*S*-hydroxy-7-(tritylthio)hept-4*E*-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (39)

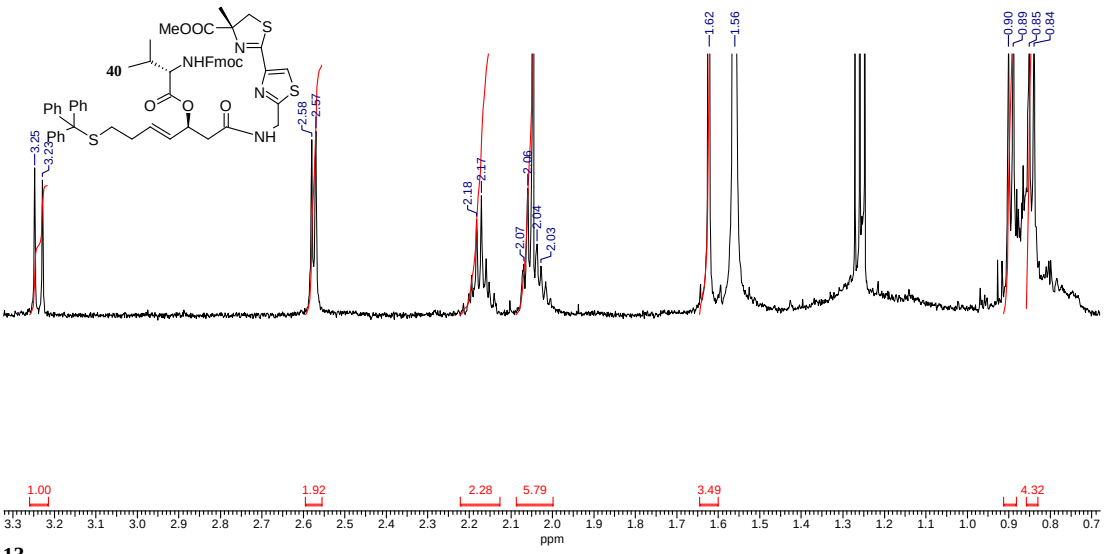




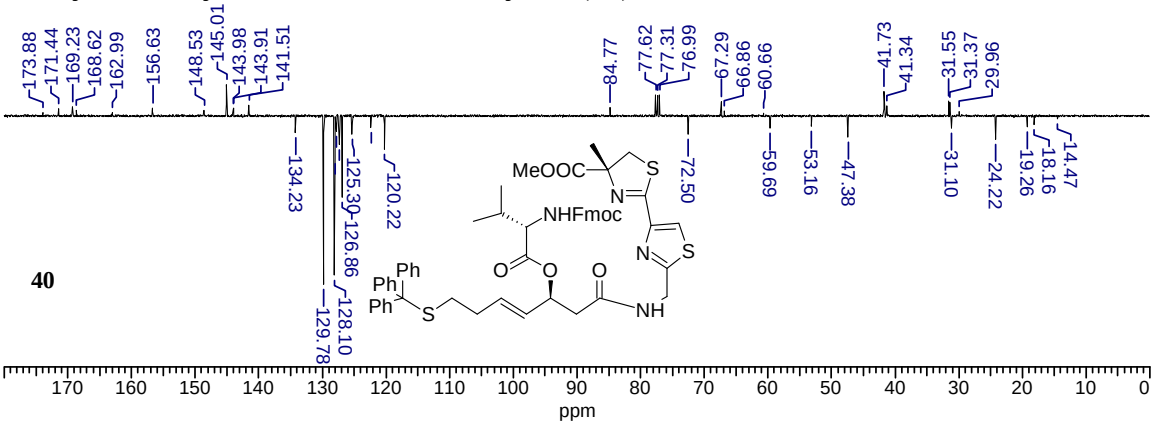

84.63
77.54
77.23
76.91
66.70
61.69
53.13
42.88
41.64
40.89
31.56
31.43
24.14
MeOOC
S
N
N
S
39
Ph
Ph
Ph
S
OH
O
NH
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

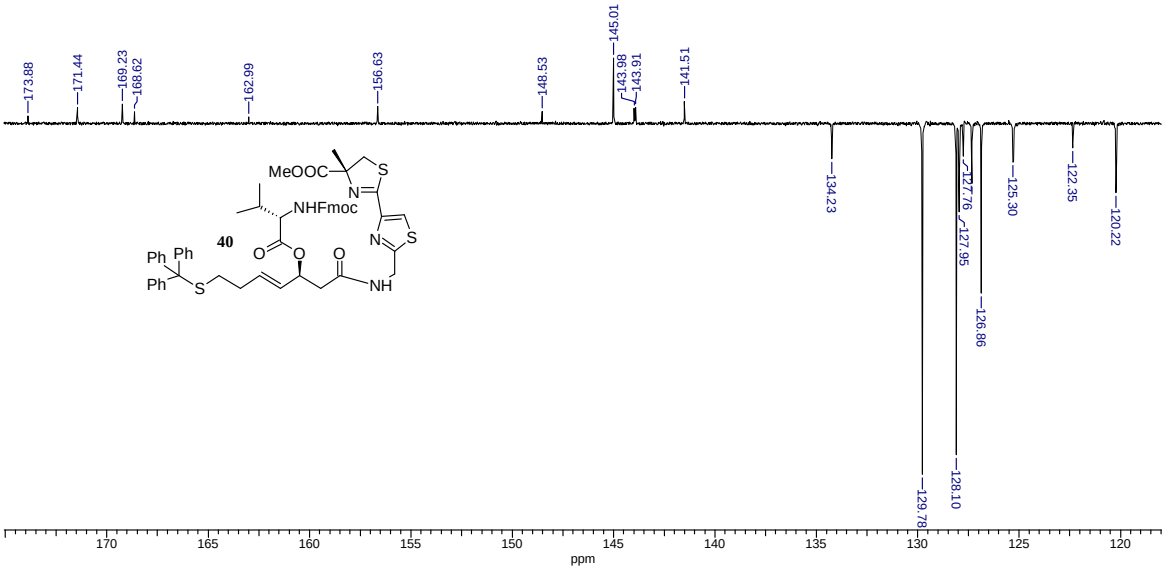
^{1}H NMR of (4*R*)-methyl 2-(2-((8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)-but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (40)

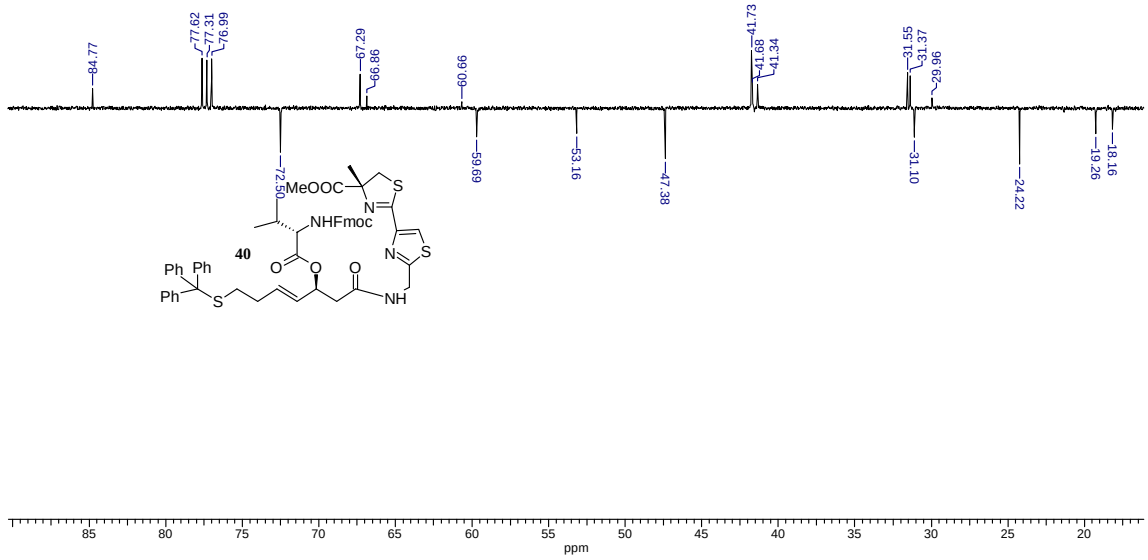




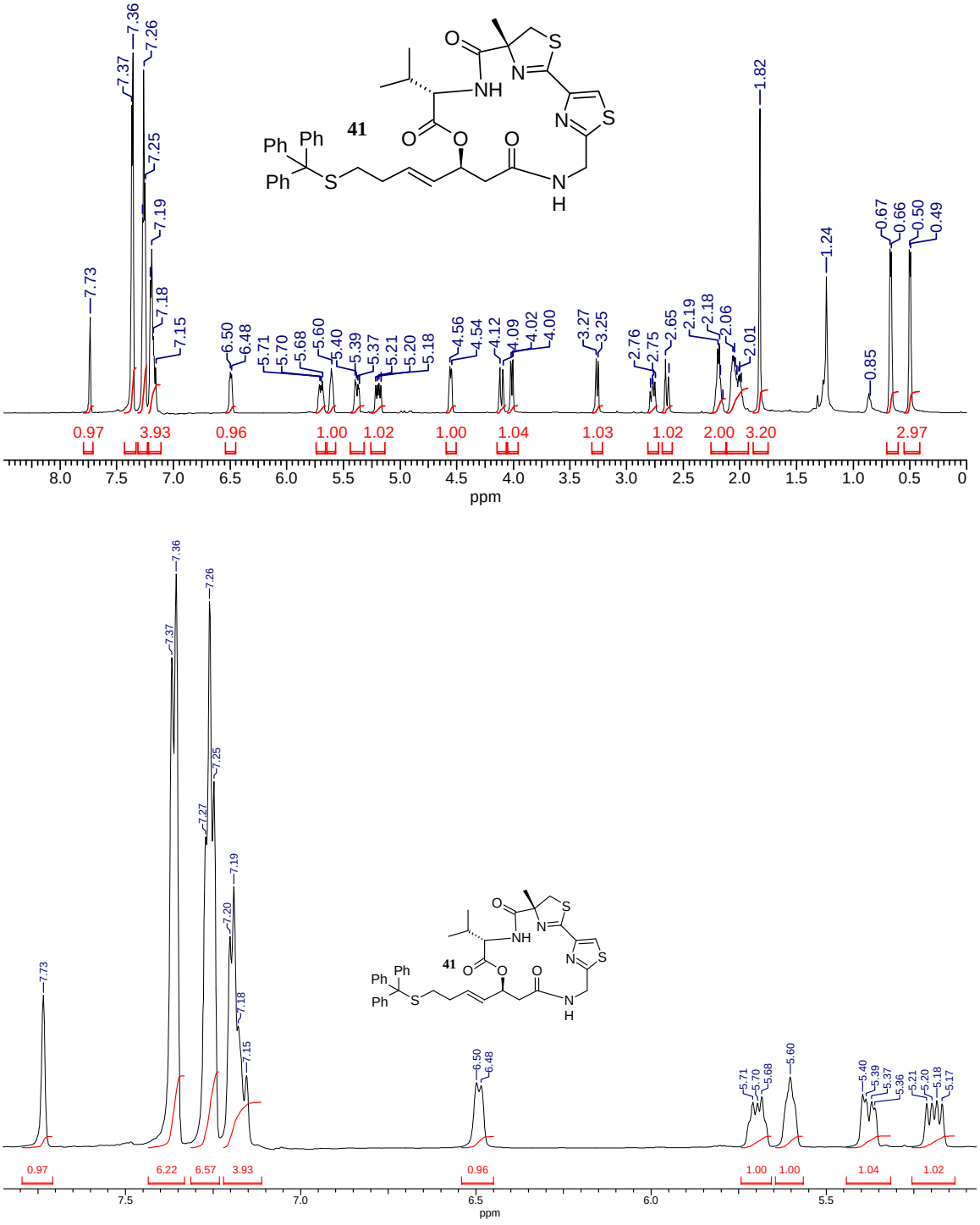
^{13}C NMR of (4*R*)-methyl 2-(2-((8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)-but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (40)

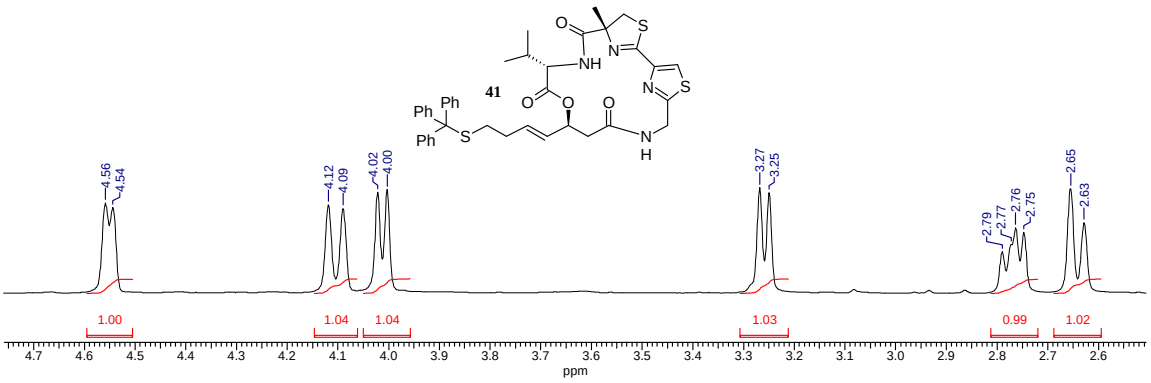


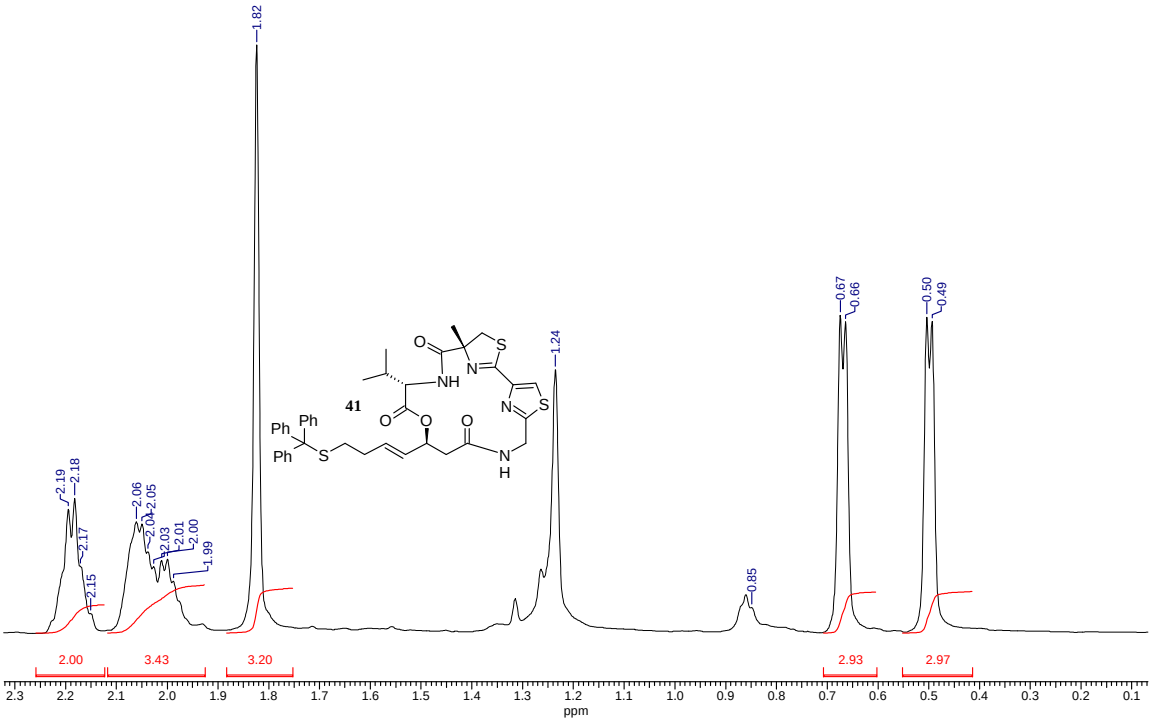

173.88
171.44
169.23
168.62
162.99
156.63
148.53
145.01
143.98
143.91
141.41
134.23
129.78
128.10
127.95
127.76
126.86
125.30
122.35
120.22
MeOOC
NHFmoc
40
Ph
Ph
Ph
S
N
S
N
S
O
O
O
NH
170
165
160
155
150
145
140
135
130
125
120
ppm


84.77
77.62
77.31
76.99
67.29
66.98
60.69
59.69
53.16
47.38
41.73
41.68
41.34
31.55
31.37
31.10
96.62
24.22
19.26
18.16
72.50
MeOOC
NHFmoc
40
Ph
Ph
Ph
S
N
S
N
S
O
O
O
NH
85
80
75
70
65
60
55
50
45
40
35
30
25
20
ppm

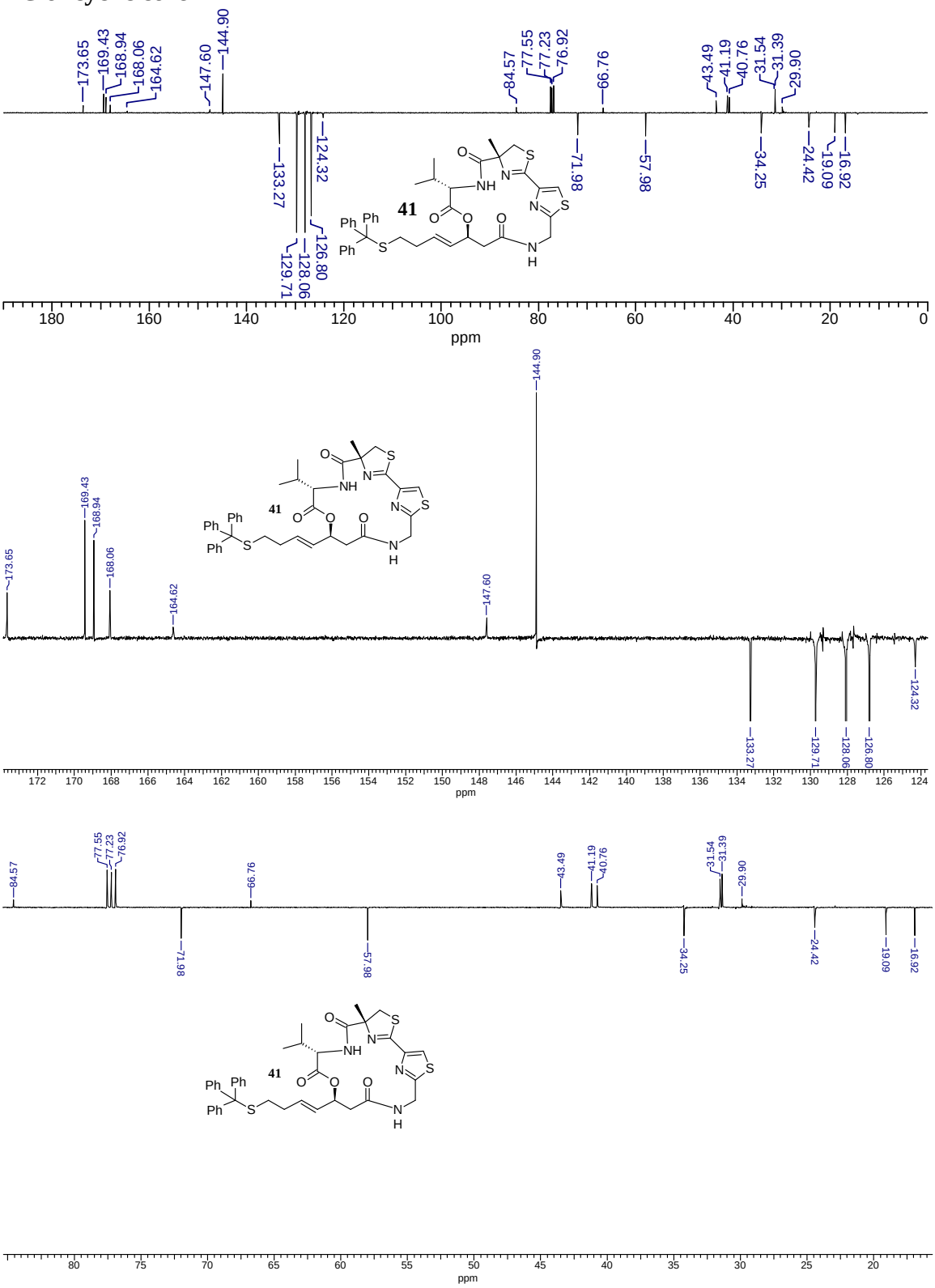
^{1}H NMR of cyclic core 41



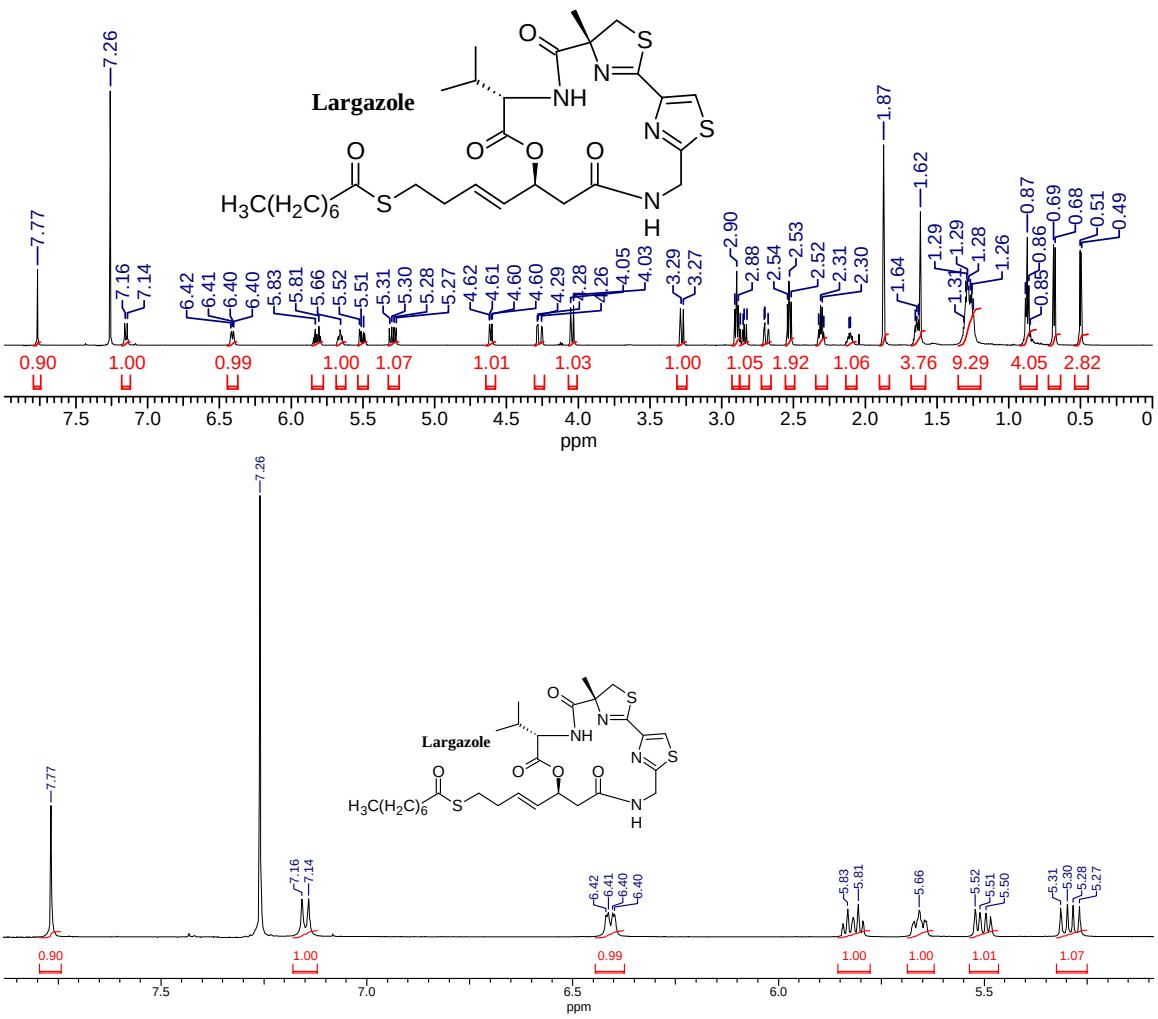

41
4.56
4.54
4.12
4.09
4.02
4.00
3.27
3.25
2.79
2.77
2.76
2.75
2.65
2.63
1.00
1.04
1.04
1.03
0.99
1.02
ppm

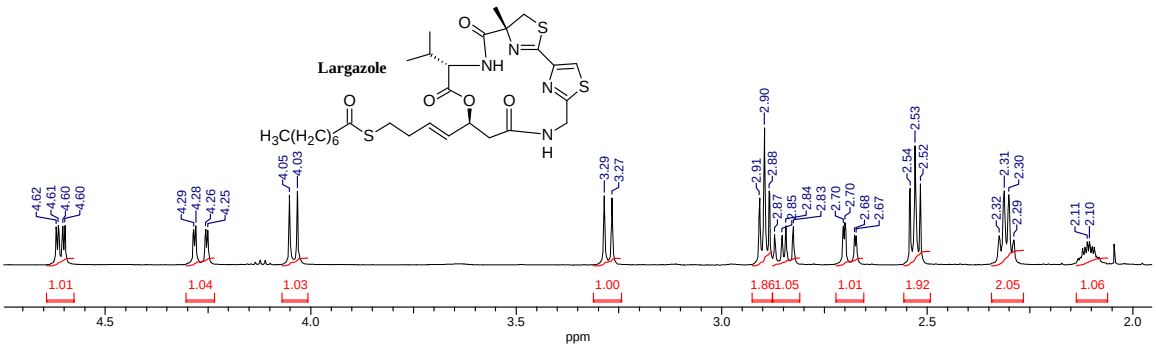

41
1.82
1.24
2.19
2.18
2.17
2.15
2.06
2.05
2.04
2.03
2.01
2.00
1.99
0.85
0.67
0.66
0.50
0.49
2.00
3.43
3.20
2.93
2.97
ppm

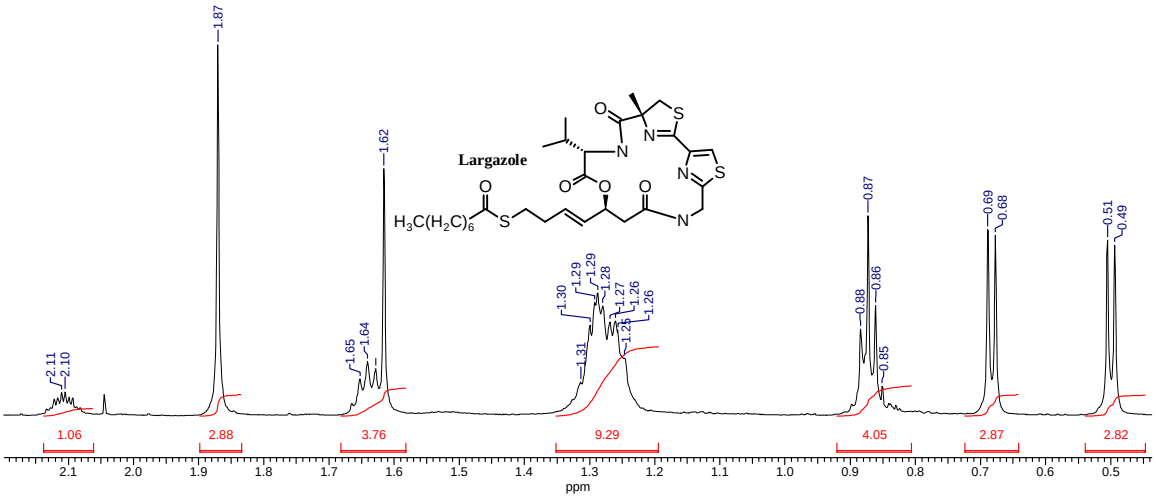
^{13}C of cyclic core 41



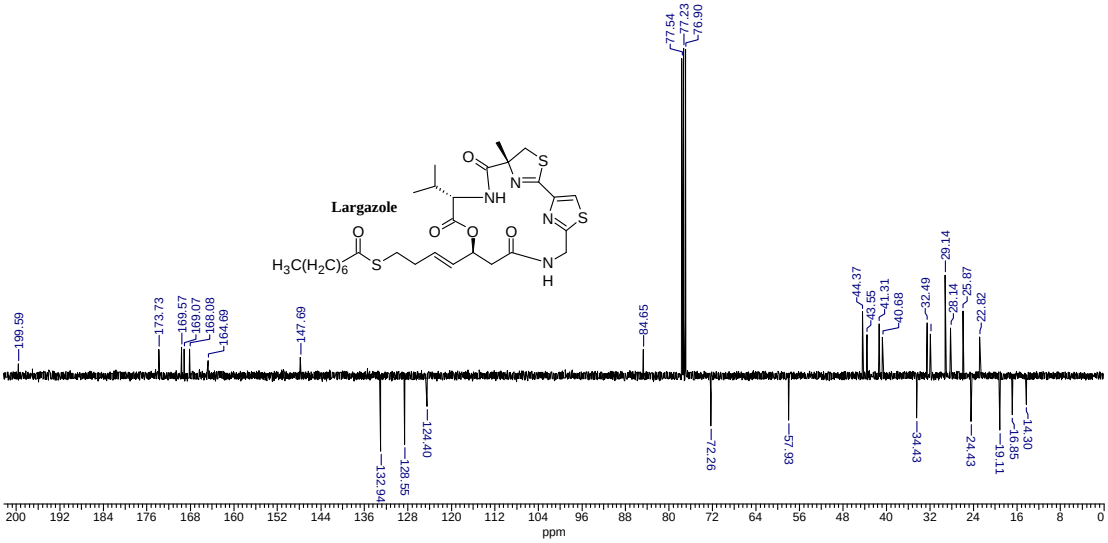
^{1}H NMR of largazole (1)

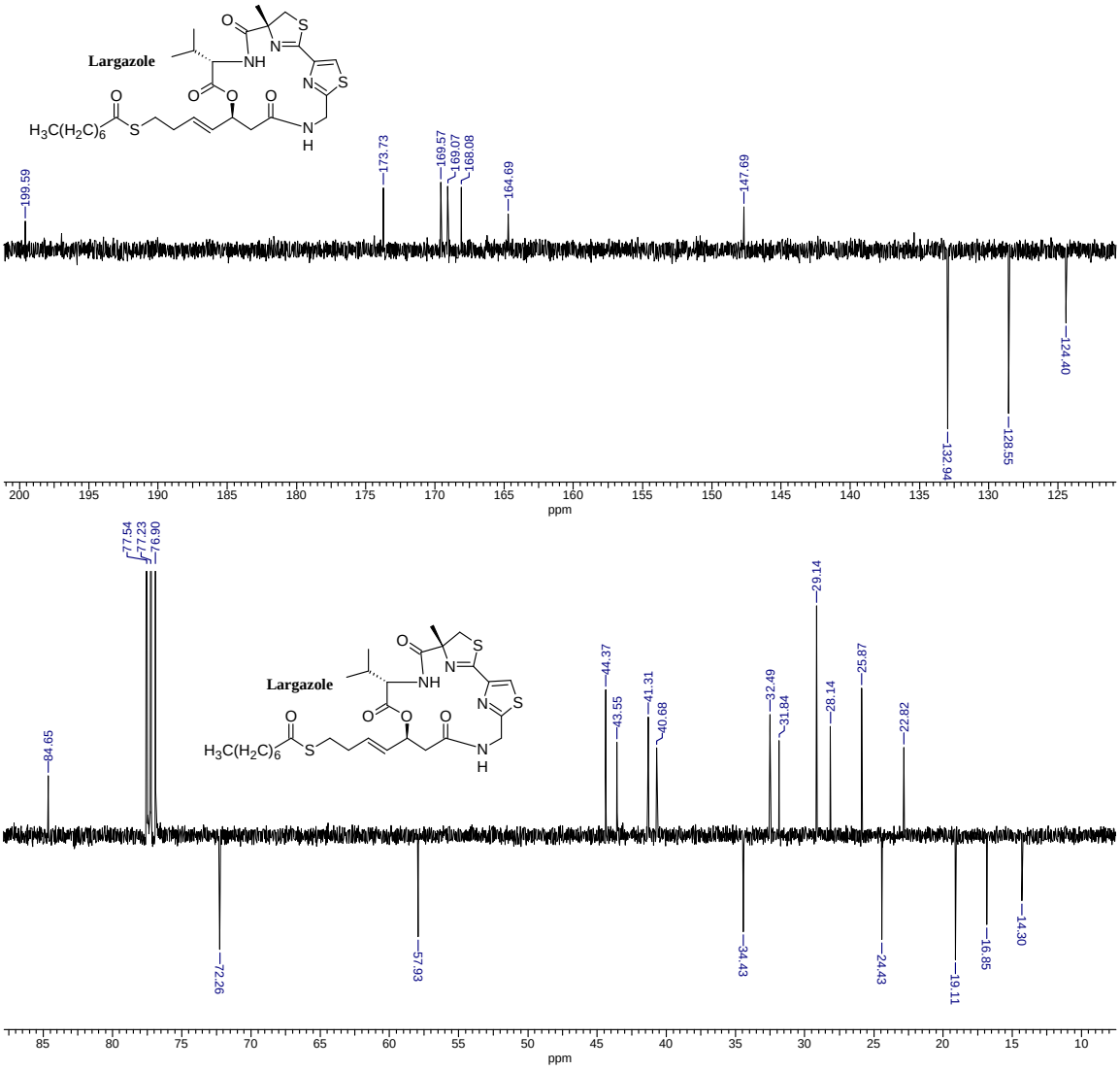



Largazole
4.62
4.61
4.60
4.60
4.29
4.28
4.26
4.25
4.05
4.03
3.29
3.27
2.91
2.90
2.88
2.87
2.85
2.84
2.83
2.70
2.70
2.68
2.67
2.54
2.53
2.52
2.32
2.31
2.30
2.29
2.11
2.10
1.01
1.04
1.03
1.00
1.86
1.05
1.01
1.92
2.05
1.06
4.5
4.0
3.5
3.0
2.5
2.0
ppm

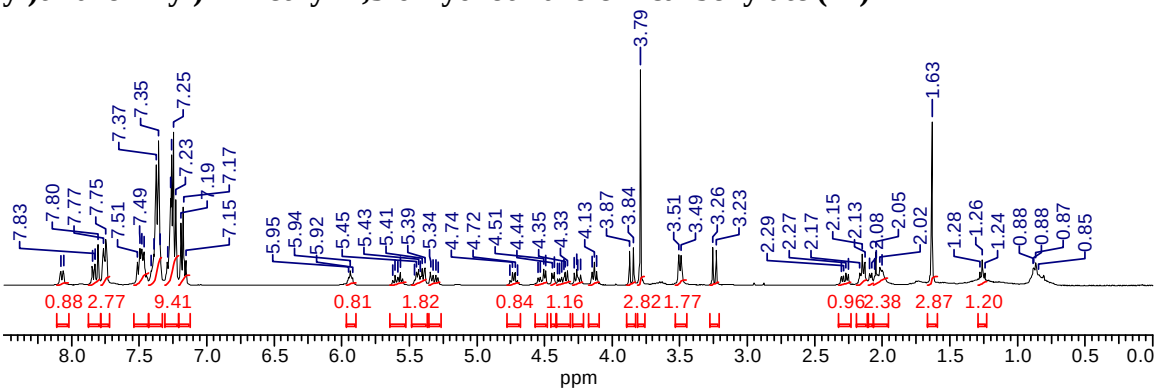

Largazole
1.87
1.62
1.65
1.64
1.30
1.29
1.29
1.28
1.27
1.26
1.26
1.25
1.31
0.88
0.87
0.86
0.85
0.69
0.68
0.51
0.49
2.11
2.10
1.06
2.88
3.76
9.29
4.05
2.87
2.82
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
ppm

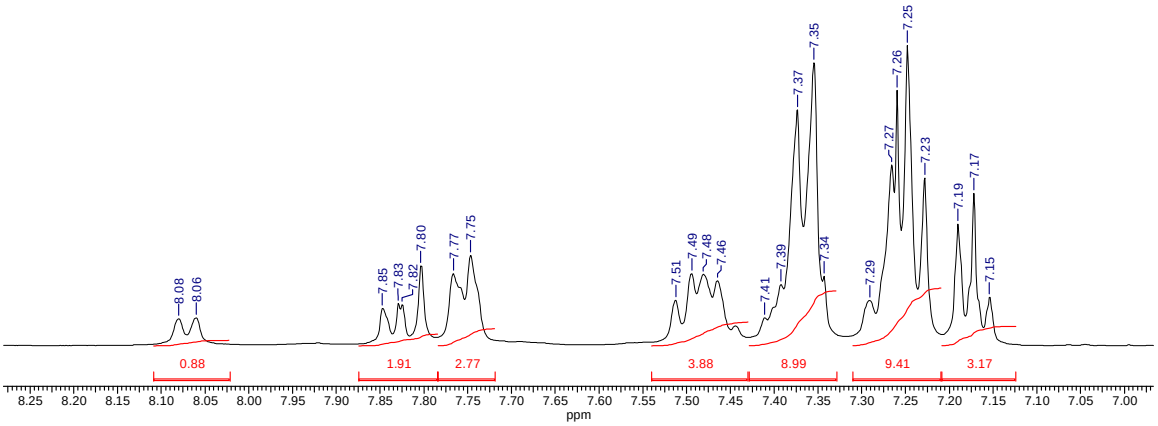
^{13}C NMR of largazole (1)

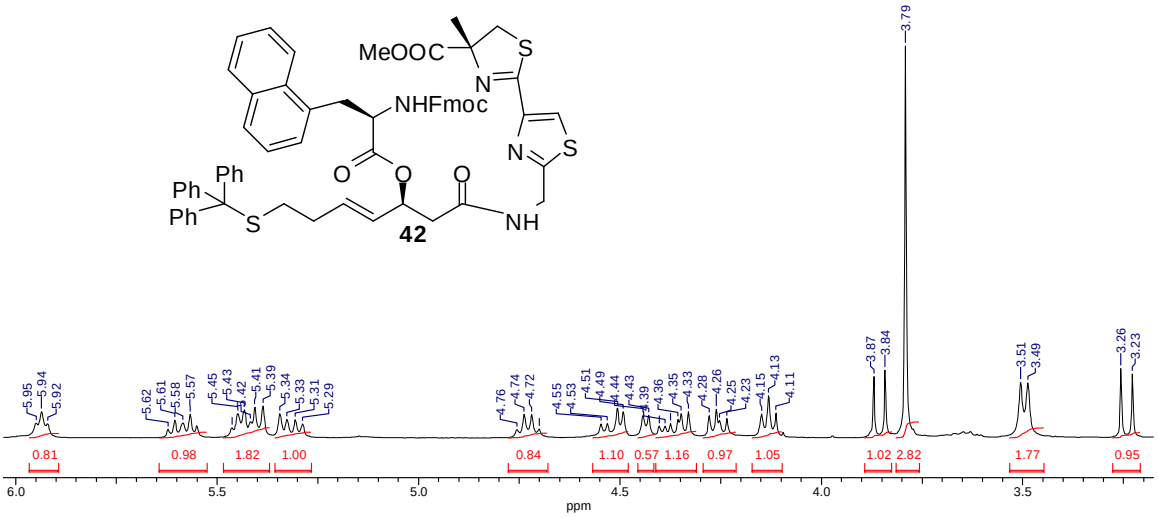


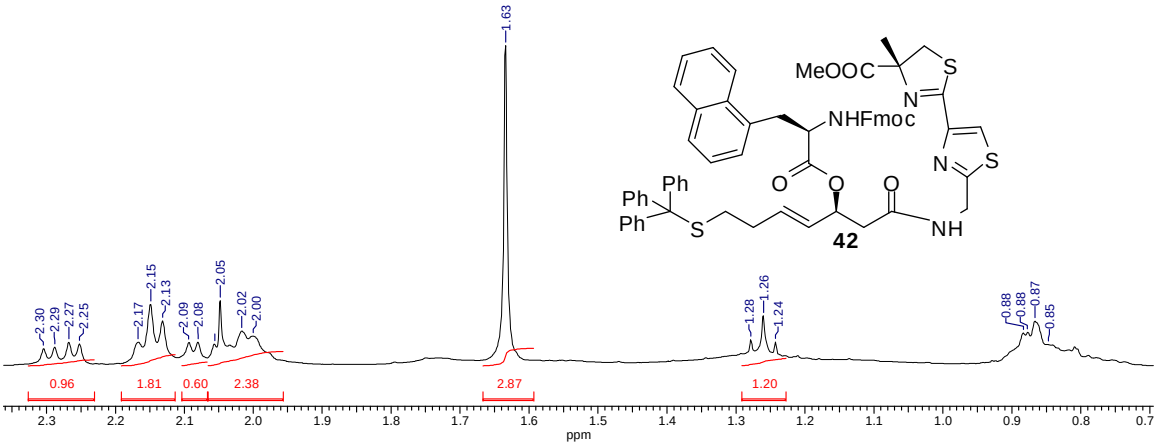

Largazole
199.59
173.73
169.57
169.07
168.08
164.69
147.69
132.94
128.55
124.40
ppm
Largazole
77.54
77.23
76.90
84.65
72.26
57.93
44.37
43.55
41.31
40.68
34.43
32.49
31.84
29.14
28.14
25.87
24.43
22.82
19.11
16.85
14.30
ppm

^{1}H NMR of (*R*)-methyl 2-(2-((5*R*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (42)

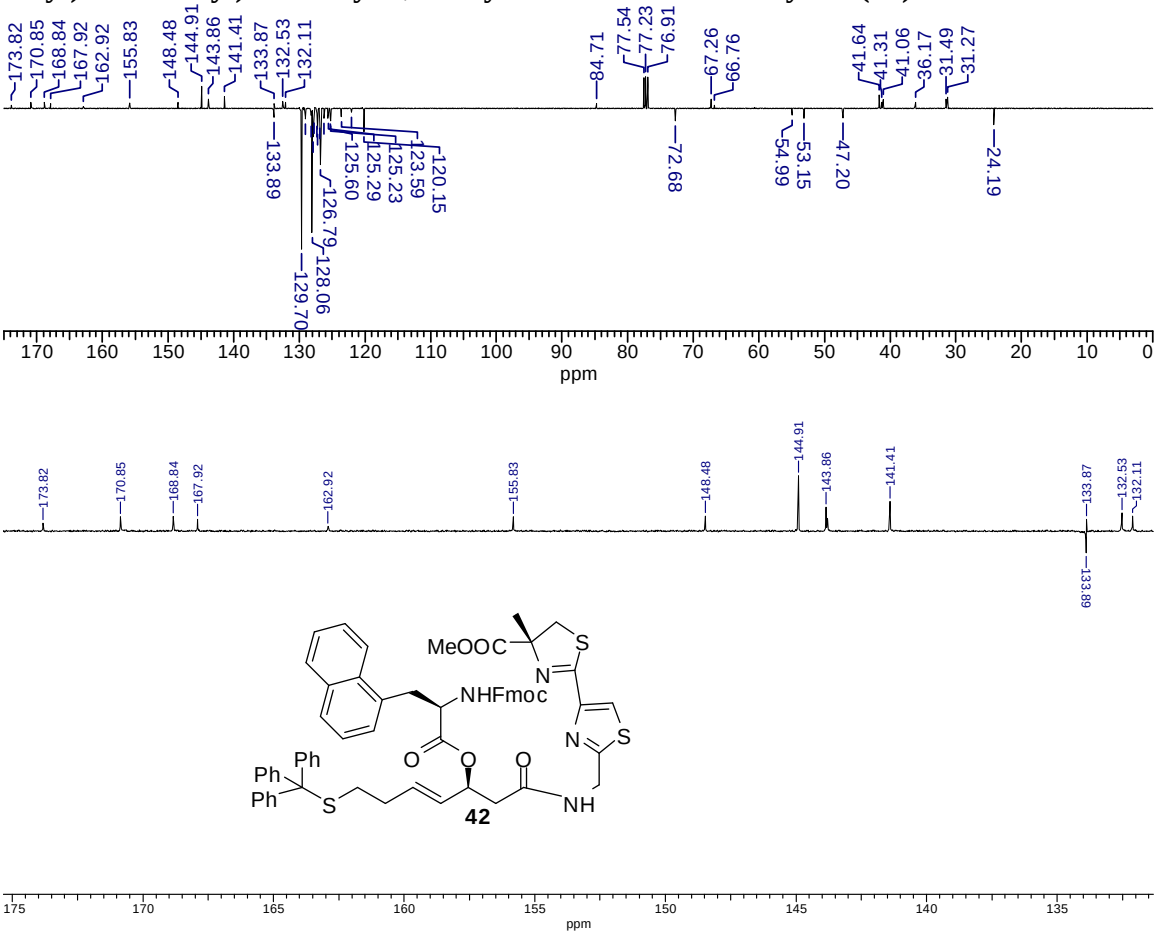


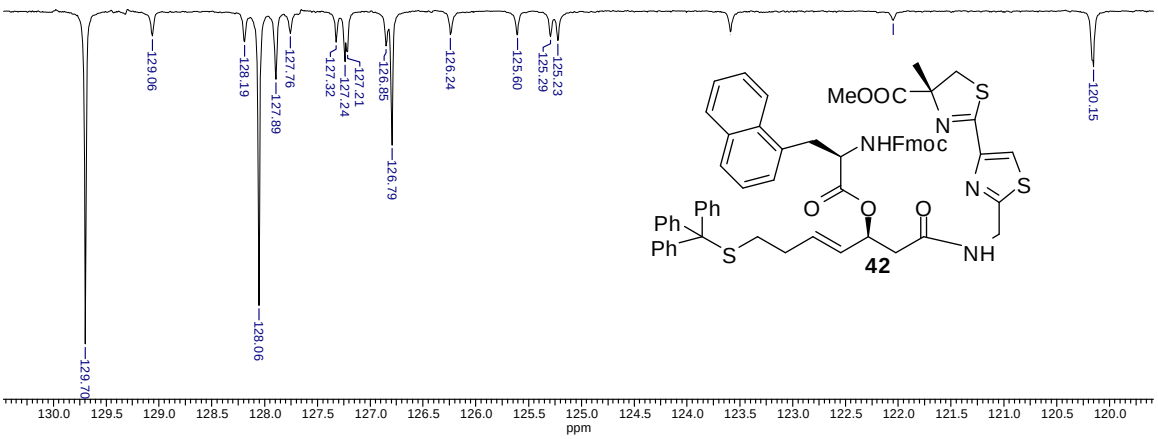




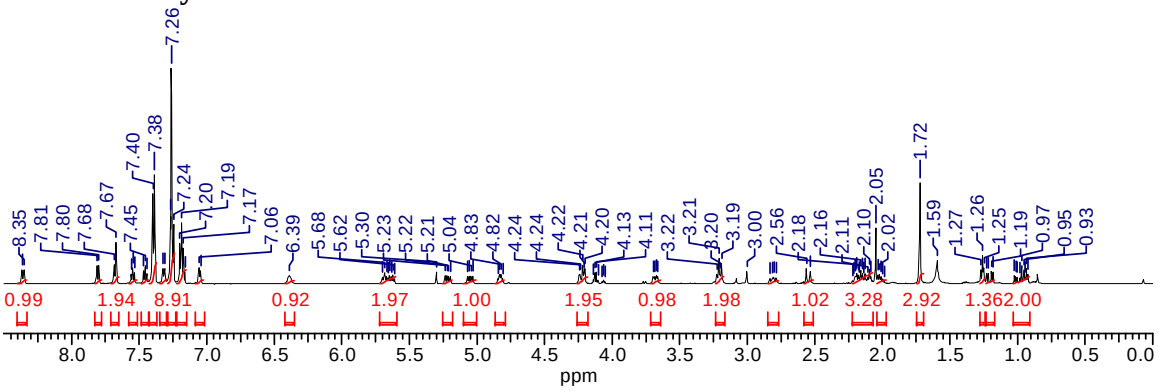


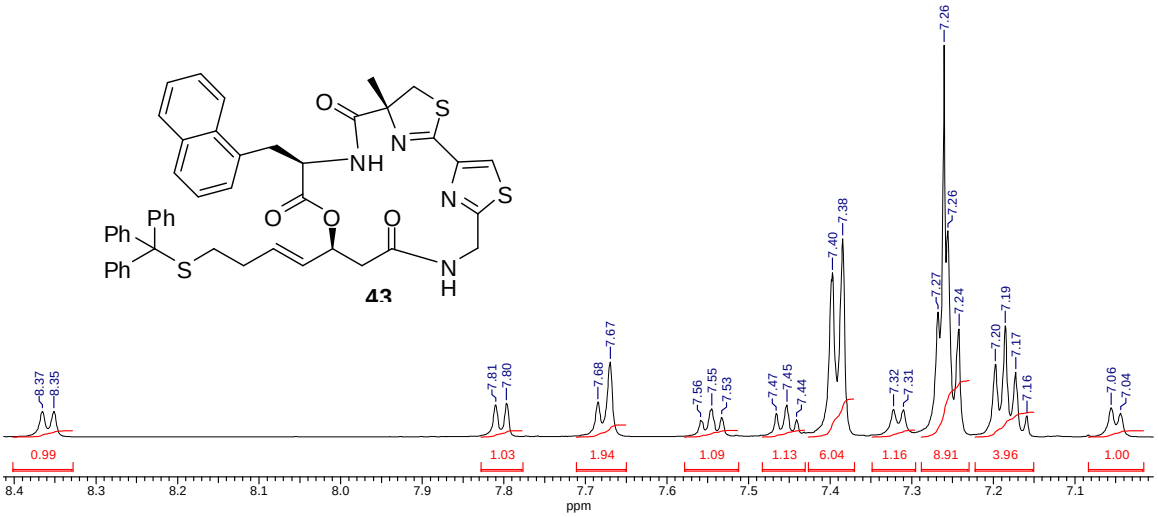
^{13}C NMR of (*R*)-methyl 2-(2-((5*R*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (42)

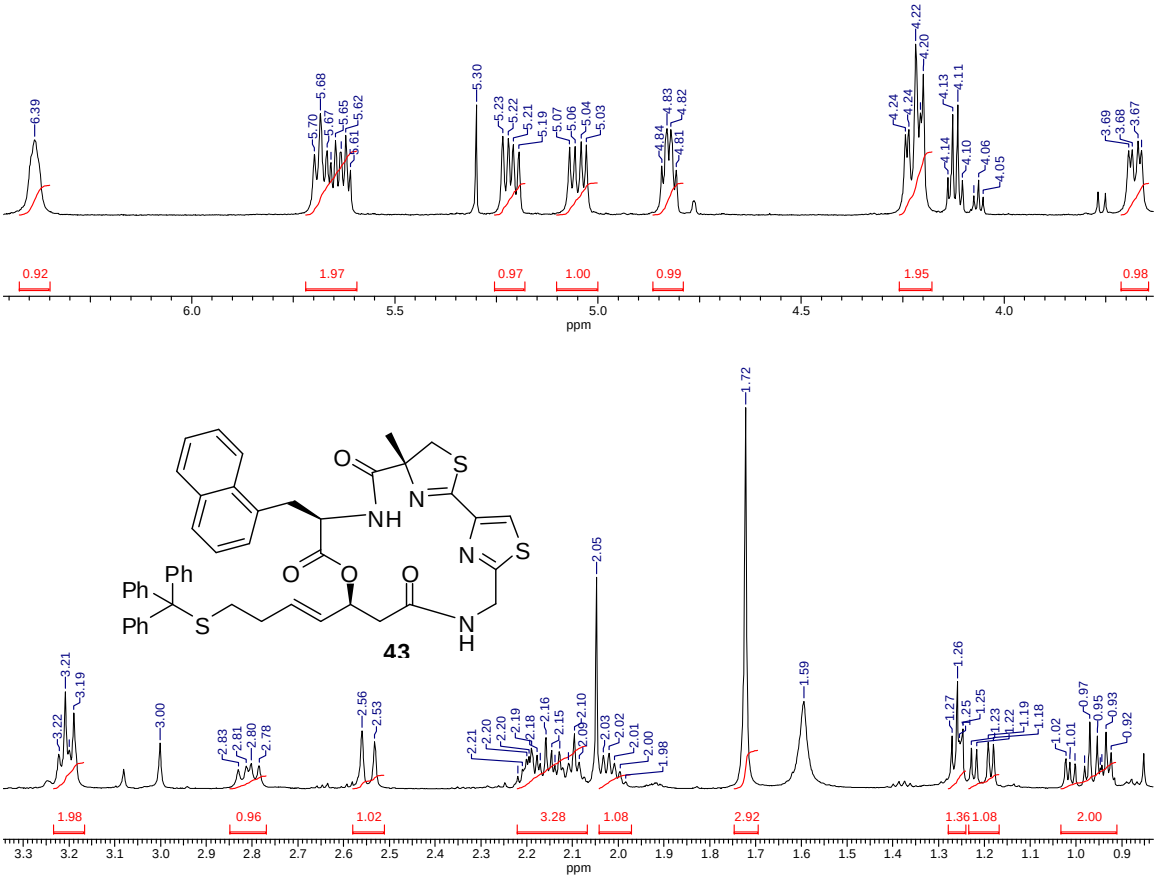




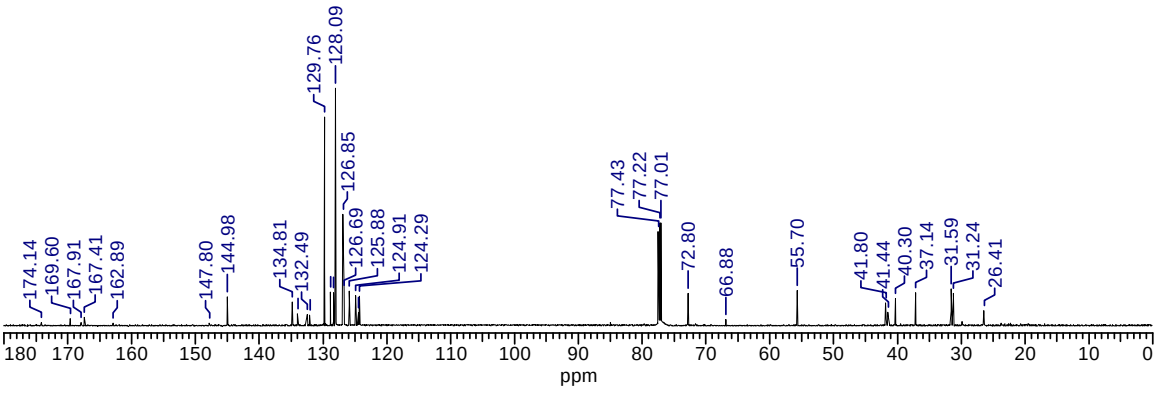
^{1}H NMR of cyclic core 43

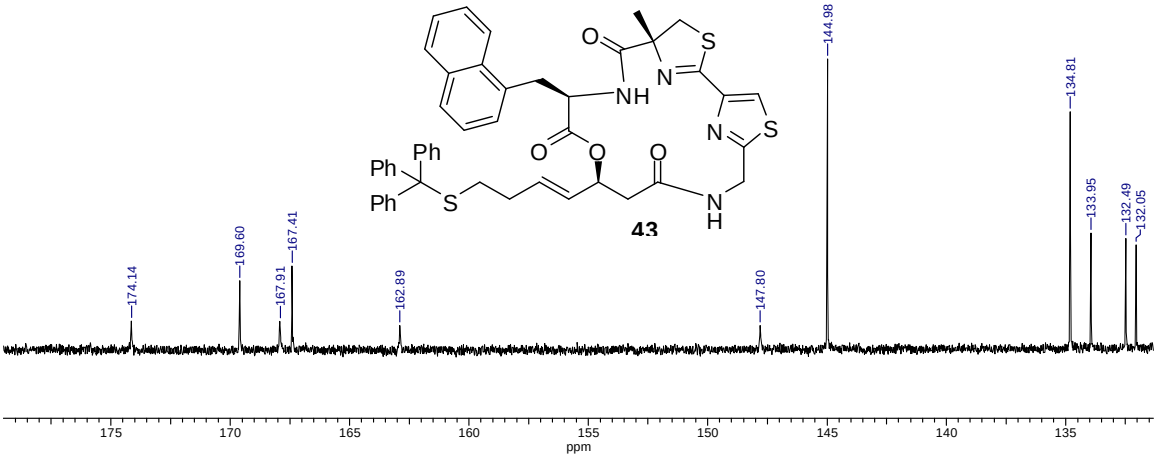


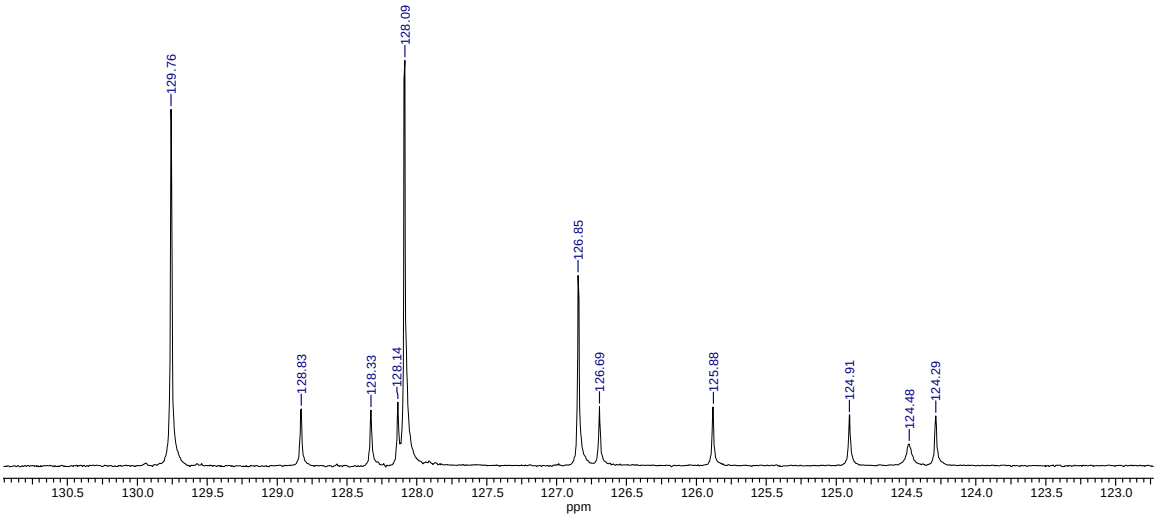


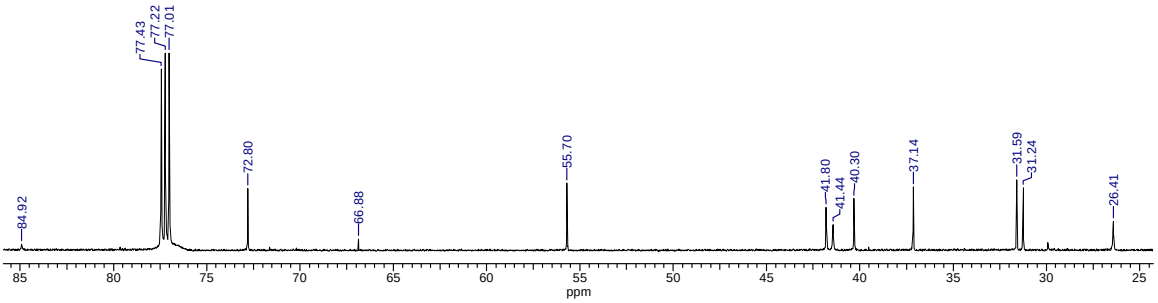


^{13}C NMR of cyclic core 43

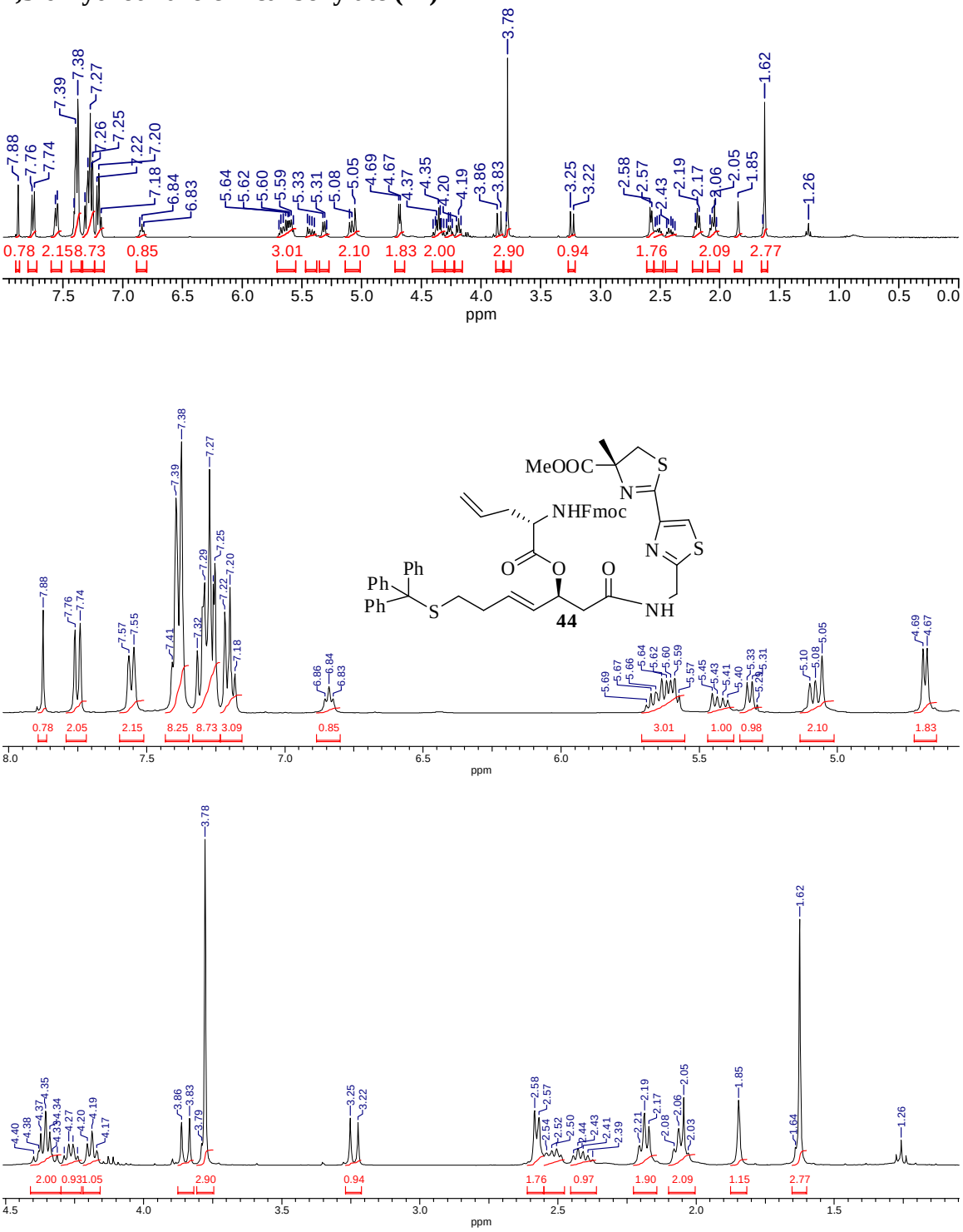



O
S
N
NH
N
S
O
O
O
Ph
Ph
Ph
S
N
H
43
144.98
134.81
133.95
132.49
132.05
174.14
169.60
167.41
167.91
162.89
147.80
175
170
165
160
155
150
145
140
135
ppm

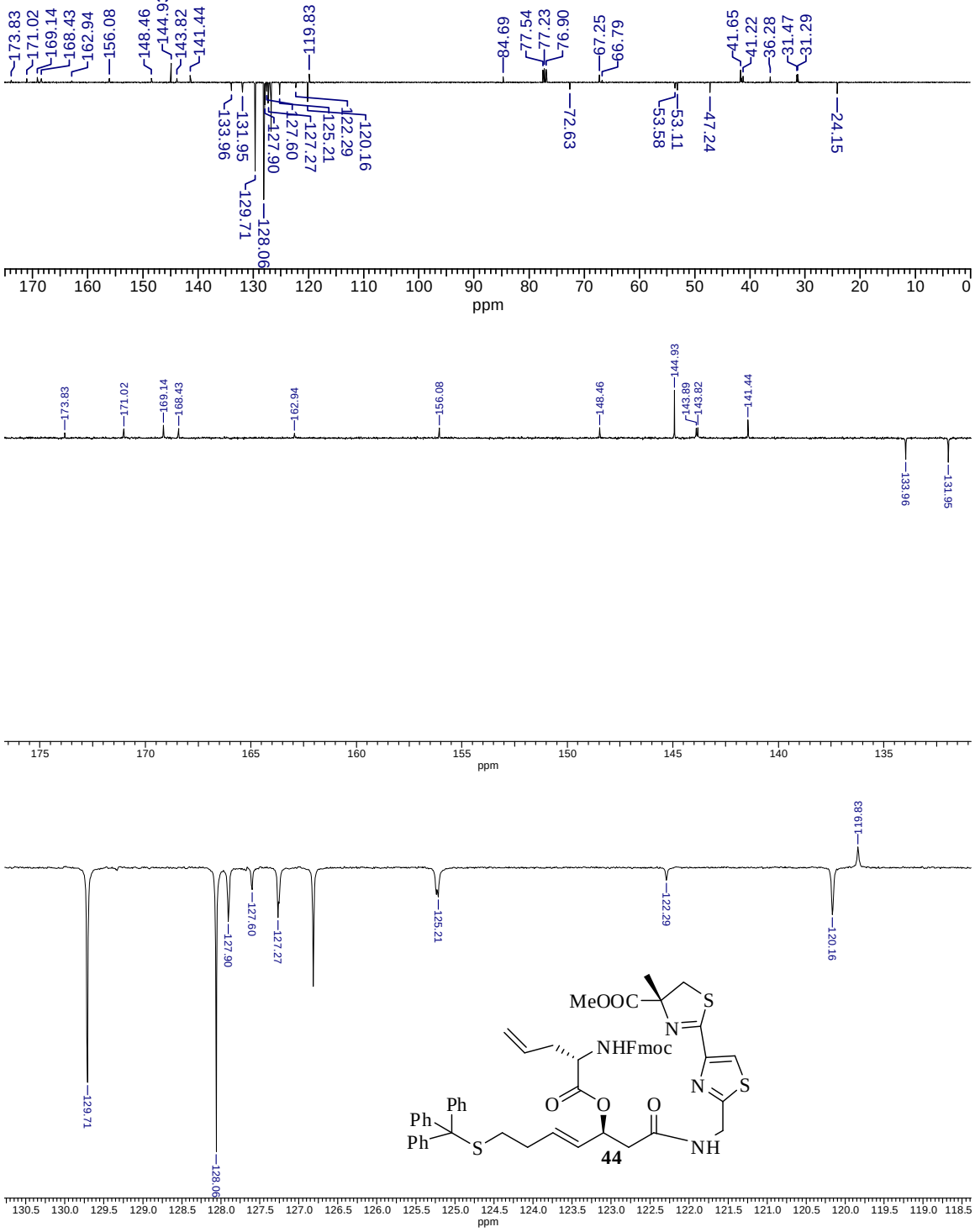

129.76
128.09
126.85
128.83
128.33
128.14
126.69
125.88
124.91
124.48
124.29
130.5
130.0
129.5
129.0
128.5
128.0
127.5
127.0
126.5
126.0
125.5
125.0
124.5
124.0
123.5
123.0
ppm

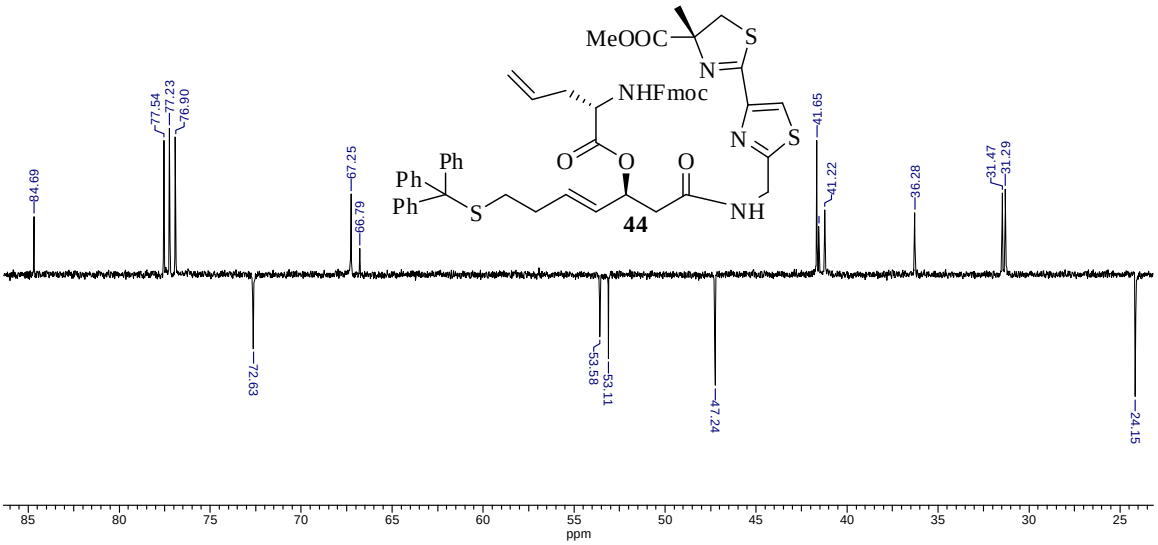

77.43
77.22
77.01
72.80
84.92
66.88
55.70
41.80
41.44
40.30
37.14
31.59
31.24
26.41
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-5-allyl-1-(9*H*-fluoren-9-yl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (44)

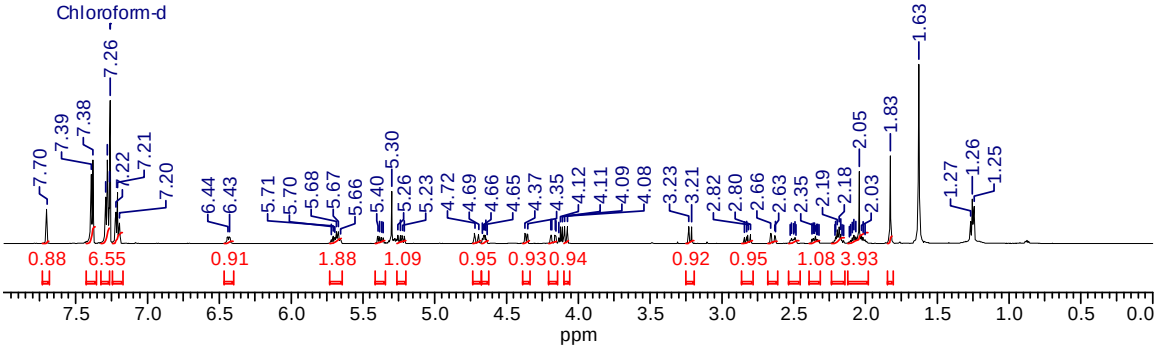


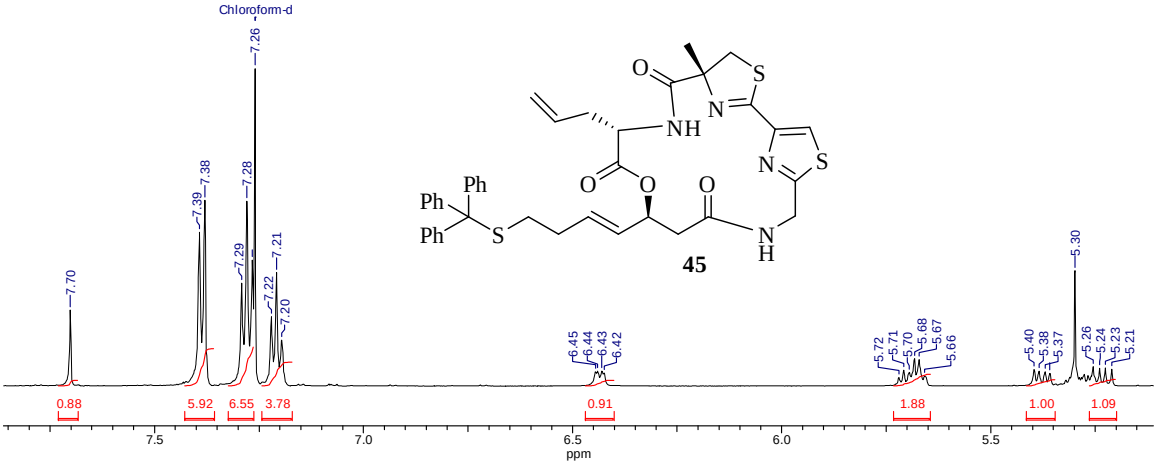
^{13}C NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-5-allyl-1-(9*H*-fluoren-9-yl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (44)

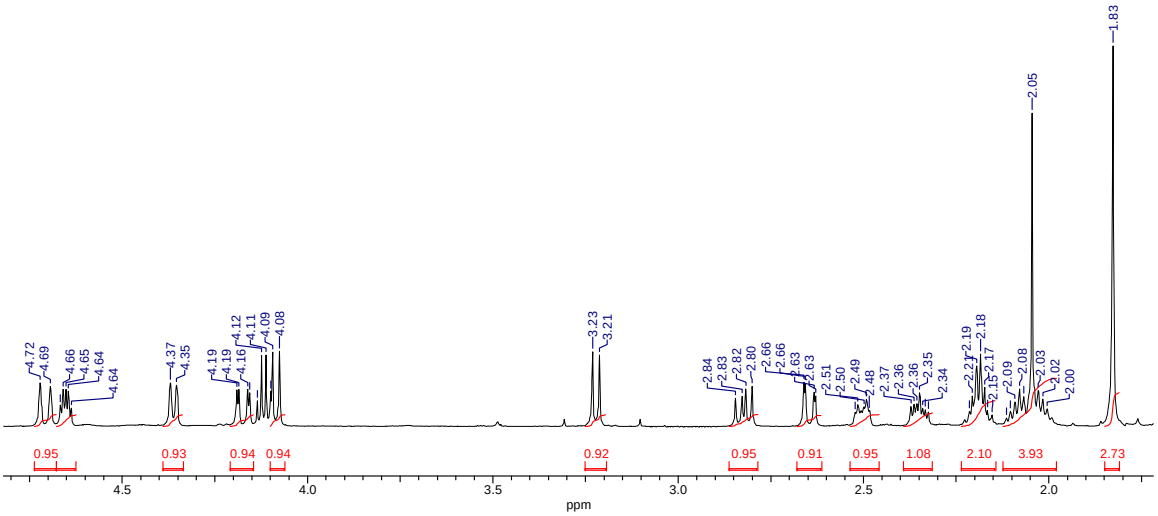



MeOOC
S
N
NHFmoc
N
S
O
O
O
Ph
Ph
Ph
S
NH
44
84.69
77.54
77.23
76.90
67.25
66.79
72.63
53.58
53.11
47.24
41.65
41.22
36.28
31.47
31.29
24.15
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

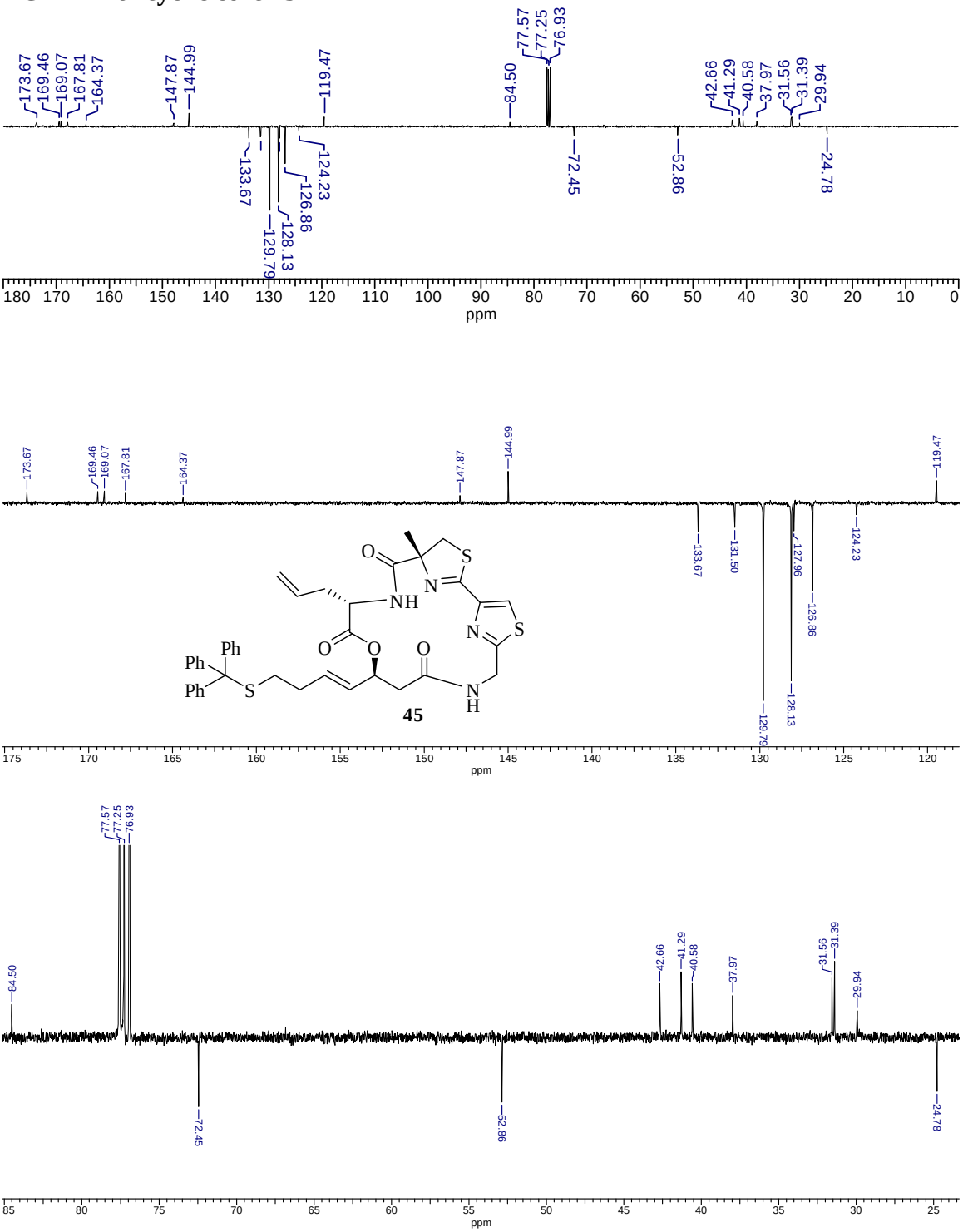
^{1}H NMR of cyclic core 45



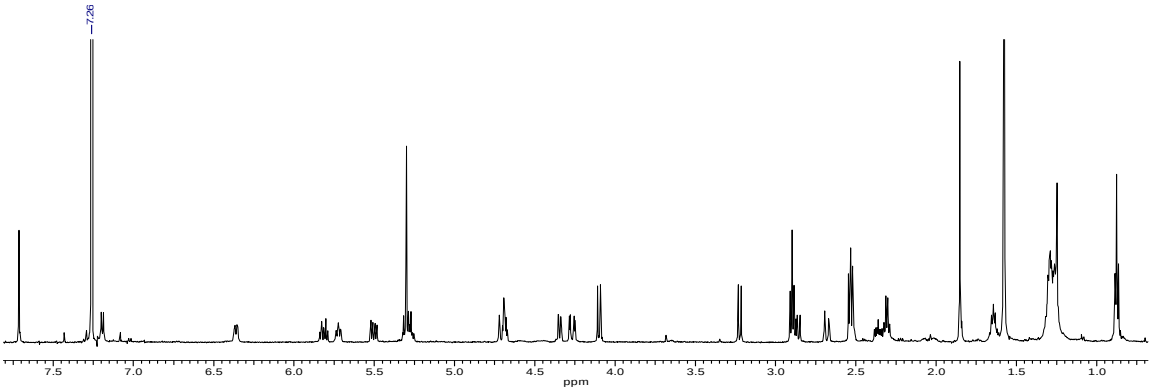


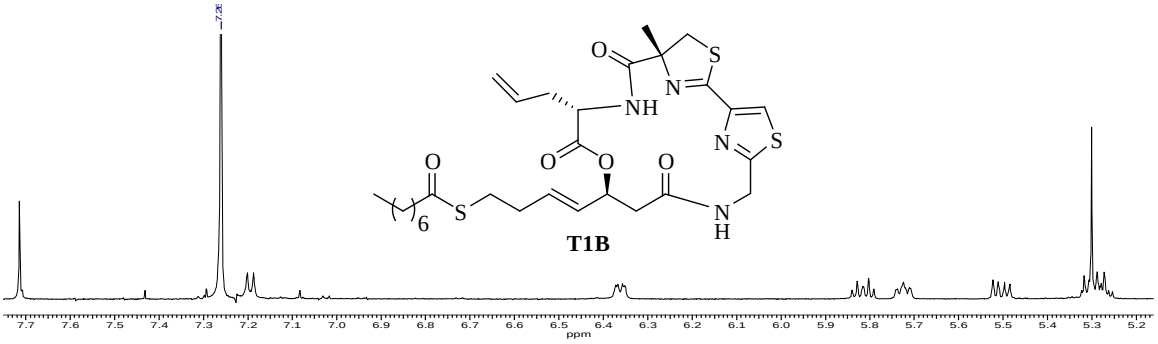


^{13}C NMR of cyclic core 45

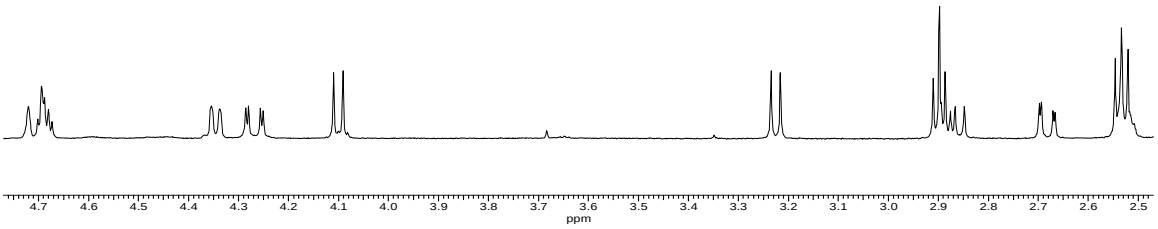


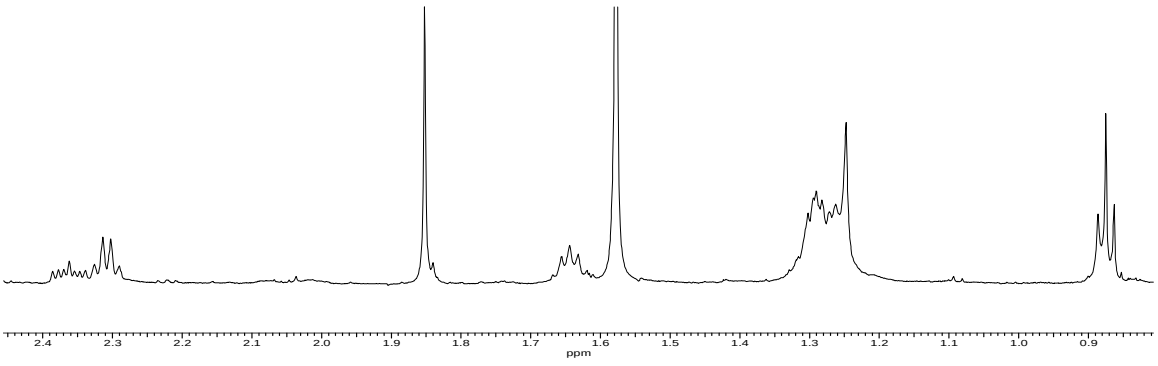
^{1}H NMR of analog T1B


7.26
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

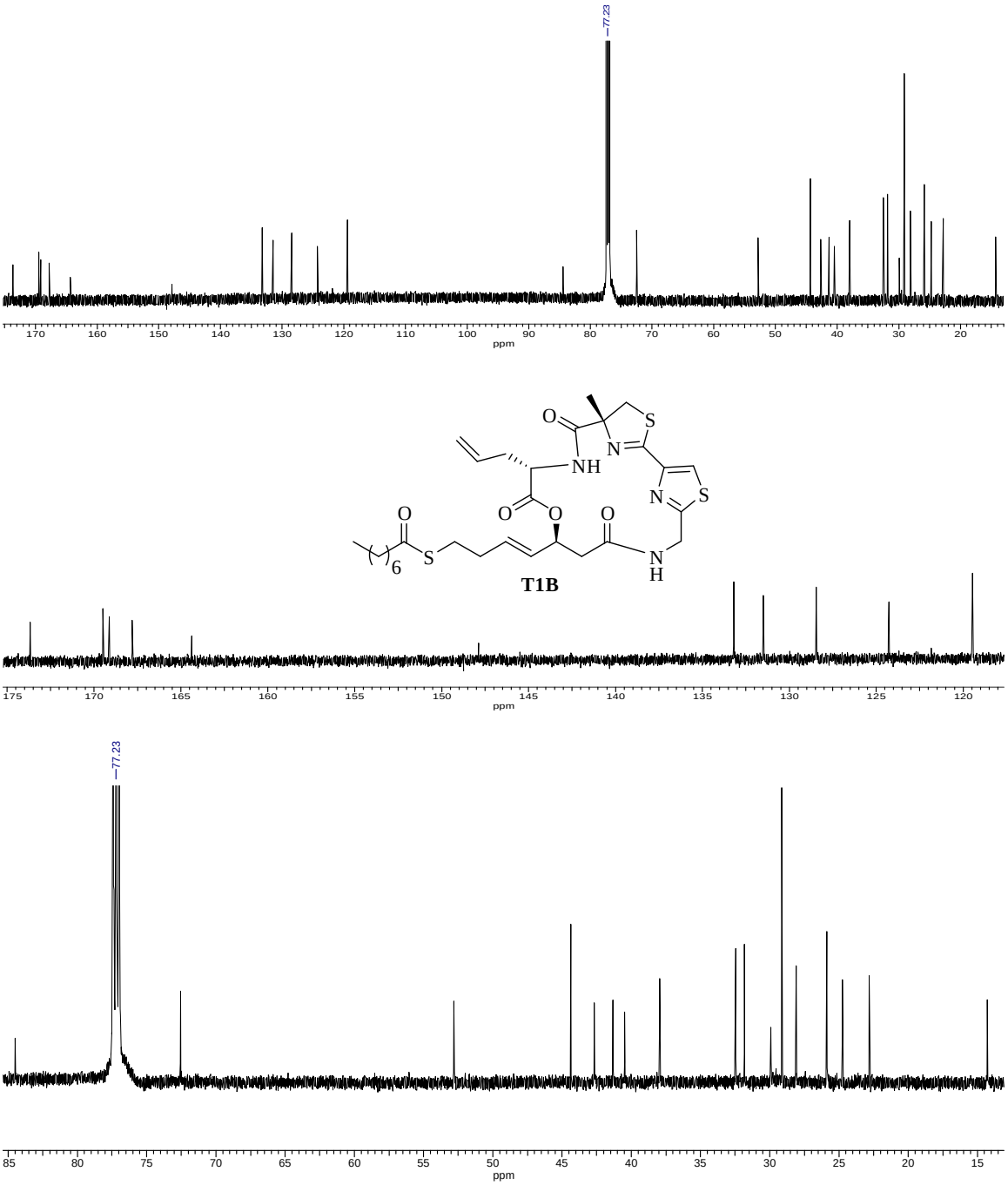

7.26
O
S
N
NH
N
S
O
O
O
O
6
S
N
H
7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2
ppm

T1B

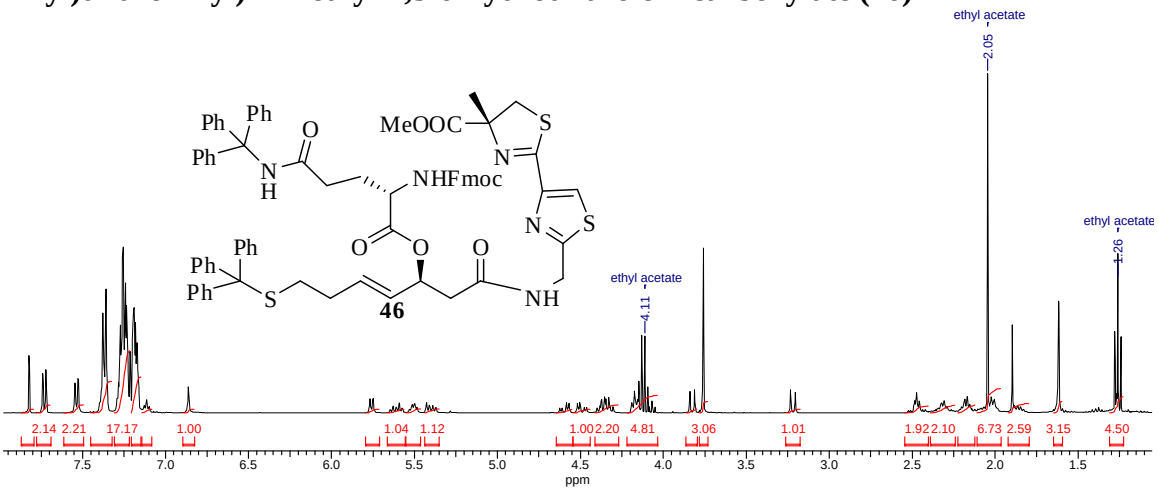

4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5
ppm

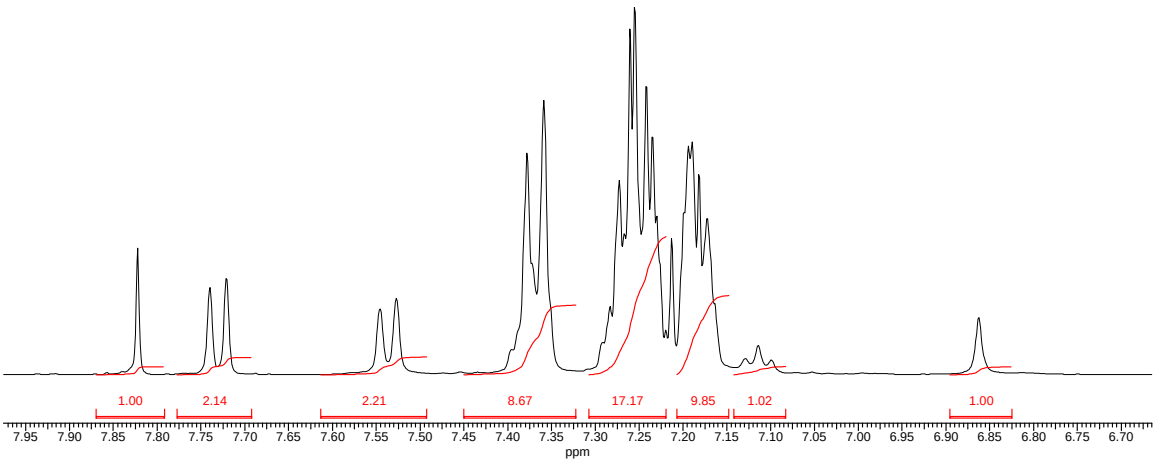

2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9
ppm

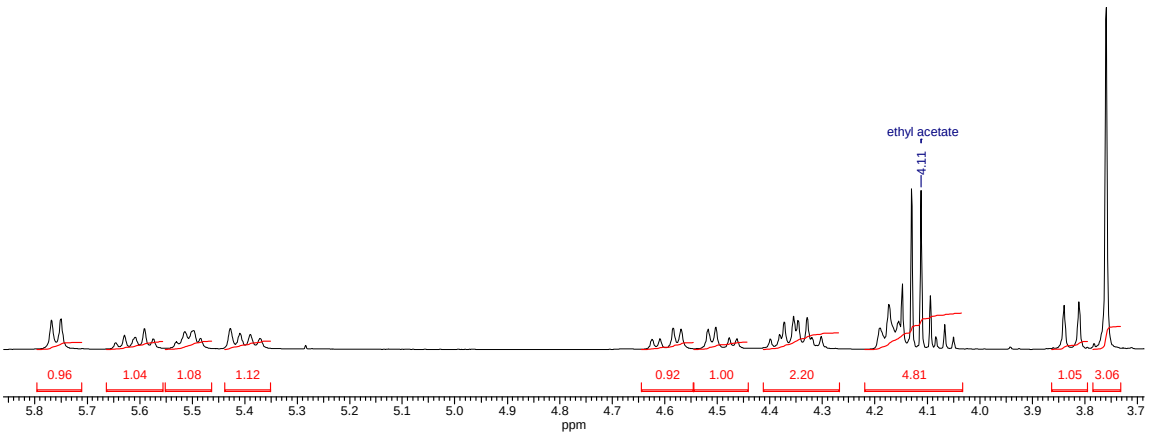
^{13}C NMR of T1B

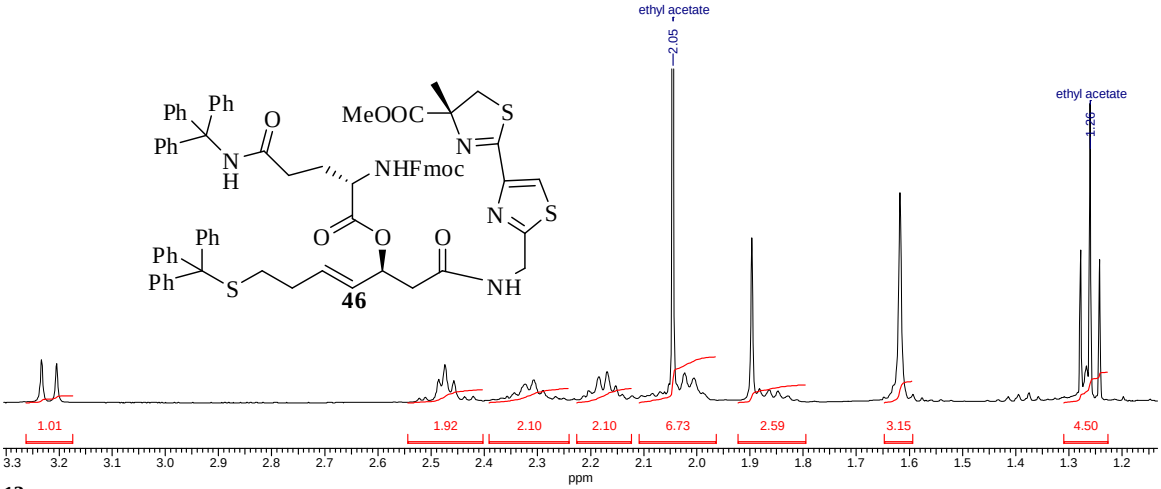


^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-3,6,10-trioxo-5-(3-oxo-3-(tritylamino)propyl)-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (46)

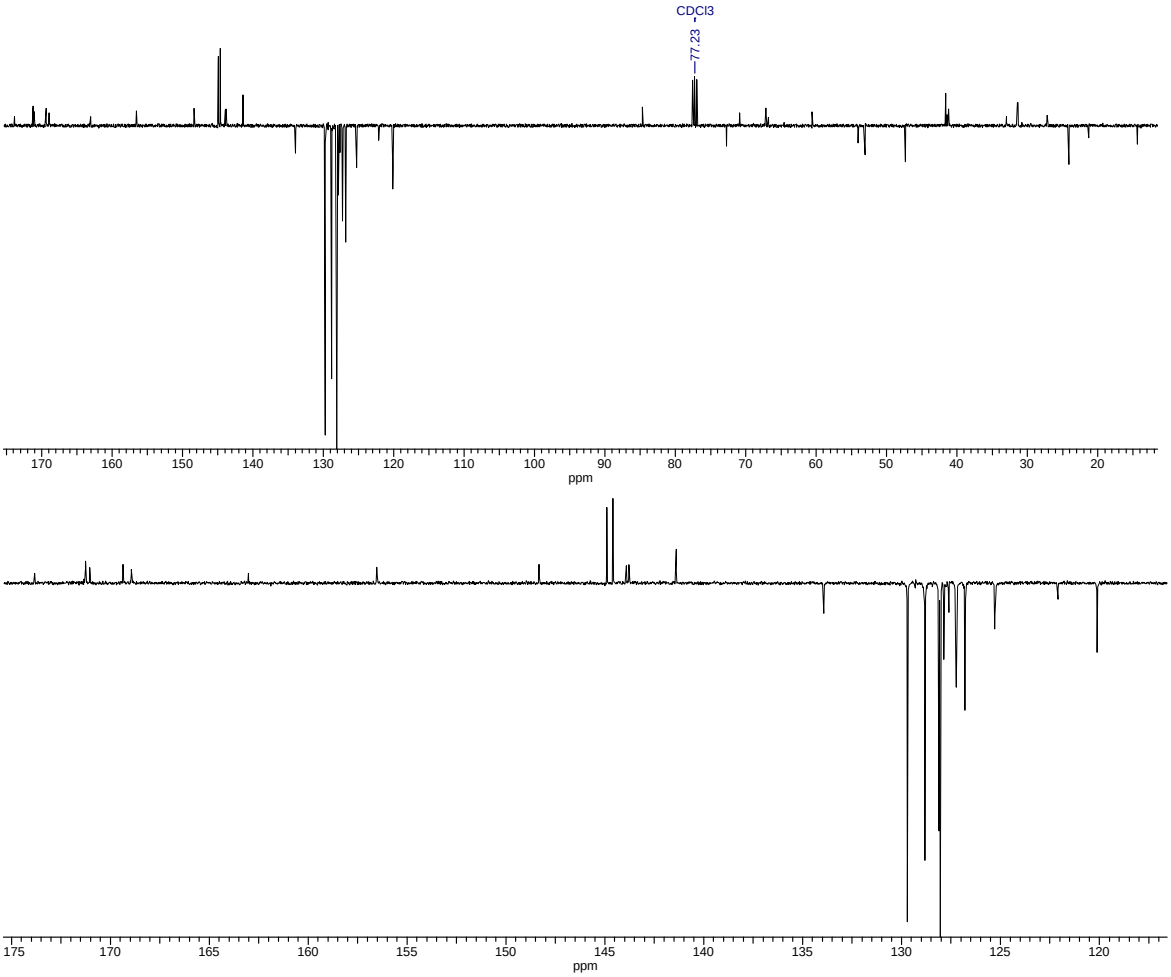


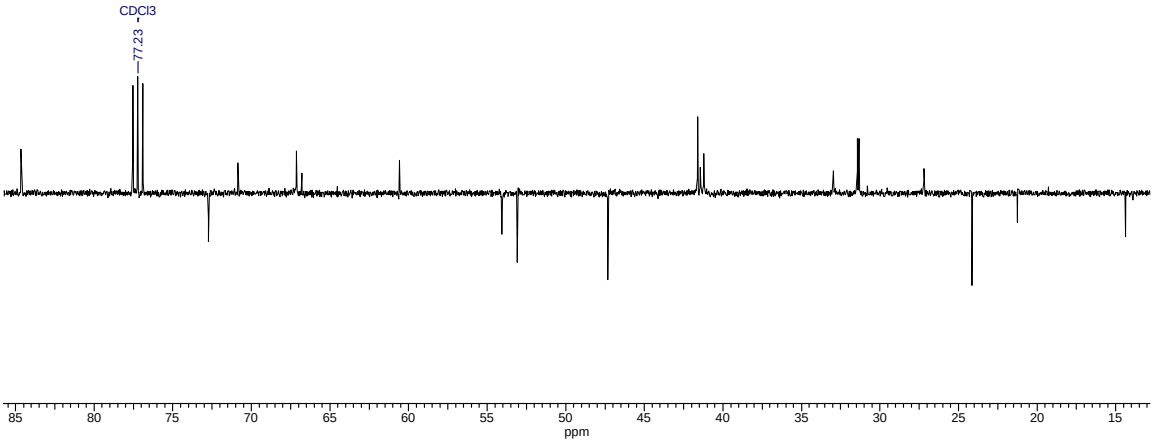




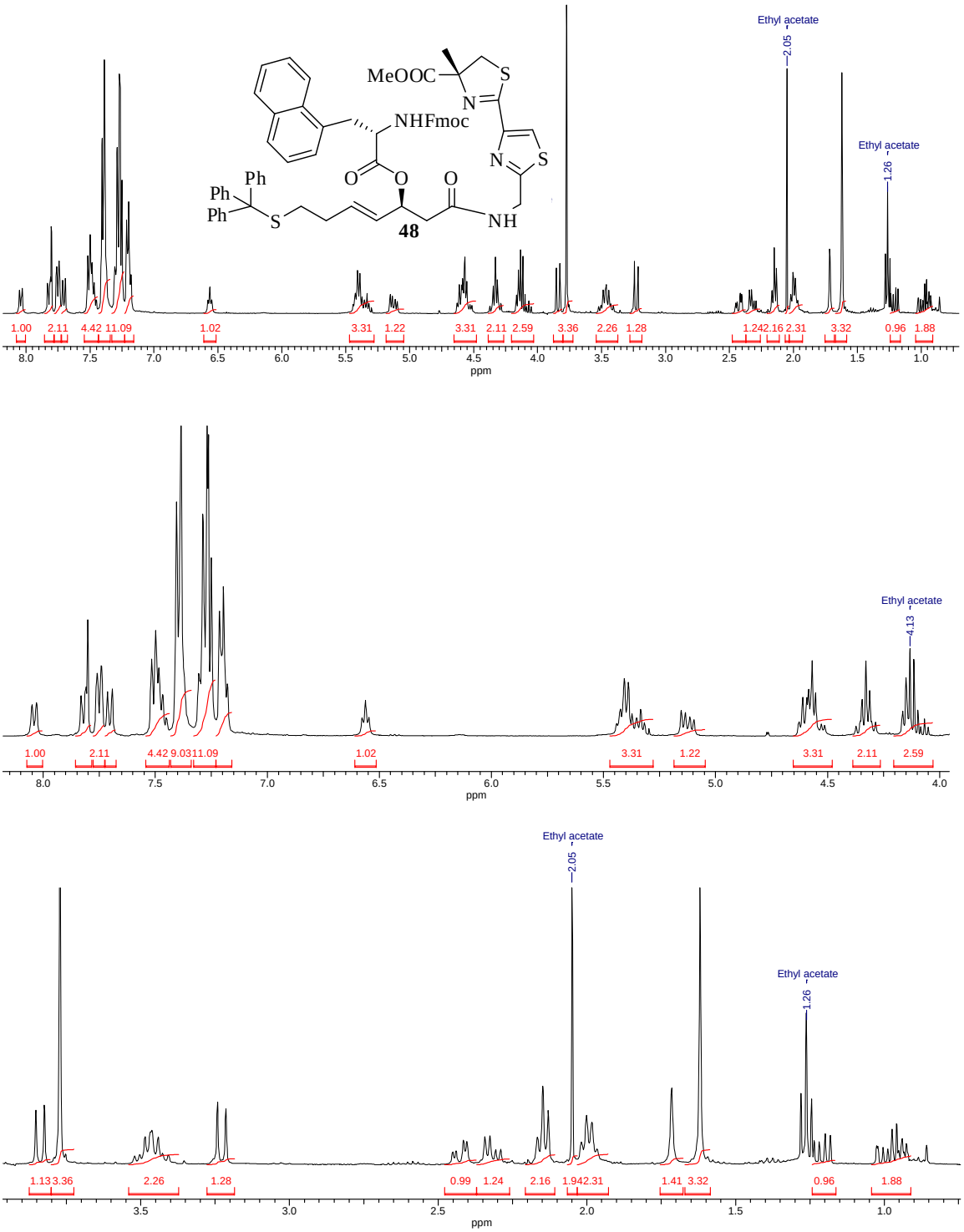


^{13}C NMR of (*R*)-methyl 2-(2-((5S,8S)-1-(9H-fluoren-9-yl)-3,6,10-trioxo-5-(3-oxo-3-(tritylamino)propyl)-8-((E)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (46)

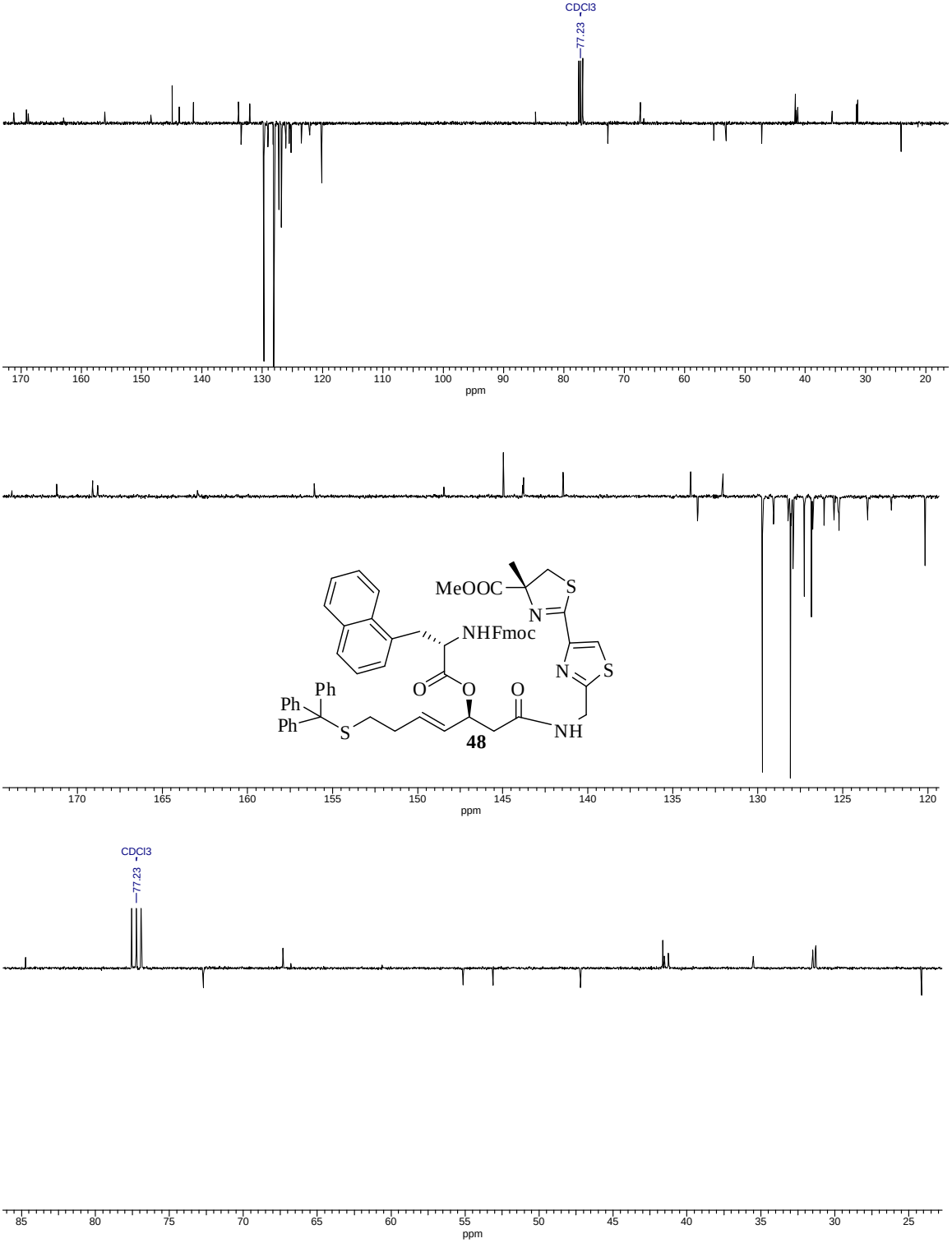



CDCl3
77.23
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
ppm

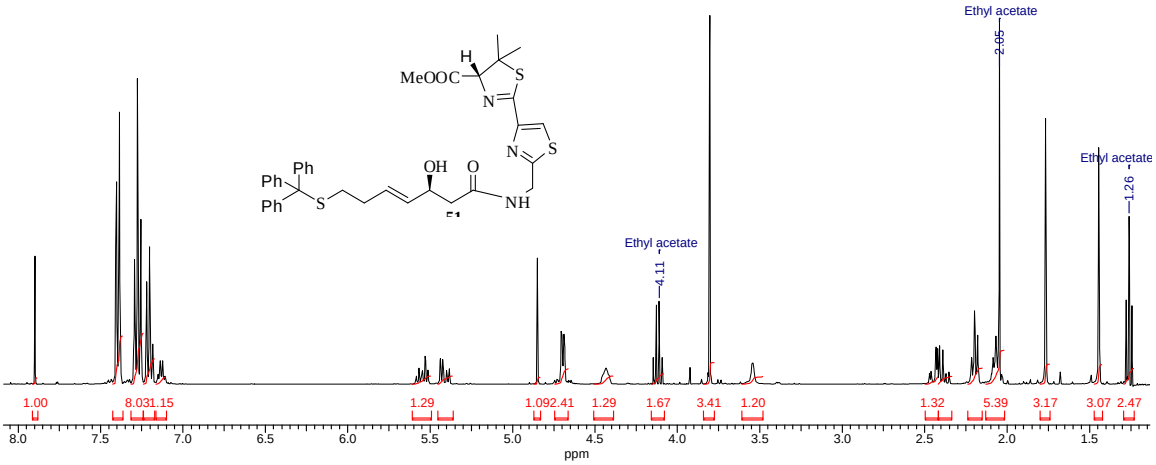
^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (48)

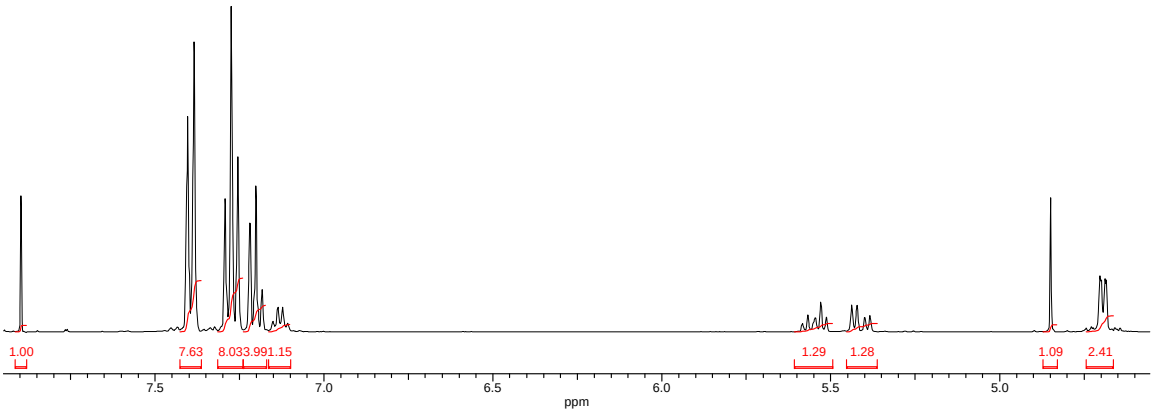


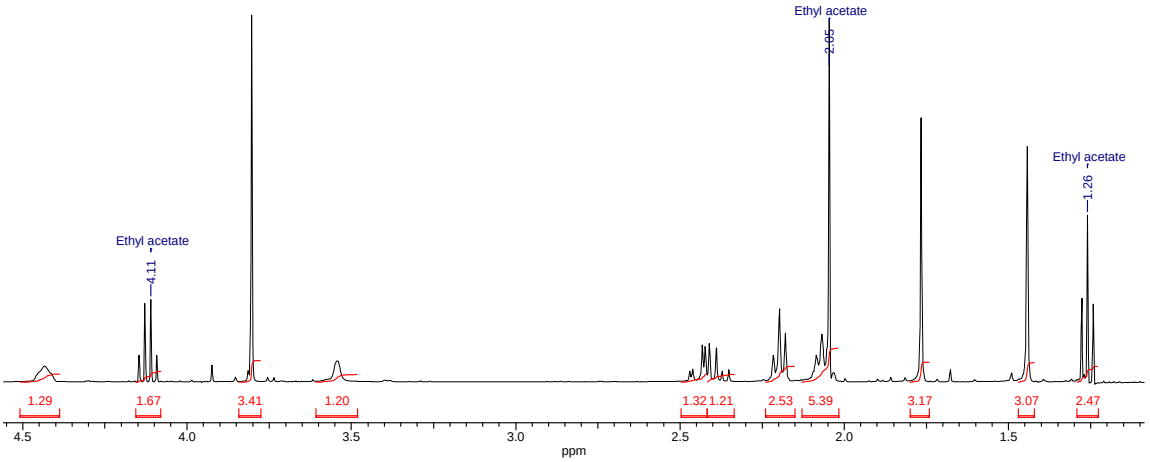
^{13}C NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-(naphthalen-1-ylmethyl)-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (48)



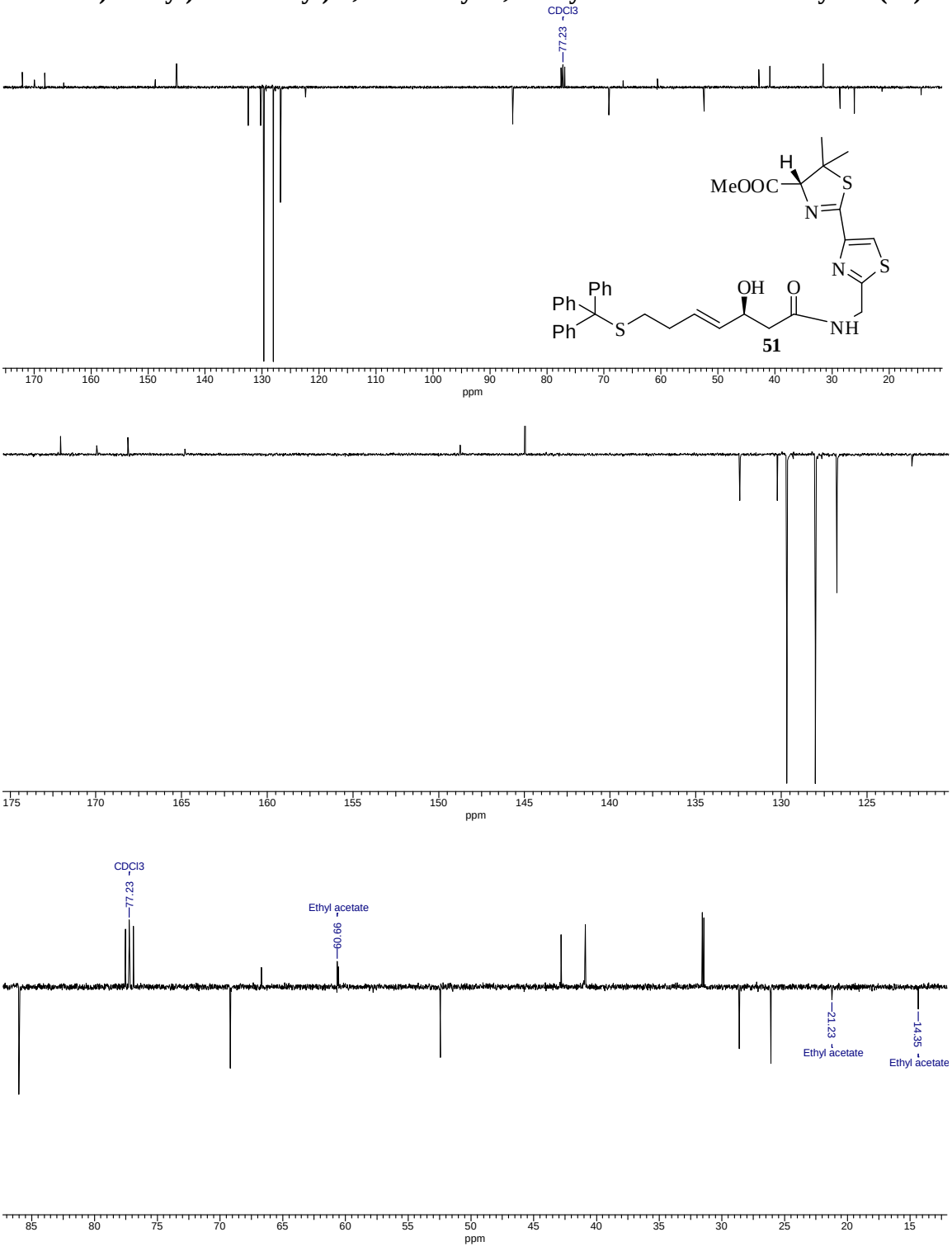
^{1}H NMR of (*R*)-methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (51)



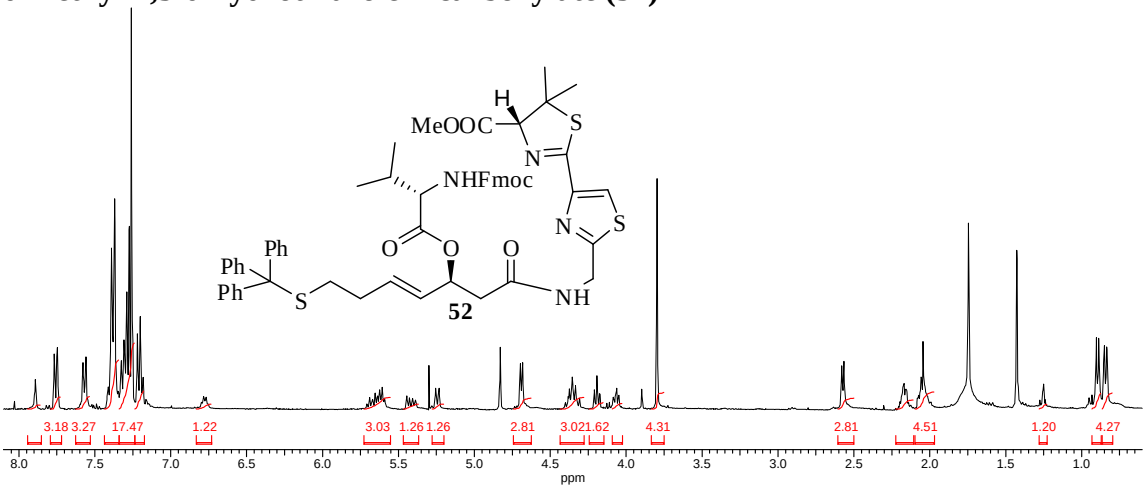


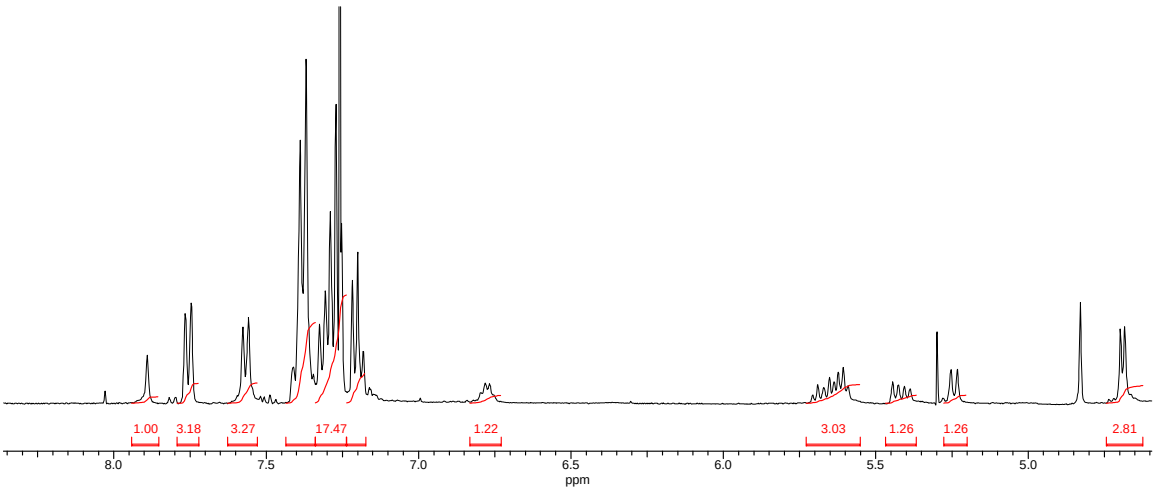


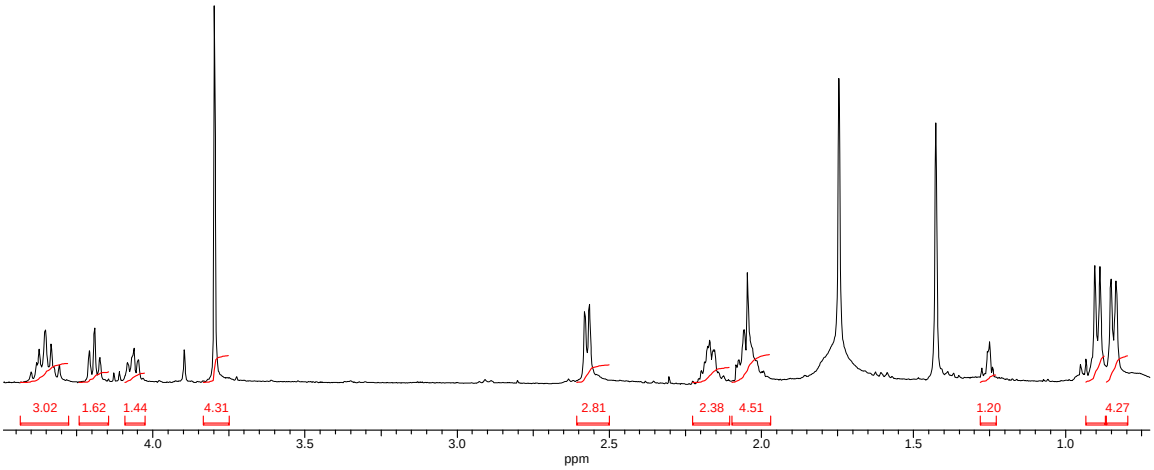
^{13}C NMR of (*R*)-methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (51)



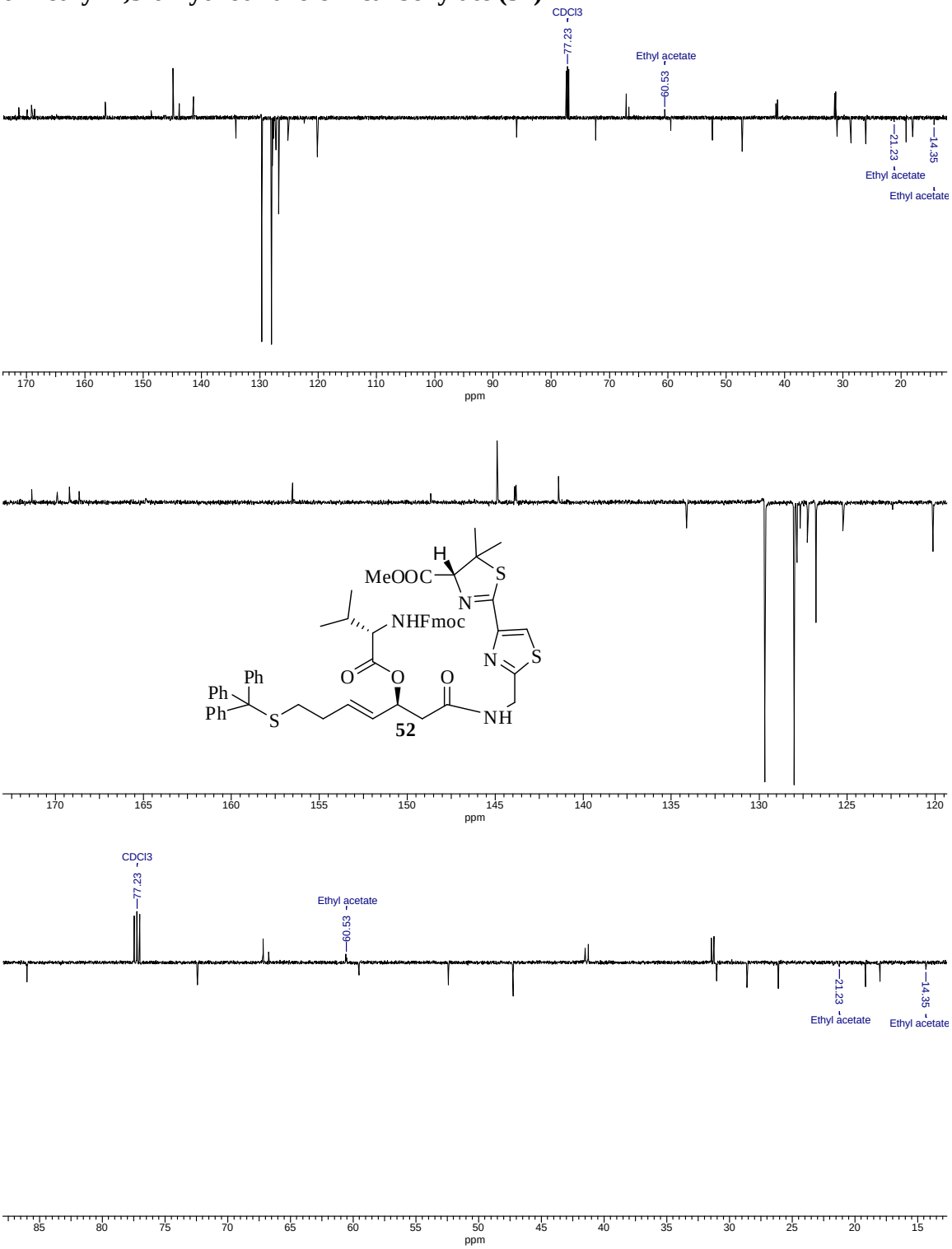
^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (52)



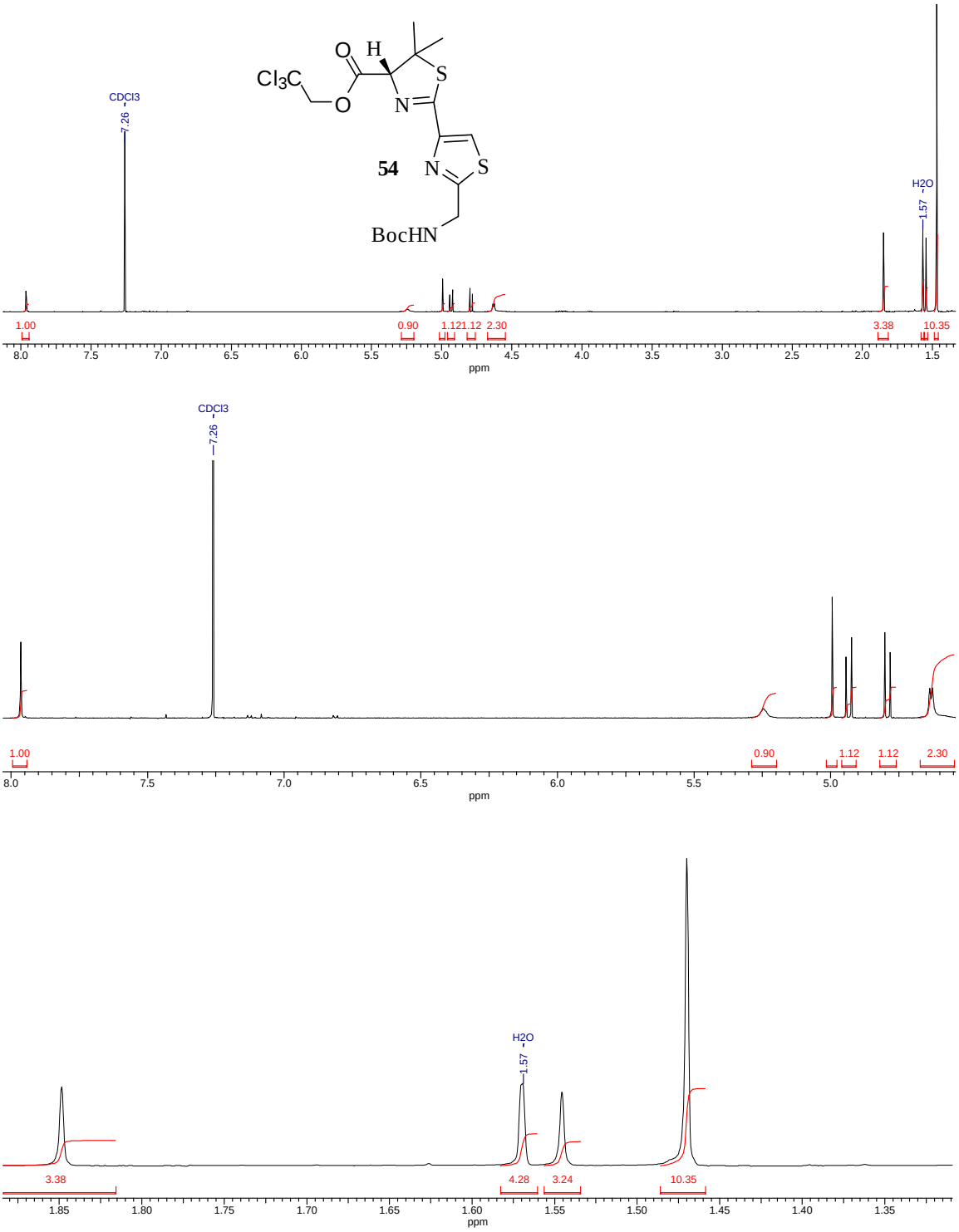




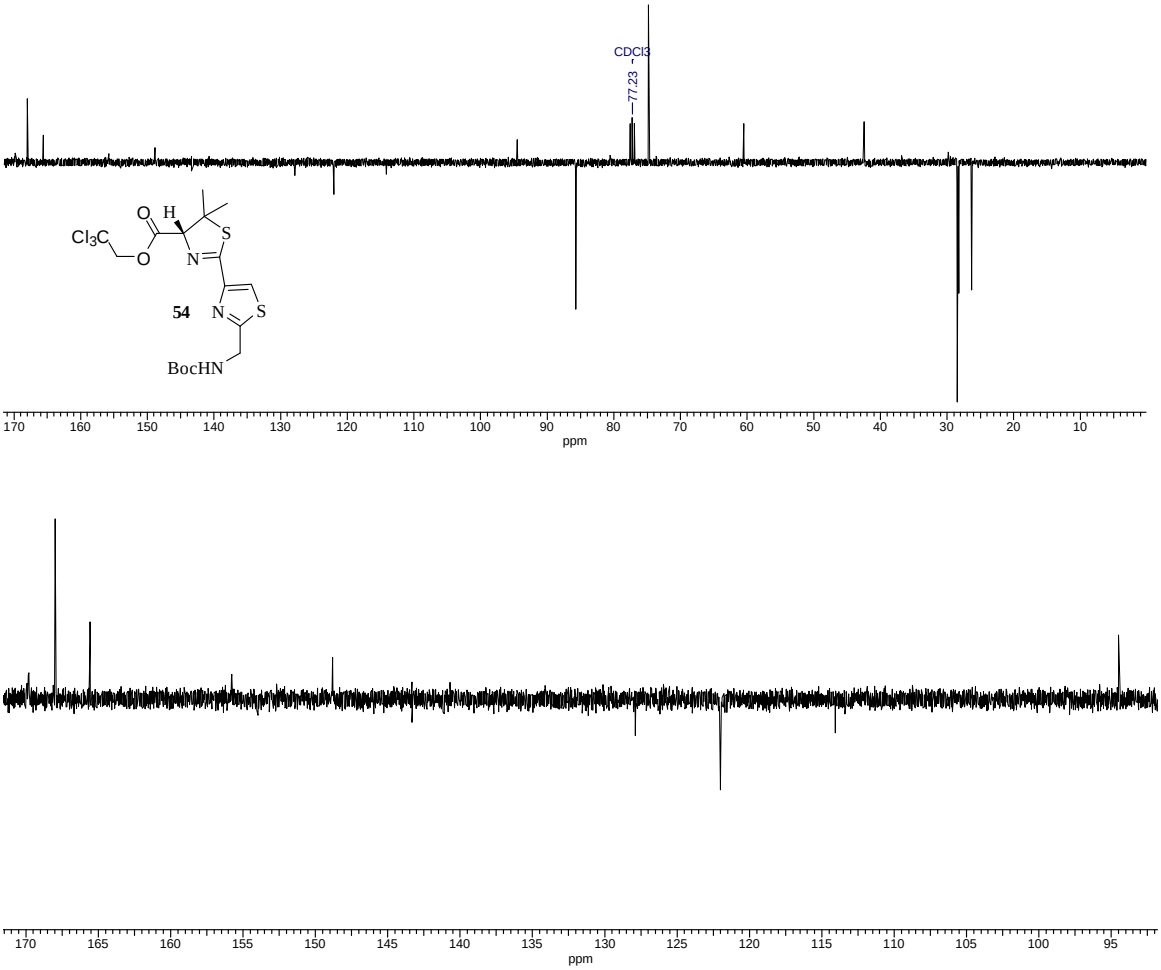
^{13}C NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (52)



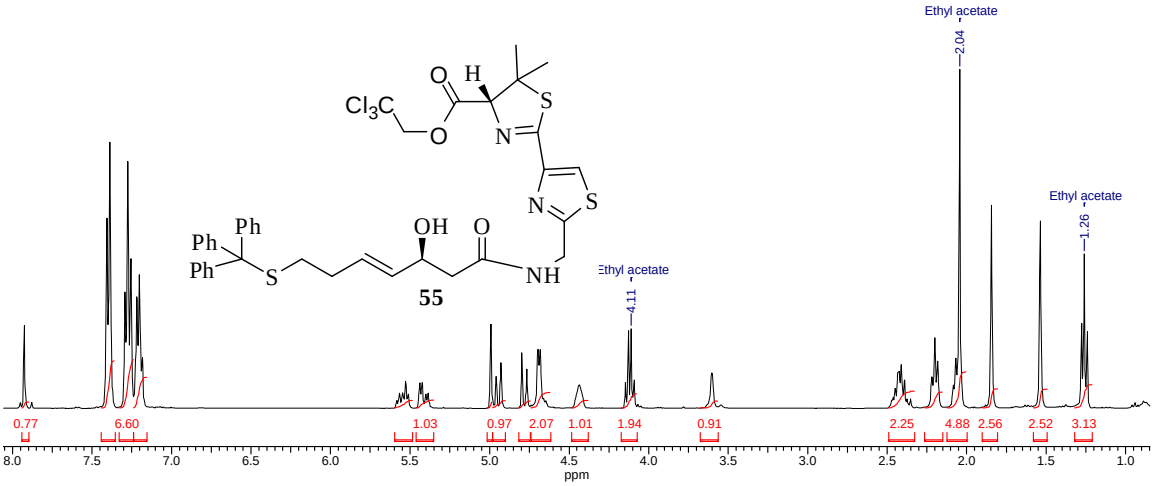
^{1}H NMR of (*R*)-2,2,2-trichloroethyl 2-(2-((*tert*-butoxycarbonylamino)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (54)

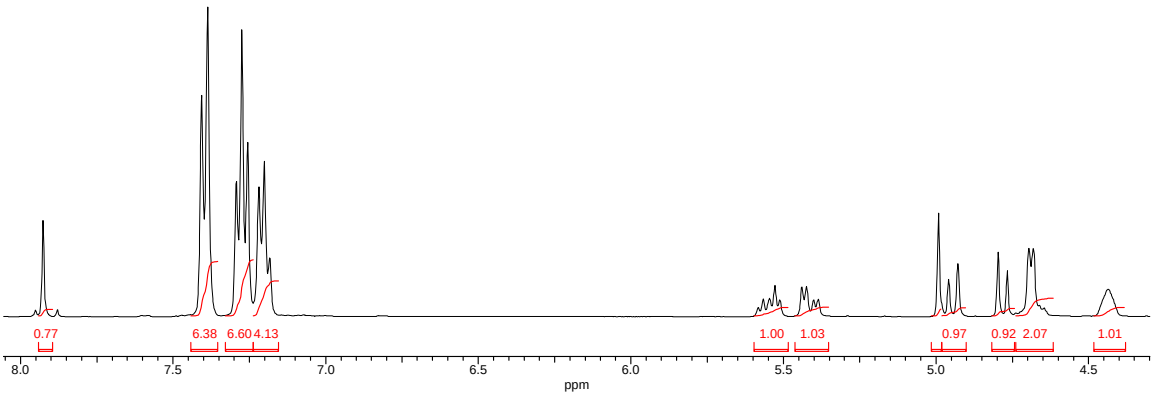


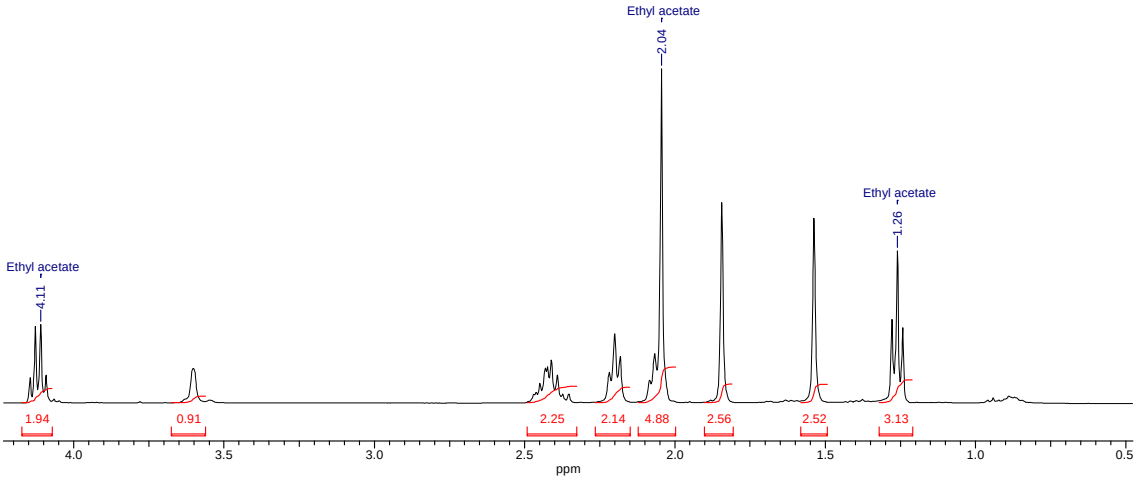
^{13}C NMR of (*R*)-2,2,2-trichloroethyl 2-(2-((*tert*-butoxycarbonylamino)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (54)



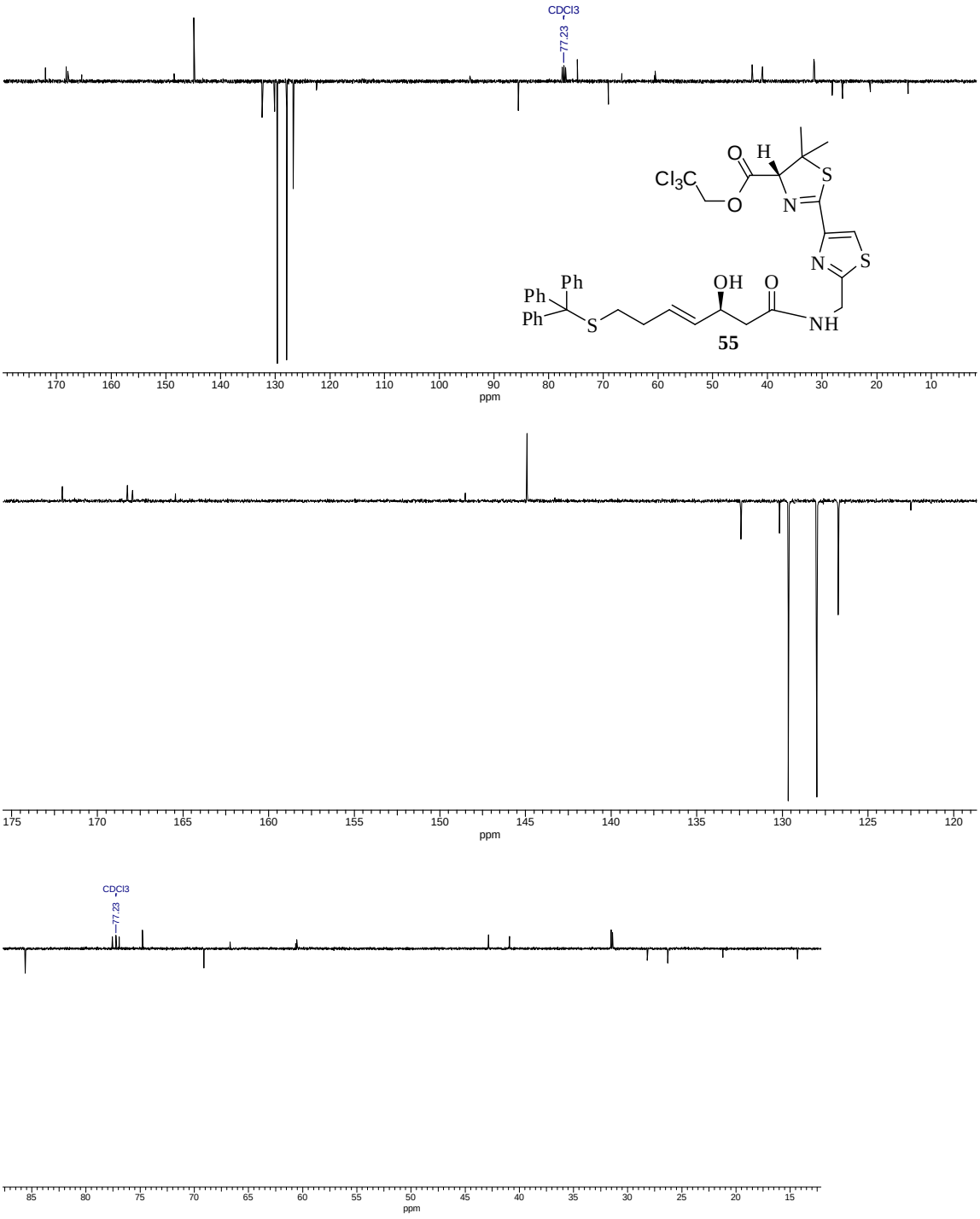
^{1}H NMR of (*R*)-2,2,2-trichloroethyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (55)



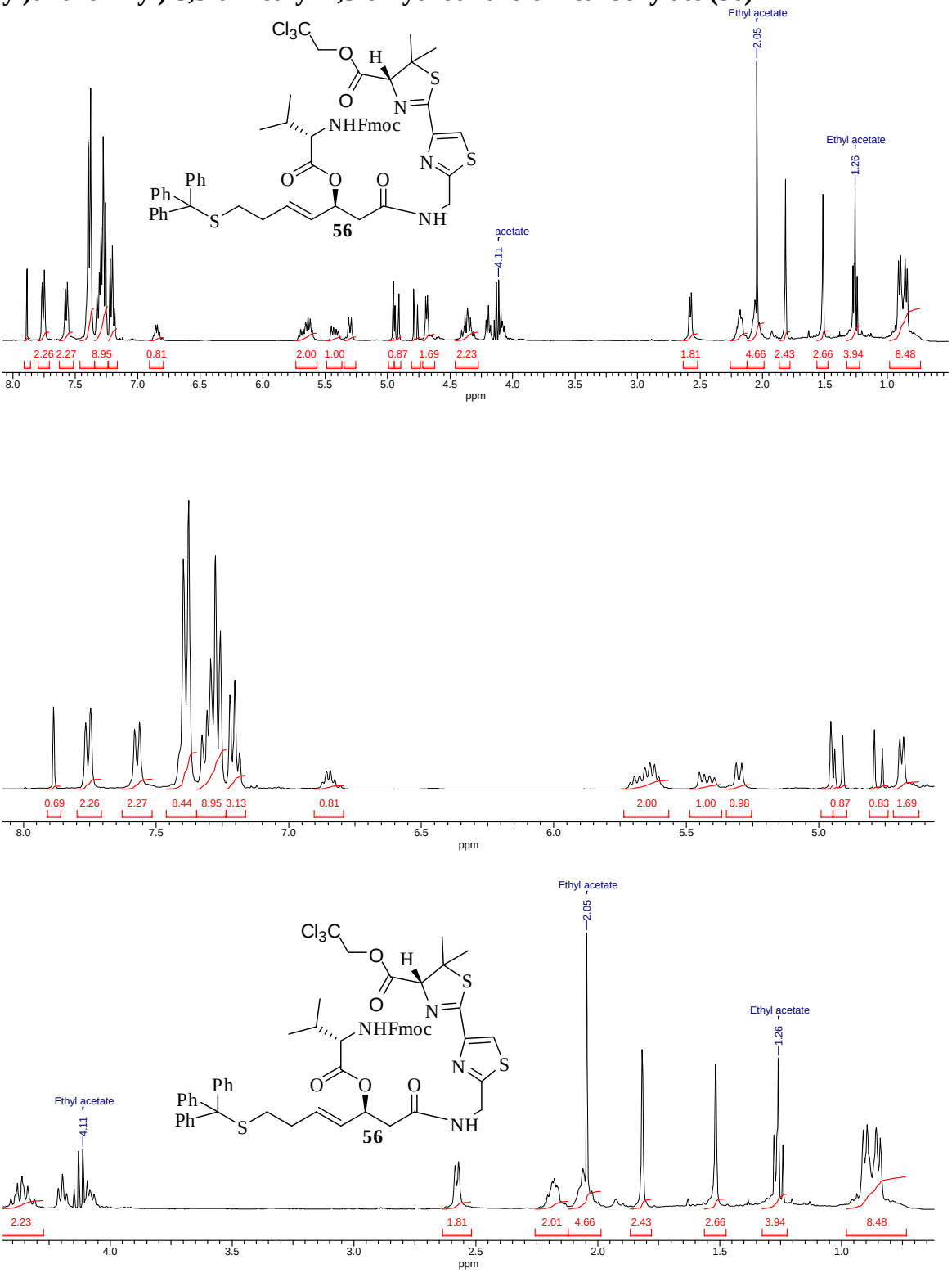




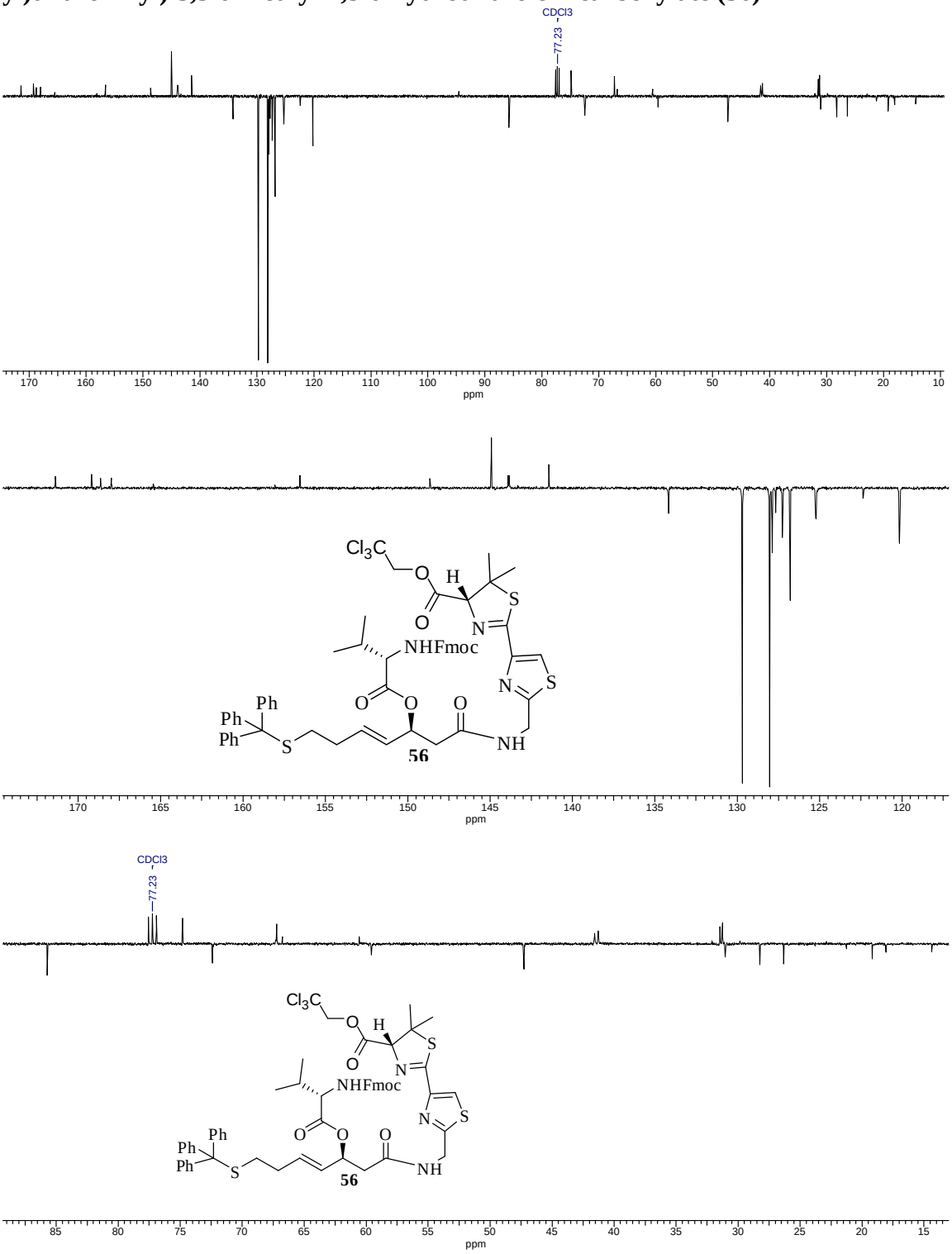
^{13}C NMR of (*R*)-2,2,2-trichloroethyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (55)



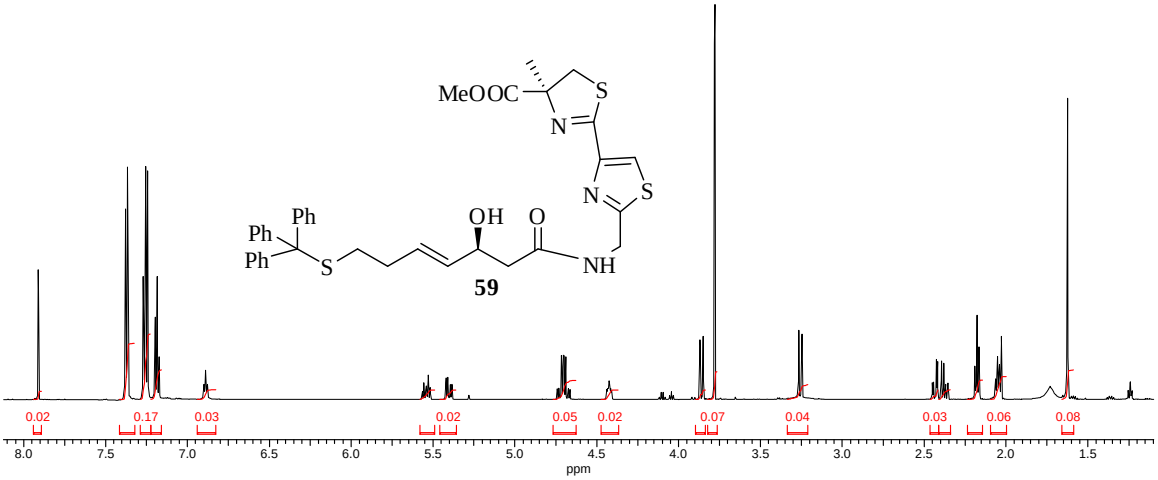
^{1}H NMR of (*R*)-2,2,2-trichloroethyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (56)

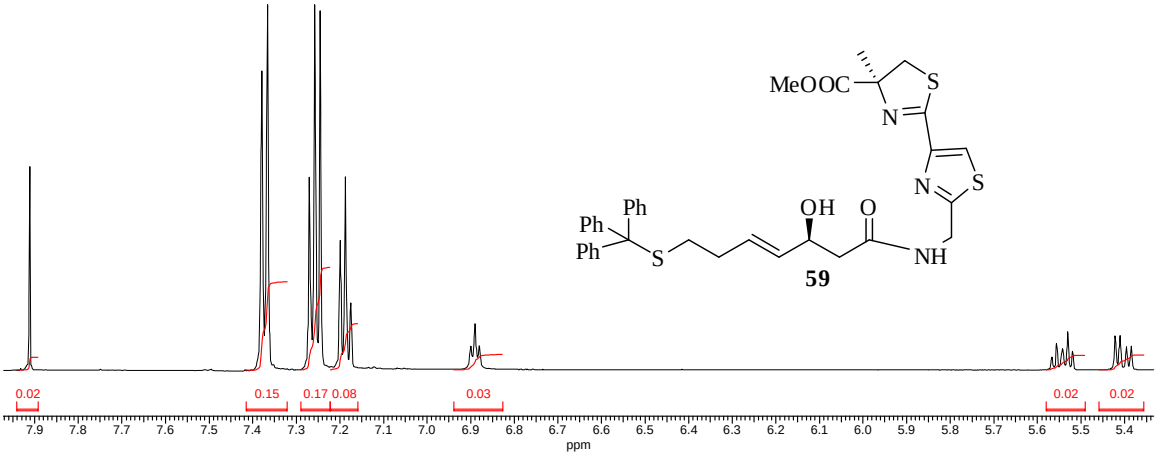


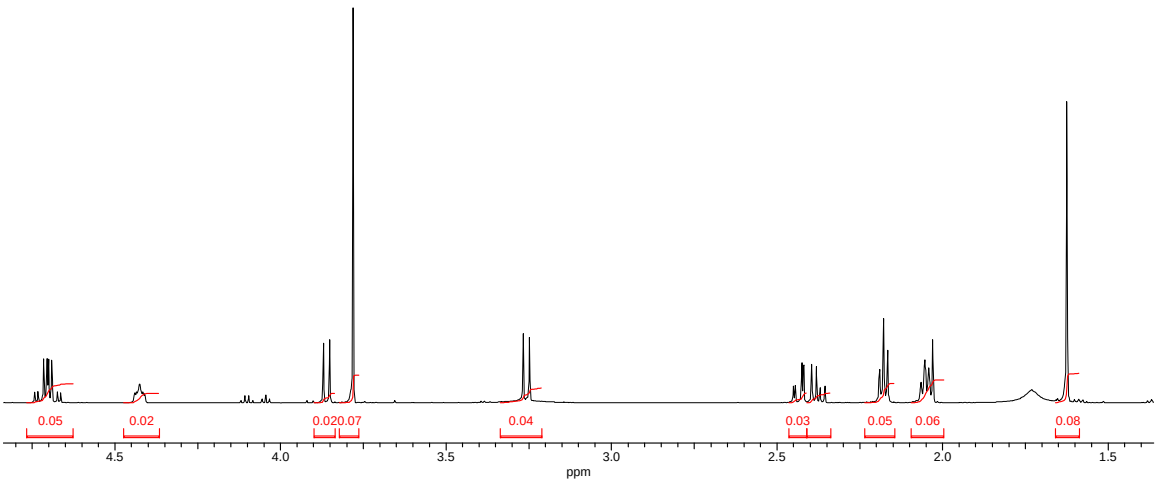
^{13}C NMR of (*R*)-2,2,2-trichloroethyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-5,5-dimethyl-4,5-dihydrothiazole-4-carboxylate (56)



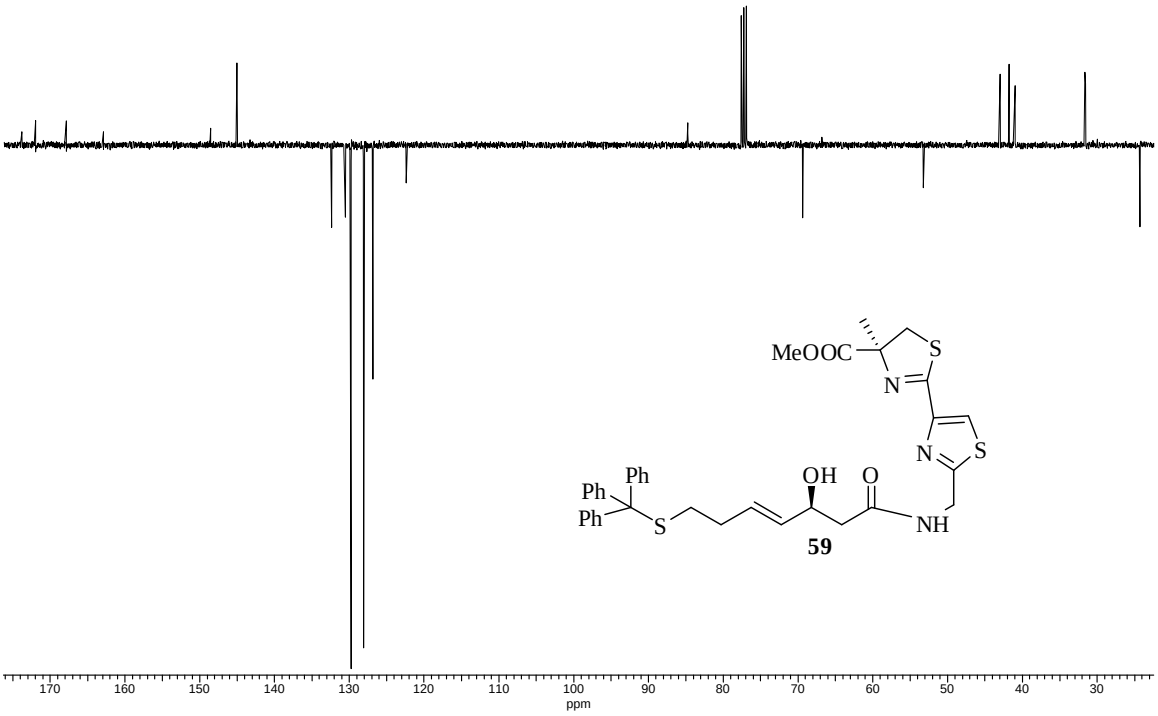
^{1}H NMR of (*S*)-methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (59)

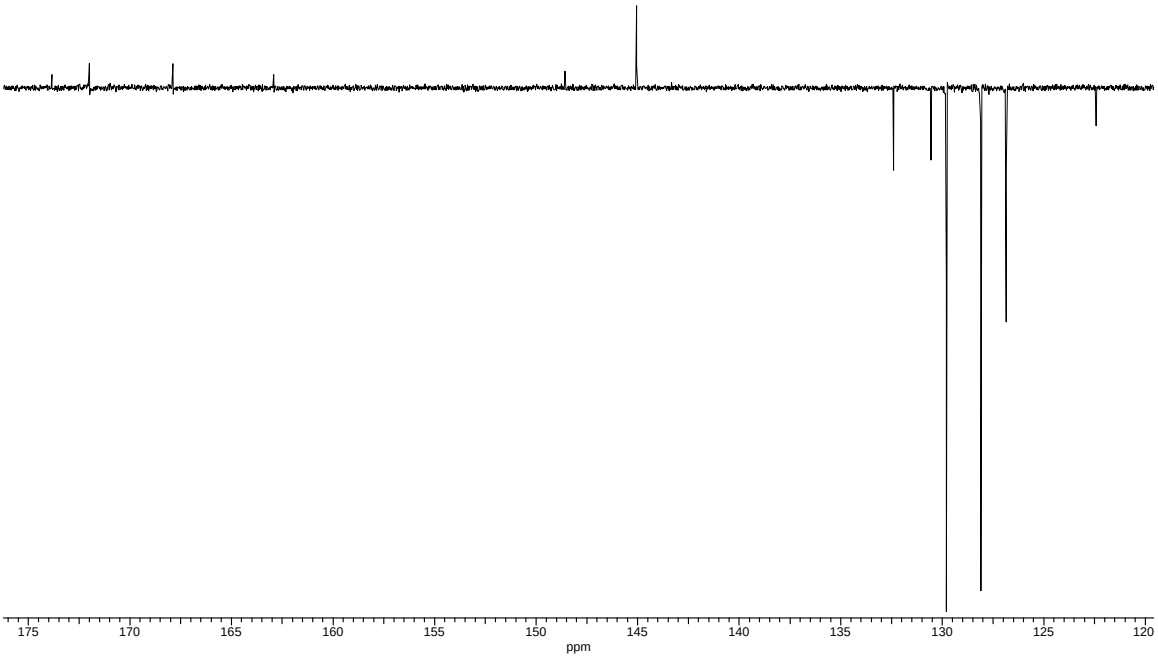


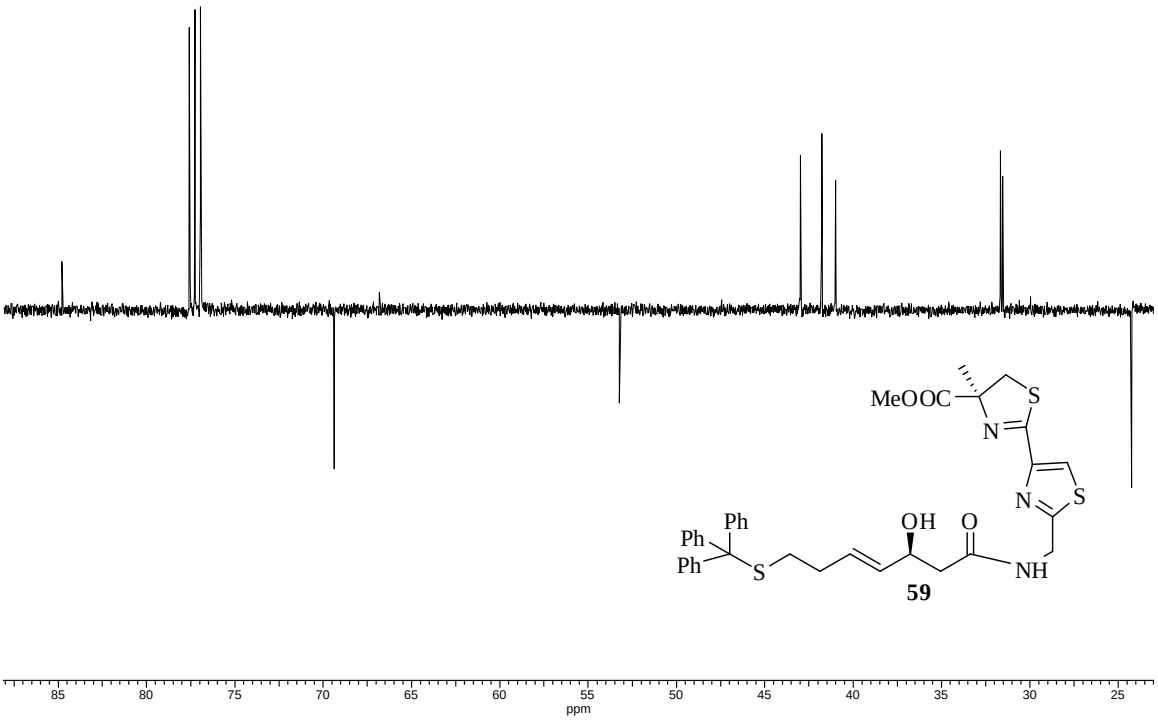




^{13}C NMR of (*S*)-methyl 2-(2-(((*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (59)

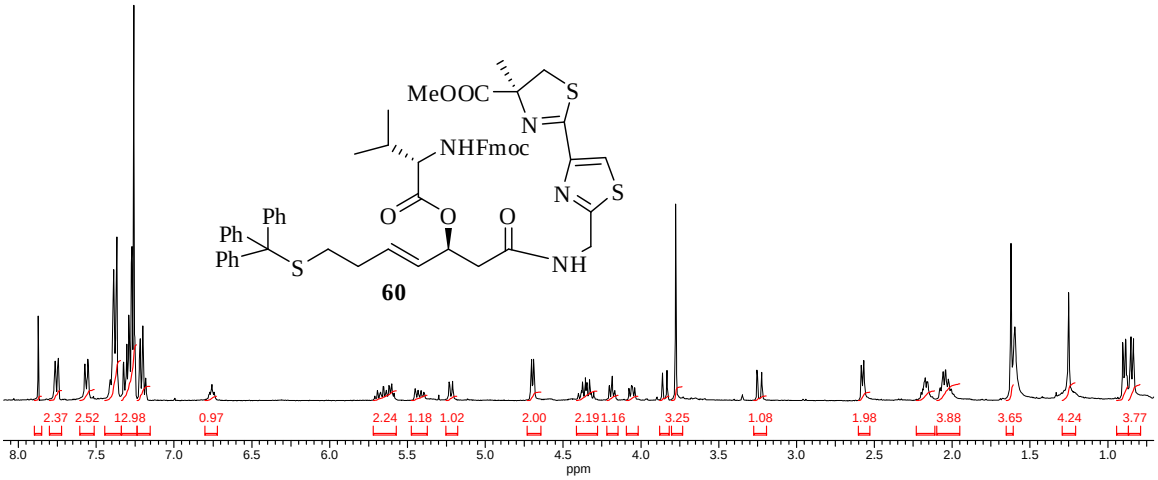


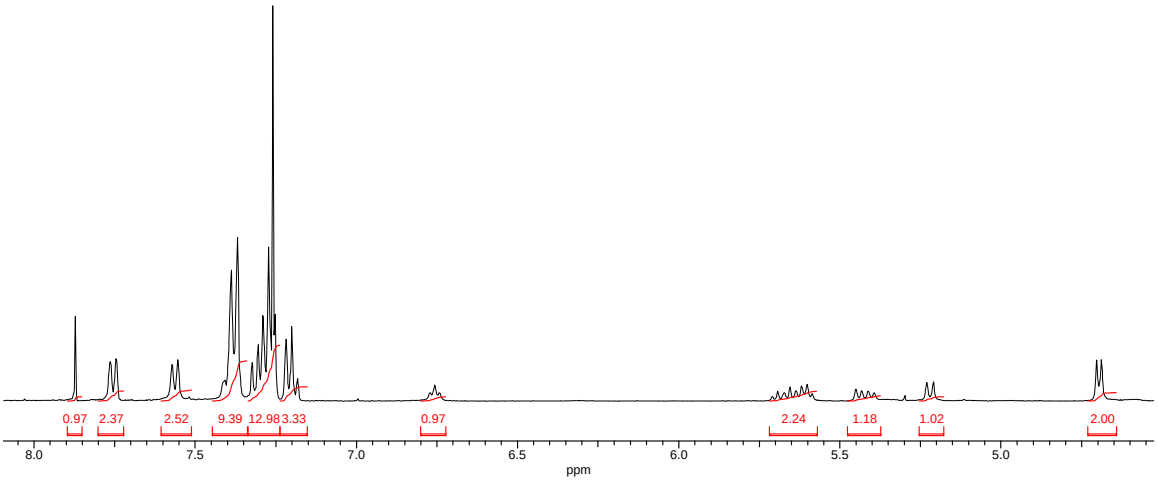


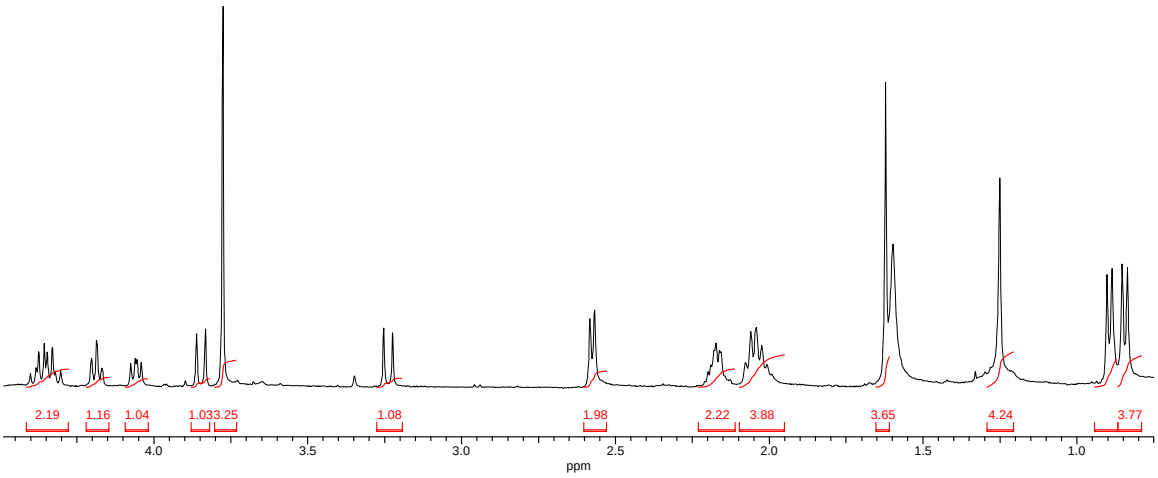

MeOOC
S
N
N
S
Ph
Ph
Ph
S
OH
O
NH
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

59

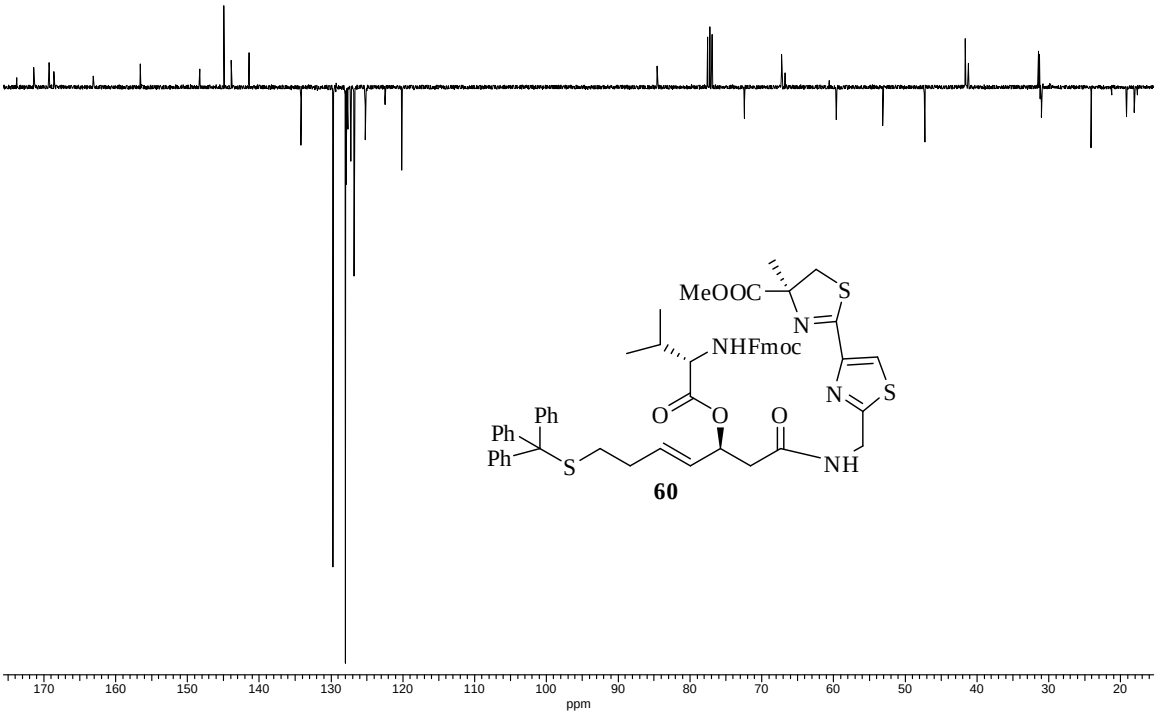
^{1}H NMR of (*S*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (60)



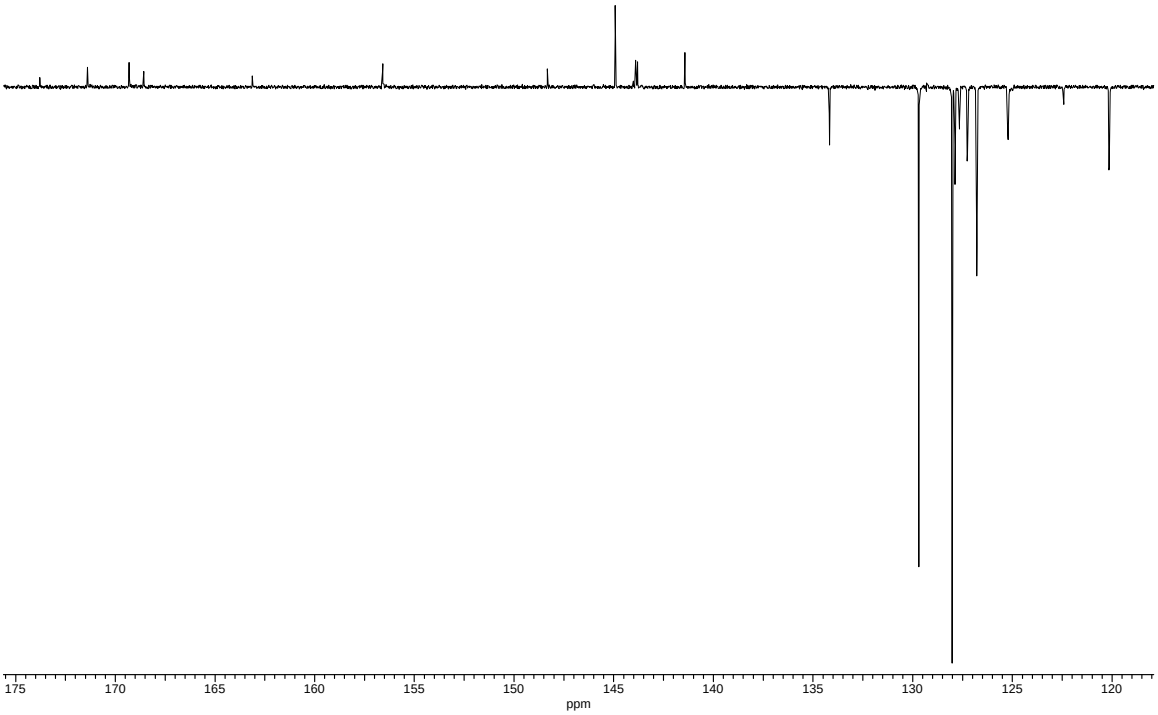


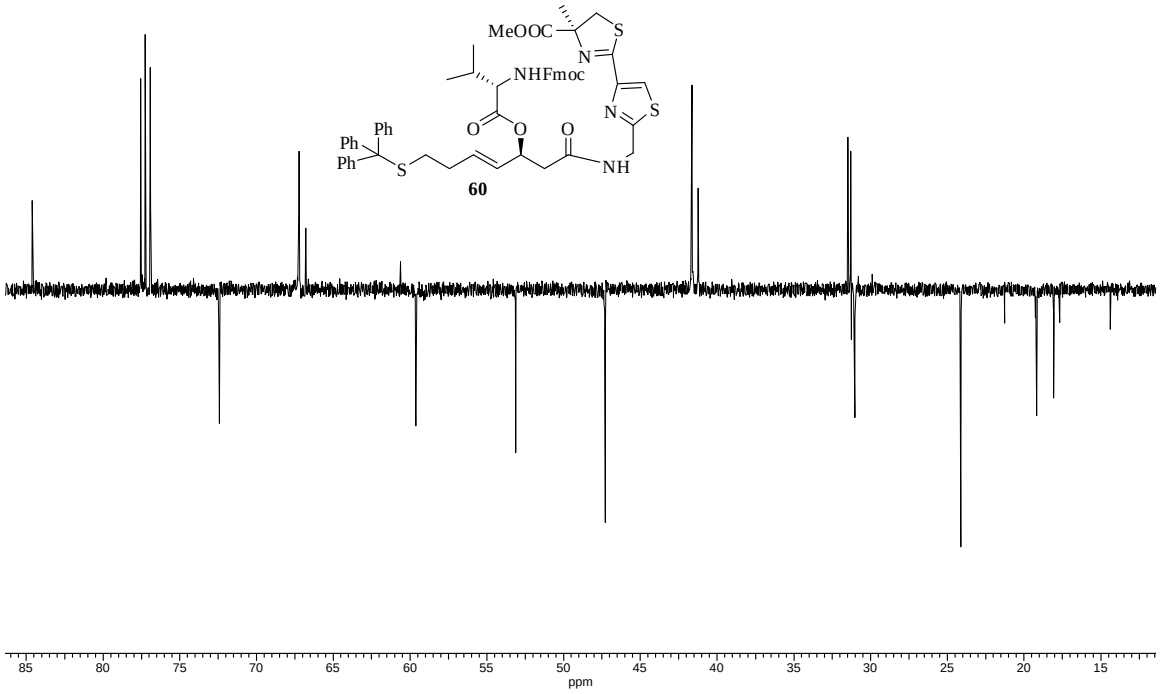


^{13}C NMR of (*S*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (60)

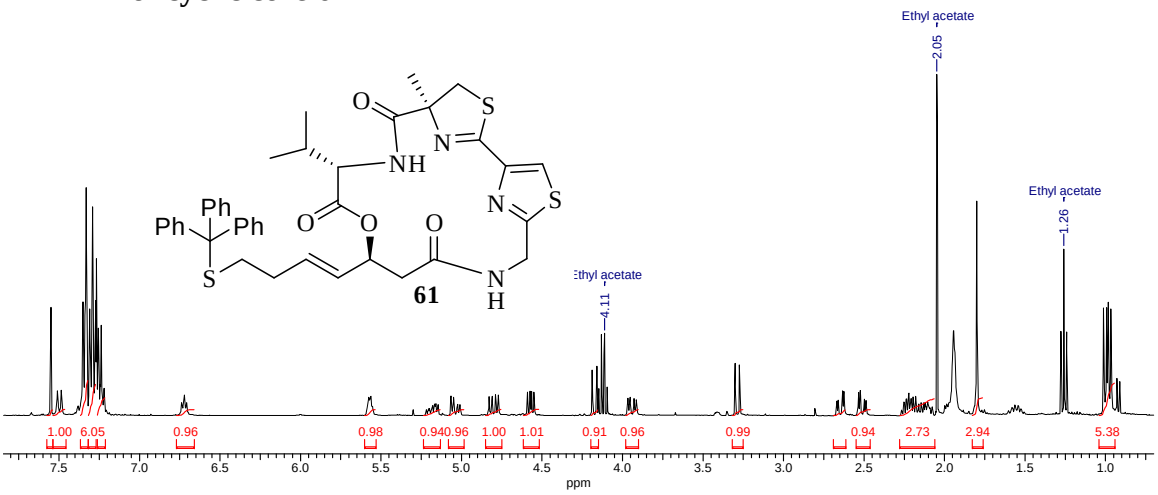


60

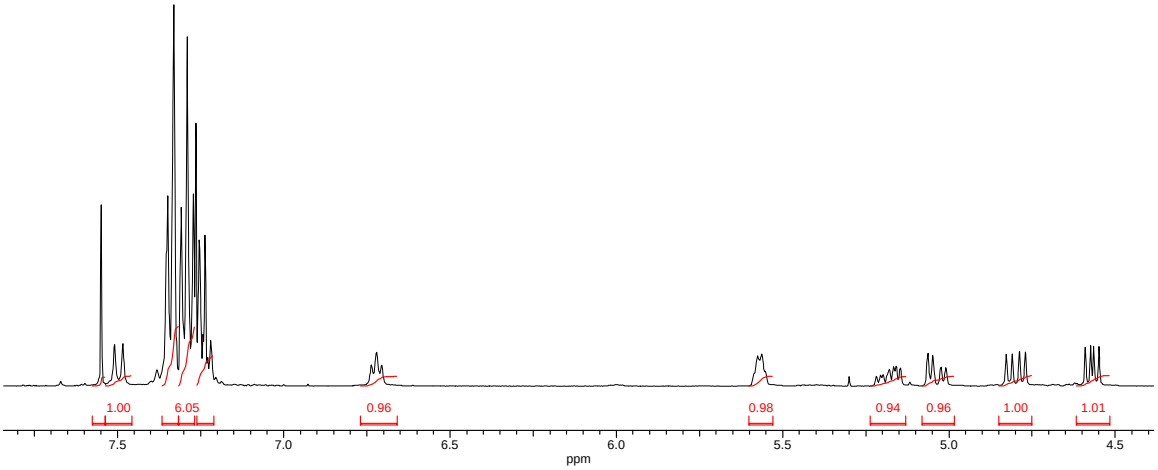


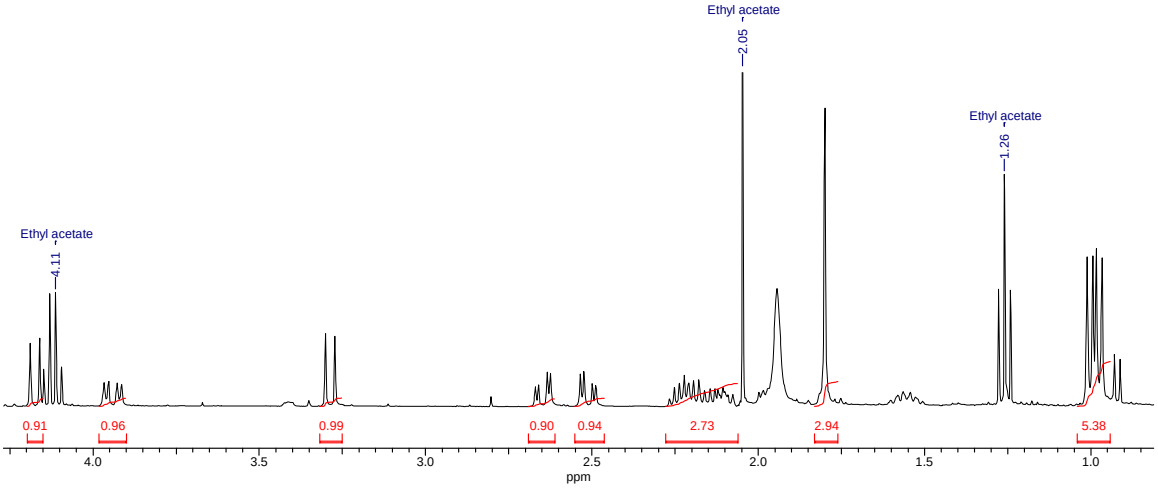

MeOOC
S
N
NHFmoc
N
S
O
O
O
Ph
Ph
S
NH
60
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
ppm

^{1}H NMR of cyclic core 61

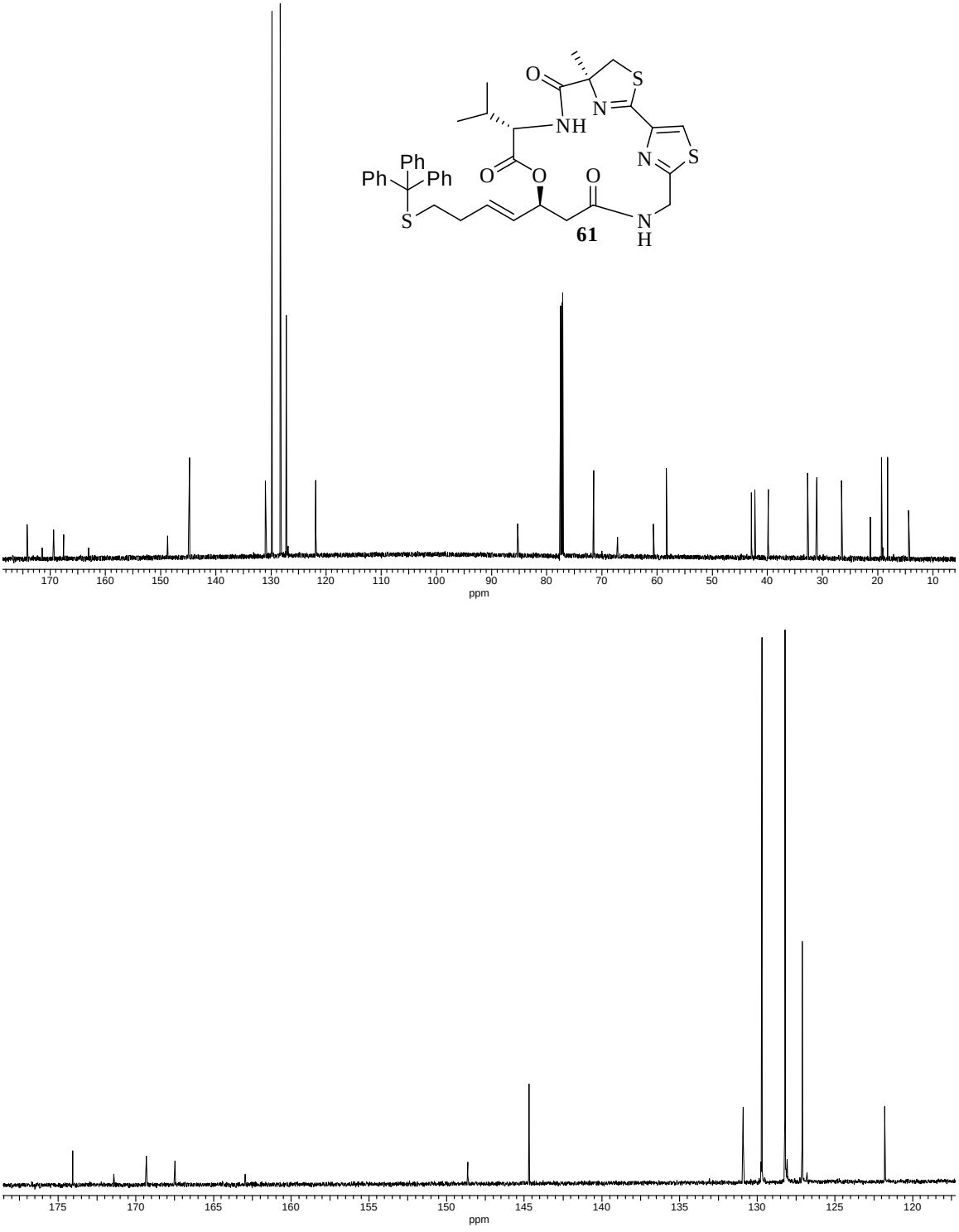


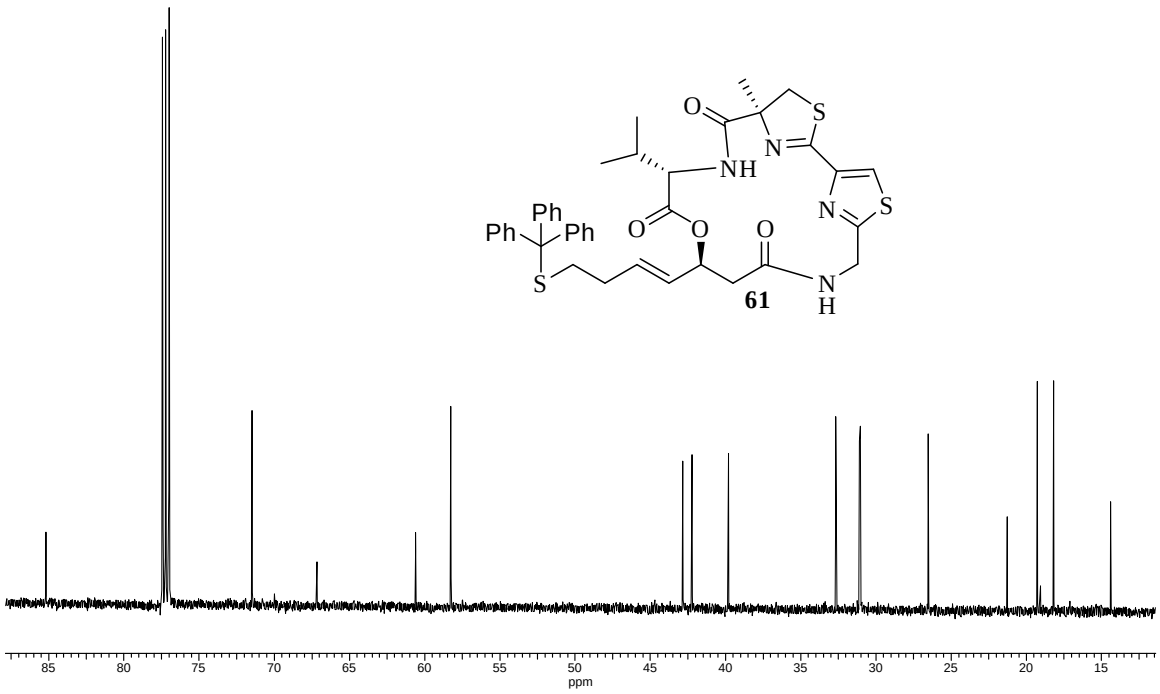
61



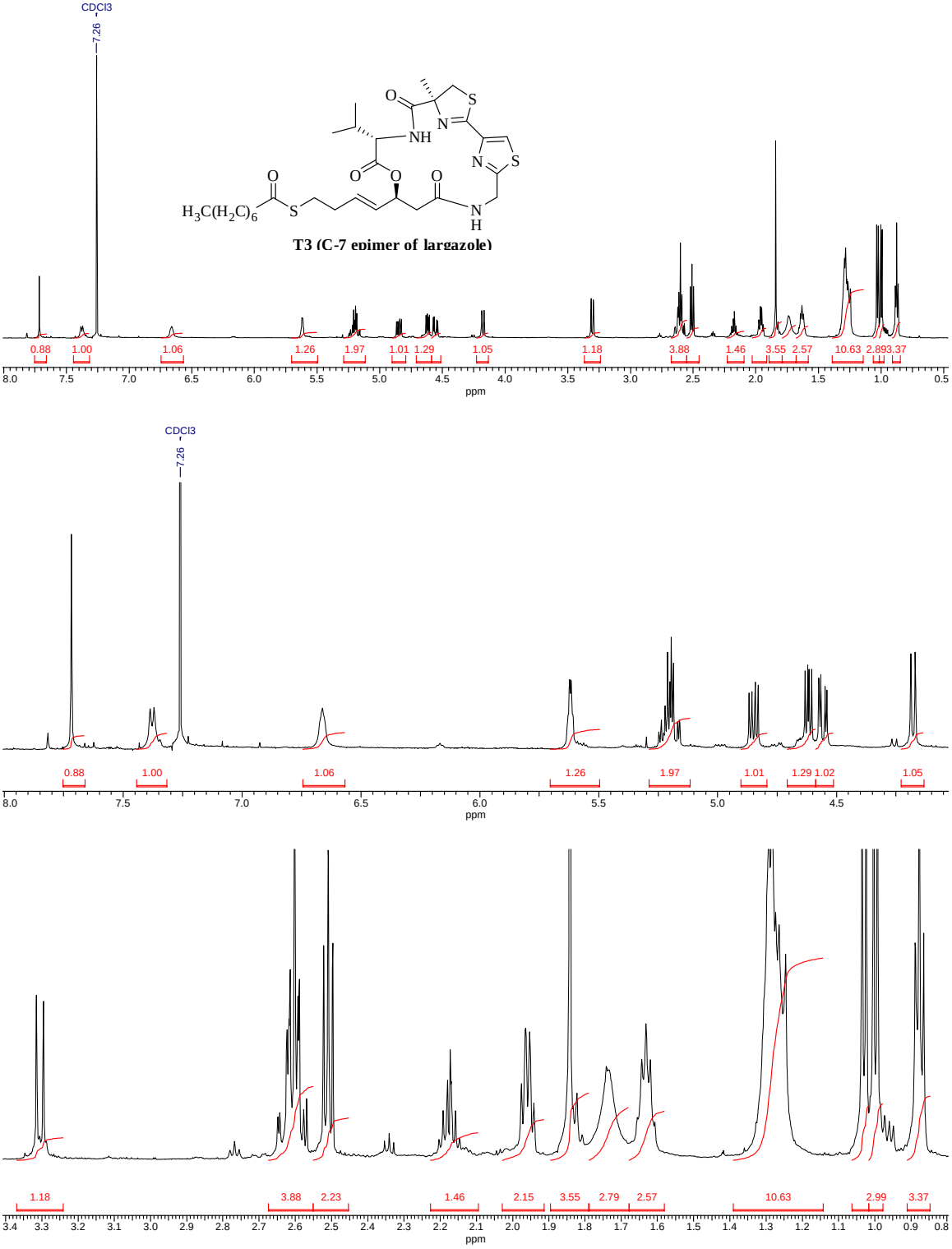


^{1}H NMR of cyclic core 61

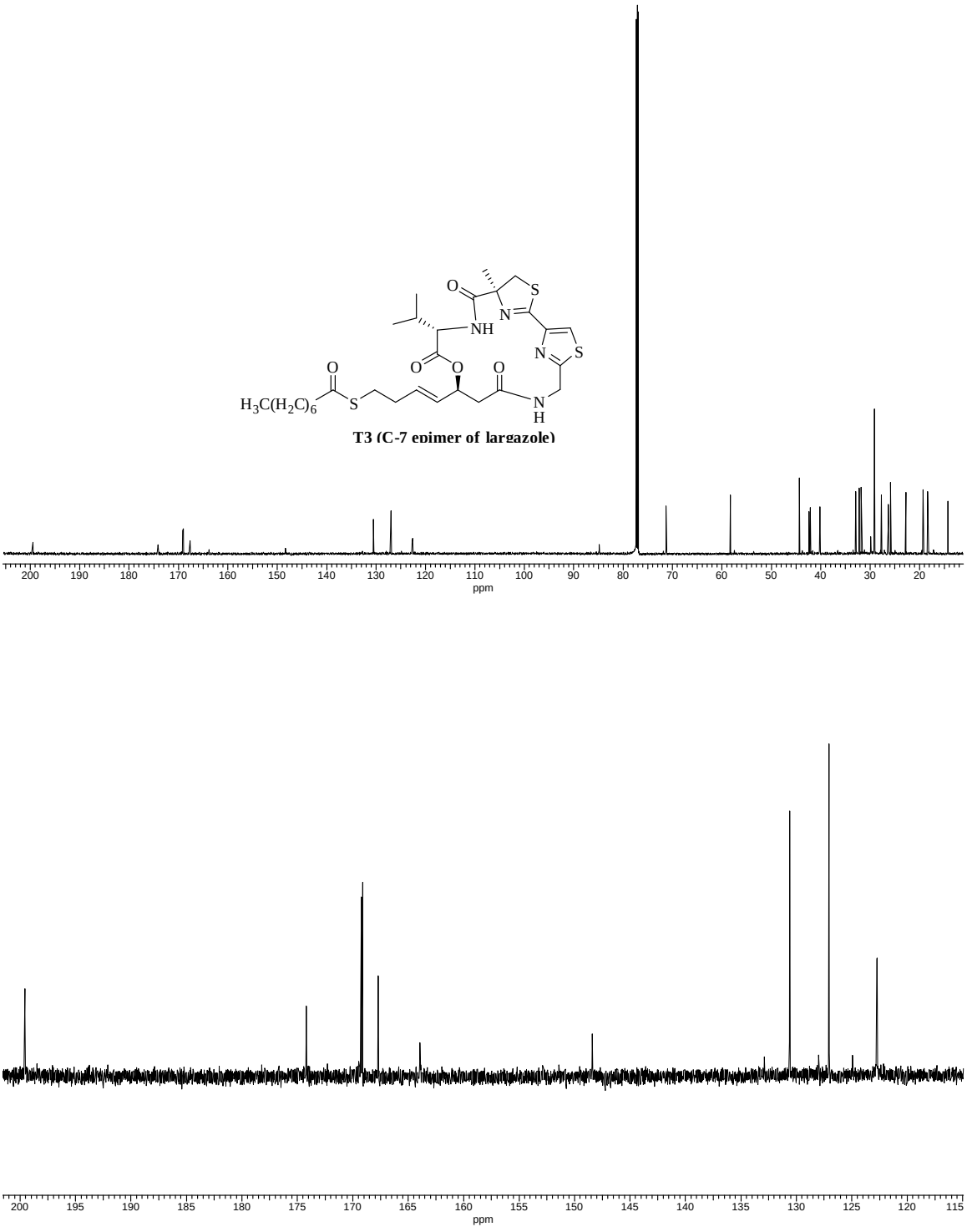



O
S
N
NH
N
S
Ph
Ph
Ph
O
O
O
S
N
H
61
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
ppm

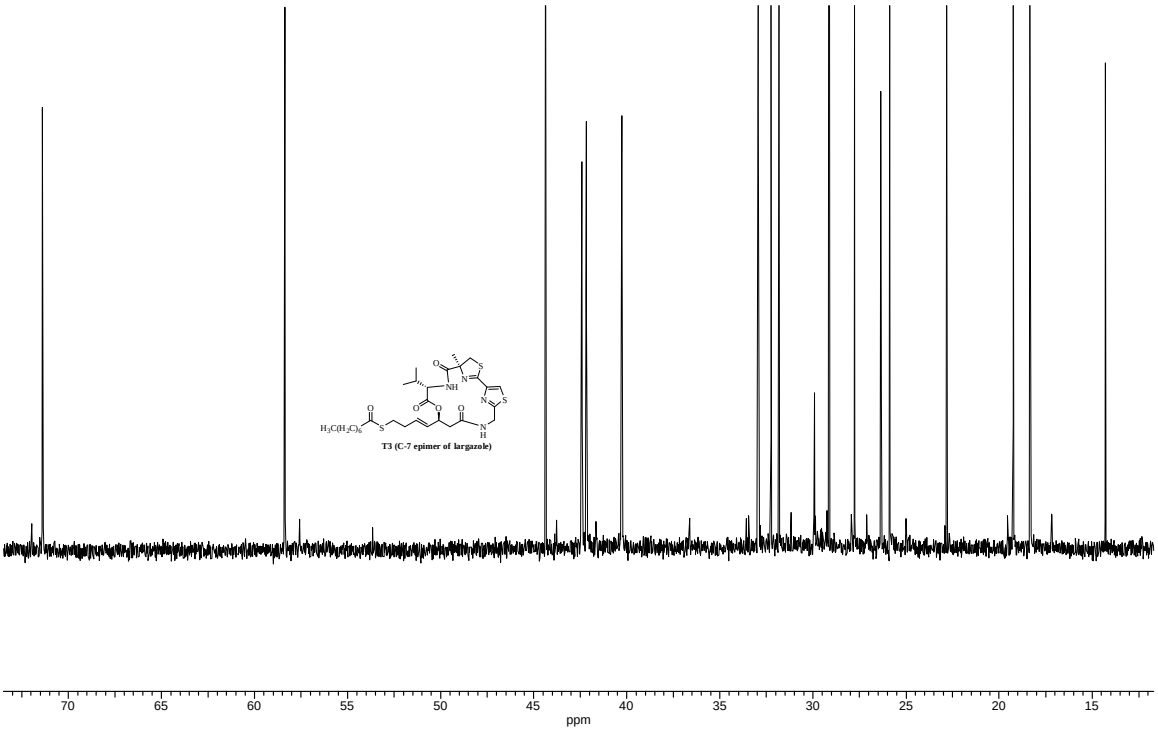
^{1}H NMR of analog T3 (C-7 largazole epimer)



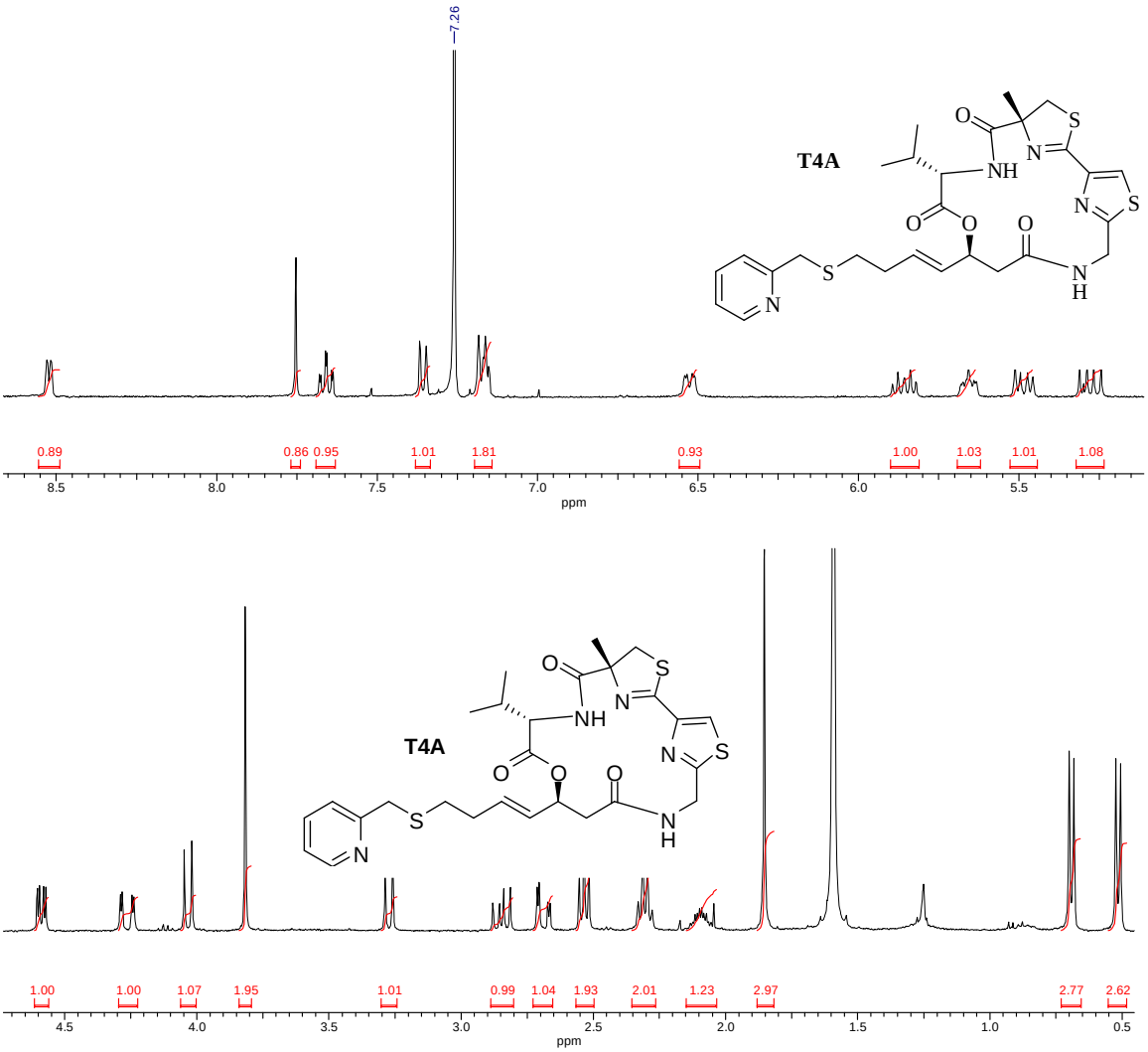
^{13}C NMR of analog T3 (C-7 largazole epimer)



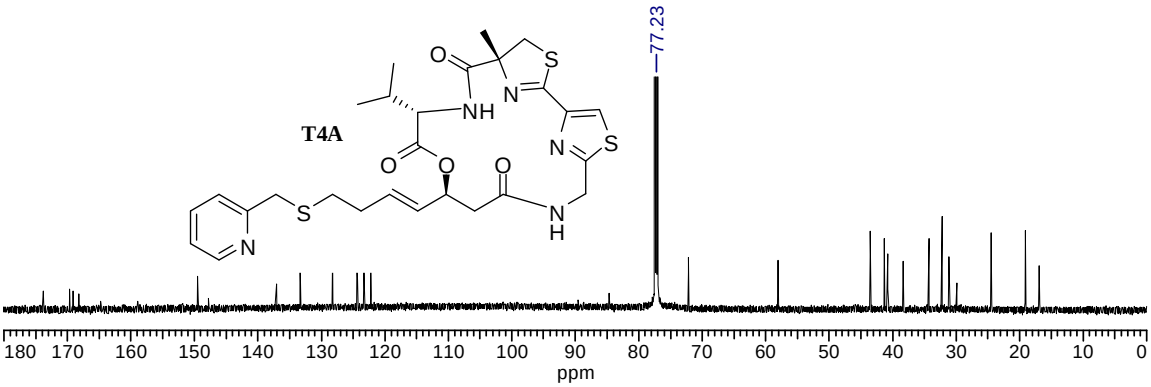
T3 (C-7 epimer of largazole)

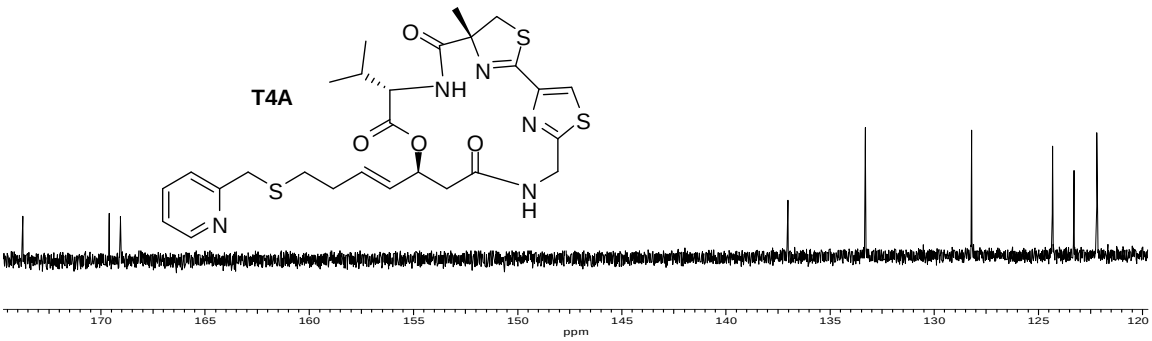

T3 (C-7 epimer of largazole)
70
65
60
55
50
45
40
35
30
25
20
15
ppm

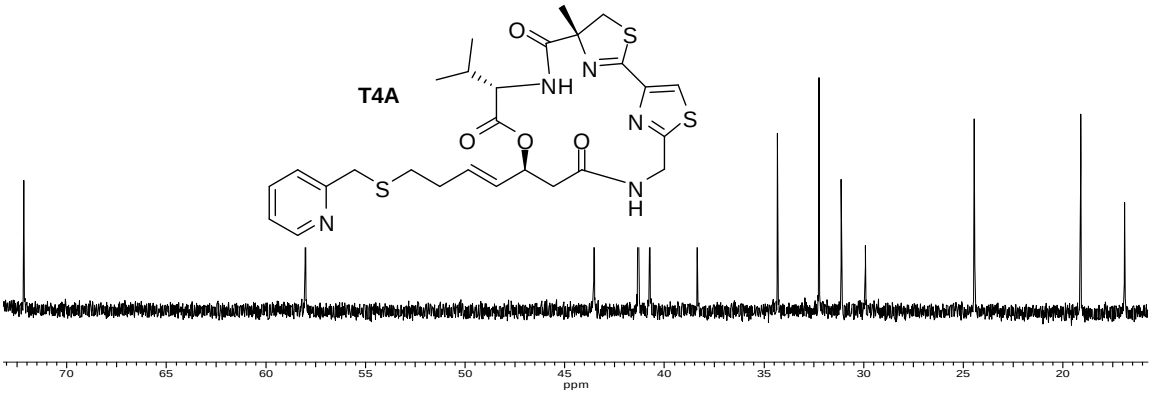
^{1}H NMR of analog T4A



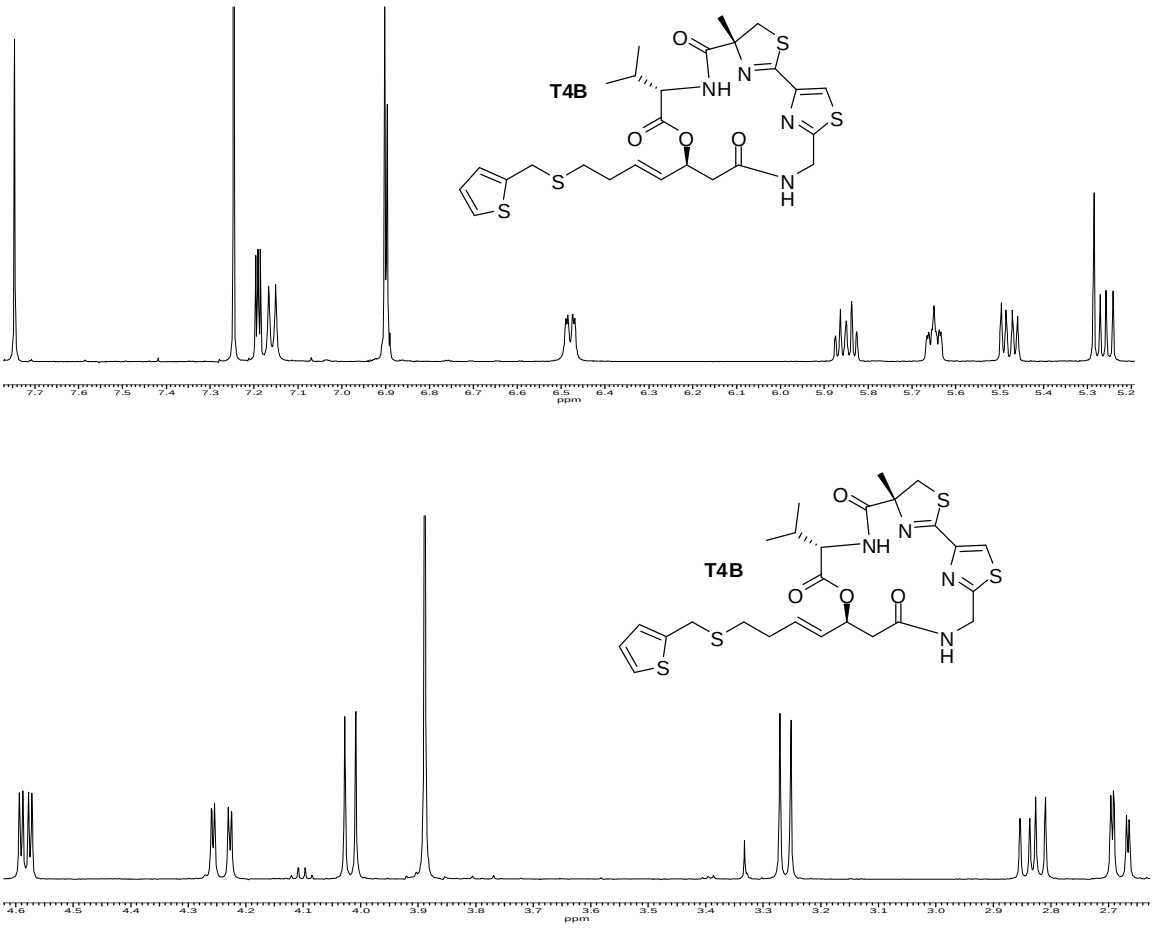
^{13}C NMR of analog T4A



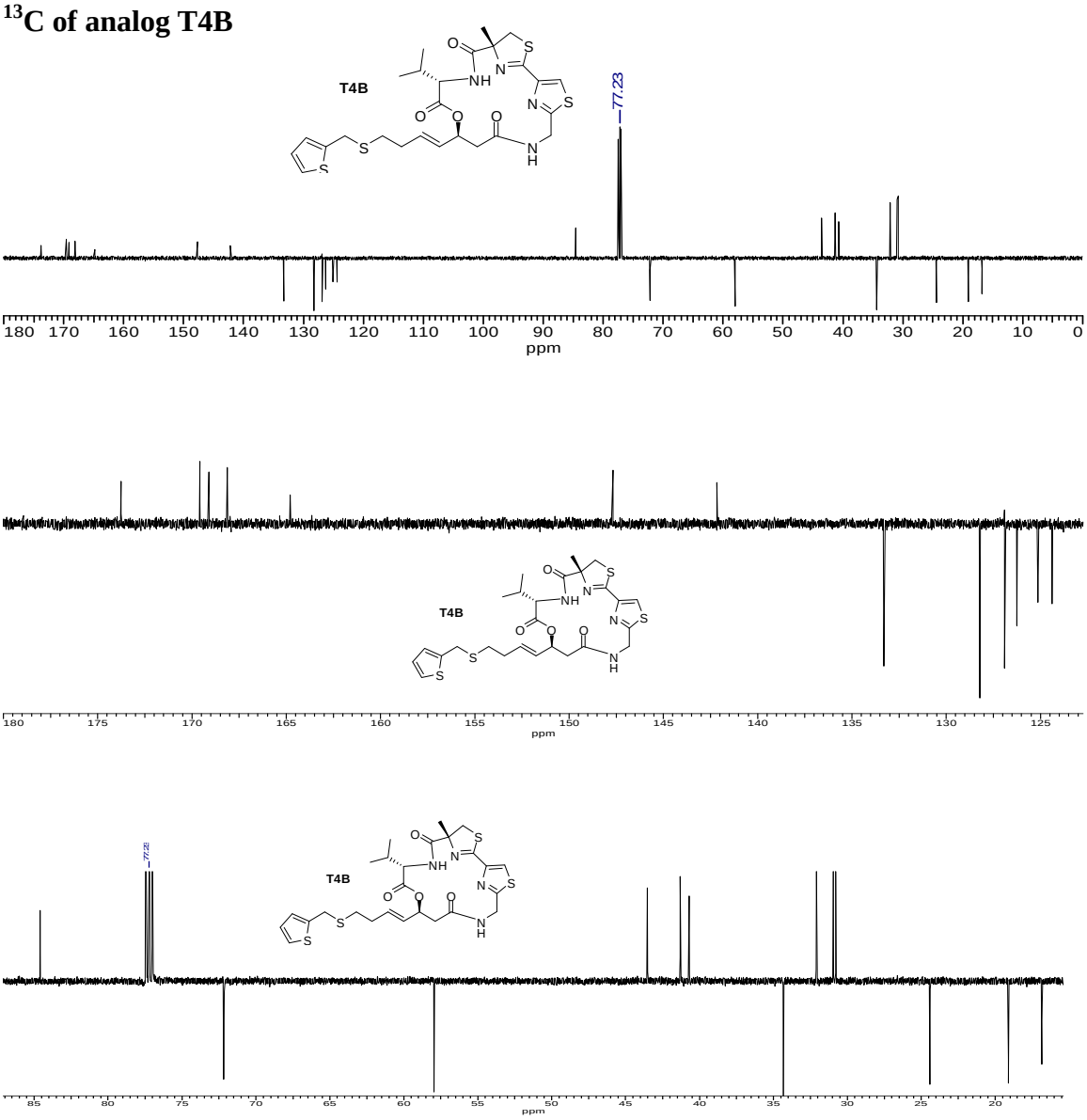




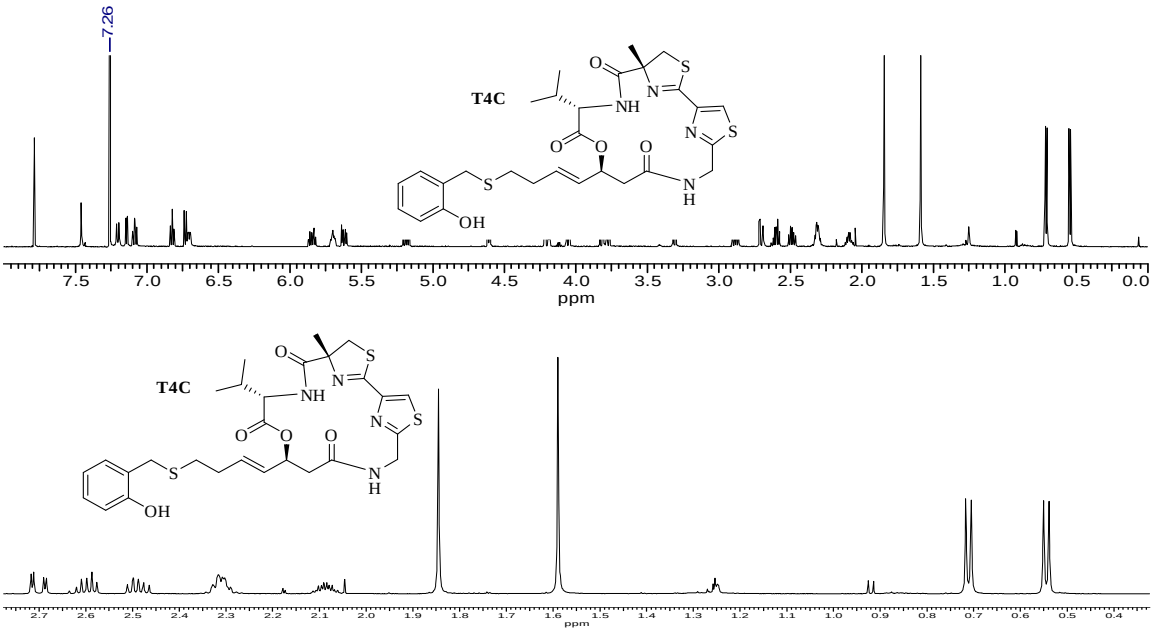
^{1}H NMR of analog T4B



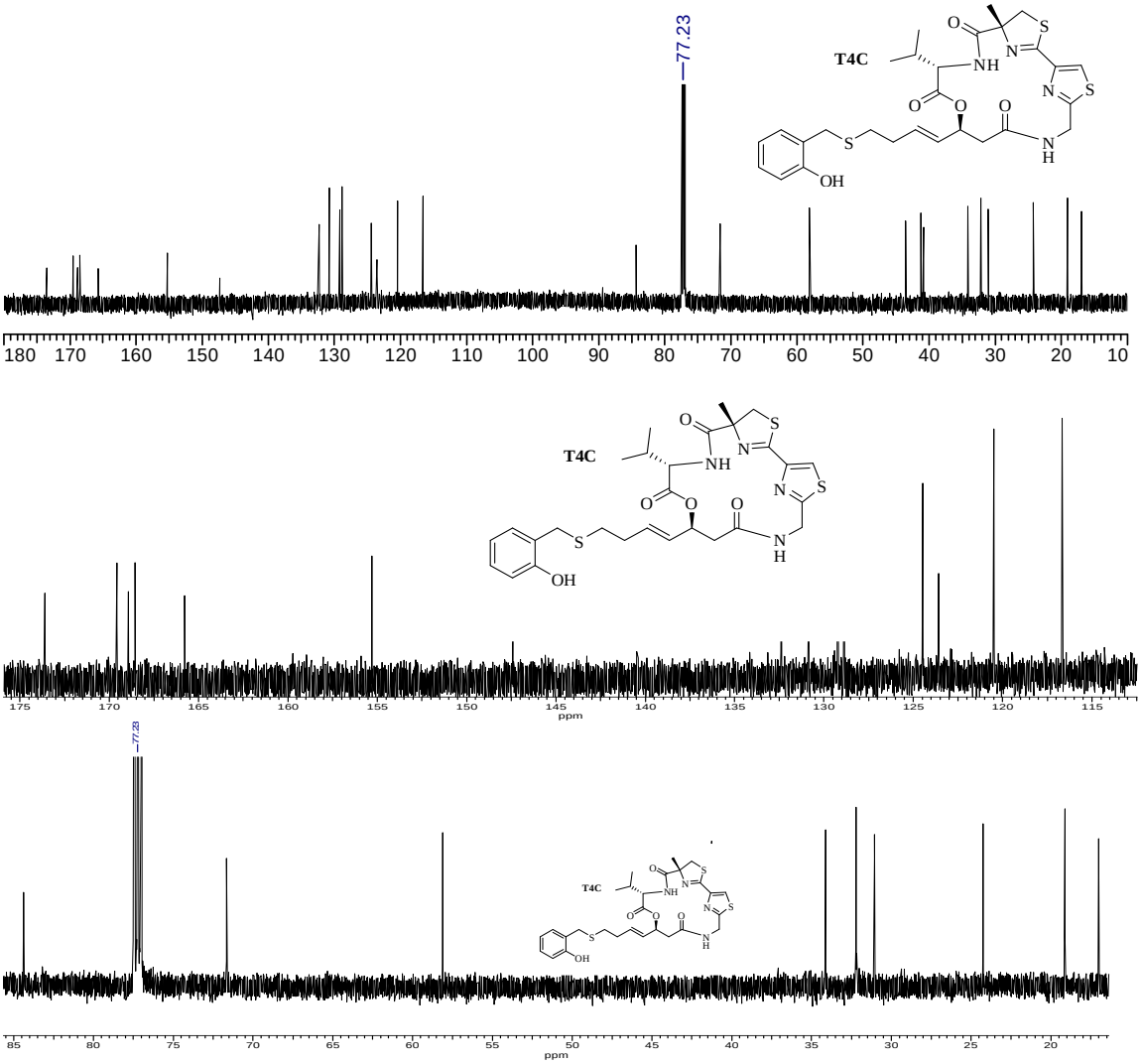
^{13}C of analog T4B



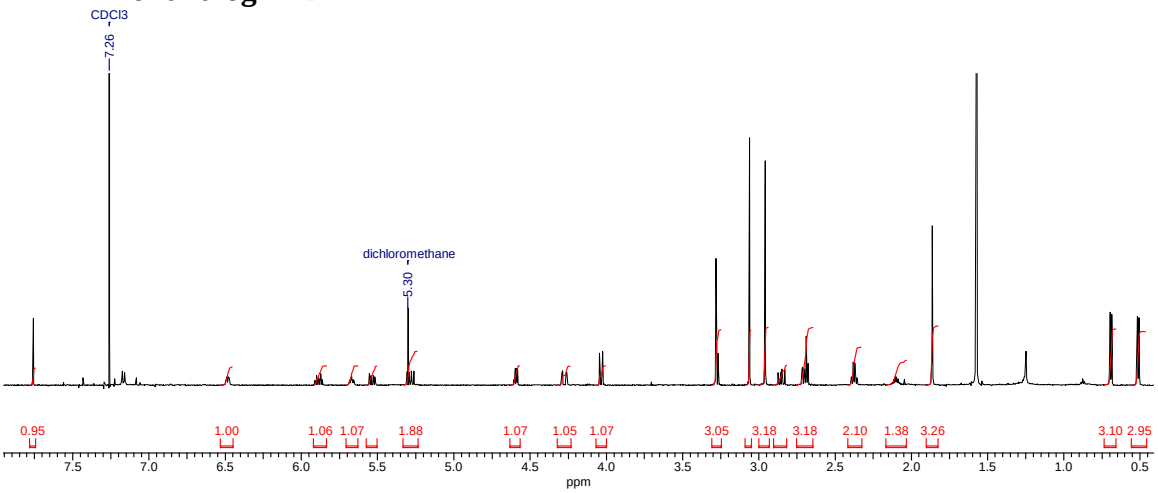
^{1}H NMR of analog T4C

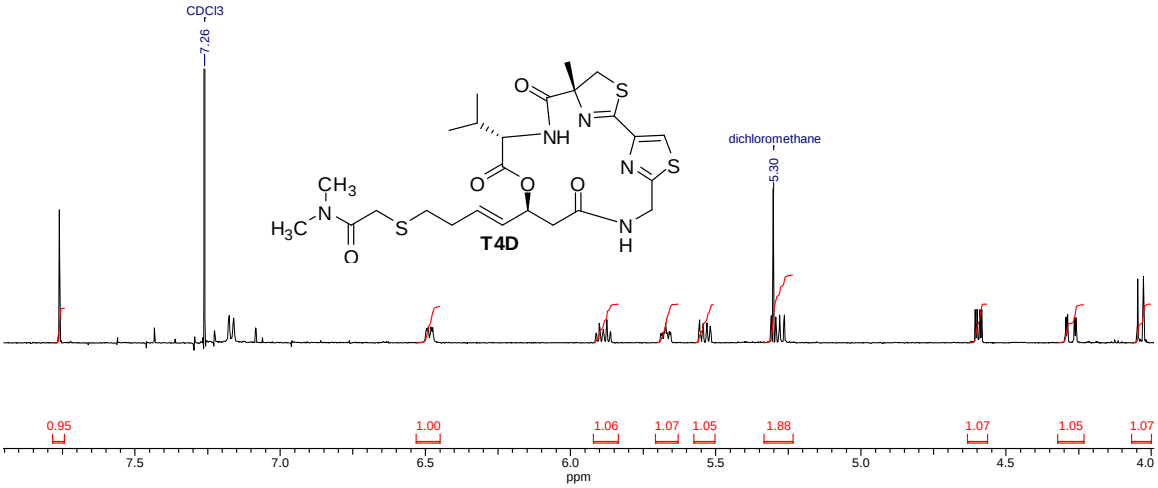


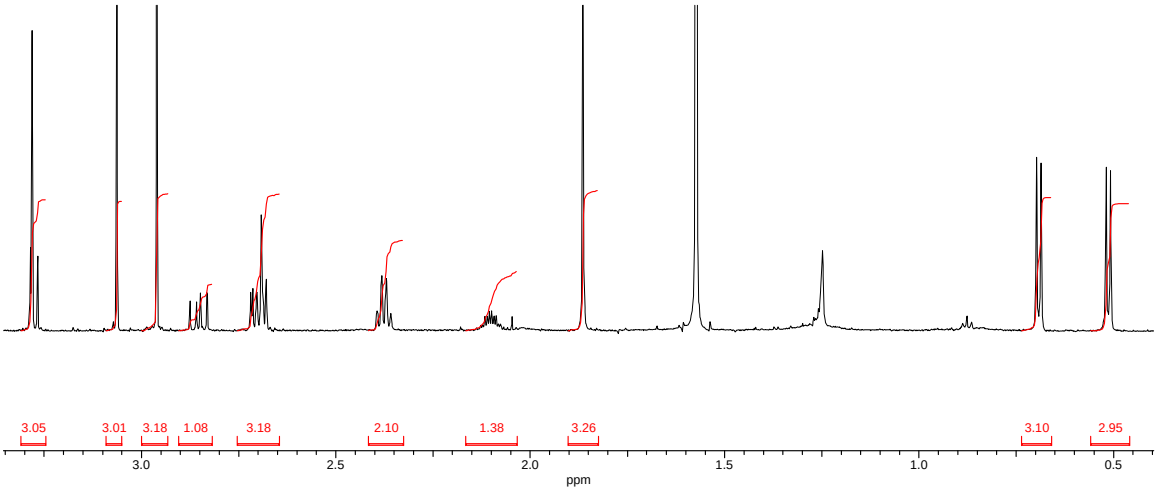
^{13}C NMR of analog T4C



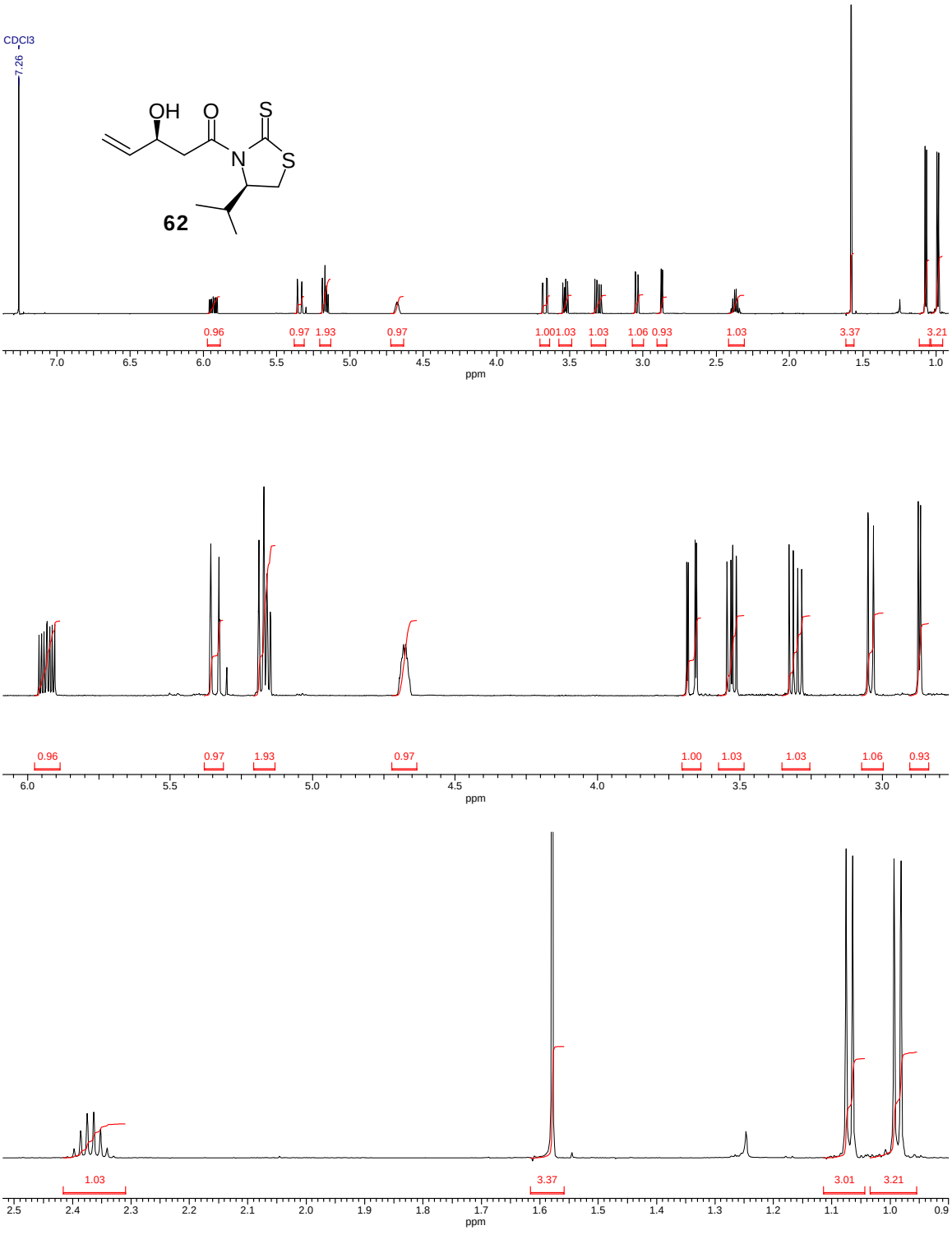
^{1}H NMR of analog T4D



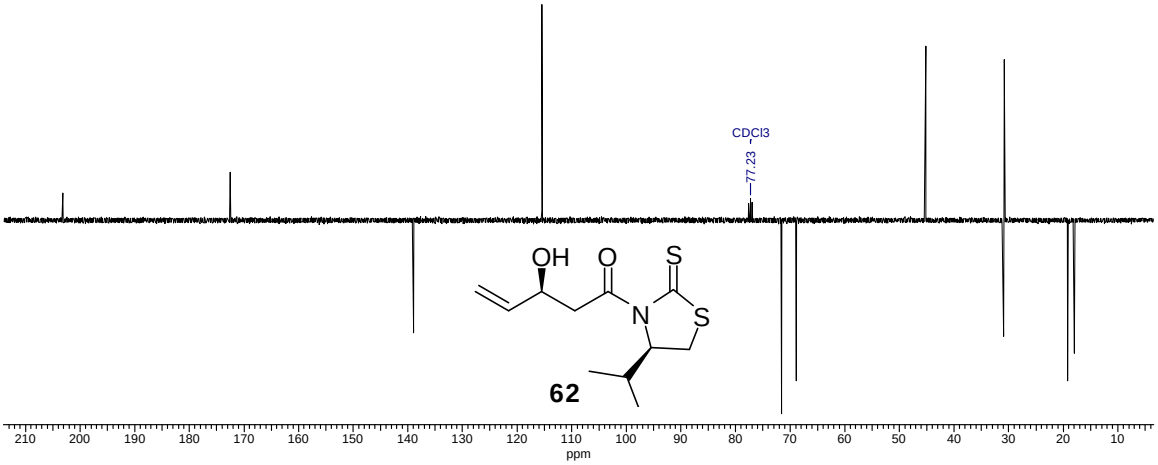




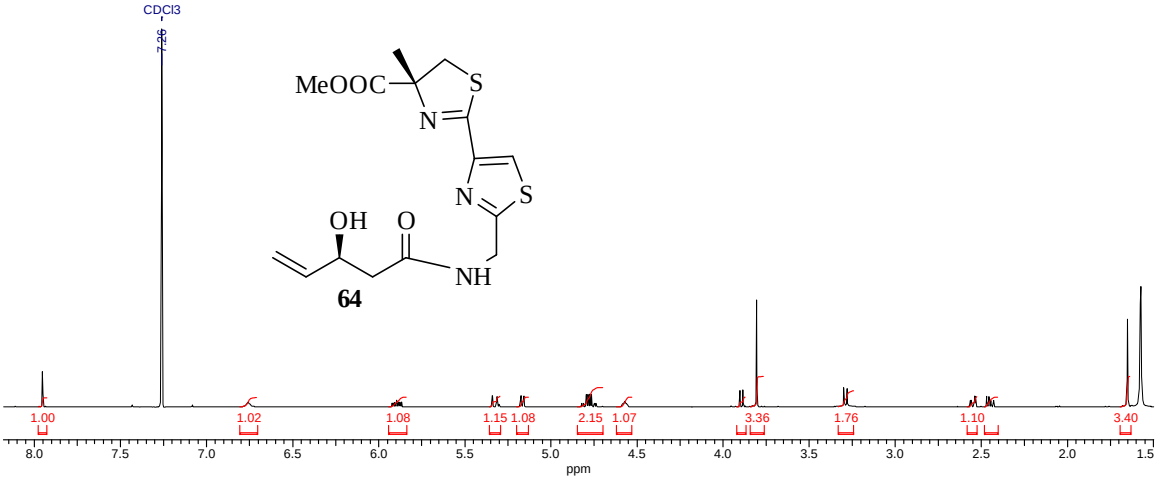
^{1}H NMR of (*S*)-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)pent-4-en-1-one (62)

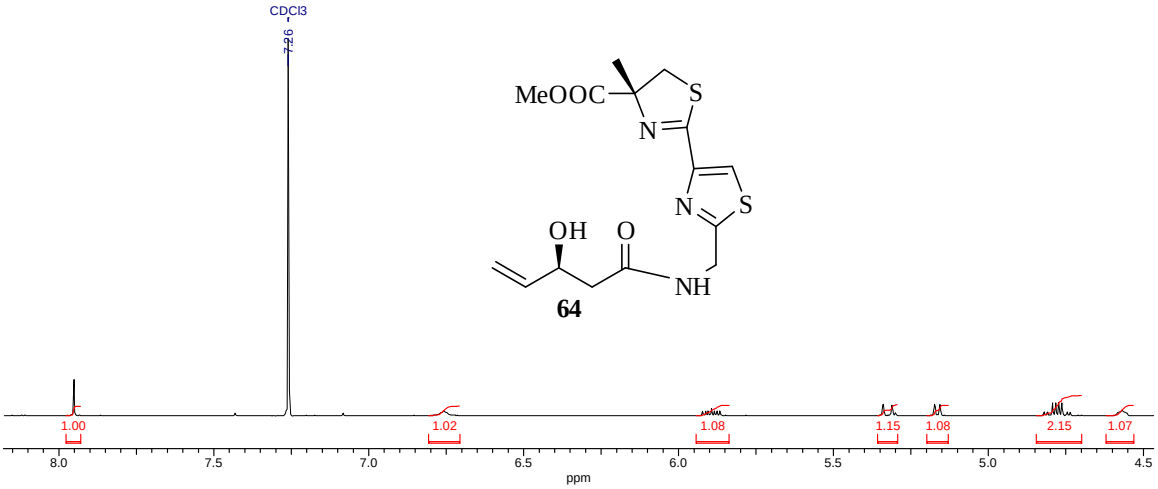


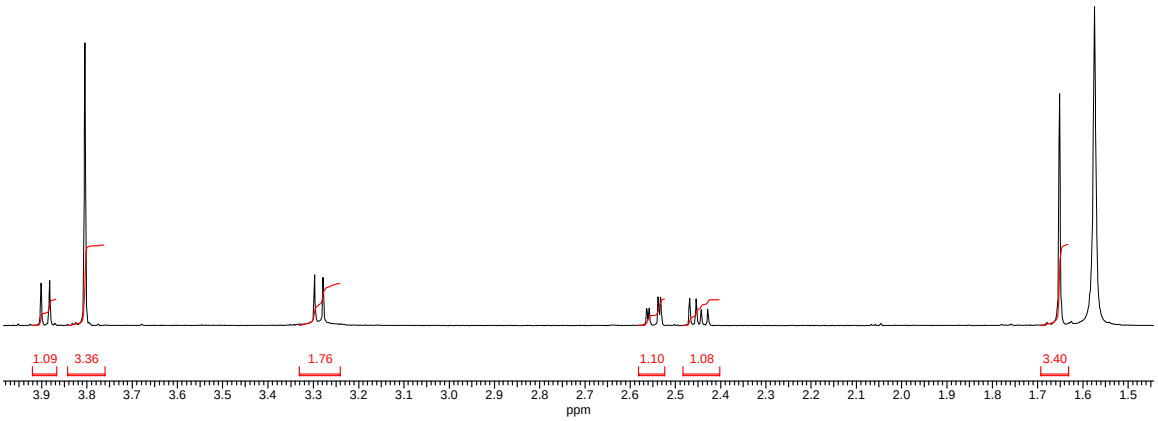
^{13}C NMR of (*S*)-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)pent-4-en-1-one (62)



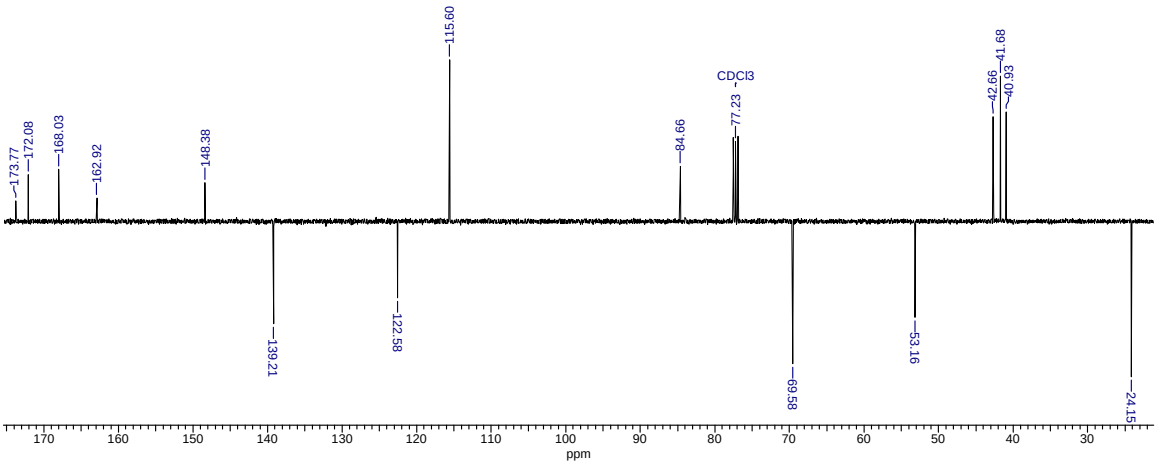
^{1}H NMR of (*R*)-methyl 2-(2-(((*S*)-3-hydroxypent-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (64)



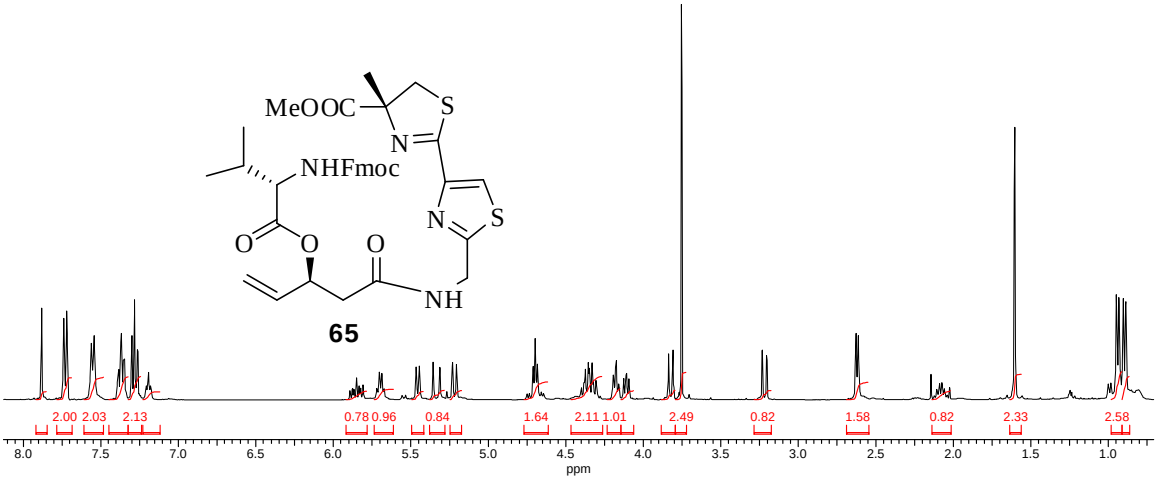


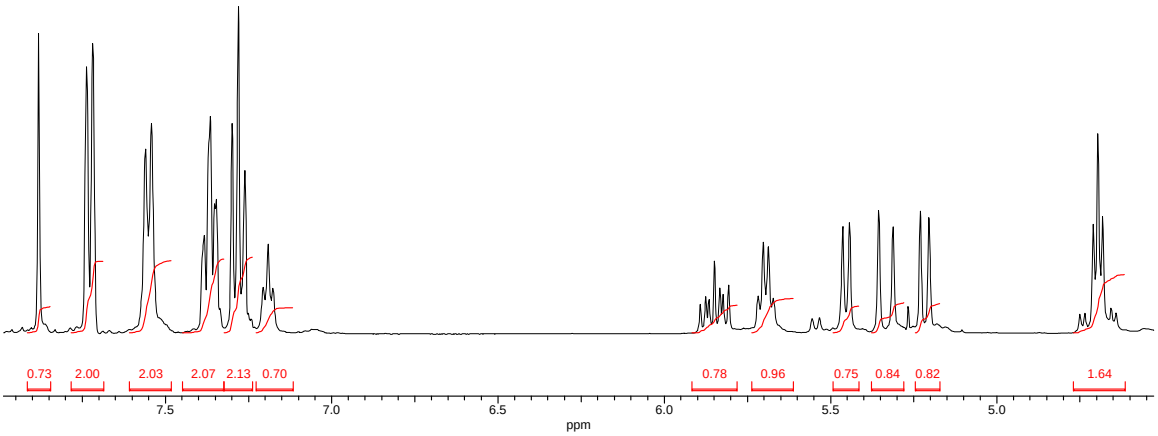


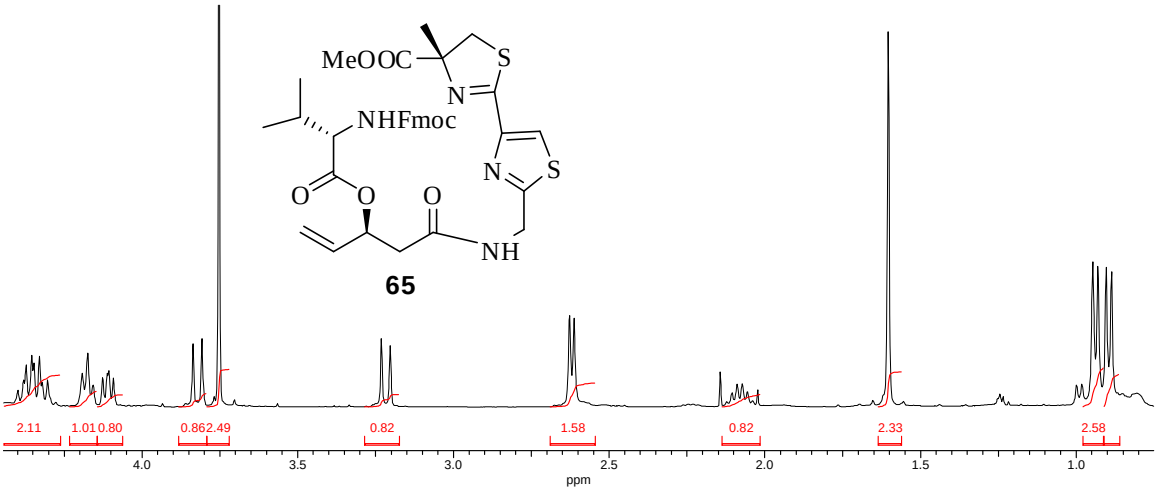
^{13}C NMR of (*R*)-methyl 2-(2-(((*S*)-3-hydroxypent-4-enamido)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (64)



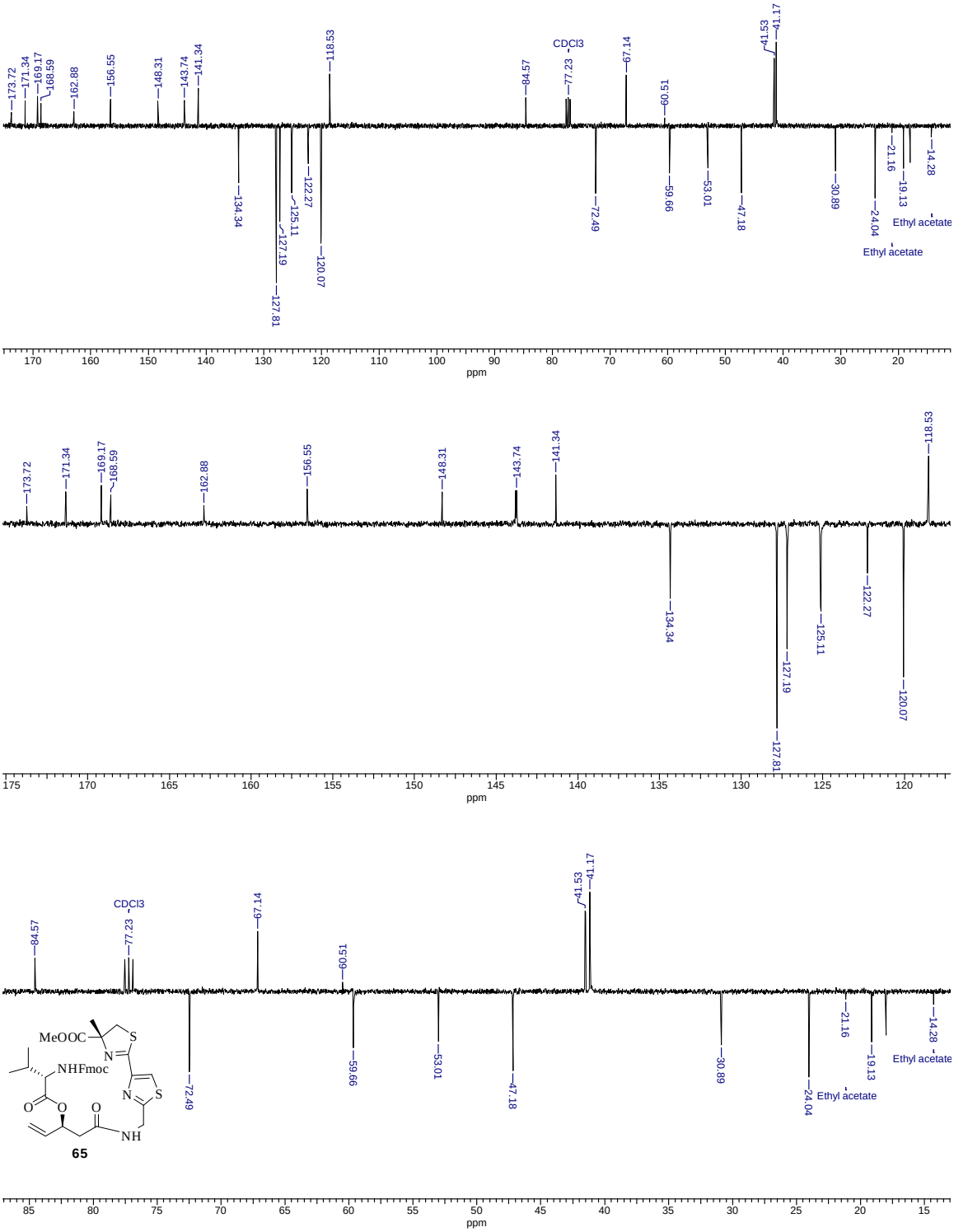
^{1}H NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-vinyl-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (65)



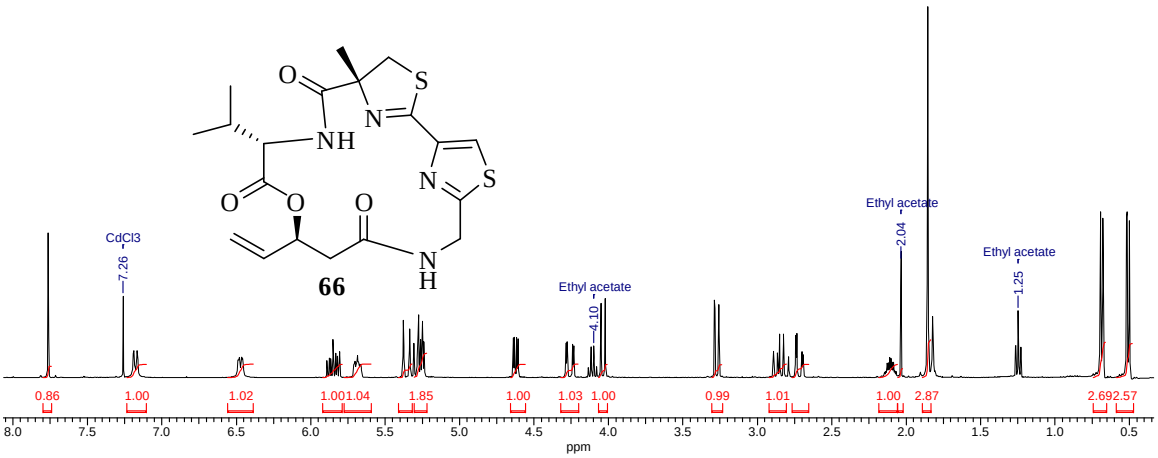




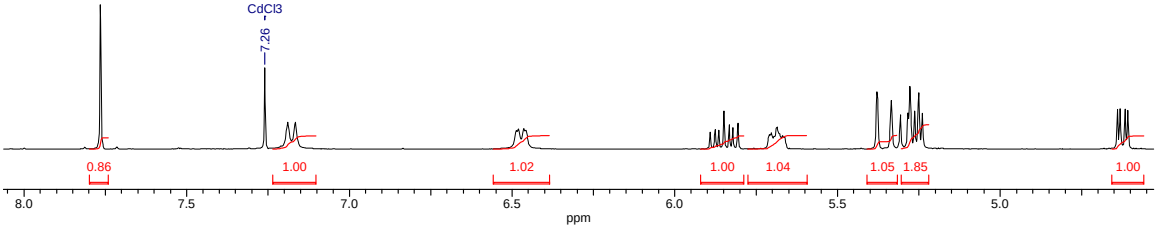
^{13}C NMR of (*R*)-methyl 2-(2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-vinyl-2,7-dioxa-4,11-diazadodecan-12-yl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylate (65)

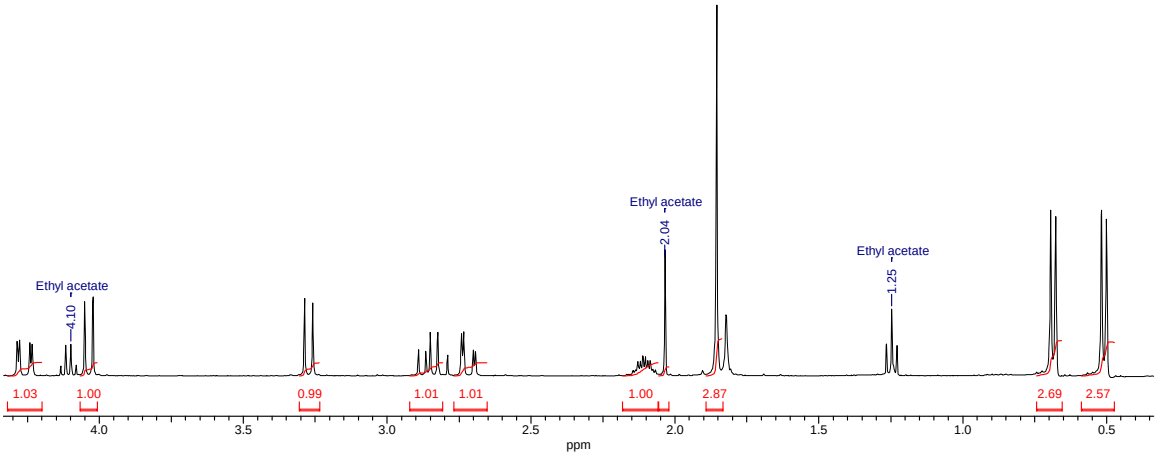


^{1}H NMR of cyclic core 66

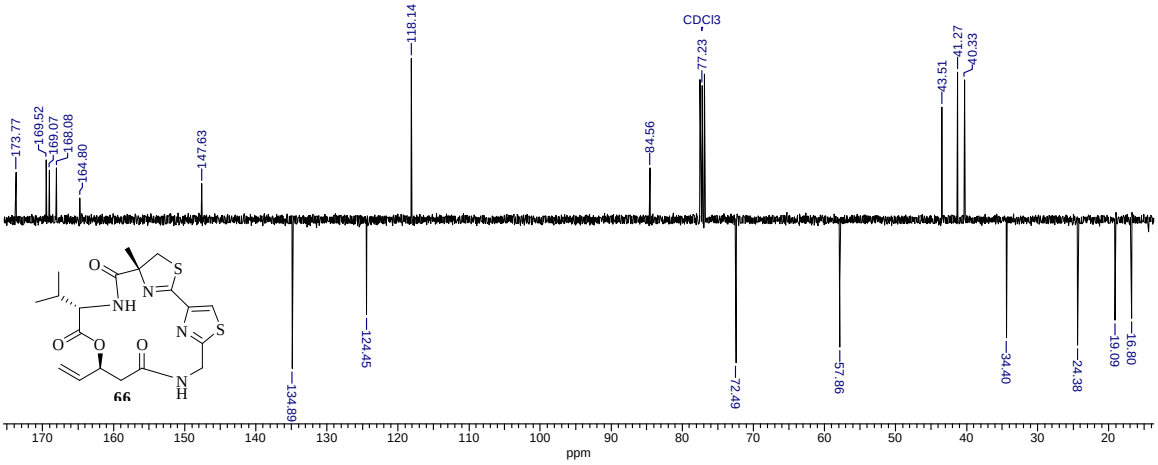


66

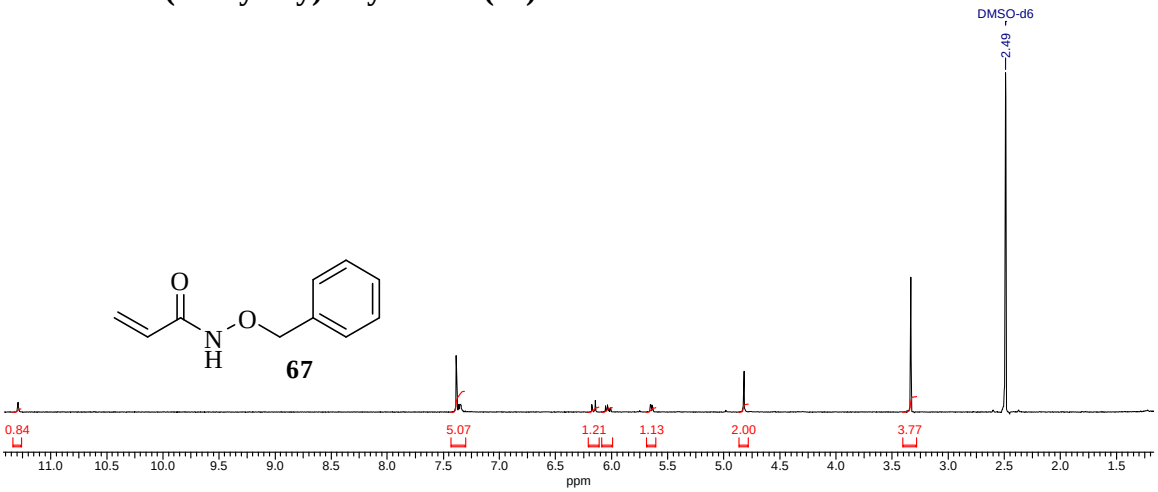


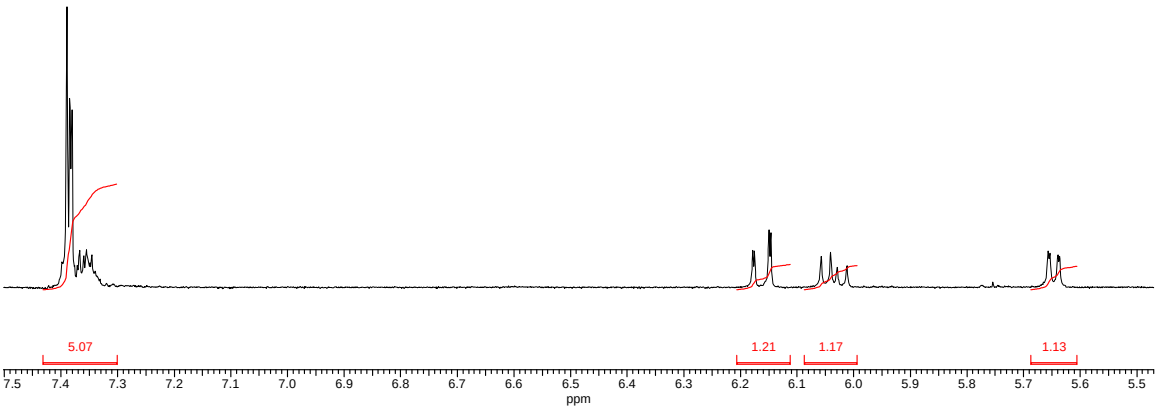


^{13}C NMR of cyclic core 66

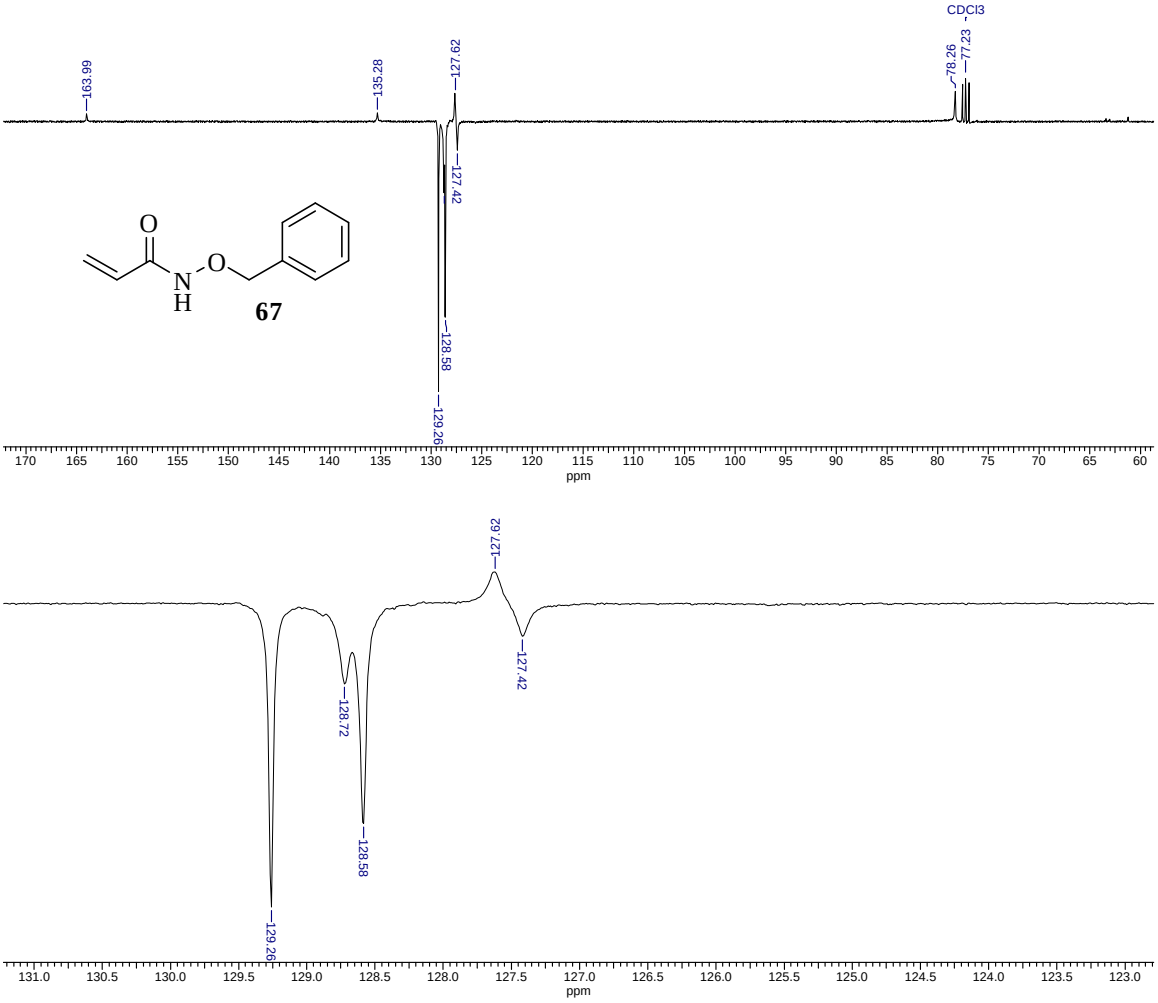


^{1}H NMR N-(benzyloxy)acrylamide (67)

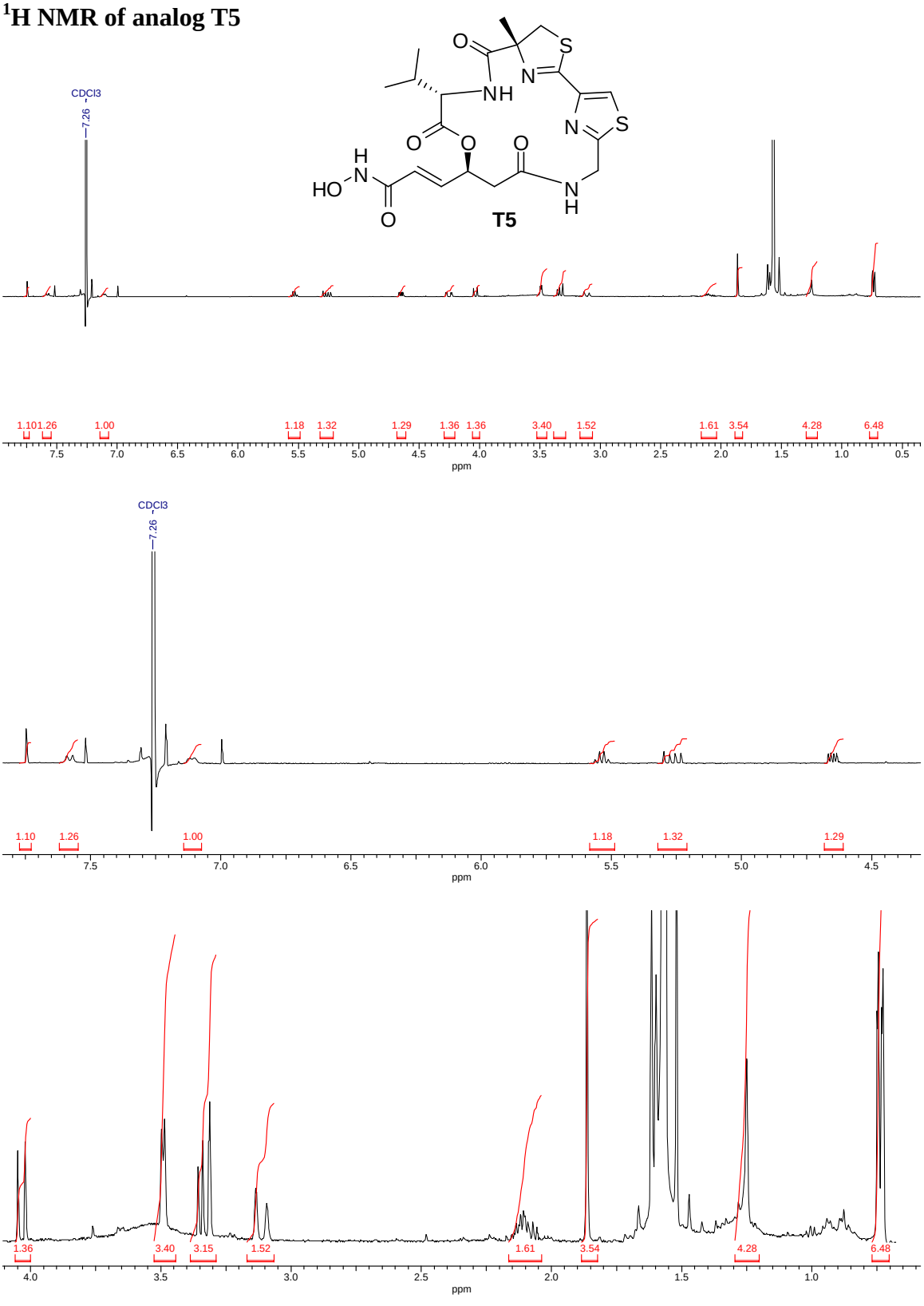




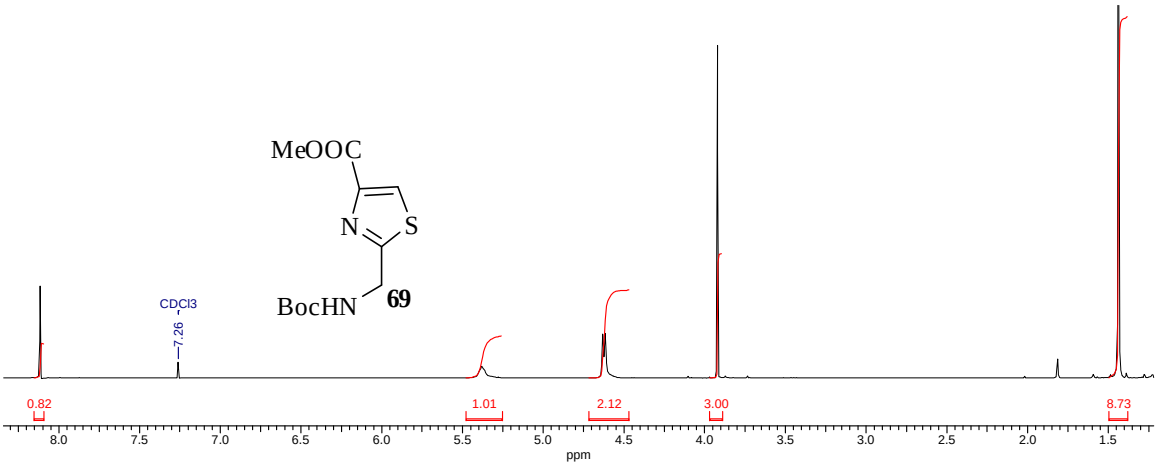
^{13}C NMR N-(benzyloxy)acrylamide (67)



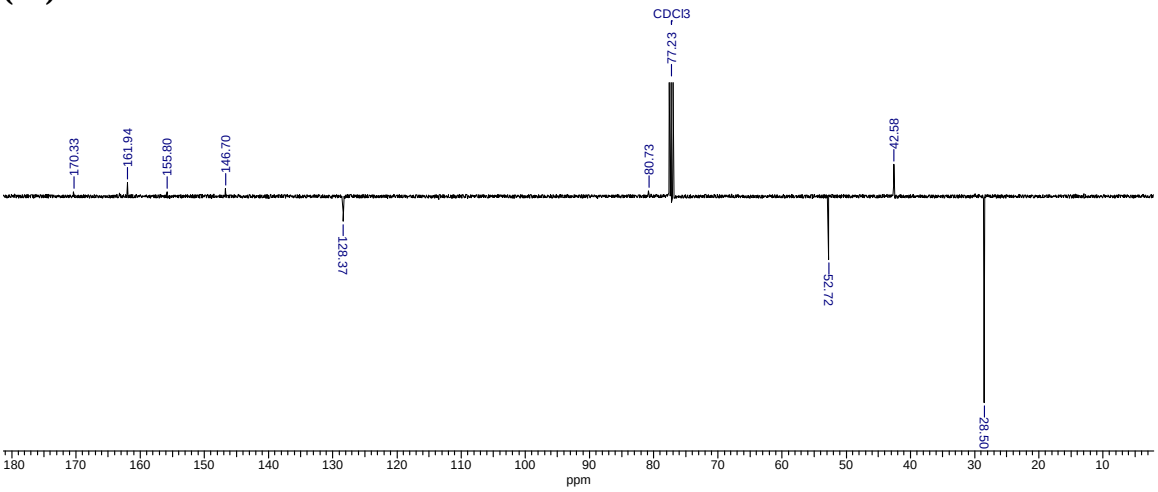
^{1}H NMR of analog T5



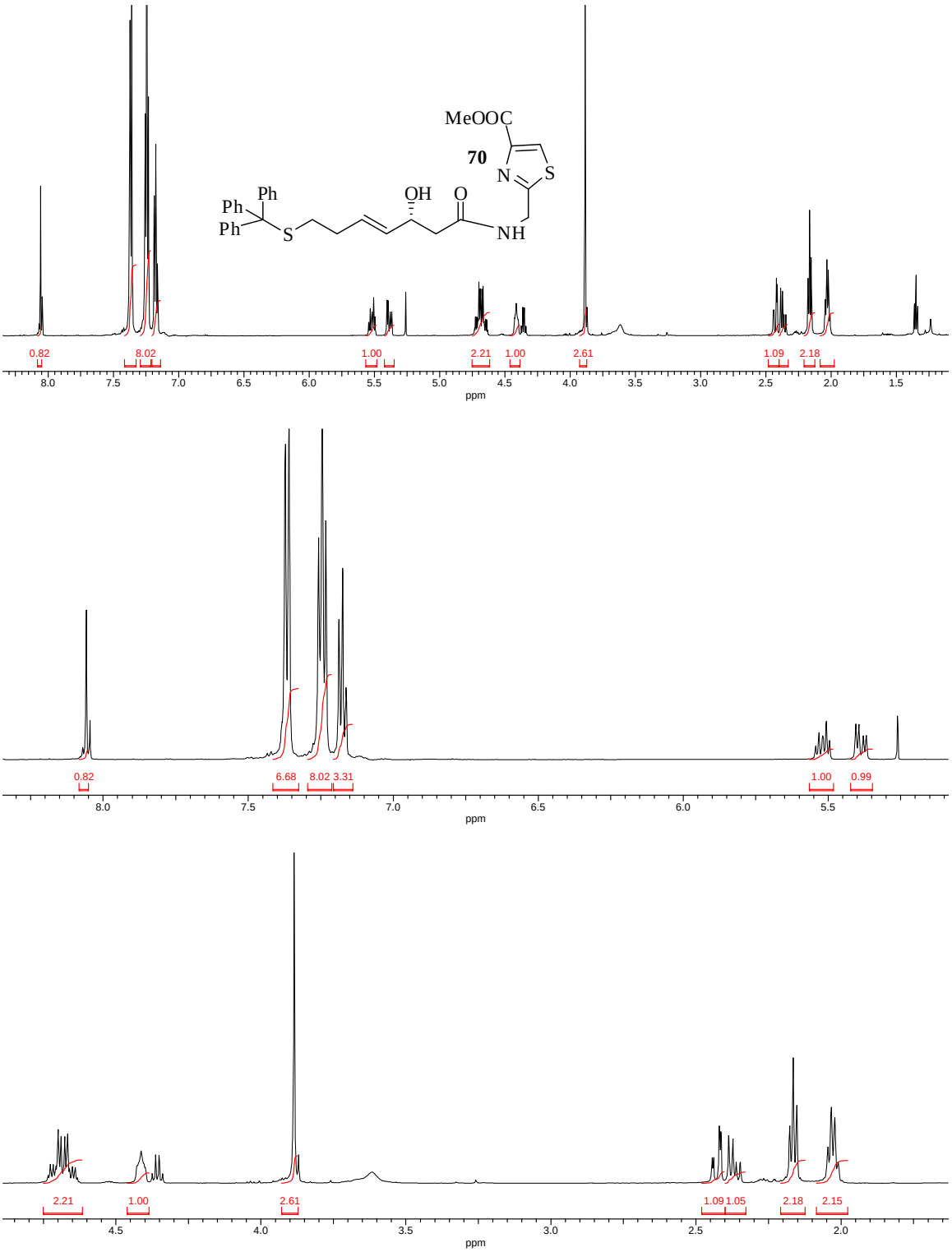
^{1}H NMR of methyl 2-((*tert*-butoxycarbonylamino)methyl)thiazole-4-carboxylate (69)



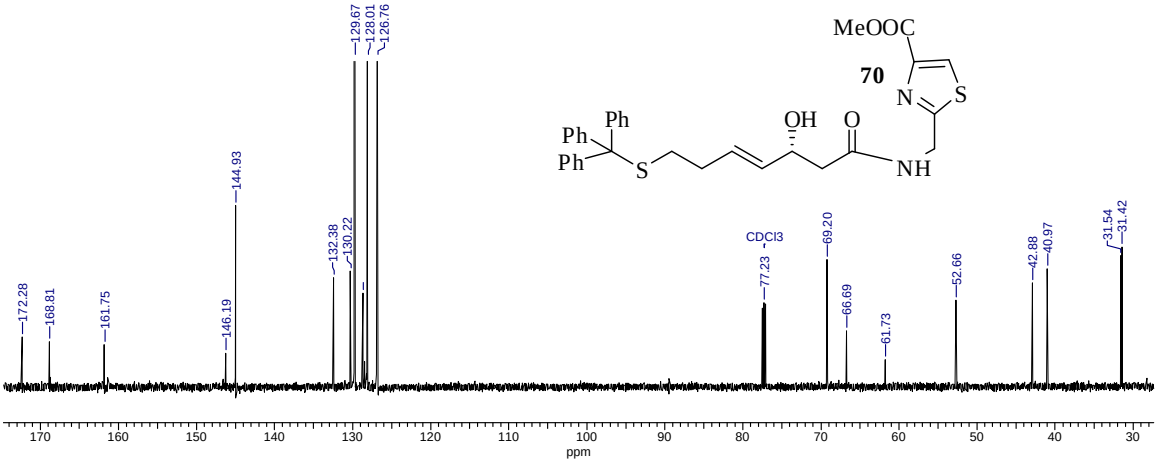
^{13}C NMR of methyl 2-((*tert*-butoxycarbonylamino)methyl)thiazole-4-carboxylate (69)

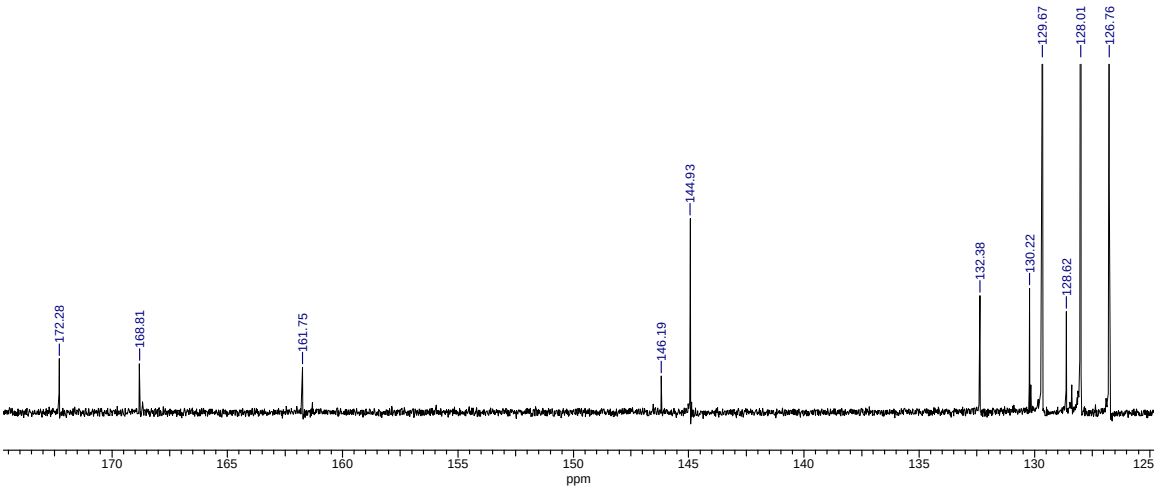


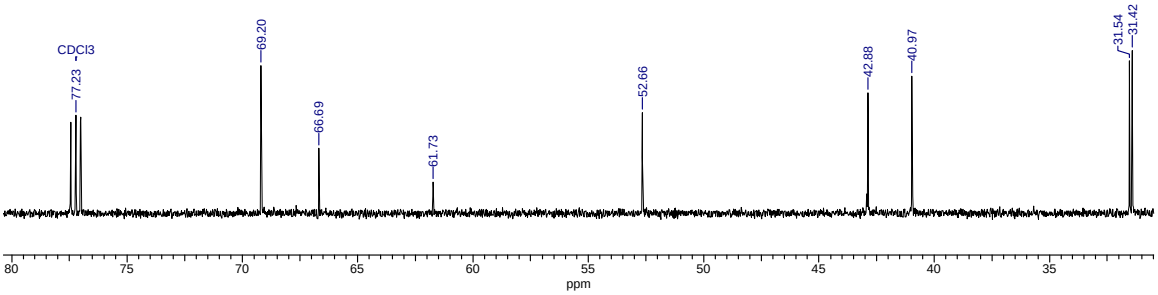
^{1}H NMR of (*R*,*E*)-methyl 2-((3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazole-4-carboxylate (70)



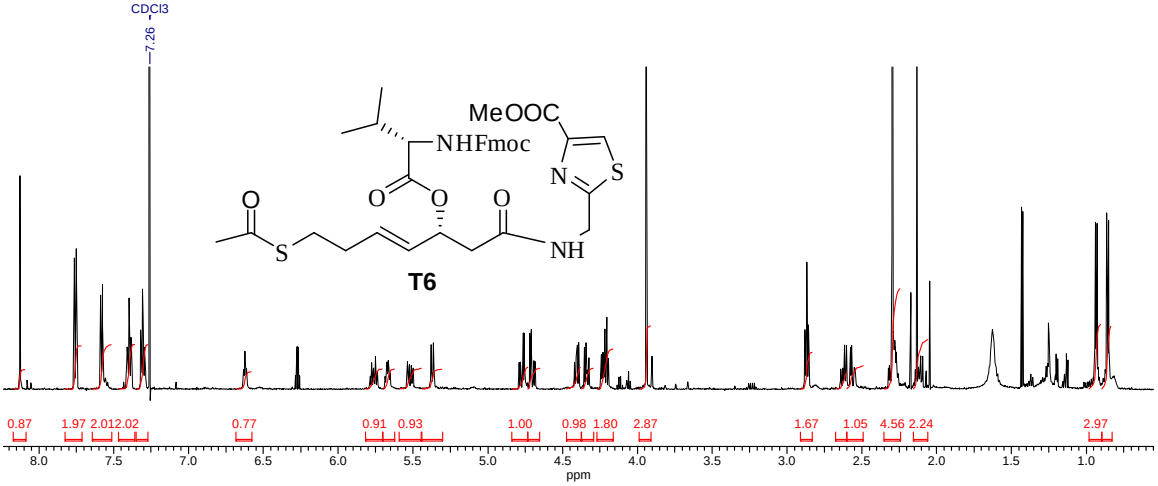
^{13}C NMR of (*R*,*E*)-methyl 2-((3-hydroxy-7-(tritylthio)hept-4-enamido)methyl)thiazole-4-carboxylate (70)

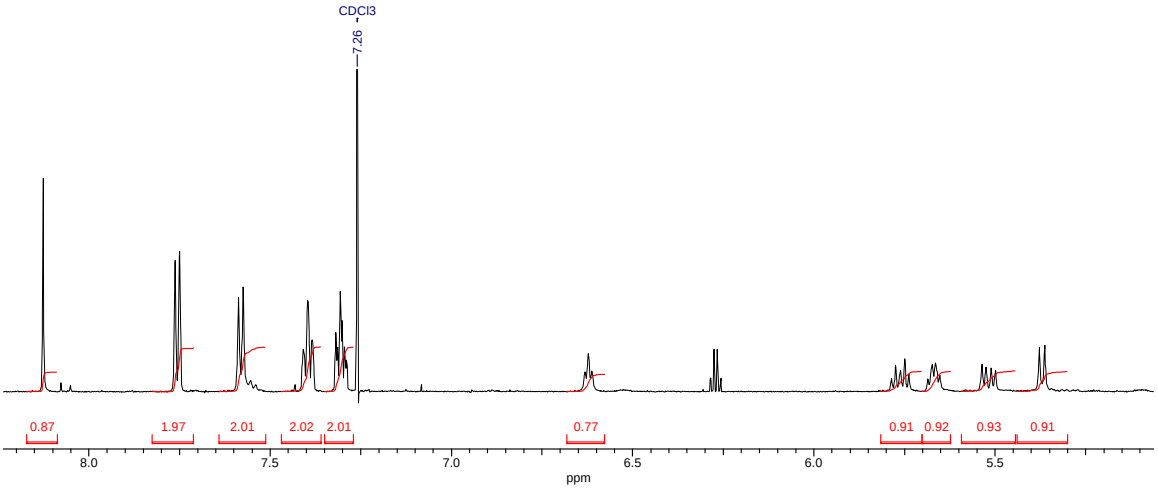


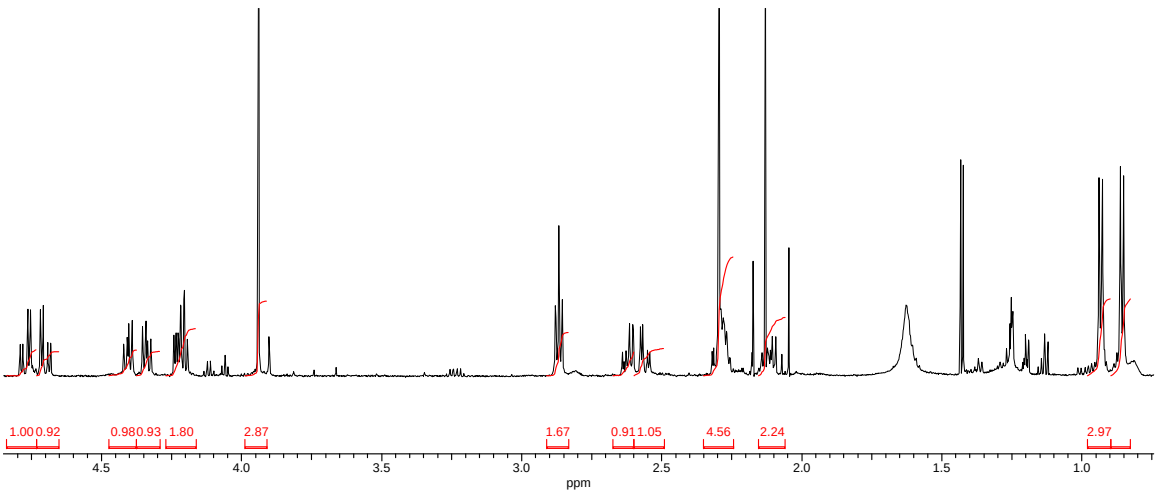




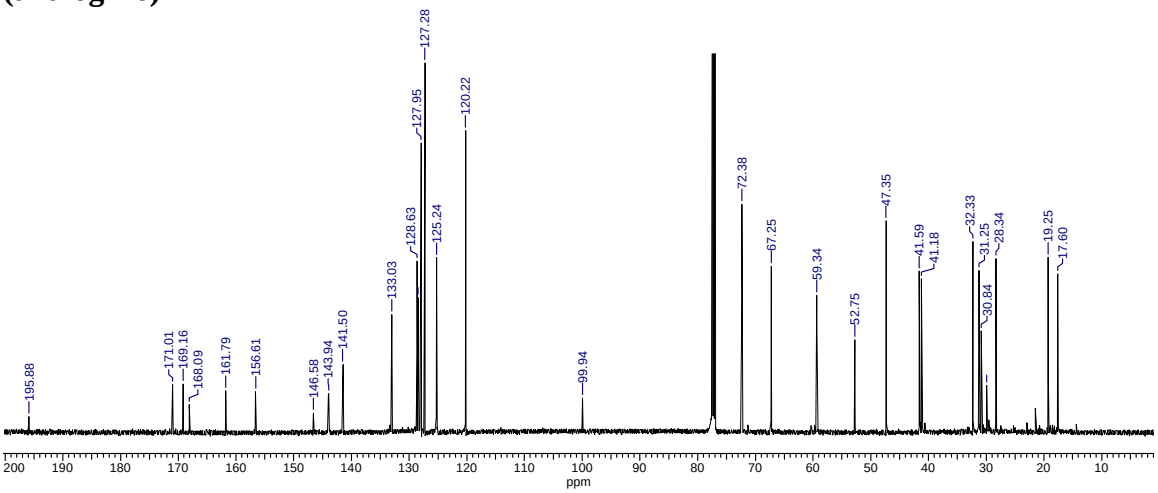
^{1}H NMR of methyl 2-((5*S*,8*R*)-8-((*E*)-4-(acetylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (analog T6)

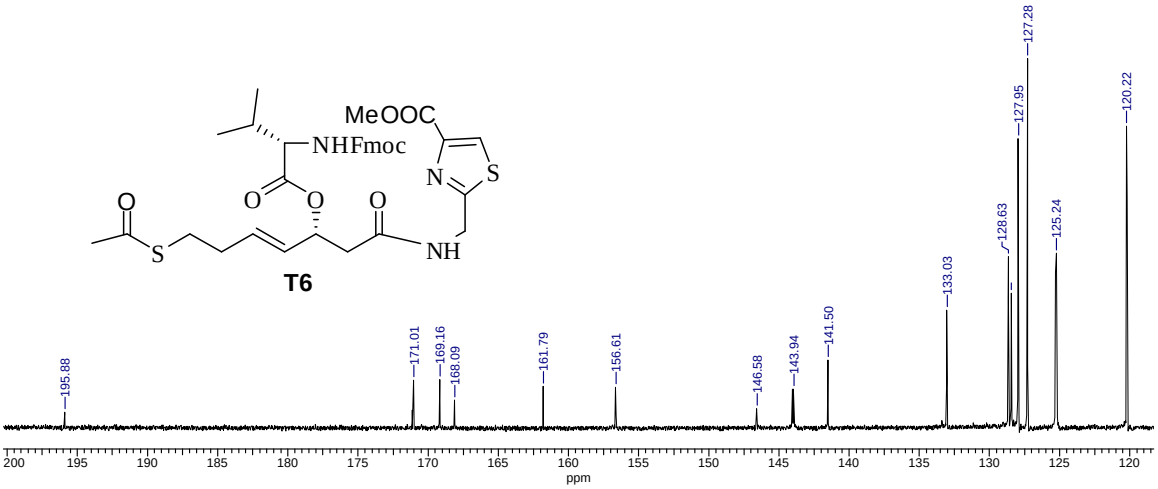


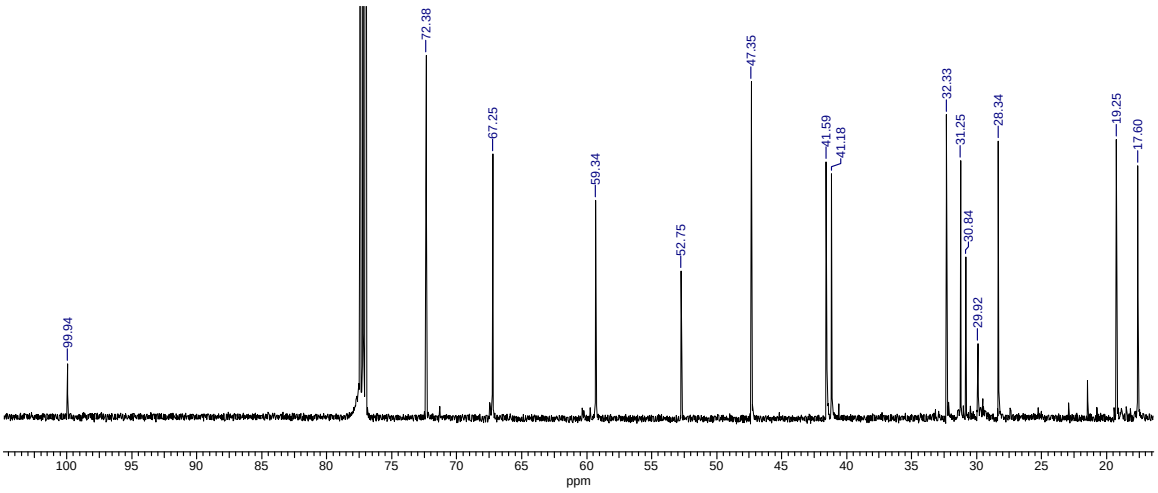




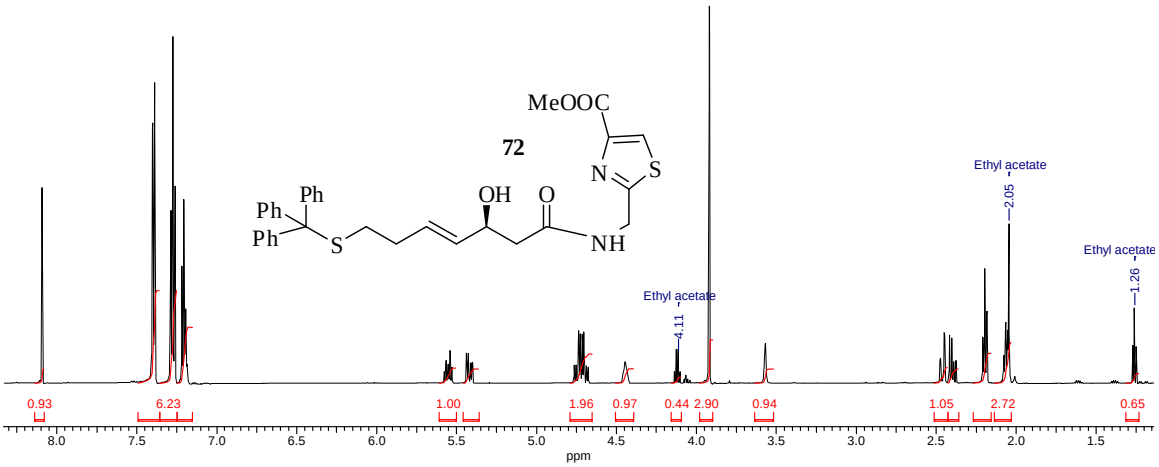
^{13}C NMR of methyl 2-((5*S*,8*R*)-8-((*E*)-4-(acetylthio)but-1-enyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (analog T6)

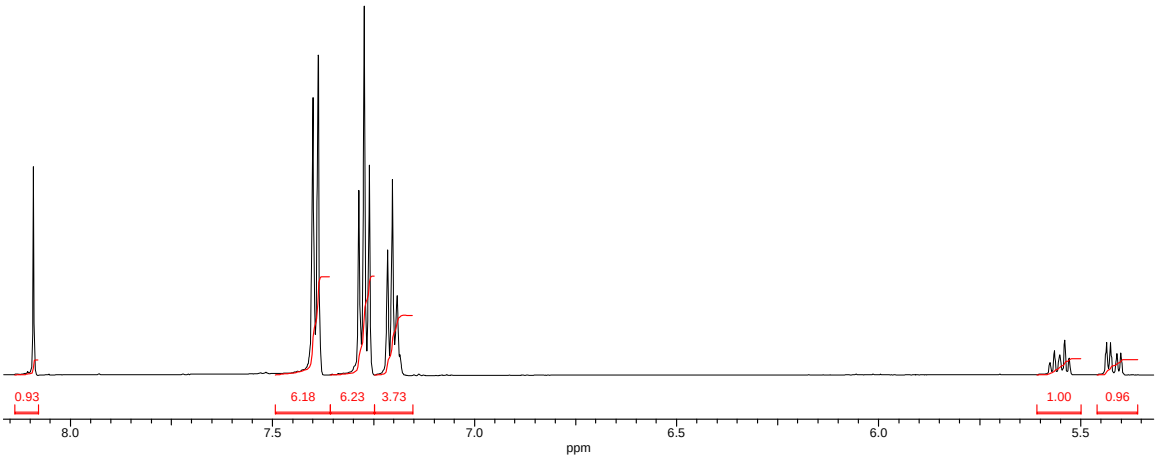


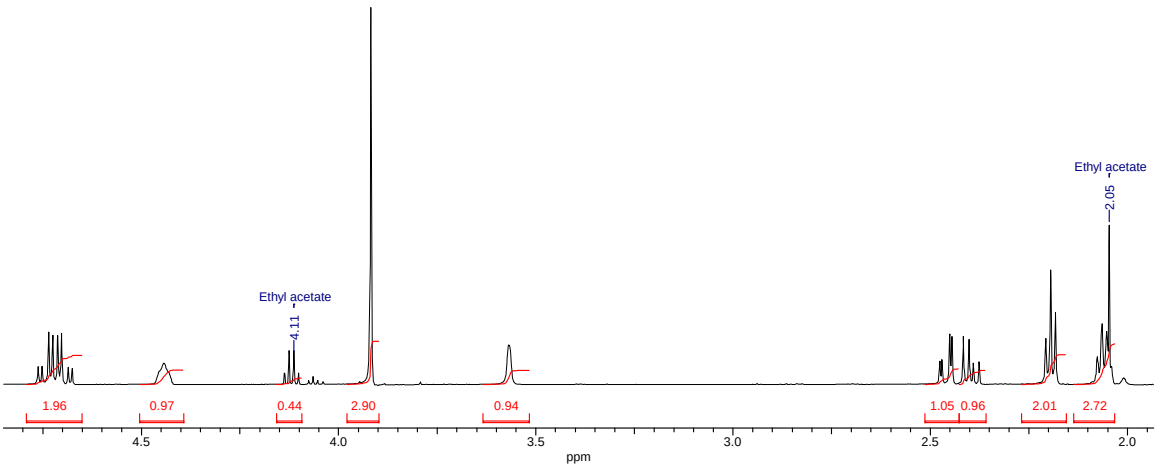




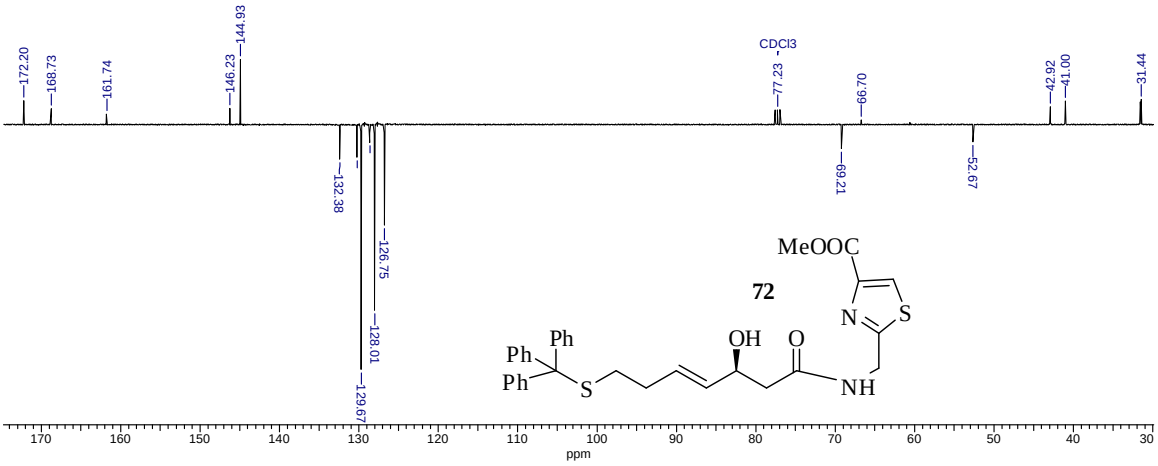
^{1}H NMR of (*S*, *E*)-methyl 2-((3-hydroxy-7-(tritylthio)hept-4enamido)methyl)thiazole-4-carboxylate (72)

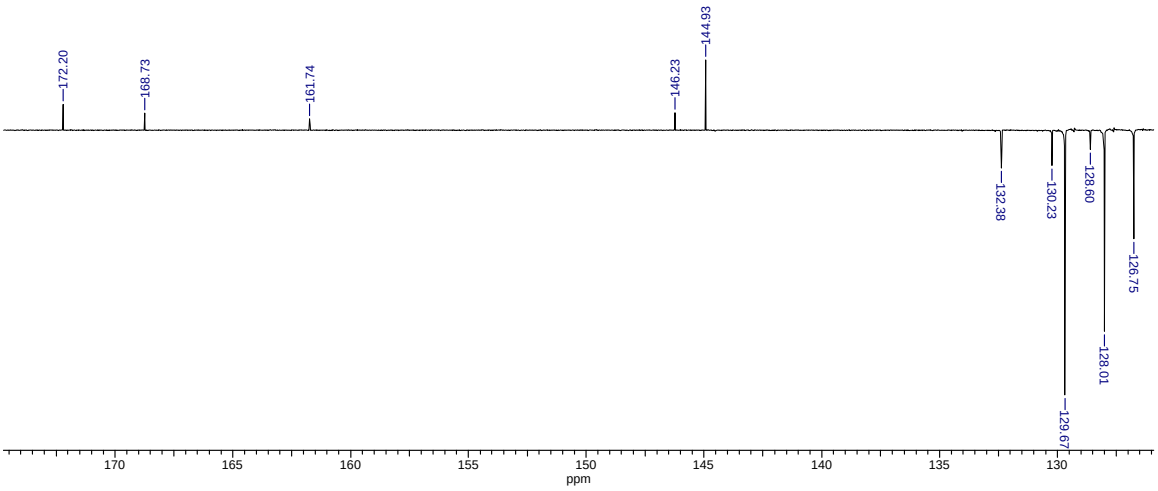


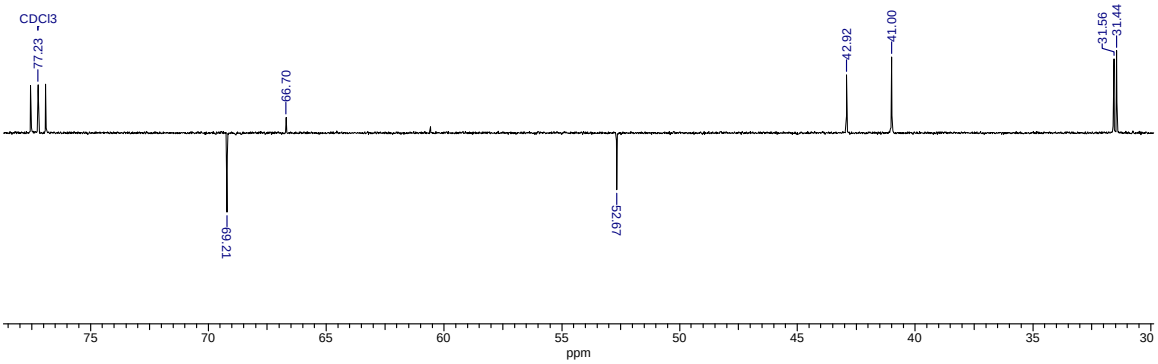




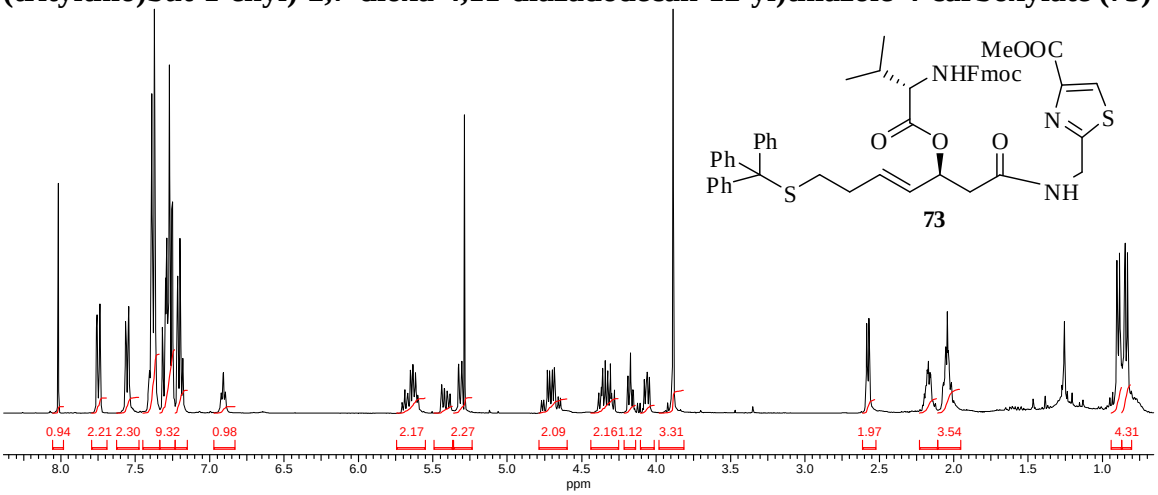
^{13}C NMR of (*S*, *E*)-methyl 2-((3-hydroxy-7-(tritylthio)hept-4enamido)methyl)thiazole-4-carboxylate (72)



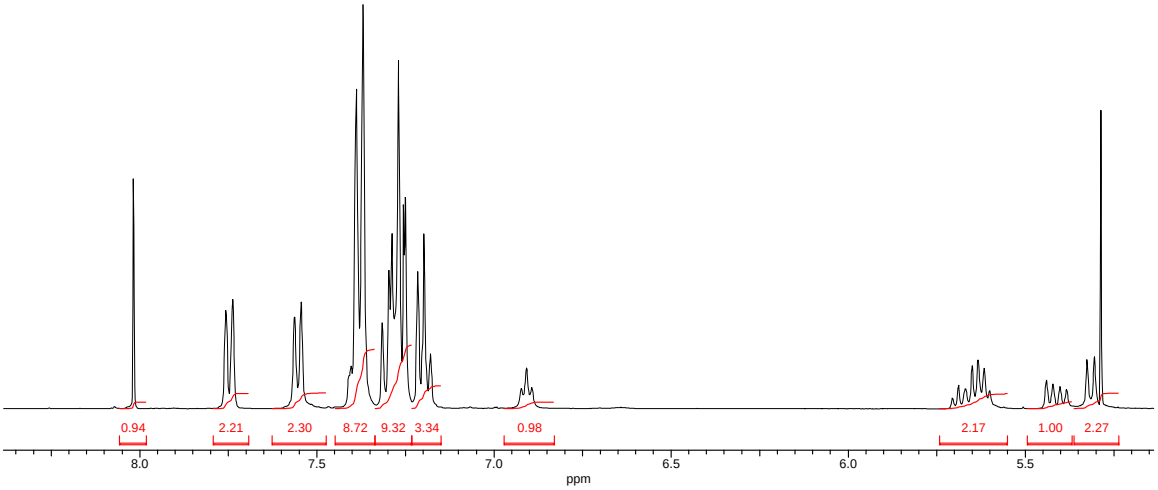


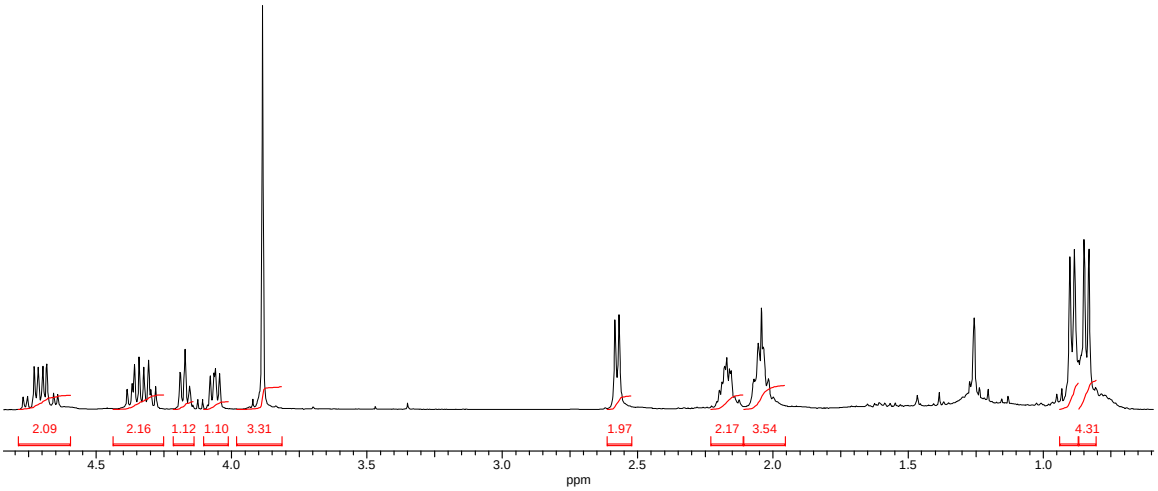


^{1}H NMR of methyl 2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (73)

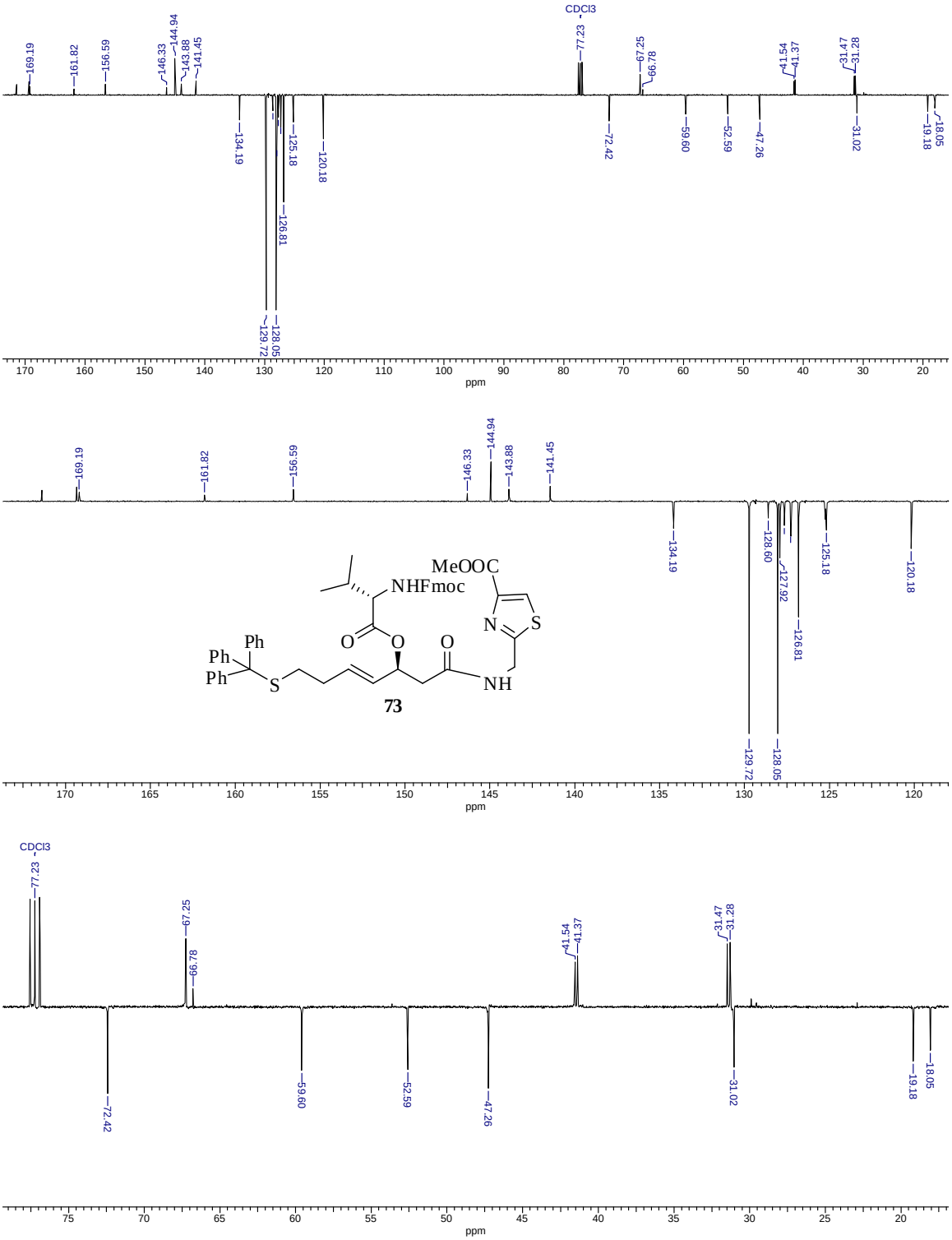


73

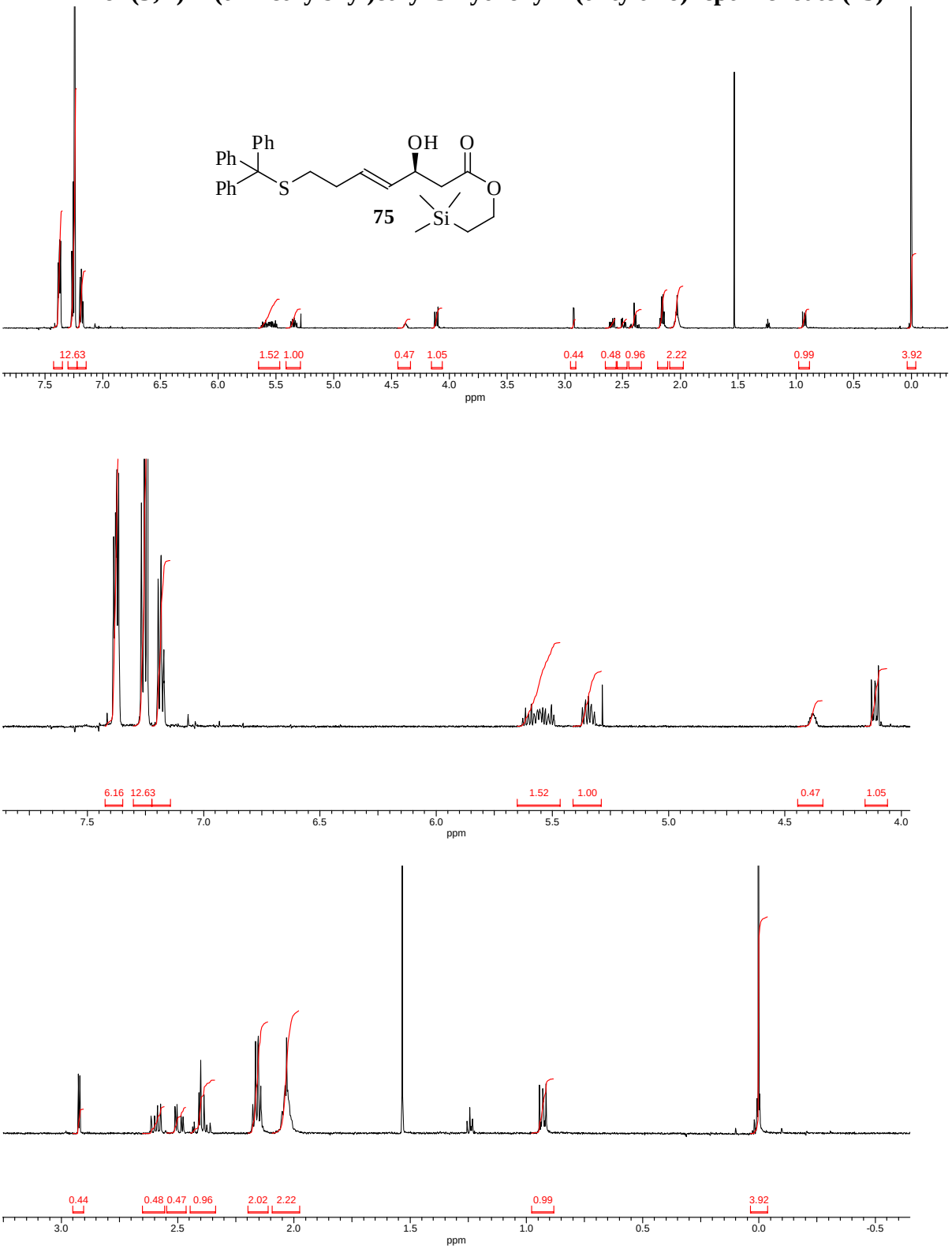




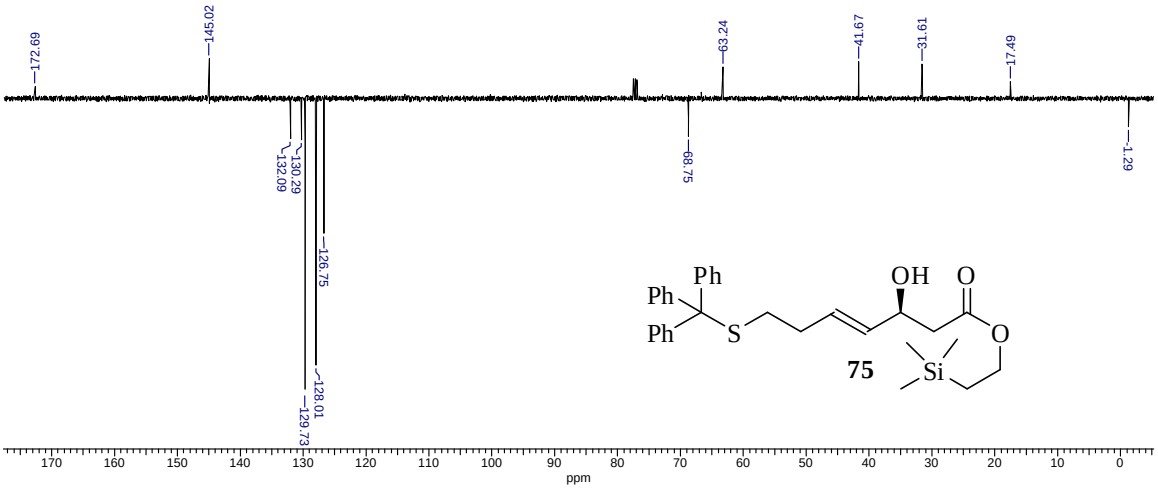
^{13}C NMR of methyl 2-((5*S*,8*S*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-3,6,10-trioxo-8-((*E*)-4-(tritylthio)but-1-enyl)-2,7-dioxa-4,11-diazadodecan-12-yl)thiazole-4-carboxylate (73)



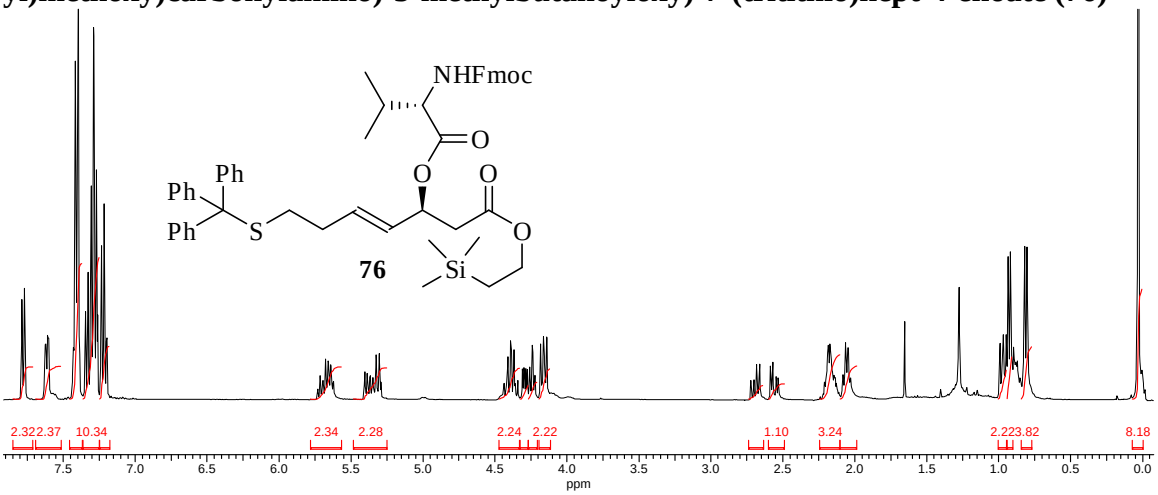
^{1}H NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-hydroxy-7-(tritylthio)hept-4-enoate (75)

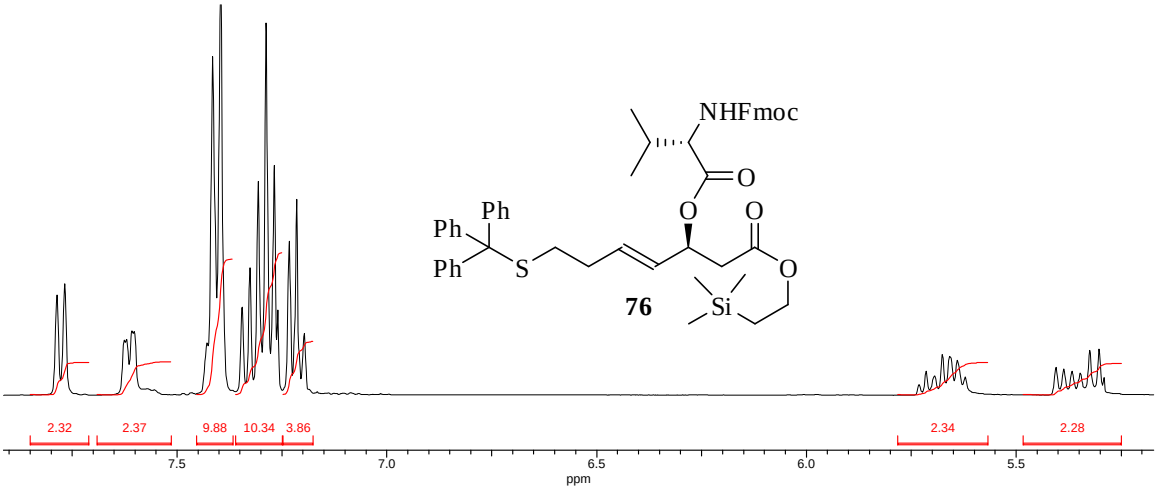


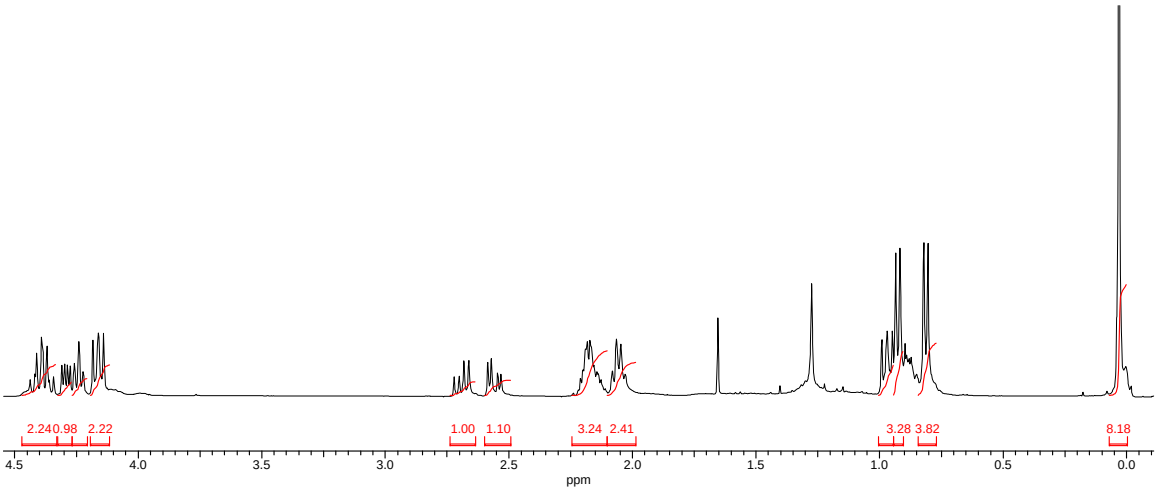
^{13}C NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-hydroxy-7-(tritylthio)hept-4-enoate (75)



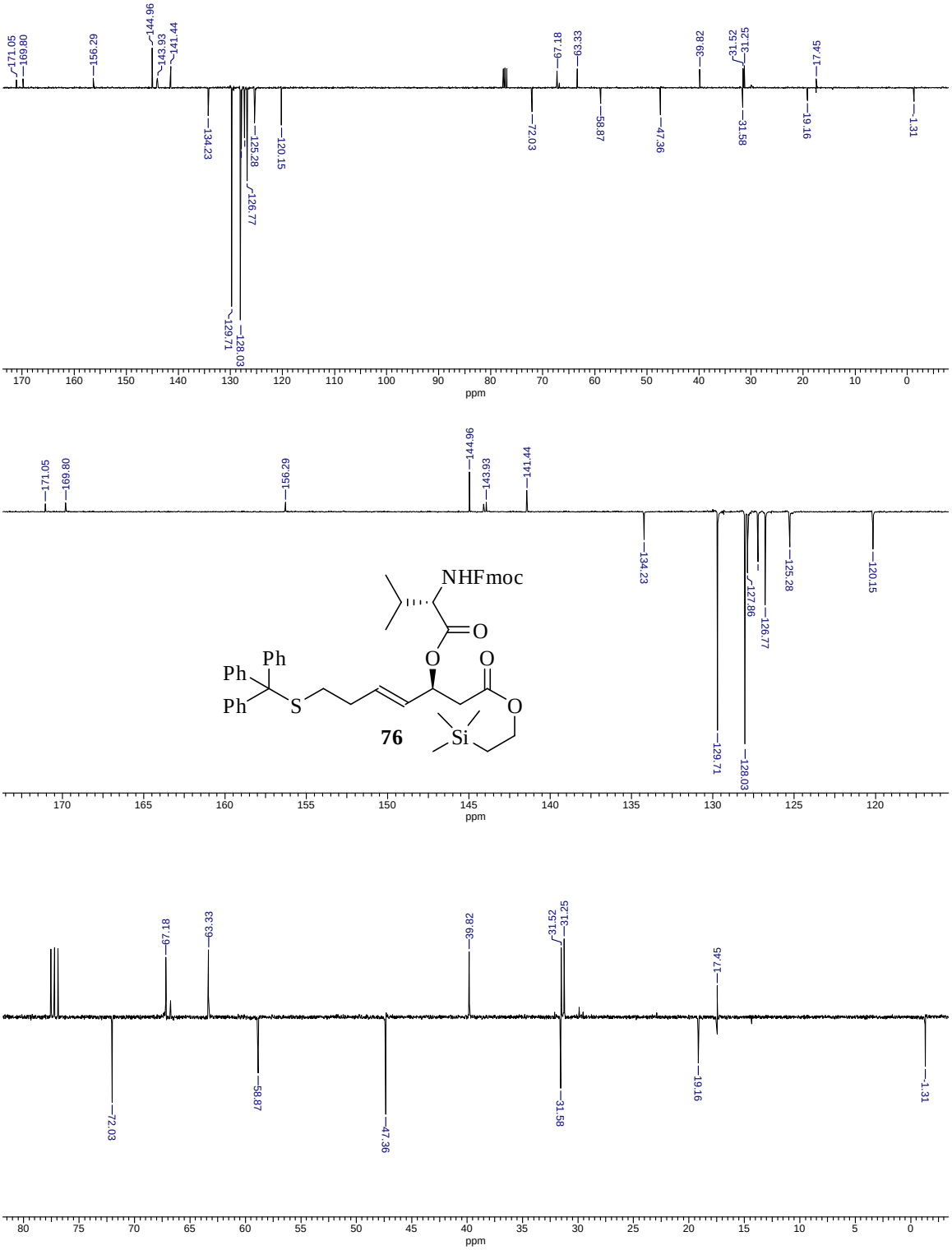
^{1}H NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-7-(tritlthio)hept-4-enoate (76)



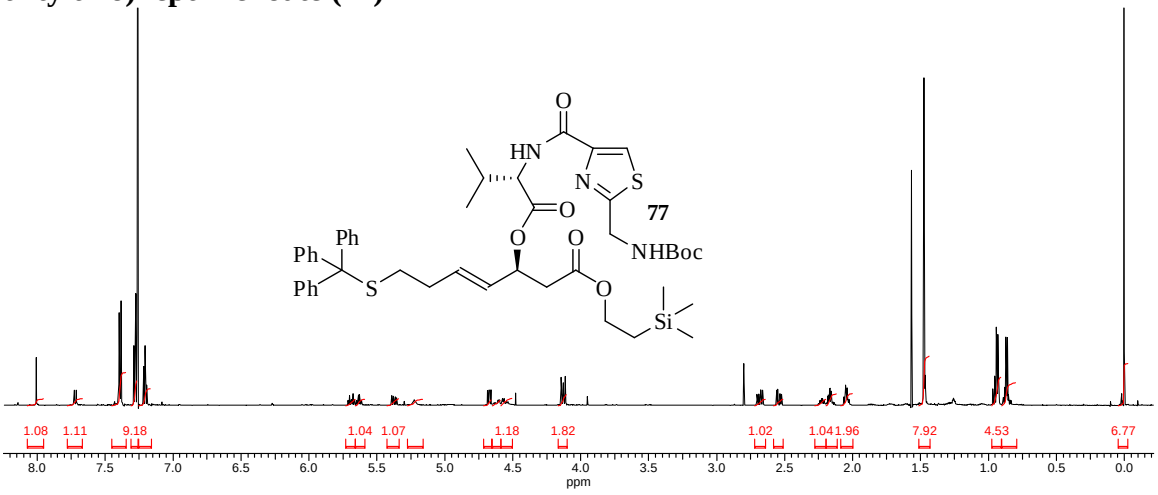


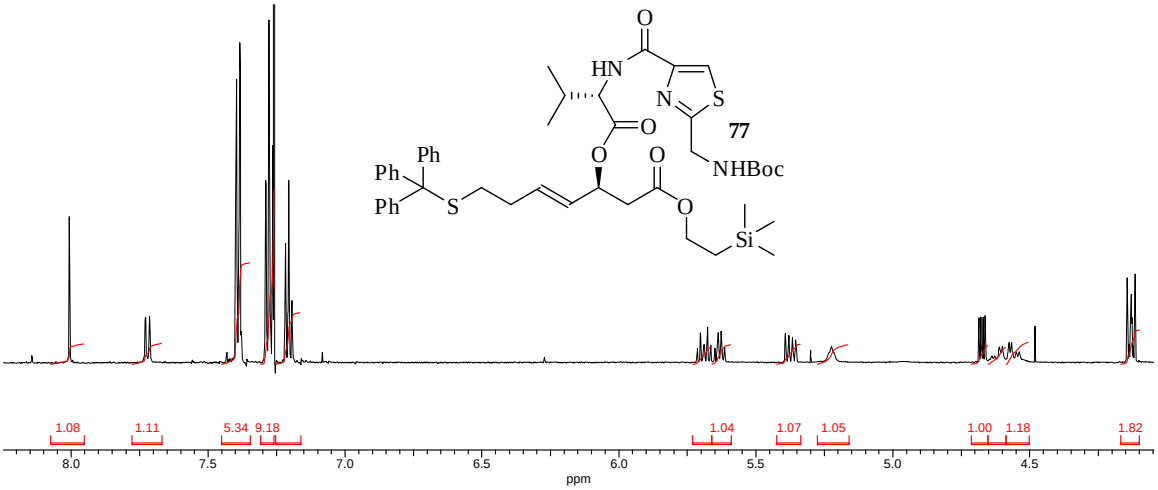


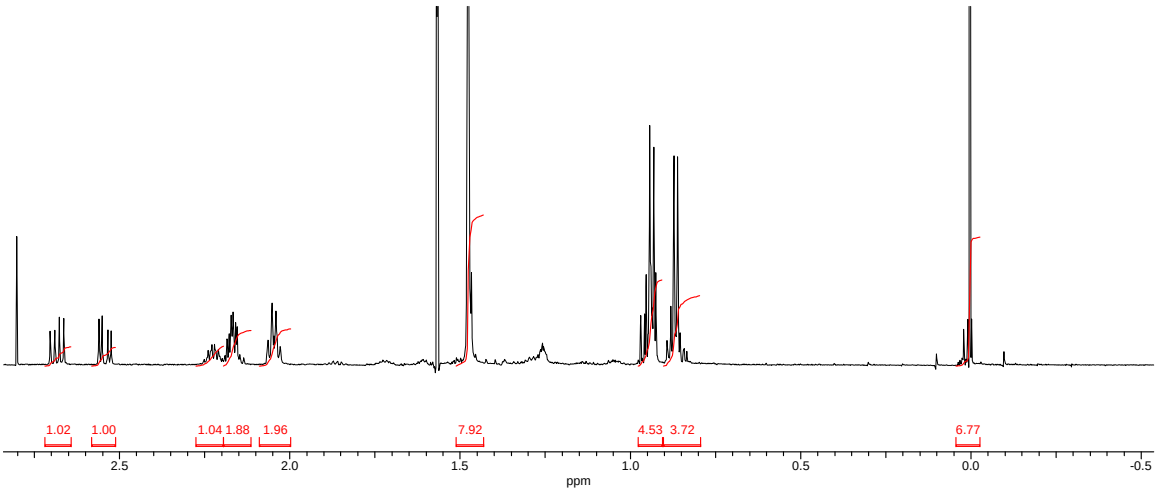
^{13}C NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-7-(tritlthio)hept-4-enoate (76)



^{1}H NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(2-((*tert*-butoxycarbonylamino)methyl)thiazole-4carboxamido)-3-methylbutanoyloxy)-7-tritylthio)hept-4-enoate (77)







^{13}C NMR of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(2-((*tert*-butoxycarbonylamino)methyl)thiazole-4carboxamido)-3-methylbutanoyloxy)-7-tritylthio)hept-4-enoate (77)