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The Design And Synthesis Of Allosteric Effectors Of Carbon Monoxide Binding To Hemoglobin

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THE DESIGN AND SYNTHESIS OF ALLOSTERIC EFFECTORS OF CARBON MONOXIDE BINDING TO HEMOGLOBIN

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THE DESIGN AND SYNTHESIS OF ALLOSTERIC EFFECTORS OF CARBON MONOXIDE BINDING TO HEMOGLOBIN

In Loving Memory of Donjie Cooper and Alexandra Knöppel

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confidence that I could do anything, a belief that, until recently, I had all but taken for granted. Your confidence in me has sustained me through the toughest times. Ben, Laura Mae, you are the best siblings a girl could have. You are both so smart, confident and beautiful. I can't wait to see what you do with your lives. I can't say how much it means to me to have such a loving and supporting family, including my entire extended family. Especially, Granny, Poppy, Josh and Laura, you have all supported me so much during this journey. I am so glad that we have had the opportunity to spend so much time together while I have lived in Philly.

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ABSTRACT

THE DESIGN AND SYNTHESIS OF ALLOSTERIC EFFECTORS OF CARBON MONOXIDE BINDING TO HEMOGLOBIN

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The development of small molecule allosteric inhibitors of carbon monoxide (CO) binding to hemoglobin (Hb) is important for the treatment of CO poisoning. We have found that the synthetic peptide IRL 2500 leads to inhibition of CO binding, but with concomitant hemolytic activity. We describe herein the design, synthesis and biological evaluation of analogs of IRL 2500 that inhibit CO binding without hemolysis. The most potent compounds that we have prepared to date contain heteroaromatic biaryls in place of the biphenyl moiety of IRL 2500. These compounds show improved solubility and reduced hemolytic activity. We also describe the synthesis of conformationally constrained analogs of IRL 2500 based on a piperazine-derived scaffold.

LIST OF ABBREVIATIONS

Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
AIBN	2,2-azo *bis*isobutyronitrile
BBN (9-BBN)	9-borabicyclo[3.3.1]nonane
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BEP reagent	2-bromo-1-ethyl pyridinium tetrafluoroborate
BOP reagent	benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate
Bz	benzoyl
BuLi	butyllithium
BMS	borane dimethylsulfide
Cbz	benzyloxycarbonyl
CO	carbon monoxide
CO_2	carbon dioxide
CO	carboxyhemoglobin
CO_2Hb	carbaminohemoglobin
CoA	co-enzyme A
Collidine	2,4,6-trimethylpyridine
18-crown-6	1,4,7,10,13,16-hexaoxacyclooctadecane

CSA	camphorsulfonic acid
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicycol[5.4.0]undec-7-ene
DCB	1,4-dicyanobenzene
DCM	dichloromethane
DCC	dicyclohexylcarbodimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azocarboxylate
DIBAL	diisobutylaluminum hydride
DIPEA	diisopropylethylamine
dHb	deoxyhemoglobin
DKP	diketopiperazine
DPG	2,3-diphosphoglycerate
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMS	dimethylsulfide
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
2,6-DTBP	2,6-di-*tert*-butylpyridine
Et	ethyl
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
Fe	iron

Fmoc	fluorenylmethyloxycarbonyl
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HHb	protohemoglobin
Hb	hemoglobin
IHP	inositol hexaphosphate
Im	imidazole
IPP	inositol pentaphosphate
KHMDS	potassium (bis)trimethylsilyl amide
LDA	lithium diisopropylamine
LHMDS	lithium (bis)trimethylsilyl amide
2,6-lutidine	2,6-dimethylpyridine
*m*CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MeI	methyl iodide
MeOH	methanol
$MgCl_2$	magnesium chloride
Ms	methanesulfonyl (mesylate)
MsCl	methanesulfonyl chloride
$NaCNBH_4$	sodium cyanoborohydride
NBS	*N*-bromosuccinimide
NaHMDS	sodium (bis)trimethylsilyl amide
NMM	*N*-methyl morpholine

O_2	oxygen
O_2Hb	oxyhemoglobin
ODC	oxygen dissociation curve
Phen	phenanthrene
PMB	*p*-methoxybenzyl
PPTS	pyridinium *p*-toluenesulfonate
PTLC	preparative thin layer chromatography
i-Pr	isopropyl
Py. or Pyr	pyridine
Red-Al	sodium bis(2-methoxyethoxy)-aluminum hydride
RBC	red blood cell
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
t-DDSH	*tert*-dodecanethiol
t-BuOK	potassium *tert*-butoxide
TEA	triethylamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TfN_3	trifluoromethanesulfonyl azide
Tf_3O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography

TMS	trimethylsilyl
TMSI	trimethylsilyl iodide
TMSCl	trimethylsilyl chloride

TABLE OF CONTENTS

TABLE OF FIGURES, SCHEMES AND TABLES

CHAPTER 1 INTRODUCTION

SECTION 1.1 INTRODUCTION TO HEMOGLOBIN

Hemoglobin (Hb), an $\alpha_2\beta_2$ tetrameric globular protein found in red blood cells (RBCs),[1,2] is arguably the most well studied and understood allosteric protein.[3] In fact, Hb followed shortly after myoglobin (Mb) as the first protein x-ray crystal structures to be elucidated in 1958 and 1960, respectively.[1,4] Although Hb does not catalyze a reaction, it has been labeled an honorary enzyme due to its unique ability to bind oxygen and other small gaseous molecules.[1] Hb's primary function is to transport oxygen from the lungs to the outlying muscles and tissue, and then to carry carbon dioxide from these tissues to the lungs for export.[5]

SECTION 1.2 STRUCTURAL FEATURES OF HEMOGLOBIN

Hb contains four hemes, pentacoordinate iron (II) chelated porphyrins, which bind reversibly to oxygen thereby carrying it throughout the bloodstream.[1,2] Hb has two main conformational states. The relaxed (R) state occurs when Hb is ligated at the heme and the tense (T) state occurs when Hb is unligated at the heme.[1,3] The T and R state conformations both contain a 2-fold axis of symmetry around a central water cavity (see **Figure 1.1**, below). This central water cavity is particularly large in the T state with a distance of about 20 Å across along the entire 50 Å length of the protein. The α subunits and β subunits meet their respective counterparts across this distance and are known as the α and β clefts, respectively. Upon conformational change from the T state to the R state, there is about a 15° rotation and 1 Å translation of the $\alpha_1\beta_1$ interface with respect to $\alpha_2\beta_2$.[1]

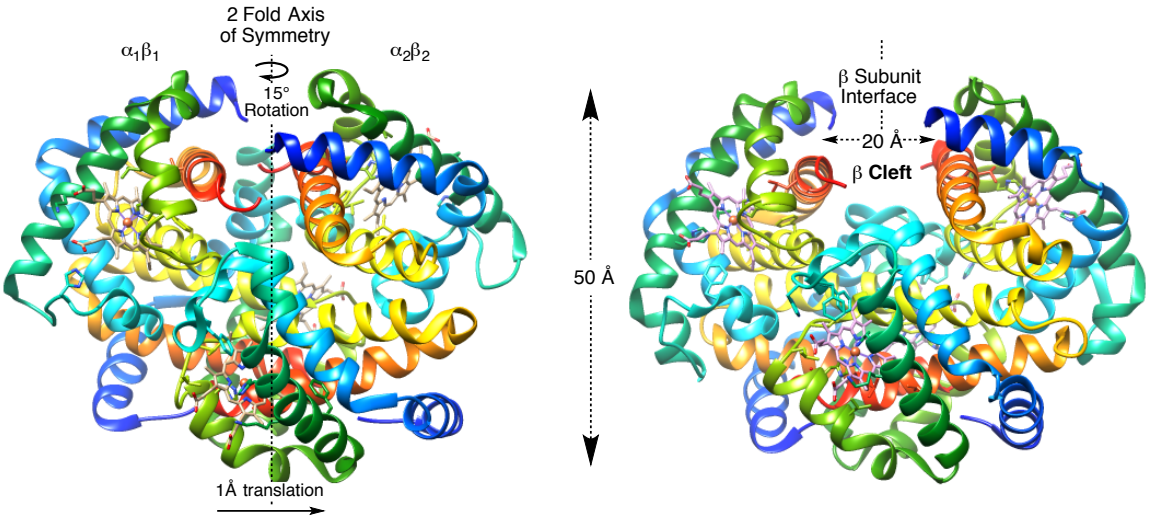


Figure 1.1 Comparison of oxy (R state) and deoxyhemoglobin (T state) crystal structures.

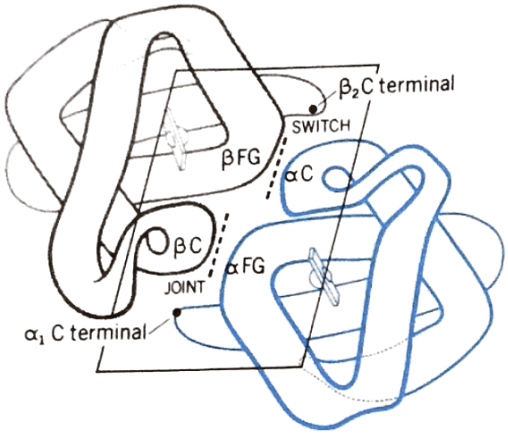


Figure 1.2 Representation of switch and joint regions of Hb.[1]

Conformational change from the T to R state induces the cleavage of several T state hydrogen-bond and salt bridge interactions. These changes particularly occur between the αC and βFG regions, known as the switch, while the joint, containing the symmetrical βC and αFG regions undergoes only a small conformational change and reorganization (see **Figure 1.2**).[1,3] Additionally the C-terminal Arg141α breaks two salt bridges in the transition (not shown), which have been shown to be crucial to binding cooperativity.[1,3]

SECTION 1.3 SMALL MOLECULE INTERACTIONS WITH HEMOGLOBIN

Small molecules are known to bind to Hb in various ways. In addition to the binding of small gaseous ligands at the heme, there are a number of compounds that bind to Hb and allosterically affect function. These ligands can have large effects on the stability of different conformational states.

Section 1.3.1 Binding of Ligands to Heme

The heme consists of four pyrrole rings linked by methylenes, forming the porphyrin unit, which in the case of Hb is protoporphyrin IX, due to the specific substituents attached to the pyrrole rings (see **Figure 1.3A**, below). The centrally bound iron, in the ferrous (II) state, is responsible for the distinct color of Hb and RBCs.[1] In the deoxy (or unligated) state, the iron is five-coordinate and has square pyramidal geometry among the four nitrogens of the porphyrin and a nitrogen of the proximal histidine (His). Upon the binding of a ligand, specifically oxygen, the iron becomes octahedrally coordinated.[1] This binding event changes the electronics of the iron, accounting for the dark blue color of venous blood, while arterial blood is red (see **Figure 1.3B**).[1] This color change can be quantified in the visible light spectrum, where the absorption of Hb shifts from a λ_{max} of 559 nm in the deoxy state, to a λ_{max} of 575 nm in the oxy state (see **Figure 1.3C**). Additionally, there is a strong diagnostic signal in the near-UV known as the Soret band (λ_{max} of 400-436 nm), which indicates the presence of a porphyrin.[6] The Soret band shifts when a ligand binds to the heme and also with a change in pH or oxidation state of the iron.

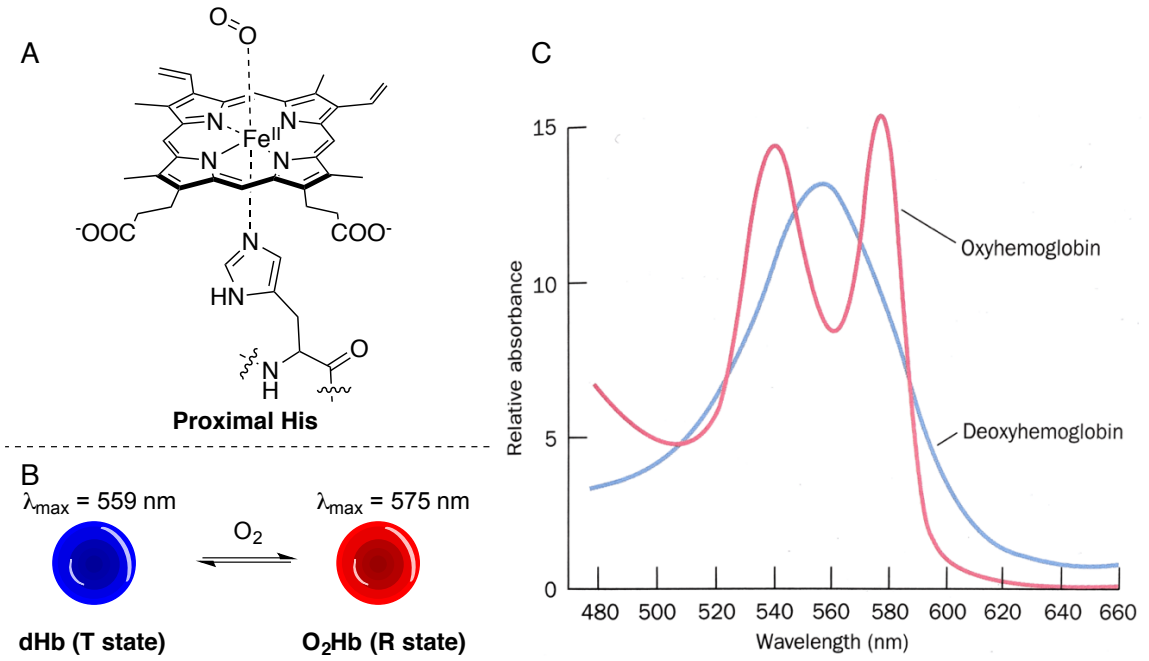


Figure 1.3 A. Heme bound to oxygen. B. λ_{max} of oxy and dHb. C. Visible light spectra of oxy and dHb.[1]

It is hypothesized that the T to R conformational change is a result of the changing stereoelectronic demands of the iron. Closer investigation of the pocket surrounding the heme explains the sterics of gaseous ligand binding during the T to R transition. The most widely recognized steric effects are the proximal push and proximal pull (see **Figure 1.4**, right).[7] Proximal push is a process involving heme deformation when the iron is forced towards the gaseous ligand, resulting in a 0.6 Å shift in the proximal histidine's position. This is thought to catalyze the

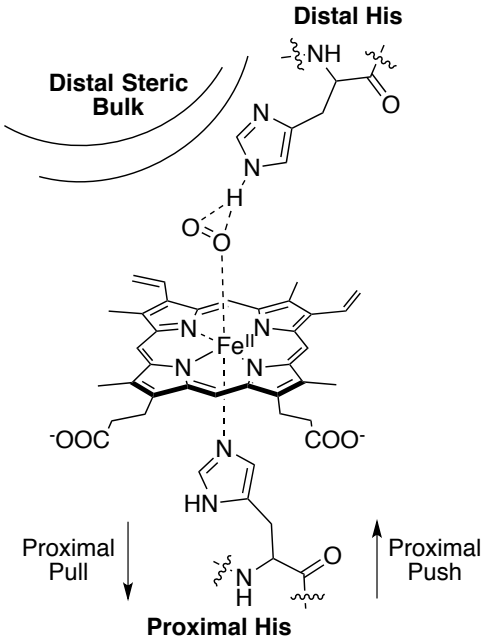


Figure 1.4. Representation of proximal pull and push.[7]

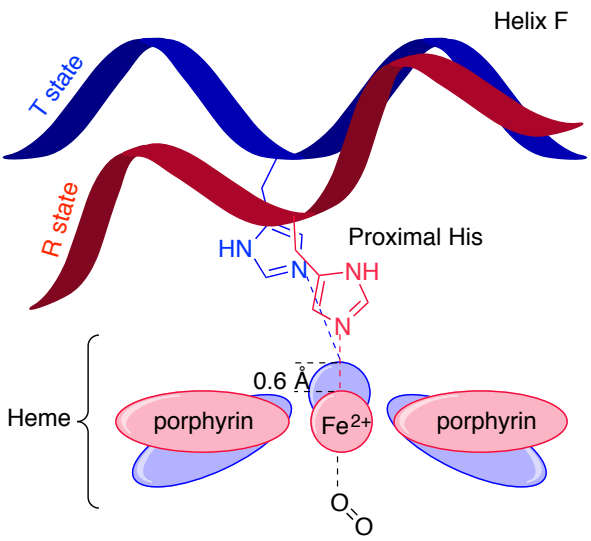


Figure 1.5 Shift in proximal his and helix F upon oxygen binding to Hb.[1]

restructuring of the Hb from T to R.[1] Proximal pull is the deformation when the iron is forced away from the ligand. When there is no ligand bound to the heme, the iron-porphyrin bonds (Fe – $N_{porphyrin}$) are unable to adopt a planar binding mode with the iron center. However, when a ligand binds to the heme, the electronics surrounding the iron change, promoting a 0.1 Å shortening of the Fe – $N_{porphyrin}$ bonds, thereby bringing the iron in plane with the porphyrin (see **Figure 1.5**, left). In addition, there are some distal steric effects that limit the ligand's access to the heme and may also play a role in binding cooperativity.[7] It is important to note that these effects differ widely by gaseous ligand. Specifically, while the proximal pull only increases the rate of oxygen dissociation from the heme, it both the decreases the rate of association and increases the rate of dissociation of carbon monoxide. This information could prove useful in differentiating the binding of carbon monoxide and oxygen.

As previously discussed, the transition between the T and R states of Hb has large implications on the ability of oxygen and other ligands to bind to Hb. Although there are four hemes in Hb, the binding of oxygen at a single heme leads to the T to R conformational change and, due to decreased steric bulk and proximal push, oxygen subsequently binds to the other three hemes of the R state of Hb. This increase in oxygen affinity upon conformational change to the R state leads to the Hb's unique ability to have

a very high saturation (Y_{O2}) for oxygen at high partial pressures (pO_2) of oxygen and a low saturation at low partial pressures of oxygen (see **Figure 1.6**, below).[1] This property is defined as binding cooperativity, and leads to the canonical sigmoidal oxygen binding curve of Hb (see **Figure 1.6**, represented by purple line). If instead, Hb were only able to access the T state, oxygen would have a very low affinity for Hb and its binding curve would have a shallow, linear slope (see blue line below). In contrast, if Hb were only able to access the R state, Hb's affinity for oxygen would always be high and would exhibit a logarithmic binding curve (see red line below). As it is, Hb is able to access both conformations, therefore having a binding curve (purple line) that at lower partial pressures of oxygen more closely resembles the binding curve of the T state (blue line) and at higher partial pressures of oxygen more closely resembles the R state (red line).

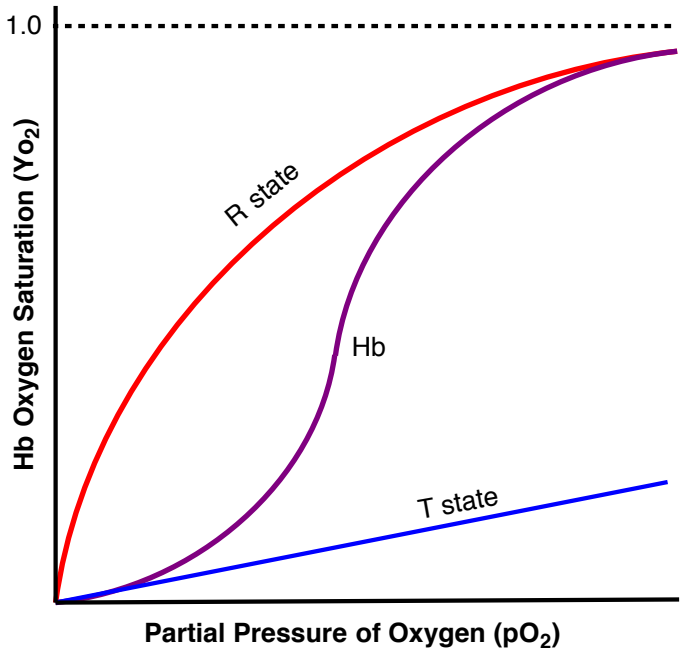


Figure 1.6 The oxygen dissociation curve of Hb. Y_{O2} refers to the saturation of oxygen on the heme. pO_2 is the partial pressure of oxygen.[1]

Cooperativity explains Hb's unique ability to transport oxygen in the bloodstream. In the lungs, where the partial pressure of oxygen is high, Hb becomes saturated with oxygen. When Hb reaches the capillaries, where the partial pressure of oxygen is low, Hb releases the stored oxygen, allowing it to diffuse into tissue.[1,5]

Section 1.3.2 Allosteric Effectors of Binding

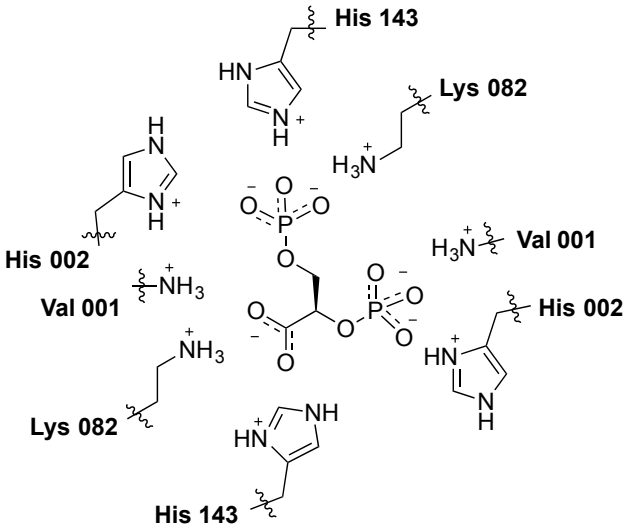


Figure 1.7 Representation of DPG bound in the β cleft.

In addition to binding cooperativity, some molecules bind allosterically to Hb and induce a shift in the oxygen dissociation curve (ODC). In 1967, Benesch and Benesch reported the discovery of the first allosteric effector of human Hb.[8] It had previously been shown, as early as 1909, that the concentration of certain salts can affect the ODC.[9,10] However, Benesch and Benesch noticed that the ODC was strongly affected by the concentration of inorganic phosphate and hypothesized that 2,3-diphosphoglycerate (DPG, see **Figure 1.7**, above), which has multiple phosphates and was known to be present in RBCs at a concentration of about 3.6-5 mM,[5,8] might induce a similar shift. Not only did DPG affect the ODC, but it greatly increased (right shifted) the oxygen dissociation relative even to inorganic phosphate (see **Figure 1.8**, below). They further found that the use of a nonspecific source of anionic charge (potassium ferricyanide) did not affect the ODC, which led to the proposal that DPG was acting allosterically to regulate the ODC.[8] In 1972, five years after

the Benesch hypothesis, a crystal structure of DPG bound to Hb was first published by Arnone and coworkers and the mechanism of action was resolved.[11] Arnone showed that DPG binds to the β cleft, making interactions with six histidines, two lysines and the two N-terminal valines (see **Figure 1.7**, above). Additionally, he demonstrated that DPG had a stabilizing effect on deoxyhemoglobin (dHb)/T state Hb, slightly deforming the structure of dHb and shortening the distance between Val $\beta_1$006 and Val $\beta_2$006 by about 2Å, which he postulated would affect Hb solubility.

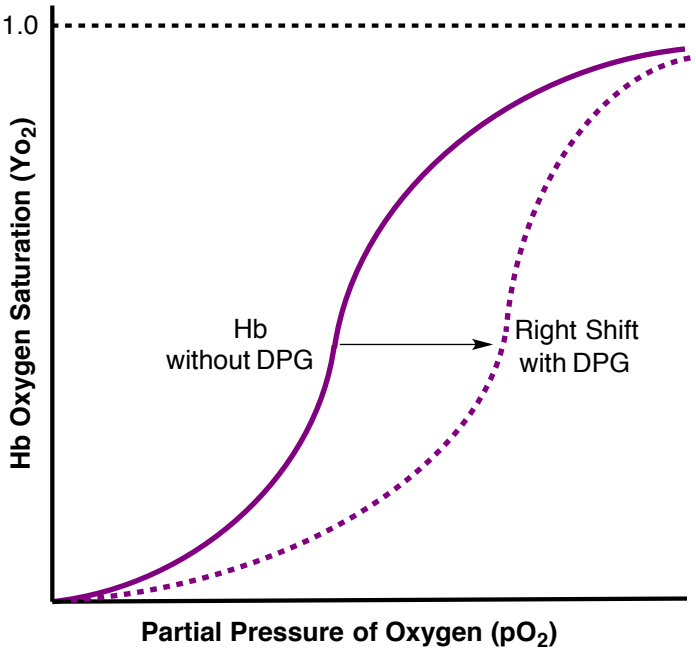


Figure 1.8 The effect of DPG on the ODC.[8]

One might further extrapolate that molecules containing a greater number of phosphates than DPG would induce an even greater effect on the ODC. Benesch and coworkers used three inositol esters, inositol hexaphosphate (IHP) and inositol pentaphosphate (IPP) as well as DPG to establish a positive correlation between the number of polyanions and the

ODC.[12] Unfortunately, measuring these effects *in vivo* is challenging due to the polyanions' inability to cross the phospholipid bilayer.[13] However, Teisseire *et al.* were able to demonstrate that piglets treated with erythrocytes preloaded with IHP had a lower cardiac output and increased oxygen release capacity for the lifespan of the erythrocytes. This indicates that treatment with a natural or synthetic ODC right-shifter (referring to the ability for the compound to shift the ODC towards the right, stabilizing the T state) might be a useful tool for tissue reoxygenation in the case of interrupted or poor blood flow.[13]

In addition to organophosphates, there has been an ongoing search for organic compounds that can bind allosterically to affect an ODC shift. In the search for these compounds, Perutz and coworkers discovered clofibrate (**1**, see **Figure 1.9**, below), an aromatic propionate that shifted the ODC to the right, stabilizing the T state.[3] These and other propionates were crystallized with Hb to determine their mechanism of action. It was found that clofibrate and bezafibrate (**2**) bind to Hb in a 2:1 ratio at a site within the water cavity about 20 Å away from the binding site of DPG.[3,14] L35, an analog of benzafibrate (**3**), was then synthesized and greatly increased the effect on the ODC relative to its progenitor. They were proposed as radiation enhancing compounds for the treatment of hypoxic tumors, as the compounds could help to reoxygenate the tumor mass during radiation therapy. The efficacy of these initial compounds was thwarted by their strong binding affinity to serum albumin,[3] but Efaproxiral (**4**), a compound introduced by Allos Therapeutics, was able to resolve this issue by eliminating the urea linkage present in L35. Although Efaproxiral advanced to phase III clinical trials as a radiation enhancing compound, it was found through meta-analysis that it was likely not efficacious.[3]

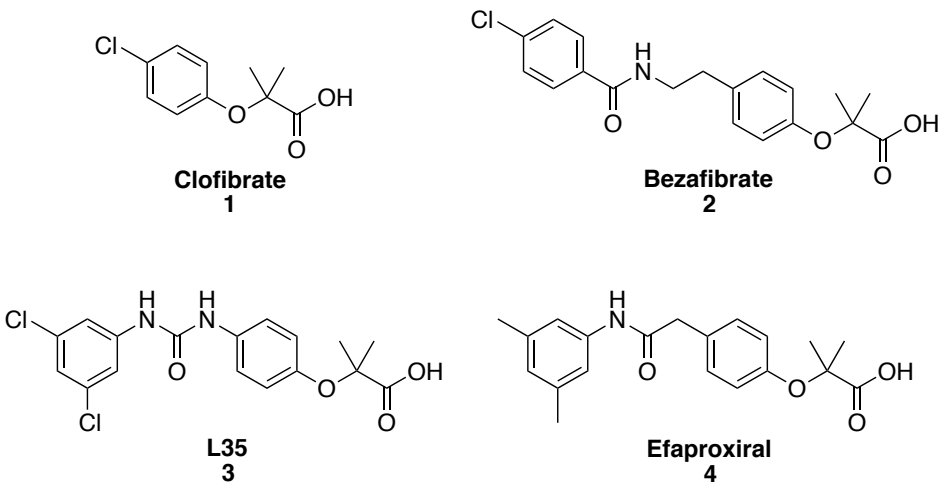
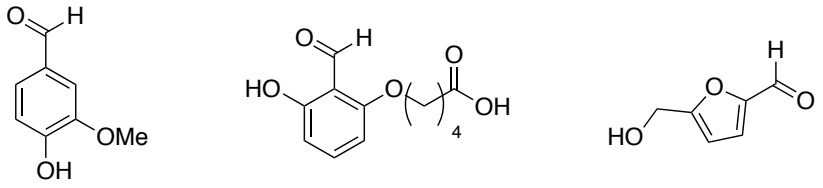


Figure 1.9 Organic right-shifters.

In addition to organic right-shifters, several organic left-shifters have also been discovered. These compounds all contain an electrophilic warhead, normally in the form of an aromatic aldehyde, that acts as a Schiff base, attaching to the α-N-terminal valines.[3,15] Vanillin (**5**, see **Figure 1.10**, below) was the first aldehyde discovered with this ability, followed by Tucaresol (**6**). Tucaresol was the first left-shifter to be crystallized with Hb, confirming the mechanism of action to be Schiff base formation.[3,16] The acidic moiety of Tucaresol was shown to disrupt the αArg141 salt bridges that are crucial for T state stability. Due to their left-shifting ability, these compounds showed promise for the treatment of sickle cell disease. Unfortunately, large doses of these compounds were needed clinically to overcome poor their bioavailability and they were abandoned as potential therapeutics. More recently, 5HMF (**7**) was discovered as an even more potent left-shifter with improved bioavailability. 5HMF is currently undergoing pre-clinical and phase I clinical antisickling studies.[3]



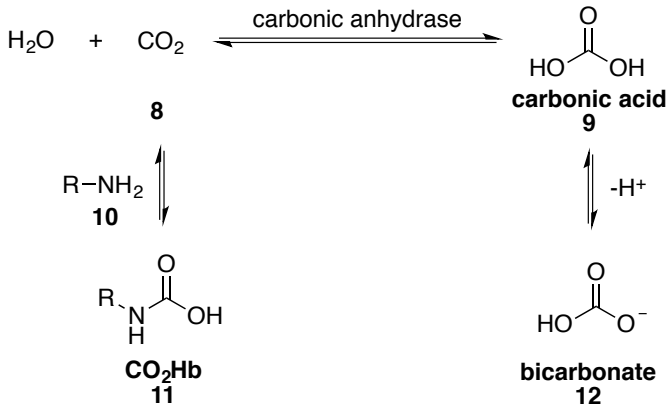
Vanillin
5

Tucaresol
6

5HMF
7

Figure 1.10 Organic left-shifters.

Section 1.3.3 CO_2Hb and the Bohr Effect



8

carbonic acid
9

10

CO_2Hb
11

bicarbonate
12

Scheme 1.1 Formation of CO_2Hb and carbonic acid from CO_2.

As indicated previously, the environment in which Hb exists can dramatically affect its function. Specifically, concentration, ionic strength, pH and allosteric effectors all play a great role in the conformational state of Hb and thus its ability to bind ligands. The binding of carbon dioxide to Hb differs greatly from its mechanism of binding oxygen. In fact, carbon dioxide does not bind to the heme at all, but rather to the α-N-terminal amino groups (**10**) in a process known as carbamoylation.[1,5] In tissue, carbon dioxide (CO_2) is rapidly converted to carbonic acid (**9**) by carbonic anhydrase (see **Scheme 1.1**). This causes the pH of carbon dioxide rich tissue to drop. Hb can be protonated by carbonic acid, which allows it to transport carbon dioxide as bicarbonate ion pairs with Hb.[1,5] In addition, carbon dioxide (**8**) can also undergo nucleophilic attack by N-terminal Val (**10**), leading to the formation of carbaminohemoglobin (CO_2Hb, **11**). These processes are driven significantly by the pH of the system.

To further complicate this issue, pH also has a great effect on the oxygen affinity of Hb, a property first discovered by Christian Bohr, which is named the Bohr effect.[5,17] The oxygen affinity begins to increase at a pH greater than 6 and plateaus at 7.4. This effect is mainly attributed to three residues, in the absence of DPG: Val α001, Lys β082 and His β146, which liberate their protons at high pH.[18] It is hypothesized that the T state has a higher affinity for protons than the R state.[17] Therefore, at physiological pH, protohemoglobin (HHb) is normally in equilibrium with oxyHb. This allows HHb to liberate its proton in the presence of high concentrations of oxygen also liberating carbon dioxide which is trapped as a bicarbonate ion pair. Conversely, in tissues with high concentrations of carbon dioxide and low concentrations of oxygen, the reverse reaction occurs, liberating oxygen from Hb and trapping carbon dioxide as a bicarbonate ion pair. Bicarbonate ion pairs account for 60-70% of all carbon dioxide transport.

SECTION 1.4 CARBON MONOXIDE POISONING

Another molecule known to cause a left shift in the ODC is carbon monoxide, a toxic gas which has a 240 times greater affinity for Hb than oxygen. Carbon monoxide is an odorless, tasteless and colorless gas responsible for 40,000 emergency room visits and 5000 to 6000 deaths per year in the United States.[19–21] While nonsmokers have up to 3 percent carboxyhemoglobin (COHb), smokers may have up to 10 to 15 percent concentration in their blood.[19] Upon binding to the heme, CO induces a conformation similar to the R state, which increases the oxygen affinity of the three remaining heme sites, but will not allow oxygen to be released in low partial pressures.[22] This effect causes a leftward shift in the ODC, making oxygen less available to the tissues (see **Figure 1.11**).

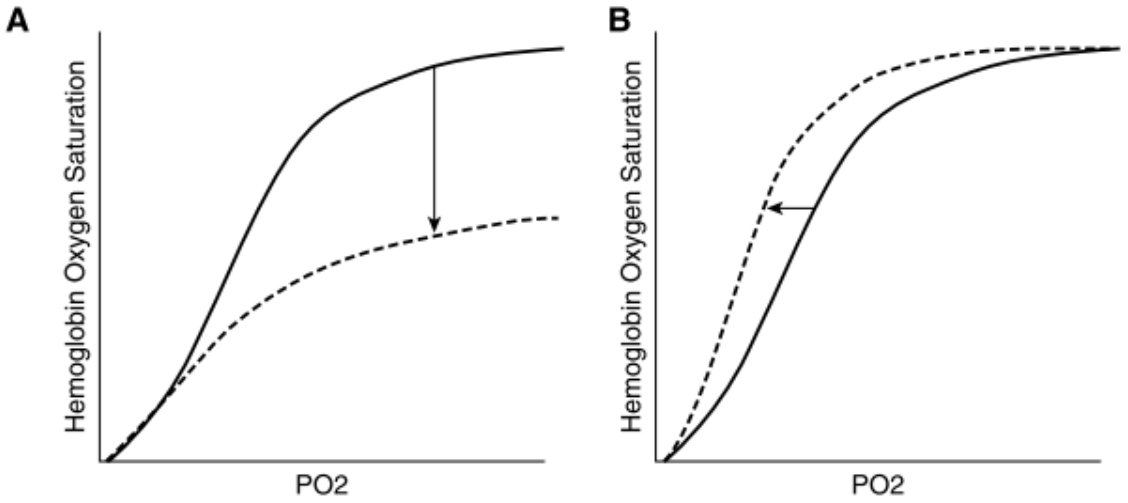


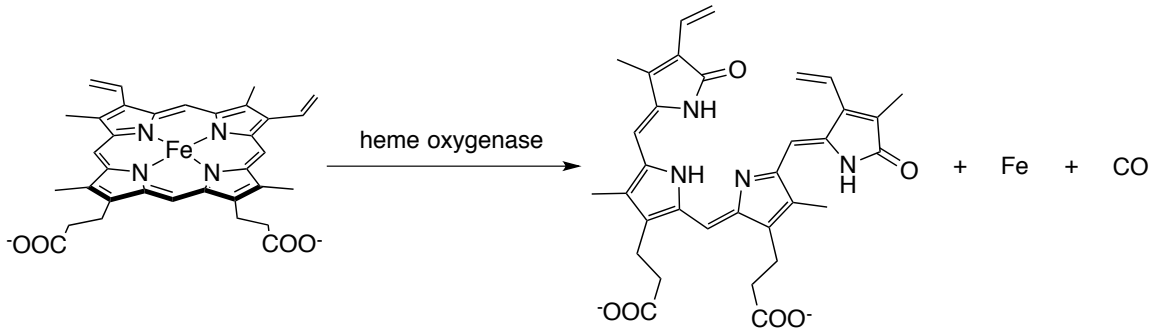
Figure 1.11 A. Carbon monoxide depresses the ODC by reducing the total amount of Hb able to be saturated. B. CO also causes a left-shift of ODC by inducing the R state.[22]

Treatment of carbon monoxide poisoning generally involves the use of either a high-flow oxygen mask or a hyperbaric oxygen chamber.[19] Normally, the half-life ($T_{1/2}$) of COHb in the human body is 300 minutes, but with the use of a high-flow oxygen mask, this can be lowered to 90 minutes, and in a pressurized hyperbaric oxygen chamber, the $T_{1/2}$ is further lowered to 30 minutes. Generally, hyperbaric oxygen is only used when the concentration of COHb in the blood is greater than 25 percent. Only about 1500 patients are treated with hyperbaric oxygen in the US yearly, mainly due to the low availability of these chambers of which there are only about 250 in the United States.

Section 1.4.1 Carbon monoxide as a neurotransmitter

Carbon monoxide is already present in very small concentrations in the body, and plays an important role in cellular function.[23–25] Carbon monoxide is endogenously produced by two means: the recycling of heme by heme oxygenase and the peroxidation of liposomal

lipids. Heme oxygenase produces most of the endogenous carbon monoxide when it reacts with heme (**13**) to release iron, biliverdin (**14**), and a molecule of carbon monoxide (see **Scheme 1.2**). Biliverdin is thought to then block further heme oxygenase activity through feedback inhibition. Secondarily, a lesser source of carbon monoxide has been observed in the brain, kidney, lung, spleen and blood during lipid peroxidation.

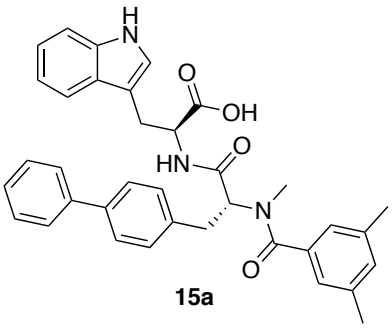


13 **biliverdin 14**

Scheme 1.2 Degradation of heme by heme oxygenase.

Some amount of the endogenous carbon monoxide is exhaled and the rest is stored as carboxyhemoglobin (COHb).[25] This carbon monoxide is thought to inhibit enzymes with Fe-S centers and also upregulate the production of cyclic GMP, through activation of guanylyl cyclase. Endogenous carbon monoxide is also thought to act as a neurotransmitter in conjunction with nitrous oxide.[23,26] While heme oxygenase in peripheral tissue is present in its inducible isoform (HO1), heme oxygenase (HO2) in nerve tissue is constitutively expressed, and it is believed that endogenous CO is necessary for nerve function.[26] Furthermore, mice with a genomic deletion of either HO2, NO2 (the nitrous oxide equivalents to HO2) or both HO2 and NO2, all show decreased function in gastrointestinal neurotransmission. Additionally, loss of function in mouse smooth muscle tissue due to HO2 inhibition can be rescued with exogenous CO.

SECTION 1.5 LEAD COMPOUNDS AND BACKGROUND



15a

Figure 1.12 IRL 2500.

Although CO plays an important role in intercellular function, exposure to large volumes of CO can be deadly, and there are very few current treatment methods available for CO exposure. To address this need, we, in collaboration with Professor Warren Zapol at Massachusetts General Hospital, have designed several small molecules that allosterically inhibit the binding of CO to Hb. Our lead compound, IRL 2500 (**Figure 1.12**, **15a**) lowers the half-life of CO bound Hb (COHb) *in vitro*.[27]

Section 1.5.1 IRL 2500

X-ray crystallographic analysis shows that IRL 2500 and DPG both bind to the β cleft of Hb in a similar position (**Figure 1.13**).[28] Since DPG and IRL 2500 both bind to the β cleft and DPG is known to stabilize deoxy-Hb (dHb),[28,29] we propose that IRL 2500 is

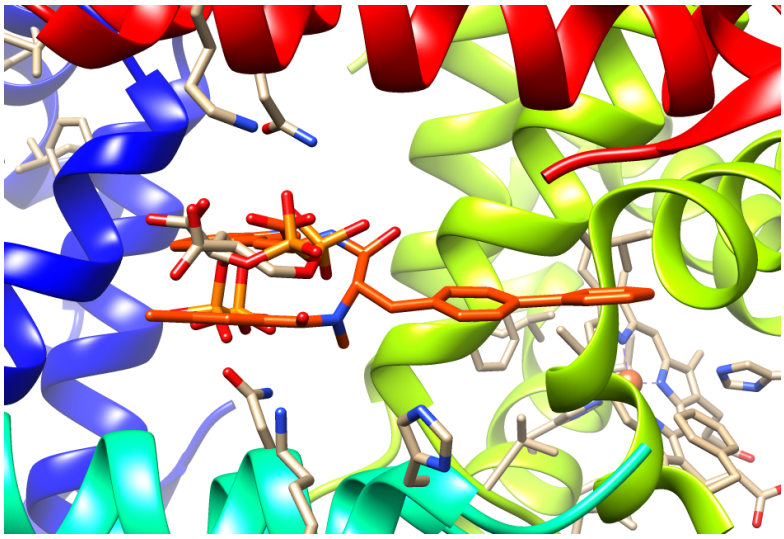
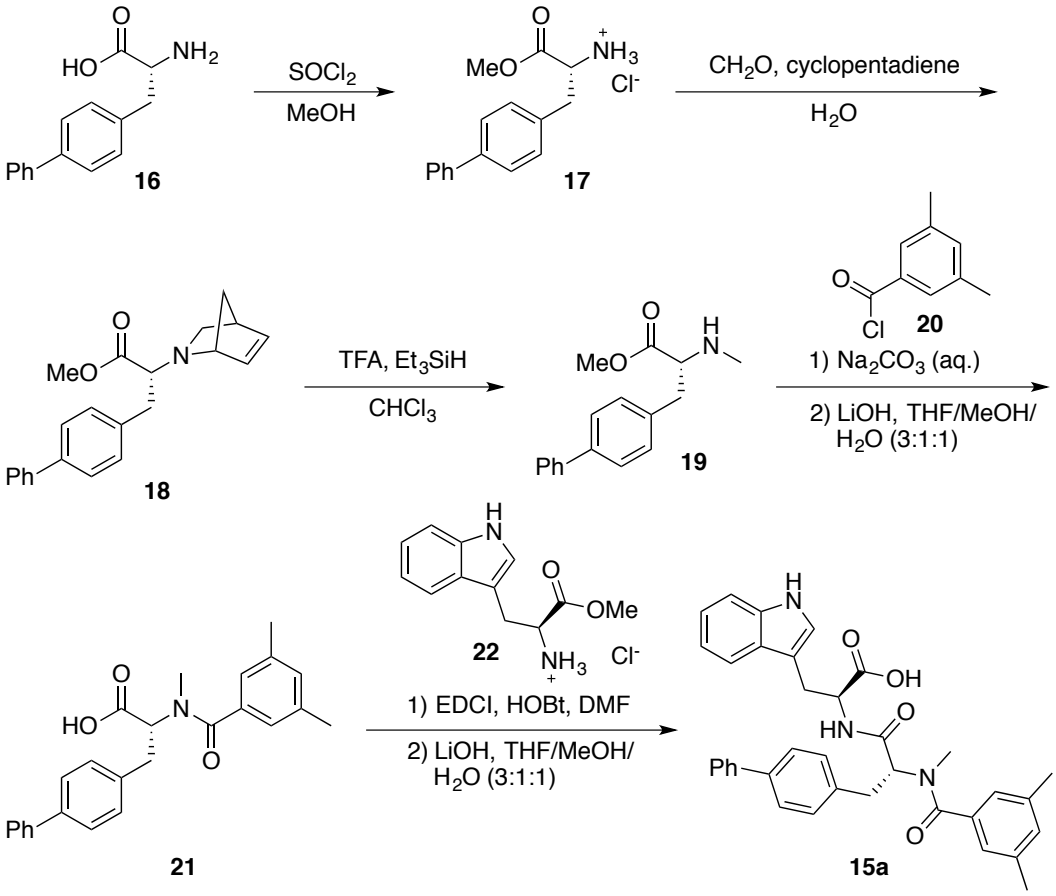


Figure 1.13 IRL 2500 (orange) and DPG (tan) bound in the β cleft.

able to similarly enforce the dHb conformation (T state) and block CO and other ligands from binding to the heme. Unfortunately, IRL 2500 induces hemolysis, making it incompatible with *in vivo* studies.[27]

IRL 2500 (**15a**) was first reported by Fruh *et al.* in 1996 as a selective ET_B endothelin antagonist.[30] Endothelins are a family of vasoactive peptides that play a role in a number of diseases including renal failure, vasospasm, hypertension and asthma. These peptides act on two receptors, ET_A and ET_B and various endothelins show selectivity for one over the other. IRL 2500 was discovered through an SAR screen of the C-terminal dodecapeptide of ET-1 and has a 1 nM affinity for ET_B and a 440 nM affinity for ET_A.

The first synthesis of IRL 2500 was reported in a 1995 patent by Fruh, *et al.* shortly before the publication of their subsequent paper (see **Scheme 1.3**, below).[31] IRL 2500 was constructed in six steps from (*D*)-3-(biphenyl-4-yl)-alanine (**16**). Following esterification of acid **16**, ester **17** is treated with formaldehyde and freshly distilled cyclopentadiene leading to hetero Diels Alder adduct **18** as masking group for the imine. Following isolation, the bicyclic intermediate is treated with trifluoroacetic acid in triethylsilane to induce the retro Diels-Alder and subsequent reduction of the imine to N-methyl ester **19**. Reaction with benzoyl chloride **20** followed by saponification gives acid **21** which is then coupled to *L*-tryptophan methyl ester **22**, followed by a final saponification to give IRL 2500. No yields for the initial synthesis were reported.



Scheme 1.3 First reported synthesis of IRL 2500.

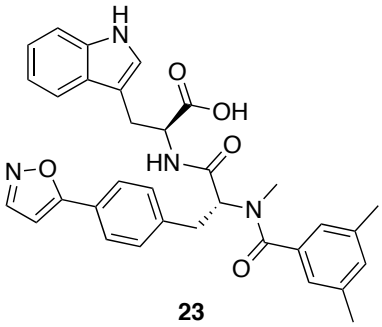
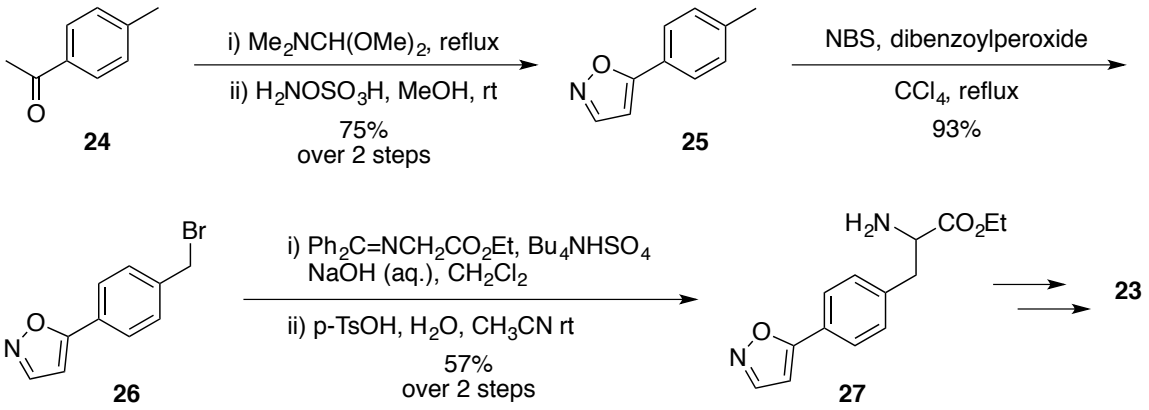


Figure 1.14 IRL 3461, an analog of IRL 2500.

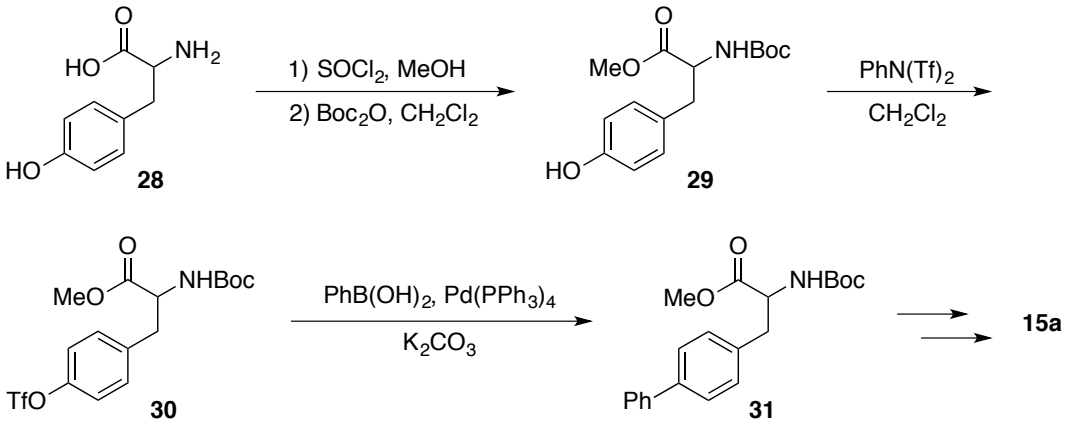
Unfortunately, their initial route did not allow them to readily access a variety of analogs, particularly derivatives of the biphenyl moiety. Therefore, each analog was synthesized individually. This proved a hindrance for the synthesis of analogs for which precursors corresponding to **16** were commercially unavailable. For example, in the synthesis of IRL 3461 (**23**, **Figure 1.14**), the isoxazole amino ester **27** had to be synthesized prior to the commencing the synthesis of the analog.[32] This required beginning with ketone **24**, which was converted to isoxazole **25** in

two steps (see **Scheme 1.4**, below). Subsequent bromination of the benzylic methyl group allowed for installation of the amino ester moiety in two steps. First, bromo isoxazole **26** was reacted with *N*-(diphenylmethylene)glycine ethyl ester. Subsequently, hydrolysis yielded amino ester **27**. Prior to completion of the ten-step synthesis, the diastereomers of isoxazole **23** had to be separated, leading to the effective loss of half of the final compound.



Scheme 1.4 Synthesis of biaryl amino ester towards the synthesis of IRL 3461.

In July of 2015, Zapol and coworkers reported an improved approach to the synthesis of IRL 2500 that allowed for diversification at various stages of the route and used either racemic or enantiopure *D*-tyrosine amino acid starting material (**28**, see **Scheme 1.5**).[33] By converting the phenol of tyrosine to a triflate (**30**), Zapol and coworkers were able to install the biphenyl moiety through a Suzuki-Miyaura cross-coupling. It is important to note, however, that they chose to install the biphenyl early in the synthesis, which makes diversification step-intensive. Furthermore, if tyrosine were to be used as the functional handle for installation of the biphenyl later in the synthetic route, it would be necessary to protect the free phenol, as phenols are often incompatible with coupling conditions.

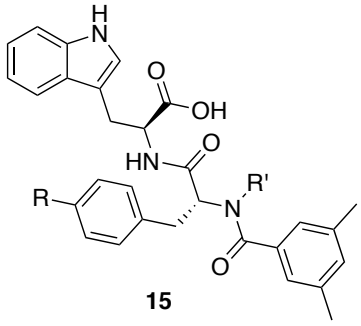


Scheme 1.5 Updated synthesis of IRL 2500.

Section 1.5.2 High-throughput Screening

Due to IRL 2500's propensity to cause hemolysis, our collaborators at Massachusetts General Hospital undertook a screen of structurally similar analogs to find a compound with similar potency but with no hemolytic activity.[27] Shown on the next page are the results of the screen (**Table 1.1**).

Table 1.1 Original HTS of IRL analogs.



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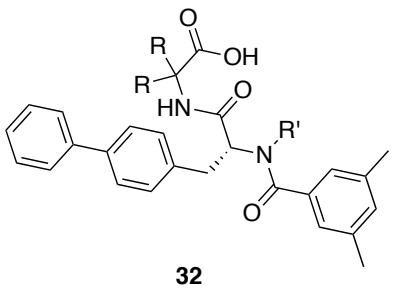

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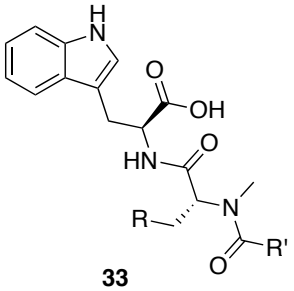

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entry	Compound (2-10x tetramer)	R	R'	$T_{1/2}$ of COHb (min)	Hemolysis
1	DMSO	-	-	19	No
2	**IRL 2500 (15a)**	**Ph**	**Me**	**17**	**Yes (major)**
3	**IRL 1 (15b)**	**H**	**Me**	**17**	**No**
4	**15c**	H	H	Not tested	No
5	**32a**	H	H	16	Yes
6	**32b**	H	Me	17	Yes
7	**32c**	Me	H	16	Yes
8	**33a**	Me	Me	20	No
9	**33b**	Me	3,5-DiMe-C_6H_4	19	No
10	**33c**	Me	4-Ph-Ph	18	No

Through the screen, they discovered IRL 1 (**15b, Table 1.1**), which proved effective in lowering the half-life of CO without inducing hemolysis. We decided to proceed with a more robust SAR screen using **15b** as the framework upon which to design derivatives.

SECTION 1.6 GOALS OF PROJECT

We chose **15b** as the lead compound for our SAR studies because it did not cause hemolysis. We also wished to design an analog that would reduce the COHb half-life at least as much as the original lead compound IRL 2500 (**15a**). Finally, we set out to design a compound that would be significantly more soluble than **15b** in aqueous media. It is important to note that we anticipated problems with the solubility of **15b** analogs, but we believed we could overcome these issues by introduction of solubilizing groups.

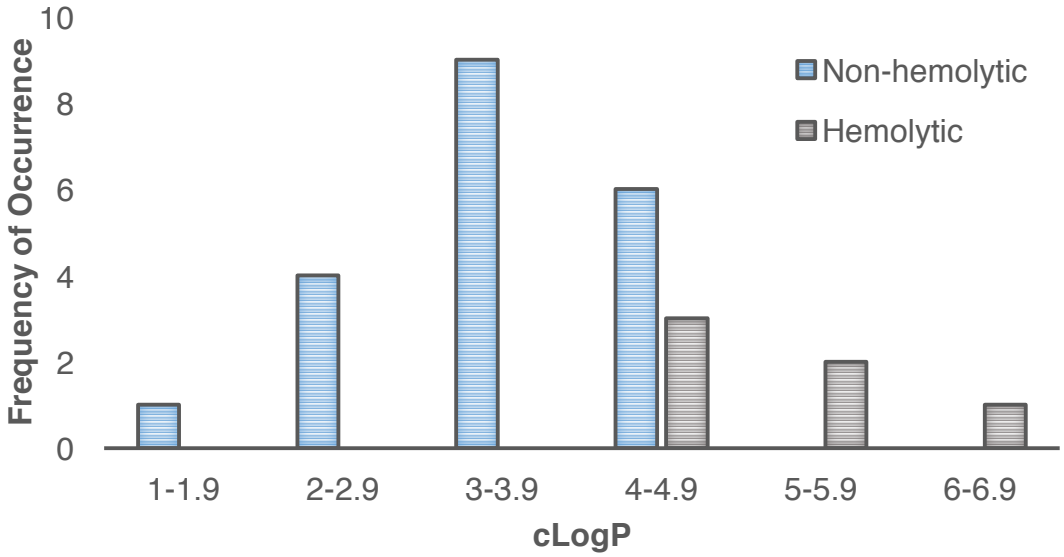
SECTION 1.7 CORRELATION BETWEEN HEMOLYSIS AND CLOGP

In order to appropriately design our analogs, we wanted to develop a simple metric for evaluating solubility. For this, we decided to examine cLogP, the calculated logarithmic value of the partitioning coefficient between octanol and water. cLogP has been popularized for evaluating drug candidates for solubility and cell permeability. Most notably, cLogP, or Log P (in its experimentally determined form), is included as one of Lipinski's "rule of five", where no drug candidate should have a Log P greater than 5. This means that the given compound has no more than 100,000 times greater solubility in octanol than water.[34] Currently, we calculate a Log P (cLogP) using BioByte in Chemdraw®, which gives us a metric for the relative aqueous solubilities of our analogs. If we compare, for instance, IRL 2500 and IRL 1, there is a notable difference between their respective cLogPs of 6.6 and 4.7. This difference tells us that IRL 1 should have significantly greater aqueous solubility (almost 100x) than IRL 2500. We, in fact, observe this experimentally, where IRL 1 can be dissolved at 400 μM in DPBS with 5 volume percent DMSO, whereas IRL 2500 can only be evaluated at 200 μM in DPBS with 5 volume percent DMSO.

While evaluating the cLogPs of the previously screened compounds, we observed a trend in which the compounds with lower cLogP are less likely to cause hemolysis. To quantify this relationship, we did a retrospective study of the previously screened analogs to determine whether cLogP could be used as a predictor of hemolytic activity. We observed a direct correlation between the cLogP the IRL analogs and their hemolytic activity. Summarized in **Table 1.2**, we established a correlation between hemolytic activity and the

cLogP of the ligands, where compounds with a cLogP lower than 4.8 did not cause hemolysis while those higher than 5 caused hemolysis. This metric allows for rational design of candidates going forward that are less likely to affect hemolysis.

Table 1.2 Comparison of cLogP and hemolytic activity.



SECTION 1.8 PROPOSED STUDIES

Starting with IRL 1 (**15b**) as our lead compound, we decided to investigate the SAR of three distinct regions of IRL (see **Figure 1.15**, below): the region derived from *L*-tryptophan (described in **Chapter 2**), the region derived from *D*-phenylalanine (described in **Chapter 3**) and the region derived from 3,5-dimethylbenzoic acid (described in **Chapter 2**). To improve the expediency of synthesis, we imagined that the analogs of each of these regions would come from a common intermediate, which will be described in detail in the following chapters.

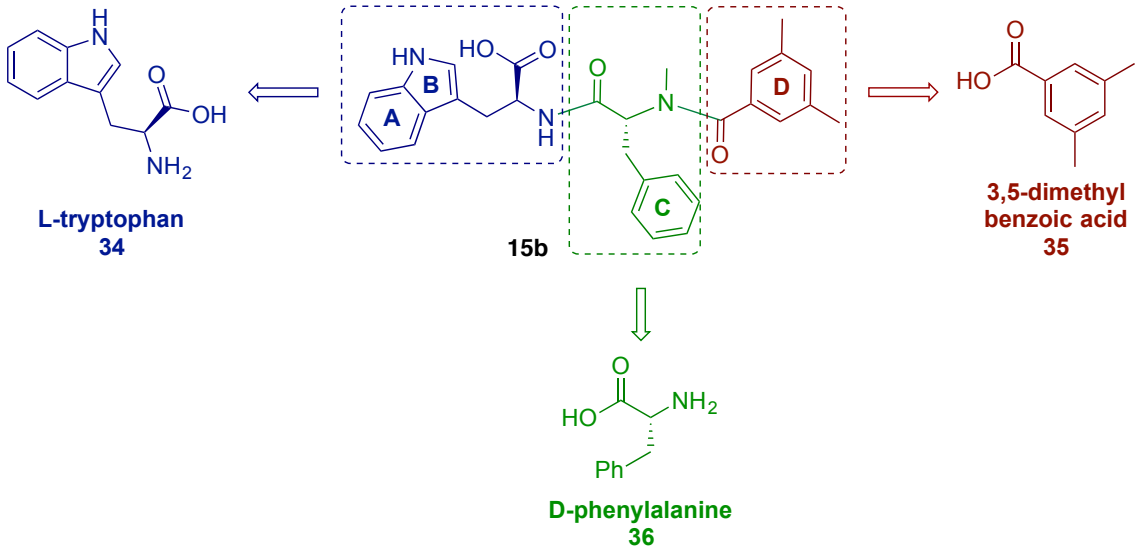


Figure 1.15 Deconstruction of IRL 1 for SAR.

Given our goal of designing a compound with greater CO half-life lowering activity than IRL 1 and with greater aqueous solubility and a lower cLogP than IRL 2500, we decided to prepare compounds that had a greater number of solubilizing functional groups. Additionally, we wished to expand the scope of SAR that had been previously explored. We expected to use the crystal structure for the rational design of analogs that would be

able to pick up on further interactions in the β cleft. Particularly, given the rich cation density in the cleft, and the history of polyanionic allosteric effectors that bind to the cleft, we believed that the design of polyanionic molecules would lead to improved activity and solubility. With these ideas in mind, we set about the synthesis of our first generation of IRL derivatives.

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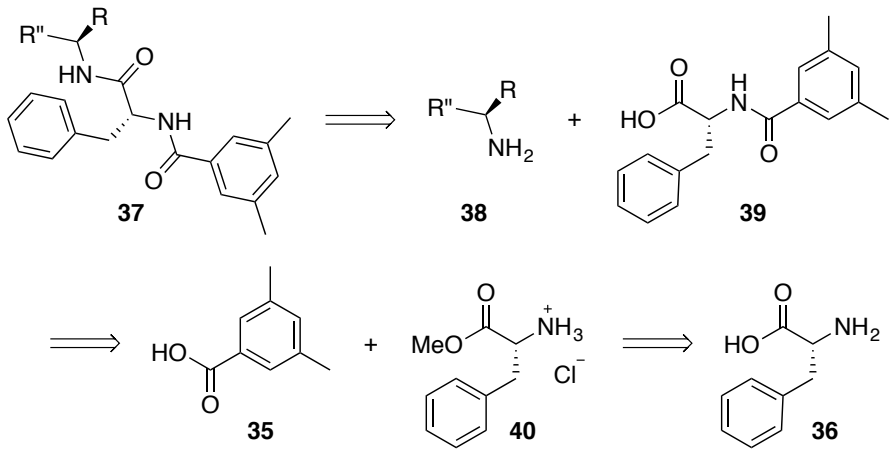
CHAPTER 2 FIRST GENERATION DERIVATIVES

SECTION 2.1 TRYPTOPHAN DERIVATIVES

As described in **Chapter 1**, **Chapter 2** will be divided into two parts to establish the structure activity relationships (SAR) for IRL 1: the *L*-tryptophan (*L*-Trp) derived region and the 3,5-dimethylbenzoic acid-derived region. Retrosynthetically, one could imagine all analogs of *L*-Trp **37** (where R=CO_2H, R"= 3-CH_2-1*H*-indole) arising from an intermediate, acid **39**, which can be synthesized by coupling 3,5-dimethylbenzoic acid (**35**) and *D*-Phe ester **40**, derived from amino acid **36** (see **Scheme 2.1**). The analogs would be accessed by coupling this intermediate with various amines (**38**), to generate *L*-Trp analogs (**37**).



Scheme 2.1 Retrosynthesis of *L*-Trp derivatives.

Section 2.1.1 Phosphorylated analogs

Close investigation of the crystal structure indicates that IRL 2500 is bound in the β cleft in a similar manner to DPG. We therefore hypothesized that a hybridized IRL/DPG, where the charge-bearing region of IRL 1 is replaced with a phosphate, might improve the potency and aqueous solubility. The proposed IRL 1/DPG hybrid is shown below (**41**, see

Figure 2.1, below). We hypothesized that this substitution would provide multiple improvements to IRL 1. Specifically, the polyanionic character of the phosphate would form a stronger interaction with the highly cationic β cleft (where IRL 1 is only able to make a salt bridge with one Lys β082). Additionally, because of its lower pKa,[1] we expected the phosphate to be charged at physiological pH, which would greatly improve solubility. Finally, the phosphate was estimated to have a cLogP of 3.9, which is significantly lower than the IRL 1 value of 4.7. We hypothesized that this decrease in cLogP would improve the aqueous solubility and decrease the likelihood that the analog would cause hemolysis. Additionally, we proposed the synthesis of alcohol **42** as a negative control to confirm the necessity for an anionic charge source in the IRL series. Alcohol **42** is also the precursor to phosphate **41**, giving us access to both analogs in one route.

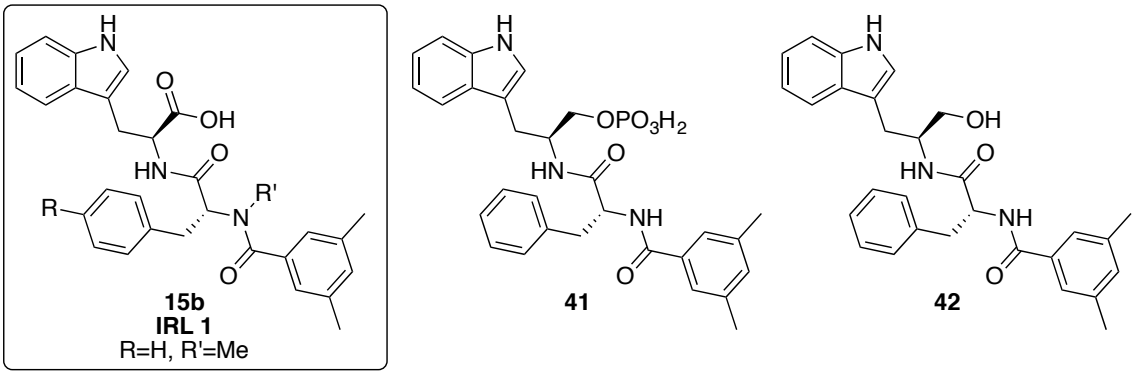
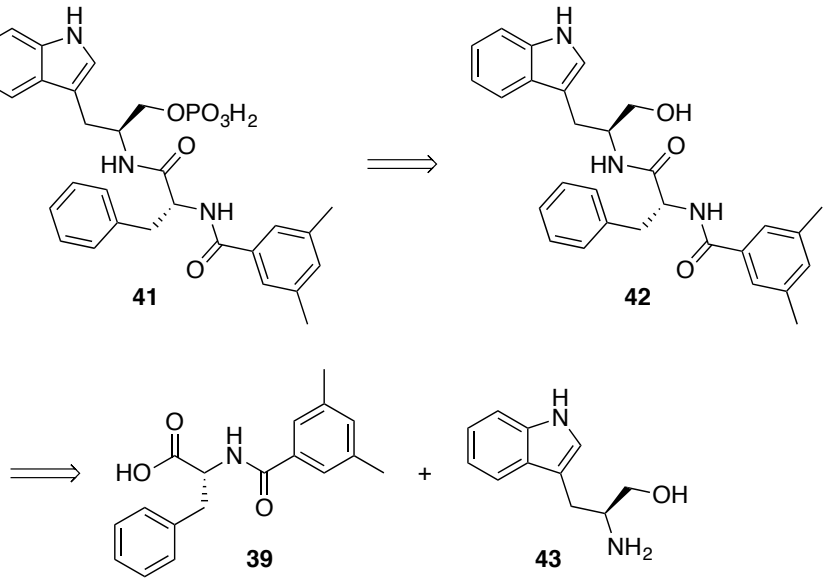


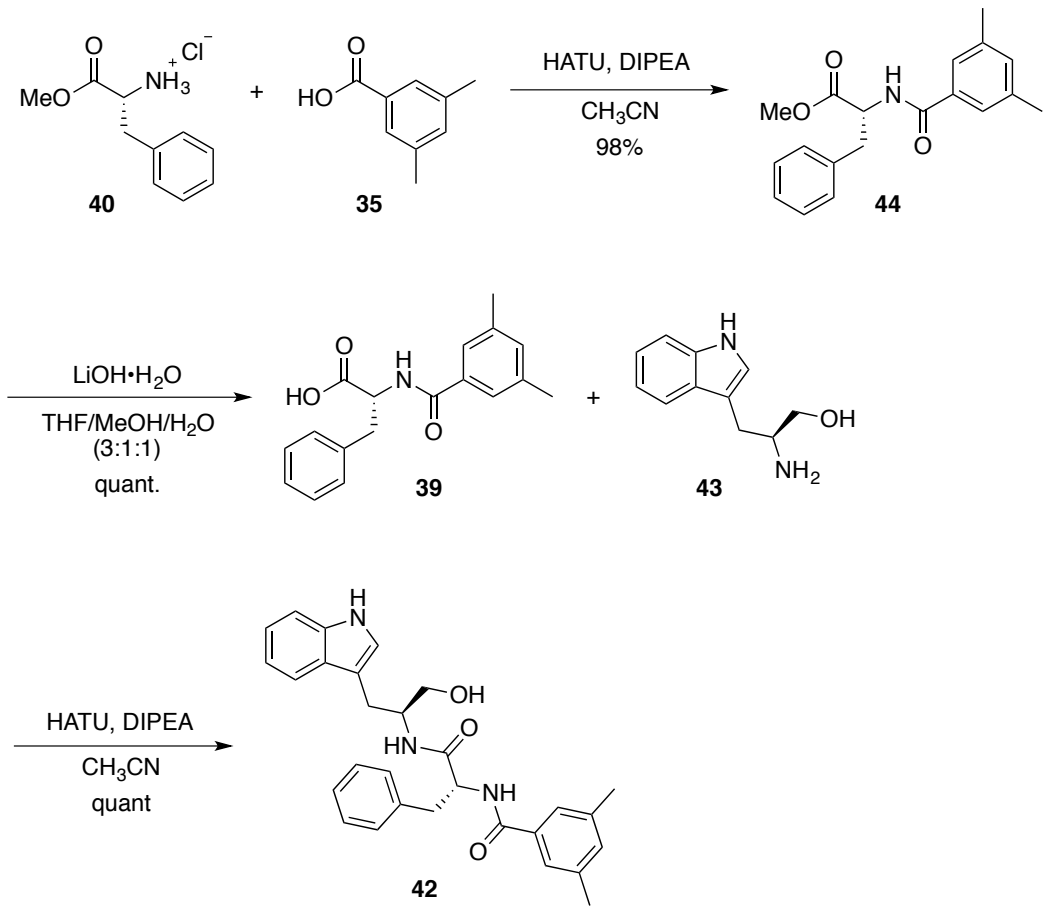
Figure 2.1 IRL/DPG hybrid structure and control.

This alcohol would be derived from the coupling of intermediate **39** with *L*-tryptophanol **43** (see **Scheme 2.2**, below).



Scheme 2.2 Retrosynthesis of phosphate analogs.

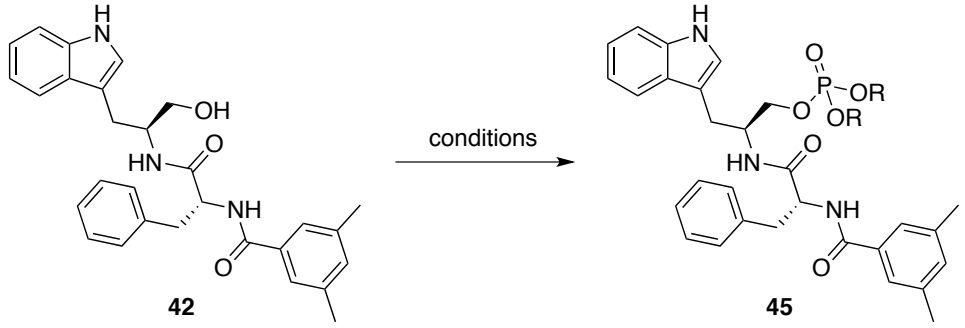
In the forward sense, alcohol **42**, the penultimate compound in the synthesis of phosphate **47** was produced in relatively short order (see **Scheme 2.3**, below). Ester **40**, synthesized in one step from *D*-Phe, was coupled with 3,5-dimethylbenzoic acid (**35**). The resulting ester (**44**) was saponified and coupled with tryptophanol **43,** which was synthesized quantitatively in one step from *L*-Trp using known conditions.[2] The synthetic peptide **42**, the first of the analogs to be screened, was poised for phosphorylation.



Scheme 2.3 Synthesis of alcohol 42, precursor to phosphate 41.

Phosphorylation of alcohol **42** proved difficult. Initial attempts were undertaken with several chlorophosphates, summarized in **Table 2.1** below, with very little success. Screening of different bases and solvents did not improve reaction yields. The most successful phosphorylation conditions involved the use of sodium hydride to deprotonate alcohol **42** followed by treatment with diethylchlorophosphate. However, deprotection of the resulting ethyl phosphate esters proved problematic, and this route was ultimately abandoned.

Table 2.1 Initial phosphorylation conditions.

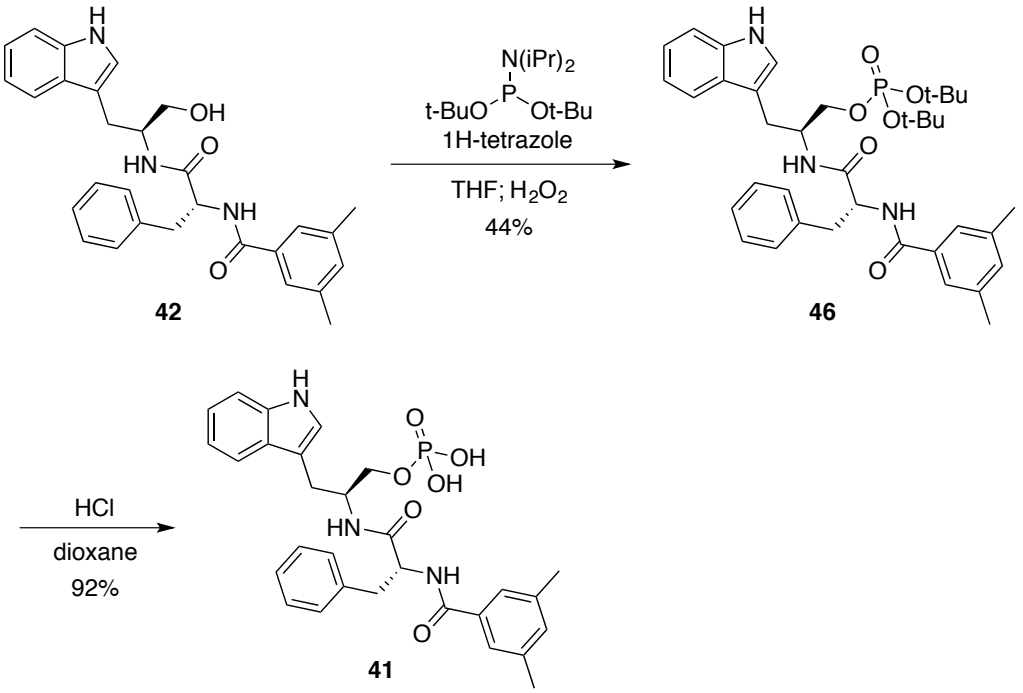


42 **45**

entry	reagent	base	solvent	temp	yield
1	di**ethyl**chlorophosphate	NaH	THF	0 °C - rt	43%
2	di**ethyl**chlorophosphate	Pyridine	Neat	0 °C - rt	N.R.
3	di**benzyl**chlorophosphate	NaH	THF	0 °C - rt	>20%
4	di**benzyl**chlorophosphate	Pyridine	Neat	0 °C - rt	N.R.
5	di**benzyl**chlorophosphite, *N*-chlorosuccinimide	NaH	THF	0 °C - rt	N.R.
6	di**benzyl**chlorophosphate	KHMDS	THF	0 °C - rt	N.R.

Further investigation with a model system showed that the tryptophanol alcohol was incredibly resistant to phosphorylation, particularly with chlorophosphates. In an attempt to improve the reactivity of the electrophile with alcohol **42**, we decided to employ phosphoramidite chemistry, which was originally developed for use in oligonucleotide synthesis in solid phase.[3,4] We had initially avoided the use of phosphoramidites due to the need to oxidize the intermediate phosphite because we believed the oxidation might prove problematic in the presence of the unprotected indole. Fortuitously, after screening conditions, di-tert-butyl-*N*,*N*-diisopropylphosphoramidite proved to be an effective phosphorylating reagent with oxidation proceeding uneventfully and selectively at the phosphine (**Scheme 2.4**). Deprotection of the resultant *tert*-butylphosphate (**46**) occurred

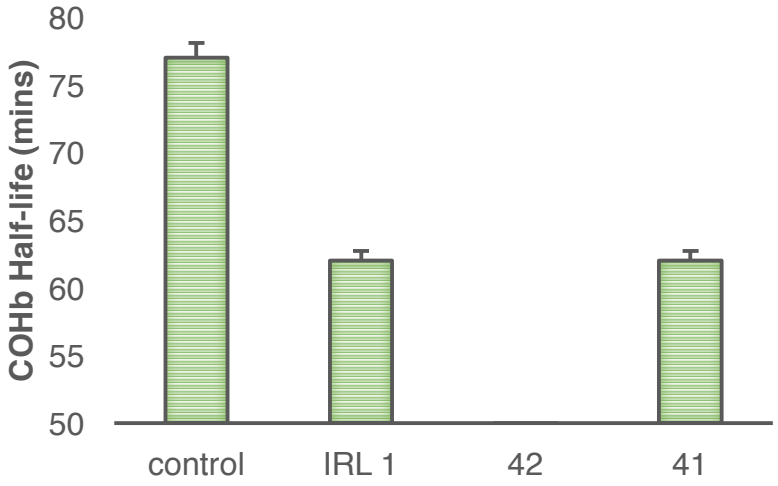
readily and in good yield, generating the final phosphate (**41**), which was submitted for biological testing.



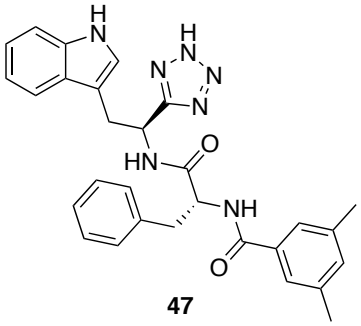
Scheme 2.4 Phosphoramidite phosphorylation.

The results of the biological screen of the COHb half-life of our two initial analogs, phosphate **41** and alcohol **42** are listed below in **Table 2.2**. Unfortunately, the insolubility of alcohol **42** precluded testing. However, we were encouraged to find that compound **41** showed identical activity to IRL 1 in the screen. Both compounds were subjected to a screen for hemolysis and neither of the compounds exhibited hemolytic activity. From these data, we concluded that there was no significant perturbation in activity by converting the carboxylic acid to the phosphate.

Table 2.2 Phosphorylated analog COHb half-life evaluation.



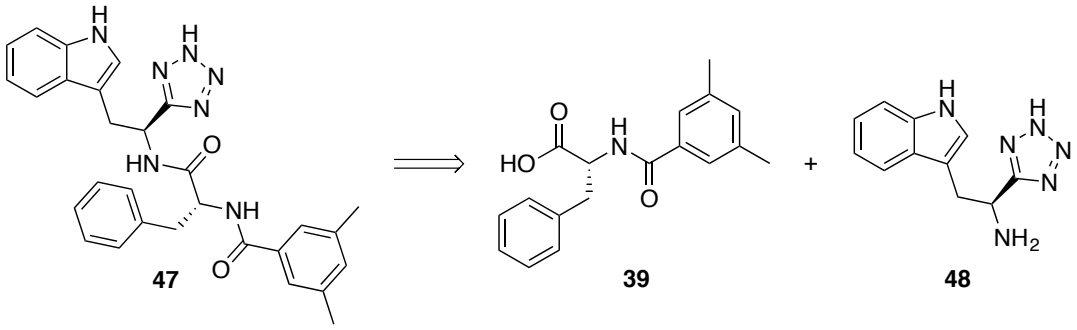
Section 2.1.2 Tetrazole analog



47

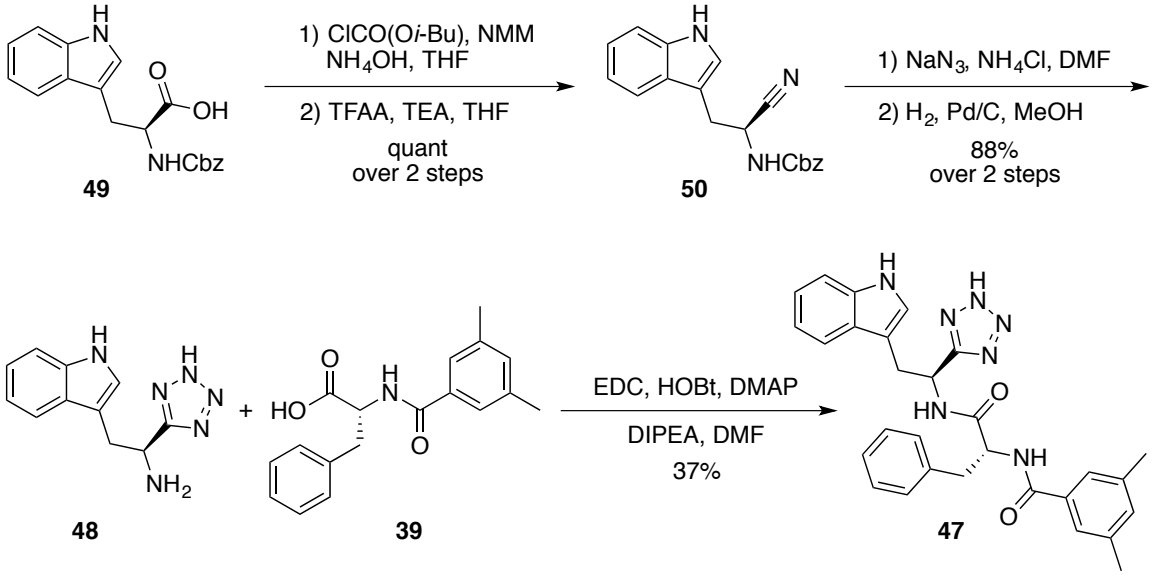
Figure 2.2 Proposed tetrazole analog.

Given our interest in developing analogs with lower cLogPs, we synthesized the IRL 1 analog containing an isosteric tetrazole (see **Figure 2.2**) in place of the carboxylate. Tetrazoles are the among the most commonly used isosteres for carboxylic acid.[5] They are favored among isosteres because they are highly planar, similar to the planar carboxylic acid, and they have a similar pKa to the carboxylic acid (~4.5–4.9). However, one drawback is that they are somewhat larger than the carboxylic acid and tend to have lower membrane permeability.[1] As alluded to above, the tetrazole analog (**47**) has a significantly depressed cLogP of 3.9 relative to IRL 1, which has a cLogP of 4.7. We chose to use **39** to synthesize tetrazole **47**, which could be generated through coupling reaction of **39** with tetrazole **48** derived from *L*-Trp (see **Scheme 2.5**).



Scheme 2.5 Retrosynthetic analysis of tetrazole 47.

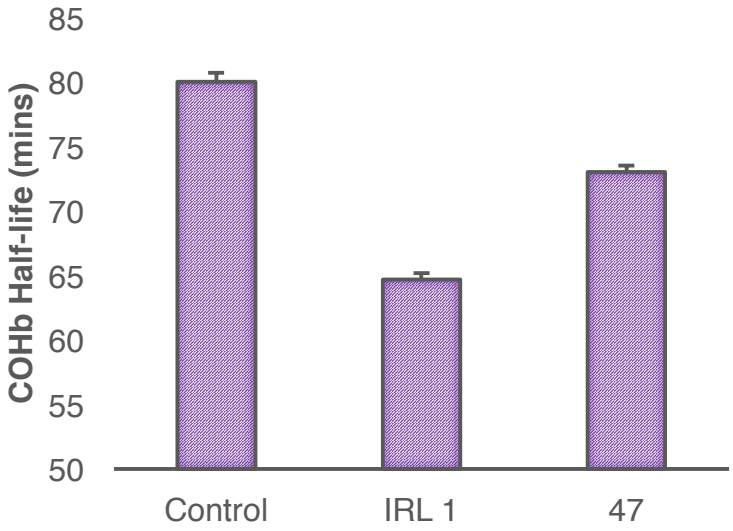
In the forward sense, beginning with *N*-Cbz *L*-tryptophan (**49**, see **Scheme 2.6**, below), we converted the carboxylic acid of **49** to the corresponding primary amide (not shown). This amide intermediate was subsequently dehydrated to generate nitrile **50**. The nitrile was then subjected to cycloaddition conditions with sodium azide to provide the Cbz-protected tetrazole, which upon hydrogenation liberated amino tetrazole **48**. The resulting amino tetrazole was then coupled to **39** using EDC to generate IRL tetrazole **47**.



Scheme 2.6 Synthesis of tetrazole 47.

Biological results for COHb half-life lowering activity indicated that the presence of the tetrazole led to significantly reduced activity relative to IRL 1 (see **Table 2.3**, below). This suggests that in the case of the IRL analogs, the tetrazole is not a good isostere for the carboxylate. The difference in activity might be due to the increased size of the tetrazole making it more difficult to bind to the β cleft, or the increased hydrophilicity of the tetrazole may make it less able to bind to the hydrophobic β cleft.

Table 2.3 COHb half-life lowering activity for tetrazole 53.



Section 2.1.3 A/B ring analogs

To further explore the SAR of IRL 1, we wished to determine whether the indole (A/B ring) was necessary for potency (**Figure 2.3**). Changing the A/B ring system would allow us to understand the role of the indole in binding, as a function of aromaticity and hydrogen bond (H-bond) donation. Thus,

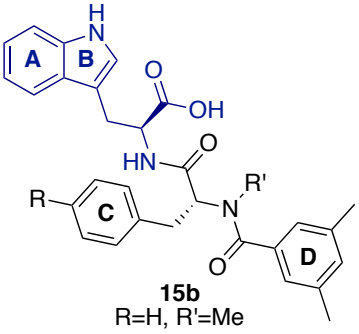


Figure 2.3 IRL 1 with rings labeled.

we chose to explore three analogs: a *des*-indole analog (**51a**, see **Figure 2.4**), a *des*-pyrrole analog (**51b**) and an imidazole analog (**51c**).

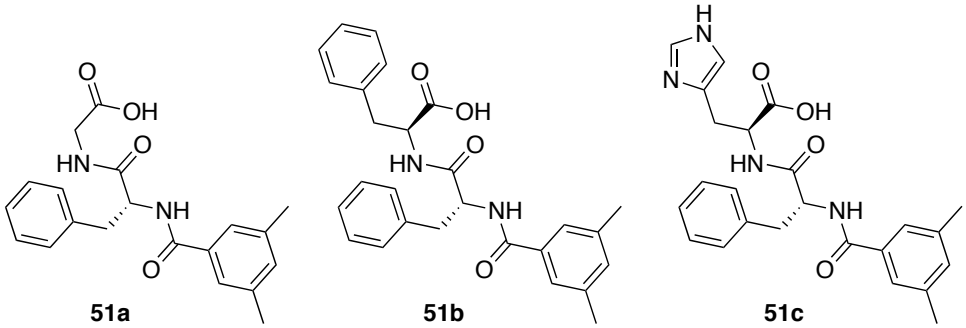
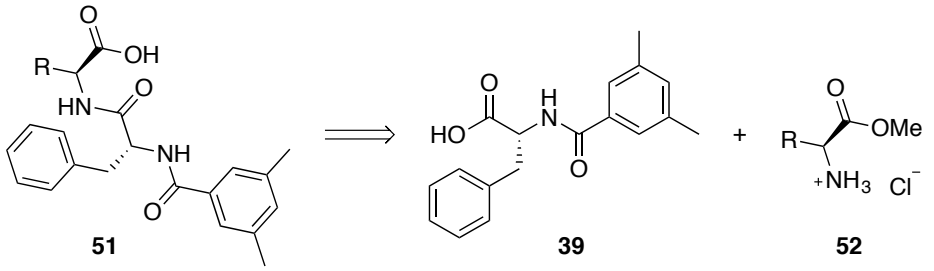


Figure 2.4 Proposed A/B ring analogs.

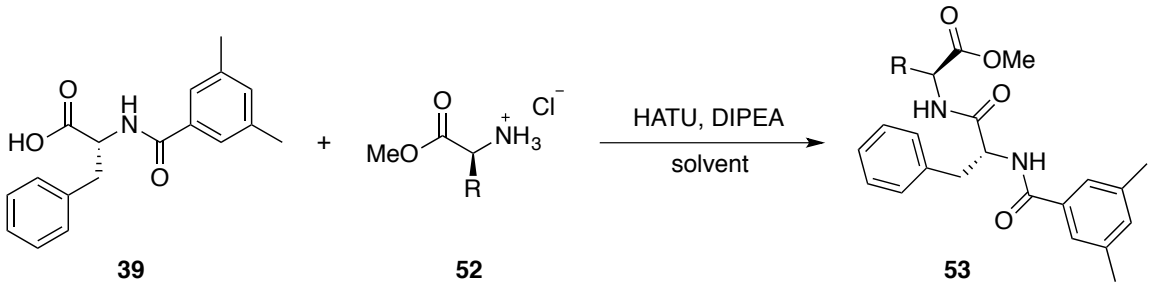
To synthesize the proposed analogs, we planned to couple various amino esters **52** with the same intermediate **39** that was used in the synthesis of phosphate **41** and tetrazole **47** (see **Scheme 2.7**).



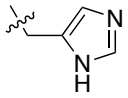
Scheme 2.7 Retrosynthesis of A/B ring analogs using intermediate 39.

In the forward sense, the synthesis of **51a**, **b** and **c** was achieved, readily generating two analogs for testing. First, various amino esters **52** were coupled with intermediate **39** (see **Table 2.4**, below).

Table 2.4 Synthesis of A/B ring esters.

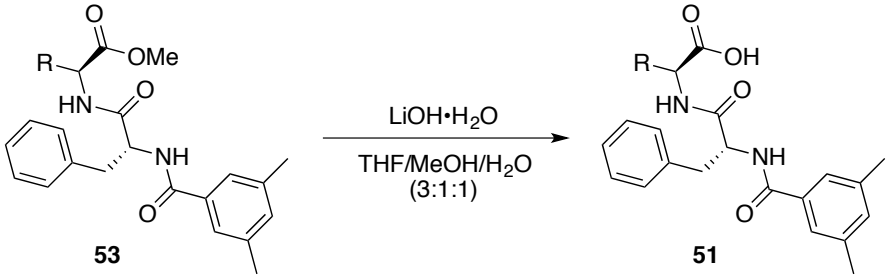


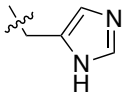
39 **52** **53**

entry	R	solvent	52	53	yield (%)
1	H	CH_3CN	**52a**	**53a**	61
2	Bn	DMF	**52b**	**53b**	69
3		DMF	**52c**	**53c**	71

The resulting analogs **53a**, **b** and **c** were then saponified to their corresponding acids **51a**, **b** and **c** (**Table 2.5**). Unfortunately, while the coupling of acid **39** to *L*-His methyl ester **52c** proved fruitful, attempts at saponification were unsuccessful, possibly due to the formation of a zwitterion resulting from deprotection of the acid. We plan further exploration of this analog in the future.

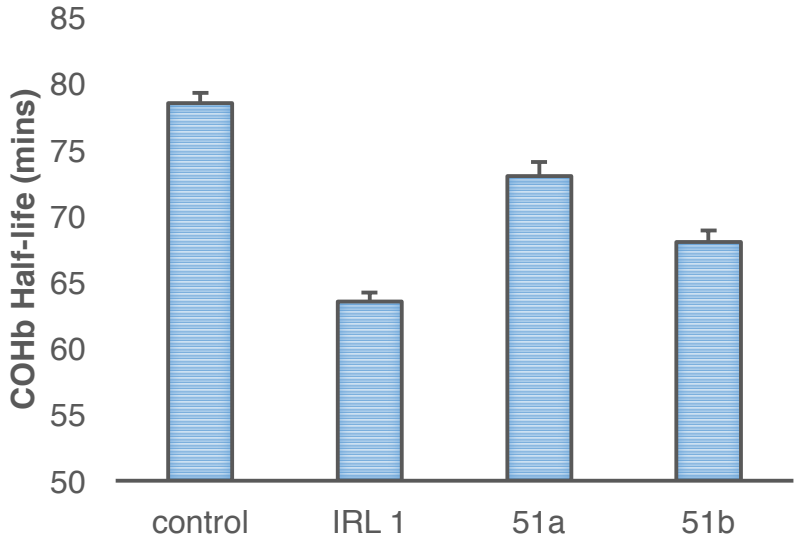
Table 2.5 Hydrolysis of A/B ring esters.



entry	R	53	51	yield (%)
1	H	**53a**	**51a**	100
2	Bn	**53b**	**51b**	100
3		**53c**	**51c**	0

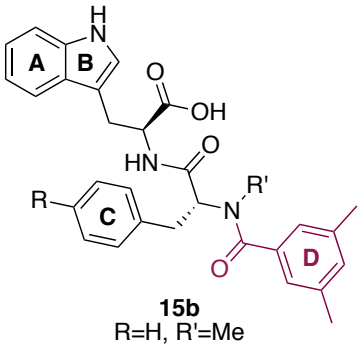
This second series of analogs was evaluated for activity in the COHb half-life assay as well as in a hemolysis screen (see **Table 2.6**, below). We found that both analogs had longer COHb half-lives relative to IRL 1 but that neither caused hemolysis. Eliminating the indole ring completely (**51a**) significantly increased the half-life, indicating the importance of a substituent in that position. Furthermore, simple replacement of the indole ring with a phenyl ring (**51b**) did slightly decrease the activity. This allows us to conclude that not only is it necessary to have an aromatic substituent in this position, but it is also important to have the pyrrole ring of the indole. It is possible that the indole is making some H-bond interactions or helping to improve aqueous solubility. To further investigate this, we would like to continue to pursue the synthesis of imidazole **51c**, while also exploring the replacement of the *L*-Trp moiety with other amino acids, such as *L*-tyrosine, *L*-lysine and *L*-alanine.

Table 2.6 Evaluation of COHb half-life of A/B ring analogs



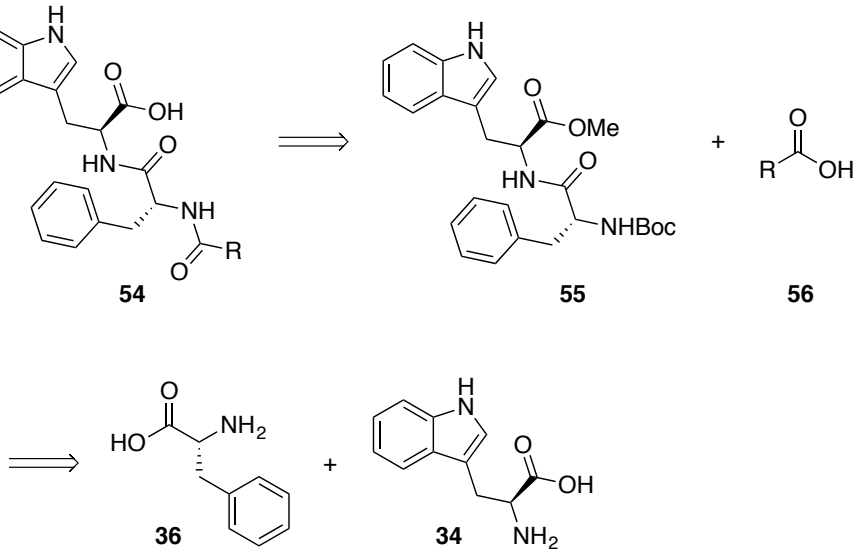
SECTION 2.2 BENZOYL ANALOGS

The most readily modified region of IRL 1 is arguably the D ring (see **Figure 2.5**), where one could imagine readily substituting other benzoyl groups for the 3,5-dimethyl benzoyl moiety. We chose to explore different analogs of the D ring, beginning with the introduction of H-bond donors and acceptors, to tease out any further SAR that could be exploited for the improvement of our analogs. Retrosynthetically, we planned to introduce variations to the D ring using Boc-protected amino ester **55** as an intermediate (see **Scheme 2.8**, below), which would be derived from *L*-Trp (**34**) and *D*-Phe (**36**).



15b
R=H, R'=Me

Figure 2.5 IRL 1 with highlighted D ring.



Scheme 2.8 Retrosynthetic analysis of D ring analogs.

Section 2.2.1 Hydrogen bond donors and acceptors

There are several potential H-bonding partners surrounding the binding site of IRL 1/2500 that we hoped to recognize with suitably substituted benzoyl analogs **54**. Specifically, there are several histidines (His β143 and His β002) in proximity of the D ring with which suitably substituted analogs could interact (see **Figure 2.6**, below).

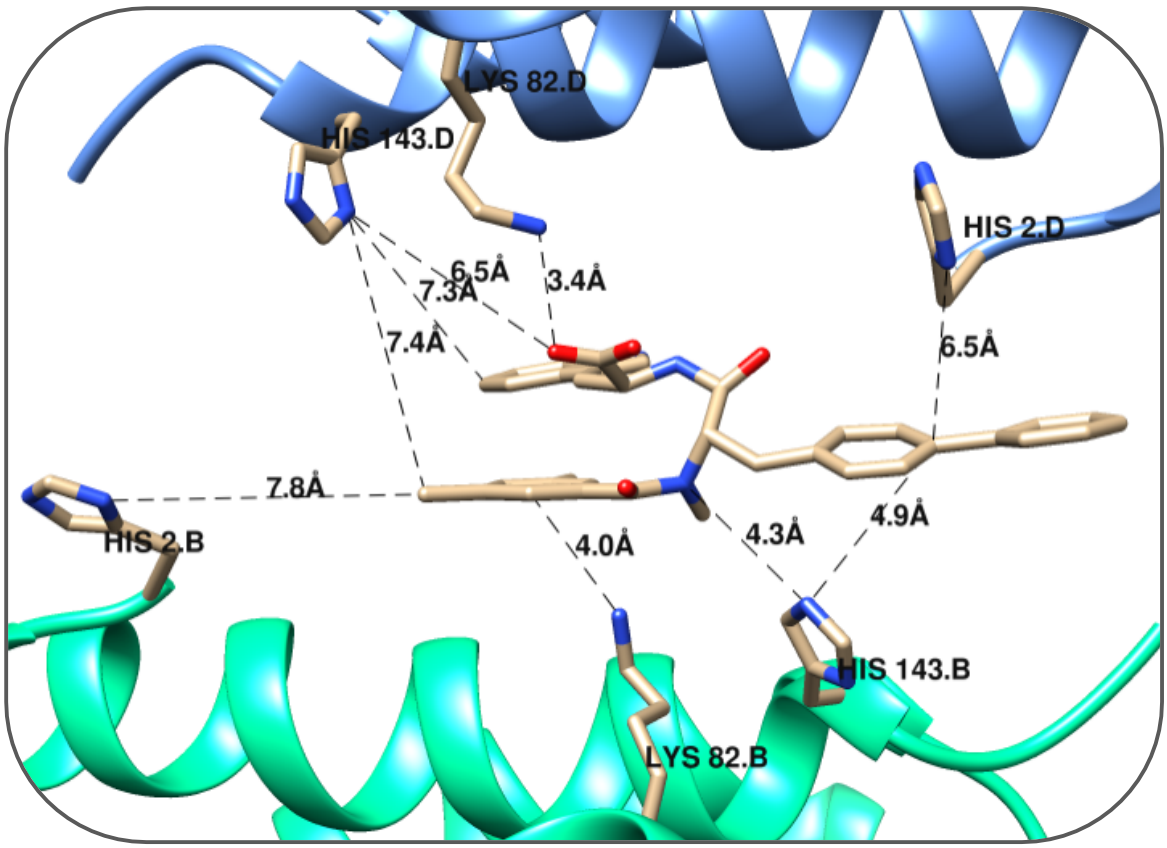


Figure 2.6 IRL 2500 in β cleft surrounded by labeled cationic residues.

Additionally, increasing the number of heteroatoms in IRL would likely increase the aqueous solubility and decrease the cLogP. Further, we wished to explore the removal of the methyl substituents on the D ring, which we expected would improve solubility and lead to decreased cLogP values. Finally, we wanted to explore the functional group tolerance of the IRL D ring. Due to the ready availability of substituted benzoic acid precursors, we chose to look at a series of pyridyl D rings, including picolinyl peptide **54c**, nicotinyl peptide **54d** and isonicotinyl peptide **54e**, for our H-bond acceptors (see **Figure 2.7**, below). For H-bond donors/acceptors, we chose to look at a series of phenolic D rings, specifically salicyl peptide **54f** and 3 and 4-hydroxy benzoyl peptides (**54g** and **54h**, respectively). In addition to these derivatives' ability to make H-bonds, they allow us to

explore the effect of both electron donating and electron withdrawing groups on this ring. Additionally, we decided to look at the 3,5-dimethylbenzoyl derivative (**54b**) lacking IRL 1's *N*-methyl group to determine whether this perturbation would affect activity. We also decided to explore the bare benzoyl ring (**54a**).

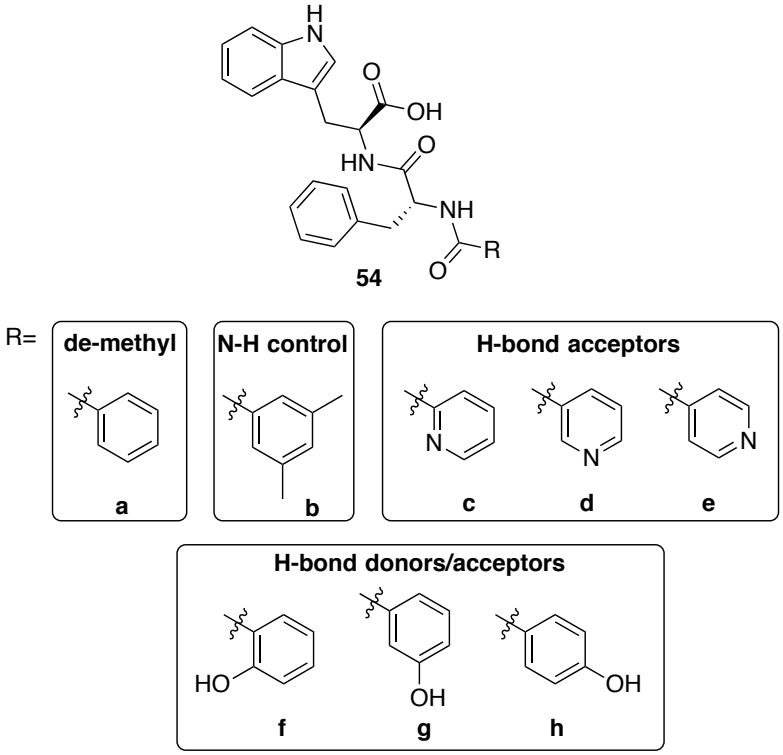
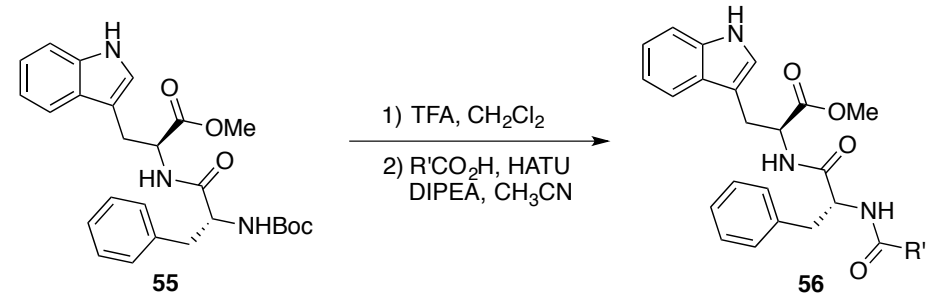


Figure 2.7 Initially proposed D ring analogs.

Synthesis of dipeptide **55** proceeded quantitatively in three steps from the starting amino acids (see **Table 2.7**, below). The Boc-group of intermediate **55** was then deprotected and the resulting amine was coupled with the corresponding benzoic acids. While the benzoyl and picolinoyl analogs (**56a-e**) were obtained in good yield, the phenolic acids were less efficient partners in the coupling reaction, specifically for the 2- and 4-hydroxybenzoyl analogs.

Table 2.7 Coupling of dipeptide 61 with benzoic acids.



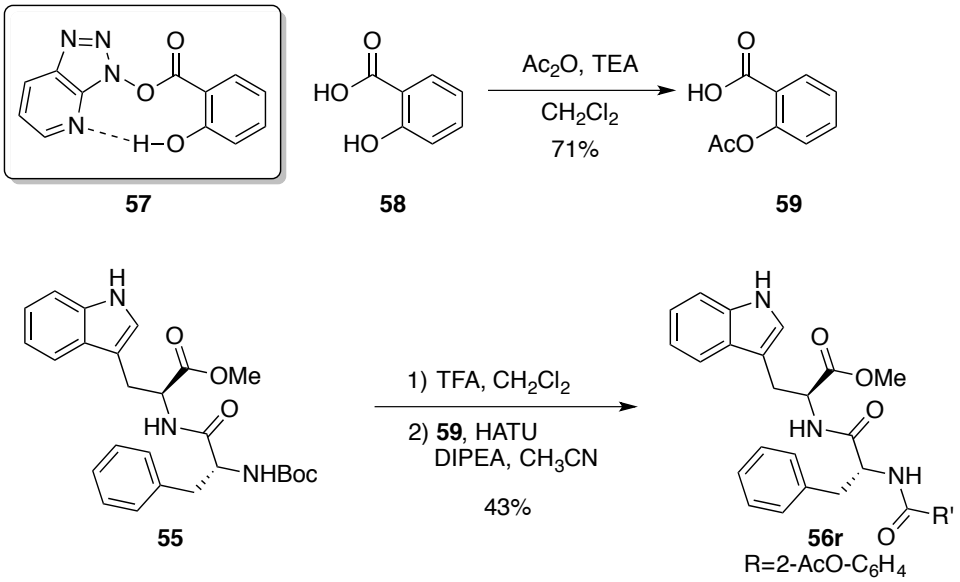
55

3 steps from ***L*-Trp** and ***D*-Phe**
quant

56

entry	R'	56	yield (%)
1	Ph	**56a**	82
2	3,5-di-Me-C_6H_3	**56b**	82
3	2-pyridyl	**56c**	100
4	3-pyridyl	**56d**	100
5	4-pyridyl	**56e**	100
6	2-HO-C_6H_4	**56f**	0
7	3-HO-C_6H_4	**56g**	86
8	4-HO-C_6H_4	**56h**	30

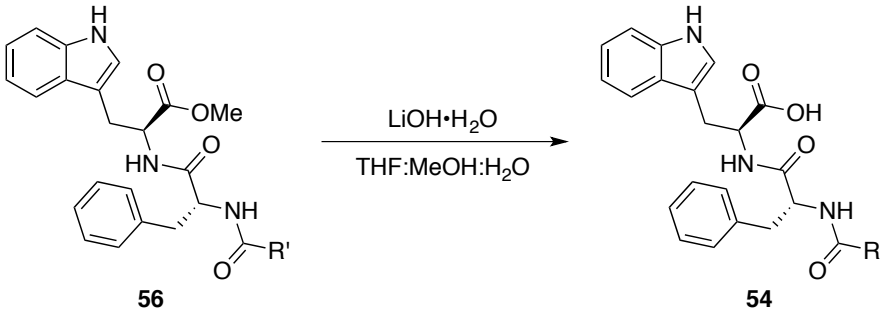
We hypothesize that this is due to the lower electrophilicity of the phenol due to its ability to donate electron density into the ring (see **Scheme 2.9**, below). We also believe that the coupling of salicylic acid may have also been problematic because of the phenol's ability to coordinate with the HATU intermediates and hinder reactivity (see **57**). To resolve this, we decided to protect the salicyl phenol (**58**) as an acetate group prior to coupling (**59**), which allowed us to generate acetate **56r** in modest yield.



Scheme 2.9 Synthesis of modified salicylic acid 56r.

Saponification of the methyl esters proceeded smoothly for all analogs (see **Table 2.8**, below).

Table 2.8 Saponification of D ring derivatives.



56 54

entry	R'	R	56	54	yield (%)
1	Ph	Ph	**56a**	**54a**	90
2	3,5-di-Me-C_6H_3	3,5-di-Me-C_6H_3	**56b**	**54b**	81
3	2-pyridyl	2-pyridyl	**56c**	**54c**	92
4	3-pyridyl	3-pyridyl	**56d**	**54d**	100
5	4-pyridyl	4-pyridyl	**56e**	**54e**	68
6	2-AcO-C_6H_4	2-HO-C_6H_4	**56r**	**54f**	80
7	3-HO-C_6H_4	3-HO-C_6H_4	**56g**	**54g**	100
8	4-HO-C_6H_4	4-HO-C_6H_4	**56h**	**54h**	96

The COHb half-life activity proved very interesting for this series. Specifically, **54b** was equipotent with IRL 1, which established that the *N*-methyl group is unnecessary for activity (see **Table 2.9**, below). As a result, the analogs were all prepared as the secondary amides. The next most potent analog was **54f**. We noticed an interesting trend among the H-bond donors and acceptors where the most potent of both the pyridyl (**54c-54e**) and phenolic series

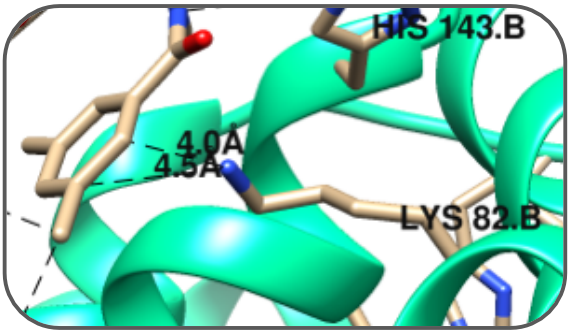
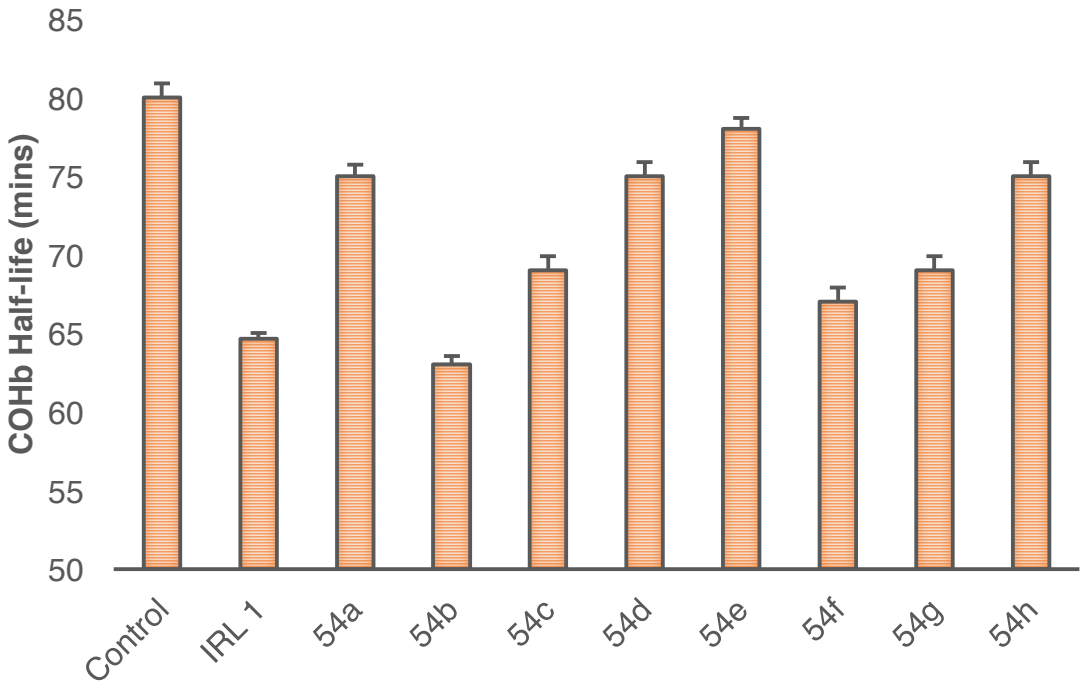


Figure 2.8 Close up of D ring contacts with Lys β082.

(**54f-54h**) had substitution in the 2-position (**54c** and **54f**, respectively). The next most potent in this series is the analog with substitution in the 3-position (**54d** and **54g**, respectively) and the least potent analog has substitution at the 4-position (**54e** and **54h**, respectively). We hypothesized that this might be occurring due to a H-bond between the substituents at the 2- and 3-position and Lys β082, which have distances of 4 (shown in **Figure 2.9**) and 4.5 Å, respectively. The difference between the activity of the analogs with substitution at the 2-position and the 3-position, respectively, might be due to the increasing distance from the Lys as the substituent moves from the 2- to the 3- to the 4-position (see **Figure 2.8**, above). Finally, it is interesting to note that the bare benzoyl analog **54a** showed significantly decreased activity relative to IRL 1, likely due to the reduced hydrophobicity of this analog.

Table 2.9 Results of biological screen of initial D ring analogs



Section 2.2.2 Polyacid analogs

Based on the results of our previous biological study, indicating possible H-bonding with a Lys residue in the β cleft, we designed derivatives of IRL 1 that would allow us to further exploit these proposed interactions. As discussed in **Section 2.2.1**, there are several cationic residues in the β cleft surrounding the binding site of DPG and IRL 2500 (see **Figure 2.10**) and some are in close proximity to IRL 2500 (see **Figure 2.9,** above). Using the crystal structure, we designed several carboxylic acid containing analogs that would allow us to pick up interactions with these residues. Specifically, His β002 and His β143 in reasonable proximity to (7.4-7.8 Å) the D ring.

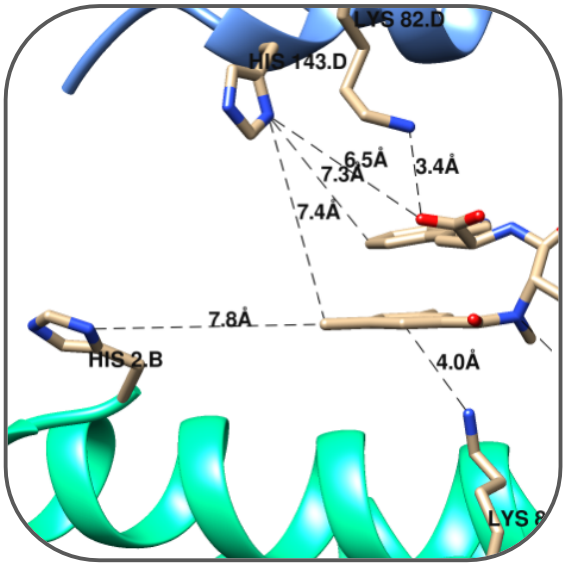


Figure 2.9 Close up of D ring contacts with β His002 and β His143.

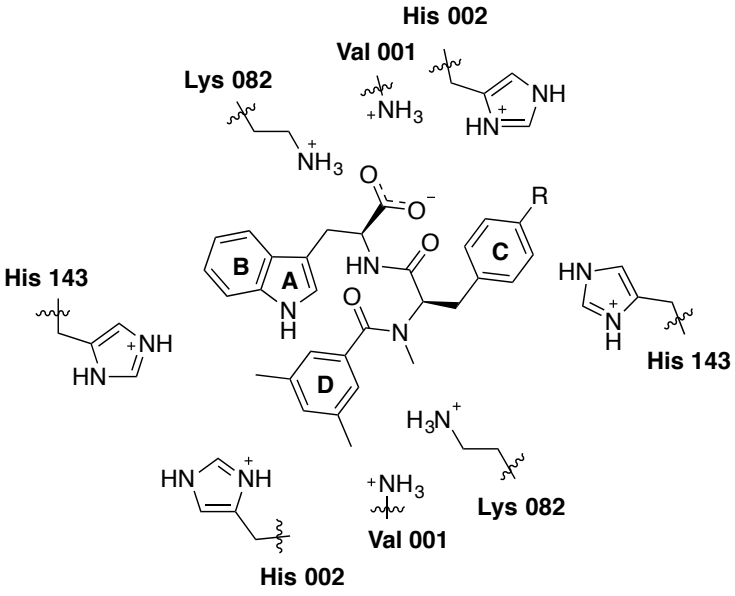


Figure 2.10 Pictorial representation of IRL in binding site.

To target these residues, we chose to synthesize carboxylic acids with varying distances from the D ring (see **Figure 2.11**, below). However, based on length and position, we expected that the derivatives **54i** and **54k** to be most successful. This is because the 2-position of the D ring appeared most poised to reach the His β002, with a 7.4 Å distance from the methyl group of IRL 2500 (see **Figure 2.9**, previous page and **Figure 2.10**, above). Examining analogs with carboxylic acids either directly attached to the D ring or alkylated to a phenol also allowed us to further explore the effect of electron donating and withdrawing substituents on the ring. We also decided to explore an analog of the IRL C ring (**60**), which also is closely positioned to two His residues, as illustrated in **Figure 2.9** and **2.10**.

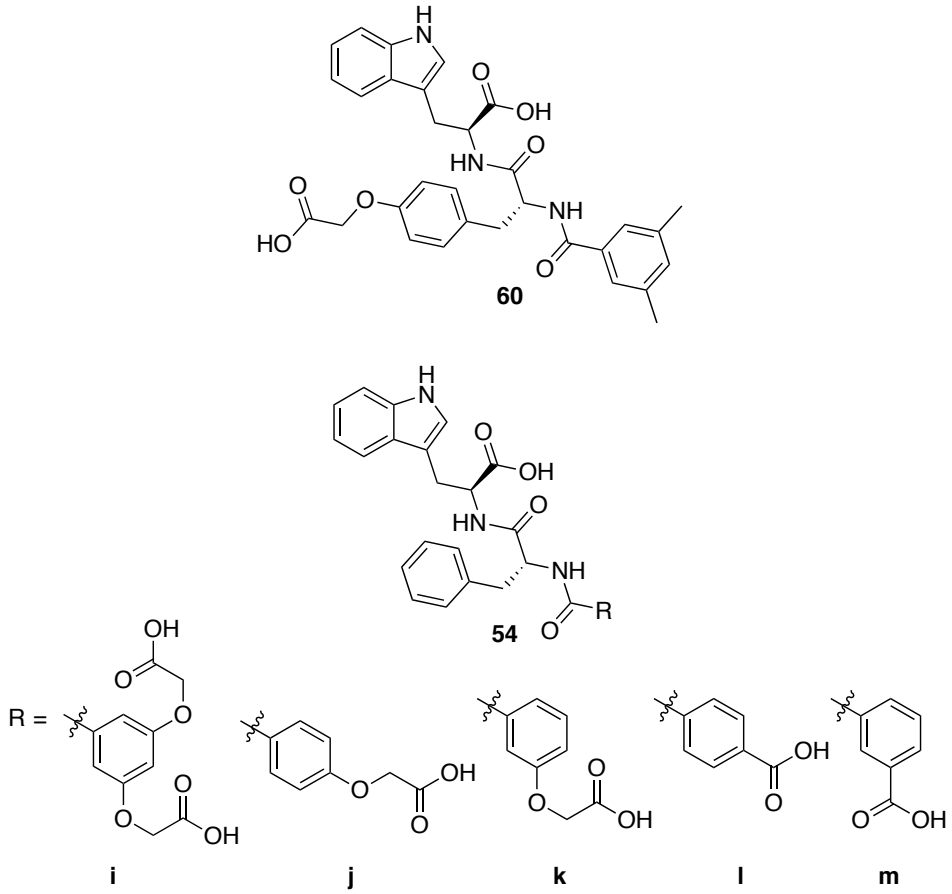
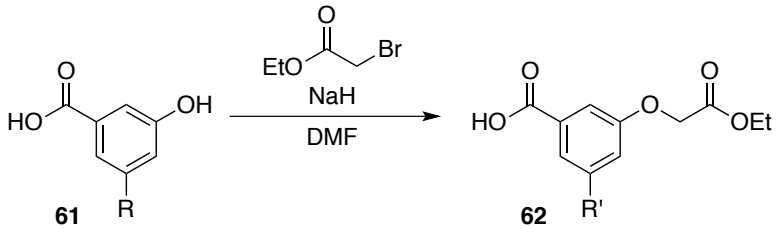
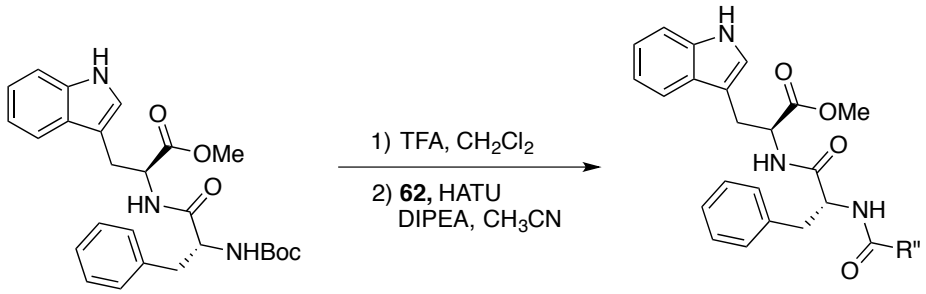


Figure 2.11 Initially proposed polyacid derivatives.

To synthesize **54i** and **54k**, we began with their parent D ring hydroxybenzoic acids (**61**, see **Table 2.10**, below). Following deprotonation with excess sodium hydride, the acids were treated with ethyl bromoacetate to alkylate the resultant phenoxides.

Table 2.10 Synthesis of *m*-carboxymethyl esters.

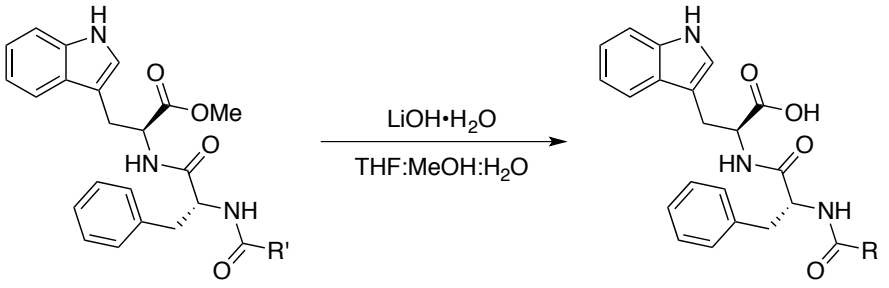




entry	R	R'	R"	61	62	56	yield (%) over 3 steps
1	H	H	3-(EtOOCCH$_2$O)-C$_6$H$_4$	**61i**	**62i**	**56i**	20
2	OH	OCH$_2$OOEt	3,5-di(EtOOCCH$_2$O)-C$_6$H$_4$	**61k**	**62k**	**56k**	28

The resulting alkylated acids (**62**) were then subjected to standard coupling conditions to generate the alkylated esters **56**. Saponification under our standard conditions produced polyacids **54i** and **54k** (see **Table 2.11**, below). Similar conditions were initially attempted in the synthesis of **54j** but were unsuccessful.

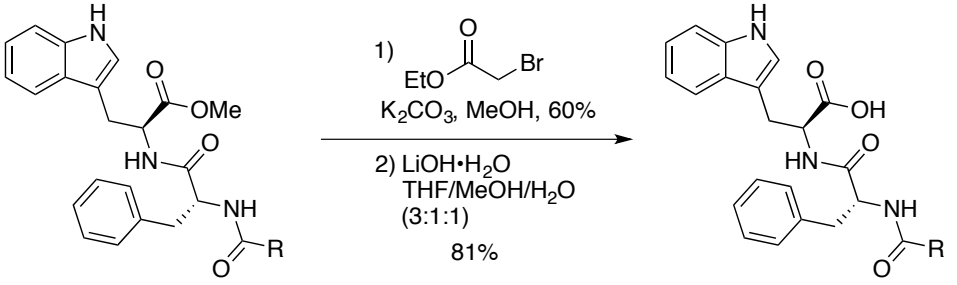
Table 2.11 Synthesis of *m*-carboxymethyl acids.



56 **54**

entry	R'	R	56	54	yield (%)
1	3-(EtOOCCH$_2$O)-C$_6$H$_4$	3-(HOOCCH$_2$O)-C$_6$H$_4$	**56i**	**54i**	83
2	3,5-di(EtOOCCH$_2$O)-C$_6$H$_4$	3,5-di(HOOCCH$_2$O)-C$_6$H$_4$	**56k**	**54k**	49

The synthesis of **54j** was undertaken beginning from **54h**, synthesized in our previous screen (see **Table 2.7**, above). Alkylation with ethyl bromoacetate, followed by saponification, yielded compound **54j** in good yield (**Scheme 2.10**).



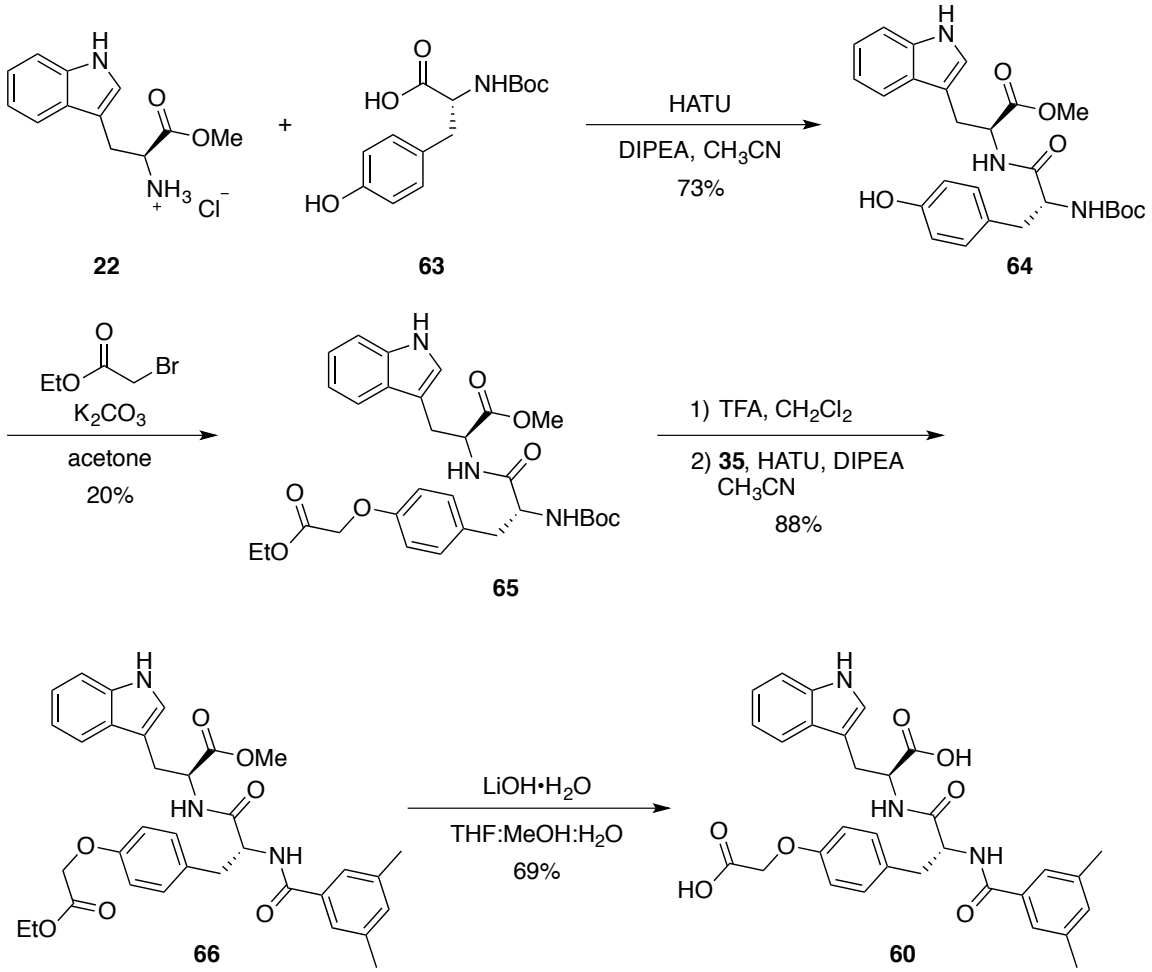
56h
R=4-HO-C$_6$H$_4$OH

54j
R=4-(HOOCCH$_2$O)-C$_6$H$_4$

Scheme 2.10 Synthesis of 54j.

The C ring derivative **60** was synthesized similarly to the D ring analogs (see **Scheme 2.11**, below). Initially, to build the dipeptide scaffold we coupled *L*-Trp methyl ester **22** and Boc protected *D*-Tyr (**63**). The resulting phenol (**64**) was then alkylated with ethyl bromoacetate. Amino ester **65** was then deprotected and coupled to 3,5-dimethybenzoic

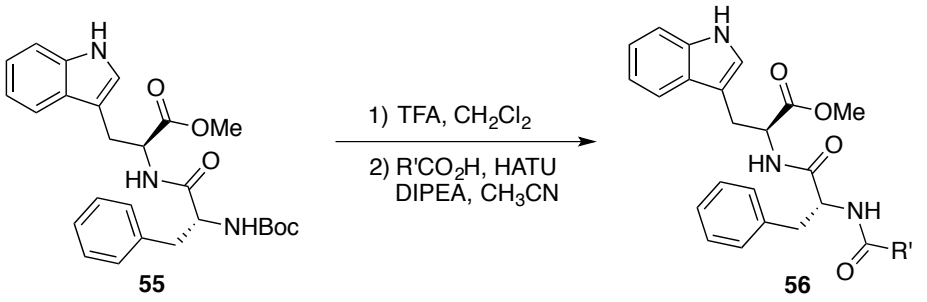
acid (**35**) to generate the full-length scaffold **66**, which was saponified to generate the final compound (**60**).



Scheme 2.11 Synthesis of 60.

Next, we synthesized the two phthalic acid analogs (**54m** and **54l**). Using intermediate **55** from previous syntheses, we first coupled the mono methyl ester phthalic acids to the IRL scaffold (see **Table 2.12**, below).

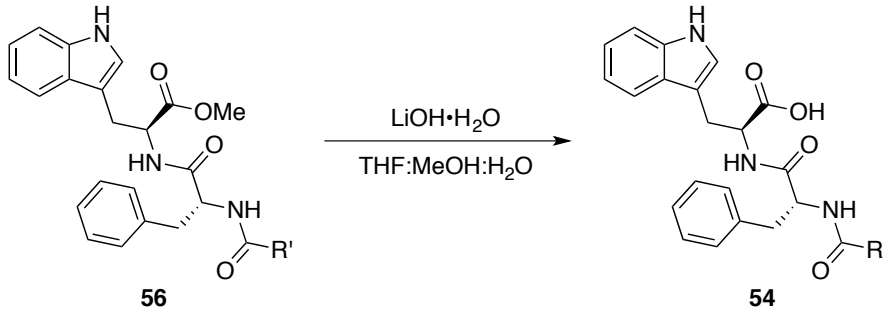
Table 2.12 Coupling of diesters 56m and l.



entry	R'	56	yield (%)
1	*m*-MeOOC-C_6H_4	**56m**	49
2	*p*-MeOOC-C_6H_4	**56l**	34

We then saponified the resulting esters (**56m** and **56l**) to generate the final two dicarboxylic acid analogs (**Table 2.13**).

Table 2.13 Saponification of diacids 54m and l.

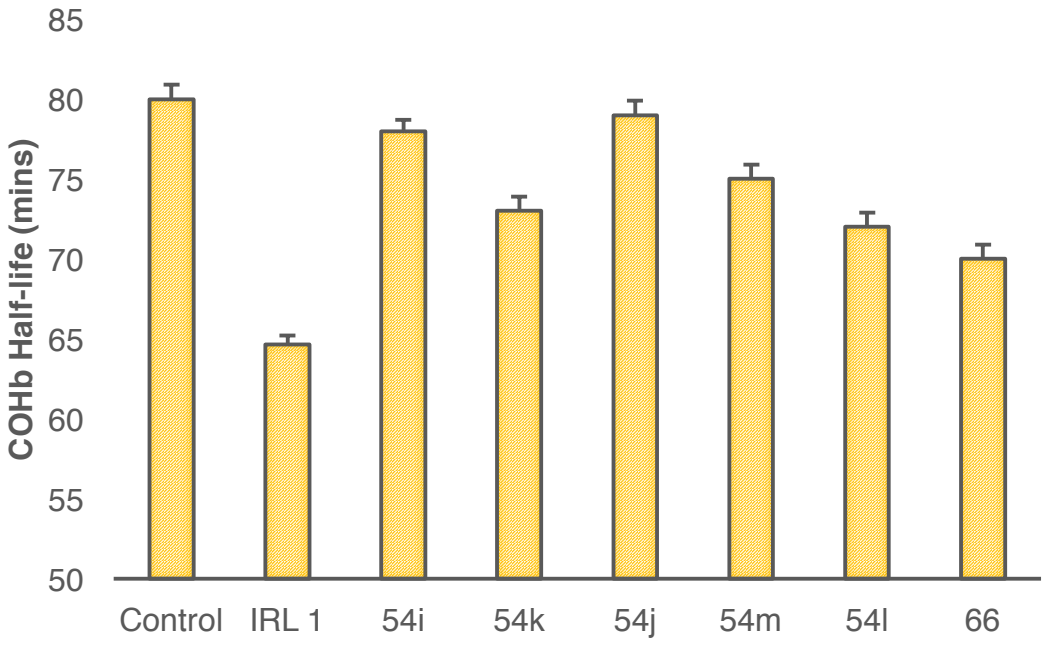


entry	R'	R	56	54	yield (%)
1	*m*-MeOOC-C_6H_4	*m*-HOOC-C_6H_4	**56m**	**54m**	49
2	*p*-MeOOC-C_6H_4	*p*-HOOC-C_6H	**56l**	**54l**	34

This series was analyzed for its ability to reduce COHb half-life. Interestingly, none of the analogs were more potent than IRL 1 (see **Table 2.14**, below). In fact, none of the screened analogs had potency even approaching IRL 1. This led us to conclude that not

only were these analogs unable to pick up interactions within the β cleft of Hb, but these analogs might not even be effectively binding to Hb at all. We hypothesized that this decrease in activity might be due to increased negative charge, which could make it more difficult for our analogs to leave the aqueous environment and bind in the hydrophobic pocket.

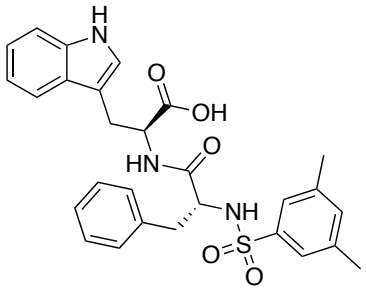
Table 2.14 Biological activity of polyacid analogs.



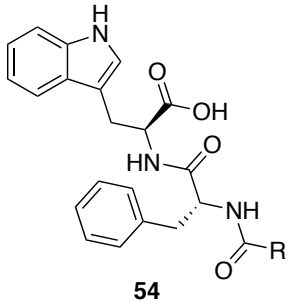
Section 2.2.3 Hydrophobic IRL analogs

Based on this and previous results, we hypothesized that our binding affinity might be affected significantly by hydrophobicity. Due to the reduced activity of the polyacid analogs, we decided to explore the effect of adding more hydrophobic groups to the D ring (see **Figure 2.12**, below). To that end, we synthesized an IRL 1 D ring sulfonamide isostere (**67**), a bis-trifluoromethyl isostere (**54n**), two naphthyl derivatives (**54o** and **54p**)

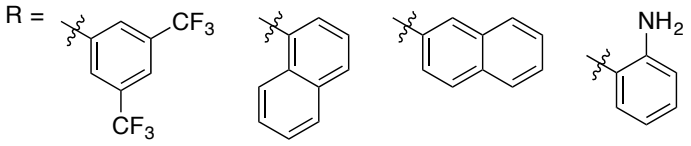
and finally an ortho aniline (**54q**), which we wished to explore based on the relative success of the ortho phenol analogs (**54f**).



67



54

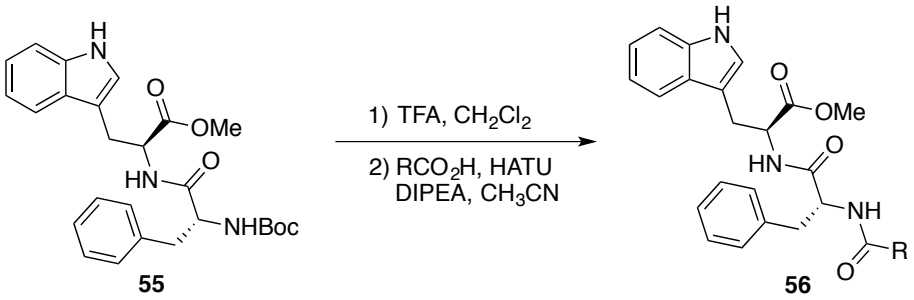


n **o** **p** **q**

Figure 2.12 Hydrophobic analogs.

The synthesis of analogs **54n-q**, was similar to the previous D ring analogs. Intermediate **55** was first deprotected and then coupled to the benzoyl derivatives (see **Table 2.15**, below).

Table 2.15 Coupling of hydrophobic analogs.

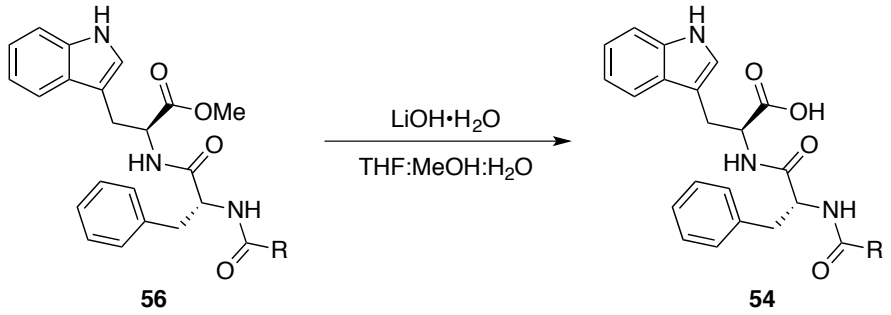


55 **56**

entry	R	56	yield (%)
1	3,5-di-CF_3-C_6H_3	**56n**	70
2	α-naphthyl	**56o**	100
3	β-naphthyl	**56p**	85
4	*o*-BocNH-C_6H_4	**56q**	88

These analogs were then saponified to yield the products (see **Table 2.16**).

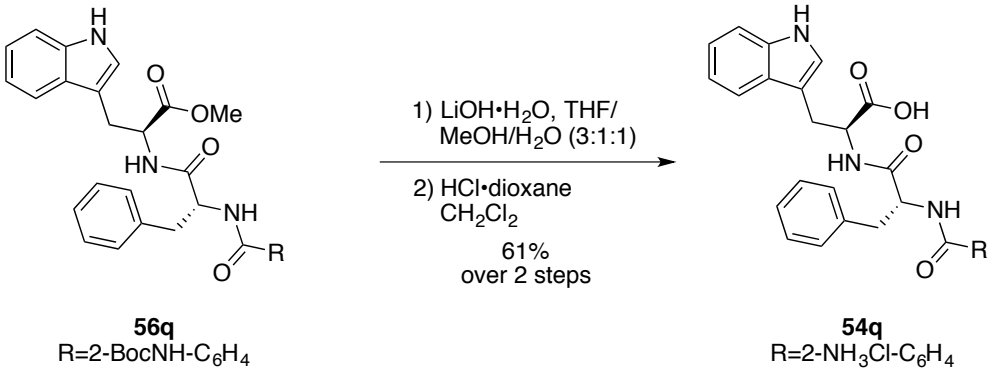
Table 2.16 Saponification of hydrophobic analogs.



56 **54**

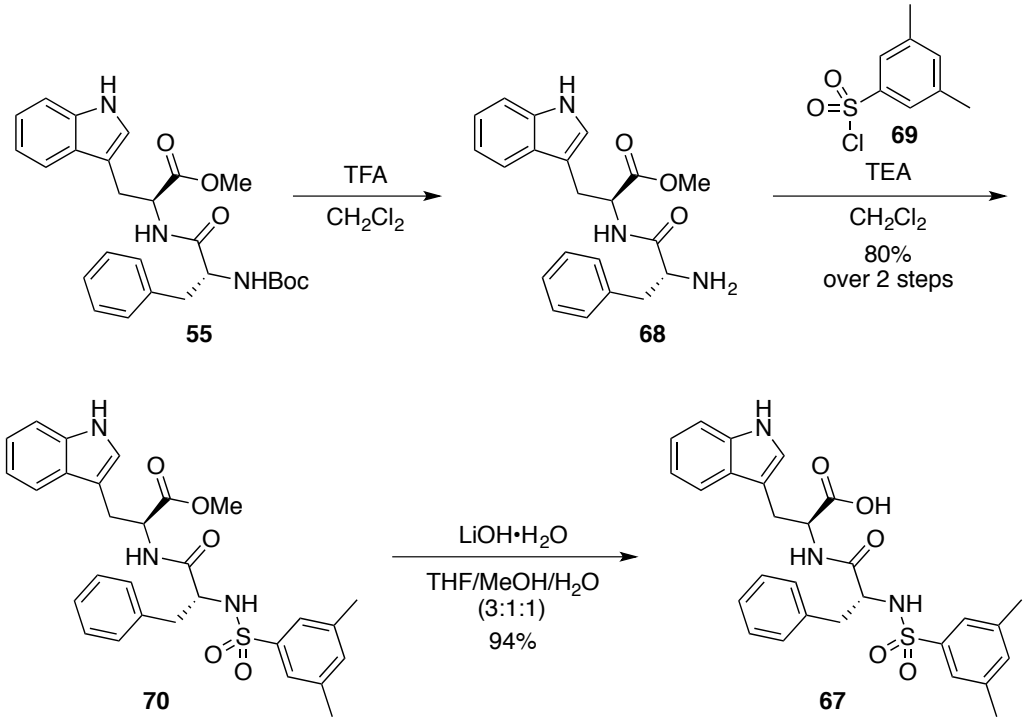
entry	R	56	54	yield (%)
1	3,5-di-CF_3-C_6H_3	**56n**	**54n**	92
2	α-naphthyl	**56o**	**54o**	76
3	β-naphthyl	**56p**	**54p**	86

In the case of **54q**, where the resulting carbamate acid was then Boc deprotected to yield the ammonium salt (**54q**) (**Scheme 2.12**).



Scheme 2.12 Deprotection of amine 54q.

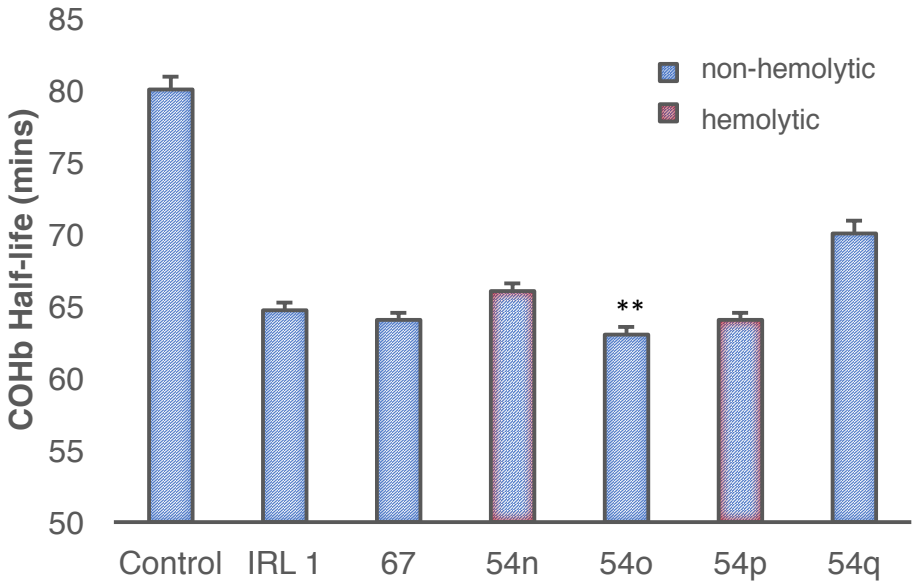
Sulfonamide **67** was synthesized from the same intermediate **55**, but the coupling reaction was performed with sulfonyl chloride **69** to generate ester **70** which was ultimately saponified (see **Scheme 2.13**).



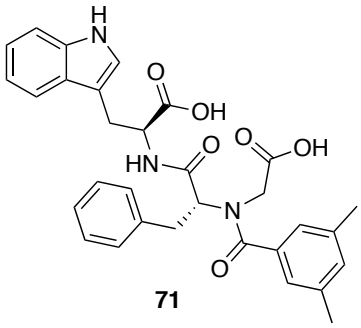
Scheme 2.13 Synthesis of sulfonamide isostere.

This final series of D ring analogs was analyzed for COHb half-life lowering activity. Interestingly, each of these analogs, except for **54q**, had activity that was extremely close to that of IRL 1 (see **Table 2.17**). In fact, one of the naphthyl analogs (**54o**) had significantly improved activity relative to IRL 1. Additionally, two of the analogs, bis-trifluoromethyl benzamide acid **54n** and naphthyl acid **54p**, caused hemolysis when screened. This was not unexpected given their cLogPs of 5.9 and 4.8, respectively, but it does highlight a significant conundrum. Based on these data, the pocket containing the D ring appears to best accommodate highly hydrophobic moieties, which lead to undesired hemolytic activity.

Table 2.17 COHb Half-life for hydrophobic D ring analogs.
Significance relative to IRL 1 * where P ≤ 0.05, ** where P ≤ 0.01, * where P ≤ 0.001**



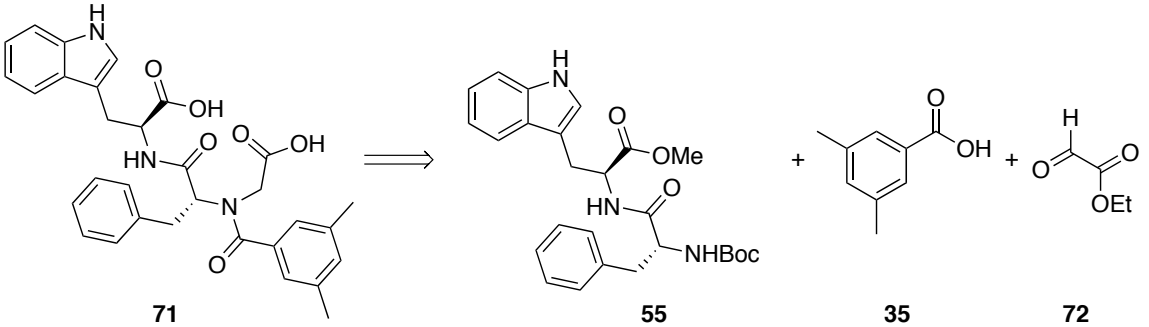
SECTION 2.3 *N*-SUBSTITUTED ANALOG



71

Figure 2.13 *N*-substituted IRL analog.

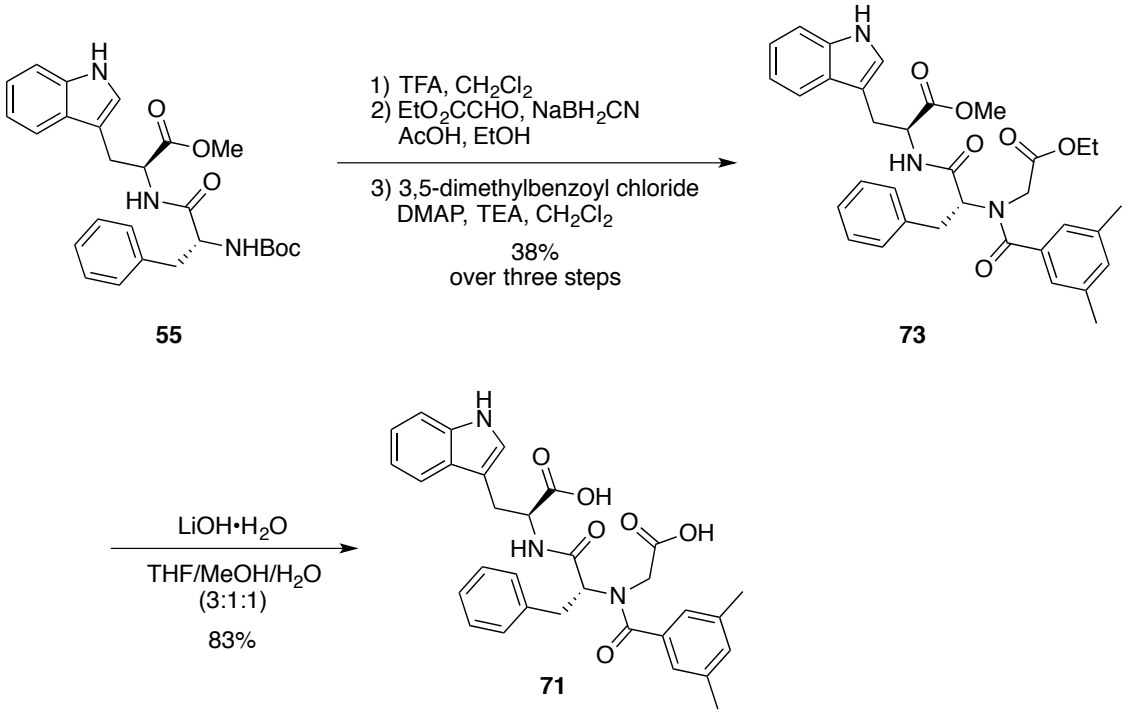
Given the poor success introducing solubilizing groups to the D ring, we decided to explore the introduction of solubilizing groups in place of the *N*-methyl group in IRL 1 (**Figure 2.13**). Because the *N*-methyl group is not necessary for activity (see **Figure 2.7**, **54b**), we expected that the addition of further functionality to this position would not have a deleterious effect on activity. To explore this, we decided to introduce a carboxy methyl group in this position to further improve solubility. We imagined that we could access this analog through reductive amination on the *N*-Boc deprotected intermediate **55** (see **Scheme 2.14**, below).



71 **55** **35** **72**

Scheme 2.14 Retrosynthetic analysis of *N*-substituted analog.

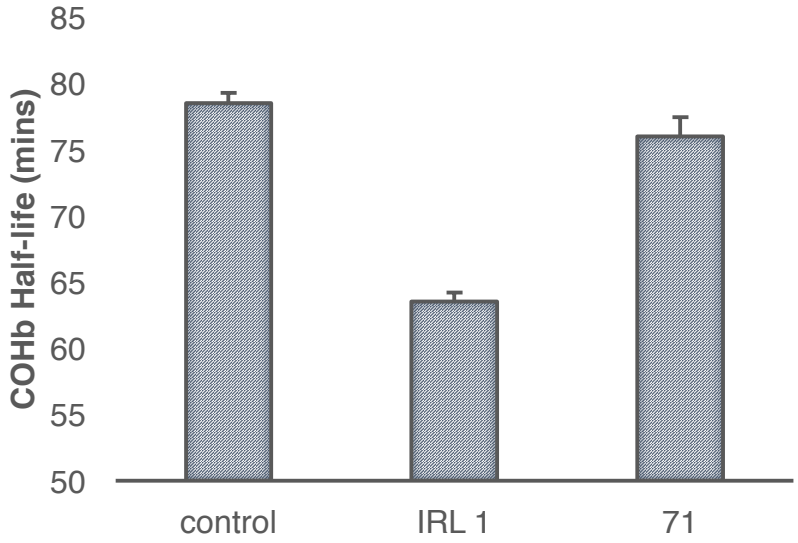
In the forward sense, from dipeptide **55** (see **Scheme 2.15**), we first deprotected the Boc group, then performed a reductive amination with ethyl glyoxylate to generate the disubstituted amine, which was benzoylated with 3,5-dimethylbenzoyl chloride to generate ester **73**. This ester was then saponified to generate the target diacid **71**.



Scheme 2.15 Synthesis of N-carboxymethylated analog.

We submitted this analog for biological testing and hemolytic assay (see **Table 2.18**). Although the *N*-carboxymethylated compound did not cause hemolysis, it showed almost no activity against COHb and it was concluded that further alterations at this nitrogen would likely not retain or improve activity.

Table 2.18 COHb half-life lowering activity of *N*-carboxymethylated IRL analog.



SECTION 2.4 CONCLUSIONS

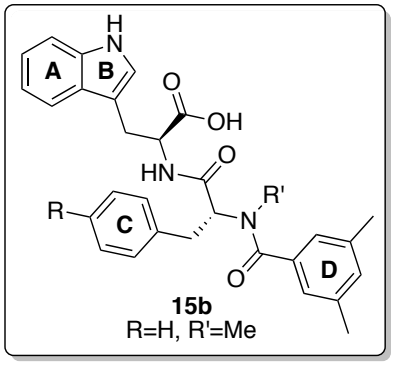


Figure 2.14 IRL 1 with labeled rings.

To summarize, our first generation of IRL 1 derivatives include substitution in both the *L*-Trp (**A/B**) and the dimethyl benzoyl regions (**D**). For the *L*-Trp derivatives, we explored the substitution of the carboxylic acid with either a phosphate or tetrazole. We also probed the functional group tolerance of the *L*-Trp sidechain (see **Figure 2.15**, below). We found that substitution of the phosphate for a carboxyl had no effect on activity, while the tetrazole significantly decreased activity, suggesting that introduction of a phosphate to further IRL derivatives might improve the solubility and cLogP without adversely affecting activity. Assay of the A/B ring analogs indicates that the indole is important for COHb half-life lowering activity, but there is still the potential for further exploration in this region.

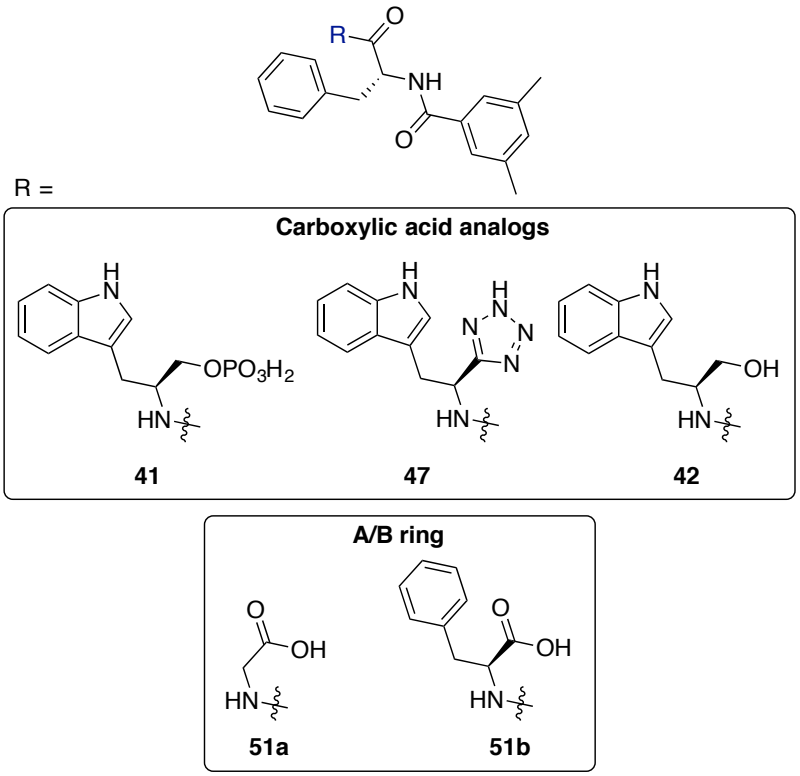


Figure 2.15 Summary of *L*-Trp analogs.

The dimethyl benzoyl region (or D ring) shows very poor functional group tolerance, particularly for the introduction of heteroatoms and other hydrophilic groups (see **Figure 2.16**, below). The most well tolerated analogs appear to be those that introduce a greater degree of hydrophobicity into the ring system. One of our most hydrophobic analogs, naphthyl **54o**, is significantly more potent than IRL 1. This leads us to conclude that greater activity can be obtained by increasing the hydrophobic surface area of the D ring. Unfortunately, it also presents a conundrum given that both aqueous solubility and hemolytic activity are negatively affected by increasing hydrophobicity. To overcome this in our second generation derivatives, we decided to explore analogs that introduce a

greater number of aromatic substituents, while also introducing more heteroatoms to mitigate the adverse effect on cLogP, which we will discuss in **Chapter 3**.

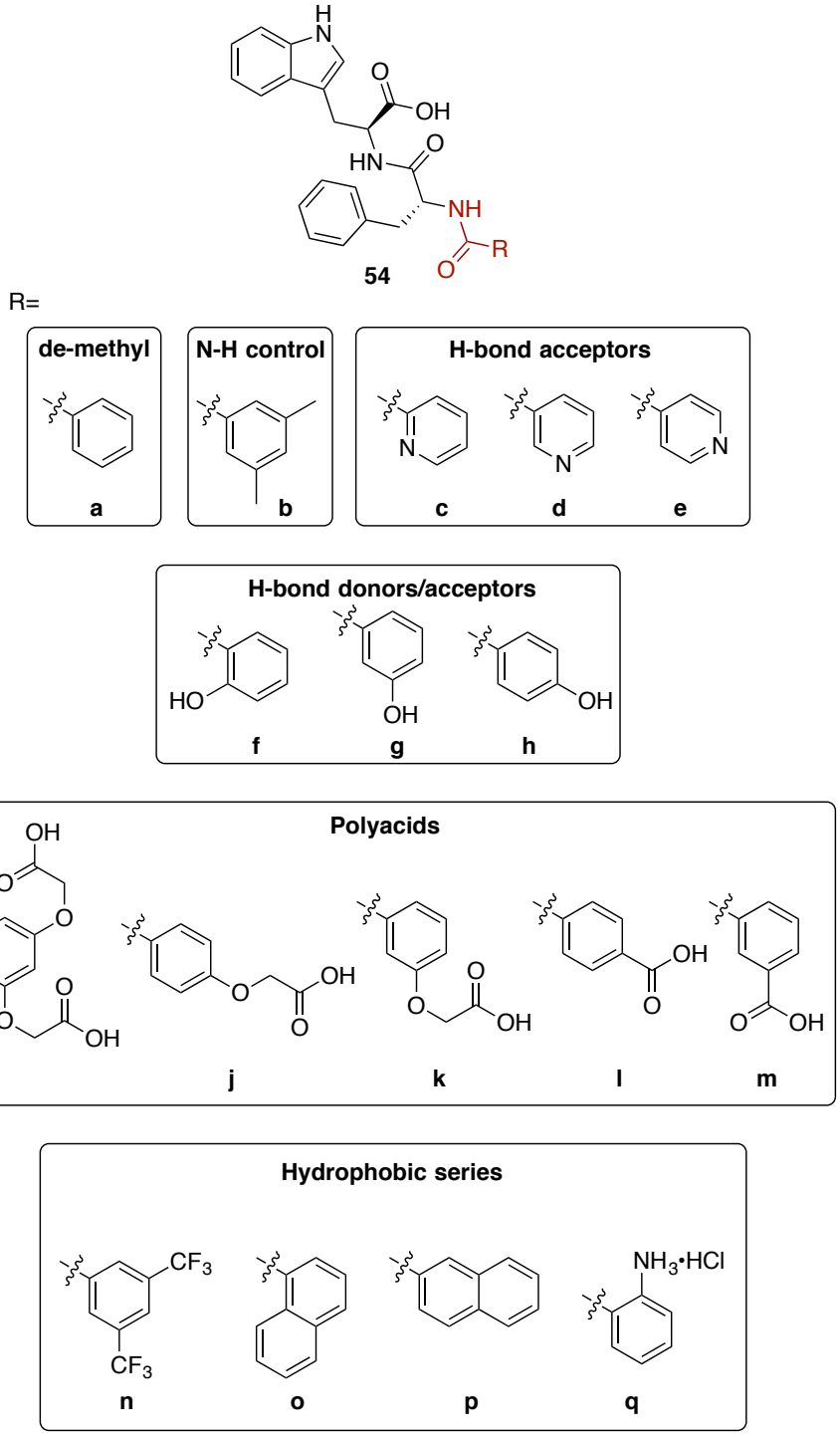


Figure 2.16 Summary of D ring analogs.

SECTION 2.5 REFERENCES

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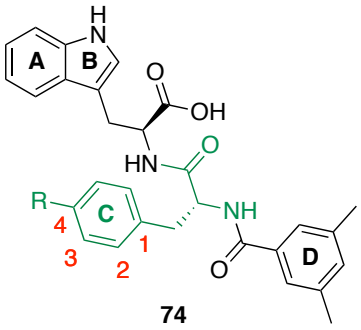
(4) Kosuri, S.; Church, G. M. *Nat. Methods* **2014**, *11*, 499.

(5) Ballatore, C.; Huryn, D. M.; Smith, A. B. *ChemMedChem* **2013**, *8* (3), 385.

CHAPTER 3 SECOND GENERATION DERIVATIVES

SECTION 3.1 BIARYL DERIVATIVES



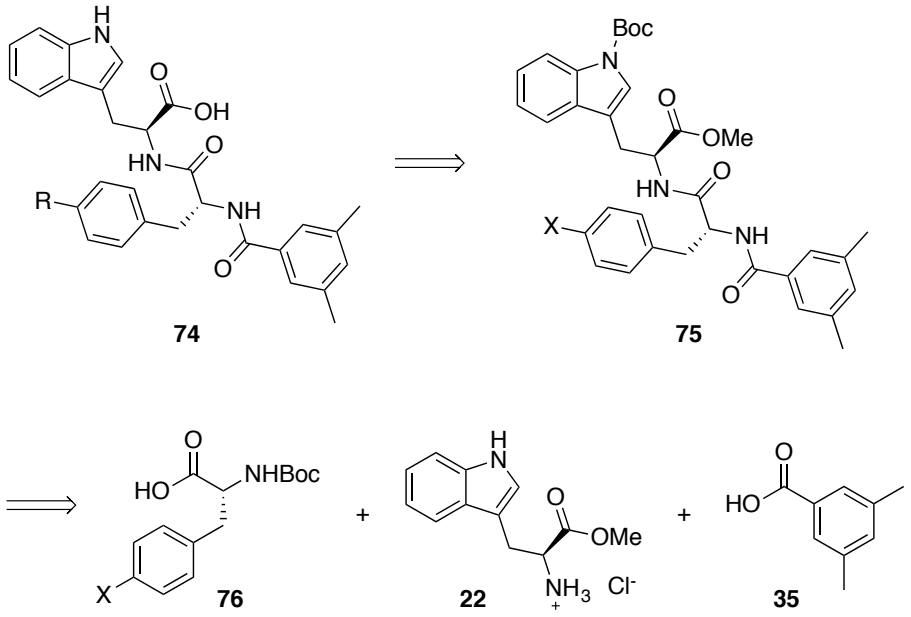
74

Figure 3.1 IRL C ring analogs.

Following the SAR survey of the *L*-Trp derived region and the benzoyl moiety of IRL 1, we concluded that the most effective analogs also had the greatest hydrophobic surface area. This conclusion led to a conundrum. Based on the analysis that we had performed, we determined that analogs with greater hydrophilicity would be less likely to cause hemolysis and would also have better solubility for *in vivo* studies. This directly contradicts the COHb half-life data, which indicated that the more hydrophobic compounds had better activity. For this reason, we decided to explore alterations to the biphenyl ring (see **Figure 3.1**). Specifically, we wanted to introduce different heterocycles at the 4-position of the *D*-Phe derived ring (C ring) to try to improve the solubility and cLogP, while maintaining a large hydrophobic surface area.

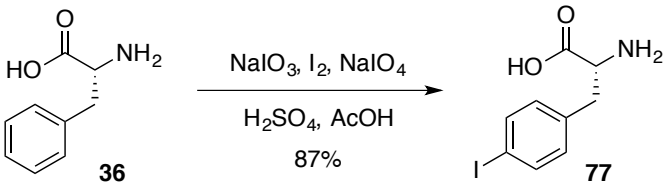
Section 3.1.1 Synthesis of biaryl precursor

We envisioned accessing the biaryl analogs through Suzuki cross-coupling on a full length IRL scaffold (see **Scheme 3.1**, **75**, where X = Hal or OTf). We planned to derive this intermediate from amino acid **76** (where X = Hal or OTf) and coupling partners **22** and **35**.



Scheme 3.1 Retrosynthetic analysis of biaryl analogs.

In the forward sense, *D*-Phe was subjected to oxidative iodination conditions to install iodine in the *para* position, generating *para*-iodo-*D*-Phe in good yield (see **Scheme 3.2**, below). This reaction occurs through a well-precedented acid-catalyzed oxidative iodination, whereby periodate, in the presence of strong acid, forms a reactive protoperiodate (HIO_4), which subsequently generates the iodosulfate ion pair ($IOSO_3H$).[1] In such strongly ionizing conditions, the iodosulfate functions as a source I^+, which is able to promote electrophilic aromatic substitution (S_EAr) on the arene substrate, generating the final iodinated product **77**.



$3\ I_2 + I(VII) \rightarrow 7\ I^+$ (*preliminary stoichiometry*)

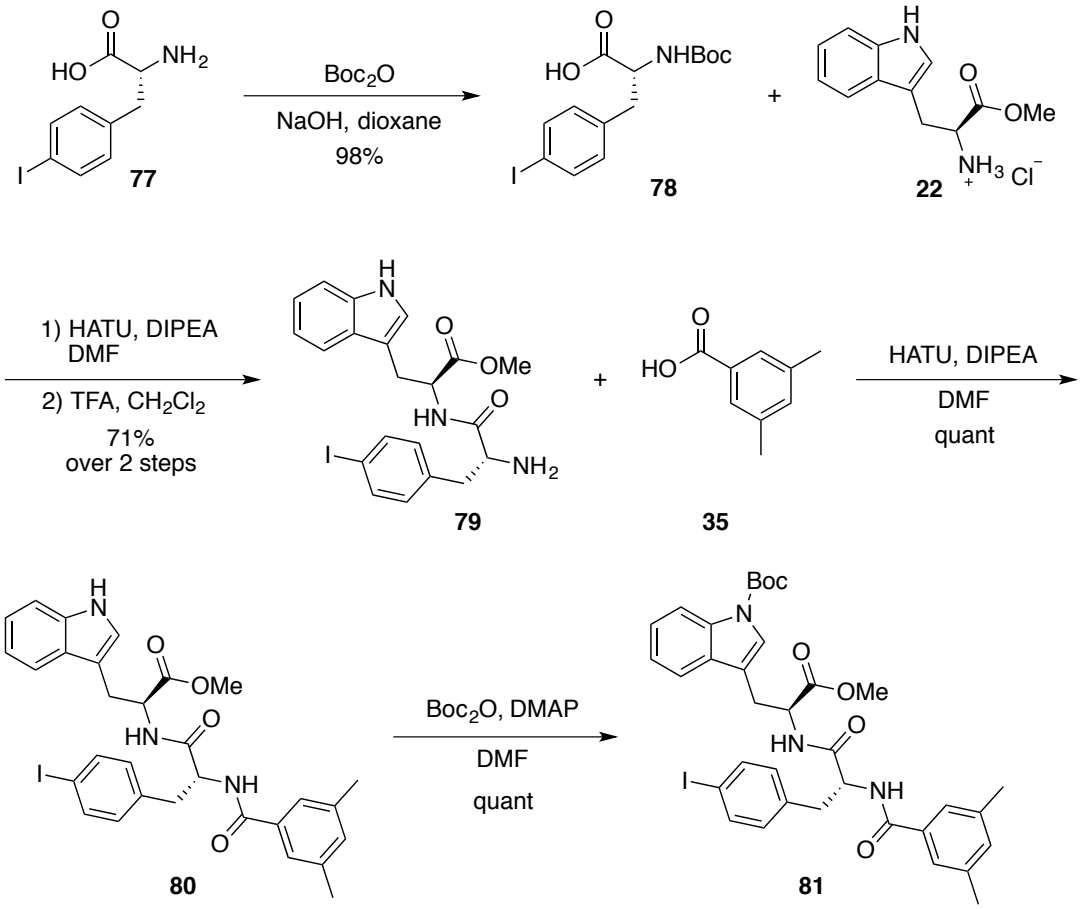
$NaIO_4 + H_2SO_4 \rightarrow NaHSO_4 + HIO_4$ (*true oxidant in the system*)

$7\ ArH + 7\ IOSO_3H \rightarrow 7\ ArI + 7\ H_2SO_4$ (*arenium ion mechanism, S_E2*)

$7\ ArH + 3I_2 + NaIO_4 + H_2SO_4 \rightarrow$ **7 ArI** $+ NaHSO_4 + 4\ H_2O$

Scheme 3.2 Oxidative iodination.[1]

Iodide **77** was then Boc protected and the resulting carbamate was coupled to amino ester **22**. The resulting amino ester was Boc deprotected to generate dipeptide **79** (see **Scheme 3.3**, below). Amine **79** was then coupled to benzoic acid **35** to generate the penultimate full-length scaffold **80**. The resulting indole **80** was Boc protected to prepare intermediate **81** to be used in Suzuki cross-coupling reactions. Overall, the synthesis of iodo coupling partner **81** was completed in six steps with a 61% overall yield.

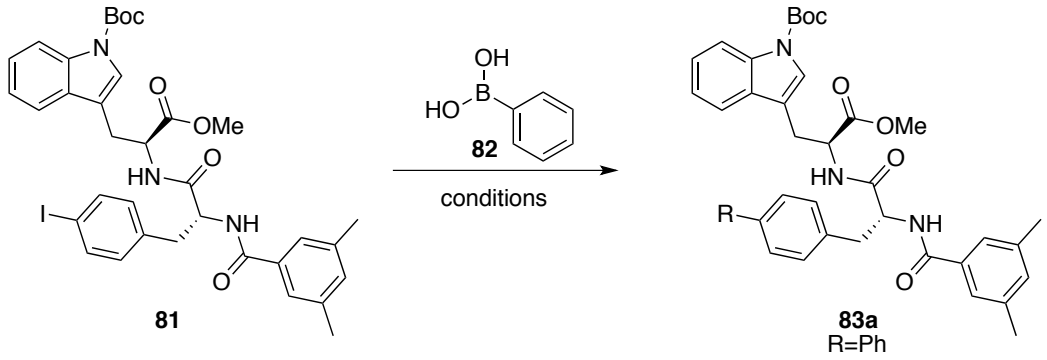


Scheme 3.3 Synthesis of iodide Suzuki precursor.

Section 3.1.2 Optimization of Suzuki cross-coupling

Initially, we screened coupling conditions using the simplest aryl boronic acid: phenyl boronic acid (**82**) to allow correlation with IRL 2500. After screening two palladium sources and several different bases and solvents, summarized in **Table 3.1** below, we determined the optimal conditions to be $PdCl_2(PPh_3)_2$ and potassium acetate in dioxane (**entry 4**). With our most successful conditions in hand, we then began to screen other boronic acids using these coupling conditions.

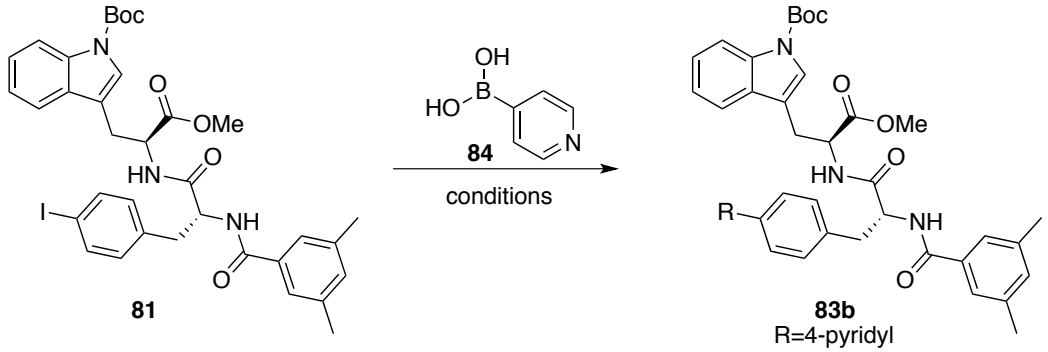
Table 3.1 Initial Suzuki cross-coupling optimization on phenyl boronic acid.



entry	palladium source	base	solvent	time	temp (°C)	yield
1	$Pd(PPh_3)_4$	Cs_2CO_3	dioxane	24h	80	24%
2	$Pd(PPh_3)_4$	Cs_2CO_3	toluene	24h	80	59% (inseparable mixture)
3	$Pd(PPh_3)_4$	Na_2CO_3	tol/MeOH	24h	80	36%
4	**$PdCl_2(PPh_3)_2$**	**KOAc**	**dioxane**	**24h**	**80**	**58%**

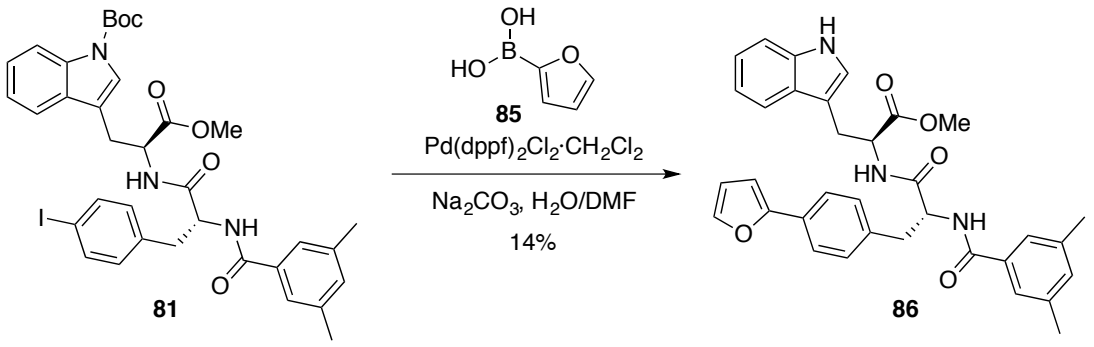
We next tested these conditions on a heteroaromatic system, 4-pyridylboronic acid (**84**, see **Table 3.2**). Unfortunately, the conditions identified in our previous screen yielded no desired product. After screening further, we found that $PdCl_2(dppf)\cdot CH_2Cl_2$ and sodium carbonate in a mixture of DMF and water at 80 °C gave us a moderate yield of pyridine **83b** (**entry 6**).

Table 3.2 Optimization of Suzuki cross-coupling on heteroaryl boronic acid.



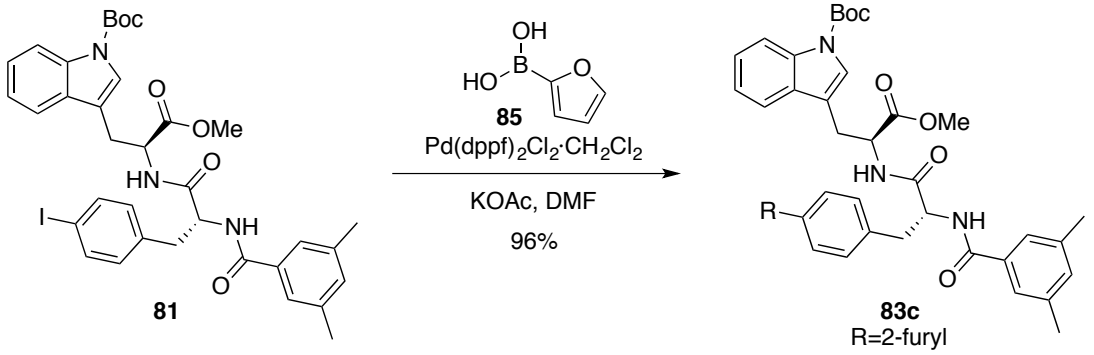
entry	palladium source	base	solvent	time	temp (°C)	yield
1	$PdCl_2(PPh_3)_2$	KOAc	dioxane	48h	80-110	No rxn
2	$PdCl_2(PPh_3)_2$	KOAc	toluene	48h	110	No rxn
3	$PdCl_2(PPh_3)_2$	KOAc	tol/MeOH	48h	110	10%
4	$Pd(PPh_3)_4$	Na_2CO_3	dioxane	48h	110	No rxn
5	$Pd(PPh_3)_4$	Cs_2CO_3	dioxane	48h	80	No rxn
6	**$PdCl_2(dppf)\cdot CH_2Cl_2$**	**Na_2CO_3**	**DMF/H_2O**	**24h**	**80**	**34%**

We applied our optimized coupling conditions from the synthesis of **83b** to the coupling of the iodo-scaffold (**81**) with 2-furyl boronic acid (**85**, see **Scheme 3.4**, below). Unfortunately, these conditions were only modestly successful and gave a low yield of product with concomitant Boc deprotection.



Scheme 3.4 Further optimization of Suzuki cross-coupling.

We hypothesized that the low yield might be due to hydrolysis of the ester. For this reason, we decided to switch to a weaker base (potassium acetate) in dry *N,N*-dimethylformamide, which should be less likely to cause hydrolysis. These conditions proved very successful, yielding furan **83c** in a 96% yield, without loss of the Boc group (see **Scheme 3.5**).



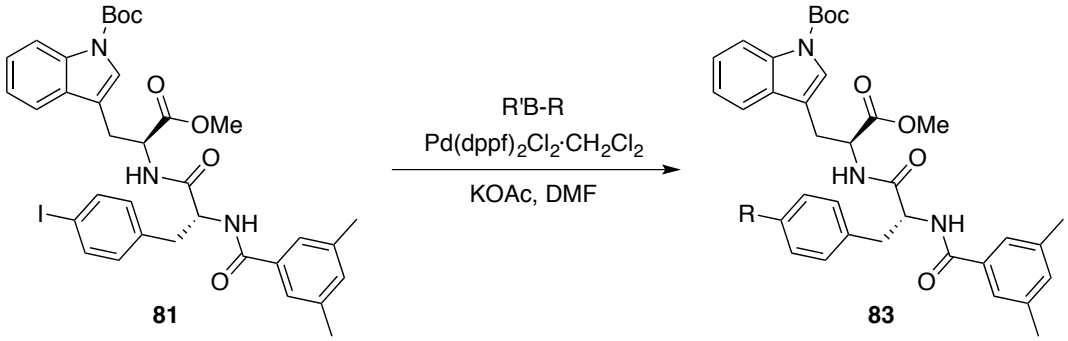
Scheme 3.5 Final conditions for Suzuki cross-coupling.

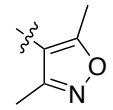
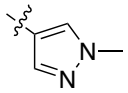
Section 3.1.3 Application of Suzuki cross-coupling to SAR

For SAR purposes, we wanted to explore heterocycles containing both nitrogen and oxygen and a combination of each. Beginning from inexpensive, commercially available

heterocyclic boronic esters and acids, we applied the optimized conditions from **Scheme 3.5** to the coupling reaction and they proved very robust (see **Table 3.3**).

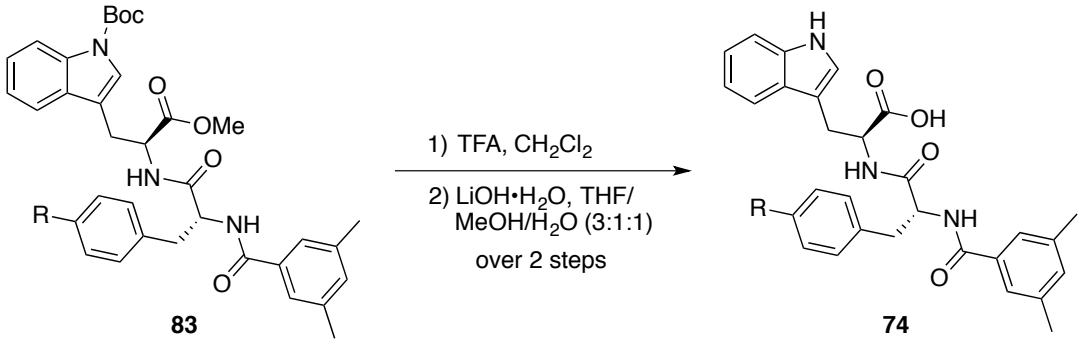
Table 3.3 Scope of Suzuki cross-coupling.

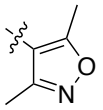
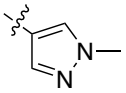


entry	R	R'	83	yield (%)
1	Ph	$(OH)_2$	**83a**	76
2	5-pyrimidyl	$(OH)_2$	**83d**	78
3		pin	**83e**	75
4		pin	**83f**	80

These Boc esters were then deprotected and saponified to yield the final biaryl analogs **74a-f** (**Table 3.4**, below).

Table 3.4 Deprotection of biaryl analogs.



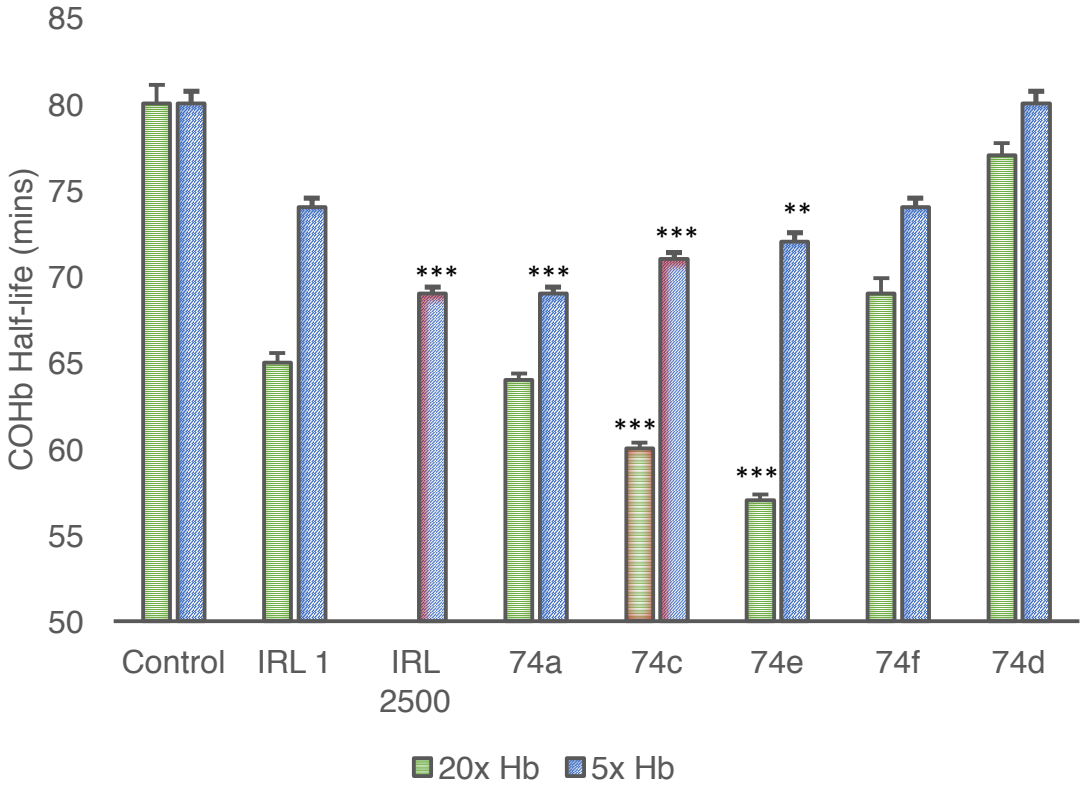
entry	R	83	74	yield (%)
1	Ph	**83a**	**74a**	45
2	2-furyl	**83c**	**74c**	43
3	5-pyrimidyl	**83d**	**74d**	56
4		**83e**	**74e**	70
5		**83f**	**74f**	70

Section 3.1.4 Biological results of initial biaryl derivatives

With these analogs in hand, we tested the COHb half-life. After initially screening the biaryl analogs at 20 times the concentration of Hb (20x), we found that both furan **74c** and isoxazole **74e**, as well as biphenyl **74a** had significantly improved activity compared to IRL 1 (see **Table 3.5**). Due to their increased activity, we decided to also screen the compounds at five times the concentration (5x) of Hb, the highest concentration at which IRL 2500 can be screened. At 5x, biphenyl **74a**, isoxazole **74e** and furan **74c**, were all significantly more potent than IRL 1. The furan **74c** was found to have slight hemolytic activity (1-2% of total Hb at 5x Hb), which tracks with its cLogP of 5.9. It is important to note that although biphenyl **74a** did not cause hemolysis, it was screened for hemolysis

at a much lower concentration (2x Hb) because of its poor solubility in the assay. IRL 2500 caused significant hemolytic activity (>10% of total Hb at 4x Hb). Pyrazole **74f**, pyrimidine **74d** and furan **74c** are the only three compounds screened that had aqueous solubility at a concentration that would allow for *in vivo* experiments (2.5 mM in 1% DMSO). Based on these data, the two most promising analogs are isoxazole **74e** and furan **74c**. However, each showed problems, **74e** with solubility and **74c** with hemolytic activity.

Table 3.5 Biological results for initial biaryl analogs.
Significance relative to IRL 1 * where P ≤ 0.05, ** where P ≤ 0.01, * where P ≤ 0.001**



Section 3.1.5 Further biaryl design optimization

Although the aforementioned analogs significantly improved potency relative to our lead compound, IRL 1, we believed there was still room for further improvement. Specifically, we wanted to develop an analog that retained the COHb half-life of the other analogs, but had improved solubility and no hemolytic activity. We proposed the synthesis of a second series of biaryl analogs derived from the best hits of the previous series. These analogs contain added solubilizing groups that we believed would reduce hemolytic activity while increasing solubility (furan **74g**, isoxazole **74h**, and alcohol **74i**, see **Figure 3.2**, above). The isoxazole boronic ester precursors were not commercially available and therefore needed to be synthesized. The synthesis of the isoxazole boronic esters was undertaken starting from acid **87**, which was first esterified to produce ester **88a** using known conditions[2,3] (see **Scheme 3.8**).

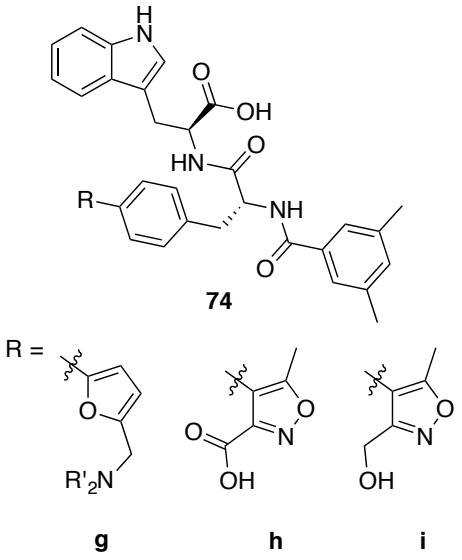
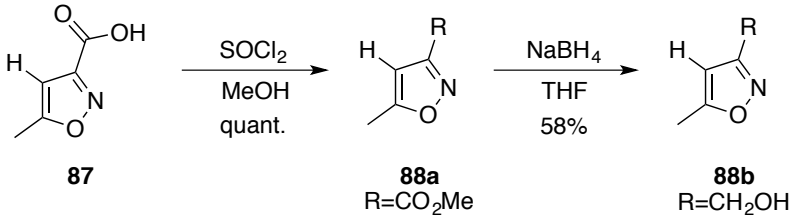


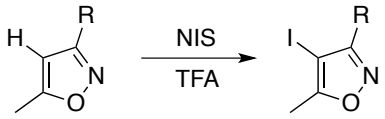
Figure 3.2 Design of solubilizing isoxazoles and furans.



Scheme 3.6 Synthesis of isoxazole precursors to iodination.

This ester was either reduced to the alcohol **88b** then iodinated or directly iodinated to produce iodo compounds **89a** and **89b** (**Table 3.6**, below).

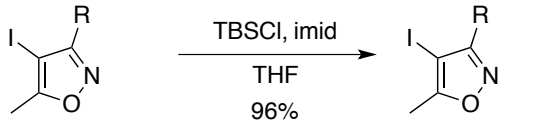
Table 3.6 Iodination of isoxazoles.



88 **89**

entry	R	88	89	yield (%)
1	CO_2Me	**88a**	**89a**	100
2	CH_2OH	**88b**	**89b**	73

Iodide **91b** was then silyl protected to produce iodo precursor **89c** (**Scheme 3.7**).



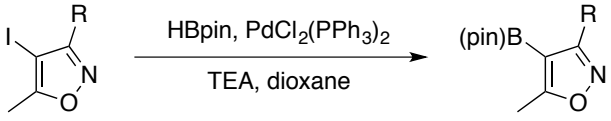
89b
R=CH_2OH

89c
R=CH_2OTBS

Scheme 3.7 Silyl protection of isoxazole 89b.

These iodides were then subjected to Miyaura borylation conditions to generate the corresponding boronic esters **90a** and **c** (**Table 3.7**).

Table 3.7 Miyaura borylation of isoxazoles.



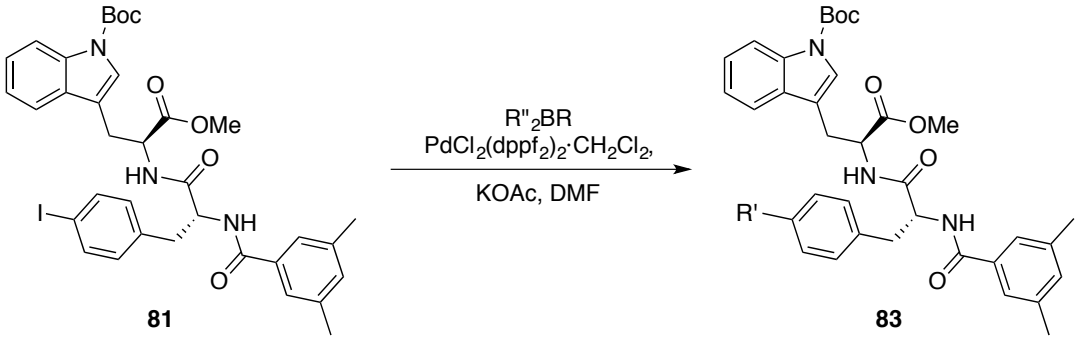
89 **90**

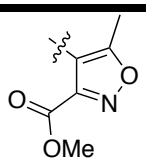
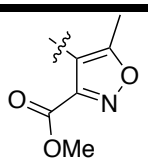
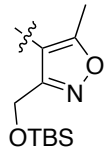
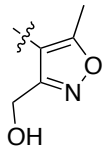
entry	R	89	90	yield (%)
1	CO_2Me	**89a**	**90a**	100
2	CH_2OH	**89c**	**90c**	100[a]

[a]product carried forward to the next reaction without further purification.

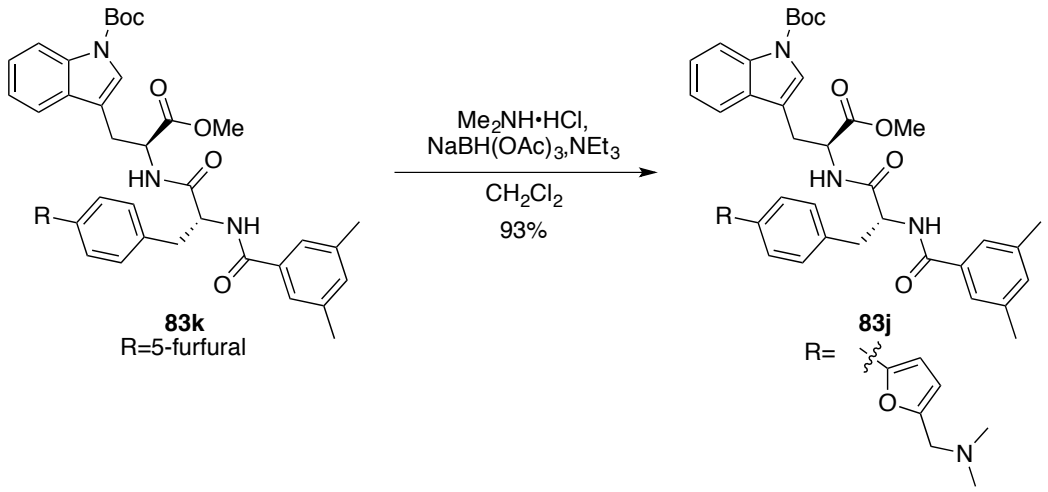
The boronic esters **90a** and **c**, and 2-furyl-5-formyl boronic acid, which is commercially available, were then coupled to the iodo-IRL scaffold **81** (see **Table 3.8**). In the case of silyl alcohol **93c**, coupling occurred with concomitant silyl ether deprotection to generate ester **83i**.

Table 3.8 Coupling of biaryl analogs 83h, i and k.



entry	R	R'	R"	83	yield (%)
1			pin	**83h**	32
2			pin	**83i**	30
3	5-furfural	5-furfural	$(OH)_2$	**83k**	59

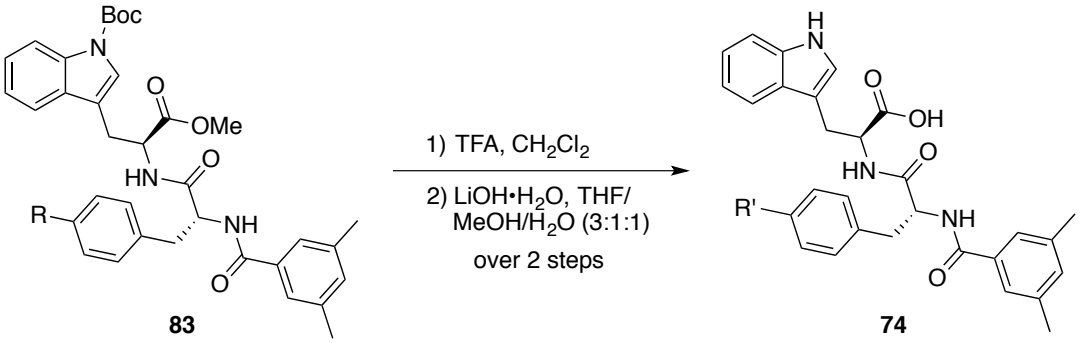
Aldehyde **83k** was further subjected to reductive amination conditions to generate dimethyl amine **83j** (**Scheme 3.8**, below).

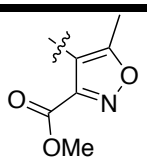
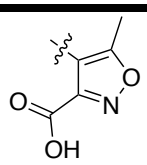
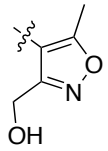
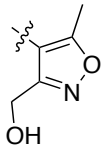
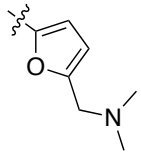
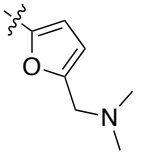


Scheme 3.8 Reductive amination of biaryl furfural.

All three analogs were then Boc deprotected and saponification to yield the second generation hetero-biaryl analogs (**74h, i** and **j**, see **Table 3.9**).

Table 3.9 Biaryl analog deprotection.

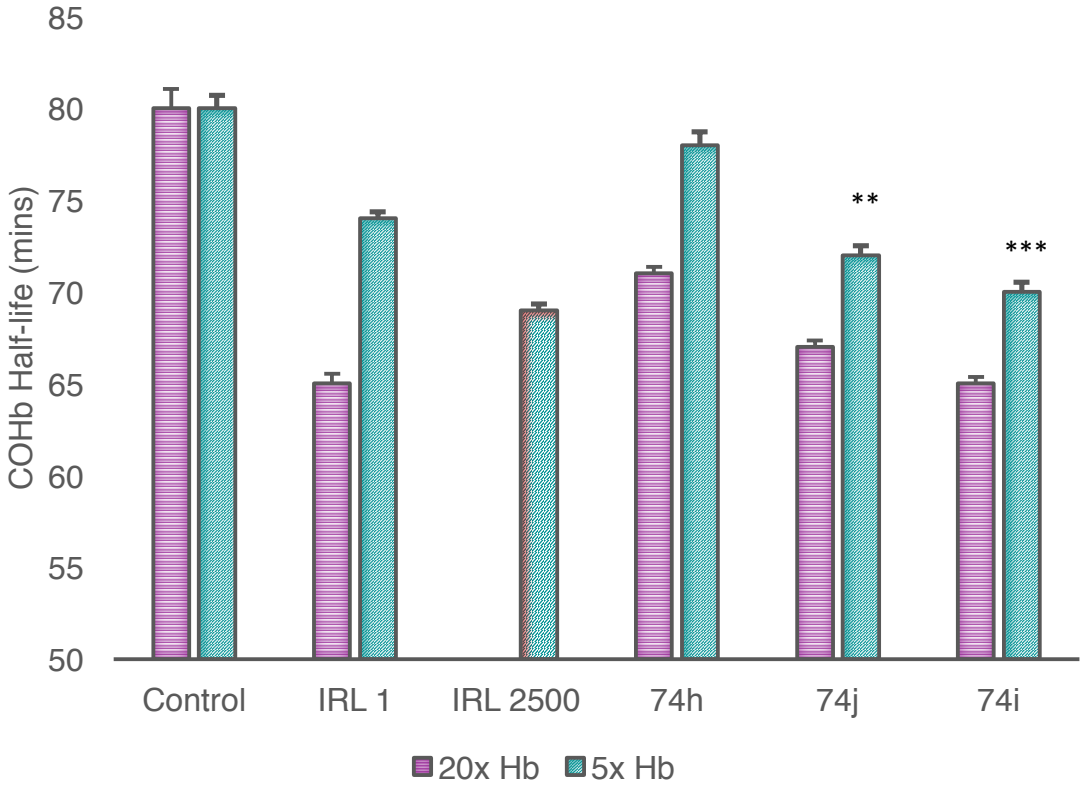


entry	R	R'	83	74	yield (%)
1			**83h**	**74h**	88
2			**83i**	**74i**	35
3			**83j**	**74j**	76

Section 3.1.6 Biological activity of next generation of biaryl analogs

These optimized biaryl analogs were then screened for COHb half-life lowering activity, solubility and hemolytic activity (see **Table 3.10**). Both alcohol **74i** and furan **74j** were significantly more potent than IRL 1. Of particular interest, alcohol **74i** had activity on the order of the other most potent compounds, including IRL 2500, and did not cause hemolysis at high concentrations relative to Hb (5x). Additionally, **74i** is one of the most soluble compounds we have prepared, with solubility well within the threshold for *in vivo* experiments (2.5 mM in 1% DMSO), making it an excellent candidate to advance for testing in mouse models.

Table 3.10 Biological results for optimized biaryls.
Significance relative to IRL 1 * where P ≤ 0.05, ** where P ≤ 0.01, * where P ≤ 0.001**



SECTION 3.2 *NOVEL* STRUCTURALLY CONSTRAINED DERIVATIVES

Upon examination of the crystal structure of IRL 2500 in the β cleft, we observed that IRL 2500 is bound in a relatively high energy conformation (see **Figure 3.3**, below). The constrained nature of this conformation is highlighted by the fact that the two opposite ends of the molecule, the D ring and the A/B rings, are proximally positioned, while the C ring is arrayed in the opposite direction in the cleft. This led us to hypothesize that if we were to tether IRL in such an orientation, we might be able to pre-organize the molecule in its optimal binding mode, thus improving its activity.

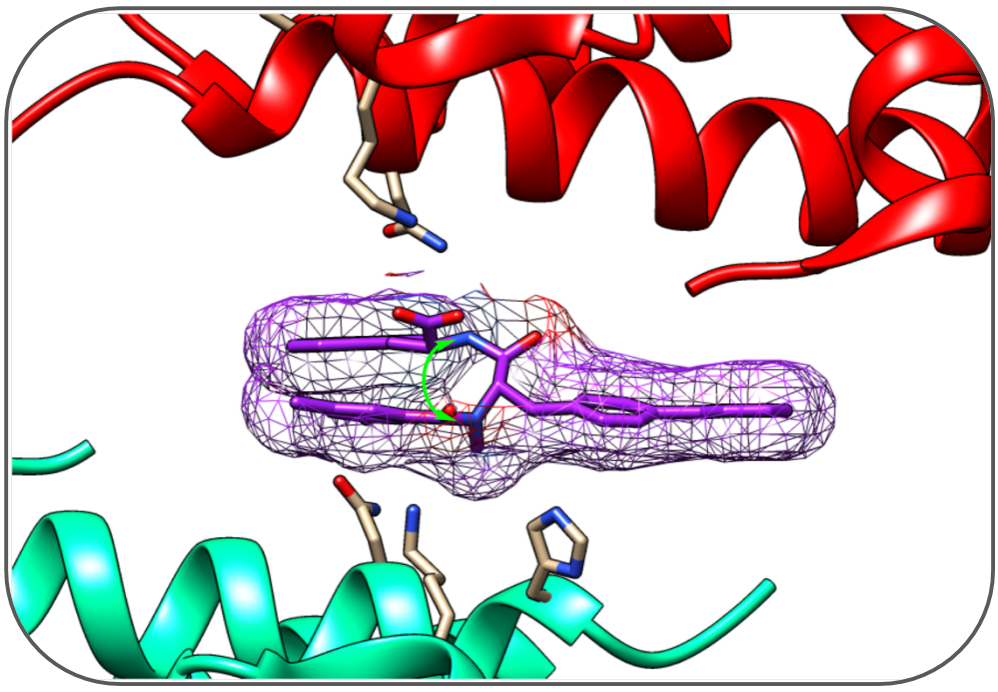
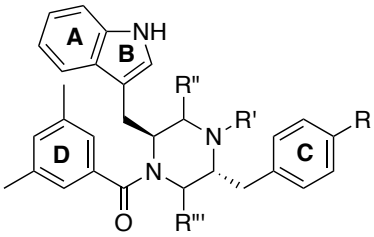


Figure 3.3 Space filling model of IRL 2500 in cleft with green arrow to indicate tether point.

Section 3.2.1 Diketopiperazine derivatives



91

R=H or Ar
R'=H or Me
R"=CO_2Me or CH_2CO_2Me
R"'=H, CO_2Me or CH_2CO_2Me

Figure 3.4 Piperazine analogs derived from diketopiperazines.

We decided that piperazine scaffold **91** would provide the best overlap with IRL 2500 in the crystal structure (see **Figure 3.4**). As initially designed the piperazine would contain either one or two acid moieties (**91**, R" and R"') positioned to interact with the Lys β082 on either side of the cleft mimicking the acid in IRL 2500 (see **Figure 3.5**, below). Also, it is important to note that all the rings align in the appropriate manner, with the A/B and D rings oriented to the left side of the central piperazine and the C ring positioned to the right. To position the D ring in the appropriate position, it is necessary to differentiate the two sides

of the diketopiperazine ring by either *N*-methylating the R' group of **91** or protecting it during the synthesis. Furthermore, the C ring of **91** would either contain an aryl ring in the *para*-position at the R, or a hydrogen, mimicking either IRL 1 or IRL 2500.

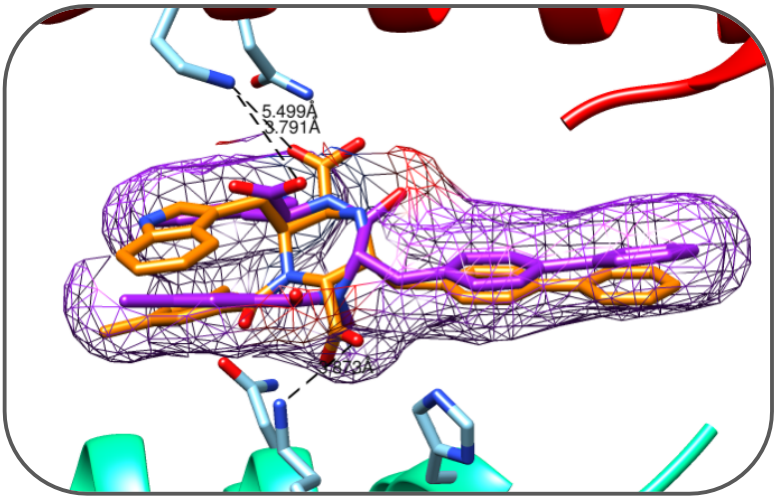
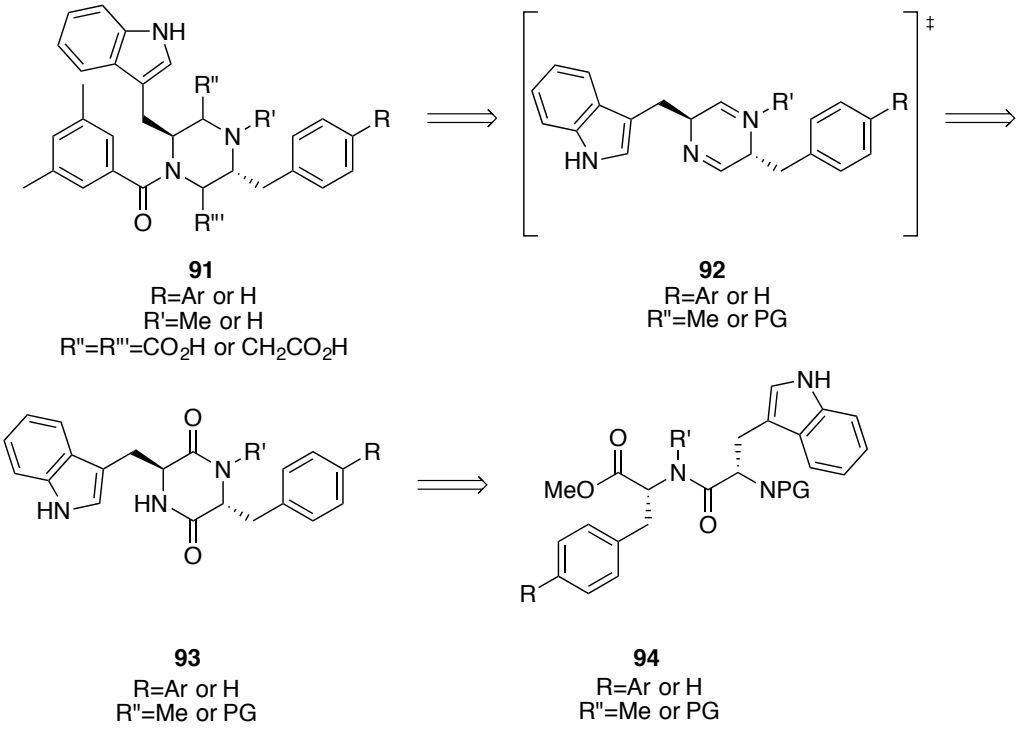


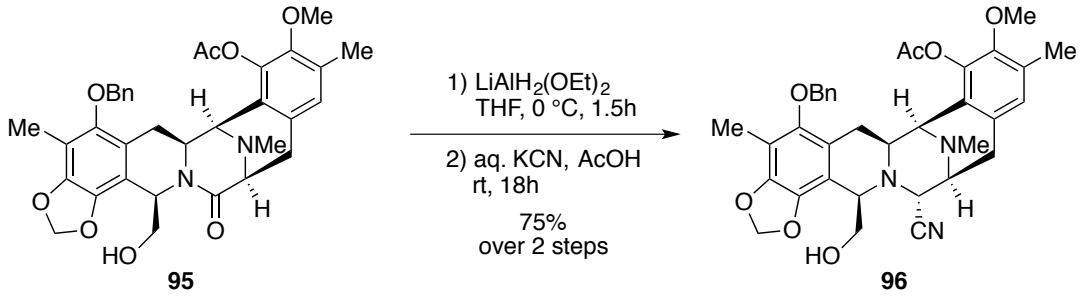
Figure 3.5 Overlay of piperazine 91 (orange, where R=Ph, R'=H and R"=R"'=CO_2H) on IRL 2500 (purple).

We envisioned the synthesis of piperazine **91** occurring via nucleophilic addition of a carboxylate surrogate onto the imine **92**, which would be generated *in situ* through partial reduction of diketopiperazine **93** (see **Scheme 3.9**, below). Diketopiperazine **93**, in turn, would be derived from cyclization of the linear precursor **94**.



Scheme 3.9 Retrosynthetic analysis of piperazine analog derived from diketopiperazine.

These types of *in situ* nucleophilic additions have been performed on similar systems. We decided to use cyanide as our nucleophile, given its broad use and ability to be hydrolyzed to the acid. Recently, in the synthesis of (–)-renieramycin T, Yokoya, *et. al.* employed reductive cyanation to stereoselectively install the amino nitrile **96** of (–)-renieramycin, which is present in many 1,2,3,4-tetrahydroisoquinoline marine natural products (see **Scheme 3.10**, below).[4] Additionally, reductive cyanations have been used in the total synthesis of aspeverin,[5] (–)-lemonomycin,[6] (–)-jorumycin,[7] and others. As far as we are aware, reductive cyanation has never been attempted on a diketopiperazine.



Scheme 3.10 Reductive cyanation to generate advanced intermediate 96 in the synthesis of (–)-Renieramycin.[4]

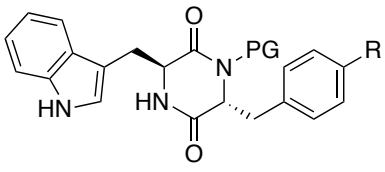
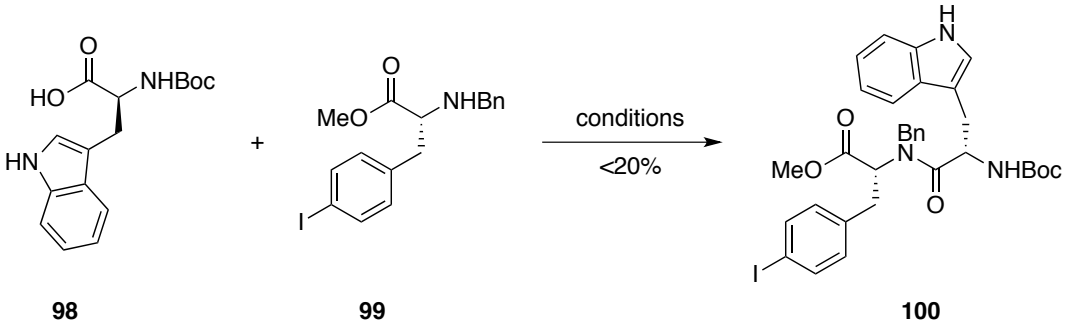


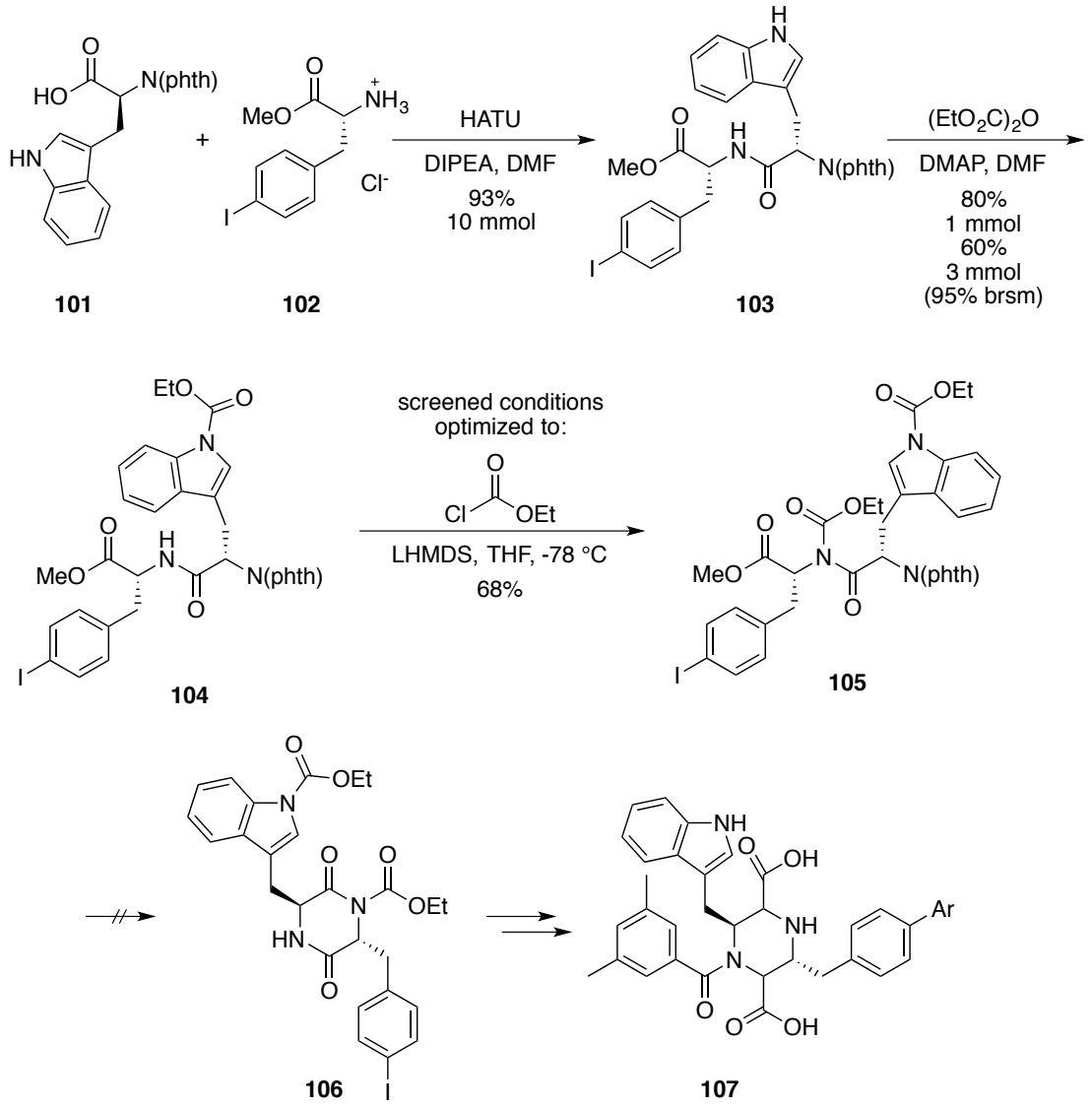
Figure 3.6 Protected diketopiperazine target.

We first decided to access a diketopiperazine with a protecting group (PG) on the amide nitrogen that could be unmasked to the free amide/amine at the end of the synthesis (see **Figure 3.6**, **97**). This would allow us to differentially benzoylate the advanced diketopiperazine. Initially, we chose to use an *N*-benzyl protecting group (**97**, where PG = -CH_2Ph), which we planned to hydrogenate at the end of the synthesis. However, attempts to couple benzyl protected secondary amine **99** to *N*-Boc tryptophan proved very low yielding under a variety of coupling conditions (**Scheme 3.11**).

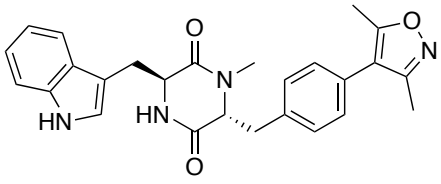


Scheme 3.11 Attempted coupling of *N*-benzyl secondary amine.

We then decided we might have better success coupling a primary amine and protecting it after amide formation. Thus, *N*-phthaloyl tryptophan **101** and *para*-iodophenylalanine methyl ester **102** were coupled to generate dipeptide **103** (see **Scheme 3.12**, below). After initially attempting the ethyl carbamate protection of the amide and the indole in one step, we screened conditions to first protect the indole generating monocarbamate **104** and then to protect the amide generating the desired dicarbamate **105**. Following protection, we attempted phthalamide deprotection and diketopiperazine formation, but were unable to achieve this transformation, in either a single or stepwise manner.



Scheme 3.12 Progress towards synthesis of bis carbamoyl diketopiperazine.

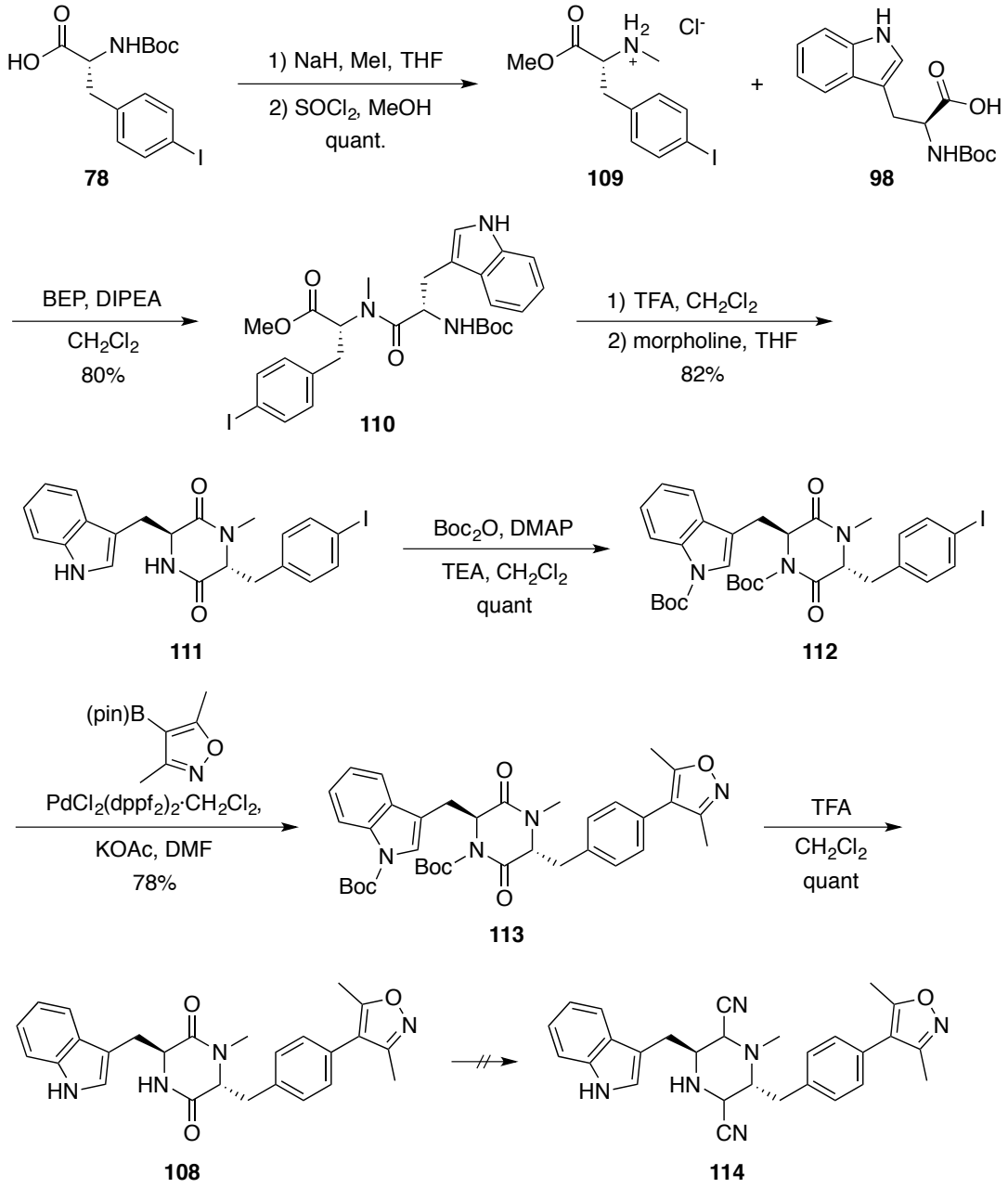


108

Figure 3.7 *N*-methylated diketopiperazine target 108.

We next pursued the *N*-methylated amino analog, which would simplify the protection scheme and allow for a more easily removed protecting group on the terminal nitrogen prior to diketopiperazine formation (see **Figure 3.7**). Beginning from the Boc protected *para*-iodophenylalanine (**78**), which can be accessed in two steps from *D*-Phe,

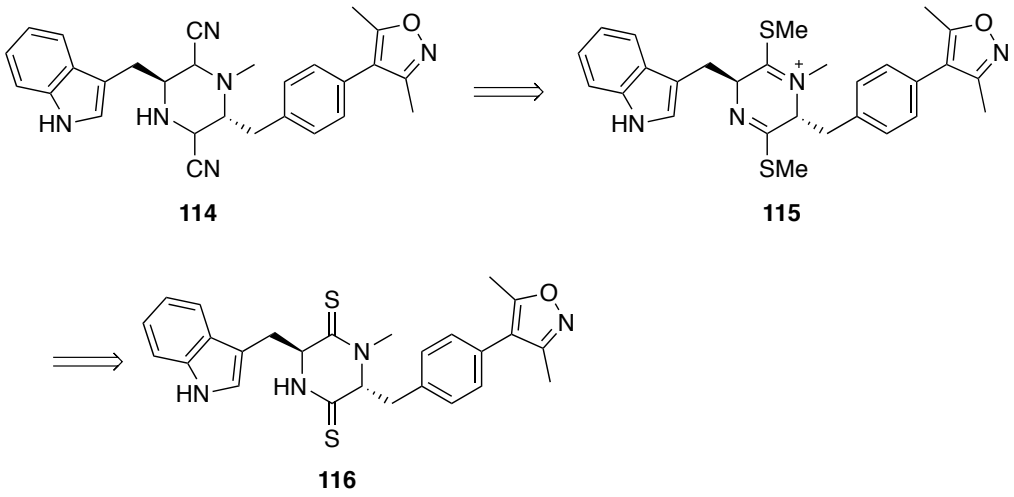
we *N*-methylated, then esterified and deprotected the resulting Boc-protected amino acid to yield *N*-methyl amino ester **109** (see **Scheme 3.13**, below). This ester was then coupled to *N*-Boc *L*-Trp, to yield the linear precursor **110**, which was subsequently cyclized to the diketopiperazine **111**. The diketopiperazine was then protected as the di-Boc to yield the precursor to Suzuki cross-coupling **112**. Suzuki cross-coupling proved successful and the resulting isoxazole **113** was deprotected to yield the precursor to reductive cyanation **108**. Unfortunately, we screened variety of cyanation conditions, but were never able to access bis-nitrile **114**.



Scheme 3.13 Synthesis of isoxazole diketopiperazine 108.

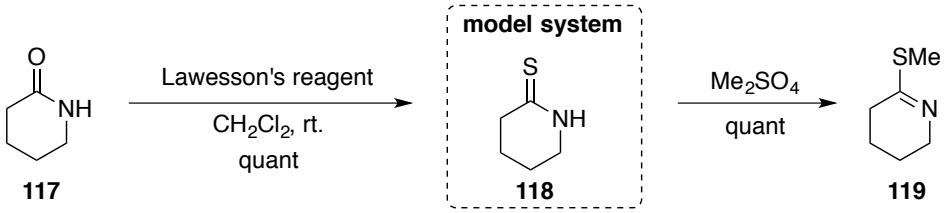
At this point, we decided to attempt the reductive cyanation stepwise through the bis-thiolactam **116**, which could be *S*-methylated to generate thioimidate **115**. After

nucleophilic displacement with cyanide followed by reduction, we could access nitrile **114** (see **Scheme 3.14**).



Scheme 3.14 Retrosynthesis of piperazine 114 through *S*-methyl 116.

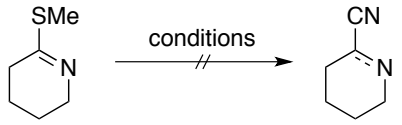
The electrophilicity of thioimidates has been used previously, particularly with Grignard reagents, such as in the synthesis of histrionicotoxin by Evans and coworkers.[8] However, displacement of the thioimidate with cyanide is unprecedented. We decided to test this reaction with a model system. Beginning with δ-valerolactam **117**, the thiolactam model system was made with Lawesson's reagent yielding thiolactam **118**. This thioamide was then quantitatively converted to thioimidate **119**, which was ready for displacement screening (**Scheme 3.15**).



Scheme 3.15 Synthesis of reductive cyanation model system.

Unfortunately, although several cyanation conditions were attempted (summarized in **Table 3.11**, below), we never observed the formation of the desired nitrile addition product. For this reason, we decided to forgo attempts to try this reaction with our advanced precursor **116**.

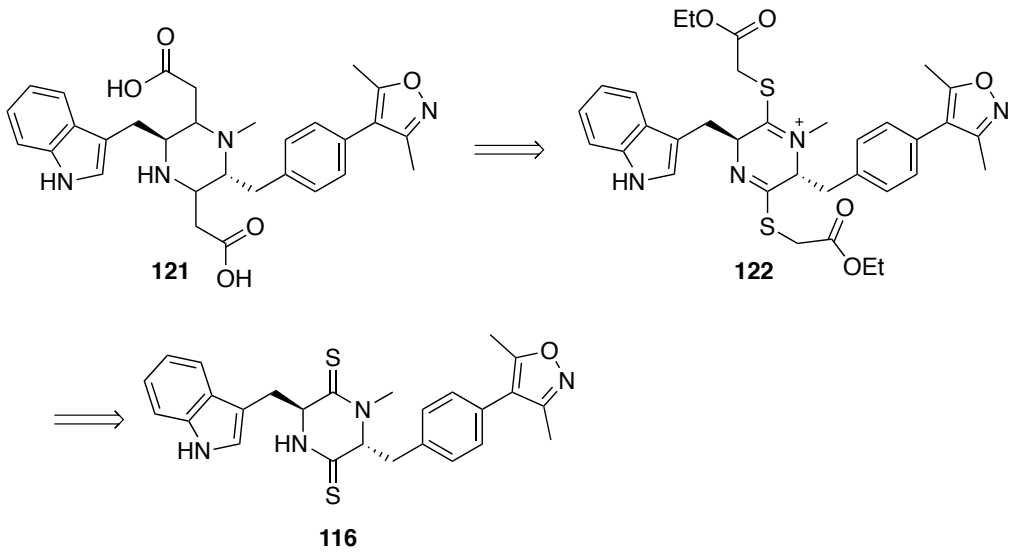
Table 3.11 Reductive cyantion conditions.



119 **120**

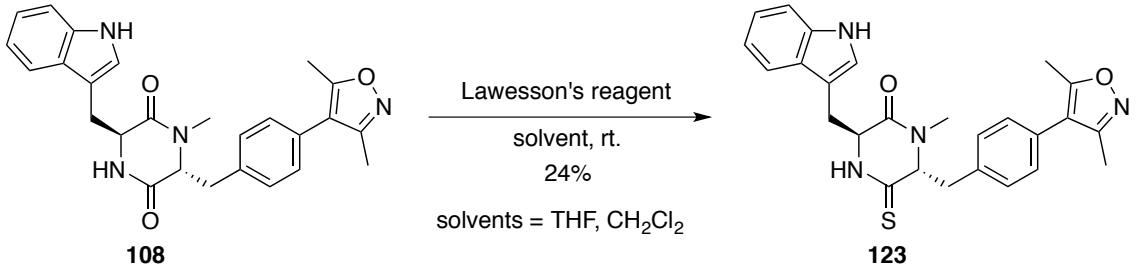
entry	reagents	solvent	temperature	result
1	KCN, 18-crown-6	DMF	100 °C	recovered S.M.
2	KCN, 18-crown-6	MeOH	60 °C	MeOH addition product
3	TMSCN, neat; $NaBH_3CN$	THF	r.t.	decomposition
4	TMSCN, $TiCl_4$, Et_2O, CH_2Cl_2; $NaBH_4$	THF	r.t.	ring opened products
5	*t* -BuNC	CH_2Cl_2	r.t. to reflux	recovered S.M.
6	4N HCl/dioxane, *t*-BuNC	THF	r.t.	decomposition

We next attempted to generate the carboxymethyl piperazine analog (**121**, see **Scheme 3.16**) through *S*-alkylation with bromoacetate (**122**) followed by rearrangement and sulfur extrusion to give the unsaturated β-aminoester (not shown).[9,10] As far as we are aware, this rearrangement has never been attempted on a bis-thiolactam.



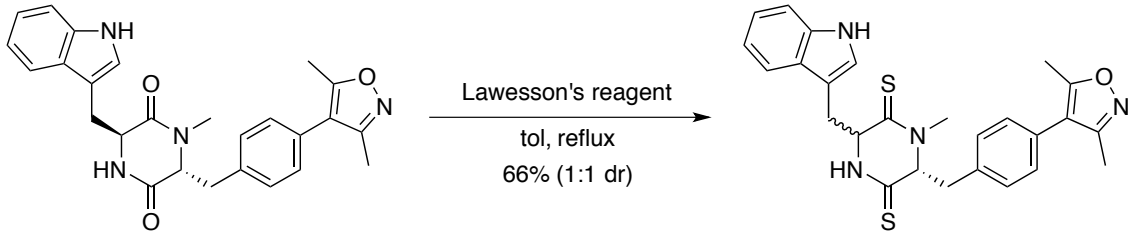
Scheme 3.16 Retrosynthesis of carboxymethylpiperazine 121 through *S*-alkylated 122.

To do this, we needed the bis-thioamide of diketopiperazine **108**. Unfortunately, thioamidation proved to be more problematic than originally anticipated. Treatment of **108** with Lawesson's reagent at room temperature in either tetrahydrofuran or dichloromethane only ever led to partial conversion to the mono-thiolactam (see **Scheme 3.17**, **123**), which NMR analysis suggested was regioisomer **123**.



Scheme 3.17 Partial thiolactam formation.

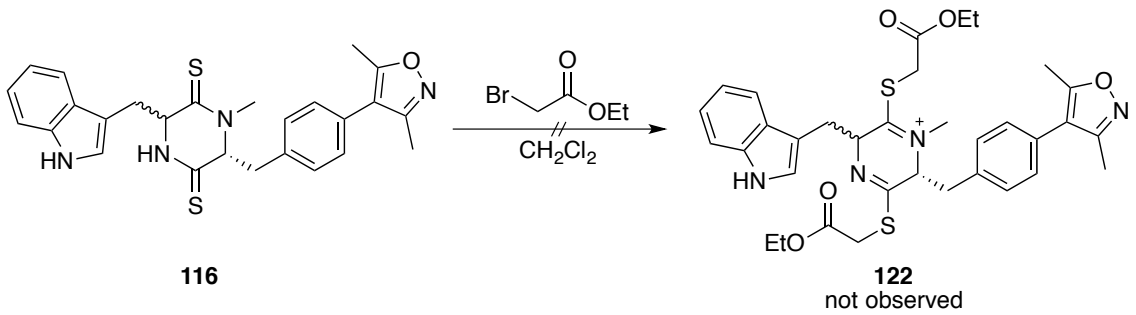
With more forcing conditions, we achieved conversion to the bis-thiolactam **116**, but with concomitant epimerization (see **Scheme 3.18**). This result led us to conclude that access to the second lactam of diketopiperazine **108** is likely very hindered.



108 **116**

Scheme 3.18 Conversion to the bis-thiolactam with concomitant epimerization.

We proceeded to take one epimer of thiolactam **116** and subject it to the bromoacetate alkylation conditions, however, we only observed decomposition of the starting material (see **Scheme 3.19**, below).



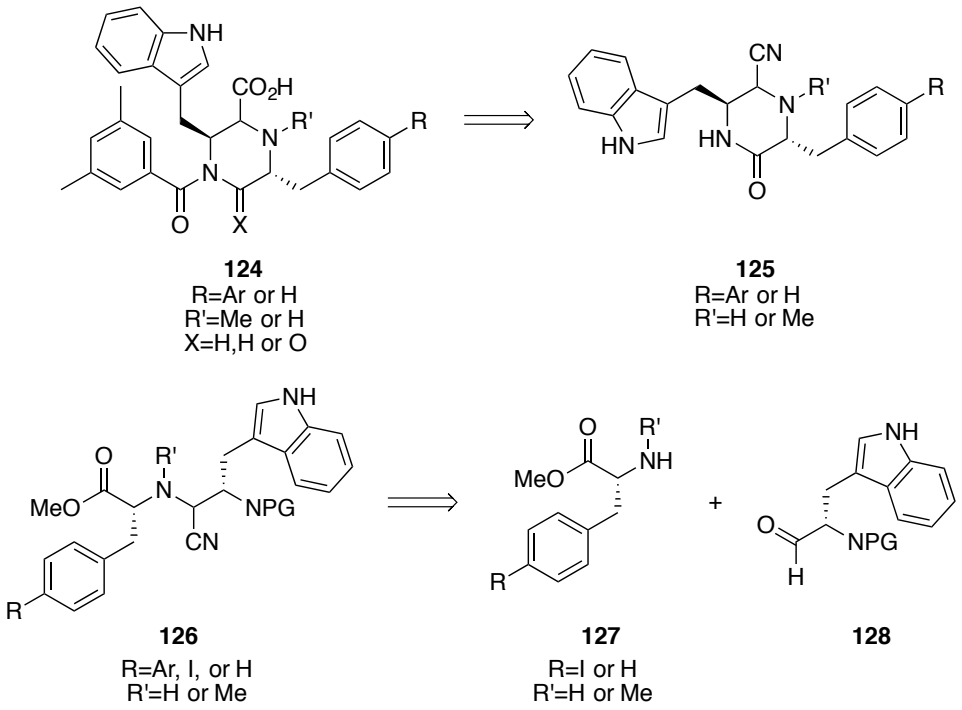
116 **122**
not observed

Scheme 3.19 Attempted thiolactam alkylation with ethyl bromoacetate.

Section 3.2.2 Piperazine derivatives

At this point, we decided to explore other compounds that might similarly overlay with IRL 2500 in the cleft. The most logical analogs we could imagine were mono-acid piperazines **124**, which could be generated from hydrolysis of the nitrile **125**, which could result from

cyclization of nitrile **126** (see **Scheme 3.20**). Nitrile **126** could be derived from a Strecker reaction between *L*-Trp aldehyde **128** and a *D*-Phe amino ester derivative **127**.



124
R=Ar or H
R'=Me or H
X=H,H or O

125
R=Ar or H
R'=H or Me

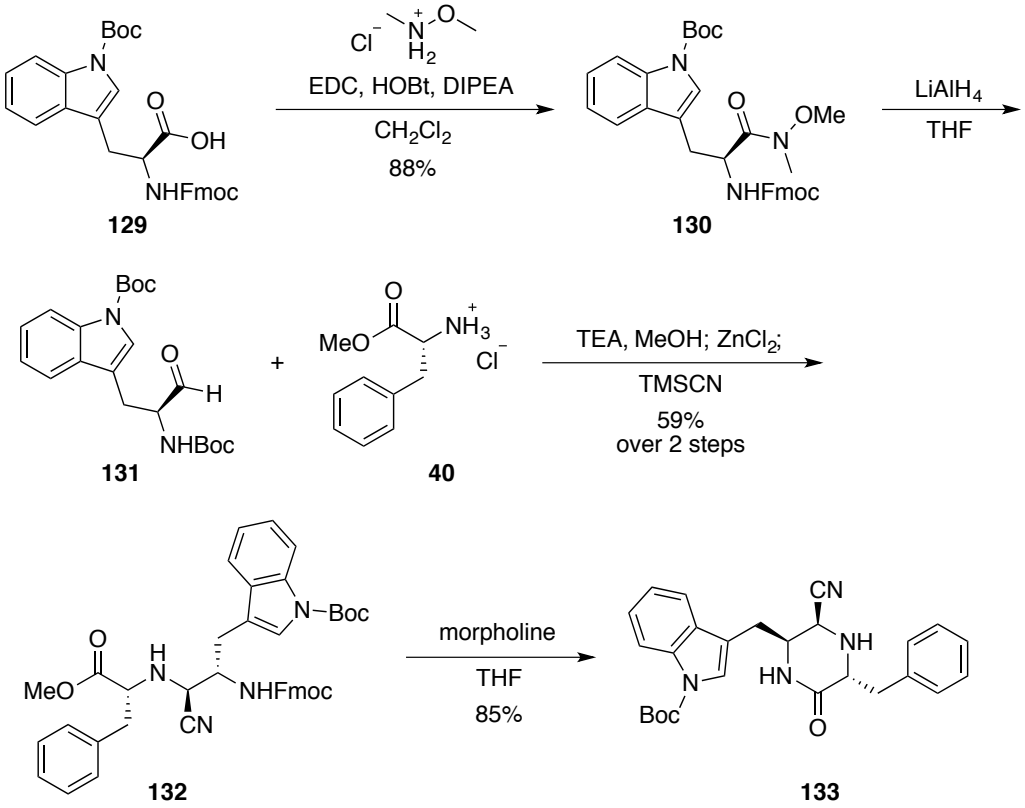
126
R=Ar, I, or H
R'=H or Me

127
R=I or H
R'=H or Me

128

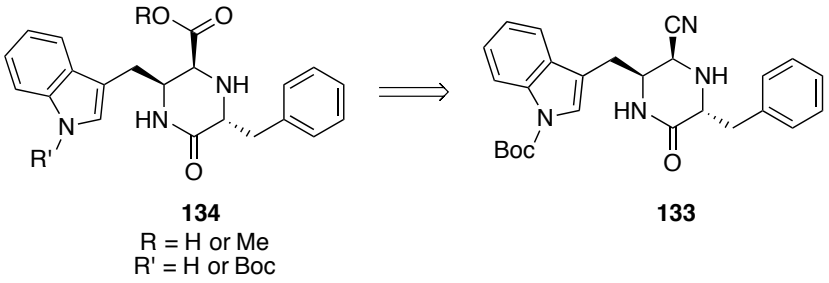
Scheme 3.20 Retrosynthetic analysis of mono acid 124 derived from a Strecker reaction.

Starting from acid **129**, we generated Weinreb amide **130** which was subsequently reduced to aldehyde **131** using known conditions (see **Scheme 3.21**).[11] Aldehyde **131** was taken directly to the Strecker reaction with *D*-Phe methyl ester to generate linear nitrile **132** as a single diastereomer. This pseudo-peptide was then cyclized with morpholine in tetrahydrofuran, to generate ketopiperazine **133**.



Scheme 3.21 Synthesis of cyclized cyano-piperazinone 133.

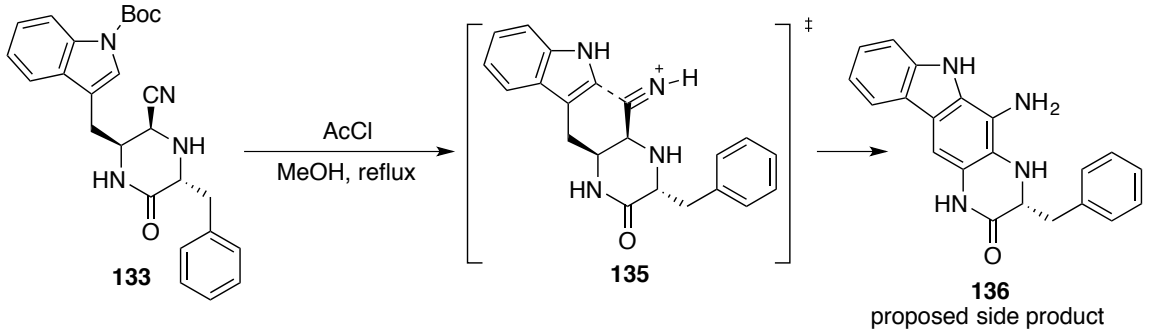
We next decided to screen conditions for the hydrolysis of nitrile **133** to either an ester or acid (**134**, see **Scheme 3.22**). The most well-precedented approach to hydrolyzing nitriles uses strongly acidic conditions.[12,13]



Scheme 3.22 Retrosynthesis of carboxylic acid/ester 134 from nitrile 133.

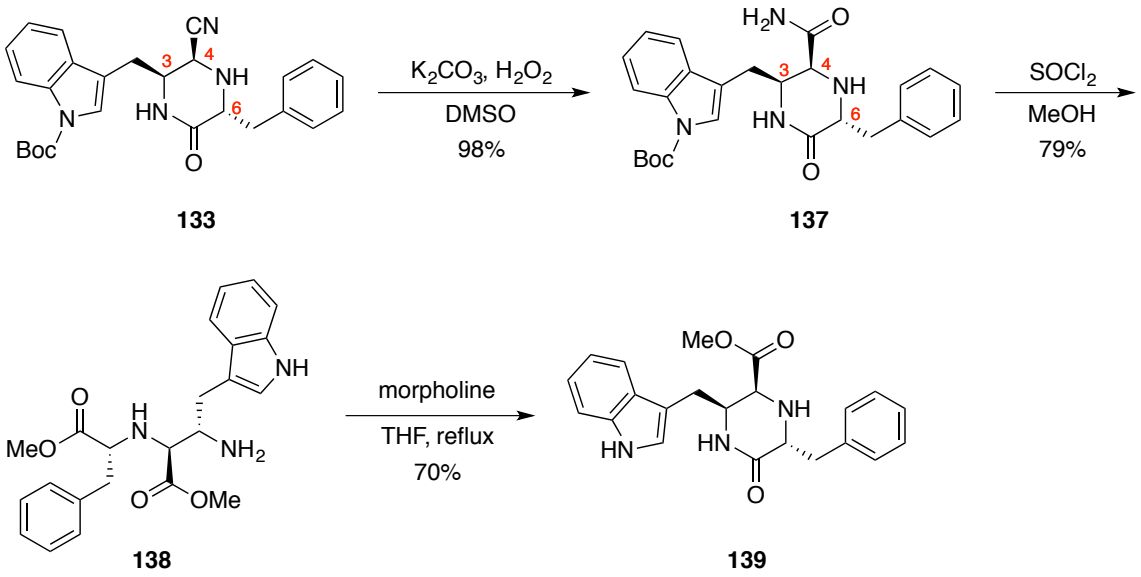
Because of this, we expected that the Boc group would also be deprotected in the same step. For ease of purification, we decided to synthesize the ester instead of the acid. Normally nitrile hydrolysis to the ester is more mild than hydrolysis to the acid. These conditions typically call for a source of hydrochloric acid (HCl) (either thionyl chloride, acetyl chloride or aqueous HCl) and use ethanol or methanol as the solvent.[14]

We employed these conditions with our substrate, but found them to be unsuccessful in hydrolyzing nitrile **133** (see **Scheme 3.23**, below). We instead only observed an inseparable mixture of de-Boc starting material and a side product, which we believed to be the result of indole cyclization and aromatization based on the absence of aliphatic peaks observed in the proton NMR (see proposed side product **136**, **Scheme 3.23**). The proto-nitrile intermediate **135** that is formed during hydrolysis is poised to undergo cyclization which could further undergo aromatization to a β-carboline, leading to several possible undesired side products. Unfortunately, the side product that was observed was not stable to isolation. We therefore decided that acid hydrolysis might prove challenging and that we would pursue alternate methods of hydrolysis.



Scheme 3.23 Attempted acid hydrolysis of nitrile 132 yielded undesired side product tentatively assigned as 135, via intermediate 134.

Base hydrolysis of nitriles to acids is not nearly as well precedented, with relatively few examples in the chemical literature,[15–17] and our attempts at hydrolysis under basic conditions only led to decomposition. Given that direct hydrolysis to the acid or ester was problematic, we hypothesized that hydrolysis to the amide, followed by further hydrolysis to the acid in two discreet steps might be less likely to generate the reactive intermediate **135** observed in **Scheme 3.23**. To that end, we first hydrolyzed nitrile **133** to the amide **137**, which proceeded smoothly (see **Scheme 3.24**, below). Using forcing acid hydrolysis conditions in a sealed vessel, we hydrolyzed the primary amide to the ester, but with concomitant hydrolysis of the secondary amide to generate linear diester **138**. While we were unable to affect selective hydrolysis, re-cyclization of diester **138** led to formation of hydrolysis product **139** of the original nitrile **133**.



Scheme 3.24 Hydrolysis of nitrile 132 to ester 138.

As stated previously, the Strecker reaction leads to the formation of only one of two possible diastereomers. Although the three methine protons of nitrile **133** (3, 4 and 5,

Scheme 3.24) were not well resolved, we observed excellent resolution between the three methines of amide **137** (see **Figure 3.8A**). Based on the observed splitting of methine 4 (J = 4.1 Hz) we deduced that the two adjacent protons 3 and 4 were likely on the same face of the ring. Using NOESY, we observed a non-sequential correlation between methine 4 and one of the protons of methylene 7, further validating this observation. We have used these data to assign the relative stereochemistry of compound **137**, and by reference **132**, **133**, **138** and **139**, as indicated in **Figure 3.8B**, below.

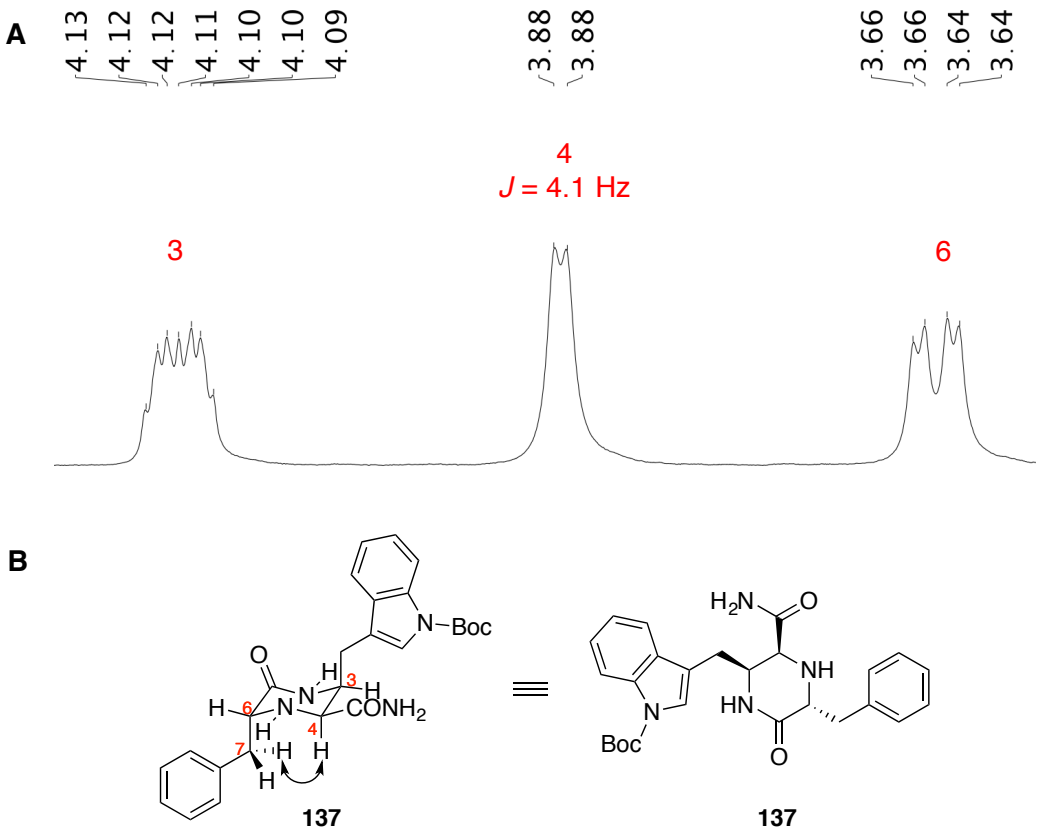
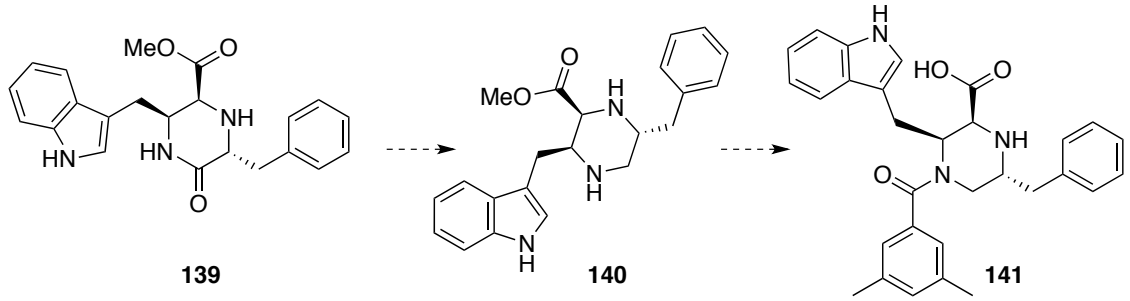


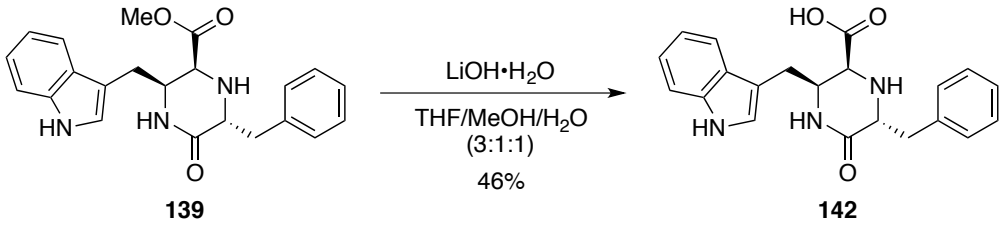
Figure 3.8 A. ^{1}HNMR indicating methine protons. B. NOE observed between methine 4 and methylene 7.

We are currently screening conditions for the reduction of lactam **139** to secondary amine **140**, in order to advance ester **140** to the benzoylated analog **141** (**Scheme 3.25**).



Scheme 3.25 Advancement to tethered analog 141.

However, we also saponified the diaryl ester **139** to acid **142**, to generate another analog, which was sent for biological evaluation (see **Scheme 3.26**, below).



Scheme 3.26 Completion of acid 141 for biological evaluation.

We have modeled acid **142** in the Hb binding site, overlaid on IRL 2500 and it shows good overlap with the indole and biphenyl substituents (see **Figure 3.9**). However, it lacks the dimethylbenzoyl functionality of IRL 1 and 2500.

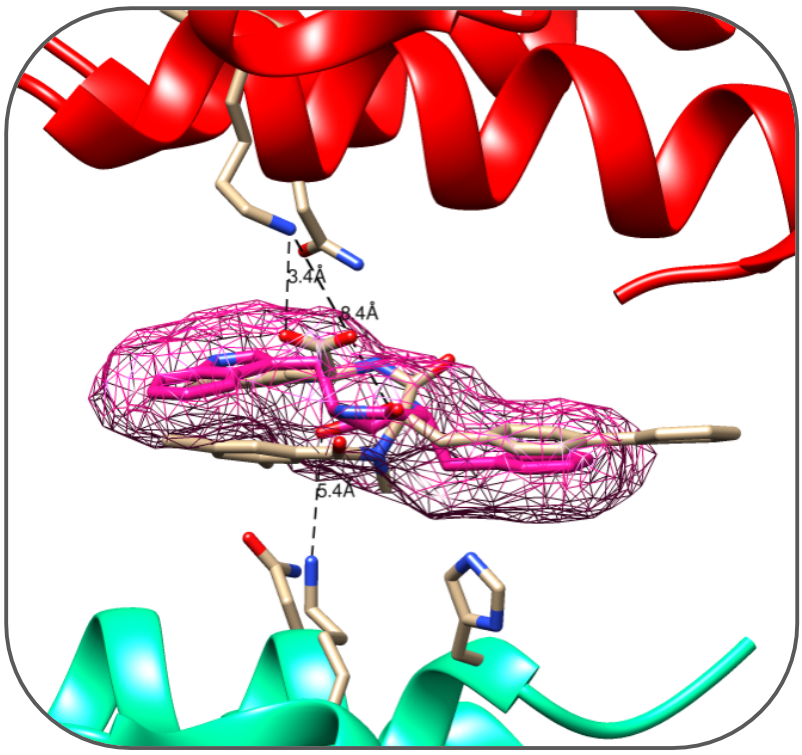
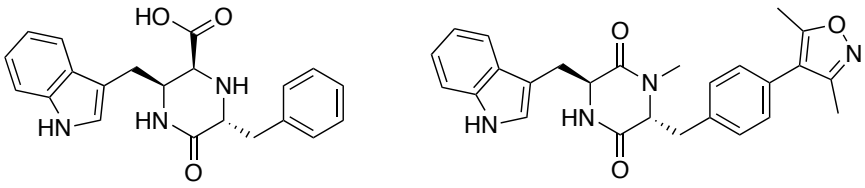


Figure 3.9 Overlay of acid 142 (purple) with IRL 2500 (tan) in β cleft.

Section 3.2.3 Biological results for de novo analogs

We have submitted two piperazine analogs, diketopiperazine **108** and ketopiperazine **142**, for biological evaluation, while working to complete the synthesis of our triaryl acid **141**.



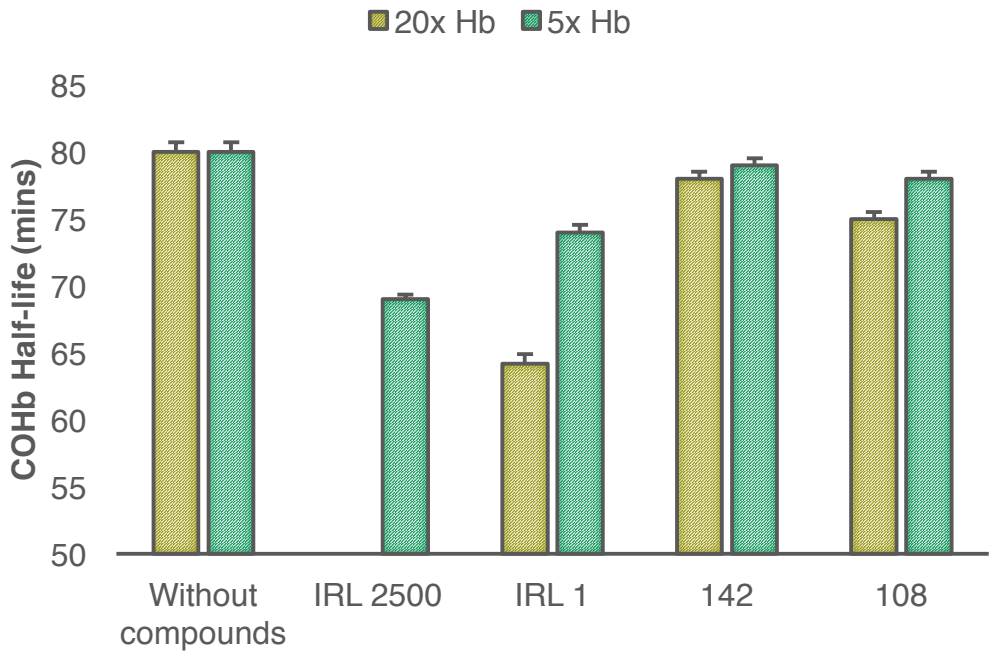
142 **108**

Figure 3.10 Novel analogs screened for biological activity.

Biological assay of these analogs indicate that the cyclic analogs have almost no half-life lowering activity (see **Table 3.12**, below). Given that acid **142** contains the same

carboxylic acid present in IRL 1, but does not show any half-life lowering activity, it is likely that it is unable to interact with the Lys β082 in the cleft. However, the addition of the dimethylbenzoyl ring in the proposed triaryl analog (**141**) may overcome the shortcomings of acid **142**. Diketopiperazine **108** did not cause hemolysis but was not aqueous soluble at the concentration necessary for *in vivo* work. The hemolysis and solubility screening of ketopiperazine **142** is still underway as of this writing.

Table 3.12 COHb half-life results for current *de novo* analogs.
Significance relative to IRL 1 * where P ≤ 0.05, ** where P ≤ 0.01, * where P ≤ 0.001**



SECTION 3.3 CARBON MONOXIDE ON AND OFF RATES

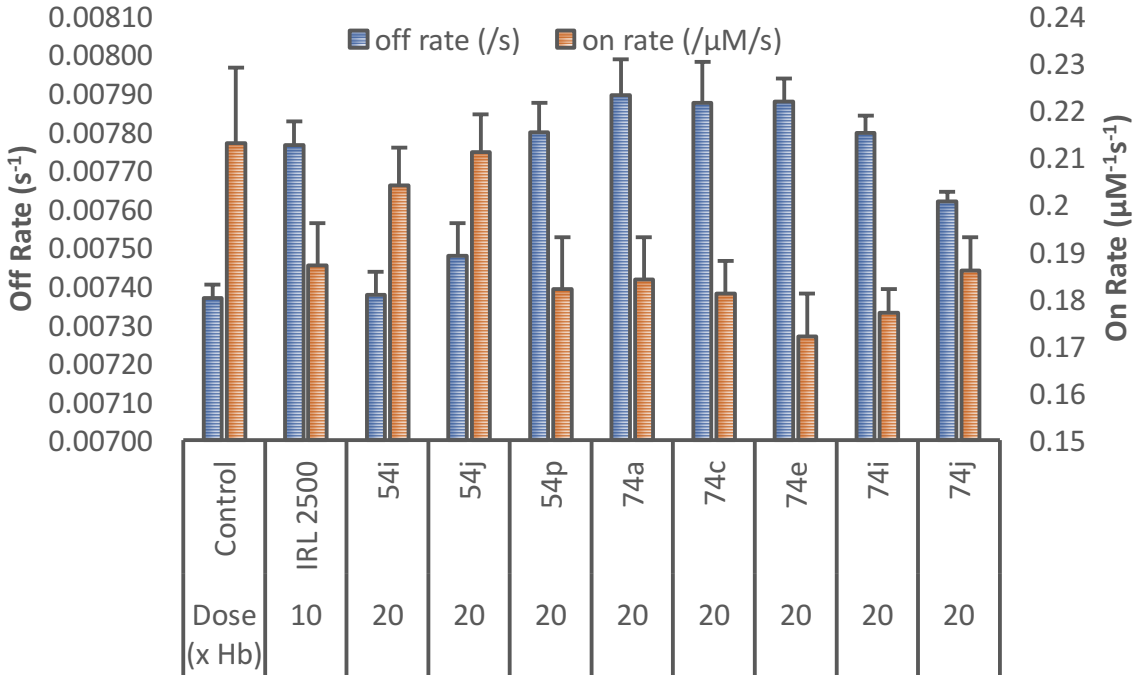
Although we know that the IRL compounds promote the conversion of COHb to oxyHb, we did not know how these compounds affected the kinetics of CO binding to Hb (see **Scheme 3.26**).[18]

$$Hb + CO \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} COHb$$

Scheme 3.27 Reversible binding of CO to Hb.[18]

To determine this, the on and off rates of CO binding to Hb using stop flow spectrometry were measured in the Zapol Laboratory. The CO on rate (k_{on}) and CO off rate (k_{off}) were measured with most potent analogs (**54p**, **74a**, **74c**, **74e**, **74i** and **74j**) and the least potent analogs (**54i** and **54j**) from our COHb half-life assay. The results of this assay indicate that both k_{on} and k_{off} are affected by treatment with the IRL analogs (see **Table 3.13**).

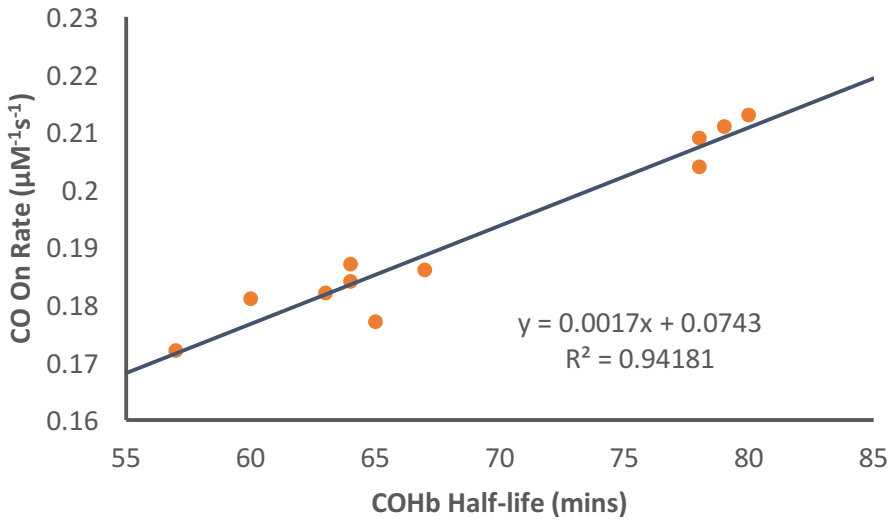
Table 3.13 Summary of CO on and off rates.



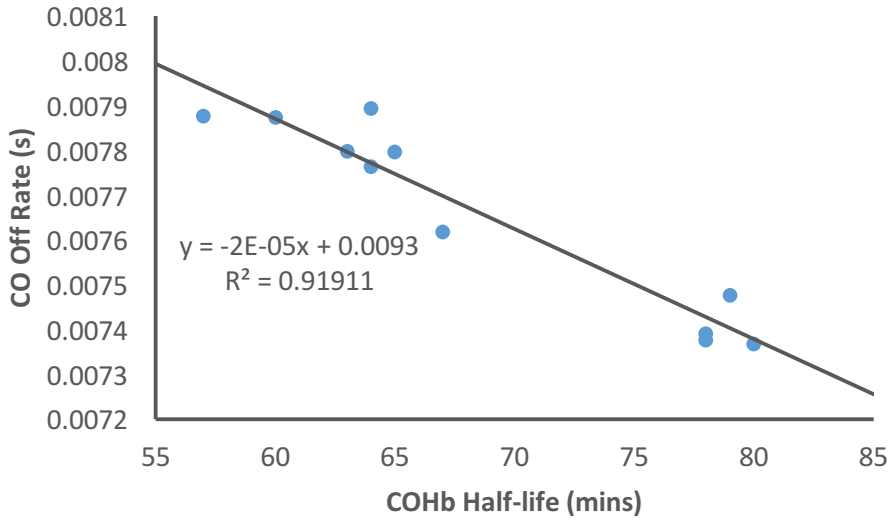
Furthermore, we correlated k_{on} and k_{off} with the COHb half-life data and found that in both cases the R-squared coefficient is above 0.9, indicating a good linear correlation between these two measures of efficacy (see **Table 3.14A** and **B**).

Table 3.14 A. Correlation between COHb half-life and K_{on}. B. Correlation between COHb half-life and K_{off}.

A

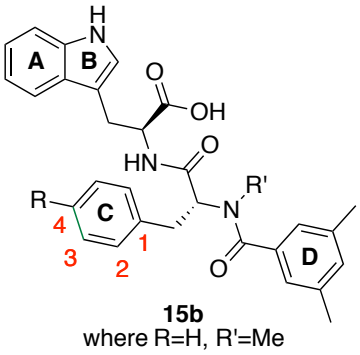


B



These new data further validate our initial COHb half-life results using a different measure of CO binding Hb. In addition, these data indicate that the IRL analogs can both decrease the rate that CO binds to Hb, and also to increase the rate that CO dissociates from Hb.

SECTION 3.4 CONCLUSIONS



15b
where R=H, R'=Me

Figure 3.11 IRL 1

We have prepared 35 analogs, exploring modifications to the carboxylic acid, the A/B rings, the C ring, the D ring and the *N*-methyl amide (see **Figure 3.11**). We chose to explore the functional group tolerance across the entire scaffold, with a focus on decreasing the cLogP to improve aqueous solubility and prevent hemolysis. Our results indicate a delicate balance between adequate hydrophilicity to improve aqueous solubility and prevent hemolysis, and sufficient hydrophobicity to afford potency. For this reason, in our second generation, we have pursued compounds in which the 4-position of the C ring is modified by adding heteroaromatic rings that aid in solubility while retaining the hydrophobic surface area necessary for potency.

With the introduction of the second generation, we demonstrated that we can reduce the COHb half-life significantly relative to IRL 1, without causing hemolysis. Five of the biaryl analogs we assayed, biphenyl **74a**, furan **74c**, isoxazole **74e**, alcohol **74i** and dimethylamine **74j**, are significantly more potent than IRL 1 (see **Figure 3.12**). Additionally, three analogs, biphenyl **74a**, furan **74c** and alcohol **74i** are within the potency range desired to forward them to *in vivo* studies. Of these analogs, furan **74c** and alcohol **74i** also exhibit the solubility necessary for further studies, and alcohol **74i** does not cause hemolysis at high concentrations relative to Hb (5x). For this reason, we are planning to forward alcohol **74i** to a mouse model of carbon monoxide poisoning.

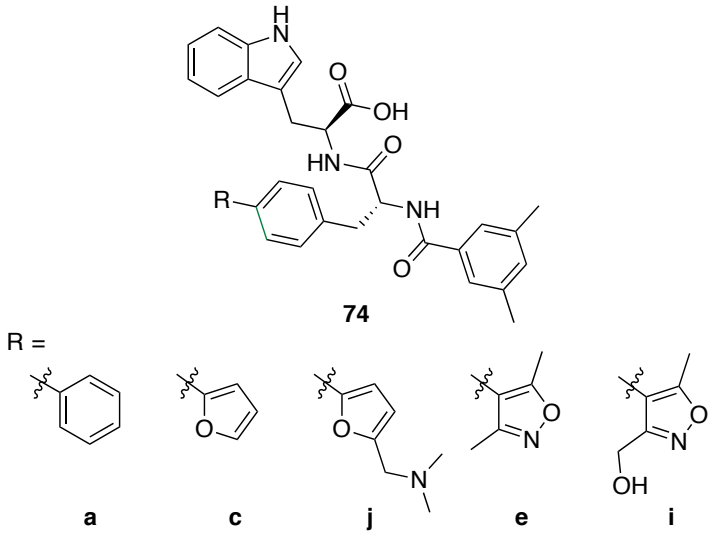


Figure 3.12 Second generation analogs with greater potency than IRL 1.

In addition to these modified IRL analogs, we explored the synthesis of a several novel piperazine-derived analogs that demonstrated good modeled structural overlap with IRL 2500 in the crystal structure. The intermediates in our proposed reaction sequence do not achieve potency comparable to that seen with IRL 1. We hypothesize that introduction of a third ring, mimicking the D ring of IRL 2500, will lead to increased potency. Synthesis of this series is ongoing.

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APPENDIX A. GENERAL METHODS AND EXPERIMENTAL PROCEDURES

SECTION A.1 GENERAL METHODS

Section A.1.1 Measurement of COHb half-life

Carboxyhemoglobin (COHb) was prepared by mixing oxyhemoglobin with CO saturated Dulbecco's phosphate buffered saline (DPBS) buffer. CO buffer was prepared by bubbling DPBS with 1% CO gas (balanced with nitrogen) for 30 minutes. COHb and compounds (dissolved in dimethyl sulfoxide) were mixed in Falcon tubes and then dispensed to a 384-well plate. The final concentration of hemoglobin was 10 μM and dimethyl sulfoxide (DMSO) was 5 vol % in DPBS. The final concentration of tested compound was 200 μM (20 fold excess) or 50 μM (5 fold excess).

The prepared 384 well plate was placed in an inflatable polyethylene chamber (AtmosBag, Sigma) and the chamber was inflated with 1% CO (balanced with nitrogen). The plate was shaken at 2000/minute for 1 hour to equilibrate CO gas with the COHb solution. The plate was then sealed with a plastic seal (Cag. 60941-120, VWR) and centrifuged at 2000g for 10 second. The plastic seal was removed before the plate was transported to the plate reader. Absorption spectrum of solution in each well was measured at from 500nm to 700nm every 15 minutes for 90 minutes using a plate reader (Multiskan Go, Thermo Scientific). COHb percentage of each solution at each time point was calculated by deconvoluting the spectrum. The COHb half-life for each solution was calculated by fitting the COHb percentages at all time points with a single exponential decay equation. Method

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Section A.1.2 Hemolysis assay

Human whole blood was collected under the approval by the IRB of Partners Human Research Committee. After obtaining informed consent from volunteers, blood was drawn from the volunteers into tubes coated with EDTA. The concentration of hemoglobin tetramer of the blood was determined using a blood gas analyzer (ABL 800 FLEX, Radiometer Medical). The blood was diluted using TES buffer and mixed with DMSO and/or compound. The final concentration of hemoglobin tetramer and compound was 50 μM and 1 mM in 0.1 vol % DMSO respectively. The mixture was incubated at 37 °C for 60 minand then the mixture was centrifuged at 2,800g for 20 min. After the centrifugation, the absorption of the supernatant was measured at wavelength of 415 nm and compared to the absorption of 0.5 μM hemoglobin ($A_{415ctrl}$, 0.5 μM of hemoglobin tetramer corresponds to 1% hemolysis of RBCs). If $A_{415sample}$ was smaller than $A_{415ctrl}$, we concluded that hemolysis caused by the tested compound was below 1%. Method provided and used with permission by Liu, C.; Nakagawa, A.; Zapol, W. M.; **2016.** Massachusetts General Hospital.

Section A.1.3 Determination of k_{on}(CO) and k_{off}(CO)

The association and dissociation rate constants of carbon monoxide for hemoglobin k_{on}(CO) and k_{off}(CO) were measured using a stopped flow apparatus (SX20 stopped flow spectrometer, Applied Photophysics, Surrey, UK) at 22^{o}C.

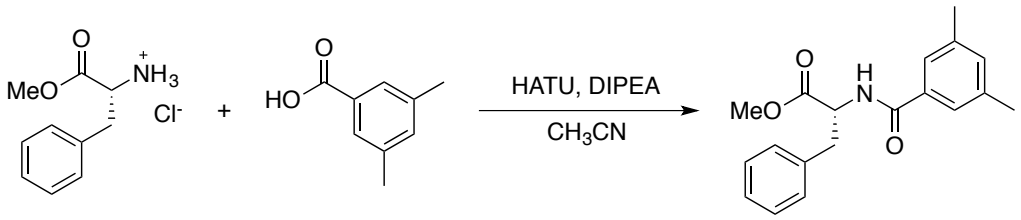
In the association rate constant measurement, the stopped flow instrument was loaded with deoxygenated hemoglobin solution (deoxyHb, 2.5μM in hemoglobin tetramer) and carbon monoxide solution (CO concentration was about 200 μM). A stock solution of deoxyHb (400μM in hemoglobin tetramer) was prepared by purging nitrogen gas to oxyhemoglobin solution for at least 2 hours. Carbon monoxide solution was prepared by bubbling PBS solution using 20% carbon monoxide gas balanced with nitrogen for at least 1 hour. The stopped flow instrument was deoxygenated by flushing the system using deoxygenated PBS for at least three times before using. Before mixing, CO concentration in the CO solution was determined by mixing CO solution with the deoxyHb stock and collecting the spectrum of the mixture from 500nm to 700nm. The CO concentration in the CO solution was calculated using the COHb concentration in the mixture that was determined from deconvolution of the spectrum. Before mixing, PBS was added to one of the syringes and deoxygenated for at least 30 minutes. The deoxyHb stock and compound/DMSO were added to the deoxygenated PBS in this syringe to get 2.5μM deoxyHb, 50μM compound (25μM for IRL2500) with 2vol% DMSO. CO solution was loaded to the other syringe. The loaded deoxyHb solution and CO solution were mixed and the absorbance of the measurement at 436 nm (A436) was recorded for 0.3 seconds. Three mixings were recorded for each repeat and four repeats were measured for each compound or control. The value of apparent rate was determined by fitting the time course

of A436 with a single exponential equation using Pro-Data Viewer (version 4.2.16, Applied Photophysics, Surrey, UK). The kon(CO) was obtained by dividing the apparent rate by the CO concentration after mixing (half of the CO concentration in the CO saturated solution).

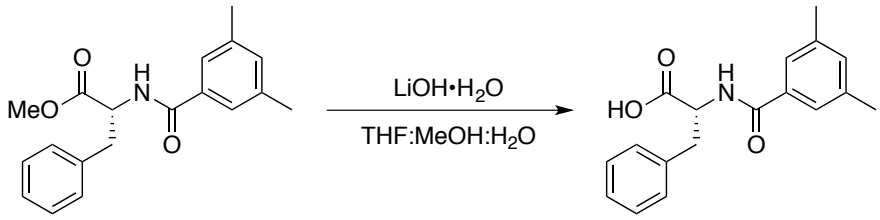
In the dissociation rate constant measurement, the stopped flow instrument was loaded with carboxyhemoglobin solution (COHb, 20μM in hemoglobin tetramer) and nitric oxide (NO) solution. Compound or DMSO was added to PBS solution and bubbled using 10% CO gas (balanced with nitrogen) for 30 minutes at room temperature. Oxyhemoglobin was added to the CO saturated PBS solution to obtain COHb. The final DMSO percentage was 2 vol% and concentrations of hemoglobin and compound before mixing were 20μM and 400μM (200μM for IRL 2500), respectively. The absorption spectrum of the sample was measured from 500 to 700nm and the formation of COHb was confirmed by deconvoluting the measured spectrum. Aqueous solution of NO was obtained by adding MAHMANONOate (50μL, 20mg/mL in 0.01M NaOH) to 3 mL deoxygenated DPBS solution right before the mixing. The loaded COHb solution and NO solution were mixed and the absorbance of the measurement at 584 nm (A584) was recorded for 600 seconds. Three mixings were recorded for each repeat and four repeats were measured for each compound or control. The value of k_{off}(CO) was determined by fitting the time course of A584 using single exponential equation using Pro-Data Viewer (version 4.2.16, Applied Photophysics, Surrey, UK). Method provided and used with permission by Liu, C.; Nakagawa, A.; Zapol, W. M.; **2016.** Massachusetts General Hospital.

SECTION A.2 EXPERIMENTAL PROCEDURES

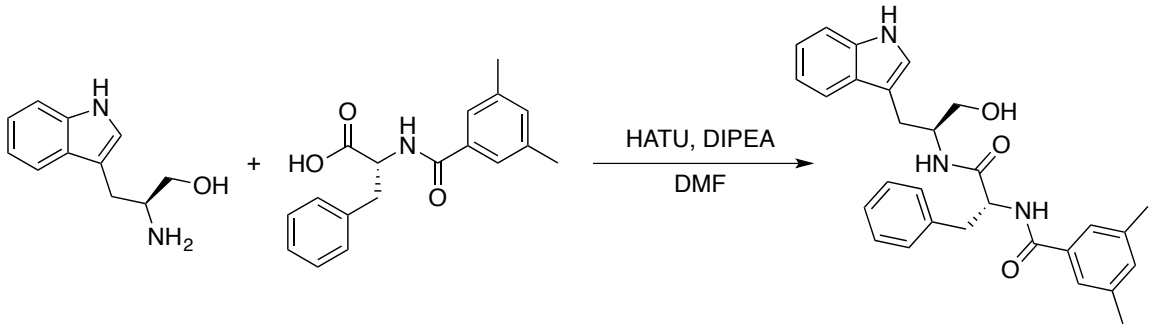
General. Solvents used for extraction and purification were HPLC grade from Fisher. Unless otherwise indicated, all reactions were run under an inert atmosphere of argon. Room temperature is considered 20 °C, unless otherwise noted. Merck pre-coated silica gel plates (250 mm, 60 F254) were used for analytical TLC. Spots were visualized using 254 nm ultraviolet light. Chromatographic purifications were performed on Sorbent Technologies silica gel (particle size 32-63 microns). ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, in d6-DMSO, $CDCl_3$ and MeOD on a Bruker AM-500 or a DRX-500 spectrometer. Chemical shifts are reported relative to internal d6-DMSO (δ 2.54 for ^{1}H), $CDCl_3$ (δ 7.26 for ^{1}H), MeOD (δ 3.34 for ^{1}H). Infrared spectra were recorded as a solid using a Perkin-Elmer 1600 series Fourier transform spectrometer. UV/VIS spectra were obtained using a Varian-530. High resolution mass spectra were obtained by Dr. Rakesh Kohli and Dr. Charles Ross at the University of Pennsylvania Mass Spectrometry Service Center on an Autospec high resolution double-focusing electrospray ionization/chemical ionization spectrometer with either DEC 11/73 or OPUS software data system. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected.



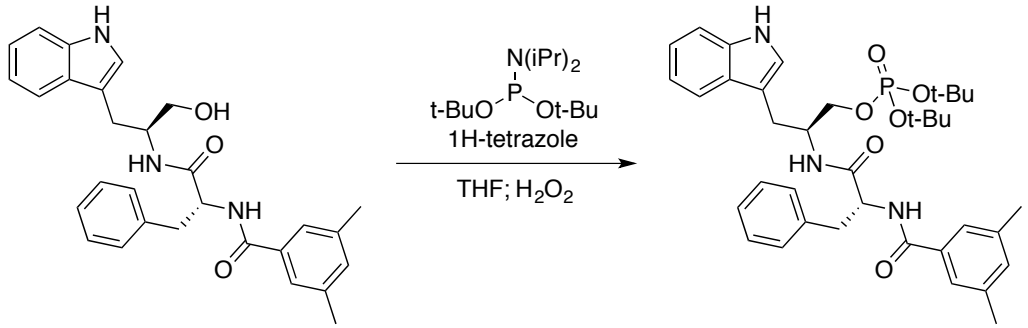
Methyl (3,5-dimethylbenzoyl)-D-*phenylalaninate* (**44**). To *D*-phenylalanine methyl ester[1] (1.0 g, 5 mmol, 1 eq) was added 3,5-dimethylbenzoic acid (0.75 g, 5 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (4.4 mL, 25 mmol, 5 eq) and anhydrous acetonitrile (16.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (2.9 g, 7.5 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as an orange foam. This residue was purified by flash chromatography (20% EtOAc/Hexanes) to afford the product as an off-white foam (1.53 g, 98%). IR (solid) 1737, 1643, 1604, 1534, 1495 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.32 (d, *J* = 1.5 Hz, 2H), 7.31 – 7.26 (m, 2H), 7.13 (dt, *J* = 5.5, 1.6 Hz, 3H), 6.53 (d, *J* = 7.6 Hz, 1H), 5.09 (dt, *J* = 7.6, 5.6 Hz, 1H), 3.77 (s, 3H), 3.29 (dd, *J* = 13.8, 5.8 Hz, 1H), 3.22 (dd, *J* = 13.8, 5.3 Hz, 1H), 2.34 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.28, 167.28, 138.46, 136.04, 134.02, 133.53, 129.53, 128.72, 127.31, 124.91, 53.62, 52.55, 38.11, 21.38. HRMS (ESI) m/z calc'd for $C_{20}H_{22}N_2O_4$ $[M+Na]^+$ 377.1461, found 377.1467.



(3,5-Dimethylbenzoyl)-D-*phenylalanine* (**39**). To a stirring solution of **44** (311 mg, 1 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 15 mL, 0.067 M), lithium hydroxide monohydrate (419 mg, 10 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 1N hydrochloric acid was added until a pH of 4 was reached. The aqueous was two times extracted with ethyl acetate (30 mL). The organic layer was washed twice with brine (30 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the product as a white foam (300 mg, quant). IR (solid) 1709, 1643, 1601, 1532, 1496 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.42 (s, 1H), 7.34 – 7.23 (m, 5H), 7.20 (d, *J* = 7.2 Hz, 2H), 7.13 (s, 1H), 6.65 – 6.55 (m, 1H), 5.08 (q, *J* = 7.4, 5.8 Hz, 1H), 3.37 (dd, *J* = 14.0, 5.6 Hz, 1H), 3.26 (dd, *J* = 14.0, 5.7 Hz, 1H), 2.32 (d, *J* = 2.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.13, 168.30, 138.55, 135.85, 133.83, 133.53, 129.61, 128.80, 127.40, 124.99, 53.81, 37.46, 21.34. HRMS (ESI) m/z calc'd for $C_{18}H_{19}NO_3$ $[M+H]^+$ 298.1443, found 298.1440.

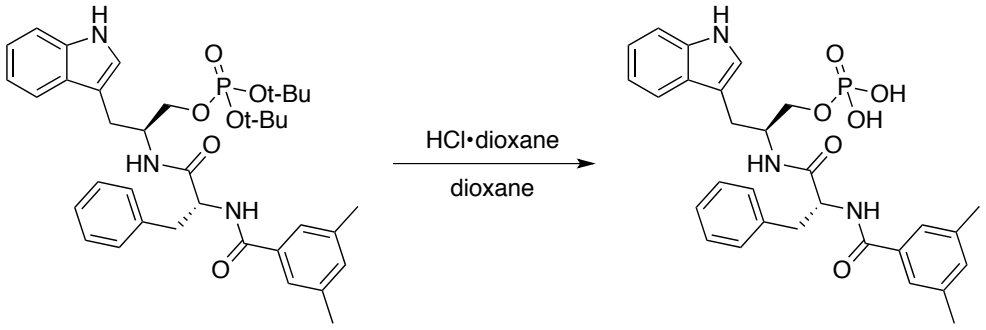


N-*((*R*)-1-(((*S*)-1-hydroxy-3-(1*H*-indol-3-yl)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-3,5-dimethylbenzamide* (**42**). To *L*-tryptophanol[4,5] (95 mg, 0.5 mmol, 1 eq) was added **44** (148 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (440 μL, 2.5 mmol, 5 eq) and anhydrous *N,N*-dimethylformamide (1.7 mL, 0.3 M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 0.75 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over cold water (100 mL) and filtered. The solid was then dissolved in dichloromethane. Organic was then concentrated to dryness *in vacuo* to afford the product an off-white foam (240 mg, quant). IR (thin film) 3390, 2922, 1627, 1598, 1453 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.61 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.29 (s, 2H), 7.15 (dd, J = 5.5, 1.7 Hz, 4H), 7.10 – 7.04 (m, 3H), 7.03 (s, 1H), 7.00 (t, J = 7.3 Hz, 1H), 4.75 (dd, J = 8.3, 6.0 Hz, 1H), 4.22 (p, J = 6.0 Hz, 1H), 3.61 – 3.53 (m, 2H), 2.99 (td, J = 15.2, 14.5, 6.3 Hz, 2H), 2.84 (ddd, J = 17.0, 14.1, 8.0 Hz, 2H), 2.31 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 172.08, 169.00, 138.10, 137.08, 136.87, 133.90, 132.97, 129.13, 128.06, 127.71, 126.42, 124.79, 122.95, 121.05, 118.42, 118.21, 114.65, 110.97, 63.01, 55.30, 52.27, 37.76, 26.27, 19.96. HRMS (ESI) m/z calc'd for $C_{29}H_{31}N_3O_3$ $[M+H]^+$ 470.2444, found 470.2430.

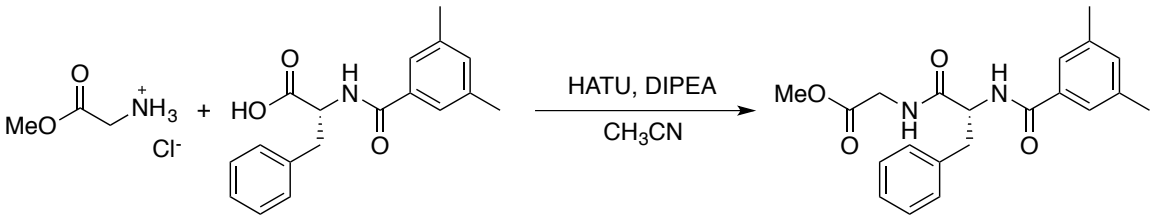


Di-tert-*butyl ((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-phenylpropanamido)-3-(*1H*-indol-3-yl)propyl) phosphate* (**46**) To a stirring solution of alcohol **42** (235 mg, 0.5 mmol, 1 eq) in anhydrous tetrahydrofuran (3 mL, 0.18 M), 1*H*-tetrazole (3 wt%, 3 mL, 1 mmol, 2 eq) was added followed by di-*tert*-butyl *N,N*-diisopropylphosphoramidite (240 μL, 0.75 mmol, 1.5 eq). After stirring for 18 hours at room temperature (20 °C), reaction was cooled to 0 °C and hydrogen peroxide (30 % wt/vol in water, 250 μL, 2.2 mmol, 4.4 eq) was added. Reaction was warmed to room temperature for one hour, then quenched with 10% sodium sulfite solution (6 mL). Partitioned between ethyl acetate (10 mL) and water (10 mL), the organic layer was then washed with brine, dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the product as a crude off-white foam. This residue was purified by flash chromatography (1-3% MeOH/CH_2Cl_2) followed by a prep plates (2-4% MeOH/CH_2Cl_2) to afford the product as an off-white foam (146 mg, 44%). IR (thin film) 3280, 3060, 2980, 1652, 1602 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.22 (s, 1H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.40 – 7.32 (m, 3H), 7.23 – 7.15 (m, 6H), 7.15 – 7.08 (m, 2H), 7.03 (s, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 6.89 (s, 1H), 4.90 (q, *J* = 7.7, 6.7 Hz, 1H), 4.37 (ddt, *J* = 12.2, 8.0, 4.3 Hz, 1H), 3.97 – 3.83 (m, 2H), 3.18 (d, *J* = 6.6 Hz, 2H), 3.01 (dd, *J* = 14.4, 5.3 Hz, 1H), 2.85 (dd, *J* = 14.4, 9.0 Hz, 1H), 2.33 (s, 6H), 1.44 (d, *J* = 6.4 Hz, 18H). ^{13}C NMR (126 MHz, CDCl3) δ 170.63, 167.23, 138.26, 136.77, 136.41, 134.12, 133.33, 129.66, 128.67, 127.63, 127.07, 125.05, 123.20, 122.29, 119.78, 119.06, 111.38, 111.30,

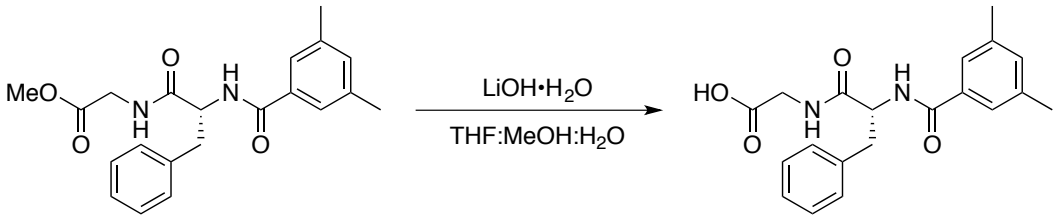
83.01 (dd, *J* = 7.4, 3.4 Hz), 66.69, 54.92, 50.25 (d, *J* = 5.6 Hz), 39.14, 29.98 (d, *J* = 4.0 Hz), 26.50, 21.33. ^{31}P NMR (203 MHz, $CDCl_3$) δ -8.66. HRMS (ESI) m/z calc'd for $C_{37}H_{48}N_3O_6P$ $[M+H]^+$ 662.3359, found 662.3392.



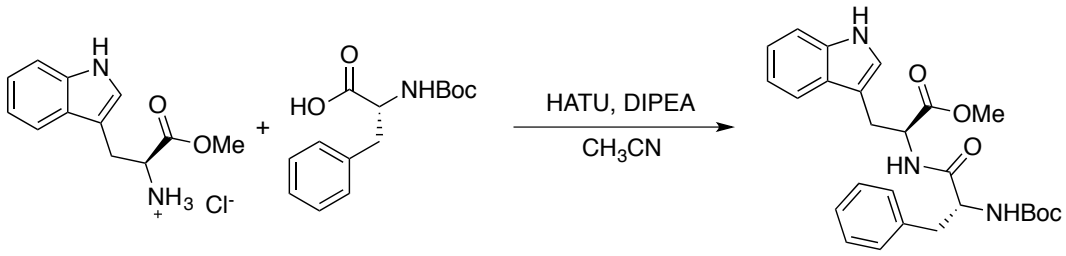
*(*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-phenylpropanamido)-3-(*1H*-indol-3-yl)propyl dihydrogen phosphate* (**41**). To a flask containing phosphate ester **46** (73 mg, 0.1 mmol, 1 eq) in 1,4-dioxane (200 μL, 0.55 M), HCl in dioxane (4 M, 67 μL, 0.24 mmol, 2.4 eq) was added. After four hours stirring at room temperature (20 °C), a precipitate had formed. The suspension was concentrated *in vacuo*, resuspended in dichloromethane and concentrated *in vacuo* a second time to yield the product as an off-white film (56 mg, 92%) without further purification. IR (solid) 3279, 2923, 1636, 1602, 1515 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.60 (d, *J* = 7.9 Hz, 1H), 7.37 – 7.23 (m, 3H), 7.20 – 6.93 (m, 9H), 4.78 (s, 1H), 4.38 (s, 1H), 4.00 (d, *J* = 5.3 Hz, 2H), 2.96 (dd, *J* = 67.1, 10.8 Hz, 4H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.39, 170.31, 139.35, 138.26, 138.12, 135.07, 134.24, 130.38, 129.34, 128.82, 127.69, 126.06, 124.47, 122.39, 119.81, 119.38, 112.29, 111.49, 68.11, 56.55, 51.66, 39.02, 27.36, 21.23. ^{31}P NMR (203 MHz, $CDCl_3$) δ 0.66. HRMS (ESI) m/z calc'd for $C_{29}H_{32}N_3O_6P$ $[M-H]^-$ 548.1950, found 548.1969.



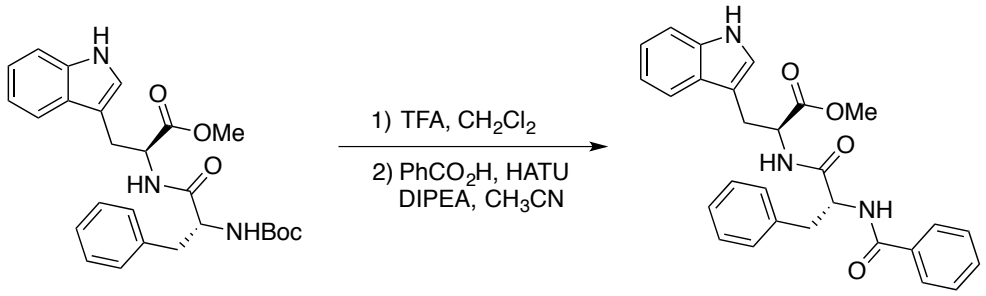
Methyl (3,5-dimethylbenzoyl)-D-*phenylalanylglycinate* (**53a**) To **39** (297 mg, 1 mmol, 1 eq) was added glycine methyl ester hydrochloride (125 mg, 1 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (870 μL, 5 mmol, 5 eq) and anhydrous acetonitrile (3 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (570 mg, 1.5 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as an orange foam. This residue was purified by flash chromatography (10-50% EtOAc/Hexanes) to afford the product as an off-white foam (114 mg, 31%). IR (solid) 1746, 1658, 1633, 1600, 1570 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.29 (q, *J* = 7.1 Hz, 6H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.12 (s, 1H), 6.73 (s, 1H), 6.45 (s, 1H), 4.90 (q, *J* = 7.2 Hz, 1H), 4.07 – 3.89 (m, 2H), 3.72 (d, *J* = 1.6 Hz, 3H), 3.31 – 3.12 (m, 2H), 2.33 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.27, 169.79, 167.81, 138.47, 136.65, 133.75, 133.60, 129.49, 128.88, 127.26, 124.93, 54.78, 52.49, 41.37, 38.37, 21.32. HRMS (ESI) m/z calc'd for $C_{21}H_{24}N_2O_4$ $[M+H]^+$ 369.1814, found 369.1818.



SRG-III-091: *(3,5-dimethylbenzoyl)*-D-*phenylalanylglycine* (**51a**). To a stirring solution of **53a** (60 mg, 0.16 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 0.7 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 1N hydrochloric acid was added until a pH of 4 was reached. The aqueous was then extracted two times with ethyl acetate (10 mL). The organic layer was washed twice with brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the product as a white foam (57.7 mg, quant). IR (thin film) 3292, 2922, 1726, 1633, 1600 cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ 7.32 – 7.28 (m, 4H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.20 – 7.15 (m, 1H), 7.12 (s, 1H), 4.90 (dd, *J* = 9.7, 5.1 Hz, 1H), 3.93 (s, 2H), 3.35 – 3.31 (m, 1H), 3.05 (dd, *J* = 13.9, 9.7 Hz, 1H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.19, 172.88, 170.45, 139.27, 138.74, 135.10, 134.19, 130.35, 129.39, 127.69, 126.14, 56.34, 41.97, 38.73, 21.25. HRMS (ESI) m/z calc'd for $C_{20}H_{22}N_2O_4$ $[M+Na]^+$ 377.1461, found 377.1467.

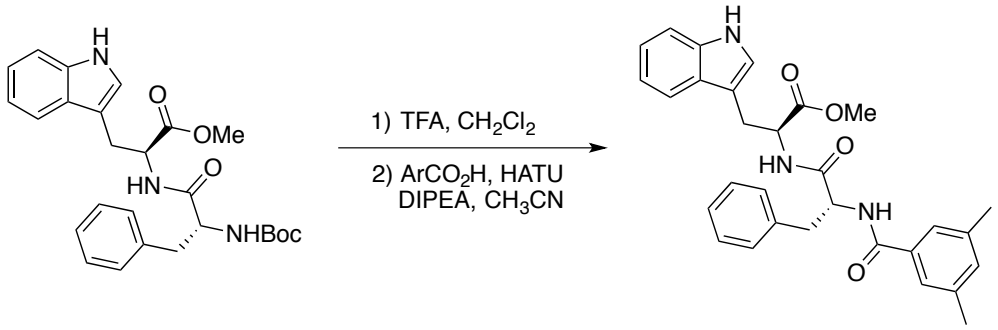


Methyl (tert-butoxycarbonyl)-D-*phenylalanyl*-L-*tryptophanate* (**55**). To (tert-butoxycarbonyl)-*D*-phenylalanine[3] (2.65 g, 10 mmol, 1 eq) was added tryptophan methyl ester hydrochloride[2] (2.54 g, 10 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (9 mL, 50 mmol, 5 eq) and anhydrous acetonitrile (33 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (5.7 g, 15 mmol, 1.5 eq) was added. The reaction mixture was quenched with saturated ammonium chloride (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed twice with deionized water (50 mL), followed by brine (50 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as an orange foam. This residue was purified through a plug of silica (20% EtOAc/Hexanes) to afford the product as a white foam (4.65 g, quant). IR (solid) 3342, 1733, 1684, 1658, 1544 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.22 (dd, *J* = 12.6, 6.9 Hz, 3H), 7.17 (t, *J* = 7.7 Hz, 1H), 7.15 – 7.04 (m, 3H), 6.76 (s, 1H), 6.45 (s, 1H), 4.97 (s, 1H), 4.85 (q, *J* = 6.3 Hz, 1H), 4.35 (s, 1H), 3.61 (s, 3H), 3.25 (dd, *J* = 15.1, 5.6 Hz, 1H), 3.13 (dd, *J* = 14.8, 5.6 Hz, 1H), 3.05 (dd, *J* = 13.8, 7.0 Hz, 1H), 2.98 (s, 1H), 1.36 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.12, 171.03, 155.47, 136.80, 136.25, 129.48, 128.75, 127.49, 127.03, 122.99, 122.39, 119.84, 118.58, 111.37, 109.81, 80.28, 55.96, 52.80, 52.46, 38.57, 28.36, 27.75. HRMS (ESI) m/z calc'd for $C_{26}H_{31}N_3O_5$ $[M+H]^+$ 466.2342, found 466.2361.



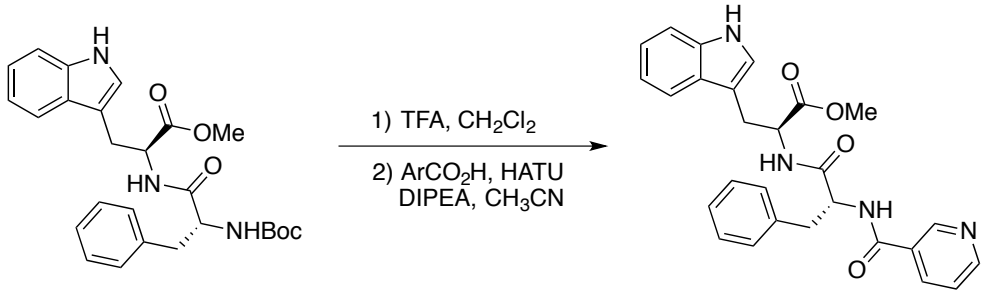
Methyl benzoyl-D-*phenylalanyl*-L-*tryptophanate* (**56a**). To a stirring solution of **55** (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.04 M), trifluoroacetic acid (160 μL, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added benzoic acid (26 mg, 0.2 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (220 μL, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (83 mg, 82%). IR (thin film) 3515, 3445, 1735, 1632, 1530 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.15 (s, 1H), 7.64 (d, *J* = 6.7 Hz, 2H), 7.46 (dd, *J* = 13.6, 7.8 Hz, 2H), 7.37 (q, *J* = 6.9 Hz, 2H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.20 (s, 3H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.13 – 7.04 (m, 3H), 6.76 (d, *J* = 2.4 Hz, 2H), 6.59 (s, 1H), 4.90 – 4.80 (m, 2H), 3.60 (s, 3H), 3.25 (dd, *J* = 14.8, 5.4 Hz, 1H), 3.19 – 3.08 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.87, 170.46, 167.10, 136.33, 136.03, 133.62, 131.69,

129.29, 128.56, 128.48, 127.16, 126.94, 126.89, 122.89, 122.19, 119.60, 118.30, 111.20, 109.50, 54.70, 52.63, 52.28, 38.24, 27.39. HRMS (ESI) m/z calc'd for $C_{28}H_{27}N_3O_4$ $[M+H]^+$ 470.2080, found 470.2085.



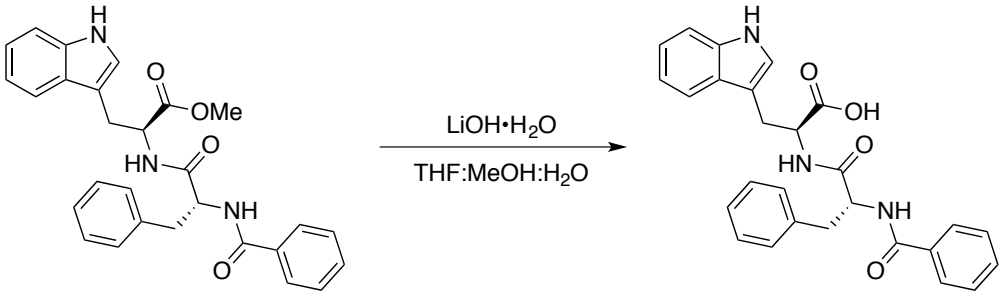
Methyl (3,5-dimethylbenzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56b**). To a stirring solution of **55** (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.04 M), trifluoroacetic acid (160 μL, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 3,5-dimethylbenzoic acid (33 mg, 0.2 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (220 μL, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (88 mg, 82%). IR (thin film) 3290, 1734, 1654, 1636, 1602 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 8.38

(s, 1H), 7.49 – 7.40 (m, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.25 (s, 2H), 7.21 – 7.12 (m, 4H), 7.12 – 7.02 (m, 4H), 6.84 (t, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 2.3 Hz, 1H), 4.90 (q, *J* = 7.0 Hz, 1H), 4.83 (q, *J* = 6.5 Hz, 1H), 3.59 (s, 3H), 3.24 (dd, *J* = 14.7, 6.5 Hz, 1H), 3.14 (ddd, *J* = 24.2, 12.0, 5.9 Hz, 3H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.16, 170.88, 167.74, 138.33, 136.58, 136.28, 133.81, 133.46, 129.50, 128.66, 127.35, 127.05, 124.89, 123.26, 122.25, 119.68, 118.45, 111.45, 109.65, 54.83, 52.95, 52.45, 38.49, 27.58, 21.25. HRMS (ESI) m/z calc'd for $C_{30}H_{31}N_3O_4$ $[M+H]^+$ 498.2393, found 498.2396.

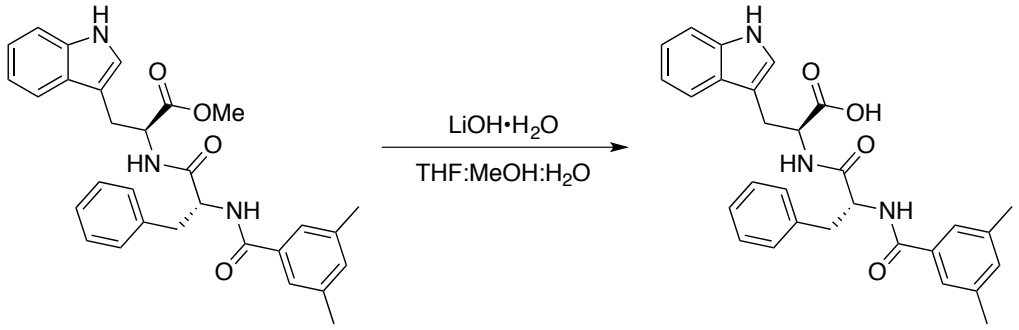


Methyl nicotinoyl-D-*phenylalanyl*-L-*tryptophanate* (**56d**). To a stirring solution of **55** (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.04 M), trifluoroacetic acid (160 μL, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added nicotinic acid (26 mg, 0.2 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (220 μL, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over

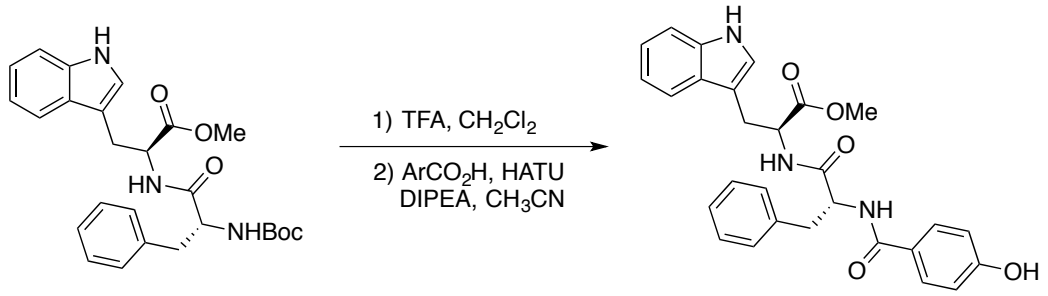
anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (101 mg, quant.). IR (solid) 3287, 1733, 1660, 1634, 1595 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.88 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.73 (d, *J* = 8.7 Hz, 1H), 8.67 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.63 (d, *J* = 7.9 Hz, 1H), 8.07 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.46 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.27 (d, *J* = 7.4 Hz, 3H), 7.21 (t, *J* = 7.5 Hz, 2H), 7.19 – 7.10 (m, 2H), 7.05 (t, *J* = 7.6 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 4.77 (d, *J* = 9.8 Hz, 1H), 4.63 – 4.55 (m, 1H), 3.62 (s, 3H), 3.19 (dd, *J* = 14.5, 5.2 Hz, 1H), 3.05 (dd, *J* = 14.5, 8.7 Hz, 1H), 2.92 – 2.85 (m, 1H), 2.80 – 2.71 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 172.85, 171.71, 165.32, 152.49, 149.06, 138.72, 136.71, 135.63, 130.12, 129.76, 128.58, 127.62, 126.83, 124.51, 123.93, 121.56, 119.01, 118.60, 112.01, 109.86, 55.15, 53.58, 52.52, 37.90, 27.90. HRMS (ESI) m/z calc'd for $C_{27}H_{26}N_4O_4$ $[M+H]^+$ 471.2032, found 471.2035.



Benzoyl-D-phenylalanyl-L-tryptophan (**54a**). To a stirring solution of **56a** (83 mg, 0.17 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 0.7 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (72.8 mg, 90%). m.p. = 110-120 °C. IR (solid) 3300, 2925, 1721, 1634, 1520 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.05 (d, J = 7.9 Hz, 1H), 7.91 – 7.83 (m, 2H), 7.62 (d, J = 8.4 Hz, 1H), 7.59 – 7.42 (m, 4H), 7.39 (t, J = 7.7 Hz, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.10 (dd, J = 4.9, 1.9 Hz, 3H), 7.06 (t, J = 8.1 Hz, 1H), 7.03 – 6.99 (m, 2H), 6.96 (t, J = 7.9 Hz, 1H), 4.89 – 4.80 (m, 0H), 4.75 (td, J = 8.0, 4.9 Hz, 1H), 3.37 – 3.32 (m, 1H), 3.18 (dd, J = 14.7, 8.1 Hz, 1H), 3.08 (dd, J = 13.8, 5.5 Hz, 1H), 2.85 (dd, J = 8.6, 5.3 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.67, 171.94, 168.57, 136.90, 133.78, 131.57, 131.43, 128.95, 128.14, 128.12, 127.34, 126.94, 126.30, 123.29, 121.13, 118.59, 117.87, 111.01, 109.28, 54.93, 54.89, 53.16, 37.43, 27.10. HRMS (ESI) m/z calc'd for $C_{27}H_{25}N_3O_4$ $[M+H]^+$ 4561923, found 456.1938.

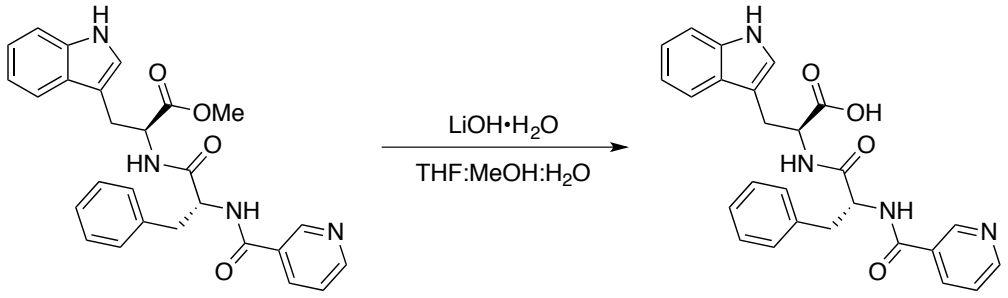


(3,5-Dimethylbenzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54b**). To a stirring solution of **56b** (88 mg, 0.18 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 0.7 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (85.5 mg, 81%). m.p. = 110-120 °C.IR (solid) 2918, 1633, 1601, 1520, 1456 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.55 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.21 (s, 2H), 7.14 – 6.90 (m, 10H), 4.76 (p, J = 4.0 Hz, 1H), 3.52 – 3.43 (m, 1H), 3.37 – 3.30 (m, 1H), 3.18 (dd, J = 14.9, 8.1 Hz, 1H), 3.08 (dd, J = 14.0, 5.5 Hz, 1H), 2.84 (dt, J = 13.3, 5.3 Hz, 1H), 2.27 (t, J = 2.6 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 175.02, 173.30, 170.24, 139.31, 138.27, 138.05, 135.11, 134.20, 130.33, 129.27, 128.72, 127.62, 126.01, 124.63, 122.44, 119.94, 119.21, 112.32, 110.65, 66.87, 56.13, 38.80, 28.46, 21.24. HRMS (ESI) m/z calc'd for $C_{29}H_{29}N_3O_4$ $[M+H]^+$ 484.2236, found 484.2226.



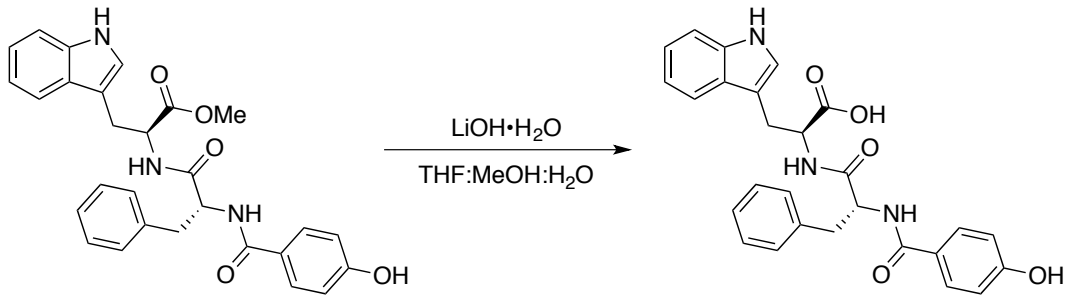
Methyl (4-hydroxybenzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56h**). To a stirring solution of **55** (200 mg, 0.4 mmol, 1 eq) in anhydrous dichloromethane (10 mL, 0.04 M), trifluoroacetic acid (320 μL, 4 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 4-hydroxybenzoic acid (60 mg, 0.4 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (440 μL, 2.6 mmol, 6 eq) and anhydrous acetonitrile (1.4 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (240 mg, 0.6 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed twice with deionized water (20 mL), followed by brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (4% MeOH/CH_2Cl_2) to afford the product as an off-white foam (63 mg, 30%). IR (thin film) 3267, 3024, 2921, 1732, 1635, 1606 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.13 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 4H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.21 (td, *J* = 7.0, 3.6 Hz, 3H), 7.14 (t, *J* = 7.2 Hz, 3H), 7.09 – 7.04 (m, 1H), 4.92 – 4.76 (m, 2H), 3.61 (s, 3H), 3.28 (dd, *J* = 14.9, 5.8 Hz, 1H), 3.21 – 3.05 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.21, 172.03, 168.17, 160.81, 136.82, 136.59, 128.86, 128.81, 127.82, 127.06,

126.16, 124.36, 123.11, 121.00, 118.43, 117.58, 114.56, 110.88, 108.87, 54.66, 53.16, 51.24, 37.32, 26.96. HRMS (ESI) m/z calc'd for $C_{28}H_{27}N_3O_5$ $[M+H]^+$ 486.2029, found 486.2006.

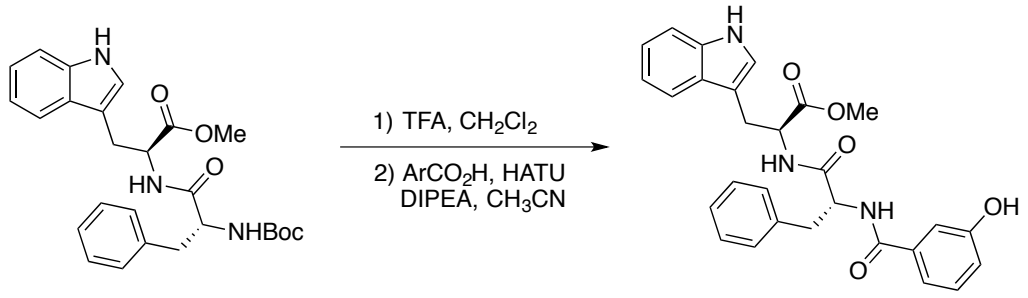


Nicotinoyl-D-*phenylalanyl*-L-*tryptophan* (**54d**). To a stirring solution of **56d** (108 mg, 0.2 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3.5 mL, 0.067 M), lithium hydroxide monohydrate (38 mg, 0.9 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (96 mg, quant.). m.p. = 148-158 °C. IR (solid) 3061, 2926, 1723, 1632, 1539 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.76 (s, 1H), 8.66 (d, J = 5.0 Hz, 1H), 8.53 (d, J = 8.2 Hz, 1H), 8.07 (t, J = 9.8, 8.9 Hz, 1H), 7.56 – 7.46 (m, 2H), 7.29 (d, J = 8.1 Hz, 1H), 7.13 (dd, J = 5.2, 1.9 Hz, 3H), 7.09 – 7.00 (m, 4H), 6.95 (t, J = 7.7 Hz, 1H), 4.87 (ddd, J = 8.2, 5.6, 2.5 Hz, 1H), 4.77 (td, J = 8.0, 5.0 Hz, 1H), 3.38 – 3.32 (m, 1H), 3.19 (dd, J = 14.7, 8.0 Hz, 1H), 3.07 (dd, J = 14.0, 5.5 Hz, 1H), 2.82 (dd, J = 13.9, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.72, 171.86, 165.90, 150.53, 147.14, 137.03, 136.78, 136.67, 129.03, 128.10, 127.49, 126.47, 123.38, 121.20, 118.68, 117.97, 111.09, 109.39,

100.11, 55.12, 53.39, 37.49, 27.16. HRMS (ESI) m/z calc'd for $C_{26}H_{24}N_4O_4$ $[M+H]^+$ 457.1876, found 457.1883.

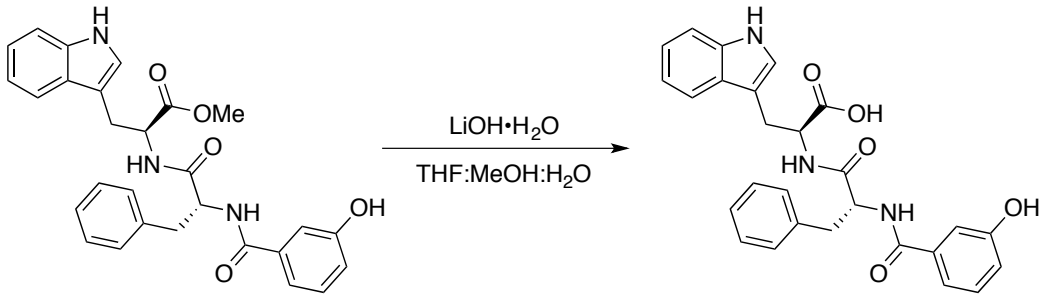


(4-Hydroxybenzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54h**). To a stirring solution of **56h** (60 mg, 0.12 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2 mL, 0.067 M), lithium hydroxide monohydrate (21 mg, 0.5 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water, then dissolved with dichloromethane. Organic was then concentrated to dryness *in vacuo* to afford the product as a white foam (56 mg, 96%). IR (thin film) 3301, 2924, 1607, 1539, 1501 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 10.35 – 10.15 (m, 0H), 7.96 (dd, J = 60.4, 8.0 Hz, 1H), 7.54 (t, J = 8.7 Hz, 3H), 7.30 (d, J = 8.1 Hz, 1H), 7.15 – 7.03 (m, 4H), 7.03 – 6.93 (m, 4H), 6.76 (d, J = 8.7 Hz, 2H), 4.86 – 4.79 (m, 6H), 4.75 (ddd, J = 7.9, 6.5, 3.8 Hz, 1H), 3.34 (d, J = 1.6 Hz, 1H), 3.18 (dd, J = 14.7, 8.1 Hz, 1H), 3.07 (dd, J = 13.8, 5.4 Hz, 1H), 2.84 (dd, J = 13.9, 8.5 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 175.02, 173.45, 169.62, 162.18, 138.31, 138.07, 130.34, 130.29, 129.27, 128.69, 127.60, 125.90, 124.62, 122.45, 119.93, 119.21, 116.03, 112.32, 110.62, 56.18, 54.50, 38.83, 28.47. HRMS (ESI) m/z calc'd for $C_{27}H_{25}N_3O_5$ $[M+H]^+$ 472.1872, found 472.1873.



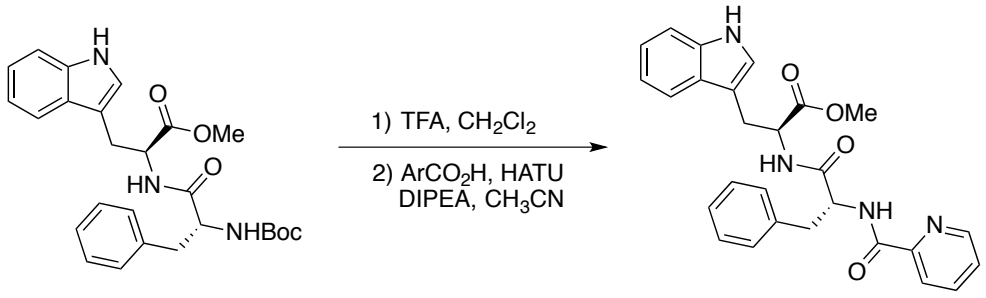
Methyl (3-hydroxybenzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56g**). To a stirring solution of **55** (200 mg, 0.4 mmol, 1 eq) in anhydrous dichloromethane (10 mL, 0.04 M), trifluoroacetic acid (320 μL, 4 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 3-hydroxybenzoic acid (60 mg, 0.4 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (440 μL, 2.6 mmol, 6 eq) and anhydrous acetonitrile (1.4 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (240 mg, 0.6 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed twice with deionized water (20 mL), followed by brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (170 mg, 86%). IR (solid) 3294, 1725, 1663, 1635, 1584 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 10.86 (d, J = 2.4 Hz, 1H), 9.59 (s, 1H), 8.55 (d, J = 7.9 Hz, 1H), 8.28 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.25 – 7.10 (m, 9H), 7.05 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H), 6.88 (dt, J = 7.6, 2.1 Hz, 1H), 4.73 (td, J = 9.2, 8.6, 4.1 Hz,

1H), 4.58 (td, *J* = 8.2, 5.3 Hz, 1H), 3.62 (s, 3H), 3.19 (dd, *J* = 14.5, 5.4 Hz, 1H), 3.06 (dd, *J* = 14.5, 8.6 Hz, 1H), 2.87 (dd, *J* = 13.7, 4.1 Hz, 1H), 2.80 (dd, *J* = 13.7, 10.3 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.64, 171.72, 166.50, 157.55, 138.59, 136.49, 136.00, 135.87, 129.54, 128.30, 127.38, 126.51, 124.29, 121.33, 118.80, 118.52, 118.37, 118.20, 114.68, 111.79, 109.64, 54.83, 53.36, 52.27, 37.54, 27.71. HRMS (ESI) m/z calc'd for $C_{28}H_{27}N_3O_5$ $[M+Na]^+$ 508.1848, found 508.1850.



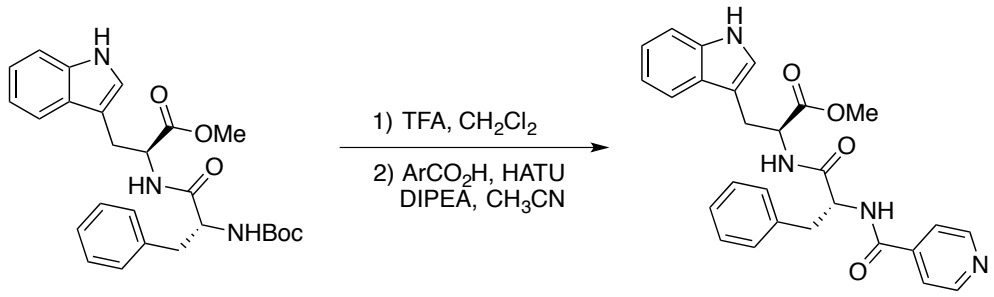
(3-Hydroxybenzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54g**). To a stirring solution of **SRG-III-273** (100 mg, 0.2 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3 mL, 0.067 M), lithium hydroxide monohydrate (35 mg, 0.8 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (98 mg, quant). m.p. = 120-148 °C. IR (solid) 3060, 1722, 1635, 1583, 1520 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 10.27 (s, 1H), 8.05 (dd, *J* = 19.9, 8.0 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.13 – 7.00 (m, 7H), 6.97 (t, *J* = 7.5 Hz, 3H), 6.91 (ddd, *J* = 8.1, 2.4, 1.1 Hz, 1H), 3.36 – 3.32 (m, 1H), 3.18 (dd, *J* = 14.7, 8.3 Hz, 1H), 3.06 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.82 (dd, *J* = 13.8, 8.5 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 174.03, 171.94, 168.65, 157.49,

136.95, 136.83, 135.34, 129.33, 129.06, 128.04, 127.50, 127.47, 126.38, 123.36, 121.21, 118.69, 118.52, 117.94, 113.93, 111.08, 109.52, 54.90, 53.47, 37.58, 27.27. HRMS (ESI) m/z calc'd for $C_{27}H_{25}N_3O_5$ $[M+H]^+$ 472.1872, found 472.1882.



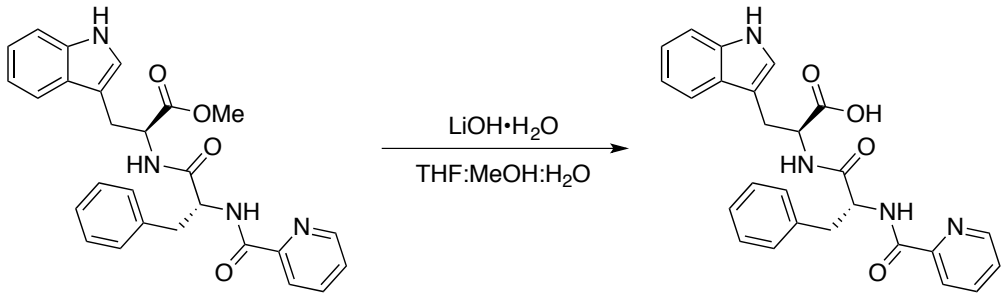
Methyl picolinoyl-D-*phenylalanyl*-L-*tryptophanate* (**56c**). To a stirring solution of **55** (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (1 mL, 0.2 M), trifluoroacetic acid (160 μL, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added picolinic acid (26 mg, 0.2 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (220 μL, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (110 mg, quant). IR (solid) 3059, 2951, 1739, 1655, 1591 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.55 – 8.42 (m, 2H), 8.12 (s, 1H), 8.08 (dt,

J = 7.8, 1.1 Hz, 1H), 7.79 (td, *J* = 7.7, 1.5 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.28 – 7.25 (m, 1H), 7.25 – 7.16 (m, 5H), 7.14 – 7.08 (m, 1H), 7.04 – 6.98 (m, 1H), 6.70 (d, *J* = 2.3 Hz, 1H), 6.57 (d, *J* = 7.8 Hz, 1H), 4.91 – 4.79 (m, 2H), 3.56 (d, *J* = 0.9 Hz, 3H), 3.25 (dd, *J* = 14.9, 5.7 Hz, 1H), 3.23 – 3.17 (m, 1H), 3.17 – 3.08 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.00, 170.46, 164.49, 149.25, 148.41, 137.36, 136.82, 136.18, 129.55, 128.75, 127.48, 127.05, 126.53, 123.12, 122.32, 122.26, 119.73, 118.51, 111.32, 109.72, 54.99, 52.90, 52.43, 38.41, 27.65. HRMS (ESI) m/z calc'd for $C_{27}H_{26}N_4O_4$ $[M+H]^+$ 471.2032, found 471.2054.



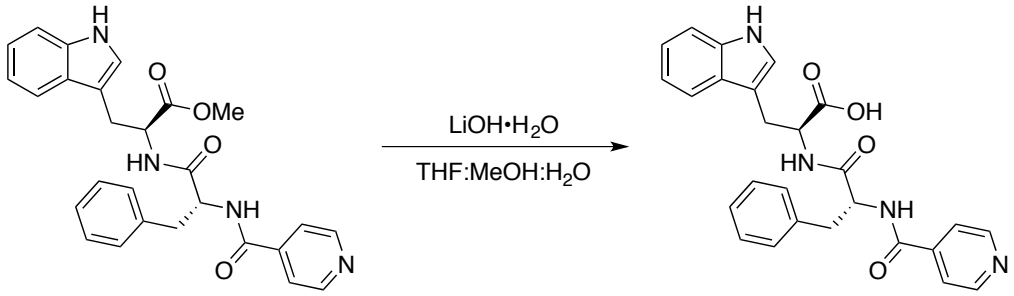
Methyl isonicotinoyl-D-*phenylalanyl*-L-*tryptophanate* (**56e**) To a stirring solution of **55** (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (1 mL, 0.2 M), trifluoroacetic acid (160 *μ*L, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added isonicotinic acid (26 mg, 0.2 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (220 *μ*L, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized

water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (104 mg, quant). IR (solid) 3059, 1737, 1641, 1532, 1456 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.63 (d, *J* = 4.4 Hz, 2H), 8.28 (s, 1H), 7.49 – 7.34 (m, 3H), 7.31 (d, *J* = 7.3 Hz, 1H), 7.23 – 7.12 (m, 4H), 7.14 – 7.02 (m, 4H), 6.76 (d, *J* = 2.4 Hz, 1H), 6.61 (s, 1H), 4.84 (dq, *J* = 12.0, 6.5, 5.8 Hz, 2H), 3.63 (s, 3H), 3.26 (dd, *J* = 14.9, 6.2 Hz, 1H), 3.16 (dd, *J* = 14.9, 5.4 Hz, 1H), 3.09 (d, *J* = 6.9 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 172.44, 171.69, 166.30, 149.56, 142.15, 136.93, 136.81, 129.03, 128.12, 127.36, 126.51, 123.32, 121.72, 121.62, 121.26, 118.67, 117.84, 117.78, 111.13, 109.14, 55.04, 53.44, 51.47, 37.47, 27.15. HRMS (ESI) m/z calc'd for $C_{27}H_{26}N_4O_4$ $[M+H]^+$ 471.2032, found 471.2044.



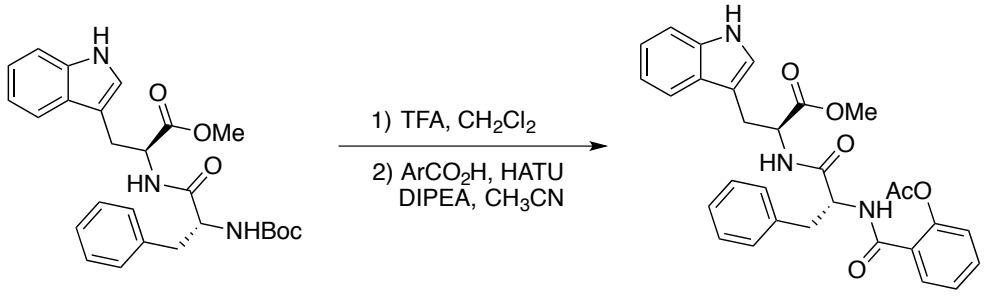
Picolinoyl-D-*phenylalanyl*-L-*tryptophan* (**54c**). To a stirring solution of **56c** (74.5 mg, 0.16 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (27 mg, 0.6 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to

produce a white solid (72 mg, 92%). m.p. = 105-108 °C. IR (solid) 3302, 2925, 1726, 1652, 1591 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.52 (d, J = 4.9 Hz, 1H), 8.27 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.88 (t, J = 7.7 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.48 (dd, J = 7.7, 4.7 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.10 – 7.00 (m, 3H), 6.96 (t, J = 7.5 Hz, 3H), 6.82 (d, J = 7.2 Hz, 2H), 4.86 (m, J = 3.6 Hz, 1H), 4.78 – 4.68 (m, 1H), 3.35 (dd, J = 14.7, 4.6 Hz, 1H), 3.15 (dd, J = 14.7, 8.9 Hz, 1H), 3.05 (dd, J = 13.7, 5.6 Hz, 1H), 2.86 (dd, J = 13.7, 7.3 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.85, 171.66, 164.61, 148.46, 137.47, 136.85, 136.32, 129.12, 127.97, 127.38, 126.64, 126.39, 123.38, 121.81, 121.23, 118.68, 117.94, 111.13, 109.55, 54.34, 53.46, 38.12, 27.21. HRMS (ESI) m/z calc'd for $C_{26}H_{24}N_4O_4$ $[M-H]^-$ 455.1719, found 455.1713.



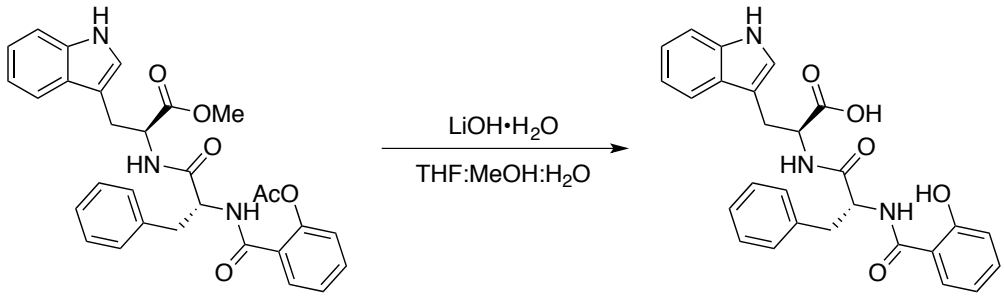
Isonicotinoyl-D-*phenylalanyl*-L-*tryptophan* (**54e**). To a stirring solution of **56e** (74.2 mg, 0.16 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (27 mg, 0.6 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (48.7 mg, 68%). m.p. = 146-166 °C. IR (solid) 3279, 3062, 2924, 1721, 1643 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.68 (s, 2H), 7.71 (d, J = 6.4 Hz, 1H),

7.55 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.12 (dd, *J* = 5.0, 1.9 Hz, 2H), 7.08 – 6.99 (m, 4H), 6.96 (td, *J* = 7.5, 7.0, 1.0 Hz, 1H), 4.88 – 4.85 (m, 1H), 4.81 – 4.72 (m, 1H), 3.34 (dd, *J* = 14.7, 5.0 Hz, 1H), 3.18 (dd, *J* = 14.7, 8.2 Hz, 1H), 3.04 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.80 (dd, *J* = 13.9, 9.2 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.70, 171.55, 165.22, 147.28, 144.73, 136.91, 136.78, 129.02, 128.11, 127.47, 126.49, 123.36, 122.81, 121.20, 118.66, 117.98, 111.09, 109.43, 55.13, 53.29, 37.49, 27.15. HRMS (ESI) m/z calc'd for $C_{26}H_{24}N_4O_4$ $[M+H]^+$ 471.2044, found 471.2032.



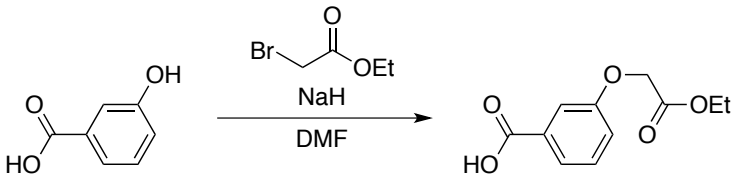
Methyl (2-acetoxybenzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56r**). To a stirring solution of **55** (233 mg, 0.5 mmol, 1 eq) in anhydrous dichloromethane (2.5 mL, 0.2 M), trifluoroacetic acid (390 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 2-acetoxybenzoic acid[6] (90 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (530 μL, 3 mmol, 6 eq) and anhydrous acetonitrile (1.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 1 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic

was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (112 mg, 43%). IR (solid) 1731, 1661, 1637, 1532, 1480 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.58 – 7.53 (m, 2H), 7.48 (td, *J* = 7.8, 1.7 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.15 – 7.08 (m, 3H), 7.08 – 7.05 (m, 2H), 7.05 – 6.99 (m, 3H), 6.81 (d, *J* = 7.5 Hz, 2H), 4.84 (d, *J* = 6.4 Hz, 1H), 4.74 (dd, *J* = 8.8, 5.1 Hz, 1H), 3.68 (s, 3H), 3.33 (d, *J* = 5.5 Hz, 1H), 3.17 – 3.03 (m, 2H), 2.83 (d, *J* = 7.3 Hz, 1H), 1.99 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.30, 171.18, 169.16, 165.89, 148.12, 136.69, 136.23, 131.64, 129.13, 128.94, 127.82, 127.24, 127.03, 126.18, 125.57, 123.18, 122.98, 121.07, 118.46, 117.66, 110.99, 109.15, 54.29, 53.27, 51.26, 37.32, 27.02, 19.34. HRMS (ESI) m/z calc'd for $C_{30}H_{29}N_3O_6$ $[M+H]^+$ 528.2135, found 528.2130.

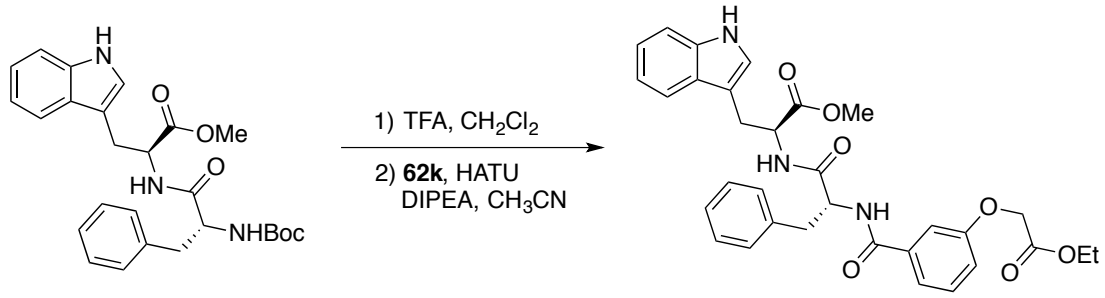


(2-Hydroxybenzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54f**). To a stirring solution of **56r** (96 mg, 0.18 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (62 mg, 1.5 mmol, 8 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was

dried to produce a white solid (68.7 mg, 80%). m.p. = 135-140 °C. IR (solid) 3282, 3060, 2920, 1739, 1635 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.68 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.33 (ddd, *J* = 8.6, 7.3, 1.7 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.08 – 7.00 (m, 3H), 6.96 (t, *J* = 6.9 Hz, 1H), 6.91 (d, *J* = 6.6 Hz, 1H), 6.88 – 6.80 (m, 2H), 4.86 (s, 1H), 4.72 (dd, *J* = 8.4, 4.8 Hz, 1H), 3.34 (s, 1H), 3.16 (dd, *J* = 14.7, 8.4 Hz, 1H), 3.05 (dd, *J* = 13.8, 5.5 Hz, 1H), 2.85 (dd, *J* = 13.8, 7.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.08, 171.54, 168.07, 158.65, 136.57, 136.46, 133.22, 128.86, 128.35, 127.75, 127.24, 126.12, 123.12, 120.92, 118.76, 118.39, 117.74, 116.57, 116.04, 110.81, 109.38, 54.43, 53.42, 37.55, 27.06. HRMS (ESI) m/z calc'd for $C_{27}H_{25}N_3O_5$ $[M+Na]^+$ 472.1872, found 472.1871.

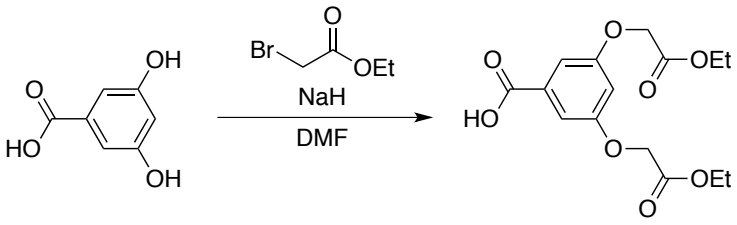


3-(2-Ethoxy-2-oxoethoxy)benzoic acid (**62k**). To a solution of 3-hydroxybenzoic acid (140 mg, 1 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (3 mL, 0.33 M), sodium hydride (60% dispersion, 120 mg, 3 mmol, 3 eq) was added at 0 °C. After stirring for 30 minutes, ethyl bromoacetate (130 μL, 1.2 mmol, 1.2 eq) was added and the reaction mixture was allowed to warm to room temperature for 18 h. The reaction mixture was quenched with water at 0 °C then acidified with 1N HCl. The reaction mixture was then extracted with ethyl acetate (20 mL) and washed five times with water (20 mL), followed by brine (20 mL). The organic layer was then sodium sulfate and concentrated *in vacuo* to yield a crude oil, which was used in the next step without purification.

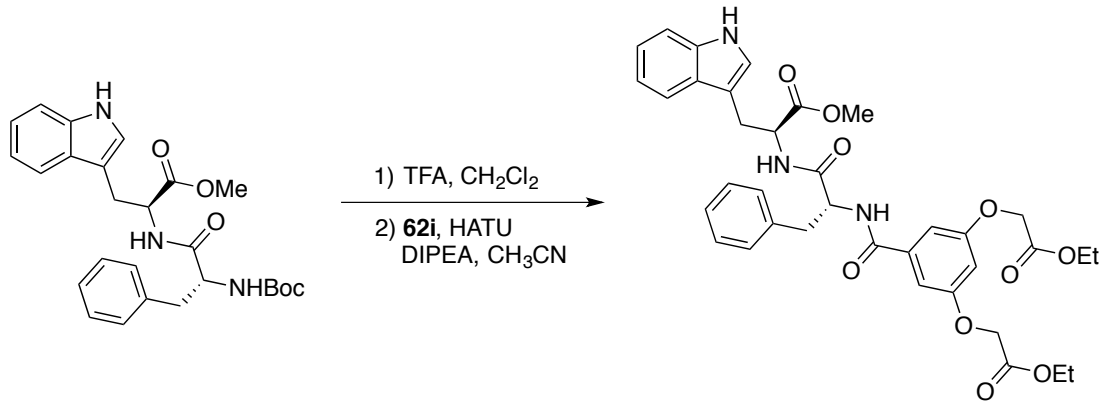


Methyl (3-(2-ethoxy-2-oxoethoxy)benzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56k**). To a stirring solution of **55** (465 mg, 1 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.2 M), trifluoroacetic acid (780 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added crude acid **62k** (1 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (1 mL, 5 mmol, 5 eq) and anhydrous acetonitrile (3.3 mL, 0.3 M). The reaction mixture was then cooled to 0 °C and HATU (570 mg, 1.5 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed twice with deionized water (20 mL), followed by brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (40-60% EtOAc/Hex) to afford the product as an off-white foam (110 mg, 20% over three steps). IR (solid) 3287, 1739, 1636, 1583, 1520 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.27 (dt, *J* = 11.7, 4.1 Hz, 3H), 7.20 (d, *J* = 5.0 Hz, 3H), 7.17 – 7.12 (m, 2H), 7.10 (dd, *J* = 7.9, 5.2 Hz, 2H), 7.08 – 7.01 (m, 2H), 6.80 – 6.71 (m, 2H), 6.67 (d, *J* = 7.8 Hz, 1H), 4.92 – 4.78 (m, 2H), 4.64 (s, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 3.60 (s, 3H), 3.29 (dd, *J* = 14.8, 5.9 Hz, 1H), 3.20 – 3.04 (m, 3H), 1.29

(t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.15, 170.63, 168.97, 166.86, 158.11, 136.53, 136.36, 135.37, 129.95, 129.47, 128.82, 127.33, 127.19, 123.30, 122.31, 119.99, 119.70, 118.60, 118.51, 113.47, 111.51, 109.42, 65.38, 61.75, 54.95, 52.60, 52.53, 38.21, 27.57, 14.28. HRMS (ESI) m/z calc'd for $C_{32}H_{33}N_3O_7$ $[M+Na]^+$ 594.2216, found 594.2222.

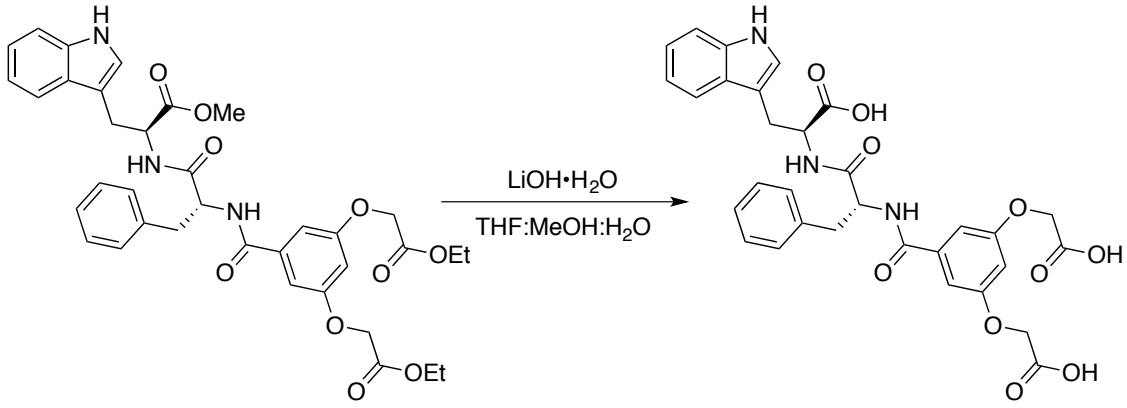


3,5-bis(2-ethoxy-2-oxoethoxy)benzoic acid (**62i**). To a solution of 3,5-hydroxybenzoic acid (154 mg, 1 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (3 mL, 0.33 M), sodium hydride (60% dispersion, 160 mg, 3 mmol, 4 eq) was added at 0 °C. After stirring for 30 minutes, ethyl bromoacetate (260 μL, 2.4 mmol, 2.4 eq) was added and the reaction mixture was allowed to warm to room temperature for 18 h. The reaction mixture was quenched with water at 0 °C then acidified with 1N HCl. The reaction mixture was then extracted with ethyl acetate (20 mL) and washed five times with water (20 mL), followed by brine (20 mL). The organic layer was then sodium sulfate and concentrated *in vacuo* to yield a crude oil, which was used in the next step without purification.



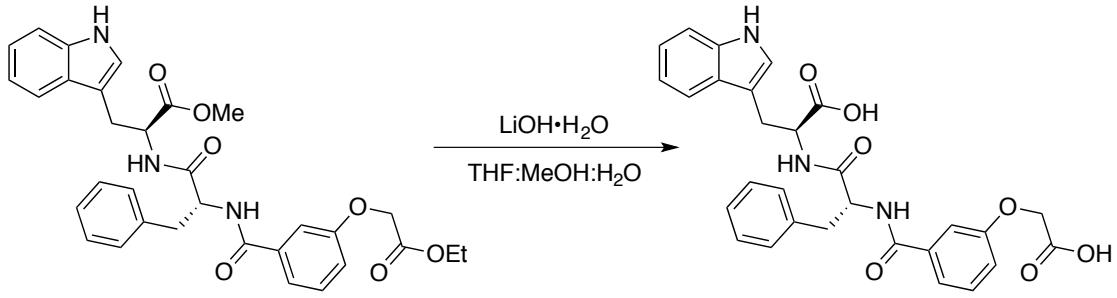
*Diethyl 2,2'-((5-(((*R*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-1,3-phenylene)bis(oxy))diacetate* (**56i**). To a stirring solution of **55** (465 mg, 1 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.2 M), trifluoroacetic acid (780 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness in vacuo. To the crude amine salt was added crude acid **62i** (1 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (1 mL, 5 mmol, 5 eq) and anhydrous acetonitrile (3.3 mL, 0.3 M). The reaction mixture was then cooled to 0 °C and HATU (570 mg, 1.5 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed twice with deionized water (20 mL), followed by brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as an orange foam. This residue was purified by flash chromatography (40-60% EtOAc/Hex) to afford the product as an off-white foam (191 mg, 28% over three steps). IR (solid) 1756, 1740, 1625, 1594, 1521 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.55 (s, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.24 (d, J = 4.2 Hz, 1H), 7.18 (d, J = 7.3 Hz, 3H), 7.12 – 7.04 (m, 3H), 7.01 (t, J = 7.4 Hz, 1H), 6.74 (dd, J = 18.9, 2.3 Hz, 3H), 6.65 (t, J = 7.6 Hz, 2H),

6.58 (s, 1H), 4.89 – 4.75 (m, 2H), 4.54 (d, J = 3.5 Hz, 4H), 4.23 (q, J = 7.2 Hz, 4H), 3.60 (s, 3H), 3.30 (dd, J = 14.9, 5.5 Hz, 1H), 3.08 (ddt, J = 34.0, 13.9, 7.2 Hz, 3H), 1.27 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.22, 170.61, 168.71, 166.59, 159.09, 136.58, 136.44, 136.20, 129.44, 128.82, 127.27, 127.18, 123.38, 122.25, 119.61, 118.49, 111.57, 109.19, 106.68, 105.42, 65.41, 61.79, 54.94, 52.55, 52.34, 37.94, 27.49, 14.27. HRMS (ESI) m/z calc'd for $C_{36}H_{39}N_3O_{10}$ $[M+H]^+$ 674.2714, found 674.2713.



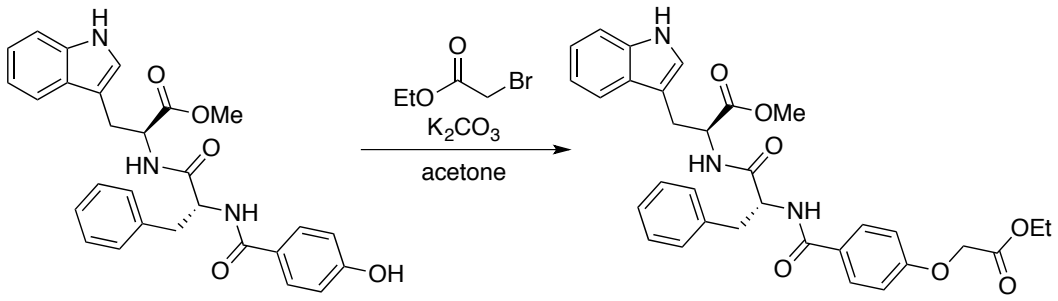
*2,2'-((5-(((*R*)-1-(((*S*)-1-Carboxy-2-(1*H*-indol-3-yl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-1,3-phenylene)bis(oxy))diacetic acid* (**54i**). To a stirring solution of **56i** (123 mg, 0.2 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3 mL, 0.067 M), lithium hydroxide monohydrate (101 mg, 2.4 mmol, 12 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (59 mg, 49%). m.p. = 125-148 °C. IR (solid) 3279, 2923, 1729, 1634, 1591 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.92 (d, J = 7.9 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.19 – 7.10 (m, 3H), 7.10 – 7.03 (m, 3H), 7.01 (s, 1H), 6.97 (t, J = 7.4 Hz, 1H), 6.83 (d, J = 2.3 Hz, 2H), 6.70 (t, J = 2.4 Hz, 1H), 4.81 (d,

J = 5.5 Hz, 1H), 4.79 – 4.73 (m, 1H), 4.66 (s, 4H), 3.35 (t, J = 4.7 Hz, 1H), 3.22 (dd, J = 14.7, 7.6 Hz, 1H), 3.10 (dd, J = 13.9, 5.3 Hz, 1H), 2.84 (dd, J = 13.9, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.99, 173.24, 172.25, 169.23, 160.50, 138.37, 138.09, 137.38, 130.28, 129.39, 128.65, 127.71, 124.69, 122.49, 119.95, 119.24, 112.37, 110.48, 107.65, 106.39, 65.99, 56.41, 54.30, 38.65, 28.40. HRMS (ESI) m/z calc'd for $C_{31}H_{29}N_3O_{10}$ $[M+Na]^+$ 626.1756, found 626.1754.



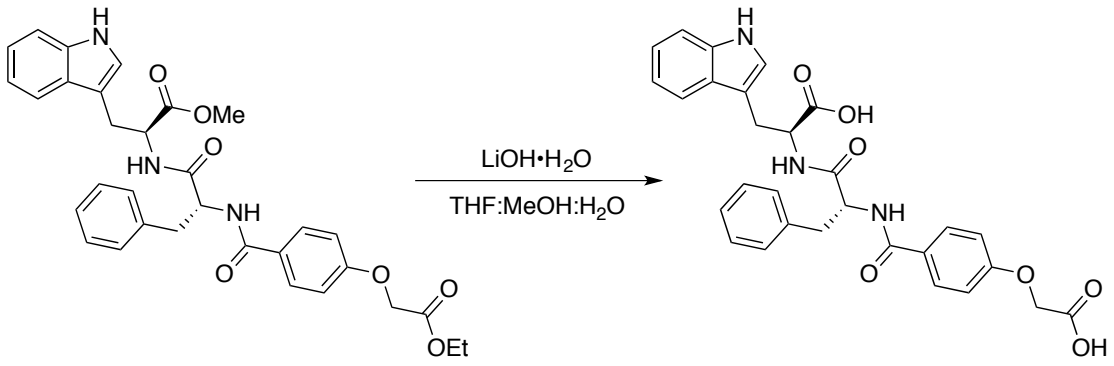
(3-(carboxymethoxy)benzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54k**). To a stirring solution of **56k** (75 mg, 0.13 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M), lithium hydroxide monohydrate (44 mg, 1 mmol, 8 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (57 mg, 83%). m.p. = 130-145 °C. IR (solid) 3289, 2923, 1725, 1636, 1582 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 7.9 Hz, 1H), 7.31 (dt, *J* = 7.9, 3.9 Hz, 2H), 7.27 – 7.17 (m, 2H), 7.17 – 7.11 (m, 3H), 7.11 – 7.05 (m, 2H), 7.05 – 7.00 (m, 3H), 6.97 (t, *J* = 7.4 Hz, 1H), 4.85 – 4.82 (m, 1H), 4.77 (dd, *J* = 7.9, 5.1 Hz, 1H), 4.68 (s, 2H), 3.35 (d, *J* = 5.0 Hz, 1H), 3.21 (dd, *J* = 14.8, 7.9 Hz, 1H), 3.09 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.85 (dd, *J* = 13.9, 8.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.00,

173.25, 172.40, 169.53, 159.43, 138.32, 138.09, 136.65, 130.70, 130.29, 129.35, 128.69, 127.68, 124.66, 122.48, 121.30, 119.95, 119.27, 119.24, 114.44, 112.36, 110.58, 65.88, 56.32, 54.41, 38.73, 28.44. HRMS (ESI) m/z calc'd for $C_{29}H_{27}N_3O_7$ $[M+H]^+$ 530.1909, found 530.1921.



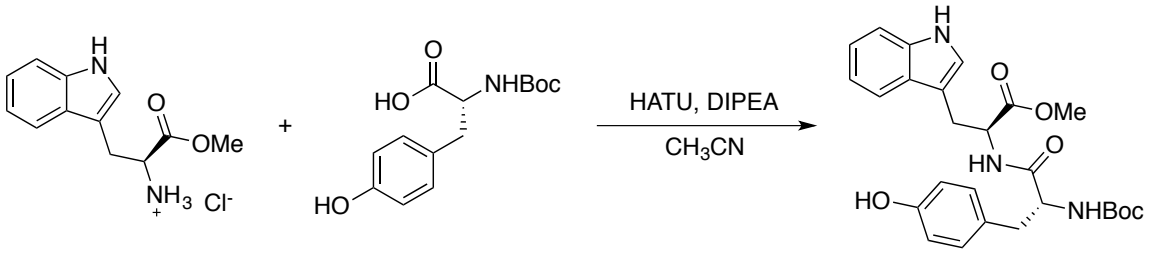
Methyl (4-(2-ethoxy-2-oxoethoxy)benzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56j**). A stirring solution of **56h** (74 mg, 0.15 mmol, 1 eq), ethyl bromoacetate (17 μL, 0.15 mmol, 1 eq) and potassium carbonate (63 mg, 0.45 mmol, 3 eq) in a acetone (1.5 mL, 0.1 M) was heated to 60 °C in a sealed tube. The reaction mixture was stirred for 18 h. The reaction mixture was then concentrated to remove the organic solvents. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (52.4 mg, 60%). IR (thin film) 3304, 3060, 1742, 1634, 1606 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.46 – 7.41 (m, 1H), 7.30 – 7.25 (m, 1H), 7.20 – 7.15 (m, 3H), 7.12 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.08 – 7.01 (m, 3H), 6.84 (dd, *J* = 22.6, 8.3 Hz, 4H), 6.73 (d, *J* = 2.4 Hz, 1H), 4.89 (q, *J* = 7.0 Hz, 1H), 4.81 (dt, *J* = 7.9, 5.8 Hz, 1H), 4.62 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.58 (s, 3H), 3.24 (dd, *J* = 14.8, 6.3 Hz, 1H), 3.17 – 3.06 (m, 3H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.21, 170.94, 168.51, 166.65, 160.57, 136.60, 136.28, 129.46, 129.05, 128.66, 127.31, 127.10, 127.02, 123.29, 122.20, 119.62, 118.43, 114.47, 111.47, 109.49, 65.25,

61.68, 54.79, 52.45, 38.31, 29.37, 27.53, 14.24. HRMS (ESI) m/z calc'd for $C_{32}H_{33}N_3O_7$ $[M+H]^+$ 572.2397, found 572.2390.



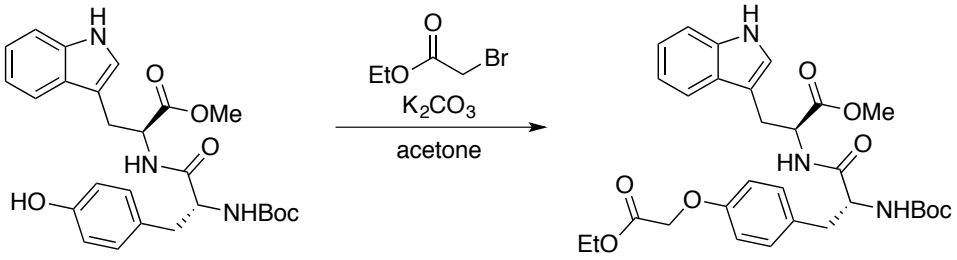
(4-(carboxymethoxy)benzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54j**). To a stirring solution of **56j** (52.4 mg, 0.09 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.4 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 1 mmol, 8 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (39 mg, 81%). IR (solid) 3322, 2923, 1726, 1605, 1536 cm^{-1}. m.p. = 155-165 °C. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.62 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 3.5 Hz, 3H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.04 – 6.99 (m, 3H), 6.96 (dd, *J* = 15.1, 8.1 Hz, 3H), 4.83 (d, *J* = 9.2 Hz, 1H), 4.75 (ddd, *J* = 7.7, 5.1, 2.6 Hz, 1H), 4.72 (s, 2H), 3.34 (d, *J* = 5.1 Hz, 1H), 3.20 (dd, *J* = 14.7, 8.0 Hz, 1H), 3.09 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.85 (dd, *J* = 13.9, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.01, 173.39, 172.26, 169.29, 162.25, 138.35, 138.06, 130.27, 130.22, 129.29, 128.68, 127.98, 127.62, 124.63, 122.48, 119.94, 119.22, 115.37, 112.34, 110.56,

65.85, 56.25, 54.42, 38.75, 28.42. HRMS (ESI) m/z calc'd for $C_{29}H_{27}N_3O_7$ $[M-H]^-$ 528.1771, found 528.1755.

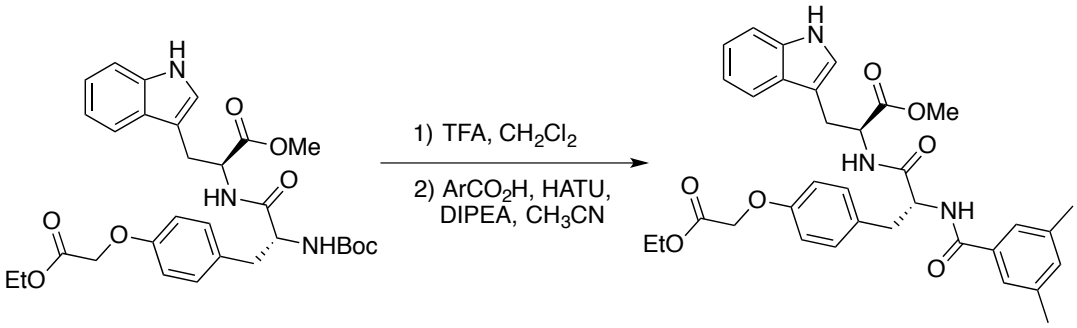


*Methyl (*tert-*butoxycarbonyl)*-D-*tyrosyl*-L-*tryptophanate* (**64**). To (*tert*-butoxycarbonyl)-*D*-tyrosine (300 mg, 1 mmol, 1 eq) was added tryptophan methyl ester hydrochloride (260 mg, 1 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (1 mL, 5 mmol, 5 eq) and anhydrous acetonitrile (3.3 mL, 0.3 M). The reaction mixture was then cooled to 0 °C and HATU (570 mg, 1.5 mmol, 1.5 eq) was added. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography plug (40% EtOAc/Hexanes) to afford the product as an off-white foam (350 mg, 73%). IR (solid) 3441, 2955, 1668, 1527, 1503 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.45 (s, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.32 – 7.24 (m, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 2H), 6.63 – 6.56 (m, 3H), 5.19 (d, *J* = 8.2 Hz, 1H), 4.81 (q, *J* = 6.5 Hz, 1H), 4.30 (d, *J* = 8.9 Hz, 1H), 3.59 (s, 3H), 3.28 – 3.07 (m, 2H), 2.97 – 2.77 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.44, 171.61, 155.73, 155.27, 136.32, 130.53, 127.98, 127.38, 123.20, 122.28, 119.71, 118.42, 115.74,

111.53, 109.37, 80.59, 56.20, 52.95, 52.61, 37.86, 28.39, 27.63. HRMS (ESI) m/z calc'd for $C_{26}H_{31}N_3O_6$ $[M-H]^-$ 480.2135, found 480.2127.

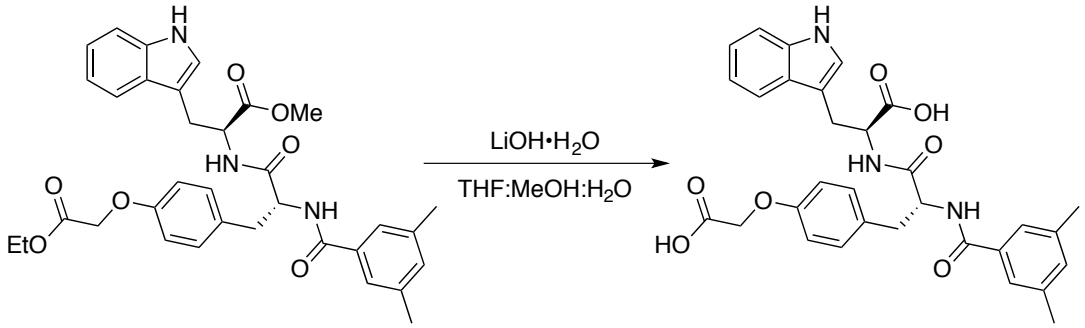


*Methyl ((*R*)-2-((tert-butoxycarbonyl)amino)-3-(4-(2-ethoxy-2-oxoethoxy)phenyl)propanoyl)*-L-*tryptophanate* (**65**). To a stirring solution of **64** (200 mg, 0.4 mmol, 1 eq), ethyl bromoacetate (55 μL, 0.5 mmol, 1.2 eq) and potassium carbonate (172 mg, 1.2 mmol, 3 eq) in acetone (4 mL, 0.1 M) was heated to 60 °C in a sealed tube. The reaction mixture was stirred for 18 h. The reaction mixture was then concentrated to remove the organic solvents. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (46.2 mg, 20%). IR (thin film) 3304, 3060, 1742, 1634, 1606 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.64 (s, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.15 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.06 (dd, *J* = 14.2, 8.3 Hz, 3H), 6.73 (d, *J* = 8.2 Hz, 2H), 6.43 (s, 1H), 6.30 (d, *J* = 7.4 Hz, 1H), 5.10 (d, *J* = 8.3 Hz, 1H), 4.82 – 4.74 (m, 1H), 4.60 (s, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 3.63 (s, 3H), 3.16 (td, *J* = 16.3, 15.0, 5.7 Hz, 2H), 2.93 (d, *J* = 7.2 Hz, 2H), 1.39 (s, 6H), 1.32 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.10, 170.99, 169.67, 156.96, 136.29, 130.61, 130.07, 127.35, 123.12, 122.07, 119.51, 118.37, 114.84, 111.51, 109.17, 65.51, 61.73, 53.96, 52.50, 29.41, 28.41, 27.53, 14.30. HRMS (ESI) m/z calc'd for $C_{30}H_{37}N_3O_8$ $[M+H]^+$ 568.2659, found 568.2669.



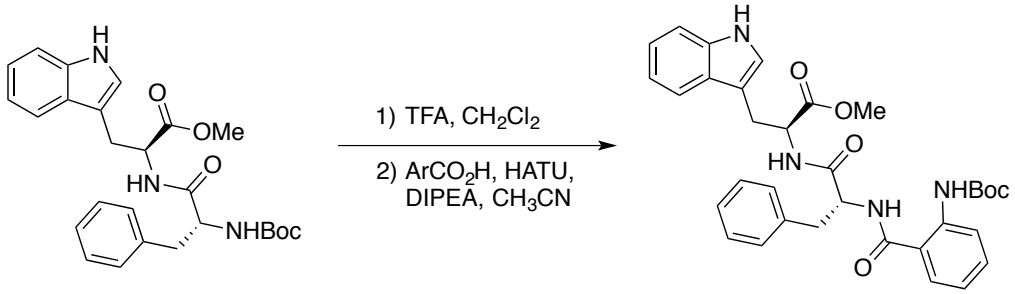
*Methyl ((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(2-ethoxy-2-oxoethoxy)phenyl)propanoyl)*-L-*tryptophanate* (**66**). To a stirring solution of **65** (46.2 mg, 0.08 mmol, 1 eq) in anhydrous dichloromethane (0.4 mL, 0.2 M), trifluoroacetic acid (70 μL, 0.8 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 3,5-dimethylbenzoic acid (12 mg, 0.08 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (60 μL, 0.33 mmol, 4 eq) and anhydrous acetonitrile (2.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (46 mg, 0.12 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (42 mg, 88%). IR (solid) 3300, 2922, 1739, 1637, 1603 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.74 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.32 (s, 3H), 7.18 – 7.10 (m, 2H), 7.04 (dd, *J* = 14.3, 7.6 Hz, 3H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 2H), 6.56 (d, *J* = 7.4 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 4.86 – 4.74 (m, 2H), 4.59 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.62 (s, 3H), 3.27 – 3.14 (m, 2H), 3.03 (h, *J* = 8.2

Hz, 2H), 2.32 (s, 6H), 1.32 (t, *J* = 7.2 Hz, 4H). ^{13}C NMR (126 MHz, CDCl3) δ 172.04, 170.88, 169.71, 167.45, 157.00, 138.39, 136.35, 133.82, 133.52, 130.68, 129.97, 127.27, 124.96, 123.31, 122.06, 119.49, 118.36, 114.82, 111.57, 109.13, 65.48, 61.75, 55.32, 52.92, 52.55, 38.08, 27.44, 21.32, 14.30. HRMS (ESI) m/z calc'd for $C_{33}H_{36}N_4O_6$ $[M+H]^+$ 600.2710, found 600.2710.



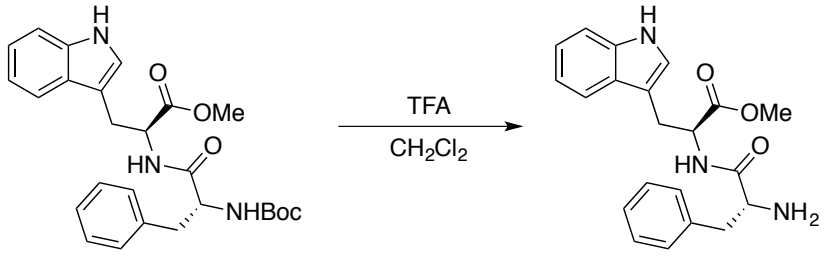
*((*R*)-3-(4-(carboxymethoxy)phenyl)-2-(3,5-dimethylbenzamido)propanoyl)*-L-*tryptophan* (**60**). To a stirring solution of **66** (42 mg, 0.07 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1 mL, 0.067 M), lithium hydroxide monohydrate (12 mg, 0.28 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (27 mg, 69%). m.p. = 169-175 °C. IR (solid) 2921, 1726, 1635, 1511, 1439 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.57 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 1.6 Hz, 2H), 7.15 (s, 1H), 7.08 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (s, 1H), 6.98 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.62 (d, *J* = 8.6 Hz, 2H), 4.83 – 4.79 (m, 1H), 4.75 (dd, *J* = 8.6, 4.6 Hz, 1H), 4.52 (d, *J* = 1.8 Hz, 2H), 3.36 (dd, *J* = 14.8, 4.7 Hz, 1H), 3.18 (dd, *J* = 14.8, 8.6 Hz, 1H), 3.02 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.79 (dd, *J* = 13.5, 7.3 Hz, 1H), 2.31 (s, 6H). ^{13}C NMR

(126 MHz, MeOD) δ 175.11, 173.23, 170.14, 158.29, 139.40, 138.10, 135.17, 134.23, 131.40, 130.96, 126.36, 126.02, 124.64, 122.48, 119.96, 119.23, 115.44, 112.41, 110.75, 66.07, 56.16, 54.54, 38.01, 28.45, 21.24. HRMS (ESI) m/z calc'd for $C_{31}H_{31}N_3O_7$ $[M+Na]^+$ 580.2060, found 580.2057.

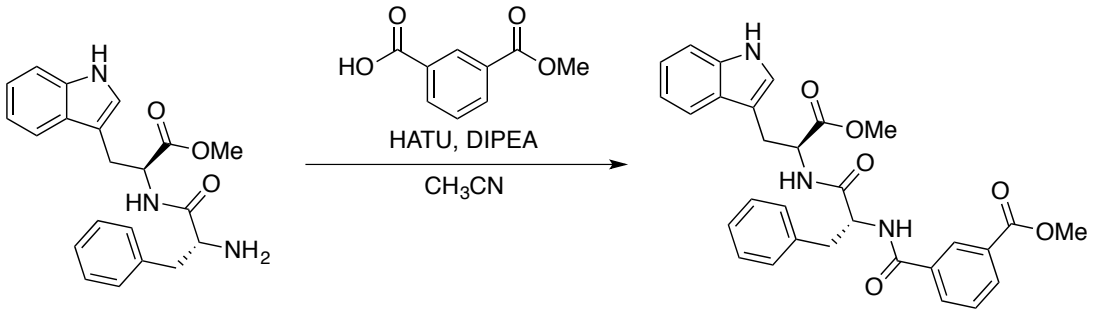


Methyl (2-((tert-butoxycarbonyl)amino)benzoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56q**). To a stirring solution of **55** (233 mg, 0.5 mmol, 1 eq) in anhydrous dichloromethane (2.5 mL, 0.2 M), trifluoroacetic acid (390 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 2-((tert-butoxycarbonyl)amino)benzoic acid (119 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (350 μL, 2 mmol, 4 eq) and anhydrous acetonitrile (1.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 1 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to

afford the product as an off-white foam (292 mg, 88%). IR (thin film) 3336, 2979, 1729, 1634, 1587 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.04 (s, 1H), 8.32 (d, *J* = 8.4 Hz, 1H), 8.26 (s, 1H), 7.46 – 7.38 (m, 2H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 7.1 Hz, 4H), 7.17 – 7.12 (m, 1H), 7.12 – 7.09 (m, 2H), 7.09 – 7.04 (m, 1H), 6.94 – 6.86 (m, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 2.3 Hz, 1H), 6.47 (d, *J* = 7.7 Hz, 1H), 4.96 – 4.68 (m, 2H), 3.62 (s, 3H), 3.27 (dd, *J* = 14.9, 5.9 Hz, 1H), 3.20 – 3.04 (m, 3H), 1.52 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.10, 170.42, 168.71, 153.16, 140.39, 136.34, 136.27, 132.95, 129.41, 128.84, 127.32, 127.27, 126.95, 123.12, 122.42, 121.65, 119.99, 119.80, 119.06, 118.43, 111.47, 109.47, 80.70, 54.79, 52.75, 52.55, 38.32, 28.48, 27.55. HRMS (ESI) m/z calc'd for $C_{33}H_{36}N_4O_6$ $[M+H]^+$ 585.2713, found 585.2718.

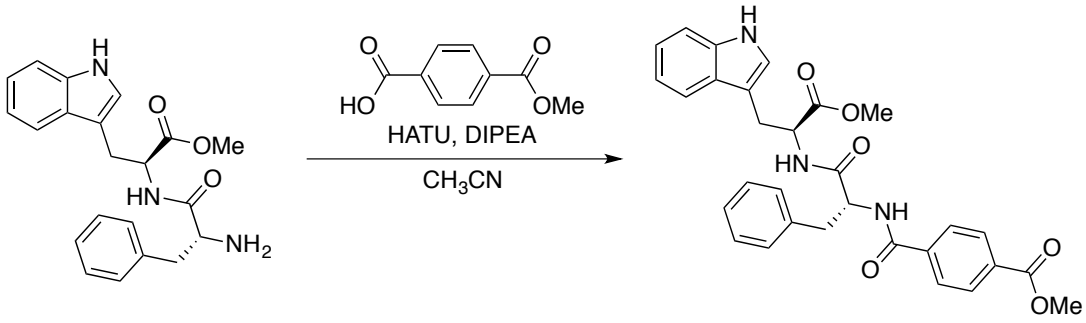


Methyl D-*phenylalanyl*-L-*tryptophanate* (**68**). To a stirring solution of **55** (930 mg, 2 mmol, 1 eq) in anhydrous dichloromethane (10 mL, 0.2 M), trifluoroacetic acid (1.6 mL, 20 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam (730 mg, quant.). This residue was carried forward without further purification.



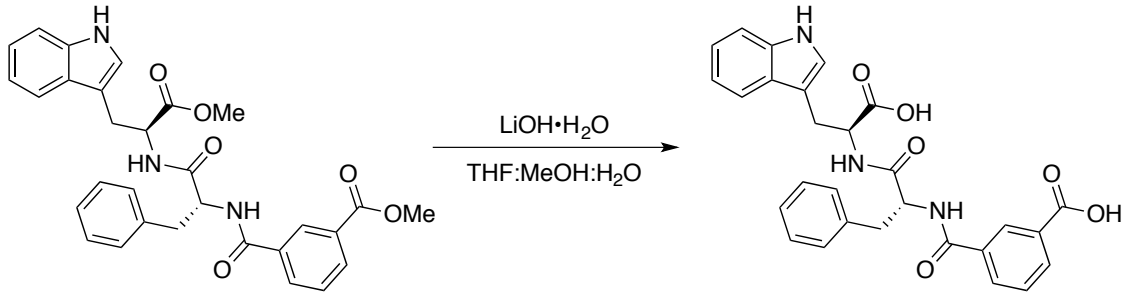
*Methyl 3-(((*R*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoate* (**56m**). To the crude amine **68** (183 mg, 0.5 mmol, 1 eq)was added 3-(methoxycarbonyl)benzoic acid (90 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (350 μL, 2 mmol, 4 eq) and anhydrous acetonitrile (1.8 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 1 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (130 mg, 49%). IR (solid) 1742, 1715, 1629, 1424 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.27 (t, J = 1.8 Hz, 1H), 8.08 (dt, J = 7.7, 1.3 Hz, 1H), 7.83 (ddd, J = 7.8, 1.9, 1.2 Hz, 1H), 7.49 (dt, J = 7.9, 1.0 Hz, 1H), 7.48 – 7.43 (m, 1H), 7.30 (dt, J = 8.1, 0.9 Hz, 1H), 7.17 – 7.07 (m, 5H), 7.04 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 6.99 (s, 1H), 6.96 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 4.90 (dd, J = 8.9, 5.7 Hz, 2H), 4.78 (dd, J = 7.9, 5.5 Hz, 2H), 3.88 (s, 2H), 3.64 (s, 1H), 3.35 (s, 3H), 3.29 – 3.21 (m, 1H), 3.15 (dd, J = 14.8, 8.0 Hz, 1H), 3.08 (dd, J = 13.8, 5.7 Hz, 1H), 2.89 (dd, J = 13.8, 8.9 Hz, 1H), 2.78 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.70, 173.25, 168.74, 167.59, 138.30,

138.01, 135.64, 133.36, 132.78, 131.62, 130.33, 129.79, 129.42, 129.34, 128.58, 127.69, 124.60, 122.48, 119.92, 119.08, 112.38, 110.38, 56.33, 54.70, 52.79, 52.75, 49.85, 38.85, 28.44. HRMS (ESI) m/z calc'd for $C_{30}H_{29}N_3O_6$ $[M+H]^+$ 528.2135, found 528.2134.



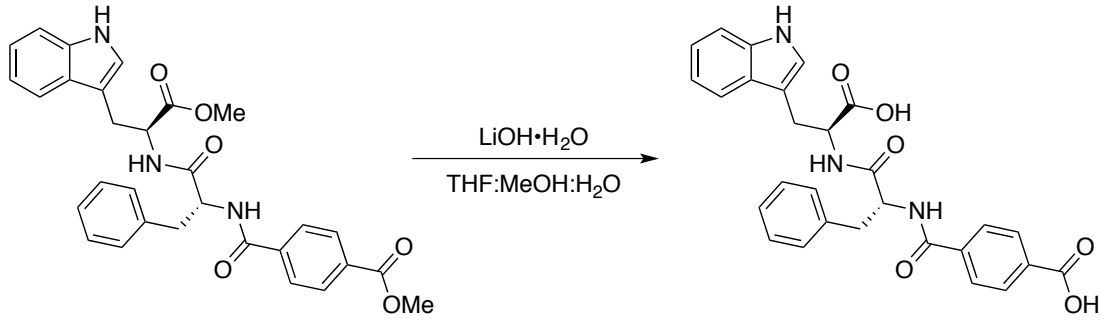
*Methyl 4-(((*R*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoate* (**56l**). To the crude amine **68** (183 mg, 0.5 mmol, 1 eq)was added 4-(methoxycarbonyl)benzoic acid (90 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (350 μL, 2 mmol, 4 eq) and anhydrous acetonitrile (1.8 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 1 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (89 mg, 34%). IR (solid) 1723, 1662, 1635, 1531, 1497 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.01 (dd, J = 8.3, 2.5 Hz, 2H), 7.69 (dd, J = 8.4, 2.6 Hz, 2H), 7.49 (dd, J = 7.6, 2.5 Hz, 1H), 7.31 (dd, J = 8.3, 2.5 Hz, 1H), 7.13 (s, 3H), 7.11 – 7.03 (m, 3H), 7.01 – 6.93 (m, 2H), 4.90 – 4.85 (m, 1H), 4.76 (t, J = 6.7, 6.2 Hz,

1H), 3.91 (s, 3H), 3.66 (s, 3H), 3.29 – 3.21 (m, 1H), 3.15 (ddd, *J* = 14.8, 8.1, 2.6 Hz, 1H), 3.07 (dd, *J* = 13.0, 7.1 Hz, 1H), 2.91 – 2.82 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.21, 171.68, 167.49, 166.15, 137.88, 136.78, 136.58, 132.50, 129.02, 128.81, 127.87, 127.10, 127.05, 126.24, 123.11, 121.01, 118.43, 117.60, 110.89, 108.88, 54.83, 53.18, 51.35, 51.26, 37.26, 26.94. HRMS (ESI) m/z calc'd for $C_{30}H_{29}N_3O_6$ $[M+H]^+$ 528.2135, found 528.2136.



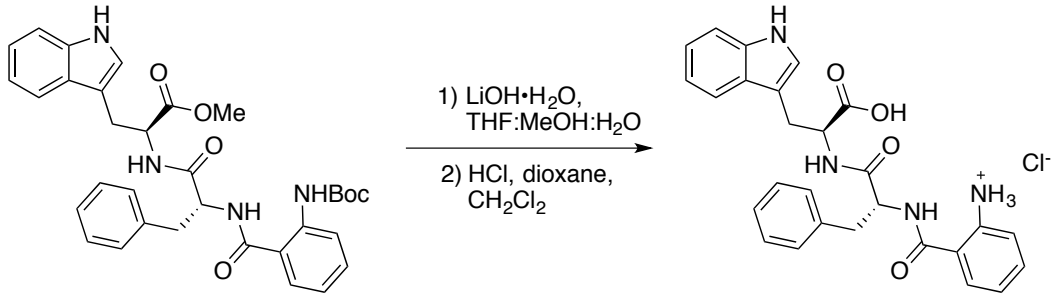
*3-(((*R*)-1-(((*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoic acid* (**54m**). To a stirring solution of **56m** (130 mg, 0.25 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3.8 mL, 0.067 M), lithium hydroxide monohydrate (42 mg, 1 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (114 mg, 93%). m.p. = 161-171 °C. IR (solid) 3283, 2923, 1704, 1635, 1538 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.31 (d, *J* = 1.9 Hz, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.13 (dt, *J* = 5.9, 2.9 Hz, 3H), 7.09 – 7.00 (m, 5H), 6.96 (t, *J* = 7.5 Hz, 1H), 4.91 – 4.87 (m, 1H), 4.77 (dd, *J* = 8.0, 4.9 Hz, 1H), 3.38 – 3.33 (m, 1H), 3.20 (dd, *J* = 14.7, 8.0

Hz, 1H), 3.10 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.86 (dd, *J* = 13.9, 9.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.10, 173.18, 169.04, 168.91, 138.32, 138.06, 135.69, 133.66, 132.62, 130.31, 129.73, 129.66, 129.35, 128.73, 127.68, 124.61, 122.45, 119.94, 119.20, 112.32, 110.65, 101.38, 56.39, 54.59, 38.78, 28.41. HRMS (ESI) m/z calc'd for $C_{28}H_{25}N_3O_6$ $[M+Na]^+$ 522.1641, found 522.1645.



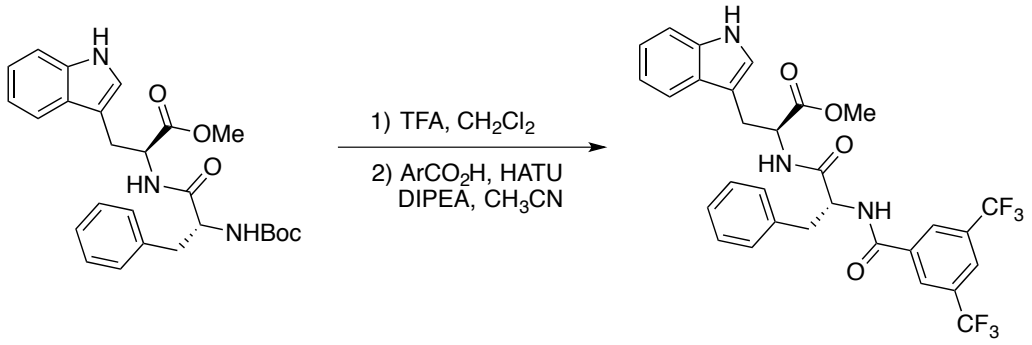
*4-(((*R*)-1-(((*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoic acid* (**54l**). To a stirring solution of **56l** (79 mg, 0.15 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.3 mL, 0.067 M), lithium hydroxide monohydrate (26 mg, 0.6 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (70 mg, 93%). m.p. = 139-145 °C. IR (solid) 3286, 2926, 1703, 1631, 1529 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.99 (t, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 2.0 Hz, 3H), 7.07 – 6.98 (m, 4H), 6.96 (t, *J* = 7.4 Hz, 1H), 4.88 – 4.83 (m, 1H), 4.77 (dd, *J* = 8.1, 4.8 Hz, 1H), 3.34 (dd, *J* = 14.7, 5.1 Hz, 1H), 3.19 (dd, *J* = 14.6, 8.0 Hz, 1H), 3.07 (ddd, *J* = 14.1, 5.6, 3.2 Hz, 1H), 2.83 (ddd, *J* = 14.0, 9.0, 2.6 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.82, 171.88,

167.83, 167.63, 137.88, 137.04, 136.79, 129.46, 129.24, 129.04, 128.08, 127.47, 127.26, 127.17, 126.43, 123.36, 121.21, 118.68, 117.98, 111.08, 55.07, 53.29, 51.61, 37.50, 27.17. HRMS (ESI) m/z calc'd for $C_{28}H_{25}N_3O_6$ $[M+Na]^+$ 522.1641, found 522.1645.



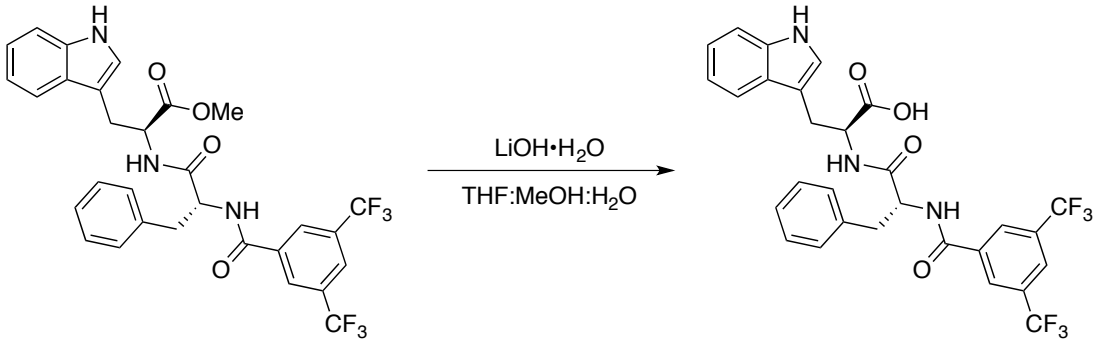
*2-(((*R*)-1-(((*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzenaminium chloride* (**54q**). To a stirring solution of **56q** (250 mg, 0.4 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 6 mL, 0.067 M), lithium hydroxide monohydrate (72 mg, 1.6 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (149 mg, 61%). m.p. = 195-203 °C. This solid was carried forward to the next step. To a stirring solution of **SRG-IV-086** (100 mg, 0.17 mmol, 1 eq) in anhydrous dichloromethane (900 μL, 0.2 M), hydrochloric acid (4N in dioxane, 440 μL, 1.7 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo* to produce an off-white solid (88.7 mg, quant). IR (solid) 2923, 2854, 1716, 1645, 1532 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.58 (dd, J = 15.5, 7.7 Hz, 3H), 7.40 – 7.30 (m, 3H), 7.14 (dd, J = 5.2, 1.8 Hz, 3H), 7.09 – 7.03 (m, 4H), 6.99 (t, J = 7.5 Hz, 1H), 4.78 (dd, J = 8.4, 5.0

Hz, 1H), 3.34 (dd, *J* = 14.7, 5.0 Hz, 1H), 3.18 (dd, *J* = 14.7, 8.3 Hz, 1H), 3.02 (dd, *J* = 13.8, 5.6 Hz, 1H), 2.83 (ddd, *J* = 13.9, 9.1, 2.0 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.82, 171.90, 167.16, 137.01, 136.83, 132.65, 129.04, 128.63, 128.13, 127.47, 127.39, 126.51, 123.57, 123.38, 121.22, 118.64, 117.99, 111.11, 109.49, 54.82, 53.33, 37.33, 27.27. HRMS (ESI) m/z calc'd for $C_{27}H_{26}N_4O_4$ $[M+H]^+$ 471.2032, found 471.2031.



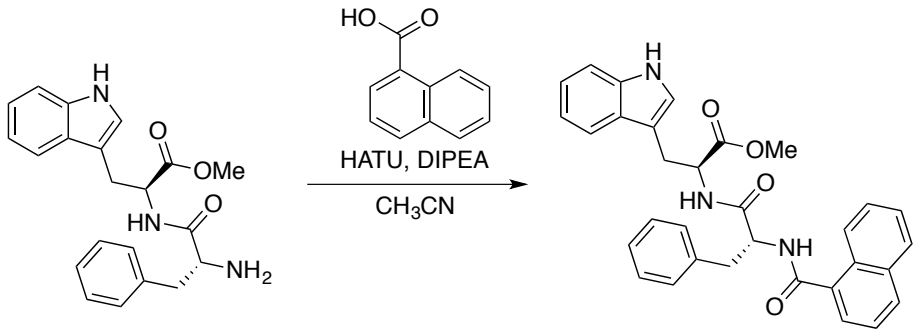
Methyl (3,5-bis(trifluoromethyl)benzoyl)-D-phenylalanyl-L-tryptophanate (**56n**). To a stirring solution of **55** (250 mg, 0.5 mmol, 1 eq) in anhydrous dichloromethane (2.7 mL, 0.2 M), trifluoroacetic acid (410 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 3,5-bis(trifluoromethyl)benzoic acid (138 mg, 0.5 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (560 μL, 3 mmol, 6 eq) and anhydrous acetonitrile (1.8 mL, 0.3 M). The reaction mixture was then cooled to 0 °C and HATU (306 mg, 0.8 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed twice with deionized water (20 mL), followed by brine (20 mL). The organic was then dried over anhydrous

sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (210 mg, 70% over two steps). IR (solid) 3284, 1738, 1644, 1537, 1456 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.08 (d, *J* = 10.8 Hz, 3H), 7.97 (s, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.24 – 7.20 (m, 3H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.11 – 7.04 (m, 3H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.75 (s, 1H), 6.40 (d, *J* = 8.0 Hz, 1H), 4.85 (dtd, *J* = 14.2, 7.9, 7.1, 3.1 Hz, 2H), 3.65 (s, 3H), 3.26 (dd, *J* = 14.9, 6.0 Hz, 1H), 3.16 (dd, *J* = 15.0, 5.6 Hz, 1H), 3.13 – 3.05 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.02, 170.27, 164.22, 136.25, 136.18, 135.98, 132.43, 132.15, 129.48, 128.90, 127.52, 127.43, 125.34, 124.04, 122.84, 122.59, 119.99, 118.45, 111.44, 109.71, 55.20, 53.02, 52.62, 38.57, 27.57. ^{19}F NMR (471 MHz, C_6D6) δ -62.91. HRMS (ESI) m/z calc'd for $C_{30}H_{25}F_6N_3O_4$ $[M+H]^+$ 606.1828, found 606.1830.



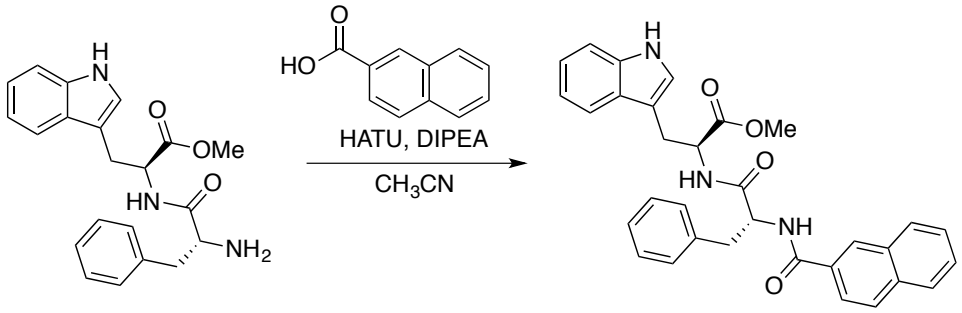
(3,5-Bis(trifluoromethyl)benzoyl)-D-*phenylalanyl*-L-*tryptophan* (**54n**). To a stirring solution of **56n** (130 mg, 0.2 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3 mL, 0.067 M), lithium hydroxide monohydrate (36 mg, 0.9 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product

was dried to produce a white solid (117 mg, 92%). m.p. = 143-165 °C. IR (solid) 3413, 3286, 3064, 1736, 1644 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.16 (s, 2H), 8.10 (s, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.20 – 7.12 (m, 3H), 7.12 – 7.06 (m, 2H), 7.03 (s, 1H), 6.98 (t, *J* = 7.8 Hz, 1H), 6.91 (t, *J* = 7.4 Hz, 1H), 4.91 (dd, *J* = 9.4, 5.4 Hz, 1H), 4.78 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.35 (d, *J* = 5.1 Hz, 1H), 3.21 (dd, *J* = 14.8, 7.7 Hz, 1H), 3.11 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.84 (dd, *J* = 13.9, 9.4 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 175.38, 172.86, 166.56, 138.39, 137.97, 137.69, 133.01, 132.75, 130.28, 129.36, 129.07, 128.86, 127.73, 124.61, 122.31, 119.89, 119.16, 112.26, 110.70, 56.52, 54.92, 38.71, 28.38. ^{19}F NMR (471 MHz, MeOD) δ -64.38. HRMS (ESI) m/z calc'd for $C_{29}H_{23}F_6N_3O_4$ $[M+H]^+$ 592.1671, found 592.1673.



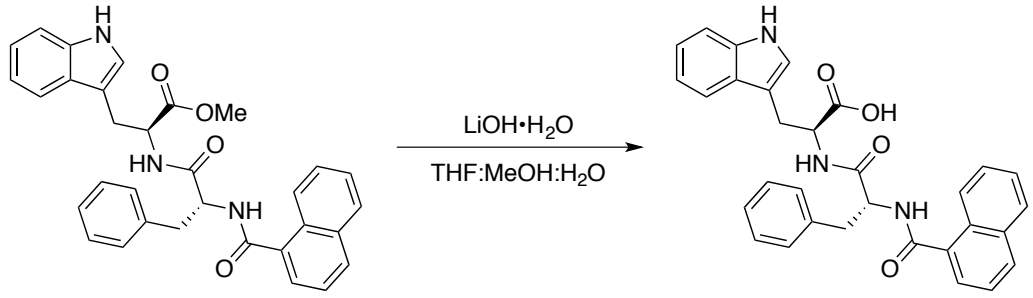
Methyl (1-naphthoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56o**). To the crude amine **68** (90 mg, 0.25 mmol, 1 eq) was added 1-naphthoic acid (43 mg, 0.25 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (180 μL, 1 mmol, 4 eq) and anhydrous acetonitrile (0.9 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (142 mg, 0.4 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over

anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (130 mg, quant.). IR (solid) 3284, 1736, 1661, 1635, 1592 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.07 (s, 1H), 7.89 (dd, *J* = 18.0, 8.4 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.37 – 7.30 (m, 3H), 7.30 – 7.27 (m, 2H), 7.22 – 7.18 (m, 3H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.73 (d, *J* = 7.8 Hz, 1H), 6.46 (d, *J* = 8.0 Hz, 1H), 5.01 (q, *J* = 7.5 Hz, 1H), 4.89 (dt, *J* = 7.7, 5.6 Hz, 1H), 3.62 (s, 3H), 3.32 (dd, *J* = 15.0, 5.8 Hz, 1H), 3.24 (td, *J* = 14.1, 6.1 Hz, 2H), 3.11 (dd, *J* = 14.0, 7.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.07, 170.56, 169.64, 136.69, 136.30, 133.70, 133.53, 131.03, 130.09, 129.50, 128.90, 128.34, 127.49, 127.33, 127.22, 126.55, 125.32, 125.25, 124.76, 123.25, 122.45, 119.87, 118.64, 111.44, 109.72, 54.85, 52.86, 52.55, 38.02, 27.64. HRMS (ESI) m/z calc'd for $C_{32}H_{29}N_3O_4$ $[M+Na]^+$ 542.2056, found 542.2041.



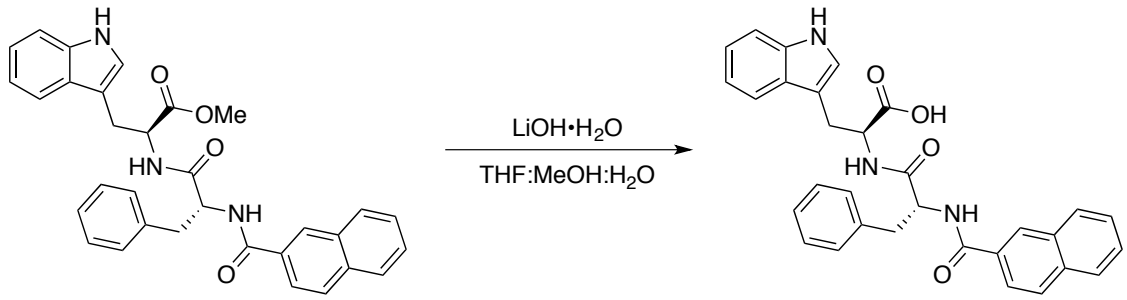
Methyl (2-naphthoyl)-D-*phenylalanyl*-L-*tryptophanate* (**56p**). To the crude amine **68** (90 mg, 0.25 mmol, 1 eq) was added 2-naphthoic acid (43 mg, 0.25 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (180 μL, 1 mmol, 4 eq) and anhydrous acetonitrile (0.9 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (142 mg, 0.4 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (110 mg, 85%). IR (solid) 3278, 1739, 1659, 1636, 1528 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.64 – 8.58 (m, 1H), 8.36 (s, 1H), 7.95 (dd, J = 19.7, 8.4 Hz, 3H), 7.83 (dd, J = 8.6, 1.7 Hz, 1H), 7.58 (td, J = 6.9, 1.5 Hz, 2H), 7.51 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.27 (d, J = 7.5 Hz, 2H), 7.18 (dd, J = 14.3, 6.9 Hz, 4H), 7.12 (d, J = 7.3 Hz, 1H), 7.03 (t, J = 7.7 Hz, 1H), 6.94 (t, J = 7.5 Hz, 1H), 4.82 (dd, J = 10.7, 4.4 Hz, 1H), 4.61 – 4.55 (m, 1H), 3.61 (s, 3H), 3.18 (dd, J = 14.4, 5.3 Hz, 1H), 3.05 (dd, J = 14.5, 8.7 Hz, 1H), 2.93 – 2.87 (m, 1H), 2.86 – 2.78 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 172.87, 171.95, 166.70, 138.82, 136.72, 134.73, 132.62, 131.93, 129.80, 129.42, 128.56, 128.34, 128.24, 128.21, 128.18, 127.62,

127.30, 126.78, 124.80, 124.52, 121.55, 119.02, 118.60, 112.02, 109.87, 55.49, 53.60, 52.51, 37.94, 27.92. HRMS (ESI) m/z calc'd for $C_{32}H_{29}N_3O_4$ $[M+H]^+$ 520.2236, found 520.2234.

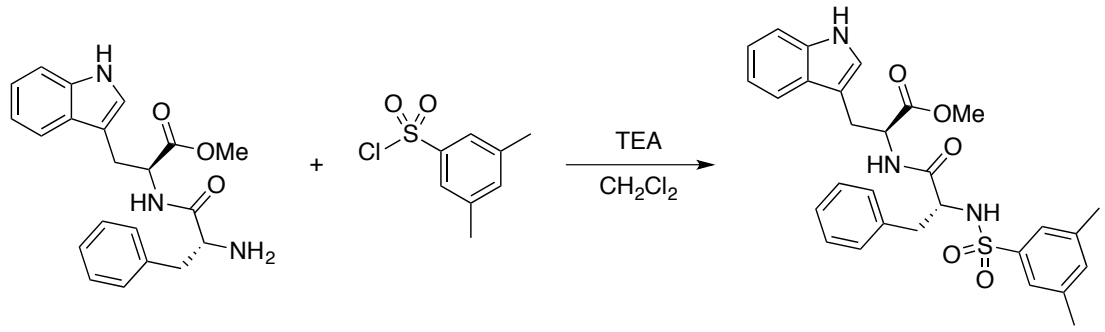


(1-Naphthoyl)-D-*phenylalanyl*-L-*tryptophan* (**54o**) To a stirring solution of **56o** (130 mg, 0.25 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 4 mL, 0.067 M), lithium hydroxide monohydrate (104 mg, 2.5 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (96.3 mg, 76%). m.p. = 126-140 °C. IR (solid) 3278, 3057, 1725, 1636, 1591 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.88 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.28 (t, J = 7.8 Hz, 1H), 7.24 – 7.20 (m, 3H), 7.20 – 7.13 (m, 3H), 7.10 – 7.05 (m, 2H), 7.00 (t, J = 7.5 Hz, 1H), 5.03 (dd, J = 10.2, 4.8 Hz, 1H), 4.80 (dd, J = 7.6, 4.8 Hz, 1H), 3.40 (dd, J = 14.8, 4.8 Hz, 1H), 3.28 – 3.21 (m, 1H), 3.15 (d, J = 14.1 Hz, 1H), 2.79 (dd, J = 13.9, 10.2 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.79, 171.98, 170.88, 137.34, 136.83, 133.91, 133.61, 130.13, 129.85, 129.21, 128.23, 127.84, 126.66, 126.51, 126.04, 125.10, 124.91, 124.49, 123.60, 123.43, 121.25, 118.72, 118.12, 111.13,

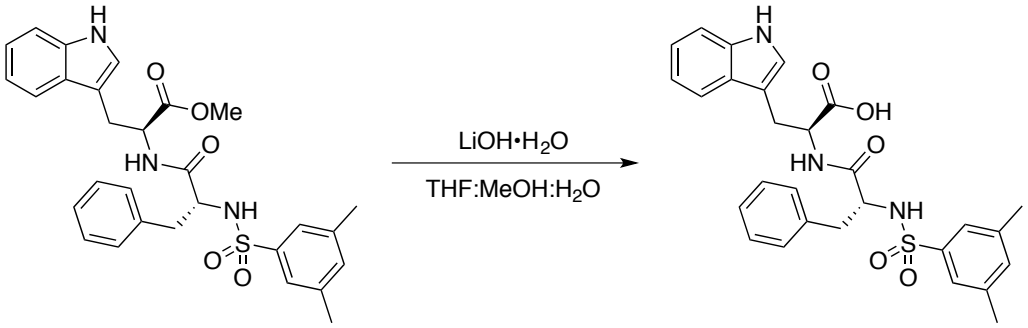
109.40, 54.92, 53.31, 37.46, 27.26. HRMS (ESI) m/z calc'd for $C_{31}H_{27}N_3O_4$ $[M+H]^+$ 506.2080, found 506.2089.



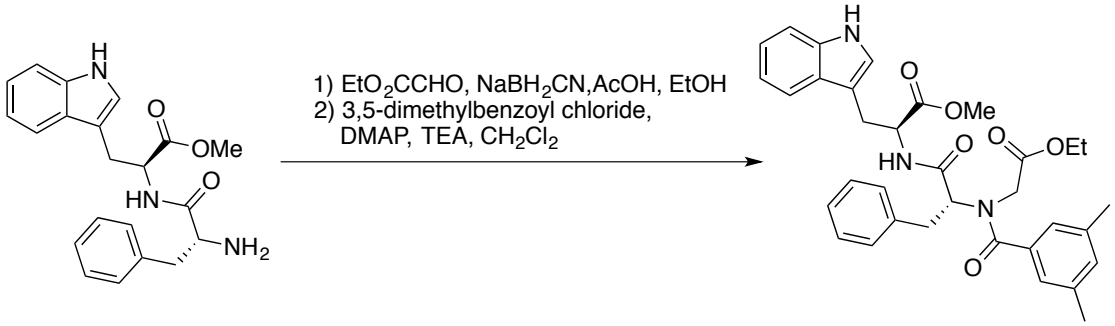
(2-Naphthoyl)-D-*phenylalanyl*-L-*tryptophan* (**54p**). To a stirring solution of **56p** (99.5 mg, 0.2 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3 mL, 0.067 M), lithium hydroxide monohydrate (33 mg, 0.8 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (93.5 mg, 86%). m.p. = 150-159 °C. IR (solid) 3060, 1728, 1640, 1520, 1457 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.08 (s, 1H), 7.91 – 7.82 (m, 3H), 7.64 (dd, J = 8.6, 1.8 Hz, 1H), 7.59 – 7.50 (m, 3H), 7.22 (d, J = 7.9 Hz, 1H), 7.14 – 7.09 (m, 3H), 7.09 – 7.05 (m, 2H), 7.04 (s, 1H), 6.95 (t, J = 7.4 Hz, 1H), 6.90 (t, J = 7.4 Hz, 1H), 4.92 (dd, J = 9.1, 5.2 Hz, 1H), 4.74 (dd, J = 7.4, 4.9 Hz, 1H), 3.37 – 3.32 (m, 1H), 3.26 – 3.14 (m, 2H), 2.89 (dd, J = 13.9, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 174.50, 171.97, 168.67, 137.18, 136.77, 134.98, 132.61, 131.08, 129.12, 128.77, 128.13, 128.08, 127.97, 127.65, 127.58, 127.45, 126.50, 126.41, 123.56, 123.40, 121.15, 118.69, 118.06, 111.06, 109.58, 55.15, 53.76, 51.55, 37.65, 27.34. HRMS (ESI) m/z calc'd for $C_{31}H_{27}N_3O_4$ $[M+H]^+$ 506.2080, found 506.2099.



Methyl ((3,5-dimethylphenyl)sulfonyl)-D-*phenylalanyl*-L-*tryptophanate* (**70**) To the crude amine **68** (91 mg, 0.25 mmol, 1 eq) and triethylamine (90 μL, 0.6 mmol, 2.4 eq) in anhydrous dichloromethane (1.25 mL, 0.2 M) was added 3,5-dimethylbenzenesulfonyl chloride (62 mg, 0.3 mmol, 1.2 eq) at 0 °C. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was then quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (107 mg, 80%). IR (solid) 1738, 1658, 1520, 1456, 1436 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.21 – 7.16 (m, 3H), 7.16 – 7.08 (m, 6H), 7.05 (t, *J* = 7.5 Hz, 2H), 6.78 (d, *J* = 7.4 Hz, 2H), 5.07 (d, *J* = 6.1 Hz, 1H), 4.89 (dt, *J* = 8.4, 5.3 Hz, 1H), 3.91 (dt, *J* = 8.6, 5.7 Hz, 1H), 3.59 (s, 3H), 3.41 (dd, *J* = 14.8, 5.1 Hz, 1H), 3.21 (dd, *J* = 14.8, 5.6 Hz, 1H), 2.95 (dd, *J* = 14.1, 5.4 Hz, 1H), 2.74 (dd, *J* = 14.2, 8.6 Hz, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.00, 170.36, 139.27, 138.19, 136.34, 135.16, 134.80, 129.05, 128.69, 127.40, 127.16, 124.69, 124.01, 122.18, 119.63, 118.57, 111.47, 109.16, 58.24, 53.54, 52.45, 38.08, 27.68, 21.26. HRMS (ESI) m/z calc'd for $C_{29}H_{31}N_3O_5S$ $[M+H]^+$ 556.1882, found 556.1872.

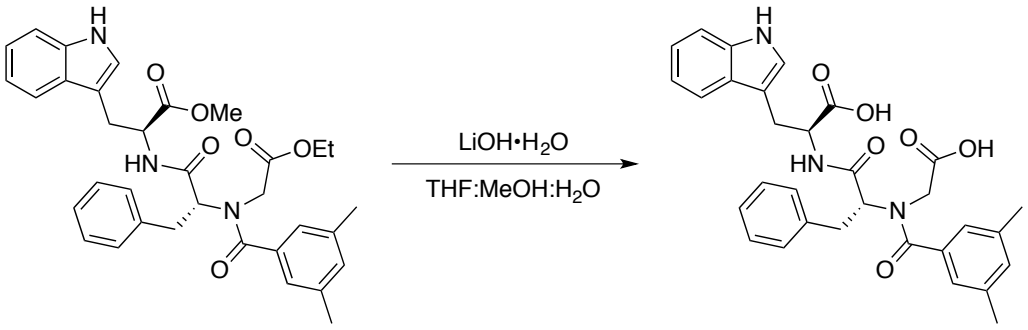


((3,5-Dimethylphenyl)sulfonyl)-D-*phenylalanyl*-L-*tryptophan* (**67**). To a stirring solution of **70** (91 mg, 0.17 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 0.7 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (83 mg, 94%). m.p. = 100-110 °C. IR (solid) 3320, 2923, 1728, 1656, 1525 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 10.29 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.21 (s, 2H), 7.11 (s, 1H), 7.08 – 6.96 (m, 6H), 6.90 – 6.84 (m, 2H), 4.56 (t, J = 6.1 Hz, 1H), 3.98 (dd, J = 9.0, 5.2 Hz, 1H), 3.17 (qd, J = 14.7, 6.1 Hz, 2H), 2.81 (dd, J = 13.9, 5.2 Hz, 1H), 2.55 (dd, J = 13.9, 9.0 Hz, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.40, 171.58, 139.91, 138.90, 136.38, 133.93, 128.99, 127.83, 126.39, 124.20, 123.81, 123.64, 121.08, 118.53, 118.14, 111.00, 110.95, 58.23, 53.02, 38.51, 27.45, 20.01. HRMS (ESI) m/z calc'd for $C_{28}H_{29}N_3O_5S$ $[M+H]^+$ 520.1906, found 520.1901.



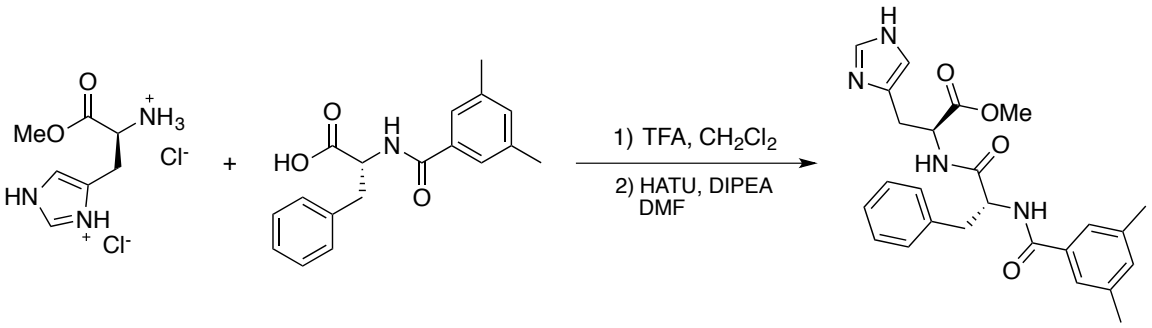
Methyl N-*(3,5-dimethylbenzoyl)*-N-*(2-ethoxy-2-oxoethyl)*-D-*phenylalanyl*-L-*tryptophanate* (**73**). To amine **68** (180 mg, 0.5 mmol, 1 eq) in absolute ethanol (1 mL, 0.5 M), ethyl glyoxylate (50% w/v in tol, 160 μL, 0.75 mmol, 1.5 eq). After 1 hour, sodium cyanoborohydride (40 mg, 0.6 mmol, 1.2 eq) and glacial acetic acid (1 drop, catalytic) were added. After stirring for 18 h, the reaction mixture was concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (160 mg, 0.4 mmol). To this product and triethylamine (120 μL, 0.9 mmol, 2.4 eq) in anhydrous dichloromethane (1.8 mL, 0.2 M) 3,5-dimethylbenzyl chloride (70 μL, 0.3 mmol, 1.2 eq) was added at 0 °C. The reaction mixture was allowed to warm to room temperature (20 °C). After one hour 4-dimethylaminopyridine (5 mg, 0.04 mmol, 0.1 eq) was added to the reaction and it was heated to reflux for 18 h. The reaction mixture was then quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as a yellow foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (79.3 mg, 38% over three steps). IR (solid) 3315, 2952, 1736, 1642, 1603 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 6.8 Hz, 1H), 8.45 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.26 – 7.17 (m, 4H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 6.9 Hz, 2H), 6.78 (s,

1H), 6.04 (s, 2H), 4.78 (ddd, *J* = 11.1, 6.8, 3.8 Hz, 1H), 4.50 (dd, *J* = 11.6, 3.5 Hz, 1H), 4.36 (d, *J* = 15.9 Hz, 1H), 4.28 (qd, *J* = 7.2, 1.7 Hz, 2H), 3.86 (d, *J* = 16.1 Hz, 1H), 3.82 (s, 3H), 3.48 (dd, *J* = 14.7, 3.7 Hz, 2H), 3.19 (dd, *J* = 14.6, 11.6 Hz, 1H), 2.64 (dd, *J* = 14.7, 11.6 Hz, 1H), 1.89 (s, 6H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.73, 172.69, 170.98, 170.10, 137.71, 136.75, 136.44, 133.43, 131.40, 129.11, 128.56, 126.82, 126.70, 123.72, 123.64, 122.06, 119.51, 118.42, 111.45, 110.35, 65.20, 62.00, 53.92, 52.35, 46.28, 35.48, 26.82, 20.78, 13.98. HRMS (ESI) m/z calc'd for $C_{34}H_{37}N_3O_6$ $[M+Na]^+$ 606.2580, found 606.2589.



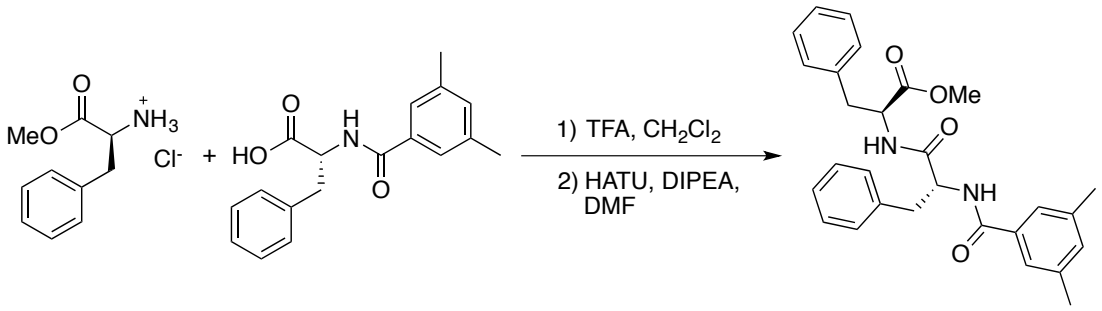
N-*(carboxymethyl)*-N-*(3,5-dimethylbenzoyl)*-D-*phenylalanyl*-L-*tryptophan* (**71**). To a stirring solution of **73** (75 mg, 0.13 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2 mL, 0.067 M), lithium hydroxide monohydrate (54 mg, 0.7 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water, then dissolved in dichloromethane. Organic was then concentrated to dryness in vacuo to produce an off-white foam (59 mg, 83%). IR (solid) 3301, 2923, 1727, 1634, 1538 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.62 (dd, *J* = 30.0, 7.8 Hz, 1H), 7.35 – 7.27 (m, 3H), 7.25 – 7.20 (m, 3H), 7.10 (d, *J* = 7.4 Hz, 1H), 7.06 – 6.95 (m, 2H), 6.91 – 6.82 (m,

2H), 6.44 (s, 1H), 6.03 (s, 1H), 4.61 (ddd, *J* = 57.3, 11.4, 3.6 Hz, 1H), 4.40 – 4.20 (m, 1H), 4.20 – 3.98 (m, 1H), 3.45 (ddd, *J* = 14.1, 9.6, 3.4 Hz, 1H), 3.16 (ddd, *J* = 23.2, 11.4, 3.4 Hz, 1H), 2.83 – 2.69 (m, 1H), 2.26 – 2.11 (m, 2H), 1.89 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.39, 173.55, 172.35, 170.67, 138.33, 137.90, 136.87, 133.54, 131.07 (d, *J* = 10.3 Hz), 128.95 (d, *J* = 20.8 Hz), 128.26 (d, *J* = 11.1 Hz), 126.90 (d, *J* = 4.7 Hz), 126.48 (d, *J* = 13.8 Hz), 123.47 (d, *J* = 20.3 Hz), 122.87, 121.12, 118.58 (d, *J* = 7.3 Hz), 117.59 (d, *J* = 12.2 Hz), 111.22, 109.68, 64.69 (d, *J* = 178.7 Hz), 54.15 (d, *J* = 14.6 Hz), 45.73, 34.35 (d, *J* = 131.6 Hz), 26.50 (d, *J* = 26.6 Hz), 19.51 (d, *J* = 16.3 Hz). HRMS (ESI) m/z calc'd for $C_{31}H_{31}N_3O_6$ $[M+H]^+$ 542.2291, 542.2297 found.



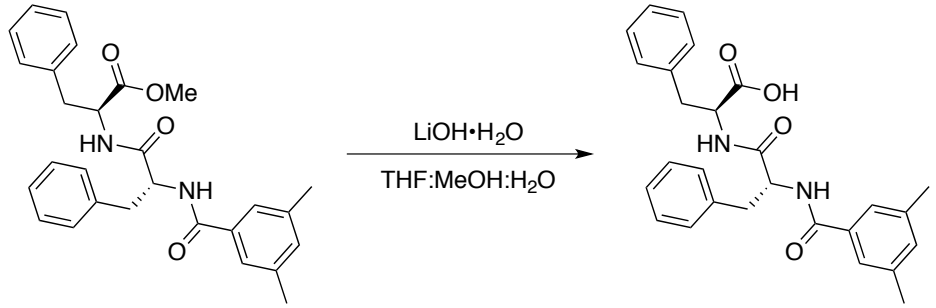
Methyl (3,5-dimethylbenzoyl)-D-*phenylalanyl*-L-*histidinate* (**53c**). To the amino ester (121 mg, 0.5 mmol, 1 eq) was added acid dipeptide (148 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (440 μL, 2.5 mmol, 5 eq) and anhydrous *N,N*-dimethylformamide (1.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 0.75 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over 100 mL ice water. After stirring for 5 mins, precipitate collected and dried *in vacuo* to afford the product as an off-white solid (158 mg, 71%). m.p. = 178-185 °C. IR (solid) 3277, 3183, 1741, 1634, 1601 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.56 (s, 1H), 7.44 (s, 1H), 7.33 – 7.25 (m, 5H), 7.22 (t, *J* = 7.7 Hz, 3H), 7.11 (s, 1H), 6.85 (s, 1H), 6.71 (s, 1H), 4.84 (t, *J* = 7.1 Hz, 1H), 4.70 (dd, *J* = 7.2,

4.8 Hz, 1H), 3.64 (s, 3H), 3.26 (dd, *J* = 13.8, 6.5 Hz, 1H), 3.16 (dd, *J* = 14.0, 7.1 Hz, 1H), 3.12 – 2.98 (m, 2H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.58, 171.54, 168.47, 138.41, 136.63, 134.99, 133.70, 133.49, 131.62, 129.45, 128.73, 127.13, 125.04, 117.96, 55.34, 52.85, 52.65, 38.00, 28.22, 21.24. HRMS (ESI) m/z calc'd for $C_{25}H_{28}N_4O_4$ $[M+H]^+$ 449.2189, found 449.2197.

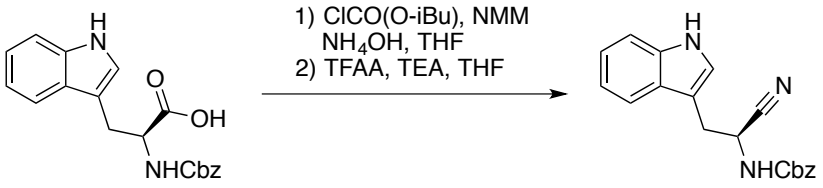


Methyl (3,5-dimethylbenzoyl)-D-*phenylalanyl*-L-*phenylalaninate* (**53b**). To the amino ester (107 mg, 0.5 mmol, 1 eq) was added acid dipeptide (148 mg, 0.5 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (440 *μ*L, 2.5 mmol, 5 eq) and anhydrous *N,N*-dimethylformamide (1.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (285 mg, 0.75 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over 100 mL ice water. After stirring for 5 mins, precipitate collected and dried *in vacuo* to afford the product as an off-white solid (158 mg, 69%). m.p. = 170-188 °C. IR (solid) 3275, 1739, 1663, 1634, 1600 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.33 – 7.26 (m, 5H), 7.24 – 7.20 (m, 3H), 7.20 – 7.15 (m, 2H), 7.13 (s, 1H), 6.98 – 6.93 (m, 2H), 6.74 (d, *J* = 7.6 Hz, 1H), 6.56 (d, *J* = 8.1 Hz, 1H), 4.89 (q, *J* = 7.1 Hz, 1H), 4.83 (dt, *J* = 7.9, 6.1 Hz, 1H), 3.64 (s, 3H), 3.14 (d, *J* = 6.9 Hz, 2H), 3.03 (dd, *J* = 13.8, 6.4 Hz, 1H), 2.96 (dd, *J* = 13.9, 5.8 Hz, 1H), 2.33 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.33, 170.53, 167.41, 138.16, 136.42, 135.54,

133.59, 133.30, 129.32, 129.06, 128.59, 128.54, 127.06, 126.99, 124.72, 54.65, 53.13, 52.18, 38.33, 37.77, 21.08. HRMS (ESI) m/z calc'd for $C_{28}H_{30}N_2O_4$ $[M+H]^+$ 459.2284, found 459.2282.

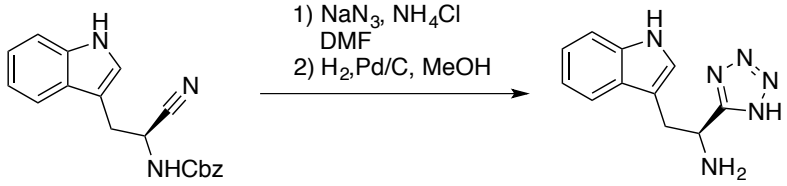


N-*(carboxymethyl)*-N-*(3,5-dimethylbenzoyl)*-D-*phenylalanyl*-L-*tryptophan* (**51b**). To a stirring solution of **53b** (100 mg, 0.22 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 3.3 mL, 0.067 M), lithium hydroxide monohydrate (93 mg, 2.2 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce a white solid (97 mg, quant). m.p. = 105-112 °C. IR (solid) 3280, 3031, 2920, 1717, 1635 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.29 – 7.10 (m, 13H), 4.70 (dd, *J* = 8.0, 5.1 Hz, 1H), 3.15 (ddd, *J* = 18.7, 13.9, 5.2 Hz, 2H), 3.01 (dd, *J* = 13.8, 8.0 Hz, 1H), 2.88 (dd, *J* = 13.9, 9.2 Hz, 1H), 2.32 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.26, 171.77, 168.80, 137.85, 137.02, 136.70, 133.64, 132.71, 128.94, 128.81, 127.98, 127.87, 126.29, 126.20, 124.60, 54.85, 53.66, 37.28, 37.01, 19.73. HRMS (ESI) m/z calc'd for $C_{27}H_{28}N_2O_4$ $[M+H]^+$ 445.2127, found 445.2138.



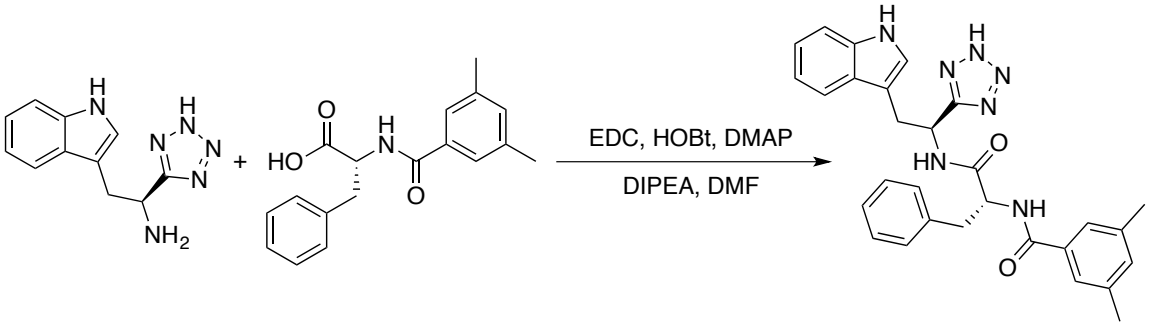
*Benzyl (*S*)-(1-cyano-2-(1*H*-indol-3-yl)ethyl)carbamate* (**50**). To a solution of *N*-Cbz-*L*-trp (1.35 g, 4 mmol, 1 eq) in anhydrous tetrahydrofuran (20 mL, 0.2 M) at -10 °C, *N*-methylmorpholine (490 μL, 4.4 mmol, 1.1 eq) was added dropwise, followed by isobutyl chloroformate (570 μL, 4.4 mmol, 1.1 eq). After the reaction had been stirring 20 mins, aqueous ammonium hydroxide (1.6 mL, 2.5 M) was added and the reaction was allowed to warm to room temperature (20 °C) for 18 h. The reaction was then diluted with ethyl acetate (20 mL) and partitioned with saturated sodium bicarbonate (20 mL). The aqueous was further extracted one time with ethyl acetate (20 mL). The combined organic was washed with saturated sodium bicarbonate (20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The organic was then concentrated to dryness *in vacuo* to afford the product as a white solid (1.35 g, quant). This solid was then suspended in anhydrous tetrahydrofuran (20 mL, 0.2 M) and anhydrous triethylamine (1.7 mL, 12 mmol, 3 eq). Trifluoroacetic anhydride (840 μL, 6 mmol, 1.5 eq) was then added dropwise at -10 °C. The reaction was allowed to warm to room temperature for 18 h. The reaction was quenched with sodium bicarbonate until gas stopped evolving. The reaction mixture was then diluted with ethyl acetate (20 mL) and partitioned. The organic layer was washed with brine (20 mL), dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the product as a white solid (1.3 g, quant over two steps). m.p. = 160-162 °C. IR (solid) 1691, 1531, 1460, 1346, 1312 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.17 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.35 (q, *J* = 8.1, 7.5 Hz, 5H), 7.24 (s, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 5.13 (s, 2H), 5.10 (s, 1H), 4.99 (s, 1H), 3.30 (d, *J* = 5.9

Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.19, 136.55, 136.36, 127.99, 127.60, 127.36, 126.89, 123.58, 121.13, 118.74, 118.59, 117.51, 110.92, 107.85, 66.50, 43.80, 28.69. HRMS (ESI) m/z calc'd for $C_{19}H_{17}N_3O_2$ $[M+H]^+$ 320.1399, found 320.1406.



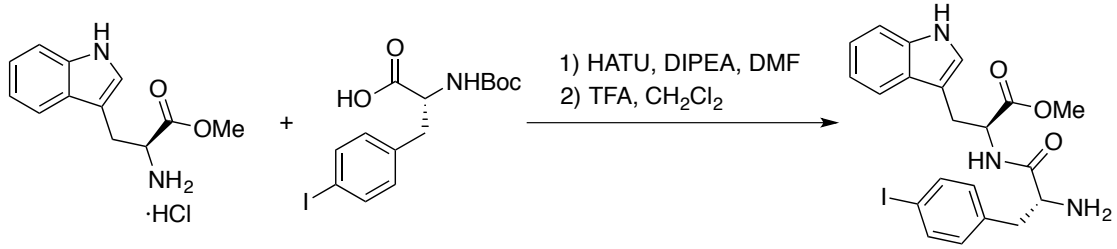
*(*S*)-2-(1*H*-indol-3-yl)-1-(1*H*-tetrazol-5-yl)ethan-1-amine* (**48**). A sealed vessel containing **50** (320 mg, 1 mmol, 1 eq), sodium azide (72 mg, 1.1 mmol, 1.1 eq) and ammonium chloride (62 mg, 1.15 mmol, 1.15 eq in anhydrous *N,N*-dimethylformamide (3.3 mL, 0.3 M) was heated to 90 °C for 18 h. The reaction mixture was then cooled and poured over ice water (50 mL). The aqueous was adjusted to pH ~4 with saturated sodium bisulfate, then extracted three times with ethyl acetate (30 mL). The organic layer was washed four times with water (30 mL), once with brine (30 mL), dried over anhydrous sodium sulfate and then concentrated to dryness *in vacuo* to afford the product as a yellow solid (320 mg, 0.88 mmol, 1 eq). This solid was taken up in methanol (8.8 mL, 1 M) and palladium on carbon (32 mg, 10% w/w) was added. The reaction mixture was then sparged with argon for 15 mins, followed by hydrogen for 15 mins. The reaction was then left to stir under a hydrogen atmosphere for 18 h. The reaction mixture was then filtered through celite with an excess of methanol and concentrated to dryness to afford the product was a yellow solid (214 mg, 88% over two steps). m.p. = 195-210 °C. IR (solid) 2918, 1616, 1491, 1456, 1418 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.49 (d, J = 8.2 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.10 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.02 (ddd, J = 8.1, 7.0, 1.0 Hz, 1H), 6.99 (s, 1H), 4.89 (dd, J = 7.7, 6.8 Hz, 1H), 3.59 (dd, J = 14.7, 6.8 Hz, 1H), 3.43 (dd, J = 14.7, 7.7

Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 159.53, 136.93, 127.13, 124.07, 121.43, 118.88, 117.69, 111.20, 107.64, 48.48, 29.73. HRMS (ESI) m/z calc'd for $C_{11}H_{12}N_6$ $[M+H]^+$ 229.1202, found 229.1199.



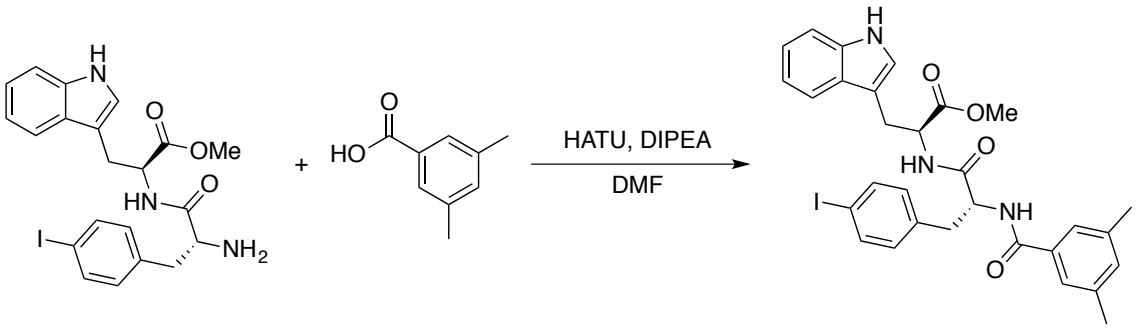
N-*((*R*)-1-(((*S*)-2-(1*H*-Indol-3-yl)-1-(2*H*-tetrazol-5-yl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)-3,5-dimethylbenzamide* (**47**). To a flask containing **39** (156mg, 0.5 mmol, 1 eq), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (115 mg, 0.6 mmol, 1.2 eq) and hydroxybenzotriazole (81 mg, 0.6 mmol, 1.2 eq), anhydrous *N,N*-dimethylformamide (3 mL, 0.17 M) was added. After 10 mins, **48** (115 mg, 0.5 mmol, 1 eq) was added, followed by *N,N*-diisopropylethylamine (180 μL, 1 mmol, 2 eq). The reaction mixture was stirred at room temperature (20 °C) for 18 h, then poured over ice water (10 mL). The precipitate was filtered and purified by flash chromatography (5% MeOH/CH_2Cl_2) to afford the product as an off-white solid (94 mg, 37%). m.p. = 199-209 °C. IR (solid) 2919, 1636, 1600, 1513, 1456 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 16.24 (s, 1H), 10.84 (s, 1H), 8.92 (dd, *J* = 40.0, 7.7 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 7.52 (dd, *J* = 55.3, 7.9 Hz, 1H), 7.37 (d, *J* = 11.3 Hz, 2H), 7.31 (dd, *J* = 8.1, 5.2 Hz, 1H), 7.26 (d, *J* = 7.0 Hz, 1H), 7.23 – 7.16 (m, 3H), 7.13 (dd, *J* = 8.4, 6.3 Hz, 2H), 7.06 (td, *J* = 8.0, 7.1, 2.1 Hz, 2H), 6.96 (dt, *J* = 14.8, 7.5 Hz, 1H), 5.49 – 5.40 (m, 1H), 4.79 – 4.70 (m, 1H), 3.36 (dd, *J* = 11.8, 7.5 Hz, 1.5H), 3.25 (dd, *J* = 14.4, 8.2 Hz, 0.5H), 3.06 (dd, *J* = 13.9, 4.2 Hz, 0.5H), 2.91 (dd, *J* = 14.1, 10.2 Hz, 1H),

2.85 – 2.76 (m, 0.5H), 2.28 (d, J = 10.2 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.97 (d, J = 24.4 Hz), 167.05 (d, J = 27.9 Hz), 158.07, 138.73 (d, J = 12.9 Hz), 137.87, 136.69 (d, J = 10.7 Hz), 134.66 (d, J = 10.0 Hz), 133.11, 129.75 (d, J = 10.9 Hz), 128.57 (d, J = 4.5 Hz), 127.62, 126.79, 125.71 (d, J = 8.1 Hz), 124.50 (d, J = 21.7 Hz), 121.61, 119.07, 118.66 (d, J = 17.8 Hz), 112.04, 109.78 (d, J = 26.6 Hz), 55.10 (d, J = 8.9 Hz), 45.61 (d, J = 53.3 Hz), 37.80 (d, J = 40.4 Hz), 30.05, 21.42 (d, J = 3.5 Hz). HRMS (ESI) m/z calc'd for $C_{29}H_{29}N_7O_2$ $[M+H]^+$ 508.2461, found 508.2454.

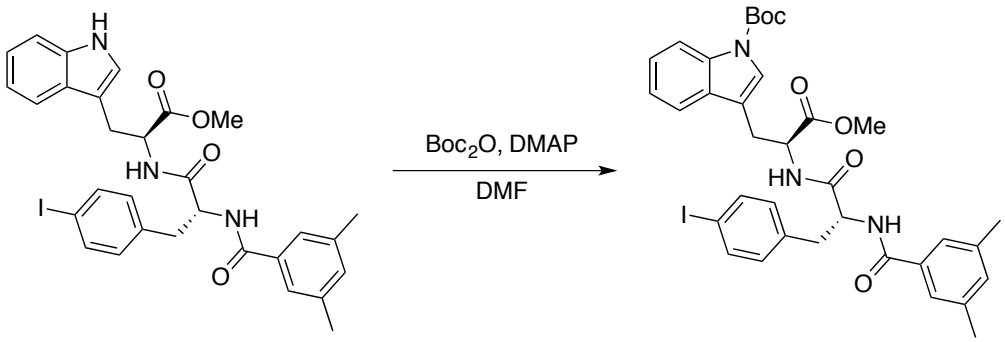


*Methyl ((*R*)-2-amino-3-(4-iodophenyl)propanoyl)*-L-*tryptophanate* (**79**). To the tryptophan methyl ester (7.6 g, 30 mmol, 1 eq) and iodide **78**[7] (11.7 g, 30 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (100 mL, 0.3M) was added *N,N*-diisopropylethylamine (25 mL, 150 mmol, 5 eq). The reaction mixture was then cooled to 0 °C and HATU (17 g, 45 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over ice water (500 mL), filtered and dried *in vacuo* to afford the crude product as a purple solid. The crude product was then dissolved in anhydrous dichloromethane (150 mL, 0.2 M) and trifluoroacetic acid (20 mL, 300 mmol, 10 eq) was added. After stirring for 18 h at room temperature (20 °C), the reaction was quenched slowly with saturated sodium bicarbonate (200 mL). Layers were then partitioned and the aqueous was extracted a further two times with dichloromethane. The combined organic was washed with brine (100 mL) and dried over sodium sulfate.

The reaction was concentrated *in vacuo* to yield the crude product as an orange foam. This residue was purified by flash chromatography (1-2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (10.5 g, 71% over two steps). IR (solid) 3300, 2949, 1737, 1651, 1511 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.04 (s, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.17 (t, *J* = 7.1 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 6.98 – 6.89 (m, 3H), 4.92 (dt, *J* = 8.2, 5.6 Hz, 1H), 3.70 (s, 3H), 3.50 (dd, *J* = 9.3, 4.2 Hz, 1H), 3.39 – 3.25 (m, 2H), 3.15 (dd, *J* = 13.9, 4.2 Hz, 1H), 2.61 (dd, *J* = 13.8, 9.3 Hz, 1H), 1.10 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.94, 172.40, 137.83, 137.67, 136.25, 131.45, 127.80, 122.79, 122.35, 119.66, 118.66, 111.47, 110.32, 92.22, 56.43, 52.91, 52.51, 40.39, 27.75. HRMS (ESI) m/z calc'd for $C_{21}H_{22}IN_3O_3$ $[M+H]^+$ 492.0784, found 492.0789.

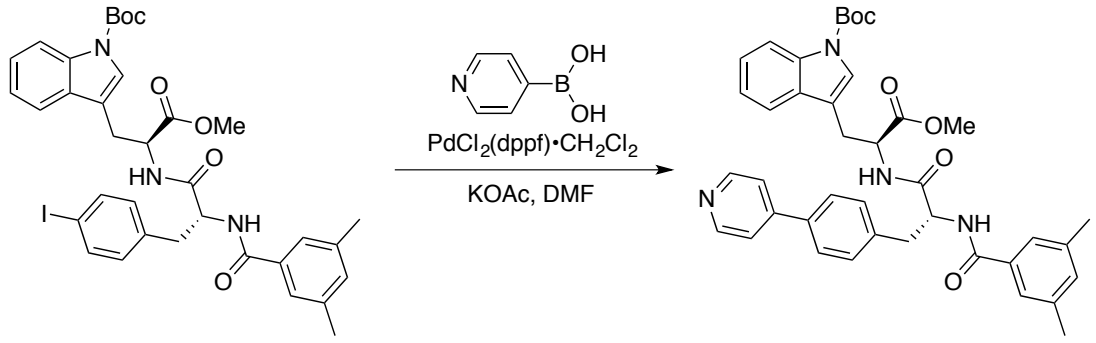


*Methyl ((*R*)-2-(3,5-dimethylbenzamido)-3-(4-iodophenyl)propanoyl)*-L-*tryptophanate* (**80**). To the amine **79** (1.15 g, 2.3 mmol, 1 eq) and 3,5-dimethylbenzoic acid (350 mg, 2.3 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (7.5 mL, 0.3 M) was added *N,N*-diisopropylethylamine (1.6 mL, 9 mmol, 4 eq). The reaction mixture was then cooled to 0 °C and HATU (1.3 g, 3.4 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over ice water (200 mL), filtered and dried *in vacuo* to afford the product as an off white solid (1.5 g, quant). m.p. = 128-142 °C. IR (solid) 1758, 1662, 1637, 1600, 1528 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.63 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 7.52 (dd, *J* = 7.9, 5.2 Hz, 3H), 7.36 (s, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 2.4 Hz, 1H), 7.13 (s, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 4.74 (td, *J* = 10.1, 9.6, 3.9 Hz, 1H), 4.57 (q, *J* = 8.0 Hz, 1H), 3.62 (s, 3H), 3.19 (dd, *J* = 14.5, 5.3 Hz, 1H), 3.04 (dd, *J* = 14.5, 8.9 Hz, 1H), 2.78 (dd, *J* = 13.6, 4.1 Hz, 1H), 2.71 (dd, *J* = 11.7, 9.3 Hz, 1H), 2.28 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.85, 171.74, 166.88, 138.63, 137.89, 137.26, 136.72, 134.51, 133.14, 132.23, 127.61, 125.64, 124.54, 121.57, 119.05, 118.62, 112.03, 109.86, 92.72, 54.66, 53.57, 52.53, 37.36, 27.93, 21.41. HRMS (ESI) m/z calc'd for $C_{30}H_{30}IN_3O_4$ $[M+H]^+$ 624.1359, found 624.1361.



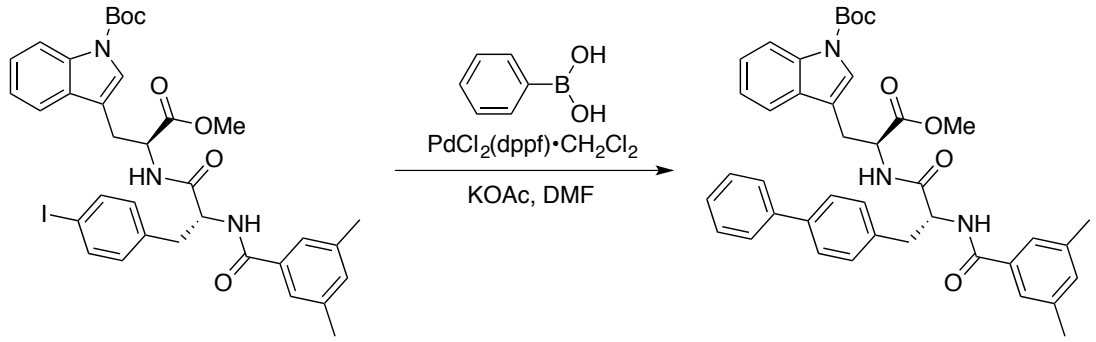
Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-iodophenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**81**). To a stirring solution of **80** (215 mg, 0.35 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (1.4 mL, 0.1 M), di-*tert*-butyl dicarbonate (94 mg, 0.43 mmol, 1.3 eq) was added portionwise, followed by *N,N*-dimethylamino pyridine (4 mg, 0.04 mmol, 0.1 eq) at 0 °C. Allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL) two times. The organic layer was washed with water (10 mL) ten times, brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (250 mg, quant). IR (solid) 3277, 2977, 1732, 1634, 1602 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.53 – 7.41 (m, 4H), 7.38 – 7.29 (m, 1H), 7.27 (d, *J* = 4.6 Hz, 2H), 7.26 – 7.16 (m, 2H), 7.11 (s, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.76 (d, *J* = 8.1 Hz, 2H), 4.98 (q, *J* = 6.9 Hz, 1H), 4.88 (td, *J* = 7.4, 5.7 Hz, 1H), 3.62 (s, 3H), 3.19 (dd, *J* = 14.7, 5.7 Hz, 1H), 3.12 (dt, *J* = 14.2, 7.3 Hz, 2H), 3.02 (dd, *J* = 13.9, 6.2 Hz, 1H), 2.31 (s, 6H), 1.64 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.84, 170.87, 167.77, 149.63, 138.43, 137.64, 136.12, 133.68, 133.63, 131.50, 130.37, 124.94, 124.83, 124.33, 122.87, 118.84, 115.55, 115.13,

100.11, 92.58, 83.96, 54.26, 52.75, 52.62, 37.98, 28.33, 27.66, 21.34. HRMS (ESI) m/z calc'd for $C_{35}H_{38}IN_3O_6$ $[M+H]^+$ 724.1884, found 724.1882.



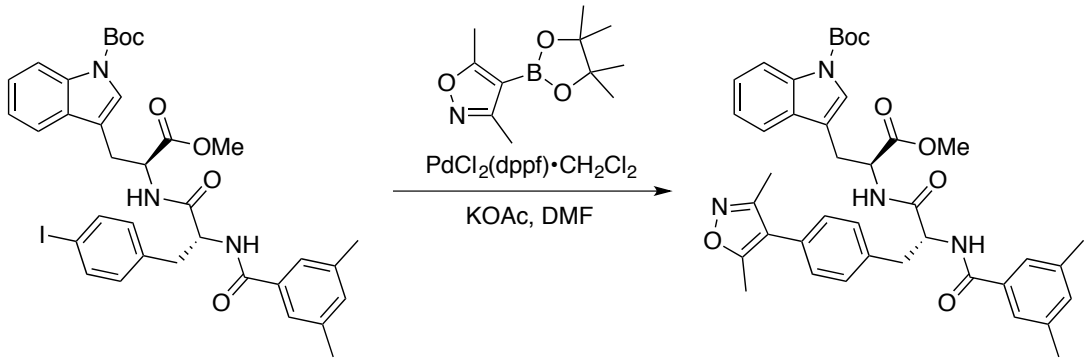
Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(pyridin-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83b**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), 4-pyridyl boronic acid (69 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and sodium carbonate (240 mg, 2.2 mmol, 8 eq) was added in deionized water (1.1 mL, 2 M {relative to Na_2CO_3}) and N,N-dimethylformamide (7 mL, 0.04 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (64 mg, 34%). IR (solid) 2926, 1732, 1637, 1599, 1537 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.59 (d, J = 5.1 Hz, 2H), 8.08 (s, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.45 – 7.36 (m, 5H), 7.28 (s, 3H), 7.20 (d, J = 7.4 Hz, 1H), 7.16 (d, J = 7.9 Hz, 2H), 7.10 (s, 2H), 6.90

(d, J = 7.7 Hz, 1H), 5.01 (q, J = 6.9 Hz, 1H), 4.91 (q, J = 6.7 Hz, 1H), 3.61 (s, 3H), 3.28 – 3.17 (m, 2H), 3.15 (dd, J = 6.4, 2.9 Hz, 2H), 2.29 (s, 6H), 1.61 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.79, 170.92, 167.69, 150.31, 150.27, 147.93, 138.40, 137.75, 136.76, 133.78, 133.58, 130.41, 130.26, 127.23, 127.17, 124.95, 124.81, 124.26, 122.81, 121.54, 118.82, 115.52, 115.08, 83.92, 54.53, 52.62, 52.59, 38.26, 28.28, 27.72, 21.30. HRMS (ESI) m/z calc'd for $C_{40}H_{42}N_4O_6$ $[M+H]^+$ 675.3183, found 675.3174.



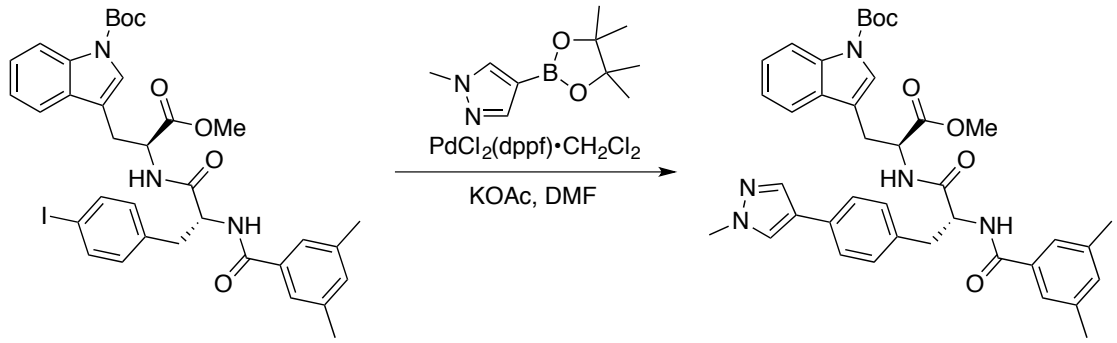
Tert-*butyl 3-((*S*)-2-((*R*)-3-([1,1'-biphenyl]-4-yl)-2-(3,5-dimethylbenzamido)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83a**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), phenyl boronic acid (67 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous *N,N*-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as a brown foam. This residue was purified by flash chromatography (30% EtOAc/Hex) to afford the product as

an off-white foam (141 mg, 76%). IR (solid) 3276, 1732, 1635, 1602, 1537 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 3H), 7.45 (s, 1H), 7.44 – 7.37 (m, 4H), 7.35 – 7.29 (m, 4H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 7.10 (s, 1H), 7.01 (d, *J* = 7.7 Hz, 1H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.94 (dt, *J* = 8.0, 6.4 Hz, 1H), 3.60 (s, 3H), 3.28 – 3.19 (m, 2H), 3.15 (d, *J* = 6.4 Hz, 2H), 2.30 (s, 6H), 1.63 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.86, 171.21, 167.84, 149.66, 140.86, 139.96, 138.37, 135.65, 133.91, 133.53, 130.44, 129.97, 128.85, 127.36, 127.32, 127.11, 125.04, 124.77, 124.34, 122.82, 118.91, 115.52, 115.22, 83.84, 54.71, 52.71, 52.54, 38.31, 28.30, 27.77, 21.32. HRMS (ESI) m/z calc'd for $C_{41}H_{43}N_3O_6$ $[M+H]^+$ 674.3230, found 674,3233.



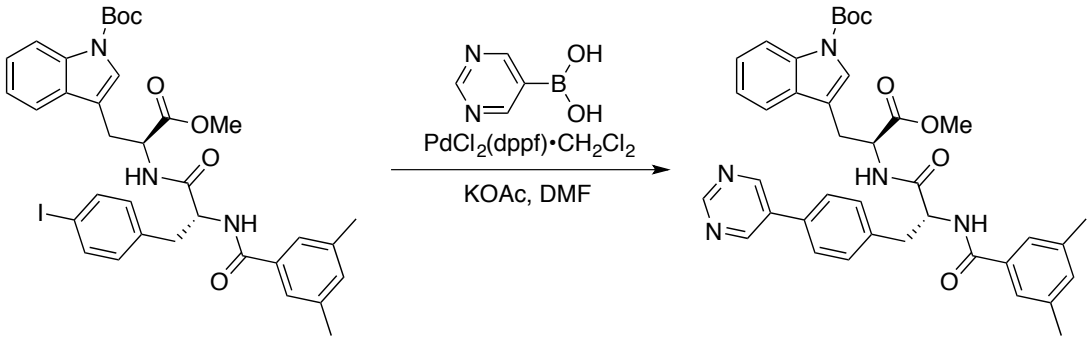
Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(3,5-dimethylisoxazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83e**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), 3,5-dimethylisooxazole-4-boronic ester (123 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The

reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (30% EtOAc/Hex) to afford the product as an off-white foam (144 mg, 75%). IR (solid) 3280, 2928, 1732, 1635, 1603 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.09 (s, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.43 (s, 1H), 7.28 (d, J = 9.7 Hz, 3H), 7.21 (t, J = 7.4 Hz, 1H), 7.13 (d, J = 8.9 Hz, 3H), 7.06 (d, J = 7.5 Hz, 2H), 6.99 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 5.00 (q, J = 7.0 Hz, 1H), 4.90 (q, J = 6.8 Hz, 1H), 3.63 (s, 3H), 3.23 – 3.10 (m, 4H), 2.32 (d, J = 7.1 Hz, 9H), 2.19 (s, 3H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.81, 170.90, 167.73, 165.23, 158.71, 149.61, 138.41, 135.90, 135.47, 133.82, 133.58, 130.37, 129.94, 129.26, 124.92, 124.82, 124.33, 122.83, 118.81, 116.31, 115.51, 115.03, 83.94, 54.50, 52.72, 52.59, 38.17, 28.28, 27.74, 21.31, 21.27, 11.64, 10.89. HRMS (ESI) m/z calc'd for $C_{40}H_{44}N_4O_7$ $[M+H]^+$ 693.3288, found 693.3295.



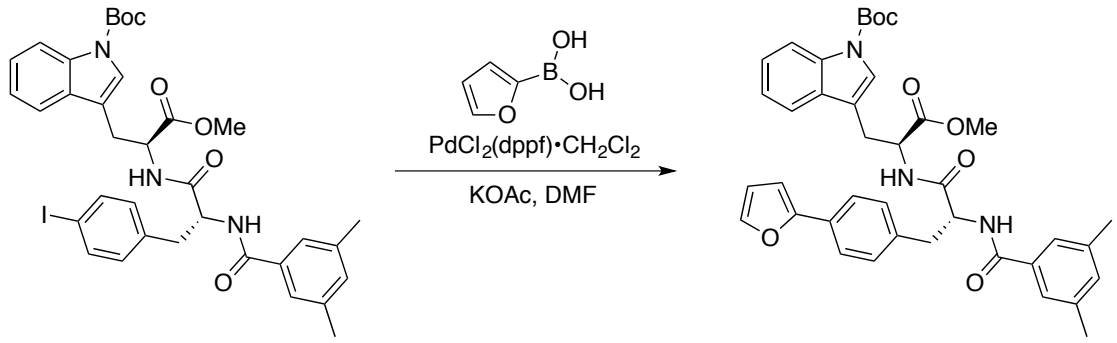
Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83f**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), 1-methyl-4-pyrazole boronic ester (115 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the product as an off-white foam (149 mg, 80%). IR (solid) 1732, 1636, 1602, 1532, 1452 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.66 (s, 1H), 7.48 (s, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.37 (s, 1H), 7.31 – 7.23 (m, 5H), 7.19 (td, J = 7.5, 2.6 Hz, 1H), 7.08 (d, J = 8.7 Hz, 3H), 6.84 (t, J = 7.9 Hz, 2H), 4.98 – 4.85 (m, 2H), 3.89 (d, J = 2.2 Hz, 3H), 3.59 (d, J = 2.0 Hz, 3H), 3.12 (ddd, J = 13.9, 6.8, 4.2 Hz, 4H), 2.29 (s, 6H), 1.63 (s, 9H). ^{13}C NMR (126

MHz, $CDCl_3$) δ 171.78, 171.01, 167.68, 149.63, 138.37, 136.77, 135.46, 134.52, 133.85, 133.51, 131.50, 130.39, 129.94, 126.99, 125.76, 124.96, 124.73, 124.23, 122.93, 122.78, 118.83, 115.47, 115.03, 83.88, 54.74, 52.55, 39.16, 38.29, 28.30, 27.72, 24.99, 21.30. HRMS (ESI) m/z calc'd for $C_{39}H_{43}N_5O_6$ $[M+H]^+$ 678.3292, found 678.3291.



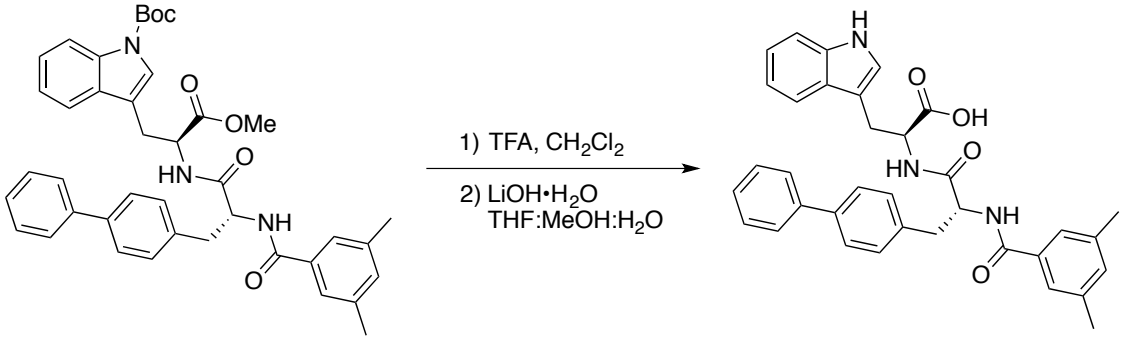
Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(pyrimidin-5-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83d**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), pyrimidin-5-ylboronic acid (68 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (50% EtOAc/Hex) to afford the product as an off-white foam (146 mg, 78%). IR (solid) 1731, 1636, 1602, 1533, 1451 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 9.10 (s, 1H), 8.76 (d, J

= 1.5 Hz, 2H), 8.04 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.45 (s, 1H), 7.28 (s, 3H), 7.26 – 7.23 (m, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.10 (d, J = 7.7 Hz, 2H), 7.05 (s, 1H), 5.13 (q, J = 6.9 Hz, 1H), 4.92 (q, J = 7.2 Hz, 1H), 3.59 (s, 3H), 3.33 – 3.05 (m, 4H), 2.24 (s, 6H), 1.56 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.99, 171.15, 167.77, 157.37, 154.75, 149.61, 138.32, 137.75, 135.42, 133.91, 133.74, 133.56, 132.69, 130.59, 130.35, 126.91, 124.99, 124.80, 124.39, 122.81, 118.88, 115.52, 115.29, 83.90, 54.40, 52.59, 38.25, 28.24, 27.70, 21.29. HRMS (ESI) m/z calc'd for $C_{39}H_{41}N_5O_6$ $[M+H]^+$ 676.3135, found 676.3132.



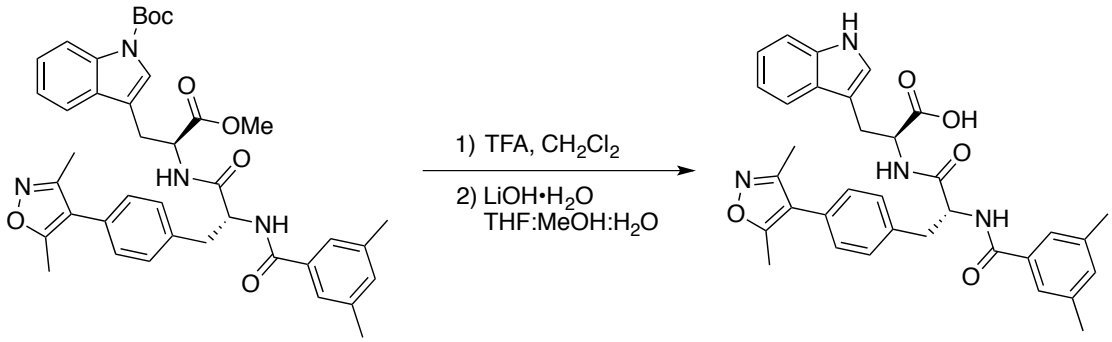
Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(furan-2-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83c**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), 2-furyl boronic acid (61 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous *N,N*-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed four times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous

sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as a brown foam. This residue was purified by flash chromatography (30% EtOAc/Hex) to afford the product as an off-white foam (176 mg, 96%). IR (thin film) 3280, 2979, 1735, 1637, 1602 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 7.47 (d, *J* = 7.9 Hz, 3H), 7.45 – 7.39 (m, 2H), 7.35 – 7.27 (m, 3H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 7.13 – 7.02 (m, 3H), 6.91 (d, *J* = 7.6 Hz, 1H), 6.55 (d, *J* = 3.3 Hz, 1H), 6.43 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.03 (q, *J* = 6.9 Hz, 1H), 4.95 – 4.82 (m, 1H), 3.59 (s, 3H), 3.22 – 3.07 (m, 4H), 2.28 (s, 6H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.85, 171.06, 167.75, 153.86, 149.63, 142.05, 138.37, 135.63, 135.47, 133.83, 133.53, 130.40, 129.82, 129.77, 124.98, 124.75, 124.29, 124.05, 122.81, 118.87, 115.50, 115.17, 111.75, 105.02, 83.83, 54.59, 52.71, 52.56, 38.37, 28.30, 27.72, 21.31. HRMS (ESI) m/z calc'd for $C_{39}H_{41}N_3O_7$ $[M+Na]^+$ 686.2842, found 686.2845.



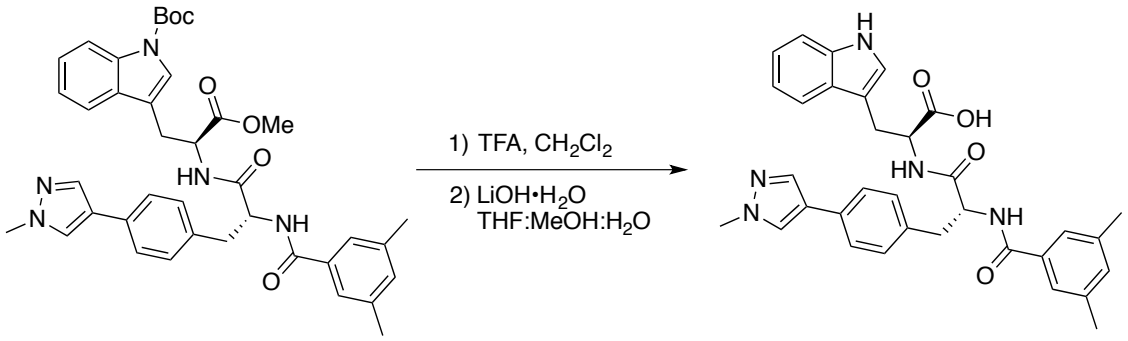
*((*R*)-3-([1,1'-biphenyl]-4-yl)-2-(3,5-dimethylbenzamido)propanoyl)*-L-*tryptophan* (**74a**). To a stirring solution of **83a** (161 mg, 0.23 mmol, 1 eq) in anhydrous dichloromethane (1.2 mL, 0.2 M), trifluoroacetic acid (370 μL, 2.7 mmol, 20 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then

concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (61 mg, 0.1 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M) and lithium hydroxide monohydrate (45 mg, 1 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (59 mg, 45% over 2 steps). m.p. = 145-150 °C. IR (solid) 3288, 2920, 1723, 1634, 1600 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.1 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.33 – 7.27 (m, 4H), 7.23 (s, 2H), 7.14 (s, 1H), 7.08 (s, 1H), 7.06 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.97 (t, *J* = 7.8 Hz, 1H), 4.92 – 4.86 (m, 1H), 4.78 (dd, *J* = 8.1, 4.9 Hz, 1H), 3.37 (dd, *J* = 14.8, 4.8 Hz, 1H), 3.21 (dd, *J* = 14.7, 8.1 Hz, 1H), 3.15 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.89 (dd, *J* = 13.9, 8.2 Hz, 1H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.63, 173.06, 170.27, 142.13, 140.77, 139.37, 138.11, 137.37, 135.19, 134.22, 130.87, 129.74, 128.12, 127.80, 126.10, 126.02, 124.67, 122.43, 119.96, 119.34, 112.33, 110.92, 101.39, 56.04, 54.91, 38.50, 28.61, 21.24. HRMS (ESI) m/z calc'd for $C_{35}H_{33}N_3O_4$ $[M+H]^+$ 560.2549, found 560.2552.



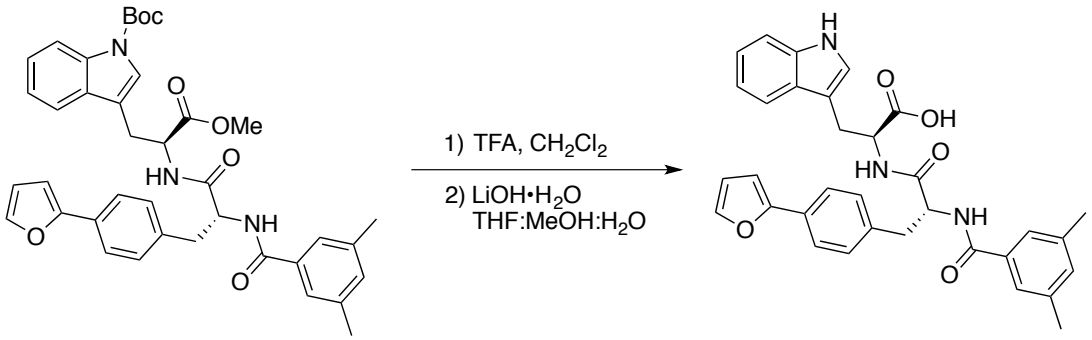
*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(3,5-dimethylisoxazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**74e**). To a stirring solution of **83e** (100 mg, 0.14 mmol, 1 eq) in anhydrous dichloromethane (0.7 mL, 0.2 M), trifluoroacetic acid (110 μL, 1.5 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (10 mL) and partitioned. The organic layer was washed with brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (60.3 mg, 0.1 mmol, 70%). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M) and lithium hydroxide monohydrate (42 mg, 1 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (58.9 mg, 70% over two steps). m.p. = 140-150 °C. IR (solid) 3302, 2923, 1726, 1635, 1602 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 10.33 (s, 1H), 8.22 – 8.01 (m, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.25 (s, 2H), 7.17 – 7.10 (m, 1H), 7.10 – 7.01 (m, 2H), 6.97 (s, 5H), 4.78 (s, 1H), 3.36 (dd, J = 15.5, 4.2 Hz, 1H), 3.15 (ddd, J = 20.9, 14.5, 7.1 Hz, 2H), 2.87 (dd, J = 13.9, 7.7 Hz, 1H), 2.28 (s, 9H), 2.12

(s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 173.91, 171.74, 168.86, 165.43, 158.66, 138.12, 136.51, 133.01, 129.70, 128.73, 128.34, 127.47, 124.76, 123.62, 123.46, 121.22, 118.72, 118.03, 116.35, 111.17, 111.11, 109.61, 54.60, 53.34, 37.34, 27.32, 19.99, 10.11, 9.41. HRMS (ESI) m/z calc'd for $C_{34}H_{34}N_4O_5$ $[M+Na]^+$ 601.2451, found 601.2429.



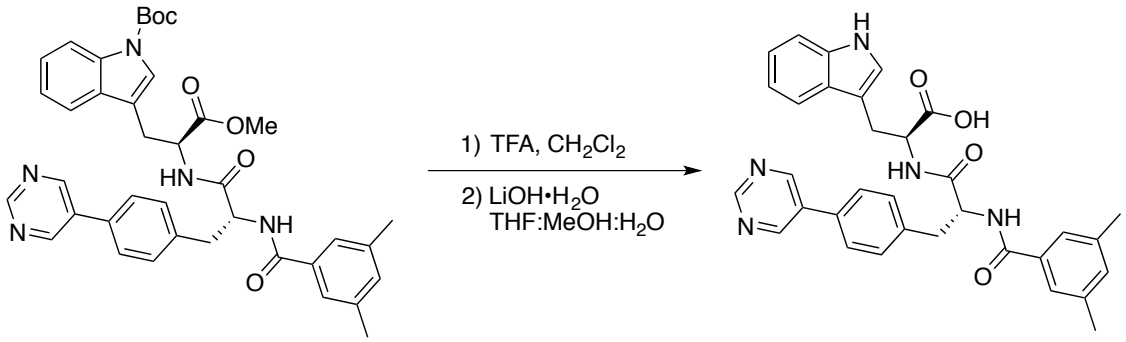
*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**74f**). To a stirring solution of **83f** (100 mg, 0.14 mmol, 1 eq) in anhydrous dichloromethane (0.7 mL, 0.2 M), trifluoroacetic acid (110 *μ*L, 1.5 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (10 mL) and partitioned. The organic layer was washed with brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (3% MeOH/CH_2Cl_2) to afford the product as an off-white foam (62.4 mg, 0.11 mmol, 73%). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M) and lithium hydroxide monohydrate (42 mg, 1 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to

produce an off-white solid (60.9 mg, 70% over two steps). m.p. = 132-145 °C. IR (solid) 3300, 2922, 1726, 1643, 1602 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.77 (s, 1H), 7.67 (s, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.26 – 7.15 (m, 4H), 7.12 (s, 1H), 7.10 – 7.00 (m, 3H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 2H), 4.86 – 4.82 (m, 1H), 4.77 (dd, *J* = 8.3, 4.9 Hz, 1H), 3.86 (s, 3H), 3.34 (dd, *J* = 14.8, 5.0 Hz, 1H), 3.18 (dd, *J* = 14.7, 8.2 Hz, 1H), 3.07 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.83 (dd, *J* = 13.8, 8.3 Hz, 1H), 2.28 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.87, 171.92, 168.93, 138.11, 136.84, 135.95, 135.03, 132.97, 130.87, 129.60, 127.64, 127.49, 124.96, 124.81, 124.76, 123.40, 123.00, 121.18, 118.70, 118.03, 111.11, 109.50, 54.79, 53.30, 37.61, 37.26, 27.25, 19.97. HRMS (ESI) m/z calc'd for $C_{34}H_{34}N_4O_5$ $[M+Na]^+$ 586.2430, found 586.2430.



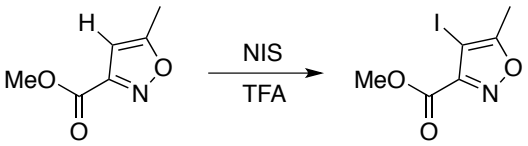
*((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(furan-2-yl)phenyl)propanoyl)-*L*-tryptophan* (**74c**). To a stirring solution of **83c** (176 mg, 0.27 mmol, 1 eq) in anhydrous dichloromethane (1.4 mL, 0.2 M), trifluoroacetic acid (200 μL, 2.7 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (50% EtOAc/Hexanes) to afford the product

as an off-white foam (61 mg, 0.1 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M) and lithium hydroxide monohydrate (45 mg, 1 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (63 mg, 43% over 2 steps). m.p. = 168-170 °C. IR (solid) 2920, 1720, 1633, 1600, 1516 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.56 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 7.8 Hz, 3H), 7.11 (s, 1H), 7.07 (q, J = 7.8, 5.9 Hz, 2H), 6.96 (dd, J = 11.2, 7.6 Hz, 3H), 6.61 (d, J = 3.1 Hz, 1H), 6.44 (s, 1H), 4.90 – 4.83 (m, 1H), 4.77 (dd, J = 8.2, 4.8 Hz, 1H), 3.36 (dd, J = 14.8, 4.9 Hz, 1H), 3.19 (dd, J = 14.7, 8.3 Hz, 1H), 3.08 (dd, J = 13.9, 5.5 Hz, 1H), 2.88 – 2.79 (m, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.97, 171.90, 169.01, 153.92, 141.89, 138.10, 136.98, 136.17, 133.86, 132.97, 129.48, 127.53, 124.76, 123.57, 123.41, 123.34, 121.22, 118.75, 118.02, 111.34, 111.17, 109.58, 104.47, 54.69, 53.42, 37.34, 27.27, 19.98. HRMS (ESI) m/z calc'd for $C_{33}H_{31}N_3O_5$ $[M+Na]^+$ 572.2185, found 572.2166.

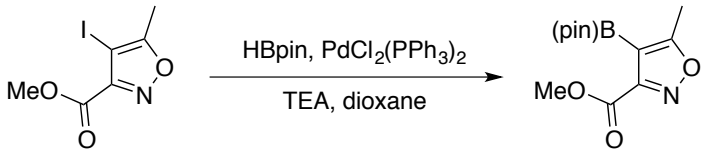


*((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(pyrimidin-5-yl)phenyl)propanoyl)*-L*-tryptophan* (**74d**). To a stirring solution of **83d** (100 mg, 0.15 mmol, 1 eq) in anhydrous dichloromethane (750 μL, 0.2 M), trifluoroacetic acid (120 *μ*L, 1.5 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (4% MeOH/CH_2Cl_2) to afford the product as an off-white foam (51.4 mg, 0.09 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.5 mL, 0.067 M) and lithium hydroxide monohydrate (38 mg, 0.9 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (47 mg, 56% over 2 steps). m.p. = 175-180 °C. IR (solid) 3301, 2926, 2467, 1722, 1634 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 9.05 (s, 1H), 8.87 (s, 2H), 8.09 (dd, *J* = 34.1, 7.9 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.21 (m, 5H), 7.14 – 7.01 (m, 5H), 6.96 (t, *J* = 7.5 Hz, 1H), 4.96 – 4.88 (m, 1H), 4.80 – 4.73 (m, 1H), 3.37 (dd,

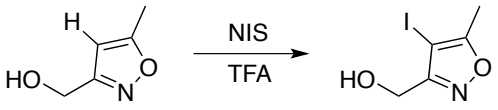
J = 14.8, 4.6 Hz, 1H), 3.17 (ddd, *J* = 19.6, 14.3, 7.0 Hz, 2H), 2.90 (dd, *J* = 13.8, 8.0 Hz, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.14, 171.62, 168.82, 156.35, 154.53, 138.40, 138.14, 136.85, 133.81, 133.01, 132.04, 130.25, 127.57, 126.52, 124.84, 124.75, 123.40, 121.19, 118.71, 118.09, 111.11, 109.71, 54.58, 53.54, 37.29, 27.31, 19.97. HRMS (ESI) m/z calc'd for $C_{33}H_{31}N_5O_4$ $[M+H]^+$ 562.2454, found 562.2451.



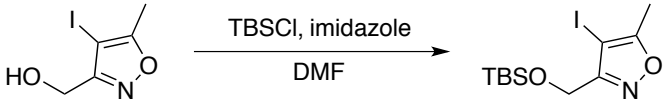
Methyl 4-iodo-5-methylisoxazole-3-carboxylate (**89a**). To a flask containing isoxazole **88a**[9] (212 mg, 1.5 mmol, 1 eq) and *N*-iodosuccinamide (405 mg, 1.8 mmol, 1.2 eq), trifluoroacetic acid (8.5 mL, 0.18 M) was added at room temperature (20 °C). After stirring for 18 h, the reaction mixture was concentrated *in vacuo*, diluted with water (20 mL) and extracted with diethyl ether (20 mL). The organic layer was then washed with 1N NaOH (20 mL), 5% $NaHSO_4$ (20 mL) and brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford the product as an off-white solid (410 mg, quant). m.p. = 57-60 °C. IR (solid) 2964, 1730, 1575, 1465, 1418 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.99 (s, 3H), 2.56 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.59, 159.74, 155.28, 56.74, 53.04, 12.81. HRMS (ESI) m/z calc'd for $C_6H_6INO_3$ $[M+H]^+$ 267.9471, found 267.9481.



Methyl 5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole-3-carboxylate (**90a**). To a flask containing iodide **89a** (410 mg, 1.5 mmol, 1 eq) and bis(triphenylphosphine)palladium(II) dichloride (53 mg, 0.075 mmol, 0.05 eq) in degassed anhydrous dioxane (2.8 mL, 0.53 M) was added pinacol borane (330 μL, 2.3 mmol, 1.5 eq) followed by degassed anhydrous triethylamine (630 μL, 4.5 mmol, 3 eq) at room temperature (20 °C). The reaction mixture was then heated to reflux for one hour. The reaction mixture was then cooled to room temperature and concentrated *in vacuo* to afford the crude product as a yellow oil. This was taken up in diethyl ether, run through Celite, concentrated *in vacuo* and triturated with hexane. The hexane was concentrated *in vacuo* to yield the product as a yellow solid (410 mg, quant). m.p. = 100-105 °C. IR (solid) 1737, 1596, 1470, 1456, 1383 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.94 (s, 3H), 2.56 (s, 3H), 1.35 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.05, 161.23, 158.88, 84.25, 52.73, 24.85, 12.87 (carbon bearing boron not observed). ^{11}B NMR (128 MHz, $CDCl_3$) δ 29.02. HRMS (ESI) m/z calc'd for $C_{12}H_{18}BNO_5$ $[M+Na]^+$ 290.1176, found 290.1179.

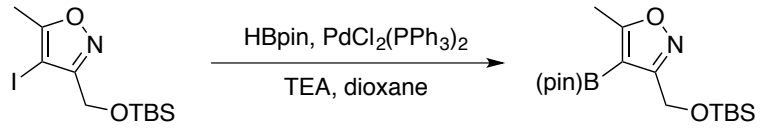


(4-Iodo-5-methylisoxazol-3-yl)methanol (**89b**). To a flask containing isoxazole **88b**[10] (500 mg, 4.4 mmol, 1 eq) and *N*-iodosuccinamide (1.2 g, 5.3 mmol, 1.2 eq), trifluoroacetic acid (24 mL, 0.18 M) was added at room temperature (20 °C). After stirring for 18 h, the reaction mixture was concentrated *in vacuo*, diluted with water (20 mL) and extracted with diethyl ether (20 mL). The organic layer was then washed with 1N NaOH (20 mL), 5% $NaHSO_4$ (20 mL) and brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a crude solid. This solid purified by flash chromatography (10% EtOAc/Hexanes) to afford the product as an off-white solid (773 mg, 73%). m.p. = 78-80 °C. IR (solid) 3340, 1584, 1485, 1408, 1378 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 4.67 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.18, 163.56, 100.11, 57.51, 12.44. HRMS (ESI) m/z calc'd for $C_5H_6INO_2$ $[M+H]^+$ 239.9522, found 239.9529.

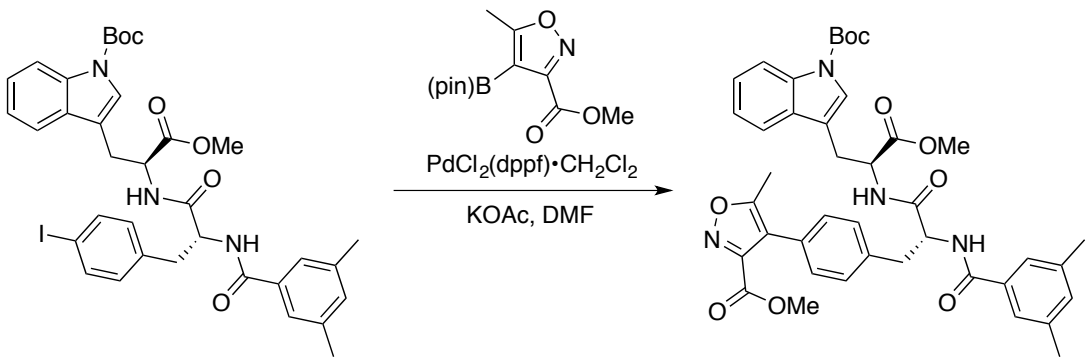


*3-(((*Tert*-butyldimethylsilyl)oxy)methyl)-4-iodo-5-methylisoxazole* (**89c**) To a flask containing isoxazole **89b** (500 mg, 2.1 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (2.1 mL, 1 M), imidazole (356 mg, 5.2 mmol, 2.5 eq) was added, followed by *tert*-butyldimethylsilyl chloride (380 mg, 2.5 mmol, 1.2 eq) at room temperature (20 °C). After stirring for two hours, the reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted two times with ethyl acetate (20 mL). The combined organic was then washed four times with water (20 mL) and brine (20 mL). The organic was then

dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a crude oil. This oil purified by flash chromatography (10% EtOAc/Hexanes) to afford the product as a clear oily solid (710 mg, 96%). IR (solid) 2956, 2928, 2857, 1592, 1485 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.66 (s, 2H), 2.45 (s, 3H), 0.92 (s, 9H), 0.12 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.86, 163.12, 57.56, 57.37, 25.98, 18.49, 12.45, -5.19. HRMS (ESI) m/z calc'd for $C_{11}H_{20}INO_2Si$ $[M+H]^+$ 354.0386, found 354.0380.

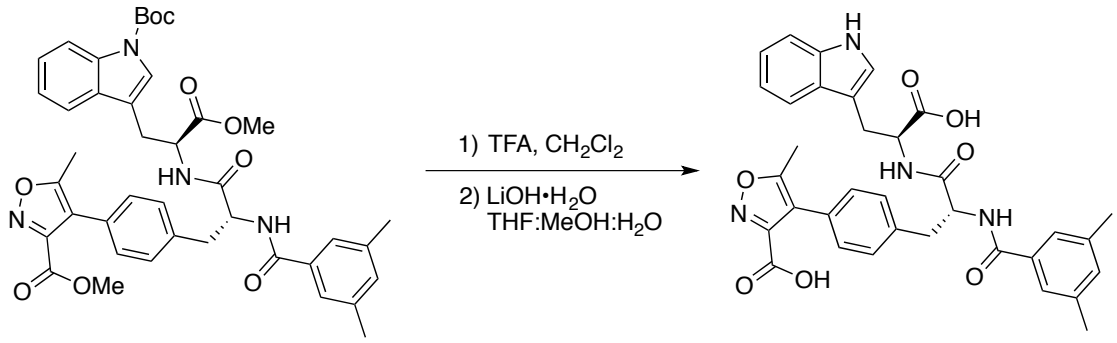


Methyl 5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole-3-carboxylate (**90c**). To a flask containing iodide **89c** (353 mg, 1 mmol, 1 eq) and bis(triphenylphosphine)palladium(II) dichloride (35 mg, 0.075 mmol, 0.05 eq) in degassed anhydrous dioxane (1.9 mL, 0.53 M) was added pinacol borane (220 μL, 2.3 mmol, 1.5 eq) followed by anhydrous triethylamine (420 μL, 4.5 mmol, 3 eq) at room temperature (20 °C). The reaction mixture was then heated to reflux for one hour. The reaction mixture was then cooled to room temperature and concentrated *in vacuo* to afford the crude product as a yellow oil. This was taken up in diethyl ether, run through celite, concentrated *in vacuo* and triturated with hexane. The hexane was concentrated *in vacuo* to yield the crude product as a yellow solid (226 mg, quant). Carried forward without further purification.



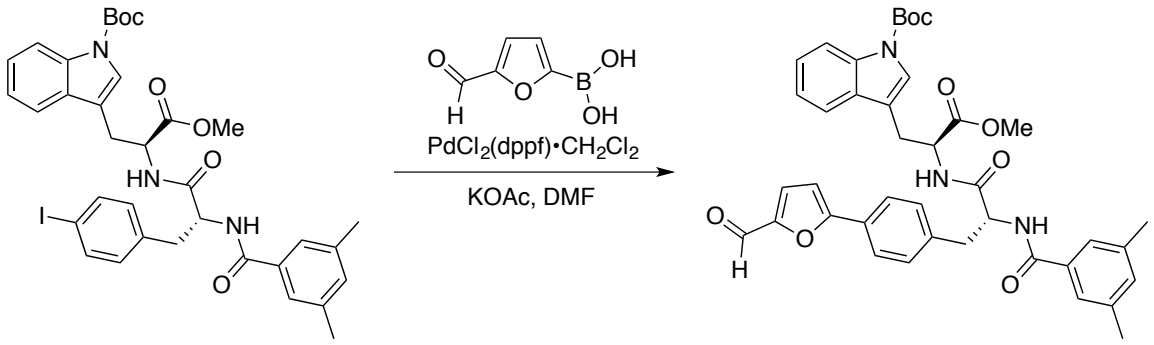
*Methyl 4-(4-((*R*)-3-(((*S*)-3-(1-(tert-butoxycarbonyl)-1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-(3,5-dimethylbenzamido)-3-oxopropyl)phenyl)-5-methylisoxazole-3-carboxylate* (**83h**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), boronic ester **90a** (145 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed 4 times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (20-40% EtOAc/Hex) to afford the product as an off-white foam (63 mg, 32%). IR (solid) 3278, 2924, 1733, 1635, 1602 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.07 (s, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.28 (d, J = 6.3 Hz, 3H), 7.17 (qd, J = 7.8, 4.1 Hz, 5H), 7.11 (s, 1H), 6.83 (t, J = 7.3 Hz, 2H), 4.96 (q, J = 7.1 Hz, 1H), 4.88 (q, J = 6.7 Hz, 1H), 3.78 (s, 3H), 3.60 (s, 3H), 3.18 (dd, J = 6.9, 4.0 Hz, 2H), 3.14 (d, J = 6.4 Hz, 2H), 2.35 (s, 3H), 2.31 (s, 6H), 1.62 (s, 9H). ^{13}C NMR (126

MHz, $CDCl_3$) δ 171.80, 170.87, 168.37, 167.73, 160.74, 153.84, 149.63, 138.41, 136.68, 135.46, 133.81, 133.56, 130.41, 130.15, 129.50, 127.43, 124.94, 124.76, 124.30, 122.78, 118.83, 117.31, 115.47, 115.06, 83.92, 54.57, 52.76, 52.56, 38.23, 28.30, 27.72, 21.30, 11.52. HRMS (ESI) m/z calc'd for $C_{41}H_{44}N_5O_9$ $[M+Na]^+$ 759.3006, found 759.3018.



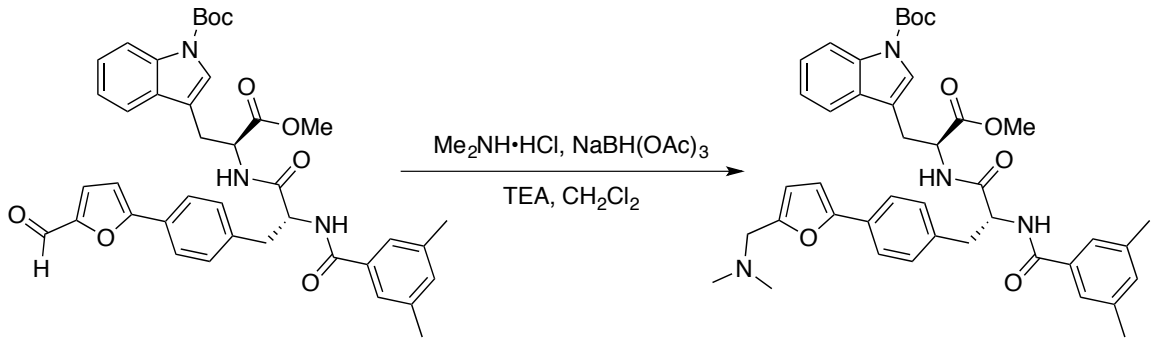
*4-(4-((*R*)-3-(((*S*)-1-Carboxy-2-(1H-indol-3-yl)ethyl)amino)-2-(3,5-dimethylbenzamido)-3-oxopropyl)phenyl)-5-methylisoxazole-3-carboxylic acid* (**74h**). To a stirring solution of **83h** (63 mg, 0.09 mmol, 1 eq) in anhydrous dichloromethane (430 μL, 0.2 M), trifluoroacetic acid (70 μL, 0.9 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness in vacuo to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (49.3 mg, 0.08 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1 mL, 0.067 M) and lithium hydroxide monohydrate (33 mg, 0.8 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5%

$NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (45.7 mg, 88% over 2 steps). m.p. = 130-142 °C. IR (solid) 3331, 2924, 1722, 1634, 1601 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 8.09 (dd, J = 38.3, 8.0 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.25 (s, 2H), 7.13 (s, 1H), 7.07 – 7.01 (m, 4H), 7.01 – 6.90 (m, 3H), 4.79 (td, J = 8.1, 4.9 Hz, 2H), 3.35 (dd, J = 14.7, 4.9 Hz, 1H), 3.18 (dd, J = 14.7, 8.3 Hz, 1H), 3.11 (dd, J = 13.9, 5.7 Hz, 1H), 2.89 (dd, J = 13.8, 7.9 Hz, 1H), 2.28 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ 173.77, 171.95, 171.87, 169.03, 168.26, 161.91, 138.14, 136.86, 136.83, 133.89, 132.99, 129.44, 129.12, 127.46, 127.27, 124.78, 123.43, 121.23, 118.72, 118.00, 116.76, 111.13, 109.48, 54.69, 53.33, 53.24, 37.30, 27.28, 19.99, 10.05. HRMS (ESI) m/z calc'd for $C_{34}H_{32}N_4O_7$ $[M+H]^+$ 609.2349, found 609.2372.

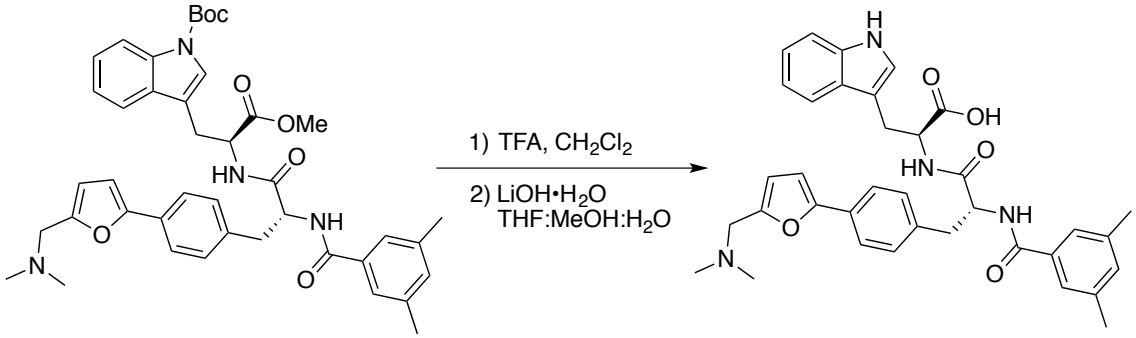


Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(5-formylfuran-2-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83k**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), (5-formylfuran-2-yl)boronic acid (77 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The

reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed four times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (40% EtOAc/Hex) to afford the product as an off-white foam (145 mg, 76%). IR (solid) 1732, 1670, 1636, 1603, 1530 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 9.60 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.33 – 7.24 (m, 4H), 7.20 (t, J = 7.5 Hz, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.09 (s, 1H), 7.02 (s, 1H), 6.85 (s, 1H), 6.72 (d, J = 3.6 Hz, 1H), 4.99 (s, 1H), 4.89 (q, J = 6.7 Hz, 1H), 3.61 (s, 3H), 3.24 – 3.08 (m, 4H), 2.29 (s, 6H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.29, 171.77, 170.81, 167.71, 159.28, 152.09, 149.60, 138.43, 135.47, 133.69, 133.62, 130.36, 130.07, 127.84, 125.56, 124.91, 124.79, 124.22, 122.81, 118.78, 115.51, 115.00, 107.77, 83.93, 54.46, 53.55, 52.62, 38.41, 28.30, 27.70, 21.30. HRMS (ESI) m/z calc'd for $C_{40}H_{41}N_3O_8$ $[M+H]^+$ 692.2972, found 692.2979.

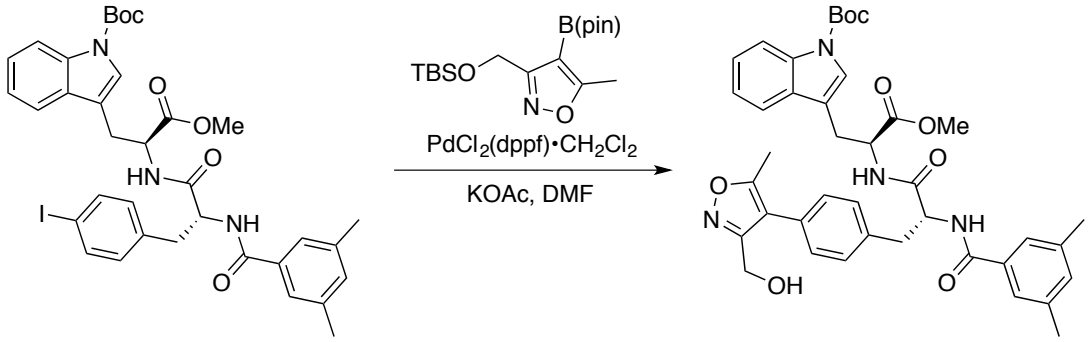


Tert-*butyl* *3-((*S*)-2-((*R*)-3-(4-(5-((dimethylamino)methyl)furan-2-yl)phenyl)-2-(3,5-dimethylbenzamido)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83j**). To a flask containing **83k** (70 mg, 0.1 mmol, 1 eq), dimethylamine hydrochloride (27 mg, 0.3 mmol, 3 eq) and sodium triacetoxyborohydride (26 mg, 0.12 mmol, 1.15 eq) in dichloromethane (560 μL, 0.2 M) at room temperature (20 °C), triethylamine (40 μL, 0.26 mmol, 2.4 eq) was added. After four hours, the reaction mixture was diluted with ethyl acetate and then quenched with water (10 mL). The organic layer was extracted and washed with brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the product as an orange foam (68 mg, 93%). IR (solid) 3268, 2934, 1733, 1636, 1602 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.09 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 7.8 Hz, 1H), 7.41 (s, 1H), 7.32 – 7.24 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.14 – 7.05 (m, 3H), 6.91 (d, J = 7.9 Hz, 1H), 6.79 (d, J = 7.6 Hz, 1H), 6.50 (d, J = 3.2 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 4.95 (q, J = 6.9 Hz, 1H), 4.92 – 4.83 (m, 1H), 3.59 (s, 3H), 3.51 (s, 2H), 3.13 (t, J = 7.1 Hz, 4H), 2.28 (d, J = 5.2 Hz, 12H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.78, 170.97, 167.74, 153.31, 152.05, 149.61, 138.37, 135.50, 135.40, 133.83, 133.52, 130.38, 129.86, 129.71, 124.94, 124.73, 124.28, 124.02, 122.80, 118.83, 115.47, 115.06, 110.77, 105.67, 83.83, 55.93, 54.61, 52.69, 52.54, 45.03, 38.26, 28.30, 27.73, 21.30. HRMS (ESI) m/z calc'd for $C_{40}H_{44}N_4O_8$ $[M+H]^+$, found.



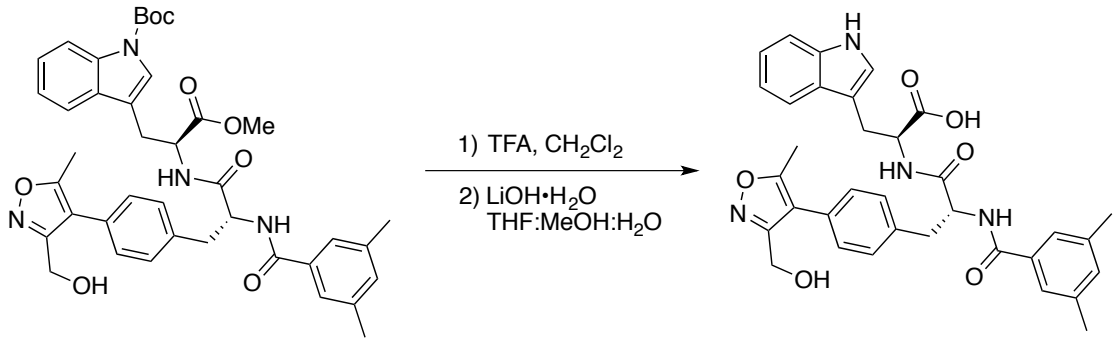
*((*R*)-3-(4-(5-((dimethylamino)methyl)furan-2-yl)phenyl)-2-(3,5-dimethylbenzamido)propanoyl)*-L-*tryptophan* (**74j**). To a stirring solution of **83j** (67 mg, 0.09 mmol, 1 eq) in anhydrous dichloromethane (450 μL, 0.2 M), trifluoroacetic acid (70 *μ*L, 0.9 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by preparative thin layer chromatography (4% MeOH/CH_2Cl_2) to afford the product as an off-white foam (33 mg, 0.05 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 750 μL, 0.067 M) and lithium hydroxide monohydrate (22 mg, 0.5 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (33 mg, 59% over 2 steps). m.p. = 152-169 °C. IR (solid) 3288, 2922, 1644, 1602, 1498 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.61 – 7.57 (m, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.15 (s, 2H),

7.08 (d, *J* = 11.2 Hz, 2H), 7.01 (dd, *J* = 17.7, 7.8 Hz, 3H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.68 (s, 2H), 4.70 (dd, *J* = 7.1, 4.8 Hz, 1H), 4.29 – 4.19 (m, 2H), 3.35 (dd, *J* = 14.6, 5.0 Hz, 1H), 3.21 (ddd, *J* = 23.2, 14.3, 6.1 Hz, 2H), 2.87 (dd, *J* = 13.9, 8.8 Hz, 1H), 2.72 (s, 6H), 2.24 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.75, 171.06, 171.03, 155.78, 137.75, 137.35, 136.48, 133.53, 132.64, 129.42, 128.17, 127.65, 124.50, 123.43, 123.14, 120.77, 118.35, 118.00, 115.52, 115.41, 110.73, 109.82, 105.35, 54.58, 52.68, 41.41, 41.30, 37.05, 27.27, 19.71. HRMS (ESI) m/z calc'd for $C_{36}H_{38}N_4O_5$ $[M+H]^+$ 607.2920, found 607.2913.



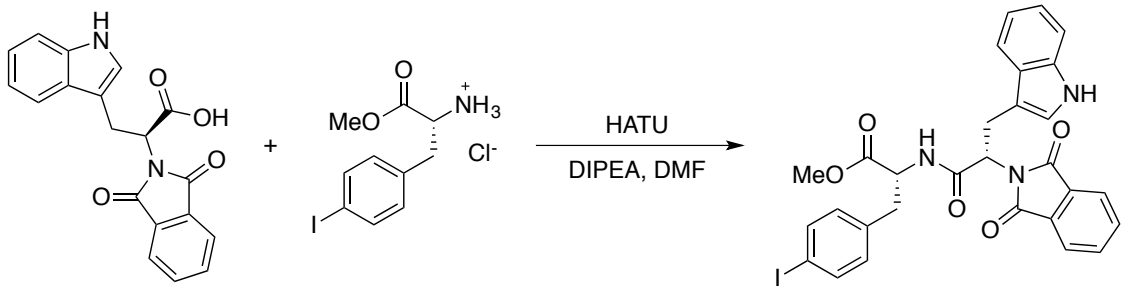
Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(3-(hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**83i**). To a sealed vessel containing iodide **81** (200 mg, 0.28 mmol, 1 eq), **90c** (194 mg, 0.55 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (23 mg, 0.03 mmol, 0.1 eq) and potassium acetate (135 mg, 1.4 mmol, 5 eq) was added in anhydrous *N,N*-dimethylformamide (2.8 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (10 mL). The organic layer was extracted and washed four times with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude

product as a brown foam. This residue was purified by flash chromatography (40-50% EtOAc/Hex) to afford the product as an off-white foam (59 mg, 30% over 2 steps). IR (thin film) 3289, 2925, 1733, 1637, 1603 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.41 (s, 1H), 7.29 (s, 3H), 7.20 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 7.9 Hz, 2H), 7.11 (d, J = 8.4 Hz, 3H), 7.04 (d, J = 7.8 Hz, 1H), 6.94 (d, J = 7.8 Hz, 1H), 4.99 (q, J = 7.0 Hz, 1H), 4.88 (q, J = 6.4 Hz, 1H), 4.61 (d, J = 4.5 Hz, 2H), 3.61 (s, 3H), 3.20 – 3.08 (m, 4H), 2.86 (s, 1H), 2.34 (s, 3H), 2.29 (s, 6H), 1.60 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.96, 170.98, 167.77, 166.08, 161.58, 149.66, 138.40, 136.27, 135.49, 133.77, 133.58, 130.39, 130.03, 129.28, 128.47, 124.94, 124.82, 124.34, 122.84, 118.84, 115.65, 115.49, 115.09, 83.99, 55.94, 54.51, 52.76, 52.64, 38.27, 28.27, 27.68, 21.30, 11.64. HRMS (ESI) m/z calc'd for $C_{40}H_{44}N_4O_8$ $[M+H]^+$ 709.3237, found 709.3250.



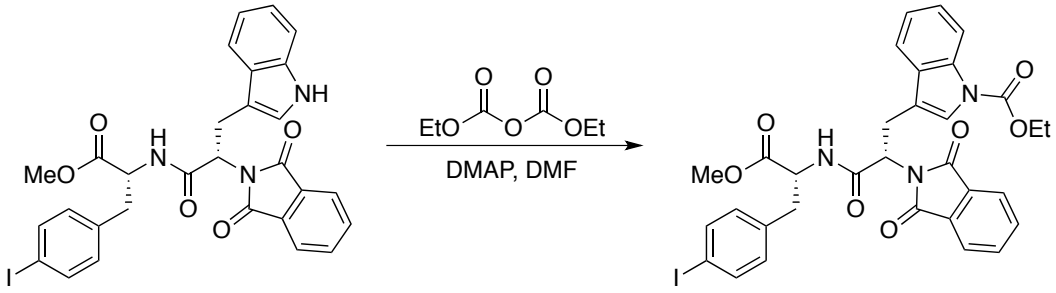
*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(3-(hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**74i**). To a stirring solution of **83i** (58 mg, 0.08 mmol, 1 eq) in anhydrous dichloromethane (400 μL, 0.2 M), trifluoroacetic acid (70 *μ*L, 0.9 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by preparative thin layer chromatography (4% MeOH/CH_2Cl_2) to afford the product as an off-white foam (15.6 mg, 0.03 mmol). This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 400 μL, 0.067 M) and lithium hydroxide monohydrate (11 mg, 0.3 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to produce an off-white solid (24.3 mg, 35% over 2 steps). m.p. = 160-175 °C. IR (solid) 3302, 2923, 1640, 1602, 1516 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 2H), 7.13 (d, *J* = 8.1 Hz, 3H), 7.07 (s, 1H), 7.02 (t, *J* = 8.3 Hz, 3H), 6.94 (t, *J* = 7.5 Hz, 1H), 4.90 (dd, *J* =

8.3, 5.4 Hz, 1H), 4.75 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.52 (s, 2H), 3.36 (dd, *J* = 14.7, 4.8 Hz, 1H), 3.25 – 3.14 (m, 2H), 2.89 (dd, *J* = 13.8, 8.3 Hz, 1H), 2.33 (s, 3H), 2.30 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.26, 168.68, 165.75, 162.51, 161.20, 139.53, 137.85, 136.51, 133.66, 132.71, 129.37, 128.62, 127.78, 127.43, 124.51, 123.14, 120.84, 118.41, 117.88, 115.54, 110.72, 109.61, 54.49, 54.25, 53.89, 37.12, 27.23, 19.74, 9.92. HRMS (ESI) m/z calc'd for $C_{34}H_{34}N_4O_6$ $[M+H]^+$ 595.2557, found 595.2542.



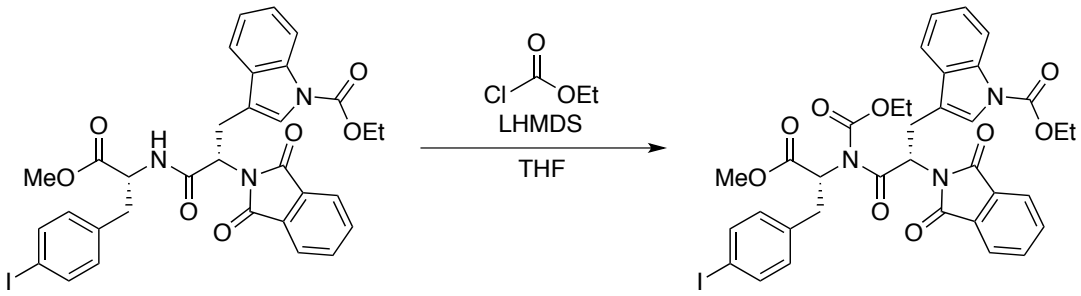
*Methyl (*R*)-2-((*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-(1*H*-indol-3-yl)propanamido)-3-(4-iodophenyl)propanoate* (**103**). To the amino ester (3.41 g, 10 mmol, 1 eq) was added acid phthalate[11] (3.4 g, 10 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (8.7 mL, 50 mmol, 5 eq) and anhydrous *N*,*N*-dimethylformamide (33 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (5.7 mg, 15 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over 200 mL ice water. After stirring for 5 mins, precipitate collected and purified by flash chromatography (40% EtOAc/Hexanes) to afford the product as an off-white foam (5.8 mg, 93%). IR (solid) 1740, 1708, 1668, 1524, 1484 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.23 (s, 1H), 7.76 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.67 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.17 – 7.12 (m, 1H), 7.12 – 7.06 (m, 1H), 6.86 – 6.81 (m, 2H), 6.78 (d, *J* = 8.2 Hz, 2H), 5.28 (t, *J*

= 7.9 Hz, 1H), 4.81 (q, *J* = 7.1, 5.8, 5.3 Hz, 1H), 3.73 (dd, *J* = 15.0, 7.8 Hz, 1H), 3.64 (s, 3H), 3.57 (dd, *J* = 15.0, 8.2 Hz, 1H), 3.12 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.00 (dd, *J* = 13.9, 5.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.32, 168.56, 168.16, 137.63, 136.44, 135.52, 134.39, 131.69, 131.58, 126.88, 123.66, 123.05, 122.52, 119.92, 118.71, 111.48, 110.75, 92.77, 54.55, 53.47, 52.61, 36.98, 25.81. HRMS (ESI) m/z calc'd for $C_{29}H_{24}IN_3O_5$ $[M+H]^+$ 622.0839, found 622.0822.



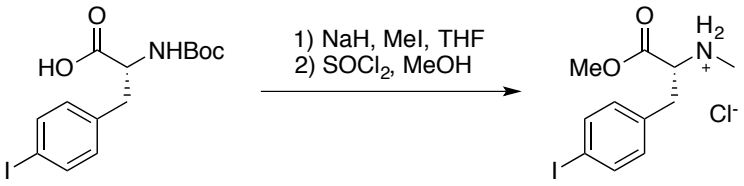
*Ethyl 3-((*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-(((*R*)-3-(4-iodophenyl)-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate* (**104**). To a stirring solution of **103** (1.9 g, 3 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (6 mL, 0.5 M), di-diethyl dicarbonate (520 μL, 3.6 mmol, 1.2 eq) was added portionwise, followed by N,N-dimethylamino pyridine (40 mg, 0.3 mmol, 0.1 eq) at 0 °C. Allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL) two times. The organic layer was washed with water (10 mL) ten times, brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness in vacuo to afford the crude product as an off-white foam. This residue was purified by flash chromatography (30% EtOAc/Hex) to afford the product as an off-white foam (1.2 g, 60%, 95% brsm). IR (thin film) 3358, 2952, 1714, 1527, 1455 cm^{-1}. ^{1}H NMR (500 MHz,

Chloroform-d) δ 8.09 (d, J = 8.3 Hz, 1H), 7.79 (dd, J = 5.4, 3.1 Hz, 2H), 7.71 (dd, J = 5.6, 2.9 Hz, 2H), 7.55 (d, J = 8.3 Hz, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.33 (s, 1H), 7.29 (t, J = 7.7 Hz, 2H), 7.23 (t, J = 7.5 Hz, 1H), 6.78 (dd, J = 14.7, 7.6 Hz, 3H), 5.23 (dd, J = 9.1, 7.1 Hz, 1H), 4.82 (q, J = 6.9, 5.9, 5.7 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 3.68 (s, 3H), 3.61 (qt, J = 9.3, 7.5, 7.0, 5.9, 3.1 Hz, 2H), 3.12 (dd, J = 13.9, 5.8 Hz, 1H), 3.01 (dd, J = 13.9, 5.3 Hz, 1H), 1.38 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.25, 168.06, 167.98, 150.78, 137.63, 135.67, 135.44, 134.56, 131.54, 131.44, 129.74, 125.08, 123.92, 123.80, 123.17, 118.97, 116.31, 115.40, 92.77, 63.25, 54.16, 53.41, 52.65, 36.99, 25.13, 14.54. HRMS (ESI) m/z calc'd for $C_{32}H_{28}IN_3O_7$ $[M+H]^+$ 694.1050, found 694.1066.



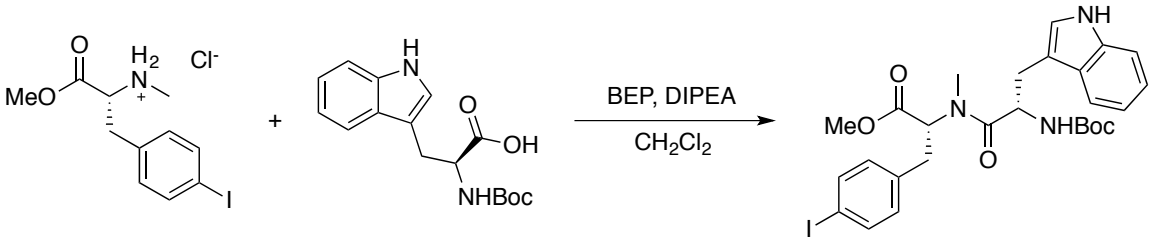
*Ethyl 3-((*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-((ethoxycarbonyl)((*R*)-3-(4-iodophenyl)-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate* (**105**). To a stirring solution of **104** (280 mg, 0.4 mmol, 1 eq) in anhydrous tetrahydrofuran (1.3 mL, 0.3 M) at -78 °C, LHMDS (500 μL, 0.48 mmol, 1.2 eq) was added slowly dropwise down the side of the flask. After 15 mins stirring at -78 °C, ethyl chloroformate (100 μL, 1 mmol, 2.5 eq) was added slowly dropwise down the side of the flask. After stirring for one hour reaction was slowly warmed to room temperature (20 °C). The reaction mixture was opened to the air and quenched for 30 mins. The reaction was then diluted with ethyl acetate (50 mL) then quenched for of water (10 mL). The organic layer was washed with

then washed with brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness in vacuo to afford the crude product as an off-white foam. This residue was purified by flash chromatography (20% EtOAc/Hex) to afford the product as an white foam (210 g, 68%). IR (solid) 1734, 1717, 1611, 1455, 1379 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.82 – 7.73 (m, 3H), 7.73 – 7.61 (m, 4H), 7.43 (s, 1H), 7.32 – 7.23 (m, 2H), 6.95 (d, J = 8.3 Hz, 2H), 6.45 (dd, J = 11.5, 4.4 Hz, 1H), 5.46 (dd, J = 10.2, 5.7 Hz, 1H), 4.46 – 4.33 (m, 2H), 4.31 – 4.14 (m, 2H), 3.76 (s, 3H), 3.50 (dd, J = 14.3, 5.8 Hz, 2H), 3.22 (dd, J = 14.3, 10.1 Hz, 1H), 3.08 – 2.96 (m, 1H), 1.40 (t, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.85, 170.47, 169.64, 167.74, 152.68, 137.72, 136.63, 133.92, 133.84, 131.61, 131.30, 124.51, 123.98, 123.27, 122.84, 119.36, 117.75, 116.30, 114.90, 92.13, 63.98, 62.87, 55.44, 52.54, 35.50, 23.93, 14.30, 13.91, 13.54. HRMS (ESI) m/z calc'd for $C_{35}H_{32}IN_3O_9$ $[M+Na]^+$ 788.1081, found 788.1088.



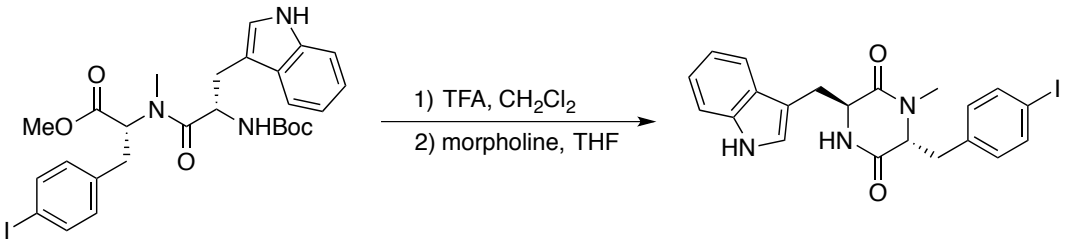
*(*R*)-3-(4-iodophenyl)-1-methoxy-*N*-methyl-1-oxopropan-2-aminium chloride* (**109**). To a solution of (*R*)-2-((tert-butoxycarbonyl)amino)-3-(4-iodophenyl)propanoic acid (1.62 g, 4.1 mmol, 1 eq) in anhydrous tetrahydrofuran (11.5 mL, 0.36 M) at 0 °C was added sodium hydride (60% disp, 660 mg, 16 mmol, 4 eq). After 15 minutes, iodomethane (770 μL, 12 mmol, 3 eq) was added and the reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction was then cooled to 0 °C and quenched with water until no further gas evolved. This solution was then extracted with two times ethyl acetate (50 mL).

The combined organic was washed with brine (50 mL), dried over sodium sulfate and concentrated to dryness *in vacuo* to afford the methylated product as a white foam (1.67 g). The product from step one was taken up in methanol (9 mL, 0.45 M) and cooled to 0 °C. Thionyl chloride (1.5 mL, 20 mmol, 5 eq) was then added dropwise. Heated to reflux for 2 hours. When cooled methanol was extracted with hexanes to remove oil. concentrated to dryness *in vacuo* and triturated with ether. Concentrated to dryness *in vacuo* to afford the product as a yellow foam (1.47 g, quant). IR (solid) 2949, 2691, 1752, 1741, 1588 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.67 (s, 1H), 9.52 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.04 (d, *J* = 8.2 Hz, 2H), 4.01 (s, 1H), 3.71 (s, 3H), 3.55 (dd, *J* = 13.9, 5.0 Hz, 1H), 3.33 (dd, *J* = 14.0, 9.1 Hz, 1H), 2.76 (t, *J* = 5.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.22, 138.07, 134.06, 131.43, 93.46, 62.24, 53.30, 35.36, 32.39. HRMS (ESI) m/z calc'd for $C_{11}H_{15}ClINO_2$ $[M+H]^+$ 320.0148, found 320.0145.



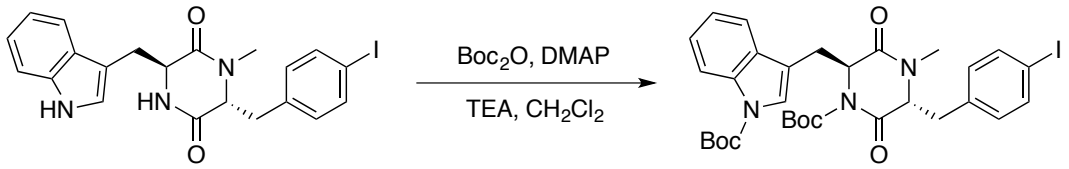
*Methyl (*R*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(1*H*-indol-3-yl)*-N*-methylpropanamido)-3-(4-iodophenyl)propanoate* (**110**). To the amine **109**[8] (200 mg, 0.56 mmol, 1 eq), *N*-boc trp (OH)[3] (172 mg, 0.56 mmol, 1 eq) and 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP, 200 mg, 0.73 mmol, 1.3 eq) in anhydrous dichloromethane (5.6 mL, 0.1 M) at 0 °C, was added N,N-diisopropylethylamine (300 μL, 1.7 mmol, 3 eq) and anhydrous acetonitrile (0.9 mL, 0.3M). The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was concentrated to dryness *in vacuo*

and taken up in ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an orange foam. This residue was purified by flash chromatography (40% EtOAc/Hex) to afford the product as a yellow foam (266 mg, 79%). IR (solid) 3316, 2975, 1738, 1697, 1640 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 7.58 (dd, *J* = 14.0, 8.0 Hz, 3H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.90 (dd, *J* = 21.4, 8.1 Hz, 2H), 6.71 (d, *J* = 2.4 Hz, 1H), 5.36 (d, *J* = 9.0 Hz, 1H), 5.12 (dd, *J* = 10.0, 5.9 Hz, 1H), 4.92 (q, *J* = 7.4 Hz, 1H), 3.67 (s, 3H), 3.20 – 3.10 (m, 1H), 2.96 – 2.86 (m, 2H), 2.72 (dd, *J* = 14.4, 10.0 Hz, 1H), 2.64 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.34, 170.74, 155.41, 137.74, 136.87, 136.15, 131.23, 127.74, 122.89, 122.17, 119.72, 118.83, 111.31, 110.39, 92.22, 79.82, 58.80, 52.52, 51.01, 34.33, 33.02, 29.18, 28.50. HRMS (ESI) m/z calc'd for $C_{27}H_{32}IN_3O_5$ $[M+H]^+$ 606.1465, found 606.1464.

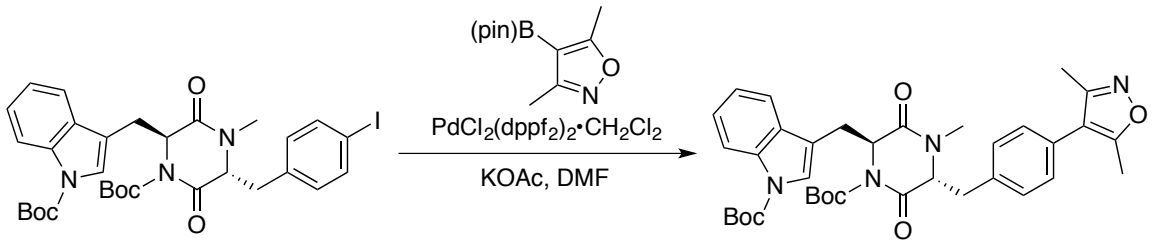


*(3*S*,6*R*)-3-((1*H*-indol-3-yl)methyl)-6-(4-iodobenzyl)-1-methylpiperazine-2,5-dione* (**111**). To a stirring solution of **110** (300 mg, 0.5 mmol, 1 eq) in anhydrous dichloromethane (2.5 mL, 0.2 M), trifluoroacetic acid (390 μL, 5 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (10 mL) and partitioned. The organic layer was washed with brine (10 mL) and dried over anhydrous sodium sulfate. The organic layer was then

concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was then dissolved in anhydrous tetrahydrofuran (8 mL, 0.063 M) and morpholine (1.5 mL, 0.33 M) was added dropwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then partitioned between ethyl acetate (10 mL) and saturated ammonium chloride (10 mL). The organic layer was washed with water (10 mL), followed by brine (10 mL) and dried over sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as a white foam (197 mg, 82% over two steps). IR (solid) 3402, 1677, 1639, 1484, 1457 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.08 (s, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.38 (dt, *J* = 7.5, 1.1 Hz, 1H), 7.27 (d, *J* = 4.0 Hz, 2H), 7.25 – 7.22 (m, 1H), 6.97 (d, *J* = 2.3 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 2H), 5.49 (s, 1H), 4.12 (t, *J* = 4.0 Hz, 1H), 3.51 (dd, *J* = 14.8, 3.8 Hz, 1H), 3.17 (dd, *J* = 14.0, 3.5 Hz, 1H), 3.11 – 3.03 (m, 4H), 2.87 (dd, *J* = 11.2, 3.7 Hz, 1H), 2.70 (dd, *J* = 14.7, 11.3 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.28, 166.84, 138.14, 136.77, 134.27, 131.97, 126.44, 123.71, 122.91, 120.43, 118.89, 111.56, 109.51, 93.59, 63.90, 52.17, 36.37, 32.98, 29.03. HRMS (ESI) m/z calc'd for $C_{21}H_{20}IN_3O_2$ $[M+H]^+$ 474.0679, found 474.0695.

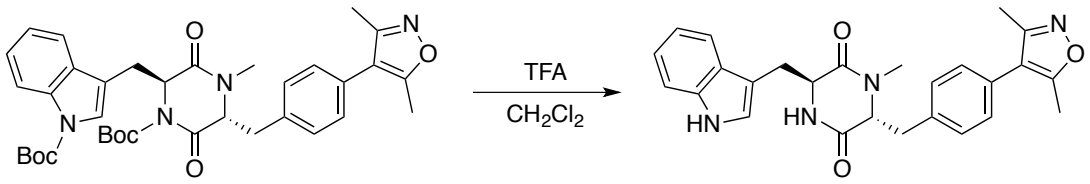


Tert-*butyl 3-(((2*S*,5*R*)-1-(*tert*-butoxycarbonyl)-5-(4-iodobenzyl)-4-methyl-3,6-dioxopiperazin-2-yl)methyl)-1*H*-indole-1-carboxylate* (**112**). To a stirring solution of **111** (200 mg, 0.4 mmol, 1 eq) and anhydrous triethylamine (150 μL, 1 mmol, 2.5 eq) in anhydrous dichloromethane (3.2 mL, 0.13 M), di-*tert*-butyl dicarbonate (280 mg, 1.3 mmol, 3 eq) was added portionwise, followed by *N,N*-dimethylamino pyridine (16 mg, 0.13 mmol, 0.3 eq) at room temperature (20 °C). After stirring for 3 hours, reaction was complete by TLC. The reaction mixture was then concentrated to dryness *in vacuo* to afford the crude product as an yellow foam. This residue was purified by flash chromatography (10-15% EtOAc/Hex) to afford the product as an off-white foam (290 mg, quant). IR (solid) 1776, 1725, 1661, 1485, 1454 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 8.2 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.32 – 7.25 (m, 1H), 7.25 – 7.15 (m, 2H), 6.71 (d, *J* = 7.9 Hz, 2H), 4.46 (s, 1H), 3.40 (dd, *J* = 14.8, 2.9 Hz, 1H), 3.33 (dd, *J* = 14.7, 5.0 Hz, 1H), 3.03 (dd, *J* = 20.7, 3.8 Hz, 2H), 2.89 (dd, *J* = 14.2, 4.2 Hz, 1H), 2.70 (s, 3H), 1.65 (s, 10H), 1.52 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.28, 166.13, 149.97, 149.48, 137.64, 135.39, 133.73, 131.63, 130.11, 126.59, 124.86, 122.77, 119.55, 115.25, 113.54, 93.06, 84.32, 84.00, 62.48, 58.53, 37.20, 32.08, 29.60, 28.32, 28.08. HRMS (ESI) m/z calc'd for $C_{31}H_{36}IN_3O_6$ $[M+H]^+$ 674.1727, found 674.1736.

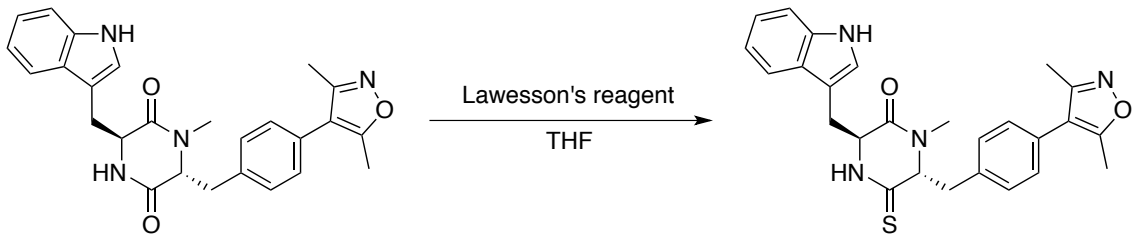


Tert-*butyl 3-(((2*S*,5*R*)-1-(*tert-*butoxycarbonyl)-5-(4-(3,5-dimethylisoxazol-4-yl)benzyl)-4-methyl-3,6-dioxopiperazin-2-yl)methyl)-1*H*-indole-1-carboxylate* (**113**). To a sealed vessel containing iodide **112** (600 mg, 0.9 mmol, 1 eq), 3,5-dimethylisooxazole-4-boronic ester (398 mg, 1.8 mmol, 2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (73 mg, 0.09 mmol, 0.1 eq) and potassium acetate (440 mg, 4.5 mmol, 5 eq) was added in anhydrous N,N-dimethylformamide (9 mL, 0.1 M) at room temperature (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was then diluted with ethyl acetate and then quenched with saturated ammonium chloride (30 mL). The organic layer was extracted and washed 5 times with deionized water (30 mL), followed by brine (30 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as a brown foam. This residue was purified by flash chromatography (20% EtOAc/Hex) to afford the product as an off-white foam (447 mg, 78%). IR (solid) 2979, 2933, 1775, 1728, 1662 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.08 (d, J = 8.3 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.31 (t, J = 8.0, 7.3 Hz, 1H), 7.29 – 7.22 (m, 3H), 7.16 (d, J = 7.7 Hz, 2H), 7.08 (d, J = 7.8 Hz, 2H), 4.95 (dd, J = 7.2, 4.7 Hz, 1H), 4.24 (t, J = 5.2 Hz, 1H), 2.90 (ddd, J = 13.7, 7.9, 4.8 Hz, 2H), 2.85 (s, 3H), 2.68 (dd, J = 14.2, 5.9 Hz, 1H), 2.34 (dd, J = 15.7, 8.4 Hz, 1H), 2.22 (s, 3H), 2.10 (s, 3H), 1.63 (s, 9H), 1.25 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.24, 165.86, 165.36, 158.61, 150.00, 149.52, 135.83, 135.53, 130.41, 130.37, 129.99, 129.75, 124.96, 124.89, 123.04, 119.40, 116.10, 115.51,

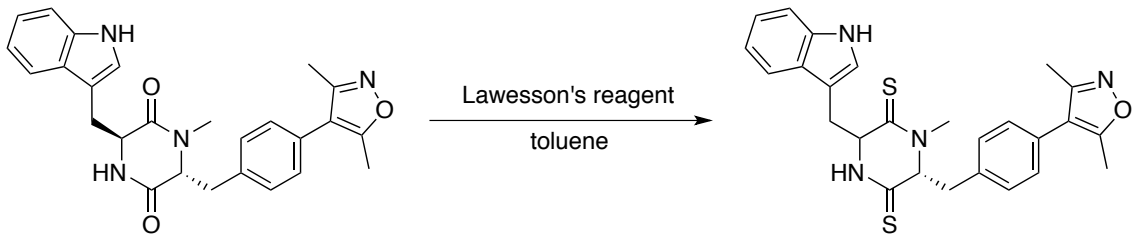
115.21, 84.65, 83.95, 65.67, 58.79, 38.19, 33.43, 30.54, 28.29, 28.26, 27.57, 11.49, 10.74. HRMS (ESI) m/z calc'd for $C_{36}H_{42}N_4O_7$ $[M+Na]^+$ 665.2951, found 665.2958.



(3S,6R)-3-((1H-indol-3-yl)methyl)-6-(4-(3,5-dimethylisoxazol-4-yl)benzyl)-1-methylpiperazine-2,5-dione (**108**). To a stirring solution of **113** (94.5 mg, 0.15 mmol, 1 eq) in anhydrous dichloromethane (750 μL, 0.2 M), trifluoroacetic acid (110 μL, 1.5 mmol, 10 eq) was added dropwise at room temperature (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate (20 mL) and partitioned. The organic layer was washed with brine (20 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by preparative TLC (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (46 mg, 71%). IR (thin film) 3274, 2922, 1675, 1458, 1333 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.48 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.27 – 7.20 (m, 4H), 7.16 (t, *J* = 7.9, 7.0 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.70 (s, 1H), 5.93 (s, 1H), 4.21 (t, *J* = 4.2 Hz, 1H), 3.99 (dt, *J* = 11.2, 2.7 Hz, 1H), 3.24 – 3.04 (m, 6H), 2.20 (s, 3H), 2.08 (s, 3H), 1.20 (dd, *J* = 13.9, 10.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.93, 165.90, 165.54, 158.72, 136.68, 134.73, 131.03, 130.13, 129.84, 126.56, 123.57, 122.65, 119.97, 118.79, 116.18, 111.64, 109.76, 63.19, 55.71, 36.65, 33.35, 31.47, 11.45, 10.71. HRMS (ESI) m/z calc'd for $C_{26}H_{26}N_4O_3$ $[M+H]^+$ 443.2083, found 443.2082.

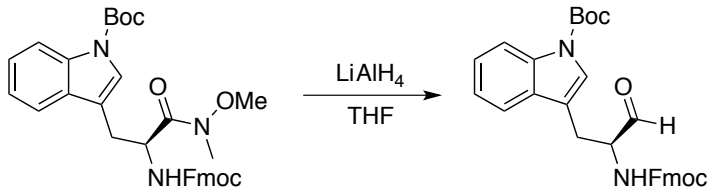


(3S,6R)-3-((1H-indol-3-yl)methyl)-6-(4-(3,5-dimethylisoxazol-4-yl)benzyl)-1-methyl-5-thioxopiperazin-2-one (**123**). To a stirring solution of **108** (200 mg, 0.45 mmol, 1 eq) in anhydrous tetrahydrofuran (2.3 mL, 0.2 M), Lawesson's reagent (220 mg, 0.54 mmol, 1.2 eq) was added at room temperature (20 °C). After stirring for 18 h, anhydrous tetrahydrofuran (2.3 mL, 0.2 M) and Lawesson's reagent (220 mg, 0.54 mmol, 1.2 eq) were added. After stirring for 18 h, the reaction mixture was concentrated to dryness *in vacuo*. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) followed by preparative TLC (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (50 mg, 24%). IR (thin film) 3280, 3059, 2928, 2797, 2244 ($CDCl_3$), 1643 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.30 (s, 1H), 7.87 (s, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.19 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.10 (t, *J* = 7.8, 7.1 Hz, 1H), 6.74 (d, *J* = 2.3 Hz, 1H), 4.75 (t, *J* = 4.3 Hz, 1H), 3.96 (dt, *J* = 11.4, 3.2 Hz, 1H), 3.63 (dd, *J* = 14.0, 3.8 Hz, 1H), 3.36 (dd, *J* = 14.0, 4.7 Hz, 1H), 3.18 – 3.07 (m, 4H), 2.19 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.90, 165.53, 164.72, 158.70, 136.67, 134.43, 131.23, 130.38, 129.86, 126.37, 123.53, 122.88, 120.20, 118.79, 116.14, 111.69, 109.40, 70.11, 58.03, 39.51, 33.43, 30.35, 11.44, 10.71. HRMS (ESI) m/z calc'd for $C_{26}H_{26}N_4O_2S$ $[M+H]^+$ 459.1855, found 459.1857.

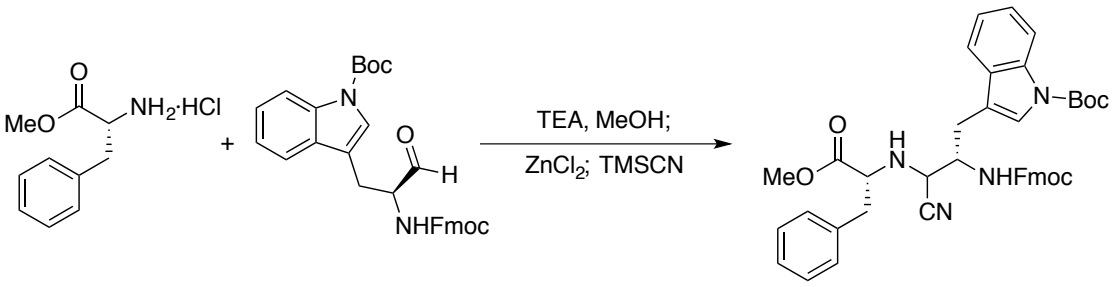


(6R)-3-((1H-indol-3-yl)methyl)-6-(4-(3,5-dimethylisoxazol-4-yl)benzyl)-1-methylpiperazine-2,5-dithione (**116**). To a suspension of **108** (100 mg, 0.23 mmol, 1 eq) in anhydrous toluene (1.2 mL, 0.2 M), Lawesson's reagent (110 mg, 0.27 mmol, 1.2 eq) was added at room temperature (20 °C). Heated to reflux for 1h. The reaction mixture was cooled and run through a plug of Celite with excess ethyl acetate. The filtrate was concentrated to dryness *in vacuo*. This residue was purified by flash chromatography (20% EtOAc/Hex) followed by preparative TLC (20% EtOAc/Hex) to afford the two epimeric products as orange films (top spot: 35 mg, bottom spot: 36 mg, 66% 1:1 dr). **Top spot**: IR (thin film) 3316, 3049, 2926, 1630, 1512 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.26 (s, 1H), 7.84 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.21 – 7.14 (m, 3H), 7.14 – 7.08 (m, 3H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 4.95 (t, *J* = 4.3 Hz, 1H), 4.18 (ddd, *J* = 14.8, 3.7, 1.1 Hz, 1H), 3.57 – 3.54 (m, 1H), 3.53 (s, 3H), 3.38 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.97 (dd, *J* = 11.5, 3.7 Hz, 1H), 2.76 (dd, *J* = 14.9, 11.3 Hz, 1H), 2.40 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.47, 193.96, 165.56, 158.58, 136.81, 133.14, 130.83, 130.67, 129.37, 126.30, 123.73, 123.26, 119.95, 118.57, 116.00, 111.80, 109.73, 73.77, 60.72, 42.33, 39.49, 33.25, 11.98, 11.18. HRMS (ESI) m/z calc'd for $C_{26}H_{26}N_4O_1S_2$ $[M+H]^+$ 475.1626, found 475.1627. **Bottom spot**: IR (thin film) 3324, 3061, 2927, 1519, 1423 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.84 (s, 1H), 7.63 – 7.58 (m, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 7.22 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.14 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 4.90

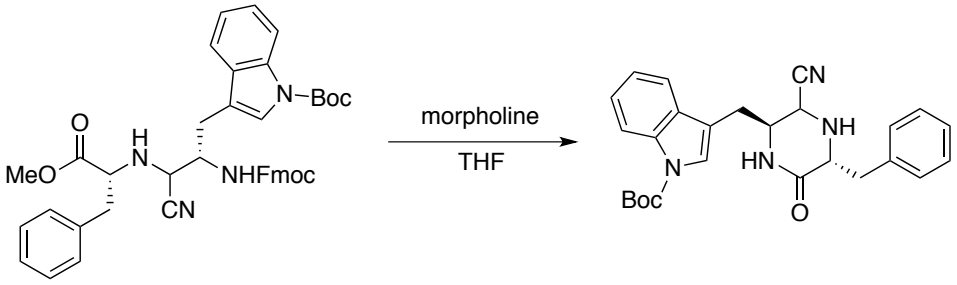
(td, *J* = 4.4, 1.3 Hz, 1H), 4.51 (d, *J* = 11.1 Hz, 1H), 3.72 (dd, *J* = 14.1, 4.2 Hz, 1H), 3.65 – 3.59 (m, 1H), 3.58 (s, 3H), 3.49 – 3.45 (m, 1H), 2.22 (s, 3H), 2.09 (s, 3H), 1.05 (dd, *J* = 14.1, 11.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 193.00, 192.84, 165.57, 158.69, 136.63, 134.04, 131.21, 130.63, 129.97, 126.48, 123.49, 122.95, 120.27, 119.06, 116.09, 111.64, 109.89, 72.25, 65.24, 42.44, 40.22, 35.02, 11.49, 10.75. HRMS (ESI) m/z calc'd for $C_{26}H_{26}N_4O_1S_2$ $[M+H]^+$ 475.1626, found 475.1617.



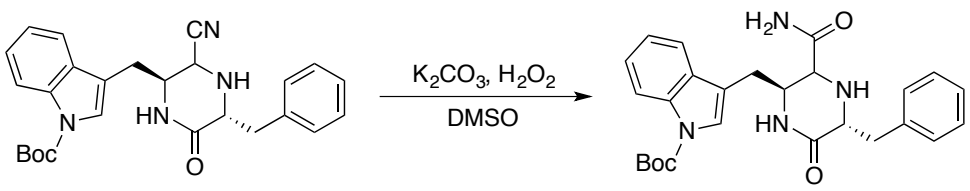
Tert-*butyl (*S*)-3-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate* (**131**). To the weinreb amide[13] (445 mg, 0.78 mmol, 1 eq) in anhydrous tetrahydrofuran (3.2 mL, 0.25 M) at 0 °C, lithium aluminum hydride (77 mg, 2 mmol, 2.6 eq) was added slowly over ten minute. The reaction mixture was allowed to stir for 30 mins then slowly quenched with 10% citric acid until gas stopped evolving. This mixture was stirred for another 10 minutes at 0 °C. This solution was diluted with diethyl ether (10 mL) and partitioned. The aqueous was extracted three more times with diethyl ether (10 mL) and the combined organic was washed with saturated sodium bicarbonate (10 mL), water (10 mL), 10% citric acid (10 mL), water (10 mL), then brine (10 mL). The organic was then dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an off-white foam which was used in the next step without further purification.



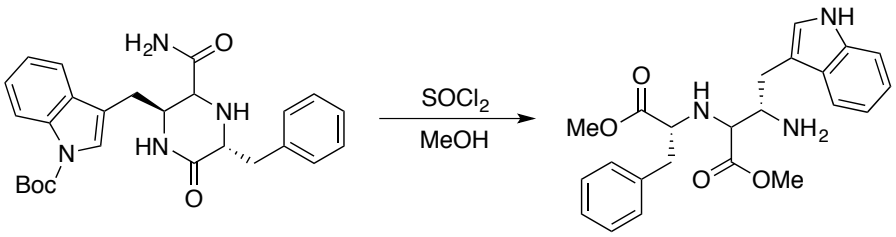
Tert-*butyl 3-((2*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-cyano-3-(((*R*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)propyl)-1*H*-indole-1-carboxylate* (**132**). To the *D*-Phe methyl ester (337 mg, 1.6 mmol, 2 eq) in methanol (3 mL), triethylamine (220 μL, 1.6 mmol, 2 eq) was added at room temperature (20 °C). The reaction mixture was allowed to stir for 20 mins then cooled to -20 °C and then an ethereal solution of zinc chloride (1 M, 780 μL, 0.78 mmol, 1 eq) was added, followed by aldehyde **131** (0.78 mmol, 1 eq) in methanol (4.8 mL, total vol 0.1 M). The reaction mixture was stirred at -20 °C for one hour, then trimethylsilylcyanide (180 μL, 1.4 mmol, 1.8 eq) was added dropwise. The reaction mixture was allowed to warm to room temperature for 18 h. The reaction was then filtered to afford the product as a white solid (324 mg, 59%, one diastereomer). m.p. = 155-160 °C. IR (solid) 1740, 1730, 1683, 1520, 1464 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.15 (d, J = 8.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 2H), 7.54 (t, J = 9.5 Hz, 3H), 7.44 – 7.27 (m, 7H), 7.06 (s, 5H), 4.89 (d, J = 8.6 Hz, 1H), 4.42 (d, J = 6.9 Hz, 2H), 4.29 (s, 1H), 4.21 (s, 1H), 3.71 (s, 3H), 3.50 (d, J = 8.1 Hz, 1H), 3.35 (d, J = 10.8 Hz, 1H), 3.05 – 2.86 (m, 3H), 2.76 (ddd, J = 13.5, 8.3, 1.9 Hz, 1H), 2.36 (s, 1H), 1.66 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.48, 149.61, 143.85, 143.77, 141.46, 136.85, 135.71, 130.04, 129.23, 128.48, 127.91, 127.26, 126.96, 125.18, 124.92, 124.25, 123.00, 120.16, 118.97, 117.97, 115.57, 114.95, 83.98, 67.30, 61.73, 53.30, 53.19, 52.36, 47.29, 40.03, 28.34, 27.75. HRMS (ESI) m/z calc'd for $C_{42}H_{42}N_4O_6$ $[M+H]^+$ 699.3183, found 699.3190.



Tert-*butyl 3-(((2*S*,5*R*)-5-benzyl-3-cyano-6-oxopiperazin-2-yl)methyl)*-1H-*indole-1-carboxylate* (**133**). To a stirring solution of **132** (300 mg, 0.4 mmol, 1 eq) in anhydrous tetrahydrofuran (7 mL, 0.063 M), morpholine (1.3 mL, 0.33 M) was added dropwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then partitioned between ethyl acetate (10 mL) and saturated ammonium chloride (10 mL). The organic layer was washed with water (10 mL), followed by brine (10 mL) and dried over sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (20-30% EtOAc/Hex) to afford the product as a white foam (162 mg, 85%). IR (solid) 1732, 1669, 1475, 1453, 1367 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 8.3 Hz, 1H), 7.56 (s, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.30 (q, *J* = 6.3, 5.0 Hz, 2H), 7.27 – 7.20 (m, 4H), 6.60 (s, 1H), 4.11 (td, *J* = 7.6, 3.9 Hz, 1H), 4.05 (s, 1H), 3.94 (dt, *J* = 10.3, 3.2 Hz, 1H), 3.55 (dd, *J* = 13.8, 3.2 Hz, 1H), 3.06 (d, *J* = 8.4 Hz, 2H), 2.74 (dd, *J* = 13.7, 10.4 Hz, 1H), 2.01 (s, 1H), 1.68 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.55, 149.45, 137.25, 135.91, 129.47, 129.42, 129.02, 127.20, 125.23, 124.86, 123.03, 118.75, 116.11, 115.76, 113.35, 84.31, 56.50, 53.40, 49.05, 38.13, 28.64, 28.33. HRMS (ESI) m/z calc'd for $C_{26}H_{28}N_4O_3$ $[M+H]^+$ 445.2240, found 445.2253.

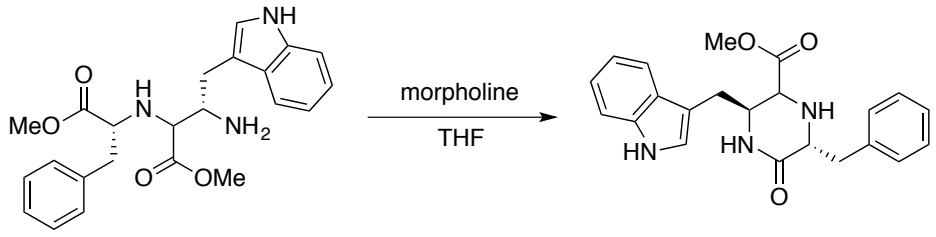


Tert-*butyl 3-(((2*S*,5*R*)-5-benzyl-3-carbamoyl-6-oxopiperazin-2-yl)methyl)-1*H*-indole-1-carboxylate* (**137**). To a stirring solution of **133** (110 mg, 0.25 mmol, 1 eq) and potassium carbonate (7 mg, 0.05 mmol, 0.2 eq) in anhydrous dimethylsulfoxide (1.3 mL, 0.2 M), hydrogen peroxide (130 μL, 1 mmol, 4.5 eq) was added dropwise at 0 °C. Reaction mixture was warmed to 40 °C for 18 h, then cooled to room temperature (20 °C), diluted with ethyl acetate (10 mL), washed five times with water (10 mL) and one time with brine (10 mL).. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as an off-white foam (113 mg, 98%). IR (solid) 3314, 2979, 1728, 1660, 1452 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.10 (s, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.46 (s, 1H), 7.33 – 7.28 (m, 3H), 7.22 (dt, J = 7.4, 5.6 Hz, 4H), 6.75 (d, J = 4.1 Hz, 1H), 6.51 (s, 1H), 6.15 (d, J = 4.1 Hz, 1H), 4.11 (hept, J = 4.1, 3.7, 3.4, 2.8, 2.8 Hz, 1H), 3.88 (d, J = 4.1 Hz, 1H), 3.65 (dd, J = 10.5, 3.7 Hz, 1H), 3.18 (dd, J = 14.0, 3.7 Hz, 1H), 3.05 (ddd, J = 13.9, 2.7, 1.2 Hz, 1H), 2.93 (dd, J = 14.0, 10.5 Hz, 1H), 2.84 (dd, J = 13.9, 10.2 Hz, 1H), 2.01 (s, 1H), 1.64 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.45, 171.42, 149.73, 138.48, 135.75, 130.06, 129.56, 128.73, 126.91, 124.94, 124.39, 123.00, 119.39, 115.63, 115.50, 84.09, 58.79, 54.26, 52.83, 37.72, 28.33, 27.25. HRMS (ESI) m/z calc'd for $C_{26}H_{30}N_4O_4$ $[M+H]^+$463.2345, found 463.2352.

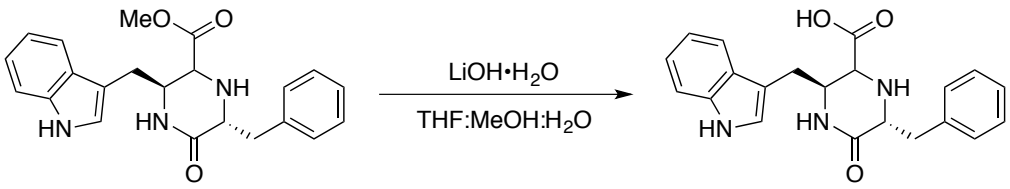


*Methyl (3*S*)-3-amino-4-(1*H*-indol-3-yl)-2-(((*R*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)butanoate* (**138**). To a stirring solution of **137** (265 mg, 0.57 mmol, 1 eq) in anhydrous methanol (8.5 mL, 0.067 M), thionyl chloride (1.5 mL, 20 mmol, 35 eq) was added dropwise at 0 °C. Reaction vessel was sealed and heated to 60 °C over two days. Upon cooling to 0 °C, the flask was opened and TLC indicated incomplete conversion. Reaction mixture was concentrated to dryness *in vacuo.* The residue was redissolved in anhydrous methanol (8.5 mL, 0.067 M) and thionyl chloride (1.5 mL, 20 mmol, 35 eq) was added dropwise at 0 °C. Reaction vessel was sealed and heated to 60 °C for 18 h. Upon cooling to 0 °C, the reaction mixture was diluted with 20 mL dichloromethane and saturated sodium bicarbonate was added to quench. The organic layer was partitioned and washed an additional time with saturated sodium bicarbonate (30 mL) and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as a brown oil. This residue was purified by flash chromatography (5% MeOH/CH_2Cl_2) to afford the product as brown film (185 mg, 79%). IR (thin film) 3347, 3029, 2951, 1735, 1495 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.41 (s, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.26 – 7.22 (m, 2H), 7.22 – 7.15 (m, 2H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.88 (d, *J* = 2.2 Hz, 1H), 3.66 (d, *J* = 5.1 Hz, 6H), 3.54 (q, *J* = 7.1, 6.5 Hz, 1H), 3.30 – 3.21 (m, 2H), 3.05 (dd, *J* = 13.6, 5.7 Hz, 1H), 2.96 – 2.84 (m, 2H), 2.58 (dd, *J* = 14.3, 8.9 Hz, 1H), 2.43 (s, 1H), 1.51 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.80, 174.00, 137.94, 136.56, 129.62, 128.49, 127.72,

126.80, 123.02, 122.09, 119.41, 118.98, 112.50, 111.39, 65.70, 63.25, 54.33, 51.96, 51.94, 40.21, 29.78. HRMS (ESI) m/z calc'd for $C_{23}H_{27}N_3O_4$ $[M+H]^+$ 410.2080, found 410.2084.



Methyl (3S,6R)-3-((1H-indol-3-yl)methyl)-6-benzyl-5-oxopiperazine-2-carboxylate (**139**). To a stirring solution of **138** (185 mg, 0.45 mmol, 1 eq) in anhydrous tetrahydrofuran (7 mL, 0.063 M), morpholine (1.4 mL, 0.33 M) was added dropwise. The reaction mixture was refluxed for 18 h. The reaction mixture was then partitioned between ethyl acetate (10 mL) and saturated ammonium chloride (10 mL). The organic layer was washed with water (10 mL), followed by brine (10 mL) and dried over sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as a white foam (120 mg, 70%). IR (solid) 3282, 2924, 1732, 1652, 1455 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.28 – 7.18 (m, 4H), 7.13 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 6.95 (d, *J* = 2.3 Hz, 1H), 5.93 (s, 1H), 4.03 – 3.95 (m, 3H), 3.79 (s, 3H), 3.28 (dd, *J* = 13.7, 3.5 Hz, 1H), 3.01 – 2.95 (m, 2H), 2.92 (dd, *J* = 13.7, 10.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.25, 170.87, 137.91, 136.75, 129.46, 128.96, 127.11, 127.01, 123.55, 122.49, 119.80, 118.54, 111.72, 110.22, 58.04, 54.62, 53.63, 52.36, 38.34, 27.93. HRMS (ESI) m/z calc'd for $C_{22}H_{23}N_3O_3$ $[M+H]^+$ 378.1818, found 378.1834.



*(3*S*,6*R*)-3-((1*H*-indol-3-yl)methyl)-6-benzyl-5-oxopiperazine-2-carboxylic acid* (**142**). To a stirring solution of **139** (30 mg, 0.08 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 1.2 mL, 0.067 M), lithium hydroxide monohydrate (33 mg, 0.8 mmol, 10 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The aqueous mixture extracted with four times with ethyl acetate (5 mL). The combined organic was then washed with brine and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as an off-white solid, which was triturated in a mixture of acetone and dichloromethane to yield the product as a white solid (13.2 mg, 46%). m.p. = 188-194 °C. IR (solid) 1680, 1635, 1456, 1406, 1367 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.54 – 7.39 (m, 2H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.26 – 7.16 (m, 2H), 7.12 (s, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 3.70 (s, 3H), 2.98 (d, *J* = 13.5 Hz, 1H), 2.95 – 2.78 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.05, 170.02, 138.97, 136.26, 129.45, 128.22, 127.38, 126.18, 123.73, 120.90, 118.29, 118.16, 111.42, 109.83, 57.17, 54.25, 53.45, 37.88, 27.26. HRMS (ESI) m/z calc'd for $C_{21}H_{21}N_3O_3$ $[M+H]^+$ 364.1661, found 364.1676.

SECTION A.3 REFERENCES

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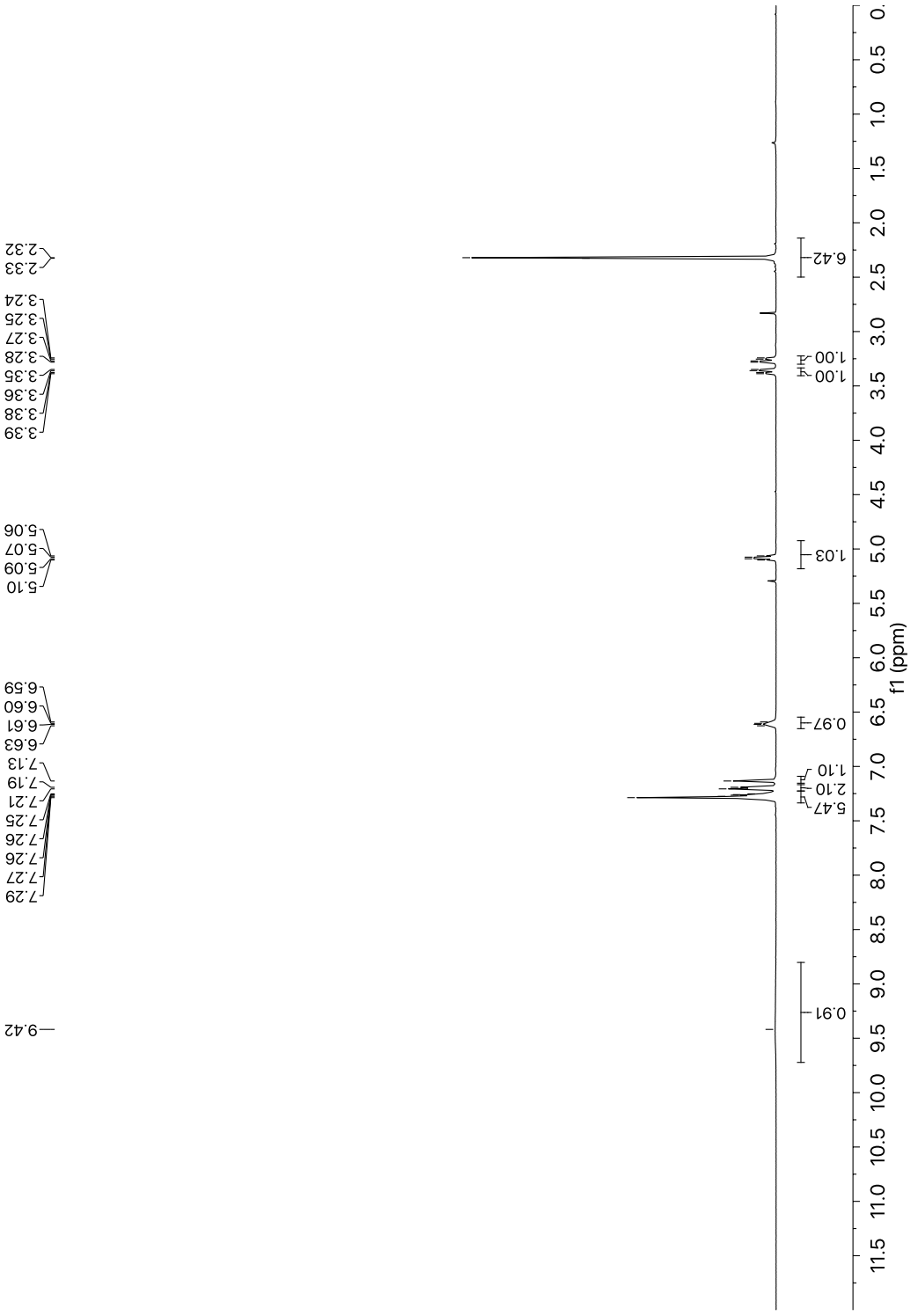
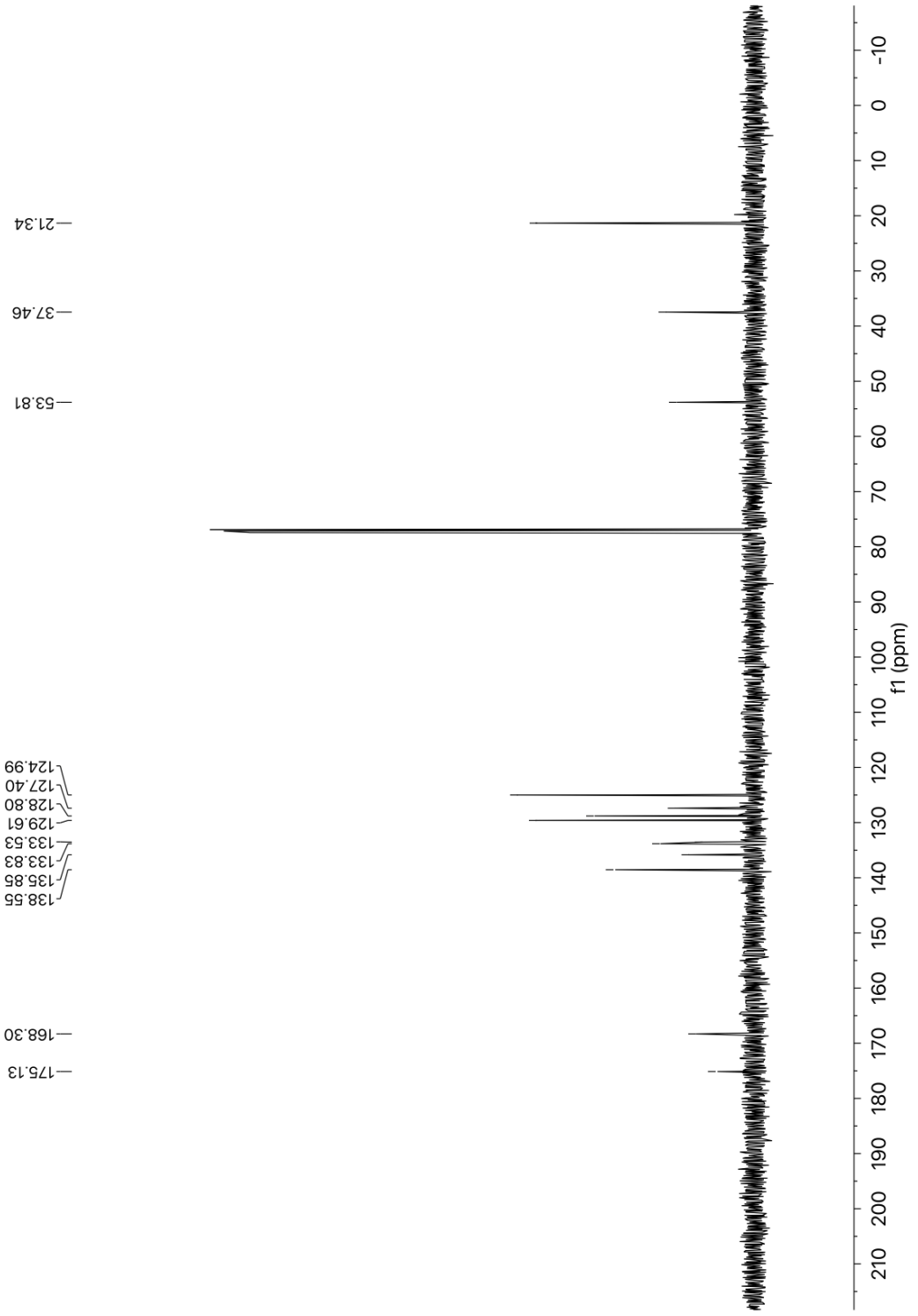
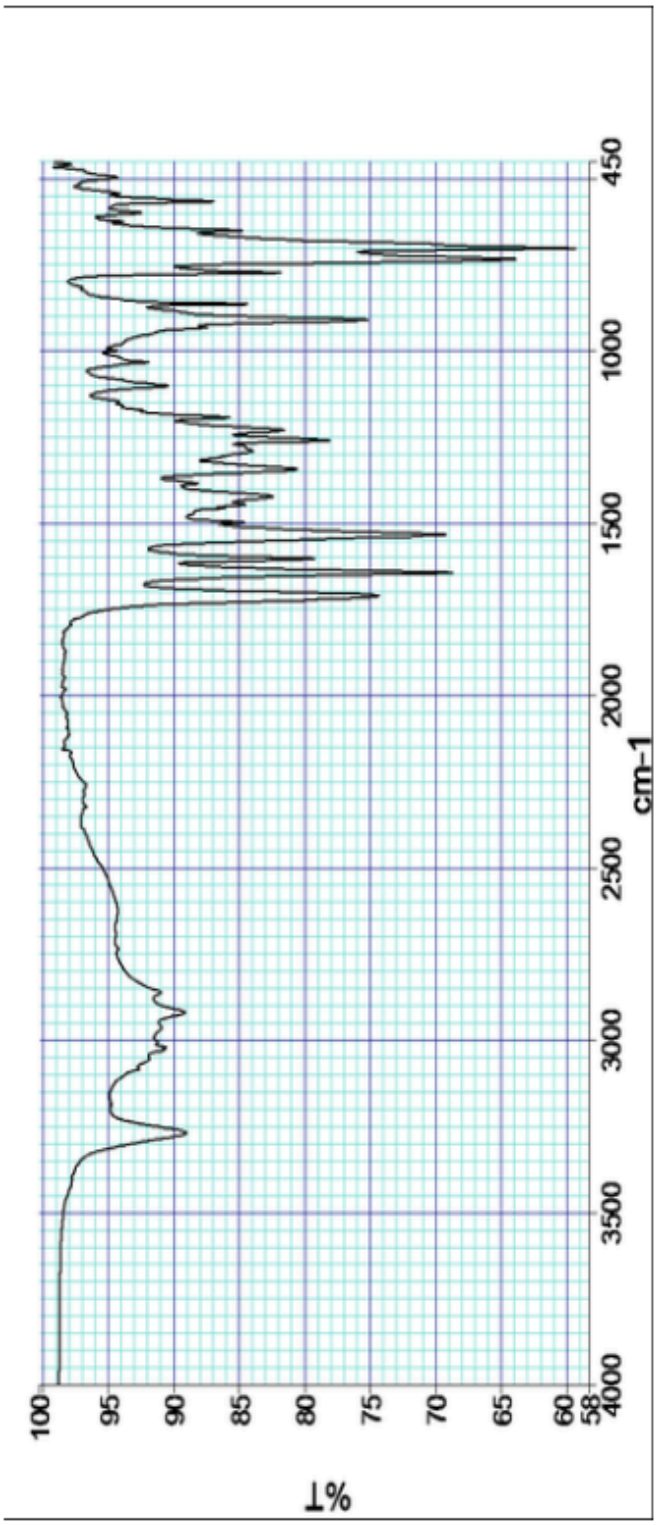
APPENDIX B. SPECTRAL IMAGES



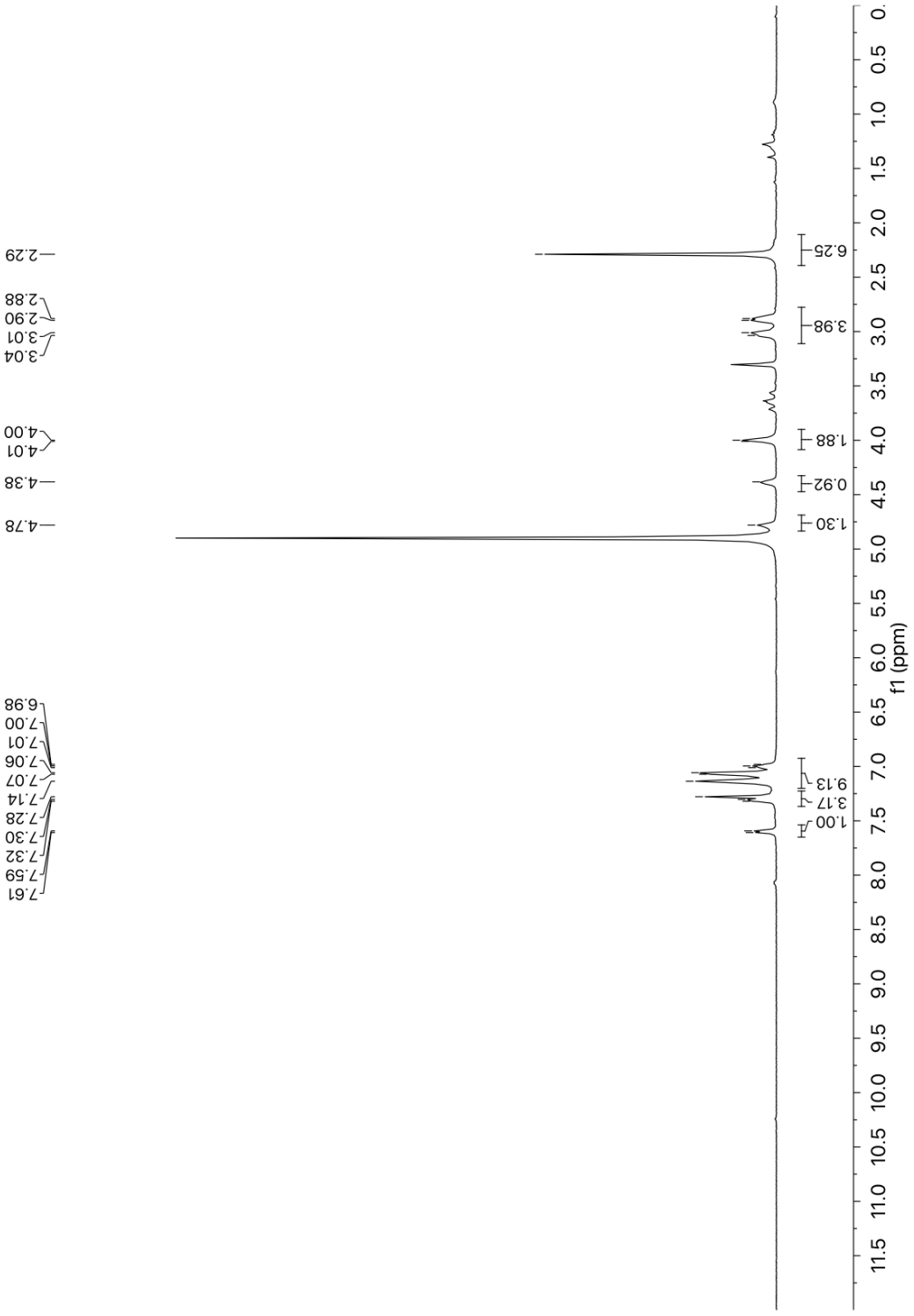
^{1}HNMR spectrum for compound **39**



^{13}CNMR spectrum for compound **39**



IR spectrum for compound **39**



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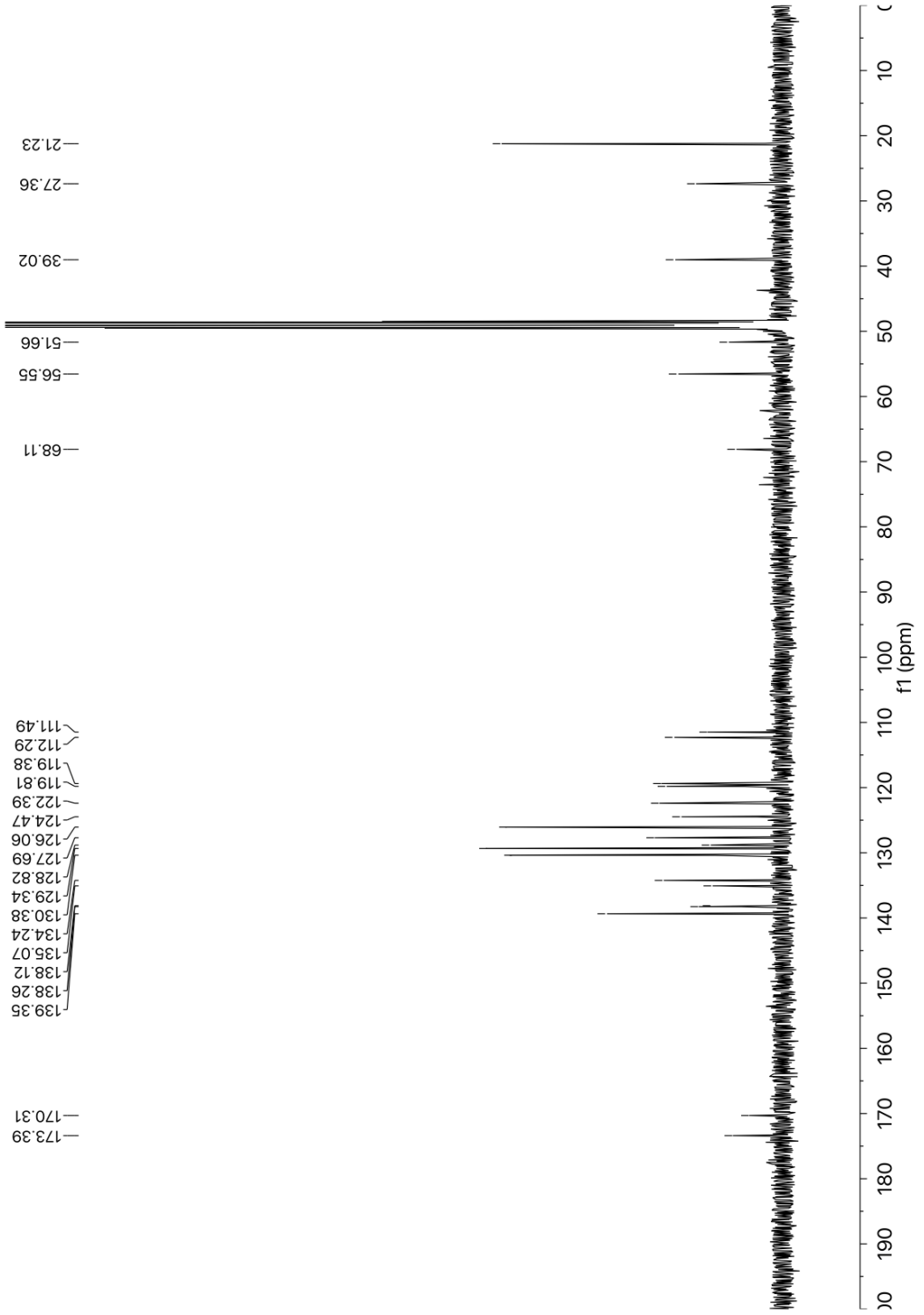

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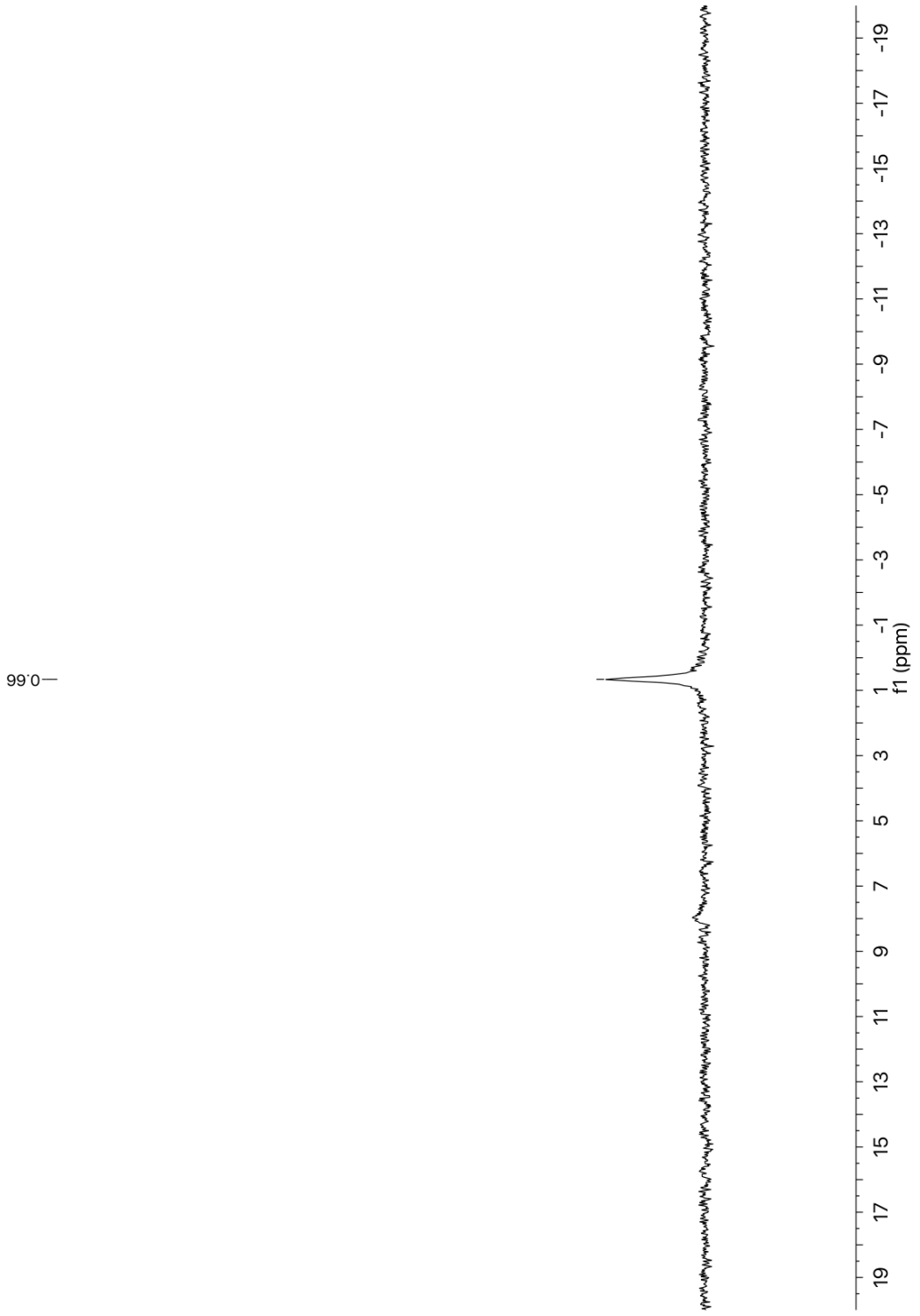
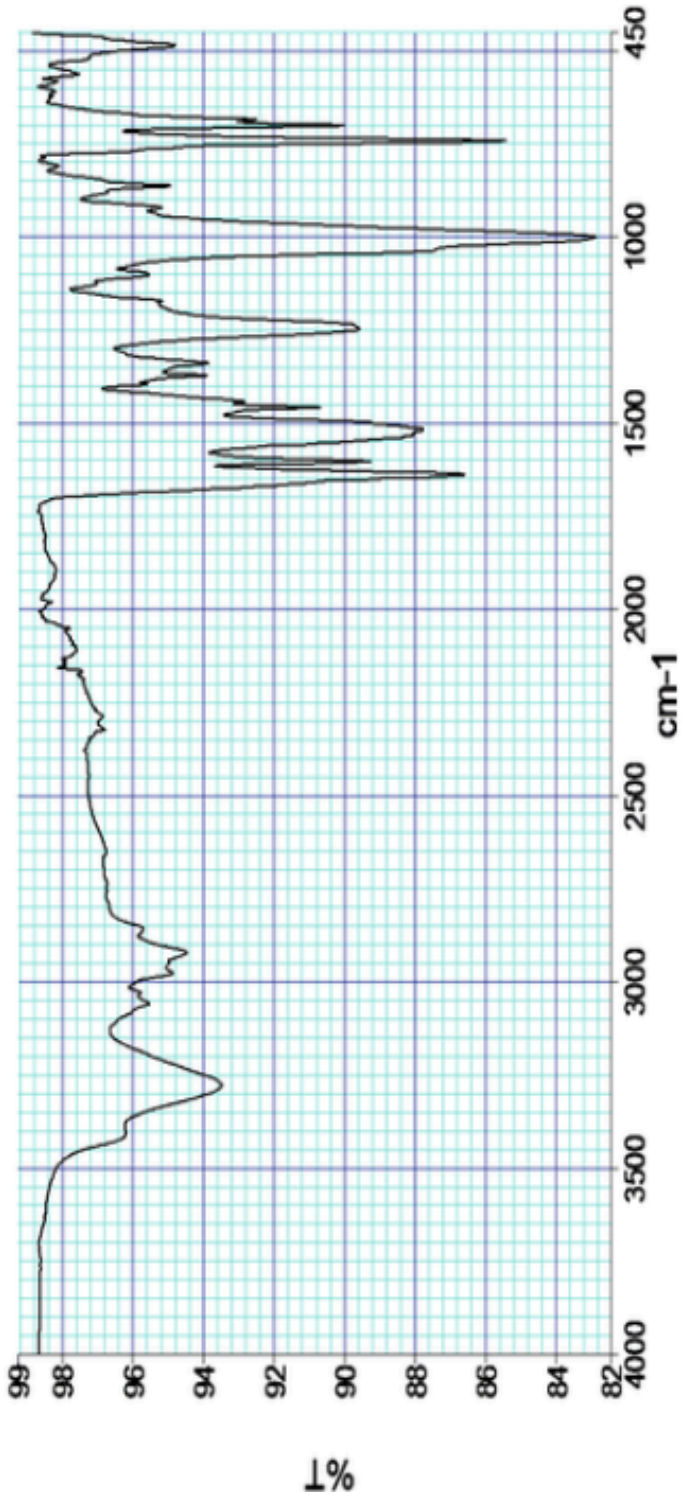
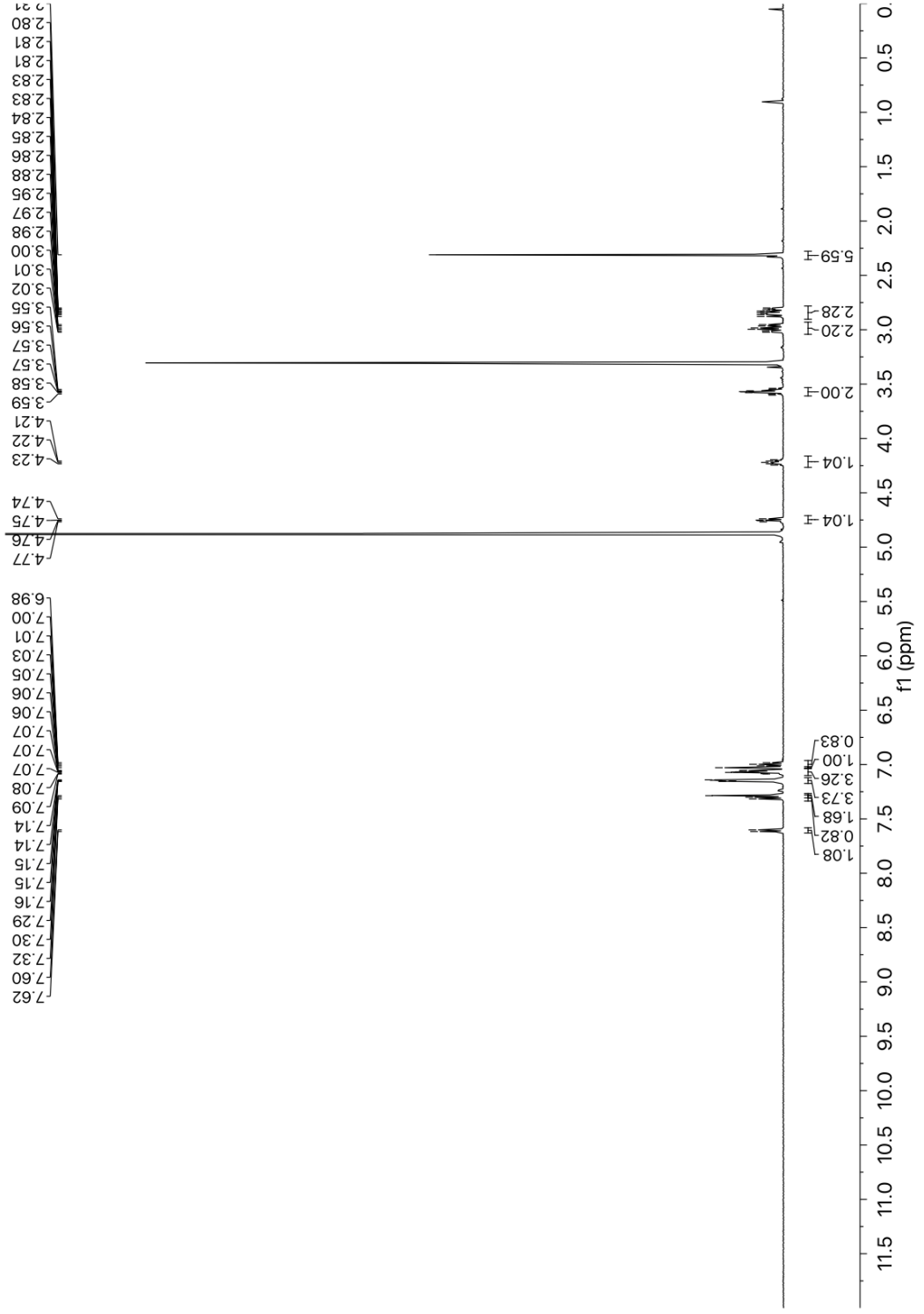


^{31}PNMR spectrum for compound **41**



IR spectrum for compound **41**



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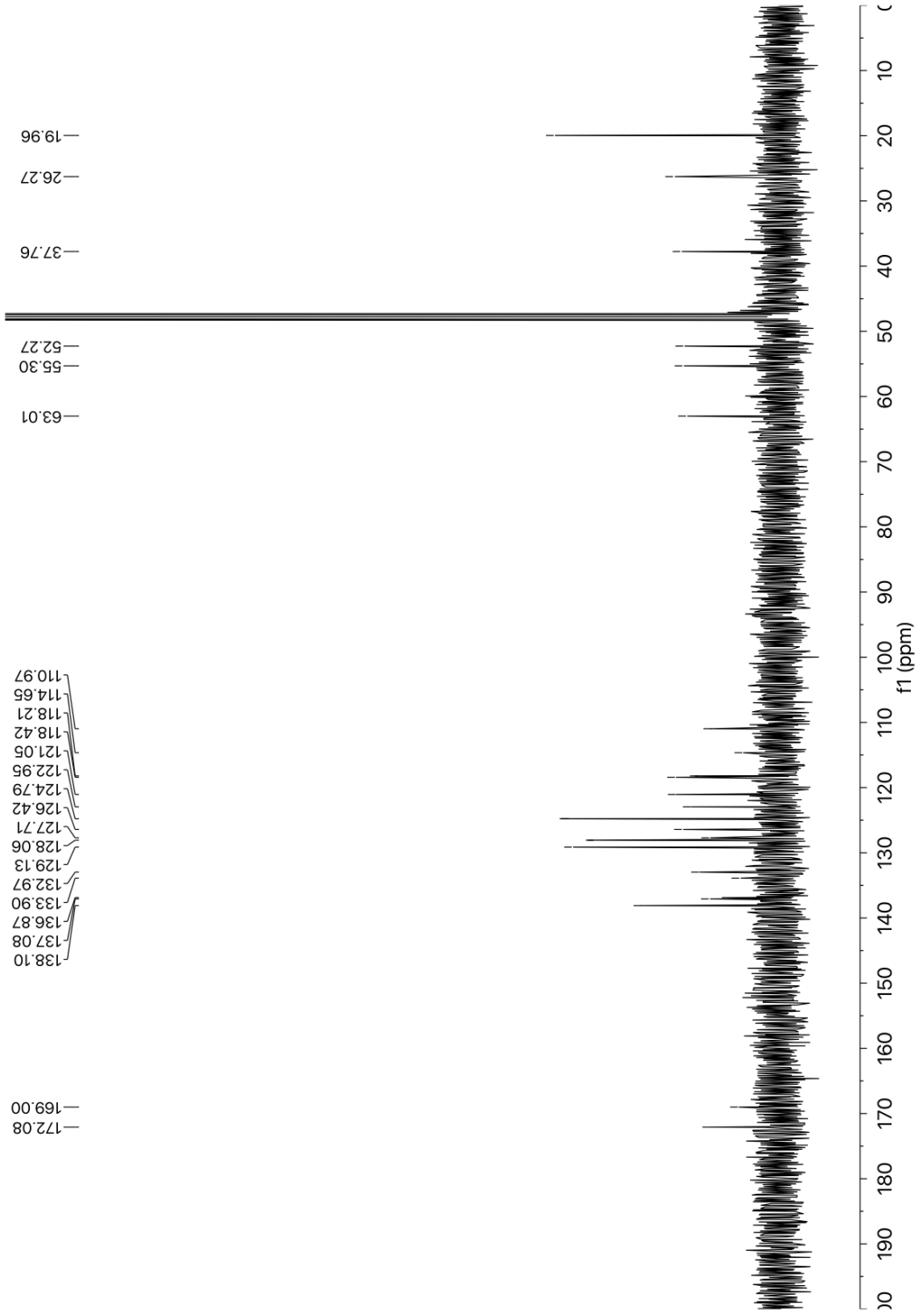

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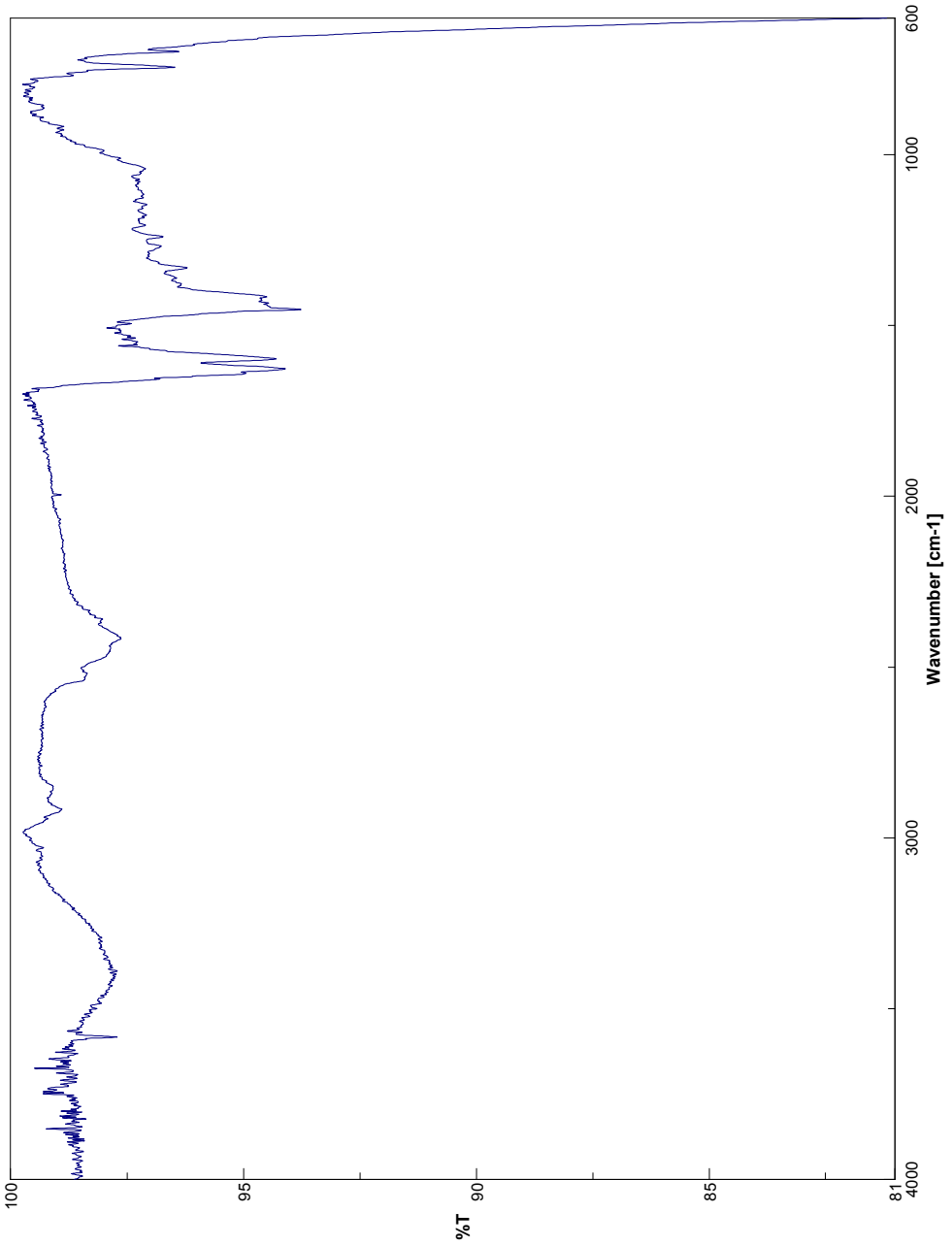
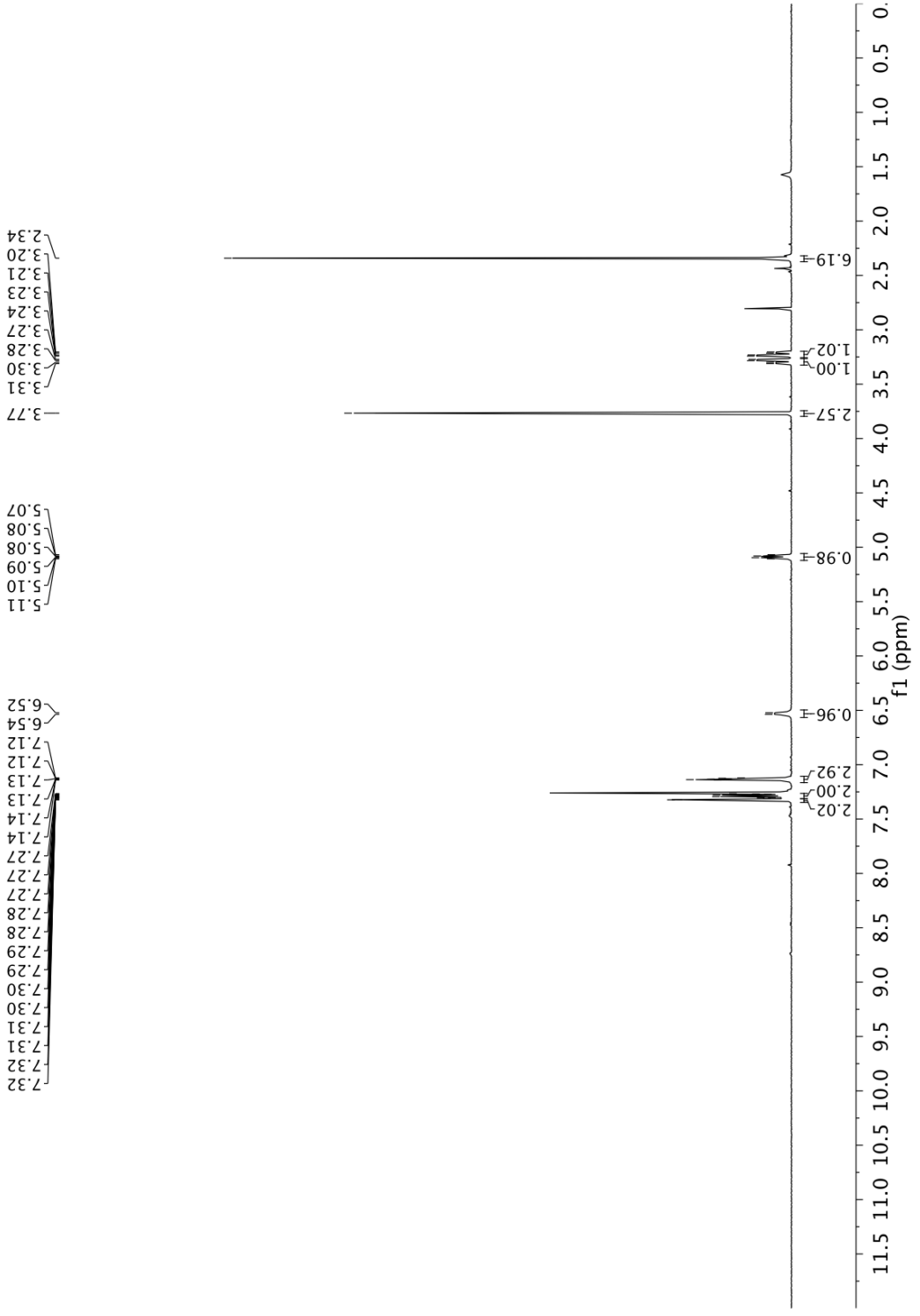
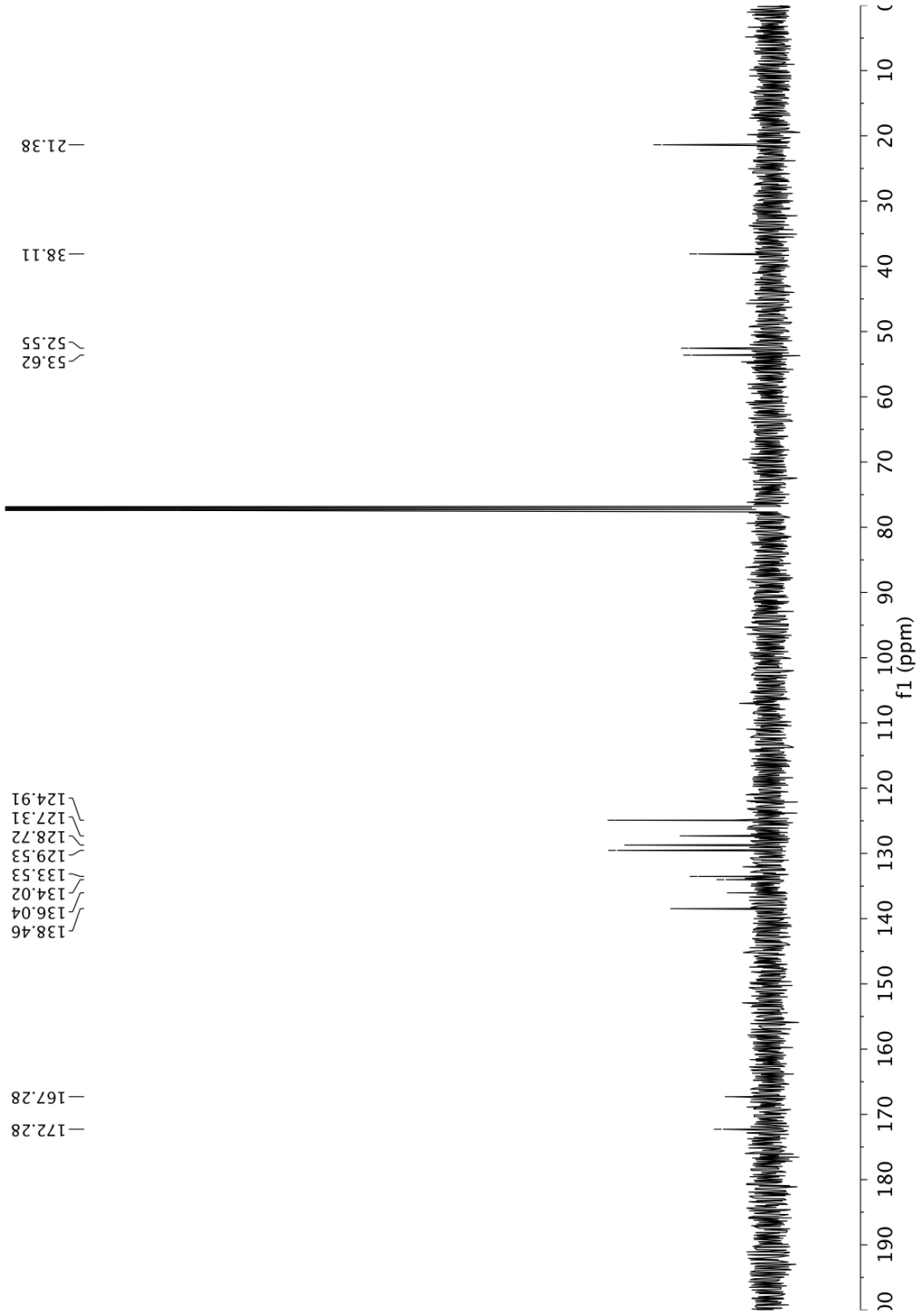


IR spectrum for compound **42**



[1]HNMR spectrum for compound **44**



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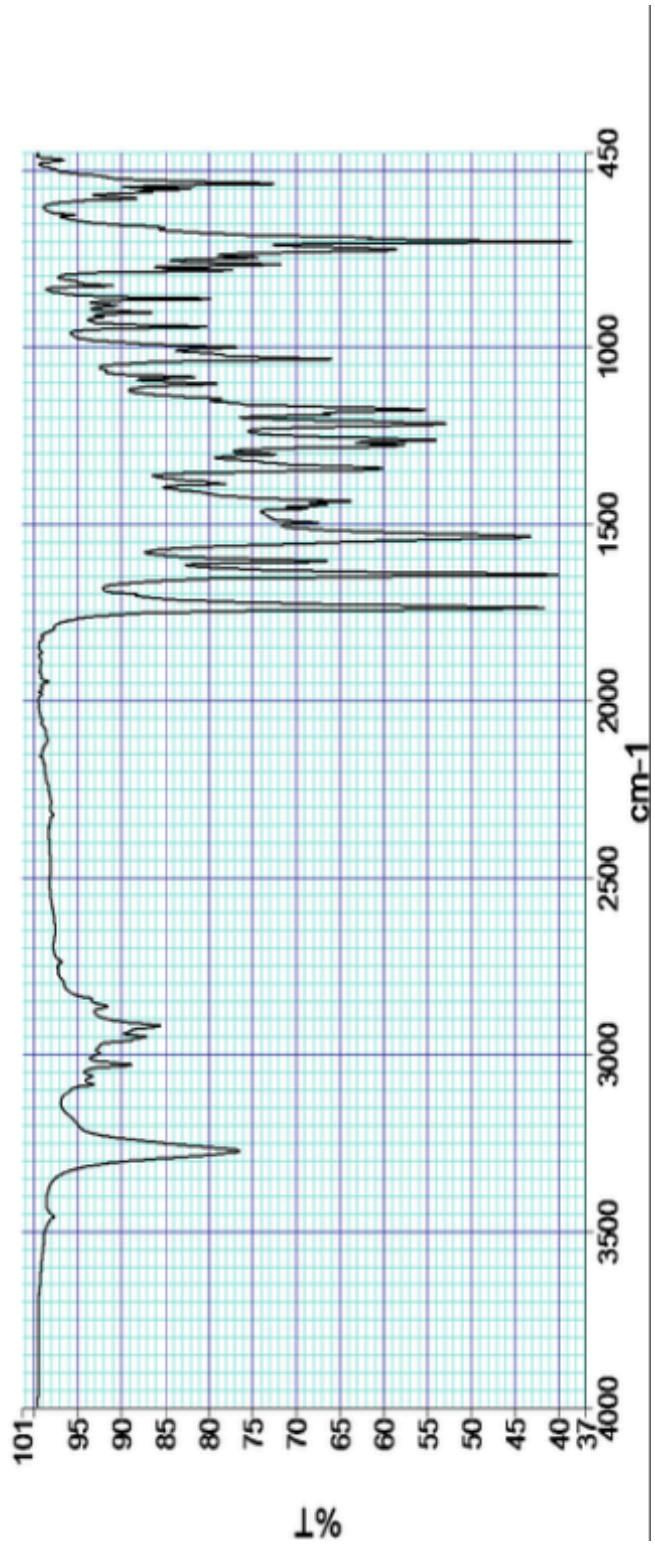
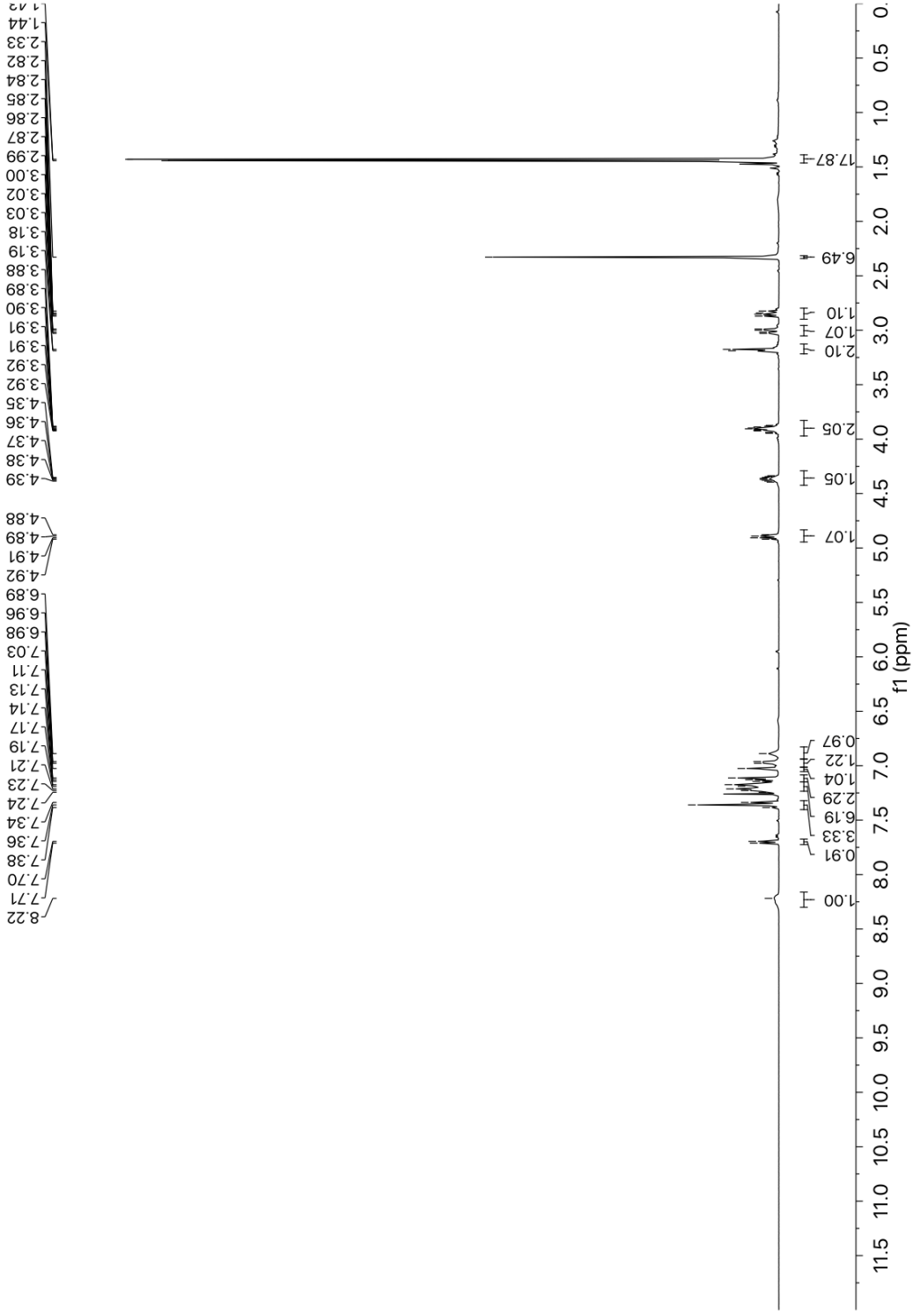
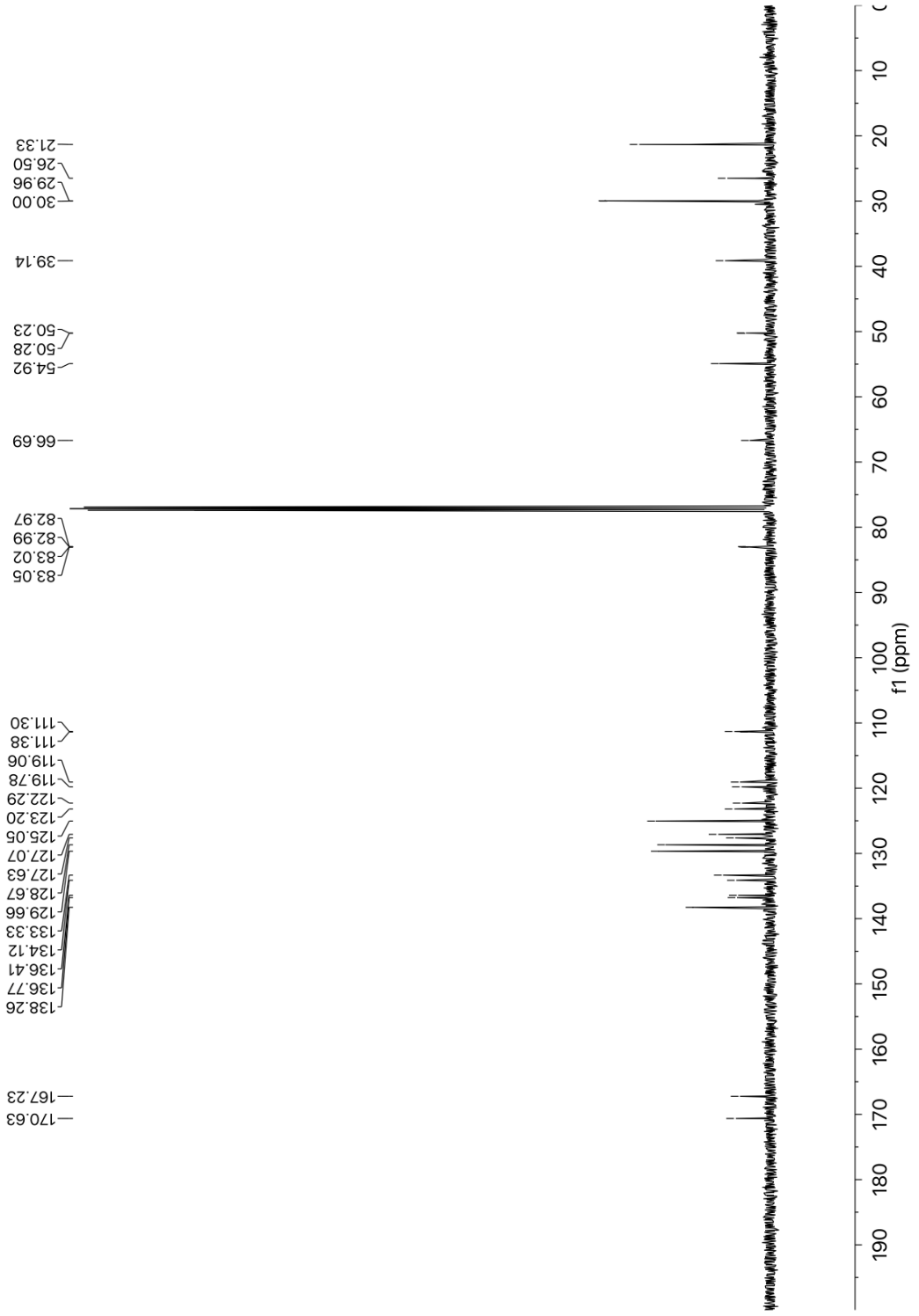


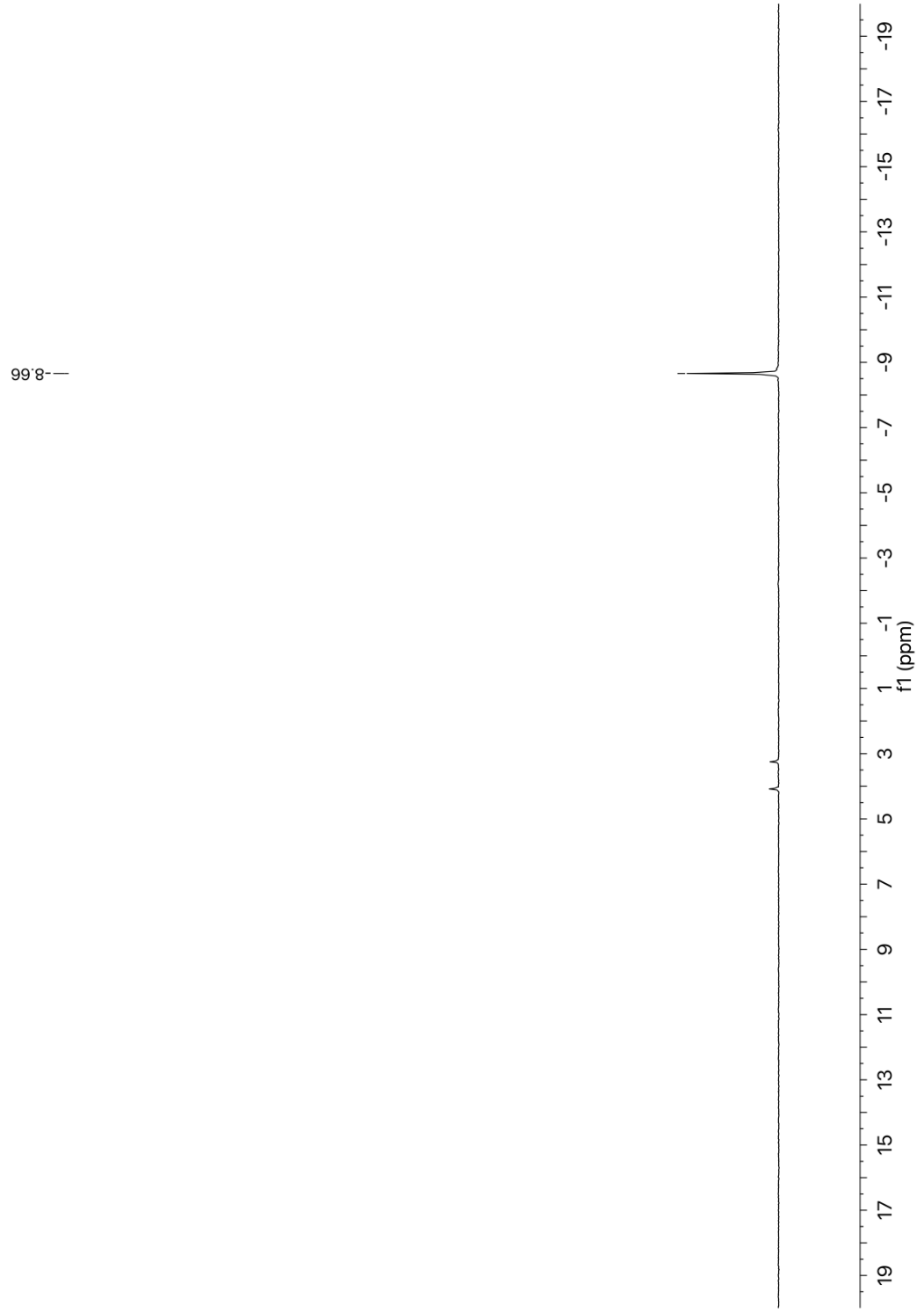
IR spectrum for compound **44**



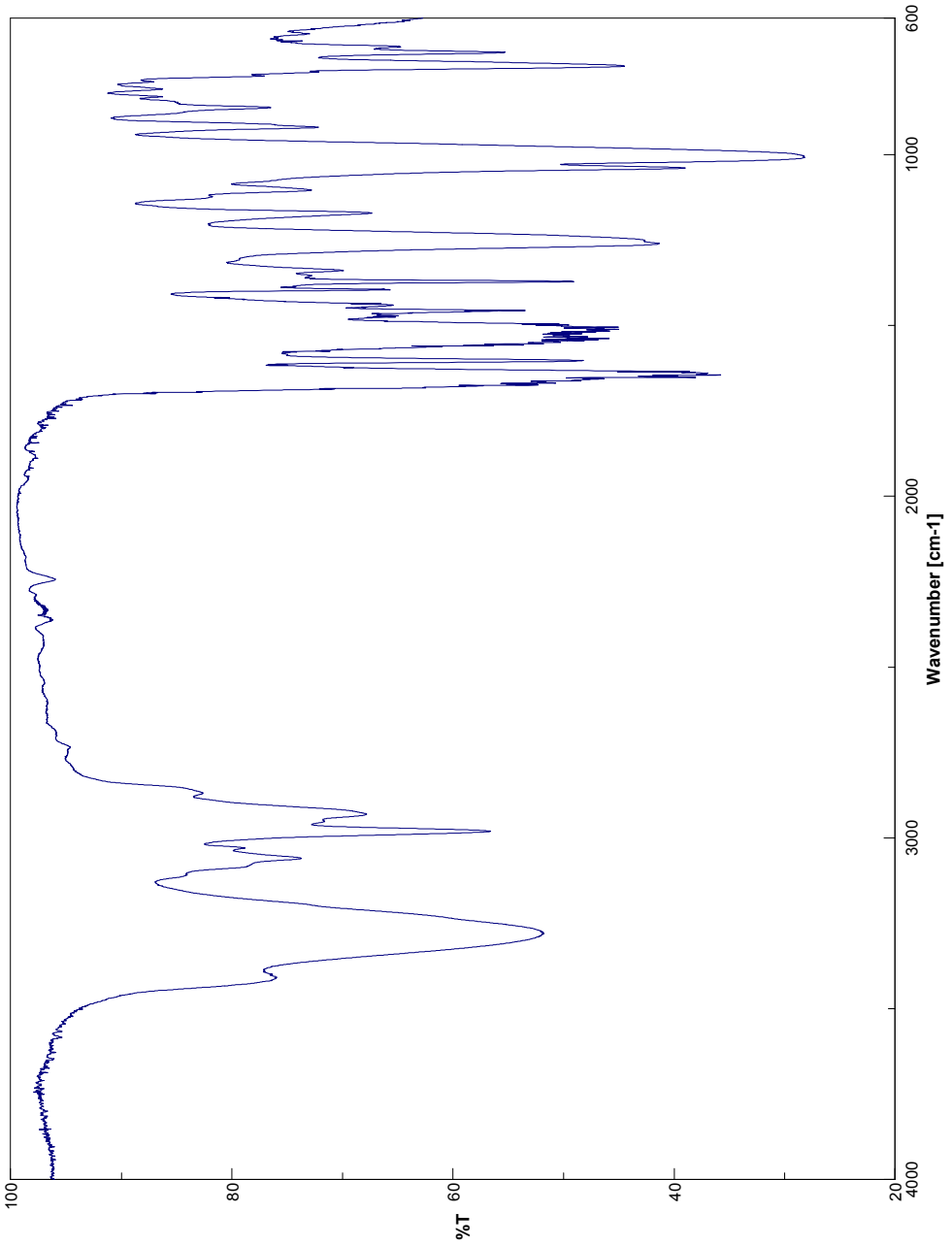
[1]HNMR spectrum for compound **46**



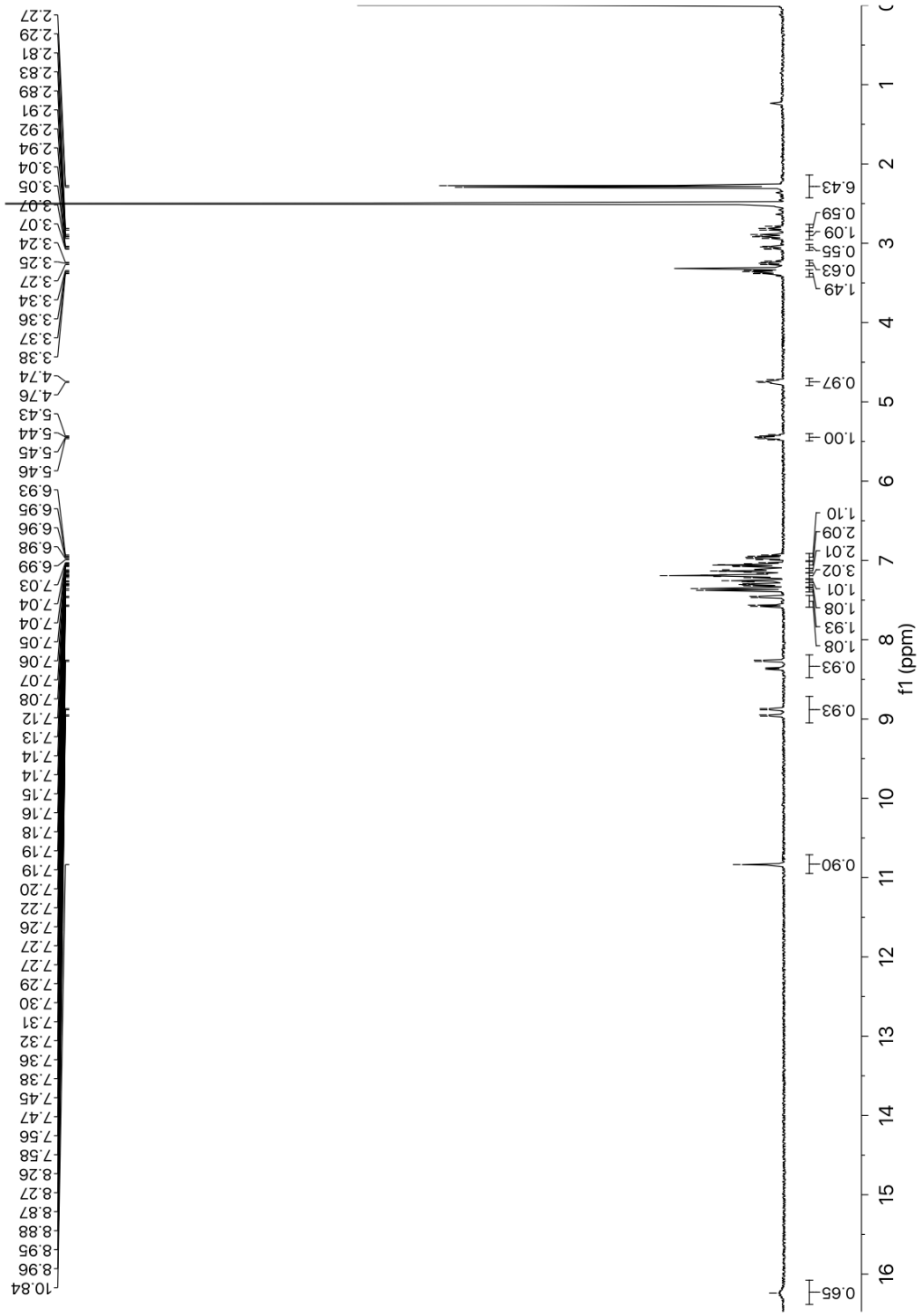
^{13}CNMR spectrum for compound **46**



^{31}PNMR spectrum for compound **46**



IR spectrum for compound **46**



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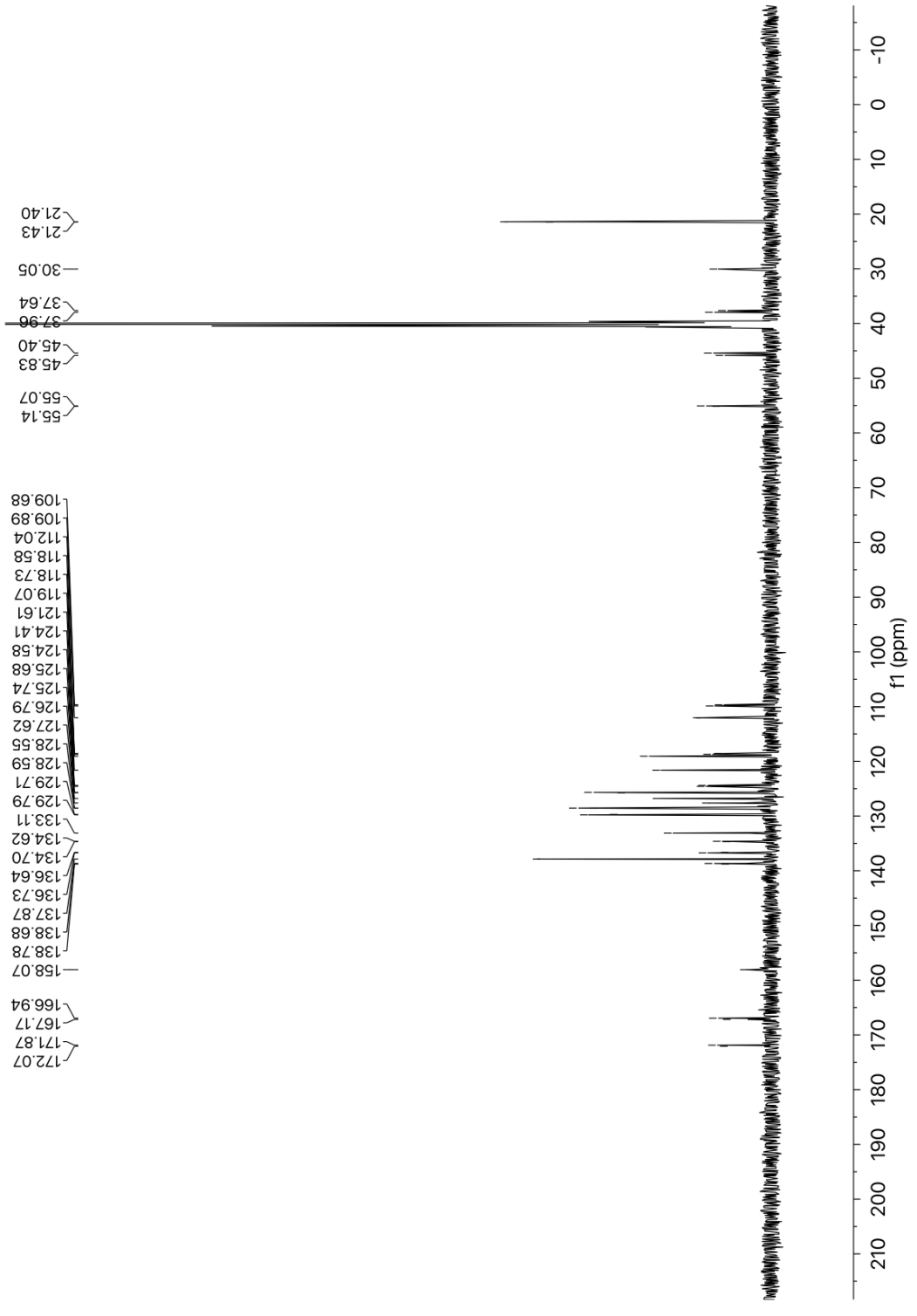

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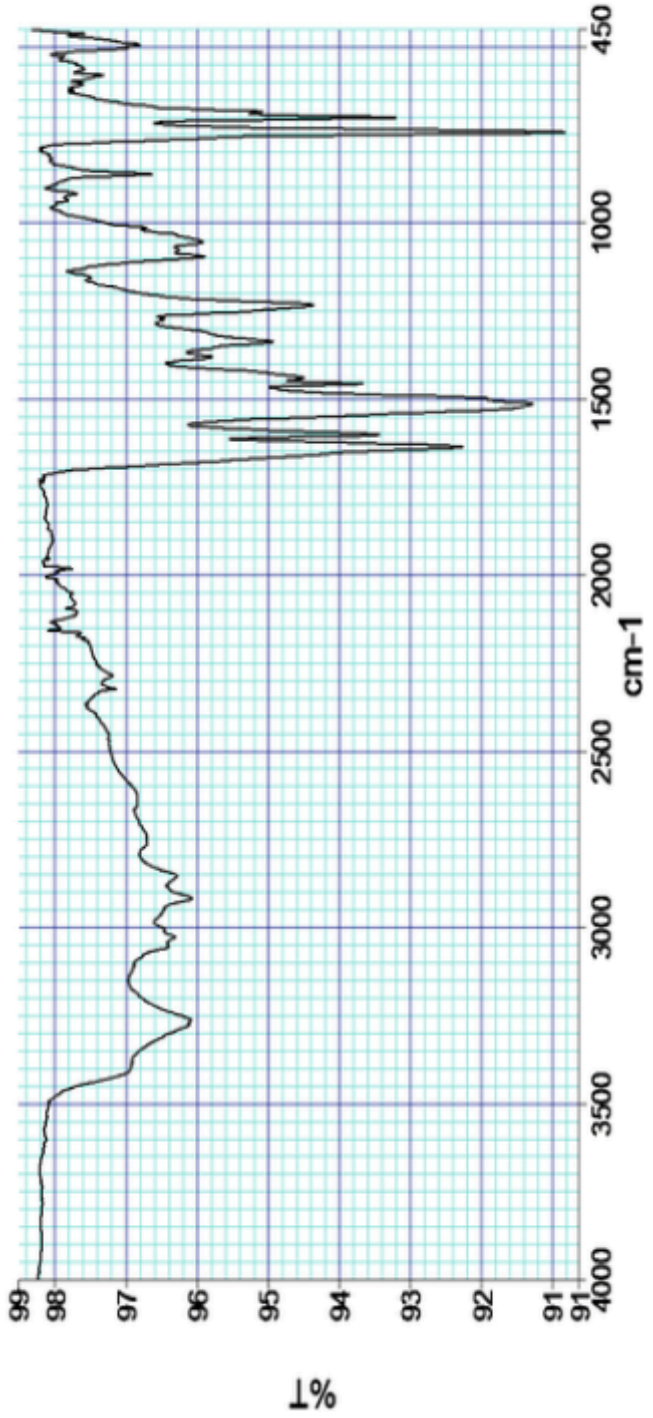
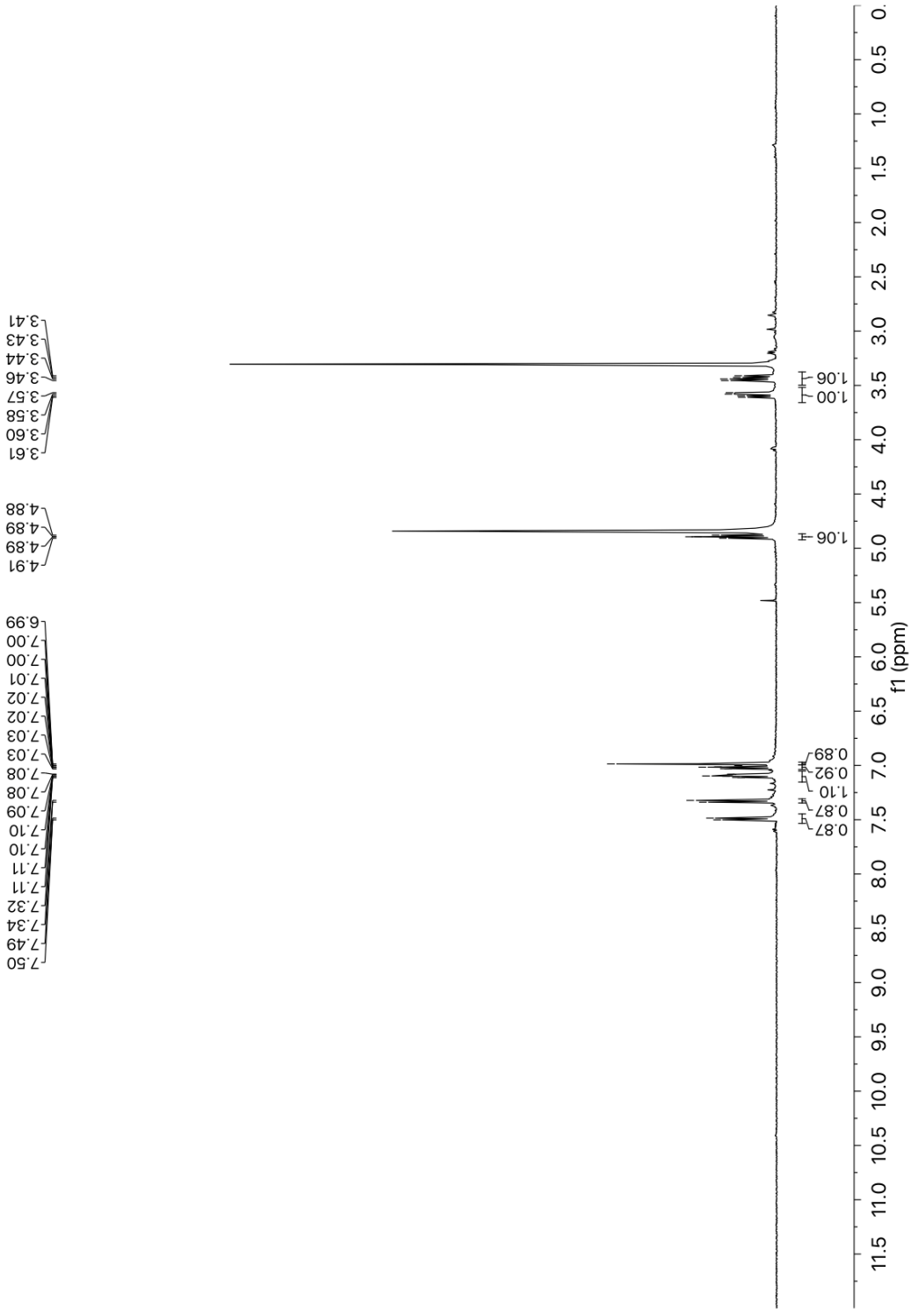
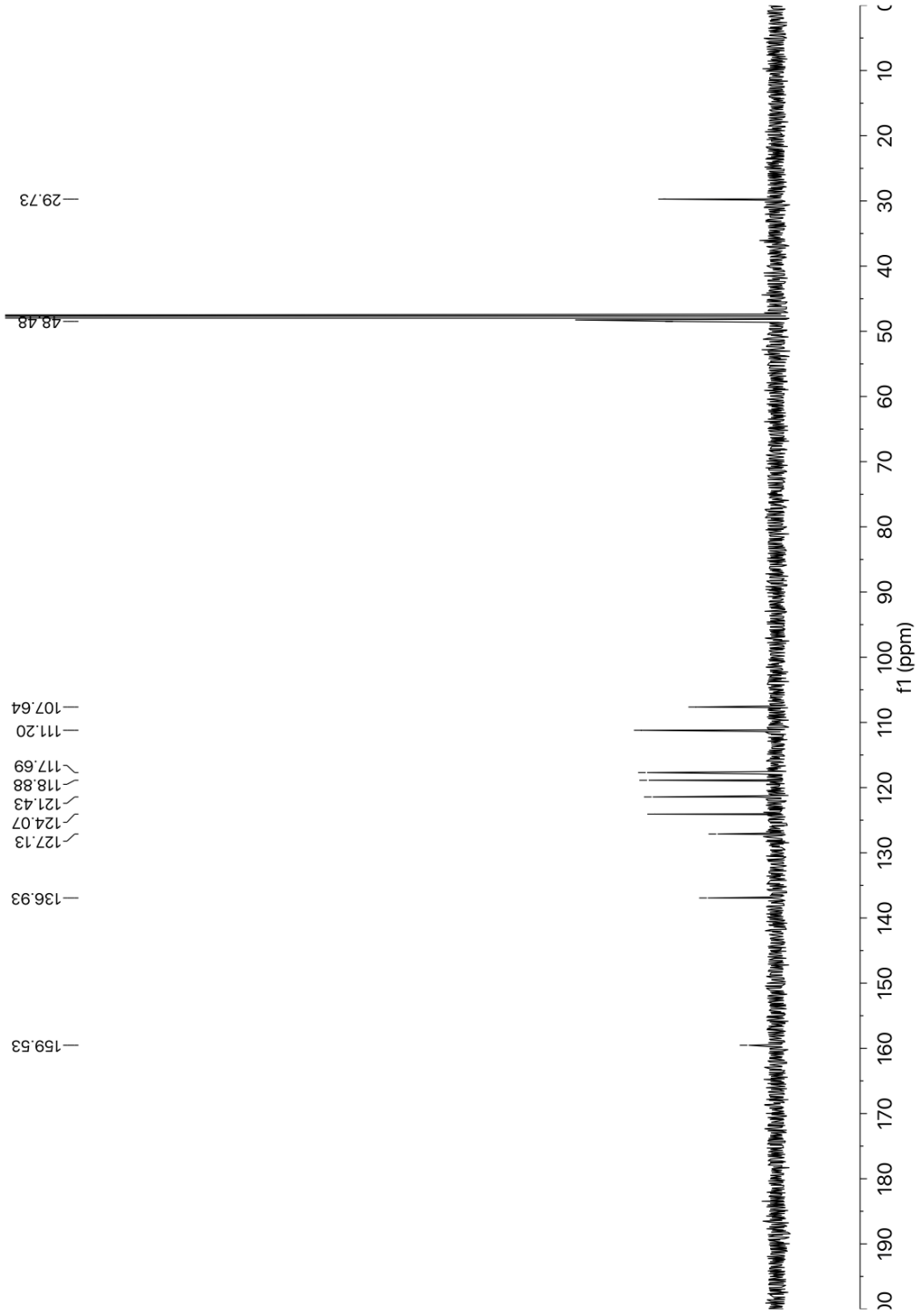


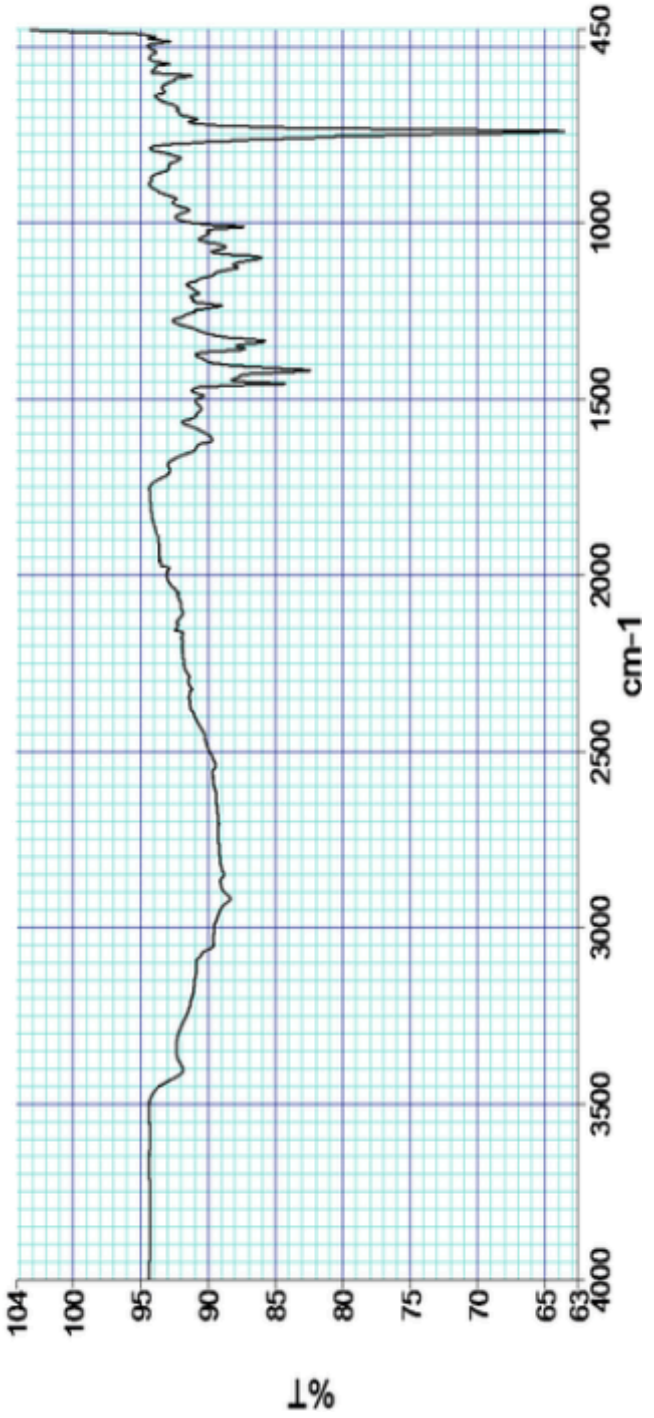
IR spectrum for compound **47**



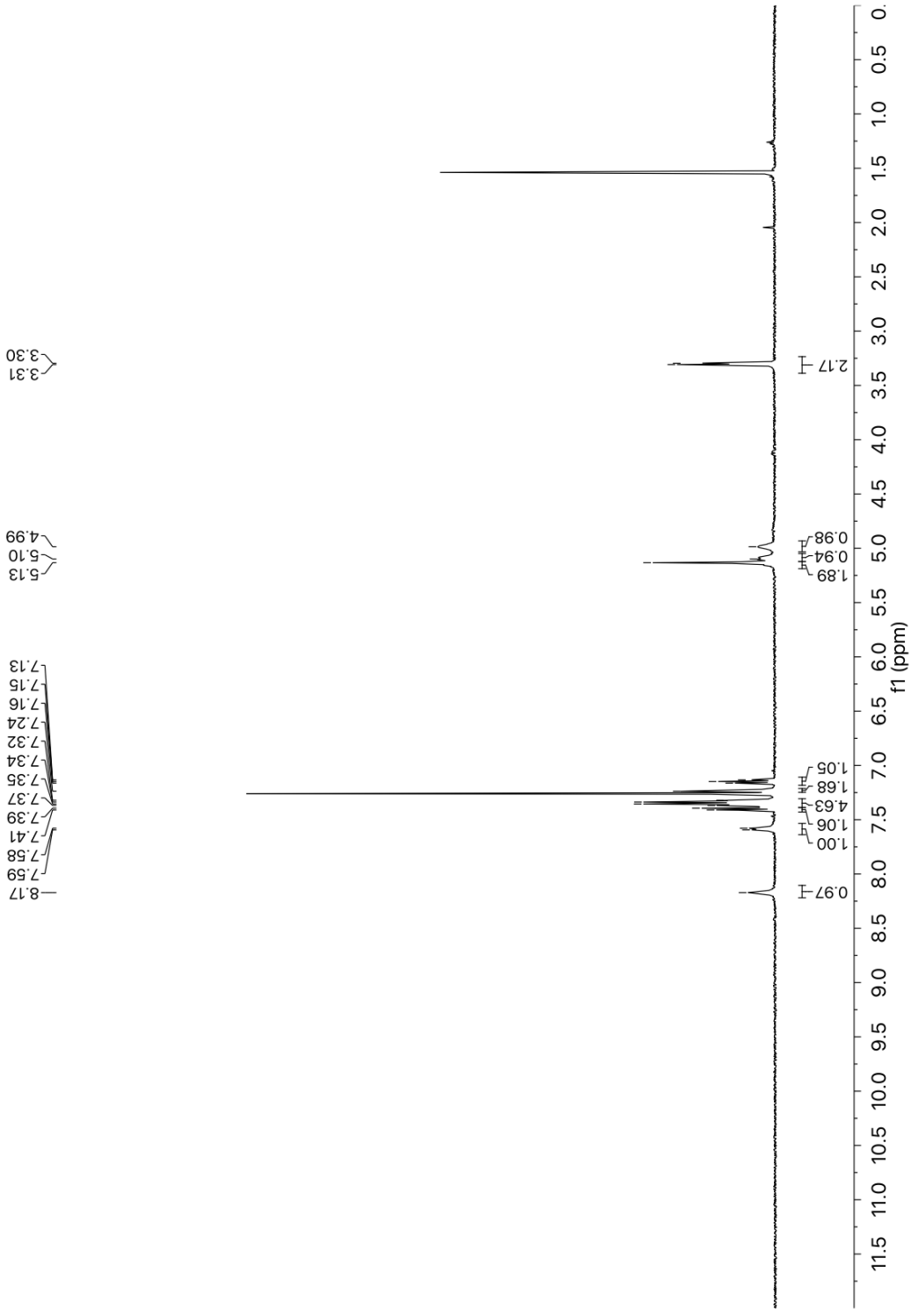
[1]HNMR spectrum for compound **48**



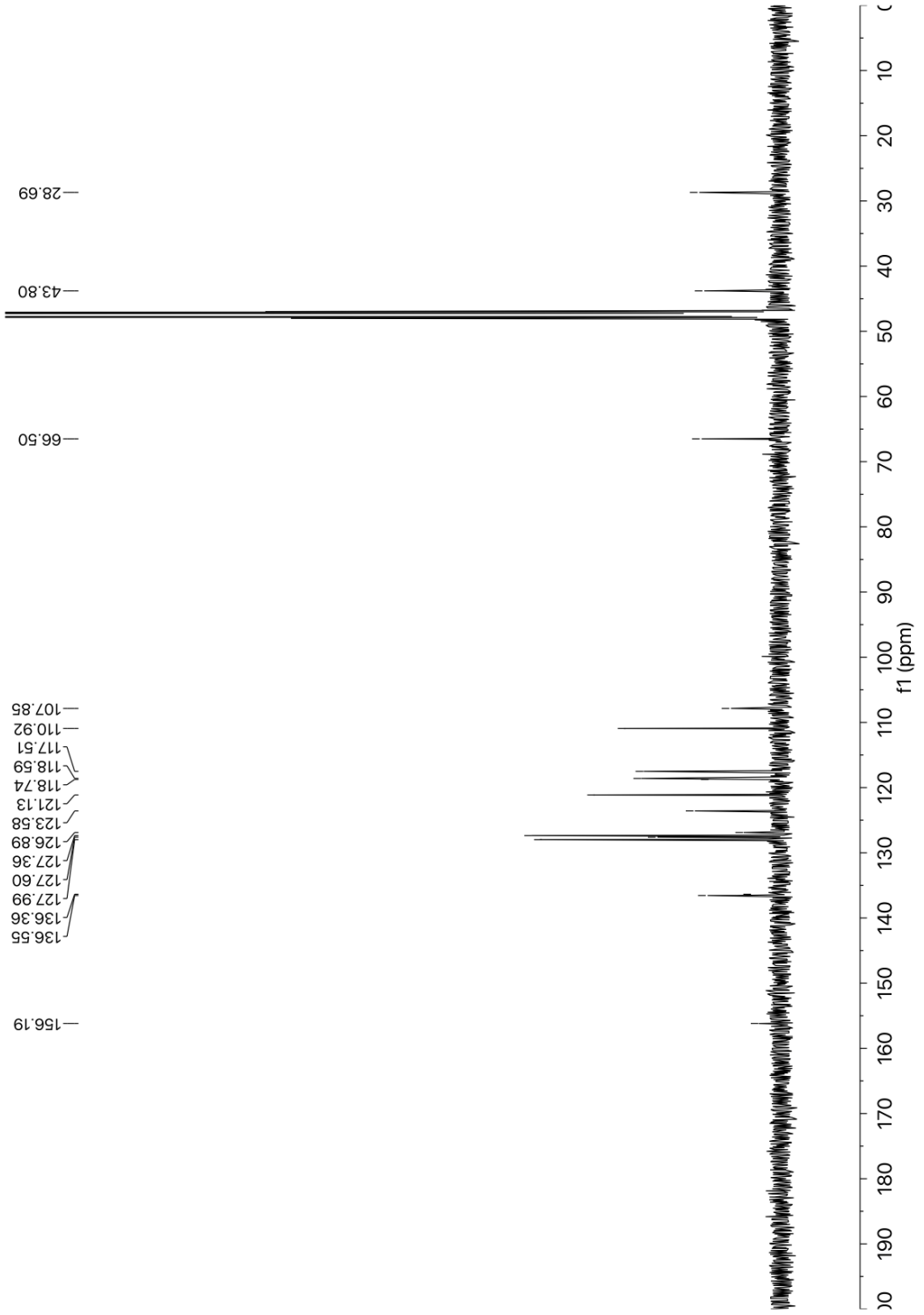
^{13}CNMR spectrum for compound **48**



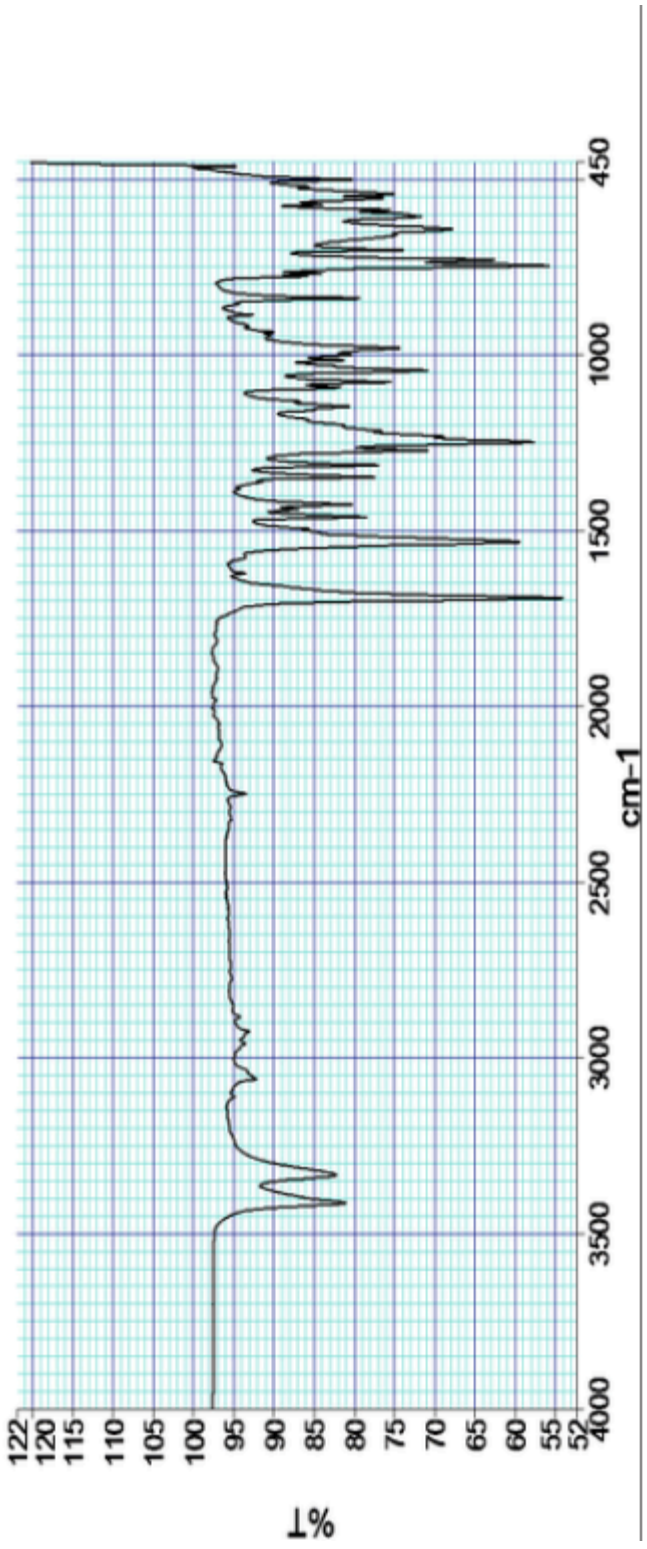
IR spectrum for compound **48**



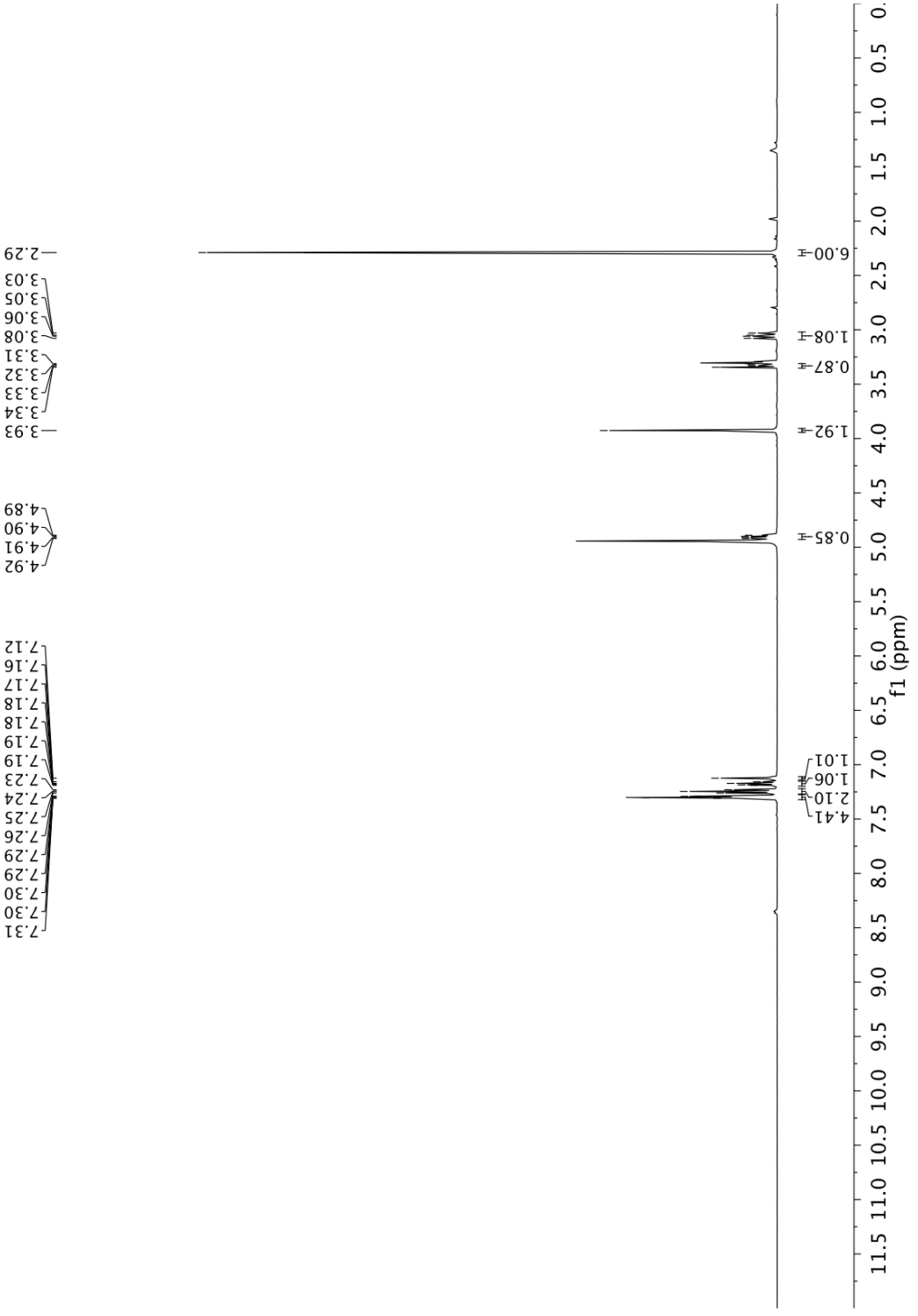
^{1}HNMR spectrum for compound **50**



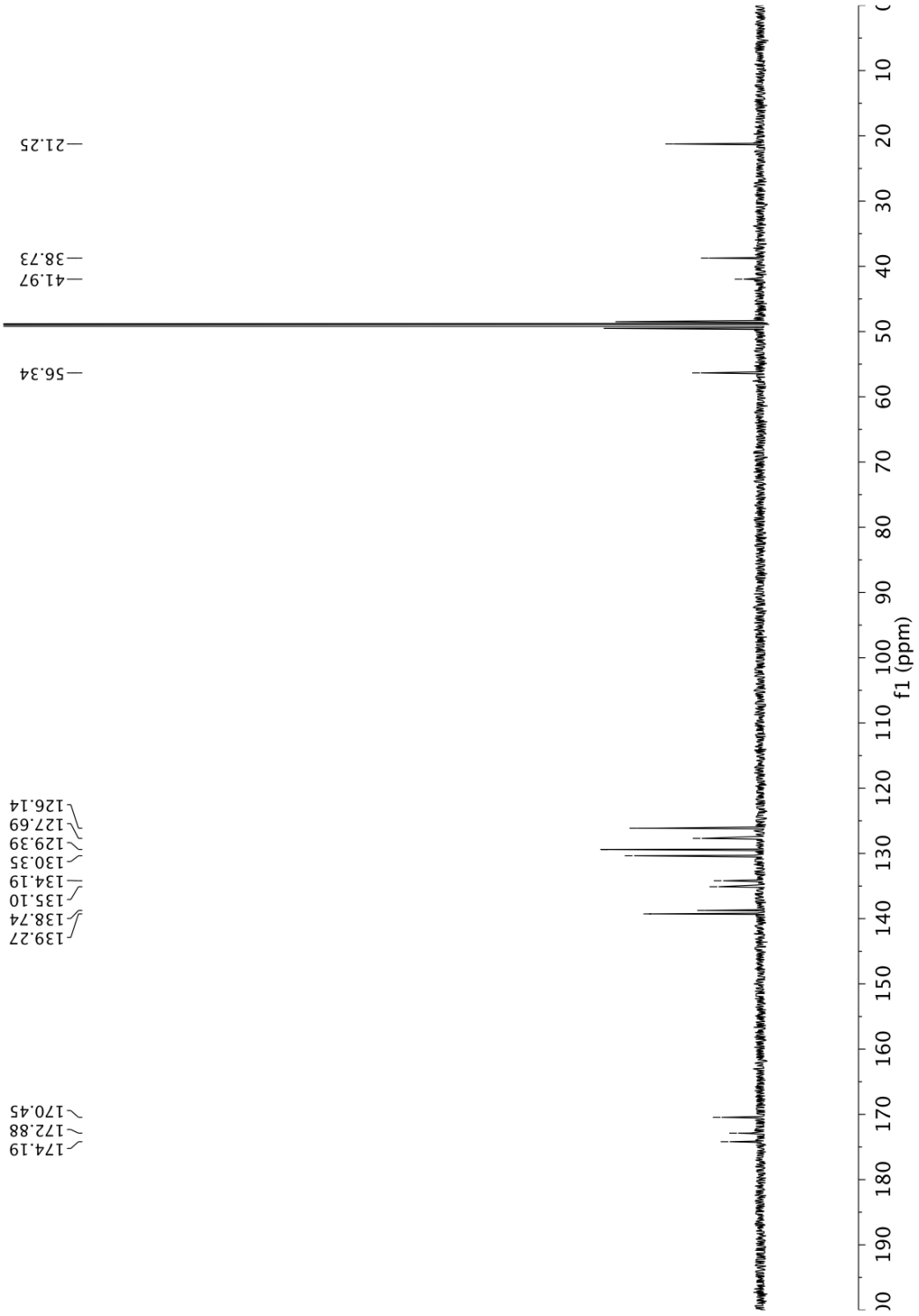
^{13}CNMR spectrum for compound **50**



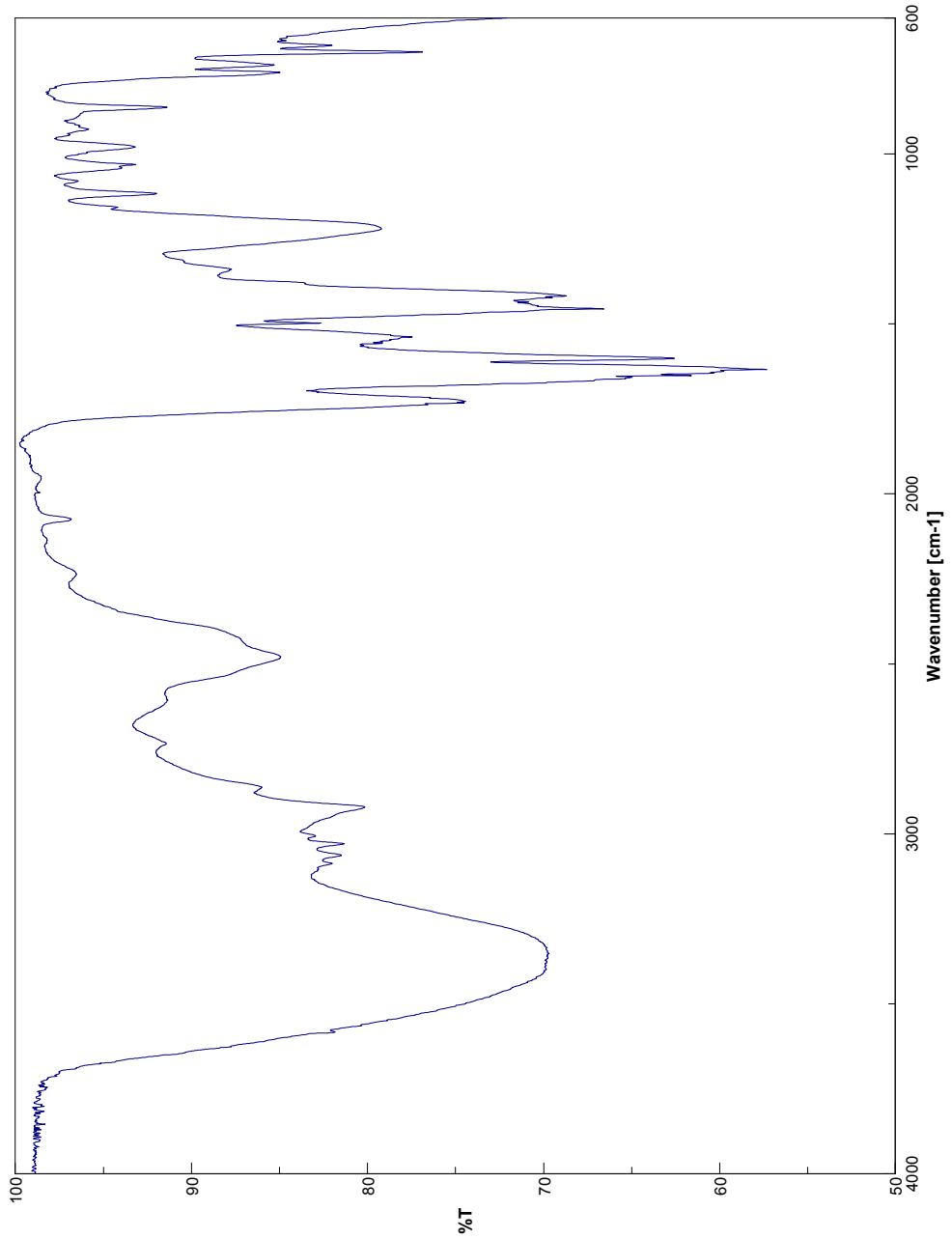
IR spectrum for compound **50**



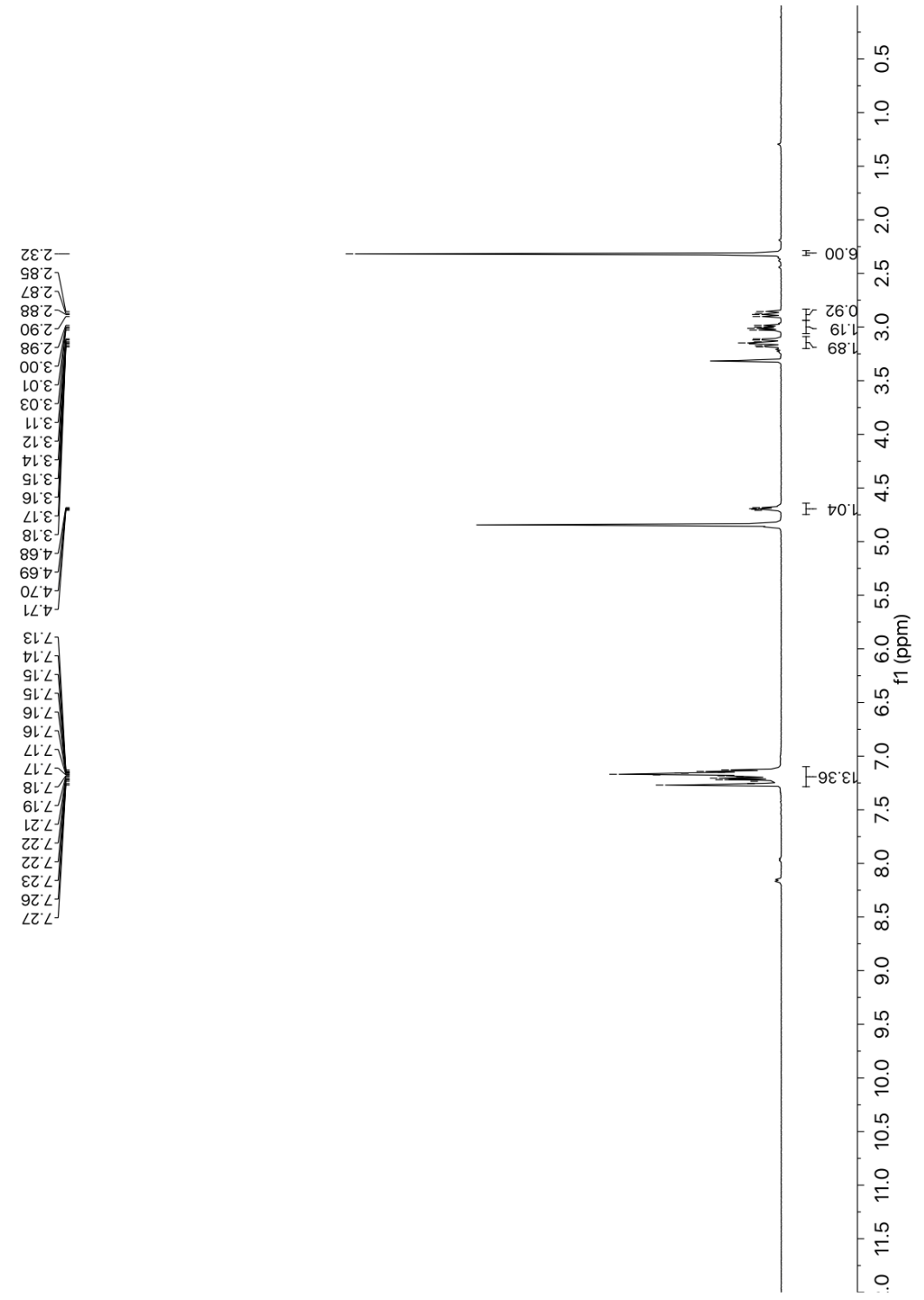
[1]HNMR spectrum for compound **51a**



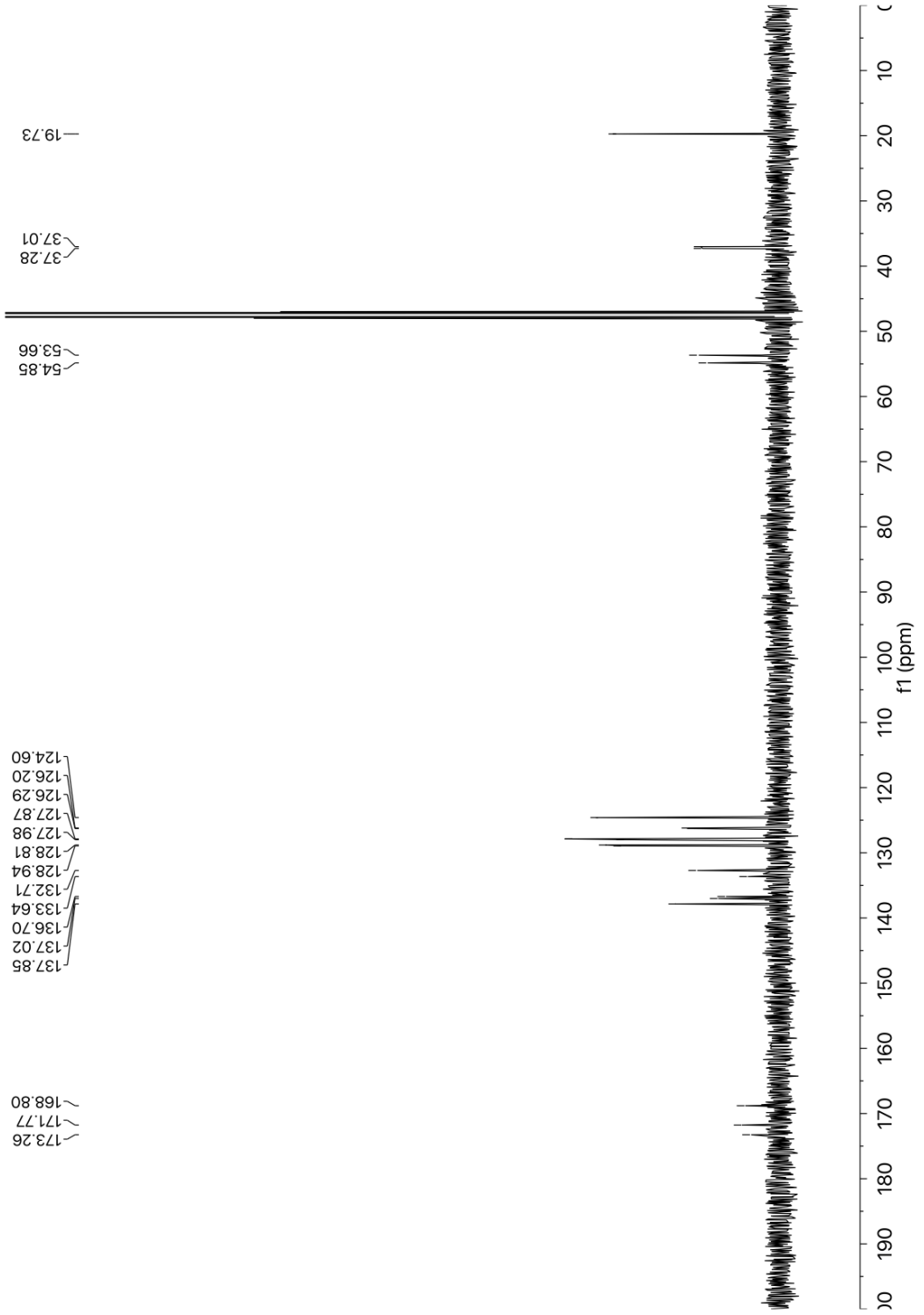
^{13}CNMR spectrum for compound **51a**



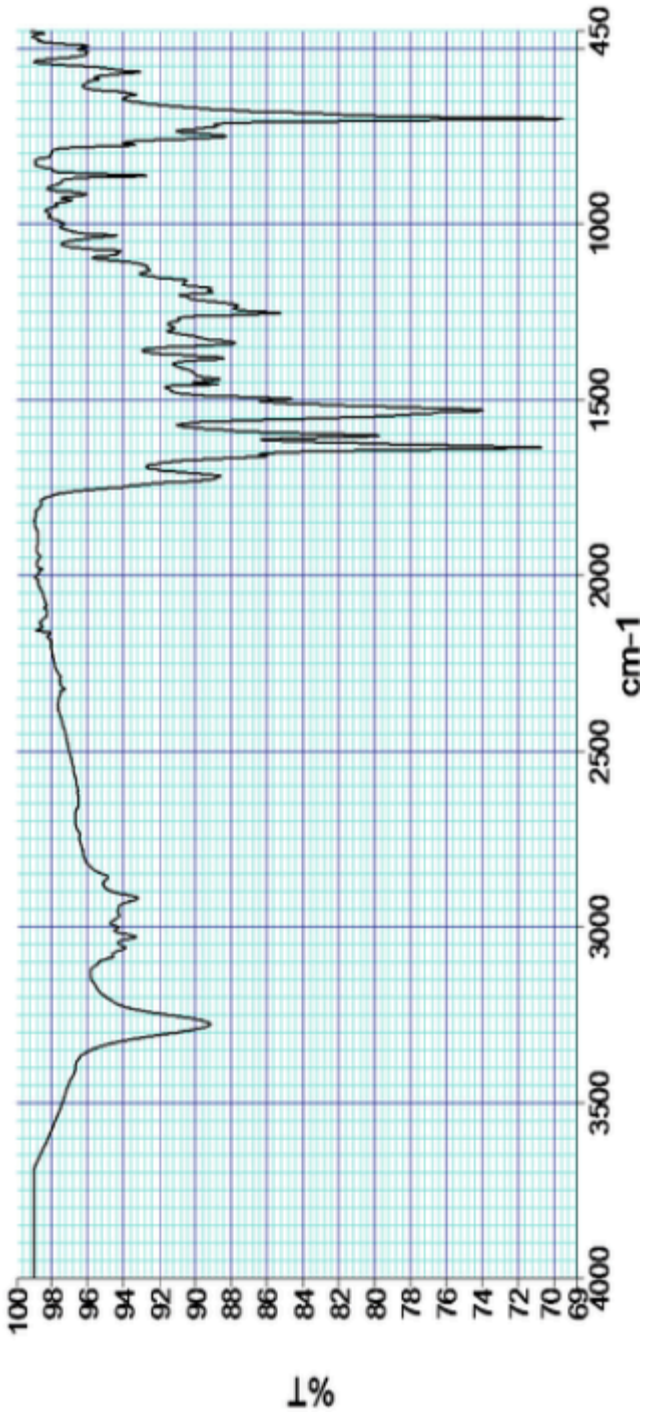
IR spectrum for compound **51a**



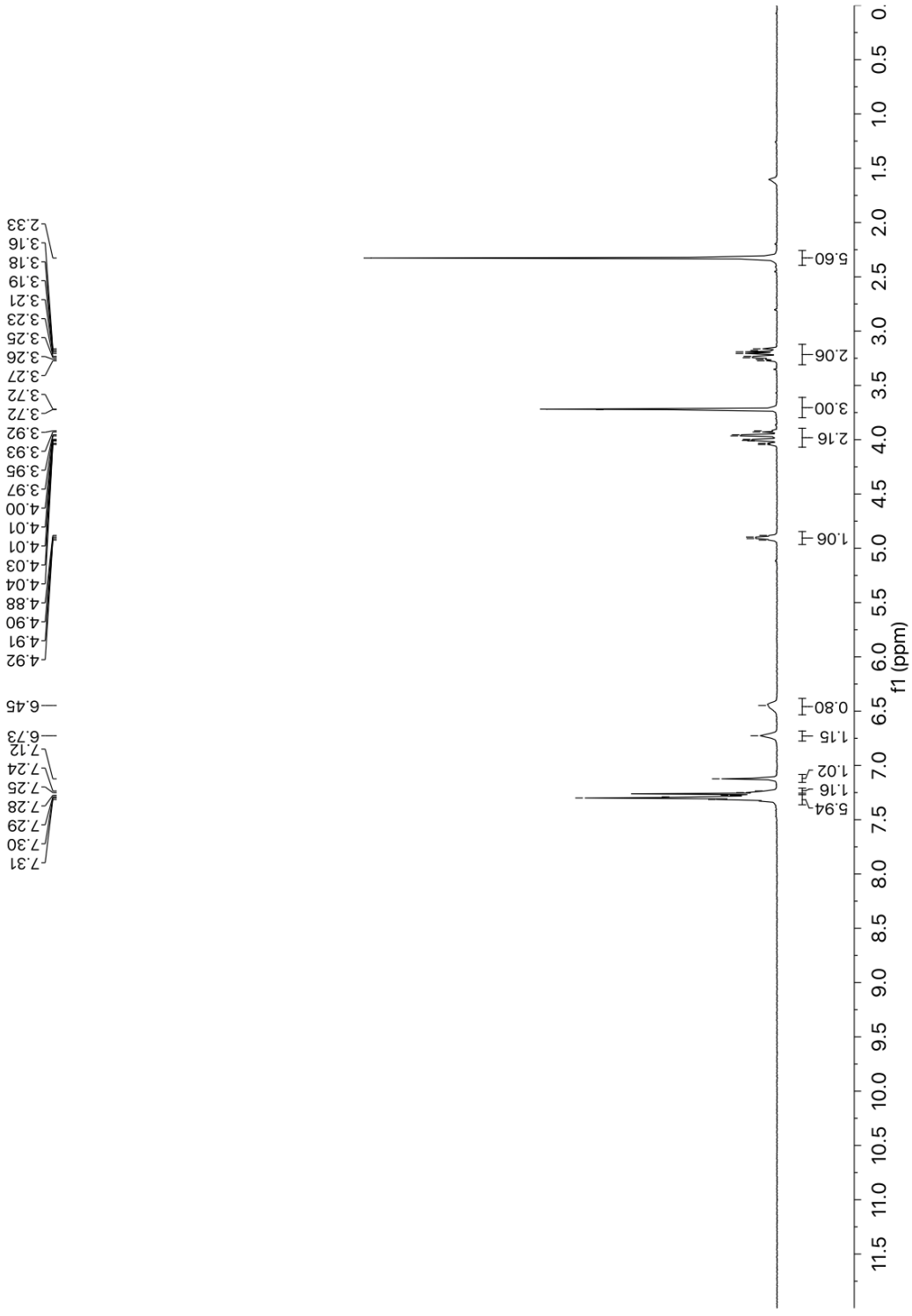
[1]HNMR spectrum for compound **51b**



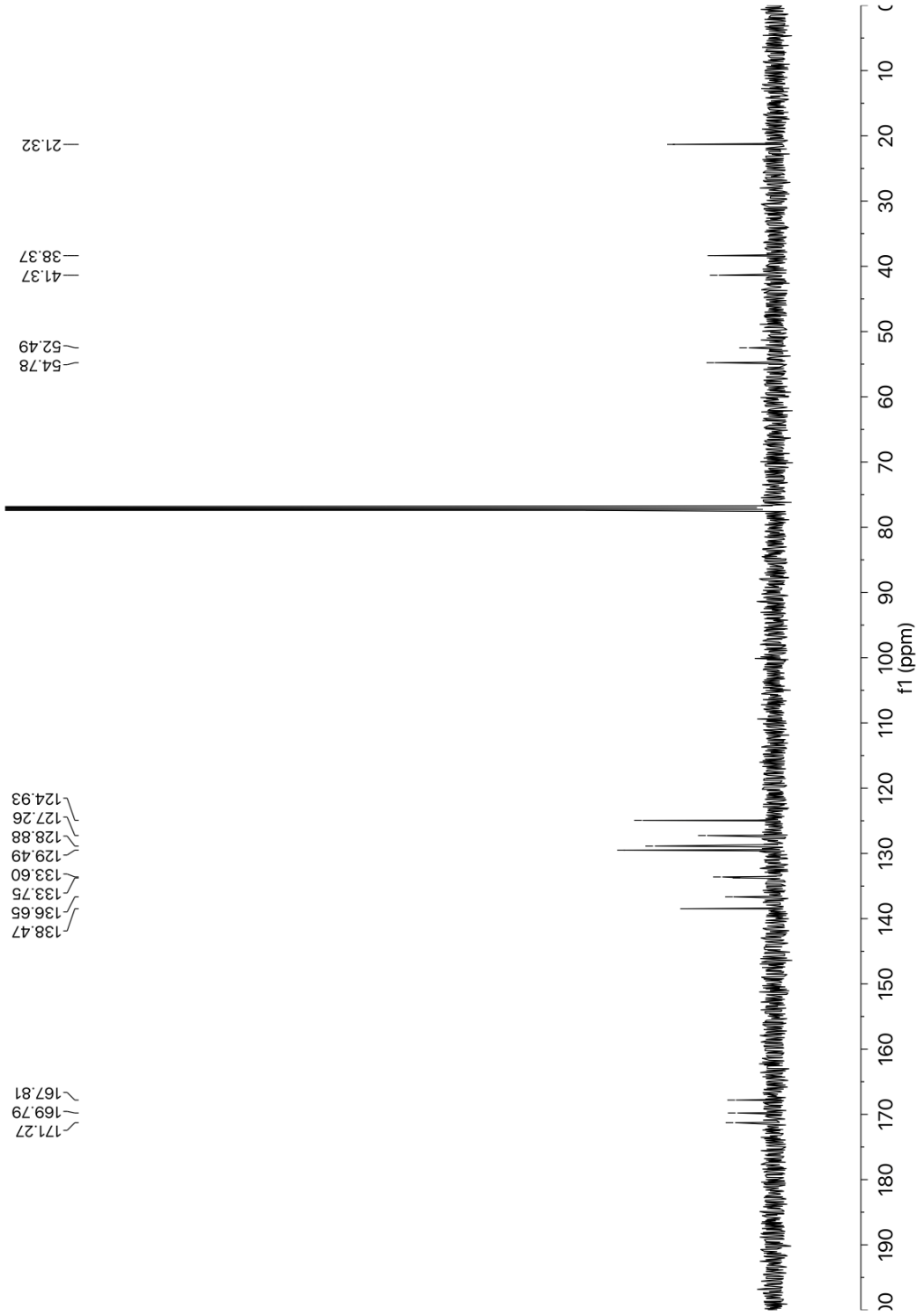
^{13}CNMR spectrum for compound **51b**



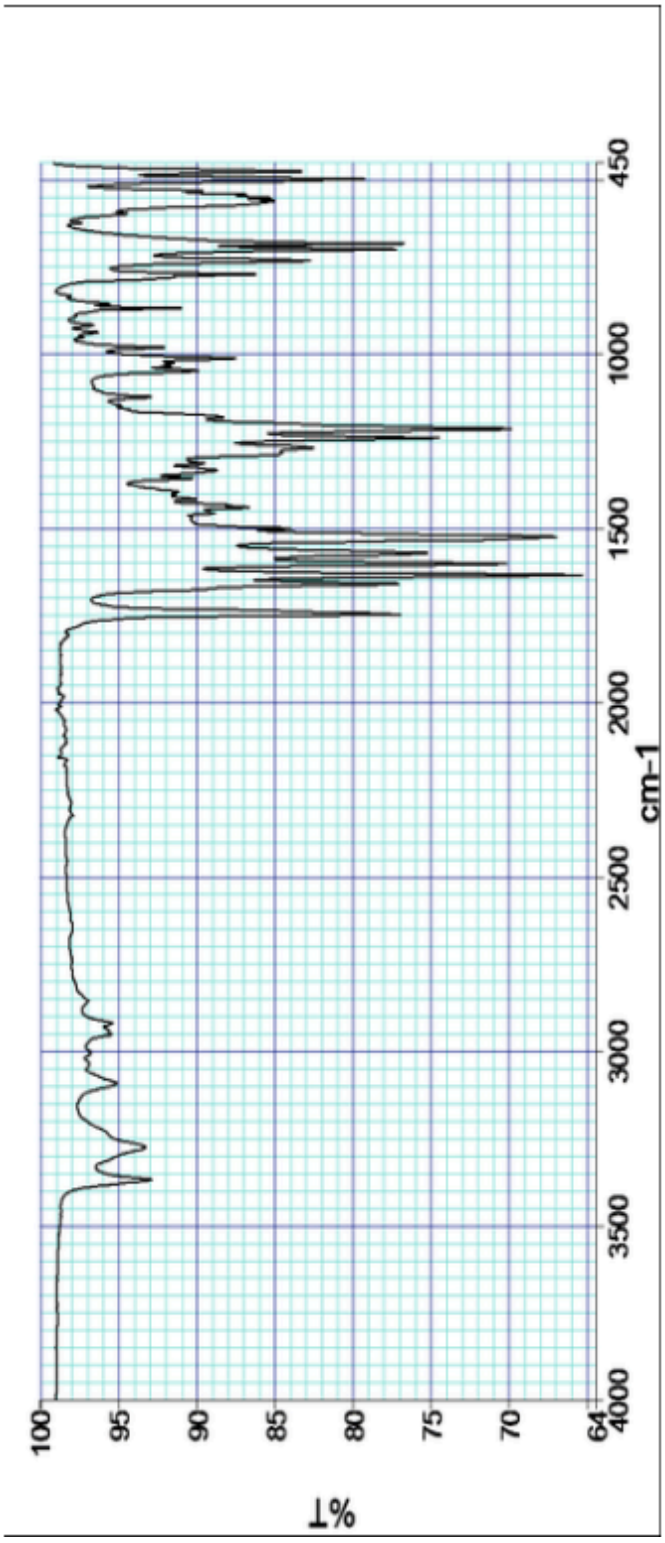
IR spectrum for compound **51b**



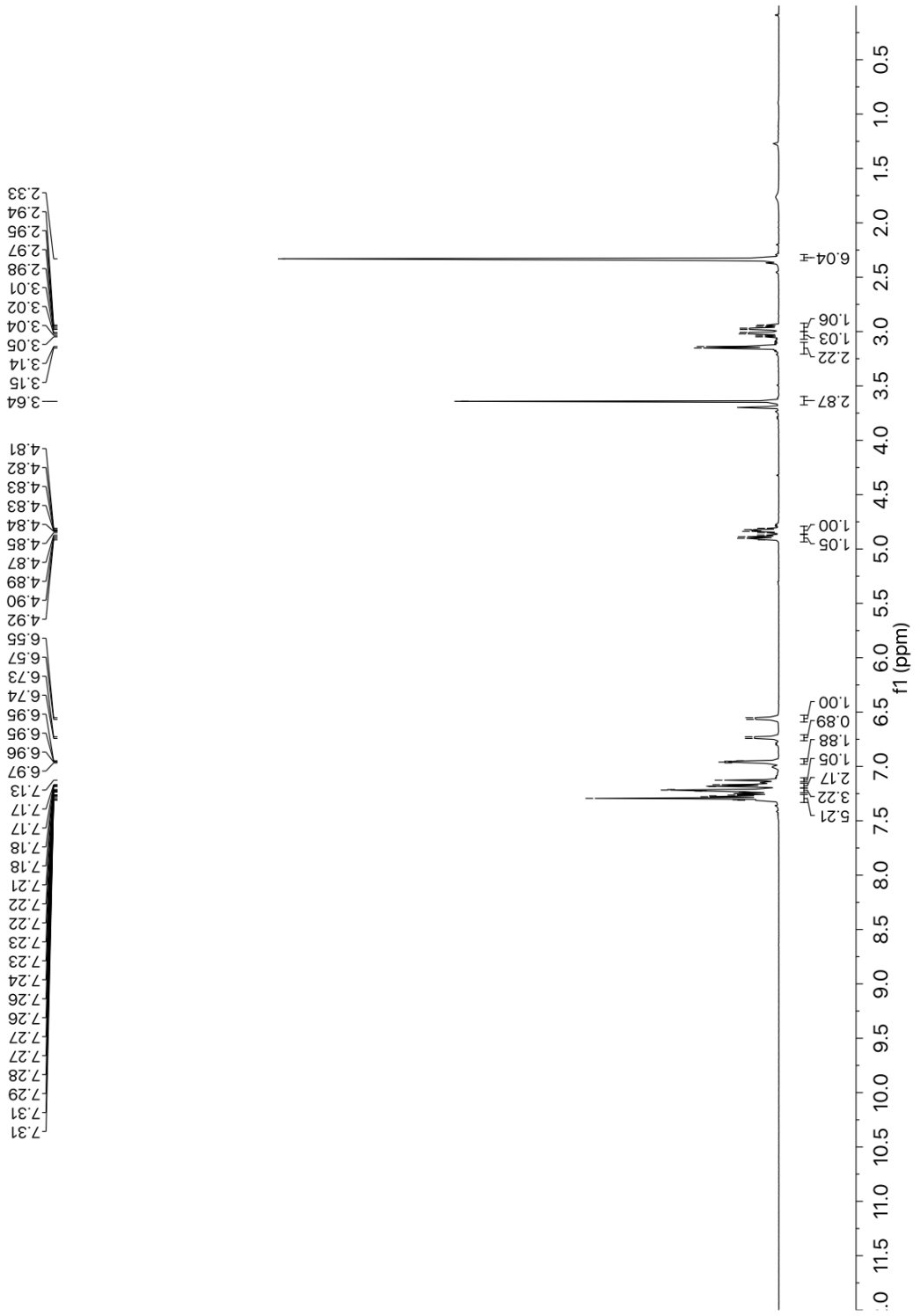
[1]HNMR spectrum for compound **53a**



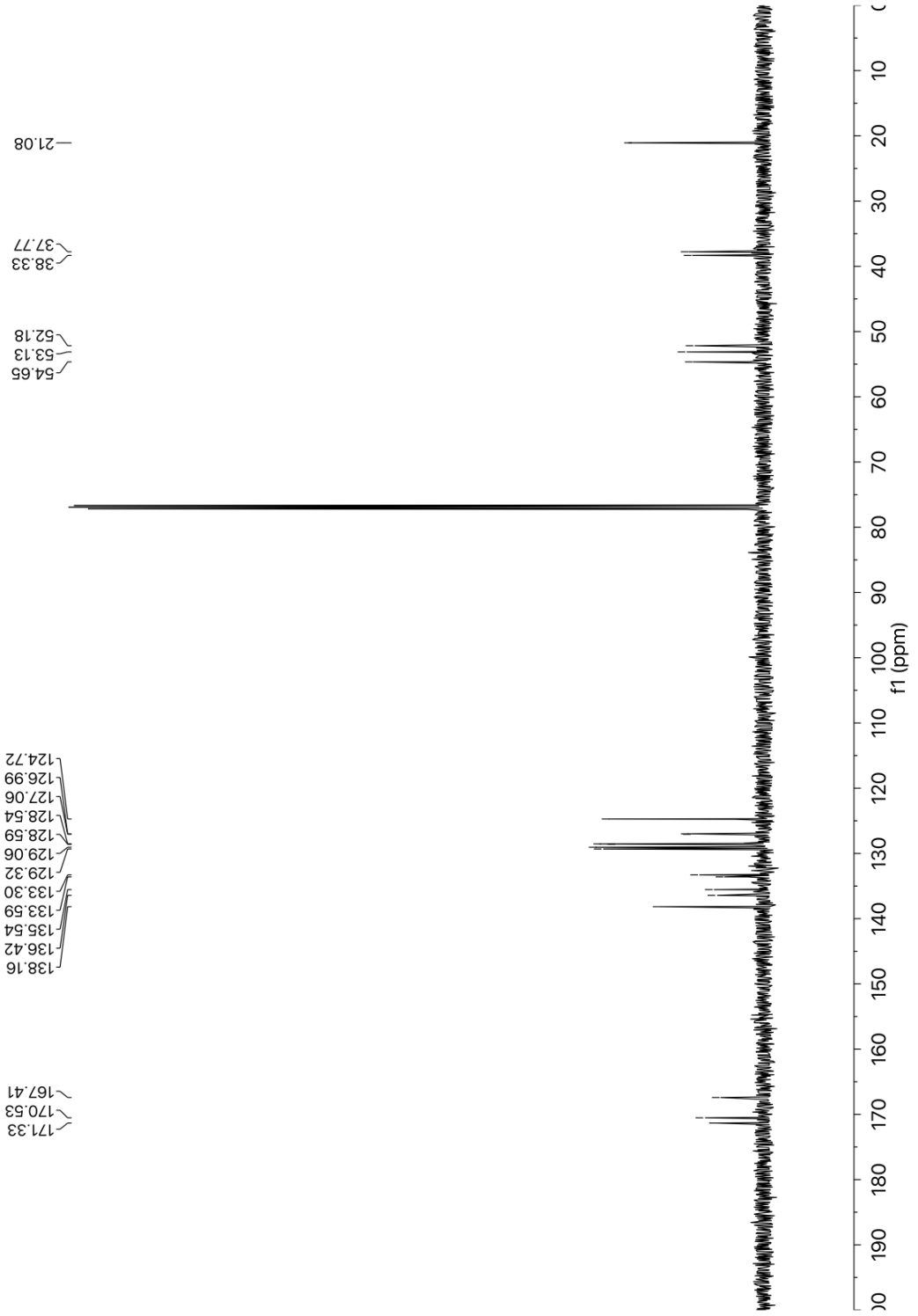
^{13}CNMR spectrum for compound **53a**



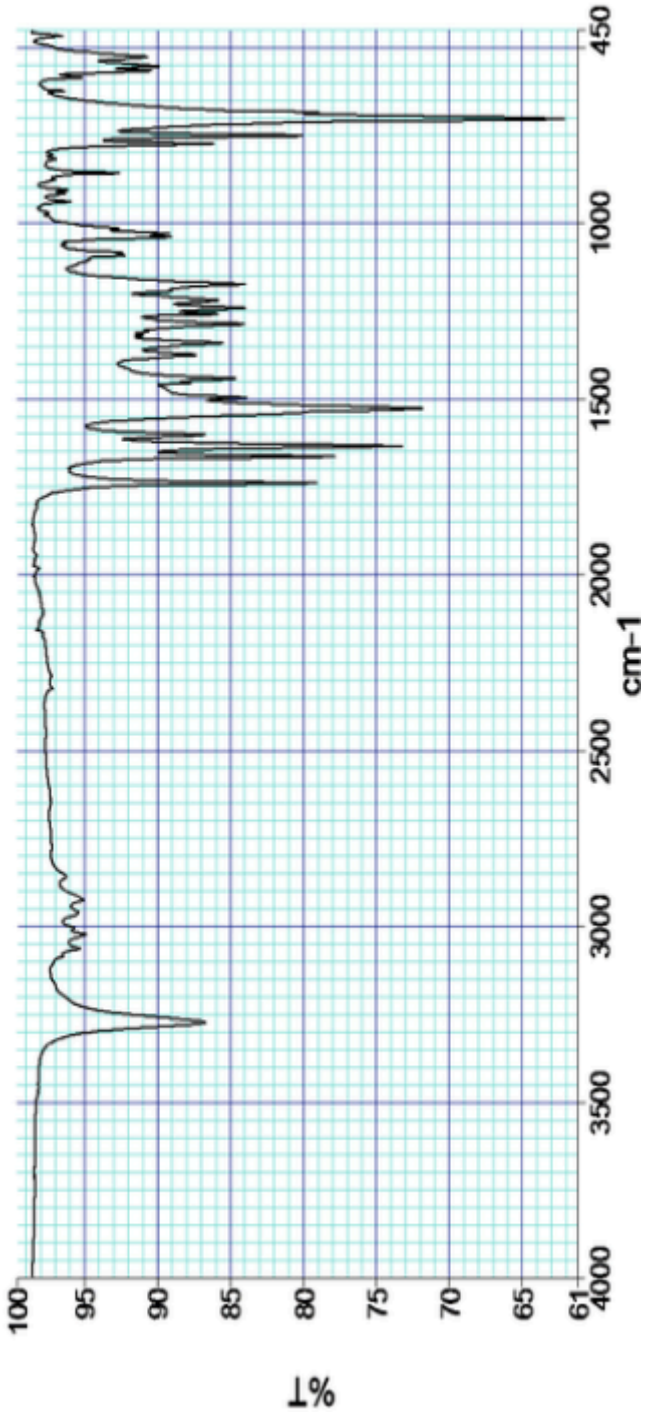
IR spectrum for compound **53a**



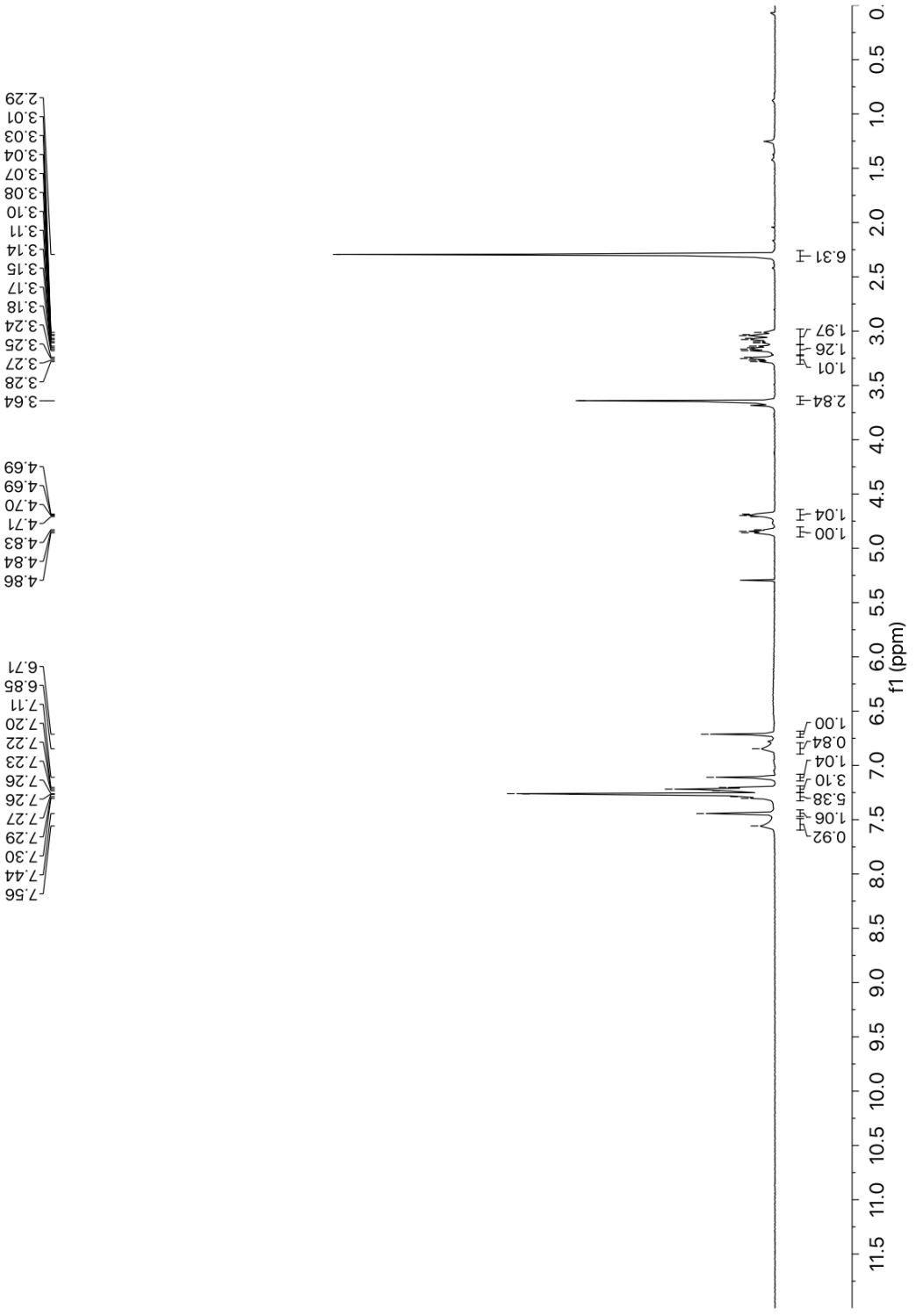
[1]HNMR spectrum for compound **53b**



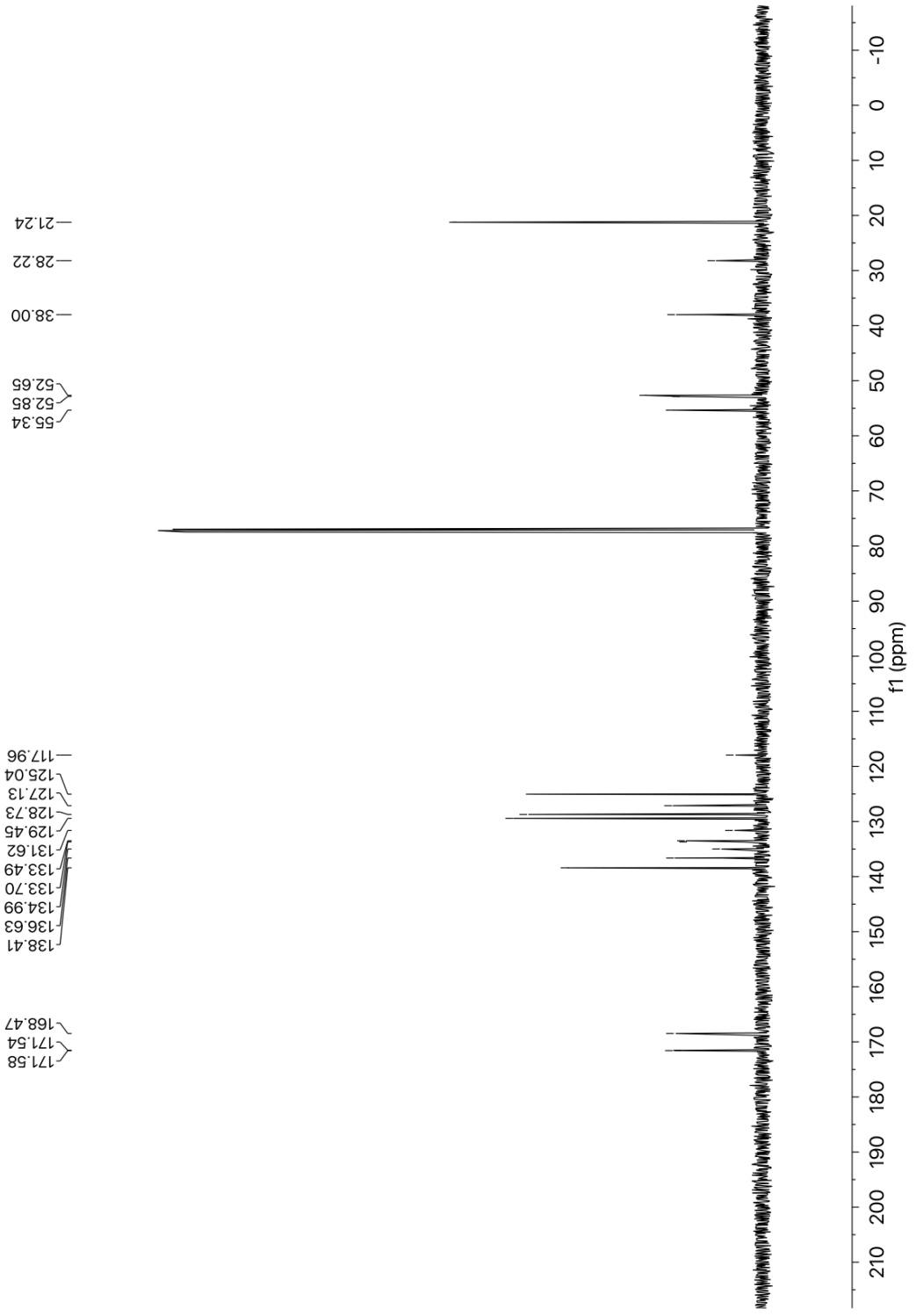
^{13}CNMR spectrum for compound **53b**



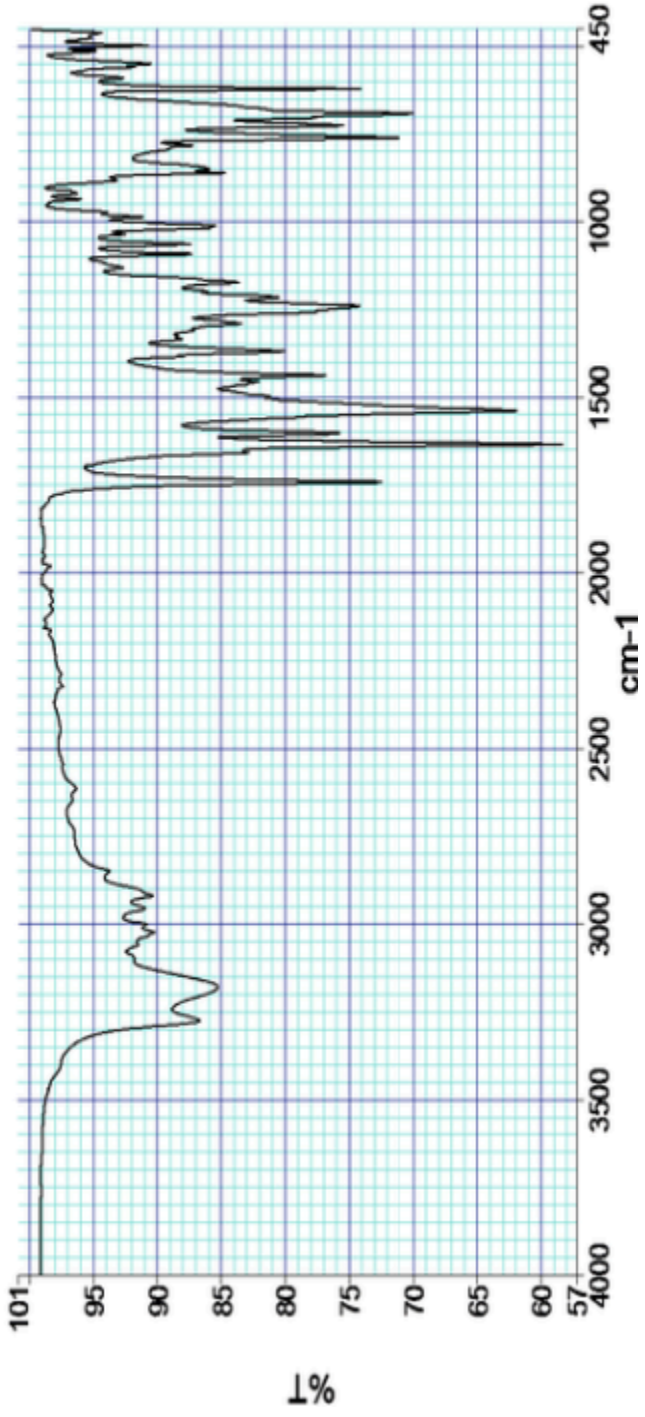
IR spectrum for compound **53b**



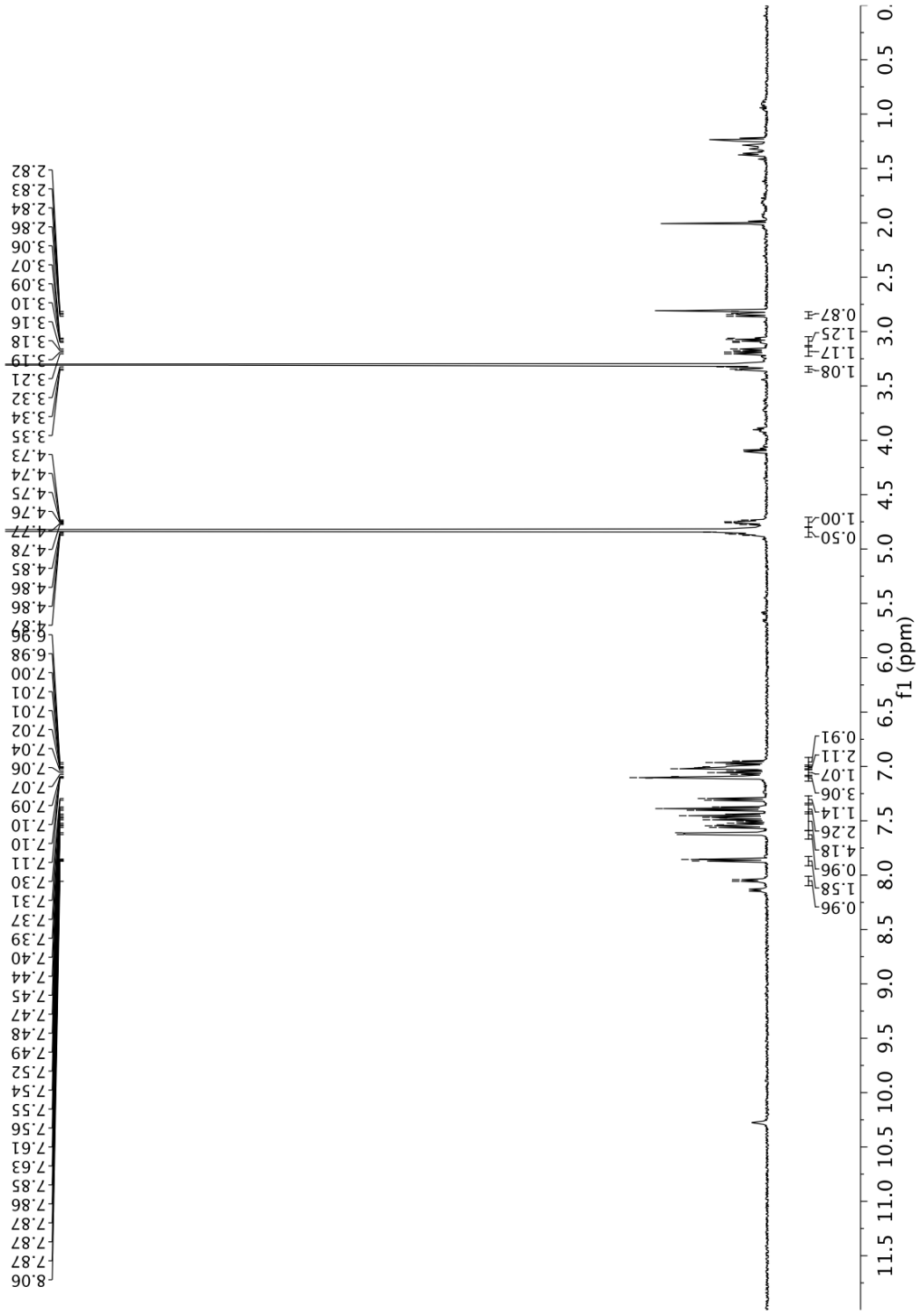
[1]HNMR spectrum for compound **53c**



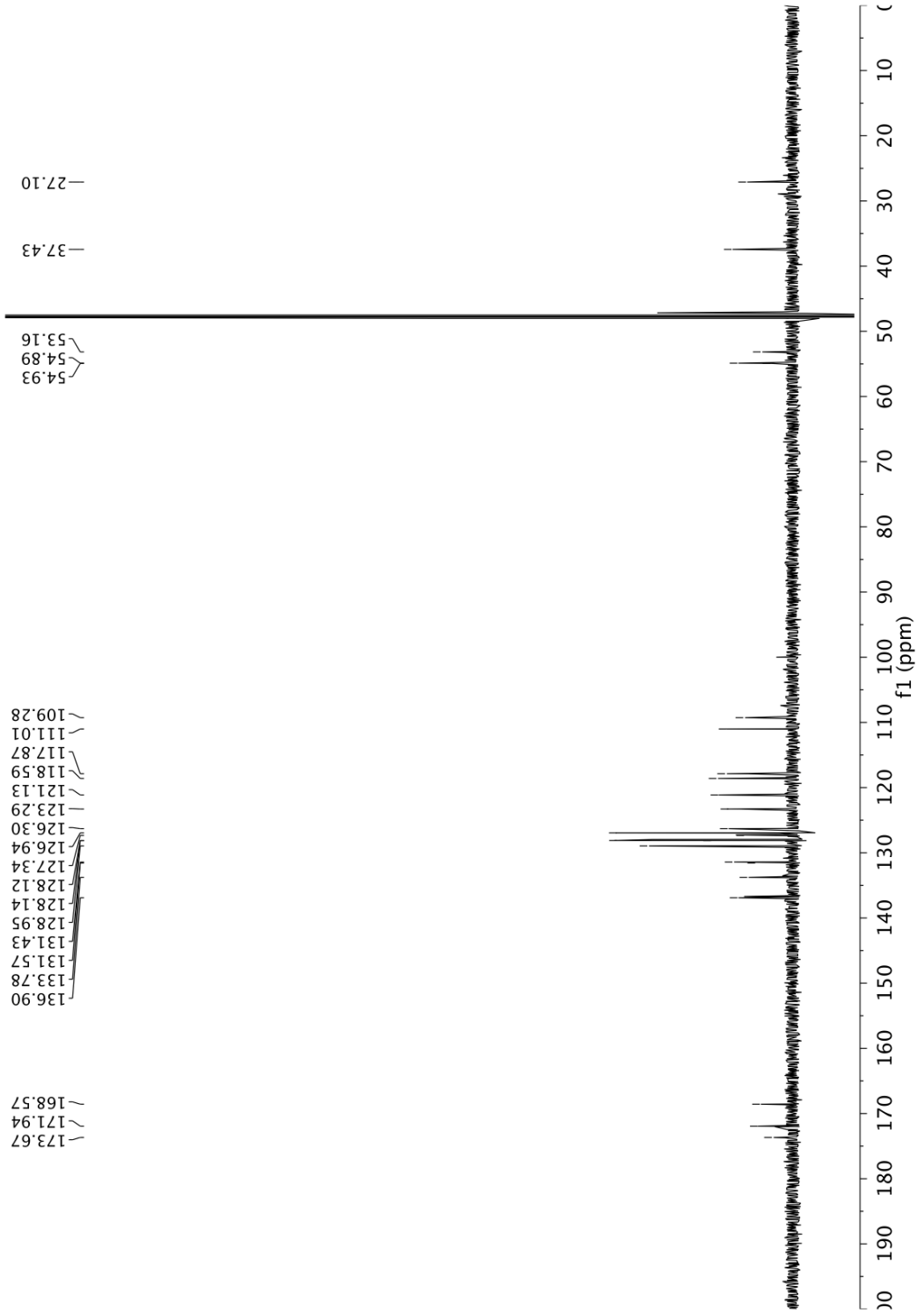
^{13}CNMR spectrum for compound **53c**



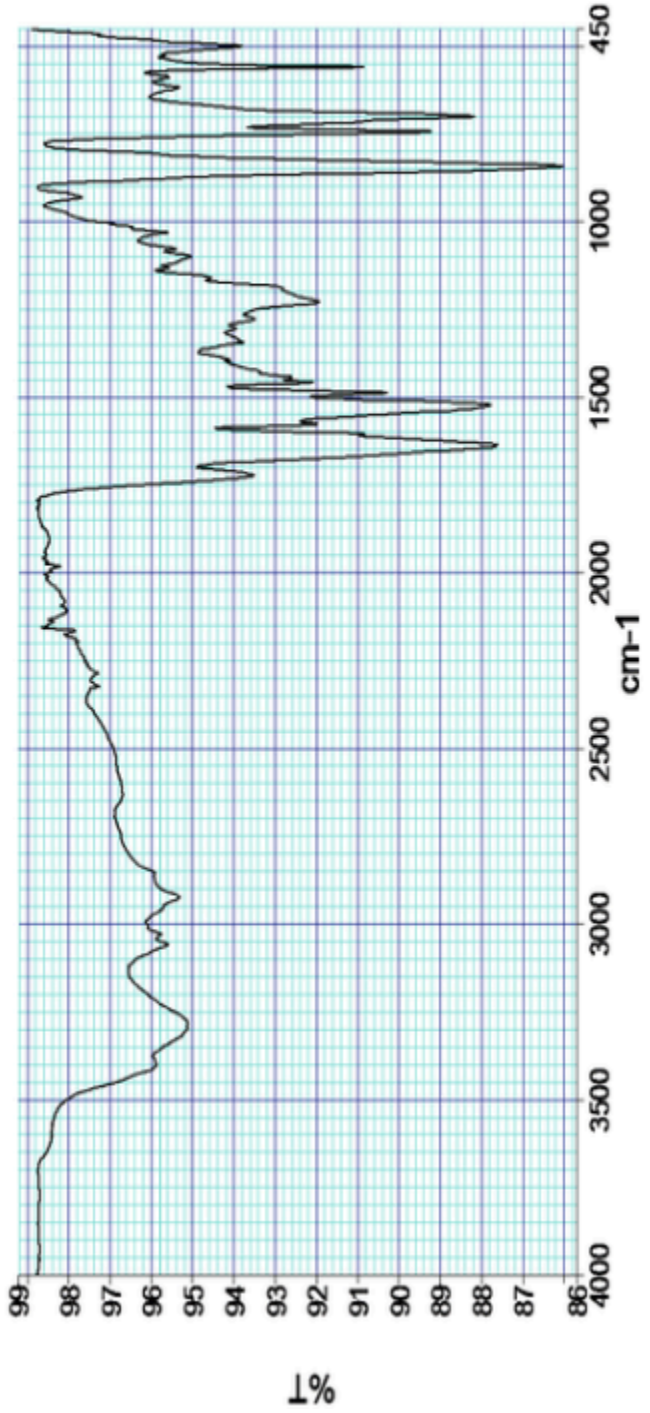
IR spectrum for compound **53c**



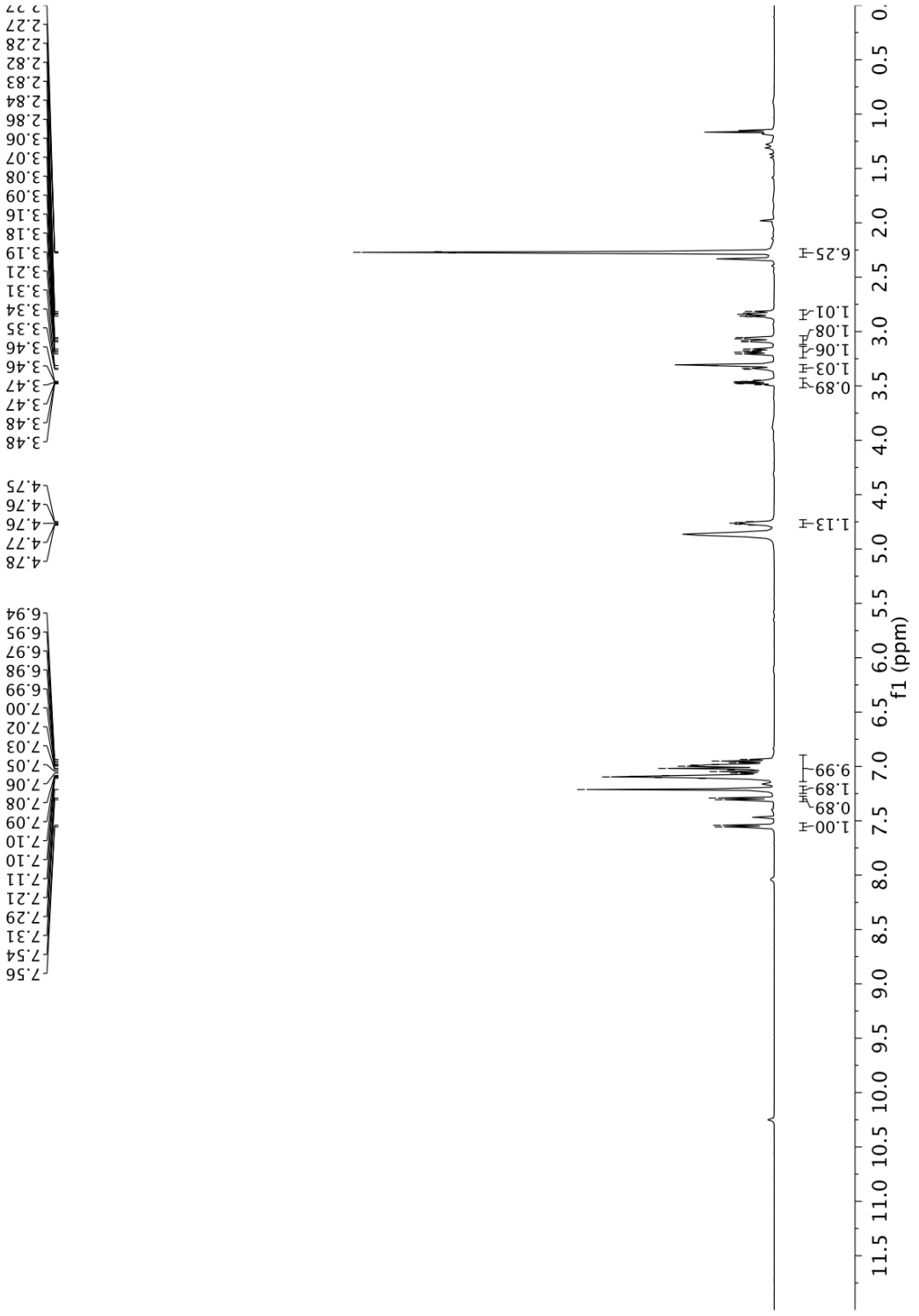
^{1}HNMR spectrum for compound **54a**



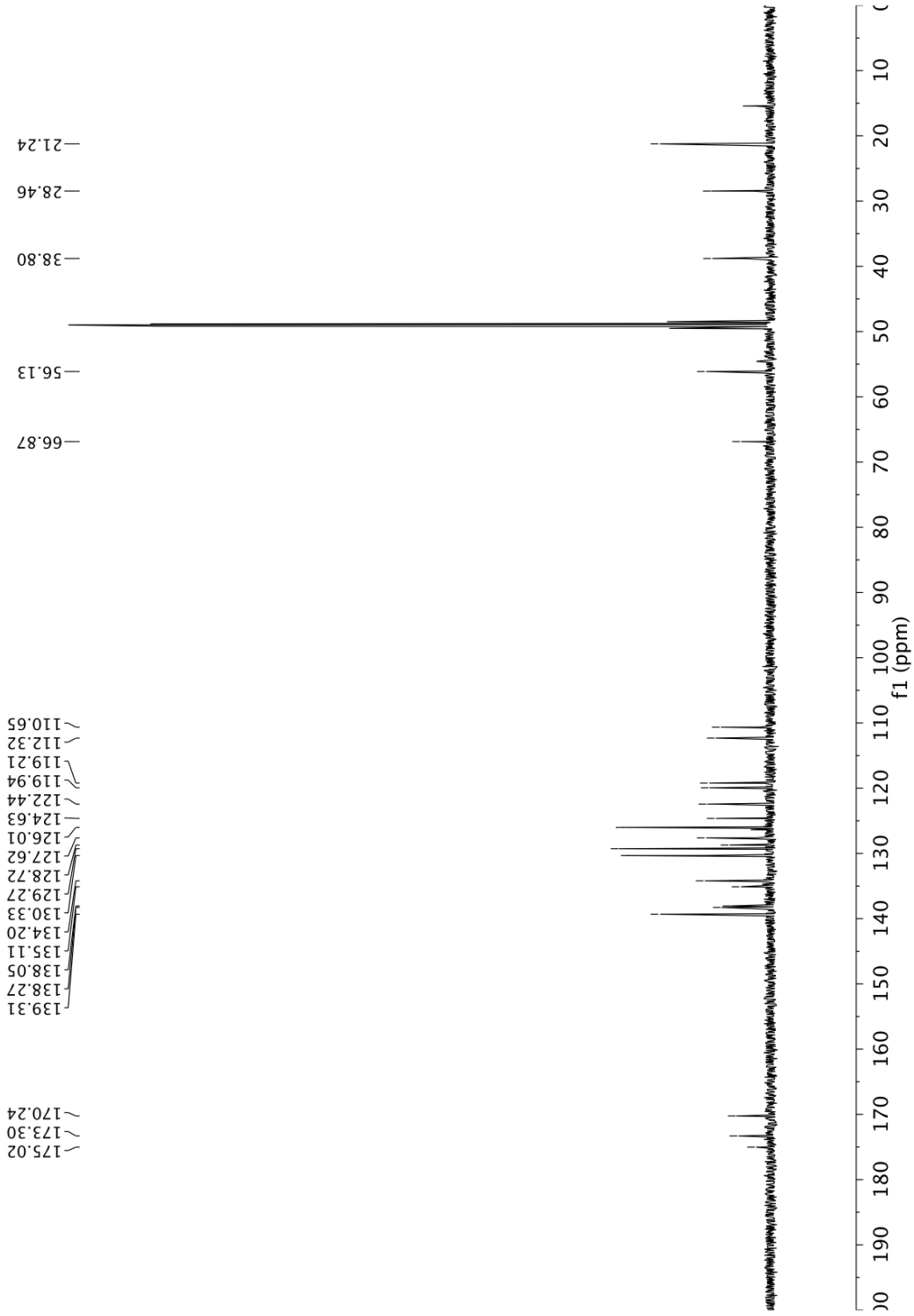
^{13}CNMR spectrum for compound **54a**



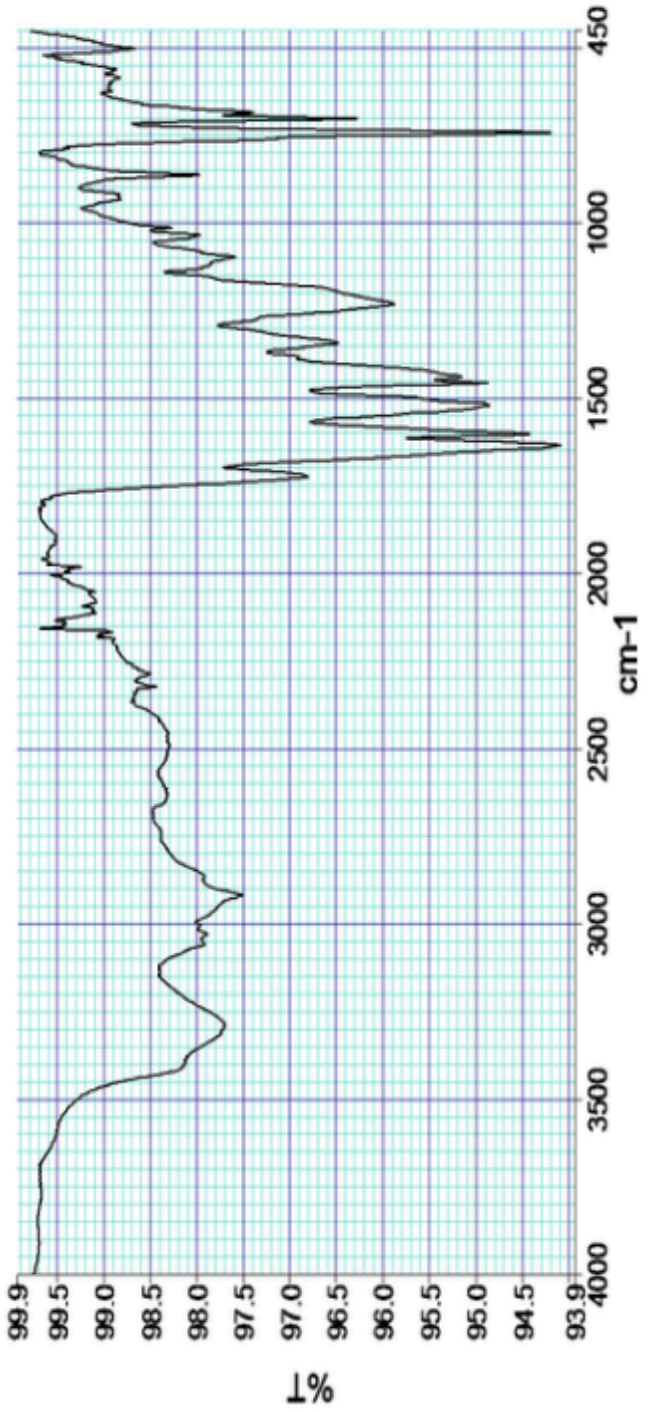
IR spectrum for compound **54a**



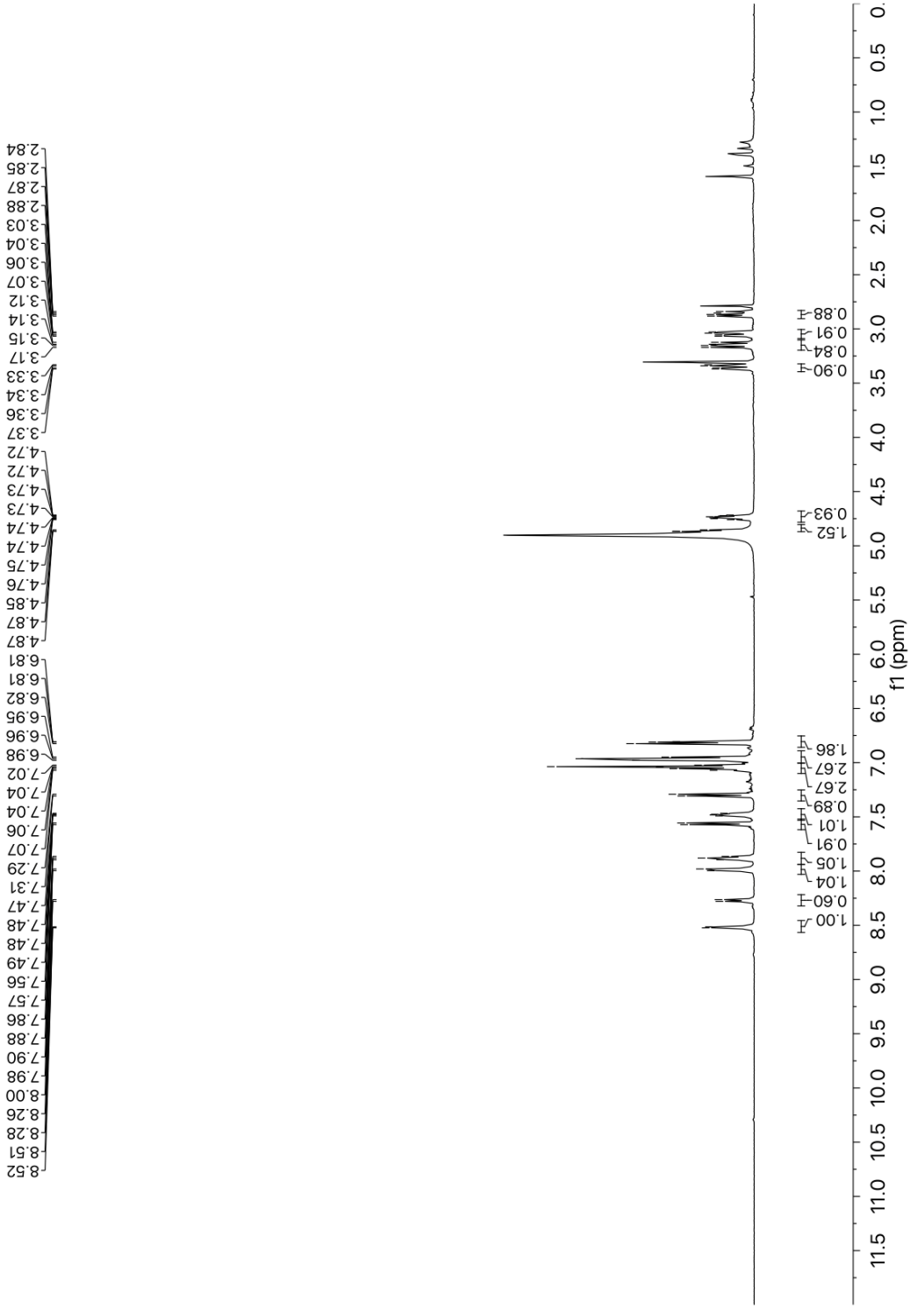
[1]HNMR spectrum for compound **54b**



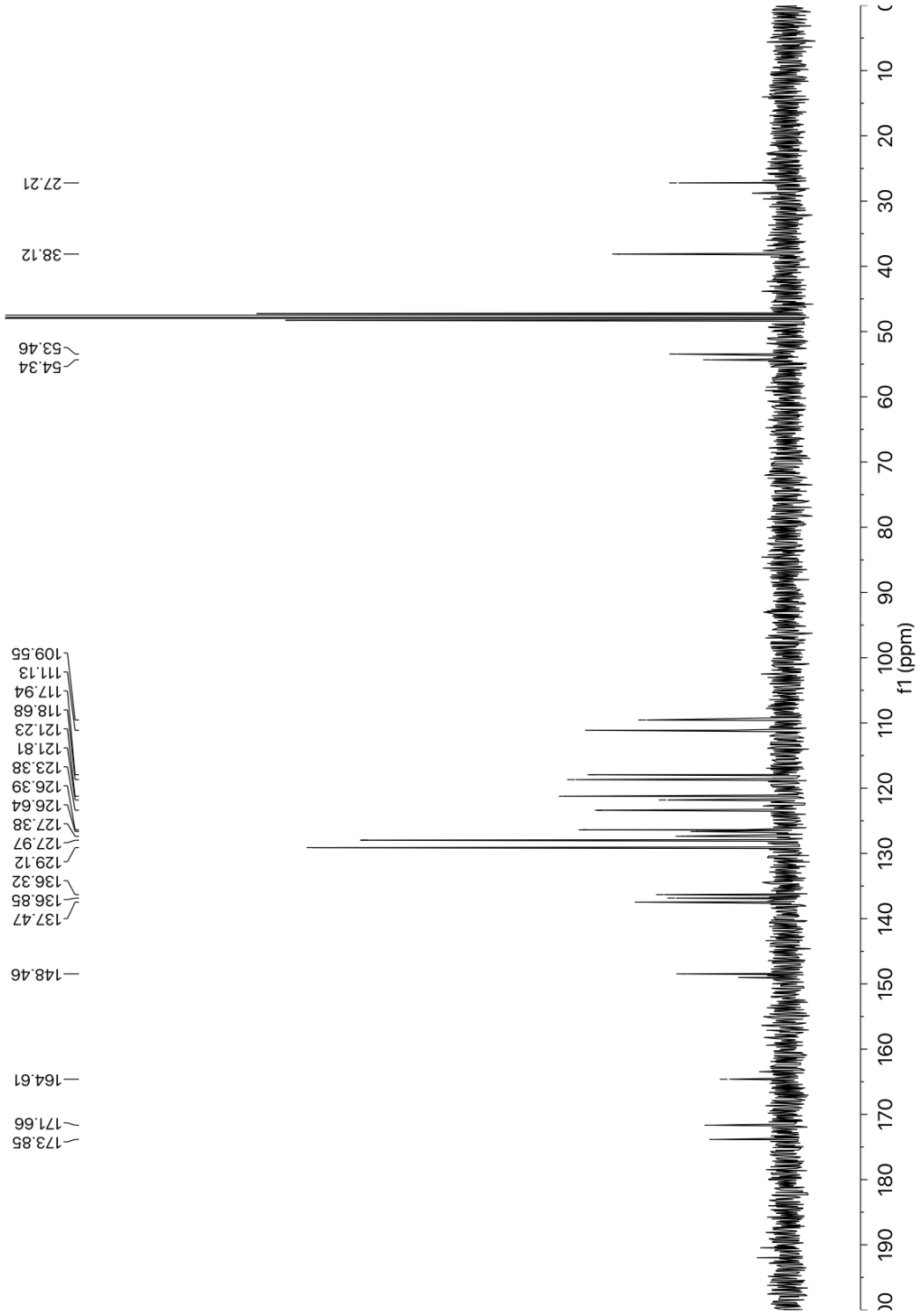
^{13}CNMR spectrum for compound **54b**



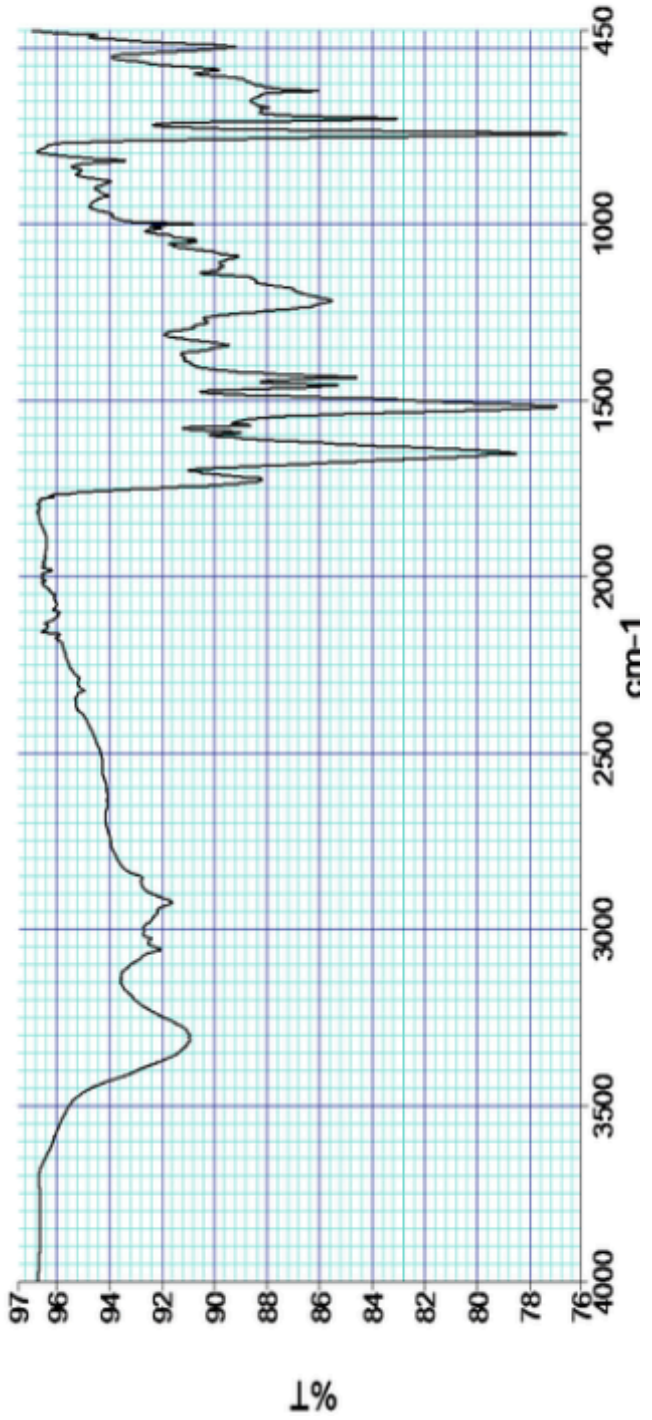
IR spectrum for compound **54b**



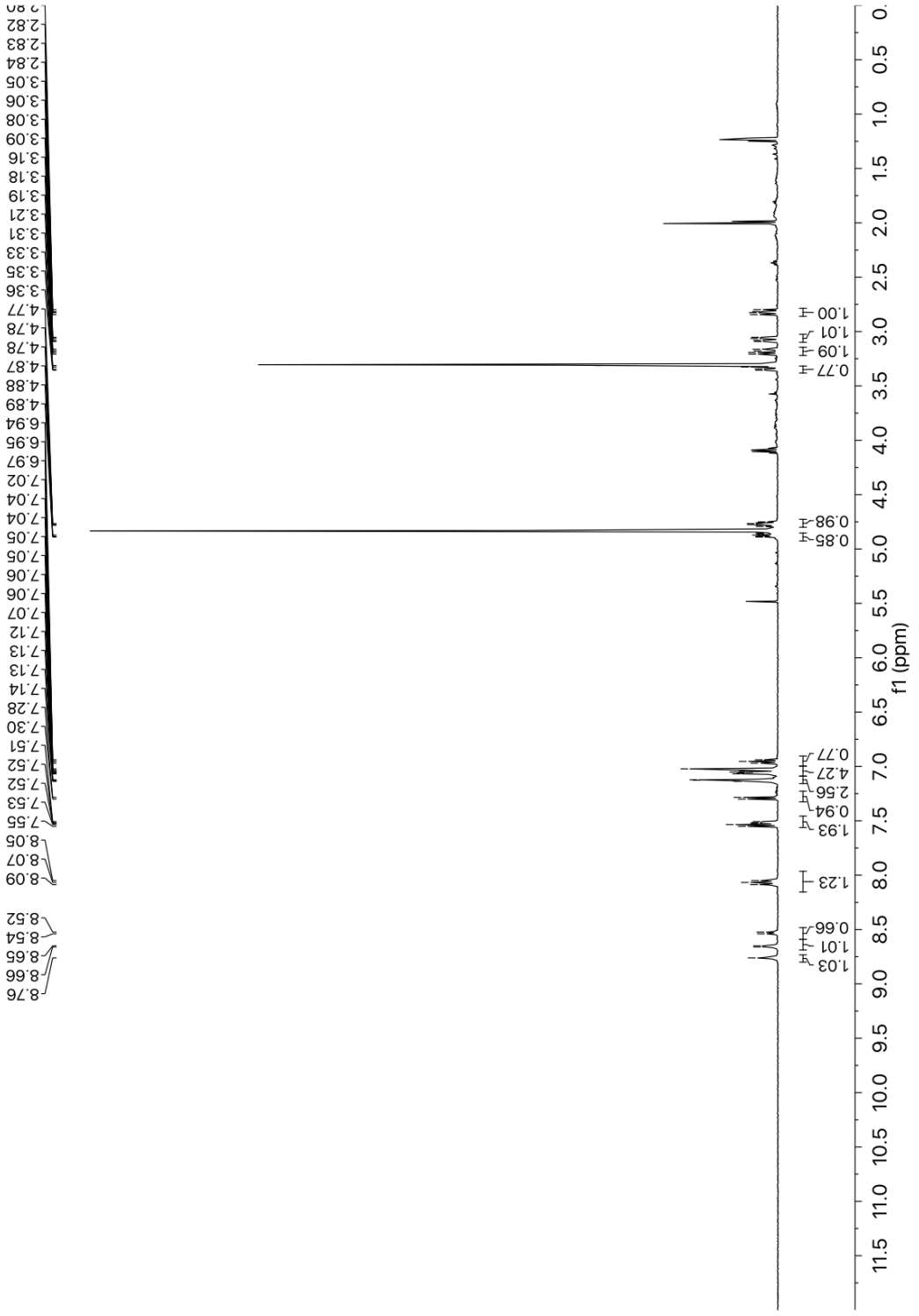
[1]HNMR spectrum for compound **54c**



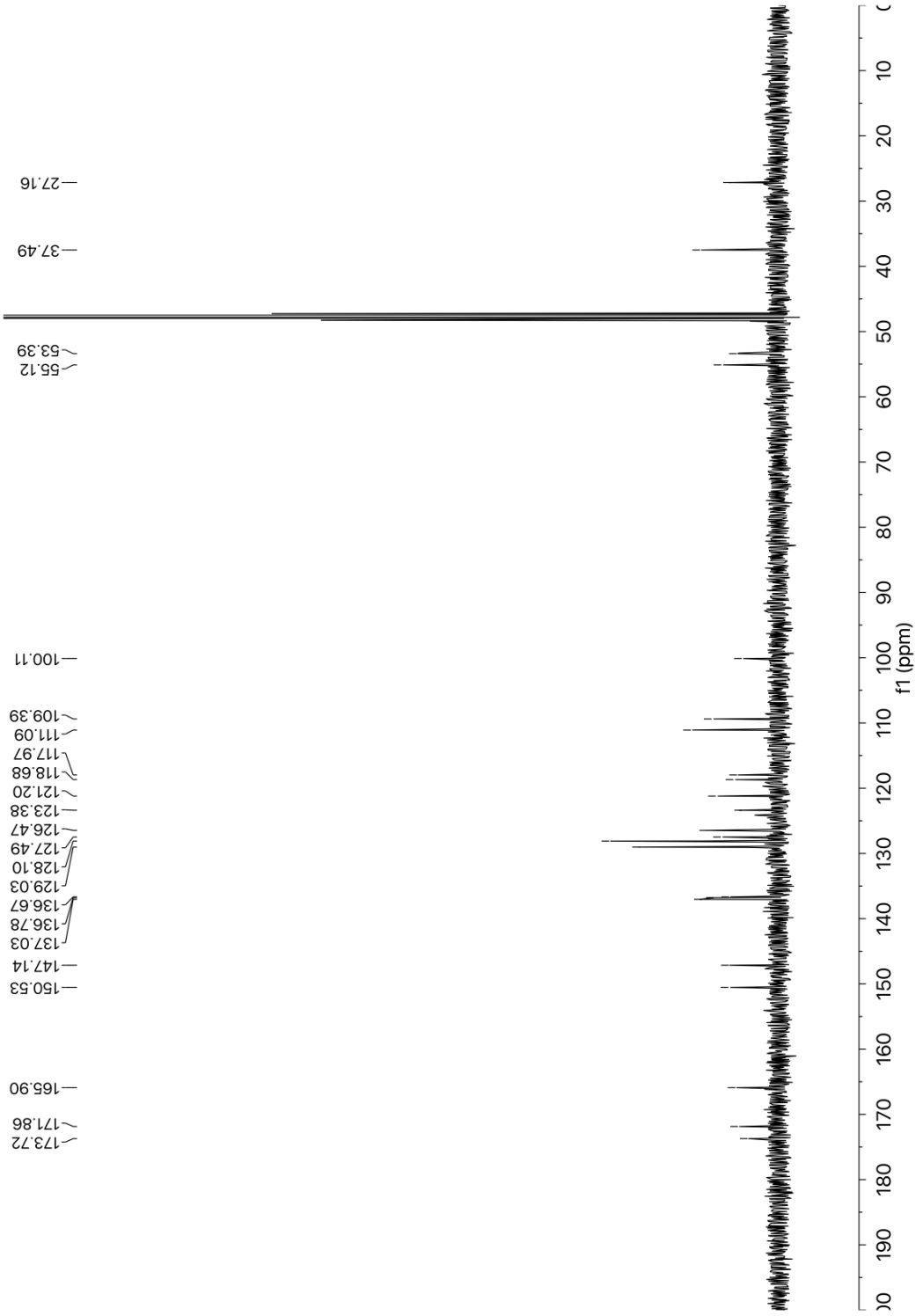
^{13}CNMR spectrum for compound **54c**



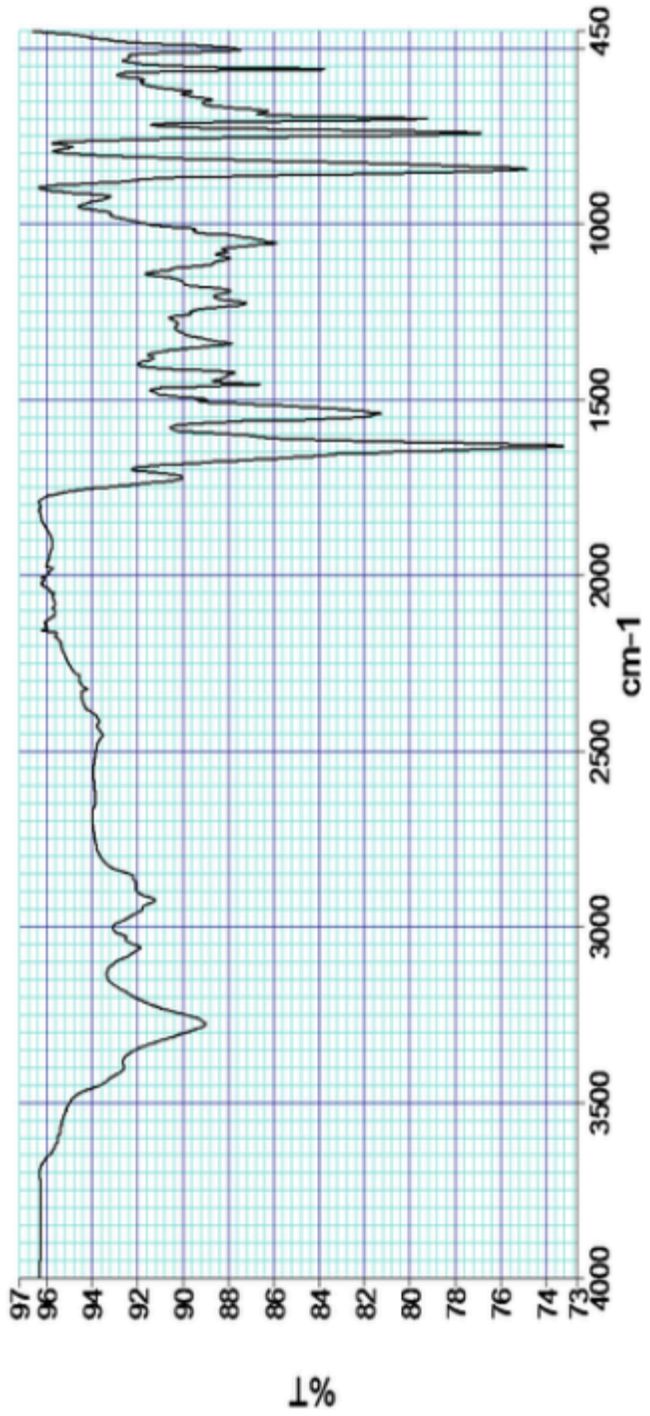
IR spectrum for compound **54c**



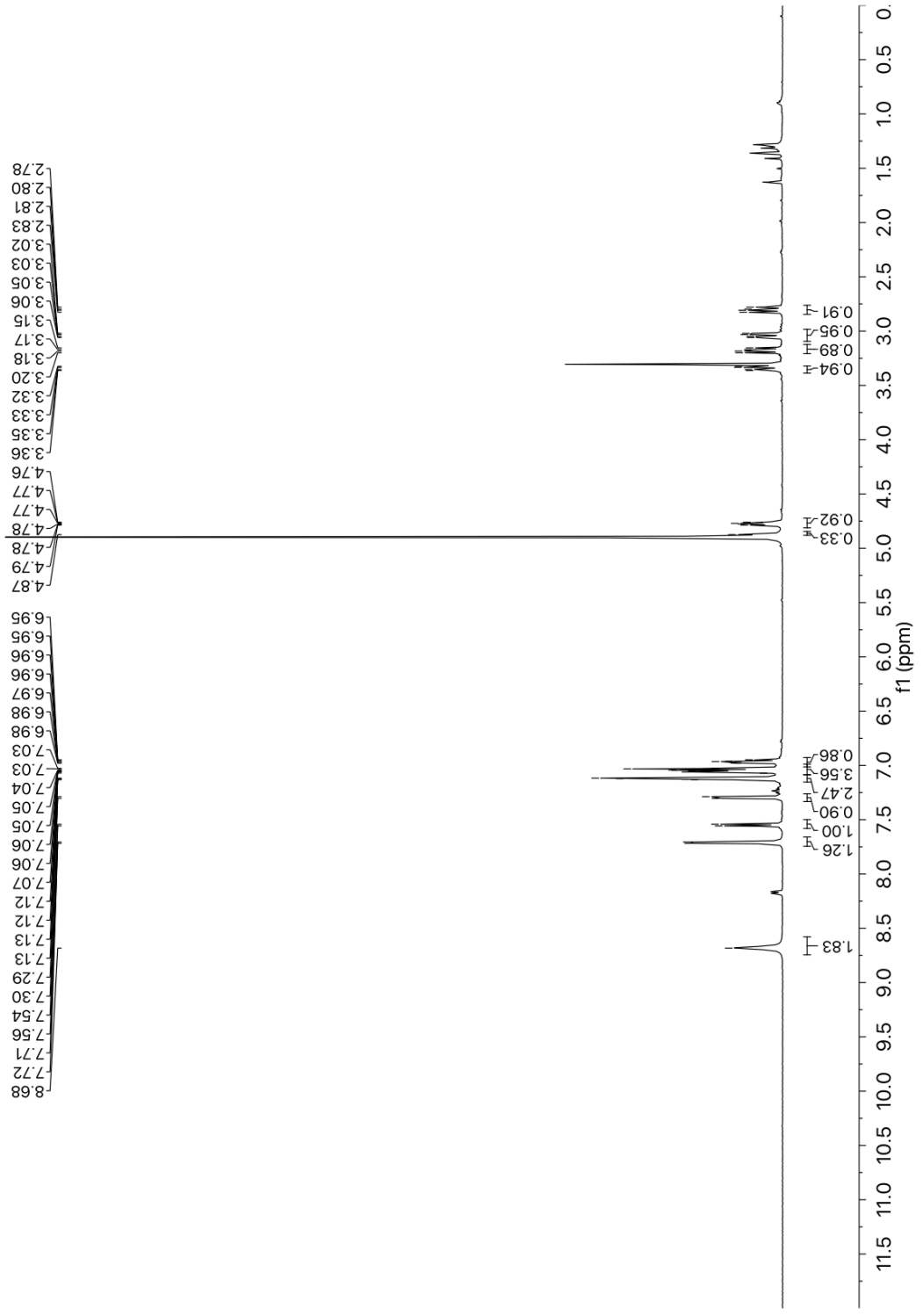
[1]HNMR spectrum for compound **54d**



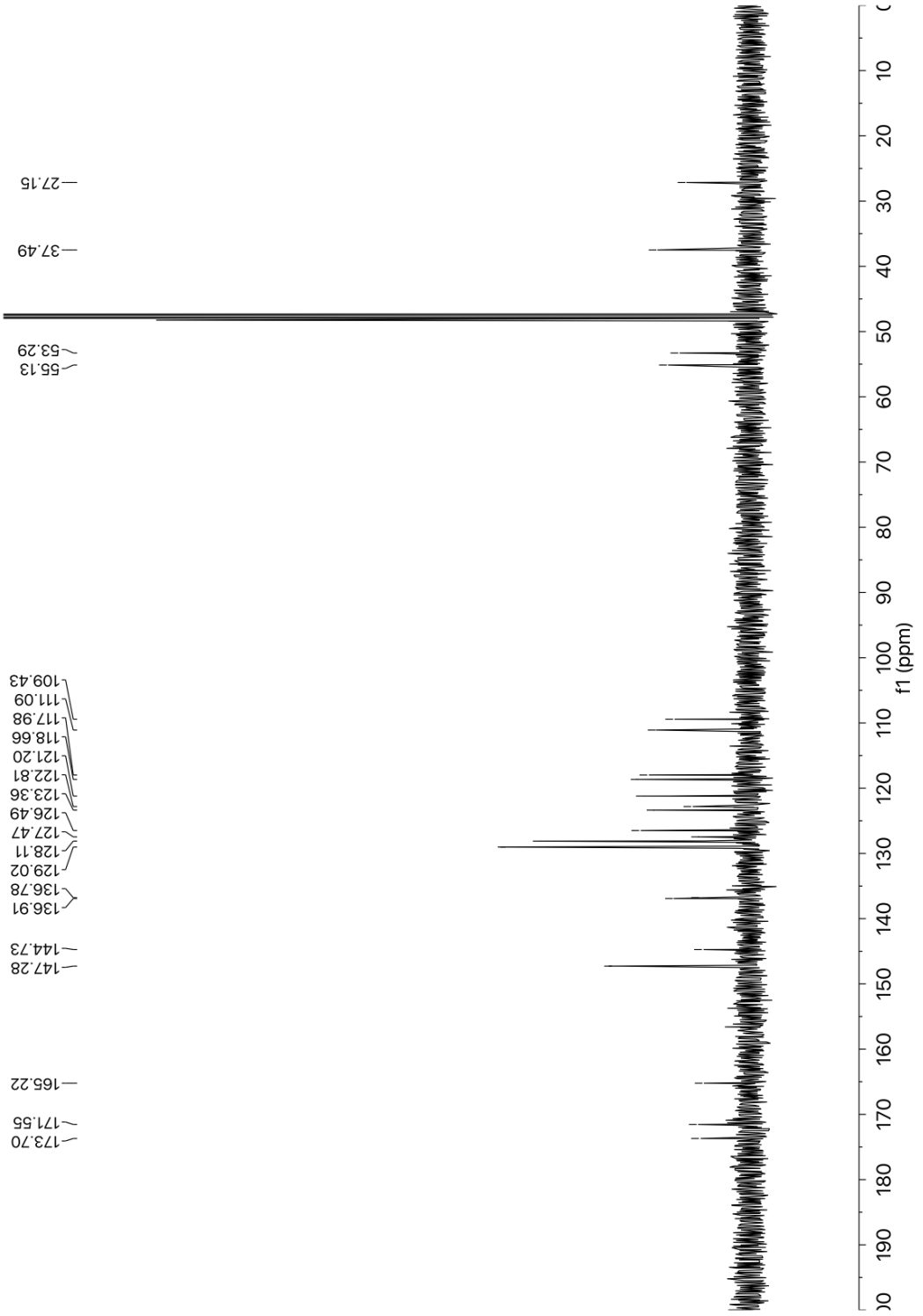
^{13}CNMR spectrum for compound **54d**



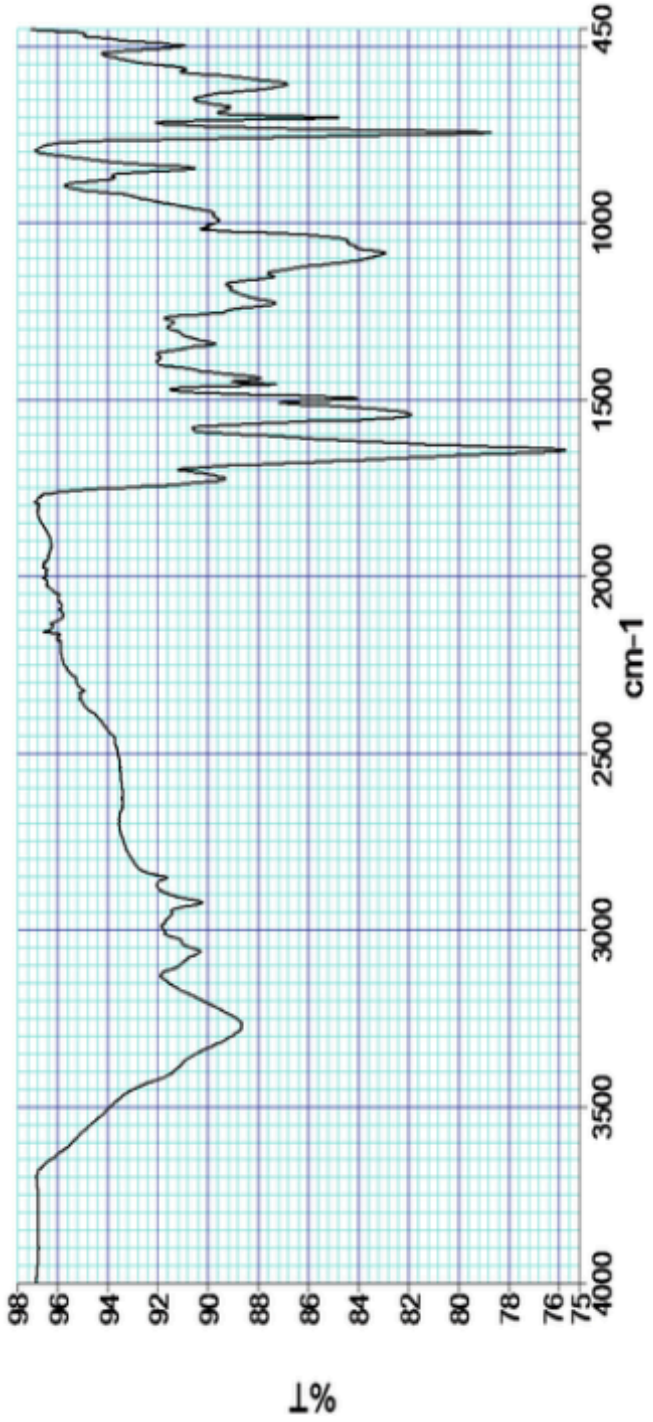
IR spectrum for compound **54d**



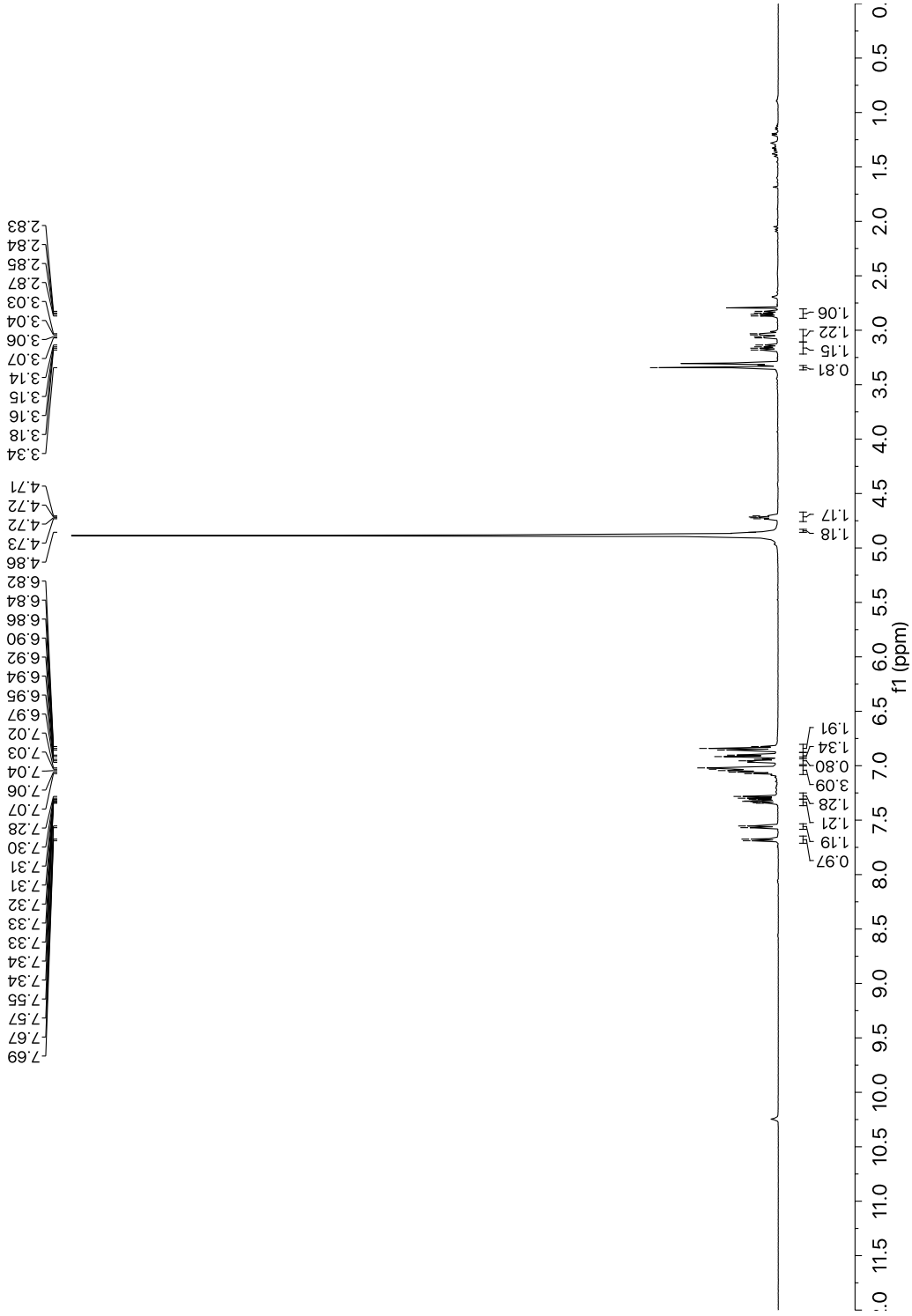
[1]HNMR spectrum for compound **54e**



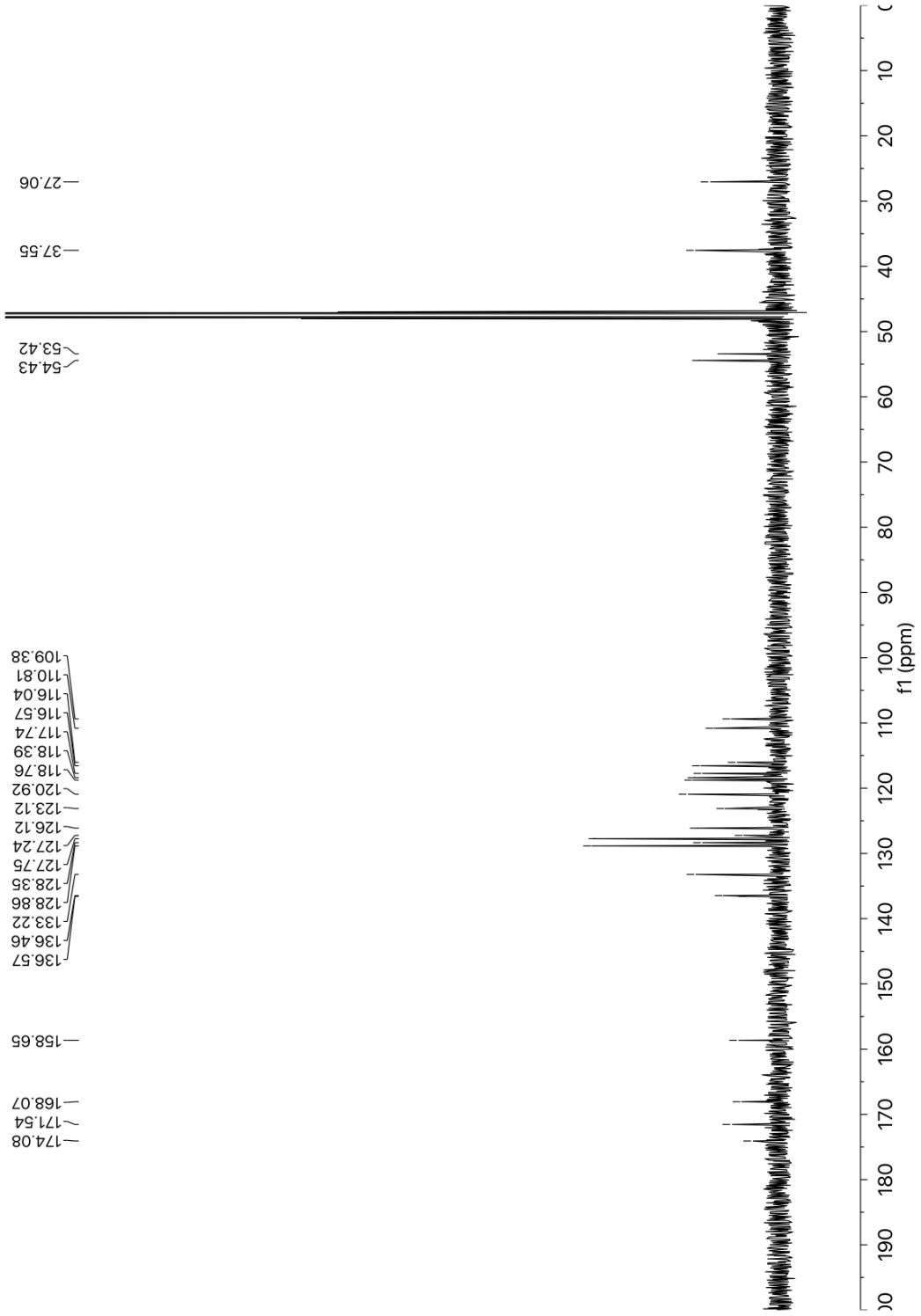
^{13}CNMR spectrum for compound **54e**



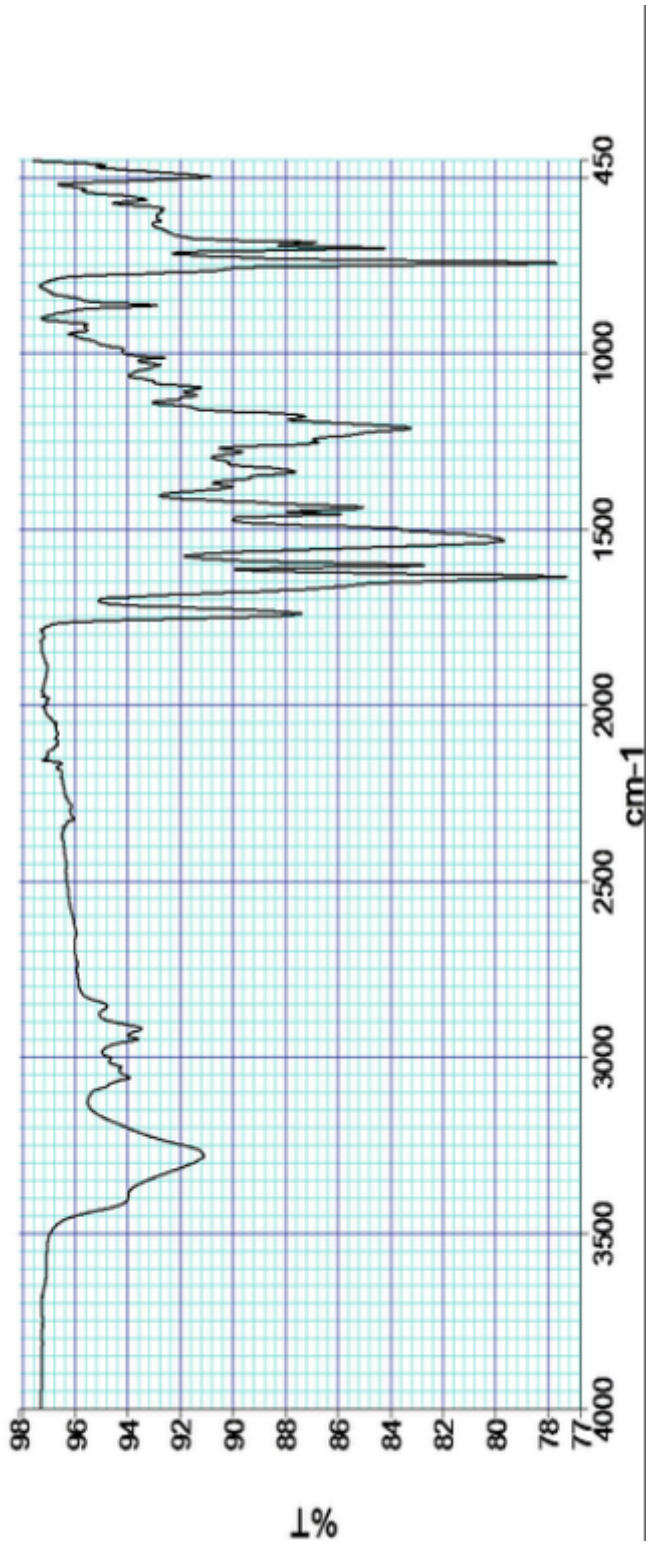
IR spectrum for compound **54e**



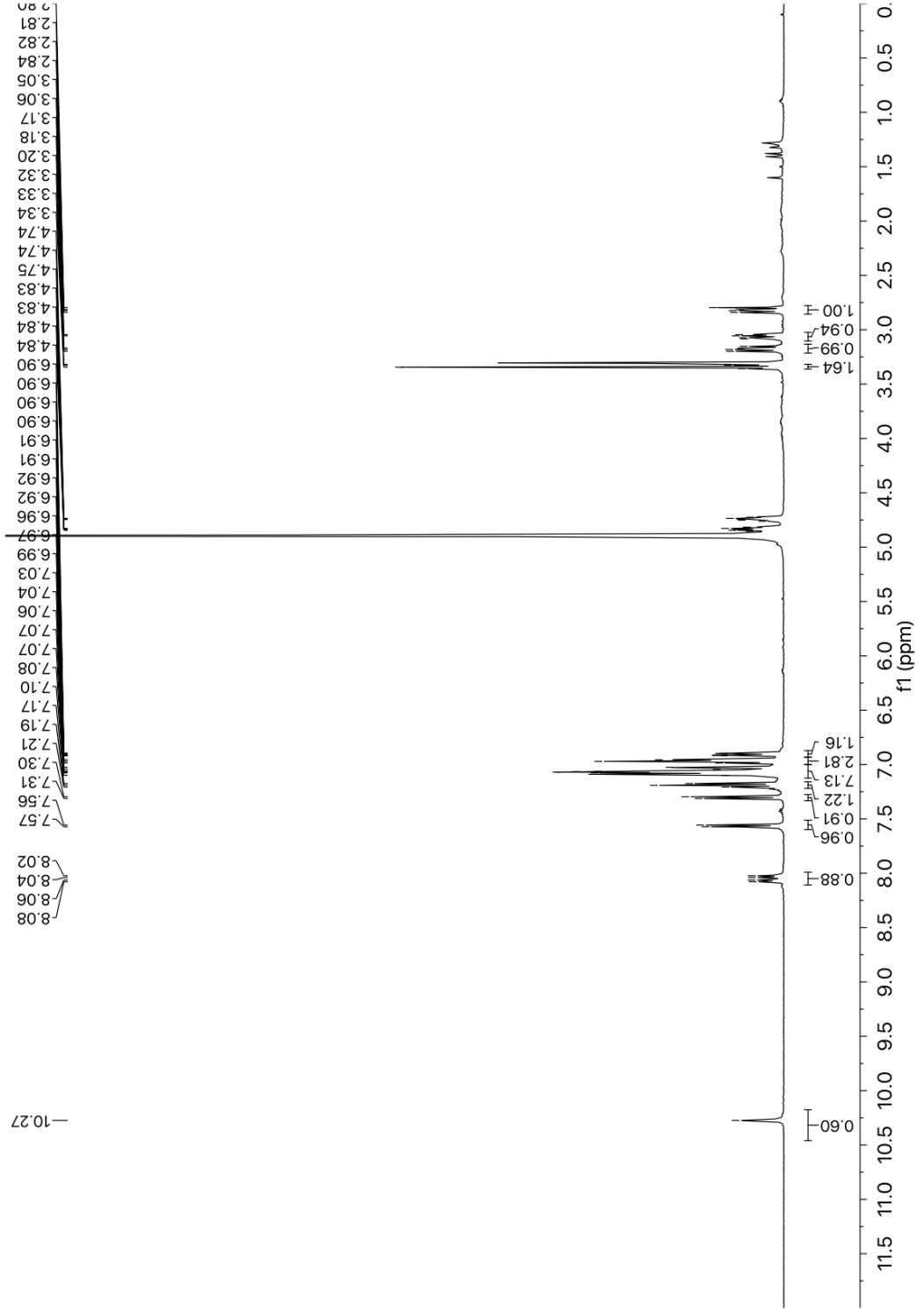
[1]HNMR spectrum for compound **54f**



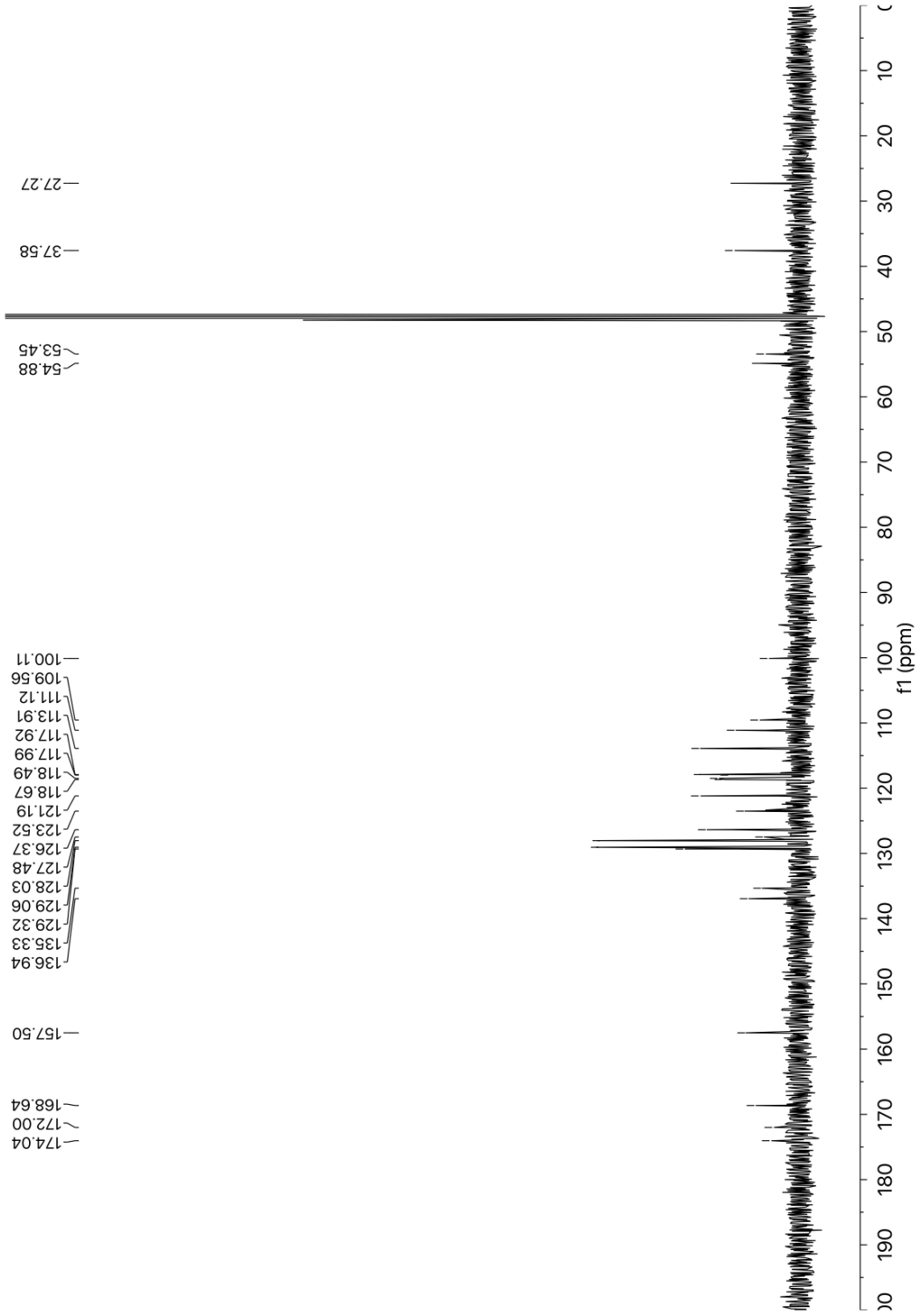
^{13}CNMR spectrum for compound **54f**



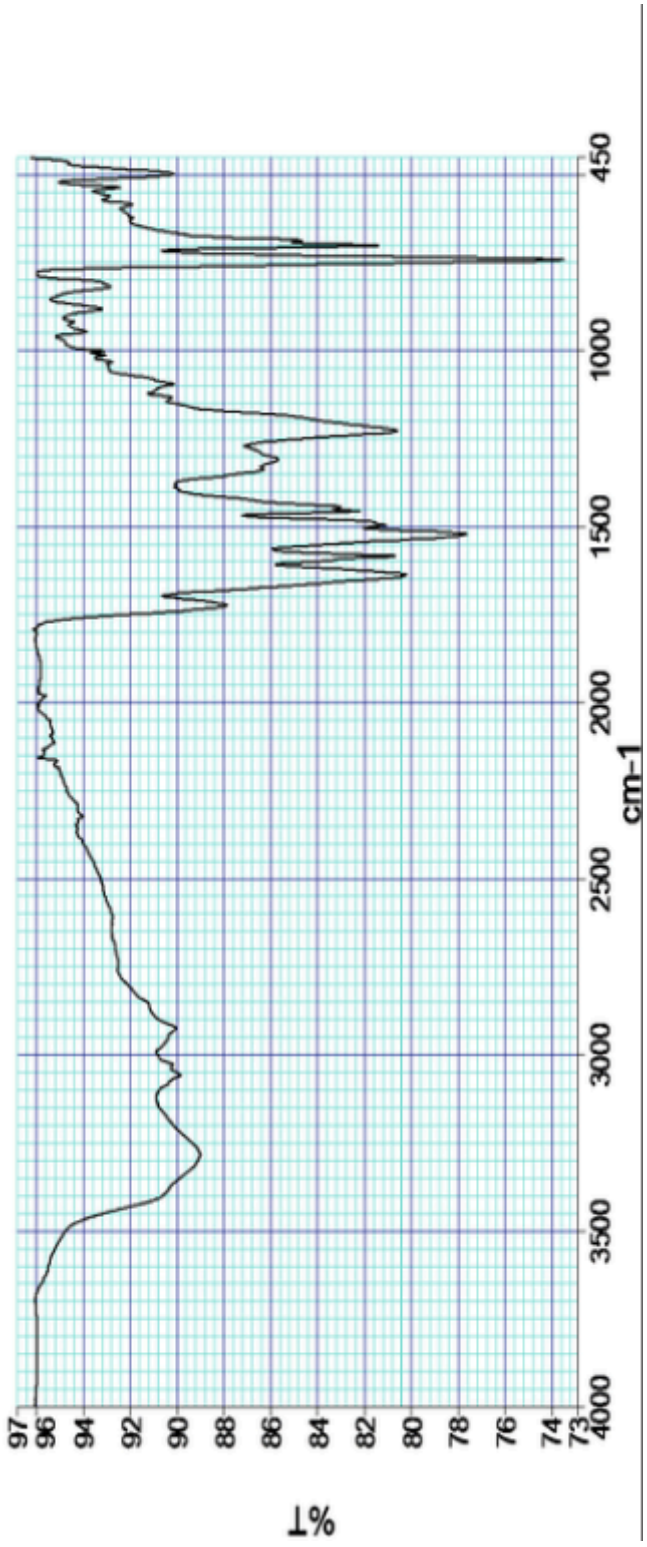
IR spectrum for compound **54f**



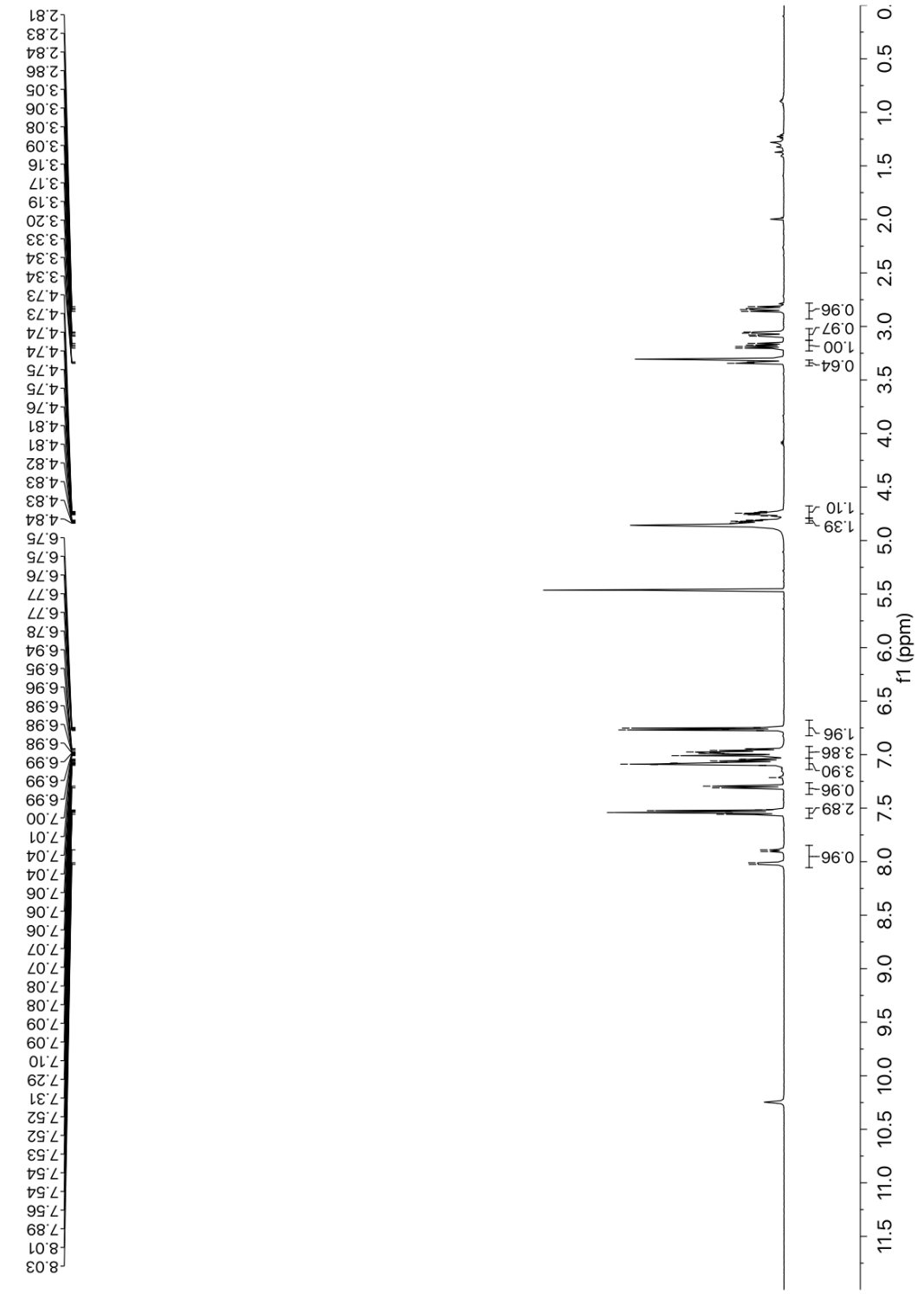
[1]HNMR spectrum for compound **54g**



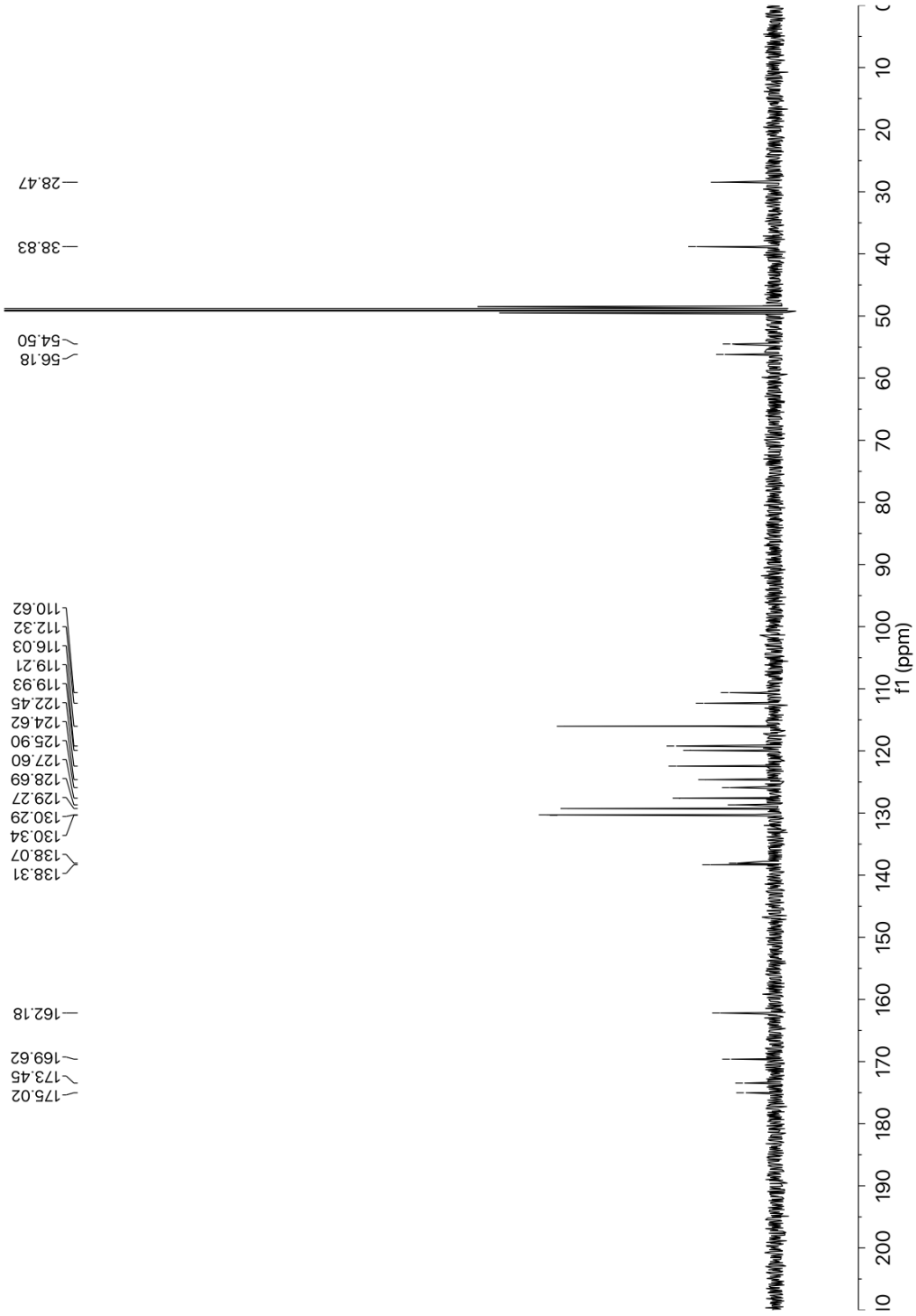
^{13}CNMR spectrum for compound **54g**



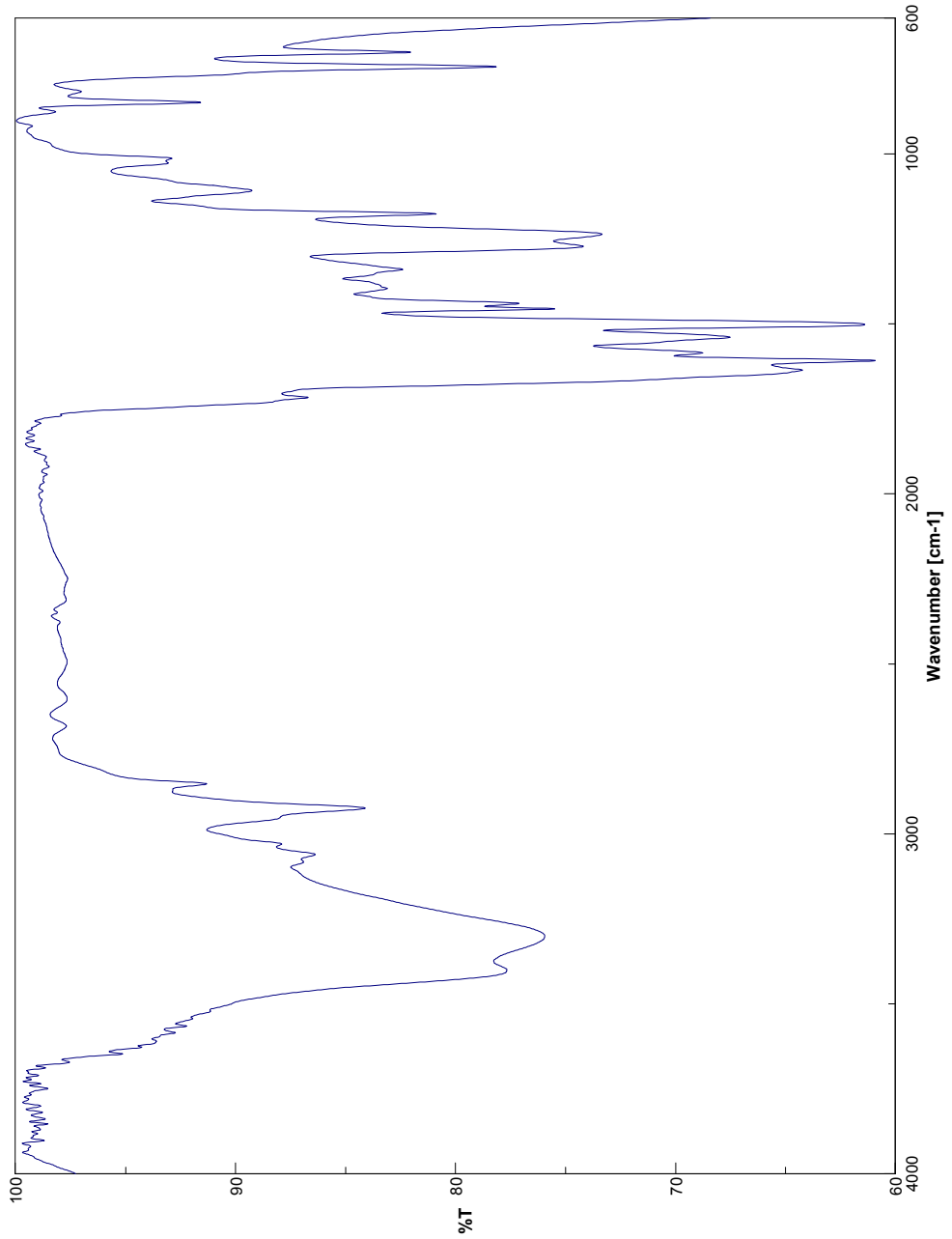
IR spectrum for compound **54g**



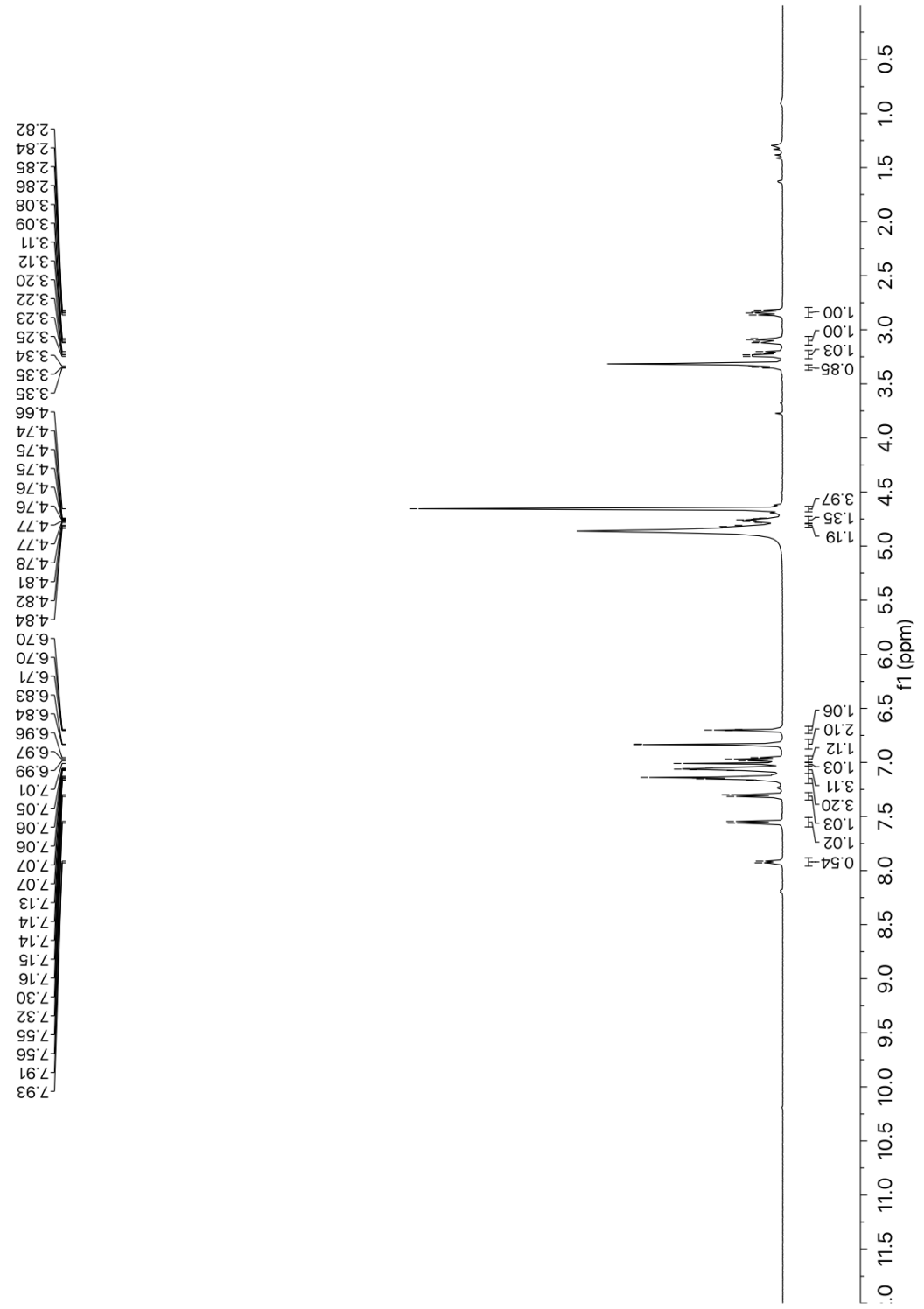
IR spectrum for compound **54h**



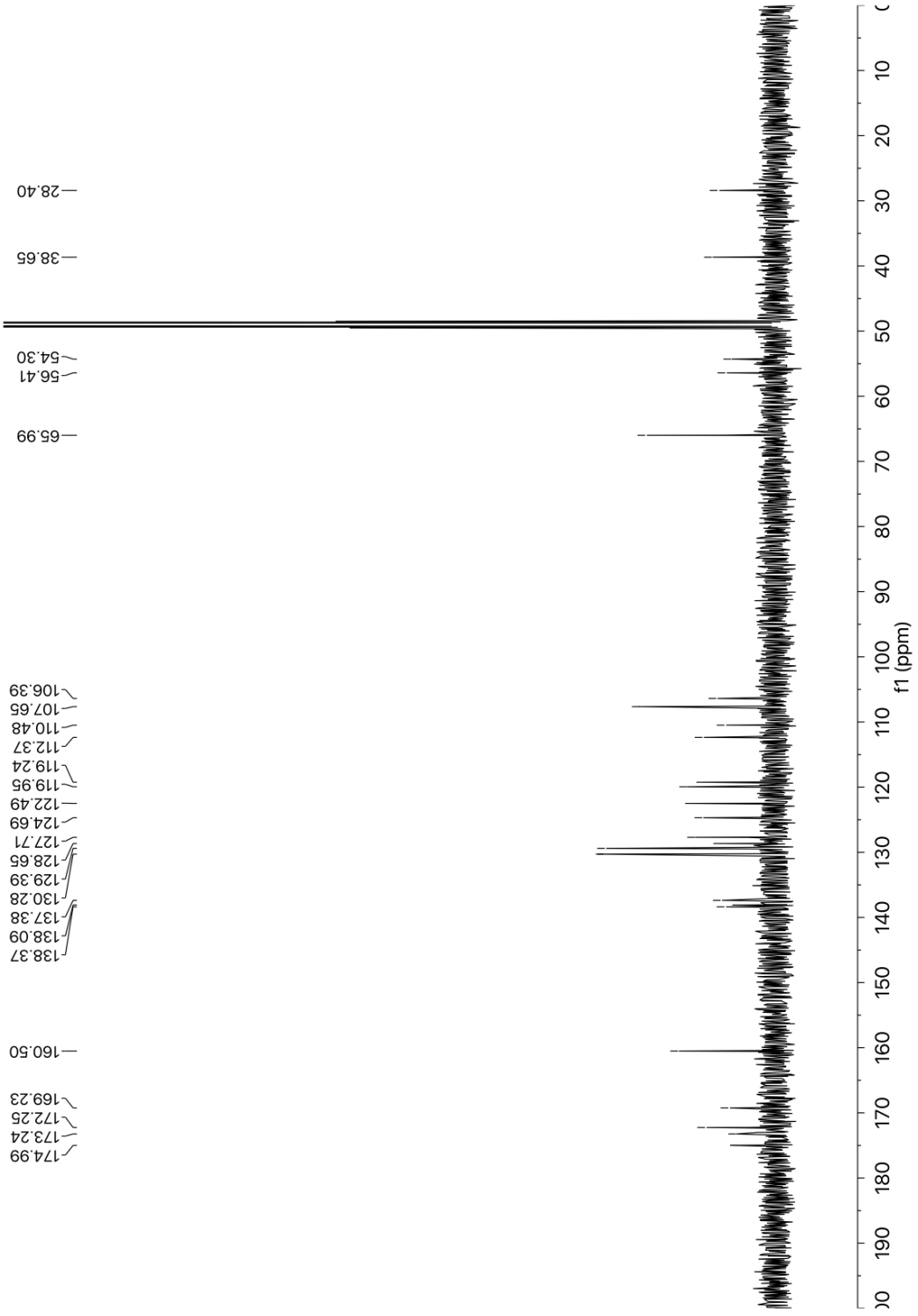
^{13}CNMR spectrum for compound **54h**



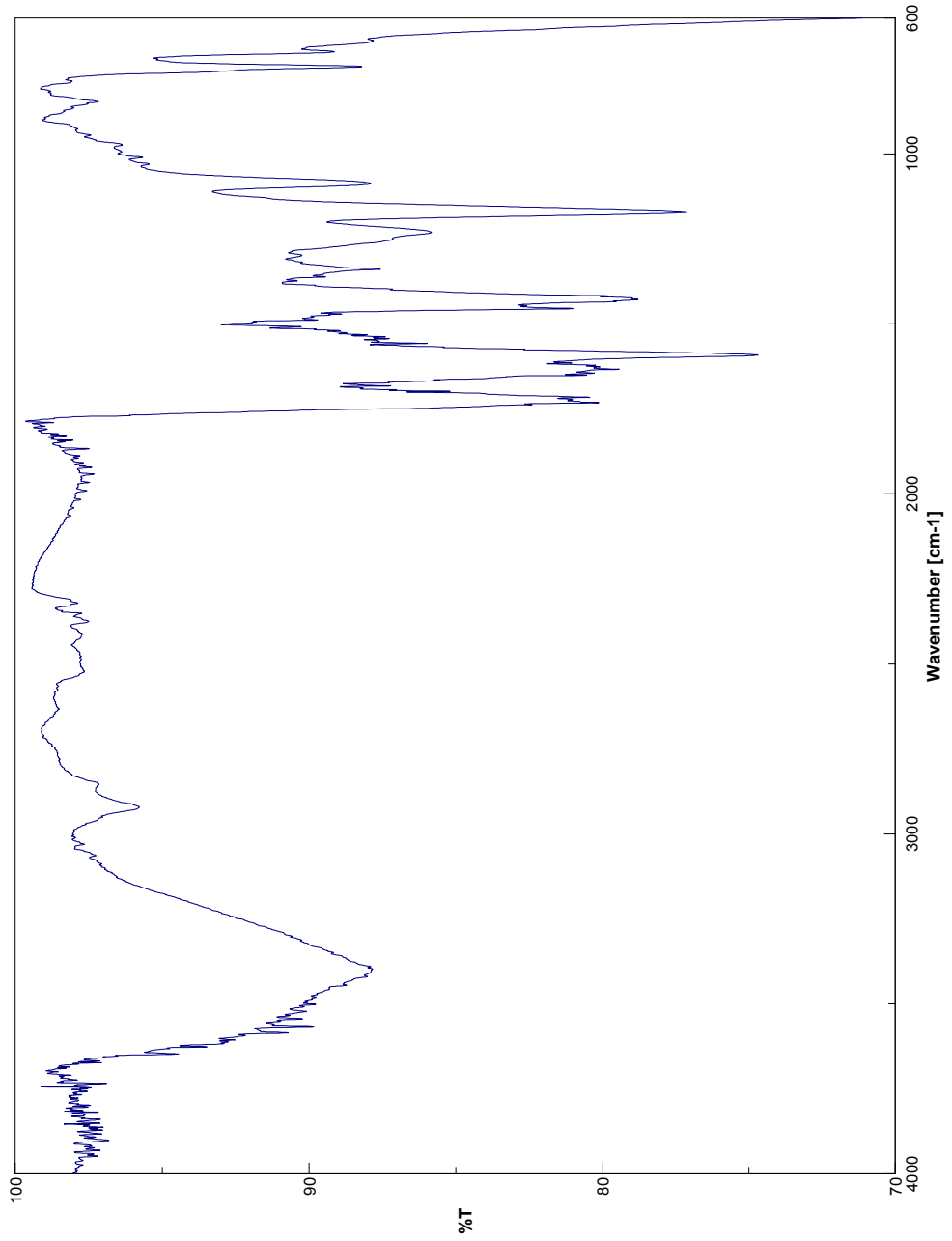
IR spectrum for compound **54h**



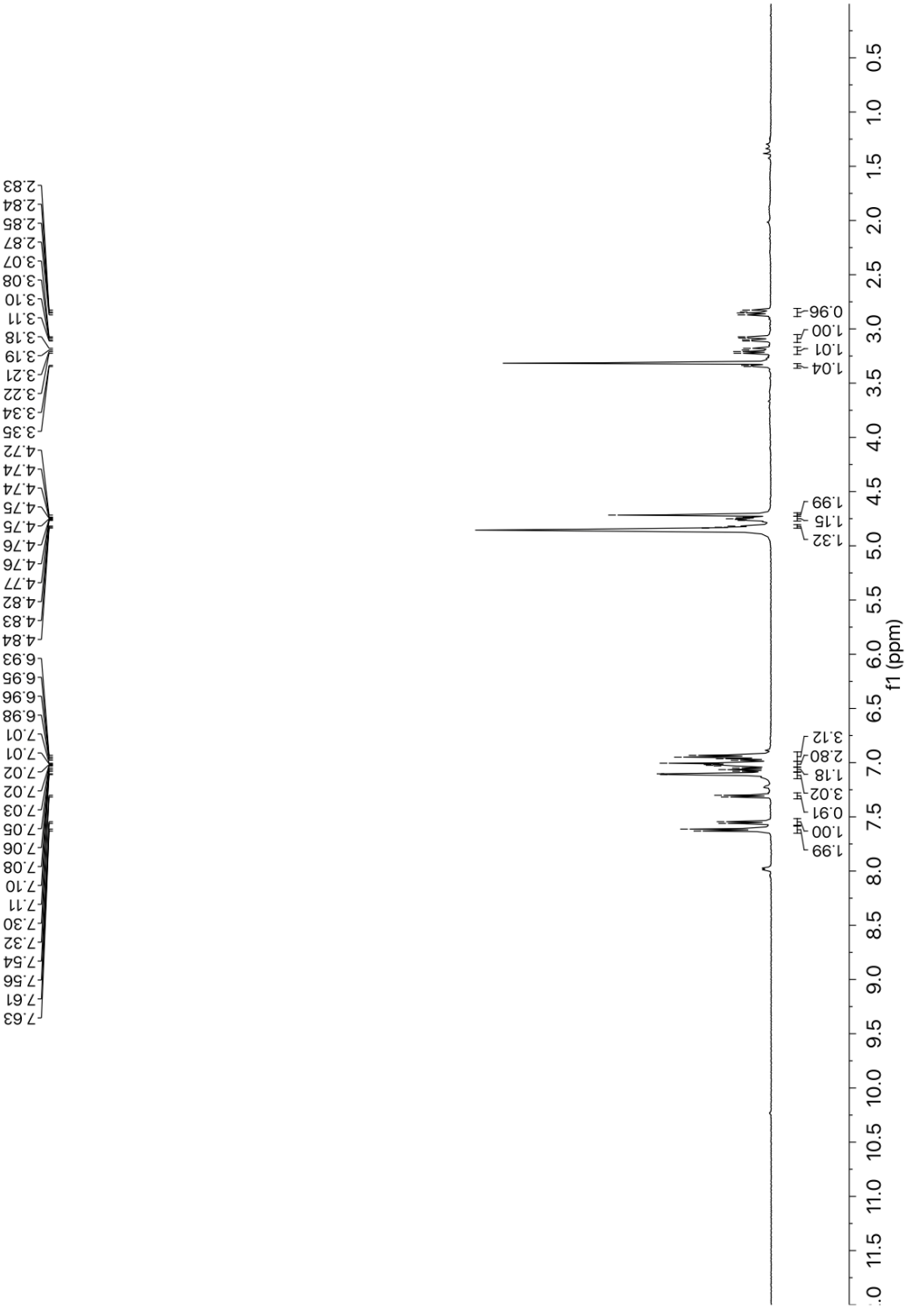
[1]HNMR spectrum for compound **54i**



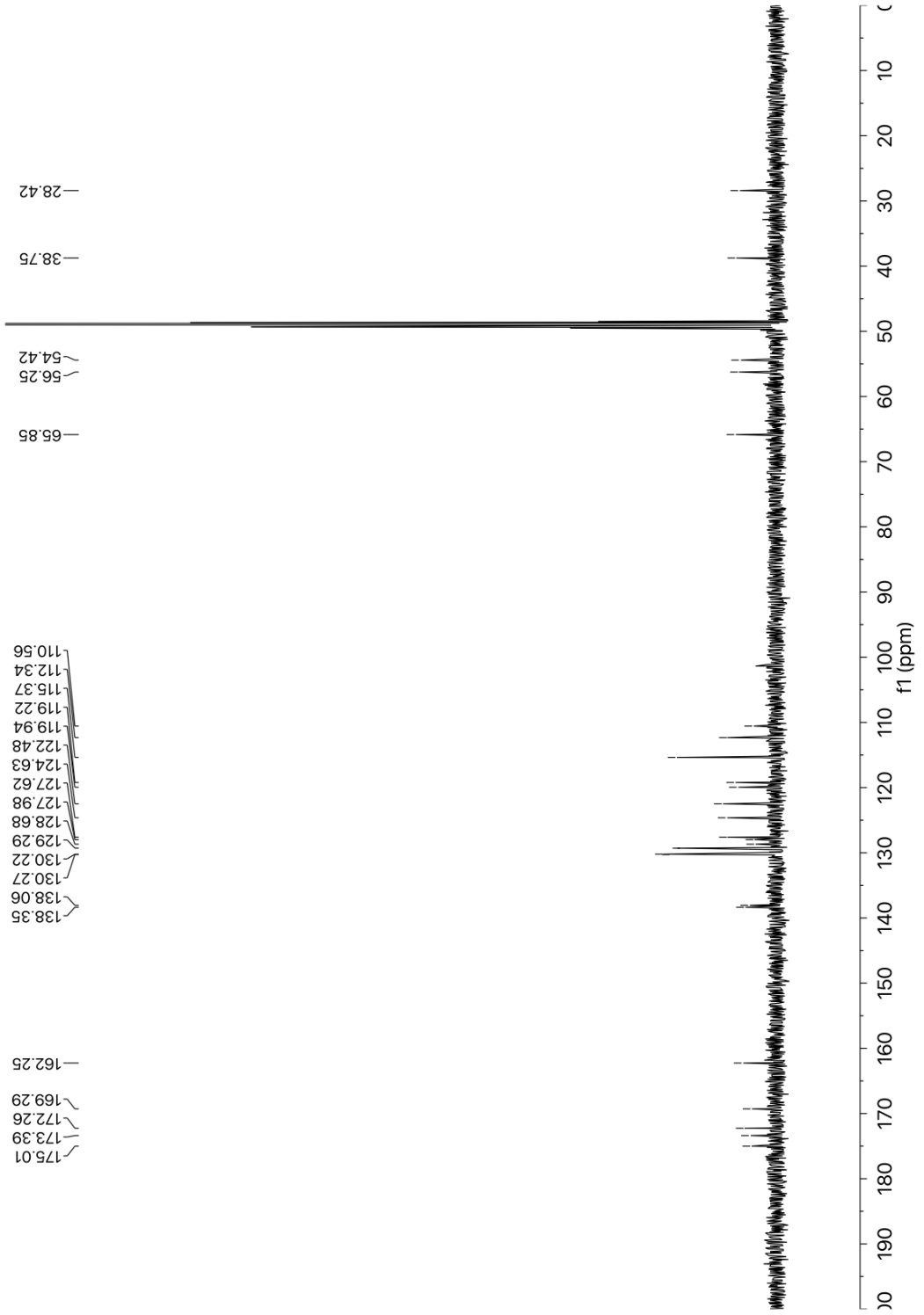
^{13}CNMR spectrum for compound **54i**



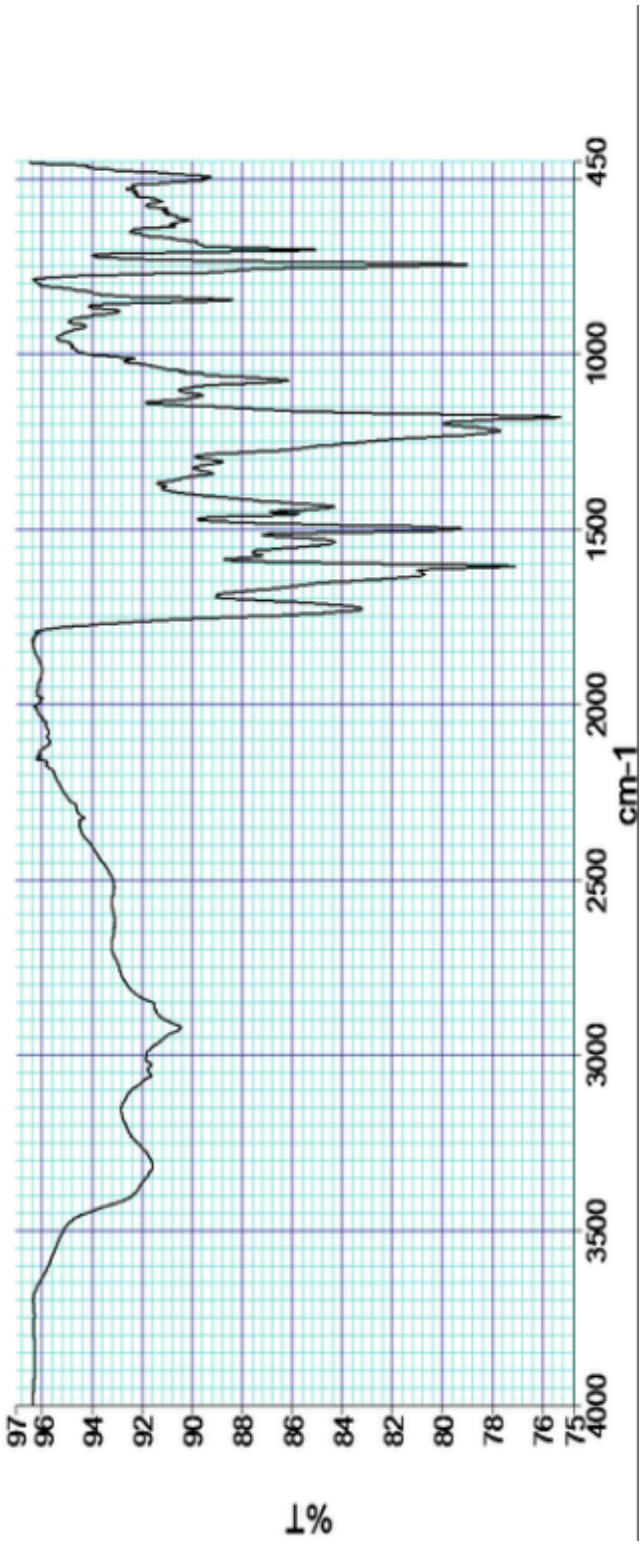
IR spectrum for compound **54i**



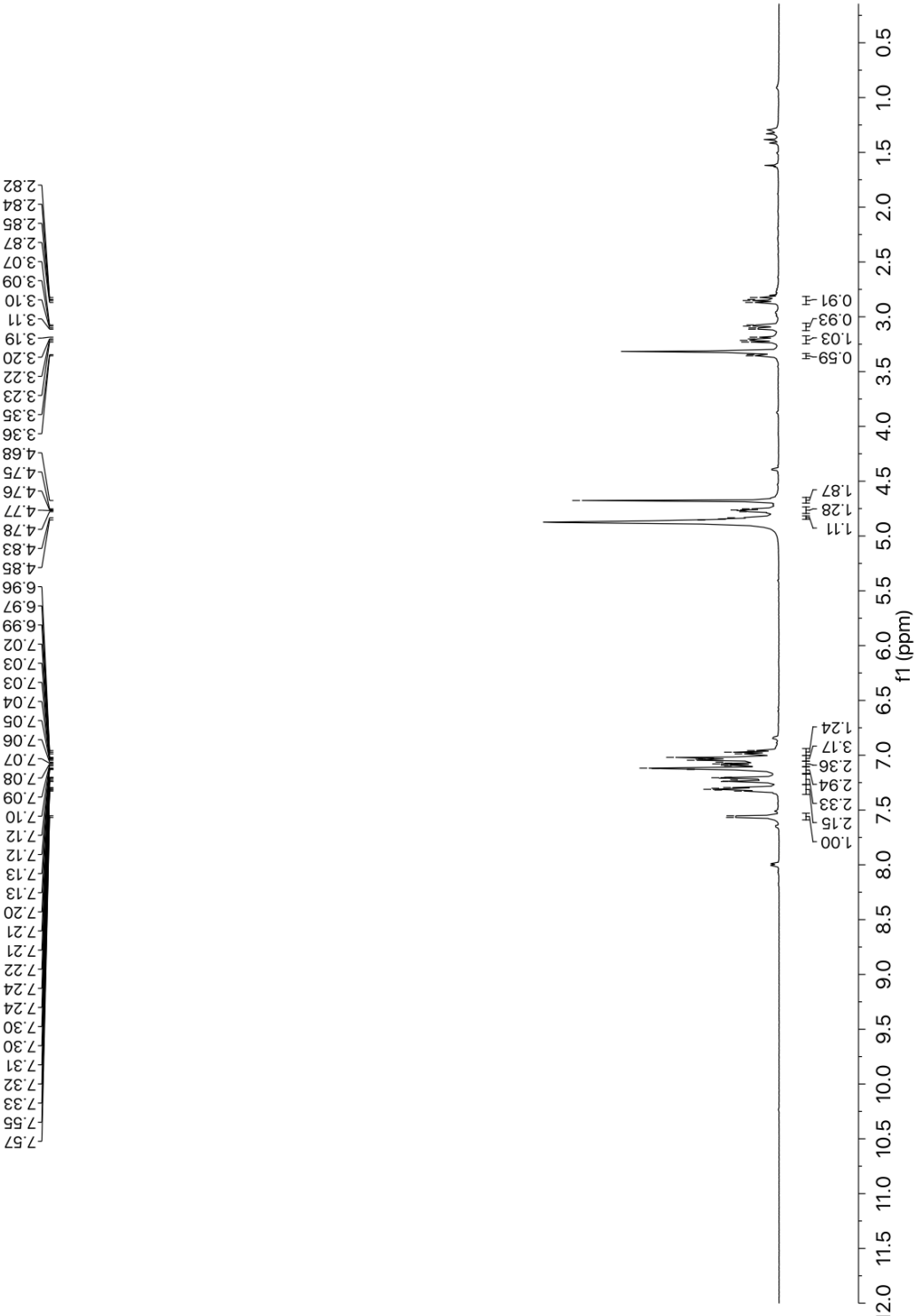
[1]HNMR spectrum for compound **54j**



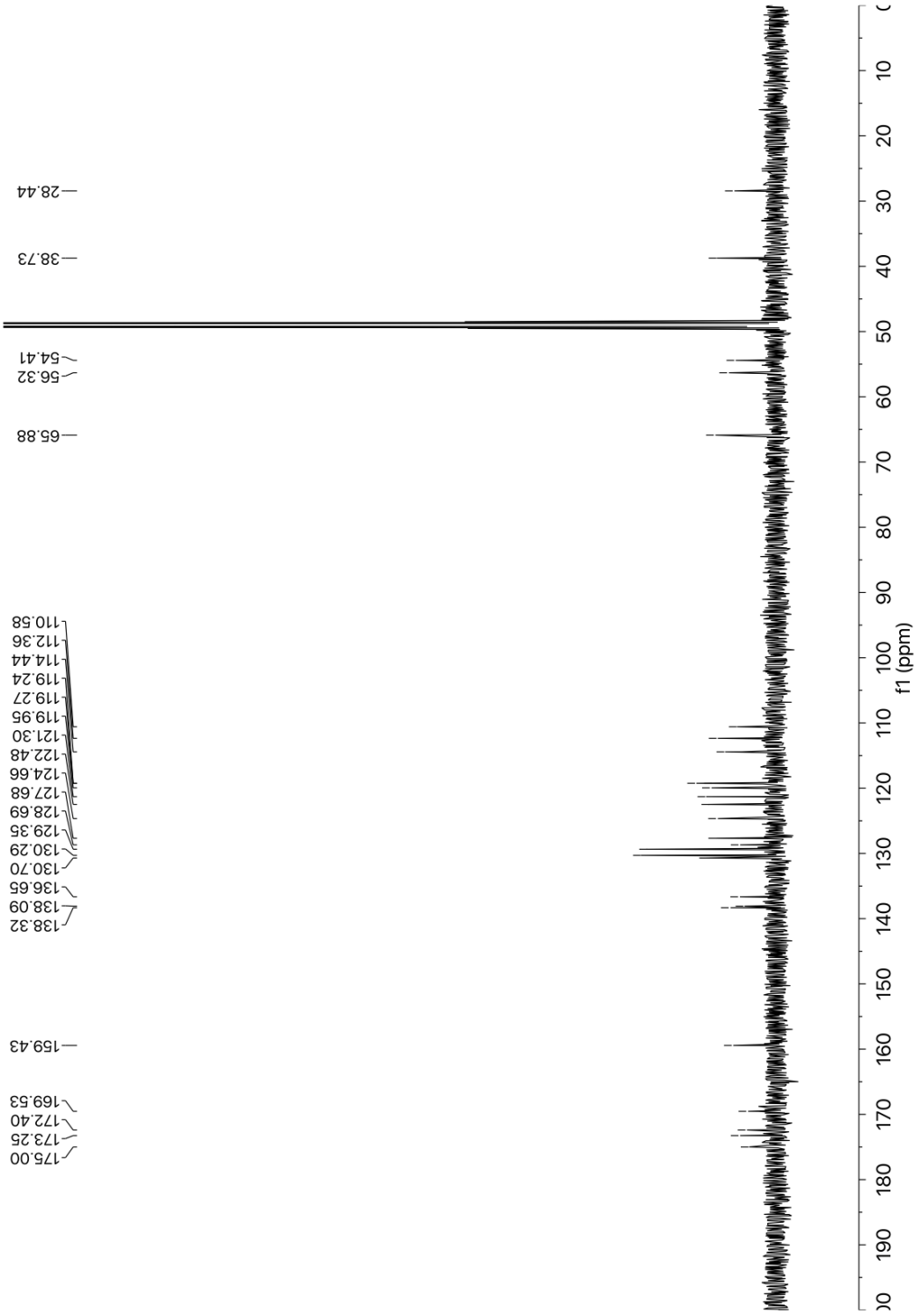
^{13}CNMR spectrum for compound **54j**



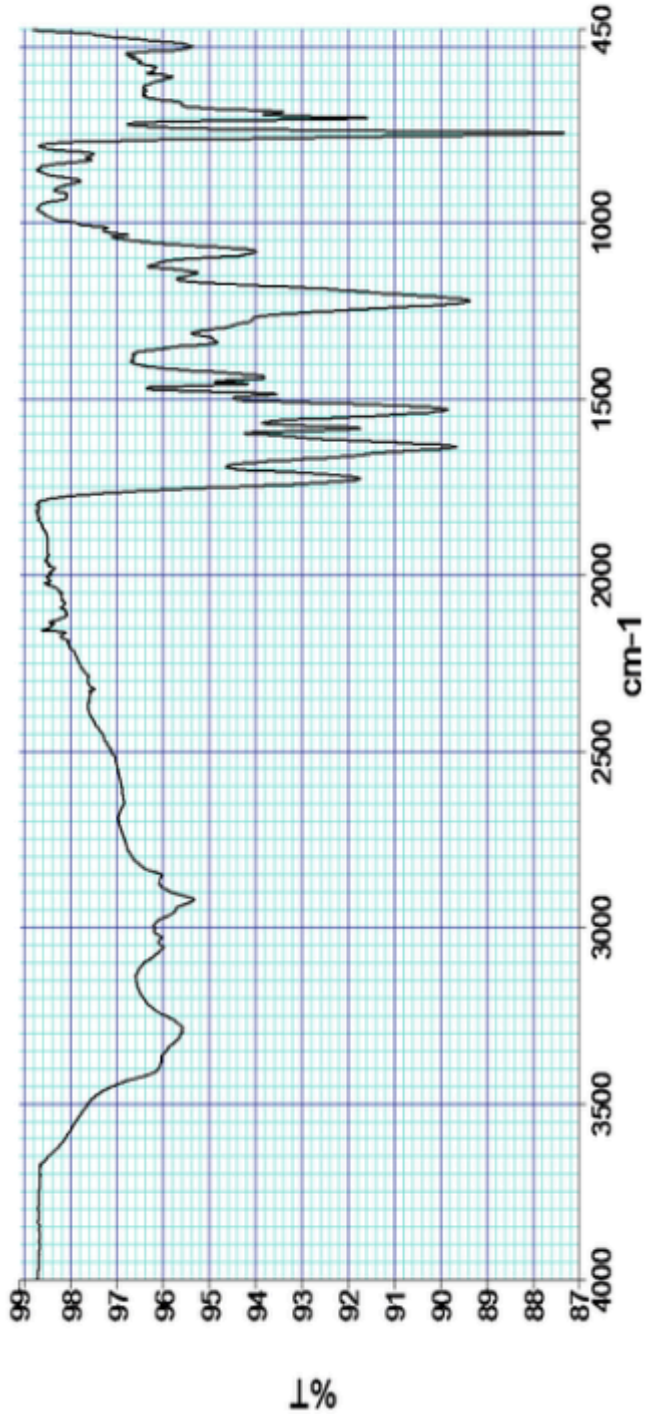
IR spectrum for compound **54j**



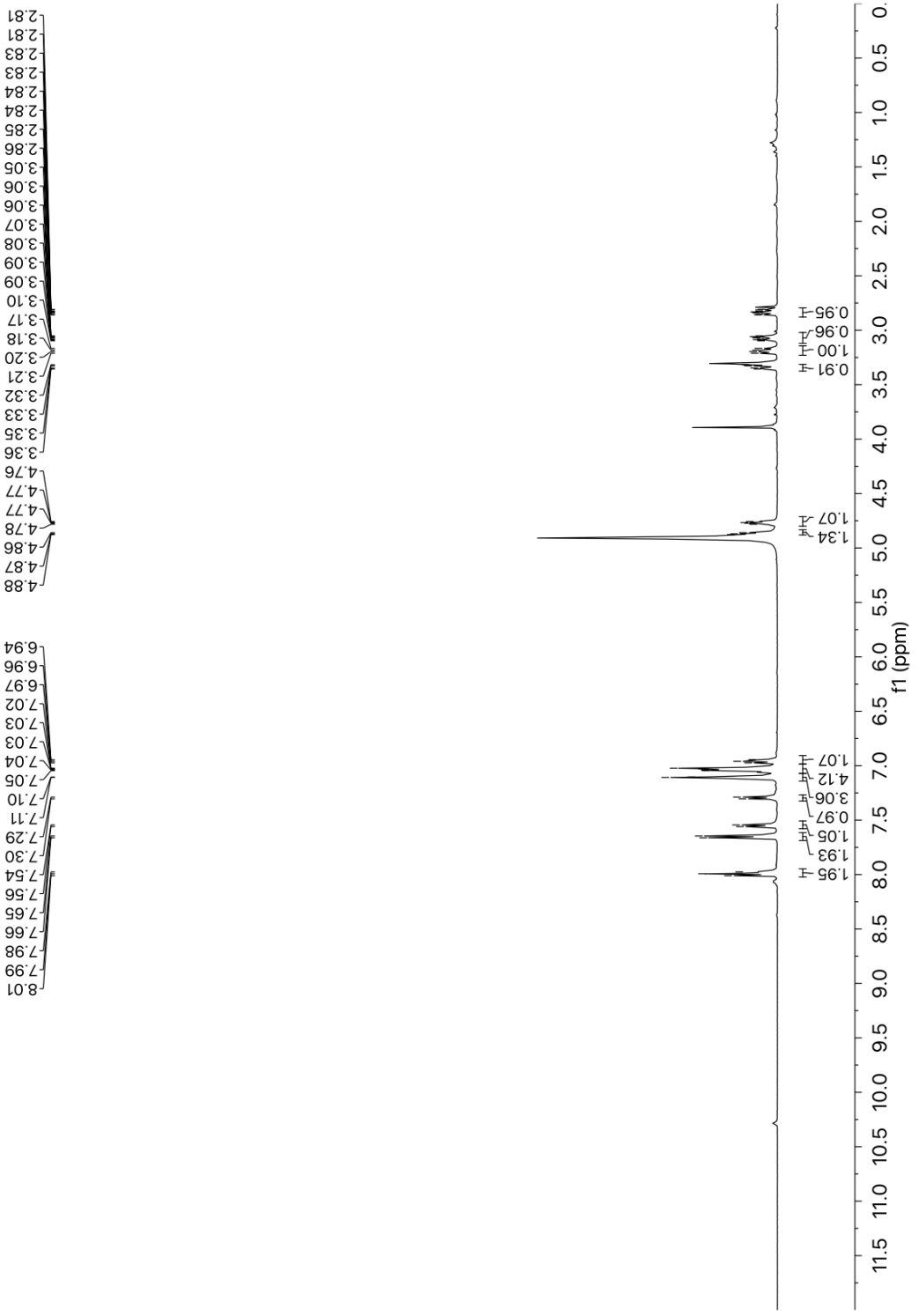
^{1}HNMR spectrum for compound **54k**



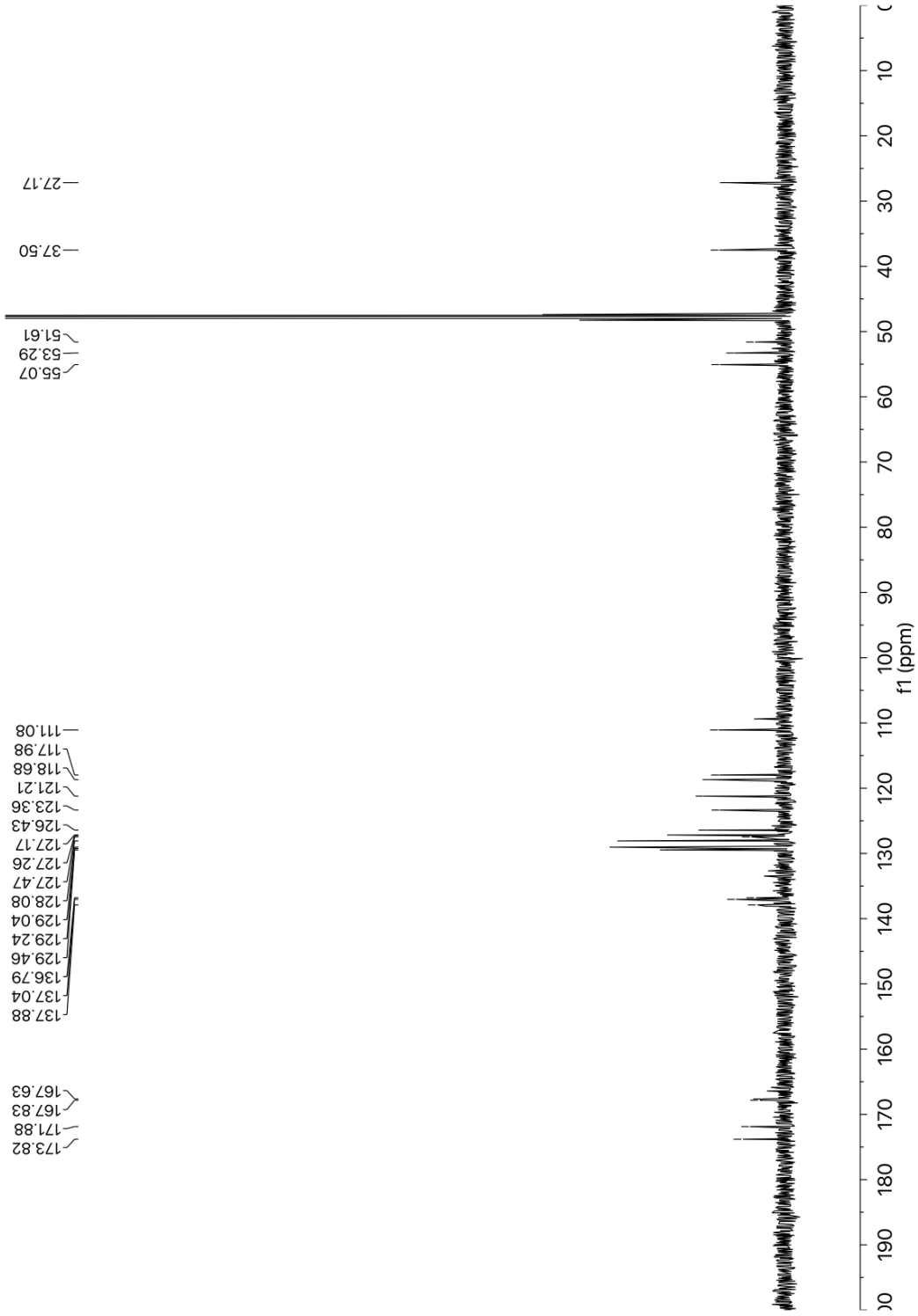
^{13}CNMR spectrum for compound **54k**



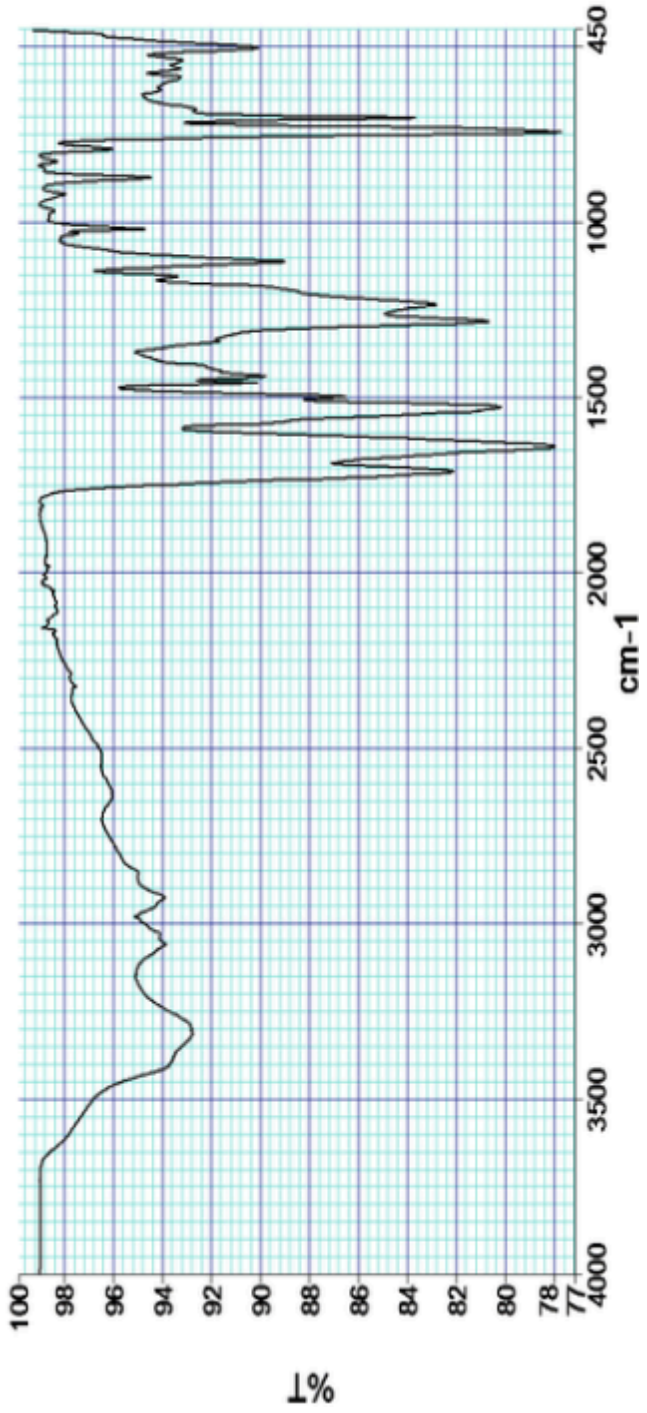
IR spectrum for compound **54k**



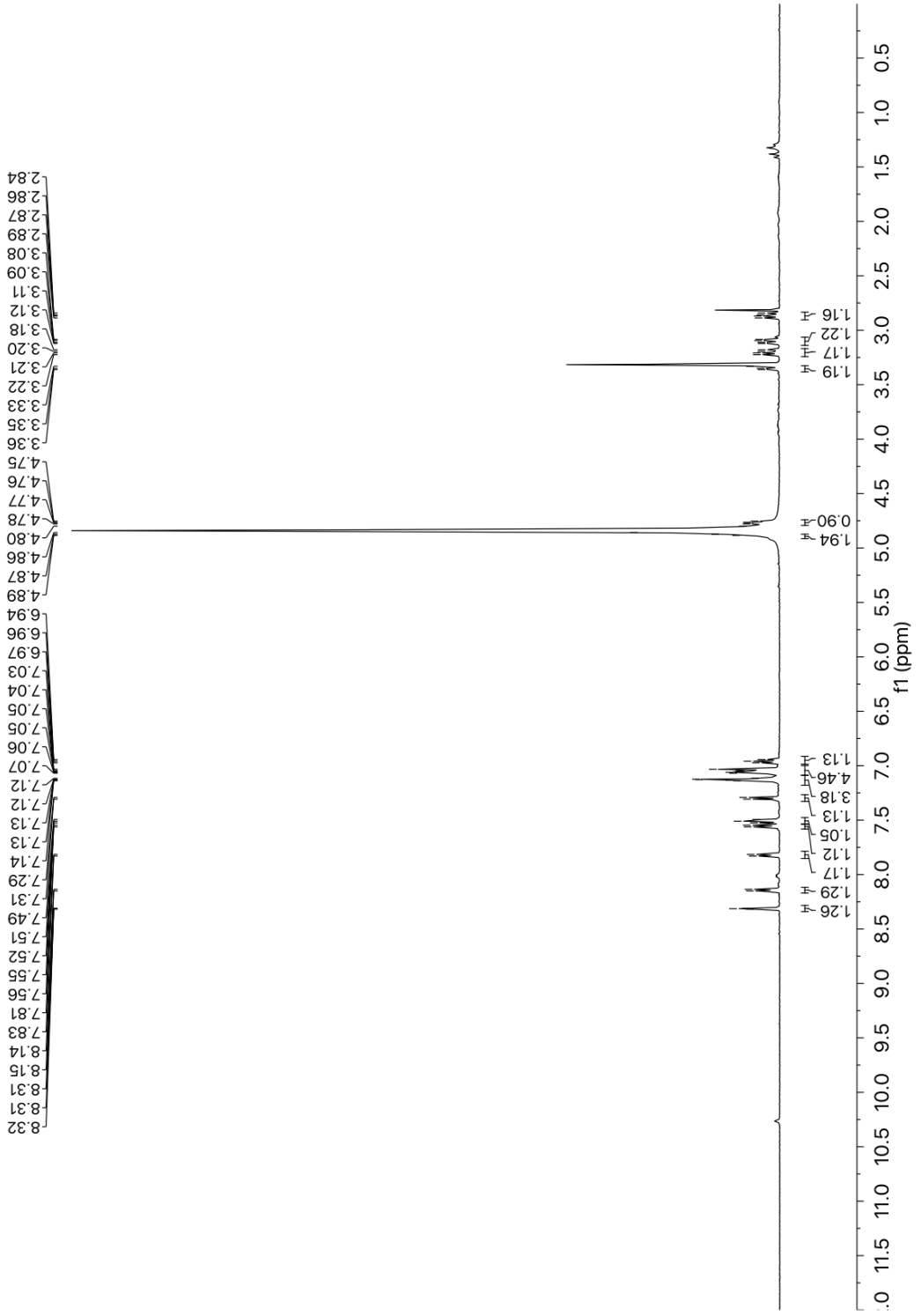
[1]HNMR spectrum for compound **54l**



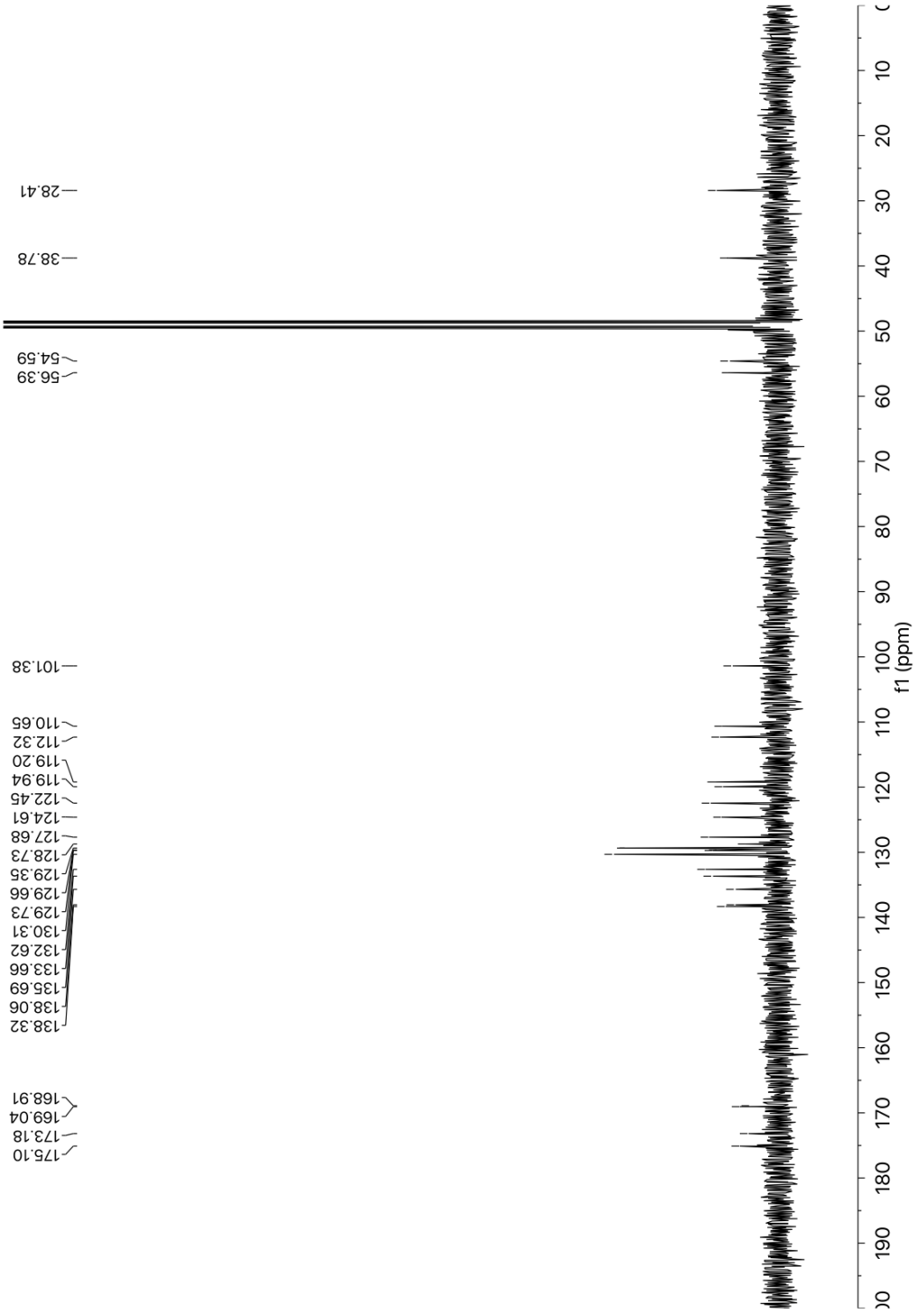
^{13}CNMR spectrum for compound **54l**



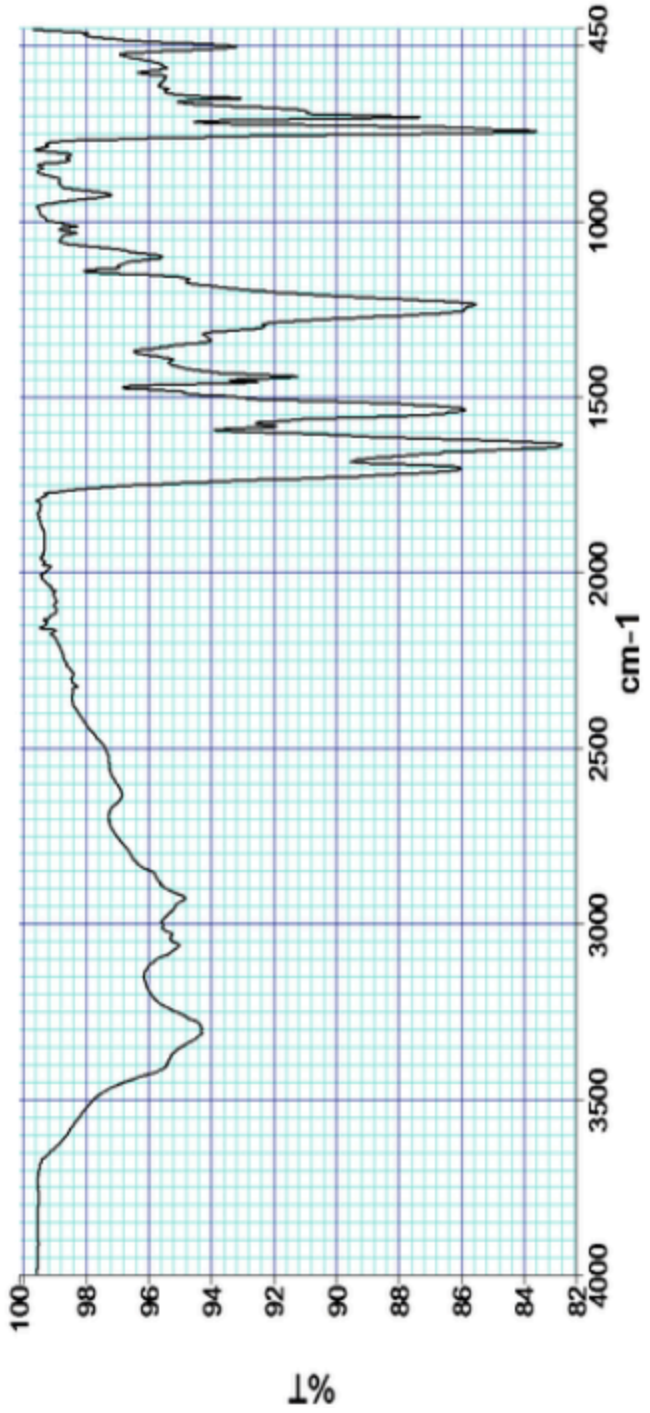
IR spectrum for compound **54l**



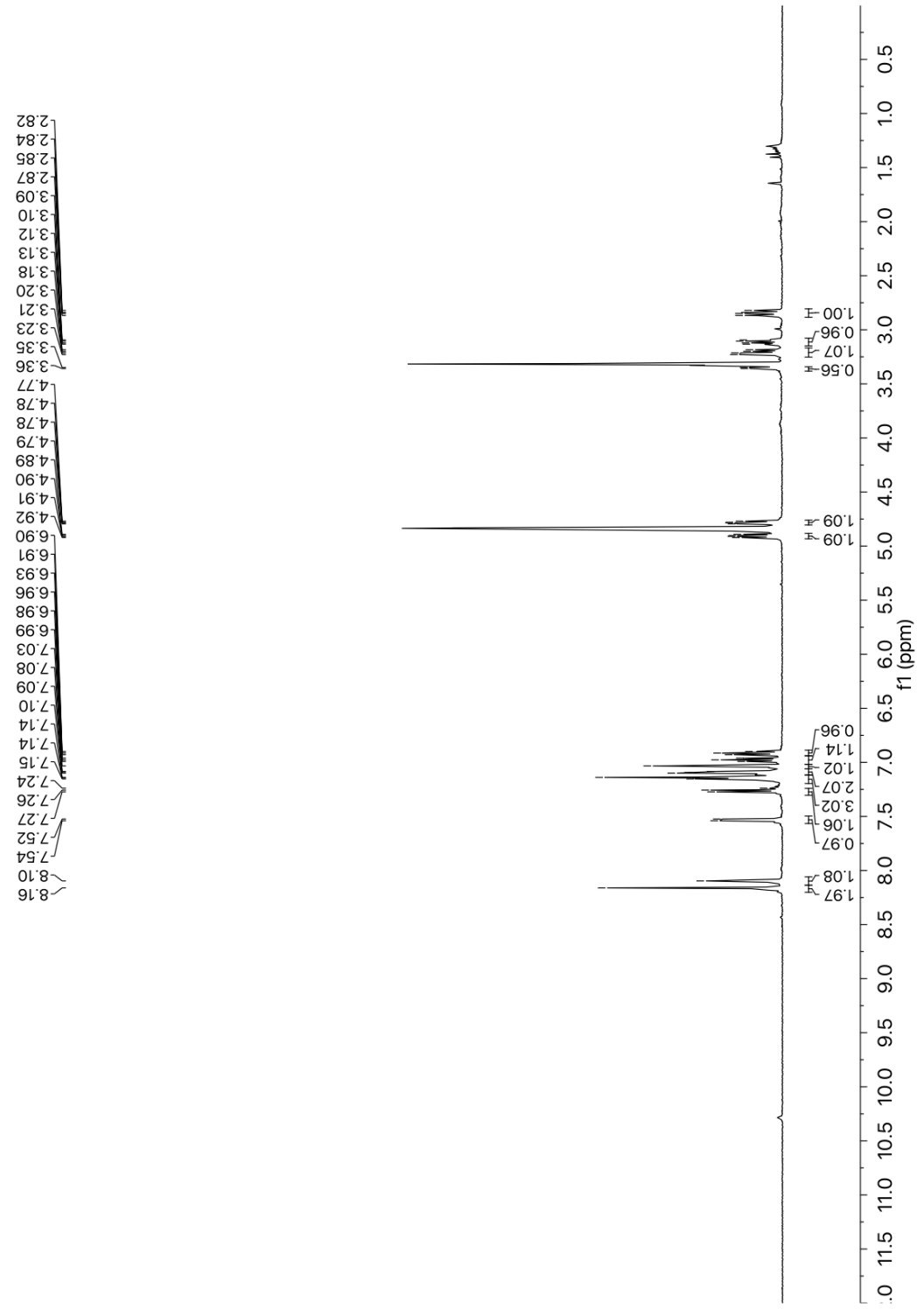
[1]HNMR spectrum for compound **54m**



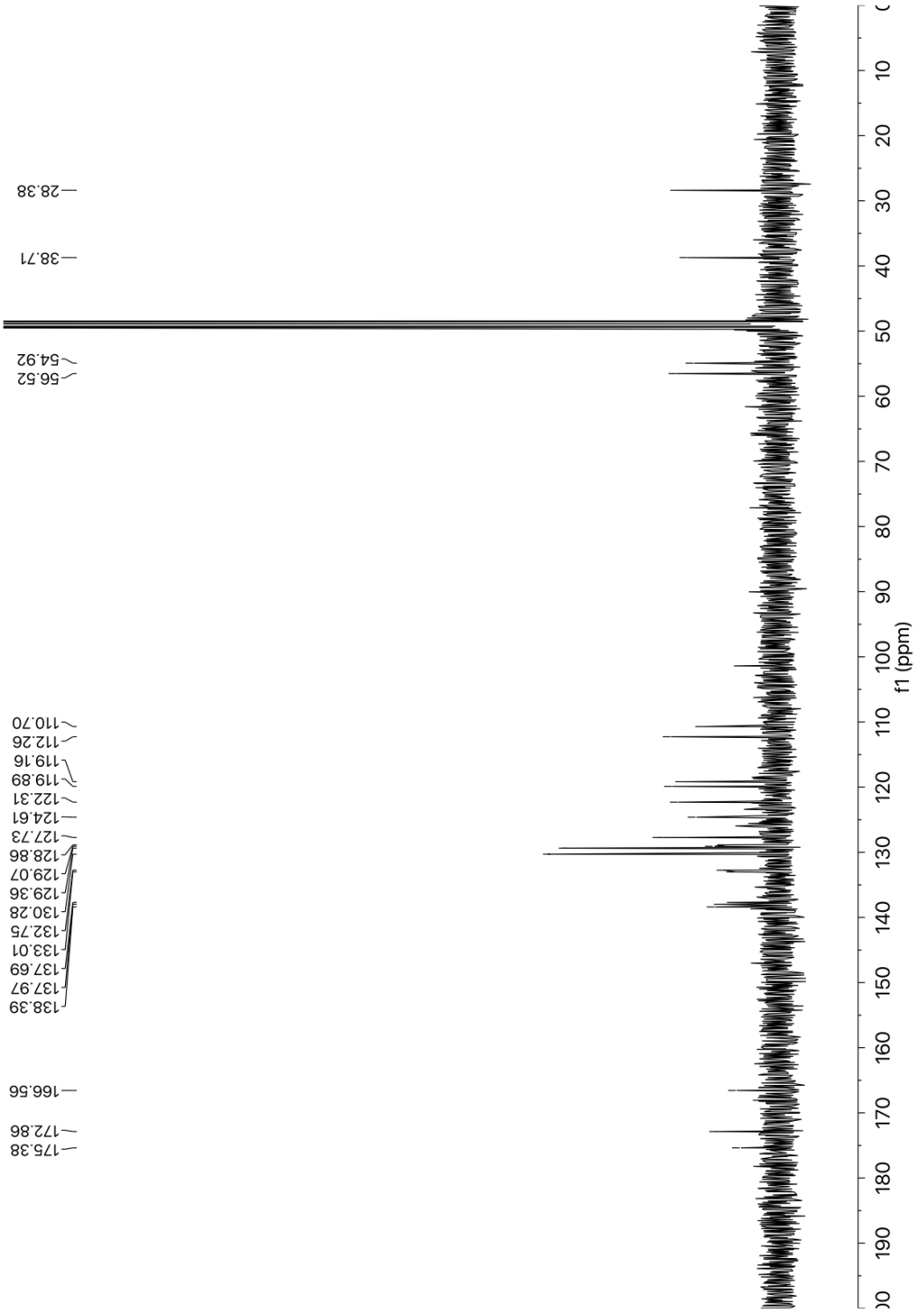
^{13}CNMR spectrum for compound **54m**



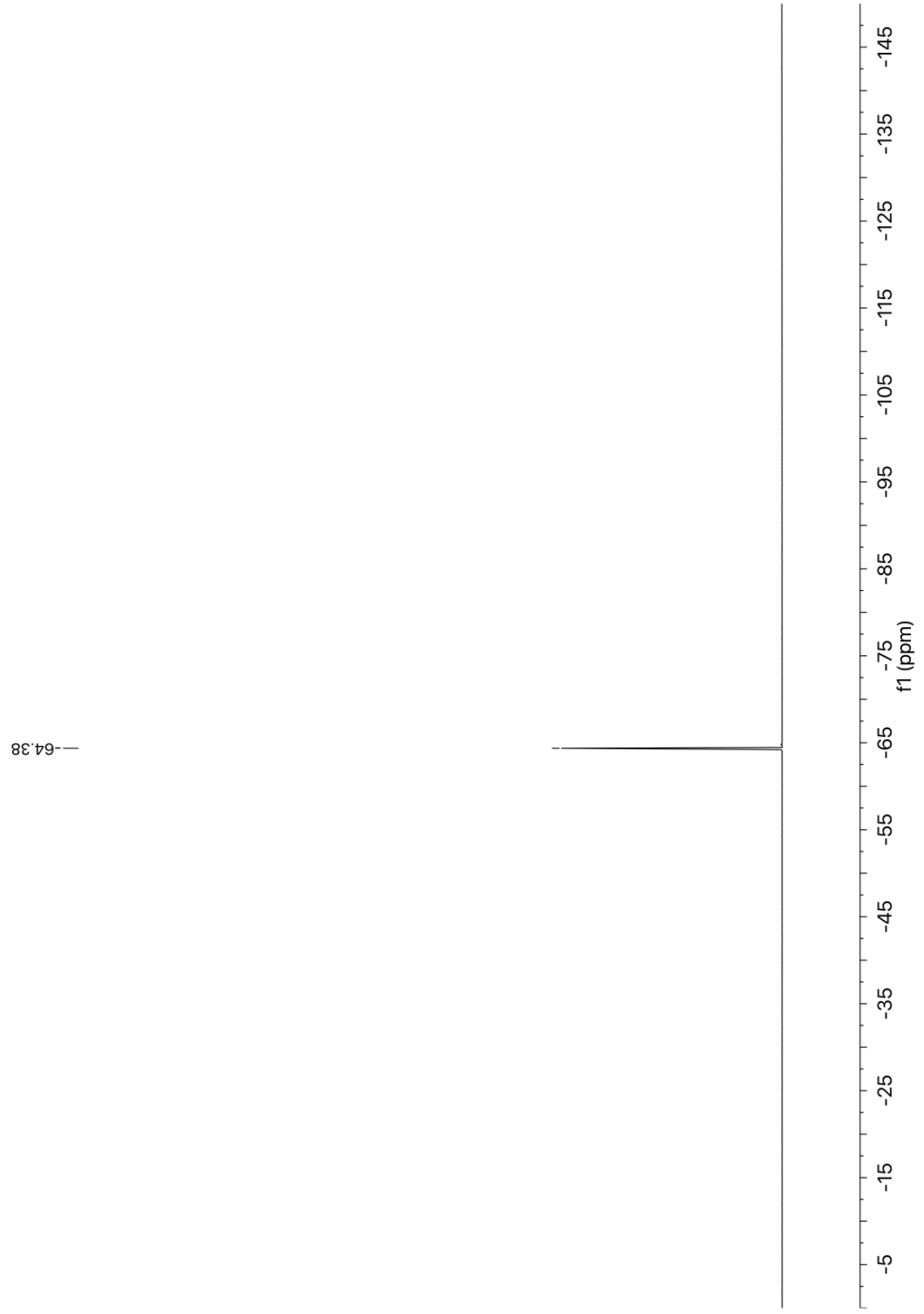
IR spectrum for compound **54m**



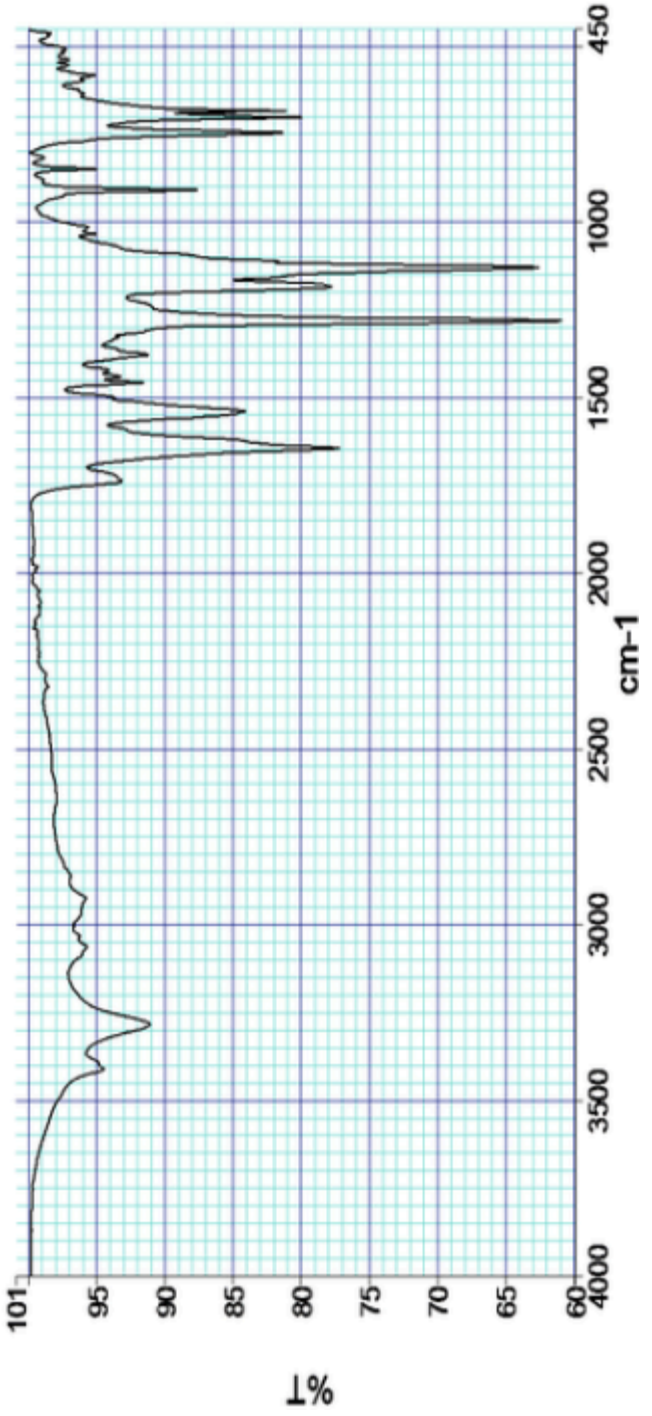
[1]HNMR spectrum for compound **54n**



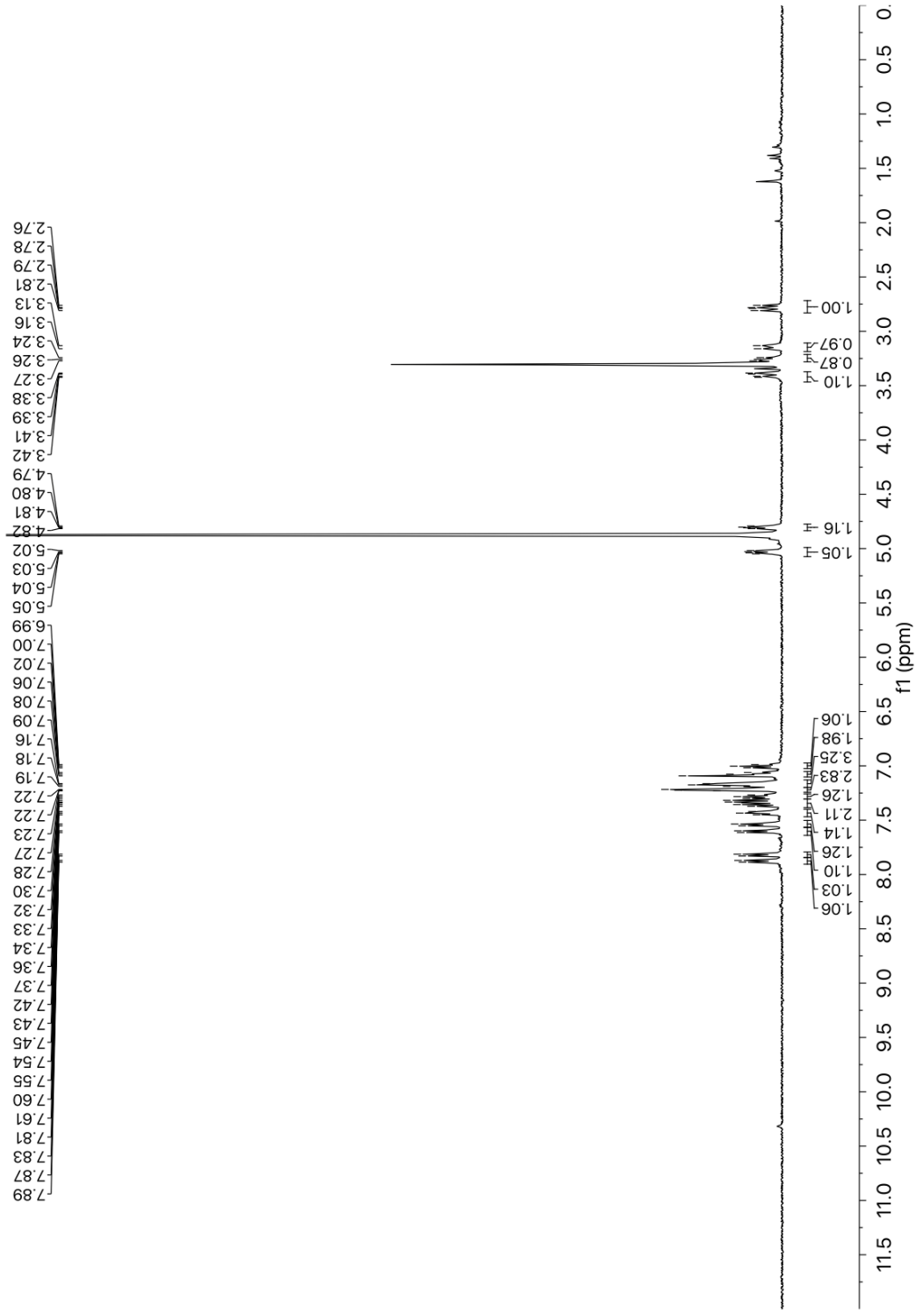
^{13}CNMR spectrum for compound **54n**



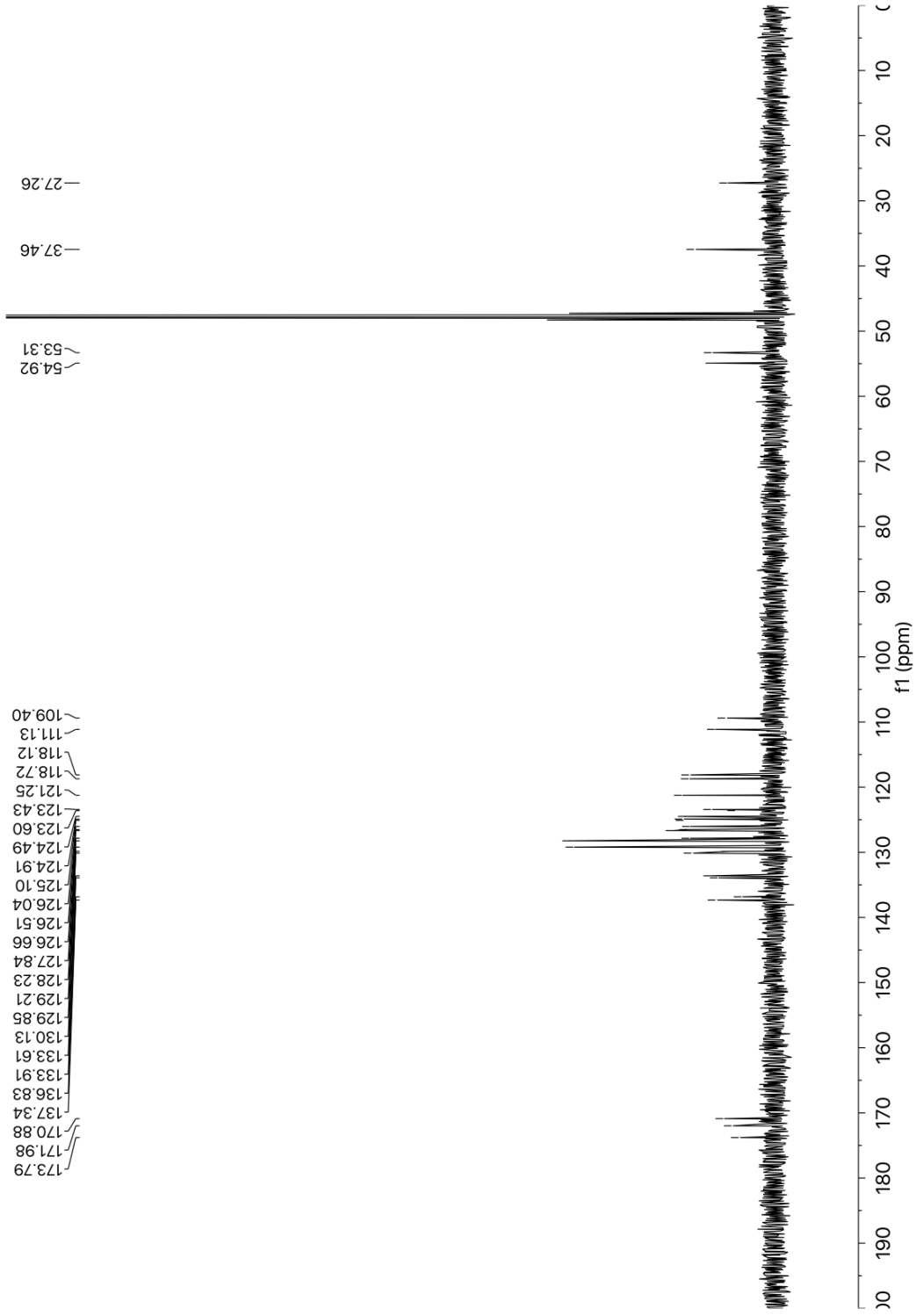
^{19}FNMR spectrum for compound **54n**



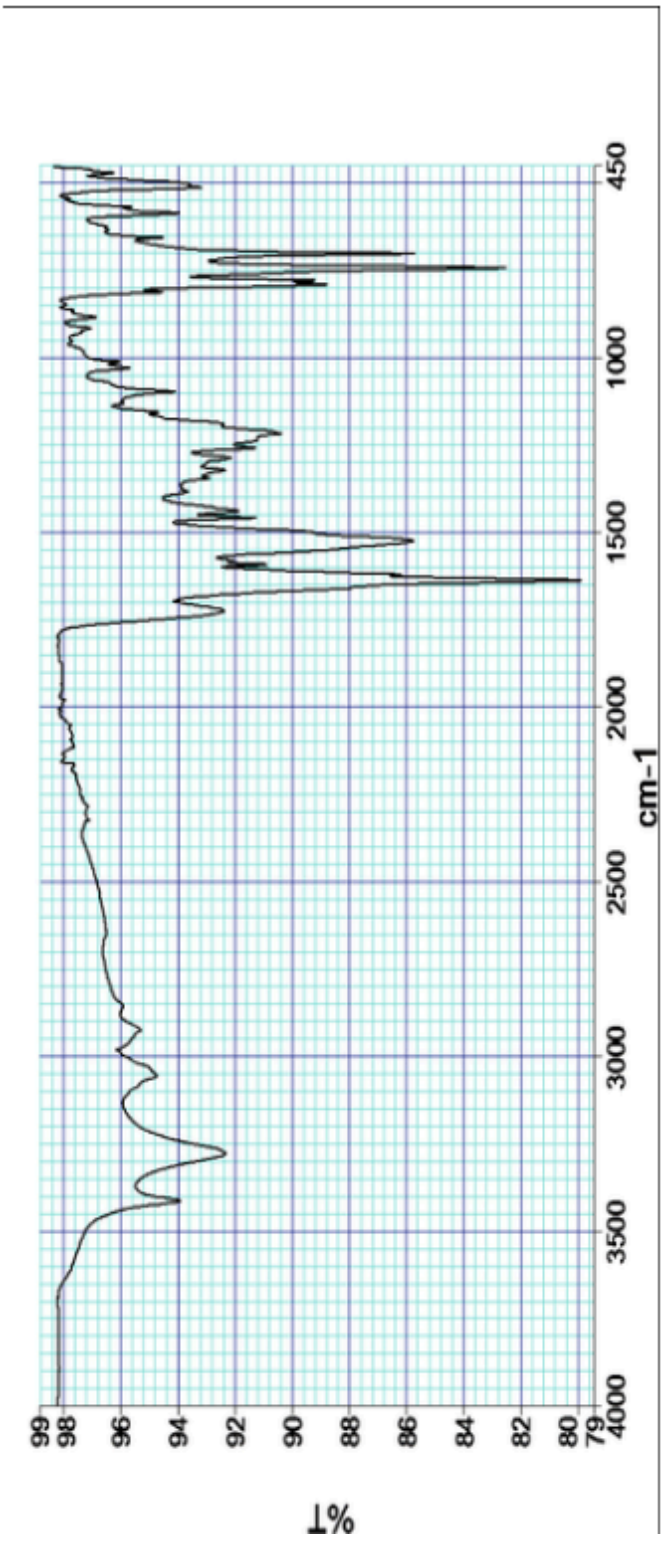
IR spectrum for compound **54n**



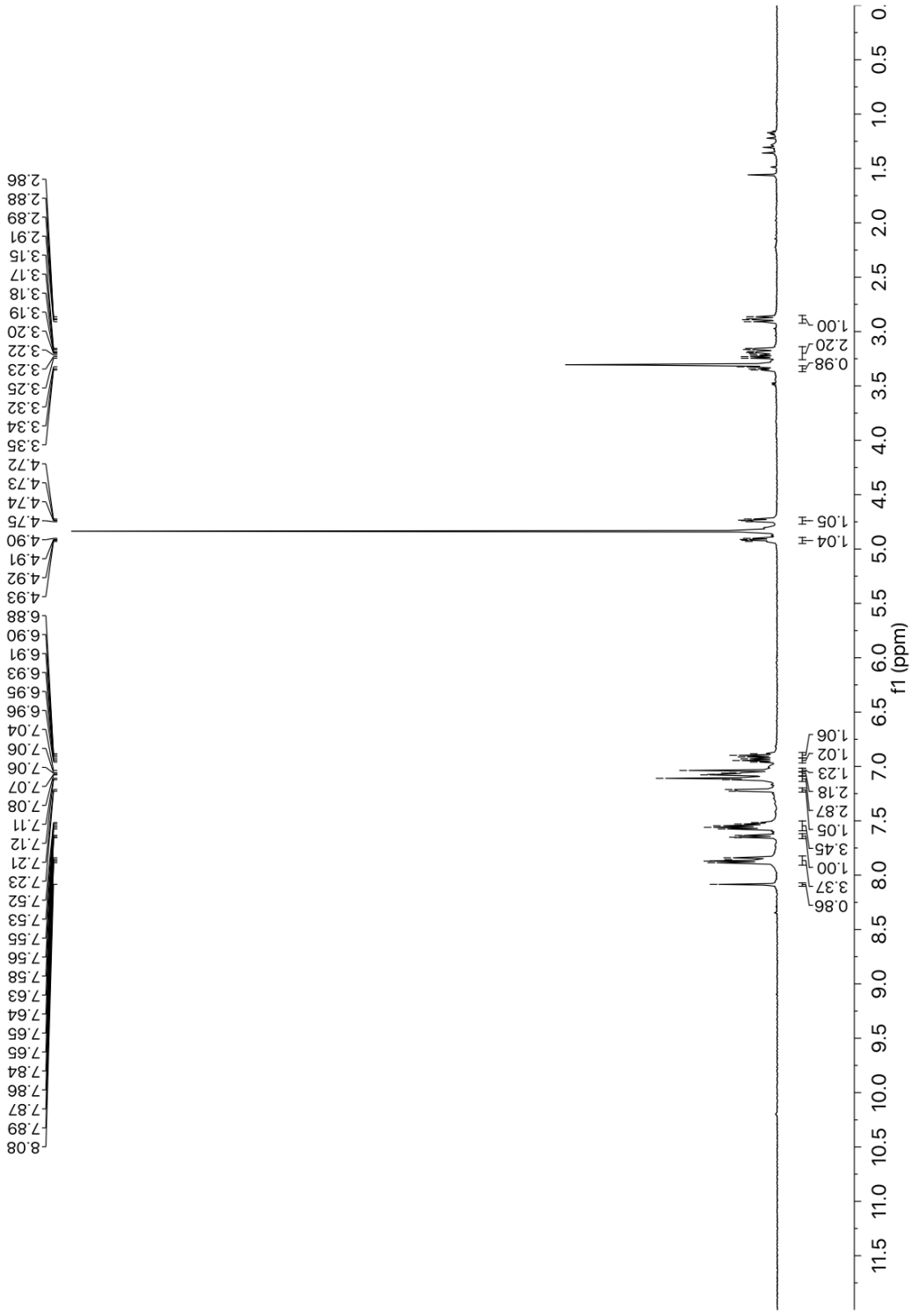
^{1}HNMR spectrum for compound **54o**



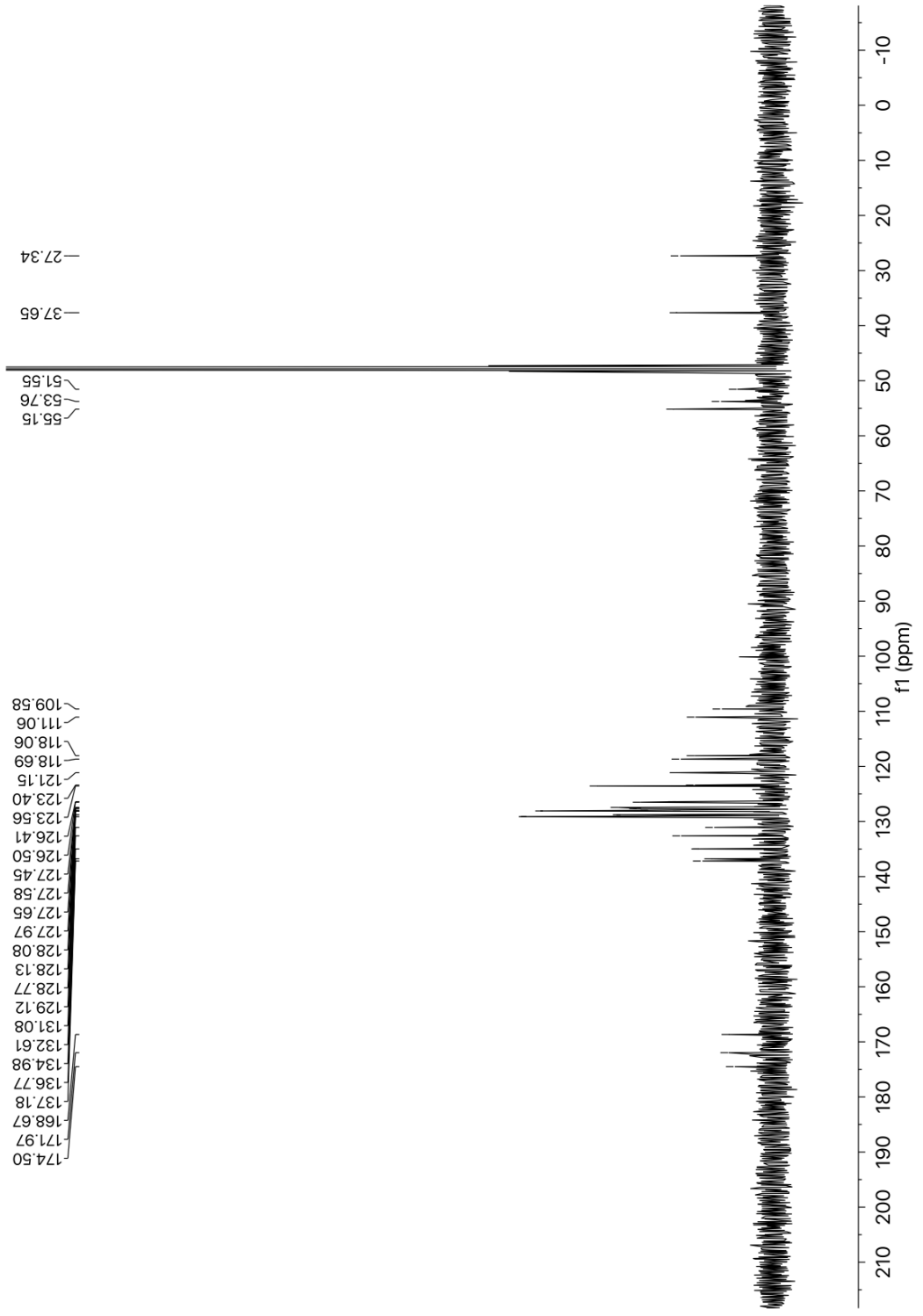
^{13}CNMR spectrum for compound **54o**



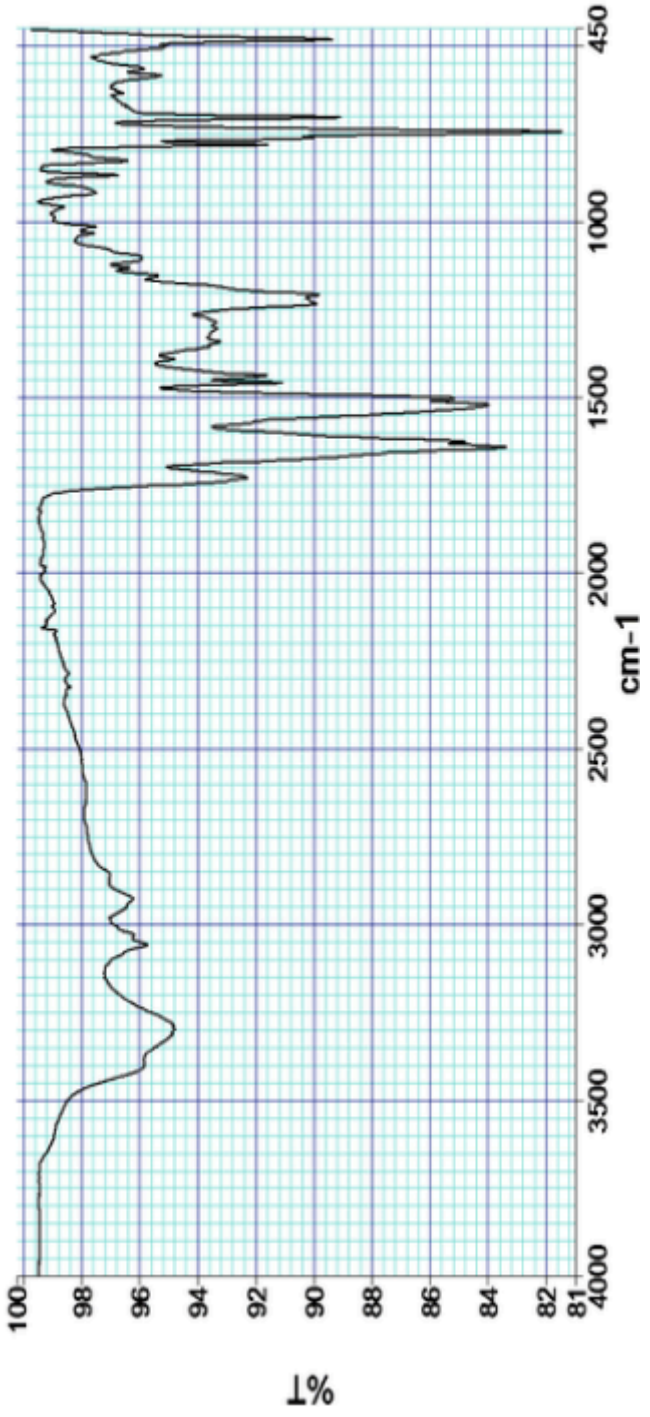
IR spectrum for compound **54o**



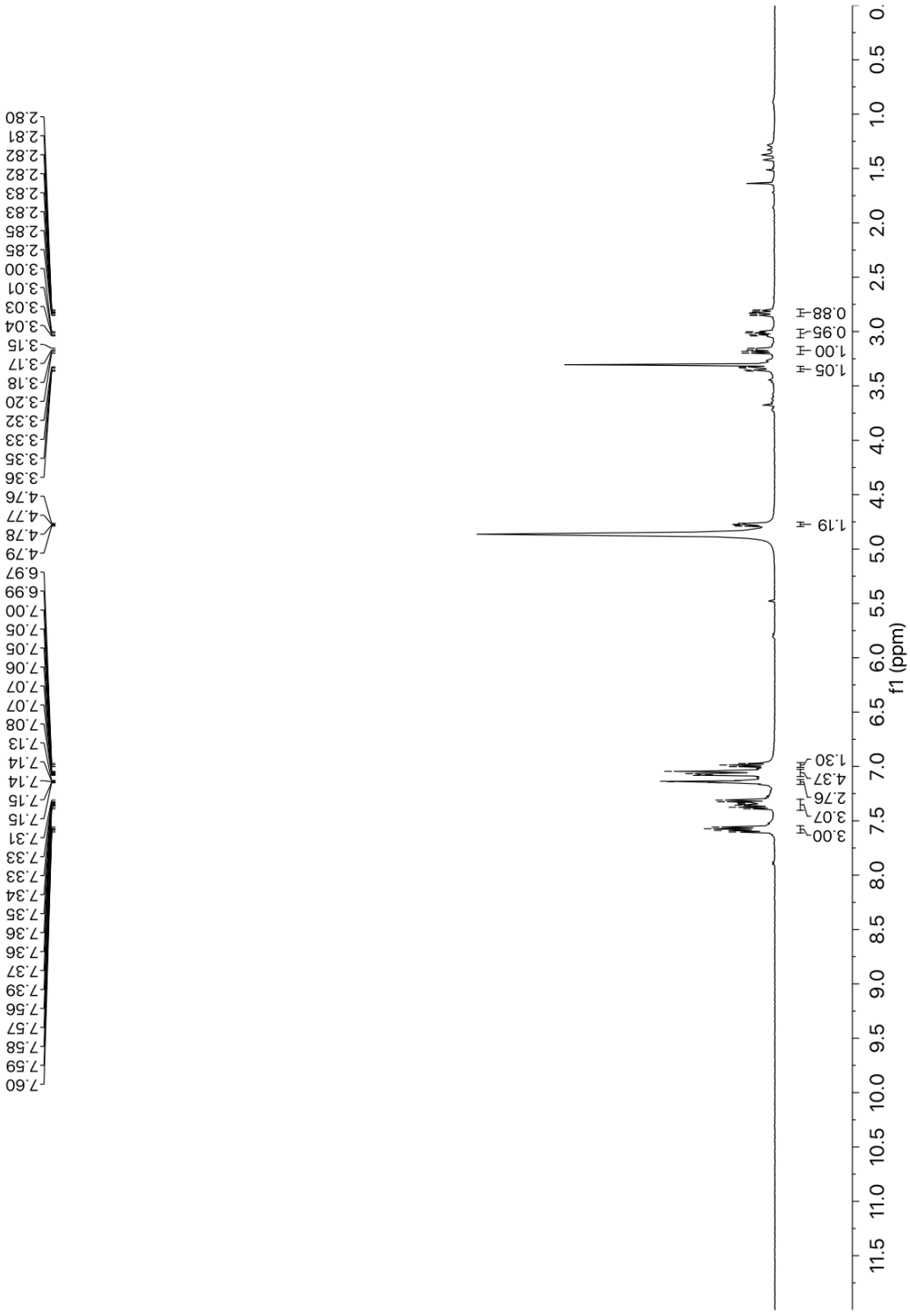
[1]HNMR spectrum for compound **54p**



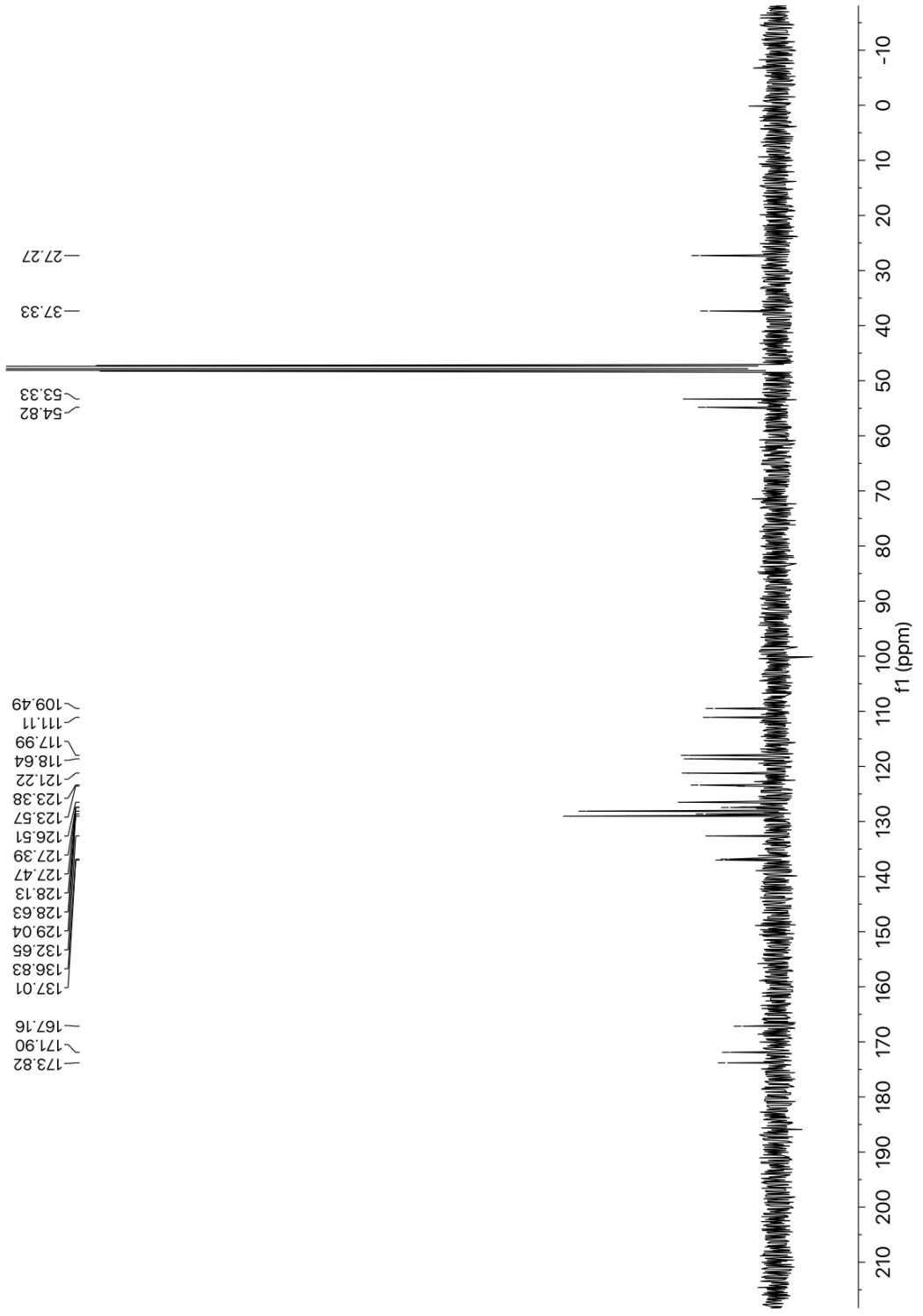
^{13}CNMR spectrum for compound **54p**



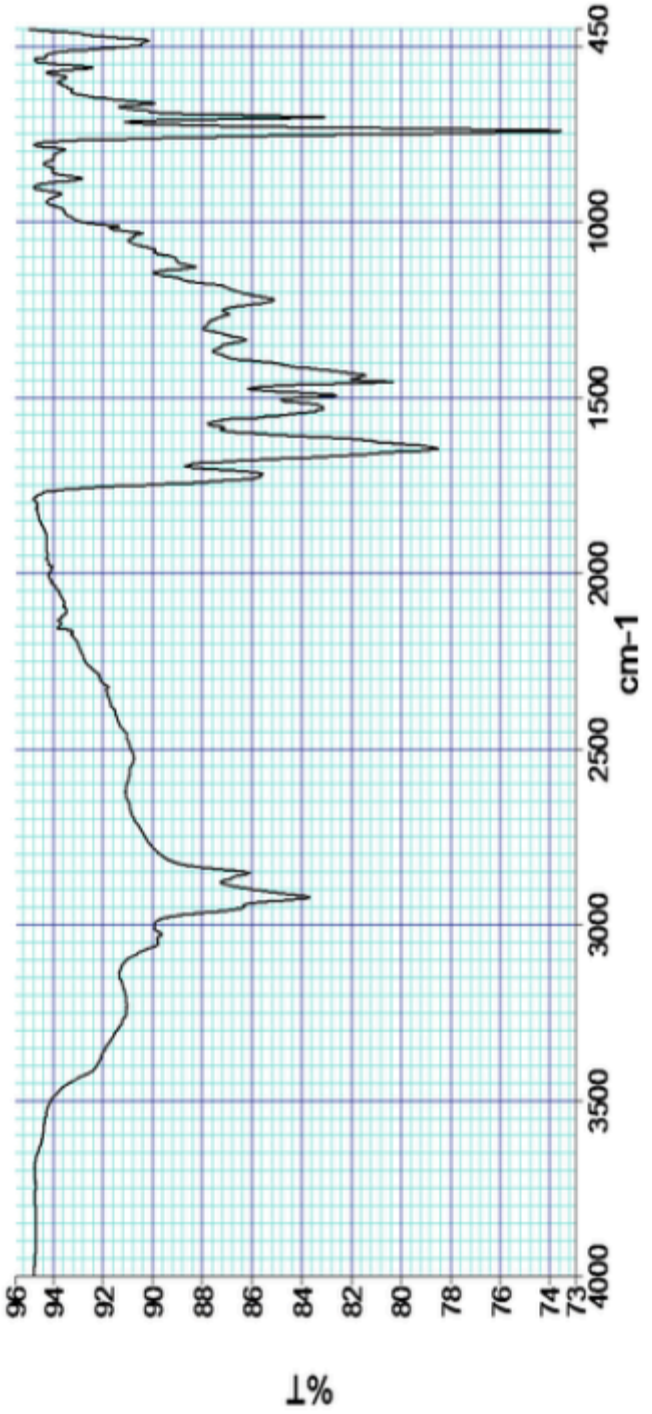
IR spectrum for compound **54p**



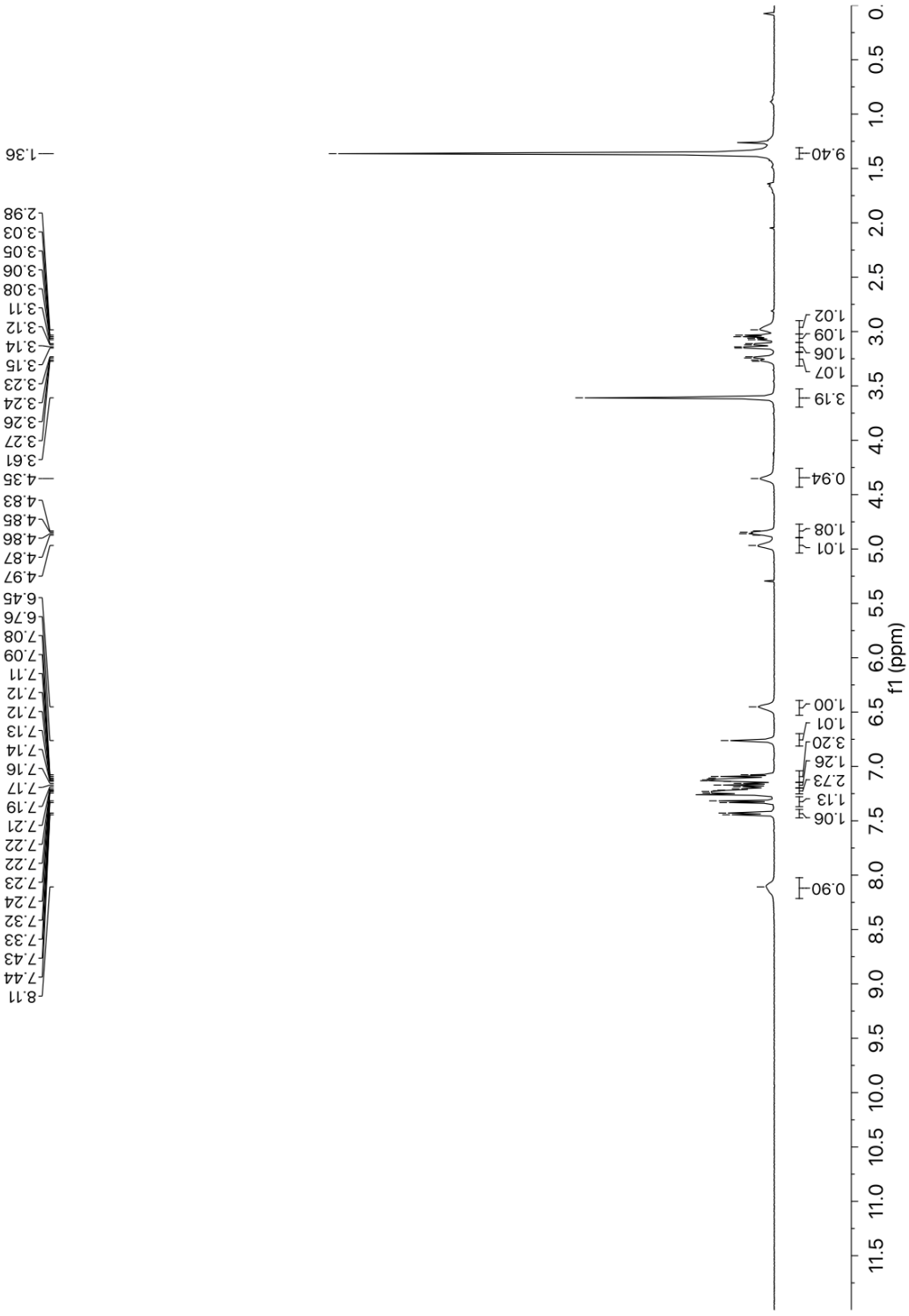
[1]HNMR spectrum for compound **54q**



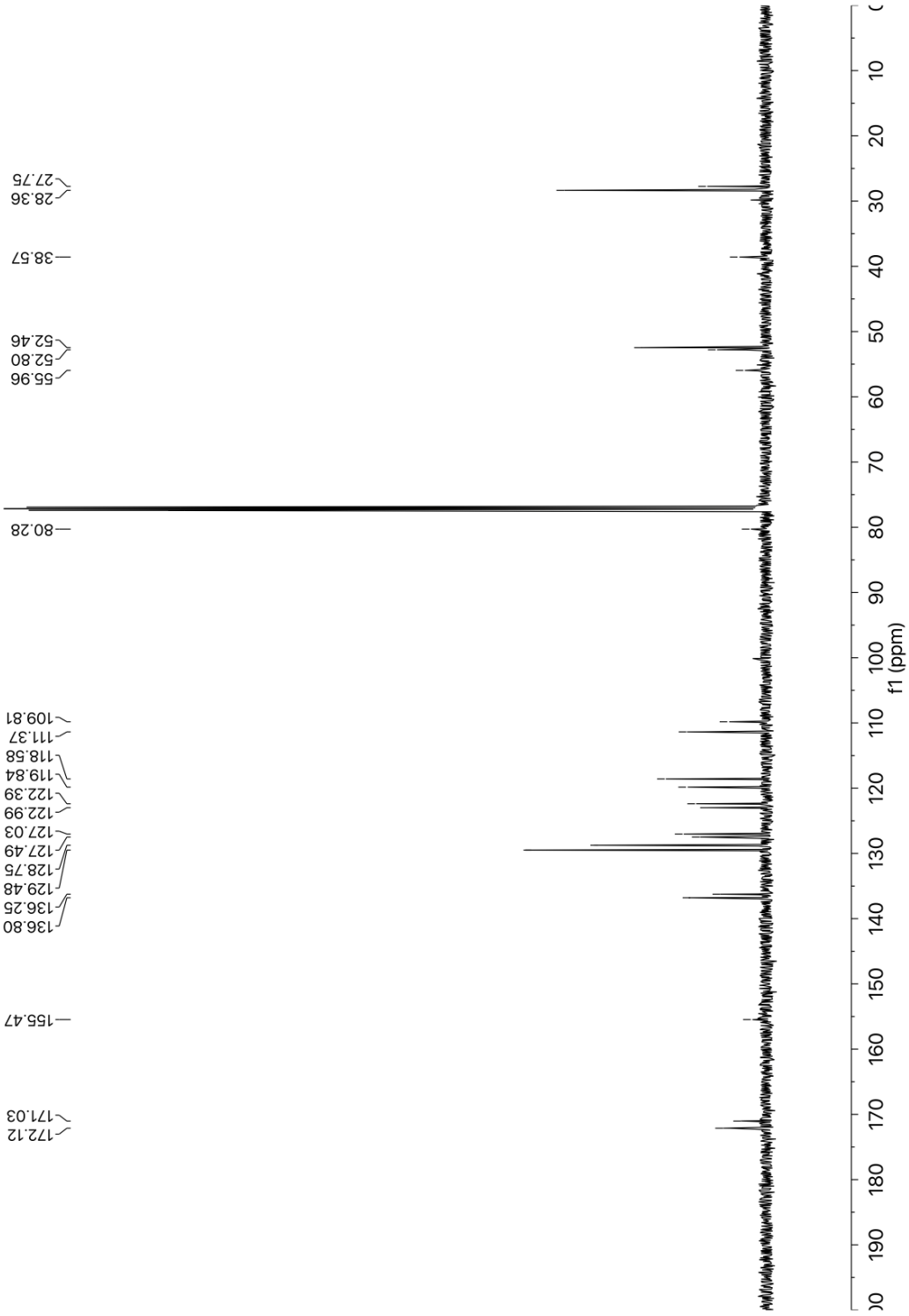
^{13}CNMR spectrum for compound **54q**



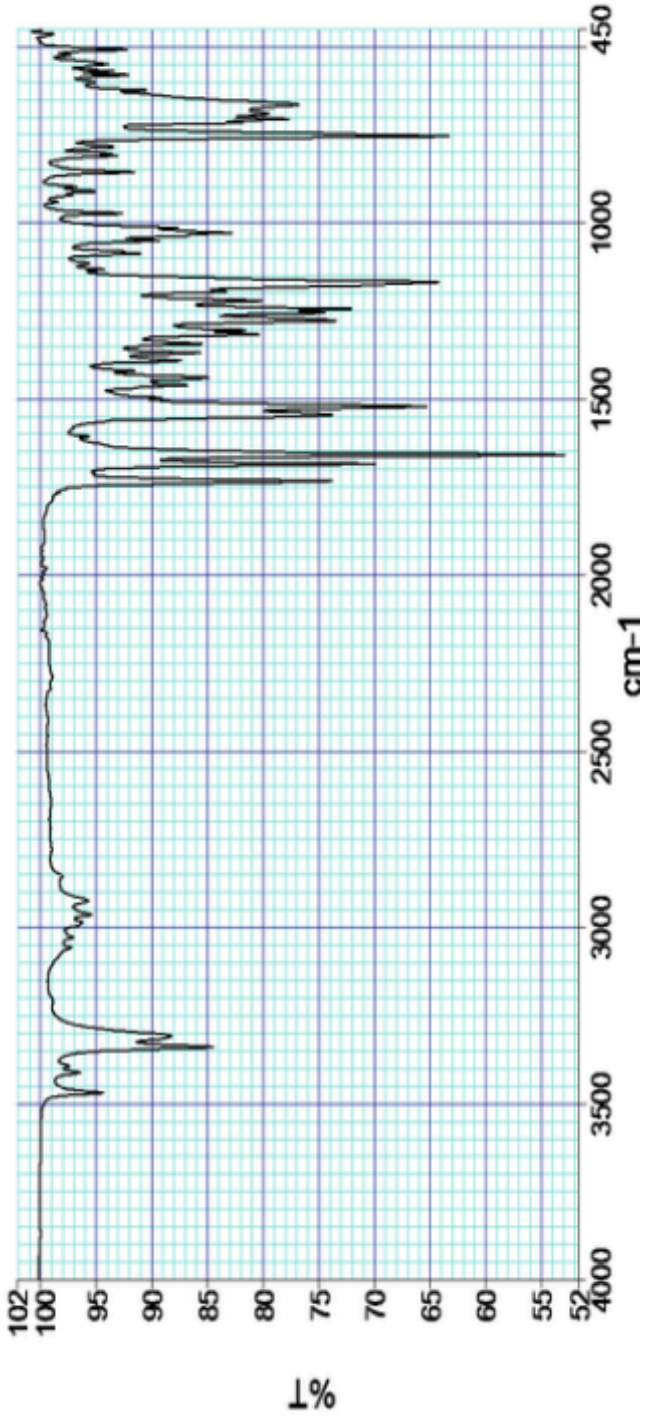
IR spectrum for compound **54q**



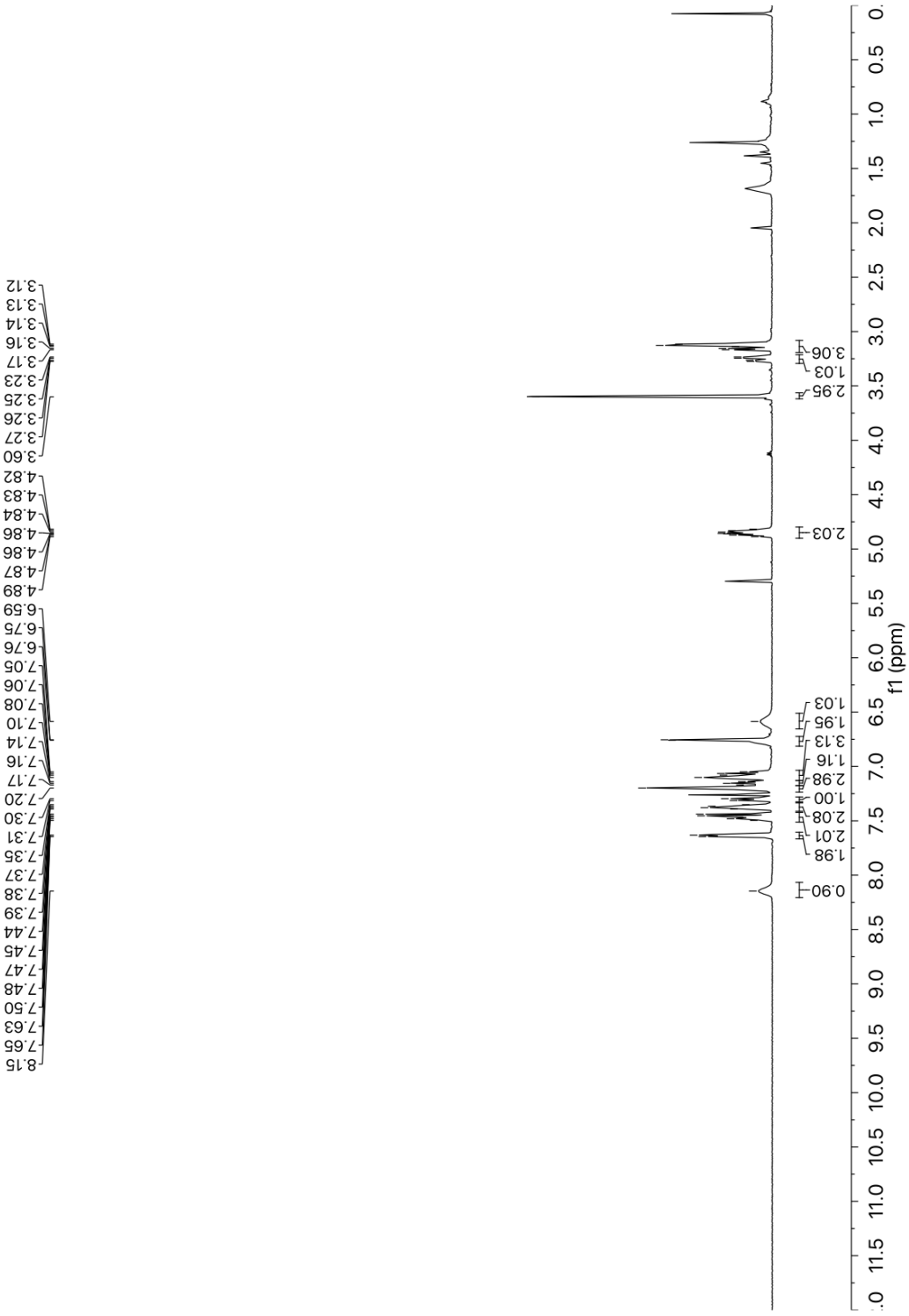
^{1}HNMR spectrum for compound **55**



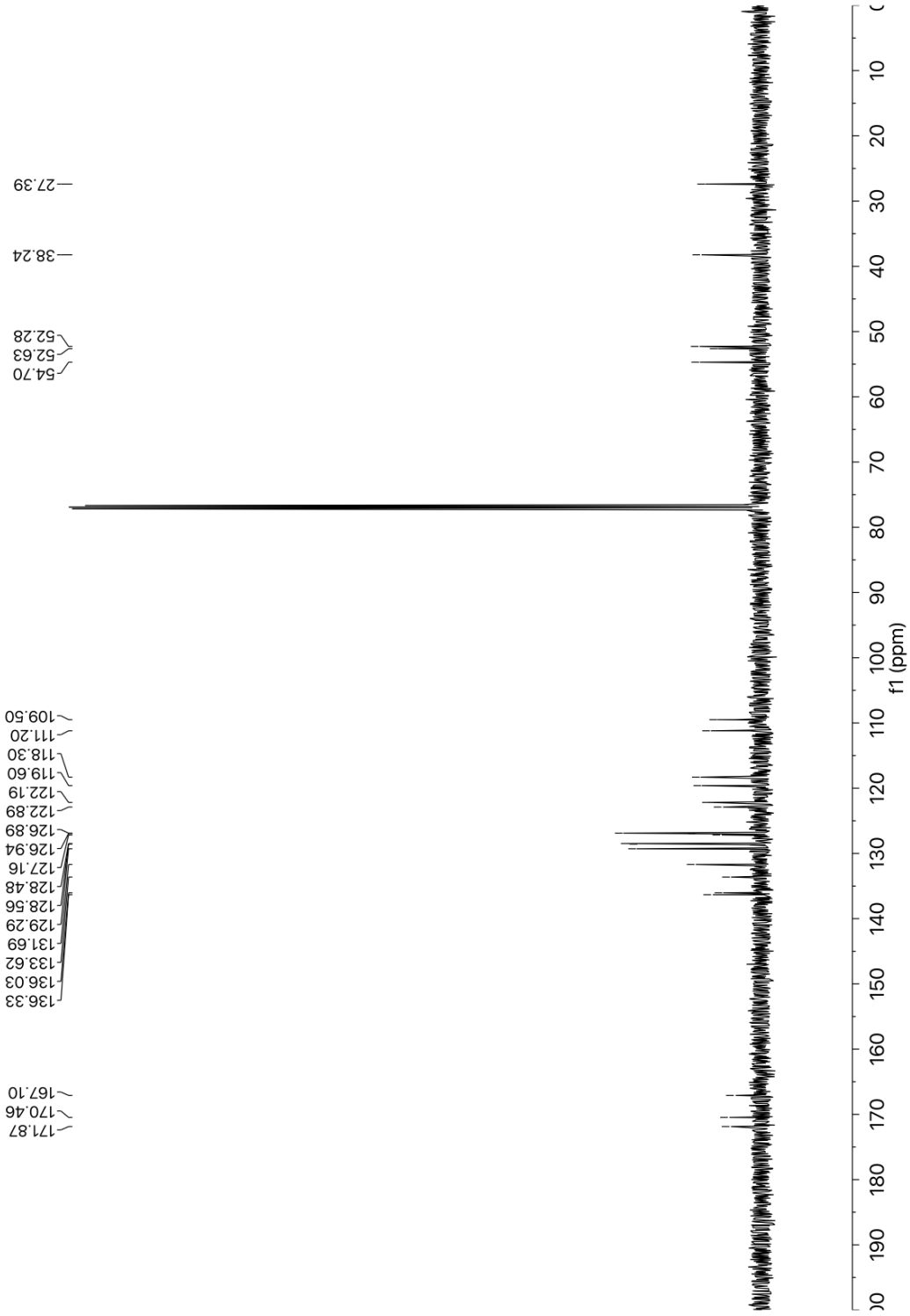
^{13}CNMR spectrum for compound **55**



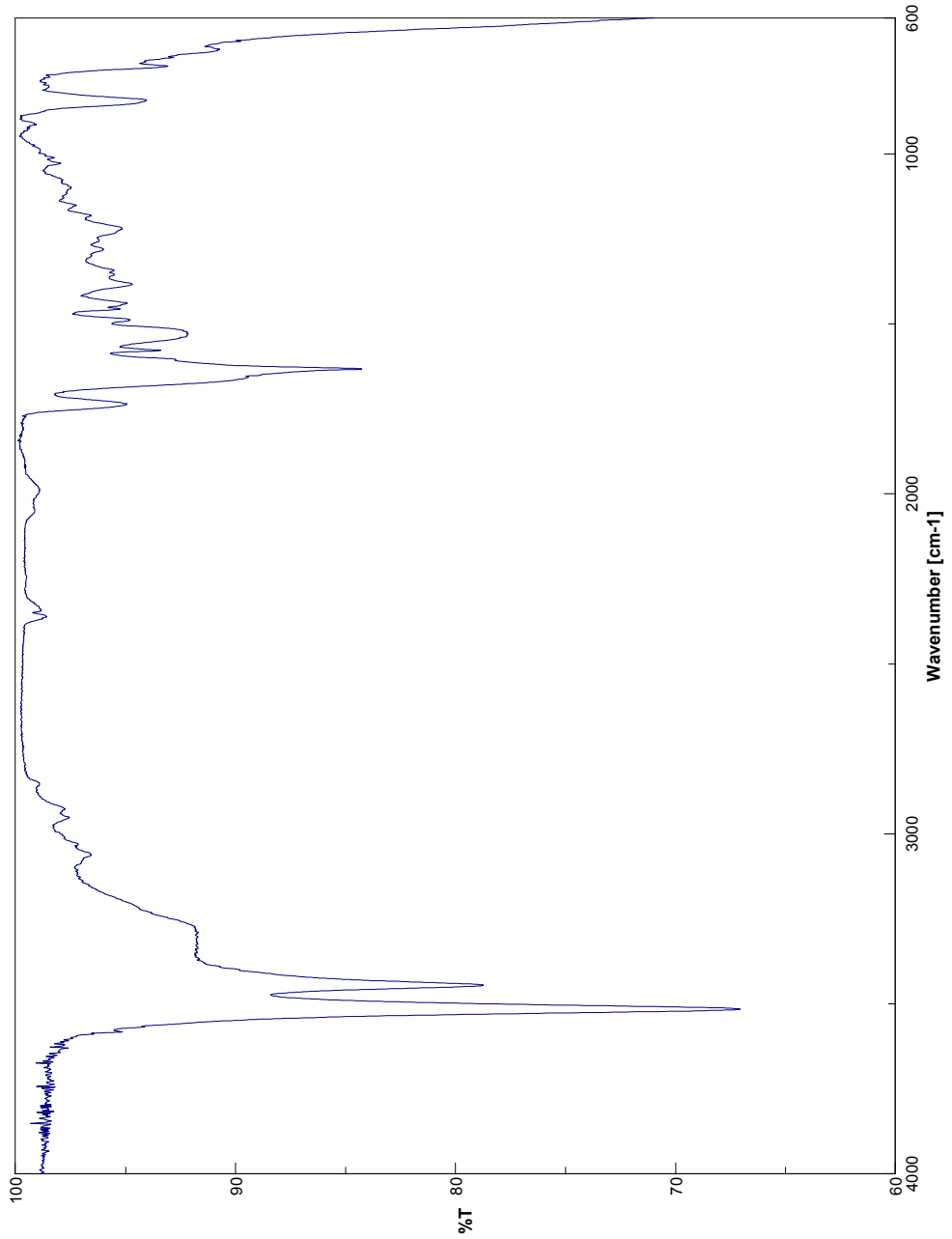
IR spectrum for compound **55**



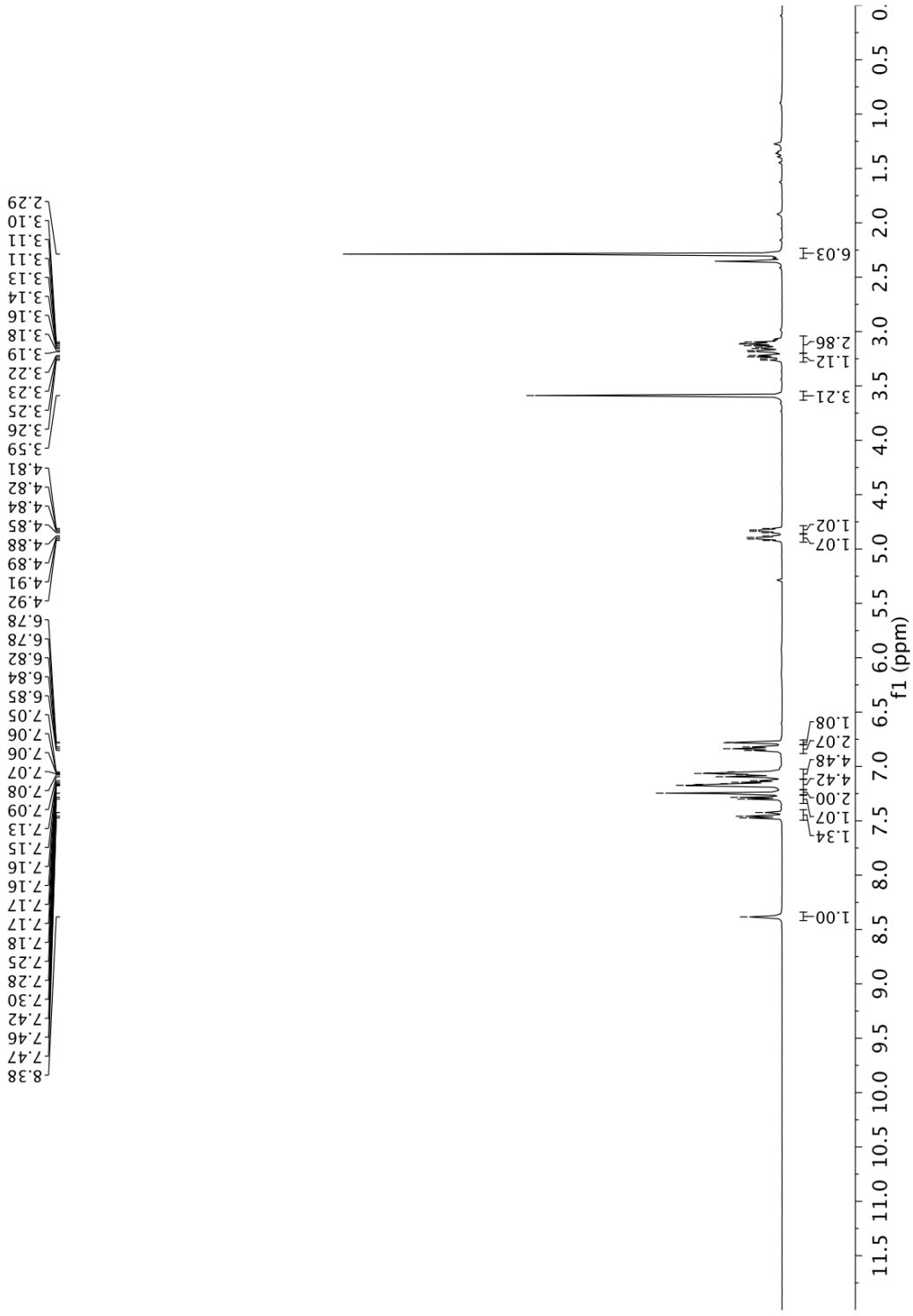
[1]HNMR spectrum for compound **56a**



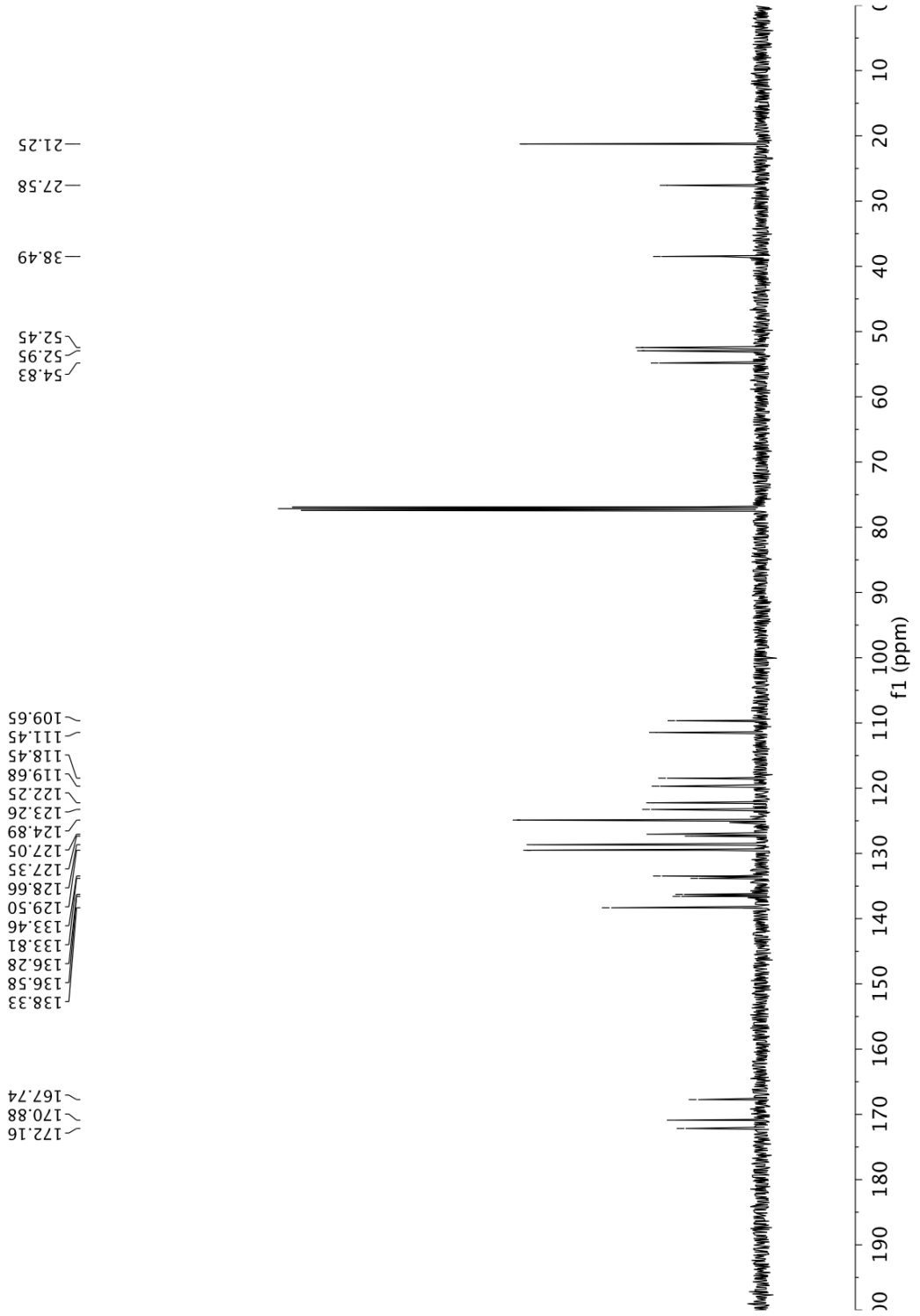
^{13}CNMR spectrum for compound **56a**



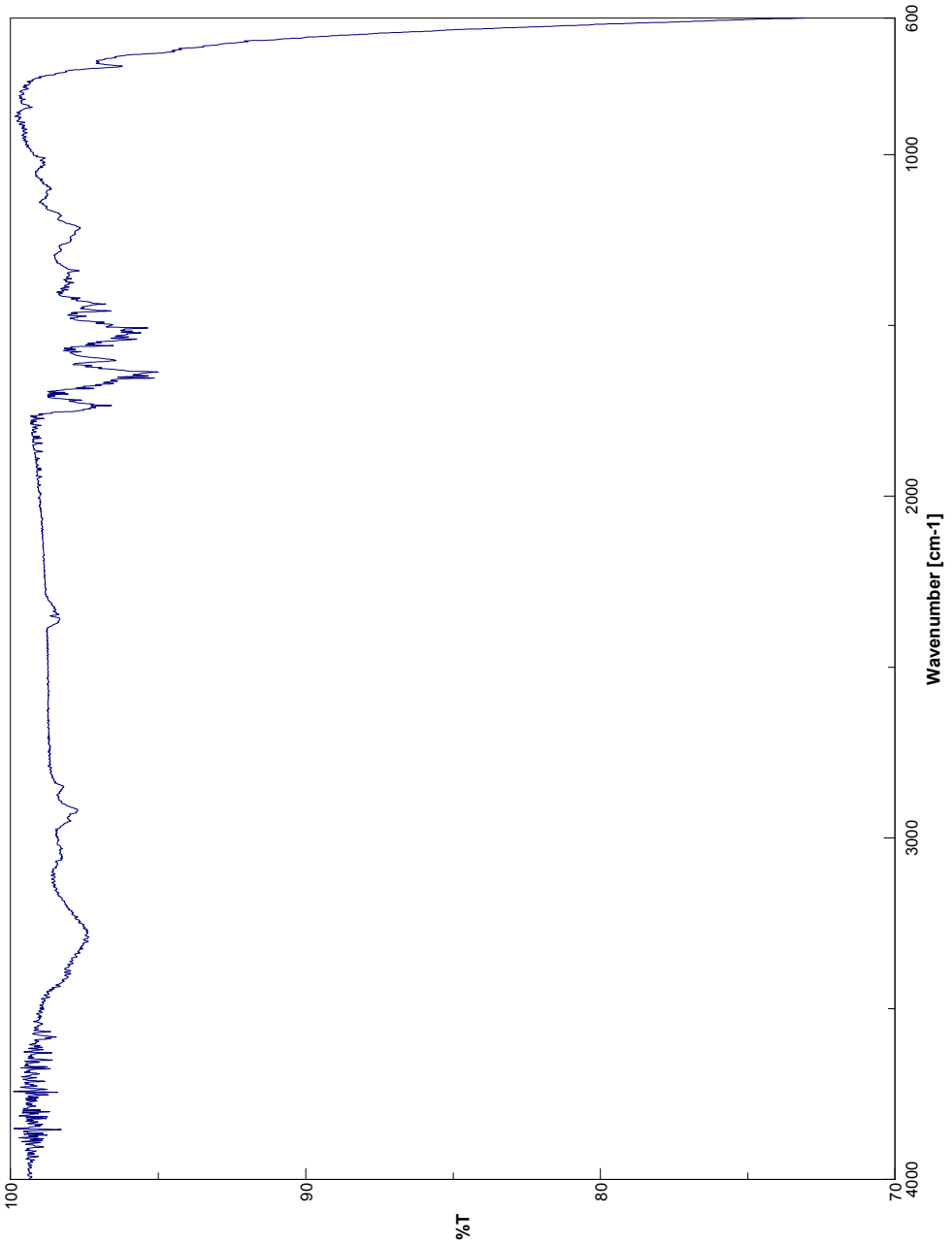
IR spectrum for compound **56a**



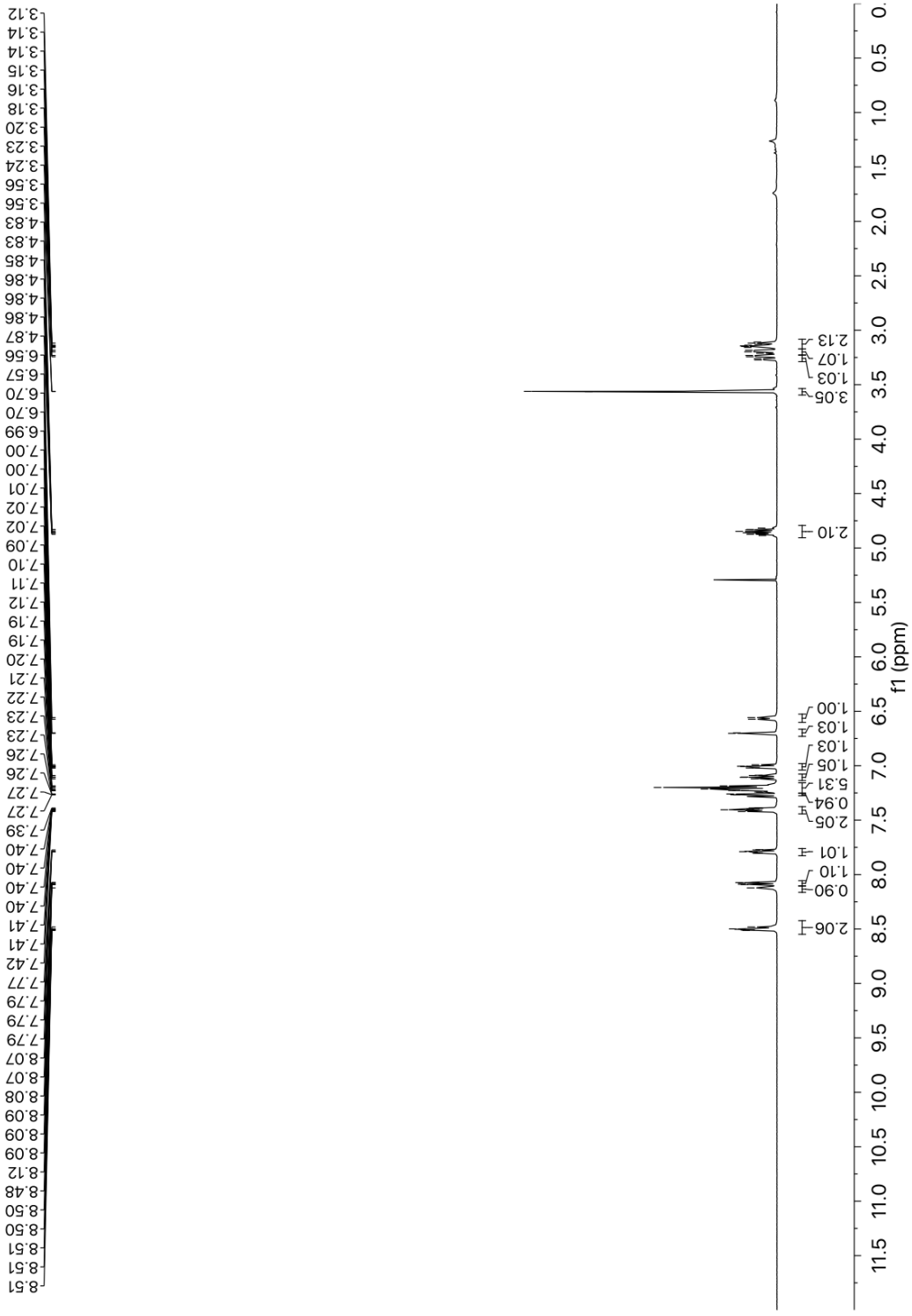
[1]HNMR spectrum for compound **56b**



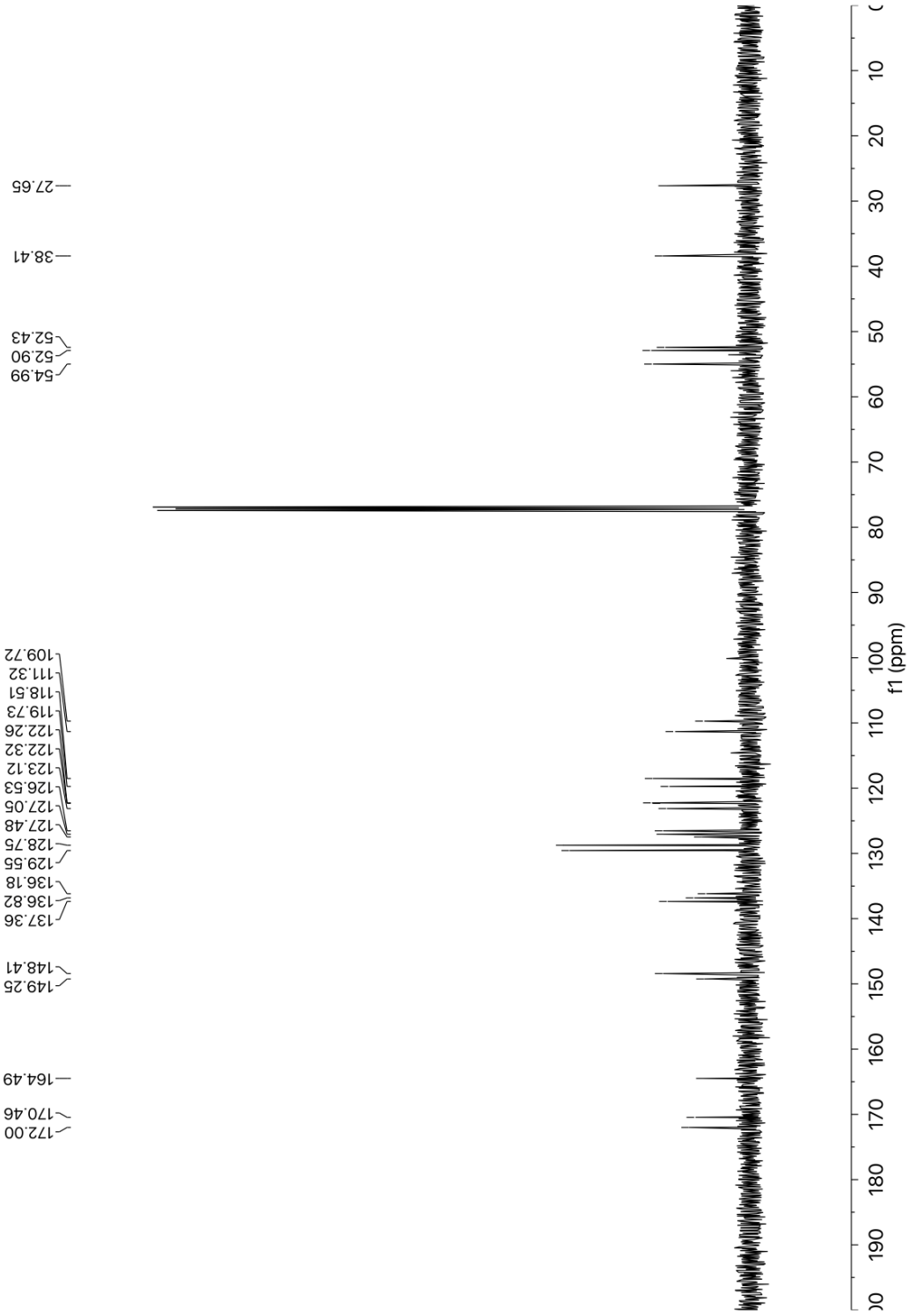
^{13}CNMR spectrum for compound **56b**



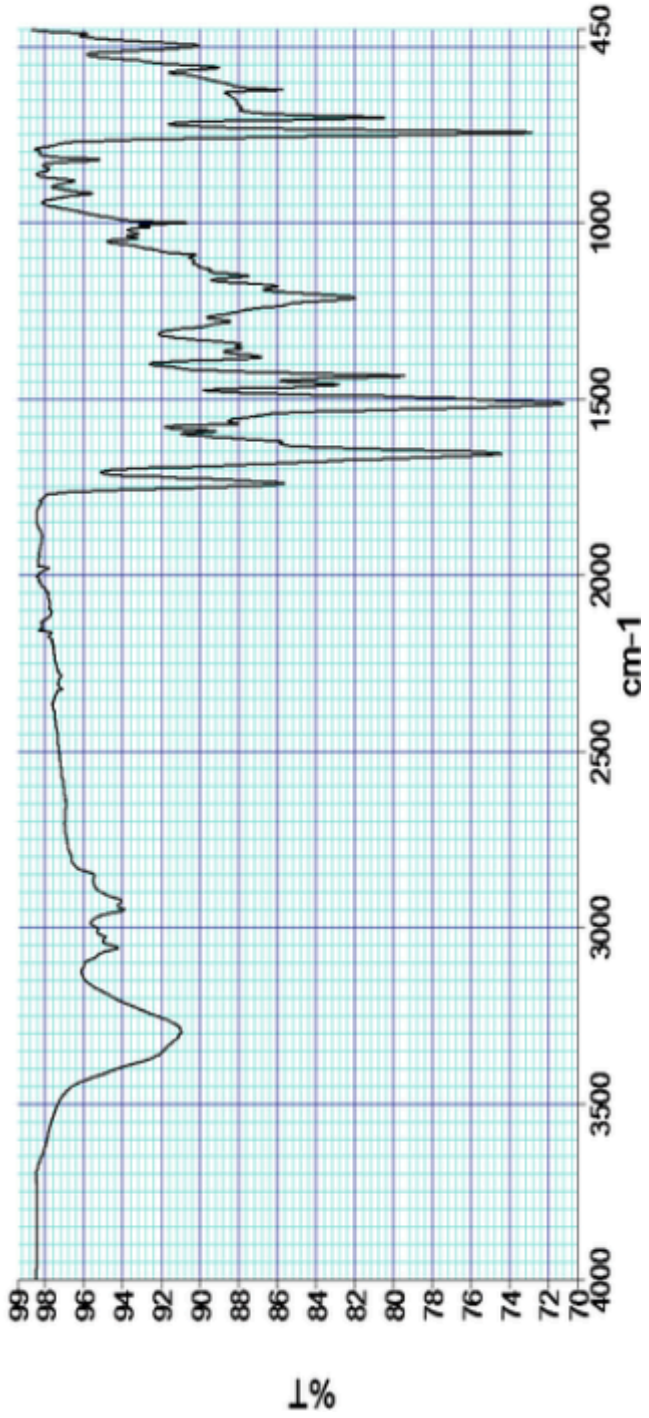
IR spectrum for compound **56b**



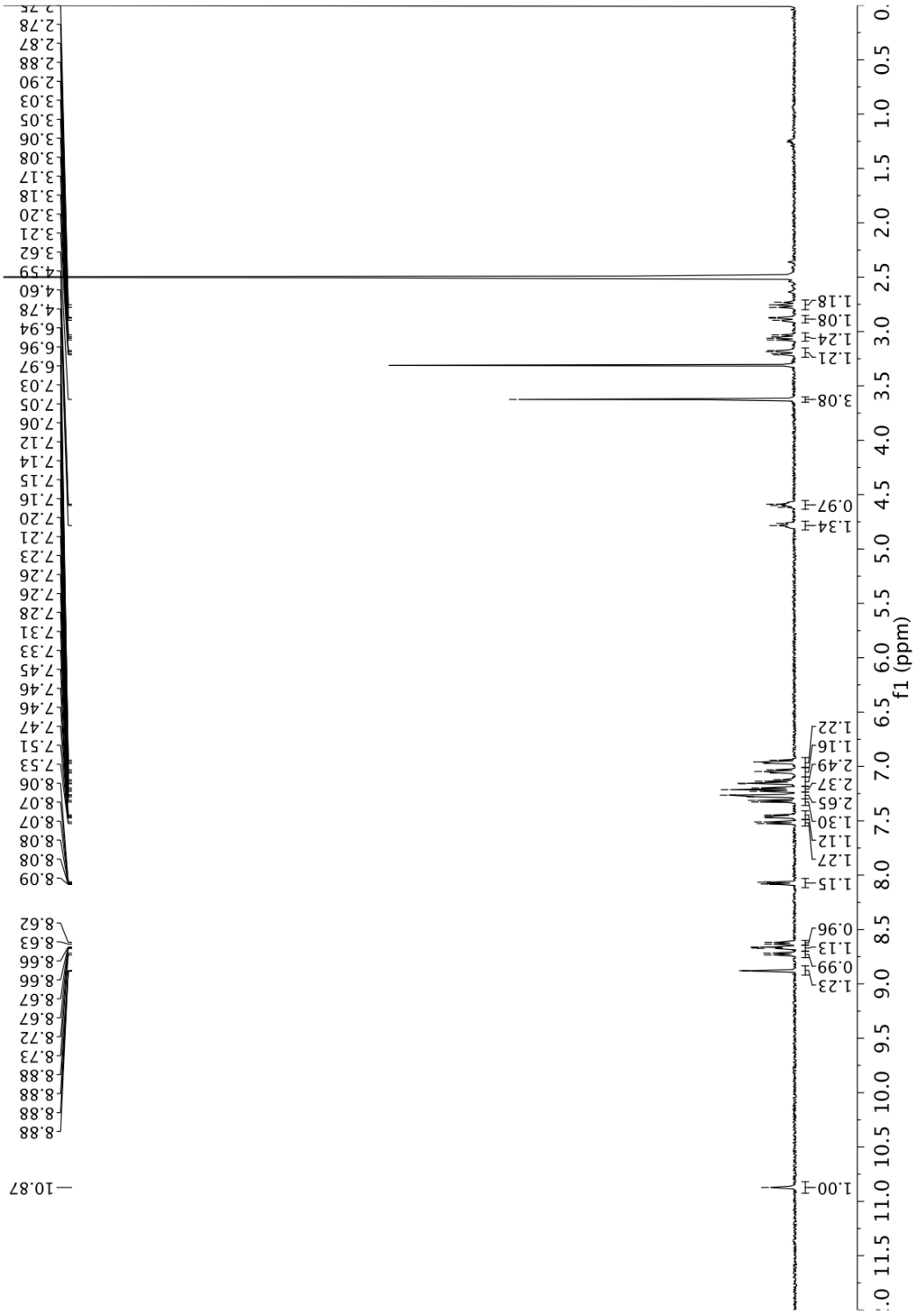
^{1}HNMR spectrum for compound **56c**



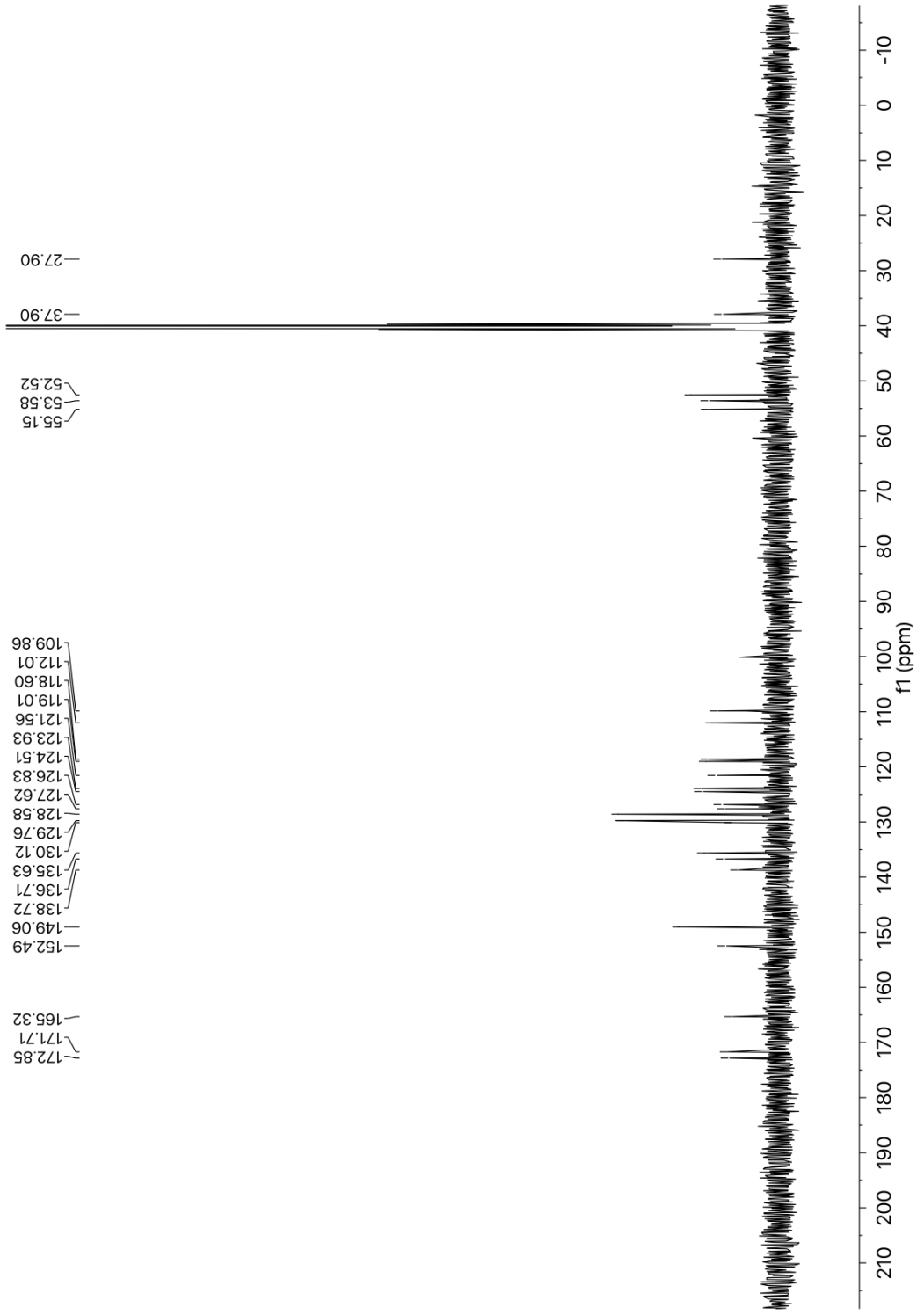
^{13}CNMR spectrum for compound **56c**



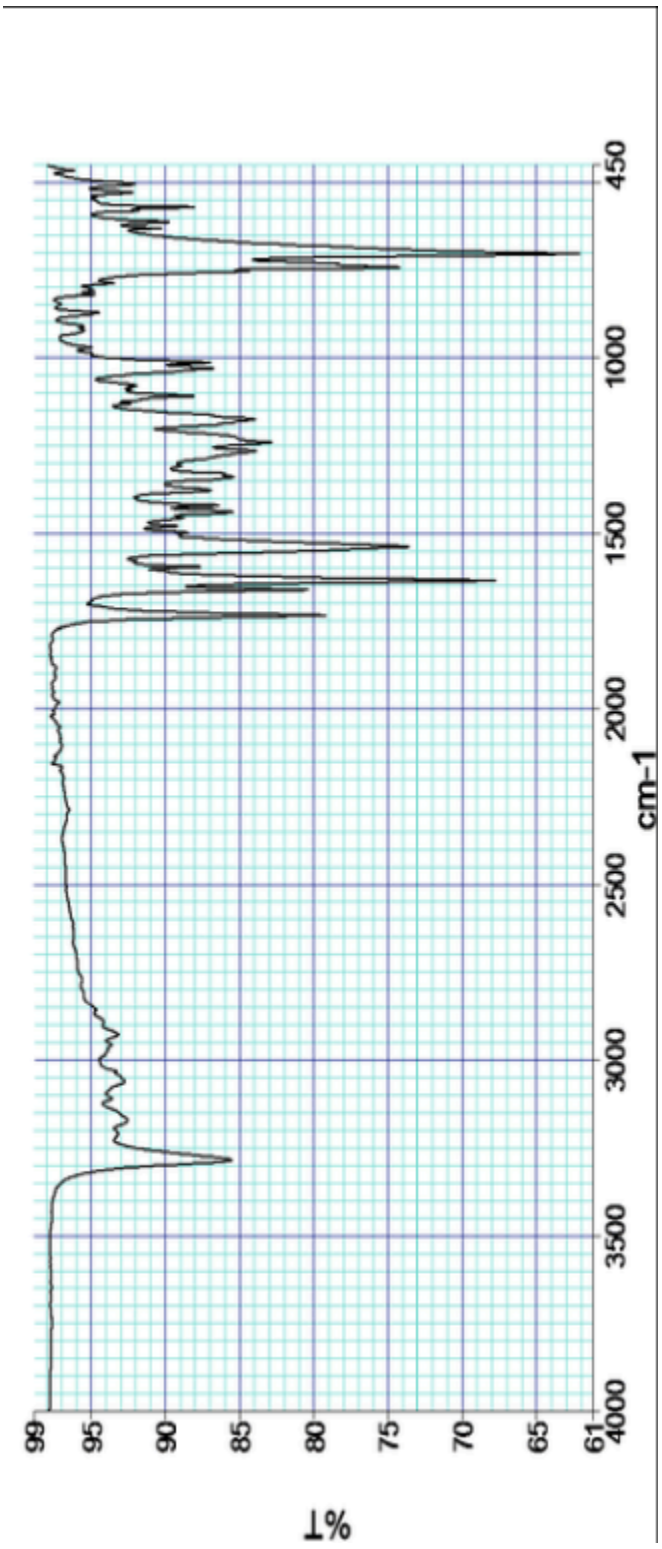
IR spectrum for compound **56c**



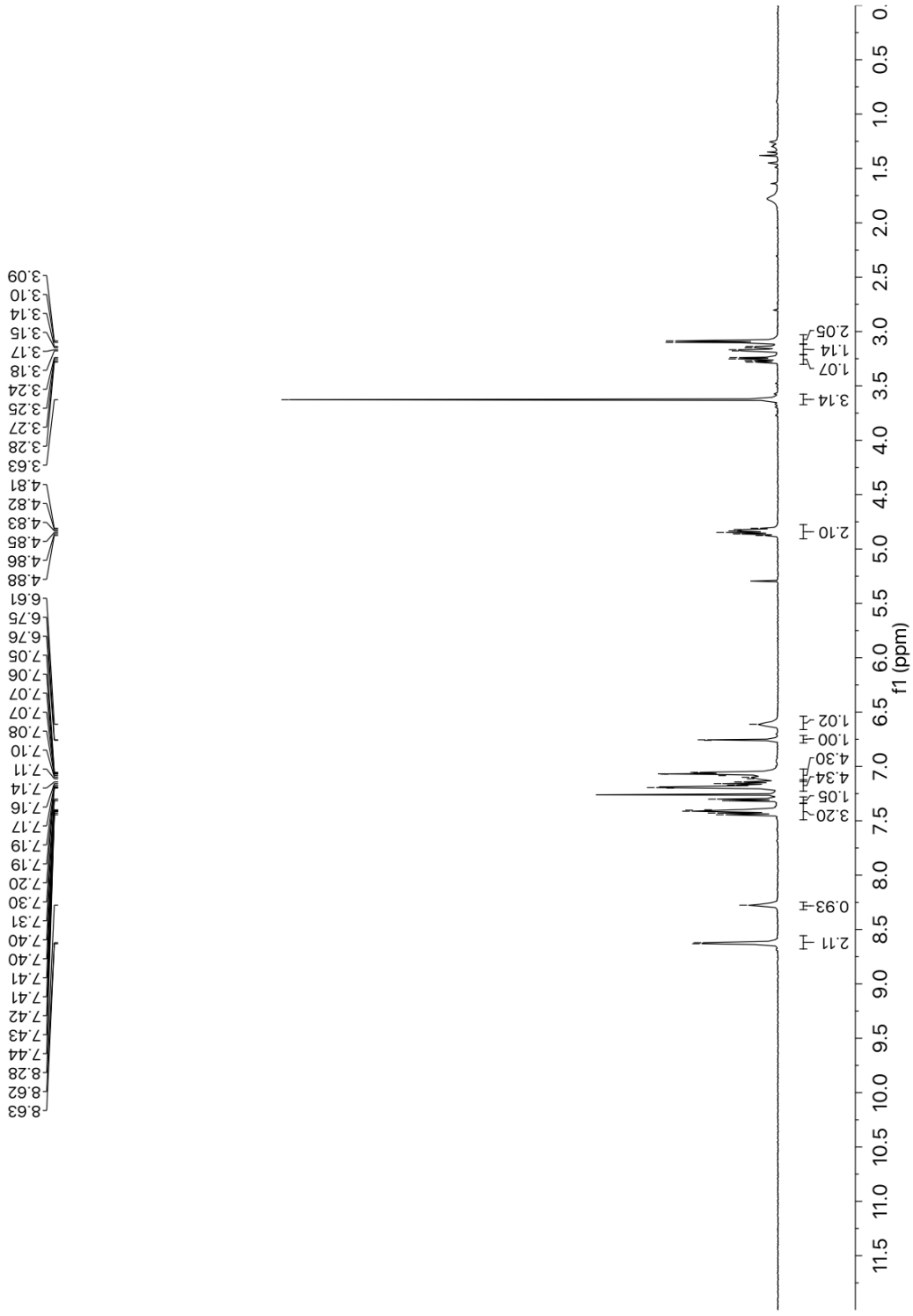
[1]HNMR spectrum for compound **56d**



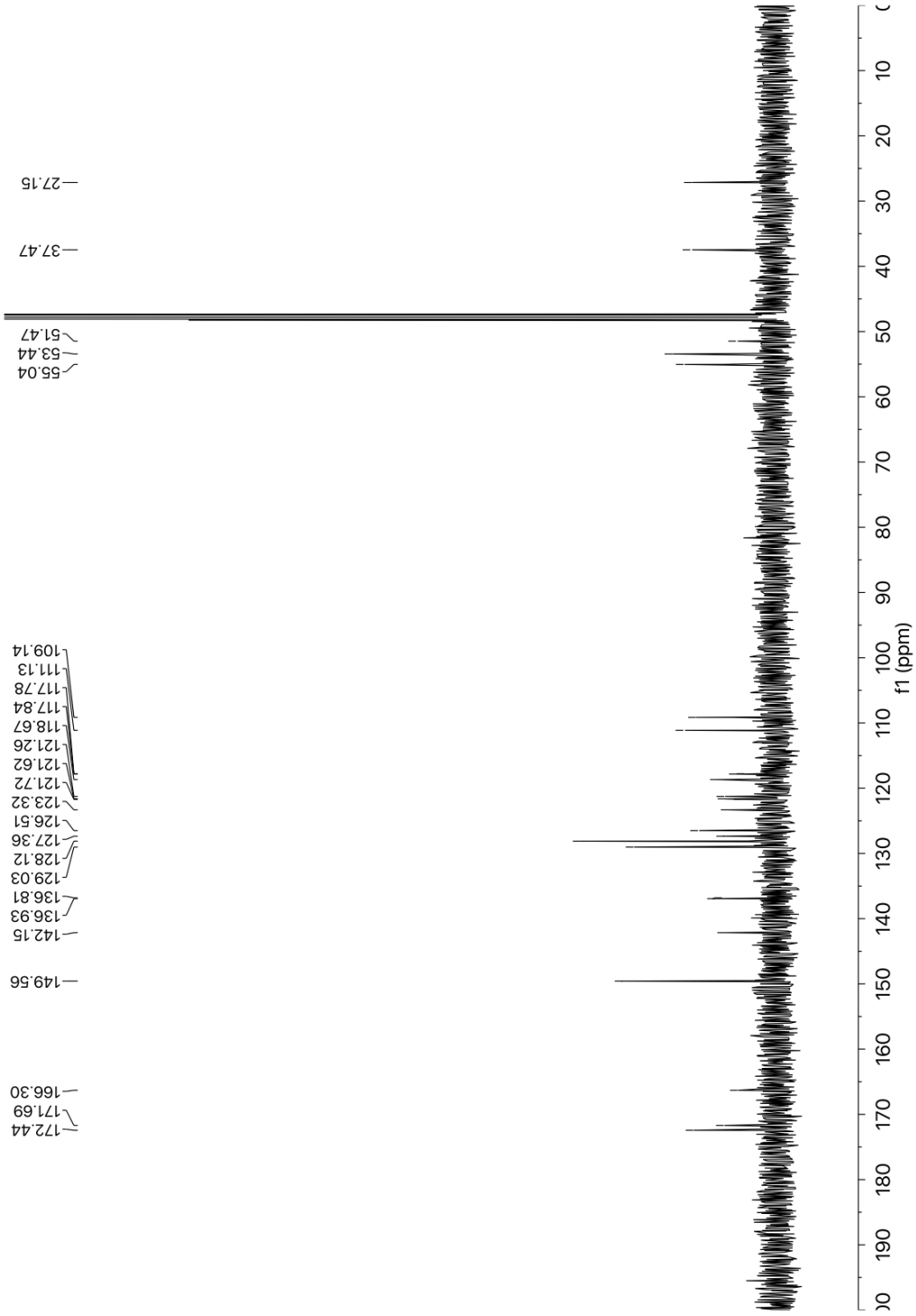
^{13}CNMR spectrum for compound **56d**



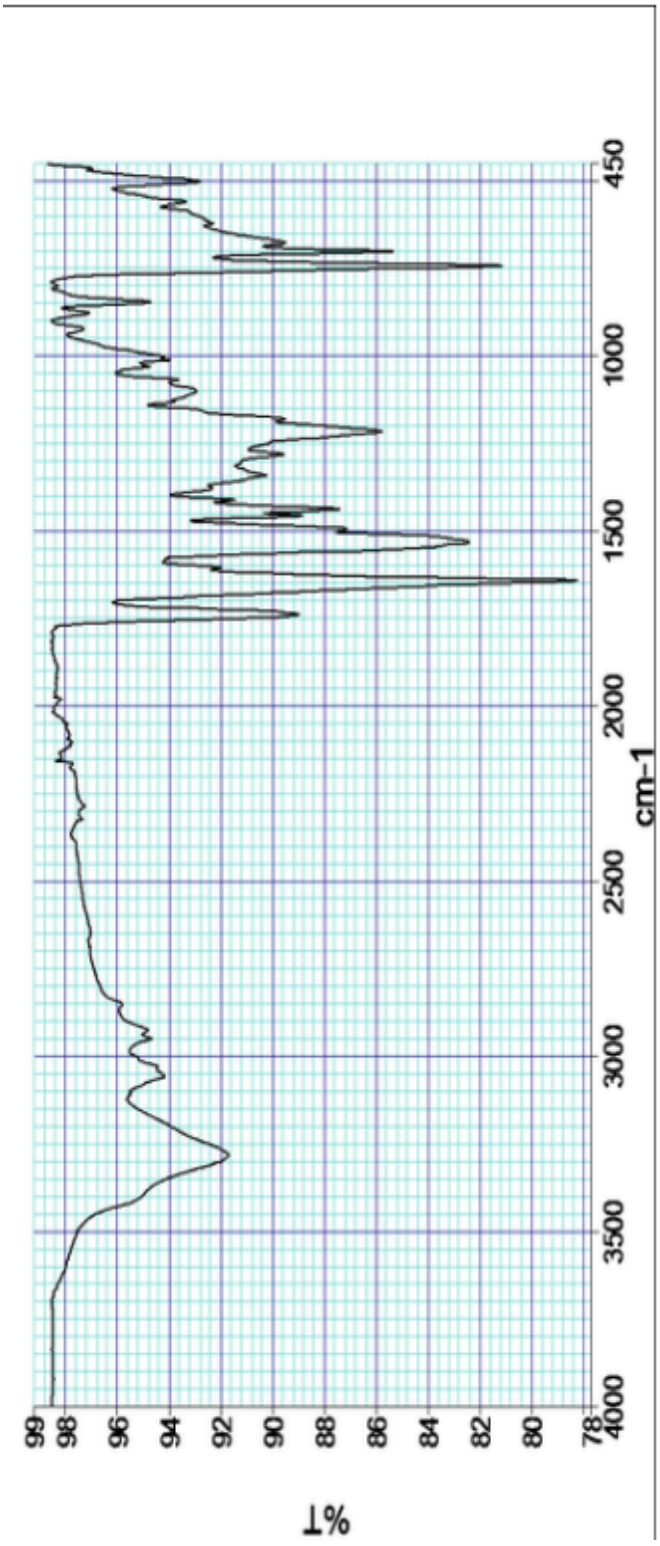
IR spectrum for compound **56d**



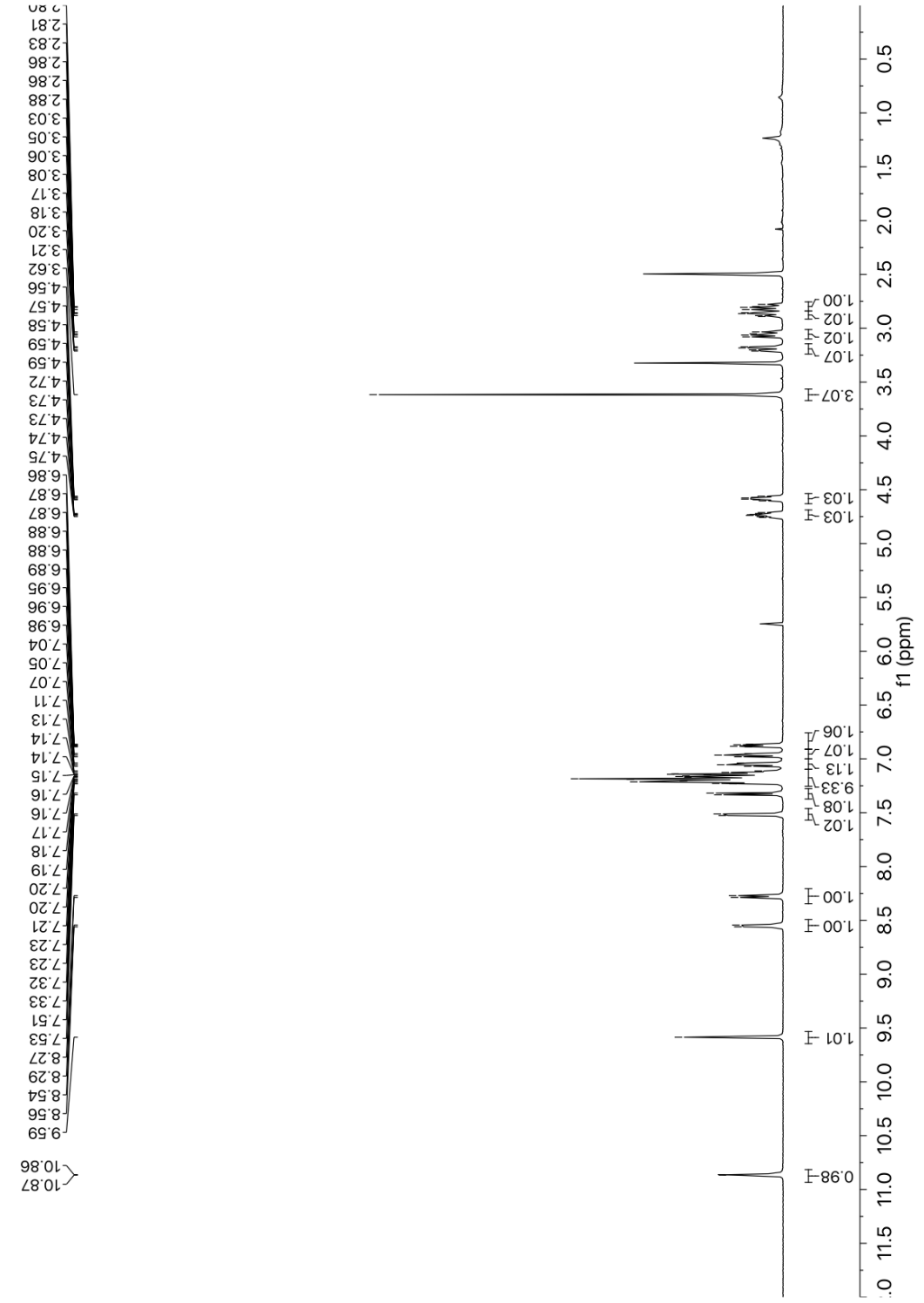
[1]HNMR spectrum for compound **56e**



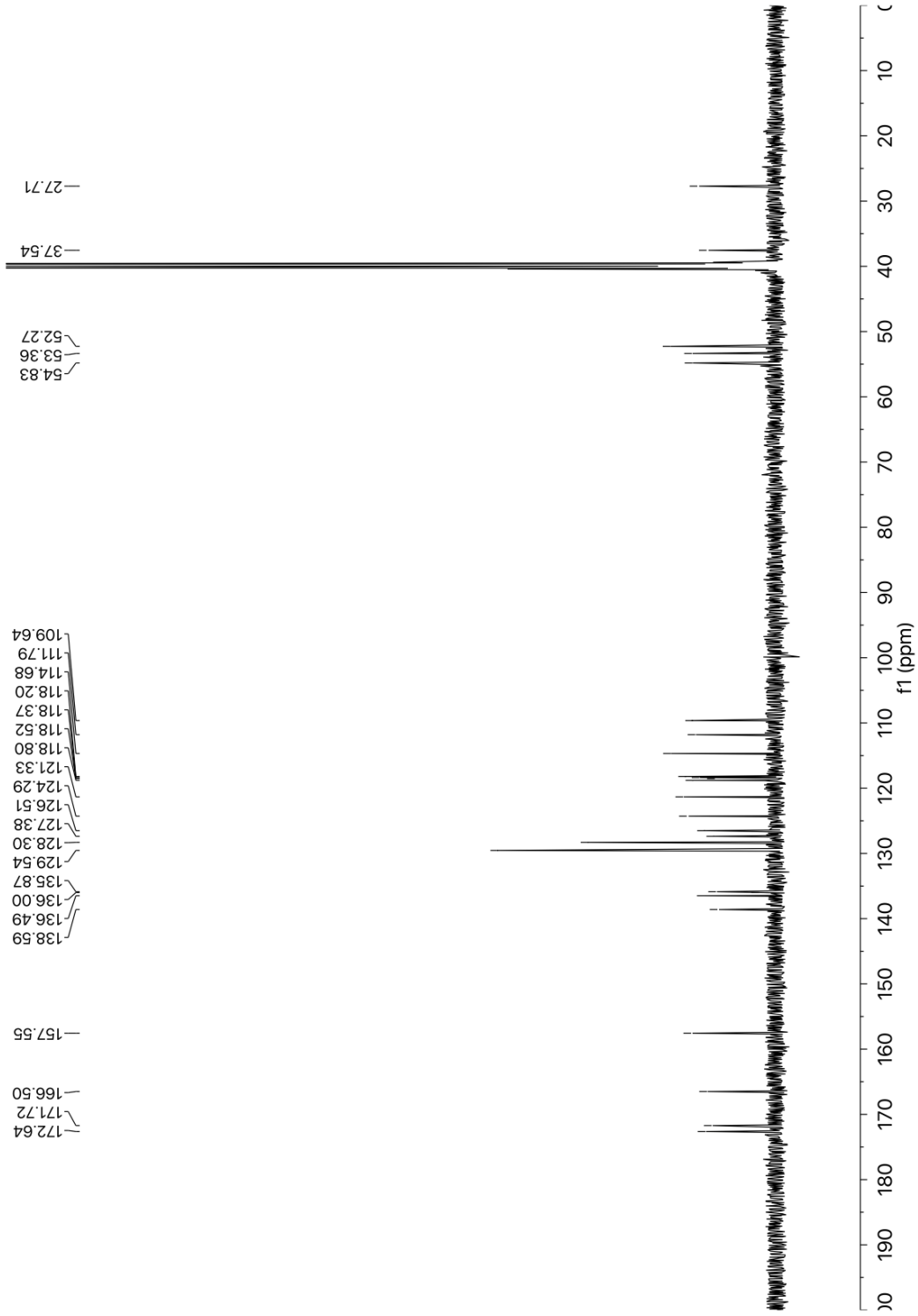
^{13}CNMR spectrum for compound **56e**



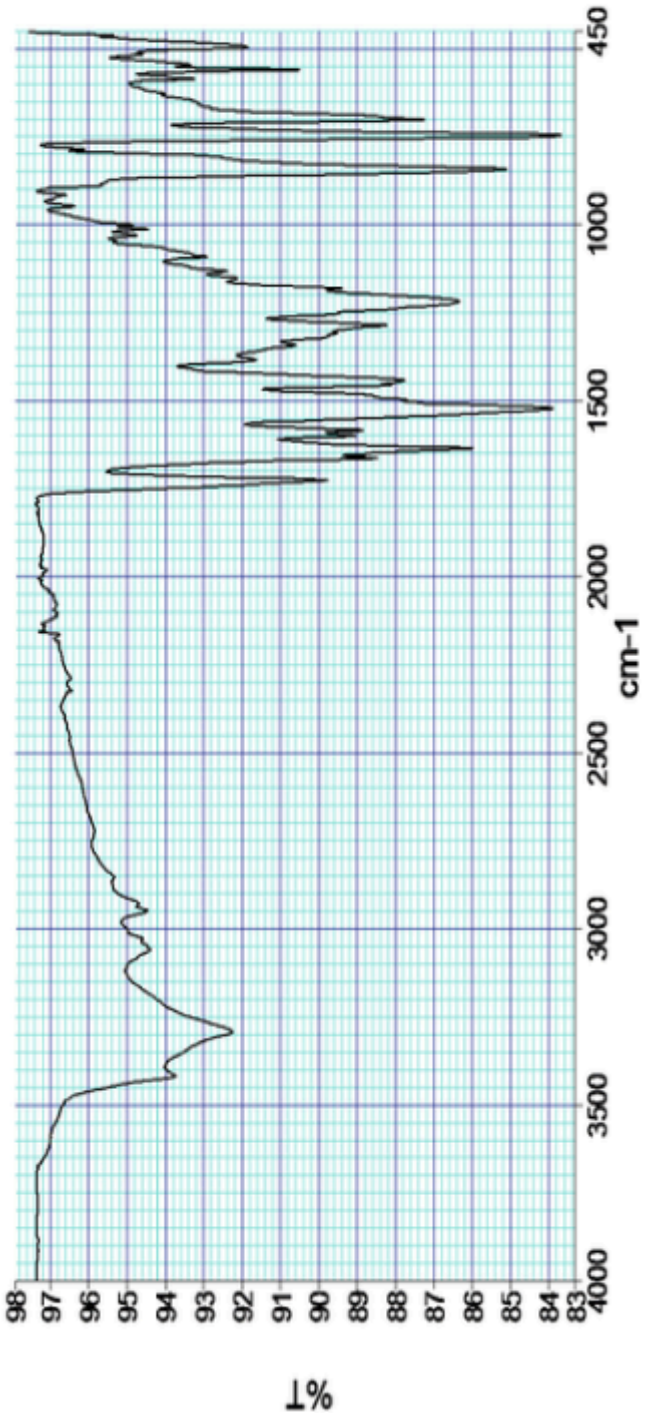
IR spectrum for compound **56e**



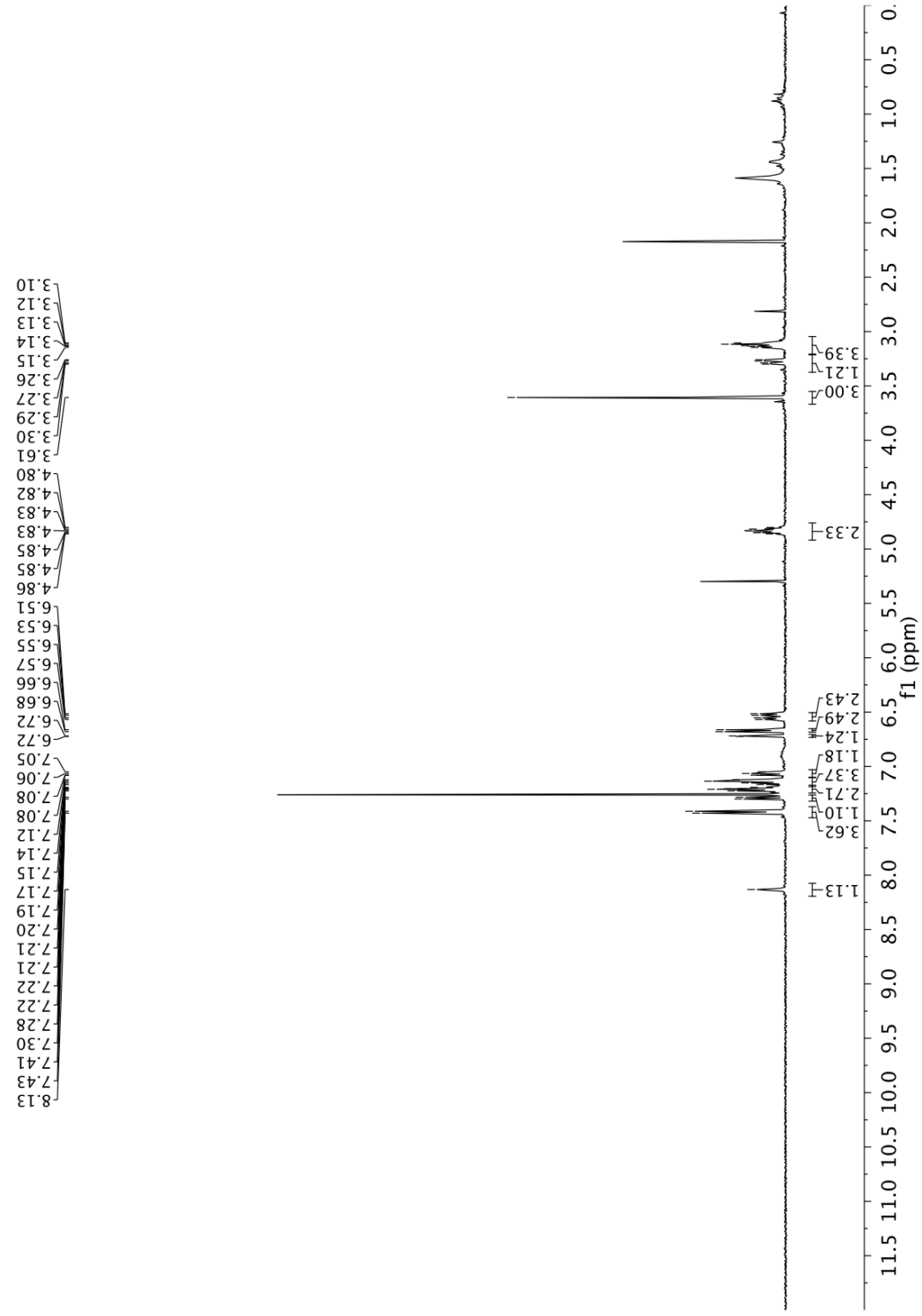
[1]HNMR spectrum for compound **56g**



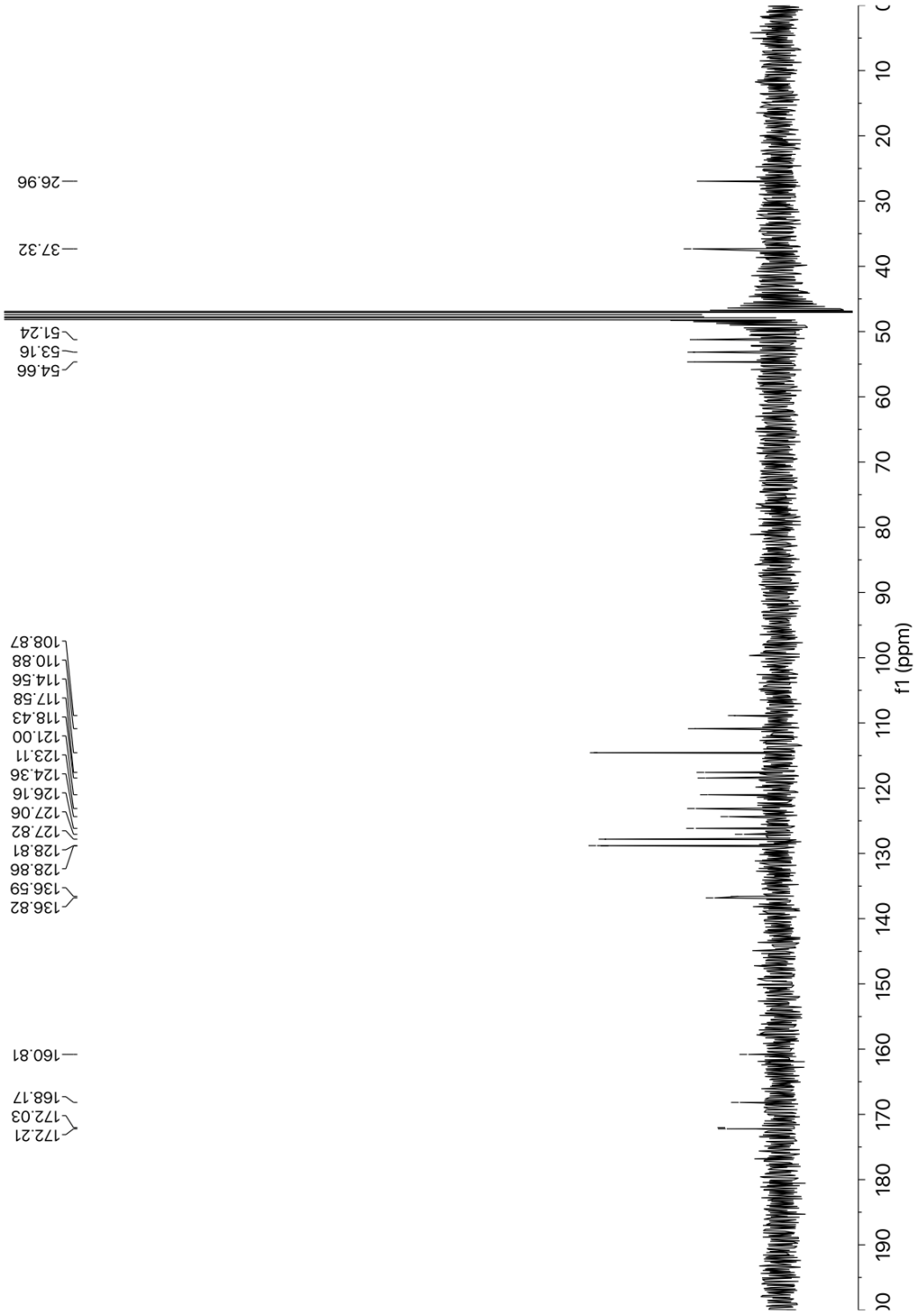
^{13}CNMR spectrum for compound **56g**



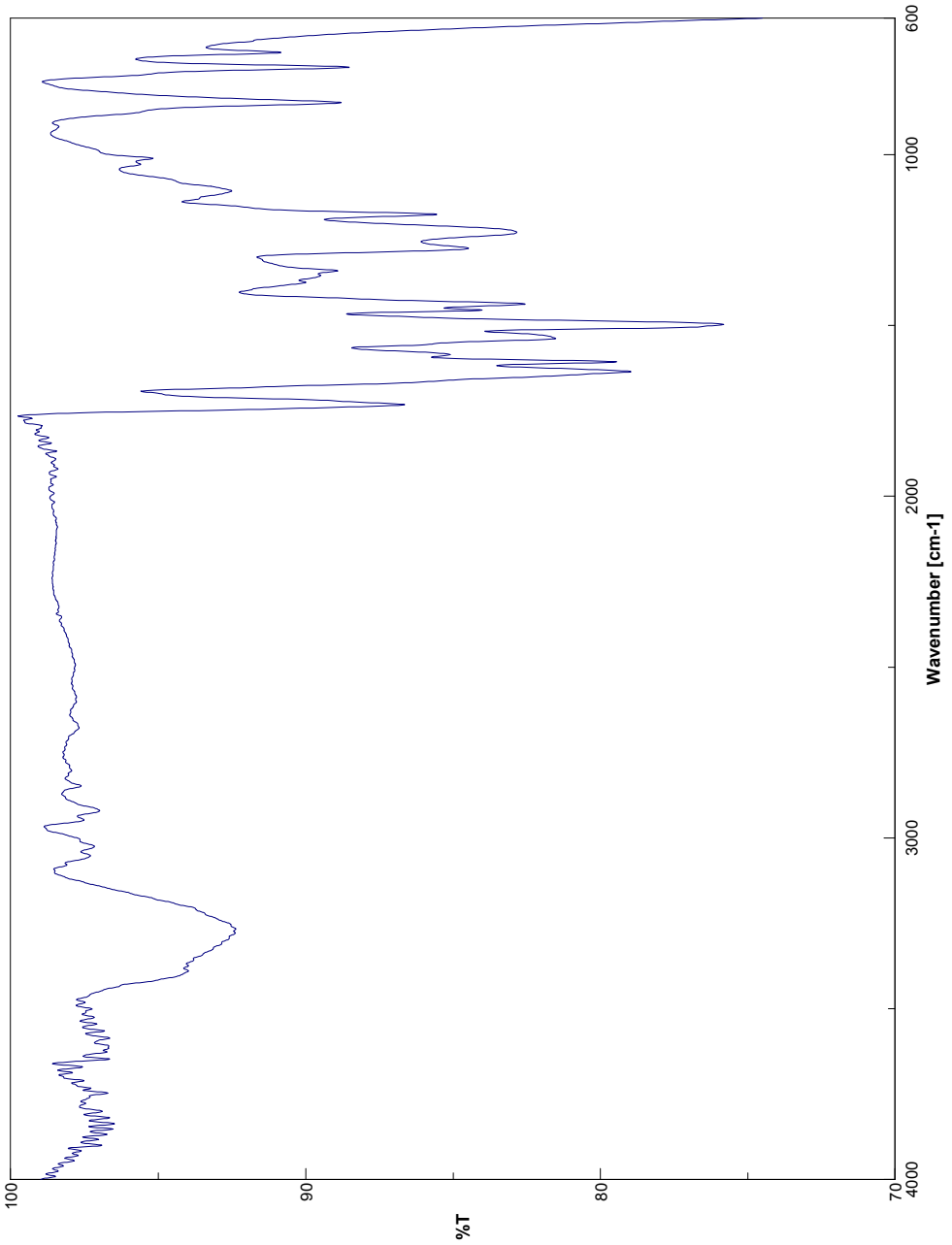
IR spectrum for compound **56g**



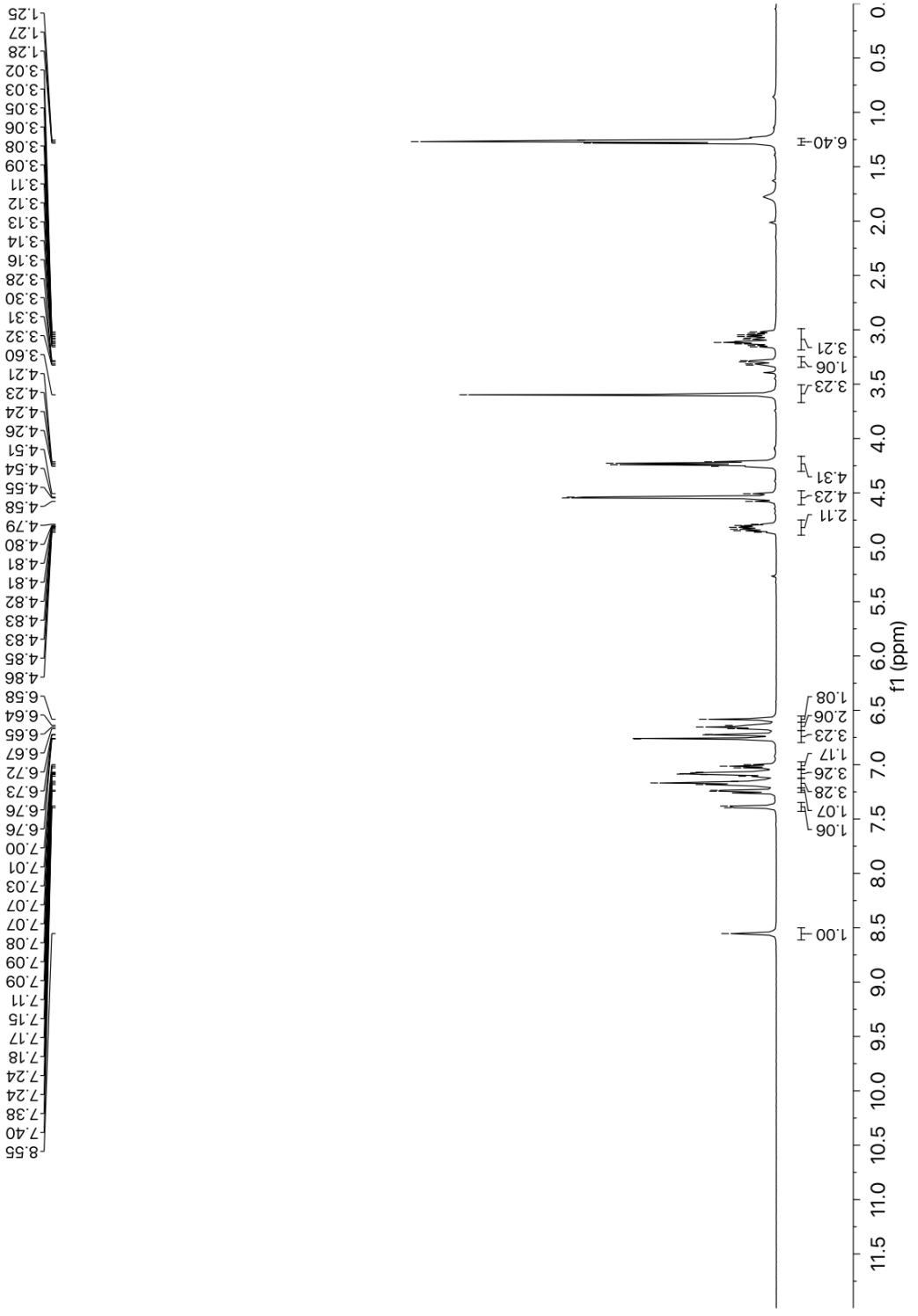
[1]HNMR spectrum for compound **56h**



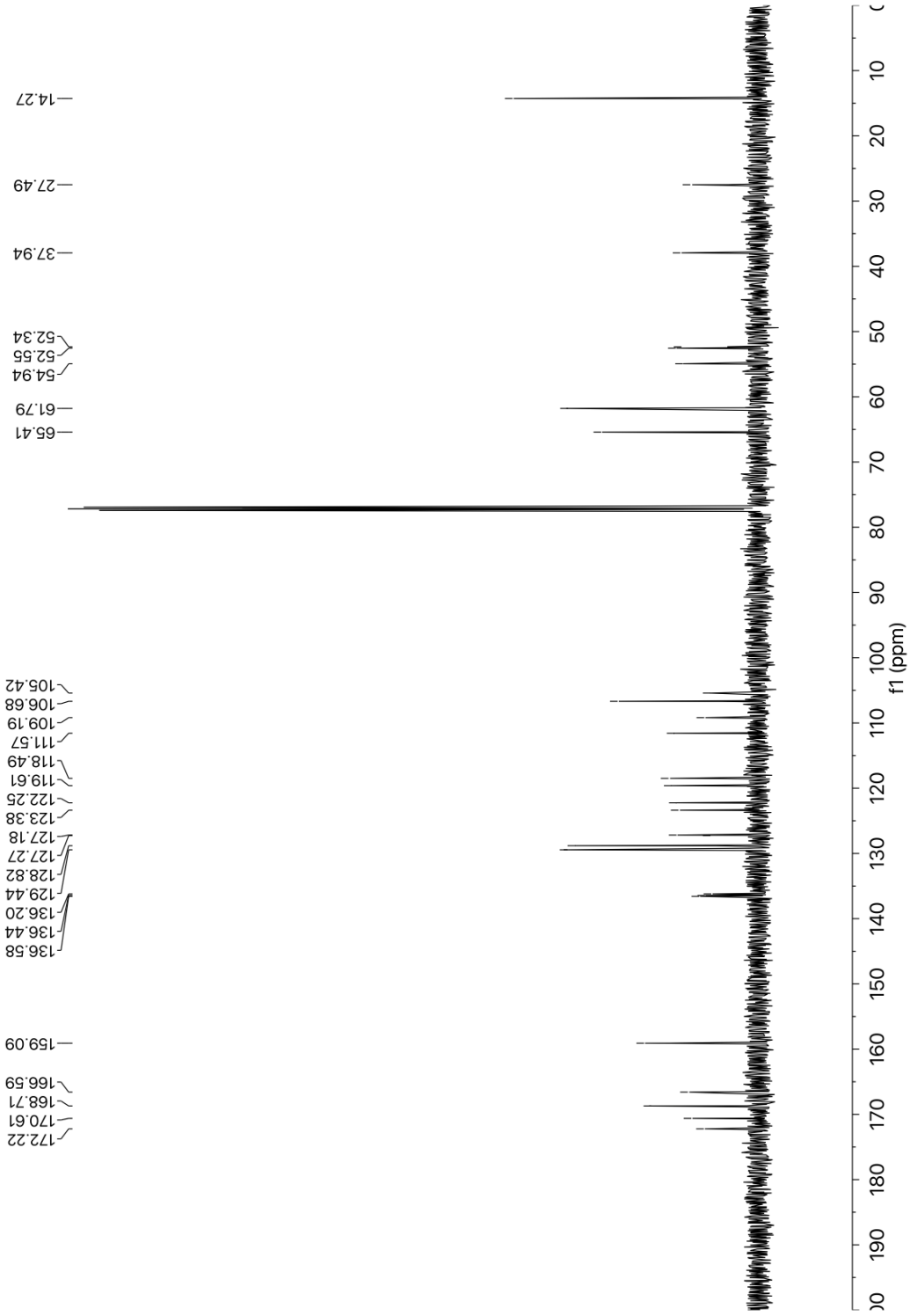
^{13}CNMR spectrum for compound **56h**



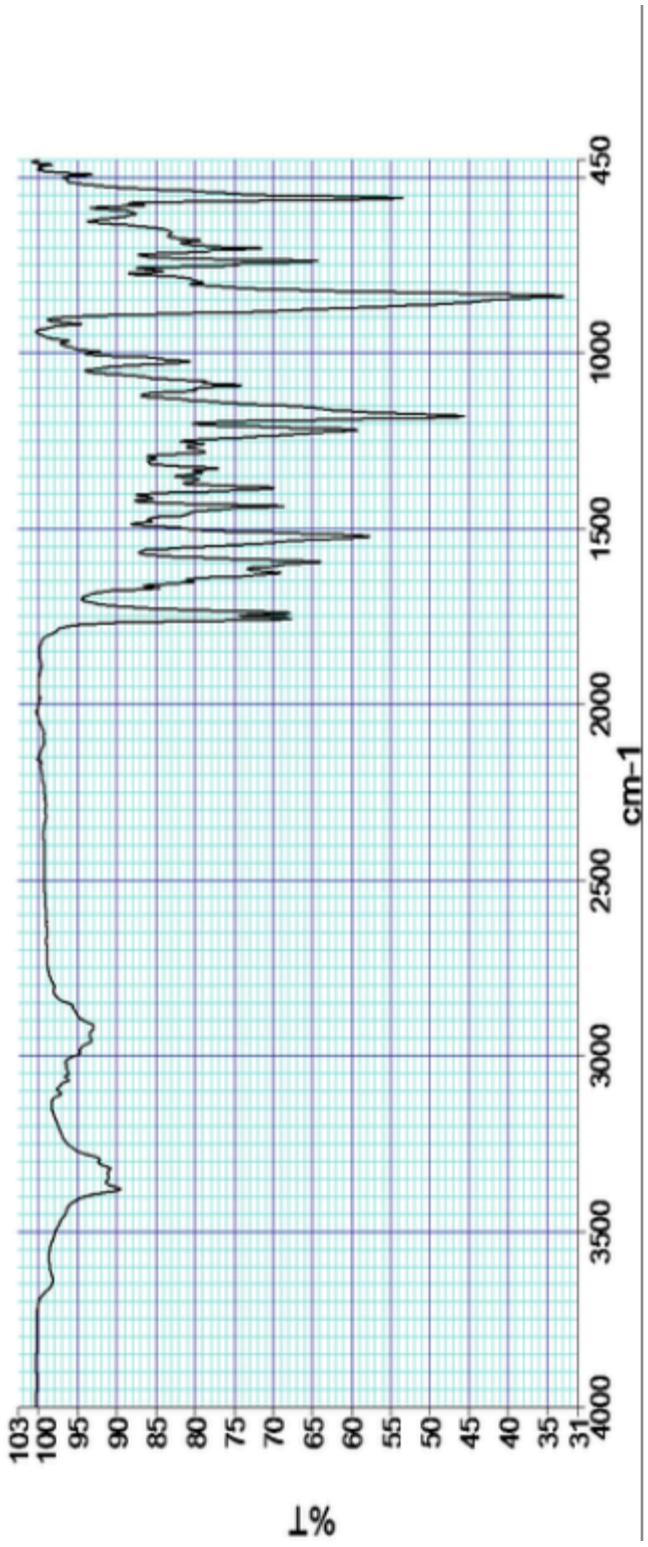
IR spectrum for compound **56h**



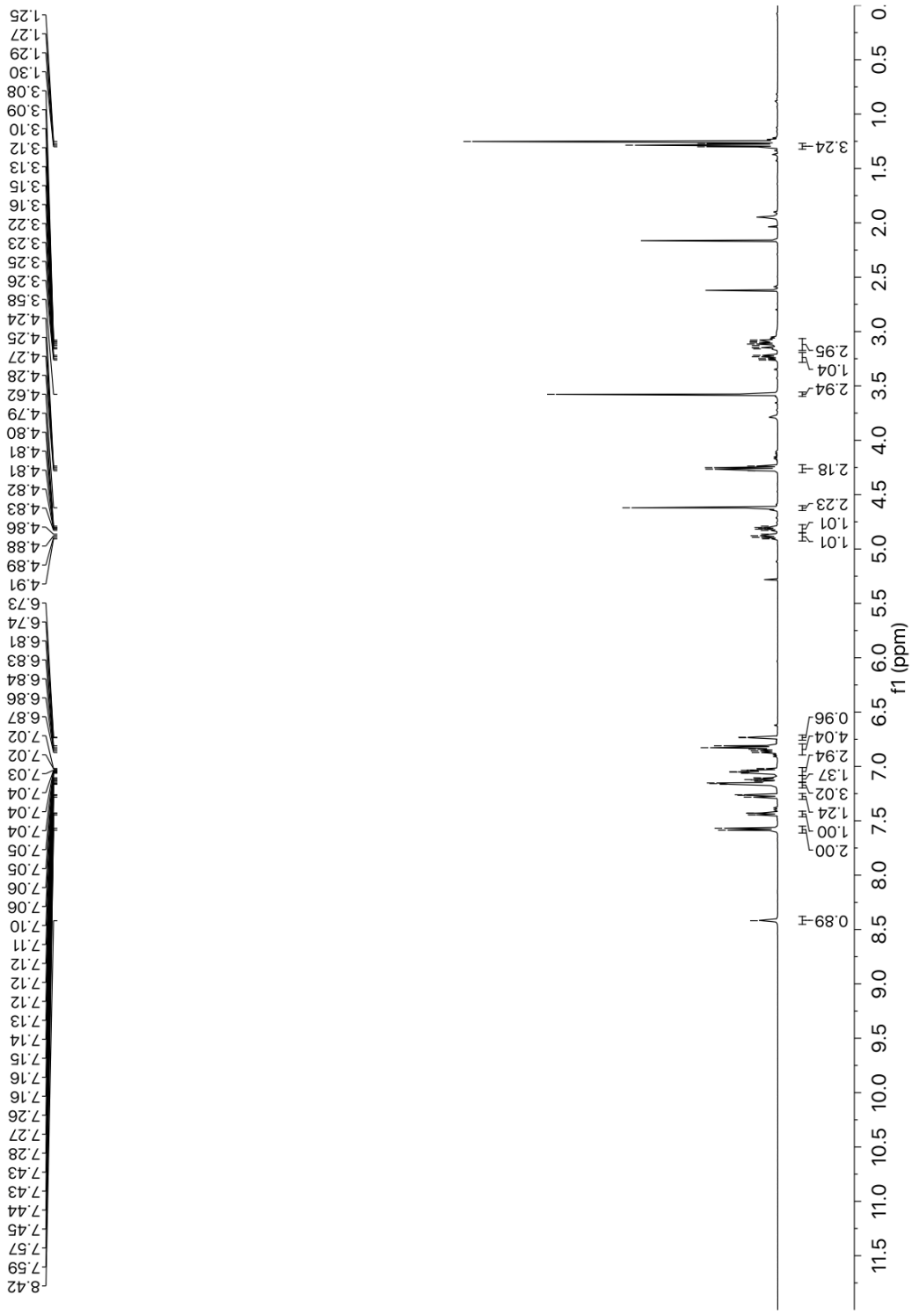
[1]HNMR spectrum for compound **56i**



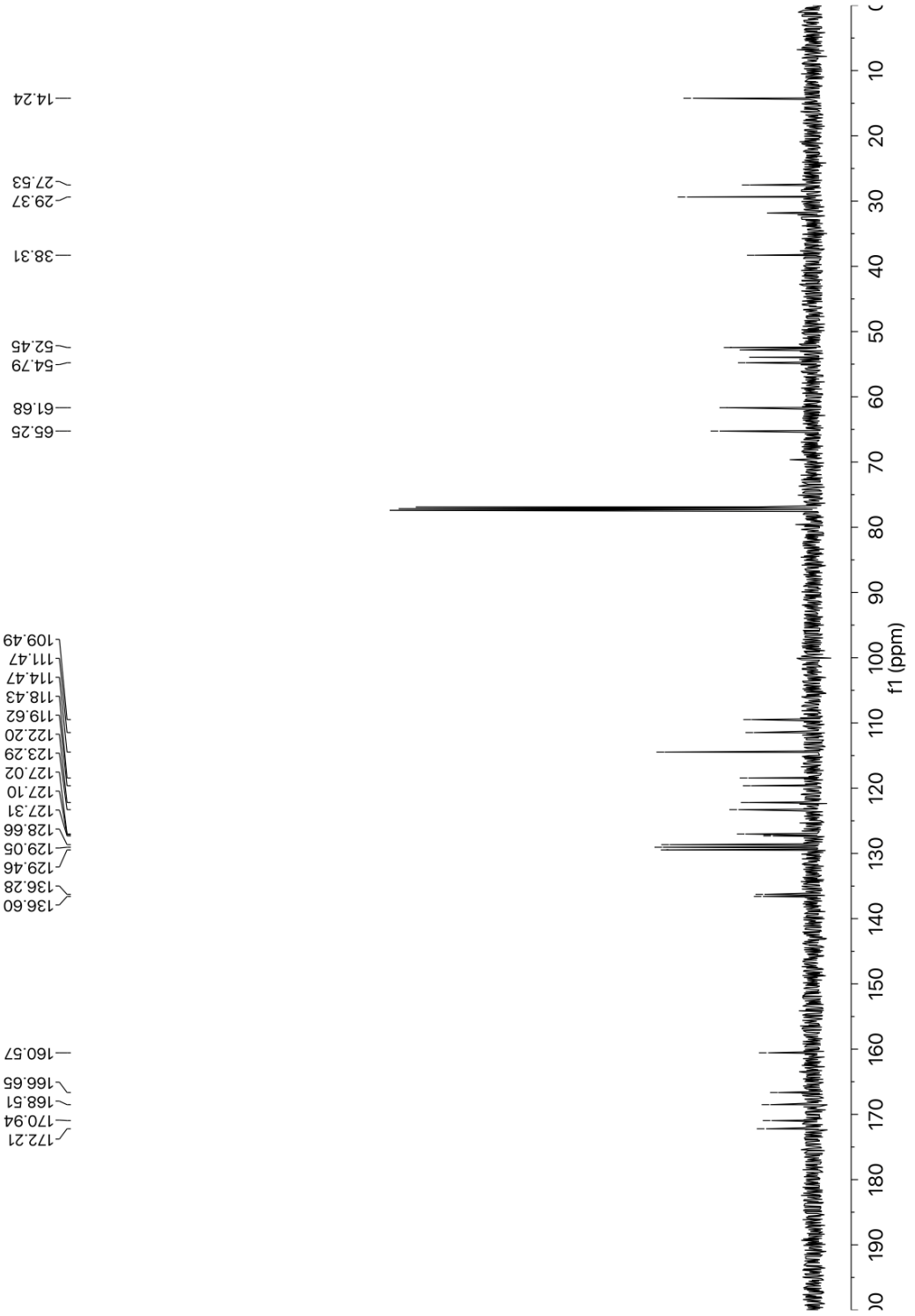
^{13}CNMR spectrum for compound **56i**



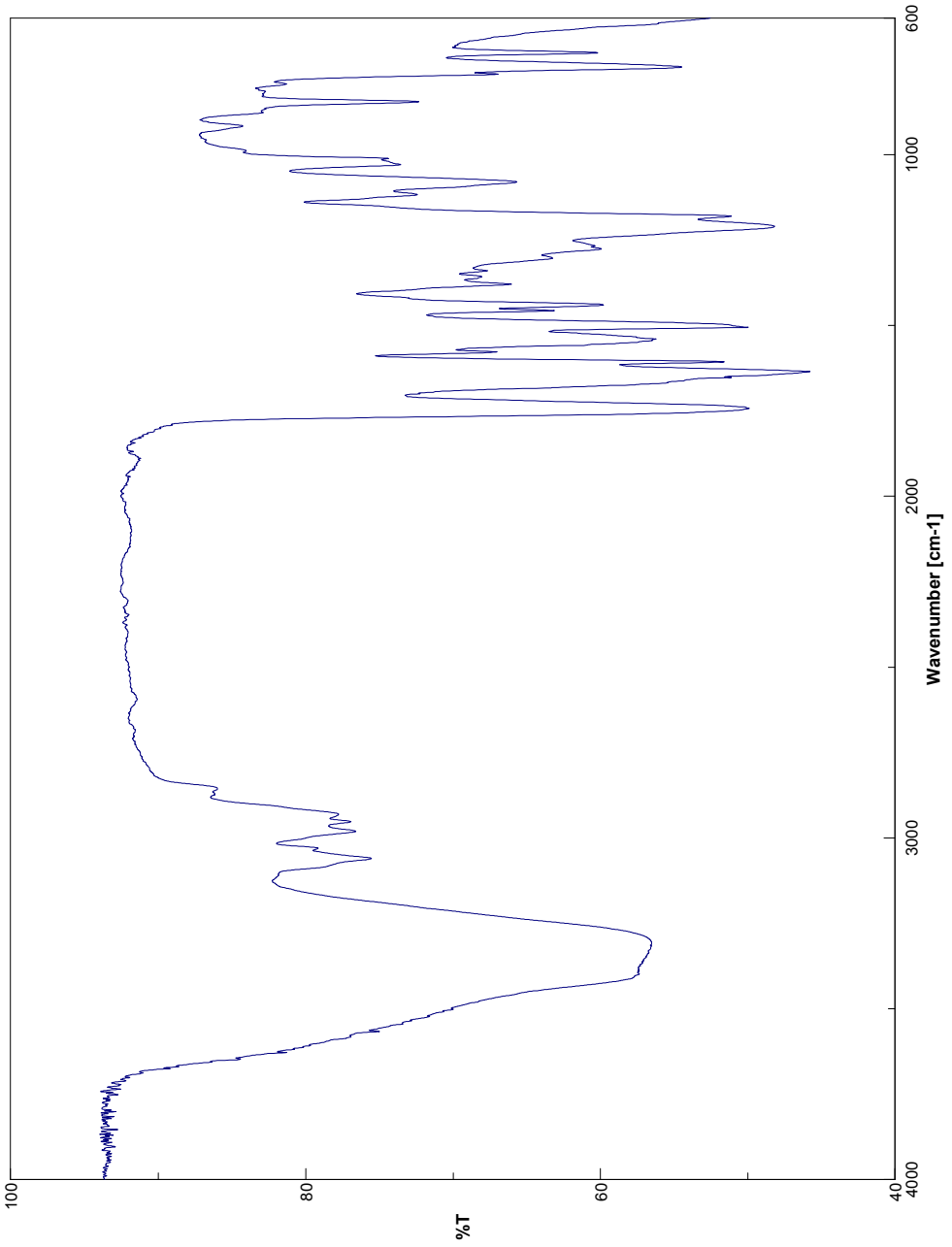
IR spectrum for compound **56i**



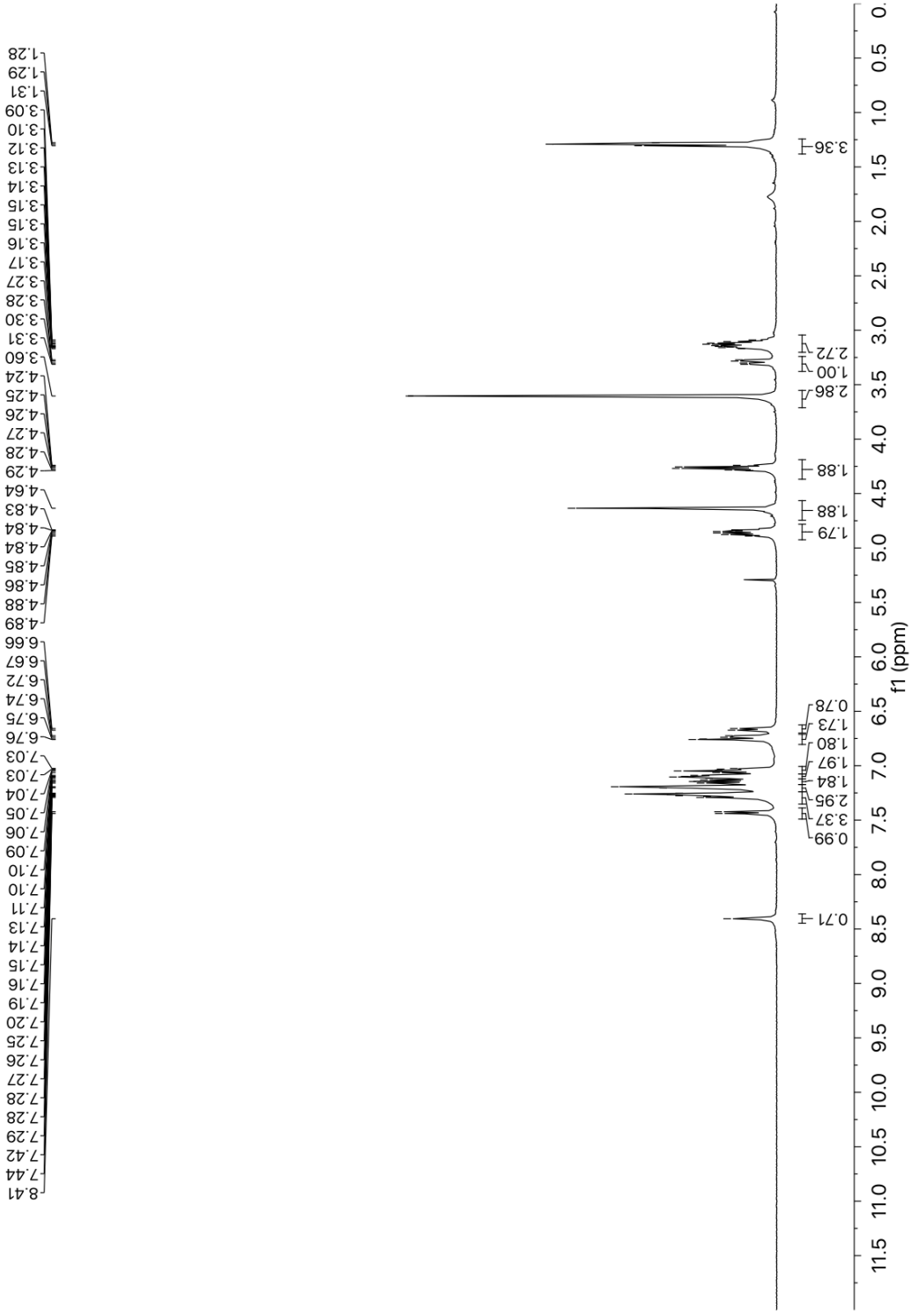
[1]HNMR spectrum for compound **56j**



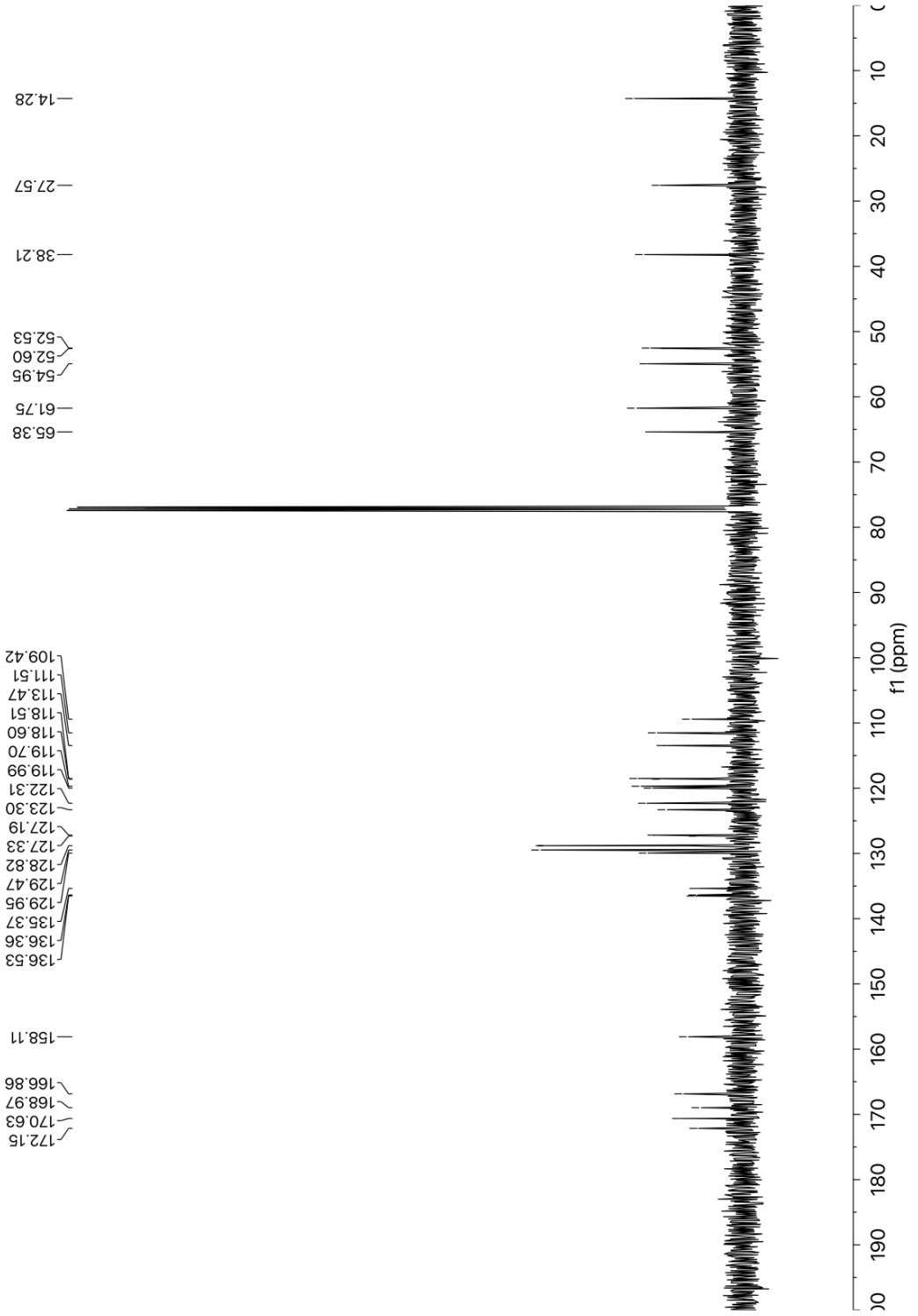
^{13}CNMR spectrum for compound **56j**



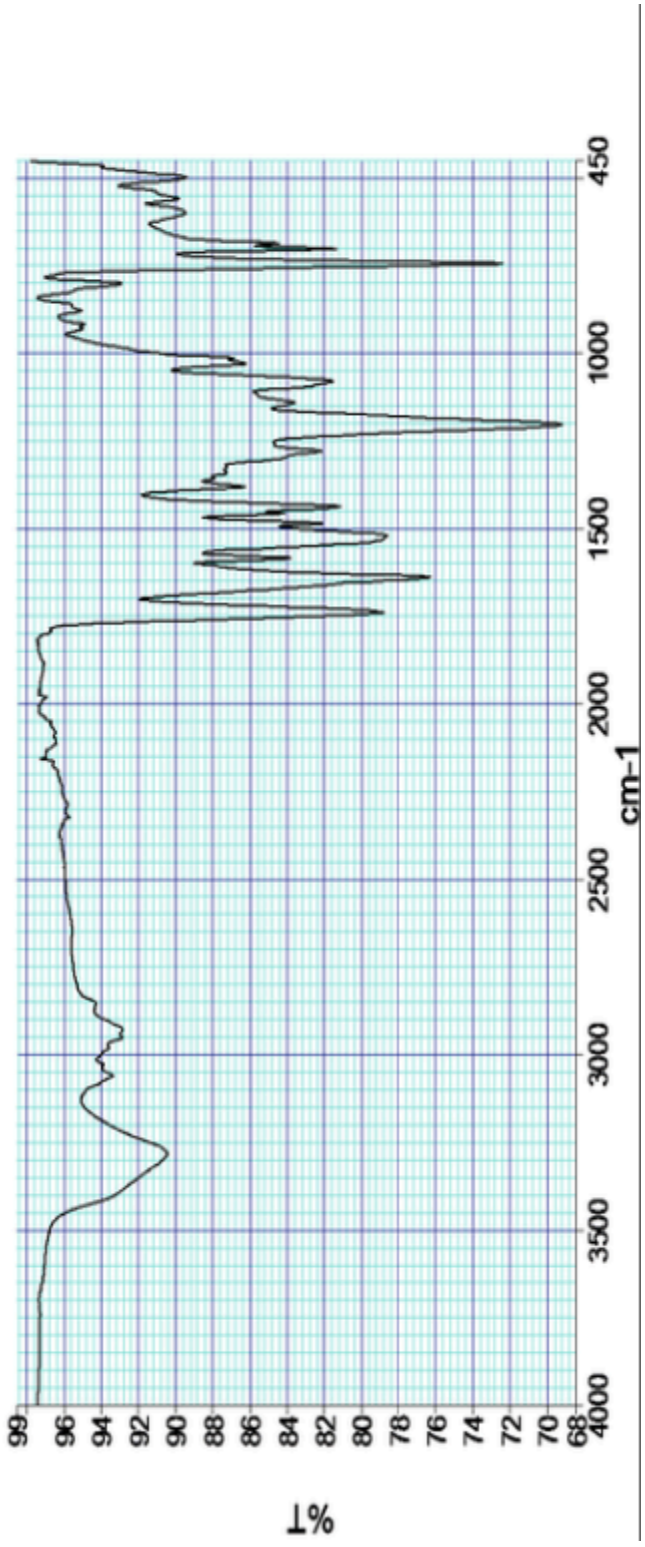
IR spectrum for compound **56j**



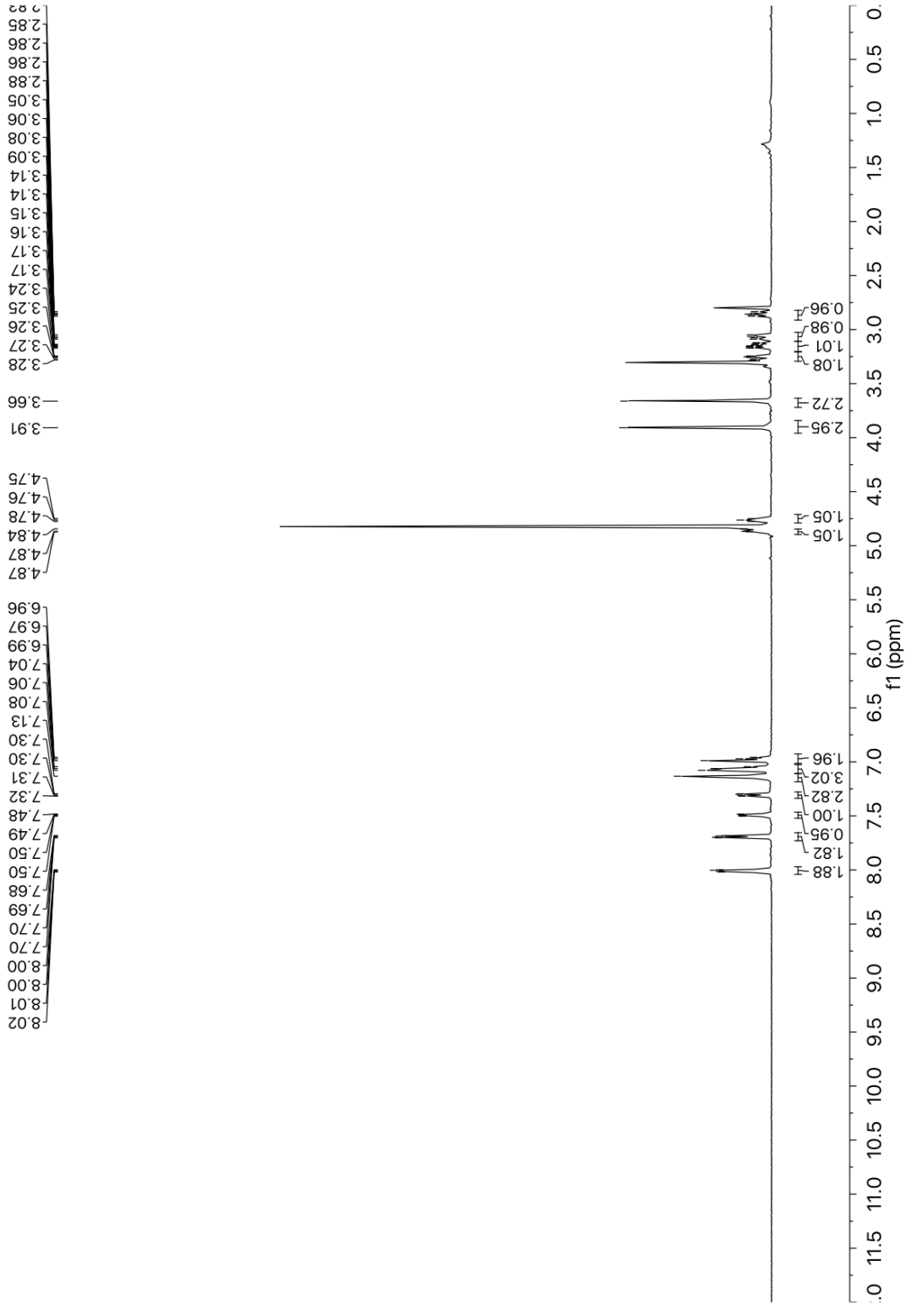
[1]HNMR spectrum for compound **56k**



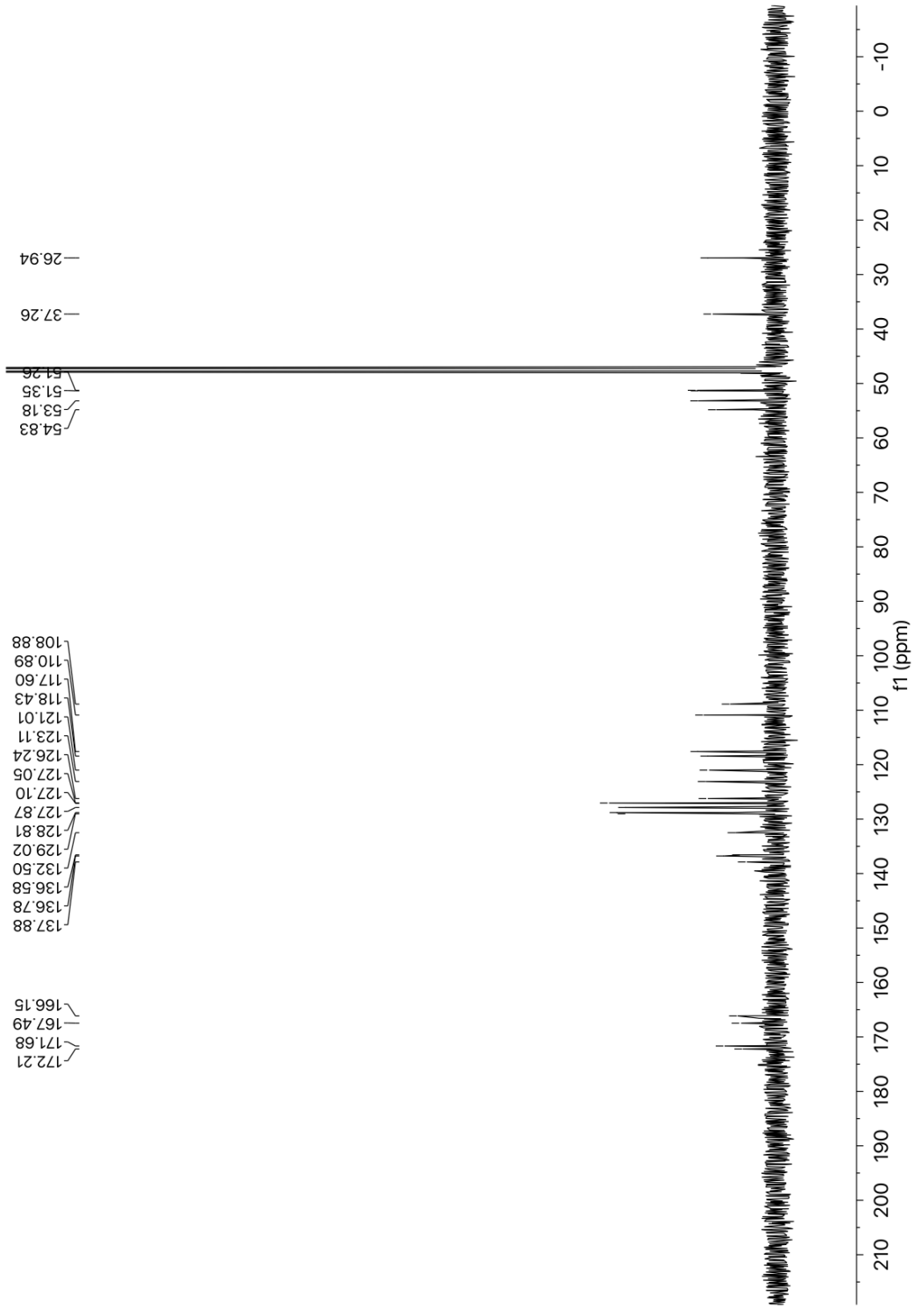
^{13}CNMR spectrum for compound **56k**



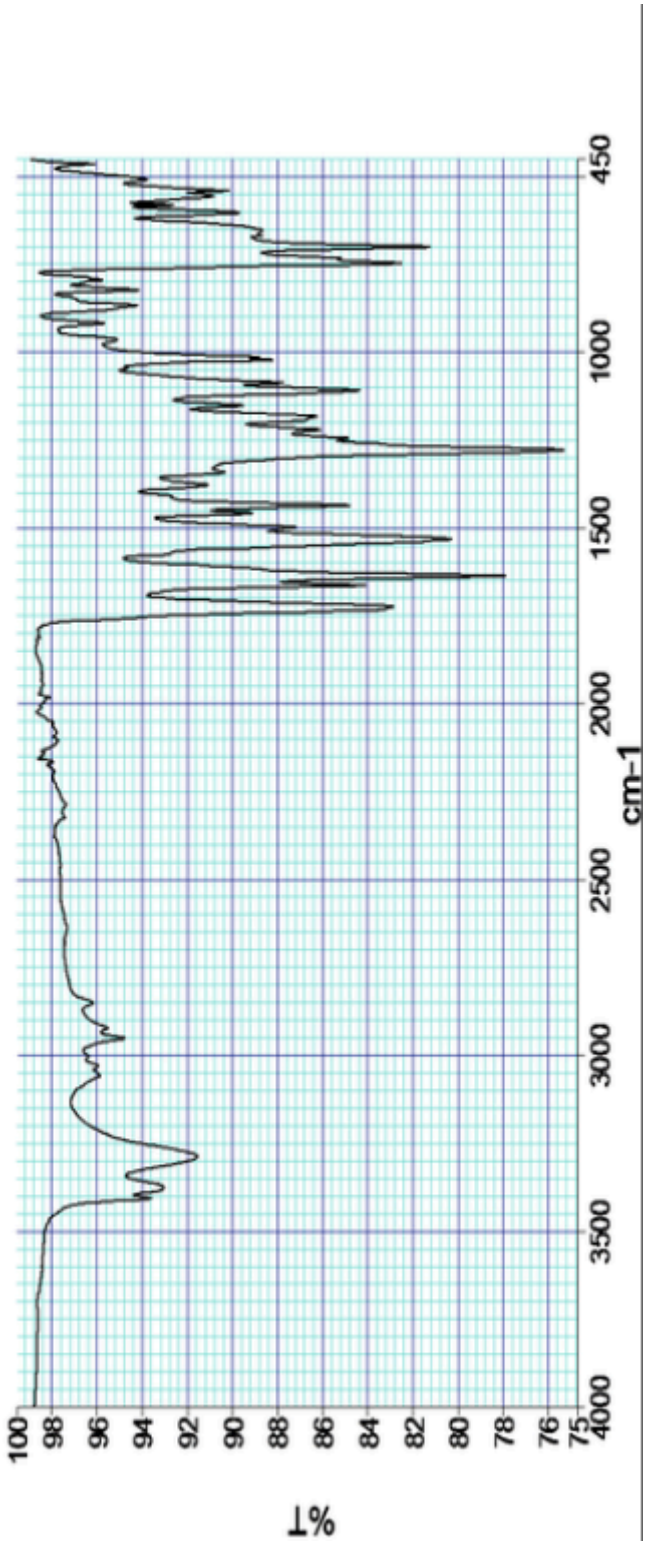
IR spectrum for compound **56k**



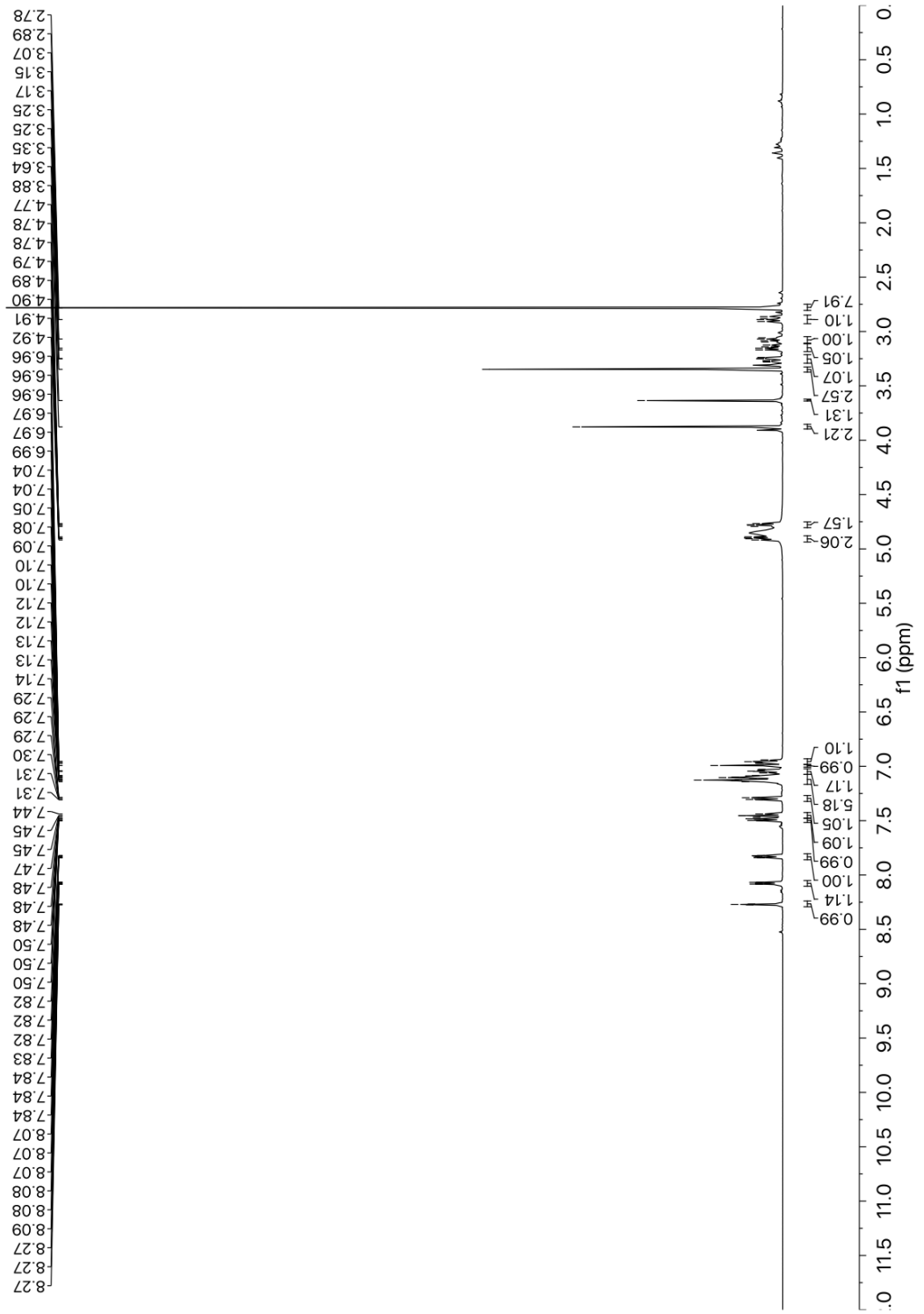
[1]HNMR spectrum for compound **56l**



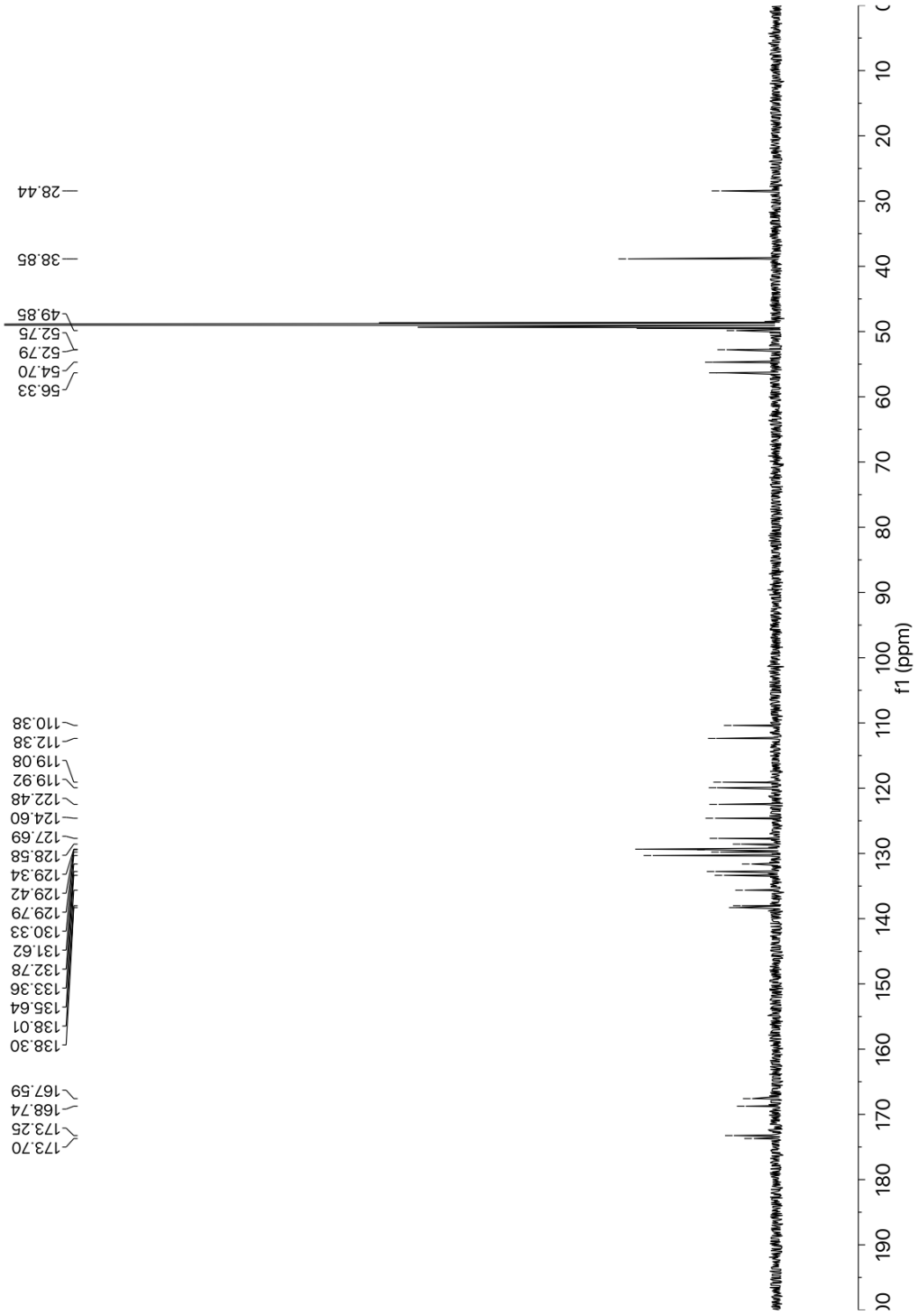
^{13}CNMR spectrum for compound **56l**



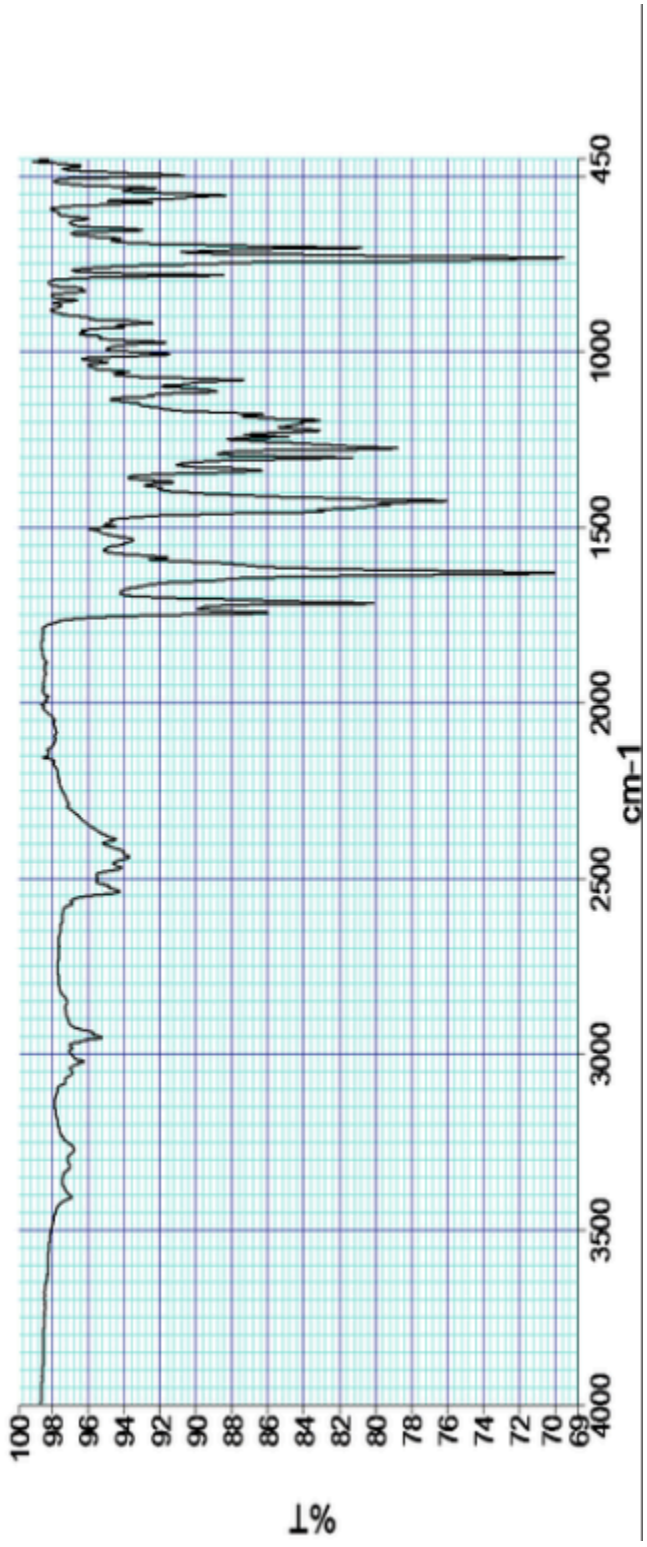
IR spectrum for compound **56l**



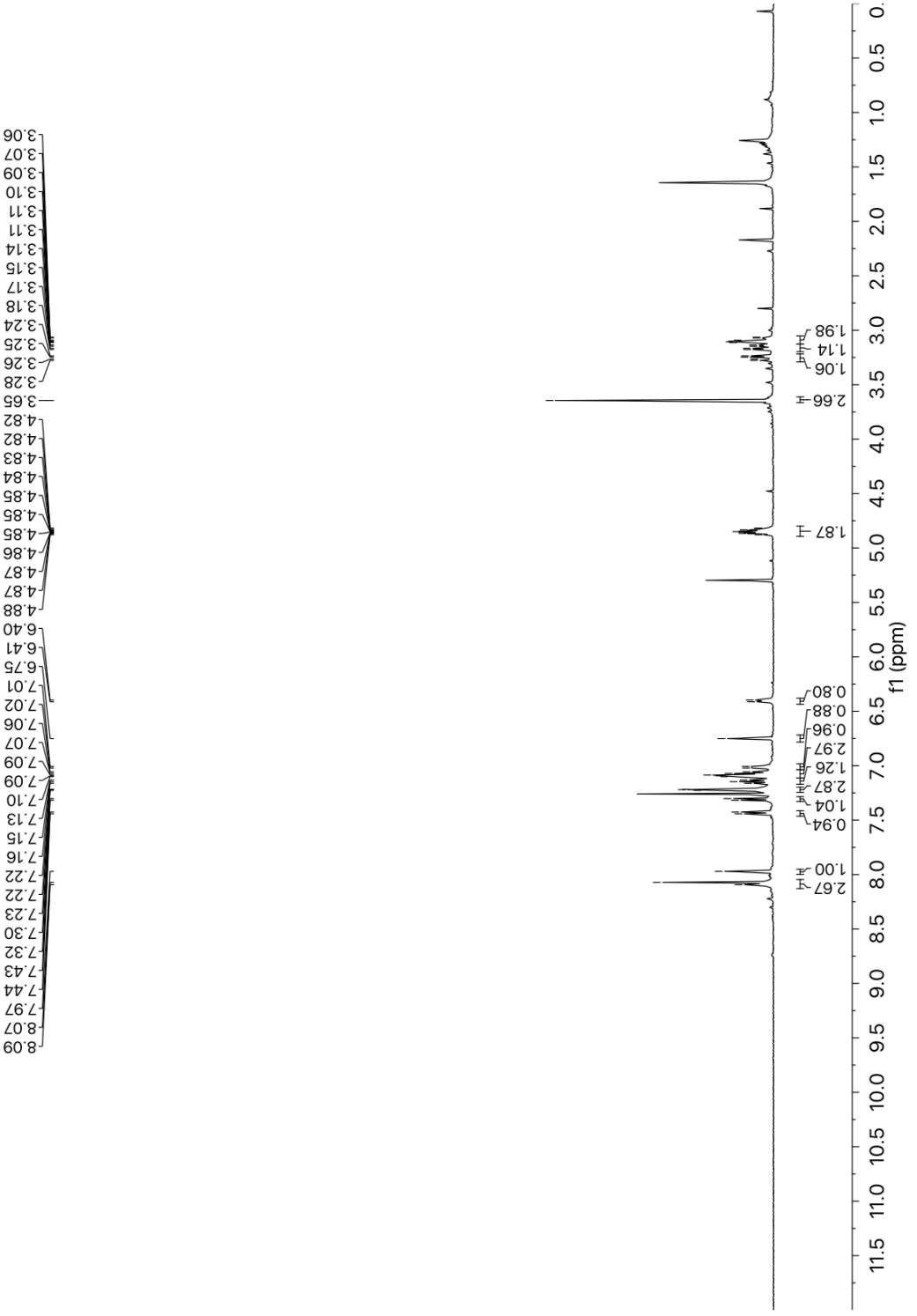
[1]HNMR spectrum for compound **56m**



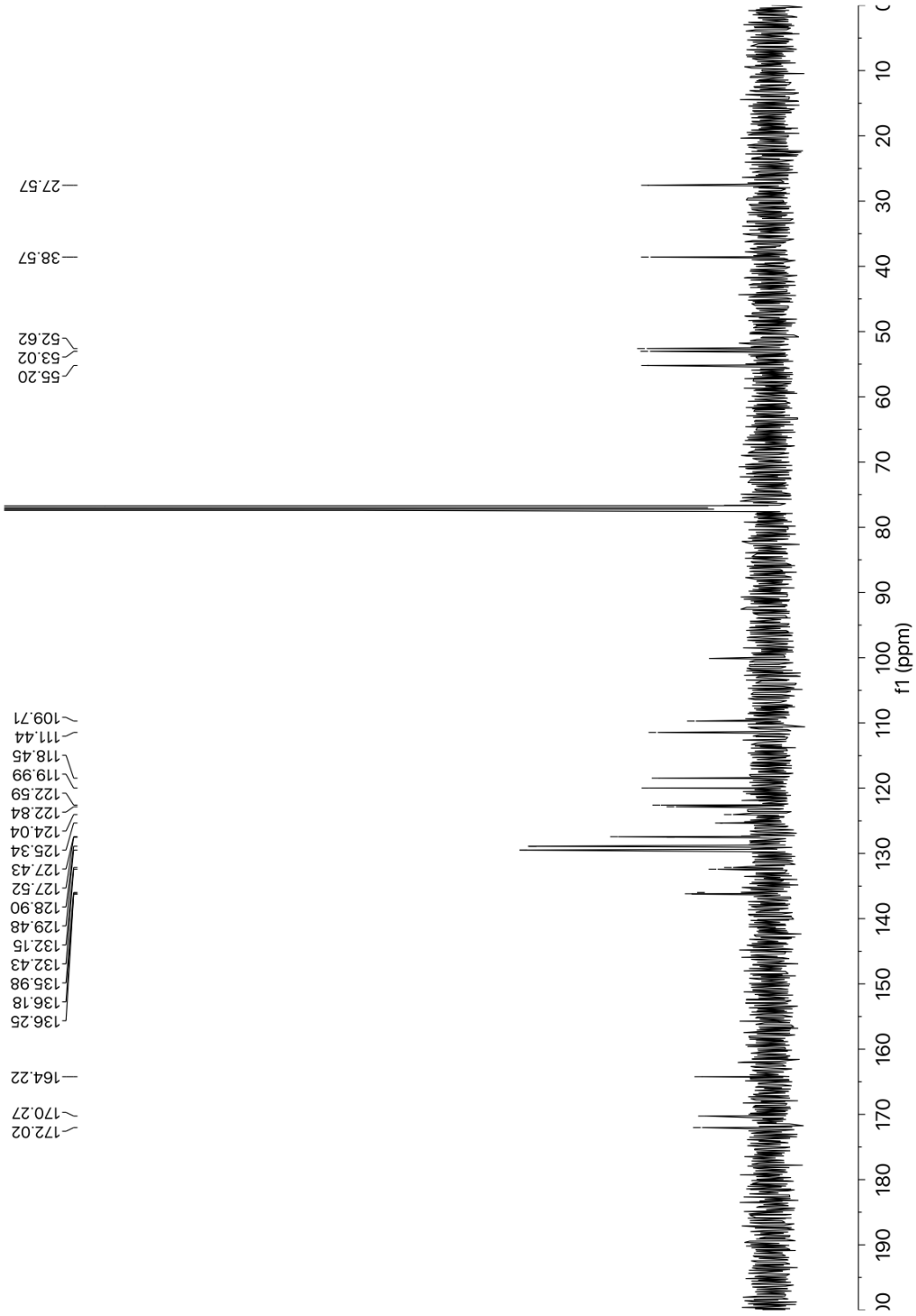
^{13}CNMR spectrum for compound **56m**



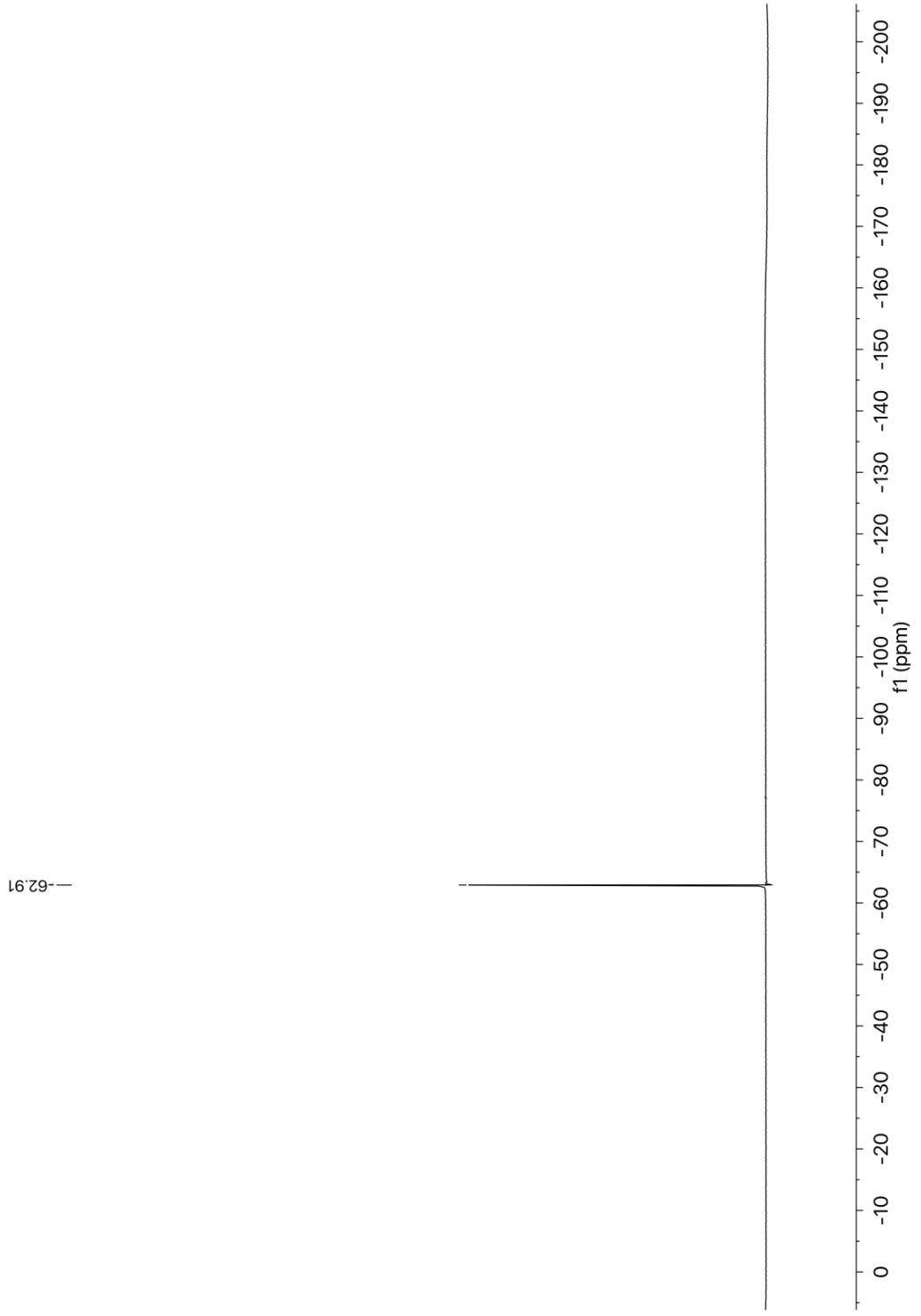
IR spectrum for compound **56m**



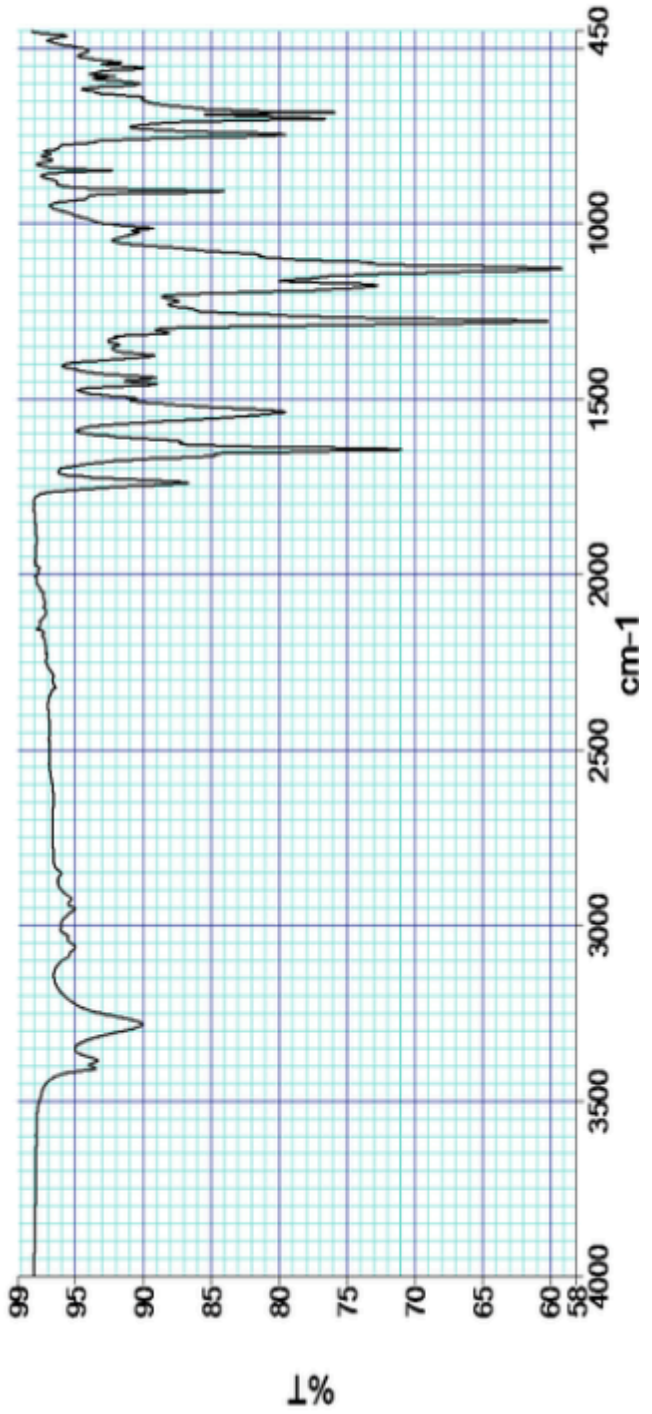
^{1}HNMR spectrum for compound **56n**



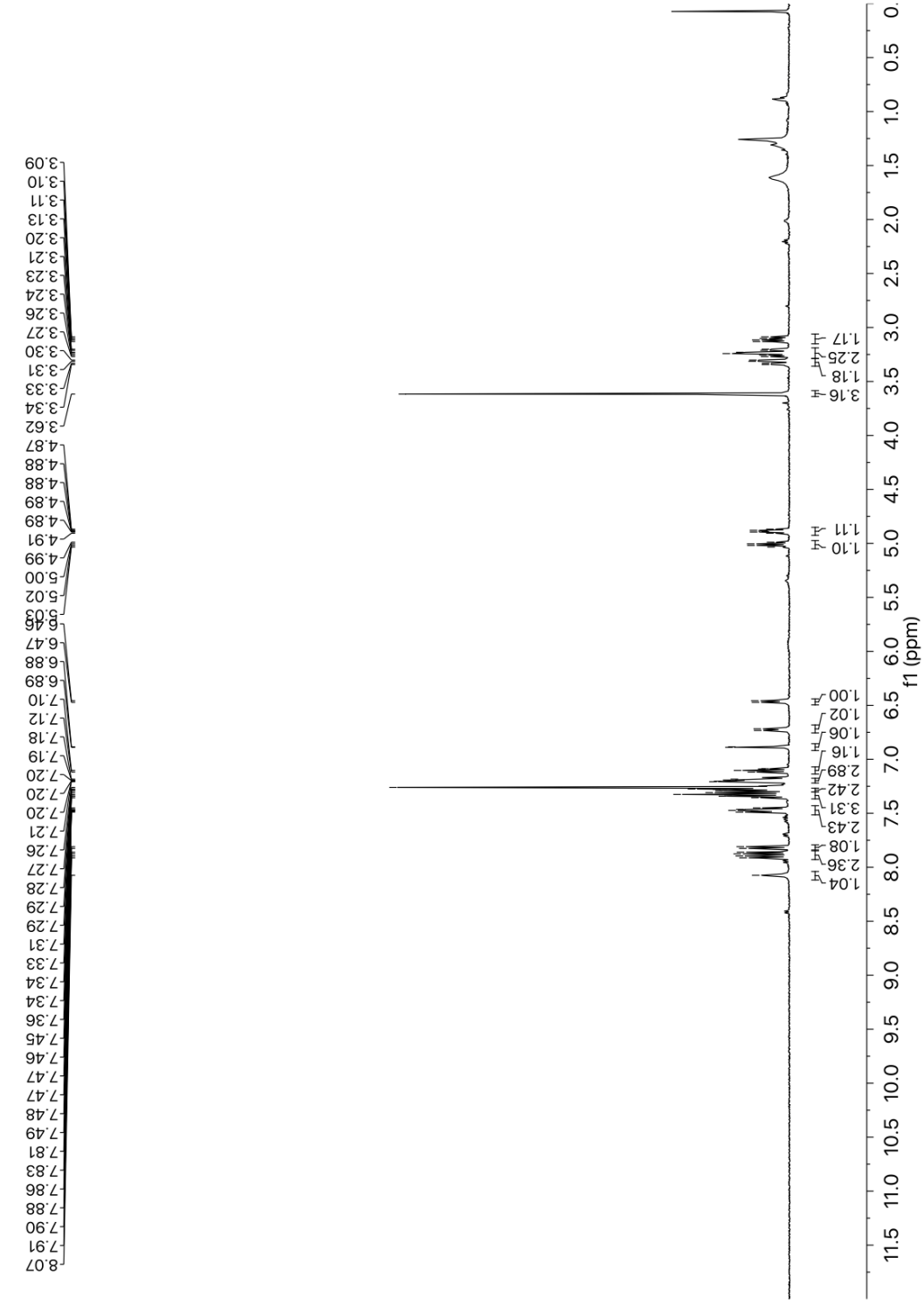
^{13}CNMR spectrum for compound **56n**



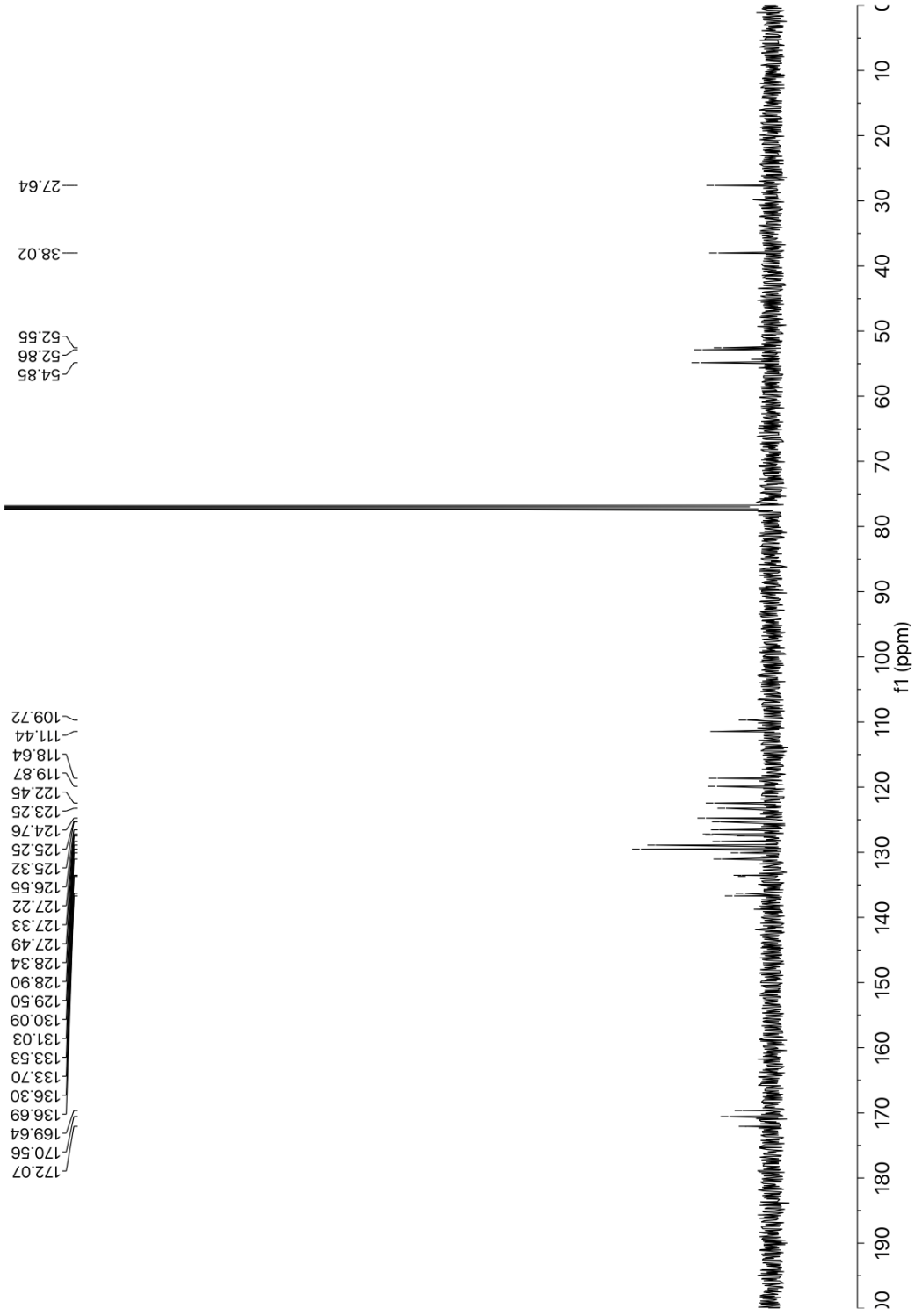
^{19}FNMR spectrum for compound **56n**



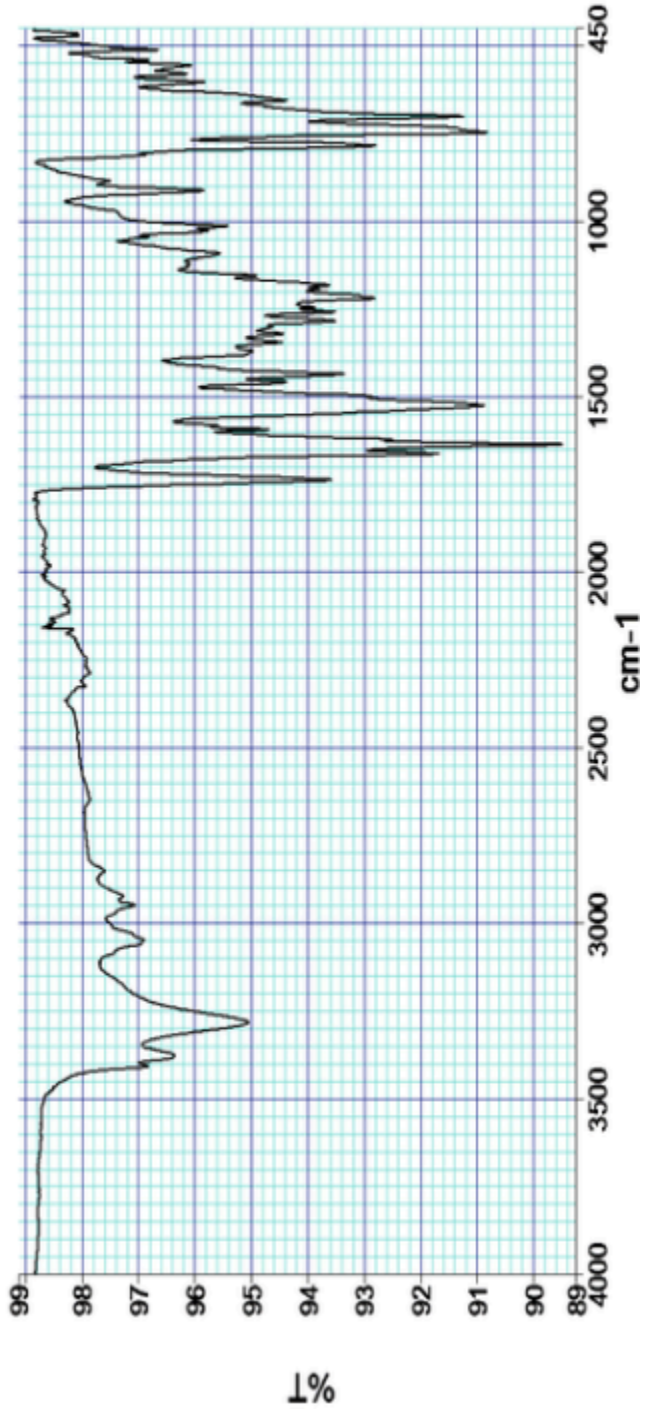
IR spectrum for compound **56n**



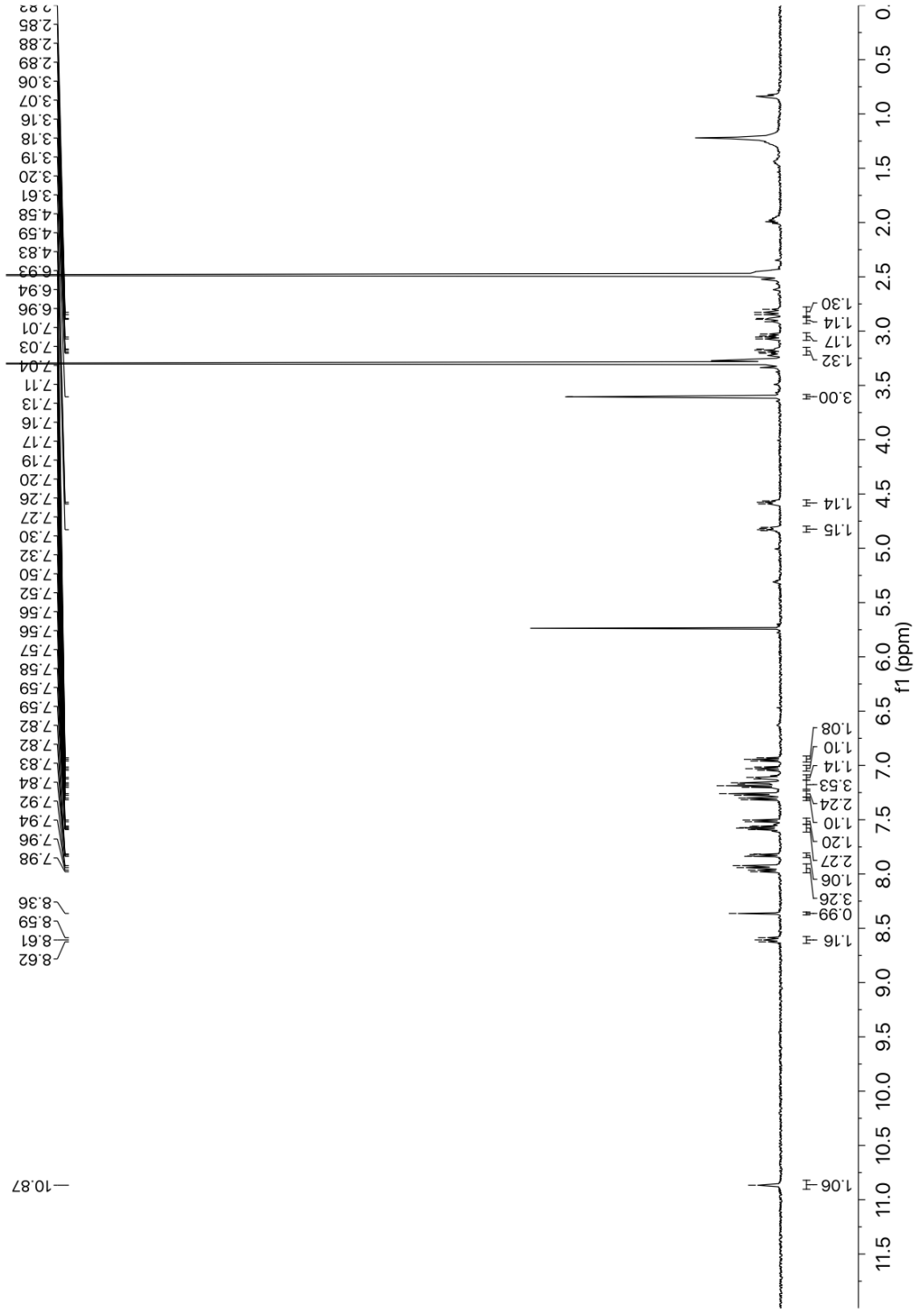
[1]HNMR spectrum for compound **56o**



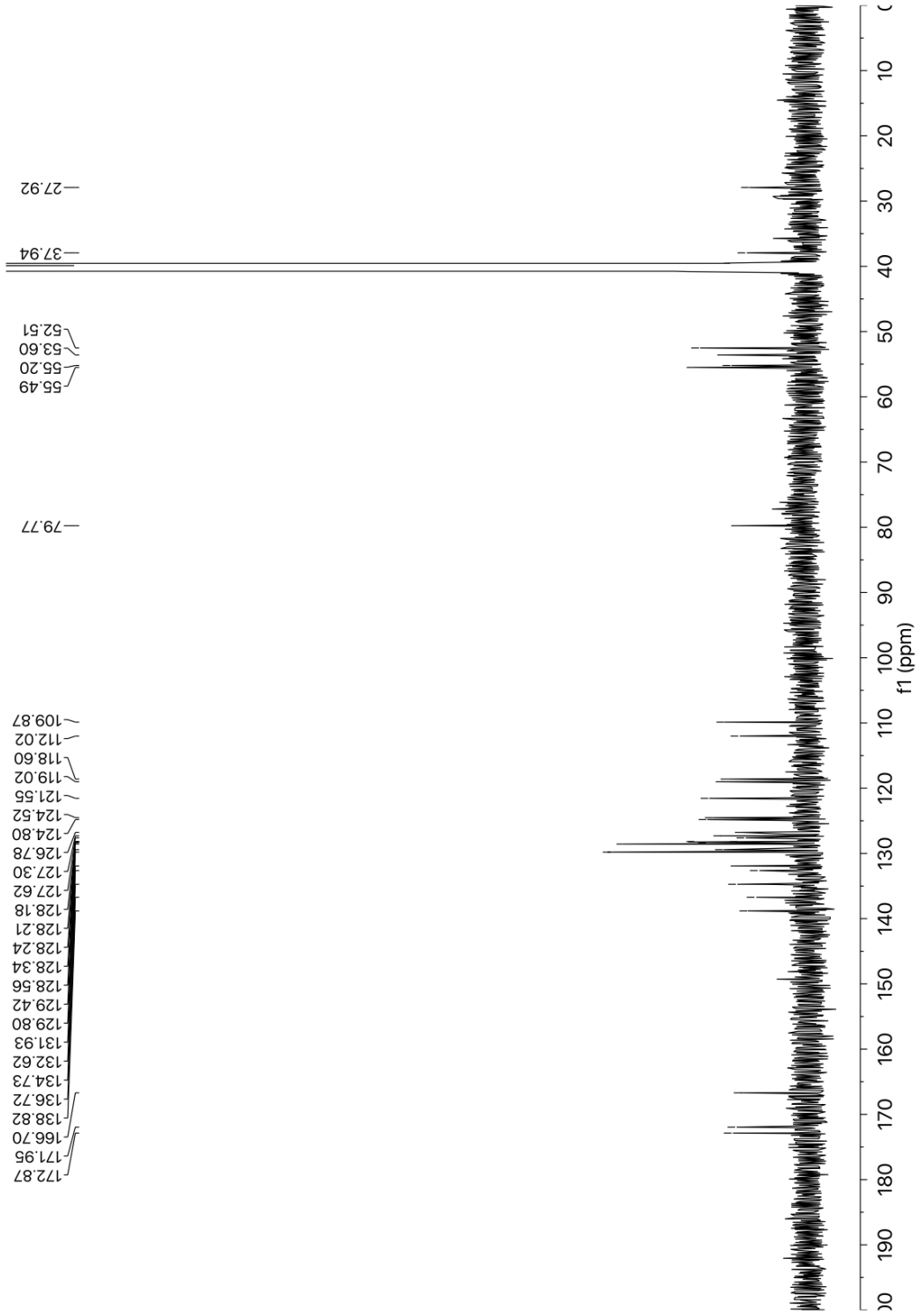
^{13}CNMR spectrum for compound **56o**



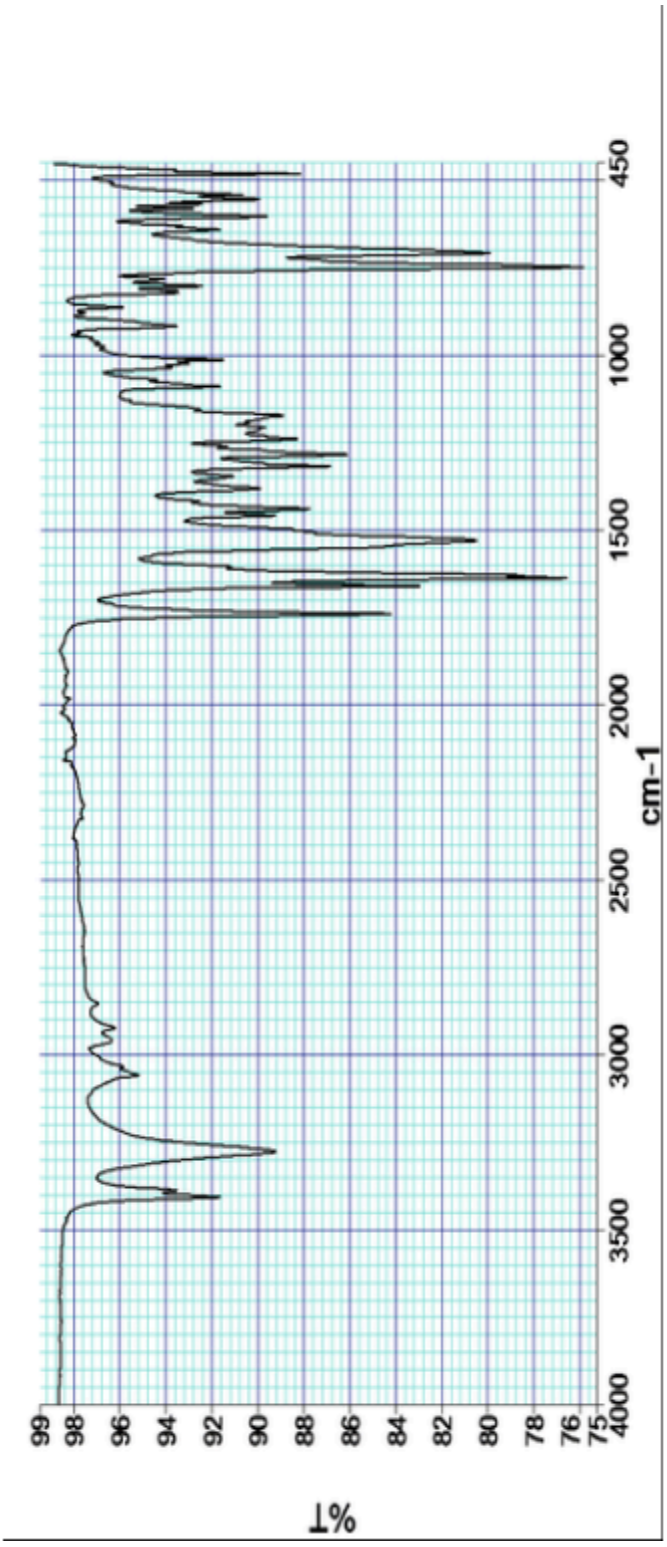
IR spectrum for compound **56o**



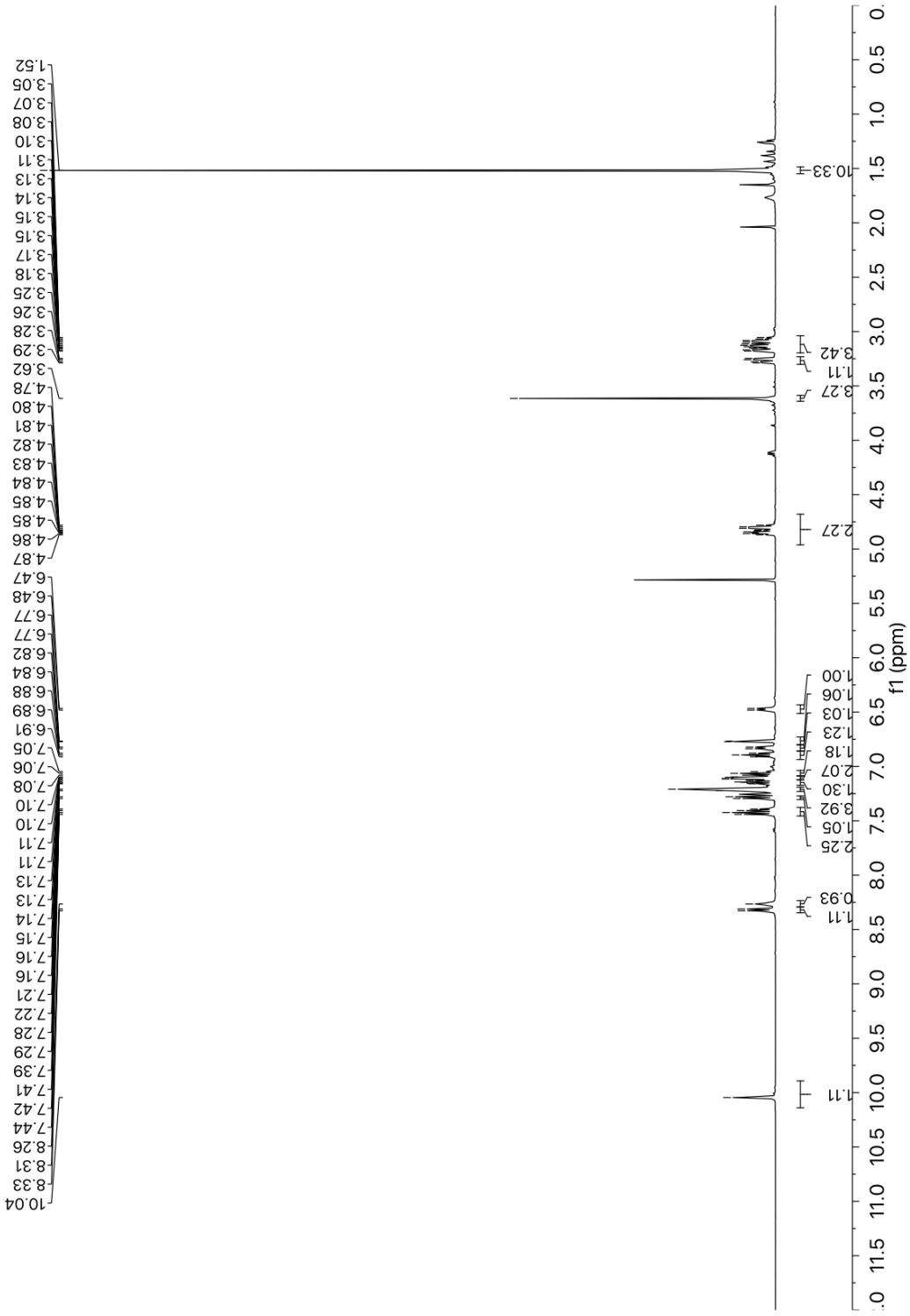
[1]HNMR spectrum for compound **56p**



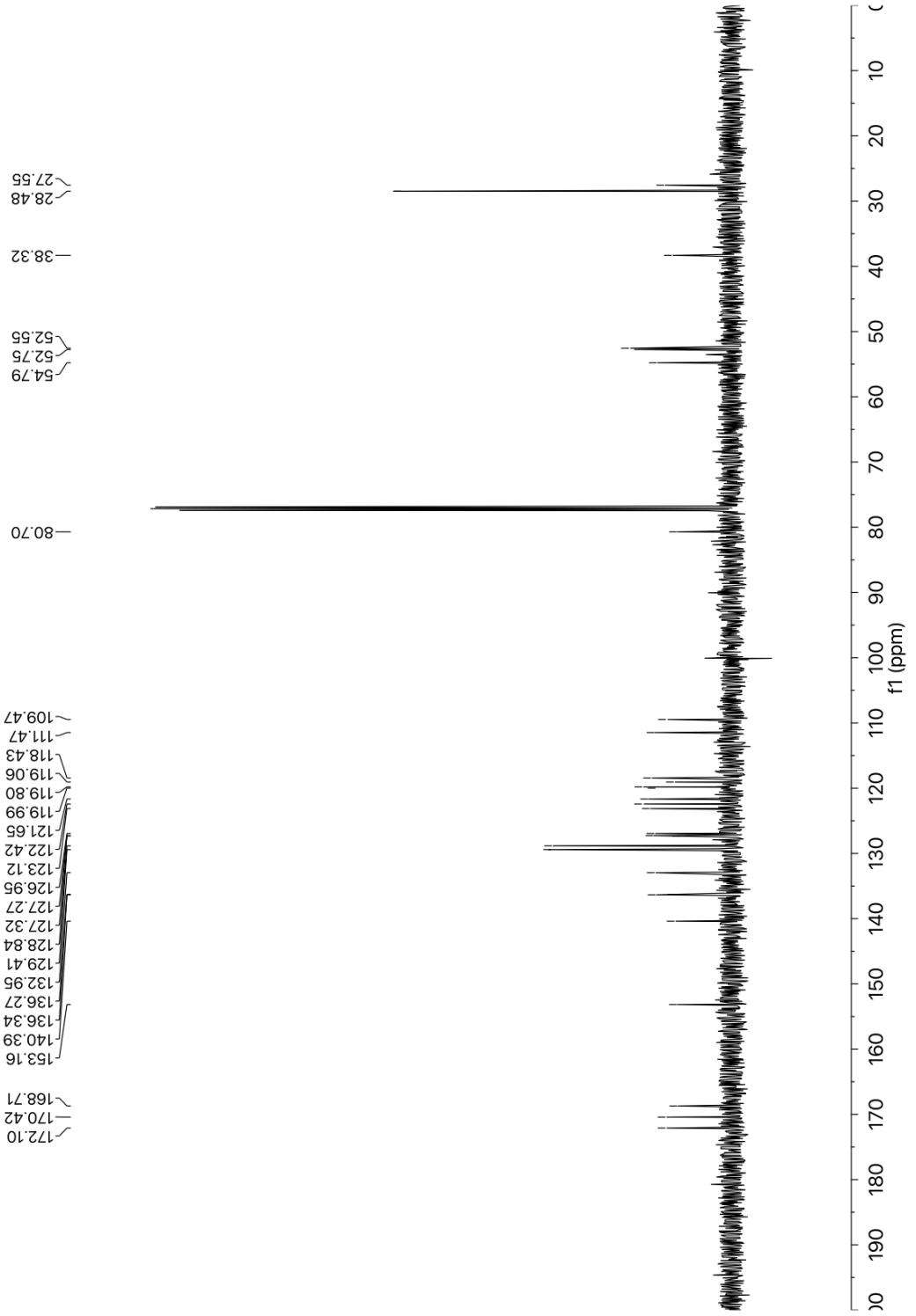
^{13}CNMR spectrum for compound **56p**



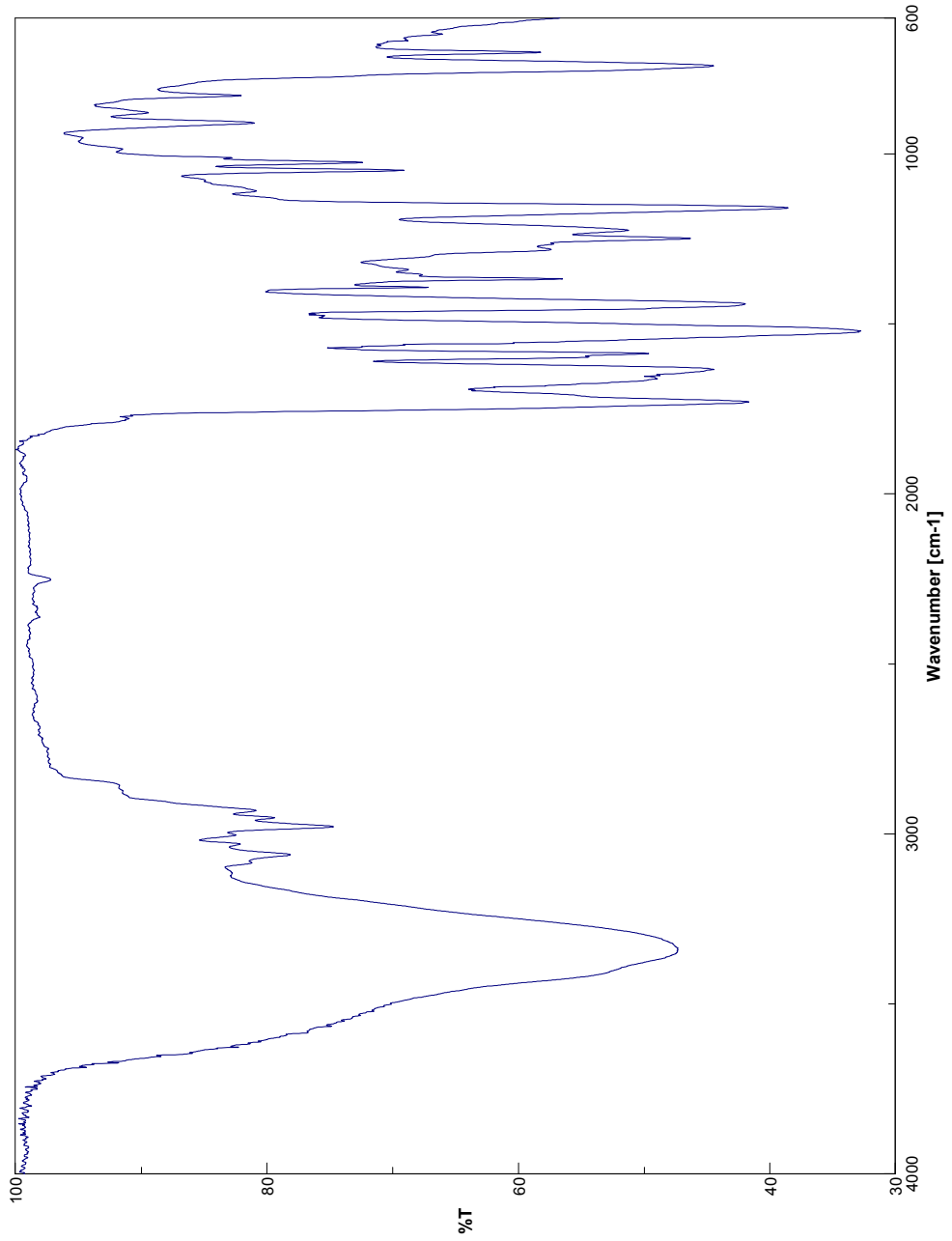
IR spectrum for compound **56p**



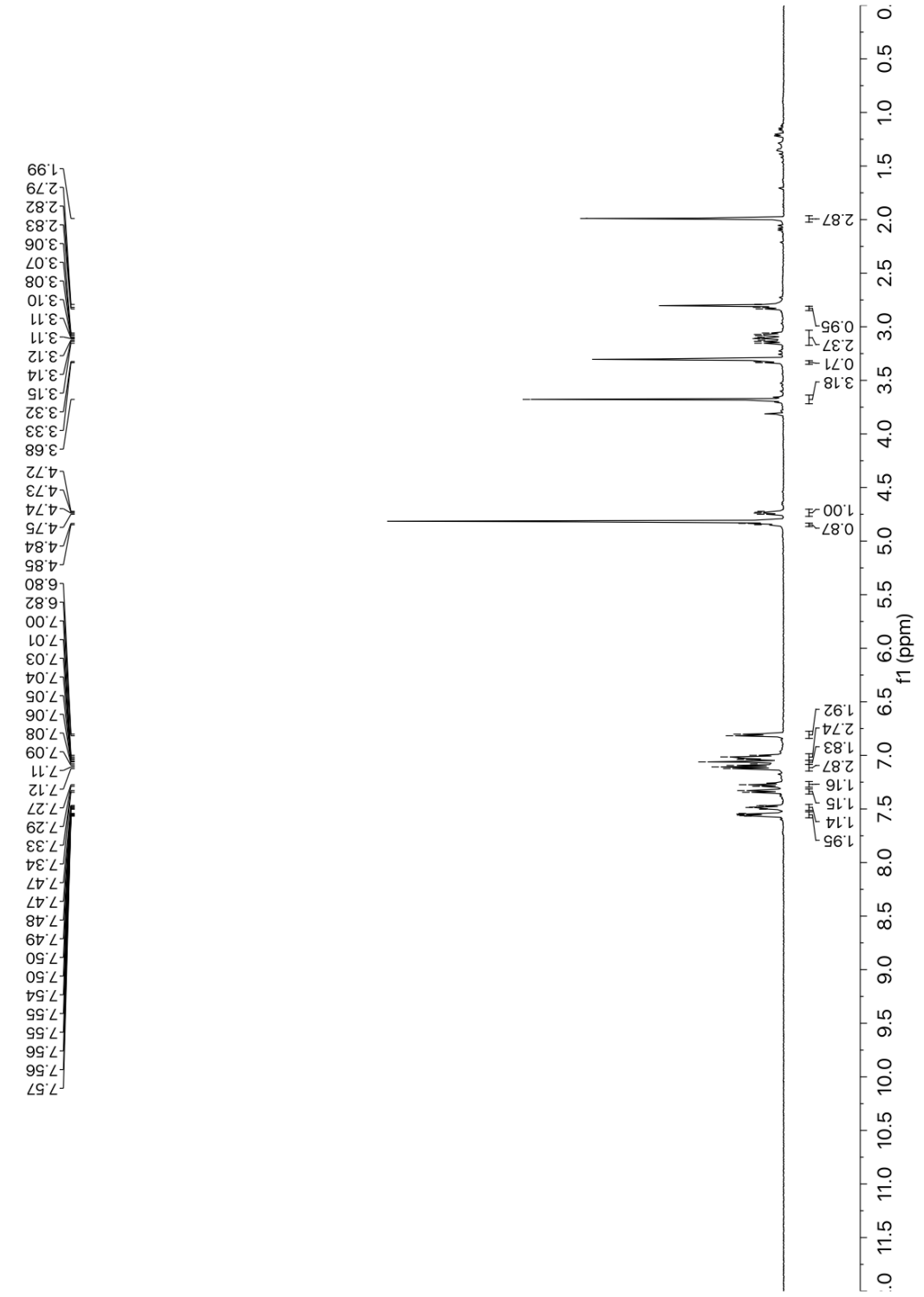
[1]HNMR spectrum for compound **56q**



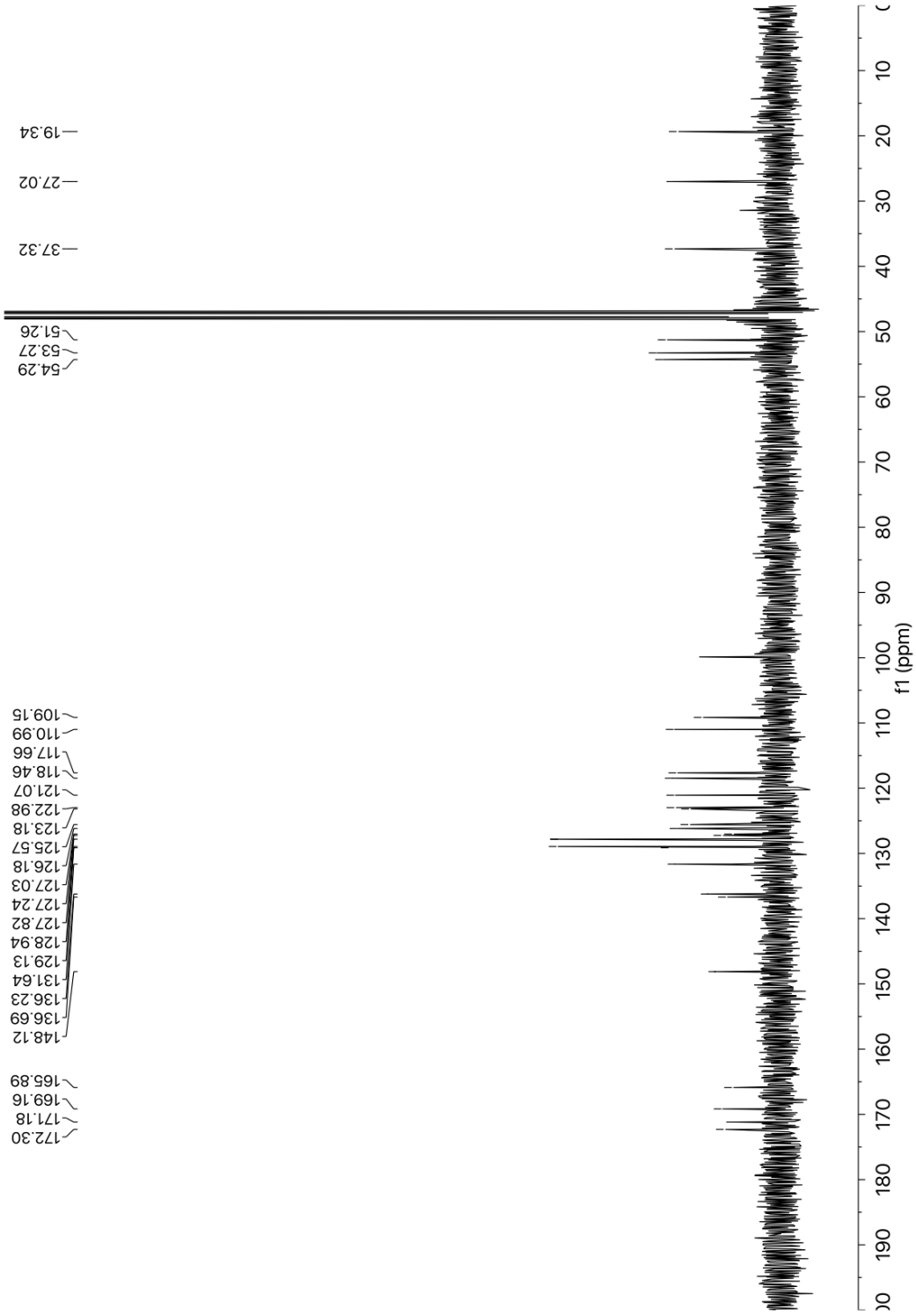
^{13}CNMR spectrum for compound **56q**



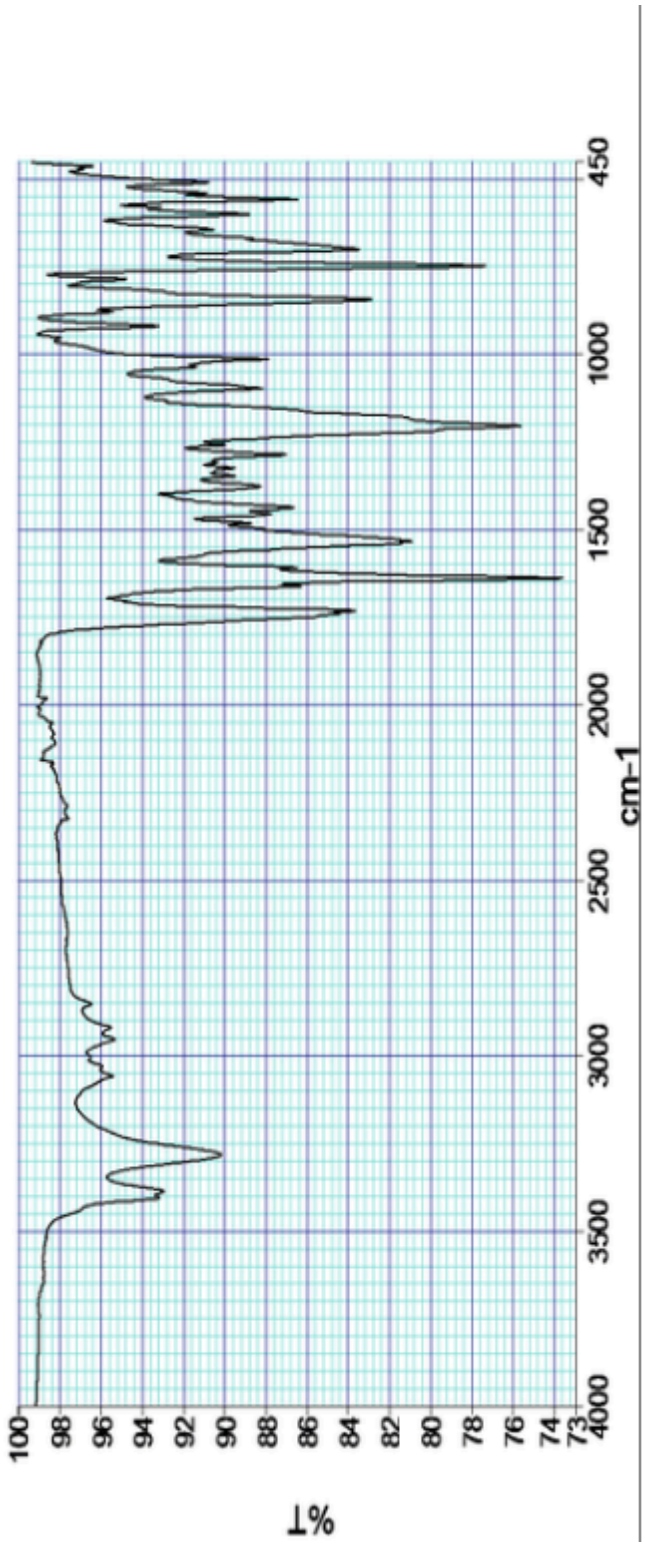
IR spectrum for compound **56q**



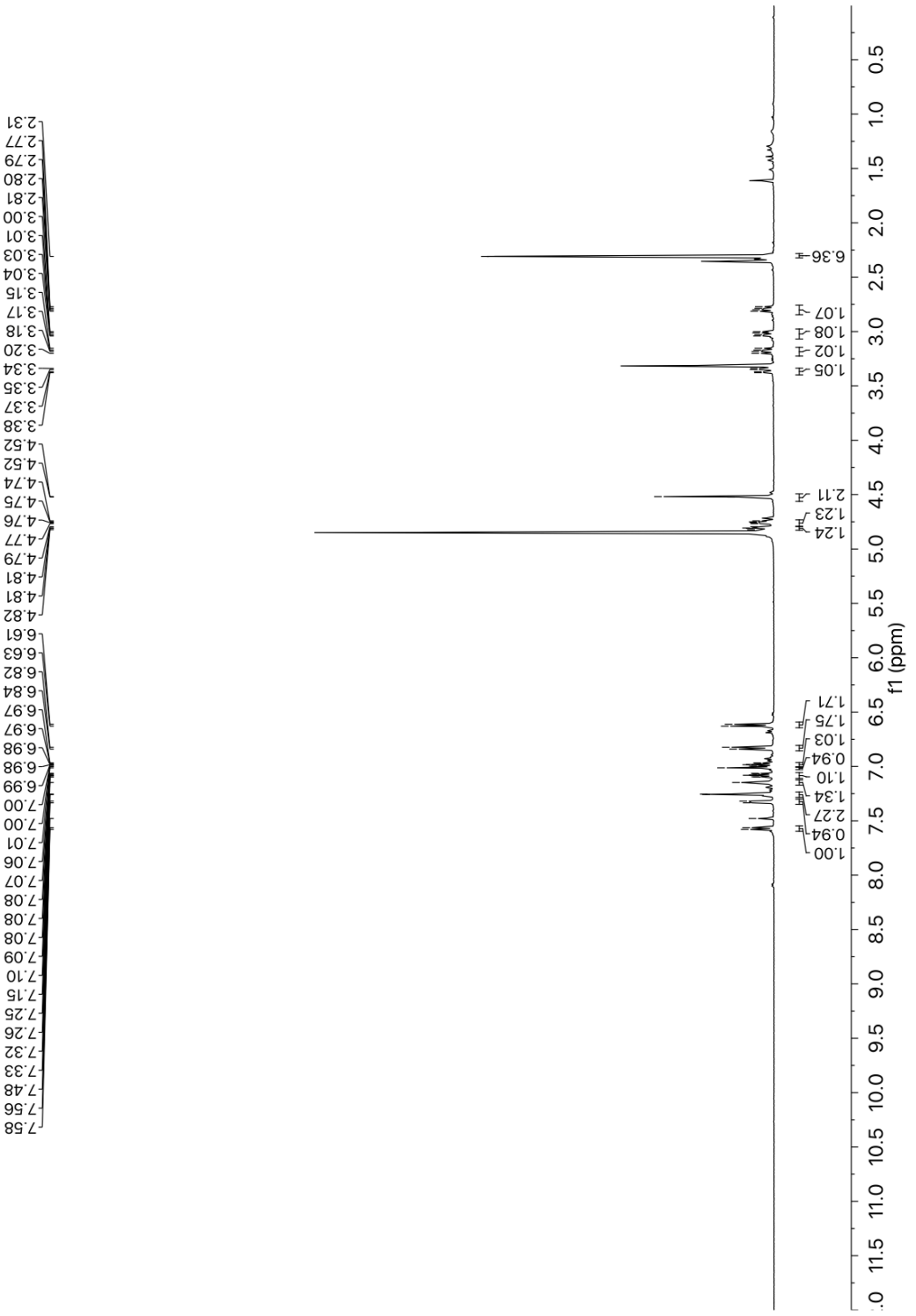
^{1}HNMR spectrum for compound **56r**



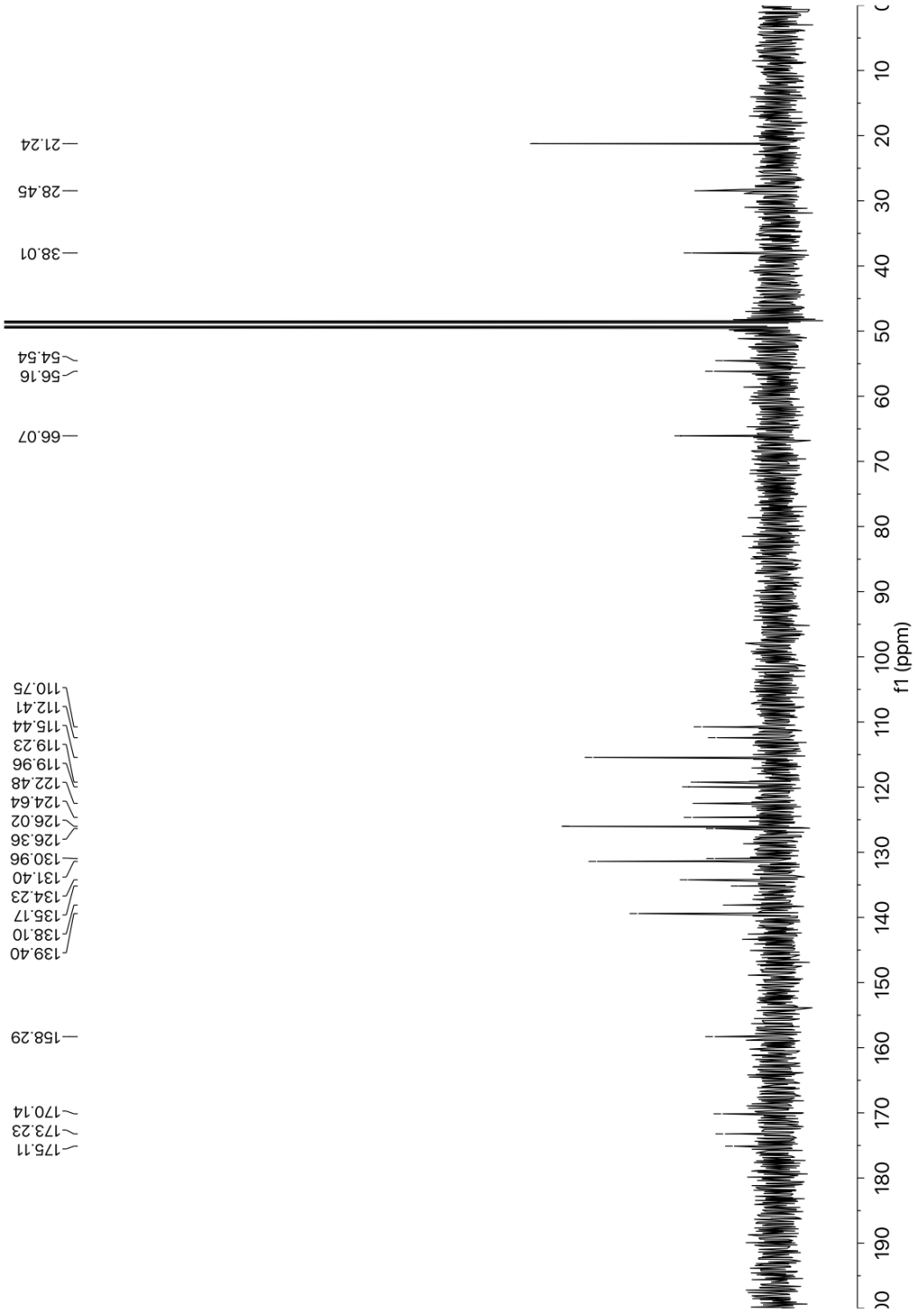
^{13}CNMR spectrum for compound **56r**



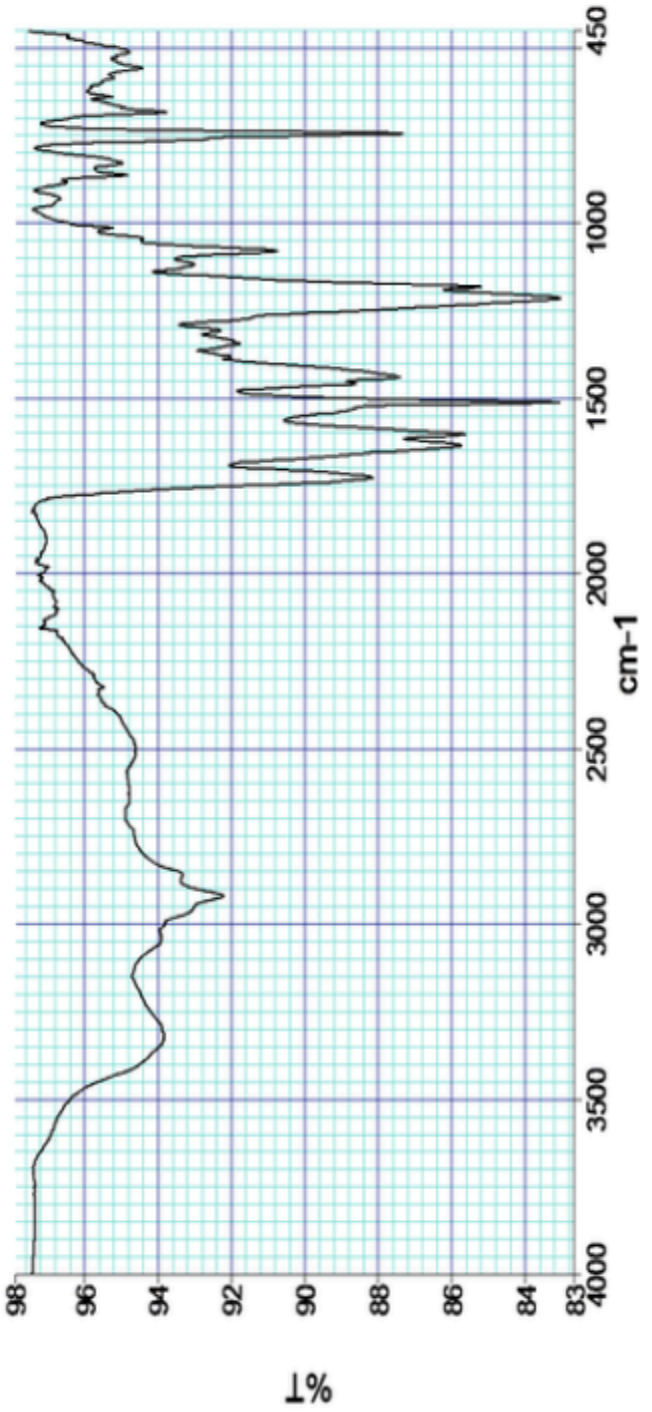
IR spectrum for compound **56r**



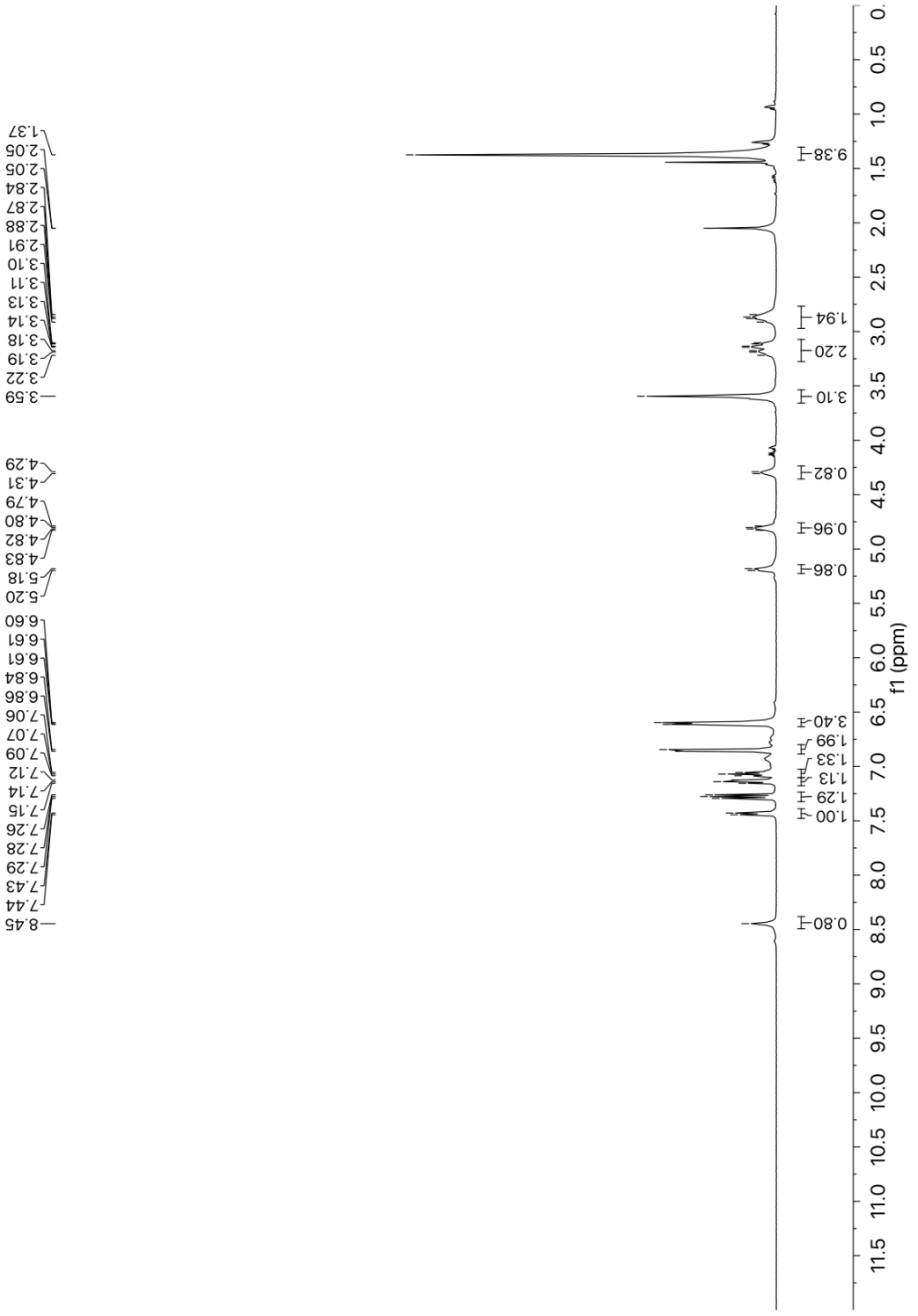
[1]HNMR spectrum for compound **60**



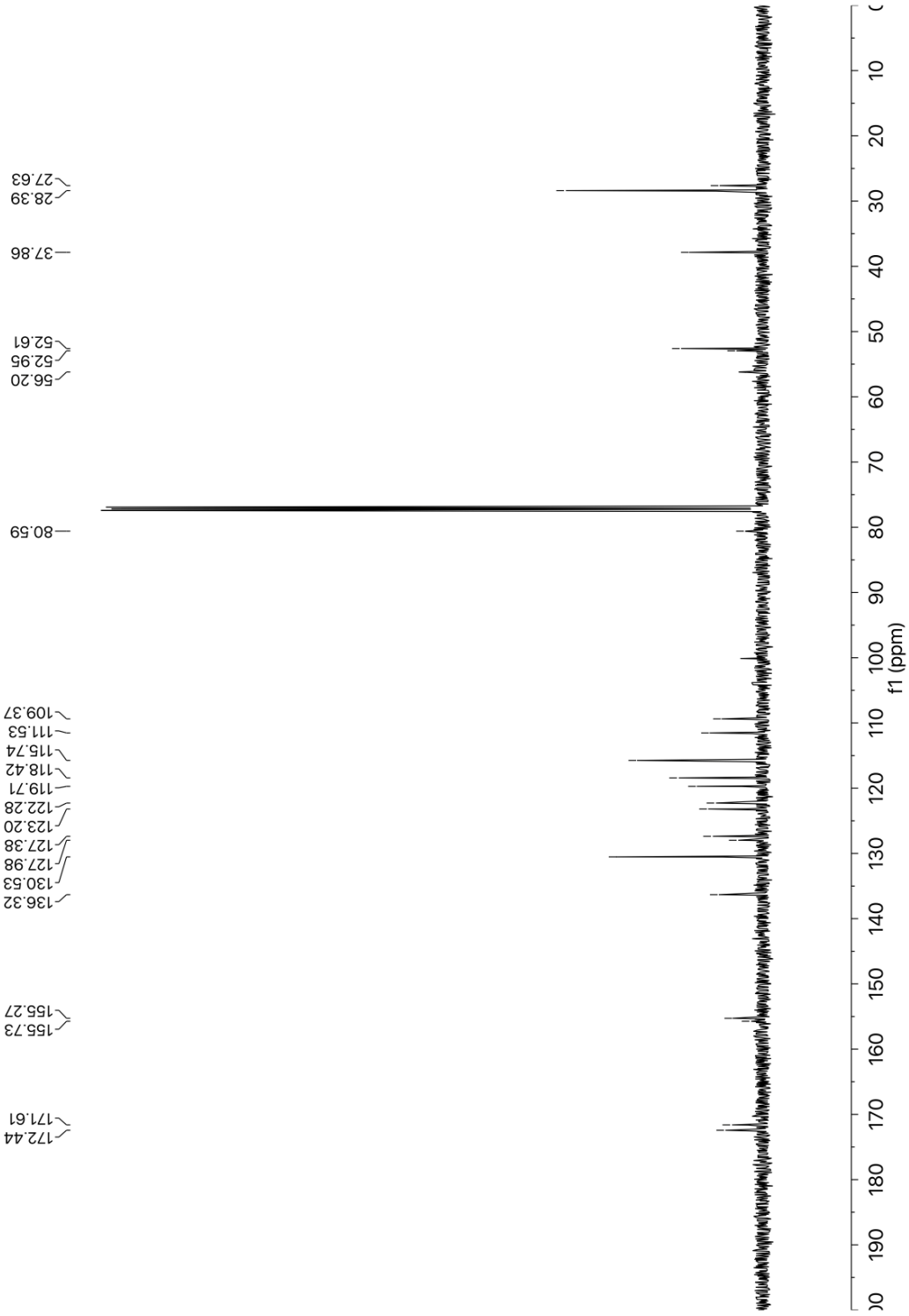
^{13}CNMR spectrum for compound **60**



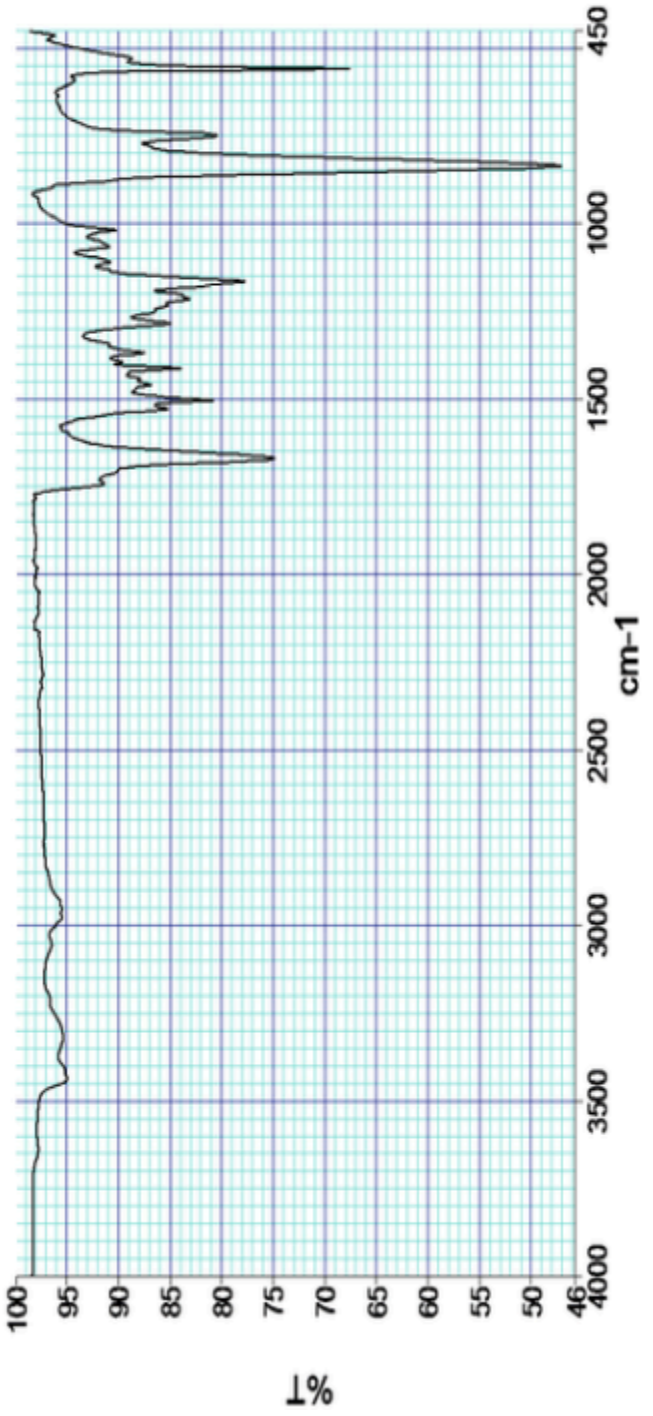
IR spectrum for compound **60**



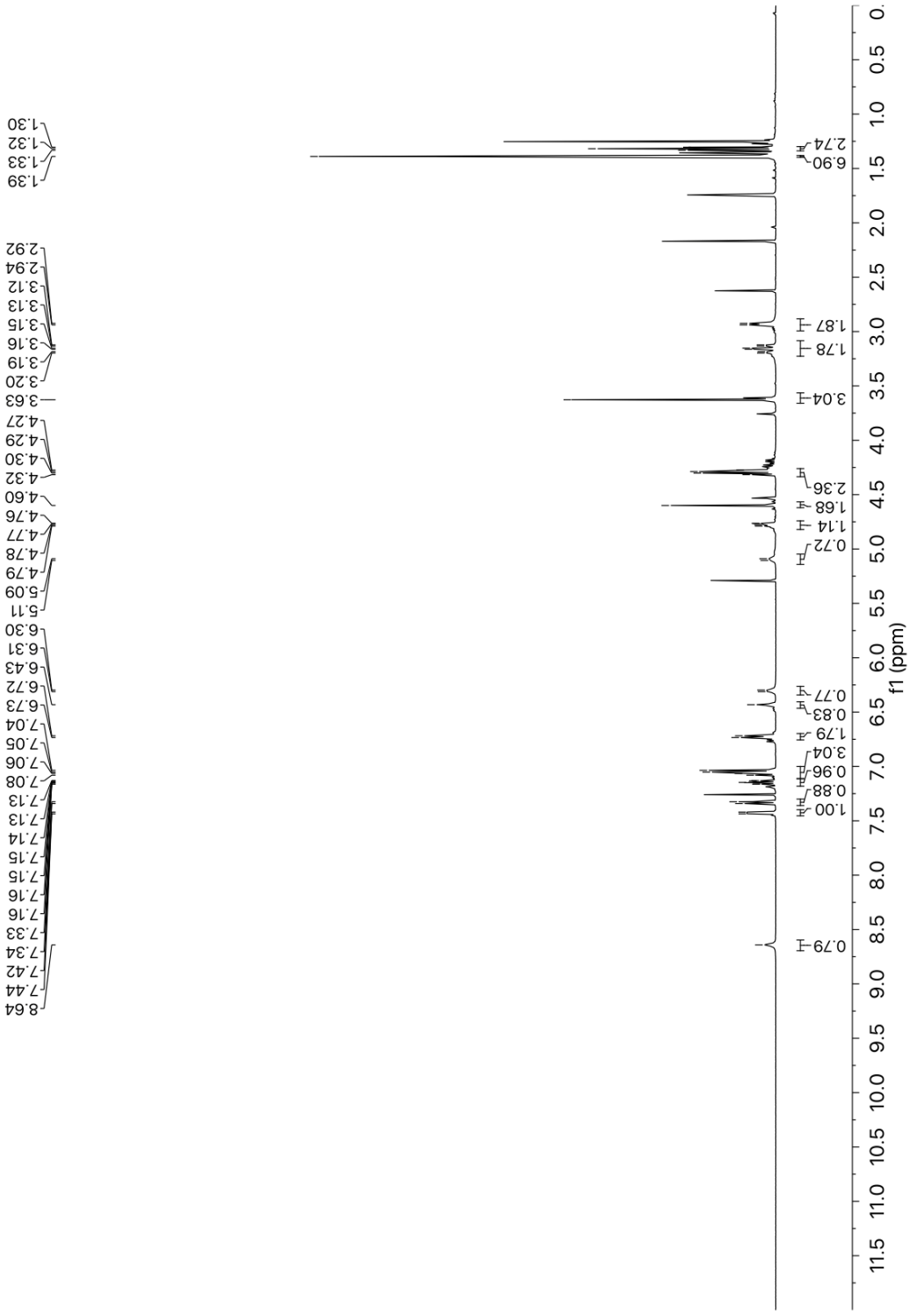
^{1}HNMR spectrum for compound **64**



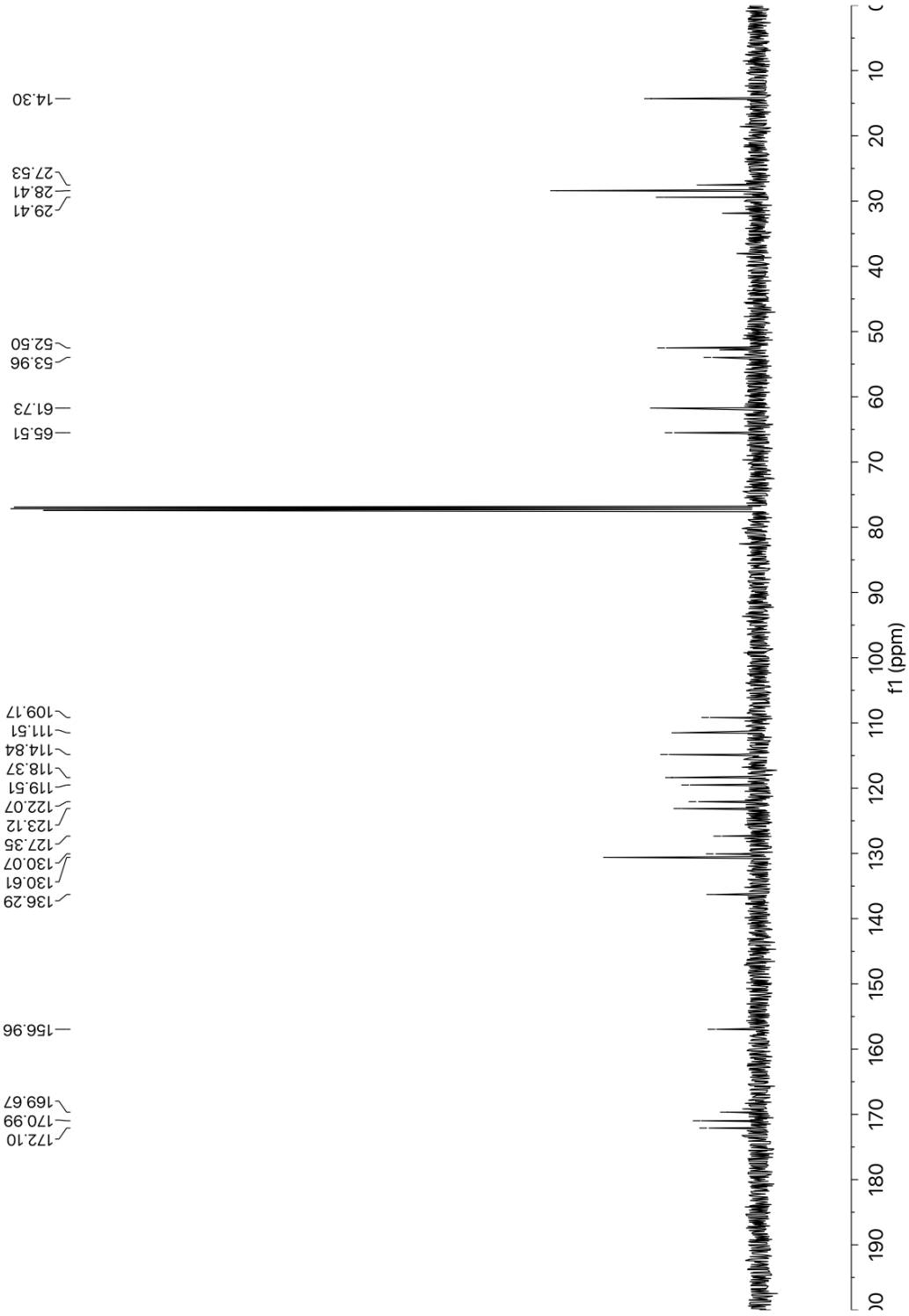
^{13}CNMR spectrum for compound **64**



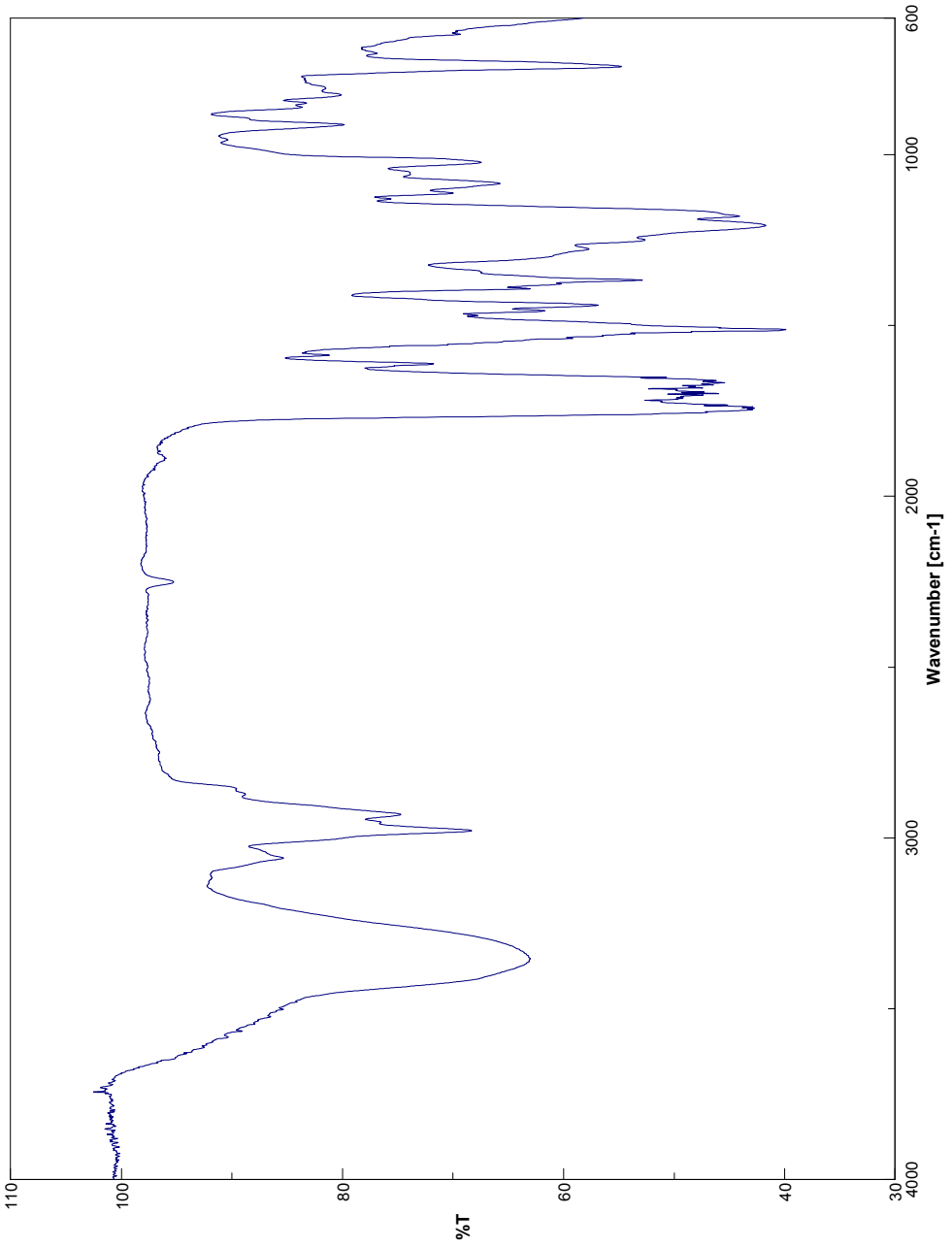
IR spectrum for compound **64**



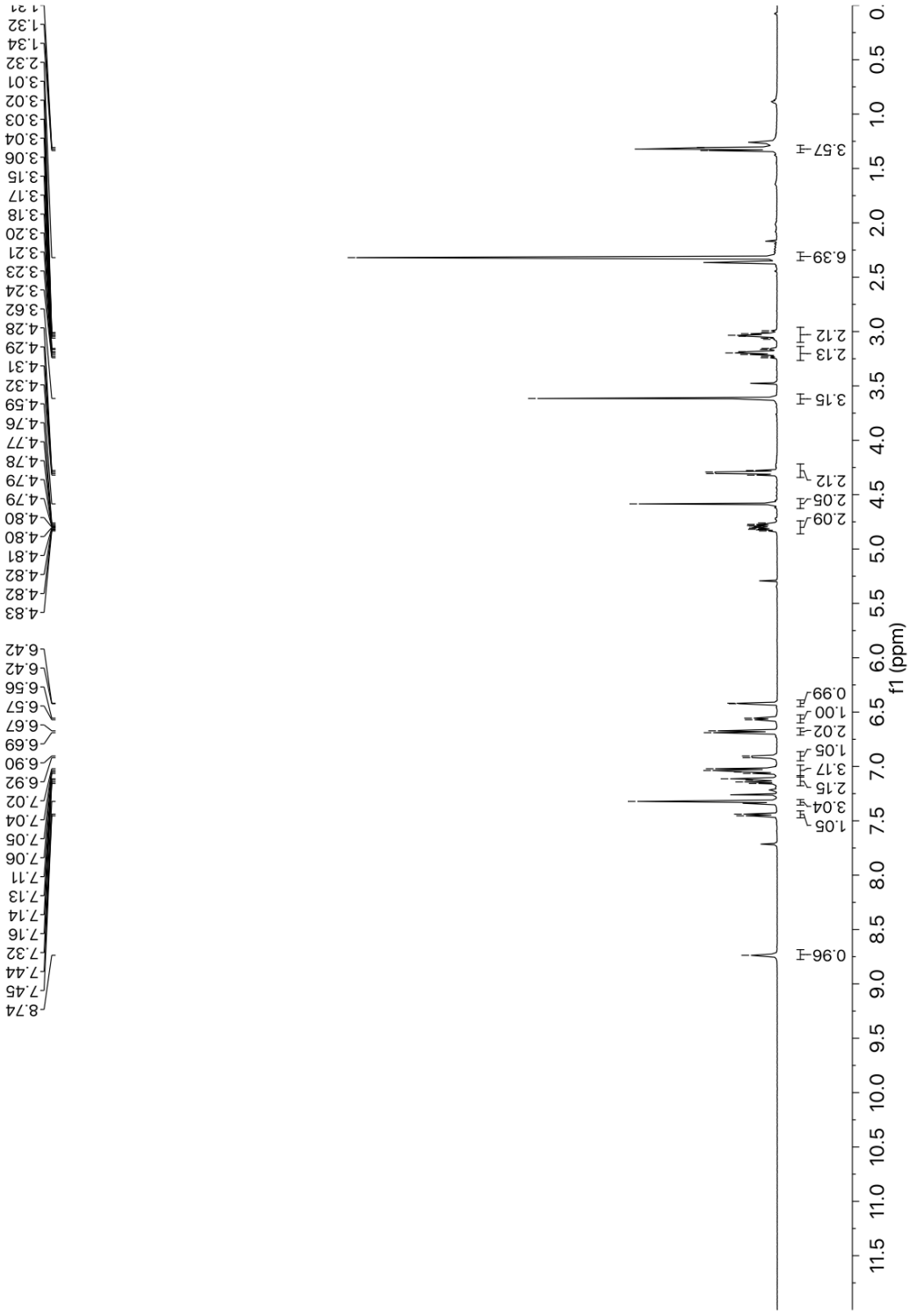
[1]HNMR spectrum for compound **65**



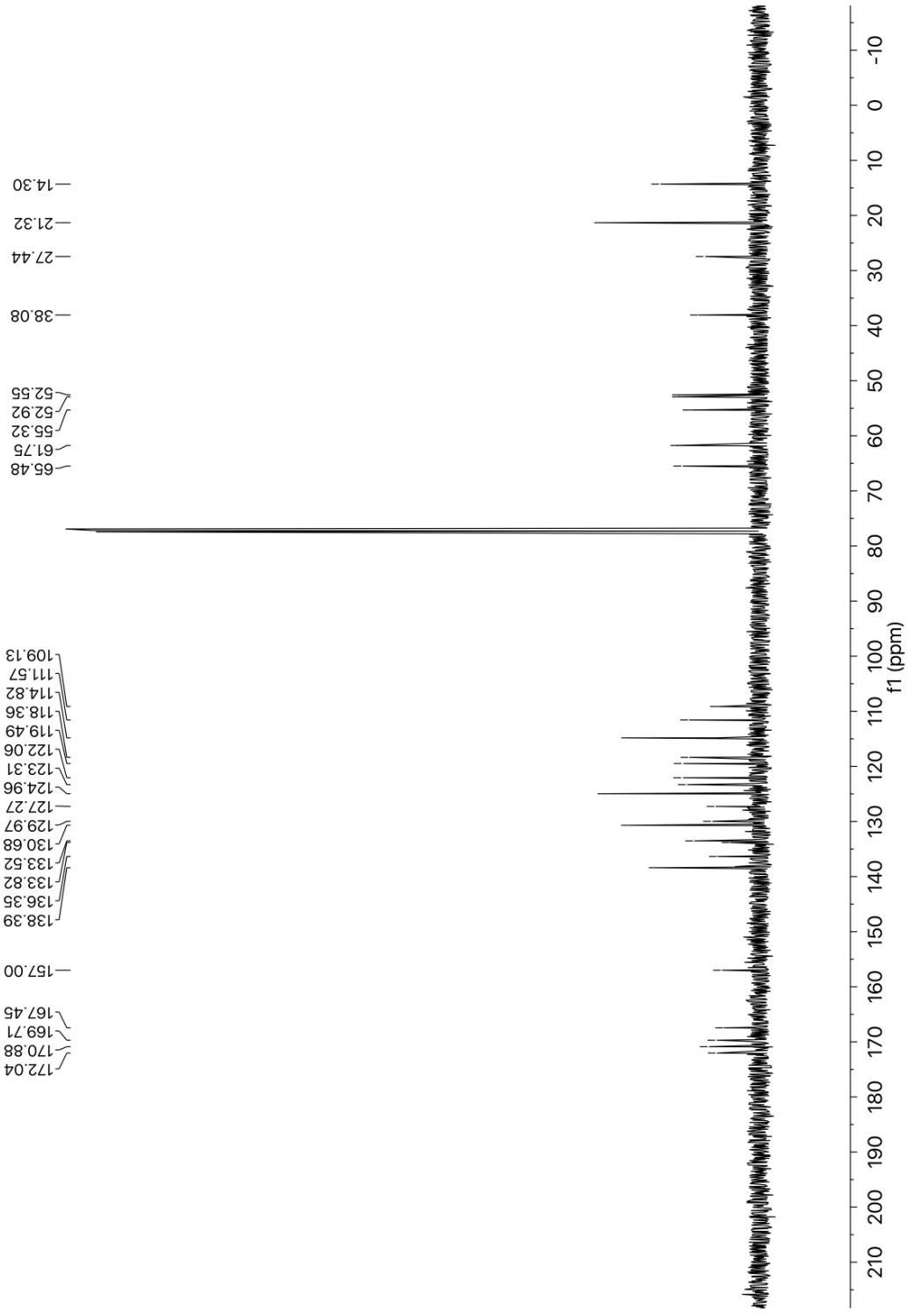
^{13}CNMR spectrum for compound **65**



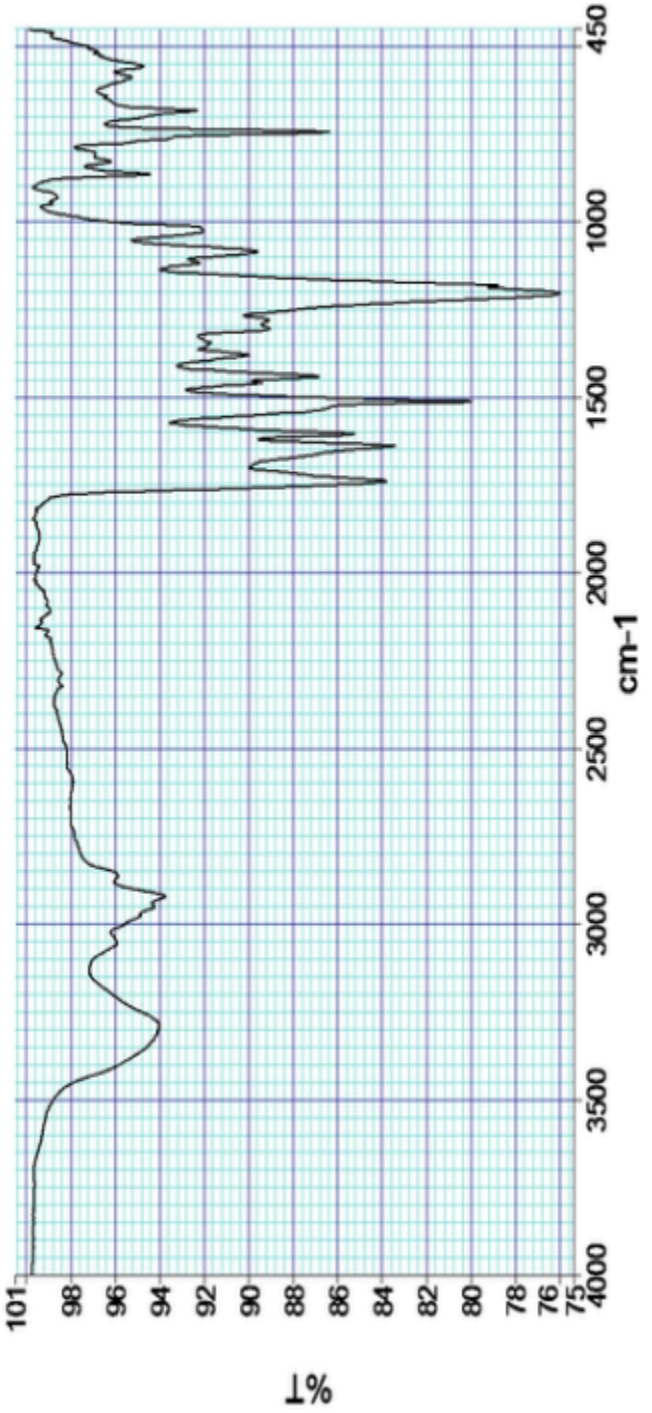
IR spectrum for compound **65**



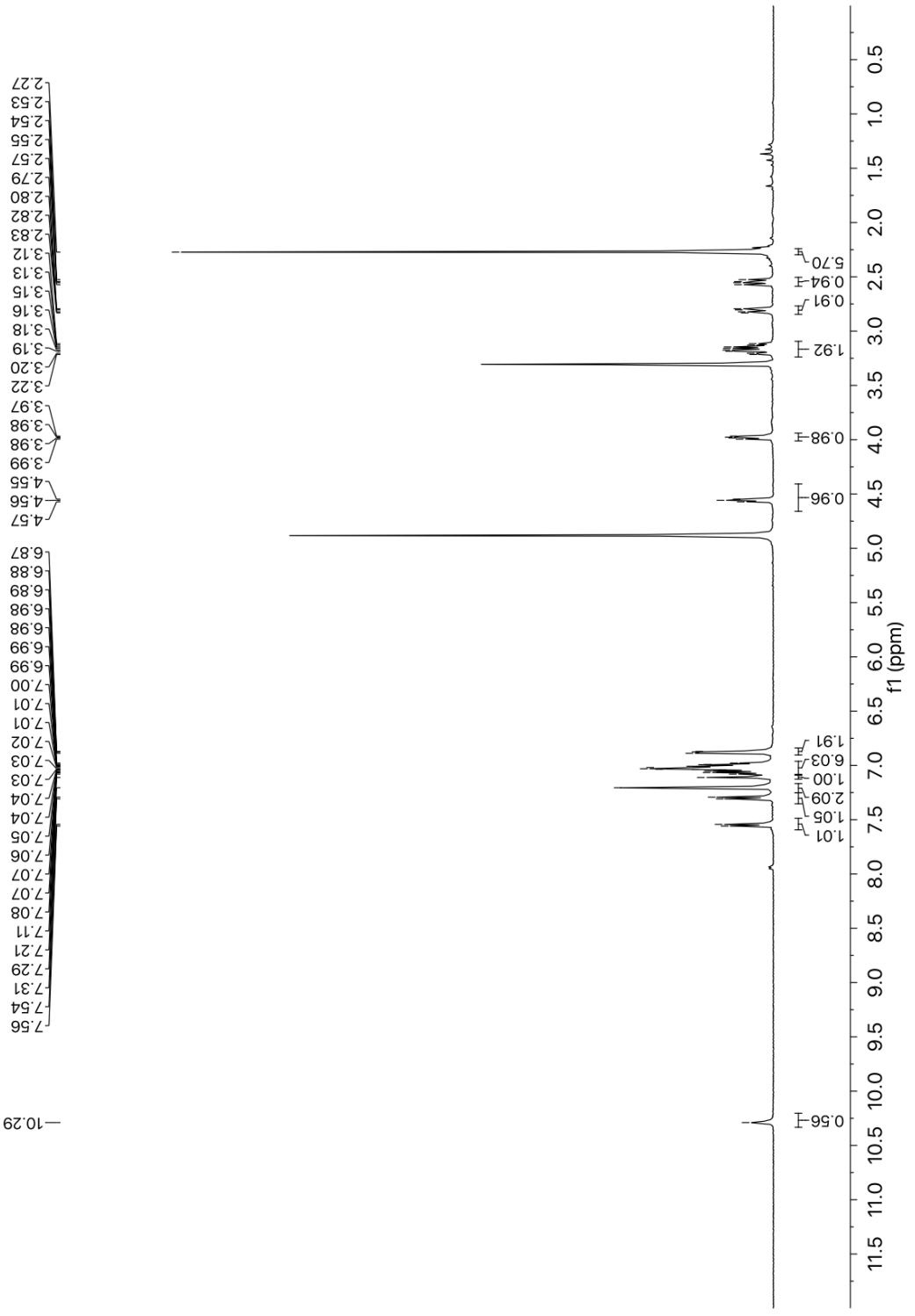
[1]HNMR spectrum for compound **66**



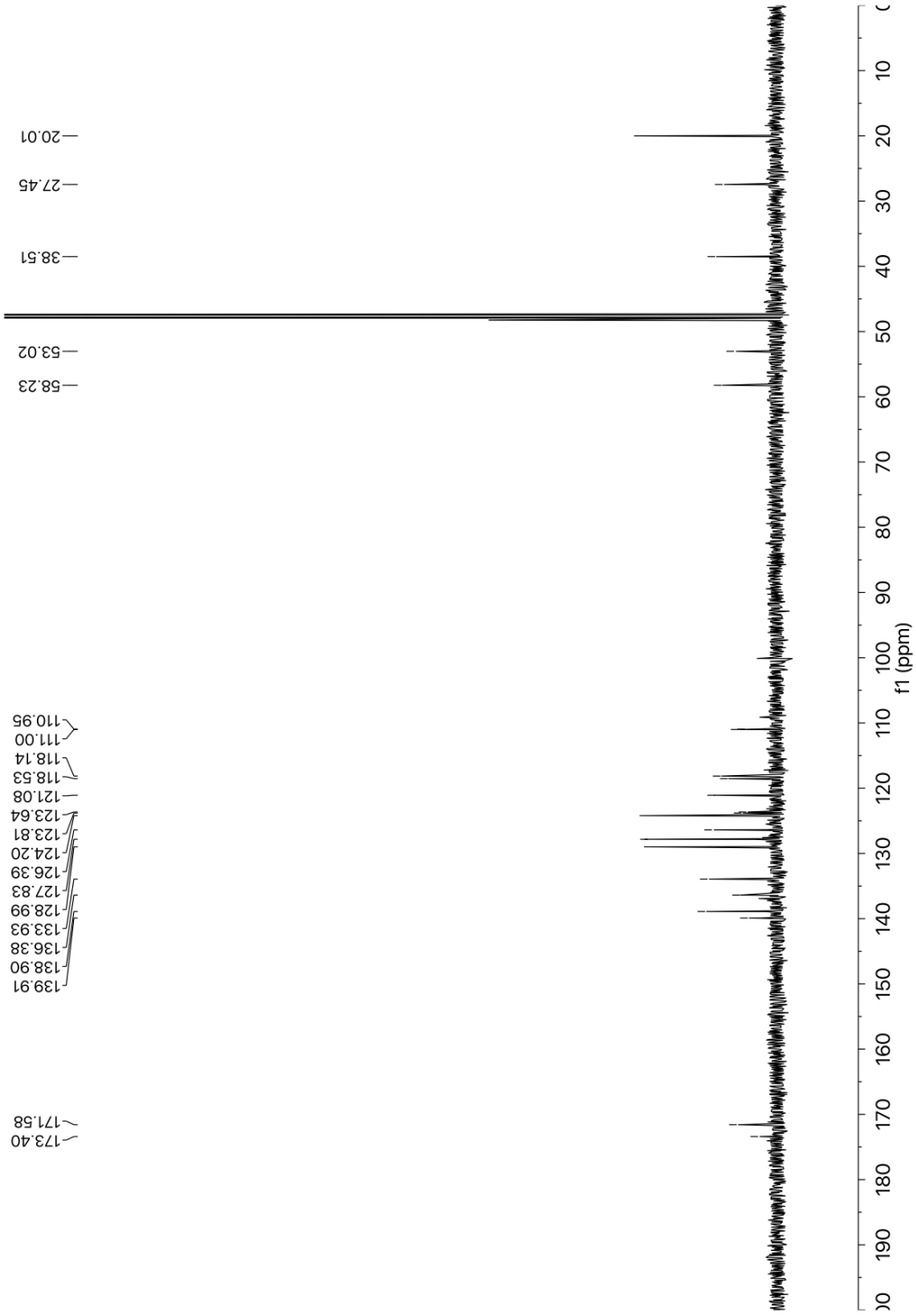
^{13}CNMR spectrum for compound **66**



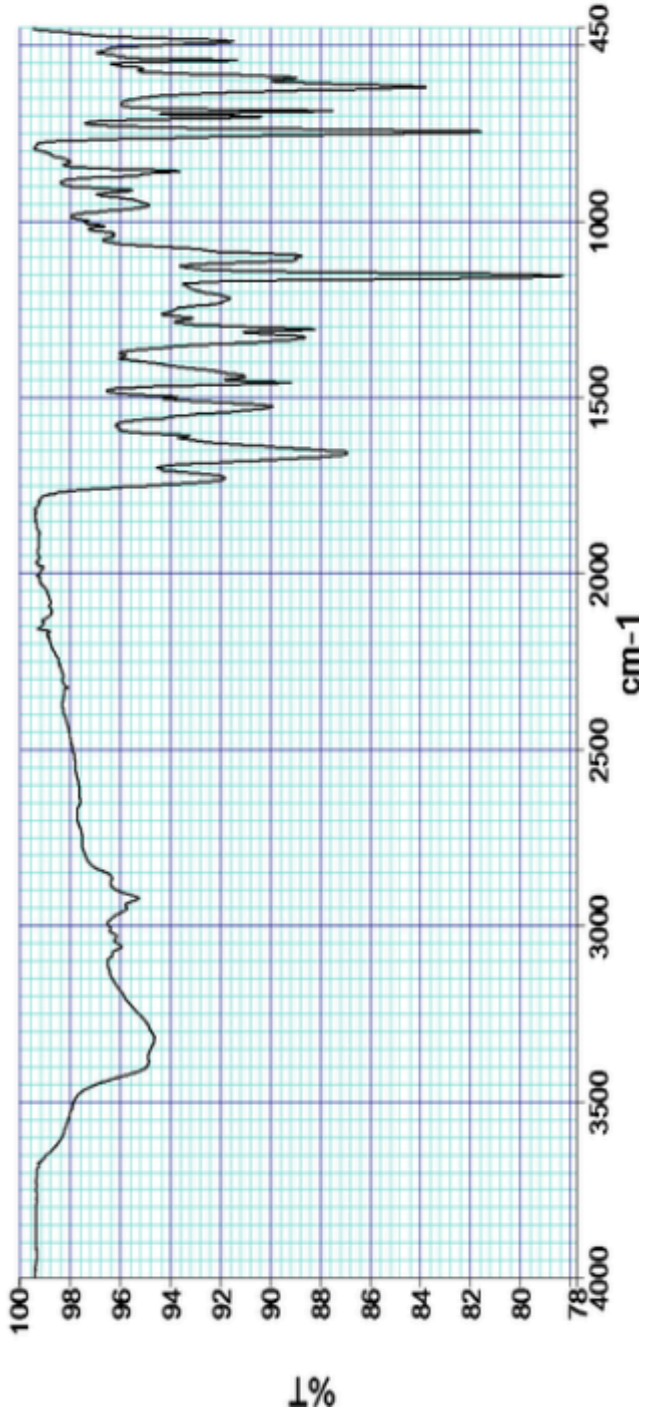
IR spectrum for compound **66**



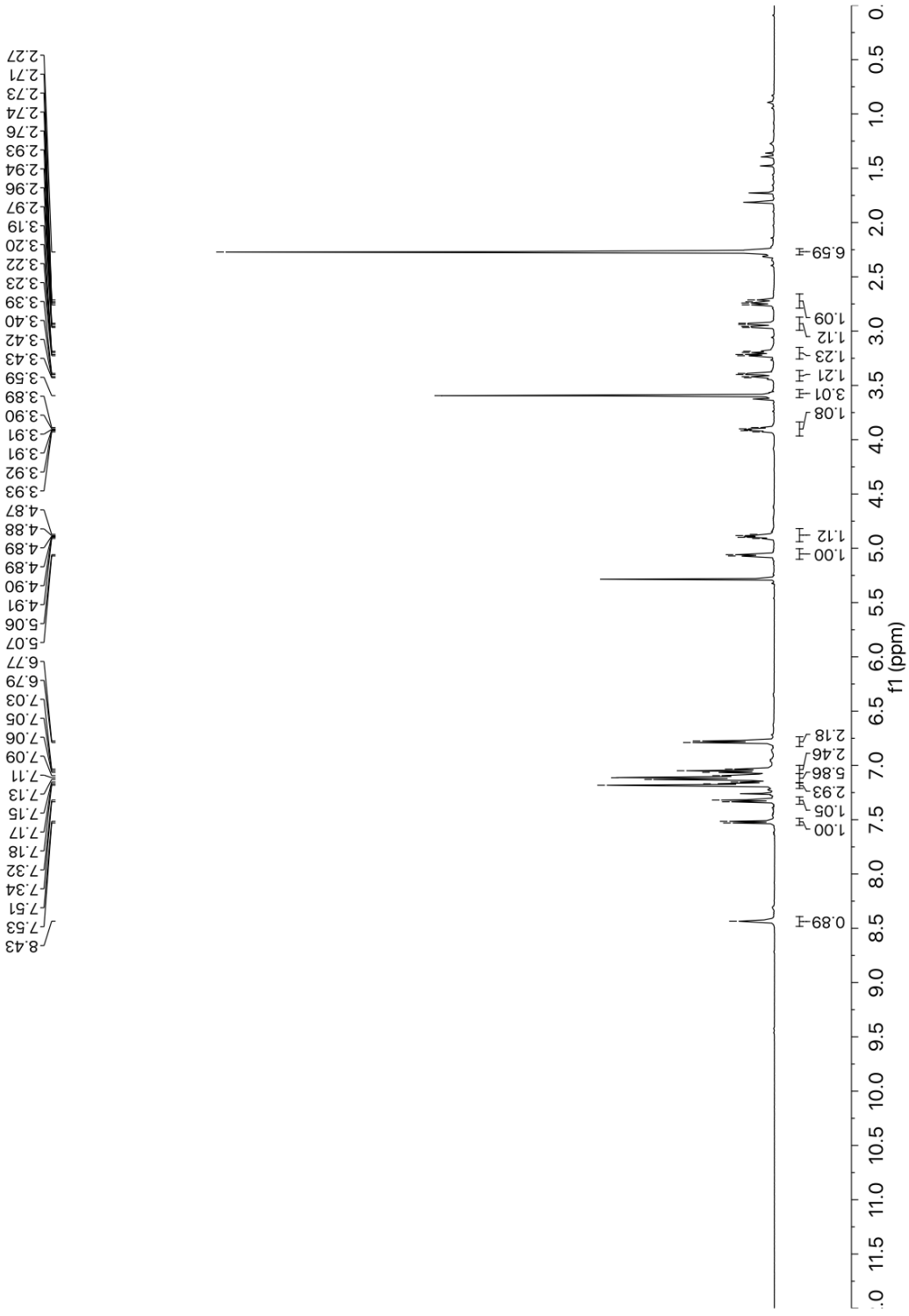
^{1}HNMR spectrum for compound **67**



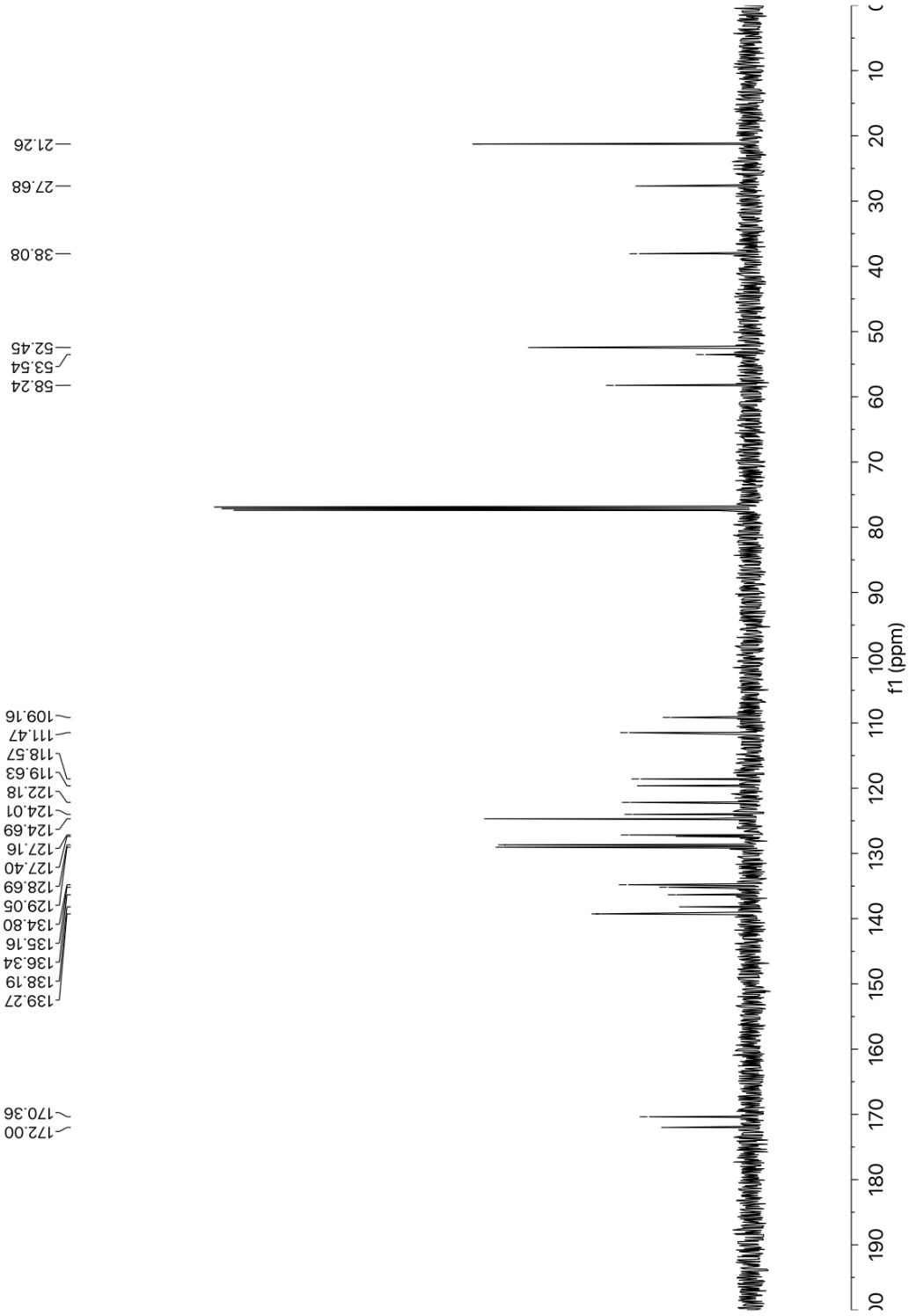
^{13}CNMR spectrum for compound **67**



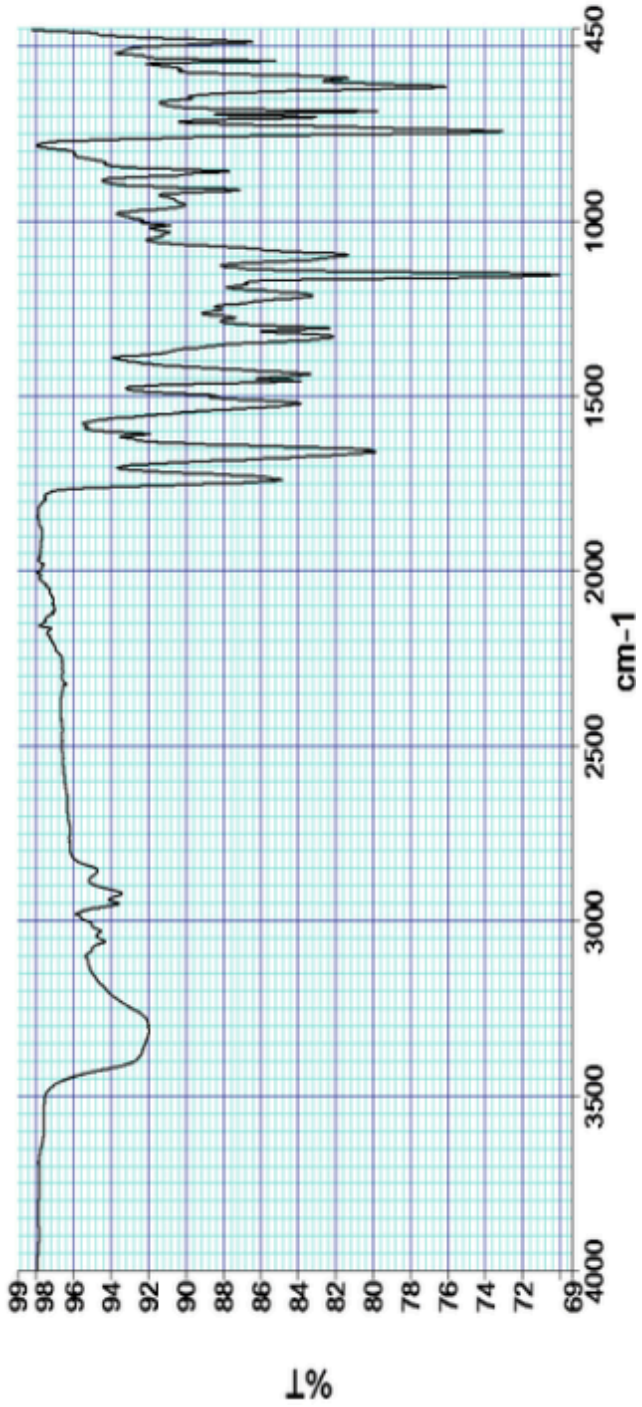
IR spectrum for compound **67**



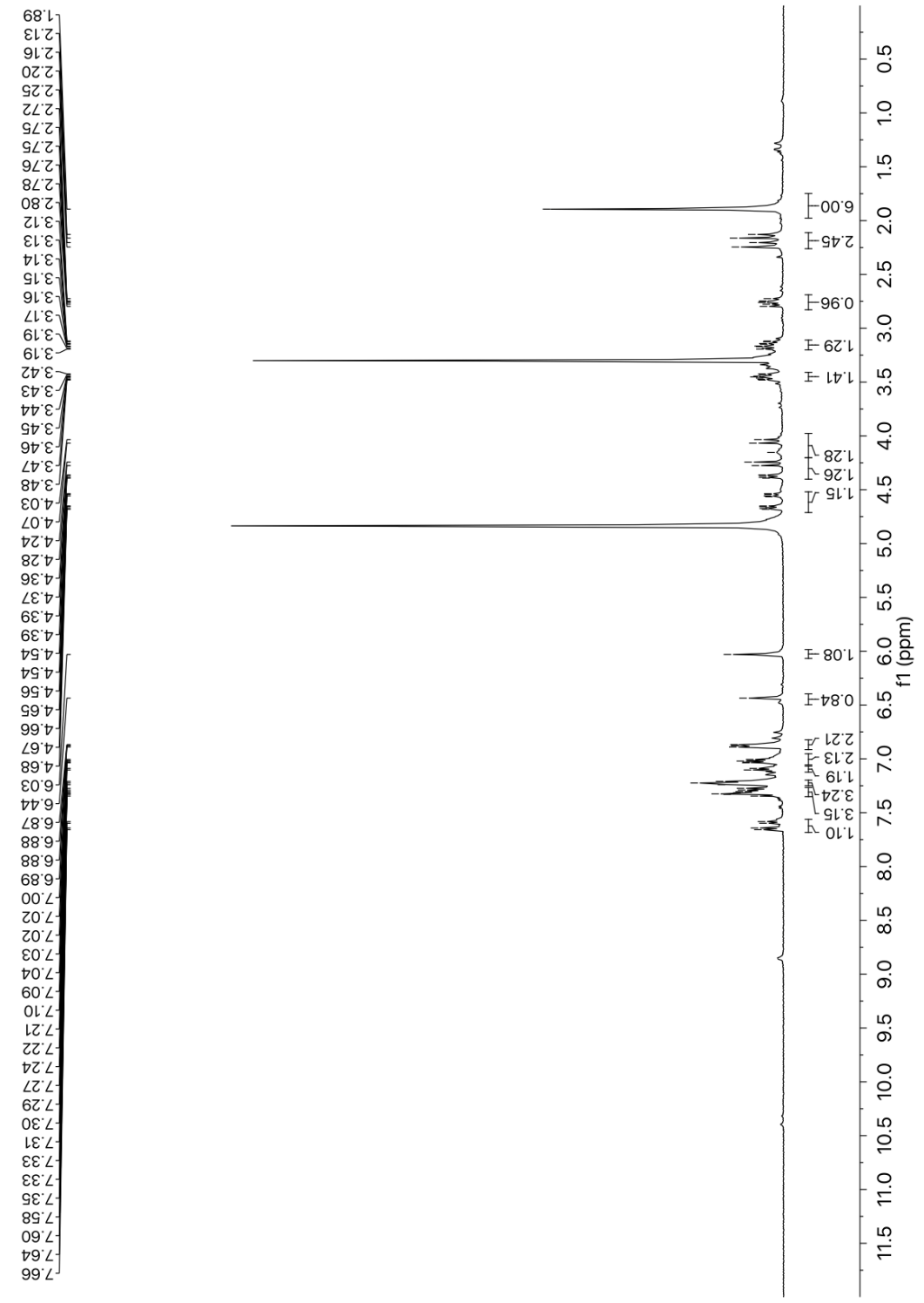
[1]HNMR spectrum for compound **70**



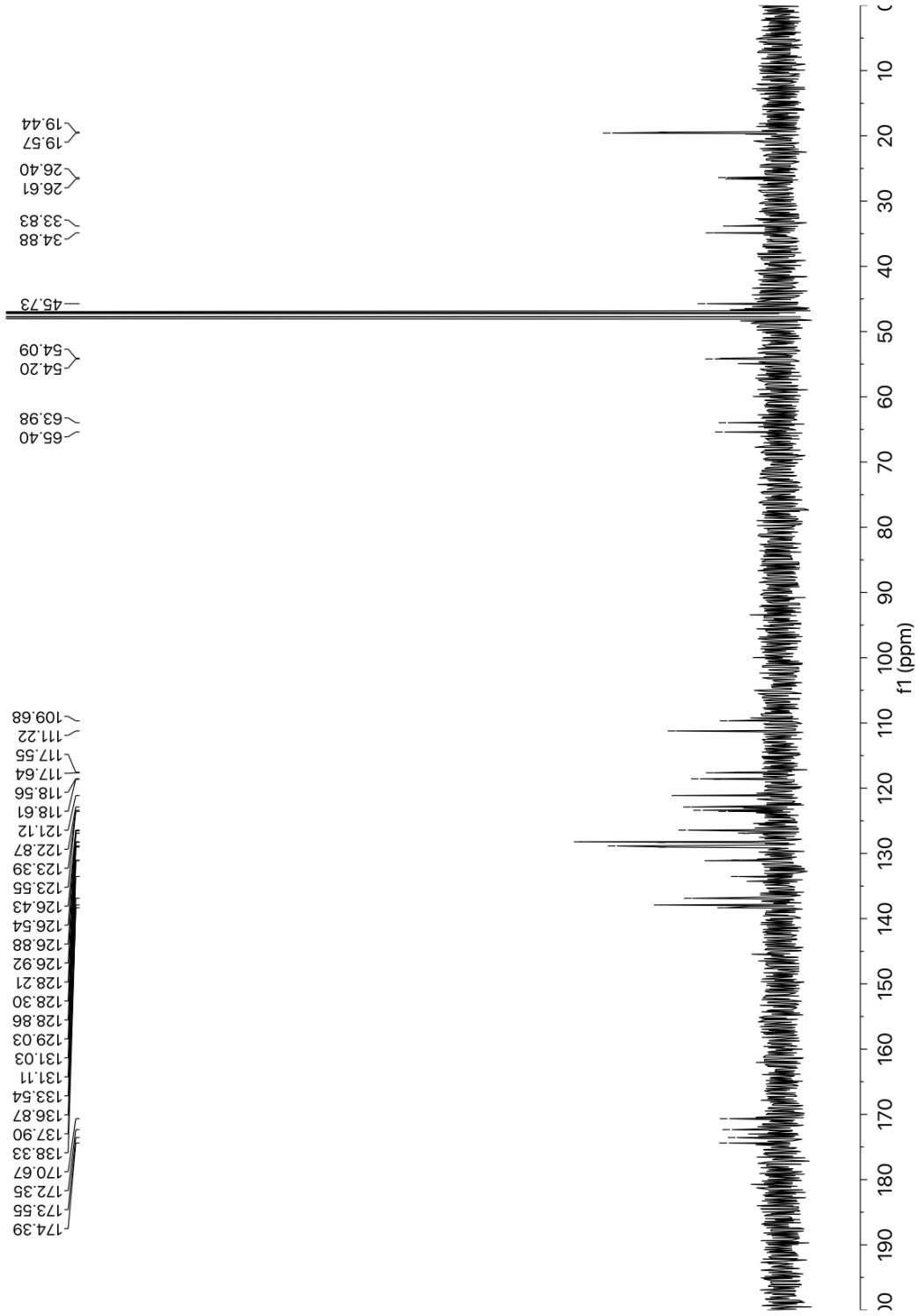
^{13}CNMR spectrum for compound **70**



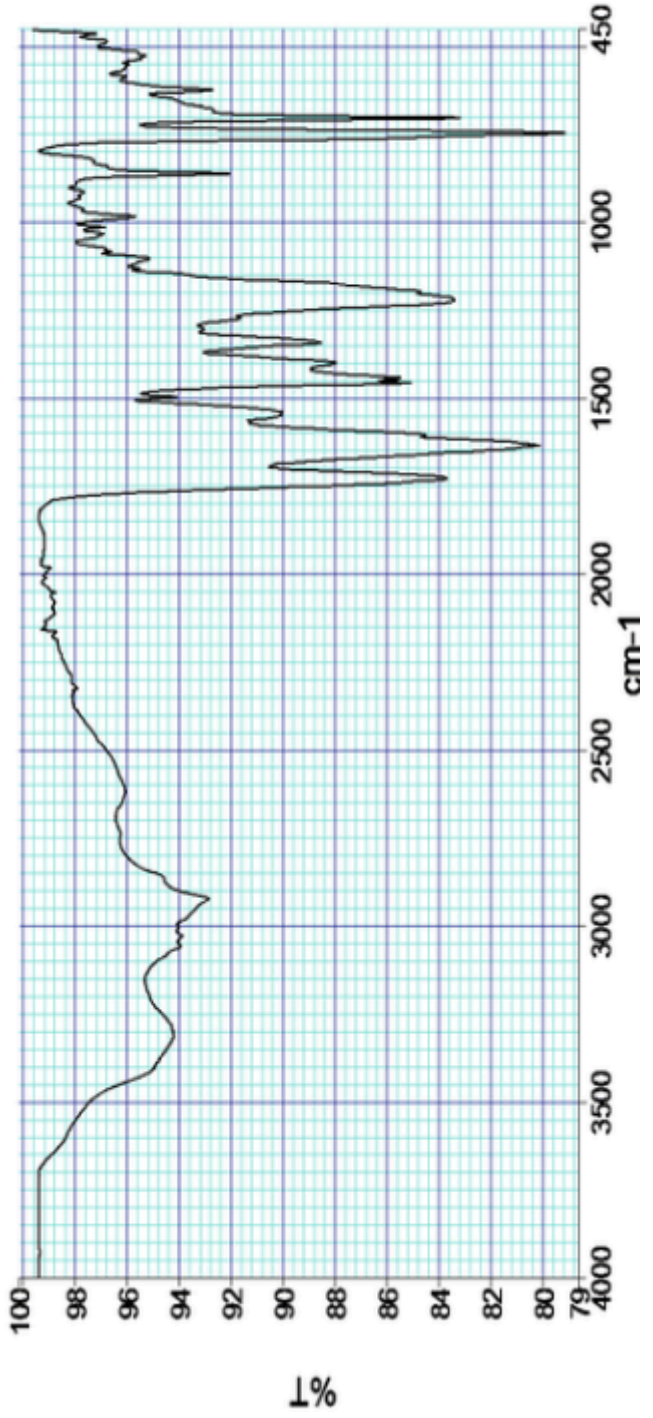
IR spectrum for compound **70**



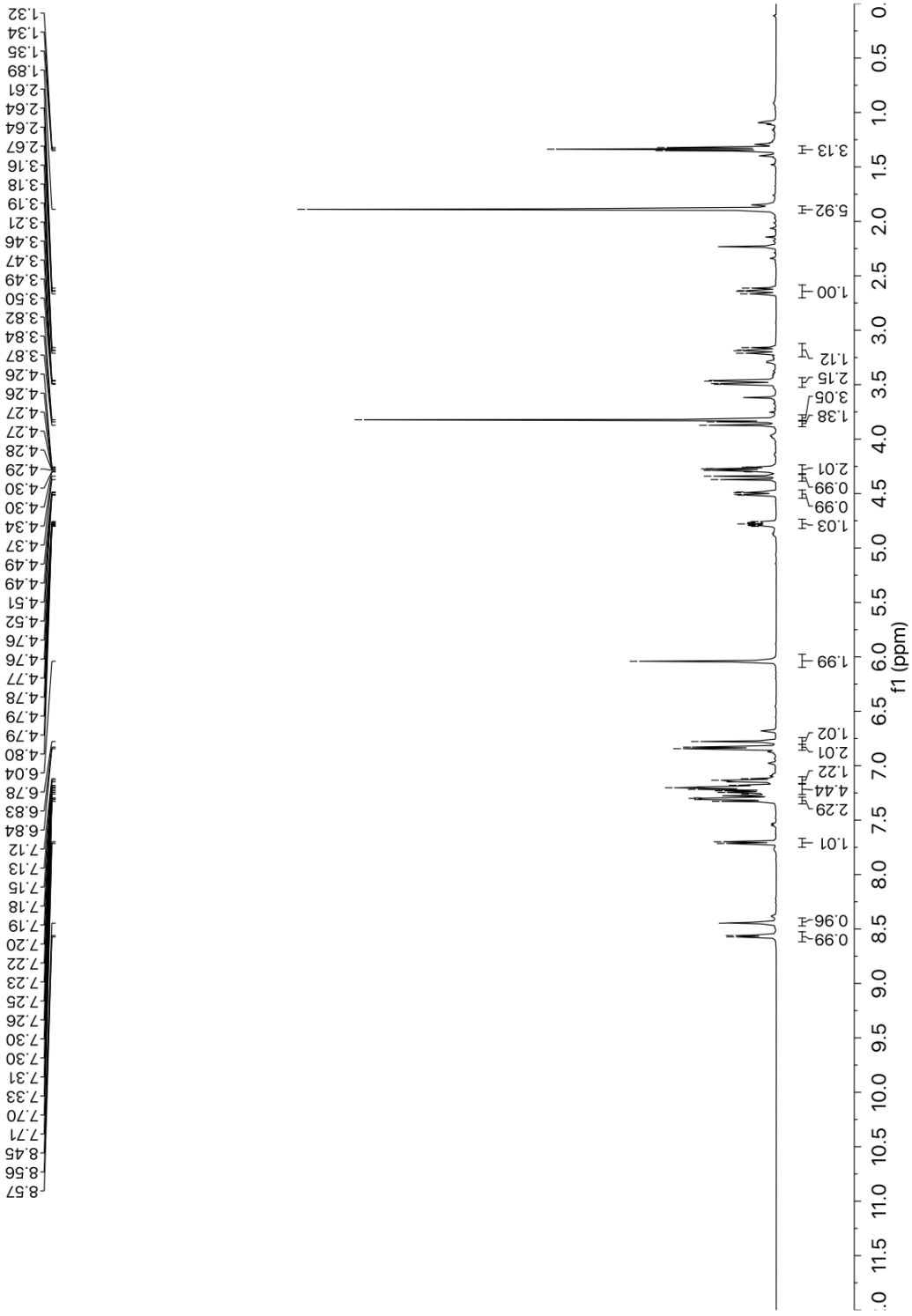
^{1}HNMR spectrum for compound **71**



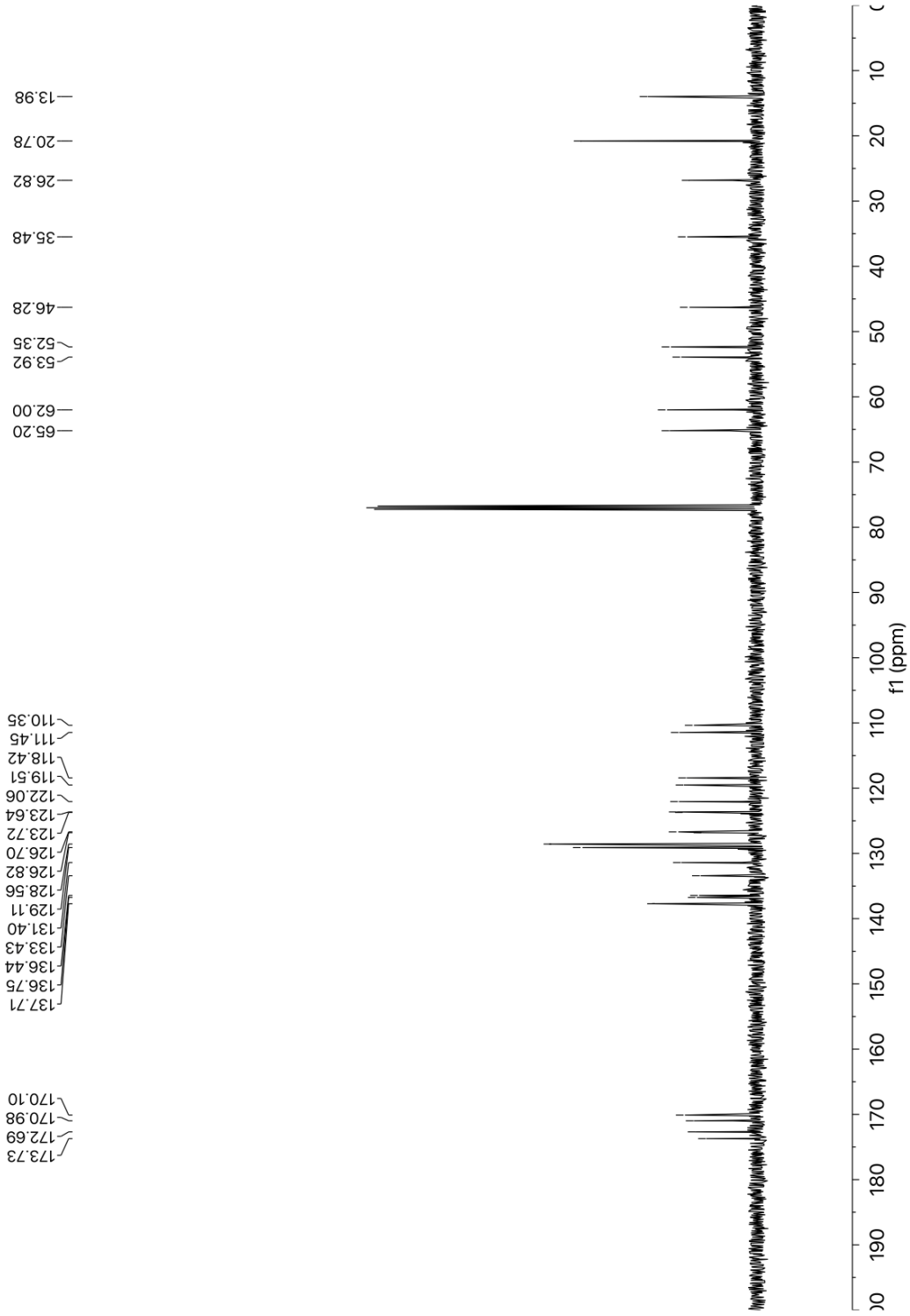
^{13}CNMR spectrum for compound **71**



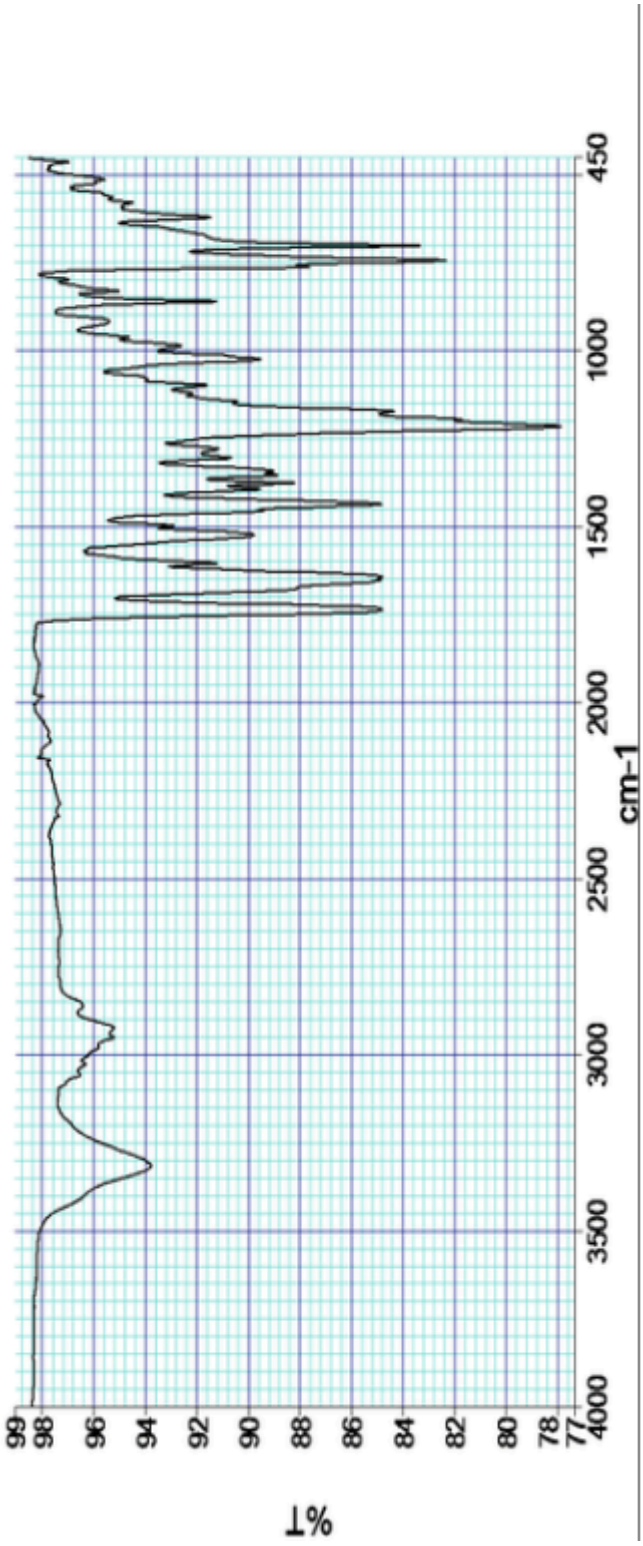
IR spectrum for compound **71**



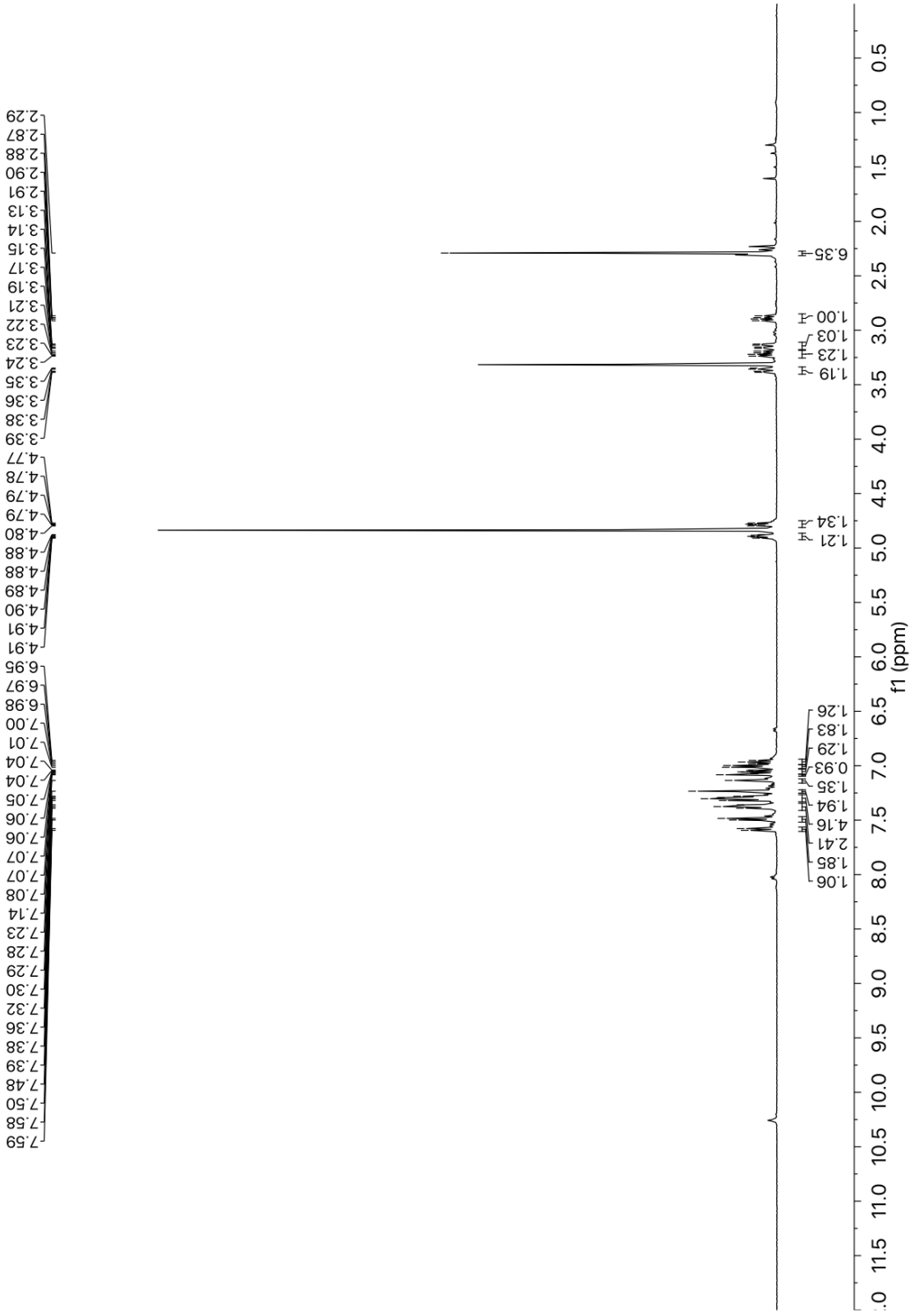
^{1}HNMR spectrum for compound **73**



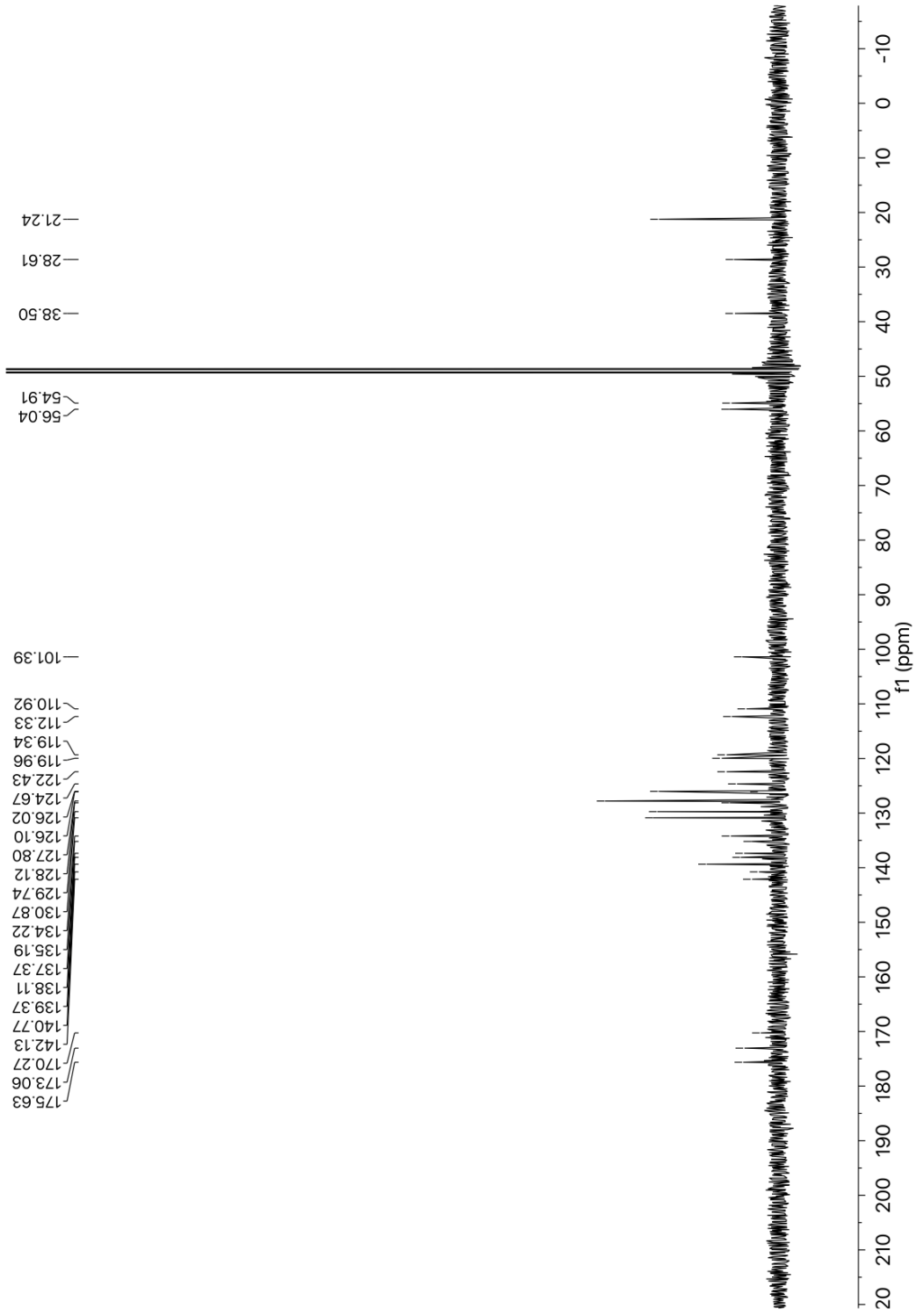
^{13}CNMR spectrum for compound **73**



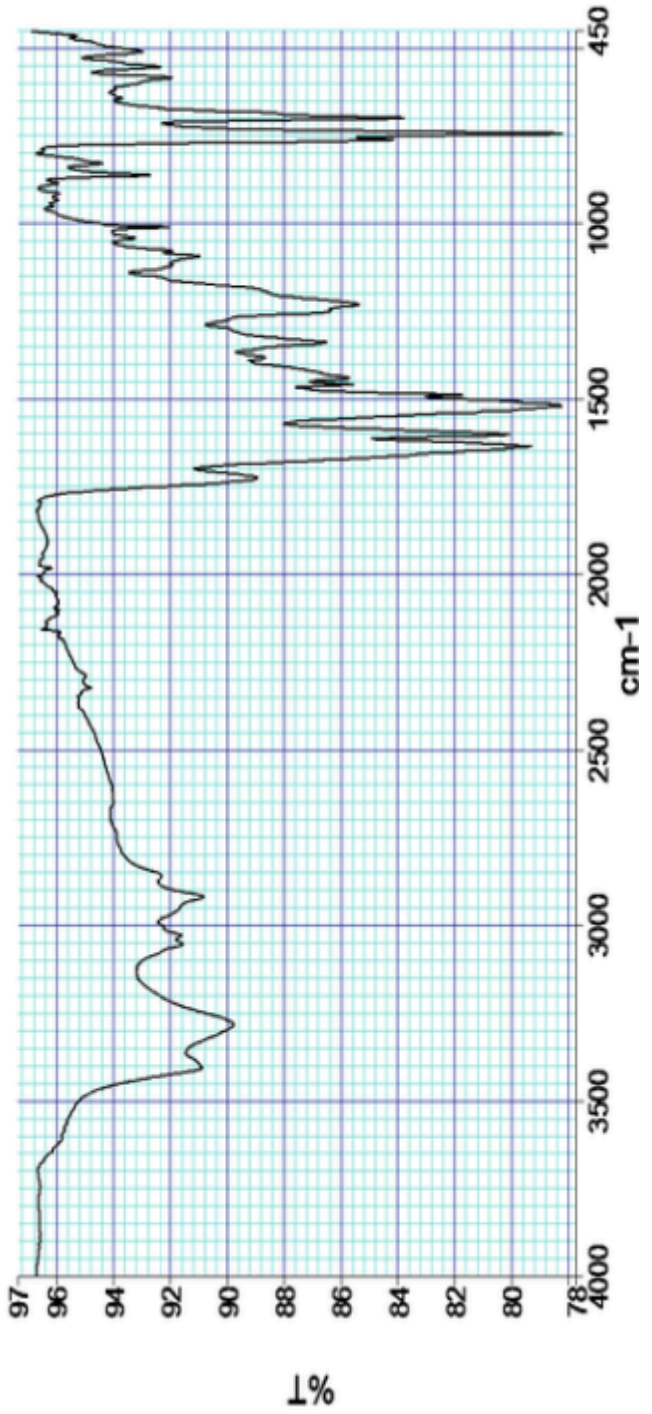
IR spectrum for compound **73**



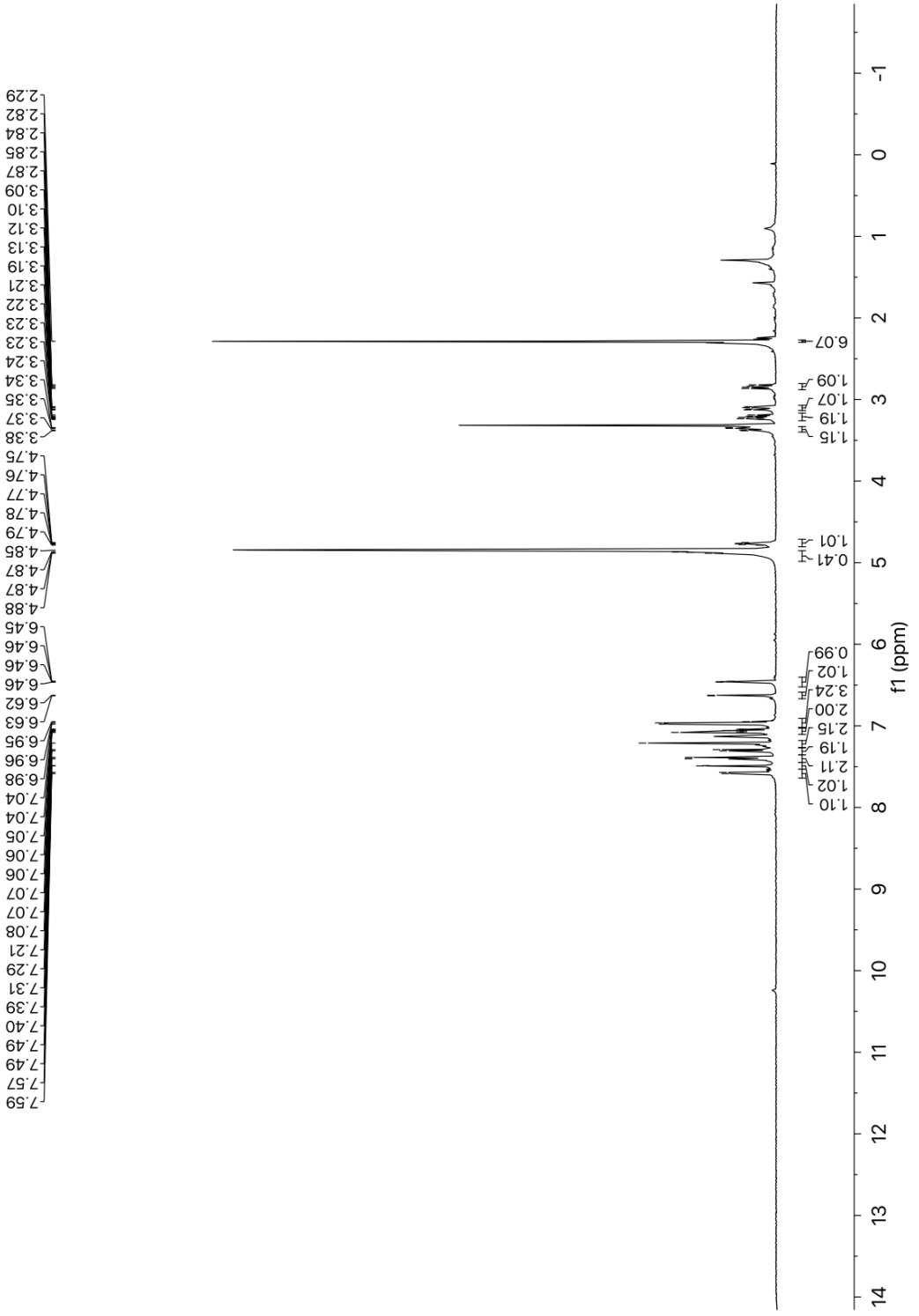
[1]HNMR spectrum for compound **74a**



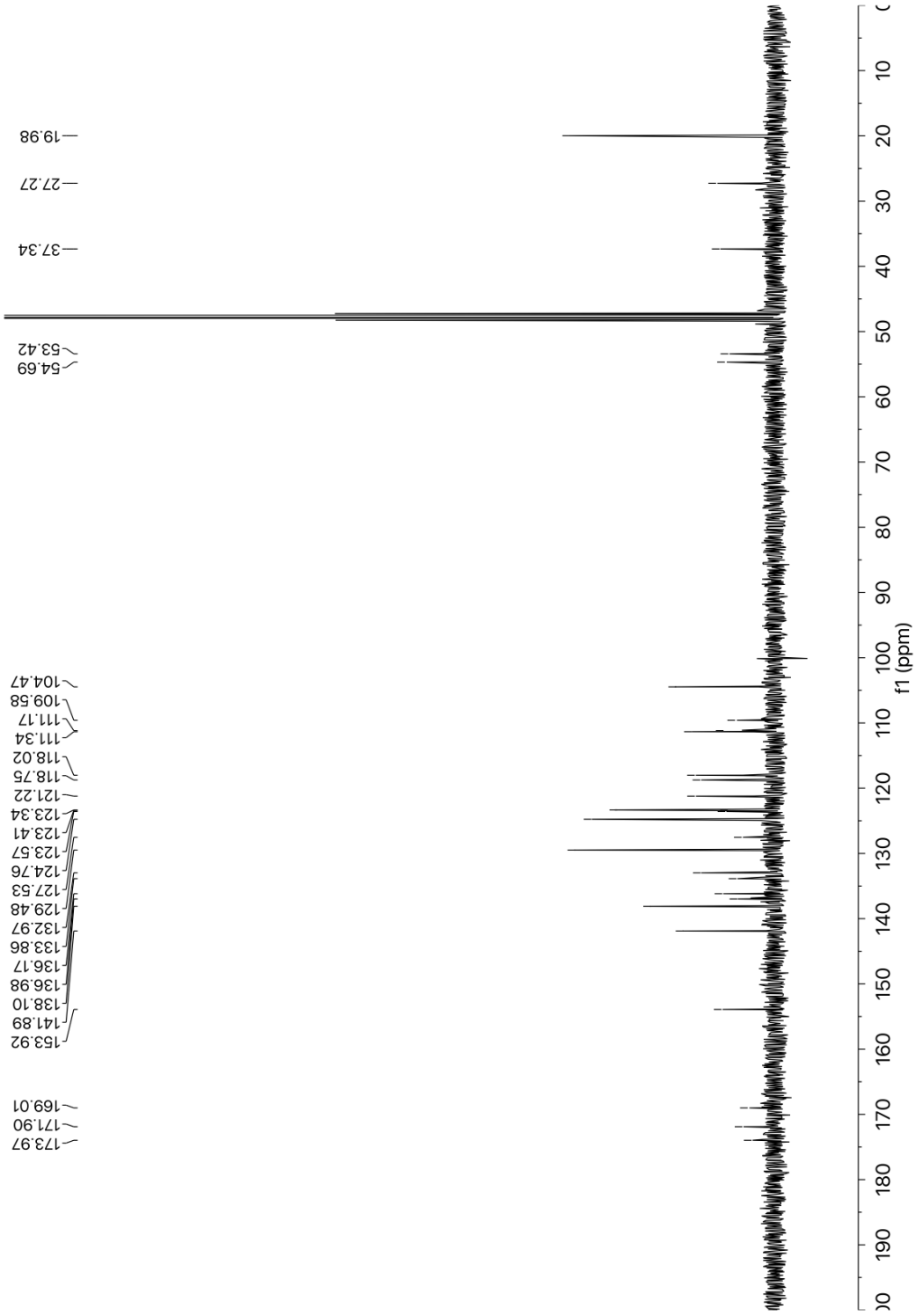
^{13}CNMR spectrum for compound **74a**



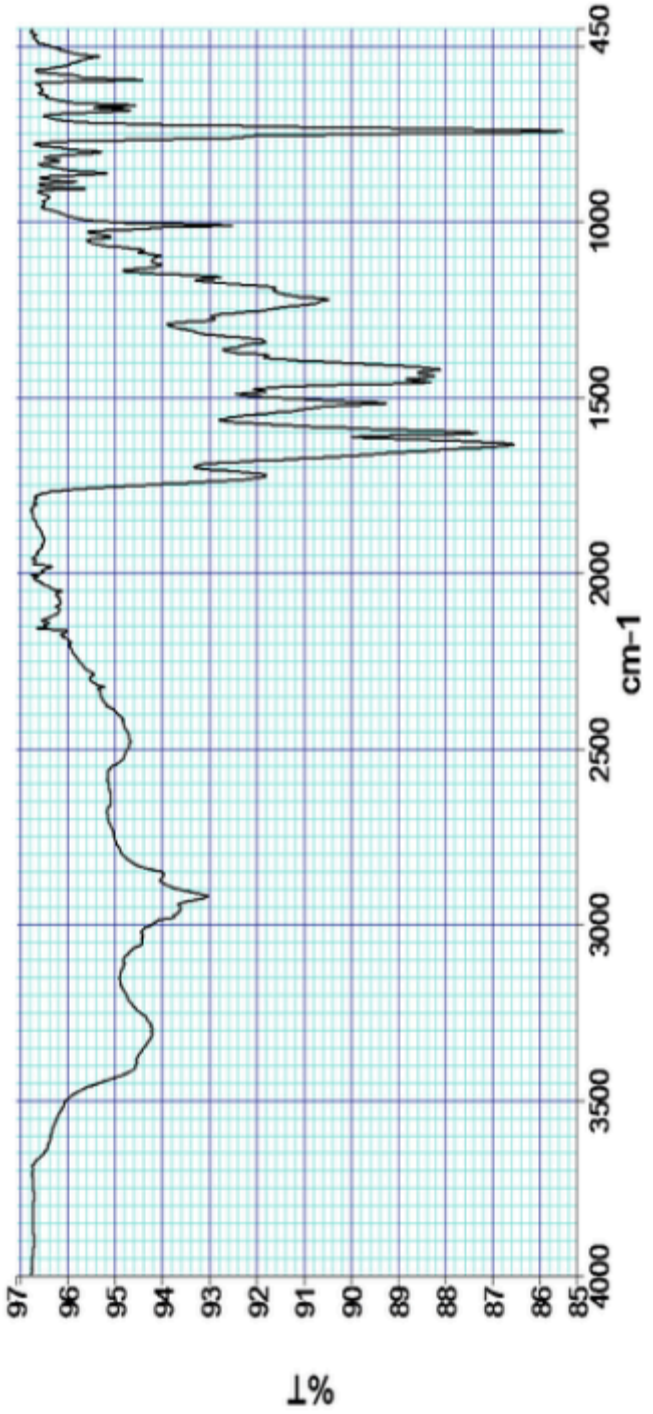
IR spectrum for compound **74a**



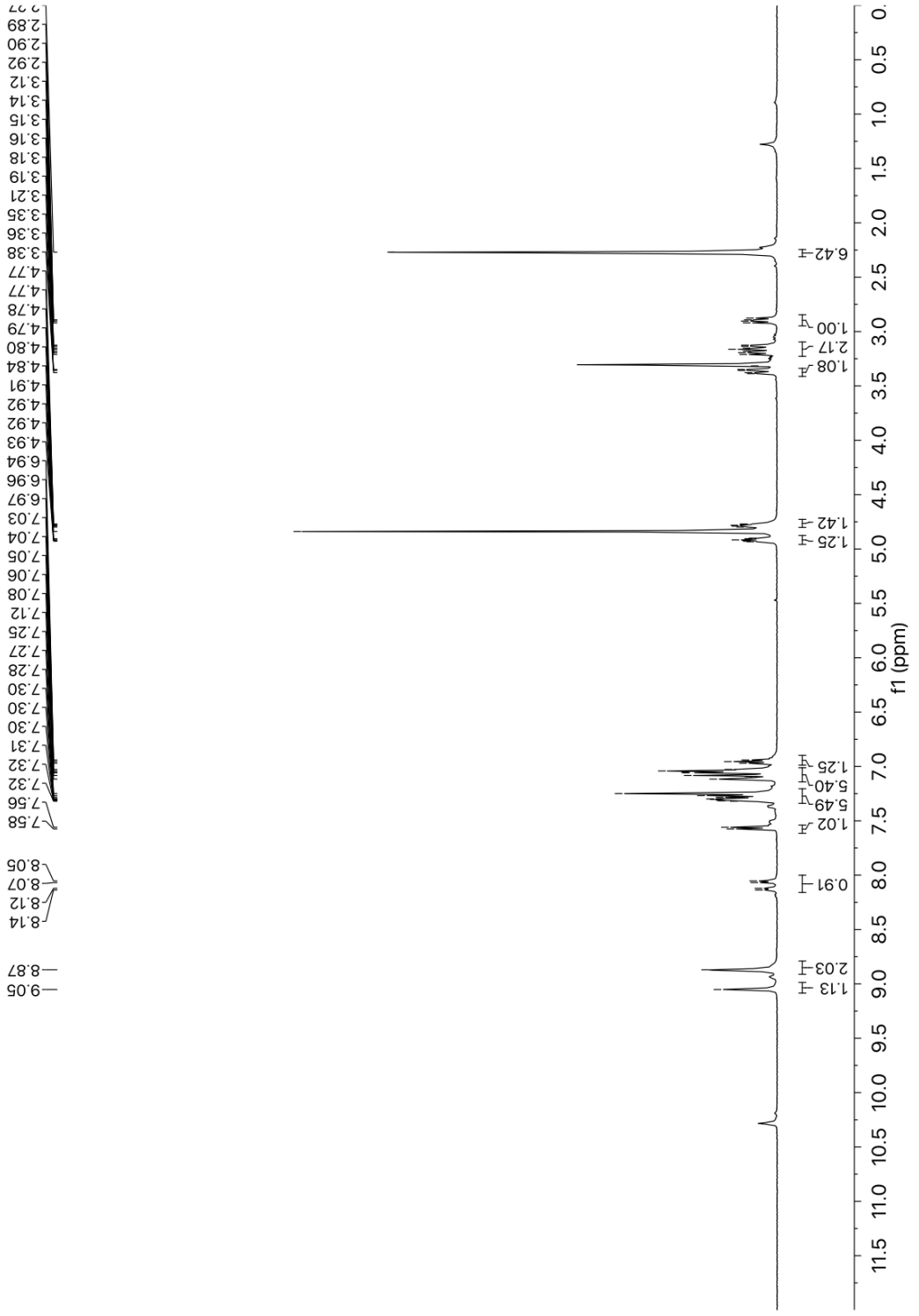
[1]HNMR spectrum for compound **74c**



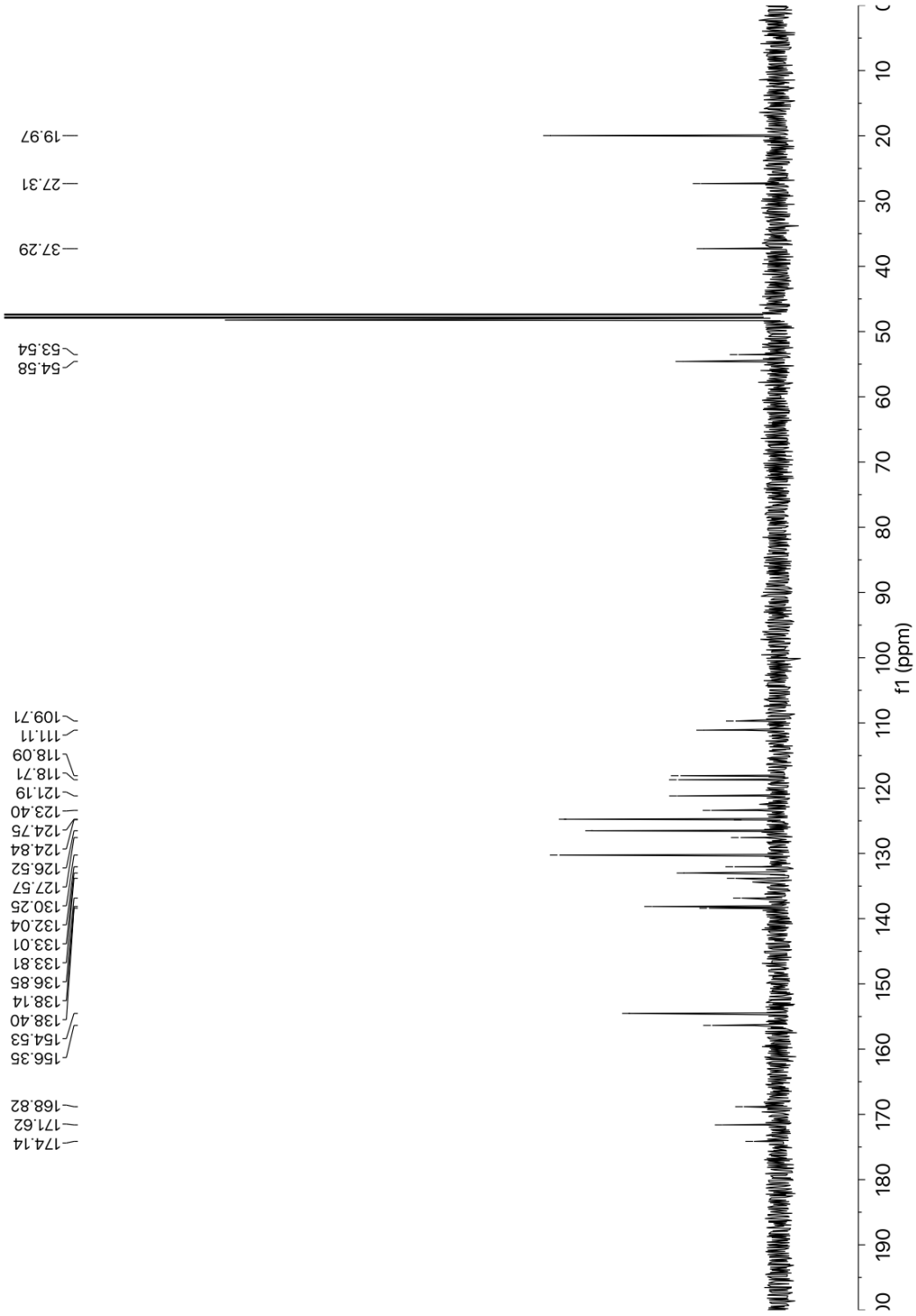
^{13}CNMR spectrum for compound **74c**



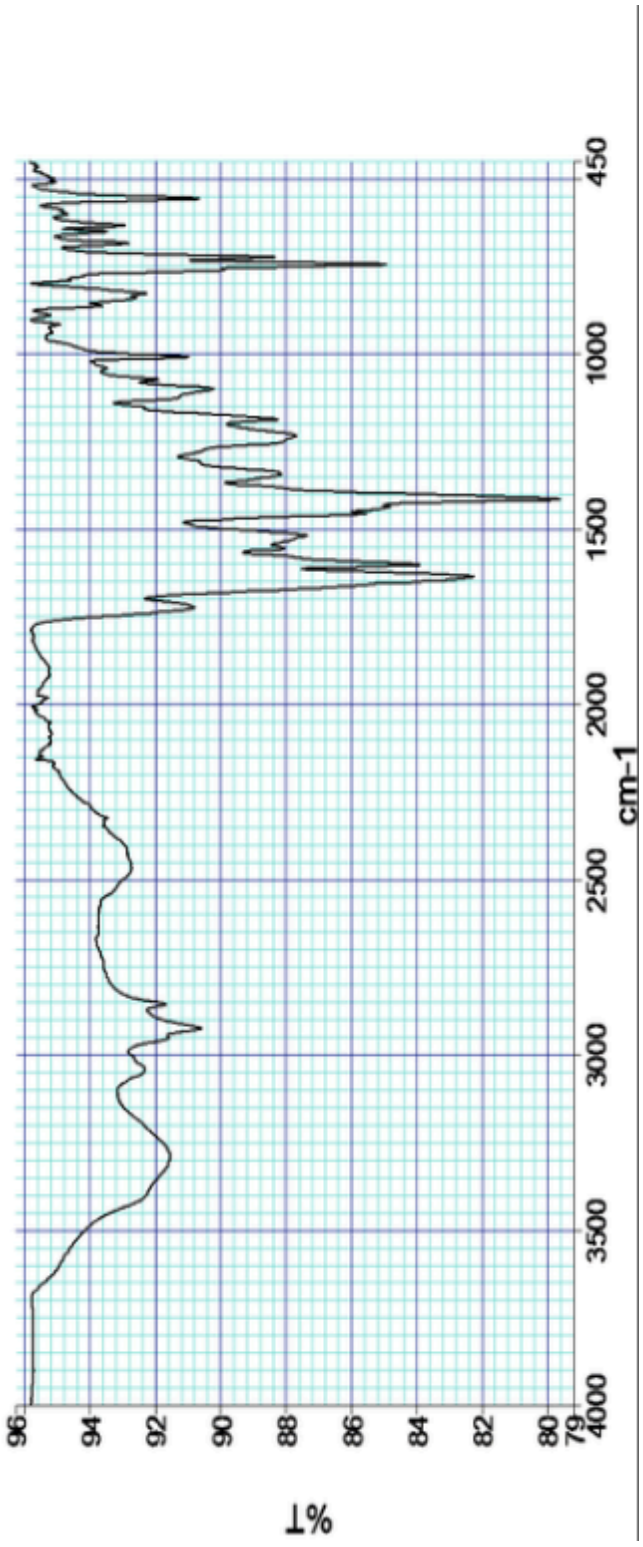
IR spectrum for compound **74c**



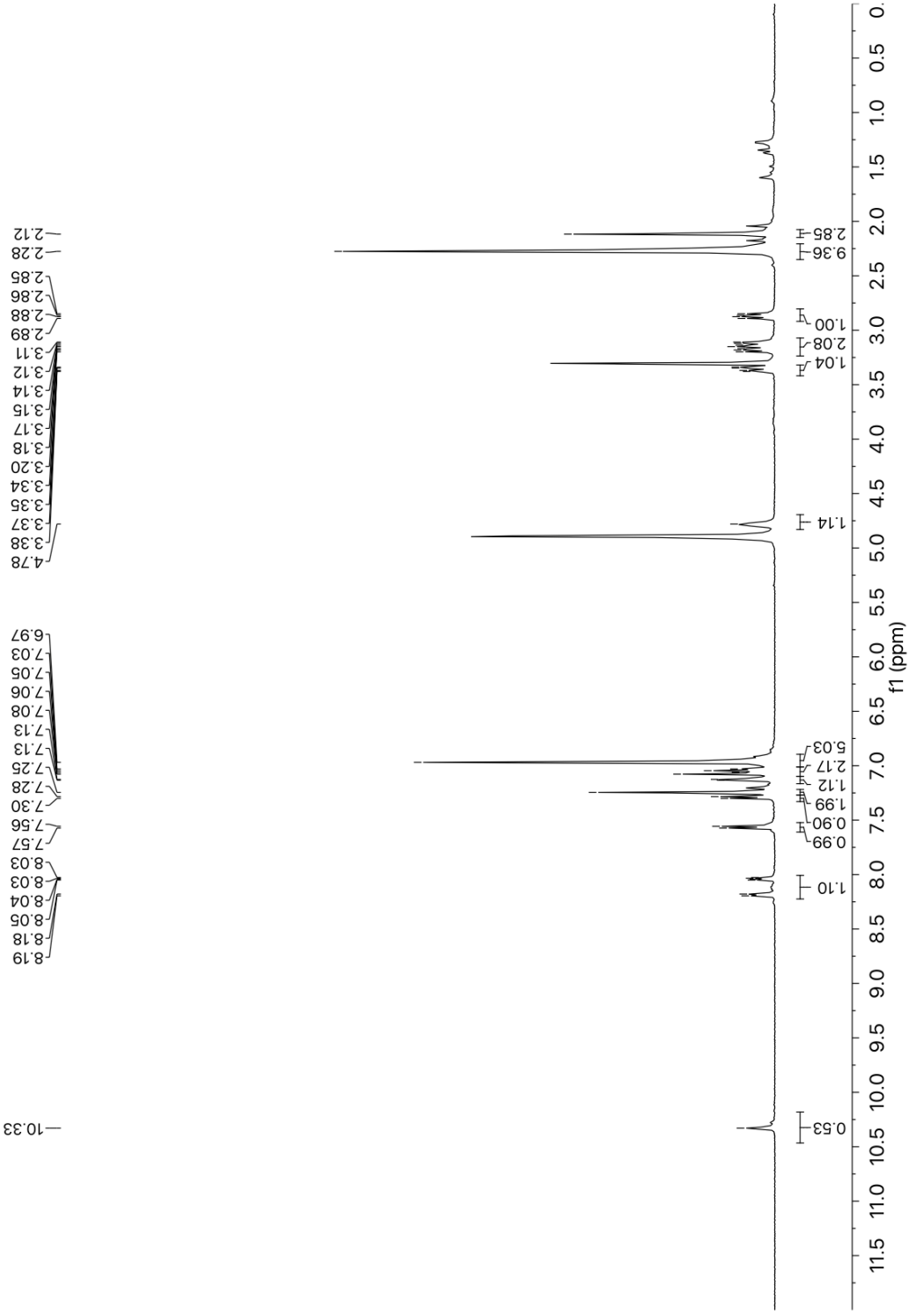
[1]HNMR spectrum for compound **74d**



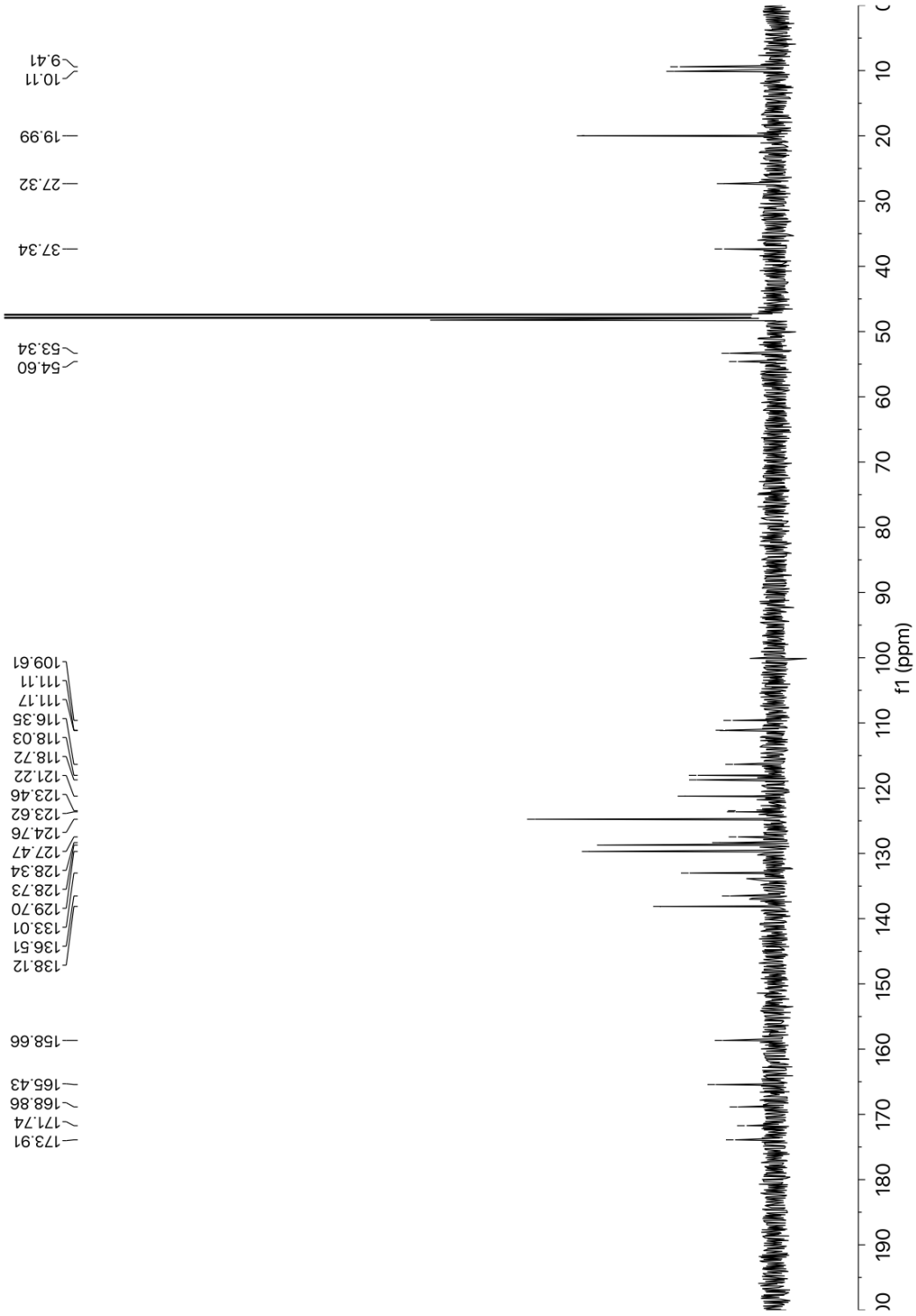
^{13}CNMR spectrum for compound **74d**



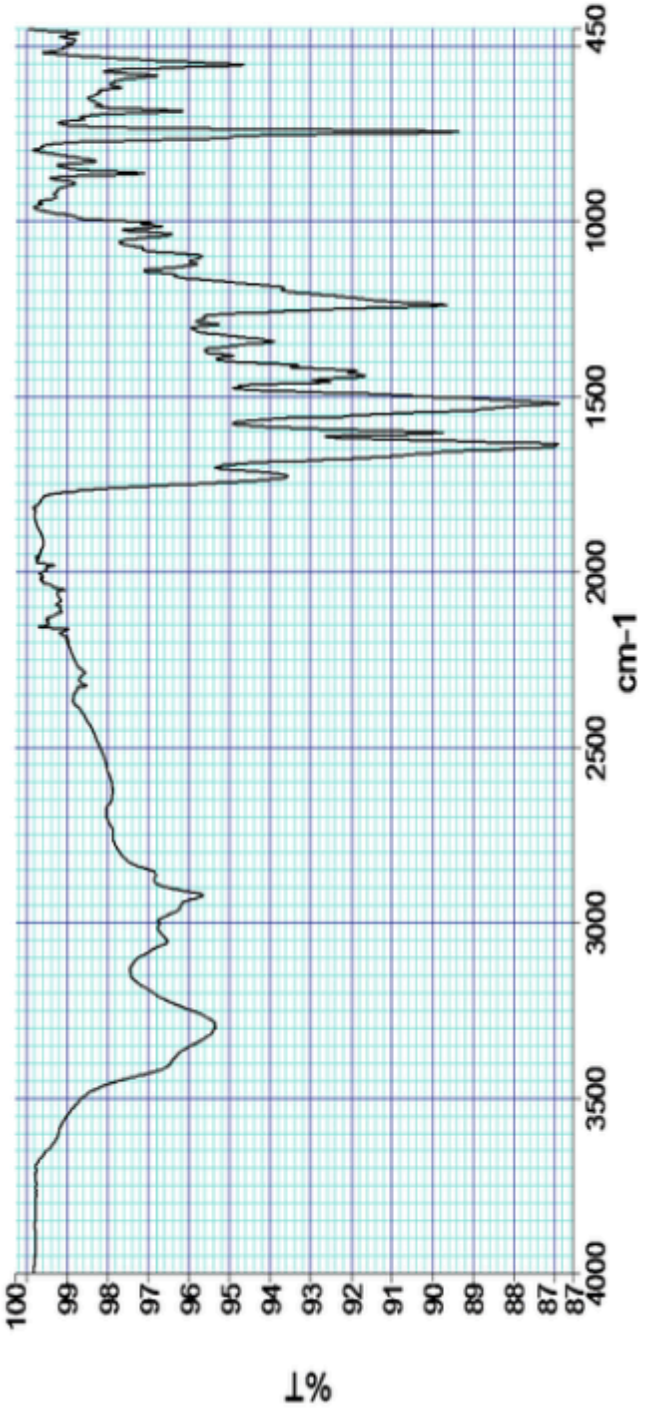
IR spectrum for compound **74d**



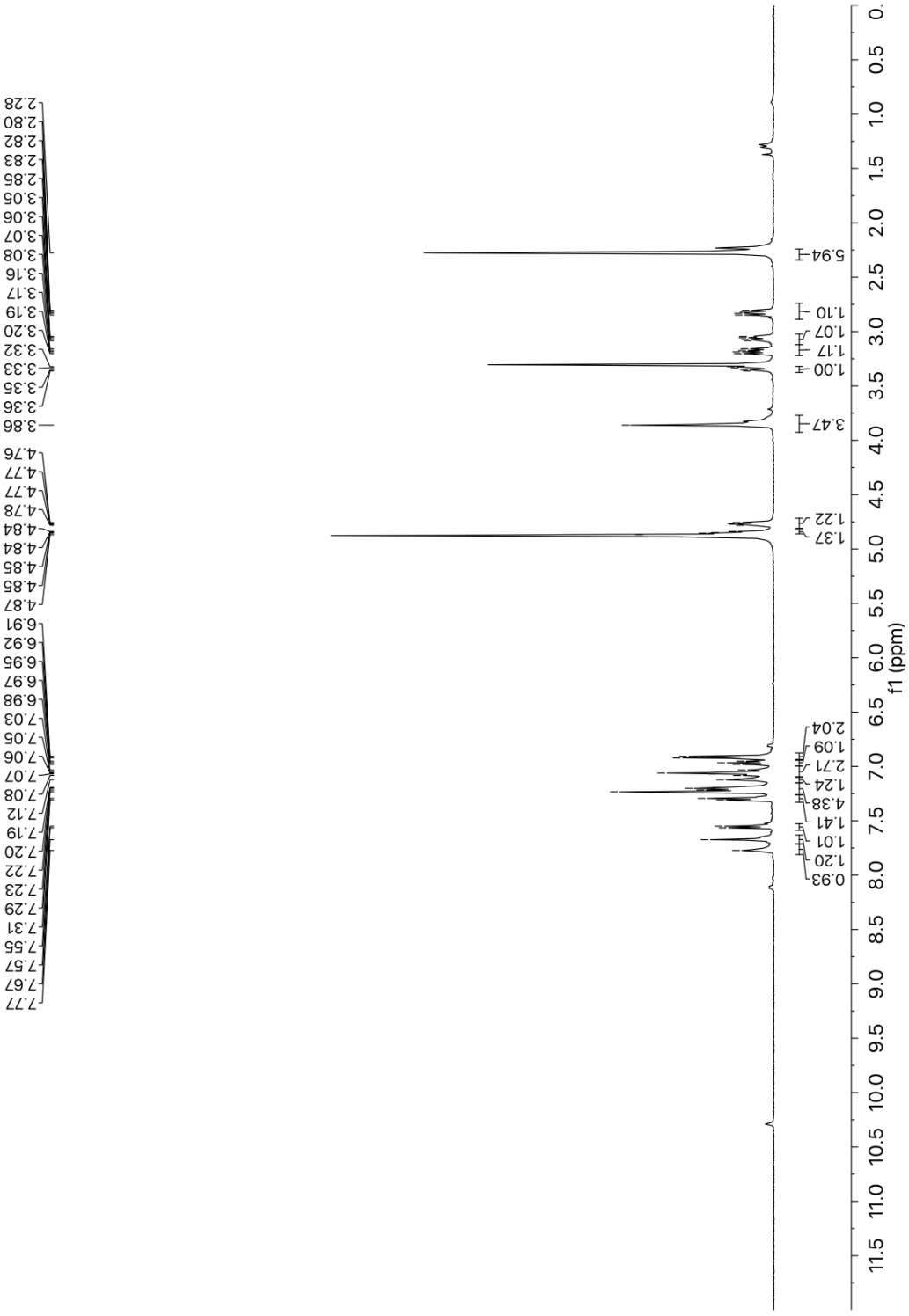
[1]HNMR spectrum for compound **74e**



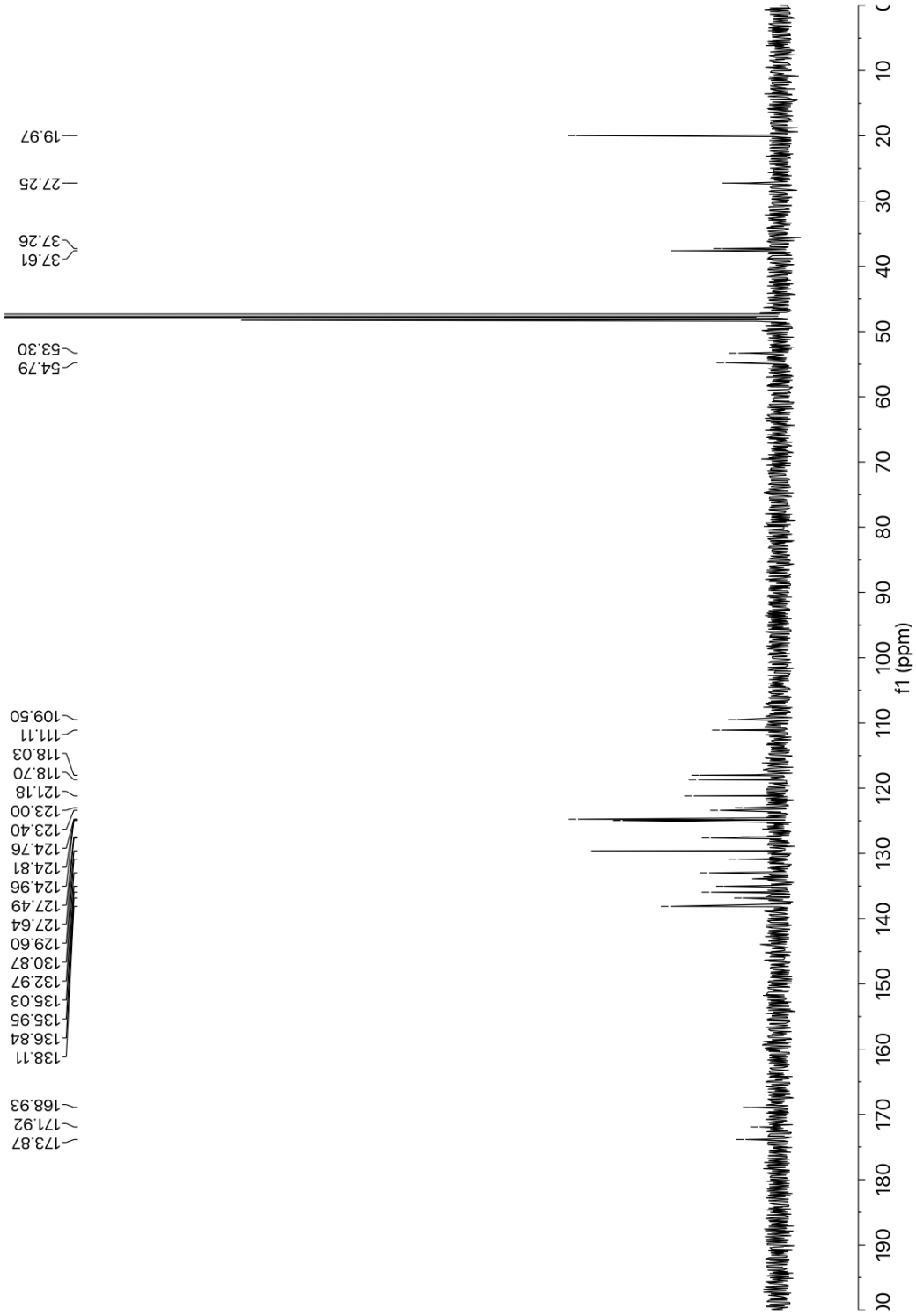
^{13}CNMR spectrum for compound **74e**



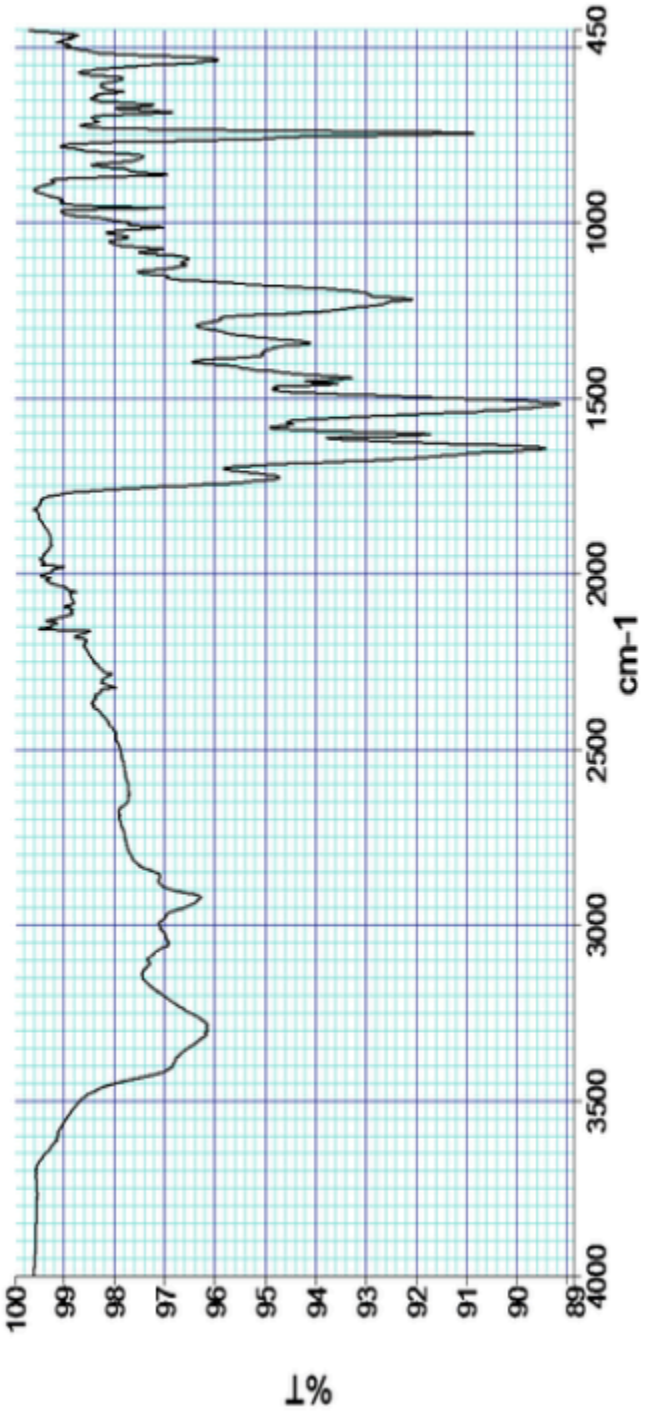
IR spectrum for compound **74e**



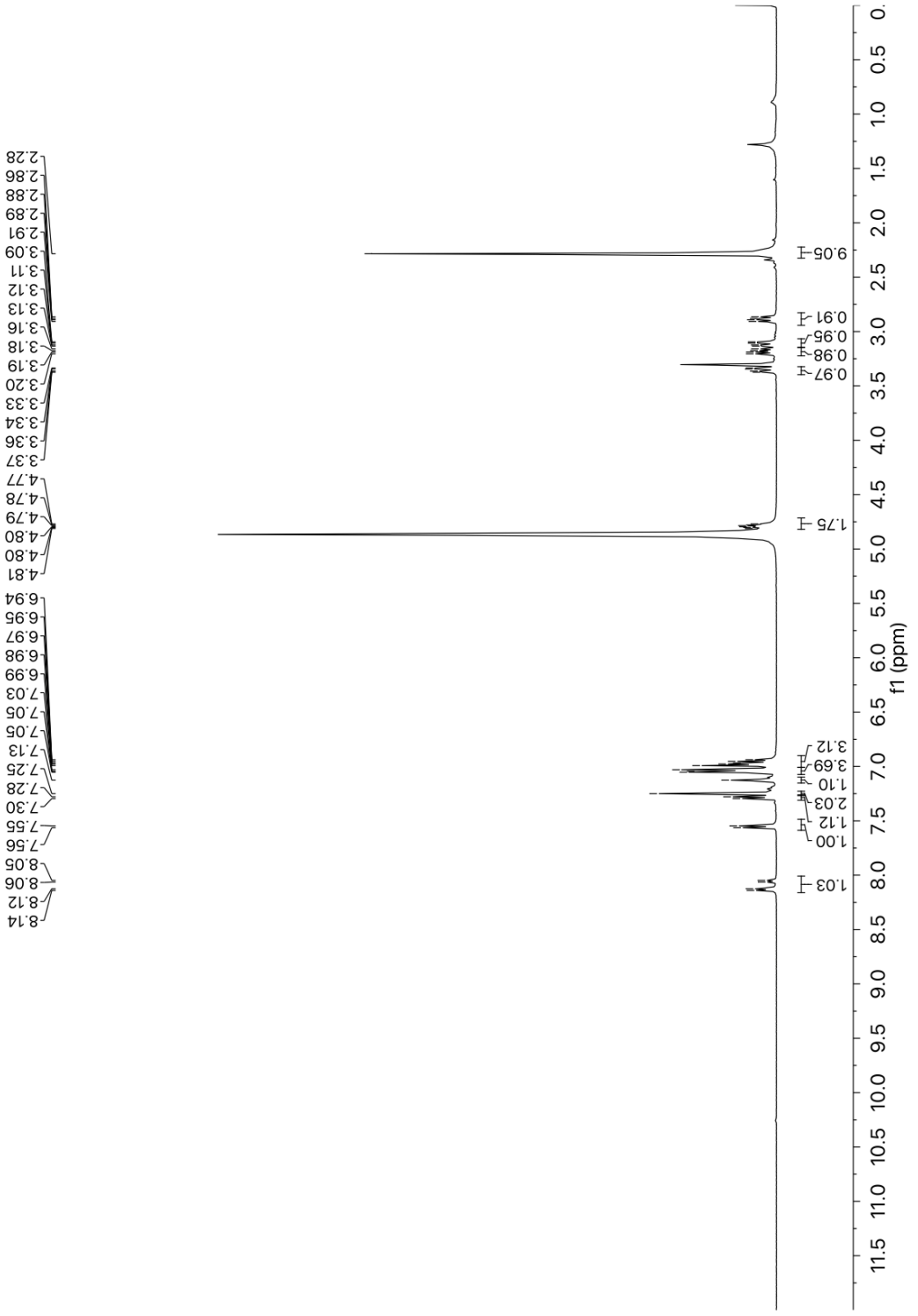
[1]HNMR spectrum for compound **74f**



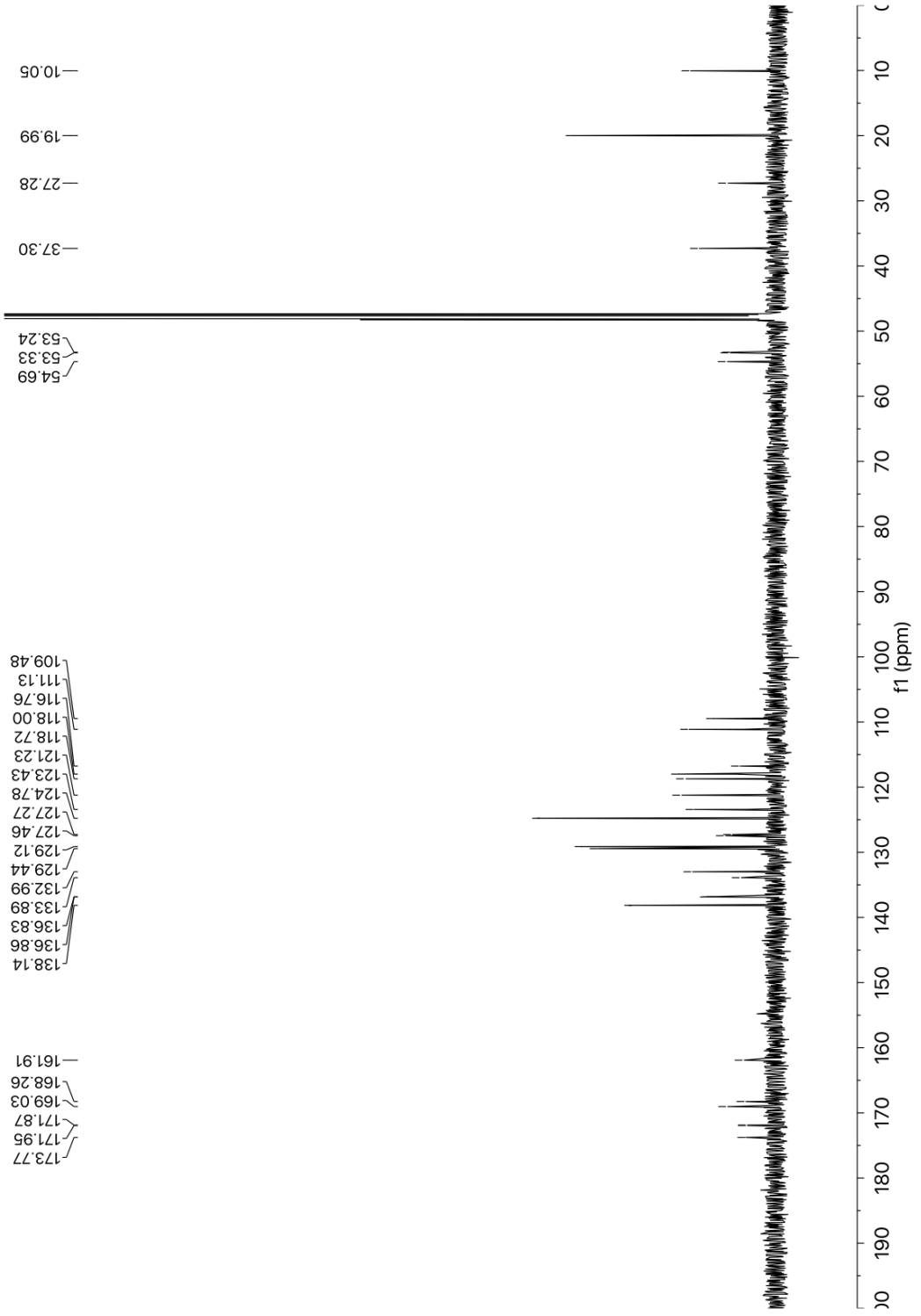
^{13}CNMR spectrum for compound **74f**



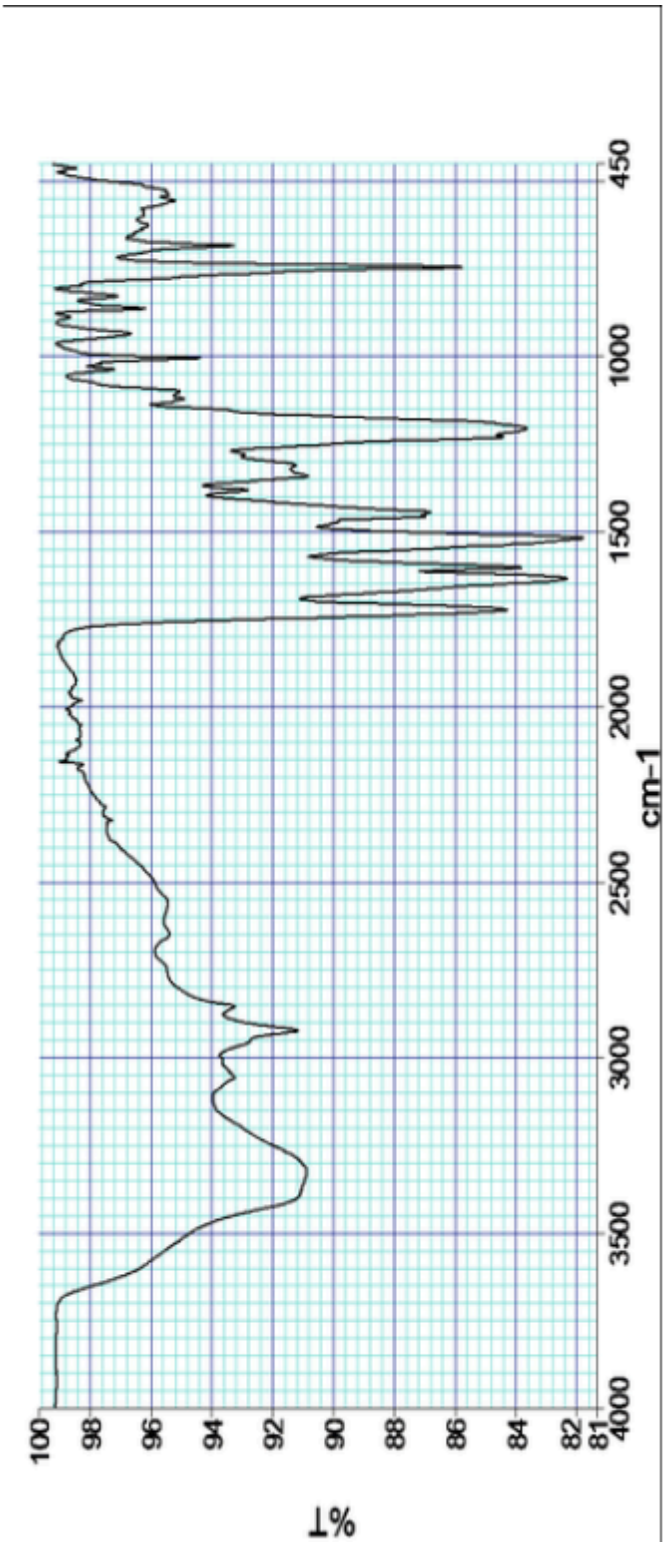
IR spectrum for compound **74f**



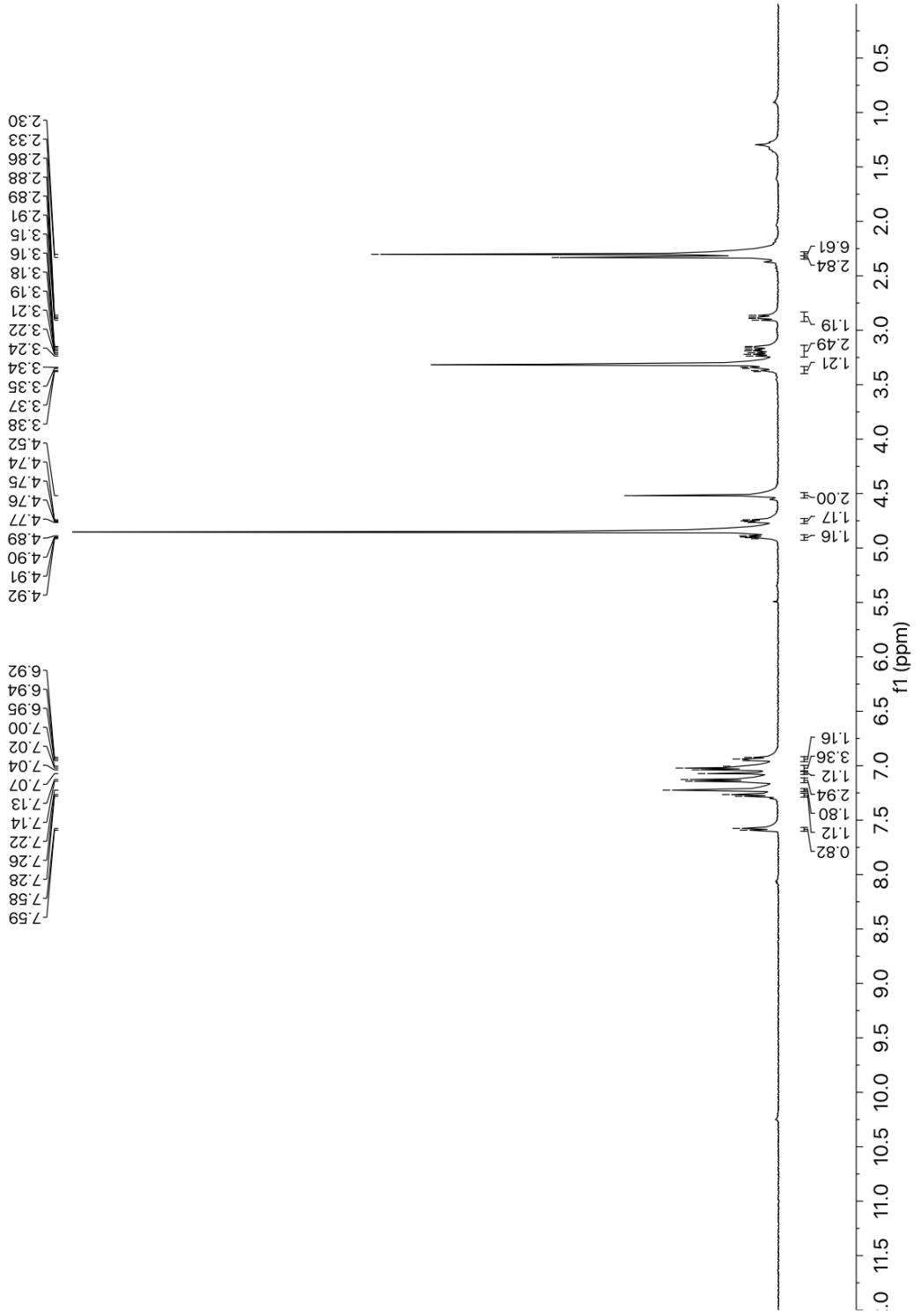
[1]HNMR spectrum for compound **74h**



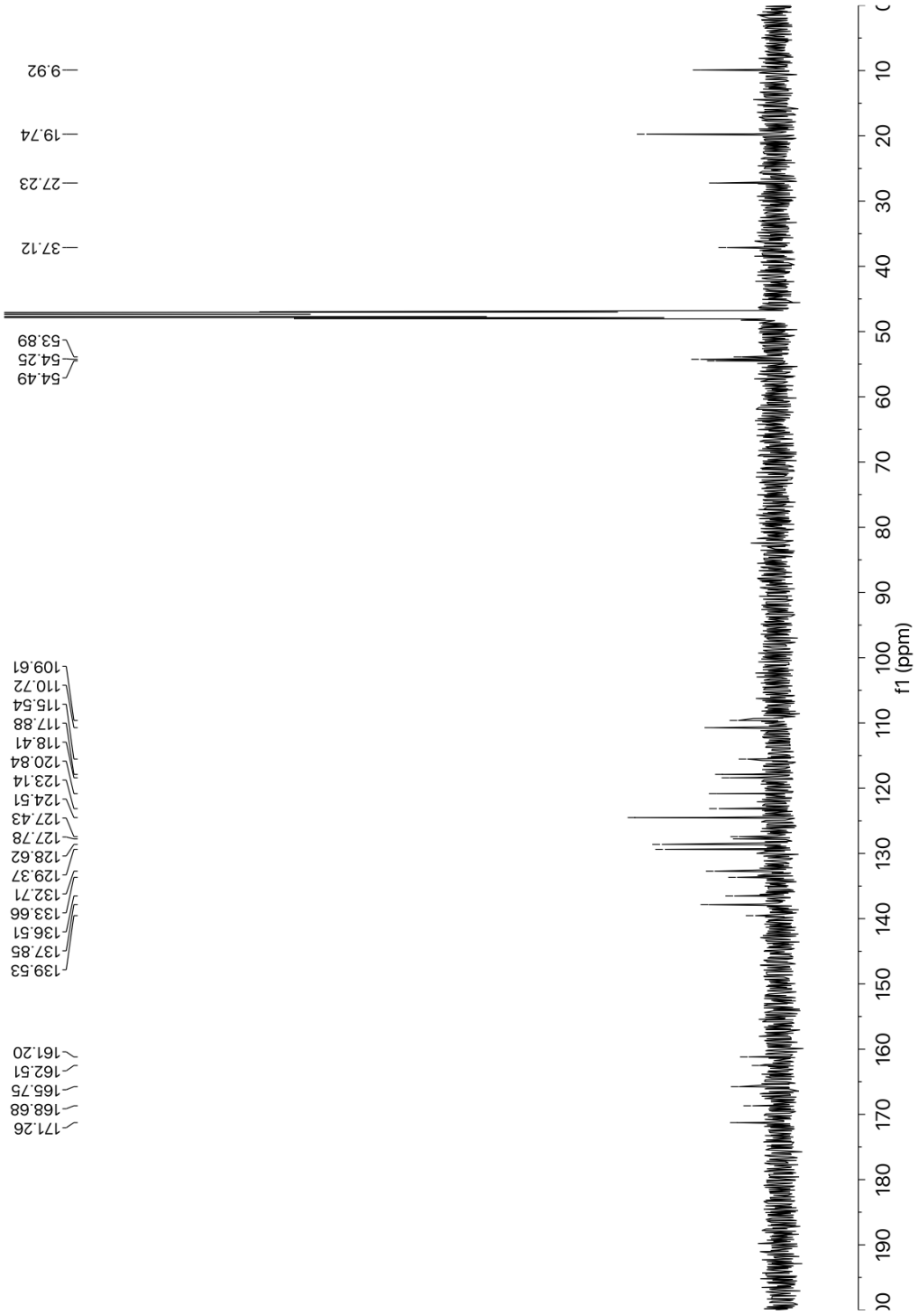
^{13}CNMR spectrum for compound **74h**



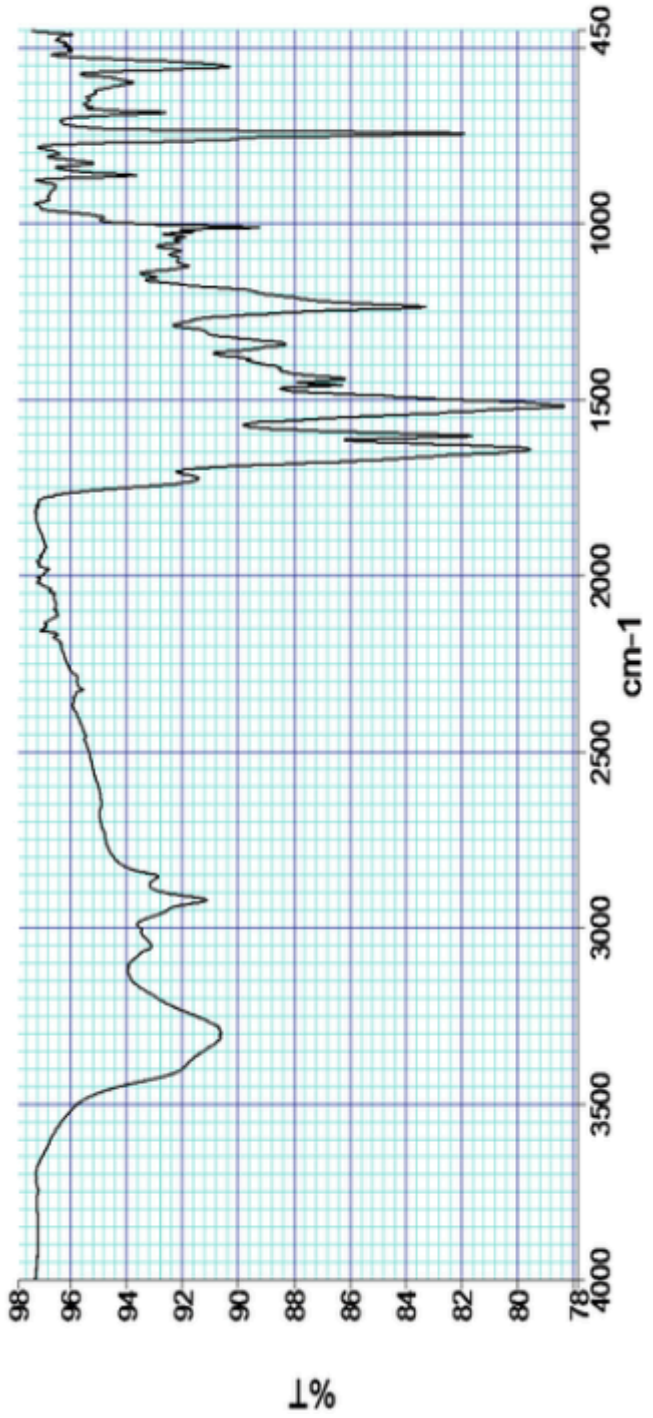
IR spectrum for compound **74h**



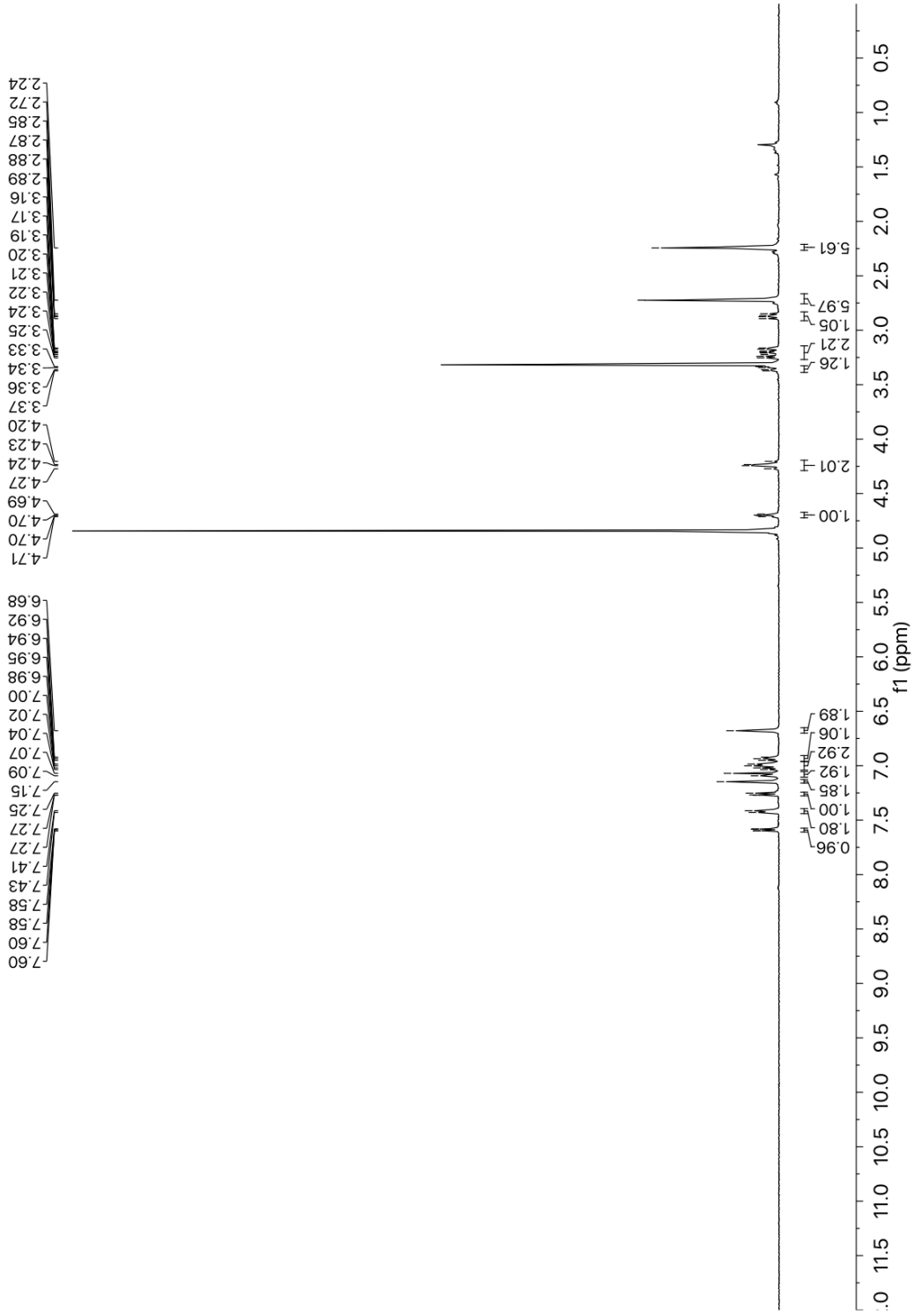
[1]HNMR spectrum for compound **74i**



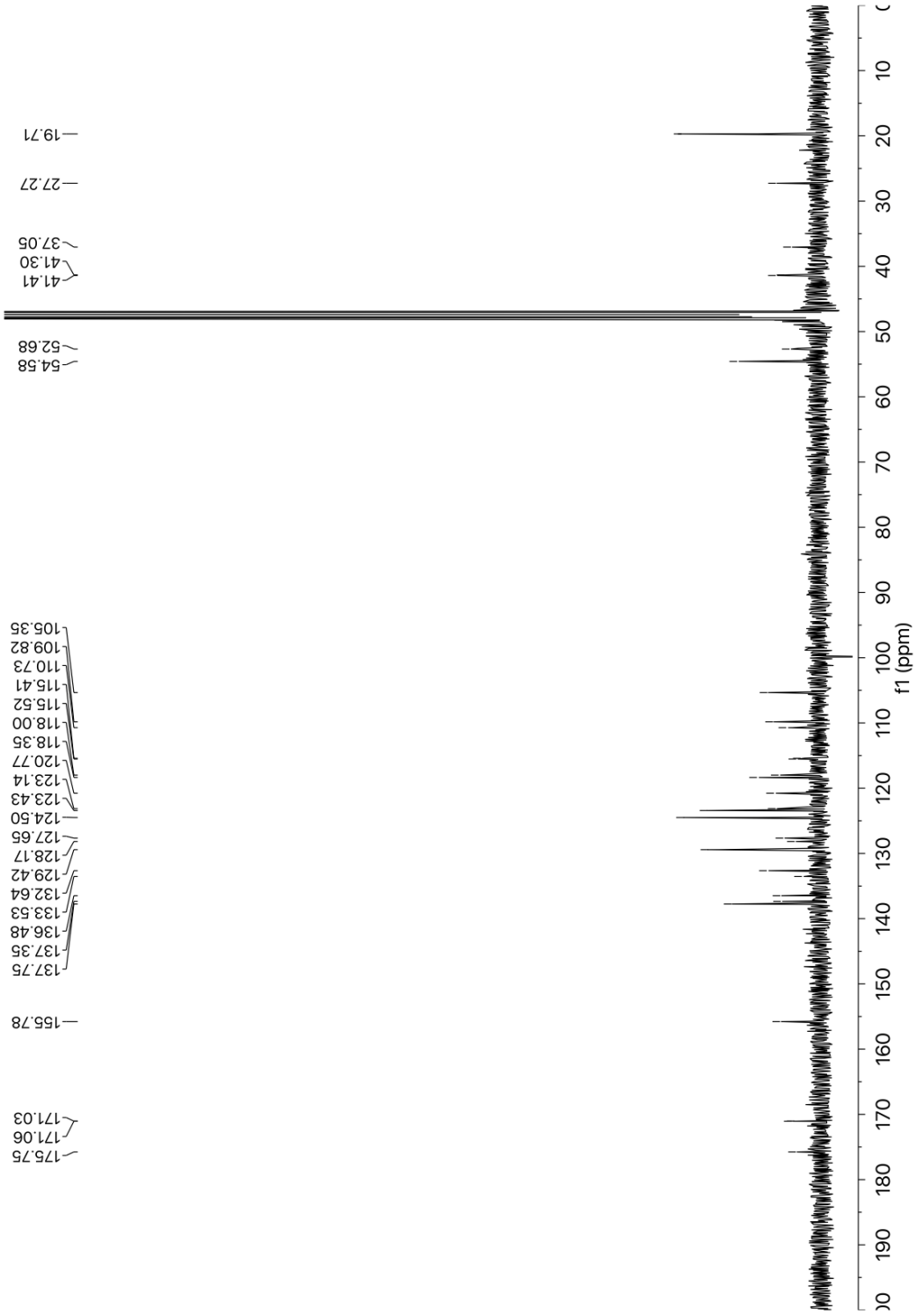
^{13}CNMR spectrum for compound **74i**



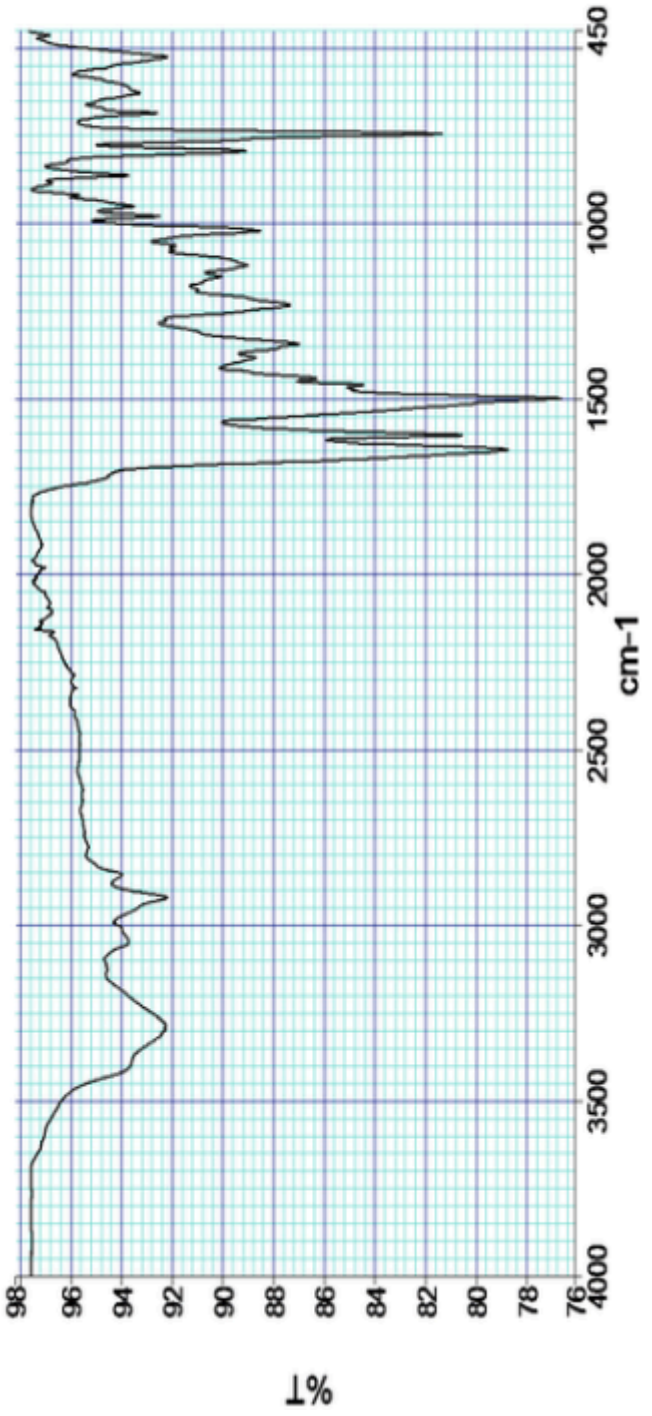
IR spectrum for compound **74i**



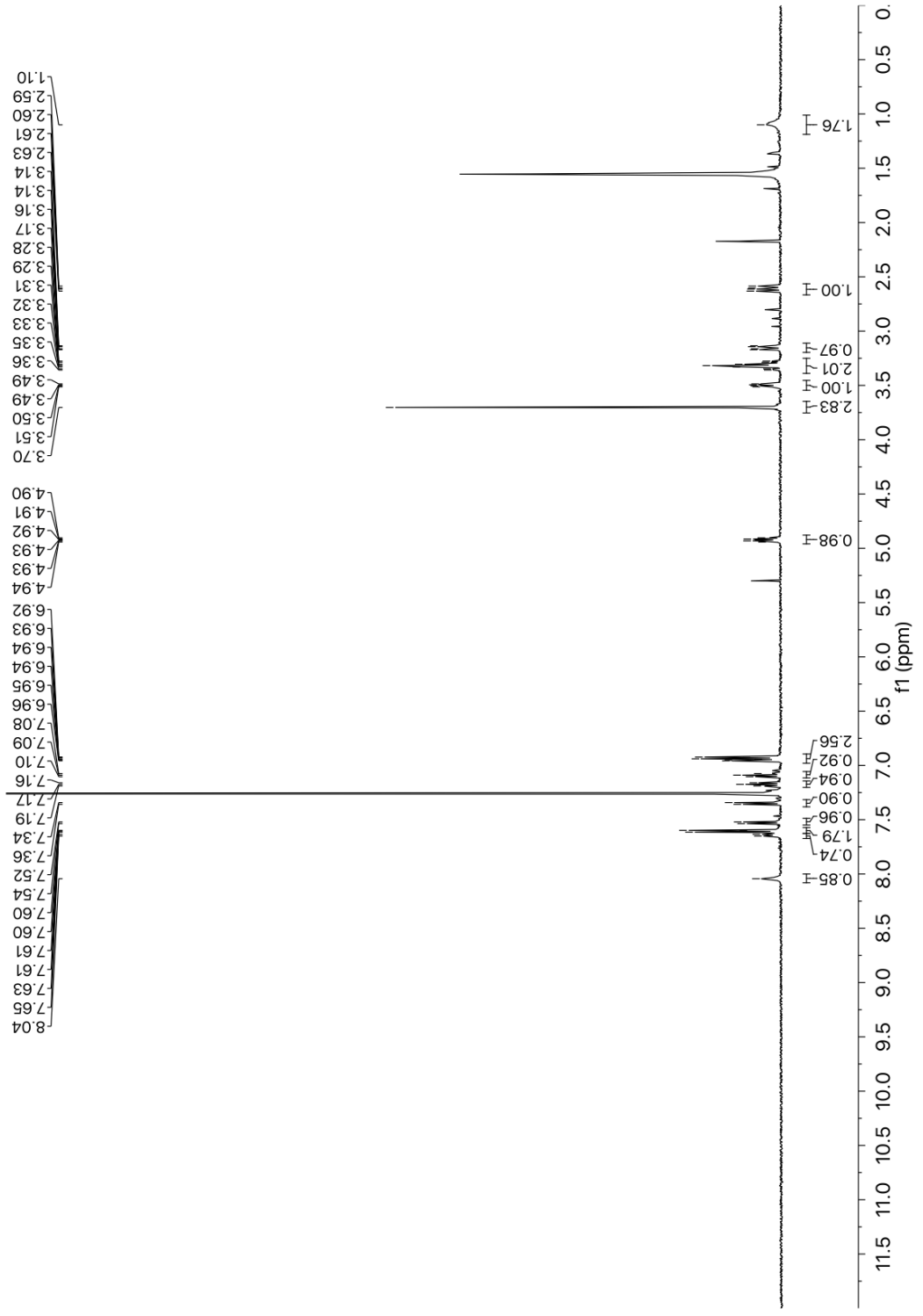
[1]HNMR spectrum for compound **74j**



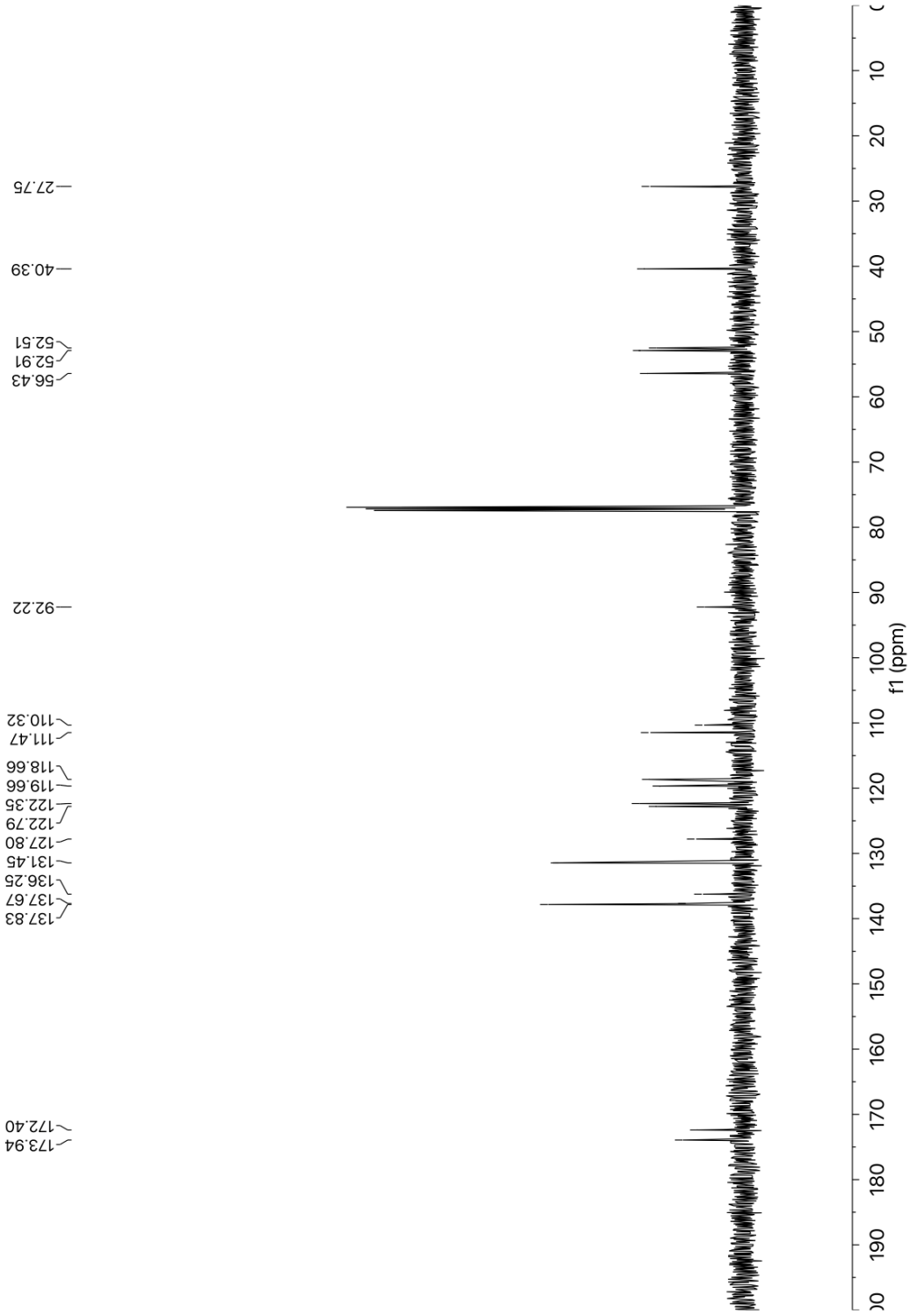
^{13}CNMR spectrum for compound **74j**



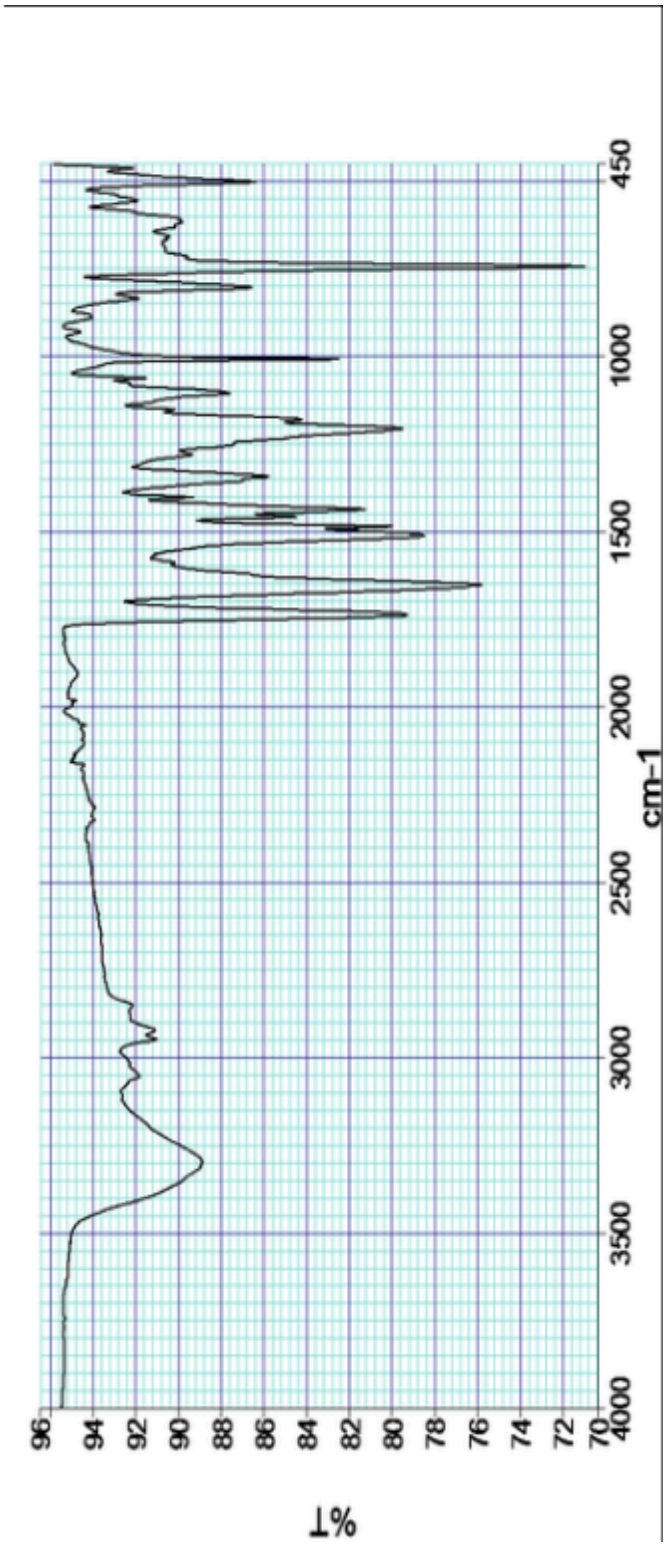
IR spectrum for compound **74j**



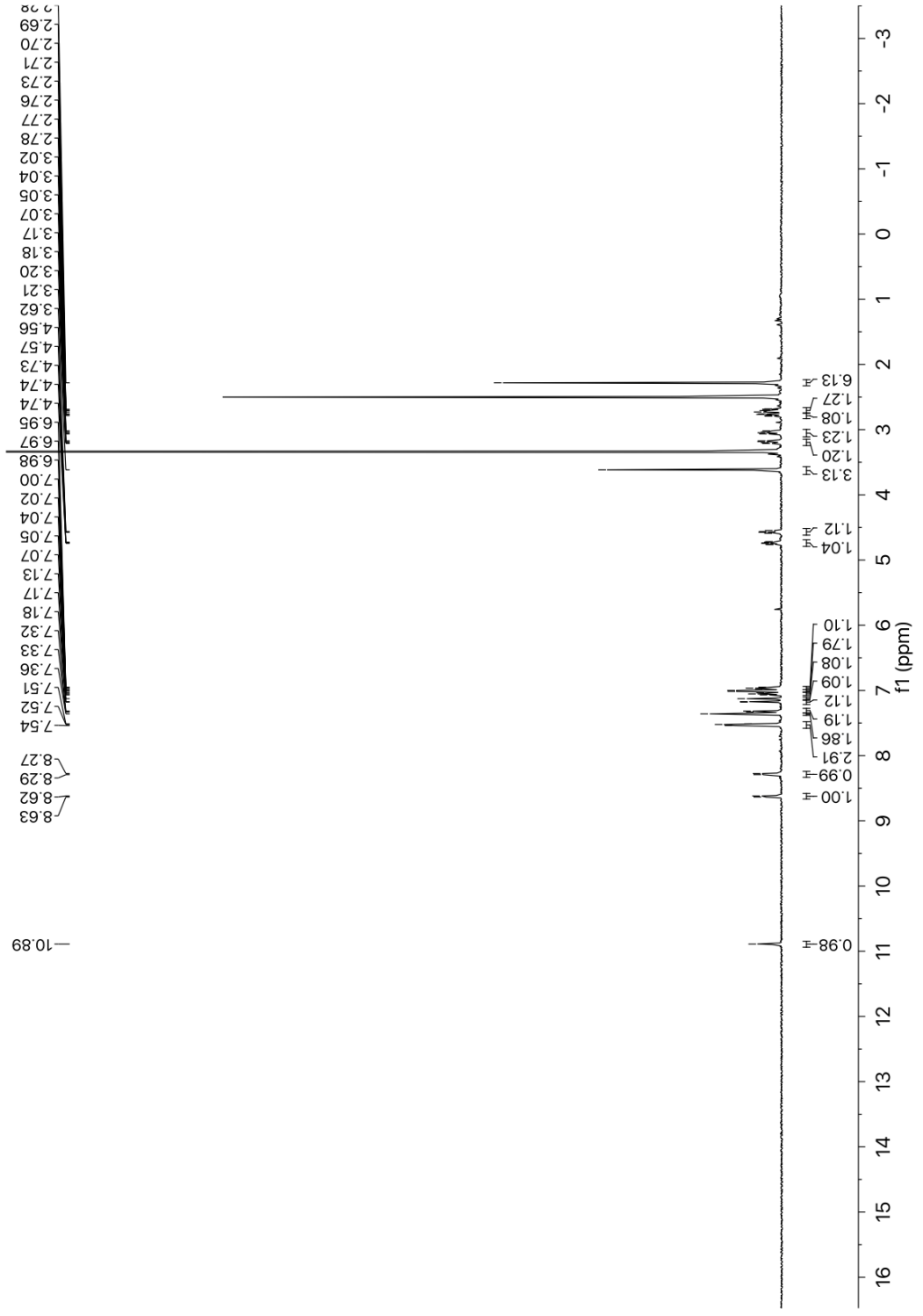
^{1}HNMR spectrum for compound **79**



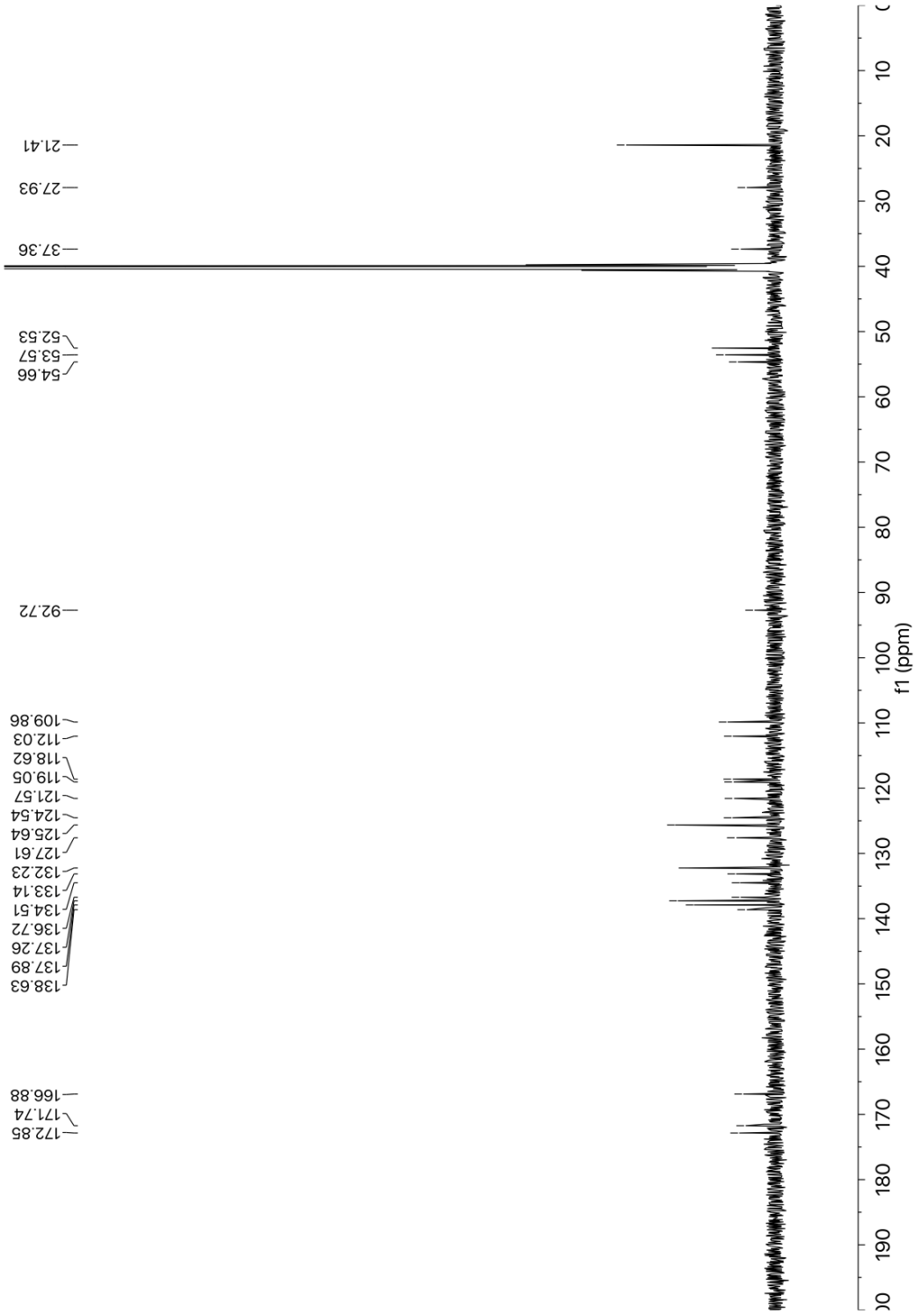
^{13}CNMR spectrum for compound **79**



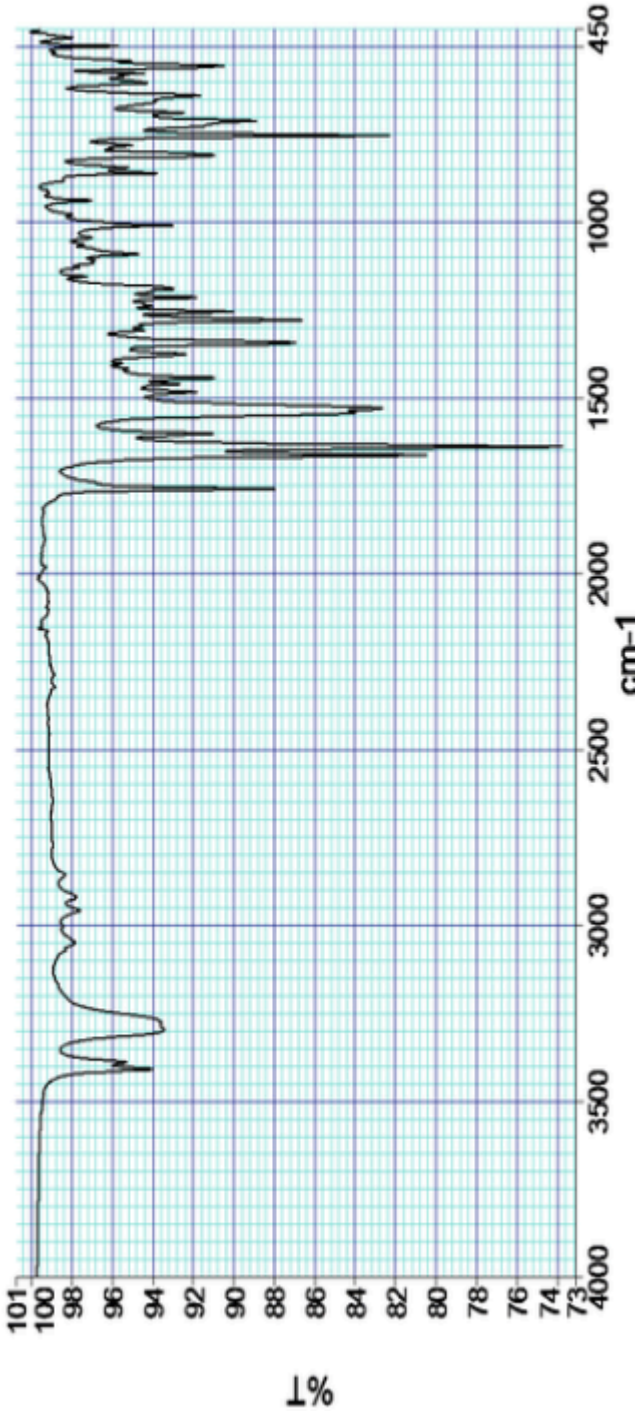
IR spectrum for compound **79**



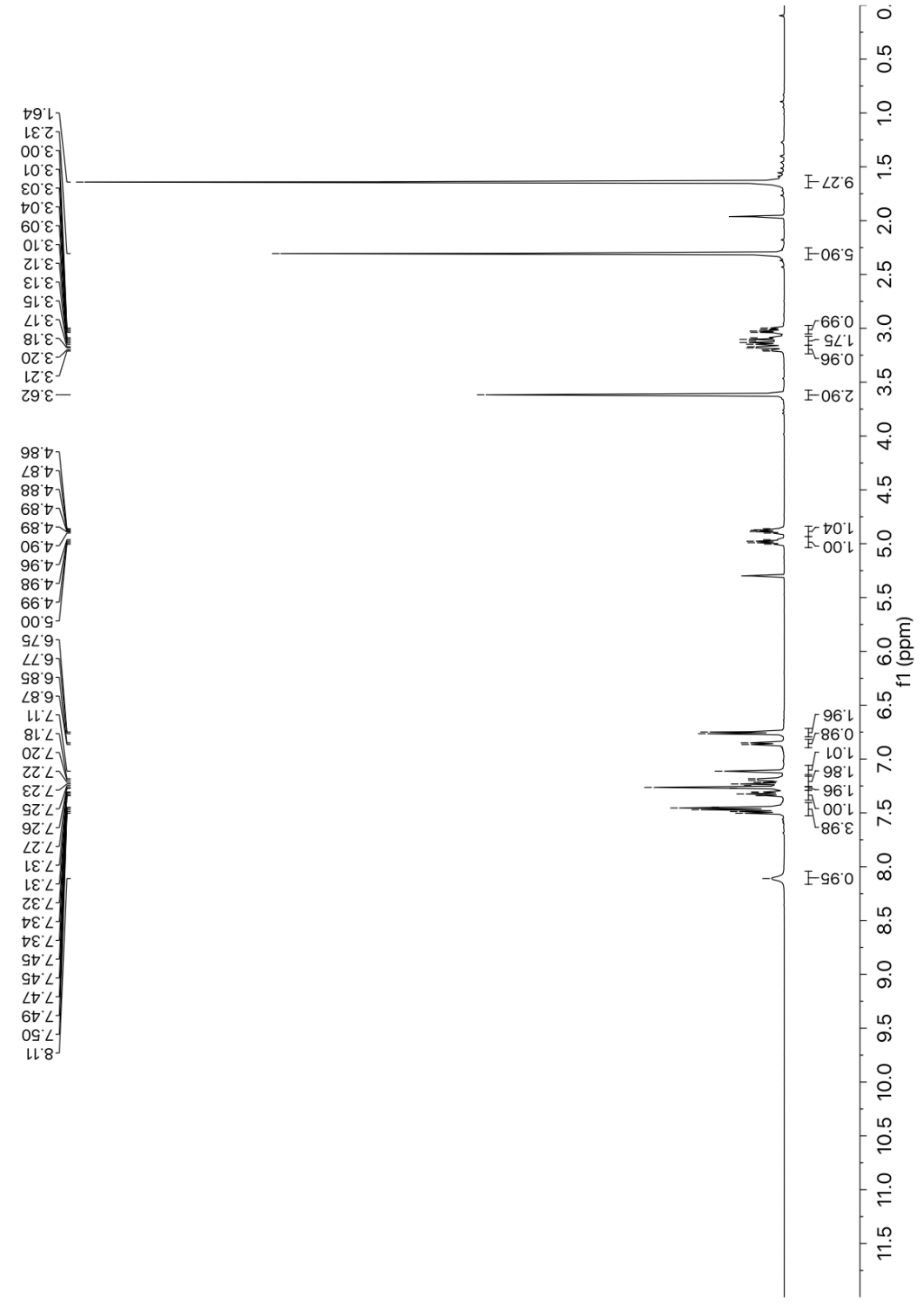
^{1}HNMR spectrum for compound **80**



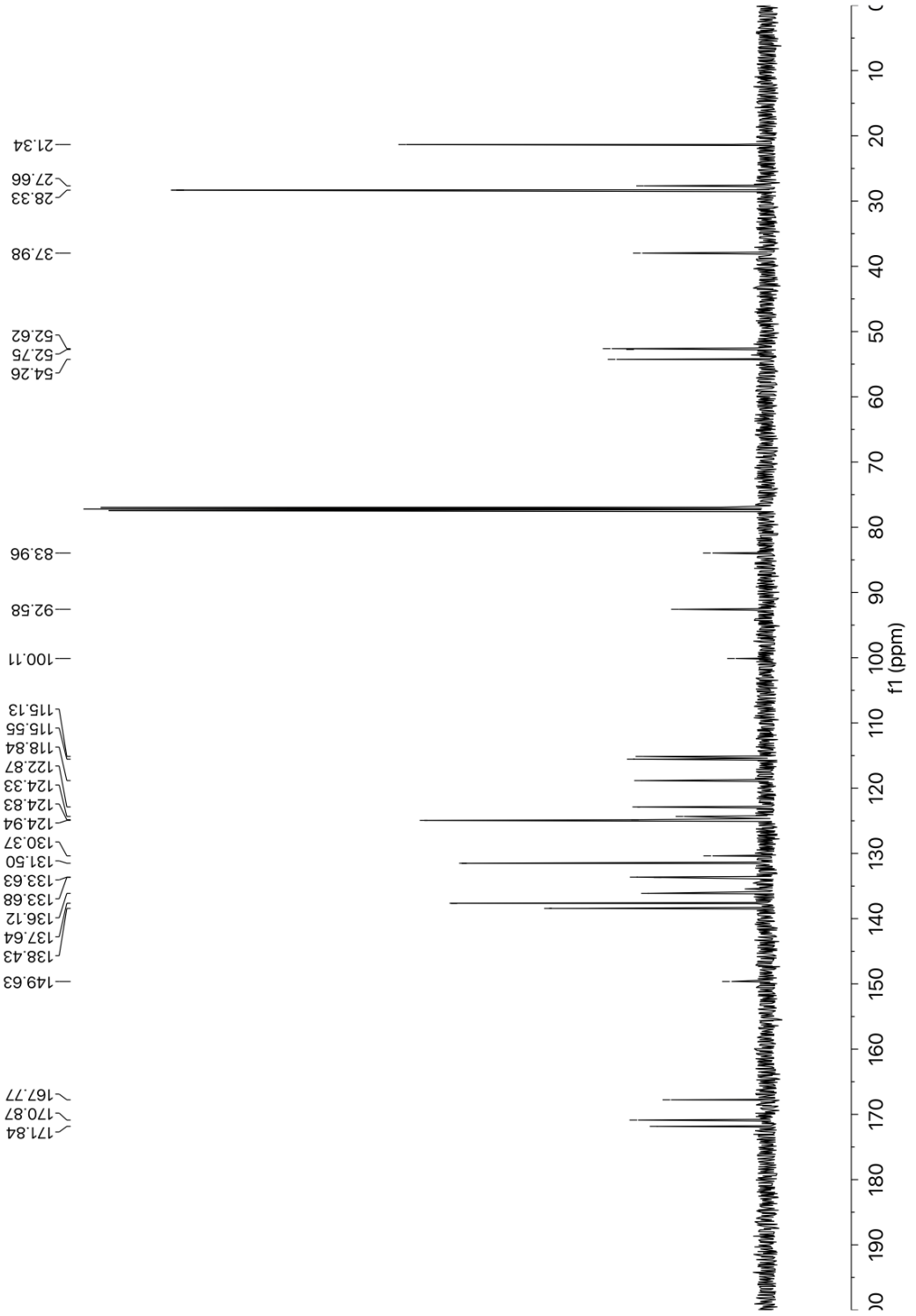
^{13}CNMR spectrum for compound **80**



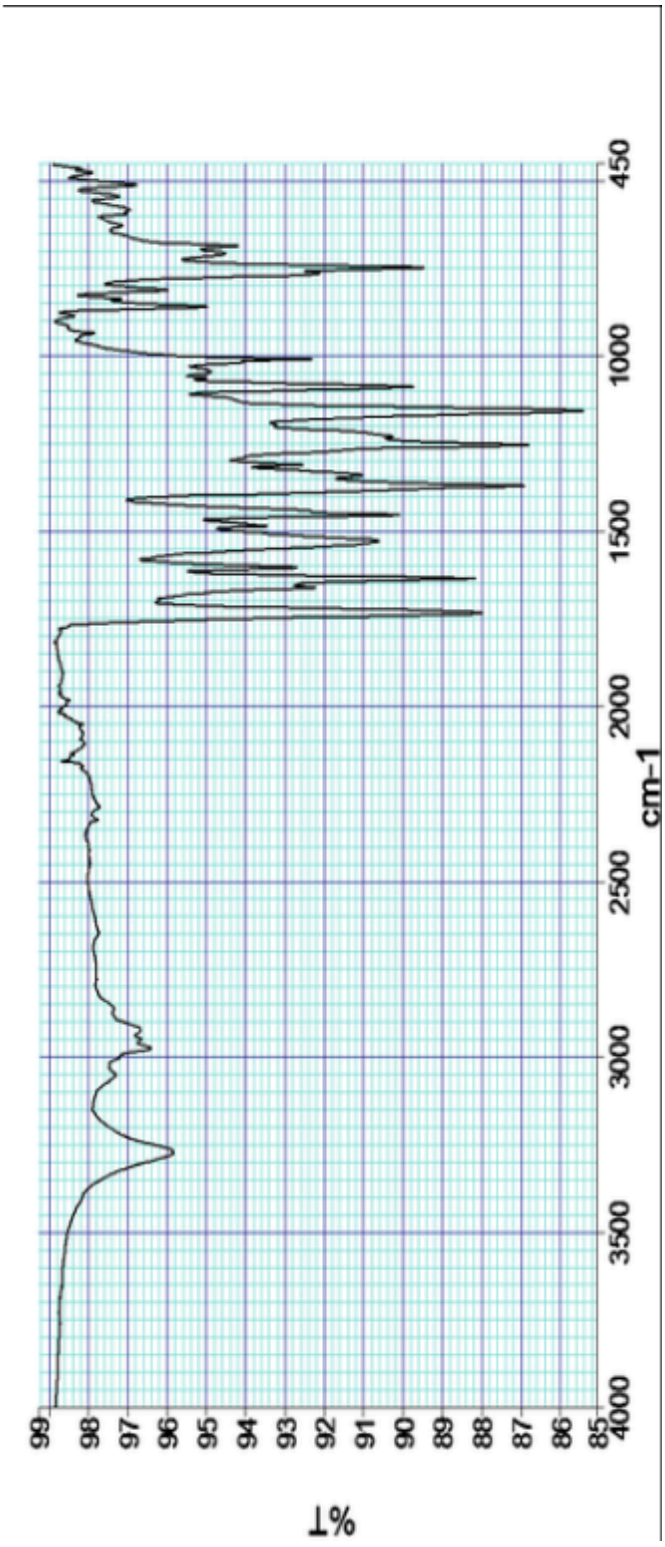
IR spectrum for compound **80**



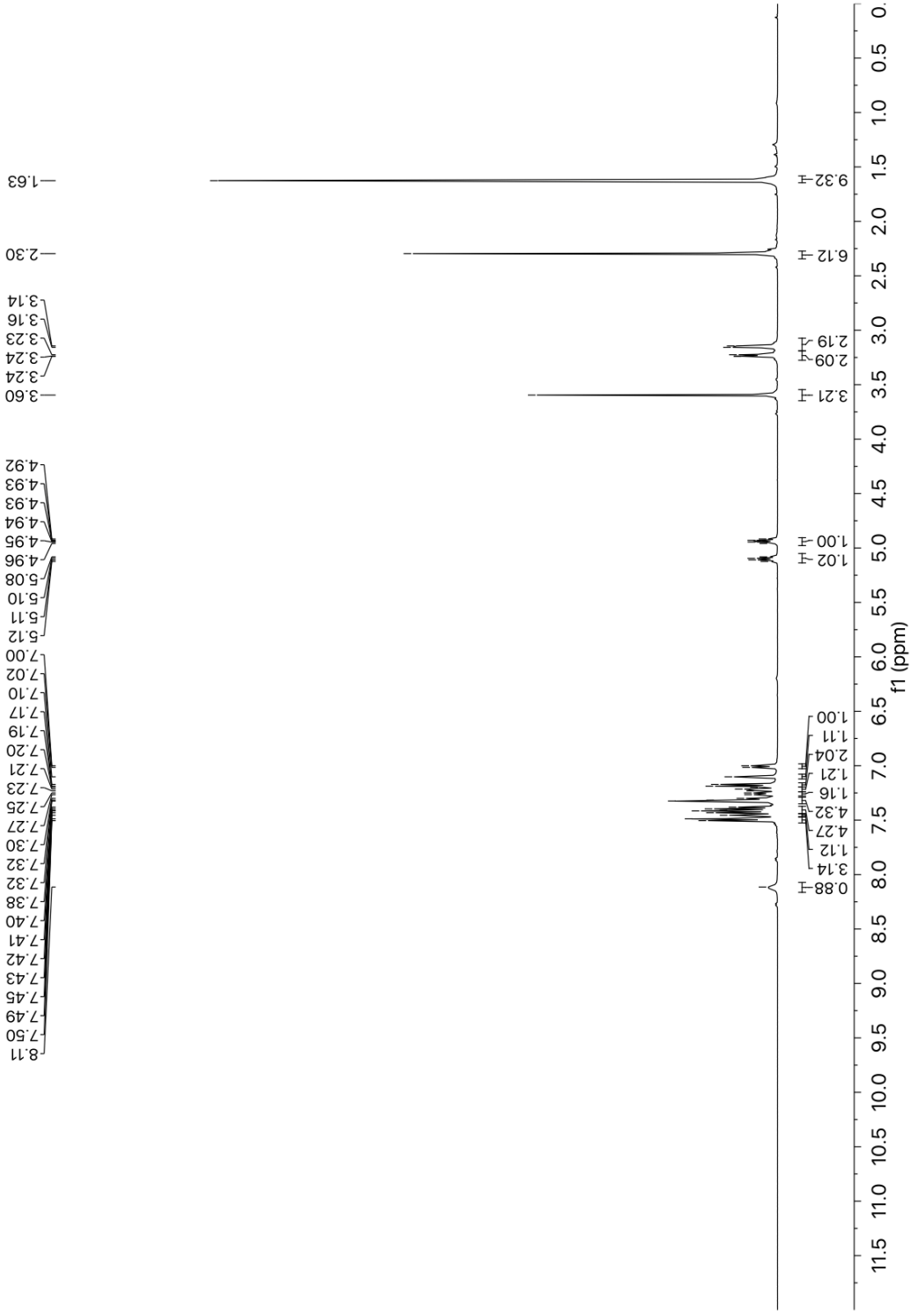
^{1}HNMR spectrum for compound **81**



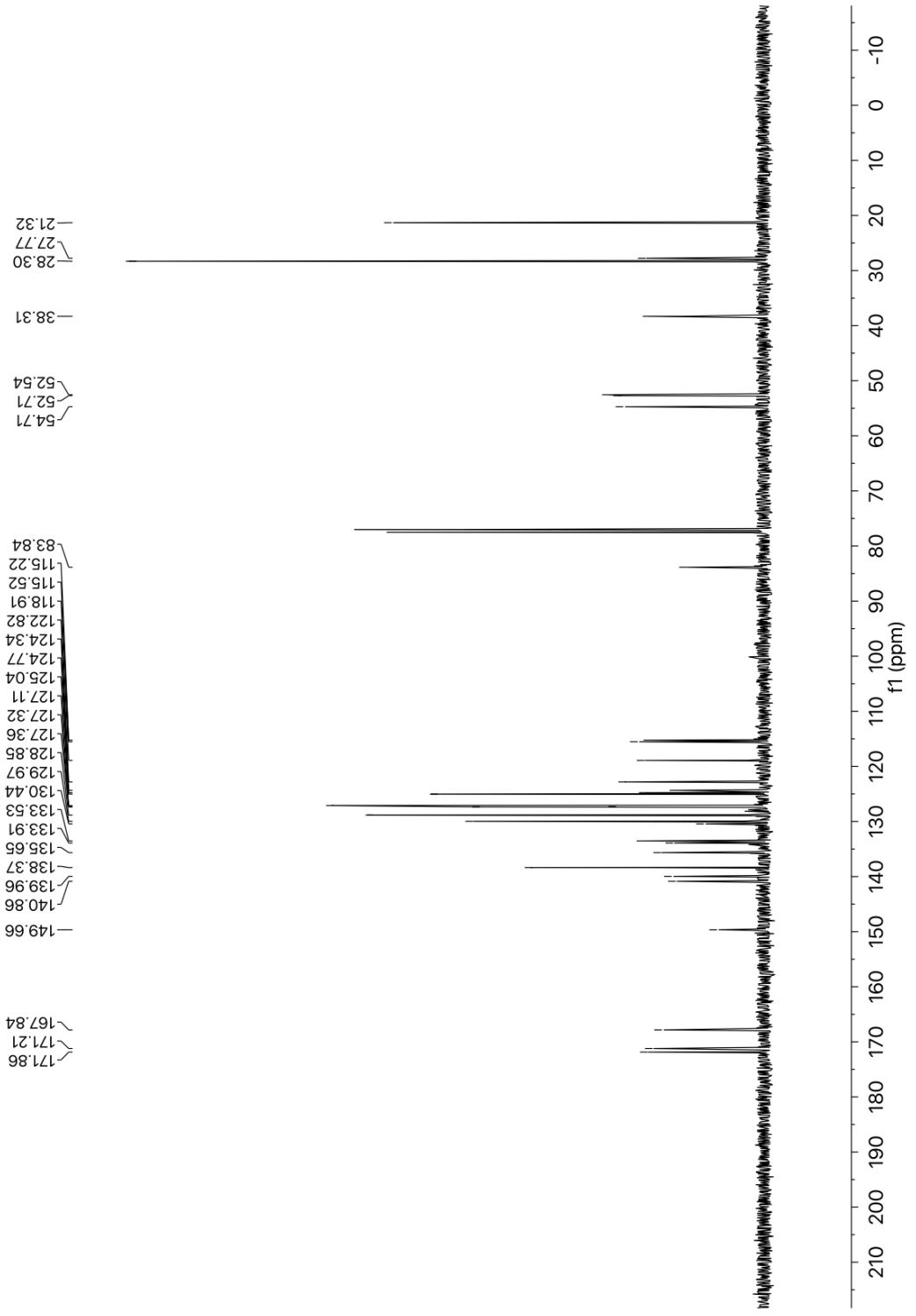
^{13}CNMR spectrum for compound **81**



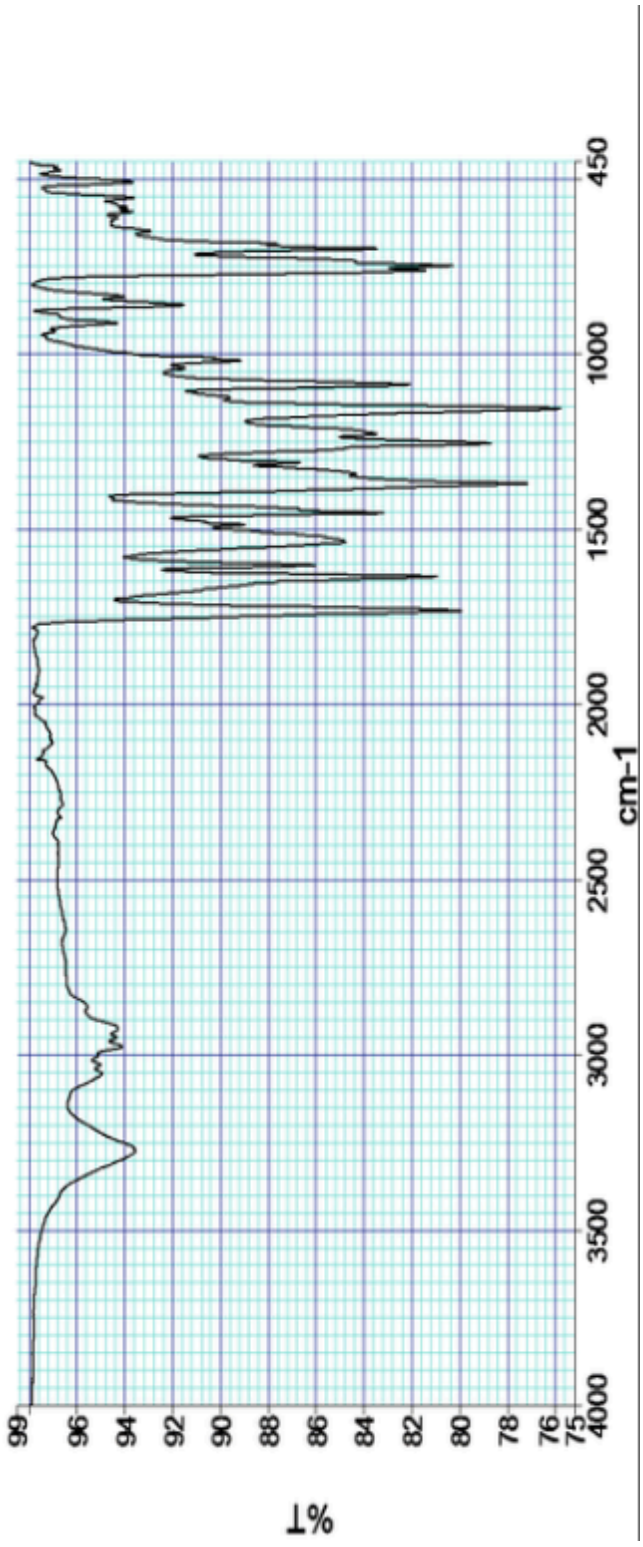
IR spectrum for compound **81**



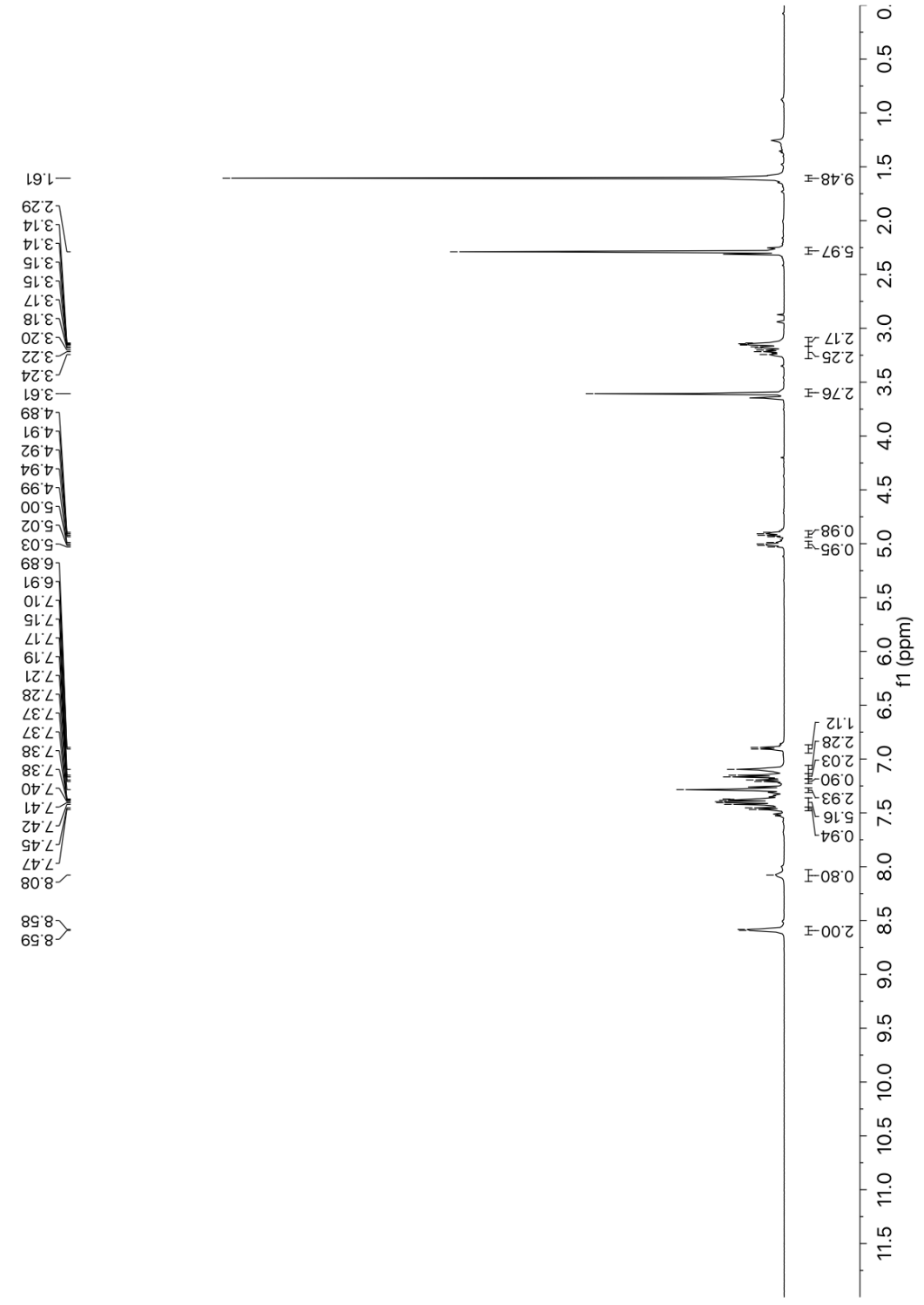
[1]HNMR spectrum for compound **83a**



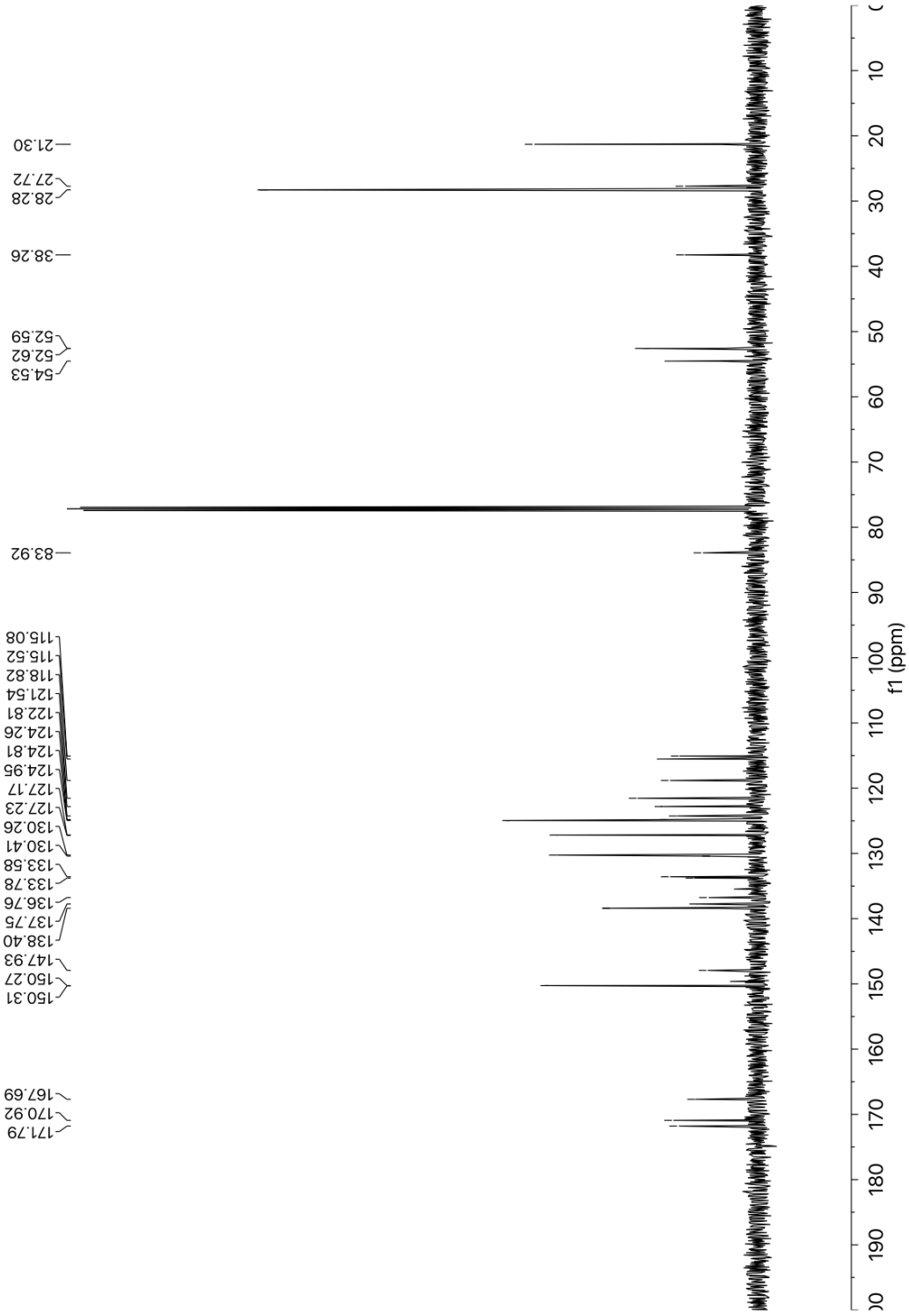
^{13}CNMR spectrum for compound **83a**



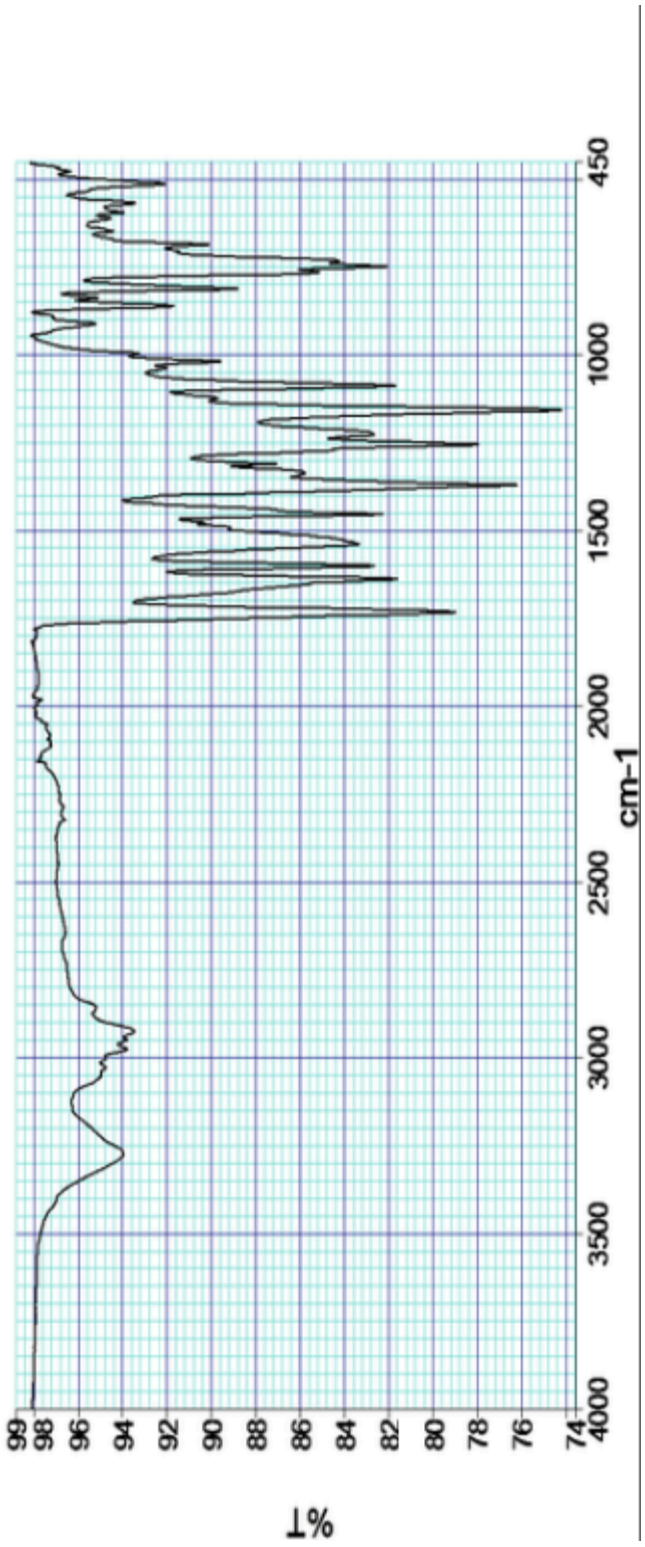
IR spectrum for compound **83a**



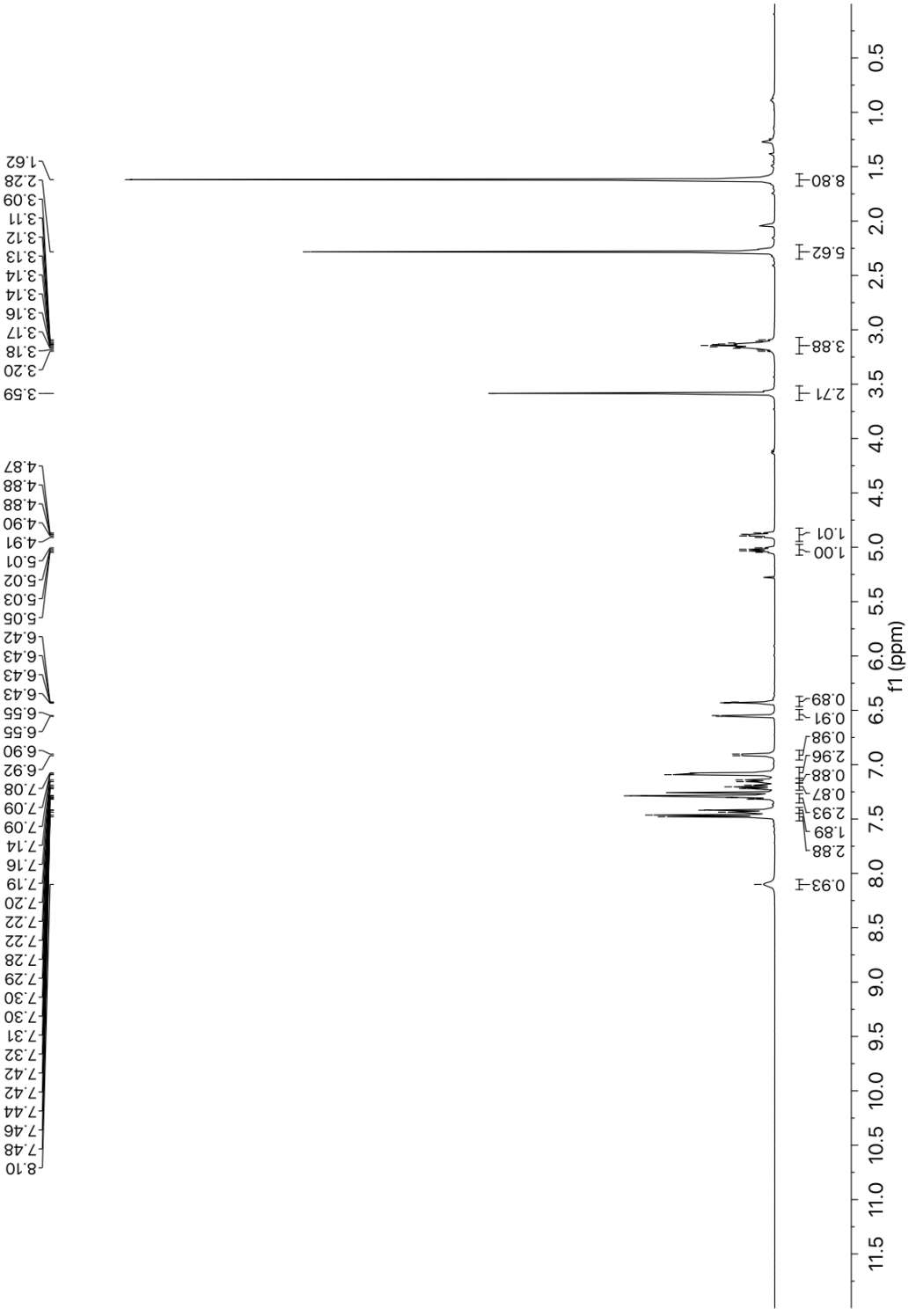
^{1}HNMR spectrum for compound **83b**



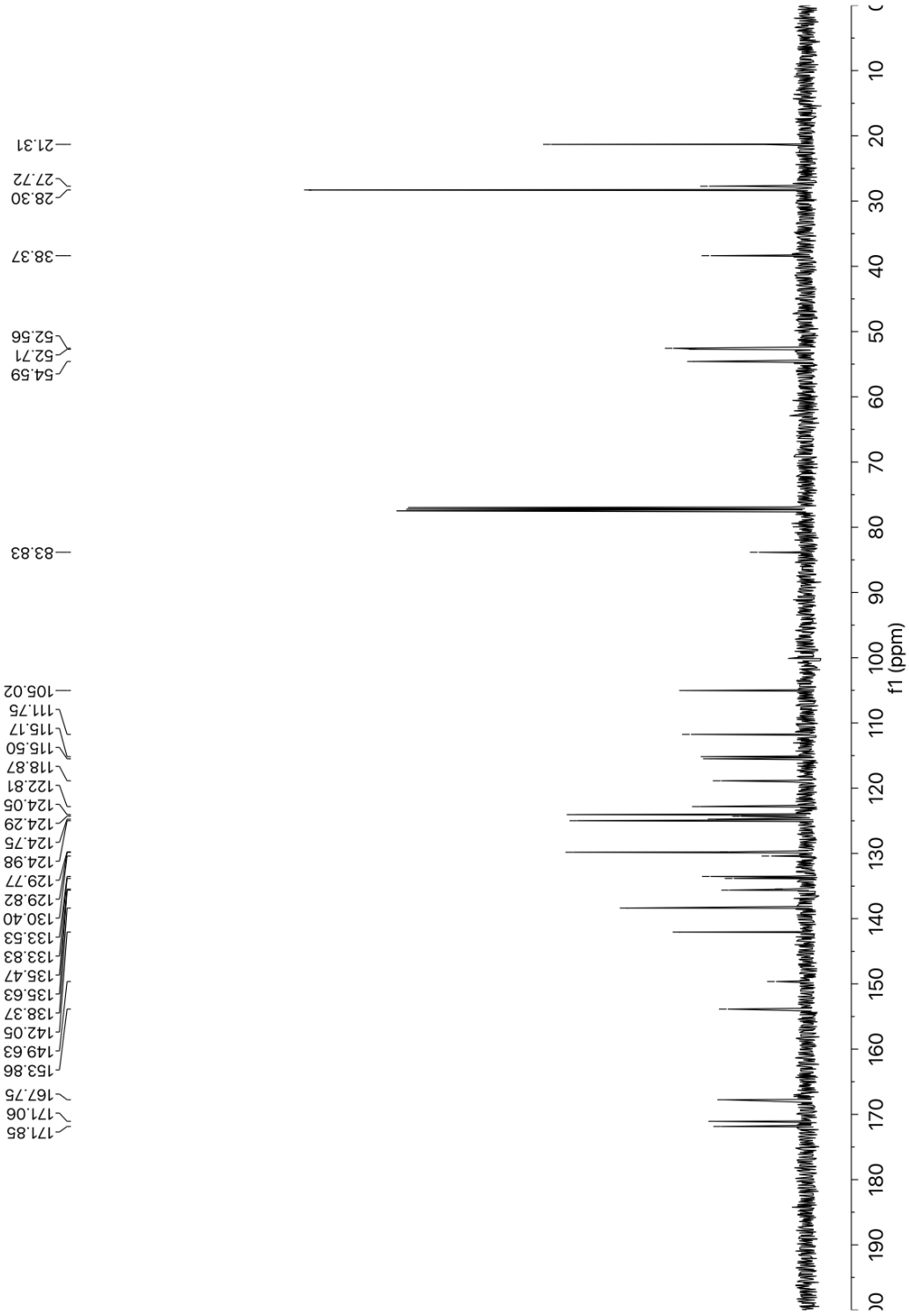
^{13}CNMR spectrum for compound **83b**



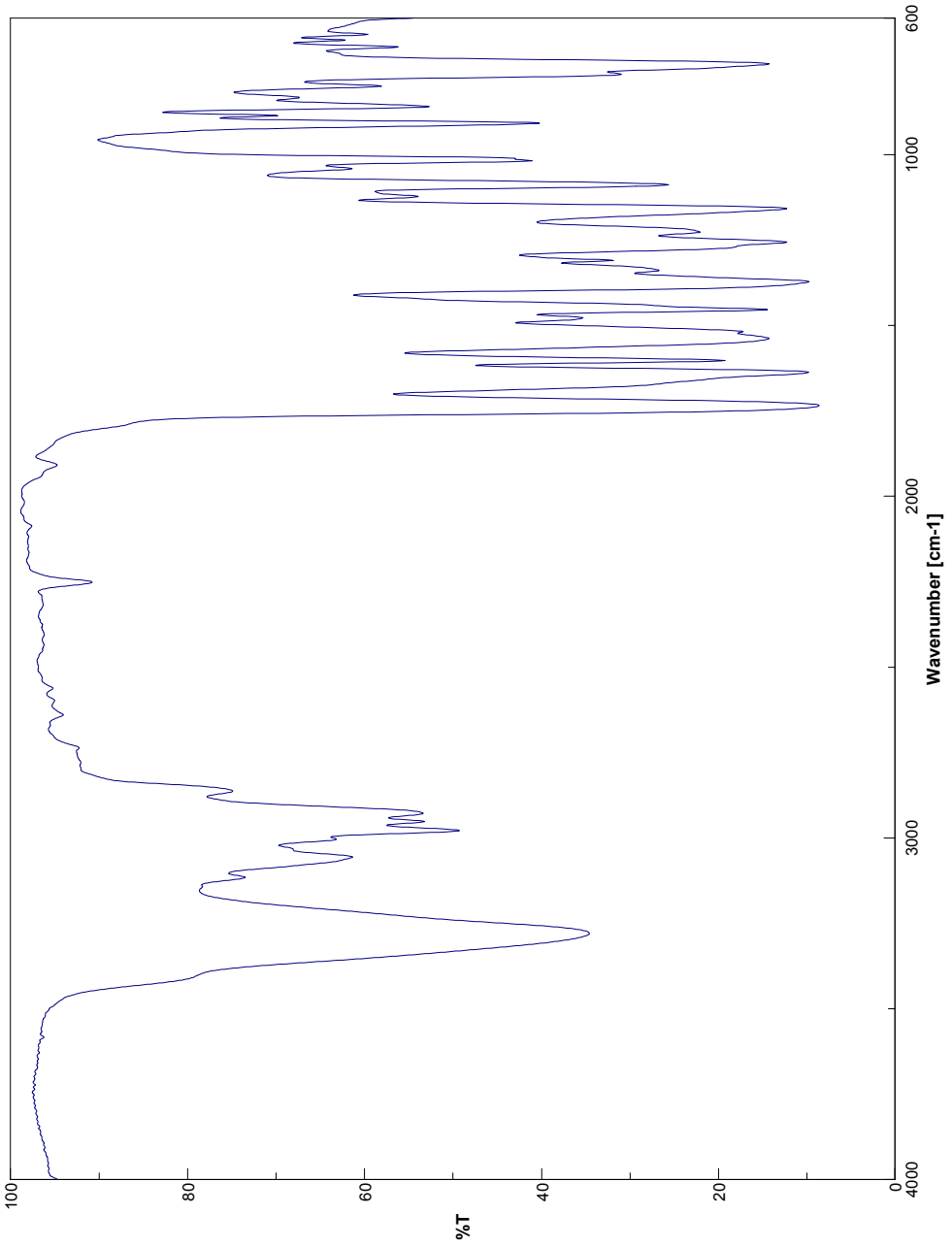
IR spectrum for compound **83b**



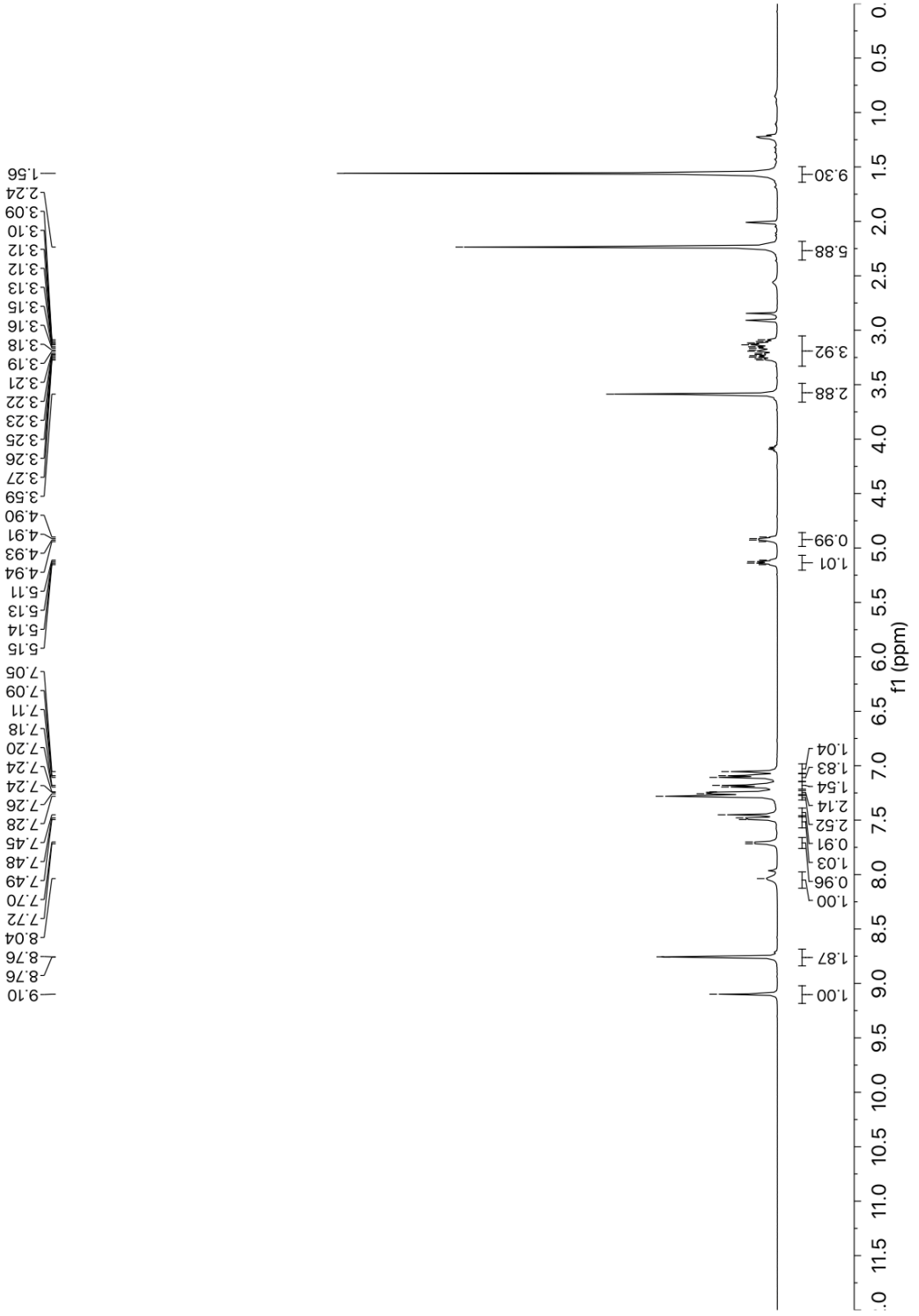
[1]HNMR spectrum for compound **83c**



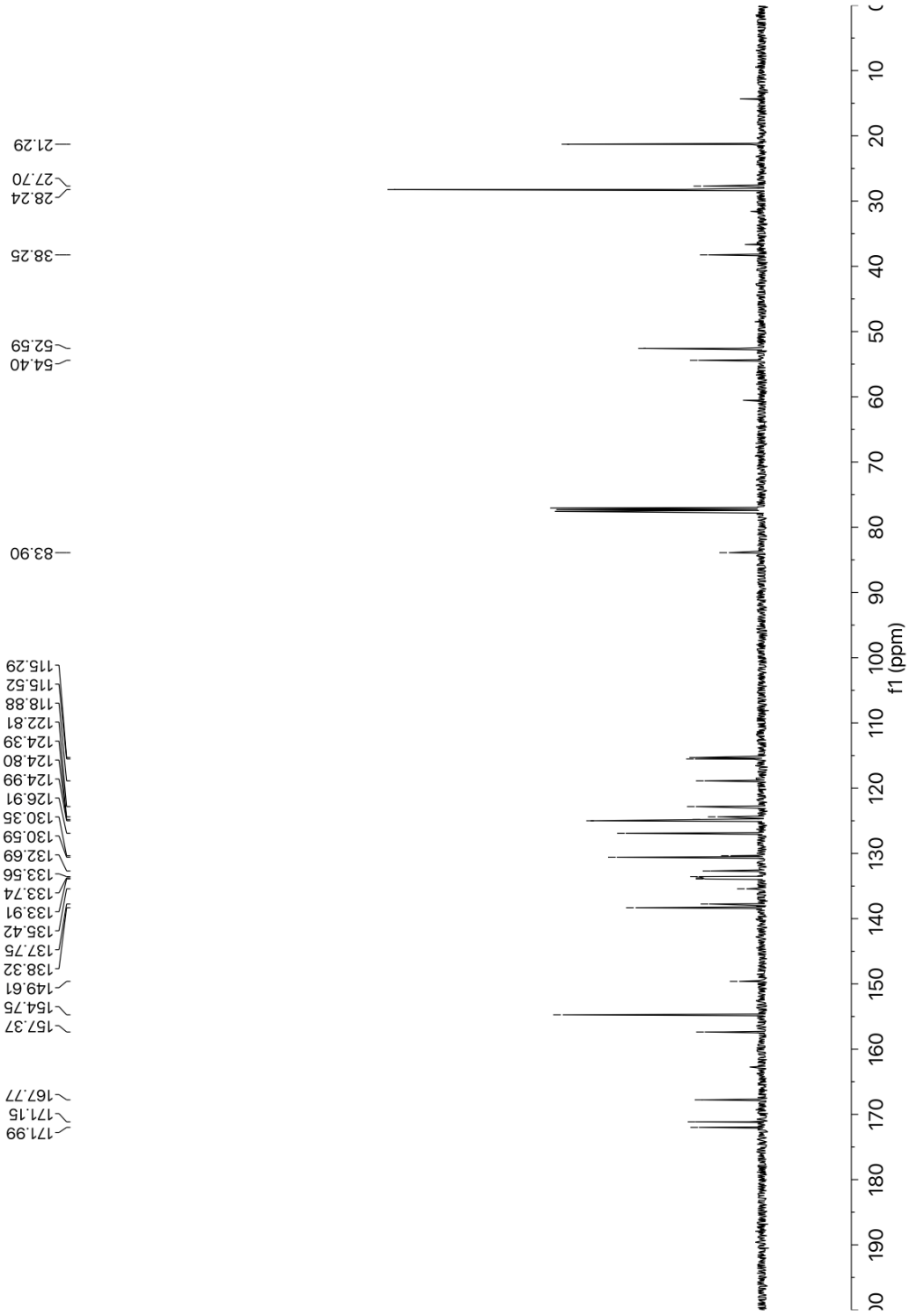
^{13}CNMR spectrum for compound **83c**



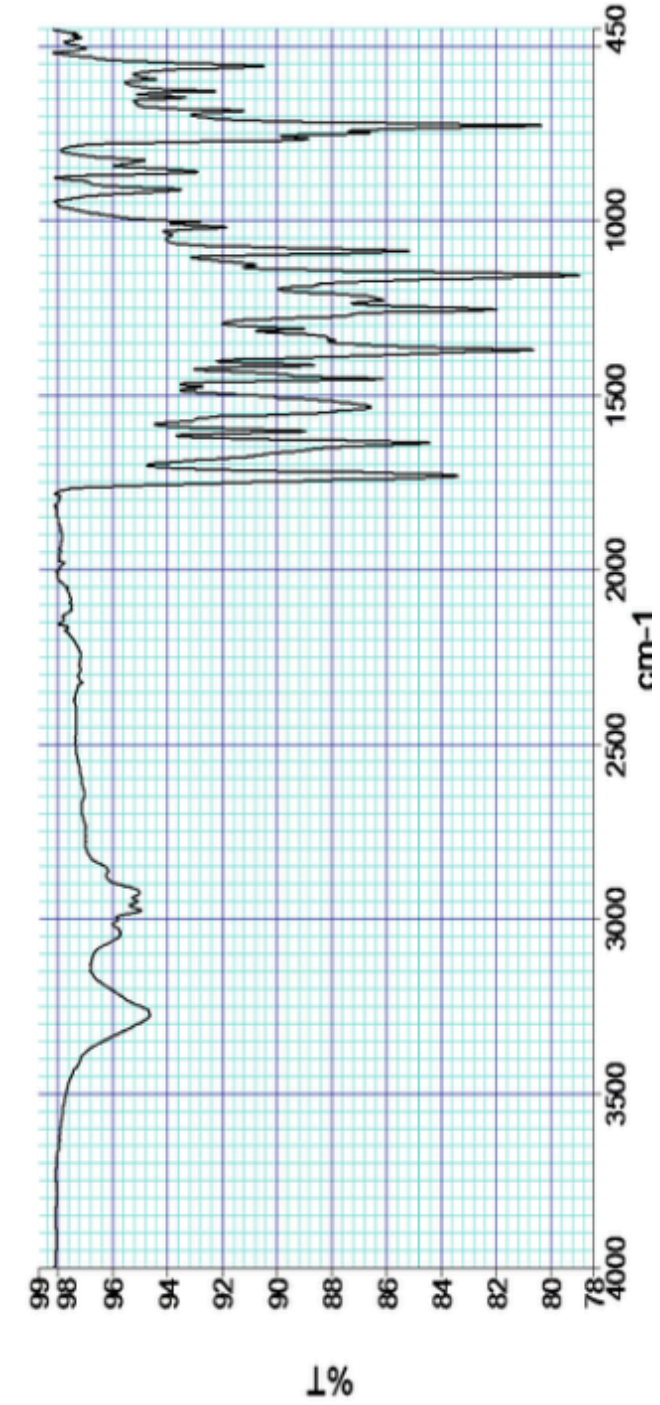
IR spectrum for compound **83c**



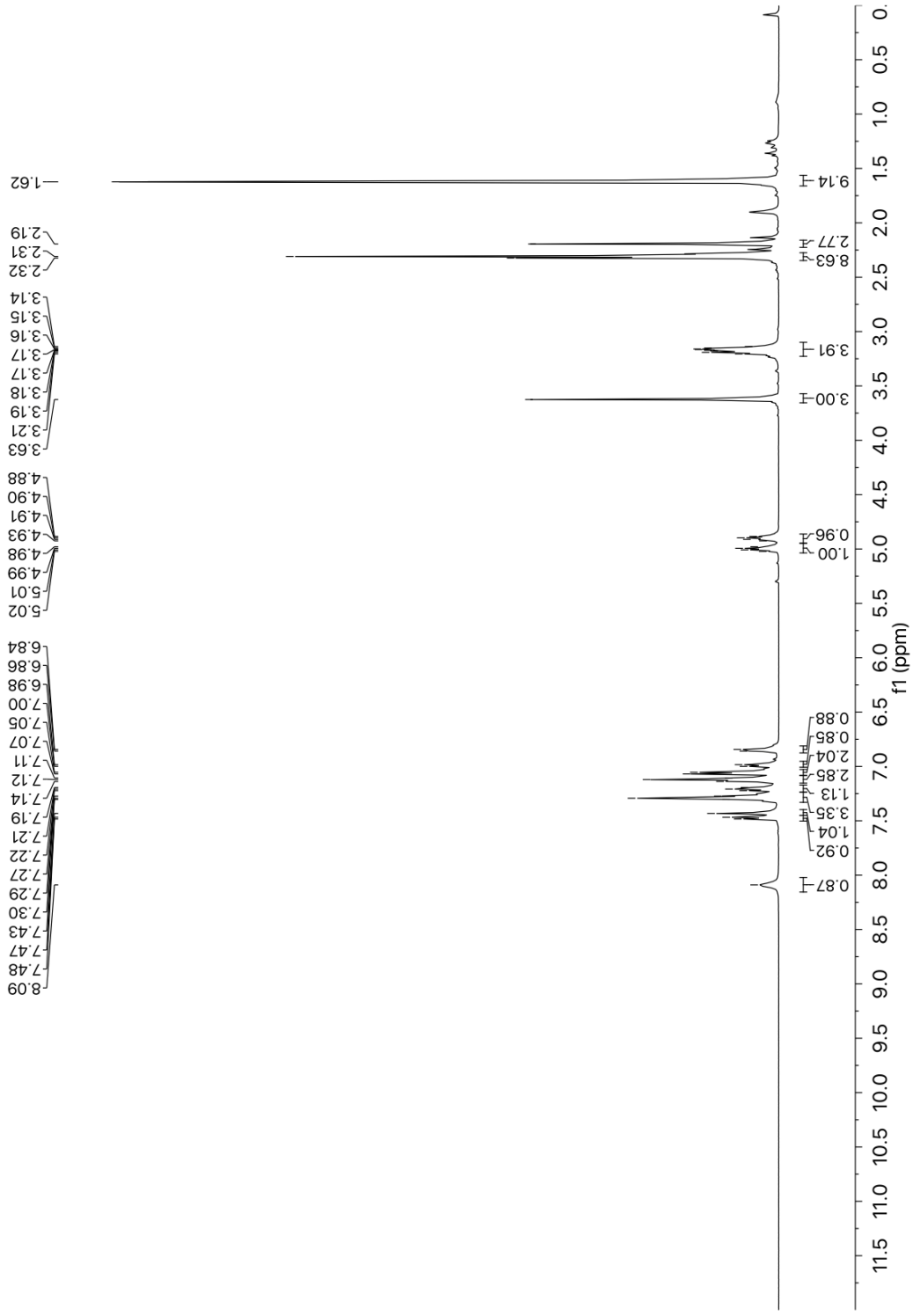
[1]HNMR spectrum for compound **83d**



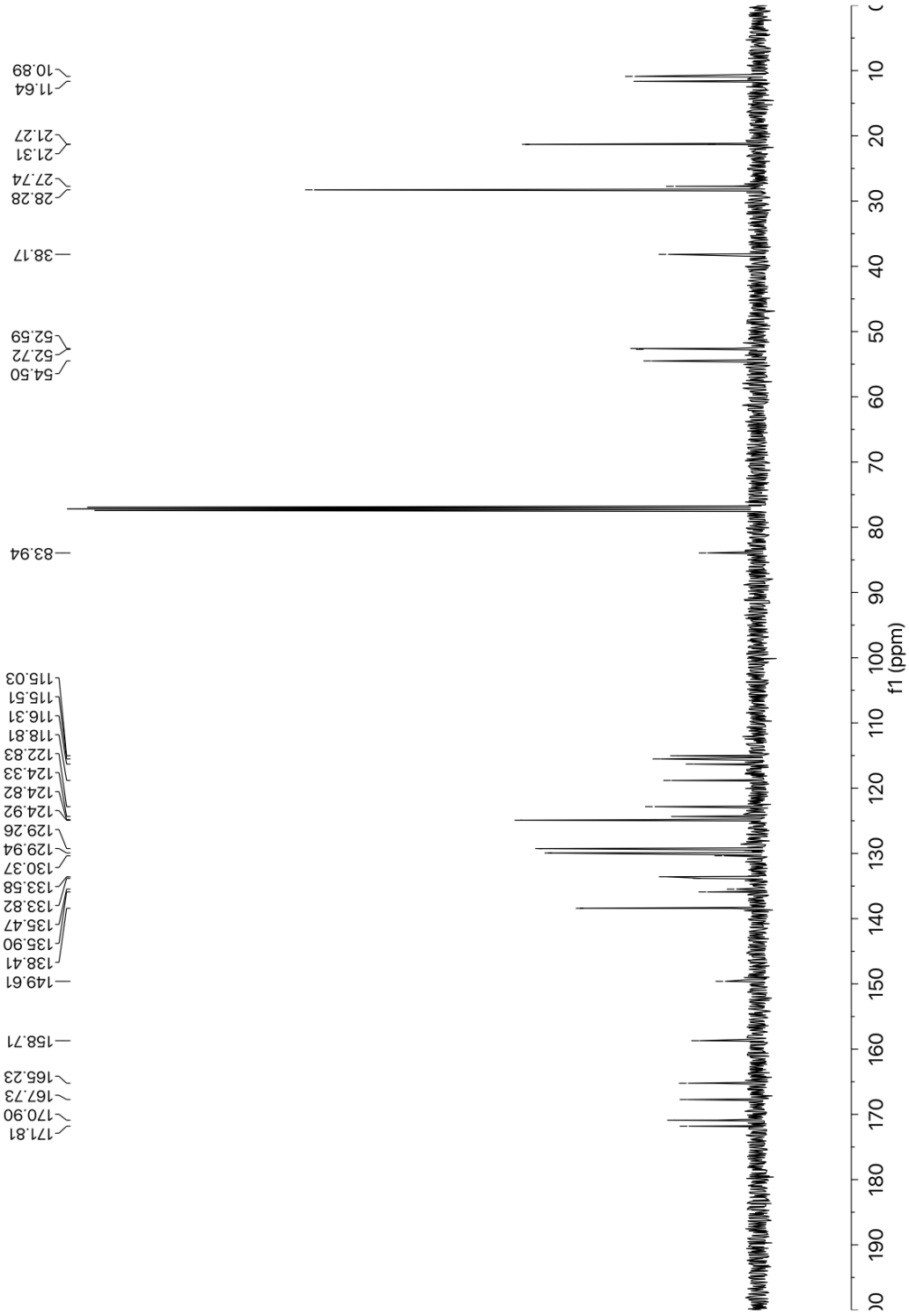
^{13}CNMR spectrum for compound **83d**



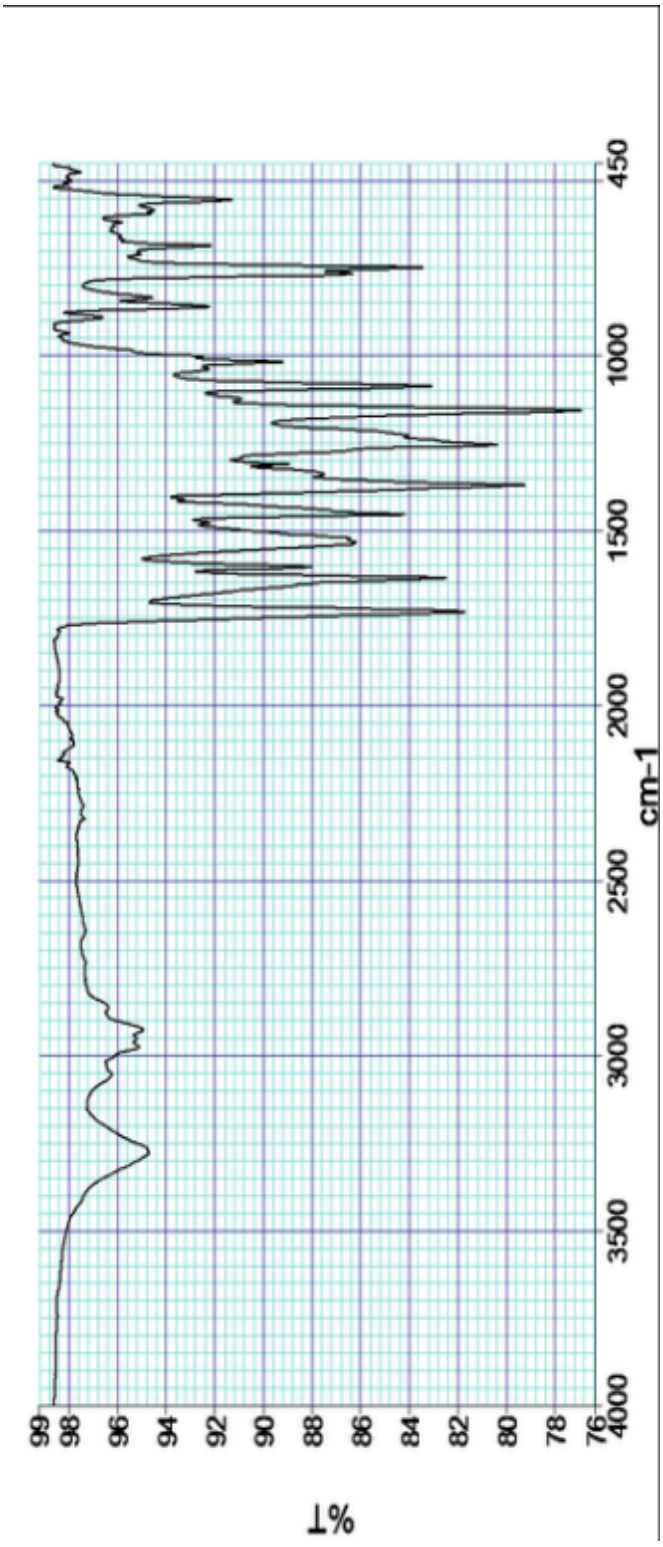
IR spectrum for compound **83d**



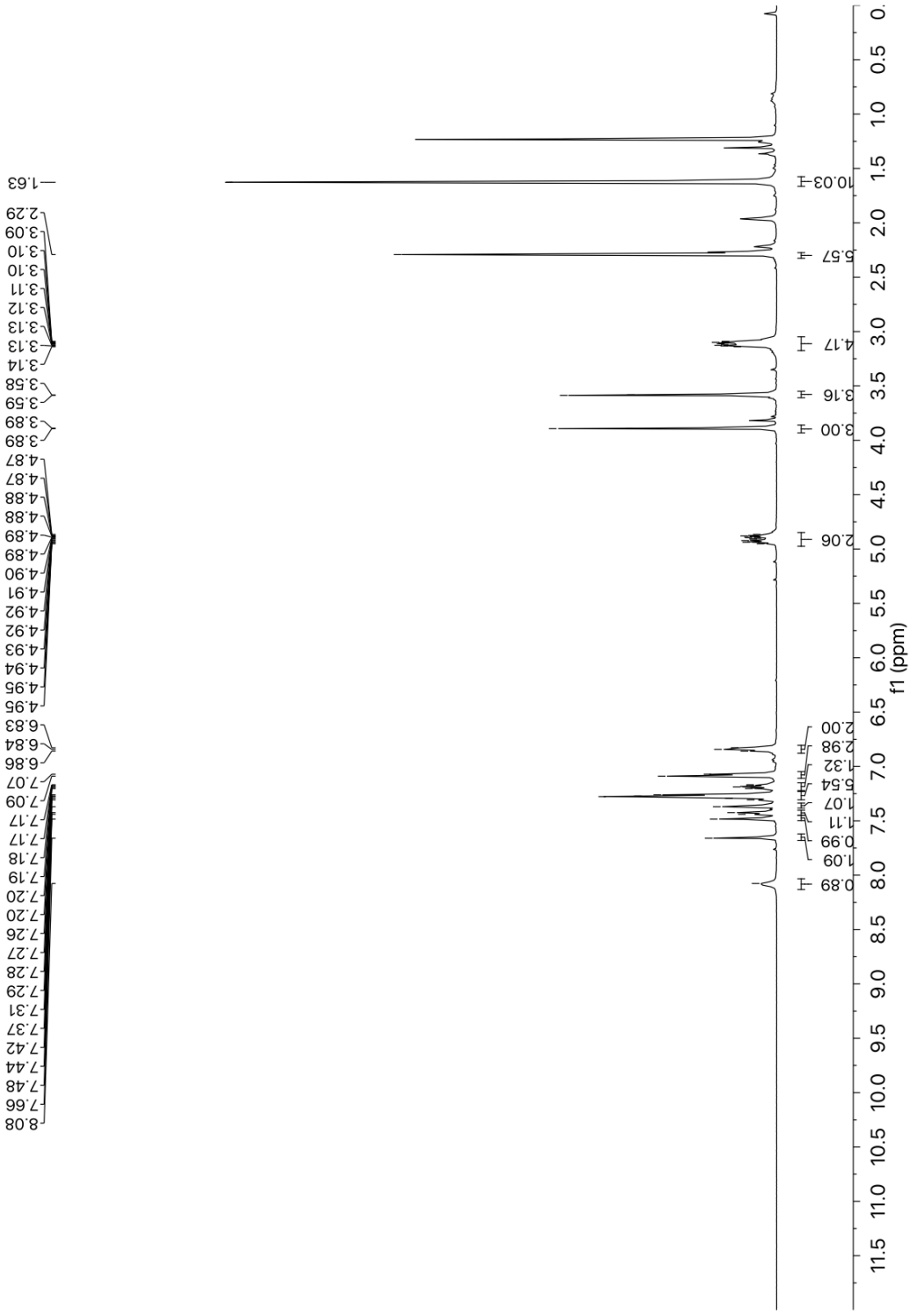
[1]HNMR spectrum for compound **83e**



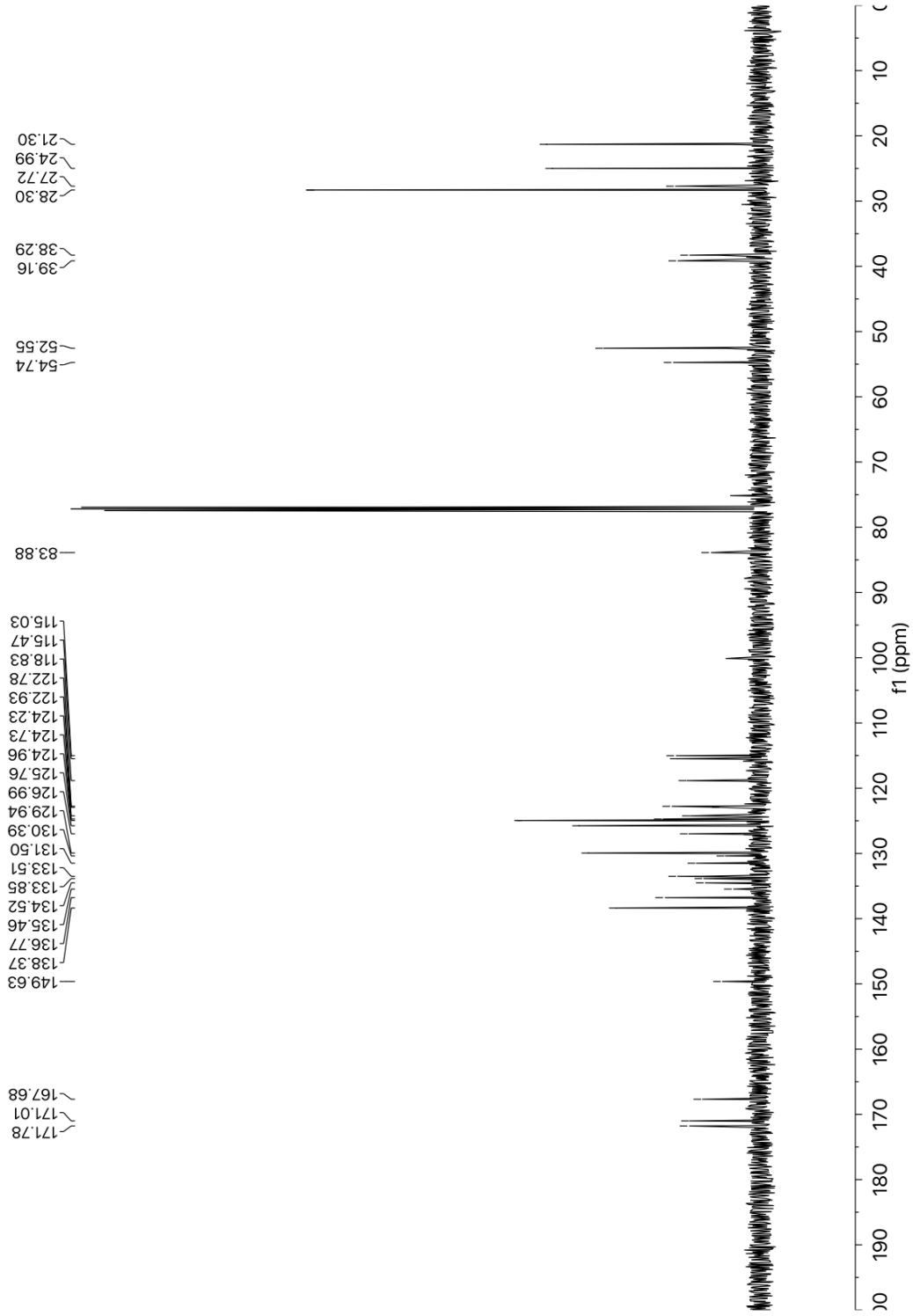
^{13}CNMR spectrum for compound **83e**



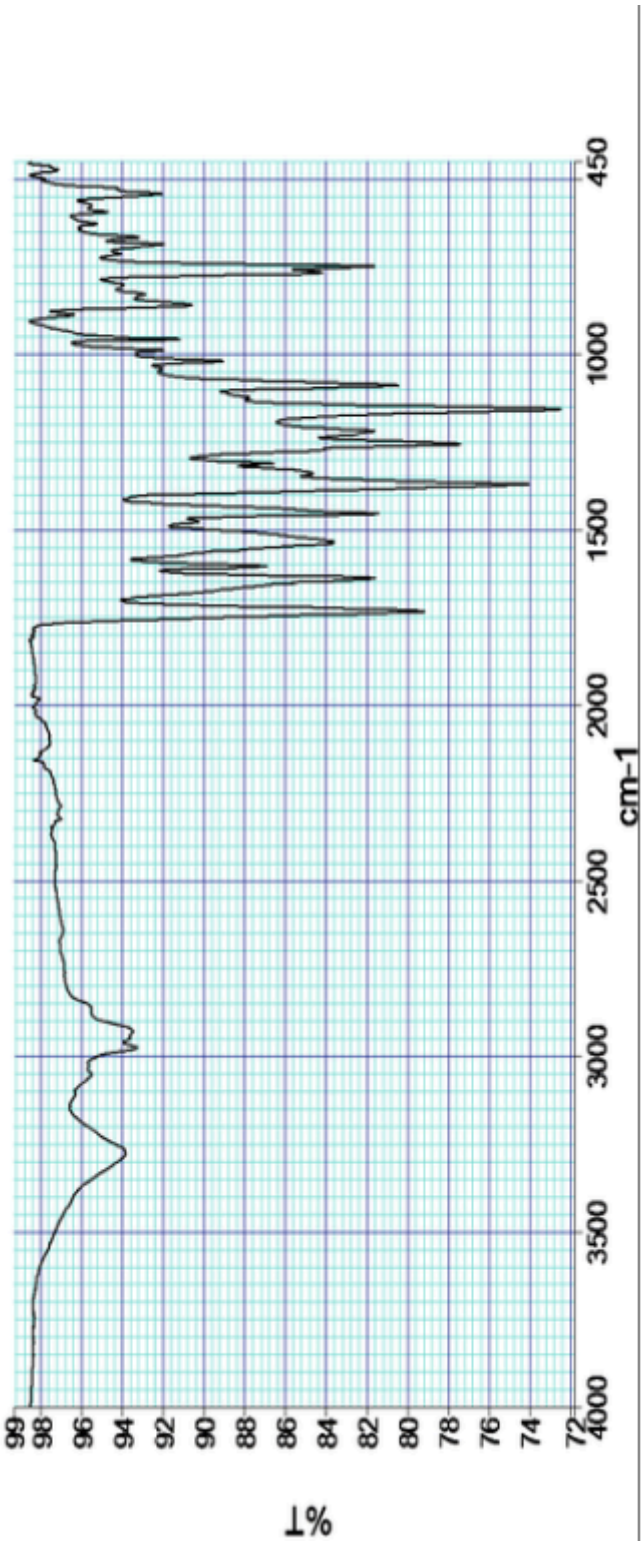
IR spectrum for compound **83e**



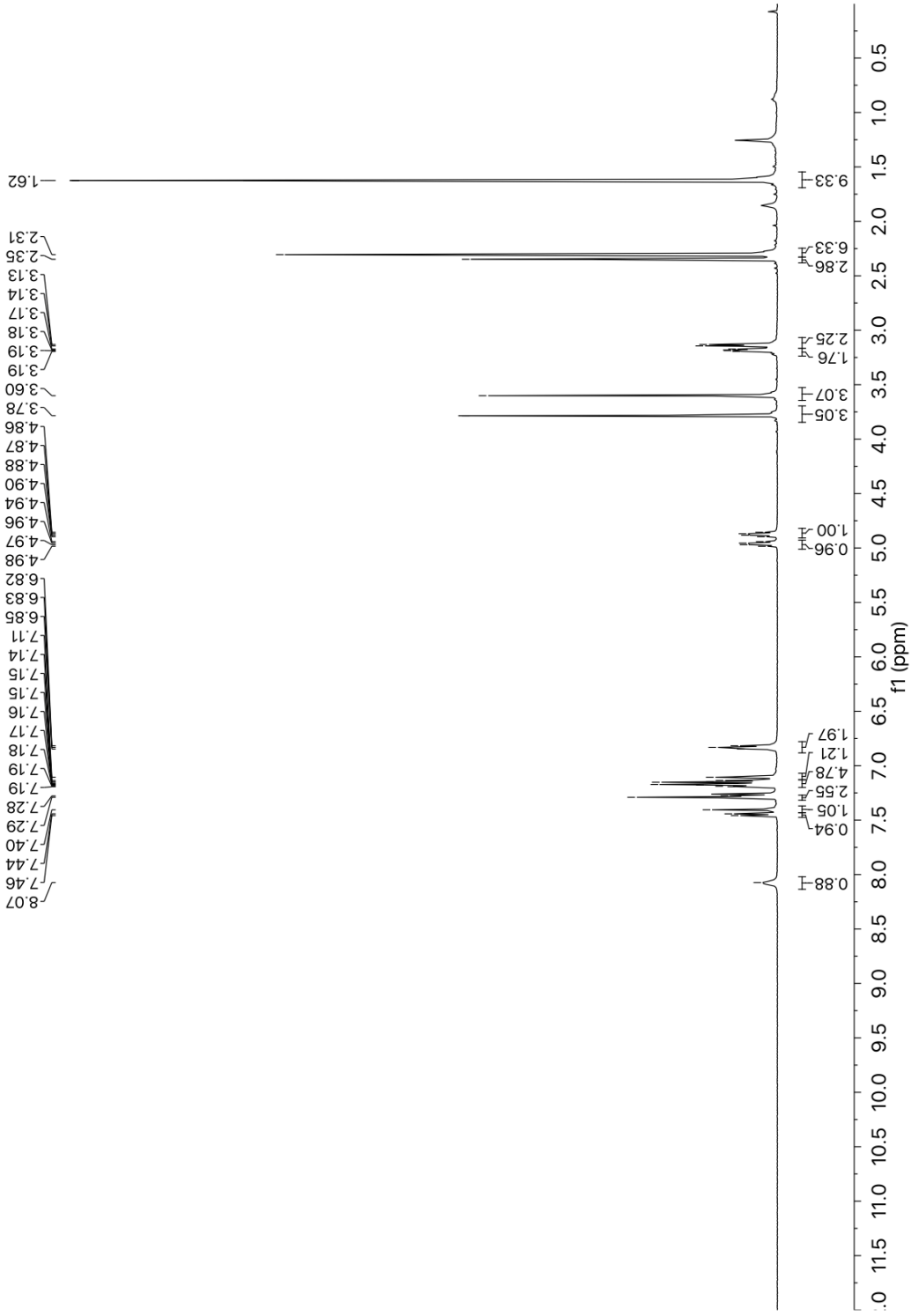
[1]HNMR spectrum for compound **83f**



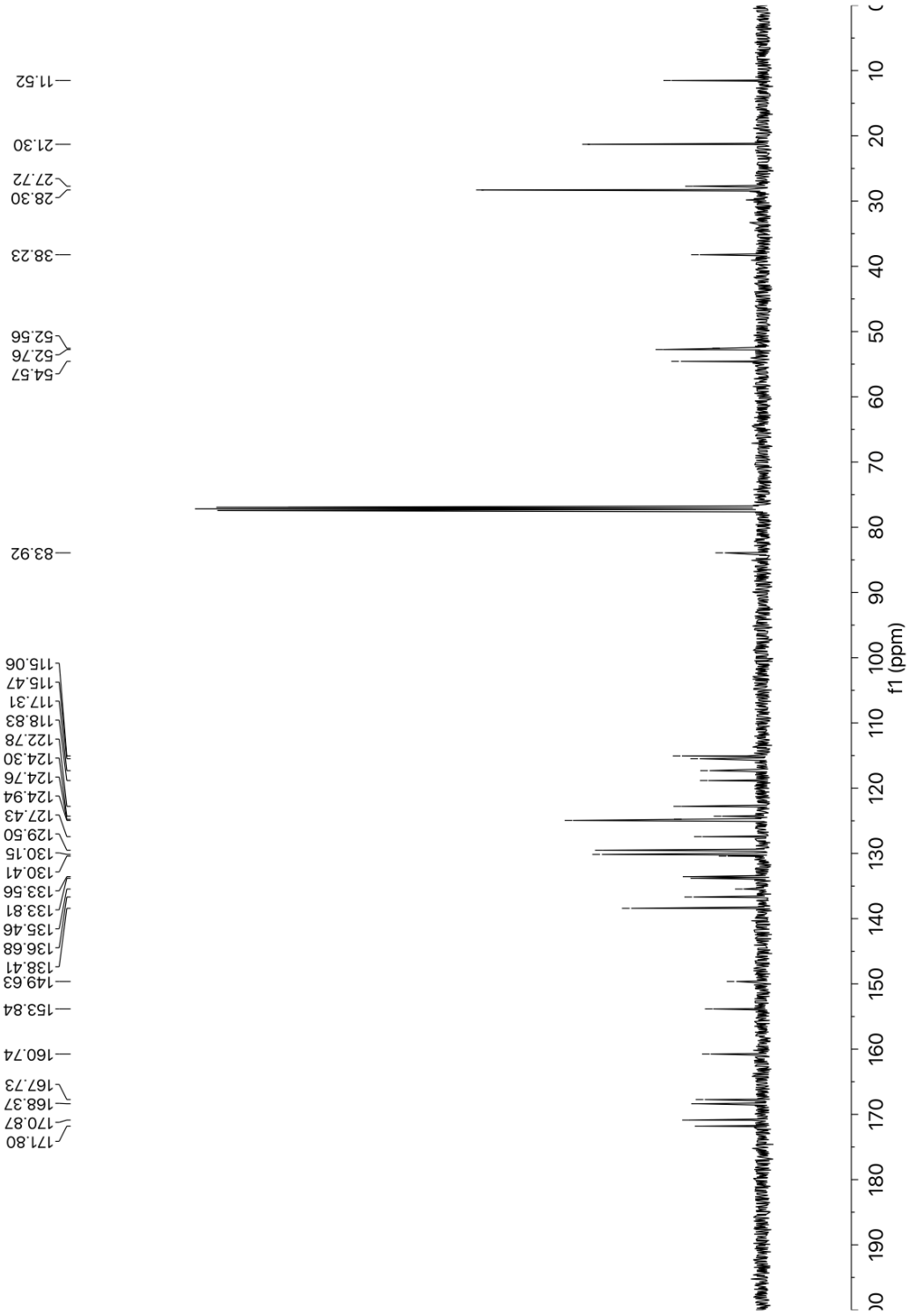
^{13}CNMR spectrum for compound **83f**



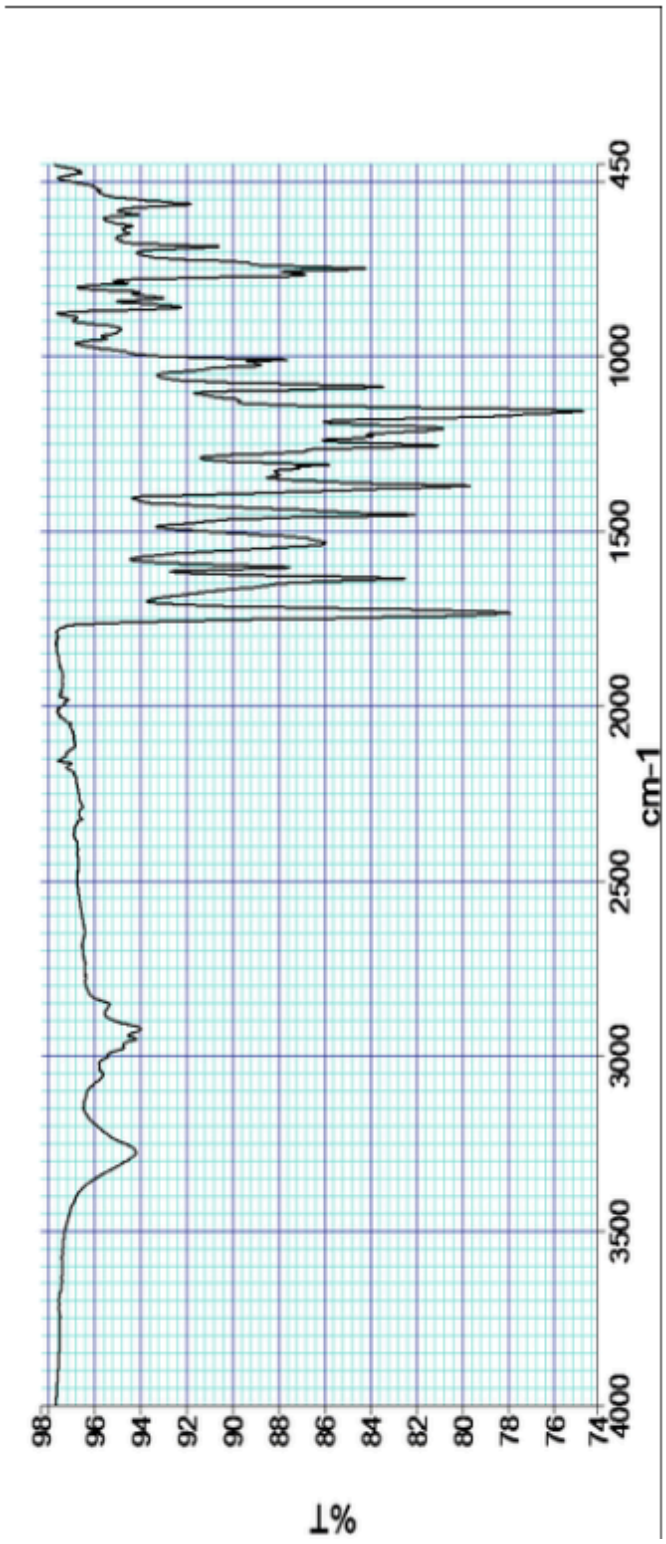
IR spectrum for compound **83f**



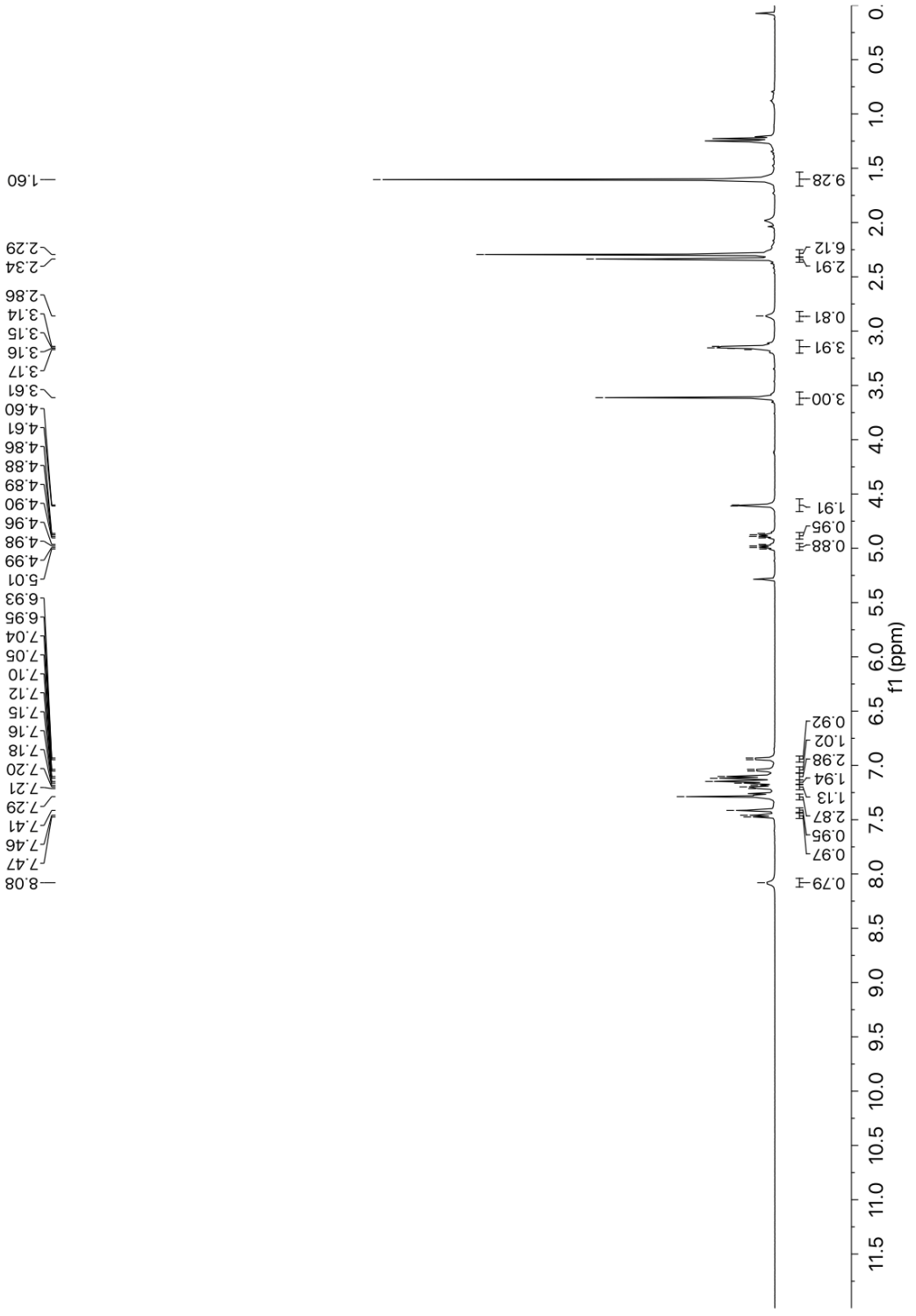
[1]HNMR spectrum for compound **83h**



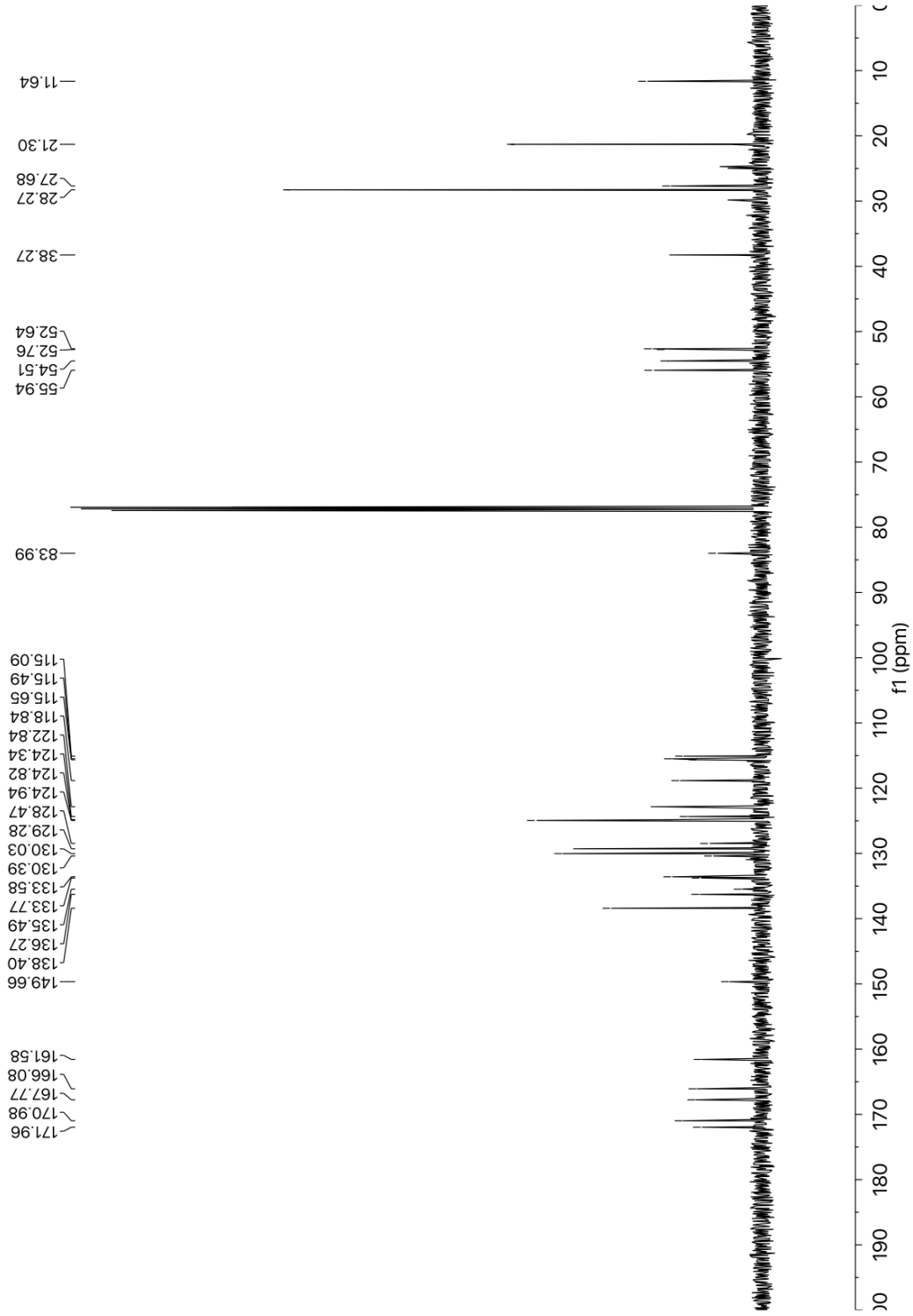
^{13}CNMR spectrum for compound **83h**



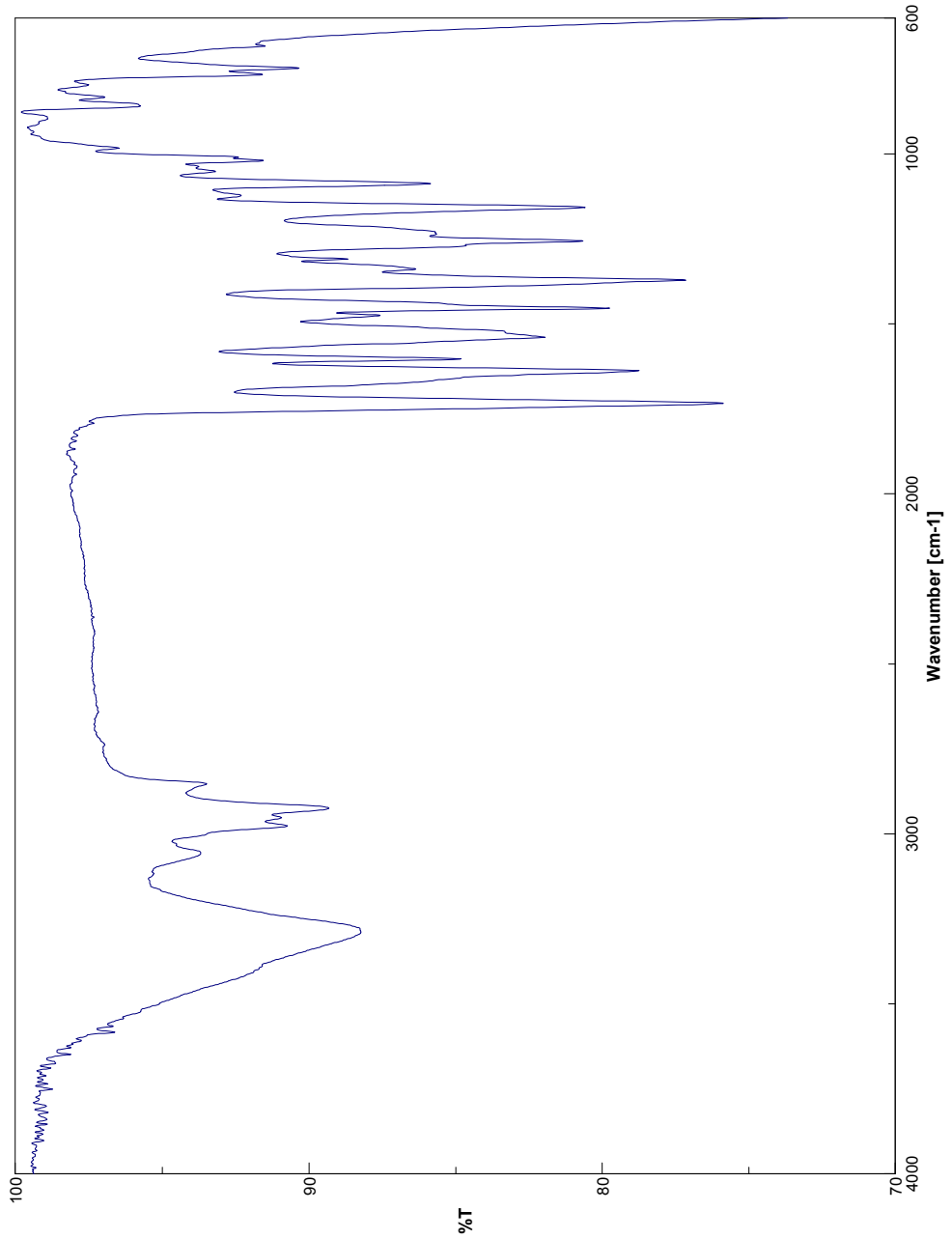
IR spectrum for compound **83h**



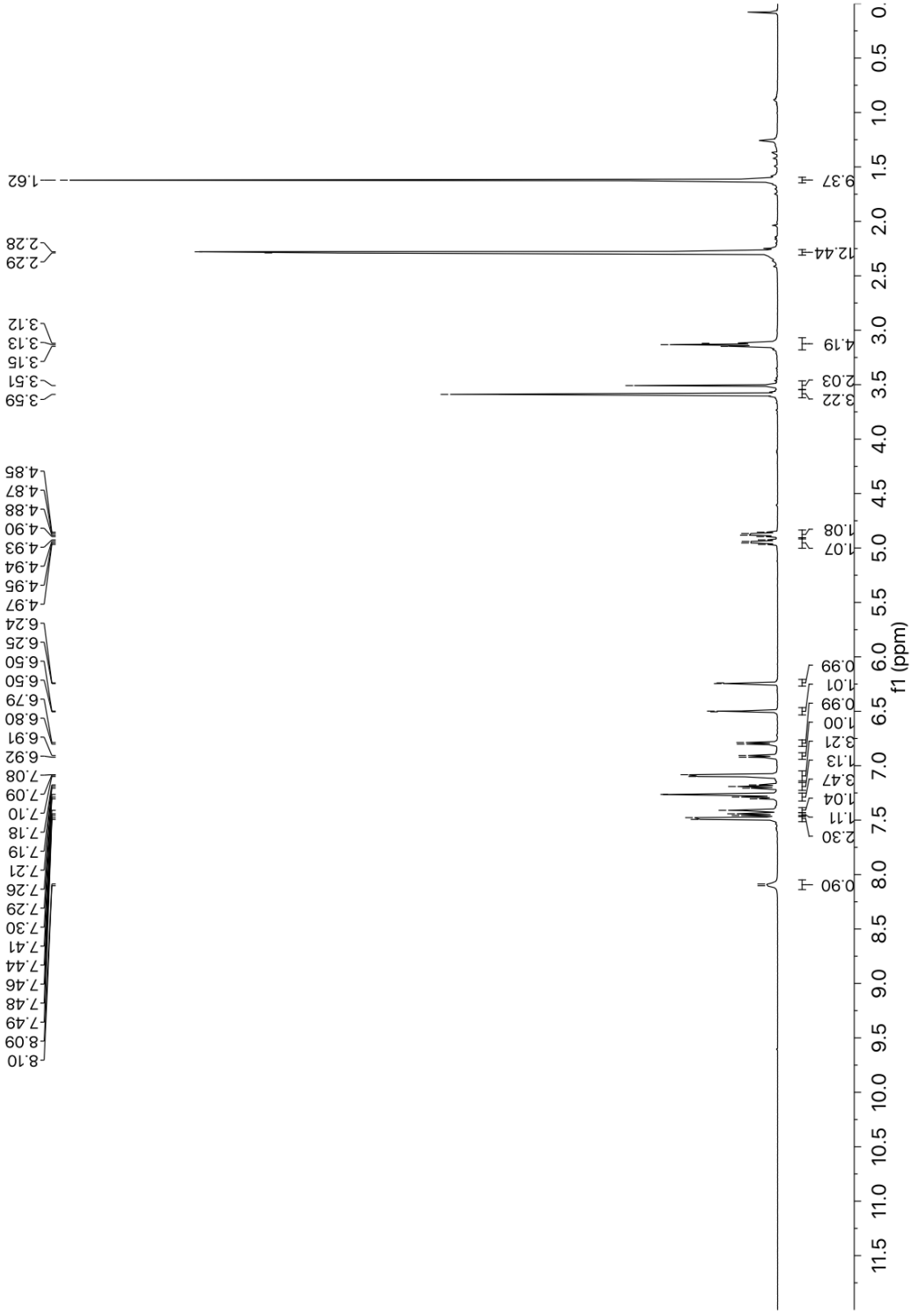
^{1}HNMR spectrum for compound **83i**



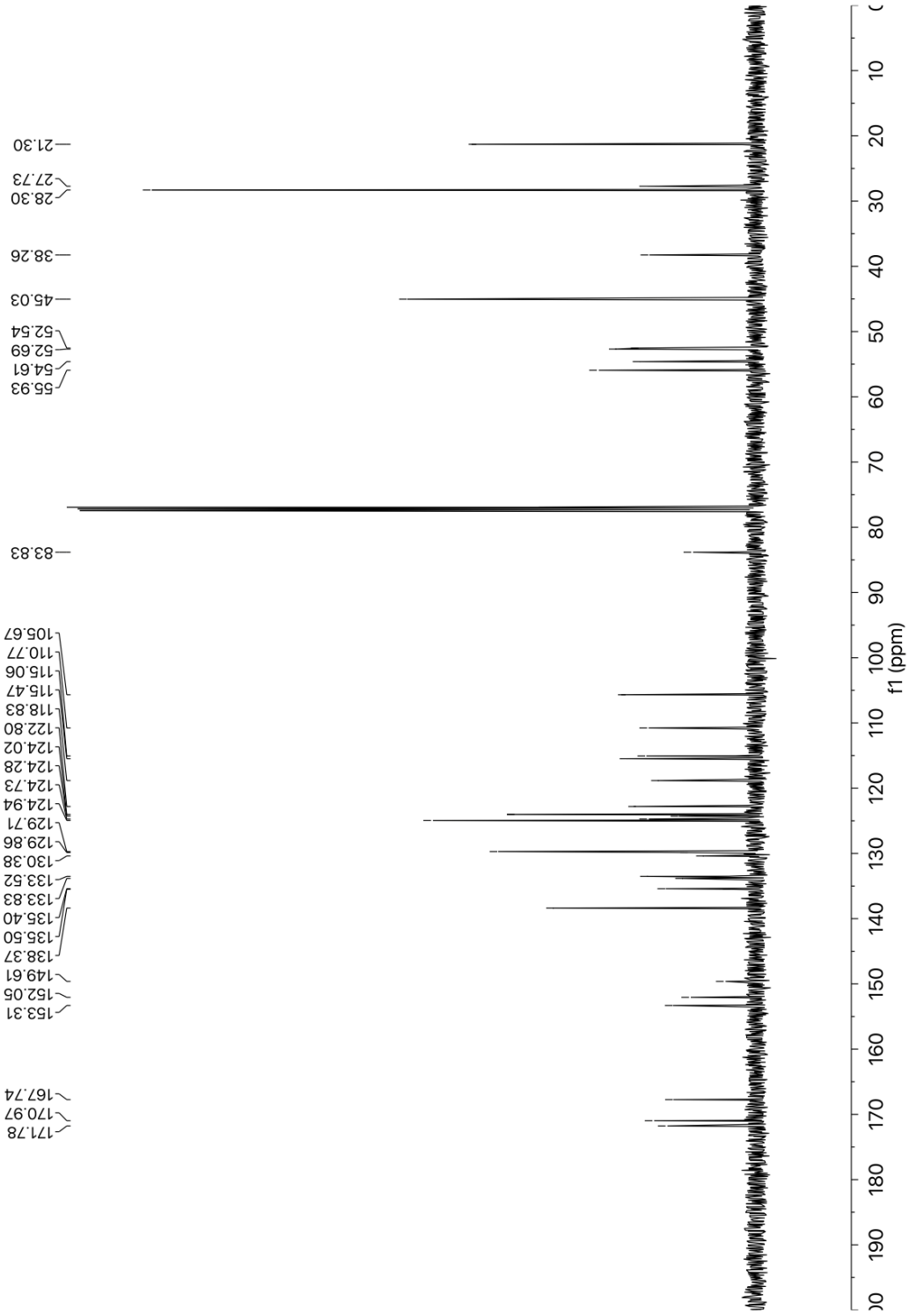
^{13}CNMR spectrum for compound **83i**



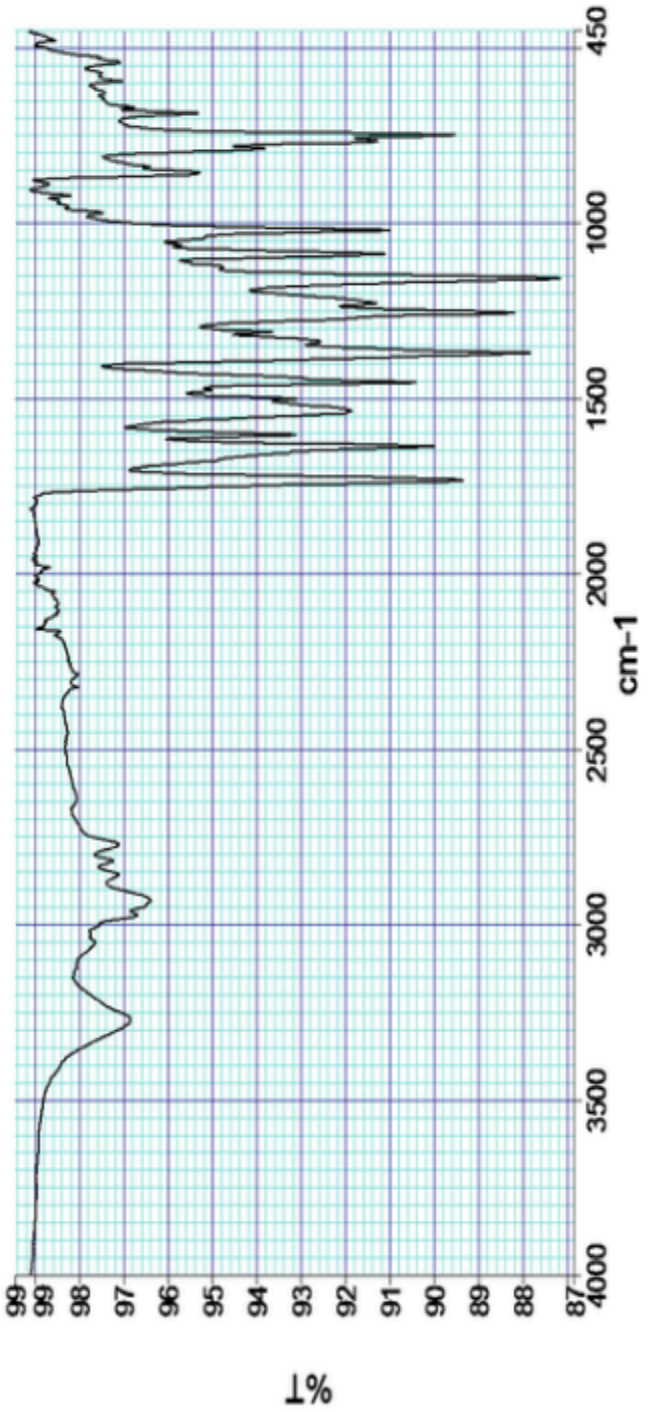
IR spectrum for compound **83i**



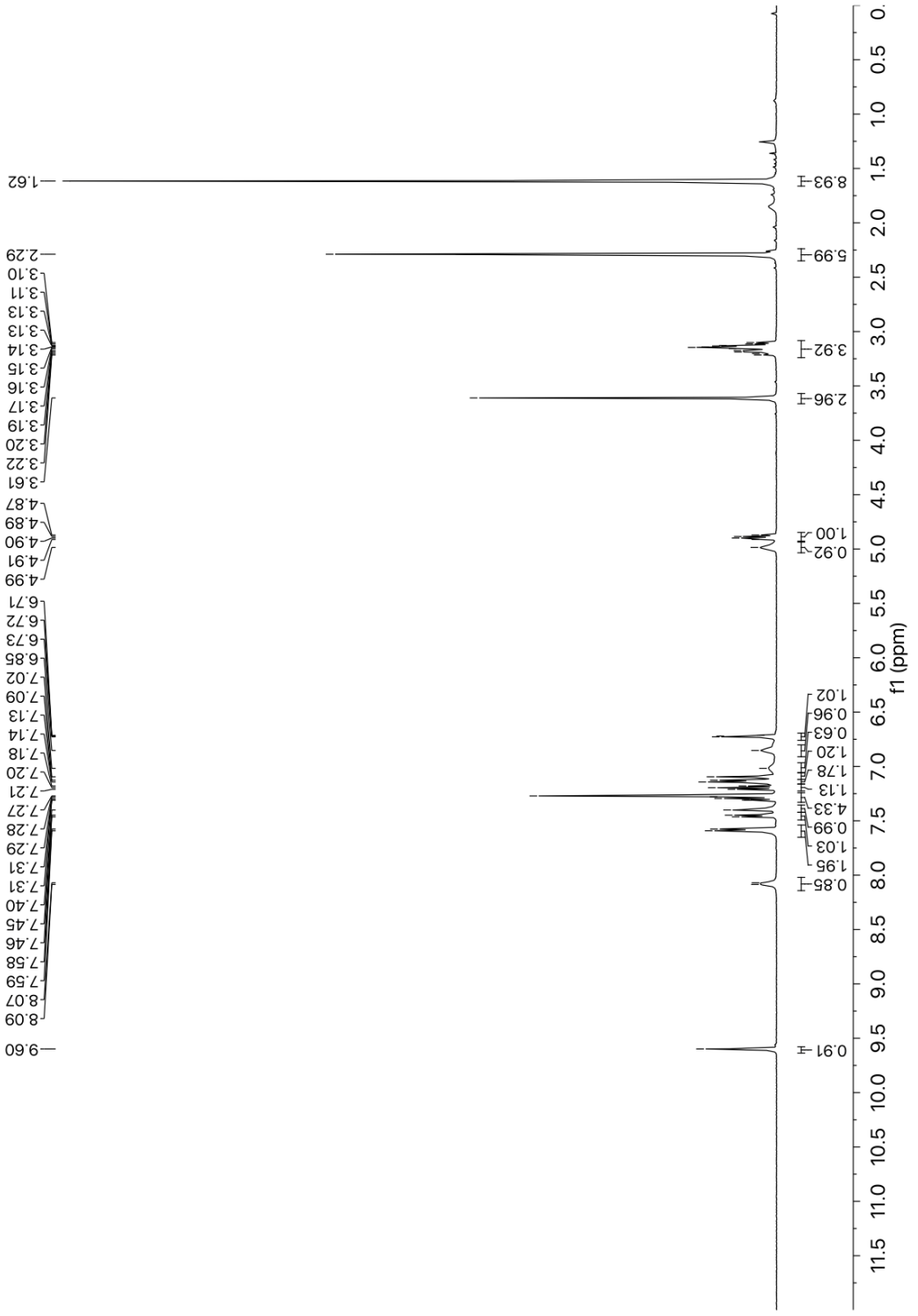
^{1}HNMR spectrum for compound **83j**



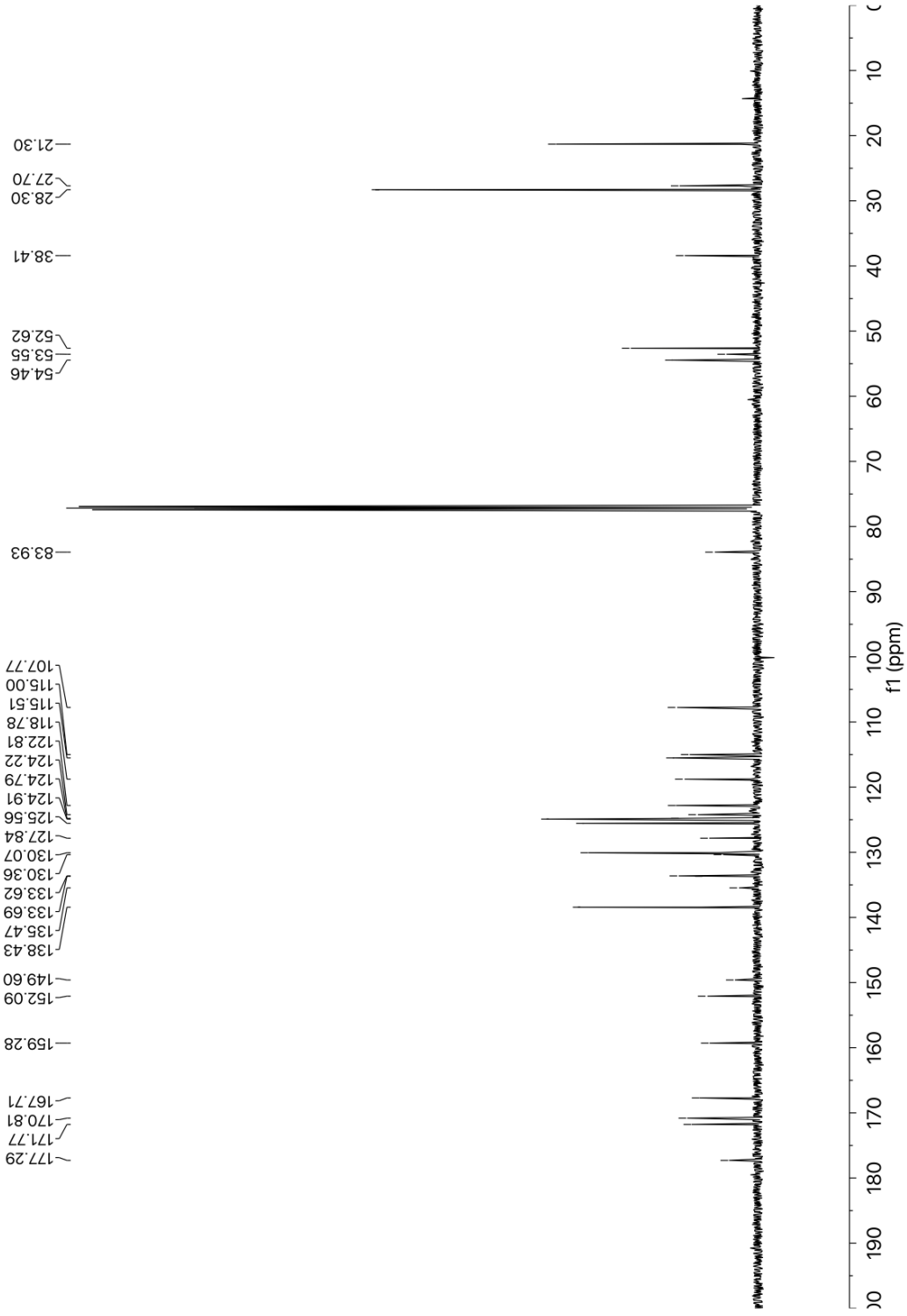
^{13}CNMR spectrum for compound **83j**



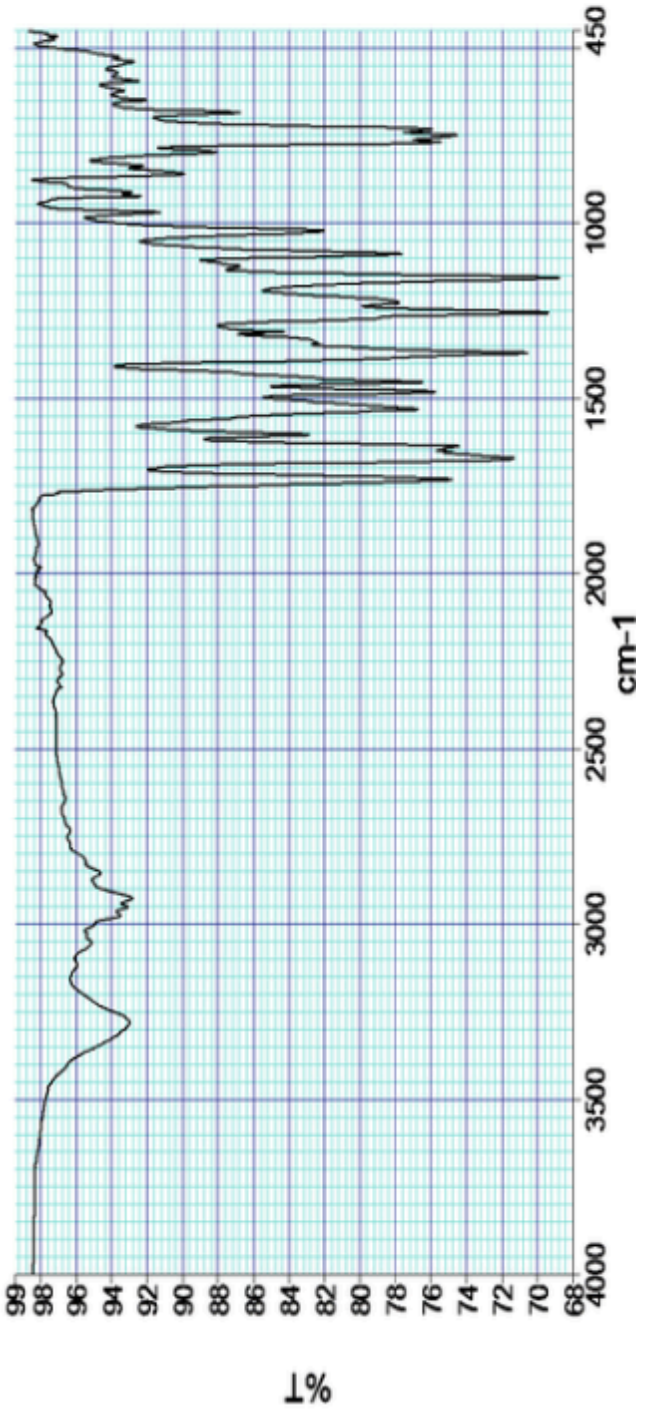
IR spectrum for compound **83j**



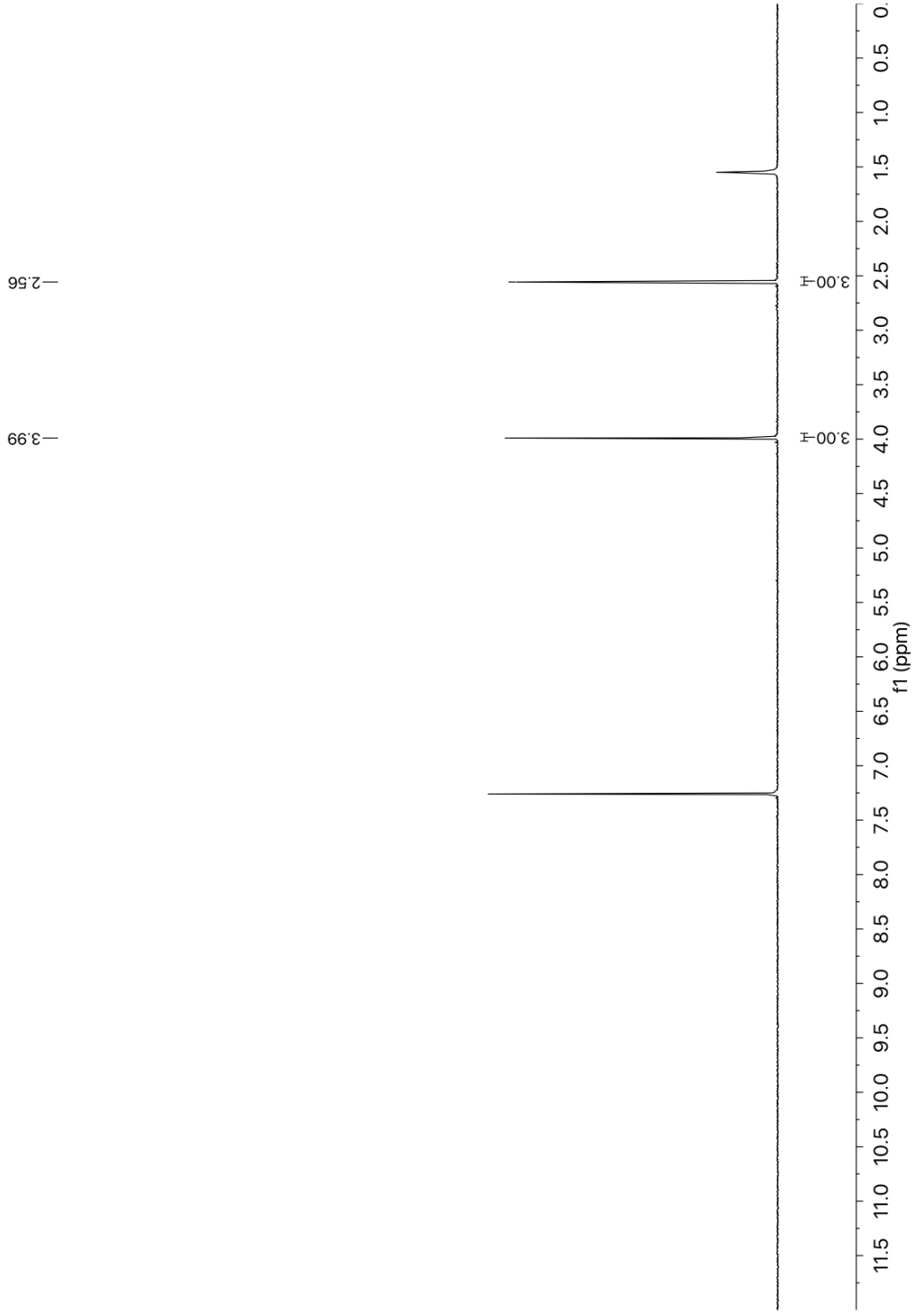
[1]HNMR spectrum for compound **83k**



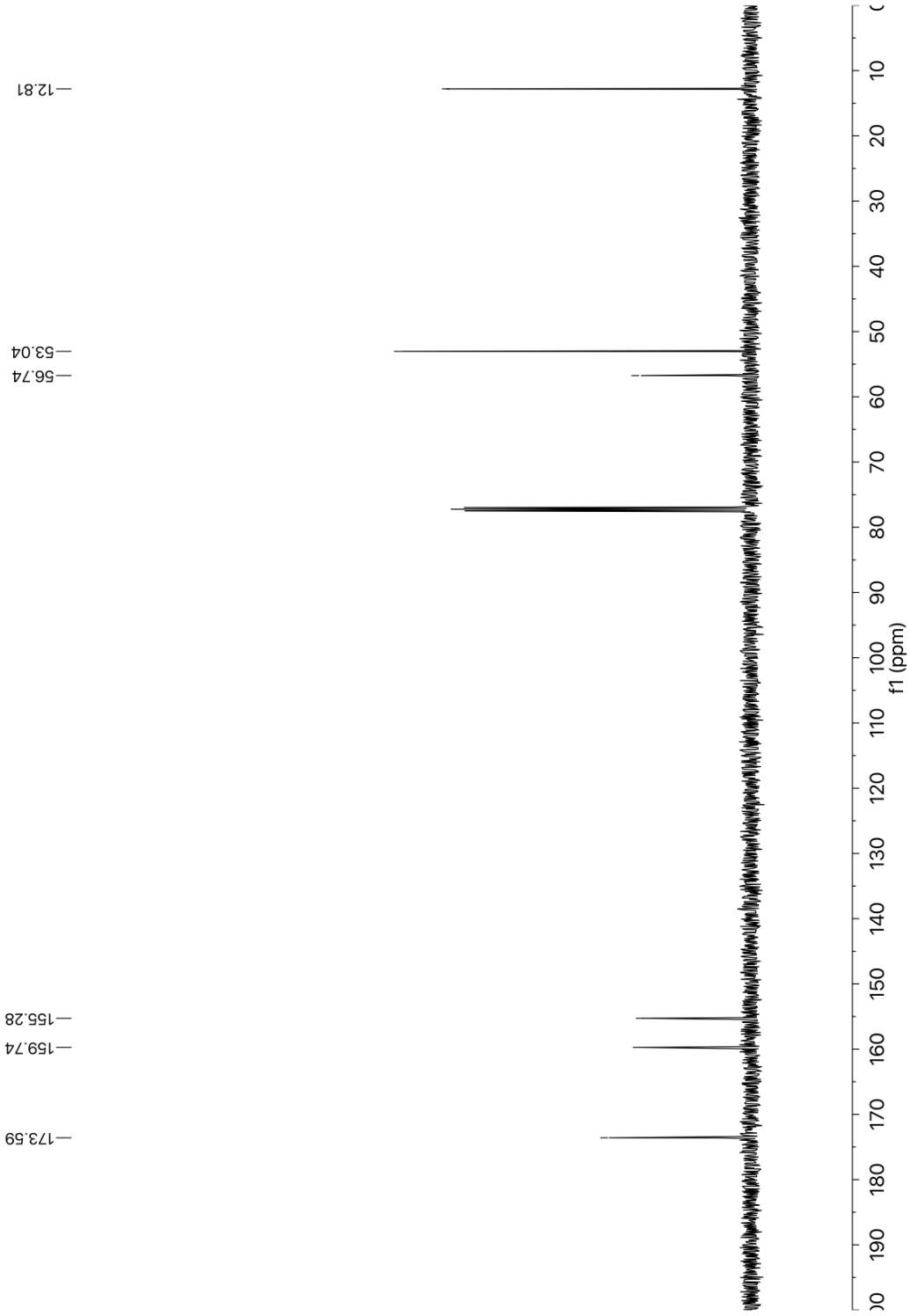
^{13}CNMR spectrum for compound **83k**



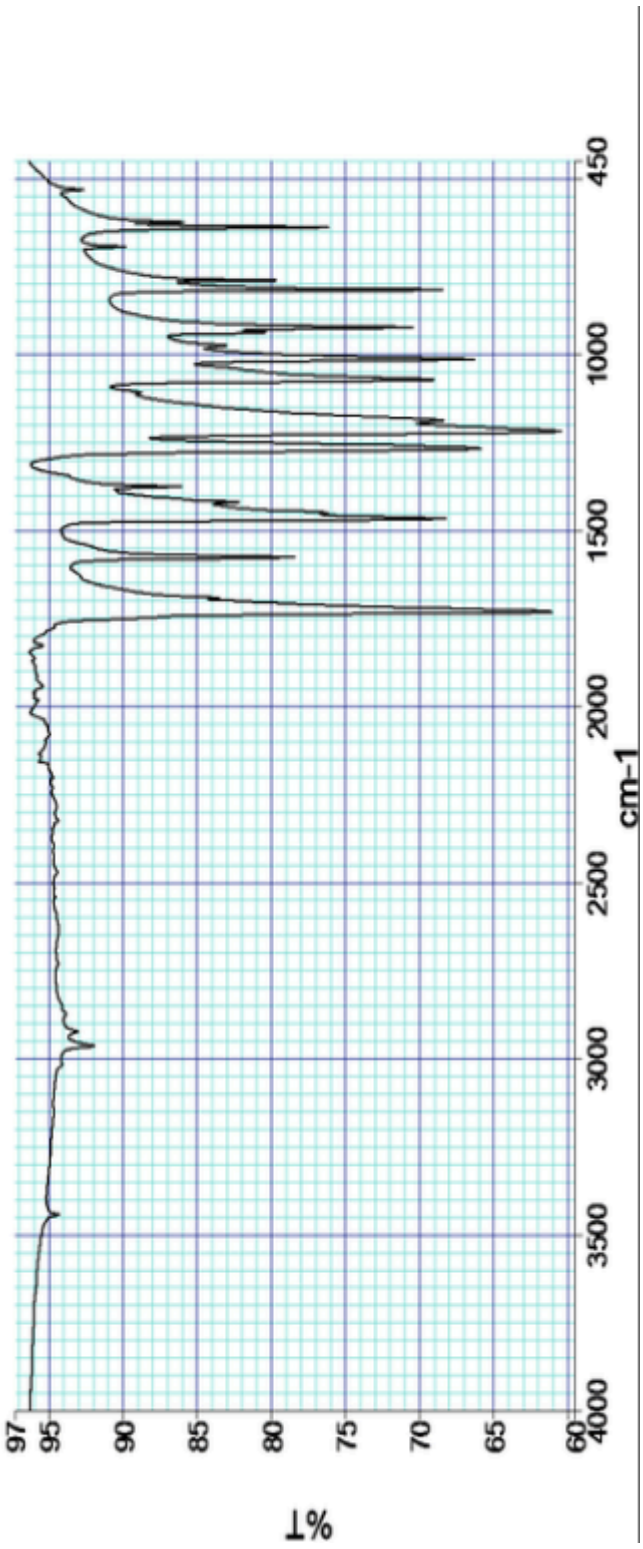
IR spectrum for compound **83k**



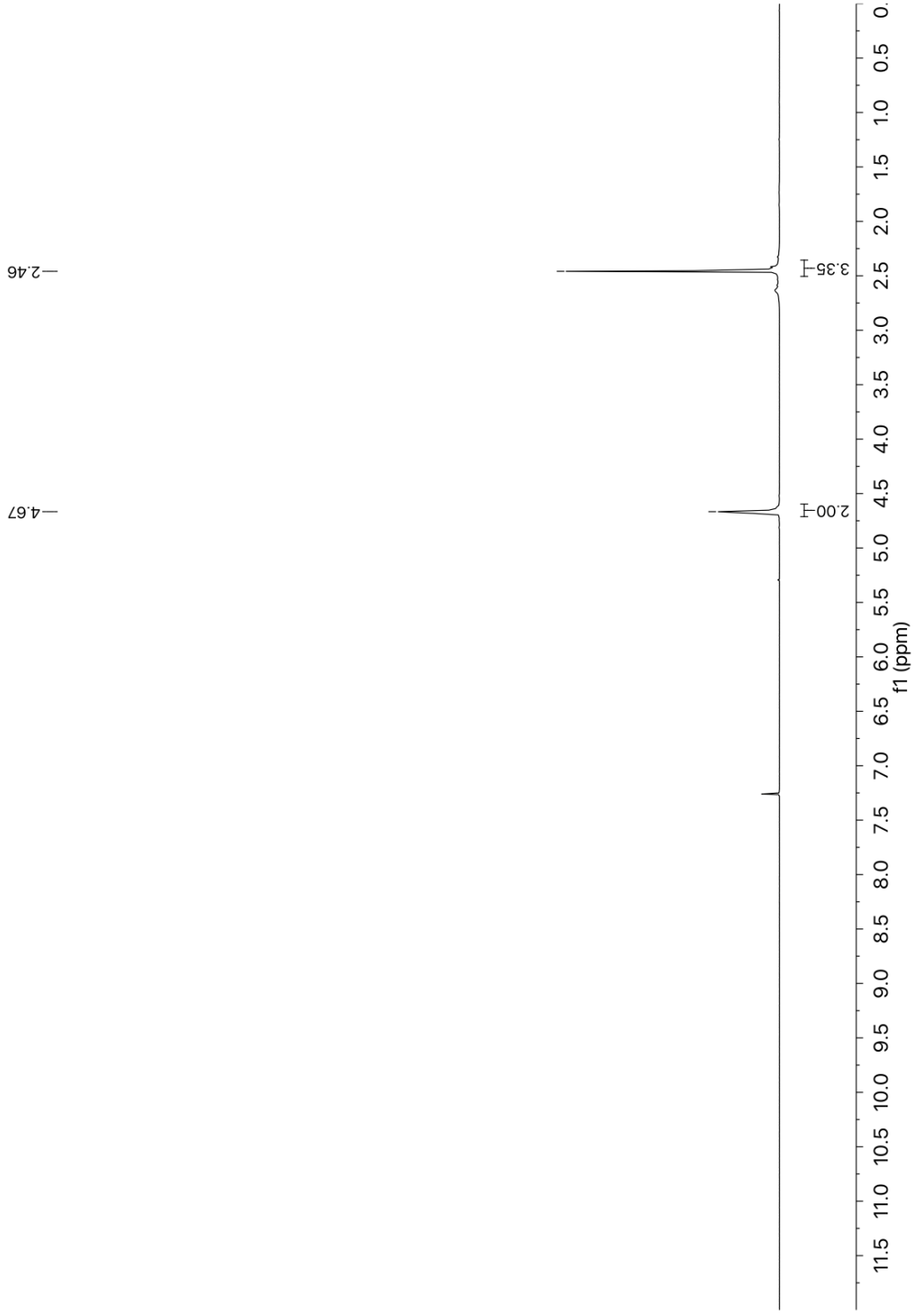
^{1}HNMR spectrum for compound **89a**



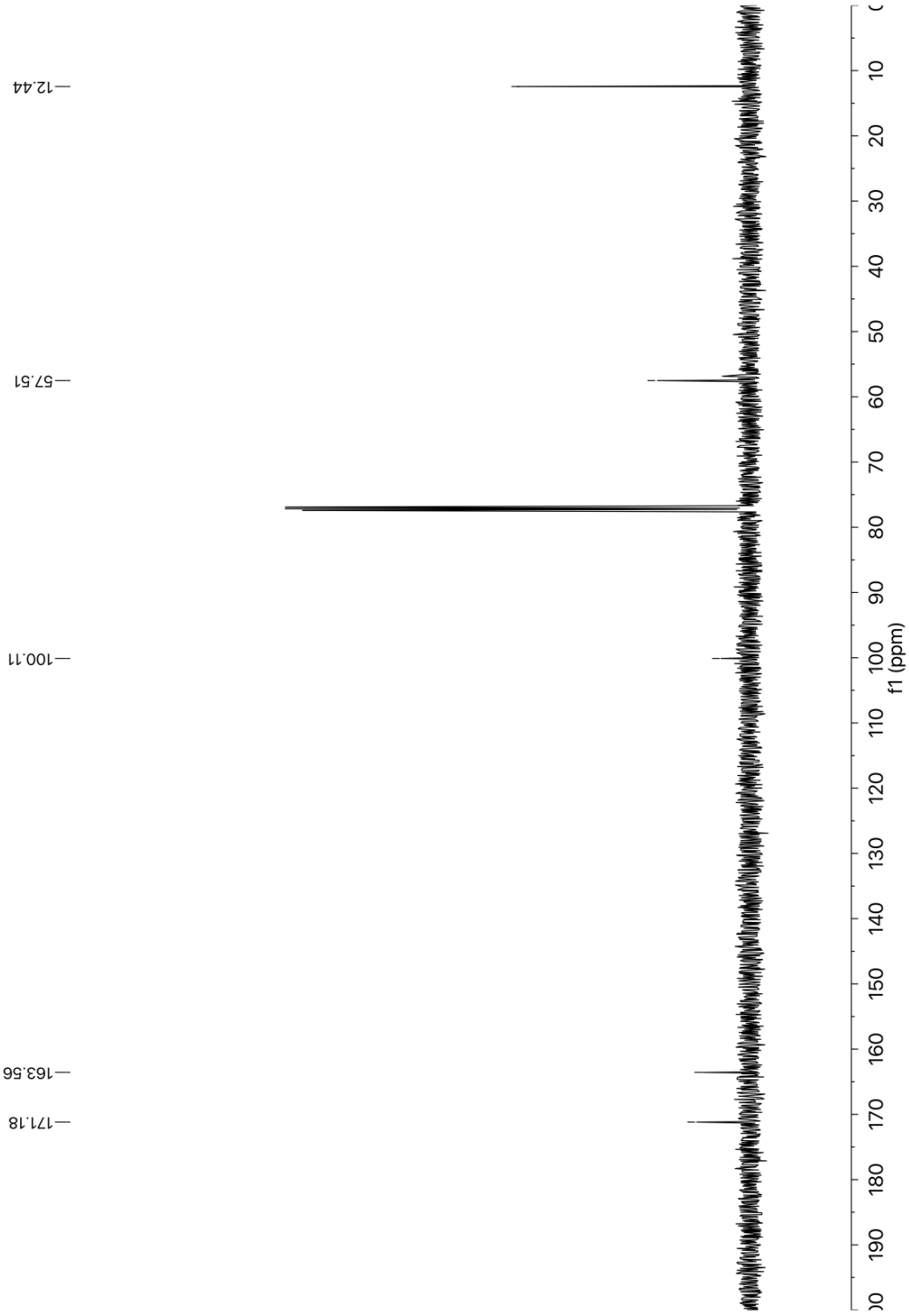
^{13}CNMR spectrum for compound **89a**



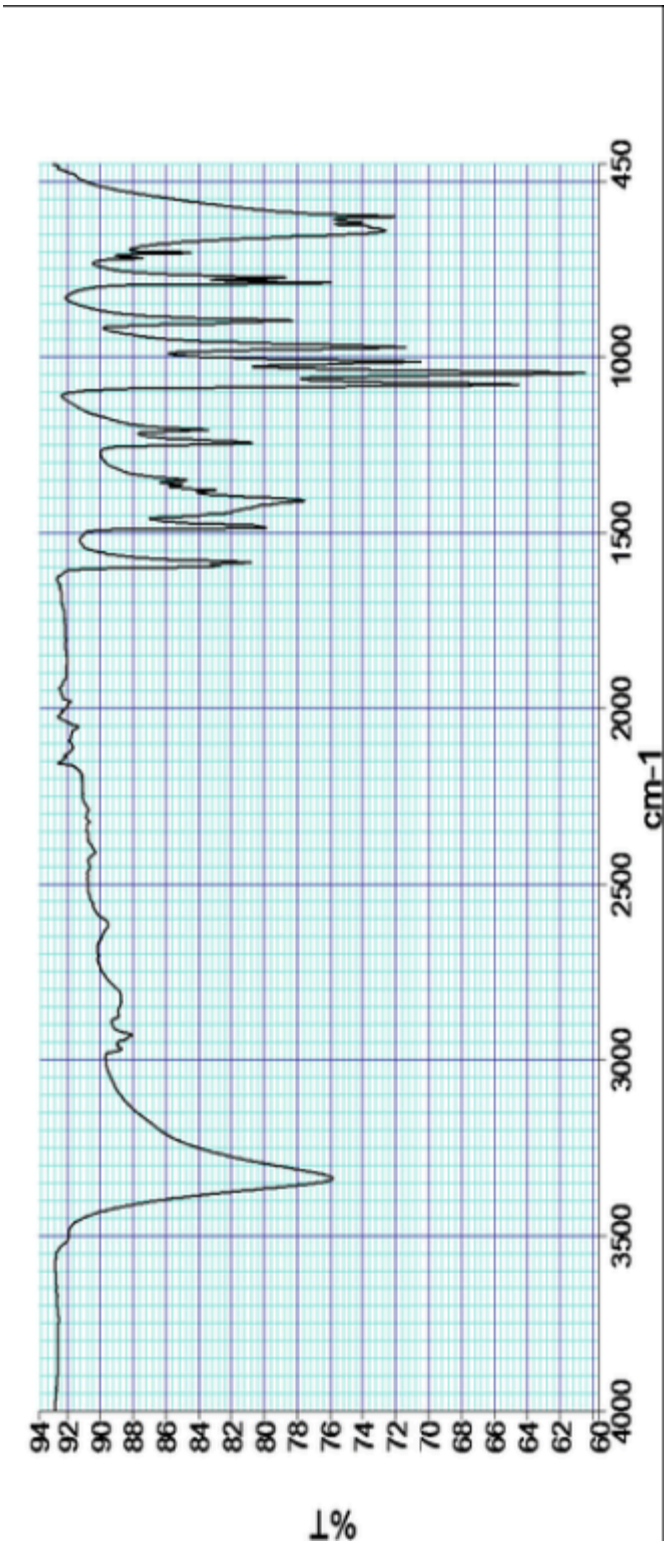
IR spectrum for compound **89a**



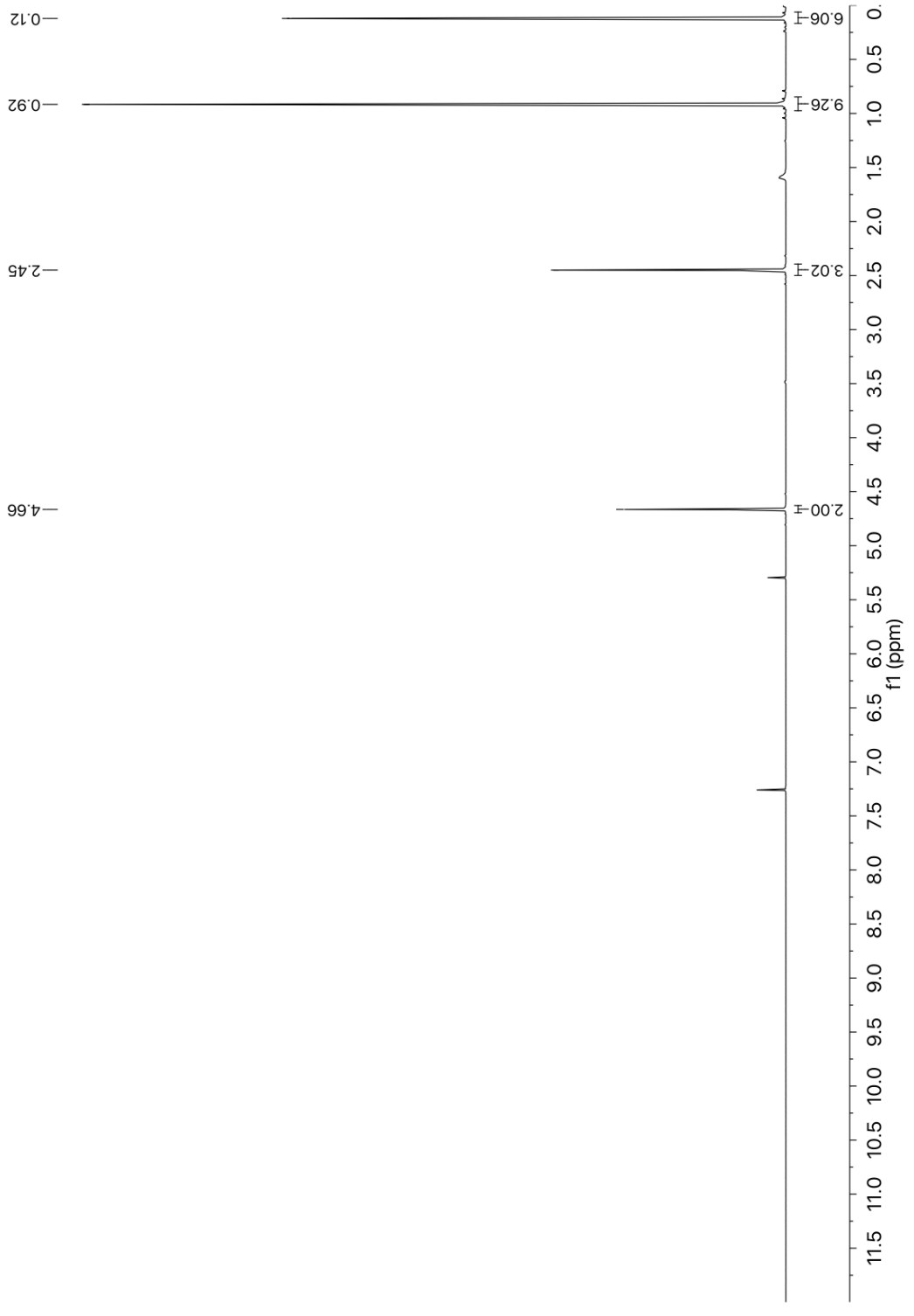
^{1}HNMR spectrum for compound **89b**



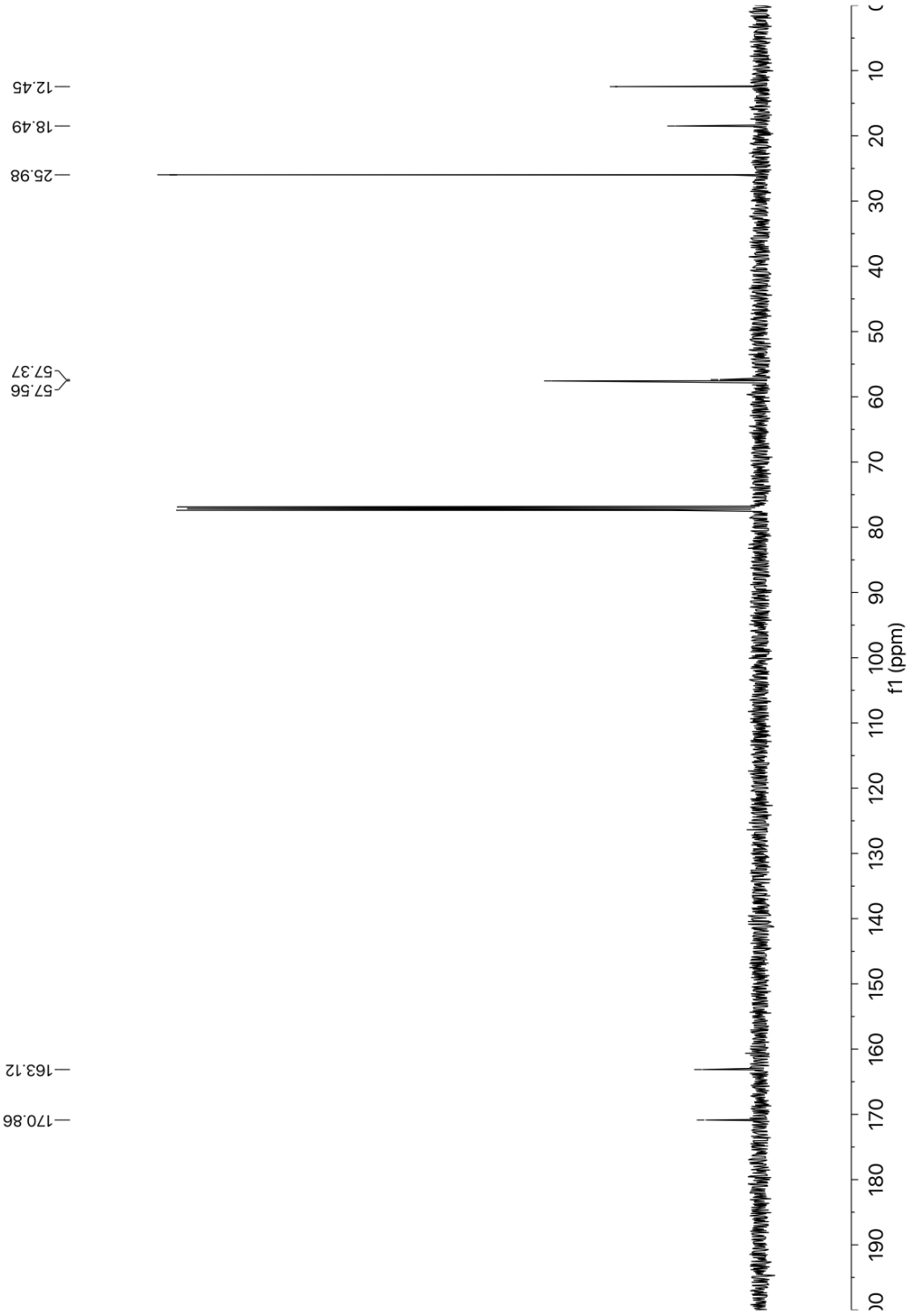
^{13}CNMR spectrum for compound **89b**



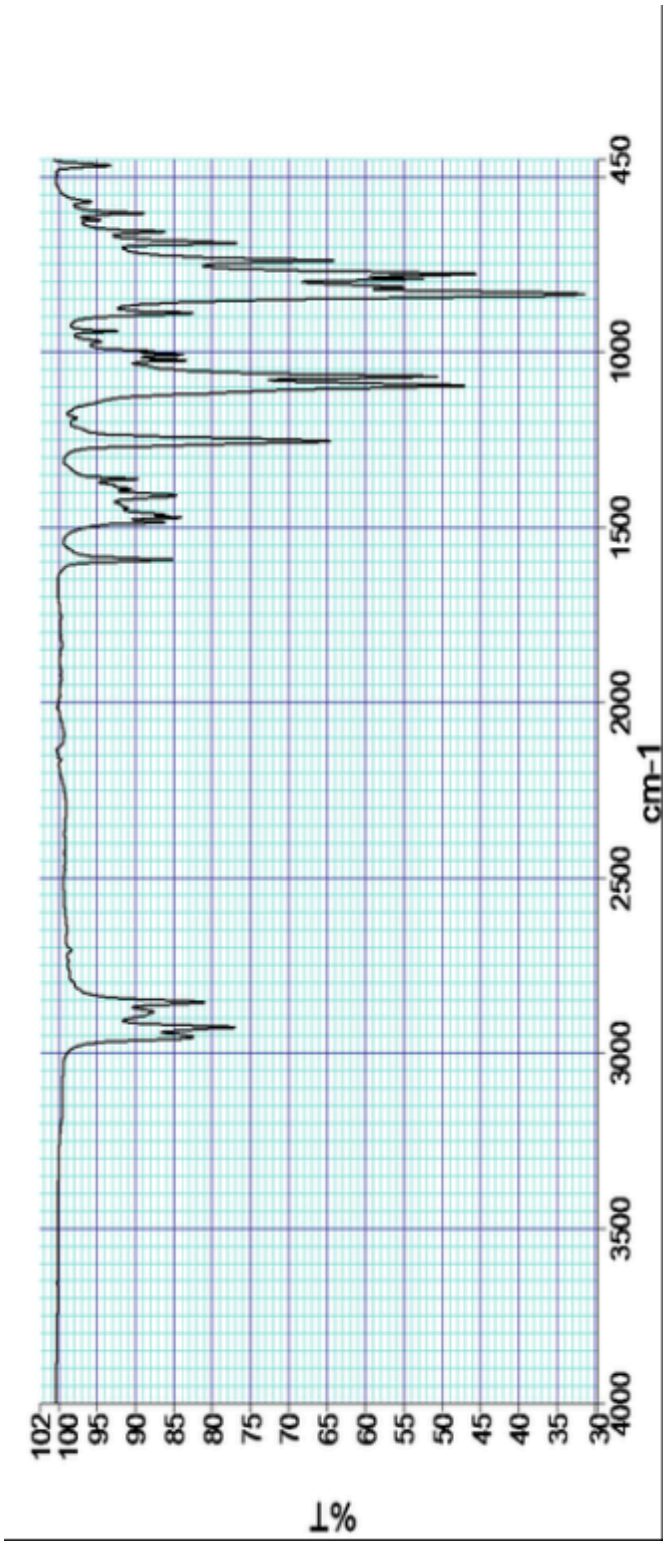
IR spectrum for compound **89b**



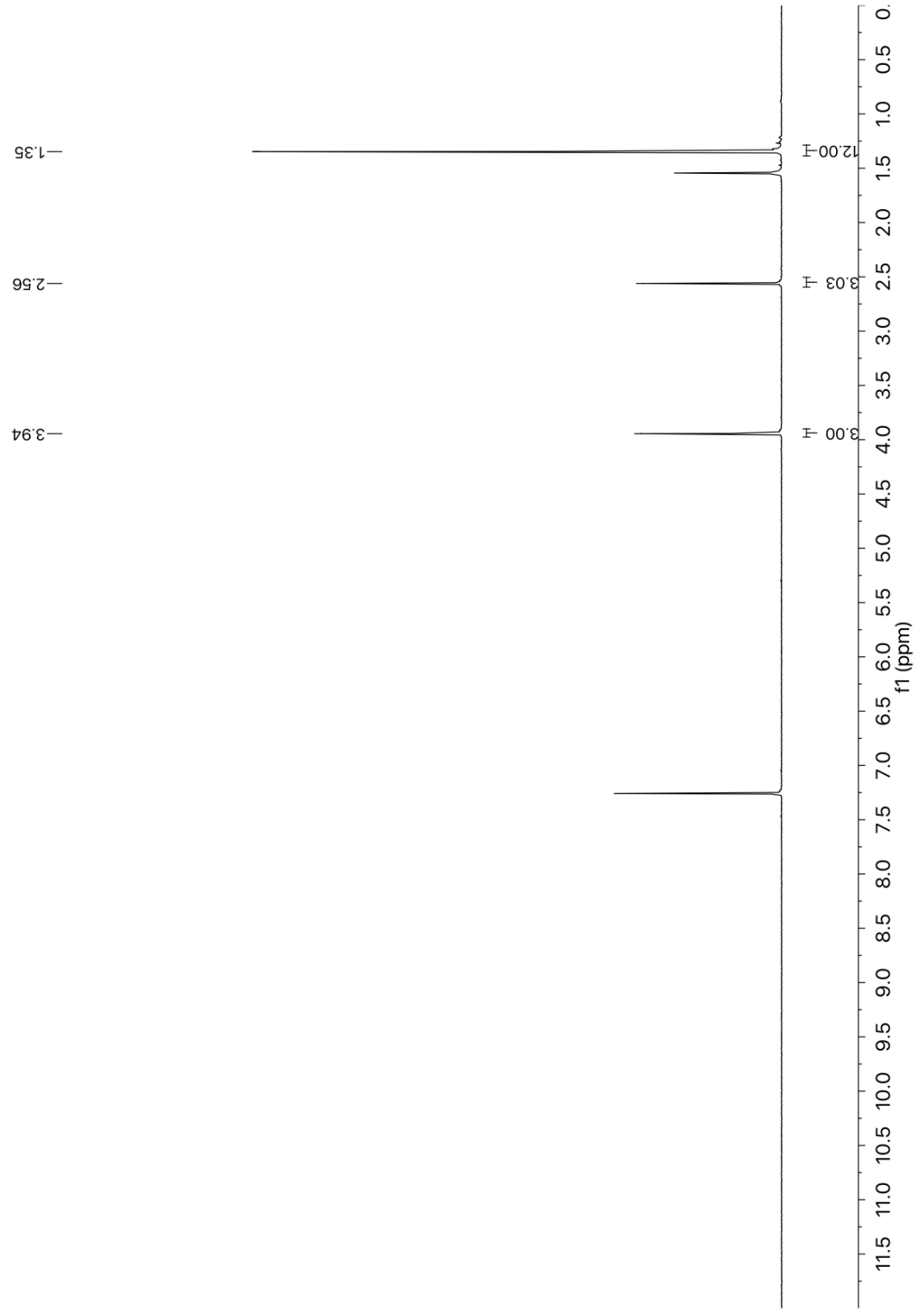
[1]HNMR spectrum for compound **89c**



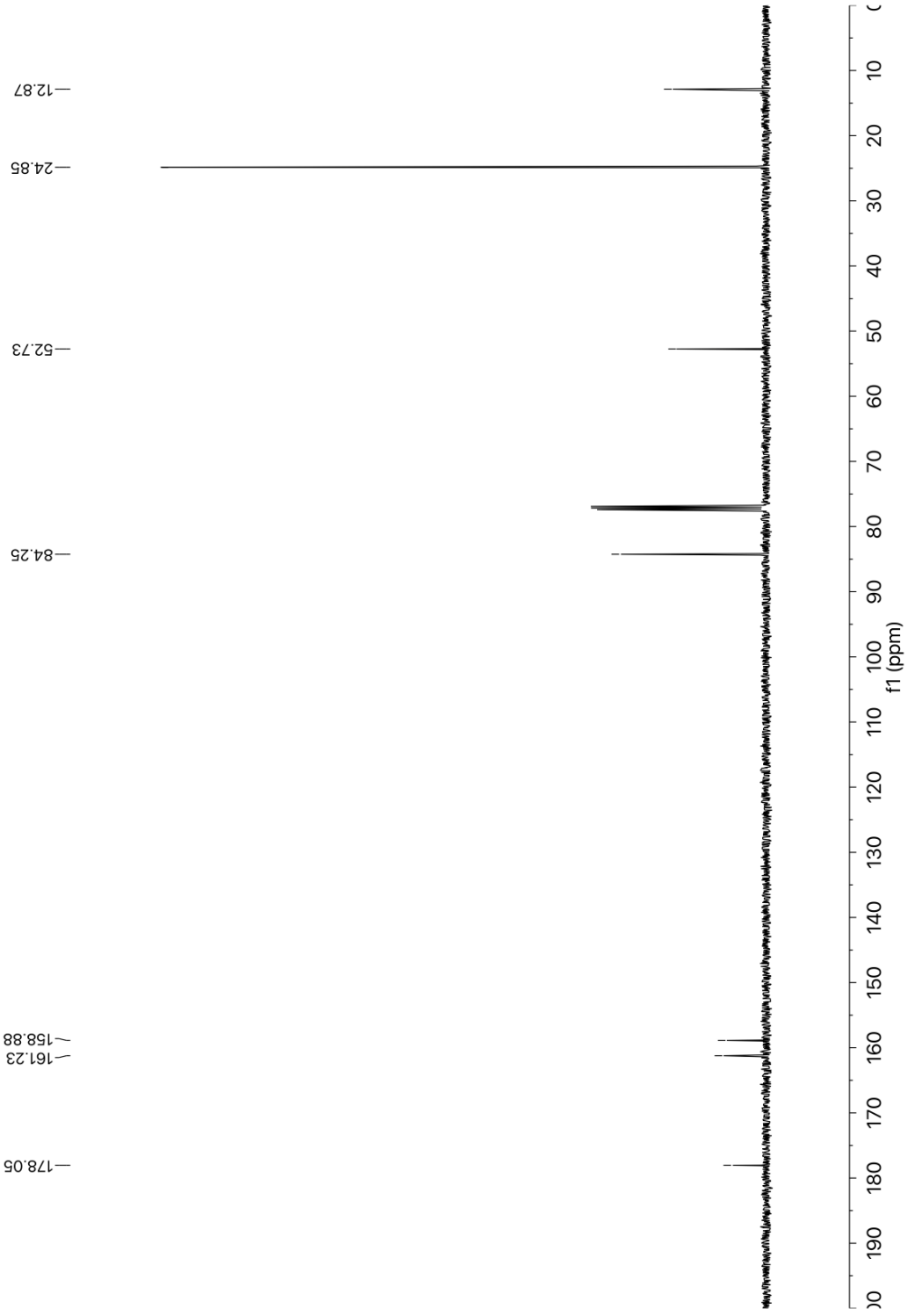
^{13}CNMR spectrum for compound **89c**



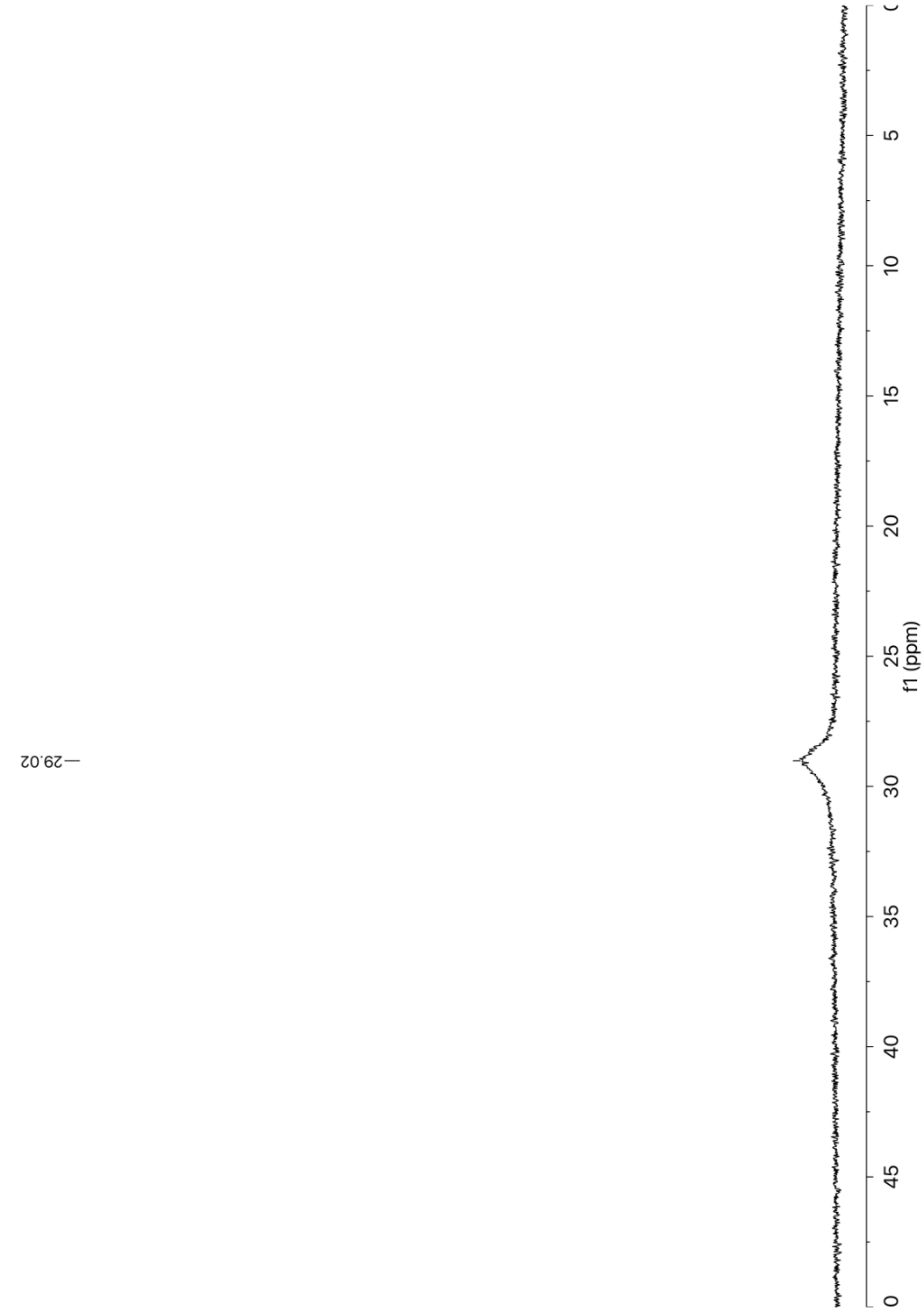
IR spectrum for compound **89c**



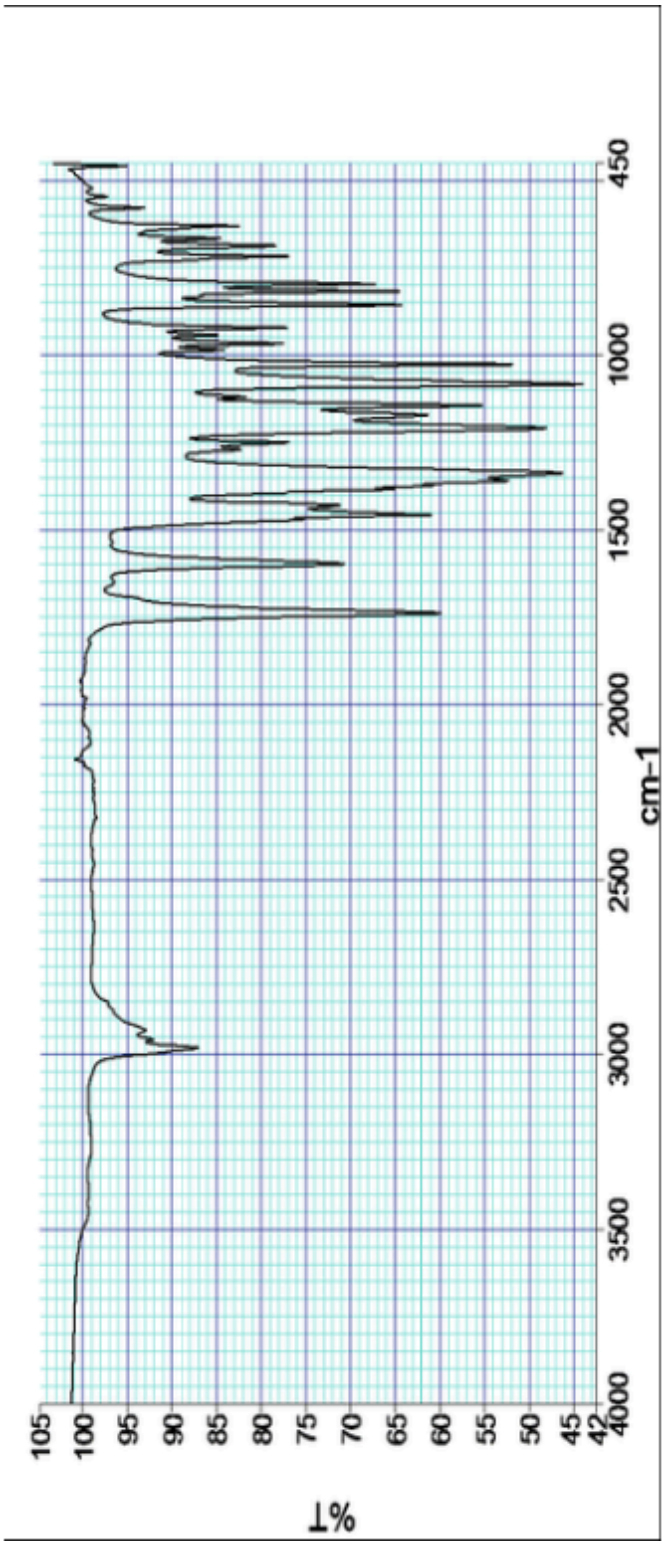
^{1}HNMR spectrum for compound **90a**



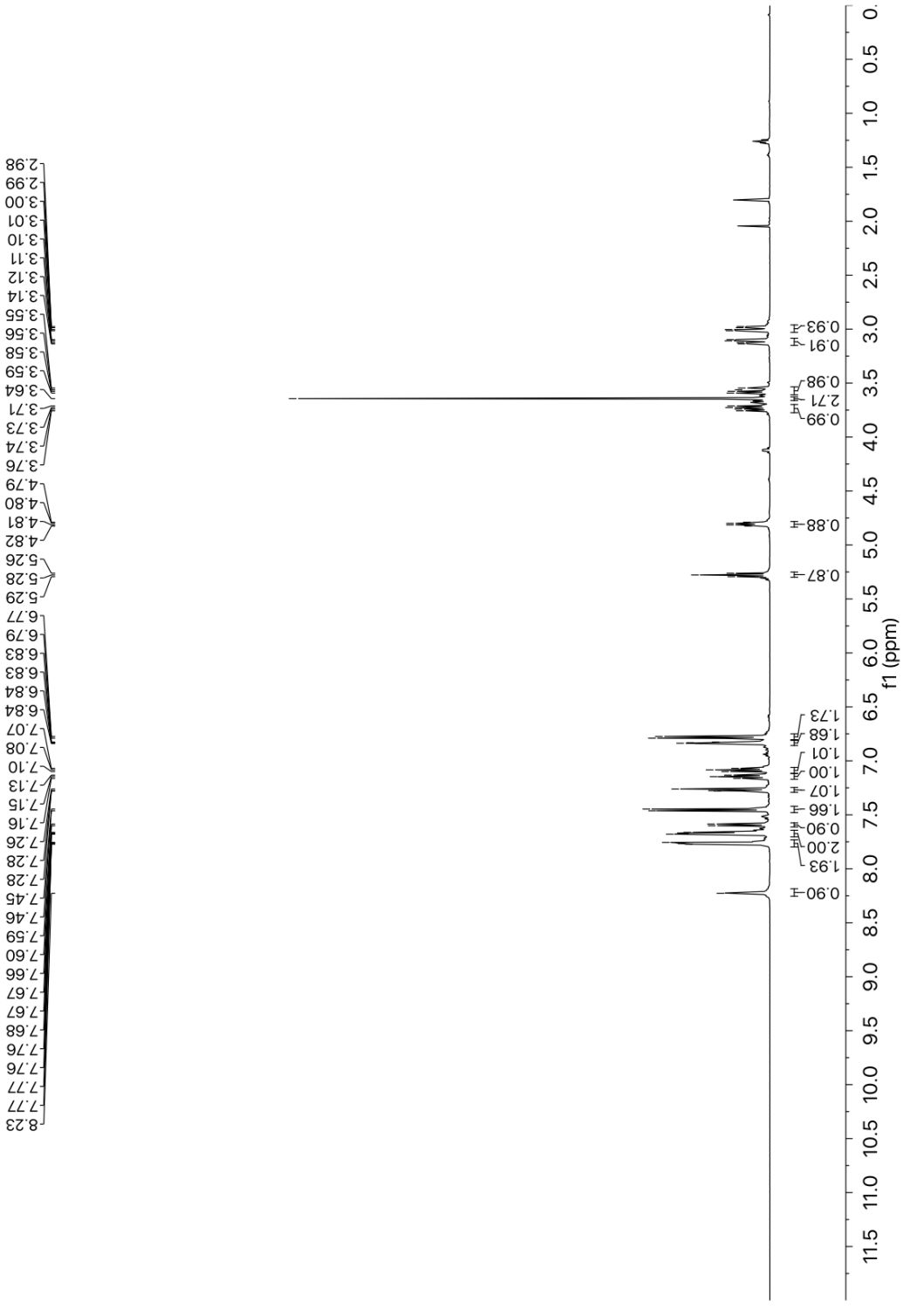
^{13}CNMR spectrum for compound **90a**



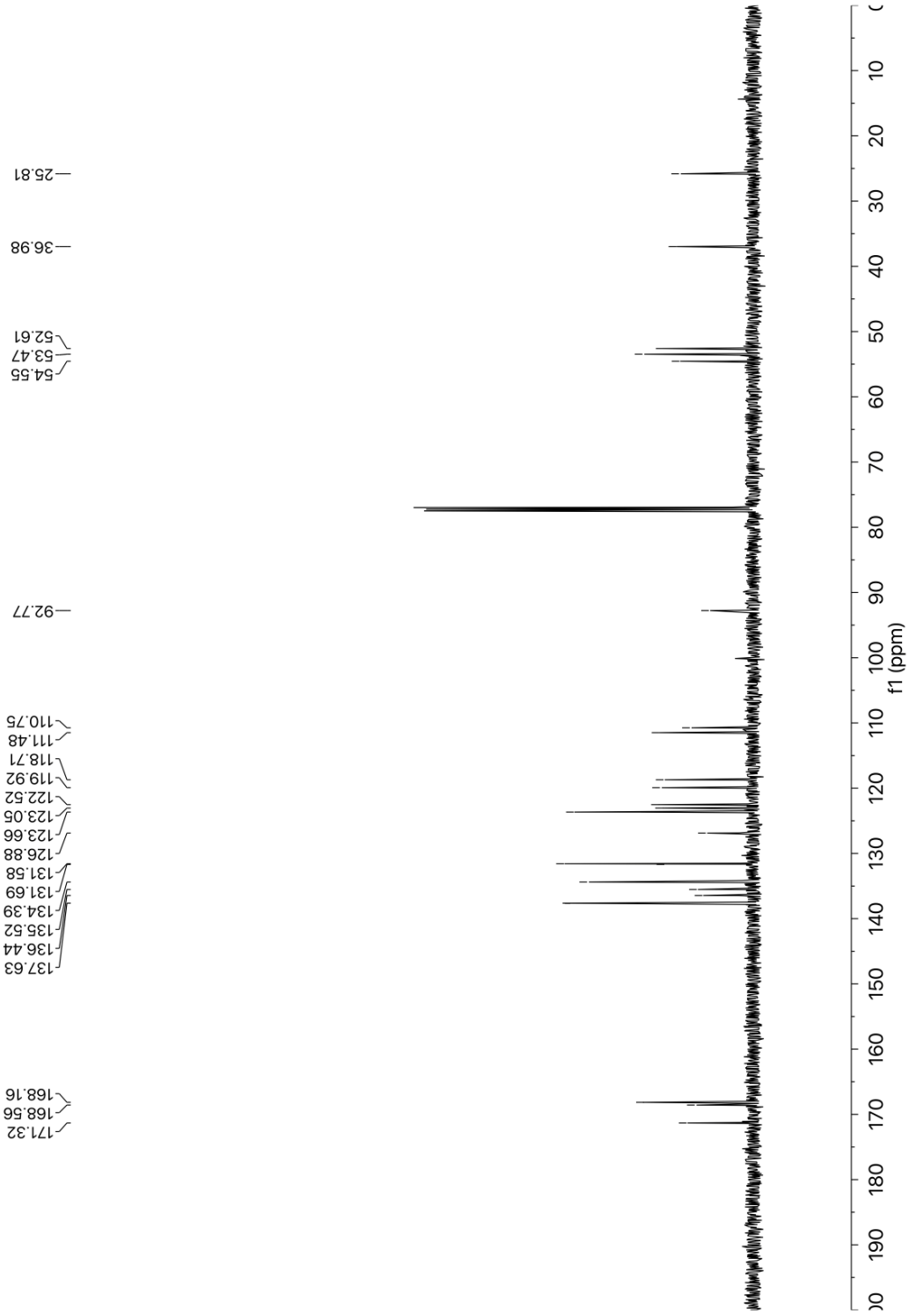
^{11}BNMR spectrum for compound **90a**



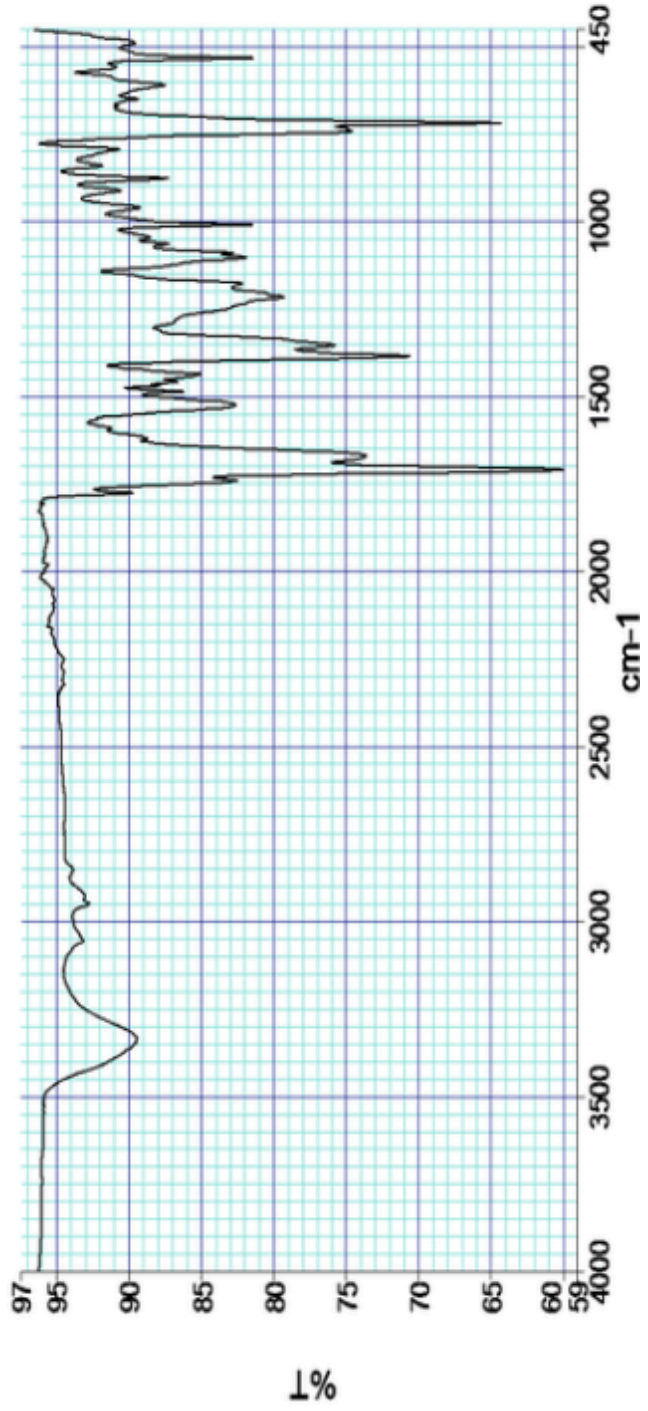
IR spectrum for compound **90a**



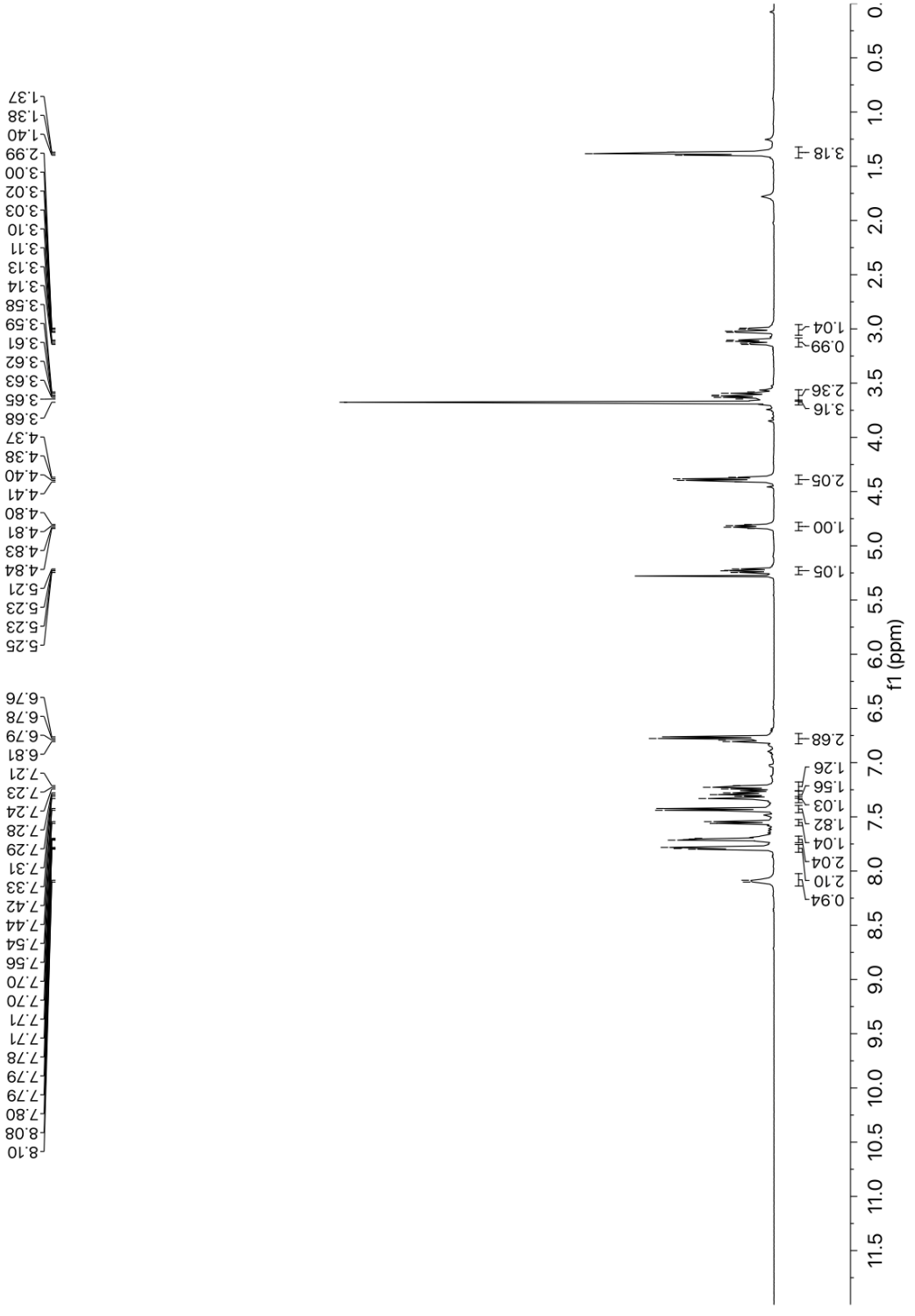
^{1}HNMR spectrum for compound **103**



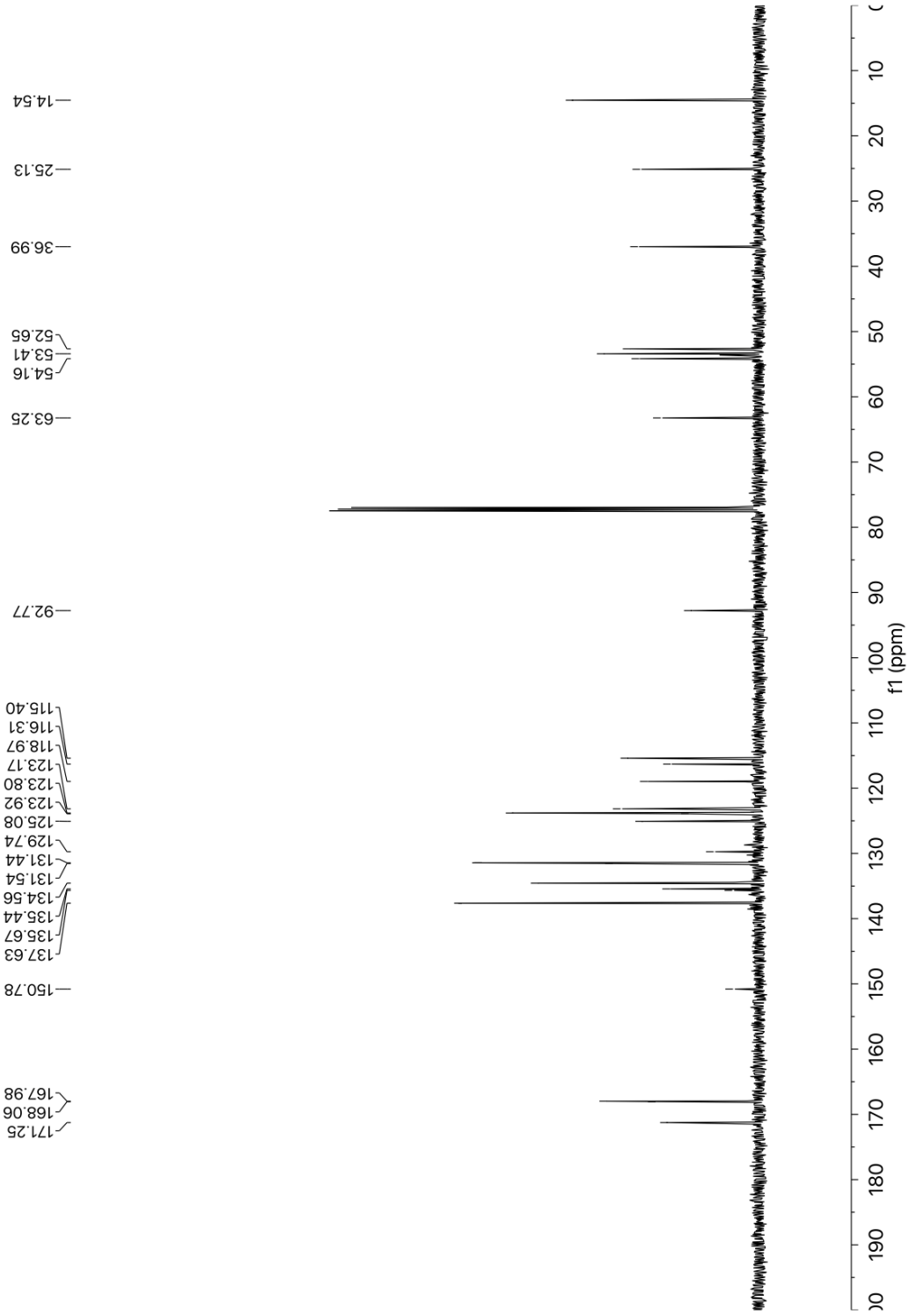
^{13}CNMR spectrum for compound **103**



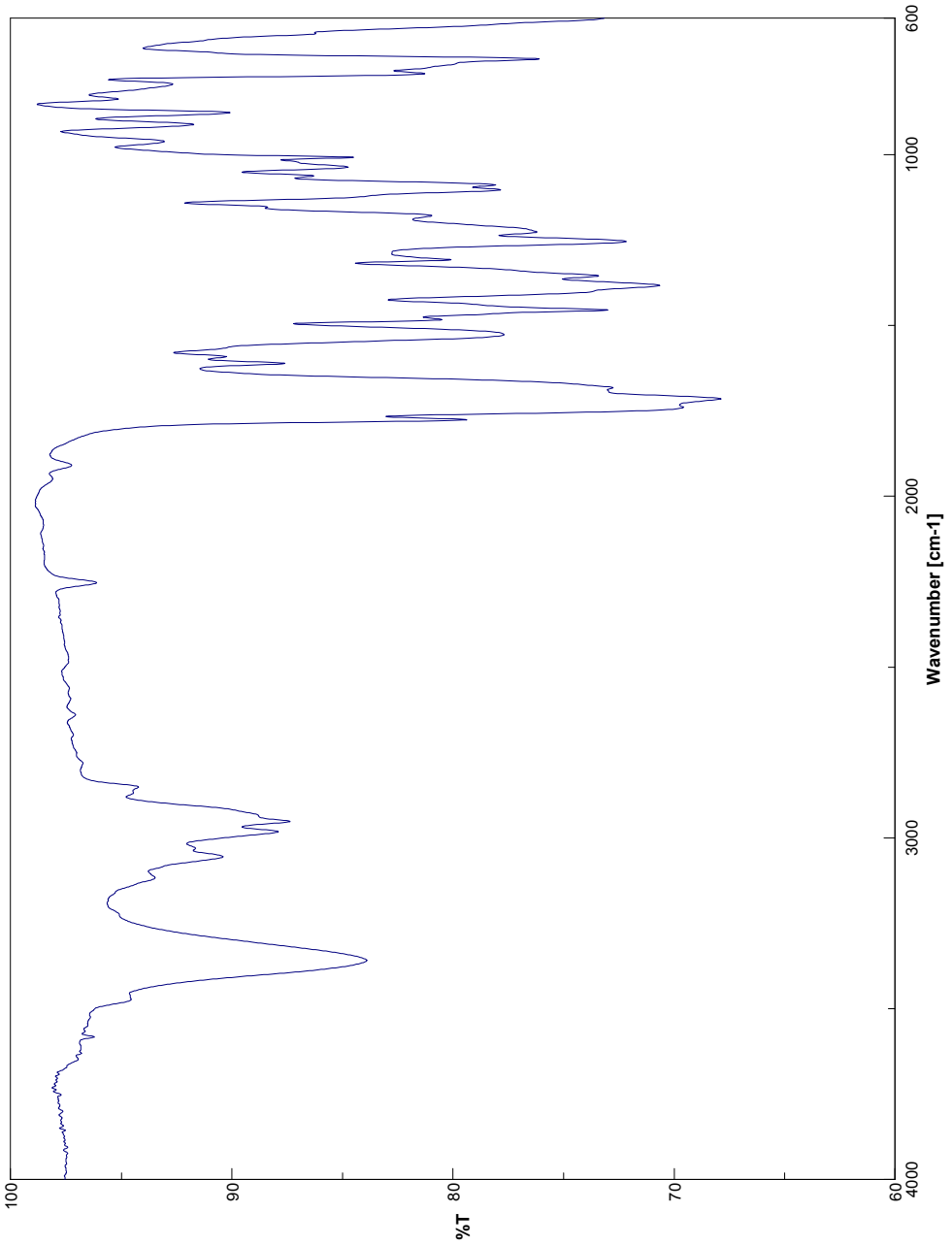
IR spectrum for compound **103**



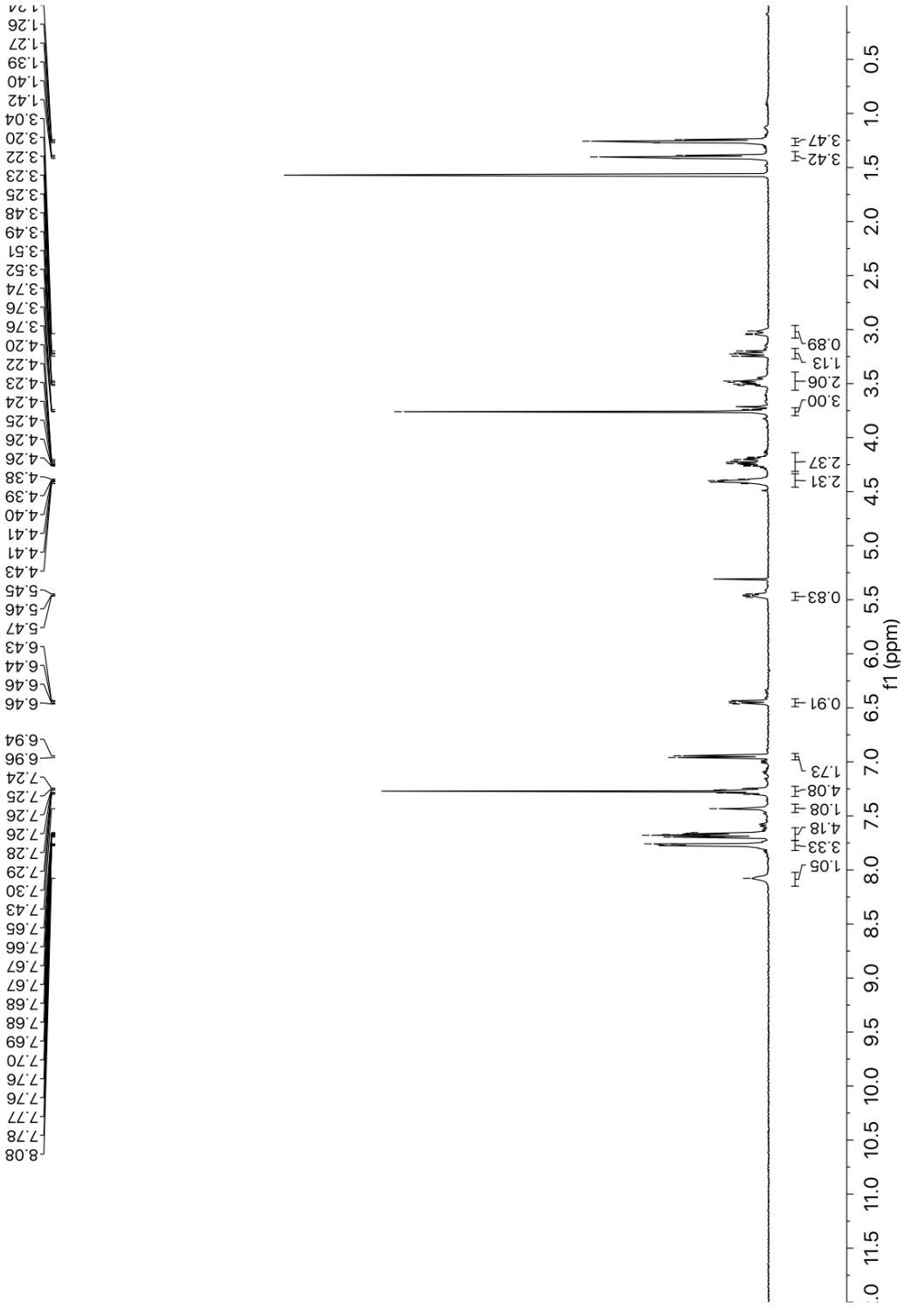
^{1}HNMR spectrum for compound **104**



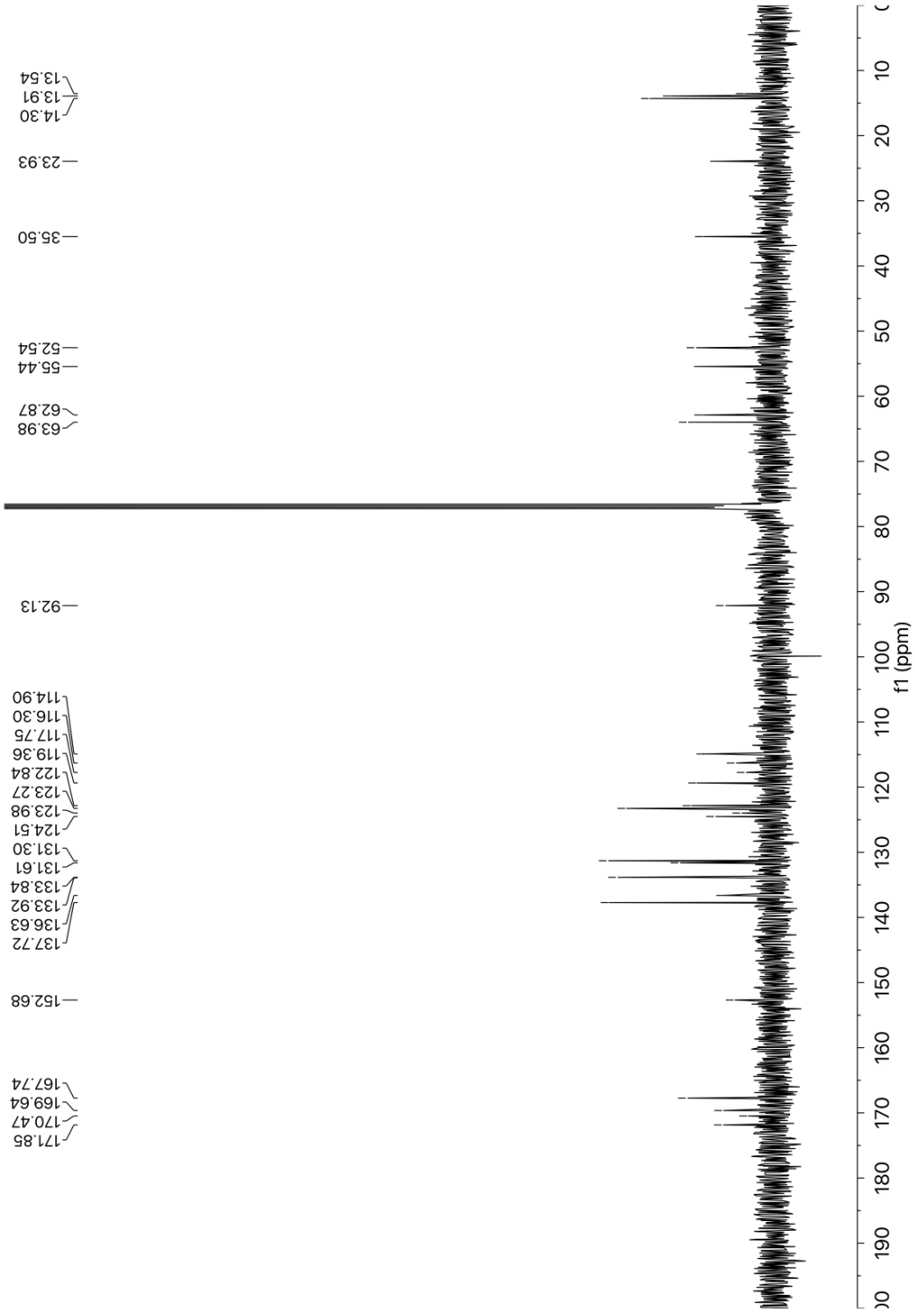
^{13}CNMR spectrum for compound **104**



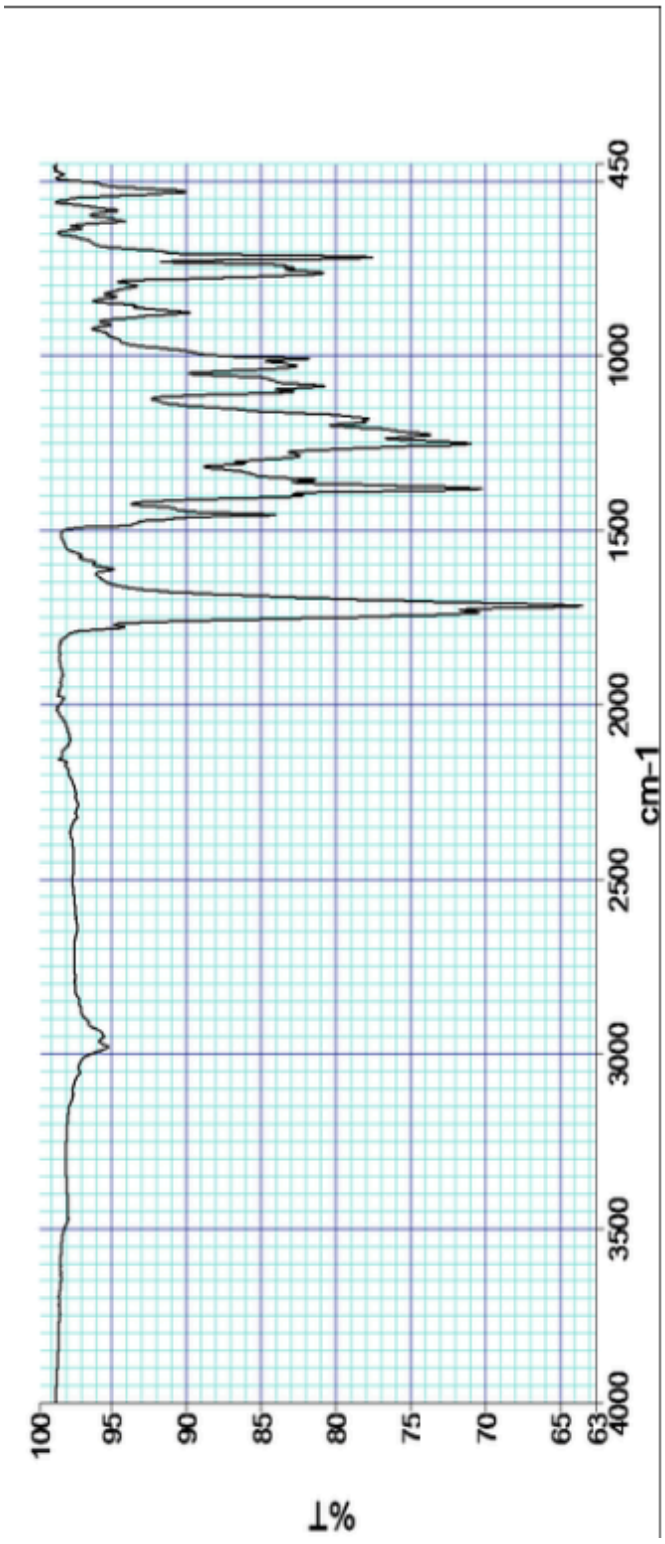
IR spectrum for compound **104**



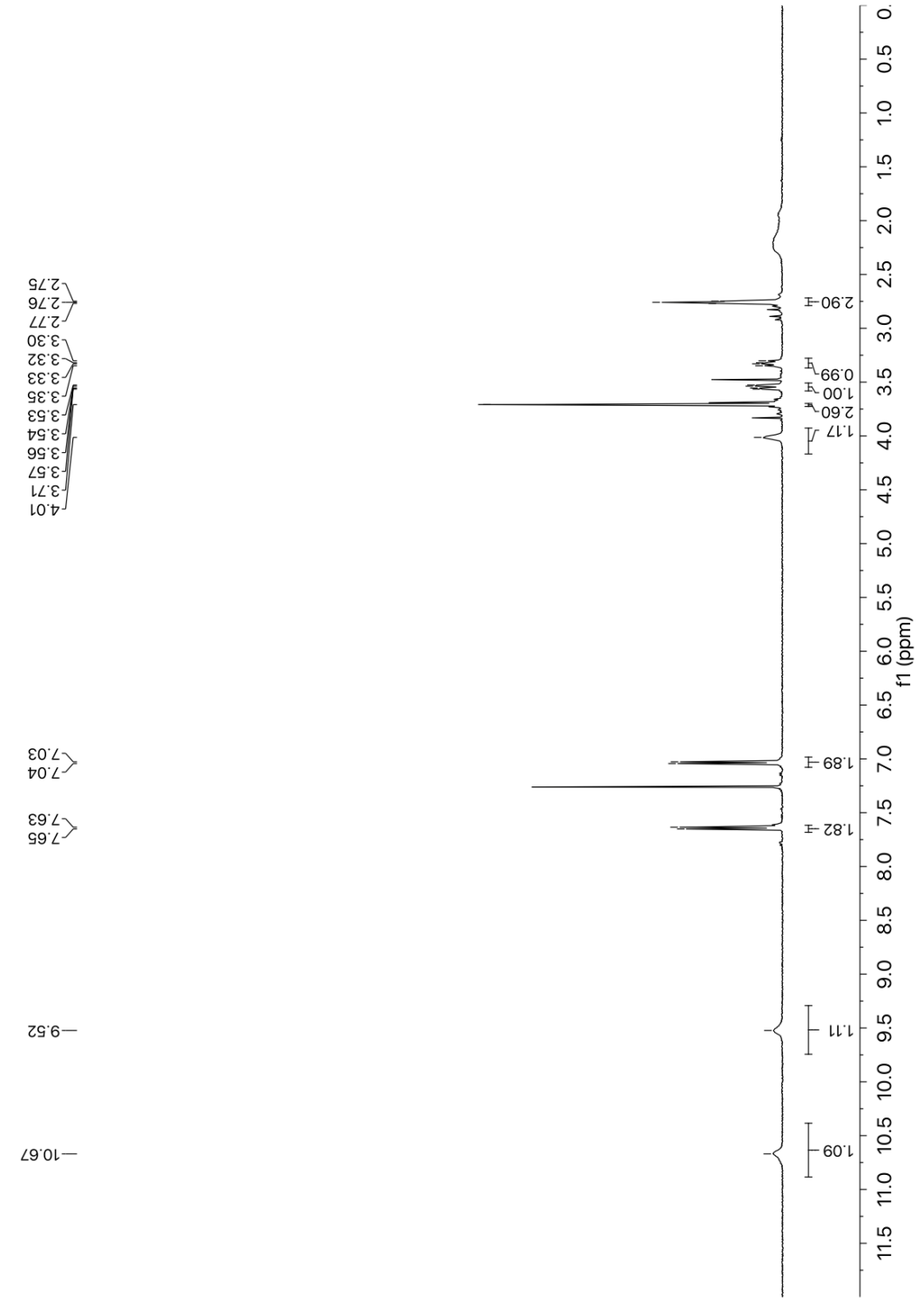
^{1}HNMR spectrum for compound **105**



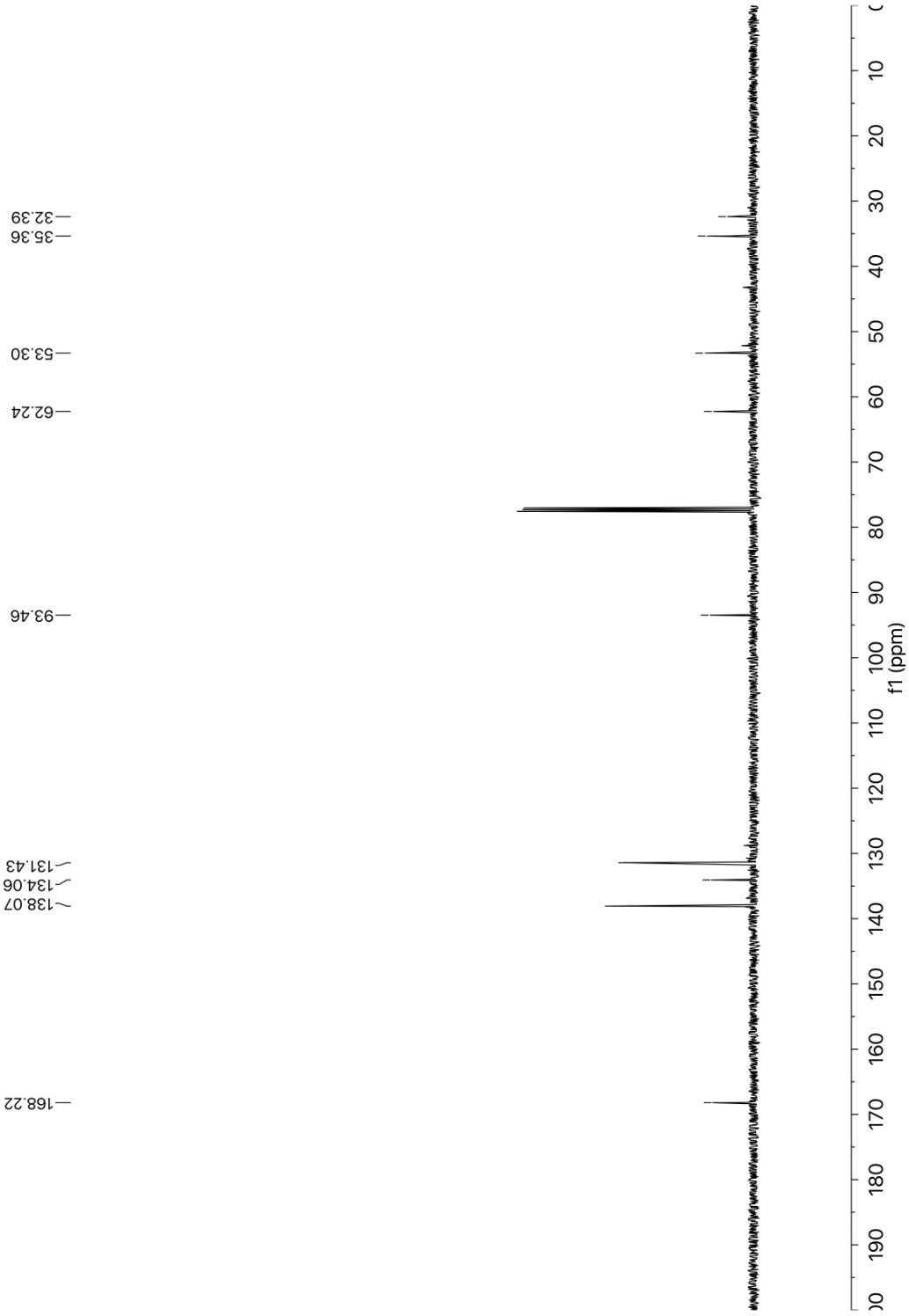
^{13}CNMR spectrum for compound **105**



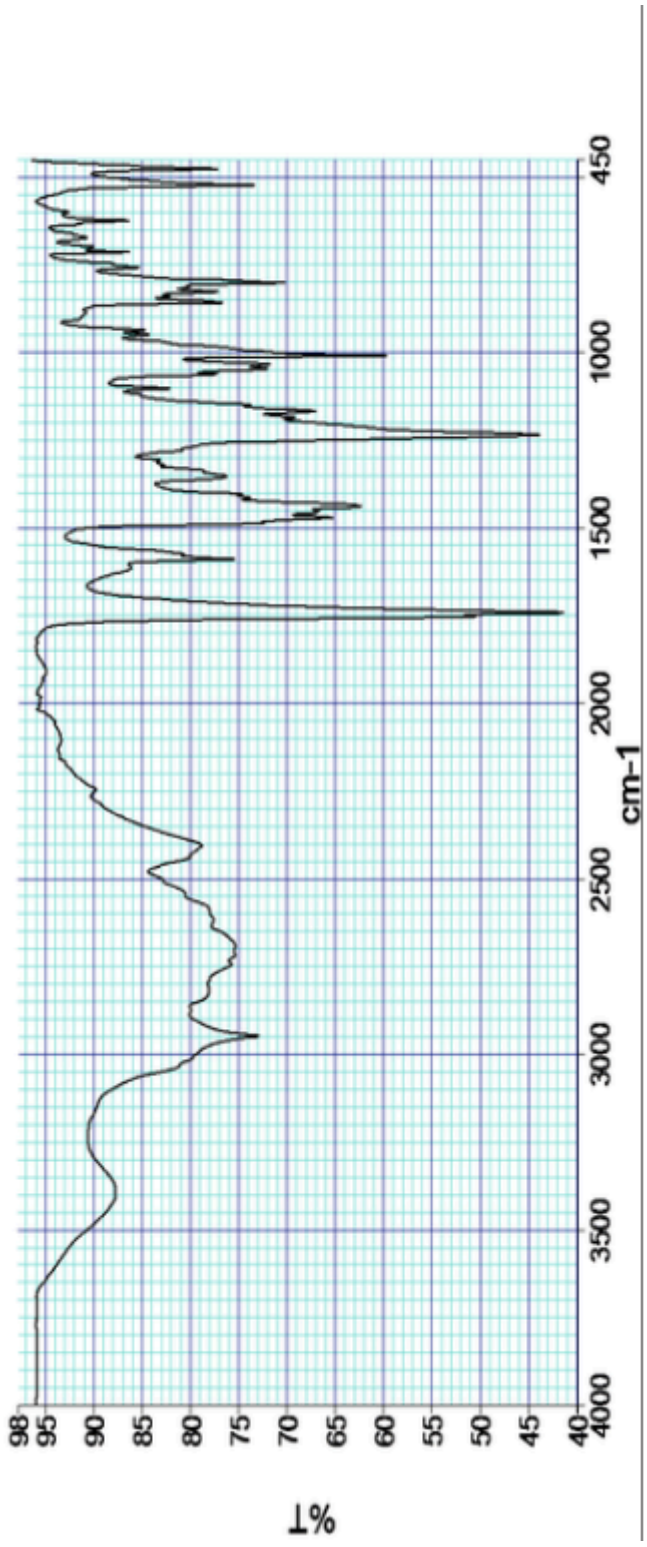
IR spectrum for compound **105**



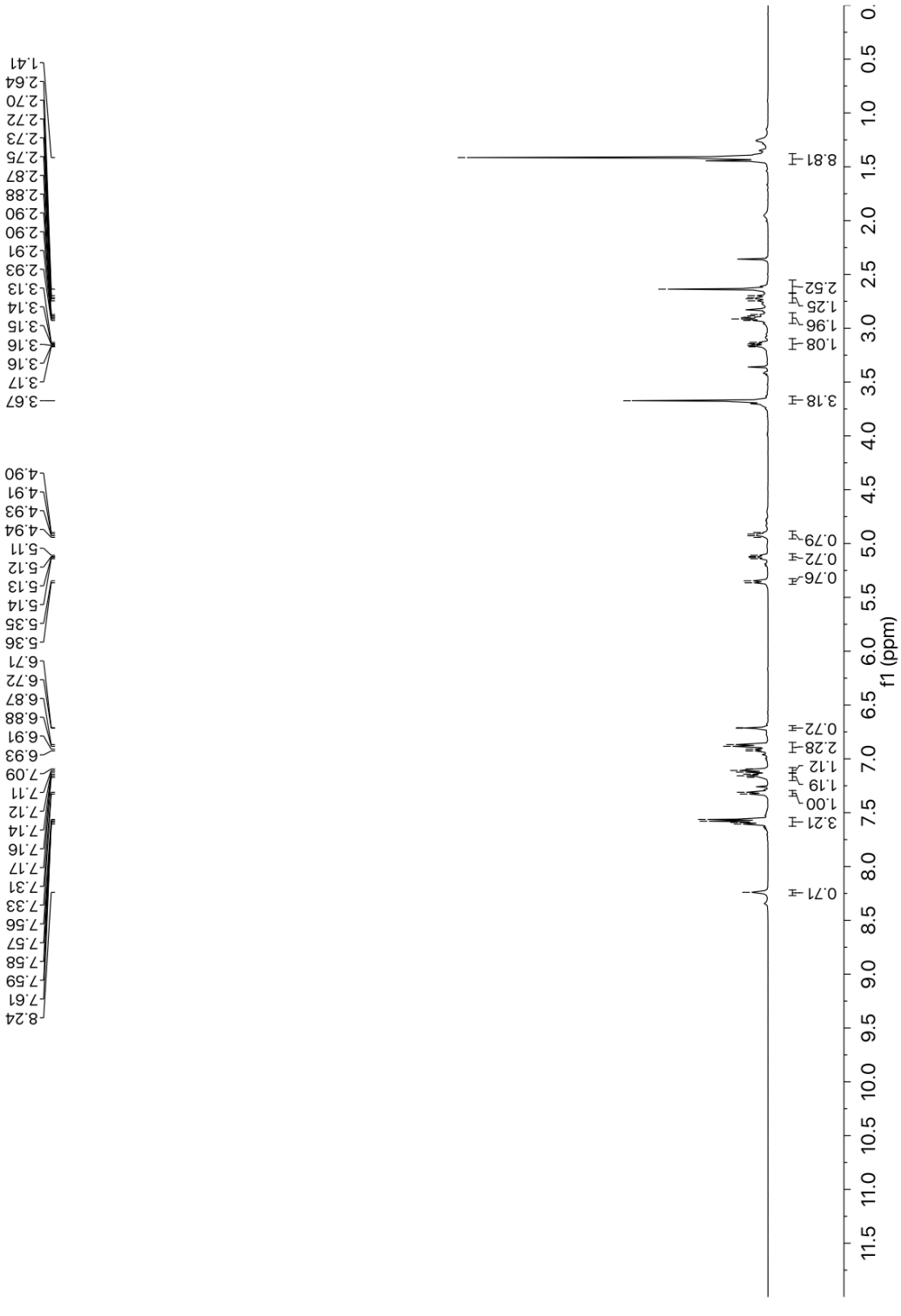
[1]HNMR spectrum for compound **109**



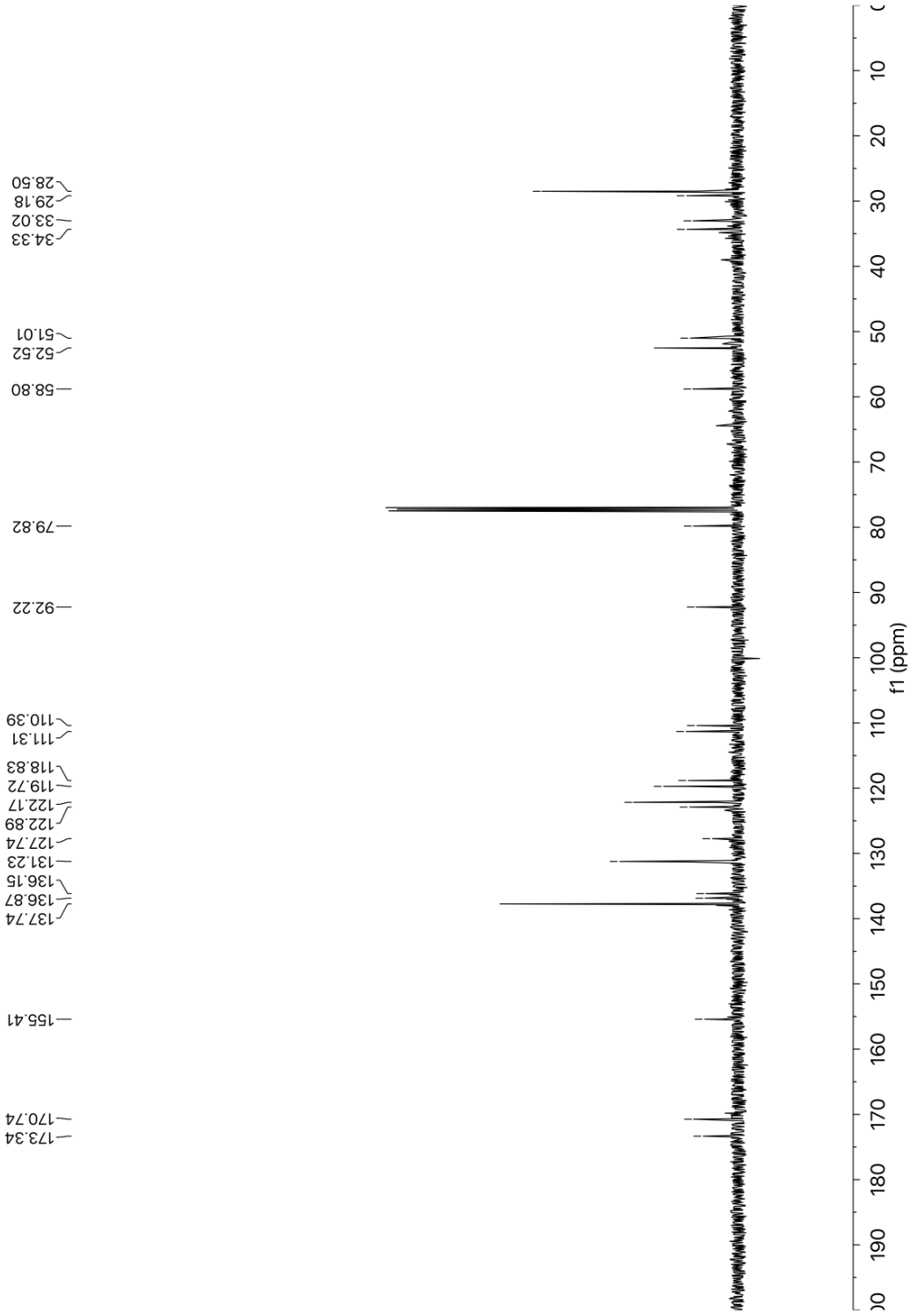
^{13}CNMR spectrum for compound **109**



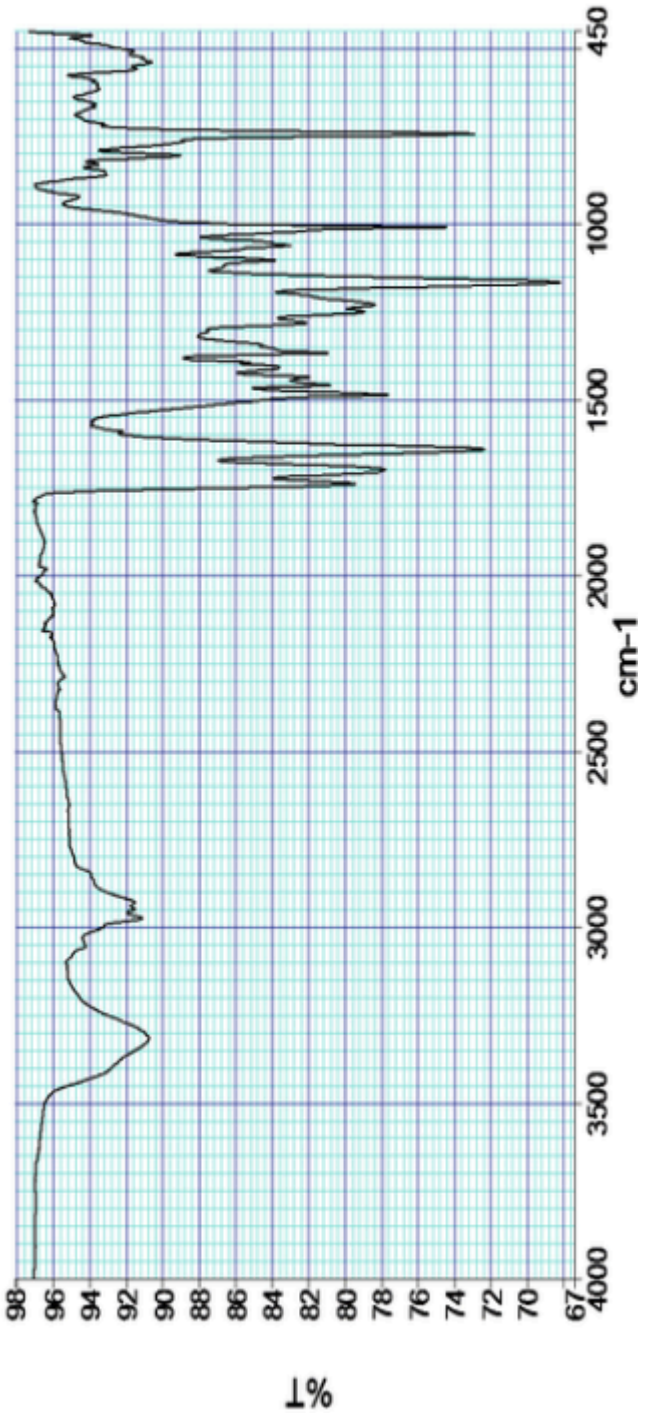
IR spectrum for compound **109**



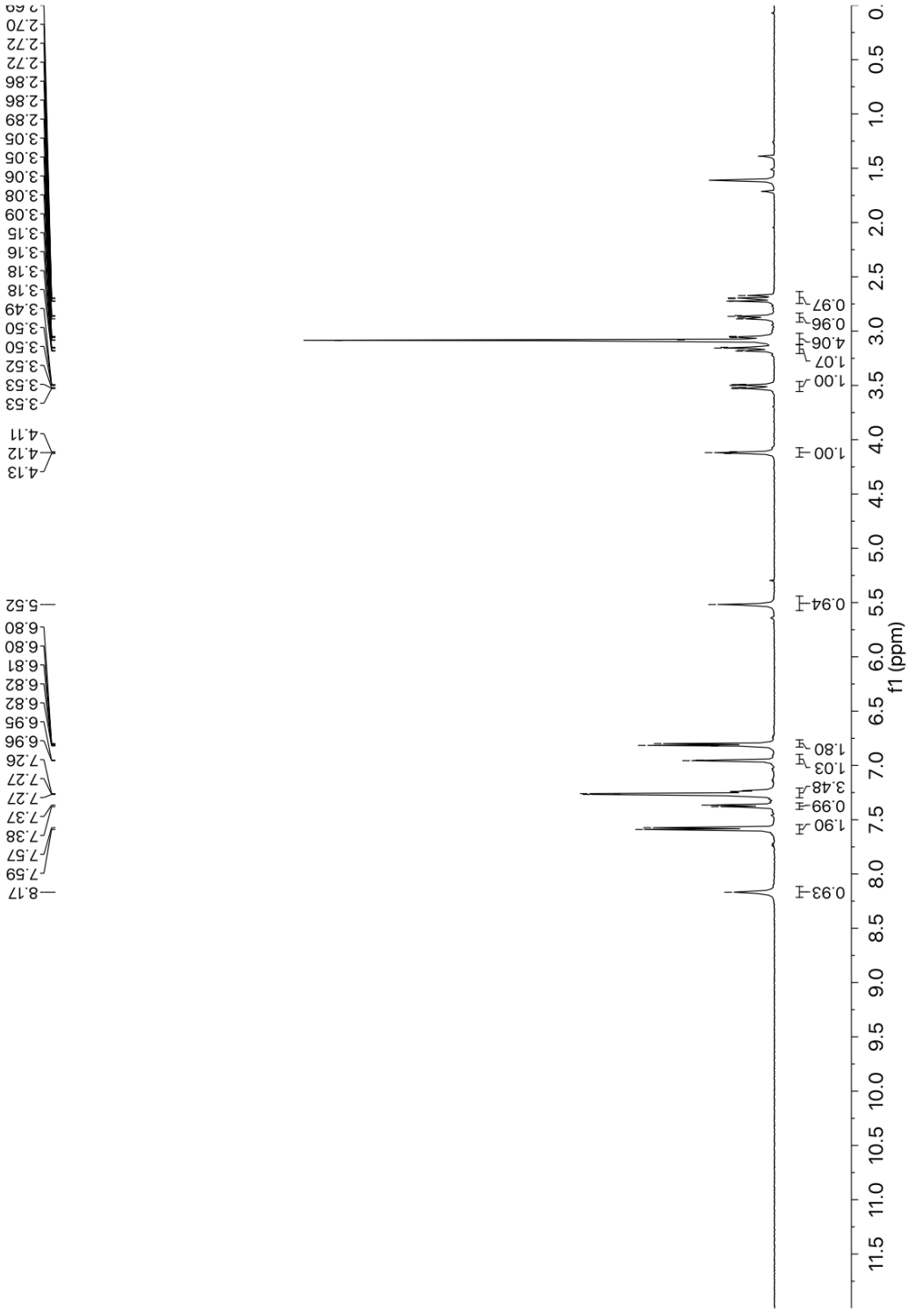
[1]HNMR spectrum for compound **110**



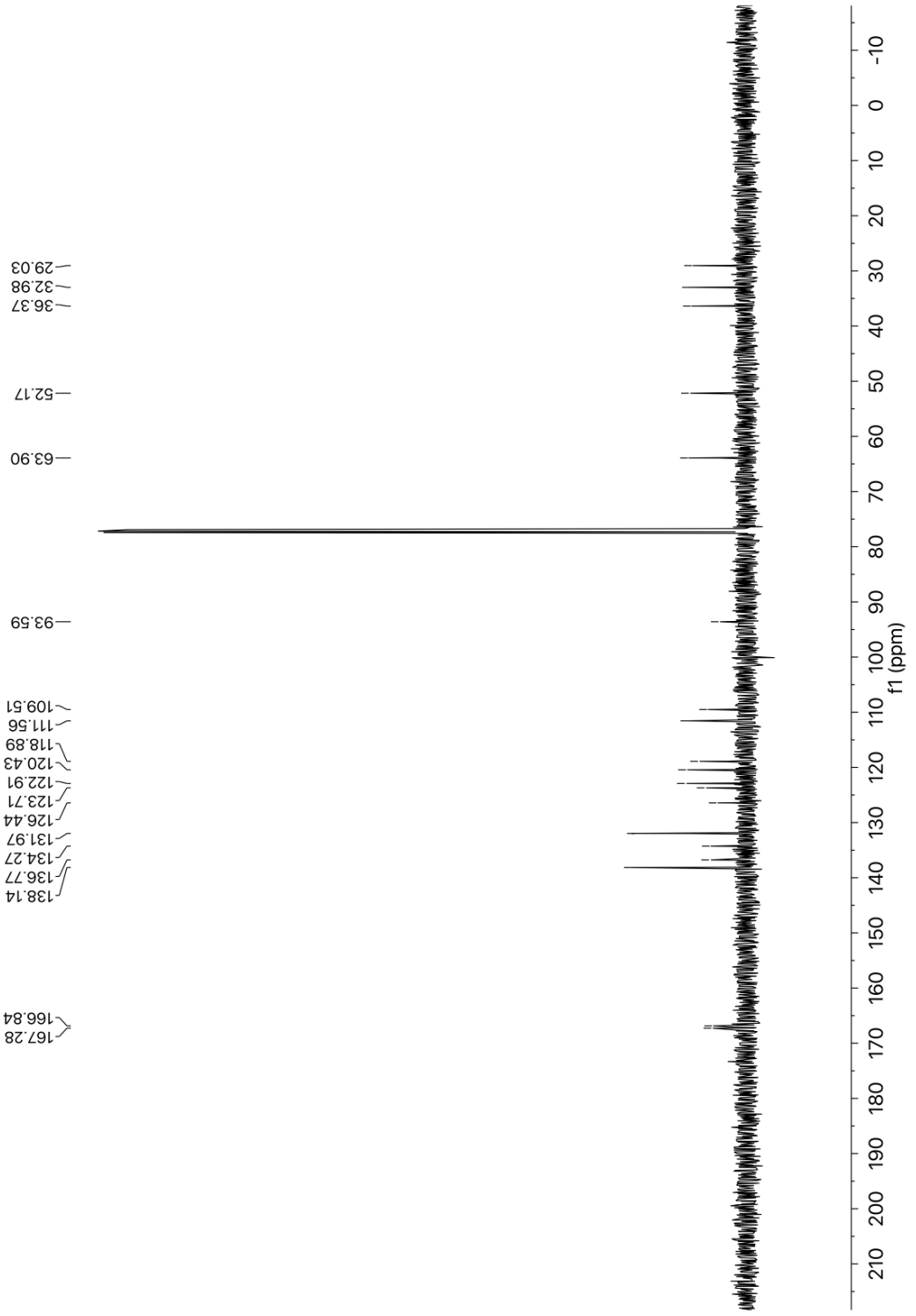
^{13}CNMR spectrum for compound **110**



IR spectrum for compound **110**



^{1}HNMR spectrum for compound **111**



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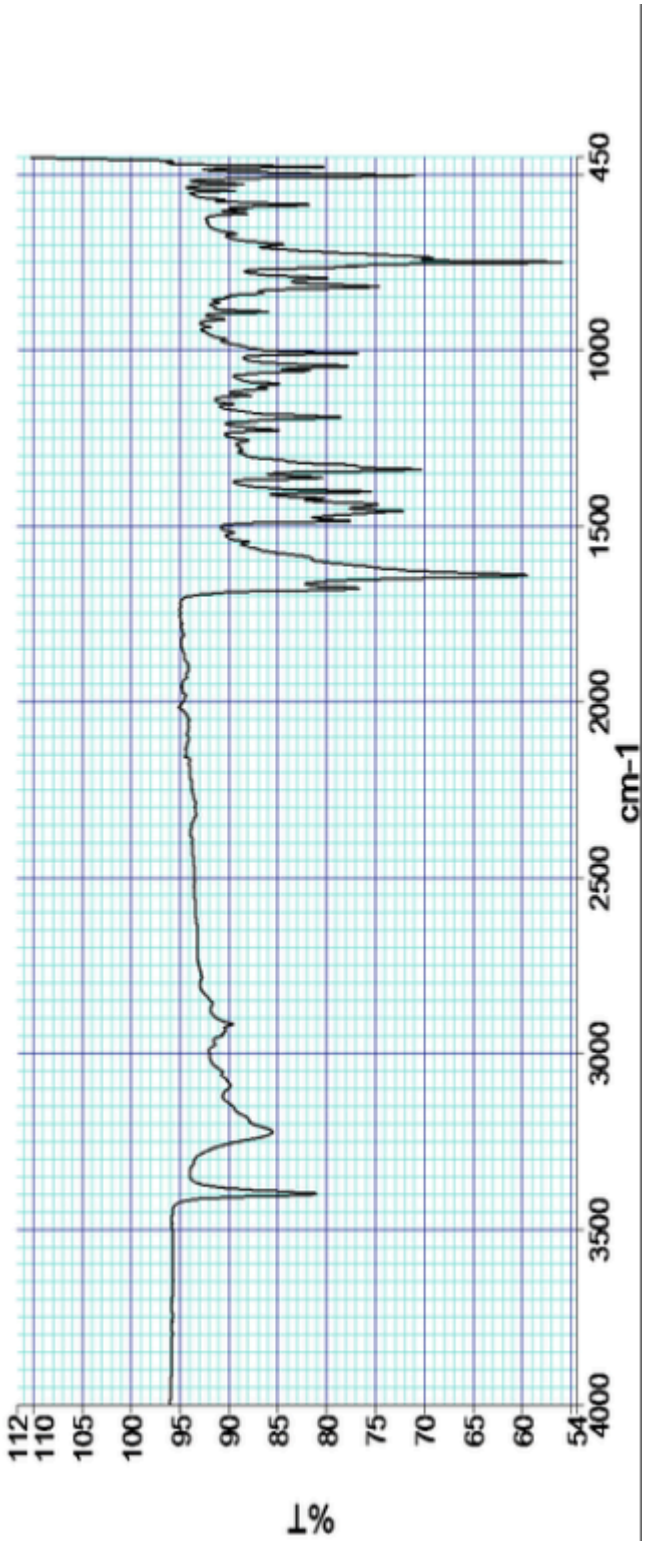
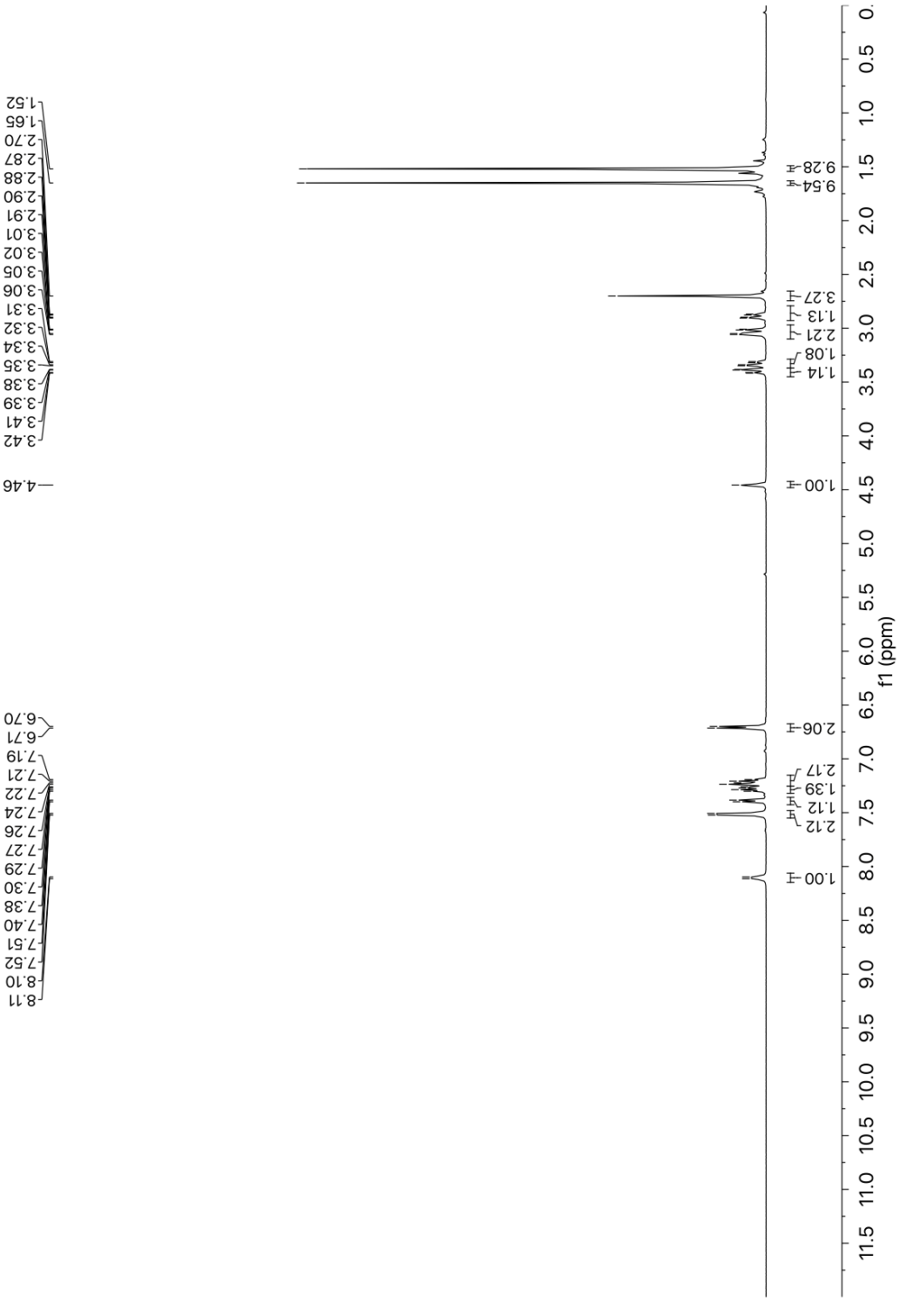
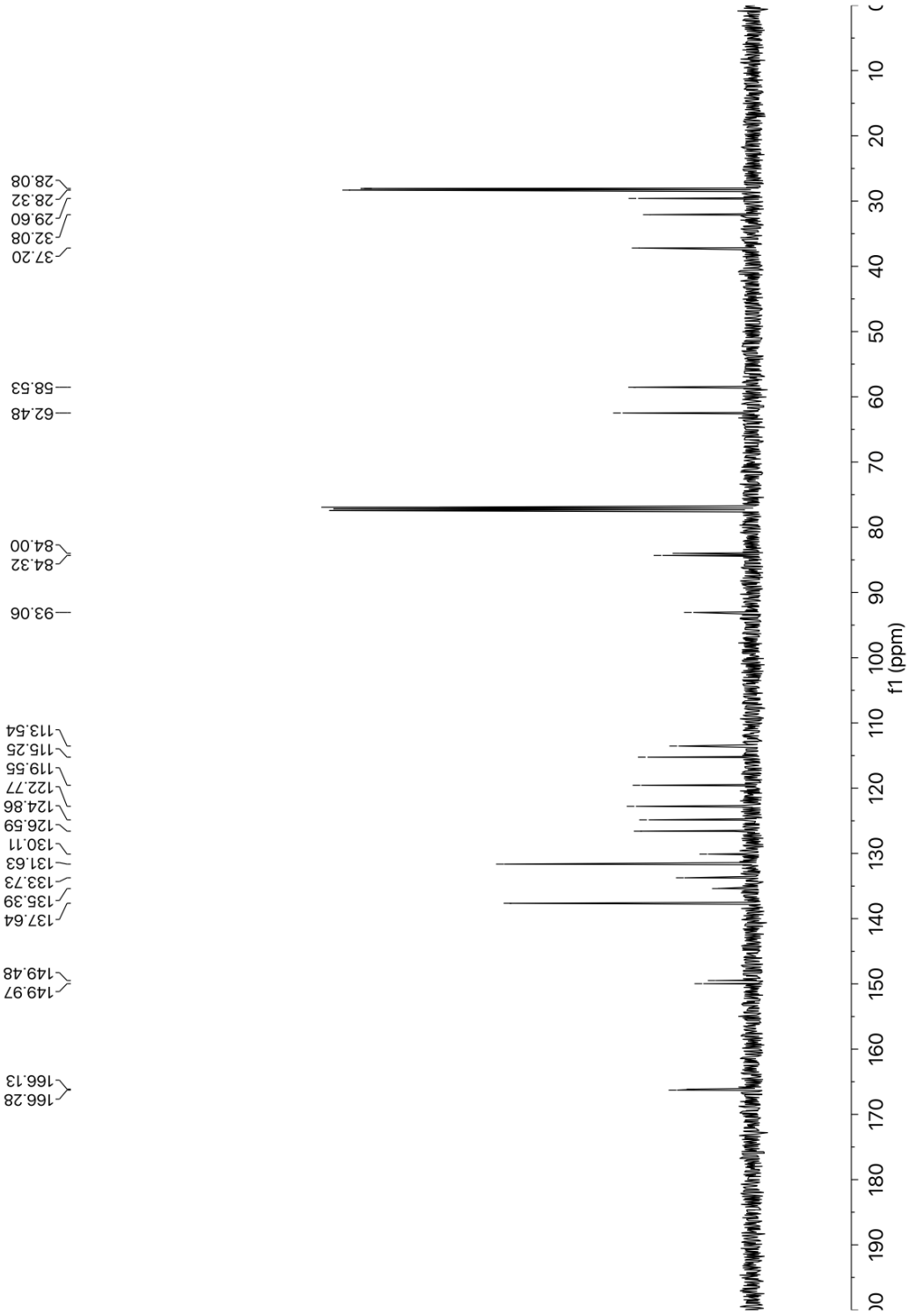


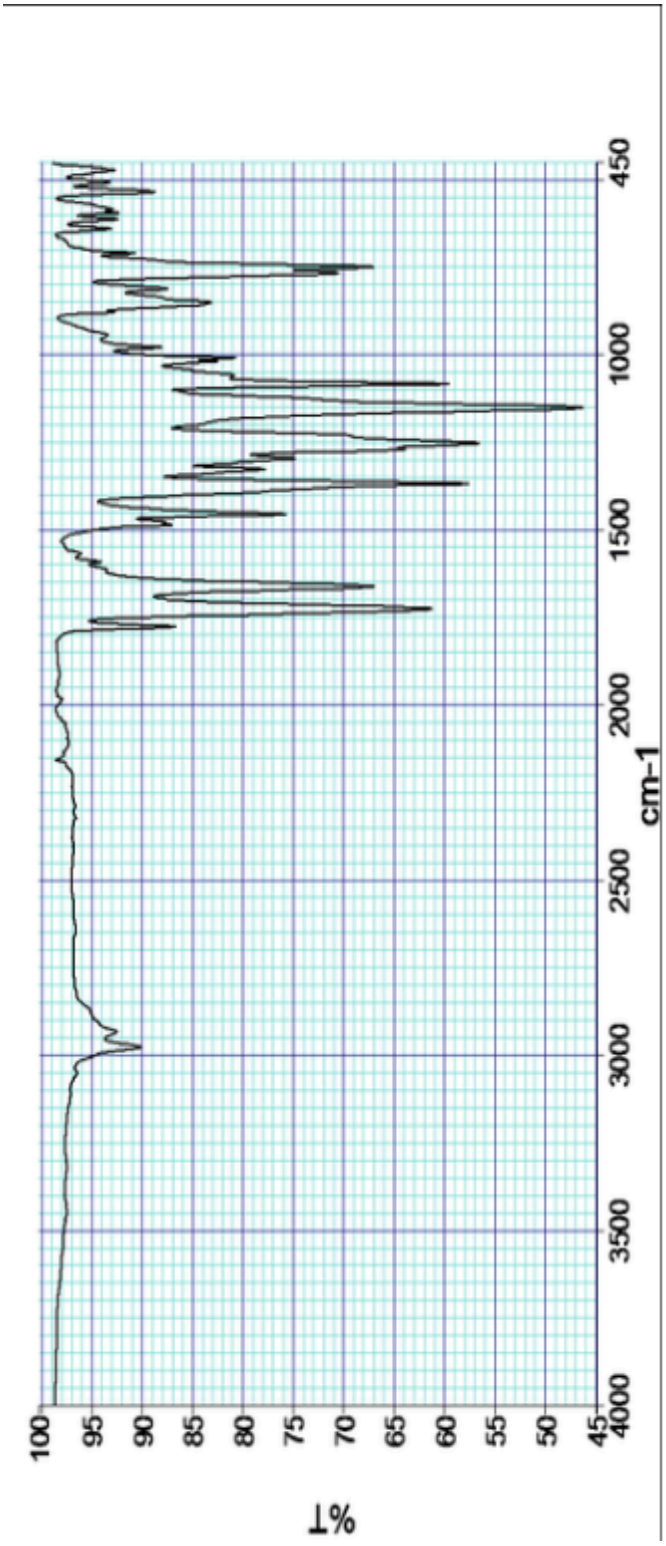
IR spectrum for compound **111**



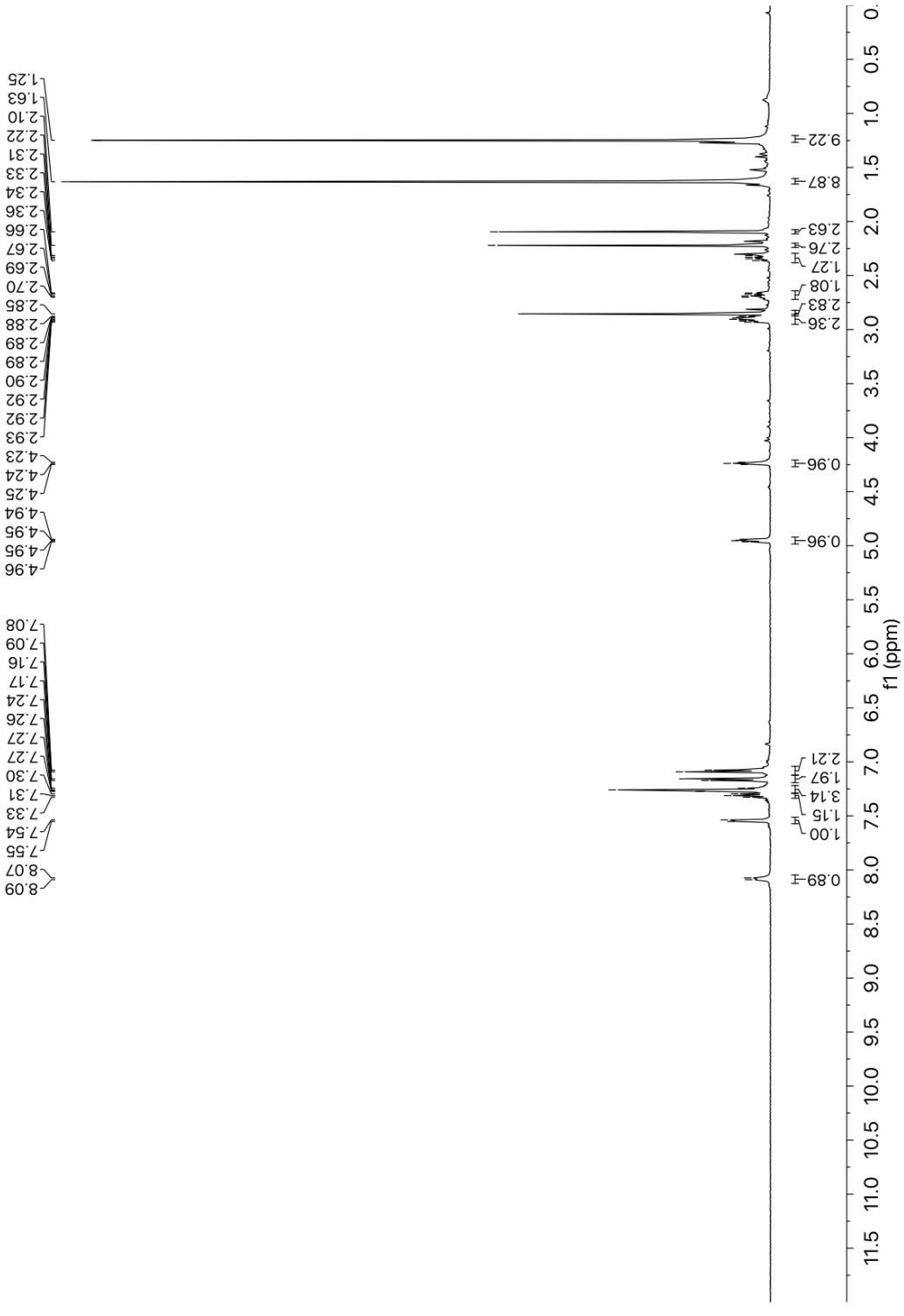
^{1}HNMR spectrum for compound **112**



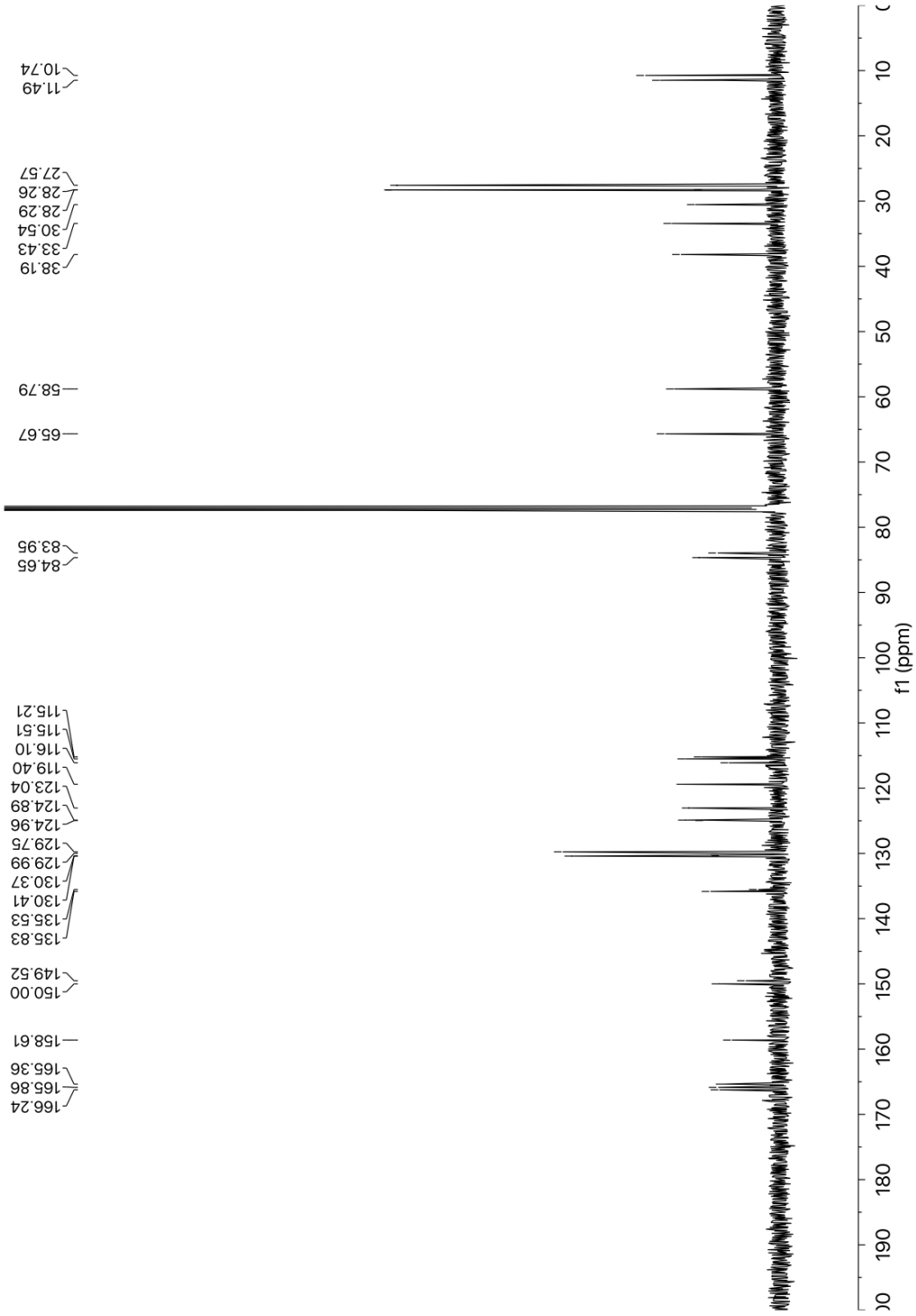
^{13}CNMR spectrum for compound **112**



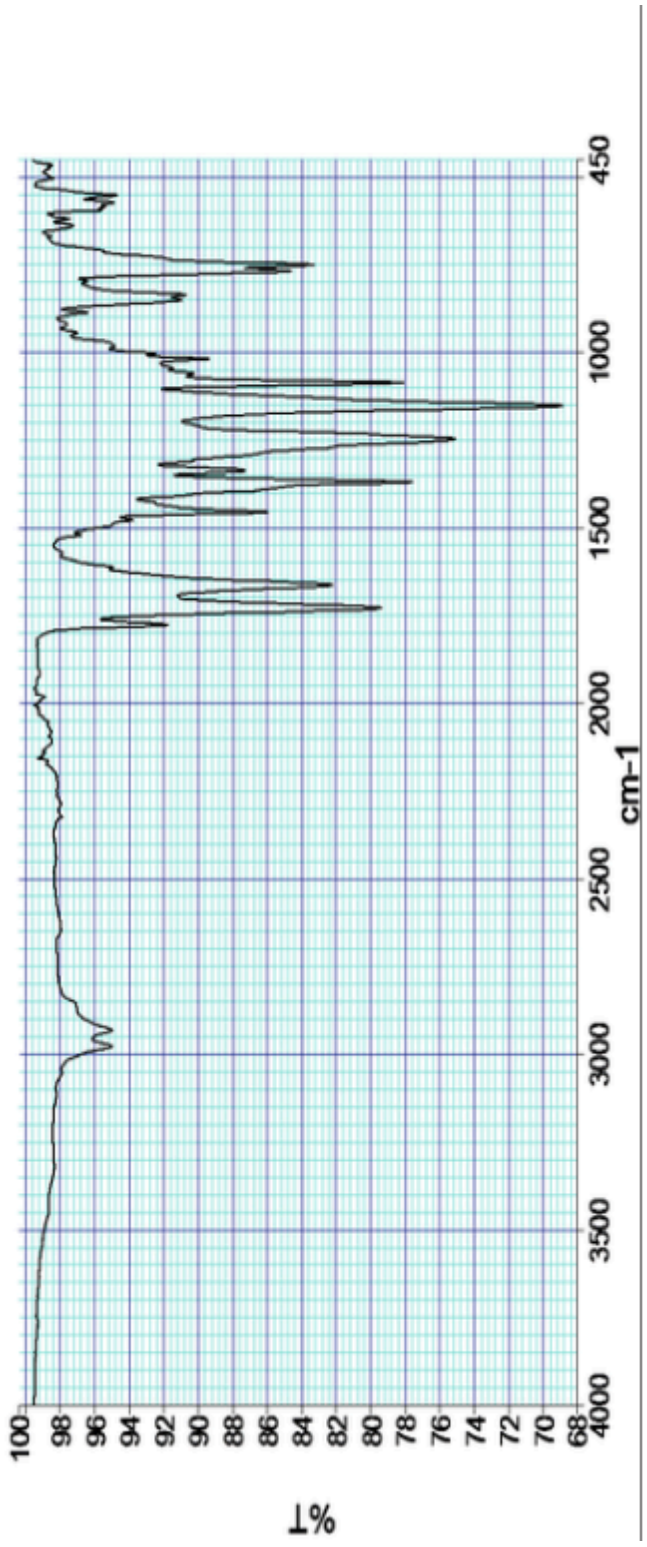
IR spectrum for compound **112**



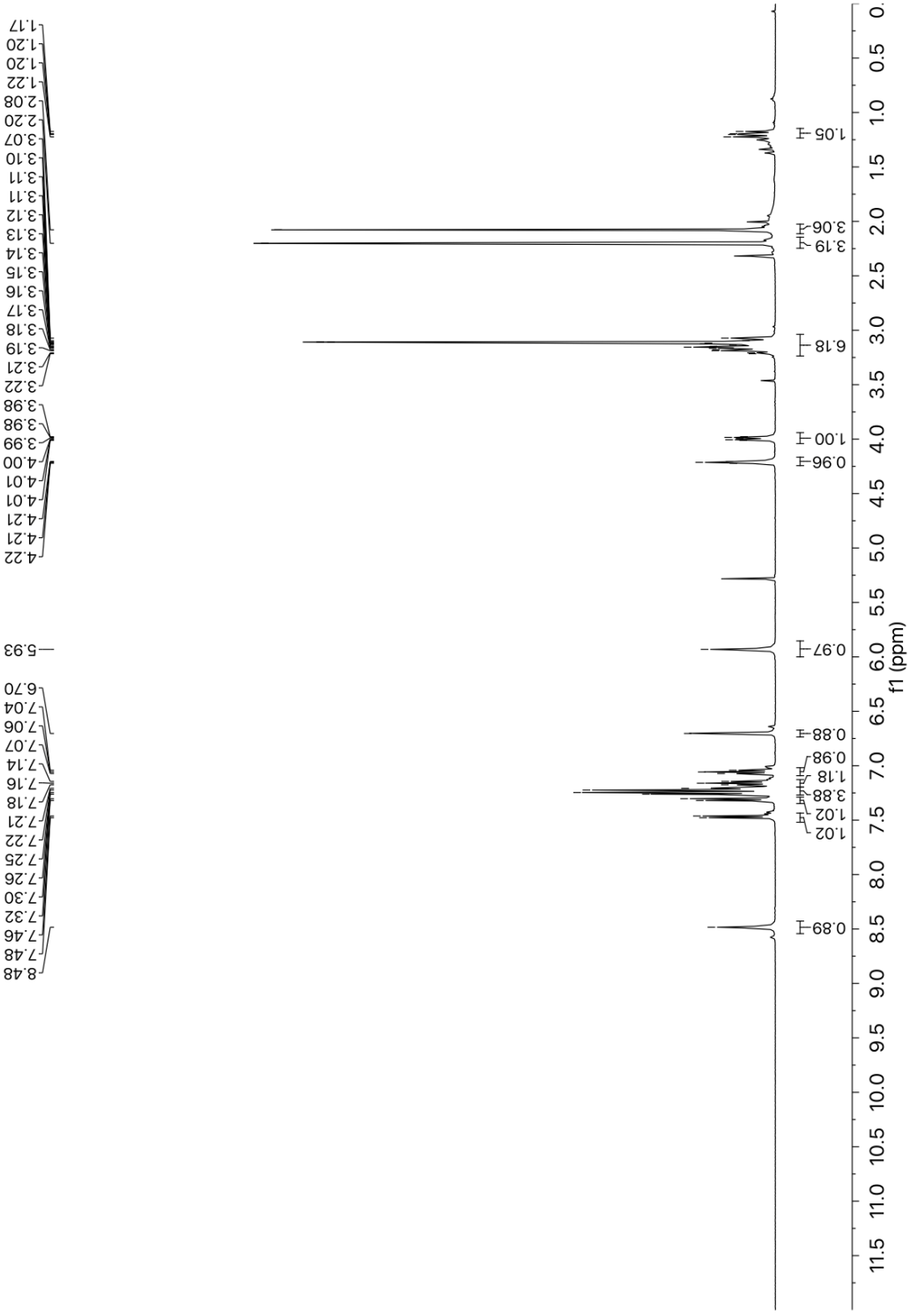
[1]HNMR spectrum for compound **113**



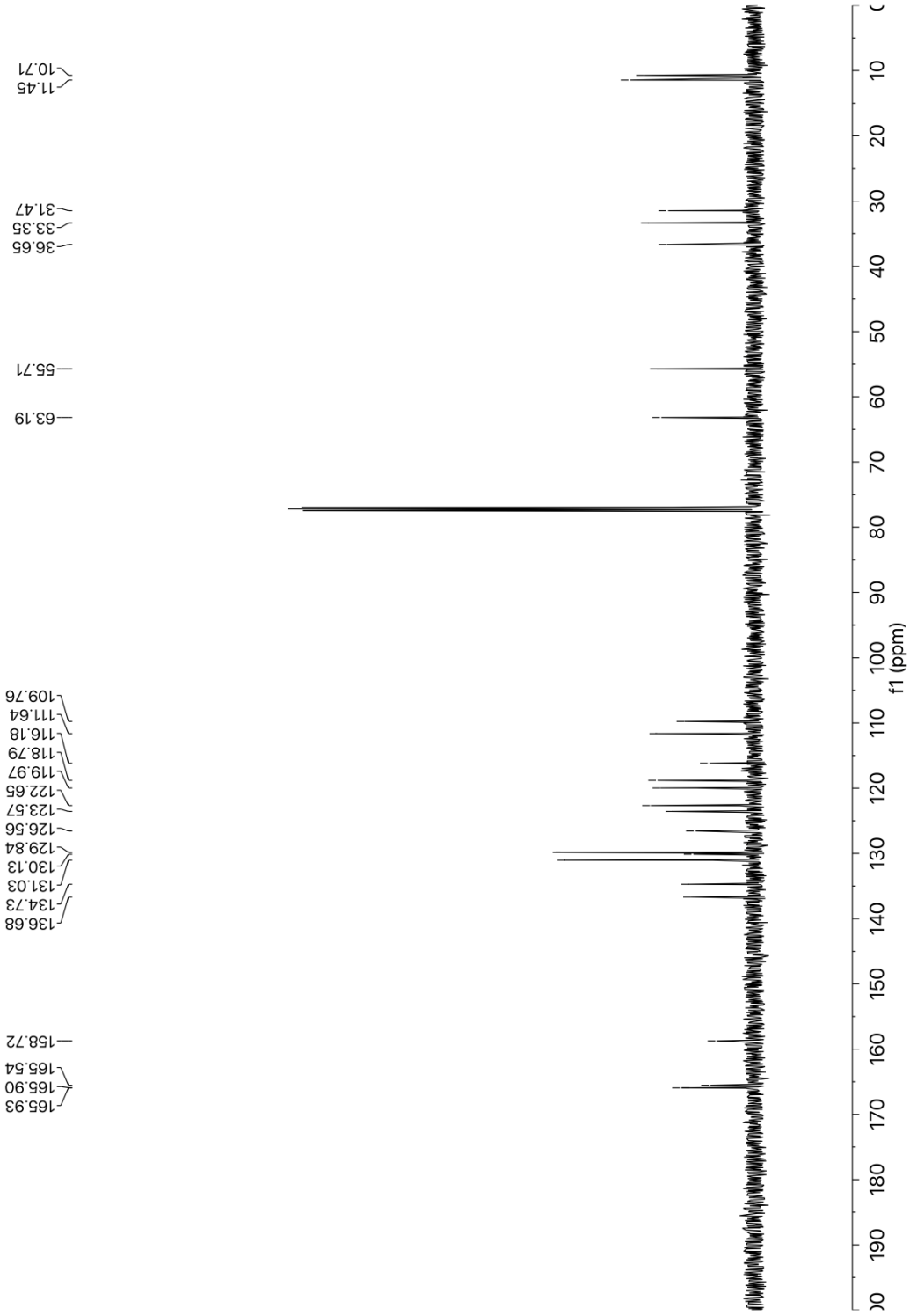
^{13}CNMR spectrum for compound **113**



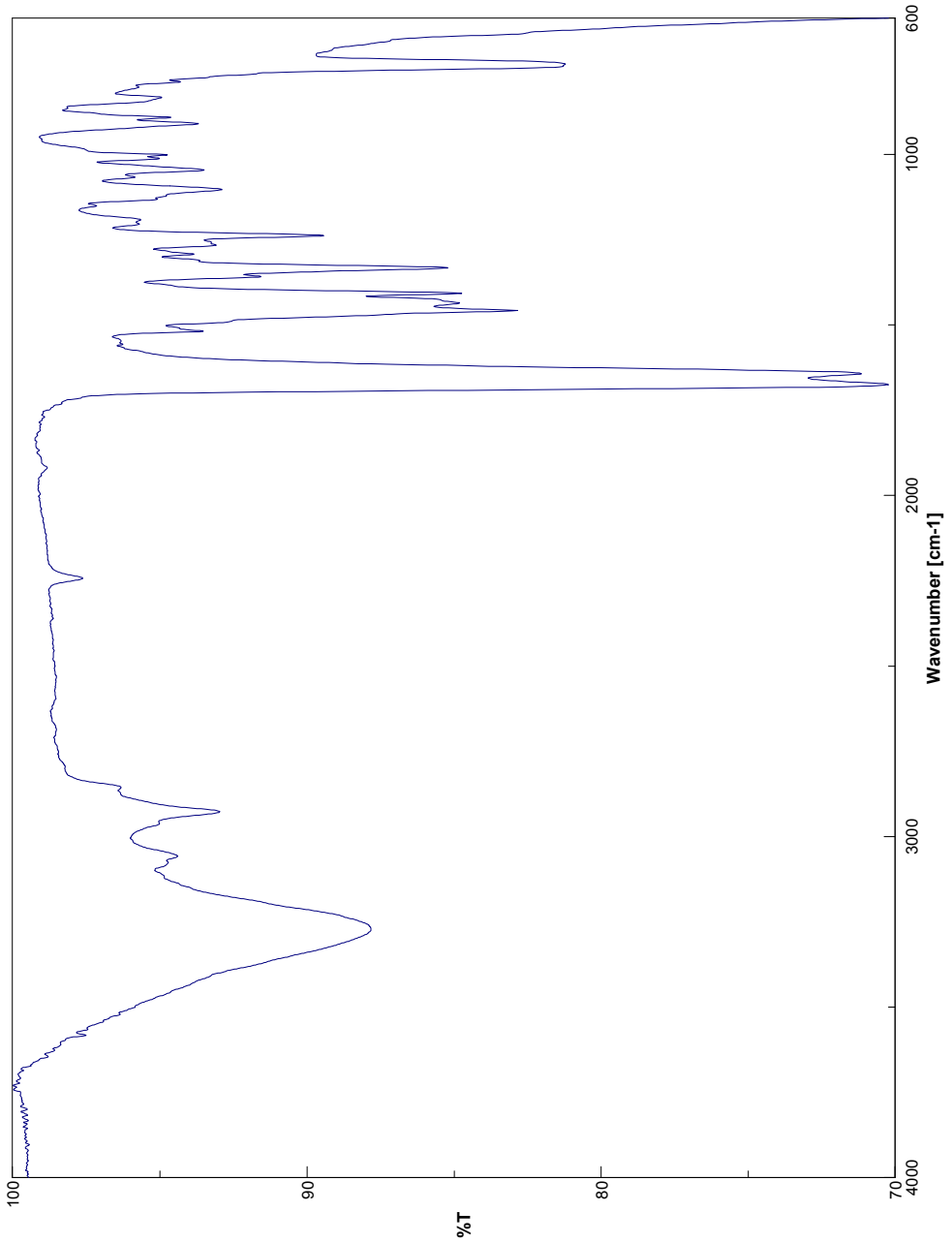
IR spectrum for compound **113**



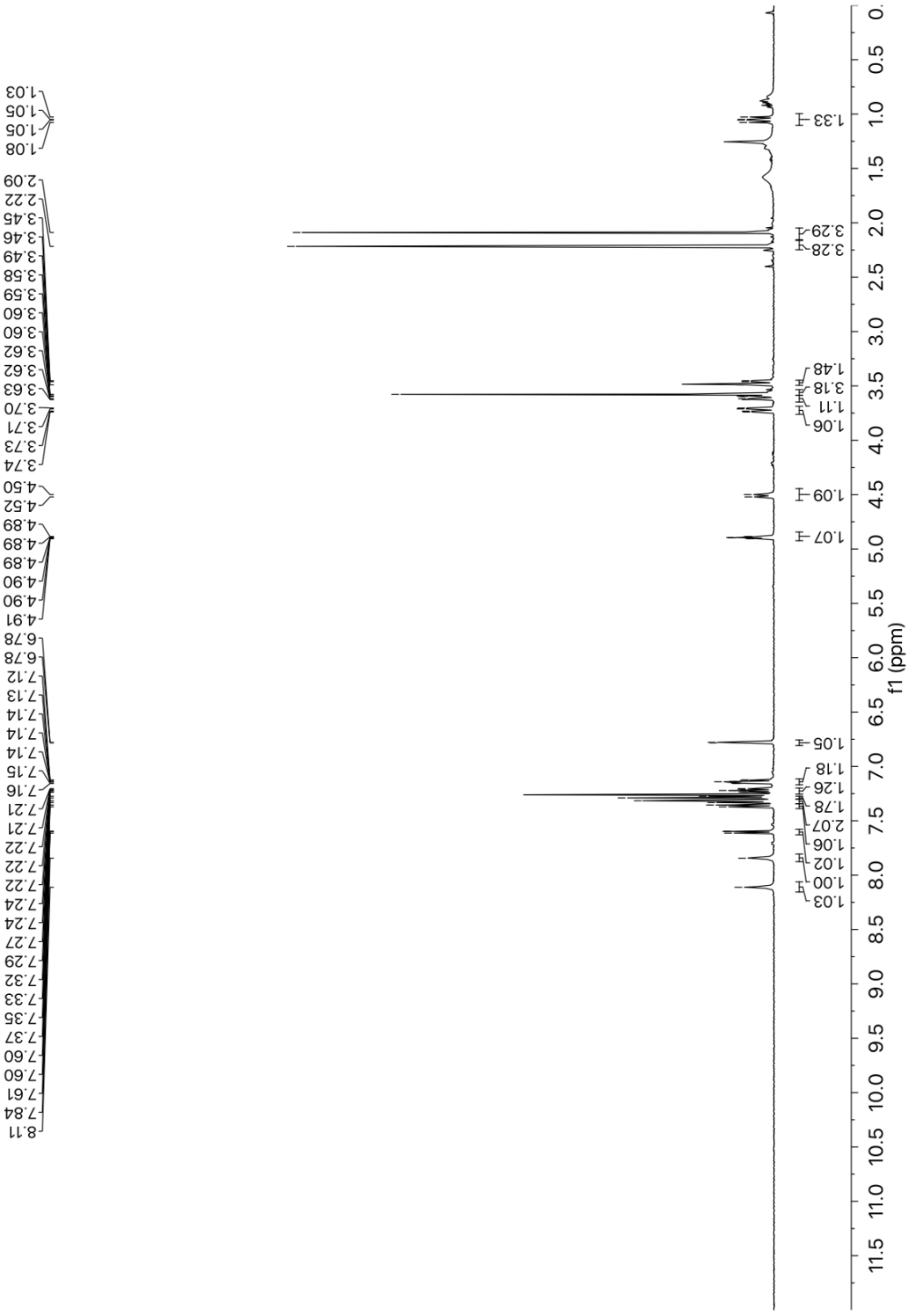
^{1}HNMR spectrum for compound **108**



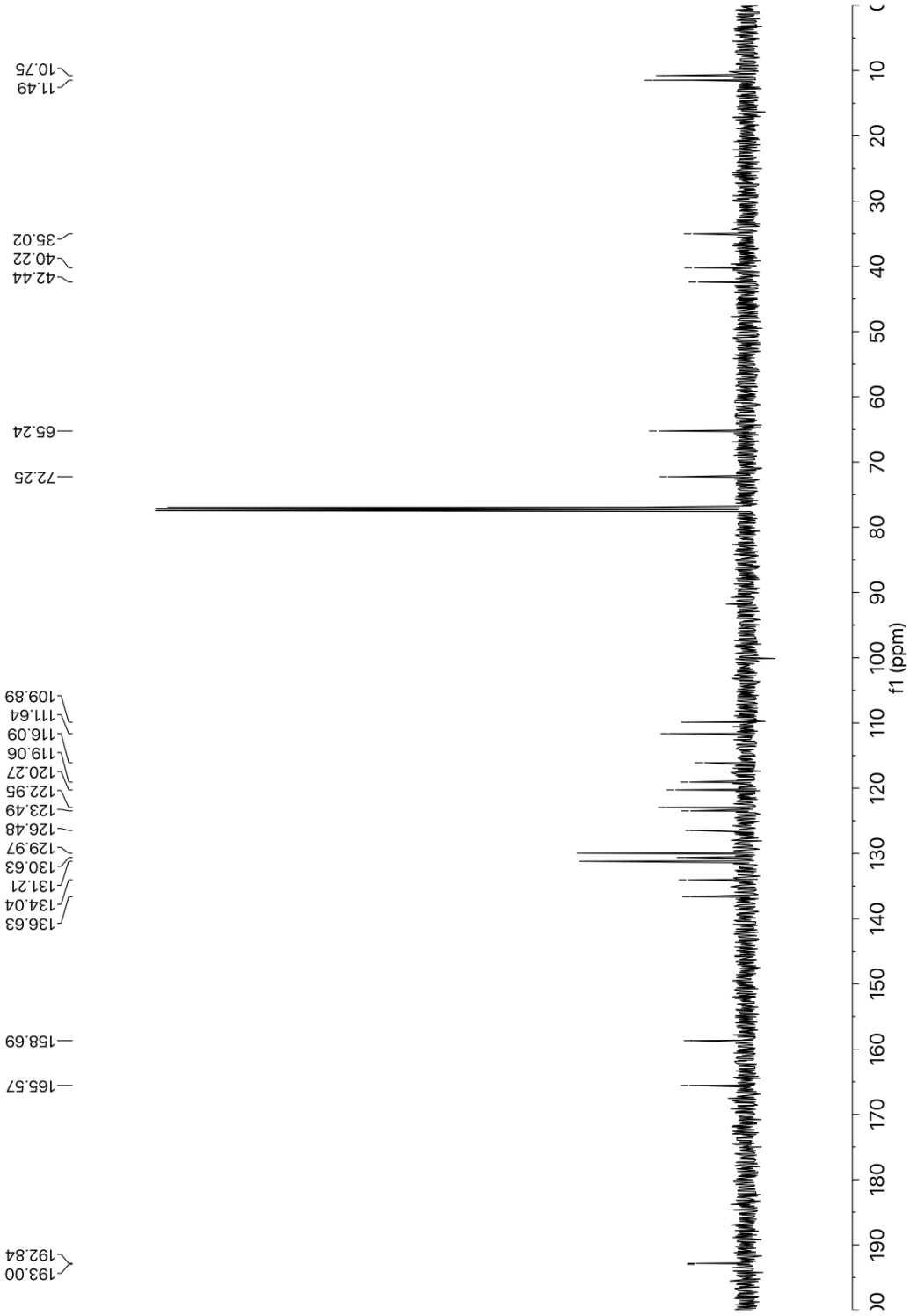
^{13}CNMR spectrum for compound **108**



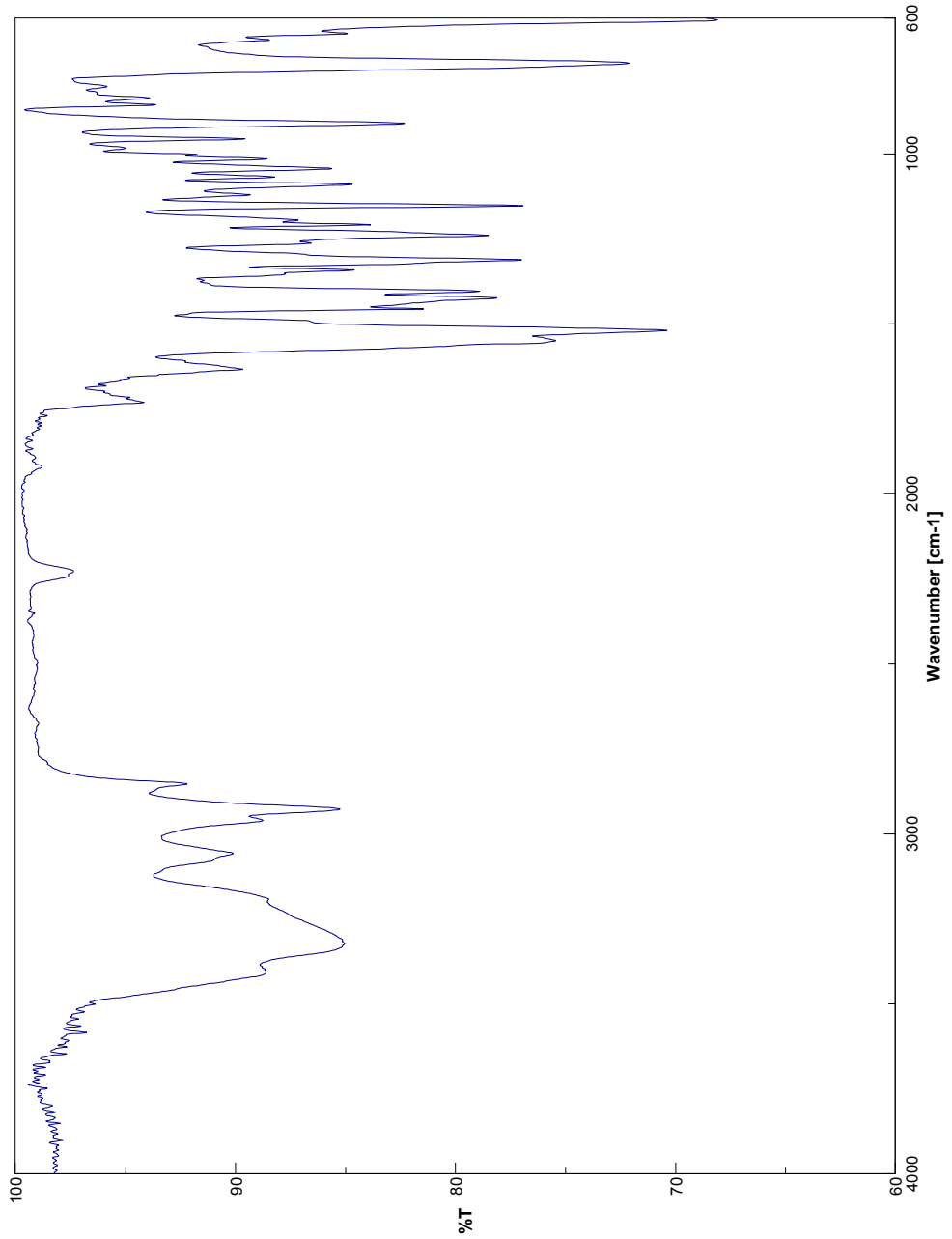
IR spectrum for compound **108**



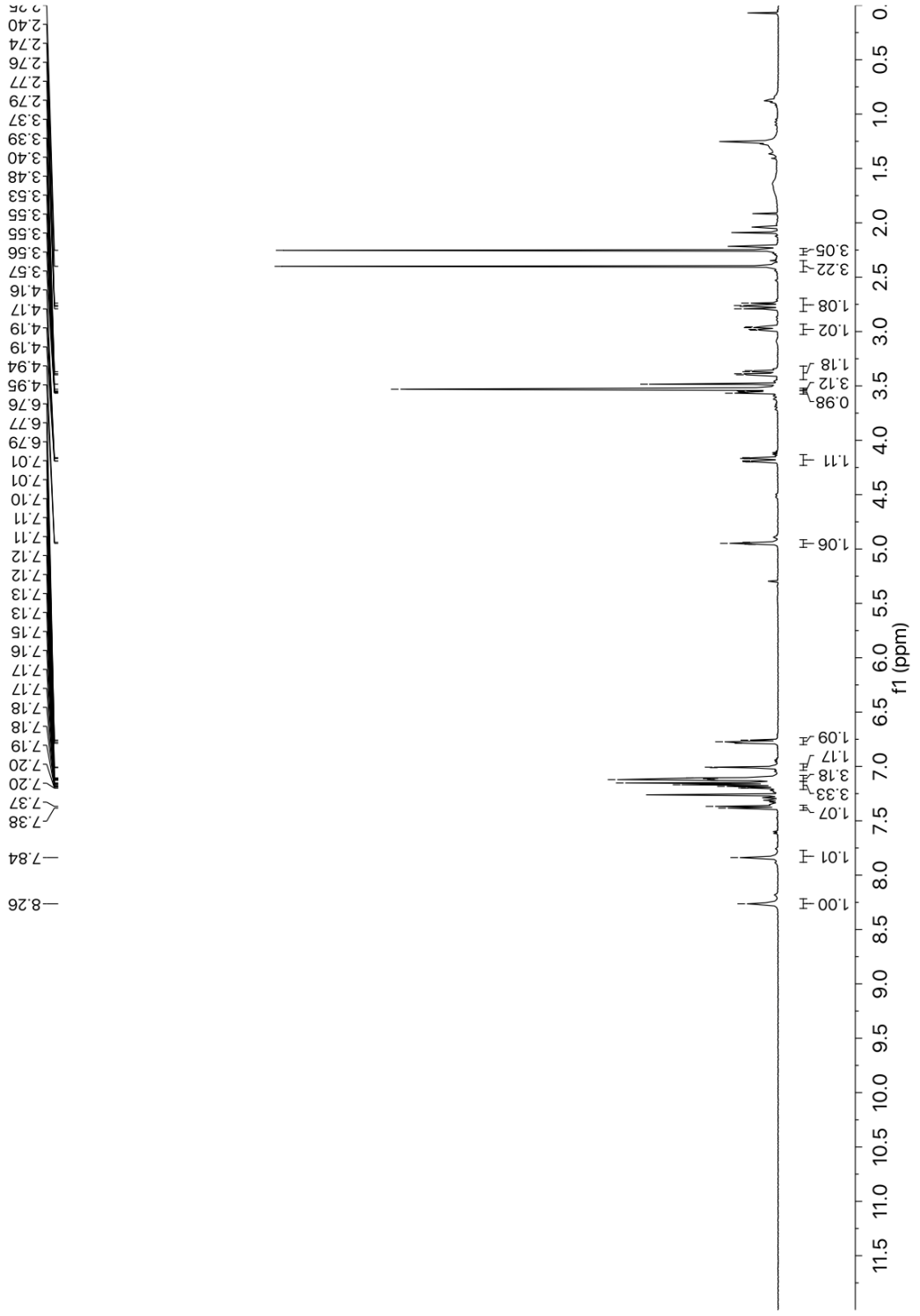
[1]HNMR spectrum for compound **116bottom**



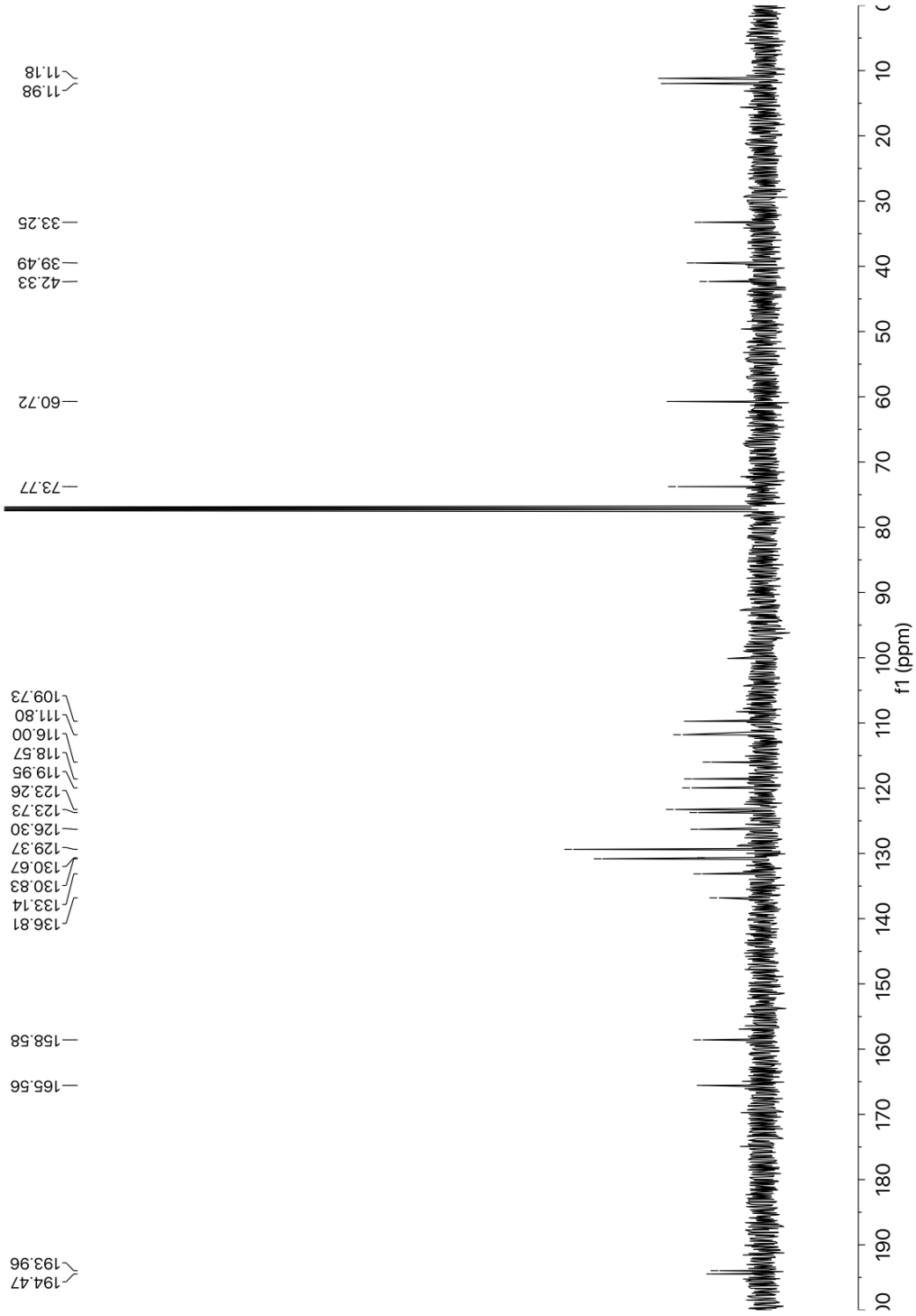
^{13}CNMR spectrum for compound **116bottom**



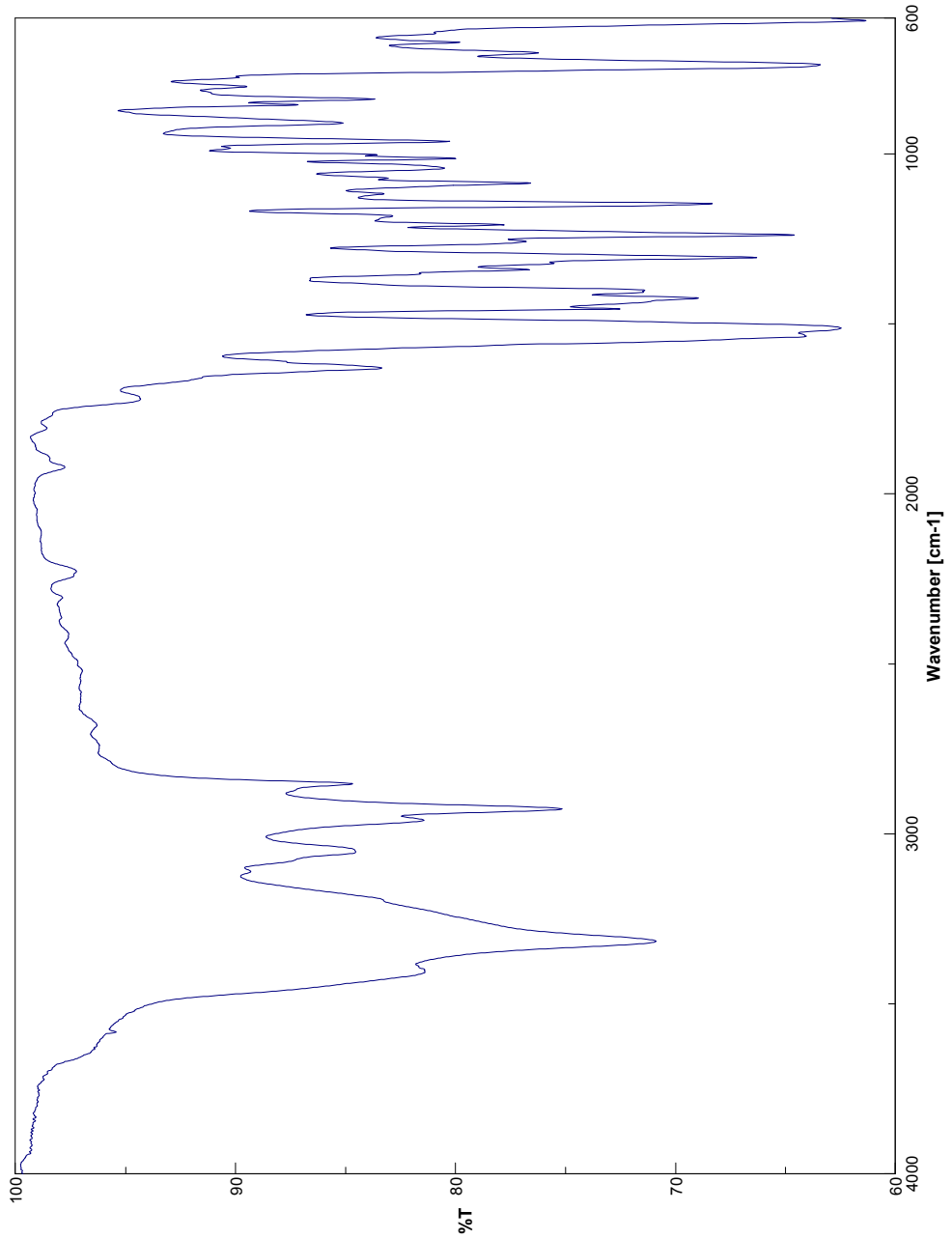
IR spectrum for compound **116bottom**



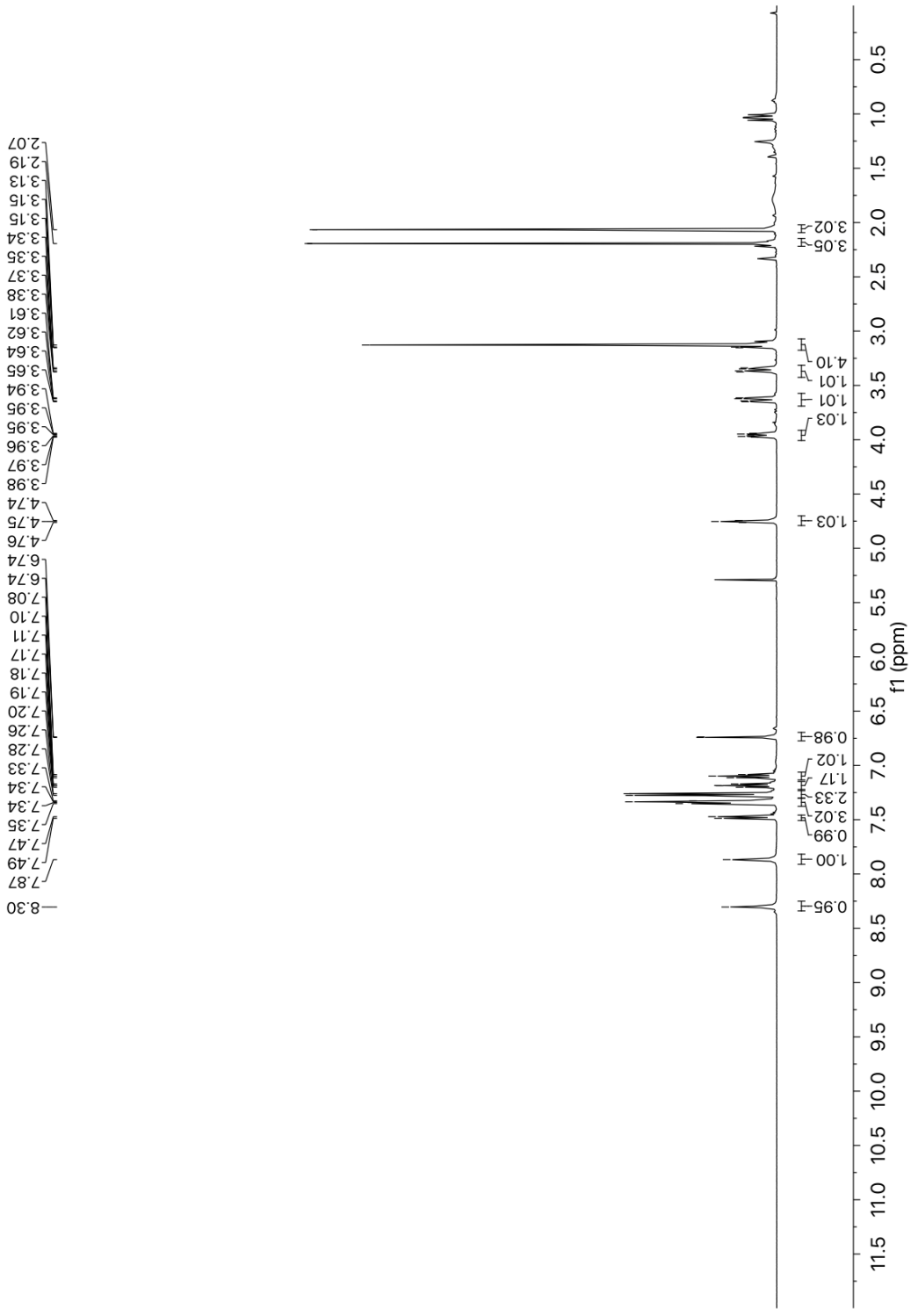
[1]HNMR spectrum for compound **116top**



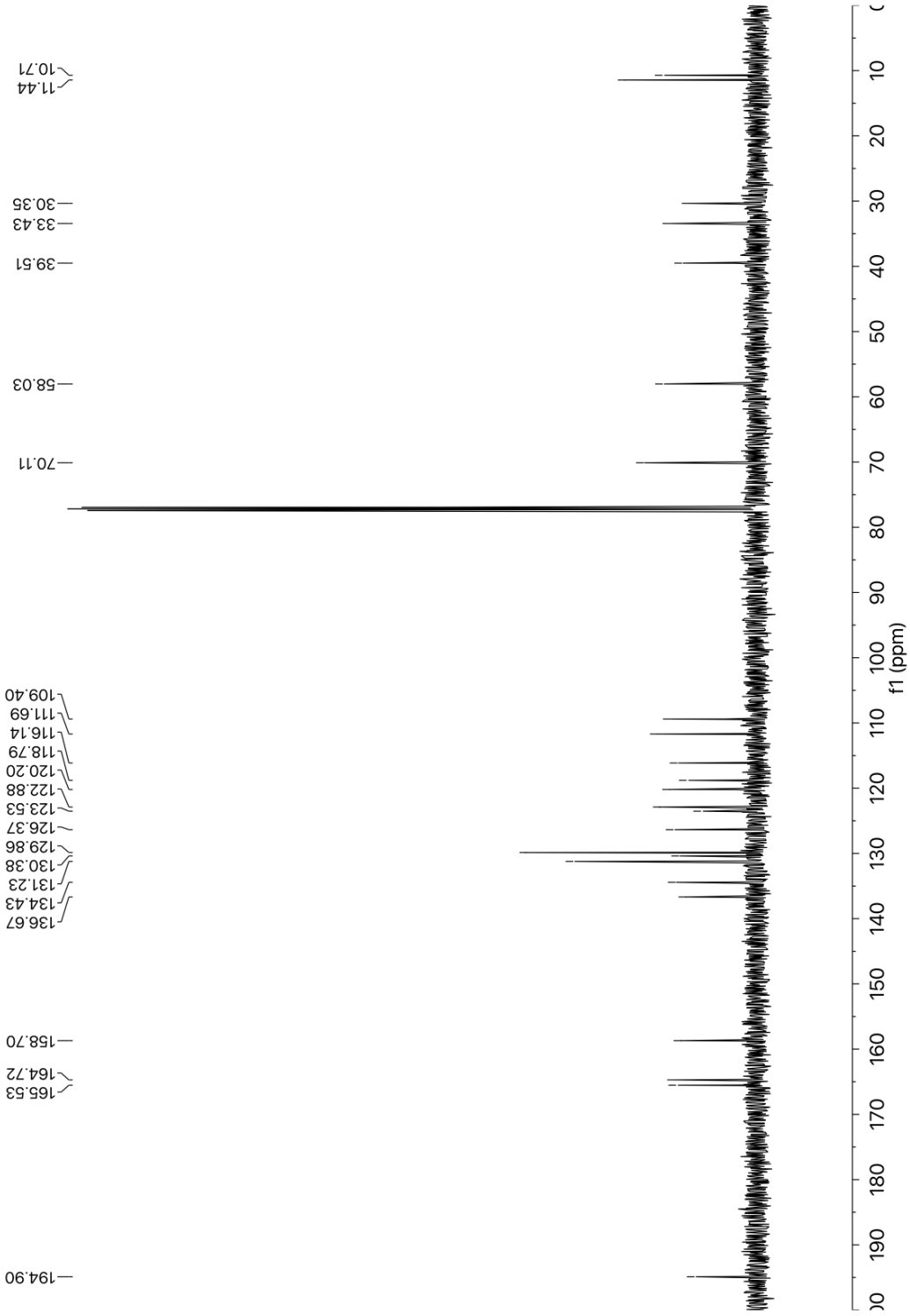
^{13}CNMR spectrum for compound **116top**



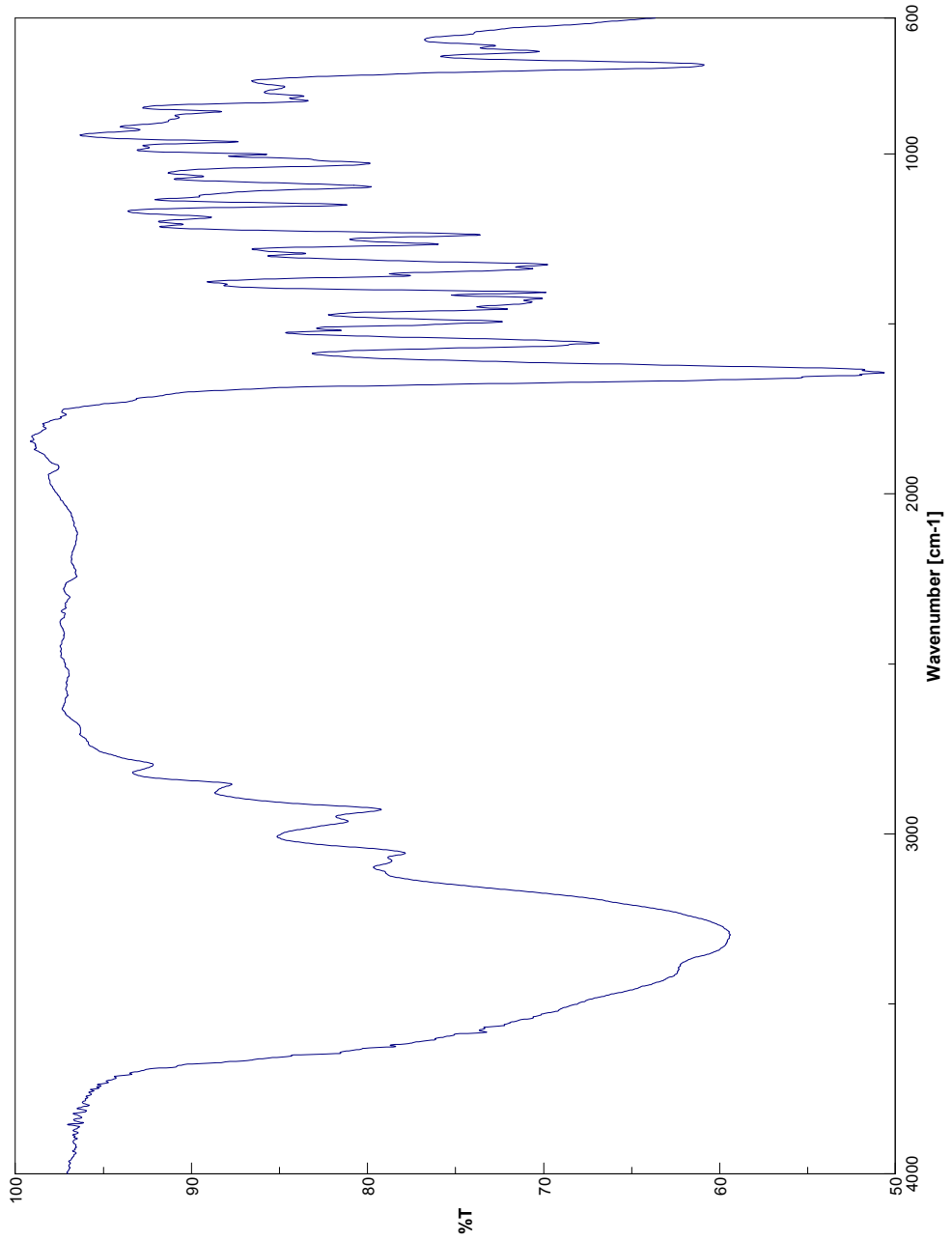
IR spectrum for compound **116top**



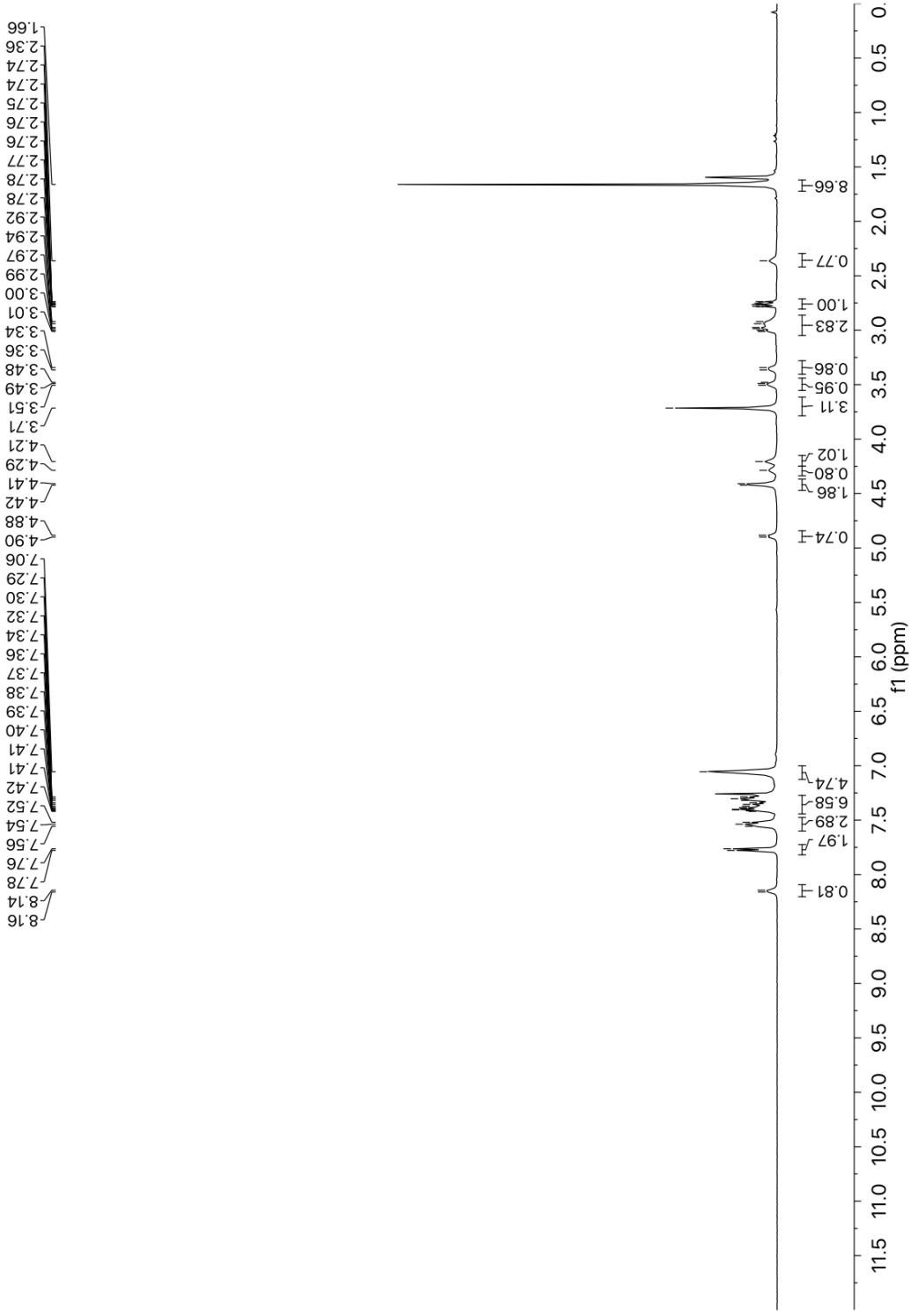
[1]HNMR spectrum for compound **123**



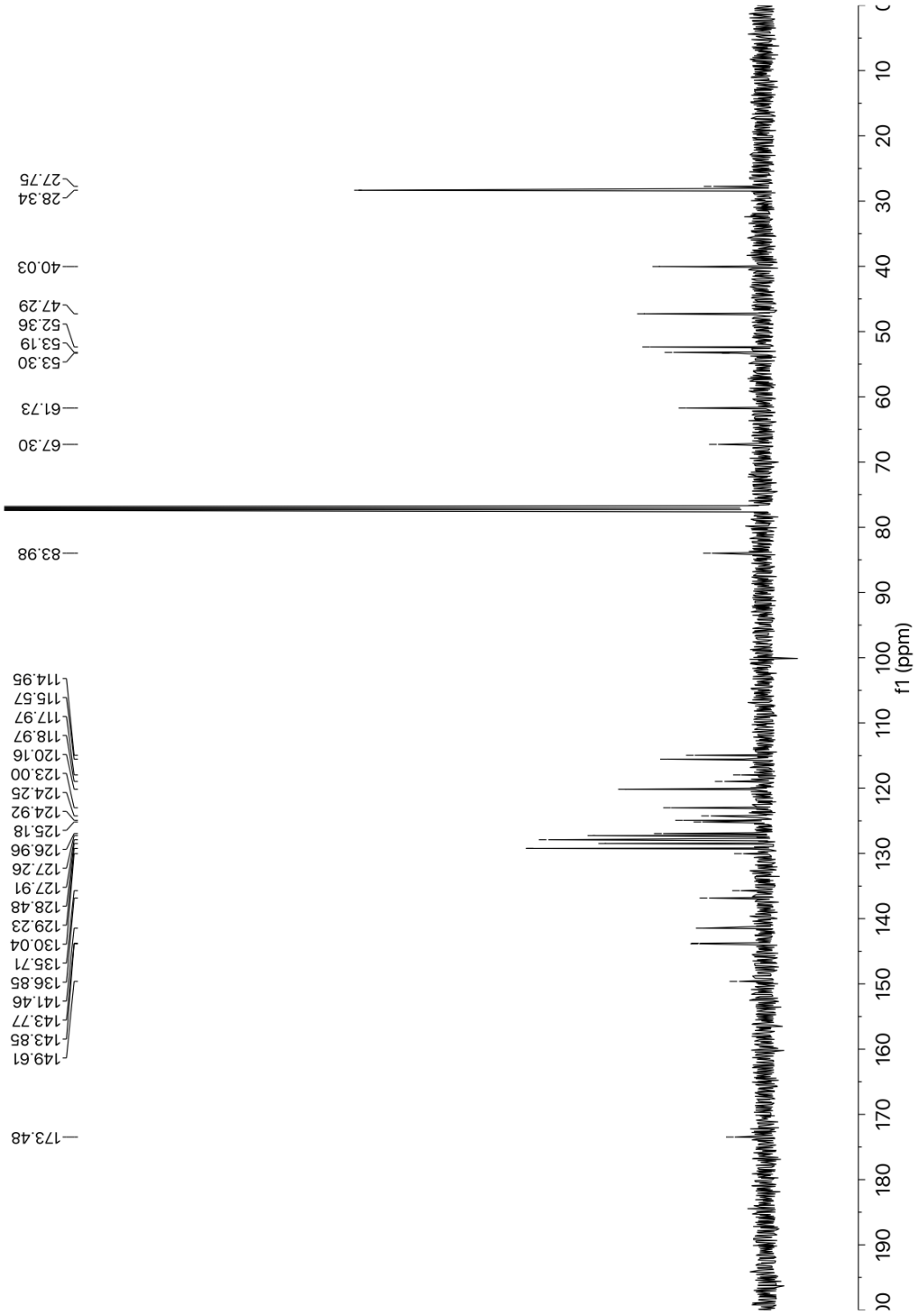
^{13}CNMR spectrum for compound **123**



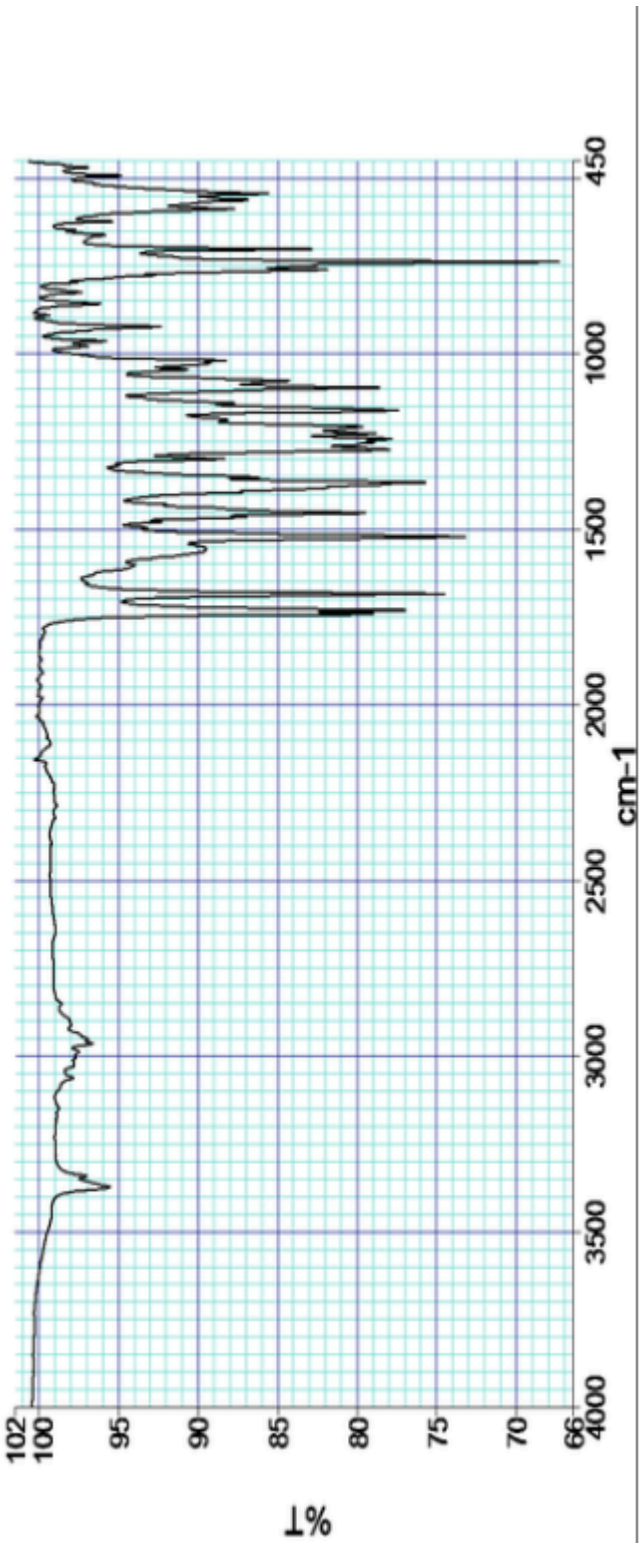
IR spectrum for compound **123**



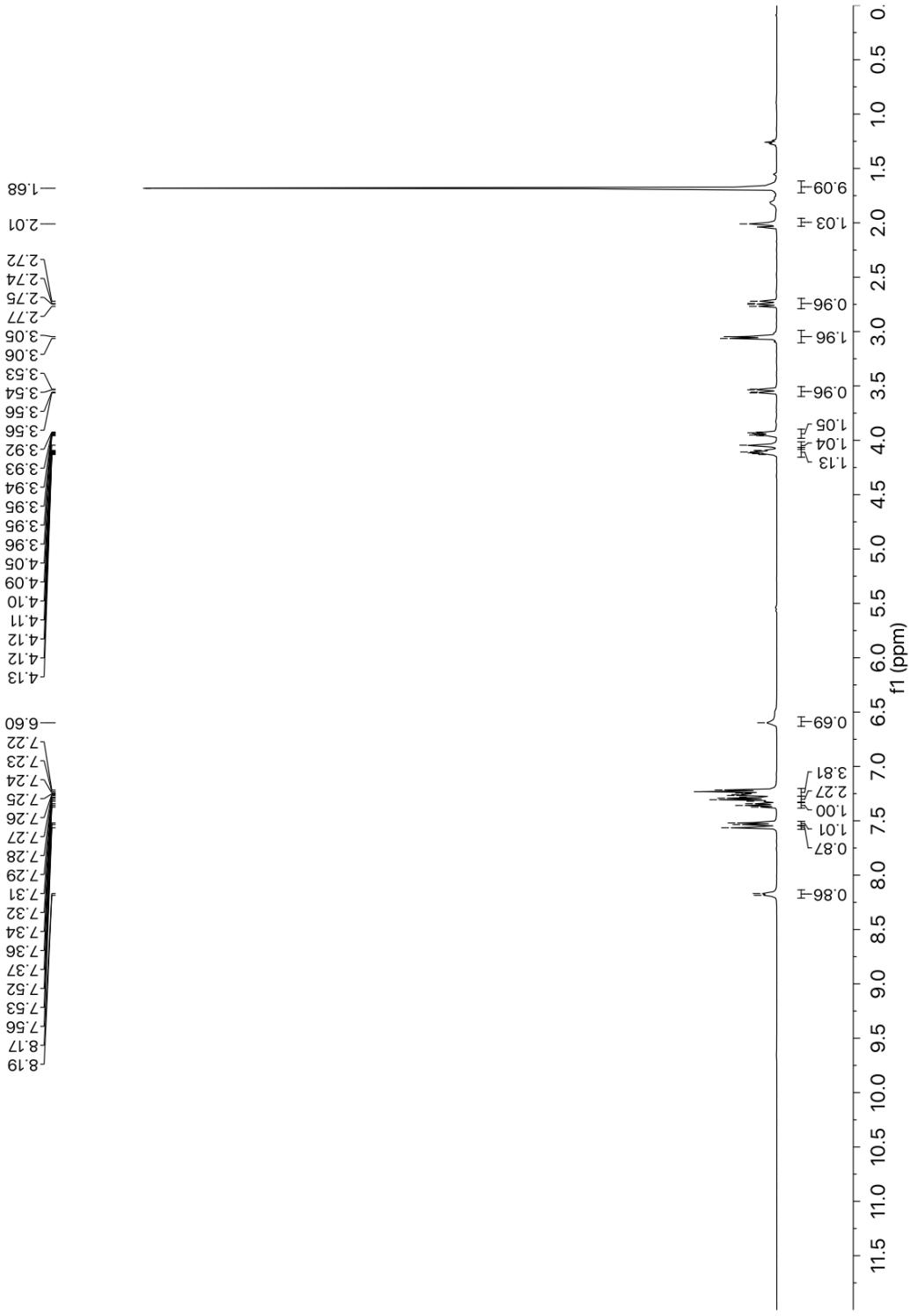
^{1}HNMR spectrum for compound **132**



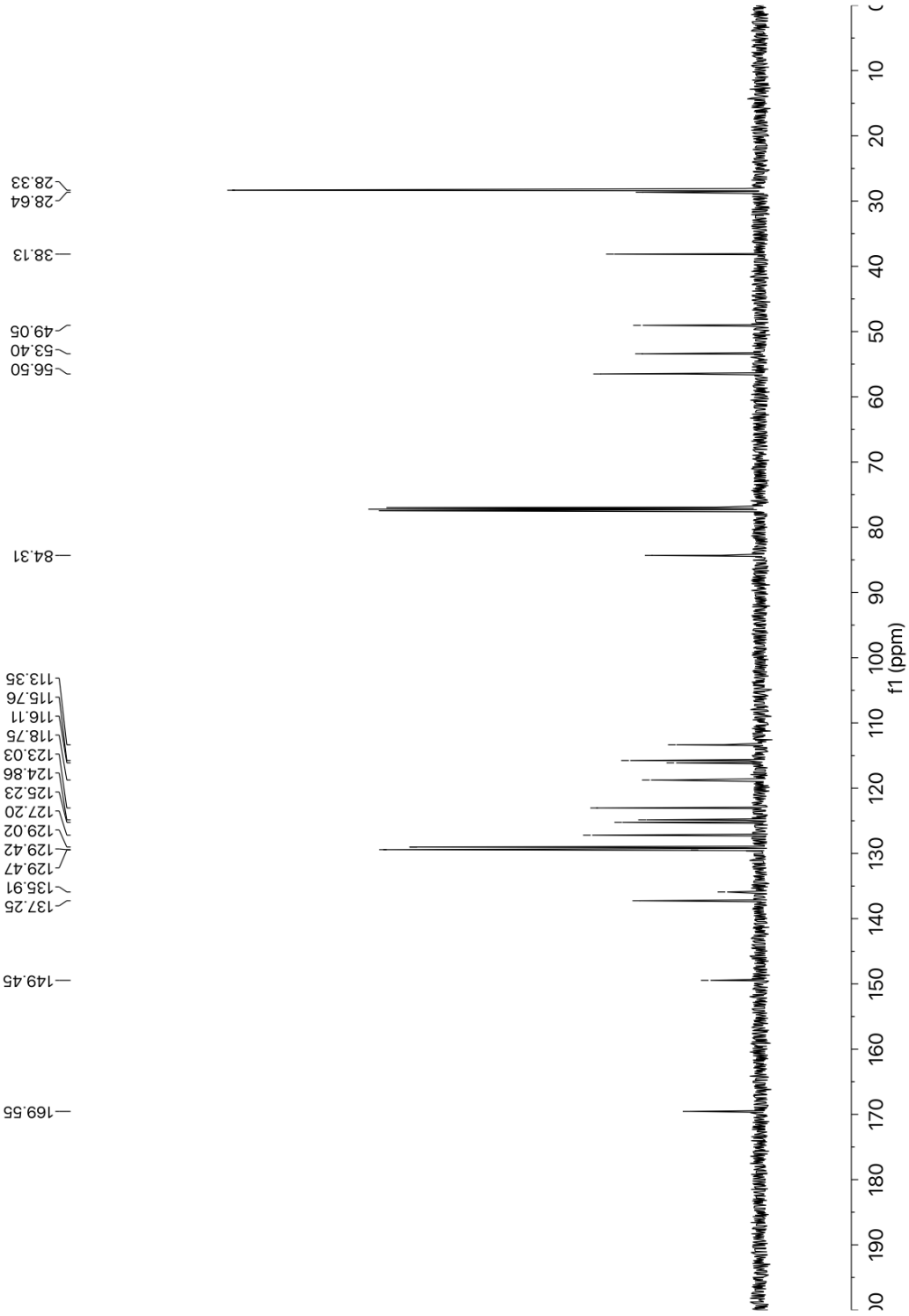
^{13}CNMR spectrum for compound **132**



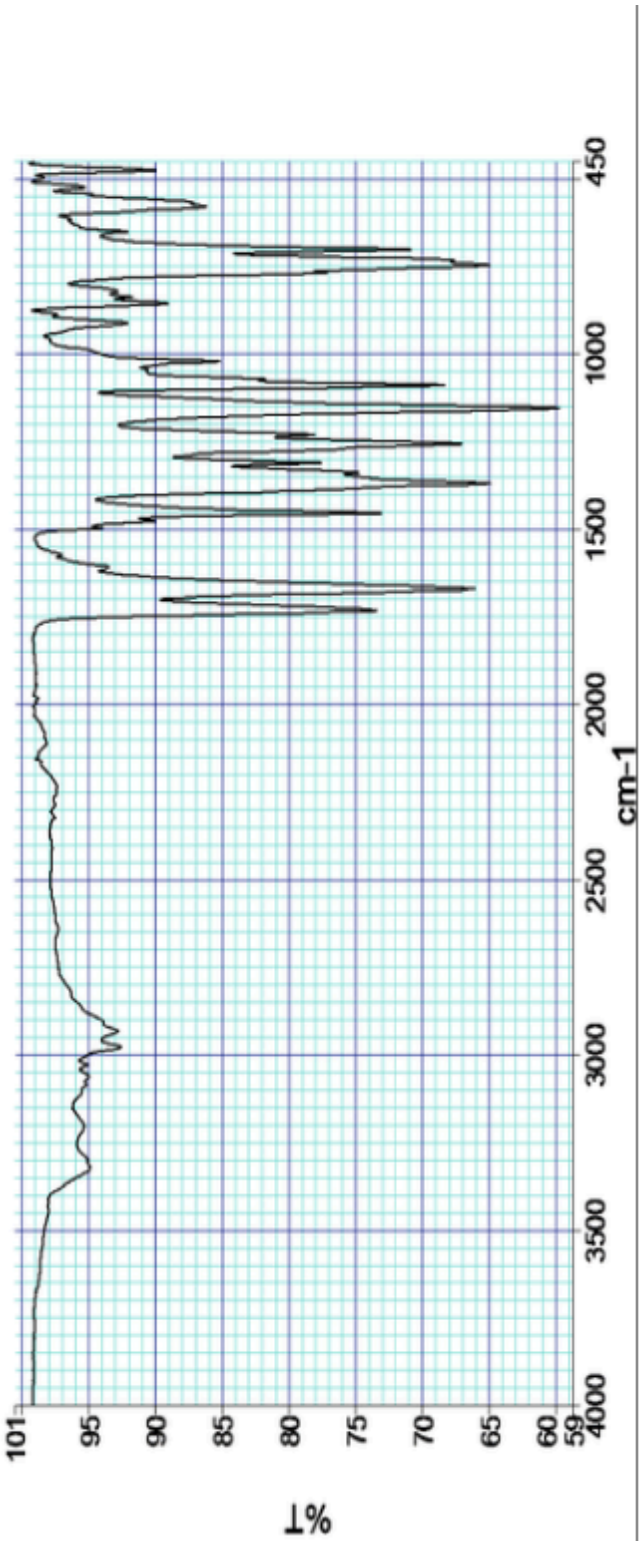
IR spectrum for compound **132**



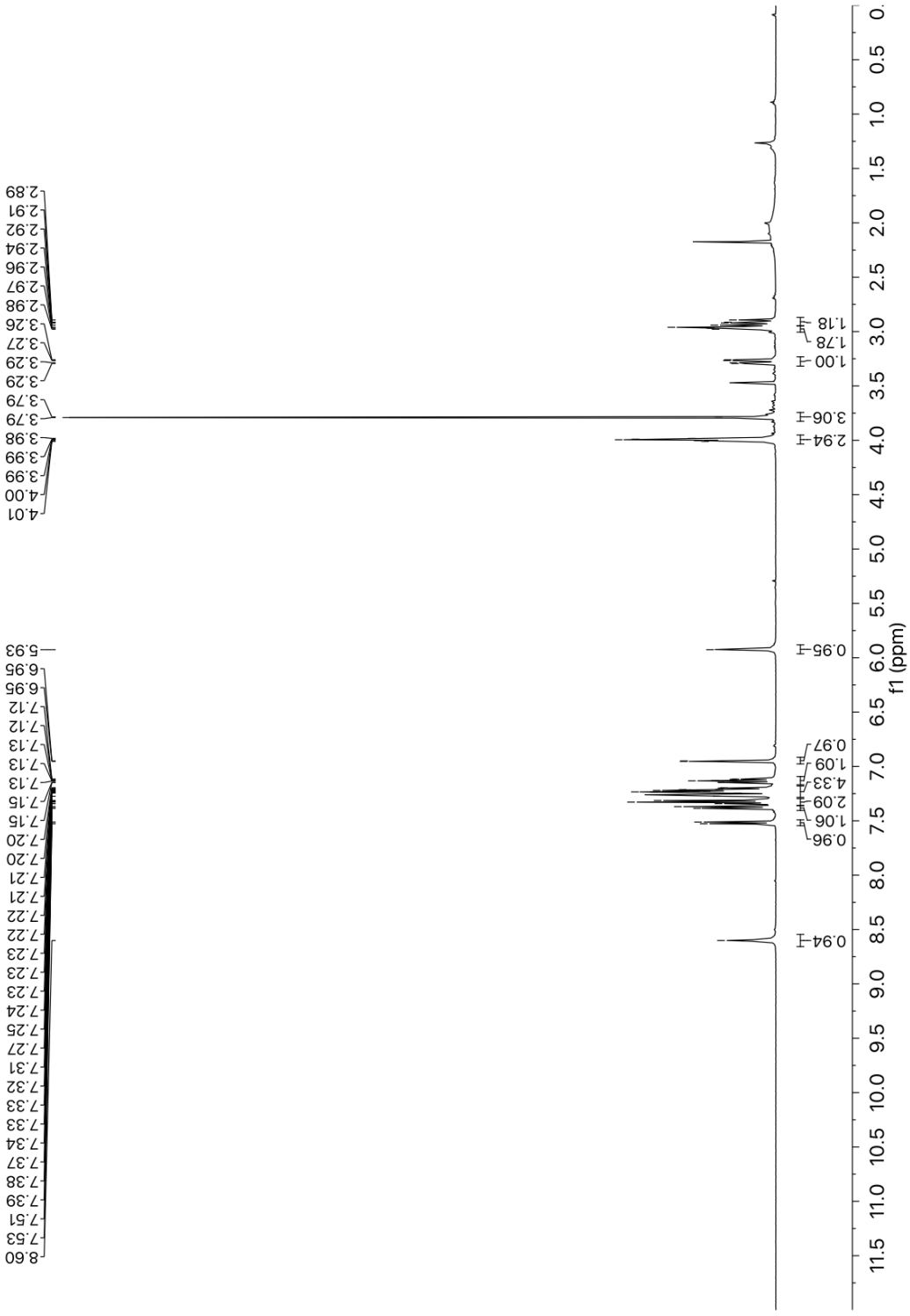
[1]HNMR spectrum for compound **133**



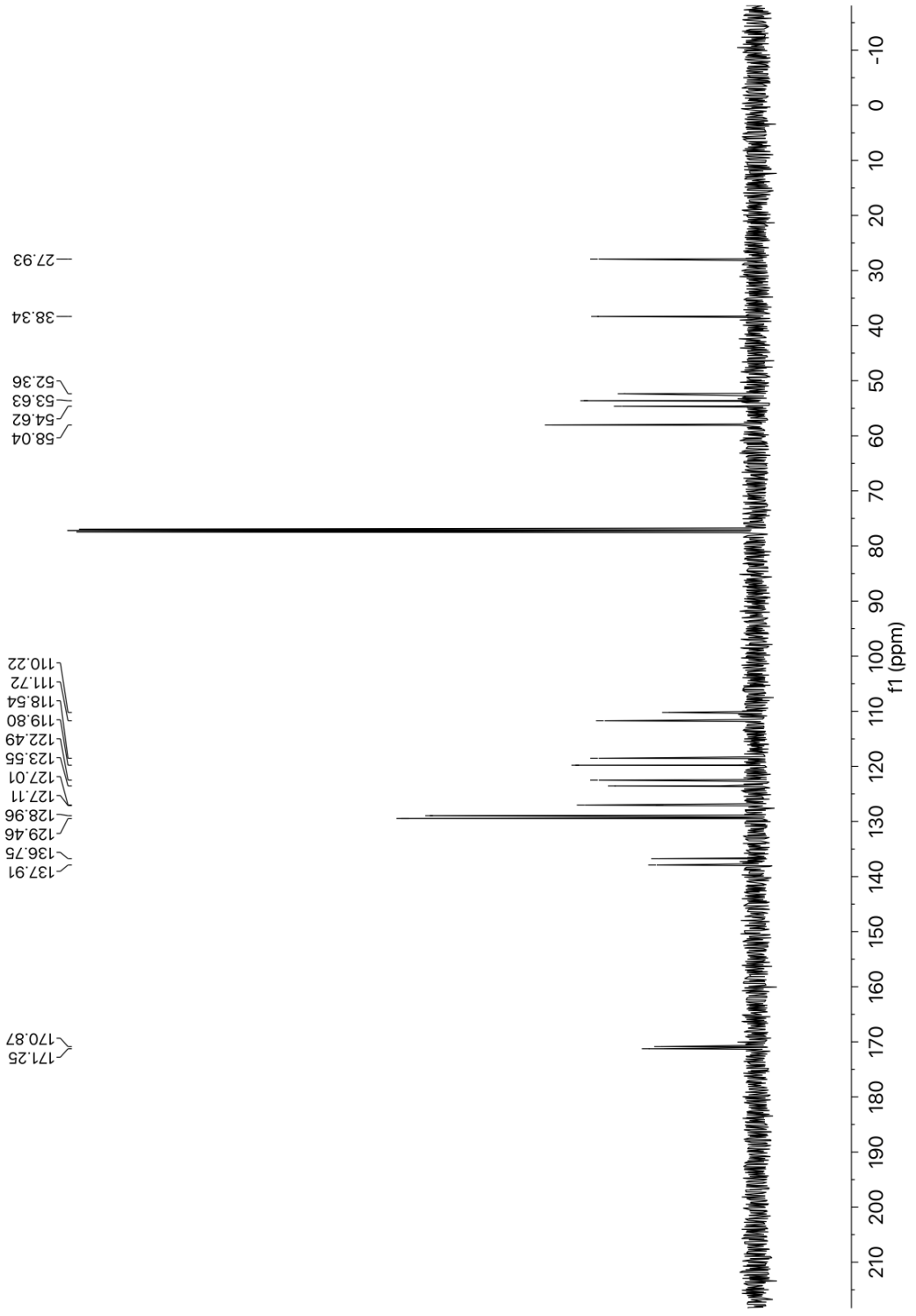
^{13}CNMR spectrum for compound **133**



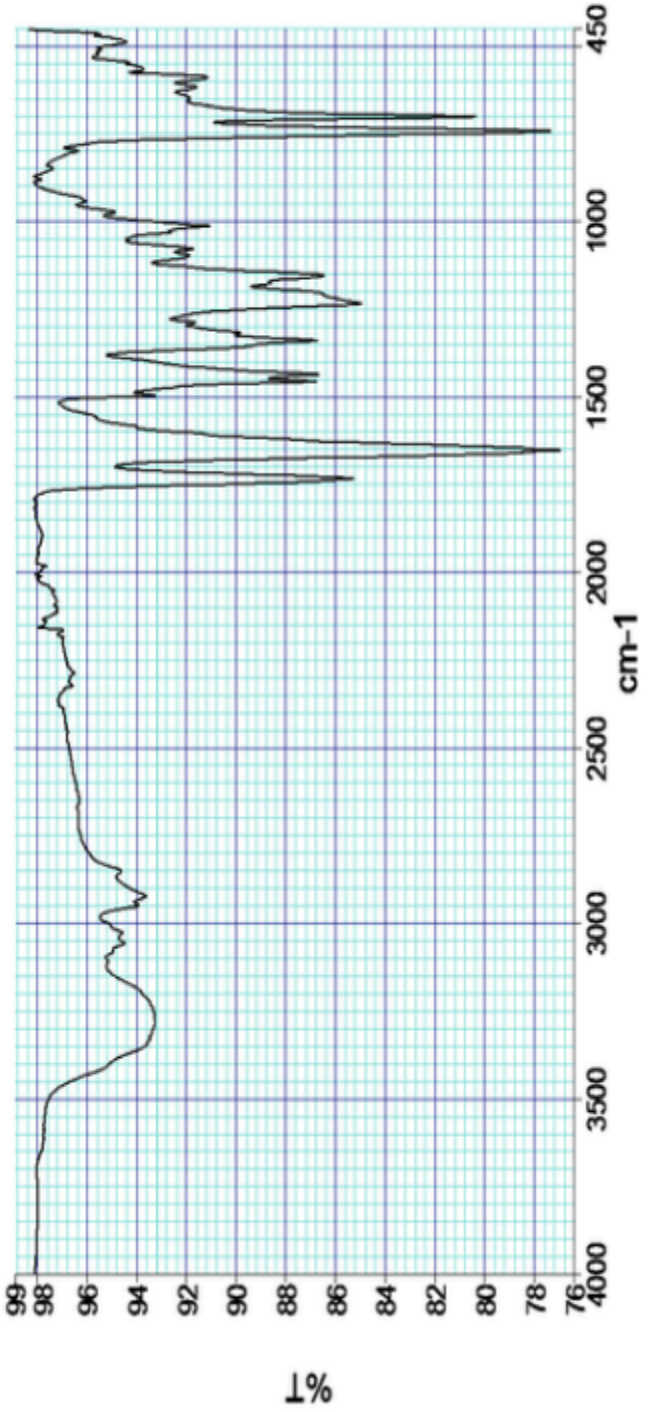
IR spectrum for compound **133**



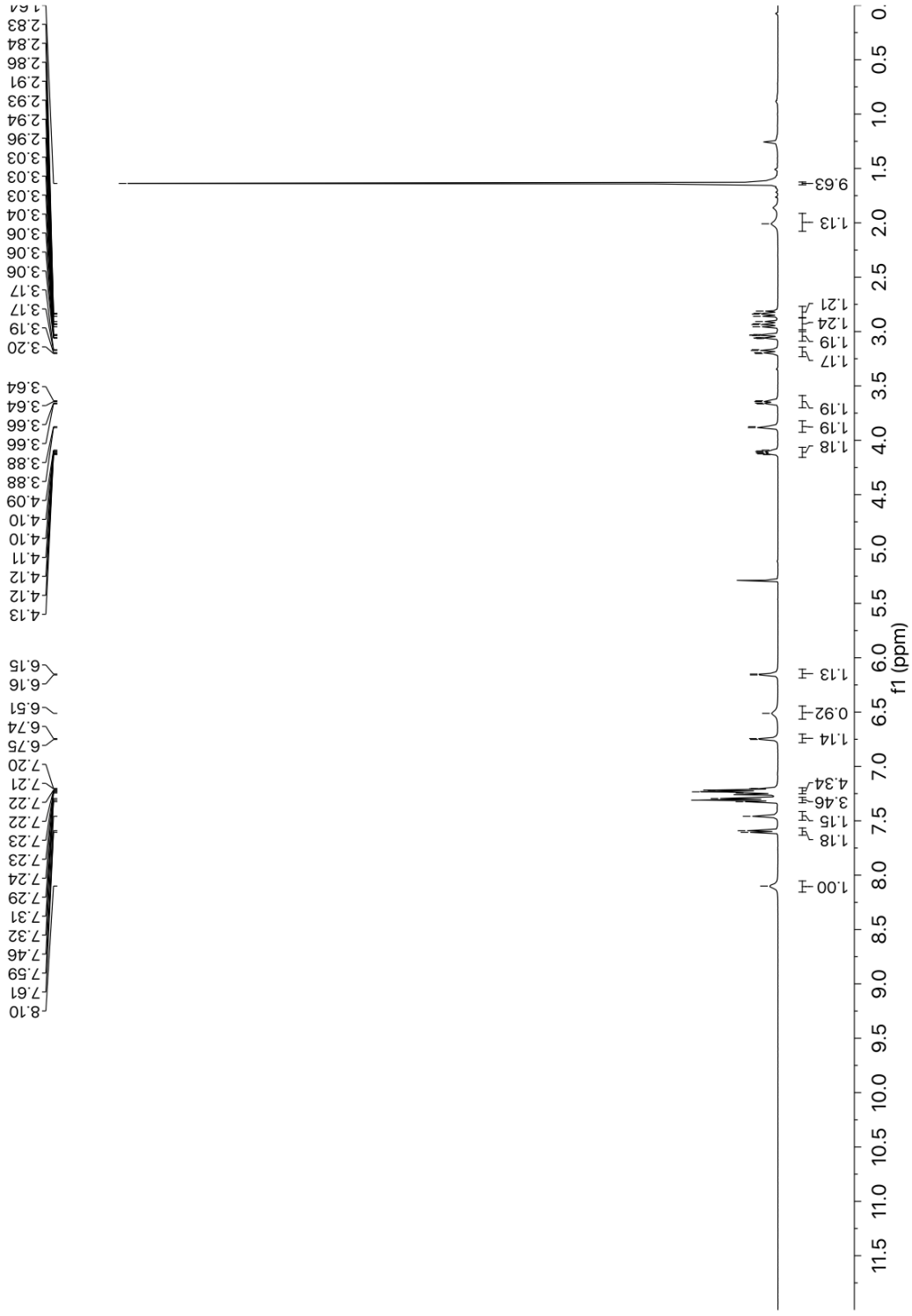
[1]HNMR spectrum for compound **139**



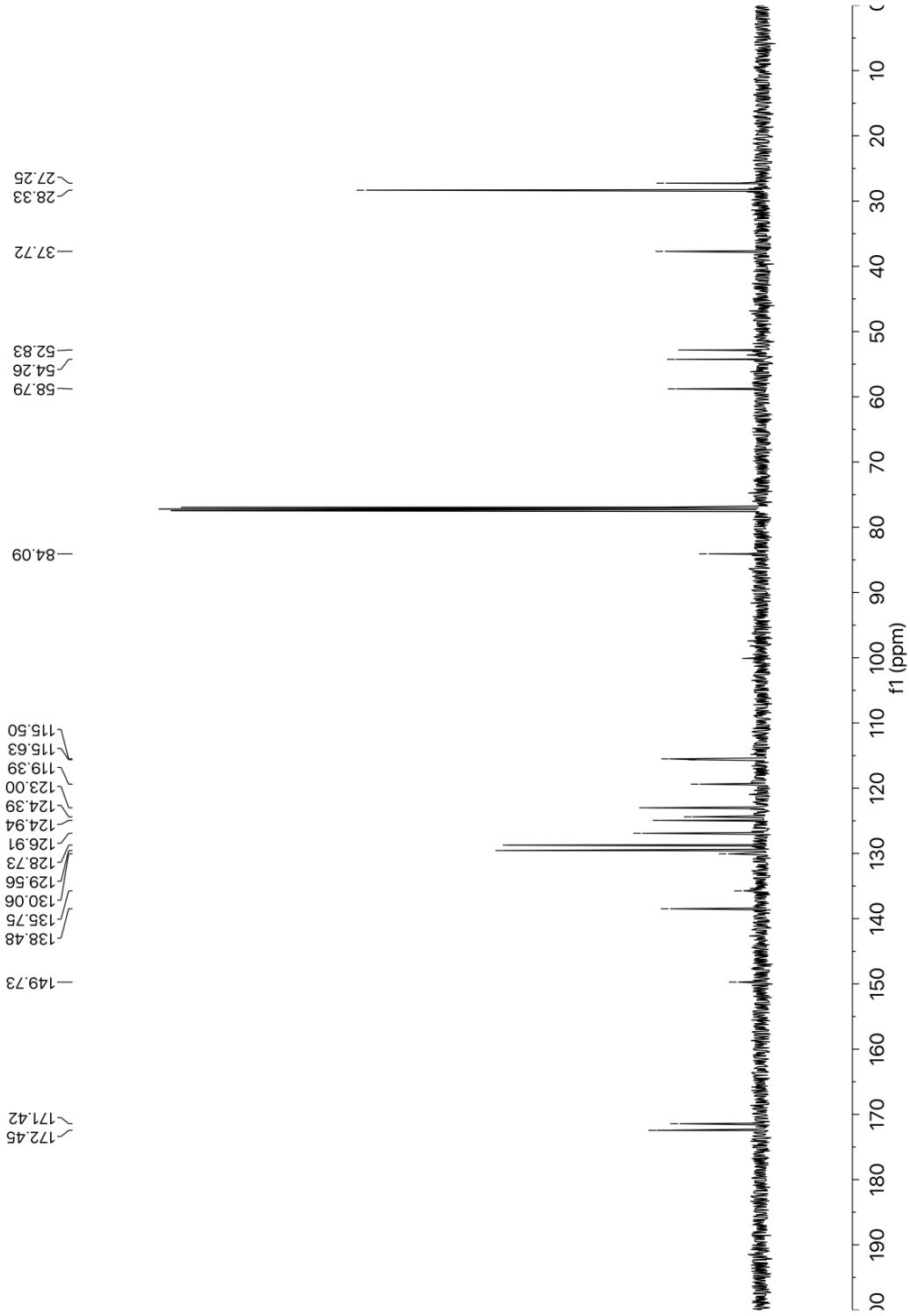
^{13}CNMR spectrum for compound **139**



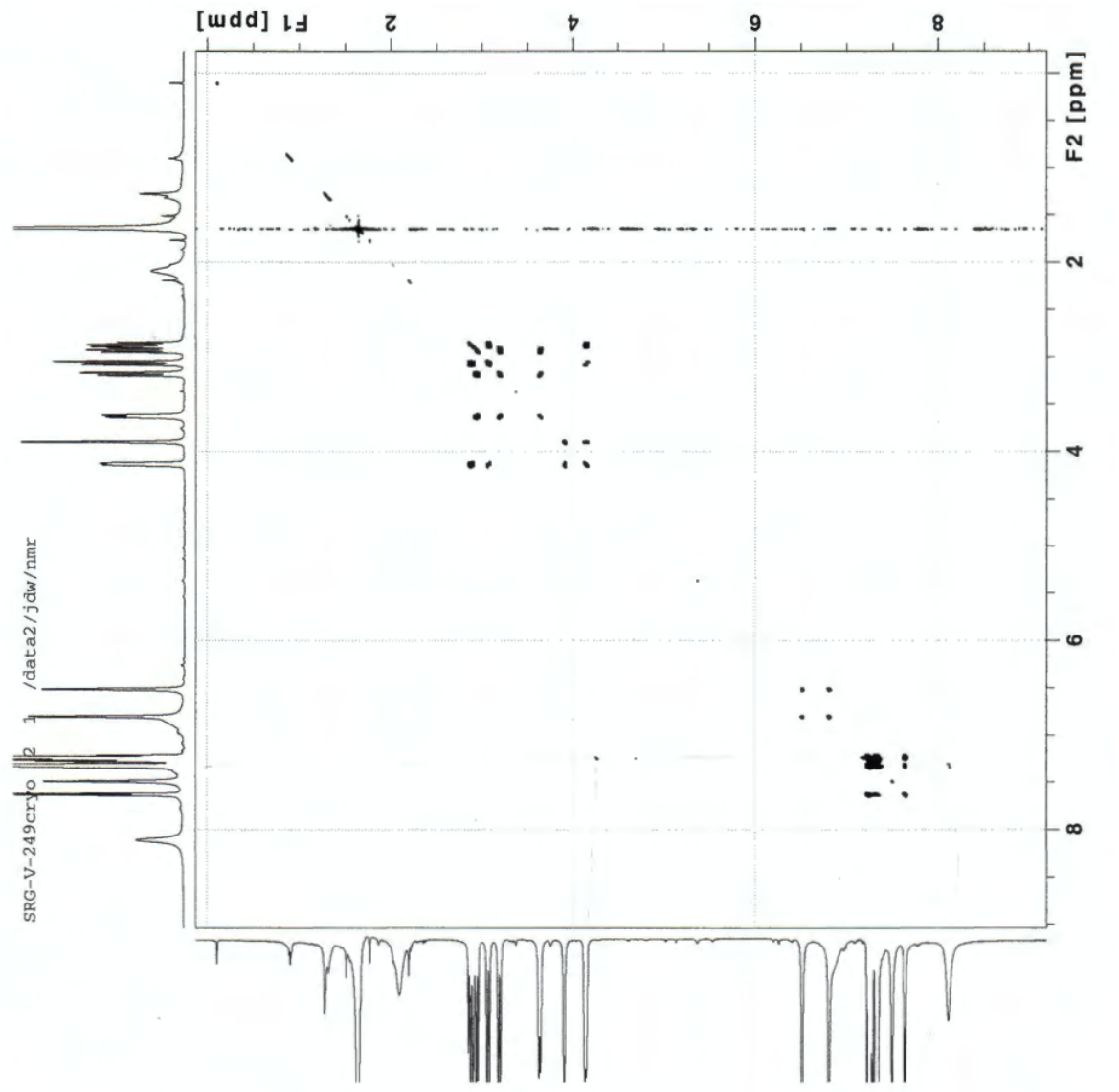
IR spectrum for compound **139**



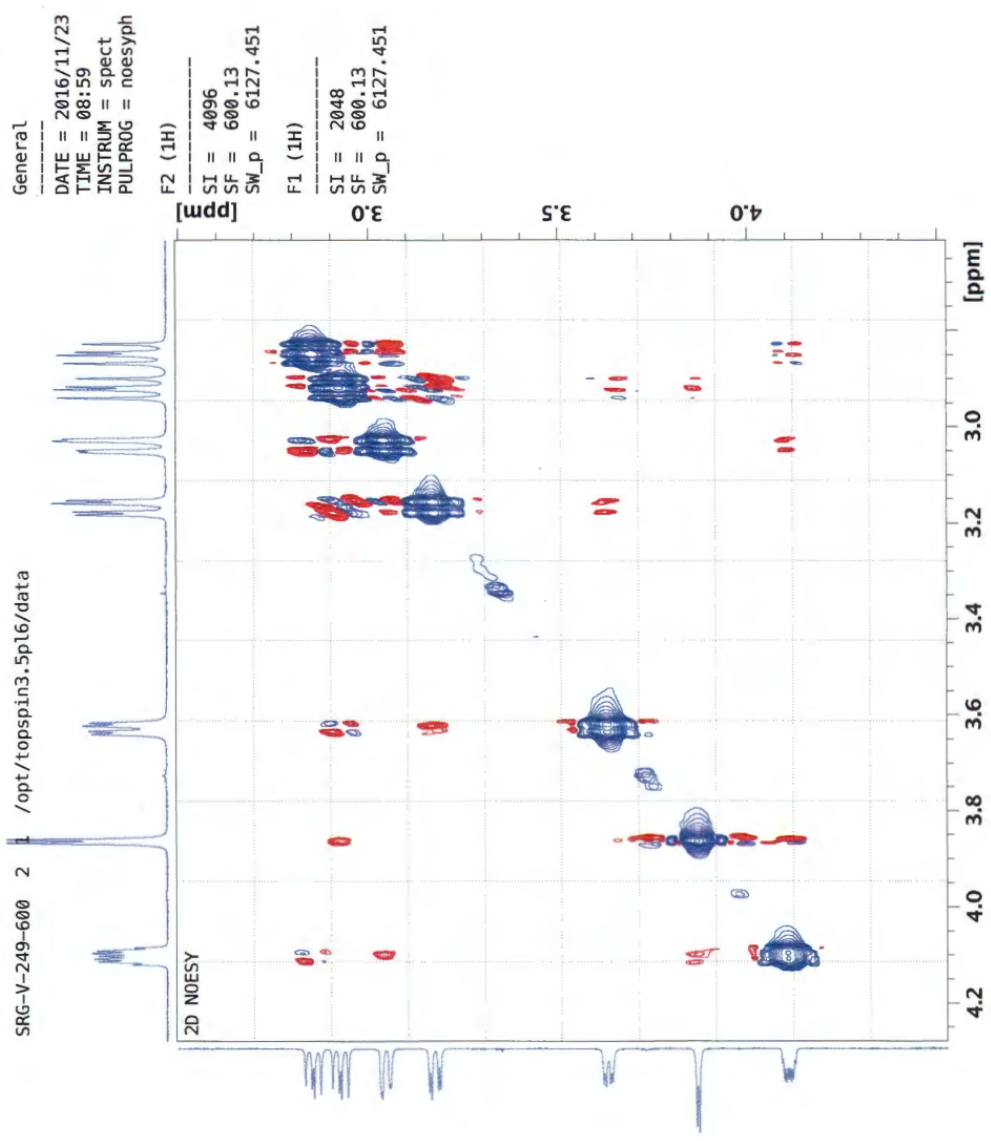
[1]HNMR spectrum for compound **137**



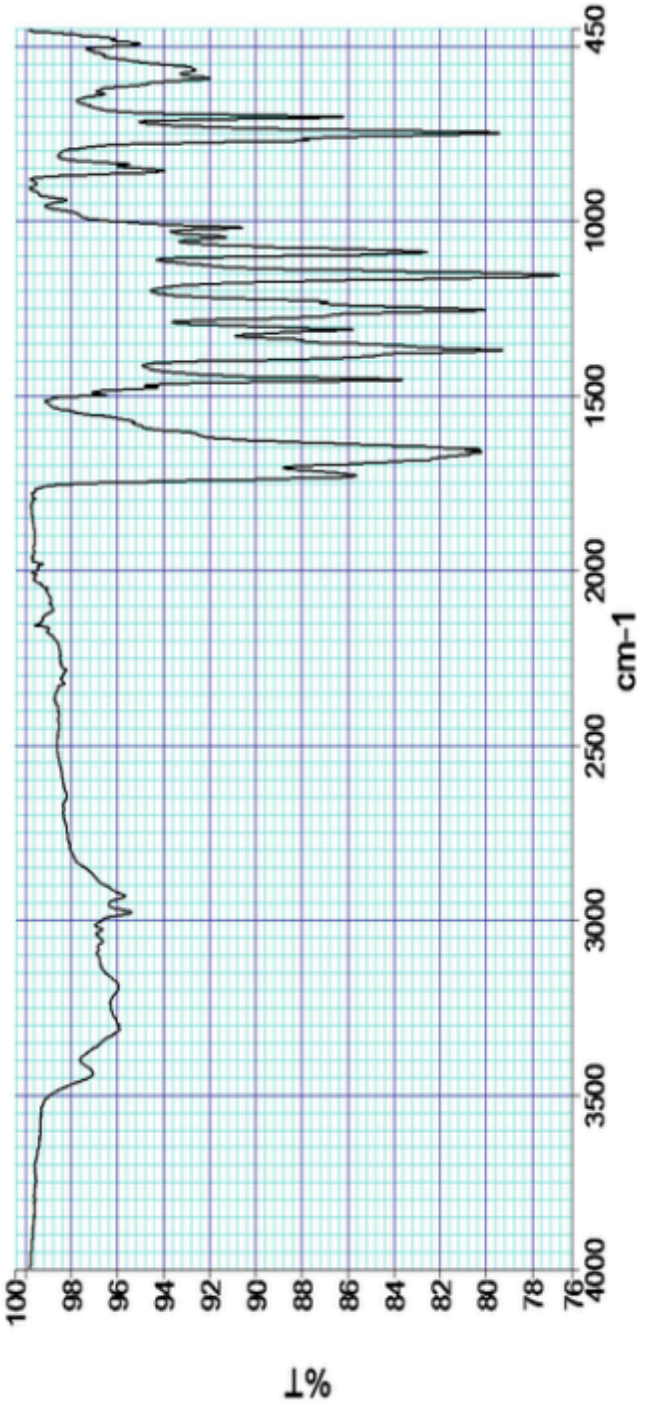
^{13}CNMR spectrum for compound **137**



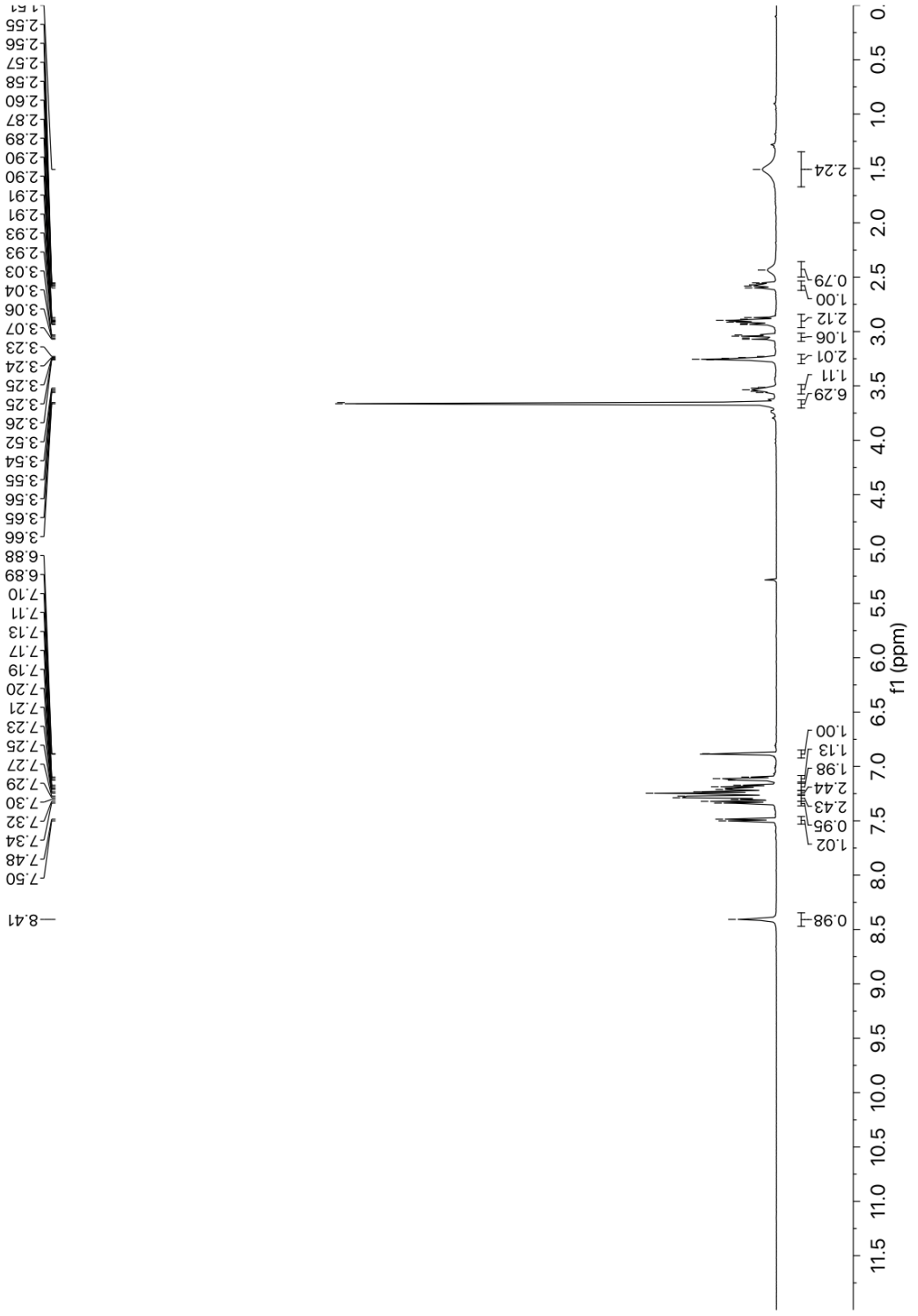
COESY spectrum for compound **137**



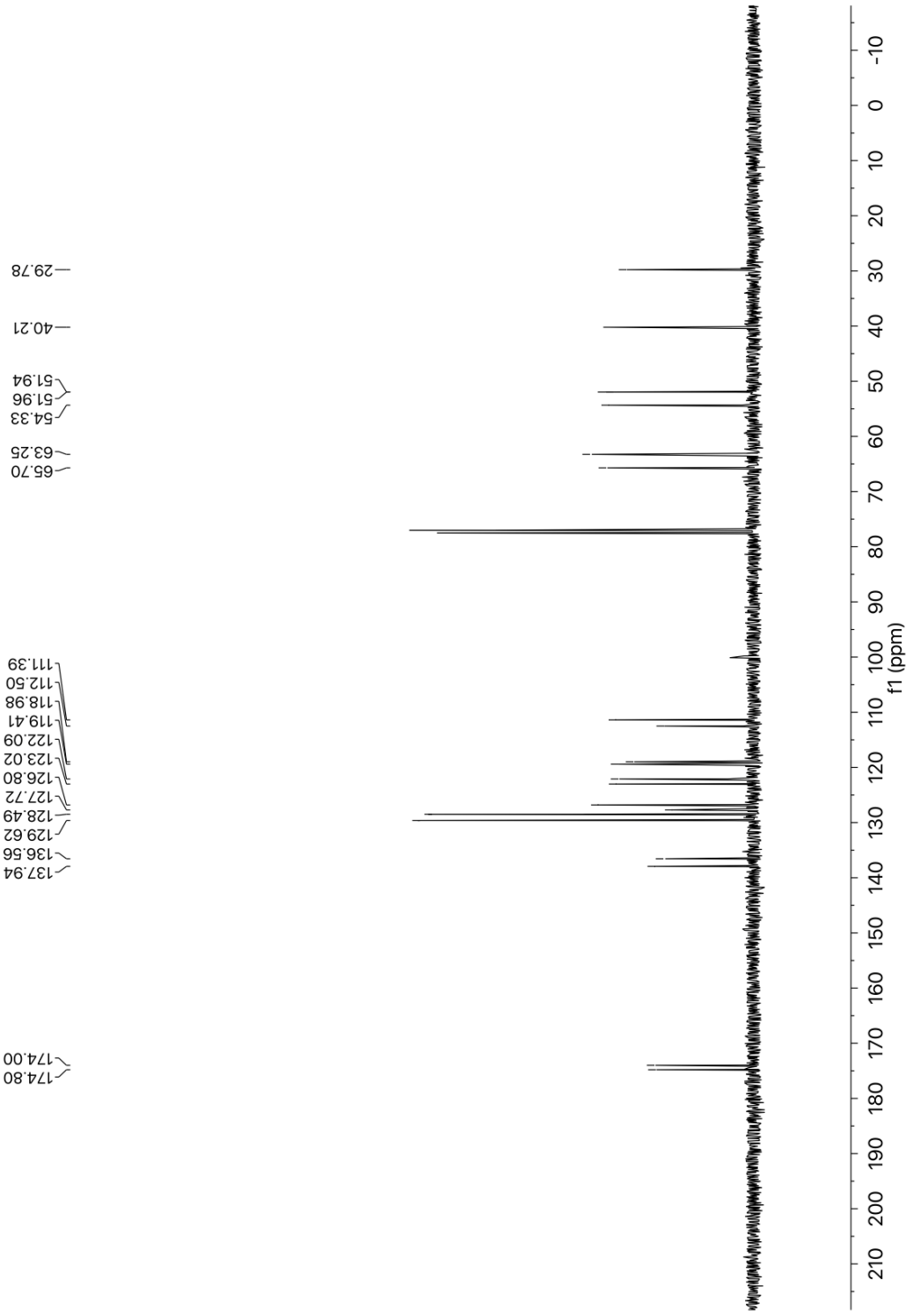
NOESY spectrum for compound **137**



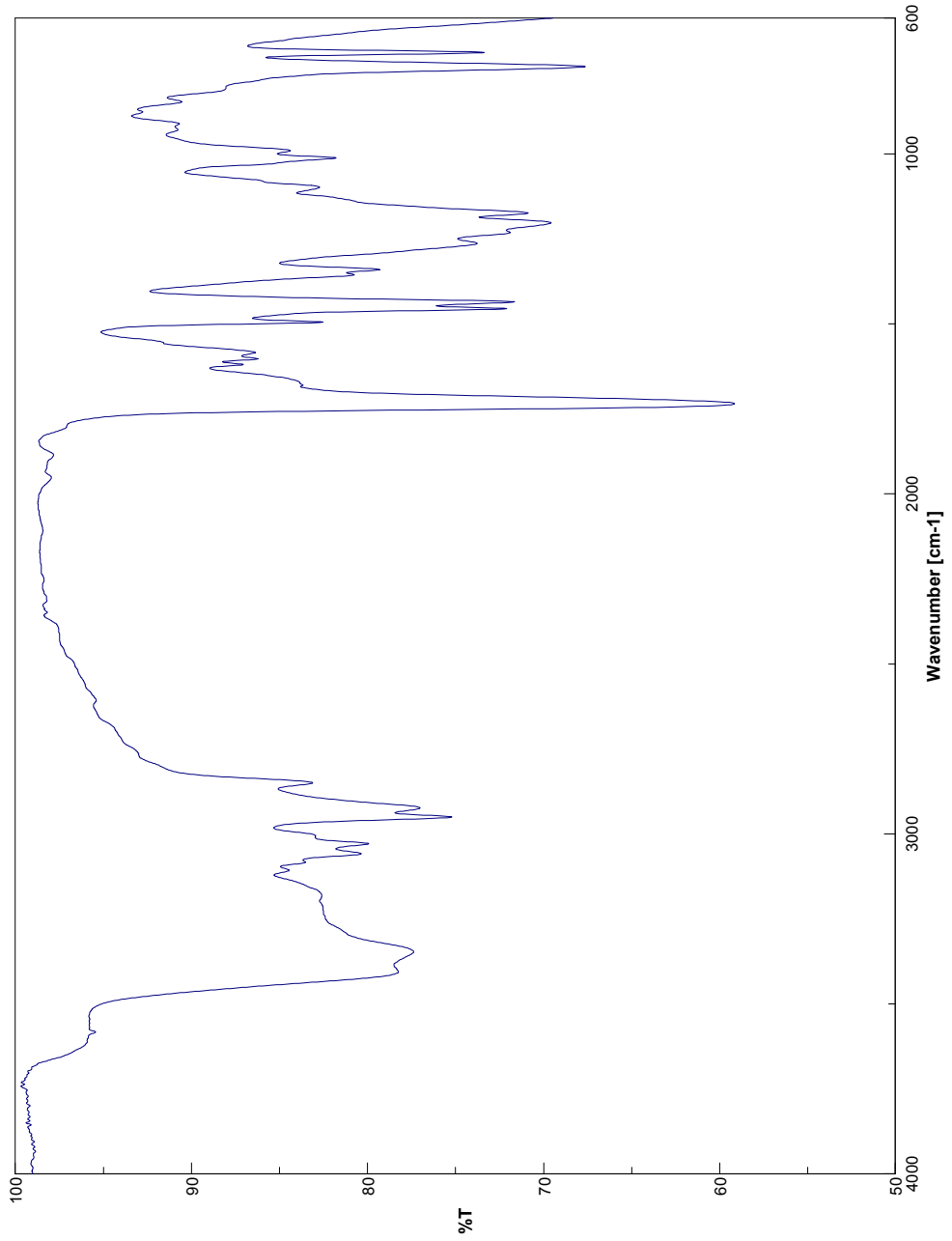
IR spectrum for compound **137**



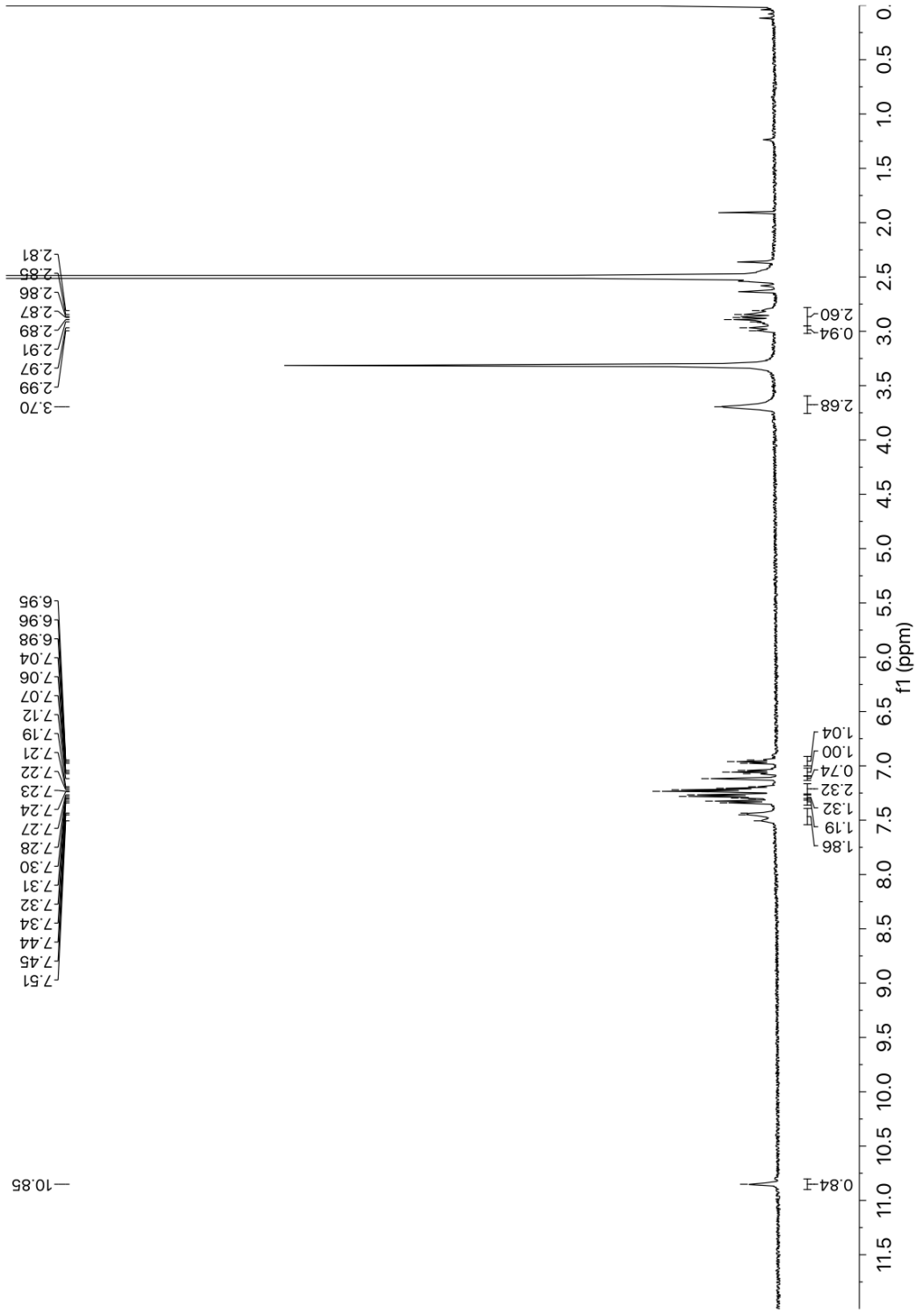
[1]HNMR spectrum for compound **138**



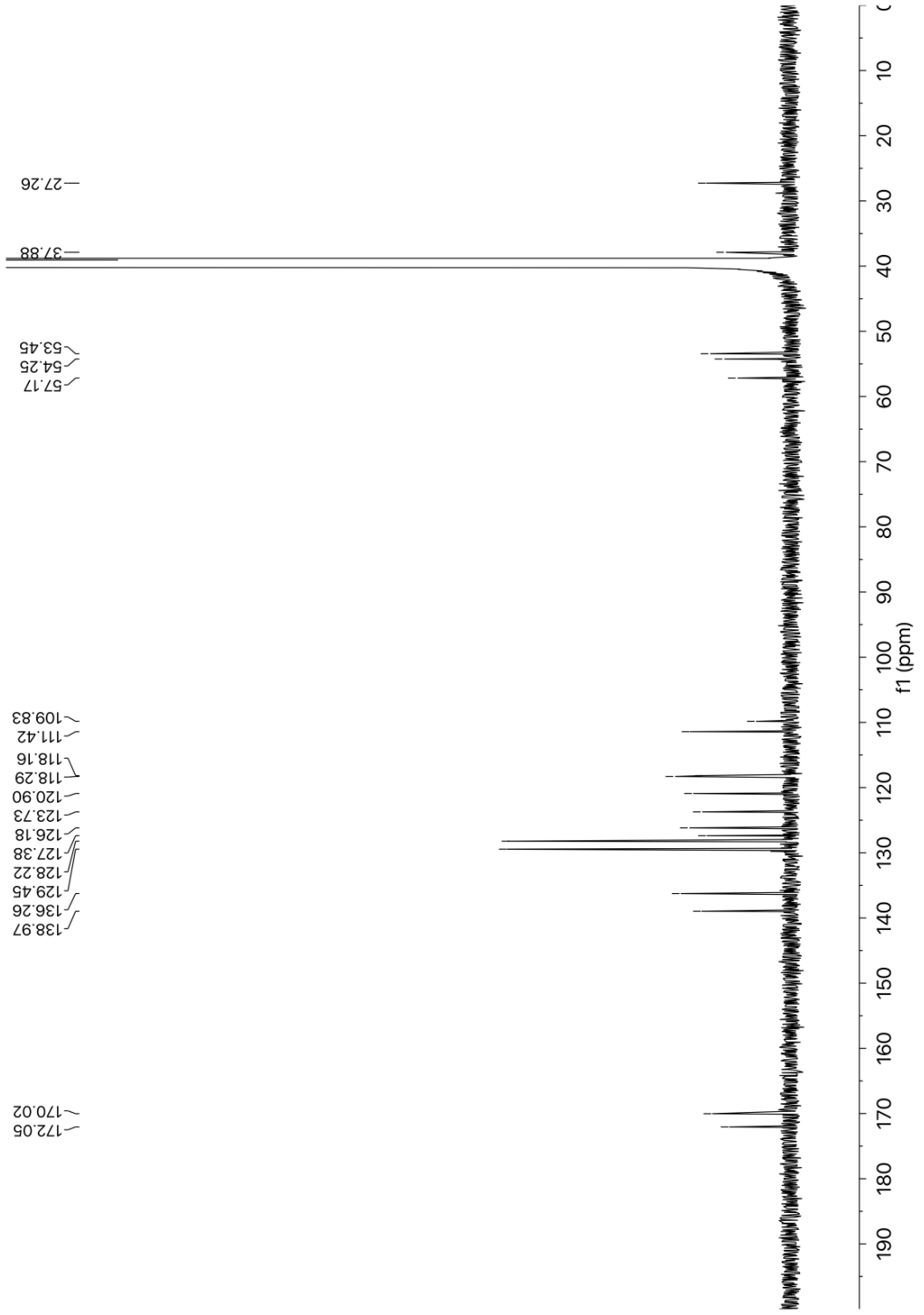
^{13}CNMR spectrum for compound **138**



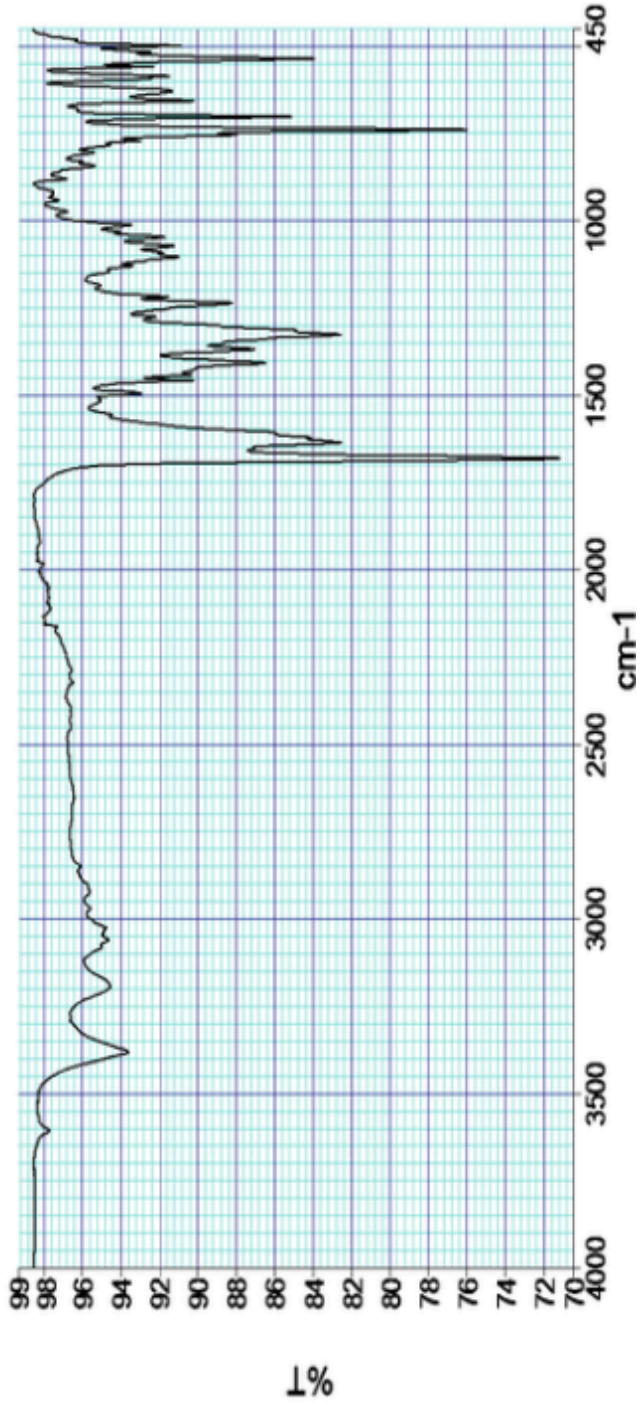
IR spectrum for compound **138**



[1]HNMR spectrum for compound **142**



^{13}CNMR spectrum for compound **142**



IR spectrum for compound **142**

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