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INDOLE BASED ANTIOXIDANTS FOR THE TREATMENT OF ISCHEMIA
REPERFUSION INJURY

By

Andrew Chapp

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

(Chemistry)

MICHIGAN TECHNOLOGICAL UNIVERSITY

2012

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This thesis, "Indole Based Antioxidants for the Treatment of Ischemia Reperfusion Injury," is hereby approved in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in CHEMISTRY.

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Date

Dedication

“To my family and friends who have always supported me”

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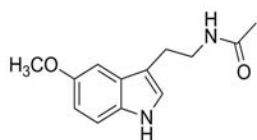
Abstract

With the number of ischemia reperfusion (I/R) injuries on the rise, and a lack of pharmacological intervention aimed at reducing free radical damage associated with I/R, we have developed 30 indole phenolic antioxidants that were synthesized by click chemistry to couple our indole with a phenolic or anisole derivative. The total antioxidant activity of the analogues was tested *in vitro* using the ferric thiocyanate lipid emulsion method. Compounds containing hydroxyl or methoxy aromatics at the 3 or 4 position on the aromatic coupled to the indole exhibited increased antioxidant scavenging. 4-methoxyindole derivatives (**8a-e**) exhibited increased scavenging ($p < 0.05$) compared to the known antioxidant butylated hydroxyanisole (BHA).

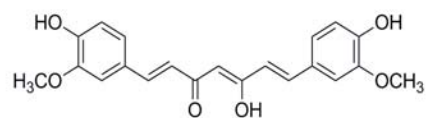
Chapter 1 Introduction:

Ischemia/reperfusion (I/R) injury constitutes one of the most common pathophysiological responses encountered during surgical procedures and in diseased states such as: cardiac arrest, stroke, organ procurement, organ transplantation, aneurysm and arterial injury.¹⁻¹⁰ The subsequent damage and cell death in response to reperfusion is unavoidable and can produce additional complications both localized and systemic including: necrosis, edema, respiratory distress syndrome and organ dysfunction.¹¹ With the number one and four leading causes of death in the United States, according to the CDC being heart disease and cerebrovascular disease, and their high association with I/R injury; there is a need for pharmacological intervention with antioxidant therapy.¹²

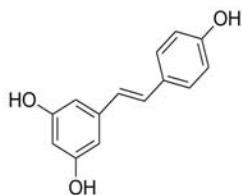
Primary literature suggests the role reactive oxygen and nitrogen species (superoxide (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), nitric oxide radical (NO) and peroxynitrite ($ONOO^-$)) (RONS) and inflammatory leukocytes play in I/R injuries.¹³ Most notably, RONS act as secondary messengers and play an important role in the regulation of apoptosis.¹⁴ As such, administration of antioxidants that inhibit reperfusion induced cellular damage and death is of therapeutic importance. The use of endogenous antioxidant therapeutics such as melatonin has provided crucial preliminary data on its ability to directly scavenge RONS and have a significant protective effect following I/R injury.^{9,15} Likewise, phenolic compounds such as resveratrol, butylated hydroxyl anisole (BHA) and polyphenols isolated from berries, vegetables and other food sources provide an additional source of antioxidants that can be utilized for therapeutic purposes (chart 1.1).¹⁶⁻²⁰



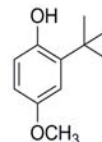
(A)



(B)



(C)



(D)

Chart 1.1. Common antioxidants; melatonin (A), curcumin (B), resveratrol (C), butylated hydroxyanisole (D).

1.1 Ischemia and the physiological response:

Ischemia is a complex physiological response caused by reduction and or stoppage of oxygen (hypoxia) and other vital nutrients to cells.⁹ Under normal aerobic conditions, oxygen (O_2) is utilized in concert with glucose in glycolysis to produce energy for the cell in the form of adenosine triphosphate (ATP).²¹ The end product of glycolysis is pyruvate which can then be utilized in the citric acid cycle (TCA), essentially re-oxidizing nicotinamide adenine dinucleotide (NAD^+) to NADH (figure 1.1). Each step in aerobic respiration transfers electrons in a sequential step-down, of energy producing reactions within the mitochondrial electron transport chain (METC), which provide ATP in response to the energy demands of the cell (figure 1.2).²¹

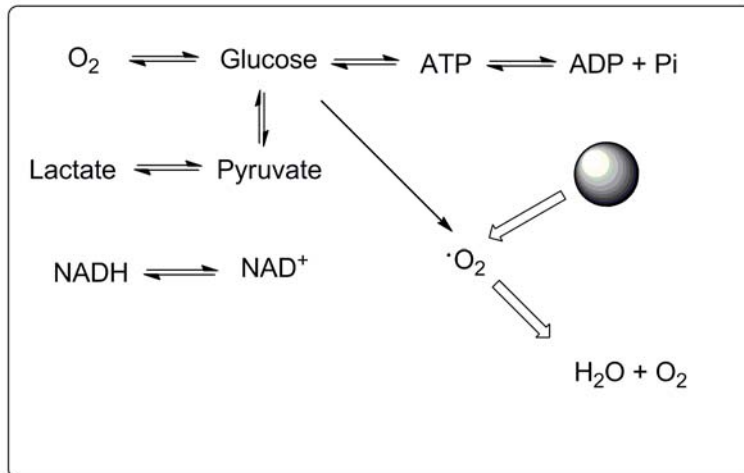


Figure 1.1. Normal aerobic respiration maintains a fine equilibrium between O_2 consumption, glycolysis, ATP production, TCA and re-oxidation of NAD^+ to NADH, while keeping reactive oxygen and nitrogen species (RONS) under control with free radical scavengers (gray circle).

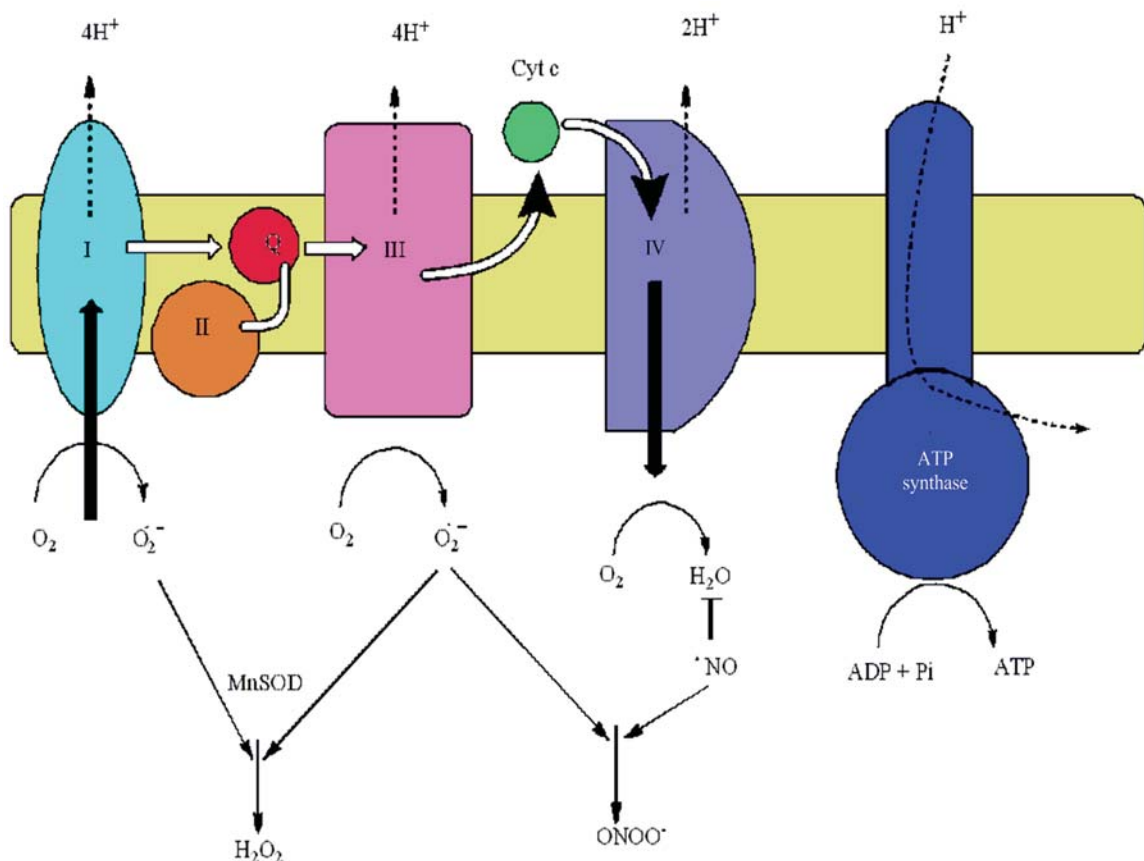


Figure 1.2. In METC cycling, *in situ* generated free radicals are detoxified by free radical scavengers like superoxide dismutase and converted to less harmful by-products.

Upon marked decrease or halting of blood flow to the cell, a number of changes occur intracellularly. First, a drop in O_2 causes a depletion of ATP stores within the cell leading to energy production via anaerobic respiration instead of aerobic respiration. Second, within the TCA under anaerobic conditions, re-oxidation of NAD^+ to NADH is drastically reduced, lactate builds in excess, damage to mitochondrial free radical scavenging enzymes increases, and electron leaking from the METC begins generating excess reactive oxygen and nitrogen species (RONS) (figure 1.3).^{7,21,22} Under aerobic conditions xanthine dehydrogenase oxidizes hypoxanthine to uric acid using NAD^+ as an electron acceptor forming NADH.²³ During anaerobic metabolism, xanthine dehydrogenase is converted to xanthine oxidase, which has detrimental effects during the reperfusion phase as will be discussed later.^{7,23}

It is particularly important to mention ischemia related to the brain. RONS generation within the brain is markedly increased during ischemia as high iron concentrations in brain tissue lead to a greater production of the hydroxyl radical via the Fenton reaction (scheme 1.1).^{21,24} Furthermore, brain ischemia exacerbates neuronal death and the modulation of cellular functions of astrocytes and microglia that can lead to neurodegenerative diseases, leading to systemic pathologies.¹⁴

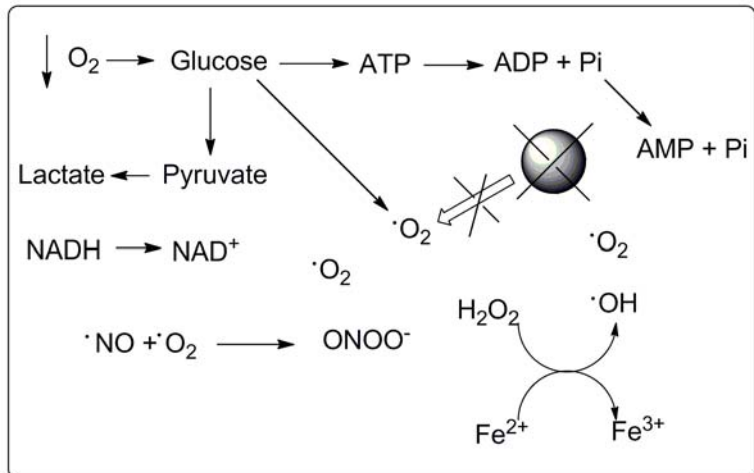


Figure 1.3. Ischemia and anaerobic respiration causes a depletion of O₂, ATP, and ADP levels. TCA re-oxidation of NAD⁺ to NADH decreases with an increase in lactate. Free radical scavengers are damaged or used up, resulting in the generation of RONS and the beginning of cellular damage.



Scheme 1.1. The Haber-Weiss (reaction 1) and Fenton (reaction 2) generates the highly reactive hydroxyl radical via the reduction of Fe³⁺ by the superoxide radical (reaction 1), and, oxidation of Fe²⁺ by H₂O₂ (reaction 2).

1.2 Reperfusion and the physiological response:

Reperfusion is the return of blood flow, O₂ and nutrients following ischemia induced from shock, cardiac arrest, hemorrhage, vascular, hepatic and other surgeries.^{1,2,6-10,25} The reperfusion stage can be broken up into two phases. First, initial return of blood flow to ischemic tissue results in the return of aerobic respiration. With the depletion of

NADH and antioxidant capabilities of the cell drastically decreased, electron transfer to O_2 due to leaks in METC generates a relatively quick increase in the superoxide radical further overwhelming cellular RONS defenses.^{21,22,26} Oxidation of the mitochondrial membrane increases and cellular processes are further disrupted.

The second phase of reperfusion involves neutrophil infiltration, histamine release and the inflammatory response.^{2,5,27} Due to mitochondrial damage, the second phase of reperfusion results in high levels of apoptosis, leading to large increases in cellular debris and a further increase in inflammation.^{2,5,21,27} Administration of RONS scavenging compounds, particularly melatonin, has drastically decreased apoptosis, RONS and inflammation due to ischemia/reperfusion injuries.

1.3 Melatonin and indoles as pharmacological agents:

Melatonin is a hormone that is produced by the pineal gland and in the digestive tract.²⁸ Its main function is in the regulation of circadian rhythms with peak blood plasma levels reached at night. Recently, melatonin has been identified as a potent antioxidant that is capable of crossing the blood brain barrier and scavenging RONS in both fatty and aqueous environments.¹⁴ The unique lipophilic and hydrophilic nature of melatonin not only provides the ability to cross the blood brain barrier, but also allows it to prevent oxidative stress in lipid membranes, and aqueous environments including, but not limited to: blood plasma, lysosomes and cytoplasm.¹⁴ As such, the free radical scavenging abilities and reduction of oxidative stress due to melatonin are considered superior to many common antioxidants, including vitamin C, glutathione and mannitol. Melatonin is capable of detoxifying two ($\cdot OH$) radicals without begetting radicals. Simply put,

melatonin is a 'suicidal antioxidant', irreversibly detoxifying radicals without recycling of the molecule and propagation of other radicals.²

Studies involving the use of melatonin related to I/R injuries have proved promising.^{2,4,5} Melatonin was found to directly scavenge RONS *in vitro* and *in vivo* by inhibiting lipid peroxidation and preventing DNA degradation.^{14,29} Administration of melatonin following surgery reduced morbidity and mortality following post-surgical induced oxidative stress, and reduced cytokines and nitrate/nitrite levels significantly.¹⁴ More importantly, melatonin significantly reduced I/R injuries in the brain where as much as 20 % of total body oxygen is consumed.³⁰ This is significant because the high oxygen consumption in the brain makes it more susceptible to RONS and oxidative damage.

With regards to cardiac protection, melatonin reduced cellular damage and exhibited antiapoptotic properties during I/R injuries. Furthermore, it has been implicated in the prevention of hypertension; presumably by inhibiting lipid oxidation in arteries and veins and reducing the build-ups of plaques seen in arteriosclerosis.¹⁴

Additionally, melatonin has been found to bind to calcium binding proteins like calmodulin.³⁰ It is well known that calmodulin binds to the small conductance calcium activated potassium channel (SK)³¹, and melatonin's interaction not only with calmodulin but possibly SK channels can either downregulate or upregulate SK channel activity. SK channels are intrinsically tied to sympathetic nerve activity which is directly related to hypertension. It is therefore possible, that in addition to reducing oxidative stress in

cardiac I/R injuries, melatonin also provides slight regulation on sympathetic nerve activity, aiding in cardioprotective effects.

Despite the seemingly endless positives associated with melatonin treatment in I/R injuries, the use of melatonin as a therapeutic antioxidant for diseases associated with I/R have limitations. First, melatonin has a high first pass metabolism with a blood plasma half-life of roughly twenty minutes.³⁰ Even though, melatonin has better antioxidant capabilities than most classical antioxidants, the quick metabolism of melatonin make it less viable for treating I/R injuries.¹⁴ To address the drawbacks of melatonin on the absorption, distribution, metabolism and excretion (ADME), analogues have been designed that retain the indole moiety and give roughly the same distribution as melatonin, but have a longer half-life.

Structure activity relationships (SAR) on the analogues identified a number of functional groups that provide the antioxidant potency seen with melatonin. The methoxy group on the indole ring was important, but not completely necessary for activity.^{13,14} Conversion of the methoxy group to the hydroxyl group was actually found to increase the amount of hydroxyl radical production, but hydroxyl coupled with the *N*-acyl group showed improved potency compared with melatonin.^{14,29} Bi and colleagues also confirmed the pharmacophore of the 5-methoxy functional group on the indole ring as being significant for potency.

To this end, significant research has been devoted to the synthesis of indole derivatives and their use as pharmacological agents. Synthetically, indole containing structures present a unique and chemically tunable backbone that can give rise to

numerous and sometimes dual acting lead compounds for various diseases and conditions including: anti-inflammatory, antioxidant, anti-cancer, cardio and neuroprotective agents.

As anti-inflammatory agents, indole backbones have been utilized in two well-known non-steroidal anti-inflammatory drugs (NSAID) namely, Indomethacin and Acemetacin (chart 1.2). Indomethacin and Acemetacin have been extensively studied as NSAIDs, yet SAR studies for the indole class of NSAIDs has been lacking. With the discovery of indoles as efficient scavengers of free radicals, the anti-inflammatory properties of indoles were hypothesized to be attributed to the above observation. Indomethacin and Acemetacin were found to be effective scavengers of RONS, reducing neutrophil infiltration, histamine release and apoptosis which may contribute to their anti-inflammatory activity.^{32,33}



Chart 1.2. NSAIDs Indomethacin (left), Acemetacin (right).

Likewise, transient brain ischemia has long been known to produce ROS that lead to neuron death post-ischemia. Indomethacin was found to rescue neurons during brain ischemia and also inhibit COX1 and COX2 isoforms, drastically reducing oxidative stress, DNA oxidation, and cellular death following reperfusion.³⁴ Despite its excellent capabilities as an NSAID, Indomethacin is not well tolerated in the gastrointestinal tract,

and analogues have been developed that still possess the NSAID activity with decreased side effects.

As neuroprotective agents, indole derivatives are capable of detoxifying free radicals associated with brain ischemia injuries and preventing the drastic and detrimental death of neurons in the brain. Carvedilol and 1,2,3,4-tetrahydro- β -carboline derivatives show potent antioxidant scavenging in addition to exhibiting neuroprotective effects (chart 1.3).³⁵⁻⁴⁰ Additionally, Biradar and colleagues synthesized 2,5-disubstituted indoles via a Knoevenagel condensation that were potent scavengers of free radicals (chart 1.4).³³

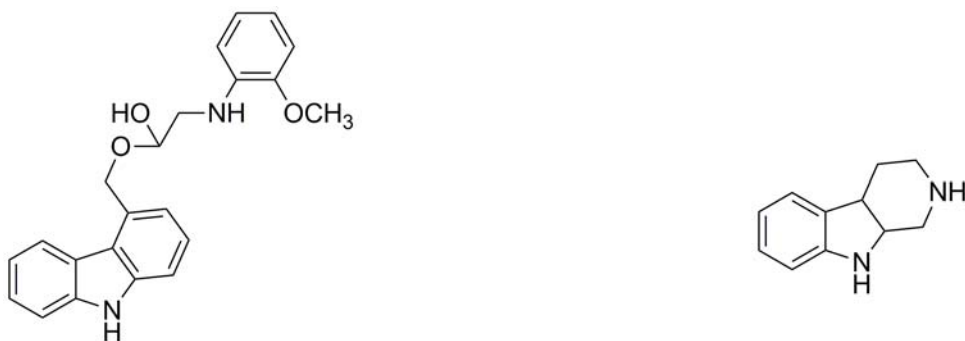


Chart 1.3. Indole derivatives Carvedilol (left), 1,2,3,4-tetrahydro- β -carboline (right).

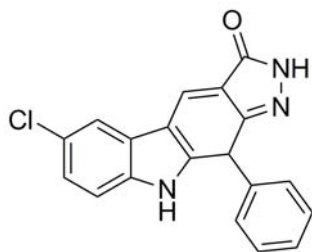


Chart 1.4. 6-chloro-10-phenyl-9,10-dihydropyrazolo[3,4-b]carbazol-3(2H)-one as an antioxidant and cytotoxic agent.

As cardioprotective agents, certain indole containing compounds have been found to prevent lipid peroxidation, especially LDL, which is thought to be a major mechanism in the development of arteriosclerosis. Andreadou and colleagues specifically examined the antioxidant activity of an indole containing compound C6458 (chart 1.5) and found it to be an efficient scavenger of the hydroxyl radical. Additionally, C6458 is protective against myocardium injury from I/R and reduced infarct size in rabbits. Presumably this works by decreasing lipid peroxidation due to free radicals evident by the reduction in malondialdehyde levels, a well-known marker of oxidative stress.⁴¹

Similarly, Stobadine was found to not only reduce ROS, and diminish impairment of myocardium muscle from oxidative stress, but also possessed neuroprotective effects via a similar free radical scavenging mechanism as C6458. Stobadine, like melatonin, has similar lipophilic and hydrophilic characteristics that make it a suitable candidate for both aqueous and lipid environments. Pharmacokinetically, Stobadine is readily absorbed by the gastrointestinal tract, may be administered by IV and can easily penetrate the blood brain barrier. Slightly different from C6458, Stobadine has been found to more effectively scavenge lipid radicals during the propagation stage rather than scavenging during the initiation stage. This prevention of LDL oxidation is thought to directly correlate to the cardioprotective effects exhibited by Stobadine.⁴²



Chart 1.5. Cardioprotective indole derivatives Stobadine (left), C6458 (right).

1.4 Coupling of indoles with known compounds for use as treatment of I/R:

With the identification of melatonin as a possible lead, manipulation of the indole ring structure from that of melatonin has been found to lengthen metabolism time, and increase antioxidant potency. While some research groups have focused on melatonin analogues alone (chart 1.6), a common and more popular method is coupling either melatonin or an indole variant with a known antioxidant or anti-inflammatory compound. Bi and colleagues have successfully synthesized a series of melatonin analogues with different anti-inflammatory/antioxidant properties that reduce cellular damage associated with I/R injuries.^{13,36-38,43}

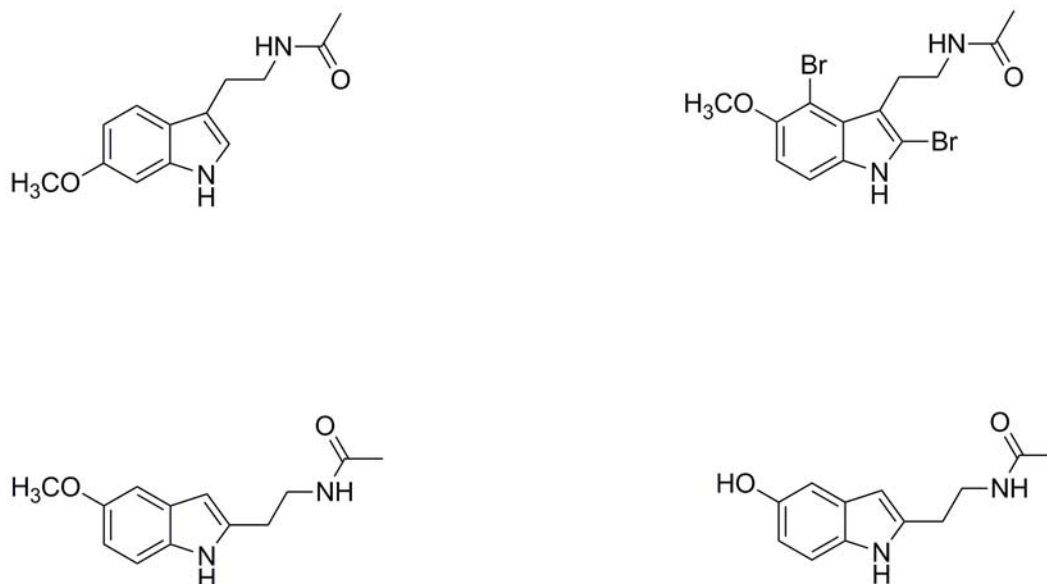


Chart 1.6. Melatonin analogues with variations on the indole ring, and amine chain.

Two well-known derivatives of melatonin-related compounds coupled with an antioxidant include DTBHB and GWC20. Both were found to inhibit free radical induced

activity dependent on the attachment point for the triazole functional group. Additionally, cardioprotective effects were seen with triazole containing indoles by the reduction of malondialdehyde in rabbits during I/R injuries.²⁹

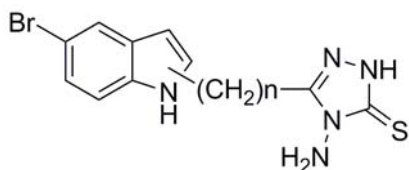


Chart 1.9. Chemical structure of a melatonin analogue containing a triazole moiety.

1.5 Problem Statement:

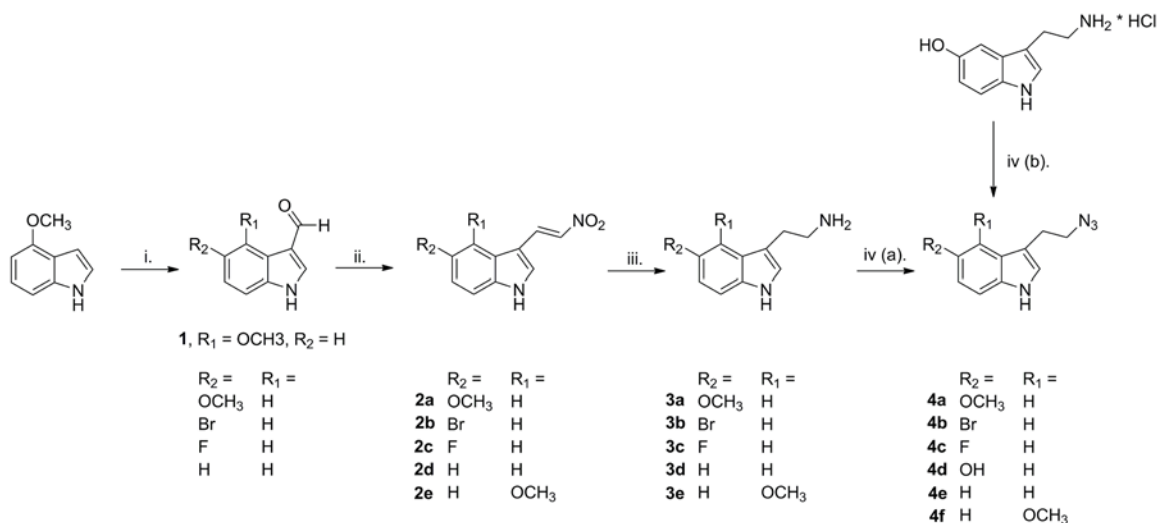
As discussed above, a wide array of compounds can be coupled with melatonin and indole based backbones to tailor new pharmaceutical agents possessing anti-inflammatory, antioxidant, cardioprotective or dual acting effects. The degree of variation seen on new indole or melatonin based compounds is only limited by creativity and the desire to produce new, more effective compounds falling under different classifications for their therapeutic use. To this end, we sought to couple the intrinsic antioxidant capabilities of melatonin and indole derivatives with phenolic/anisole derivatives to achieve extremely potent antioxidants for the treatment of I/R injuries.

Melatonin and melatonin analogues have been shown to act synergistically with the native antioxidant defense system, which includes superoxide dismutase (SOD), vitamin C, vitamin E, glutathione, mannitol, etc. to mitigate oxidative stress and damage.^{2,8,23} Using this knowledge, we designed thirty compounds based on a strategy of coupling structural indole analogues of melatonin via a click reaction with phenols or anisoles. Structurally speaking, this approach gave three moieties capable of scavenging

free radicals; the indole moiety, the triazole moiety and the phenolic/anisole moiety with the added possibility of cardioprotective effects from the triazole moiety. These compounds were tested for their total antioxidant capabilities as will be discussed later.

Chapter 2 Results and Discussion:

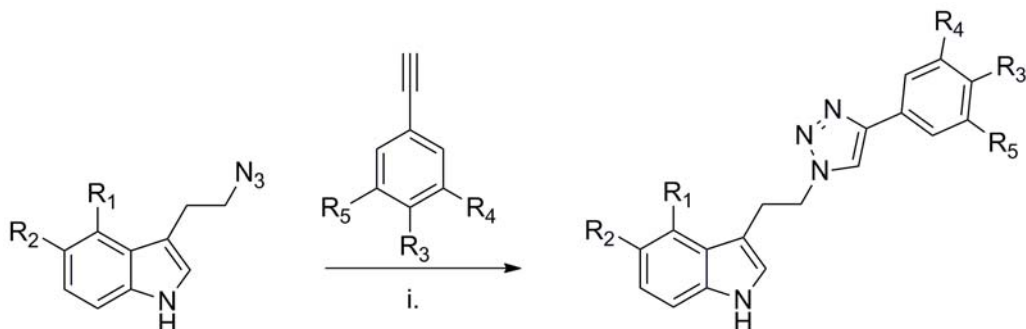
2.1 Synthesis:



Scheme 2.1. Reagents and conditions: (i) (1) DMF, POCl₃ 0 °C, 30 min. 40 °C 1 h., (2) NaOH, reflux 1 h.; (ii) NH₄OAc, CH₃NO₂, reflux 1 h.; (iii) (1) LiAlH₄, THF, reflux 1 h., (2) Rochelle's salts, ether, 12 h.; (iv) (a) TfN₃, CuSO₄(cat), K₂CO₃, 2:1 MeOH:H₂O, (b) TfN₃, ZnCl₂(cat), Et₃N, 3:7 H₂O:acetonitrile, 5 min. RT.

Starting with 4-methoxyindole, a Vilsmeier-Haack reaction converted the indole to the desired indole-3-carboxaldehyde **1** that subsequently underwent a Henry reaction to afford the corresponding indole-nitroolefins **2a-e**. After reduction with lithium aluminum hydride the desired tryptamines **3a-e** were afforded in relatively high yield. Conversion of the tryptamine amino group to the corresponding azides **4a-f** followed one of two routes. The first route involved solubilizing the desired tryptamine in a 2:1 mixture of methanol:water with a catalytic amount of copper (II) sulfate and potassium carbonate. Freshly prepared triflic azide in dichloromethane was added and the conversion time, anywhere between 2-24 hours, depended on the substituent off the indole ring. The conversion of serotonin hydrochloride required an alternative approach.

Serotonin hydrochloride is unstable in aqueous base and decomposed at an accelerated rate when attempting to convert the amino group to azide. Serotonin in itself degrades quickly when exposed to light and moisture, so a quick conversion method from amine to azide needed to be employed. Previously, dopamine hydrochloride had been converted successfully to the desired azide utilizing an alternative method. Using serotonin hydrochloride treating it with a 3:7 solution of acetonitrile:water with triethylamine and a catalytic amount of zinc (II) chloride, and triflic azide, the serotonin amine was converted to the desired azide **4d** in five minutes. It should be noted that once converted to the azide, serotonin continues to degrade at an appreciable rate, requiring a quick workup and purification.



Scheme 2.2. Reagents and conditions: (i) CuI, 2:1 PEG-400:DMF, RT 12 h.

Table 2.1 Indole/Phenolic prepared analogues via click reactions.

Compound	R ₁	R ₂	R ₃	R ₄	R ₅
5a	H	F	OCH ₃	H	H
5b	H	F	H	OCH ₃	H
5c	H	F	H	OH	H
5d	H	F	H	OCH ₃	OCH ₃
5e	H	F	F	H	H
6a	H	OH	H	OCH ₃	OCH ₃
6b	H	OH	H	OCH ₃	H
6c	H	OH	OCH ₃	H	H
6d	H	OH	F	H	H
6e	H	OH	H	OH	H
7a	H	OCH ₃	H	OCH ₃	OCH ₃
7b	H	OCH ₃	H	OCH ₃	H
7c	H	OCH ₃	H	OH	H
7d	H	OCH ₃	OCH ₃	H	H
7e	H	OCH ₃	F	H	H
8a	OCH ₃	H	F	H	H
8b	OCH ₃	H	OCH ₃	H	H
8c	OCH ₃	H	H	OCH ₃	OCH ₃
8d	OCH ₃	H	H	OCH ₃	H
8e	OCH ₃	H	H	OH	H
9a	H	Br	H	OCH ₃	H
9b	H	Br	OCH ₃	H	H
9c	H	Br	H	OCH ₃	OCH ₃
9d	H	Br	F	H	H
9e	H	Br	H	OH	H
10a	H	H	OCH ₃	H	H
10b	H	H	H	OH	H
10c	H	H	H	OCH ₃	OCH ₃
10d	H	H	F	H	H
10e	H	H	H	OCH ₃	H

With the azides in hand, we explored different click reaction conditions to couple the indoles with aromatic alkynes. Attempts utilizing various solvents in a 1:1 ratio like; MeOH:H₂O, EtOH:H₂O, t-butanol:H₂O with copper (II) sulfate and sodium ascorbate, proved fruitless. Likewise, using copper (I) with acetonitrile and DMF as solvents also gave very low yields or no product. Reviewing the literature involving click reactions with indoles, we were able to find one account reporting similar difficulties involving indoles and click reactions. Their solution was using copper (I) and PEG-400 as the solvent.⁴⁵ Utilizing this solvent system, with a little experimentation, we finally achieved the optimal solvent system (2:1 PEG-400:DMF) capable of solubilizing the indole and aromatic alkyne, to carry out the desired click reaction affording analogues **5-10**.

It is interesting to note that certain click coupling reactions of indole with aromatic alkyne resulted in a chelation effect with the copper in solution. This phenomenon was evident by the emulsion formed upon aqueous workup and low yields. To reduce the emulsions formed and free the desired product from copper, certain click products having the above observations were stirred with disodium EDTA overnight in a 1:1 mixture of ethyl acetate:water, followed by an aqueous work-up and purification.

2.2 Total Antioxidant Scavenging:

To test the antioxidant capabilities of the newly synthesized compounds, total antioxidant scavenging using the iron thiocyanate method with linoleic acid emulsion was employed at 37 °C. Linoleic acid naturally oxidizes forming lipid peroxides that,

when treated with iron (II), oxidize iron (II) to iron (III) to form complexes with thiocyanate giving a red hue with a maximum absorption at 500 nm. Incubation of the linoleic acid emulsion with antioxidants reduces the degree of lipid peroxidation and subsequently reduces the formation of the iron (III) thiocyanate complex as measured by UV-VIS spectroscopy.

Originally, the goal of this strategy was to compare the new indole analogue antioxidants with the parent antioxidant, melatonin. Upon running several trials with melatonin, it was apparent that the results of the total antioxidant capability of melatonin varied. Literature searches explained the discrepancy. Melatonin has been documented as having the capabilities of chelating transition metals; copper and iron to name a few, and our subsequent hypothesis for the varied results during the total antioxidant tests were confirmed based on this characteristic.^{46,47}

In order for the test to have validity, we needed another well-known antioxidant as a comparison and therefore attempted a trial with vitamin C. Vitamin C, also like melatonin proved to be a poor standard as vitamin C is used as a reducing agent in click reactions, reducing copper (II) to copper (I).⁴⁸ Likewise, once iron (II) and thiocyanate were added to the linoleic acid emulsion with vitamin C, any iron (III) generated was quickly reduced back to iron (II) and gave 100 % inhibition.

Finally, butylated hydroxyanisole (BHA) was tested and gave consistent results with the total antioxidant method through a number of trials. BHA is a well-known antioxidant that is used in foods and food packaging.⁴⁹ The antioxidant capabilities of

BHA are quite remarkable and provided a reasonable comparison for our newly synthesized analogues.

With our suitable standard identified, we turned towards the analogues. Original protocol from the literature indicated solubilizing the desired antioxidants in a phosphate buffer with subsequent addition of this solution to a linoleic acid emulsion in the same buffer. This however was impractical as almost all compounds were virtually insoluble in aqueous solutions. To increase solubility, samples were dissolved in DMSO and added to a linoleic acid emulsion in phosphate buffer. While this increased solubility, certain compounds were observed to be slightly less soluble than others, indicating that 2nd generation compounds should be prepared with the goal of increasing aqueous solubility.

Once incubated at physiological temperature for 24 hours, the solutions were measured for their ability to inhibit lipid peroxidation. A number of compounds showed comparable antioxidant capabilities to BHA with a few showing significant improvement compared to BHA; namely **8a-e**. From the results, the following properties of the possible pharmacophore for antioxidant capabilities were identified.

Of the phenolic analogues that were added to the indoles via click reaction, the 3-methoxy, 4-methoxy and 3-hydroxyl aromatic rings proved the most effective. This is consistent with literature data involving anisoles, phenols and polyphenols as antioxidants.⁵⁰⁻⁵² Secondly, the indole substituents were also crucial to the antioxidant activity. Serotonin analogues containing the 3-methoxy and 4-methoxy coupled aromatics (**6b**, **6c**, **6e**) had comparable inhibition of lipid peroxidation to that seen with BHA. This finding is consistent with melatonin SAR studies.¹⁴ The 5-bromo substituted indole,

coupled with 3-methoxy, 4-methoxy and 3-hydroxy aromatics (**9a**, **9b**, **9e**) also had comparable inhibition of lipid peroxidation to BHA. The 4-methoxy substituent however had a marked increase in inhibition of lipid peroxidation compared with BHA (figure 2.7). Furthermore, all analogues containing the 4-methoxy substituent on the indole ring, regardless of the aromatic functional group that was coupled to it, showed an increase in inhibition of lipid peroxidation compared to similar indoles without the 4-methoxy functional group. This strongly suggests that the 4-methoxy functional group on the indole ring may add increased antioxidant potency especially in the prevention of lipid peroxidation.

Indole analogues containing the fluoro group at the 5 position on the indole (**5a-e**, figure 2.1) had very poor total antioxidant activity. It should be noted that the 5-fluoroindoles once coupled with their corresponding aromatic phenol or anisole had very poor solubility and may not have been effective at scavenging free radicals under conditions in the test employed.

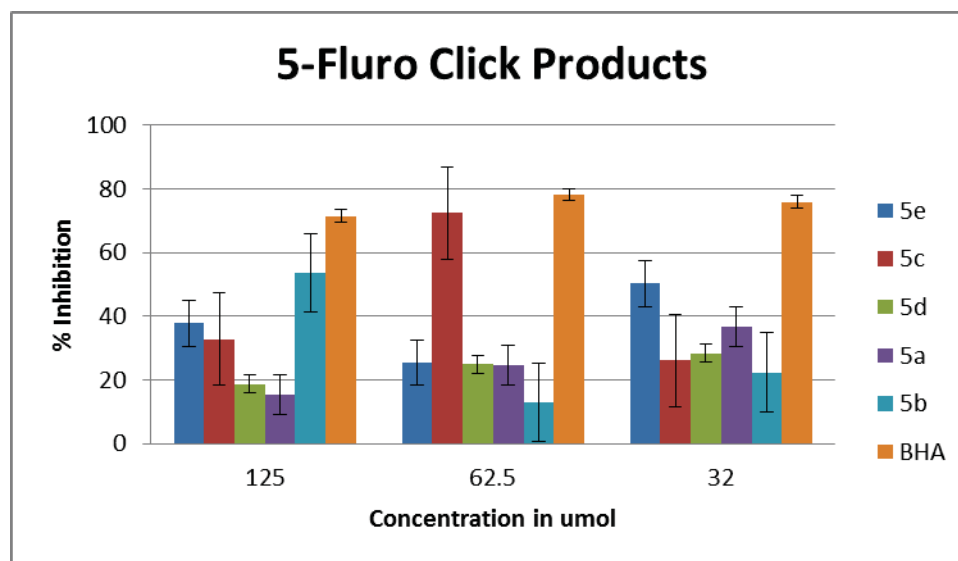


Figure 2.1. 4. Total antioxidant scavenging of 5-fluoroindole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

Indole analogues having the bromine at the 5 position on the indole (**9a-e**, figure 2.3) possessed similar antioxidant scavenging compared to BHA with analogue **9b** having improved scavenging. **9b** contains the aromatic anisole with the methoxy group at the 4 position on the aromatic ring. As many SAR studies of melatonin and their analogues used for antioxidant purposes have indicated, the pyrrole has been observed to be important in forming a stable structure once the free radical has been neutralized. Given the orientation of the pyrrole with regards to the 4-methoxyanisole moiety, electron delocalization from the methoxy group towards the *para* position could possibly activate the triazole towards detoxification of free radicals. Coupling this with the close proximity of the pyrrole and its use in forming cyclic structures after detoxification of radicals (figure 2.2), it seems resonable to propose such a hypothesis, however, metabolites would need to be identified to support this theory.

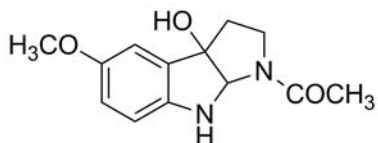


Figure 2.2. Cyclic-3-hydroxymelatonin

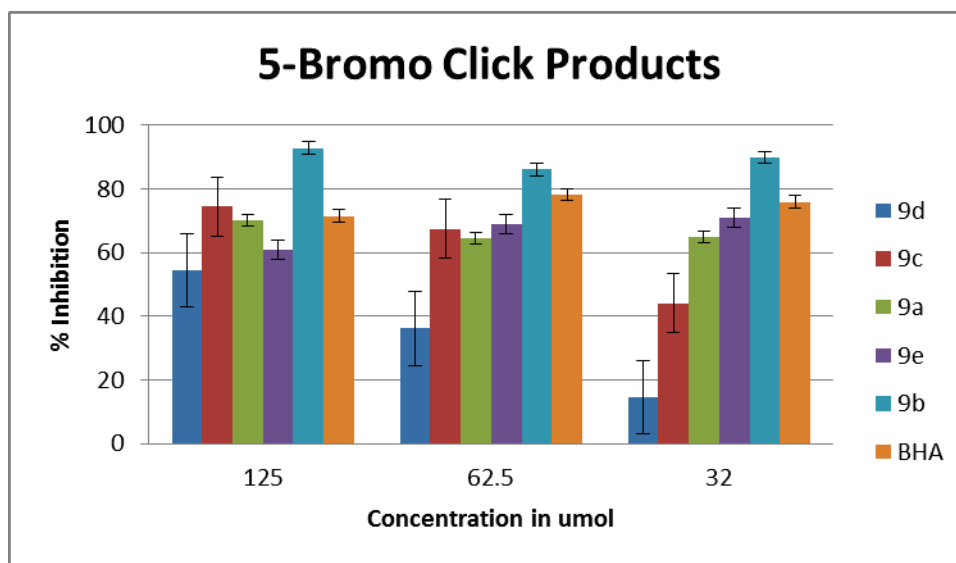


Figure 2.3. 6. Total antioxidant scavenging of 5-bromoindole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

The indole analogues containing the hydroxyl group at the 5 position on the indole (**6a-e**, figure 2.4) had comparable antioxidant scavenging relative to BHA with **6e** having increased scavenging. **6e** contains the phenol moiety at the 3 position on the aromatic ring relative to the connection of the triazole. Phenolic compounds such as resveratrol have been documented as detoxifying free radicals, and we hypothesize that the increased antioxidant capability may be due to the phenolic substituent connected to the indole.

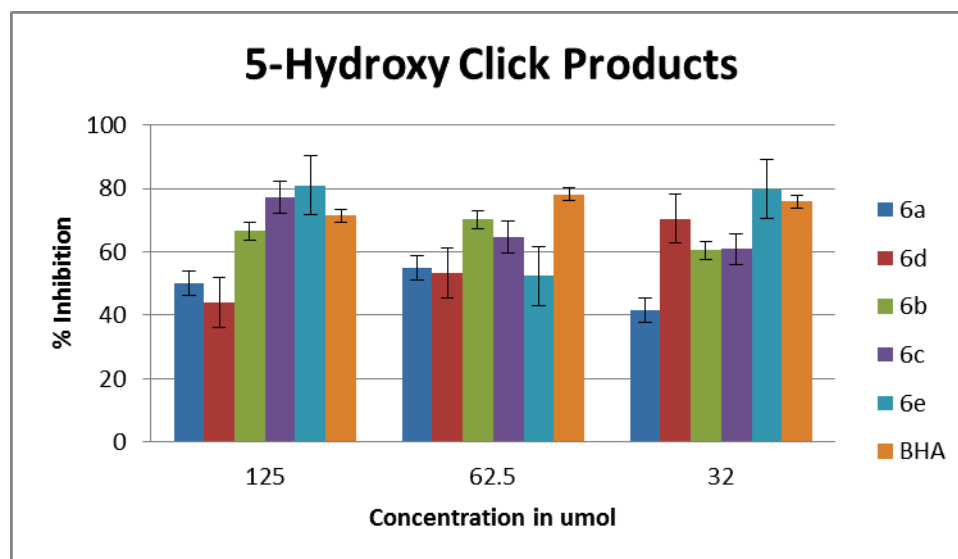


Figure 2.4. 7. Total antioxidant scavenging of 5-hydroxyindole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

Analogues containing the indole backbone and also the methoxy functional group at the 5 position on the indole (**10a-e**, **7a-e**) had comparable antioxidant scavenging to BHA (figures 2.5 and 2.6). It is interesting to note that the 5-methoxyindole derivatives did not perform as well as literature had indicated based on the SAR studies involving melatonin. While the 5-methoxyindole derivatives were somewhat effective, notable 125 μmol , their effectiveness tailed off in the lower concentration range of 32 μmol (figure 2.6).

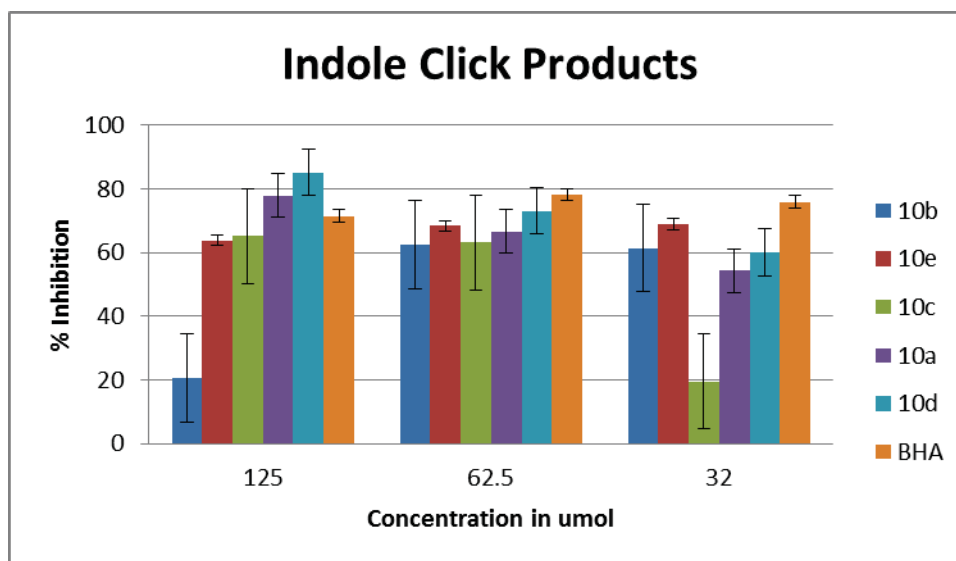


Figure 2.5. 8. Total antioxidant scavenging of indole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

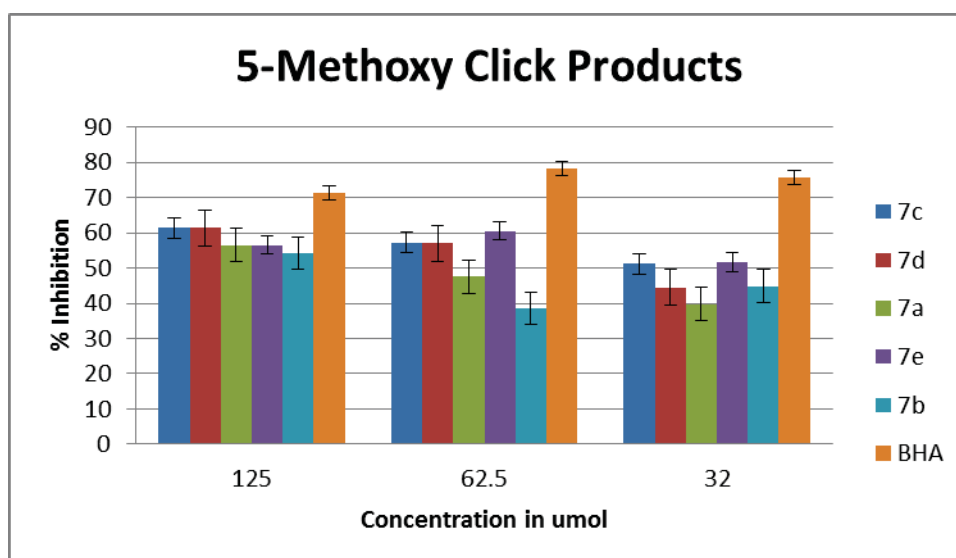
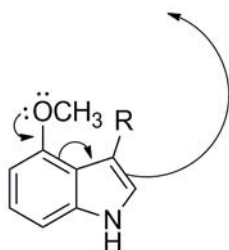


Figure 2.6. 9. Total antioxidant scavenging of 5-methoxyindole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

The 4-methoxyindole containing analogues **8a-e** all showed an increased ability to scavenge radicals compared to BHA (figure 2.7). As discussed earlier, the pyrrole has been identified as being crucial to radical detoxification. The 4-methoxy substituent on the indole ring through electron delocalization can make the pyrrole ring more electron rich, increasing the ability to react with free radicals produced from the lipid peroxidation (scheme 2.3). This hypothesis seems reasonable, and the 4-methoxy functional group on the indole backbone appears as though it may be more potent, at least at prevention of lipid oxidation compared to all other analogues tested.

Detoxification of free radicals



Scheme 2.3. Detoxification of free radicals with 4-methoxyindole analogues.

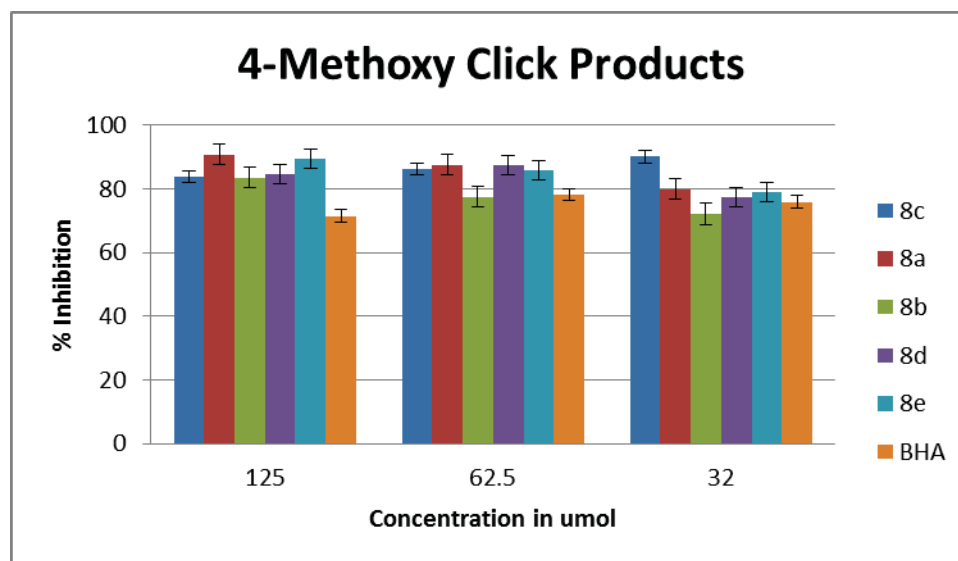


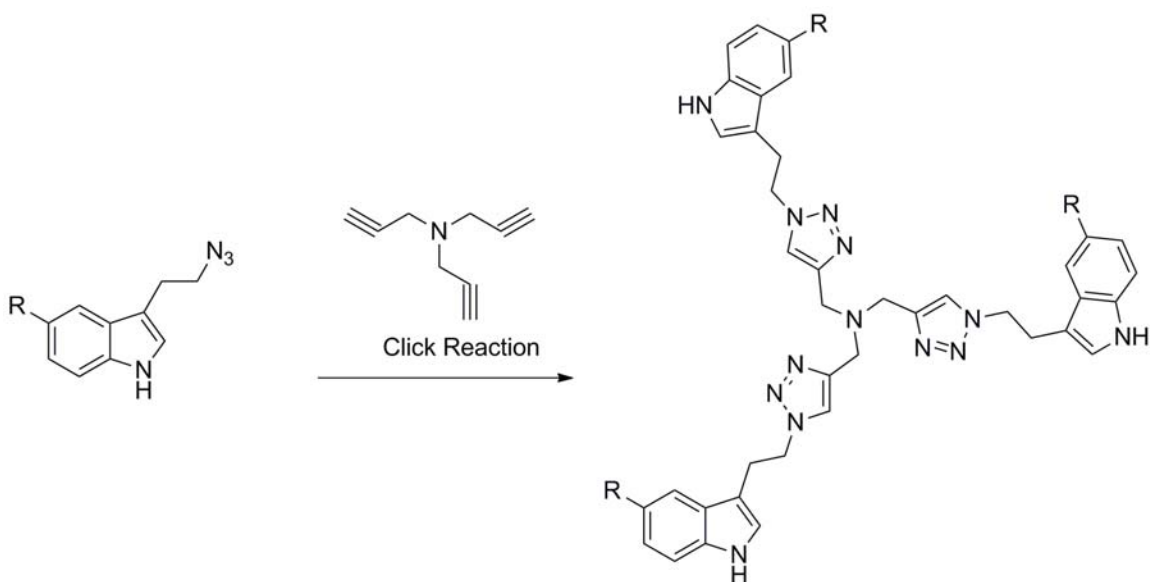
Figure 2.7. 10. Total antioxidant scavenging of 4-methoxyindole click products with corresponding phenol/anisole and standard deviation bars. The data are the average of triplicates.

2.3 Conclusions and Future Work:

Based on the preliminary results from the total antioxidant scavenging using the ferric thiocyanate method, a number of analogues possessed a marked improvement compared to the known antioxidant standard BHA. All 4-methoxyindole containing analogues **8a-e**, and analogues **6e** and **9b** were capable of inhibiting lipid oxidation better than BHA, suggesting that the 4-methoxy substituent on the indole ring is crucial to the detoxification of radicals. Additionally, aromatic anisoles and phenols were identified across all synthesized analogues as having the greatest capability at preventing lipid oxidation, suggesting that anisoles and phenols also possess radical scavenging capabilities. To this end, 2nd generation analogues should, if possible contain the 4-

methoxy substituent on the indole ring coupled to one or more anisoles or phenolic compounds.

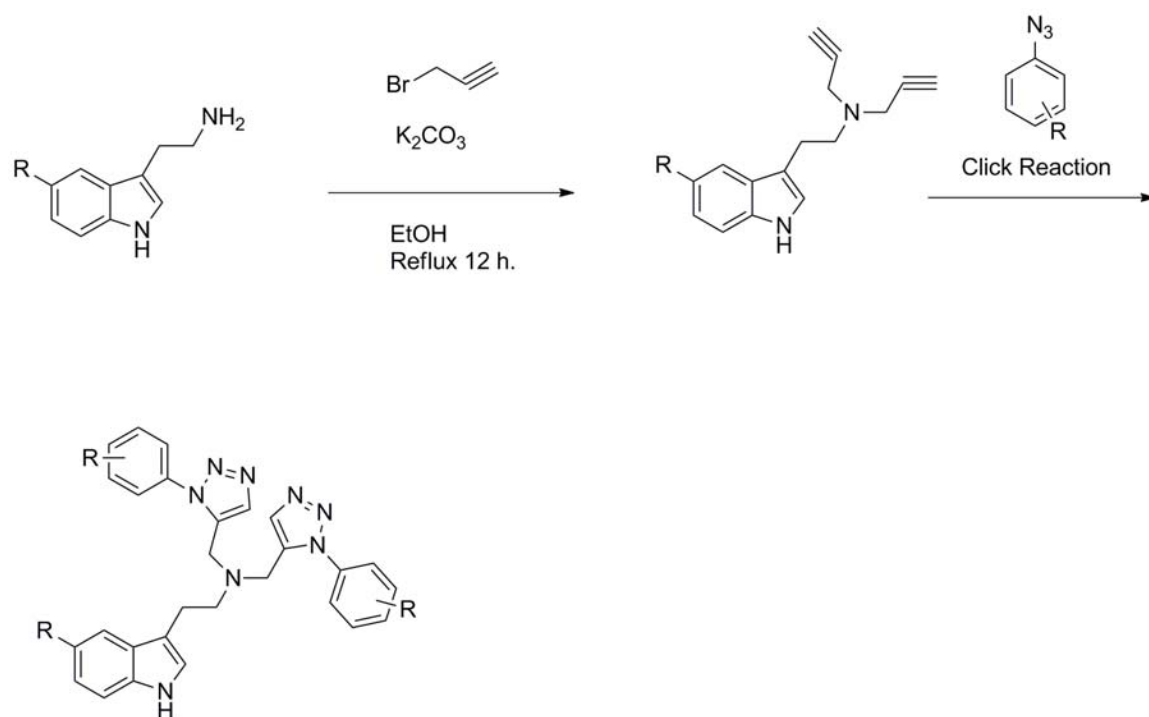
Synthetically speaking, we began to explore new 2nd generation compounds containing di-phenolic and di-anisole containing aromatics coupled to the indole backbone via a double click reaction. However, the synthesis proved complicated as our first approach utilized tripropargylamine and the corresponding indole azide (scheme 2.4). As experimentation proved, we had little control over the degree of substitution on the tripropargyl functional groups and therefore produced a poly substituted click analogue containing the indole azides.



Scheme 2.4. Initial approach to synthesizing diphenolic indole antioxidants.

We then explored conversion of the tryptamine to a di-substituted propargyl amine via a double nucleophilic substitution reaction (scheme 2.5). This reaction proved to only work for the tryptamine and resulted in numerous products that were unable to be

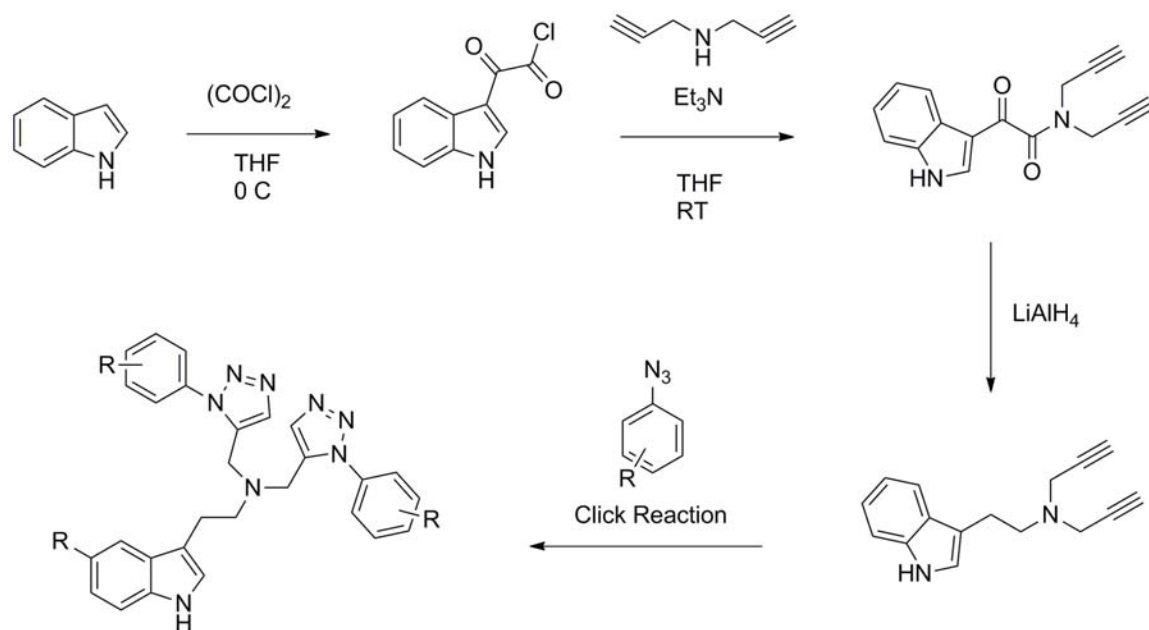
purified when working with substituted indoles. With the dipropargyl indole, we had to work out a new solvent system for the click reaction as the traditional solvent system employed from the 1st generation analogues, (2:1 DMF:PEG-400) did not produce the desired results. The final solvent system that appeared to give good yields was (2:1:1 PEG-400:H₂O:MeOH) followed by chelation of copper with EDTA.



Scheme 2.5. Synthesis of diphenolic indole antioxidants via nucleophilic substitution with 2 eq. of propargyl bromide.

Due to the sensitive nature of substituted indoles in the presence of base, an alternative approach may need to be employed to couple the desired indole with aromatic anisoles or phenols. A common method used to synthesize tryptamines involves the use of oxalyl chloride followed by reaction with an amine and reduction of the ketone functional groups (scheme 2.6). This method seems to be a plausible solution to the

above problem noticed when using substituted indoles subjected to the conditions shown in scheme 6.



Scheme 2.6. Proposed synthesis for diphenolic indole antioxidants.

Additionally, to get a better picture of how well the 1st generation analogues performed for antioxidant scavenging, it may be beneficial to subject the compounds to additional *in vitro* testing to more conclusively identify lead compounds. Furthermore, *in vivo* testing of 1st generation lead compounds would solidify their antioxidant scavenging capabilities in animal models and generate stronger evidence in the design of 2nd generation compounds.

Chapter 3 Experimental:

3.1 Synthesis of starting materials:

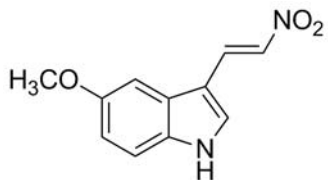
All starting materials and solvents were purchased from commercial sources unless otherwise stated. Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates 0.25 μm thickness. Flash chromatography was performed using Alfa Aesar silica gel, particle size 230-400 mesh. ^1H , ^{19}F and ^{13}C NMR were recorded on a Varian UNITY INOVA instrument at 400 MHz and 100 MHz respectively. UV-vis spectra were recorded on a Perkin Elmer Lambda 35 UV/vis.

4-Methoxyindole-3-carboxaldehyde (1) was synthesized according to a previously published procedure.⁵³ Briefly, 1.42 g (9.65 mmol) 4-Methoxyindole was dissolved in 4.5 mL (57.76 mmol) DMF and 1.9 mL (13.86 mmol) POCl_3 added with stirring at 0 $^\circ\text{C}$. The mixture was stirred for 30 min. at 0 $^\circ\text{C}$ and then at 40 $^\circ\text{C}$ for 1 h. 60 mL (2 M) NaOH was added with stirring over ice and the mixture heated at reflux for 1 h. The mixture was cooled and diluted with 60 mL of water and extracted with 3x 50 mL portions of ethyl acetate. The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation. The product, 4-Methoxyindole-3-carboxaldehyde was used without further purification.

3.2 Synthesis of Indole nitro olefins:

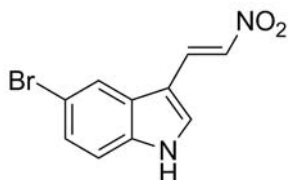
The different substituted indole nitro olefins compounds were synthesized according to a previously published procedure.⁵⁴

(E)-5-methoxy-3-(2-nitrovinyl)-1H-indole (2a):



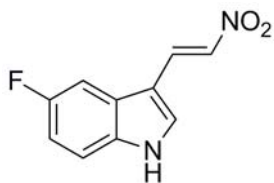
2.0 g (11.42 mmol) 5-Methoxyindole-3-carboxaldehyde and 500 mg NH_4OAc were added to 40 mL nitromethane and heated at reflux with stirring for 1 h. The solvent was removed by rotary evaporation and the product collected by vacuum filtration, washed with copious amounts of water and air dried overnight. The product, (E)-5-methoxy-3-(2-nitrovinyl)-1H-indole was used without further purification.

(E)-5-bromo-3-(2-nitrovinyl)-1H-indole (2b):



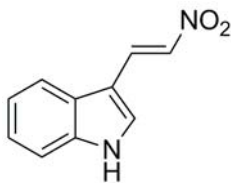
1.48 g (6.6 mmol) 5-Bromoindole-3-carboxaldehyde and 400 mg NH_4OAc were added to 20 mL nitromethane and heated at reflux with stirring for 1 h. The solvent was removed by rotary evaporation and the product collected by vacuum filtration, washed with copious amounts of water and air dried overnight. The product, (E)-5-bromo-3-(2-nitrovinyl)-1H-indole was used without further purification.

(E)-5-fluoro-3-(2-nitrovinyl)-1H-indole (2c):



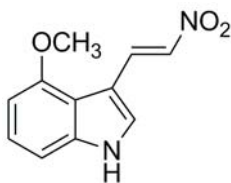
2.20 g (13.49 mmol) 5-Fluoroindole-3-carboxaldehyde and 900 mg NH_4OAc were added to 40 mL nitromethane and heated at reflux with stirring for 1 h. The solvent was removed by rotary evaporation and the product collected by vacuum filtration, washed with copious amounts of water and air dried overnight. The product, (E)-5-fluoro-3-(2-nitrovinyl)-1H-indole was used without further purification.

(E)-3-(2-nitrovinyl)-1H-indole (2d):



1.23 g (8.48 mmol) Indole-3-carboxaldehyde and 300 mg NH_4OAc were added to 20 mL nitromethane and heated at reflux with stirring for 1 h. The solvent was removed by rotary evaporation and the product collected by vacuum filtration, washed with copious amounts of water and air dried overnight. The product, (E)-3-(2-nitrovinyl)-1H-indole was used without further purification.

(E)-4-methoxy-3-(2-nitrovinyl)-1H-indole (2e):

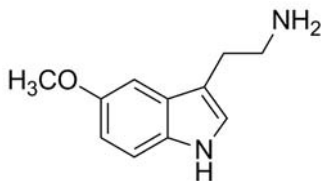


1.0 g (5.71 mmol) 4-Methoxyindole-3-carboxaldehyde and 760 mg NH_4OAc were added to 30 mL nitromethane and heated at reflux with stirring for 1 h. The solvent was removed by rotary evaporation and the product collected by vacuum filtration, washed with copious amounts of water and air dried overnight. The product, (E)-4-methoxy-3-(2-nitrovinyl)-1H-indole was used in without further purification.

3.3 Synthesis of Tryptamines:

The conversion to tryptamines from the nitro olefin indoles were carried out following a previously published procedure.⁵⁴

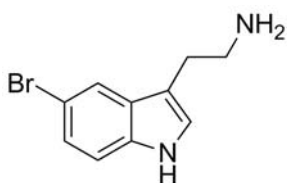
5-Methoxytryptamine (3a):



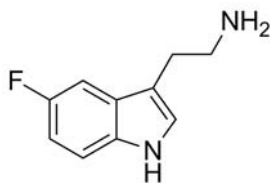
2.60 g (11.93 mmol) (E)-5-methoxy-3-(2-nitrovinyl)-1H-indole was dissolved in 20 mL anhydrous THF and added to a slurry of 3 g LiAlH_4 in 40 mL anhydrous THF with stirring at 0 °C. The mixture was heated at reflux for 1 h. Excess LiAlH_4 was quenched with water at 0 °C and 80 mL of saturated Rochelle's salts and 100 mL ether was added. The mixture was stirred at room temperature overnight. The aqueous layer was removed

and the organic layer extracted with 3x 40 mL portions of 1M HCl. The acidic layers were combined and basified with KOH with stirring over ice. The basic solution was then extracted with 3x 40 mL portions of ether. The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation. The product, 5-Methoxytryptamine was used without further purification.

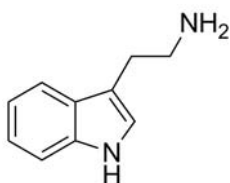
5-Bromotryptamine (3b):



1.48 g (5.54 mmol) (E)-5-bromo-3-(2-nitrovinyl)-1H-indole was dissolved in 10 mL anhydrous THF and added to a slurry of 1.55 g LiAlH_4 in 40 mL anhydrous THF with stirring at 0 °C. The mixture was heated at reflux for 1 h. Excess LiAlH_4 was quenched with water at 0 °C and 80 mL of saturated Rochelle's salts and 100 mL ether was added. The mixture was stirred at room temperature overnight. The aqueous layer was removed and the organic layer extracted with 3x 40 mL portions of 1M HCl. The acidic layers were combined and basified with KOH with stirring over ice. The basic solution was then extracted with 3x 40 mL portions of ether. The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation. The product, 5-Bromotryptamine was used without further purification.

5-Fluorotryptamine (3c):

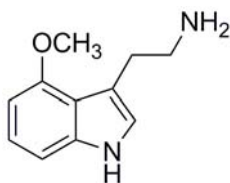
2.71 g (13.15 mmol) (E)-5-fluoro-3-(2-nitrovinyl)-1H-indole was dissolved in 20 mL anhydrous THF and added to a slurry of 3.15 g LiAlH_4 in 40 mL anhydrous THF with stirring at 0 °C. The mixture was heated at reflux for 1.5 h. Excess LiAlH_4 was quenched with water at 0 °C and 80 mL of saturated Rochelle's salts and 100 mL ether was added. The mixture was stirred at room temperature overnight. The aqueous layer was removed and the organic layer extracted with 3x 40 mL portions of 1M HCl. The acidic layers were combined and basified with KOH with stirring over ice. The basic solution was then extracted with 3x 40 mL portions of ether. The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation. The product, 5-Fluorotryptamine was used without further purification.

Tryptamine (3d):

1.77 g (9.41 mmol) (E)-3-(2-nitrovinyl)-1H-indole was dissolved in 20 mL anhydrous THF and added to a suspension of 2.00 g LiAlH_4 in 30 mL anhydrous THF with stirring at 0 °C. The mixture was heated at reflux for 1 h. Excess LiAlH_4 was quenched with water at 0 °C and 80 mL of saturated Rochelle's salts and 100 mL ether was added. The

mixture was stirred at room temperature overnight. The aqueous layer was removed and the organic layer extracted with 3x 40 mL portions of 1M HCl. The acidic layers were combined and basified with KOH with stirring over ice. The basic solution was then extracted with 3x 40 mL portions of ether. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The product, Tryptamine was used without further purification.

4-Methoxytryptamine (3e):



2.57 g (11.79 mmol) (E)-4-methoxy-3-(2-nitrovinyl)-1H-indole was dissolved in 15 mL anhydrous THF and added to 2.74 g LiAlH₄ suspended in 40 mL anhydrous THF with stirring at 0 °C. The mixture was heated at reflux for 1.5 h. Excess LiAlH₄ was quenched with water at 0 °C and 80 mL of saturated Rochelle's salts and 100 mL ether was added. The mixture was stirred at room temperature overnight. The aqueous layer was removed and the organic layer extracted with 3x 40 mL portions of 1M HCl. The acidic layers were combined and basified with KOH with stirring over ice. The basic solution was then extracted with 3x 40 mL portions of ether. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The product, 4-Methoxytryptamine was used without further purification.

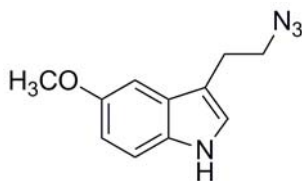
3.4 Conversion of Tryptamines to Tryptamine azides:

The conversion of Tryptamine, 4-Methoxytryptamine, 5-Methoxytryptamine, 5-Bromotryptamine and 5-Fluorotryptamine to their corresponding azides were accomplished according to a previously published procedure.⁵⁵

Preparation of Triflic azide:

5.65 g (86.9 mmol) NaN_3 was dissolved in 40 mL 1:1 CH_2Cl_2 : H_2O . 3 mL, (17.84 mmol) Triflic anhydride was added with stirring at 0 °C for 2 h. The organic layer was separated and the aqueous layer extracted with 20 mL CH_2Cl_2 . The organic layers were combined and washed with 40 mL saturated Na_2CO_3 . The Triflic azide in 40 mL CH_2Cl_2 was used without further purification.

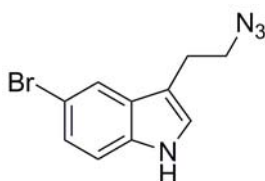
3-(2-azidoethyl)-5-methoxy-1H-indole (4a):



2.18 g (11.47 mmol) 5-Methoxytryptamine was dissolved in 90 mL 2:1 MeOH : H_2O and 150 mg CuSO_4 and 1.50 g K_2CO_3 added. The freshly prepared TfN_3 in 40 mL CH_2Cl_2 was added and the mixture stirred at room temperature overnight. The solvent was removed by rotary evaporation and the crude dissolved in 80 mL 1:1 EtOAc : H_2O . The mixture was extracted with 2x 40 mL portions of EtOAc . The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation.

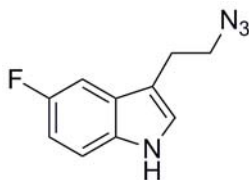
The crude was purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

3-(2-azidoethyl)-5-bromo-1H-indole (4b):



1.36 g (5.69 mmol) 5-Bromotryptamine was dissolved in 90 mL 2:1 MeOH:H₂O and 100 mg CuSO₄ and 1.30 g K₂CO₃ added. The freshly prepared TfN₃ in 40 mL CH₂Cl₂ was added and the mixture stirred at room temperature overnight. The solvent was removed by rotary evaporation and the crude dissolved in 80 mL 1:1 EtOAc:H₂O. The mixture was extracted with 2x 40 mL portions of EtOAc. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

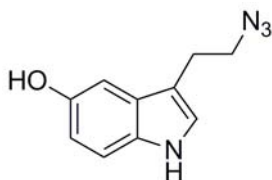
3-(2-azidoethyl)-5-fluoro-1H-indole (4c):



900 mg (mmol) 5-Fluorotryptamine was dissolved in 90 mL 2:1 MeOH:H₂O and 100 mg CuSO₄ and 900 mg K₂CO₃ added. The freshly prepared TfN₃ in 40 mL CH₂Cl₂ was added and the mixture stirred at room temperature overnight. The solvent was removed

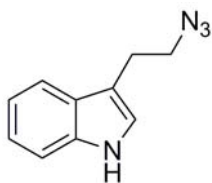
by rotary evaporation and the crude dissolved in 80 mL 1:1 EtOAc:H₂O. The mixture was extracted with 2x 40 mL portions of EtOAc. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

3-(2-aminoethyl)-1H-indol-5-ol (4d):



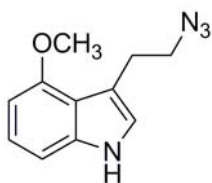
The conversion of serotonin hydrochloride to the corresponding azide was accomplished according to a previously published procedure with modifications.⁵⁶ Briefly, 1.09 g (16.8 mmol) NaN₃, 2.30 mL (14.0 mmol) triflic anhydride was added to 15 mL of anhydrous acetonitrile with stirring at 0 °C. The mixture was stirred for 2 h. 2.05 g (mmol) Serotonin hydrochloride, 160 mg ZnCl₂ and 4.8 mL (36.6 mmol) Et₃N dissolved in 30 mL 3:7 H₂O:acetonitrile was added and stirred for 5 minutes. The solvent was removed and the residue diluted with 40 mL of water. The mixture was extracted with 3x 40 mL portions of EtOAc. The organic layers were combined, dried over Na₂SO₄, decanted, and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

3-(2-azidoethyl)-1H-indole (4e):



1.29 g (8.06 mmol) Tryptamine was dissolved in 90 mL 2:1 MeOH:H₂O and 100 mg CuSO₄ and 1.30 g K₂CO₃ added. The freshly prepared TfN₃ in 40 mL CH₂Cl₂ was added and the mixture stirred at room temperature overnight. The solvent was removed by rotary evaporation and the crude dissolved in 80 mL 1:1 EtOAc:H₂O. The mixture was extracted with 2x 40 mL portions of EtOAc. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

3-(2-azidoethyl)-4-methoxy-1H-indole (4f):

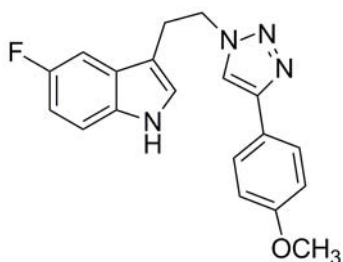


1.91 g (10.05 mmol) 4-Methoxyindole was dissolved in 90 mL 2:1 MeOH:H₂O and 190 mg CuSO₄ and 1.91 g K₂CO₃ added. The freshly prepared TfN₃ in 40 mL CH₂Cl₂ was added and the mixture stirred at room temperature for 1.5 h. The solvent was removed by rotary evaporation and the crude dissolved in 80 mL 1:1 EtOAc:H₂O. The mixture was extracted with 2x 40 mL portions of EtOAc. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was

purified by flash column chromatography, eluting with 50:50 EtOAc:Hexanes. The title compound was used immediately after purification.

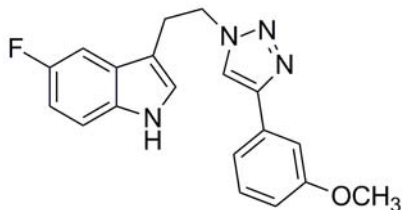
3.5 Synthesis of Melatonin/Phenolic compounds:

5-fluoro-3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (5a):



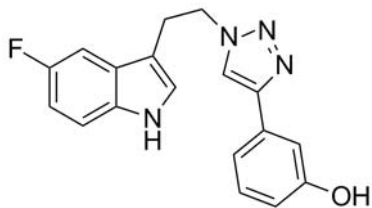
973 mg (4.82 mmol) 3-(2-azidoethyl)-5-fluoro-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.685 mL (5.30 mmol) 4-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 490 mg (30 % yield) of a light brown crystals. ¹H NMR (CDCl₃) δ 8.67 (br s, 1H), 7.63 (d, *J* = 2.0 Hz, 2H), 7.40 (s, 1H), 7.23 (dd, *J* = 8.6 Hz, 4.4 Hz, 1H), 7.14 (dd, *J* = 9.6 Hz, 2.1 Hz, 1H), 6.88 (m, 4H), 4.59 (t, *J* = 7 Hz, 2H), 3.78 (s, 3H), 3.29 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.5, 157.75 (d, *J* = 235 Hz, 1C), 147.3, 132.7, 127.09 (d, *J* = 9.1 Hz, 1C), 127.0, 124.6, 123.1, 119.4, 114.2, 112.18 (d, *J* = 9.1 Hz, 1C), 110.96 (d, *J* = 4.6 Hz, 1C), 110.45 (d, *J* = 27.4 Hz, 1C), 102.96 (d, *J* = 22.8 Hz, 1C), 55.2, 50.5, 26.4; ¹⁹F NMR (CDCl₃) δ -124.73 (sextet, *J* = 4.6 Hz, 1F). (TLC Rf 70:30 ethyl acetate:hexanes = 0.48). FAB-HRMS *m/z* 337.1478 [M+H]⁺.

5-fluoro-3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (5b):



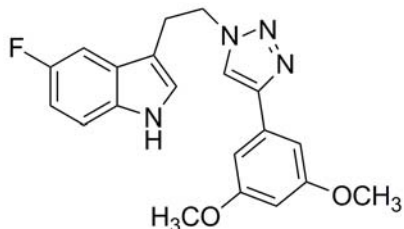
973 mg (4.82 mmol) 3-(2-azidoethyl)-5-fluoro-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.670 mL (5.30 mmol) 3-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 680 mg (42 % yield) of a light brown solid. ¹H NMR (CDCl₃) δ 8.64 (br s, 1H), 7.47 (s, 1H), 7.33 (m, 1H), 7.24 (m, 3H), 7.14 (dd, *J* = 9.6 Hz, 2.5 Hz, 1H), 6.90 (td, *J* = 9 Hz, 2.4 Hz, 1H), 6.83 (m, 2H), 4.61 (t, *J* = 6.8 Hz, 2H), 3.77 (s, 3H), 3.30 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.9, 157.77 (d, *J* = 235 Hz, 1C), 147.3, 132.7, 131.7, 129.8, 127.08 (d, *J* = 9.2 Hz, 1C), 124.6, 120.4, 118.1, 114.1, 112.20 (d, *J* = 9.1 Hz, 1C), 110.90 (d, *J* = 4.6 Hz, 1C), 110.8, 110.51 (d, *J* = 26.0 Hz, 1C), 102.96 (d, *J* = 24.4 Hz, 1C), 55.2, 50.6, 26.4; ¹⁹F NMR (CDCl₃) δ -124.65 (sextet, *J* = 4.6 Hz, 1F). (TLC R_f 70:30 ethyl acetate:hexanes = 0.48). FAB-HRMS *m/z* 337.1457 [M+H]⁺.

3-(1-(2-(5-fluoro-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)phenol (5c):



973 mg (4.82 mmol) 3-(2-azidoethyl)-5-fluoro-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.580 mL (5.30 mmol) 3-Hydroxyphenylacetylene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 620 mg (40 % yield) of a yellow powder. ¹H NMR (DMSO) δ 10.93 (br s, 1H), 9.48 (s, 1H), 8.46 (s, 1H), 7.30 (m, 2H), 7.19 (m, 4H), 6.87 (td, *J* = 10.7 Hz, 2.4 Hz, 1H), 6.69 (m, 1H), 4.61 (t, *J* = 7.3 Hz, 2H), 3.26 (t, *J* = 7.1 Hz, 2H); ¹³C NMR (DMSO) δ 158.4, 157.44 (d, *J* = 230.4 Hz, 1C), 146.9, 133.5, 132.8, 130.6, 127.84 (d, *J* = 10.6 Hz, 1C), 126.1, 122.0, 116.6, 115.5, 113.02 (d, *J* = 10.8 Hz, 1C), 112.5, 111.00 (d, *J* = 5.9 Hz, 1C), 109.85 (d, *J* = 26.0 Hz, 1C), 103.65 (d, *J* = 22.9 Hz, 1C), 50.7, 26.4; ¹⁹F NMR (DMSO) δ -125.80 (sextet, *J* = 4.5 Hz, 1F). (TLC R_f 70:30 ethyl acetate:hexanes = 0.43). FAB-HRMS *m/z* 323.1300 [M+H]⁺.

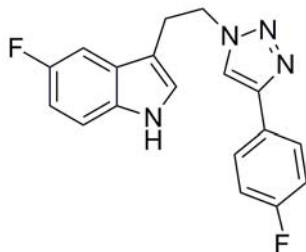
3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-5-fluoro-1H-indole (5d):



1.42 g (6.94 mmol) 3-(2-azidoethyl)-5-fluoro-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 1.35 g (8.32 mmol) 1-Ethynyl-3,5-dimethoxybenzene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by dissolving the solid in hot ethyl acetate and reprecipitating by the addition of hexanes to give 700 mg (28 % yield) of a tan powder.

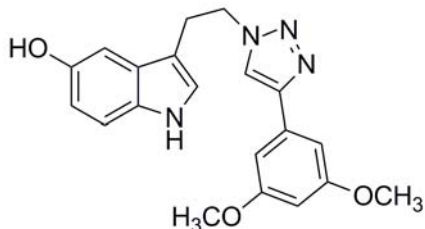
¹H NMR (DMSO) δ 10.92 (br s, 1H), 8.57 (s, 1H), 7.30 (m, 2H), 7.14 (d, J = 2.5 Hz, 1H), 6.94 (d, J = 2.3 Hz, 2H), 6.87 (td, J = 9.1 Hz, 2.6 Hz, 1H), 6.42 (t, J = 2.3 Hz, 1H), 4.61 (t, J = 7.3 Hz, 2H), 3.75 (s, 6H), 3.26 (t, J = 7.2 Hz, 2H); ¹³C NMR (DMSO) δ 161.5, 158.47 (d, J = 230.4 Hz, 1C), 146.8, 133.44 (d, J = 4.6 Hz, 1C), 127.86 (d, J = 10.7 Hz, 1C), 126.1, 122.5, 113.04 (d, J = 9.2 Hz, 1C), 110.95 (d, J = 4.6 Hz, 1C), 109.87 (d, J = 25.9 Hz, 1C), 103.7, 103.5, 100.5, 55.9, 50.8, 26.4. ¹⁹F NMR (DMSO) δ -125.83 (sextet, J = 4.5 Hz, 1F). (TLC R_f 70:30 ethyl acetate:hexanes = 0.42). FAB-HRMS m/z 367.1563 [M+H]⁺.

5-fluoro-3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (5e):



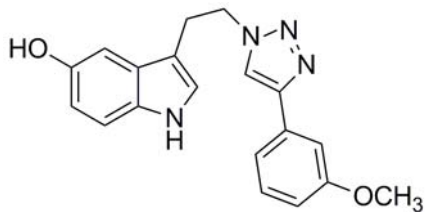
1.42 g (6.94 mmol) 3-(2-azidoethyl)-5-fluoro-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 0.955 mL (8.32 mmol) 1-Ethynyl-4-fluorobenzene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 500 mg (22% yield) of a light yellow solid. ¹H NMR (CDCl₃) δ 8.20 (br s, 1H), 7.68 (m, 2H), 7.42 (s, 1H), 7.25 (m, 1H), 7.17 (dd, *J* = 9.5 Hz, 2.5 Hz, 1H), 7.06 (m, 2H), 6.94 (td, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 7.65 (t, *J* = 6.2 Hz, 2H), 3.35 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (CDCl₃) δ 162.8 (d, *J* = 247.2 Hz, 1C), 158.1 (d, *J* = 235.0 Hz, 1C), 146.8, 132.9, 127.6 (d, *J* = 7.7 Hz, 1C), 127.4 (d, *J* = 10.7 Hz, 1C), 127.0 (d, *J* = 3.1 Hz, 1C), 124.7, 120.0, 116.0 (d, *J* = 21.4 Hz, 1C), 112.4 (d, *J* = 9.2 Hz, 1C), 111.5 (d, *J* = 4.6 Hz, 1C), 111.0 (d, *J* = 25.9 Hz, 1C), 103.4 (d, *J* = 22.9 Hz, 1C), 50.8, 26.7. ¹⁹F NMR (CDCl₃) δ -114.04 (septet, *J* = 4.6 Hz, 1F), -124.48 (sextet, *J* = 4.4 Hz, 1F). (TLC R_f 70:30 ethyl acetate:hexanes = 0.5). FAB-HRMS *m/z* 325.1254 [M+H]⁺.

3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indol-5-ol (6a):



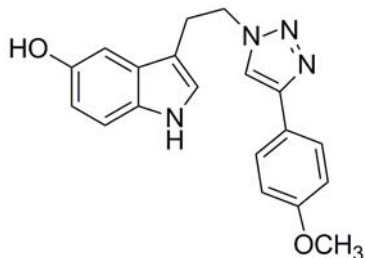
1.62 g (8.1 mmol) 3-(2-azidoethyl)-1H-indol-5-ol was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 1.53 g (8.9 mmol) 1-Ethynyl-3,5-dimethoxybenzene added. The mixture was stirred at room temperature for 12 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 940 mg (32 % yield) of an off-white powder. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 7.98 (br s, 1H), 7.35 (s, 1H), 7.17 (d, 8.7 Hz, 1H), 6.94 (m, 1H), 6.87 (m, 2H), 6.78 (m, 2H), 6.38 (t, *J* = 2.3 Hz, 1H), 6.10 (br s, 1H), 4.56 (t, *J* = 6.8 Hz, 2H), 3.76 (s, 6H), 3.25 (t, *J* = 7.1 Hz, 2H); ¹³C NMR (CDCl₃) δ 161.1, 150.0, 147.2, 132.3, 131.4, 127.5, 123.7, 120.7, 112.4, 112.1, 110.4, 103.7, 102.8, 100.5, 55.4, 50.8, 26.6. (TLC R_f 70:30 EtoAc:hexanes = 0.30). FAB-HRMS *m/z* 365.1595 [M+H]⁺.

3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indol-5-ol (6b):



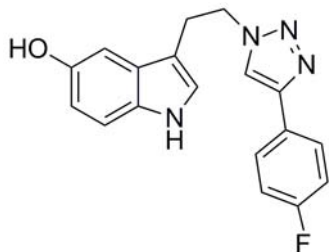
1.18 g (5.86 mmol) 3-(2-azidoethyl)-1H-indol-5-ol was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.815 mL (6.45 mmol) 3-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 680 mg (35 % yield) of an off-white powder. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (DMSO) δ 10.5 (br s, 1H), 8.64 (s, 1H), 8.53 (s, 1H), 7.32 (m, 3H), 7.09 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.85 (m, 2H), 6.57 (dd, *J* = 8.7 Hz, 2.3 Hz, 1H), 4.59 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 3.20 (t, *J* = 7.2 Hz, 2H); ¹³C NMR (DMSO) δ 160.3, 151.0, 146.7, 132.9, 131.4, 130.7, 128.3, 124.3, 122.2, 118.1, 114.2, 112.5, 112.1, 111.0, 109.6, 102.7, 55.8, 50.7, 26.6. (TLC R_f 70:30 EtoAc:hexanes = 0.33). FAB-HRMS *m/z* 335.1497 [M+H]⁺.

3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indol-5-ol (6c):



1.74 g (8.61 mmol) 3-(2-azidoethyl)-1H-indol-5-ol was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 1.23 mL (9.48 mmol) 4-Ethynylanisole added. The mixture was stirred under nitrogen at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by partially solvating the solid in hot ethyl acetate and vacuum filtration to give 870 mg (30 % yield) of tan crystals. ¹H NMR (DMSO) δ 10.51 (br s, 1H), 8.62 (s, 1H), 8.41 (s, 1H), 7.71 (m, 2H), 7.11 (d, *J* = 8.6 Hz, 1H), 6.96 (m, 3H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.60 (dd, *J* = 8.5 Hz, 2.3 Hz, 1H), 4.59 (t, *J* = 7.3 Hz, 2H), 3.74 (s, 3H), 3.21 (t, *J* = 7.5 Hz, 2H); ¹³C NMR (DMSO) δ 159.6, 151.1, 146.8, 131.4, 128.3, 127.1, 124.3, 124.2, 121.0, 115.0, 112.5, 112.2, 109.7, 102.8, 55.8, 50.6, 26.7. (TLC R_f 70:30 EtoAc:hexanes = 0.49). FAB-HRMS *m/z* 335.1512 [M+H]⁺.

3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indol-5-ol (6d):



1.70 g (8.42 mmol) 3-(2-azidoethyl)-1H-indol-5-ol was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 1.10 mL (9.61 mmol) 1-Ethynyl-4-fluorobenzene added. The mixture was stirred at room temperature for 12 h under nitrogen. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was washed with 40 mL of 70:30 ethyl acetate:hexanes and collected by vacuum filtration to give 1.0 g (37 % yield) of tan crystals. ¹H NMR (DMSO) δ 10.52 (br s, 1H), 8.63 (s, 1H), 8.51 (s, 1H), 7.83 (m, 2H), 7.23 (tt, *J* = 8.9 Hz, 2.2 Hz, 2H), 7.12 (d, *J* = 8.7 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.61 (dd, *J* = 8.5 Hz, 2.3 Hz, 1H) 4.62(t, *J* = 7.2 Hz, 2H), 3.21 (t, *J* = 7.3 Hz, 2H); ¹³C NMR (DMSO) δ 162.40 (d, *J* = 244.2 Hz, 1C), 151.10, 146.00, 131.43, 128.30, 128.16 (d, *J* = 3.0 Hz, 1C), 127.77 (d, *J* = 7.6 Hz, 1C), 124.30, 121.90, 116.45 (d, *J* = 21.3 Hz, 1C), 112.50, 112.20, 109.60, 102.80, 50.72, 26.70. ¹⁹F NMR (DMSO) δ -114.65 (septet, *J* = 4.6 Hz, 1F). FAB-HRMS *m/z* 323.1314 [M+H]⁺.

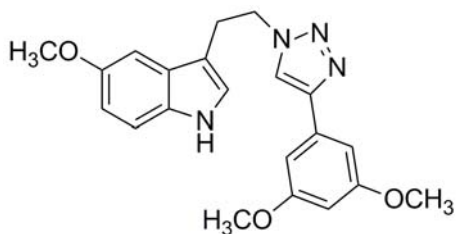
3-(2-(4-(3-hydroxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indol-5-ol (6e):



1.70 g (8.41 mmol) 3-(2-azidoethyl)-1H-indol-5-ol was dissolved in 8 mL 2:1 PEG-400:DMF and 1.16 g CuI and 1.03 mL (9.47 mmol) 3-Hydroxyphenylacetylene added. The mixture was stirred under nitrogen at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 960 mg (36 % yield) as a brown solid. Residual solvent and DMF was removed in vacuo at 60 °C ¹H NMR (DMSO) δ 10.51 (br s, 1H), 9.51 (s, 1H), 8.63 (s, 1H), 8.45 (s, 1H), 7.25 (m, 1H), 7.19 (m, 2H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.71 (m 1H), 6.62 (dd, *J* = 8.7 Hz, 2.3 Hz, 1H), 4.61 (t, *J* = 7.3 Hz, 2H), 3.22 (t, *J* = 7.3 Hz, 2H); ¹³C NMR (DMSO) δ 158.4, 151.1, 146.9, 132.8, 131.4, 130.6, 128.3, 124.3, 121.9, 116.7, 115.5, 112.53, 112.47, 112.2, 109.6, 102.8, 50.6, 26.7. (TLC R_f 70:30 EtoAc:hexanes = 0.20). FAB-HRMS *m/z* 321.1348 [M+H]⁺.

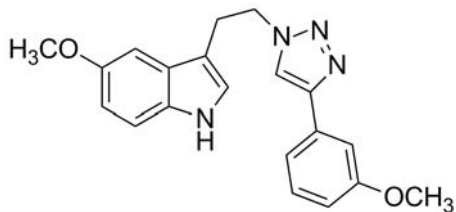
3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-5-methoxy-1H-indole

(7a):



1.11 g (5.14 mmol) 3-(2-azidoethyl)-5-methoxy-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 920 mg (5.67 mmol) 1-Ethynyl-3,5-dimethoxybenzene added. The mixture was stirred at room temperature for 12 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 670 mg (34 % yield) of a light brown solid. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.59 (br s, 1H), 7.44 (s, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.88 (m, 3H), 6.80 (m, 2H), 6.40 (t, *J* = 2.3 Hz, 1H), 4.59 (t, *J* = 6.8 Hz, 2H), 3.79 (s, 3H), 3.74 (s, 6H), 3.30 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 161.0, 153.9, 147.1, 132.3, 131.3, 127.1, 123.4, 120.5, 112.1, 110.5, 103.5, 100.2, 99.8, 55.7, 55.2, 50.7, 26.3. (TLC R_f 70:30 EtoAc:hexanes = 0.43). FAB-HRMS *m/z* 379.1787 [M+H]⁺.

5-methoxy-3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (7b):



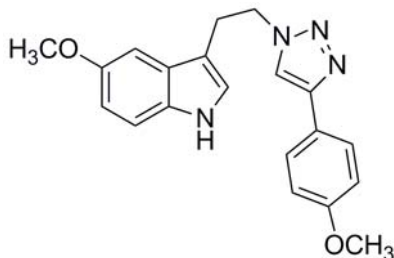
1.11 g (5.14 mmol) 3-(2-azidoethyl)-5-methoxy-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.72 mL (5.67 mmol) 3-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was added to 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 1.37 g (77 % yield) of an orange oil. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.60 (br s, 1H), 7.45 (s, 1H), 7.34 (m, 1H), 7.21 (m, 3H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.82 (m, 3H), 4.60 (t, *J* = 6.9 Hz, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.30 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.8, 153.9, 147.1, 131.8, 131.3, 129.7, 127.1, 123.4, 120.4, 117.9, 113.9, 112.1, 110.7, 110.5, 99.6, 55.7, 55.1, 50.7, 26.4. (TLC R_f 70:30 EtoAc:hexanes = 0.42). FAB-HRMS *m/z* 349.1653 [M+H]⁺.

3-(1-(2-(5-methoxy-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)phenol (7c):



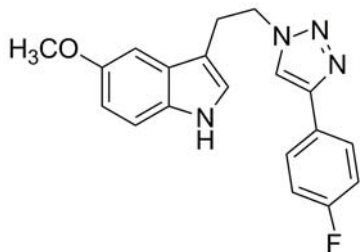
1.15 g (5.32 mmol) 3-(2-azidoethyl)-5-methoxy-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.64 mL (5.85 mmol) 3-Hydroxyphenylacetylene added. The mixture was stirred at room temperature for 12 h. The mixture was added to 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 740 mg (42 % yield) of an off-white crystalline solid. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.0 (br s, 1H), 7.41 (m, 1H), 7.31 (s, 1H), 7.20 (m, 3H), 7.10 (br s, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.82 (m, 2H), 6.75 (d, *J* = 2.4 Hz, 1H), 4.62 (t, *J* = 6.9 Hz, 2H), 3.81 (s, 3H), 3.32 (t, *J* = 6.4 Hz, 2H); ¹³C NMR (CDCl₃) δ 156.7, 154.2, 147.1, 131.6, 131.4, 130.1, 127.2, 123.5, 120.6, 117.7, 115.6, 112.8, 112.5, 112.2, 110.8, 100.1, 56.0, 50.9, 26.5. (TLC R_f 70:30 EtoAc:hexanes = 0.31). FAB-HRMS *m/z* 335.1513 [M+H]⁺.

5-methoxy-3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (7d):



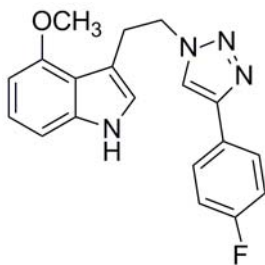
800 mg (4.21 mmol) 3-(2-azidoethyl)-5-methoxy-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.12 g CuI and 0.59 mL (4.63 mmol) 4-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was added to 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 600 mg (41 % yield) of an orange oil. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.59 (br s, 1H), 7.61 (m, 2H), 7.38 (s, 1H), 7.2 (d, *J* = 8.8 Hz, 1H), 6.88 (m, 5H), 4.59 (t, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 3.30 (t, *J* = 6.8 Hz, 2H) ; ¹³C NMR (CDCl₃) δ 159.4, 154.0, 147.1, 131.3, 127.2, 126.8, 123.4, 123.2, 119.5, 114.1, 112.2, 110.6, 105.4, 99.9, 55.8, 55.1, 50.7, 26.4. (TLC R_f 70:30 EtoAc:hexanes = 0.33). FAB-HRMS *m/z* 349.1659 [M+H]⁺.

3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-5-methoxy-1H-indole (7e):



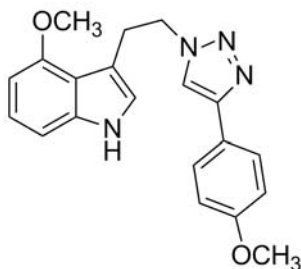
1.15 g (5.32 mmol) 3-(2-azidoethyl)-5-methoxy-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.67 mL (5.85 mmol) 1-Ethynyl-4-fluorobenzene added. The mixture was stirred at room temperature for 12 h. The mixture was added to 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 560 mg (31 % yield) of light brown viscous oil. Residual DMF was removed by heating to 70 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.40 (br s, 1H), 7.63 (m, 2H), 7.39 (s, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 7.02 (m, 2H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.84 (dd, *J* = 8.7 Hz, *J* = 2.4 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 4.62 (t, *J* = 7.0 Hz, 2H), 3.80 (s, 3H), 3.33 (t, *J* = 6.7 Hz, 2H); ¹³C NMR (CDCl₃) δ 162.47 (d, *J* = 247.2 Hz, 1C), 154.1, 146.4, 131.4, 127.29 (d, *J* = 9.2 Hz, 1C), 127.2, 126.72 (d, *J* = 3.0 Hz, 1C), 123.4, 120.0, 115.68 (d, *J* = 21.4 Hz, 1C), 112.3, 112.2, 110.7, 100.0, 55.8, 50.8, 26.5; ¹⁹F NMR (CDCl₃) δ -113.98 (septet, *J* = 4.6 Hz, 1F). (TLC R_f 70:30 EtoAc:hexanes = 0.36). FAB-HRMS *m/z* 337.1464 [M+H]⁺.

3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-methoxy-1H-indole (8a):



980 mg (4.54 mmol) 3-(2-azidoethyl)-4-methoxy-1H-indole, 1.15 g CuI and 570 μ L (4.99 mmol) 1-Ethynyl-4-fluorobenzene 1.1 eq was added to 8 mL 2:1 PEG-400:DMF and the mixture stirred at room temperature overnight. The resulting solution was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 660 mg (43 % yield) of a brown solid. Residual DMF and solvent was removed by vacuum at 60 °C. ¹H NMR (CDCl₃) δ 8.37 (br s, 1H), 7.66 (m, 2H), 7.36 (s, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.04 (m, 2H), 6.96 (dd, *J* = 8.1 Hz, 1 Hz, 1H), 6.65 (d, *J* = 2.3 Hz, 1H), 6.51 (d, *J* = 7.8 Hz, 1H), 4.71 (t, *J* = 6.9 Hz, 2H), 3.95 (s, 3H), 3.43 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 164.4 (d, *J* = 247.2 Hz, 2C), 154.3, 146.2, 138.1, 127.3 (d, *J* = 7.6 Hz, 2C), 126.9 (d, *J* = 3.1 Hz, 2C), 123.0, 121.8, 119.9, 116.8, 115.7 (d, *J* = 22.9 Hz, 2C), 111.4, 104.8, 99.4, 55.1, 52.1, 28.4; ¹⁹F NMR (CDCl₃) δ -114.2 (pentet, *J* = 4.5 Hz, 1F). (TLC R_f 70:30 EtoAc:hexanes = 0.33). FAB-HRMS *m/z* 336 [M+H]⁺.

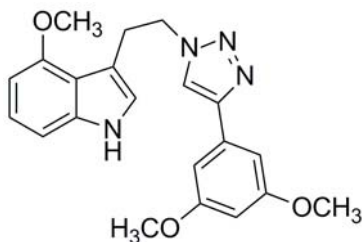
4-methoxy-3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (8b):



1.34 g (6.20 mmol) 3-(2-azidoethyl)-4-methoxy-1H-indole, 1.15 g CuI and 890 μ L (6.85 mmol) 4-ethynylanisole 1.1 eq was added to 8 mL 2:1 PEG-400:DMF and the mixture stirred at room temperature overnight. The resulting solution was diluted with 60 mL H_2O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na_2SO_4 , decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 690 mg (32 % yield) of an orange solid. Residual DMF and solvent was removed by vacuum at 60 $^{\circ}C$. 1H NMR ($CDCl_3$) δ 8.47 (br s, 1H), 7.65 (dt, J = 9.2 Hz, 2.8 Hz, 2H), 7.34 (s, 1H), 7.10 (t, J = 8.1 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 6.90 (dt, J = 9.2 Hz, 2.8 Hz, 2H), 6.63 (d, J = 2.5 Hz, 1H), 6.51 (d, J = 7.4 Hz, 1H), 4.68 (t, J = 7.0 Hz, 2H), 3.94 (s, 3H), 3.79 (s, 3H), 3.43 (t, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 159.3, 154.3, 146.9, 138.1, 126.8, 123.5, 122.9, 121.8, 119.3, 116.8, 114.1, 111.4, 104.8, 99.3, 55.2, 55.1, 52.0, 28.4. (TLC Rf: 0.42). FAB-HRMS m/z 349.1659 $[M+H]^+$.

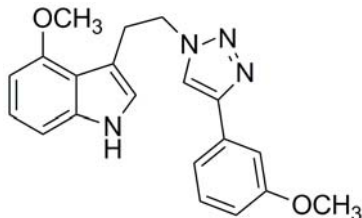
3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-4-methoxy-1H-indole

(8c):



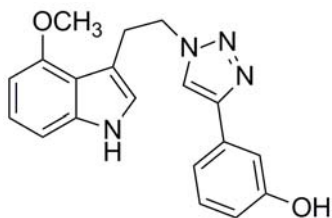
1.27 g (5.88 mmol) 3-(2-azidoethyl)-4-methoxy-1H-indole, 1.15 g CuI and 1.04 g (6.47 mmol) 1-Ethynyl-3,5-Dimethoxybenzene 1.1 eq was added to 8 mL 2:1 PEG-400:DMF and the mixture stirred at room temperature for 48 h. The resulting solution was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 750 mg (34 % yield) of an amber solid. Residual DMF and solvent was removed by vacuum at 60 °C. ¹H NMR (CDCl₃) δ 8.31 (br s, 1H), 7.38 (s, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 1.8 Hz, 2H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.50 (d, *J* = 7.8 Hz, 1H), 6.40 (t, *J* = 1.7 Hz, 1H), 4.70 (t, *J* = 6.8 Hz, 2H), 3.93 (s, 3H), 3.78, (s, 3H), 3.43 (t, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 161.3, 154.6, 147.2, 138.4, 132.9, 123.3, 122.1, 120.7, 117.0, 111.7, 105.1, 103.8, 100.6, 99.7, 55.7, 55.4, 52.4, 28.7. (TLC R_f: 0.43). FAB-HRMS *m/z* 379.1777 [M+H]⁺.

4-methoxy-3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (8d):



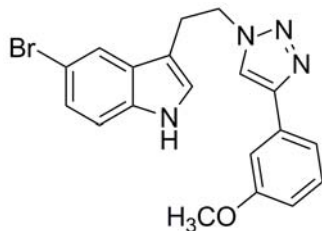
1.34 g (6.20 mmol) 3-(2-azidoethyl)-4-methoxy-1H-indole, 1.15 g CuI and 870 μ L (6.85 mmol) 3-Ethynylanisole 1.1 eq was added to 8 mL 2:1 PEG-400:DMF and the mixture stirred at room temperature for 48 h. The resulting solution was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 440 mg (20 % yield) of a light orange viscous oil. Residual DMF and solvent was removed by vacuum at 60 °C. ¹H NMR (CDCl₃) δ 8.33 (br s, 1H), 7.40 (s, 1H), 7.36 (m, 1H), 7.25 (m, 2H), 7.10 (t, J = 7.9 Hz, 1H), 6.95 (dd, J = 8.1 Hz, 1 Hz, 1H), 6.84 (dt, J = 6.8 Hz, 2.6 Hz, 1H), 6.65 (d, J = 2.3 Hz, 1H), 6.51 (d, J = 7.5 Hz, 1H), 4.70 (t, J = 7.0 Hz, 2H), 3.95 (s, 3H), 3.80 (s, 3H), 3.43 (t, J = 7.0 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.9, 154.3, 146.9, 138.1, 132.1, 129.7, 123.0, 121.8, 120.3, 118.0, 116.7, 113.9, 111.4, 110.6, 104.8, 99.4, 55.2, 55.1, 52.0, 28.4.(TLC Rf: 0.50). FAB-HRMS m/z 349.1671 [M+H]⁺.

3-(1-(2-(4-methoxy-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)phenol (8e):



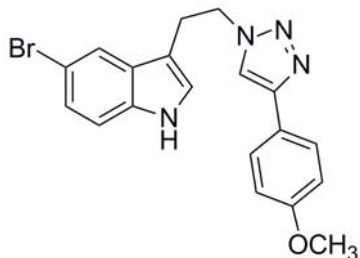
980 mg (4.54 mmol) 3-(2-azidoethyl)-4-methoxy-1H-indole, 1.15 g CuI and 540 μ L (4.99 mmol) 3-Hydroxyphenylacetylene 1.1 eq was added to 8 mL 2:1 PEG-400:DMF and the mixture stirred at room temperature for 48 h. The resulting solution was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 550 mg (36 % yield) of a light orange solid. Residual DMF and solvent was removed by vacuum at 60 °C. ¹H NMR (CDCl₃) δ 8.0 (br s, 1H), 7.45 (m, 1H), 7.30 (br s, 1H), 7.24 (s, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.10 (m, 2H), 6.93 (dd, *J* = 8.2 Hz, 1 Hz, 1H), 6.83 (ddd, *J* = 8.0 Hz, 2.4 Hz, 1.2 Hz, 1H), 6.57 (d, *J* = 2.2 Hz, 1H), 6.51 (d, *J* = 7.5 Hz, 1H), 4.69 (t, *J* = 6.7 Hz, 2H), 6.94 (s, 3H), 3.42 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (CDCl₃) δ 156.8, 154.4, 146.8, 138.1, 131.7, 130.1, 123.1, 121.9, 120.6, 117.6, 116.8, 115.5, 112.8, 111.4, 104.8, 99.6, 55.2, 52.1, 28.3. (TLC R_f: 0.34). FAB-HRMS *m/z* 334 [M+H]⁺.

5-bromo-3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (9a):



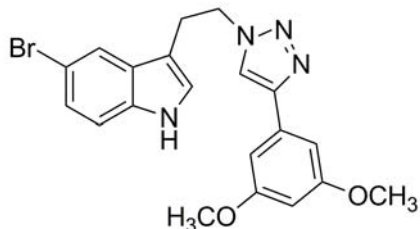
810 mg (3.06 mmol) 3-(2-azidoethyl)-5-bromo-1H-indole was dissolved in 5 mL 2:1 PEG-400:DMF and 1.28 g 2 eq. CuI and 500 μ L (3.93 mmol) 3-ethynylanisole 1.3 eq. added and the mixture stirred at room temperature overnight. The resulting solution was diluted with 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes increasing to 100% ethyl acetate to give 560 mg (46 % yield) of a dark brown syrup. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 9.4 (s, 1H), 7.58 (s, 1H), 7.54 (s, 1H), 7.38 (s, 1H), 7.20 (m, 4H), 6.80 (m, 2H), 4.51 (t, *J* = 5.8 Hz, 2H), 3.72 (s, 3H), 3.22 (t, *J* = 6.7 Hz, 2H); ¹³C NMR (CDCl₃) δ 160.2, 147.6, 135.1, 132.0, 130.1, 128.8, 125.2, 124.4, 120.9, 120.6, 118.4, 114.4, 113.3, 112.1, 111.1, 110.8, 55.5, 50.9, 26.6. (TLC R_f 70:30 EtoAc:hexanes = 0.22). FAB-HRMS *m/z* 397.0653 [M+H]⁺.

5-bromo-3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (9b):



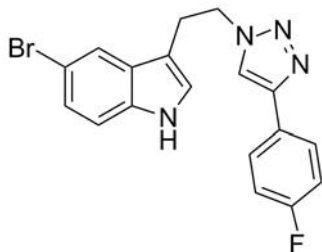
860 mg (3.24 mmol) 3-(2-azidoethyl)-5-bromo-1H-indole was dissolved in 10 mL DMF and 1.26 g 2 eq. CuI and 515 μ L (1.04 mmol) 4-ethynylanisole 1.2 eq added and the mixture stirred at room temperature overnight. The resulting solution was diluted with 160 ml H₂O and 40 mL ethyl acetate and 3.5 g disodium EDTA added with stir at room temperature for 1.5 h. The mixture was extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to 100 % ethyl acetate to give 720 mg (56 % yield) of a dark brown oil. Residual DMF and solvent was removed by vacuum at 90 °C. ¹H NMR (CDCl₃) δ 9.0 (s, 1H), 7.6 (m, 3H), 7.41 (s, 1H), 7.2 (m, 2H), 6.86 (d, J = 8.7 Hz, 2H), 6.78 (s, 1H), 4.55 (t, J = 7.3 Hz, 2H), 3.79 (s, 3H), 3.25 (t, J = 7.2 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.5, 147.3, 134.8, 128.5, 126.9, 124.8, 124.1, 123.0, 120.6, 119.4, 114.2, 113.0, 112.6, 110.4, 55.2, 50.6, 26.2. FAB-HRMS m/z 397.0667 [M+H]⁺.

5-bromo-3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (9c):



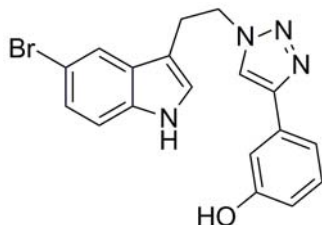
700 mg (2.64 mmol) 3-(2-azidoethyl)-5-bromo-1H-indole was dissolved in 6 mL 2:1 PEG-400:DMF and 1.0 g 2 eq. CuI and 515 mg (3.17 mmol) 1-Ethynyl-3,5-dimethoxybenzene added. The mixture was stirred at room temperature for 12 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 560 mg (50 % yield) of a light yellow powder. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (DMSO) δ 11.0 (s, 1H), 8.57 (s, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.27 (dd, *J* = 8.5 Hz, 0.5 Hz, 1H), 7.14 (dd, *J* = 8.7 Hz, 1.8 Hz, 2H), 6.95 (d, *J* = 2.3 Hz, 2H), 6.42 (t, *J* = 2.3 Hz, 1H), 4.6 (t, *J* = 7.1 Hz, 2H), 3.75 (s, 6H), 3.27 (apparent triplet, 2H); ¹³C NMR (DMSO) δ 161.5, 147, 136, 134, 130, 126, 124, 123, 122, 114, 112, 111, 104, 101, 56, 51, 26. (TLC R_f 70:30 EtoAc:hexanes = 0.30). FAB-HRMS *m/z* 427.0757 [M+H]⁺.

5-bromo-3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (9d):



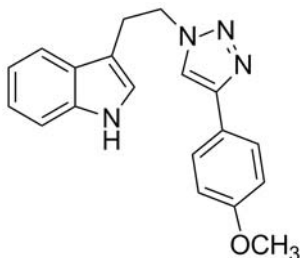
1.00 g (3.77 mmol) 3-(2-azidoethyl)-5-bromo-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.00 g CuI and 0.70 mL (6.11 mmol), 1-Ethynyl-4-fluorobenzene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude product was purified by flash column chromatography, eluting with 70:30 ethyl acetate: hexanes and residual crude purified by reprecipitation. The crude solid was dissolved in acetone and the product precipitated by the addition of H₂O. The purified product was collected by vacuum filtration to give 370 mg (25 % yield) combined of an off-white solid. ¹H NMR (DMSO) δ 11.0 (br s, 1H), 8.51 (s, 1H), 7.80 (m, 2H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.23 (m, 3H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.12 (m, 1H), 4.62 (t, *J* = 7.2 Hz, 2H), 3.28 (t, *J* = 7.2 Hz, 2H); ¹³C NMR (DMSO) δ 162.34 (d, *J* = 244.1 Hz, 1C), 146.0, 135.5, 129.5, 128.1 (d, *J* = 4.2 Hz, 1C), 127.8 (d, *J* = 9.1 Hz, 1C), 125.7, 124.2, 121.9, 121.2, 116.4 (d, *J* = 21.4 Hz, 1C), 114.1, 111.9, 110.6, 50.9, 26.3; ¹⁹F NMR (DMSO) δ (septet, *J* = 4.5 Hz, 1F) -114.65. FAB-HRMS *m/z* 385.0461 [M+H]⁺.

3-(1-(2-(5-bromo-1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)phenol (9e):



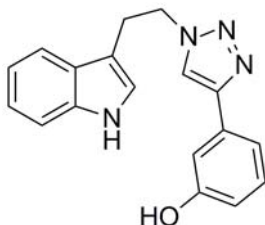
1.26 g (4.75 mmol) 3-(2-azidoethyl)-5-bromo-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 570 μ L (5.23 mmol) 3-Hydroxyphenylacetylene added and the mixture stirred at room temperature overnight. The resulting solution was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 800 mg (44 % yield) of a thick amber syrup. Residual DMF was removed by heating to 60 °C and removing in vacuo. ¹H NMR (Acetone-d₆) δ 10.28 (br s, 1H), 8.50 (s, 1H), 8.20 (s, 1H), 7.78 (d, J = 1.8 Hz, 1H), 7.39 (m, 1H), 7.36 (dd, J = 7.2 Hz, 0.7 Hz, 1H), 7.28 (dt, J = 7.6 Hz, 1.2 Hz, 1H), 7.21 (m, 2H), 7.15 (d, J = 2.5 Hz, 1H), 6.78 (ddd, J = 8.1 Hz, 2.6 Hz, 1.1 Hz, 1H), 4.73 (t, J = 7.1 Hz, 2H), 3.41 (t, J = 7.2 Hz, 2H); ¹³C NMR (Acetone-d₆) δ 158.0, 147.0, 135.6, 133.0, 130.0, 129.4, 155.1, 124.3, 121.0, 120.9, 116.9, 114.9, 113.5, 112.4, 112.0, 110.9, 50.6, 26.2. (TLC R_f 70:30 EtoAc:hexanes = 0.33). FAB-HRMS m/z 383.0501 [M+H]⁺.

3-(2-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (10a):



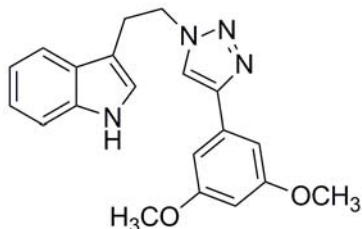
1.21 g (6.51 mmol) 3-(2-azidoethyl)-1H-indole was dissolved in 6 mL 2:1 PEG-400:DMF and 2.45 g 2 eq. CuI and 1.00 mL (7.81 mmol), 1.2 eq. 4-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 4x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 620 mg (30 % yield) of light brown powder. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.10 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.37 (m, 2H), 7.19 (apparent dt, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 1.8 Hz, 1H), 4.67 (t, *J* = 7.0 Hz, 2H), 3.81 (s, 3H), 3.39 (t, *J* = 7.1 Hz, 2H); ¹³C NMR (CDCl₃) δ 159.5, 147.2, 136.3, 127.0, 126.8, 123.4, 122.7, 122.4, 119.7, 119.3, 118.2, 114.2, 111.4, 111.2, 55.3, 50.7, 26.6. (TLC R_f 70:30 EtoAc:hexanes = 0.43). FAB-HRMS *m/z* 319.1559 [M+H]⁺.

3-(1-(2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)phenol (10b):



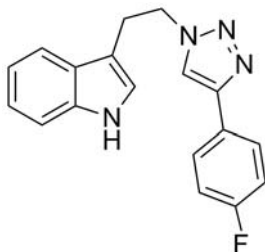
1.14 g (6.12 mmol) 3-(2-azidoethyl)-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.15 g CuI and 0.752 mL (6.90 mmol), 3-Hydroxyphenylacetylene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 620 mg (54 % yield) of light brown powder. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (DMSO) δ 10.82 (br s, 1H), 9.47 (s, 1H), 8.47 (s, 1H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.20 (m 3H), 7.06 (m, 2H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.67 (m, 1H), 4.62 (t, *J* = 7.5 Hz, 2H), 3.28 (apparent t, 2H); ¹³C NMR (DMSO) δ 158.4, 146.9, 136.8, 132.8, 130.6, 127.6, 123.9, 121.9, 121.8, 119.1, 118.8, 116.7, 115.5, 112.5, 112.1, 110.6, 50.8, 26.5. (TLC R_f 70:30 EtoAc:hexanes = 0.38). FAB-HRMS *m/z* 305.1399 [M+H]⁺.

3-(2-(4-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (10c):



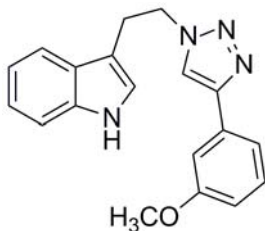
960 mg (5.16 mmol) 3-(2-azidoethyl)-1H-indole was dissolved in 6 mL 2:1 PEG-400:DMF and 960 mg 2 eq. CuI and 1.00 g (6.19 mmol), 1.2 eq. 1-Ethynyl-3,5-dimethoxybenzene added. The mixture was stirred at room temperature for 48 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 700 mg (39 % yield) of an off-white powder. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.30 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.40 (s, 1H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.18 (td, *J* = 8 Hz, 1.1 Hz, 1H), 7.13 (td, *J* = 7.8 Hz, 1.0 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 2H), 6.80 (d, *J* = 2.3 Hz, 1H) 6.40 (t, *J* = 2.3 Hz, 1H), 4.64 (t, *J* = 7.1 Hz, 2H), 3.77 (s, 6H), 3.36 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (CDCl₃) δ 161.1, 147.2, 136.2, 132.4, 126.7, 122.7, 122.2, 120.5, 119.6, 118.1, 111.5, 110.9, 103.6, 100.4, 55.4, 50.8, 26.5. (TLC R_f 70:30 EtoAc:hexanes = 0.33). FAB-HRMS *m/z* 349.1659 [M+H]⁺.

3-(2-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (10d):



1.20 g (6.45mmol) 3-(2-azidoethyl)-1H-indole was dissolved in 8 mL 2:1 PEG-400:DMF and 1.25 g CuI and 0.800 mL (10.1 mmol), 1-Ethynyl-4-fluorobenzene added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with 60 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude product was purified by reprecipitation by dissolving the solid in acetone and precipitating the product by the addition of H₂O. The purified product was collected by vacuum filtration to give 650 mg (33 % yield) of an off-white cake. ¹H NMR (CDCl₃) δ 8.06 (br s, 1H), 7.68 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.36 (m 2H), 7.24 (apparent td, 1H), 7.14 (td, *J* = 7.0 Hz, 1.0 Hz, 1H), 7.06 (td, *J* = 9.0 Hz, 0.6 Hz, 2H), 6.85 (d, *J* = 1.8 Hz, 1H), 4.69 (t, *J* = 7.0 Hz, 2H), 3.40 (t, *J* = 7.0 Hz, 2H); ¹³C NMR (CDCl₃) δ 162.4 (d, *J* = 244.2 Hz, 1C), 146.0, 136.8, 128.2 (d, *J* = 3.1 Hz, 1C), 127.8 (d, *J* = 9.1 Hz, 1C), 127.6, 123.9, 121.9, 121.8, 119.1, 118.8, 116.5 (d, *J* = 21.4 Hz, 1C), 112.1, 110.6, 50.9, 26.5; ¹⁹F NMR (CDCl₃) δ -114.2 (septet, *J* = 4.6 Hz, 1F). FAB-HRMS *m/z* 307.1364 [M+H]⁺.

3-(2-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-indole (10e):



1.15 g (6.18 mmol) 3-(2-azidoethyl)-1H-indole was dissolved in 6 mL 2:1 PEG-400:DMF and 2.35 g 2 eq. CuI and 0.940 mL (7.42 mmol), 1.2 eq. 3-Ethynylanisole added. The mixture was stirred at room temperature for 12 h. The mixture was added to 40 mL H₂O and extracted with 3x 40 mL portions of ethyl acetate. The organic layers were combined, dried over Na₂SO₄, decanted and the solvent removed by rotary evaporation. The crude was purified by flash column chromatography, eluting with 70:30 ethyl acetate:hexanes to give 710 mg (36 % yield) of a viscous maroon oil. Residual DMF was removed by heating to 90 °C and removing in vacuo. ¹H NMR (CDCl₃) δ 8.40 (s, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.42 (s, 1H), 7.26 (m, 7H), 6.84 (m, 2H), 4.66 (t, *J* = 6.8 Hz, 2H), 3.81 (s, 3H), 3.38 (t, *J* = 7.2 Hz, 2H); ¹³C NMR (CDCl₃) δ 160.2, 147.5, 136.5, 132.2, 130.0, 127.0, 123.0, 122.5, 120.6, 119.9, 118.4, 118.3, 114.3, 111.7, 111.3, 111.0, 55.5, 51.0, 27.0. FAB-HRMS *m/z* [M+H]⁺.

3.6 Determination of antioxidant activity in linoleic acid emulsion system by ferric-thiocyanate method:

The total antioxidant activity of the newly synthesized melatonin/phenolic analogues was determined and compared to a known antioxidant butylated hydroxyanisole, by the ferric-thiocyanate method from a previously published procedure,

with some modifications.^{20,57,58} Different concentrations of sample (500 μ L) (125, 62.5, 32 μ M) in DMSO were mixed with linoleic acid emulsion in potassium phosphate buffer (500 μ L) (0.02 M, pH 7.0). Ten milliliters of linoleic acid emulsion was prepared by homogenous mixing of 35 mg Tween 20, 31 μ L linoleic acid and 0.02 M phosphate buffer. The mixture was incubated in a warm water bath at 37 °C for 24 h. Aliquots of 0.1 mL were taken at 24 h. and sequentially added to ethanol (5 mL, 75% v/v), ammonium thiocyanate (0.1 mL, 30% w/v) and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl v/v) to a sample solution (0.1 mL) and the absorbance read at 500 nm. Ethanol was used as a blank. The amount of oxidation was measured and the data reported are the average of triplicates. During the incubation time, peroxides are formed from the linoleic acid that leads to the oxidation of Fe^{2+} to Fe^{3+} . The ferric ion complexes with thiocyanate, forming a red hue and this complex has a maximum absorbance at 500 nm. The inhibition of lipid peroxidation in percent can be calculated from the following equation:

$$\text{LPI (\%)} = 100 - [(A_1/A_0) \times 100],$$

where A_1 is the absorbance at 500 nm of the sample and A_0 is the absorbance of the control at 500 nm.

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Appendix-I ^1H NMR Spectra

Figure A-I.1. ^1H NMR spectra of (**5a**)

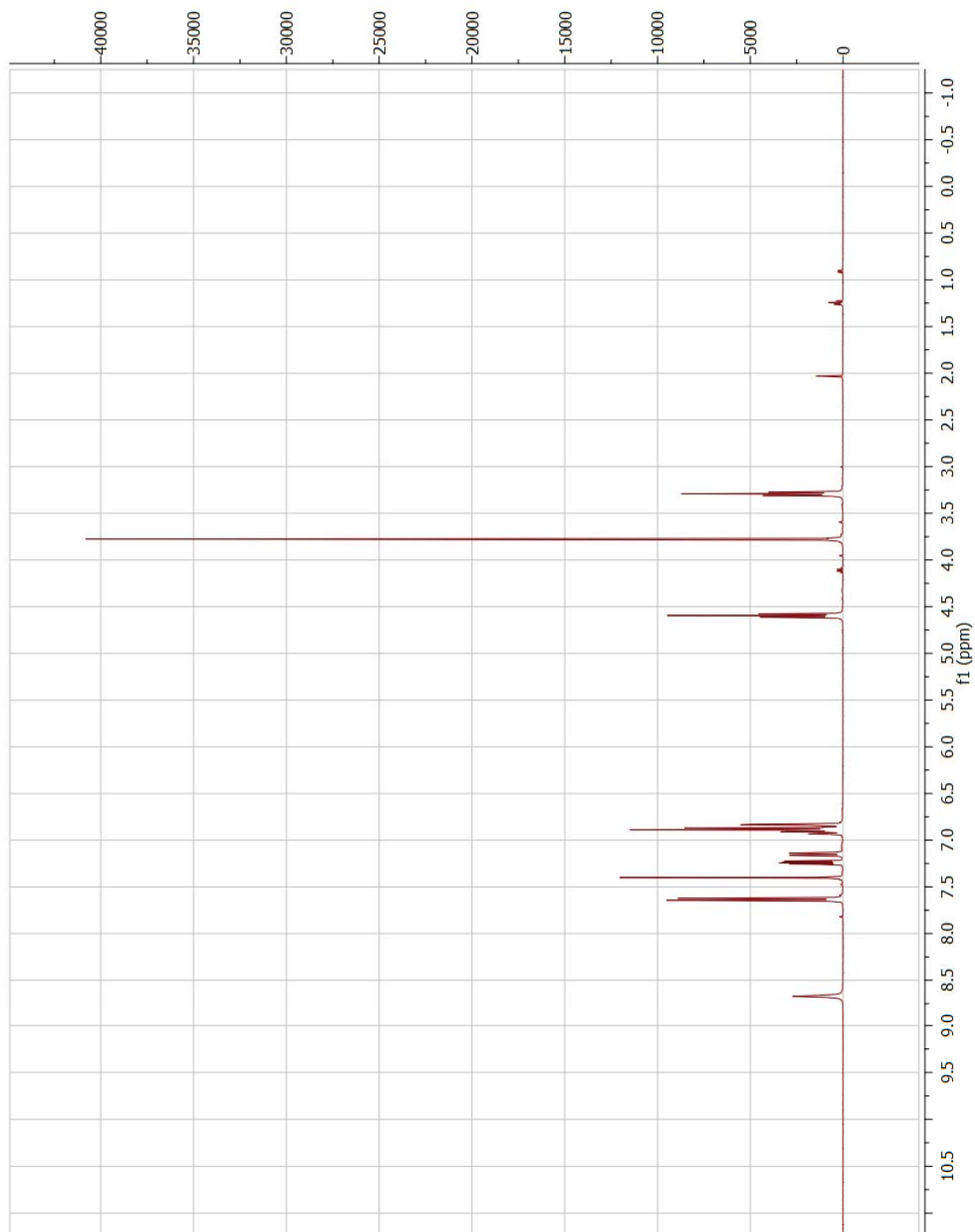


Figure A-I.2. ^1H NMR spectra of **(5b)**

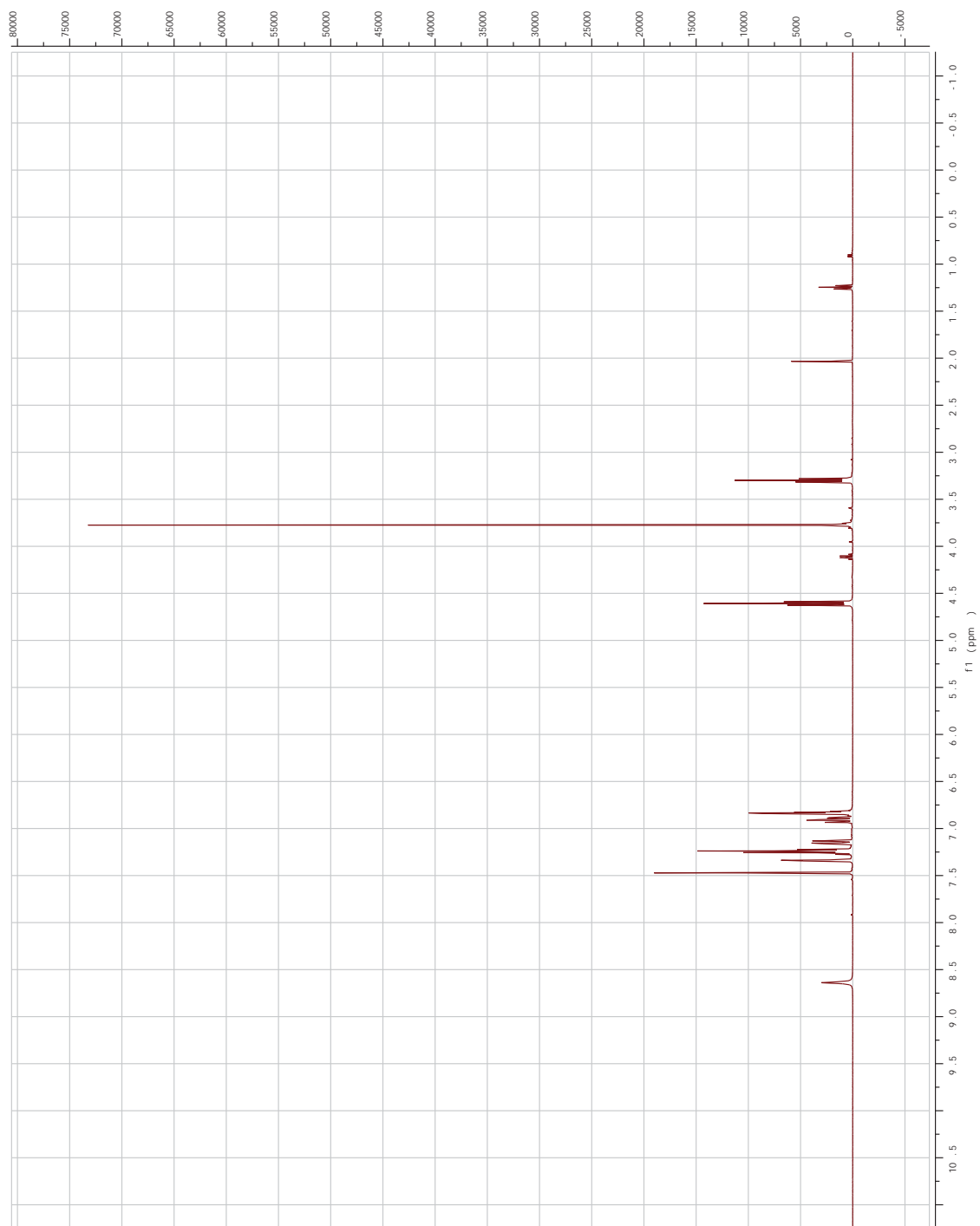


Figure A-I.3. ^1H NMR spectra of (**5c**)

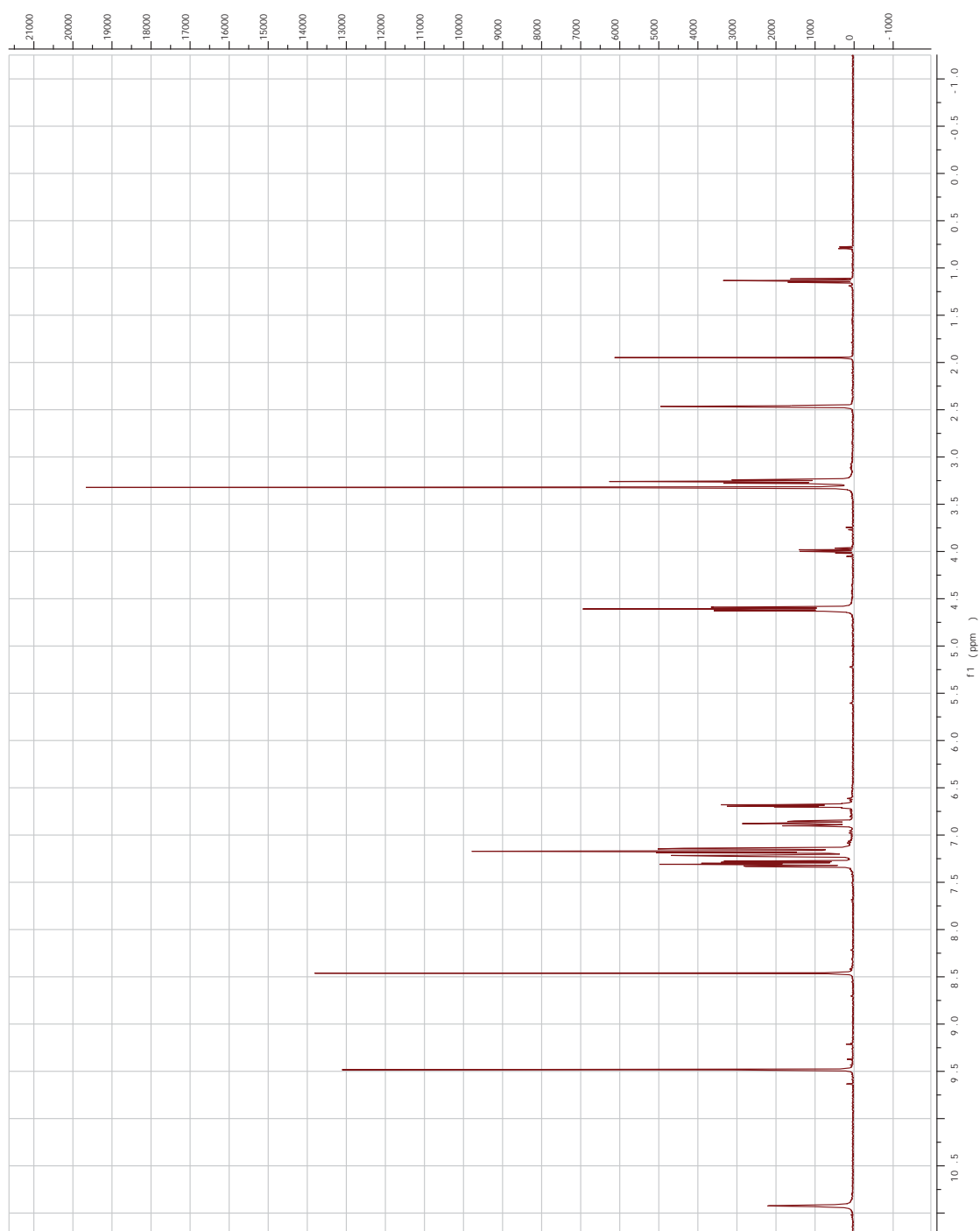


Figure A-I.4. ^1H NMR spectra of (**5d**)

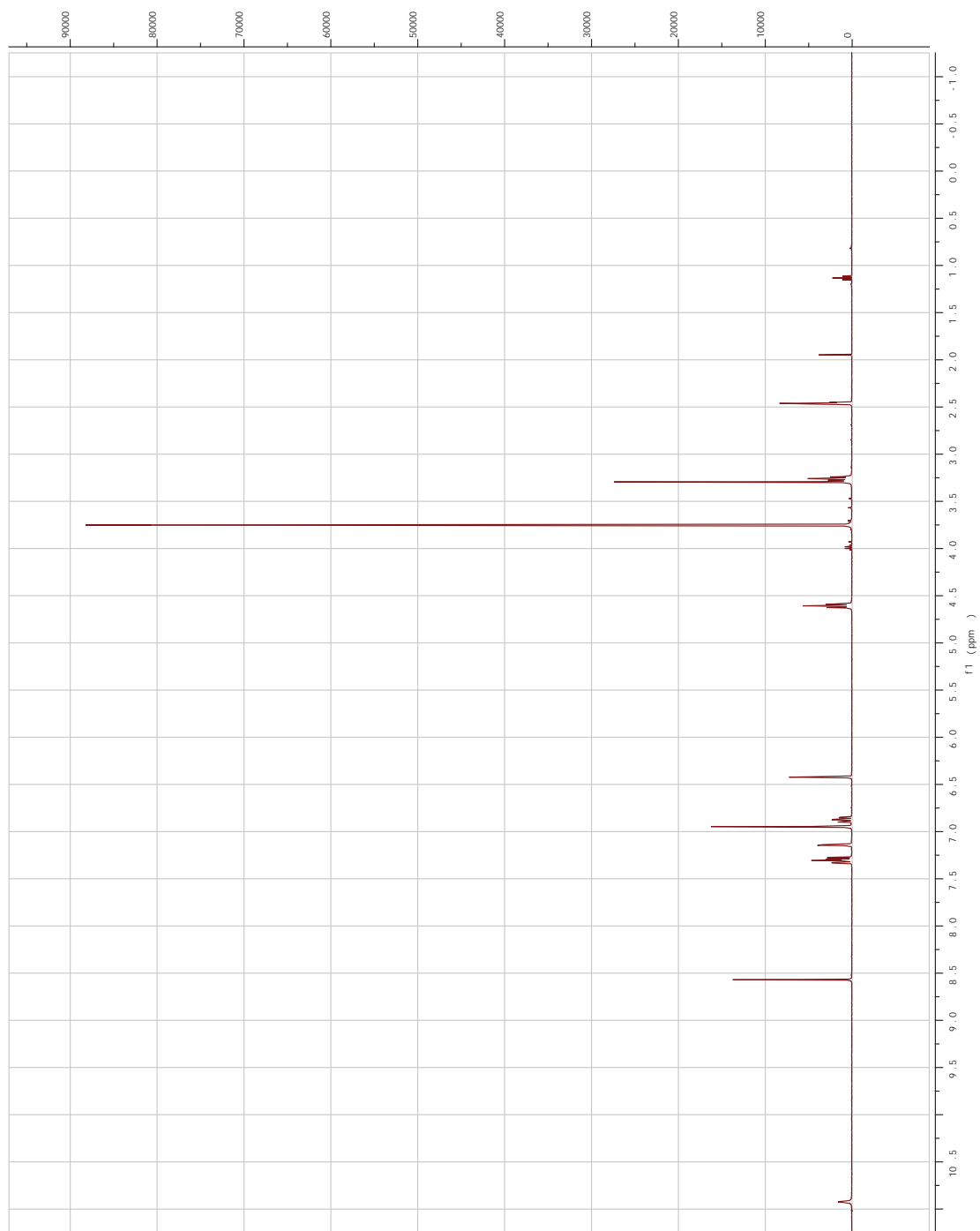


Figure A-I.5. ^1H NMR spectra of (**5e**)

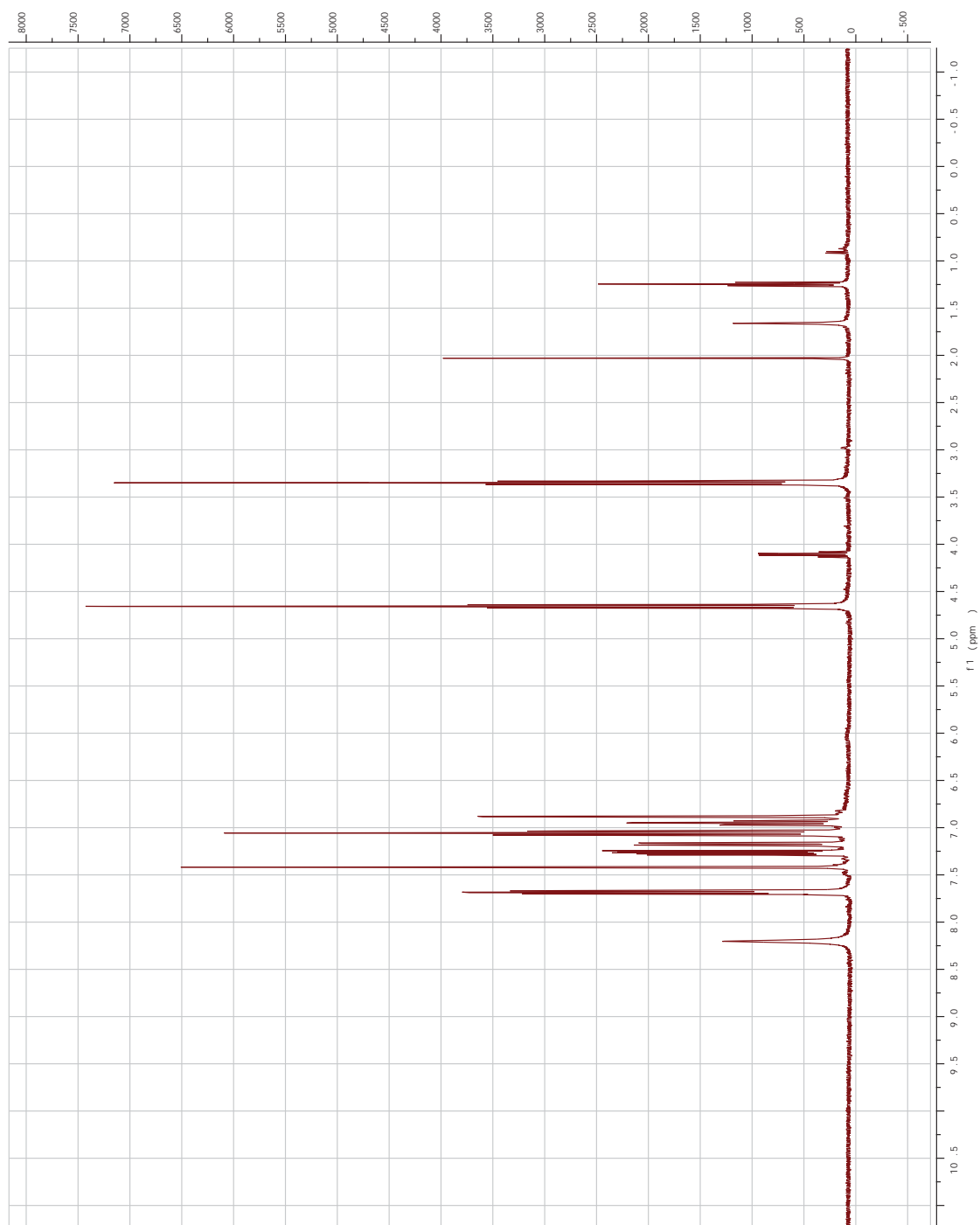


Figure A-I.6. ^1H NMR spectra of (**6a**)

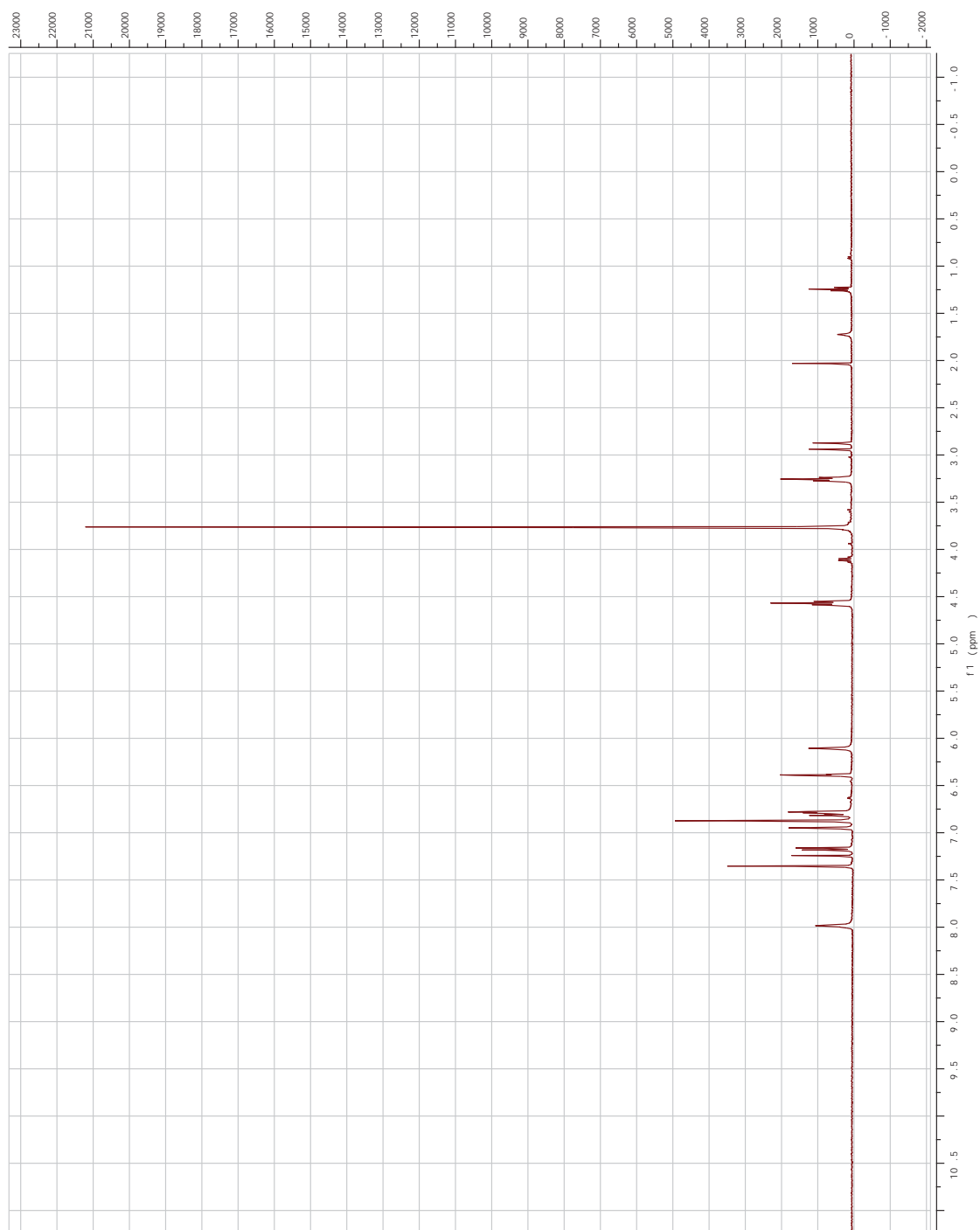


Figure A-I.7. ^1H NMR spectra of (**6b**)

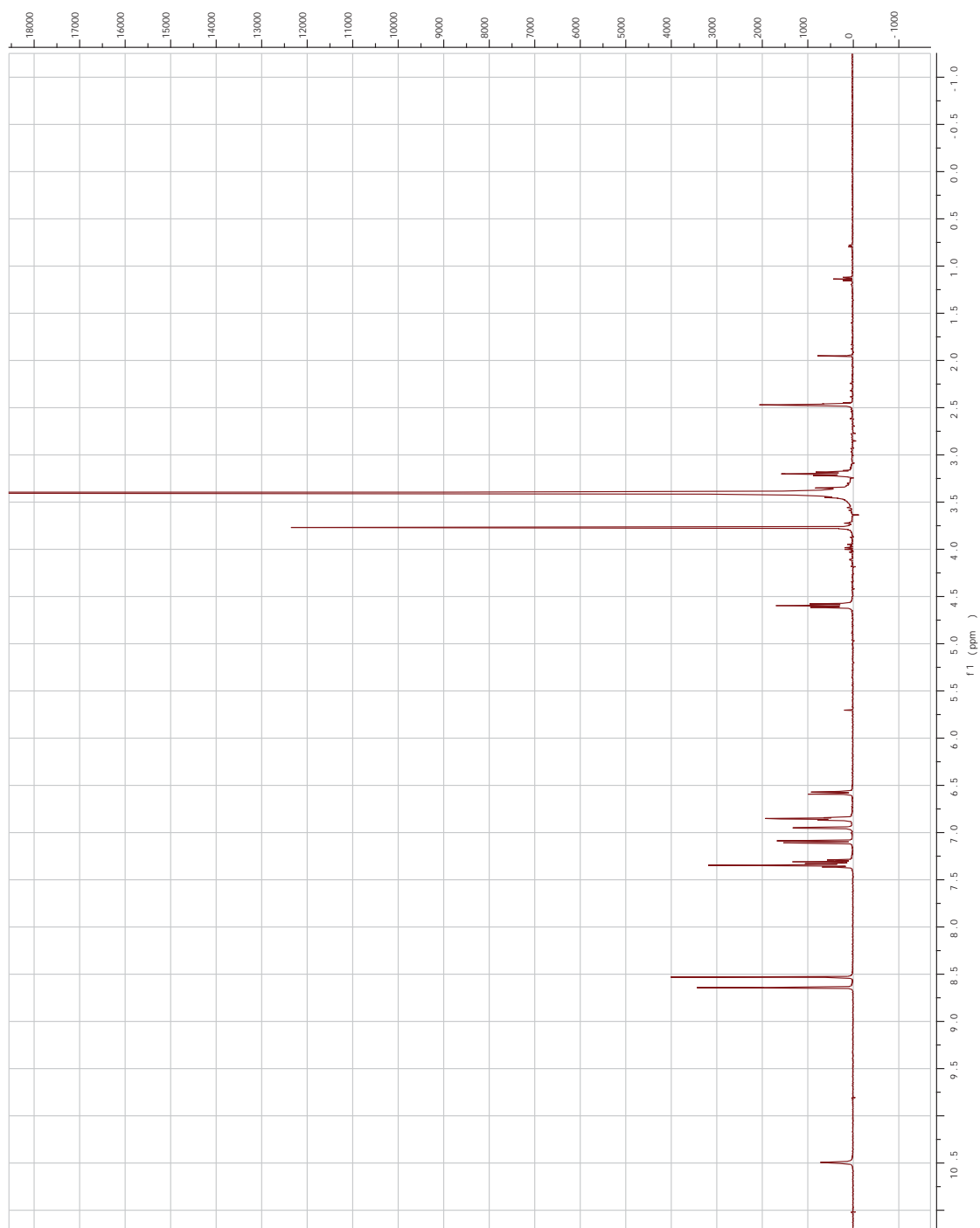


Figure A-I.8. ^1H NMR spectra of (**6c**)

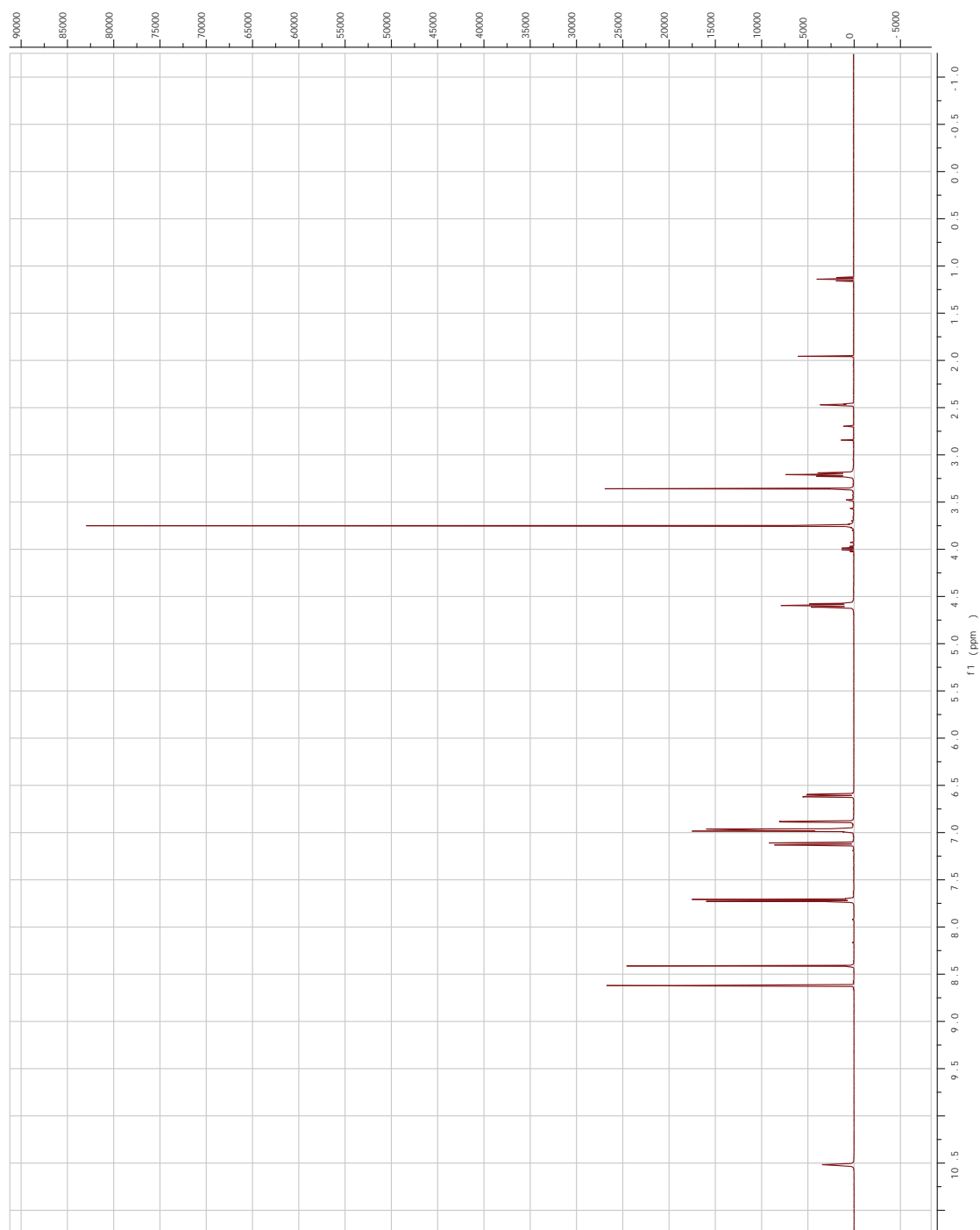


Figure A-I.9. ^1H NMR spectra of (**6d**)

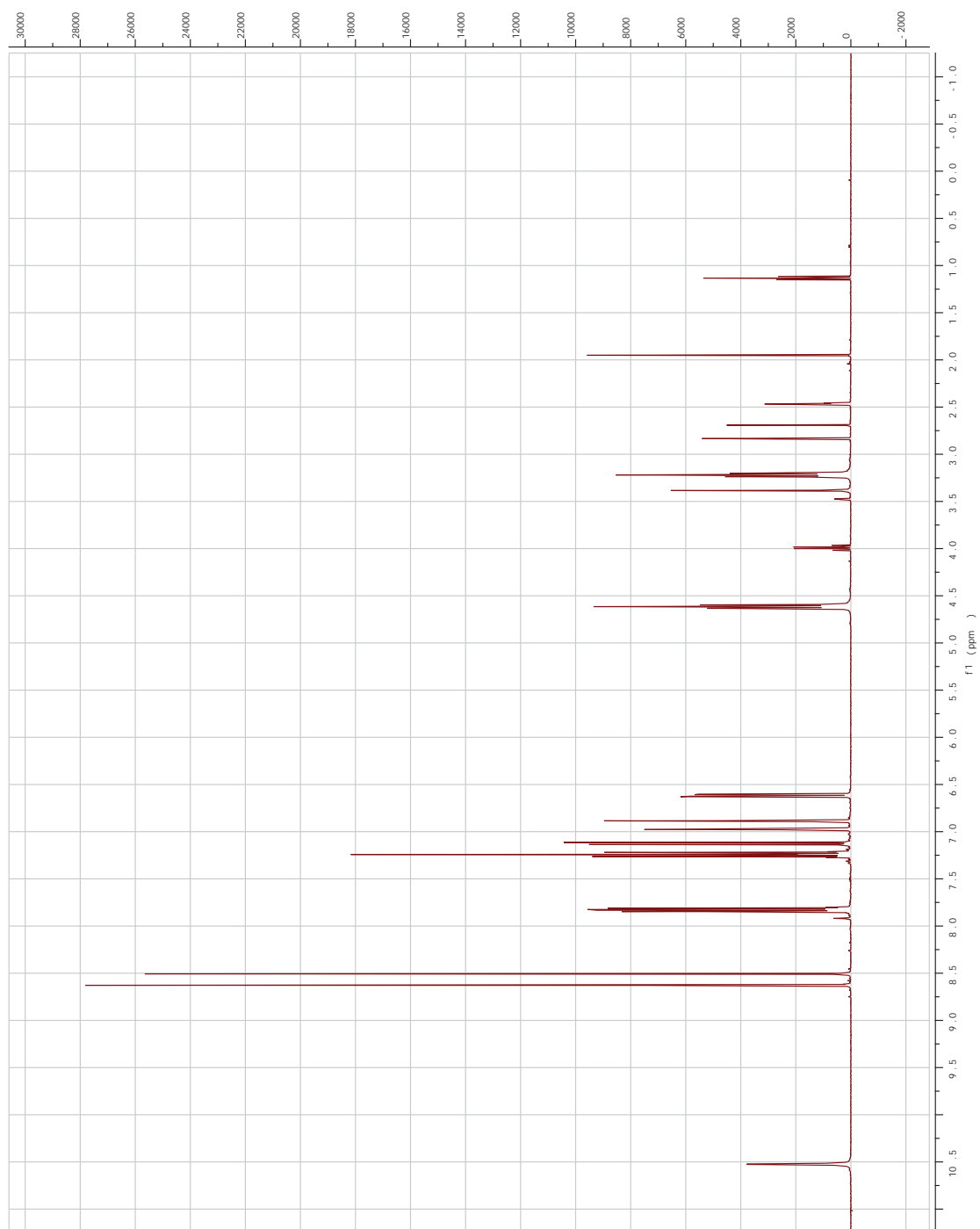


Figure A-I.10. ^1H NMR spectra of **(6e)**

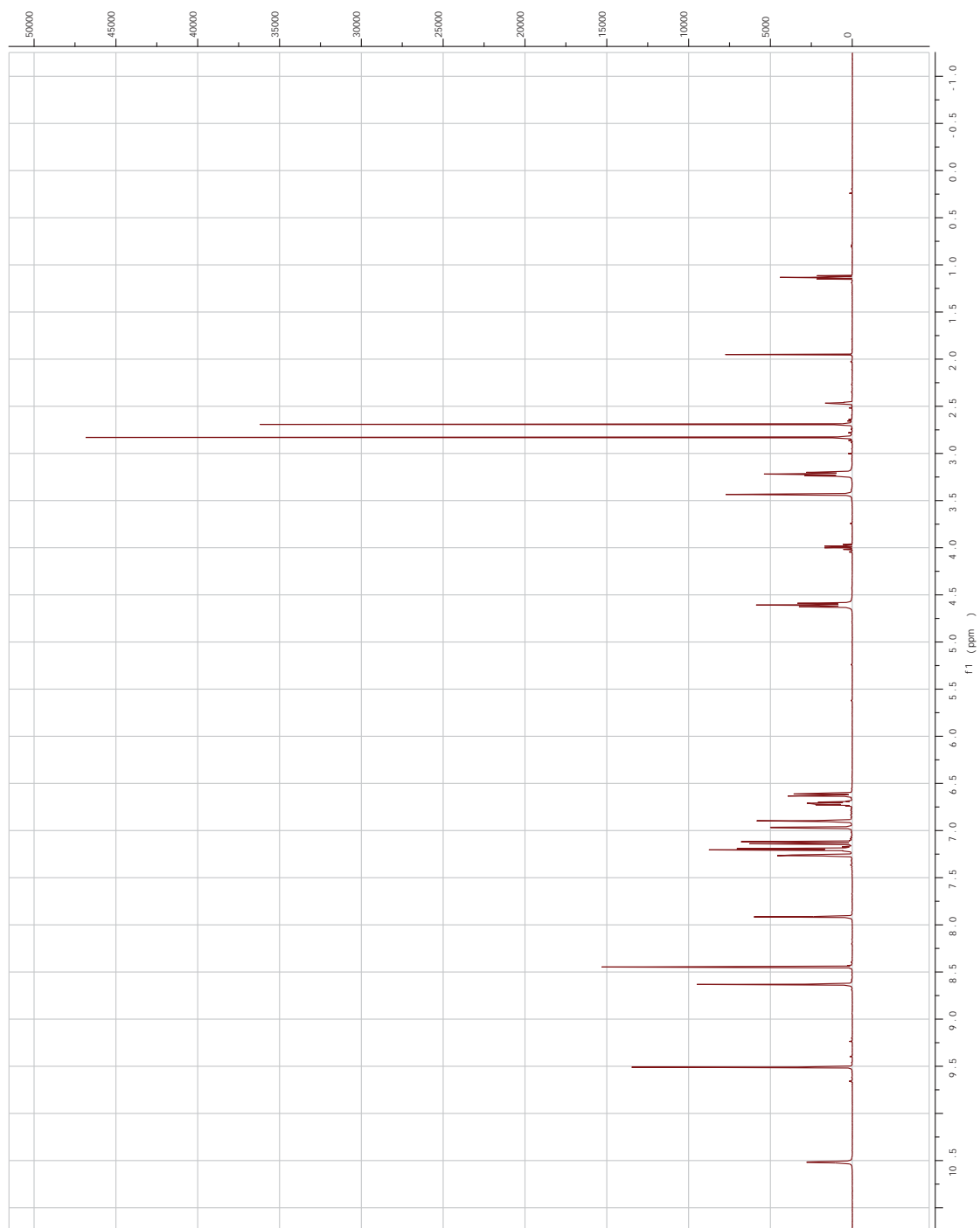


Figure A-I.11. ^1H NMR spectra of (**7a**)

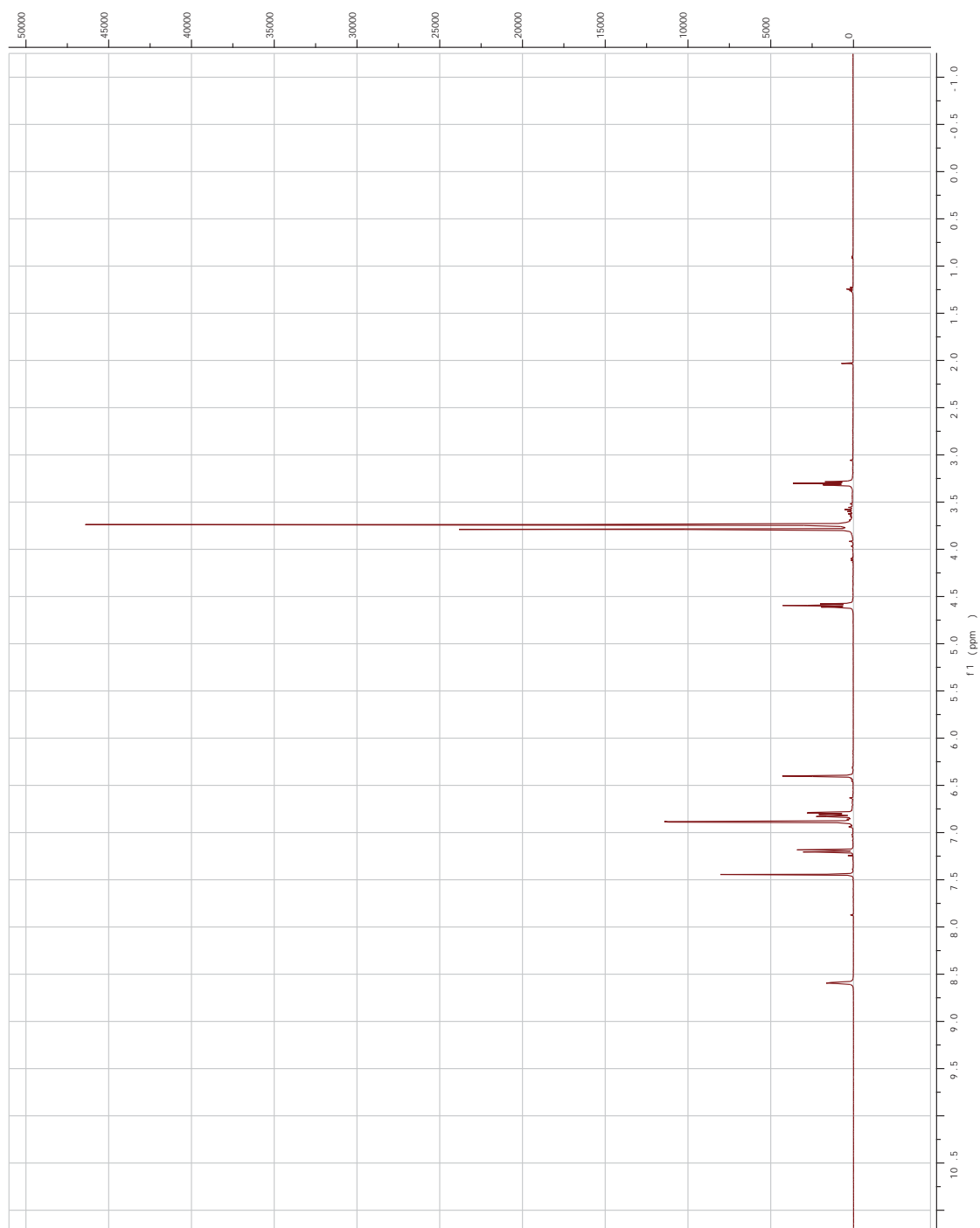


Figure A-1.12. ^1H NMR spectra of (**7b**)

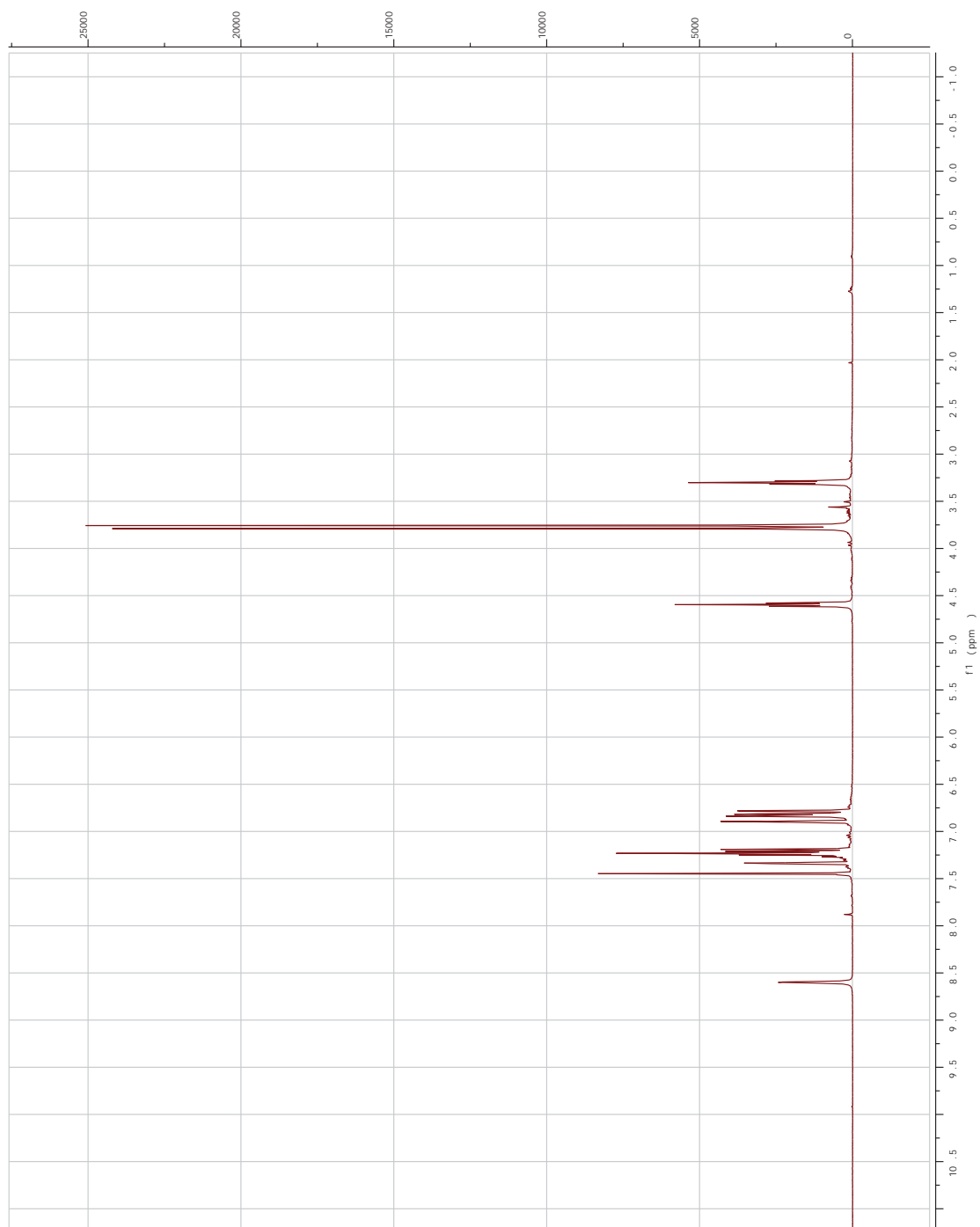


Figure A-I.13. ^1H NMR spectra of (7c)

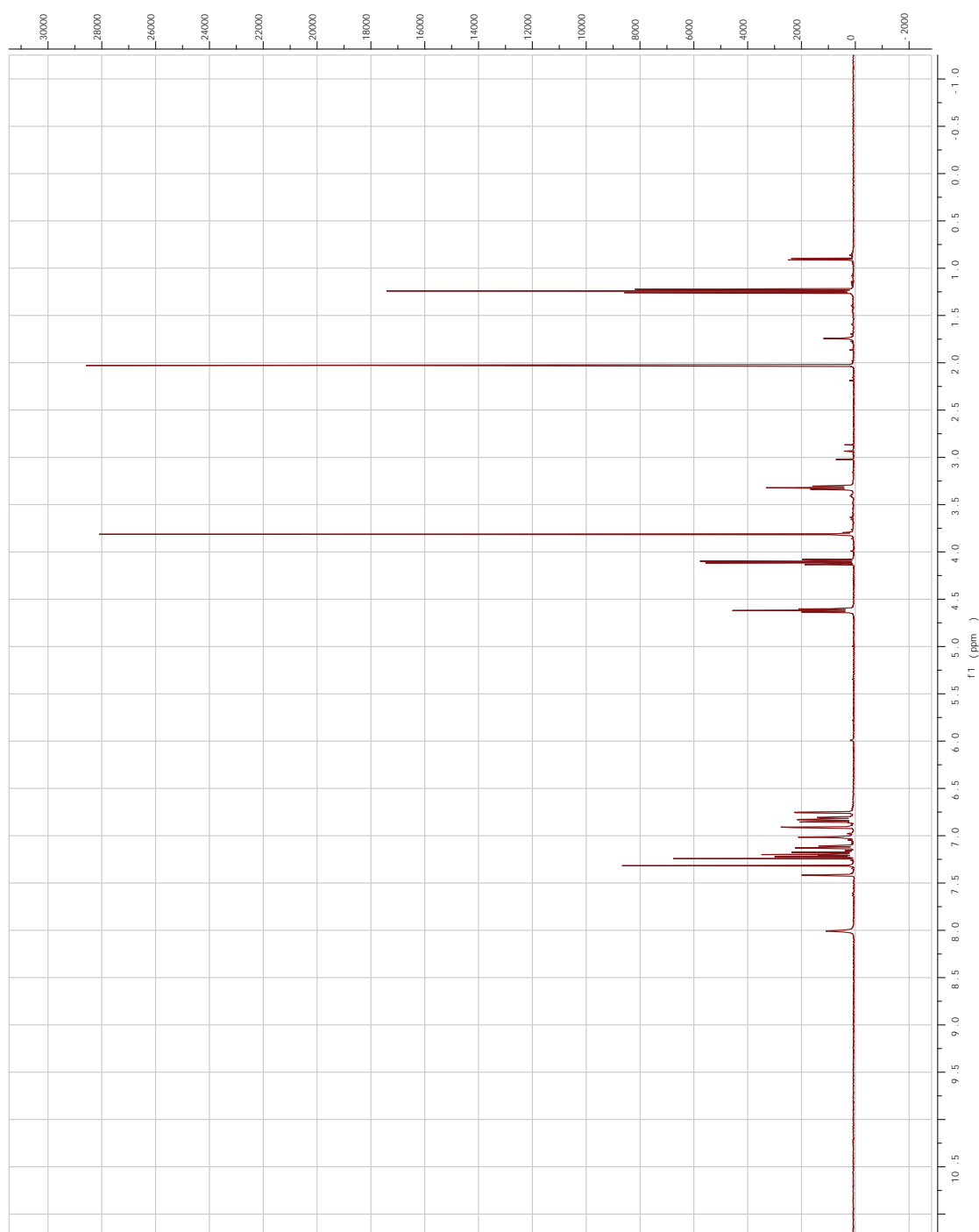


Figure A-I.14. ^1H NMR spectra of (**7d**)

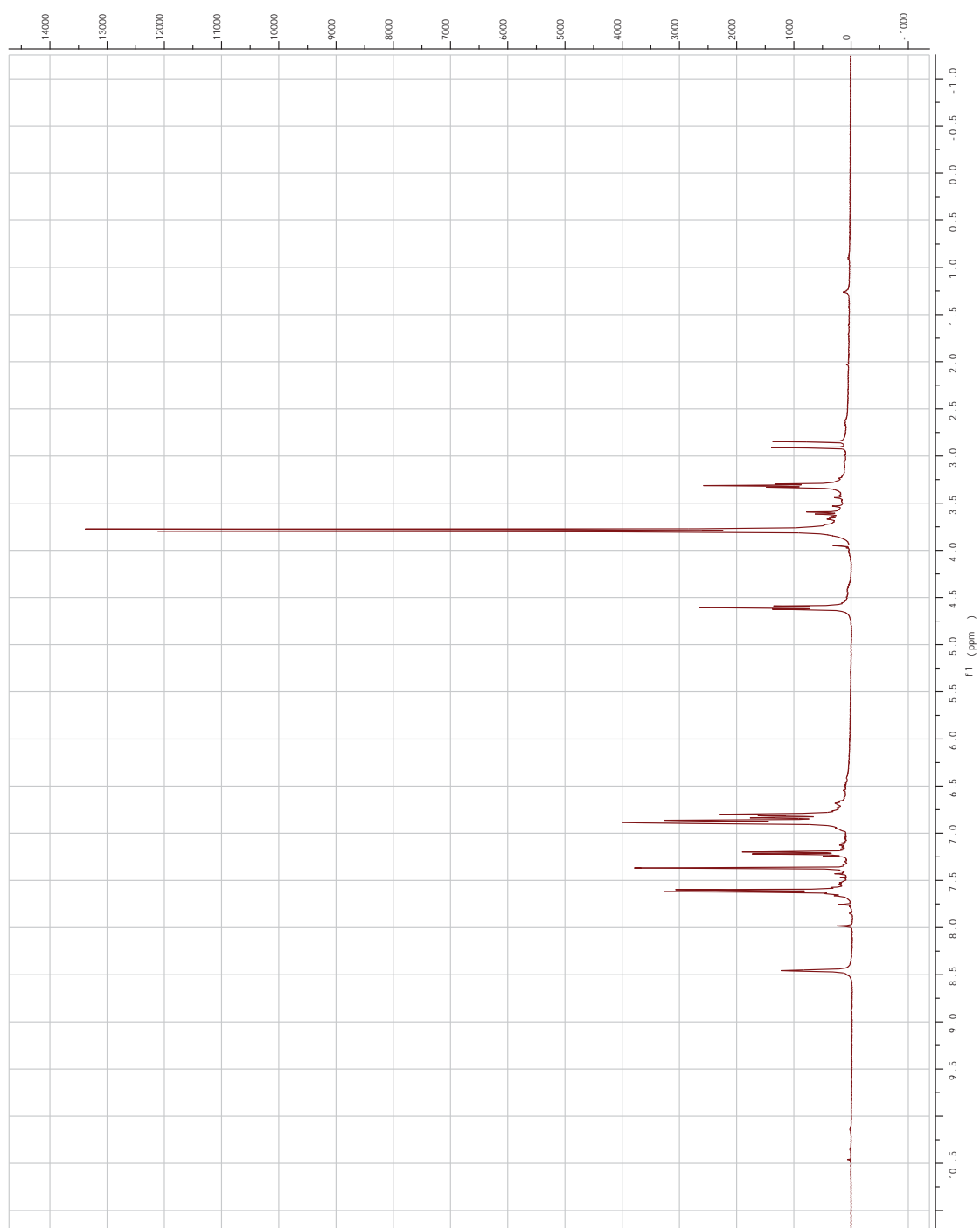


Figure A-I.15. ^1H NMR spectra of (**7e**)

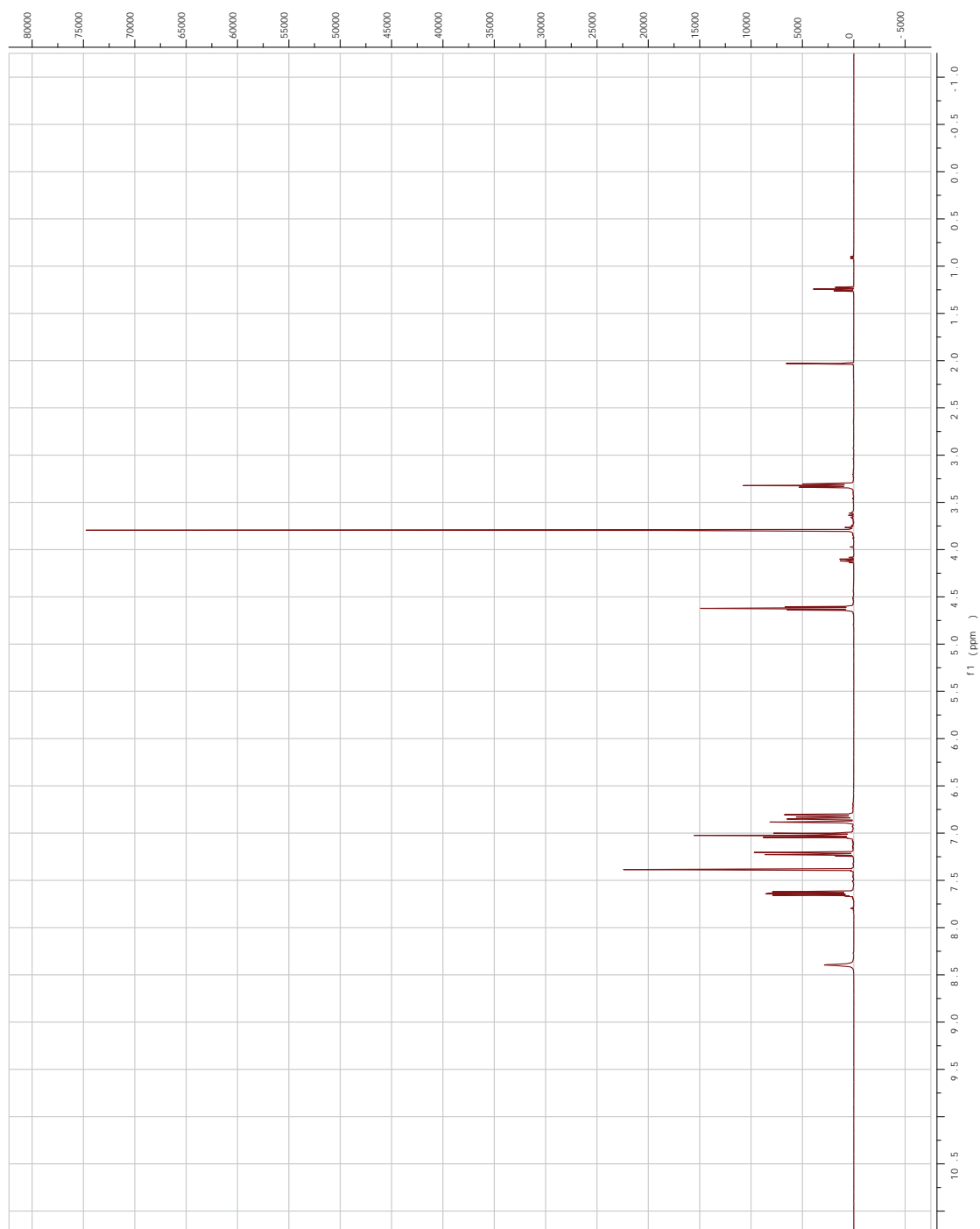


Figure A-I.16. ^1H NMR spectra of (**8a**)

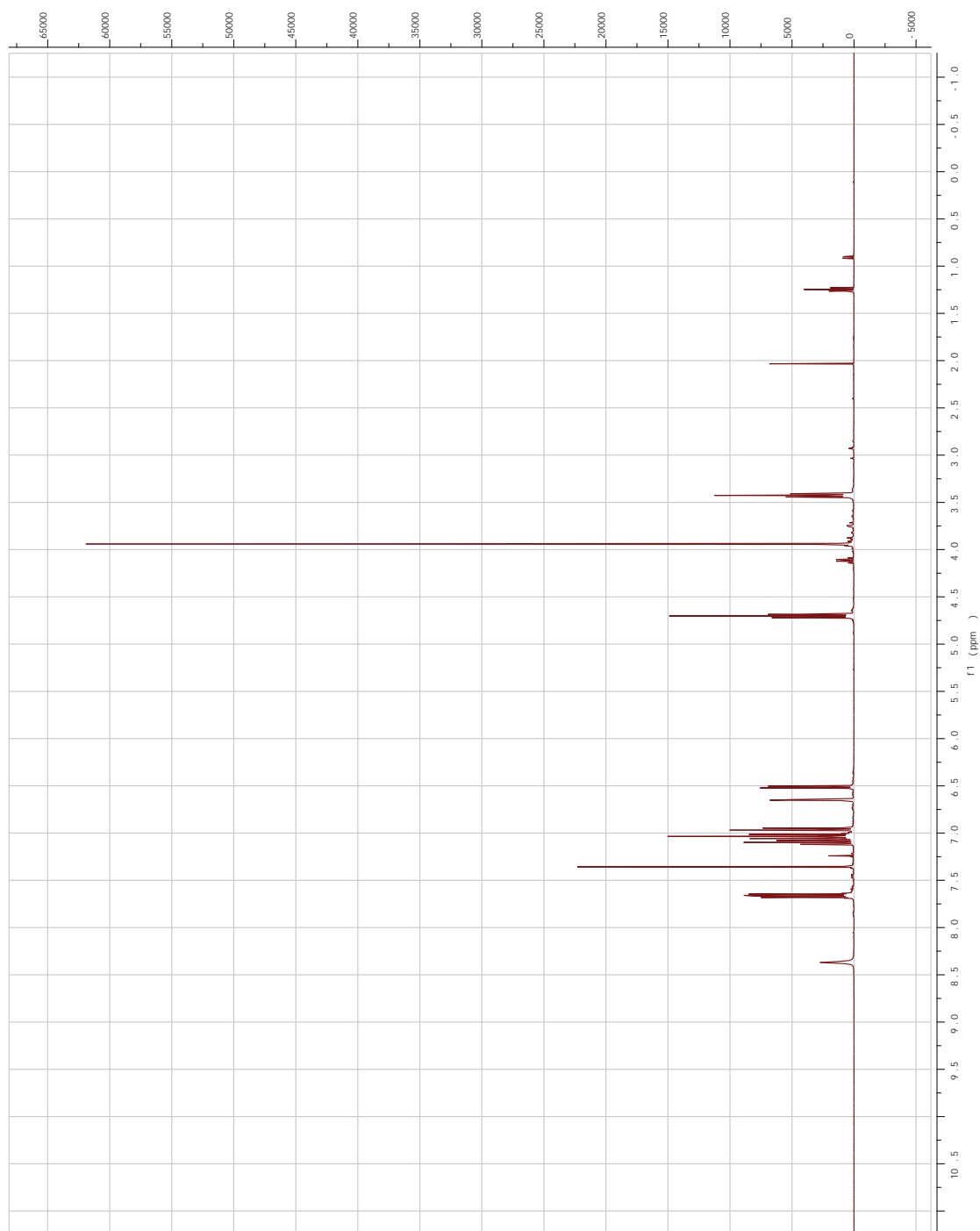


Figure A-I.17. ^1H NMR spectra of **(8b)**

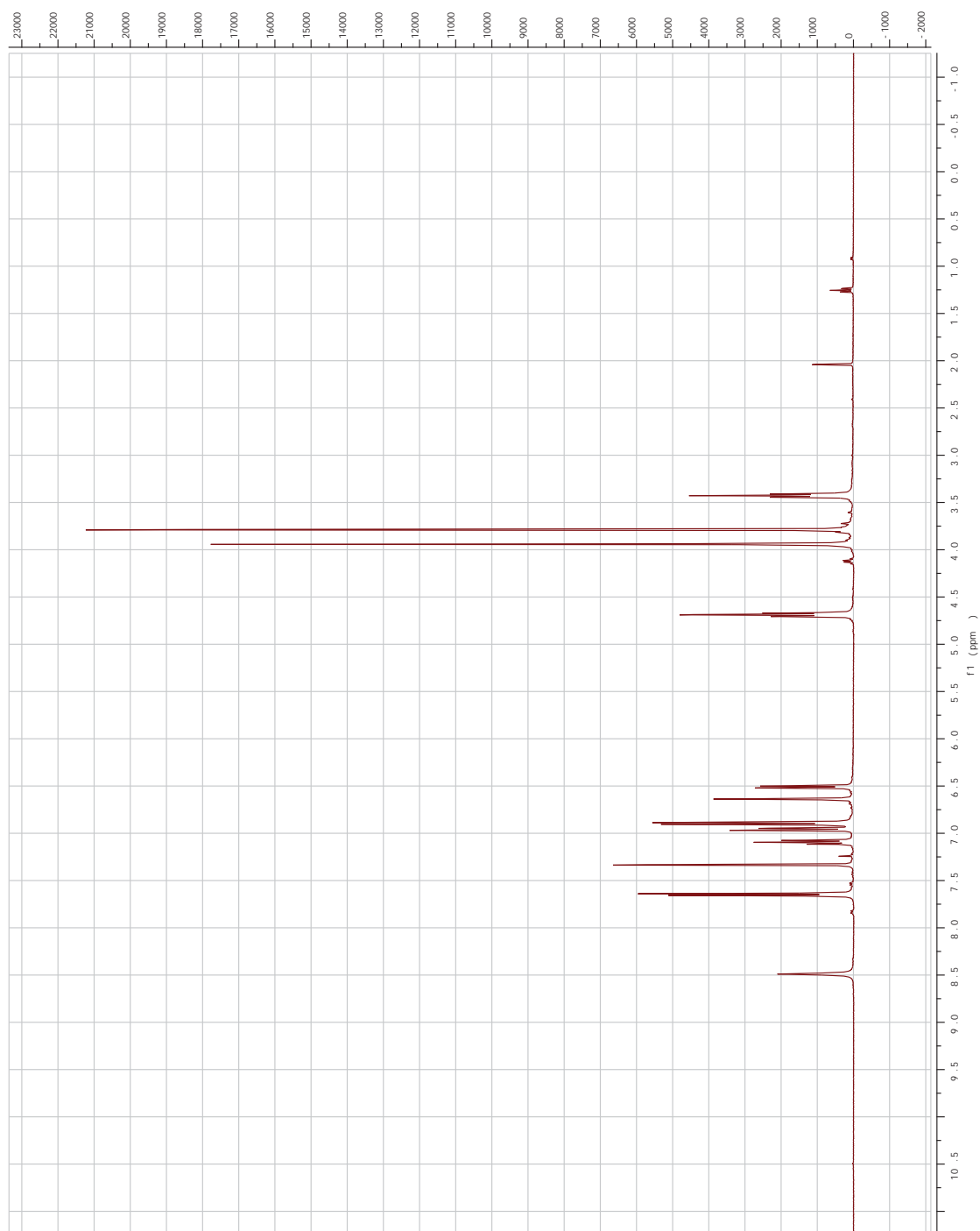


Figure A-I.18. ^1H NMR spectra of (**8c**)

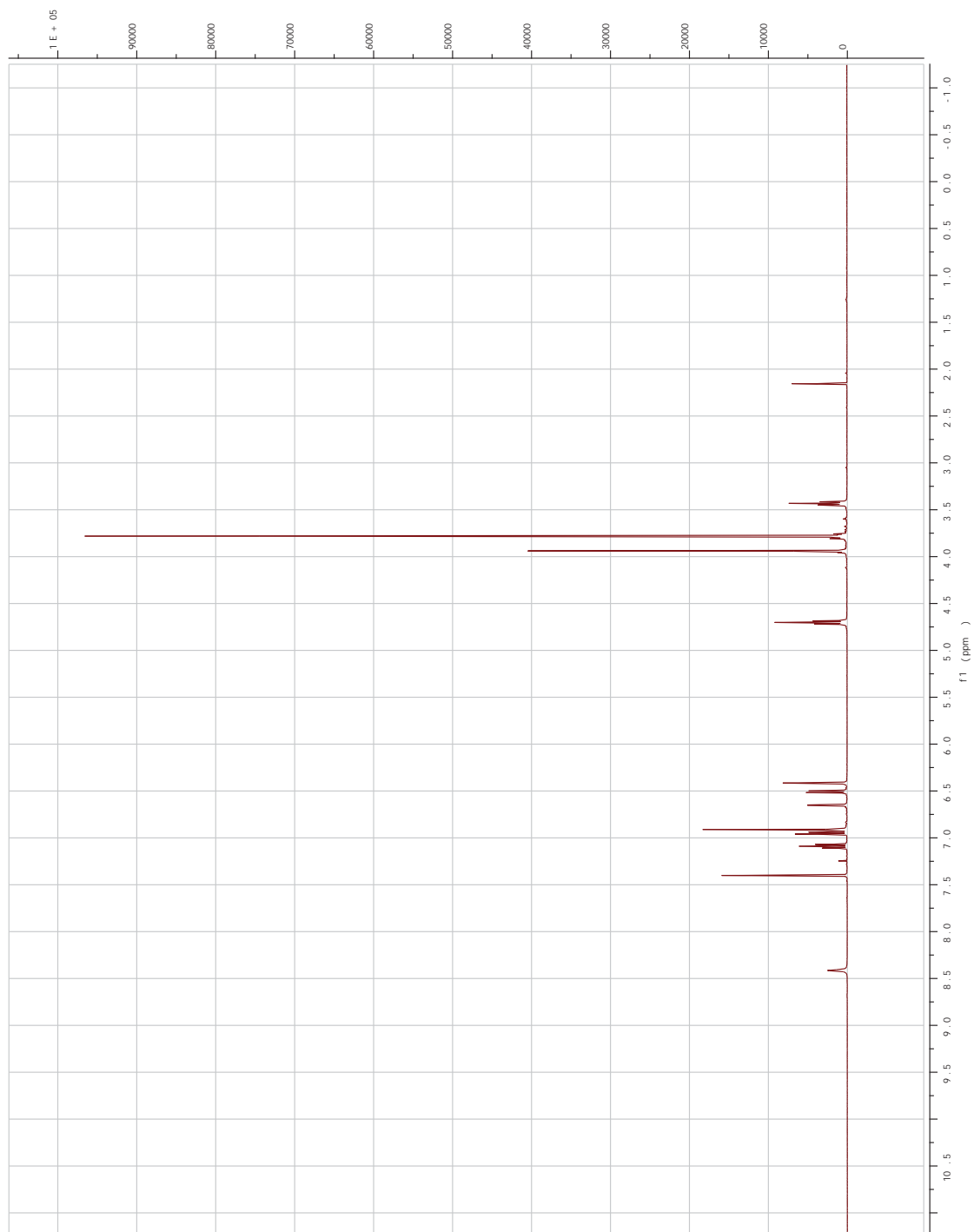


Figure A-I.19. ^1H NMR spectra of **(8d)**

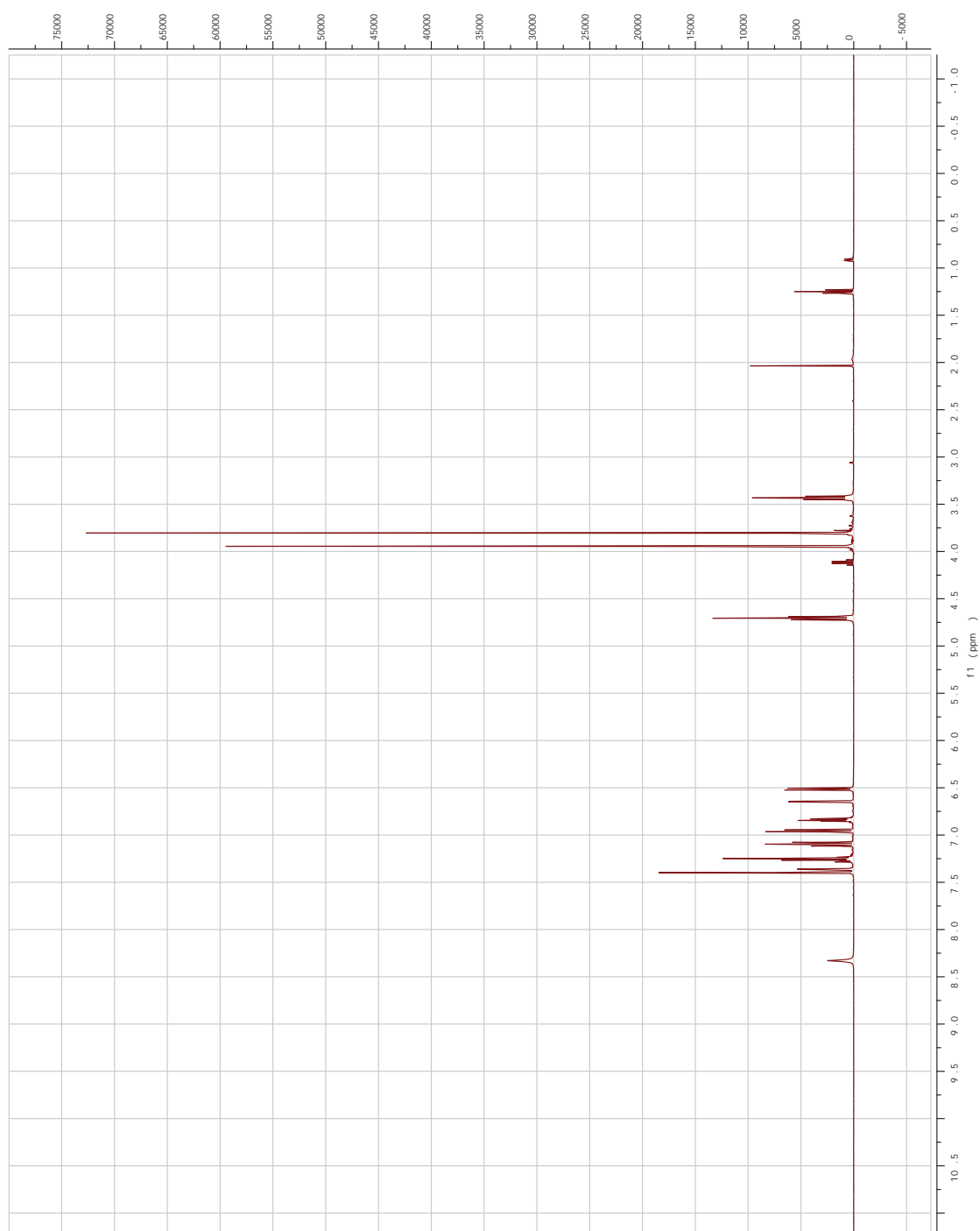


Figure A-I.20. ^1H NMR spectra of (**8e**)

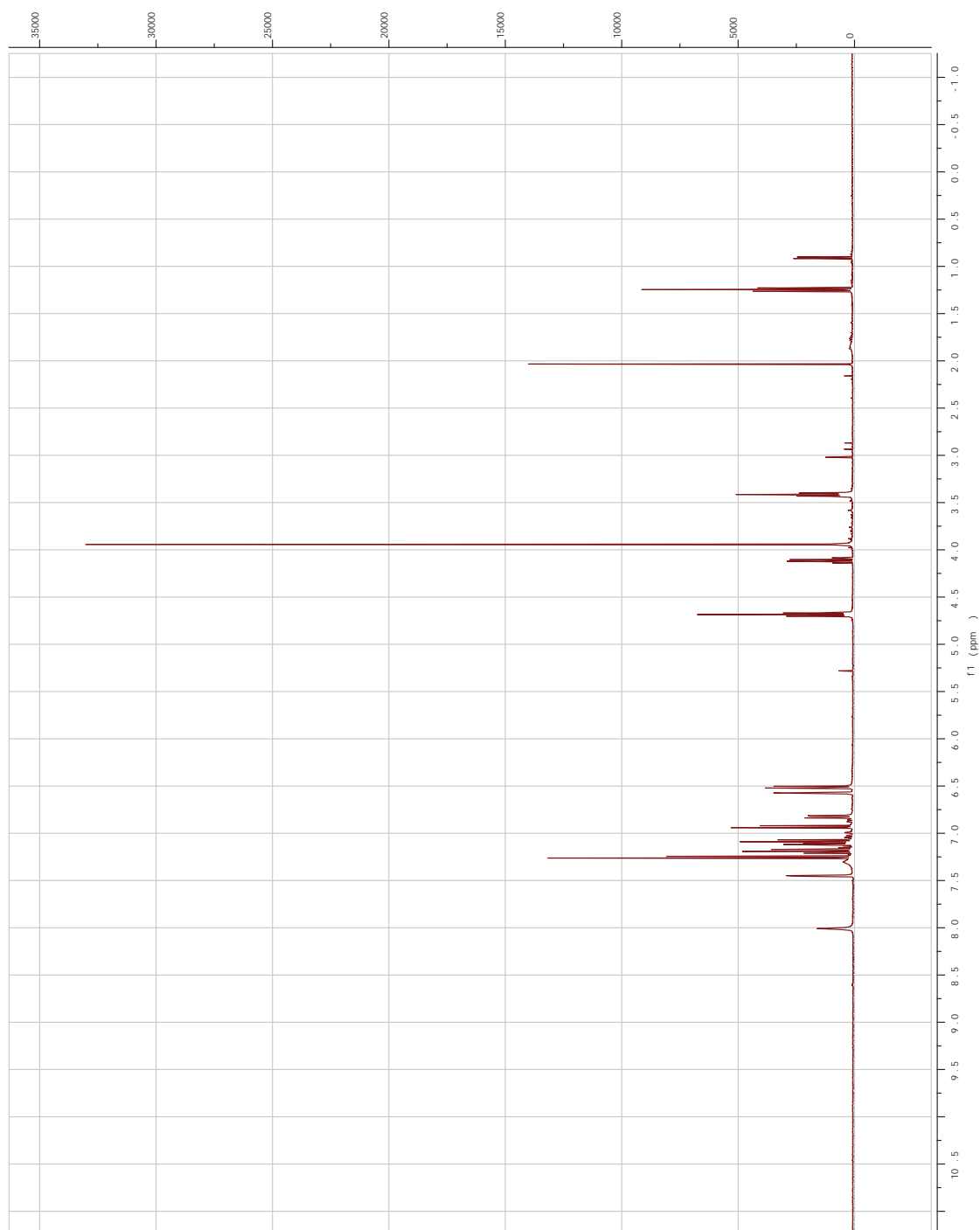


Figure A-I.21. ^1H NMR spectra of **(9a)**

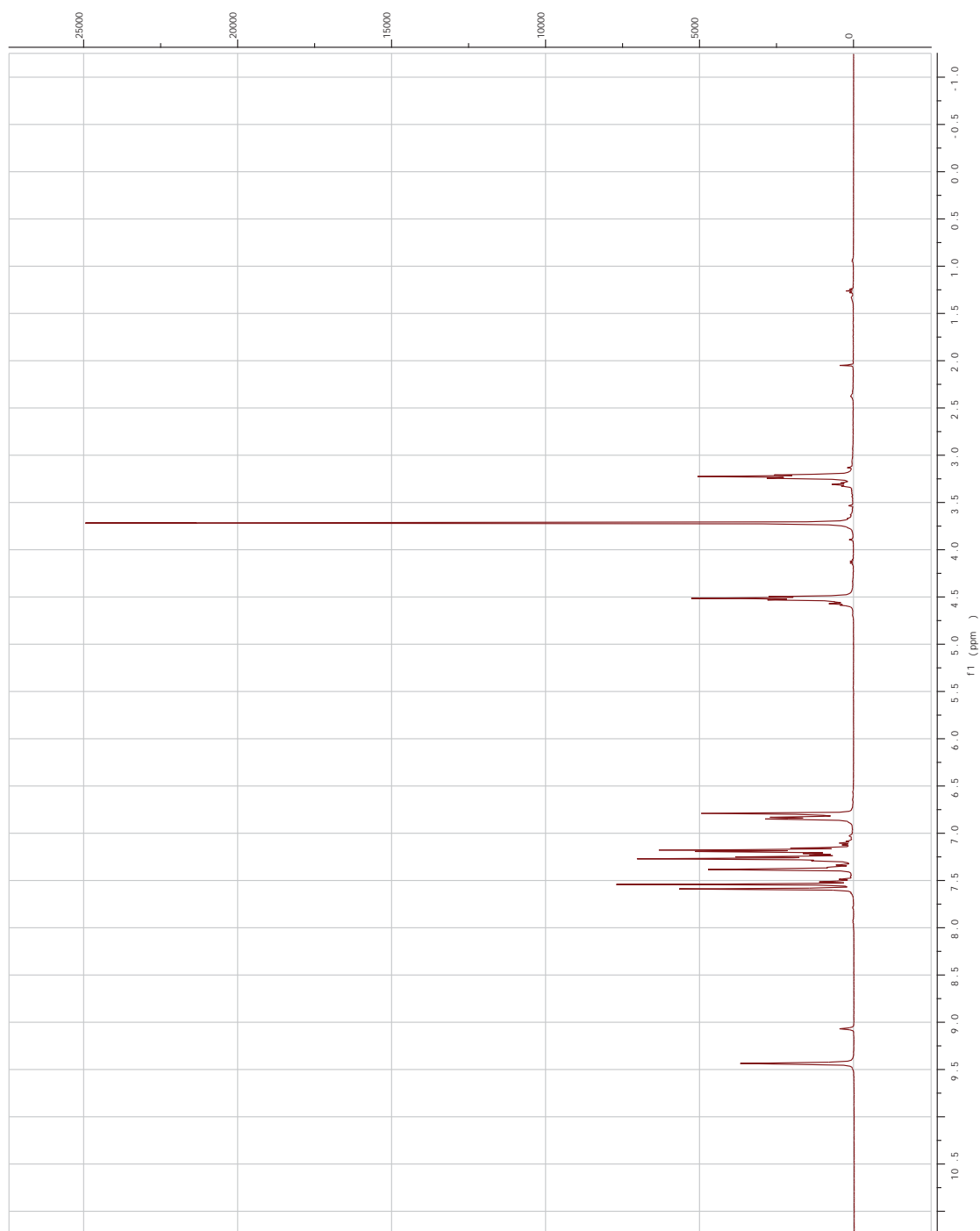


Figure A-I.22. ^1H NMR spectra of **(9b)**

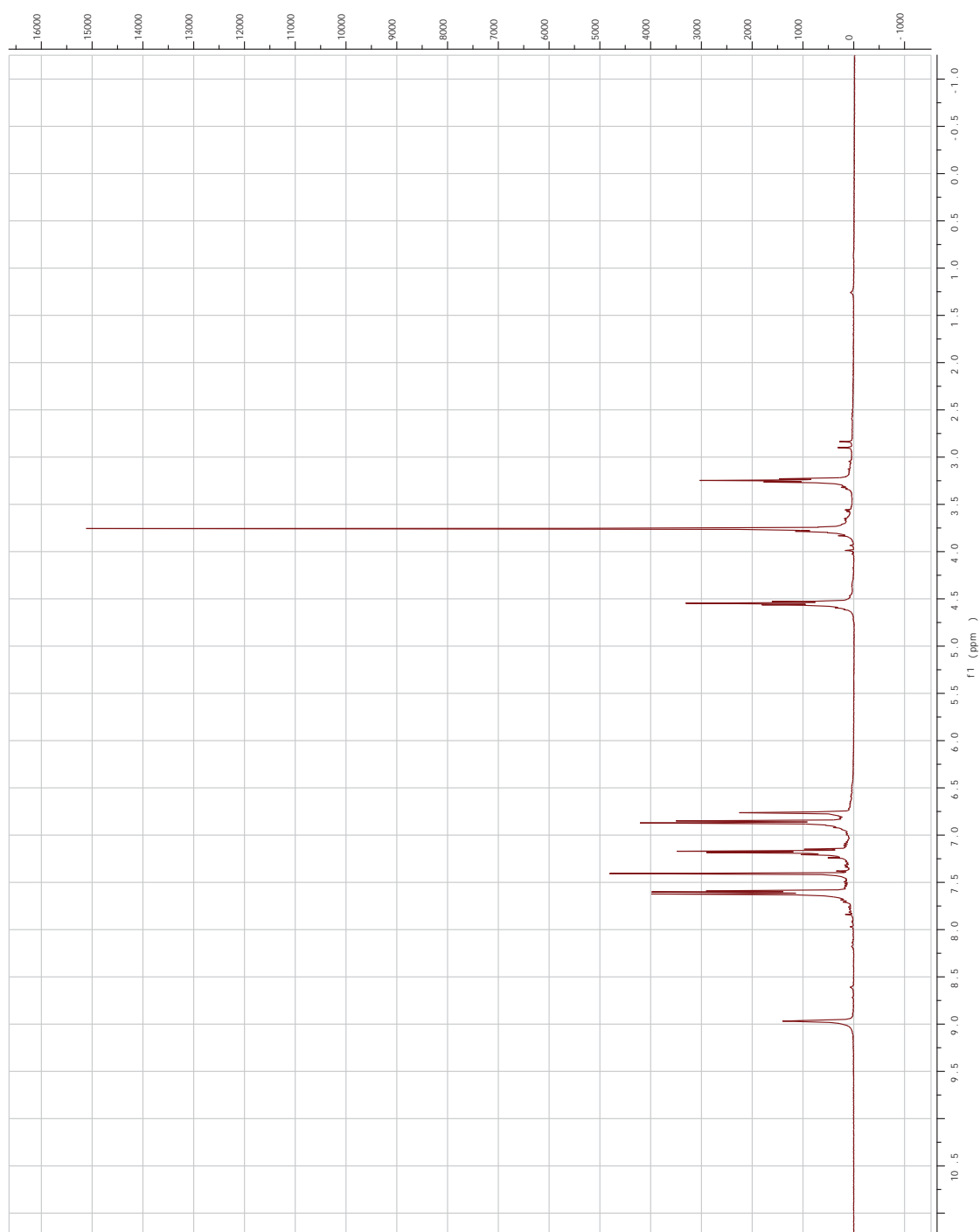


Figure A-I.23. ^1H NMR spectra of **(9c)**

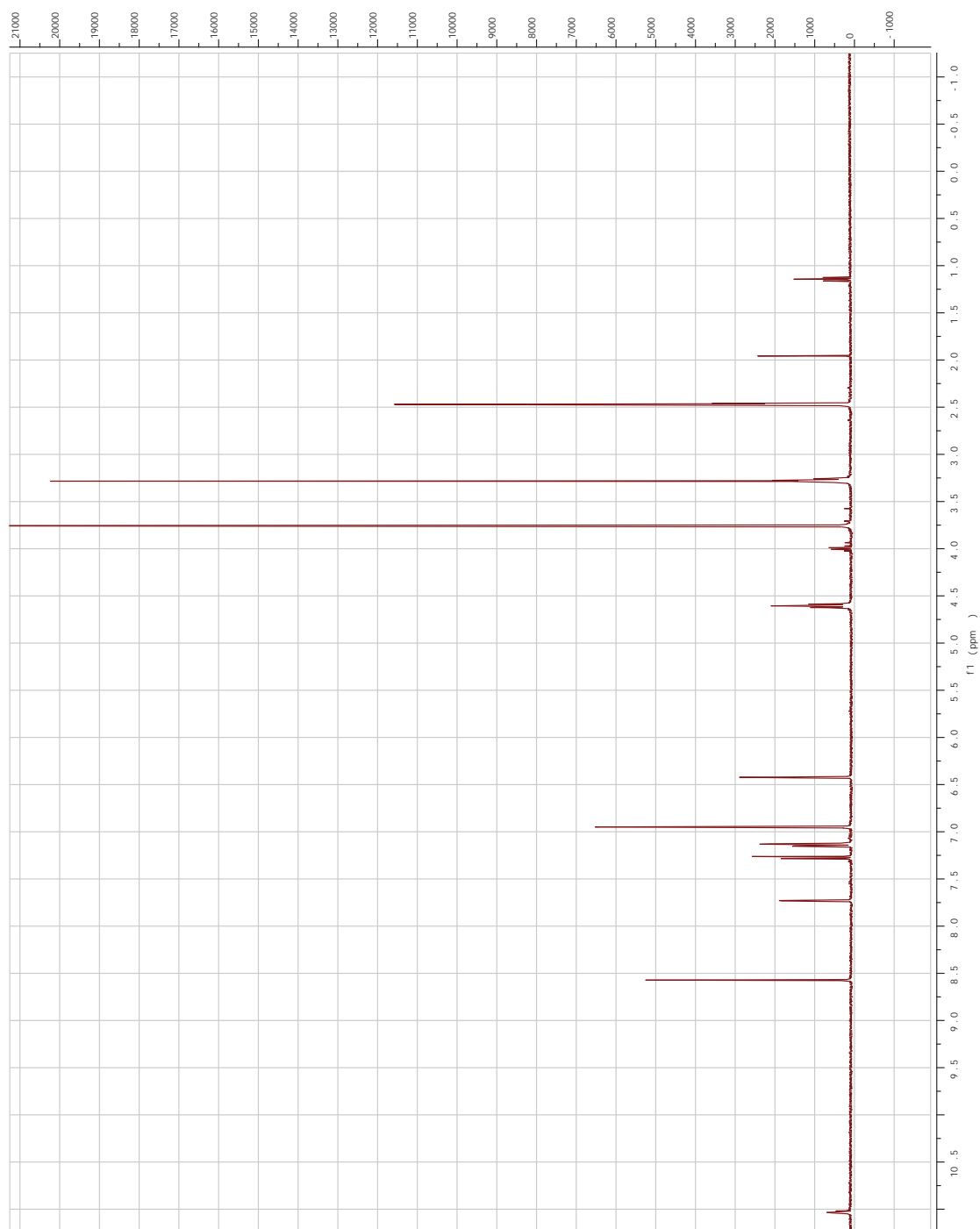


Figure A-I.24. ^1H NMR spectra of **(9d)**

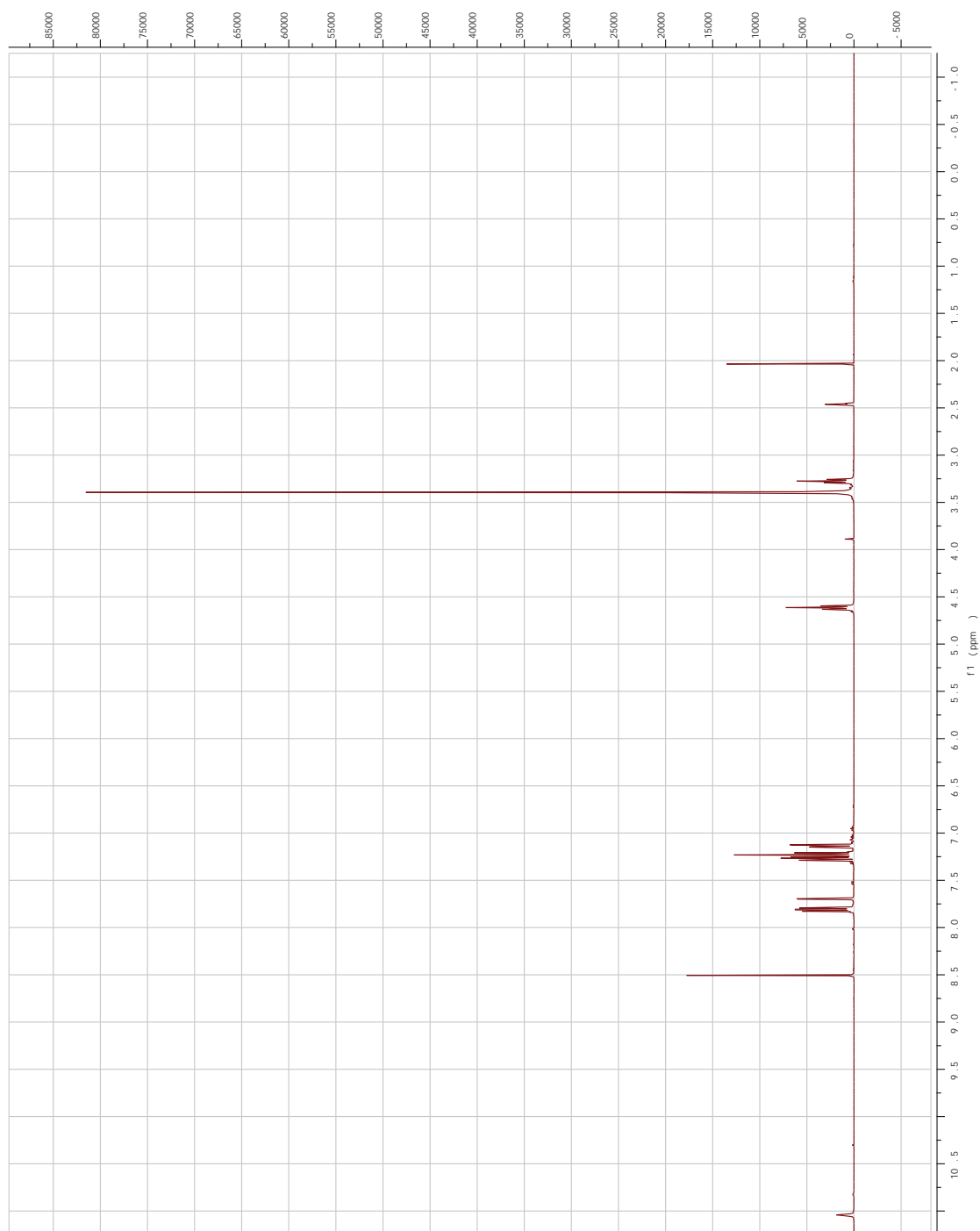


Figure A-I.25. ^1H NMR spectra of **(9e)**

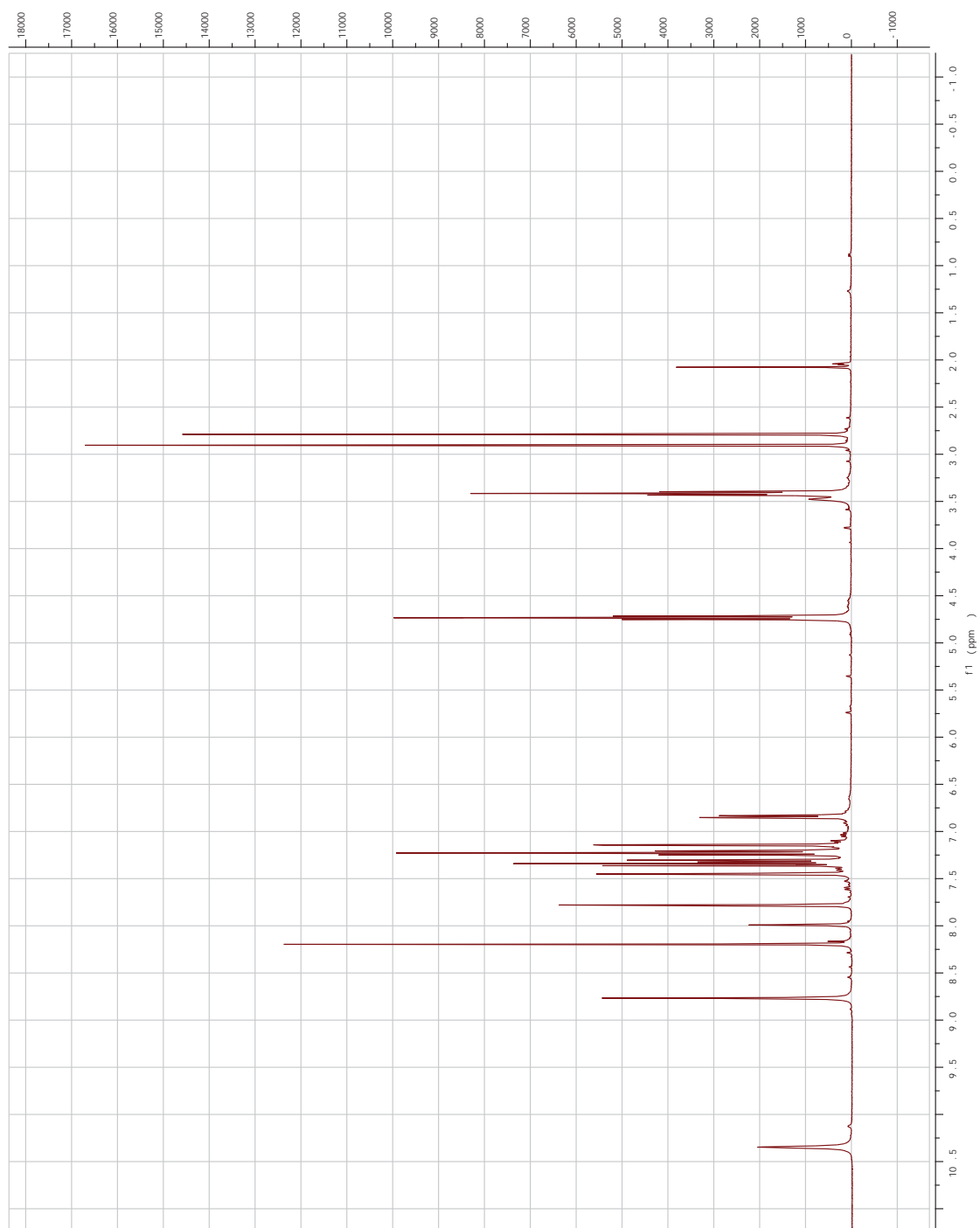


Figure A-I.26. ^1H NMR spectra of **(10a)**

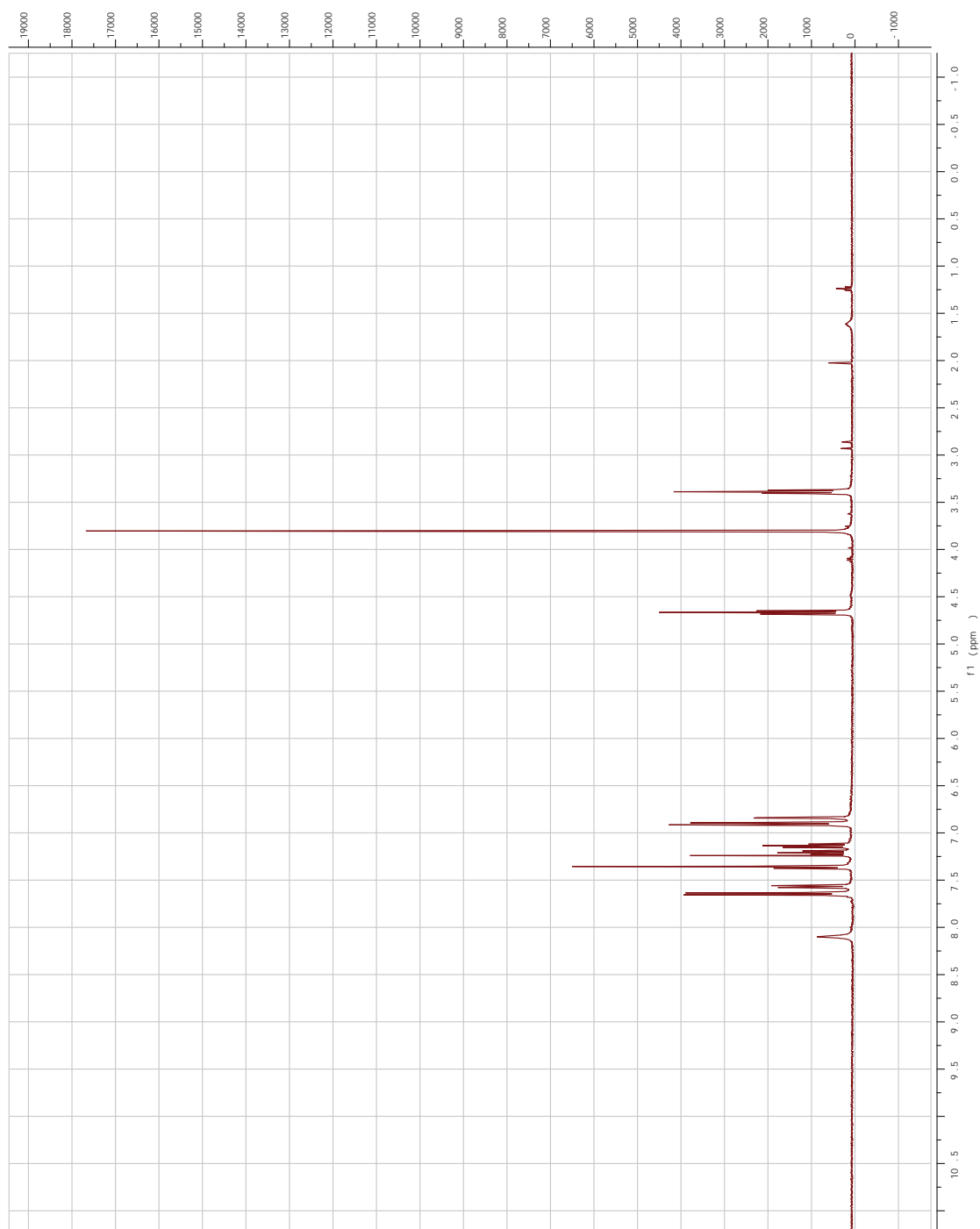


Figure A-I.27. ^1H NMR spectra of **(10b)**

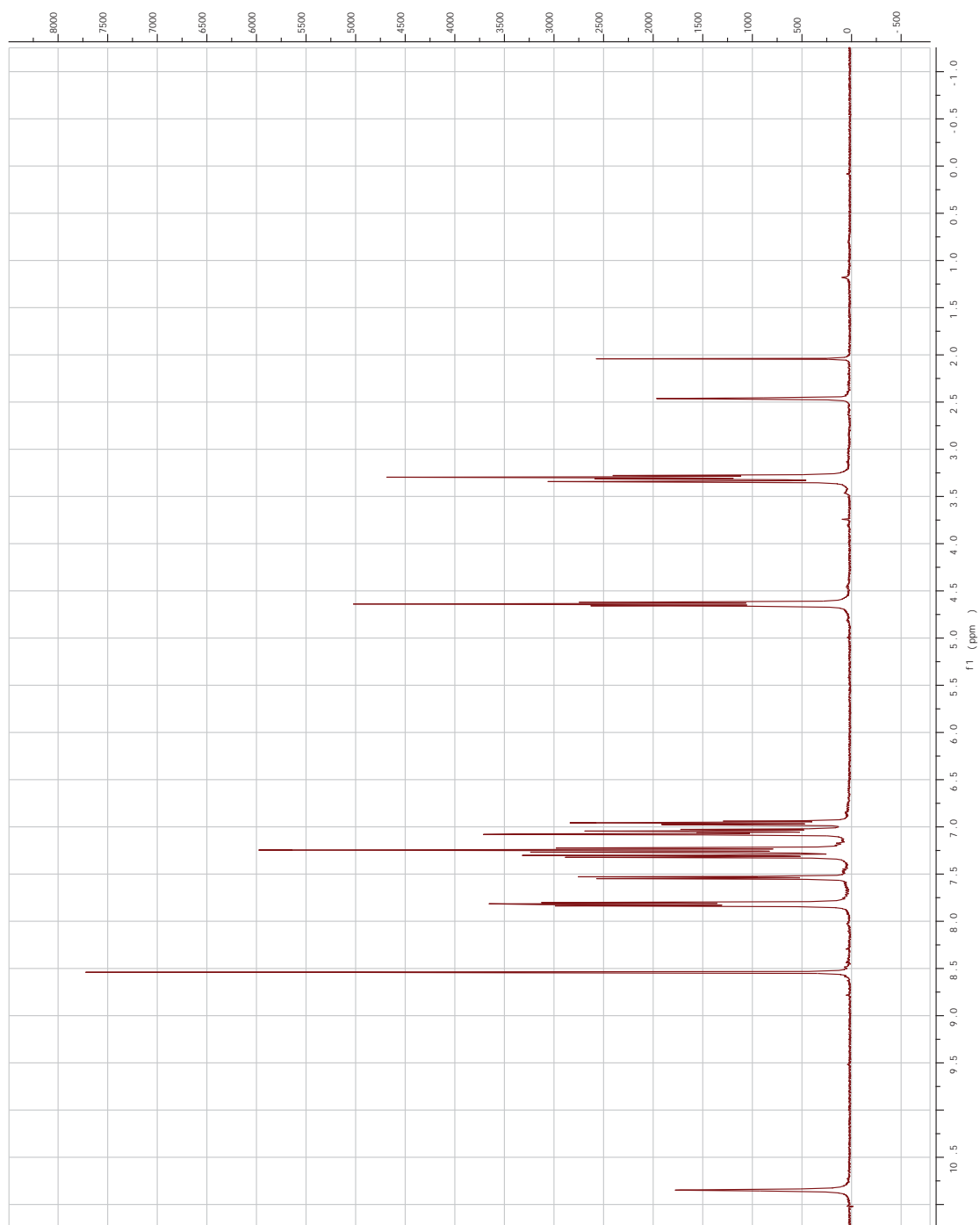


Figure A-I.28. ^1H NMR spectra of (**10c**)

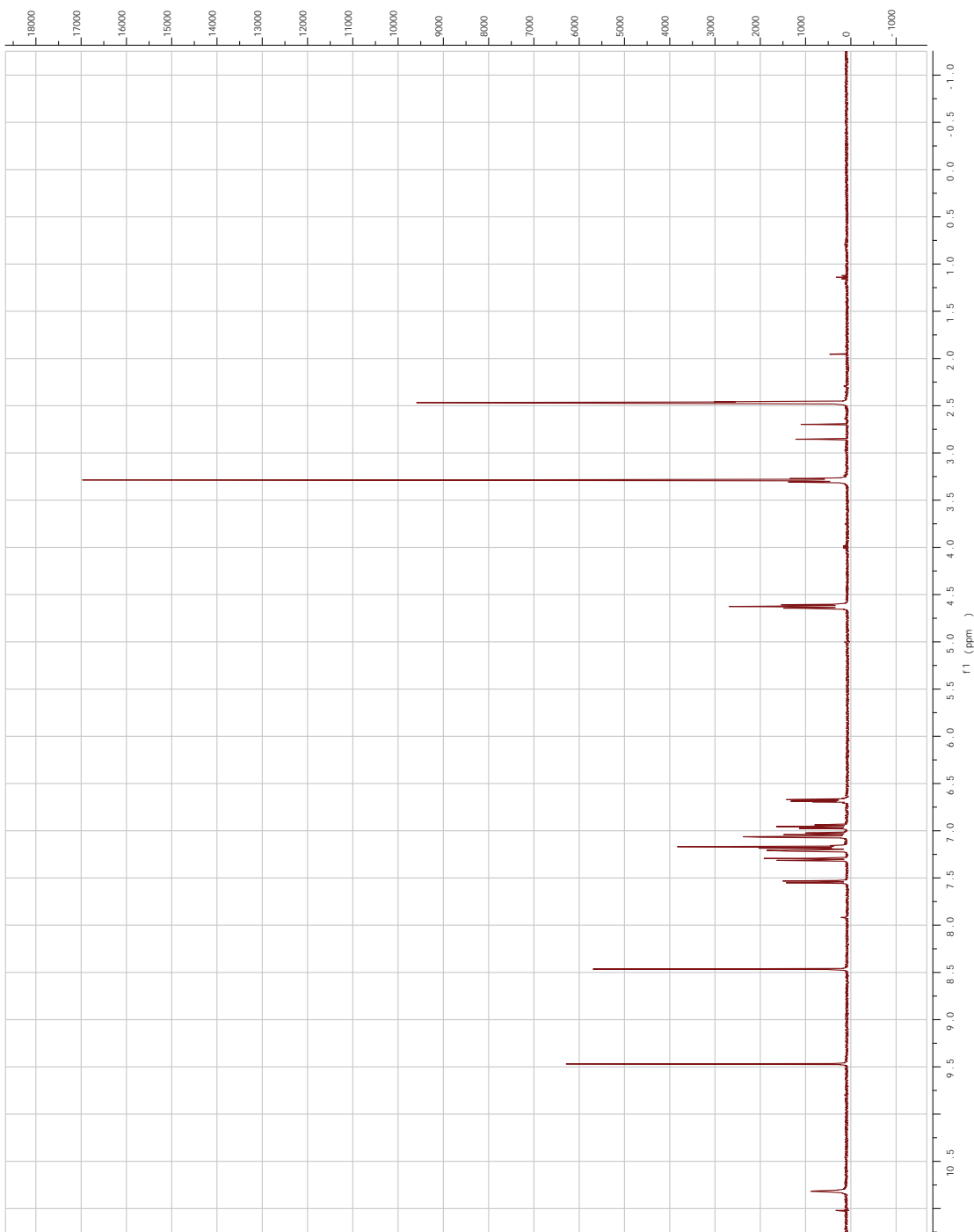


Figure A-I.29. ^1H NMR spectra of (**10d**)

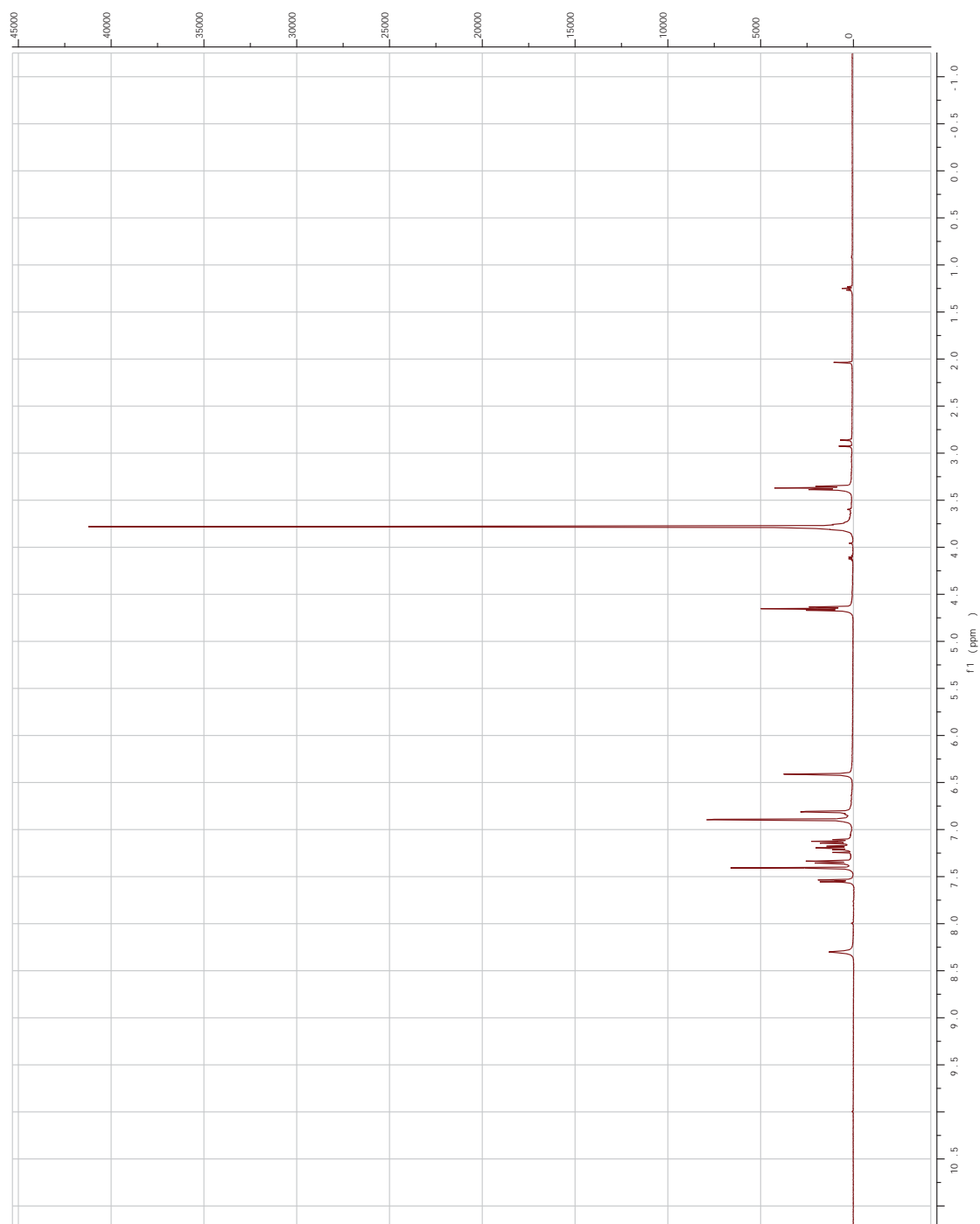
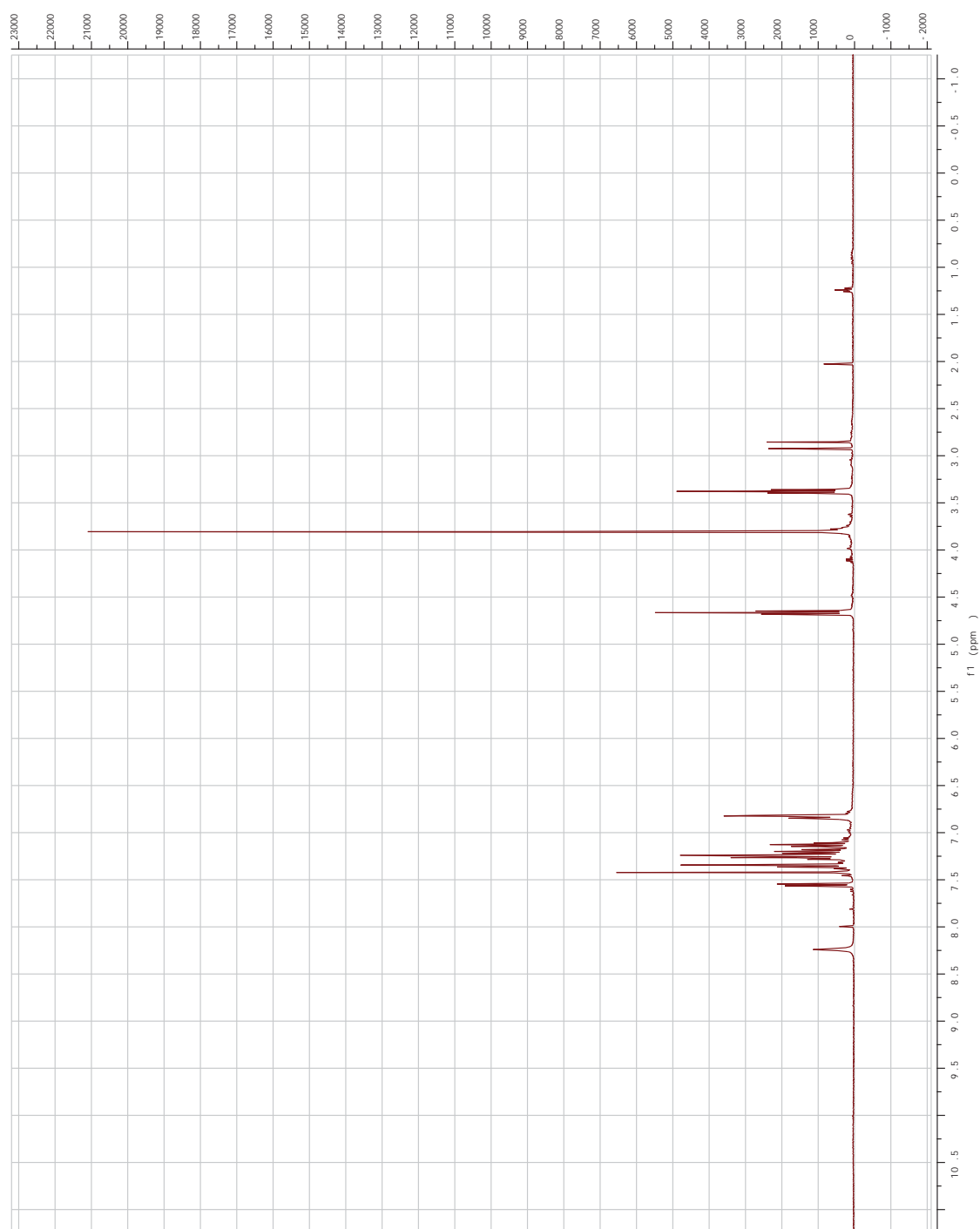


Figure A-I.30. ^1H NMR spectra of **(10e)**



Appendix-II ^{13}C NMR Spectra

Figure A-II.1. ^{13}C NMR spectra of (5a)

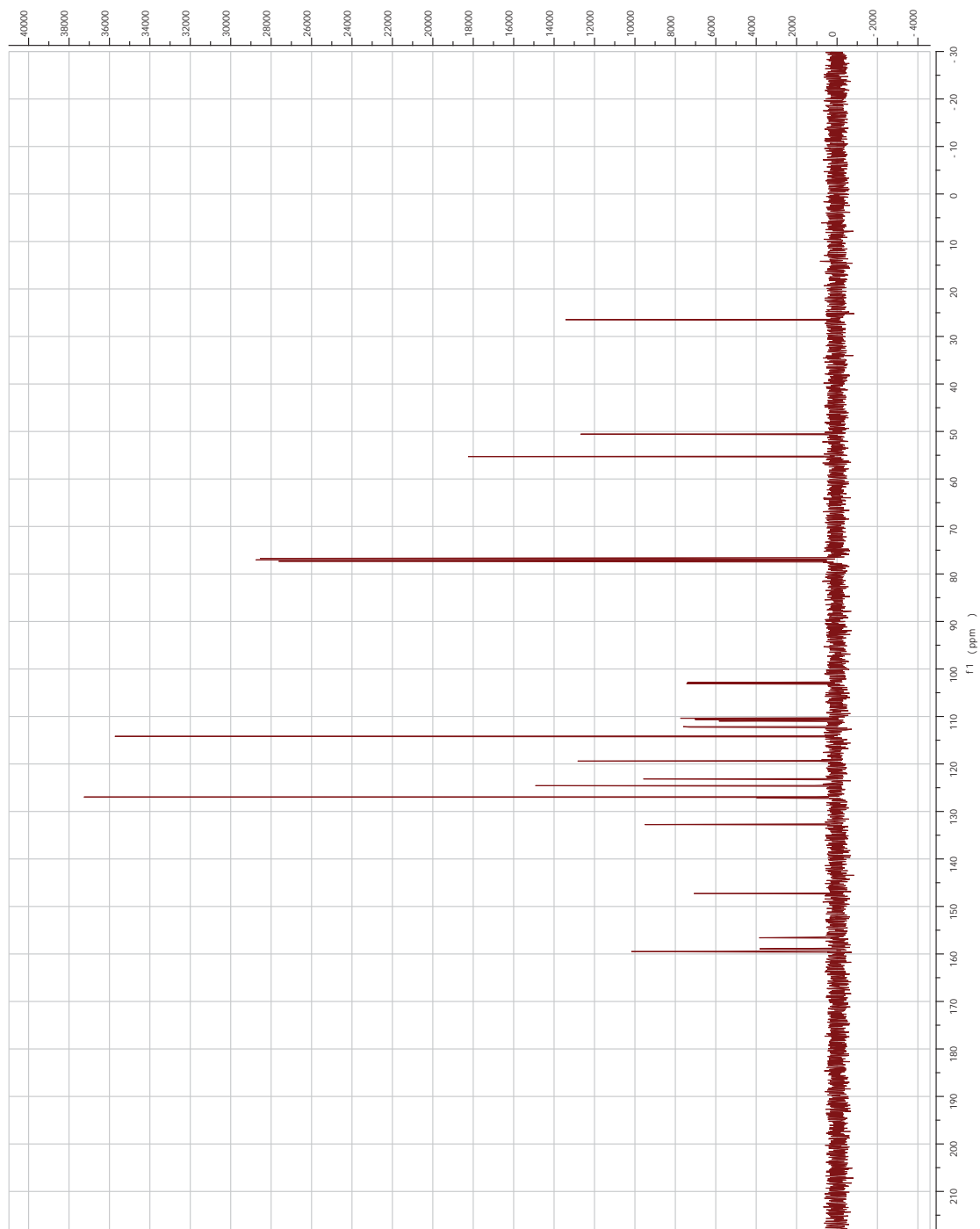


Figure A-II.2. ^{13}C NMR spectra of (**5b**)

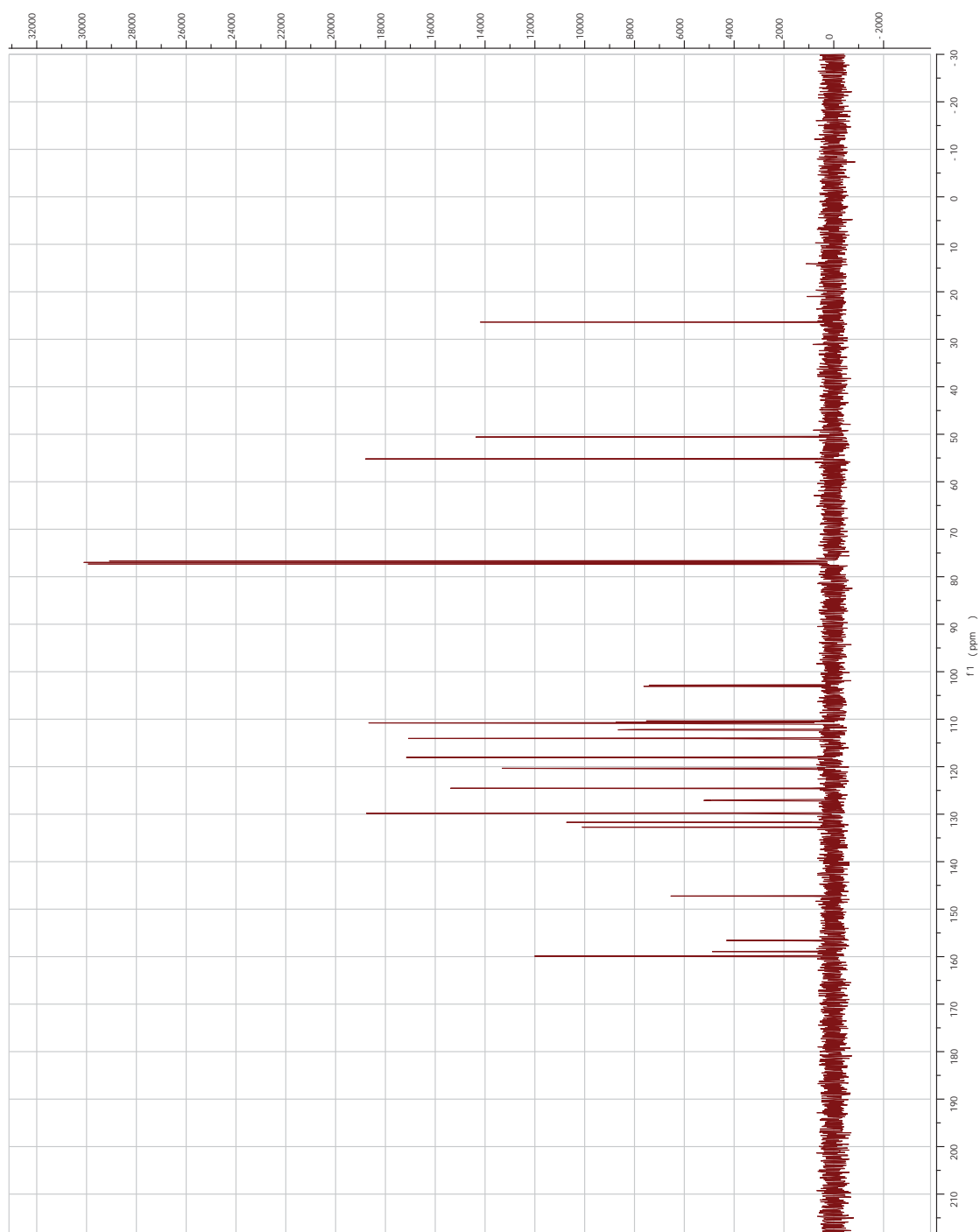


Figure A-II.3. ^{13}C NMR spectra of (**5c**)

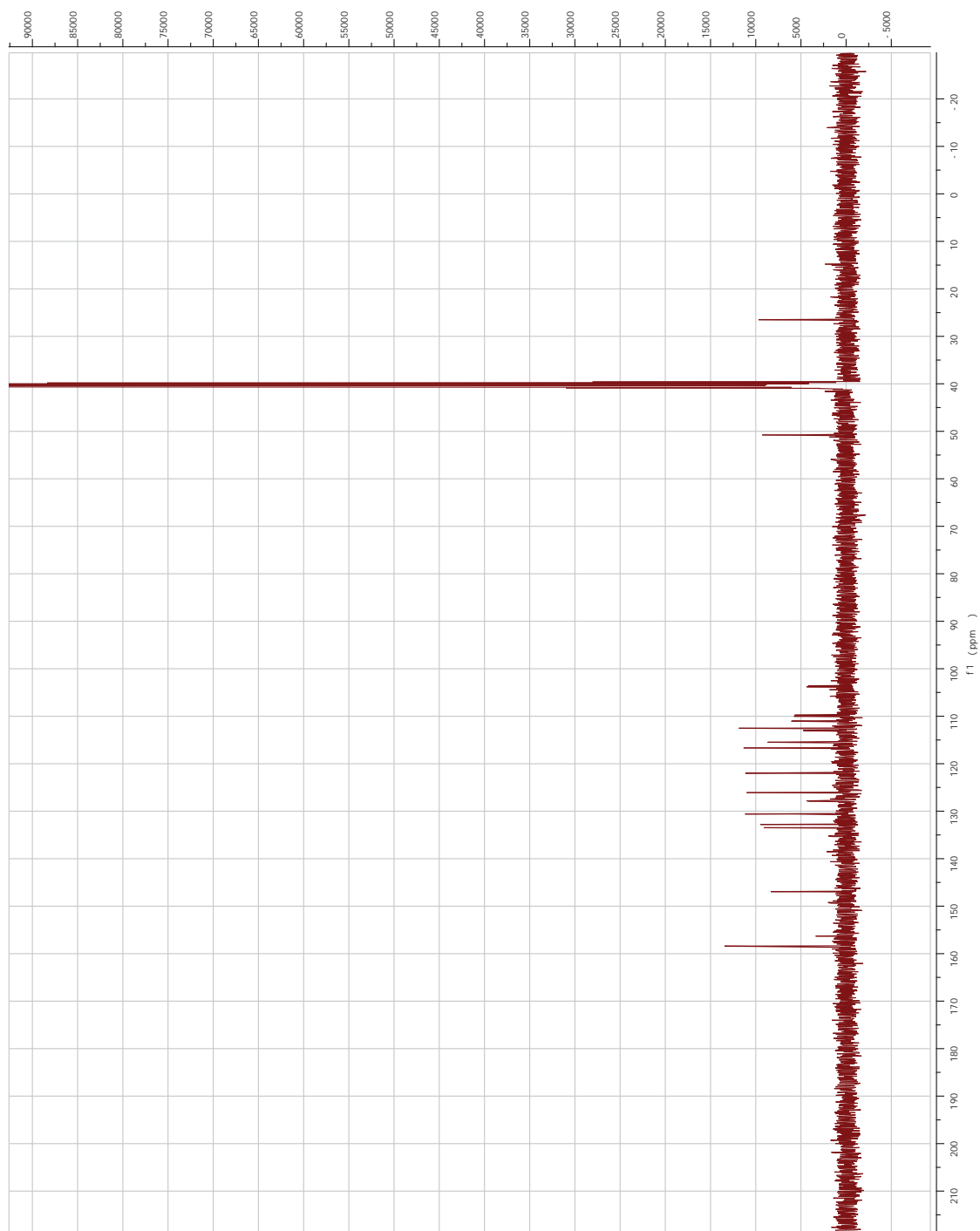


Figure A-II.4. ^{13}C NMR spectra of (**5d**)

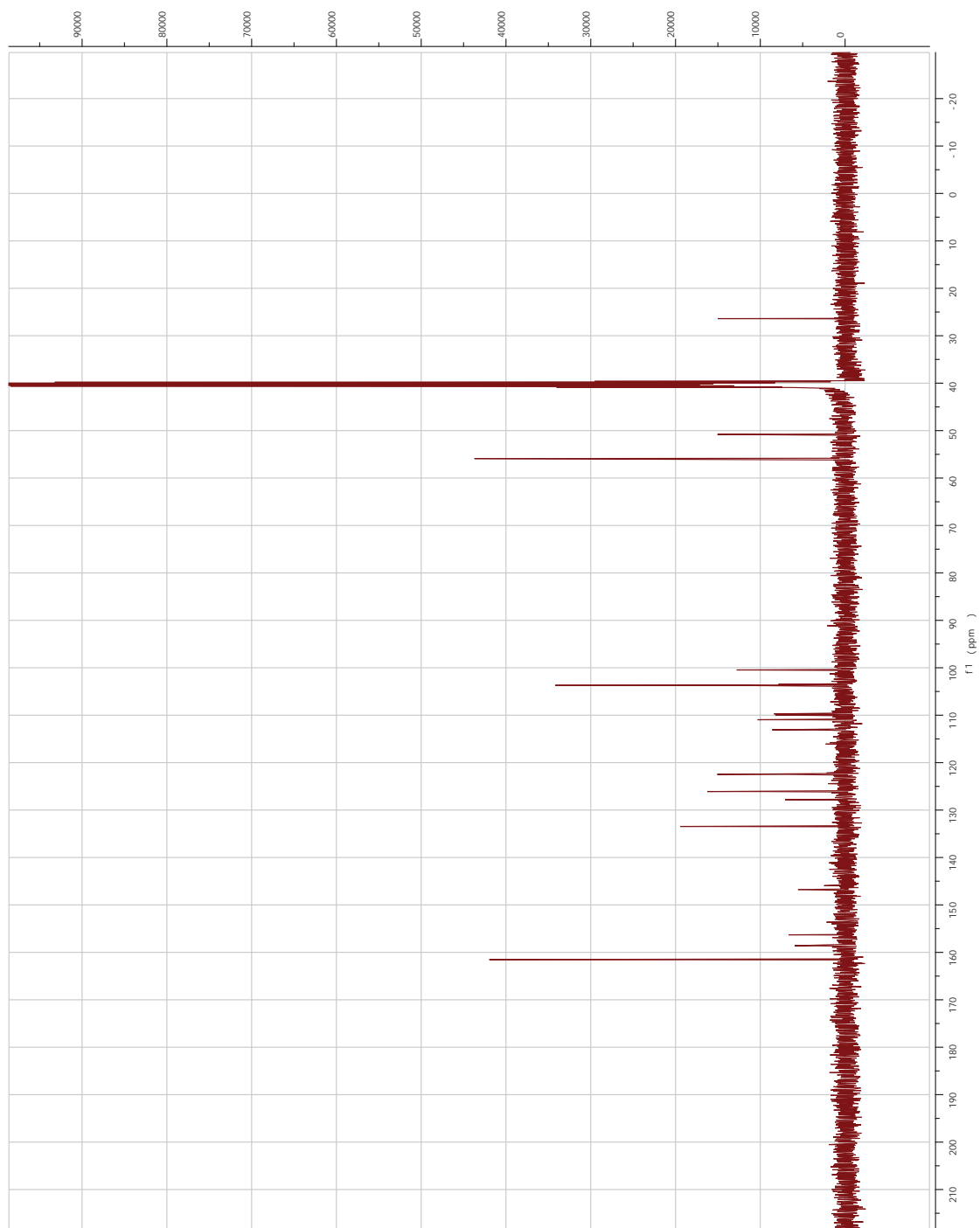


Figure A-II.5. ^{13}C NMR spectra of (**5e**)

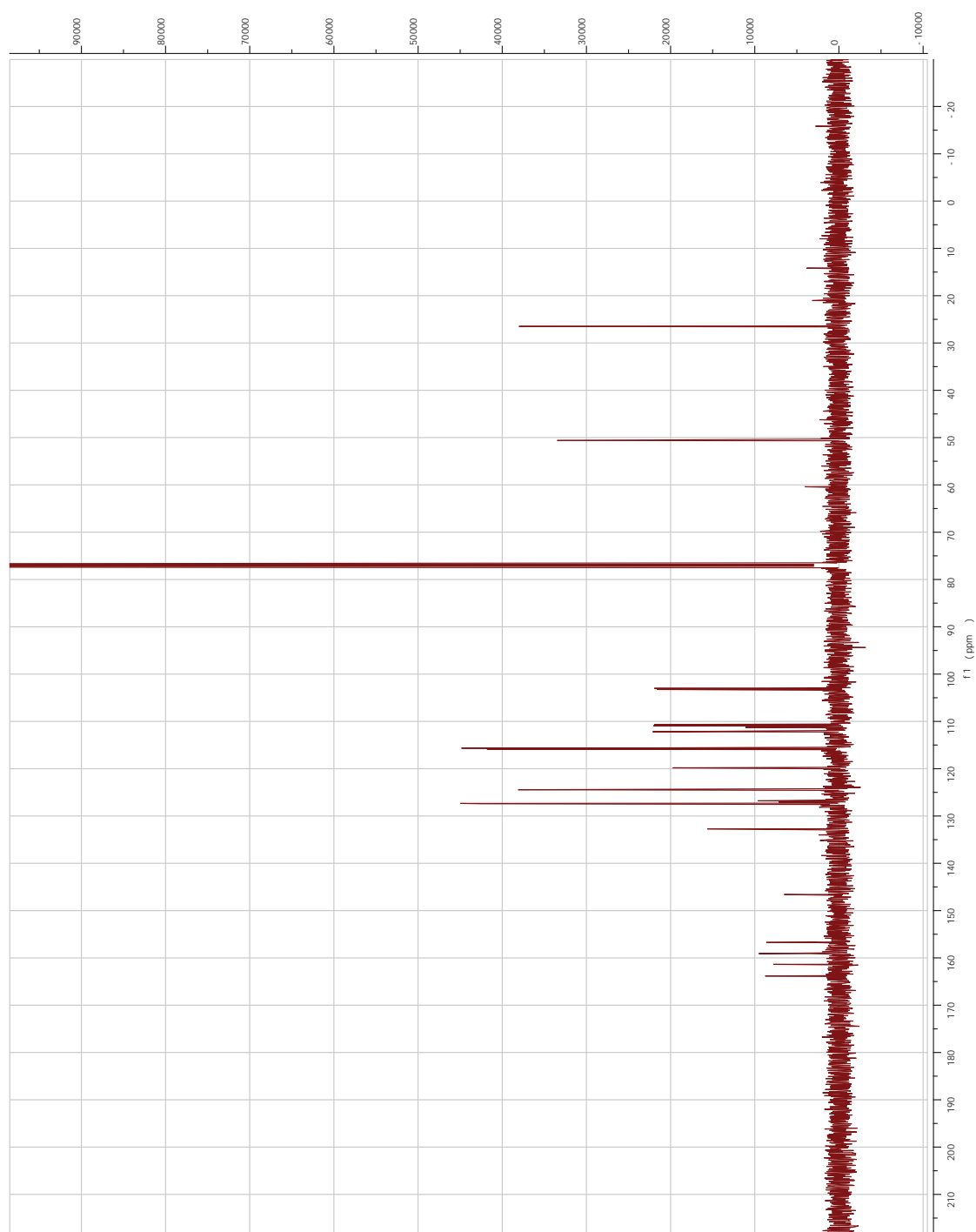


Figure A-II.6. ^{13}C NMR spectra of (6a)

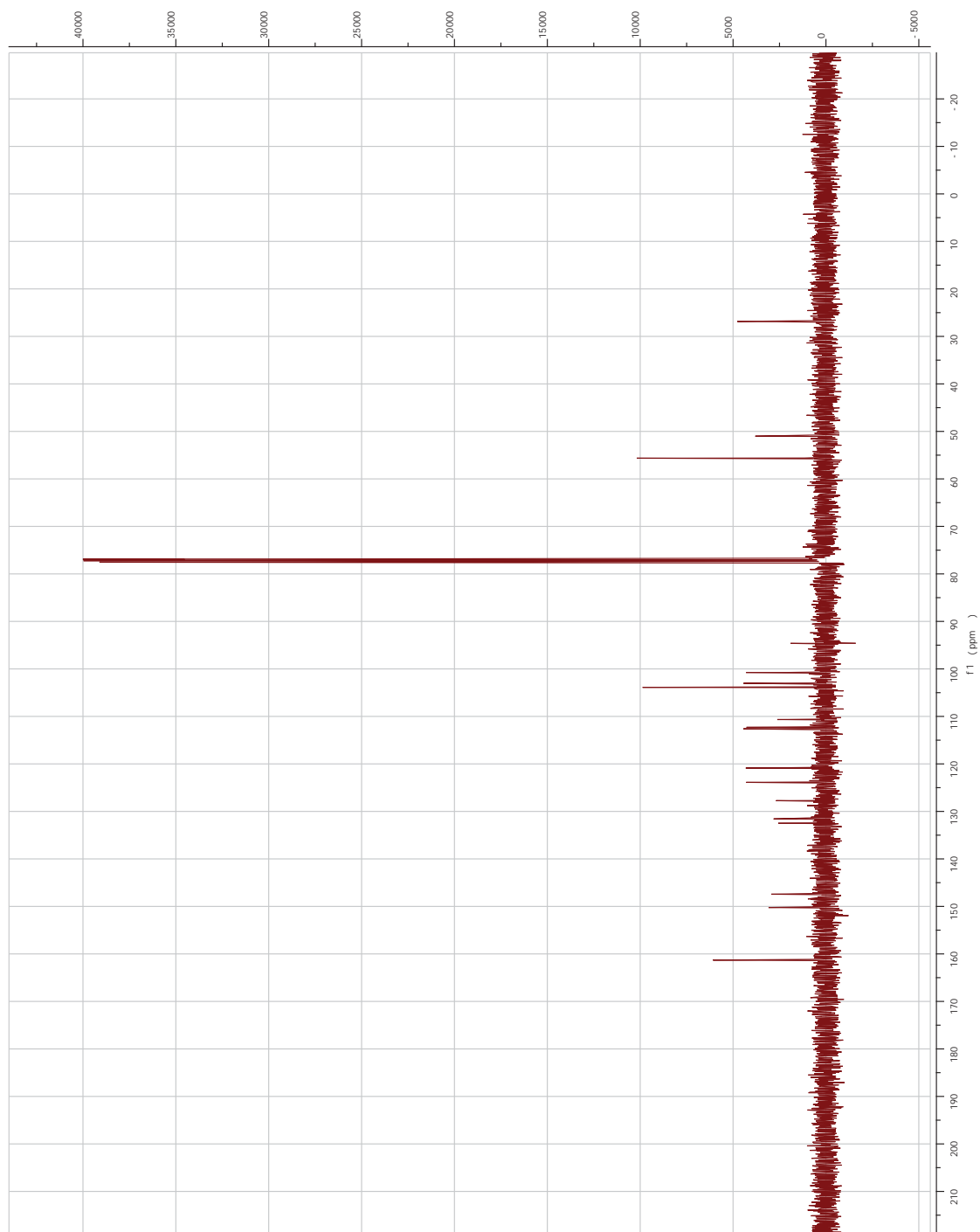


Figure A-II.7. ^{13}C NMR spectra of (**6b**)

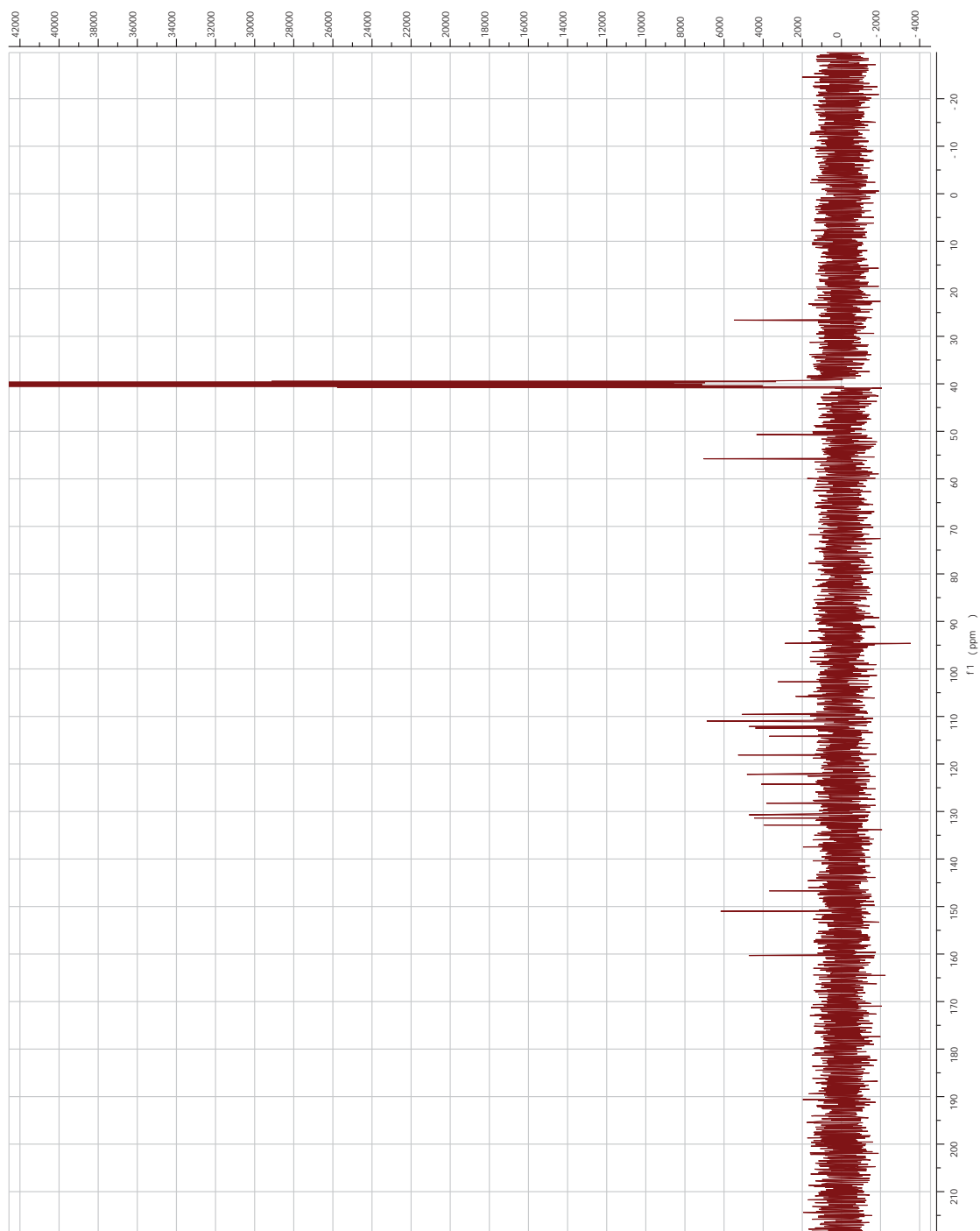


Figure A-II.8. ^{13}C NMR spectra of (6c)

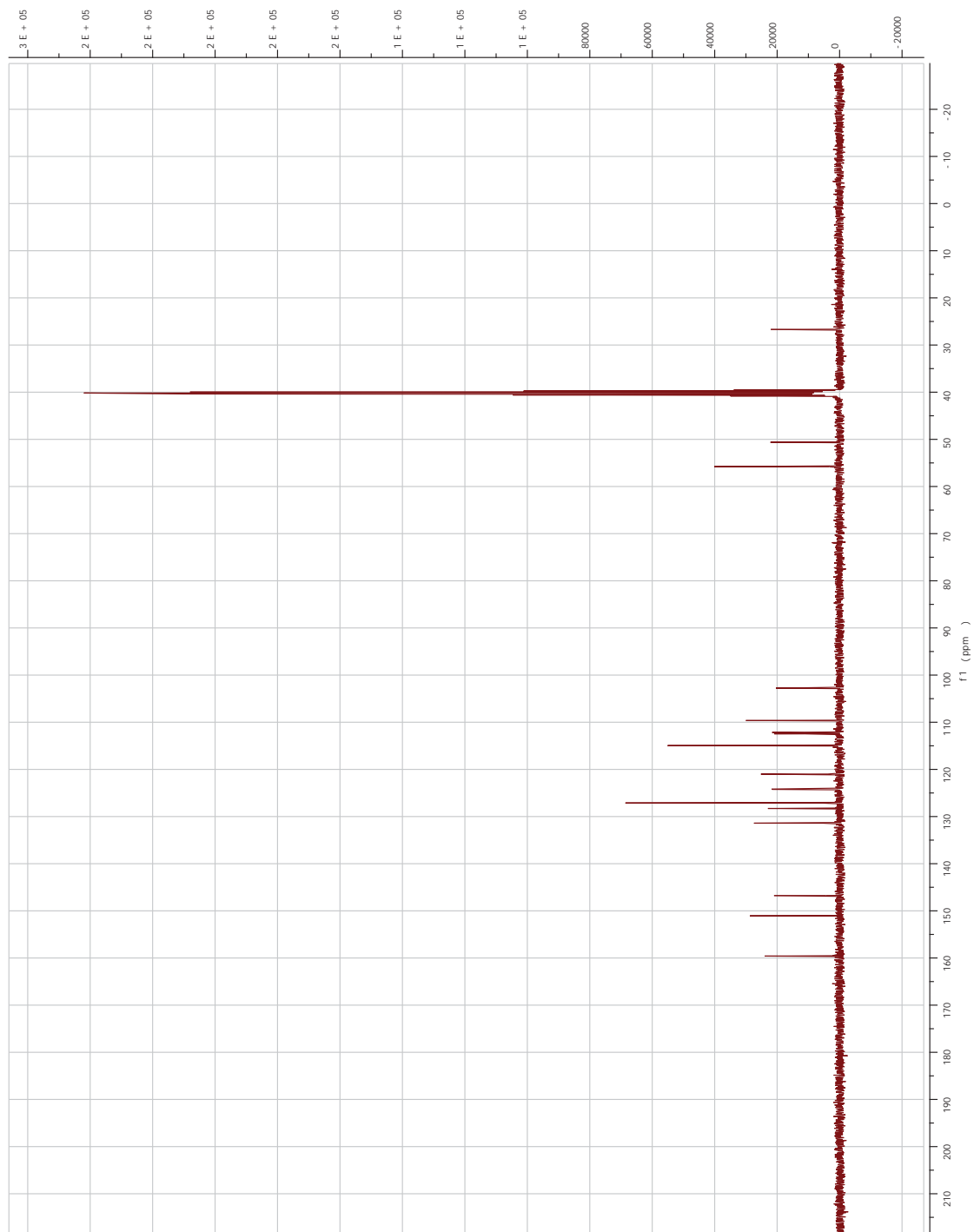


Figure A-II.9. ^{13}C NMR spectra of (**6d**)

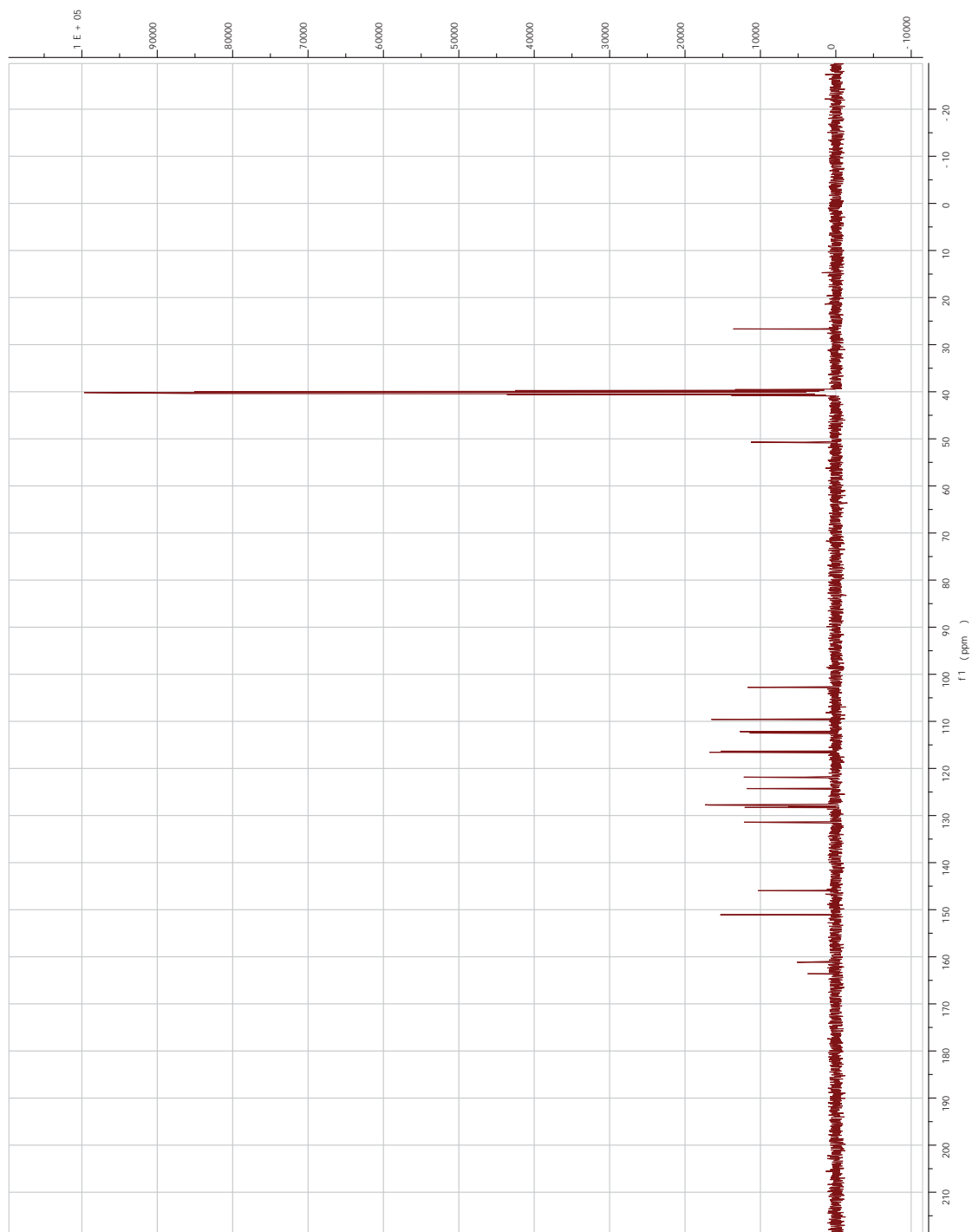


Figure A-II.10. ^{13}C NMR spectra of (6e)

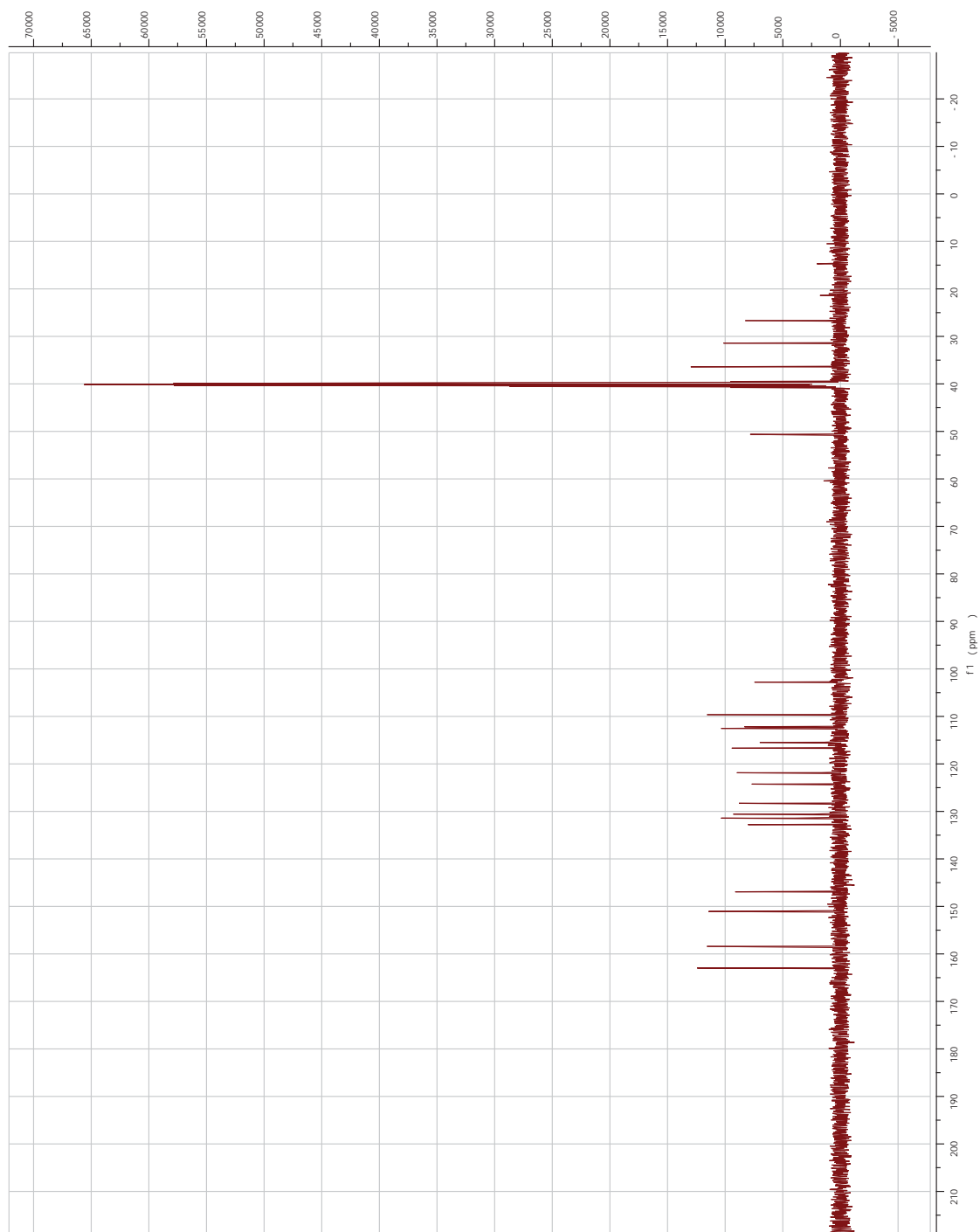


Figure A-II.11. ^{13}C NMR spectra of (**7a**)

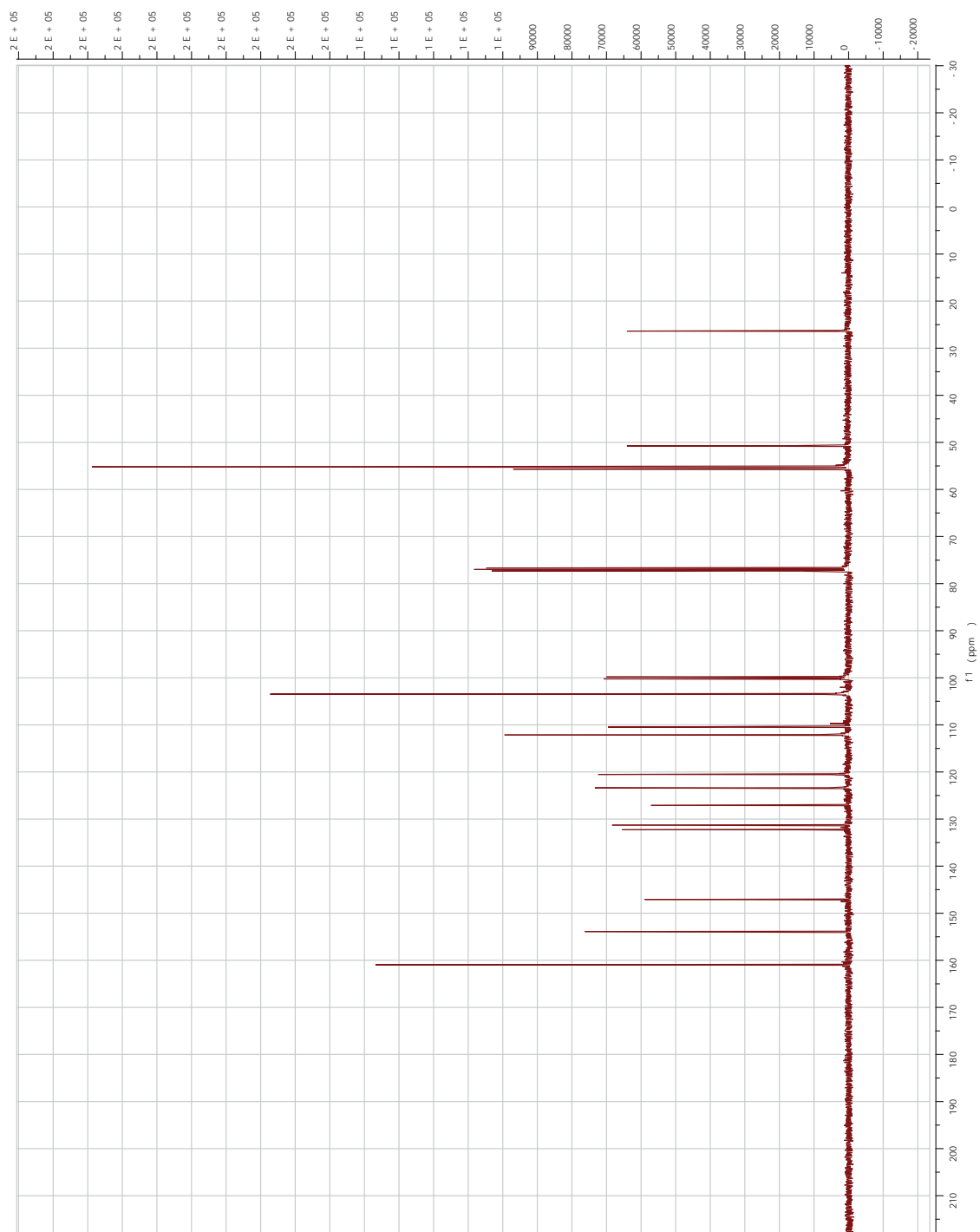


Figure A-II.12. ^{13}C NMR spectra of (**7b**)

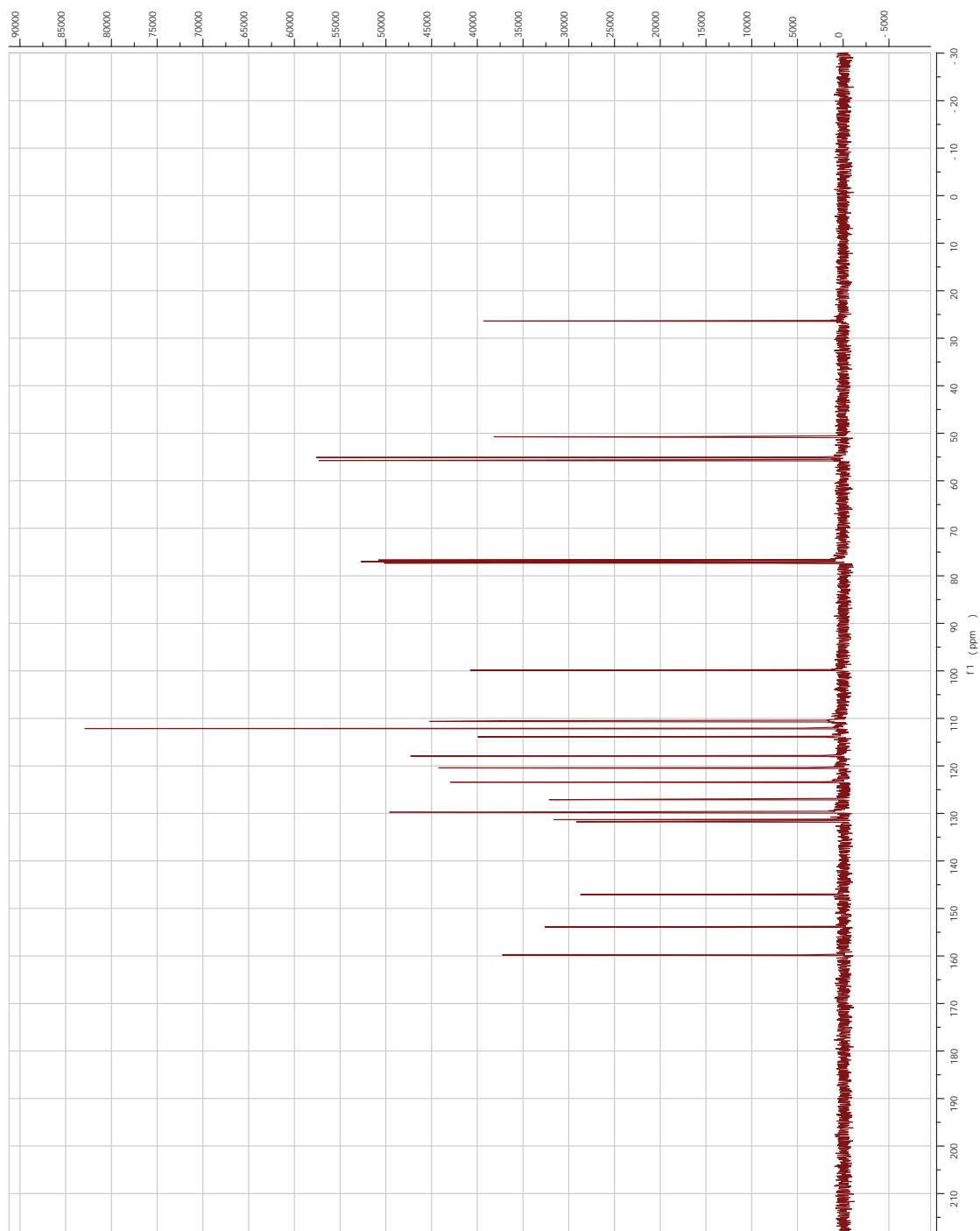


Figure A-II.13. ^{13}C NMR spectra of (7c)

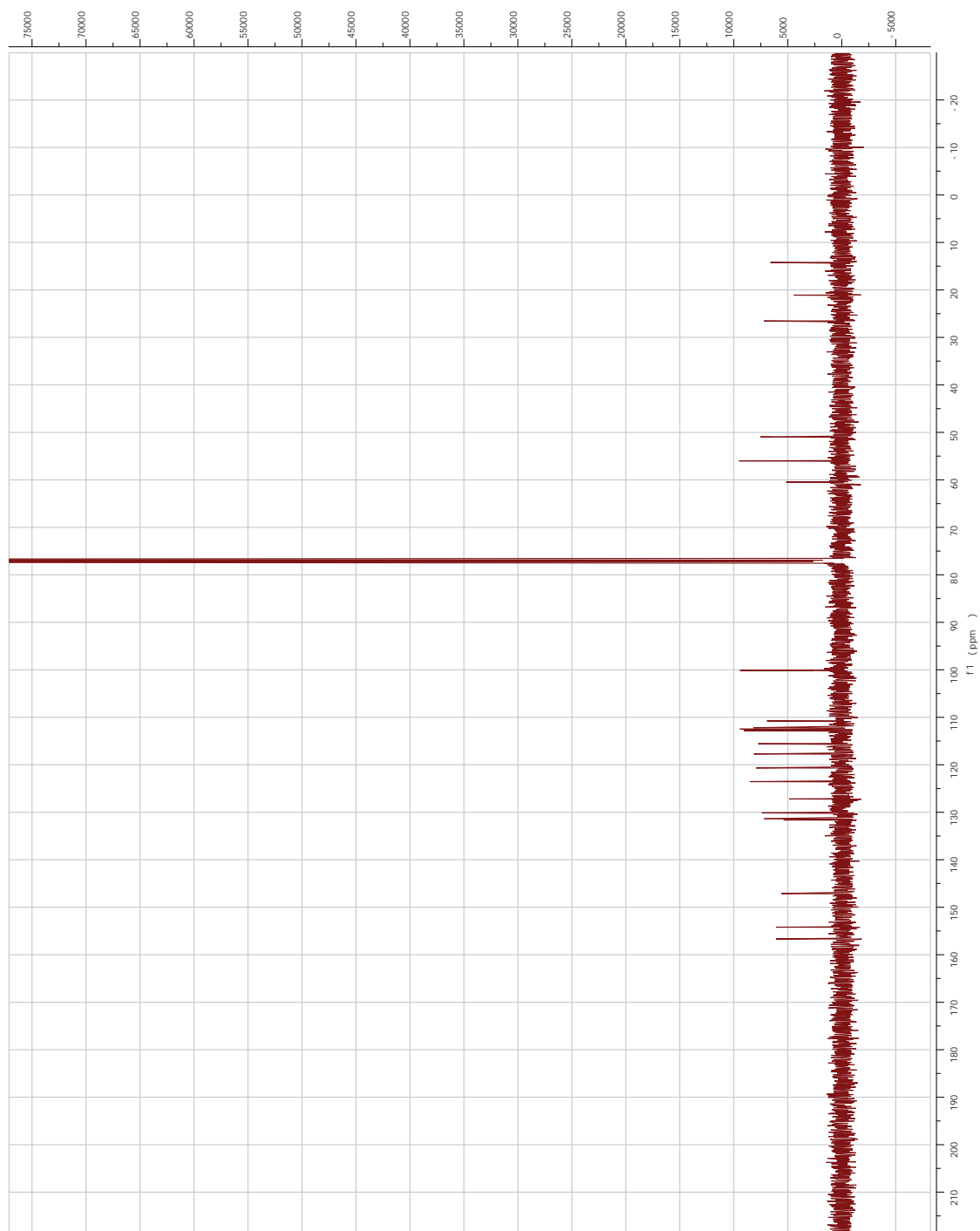


Figure A-II.14. ^{13}C NMR spectra of (**7d**)

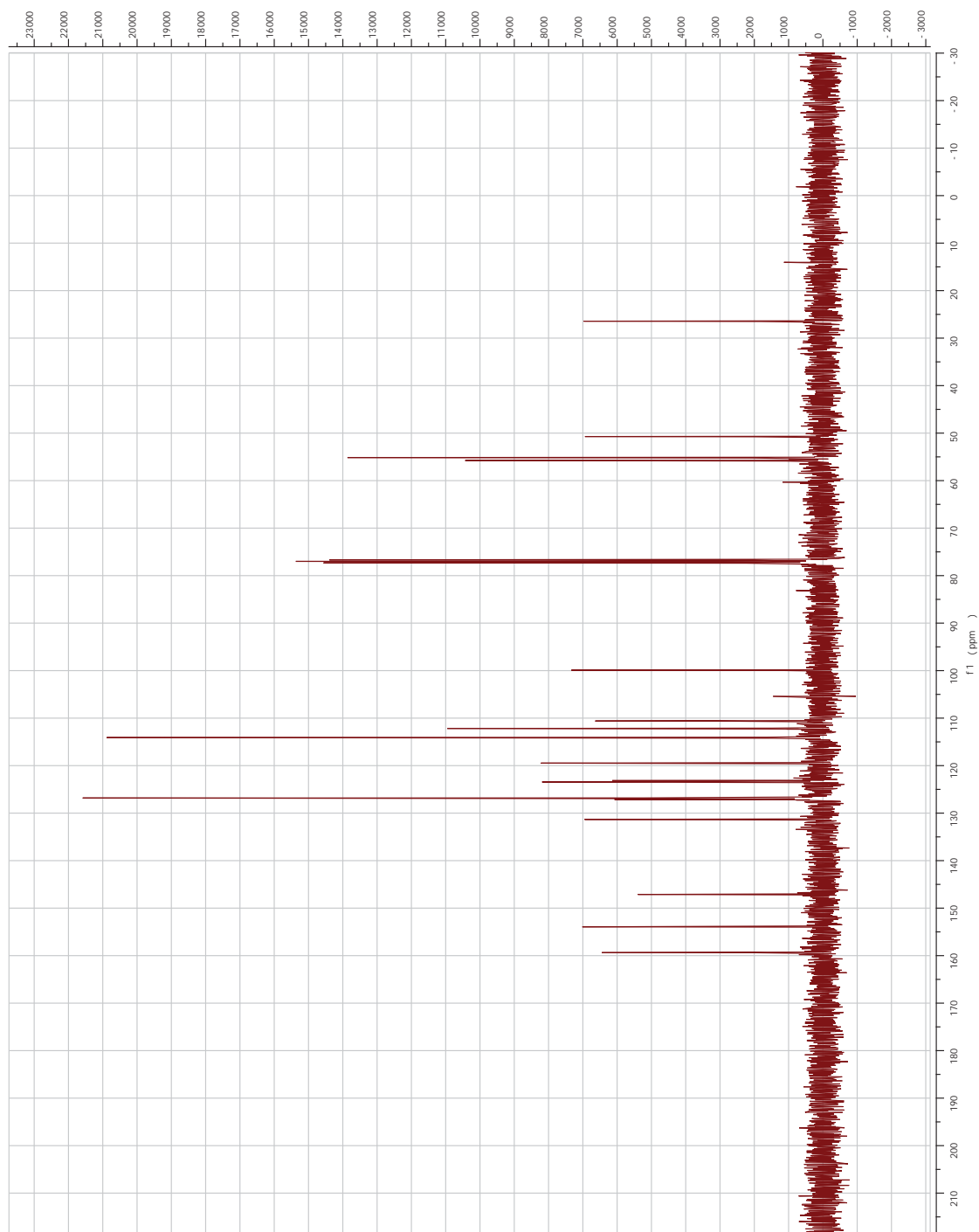


Figure A-II.15. ^{13}C NMR spectra of (7e)

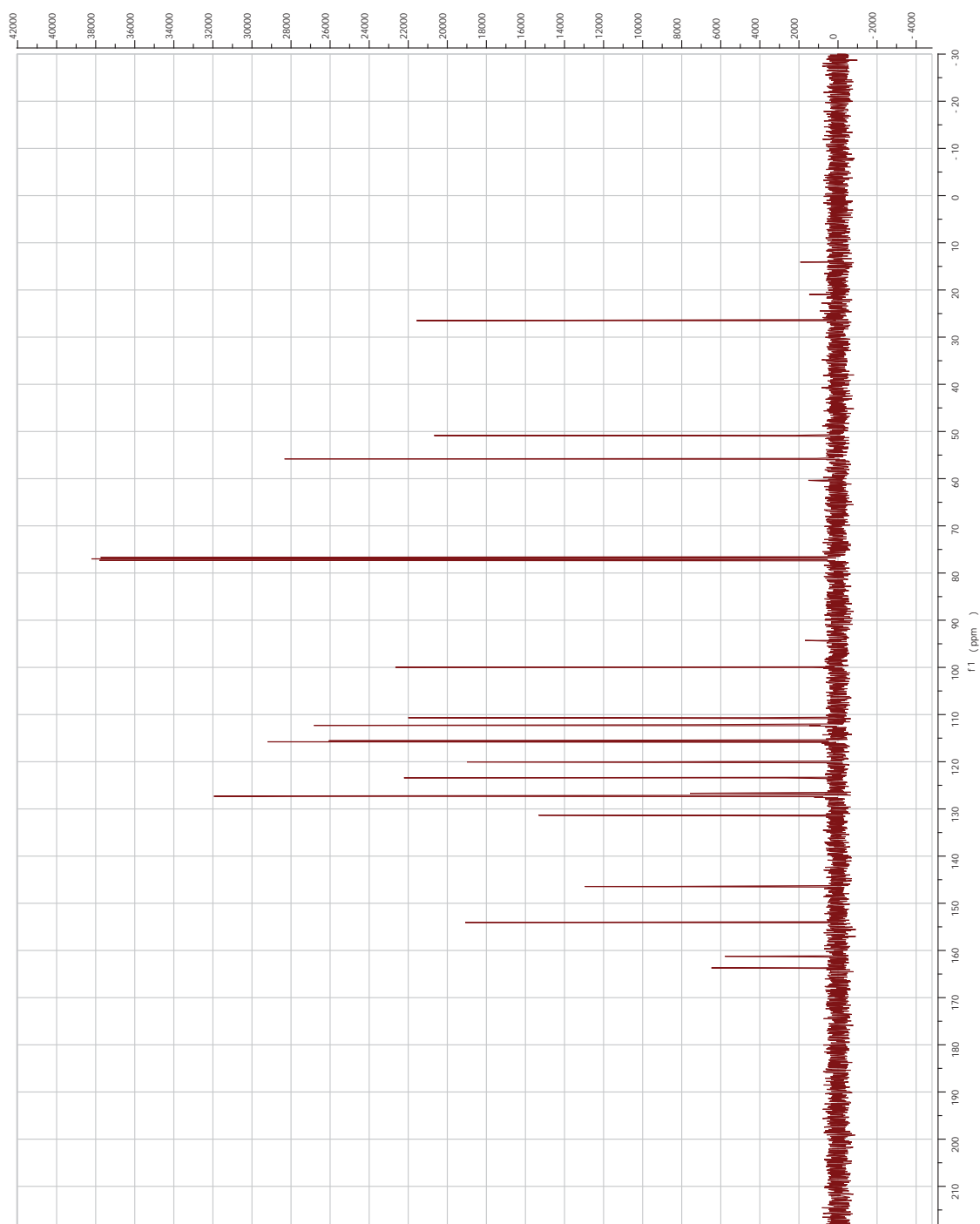


Figure A-II.16. ^{13}C NMR spectra of (**8a**)

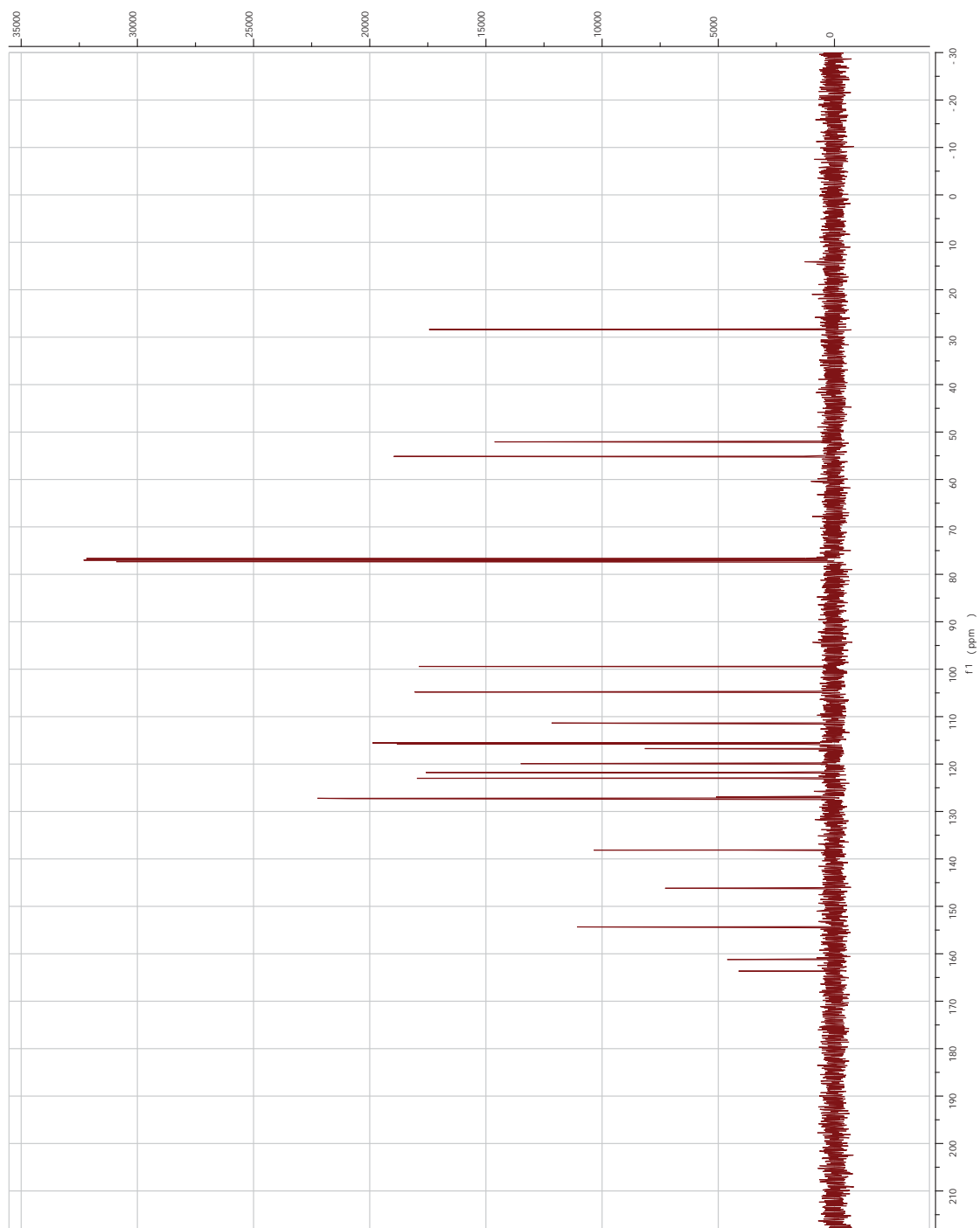


Figure A-II.17. ^{13}C NMR spectra of (8b)

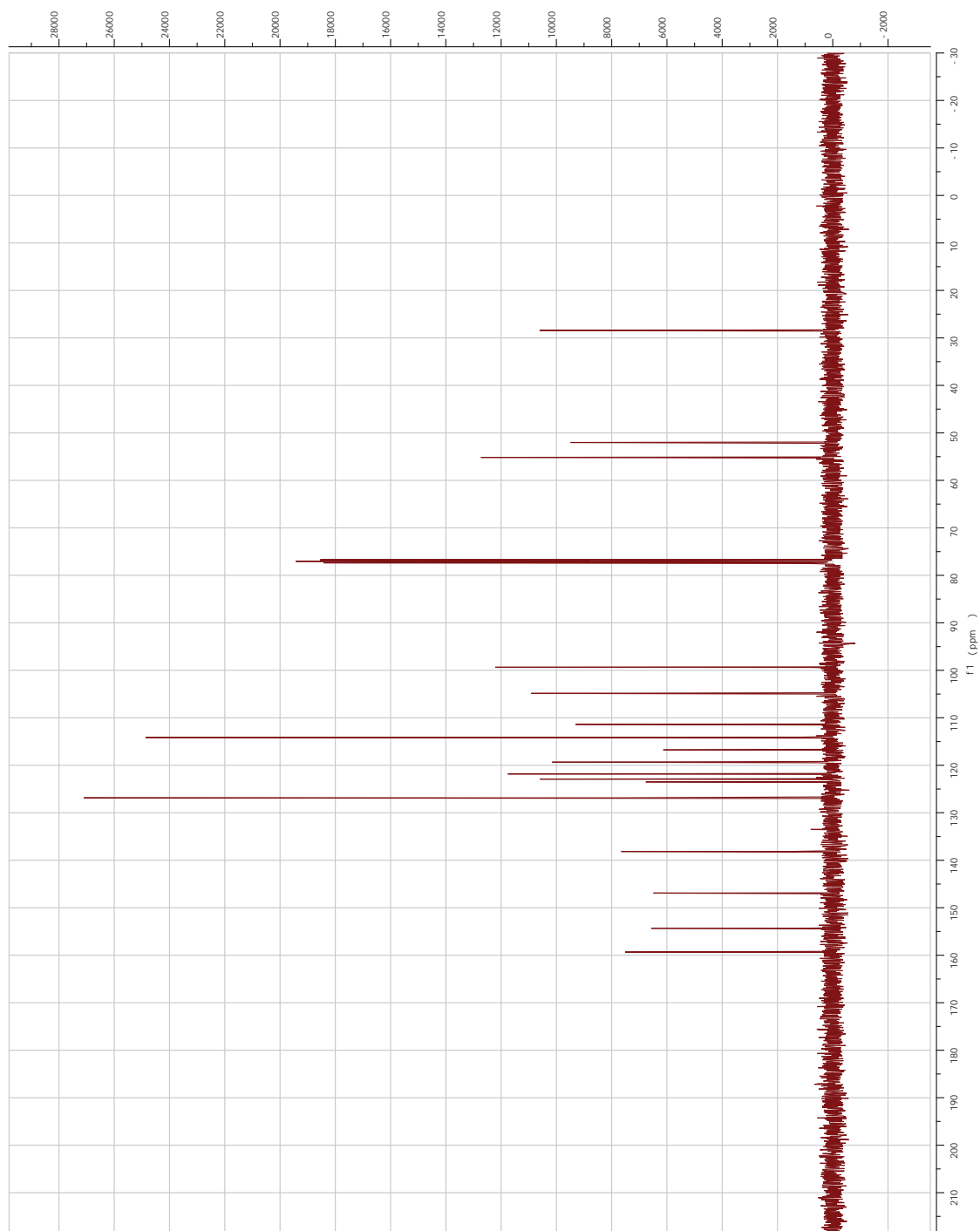


Figure A-II.18. ^{13}C NMR spectra of (8c)

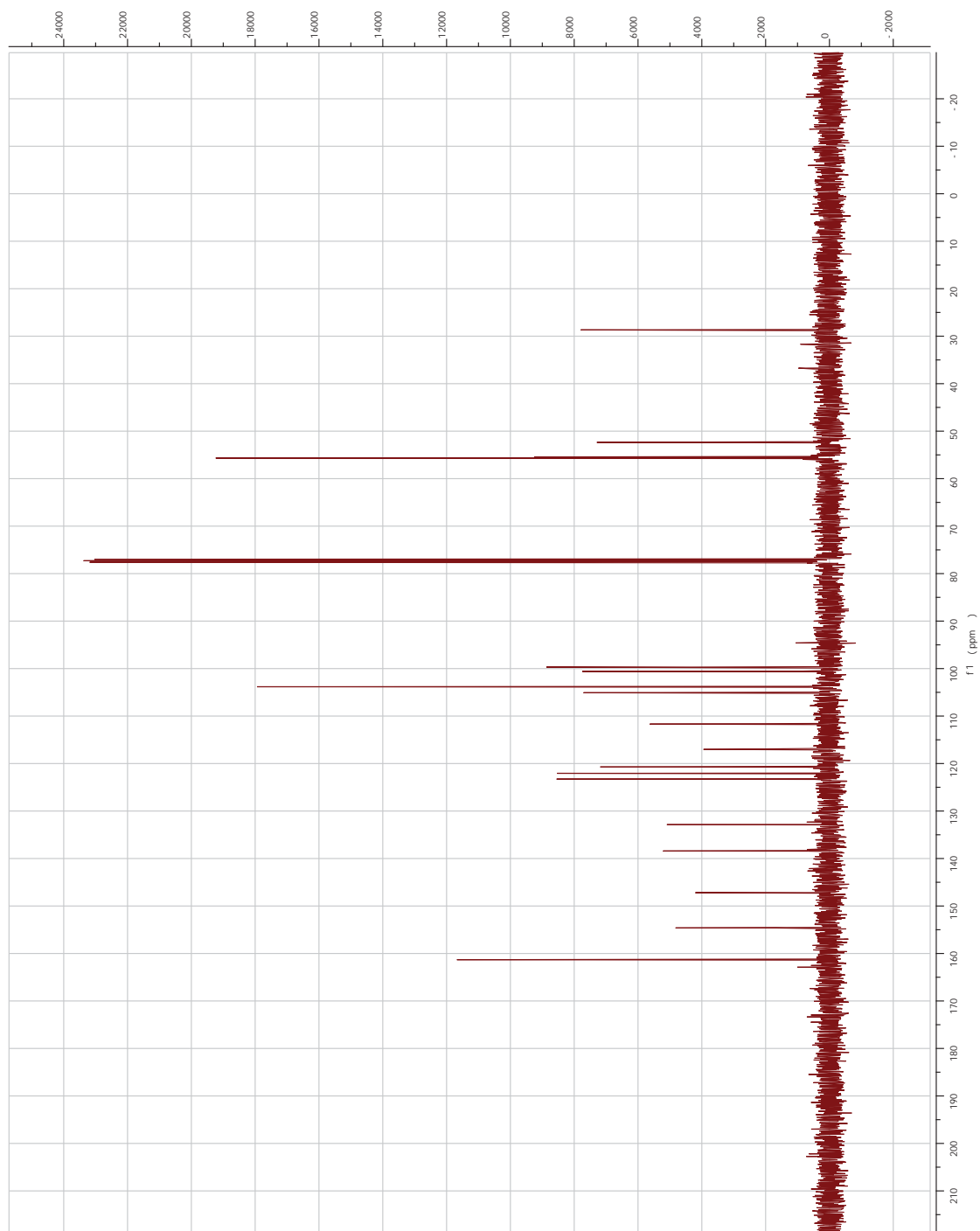


Figure A-II.19. ^{13}C NMR spectra of (**8d**)

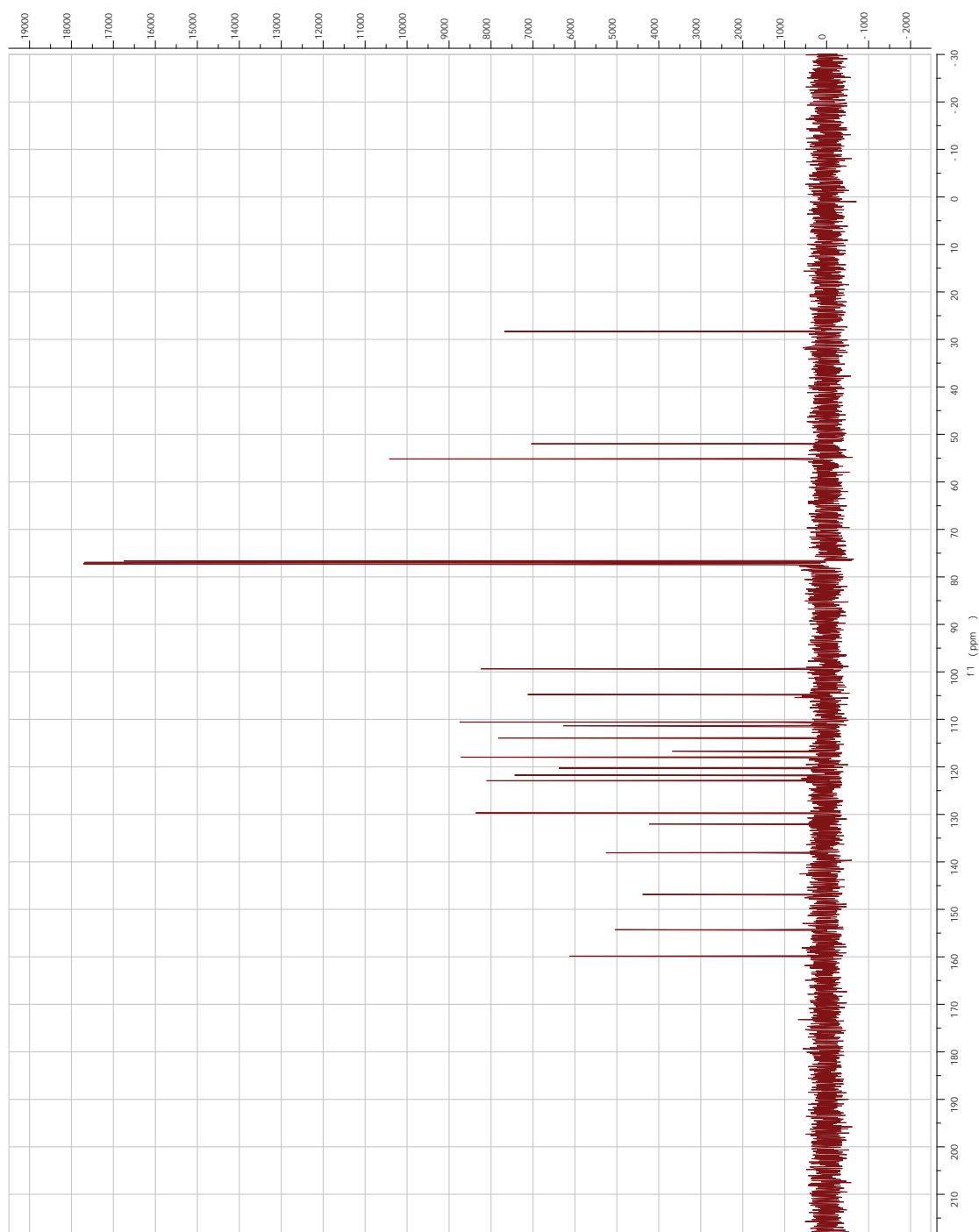


Figure A-II.20. ^{13}C NMR spectra of (**8e**)

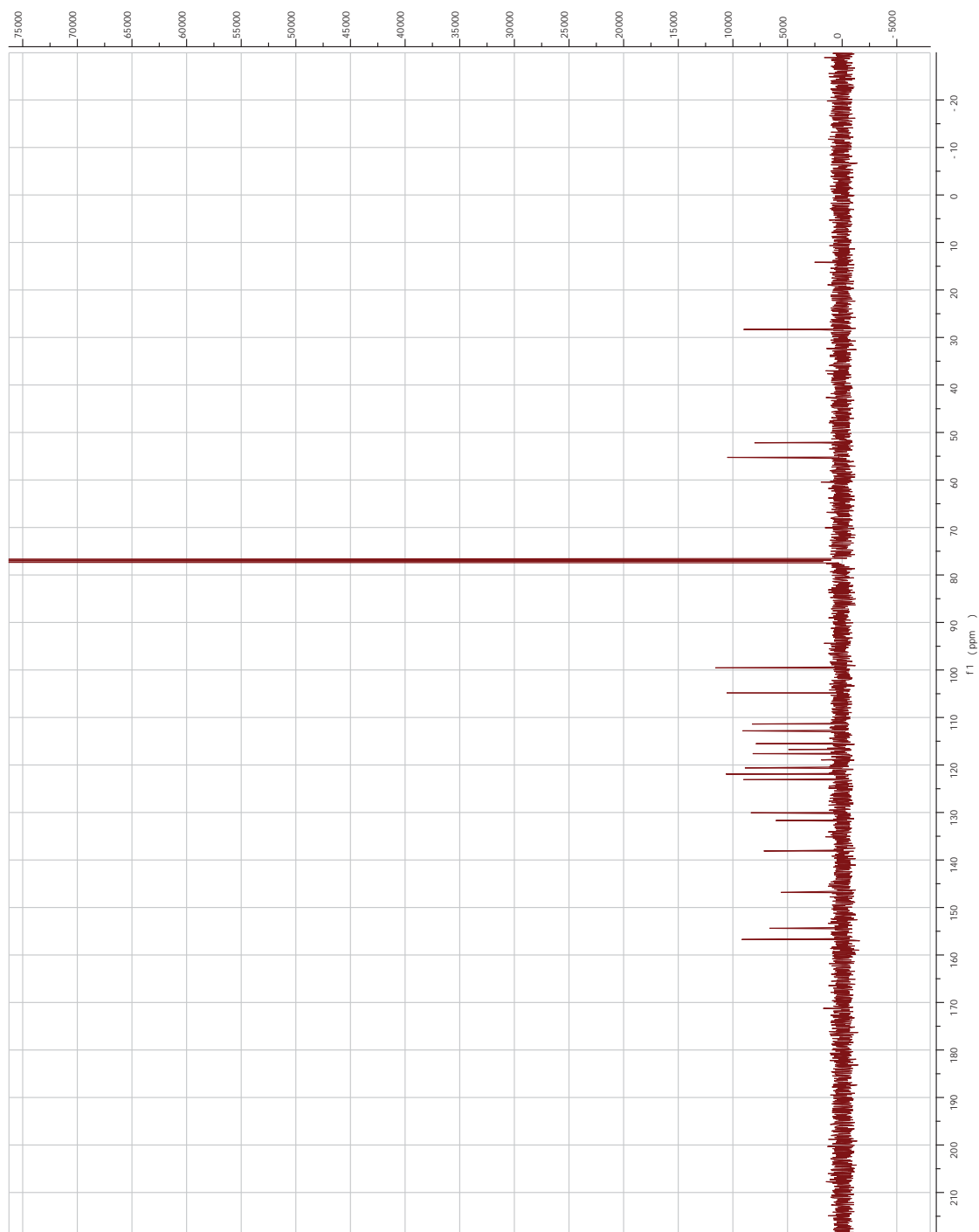


Figure A-II.21. ^{13}C NMR spectra of (9a)

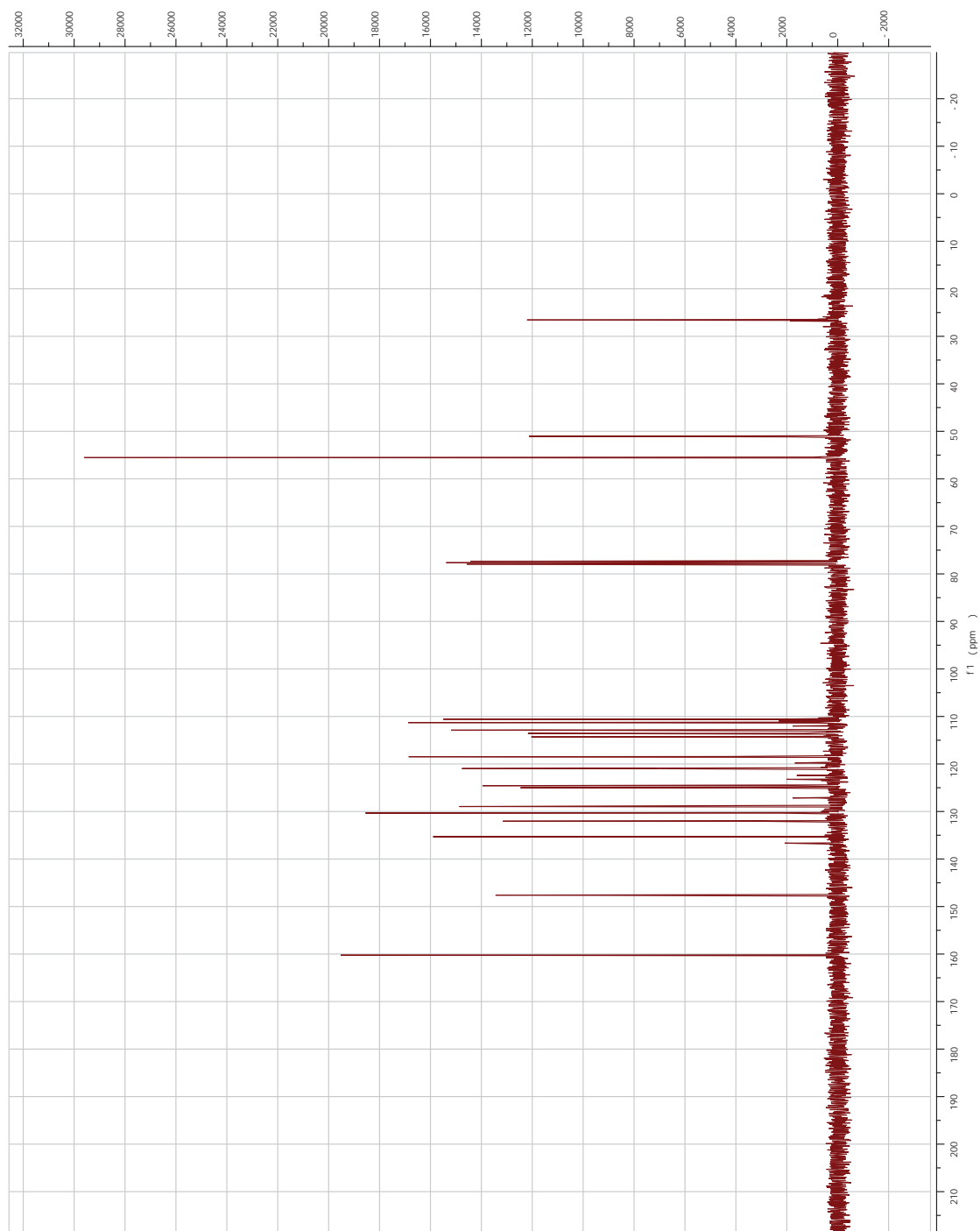


Figure A-II.22. ^{13}C NMR spectra of (**9b**)

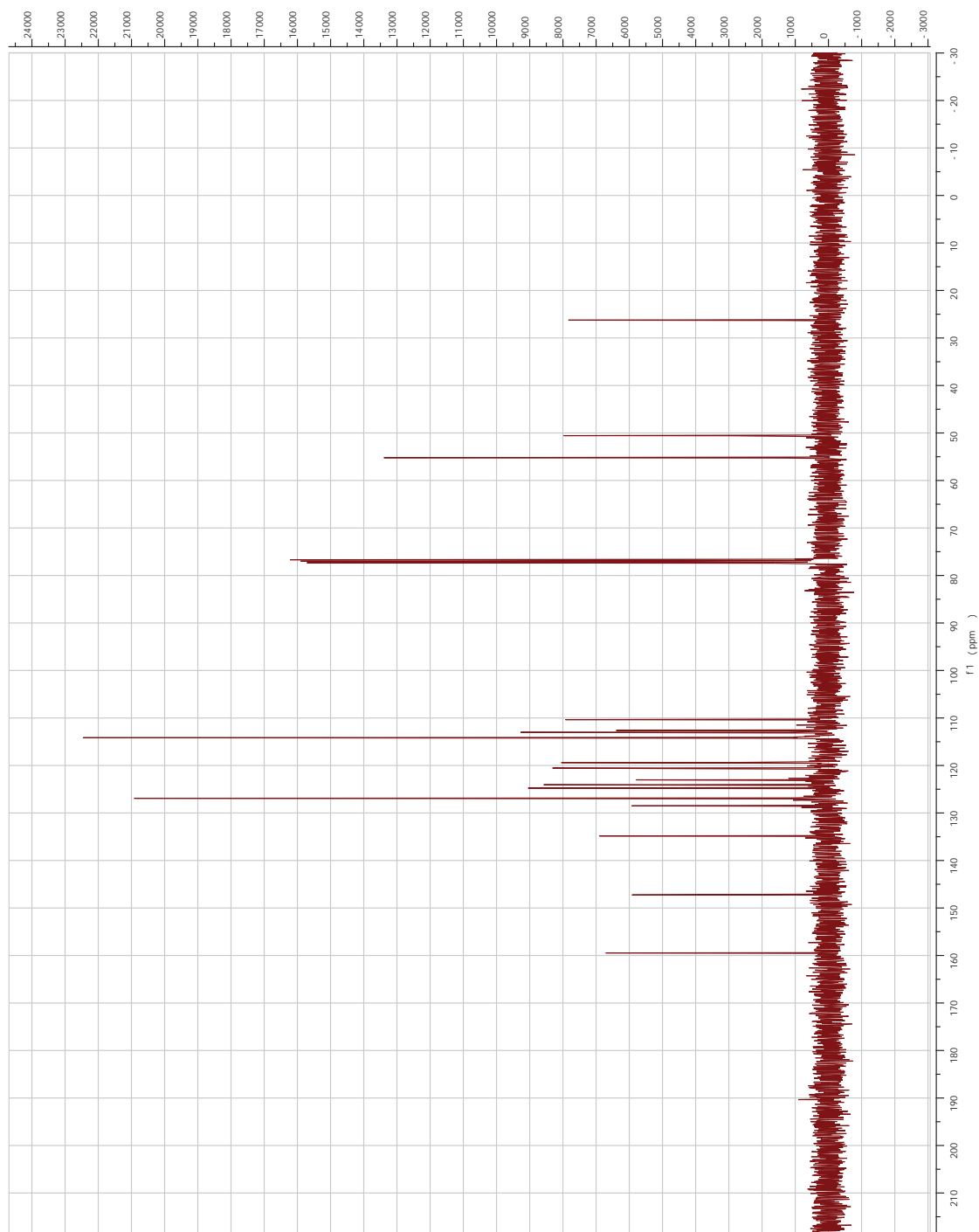


Figure A-II.23. ^{13}C NMR spectra of (9c)

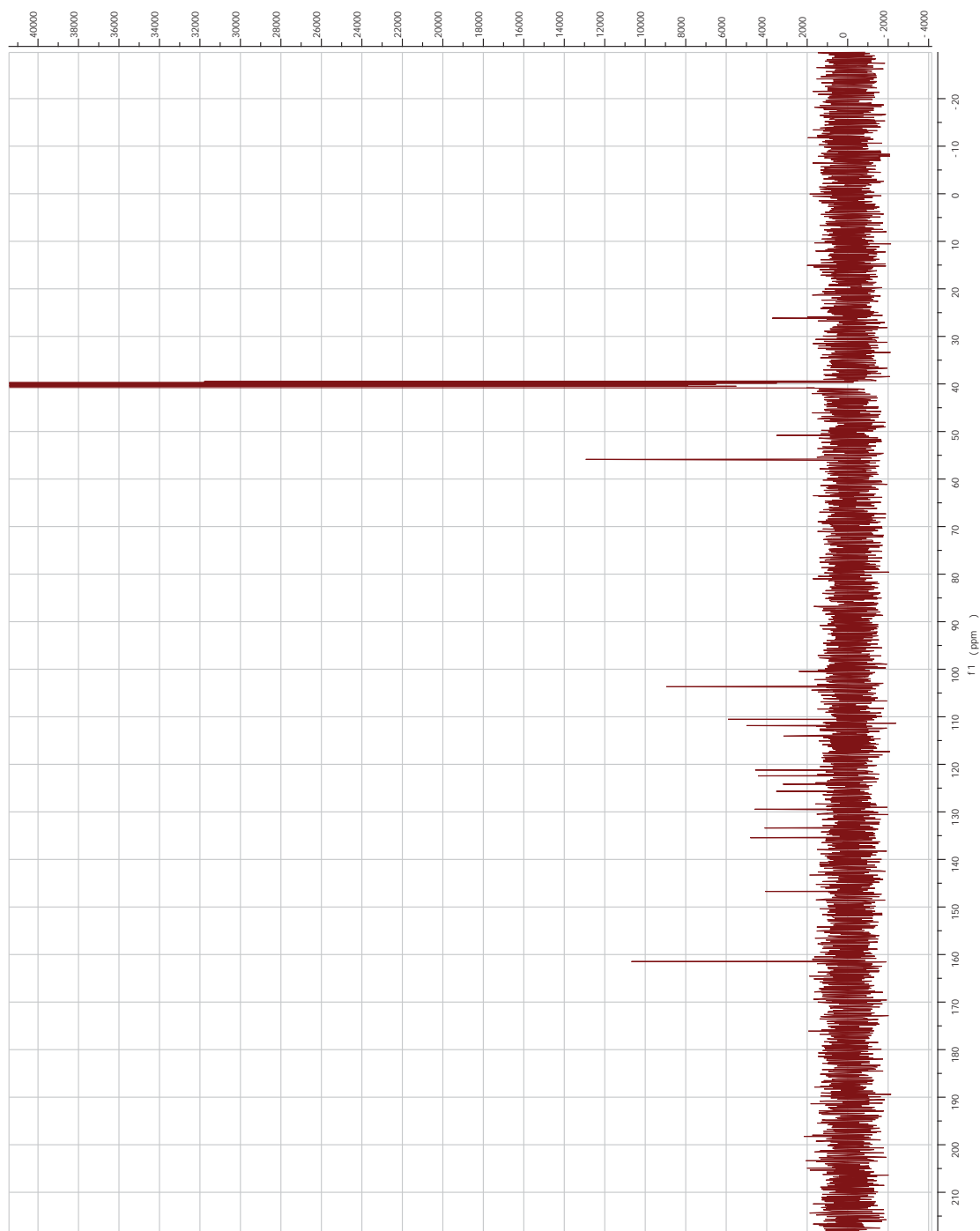


Figure A-II.24. ^{13}C NMR spectra of (**9d**)

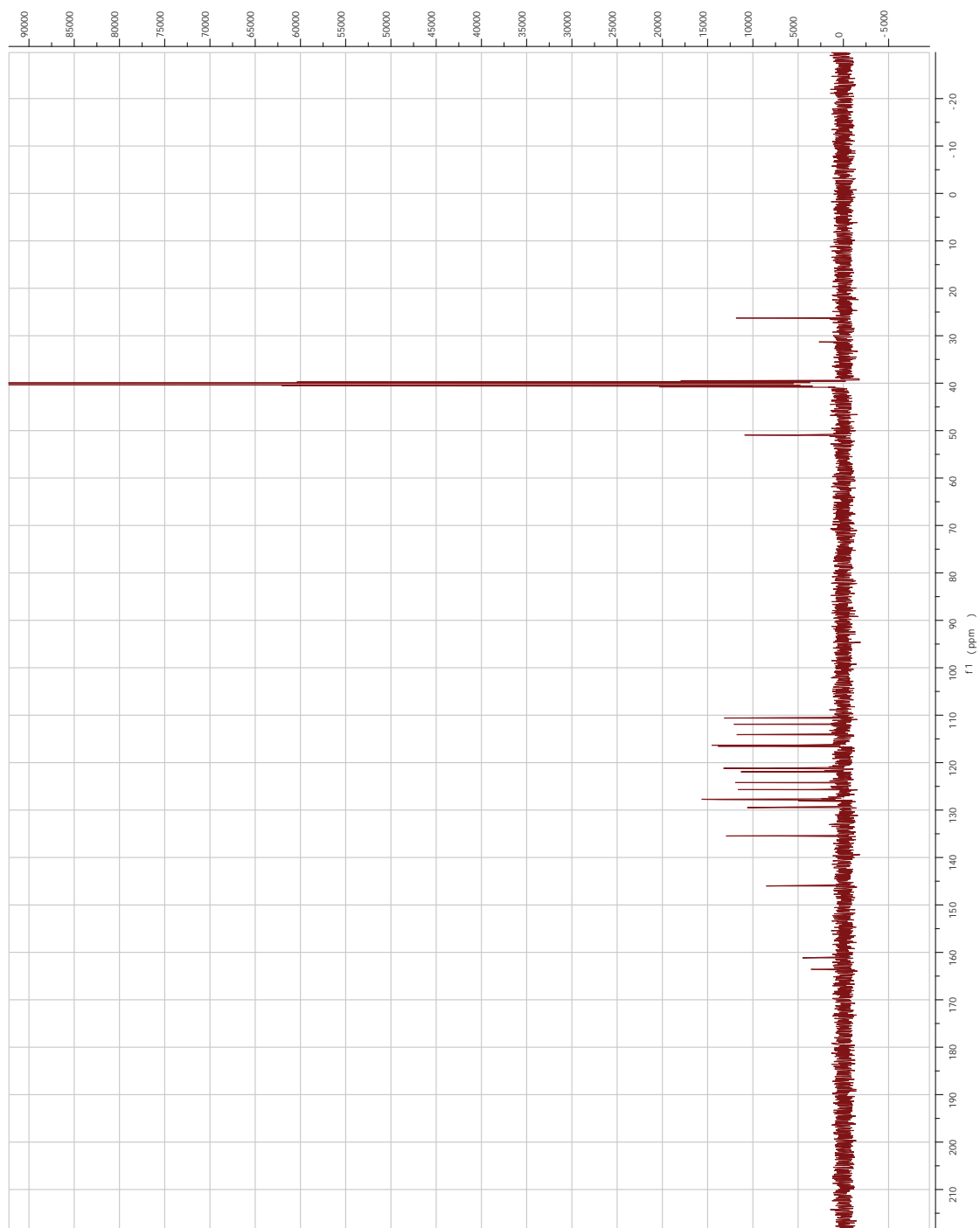


Figure A-II.25. ^{13}C NMR spectra of (9e)

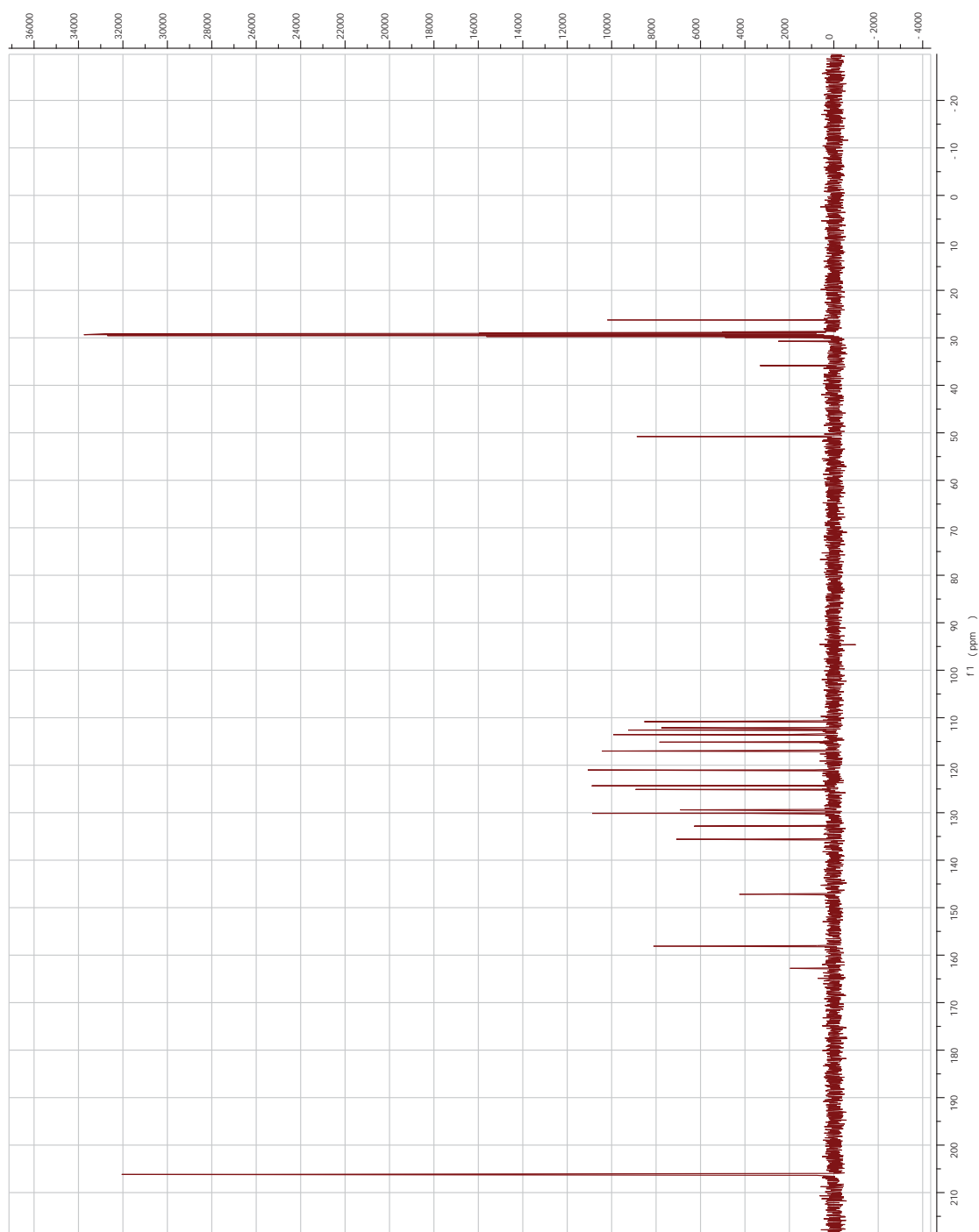


Figure A-II.26. ^{13}C NMR spectra of (10a)

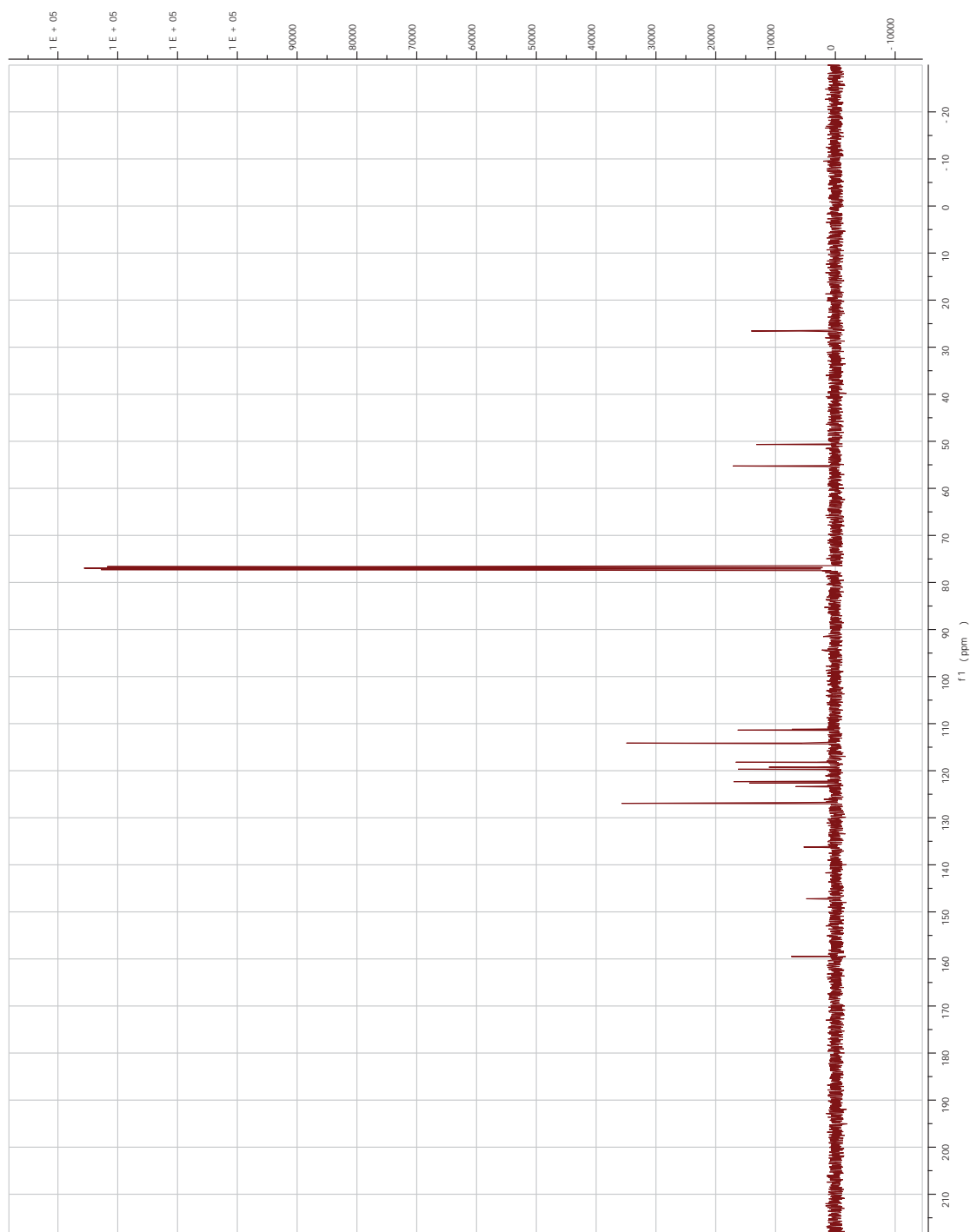


Figure A-II.27. ^{13}C NMR spectra of (10b)

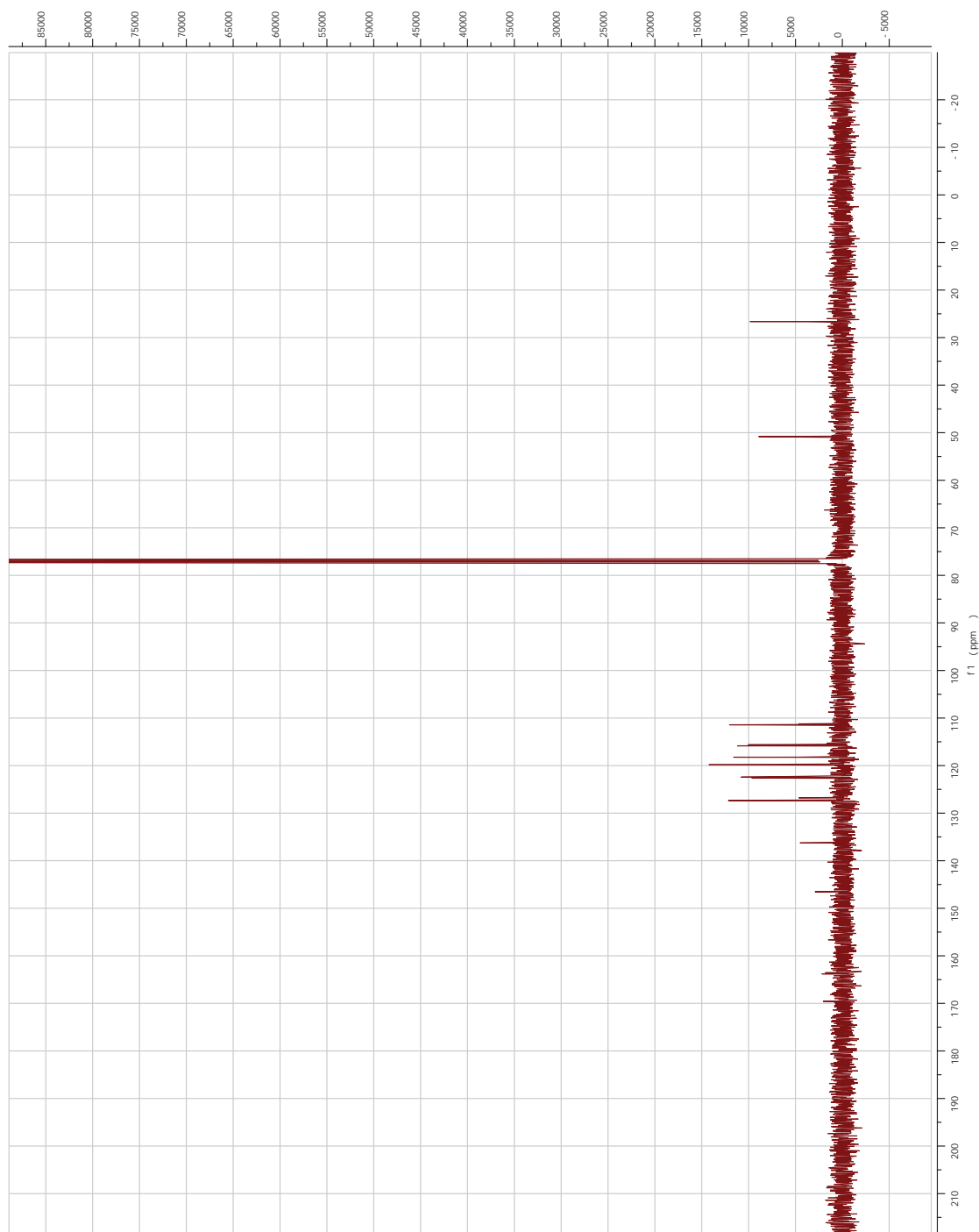


Figure A-II.28. ^{13}C NMR spectra of (10c)

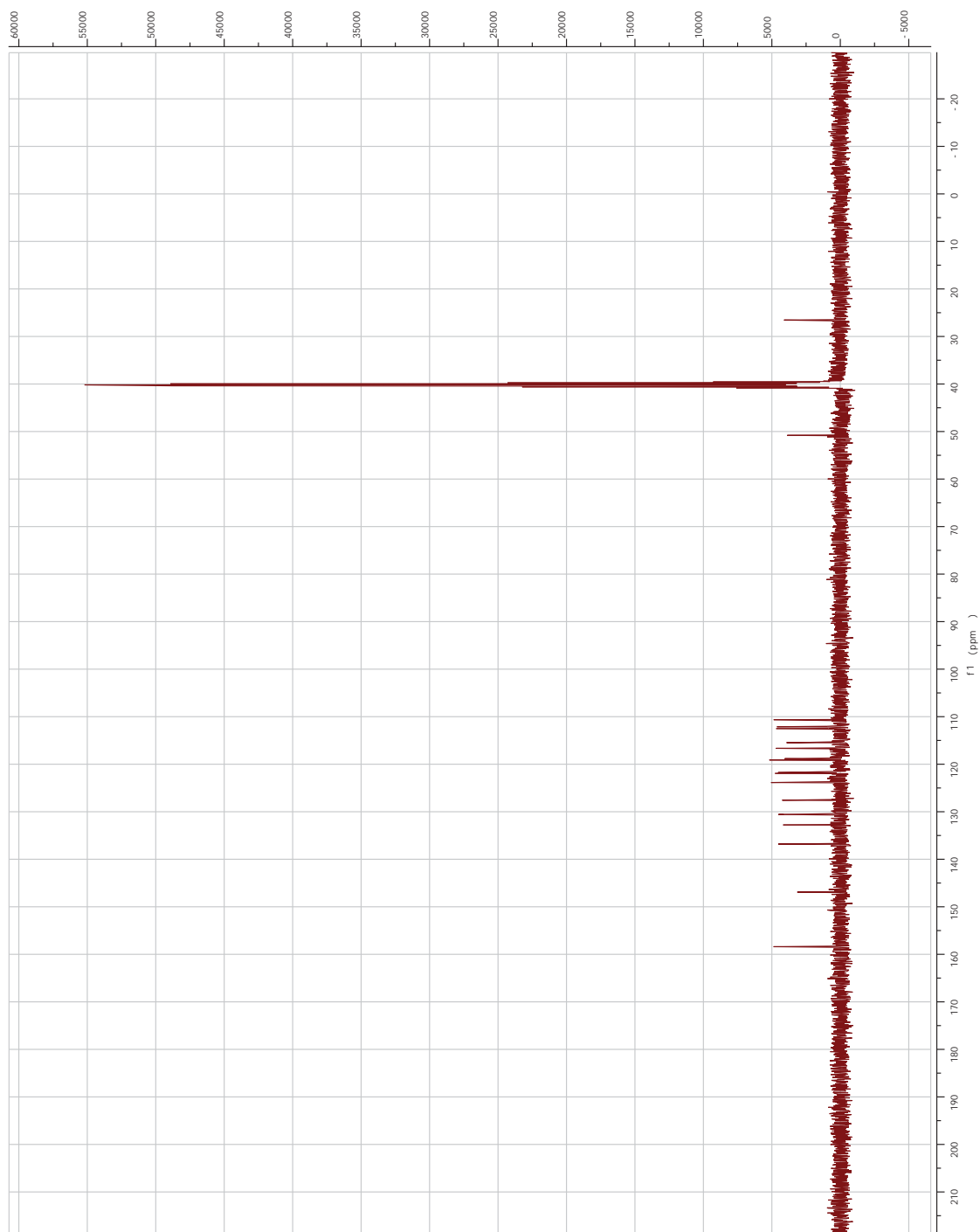


Figure A-II.29. ^{13}C NMR spectra of (**10d**)

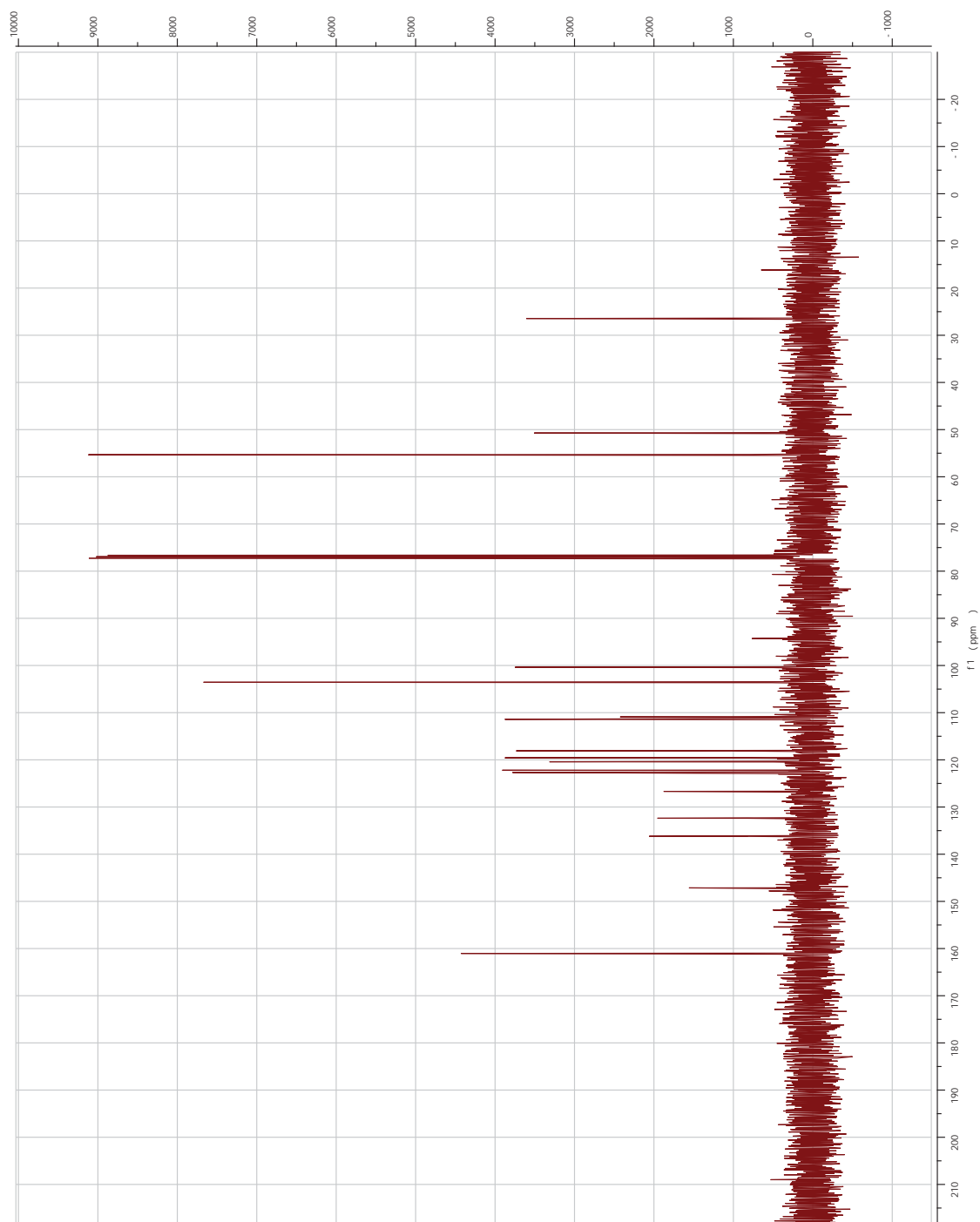
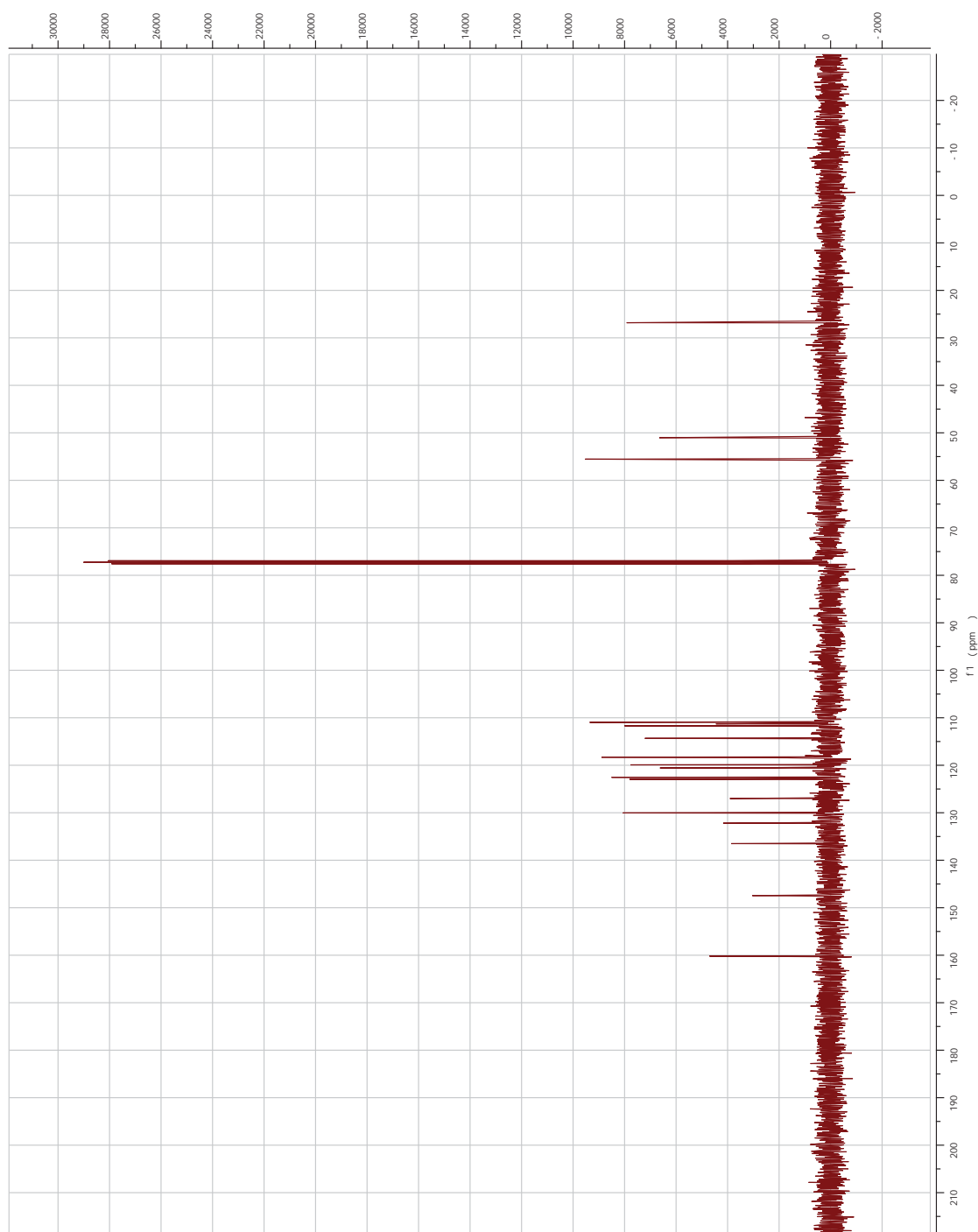


Figure A-II.30. ^{13}C NMR spectra of (**10e**)



Appendix-III ^{19}F NMR Spectra

Figure A-III.1. ^{19}F NMR spectra of (**5a**)

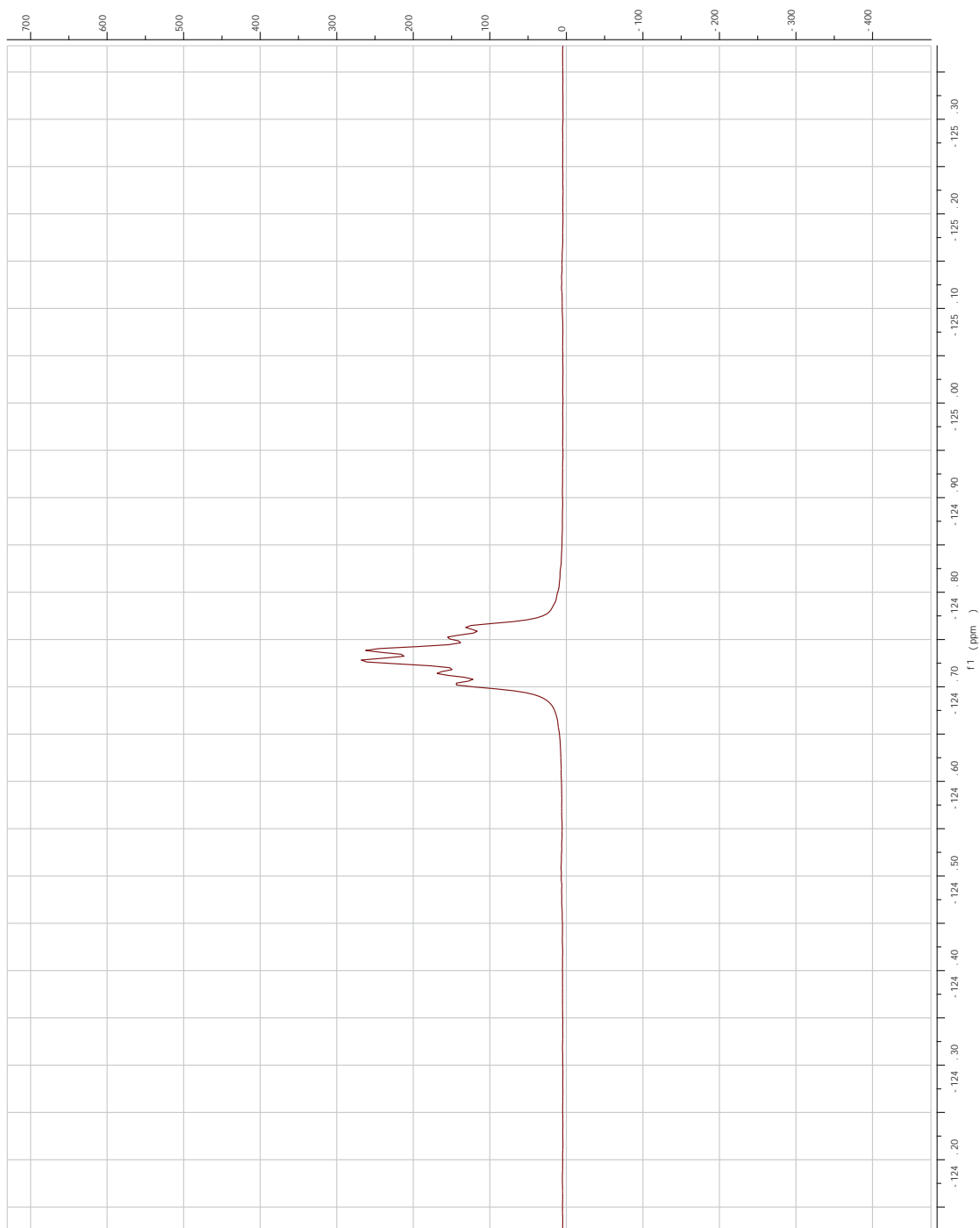


Figure A-III.2. ^{19}F NMR spectra of (**5b**)

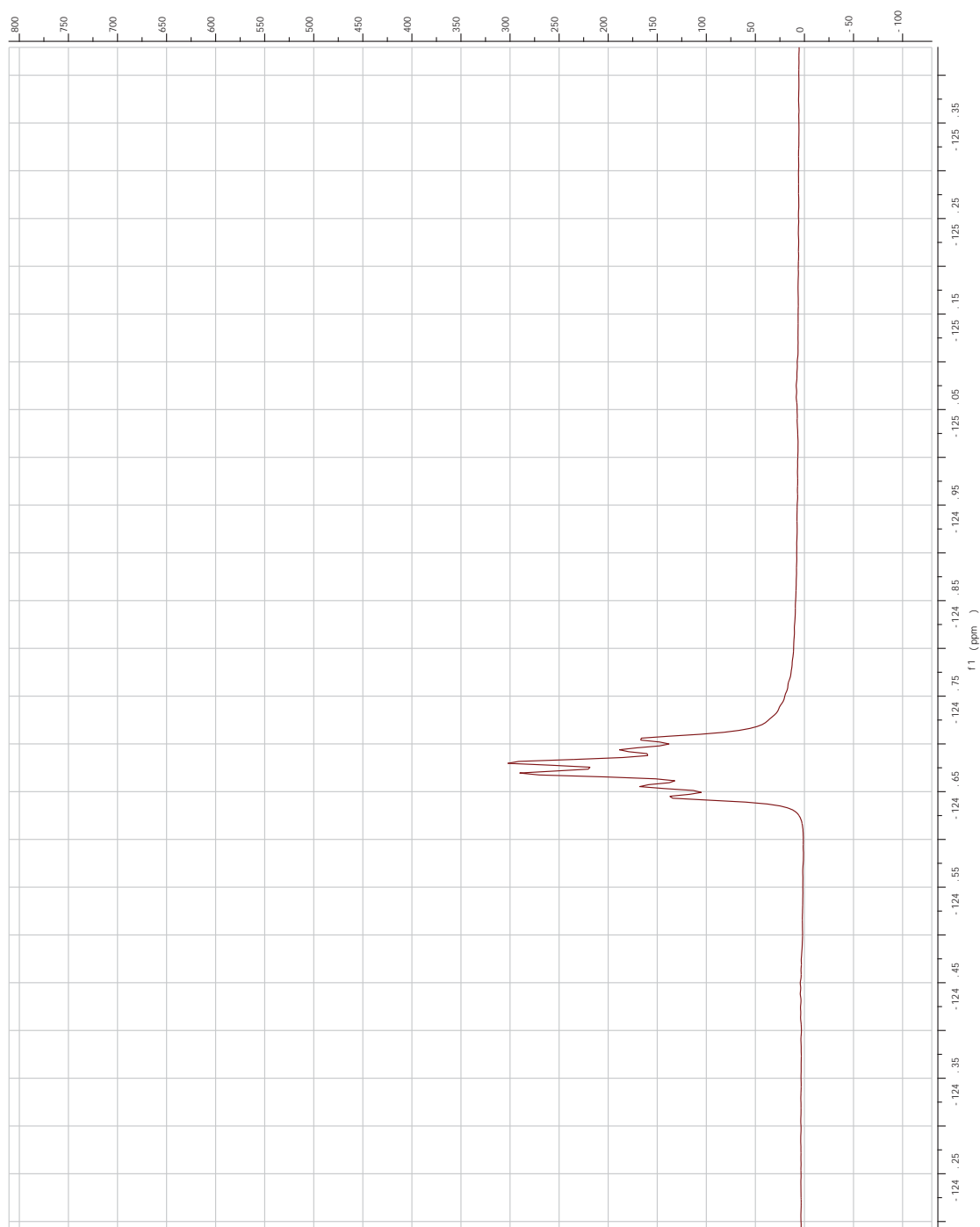


Figure A-III.3. ^{19}F NMR spectra of (**5c**)

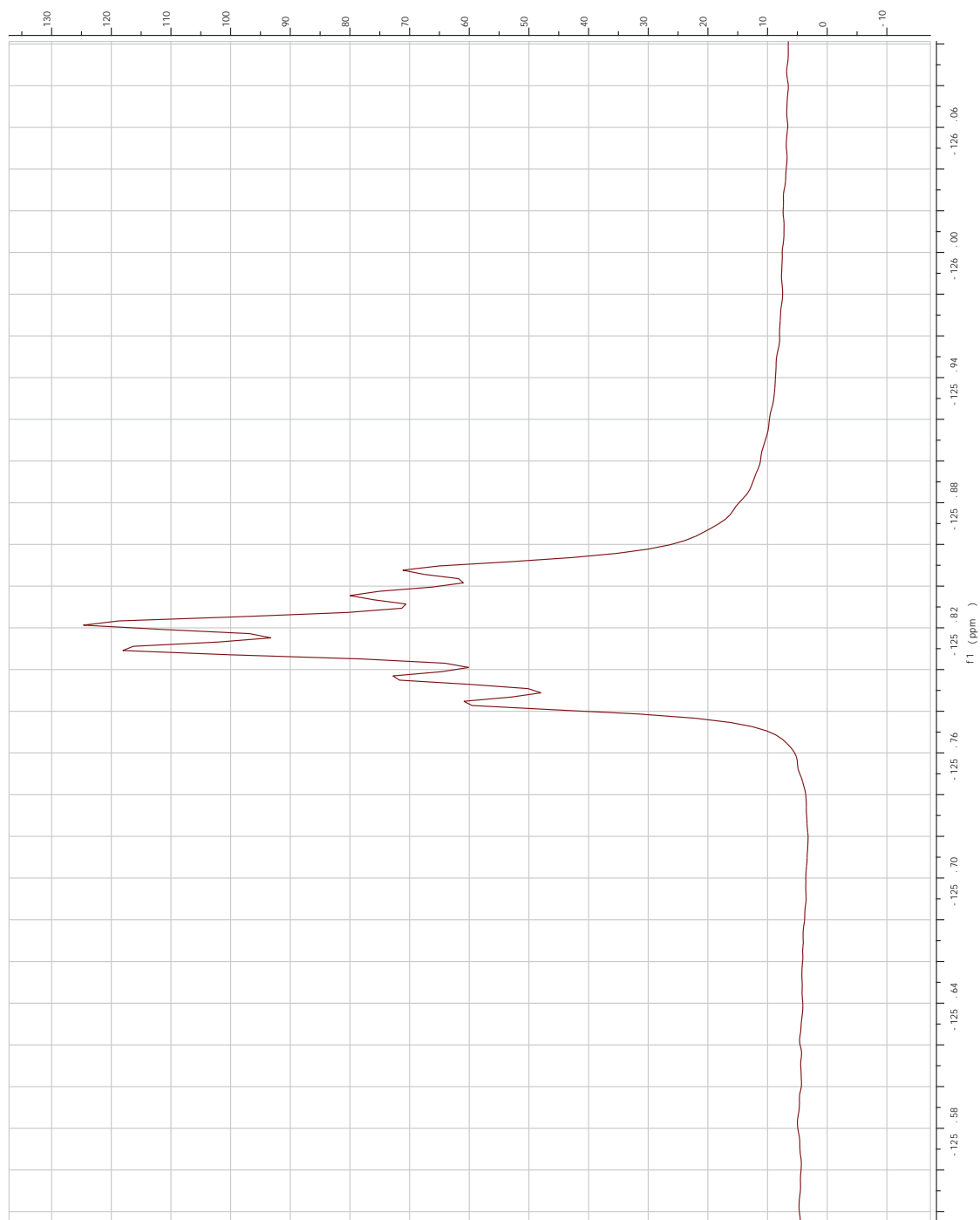


Figure A-III.4. ^{19}F NMR spectra of (**5d**)

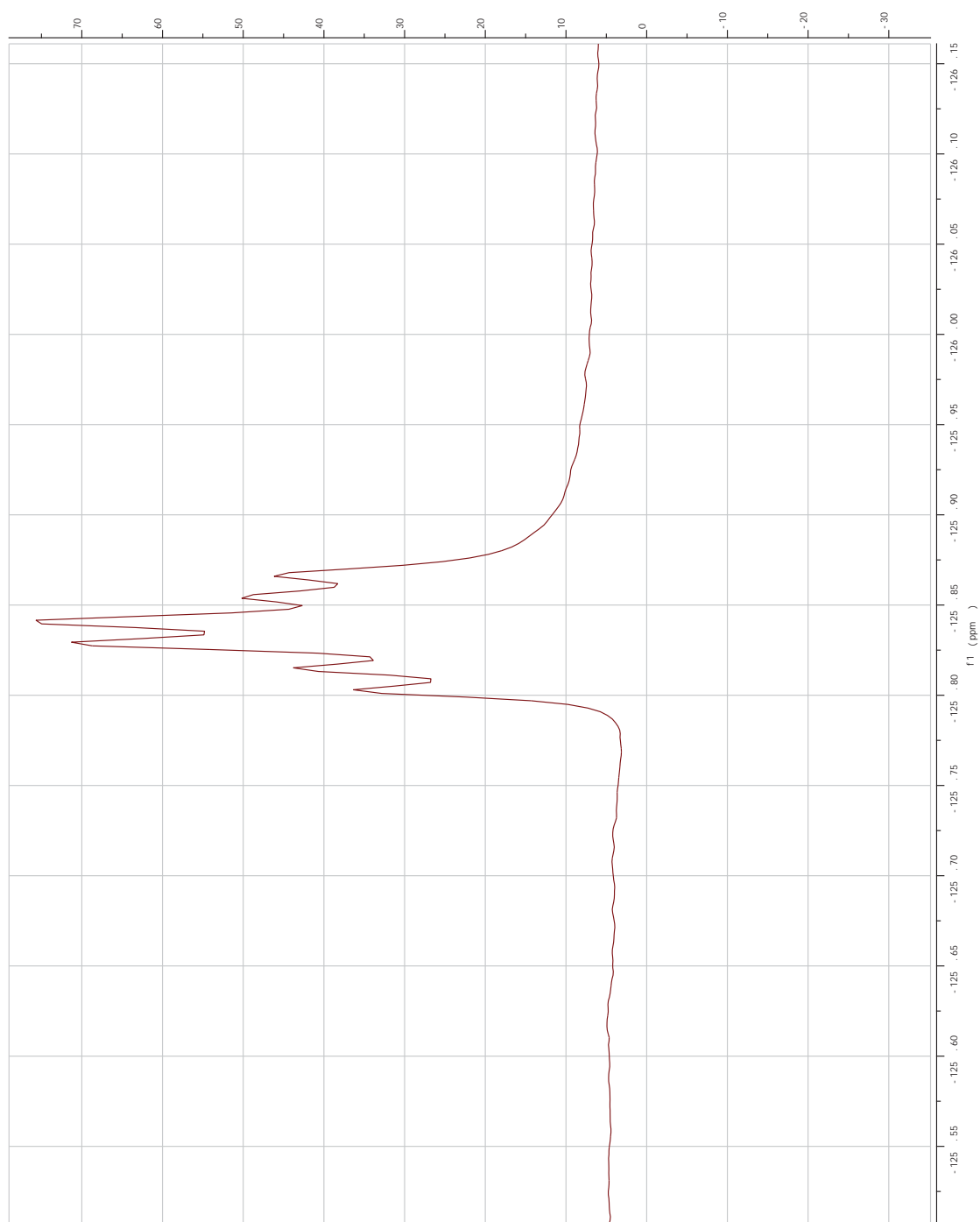


Figure A-III.5. ^{19}F NMR spectra of (**5e**)

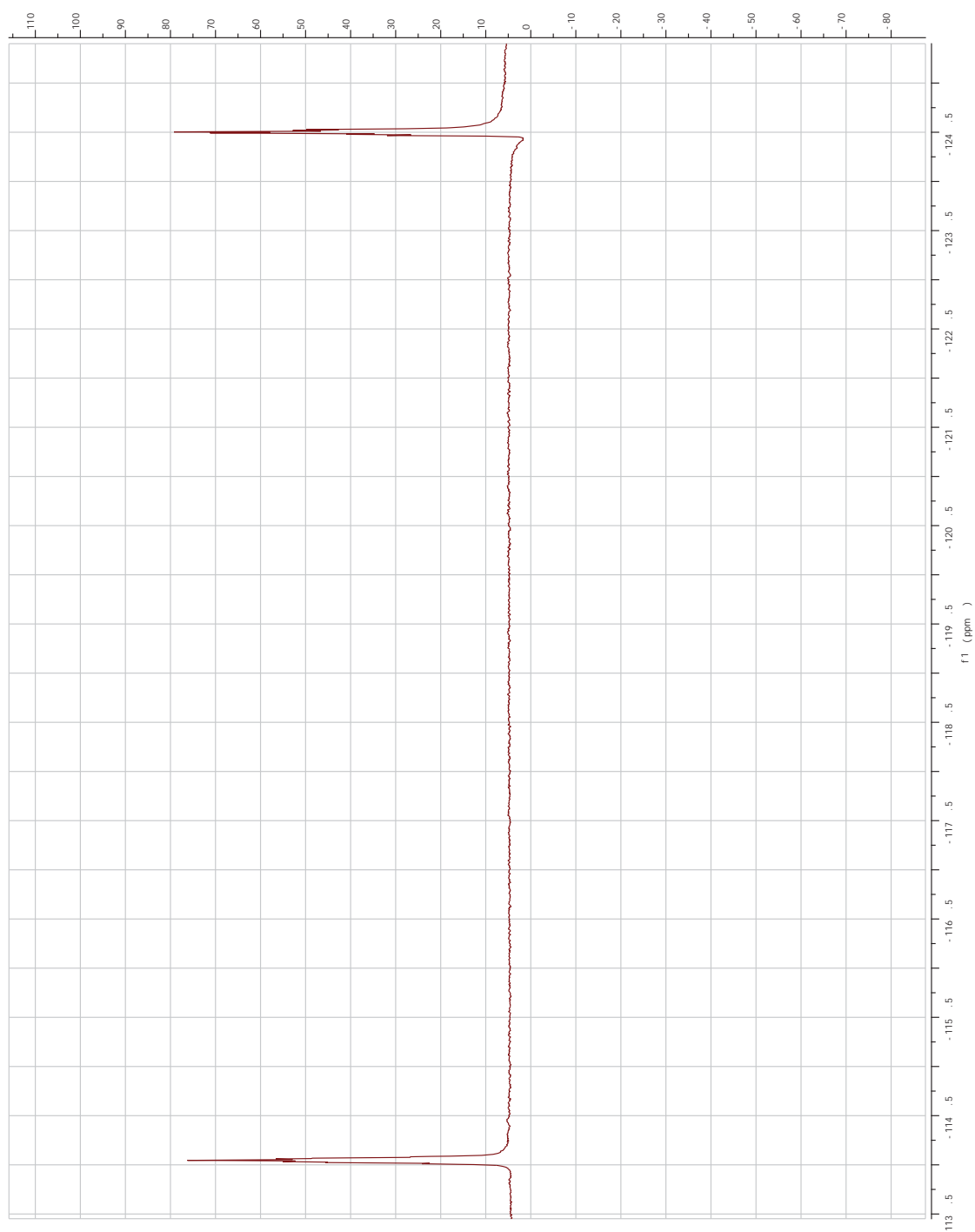


Figure A-III.6. ^{19}F NMR spectra of (**6d**)

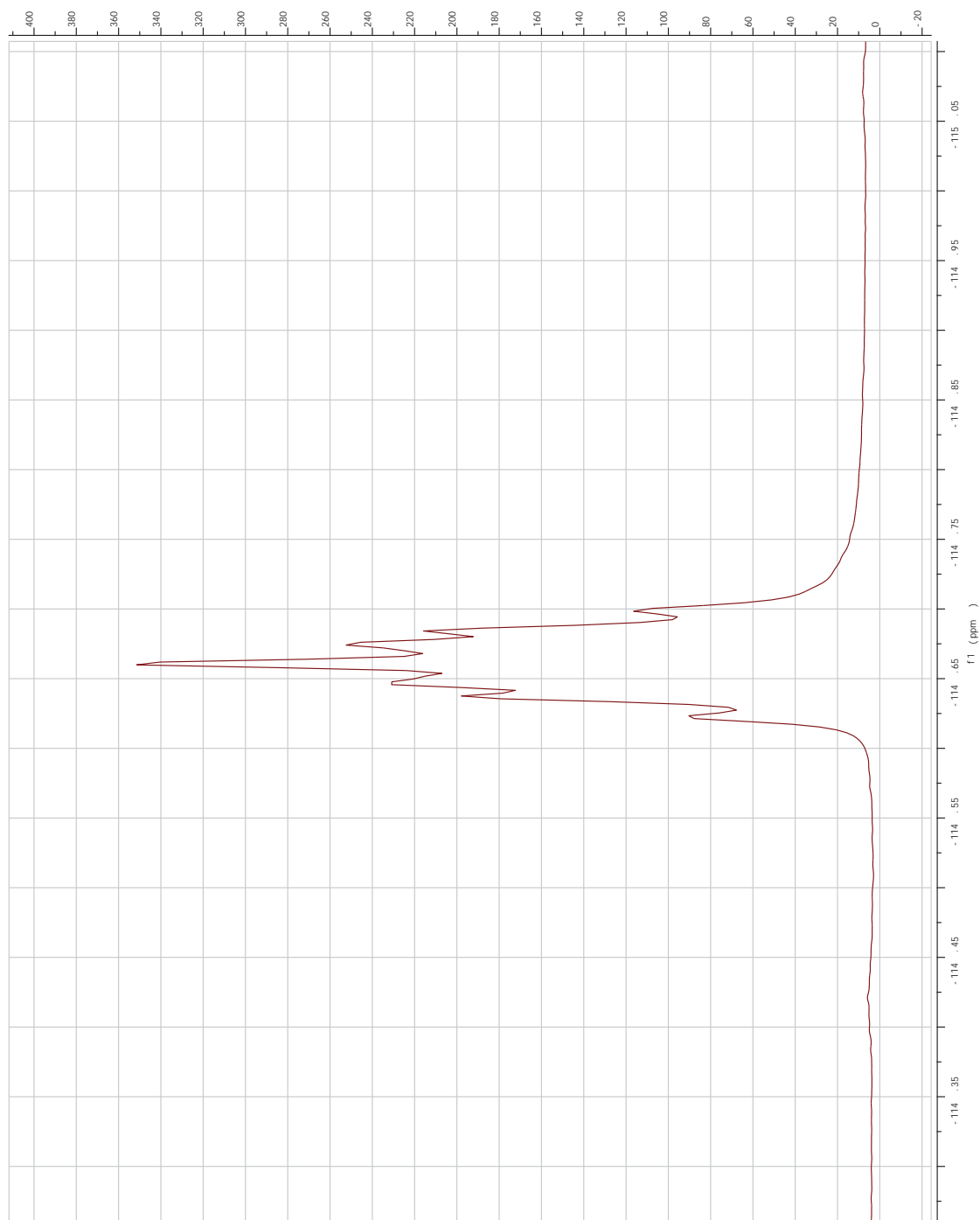


Figure A-III.7. ^{19}F NMR spectra of (**7e**)

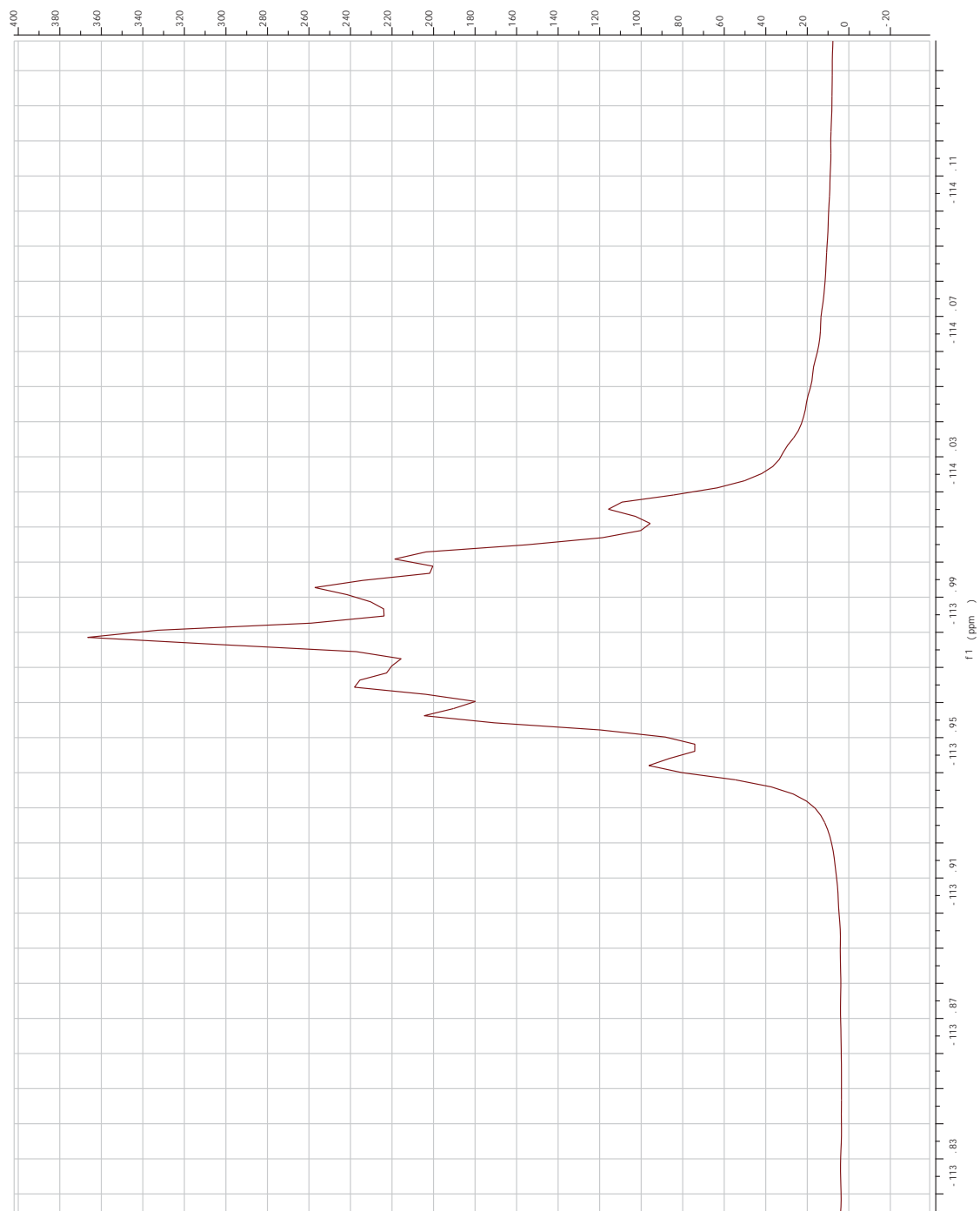


Figure A-III.8. ^{19}F NMR spectra of (**8a**)

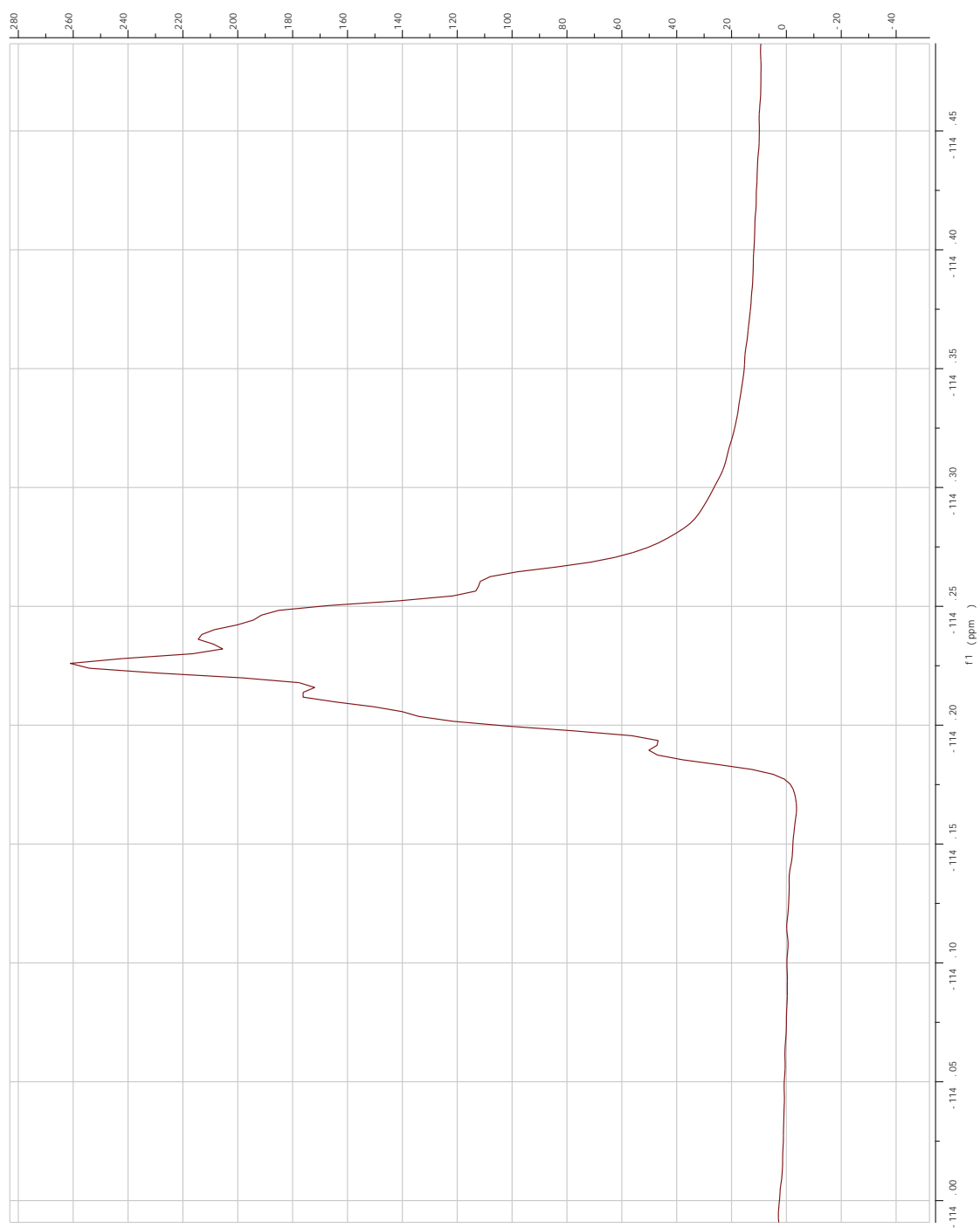


Figure A-III.9. ^{19}F NMR spectra of (**9d**)

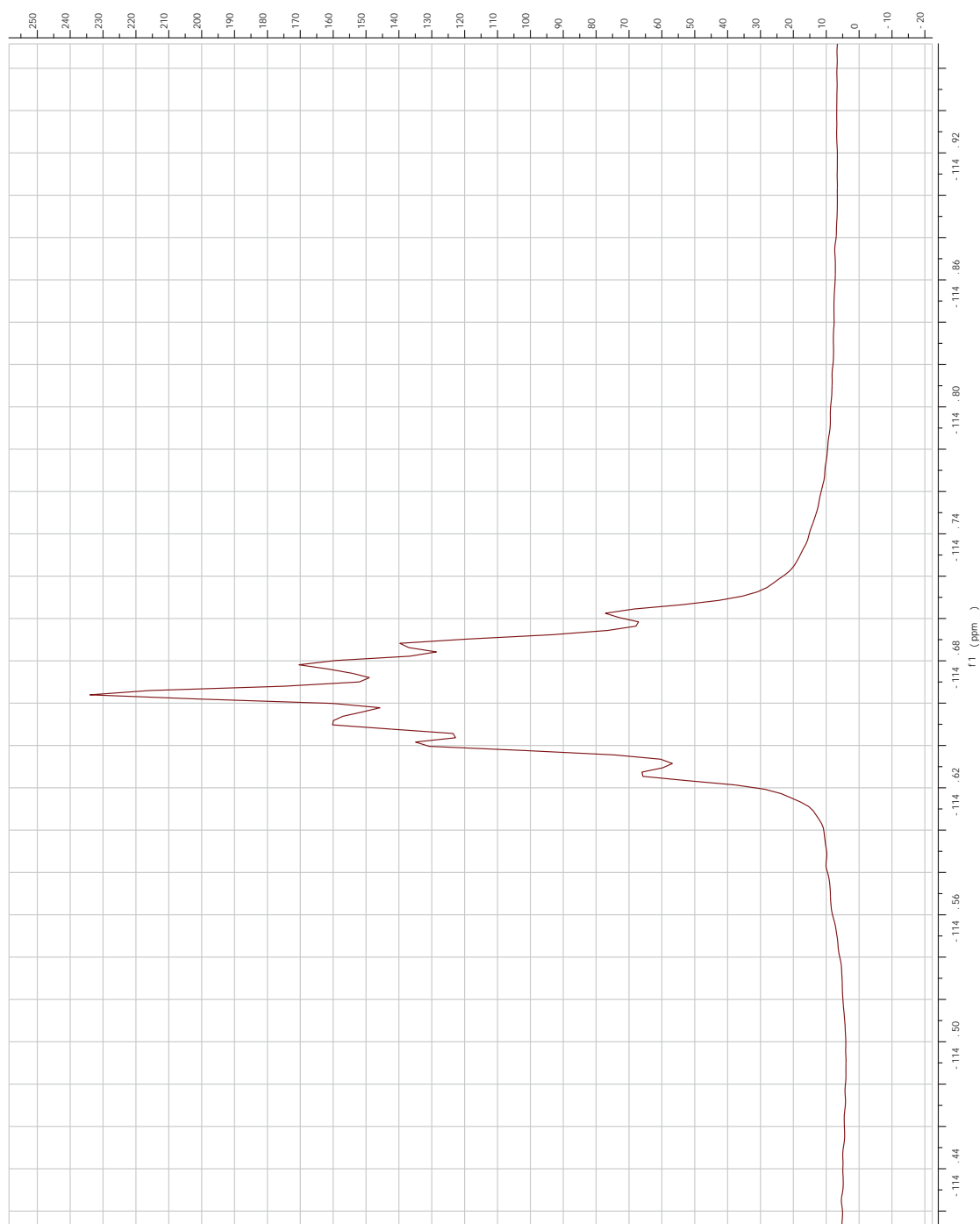


Figure A-III.10. ^{19}F NMR spectra of **(10b)**

