Synthesis of Pyrrolo[1,3]-Diazepines and Potential Poxvirus Resolvase Inhibitors

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Synthesis of Pyrrolo[1,3]-Diazepines and Potential Poxvirus Inhibitors

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This thesis describes our efforts toward the development of a dynamic combinatorial library using pyrrolo[1,3]-diazepines. During our work, I have demonstrated the ability to hydrolyze and recyclize the diazepine core via the condensation of several different aldehydes to afford novel diazepine derivatives. Additionally, I have been able to modulate the electronic and steric properties of the diazepine scaffold through substitution on the pyrrole core. My work towards finding suitable conditions for a thermodynamically controlled dynamic exchange reaction have shown that while hydrolysis of the diazepine scaffold seems to be favored, recyclization of the resulting amine intermediate appears to be disfavored. As a second project I describe our efforts toward the development of a library of pyrimidinone-based potential poxvirus resolvase inhibitors. Utilizing the multi-component Biginelli reaction, I have synthesized a small library of pyrimidinones attached to potential chelating functionalities, including the di-keto acid moiety. Biological testing has resulted in the discovery of a carboxylic acid containing pyrimidinone (**MAL1-265**) that possesses moderate fowlpox resolvase inhibitory activity (IC_{50} 16 μM).

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ABBREVIATIONS

Ac…… acetyl

Boc……...*tert*-butyloxycarbonyl

BOP…….benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate

CDC……Centers for Disease Control and Prevention

DMAD……dimethyl acetylenedicarboxylate

DMAP…….4-dimethylaminopyridine

DMF……*N*,*N*-dimethylformamide

EEV…….extracellular enveloped virus

EC_{50}…….half-maximal effective concentration

equiv……..... equivalent(s)

HIV……..human immunodeficiency virus

HRMS…….high-resolution mass spectroscopy

IR……infrared spectroscopy

K_i…….....enzyme-inhibitor dissociation constant

LC-HRMS……..liquid chromatography-high-resolution mass spectroscopy

LiHMDS…….lithium bis(trimethysilyl)amide

MS……...molecular sieves

NMR……nuclear magnetic resonance

PF……processivity factor

PPTs……pyridinium p-toluenesulfonate

p-TsOH……...p-toluenesulfonic acid

Red-Al……sodium bis(2-methoxyethoxy)aluminumhydride

RNase……..ribonuclease

rt……..room temperature

SAR…….structure activity relationship

T3P……..propylphosphonic anhydride

THF…….tetrahydrofuran

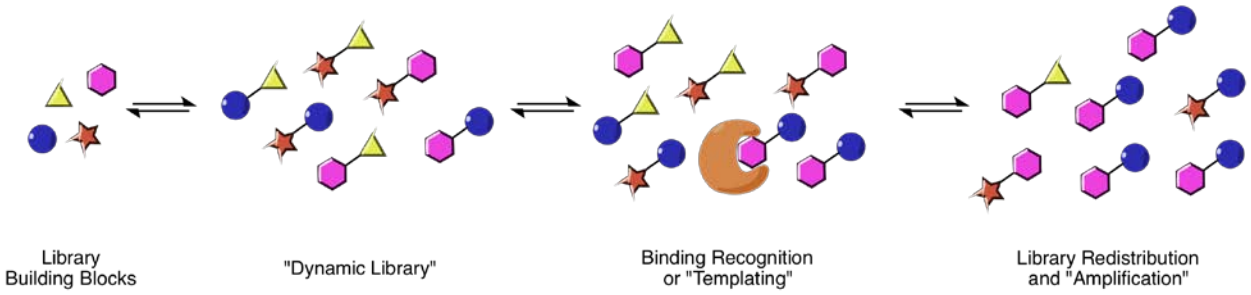
PREFACE

I would like to thank all of my friends and family for their continued support. I would also like to dedicate this document to the memory of my grandfather Raymond Dietrich.

1.0 EFFORTS TOWARD THE DEVELOPMENT OF A DYNAMIC COMBINATORIAL LIBRARY USING PYRROLO[1,3]-DIAZEPINES

1.1 INTRODUCTION

Beginning in the mid 1990's, dynamic combinatorial chemistry emerged as a powerful technique capable of creating large libraries where the composition is controlled by the relative thermodynamic stability of the components under a particular set of conditions.[1-5] In simplistic terms, dynamic combinatorial chemistry may be considered "traditional combinatorial chemistry under thermodynamic control" as the two methods share several similarities.[1] Dynamic combinatorial chemistry relies on the use of reversible reactions, be it noncovalent interactions (i.e. H-bonding or metal-ligand interactions)[6-8] or covalent bonds, in order to generate complex libraries of compounds that are continuously interconverting until the minimum free energy of the system is achieved.[1-2] One of the most useful aspects of dynamic combinatorial chemistry is that the library composition changes in response to environmental stimuli (i.e. pH or temperature), providing the opportunity to select molecules with a particular set of properties.[2] The library may also be manipulated via the addition of a template (i.e protein) in order to identify high-affinity binding molecules.[9-10] Molecules with a high-affinity for the template bind and are removed from the library equilibrium. Following Le Chatelier's principle, the library composition is redistributed to compensate for the substrate bound to the template; resulting in a net increase or amplification in the amount of the template-bound molecules (Scheme 1).

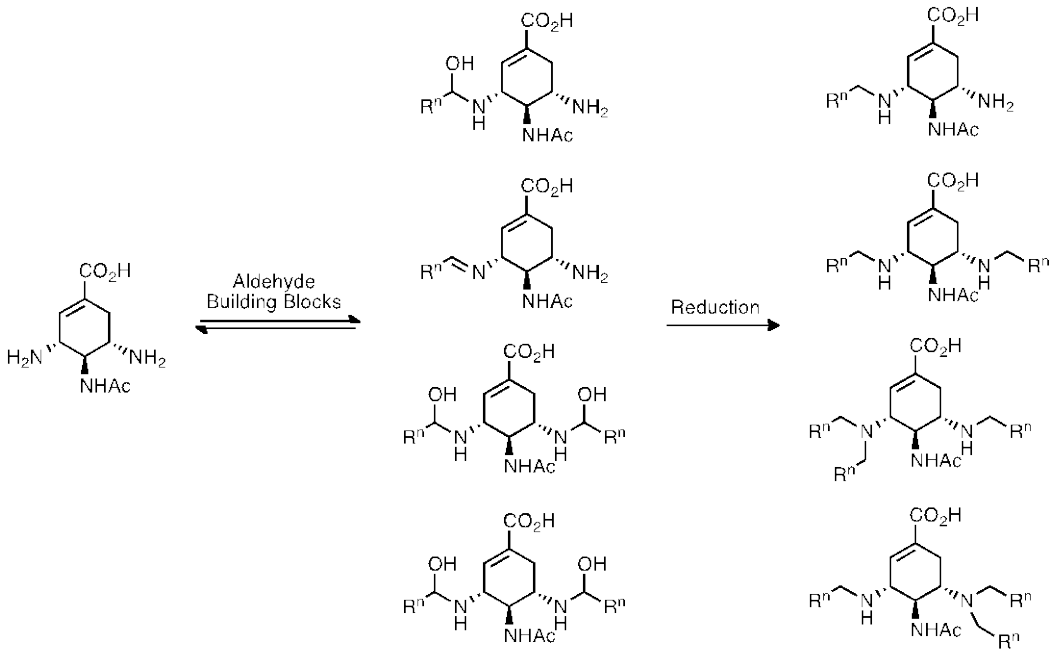


Scheme 1. Dynamic combinatorial chemistry.

One of the drawbacks in employing dynamic combinatorial chemistry is that the method relies on the use of reversible reactions in order to achieve the desired thermodynamically controlled system. While the number of viable reversible reactions found in the literature has significantly increased during the last decade, there is a plethora of irreversible reactions that may be utilized in traditional combinatorial chemistry.[1, 5] However, while dynamic combinatorial libraries require the use of reversible reactions synthesis, the identification of potential targets is extremely efficient in comparison to traditional combinatorial chemistry as targets may be identified *in situ* upon exposure to desired conditions or templates.[5] Traditional combinatorial libraries on the other hand require that each member be individually synthesized and isolated prior to testing. Moreover, as each library member must be tested separately, there is an increased amount of time between library conception and isolation of a target compound. An additional concern when creating a dynamic combinatorial library is that in order to have the library under thermodynamic control every member of the library must be soluble; otherwise shifts in library composition may occur as a result of solubility instead of thermodynamic stability.[5] Traditional combinatorial chemistry is able to avoid solubility issues as each target from a traditional library is isolated and tested separately, thus, insoluble library members have no effect on other members. Lastly, a noteworthy question is how large a dynamic combinatorial library should be in order to efficiently identify potential substrates. Generally, the larger a

traditional combinatorial library is the better the chances of finding potential targets is, however, larger dynamic combinatorial libraries are not necessarily always better.[11] With an increased dynamic library size the probability of detecting every species present in the library is lowered, but the probability of detecting amplification is also increased due to the increased odds of the library containing a high-affinity binding molecule. While there are several disadvantages to using dynamic combinatorial chemistry in the investigation of potential drug targets or catalysts the concept remains a useful method for the rapid discovery of such molecules and has been applied in the investigation of several potential targets.

A dynamic combinatorial library was employed in the study of potential inhibitors of neuraminidase, a key enzyme in the propagation of the influenza virus.[12] Utilizing an amine scaffold, a dynamic library was created via the condensation with a number of diversified aldehydes under imidazole/HCl buffered conditions (Scheme 2).



Scheme 2. Dynamic combinatorial library development using an aldehyde condensation reaction.

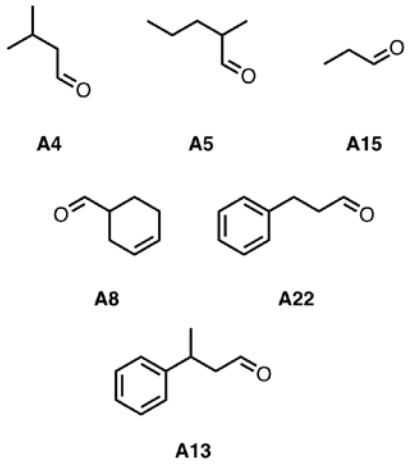


Figure 1. Selected sample library aldehydes.

While quantifying the diversity of a transient library is often difficult, HPLC-MS of a small library consisting of five (A4, A5, A8, A15, and A22) aldehydes (Figure 1) following reduction showed molecular ions representing the mono- and di-substituted derivatives; indicating that each of the proposed species was forming in the library (Figure 2).

Even as HPLC-MS of the small sample library showed the detection of the proposed species, analysis of the library upon exposure to a template (neuraminidase enzyme in this case) is often complex and difficult to quantify. In order to overcome this problem a "virtual library", or library where the individual components cannot be detected unless amplified by the presence of a template, was utilized. By having only trace amounts of the library members present only those substrates that bind to the template and are amplified as a result will be detected. Upon addition of the neuraminidase extracellular domain to the library (1:1 ratio in respect to the amine scaffold), a significant shift in the library composition was detected using HPLC-MS favoring condensation products with larger hydrophobic side-chains (A22, A13, and A8) (Figure 3) where A# represents the various aldehydes utilized). As the concentration of the library members was extremely low, only amplification of the condensation product with

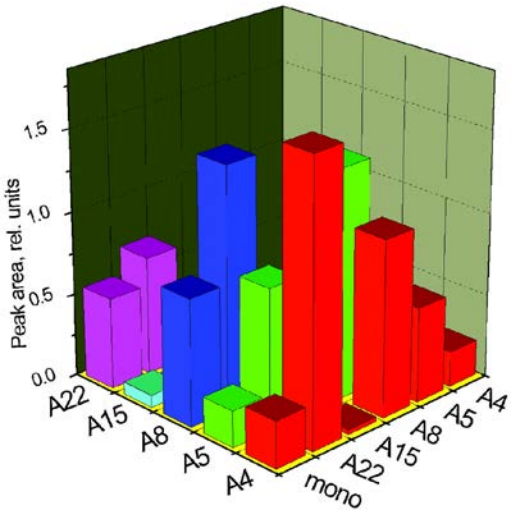
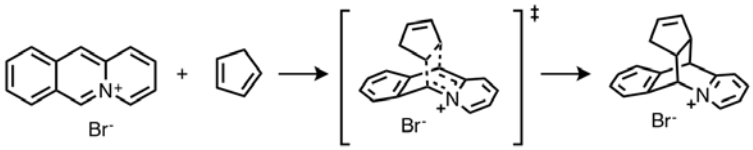


Figure 2. HPLC-MS analysis of sample library.[12] Copyright 2002 National Academy of Sciences, USA.

Figure 3. Observed library amplification.[12] Copyright 2002 National Academy of Sciences, USA.

aldehyde A22 could be quantified and was found to be amplified 120-fold in the presence of the neuraminidase enzyme (as compared to the absence of the neuraminidase). Since a "virtual library" was being utilized the detection of the condensation substrates strongly suggests that a binding interaction with the enzyme had occurred and led to amplification of the substrates. Synthesis and isolation of library hits **11**, **12**, and **13** (Figure 3) showed that **11** and **12** possessed considerably higher K_i values (2.16 and 1.64 μM, respectively) than the initial amine scaffold (31.3 μM) while **13** only showed a slightly lower K_i value (22.7 μM). The significantly better K_i values of library hits **11**and **12** further supports that the neuraminidase had bound to the



Scheme 3. Model Diels-Alder reaction.

substrates of the dynamic library and caused the subsequent amplification and detection.

In their efforts to identify a potential catalyst for the Diels-Alder reaction shown in Scheme 3, members of the Sanders group elected to explore a disulfide dynamic combinatorial library.[13] Exposure of the Diels-Alder product to a dynamic combinatorial library of disulfides resulted in a 6.5 fold amplification of macrocyclic structures **A** and **B** (Figure 4). After isolation of the macrocycles, the group found that while macrocycle **B** bound tightly to the starting material it provided no catalytic activity. However, **A** bound strongly to the product and

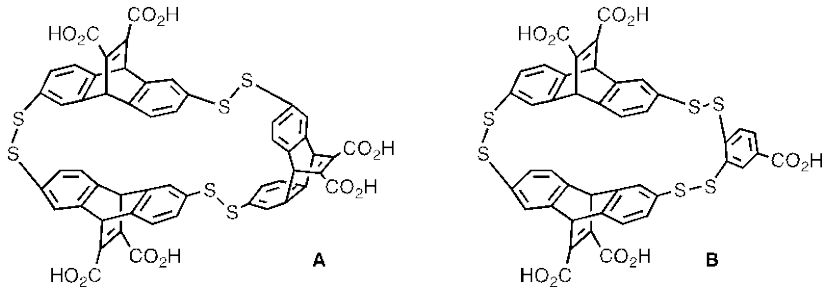
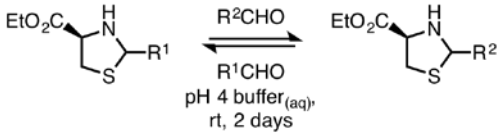


Figure 4. Macrocyclic catalysts from a dynamic combinatorial library.

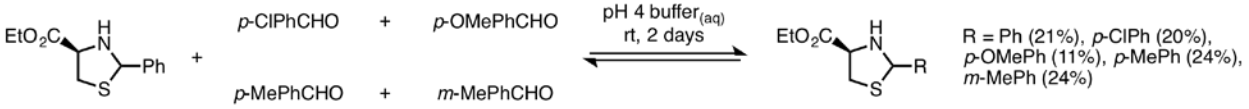
demonstrated a moderate increase in the rate of reaction. While the increase in reaction rate was modest, the potential use of a dynamic combinatorial library to identify novel catalytic systems was demonstrated. Others have gone on to employ similar methods to identify potential catalysts and have met with good success.[14-15] While there have been many examples of applying dynamic combinatorial chemistry to the discovery of potential targets, one of the main problems in the field is the lack of viable reversible reactions that may be utilized.



Scheme 4. Reversible thiazolidine exchange.

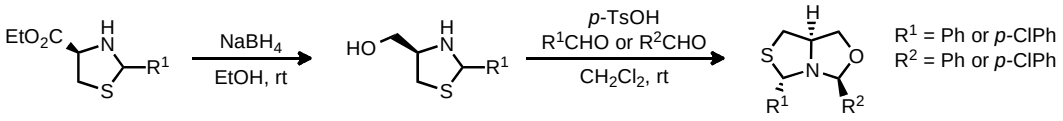
In an effort to increase the number of viable reversible reactions that may be utilized for the development of dynamic combinatorial libraries, the Wipf group designed a reversible thiazolidine exchange reaction (Scheme 4) that employs mild acetate-buffered conditions (pH 4,

rt).[16] The exchange reaction was then applied to the synthesis of a small "proof of concept" dynamic combinatorial library utilizing the thiazolidine scaffold under similarly buffered condtions. (Scheme 5).



Scheme 5. Thiazolidine dynamic library development.

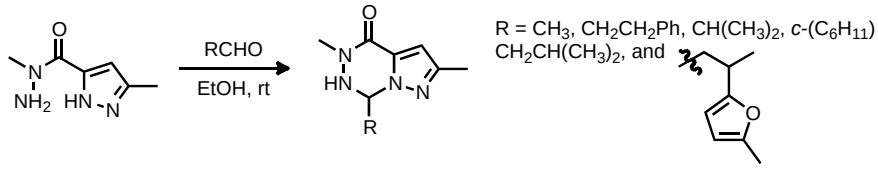
Additionally, the thiazolidine scaffold could be modified to provide a new oxazolidine substrate (Scheme 6) that could participate in a similar exchange reaction under acid-catalyzed



Scheme 6. Thiazolidine-oxazolidine synthesis and exchange reaction.

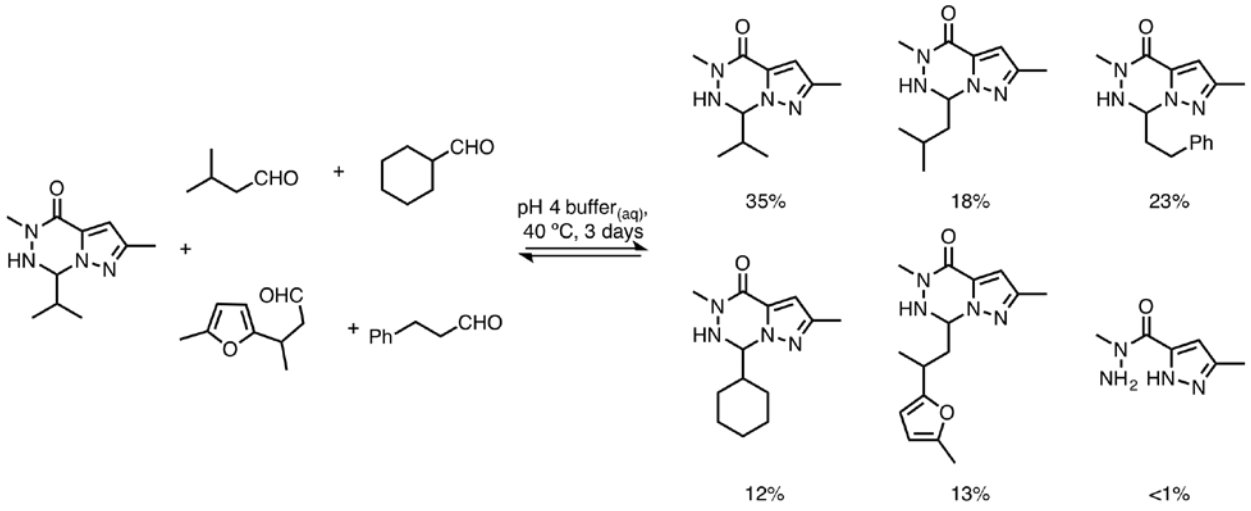
conditions, providing an additional thermodynamically controlled reaction that may be applied in the development of a dynamic combinatorial library.

In addition to developing a reversible thiazolidine reaction, the Wipf group has also utilized a pyrazolotriazinone scaffold in the generation of a small dynamic combinatorial library.[17] Condensing a hydrazide scaffold with a number of aldehydes afforded several new pyrazolotriazinone derivatives (Scheme 7).



Scheme 7. Hydrazide condensation and exchange chemistry.

Using conditions similar to those employed in the thiazolidine exchange reaction (pH 4 buffer, 40 °C), an efficient exchange reaction using the pyrazolotriazninone substrate was then developed. Exposure of the pyrazolotriazinone to the exchange conditions in the presence of several aldehydes resulted in the creation of a small dynamic combinatorial library (Scheme 8). Building on work previously completed in our group, we hoped to apply similar chemistry in the development of a dynamic combinatorial library using pyrrolo[1,3]-diazepines.

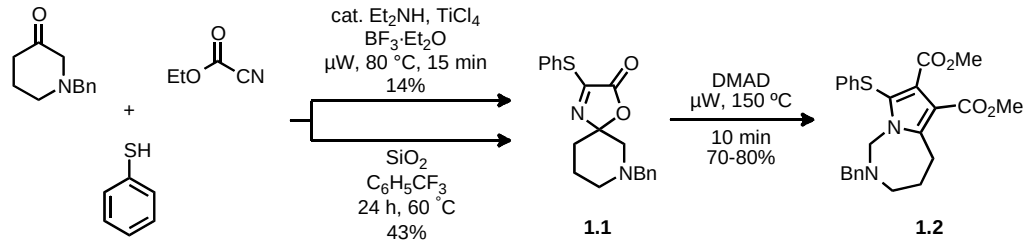


Scheme 8. Reversible exchange of the pyrazolotriazinone scaffold.

1.2 RESULTS AND DISCUSSION

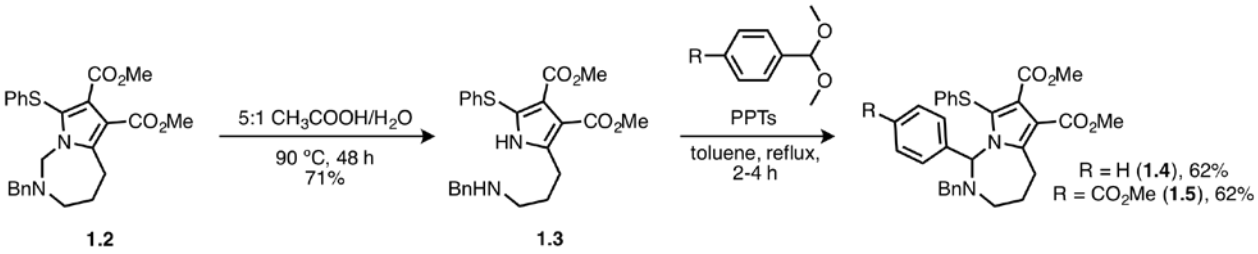
Our work began with the construction of the pyrrolo[1,3]-diazepine core using chemistry previously established in our group.[18-19] The intermediate oxazolinone **1.1** was synthesized in a "3-component, 1-pot reaction" using 1-benzyl-3-piperidone, ethyl cyanoformate, and thiophenol (Scheme 9). Initial conditions using catalytic $TiCl_4$ and BF_3-etherate were found to afford the desired oxazolinone albeit in low yield (14%), however, the use of silica gel was found to

moderately increase the yield (43%).[19] Heating **1.1** at 150 °C using microwave irradiation afforded diazepine **1.2** via a domino 1,3-dipolar cycloaddition and *retro*-Mannich cyclization in good yield.[18]



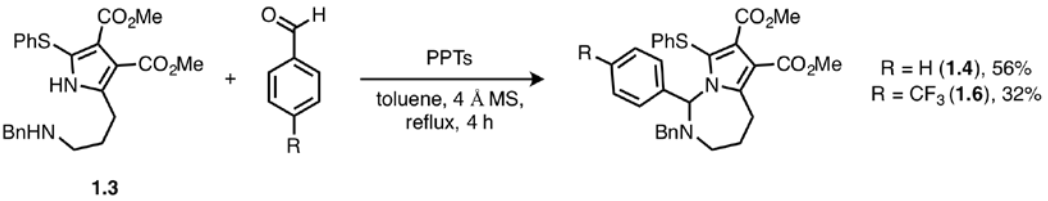
Scheme 9. Synthesis of the pyrrolo[1,3]-diazepine core.

Diazepine **1.2** was hydrolyzed using a mixture of acetic acid and water to afford amine **1.3** in a respectable yield (Scheme 10).[18] Utilizing previously developed conditions, amine **1.3** was condensed with two acetals under acidic conditions to provide functionalized diazepines **1.4**. and **1.5**.[20]



Scheme 10. Hydrolysis and acetal recyclization using the diazepine core.

Alternatively it was found that diazepines **1.4** and **1.6** could be synthesized using the corresponding aldehydes under comparable conditions although in slightly lower yields (Scheme 11) While the yields are modest, these initial results were suggested the possibility of developing the aldehyde "exchange reaction" into a dynamic combinatorial library. However, before developing our exchange chemistry we sought to functionalize the diazepine scaffold.



Scheme 11. Aldehyde condensation using the diazepine core.

In an effort to alter the electronics and sterics of the diazepine core, we sought to remove the thiophenol fragment and reduce the remaining pyrrole esters. Initial attempts to remove the thiophenol functionality using Raney-Ni afforded only trace amounts of the desired product (Table 1, Entry 1). The use of increased amounts of Raney-Ni (Table 1, Entry 2) provided **1.7** in 20% yield. Changing the solvent from EtOH to THF improved the yield considerably (69%), (Table 1, Entry 3) . Lowering the reaction temperature to 0 °C was found to increase the yield of **1.7** to 92% (Table 1, Entries 4-5). With the desulfurized material in hand we next investigated the ester reduction.

Table 1. Desulfurization of the Diazepine Core

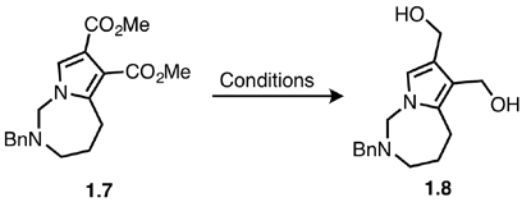
1.2 1.7

Entry	Condition	Time (h)	Result
1	Raney-Ni (11 equiv.), EtOH, 60 °C	2	trace pdt. observed
2	Raney-Ni (96 equiv.), EtOH, 60 °C	2.5	20%
3	Raney-Ni (97 equiv.), THF, rt	3	69%
4	Raney-Ni (129 equiv.), THF, 0 °C	6	85%
5	Raney-Ni (150 equiv.), THF, 0 °C	4	92%

We initially found that the use of *t*Bu_2AlH at low temperature provided the desired diol in moderate yield (44%) while $LiBH_4$ was not reactive (Table 2, Entries 1-2). Fortunately, the use

of excess Red-Al afforded diol **1.8** quantitatively (Scheme 12). Furthermore, diazepine **1.2** could be reduced under the same conditions to afford diol **1.9 (**Scheme 12**)**.

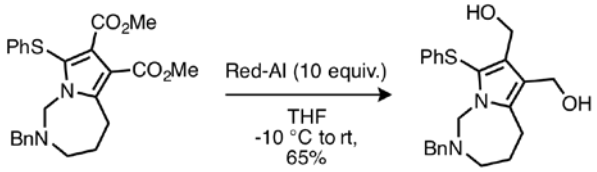
Table 2. Pyrrole Ester Reduction



1.7 1.8

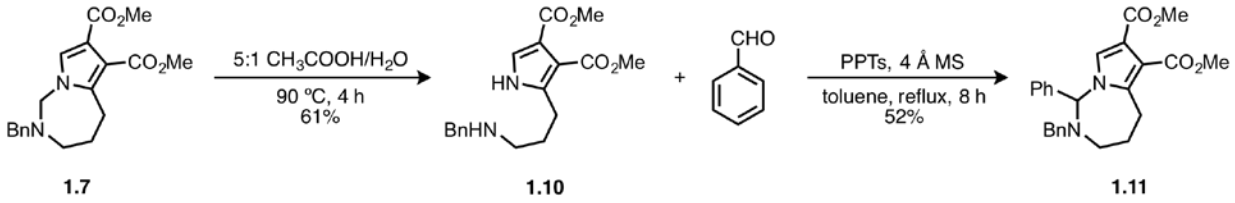
Entry	Condition	Time (h)	Result
1	tBu_2AlH (1.2 equiv.) CH_2Cl_2, 0 °C	2	44%
2	$LiBH_4$ (2 equiv.), THF, 0 °C	2	NR
3	Red-Al (10 equiv.), THF -10 °C to rt	24	>99%

We then investigated the propensity of the reduced diazepine derivatives toward hydrolysis and cyclization using conditions similar to those previously employed. Diazepine **1.7** was hydrolyzed more rapidly than the parent diazepine (**1.2**) and afforded the desired amine **1.10** in a respectable yield (61%) (Scheme 13). The resulting amine was condensed with benzaldehyde and supplied the diazepine **1.11** in moderate yield. Removal of the thiophenol portion appears to increase the reactivity of the amine species towards condensation and may be the result of decreased steric strain.



1.2 1.9

Scheme 12. Reduction of diazepine **1.2**.



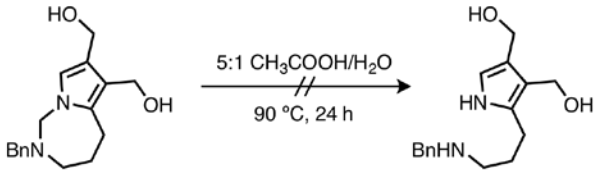
1.7 1.10 1.11

Scheme 13. Hydrolysis and recyclization of diazepine **1.7**.

Looking to further investigate the application of the hydrolysis and aldehyde condensation reaction, we attempted the hydrolysis of diol **1.8** (Scheme 14). Whereas desulfurized diazepine **1.7** was efficiently hydrolyzed, diol **1.8** was found to decompose under

the hydrolysis conditions and amine **1.11** was not observed. The increase of pyrrole electron density following ester reduction may lead to increased rates of undesired oxidative side-products and results in the decomposition of the material.[21]

Having demonstrated the ability to hydrolyze and condense amine intermediates **1.3** and

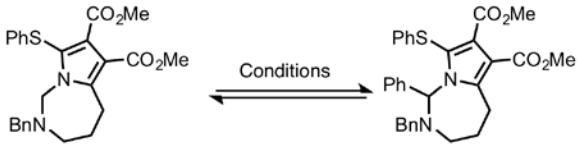


1.8 **1.12**

Scheme 14. Hydrolysis of diol **1.8**.

1.9 with several aldehydes we next sought to develop conditions that would be suitable for a reversible, thermodynamically-controlled, aldehyde "dynamic exchange reaction" that could be used in the creation of a dynamic combinatorial library (Table 3). Initially, we hoped to utilize mild, buffered conditions, unfortunately, after investigating several different buffered systems

Table 3. Dynamic Exchange of Pyrrolo[1,3]-Diazepines



1.2 **1.4**

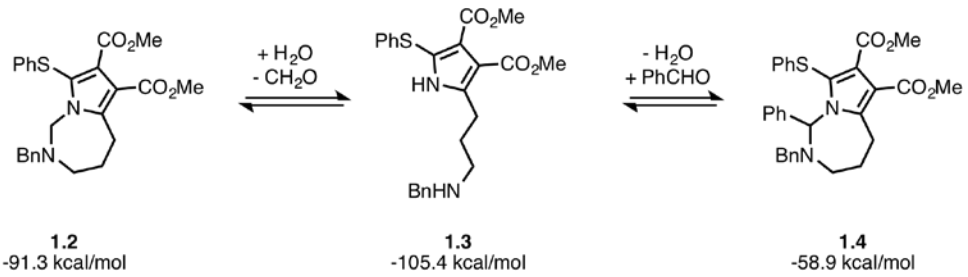
Entry	Condition	Time	Result	Entry	Condition	Time	Result
1	0.1 M phosphate-citrate buffer, pH 4, rt & 40 °C	48-72 h	no reactivity	8	Amberlite, MeOH, rt & 40 °C	24-48 h	no reactivity
2	0.1 M acetate buffer, pH 4, rt & 40 °C	48-72 h	no reactivity	9	SiO_2, CH_2Cl_2, rt	24-48 h	no reactivity
3	0.1 M acetate buffer/methanol (7:3), pH 4, rt	24-48 h	some hydrolysis, no product obs.	10	5:1 AcOH/H_2O, rt	24-48 h	hydrolysis, no product obs.
4	PPTs (0.5 equiv.), CH_2Cl_2, rt	24-48 h	no reactivity	11	3:1 methanol/1M $HCl_{(aq)}$, rt & 40 °C	24-48 h	hydrolysis, no product obs.
5	TsOH (0.5 equiv.), CH_2Cl_2, rt	24-48 h	no reactivity	12	PPTs (1.2 equiv.) benzaldehyde (5 equiv.) 0.1 M, toluene, 80 °C	67 h	hydrolysis and desired product observed
6	TsOH (5 equiv.), CH_2Cl_2, rt	24-48 h	some hydrolysis, no product obs.				
7	CF_3SO_3H (5 equiv.), CH_2Cl_2, rt	24-48 h	some hydrolysis, no product obs.				

* All reactions used benzaldehyde (1 mM, 1 equiv.) and diazepine (1 mM, 1 equiv.) except entry 12

(Table 3, Entries 1-3) we found that more vigorous conditions would be necessary. We next pursued the use of several different strong acids (Table 3, Entries 4-7).

While the starting material was found to hydrolyze under these conditions, the recyclized product was not observed. Conditions similar to those initially used for the hydrolysis of the diazepine core (Table 3, Entries 10-11) provided efficient hydrolysis, however, formation of diazepine **1.4** was not observed. Utilizing the same conditions previously used for the cyclization of the hydrolyzed amine intermediate (Table 3, Entry 12) provided the amine intermediate with a trace of the desired product.

In order to better understand the thermodynamics of our proposed dynamic exchange process, the heat of formation of each reaction component was calculated using Scigress (Scheme 15).[22] The hydrolysis of **1.2** to **1.3** was found to require 6.0 kcal/mol while the recyclization of **1.3** to **1.4** would require 3.8 kcal/mol (the heat of formation values for H_2O, CH_2O, and PhCHO were -58.1, -38.4, and -15.4 kcal/mol, respectively). The overall exchange process (**1.2** to **1.4**) should then require an overall 9.8 kcal/mol; indicating that the each individual step is endothermic as is the overall exchange reaction. While heat of formation calculations suggest that both the hydrolysis and recyclization steps are endothermic and that the hydrolysis is in fact less thermodynamically favorable our experimental data indicates that the hydrolysis is likely more facile than the recyclization. One possible rationalization is that the heat of formation calculation does not take into account the entropic effects that the hydrolysis



1.2
-91.3 kcal/mol

1.3
-105.4 kcal/mol

1.4
-58.9 kcal/mol

Scheme 15. Heat of formation comparison.

and recyclization of the diazepine ring entails. Hydrolysis of the ring system should result in an increase in the degrees of rotational freedom of the system and thus be entropically favorable whereas recyclization of **1.3** would likely be entropically unfavorable.

1.3 CONCLUSIONS

In our efforts toward the development of a dynamic combinatorial library using pyrrolo[1,3]-diazepines, we have shown the potential to hydrolyze and recyclize the diazepine core via the condensation of several different aldehydes to afford novel diazepine derivatives. Additionally, we have been able to modulate the electronics and sterics of the core through the removal of the thiophenol and reduction of the pyrrole esters. Our work towards finding suitable conditions for a thermodynamically controlled dynamic exchange reaction have shown that while hydrolysis of the diazepine core is possible, recyclization of the resulting amine intermediate appears to be unfavored.

2.0 DEVELOPMENT OF NOVEL POXVIRUS RESOLVASE INHIBITORS

2.1 INTRODUCTION

2.1.1 Poxvirus: A Bioterrorism Threat?

The poxvirus is estimated to be responsible for 300-500 million deaths during the 19th century alone, killing more people than every war combined.[23-24] The disease typically presents with high fever, muscle pain, and malaise, eventually leading to a maculopapular rash resulting in raised, fluid-filled blisters.[25] Historically, the virus has had a 30% fatality rate although the values vary depending on the subtype of the disease.[25] The poxvirus, or variola virus, exists in two major forms with the most common and deadly being the variola major. Fortunately, the virus was officially eradicated in 1980 due in most part to the creation of the poxvirus vaccine. Despite this proclamation the employment of the virus as a biochemical weapon has remained a significant risk.

One of the earliest examples of biochemical warfare using the poxvirus occurred during the siege of Fort Pitt in 1763 when contaminated blankets were used to infect the fort's occupants.[26] The first serious threat however did not occur until 1947 when the then Soviet

Union established a small chemical weapons factory designed to weaponize the virus.[27] In 1992, reports surfaced that the Soviet Union had successfully weaponized a "large-stockpile" of the virus posing a significant bioterrorism threat.[28] Russia has reportedly destroyed all but a few remaining samples of the virus, which are used strictly for research purposes. Subsequently, several reports have surfaced of the poxvirus being weaponized, however, there has been no evidence validating those stories.

The CDC has declared the poxvirus a "Category A" bioterrorism threat for the following reasons: "it can be easily spread or transmitted from person to person, it results in high death rates and has the potential for major public health impact, it may cause public panic and social disruption, and it requires special action for public health preparedness".[29] While the remaining sources of the virus are securely stored in laboratories found in the United States and Russia, the possibility of a terrorist attack using the poxvirus remains a grave threat.[24] As an increasing percentage of today's population has not been vaccinated against the virus, an attack could be devastating and has become a grim concern following recent terroristic attacks.

Although a poxvirus vaccine is available, the incubation period of the virus is 9-17 days whereas the vaccine typically must be administrated within 4 days of innoculation in order to be effective.[24] Additionally, the overall effectiveness of the vaccine has often been brought into question and implores that an alternative solution be found. After the terroristic attacks in 2001 the U.S. initiated an effort to generate large stockpiles of drugs and vaccines that could be utilized in response to a biochemical attack. Until very recently there has not been a suitable drug for the treatment of the poxvirus.[24] Fortunately, Siga Technologies® has begun the production of ST-246®, the first

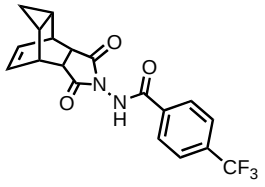


Figure 5. ST-246 (Tecovirimat).

small molecule antiviral found to effectively inhibit the poxvirus via the disruption of viral packaging (Figure 5).[30] While the compound has yet to obtain FDA consent, officials expect the drug to receive approval within the next 5 years and become commercially available shortly thereafter. Even as the production of ST-246 has started, viral mutation and resistance necessitates the availability of multiple alternative treatments.[31]

2.1.2 Poxvirus Activity and Possible Therapeutic Targets

Efforts to find an effective inhibitor of the poxvirus have led to the discovery of several potential therapeutic targets, many of which focus on the replication of viral DNA. One consideration while evaluating potential therapeutic targets is that poxvirus DNA replication occurs entirely within the host cell and many of the required replication proteins are encoded within the viral genome.[32]

One of the recent targets of interest has been the vaccinia virus processivity factor (PF) complex consisting of the E9 polymerase[33-34], A20 processivity factor[35], and D4 uracil DNA glycosylase.[36-38] The vaccinia virus, which was used to create the poxvirus vaccine, is an ideal surrogate for the variola virus due to its high sequence homology as well as improved experimental properties (i.e. solubility).[37] Although, the exact roles of A20 and D4 have not yet been determined, both appear to be necessary for efficient DNA replication and have been evaluated as potential anti-viral targets.[36-38]

A high-throughput screening campaign of 28,000 compounds afforded 5 compounds (Figure 6) that demonstrated reduced viral plaque growth with low cytotoxicity.[37] These 5 hits all showed D4 binding but only 3 showed significant reduction of viral DNA replication, indicating that while all of the compounds affected viral growth only 3 did so via the inhibition of DNA

replication. Molecular docking to the D4 glycosylase subsequently showed that all 5 substrates interacted with amino acids K126, K160, and R187, which were previously shown to be critical for D4 activity.[36] Protein mutations often create resistant viral strains that counteract the effect of anti-viral treatments and have lead to the use of "cocktails" or combinations of multiple anti-viral drugs in order to combat the infection. Thus, having a variety of potential anti-viral drugs provides alternative treatments for patients with resistant viral strains. However, an alternative method that may avoid viral mutations is the inhibition of the host cell enzymes that are necessary for viral replication.

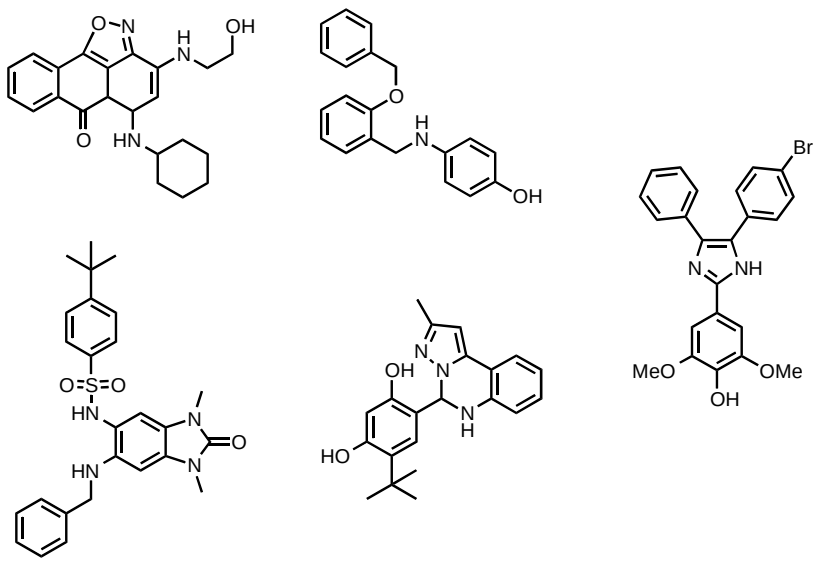


Figure 6. D4 glycosylase inhibitors.

A new series of potential targets has been the Abl-family of tyrosine kinases found within viral host cells.[39-40] In 2005, STI-571 (Gleevac), a known Abl-family tyrosine kinase inhibitor normally used in the treatment of chronic myelogenous leukemias, was found to drastically reduce viral levels (Figure 7).[40] The Src- and Abl-family kinases were found to be essential for actin motility and release of extracellular enveloped virus (EEV). In this approach, inhibition of the host machinery (Abl-kinase) using STI-571 blocked viral replication. This method is interesting in

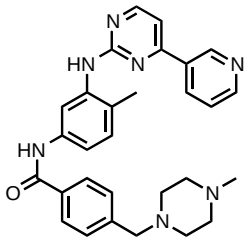


Figure 7. Tyrosine kinase inhibitor Gleevac.

that disrupting the host proteins necessary for the viral life cycle bypasses the possibility of viral mutations that may negate the effects of anti-viral medication while hopefully not affecting the host cell itself.[40] However, a significant concern with this type of approach is the possible cytotoxicity the drugs may possess. In addition to the use of STI-571 in the inhibition of EEV release, the promising anti-viral ST-246 appears to also target viral packaging.

ST-246 (Tecovirimat) was initially discovered as part of a high-throughput screening effort initiated in 2002.[41] A series of tricyclononene carboxamides were discovered within the group of 759 hits from 356,240 compounds. SAR studies of the scaffold resulted in the synthesis of ST-246 which possessed an EC_{50} of 0.04 μM.[41] Genetic resistance mapping revealed the vaccinia F13L gene, which participates in the wrapping of intracellular mature virus, to encode the target of ST-246. Compound ST-246 is believed to block the interaction of p37, a protein encoded by F13L, with Rab9 and TIP47.[41] Rab9, TIP47, and p37 form a wrapping complex necessary for the packaging of intracellular mature virus particles. While ST-246 still seeks FDA approval, other anti-viral agents are still desired.

An additional target that has been gaining more attention over the last 5 years has been the poxvirus resolvase. The resolvase enzyme belongs to the RNase H superfamily of enzymes, which also includes HIV-I integrase.[42-43] The resolvase enzyme has been recognized as a critical enzyme required for viral DNA replication.[44] The poxvirus replicates via a "rolling hairpin" mechanism resulting in a linear concatemer genome (Figure 8).[45-46] Inverted repeat sequences in the linear genome form cruciform structures known as Holliday junctions.[47] Holliday junctions are formed during the crossover and recombination of two double-stranded DNA molecules. The junctions are known to occur in many different bacteria and viruses and play a critical role in genetic replication.[48] The resolvase enzyme is highly selective for Holliday junctions and cleaves

the cruciform structures into monomeric DNA fragments. Ligation of the resulting fragments affords the characteristic DNA hairpins associated with the poxviruses. Previous work has shown that the inactivation of the resolvase enzyme results in the accumulation of uncleaved DNA and arrest of viral DNA synthesis; indicating that the resolvase is critical for viral DNA replication *in*

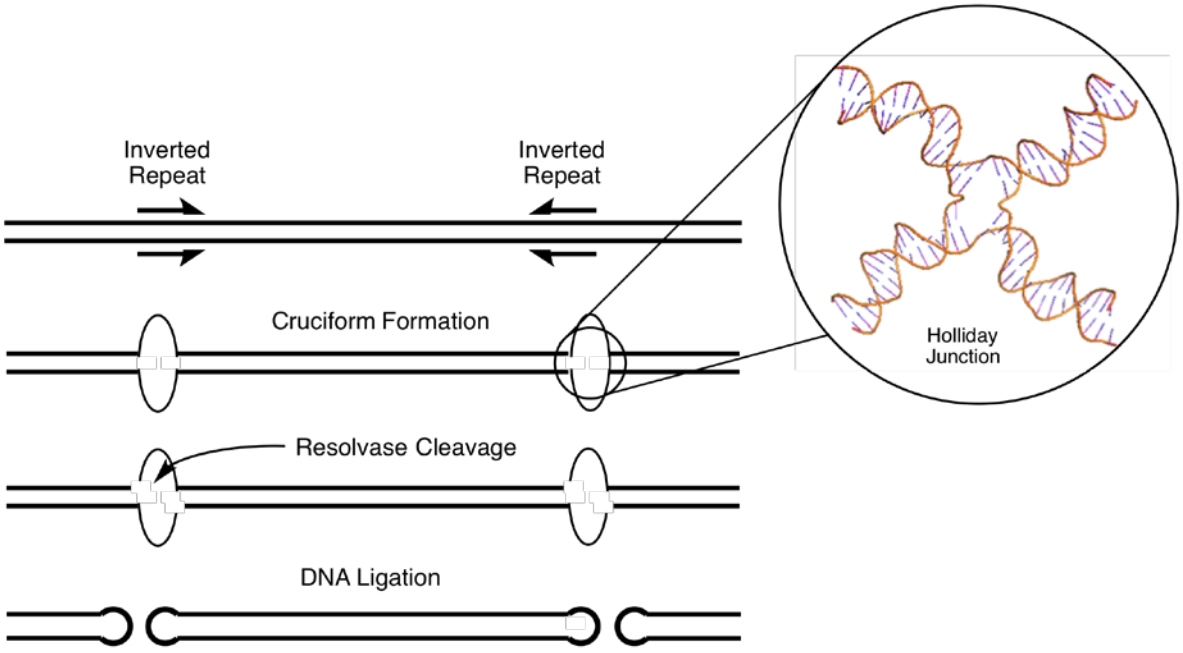


Figure 8. Cleavage of the Holliday junction by the poxvirus.

vivo.[44] Thus, inhibition of the poxvirus resolvase has been shown to prevent viral DNA replication and provides an intriguing therapeutic target.

While the resolvase enzyme has been recognized as a potential therapeutic target much is still unknown about its exact structure and mechanism. Fortunately, HIV-I integrase, which also belongs to the RNase H superfamily of enzymes, shares a great deal of homology with the poxvirus resolvase and has been studied more thoroughly. As a result, many of the potential inhibitors and strategies applied to the HIV integrase are also applicable to the resolvase enzyme.

The active site of the HIV-I integrase requires divalent metals, usually Mg^{2+} or Mn^{2+}, for activity.[49] The integrase activity is controlled by a set of essential acidic amino acids known as

the DDE motif (D64, D116, and E152) that chelate these metals.[49] The DDE motif is highly conserved over the RNase H superfamily and very similar motifs are found in the poxvirus resolvase. Studies have also demonstrated the presence of divalent metals (Mg^{2+}) in the enzymatic active site of the fowlpox resolvase.[43, 50] The metals are surrounded by 5 key acidic amino acids: D7, E60, K102, D132, and D135, resembling the DDE motif found in the HIV integrase.[42] Removal of these amino acid residues or the metals themselves resulted in the elimination of enzymatic activity, emphasizing the necessity of the DDE scaffold and active site metals.

Many of the current HIV-I integrase and RNase H inhibitor designs have focused on the ability to chelate the active site metals (Mg^{2+}) that are required for enzymatic activity. A common motif of 3 aligned heteroatoms (highlighted in red) is often observed in many of the potential metal-chelating inhibitors (Figure 9) as activity has been found to correlate to the ability of the inhibitor to bind to two Mg^{2+} atoms separated by 3.6-4.0 Å.[49] Some of the most

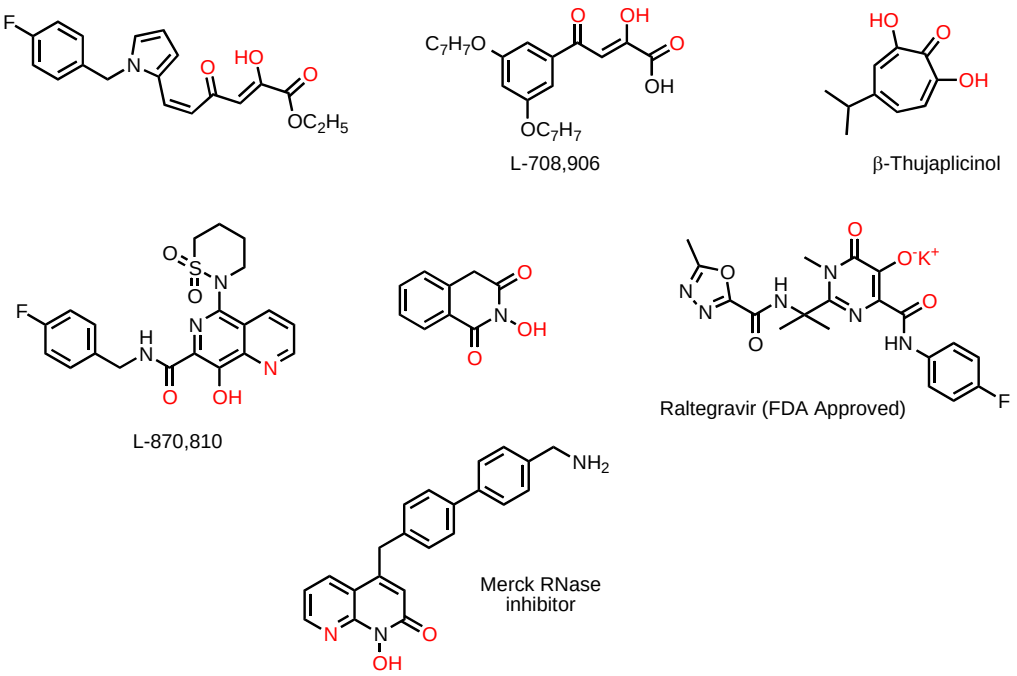


Figure 9. Current integrase inhibitors.

relevant scaffolds have been the di-keto acid (L-708,906-integrase and RNase H inhibitor)[51-52], 8-hydroxyquinoline-7-carboxamide (L-870,810-integrase inhibitor)[53], 6-hydroxy-5-oxopyrimidinecarboxamide (Raltegravir-integrase inhibitor)[54], and 2-hydroxyisoquinoline-1,3(*2H,4H*)-dione moieties (RNase H inhibitor).[55] The di-keto acid moiety has been repeatedly shown to exhibit activity against the HIV integrase enzyme and presents an interesting structure to incorporate into future work.[49, 56-58]

Several di-keto acid containing scaffolds demonstrate HIV-integrase *in vitro* and *in vivo* inhibition (IC_{50} 50-400 nm) through this active site metal-chelation mechanism (Figure 10). Specifically, the inhibitors are thought to interrupt the strand transfer process of HIV-I integrase and as a result inhibit viral DNA replication.[49] However, removal or modification of the di-keto acid scaffold significantly decreases inhibition (IC_{50} >100,000 nm) of the strand transfer process (Figure 11).

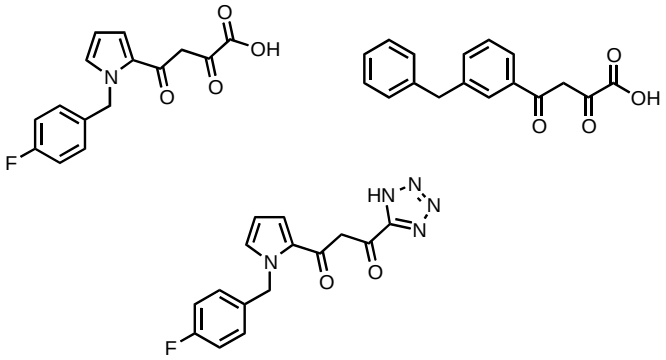


Figure 10. Di-keto acid integrase inhibitors.

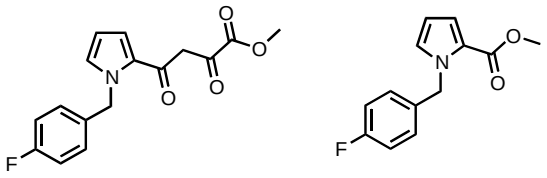


Figure 11. Removal of di-keto acid scaffold results in loss of

Related work has supported the hypothesis that di-keto acid inhibitors coordinate with the Mg^{2+} found in the integrase active site.[59] Figure 12 displays the modeled binding interactions of

the known HIV-I integrase inhibitors L-731,988 (A), L-708,906 (B), and S-1360 (C).[49, 60] Importantly, the di-keto acid moiety is found to chelate the active site Mg^{2+} (highlighted by the red arrow) in each example. Similar interactions between the di-keto acid scaffold and metals found in the active site of the poxvirus resolvase are expected due to the structural similarities

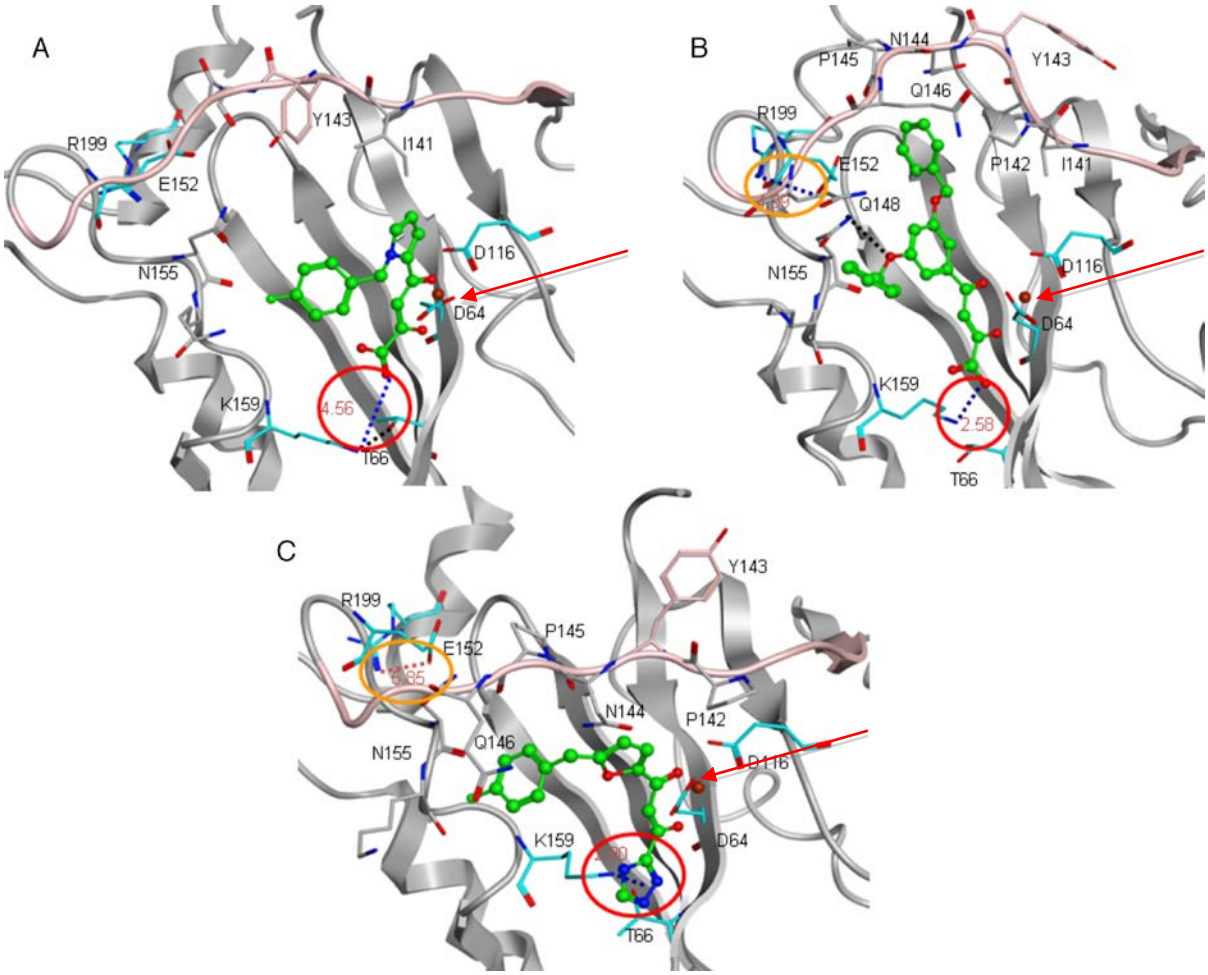


Figure 12. Binding interactions of L-731,988 (A), L-708,906 (B), and S-1360 (C).[58] Reproduced with permission from Elsevier

between the integrase and resolvase, lending to the idea that proposed HIV-I integrase inhibitors and strategies should be applicable to the resolvase enzyme.

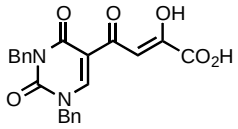


Figure 14. Nucleobase HIV integrase inhibitor.

The di-keto acid moiety has also been incorporated into other potential HIV integrase inhibitors. Efforts toward the discovery of new di-keto acid inhibitors resulted in the discovery of the novel nucleobase di-keto acid shown in Figure 14.[47] The acid was found to possess an IC_{50}

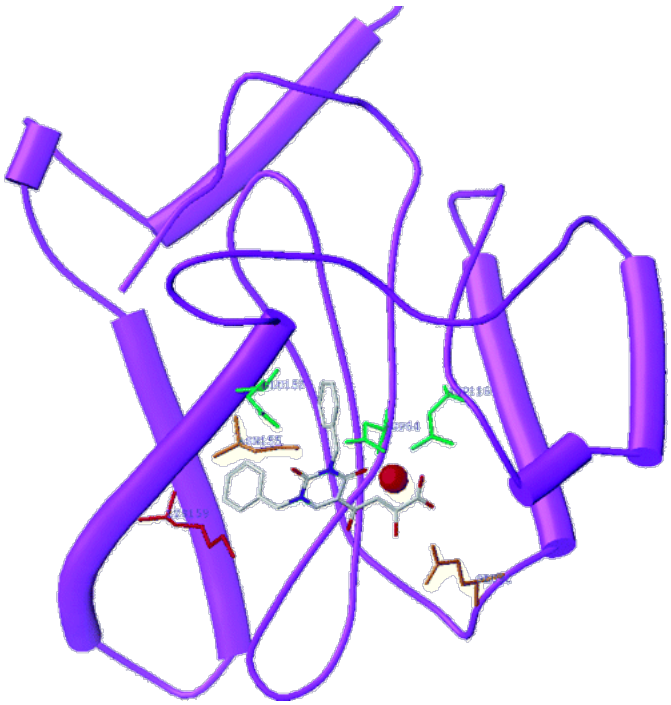


Figure 13. Molecular docking of nucleobase diketoacid to HIV-1 integrase.[46]

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value of 50 nM *in vivo* while FDA-approved HIV reverse transcriptase inhibitor AZT (Retrovir) demonstrated an IC_{50} value of 0.14 nM in similar control assays. Molecular docking of the nucleobase inhibitor to HIV-I integrase (Figure 13) strongly suggests that the uracil amide carbonyl (4-position) participates with the di-keto acid functionality in the chelation of the Mg^{2+} found in the enzyme active site. We elected to use the nucleobase di-keto acid scaffold as a starting point for the development of similar di-keto acid substrates utilizing a pyrimidinone core.

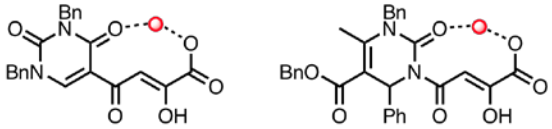


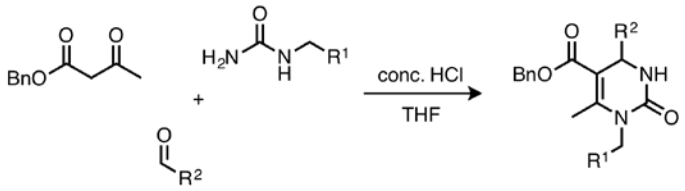
Figure 15. Proposed mode of chelation.

Focusing on the previously disclosed nucleobase scaffold we propose the incorporation of the di-keto acid and other known chelating functionalities into a pyrimidinone-based scaffold (Figure 15). Based on the predicted overlap between the known nucleobase inhibitor and our proposed di-keto acid, we anticipate the possible participation of the pyrimidinone carbonyl in the chelation of the active site metal; similar to the uracil binding mechanism proposed in the docking simlulation of the nucleobase scaffold (Figure 15).

2.2 RESULTS AND DISCUSSION

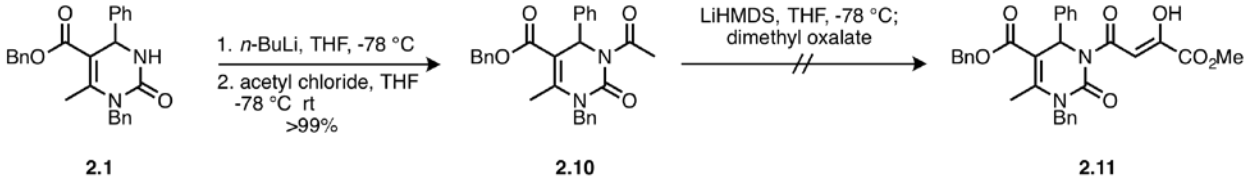
Our work began with the construction of the pyrimidinone core utilizing the Biginelli reaction. This multi-component process provided pyrimidinones **2.1**-**2.9** in good to high yields (Table 4).[61-62] Electronic and stereochemical diversity was provided via the incorporation of several different aldehydes while additional diversity was also introduced using differently substituted ureas. This approach not only provides the desired substituted heterocycles in high yield, but also incorporates opportunities for further manipulation and diversification.

Table 4. Pyrimidinone Synthesis via the Biginelli Reaction



	R^1	R^2	Yield (%)
2.1	C_6H_5	C_6H_5	70
2.2	C_6H_5	4-ClC_6H_4	88
2.3	C_6H_5	4-$CF_3C_6H_4$	81
2.4	C_6H_5	4-MeC_6H_4	73
2.5	C_6H_5	4-$OMeC_6H_4$	57
2.6	C_6H_5	naphthyl	77
2.7	4-$OMeC_6H_4$	C_6H_5	87
2.8	4-$OMeC_6H_4$	4-ClC_6H_4	85
2.9	4-$OMeC_6H_4$	4-MeC_6H_4	79

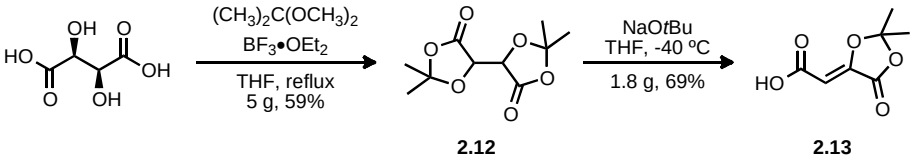
With the pyrimidinone core in hand, we attempted to install the α,γ-diketoacid chelating moiety (Scheme 16). Acetylation of **2.1** at the *N3*-position went smoothly and quantitatively afforded pyrimidinone **2.10**.[63] Treatment of **2.10** with LiHMDS and dimethyl oxalic acid did not afford the desired α-hydroxyester.[56, 64] At this point, we elected a different method to install the chelating moiety using acetonide **2.13**.



2.1 **2.10** **2.11**

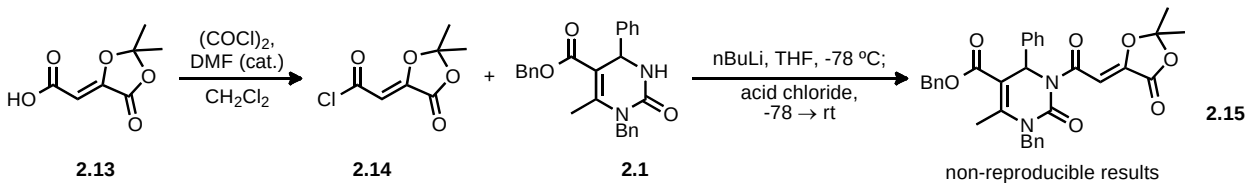
Scheme 16. Introduction of α,γ-diketoacid moiety.

The synthesis of acetonide **2.13** began with the acetalization of L-tartaric acid in the presence of 2,2-dimethoxypropane and catalytic BF_3•OEt_2 (Scheme 17).[57] Preparation of the carboxylic acid was completed using sodium *tert*-butoxide in THF to afford the desired compound in good yield (69%). With this acetonide-coupling fragment in hand we next sought methods to combine the acetonide with the pyrimidinone scaffolds.



Scheme 17. Synthesis of acetonide fragment **2.13**

Our initial attempts focused on the conversion to acid chloride **2.14** using oxalyl chloride and catalytic DMF (Scheme 18). Crude acid chloride **2.14** was directly used in the following coupling step without further purification.[57-58] Treatment of pyrimidinone **2.1** with *n*BuLi at low temperature followed by the crude acid chloride led to mixed results with only trace amounts of the desired product **2.15** and un-reacted starting material. However, the incorporation of catalytic pyridine and elongated reaction times led to a mixture of starting material, coupled product, and



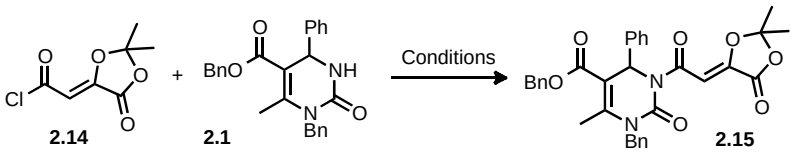
Scheme 18. Coupling with acid chloride **2.14.**

a dimeric species resulting from the nucleophilic opening of the acetonide system. Using CH_2Cl_2 as the solvent (Table 5, Entry 3) gave a 1:1 mixture of starting material and the desired product, as determined by LC-HRMS. Surprisingly, the use of DMAP and Et_3N in place of pyridine and

*n*BuLi also led to a 1:1 mixture of starting material and the desired coupled product (Table 5, Entry 4).

As a result of the amide formation in the absence of strong base, a coupling strategy using the carboxylic acid was investigated. Acetonide **2.13** has been shown to participate in "amino acid-like couplings" with simplified substrates.[57-58] This coupling most often employs the BOP reagent, but we opted to use T3P as the reagent has demonstrated high yields in a number of difficult amino acid-like couplings.[58, 65-66] While others have reported coupling reactions using acetonide **2.13**, to the best of our knowledge no one has reported using a substrate as complex as our pyrimidinone scaffold.[58]

Table 5. Optimized Acid Chloride Coupling

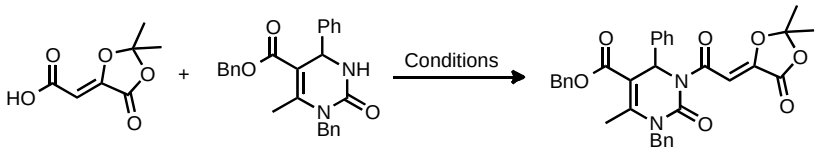


2.14 **2.1** **2.15**

Entry	Conditions	Result*
1	*n*BuLi (1.1 equiv.), THF, 1 h, -78 ºC	trace pdt.
2	*n*BuLi (1.1 equiv.), pyridine (0.2 equiv.), THF, -78 ºC- rt, overnight	2:2:1 sm:pdt:dimer
3	*n*BuLi (1.1 equiv.), pyridine (0.2 equiv.), CH_2Cl_2, -78ºC - rt, overnight	1:1 sm:pdt
4	DMAP (0.2 equiv.), Et_3N (5.0 equiv.), CH_2Cl_2, -78 ºC - rt, overnight	1:1 sm:pdt

* Ratios determined by LC-HRMS

Table 6. Acetonide Coupling Using T3P



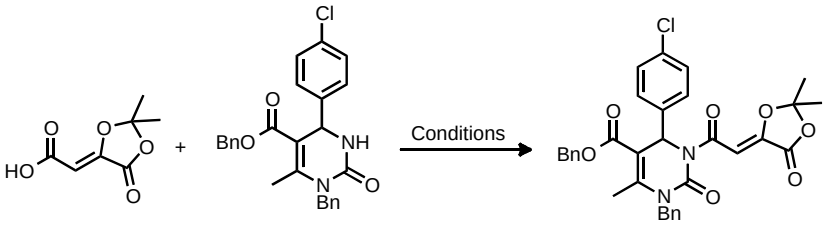
2.13 **2.1** **2.15**

Entry	Conditions	Result*
1	T3P (1.2 equiv.), acid (1.0 equiv.), DMAP (0.2 equiv.), Et_3N (5.0 equiv.), CH_2Cl_2, rt, 72 h	1:1 pdt:sm
2	T3P (2.2 equiv.), acid (2.0 equiv.), DMAP (0.4 equiv.), Et_3N (5.0 equiv.), CH_2Cl_2, rt, 48 h	82:18 pdt:sm 24% isolated
3	T3P (2.2 equiv.), acid (2.0 equiv.), DMAP (0.4 equiv.), Et_3N (5.0 equiv.), CH_2Cl_2, rt, 72 h	3:1 pdt:sm 38% isolated

* Ratios determined by LC-HRMS

Much to our delight, application of the typical amino acid coupling conditions using T3P, DMAP, and Et_3N gave a 1:1 mixture of the desired acetylation product and un-reacted pyrimidinone (Table 6, Entry 1). Increasing the amount of T3P, DMAP, and carboxylic acid drastically increased the overall conversion (Table 6, Entry 2). Elongated reaction time (72 h) did not have a significant effect on the reaction (Table 6, Entry 3). Although high reaction conversions were observed, only modest isolated yields were obtained due to difficult chromatographic purifications.

Table 7. Optimized T3P Coupling

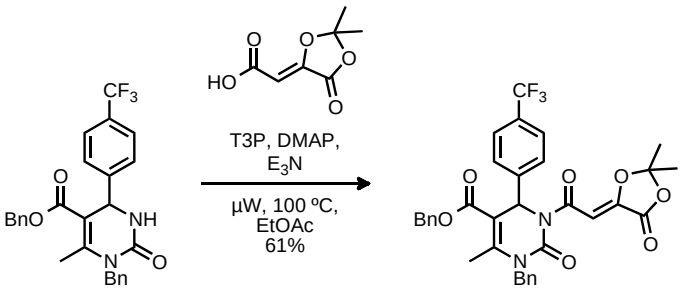


2.13 **2.2** **2.16**

Entry	Conditions	Result**
1	CH_2Cl_2, rt, 22 h	86:14 pdt:sm
2	EtOAc, μW, 100 ºC, 1 h	9:1 pdt:sm 40% isolated
3	DMF, μW, 100 ºC, 1 h	88:12 pdt:sm

* all reactions performed using T3P (2.2 equiv.), acid (2.0 equiv.), DMAP (0.4 equiv.), and Et_3N (5.0 equiv.)
** Ratios determined by LC-HRMS

In order to reduce the reaction time while also improving the overall reaction conversion, we investigated the use of microwave irradiation conditions (Table 7). An 86% conversion was obtained when the reaction was performed in CH_2Cl_2 at room temperature for 22 h using pyrimidinone **2.2** (Table 7, Entry 1). However, subjection of the reaction mixture to microwave irradiation at 100 °C in ethyl acetate for 1 h afforded the acetylation product **2.16** in 90% conversion (40% isolated yield) and similar results were observed when



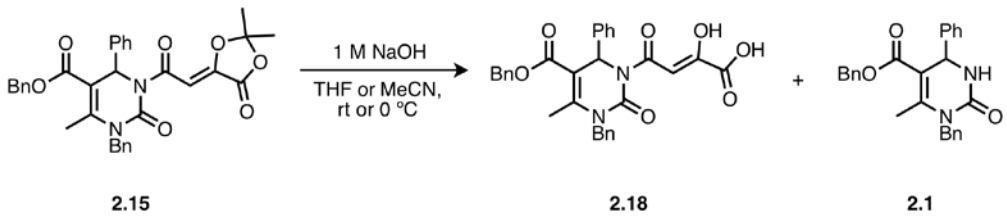
2.3 **2.17**

Scheme 19. Synthesis of acetonide **2.17.**

using DMF as the solvent (Table 7, Entries 2-3). Using the optimized conditions (Table 7, Entry 2), pyrimidinone **2.3** was used in the synthesis of acetonide **2.17** in 61% yield (Scheme 19).

With the masked α,γ-diketoacid moiety in hand, we sought to saponify the acetonide to reveal the chelating portion of the molecule (Scheme 20). Exposure of acetonide **2.15** to saponification conditions (1 M NaOH, THF) that were previously successful for analogous susbstrates resulted in the full consumption of the starting material and the desired di-keto acid as determined by LC-HRMS analysis. It became apparent, however, that isolation of the desired carboxylic acid would be problematic due to the instability of the desired carboxylic acid.[57-58] During extraction of the carboxylic acid using 1 M $HCl_{(aq)}$, the di-keto acid moiety is hydrolyzed to afford pyrimidinone **2.1**. Additionally, the di-keto acid was found to decompose in a similar manner to the pyrimidinone core in a methanol solution as shown by LC-HRMS. Performing the reaction in MeCN also provided high conversions to the desired product but did not avoid the stability issue upon isolation attempts. Saponification attempts at low temperature (0 °C) resulted in incomplete conversion.

We next switched to a slightly different set of reaction conditions (3:1 THF/H_2O) recently reported in the literature used to saponify similar acetonide substrates.[67] Utilizing the new conditions provided quantitative conversion of the acetonide, as determined by LC-HRMS, to the desired carboxylic acid; however, similar stability issues were observed. In order to reduce

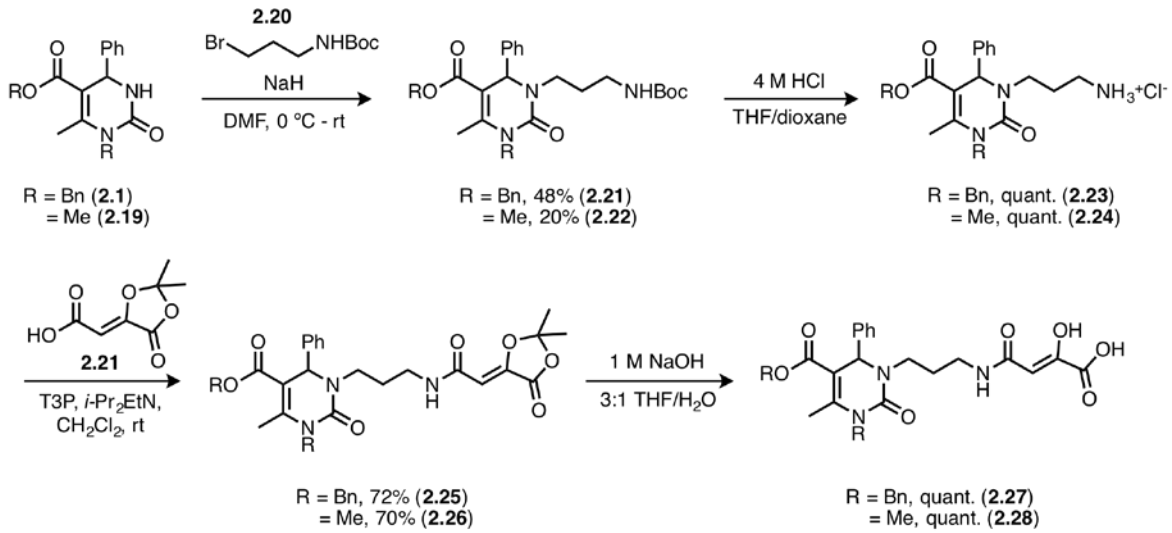


2.15 **2.18** **2.1**

Scheme 20. Saponification of acetonide **2.15.**

the amount of decomposition during extraction the crude reaction mixture was lyophilized to afford a mixture (84:16) of the sodium salt of acid **2.18** and pyrimidinone **2.1**. In spite of this, the general stability of acid **2.18** became a great concern to us and we elected to modify the scaffold in order to avoid further stability issues.

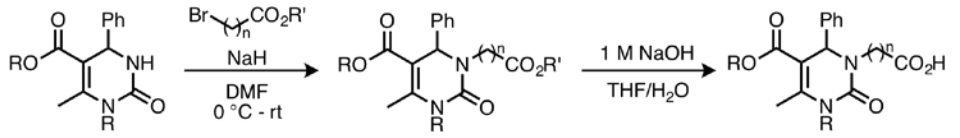
Observing that the acetylated pyrimidinone core **2.18** was generally unstable, we sought to incorporate a carbon spacer that would reduce the propensity of the di-keto acid towards hydrolysis. We began with the alkylation of pyrimidinone **2.1** and **2.19** to afford compounds **2.20** and **2.22** (Scheme 21**)**. Removal of the Boc-protecting group under traditional conditions (4 M HCl in dioxane) provided the hydrochloride salts **2.23** and **2.24** in excellent yield. Coupling of the acetonide fragment went smoothly and supplied acetonides **2.25** and **2.26**. Saponification of acetonides **2.25** and **2.26** unmasked the desired di-keto acids **2.27** and **2.28** in high yield. It is important to note that while di-keto acid **2.18** was found to be labile in aqueous environments, derivatives **2.27** and **2.28** were more stable and were not hydrolyzed during the 1 M HCl workup.



Scheme 21. Di-keto acid synthesis.

In addition to incorporating a carbon linker, we questioned whether other known chelating functionalities (i.e. carboxylic acids, tetrazoles) could be incorporated into the pyrimidinone scaffold to provide biologically active compounds. We initially investigated the installation of simple carboxylic acids at the N^3-position that could potentially chelate a metal ion with the assistance of the pyrimidinone core (Table 8). Alkylation using NaH and several bromo-esters afforded compounds **2.29**-**2.33** in moderate to good yield, however, alkylation using the ethyl 4-bromobutanoate lower reactivity. Subsequent saponification of the resulting esters smoothly provided acids **2.34**-**2.38**.

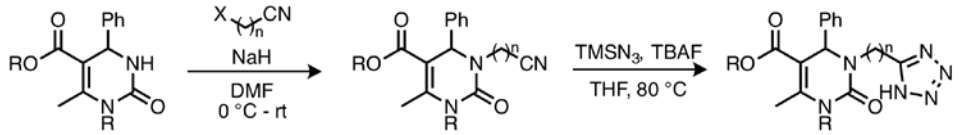
Table 8. Carboxylic Acid Derivative Synthesis



R	n	R'	Yield	Yield
-Bn	1	Et	80% (**2.29**)	94% (**2.34**)
-Bn	2	Me	64% (**2.30**)	93% (**2.35**)
-Bn	3	Et	22% (**2.31**)	73% (**2.36**)
-Me	1	Et	83% (**2.32**)	quant. (**2.37**)
-Me	2	Me	65% (**2.33**)	quant. (**2.38**)
-Me	3	Et	Et, trace	X

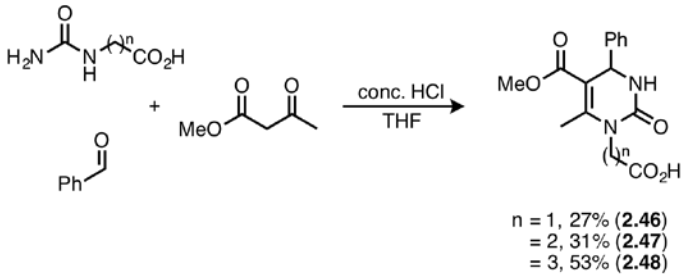
Having prepared several carboxylic acid derivatives we next sought to incorporate tetrazoles into our pyrimidinone core (Table 9). Alkylation using using NaH afforded nitriles **2.39**-**2.42** in moderate yield. As previously observed in the preparation of esters, alkyation using the longer 3-carbon linker bromide showed little reactivity. Formation of the tetrazole using $TMSN_3$ and TBAF in THF afforded the desired products **2.43**-**2.45**.

Table 9. Tetrazole Derivative Synthesis



R	n	X	Yield	Yield
-Bn	1	Cl	50% (**2.39**)	59% (**2.43**)
-Bn	2	Br	75% (**2.40**)	19% (**2.44**)
-Bn	3	Br	trace	X
-Me	1	Cl	53% (**2.41**)	48% (**2.45**)
-Me	2	Br	46% (**2.42**)	X
-Me	3	Br	trace	X

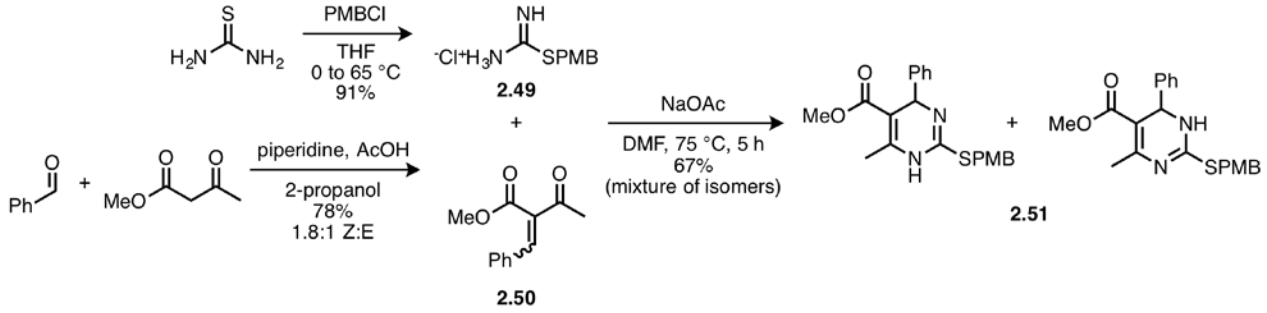
In addition to the installment of chelating functions at the N^3-position we also sought to investigate the effect of moving the chelating groups to the N^1-position of the pyrimidinone scaffold. Utilizing the corresponding carboxylic acid containing ureas in the Biginelli synthesis rapidly provided acids **2.46**-**2.48** (Scheme 22). One could envision similar chelating modes to those previously proposed although the actual active site binding could vary greatly with the chelating group residing at the N^1-position.



Scheme 22. Installation of the N^1-carboxylic acids.

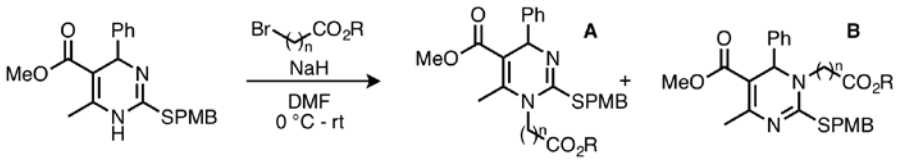
After synthesizing several substrates containing a carboxylic acid functionality at the N^1-position we became interested in replacing the urea moiety with a thiourea in order to study the possible effect on chelation (Scheme 23).[68] Protection of thiourea using PMBCl afforded the hydrochloride salt **2.49** in excellent yield. Knoevenagel condensation of benzaldeyhde and methyl acetoacetate provided the desired enone **2.50** as an inconsequential mixture of Z and E

isomers. Exposure of **2.49** and **2.50** to sodium acetate and mild heating afford the desired thiopyrimidine **2.51** as a mixture of isomers.



Scheme 23. Thiopyrimidine synthesis.

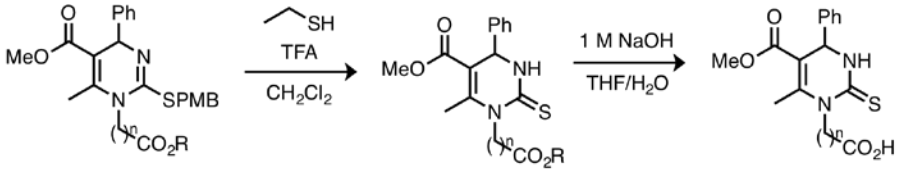
With thiopyrimidine **2.51** in hand we next sought to alkylate at either the N^1- or N^3-positions, as chelating functions would be desirable at both positions (Scheme 25). Alkylation using ethyl bromoacetate afforded **2.52** as a single regioisomer (N^1-alkylation) in 76% yield while methyl 3-bromopropionate afforded the desired product (**2.53**) in 64% as a partially separable 4.4:1 mixture of regioisomers favoring the N^1-product.



n	R'	Yield	Isomer Ratio
1	Et	64% (**2.52**)	only **A** obs.
2	Me	76% (**2.53**)	4.4:1 **A**:**B**

Scheme 24. Alkyation of thiopyrimidine **2.51**

Exposure of **2.52** and **2.53** (single N^1-isomer of each) to ethanethiol and TFA in CH_2Cl_2 efficiently removed the PMB to reveal thioureas **2.54** and **2.55** (Scheme 24). Subsequent saponification afforded the desired carboxylic acids **2.56** and **2.57** in excellent yield.



n	R'	Yield	Yield
1	Et	66% (**2.54**)	quant. (**2.56**)
2	Me	71% (**2.55**)	89% (**2.57**)

Scheme 25. Thiopyrimidine acid derivative synthesis.

In addition to the compounds presented in this document, we elected to take advantage of our access to the large number of compounds present in the University of Pittsburgh's center for Chemical Methodology and Library Development (CMLD). Based on our proposed chelation model we selected several compounds containing N^1-carboxylic acids and varying pyrimidinone

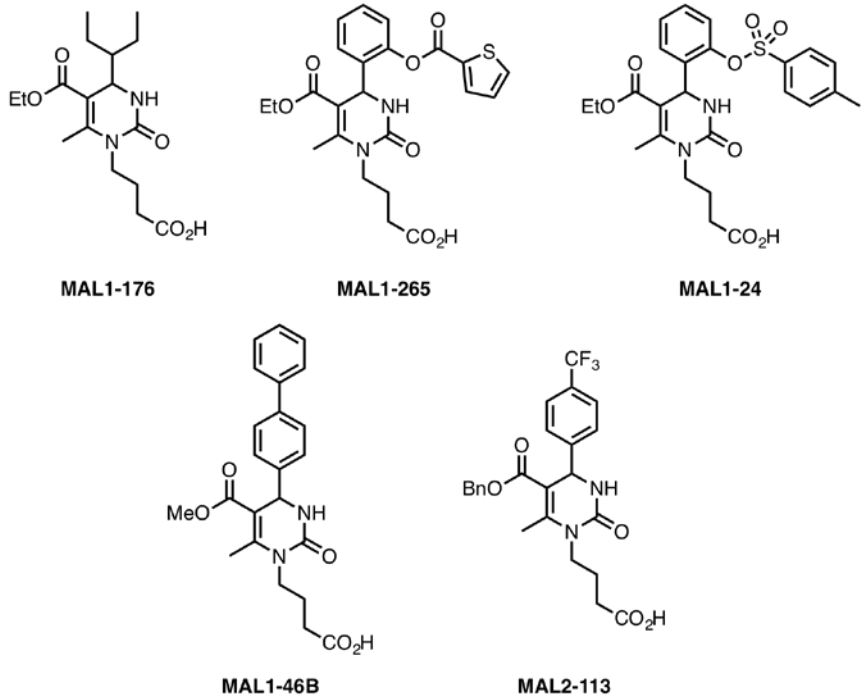


Figure 16. Selected CMLD substrates.

scaffolds for submission to biological testing in order to supplement our synthesized library (Figure 16). Biological testing was conducted by the Bushman group at the University of Pennsylvania and utilized a high-throughput fowlpox resolvase fluorescence polarization assay in which changes in fluorescence of a labeled Holliday junction were correlated to the cleavage of the junction by the fowlpox resolvase and could be used to determine resolvase activity.[69] The biological data (Table 10) shows that substrates containing chelating functions at the N^3-position, including esters, nitriles, carboxylic acids, and tetrazoles, showed no activity (IC_{50} > 300 μM). Furthermore, incorporation of the thiopyrimidine core did not afford active compounds. However, several of the compounds selected from the University of Pittsburgh CMLD possessing N^1-carboxylic acids exhibited moderate inhibitory activity (IC_{50} 16 μM). Surprisingly, compounds similar to **MAL1-265**, such as **2.46-2.48,** did not show activity. Based on our initial data, the 4-position substitution of the pyrimidinone scaffold appears to play a critical role in binding and inhibitory activity. Further studies are underway to study the effect of

Table 10. Biological Results

Compound #	IC_{50} (μM)*	Compound #	IC_{50} (μM)*
2.16	> 300	**2.42**	> 300
2.23	> 300	**2.43**	> 300
2.25	> 300	**2.44**	> 300
2.29	> 300	**2.45**	> 300
2.30	> 300	**2.46**	> 300
2.31	> 300	**2.47**	> 300
2.32	> 300	**2.48**	> 300
2.33	> 300	**2.54**	> 300
2.34	> 300	**2.55**	> 300
2.35	> 300	**2.56**	> 300
2.36	> 300	**2.57**	> 300
2.37	> 300	**MAL1-46B**	> 300
2.38	> 300	**MAL2-113**	> 300
2.39	> 300	**MAL1-176**	63
2.40	> 300	**MAL1-24**	130
2.41	> 300	**MAL1-265**	16

* Assays were conducted as reported in *Nucleic Acids Res.* **2012**, *1*.

various carboxylic acid isosteres and 4-position substituents of the pyrimidinone.

2.3 CONCLUSIONS

In our efforts toward the development of a series of novel poxvirus resolvase inhibitors, a small library of new pyrimidinone-based small molecules have been synthesized utilizing the multi-component Biginelli reaction and submitted for biological testing. While our initial attempts to directly install the di-keto acid chelating functionality onto the pyrimidinone scaffold resulted in unstable products that were readily cleaved to afford the pyrimidinone core, the incorporation of an alkyl linker has improved the stability of the di-keto acids in aqueous environments. Additional chelating functionalities (i.e. carboxylic acids and tetrazoles) have also been utilized as potential substrates while we intend to also investigate other chelating groups. Initial biological data indicated that substrates containing chelating functions at the N^3-position do not possess biological activity while moving the carboxylic acid function to the N^1-position resulted in several substrates with moderate activity. The substitution at the 4-position of the pyrimidinone scaffold appears to be critical for activity, as slight changes result in the complete loss of activity based on our current data.

3.0 EXPERIMENTAL SECTION

3.1 GENERAL

All moisture sensitive reactions were performed using syringe-septum techniques under an atmosphere of either dry N_2 or Ar unless otherwise noted. All glassware used in moisture sensitive reactions was flame-dried under an atmosphere of dry N_2 or Ar prior to use. Dry tetrahydrofuran was purified by filtration through an activated alumina column or dried by distillation over sodium/benzophenone under a dry N_2 atmosphere. Dry diethyl ether was purified by distillation over sodium/benzophenone under a dry N_2 atmosphere. Dry methylene chloride was purified by filtration through an activated alumina column or by distillation from CaH_2. All degassed solvents were prepared using the freeze/pump/thaw method (3x). Deuterated chloroform was filtered through an oven-dried alumina plug prior to use. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 µm layer thickness) and visualized by using UV lamp (254 nm) and/or by staining with a ninhydrin solution (0.6 g ninhydrin in 6 mL acetic acid and 200 mL n-butyl alcohol) or a $KMnO_4$ solution (3.0 g $KMnO_4$, 4.0 g K_2CO_3, in 200 mL H_2O and 4 mL 5% NaOH). Flash column chromatography was performed with 40-63 µm silica gel (Silicycle). Microwave reactions were performed on a Biotage Initiator microwave reactor. Infrared spectra were measured on a Smiths Detection IdentifyIR FT-IR spectrometer (ATR). All NMR data was collected at room temperature in

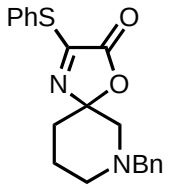
$CDCl_3$ on a 300, 400, or 500 MHz Bruker instrument. Chemical shifts (δ) are reported in parts per million (ppm) with internal $CHCl_3$ (δ 7.26 ppm for ^1H and 77.00 ppm for ^{13}C), $(CH_3)_2CO$ (δ 2.05 ppm for ^1H and 29.84 ppm for ^{13}C), or $(CH_3)_2SO$ (δ 2.50 ppm for ^1H and 39.52 ppm for ^{13}C) as the reference. ^1H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, qd = quartet of doublets, sep = septet), integration, and coupling constant(s) (*J*) in Hertz (Hz).

Heat of Formation Calculations:

Heat of formation values were calculated using Scigress. Initial conformational minimization was conducted using a CONFLEX/MM3 (extensive search) minimization. Heat of formation was then calculated using a PM3 (H_2O) method. All values are reported in kcal/mol.

3.2 EXPERIMENTAL PROCEDURES

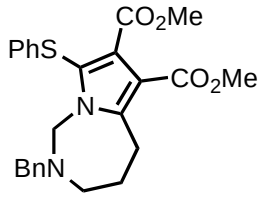
3.2.1 Chapter 1 Experimental



1.1

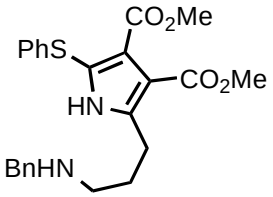
7-Benzyl-3-(phenylthio)-1-oxa-4,7-diazaspiro[4,5]dec-7-en-2-one (1.1).[18] To a solution of 1-benzyl-3-piperidone hydrochloride (376 mg, 1.60 mmol, 1.0 equiv) in water (3.1 mL, 0.5 M) was added K_2CO_3 (335 mg, 2.42 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 30 min and extracted with EtOAc (3 mL, 3x). The combined organic extracts

were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford 1-benzyl-3-piperidone (305 mg, 1.61 mmol, 97%). To a stirred solution of thiophenol (201 µL, 1.90 mmol, 1.2 equiv) in trifluorotoluene (1.9 mL, 1.0 M) in a flame-dried microwave vial under an Ar atmosphere at 0 °C was added ethyl cyanoformate (158 µL, 1.58 mmol, 1.0 equiv). The reaction mixture was stirred for 2 h at room temperature, cooled to 0 °C, and SiO_2 (162 mg, 1.80 mmol, 1.1 equiv, 11.1 mmols/g) was added. After stirring for 10 min at 0 °C a solution of 1-benzyl-3-piperidone (305 mg, 1.61 mmol, 1.0 equiv) in trifluorotoluene (1.1 mL, 1.4 M) was added drop-wise to afford a mild yellow solution, which was stirred at room temperature for 24.5 h. The reaction mixture purified using chromatography on SiO_2 (4:1, hexanes/ethyl acetate, isocratic) to afford a crude light yellow oil residue. The resulting crude residue was purified further using recrystallization (ethyl acetate/hexanes) to afford **1.1** (246 mg, 0.698 mmol, 43%) as a clear crystalline solid. Remaining crude material was recrystallized again to afford additional **1.1** (51.0 mg, 0.144 mmol, 9%) as a pale yellow crystalline solid (combined total yield of 52%): MP 92.4-97 °C; IR (neat) 2945, 2805, 1771, 1581, 1439, 1249, 1115, 1053, 973, 738, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.62-7.57 (m, 2 H), 7.47-7.41 (m, 3 H), 7.34-7.28 (m, 4 H), 7.28-7.23 (m, 1 H), 3.66, 3.56 (AB, 2 H, J = 13.5 Hz), 2.89-2.83 (m, 1 H), 2.55 (t, 2 H, J = 13.5 Hz), 2.24 (t, 1 H, J = 10.4 Hz), 2.01-1.96 (m, 2 H), 1.81-1.75 (m, 1 H), 1.67-1.60 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7, 161.5, 137.7, 133.9, 129.8, 129.5, 128.8, 128.3, 127.2, 126.4, 106.8, 61.9, 59.8, 52.1, 34.3, 22.1; HRMS (EI^+) *m/z* calcd for $C_{20}H_{21}N_2O_2S$ ($[M+H]^+$) 353.1324, found 353.1309.



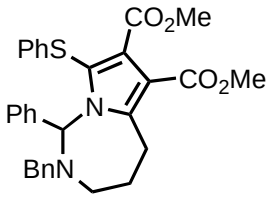
1.2

Dimethyl-2-benzyl-8-(phenylthio)-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-dicarboxylate (1.2).[18] To a solution of **1.1** (409 mg, 1.16 mmol, 1.0 equiv) in chlorobenzene (2.3 mL, 0.5 M) was added dimethyl acetylenedicarboxylate (286 µL, 2.33 mmol, 2.0 equiv) to afford a mild yellow solution that was heated at 150 °C for 10 min using microwave irradiation. The reaction mixture was directly purified using chromatography on SiO_2 (4:1, hexanes/ethyl acetate, isocratic) to afford a crude residue. The resulting residue was purified further using recrystallization (ethyl acetate) to **1.2** (267 mg, 0.593 mmol, 51%) as an off-white solid: MP 114.6-117.8 °C; IR (neat) 3027, 2937, 1722, 1702, 1581, 1493, 1450, 1212, 118, 1077, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.31-7.18 (m, 3 H), 7.12-7.06 (m, 7 H), 5.12 (s, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.39 (s, 3 H), 3.30 (bs, 2 H), 3.01 (t, 2 H, *J* = 5.0 Hz), 1.70-1.65 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 164.6, 143.6, 137.9, 136.3, 129.1, 128.4, 127.2, 126.3, 125.2, 119.8, 111.4, 65.5, 54.4, 52.7, 52.3, 51.5, 25.5, 22.1; HRMS (EI^+) *m/z* calcd for $C_{25}H_{25}N_2O_4S$ ($[M-H]^+$) 449.1535, found 449.1573.



1.3

Dimethyl-2-(3-(benzylamino)propyl)-5-(phenylthio)-1*H*-pyrrole-3,4-dicarboxylate (1.3).[18] A solution of **1.2** (167 mg, 0.370 mmol, 1.0 equiv) in acetic acid (8.4 mL) and water (1.7 mL) was heated at 95 °C for 48 h with stirring. The reaction mixture was concentrated under reduced pressure and purified using chromatography on SiO_2 (9:1 CH_2Cl_2/MeOH, isocratic) to afford **1.3** (116 mg, 0.264 mmol, 71%) as a light tan solid: MP 108-110 °C; IR (neat) 3064, 3000, 2951, 1737, 1700 1448, 1291 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22-7.09 (m, 10 H), 3.84 (s, 3 H), 3.77 (s, 3 H), 3.67 (s, 2 H), 2.95 (t, 2 H, *J* = 6.6 Hz), 2.59 (t, 2 H, *J* = 5.6 Hz), 1.99-1.89 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 166.1, 164.5, 140.8, 137.0, 132.9, 129.8, 129.2, 129.1, 129.1, 128.1, 126.5, 124.8, 118.6, 111.9, 52.4, 52.0, 51.5, 45.8, 26.1, 23.9, 23.7, 14.4.



1.4

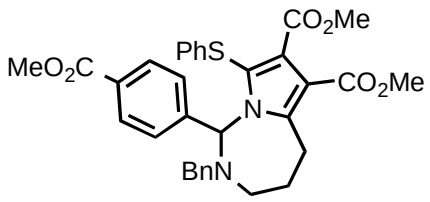
Dimethyl-2-benzyl-1-phenyl-8-(phenylthio)-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-dicarboxylate (1.4).[20] A solution of **1.2** (50.0 mg, 0.111 mmol, 1.0 equiv) in acetic acid (1.9 mL) and water (320 µL) was heated at 95 °C for 24 h. The reaction mixture was azeotropically dried using toluene (2 mL, 2x) and the resulting yellow oil residue was dissolved in toluene (2.2 mL). Pyridinium *p*-toluenesulfonate (33.0 mg, 0.131 mmol, 1.2 equiv)

and benzaldehyde (57.0 µL, 0.550 mmol, 5.0 equiv) was added followed by activated 4 Å molecular sieves. The reaction mixture was heated at reflux for 4 h, basified to a pH of 9-10 using 0.5 M Na_2CO_3, and extracted using EtOAc (2 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford an opaque oil residue. The resulting crude residue was purified using chromatography on SiO_2 (4:1, hexanes/ethyl acetate, isocratic) to afford **1.4** (33.0 mg, 0.0627 mmol, 56%) as an opaque, white solid: MP 162.2-165.2 °C; IR (neat) 3059, 2939, 1707, 1489, 1443, 1213, 1128, 744 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36-7.26 (m, 4 H), 7.18-7.14 (m, 3 H), 7.12-7.08 (m, 2 H), 7.02-6.97 (m, 3 H), 6.97-6.92 (m, 1 H), 6.87-6.82 (m, 2 H), 3.90 (s, 3 H), 3.85 (s, 2 H), 3.62, 3.56 (AB, 2 H, *J* = 14.5 Hz), 3.06 (t, 1 H, *J* = 14.3 Hz), 2.85 (d, 1 H, *J* = 15.0 Hz), 2.24 (t, 1 H, *J* = 13.9 Hz), 1.81 (q, 1 H, *J* = 13.5 Hz), 1.39-1.32 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 164.7, 143.3, 138.4, 136.3, 134.8, 129.1, 128.7, 128.4, 128.3, 127.6, 127.2, 126.7, 125.4, 122.0, 111.9, 54.8, 52.3, 51.6, 47.3, 25.1, 21.3; HRMS (EI^+) *m/z* calcd for $C_{31}H_{31}N_2O_4S$ 527.2005 ($[M+H]^+$), found 527.2018.

Compound **1.4** could also be prepared as follows:

To a stirred solution of **1.2** (52.2 mg, 0.119 mmol, 1.0 equiv) in dry toluene (3 mL, 0.04 M) was added pyridinium *p*-toluenesulfonate (34.8 mg, 0.138 mmol, 1.2 equiv) and methyl 4-(dimethoxymethyl)benzoate (141 mg, 0.671 mmol, 5.6 equiv). All reagents were azeotropically dried using toluene prior to their addition. The reaction mixture was refluxed for 4 h, concentrated under reduced pressure, and dissolved in EtOAc (5 mL). The solution was basified to a pH of 9-10 using 0.5 M Na_2CO_3 and extracted using EtOAc (5 mL, 2x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high

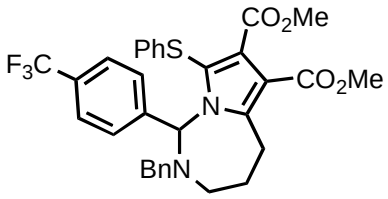
vacuum. The resulting crude residue was purified using chromatography on SiO_2 (3:1, hexanes/ethyl acetate, isocratic) to afford **1.4** (57.4 mg, 0.084 mmol, 62%) as an light yellow crystalline solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-6.85 (m, 14 H), 3.97 (s, 3 H), 3.87 (s, 3 H), 3.63 (AB, 2 H, *J* = 14 Hz), 3.03-2.86 (m, 2 H), 2.17 (t, 1 H, *J* = 15 Hz), 1.79 (q, 1 H, *J* = 11 Hz), 1.40-1.34 (m, 1 H). Other data matched that previously obtained.



1.5

Dimethyl-2-benzyl-1-(4-(methoxycarbonyl)phenyl)-8-(phenylthio)-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-dicarboxylate (1.5).[20] To a stirred solution of **1.3** (52.2 mg, 0.119 mmol, 1.0 equiv) in dry toluene (3 mL, 0.04 M) was added pyridinium *p*-toluenesulfonate (34.8 mg, 0.138 mmol, 1.2 equiv) and methyl 4-formylbenzoate dimethyl acetal (141 mg, 0.671 mmol, 5.6 equiv). All reagents were azeotropically dried using toluene prior to their addition. The reaction mixture was refluxed for 4 h, concentrated under reduced pressure, and dissolved in EtOAc (5 mL). The solution was basified to a pH of 9-10 using 0.5 M Na_2CO_3 and extracted with EtOAc (5 mL, 2x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum. The resulting crude residue was purified using chromatography on SiO_2 (3:1, hexanes/ethyl acetate, isocratic) to afford **1.5** (57.4 mg, 0.084 mmol, 62%) as an light yellow crystalline solid: IR (neat) 2932, 2951, 1726, 1499, 1435, 1279, 1210, 1141, 1122, 701 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-6.85 (m, 14 H), 3.97 (s, 3 H), 3.87 (s, 3 H), 3.63 (AB, 2 H, *J* = 14 Hz), 3.03-2.86 (m, 2 H), 2.17 (t, 1 H, *J* =

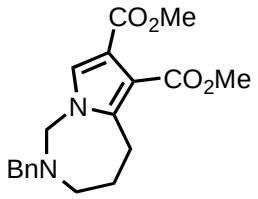
15 Hz), 1.79 (q, 1 H, J = 11 Hz), 1.40-1.34 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 166.4, 141.8, 138.3, 134.7, 129.9, 129.7, 129.7, 129.0, 128.8, 128.7, 127.7, 127.0, 122.6, 52.6, 52.4, 51.9, 48.0, 25.3, 21.5; HRMS (EI^+) *m/z* calcd for $C_{33}H_{32}N_2O_6SNa$ ($M+Na^+$) 607.1879, found 607.1873.



1.6

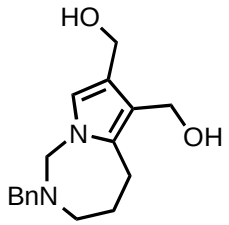
Dimethyl-2-benzyl-8-(phenylthio)-1-(4-(trifluoromethyl)phenyl)-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-dicarboxylate (1.6). A solution of **1.2** (51.0 mg, 0.113 mmol, 1.0 equiv) in acetic acid (1.9 mL) and water (320 µL) was heated at 95 °C for 23 h. The reaction mixture was azeotropically dried using toluene (2 mL, 2x) and the resulting yellow residue was dissolved in toluene (2.2 mL). Pyridinium *p*-toluenesulfonate (33.0 mg, 0.131 mmol, 1.2 equiv) and 4-(trifluoromethyl)benzaldehyde (76.0 µL, 0.557 mmol, 4.9 equiv) was added followed by activated 4 Å molecular sieves. The reaction mixture was heated at reflux for 4 h, basified to a pH of 9-10 using 0.5 M Na_2CO_3, and extracted using EtOAc (2 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford an off-yellow residue. The resulting crude residue was purified using chromatography on SiO_2 (4:1, hexanes/ethyl acetate, isocratic) to afford a yellow opaque residue which was purified further using recrystallization (hexanes) to **1.6** (21.7 mg, 0.0365 mmol, 32%) as a white, flakey solid: MP 134.7-135.4 °C.; IR (neat) 3059, 2934, 1726, 1707, 1491, 1446, 1320, 1228, 1141, 1124, 1066, 757, 701 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-

7.29 (m, 6 H), 7.06-7.04 (m, 2 H), 6.99 (s, 1 H), 6.94-6.91 (m, 3 H), 6.91-6.86 (m, 2 H), 3.94-3.91 (m, 1 H), 3.93 (s, 3 H), 3.86 (s, 3 H), 3.68, 3.62 (AB, 2 H, J = 14.0 Hz), 3.00-2.90 (m, 2 H), 2.20-2.14 (m, 1 H), 1.86-1.79 (m, 1 H), 1.42-1.39 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 142.9, 138.0, 134.3, 129.9, 128.8, 128.6, 128.5, 127.5, 127.2, 127.1, 125.4, 122.6, 112.2, 55.0, 52.5, 51.7, 48.1, 25.2, 21.4; HRMS (EI$^+$) *m/z* calcd for $C_{32}H_{30}N_2O_4F_3S$ ([M]$^+$) 594.1800, found 594.1835.



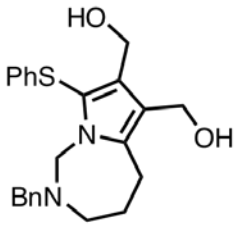
1.7

Dimethyl-2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-dicarboxylate (1.7). To a solution of **1.2** (303 mg, 0.673 mmol, 1.0 equiv) in THF (2.1 mL, 0.3 M) at 0 °C was added Raney-Nickel (5.55 g, 94.6 mmol, 140 equiv) in THF (10.5 mL) (the Raney-Nickel was prepared from a 50/50 wt. suspension of Raney-Nickel in water after rinsing with THF (5 mL, 3x)). The reaction mixture was vigorously stirred at 0 °C for 4 h. The supernatant was removed and the remaining Raney-Nickel was rinsed with THF (5 mL, 3x). The combined organic extracts were concentrated under reduced pressure and dried using high vacuum to afford **1.7** (179 mg, 0.523 mmol, 78%) as a white solid: MP 99.8-101.6 °C; IR (neat) 2937, 2852, 1685, 1530, 1441, 1208, 1120, 1062, 999, 727 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.30 (m, 2 H), 7.30-7.24 (m, 3 H), 6.91 (s, 1 H), 4.79 (s, 2 H), 3.86 (s, 3 H), 3.79 (s, 3 H), 3.48 (s, 2 H), 3.21 (t, 2 H, J = 5.0 Hz), 3.09 (bs, 2 H), 1.88-1.80 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0, 164.4, 139.7, 137.4, 128.7, 128.5, 127.5, 127.1, 113.4, 67.6, 56.9, 52.6, 51.6, 51.3, 25.1, 23.3; HRMS (EI$^+$) *m/z* calcd for $C_{19}H_{21}N_2O_4$ ([M-H]$^+$) 341.1501, found 341.1521.



1.8

(2-Benzyl-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine-6,7-diyl)dimethanol (1.8). To a stirred solution **1.7** (19.8 mg, 0.0578 mmol, 1.0 equiv) in dry THF (2.9 mL, 0.02 M) under an Ar atmosphere at -10 °C was added Red-Al (23.0 µL, 0.118 mmol, 2.0 equiv). The reaction mixture was stirred at -10 °C for 2 h after which additional of Red-Al (23.0 µL, 0.118 mmol, 2.0 equiv) was added. Additional Red-Al (69.0 µL, 0.354 mmol, 6.0 equiv) was added to the reaction mixture after 4 h and allowed to stir overnight after warming to room temperature. The reaction mixture was quenched via the addition of sat. Rochelle's salt solution (0.5 mL), stirred for 20 min, diluted with H_2O (2 mL), and extracted with CH_2Cl_2 (2 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford **1.8** (16.5 mg, 0.0576 mmol, quant) as a fine white powder: MP 107.9-109 °C; IR (neat) 3258, 2924, 2850, 1456, 1316, 1141, 977, 695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33-7.26 (m, 5 H), 6.37 (s, 1 H), 4.72 (s, 2 H), 4.58 (s, 2 H), 4.57 (s, 2 H), 3.47 (s, 2 H), 3.20 (t, 2 H, J = 5 Hz), 2.82 (bs, 2 H), 2.49 (bs, 1 H), 2.40 (bs, 1 H), 1.72-1.68 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 132.8, 128.8, 128.4, 127.3, 120.4, 119.9, 119.0, 66.7, 57.5, 57.1, 56.3, 52.6, 30.9, 24.7, 24.3; HRMS (EI^+) *m/z* calcd for $C_{17}H_{23}N_2O_2$ 287.1760, found 287.1786.



1.9

(2-Benzyl-8-(phenylthio)-2,3,4,5-tetrahydro-1H-pyrrolo[1,2-c][1,3]diazepine-6,7-diyl)dimethanol (1.19). To a solution of **1.2** (103 mg, 0.229 mmol, 1.0 equiv) in THF (11.1 mL, dry-solvent system, 0.02 M) at -10 °C was added sodium bis(2-methoxyethoxy)aluminumhydride (0.430 mL, 2.21 mmol, 9.7 equiv) as a 70% w/w toluene solution. The reaction mixture was stirred at -10 °C for 5 min, warmed to room temperature overnight, and quenched by the slow addition of a sat. Rochelle's salt solution (4 mL) followed by water (4 mL). The resulting emulsion was stirred for 15 min and extracted using EtOAc (10 mL, 3x). The combined organic extracts were washed with brine, dried (Na_2SO_4), concentrated under reduced pressure, and dried using high-vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, CH_2Cl_2/MeOH, 0-10% MeOH) to afford **1.9** (59.0 mg, 0.150 mmol, 65%) as a white foam: IR (neat) 3303, 3051, 2931, 2850, 1581, 1467, 1435, 1316, 1215, 1053, 995, 820, 814, 738, 733, 695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31-7.23 ($CDCl_3$ overlap, m, 3 H), 7.23-7.14 (m, 4 H), 7.09-7.02 (m, 1 H), 6.95-6.88 (m, 2 H), 5.10 (s, 2 H), 4.75 (s, 2 H), 4.65 (s, 2 H), 3.40 (s, 2 H), 3.01 (t, 2 H, *J* = 5.1 Hz), 2.95-2.85 (m, 2 H), 1.70-1.61 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.4, 136.9, 129.3, 129.0, 128.3, 127.0, 125.6, 125.4, 119.6, 115.5, 65.0, 57.3, 56.1, 54.6, 52.8, 30.9, 25.2, 23.4; ; HRMS (EI^+) *m/z* calcd for $C_{23}H_{27}N_2O_5S$ ($[M+H]^+$) 395.1788, found 395.1765.

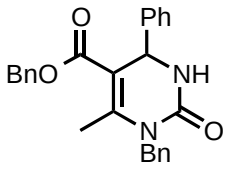
3.2.2 Chapter 2 Experimental

General Procedure A: Biginelli Substrate Synthesis[7]

To a stirred solution of urea (1.0 equiv) in THF (0.2 M) under a N_2 atmosphere was added aldehyde (2.0 equiv) and acetoacetate (2.0 equiv) sequentially. After stirring at room temperature for 5 min conc. HCl (0.7 equiv) was added drop-wise and the reaction mixture was stirred at room temperature for 24-72 h. The reaction mixture was concentrated under reduced pressure and resulting crude residue was recrystallized using a mixture of hexanes/ethyl acetate and/or *t*-butyl methyl ether to afford the desired pyrimidinone.

General Procedure B: Acetonide Coupling

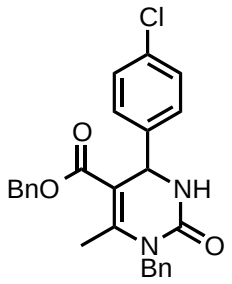
To a mixture of pyrimidinone (1.0 equiv) in EtOAc (0.44 M) was added (Z)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetic acid (2.0 equiv), T3P (2.2 equiv) as a 50% ethyl acetate solution, DMAP (0.4 equiv), and Et_3N (5.0 equiv). The reaction mixture was heated at 100 °C for 1 h in the microwave.



2.1

Benzyl-1-benzyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.1). According to General Procedure A, benzylurea (0.500 g, 3.26 mmol), benzaldehyde (576 µL, 6.56 mmol), benzyl acetoacetate (1.16 mL, 6.51 mmol), and conc. HCl (0.200 mL, 2.33 mmol) were stirred at room temperature for 72 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) and triturated using *t*-butyl methyl ether

to afford **2.1** (945 mg, 2.29 mmol, 70%) as a white solid: MP 130-132 °C; IR (neat) 3227, 3115, 3057, 3020, 2964, 1687, 1618, 1462, 1385, 1311, 1204, 1165, 1104, 1035, 958, 764, 725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.24 ($CDCl_3$ overlap, m, 9 H), 7.24-7.19 (m, 2 H), 7.15-7.13 (m, 4 H), 5.48 (d, 1 H, *J* = 20.8 Hz), 5.45 (s, 1 H), 5.19, 4.91 (AB, 2 H, *J* = 16.2 Hz), 5.07, 5.05 (AB, 2 H, *J* = 12.6 Hz), 2.47 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 153.5, 149.8, 143.0, 137.8, 135.9, 128.8, 128.7, 128.4, 128.1, 127.9, 127.2, 126.5, 126.4, 104.2, 66.1, 54.2, 16.6; HRMS (EI^+) *m/z* calcd for $C_{26}H_{25}N_2O_3$ ($[M+H]^+$) 413.1800, found 413.1855.

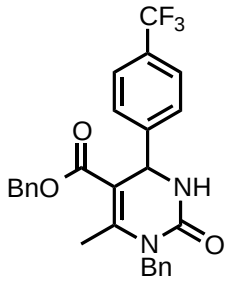


2.2

Benzyl-1-benzyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.2). According to General Procedure A, benzylurea (501 mg, 3.27 mmol), 4-chlorobenzaldehyde (927 mg, 6.53 mmol), benzyl acetoacetate (1.16 mL, 6.51 mmol) and conc. HCl (0.200 mL, 2.33 mmol) were stirred at room temperature for 67 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:2) to afford **2.2** (1.28 g, 2.86 mmol, 87%) as a pale yellow powder: MP 171-173 °C; IR (neat) 3341, 3052, 3020, 1706, 1676, 1613, 1393, 1311, 1215, 1197, 1154, 1104, 757, 720, 688 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) δ 7.35-7.20 ($CDCl_3$ overlap, m, 6 H), 7.14-7.12 (m, 2 H), 7.12-7.08 (m, 6 H), 5.52 (d, 1 H, *J* = 10.4 Hz), 5.41 (s, 1 H), 5.19, 4.89 (AB, 2 H, *J* = 16.6 Hz), 5.06, 5.02 (AB, 2 H, *J* = 12.6 Hz), 2.47 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 152.7, 150.7, 142.7, 138.5, 136.2, 132.0, 128.5, 128.4, 128.3,

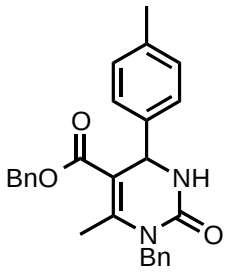
128.1, 127.8, 127.7, 126.9, 126.1, 102.5, 65.3, 51.9, 44.9, 16.0; HRMS (EI^+) *m/z* calcd for $C_{26}H_{24}ClN_2O_3$ ($[M+H]^+$) 447.9379, found 447.1465.

(Note: this compound is commercially available from Aurora Screening Library, however, characterization data has yet to be published in the literature)



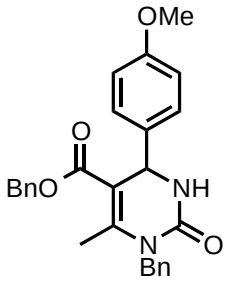
2.3

Benzyl-1-benzyl-6-methyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.3). According to General Procedure A, benzylurea (251 mg, 1.64 mmol), 4-(trifluoromethyl)benzaldehyde (446 µL, 3.27 mmol), benzyl acetoacetate (557 µL, 3.26 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 42 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.3** (0.640 g, 1.33 mmol, 81%) as a white solid: MP 177-179 °C; IR (neat) 3216, 3102, 2975, 1706, 1687, 1631, 1324, 1210, 1165, 1109, 1066, 1040, 701 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 2 H, *J* = 8.0 Hz), 7.32-7.23 (m, 8 H), 7.14-7.09 (m, 4 H), 5.61 (d, 1 H, *J* = 21.2 Hz), 5.49 (d, 1 H, *J* = 3.2 Hz), 5.20, 4.88 (AB, 2 H, *J* = 16.6 Hz), 5.12, 5.02 (AB, 2 H, *J* = 12.0 Hz), 2.49 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 153.6, 150.5, 146.9, 137.6, 135.6, 130.0 (d, *J* = 32.0 Hz), 128.7, 128.5, 128.2, 127.4, 126.4, 125.6 (t, *J* = 3 Hz), 124.0 (d, *J* = 270.0 Hz), 103.7, 66.3, 53.5, 46.0, 16.6; HRMS (EI^+) *m/z* calcd for $C_{27}H_{24}N_2O_2F_3$ ($[M+H]^+$) 481.1700, found 481.1728.



2.4

Benzyl-1-benzyl-6-methyl-2-oxo-4-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.4). According to General Procedure A, benzylurea (502 mg, 3.28 mmol), *p*-tolualdehyde (785 µL, 6.52 mmol), benzyl acetoacetate (1.16 mL, 6.51 mmol) and conc. HCl (0.200 mL, 2.33 mmol) were stirred at room temperature for 72 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.4** (1.02 g, 2.39 mmol, 73%) as a pale yellow powder: MP 170-172 °C; IR (neat) 3346, 3052, 3020, 1706, 1681, 1613, 1385, 1316, 1191, 1160, 1109, 757, 725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.20 (m, 7 H), 7.16-7.10 (m, 4 H), 7.10-7.06 (m, 4 H), 5.42 (bs, 2 H), 5.19, 4.92 (AB, 2 H, *J* = 16.4 Hz), 5.06 (t, 2 H, *J* = 13.6 Hz), 2.46 (s, 3 H), 2.34 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 152.9, 150.1, 140.8, 138.6, 136.6, 136.3, 128.9, 128.5, 128.3, 127.8, 127.7, 126.8, 126.1, 103.1, 65.2, 52.2, 44.9, 20.6 16.0; HRMS (EI^+) *m/z* calcd for $C_{27}H_{27}N_2O_3$ ($[M+H]^+$) 427.5179, found 427.2011.

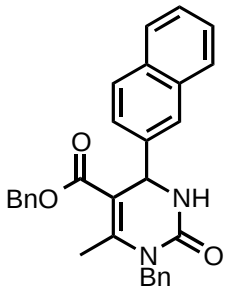


2.5

Benzyl-1-benzyl-4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-

5-carboxylate (2.5). According to General Procedure A, benzylurea (0.250 g, 1.63 mmol), 4-anisaldehyde (409 µL, 3.33 mmol), benzyl acetoacetate (557 µL, 3.26 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 42 h and provided a crude residue that was recrystallized using *t*-butyl methyl ether to afford **2.5** (411 mg, 0.929 mmol, 57%) as a red-orange solid: MP 132-134 °C; IR (neat) 3346, 3065, 3026, 2938, 2832, 1700, 1605, 1385, 1197, 1160, 1109, 1035, 757, 720, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.25 (m, 8 H), 7.19-7.10 ($CDCl_3$ overlap, m, 8 H), 6.79 (d, 2 H, J = 8.0 Hz), 5.44 (bs, 1 H), 5.41 (bs, 1 H), 5.19, 4.93 (AB, 2 H, J = 16.4 Hz), 5.09, 5.05 (AB, 2 H, J = 12.6 Hz), 3.81 (s, 3 H), 2.47 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 165.8, 159.2, 153.6, 149.4, 137.9, 135.9, 135.4, 128.7, 128.4, 128.1, 128.0, 127.7, 127.2, 126.5, 114.0, 104.5, 66.1, 55.3, 53.6, 46.0, 16.6; HRMS (EI^+) *m/z* calcd for $C_{27}H_{27}N_2O_4$ ($[M+H]^+$) 443.1900, found 443.1962.

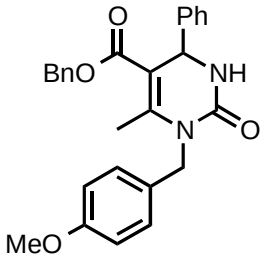
(Note: this compound is commercially available from Aurora Screening Library, however, characterization data has yet to be published in the literature)



2.6

Benzyl-1-benzyl-6-methyl-4-(naphthalen-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.6). According to General Procedure A, benzylurea (0.250 g, 1.63 mmol), 2-naphthaldehyde (0.520 g, 3.26 mmol), benzyl acetoacetate (557 µL, 3.26 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 42 h and provided a crude residue

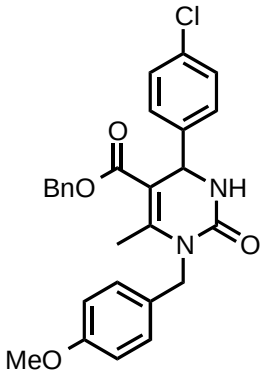
that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.6** (578 mg, 1.25 mmol, 77%) as a pale-orange solid: MP 165-167 °C; IR (neat) 3236, 3115, 3040, 1713, 1687, 1611, 1383, 1206, 1156, 1105, 1042, 738, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84-7.79 (m, 1 H), 7.74 (d, 1 H, *J* = 8.4 Hz), 7.68-7.63 (m, 1 H), 7.58 (s, 1 H), 7.56-7.45 (m, 2 H), 7.34 (d, 1 H, *J* = 8.8 Hz), 7.25-7.22 (m, 3 H), 7.22-7.14 (m, 4 H), 7.08 (d, 2 H, *J* = 7.6 Hz), 5.67 (d, 1 H, *J* = 18.0 Hz), 5.61 (s, 1 H), 5.26, 4.90 (AB, 2 H, *J* = 16.0 Hz), 5.06, 5.02 (AB, 2 H, *J* = 12.2 Hz), 2.51 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 153.5, 149.9, 140.2, 137.8, 135.8, 133.2, 128.8, 128.7, 128.4, 128.1, 128.0, 127.6, 127.3, 126.5, 126.2, 126.1, 125.3, 124.5, 104.1, 66.2, 54.4, 46.0, 16.6; HRMS (EI^+) *m/z* calcd for $C_{30}H_{27}N_2O_3$ ($[M+H]^+$) 463.1900, found 463.2016.



2.7

Benzyl-4-(4-chlorophenyl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.7). According to General Procedure A, 1-(4-methoxybenzyl)urea (299 mg, 1.56 mmol), benzaldehyde (318 µL, 3.12 mmol), benzyl acetoacetate (533 µL, 3.12 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 44 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.7** (600. mg, 1.36 mmol, 87%) as a light yellow solid: MP 138-140 °C; IR (neat) 3346, 3026, 2951, 1706, 1700, 1681, 1613, 1197, 1160, 1104, 1027, 701 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.24 (m, 4 H), 7.20-7.16 (m, 2 H), 7.14-7.10 (m, 2 H), 7.10-7.05 (m, 2

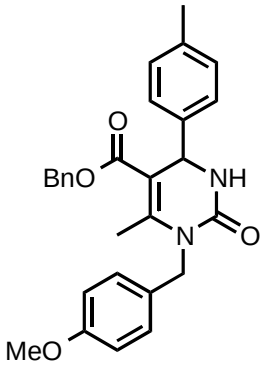
H), 6.83-6.80 (m, 2 H), 5.48 (bs, 1 H), 5.43 (d, 1 H, *J* = 2.8 Hz), 5.14, 4.82 (AB, 2 H, *J* = 16.0 Hz), 5.07, 5.04 (AB, 2 H, *J* = 12.4 Hz), 3.79 (s, 3 H), 2.48 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 158.7, 153.5, 149.8, 142.9, 135.8, 129.8, 128.7, 128.4, 128.0, 127.8, 126.4, 114.0, 104.2, 66.0, 55.2, 54.1, 16.5; HRMS (EI^+) *m/z* calcd for $C_{27}H_{27}N_2O_4$ ($[M+H]^+$) 443.1900, found 443.1963.



2.8

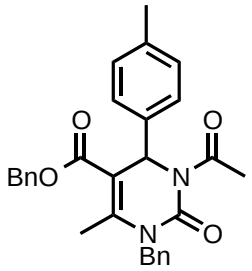
Benzyl-4-(4-chlorophenyl)-1-(4-methoxybenzyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.8). According to General Procedure A, 1-(4-methoxybenzyl)urea (302 mg, 1.58 mmol), 4-chlorobenzaldehyde (444 mg, 3.13 mmol), benzyl acetoacetate (535 µL, 3.13 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 44 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.8** (636 mg, 1.33 mmol, 85%) as a light yellow solid: MP 185-187 °C; IR (neat) 3341, 3026, 2945, 1706, 1681, 1613, 1517, 1385, 1247, 1197, 1160, 1104, 815, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.27 (m, 3 H), 7.20-7.16 (m, 2 H), 7.15-7.11 (m, 2 H), 7.09-7.03 (m, 4 H), 6.85-6.80 (m, 2 H), 5.68 (d, 1 H, *J* = 11.2 Hz), 5.39 (d, 1 H, *J* = 2.8 Hz), 5.14, 4.78 (AB, 2 H, *J* = 16.0 Hz), 5.09, 5.01 (AB, 2 H, *J* = 12.2 Hz), 3.80 (s, 3 H), 2.49 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 158.9, 153.5, 150.1, 141.6, 135.7, 133.6, 129.7, 128.8, 128.5,

128.2, 127.9, 114.1, 104.4, 66.2, 55.3, 53.5, 45.5, 16.6; HRMS (EI^+) *m/z* calcd for $C_{27}H_{26}N_2O_4Cl$ ($[M+H]^+$) 477.1500, found 477.1576.



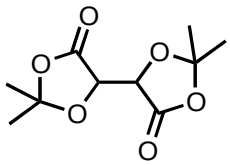
2.9

Benzyl-1-(4-methoxybenzyl)-6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.9). According to General Procedure A, 1-(4-methoxybenzyl)urea (305 mg, 1.59 mmol), *p*-tolualdehyde (377 µL, 3.13 mmol), benzyl acetoacetate (535 µL, 3.13 mmol), and conc. HCl (0.100 mL, 1.17 mmol) were stirred at room temperature for 44 h and provided a crude residue that was recrystallized using hexanes/ethyl acetate (1:1) to afford **2.9** (573 mg, 1.56 mmol, 79%) as a pale orange solid: MP 155-157 °C; IR (neat) 3346, 3001, 2932, 1706, 1681, 1613, 1393, 1197, 1160, 1104, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50-7.46 ($CDCl_3$ overlap, m, 7 H), 7.36-7.31 (m, 2 H), 7.30-7.27 (m, 4 H), 7.05-7.00 (m, 2 H), 5.63 (bs, 1 H), 5.59 (d, 1 H, *J* = 2.8 Hz), 5.34, 5.03 (AB, 2 H, *J* = 16.2 Hz), 5.26, 5.24 (AB, 2 H, *J* = 12.6 Hz), 4.00 (s, 3 H), 2.67 (s, 3 H), 2.53 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 158.8, 153.6, 149.6, 140.2, 137.6, 136.0, 130.0, 129.4, 128.4, 128.1, 128.0, 127.9, 126.4, 114.1, 104.4, 66.1, 55.3, 54.0, 45.5, 21.1, 16.6; HRMS (EI^+) *m/z* calcd for $C_{28}H_{29}N_2O_4$ ($[M+H]^+$) 457.2000, found 457.2119.



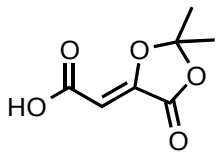
2.10

Benzyl-3-acetyl-1-benzyl-6-methyl-2-oxo-4-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.10).[63] To a suspension of **2.1** (0.200 g, 0.469 mmol, 1.0 equiv) in THF (24.0 mL, 0.02 M) in a flame-dried flask under an Ar atmosphere at -78 °C was added *n*-BuLi (325 µL, 0.520 mmol, 1.1 equiv) drop-wise to afford a clear yellow solution. The reaction mixture was warmed to room temperature, stirred for 30 min, cooled to -78 °C, and quenched via the drop-wise addition of distilled acetyl chloride (37.0 µL, 0.520 mmol, 1.1 equiv). The reaction mixture was stirred at -78 °C for 5 min and warmed to room temperature. After 20 min of stirring at room temperature the reaction mixture was washed using cold sat.'d NH_4Cl (25 mL) and extracted using ethyl acetate (25 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried under high-vacuum to afford **2.10** (223 mg, 0.476 mmol, >99%) as a yellow wax: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.40-7.28 (m, 5 H), 7.19-7.09 (m, 5 H), 6.97 (d, 2 H, *J* = 7.6 Hz), 6.83 (d, 2 H, *J* = 7.2 Hz), 6.60 (s, 1 H), 5.24, 5.18 (AB, 2 H, *J* = 12.4 Hz), 5.16, 4.76 (AB, 2 H, *J* = 16.2 Hz), 2.46 (s, 3 H), 2.41 (s, 3 H), 2.30 (s, 3 H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 171.1, 164.7, 152.0, 149.8, 137.1, 136.9, 136.0, 135.9, 129.0, 128.4, 128.3, 128.0, 127.0, 126.3, 125.8, 108.7, 65.8, 49.8, 46.0, 25.3, 20.6, 15.9; HRMS (EI^+) *m/z* calcd for $C_{29}H_{29}N_2O_4$ ($[M+H]^+$) 469.2000, found 469.2120.



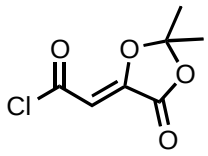
2.12

2,2,2',2'-Tetramethyl-[4,4'-bi(1,3-dioxolane)]-5,5'-dione (2.12).[57] To a solution of L-tartaric acid (5.00 g, 32.98 mmol, 1.0 equiv) in acetone (47.1 mL, 0.7 M) was added 2,2-dimethoxypropane (41.2 mL, 330. mmol, 10 equiv) followed by the drop-wise addition of boron trifluoride ether complex (835 µL, 6.60 mmol, 0.2 equiv). The reaction mixture was heated at 50 °C for 3 h and concentrated under reduced pressure to a volume of ~20 mL. Additional acetone (38 mL) and 2,2-dimethoxypropane (7.00 mL, 18.1 mmol, 0.6 equiv) was added and the reaction mixture was heated at 50 °C for 30 min. The reaction mixture was concentrated under reduce pressure to a volume of ~20 mL and additional acetone (38 mL) and 2,2-dimethoxypropane (7.00 mL, 18.1 mmol, 0.6 equiv) was added. The reaction mixture was heated at 50 °C for 30 min, concentrated under reduced pressure, and diluted using *t*-butyl methyl ether (25 mL). The reaction mixture was washed with sat.'d $NaHCO_3$ (25 mL) and resulting organic extract was diluted using heptane (25 mL). The reaction mixture was concentrated under reduced pressure to a volume of ~10 mL and diluted further using heptane (50 mL). The suspension was cooled at -20 °C for 2 h, filtered, and rinsed with heptane to afford **2.12** (3.52 g, 15.2 mmol, 46%) as a white crystalline solid. The remaining filtrate was concentrated under reduced pressure, diluted with heptane (50 mL), and stored at -20 °C overnight to afford additional **2.12** (993 mg, 4.29 mmol, 13 %, 59% overall) as a white crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.81 (s, 2 H), 1.66 (s, 6 H), 1.59 (s, 6 H).



2.13

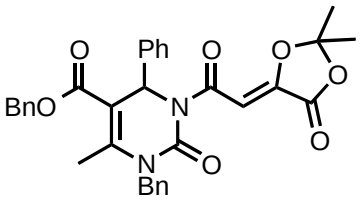
(*Z*)-2-(2,2-Dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetic acid (2.13).[57] To a solution of **2.12** (1.84 g, 7.99 mmol, 1.0 equiv) in THF (15 mL, dry - still, 0.5 M) in a flame-dried flask under an Ar atmosphere at -40 °C was added sodium *t*-butoxide (1.03 g, 10.5 mmol, 1.3 equiv) as a THF (8.0 mL, dry - still, 1.3 M) solution drop-wise over ~5 min. The reaction mixture was stirred at -40 °C for 20 min, quenched via the drop-wise addition of HCl in dioxane (3.0 mL, 12.0 mmol, 1.5 equiv), and warmed to room temperature. The reaction mixture was diluted using EtOAc (75 mL) and washed with 1 M HCl (25 mL). The the resulting organic extract was washed with water (25 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford a light brown crude residue. The resulting crude residue was triturated with *t*-butyl methyl ether to afford a white precipitate which was filtered and rinsed with additional *t*-butyl methyl ether to afford **2.13** (949 mg, 5.51 mmol, 69%) as a white powder: ^{1}H NMR (300 MHz, d_6-DMSO) δ 12.62 (s, 1 H), 5.56 (s, 1 H), 1.70 (s, 6 H).



2.14

(*Z*)-2-(2,2-Dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl chloride (2.14)[57] To a solution of **2.13** (50.0 mg, 0.290 mmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL, dry-still, 0.3 M) in a flame-dried vial under an Ar atmosphere was added distilled oxalyl chloride (27.0 µL, 0.315

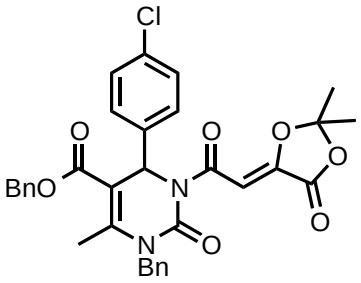
mmol, 1.1 equiv) drop-wise followed by a trace amount of DMF (1 drop, dry). The reaction mixture was stirred at room temperature for 30 min and concentrated under reduced pressure to afford **2.14** (55.0 mg, 0.289 mmol, quant) as an off-white crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (s, 1 H), 1.78 (s, 6 H).



2.15

(*Z*)-Benzyl-1-benzyl-3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.15). To a solution of **2.1** (50.0 mg, 0.121 mmol, 1.0 equiv) in CH_2Cl_2 (518 µL, 0.2 M) was added **2.13** (42.0 mg, 0.244 mmol, 2.0 equiv), T3P (159 µL, 0.267 mmol, 2.2 equiv) as a 50% ethyl acetate solution, DMAP (6.0 mg, 0.048 mmol, 0.4 equiv), and Et_3N (85.0 µL, 0.609 mmol, 5.0 equiv) sequentially. The reaction mixture was stirred at room temperature for 48 h, diluted using ethyl acetate (5 mL), washed with sat.'d $NaHCO_3$ (5 mL), and extracted with ethyl acetate (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The resulting crude residue was recrystallized using hexanes/chloroform to afford **2.15** (16.5 mg, 0.0291 mmol, 24%) as a mild brown solid: MP 206-208 °C; IR (neat) 3033, 2983, 2925, 1788, 1687, 1631, 1385, 1260, 1255, 1191, 1141, 1009, 751, 701 cm^{-1}; ^{1}H NMR (500 MHz, d_6-acetone) δ 7.36-7.24 (m, 9 H), 7.22-7.11 (m, 6 H), 6.87 (d, 2 H, *J* = 7.0 Hz), 6.78 (s, 1 H), 6.74 (s, 1 H), 5.42, 4.76 (AB, 2 H, *J* = 16.5), 5.29, 5.21 (AB, 2 H, *J* = 12.5), 2.53 (s, 3 H), 1.76 (s, 3 H), 1.74 (s, 3 H); ^{13}C NMR (125 MHz, d_6-acetone) δ 165.8, 164.2, 162.9, 153.4, 150.7, 148.0,

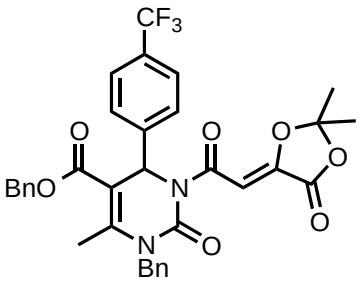
140.3, 138.0, 137.3, 129.4, 129.0, 128.9, 128.7, 128.0, 127.6, 127.4, 115.1, 110.6, 98.4, 67.0, 51.8, 47.1, 26.6, 26.5, 16.5; HRMS (EI^+) *m/z* calcd for $C_{33}H_{31}N_2O_7$ ($[M+H]^+$) 567.2179, found 567.2129.



2.16

(*Z*)-Benzyl-1-benzyl-4-(4-chlorophenyl)-3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.16). According to General Procedure B, **2.2** (51.0 mg, 0.114 mmol), **2.13** (39.4 mg, 0.229 mmol), T3P (147 µL, 0.247 mmol), DMAP (6.0 mg, 0.049 mmol), and Et_3N (79.0 µL, 0.562 mmol) were heated at 100 °C for 1 h using microwave irradiation. The reaction mixture was diluted using ethyl acetate (2 mL), washed with sat.'d $NaHCO_3$ (2 mL), and extracted using ethyl acetate (2 mL, 3x). The combined organic extracts were washed with water (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude residue was purified using chromatography on SiO_2 (ISOC-Rf, 4 g column, step-wise gradient using hexanes/ethyl acetate) to afford a pale yellow solid. The solid was triturated with cold hexanes to afford **2.16** (27.2 mg, 0.0453 mmol, 40%) as a white solid: MP 184-186 °C; IR (neat) 3039, 2970, 1795, 1687, 1631, 1493, 1385, 1247, 1210, 1186, 1096, 1027, 1003, 757, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32-7.27 ($CDCl_3$ overlap, m, 1 H), 7.24-7.10 (m, 9 H), 7.07-7.04 (m, 2 H), 6.85 (s, 1 H), 6.84 (bs, 2 H), 6.64 (s, 1 H), 5.38, 4.62 (AB, 2 H, *J* = 15.6 Hz), 5.23, 5.09 (AB, 2 H, *J* = 12.4 Hz),

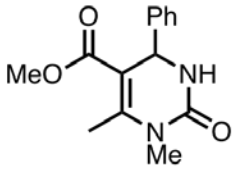
2.53 (s, 3 H), 1.76 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 163.9, 161.9, 152.2, 149.4, 147.5, 137.2, 136.1, 135.5, 133.8, 128.7, 128.6, 128.3, 128.1, 127.8, 127.2, 114.4, 109.9, 98.1, 66.6, 51.0, 46.7, 26.8, 16.4; HRMS (EI^+) *m/z* calcd for $C_{33}H_{30}N_2O_7Cl$ ($[M+H]^+$) 601.1736, found 601.1740.



2.17

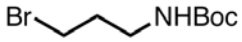
(*Z*)-Benzyl-1-benzyl-3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl)-6-methyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.17). According to General Procedure B, **2.3** (0.150 g, 0.312 mmol), **2.13** (107 mg, 0.622 mmol), T3P (409 µL, 0.687 mmol), DMAP (15.0 mg, 0.122 mmol), and Et_3N (219 µL, 1.56 mmol) were heated at 100 °C for 1 h using microwave irradiation. The reaction mixture was diluted using ethyl acetate (2 mL), washed with sat.'d $NaHCO_3$ (2 mL), and extracted using ethyl acetate (2 mL, 3x). The combined organic extracts were washed with water (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude residue was purified using chromatography on SiO_2 (ISOC-Rf, 4 g column, step-wise gradient using hexanes/ethyl acetate) to afford (Z)-benzyl 1-benzyl-3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl)-6-methyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (0.120 g, 0.190 mmol, 61%) as a off-white foam: IR (neat) 3025, 2945, 1801, 1687, 1637, 1379, 1323, 1247, 1178, 1122, 1072, 751, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, 2 H, *J* =

8.0 Hz), 7.33-7.29 (m , 3 H), 7.27-7.18 (m, 5 H), 7.16-7.10 (m, 2 H), 6.88 (s, 1 H), 6.81 (d, 2 H, *J* = 7.2 Hz), 6.74 (s, 1 H), 5.41, 4.64 (AB, 2 H, *J* = 16.0 Hz), 5.27, 5.13 (AB, 2 H, *J* = 12.4 Hz), 2.57 (s, 3 H), 1.77 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7, 163.9, 161.9, 152.2, 149.7, 148.7, 142.8, 135.9, 135.4, 130.1 (q, *J* = 32.0 Hz), 128.7, 128.6, 128.3, 128.1, 127.8, 127.0, 125.4 (t, *J* = 3.6 Hz), 123.9 (q, *J* = 27.1 Hz), 114.4, 109.7, 97.9, 66.7, 51.1, 46.7, 30.9, 26.8, 16.4; HRMS (EI^+) *m/z* calcd for $C_{34}H_{30}F_3N_2O_7$ ($[M+H]^+$) 635.2000, found 635.1987.



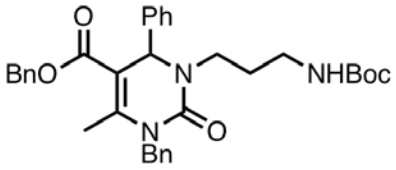
2.19

Methyl-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.19).[70] To a solution of 1-methyl urea (1.01 g, 13.2 mmol, 1.0 equiv) in THF (65.5 mL, 0.2 M, dry-solvent system) under an Ar atmosphere was added benzaldehyde (2.70 mL, 26.2 mmol, 2.0 equiv) and methyl acetoacetate (2.90 mL, 26.6 mmol, 2.0 equiv). The reaction mixture was stirred for 5 min at room temperature and conc. HCl (785 µL, 9.14 mmol, 0.7 equiv) was added drop-wise to the reaction to afford a clear solution. The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was concentrated under reduced pressure to afford a crude yellow solid. The resulting crude residue was purified via recrystallization using EtOAc to afford methyl 1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.49 g, 9.57 mmol, 72%) as an off-white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.24 ($CDCl_3$ overlap, m, 5 H), 5.63 (bs, 1 H), 5.41 (s, 1 H), 3.68 (s, 3 H), 3.26 (s, 3 H), 2.54 (s, 3 H).



2.20

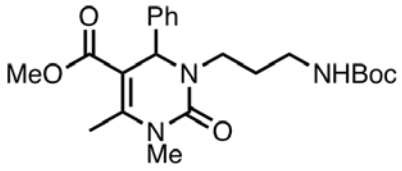
***Tert*-butyl (3-bromopropyl)carbamate (2.20).**[71] To a mixture of 1-bromopropyl amine hydrobromide (1.25 g, 5.60 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL, 0.28 M, dry-solvent system) at 0 °C was added triethylamine (0.780 mL, 5.55 mmol, 1.0 equiv) to afford a clear solution. A solution of Boc-anhydride (1.26 g, 5.72 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL, 0.11 M, dry-solvent system) was added drop-wise to the reaction mixture over 10 min. The reaction mixture was stirred at 0 °C for 2-3 min, warmed to room temperature, and stirred for 2 h. The reaction mixture was diluted using ethyl acetate (100 mL), washed with 5% citric acid (50 mL), and extracted using ethyl acetate (50 mL, 3x). The combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried under high vacuum to afford Boc-aminopropyl bromide (1.25 g, 5.25 mmol, 94%) as a very light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.65 (s, 1 H), 3.44 (t, 2 H, *J* = 6.3 Hz), 3.33 (q, 2 H, *J* = 6.5 Hz), 2.09-2.00 (m, 2 H), 1.44 (s, 9 H).



2.21

1-Benzyl-3-(3-((tert-butoxycarbonyl)amino)propyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.21). To a solution of **2.1** (201 mg, 0.487 mmol, 1.0 equiv) in DMF (5.0 mL, 0.1 M, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added NaH (30.0 mg, 0.750 mmol, 1.5 equiv) as a 60% dispersion in mineral oil. The

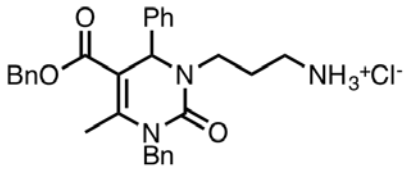
reaction mixture was stirred until bubbling stopped and **2.20** (148 mg, 0.622 mmol, 1.3 equiv) as a DMF (448 µL, 1.3 M, dry) solution was added drop-wise to the reaction mixture. The reaction mixture was stirred for 3.5 h at 0 °C, warmed to room temperature overnight, quenched with sat.'d NH_4Cl (10 mL) and water (10 mL), and extracted with EtOAc (20 mL, 3x). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3 hexanes/acetone) to afford **2.21** (134 mg, 0.235 mmol, 48%) as a white foam: IR (neat) 3353, 3033, 2969, 2932, 1700, 1668, 1625, 1493, 1448, 1392, 1254, 1204, 1165, 1135, 1077, 1034, 733, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.30 (m, 5 H), 7.30-7.13 ($CDCl_3$ overlap, m, 6 H), 7.13-7.08 (m, 4 H), 5.39 (s, 1 H), 5.21, 5.11 (AB, 2 H, *J* = 12.3 Hz), 5.23, 4.97 (AB, 2 H, *J* = 16.5), 3.91-3.88 (m, 1 H), 3.33-3.18 (m, 1 H), 3.11-2.94 (m, 2 H), 2.46 (s, 3 H), 1.77-1.64 (m, 2 H), 1.46 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 165.7, 156.0, 153.9, 149.5, 141.4, 137.9, 135.9, 128.7, 128.5, 128.4, 128.2, 127.9, 127.2, 126.8, 126.5, 104.2, 66.3, 58.9, 46.8, 28.4, 27.9, 16.7; HRMS (EI^+) *m/z* calcd for $C_{34}H_{39}N_3O_5Na$ ($[M+Na]^+$) 592.2782, found 592.2767.



2.22

Methyl-3-(3-((tert-butoxycarbonyl)amino)propyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.22). To a solution of **2.19** (201 mg, 0.772 mmol, 1.0 equiv) in DMF (7.7 mL, 0.1 M, dry) in a flame-dried flask at 0 °C under an Ar atmosphere was added sodium hydride (46.5 mg, 1.16 mmol, 1.5 equiv) as a 60% dispersion in

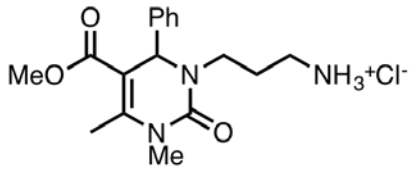
mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and **2.20** (0.220 g, 0.924 mmol, 1.2 equiv) was added drop-wise as a DMF (0.710 mL, 1.3 M, dry) solution. The reaction mixture was stirred at 0 °C for 4 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (15 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford methyl **2.22** (25.3 mg, 0.0606 mmol, 8%) as a white foam. An additional fraction of product was collected (52.4 mg, 0.109 mmol, 87% pure, 14%) with the impurity being remaining pyrimidinone starting material. The pure material was fully characterized and then combined with the impure fractions for the following de-protection step (70.9 mg, 0.155 mmol, 91% pure, 20% combined): IR (neat) 3353, 2969, 2924, 2855, 1694, 1663, 1625, 1512, 1461, 1366, 1254, 1165, 1053, 725, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33-7.18 ($CDCl_3$ overlap, m, 5 H), 5.29 (s, 1 H), 5.12 (s, 1 H), 3.81-3.70 (m, 1 H), 3.70 (s, 3 H), 3.24 (s, 3 H), 3.24-3.11 (m, 1 H), 3.02-2.89 (m, 2 H), 2.46 (s, 3 H), 1.70-1.60 (m, 2 H), 1.42 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 156.0, 154.3, 149.5, 141.6, 128.7, 127.8, 126.4, 103.8, 78.9, 58.6, 51.3, 44.2, 37.4, 31.2, 28.4, 28.0, 16.6; HRMS (EI^+) *m/z* calcd for $C_{22}H_{31}N_3O_5Na$ ($[M+Na]^+$) 440.2156, found 440.2147.



2.23

3-(3-Benzyl-5-((benzyloxy)carbonyl)-4-methyl-2-oxo-6-phenyl-2,3-dihydropyrimidin-1(6H)-yl)propan-1-aminium chloride (2.23). A solution of **2.21** (92.2 mg,

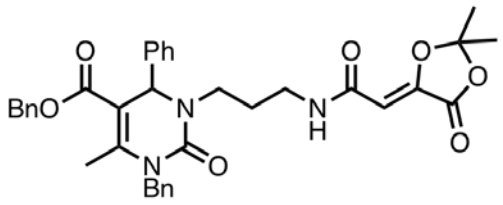
0.161 mmol, 1.0 equiv) in 4 M HCl in dioxane (0.810 mL, 3.24 mmol, 20.0 equiv) was stirred at room temperature for 27.5 h, concentrated under reduced pressure, rinsed with ether, and dried using high vacuum to afford **2.23** (81.6 mg, 0.161 mmol, quant) as an off-white foam: IR (neat) 3403, 3088, 2945, 2882, 1650, 1631, 1456, 1385, 1286, 1204, 1135, 1077, 1027, 990, 725, 695 cm^{-1}; ^{1}H NMR (300 MHz, d_6-DMSO) δ 7.38 (s, 3 H), 7.30-7.11 ($CDCl_3$ overlap, m, 15 H), 5.37 (s, 1 H), 5.14, 5.04 (AB, 2 H, *J* = 12.6 Hz), 5.09, 4.93 (AB, 2 H, *J* = 16.5 Hz), 3.69-3.57 (m, 2 H), 3.36-3.28 (m, 1 H), 3.08-2.95 (m, 1 H), 2.80-2.66 (m, 2 H), 2.36 (s, 3 H), 1.88-1.68 (m, 2 H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 165.0, 152.9, 149.5, 141.4, 138.3, 136.1, 128.5, 128.3, 127.9, 127.0, 126.6, 126.4, 103.4, 65.5, 58.4, 46.0, 44.1, 36.6, 25.8, 16.2; HRMS (EI^+) *m/z* calcd for $C_{29}H_{32}N_3O_3$ ($[M+H]^+$) 470.2438, found 470.2421.



2.24

3-(5-(Methoxycarbonyl)-3,4-dimethyl-2-oxo-6-phenyl-3,6-dihydropyrimidin-1(2H)-yl)propan-1-aminium chloride (2.24). To a solution of **2.22** (68.8 mg, 0.165 mmol, 1.0 equiv) in THF (825 uL, 0.2 M) was added 4M HCl (825 uL, 3.30 mmol, 20 equiv). The reaction mixture was stirred at room temperature overnight, concentrated under reduced pressure, rinsed with ether, and dried using high vacuum to afford **2.24** (58.6 mg, 0.151 mmol, 91% pure, 91%) as a yellow-green foam: IR (neat) 3422, 2932, 2855, 1650, 1467, 1273, 1204, 1053, 764, 725 cm^{-1}; ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.06 (bs, 1 H), 7.89 (bs, 2 H), 7.40-7.17 (m, 5 H), 5.30 (s, 1 H), 3.62 (s, 3 H), 3.47-3.33 (m, 1 H), 3.16 (s, 3 H), 3.02-2.83 (m, 1 H), 2.78-2.67 (m, 2 H), 2.45 (s, 3 H), 1.83-1.68 (m, 2 H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 166.3, 153.7, 150.7, 142.2,

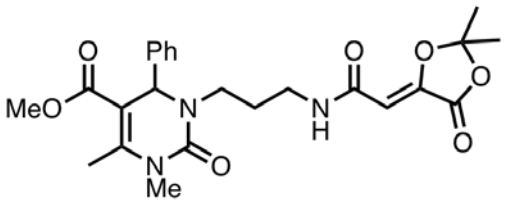
129.3, 128.4, 126.9, 103.3, 58.7, 51.8, 44.4, 37.1, 26.4, 16.7; HRMS (EI^+) *m/z* calcd for $C_{17}H_{24}N_3O_3$ ($[M+H]^+$) 318.1812, found 318.1804.



2.25

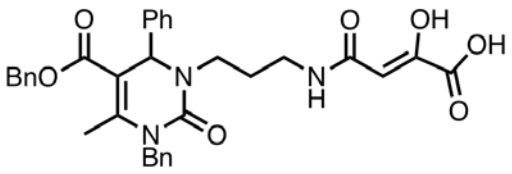
(*Z*)-Benzyl-1-benzyl-3-(3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetamido)propyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.25). To a solution of **2.23** (176 mg, 0.264 mmol, 1.0 equiv) in CH_2Cl_2 (1.9 mL, 0.1 M, dry-solvent system) under an Ar atmosphere was added **2.13** (55.2 mg, 0.321 mmol, 1.2 equiv), T3P (189 µL, 0.317 mmol, 1.2 equiv) as a 50% EtOAc solution, and N,N-diisopropylethylamine (0.230 mL, 1.32 mmol, 5.0 equiv). The reaction mixture was stirred overnight at room temperature, diluted using EtOAc (3 mL), washed with sat.'d $NaHCO_3$ (2 mL), and extracted using EtOAc (2 mL, 3x). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried under high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, hexanes/EtOAc, step-wise gradient) to afford **2.25** (118 mg, 0.189 mmol, 72%) as a white foam: IR (neat) 3316, 3070, 2995, 2945, 1795, 1668, 1625, 1385, 1260, 1210, 1135, 1008, 733, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46-7.35 (m, 1 H), 7.35-7.30 (m, 6 H), 7.30-7.15 ($CDCl_3$ overlap, m, 6 H), 7.15-7.10 (m, 4 H), 5.81 (s, 1 H), 5.32 (s, 1H), 5.18-5.98 (m, 4 H), 3.78-3.69 (m, 1H), 3.53-3.42 (m, 1 H), 3.18-3.05 (m, 2 H), 2.43 (s, 3H), 1.69-1.59 (m, 5H), 1.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 163.2, 161.9, 154.1, 149.2, 142.7, 141.6, 137.9, 135.9, 135.8, 128.8, 128.6, 128.5, 128.4, 128.2, 128.0, 127.3, 126.9, 126.2, 113.7, 104.2, 101.7,

66.4, 59.2, 47.0, 44.1, 35.7, 27.2, 26.5, 16.7; HRMS (EI^+) *m/z* calcd for $C_{36}H_{38}N_3O_7$ ($[M+H]^+$) 624.2704, found 624.2695.



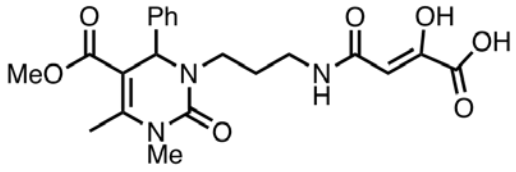
2.26

Methyl-(*Z*)-3-(3-(2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetamido)propyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.26). To a solution of **2.24** (64.1 mg, 0.138 mmol, 1.0 equiv) in CH_2Cl_2 (986 µL, 0.14 M, dry-solvent system) was added **2.13** (29.5 mg, 0.171 mmol, 1.2 equiv), T3P (99.0 µL, 0.166 mmol, 1.2 equiv) as a 50% solution in EtOAc, and N,N-diisopropylethylamine (0.120 mL, 0.689 mmol, 5.0 equiv). The reaction mixture was stirred overnight under an Ar atmosphere, diluted using EtOAc (5 mL), washed with sat.'d $NaHCO_3$ (5 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.26** (45.7 mg, 0.0969 mmol, 70%) as a white foam: IR (neat) 3295, 3001, 2945, 1795, 1663, 1631, 1536, 1467, 1385, 1254, 1204, 1008, 902, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.26 (m, 2 H), 7.26-7.23 (m, 1 H), 7.23-7.18 (m, 2 H), 5.83 (s, 1 H), 5.27 (s, 1 H), 3.79-3.67 (m, 1 H), 3.69 (s, 3 H), 3.52-3.39 (m, 1 H), 3.24 (s, 3 H), 3.12-2.98 (m, 2 H), 2.46 (s, 3 H), 1.74 (s, 6 H), 1.72-1.62 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 163.1, 162.0, 154.3, 149.3, 142.7, 141.7, 128.6, 127.9, 126.4, 113.7, 103.8, 101.5, 58.8, 51.3, 43.9, 35.7, 31.1, 27.2, 26.7, 16.5; HRMS (EI^+) *m/z* calcd for $C_{22}H_{31}N_3O_5Na$ ($[M+Na]^+$) 472.2078, found 472.2067.



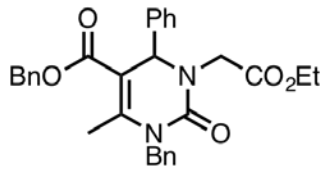
2.27

(*Z*)-4-((3-(3-Benzyl-5-((benzyloxy)carbonyl)-4-methyl-2-oxo-6-phenyl-2,3-dihydropyrimidin-1(6H)-yl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (2.27). To a solution of **2.25** (60.5 mg, 0.070 mmol, 1.0 equiv) in THF (1.8 mL) and water (603 µL, 0.04 M overall) was added 1 M NaOH (106 µL, 0.106 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature for 3.5 h, quenched using 1 M HCl (0.200 mL) and water (2 mL) and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure (no heat), and dried using high vaccum to afford **2.27** (57.5 mg, 0.0985 mmol, quant,) as a white foam (unknown impurity remained): IR (neat) 3334, 3025, 2932, 1631, 1448, 1385, 1254, 1204, 1135, 1072, 1034, 725, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.30 (m, 7 H), 7.30-7.15 ($CDCl_3$ overlap, m, 3 H), 7.15-7.05 (m , 5 H), 6.28 (s, 1 H), 5.37 (s, 1 H), 5.23-5.06 (m, 3 H), 4.92 (d, 1 H, *J* = 16.5 Hz), 3.90-3.76 (m, 1 H), 3.48-3.39 (m, 2 H), 3.18-3.03 (m, 2 H), 2.44 (s, 3 H), 1.78-1.63 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.4, 170.6, 165.4, 164.4, 156.6, 154.6, 148.8, 140.6, 137.1, 135.6, 128.9, 128.8, 128.6, 128.4, 128.3, 127.5, 126.7, 126.5, 105.2, 99.3, 66.6, 58.9, 47.2, 44.7, 35.9, 30.3, 16.7; HRMS (EI^+) *m/z* calcd for $C_{33}H_{34}N_3O_7$ ($[M+H]^+$) 584.2391, found 584.2382.



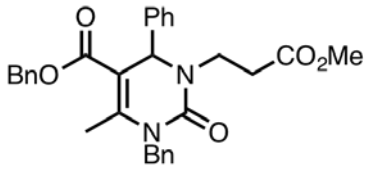
2.28

(*Z*)-2-Hydroxy-4-((3-(5-(methoxycarbonyl)-3,4-dimethyl-2-oxo-6-phenyl-3,6-dihydropyrimidin-1(2H)-yl)propyl)amino)-4-oxobut-2-enoic acid (2.28). To a solution of **2.26** (45.7 mg, 0.0969 mmol, 1.0 equiv) in THF/water (3:1, 1.8 mL THF, 0.600 mL water) at room temperature was added 1 M NaOH (116 uL, 0.116 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 2 h quenched with 1 M HCl (1 mL) and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, rinsed with ether, and dried using high vacuum to afford **2.28** (38.4 mg, 0.0890 mmol) as an impure (unknown impurity remained) off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.25 ($CDCl_3$ overlap, m, 5 H), 6.43 (s, 1 H), 5.33 (s, 1 H), 3.78-3.70 (m, 1 H), 3.71 (s, 3 H), 3.44-3.36 (m, 1 H), 3.28 (s, 3 H), 3.15-3.05 (m, 2 H), 2.47 (s, 3 H), 1.76-1.65 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 165.9, 164.7, 156.7, 155.1, 148.6, 141.0, 128.9, 128.3, 126.3, 105.0, 99.5, 58.8, 51.3, 44.8, 35.6, 31.7, 26.7, 16.5; HRMS (EI^+) *m/z* calcd for $C_{21}H_{28}N_3O_7$ ($[M+H]^+$) 432.1765, found 432.1745.



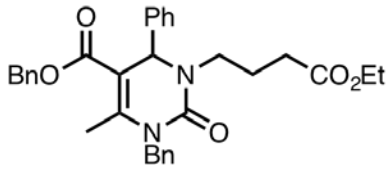
2.29

Benzyl-1-benzyl-3-(2-ethoxy-2-oxoethyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.29). To a solution of **2.1** (202 mg, 0.490 mmol, 1.0 equiv) in DMF (5.0 mL, 0.1 M, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added sodium hydride (33.0 mg, 0.825 mmol, 1.7 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and ethyl bromoacetate (60.0 µL, 0.532 mmol, 1.1 equiv) was added drop-wise as a DMF (0.410 mL, 1.3 M, dry) solution. The reaction mixture was stirred at 0 °C for 3.5 h, quenched using sat.'d NH_4Cl (10 mL) and water (10 mL) and extracted with EtOAc (20 mL, 3x). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried under high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.29** (195 mg, 0.391 mmol, 80%) as an off-white solid: MP 75-77 °C; IR (neat) 3075, 2982, 2919, 1750, 1668, 1612, 1456, 1385, 1191, 1072, 1027, 701 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.23 ($CDCl_3$ overlap, m, 5 H), 7.23-7.18 (m, 6 H), 7.18-7.09 (m, 4 H), 5.40 (s, 1 H), 5.20, 4.96 (AB, 2 H, *J* = 16.8 Hz), 5.10, 5.02 (AB, 2 H, *J* = 12.3), 4.52 (d, 1 H, *J* = 17.4 Hz), 4.24-4.07 (m, 2 H), 3.56 (d, 1H, *J* = 17.4 Hz), 2.47 (s, 3), 1.22 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 165.5, 153.2, 149.0, 140.5, 137.9, 135.9, 128.7, 128.6, 128.4, 128.2, 128.1, 127.3, 127.2, 126.6, 104.1, 66.2, 61.2, 60.3, 48.2, 46.9, 16.7, 14.1; HRMS (EI^+) *m/z* calcd for $C_{30}H_{31}N_2O_5$ ($[M+H]^+$) 499.2227, found 499.2216.



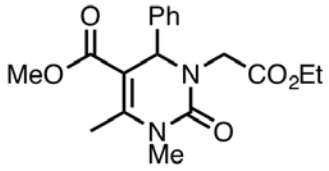
2.30

Benzyl-1-benzyl-3-(3-methoxy-3-oxopropyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.30). To a solution of **2.1** (101 mg, 0.245 mmol, 1.0 equiv) in DMF (2.7 mL, dry, 0.09 M) at 0 °C in a flame-dried flask under an Ar atmosphere was added sodium hydride (18.3 mg, 0.458 mmol, 1.9 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and methyl 3-bromopropionate (32.0 µL, 0.294 mmol. 1.2 equiv) was added drop-wise as a DMF (224 µL, dry, 1.3 M) solution. The reaction mixture was stirred at 0 °C for ~2.5 h and warmed to room temperature overnight. The reaction mixture was quenched using sat.'d NH_4Cl (2 mL) and water (2 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.30** (78.7 mg, 0.158 mmol, 64%) as an opaque oil residue: IR (neat) 3033, 2951, 1737, 1668, 1618, 1385, 1210 1135, 1040, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5 H), 7.30-7.21 ($CDCl_3$ overlap, m, 5 H), 7.21-7.10 (m, 5 H), 5.52 (s, 1 H), 5.23, 4.94 (AB, 2 H, J = 16.4 Hz), 5.18, 5.13 (AB, 2 H, J = 12.4 Hz), 3.97-3.88 (m, 1 H), 3.63 (s, 3 H), 3.41-3.32 (m, 1 H), 2.80-2.71 (m, 1 H), 2.54-2.46 (m, 1 H), 2.46 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 165.5, 153.3, 149.3, 141.3, 137.8, 135.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.1, 126.8, 126.5, 104.3, 66.1, 59.8, 51.6, 46.6, 43.9, 32.9, 16.6; HRMS (EI^+) *m/z* calcd for $C_{30}H_{31}N_2O_5$ ($[M+H]^+$) 499.2277, found 499.2220.



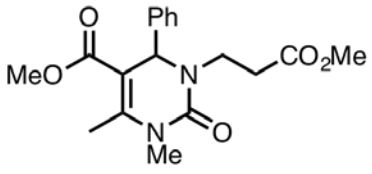
2.31

Benzyl-1-benzyl-3-(4-ethoxy-4-oxobutyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.31). To a solution of **2.1** (102 mg, 0.247 mmol, 1.0 equiv) in DMF (2.7 mL, dry, 0.09 M) at 0 °C in a flame-dried flask under an Ar atmosphere was added sodium hydride (16.8 mg, 0.420 mmol, 1.7 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and ethyl 4-bromobutyrate (42.0 µL, 0.279 mmol, 1.1 equiv) was added drop-wise as a DMF (224 µL, dry, 1.3 M) solution. The reaction mixture was stirred at 0 °C for ~2.5 h and warmed to room temperature overnight. The reaction mixture was quenched using sat.d' NH_4Cl (2 mL) and water (2 mL) and extracted using EtOAc (10 mL, 3x). The combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.31** (12.0 mg, 0.0228 mmol, 22%) as an opaque oil gum: IR (neat) 3025, 2932, 1726, 1668, 1618, 1456, 1385, 1204, 1135, 1066, 706, 701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35-7.26 (m, 6 H), 7.26-7.15 ($CDCl_3$ overlap, m, 4 H), 7.15-7.06 (m, 5 H), 5.39 (s, 1 H), 5.21, 4.90 (AB, 2 H, *J* = 16.5 Hz), 5.18, 5.09 (AB, 2 H, *J* = 12.5 Hz), 4.10 (q, 2 H, *J* = 7.0 Hz), 3.90-3.82 (m, 1 H), 2.95-2.88 (m, 1 H), 2.43 (s, 3 H), 2.38-2.26 (m, 2 H), 2.00-1.90 (m, 1 H), 1.90-1.81 (m, 1 H), 1.23 (t, 3 H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 165.7, 153.6, 149.7, 141.2, 138.0, 135.9, 128.6, 128.5, 128.2, 128.1, 127.8, 127.1, 126.8, 126.6, 104.2, 66.2, 60.4, 58.6, 46.8, 46.3, 31.5, 22.9, 16.7, 14.2; HRMS (EI^+) *m/z* calcd for $C_{32}H_{35}N_2O_5$ ($[M+H]^+$) 527.2540, found 527.2534.



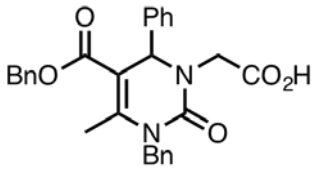
2.32

Methyl-3-(2-ethoxy-2-oxoethyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.32). To a solution of **2.19** (0.150 g, 0.576 mmol, 1.0 equiv) in DMF (6.4 mL, dry, 0.09 M) at 0 °C in a flame-dried flask under an Ar atmosphere was added sodium hydride (32.9 mg, 0.823 mmol, 1.4 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and methyl 3-bromopropionate (78.0 uL, 0.692 mmol. 1.2 equiv) was added drop-wise as a DMF (532 uL, dry, 1.3 M) solution. The reaction mixture was stirred at 0 °C for ~3.5 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (10 mL) and water (10 mL), and extracted using EtOAc (20 mL, 3x). The combined organic extracts were washed with brine (30 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue (0.240 g, 145% mass recovery). The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.32** (166 mg, 0.479 mmol, 83%) as an opaque gum: IR (neat) 2999, 2943, 1750, 1670, 1625, 1452, 1279, 1191, 1066, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34-7.15 ($CDCl_3$ overlap, m, 5 H), 5.35 (s, 1 H), 4.53, 3.50 (AB, 2 H, J = 17.4 Hz), 4.28-4.05 (m, 2 H), 3.64 (s, 3 H), 3.26 (s, 3 H), 2.48 (s, 3 H), 1.22 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 166.2, 153.5, 149.1, 140.8, 128.7, 128.1, 126.9, 103.9, 61.2, 60.0, 51.3, 47.8, 31.2, 16.7, 14.1; HRMS (EI^+) *m/z* calcd for $C_{18}H_{23}N_2O_5$ ($[M+H]^+$) 347.1601, found 347.1596.



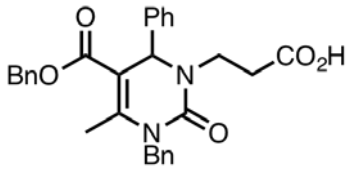
2.33

Ethyl-3-(3-methoxy-3-oxopropyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.33). To a solution of **2.19** (0.150 g, 0.576 mmol, 1.0 equiv) in DMF (6.4 mL, dry, 0.09 M) at 0 °C in a flame-dried flask under an Ar atmosphere was added sodium hydride (41.5 mg, 1.04 mmol, 1.8 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and methyl 3-bromopropionate (75.0 uL, 0.687 mmol, 1.2 equiv) was added dropwise as a DMF (532 uL, dry, 1.3 M) solution. The reaction mixture was stirred at 0 °C for 2 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (10 mL,3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.33** (121 mg, 0.349 mmol, 61%) as a clear oil: IR (neat) 3033, 2951, 1737, 1663, 1631, 1435, 1279, 1204, 1154, 1059, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34-7.15 ($CDCl_3$ overlap, m, 5 H), 5.42 (s, 1 H), 3.88-3.78 (m, 1 H), 3.69 (s, 3 H), 3.63 (s, 3 H), 3.37-3.24 (m, 1 H), 3.24 (s, 3 H), 2.75-2.63 (m, 1 H), 2.51-2.41 (m, 1 H), 2.45 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 166.1, 153.5, 149.2, 141.5, 128.5, 127.7, 126.3, 103.9, 59.6, 51.5, 51.1, 43.6, 32.8, 30.8, 16.4; HRMS (EI^+) *m/z* calcd for $C_{18}H_{23}N_2O_5$ ($[M+H]^+$) 347.1601, found 347.1584.



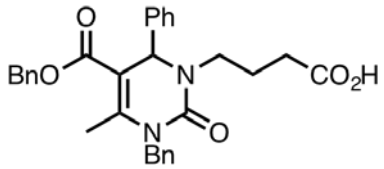
2.34

2-(3-Benzyl-5-((benzyloxy)carbonyl)-4-methyl-2-oxo-6-phenyl-2,3-dihydropyrimidin-1(6H)-yl)acetic acid (2.34). To a solution of **2.29** (57.1 mg, 0.115 mmol, 1.0 equiv) in 3:1 THF/water (2.2 mL THF, 725 µL water, 0.04 M) was added 1 M NaOH (138 µL, 0.138 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 4 h, quenched using 1 M HCl (1 mL) and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford an off-white foam. The resulting foam was rinsed with ether concentrated under reduced pressure and dried using high vacuum to afford **2.34** (45.1 mg, 0.0959 mmol, 84%) as a white foam: IR (neat) 3033, 2924, 1700, 1668, 1630, 1456, 1392, 1210, 1135, 1085, 746, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.19 ($CDCl_3$ overlap, m, 11 H), 7.17-7.11 (m, 4 H), 5.40 (s, 1 H), 5.18, 4.98 (AB, 2 H, *J* = 16.4 Hz), 5.10, 5.02 (AB, 2 H, *J* = 12.0 Hz), 4.39, 3.79 (AB, 2 H, *J* = 17.6 Hz), 2.48 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 165.4, 153.7, 148.7, 140.1, 137.5, 135.7, 128.8, 128.5, 128.4, 128.3, 128.2, 127.4, 127.3, 126.6, 104.5, 66.3, 60.8, 48.6, 47.0, 16.7; HRMS (EI^+) *m/z* calcd for $C_{28}H_{27}N_2O_5$ ($[M+H]^+$) 471.1914, found 471.1899.



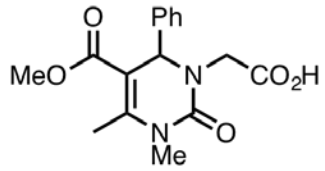
2.35

3-(3-Benzyl-5-((benzyloxy)carbonyl)-4-methyl-2-oxo-6-phenyl-2,3-dihydropyrimidin-1(6H)-yl)propanoic acid (2.35). To a solution of **2.30** (14.9 mg, 0.0300 mmol, 1.0 equiv) in THF/water (3:1, 564 µL THF, 188 µL water) was added 1 M NaOH (36.0 µL, 0.036 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 2.5 h, quenched using 1 M HCl (0.5 mL), and extracted using EtOAc (3 mL, 3x). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford **2.35** (13.4 mg, 0.0277 mmol, 93%) as a white foam: IR (neat) 3202, 3033, 2924, 1737, 1694, 1631, 1456, 1316, 1210, 1178, 1141, 1085, 738, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34-7.27 (m, 2 H), 7.26-7.10 ($CDCl_3$ overlap, m, 8 H), 7.10-7.00 (m, 5 H), 5.44 (s, 1 H), 5.15, 4.86 (AB, 2 H, *J* = 16.4 Hz), 5.10, 5.02 (AB, 2 H, *J* = 12.4 Hz), 3.82-3.75 (m, 1 H), 3.48-3.28 (m, 1 H), 2.73-2.66 (m, 1 H), 2.48-2.40 (m, 1 H), 2.38 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 165.6, 153.6, 149.3, 141.2, 137.8, 135.9, 128.7, 128.5, 128.3, 128.1, 128.0, 127.2, 126.8, 126.6, 104.6, 66.3, 60.2, 46.7, 43.8, 32.9, 16.7; HRMS (EI^+) *m/z* calcd for $C_{29}H_{29}N_2O_5$ ($[M+H]^+$) 485.2071, found 485.2061.



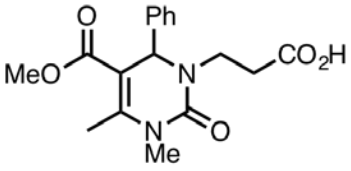
2.36

4-(3-Benzyl-5-((benzyloxy)carbonyl)-4-methyl-2-oxo-6-phenyl-2,3-dihydropyrimidin-1(6H)-yl)butanoic acid (2.36). To a solution of **2.31** (29.0 mg, 0.0551 mmol, 1.0 equiv) in THF/water (3:1, 1.0 mL THF, 345 µL water) was added 1 M NaOH (66.0 µL, 0.0661 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 3.5 h, additional 1 M NaOH (20.0 µL, 0.02 mmol, 0.4 equiv) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched using 1 M HCl (1 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford **2.36** (20.0 mg, 0.0401 mmol, 73%) as a white foam: IR (neat) 3170, 3070, 3025, 2919, 2850, 1700, 1668, 1625, 1456, 1392, 1204, 1135, 1077, 733, 695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34-7.10 ($CDCl_3$ overlap, m, 11 H), 7.10-7.00 (m, 4 H), 5.33 (s, 1 H), 5.14, 4.84 (AB, 2 H, *J* = 16.2 Hz), 5.11, 5.02 (AB, 2 H, *J* = 12.3 Hz), 3.86-3.77 (m, 1 H), 2.96-2.87 (m, 1 H), 2.36 (s, 3 H), 2.36-2.27 (m, 2 H), 1.89-1.75 (m, 2 H; ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 165.6, 153.9, 149.6, 141.1, 137.9, 135.9, 128.7, 128.6, 128.5, 128.3, 128.1, 127.9, 127.2, 127.0, 126.8, 126.6, 104.4, 66.3, 58.7, 46.8, 46.2, 31.2, 22.8, 16.7; HRMS (EI^+) *m/z* calcd for $C_{30}H_{31}N_2O_5$ ($[M+H]^+$) 499.2227, found 499.2216.



2.37

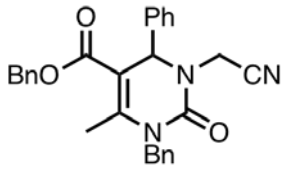
2-(5-(Methoxycarbonyl)-3,4-dimethyl-2-oxo-6-phenyl-3,6-dihydropyrimidin-1(2H)-yl)acetic acid (2.37). To a solution of **2.32** (72.0 mg, 0.208 mmol, 1.0 equiv) in THF/water (3:1, 3.9 mL THF, 1.3 mL water) was added 1 M NaOH (0.250 mL, 0.250 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 2.5 h, quenched using 1 M HCl (3 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, rinsed with ether, and dried using high vacuum to afford **2.37** (66.9 mg, 0.210 mmol, 101%) as a white foam: IR (neat) 3051, 2956, 2837, 1700, 1631, 1480, 1305, 1215, 1072, 751, 733 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.27 (m, 3 H), 7.24-7.18 (m, 2 H), 5.34 (s, 1 H), 4.49, 3.63 (AB, 2 H, *J* = 17.4 Hz), 3.66 (s, 3 H), 3.29 (s, 3 H), 2.50 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 166.2, 153.9, 148.8, 140.5, 128.9, 128.4, 126.9, 104.2, 60.4, 51.4, 48.2, 31.3, 16.8; HRMS (EI^+) *m/z* calcd for $C_{16}H_{19}N_2O_5$ ($[M+H]^+$) 319.1288, found 319.1283.



2.38

3-(5-(Methoxycarbonyl)-3,4-dimethyl-2-oxo-6-phenyl-3,6-dihydropyrimidin-1(2H)-yl)propanoic acid (2.38). To a solution of **2.33** (81.1 mg, 0.234 mmol, 1.0 equiv) in THF/water (3:1 THF/water, 4.4 mL THF, 1.5 mL water, 0.04 M) was added 1 M NaOH (281 uL, 0.281 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for ~3 h, quenched using

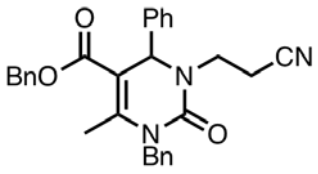
1 M HCl (4 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, rinsed with ether, and dried using high vacuum to afford **2.38** (80.6 mg, 0.243 mmol, 104%) as a white foam: IR (neat) 3316, 3070, 2951, 1694, 1625, 1461, 1310, 1191, 1172, 1122, 1027, 839, 733 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.85 (bs, 1 H), 7.35-7.15 ($CDCl_3$ overlap, m, 5 H), 5.44 (s, 1 H), 3.86-3.76 (m, 1 H), 3.68 (s, 3 H), 3.38-3.25 (m, 1 H), 3.23 (s, 3 H), 2.78-2.65 (m, 1 H), 2.54-2.45 (m, 1 H), 2.45 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 166.2, 153.9, 149.2, 141.4, 128.7, 127.9, 126.3, 104.2, 59.8, 51.3, 43.6, 32.9, 30.9, 16.5; HRMS (EI^+) *m/z* calcd for $C_{17}H_{21}N_2O_5$ ($[M+H]^+$) 333.1445, found 333.1439.



2.39

Benzyl-1-benzyl-3-(cyanomethyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.39). To a solution of **2.1** (0.100 g, 0.242 mmol, 1.0 equiv) in MECN (1.0 mL, 0.24 M, dry) and DMF (0.5 mL, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added NaH (15.0 mg, 0.375 mmol, 1.5 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred until bubbling stopped and chloroacetonitrile (19.0 µL, 0.297 mmol, 1.2 equiv) was added as a DMF (0.200 mL, 1.3 M, dry) solution. The reaction mixture was stirred at 0 C for ~2 h, warmed to room temperature for 2 h, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried under high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on

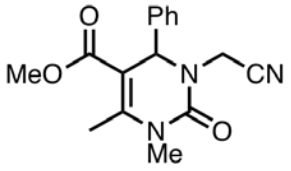
SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.39** (52.7 mg, 0.117 mmol, 48%) as a white foam: IR (neat) 3057, 2945, 2246, 1668, 1618, 1448, 1385, 1204, 1135, 1072, 1027, 733, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41-7.35 (m, 1 H), 7.35-7.26 ($CDCl_3$ overlap, m, 6 H), 7.26-7.21 (m, 4 H), 7.20-7.15 (m, 4 H), 5.53 (s, 1 H), 5.21, 4.90 (AB, 2 H, *J* = 16.5 Hz), 5.14, 5.06 (AB, 2 H, *J* = 12.3 Hz), 4.76 (d, 1 H, *J* = 17.4 Hz), 3.74 (d, 1 H, *J* = 17.4), 2.53 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 152.5, 148.6, 138.7, 137.2, 135.5, 128.9, 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 127.5, 127.2, 126.6, 114.8, 104.4, 66.4, 59.7, 47.1, 34.2, 16.6; HRMS (EI^+) *m/z* calcd for $C_{28}H_{26}N_3O_3$ ($[M+H]^+$) 452.1969, found 452.1961.



2.40

Benzyl-1-benzyl-3-(2-cyanoethyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.40). To a solution of **2.1** (0.150 g, 0.364 mmol, 1.0 equiv) in DMF (1.5 mL, 0.24 M, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added NaH (21.8 mg, 0.545 mmol, 1.5 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred until bubbling stopped and chloroacetonitrile (37.0 uL, 0.436 mmol, 1.2 equiv) was added as a DMF (335 uL, 1.3 M, dry). The reaction mixture was stirred at 0 °C for ~3.25 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.40** (127 mg, 0.273 mmol,

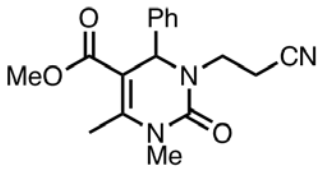
75%) as a white foam: IR (neat) 3038, 2956, 2253, 2253, 1663, 1625, 1456, 1385, 1204, 1135, 1072, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42-7.25 ($CDCl_3$ overlap, m, 10 H), 7.25-7.12 (m, 5 H), 5.49 (s, 1 H), 5.23, 4.98 (AB, 2 H, *J* = 16.5 Hz), 5.18, 5.10 (AB, 2 H, *J* = 12.3 Hz), 3.91-3.78 (m, 1 H), 3.42-3.28 (m, 1 H), 2.85-2.70 (m, 1 H), 2.50 (s, 3 H), 2.43-2.32 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 153.1, 148.9, 140.7, 137.5, 135.6, 128.7, 128.6, 128.3, 128.2, 128.0, 127.2, 126.7, 126.4, 117.6, 104.4, 66.2, 60.5, 46.6, 44.0, 16.5; HRMS (EI^+) *m/z* calcd for $C_{29}H_{28}N_3O_3$ ($[M+H]^+$) 466.2125, found 466.2119.



2.41

Methyl 3-(cyanomethyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.41). To a solution of **2.19** (151 mg, 0.580 mmol, 1.0 equiv) in DMF (2.4 mL, 0.24 M, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added NaH (36.0 mg, 0.900 mmol, 1.6 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred until bubbling stopped and chloroacetonitrile (44.2 uL, 0.691 mmol, 1.2 equiv) was added as a DMF (532 uL, 1.3 M, dry) solution. The reaction mixture was stirred at 0 °C for ~2 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue (236 mg, 130% mass recovery). The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.41** (92.7 mg, 0.310 mmol, 53%) as a white foam: IR (neat) 2956, 2913, 1705, 1676, 1637, 1456, 1286, 1210, 1066, 751, 706 cm^{-1}; ^1H NMR (400

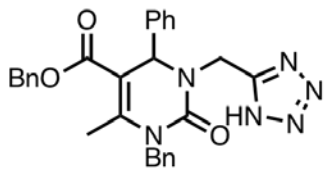
MHz, $CDCl_3$) δ 7.38-7.30 (m, 3H), 7.25-7.22 (m, 2 H), 5.45 (s, 1 H), 4.73 (d, 1 H, *J* = 17.6 Hz), 3.70 (d, 1 H, *J* = 19.2 Hz), 3.67 (s, 3 H), 3.31 (s, 3 H), 2.52 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 152.7, 148.6, 139.1, 129.1, 128.8, 126.9, 115.0, 104.3, 59.5, 51.5, 34.2, 31.3, 16.7; HRMS (EI^+) *m/z* calcd for $C_{17}H_{20}N_3O_3$ ($[M+H]^+$) 314.1499, found 314.1493.



2.42

Methyl-3-(2-cyanoethyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.42). To a solution of **2.19** (0.151 g, 0.580 mmol, 1.0 equiv) in DMF (2.4 mL, 0.24 M, dry) in a flame-dried flask under an Ar atmosphere at 0 °C was added NaH (39.0 mg, 0.975 mmol, 1.7 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred until bubbling stopped and chloroacetonitrile (58.5 uL, 0.692 mmol, 1.2 equiv) was added as a DMF (532 uL, 1.3 M, dry). The reaction mixture was stirred at 0 °C for ~2 h, warmed to room temperature overnight, quenched using sat.'d NH_4Cl (5 mL) and water (5 mL), and extracted using EtOAc (10 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue (236 mg, 130% mass recovery). The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.42** (83.9 mg, 0.268 mmol, 48%) as a clear gum: IR (neat) 3033, 2956, 2253, 1700, 1663, 1631, 1435, 1210, 1059, 1021, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.27 (m, 3 H), 7.22-7.18 (m, 2 H), 5.40 (s, 1 H), 3.80 (ddd, 1 H, *J* = 13.6, 8.0, 4.8 Hz), 3.69 (s, 3 H), 3.29 (ddd, 1 H, *J* = 14.8, 7.2, 4.8 Hz), 3.27 (s, 3 H), 2.73 (ddd, 1 H, *J* = 16.8, 8.0, 8.0 Hz), 2.36 (ddd, 1 H, *J* = 16.8, 4.8, 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 153.5,

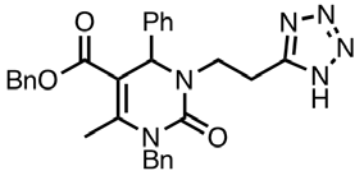
149.0, 141.2, 129.0, 128.3, 126.5, 117.8, 104.3, 60.5, 51.4, 44.1, 31.0, 16.7, 16.6; HRMS (EI^+) *m/z* calcd for $C_{16}H_{18}N_3O_3$ ($[M+H]^+$) 300.1343, found 300.1337.



2.43

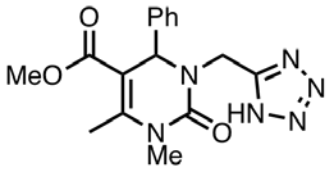
Benzyl-3-((1H-tetrazol-5-yl)methyl)-1-benzyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.43). To a solution of **2.39** (54.9 mg, 0.122 mmol, 1.0 equiv) in THF (422 µL, 0.3 M, solvent system) in a microwave vial was added trimethylsilyl azide (66.0 µL, 0.484 mmol, 4.0 equiv) and tetrabutyl ammonium fluoride (122 µL, 0.122 mmol, 1.0 equiv) as a 1.0 M THF solution. The reaction mixture was heated at 90 °C overnight in a sealed tube to afford a clear, light yellow solution. The reaction mixture was quenched using 1 M HCl (0.5 mL) and water (0.5 mL) and extracted using EtOAc (3 mL, 3x). The combined organic extracts were washed with brine (5 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude oil residue (92 mg, 153% mass recovery). The resulting crude residue was re-extracted using 1 M HCl (2 mL) and EtOAc (5 mL). The resulting organic extract was washed with water (3 mL, 2x), washed with brine (3 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high-vacuum to afford **2.43** (35.4 mg, 0.0716 mmol, 59%) as a white foam: IR (neat) 3033, 2937, 2869, 1663, 1631, 1448, 1385, 1204, 1135, 1053, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 13.70 (bs, 1 H), 7.37-7.23 ($CDCl_3$ overlap, m, 7 H), 7.23-7.10 (m, 8 H), 5.55 (s, 1 H), 5.12, 5.02 (AB, 2 H, *J* = 17.1 Hz), 5.11, 5.03 (AB, 2 H, *J* = 12.3 Hz), 4.74, 4.52 (AB, 2 H, *J* = 15.3 Hz), 2.46 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 154.4, 152.2, 148.0, 139.6, 136.9, 135.4, 128.8, 128.6, 128.5, 128.3, 128.2, 127.5, 127.1,

126.1, 105.2, 66.5, 61.1, 47.1, 40.5, 16.5; HRMS (EI^+) *m/z* calcd for $C_{28}H_{27}N_6O_3$ ($[M+H]^+$) 495.2139, found 495.2132.



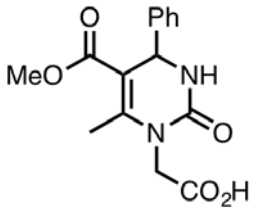
2.44

Benzyl-3-(2-(1H-tetrazol-5-yl)ethyl)-1-benzyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.44). To a solution of **2.40** (89.0 mg, 0.191 mmol, 1.0 equiv) in THF (637 uL, 0.3 M) in a microwave vial was added trimethylsilyl azide (104 uL, 0.751 mmol, 3.9 equiv) and tetrabutyl ammonium fluoride (191 uL, 0.191 mmol, 1.0 equiv) as a 1.0 M THF solution. The reaction mixture was heated at 90 °C overnight in a sealed tube, quenched using 1 M HCl (5 mL) and extracted using EtOAc (10 mL, 3x). The combined organic extracts were washed with water (10 mL, 2x) and brine (10 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude oil residue. The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, CH_2Cl_2/MeOH, 0-10% MeOH gradient) to afford **2.44** (18.6 mg, 0.0366 mmol, 19%) as a white foam and recovered starting material (28.9 mg, 0.0621 mmol, 32% recovery): IR (neat) 3144, 3033, 2924, 1668, 1631, 1456, 1385, 1204, 1135, 725, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5 H), 7.25-7.10 (m, 6 H), 7.10-7.02 (m, 4 H), 5.40 (s, 1 H), 5.16, 5.06 (AB, 2 H, *J* = 12.3 Hz), 5.12, 4.98 (AB, 2 H, *J* = 16.2 Hz), 4.02-3.91 (m, 1 H), 3.69-3.58 (m, 1 H), 3.23-3.10 (m, 1 H), 3.09-2.98 (m, 1 H), 2.41 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 155.0, 154.0, 148.2, 140.5, 137.0, 135.5, 128.9, 128.6, 128.5, 128.4, 127.5, 126.6, 126.2, 105.2, 66.6, 60.0, 47.2, 44.6, 23.2, 16.6; HRMS (EI^+) *m/z* calcd for $C_{29}H_{29}N_6O_3$ ($[M+H]^+$) 509.2296, found 509.2291.



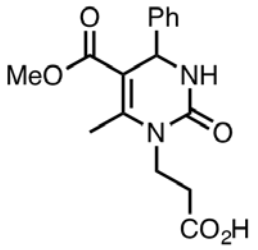
2.45

Methyl-3-((1H-tetrazol-5-yl)methyl)-1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.45). To a solution of **2.41** (83.0 mg, 0.277 mmol, 1.0 equiv) in THF (0.890 mL, 0.3 M, dry-solvent system) in a microwave vial was added trimethylsilyl azide (154 uL, 1.07 mmol, 4.0 equiv) and tetrabutyl ammonium fluoride (277 uL, 0.267 mmol, 1.0 equiv) as a 1.0 M THF solution. The reaction mixture was heated at 90 °C overnight in a sealed tube, quenched using 1 M HCl (5 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extract was washed with water (10 mL), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue (77.3 mg, 82% mass recovery). The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, CH_2Cl_2/MeOH, 0-10% MeOH gradient) to afford **2.45** (45.9 mg, 0.134 mmol, 48%) was a white foam: IR (neat) 3133, 3033, 2951, 2876, 1625, 1448, 1286, 1210, 1059, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27-7.23 ($CDCl_3$ overlap, m, 3 H), 7.19-7.15 (m, 2 H), 5.47 (s, 1 H), 4.70 (s, 2 H), 3.66 (s, 3 H), 3.34 (s, 3 H), 2.51 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 154.9, 148.1, 139.9, 129.0, 128.7, 126.7, 105.1, 61.4, 51.5, 41.2, 31.2, 16.5; HRMS (EI^+) *m/z* calcd for $C_{16}H_{19}N_6O_3$ ($[M+H]^+$) 343.1496, found 343.1496.



2.46

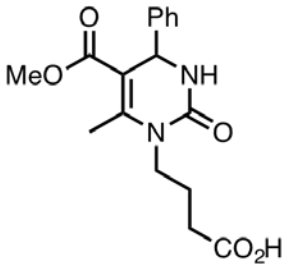
2-(5-(Methoxycarbonyl)-6-methyl-2-oxo-4-phenyl-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (2.46). According to General Procedure A, carbamoylglycine (0.500 g, 4.23 mmol, 1.0 equiv), benzaldehyde (872 uL, 8.47 mmol, 2.0 equiv), methyl acetoacetate (922 uL, 8.47 mmol, 2.0 equiv), and HCl (254 uL, 2.96 mmol, 0.7 equiv) were stirred at room temperature for ~40 h and filtered to afford **2.46** (402 mg, 1.32 mmol, 31%) as a yellow solid: MP 175-177 °C; IR (neat) 3284, 3046, 2937, 1700, 1663, 1618, 1435, 1316, 1204, 1103, 770, 701 cm^{-1}; ^{1}H NMR (300 MHz, d_6-DMSO) δ 12.87 (bs, 1 H), 8.06 (d, 1 H, *J* = 3.0 Hz), 7.37-7.22 (m, 5 H), 5.18 (d, 1 H, *J* = 3.3 Hz), 4.47, 4.33 (AB, 2 H, *J* = 18.3 Hz), 3.56 (s, 3 H), 2.40 (s, 3 H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 171.5, 166.6, 152.9, 149.9, 144.5, 128.9, 127.9, 126.9, 103.1, 53.4, 51.7, 44.5, 27.3, 16.2; HRMS (EI^+) *m/z* calcd for $C_{15}H_{17}N_2O_5$ ($[M+H]^+$) 305.1132, found 305.1124.



2.47

3-(5-(Methoxycarbonyl)-6-methyl-2-oxo-4-phenyl-3,4-dihydropyrimidin-1(2H)-yl)propanoic acid (2.47). According to General Procedure A, 3-ureidopropanoic acid (0.250 g, 1.89 mmol, 1.0 equiv), benzaldehyde (0.390 mL, 3.79 mmol, 2.0 equiv), methyl acetoacetate

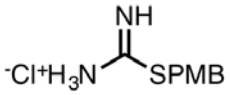
(412 uL, 3.78 mmol, 2.0 equiv), and conc. HCl (114 uL, 1.33 mmol, 0.7 equiv) were stirred at room temperature for 3.5 days and concentrated under reduced pressure. The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, CH_2Cl_2/MeOH, 0-10% MeOH gradient) to afford **2.47** (160 mg, 0.503 mmol, 27%) as pale yellow foam: IR (neat) 3258, 3051, 2945, 1681, 1650, 1430, 1385, 1191, 1103, 1046, 764, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34-7.15 ($CDCl_3$ overlap, m, 5 H), 6.51 (bs, 1 H), 5.36 (d, 1 H, *J* = 3.0 Hz), 4.11-3.90 (m, 2 H), 3.65 (s, 3 H), 2.81-2.68 (m, 1 H), 2.62-2.50 (m, 1 H), 2.54 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 166.4, 154.2, 148.3, 142.7, 128.8, 127.9, 126.1, 105.1, 53.7, 51.5, 38.6, 33.9, 16.1; HRMS (EI^+) *m/z* calcd for $C_{16}H_{19}N_2O_5$ ($[M+H]^+$) 319.1288, found 319.1284.



2.48

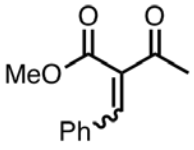
4-(5-(Methoxycarbonyl)-6-methyl-2-oxo-4-phenyl-3,4-dihydropyrimidin-1(2H)-yl)butanoic acid (2.48). According to General Procedure A, 4-ureidobutanoic acid (0.500 g, 3.42 mmol, 1.0 equiv), benzaldehyde (704 uL, 6.84 mmol, 2.0 equiv), methyl acetoacetate (745 uL, 6.84 mmol, 2.0 equiv), and conc. HCl (206 uL, 2.40 mmol, 0.7 equiv) were stirred at room temperature for ~36 h and filtered to afford **2.48** (606 mg, 1.82 mmol, 53%) as a white solid: MP 224-225 °C; IR (neat) 3284, 3221, 3064, 2951, 2505, 1700, 1644, 1474, 1417, 1236, 1191, 1115, 971, 751, 706 cm^{-1}; ^1H NMR (300 MHz, d_6-DMSO) δ 12.07 (s, 1 H), 7.96 (d, 1 H, *J* = 3.6 Hz), 7.35-7.18 (m, 5 H), 3.91-3.78 (m, 1 H), 3.57 (s, 3 H), 3.57-3.45 (m, 1 H), 2.13 (t, 2 H, *J* = 7.2

Hz), 2.09 (s, 3 H), 1.79-1.52 (m, 2 H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 207.0, 174.4, 166.6, 153.2, 150.3, 144.3, 129.0, 127.9, 126.5, 103.4, 52.7, 51.7, 41.5, 31.2, 25.1, 16.1; HRMS (EI^+) *m/z* calcd for $C_{17}H_{21}N_2O_5$ ($[M+H]^+$) 333.1445, found 333.1428.



2.49

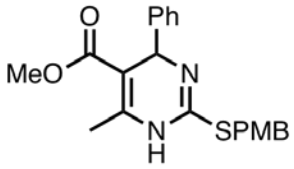
2-(4-Methoxybenzyl)isothiouronium chloride (2.49). To a mixture of thiourea (1.50 g, 19.7 mmol, 1.0 equiv) in THF (9.9 mL, 2.0 M, dry-solvent system) at 0 °C was added 4-methoxybenzyl chloride (2.73 mL, 19.7 mmol, 1.0 equiv) over ~2 min. The reaction mixture was allowed to warm to room temperature, stirred for 2 h, and then heated at 65 °C for 5 h. The resulting white solid was filtered and gently washed with ether to afford **2.49** (4.16 g, 17.9 mmol, 91%) as a white powder: ^{1}H NMR (300 MHz, d_6-DMSO) δ 9.05 (bs, 4 H), 7.34 (d, 2 H, *J* = 8.4 Hz), 6.93 (d, 2 H, *J* = 8.4 Hz), 4.42 (s, 2 H), 3.75 (s, 3 H).



2.50

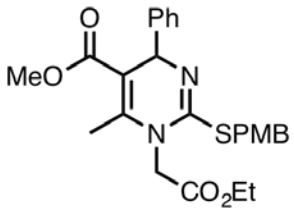
Methyl 2-benzylidene-3-oxobutanoate (2.50). To a solution of benzaldehyde (1.92 mL, 18.5 mmol, 1.0 equiv) and methyl acetoacetate (2.01 mL, 18.5 mmol, 1.0 equiv) in 2-propanol (18.7 mL, 1.0 M) was added piperidine (74.0 uL, 0.749 mmol, 0.04 equiv) and acetic acid (43.0 uL, 0.751 mmol, 0.04 equiv). The reaction mixture was stirred at room temperature for 44 h, concentrated under reduced pressure, and purified using chromatography on SiO_2 (ISCO-Rf, hexanes/ethyl acetate) to afford **2.50** (2.95 g, 14.4 mmol, 78%, 1.8:1 mixture of the Z:E isomers)

as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ Z – major isomer 7.58 (s, 1 H), 7.48-7.35 (m, 5 H), 3.84 (s, 3 H), 2.43 (s, 3 H), E – minor isomer 7.70 (s, 1 H), 7.45-7.33 (m, 5 H), 3.84 (s, 3 H), 2.35 (s, 3 H).



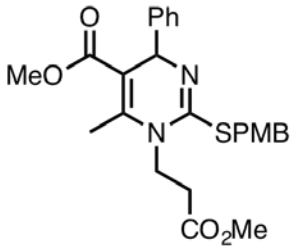
2.51

Methyl-2-((4-methoxybenzyl)thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (2.51).[68] To a solution of **2.50** (0.500 g, 2.45 mmol, 1.0 equiv) and **2.49** (0.569 g, 2.45 mmol, 1.0 equiv) in DMF (6.1 mL dry, 0.4 M) was added sodium acetate (204 mg, 2.49 mmol, 1.0 equiv). The reaction mixture was heated at 75 °C for 5 h, cooled to room temperature, quenched with water (20 mL), and extracted using EtOAc (20 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (ISCO-Rf, hexanes/EtOAc) to afford **2.51** (0.620 g, 1.62 mmol, 66%, mixture of tautomers) as a light yellow foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.20 (m, 5 H), 6.89 (d, 2 H, *J* = 8.1 Hz), 6.74 (d, 2 H, *J* = 8.1 Hz), 5.64 (bs, 1 H), 5.39 (d, 0.2 H, *J* = 2.7 Hz), 4.33, 4.09 (AB, 2 H, *J* = 13.5 Hz), 3.77 (s, 3 H), 3.63 (s, 3 H), 2.33 (s, 3 H).



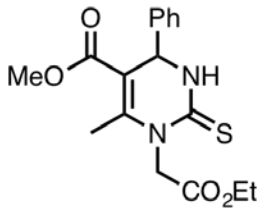
2.52

Methyl-1-(2-ethoxy-2-oxoethyl)-2-((4-methoxybenzyl)thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (2.52). To a solution of **2.51** (148 mg, 0.387 mmol, 1.0 equiv) in DMF (1.3 mL, dry, 0.3 M) under an Ar atmosphere at 0 °C was added NaH (30.0 mg, 0.750 mmol, 1.9 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and ethyl bromoacetate (53.0 uL, 0.470 mmol, 1.2 equiv) was added dropwise as a DMF (361 uL, dry, 1.3 M) solution. The reaction mixture was warmed to room temperature, stirred overnight, quenched using sat.'d NH_4Cl (3 mL) and water (3 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue (210 mg, 116% mass recovery). The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.52** (138 mg, 0.295 mmol, 76%, single isomer) as a yellow oil: IR (neat) 2937, 2837, 1737, 1687, 1599, 1499, 1417, 1241, 1185, 1085, 1027, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33-7.21 ($CDCl_3$ overlap, m, 8 H), 6.84 (d, 2 H, *J* = 8.4 Hz), 5.28 (s, 1 H), 4.42, 4.28 (AB, 2 H, *J* = 13.2 Hz), 4.12, 3.90 (AB, 2 H, *J* = 18.0 Hz), 4.13-3.95 (m, 2 H), 3.76 (s, 3 H), 3.59 (s, 3 H), 1.15 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 161.2, 158.8, 153.9, 141.8, 130.2, 128.8, 128.5, 128.1, 127.3, 113.8, 104.6, 62.4, 61.5, 55.1, 50.8, 50.7, 35.7, 30.8, 23.0, 13.9; HRMS (EI^+) *m/z* calcd for $C_{25}H_{29}N_2O_5S$ ($[M+H]^+$) 469.1792, found 469.1781.



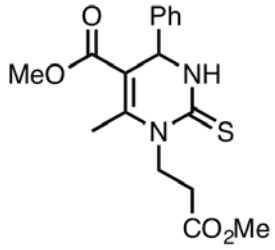
2.53

Methyl-1-(3-methoxy-3-oxopropyl)-2-((4-methoxybenzyl)thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (2.53). To a solution of **2.51** (0.150 g, 0.392 mmol, 1.0 equiv) in DMF (1.3 mL, dry, 0.3 M) under an Ar atomsphere at 0 °C was added NaH (30.3 mg, 0.758 mmol, 1.9 equiv) as a 60% dispersion in mineral oil. The reaction mixture was stirred at 0 °C until bubbling stopped and methyl 3-bromopropionate (51.0 uL, 0.467 mmol, 1.2 equiv) was added dropwise as a DMF (361 uL, dry, 1.3 M) solution. The reaction mixture was warmed to room temperature, stirred overnight, quenched using sat.'d NH_4Cl (3 mL) and water (3 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was purified using chromatography on SiO_2 (7:3, hexanes/acetone, isocratic) to afford **2.53** (118 mg, 0.252 mmol, 64%) as a yellow oil (partially separable 4.4:1 mixture of N^1- and N^3-isomers): IR (neat) 3308, 3038, 2956, 1694, 1612, 1512, 1435, 1241, 1171, 1096, 1034, 839, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.20 ($CDCl_3$ overlap, m, 7 H), 6.85 (d, 2 H, J = 8.7 Hz), 5.34 (s, 1 H), 4.46, 4.31 (AB, 2 H, J = 13.5), 3.80 (s, 3 H), 3.68-3.58 (m, 2 H), 3.65 (s, 3 H), 3.62 (s, 3 H), 2.71-2.56 (m, 1 H), 2.51-2.39 (m, 1 H), 2.38 (s, 3 H);



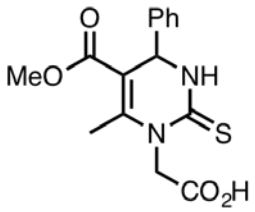
2.54

Methyl-1-(2-ethoxy-2-oxoethyl)-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.54).[68] To a solution of **2.52** (0.122 g, 0.260 mmol, 1.0 equiv) in CH_2Cl_2 (3.06 mL, dry-solvent system, 0.085 M) was added trifluoroacetic acid (100 uL, 1.31 mmol, 5.0 equiv) and ethanethiol (47.0 uL, 0.645 mmol, 2.5 equiv). The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure, and dissolved in EtOAc (10 mL). The solution was extracted using sat.'d $NaHCO_3$ (10 mL), extracted using EtOAc (10 mL, 3x), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was triturated using ether to afford **2.52** (60.0 mg, 0.172 mmol, 66%) as a pale yellow solid: MP 145-146 °C; IR (neat) 3215, 3157, 3083, 2995, 1745, 1676, 1549, 1474, 1254, 1185, 1115, 1027, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (bs, 1 H), 7.40-7.20 ($CDCl_3$ overlap, m, 5 H), 5.46 (s, 1 H), 5.26 (d, 1 H *J* = 17.4 Hz), 4.30-4.13 (m, 2 H), 3.81 (d, 1 H, *J* = 17.4 Hz), 3.67 (s, 3 H), 2.34 (s, 3 H), 1.27 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 167.6, 165.5, 142.1, 140.2, 129.0, 128.6, 127.2, 102.9, 62.2, 61.6, 52.8, 51.4, 18.4, 14.1; HRMS (EI^+) *m/z* calcd for $C_{17}H_{21}N_2O_4S$ ($[M+H]^+$) 349.1217, found 349.1211.



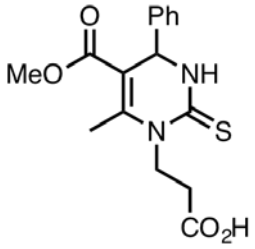
2.55

Methyl-1-(3-methoxy-3-oxopropyl)-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2.55).[68] To a solution of **2.53** (56.9 mg, 0.121 mmol, 1.0 equiv) in CH_2Cl_2 (1.4 mL, dry-solvent system, 0.085 M) was added trifluoroacetic acid (47.0 uL, 0.614 mmol, 5.0 equiv) and ethanethiol (22.0 uL, 0.302 mmol, 2.5 equiv). The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure, and dissolved in EtOAc (5 mL). The solution was extracted using sat.'d $NaHCO_3$ (5 mL), extracted using EtOAc (5 mL, 3x), dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford a crude residue. The resulting crude residue was triturated using ether to afford **2.55** (30.0 mg, 0.0861 mmol, 71%) as a pale yellow foam: IR (neat) 3321, 3020, 2945, 1732, 1663, 1525, 1435, 1241, 1197, 1165, 1109, 757, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1 H), 7.38-7.22 ($CDCl_3$ overlap, m, 5 H), 5.59 (s, 1 H), 4.37-4.26 (m, 1 H), 3.70 (s, 3 H), 3.62 (s, 3 H), 3.01-2.98 (m, 1 H), 2.65-2.55 (m, 1 H), 2.30 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 171.8, 165.6, 142.5, 140.9, 129.8, 128.9, 128.4, 126.9, 114.0, 113.8, 102.8, 62.1, 51.8, 51.4, 48.5, 31.8, 30.9; HRMS (EI^+) *m/z* calcd for $C_{17}H_{21}N_2O_4S$ ($[M+H]^+$) 349.1217, found 349.1211.



2.56

2-(5-(Methoxycarbonyl)-6-methyl-4-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (2.56). To a solution of **2.54** (40.9 mg, 0.117 mmol, 1.0 equiv) in THF/water (3:1, 2.19 mL THF, 731 uL THF, 0.04 M) was added 1 M NaOH (141 uL, 0.141 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 2 h, quenched using 1 M HCl (2 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford **2.56** (38.6 mg, 0.120 mmol, 103%) as a white foam: IR (neat) 3441, 3215, 2969, 1676, 1536, 1456, 1385, 1210, 1103, 1046, 701 cm^{-1}; ^1H NMR (300 MHz, d_4-MeOH) δ 7.39-7.26 (m, 5 H), 5.48 (s, 1 H), 5.22 (d, 1 H, *J* = 17.4 Hz), 3.81 (d, 1 H, *J* = 17.4 Hz), 3.63 (s, 3 H), 2.32 (s, 3 H); ^{13}C NMR (100 MHz, d_4-MeOH) δ 178.0, 171.4, 167.5, 145.2, 142.5, 130.0, 129.6, 128.4, 103.4, 63.1, 53.5, 49.8, 17.7; HRMS (EI^+) *m/z* calcd for $C_{15}H_{17}N_2O_4S$ ($[M+H]^+$) 321.0904, found 321.0898.



2.57

3-(5-(Methoxycarbonyl)-6-methyl-4-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoic acid (2.57). To a solution of **2.55** (20.2 mg, 0.0580 mmol, 1.0 equiv) in THF/water (3:1, 1.07 mL THF, 356 uL THF, 0.04 M) was added 1 M NaOH (69.0 uL, 0.0690 mmol, 1.2

equiv). The reaction mixture was stirred at room temperature for 2 h, quenched using 1 M HCl (1 mL), and extracted using EtOAc (5 mL, 3x). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried using high vacuum to afford **2.57** (17.0 mg, 0.0508 mmol, 88%) as a white foam: IR (neat) 3396, 3075, 2919, 1705, 1655, 1530, 1448, 1228, 1185, 1103, 1027, 807, 706 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (bs, 1 H), 7.36-7.28 (m, 5 H), 5.63 (s, 1 H), 4.33-4.25 (m, 1 H), 3.75-3.65 (m, 1 H), 3.69 (s, 3 H), 3.09-2.99 (m, 1 H), 2.67-2.59 (m, 1 H), 2.31 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 175.4, 165.6, 142.5, 140.8, 129.0, 128.5, 126.8, 102.9, 62.3, 51.5, 48.3, 31.5, 18.3; HRMS (EI^+) *m/z* calcd for $C_{16}H_{19}N_2O_4S$ ($[M+H]^+$) 335.1060, found 335.1051.

BIBLIOGRAPHY

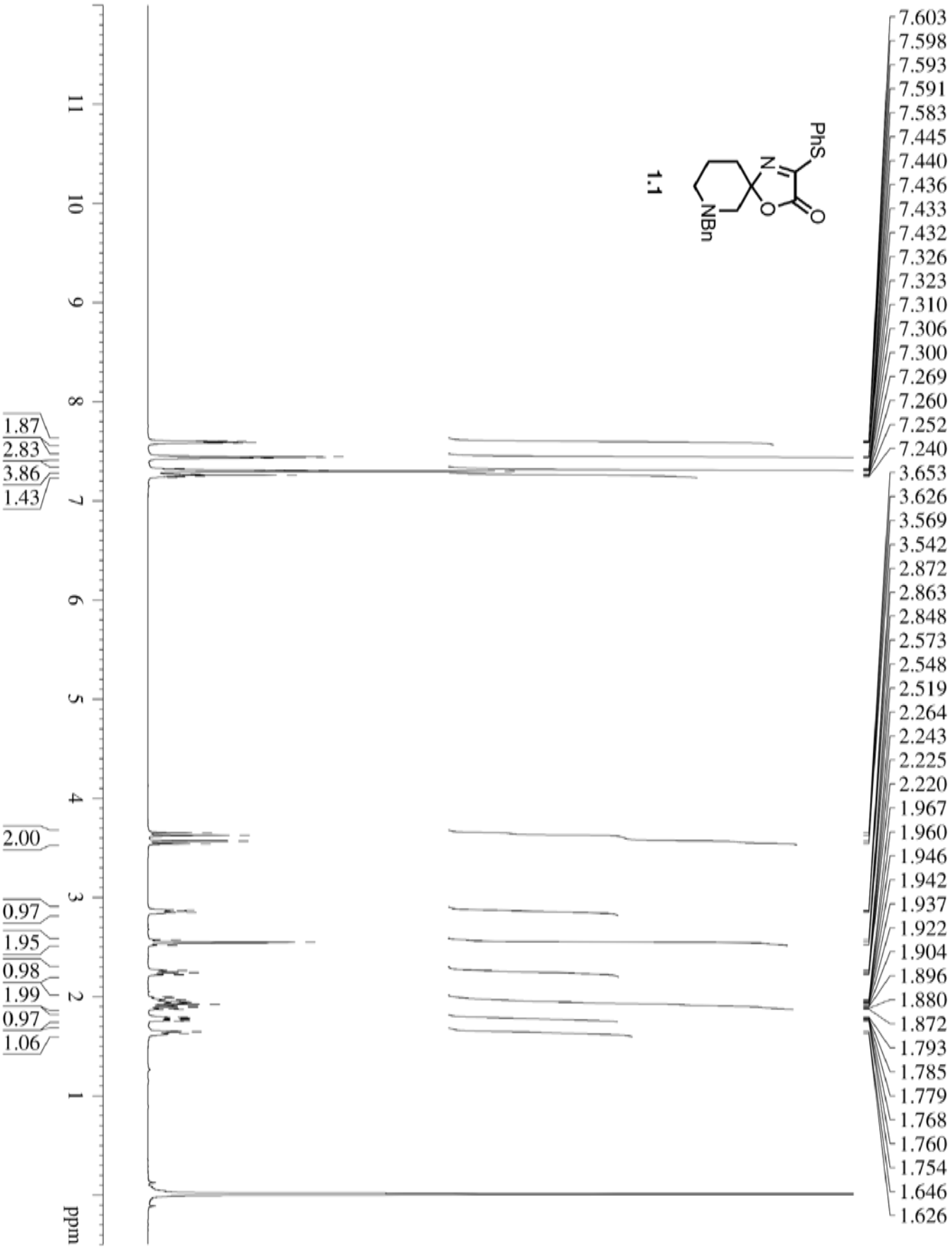
1. Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652.
2. Cougnon, F. B. L.; Sanders, J. K. M. *Acc. Chem. Res.* **2011**,
3. Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 2455.
4. Ladame, S. *Org. Biomol. Chem.* **2008**, *6*, 219.
5. Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Drug Discovery Today* **2002**, *7*, 117.
6. Hof, F.; Nuckolls, C.; Rebek, J. *J. Am. Chem. Soc.* **2000**, *122*, 4251.
7. Huc, I.; Krische, M. J.; Funeriu, D. P.; Lehn, J.-M. *Eur. J. Inorg. Chem.* **1999**, *1999*, 1415.
8. Hiraoka, S.; Kubota, Y.; Fujita, M. *Chem. Commun.* **2000**, 1509.
9. Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106.
10. Zameo, S.; Vauzeilles, B.; Beau, J.-M. *Angew. Chem. Int. Ed.* **2005**, *44*, 965.
11. Ludlow, R. F.; Otto, S. *J. Am. Chem. Soc.* **2010**, *132*, 5984.
12. Hochgürtel, M.; Kroth, H.; Piecha, D.; Hofmann, M. W.; Nicolau, C.; Krause, S.; Schaaf, O.; Sonnenmoser, G.; Eliseev, A. V. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3382.
13. Brisig, B.; Sanders, J. K. M.; Otto, S. *Angew. Chem. Int. Ed.* **2003**, *42*, 1270.
14. Vial, L.; Sanders, J. K. M.; Otto, S. *New J. Chem.* **2005**, *29*, 1001.
15. Gasparini, G.; Prins, L. J.; Scrimin, P. *Angew. Chem. Int. Ed.* **2008**, *47*, 2475.
16. Saiz, C.; Wipf, P.; Manta, E.; Mahler, G. *Org. Lett.* **2009**, *11*, 3170.
17. Wipf, P.; Mahler, S. G.; Okumura, K. *Org. Lett.* **2005**, *7*, 4483.
18. Liang, M.; Saiz, C.; Pizzo, C.; Wipf, P. *Tetrahedron Lett.* **2009**, *50*, 6810.
19. Boonya-udtayan, S.; Wipf, P., Unpublished Results.
20. Pizzo, C., Wipf, P., Unpublished Results.
21. Vaughan, W. R., Pyrrole and Pyrrole Derivatives. In *Kirk-Othmer Encyclopedia of Chemical Technology*, John Wiley & Sons, Inc.: New Jersey, 2012.
22. *Scigress*, 7.7.0.47; Fujitsu Limited: 2007.
23. Koplow, D. A., *Smallpox: The Fight to Eradicate a Global Scourge*. University of California Press: Berkeley, 2004.
24. Henderson Da, I. T. V. B. J. G.; et al. *J. Am. Med. Assoc.* **1999**, *281*, 2127.
25. Smallpox. http://www.who.int/mediacentre/factsheets/smallpox/en/ (accessed 9-19-12).
26. B., G. J. H. Colonial Germ Warfare *Colonial Willamsburg* [Online], 2003.
27. Alibek, K. H., S., *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Programs in the World - Told From the Inside by the Man Who Ran It*. Delta: New York, 1999.
28. Preston, R., *The Demon in the Freezer*. Random House: New York, 2002.

29. Emergency Preparedness and Response. http://www.bt.cdc.gov/bioterrorism/overview.asp (accessed 9-18-12).
30. Grosenbach, D. W.; Jordan, R.; Hruby, D. E. *Future Virol.* **2011**, *6*, 653.
31. Smee, D. F.; Sidwell, R. W.; Kefauver, D.; Bray, M.; Huggins, J. W. *Antimicrob. Agents Chemother.* **2002**, *46*, 1329.
32. Beaud, G. *Biochimie* **1995**, *77*, 774.
33. McDonald, W. F.; Traktman, P. *J. Biol. Chem.* **1994**, *269*, 31190.
34. McDonald, W. F.; Traktman, P. *Protein Expression Purif.* **1994**, *5*, 409.
35. Klemperer, N.; McDonald, W.; Boyle, K.; Unger, B.; Traktman, P. *J. Virol.* **2001**, *75*, 12298.
36. Druck Shudofsky, A. M.; Silverman, J. E. Y.; Chattopadhyay, D.; Ricciardi, R. P. *J. Virol.* **2010**, *84*, 12325.
37. Nuth, M.; Huang, L.; Saw, Y. L.; Schormann, N.; Chattopadhyay, D.; Ricciardi, R. P. *J. Med. Chem.* **2011**, *54*, 3260.
38. Stanitsa, E. S.; Arps, L.; Traktman, P. *J. Biol. Chem.* **2006**, *281*, 3439.
39. Langhammer, S.; Koban, R.; Yue, C.; Ellerbrok, H. *Antiviral Res.* **2011**, *89*, 64.
40. Reeves, P. M. B., B.; Lebeis, S.; McNulty, S.; Christensen, J.; Swimm, A.; Chahroudi, A.; Chavan, R.; Feinberg, M. B.; Veach, D.; Bornmann, W.; Sherman, M.; Kalman, D. *Nature Med.* **2005**, *11*, 731.
41. Jordan, R., Leeds, J. M.; Tyavanagimatte, S.; Hruby, D. E. *Viruses* **2010**, 2409.
42. Culyba, M. J.; Hwang, Y.; Hu, J. Y.; Minkah, N.; Ocwieja, K. E.; Bushman, F. D. *J. Mol. Biol.* **2010**, *399*, 182.
43. Garcia, A. D.; Aravind, L.; Koonin, E. V.; Moss, B. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8926.
44. Garcia, A. D.; Moss, B. *J. Virol.* **2001**, *75*, 6460.
45. Culyba, M. J.; Hwang, Y.; Minkah, N.; Bushman, F. D. *J. Biol. Chem.* **2009**, *284*, 1190.
46. Dickie, P.; Morgan, A. R.; McFadden, G. *J. Mol. Biol.* **1987**, *196*, 541.
47. Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.* **2005**, *49*, 445.
48. Clark, D. P., *Molecular Biology Understanding the Genetic Revolution*. Elsevier: New York, 2005.
49. Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6661.
50. Culyba, M. J.; Harrison, J. E.; Hwang, Y.; Bushman, F. D. *Virology* **2006**, *352*, 466.
51. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
52. Tramontano, E.; Esposito, F.; Badas, R.; Di Santo, R.; Costi, R.; La Colla, P. *Antiviral Res.* **2005**, *65*, 117.
53. Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453.
54. Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.

55. Klumpp, K.; Hang, J. Q.; Rajendran, S.; Yang, Y.; Derosier, A.; Wong Kai In, P.; Overton, H.; Parkes, K. E. B.; Cammack, N.; Martin, J. A. *Nucleic Acids Res.* **2003**, *31*, 6852.
56. Walker, M. A.; Johnson, T.; Ma, Z.; Zhang, Y.; Banville, J.; Remillard, R.; Plamondon, S.; Pendri, A.; Wong, H.; Smith, D.; Torri, A.; Samanta, H.; Lin, Z.; Deminie, C.; Terry, B.; Krystal, M.; Meanwell, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5818.
57. Nugent, W. A. Z., K.; Simpson, J. H.; Delaney, E. J.; Process for the Preparation of Z-5-Carboxymethylene-1,3-Dioxolane-4-ones. US 2006/0047129 A1, **2006**.
58. Banville, J.; Bouthillier, G.; Plamondon, S.; Remillard, R.; Meanwell, N. A.; Martel, A.; Walker, M. A. *Tetrahedron Lett.* **2010**, *51*, 3170.
59. Huang, M.; Grant, G. H.; Richards, W. G. *J. Mol. Graphics Modell.* **2011**, *29*, 956.
60. Marchand, C.; Johnson, A. A.; Karki, R. G.; Pais, G. C. G.; Zhang, X.; Cowansage, K.; Patel, T. A.; Nicklaus, M. C.; Burke, T. R.; Pommier, Y. *Mol. Pharmacol.* **2003**, *64*, 600.
61. Wipf, P.; Cunningham, A. *Tetrahedron Lett.* **1995**, *36*, 7819.
62. Werner, S.; Turner, D. M.; Lyon, M. A.; Huryn, D. M.; Wipf, P. *Synlett* **2006**, *2006*, 2334.
63. Singh, K.; Singh, S. *Tetrahedron Lett.* **2006**, *47*, 8143.
64. Wai, J. S.; Kim, B.; Fisher, T. E.; Zhuang, L.; Embrey, M. W.; Williams, P. D.; Staas, D. D.; Culberson, C.; Lyle, T. A.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Schleif, W. A.; Gabryelski, L. J.; Jin, L.; Chen, I. W.; Ellis, J. D.; Mallai, R.; Young, S. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5595.
65. Hiebl, J.; Baumgartner, H.; Bernwieser, I.; Blanka, M.; Bodenteich, M.; Leitner, K.; Rio, A.; Rovenszky, F.; Alberts, D. P.; Bhatnagar, P. K.; Banyard, A. F.; Baresch, K.; Esch, P. M.; Kollmann, H.; Mayrhofer, G.; Weihtrager, H.; Welz, W.; Winkler, K.; Chen, T.; Patel, R.; Lantos, I.; Stevenson, D.; Tubman, K. D.; Undheim, K. *J. Peptide Res.* **1999**, *54*, 54.
66. Escher, R.; Bünning, P. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 277.
67. Zhang, Y.; Lin, F.-Y.; Li, K.; Zhu, W.; Liu, Y.-L.; Cao, R.; Pang, R.; Lee, E.; Axelson, J.; Hensler, M.; Wang, K.; Molohon, K. J.; Wang, Y.; Mitchell, D. A.; Nizet, V.; Oldfield, E. *ACS Med. Chem. Lett.* **2012**, *3*, 402.
68. Marzabadi, M. R. W., J.; DeLeon, J.E.; Lagu, B.; Gluchowski, C.; Noble, S.; Nagarathnam, D. Selective Melanin Concentrating Hormone-1 (MCH1) Receptor Antagonists and Uses Thereof. US 2003/0069261 A1, **2003**.
69. Culyba, M.; Hwang, Y.; Attar, S.; Madrid, P. B.; Bupp, J.; Huryn, D.; Sanchez, L.; Grobler, J.; Miller, M. D.; Bushman, F. D. *Nucleic Acids Res.* **2012**,
70. Salehi, H.; Guo, Q.-X. *Chinese Journal of Chemistry* **2005**, *23*, 91.
71. Miyamoto, K. Y., Y.; Yoshioka, K; Hyaluronic Acid Derivative and Drug Containing The Same. EP 1710257 A1, **2006**.

4.0 APPENDIX A

Selected NMR Specta:


1.1
PhS
N
O
O
NBn
7.603
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7.583
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7.436
7.433
7.432
7.326
7.323
7.310
7.306
7.300
7.269
7.260
7.252
7.240
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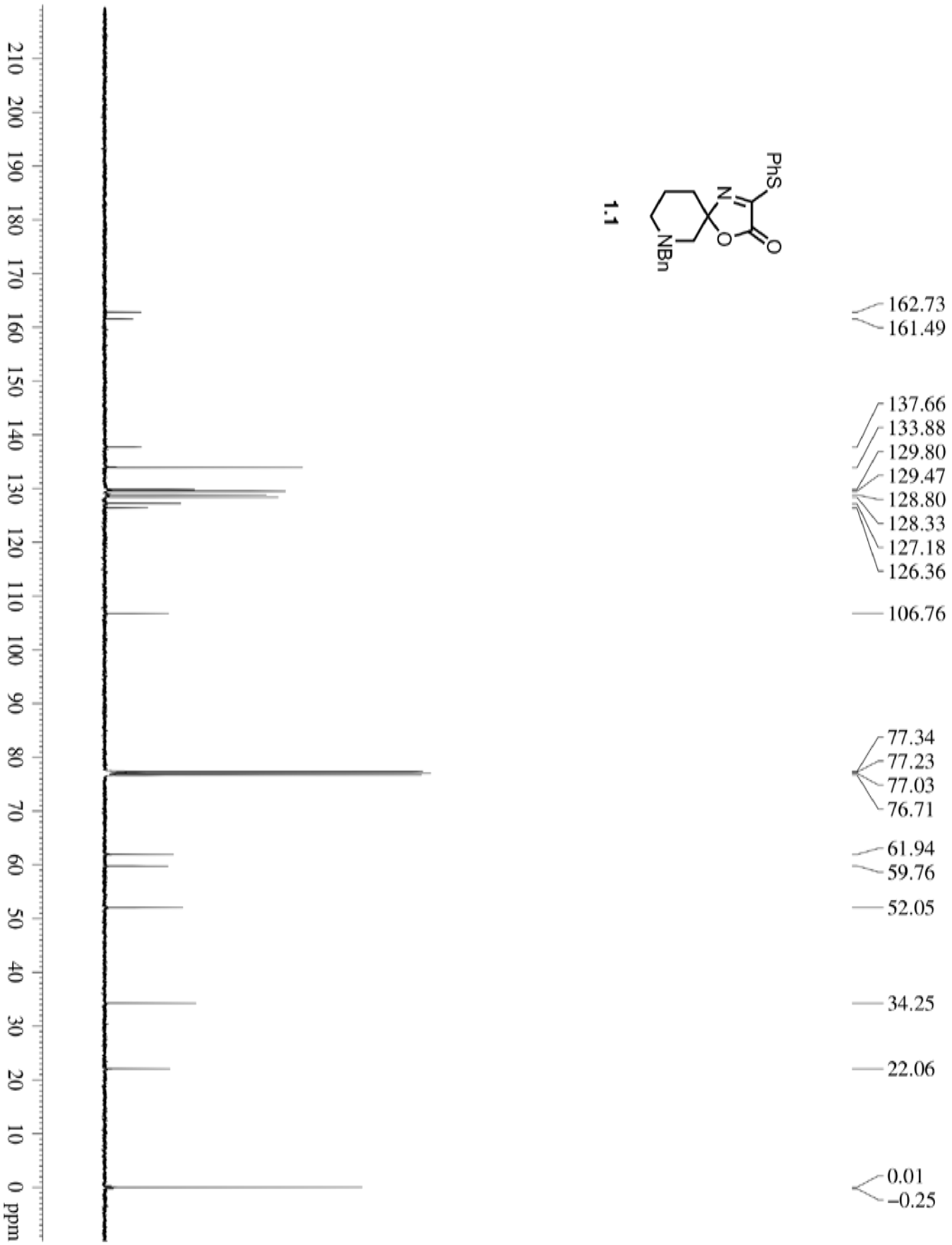
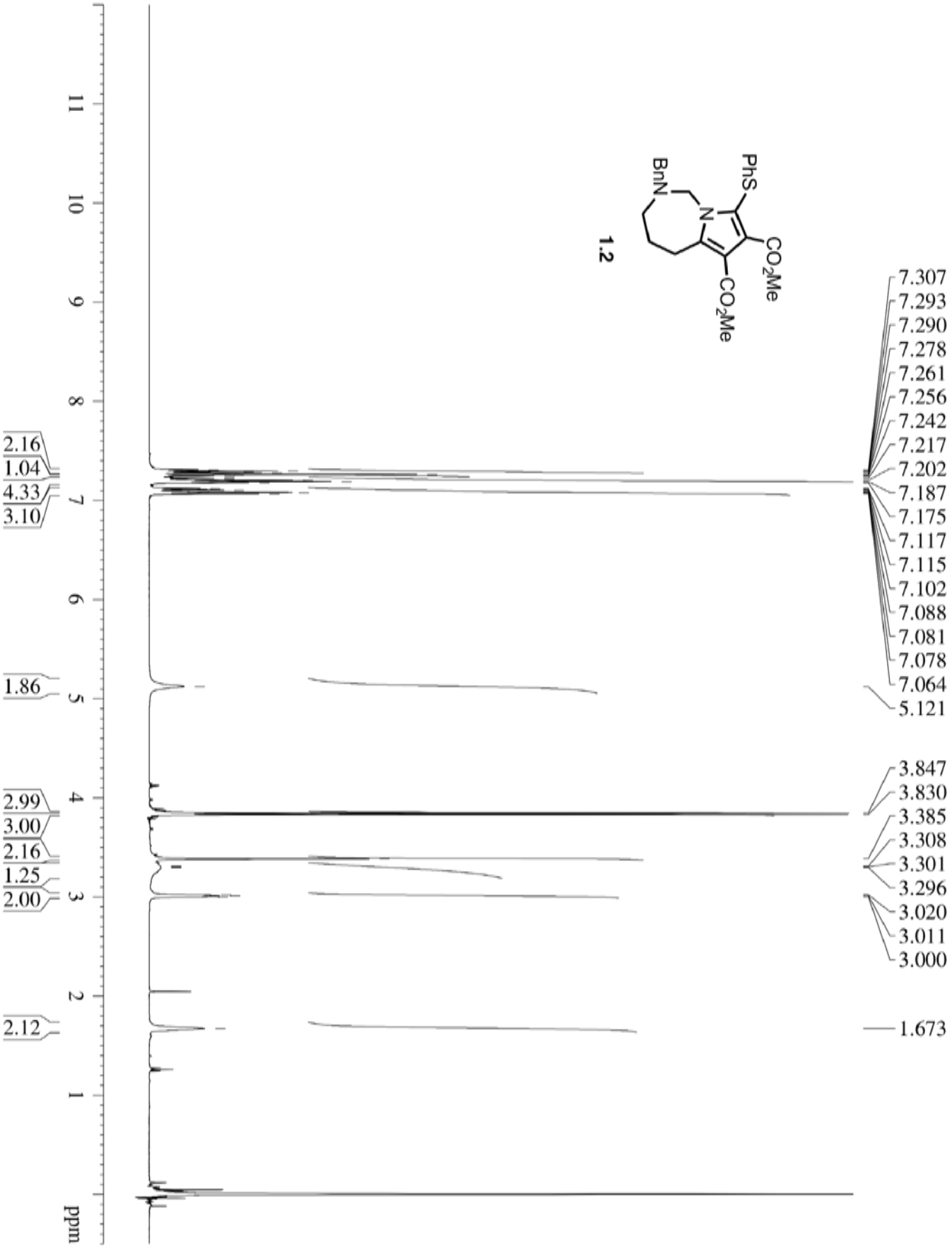
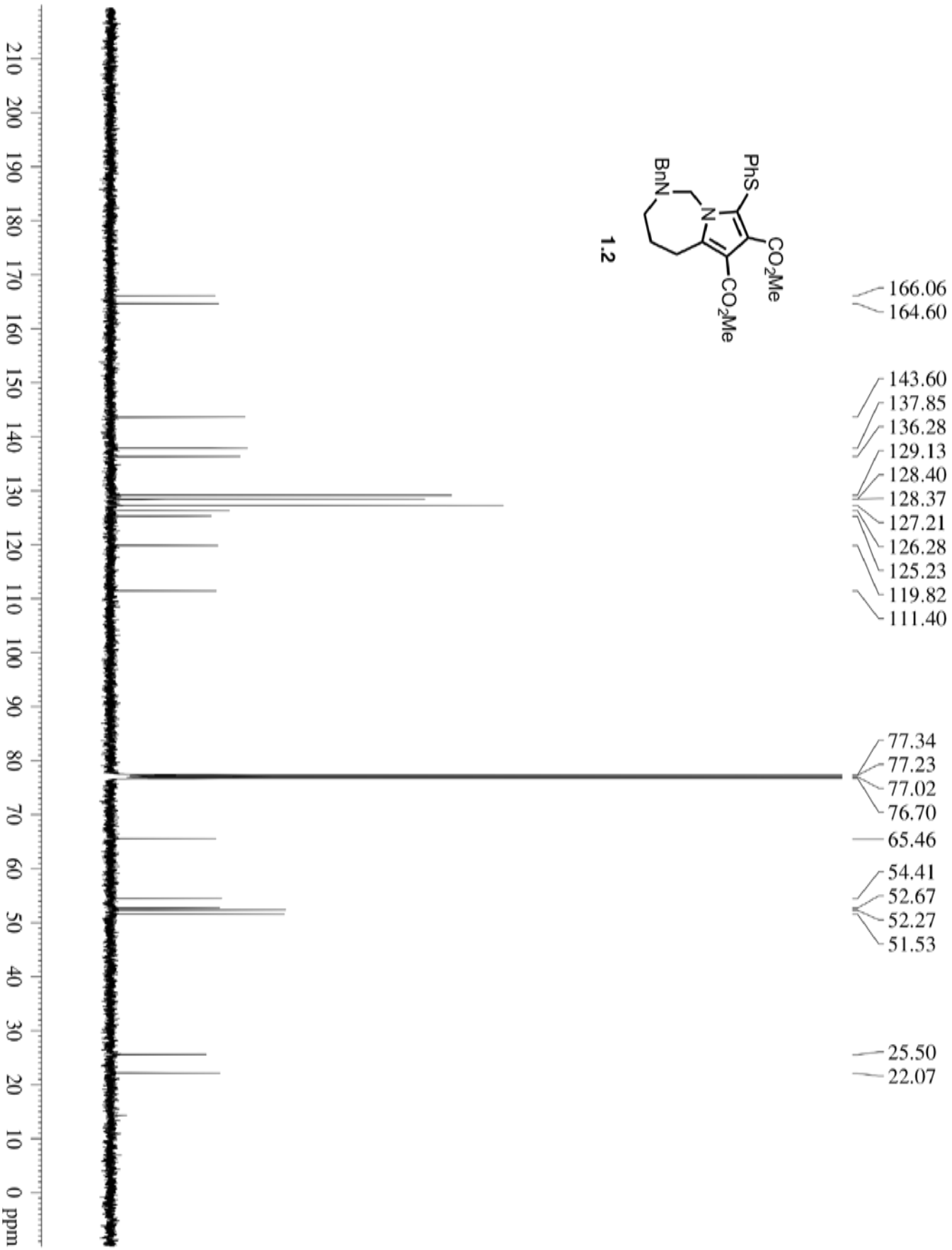
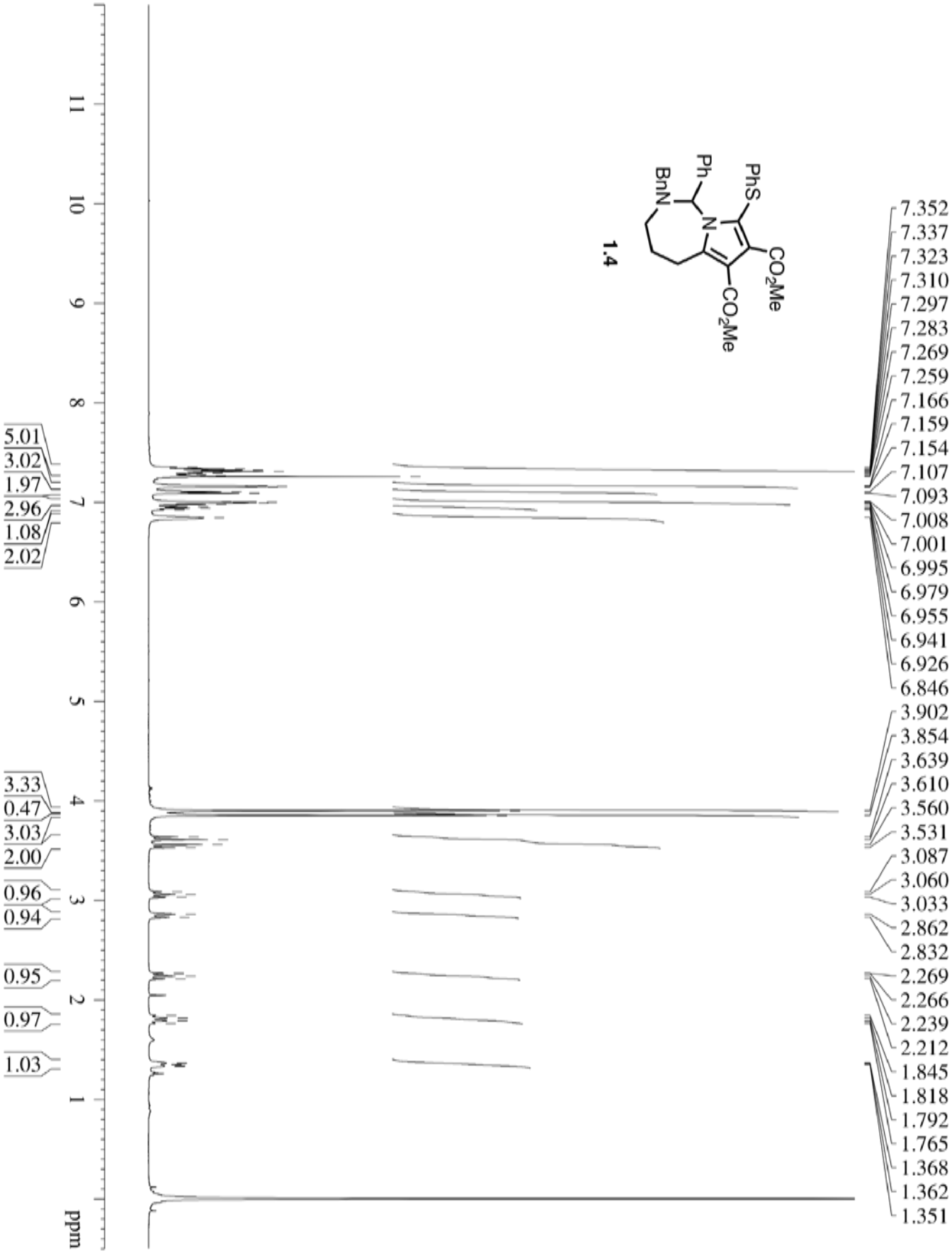
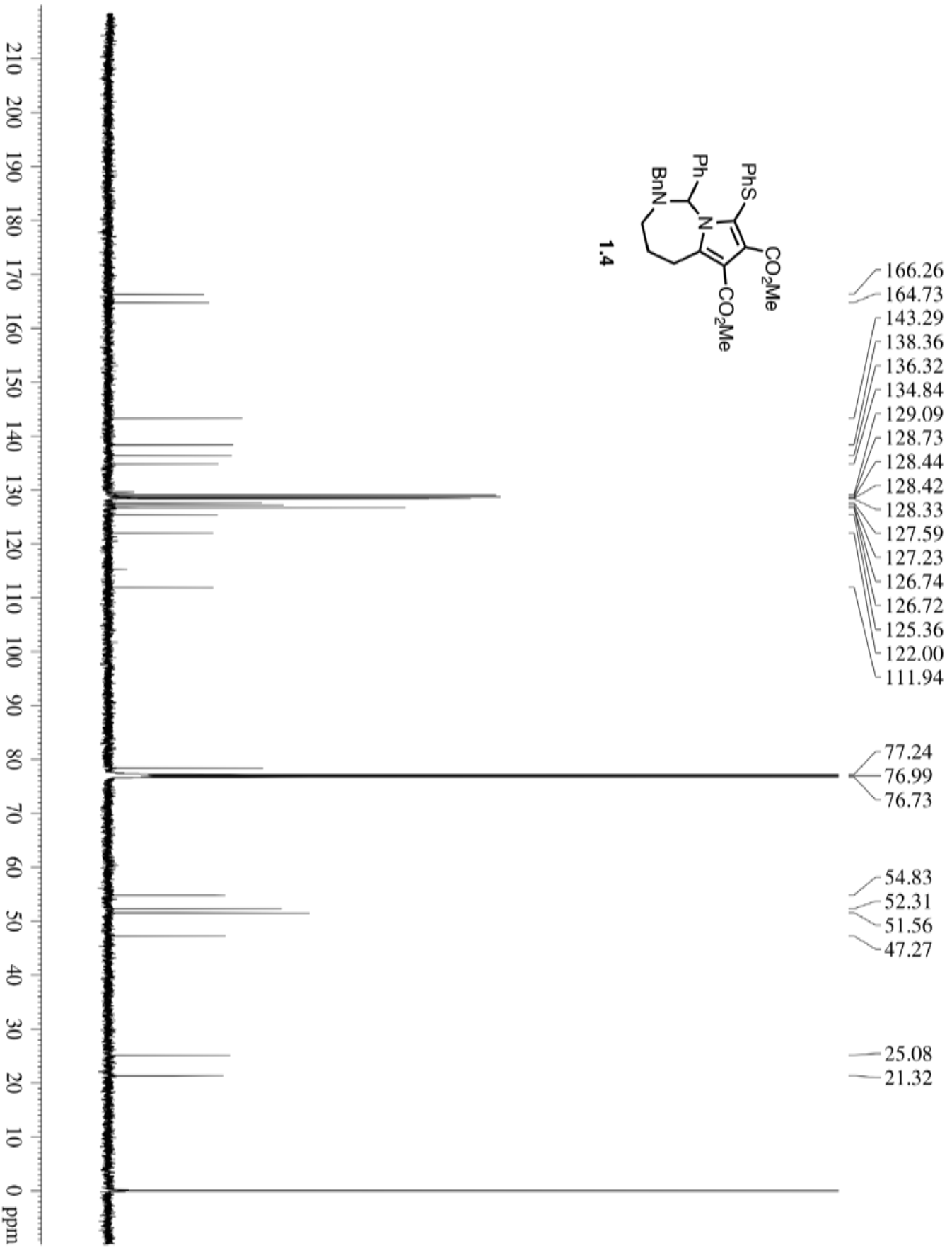
3.626
3.569
3.542
2.872
2.863
2.848
2.573
2.548
2.519
2.264
2.243
2.225
2.220
1.967
1.960
1.946
1.942
1.937
1.922
1.904
1.896
1.880
1.872
1.793
1.785
1.779
1.768
1.760
1.754
1.646
1.626
1.87
2.83
3.86
1.43
2.00
0.97
1.95
0.98
1.99
0.97
1.06
11
10
9
8
7
6
5
4
3
2
1
ppm

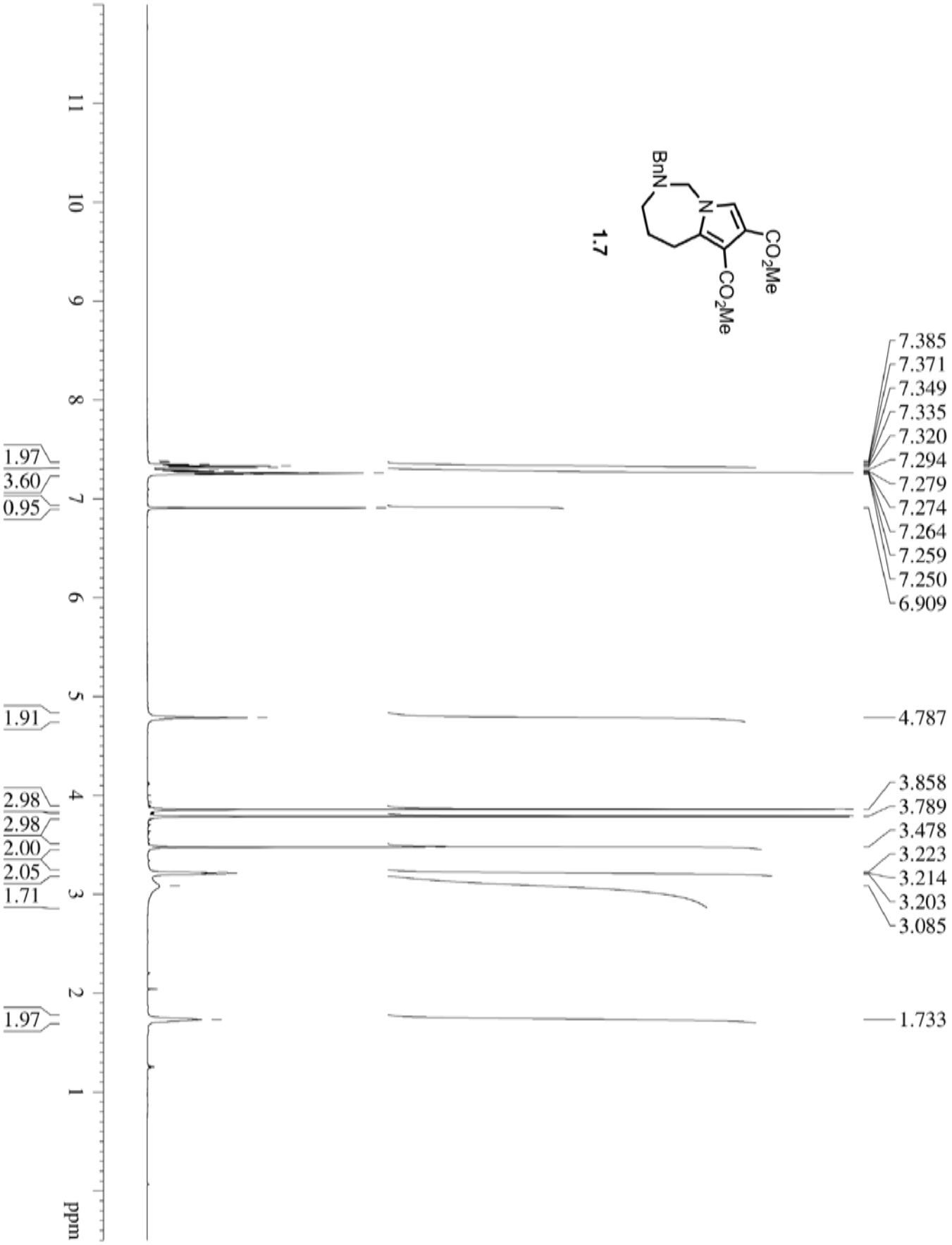

1.1
PhS
N
O
O
NBn
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
162.73
161.49
137.66
133.88
129.80
129.47
128.80
128.33
127.18
126.36
106.76
77.34
77.23
77.03
76.71
61.94
59.76
52.05
34.25
22.06
0.01
−0.25

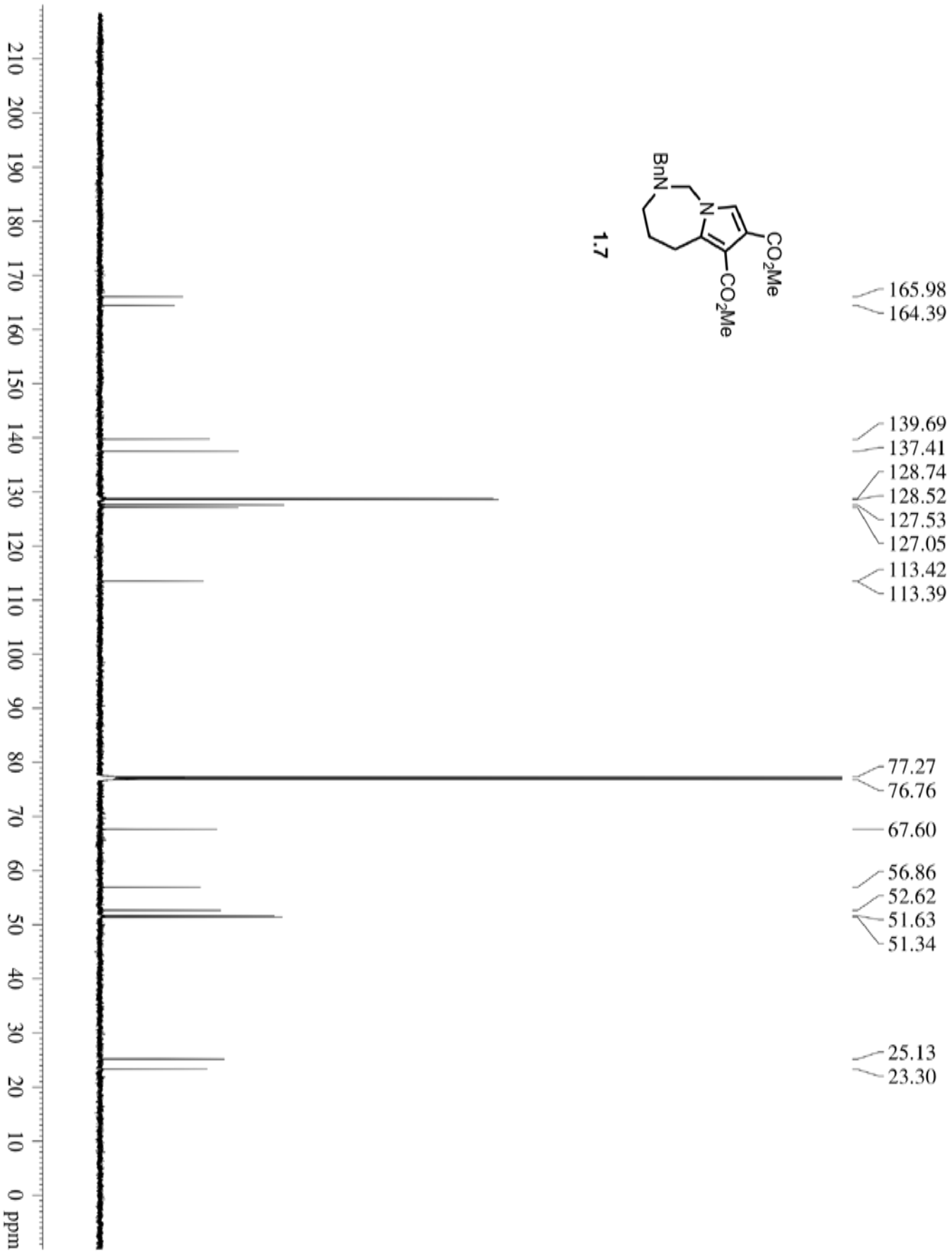

1.2
BnN
N
PhS
CO2Me
CO2Me
2.16
1.04
4.33
3.10
1.86
2.99
3.00
2.16
1.25
2.00
2.12
7.307
7.293
7.290
7.278
7.261
7.256
7.242
7.217
7.202
7.187
7.175
7.117
7.115
7.102
7.088
7.081
7.078
7.064
5.121
3.847
3.830
3.385
3.308
3.301
3.296
3.020
3.011
3.000
1.673
ppm

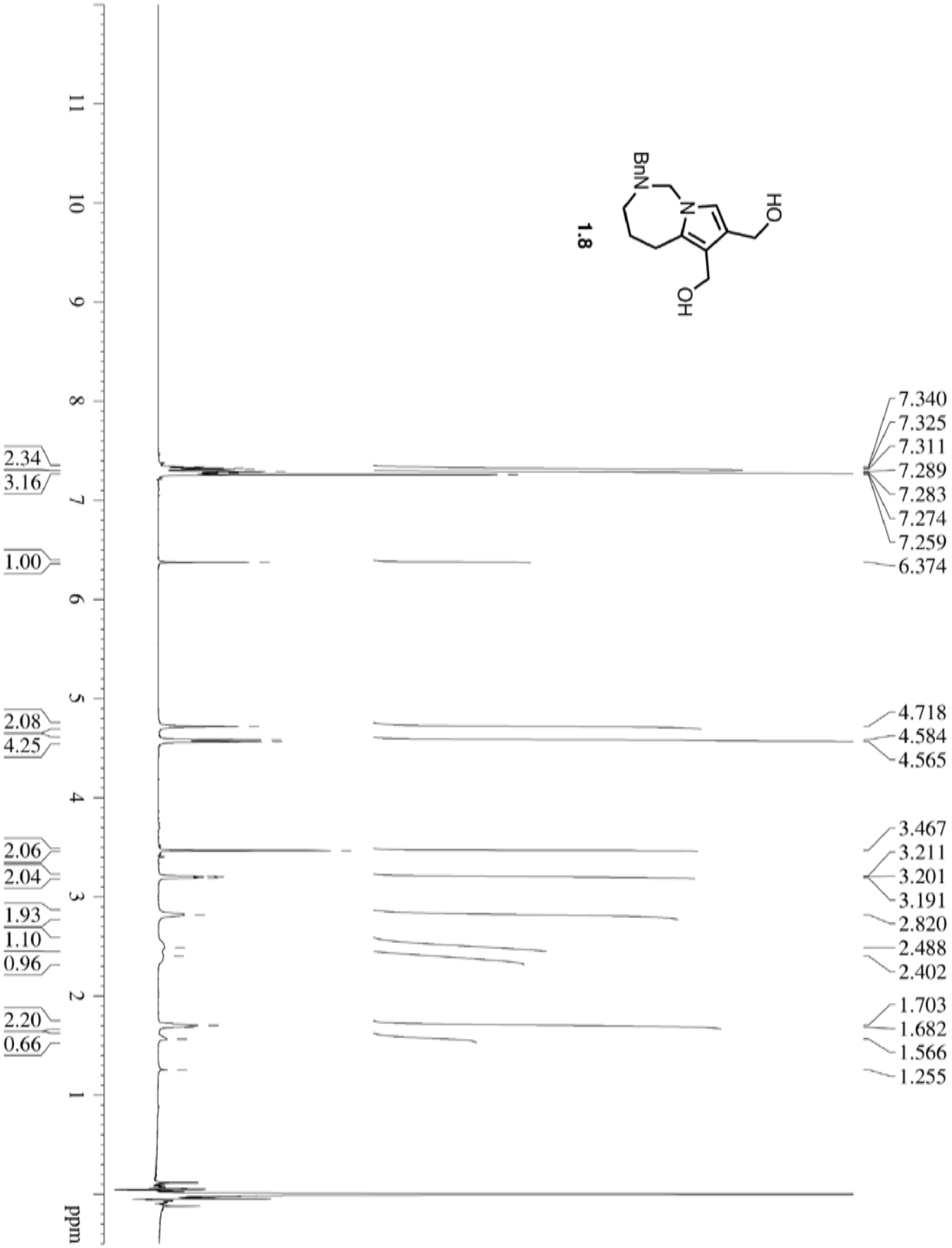

BnN
N
PhS
CO2Me
CO2Me
1.2
166.06
164.60
143.60
137.85
136.28
129.13
128.40
128.37
127.21
126.28
125.23
119.82
111.40
77.34
77.23
77.02
76.70
65.46
54.41
52.67
52.27
51.53
25.50
22.07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

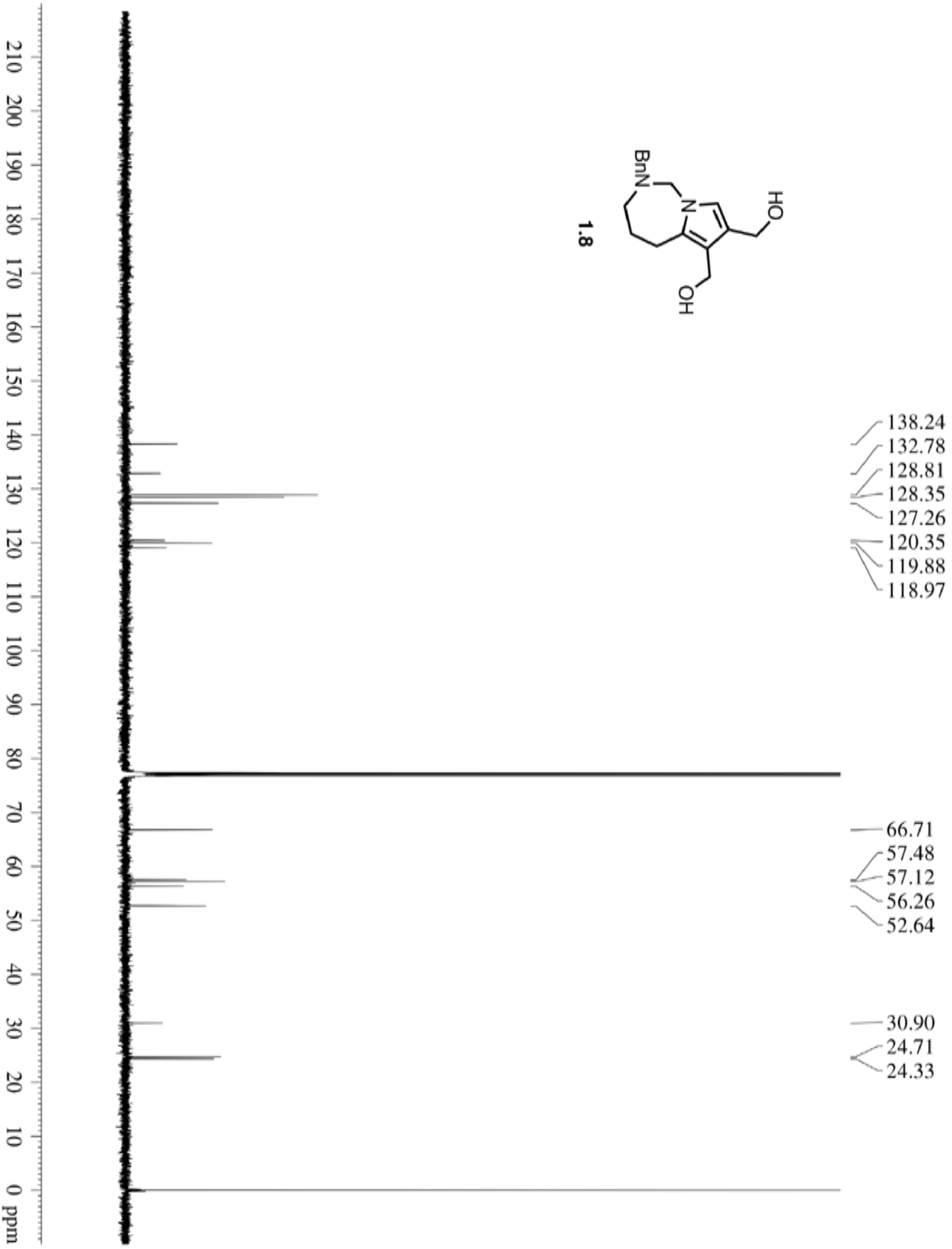

1.4
BnN
Ph
N
PhS
CO2Me
CO2Me
7.352
7.337
7.323
7.310
7.297
7.283
7.269
7.259
7.166
7.159
7.154
7.107
7.093
7.008
7.001
6.995
6.979
6.955
6.941
6.926
6.846
3.902
3.854
3.639
3.610
3.560
3.531
3.087
3.060
3.033
2.862
2.832
2.269
2.266
2.239
2.212
1.845
1.818
1.792
1.765
1.368
1.362
1.351
5.01
3.02
1.97
2.96
1.08
2.02
3.33
0.47
3.03
2.00
0.96
0.94
0.95
0.97
1.03
ppm

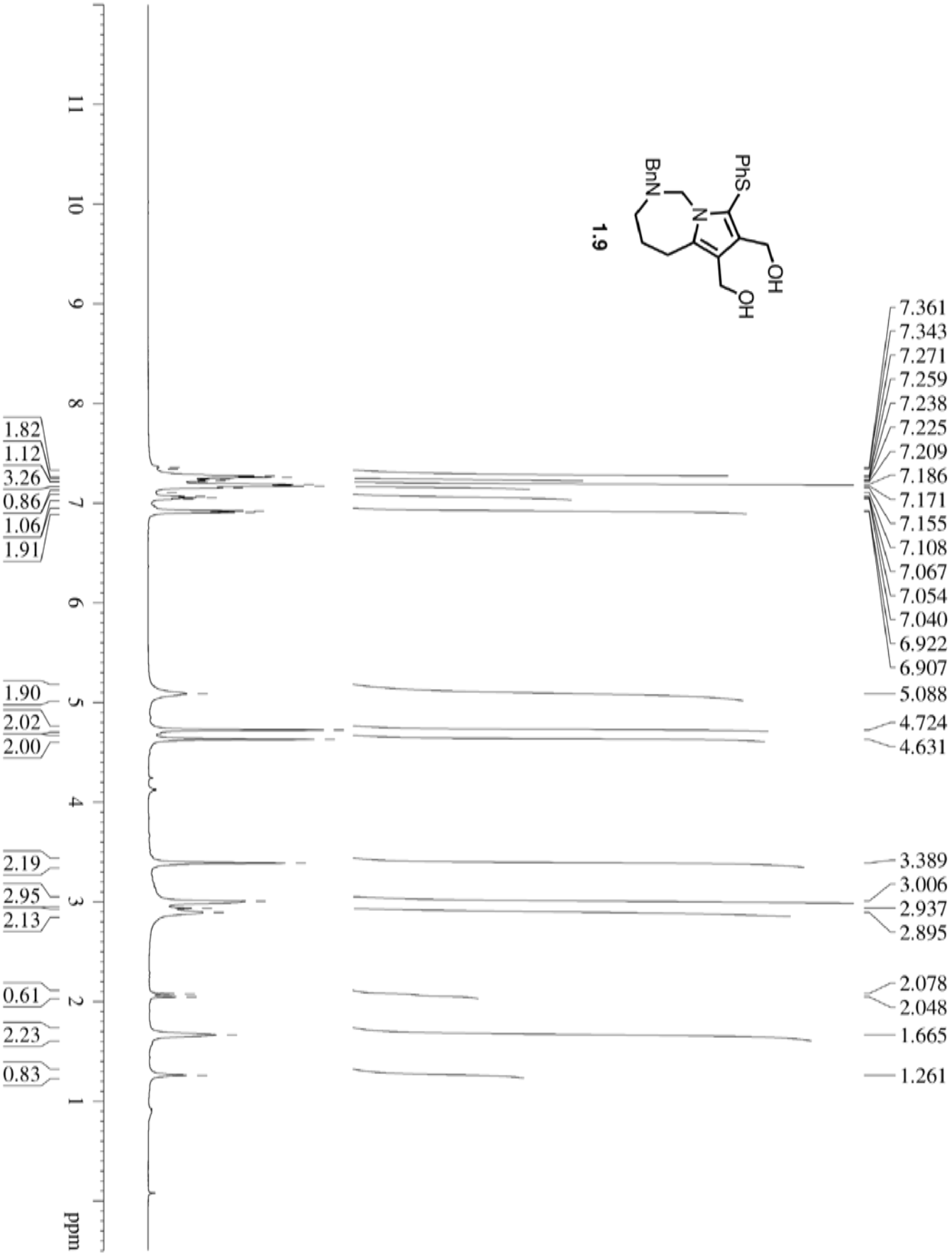

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
BnN
Ph
N
PhS
CO2Me
CO2Me
1.4
166.26
164.73
143.29
138.36
136.32
134.84
129.09
128.73
128.44
128.42
128.33
127.59
127.23
126.74
126.72
125.36
122.00
111.94
77.24
76.99
76.73
54.83
52.31
51.56
47.27
25.08
21.32

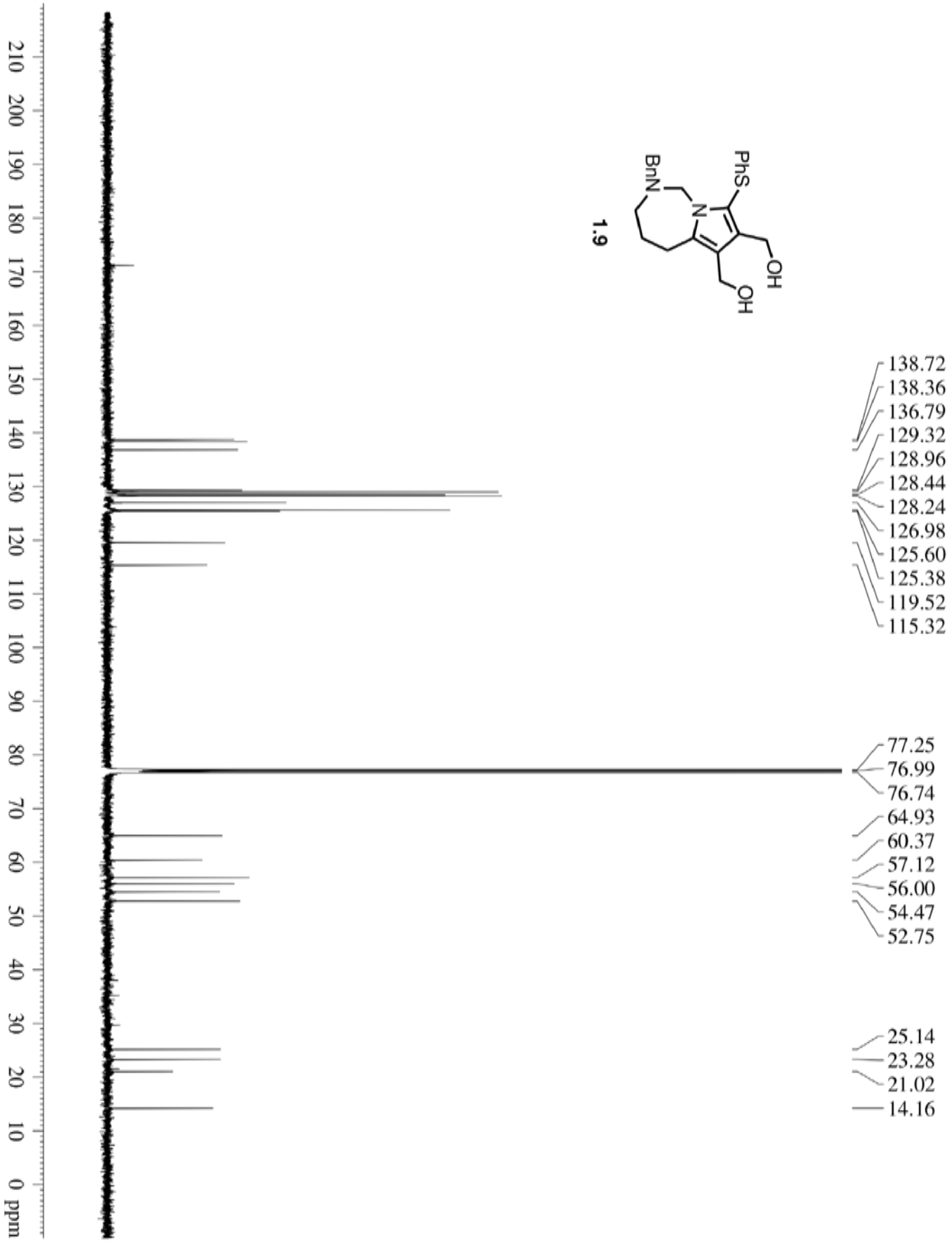

1.7
BnN
N
CO2Me
CO2Me
7.385
7.371
7.349
7.335
7.320
7.294
7.279
7.274
7.264
7.259
7.250
6.909
4.787
3.858
3.789
3.478
3.223
3.214
3.203
3.085
1.733
1.97
3.60
0.95
1.91
2.98
2.98
2.00
2.05
1.71
1.97
ppm

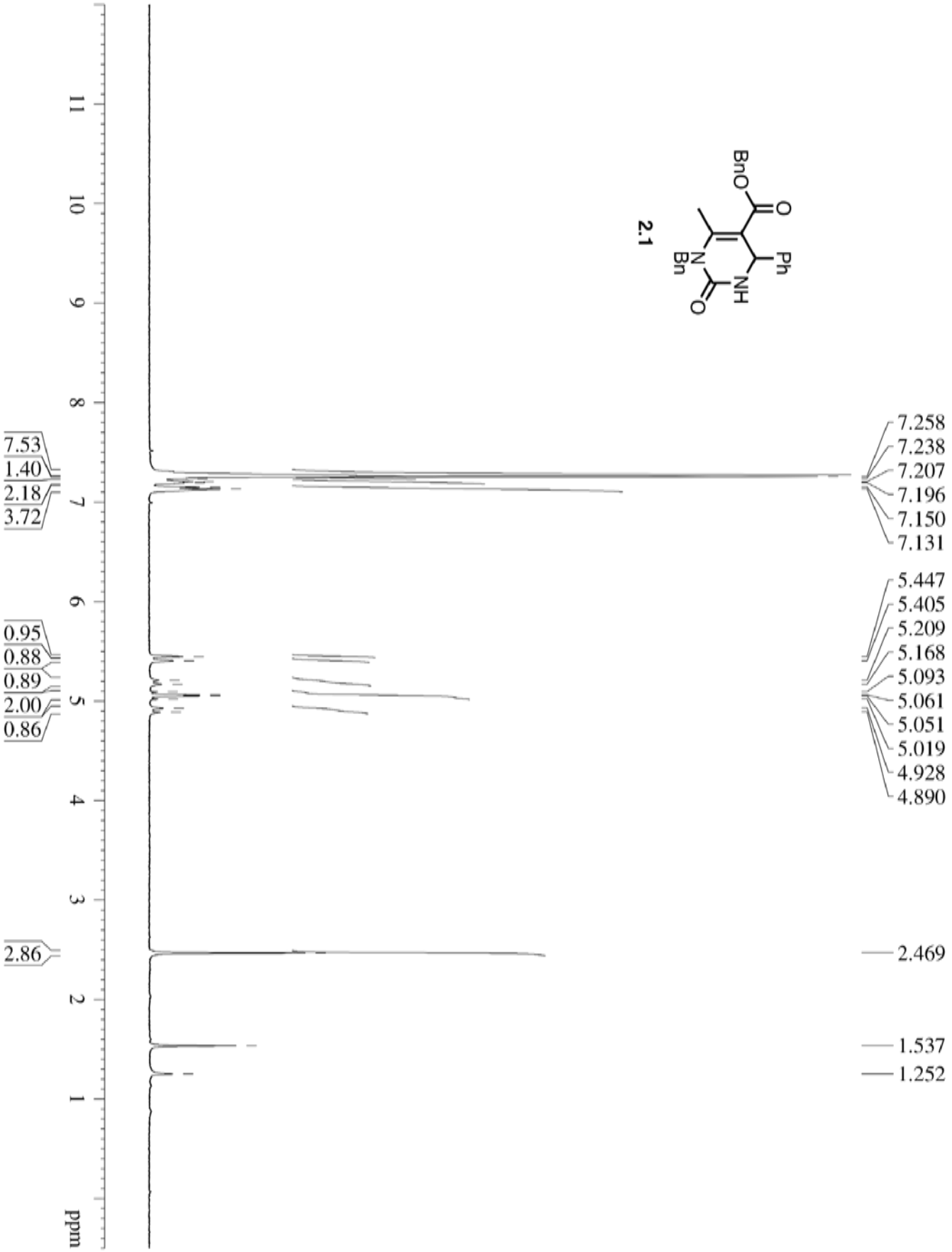

1.7
BnN
N
CO2Me
CO2Me
165.98
164.39
139.69
137.41
128.74
128.52
127.53
127.05
113.42
113.39
77.27
76.76
67.60
56.86
52.62
51.63
51.34
25.13
23.30
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

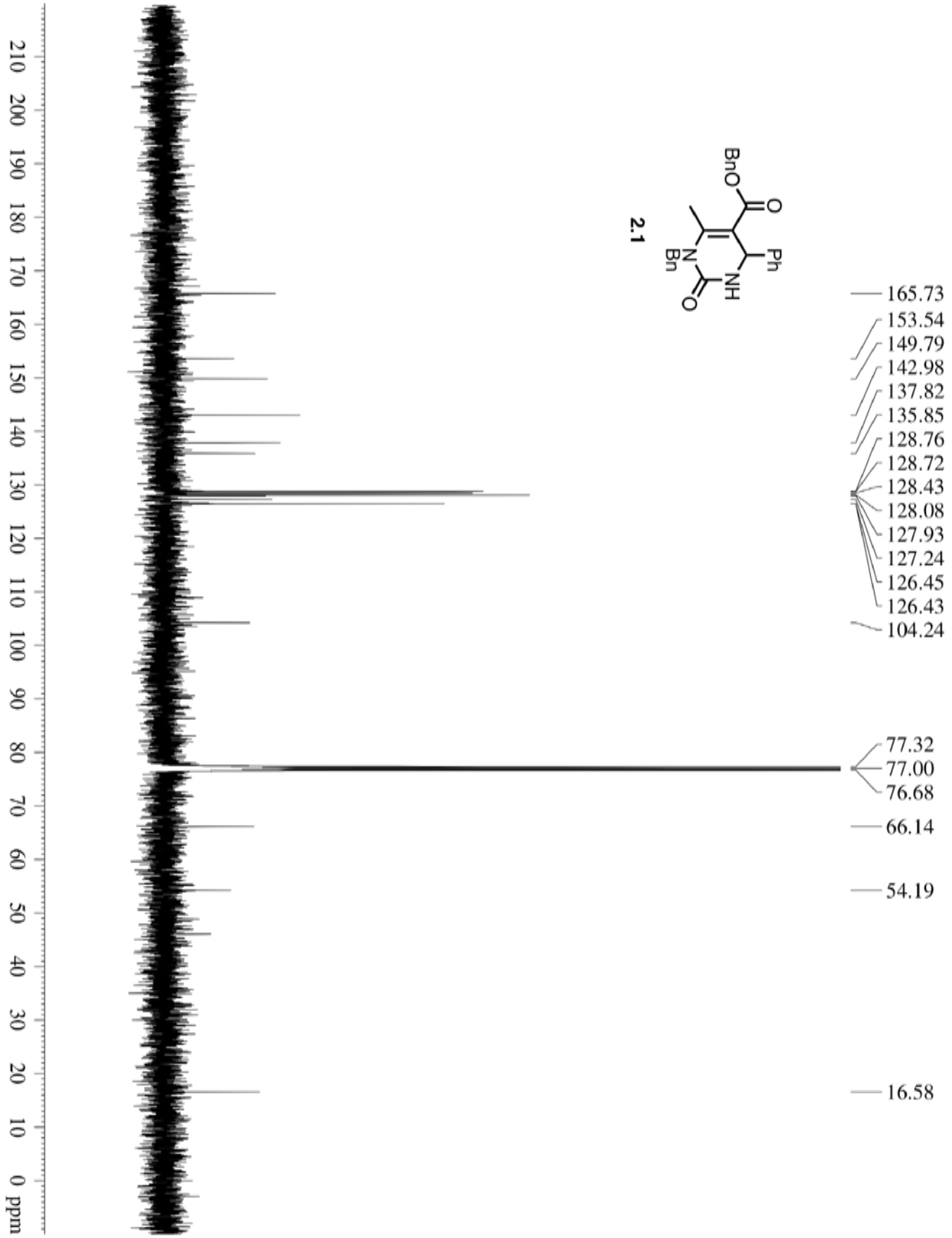

BnN
N
HO
OH
1.8
7.340
7.325
7.311
7.289
7.283
7.274
7.259
6.374
4.718
4.584
4.565
3.467
3.211
3.201
3.191
2.820
2.488
2.402
1.703
1.682
1.566
1.255
2.34
3.16
1.00
2.08
4.25
2.06
2.04
1.93
1.10
0.96
2.20
0.66
ppm

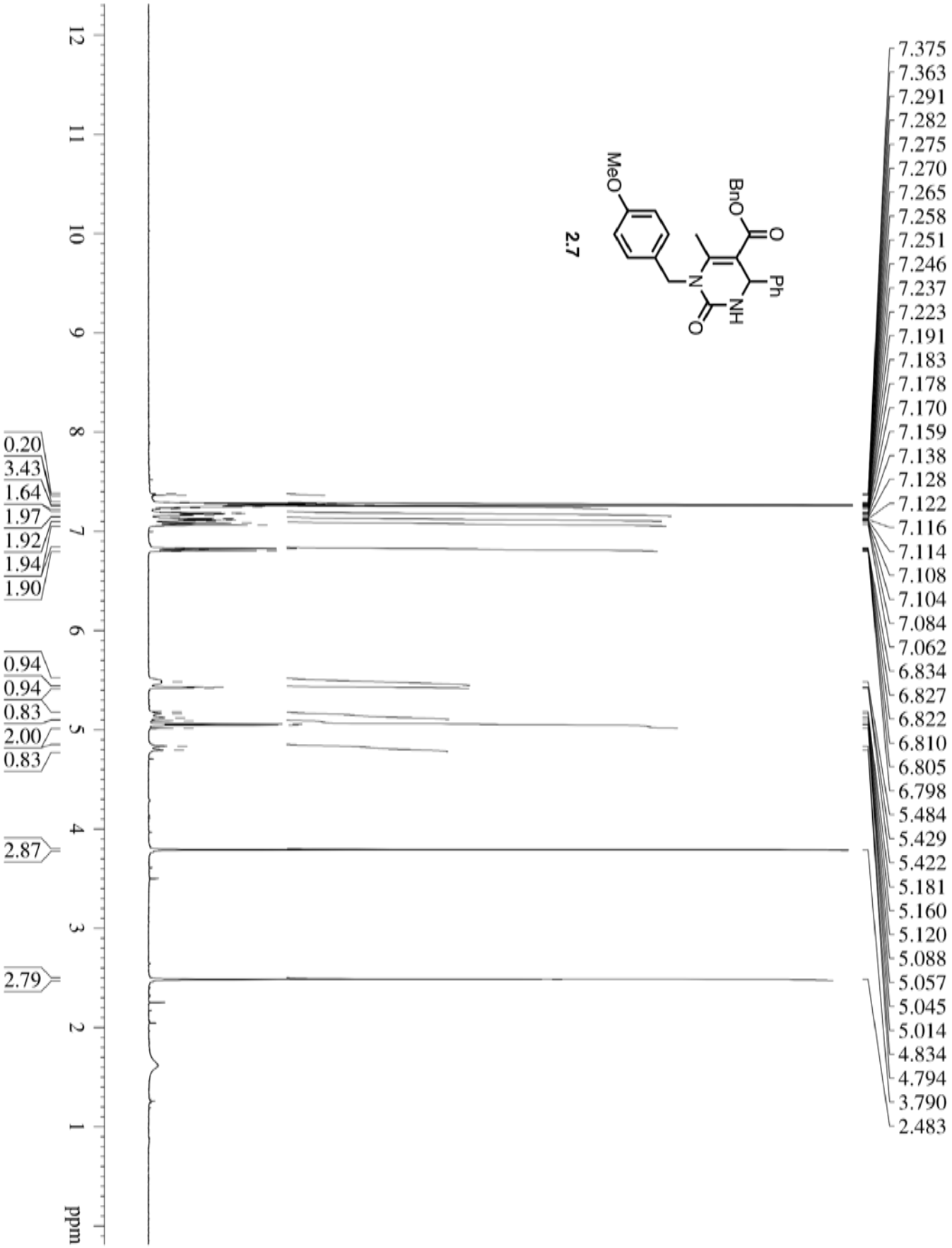

BnN
N
HO
OH
1.8
138.24
132.78
128.81
128.35
127.26
120.35
119.88
118.97
66.71
57.48
57.12
56.26
52.64
30.90
24.71
24.33
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

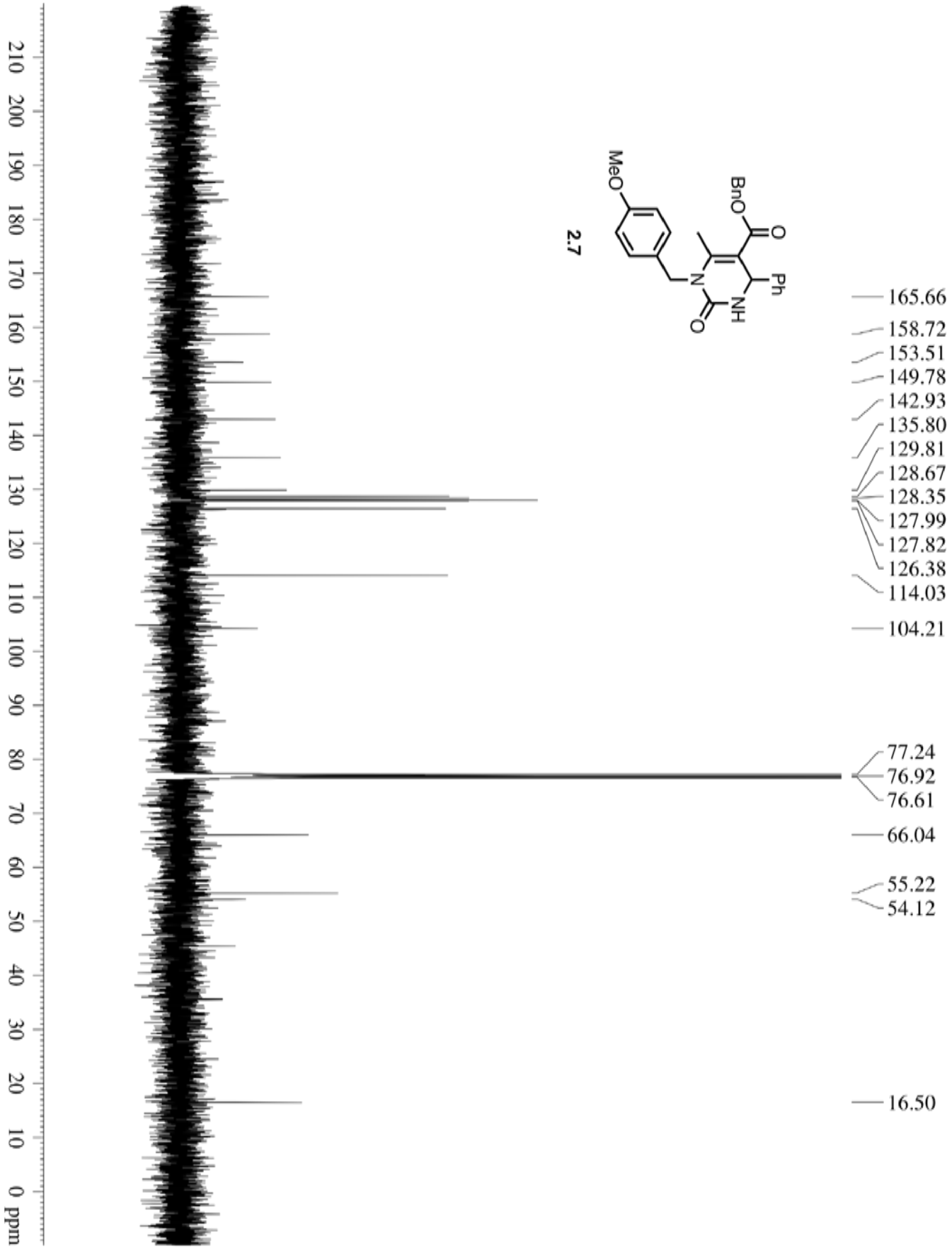

BnN
N
PhS
OH
OH
1.9
7.361
7.343
7.271
7.259
7.238
7.225
7.209
7.186
7.171
7.155
7.108
7.067
7.054
7.040
6.922
6.907
5.088
4.724
4.631
3.389
3.006
2.937
2.895
2.078
2.048
1.665
1.261
1.82
1.12
3.26
0.86
1.06
1.91
1.90
2.02
2.00
2.19
2.95
2.13
0.61
2.23
0.83
ppm

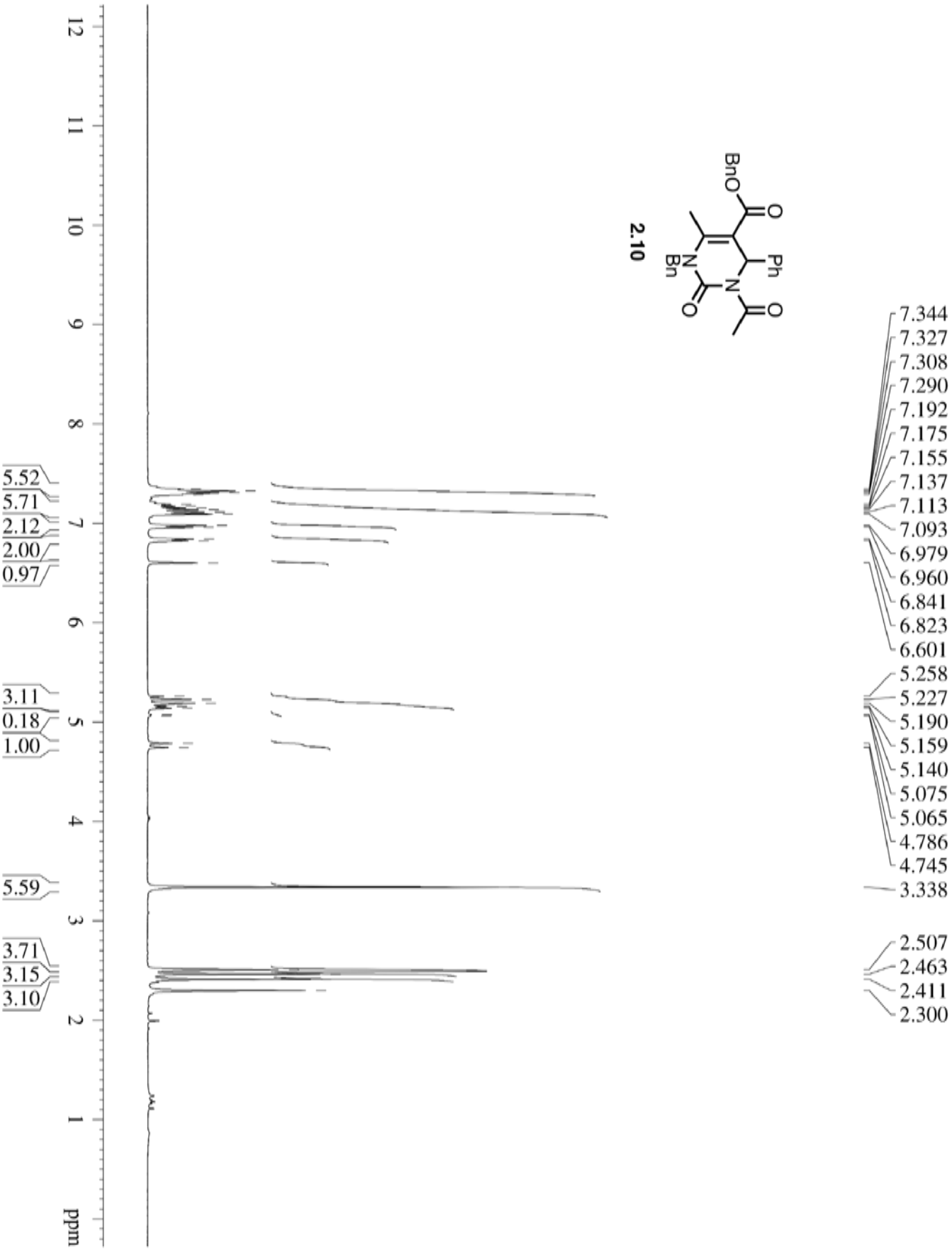

1.9
BnN
N
PhS
OH
OH
138.72
138.36
136.79
129.32
128.96
128.44
128.24
126.98
125.60
125.38
119.52
115.32
77.25
76.99
76.74
64.93
60.37
57.12
56.00
54.47
52.75
25.14
23.28
21.02
14.16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

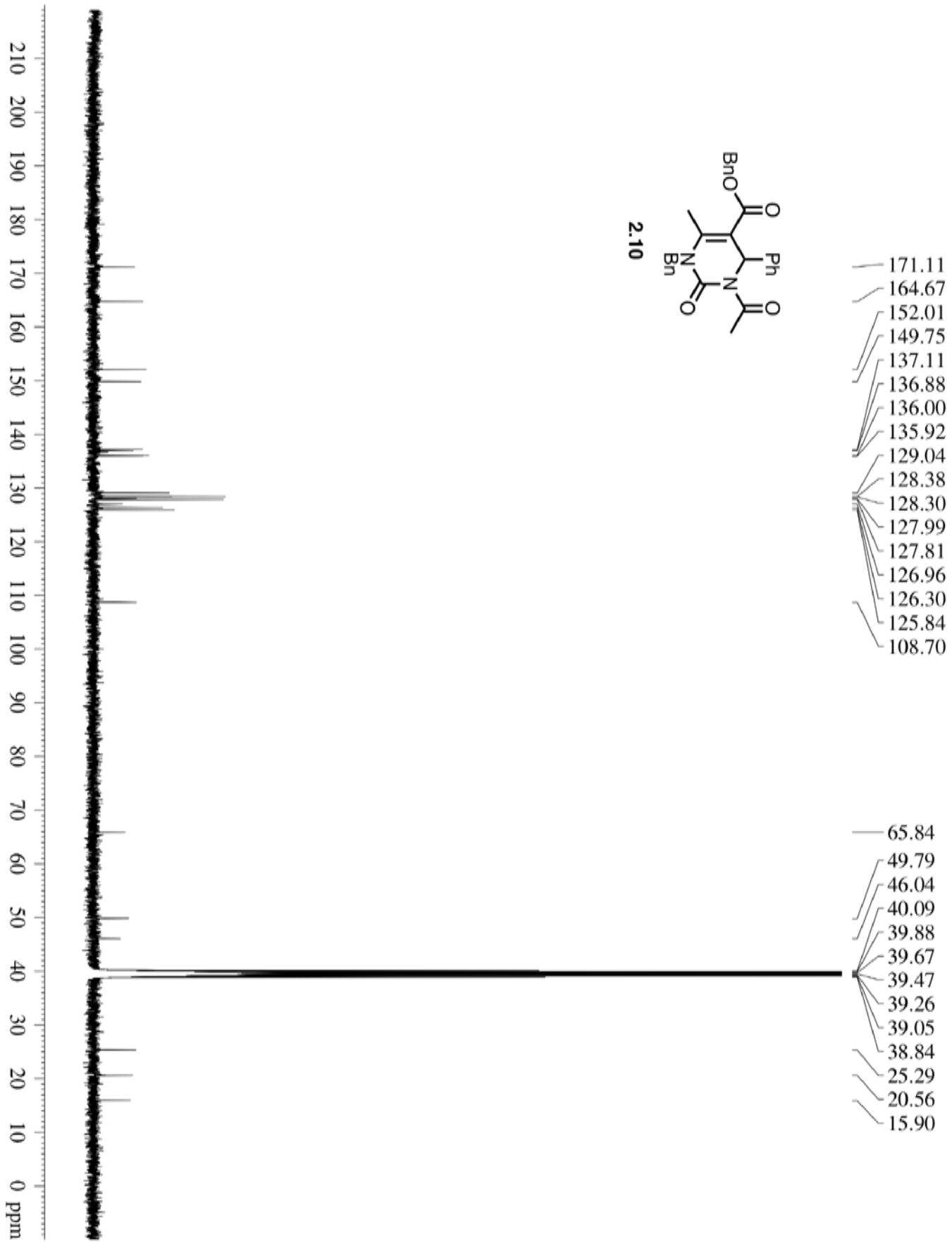

2.1
BnO
O
Ph
N
Bn
NH
O
7.53
1.40
2.18
3.72
0.95
0.88
0.89
2.00
0.86
2.86
7.258
7.238
7.207
7.196
7.150
7.131
5.447
5.405
5.209
5.168
5.093
5.061
5.051
5.019
4.928
4.890
2.469
1.537
1.252
ppm

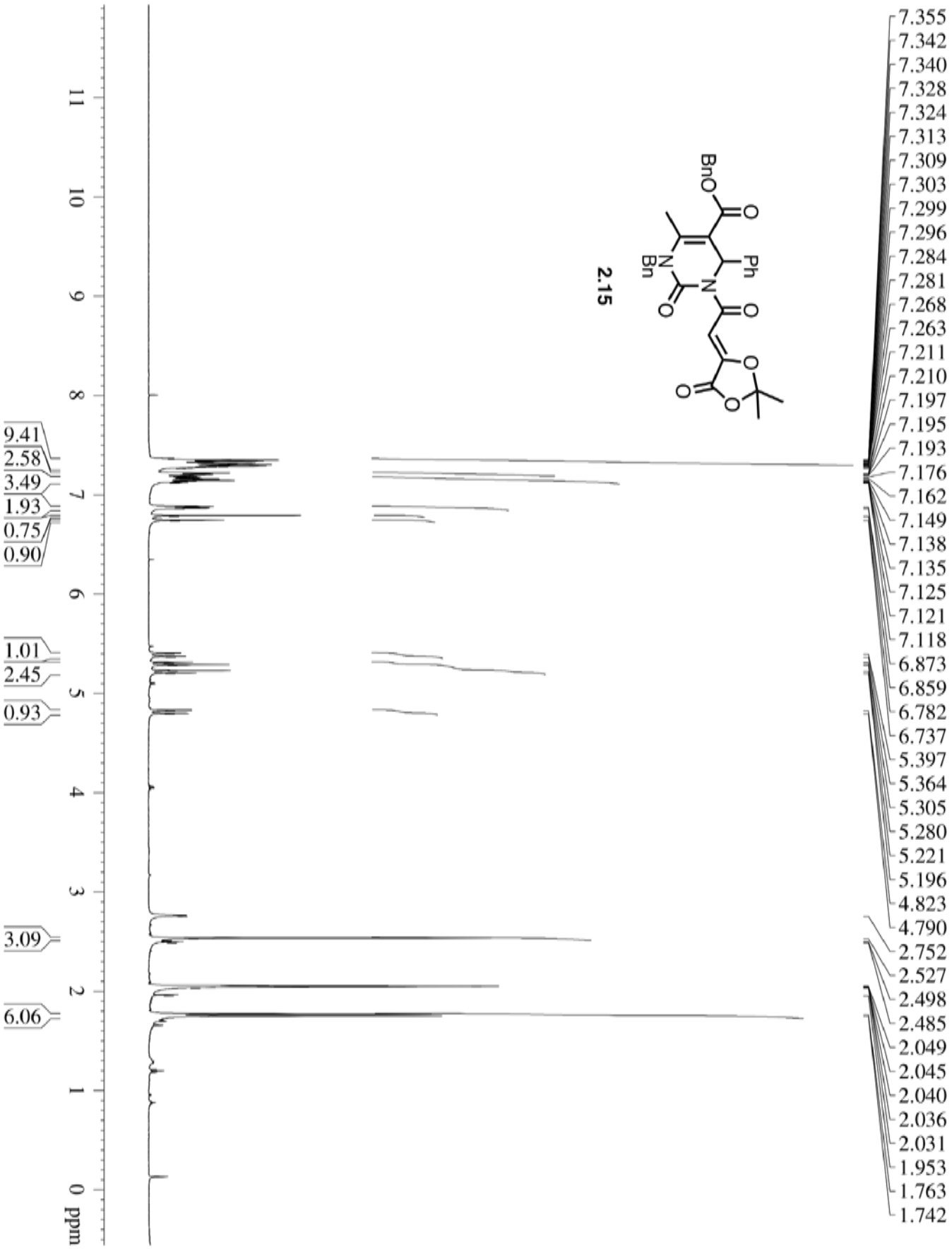

2.1
BnO
O
Ph
N
Bn
NH
O
165.73
153.54
149.79
142.98
137.82
135.85
128.76
128.72
128.43
128.08
127.93
127.24
126.45
126.43
104.24
77.32
77.00
76.68
66.14
54.19
16.58
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

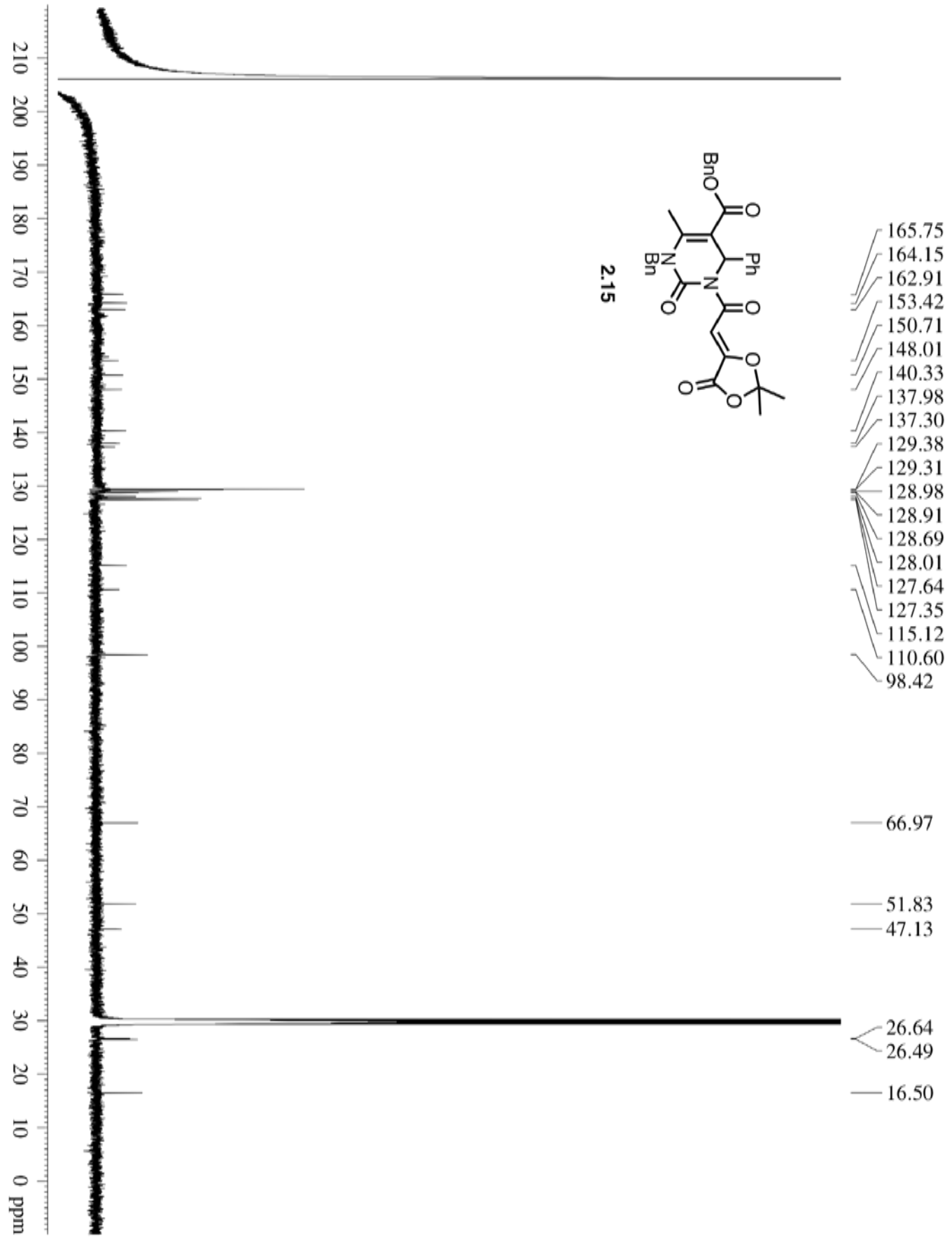

2.7
MeO
BnO
O
Ph
NH
N
O
7.375
7.363
7.291
7.282
7.275
7.270
7.265
7.258
7.251
7.246
7.237
7.223
7.191
7.183
7.178
7.170
7.159
7.138
7.128
7.122
7.116
7.114
7.108
7.104
7.084
7.062
6.834
6.827
6.822
6.810
6.805
6.798
5.484
5.429
5.422
5.181
5.160
5.120
5.088
5.057
5.045
5.014
4.834
4.794
3.790
2.483
0.20
3.43
1.64
1.97
1.92
1.94
1.90
0.94
0.94
0.83
2.00
0.83
2.87
2.79
12
11
10
9
8
7
6
5
4
3
2
1
ppm

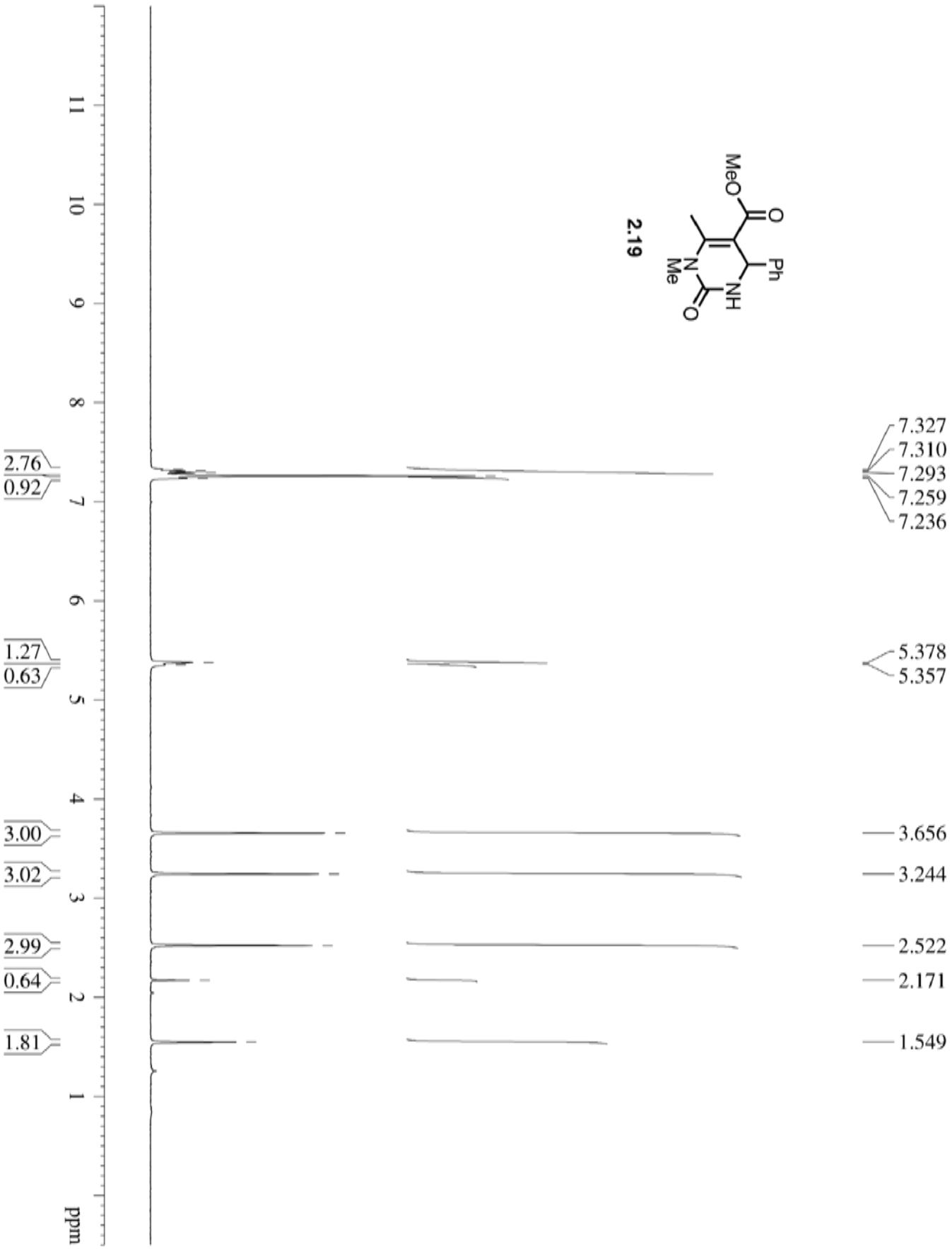

MeO
BnO
O
Ph
NH
N
O
2.7
165.66
158.72
153.51
149.78
142.93
135.80
129.81
128.67
128.35
127.99
127.82
126.38
114.03
104.21
77.24
76.92
76.61
66.04
55.22
54.12
16.50
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

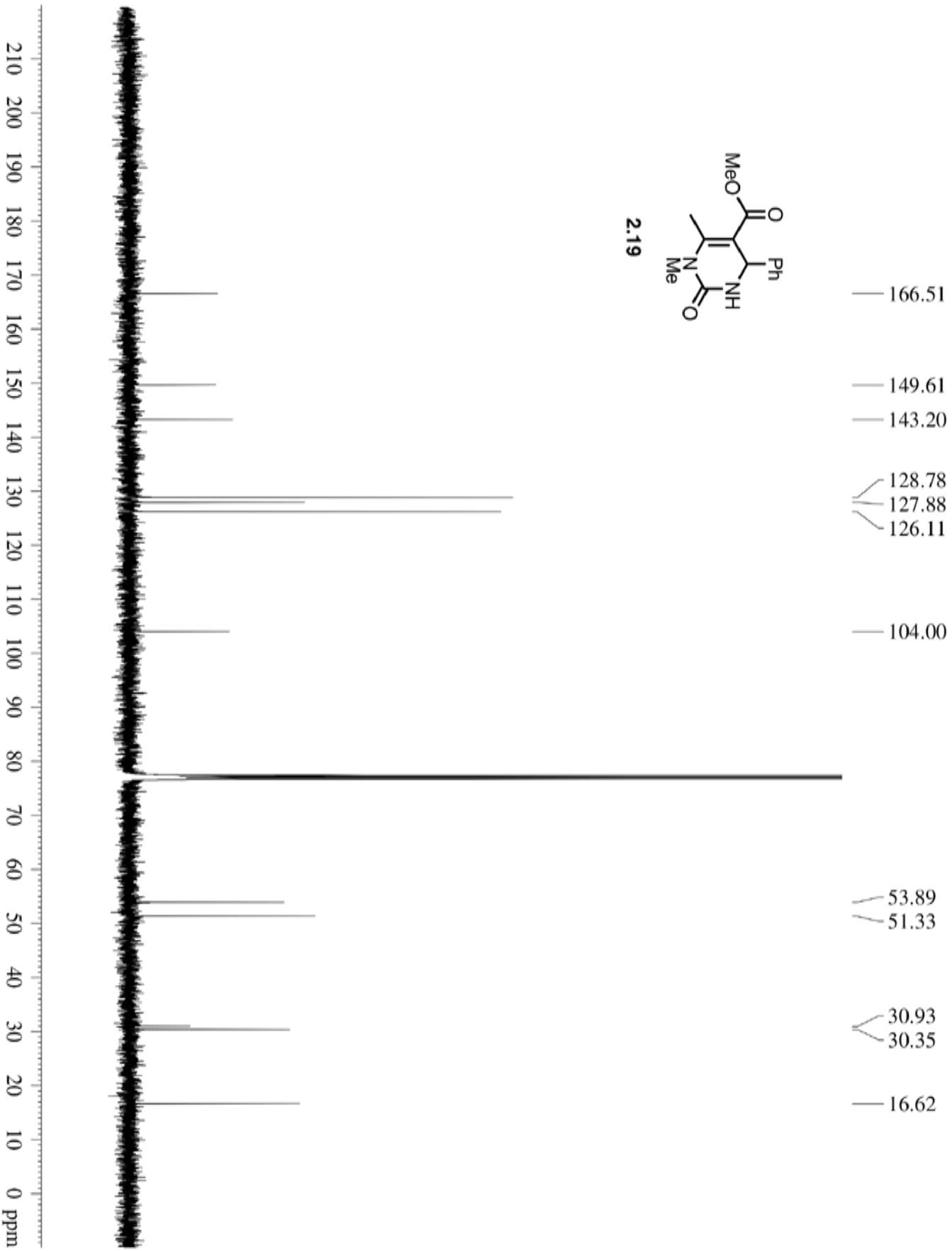

2.10
BnO
O
Ph
N
Bn
N
O
O
7.344
7.327
7.308
7.290
7.192
7.175
7.155
7.137
7.113
7.093
6.979
6.960
6.841
6.823
6.601
5.258
5.227
5.190
5.159
5.140
5.075
5.065
4.786
4.745
3.338
2.507
2.463
2.411
2.300
5.52
5.71
2.12
2.00
0.97
3.11
0.18
1.00
5.59
3.71
3.15
3.10
12
11
10
9
8
7
6
5
4
3
2
1
ppm

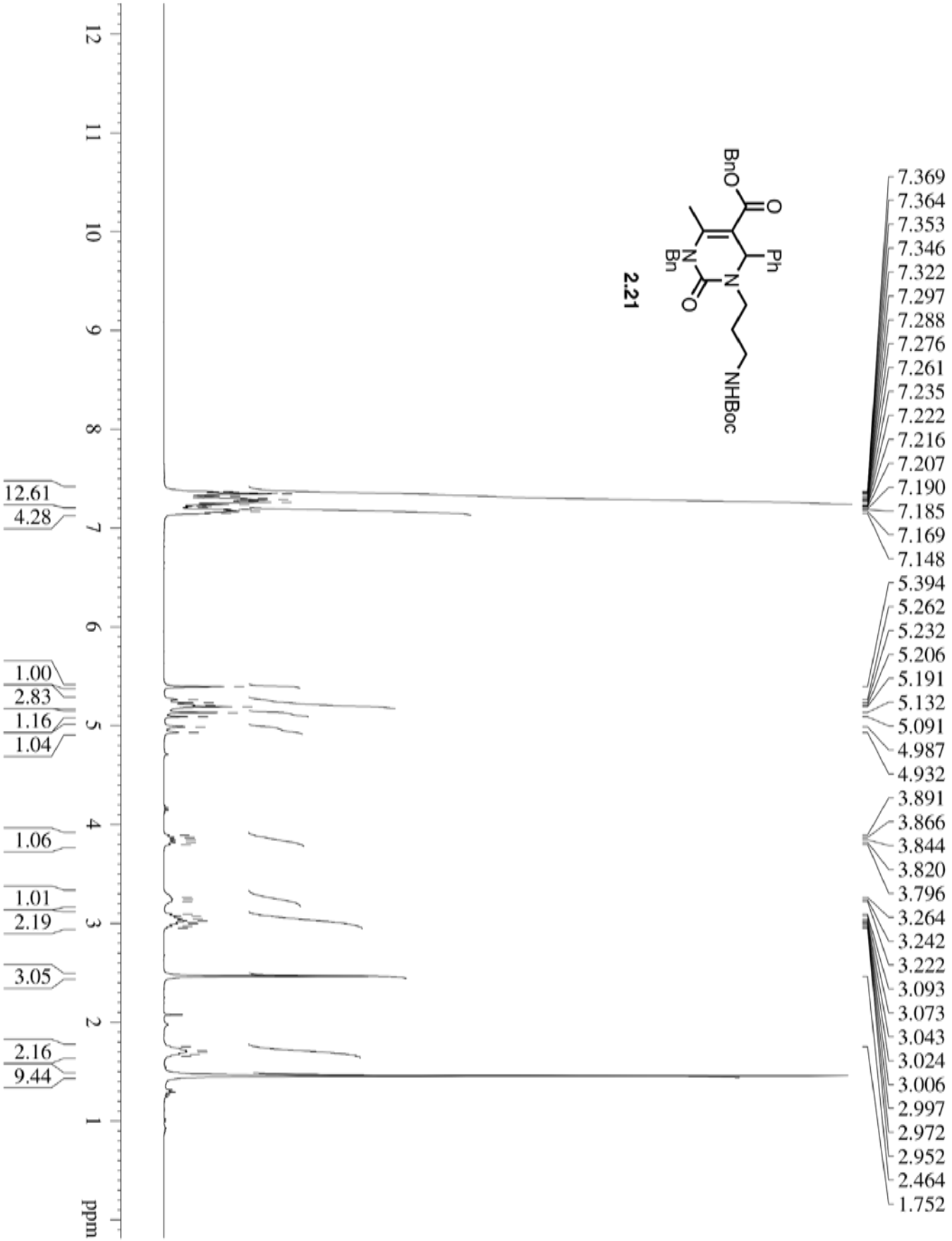

2.10
171.11
164.67
152.01
149.75
137.11
136.88
136.00
135.92
129.04
128.38
128.30
127.99
127.81
126.96
126.30
125.84
108.70
65.84
49.79
46.04
40.09
39.88
39.67
39.47
39.26
39.05
38.84
25.29
20.56
15.90
ppm

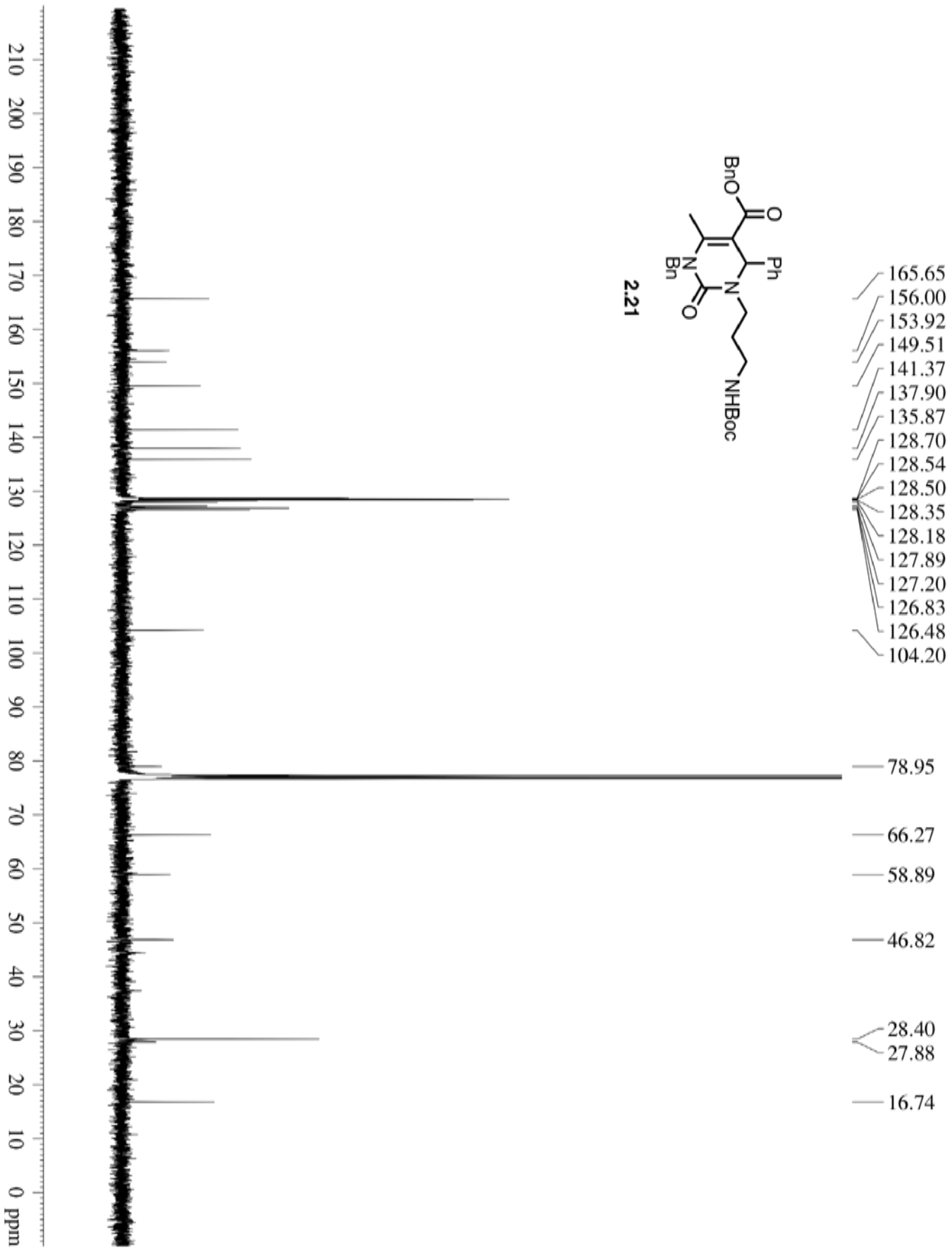

2.15
BnO
O
Ph
N
Bn
O
O
O
O
O
11
10
9
8
7
6
5
4
3
2
1
0
ppm
9.41
2.58
3.49
1.93
0.75
0.90
1.01
2.45
0.93
3.09
6.06
7.355
7.342
7.340
7.328
7.324
7.313
7.309
7.303
7.299
7.296
7.284
7.281
7.268
7.263
7.211
7.210
7.197
7.195
7.193
7.176
7.162
7.149
7.138
7.135
7.125
7.121
7.118
6.873
6.859
6.782
6.737
5.397
5.364
5.305
5.280
5.221
5.196
4.823
4.790
2.752
2.527
2.498
2.485
2.049
2.045
2.040
2.036
2.031
1.953
1.763
1.742

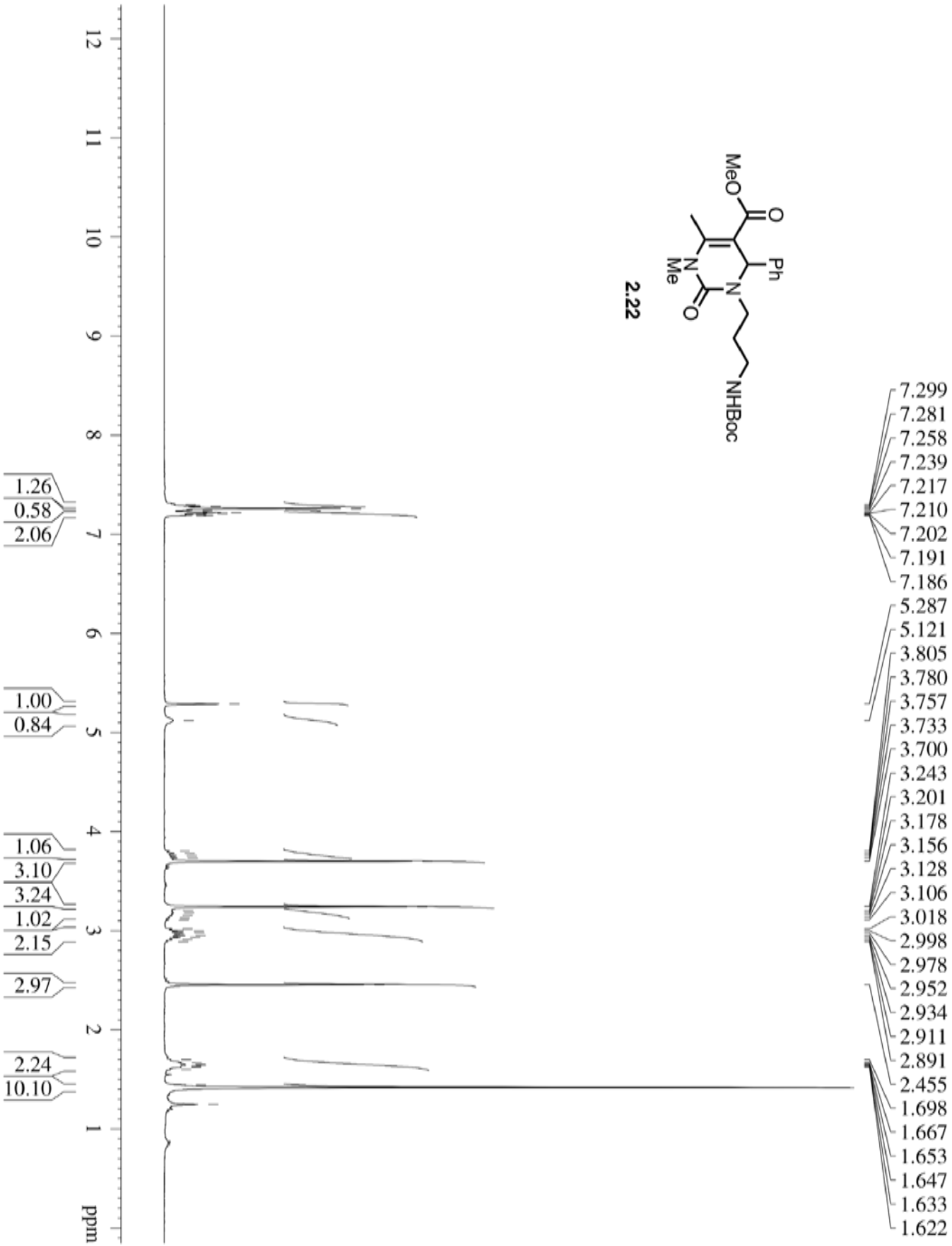

BnO
O
Ph
O
N
Bn
N
O
O
O
O
O
2.15
165.75
164.15
162.91
153.42
150.71
148.01
140.33
137.98
137.30
129.38
129.31
128.98
128.91
128.69
128.01
127.64
127.35
115.12
110.60
98.42
66.97
51.83
47.13
26.64
26.49
16.50
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

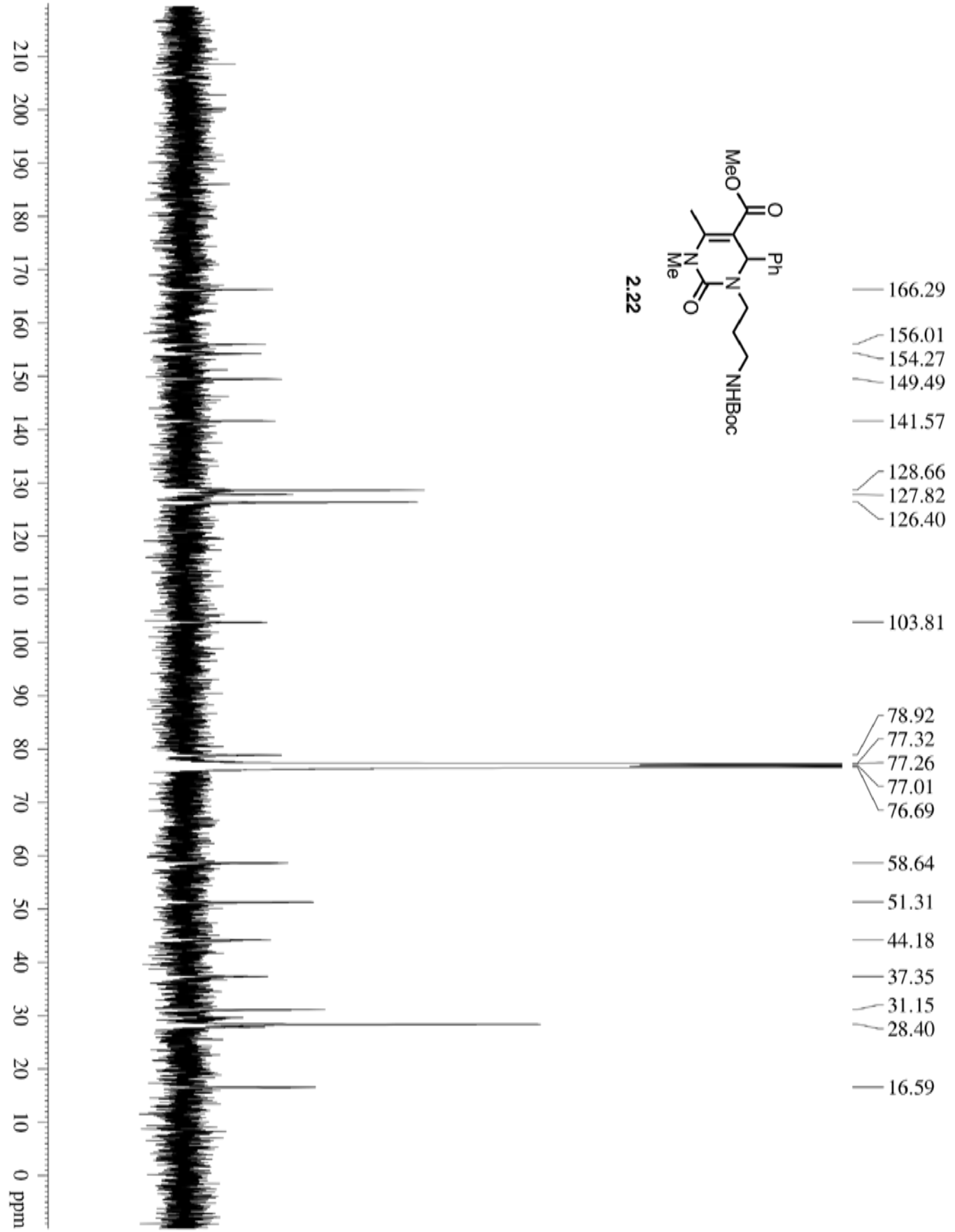

2.19
MeO
O
Ph
NH
N
Me
O
11
10
9
8
7
6
5
4
3
2
1
ppm
2.76
0.92
1.27
0.63
3.00
3.02
2.99
0.64
1.81
7.327
7.310
7.293
7.259
7.236
5.378
5.357
3.656
3.244
2.522
2.171
1.549

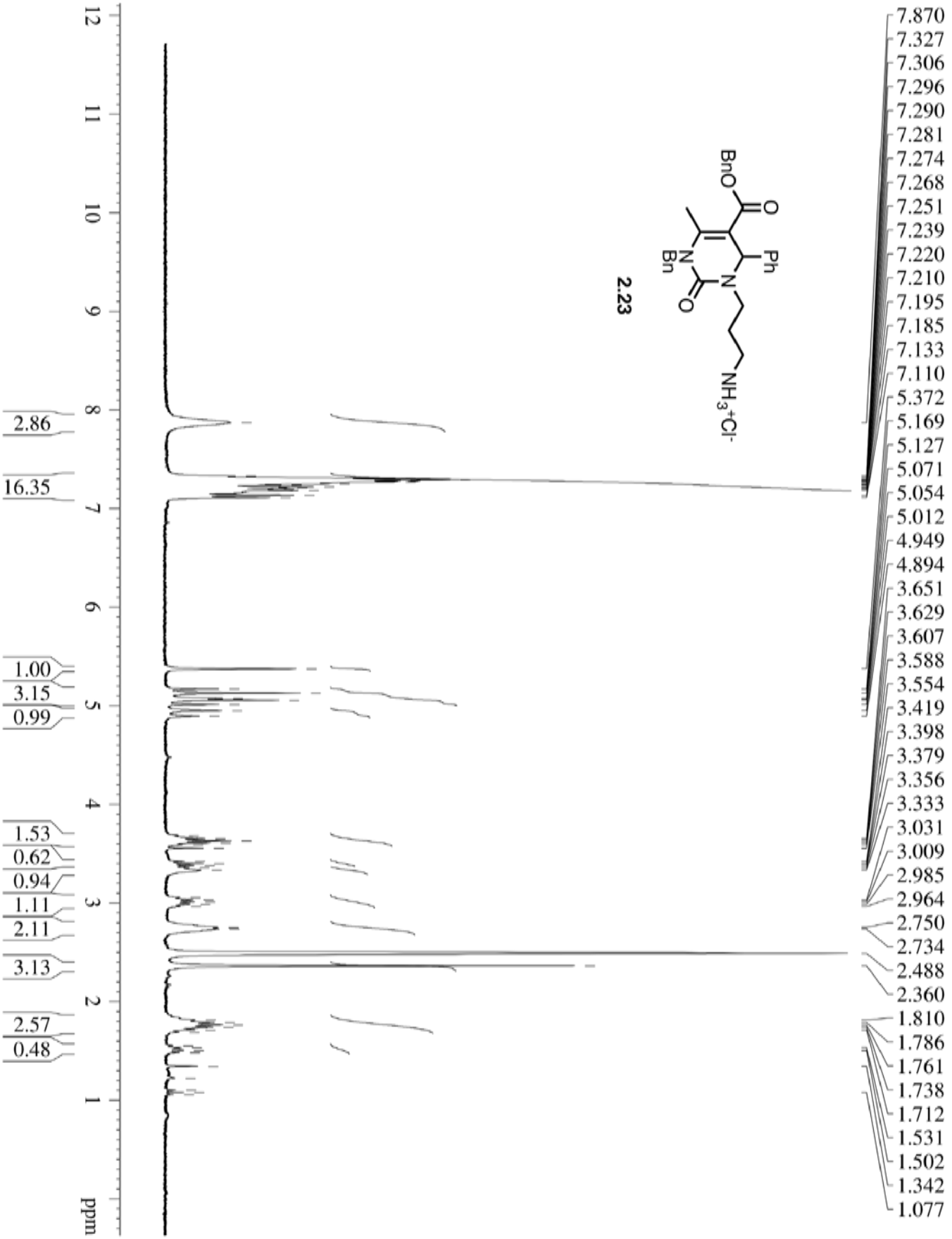

2.19
MeO
O
Ph
NH
N
Me
O
166.51
149.61
143.20
128.78
127.88
126.11
104.00
53.89
51.33
30.93
30.35
16.62
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

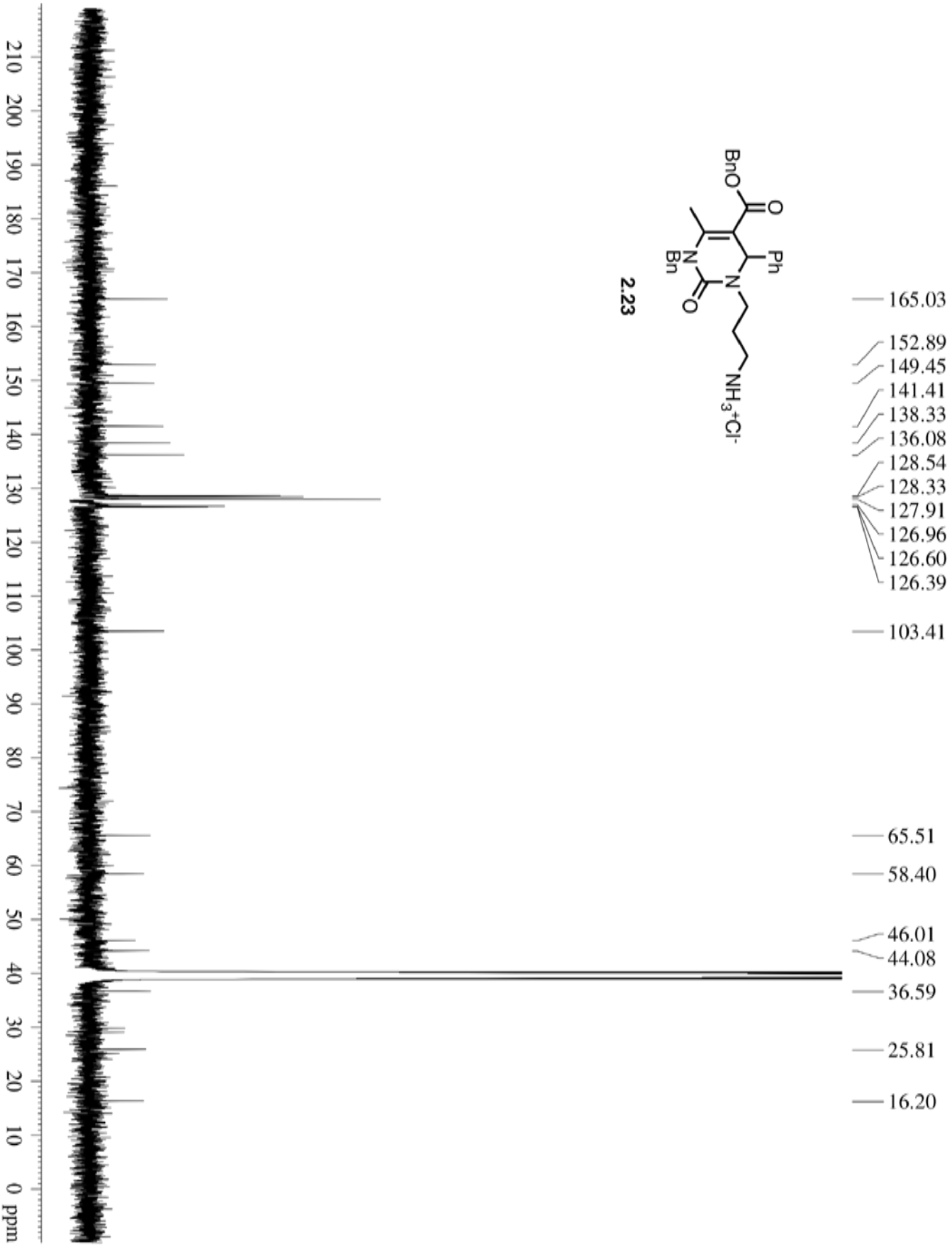

2.21
BnO
O
Ph
N
Bn
N
O
NHBoc
7.369
7.364
7.353
7.346
7.322
7.297
7.288
7.276
7.261
7.235
7.222
7.216
7.207
7.190
7.185
7.169
7.148
5.394
5.262
5.232
5.206
5.191
5.132
5.091
4.987
4.932
3.891
3.866
3.844
3.820
3.796
3.264
3.242
3.222
3.093
3.073
3.043
3.024
3.006
2.997
2.972
2.952
2.464
1.752
12.61
4.28
1.00
2.83
1.16
1.04
1.06
1.01
2.19
3.05
2.16
9.44
12
11
10
9
8
7
6
5
4
3
2
1
ppm

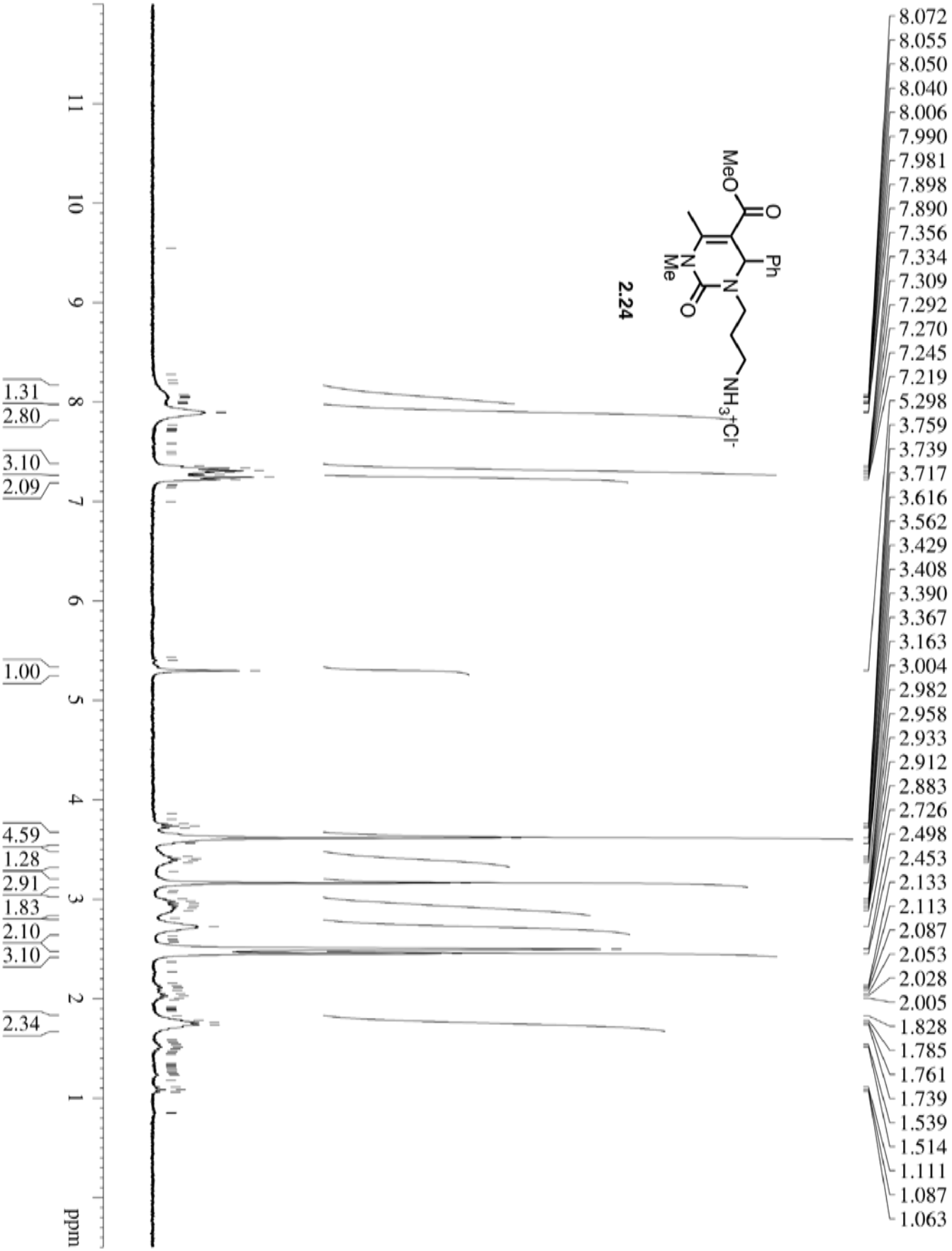

BnO
O
Ph
N
Bn
N
O
NHBoc
2.21
165.65
156.00
153.92
149.51
141.37
137.90
135.87
128.70
128.54
128.50
128.35
128.18
127.89
127.20
126.83
126.48
104.20
78.95
66.27
58.89
46.82
28.40
27.88
16.74
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

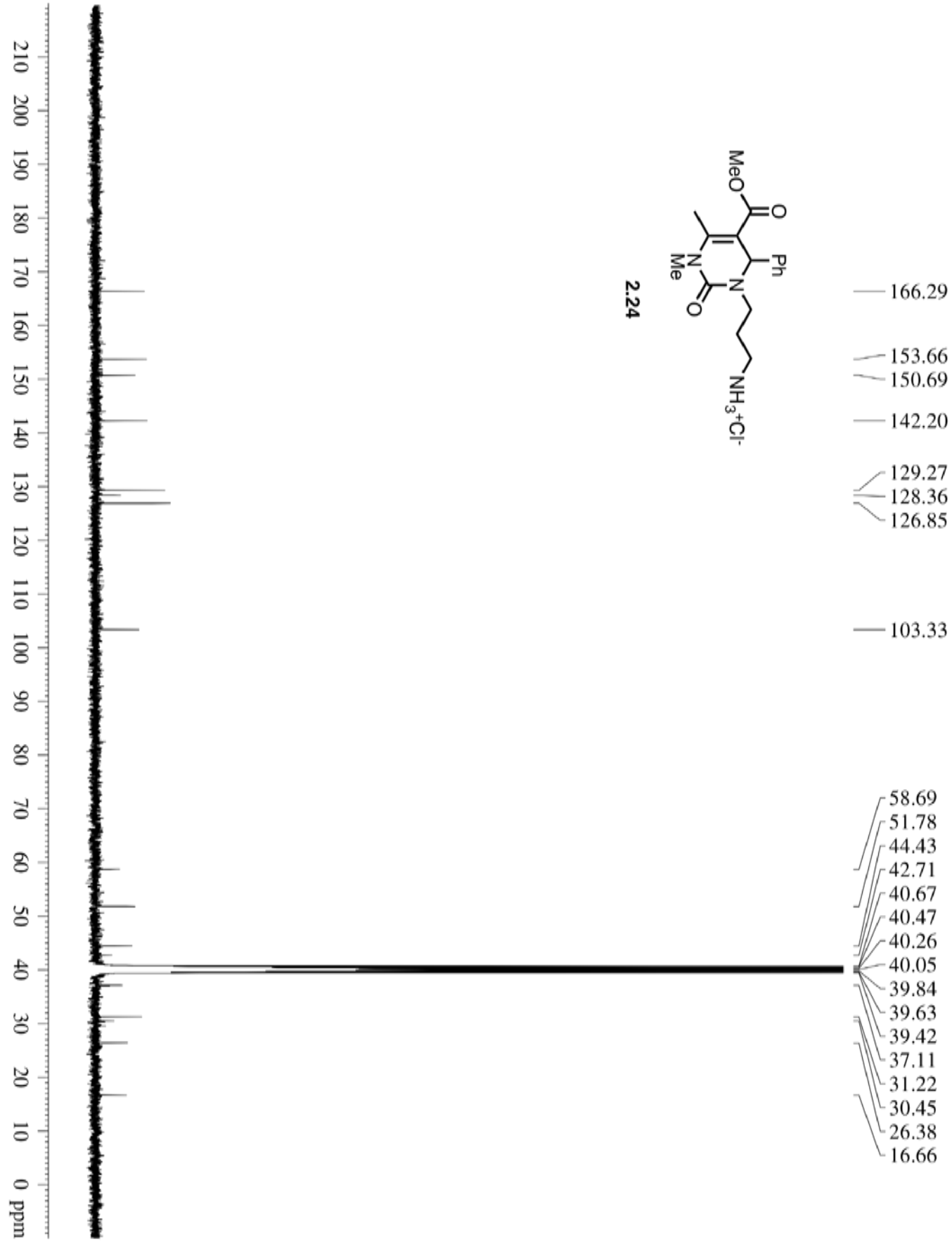

MeO
O
Ph
N
Me
N
O
NHBoc
2.22
7.299
7.281
7.258
7.239
7.217
7.210
7.202
7.191
7.186
5.287
5.121
3.805
3.780
3.757
3.733
3.700
3.243
3.201
3.178
3.156
3.128
3.106
3.018
2.998
2.978
2.952
2.934
2.911
2.891
2.455
1.698
1.667
1.653
1.647
1.633
1.622
1.26
0.58
2.06
1.00
0.84
1.06
3.10
3.24
1.02
2.15
2.97
2.24
10.10
ppm

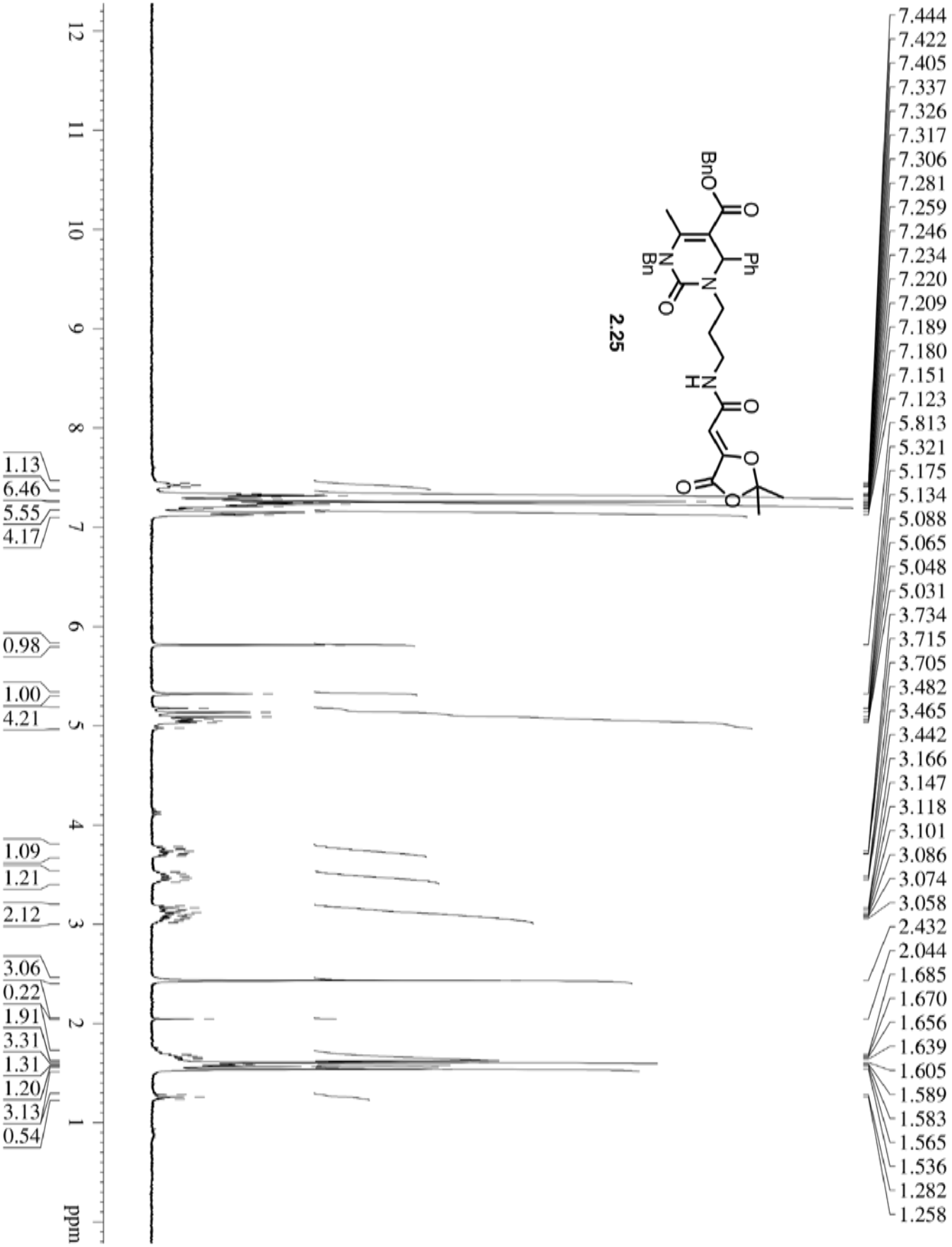

MeO
O
Ph
N
Me
N
O
NHBoc
2.22
166.29
156.01
154.27
149.49
141.57
128.66
127.82
126.40
103.81
78.92
77.32
77.26
77.01
76.69
58.64
51.31
44.18
37.35
31.15
28.40
16.59
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

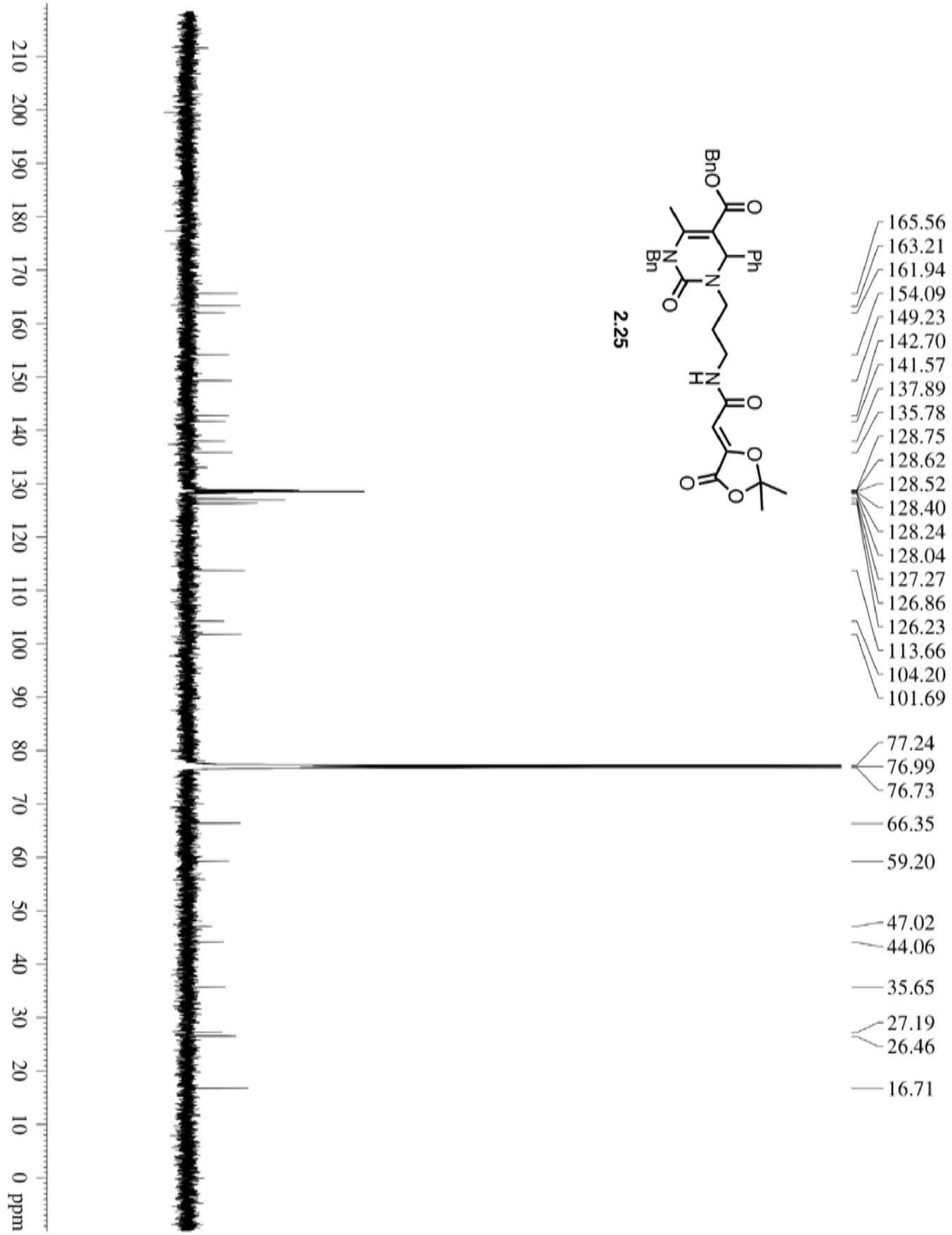

2.23
BnO
O
Ph
N
Bn
N
O
NH3+Cl-
2.86
16.35
1.00
3.15
0.99
1.53
0.62
0.94
1.11
2.11
3.13
2.57
0.48
ppm
7.870
7.327
7.306
7.296
7.290
7.281
7.274
7.268
7.251
7.239
7.220
7.210
7.195
7.185
7.133
7.110
5.372
5.169
5.127
5.071
5.054
5.012
4.949
4.894
3.651
3.629
3.607
3.588
3.554
3.419
3.398
3.379
3.356
3.333
3.031
3.009
2.985
2.964
2.750
2.734
2.488
2.360
1.810
1.786
1.761
1.738
1.712
1.531
1.502
1.342
1.077

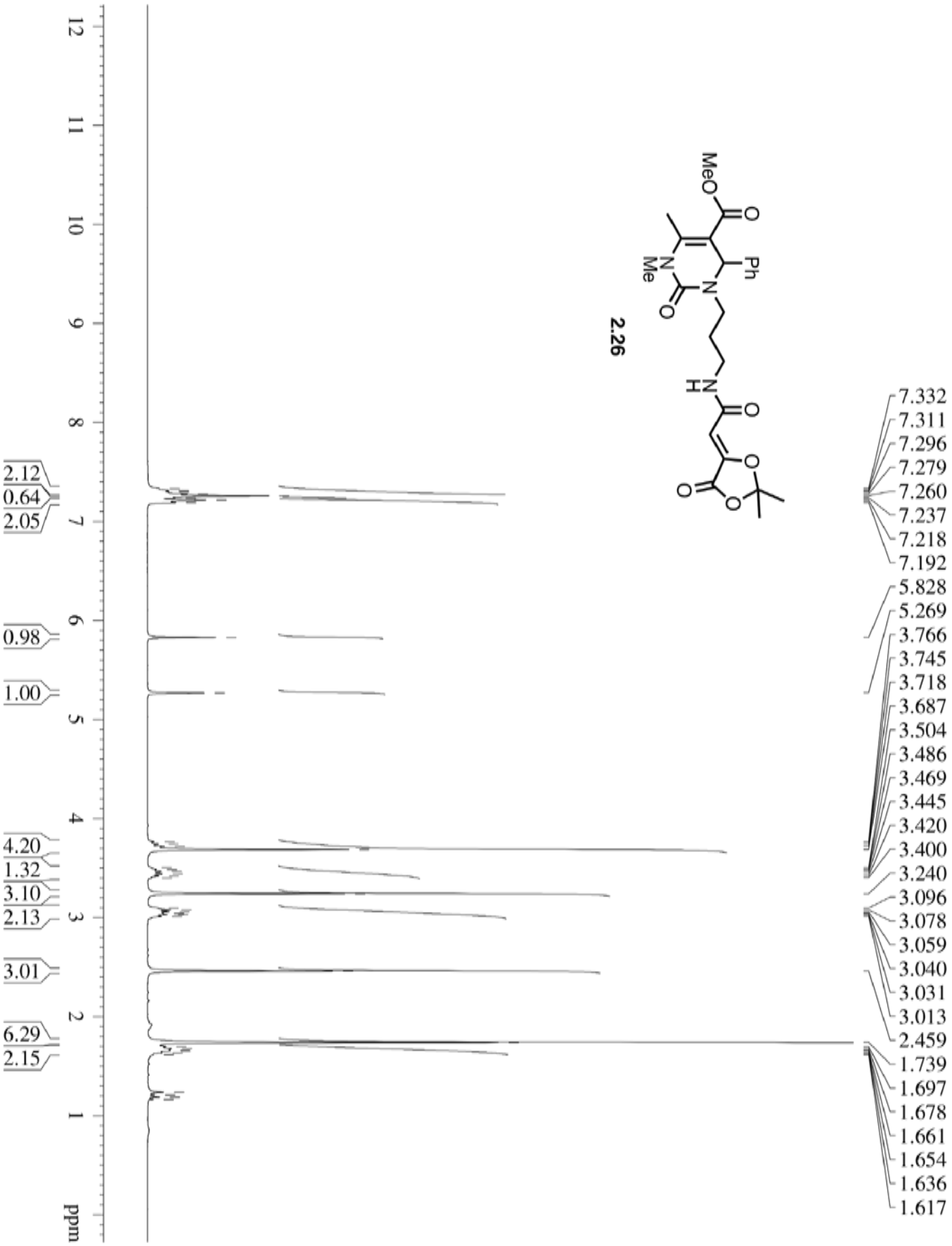

BnO
O
Ph
N
Bn
N
O
NH3+Cl-
2.23
165.03
152.89
149.45
141.41
138.33
136.08
128.54
128.33
127.91
126.96
126.60
126.39
103.41
65.51
58.40
46.01
44.08
36.59
25.81
16.20
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

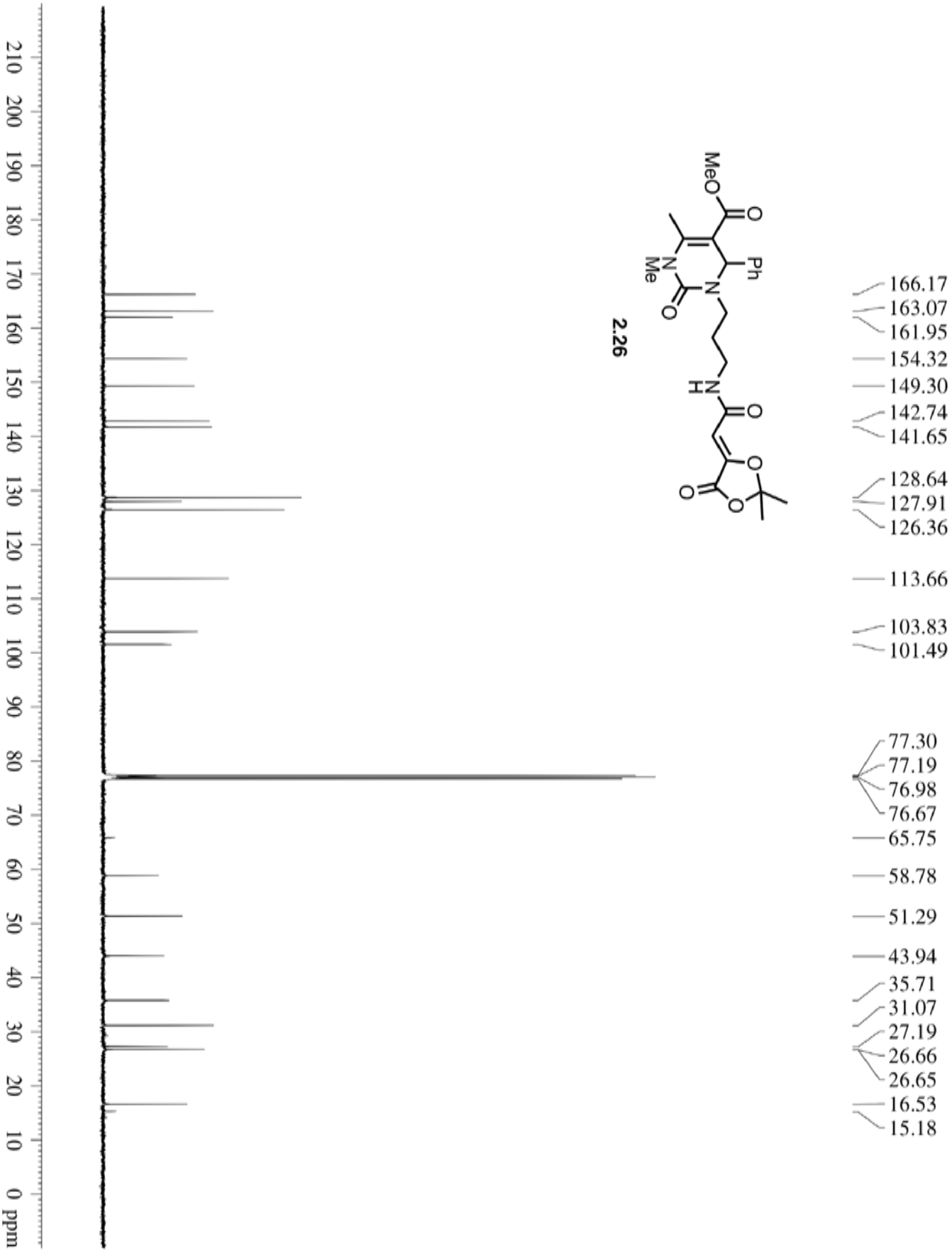

2.24
MeO
O
Ph
N
Me
N
O
NH3+Cl-
8.072
8.055
8.050
8.040
8.006
7.990
7.981
7.898
7.890
7.356
7.334
7.309
7.292
7.270
7.245
7.219
5.298
3.759
3.739
3.717
3.616
3.562
3.429
3.408
3.390
3.367
3.163
3.004
2.982
2.958
2.933
2.912
2.883
2.726
2.498
2.453
2.133
2.113
2.087
2.053
2.028
2.005
1.828
1.785
1.761
1.739
1.539
1.514
1.111
1.087
1.063
1.31
2.80
3.10
2.09
1.00
4.59
1.28
2.91
1.83
2.10
3.10
2.34
11
10
9
8
7
6
5
4
3
2
1
ppm

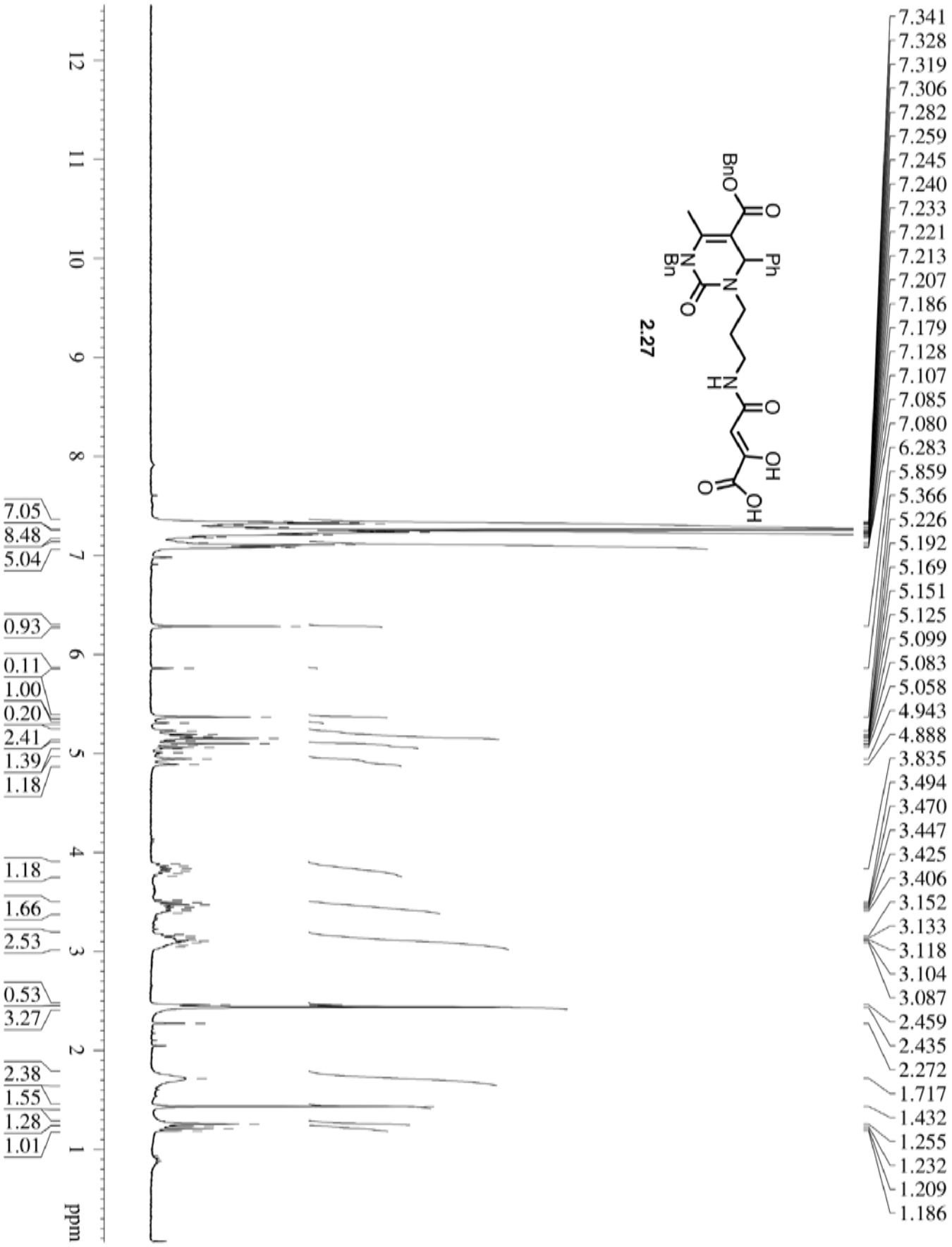

MeO
O
Ph
N
Me
N
O
NH3+Cl-
2.24
166.29
153.66
150.69
142.20
129.27
128.36
126.85
103.33
58.69
51.78
44.43
42.71
40.67
40.47
40.26
40.05
39.84
39.63
39.42
37.11
31.22
30.45
26.38
16.66
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

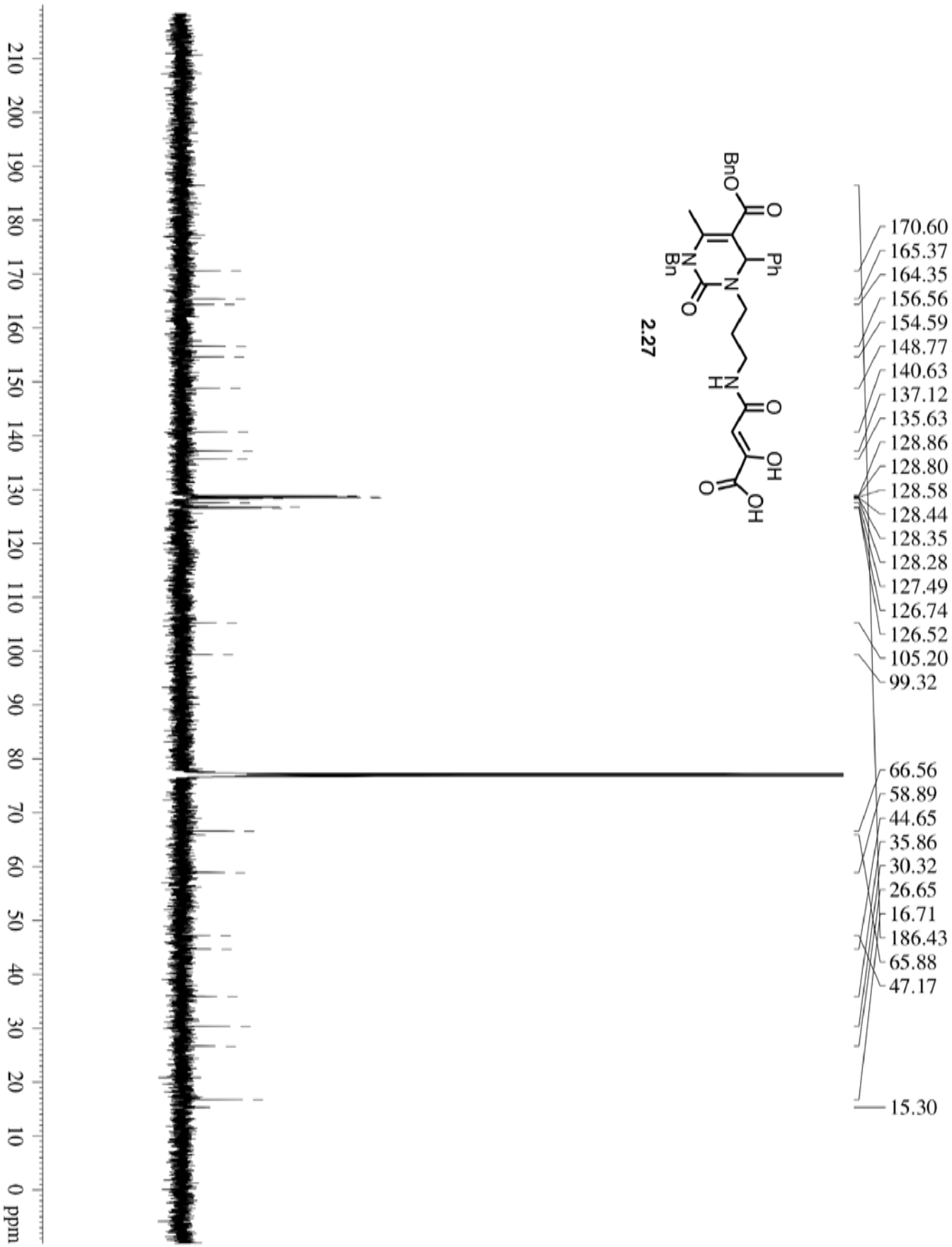

2.25
7.444
7.422
7.405
7.337
7.326
7.317
7.306
7.281
7.259
7.246
7.234
7.220
7.209
7.189
7.180
7.151
7.123
5.813
5.321
5.175
5.134
5.088
5.065
5.048
5.031
3.734
3.715
3.705
3.482
3.465
3.442
3.166
3.147
3.118
3.101
3.086
3.074
3.058
2.432
2.044
1.685
1.670
1.656
1.639
1.605
1.589
1.583
1.565
1.536
1.282
1.258
1.13
6.46
5.55
4.17
0.98
1.00
4.21
1.09
1.21
2.12
3.06
0.22
1.91
3.31
1.31
1.20
3.13
0.54
ppm

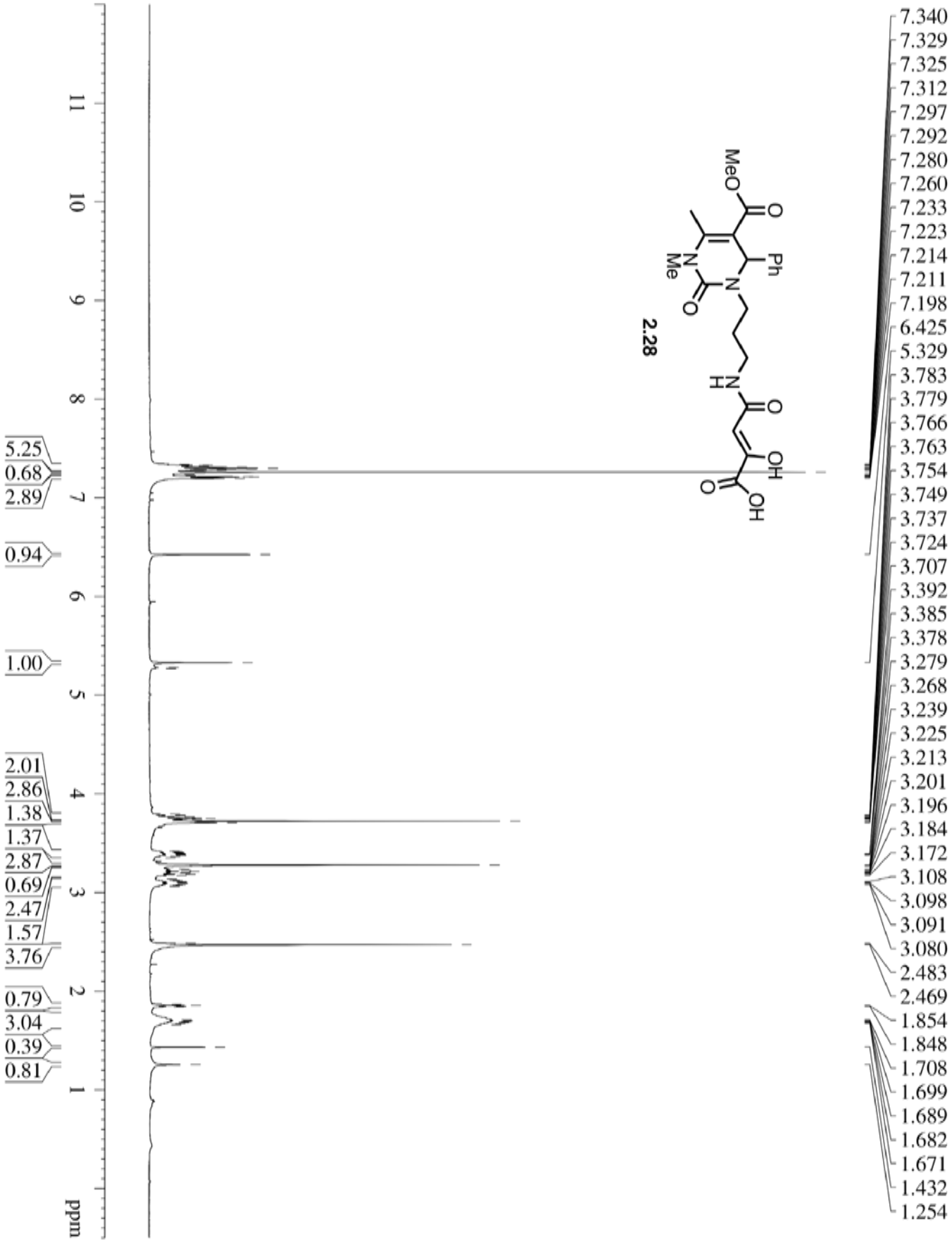

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BnO
O
Ph
N
Bn
N
O
N
H
O
O
O
O
2.25
165.56
163.21
161.94
154.09
149.23
142.70
141.57
137.89
135.78
128.75
128.62
128.52
128.40
128.24
128.04
127.27
126.86
126.23
113.66
104.20
101.69
77.24
76.99
76.73
66.35
59.20
47.02
44.06
35.65
27.19
26.46
16.71

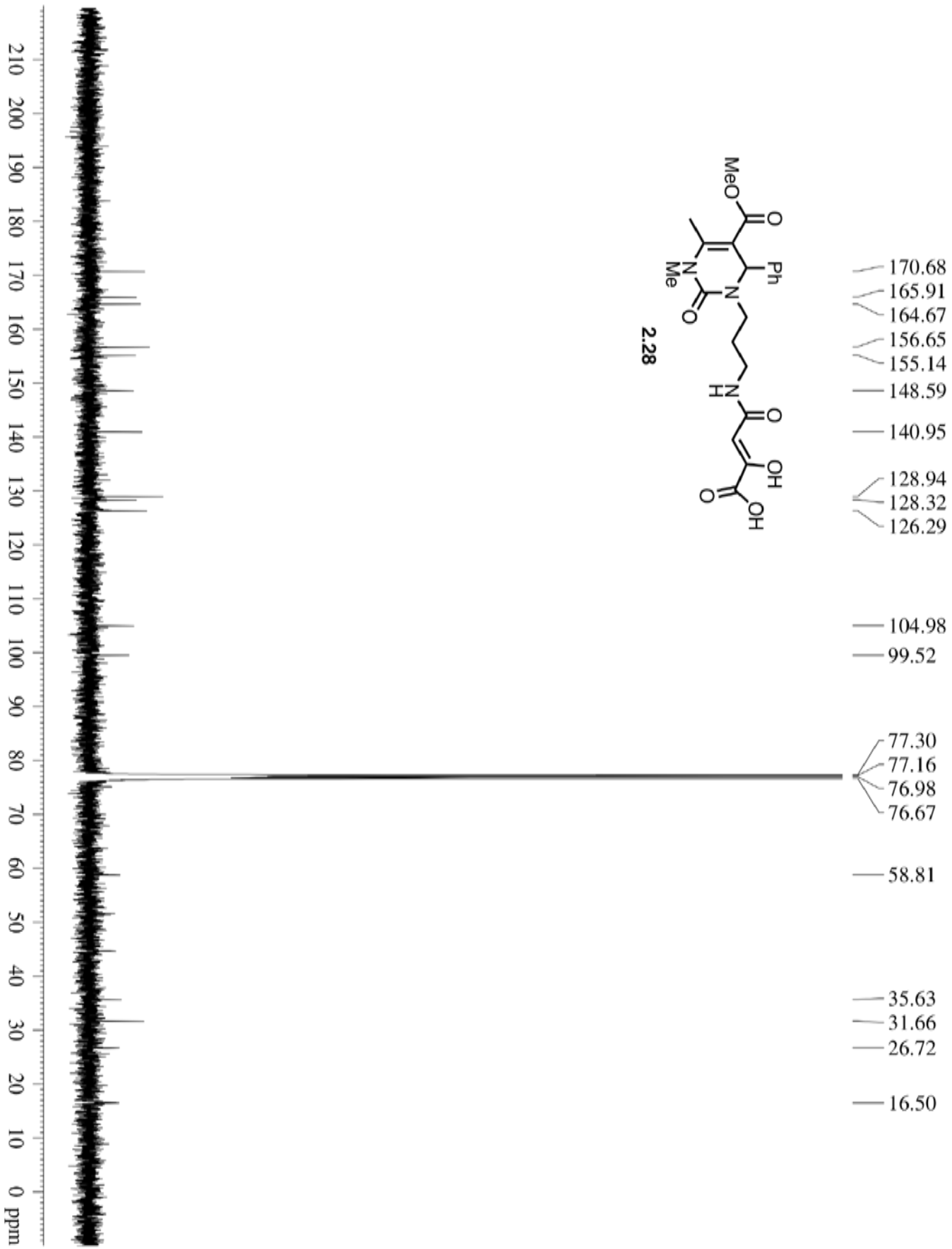

2.26
MeO
O
Ph
N
Me
N
O
N
H
O
O
O
O
12
11
10
9
8
7
6
5
4
3
2
1
ppm
2.12
0.64
2.05
0.98
1.00
4.20
1.32
3.10
2.13
3.01
6.29
2.15
7.332
7.311
7.296
7.279
7.260
7.237
7.218
7.192
5.828
5.269
3.766
3.745
3.718
3.687
3.504
3.486
3.469
3.445
3.420
3.400
3.240
3.096
3.078
3.059
3.040
3.031
3.013
2.459
1.739
1.697
1.678
1.661
1.654
1.636
1.617

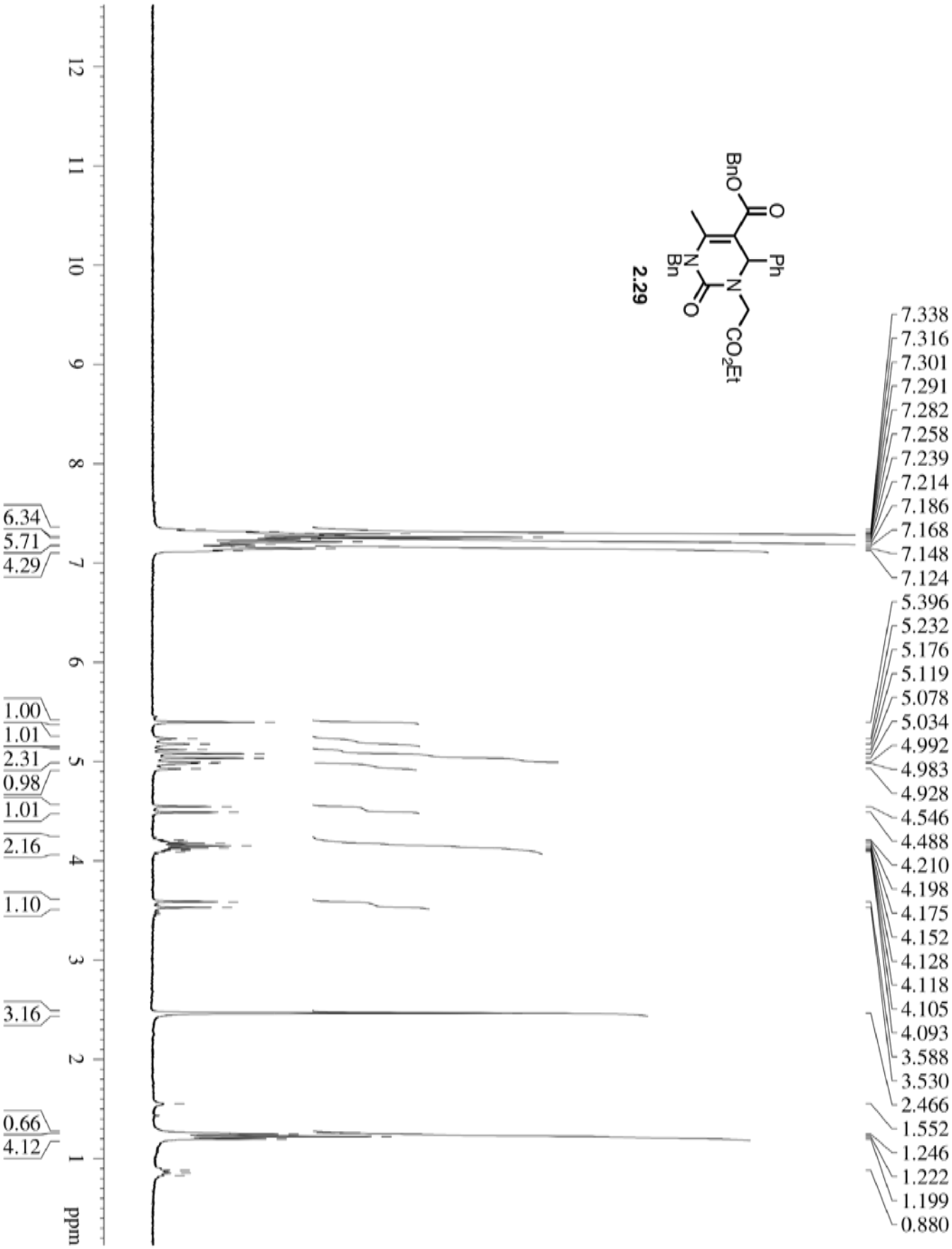

MeO
O
Ph
N
Me
N
O
N
H
O
O
O
O
2.26
166.17
163.07
161.95
154.32
149.30
142.74
141.65
128.64
127.91
126.36
113.66
103.83
101.49
77.30
77.19
76.98
76.67
65.75
58.78
51.29
43.94
35.71
31.07
27.19
26.66
26.65
16.53
15.18
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

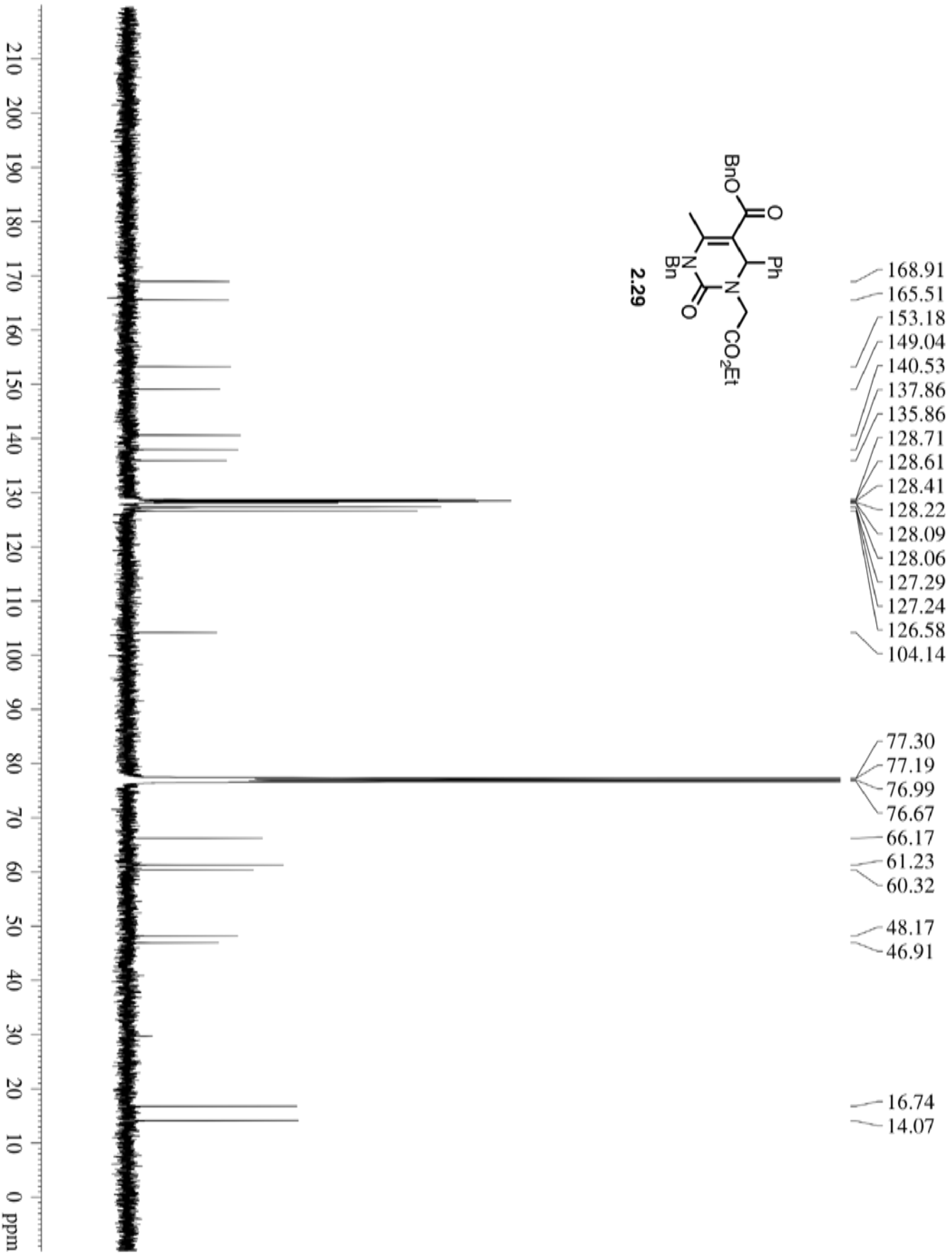

2.27
7.341
7.328
7.319
7.306
7.282
7.259
7.245
7.240
7.233
7.221
7.213
7.207
7.186
7.179
7.128
7.107
7.085
7.080
6.283
5.859
5.366
5.226
5.192
5.169
5.151
5.125
5.099
5.083
5.058
4.943
4.888
3.835
3.494
3.470
3.447
3.425
3.406
3.152
3.133
3.118
3.104
3.087
2.459
2.435
2.272
1.717
1.432
1.255
1.232
1.209
1.186
7.05
8.48
5.04
0.93
0.11
1.00
0.20
2.41
1.39
1.18
1.18
1.66
2.53
0.53
3.27
2.38
1.55
1.28
1.01
ppm

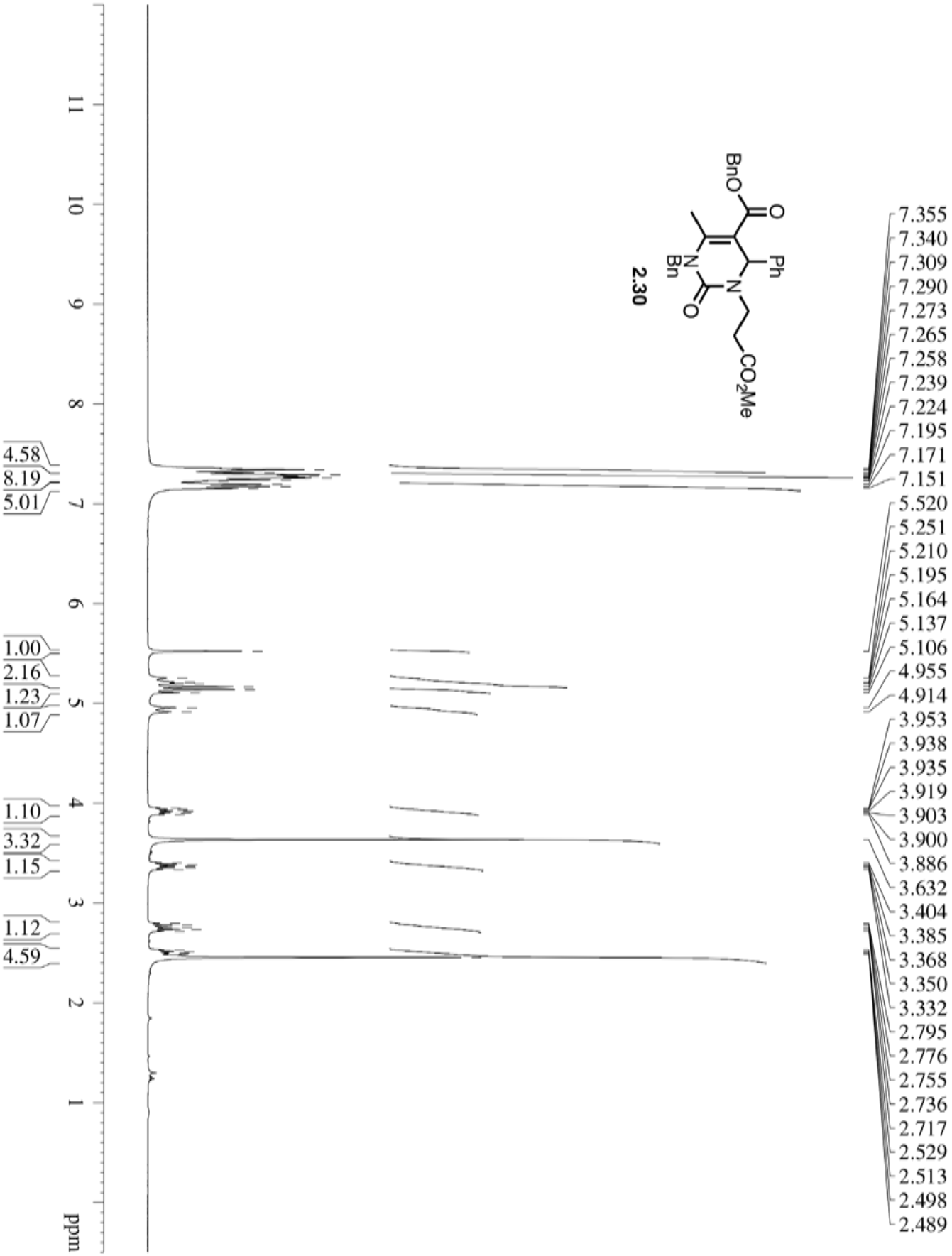

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BnO
O
Ph
N
Bn
O
N
H
O
OH
O
OH
2.27
170.60
165.37
164.35
156.56
154.59
148.77
140.63
137.12
135.63
128.86
128.80
128.58
128.44
128.35
128.28
127.49
126.74
126.52
105.20
99.32
66.56
58.89
44.65
35.86
30.32
26.65
16.71
186.43
65.88
47.17
15.30

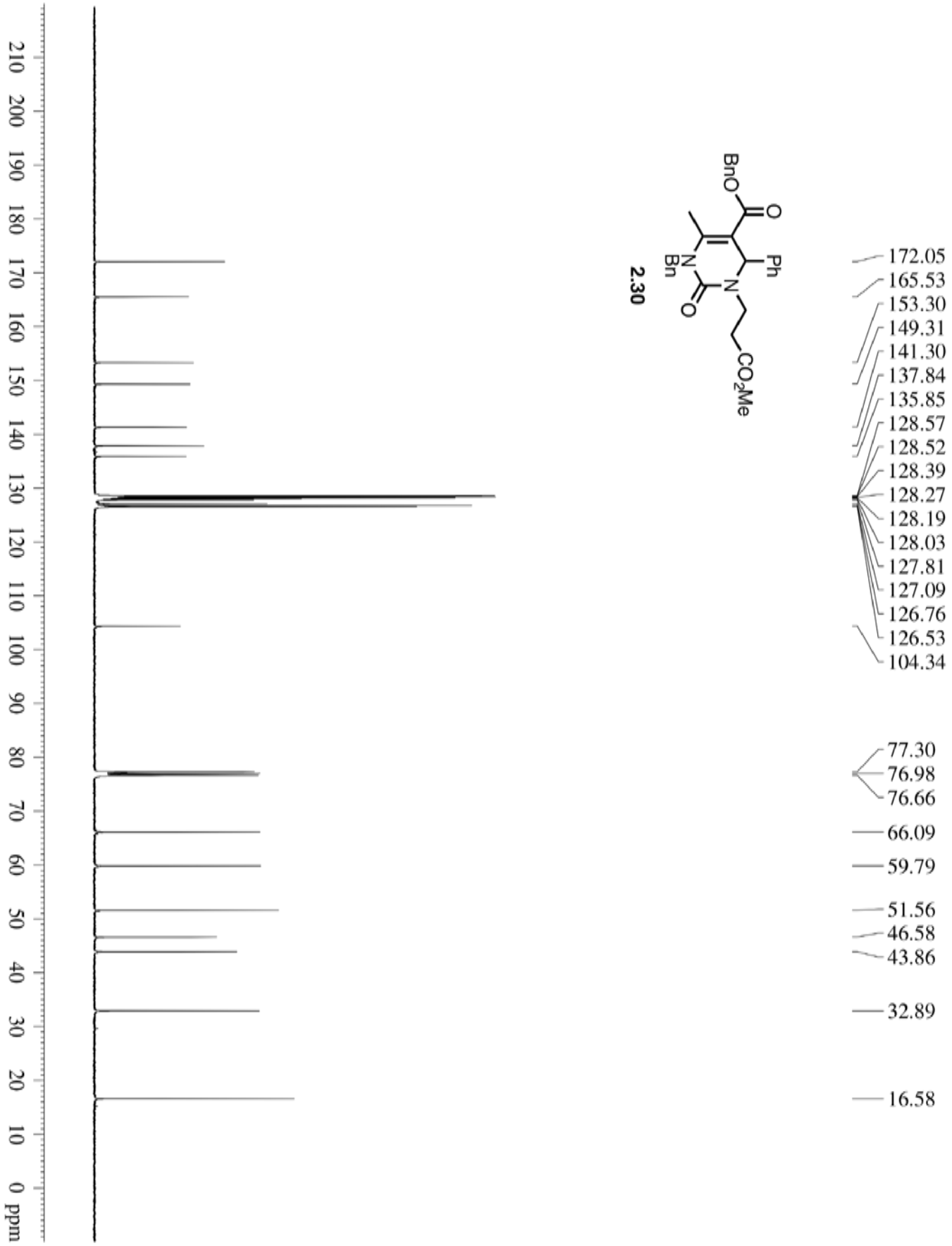

7.340
7.329
7.325
7.312
7.297
7.292
7.280
7.260
7.233
7.223
7.214
7.211
7.198
6.425
5.329
3.783
3.779
3.766
3.763
3.754
3.749
3.737
3.724
3.707
3.392
3.385
3.378
3.279
3.268
3.239
3.225
3.213
3.201
3.196
3.184
3.172
3.108
3.098
3.091
3.080
2.483
2.469
1.854
1.848
1.708
1.699
1.689
1.682
1.671
1.432
1.254
5.25
0.68
2.89
0.94
1.00
2.01
2.86
1.38
1.37
2.87
0.69
2.47
1.57
3.76
0.79
3.04
0.39
0.81
ppm
2.28

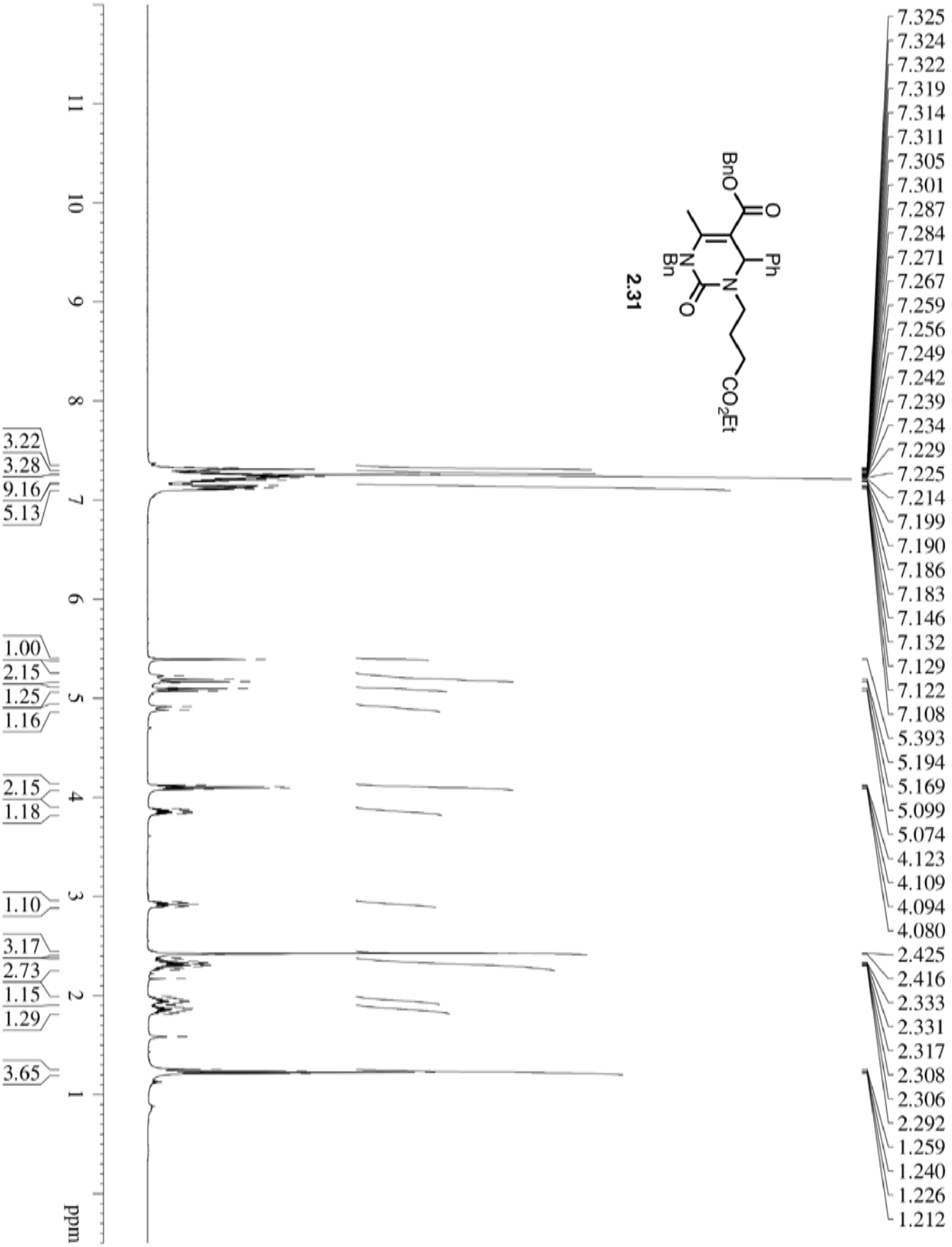

MeO
O
Ph
N
Me
N
O
N
H
O
OH
O
OH
2.28
170.68
165.91
164.67
156.65
155.14
148.59
140.95
128.94
128.32
126.29
104.98
99.52
77.30
77.16
76.98
76.67
58.81
35.63
31.66
26.72
16.50
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

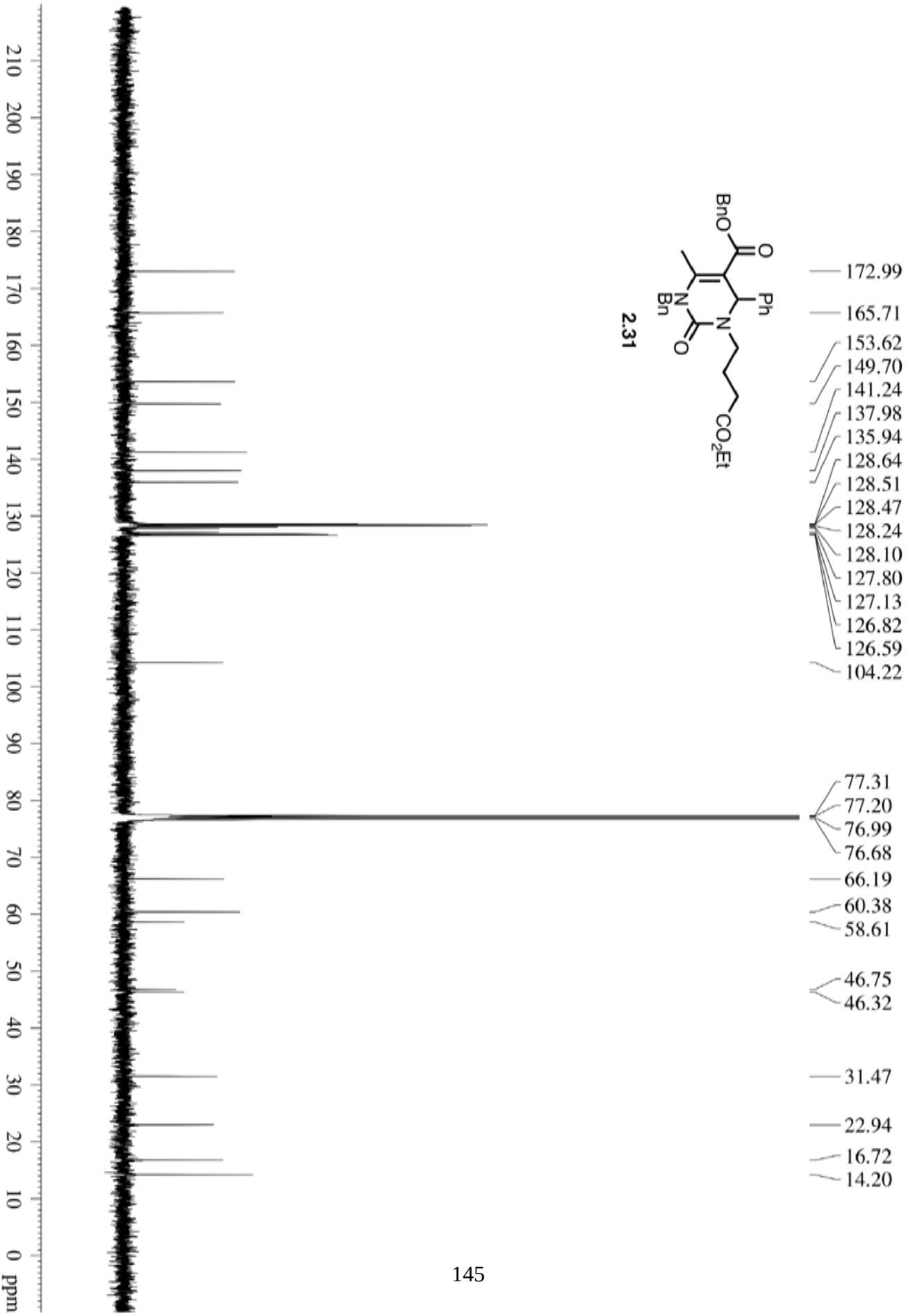

2.29
BnO
O
Ph
N
Bn
N
O
CO2Et
7.338
7.316
7.301
7.291
7.282
7.258
7.239
7.214
7.186
7.168
7.148
7.124
5.396
5.232
5.176
5.119
5.078
5.034
4.992
4.983
4.928
4.546
4.488
4.210
4.198
4.175
4.152
4.128
4.118
4.105
4.093
3.588
3.530
2.466
1.552
1.246
1.222
1.199
0.880
6.34
5.71
4.29
1.00
1.01
2.31
0.98
1.01
2.16
1.10
3.16
0.66
4.12
12
11
10
9
8
7
6
5
4
3
2
1
ppm

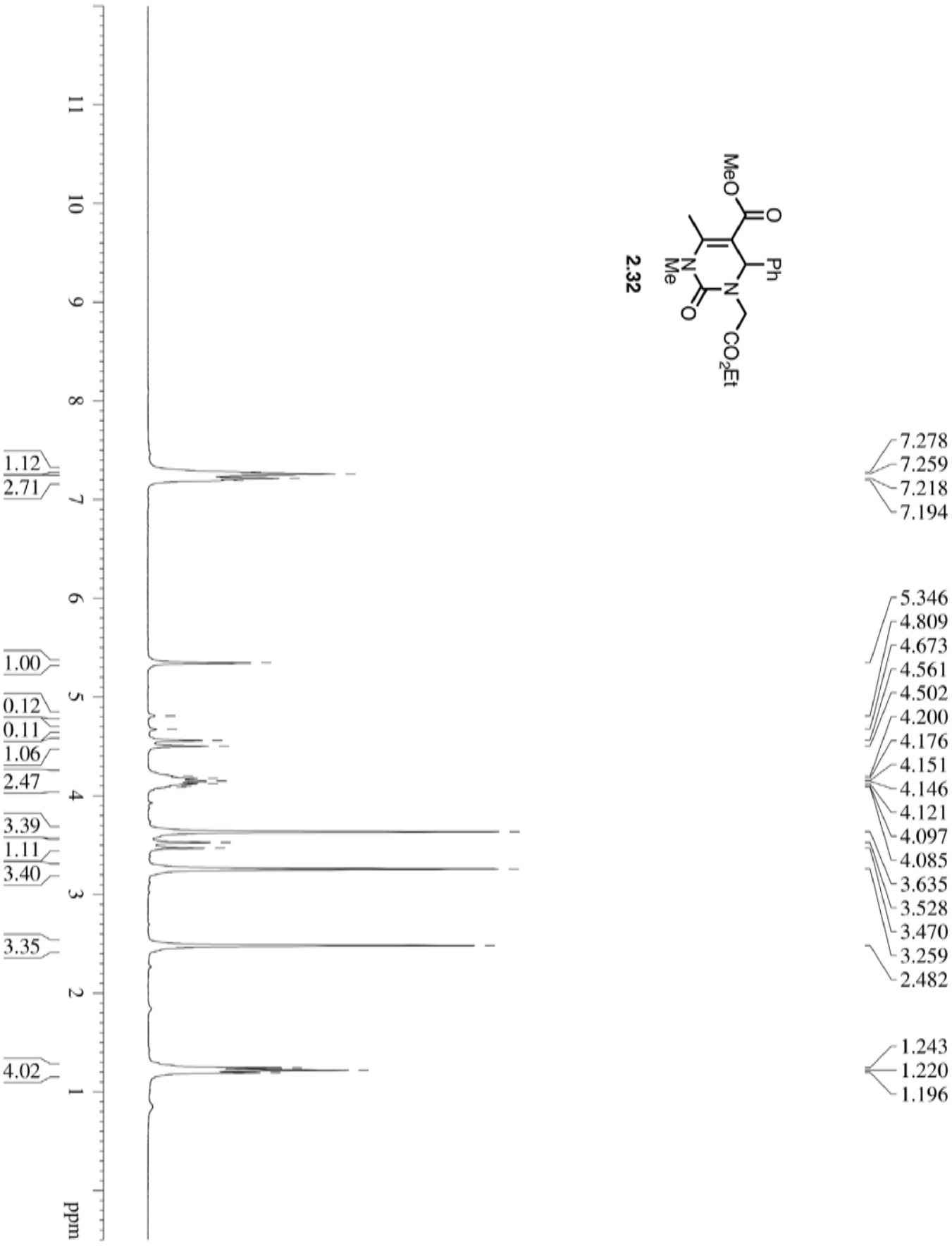

BnO
O
Ph
N
Bn
N
O
CO2Et
2.29
168.91
165.51
153.18
149.04
140.53
137.86
135.86
128.71
128.61
128.41
128.22
128.09
128.06
127.29
127.24
126.58
104.14
77.30
77.19
76.99
76.67
66.17
61.23
60.32
48.17
46.91
16.74
14.07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


BnO
O
Ph
N
Bn
N
O
CO2Me
2.30
7.355
7.340
7.309
7.290
7.273
7.265
7.258
7.239
7.224
7.195
7.171
7.151
5.520
5.251
5.210
5.195
5.164
5.137
5.106
4.955
4.914
3.953
3.938
3.935
3.919
3.903
3.900
3.886
3.632
3.404
3.385
3.368
3.350
3.332
2.795
2.776
2.755
2.736
2.717
2.529
2.513
2.498
2.489
4.58
8.19
5.01
1.00
2.16
1.23
1.07
1.10
3.32
1.15
1.12
4.59
ppm


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
BnO
O
Ph
N
Bn
N
O
CO2Me
2.30
172.05
165.53
153.30
149.31
141.30
137.84
135.85
128.57
128.52
128.39
128.27
128.19
128.03
127.81
127.09
126.76
126.53
104.34
77.30
76.98
76.66
66.09
59.79
51.56
46.58
43.86
32.89
16.58


2.31
BnO
O
Ph
N
Bn
N
O
CO2Et
7.325
7.324
7.322
7.319
7.314
7.311
7.305
7.301
7.287
7.284
7.271
7.267
7.259
7.256
7.249
7.242
7.239
7.234
7.229
7.225
7.214
7.199
7.190
7.186
7.183
7.146
7.132
7.129
7.122
7.108
5.393
5.194
5.169
5.099
5.074
4.123
4.109
4.094
4.080
2.425
2.416
2.333
2.331
2.317
2.308
2.306
2.292
1.259
1.240
1.226
1.212
3.22
3.28
9.16
5.13
1.00
2.15
1.25
1.16
2.15
1.18
1.10
3.17
2.73
1.15
1.29
3.65
11
10
9
8
7
6
5
4
3
2
1
ppm


2.31
BnO
O
Ph
N
Bn
N
O
CO2Et
172.99
165.71
153.62
149.70
141.24
137.98
135.94
128.64
128.51
128.47
128.24
128.10
127.80
127.13
126.82
126.59
104.22
77.31
77.20
76.99
76.68
66.19
60.38
58.61
46.75
46.32
31.47
22.94
16.72
14.20
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


MeO
O
Ph
N
Me
N
O
CO2Et
2.32
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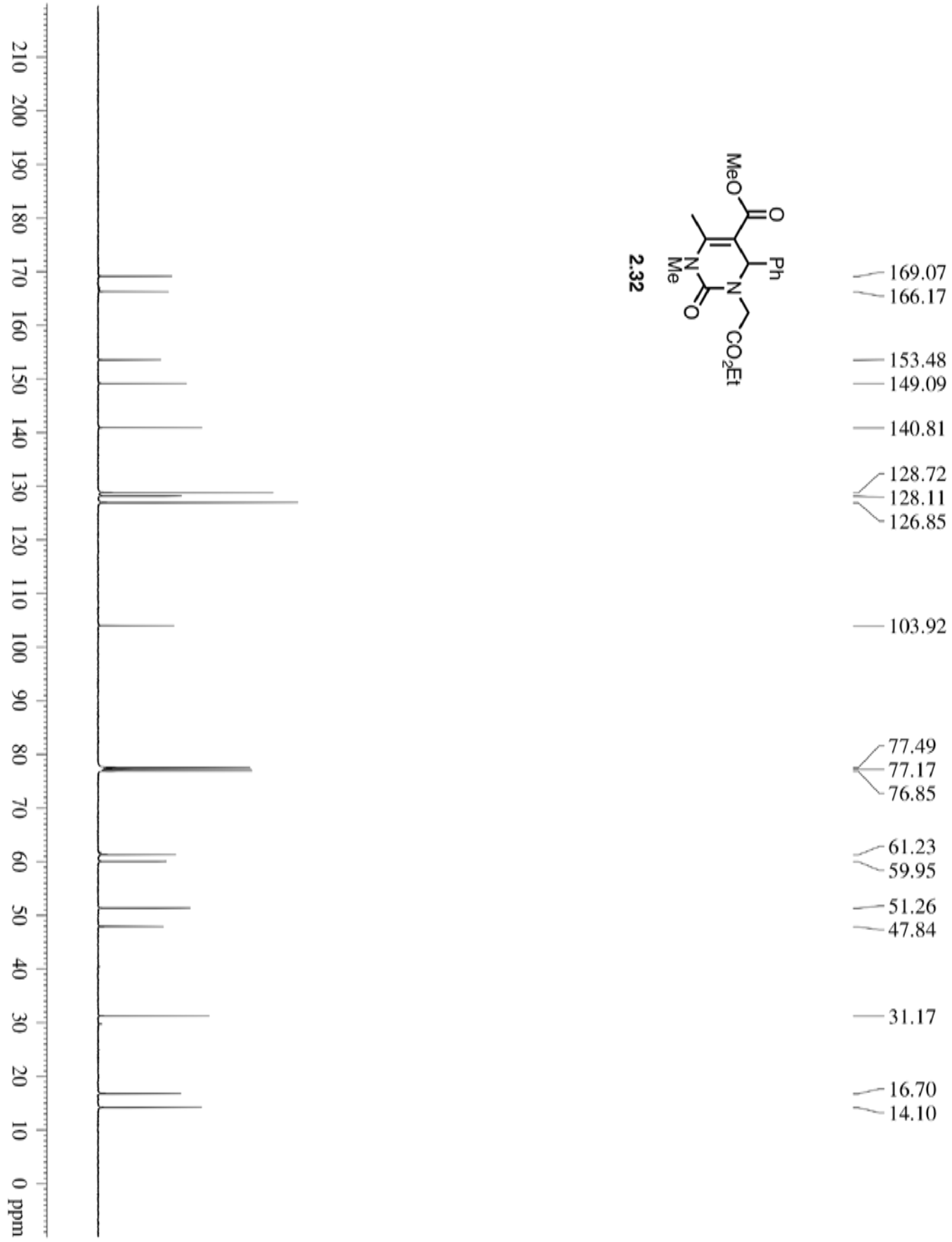
7.259
7.218
7.194
5.346
4.809
4.673
4.561
4.502
4.200
4.176
4.151
4.146
4.121
4.097
4.085
3.635
3.528
3.470
3.259
2.482
1.243
1.220
1.196
1.12
2.71
1.00
0.12
0.11
1.06
2.47
3.39
1.11
3.40
3.35
4.02
ppm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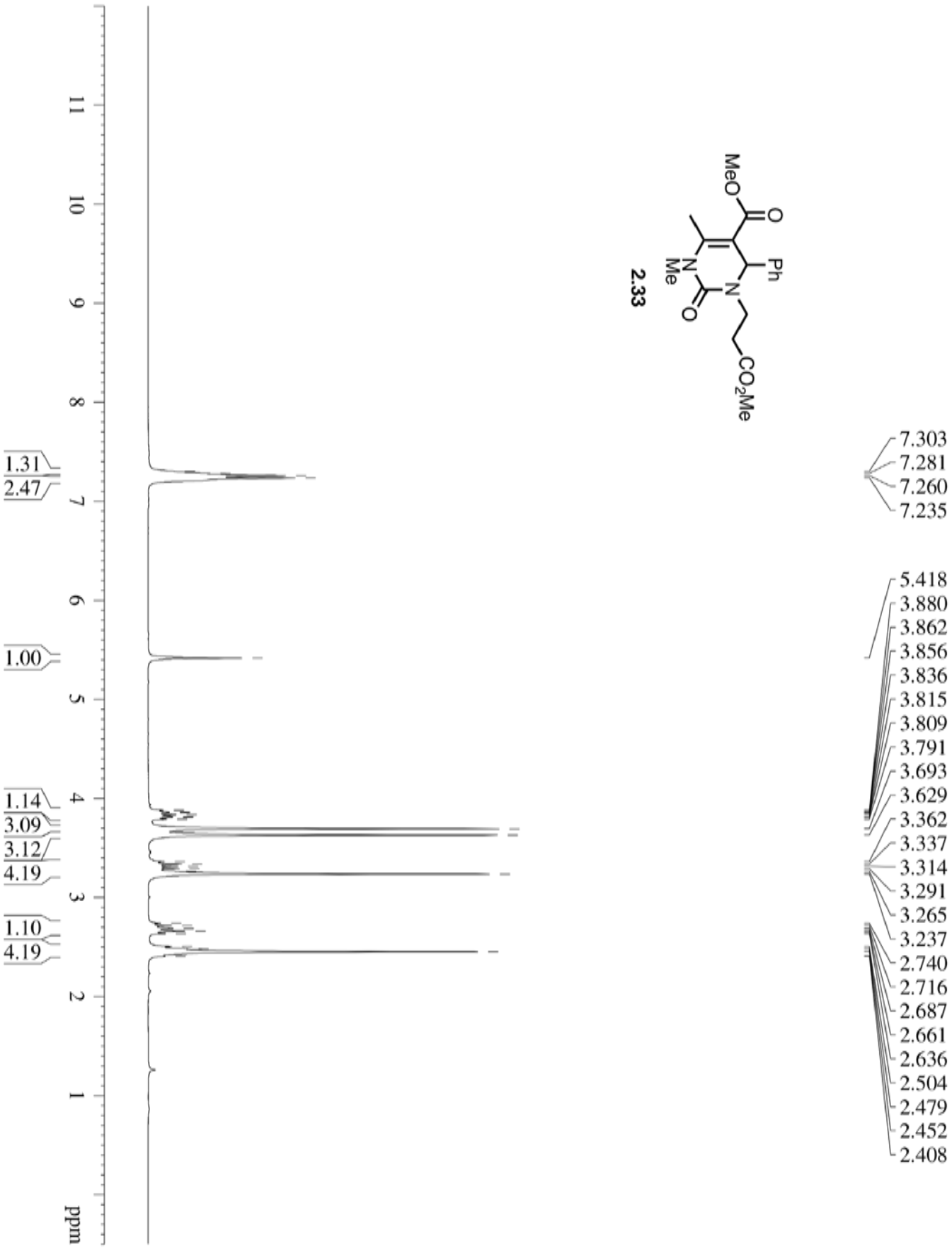
Ph
N
Me
N
O
CO2Et
2.32
169.07
166.17
153.48
149.09
140.81
128.72
128.11
126.85
103.92
77.49
77.17
76.85
61.23
59.95
51.26
47.84
31.17
16.70
14.10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
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Me
N
O
CO2Me
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7.235
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3.862
3.856
3.836
3.815
3.809
3.791
3.693
3.629
3.362
3.337
3.314
3.291
3.265
3.237
2.740
2.716
2.687
2.661
2.636
2.504
2.479
2.452
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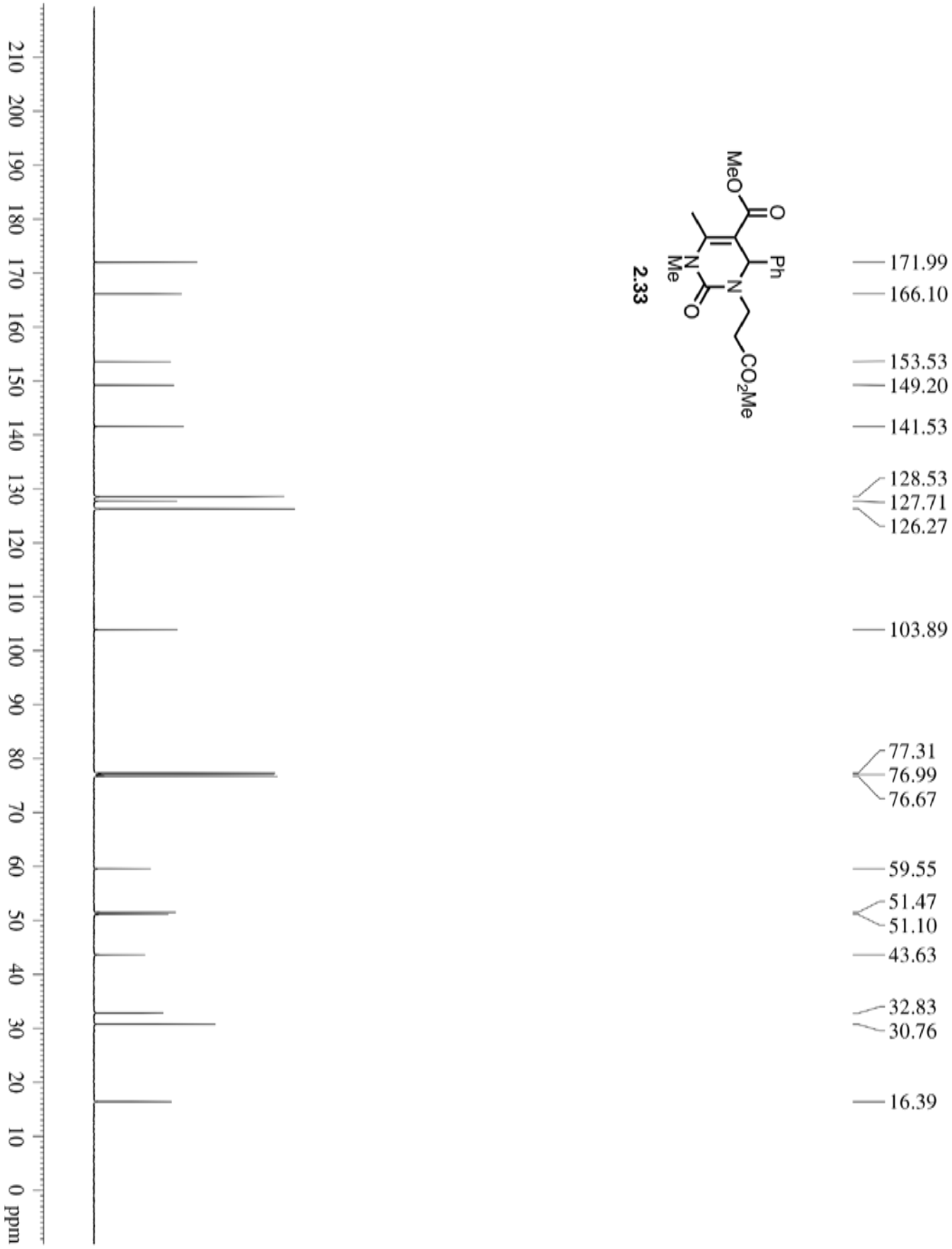
1.31
2.47
1.00
1.14
3.09
3.12
4.19
1.10
4.19
ppm


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm
MeO
O
Ph
N
Me
N
O
CO2Me
2.33
171.99
166.10
153.53
149.20
141.53
128.53
127.71
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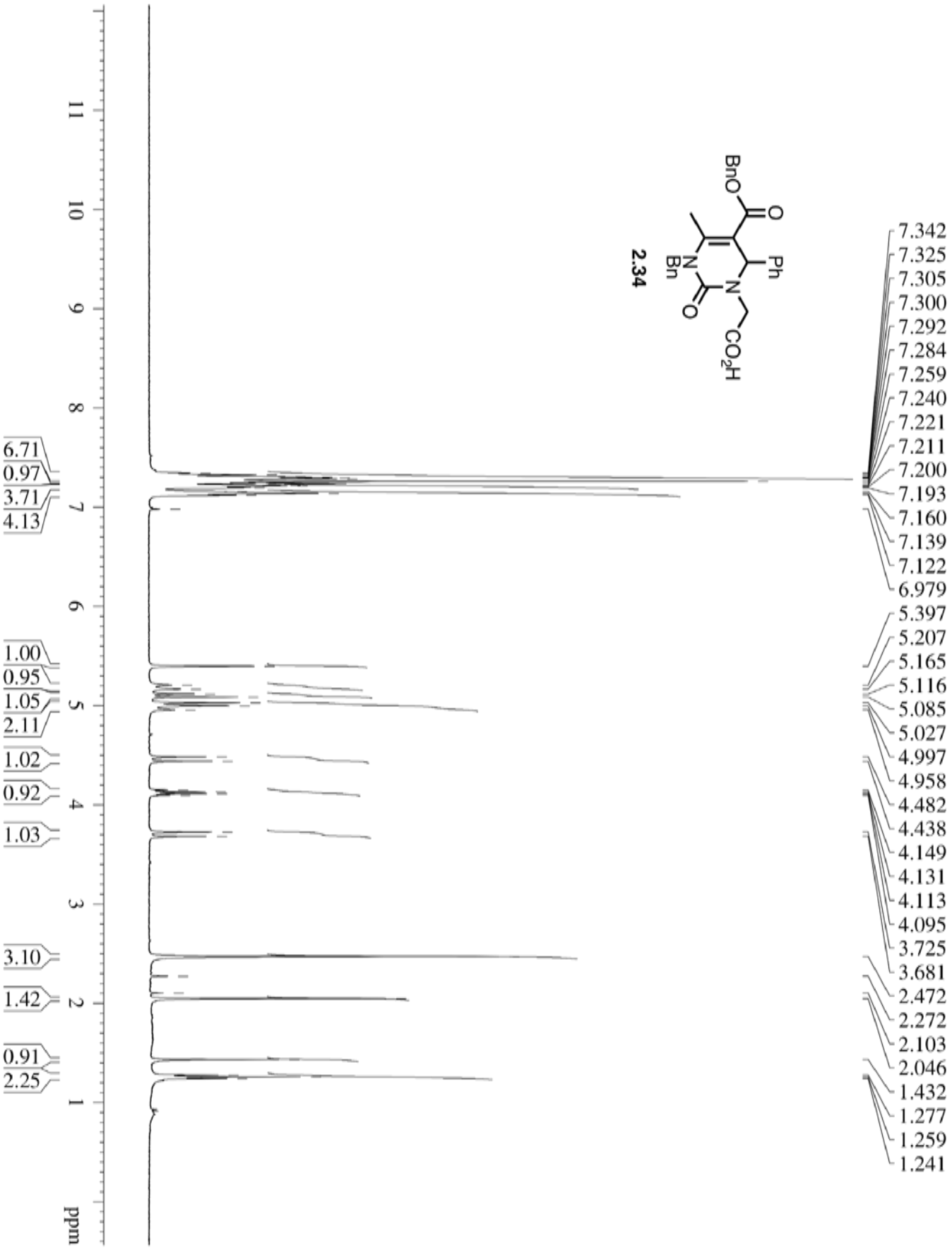
51.47
51.10
43.63
32.83
30.76
16.39


2.34
BnO
O
Ph
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Bn
N
O
CO2H
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7.325
7.305
7.300
7.292
7.284
7.259
7.240
7.221
7.211
7.200
7.193
7.160
7.139
7.122
6.979
5.397
5.207
5.165
5.116
5.085
5.027
4.997
4.958
4.482
4.438
4.149
4.131
4.113
4.095
3.725
3.681
2.472
2.272
2.103
2.046
1.432
1.277
1.259
1.241
6.71
0.97
3.71
4.13
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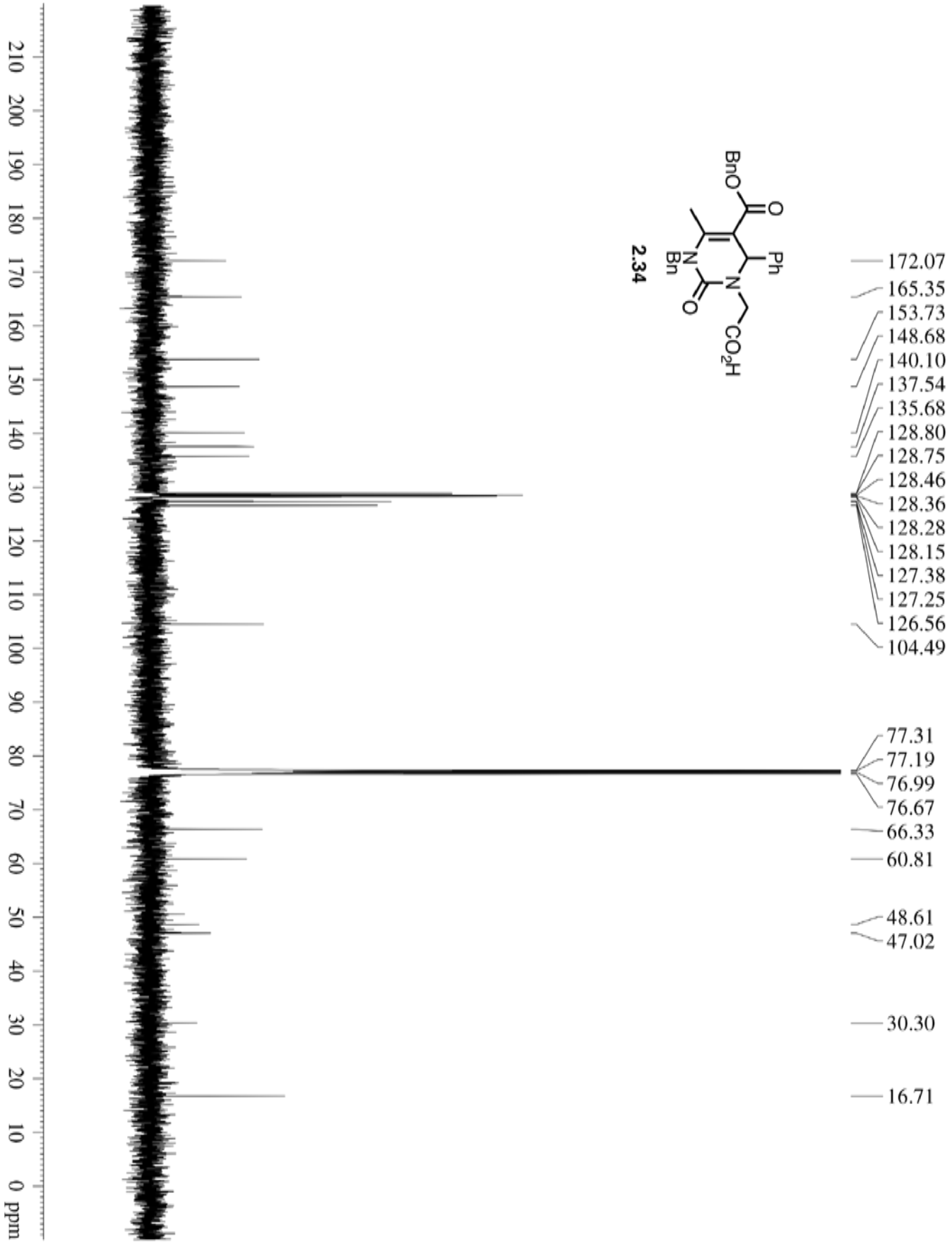
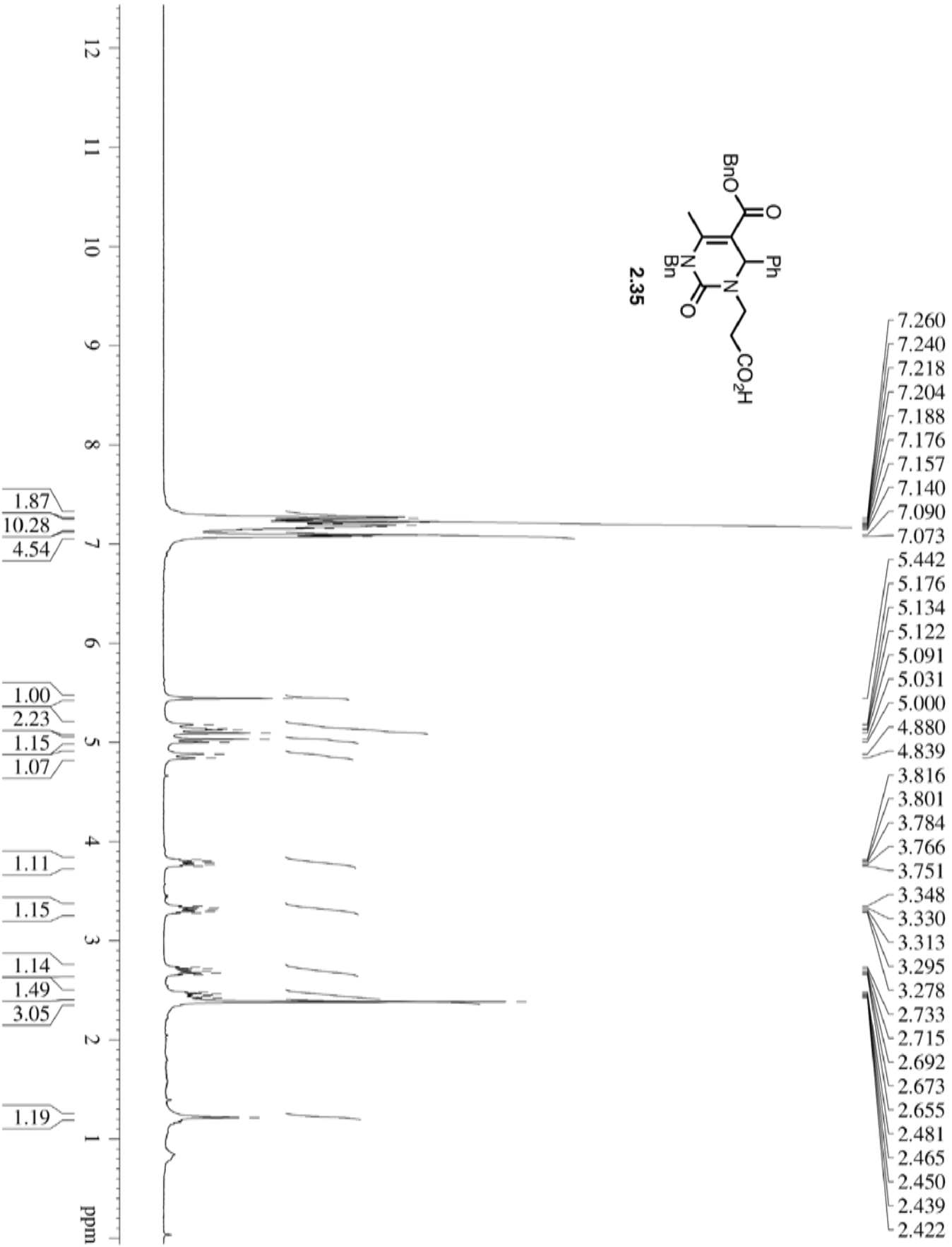
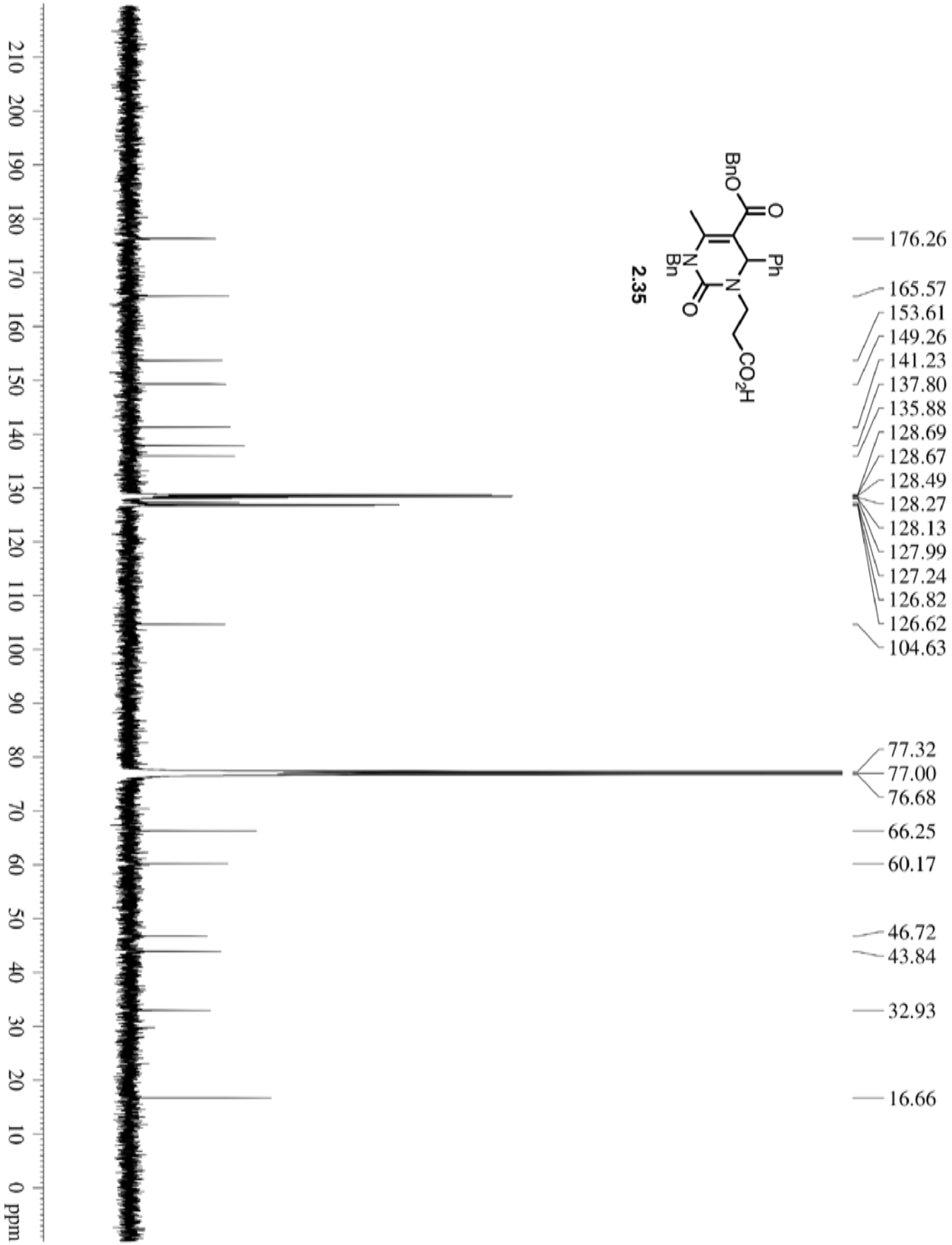
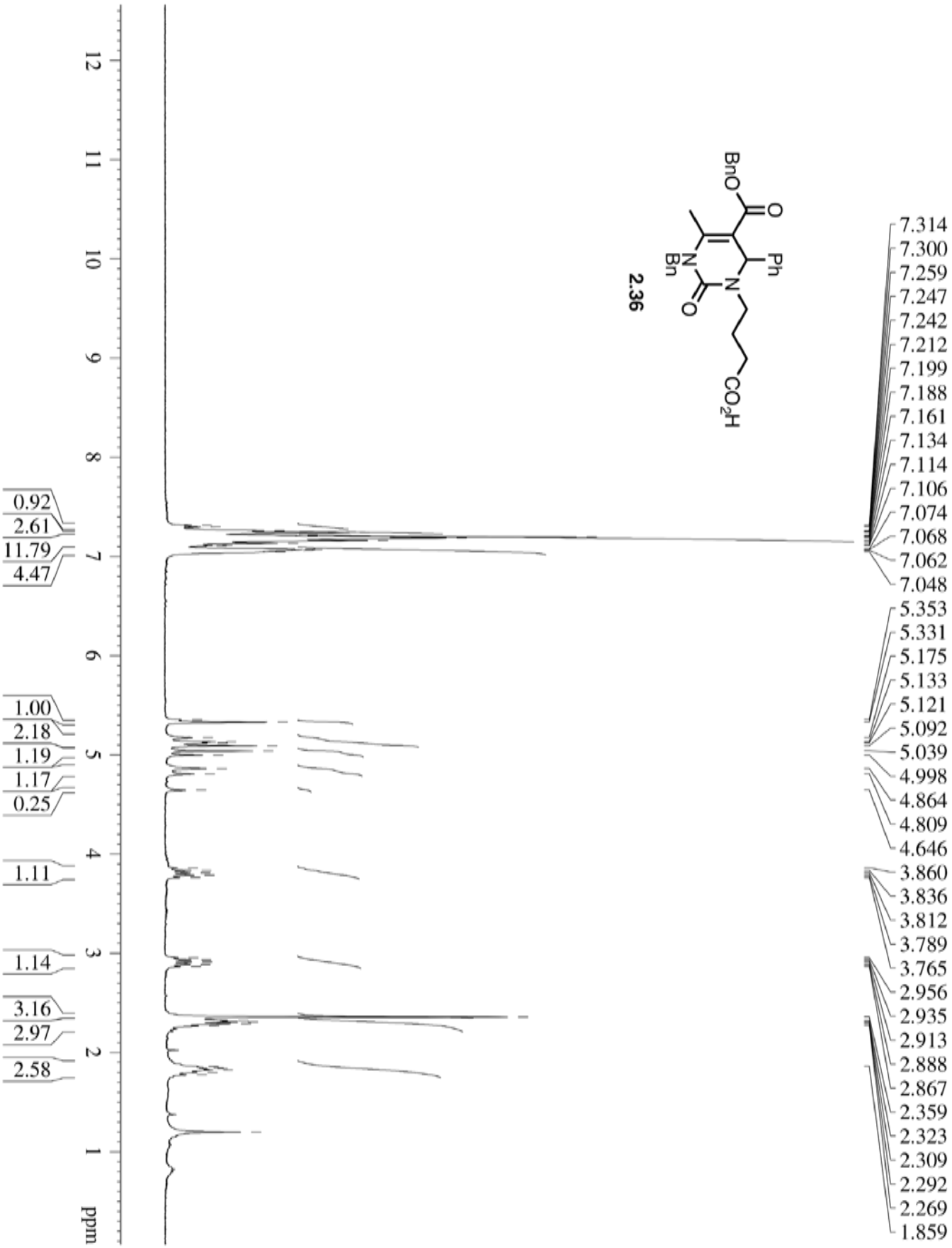
1.05
2.11
1.02
0.92
1.03
3.10
1.42
0.91
2.25
ppm


BnO
O
Ph
N
Bn
N
O
CO2H
2.34
172.07
165.35
153.73
148.68
140.10
137.54
135.68
128.80
128.75
128.46
128.36
128.28
128.15
127.38
127.25
126.56
104.49
77.31
77.19
76.99
76.67
66.33
60.81
48.61
47.02
30.30
16.71
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


2.35
BnO
O
Ph
N
Bn
N
O
CO2H
7.260
7.240
7.218
7.204
7.188
7.176
7.157
7.140
7.090
7.073
5.442
5.176
5.134
5.122
5.091
5.031
5.000
4.880
4.839
3.816
3.801
3.784
3.766
3.751
3.348
3.330
3.313
3.295
3.278
2.733
2.715
2.692
2.673
2.655
2.481
2.465
2.450
2.439
2.422
1.87
10.28
4.54
1.00
2.23
1.15
1.07
1.11
1.15
1.14
1.49
3.05
1.19
12
11
10
9
8
7
6
5
4
3
2
1
ppm


BnO
O
Ph
N
Bn
N
O
CO2H
2.35
176.26
165.57
153.61
149.26
141.23
137.80
135.88
128.69
128.67
128.49
128.27
128.13
127.99
127.24
126.82
126.62
104.63
77.32
77.00
76.68
66.25
60.17
46.72
43.84
32.93
16.66
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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BnO
O
Ph
N
Bn
N
O
CO2H
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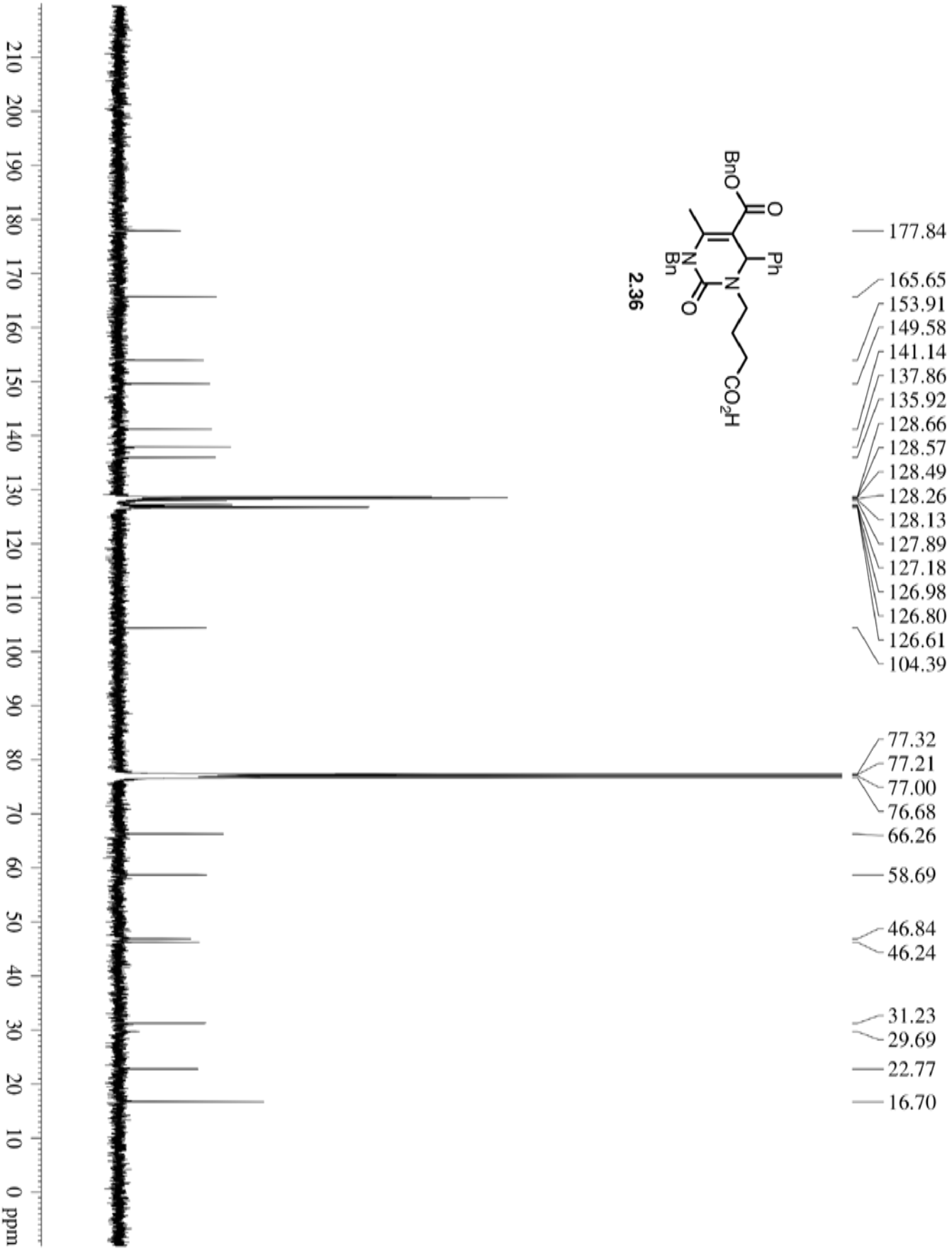
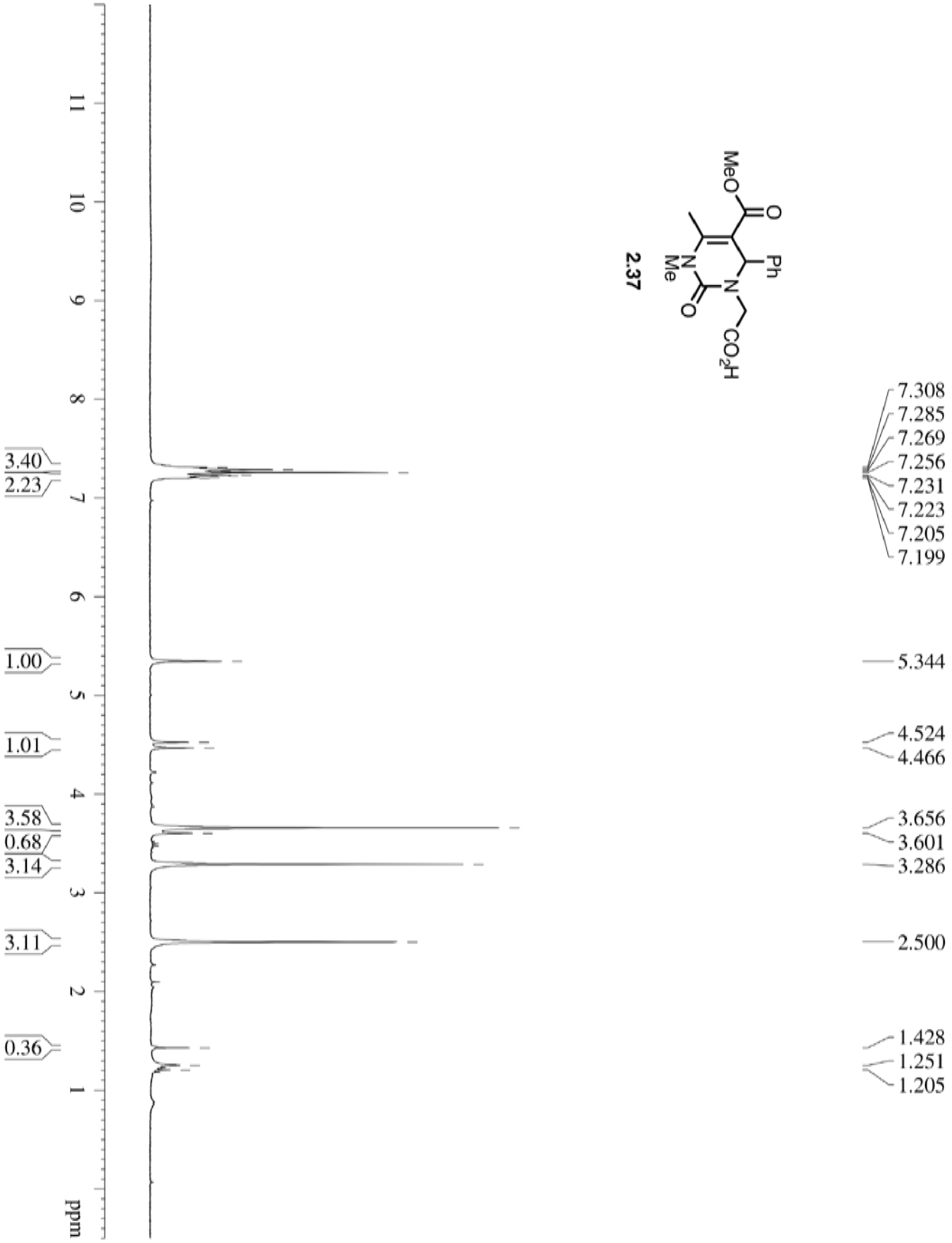
7.300
7.259
7.247
7.242
7.212
7.199
7.188
7.161
7.134
7.114
7.106
7.074
7.068
7.062
7.048
5.353
5.331
5.175
5.133
5.121
5.092
5.039
4.998
4.864
4.809
4.646
3.860
3.836
3.812
3.789
3.765
2.956
2.935
2.913
2.888
2.867
2.359
2.323
2.309
2.292
2.269
1.859
0.92
2.61
11.79
4.47
1.00
2.18
1.19
1.17
0.25
1.11
1.14
3.16
2.97
2.58
12
11
10
9
8
7
6
5
4
3
2
1
ppm


2.36
BnO
O
Ph
N
Bn
N
O
CO2H
177.84
165.65
153.91
149.58
141.14
137.86
135.92
128.66
128.57
128.49
128.26
128.13
127.89
127.18
126.98
126.80
126.61
104.39
77.32
77.21
77.00
76.68
66.26
58.69
46.84
46.24
31.23
29.69
22.77
16.70
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
N
Me
N
O
CO2H
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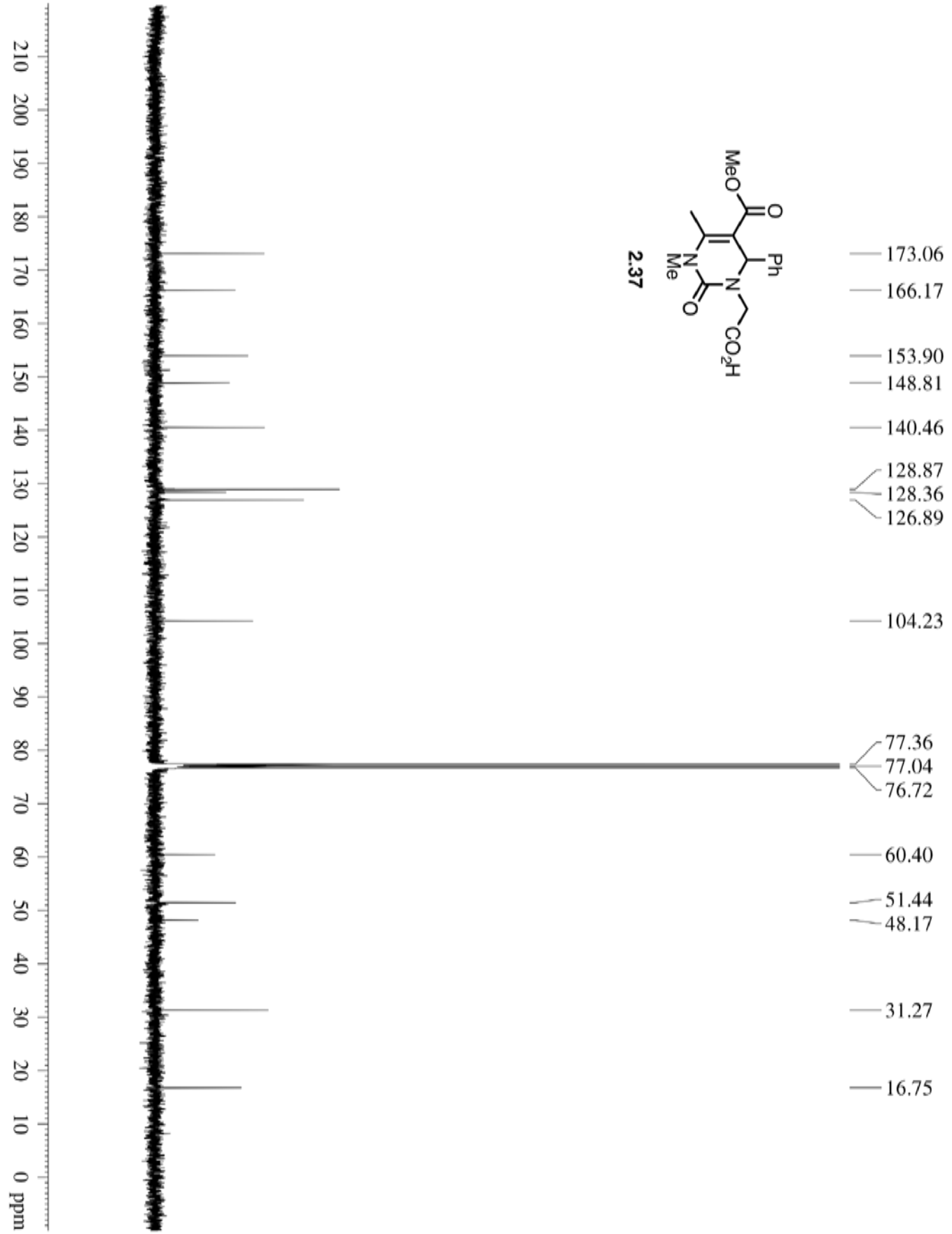
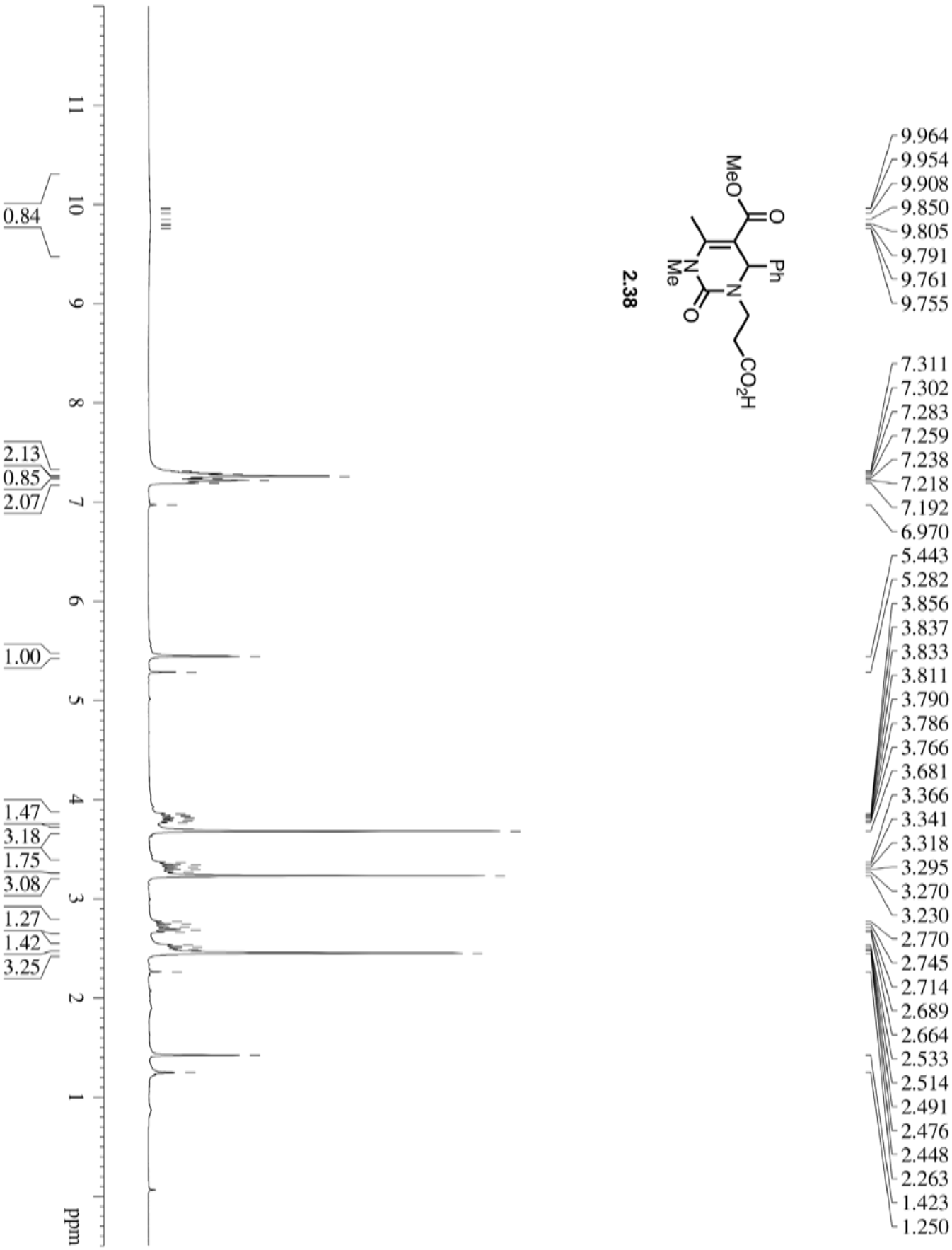
7.285
7.269
7.256
7.231
7.223
7.205
7.199
5.344
4.524
4.466
3.656
3.601
3.286
2.500
1.428
1.251
1.205
3.40
2.23
1.00
1.01
3.58
0.68
3.14
3.11
0.36
ppm


MeO
O
Ph
N
Me
N
O
CO2H
2.37
173.06
166.17
153.90
148.81
140.46
128.87
128.36
126.89
104.23
77.36
77.04
76.72
60.40
51.44
48.17
31.27
16.75
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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9.791
9.761
9.755
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7.302
7.283
7.259
7.238
7.218
7.192
6.970
5.443
5.282
3.856
3.837
3.833
3.811
3.790
3.786
3.766
3.681
3.366
3.341
3.318
3.295
3.270
3.230
2.770
2.745
2.714
2.689
2.664
2.533
2.514
2.491
2.476
2.448
2.263
1.423
1.250
MeO
O
Ph
N
N
Me
O
CO2H
2.38
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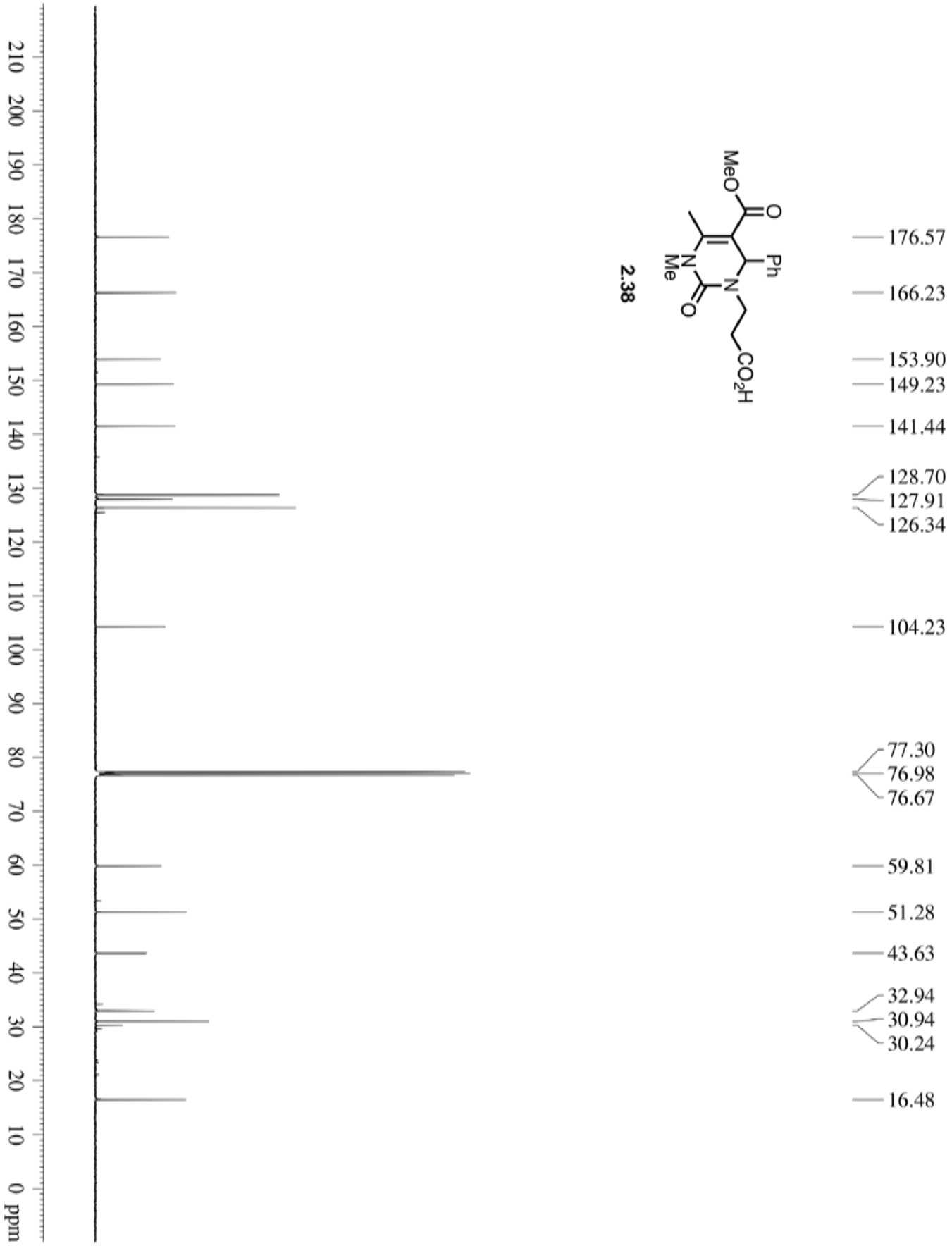
2.13
0.85
2.07
1.00
1.47
3.18
1.75
3.08
1.27
1.42
3.25
ppm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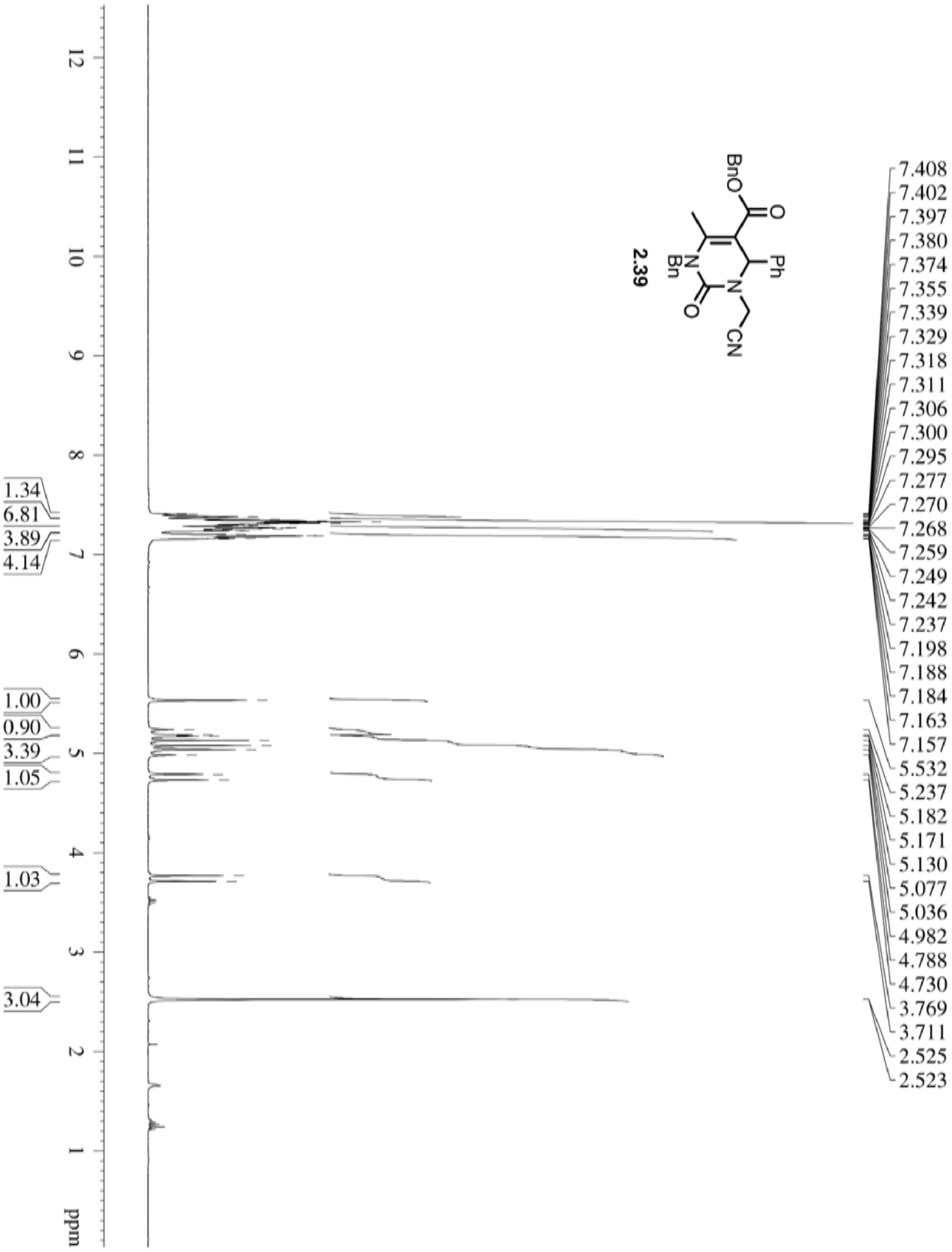
Ph
N
Me
N
O
CO2H
176.57
166.23
153.90
149.23
141.44
128.70
127.91
126.34
104.23
77.30
76.98
76.67
59.81
51.28
43.63
32.94
30.94
30.24
16.48
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
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Bn
N
O
CN
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7.339
7.329
7.318
7.311
7.306
7.300
7.295
7.277
7.270
7.268
7.259
7.249
7.242
7.237
7.198
7.188
7.184
7.163
7.157
5.532
5.237
5.182
5.171
5.130
5.077
5.036
4.982
4.788
4.730
3.769
3.711
2.525
2.523
1.34
6.81
3.89
4.14
1.00
0.90
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1.03
3.04
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11
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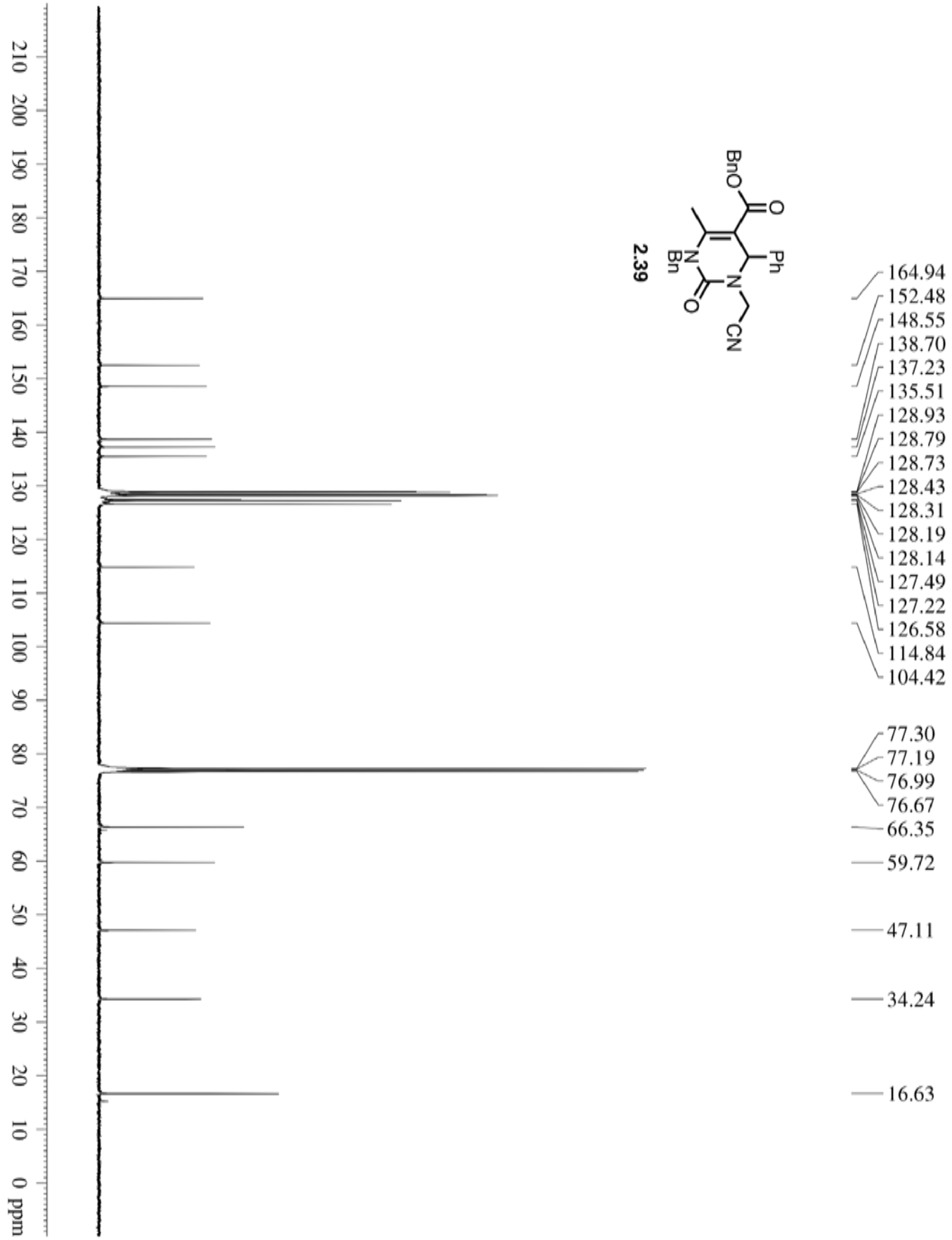
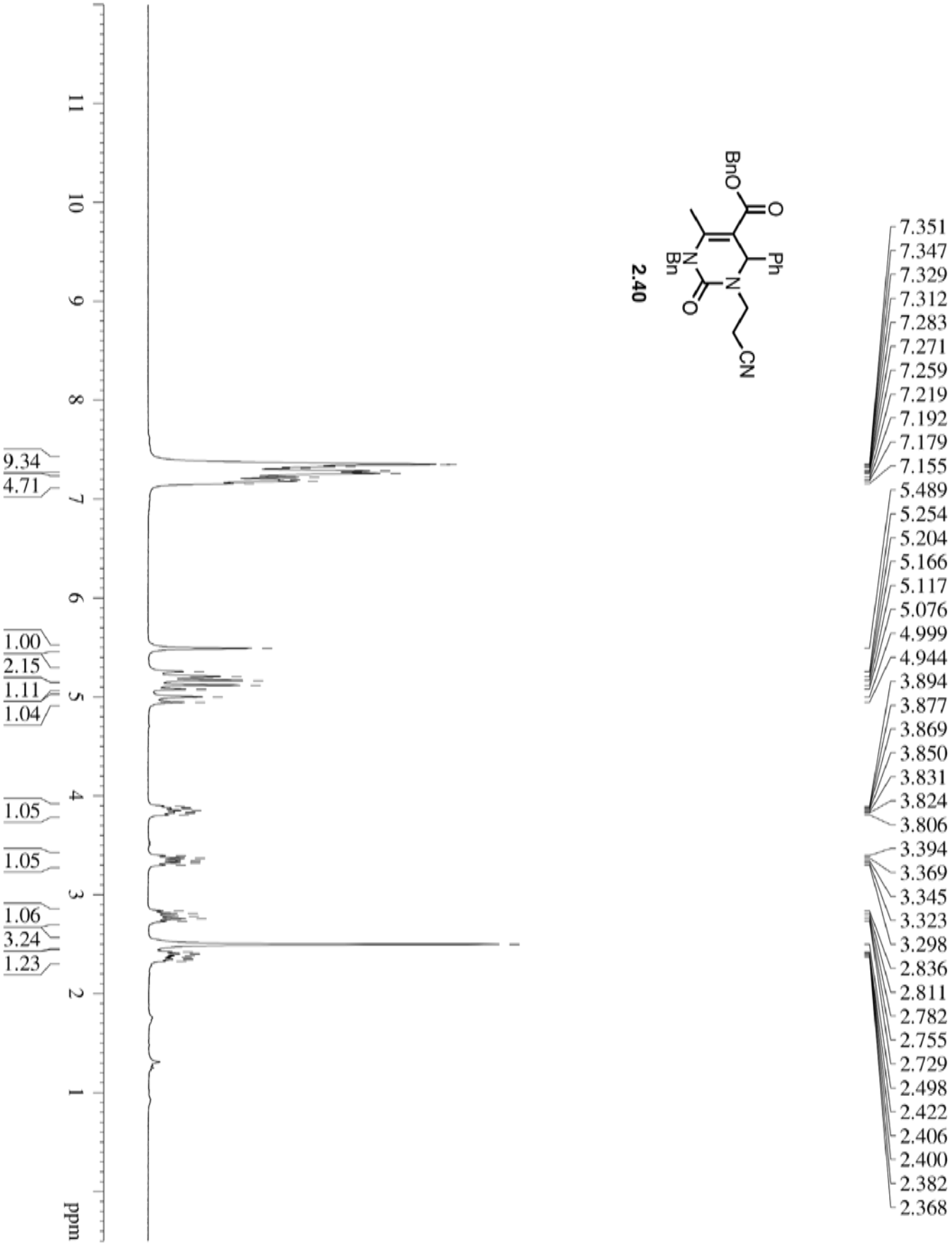
9
8
7
6
5
4
3
2
1
ppm


2.39
BnO
O
Ph
N
Bn
N
O
CN
164.94
152.48
148.55
138.70
137.23
135.51
128.93
128.79
128.73
128.43
128.31
128.19
128.14
127.49
127.22
126.58
114.84
104.42
77.30
77.19
76.99
76.67
66.35
59.72
47.11
34.24
16.63
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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BnO
O
Ph
N
Bn
N
O
CN
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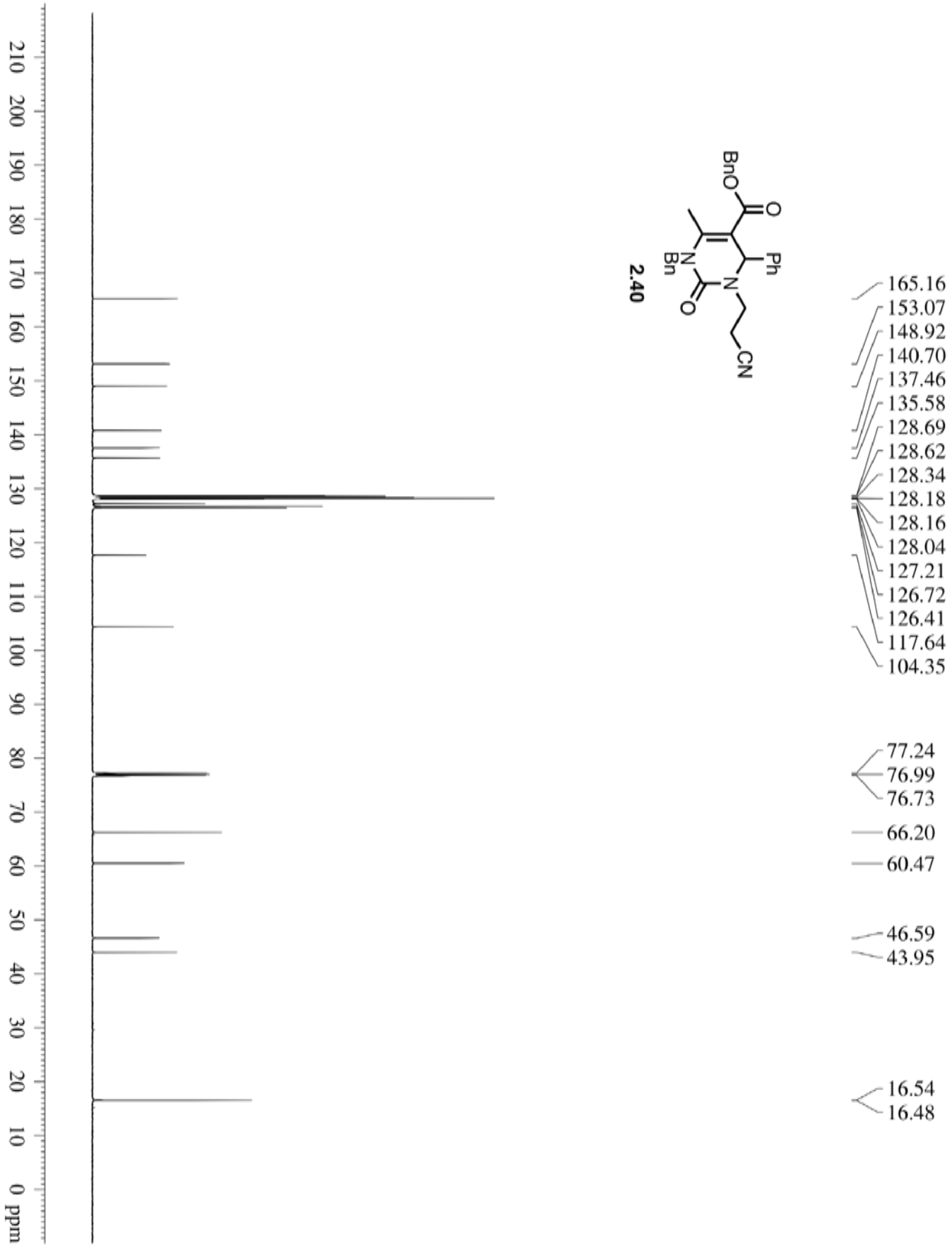
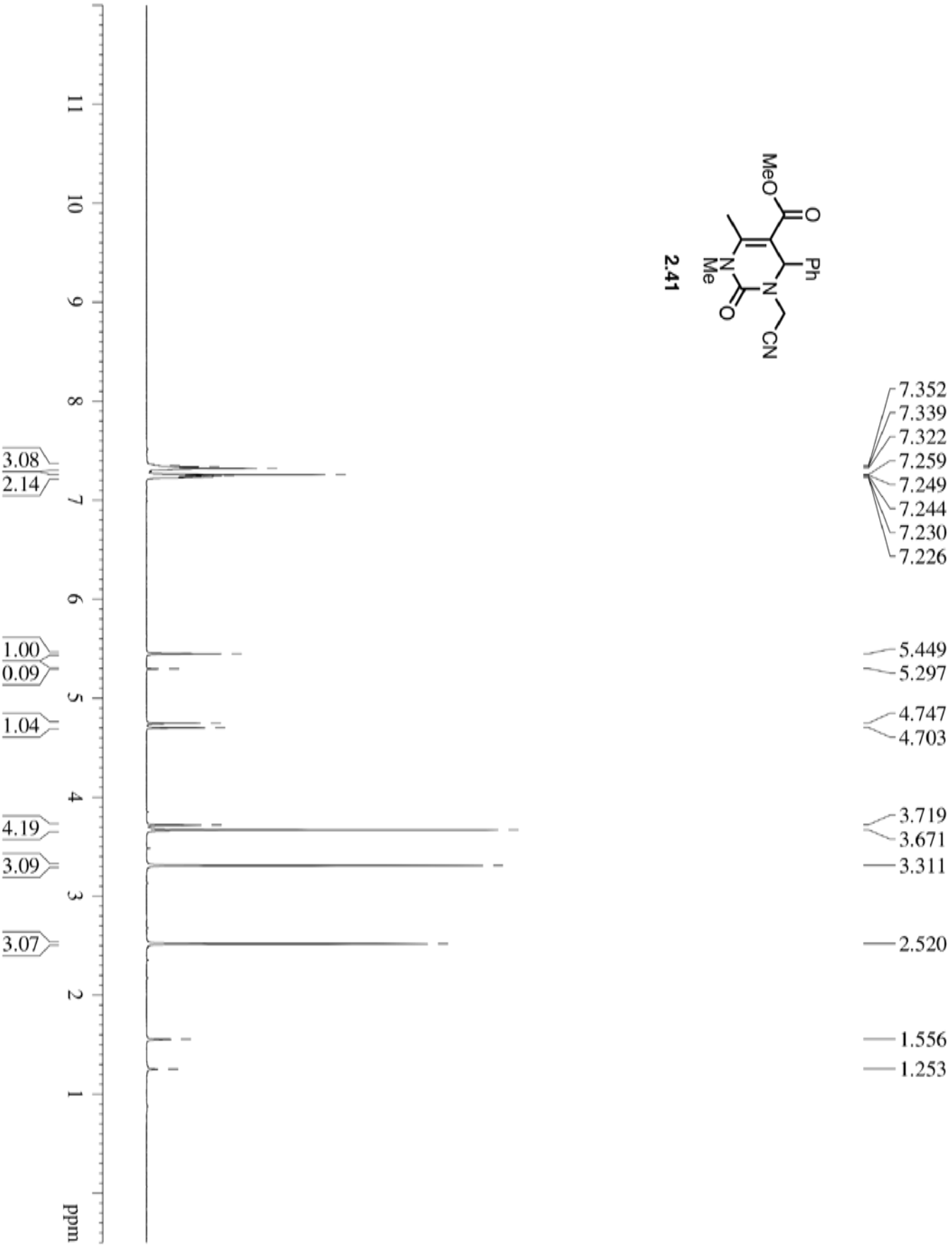
7.283
7.271
7.259
7.219
7.192
7.179
7.155
5.489
5.254
5.204
5.166
5.117
5.076
4.999
4.944
3.894
3.877
3.869
3.850
3.831
3.824
3.806
3.394
3.369
3.345
3.323
3.298
2.836
2.811
2.782
2.755
2.729
2.498
2.422
2.406
2.400
2.382
2.368
9.34
4.71
1.00
2.15
1.11
1.04
1.05
1.05
1.06
3.24
1.23
11
10
9
8
7
6
5
4
3
2
1
ppm


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm
BnO
O
Ph
N
Bn
N
O
CN
2.40
165.16
153.07
148.92
140.70
137.46
135.58
128.69
128.62
128.34
128.18
128.16
128.04
127.21
126.72
126.41
117.64
104.35
77.24
76.99
76.73
66.20
60.47
46.59
43.95
16.54
16.48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Ph
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Me
N
O
CN
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7.249
7.244
7.230
7.226
5.449
5.297
4.747
4.703
3.719
3.671
3.311
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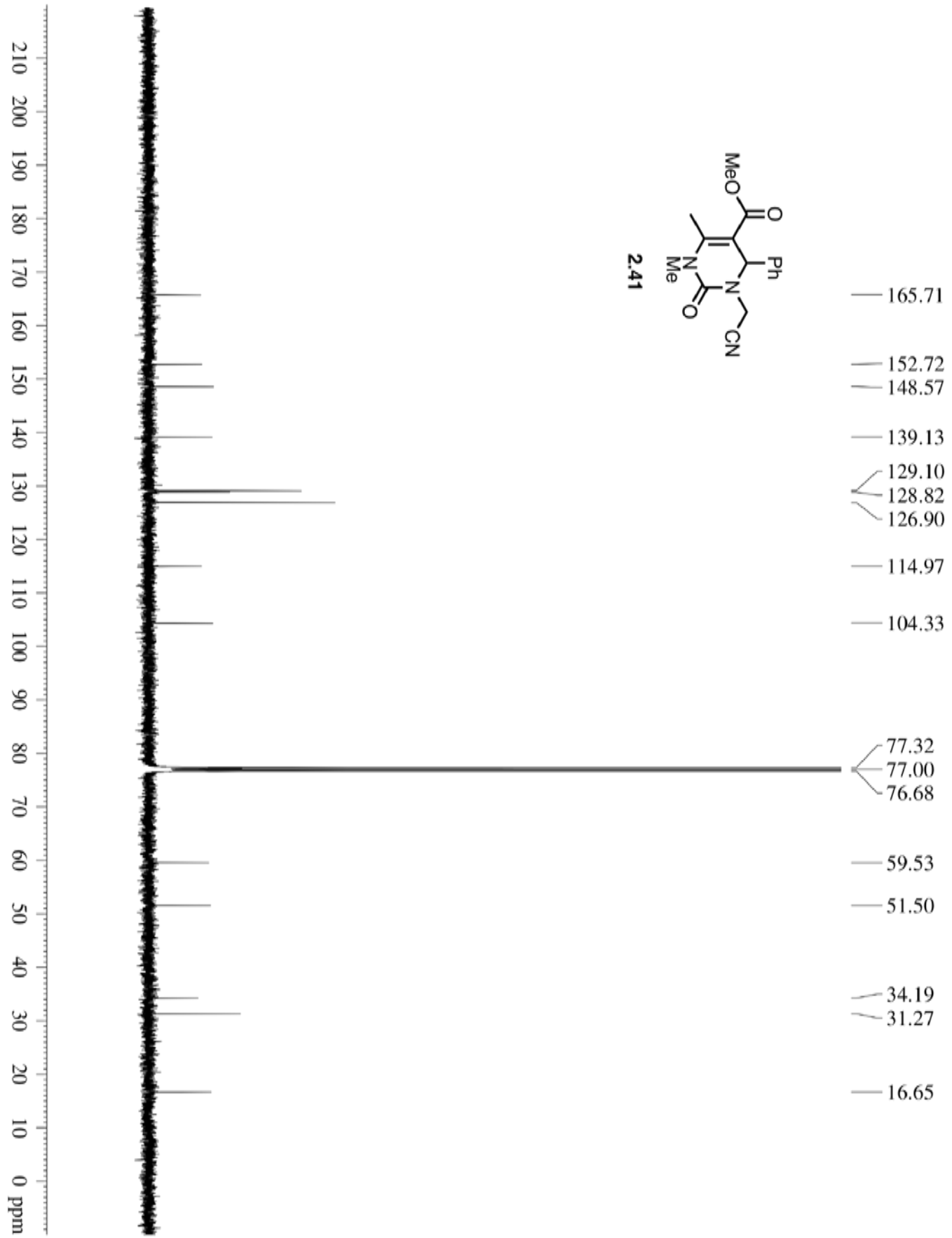
1.253
3.08
2.14
1.00
0.09
1.04
4.19
3.09
3.07
ppm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O
Ph
N
Me
N
O
CN
165.71
152.72
148.57
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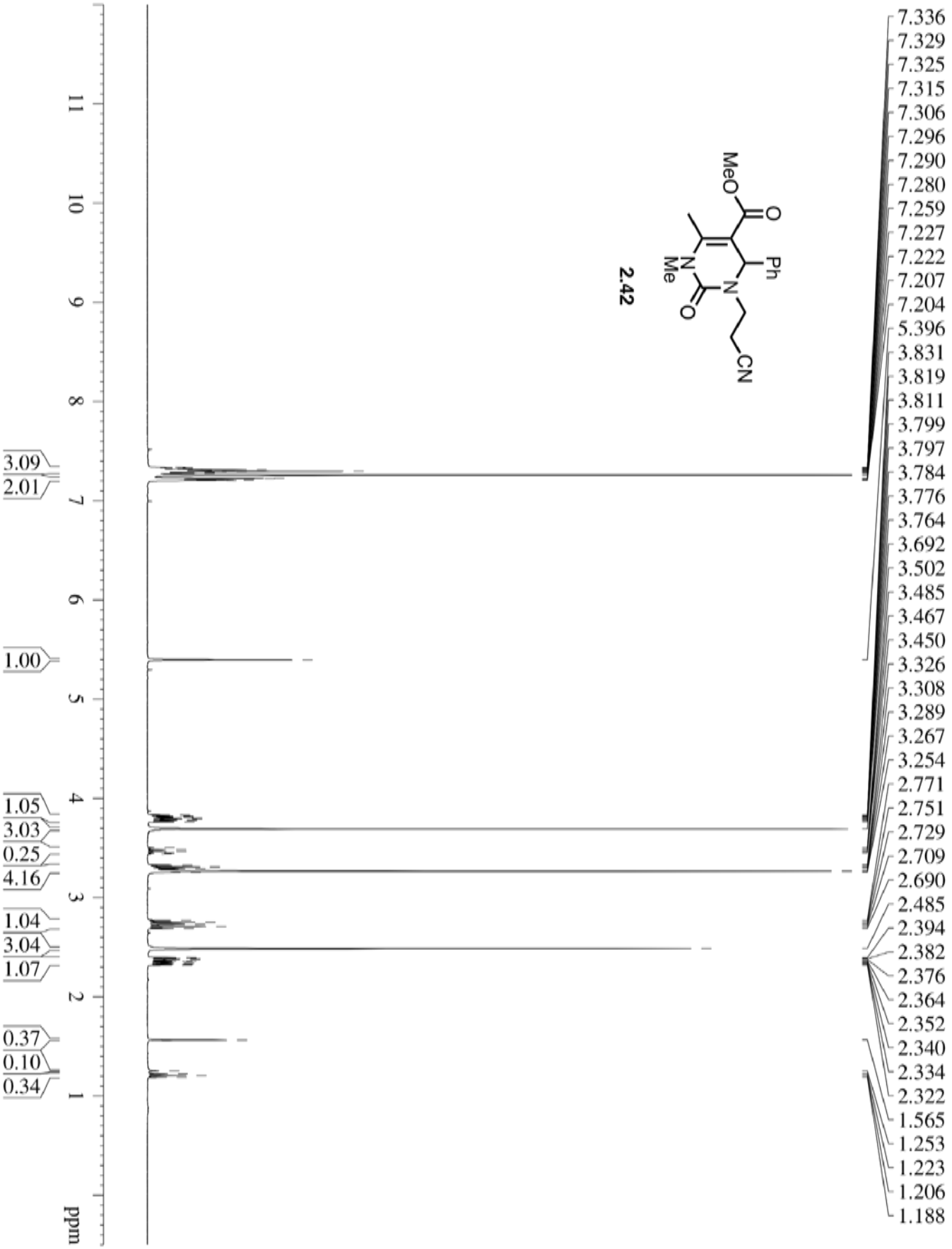
129.10
128.82
126.90
114.97
104.33
77.32
77.00
76.68
59.53
51.50
34.19
31.27
16.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
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Me
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O
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7.290
7.280
7.259
7.227
7.222
7.207
7.204
5.396
3.831
3.819
3.811
3.799
3.797
3.784
3.776
3.764
3.692
3.502
3.485
3.467
3.450
3.326
3.308
3.289
3.267
3.254
2.771
2.751
2.729
2.709
2.690
2.485
2.394
2.382
2.376
2.364
2.352
2.340
2.334
2.322
1.565
1.253
1.223
1.206
1.188
3.09
2.01
1.00
1.05
3.03
0.25
4.16
1.04
3.04
1.07
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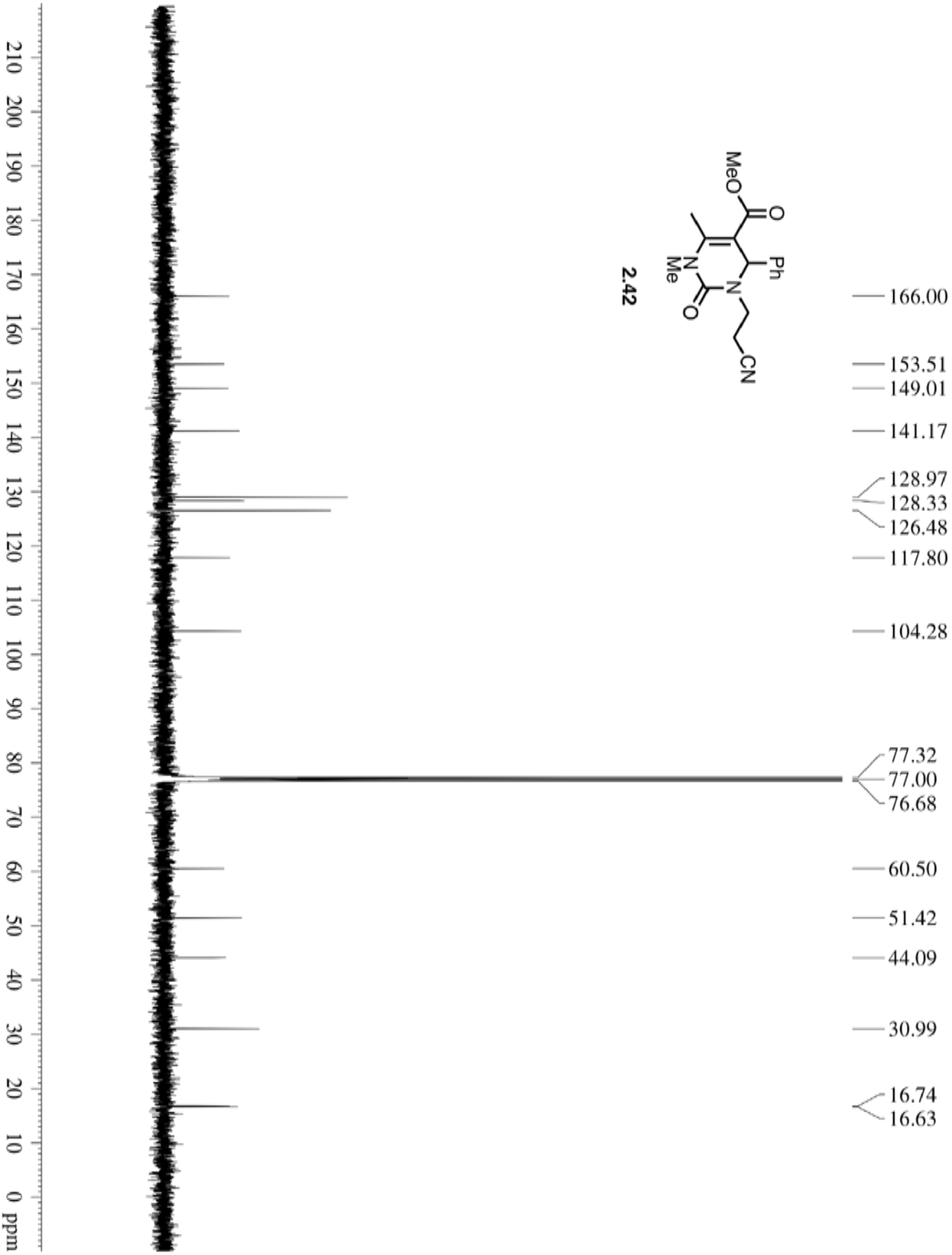
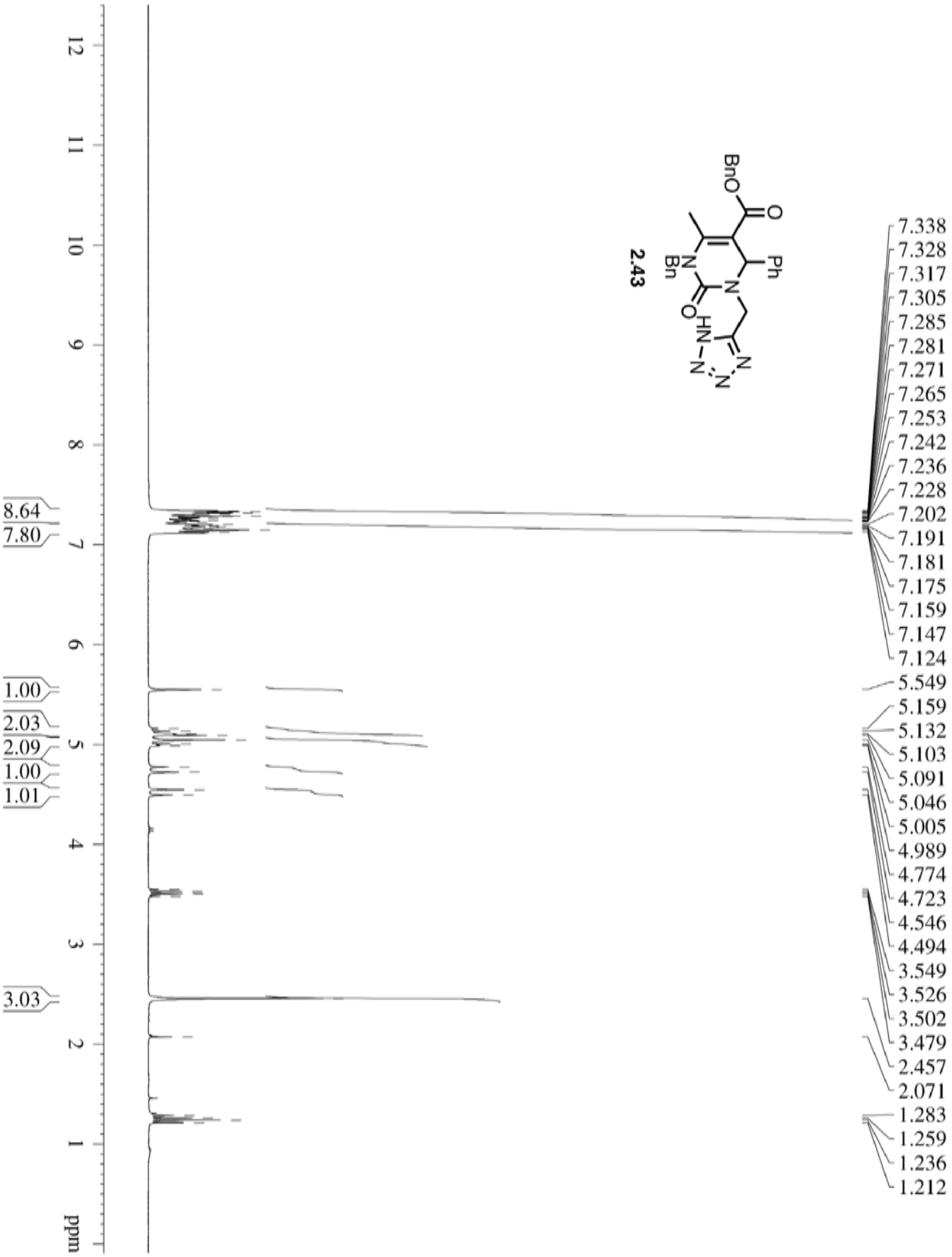
0.10
0.34
11
10
9
8
7
6
5
4
3
2
1
ppm


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
MeO
O
Ph
N
Me
N
O
CN
2.42
166.00
153.51
149.01
141.17
128.97
128.33
126.48
117.80
104.28
77.32
77.00
76.68
60.50
51.42
44.09
30.99
16.74
16.63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2.09
1.00
1.01
3.03
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7.305
7.285
7.281
7.271
7.265
7.253
7.242
7.236
7.228
7.202
7.191
7.181
7.175
7.159
7.147
7.124
5.549
5.159
5.132
5.103
5.091
5.046
5.005
4.989
4.774
4.723
4.546
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3.526
3.502
3.479
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2.071
1.283
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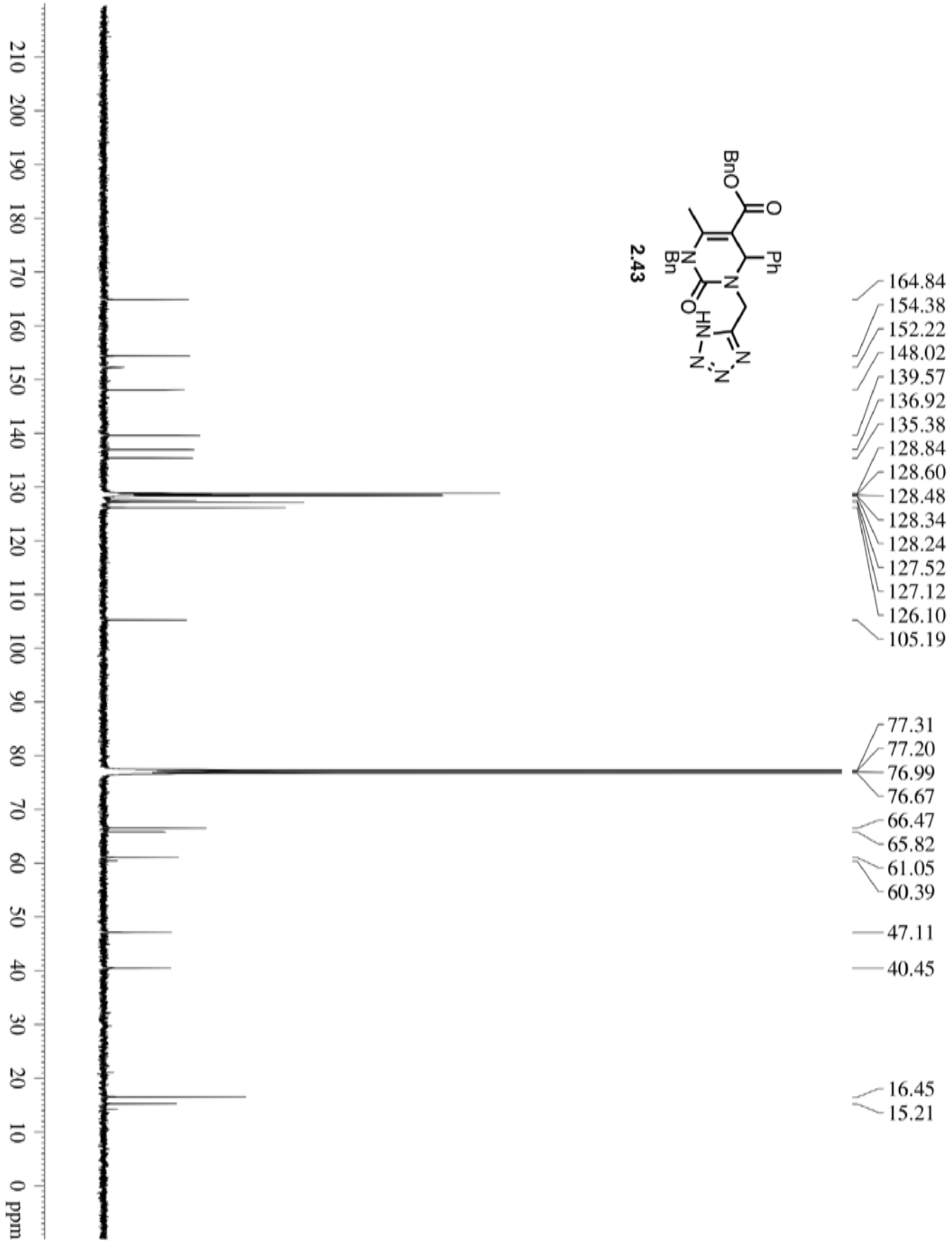
1.212
ppm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127.52
127.12
126.10
105.19
77.31
77.20
76.99
76.67
66.47
65.82
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60.39
47.11
40.45
16.45
15.21
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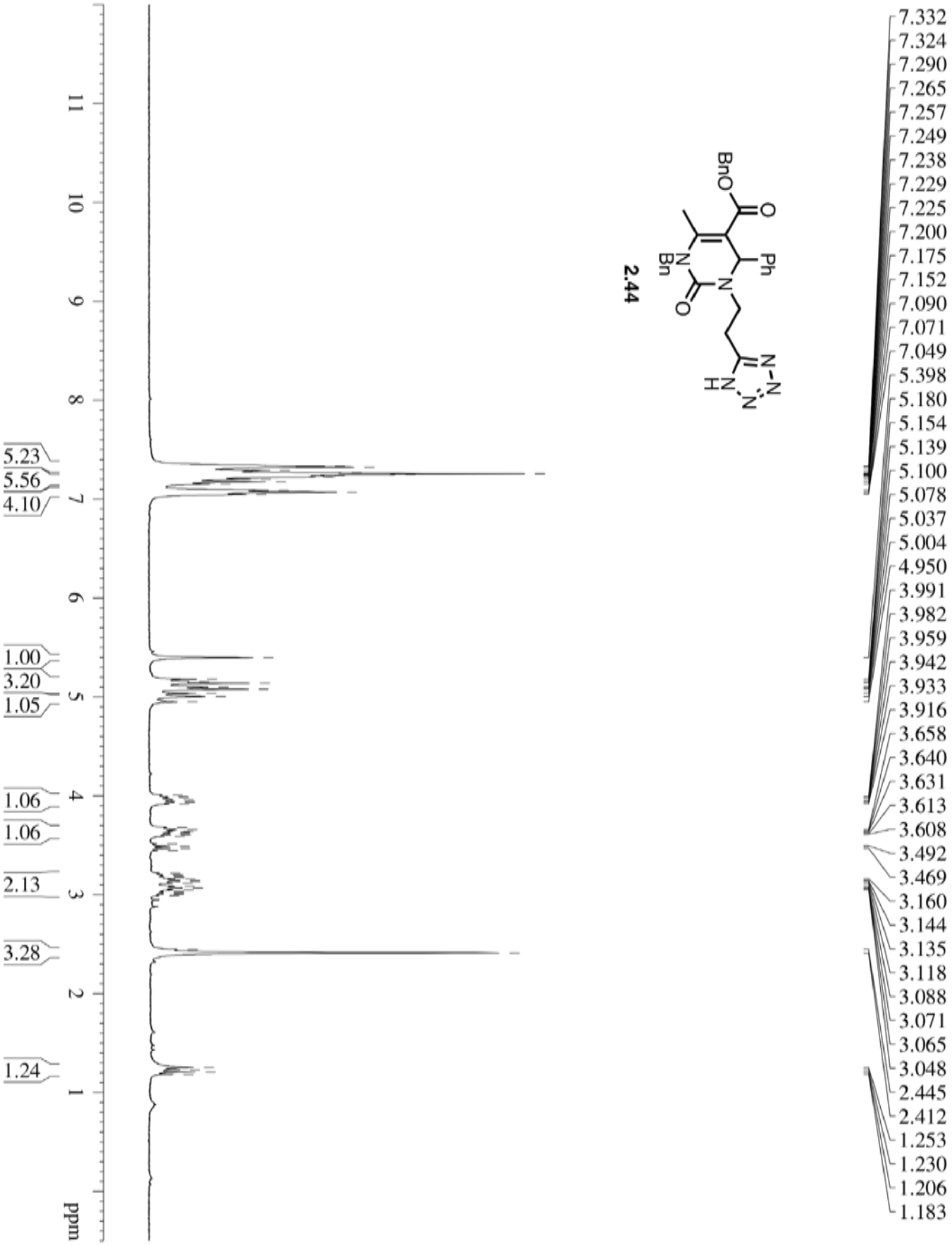
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7.229
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7.175
7.152
7.090
7.071
7.049
5.398
5.180
5.154
5.139
5.100
5.078
5.037
5.004
4.950
3.991
3.982
3.959
3.942
3.933
3.916
3.658
3.640
3.631
3.613
3.608
3.492
3.469
3.160
3.144
3.135
3.118
3.088
3.071
3.065
3.048
2.445
2.412
1.253
1.230
1.206
1.183
BnO
O
Ph
N
Bn
N
O
N-N
N
H
2.44
5.23
5.56
4.10
1.00
3.20
1.05
1.06
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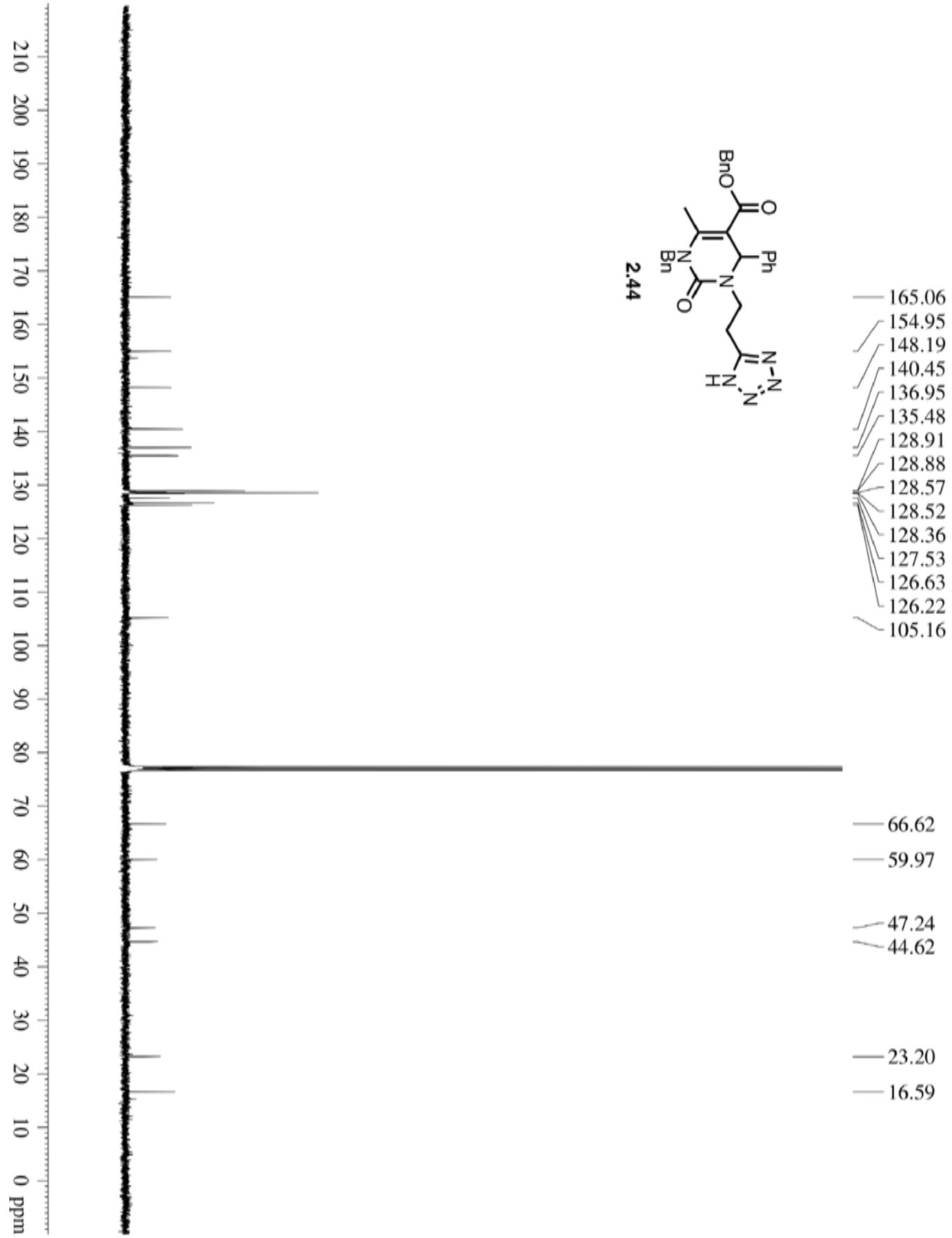
3.28
1.24
11
10
9
8
7
6
5
4
3
2
1
ppm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O
Ph
N
Bn
N
O
N-N
N
H
2.44
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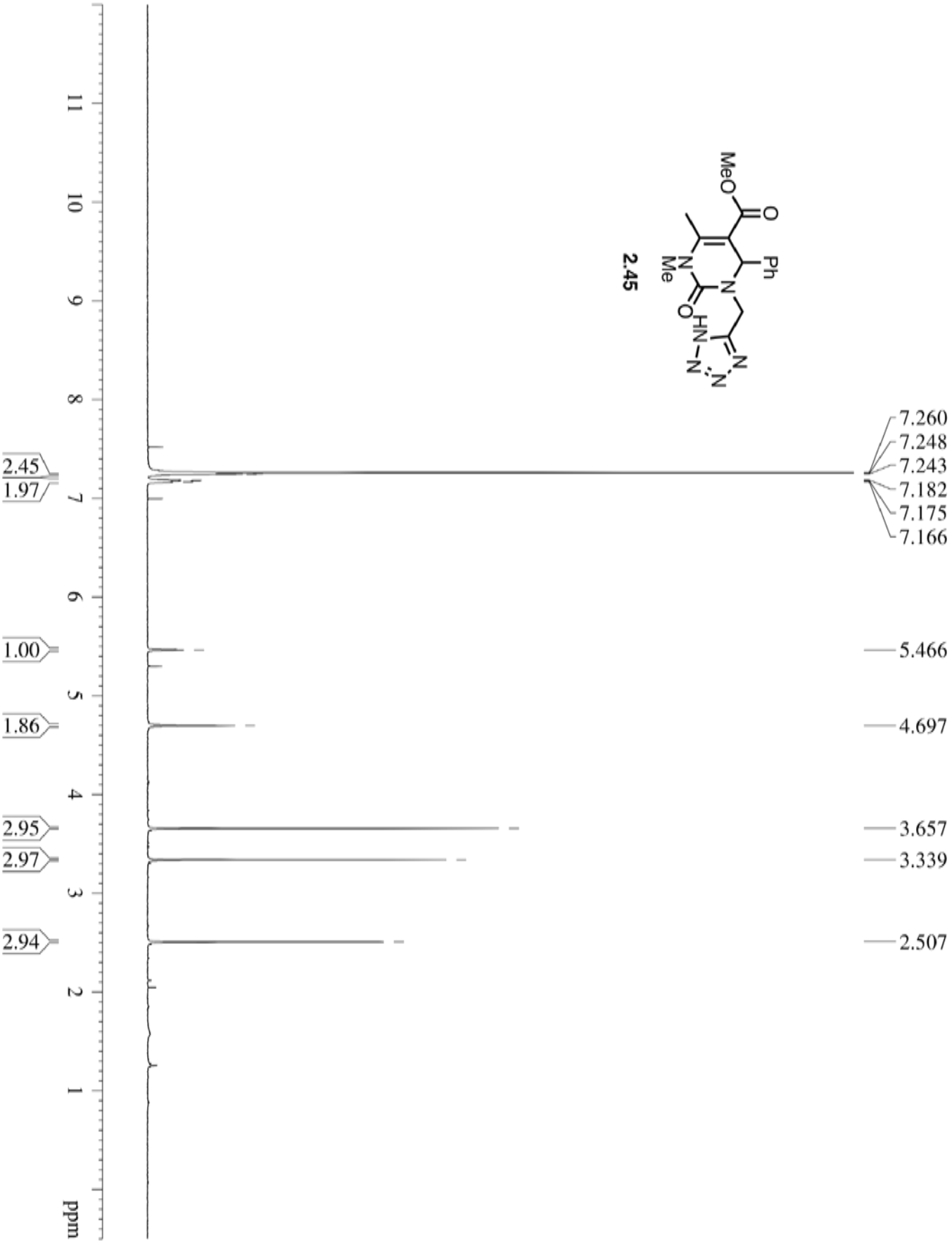
154.95
148.19
140.45
136.95
135.48
128.91
128.88
128.57
128.52
128.36
127.53
126.63
126.22
105.16
66.62
59.97
47.24
44.62
23.20
16.59
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
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Me
N
O
HN
N
N
N
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1.00
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2.97
2.94
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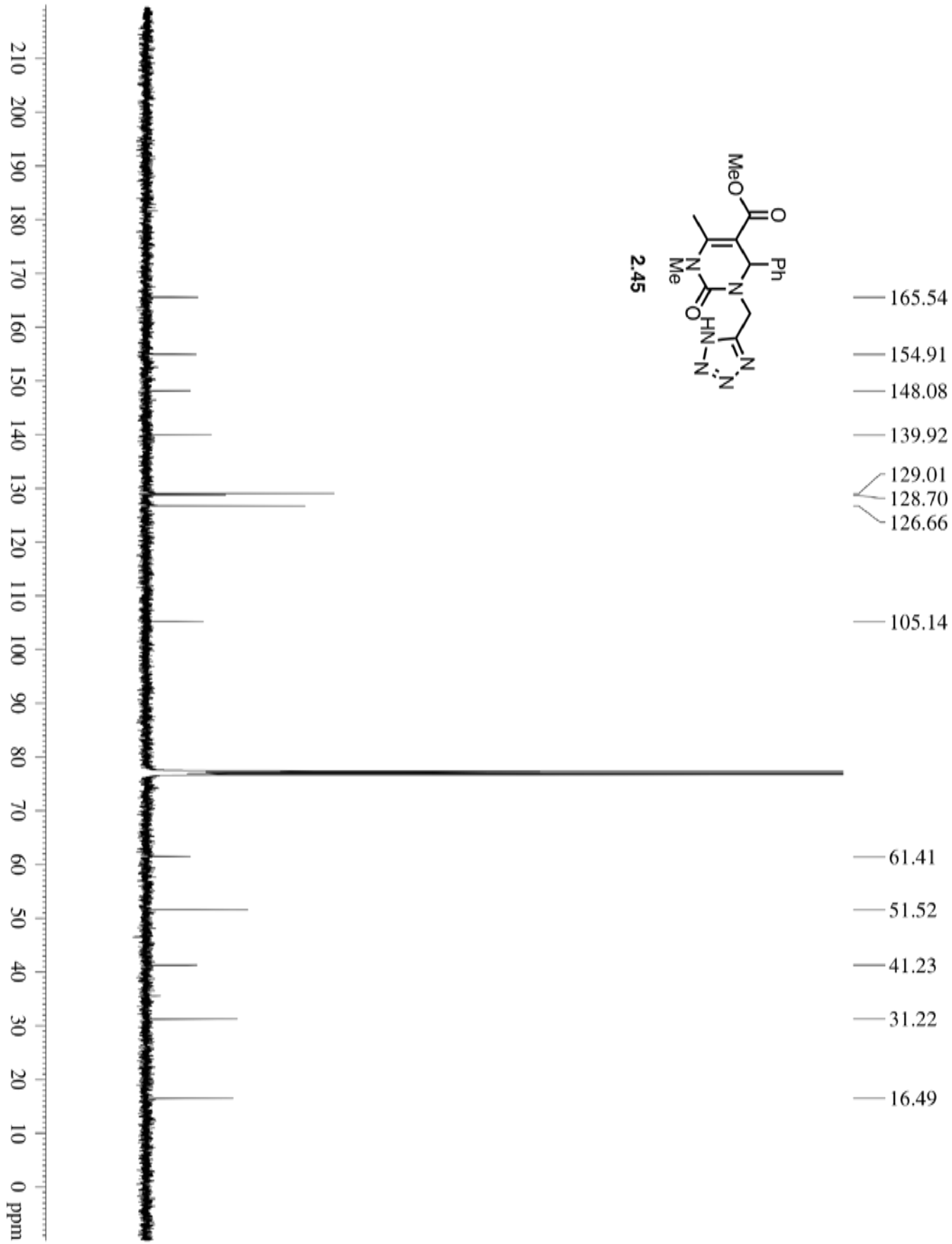
7.248
7.243
7.182
7.175
7.166
5.466
4.697
3.657
3.339
2.507
ppm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MeO
O
Ph
N
Me
HN
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148.08
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129.01
128.70
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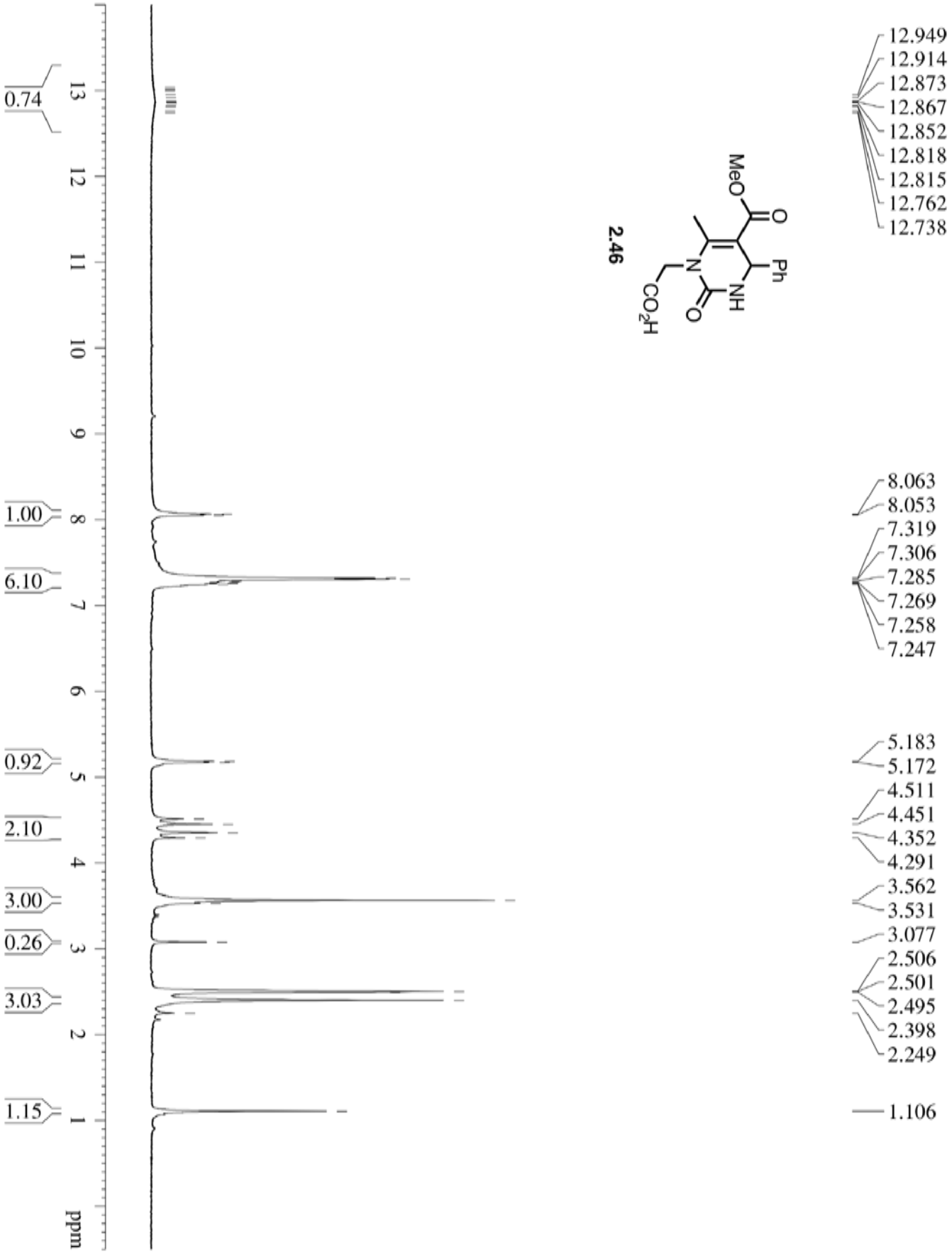
105.14
61.41
51.52
41.23
31.22
16.49
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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12.818
12.815
12.762
12.738
8.063
8.053
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7.306
7.285
7.269
7.258
7.247
5.183
5.172
4.511
4.451
4.352
4.291
3.562
3.531
3.077
2.506
2.501
2.495
2.398
2.249
1.106
0.74
1.00
6.10
0.92
2.10
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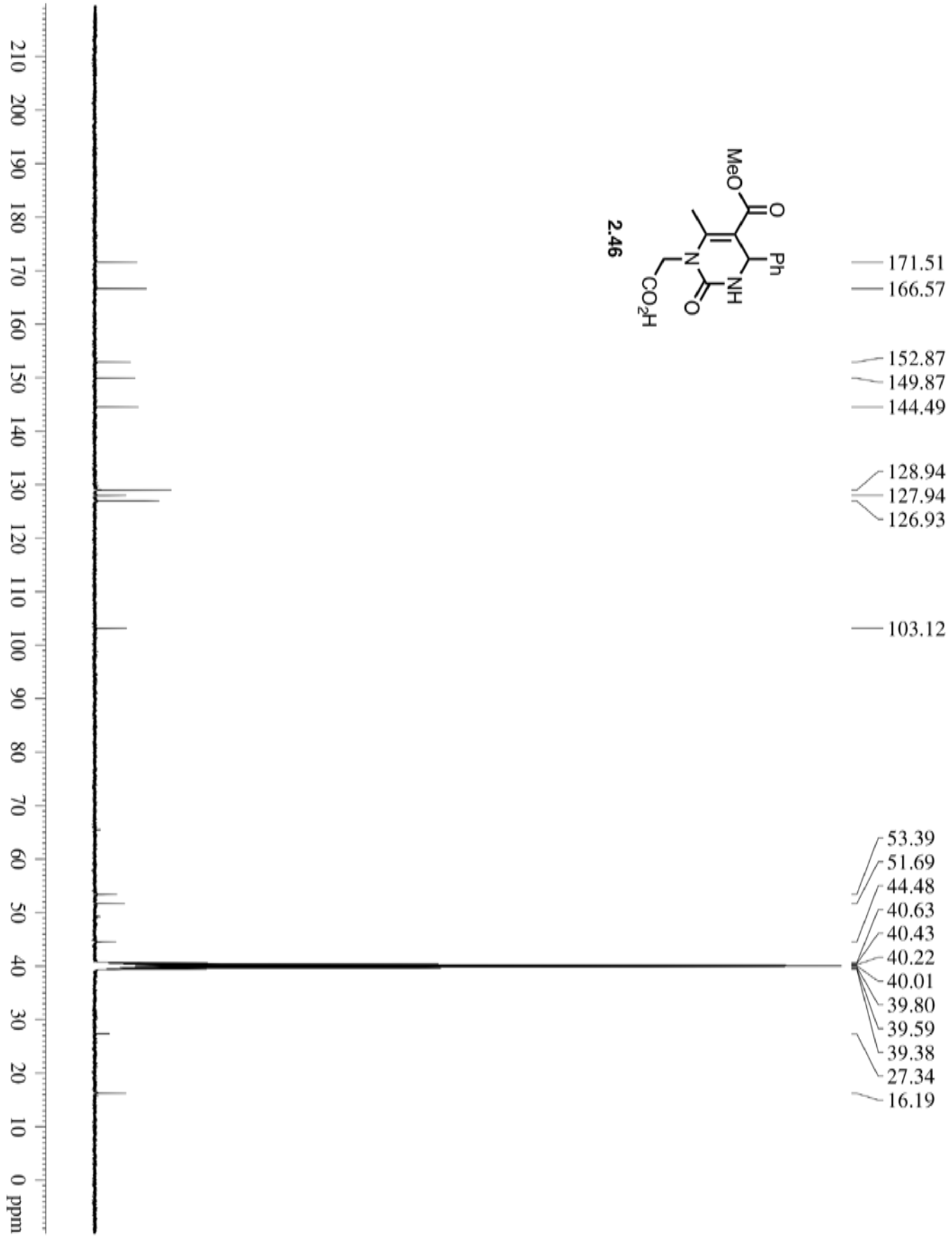
0.26
3.03
1.15
ppm
2.46
MeO
O
Ph
NH
O
N
CO2H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O
Ph
NH
O
N
CO2H
2.46
171.51
166.57
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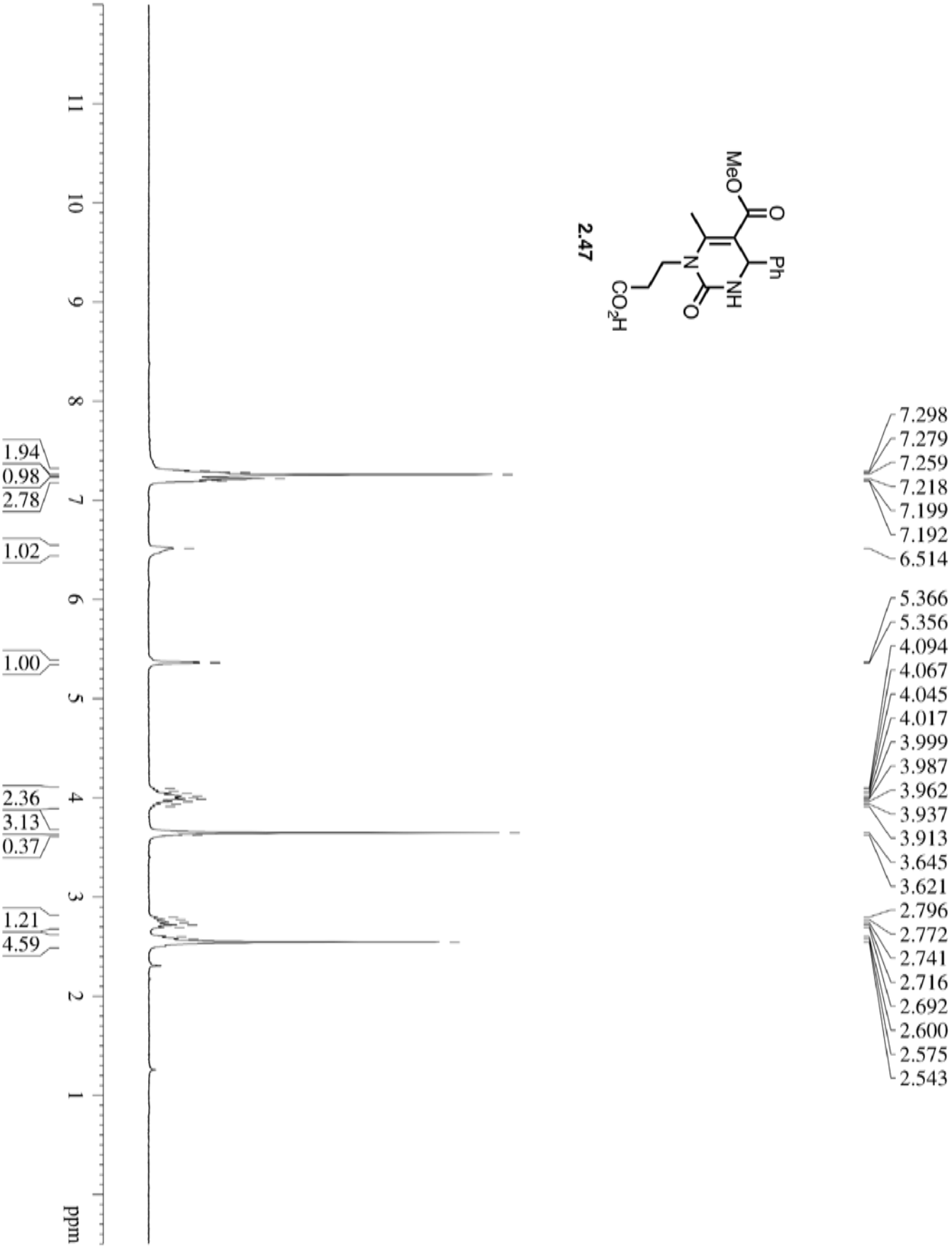
149.87
144.49
128.94
127.94
126.93
103.12
53.39
51.69
44.48
40.63
40.43
40.22
40.01
39.80
39.59
39.38
27.34
16.19
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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Ph
NH
N
O
CO2H
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7.199
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6.514
5.366
5.356
4.094
4.067
4.045
4.017
3.999
3.987
3.962
3.937
3.913
3.645
3.621
2.796
2.772
2.741
2.716
2.692
2.600
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1.02
1.00
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3.13
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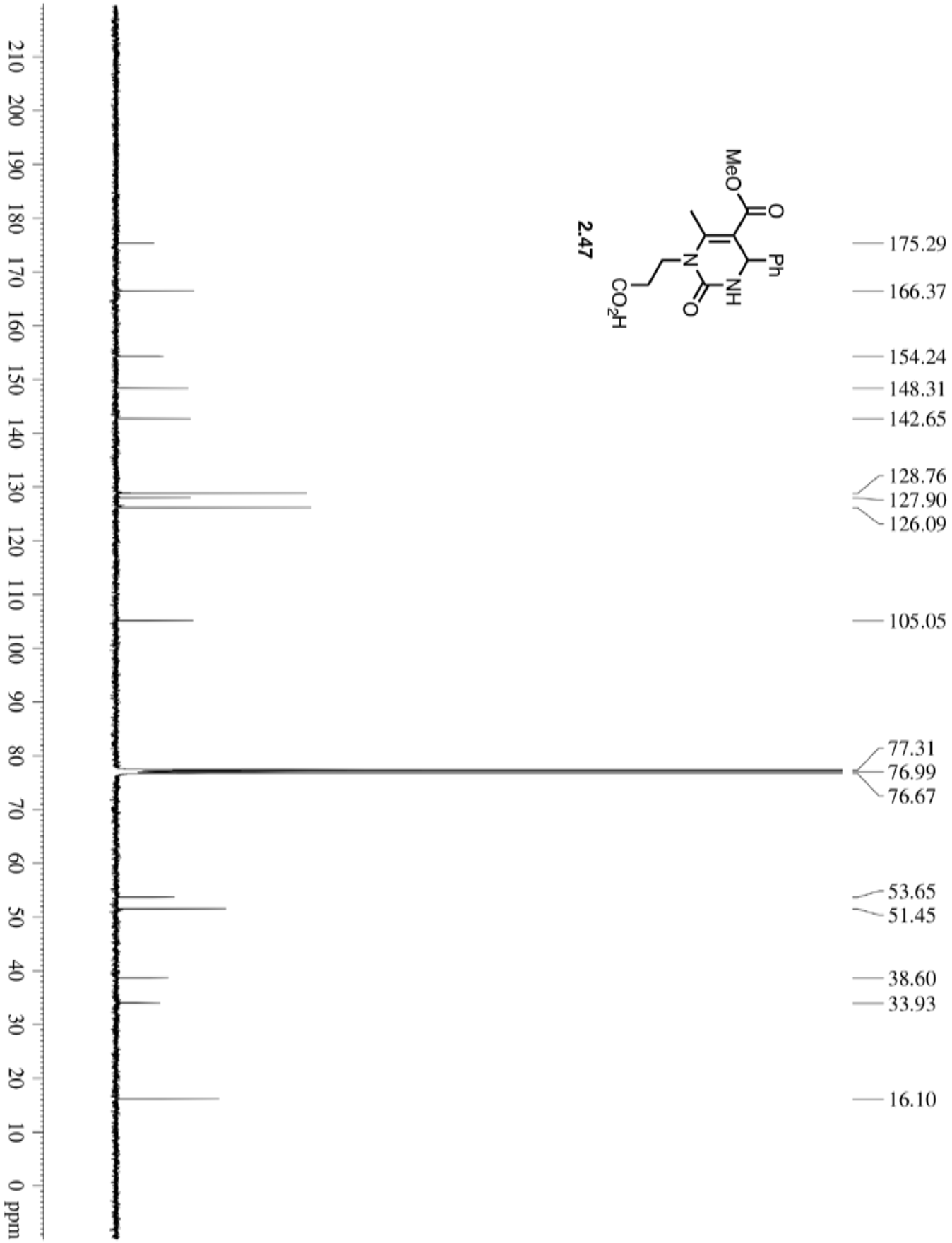
1.21
4.59
ppm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MeO
O
Ph
NH
N
O
CO2H
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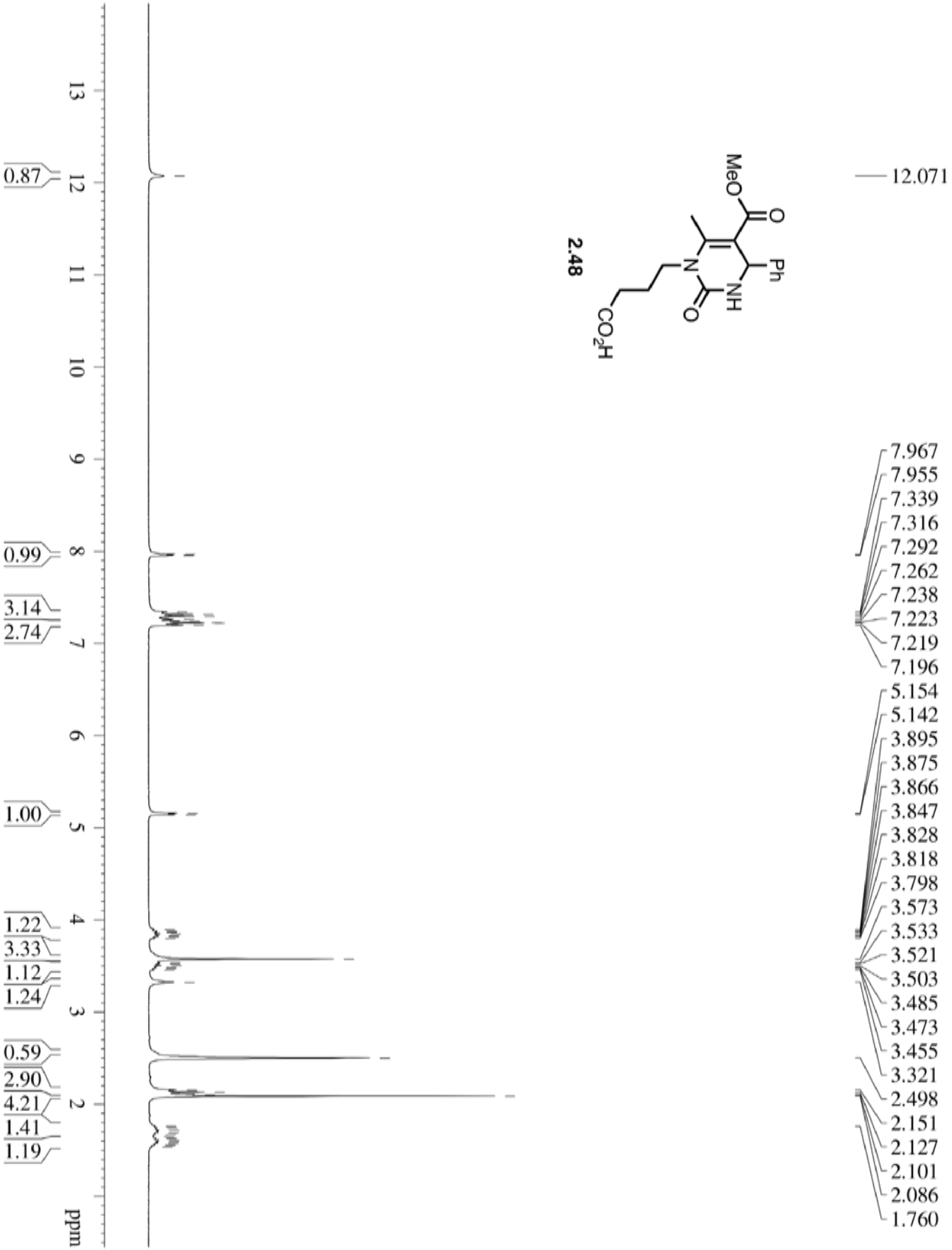
166.37
154.24
148.31
142.65
128.76
127.90
126.09
105.05
77.31
76.99
76.67
53.65
51.45
38.60
33.93
16.10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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O
Ph
NH
N
O
CO2H
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7.316
7.292
7.262
7.238
7.223
7.219
7.196
5.154
5.142
3.895
3.875
3.866
3.847
3.828
3.818
3.798
3.573
3.533
3.521
3.503
3.485
3.473
3.455
3.321
2.498
2.151
2.127
2.101
2.086
1.760
0.87
0.99
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1.22
3.33
1.12
1.24
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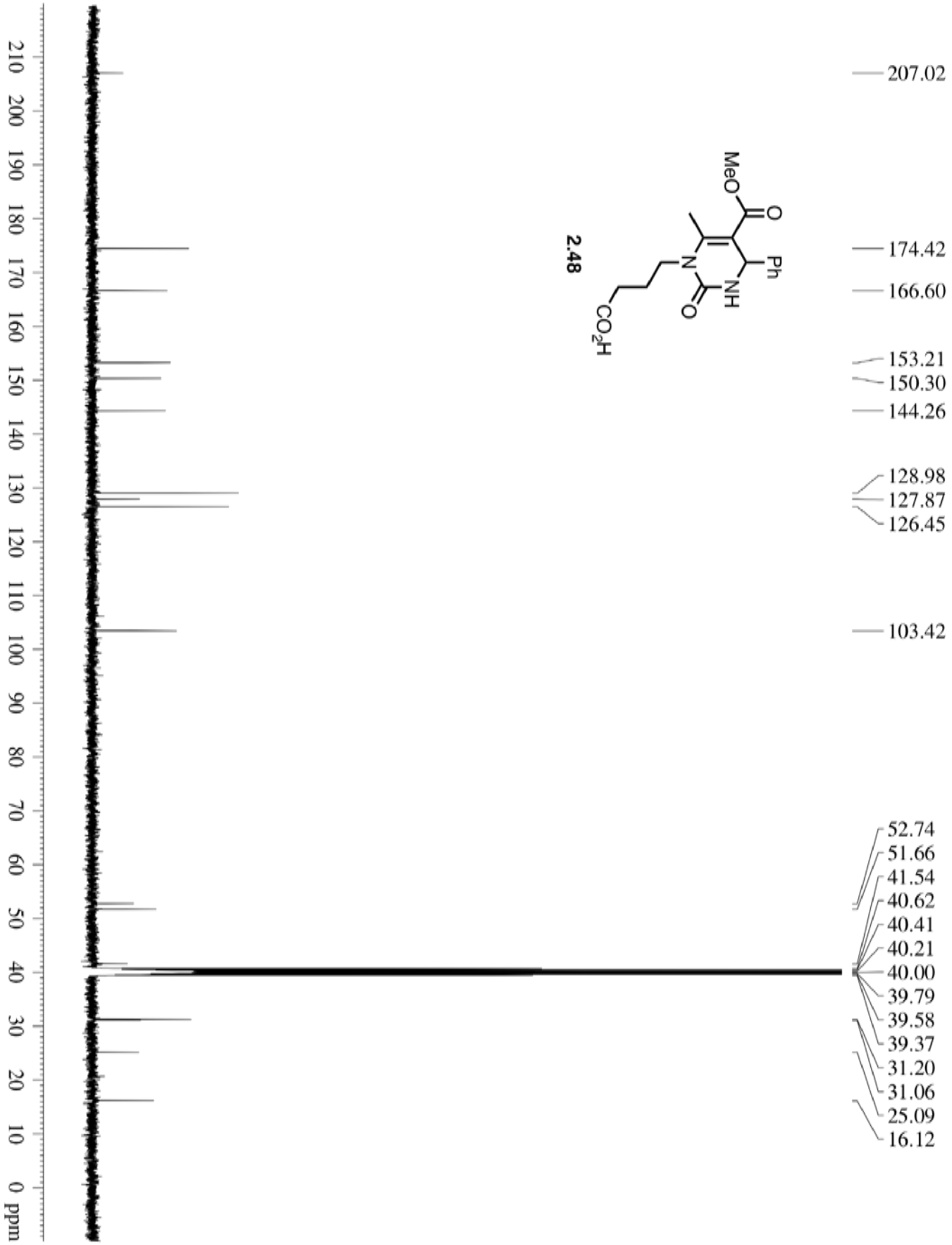
2.90
4.21
1.41
1.19
ppm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O
Ph
NH
N
O
CO2H
207.02
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150.30
144.26
128.98
127.87
126.45
103.42
52.74
51.66
41.54
40.62
40.41
40.21
40.00
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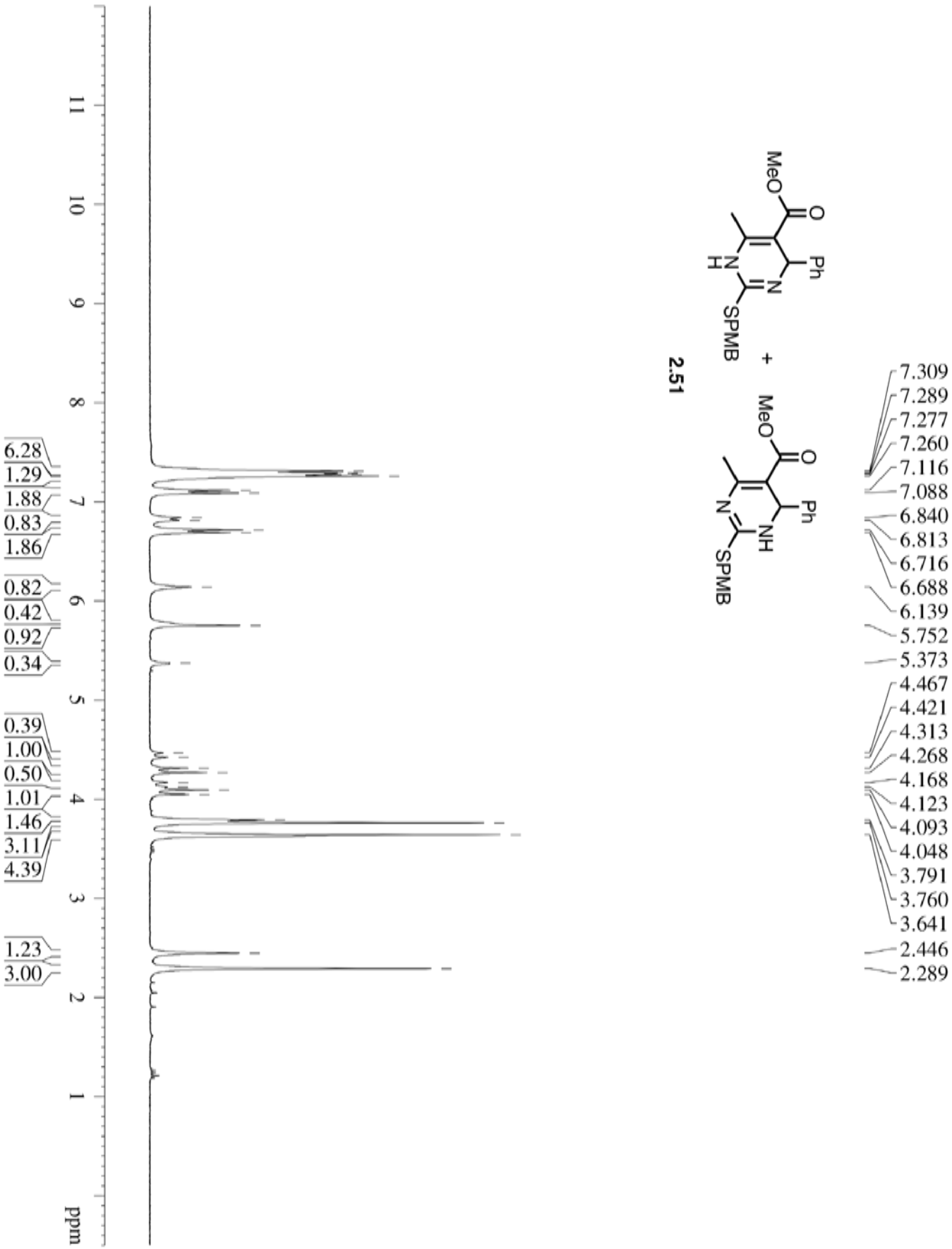
39.37
31.20
31.06
25.09
16.12
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


MeO
O
Ph
N
H
SPMB
+
MeO
O
Ph
NH
N
SPMB
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7.277
7.260
7.116
7.088
6.840
6.813
6.716
6.688
6.139
5.752
5.373
4.467
4.421
4.313
4.268
4.168
4.123
4.093
4.048
3.791
3.760
3.641
2.446
2.289
6.28
1.29
1.88
0.83
1.86
0.82
0.42
0.92
0.34
0.39
1.00
0.50
1.01
1.46
3.11
4.39
1.23
3.00
11
10
9
8
7
6
5
4
3
2
1
ppm

MeO
O
Ph
N
N
SPMB
CO2Et
2.52
7.301
7.290
7.280
7.258
6.849
6.821
5.280
4.462
4.418
4.319
4.274
4.170
4.152
4.140
4.128
4.110
4.092
4.081
4.068
4.057
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4.033
4.021
4.009
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3.948
3.888
3.794
3.615
2.415
1.257
1.194
1.170
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1.05
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3.31
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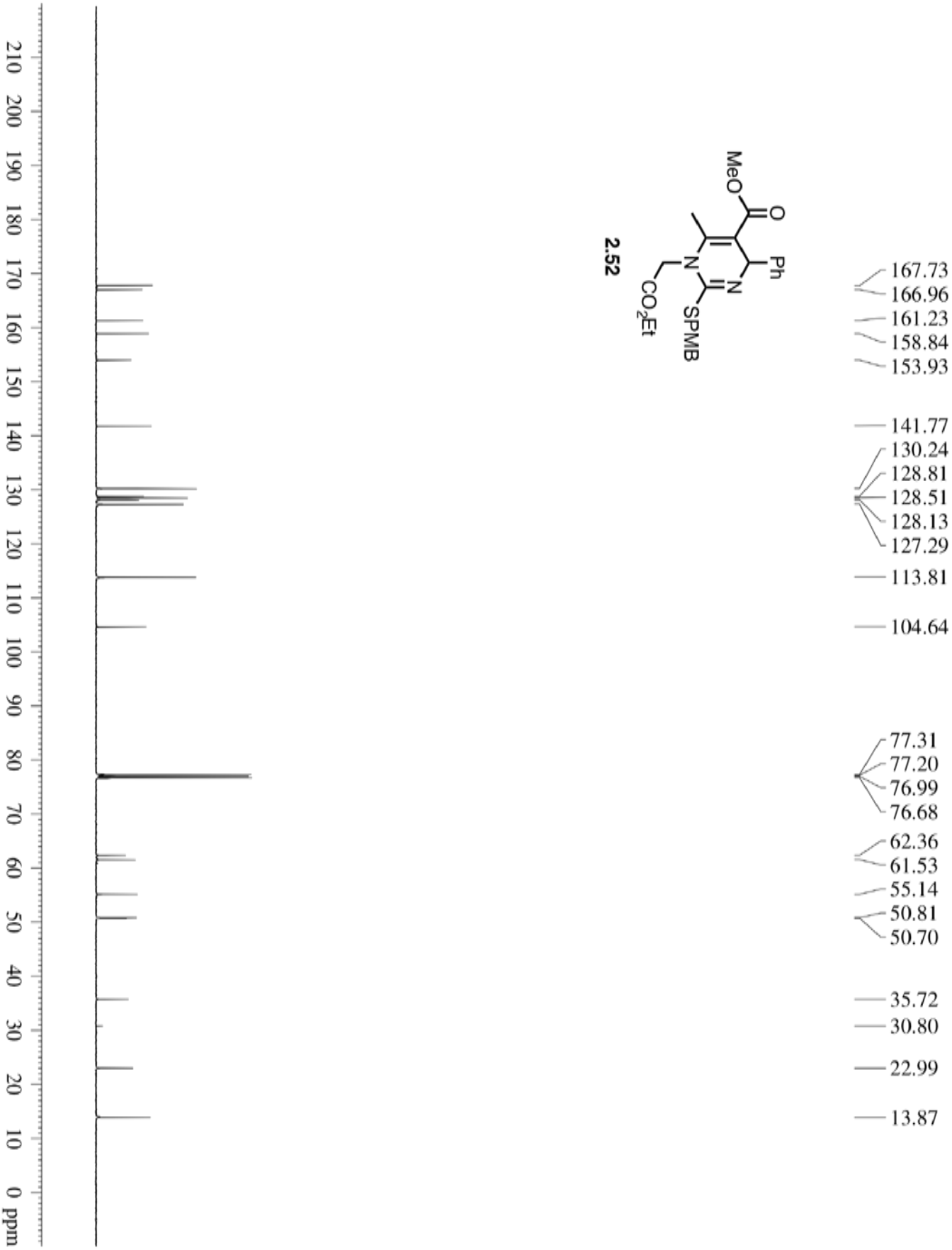
3.06
3.23
ppm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O
Ph
N
N
SPMB
CO2Et
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158.84
153.93
141.77
130.24
128.81
128.51
128.13
127.29
113.81
104.64
77.31
77.20
76.99
76.68
62.36
61.53
55.14
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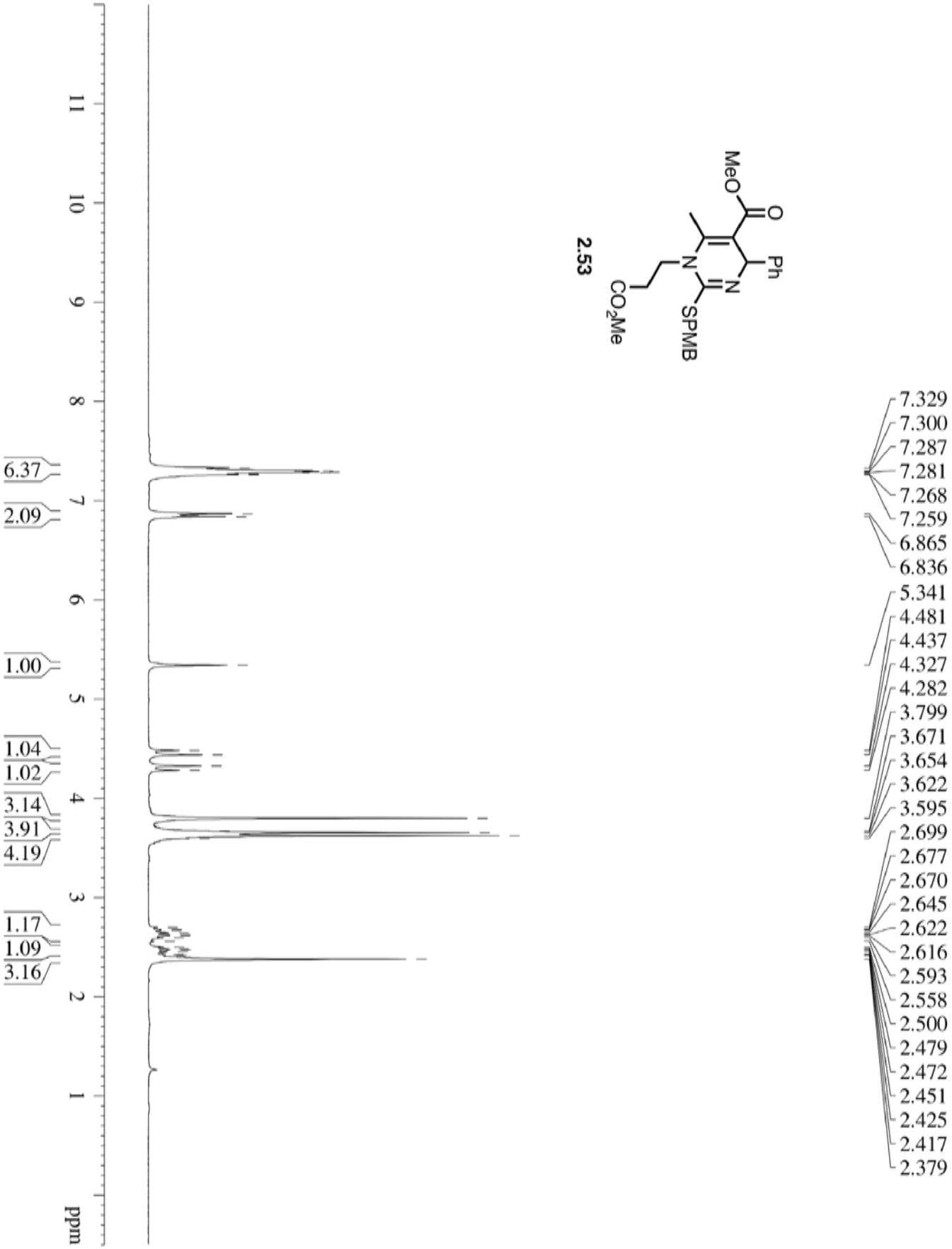
50.70
35.72
30.80
22.99
13.87
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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O
Ph
N
N
SPMB
CO2Me
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7.268
7.259
6.865
6.836
5.341
4.481
4.437
4.327
4.282
3.799
3.671
3.654
3.622
3.595
2.699
2.677
2.670
2.645
2.622
2.616
2.593
2.558
2.500
2.479
2.472
2.451
2.425
2.417
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1.04
1.02
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4.19
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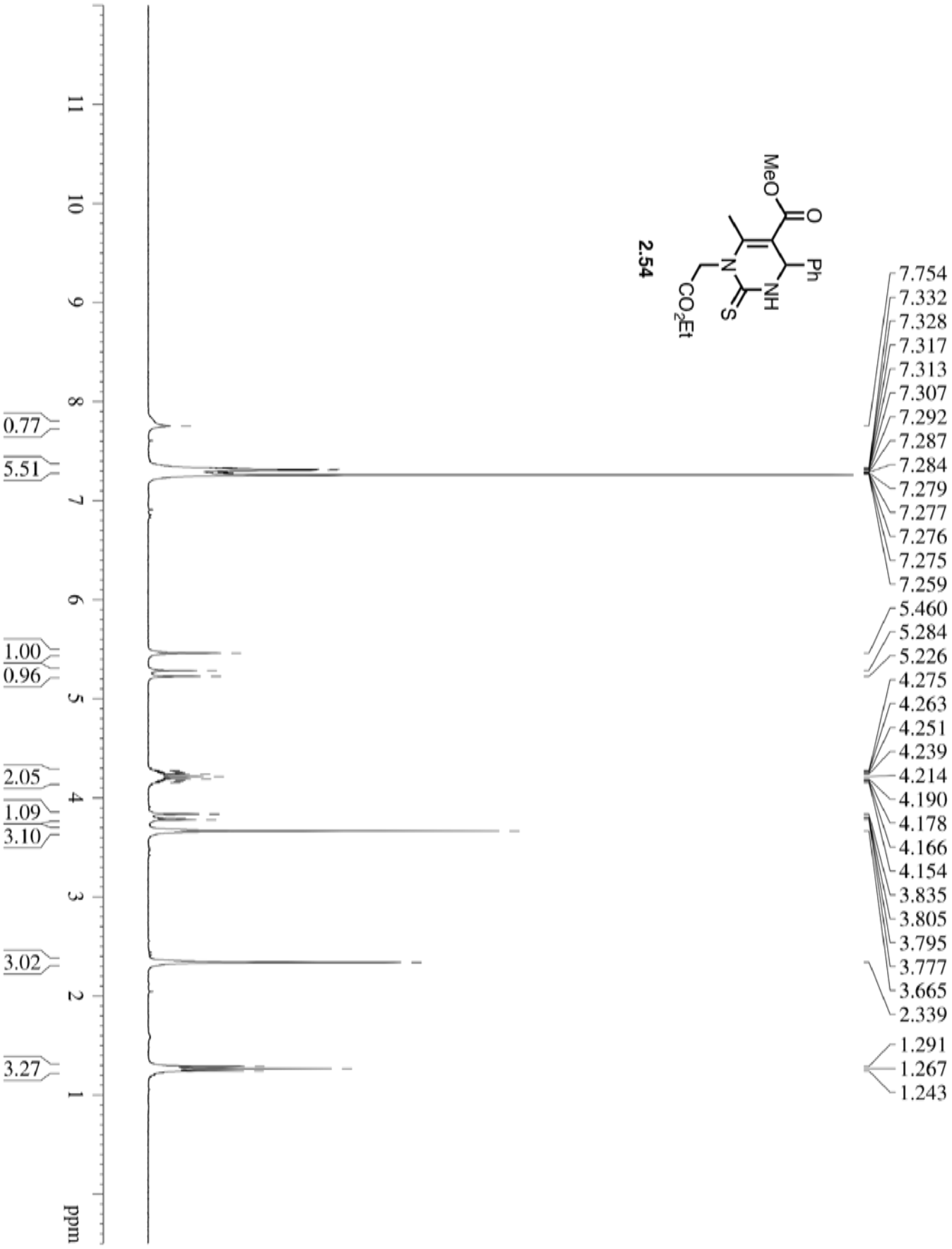
ppm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Ph
NH
S
N
CO2Et
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7.307
7.292
7.287
7.284
7.279
7.277
7.276
7.275
7.259
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5.284
5.226
4.275
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4.251
4.239
4.214
4.190
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4.154
3.835
3.805
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3.777
3.665
2.339
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1.09
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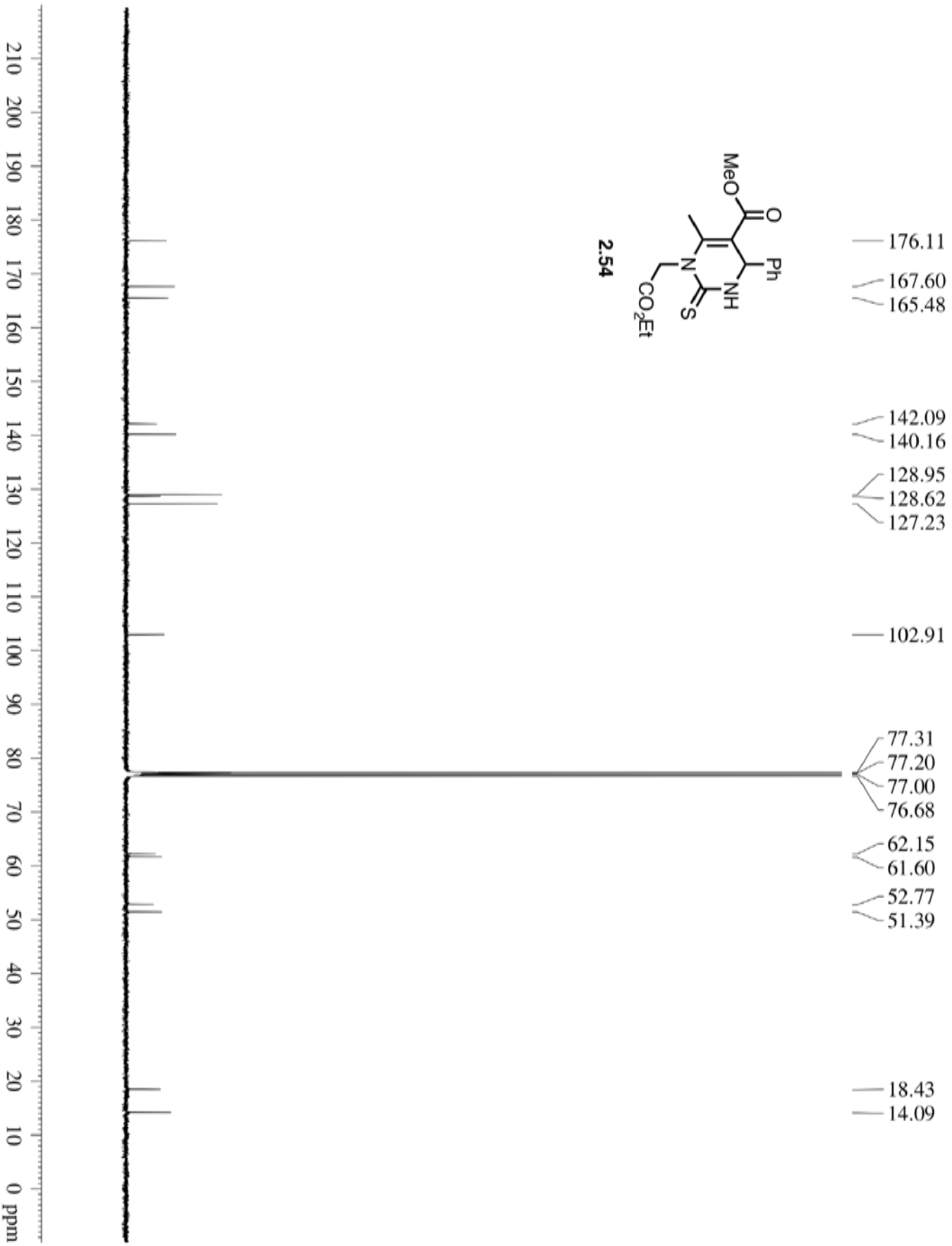
3.02
3.27
ppm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MeO
O
Ph
NH
S
N
CO2Et
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142.09
140.16
128.95
128.62
127.23
102.91
77.31
77.20
77.00
76.68
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61.60
52.77
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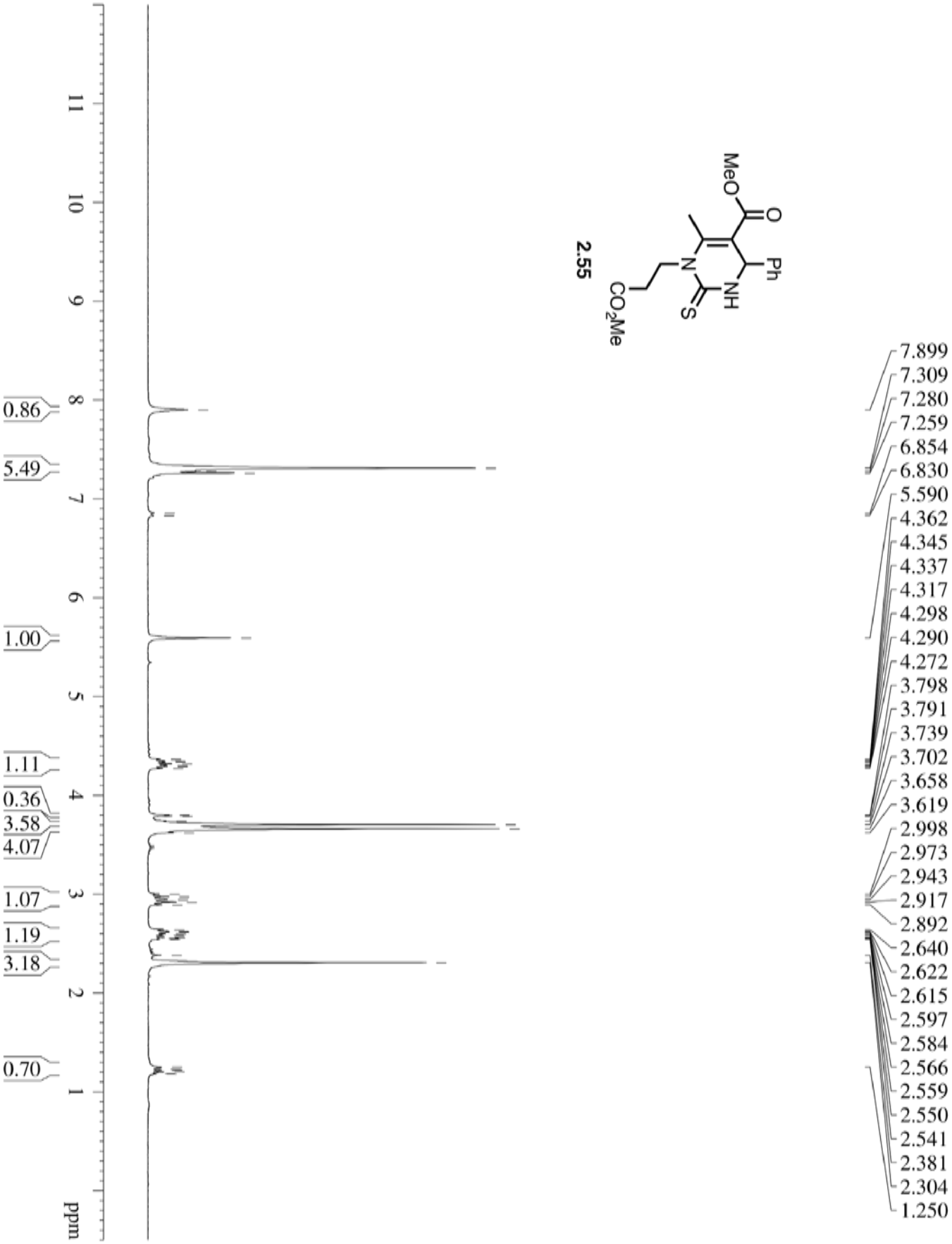
18.43
14.09
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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S
N
CO2Me
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5.590
4.362
4.345
4.337
4.317
4.298
4.290
4.272
3.798
3.791
3.739
3.702
3.658
3.619
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2.973
2.943
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2.640
2.622
2.615
2.597
2.584
2.566
2.559
2.550
2.541
2.381
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1.07
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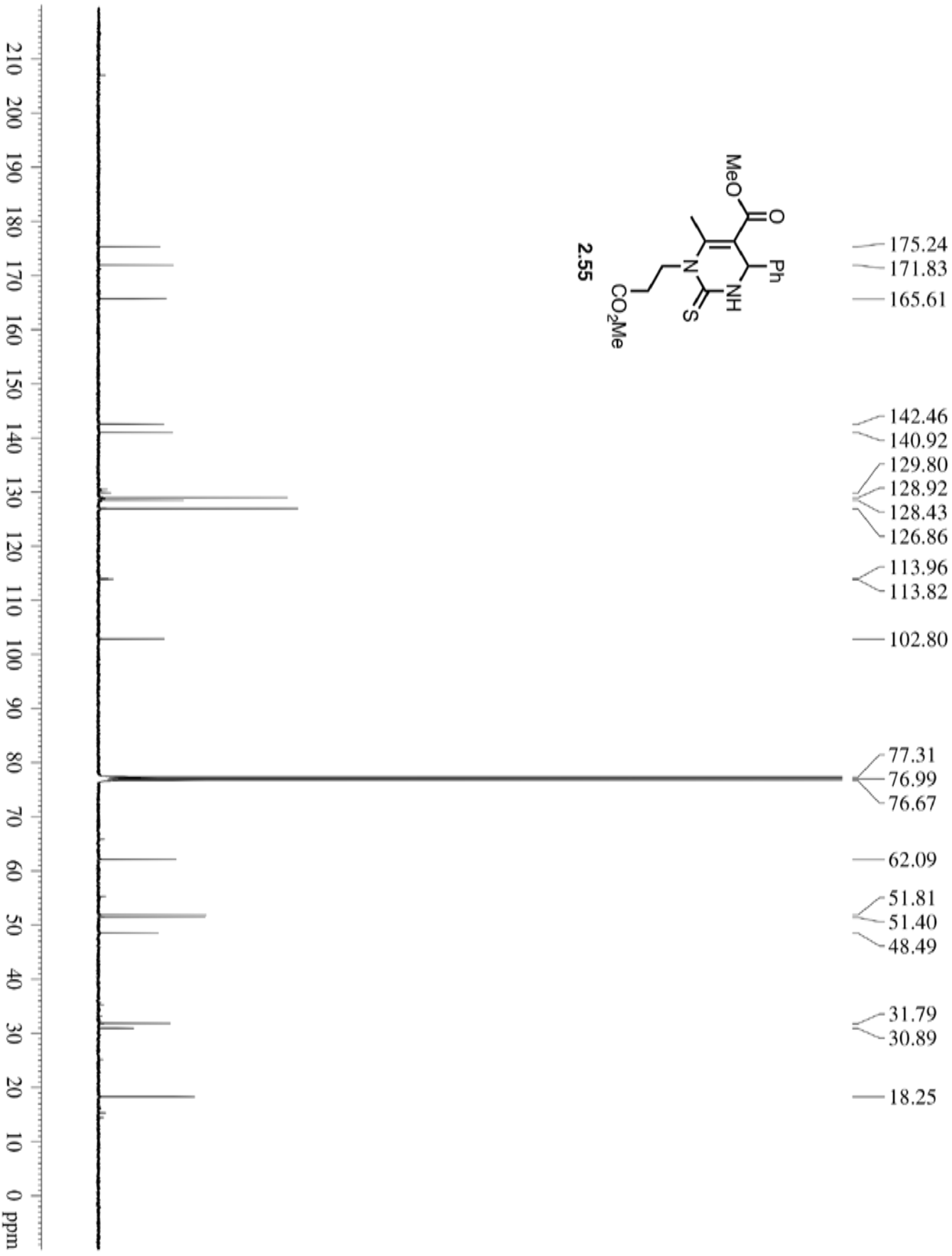
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Ph
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S
N
CO2Me
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140.92
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128.92
128.43
126.86
113.96
113.82
102.80
77.31
76.99
76.67
62.09
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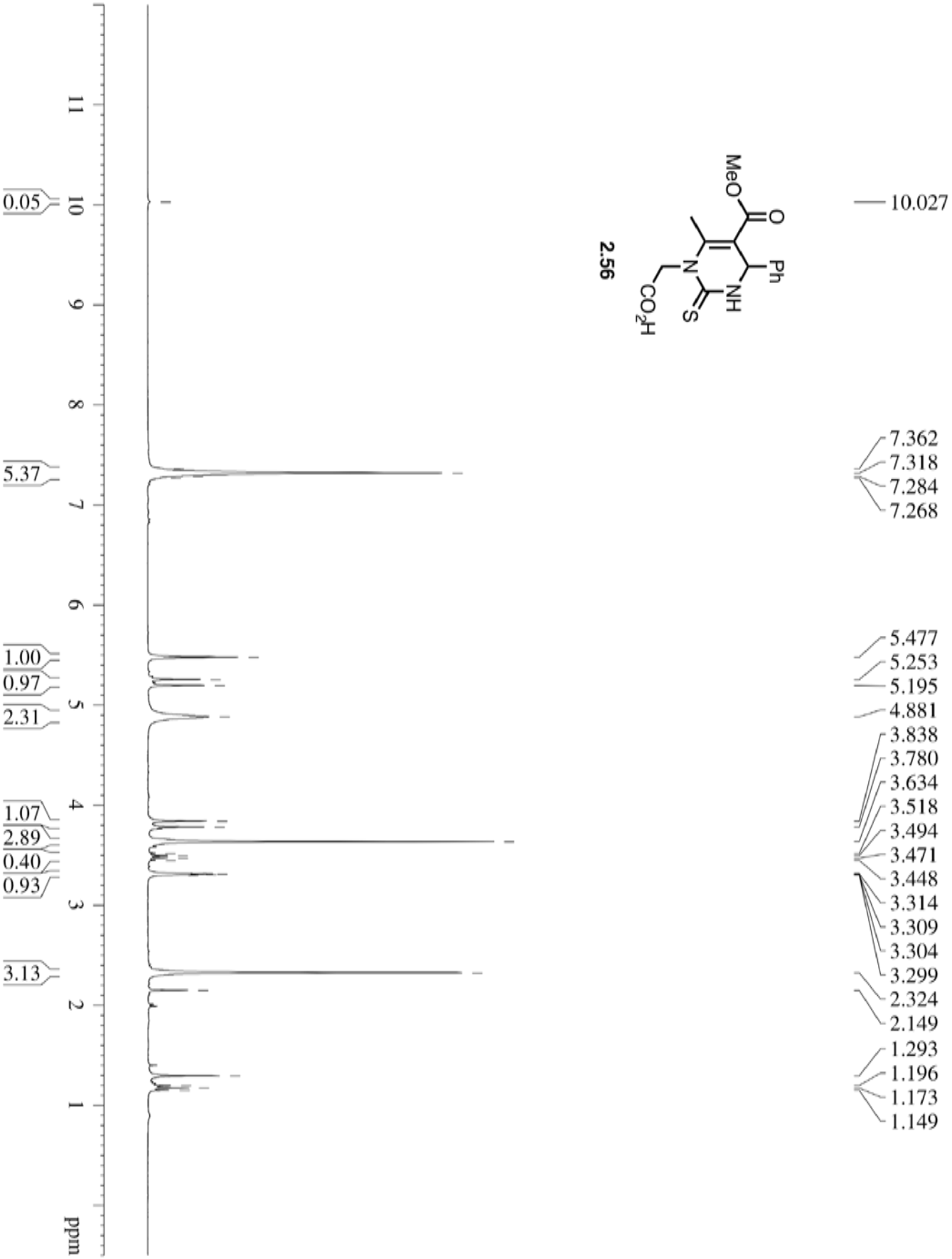
51.40
48.49
31.79
30.89
18.25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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1.07
2.89
0.40
0.93
3.13
ppm
2.56
MeO
O
Ph
NH
S
N
CO2H
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7.362
7.318
7.284
7.268
5.477
5.253
5.195
4.881
3.838
3.780
3.634
3.518
3.494
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3.309
3.304
3.299
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2.149
1.293
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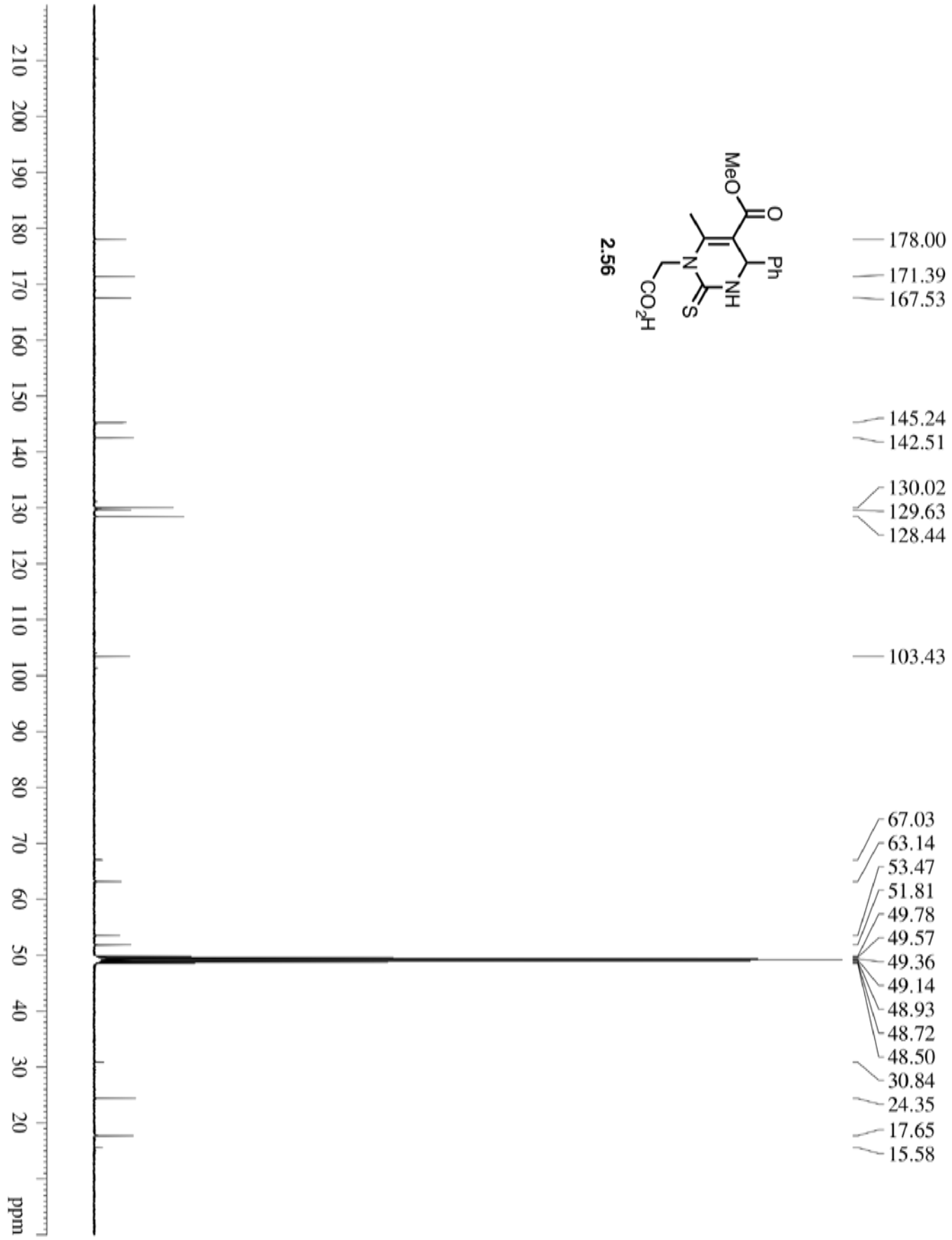
1.173
1.149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Ph
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CO2H
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130.02
129.63
128.44
103.43
67.03
63.14
53.47
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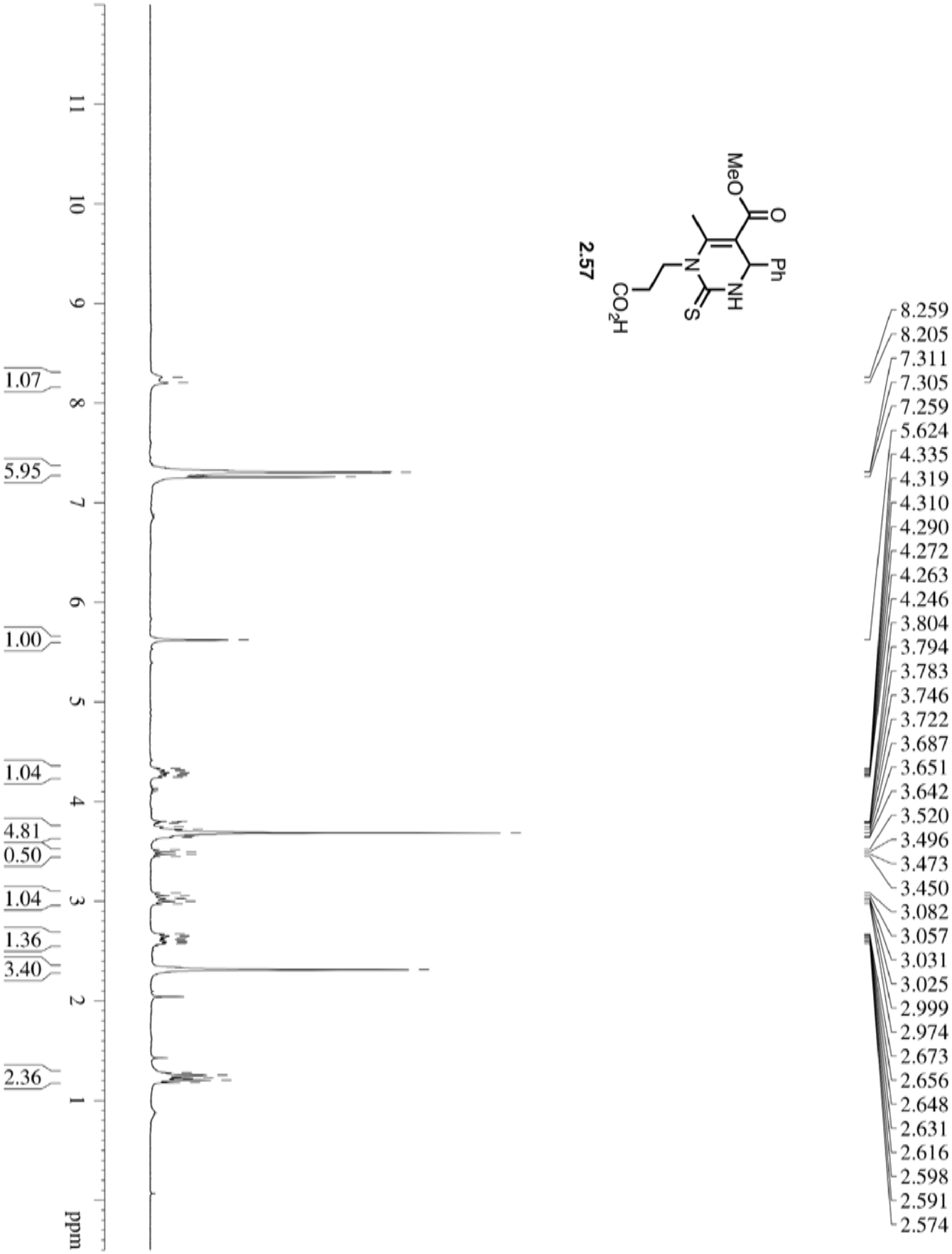
49.36
49.14
48.93
48.72
48.50
30.84
24.35
17.65
15.58
ppm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MeO
O
Ph
NH
S
N
CO2H
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8.205
7.311
7.305
7.259
5.624
4.335
4.319
4.310
4.290
4.272
4.263
4.246
3.804
3.794
3.783
3.746
3.722
3.687
3.651
3.642
3.520
3.496
3.473
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3.082
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3.031
3.025
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2.616
2.598
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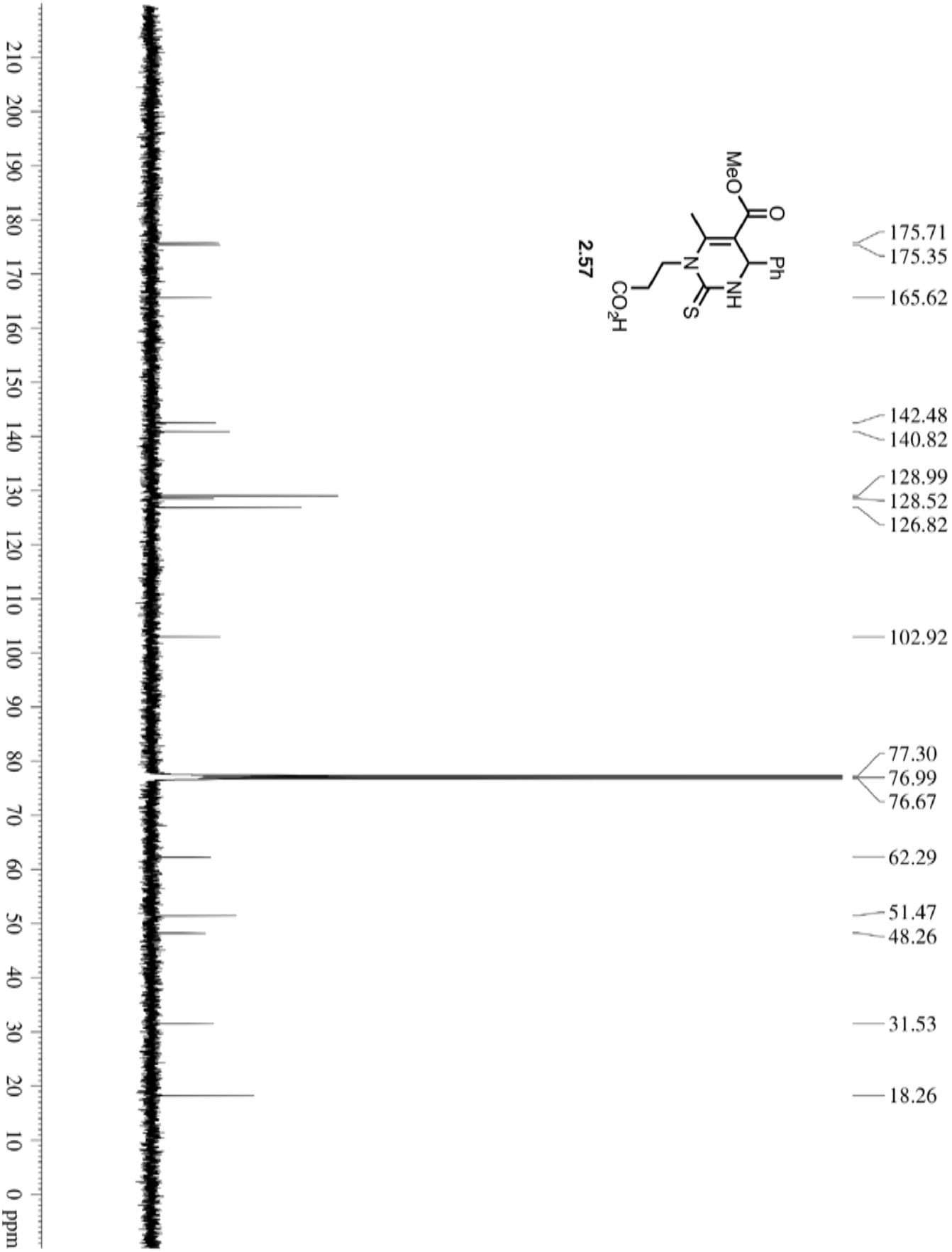
2.57
MeO
O
Ph
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S
N
CO2H
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140.82
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128.52
126.82
102.92
77.30
76.99
76.67
62.29
51.47
48.26
31.53
18.26
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm