2015

REGIOSELECTIVE CARBON-NITROGEN BOND FORMATION VIA OXIDATIVE TRANSITION METAL CATALYSIS

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REGIOSELECTIVE CARBON-NITROGEN BOND FORMATION VIA OXIDATIVE TRANSITION METAL CATALYSIS

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

UNIVERSITY OF RHODE ISLAND

2015

DOCTOR OF PHILOSOPHY DISSERTATION

OF

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2015

Abstract

The synthesis of carbon-nitrogen (C-N) bonds is an area of research that has garnered significant interest due to the ubiquity of C-N bonds in pharmaceuticals and natural products. Various methods exist for forming C-N bonds, most recognizably Buchwald-Hartwig amination, but these reactions typically require functionalization of either the C-H or N-H bond resulting in the formation of harmful byproducts. As a result, the need to explore alternative means for synthesizing C-N bonds merits consideration. An alternative means for achieving C-N bond formation lies in the oxidative cross coupling of carbon-hydrogen (C-H) and nitrogen-hydrogen (N-H) bonds. While this approach eliminates the necessity for the prefunctionalization of C-H and N-H bonds, reactions employing oxidative cross coupling can be plagued with poor regioselectivity. As a result, the use of directing groups and transition metal catalysis is typically employed to circumvent this issue.

The primary focus of this dissertation is the discussion of newly developed methodologies that enhance the regioselective control of oxidative C-N bond formation. Chapters one and two detail the use of hypervalent iodine in conjunction with a transition metal catalyst to help govern the site of amination on various electron-rich, and moderately deactivated, arene systems. Chapters three and four will deviate from C—N bond forming methodologies and instead discuss synthetic routes for the construction of a molecular probe targeting the D_2 receptor and the development of an amino acid coupling protocol to be utilized in the organic teaching laboratory.

ACKNOWLEDGMENTS

"*The journey of a thousand miles begins with a single step"*

- Lao Tzu

Every journey begins the same way; establish a goal then take that first step. I knew that the path to obtain science's highest degree would not be an easy one, but as my formal education comes to a close I am able to reflect on my time at the University of Rhode Island with an immense sense of pride. I would like to thank my major professor **Brenton DeBoef**. Over the past six years Professor DeBoef has served as a role model; leading by example and instilling valuable life lessons both inside the laboratory and out. His patience and dedication to the craft have laid the foundation for my career as a scientist and I am thoroughly grateful.

I would also like to thank Professors **Mindy Levine, Navindra Seeram, Brett Lucht,** and **Abraham Kovoor** for their time and guidance in preparation for my doctoral defense. I would like to especially thank Dr. Levine and Dr. Lucht for helpful discussions throughout the course of my graduate career. I wish to also thank my colleagues and friends **Abhishek Kantak, Ashley Porter, Nicole Serio, John Sirois,** and **Joseph Brown** whose comradery helped alleviate some of the stresses associated with graduate school.

I wish to dedicate this dissertation to two individuals:

To my loving wife, **Tanna Rose,** for her unwavering support, unconditional love, and large heart. You will always make me smile and I look forward to a lifetime of happiness.

To my mother, **Donna**, for her countless sacrifices and instilling in me the confidence I needed early on that has allowed me to pursue my dreams.

I love you both dearly.

Preface

This dissertation is presented in manuscript format. The first manuscript entitled "Regioselective Gold Catalyzed Oxidative C-N Bond Formation" discusses the effects of a gold (I) catalyst when oxidatively coupling phthalimide to simple arenes in the presence of hypervalent iodine. Mechanistic insight suggests a pathway consistent with that of electrophilic aromatic substitutionwith a preference to impart para selectivity. This manuscript has been published as a communication in *Organic Letters*.

The second manuscript entitled "Regioselective C—N Bond Formation by Aminoiodanes" employs a copper (II) reagent for an economical means of aminating various 2-phenylpyridine derivatives. The manuscript detailing this work has been published in *Chemical Communications*.

The third manuscript entitled "Efforts Towards the Synthesis of a Molecular Probe targeting the D_2 Receptor" deviates from C-N bond formation and focuses on the synthetic methodology employed for the probe's construction. Upon the completion of the synthesis this work will be submitted for publication to the *Journal of Organic and Biomolecular Chemistry.*

The final manuscript entitled "Solution-Phase Synthesis of Dipeptides: A Capstone Project that Employs Key Techniques in an Organic Laboratory Course" is an original laboratory experiment designed to serve as a final assessment of the skills acquired throughout the sophomore level organic chemistry laboratory course. This work has been accepted in the *Journal of Chemical Education*.

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Introduction

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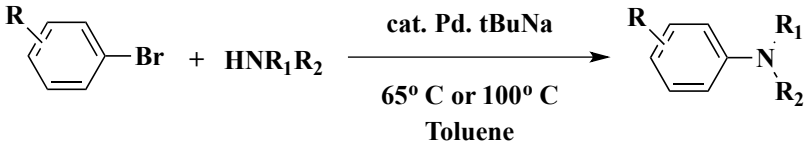
Introduction

The field of carbon–nitrogen (C–N) bond formation has generated significant attention due to the ubiquity of C–N bonds in a verity of biologically active compounds. Reactions seeking to achieve C–N bond formation can be done in one of two ways: either intramolecularly, resulting in the formation of a heterocyclic compound; or intermolecularly, wherein a nitrogen-containing compound is independently coupled to some carbon species.[1] C–N bond formation can be achieved in a myriad of ways. However, from an environmental perspective, the best way to achieve C–N bond formation is through the oxidative cross coupling of carbon–hydrogen (C–H) and nitrogen–hydrogen (N–H) bonds, as this would eliminate the need for prefunctionalization of the C–H bond and would, in principle, increase the atom economy of the overall synthesis. While the use of transition metal catalysts are not always necessary, regioselectivity often becomes an issue when attempting to perform a reaction intermolecularly using oxidative conditions.[2-4] As a result, contemporary methods employed for the oxidative construction of C–N bonds often utilize directing groups or late transition metals in order to garner control over the site of amination. Herein, a discussion highlighting contemporary methods for the construction of C–N bonds utilizing different transition metal catalysts will be presented with an emphasis on C–H prefunctionalization alternatives when applicable.

Palladium Catalysis

Perhaps the most recognized amination protocol is the one that is now taught to students in sophomore organic chemistry, the Buchwald-Hartwig reaction. In 1995

Steven Buchwald reported that the C–N bond of an N-arylamine could be formed by reacting aryl bromides with secondary amines in the presence of a base and catalytic amounts of palladium (Scheme 1).[5] While examples of C–N bond formation certainly exist before 1995, the preceding example remains one of the most widely utilized procedures today.

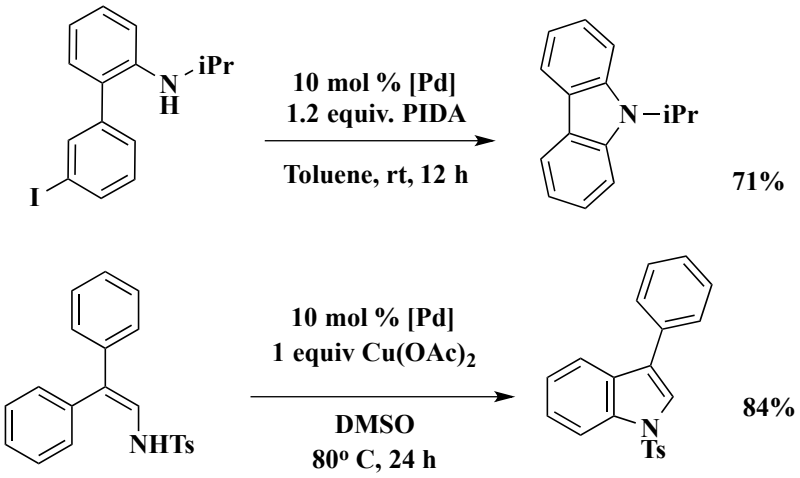


Scheme 1. Buchwald amination reaction[5]

The field of oxidative C–H amination employing a palladium catalyst is markedly less developed when compared to reactions yielding carbon-carbon (C–C) bonds.[1] Moreover, the intramolecular reaction has been more studied in comparison to its intermolecular counterpart. The syntheses of carbazoles and indoles have been extensively studied and, in the case of carbazole synthesis, demonstrated good functional group tolerance.[1] By taking advantage of the thermodynamically favored 5-membered palladacycle intermediate, the need for C–H bond functionalization is circumvented in both instances through the additional use of stoichiometric amounts of copper[6,7] or hypervalent iodine[8] (Scheme 2).

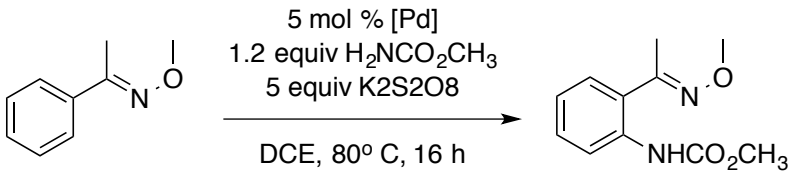
In addition to carbazoles and indoles, the synthesis of indolines and lactams have also shown to be quite amenable to palladium catalyzed reactions.[7,9] Much like the synthesis of carbazoles, reactions producing indoline derivatives also exhibited excellent functional group tolerance with considerable success being achieved across a

modest array of phenethyltriflamides.[1] Lactam synthesis also proved to be fairly fruitful as high yields were achieved in reactions producing β-, γ- , and δ-derivatives; however, the major drawback to this work was the necessity for a high degree of substitution α to the carbonyl of the amide functional group.[1,7]



Scheme 2. Intramolecular synthesis of carbazoles and indazoles[1,7-8]

Few examples of intermolecular palladium catalyzed amination reactions exist via C–H activation. In 2006 Che and associates reported the successful amination of pyridine and oxime ether derivatives.[10] Che demonstrated that catalytic amounts of palladium in the presence of excess $K_2S_2O_8$ were effective in transferring an amide moiety.[1] The electronics of the amide derivative proved to be inconsequential as electron deficient systems were demonstrated to be amenable to Che's protocol (Scheme 3).



Scheme 3. Amination of oxime ethers[10]

Most recently, Hartwig and coworkers developed a method for regioselectively coupling phthalimide to sterically encumbered arenes using catalytic amounts of palladium and hypervalent iodine.[11] While Hartwig's findings provided high yielding substrates, his methodology was limited in that regioselectivity was governed by sterics, and owing to the fact that the proposed mechanism proceeded through concerted metalation-deprotonation (CMD), little selectivity between para and meta substituted isomers were observed.

Copper Catalysis

C–N bond formation is substantially more developed with regards to copper catalysis, and an excellent review exists highlighting major accomplishments in the field.[12] For example, one of the better-known copper catalyzed amination reactions involves the arylation of arylamines, the Ullmann coupling reaction. In this reaction, aryl-halides may be coupled to aniline derivatives, or N-containing heterocycles, in the presence of a copper catalyst to yield the corresponding aryl/diarylamines (Figure 1).[12]

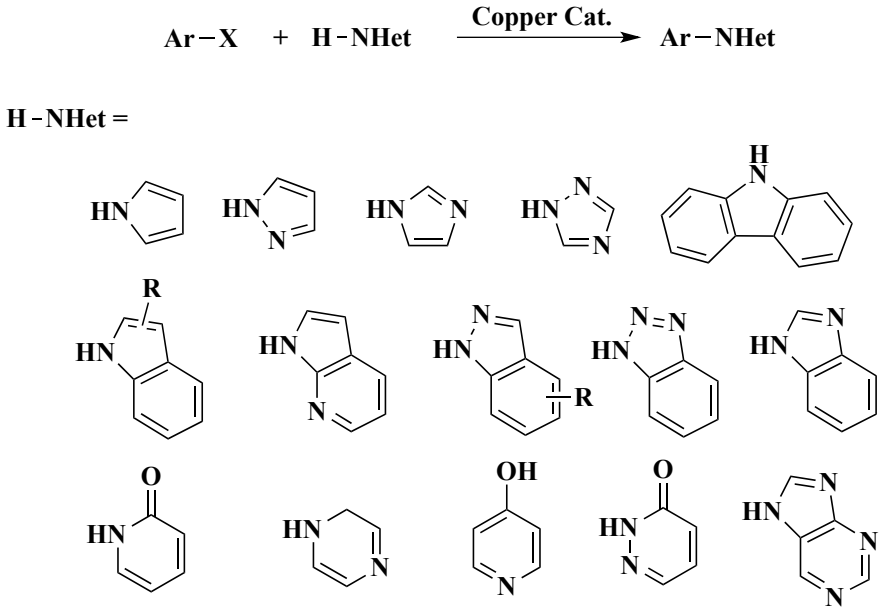
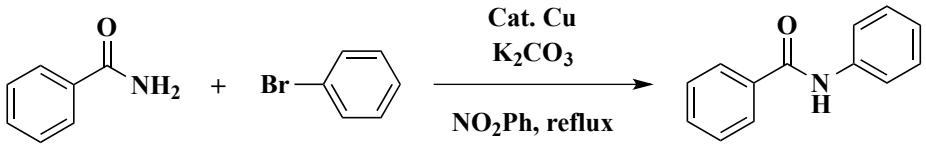


Figure 1. Arylation of N-heterocycles[12]

Ullmann coupling is typically not amenable to aliphatic systems, *i.e.* alkylamines, with the exception of chelating amines such as amino acids.[12] It was not until 2003, when Fukuyama published a communication detailing the arylation of primary amines, that copper catalyzed C–N bond formation was extended to aliphatic systems.[13]

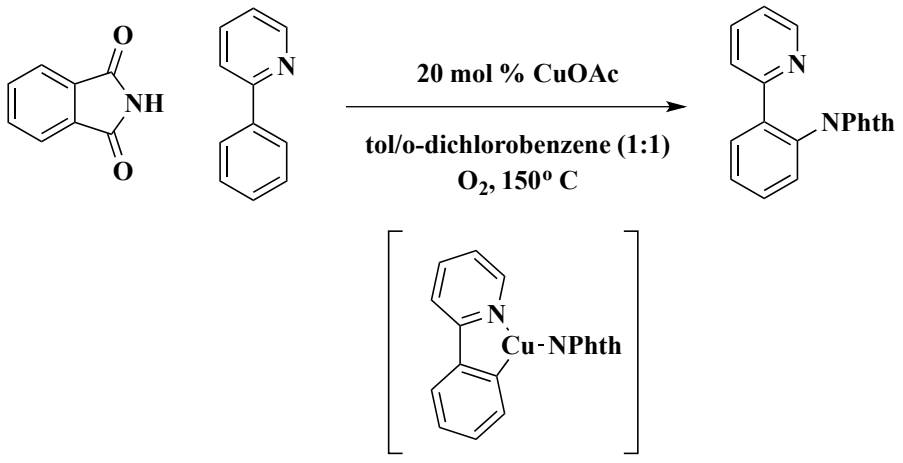
Perhaps as equally robust as the Ullmann reaction, though admittedly less advantageous, is the Goldberg condensation of primary amides. Shortly after the development of the Ullmann reaction Irma Goldberg developed a method for arylating amide and aniline substrates.[12] In this derivation of the Ullmann reaction, a primary amide and aryl halide undergo a cross coupling reaction in the presence of copper and potassium carbonate to afford the desired arylamide (Scheme 4). While the utility of

both this reaction and Ullmann coupling is extensive, attention should be diverted to the development of oxidative processes that circumvent the use of aryl halides.



Scheme 4. Representative Goldberg Condensation Reaction

Oxidative C–N bond formation via C–H activation is underdeveloped in the sphere of copper catalysis. However, over the last decade advances have been made to develop intermolecular pathways for direct C–H amination. In 2006 Yu and associates published their findings that 2-phenylpyridine derivatives, in the presence of catalytic Cu under an O_2 atmosphere, had the ability to couple nucleophilic species α to the pyridine directing group.[14] This reaction appears to be facilitated by the formation of a 5-membered ring intermediate (Scheme 5).

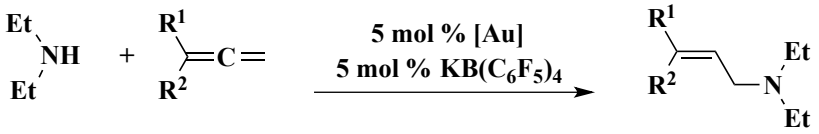


Scheme 5. Amidation of 2-phenylpyridine

Recently, Shen and associates expanded upon Yu's work and detailed the direct amination of various N-heteroarylindoles and 2-arylpyridine substrates. While the reaction is robust in most instances, with yields ranging from 20-97%, the oxidative coupling of amine/amide moieties into aromatic systems appears to be limited to substrates containing a chelating directing group.[15]

Gold Catalysis

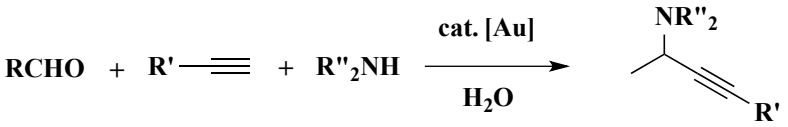
Over the course of the last two decades reactions employing a gold catalyst have been on the rise despite the long held assumption that gold was an unreactive noble metal.[16] Unlike the previously mentioned examples, most of the reactions utilizing a gold catalyst occur through a hydroamination process, *i.e.* the formal addition of a N–H bond across an unsaturated C–C bond.[16] Gold catalysis is particularly useful in the formation of allylic amines which can serve as valuable synthetic precursors (Scheme 6).



Scheme 6. Gold catalyzed allylic amine synthesis[17]

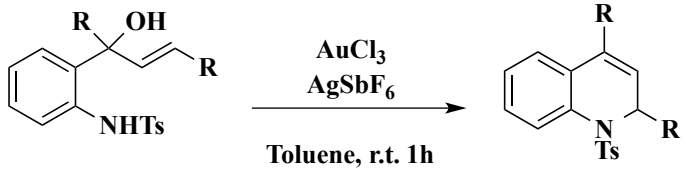
Similarly, gold can be a useful tool in the synthesis of propargyl amines. Conventional routes for synthesizing propargyl amines can become overly complicated, as procedures will typically call for the reaction of imine derivatives with water-sensitive reagents such as lithium acetylides or Grignard reagents.[16]

Alternatively, propargyl amines may be synthesized from gold salts, such as $AuBr_3$ and AuCl, by coupling an alkyne, aldehyde, and amine by C–H activation (Scheme 7). This method does however possess minor drawbacks, as gold salts have a tendency to rapidly reduce into an inert metal species upon olefin activation.[16] Despite this inevitability, considerable progress has been made towards the synthesis of propargyl amines using this reaction.[18-21]



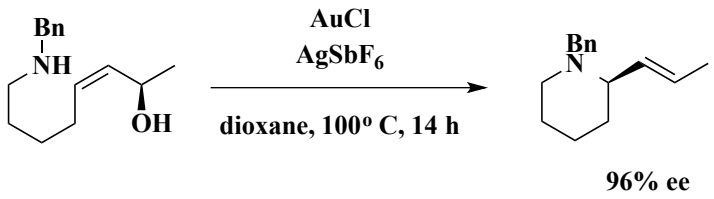
Scheme 7. Gold mediated propargyl amine synthesis

Recently, the use of gold catalysis has demonstrated effectiveness to aminate unsaturated C–C bonds intramolecularly. In 2009 Chen *et al.* reported a gold catalyzed reaction of various 2-tosylaminophenylprop-1-en-3-ol derivatives, leading to the construction of 1,2-dihydroquinolines. These reactions were also carried out in the presence of $AgSbF_6$ and produced yields ranging from 42-91% (Scheme 8).[22] This reaction was unique because of it's high functional group tolerance. The procedure was amenable to electron-withdrawing, electron-donating, and sterically encumbered substrates.



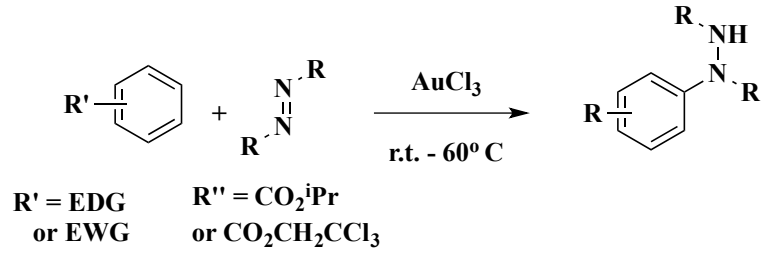
Scheme 8. Allylic amination of 2-tosylaminophenylprop-1-en-3-ols

Expanding on Chen's work, Widenhoefer employed a similar protocol for the synthesis of pyrrolidine and piperidine derivatives from alkylamine precursors.[23] Widenhoefer's methodology required increased time and temperature in comparison to Chen, but excellent enantioselectivity was observed with ee's as high as 96% (Scheme 9).



Scheme 9. Enantioselective piperidine synthesis

Few examples of gold catalyzed arene aminations exist in the literature; the first was reported by He and coworkers. In 2007 He achieved direct functionalization of aromatic C–H bonds and detailed a gold catalyzed nitrene insertion pathway that resulted in aryl and benzylic amination.[24] More recently, Zhang reported the direct amination of arenes using azodicarboxylates. His gold(III) mediated pathway represented the first catalytic system for the direct amination of electron-deficient arenes with azodicarboxylates. Zhang's conditions were mild, with temperatures not exceeding 60° C, and produced yields as high as 96% (Scheme 10).[25] With the exception of the previous two examples, and the work detailed herein, the topic of intermolecular arene amination is significantly underdeveloped with respect to gold catalysis.



Scheme 10. Direct arene amination[25]

Methods for C–N bond formation will always be an important synthetic tool in the construction of various pharmaceuticals and natural products. The necessity to develop greener, more atom efficient methods for amination protocols have only just started to take hold in the scientific community, as catalytic, oxidative alternatives begin to emerge. In order for oxidative C–N bond formation to really take root in academic and industrial practices, alternatives to reactions such as Buchwald-Hartwig coupling and Ullmann coupling need to be developed without sacrificing yield and regioselectivity. While oxidative C–H activation may still be relatively underdeveloped with respect to other contemporary amination strategies; in just a short time we have seen substantial progression in the field. It can be said with confidence that it may only be a matter of time before our contemporary methods are viewed as outdated and obsolete as oxidative catalysis begins to take hold.

References:

1. Lyons, T. W.; Sanford, M. S. *Chem. Rev.* **2010**, *110*, 1147.
2. Kantak, A.; Potavathri, S.; Barham, R. A. Romano, K.; DeBoef, B. *J. Am. Chem. Soc.* **2011**, *133*, 19960.
3. Antonchick, A. P.; Samanta, R.; Kulikov, K.; Lategahn, J. Angew. Chem., Int. Ed. **2011**, *50*, 8605.
4. Kim, H. J.; Kim, J.; Cho, S. H.; Chang, S. J. Am. Chem. Soc. **2011**, *133*, 16382.
5. Guram, A.; Renneis, R.; Buchwald, S. *Angew. Chem. Int. Engl.* **1995**, *34*, 1348.
6. Tsang, W. C. P.; Zhang, N.; Buchwald, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 14560.
7. Inamoto, K.; Saito, T.; Hiroyo, K.; Doi, T. *Synlett* **2008**, 3157.
8. Jordon-Hore, J. A.; Johamsson, C. C. C.; Gulia, M.; Beck, E. M.; Gaunt, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 16184
9. Mei, T. S.; Wang, X.; Yu, J. Q. *J. Am. Chem. Soc.* **2006**, *128*, 9048.
10. Thu, H. Y.; Yu, W. Y; Che, C. M. *J. Am. Chem. Soc.* **2006**, *128*, 9048.
11. Shrestha, R.; Mukherjee, P.; Tan, Y.; Litman, Z. C.; Hartwig, J. *J. Am. Chem. Soc.* **2013**, *135*, 8480.
12. Evano, G.; Blanchard, N.; Toumi, M. *Chem. Rev.* **2008**, *108*, 3054.
13. Okano, K.; Tokuyama, H.; Fukuyama, T. *Org. Lett.* **2003**, *5*, 4987.
14. X. Chen, X.-S. Hao, C. E. Goodhue and J.-Q. Yu, *J. Am. Chem. Soc.,* **2006**, *128*, 6790.

15. Xu, H.; Qiao, X.; Yang, S.; Shen, Z. *J. Org. Chem.* **2014**, *79*, 4414.

16. Corma, A.; Leyva-Pérez, A.; Sabater, M. J. *Chem. Rev.* **2011**, *111*, 1657.

17. Zeng, X.; Soleilhavoup, M.; Bertrand, G. *Org. Lett.* **2009**, *11,* 3166.

18. Wei, C.; Li, C. *J. Am. Chem. Soc.* **2003**, *125*, 9584.

19. Lo, V. K. Y.; Liu, Y.; Wong, M. K.; Chem, C. M. *Org. Lett.* **2006**, *8*, 1529.

20. Wei, C.; Li, Z.; Li, C. *Org. Lett.* **2003***, 5*, 4473.

21. Shi, L.; Tu, Y. Q.; Wang, M.; Zhang, F. M.; Fan, C. A. *Org. Lett.* **2004**, *6*, 100.

22. Kothandaraman, P.; Foo, S. J.; Chan, P. W. H. *J. Org. Chem.* **2009**, *74,* 5947

23. Mukherjee, P.; Widenhoefer, R. A. *Org. Lett.* **2011**, *13*, 1334.

24. Li, Z.; Capretto, D.; Rahaman, R. O.; He, C. *J. Am. Chem. Soc.* **2007**, *129*, 12058.

25. Gu, L.; Boon, S. N.; Zhang, Y. *Org. Lett.* **2011**, *13*, 1872.

MANUSCRIPT 1

Published in *Org. Lett.* **2015**, *17*, 358.

Regioselective Gold-Catalyzed Oxidative C—N Bond Formation

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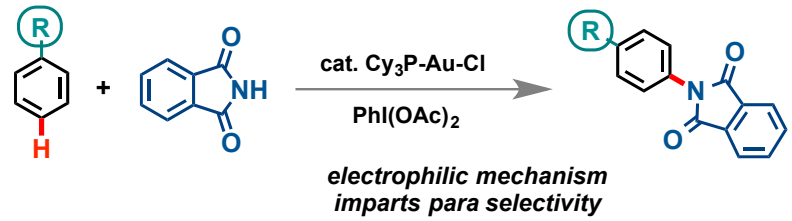
Manuscript 1

Regioselective Gold-Catalyzed Oxidative C—N Bond Formation



Abstract

A novel protocol for the regioselective intermolecular amination of various arenes has been developed. By using an I(III) oxidant in the presence of an Au(I) catalyst, a direct, and novel route for regioselectively accessing a variety of substituted aniline moieties has been achieved with yields as high as 90%. Mechanistic insight suggests that regioselectivity can be predicted based on electrophilic aromatic metalation patterns.

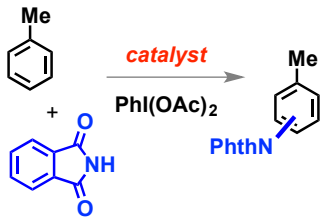
Introduction

The synthesis of carbon–nitrogen (C–N) bonds through the oxidative cross-coupling of carbon-hydrogen (C–H) and nitrogen–hydrogen (N–H) bonds is an area that has generated significant interest due to the ubiquity of C–N bonds in a variety of pharmaceuticals and natural products. The utility of late-stage transition metals, more specifically Pd, Rh, Ru and Cu, for C–H bond activation has been extensively studied and the research field has been reviewed several times.[1] Over the course of the last two decades, gold-catalyzed reactions have played a significant role in carbon–carbon

(C–C), carbon-oxygen (C–O) and C–N bond forming methodologies, despite the long-held assumption that gold was an unreactive noble metal.[2]

Recently, the use of gold catalysis has been explored for the purposes of C–H activation; however, few precedents exist.[3] The majority of the work in gold-catalyzed C–N bond formation involves hydroamination processes,[4] though one example of arene amination via nitrenes has also been reported.[3b] Herein, we report a novel gold-catalyzed reaction that regioselectively synthesizes the C–N bond of phthalimide-protected aniline moieties by oxidatively cleaving C–H and N–H bonds.

We, along with Chang and Antonchick, recently reported the metal-free synthesis of phthalimide-protected aniline derivatives utilizing phenyliodine(III) diacetate (PIDA), phthalimide, and various simple arene substrates.[5] While this reaction was high yielding, it was plagued with poor regioselectivity. In a subsequent communication, Hartwig disclosed that introducing catalytic amounts of $Pd(OAc)_2$ into the reaction greatly enhanced the regioselectivity.[6] The use of the palladium(II) catalyst also resulted in a lowering of the reaction temperature, but it required additional equivalents of the oxidant, relative to the metal-free conditions. While Hartwig was able to enhance regioselectivity, product formation was almost exclusively governed by sterics and, owing to the fact that the palladium-catalyzed reaction proceeded via a concerted metalation deprotonation (CMD) pathway, regioselectivity between meta and para-substituted regiomers was generally not observed (Scheme 1).



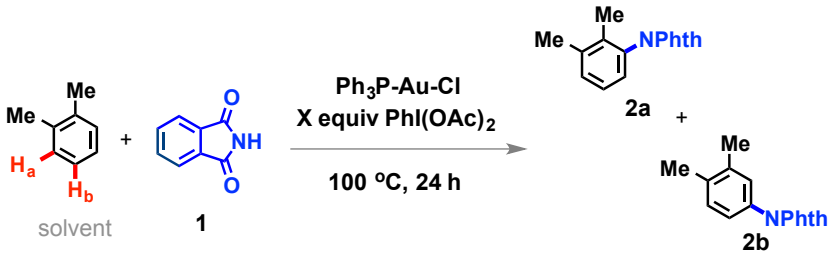
catalyst	major isomer(s)	ref
none	o/m/p mixture	5
$Pd(OAc)_2$	m/p mixture	6
Cy_3PAuCl	para	this work

Scheme 1. Regioselectivity of oxidative amination as a function of catalyst

Results and Discussion

We hypothesized that transition metal catalysts that do not metallate arenes by the CMD mechanism would further enhance the regioselectivity of our original findings, and we quickly discovered that heating a solution of chloro(triphenylphosphine)gold(I), phthalimide (**1**), and PIDA in *o*-xylene resulted in a 56% conversion of **1** to the desired phthalimide-protected aniline derivatives, **2a** and **2b**, in a 7:93 ratio, as determined by gas chromatography. Excited by this new lead, we began the optimization process by probing the role of PIDA in the reaction. By conducting control reactions and varying the loading of PIDA, several important trends were observed (Table 1).

First, the necessity for hypervalent iodine in the reaction was illustrated when PIDA was completely omitted from the reaction (Entry 1). Additionally, we observed a steady increase in the conversion of **1** to the desired phthalimide-protected aniline derivative (**2**) until the reaction plateaued at 4 equiv of PIDA (Entries 2-7).



entry[a]	PIDA (equiv)	[Au] (equiv)	% Yield (2a+2b)[b]	2a:2b[b]
1	0	0.10	2 %	100:0
2	1	0.10	24%	9:91
3	2	0.10	56%	7:93
4	3	0.10	69%	7:93
5	5	0.10	73%	8:92
6	4	0.10	77%	7:93
7[c]	4	0.10	93%	6:94
8	4	0	12%	2:1

[a] General reaction conditions: **1** (0.10 mmol), PIDA (0-5 equiv), 3.0 mL reagent grade *o*-xylene (solvent), aluminum well-plate heating at 100 °C for 24 hours. [b] Yield and product ratio obtained via GC; see Supporting Information for details. [c] Assembled in a nitrogen-containing glove box, anhydrous *o*-xylene

Table 1. Control and Optimization Results

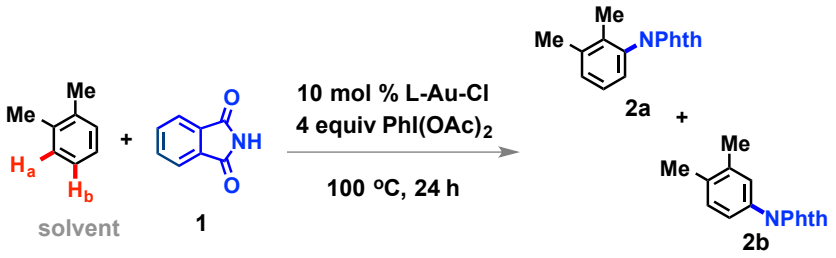
One key facet of the data that could not be ignored was the ability for the amination to proceed in the absence of a gold catalyst, albeit with a low conversion (Entry 8). It appears that the radical-mediated pathway reported in our original communication could not be completely inhibited. However, the presence of gold dramatically impacted regioselectivity (compare entries 7 and 8). As a result, we

hypothesized that two competing reaction pathways were taking place in these reactions, with metalation of the arene by the gold catalyst being favored over the non-catalyzed amination that we previously described. Other hypervalent iodine sources such as phenyliodine(III) bis(trifluoroacetate) (PIFA), and 2-iodoxybenzoicacid (IBX) were not amenable to this amination; nor were other metal-based oxidants, such as silver acetate.

Attempting to further enhance starting material conversion, we sought to determine if the reaction was stalling as a result of catalyst decomposition or oxidant consumption. A phosphorus NMR of the crude reaction mixture showed that the original gold catalyst still remained in solution. Drawing inspiration from Hartwig's Pd-catalyzed amination, we began adding additional equivalents of oxidant throughout the course of the reaction.[6] It was ultimately determined that by adding an additional 4 equiv of PIDA to the reaction after 12 hrs, a moderate increase in yield could be obtained without impacting regioselectivity. Both the gold-catalyzed reactions that are described herein and the previously described palladium catalyzed and metal-free aminations, also form biaryl side products, thus at least partially accounting for the need for additional equivalents of the oxidant.

Upon the completion of the oxidant screen, we sought to further enhance the conversion of starting material and the regioselectivity by probing the effects of different phosphine ligands (Table 2). Various gold-phosphine complexes were synthesized according to known precedures[7] and then subjected to the optimized reaction conditions. Bulky biaryl-containing ligands exhibited a drop-off in conversion, while trialkylphosphine ligands provided enhanced conversion of the

starting material and simultaneously preserved the lead reaction's regioselectivity. It was ultimately decided to continue the investigation with tricyclohexylphosphine due to its ease of handling. It is also worth mentioning that decreasing catalyst loading severely reduced the reaction rate, while an increase in catalyst loading only modestly impacted regioselectivity.



entry[a]	ligand	% Yield (2a+2b) [b]	2a:2b[b]
1	PPh_3	93%	6:94
2	Tetrahydrothiophene	54%	6:94
3	Triethyl Phosphite	73%	6:94
4	MePhos	20%	15:85
5	DavePhos	13%	46:56
6	TrixiePhos	92%	10:90
7	Cy_3P	98%	6:94
8	$(i\text{-}Pr)_3P$	97%	4:96

[a] See Table 1 for reaction conditions. Reactions assembled in a nitrogen-containing glove box. [b] Yield and product ratio obtained via GC; see Supporting Information for details.

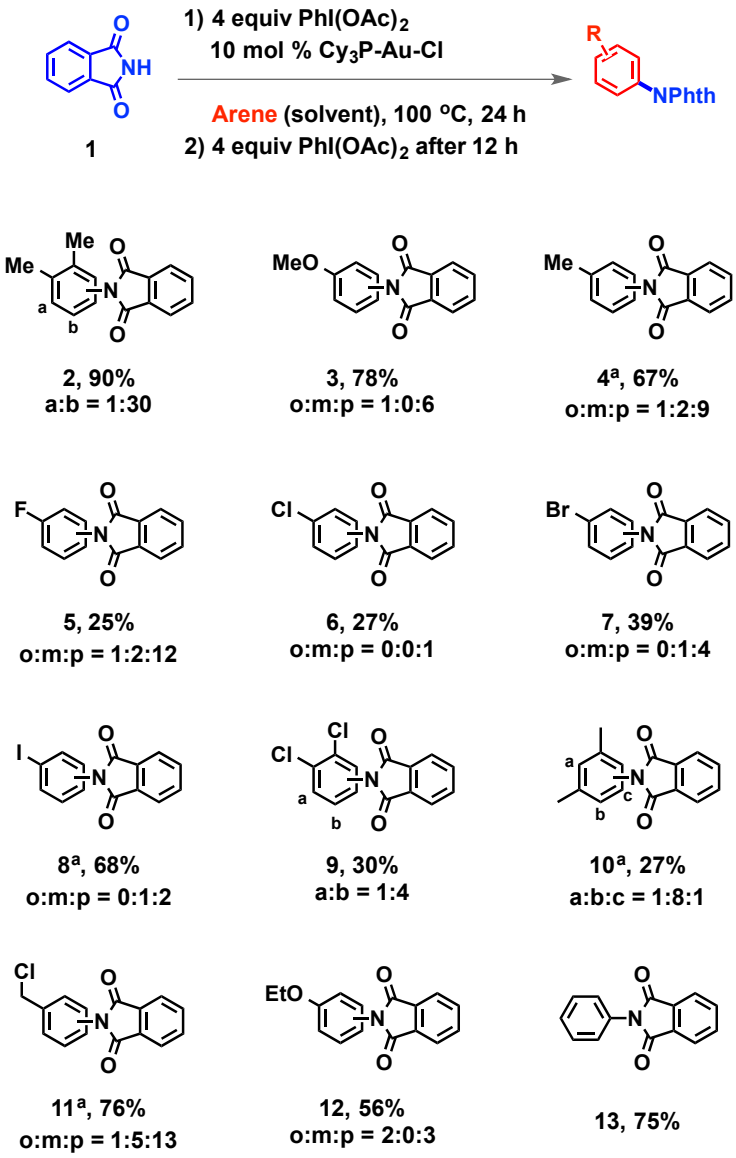
Table 2. Phosphine Ligand Synthesis

Subsequently, a variety of simple arenes with various functionalities were subjected to the optimized reaction conditions. These experiments indicated that our protocol appeared to be most amenable to electron-rich systems. As a result, our substrate scope illustrates the effects of the gold-catalyzed reaction on various halogenated arenes and arenes possessing electron-donating groups (EDG) (Table 3). The benefit of the gold catalyzed reaction lies in its significantly enhanced regioselectivity. While conducting a similar reaction with other transition metals, such as palladium, may allow for amination of more electron-deficient systems,[6] the gold-catalyzed reaction provides significantly enhanced regioselectivities favoring para-substituted isomers. We hypothesize that this is the result of an alternate mechanism that differs from our previously reported metal-free radical initiated pathway, and Hartwig's palladium-catalyzed CMD pathway.[5,6] The substitution patterns observed in the gold-catalyzed reaction appear to be governed by the same set of constraints observed in electrophilic aromatic substitution. Moreover, the predominant para-selectivity can be attributed to the large gold atom's preference to avoid positioning itself ortho to substituents.

Perhaps the most significant argument that can be made regarding whether or not the reaction is more heavily influenced by electronics or sterics is best illustrated by **10**. In the amination of *m*-xylene, a clear preference for amination to occur para with respect to either of the methyl groups is observed, rather than aminating at the less sterically encumbered position.

Minor meta-substituted products were also observed in reactions producing **4-8**, **10** and **11**, all of which are derived from less electron-rich arene substrates. The

exception to this rule is the amination of chlorobenzene (**6**), which surprisingly provided exclusive para-amination. We hypothesize that the meta-substituted products originate from a competing mechanism, the metal-free, radical-mediated reaction pathway. The meta-isomers are more often observed in less electron-rich systems, where electrophilic aromatic metalation (EAM) should be much slower. The inverse of this phenomenon is also illustrated in the reaction of the more electron-rich anisole substrate (**3**), which exhibits no meta-substitution, presumably because EAM is the dominant reaction pathway.

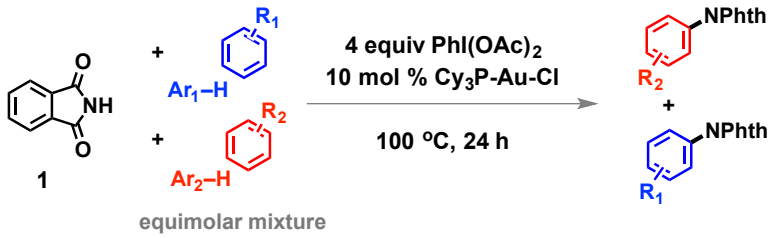


[a] Regioselectivities determined via GC/MS against standards.

Table 3. Arene Substrate Scope

Having successfully established the substrate scope, we sought to further elucidate the reaction mechanism. To do this we first probed the kinetic isotope effect by performing a competition reaction using an equimolar solution of benzene/benzene-d_6. A KIE value of 1.04 was obtained, which rules out the possibility

of a gold-mediated C-H activation contributing to the rate-determining step, and demonstrates that a CMD pathway is unlikely. In order to substantiate our claim that this reaction proceeds via EAM, additional internal competition reactions were performed (Table 4). By carrying out the amination procedure in an equimolar mixture of an electron-rich arene with a comparatively electron-deficient arene we observed that amination of the more electron-rich system was dramatically favored in both instances. These findings support the hypothesis that the observed regioselectivity patterns were likely the result of EAM and lead to the proposed mechanism detailed in Scheme 2.



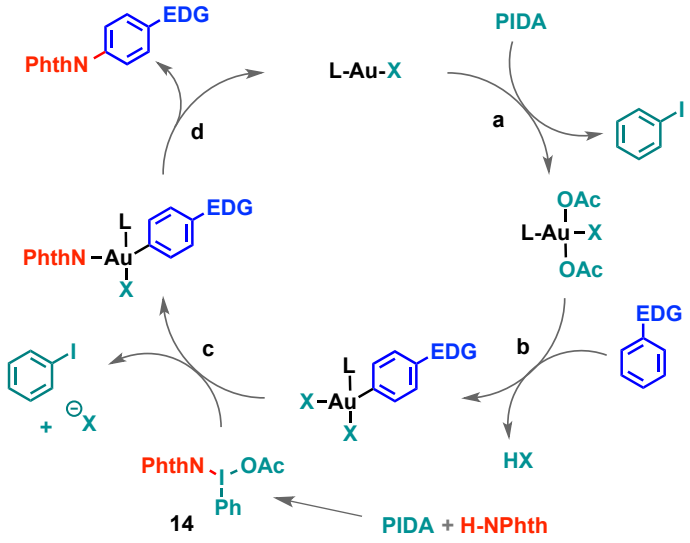
Ar_1-H	Ar_2-H	χ^a PhthN-Ar_1	χ^a PhthN-Ar_2
Anisole	Benzene	87	13
Fluorobenzene	Benzene	20	80

[a] Mole fractions determined by GC/MS

Table 4. Competition Reactions

The proposed Au(I)/Au(III) pathway is initiated by the oxidation of the Au(I) species to form Au(III) as the active catalyst. The para selectivity of this process is

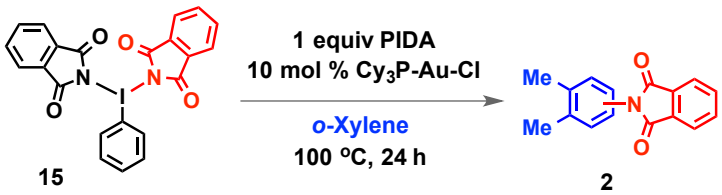
consistent with other Au-catalyzed halogenation, oxygenation and arylation reactions that have been previously reported.[3c-e] The Au(III) catalyst could then metalate either the ortho- or para-positions, with the para-position being presumably more favored. The metallated arene then proceeds to interact with an in situ-generated iodane species (**14**) via transmetalation. Once the imide reagent has been incorporated onto the gold species, the complex undergoes reductive elimination to afford the desired N-coupled product while regenerating the gold (I) catalyst.



(a) Oxidation, (b) EAM, (c) Transmetalation, (d) Reductive elimination. EDG = electron donating group, X = OAc or Cl, L = Cy_3P

Scheme 2. Proposed Reaction Mechanism

In order to determine if **14** was a plausible intermediate for this reaction, we synthesized the N,N-diphthalimidoiodane, **15**.[8] When this iodane was subjected to the reaction (instead of phthalimide), an isolated yield of 38% was observed with a regioselectivity comparable to the parent reaction (Scheme 3).



Scheme 3. Iodane-Mediated Amination

The success of this reaction indicates that transmetalation from a phthalimide-containing iodane intermediate is a viable reaction pathway. Our hypothesis for the formation of **14** is also supported by our observation of a moderate amount of the acetoxylated arene as a minor reaction product. We hypothesize that **15** allows for transfer of either the N- or O-ligand via transmetalation. Alternatively, a nucleophilic Au–arene species could directly attack the electrophilic nitrogen in **14** (not shown). Future studies will be directed towards isolating N,O-iodanes, like **14**, and studying their reactivities.

Conclusion

In conclusion, a regioselective gold-catalyzed protocol for the amination of arenes has been developed. As phthalimides can be easily converted into free amines, a direct route for regioselectively synthesizing aniline derivatives has been achieved. Future work will focus on mechanistic studies, the lowering of the arene concentration and the further enhancement of the regioselectivity by shutting off the uncatalyzed background reactions. The accomplishment of these aims will allow for the application of this reaction to the synthesis of a variety of high-value amine-containing compounds.

Acknowledgements

The authors thank URI's Chemistry Department Chairman William Euler for helpful discussions. This work was supported by the National Science Foundation (CAREER 0847222), and the National Institutes of Health (NIGMS, 1R15GM097708-01).

References

(1) For recent reviews and books describing oxidative C-H bond activation, see: (a) Lyons, T. W.; Sanford, M. S. *Chem. Rev.* **2010**, *110*, 1147. (b) Du Bois, J. *Org. Process Res. Dev.* **2011**, *15*, 758. (c) *From C–H to C–C Bonds: Cross-Dehydrogenative Coupling*; Li, C.-J., Ed. RSC Green Chemistry: London, 2014.

(2) For recent reviews of gold-catalyzed reactions, see: (a) Li, Z.; Brouwer, C.; He, C. *Chem. Rev.* **2008**, *108*, 3239; (b) Corma, A.; Leyva-Pérez, A.; Sabater, M. J. *Chem. Rev.* **2011**, *111*, 1657.

(3) For examples, see: (a) Skouta, R.; Li, C.-J. *Tetrahedron* **2008**, *64*, 4917, and references therein; (b) Li, Z.; Capretto, D. A.; Rahaman, R. O.; He, C. *J. Am. Chem. Soc.,* **2007**, *129*, 12058 (c) ; Mo, F.; Yan, J. M.; Qui, D.; Li, F.; Zhang, Y. *Angew. Chem. Int. Ed.* **2010**, *49*, 2028; (d) Pradal, A; Toullec, P. Y.; Michelet, V. *Org. Lett.* **2011**, *13*, 6086; (e) Ball, L. T.; Lloyd-Jones, G. C.; Russell, C. A. *Science* **2012**, *337*, 1644; (f) Kar, A.; Mangu, N.; Kaiser, H. M.; Tse, M. K. *J. Organomet. Chem.* **2009**, *694*, 524; (g) Kar, A.; Mangu, N.; Kaiser, H. M.; Beller, M.; Tse, M. K. *Chem. Commun.* **2008**, 386.

(4) For selected examples, see: (a) Mukherjee, P.; Widenhoefer, R. A. *Org. Lett.* **2010**, *12*, 1184; (b) Zhang, G.; Cui, L.; Wang, Y.; Zhang, L. *J. Am. Chem. Soc.* **2010**, *132*, 1474; (c) C. Brouwer, C. He, *Angew. Chem. Int. Ed.*, **2006**, *45*, 1744.

(5) The following papers simultaneously reported metal-free C–N bond formation: (a) Kantak, A.; Potavathri, S.; Barham, R. A. Romano, K.; DeBoef, B. *J. Am. Chem. Soc.* **2011**, *133*, 19960; (b) Antonchick, A. P.; Samanta, R.; Kulikov, K.; Lategahn, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 8605; (c) Kim, H. J.; Kim, J.; Cho, S. H.; Chang, S. J. Am. Chem. Soc. **2011**, *133*, 16382.

(6) Shrestha, R.; Mukherjee, P.; Tan, Y.; Litman, Z. C.; Hartwig, J. *J. Am. Chem. Soc.* **2013**, *135*, 8480.

(7) Wang, M.-Z.; Wong, M.-K.; Che, C.-M. *Chem. Eur. J.* **2008**, *14*, 8353.

(8) Hadjiarapoglou, L.; Spyroudis, S.; Varvoglis, A. *Synthesis* **1983**. *3*, 207.

Experimental Section

Reagents

Substrates, including chloro(tricyclohexylphosphine)gold(I), phthalimide, and all arenes, were purchased from Sigma-Aldrich or Fisher Scientific. Iodobenzene diacetate was purchased from Acros Chemicals. Flash chromatography was performed using a Teledyne-Isco CombiFlash Rf with Redisep Gold silica cartridges or using a Biotage Isolera with SNAP Ultra silica cartridges. All reagents were stored under an inert atmosphere before use.

Instrumentation

GC/MS analyses were performed on an Agilent Technologies 6890 GC system with a 5973 mass selective detector. NMR spectra were obtained using a Bruker Avance 300 MHz spectrometer and a Varian Inova 500 MHz spectrometer.

General Reaction Conditions

A solution of phthalimide (15 mg, 0.102 mmol), iodobenzene diacetate (0.408 mmol), and chloro(tricyclohexylphosphine)gold(I) (0.01 mmol) in 3 mL of arene was assembled in a nitrogen-filled glove box. The reaction vial was then heated on an aluminum well-plate and allowed to magnetically stir for 12 hours at 100 °C. After 12 hours, the reaction was allowed to cool to room temperature and was brought back into the nitrogen-filled glove box where an additional 0.408 mmol of iodobenzene

diacetate was added to the reaction vessel. The reaction was then allowed to stir for an additional 12 hours at 100 °C. Excess solvent was removed at reduced pressure and the crude reaction mixture was purified via flash chromatography with ethyl acetate and hexanes. The purified compound was allowed to dry in a preweighed vial overnight under high vacuum in order to determine product yield. Regioselectivities were assessed by NMR and GC/MS. In instances where NMR did not allow for definitive product identification, regioselectivities were determined via GC/MS against a set of standards.

Substrates 4, 8, 10, and 11

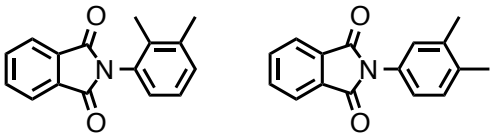
Regioselectivities were determined via comparison of GCMS retention times against independently synthesized standards as a result of overlapping signals in ^{1}H NMR. Regioselectivity was obtained by integrating the area under each product's respective signal in order to obtain an isomeric ratio.

Oxidant and Ligand Screens (Table 1 and Table 2)

Percent yield was calculated as a function of conversion of the starting material (phthalimide) into the sum of the resulting aryl coupled products (i.e. 1 - % phthalimide remaining). Analysis of phthalimide fragmentation patterns in the GC/MS suggests that the aryl-coupled product is the only phthalimide-containing species produced throughout the course of this reaction. Standard reaction conditions also

proved mild enough that isolation of unreacted starting material may be achieved leading us to conclude that minimal, if any, decomposition of the starting material occurred. This was also corroborated by the high isolated yield of **2** (90%), which is in line with the calculated GC yield (97%).

Amination of *o*-Xylene (2)[1]



2a **2b**

2a:2b = 1:30

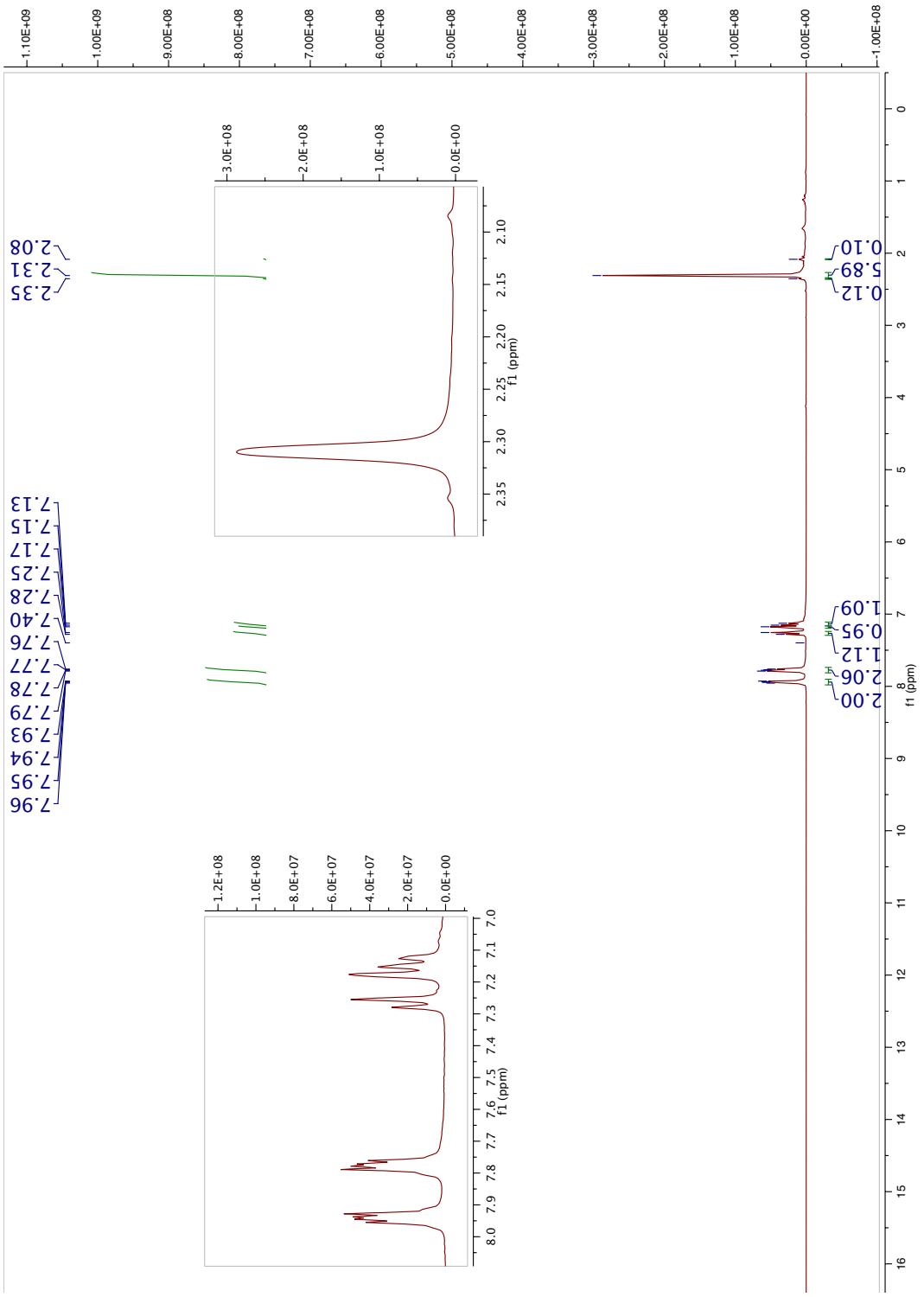
^{1}H NMR (300 MHz, $CDCl_3$):

2a: δ 2.08 (s, 3H), 2.35 (s, 3H)

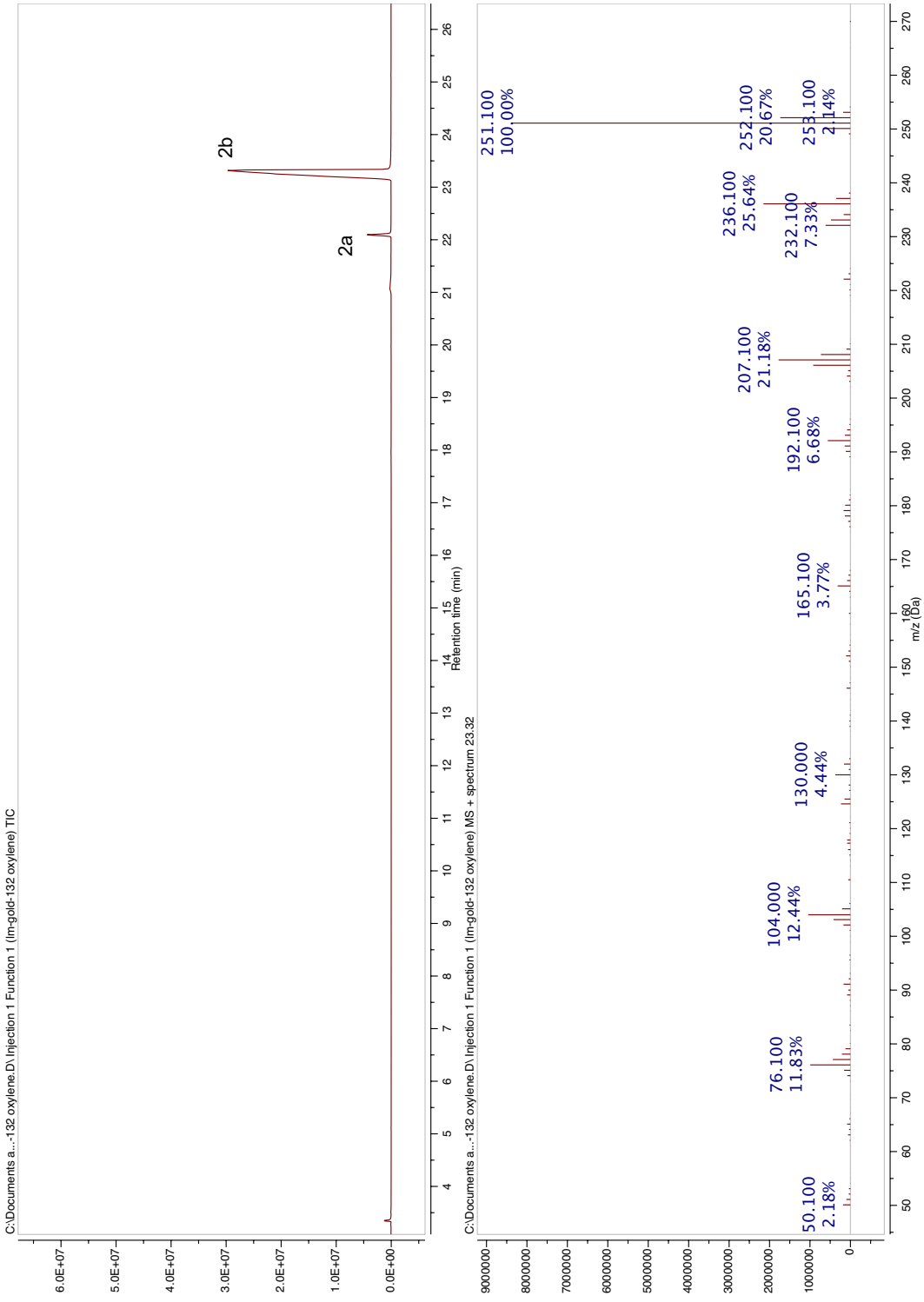
2b: δ 7.94 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.77 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.17 (s, 1H) 7.14 (d, *J* = 8.1 Hz, 1H), 2.31 (s, 6H)

*Regioselectivity determined by comparison of peaks at 2.08 ppm and 2.31 ppm

MS (m/z): calculated for $C_{16}H_{13}NO_2$ = 251.095, observed 251.100

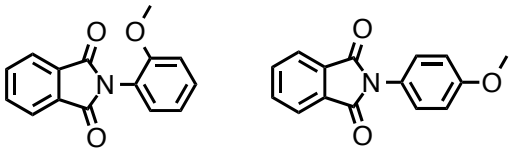


Spectrum 1: ^{1}H NMR of **2**



Spectrum 1.1: GC/MS of **2**

Figure 2: Amination of Anisole (3)[1,2]



3a **3b**

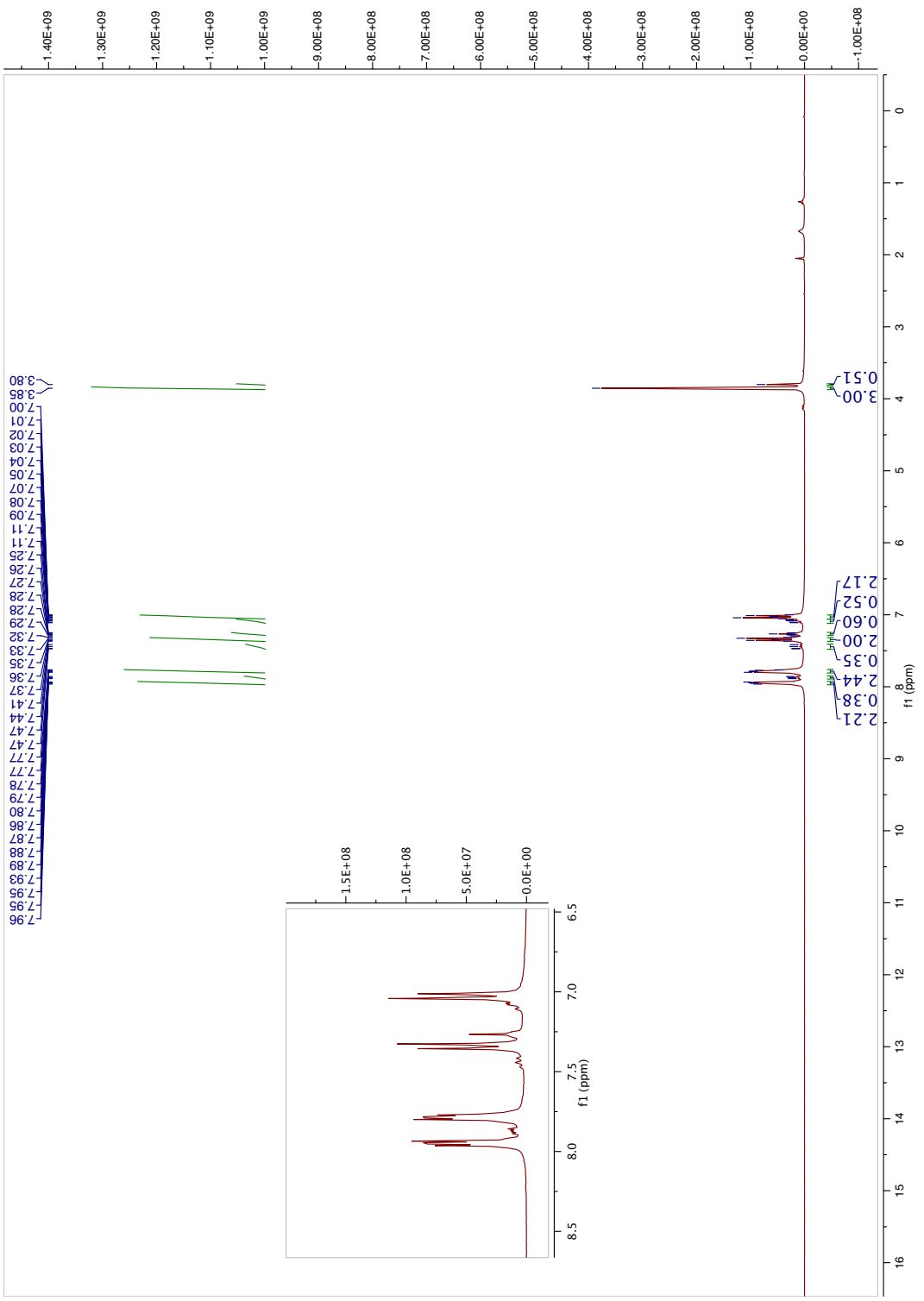
3a:3b = 1:6

^{1}H NMR (300 MHz, $CDCl_3$):

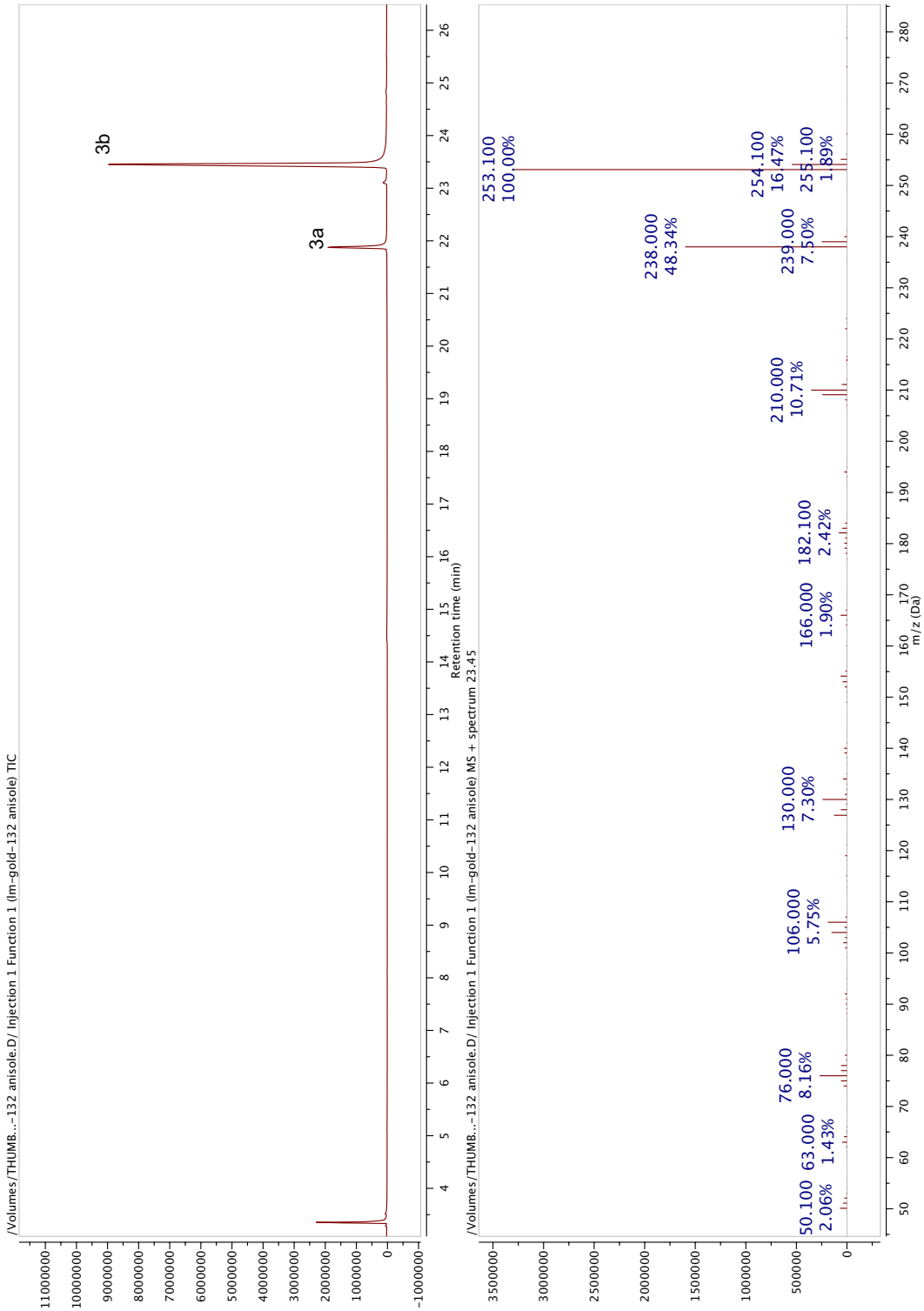
3a: δ 7.87 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.41 – 7.47 (m, 1H), 7.25 - 7.18 (m, 1H), 7.07 – 7.11 (m, 1H), 3.80 (s, 1H).

3b: δ 7.95 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.78 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.34 (d, *J* = 9.2 Hz, 2H), 7.03 (d, *J* = 9.2 Hz, 2H), 3.85 (s, 3H).

MS (m/z): calculated for $C_{15}H_{11}NO_2$ = 253.074, observed 253.100

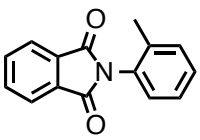


Spectrum 2: ^{1}H NMR of **3**

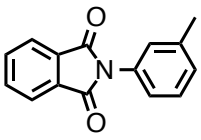


Spectrum 2.1: GC/MS of **3**

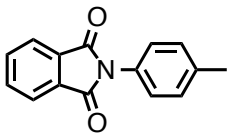
Amination of Toluene (4)[1]



4a



4b



4c

4a:4b:4c = 1:2:9

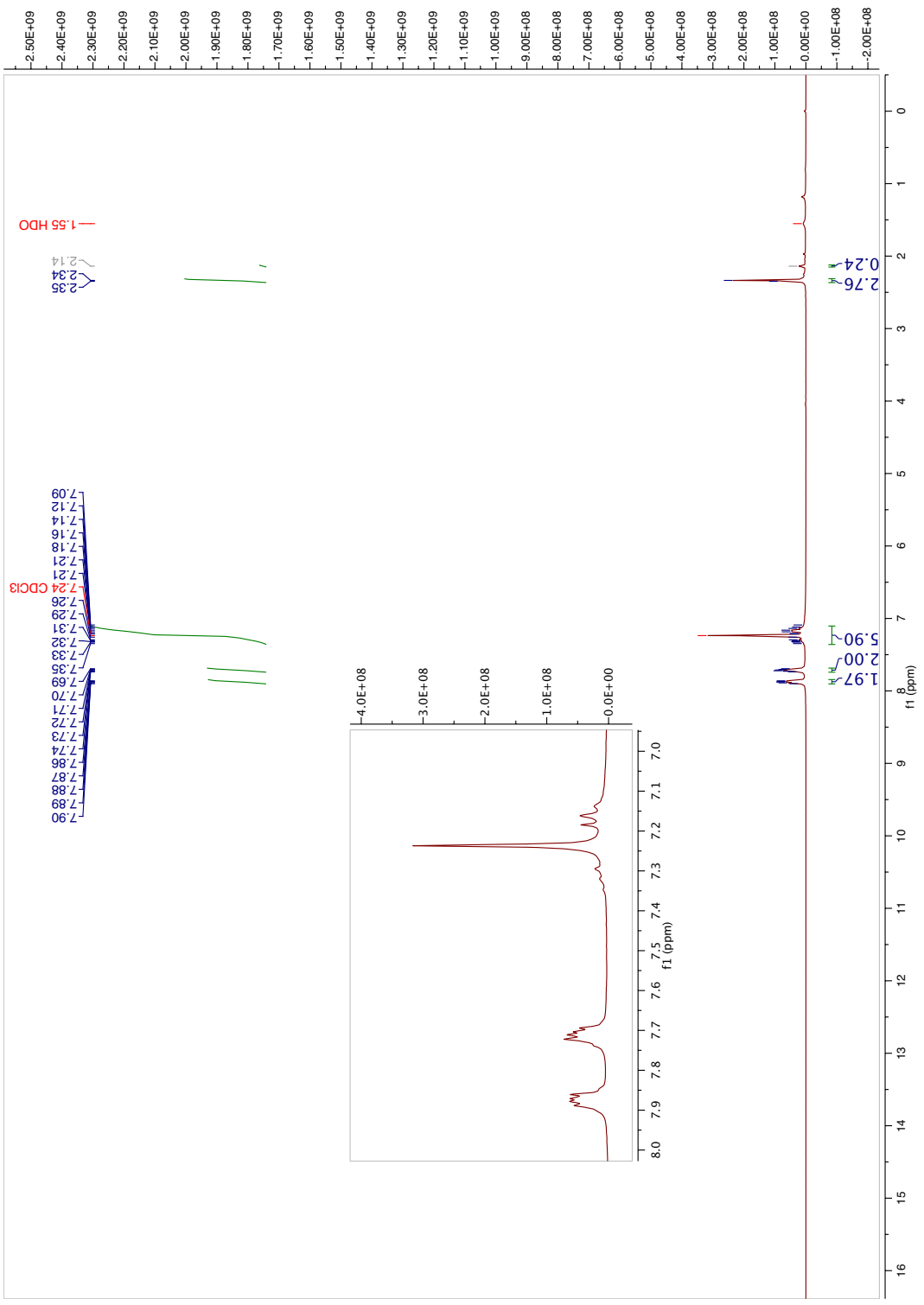
^{1}H NMR (300 MHz, $CDCl_3$):

4a: δ 7.24 (m, 6H), 2.14 (s, 3H)

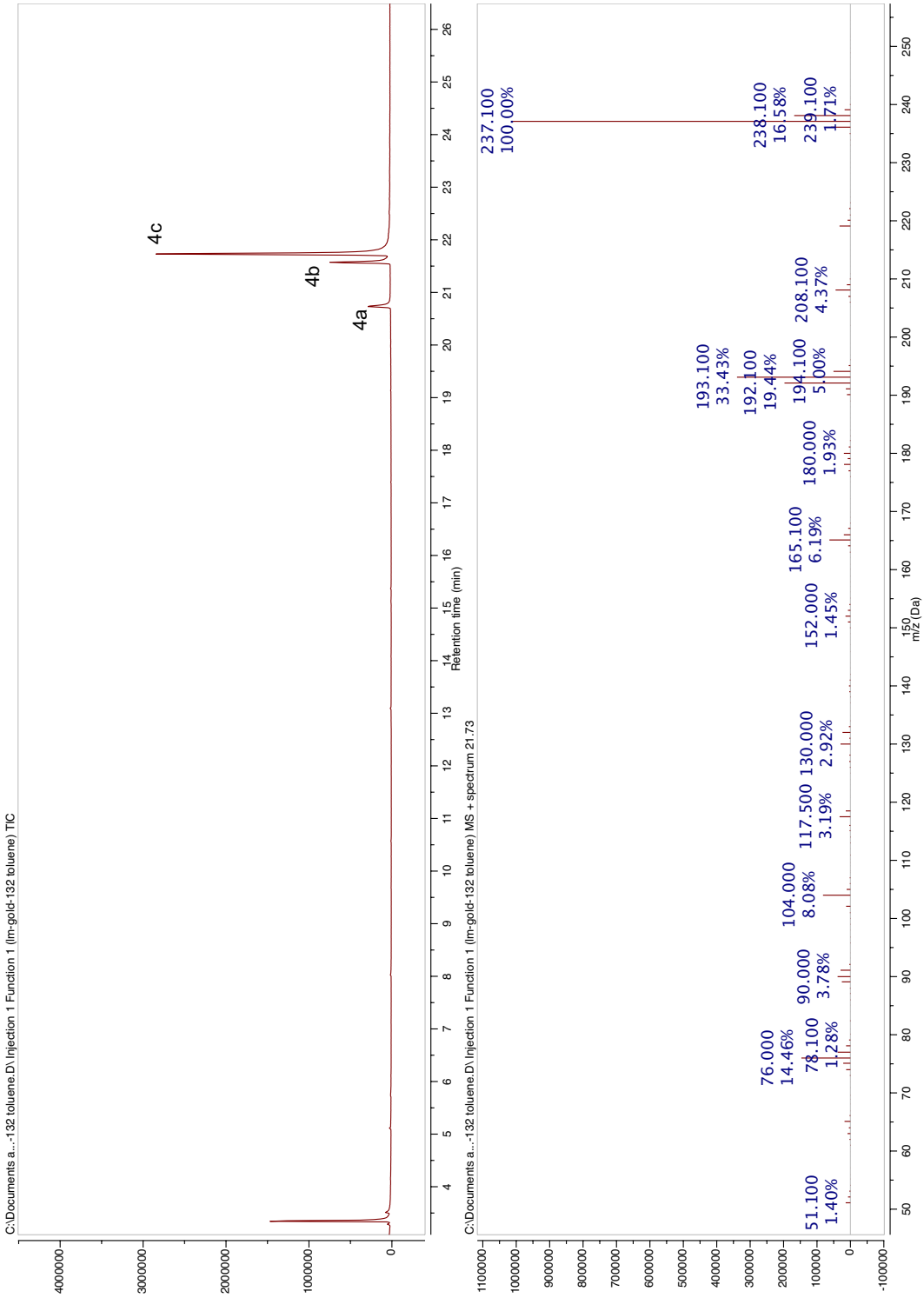
4b: δ 7.24 (m, 6H), 2.35 (s, 3H)

4c: δ 7.85 – 7.90 (m, 2H), 7.69 – 7.74 (m 2H), 7.24 (m, 6H), 2.34 (s, 3H),

MS (m/z): calculated for $C_{15}H_{11}NO_2$ = 237.079, observed 237.100

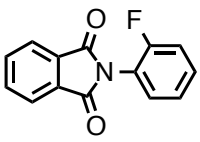


Spectrum 3: ^{1}H NMR of **4**

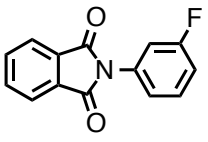


Spectrum 3.1: GC/MS of **4**

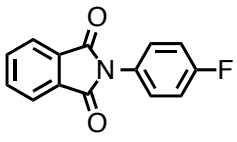
Amination of Fluorobenzene (5)[1]



5a



5b



5c

5a:5b:5c = 1:2:12

^{1}H NMR (300 MHz, $CDCl_3$):

δ 7.96 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.80 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.43 (dd, *J* = 8.5, 4.6 Hz, 2H), 7.17 (t, *J* = 8.4 Hz, 2H).

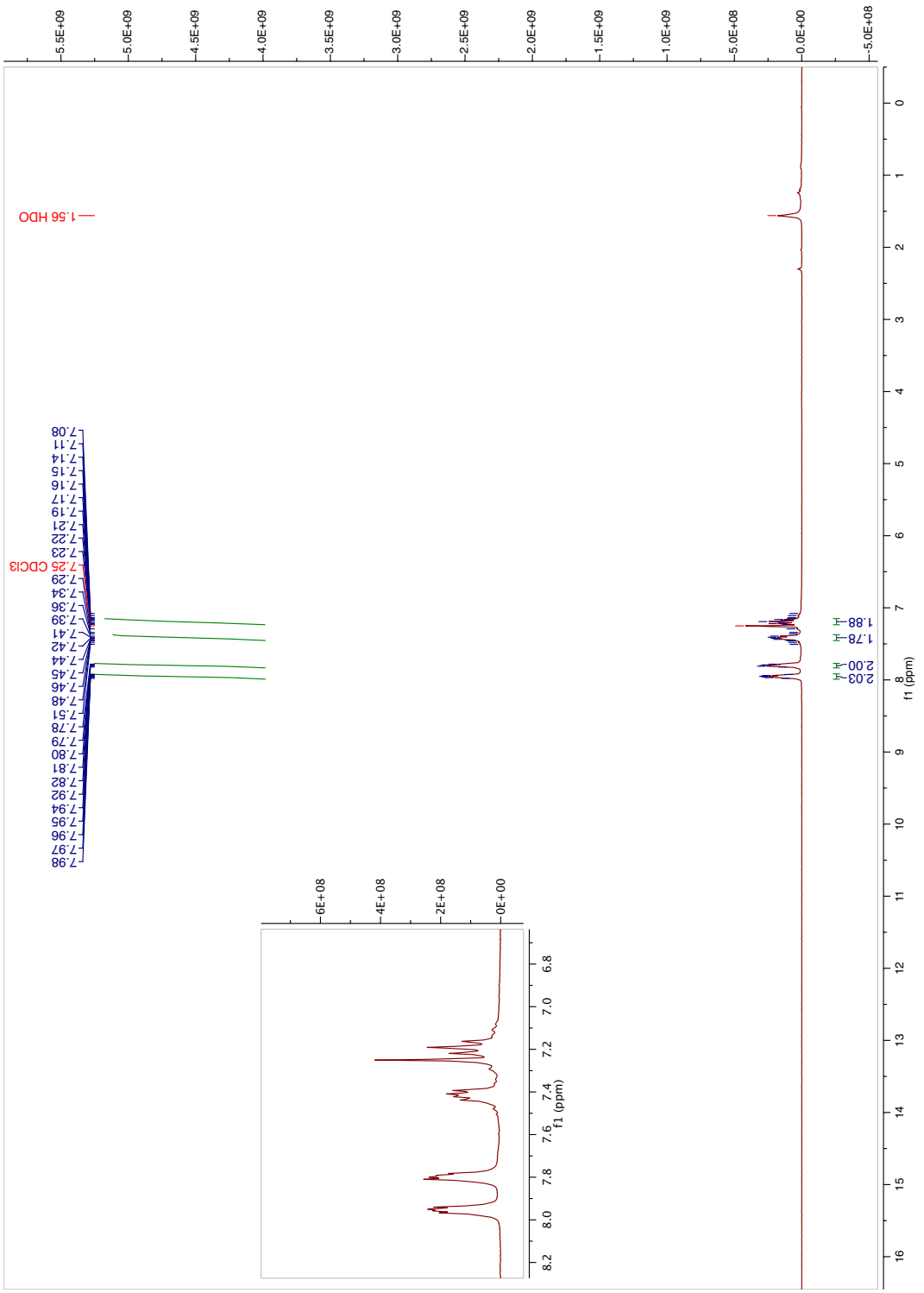
^{19}F NMR (282 MHz, $CDCl_3$):

5a: δ -118.70 (d, *J* = 10.1 Hz)

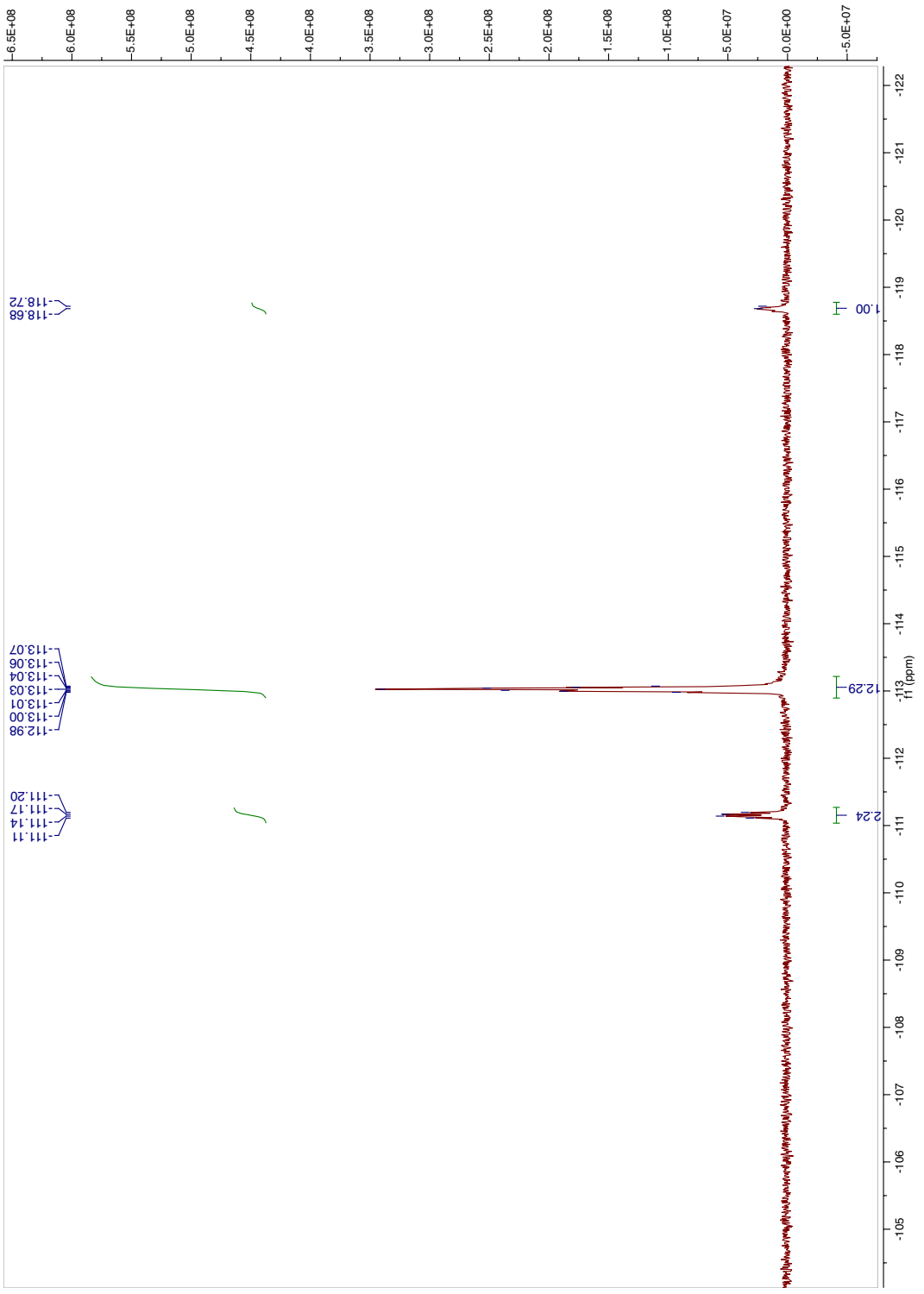
5b: δ -113.03 (tt, *J* = 8.3, 4.7 Hz)

5c: δ -111.15 (q, *J* = 8.2 Hz)

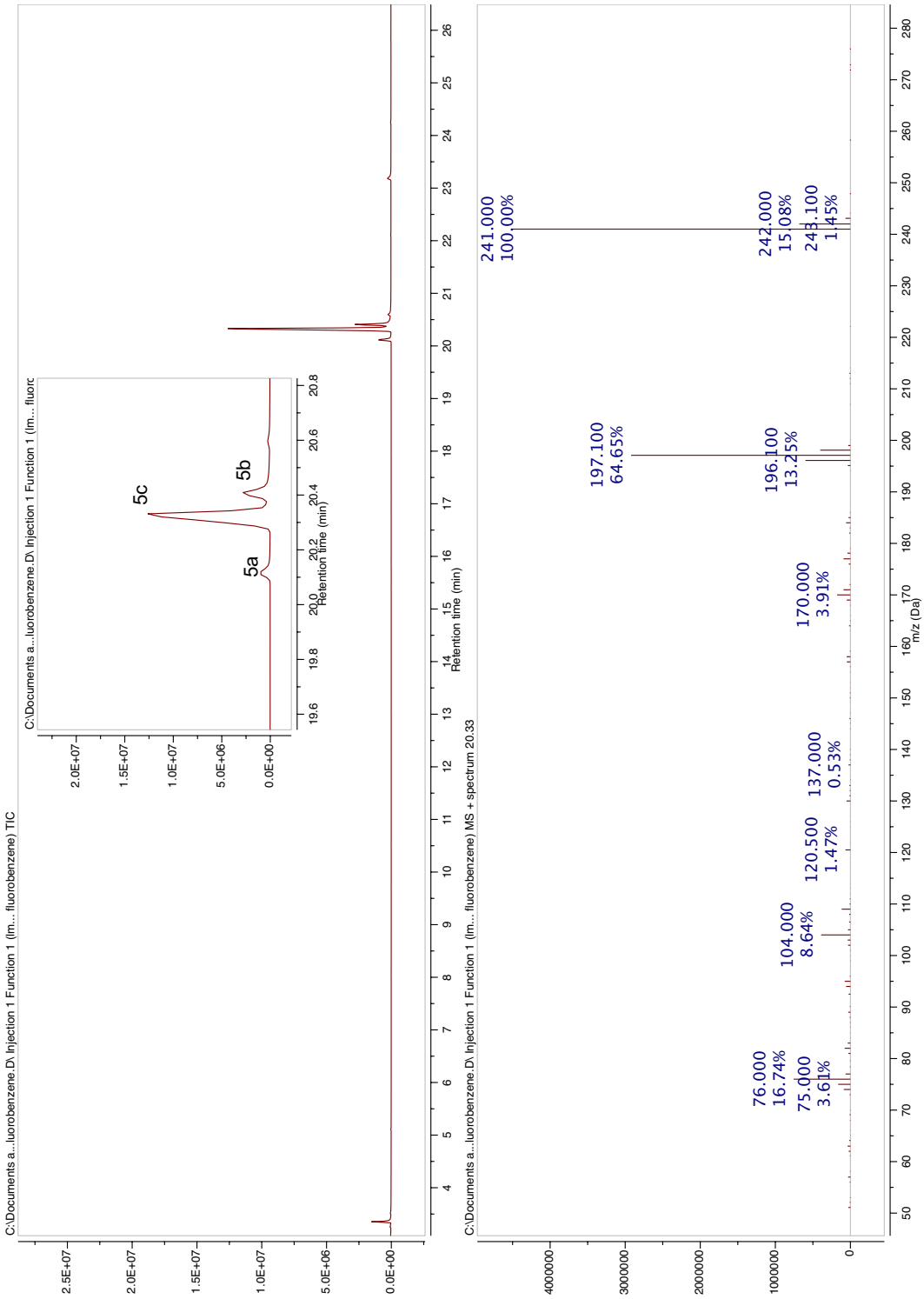
MS (m/z): calculated for $C_{14}H_8FNO_2$ = 241.054, observed 241.000



Spectrum 4: ^{1}H NMR of **5**

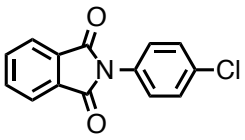


Spectrum 4.1: ^{13}C NMR of **5**



Spectrum 4.2: GC/MS of **5**

Amination of Chlorobenzene (6)[1]

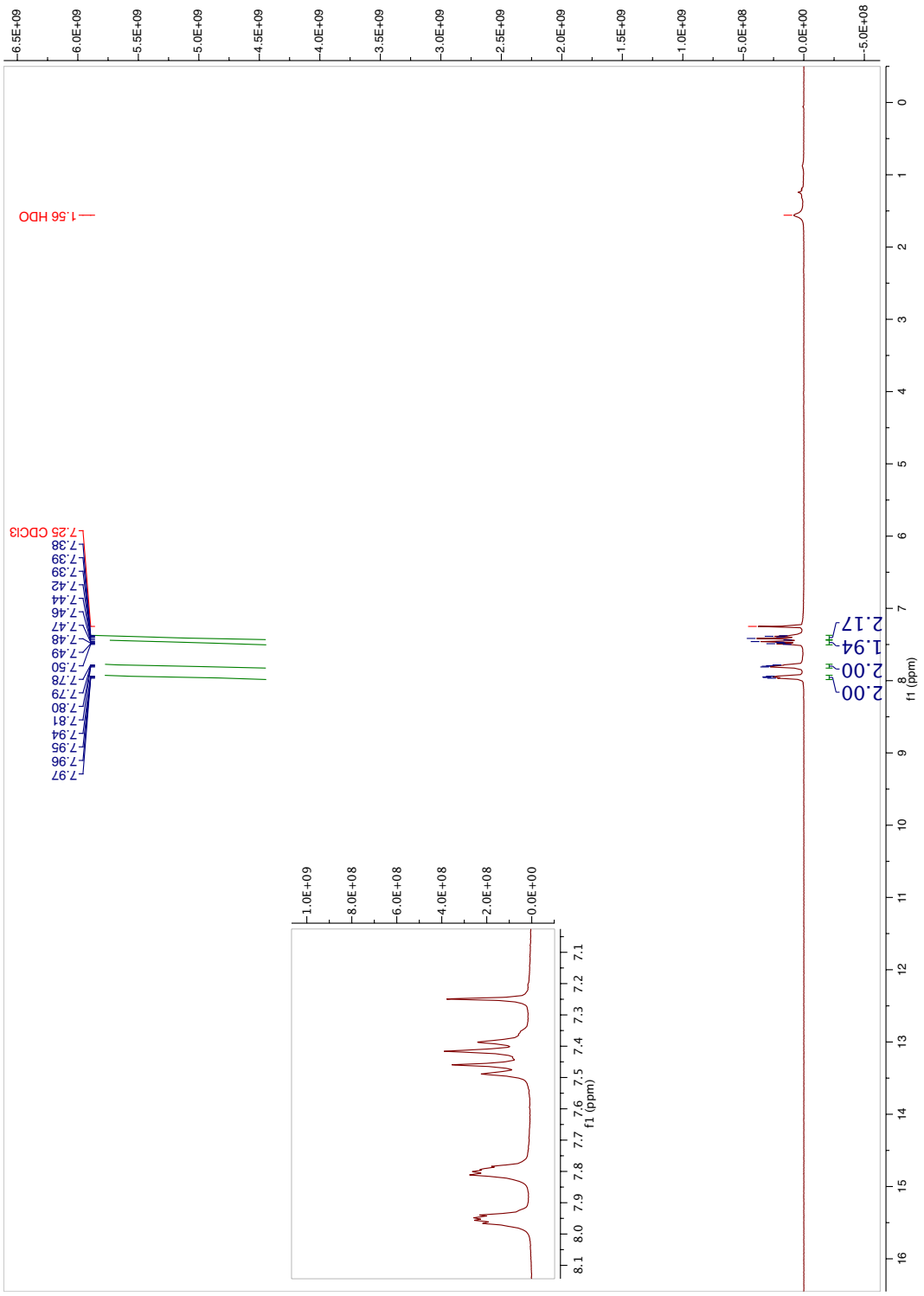


6

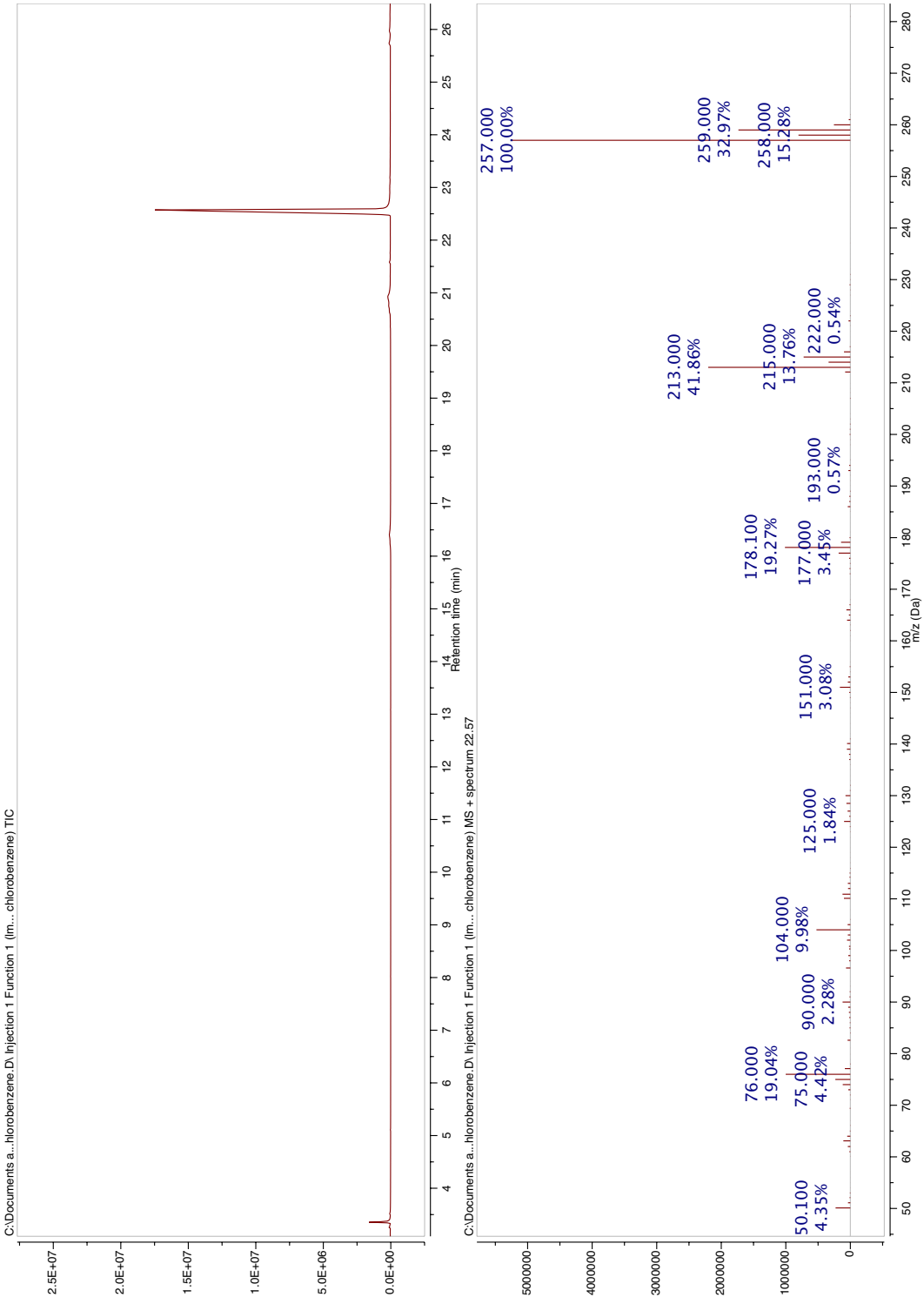
^{1}H NMR (300 MHz, $CDCl_3$):

6: δ 7.95 (dd, *J* = 5.4, 2.9 Hz, 2H), 7.80 (dd, *J* = 5.4, 2.9 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H).

MS (m/z): calculated for $C_{14}H_8ClNO_2$ = 257.024, observed 257.000

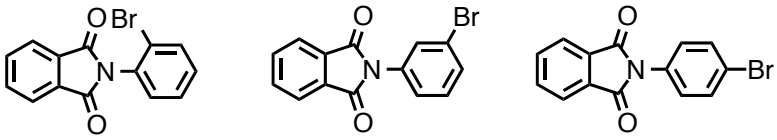


Spectrum 5: ^{1}H NMR of **6**



Spectrum 5.1: GC/MS of **6**

Amination of Bromobenzene (7)[1*]



7a **7b** **7c**

7a:7b:7c = 0:1:4

Isolated:

^{1}H NMR (300 MHz, $CDCl_3$):

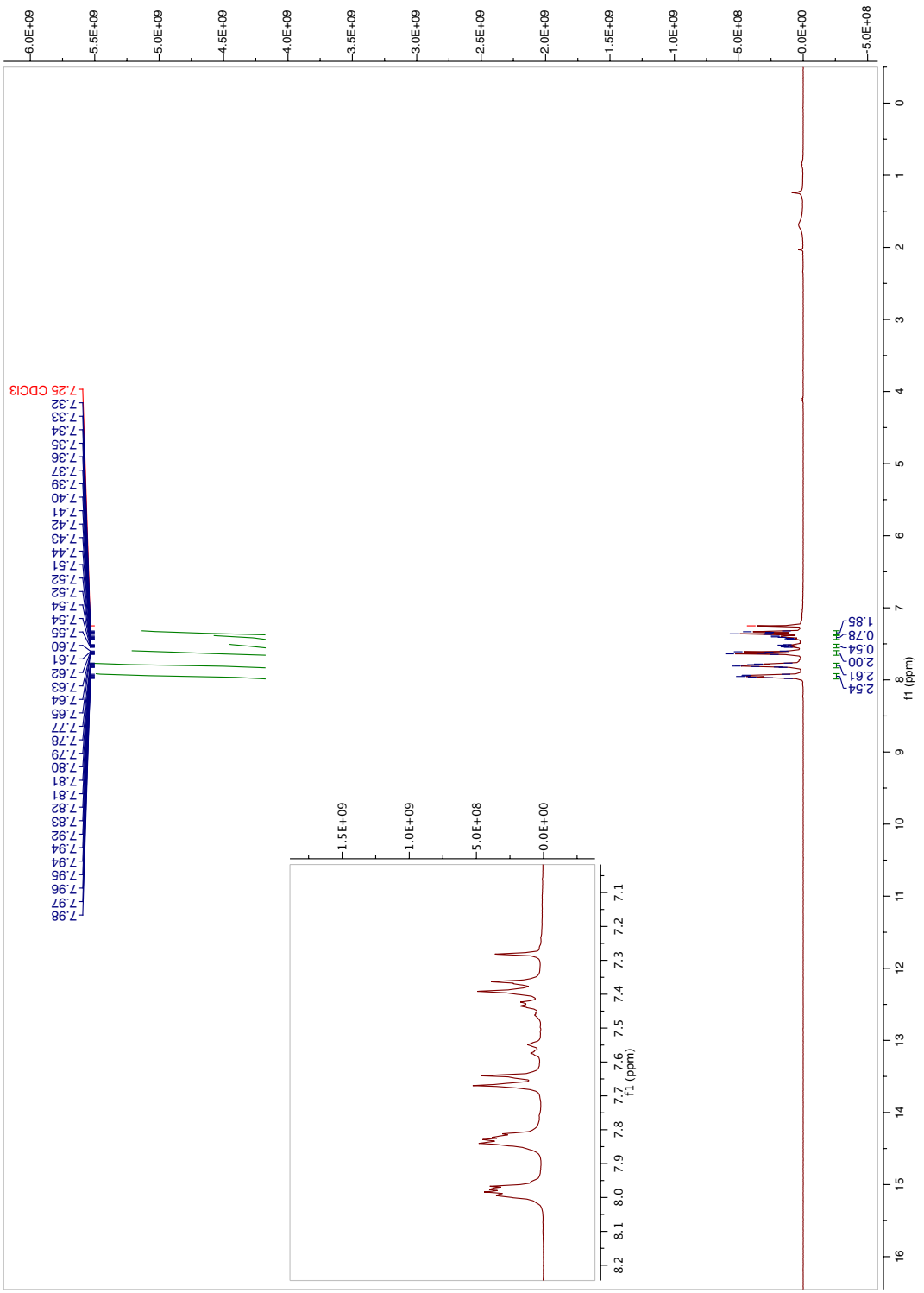
7a: Observed by GC-MS but is not detected by NMR

7b: δ 7.53 (m, 2 H), 7.40 (m, 2H)

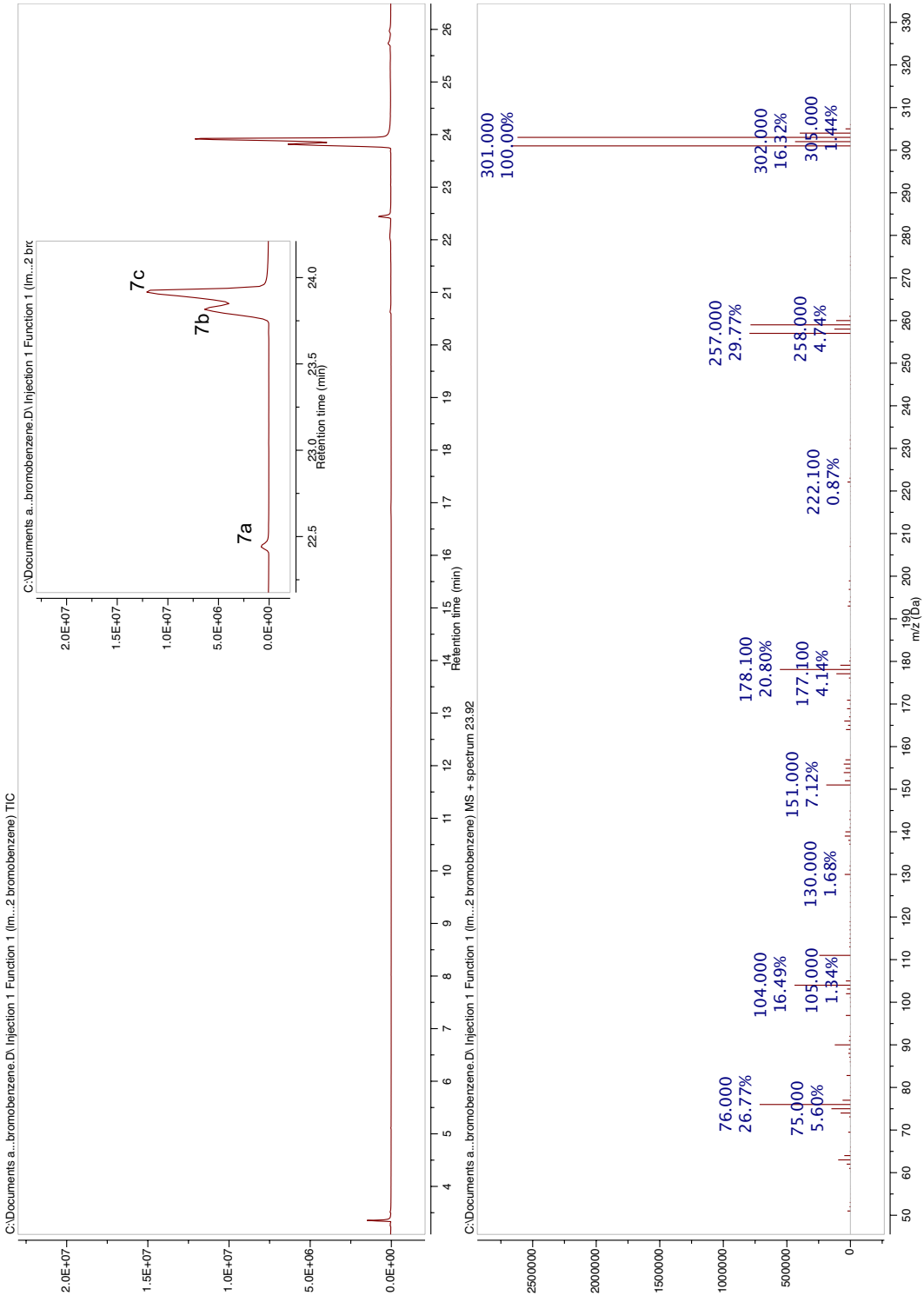
7c: δ 7.95 (m, 2H), 7.80 (m, 2H), 7.63 (dt, *J* = 8.9 Hz, 4.3 Hz, 2H), 7.35 (dt, 8.9 Hz, 4.1 Hz, 2H)

MS (m/z): calculated for $C_{14}H_8BrNO_2$ = 300.974, observed 301.000

*Reference corresponds to major isomer

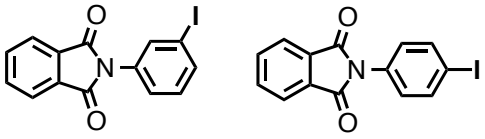


Spectrum 6: ^{1}H NMR of **7**



Spectrum 6.1: GC/MS of **7**

Amination of Iodobenzene (8)



8a **8b**

8a:8b = 1:2

^{1}H NMR (300 MHz, $CDCl_3$):

8a (distinguishable): δ 7.74 (dd, *J* = 8.1 Hz, 1.3 Hz, 1H), 7.45 (dd, *J* = 8.1 Hz, 1.3 Hz, 1H)

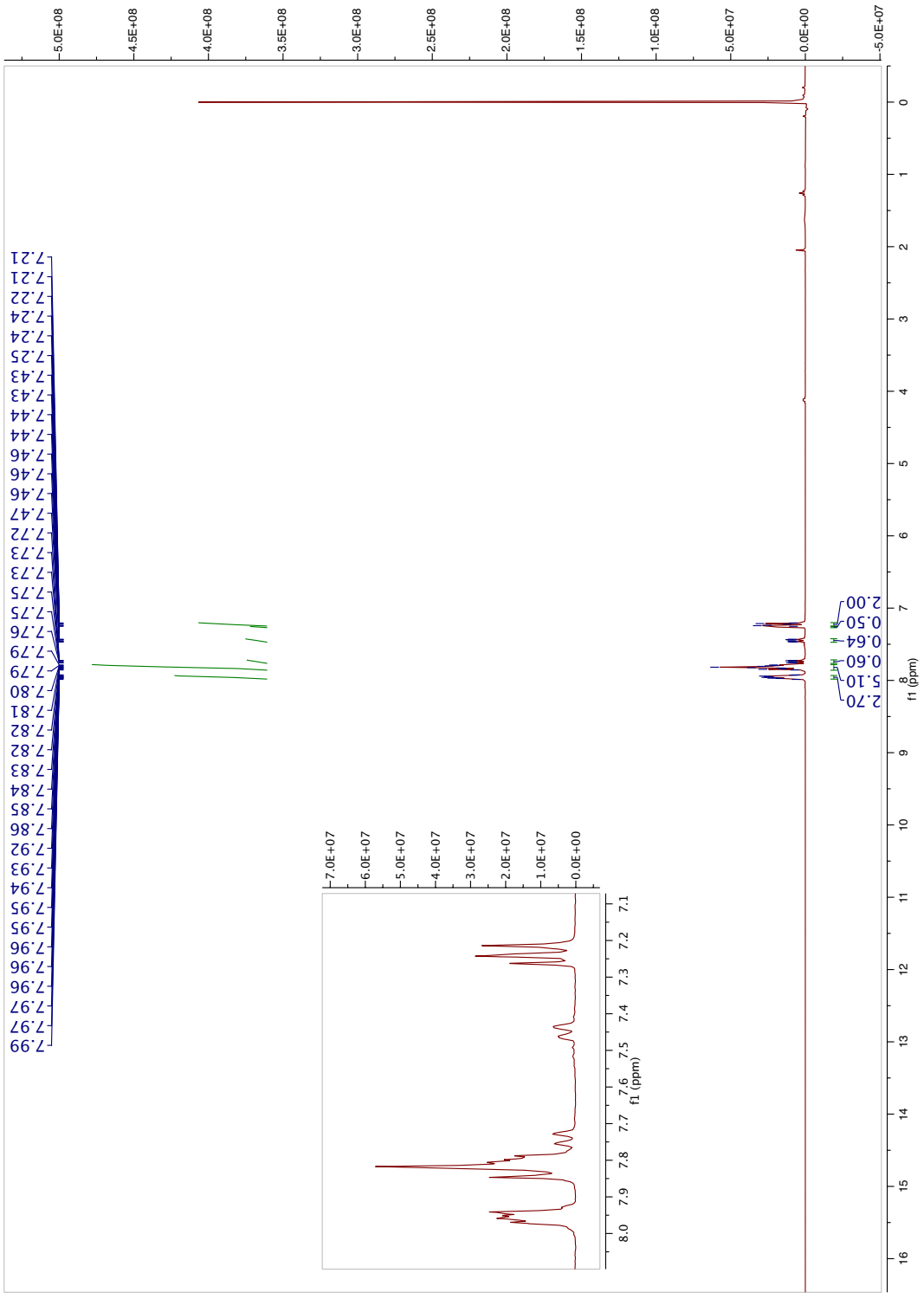
8b (distinguishable): δ 7.21 – 7.24 (d, *J* = 8.7 Hz, 2H)

Not Classifiable: δ 7.92 – 7.99 (m, 3H), 7.79 – 7.86 (m, 5H)

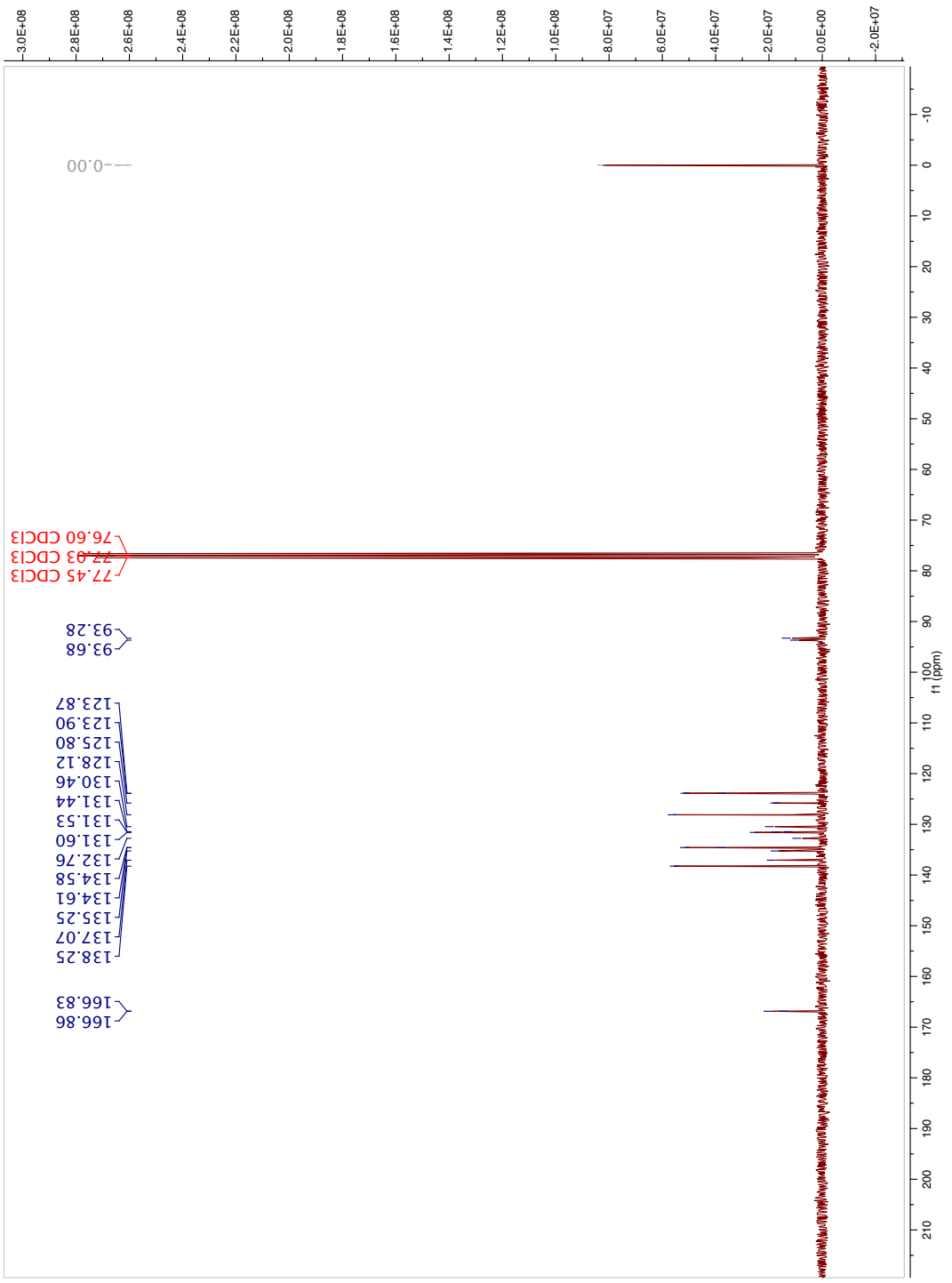
^{13}C NMR (75 MHz, $CDCl_3$):

δ 166.86, 166.83, 138.25, 137.07, 135.25, 134.61, 134.58, 132.76, 131.60, 131.53, 131.44, 130.46, 128.12, 125.80, 123.90, 123.87, 93.68, 93.28

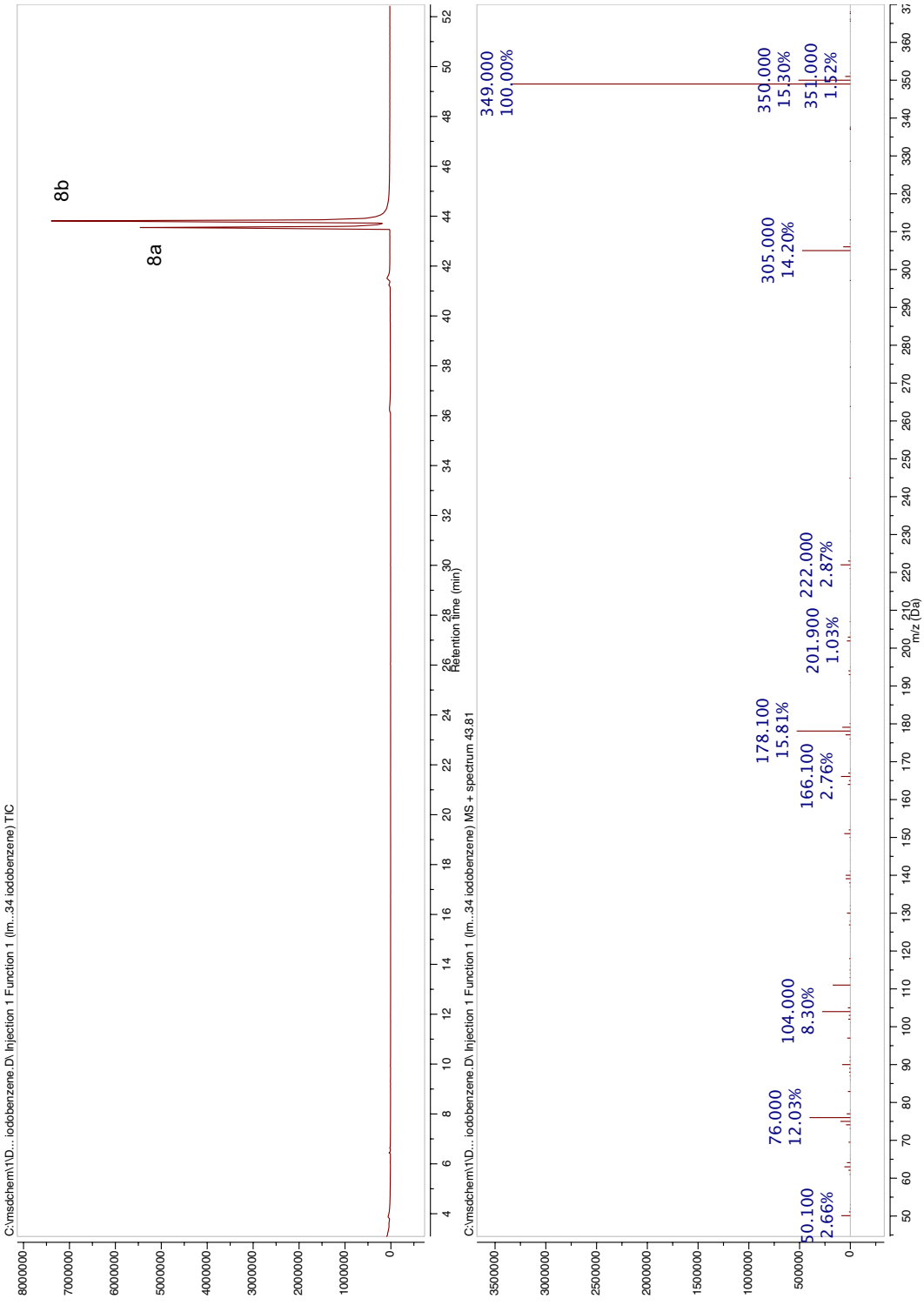
MS (m/z): calculated for $C_{14}H_8INO_2$ = 349.127, observed 349.000



Spectrum 7: ^{1}H NMR of **8**

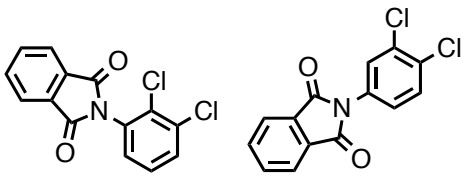


Spectrum 7.1: ^{13}C NMR of **8**



Spectrum 7.2: GC/MS of **8**

Amination of Dichlorobenzene (9)[1]



9a **9b**

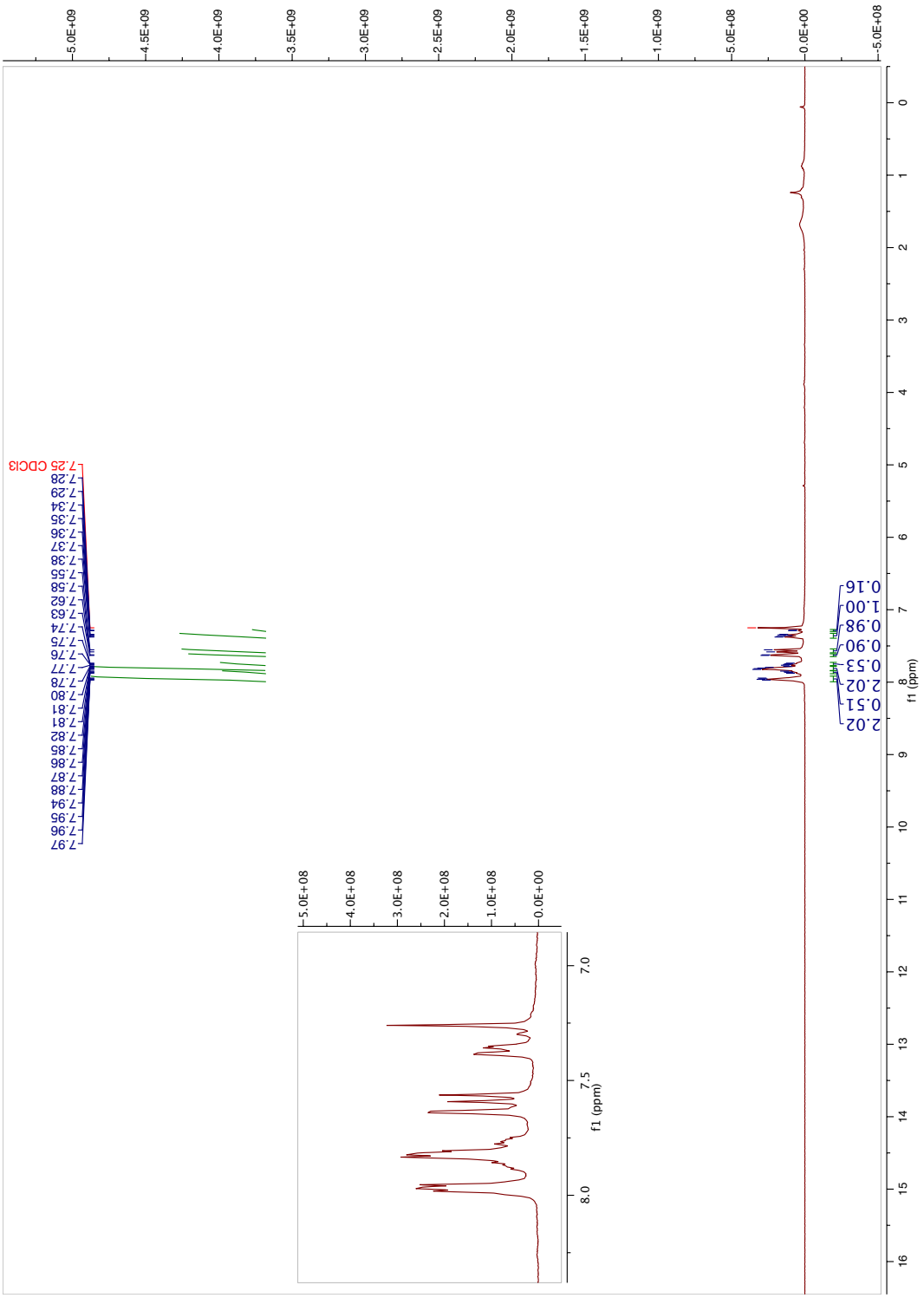
9a:9b = 1:4

^{1}H NMR (300 MHz, CDCl3):

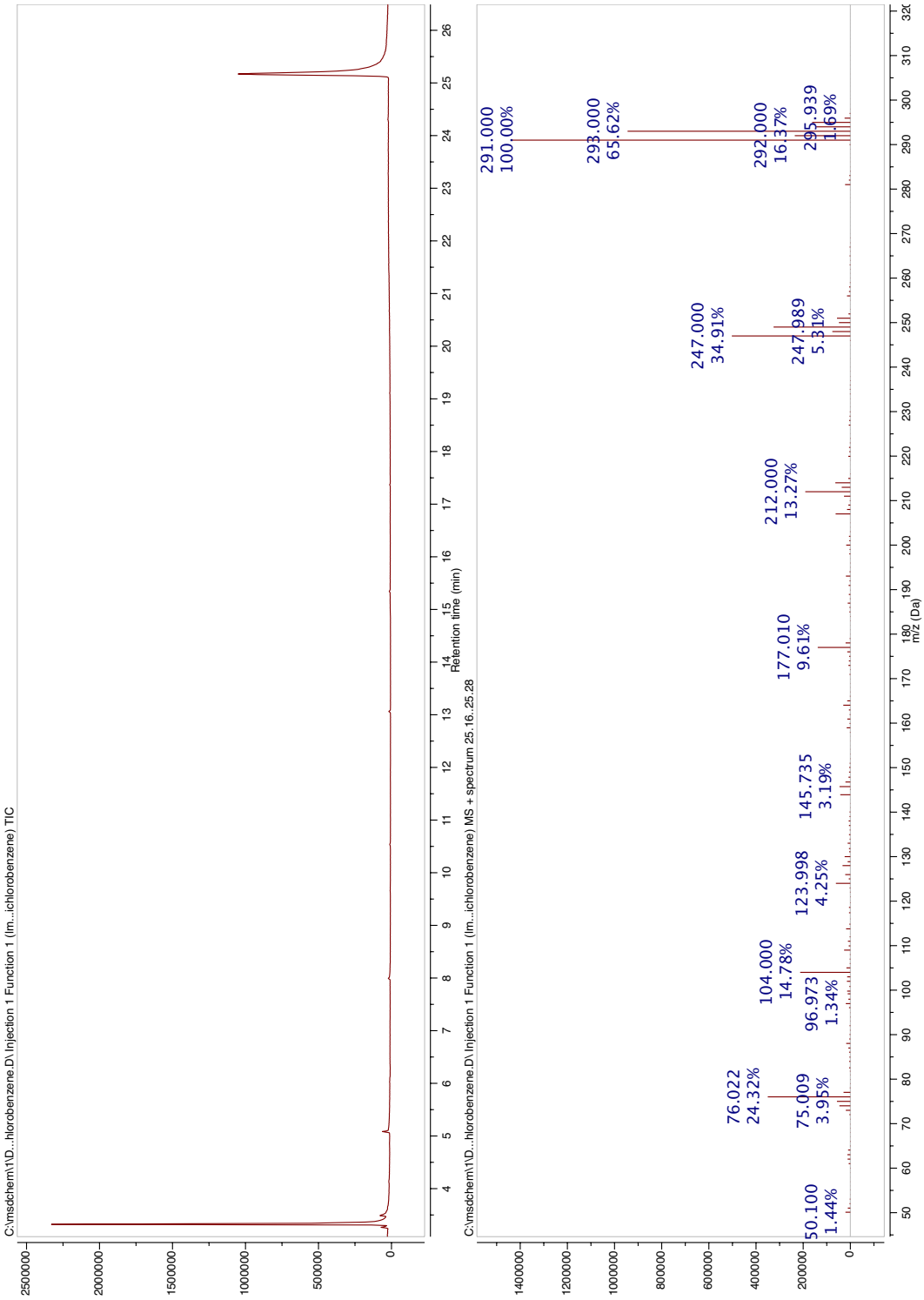
9a: δ 7.86 (dd, *J* = 5.6, 3.2 Hz 2H), 7.76 (dd, *J* = 5.6, 3.1 Hz, 2H), 7.28 – 7.29 (m 1H).

9b: δ 7.97 (dd, *J* = 5.3, 3.1 Hz, 2H), 7.81 (dd, *J* = 5.5, 3.1 Hz, 2H), , 7.63 (d, *J* = 2.4 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 1H), 7.37 (dd, *J* = 8.6, 2.5 Hz, 1H),

MS (m/z): calculated for $C_{14}H_7Cl_2NO_2$ = 290.985, observed 291.000

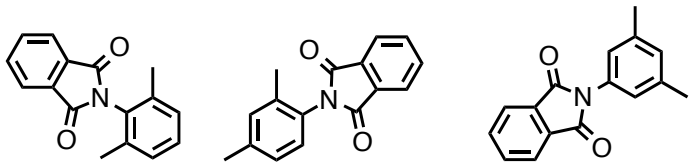


Spectrum 8: ^{1}H NMR of **9**



Spectrum 8.1: GC/MS of **9**

Amination of m-Xylene (10)[3]



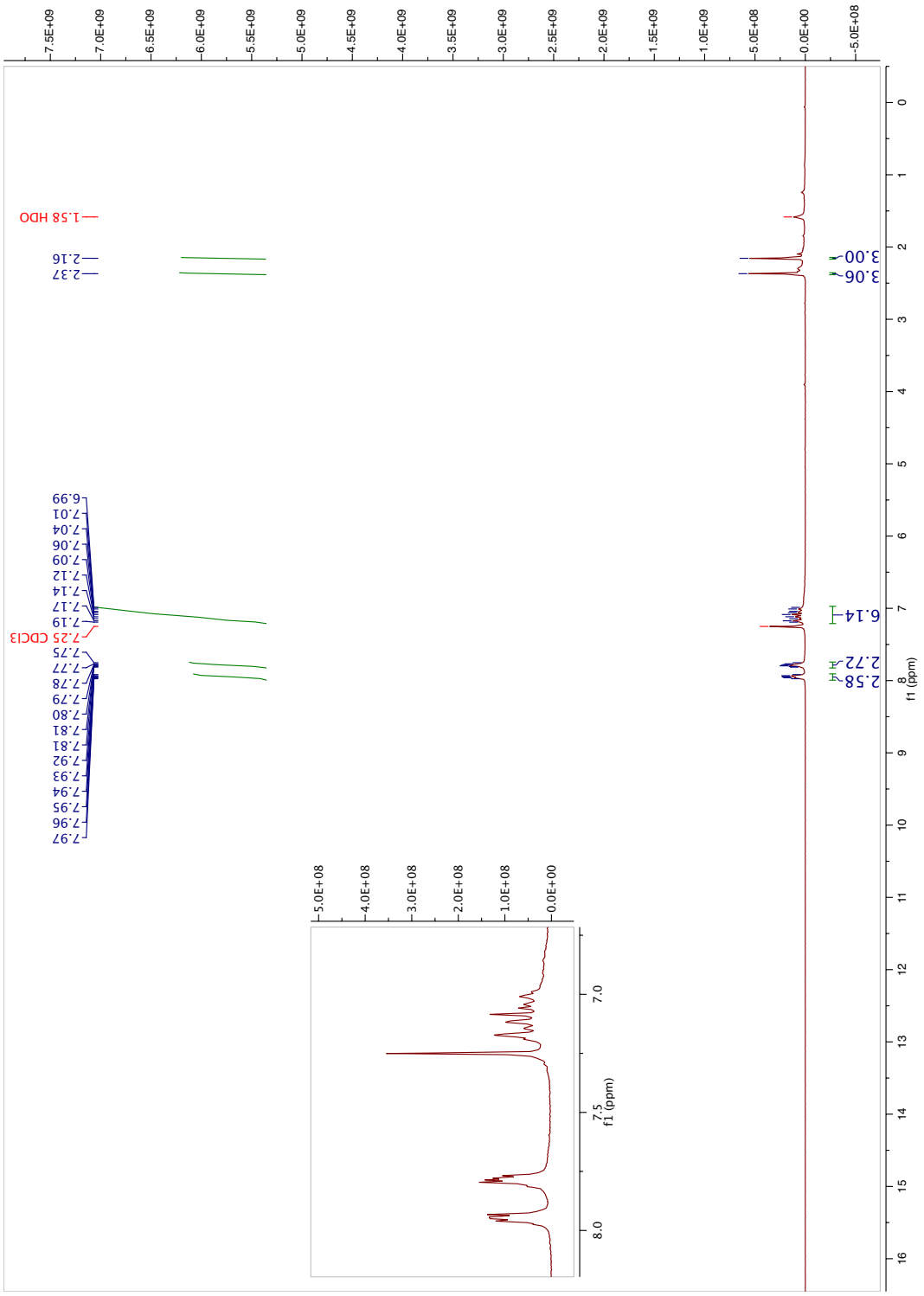
10a **10b** **10c**

10a:10b:10c = 1:8:1

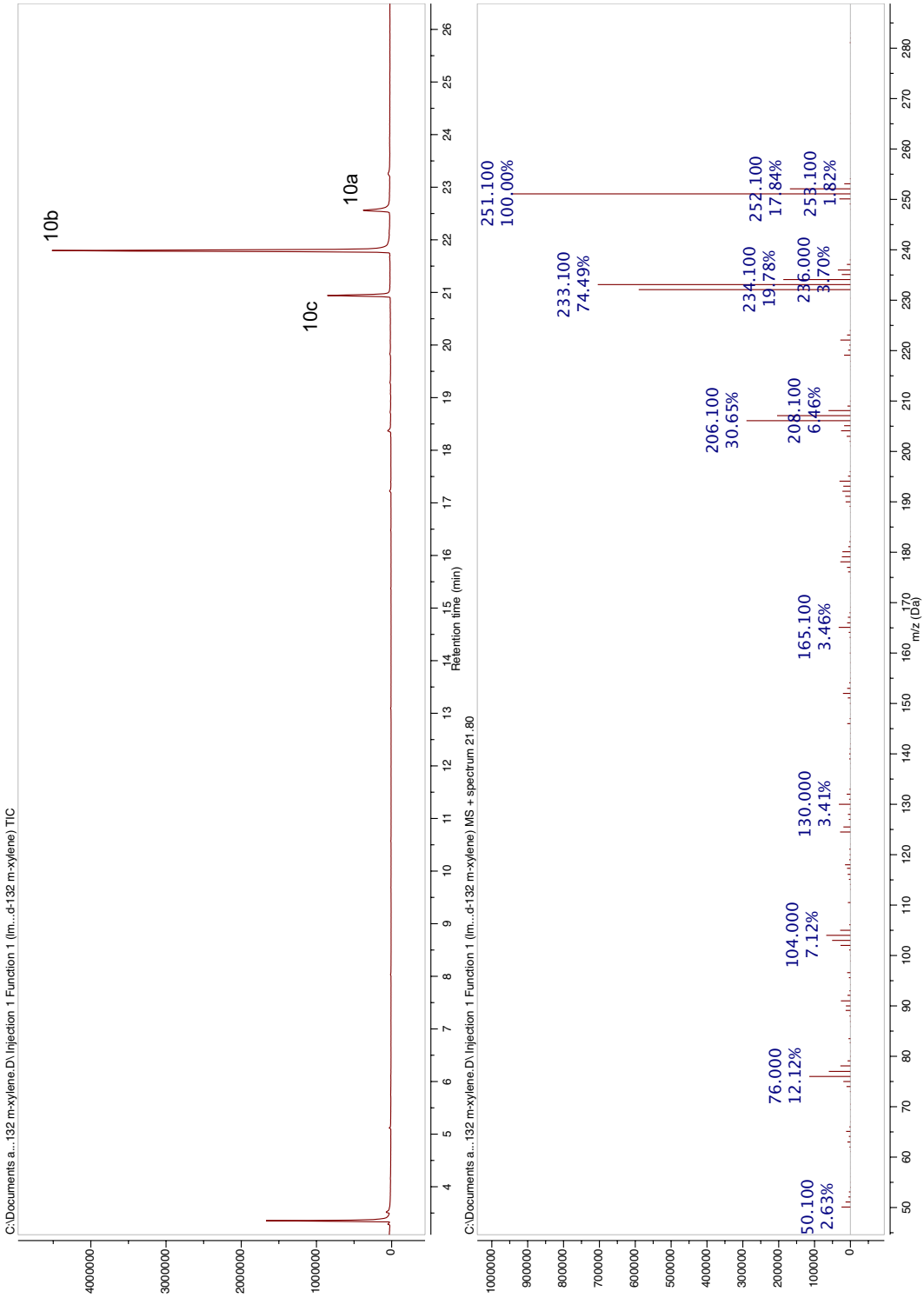
^{1}H NMR (300 MHz, $CDCl_3$):

δ 7.92 – 7.97 (m, 2H), 7.75 – 7.81 (m, 3H), 6.99 – 7.19 (m, 6H), 2.37 (s, 3H), 2.16 (s, 3H).

MS (m/z): calculated for $C_{16}H_{13}NO_2$ = 251.095, observed 251.100

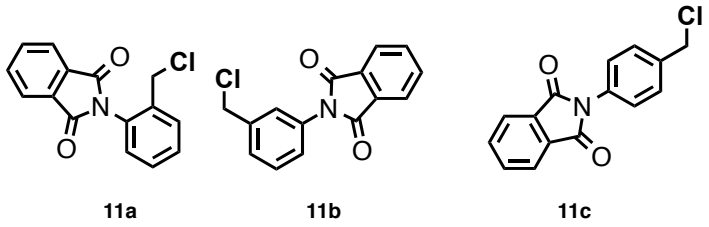


Spectrum 9: ^{1}H NMR of **10**



Spectrum 9.1: GC/MS of **10**

Amination of Benzylchloride (11)



11a 11b 11c

11a:11b:11c = 1:5:13

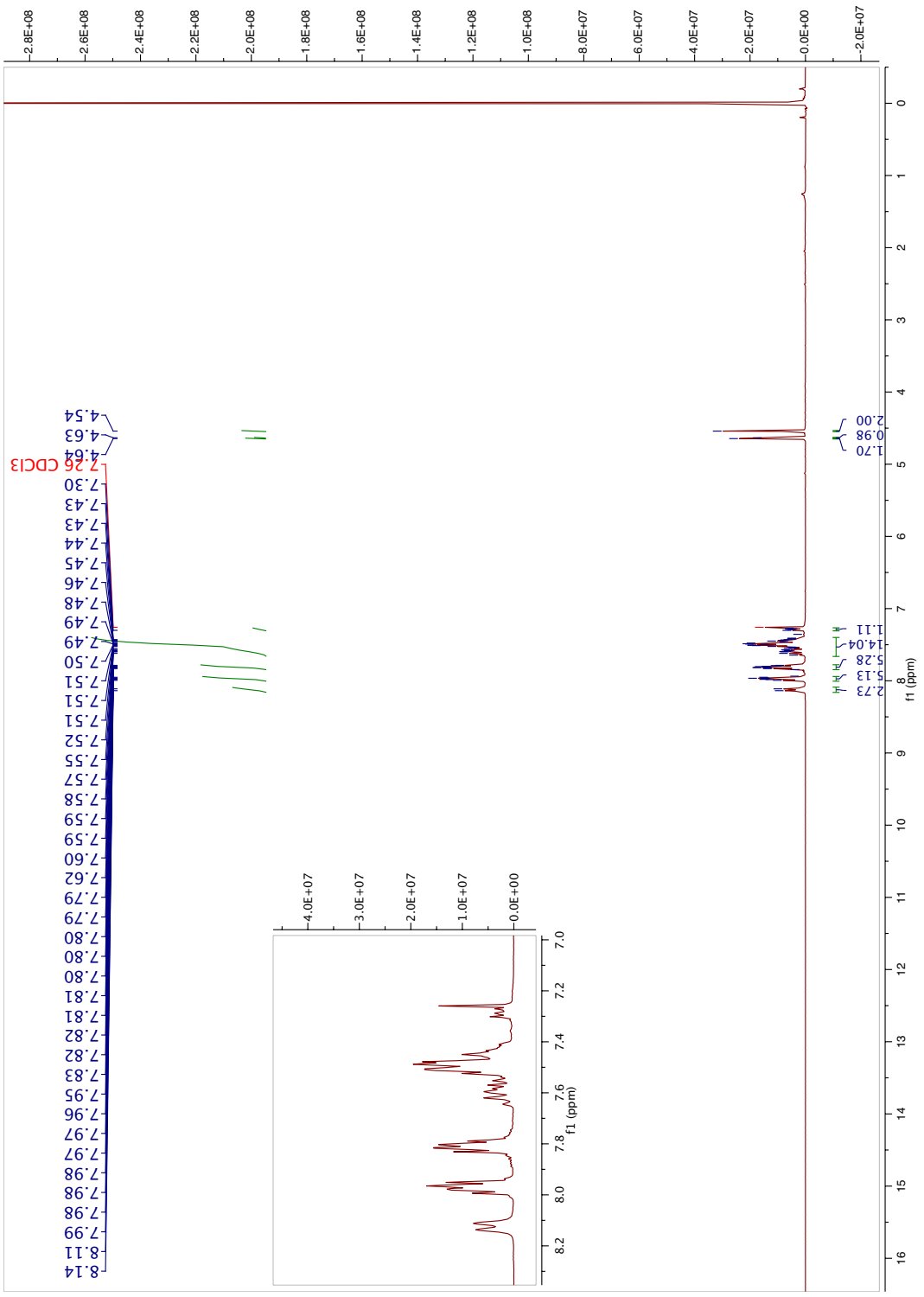
^{1}H NMR (300 MHz, $CDCl_3$):

δ 8.12 (d, *J* = 7.8 Hz, $2H_{11c}$ +$1H_{11a}$), 7.94 – 7.99 (m, 5H), 7.79 – 7.84 (m, 5H), 7.36 – 7.64 (m, 14H), 7.27 – 7.30 (m, 1H), 4.64 (s, 2H), 4.63 (s, 1H), 4.54 (s, 1H)

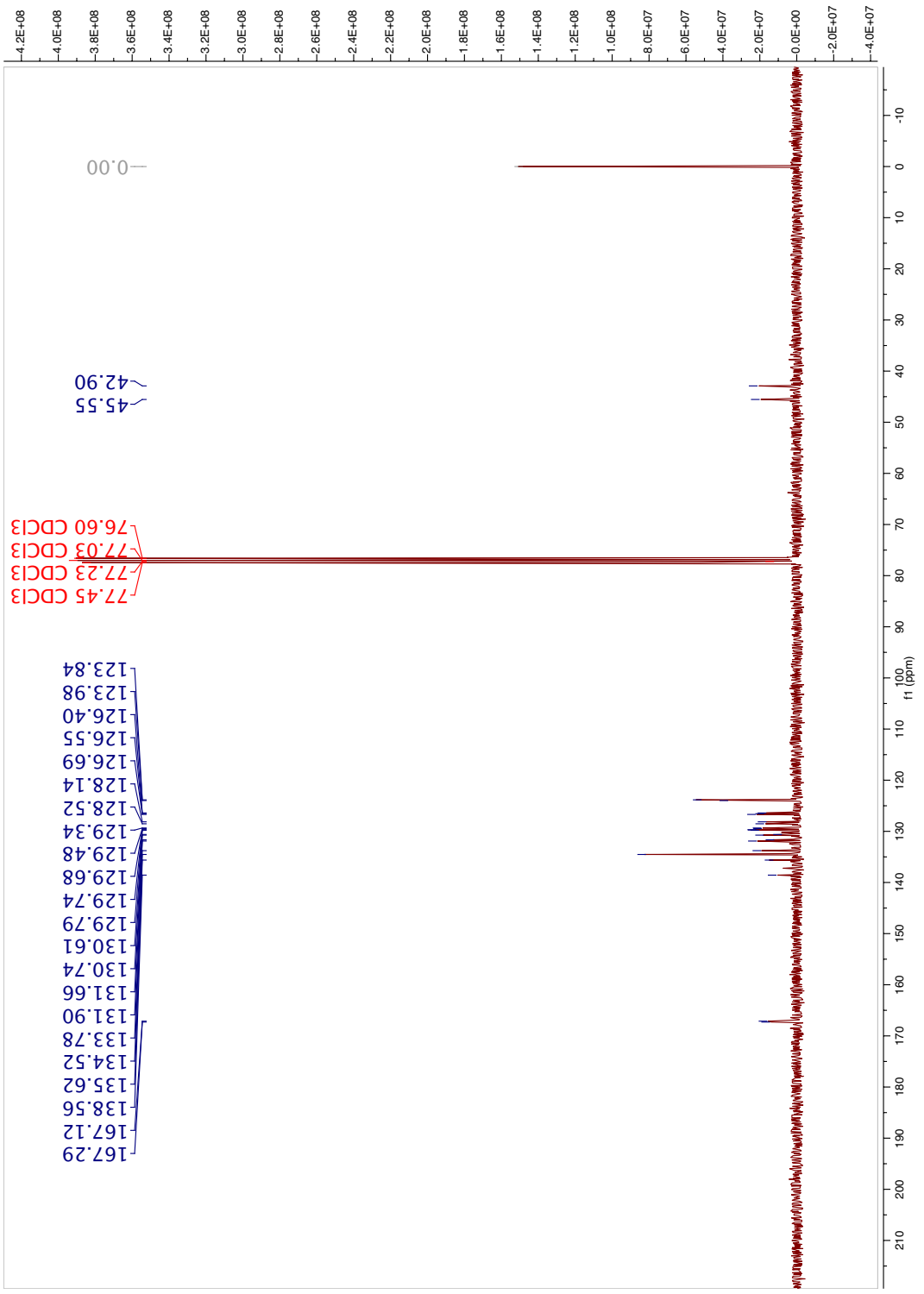
^{13}C NMR (75 MHz, $CDCl_3$):

δ 167.29, 167.12, 138.56, 135.62, 134.52, 133.78, 131.90, 131.66, 130.74, 130.61, 129.79, 129.74, 129.68, 129.48, 129.34, 128.52, 128.14, 126.69, 126.55, 126.40, 123.98, 123.84, 45.55, 42.90

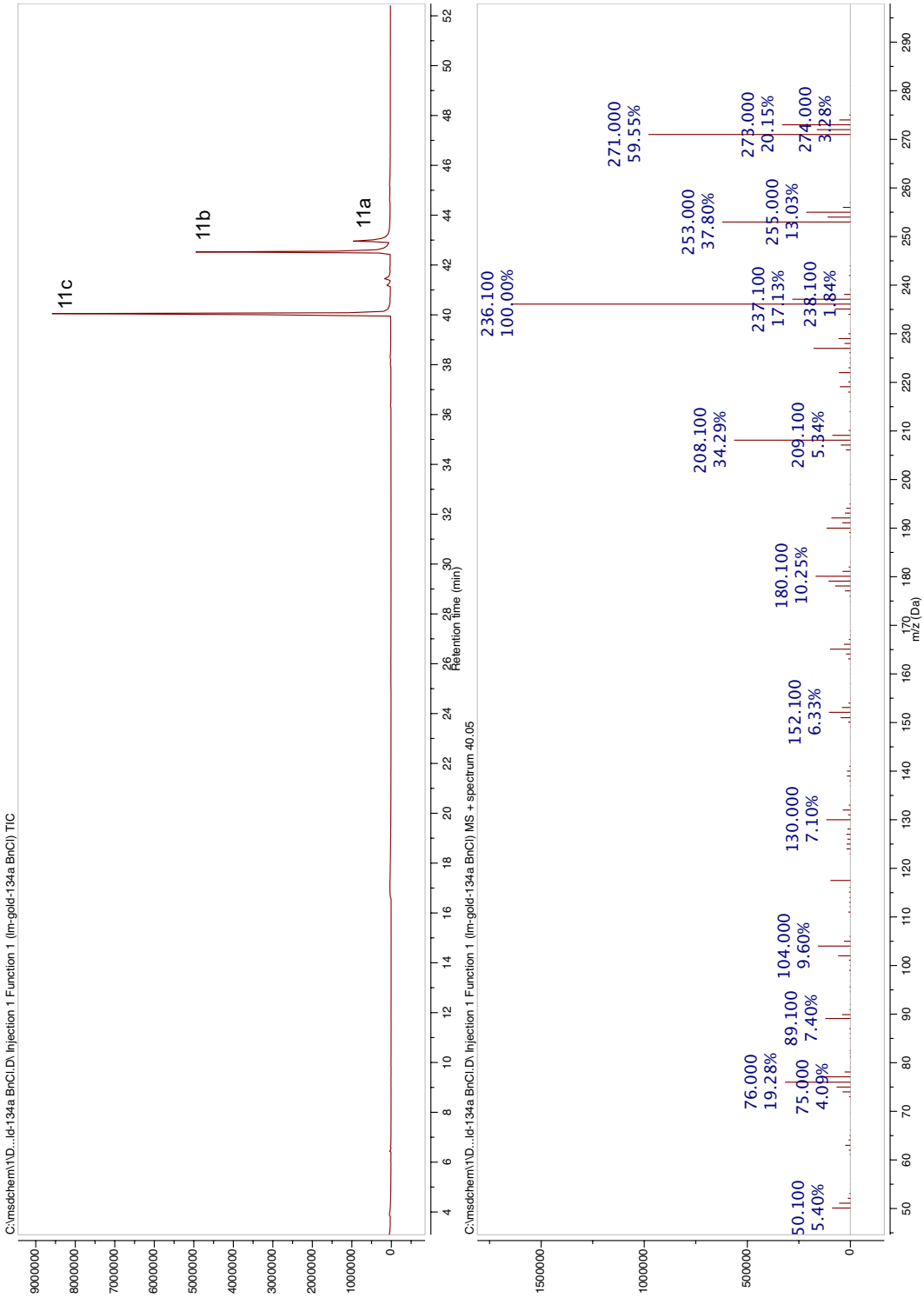
MS (m/z): calculated for $C_{15}H_{10}ClNO_2$ = 271.040, observed 271.000



Spectrum 10: ^{1}H NMR of **11**

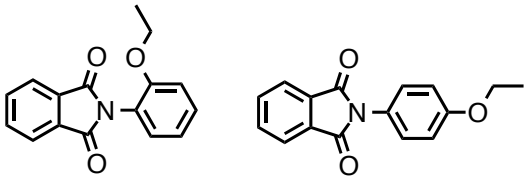


Spectrum 10.1: ^{13}C NMR of **11**



Spectrum 10.2: GC/MS of **11**

Amination of Phenetole (12)



12a **12b**

12a:12b = 2:3

^{1}H NMR (500 MHz, $CDCl_3$):

12a: δ 7.93 (dd, *J* = 5.6, 2.8 Hz, $2H_a + 2H_b$), 7.76 – 7.78 (m, $2H_a + 2H_b$), 7.40 (td, *J* = 7.8, 1.7 Hz 2H), 7.24 (d, 1.6 Hz, 1H), 6.99 – 7.06 (m, 2H) 4.05 – 4.10 (m, $2H_a + 2H_b$), 1.27 (t, 7.0 Hz, 3H)

12b: δ 7.93 (dd, *J* = 5.6, 2.8 Hz, $2H_a + 2H_b$), 7.76 – 7.78 (m, $2H_a + 2H_b$), 7.30 (dd, 6.1, 2.1 Hz, 4H), 7.00 (dd, 6.0, 2.2 Hz, 2H) (4.05 – 4.10 (m, $2H_a + 2H_b$), 1.45 (t, 6.8 Hz, 3H)

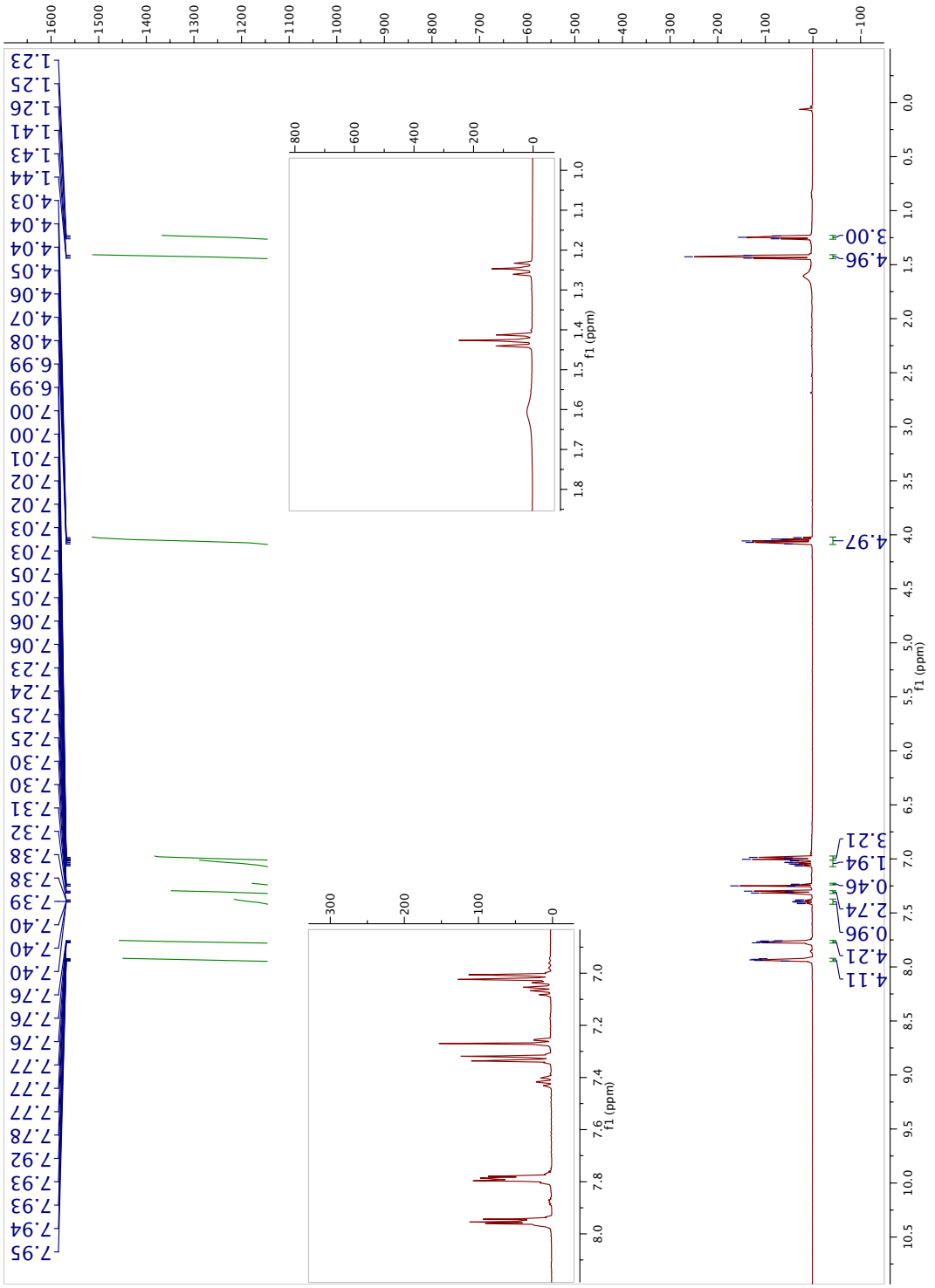
^{13}C NMR (125 MHz, $CDCl_3$):

12a (distinguishable): δ 167.38, 154.77, 64.24, 14.62

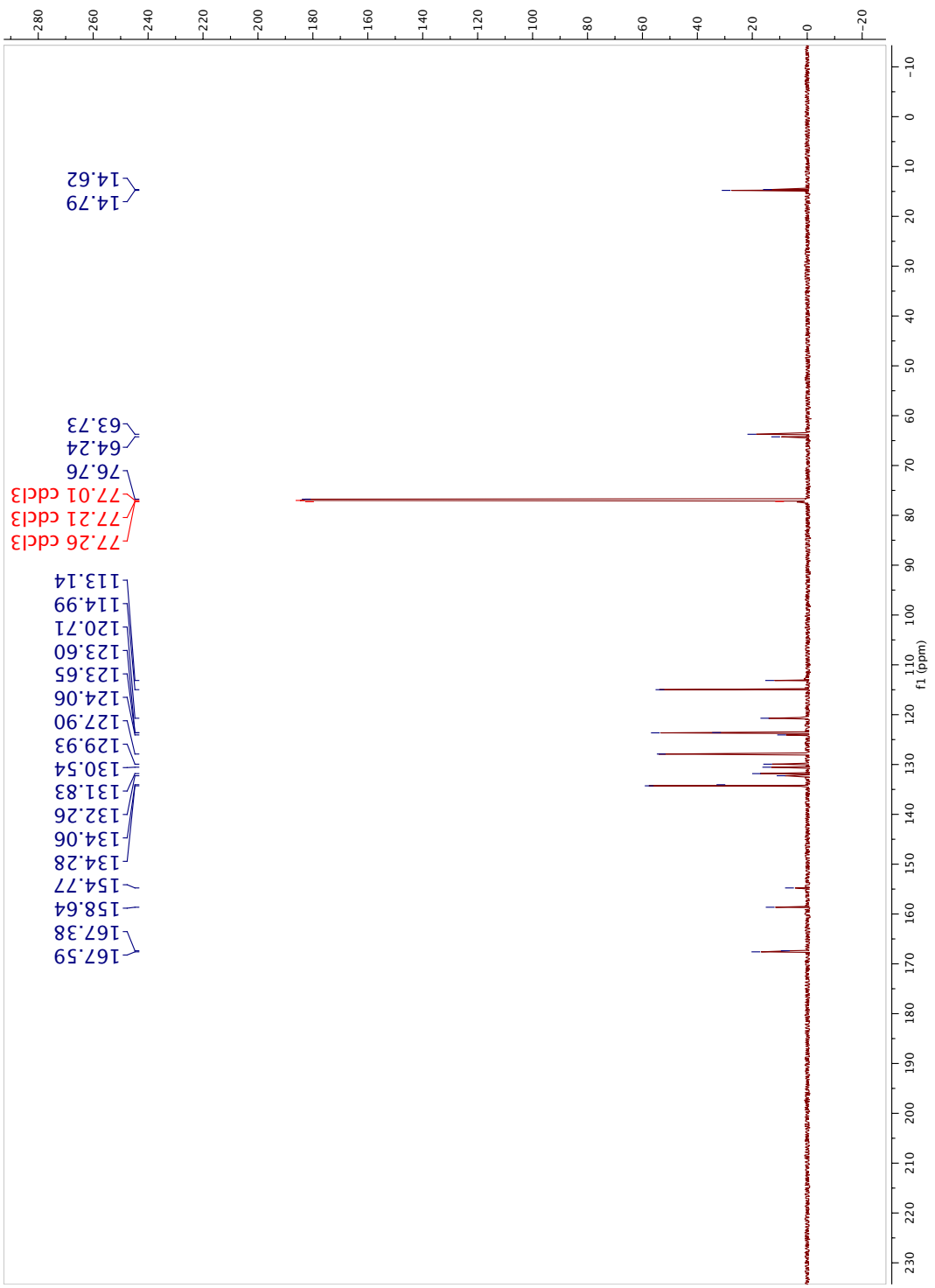
12b (distinguishable): δ 167.59, 158.64, 134.28, 63.73, 14.79

Aromatic Signals: 134.28, 134.06, 132.26, 131.83, 130.54, 129.93, 127.90, 124.06, 123.65, 123.60, 120.71, 114.99, 113.14, 77.26, 77.21, 77.01, 76.76,.

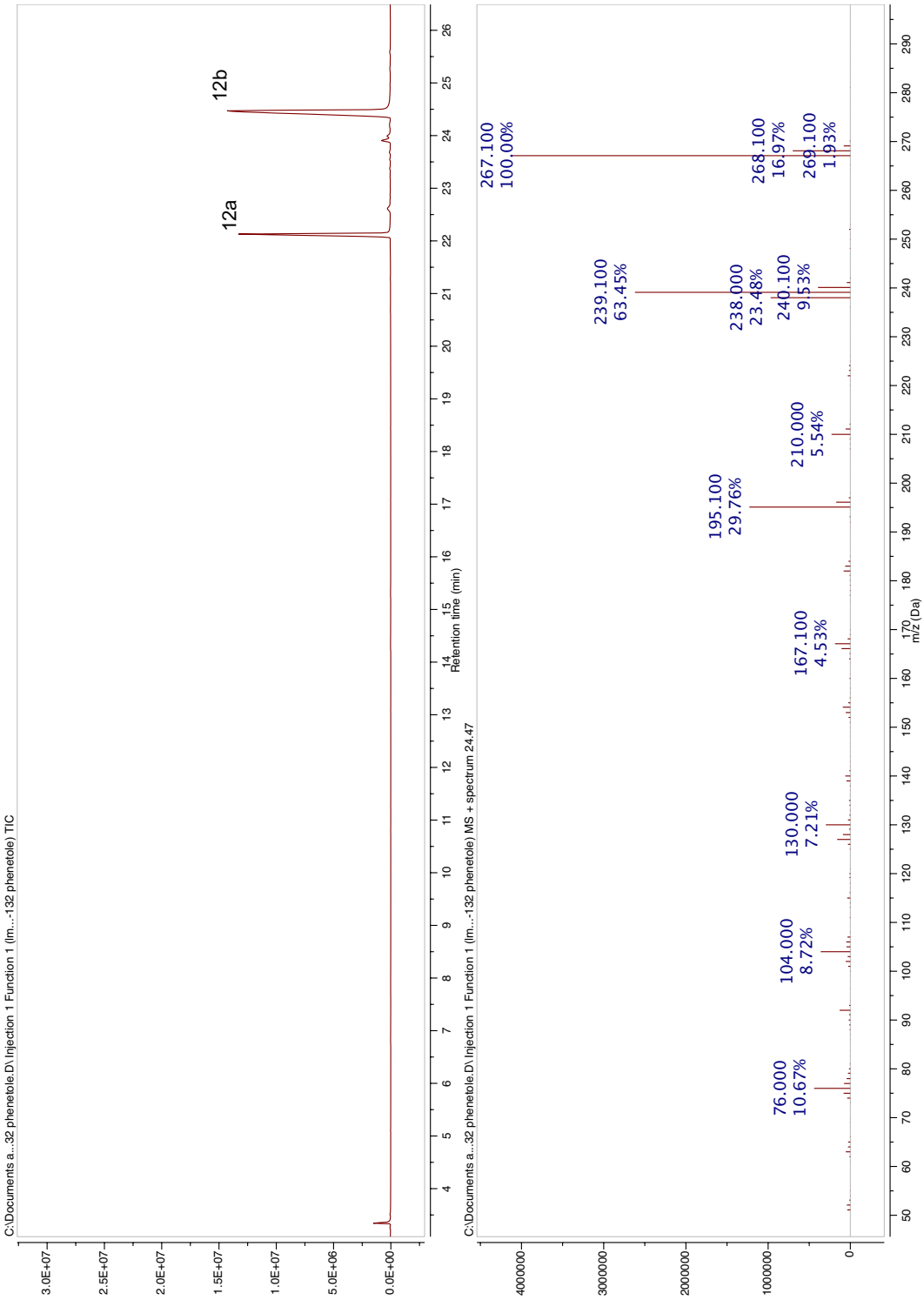
MS (m/z): calculated for $C_{16}H_{13}NO_3$ = 267.090, observed 267.100



Spectrum 11: ^{1}H NMR of **12**

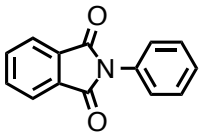


Spectrum 11.1: ^{13}C NMR of **12**



Spectrum 11.2: GC/MS of **12**

Amination of Benzene (13)

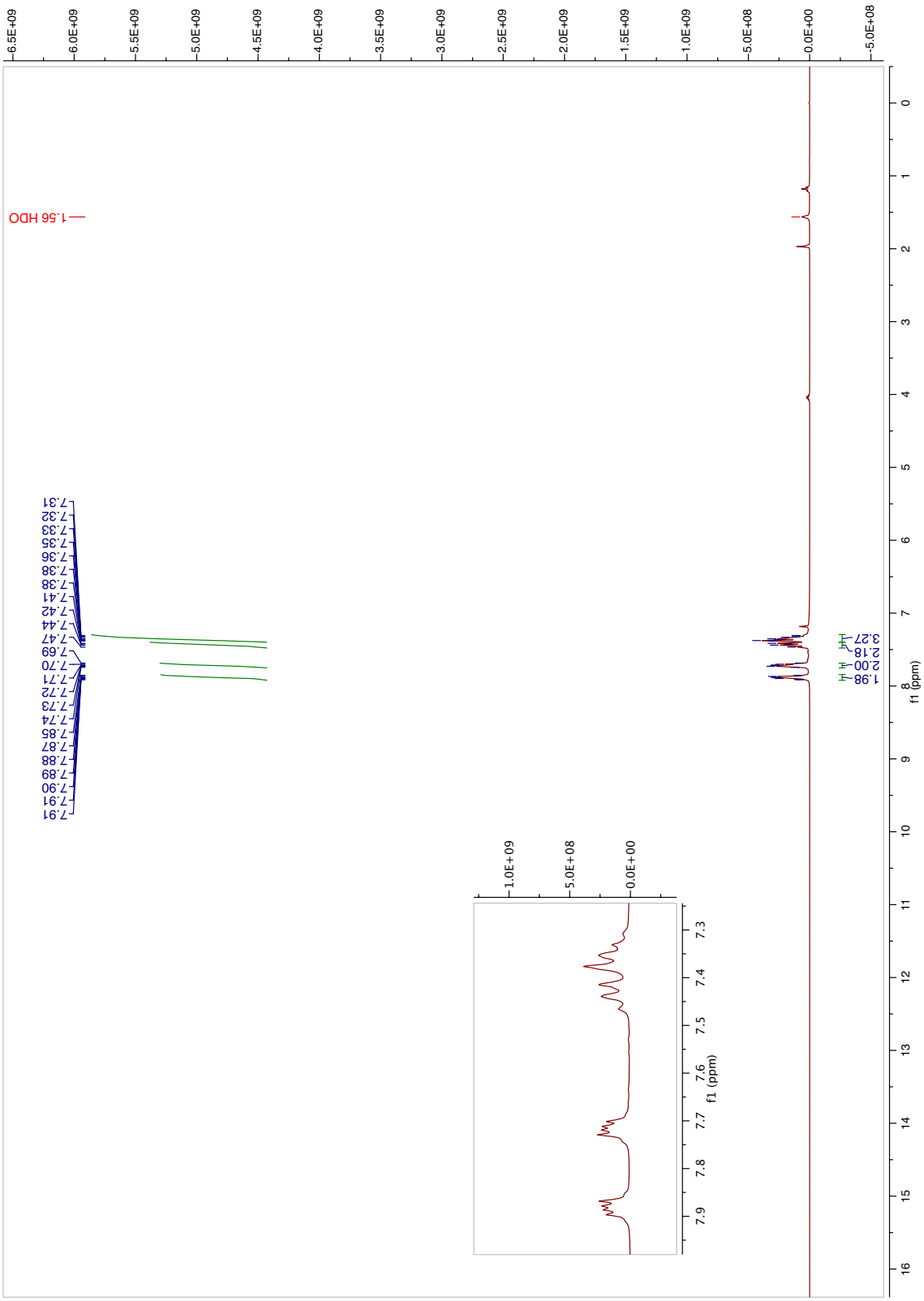


13

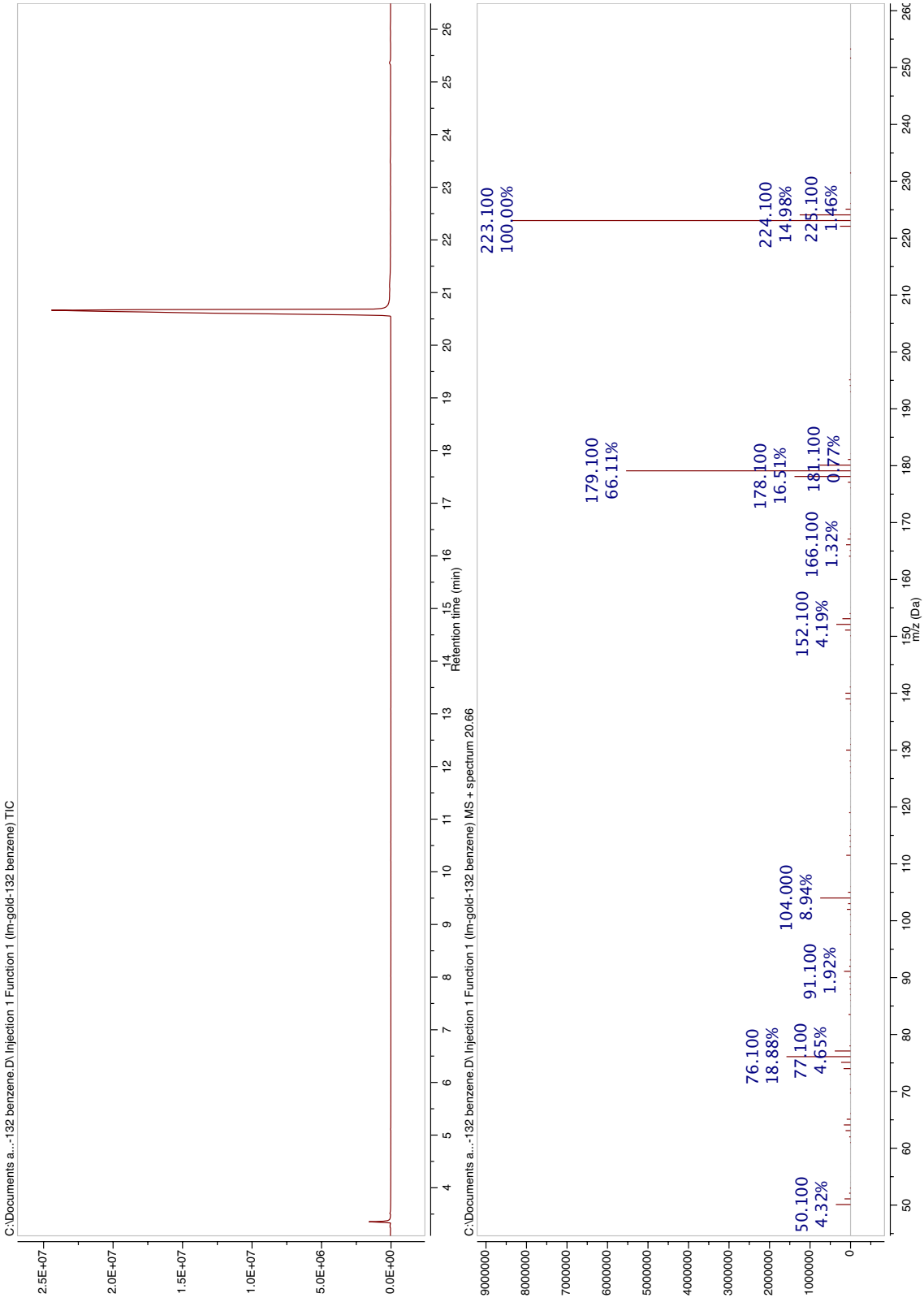
^{1}H NMR (300 MHz, CDCl3):

13: δ 7.88 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.72 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.48 - 7.40 (m, 2H), 7.40 - 7.29 (m, 3H).

MS (m/z): calculated for $C_{14}H_9NO_2$ = 223.063, observed 223.070



Spectrum 12: ^{1}H NMR of **13**



Spectrum 12.1: GC/MS of **13**

References:

1) Shrestha, R.; Mukherjee, P.; Tan, Y.; Litman, Z.; and Hartwig, J. *J. Am. Chem. Soc.* **2013**, *135*, 8480-8483.

2) Allen, L.; Cabrera, P.; Lee, M.; and Sanford, M. *J. Am. Chem. Soc.* **2014**. 136, 5607 – 5610.

3) Kantak, A.; Potavathri, S.; Barham, R.; Romano, K.; and DeBoef, B. *J. Am. Chem. Soc.* **2011**, *133*, 19960-19965.

MANUSCRIPT 2

Published in *Chem. Commun.*, **2015**, *51*, 3574.

Regioselective C—H Bond Amination By Aminoiodanes

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Manuscript 2

Regioselective C–H Bond Amination by Aminoiodanes

Abstract

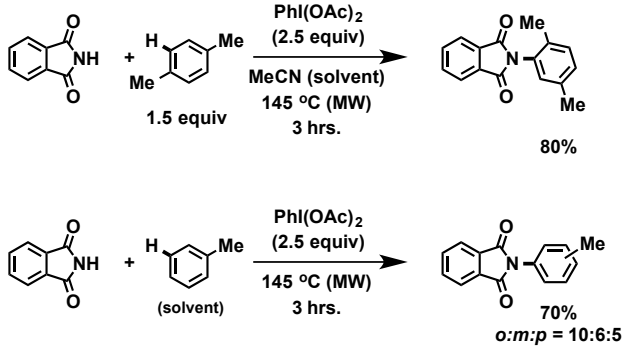
A new approach for the direct amination of 2-phenylpyridine derivatives using a diphthalimide-iodane and copper triflate has been developed. A series of different 2-phenylpyridine derivatives were aminated with yields up to 88%. Mechanistic investigations indicate that the reaction proceeds via a copper-mediated single electron transfer.

Introduction

Over the past two decades advances in transition metal catalyzed C–H functionalization have provided efficient strategies for the construction of C–C and C–heteroatom bonds.[1] During this time an extensive amount of effort has been deovted to the development of novel methodologies for the construction of C–N bonds, as they are ubiquitous in pharmaceuticals and other high value molecules. However, synthetic routes for the direct amination of the C–H bonds of arenes are relatively rare.[2] Recent examples involve the addition of nitrene intermediates into allylic and benzylic C–H bonds,[3] though methods involving radical intermediates have also been investigated.[4,5] Most of the metal-mediated directed aminations involve late transition metal catalysts, such as palladium or ruthenium,[6] though recently, efforts to discover highly efficient, economical, and environmentally benign methods for C–N bond formation have led to the development of copper-mediated C–H aminations.[7,8] Notably, Yu reported a C–N bond formation process using stoichiometric copper acetate,[2b] and Nicholas reported a

copper-catalyzed C–N bond formation process; however, this method required harsh conditions.[9a] Likewise, Li reported the copper-catalyzed dual C–H/N–H cross-coupling of 2-phenylpyridine with acetanilide.[9b]

Recently, we and both Chang and Antonchick simultaneously developed an I(III)-mediated intermolecular oxidative C–N bond formation processes for synthesizing anilines by a formal tandem C-H/N-H activation. Though these reactions provided high yields of amination products, the radical mechanism produced mixtures of regiomeric products for mono-subsituted arene substrates such as toluene (Scheme 1).[2d,2e,10] Subsequently, Hartwig reported a related palladium-catalyzed amination reaction that enhanced the regioselectivity, though both meta and para aminated products were observed for mono-substituted substrates.[11] To remedy the regioselectivity issues associated with our metal-free protocol, we proposed to develop an I(III)-mediated reaction that achieved regioselectivity via a metal-mediated C–H activation with a substrate containing a Lewis base directing group.[2d]

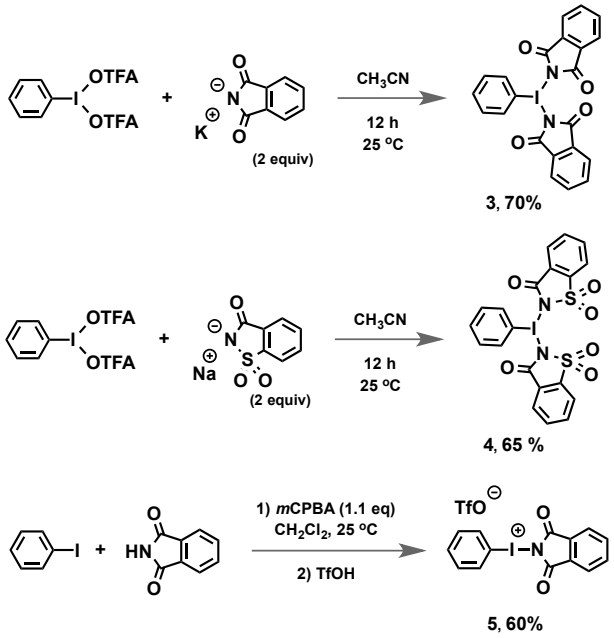


Scheme 1 Amination of arenes in the absence of a transition metal catalyst

Results and Discussion

During the course of our previous investigations, we also became interested in the synthesis of new iodane and iodonium reagents containing I–N bonds that could be used as C–H aminating agents. Recently, Muñiz reported several novel hypervalent iodine(III) reagents for metal-free intermolecular allylic amination and diamination of alkenes.[12] Furthermore, diaryliodonium salts had been used by the Sanford group to arylate C–H bonds in the presence of catalytic palladium[13] and by the Gaunt group to arylate C–H bonds in the presence of catalytic copper.[14] Consequently, we synthesized phenyl(diphthalimido)- λ^3-iodane (3) by ligand exchange from iodobenzenebis(trifluoroacetate), PIFA, using a relatively simple ligand exchange reaction.[15] We also successfully synthesized a saccharin-derived iodane (4) and the phthalimide-iodonium salt (5) by modifying a route for synthesizing diaryliodonium salts from in situ-generated iodosobenzene (Scheme 2).[16]

The new iodanes and the iodonium salt were subjected to a variety of reaction conditions mediated by palladium or copper with 2-arylpyridine substrates. We hypothesized that the I(III) species would react as an electrophilic amine, and that regioselective metalation of the ortho C_{aryl}–H would create a nucleophilic carbon. Though this initial hypothesis proved to be incorrect, we discovered a novel method to selectively aminate 2-phenylpyridines using iodanes such as 3, the results of which are presented herein.

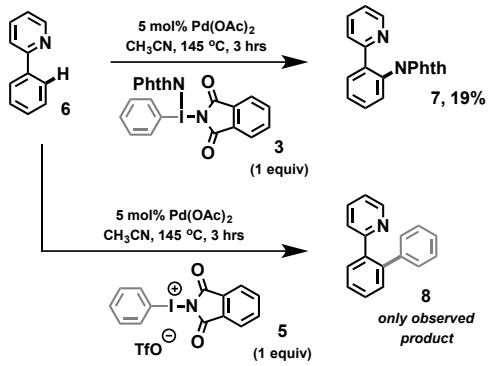


Scheme 2 Synthesis of imide-substituted iodanes and an iodonium salt

As a control, we began by heating a solution of phthalimide, 2-phenylpyridine (6), and iodobenzene diacetate (PIDA) in acetonitrile using microwave irradiation and determined that PIDA was not able to directly aminate the pyridine derivative. As a result, the addition of catalytic palladium, along with PIDA or PIFA, was also explored. While modest amination was observed, palladium catalysis did not facilitate amination with acceptable yields. Interestingly, the use of the iodane oxidant (3) provided the desired amination product (7), while the iodonium oxidant (5) exclusively provided the arylated product (8). Consequently, we concluded that the iodane structure apparently favoured C–N bond formation, while the iodonium favoured C–C bond formation. The reason for this trend is not readily apparent and will be the subject of future studies. As a result, we elected to screen copper catalysts and discovered that copper(II) triflate showed higher amounts of amination products

when compared to the previously successful experiment with palladium acetate, albeit at a higher catalyst loading (Table 1, entry 2).

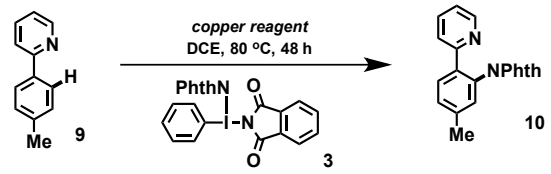
The reactions were sluggish, even at 145 °C, so as a compromise, we elected to increase the catalyst loading to 1 equiv. Such a consideration is only possible when dealing with affordable and non-toxic metals such as copper, as opposed to precious metals, like palladium.



Scheme 3 Palladium catalyzed reactions of **3** and **5**

Further optimization indicated that $Cu(OTf)_2$ was the preferred copper salt and that dichlorethane (DCE) was the preferred solvent (See supporting information). $Cu(OAc)_2$ and $CuCl_2$ provided low yeilds of 10 along with acetylated and chlorinated by-products (Table 1, entries 8 and 9). When optimizing the stoichiometry necessary for the iodane, an interesting pattern was observed. Since the I(III) species acted as both the nitrogen source and the oxidant in reactions containing substoichiometric $Cu(OTf)_2$, 3 had to be used in excess. However, when reactions containing stoichiometric $Cu(OTf)_2$, were run with 1 equiv of 3, lower yields were also observed (entry 6), so the excess iodane reagent was determined to be optimal.

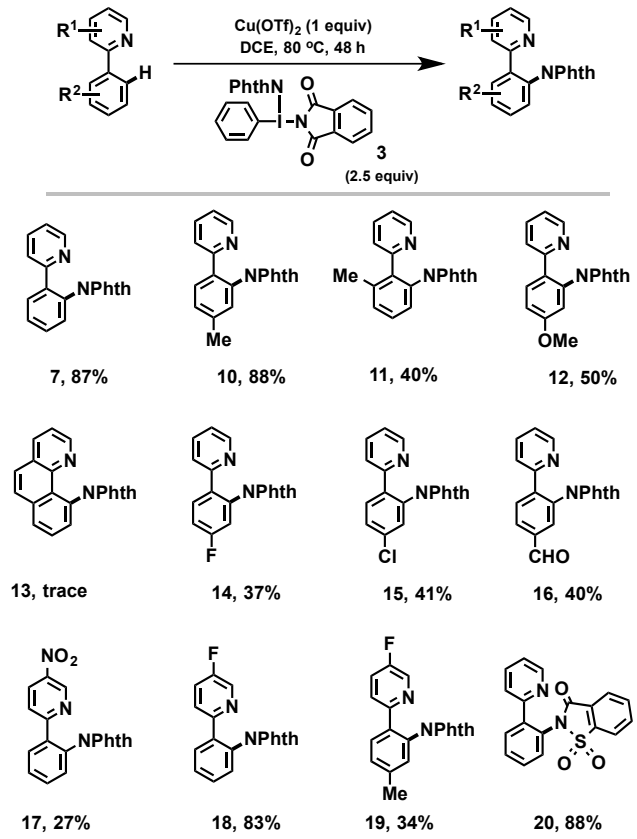
Optimized conditions required heating the iodane (3), the 2-arylpyridine substrate (9) and 1 equiv of copper triflate in dichloroethane at 80 °C for 48 h (entry 5). Amination was exclusively observed at the ortho position, relative to the pyridine substituent to yield 10, and no arylated products (*e.g.* 8) were observed. The presence of the iodane was crucial for the reactivity of the system, as reactions employing both stoichiometric copper and phtalimide produced only trace amounts of 10 (entry 7). It is also should be noted that freshly prepared iodane (3) exhibited higher yields than batches that had been stored under air at room temperature.



Entry	Cu reagent (loading)	Iodane (3) loading	Yield (%)[b]
1	None	2.5 equiv	0
2	$Cu(OTf)_2$ (25 mol%)	2.5 equiv	63[c]
3	$Cu(OTf)_2$ (25 mol%)	1 equiv	47[c]
4	$Cu(OTf)_2$ (0.5 equiv)	2.5 equiv	71[c]
5	$Cu(OTf)_2$ (1.0 equiv)	2.5 equiv	88[c]
6	$Cu(OTf)_2$ (1.0 equiv)	1 equiv	80[c]
7	$Cu(OTf)_2$ (1.0 equiv)	None[d]	2
8	$Cu(OAc)_2$ (1.0 equiv)	2.5 equiv	12
9	$CuCl_2$ (1.0 equiv)	2.5 equiv	5

[a] General reaction conditions: 9 (0.146 mmol), 3 (0.365 mmol), catalyst (0.25-1 equiv) and DCE (4 mL), heated in avial in an oil bathfor 48 h. [b] GC yield. [c] Yield of isolated products after column chromatography. [d] This reaction contained 1 equiv phthalimide.

Table 1 Lead Reaction Discovery[a]

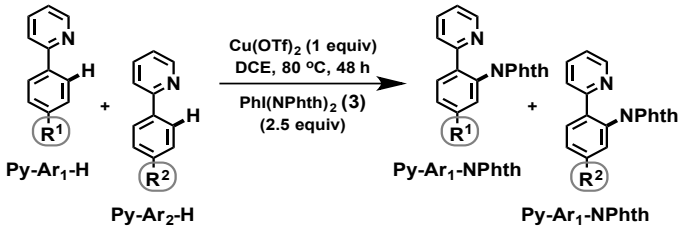


[a] Isolated yields following flash chromatography.

Table 2 Substrate scope[a]

A variety of 2-phenylpyridine substrates could be aminated using the optimal reaction conditions (Table 2). First, substrates containing different phenyl-substituted groups were screened. 4–Tolylpyridine (10) showed a comparable yield to unsubstituted 2-phenylpyridine (7); however, substrates having additional electron density, such as the 4-methoxy derivative (12), showed a drop in yield. Additionally, it appears that the central dihedral angle of the biaryl substrate may also play a part in determining the substrate's reactivity, as benzo[h]quinoline, a substrate commonly employed in C–H activation reactions, produced only trace yields of the aminated product (13). Substitution on the pyridine ring of the substrate did not appear to dramatically affect the reaction. Fluorination of the pyridine directing group did not

dramatically alter the yield of the amination, but fluorination of the phenyl ring caused the yield to drop (compare 7, 14 and 18). Additionally, the amination reaction proceeded with the saccharin iodane (4), to yield the expected aminated product (20).To elucidate the reaction mechanism, competition studies were conducted using an equimolar mixture of two different pyridine substrates in the lead reaction. In each case, amination of the more electron rich arene was favored over the electron poor arene.



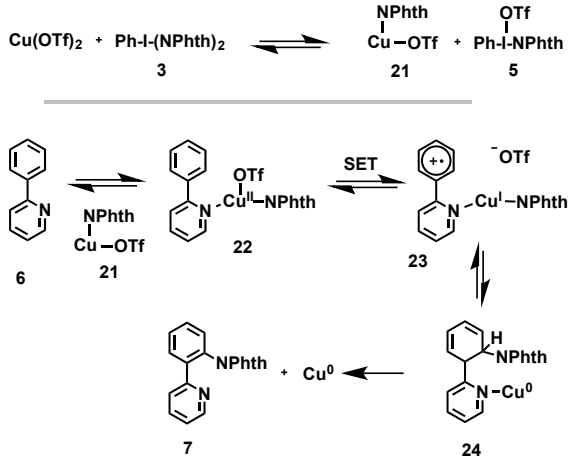
Entry	R^1 ($Py\text{-}Ar^1\text{-}H$)	R^2 ($Py\text{-}Ar^2\text{-}H$)	*X* $Py\text{-}Ar^1\text{-}NPhth$	*X* $Py\text{-}Ar^2\text{-}NPhth$
1	H	Me	42	58
2	H	F	92	8
3	Me	F	94	6

[a] Mole fractions determined by GC

Table 3 Competition Reactions[a]

The kinetic isotope effect was also studied using an intramolecular competition between a C–H bond and C–D bond in 2-phenylpyridine. The observed KIE of 1.15 indicates that C-H bond cleavage was not involved in the rate-determining step. This rules out the possibility of a metal-mediated C–H activation step via oxidative addition, sigma-bond metathesis, or concerted metalation-deprotonation, where significant isotope effects are usually observed. Additionally, the substrate scope

(Table 2), and the competition studies (Table 3) indicate that the reaction favors electron rich arenes, which could be easily oxidized. Thus, we propose a pathway mediated by a radical cation (23) to explain the data (Scheme 4).[2b]



Scheme 4 Proposed mechanism for the regioselectiveamination

First, copper triflate reacts with the iodane (3) to generate a copper-phthalimide species (21) and the iodonium (5). We have detected the formation of 5 via mass spectrometry of crude reaction mixtures, and we have independently synthesized and characterized it (Scheme 2). Thus, we propose that 21 reacts with 2-phenylpyridine (6) to generate a complex having Cu(II) coordinated to the pyridine substrate (22). A single electron transfer (SET) from the aryl ring to the coordinated Cu(II) generates the radical cation intermediate (23). Homolytic cleavage of the Cu–N bond and subsequent intramolecular anion transfer from the Cu(I) intermediate generates the ortho-aminated product (7). Competition studies show that the reaction is favored when an electron donating group is present on the aryl ring, and the SET from an aryl ring containing an electron-donating group should be faster than that

from an aryl ring containing an electron withdrawing group. We propose that SET is the rate-limiting step in this reaction.

Conclusion

In conclusion, we have developed a novel, useful and economical process for the direct amination of 2-phenylpyridine derivatives. This process requires cheap and commercially available copper triflate and works for a variety of different 2-phenylpyridine derivatives. Additionally, the process also works with a bis-saccharin iodane. Future endeavors aim to synthesize additional novel iodanes that contain I–N bonds in order to further develop this and other amination processes.

This work was supported by the National Science Foundation (CAREER 0847222), and the National Institutes of Health (NIGMS, 1R15GM097708-01).

Notes and references

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References

(1) For recent reviews describing C-H bond functionalization, see: (a) L. Ackermann, R. Vicente and A. R. Kapdi, *Angew. Chem. Int. Ed.,* **2009**, *48*, 9792; (b) D. Alberico, M. E. Scott and M. Lautens, *Chem. Rev.,* **2007**, *107*, 174; (c) X. Chen, K. M. Engle, D.-H. Wang and J.-Q. Yu, *Angew. Chem. Int. Ed.,* **2009**, *48*, 5094; (d) S. H. Cho, J. Y. Kim, J. Kwak and S. Chang, *Chem. Soc. Rev.,* **2011**, *40*, 5068; (e) D. A. Colby, R. G. Bergman and J. A. Ellman, *Chem. Rev.,* **2010**, *110*, 624; (f) O. Daugulis, H. Q. Do and D. Shabashov, *Acc. Chem. Res.,* 2009, 42, 1074; (g) C.-J. Li, *Acc. Chem. Res.,* **2009**, *42*, 335; (h) T. W. Lyons and M. S. Sanford, Chemical Reviews, **2010**, *110*, 1147; (i) G. P. McGlacken and L. M. Bateman, *Chem. Soc. Rev.,* **2009**, *38*, 2447; (j) I. V. Seregin and V. Gevorgyan, *Chem. Soc. Rev.,* **2007**, *36*, 1173; (k) C.-L. Sun, B.-J. L and Z.-J. Shi, *Chem. Commun.,* **2010**, *46*, 677; (l) C. S. Yeung and V. M. Dong, *Chem. Rev.,* **2011**, *111*, 1215.

(2) For recent examples of C-H amination , see: (a) Y. M. Badiei, A. Dinescu, X. Dai, R. M. Palomino, F. W. Heinemann, T. R. Cundari, and T. H. Warren, Angew. *Chem. Int. Ed.,* **2008**, *47*, 9961; (b) X. Chen, X.-S. Hao, C. E. Goodhue and J.-Q. Yu, *J. Am. Chem. Soc.,* **2006**, *128*, 6790; (c) M. Ichinose, H. Suematsu, Y. Yasutomi, Y. Nishioka, T. Uchida and T. Katsuki, *Angew. Chem.,* **2011**, *123*, 10058; (d) A. A. Kantak, S. Potavathri, R. A. Barham, K. M. Romano, and B. DeBoef, *J. Am. Chem. Soc.,* **2011**, *133*, 19960; (e) H. J. Kim, J. Kim, S. H. Cho and S. Chang, *J. Am. Chem. Soc,* **2011**, *133*, 16382; (f) H. Lu, V. Subbarayan, J. Tao and X. P. Zhang, *Organometallics,* **2010**, *29*, 389-393; (g) A. N. Vedernikov and K. G. Caulton, *Chem. Commun.,* **2004**, 162-163.

(3) For recent reviews describing C-N bond formation via nitreneintermediates , see: (a) H. M. L. Davies, and J. R. Manning, *Nature,* **2008**, *451*, 417; (b) P. Müller and C. Fruit, *Chem. Rev.,* **2003**, *103*, 2905; (c) C. W. Hamilton, D. S. Laitar and J. P. Sadighi, *Chem. Commun.,* **2004**, 1628.

(4) For a recent examples of arene C-H amination via nitrogen-centered radicals, see: K. Foo, E. Sella, I. Thome, M D. Eastgate and P. S. Baran, *J. Am. Chem. Soc.,* **2014**, *136*, 5279.

(5) For a review describing the reactions of nitrogen-centered radicals, see: S. Z. Zard, *Chem. Soc. Rev.,* **2008**, *37*, 1063.

(6) For recent examples Pd and Ru-catalyzedaminations, see: (a) A. R. Dick, M. S. Remy, J. W. Kampf and M. S. Sanford, *Organometallics,* **2007**, *26*, 1365; (b) J. A. Jordan-Hore, C. C. C. Johansson, M. Gulias, E. M. Beck and M. J. Gaunt, *J. Am. Chem. Soc,* **2008**, *130*, 16184; (c) K. Sun, Y. Li, T. Xiong, J. Zhang and Q. Zhang, *J. Am. Chem. Soc.,* **2011**, *133*, 1694; (d) H.-Y. Thu, W.-Y. Yu and C.-M. Che, *J. Am. Chem. Soc.,* **2006**, *128*, 9048; (e) W. C. P. Tsang, R. H. Munday, G. Brasche, N. Zheng and S. L. Buchwald, *J. Org. Chem.,* **2008**, *73*, 7603; (f) W. C. P. Tsang, N. Zheng and S. L. Buchwald, *J. Am. Chem. Soc.,* **2005**, *127*, 14560; (g) S. R. Whitfield and M. S. Sanford, *J. Am. Chem. Soc.,* **2007**, *129*, 15142; (h) B. Xiao, T.-J. Gong, J. Xu, Z.-J. Liu, L. Liu, *J. Am. Chem. Soc.,* **2011**, *133*, 1466. (i) M. Shang, S.H. Zeng, S. Z. Sun, H. X. Dai and J. Q. Yu, *Org. Lett.,* **2013**, *15*, 5286.

(7) Xu, H.; Qiao, X.; Yang, S.; Shen, Z. *J. Org. Chem.* **2014**, *79*, 4414.

(8) For recent examples of Cu-catalyzed aminations, see: (a) I. P. Beletskaya and A. V. Cheprakov, *Coordination Chem. Rev.,* **2004**, *248*, 2337; (b) G. Evano, N. Blanchard and M. Toumi, *Chem. Rev.,* **2008**, *108*, 3054; (c) T. Kawano, K. Hirano, T. Satoh and M. Miura, *J. Am. Chem. Soc.,* **2010**, *132*, 6900; (d) S. V. Ley and A. W. Thomas, *Angew. Chem. Int. Ed.,* **2003**, *42*, 5400; (e) D. Monguchi, T. Fujiwara, H. Furukawa and A. Mori, *Org. Lett.,* **2009**, *11*, 1607; (f) F. Monnier and M. Taillefer, *Angew. Chem. Int. Ed.,* **2009**, *48*, 6954; (g) Q. Wang and S. L. Schreiber, *Org. Lett.,* **2009**, *11*, 5178.

(9) (a) A. John and K. M. Nicholas, *J. Org. Chem.,* **2011**, *76*, 4158; (b) Q. Shuai, G. Deng, Z. Chua, D. S. Bohle and C.-J. Li, *Adv. Synth. Cat.,* **2010**, *352*, 632.

(10) (a) S. H. Cho, J. Yoon and S. Chang, *J. Am. Chem. Soc.,* **2011**, *133*, 5996; b) A. P. Antonchick, R. Samanta, K. Kulikov and J. Lategahn, *Angew. Chem. Int. Ed.,* **2011**, *50*, 8605.

(11) R. Shrestha, P. Mukherjee, Y. Tan, Z. C. Litman and J. F. Hartwig, *J. Am. Chem. Soc.,* **2013**, *135*, 8480.

(12) (a) C. Röben, J. A. Souto, E. C. Escudero-Adán and K. Muñiz, Organic Letters, **2013**, 15, 1008; b) J. A. Souto, C. Martínez, I. Velilla and K. Muñiz, *Angew. Chem. Int. Ed.,* **2013**, *52*, 1324.

(13) N. R. Deprez and M. S. Sanford, *J. Am. Chem. Soc.,* **2009**, *131*, 11234.

(14) R. J. Phipps and M. J. Gaunt, *Science,* **2009**, *323*, 1593.

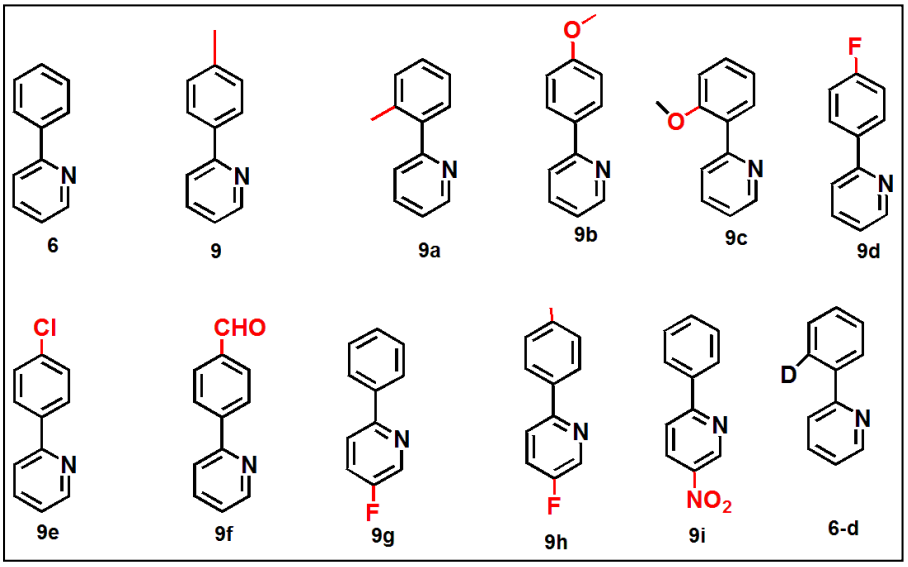
(15) L. Hadjiarapoglou, S. Spyroudis and A. Varvoglis, *Synthesis,* **1983**, 207.

(16) M. Bielawski, M. Zhu and B. Olofsson, *Adv. Synth. Cat.,* **2007**, *349,* 2610.

Experimental Section

Substrates, Reagents and Catalysts.

Potassium phthalimide, saccharin, sodium methoxide, **(**PIFA**)** Phenyliodine bis(trifluoroacetate), (PIDA**)** Iodobenzene diacetate, methanol, **(**ACN**)** acetonitrile, **(**DCM**)** dichloromethane, **(**DCE**)** dichloroethane, copper triflate, palladium acetate and other copper catalysts were purchased from Sigma Aldrich and Fisher Scientific. Flash chromatography was performed on Silicycle silica gel (60Å, 40-63 μm). All reagents were stored under an inert atmosphere before use.



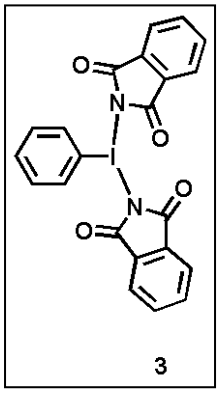
Preparation of 2- arylpyridine derivatives by literature procedures.

Substrates **6, 9** and **9f** were purchased from Sigma Aldrich. The other remaining substrates (**9a, 9b, 9c, 9d, 9g** and **9h**) were prepared via Suzuki coupling using a known procedure.[1] Substrate **9e** was prepared by literature procedure.[2] **9i** was prepared via Suzuki coupling using a literature procedure.[3] Substrate **6-*d*** was prepared by a known procedure.[4]

Instrumentation

Microwave reactions were carried out in a CEM Discover microwave. Flash chromatography was performed using CombiFlash® Rf 200. GC/MS analysis was carried out on an Agilent Technologies 6890 GC system fixed with a 5973 mass selective detector. NMR spectrum were acquired using a Bruker Avance 300MHz spectrometer.

Synthesis of phenyliodine(III) bis(phthalimidate) Iodane (3)



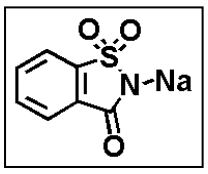
A mixture of (4.5 g, 1.04 mmol) phenyliodine(III) bis(trifluoroacetate) and potassium phthalimide (3.7 g, 2.0 mmol) in 100 mL of acetonitrile was stirred at 40 ^{0}C in an oil bath for 12 h. The off white precipitate was collected, washed with acetonitrile and dried under vaccum to obtain (3.6 g, 70 %) of the phenyliodine (III) bis(phthalimidate), Iodane **3**. The NMR matched with the one published in literature.[5]

^{1}H NMR (300 MHz, $(CD_3)_2SO$): 7.84 (s, 5H), 7.76 (s, 3H), 7.69 – 7.64 (m, 1H), 7.53 – 7.32 (m, 3H), 7.21 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 169.21, 137.08, 134.29, 132.57, 130.64, 127.67, 122.90.

LRMS EI (m/z): [M+] calc'd for $C_{14}H_9INO_2$ [M–Phthalimidate] $^+$ 349.9672, 349.1 observed m/z

Synthesis of sodium-saccharin

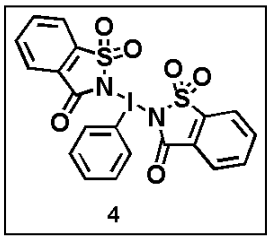


In a dry round bottom flask, a mixture of saccharin (0.25 g, 1.36 mmol) and sodium methoxide (0.073 g, 1.36 mmol) in 10 mL of methanol under nitrogen atmosphere was refluxed for 25 minutes in an oil bath. After the reflux, the flask was allowed to cool and then the excess solvent was removed under pressure to get white solid product. (0.26 g, 92.83 %)

LRMS EI (m/z): [M+] calc'd for $C_7H_4NNa_3O_3S$ 205.1663, observed 206.0 m/z.

Synthesis of phenyliodine(III) bis(saccharin)Iodane (4)

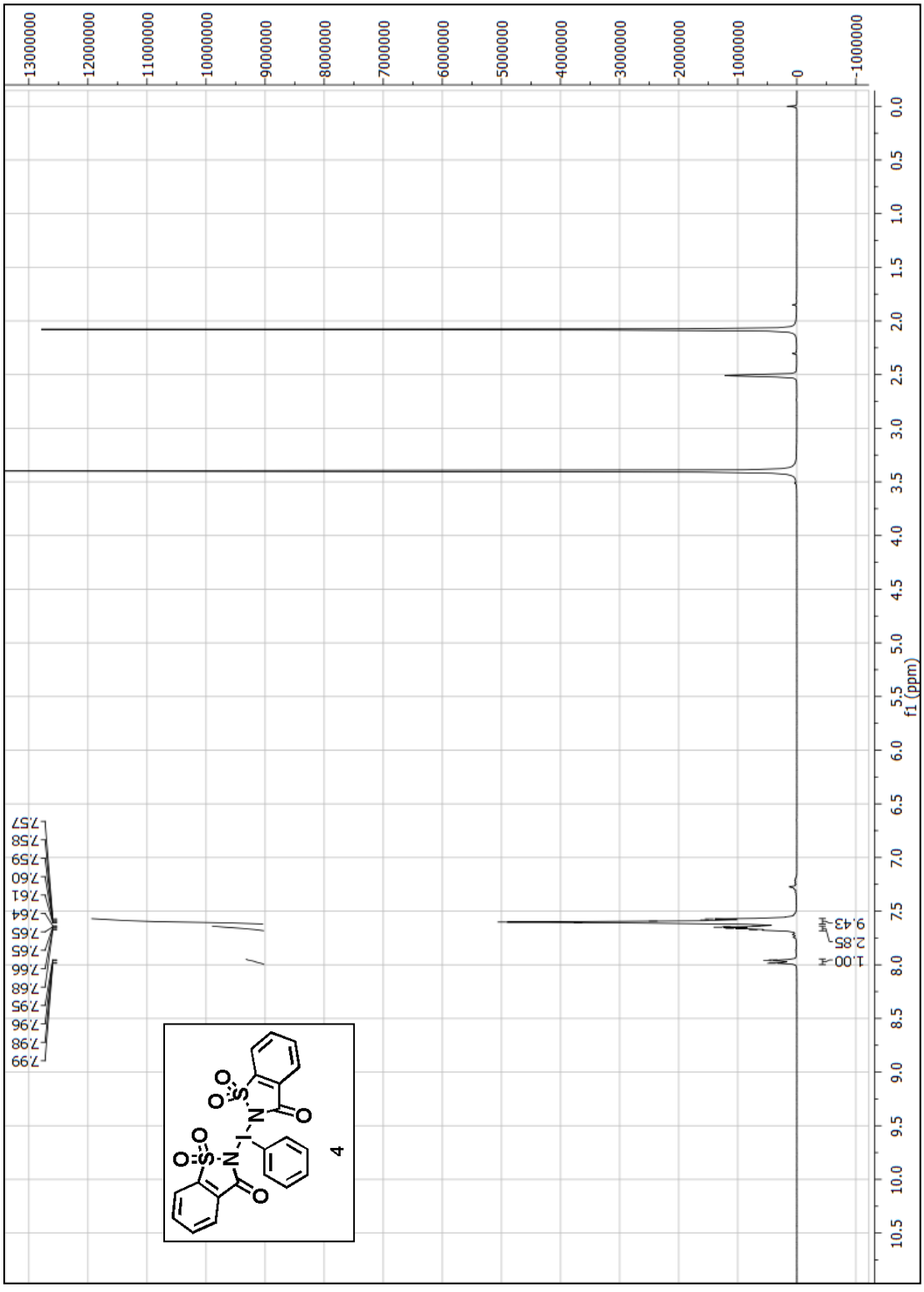
A mixture of (0.27 g, 0.633 mmol) phenyliodine(III) bis(trifluoroacetate) and sodium saccharin (0.26 g, 1.267 mmol) in 100 mL of acetonitrile was stirred at 40 ^{0}C in an oil bath for 12 h. The white precipitate was collected, washed with acetonitrile and dried under vaccum to obtain (0.234 g, 65%) of the phenyliodine(III) bis(saccharin) iodane **4**.



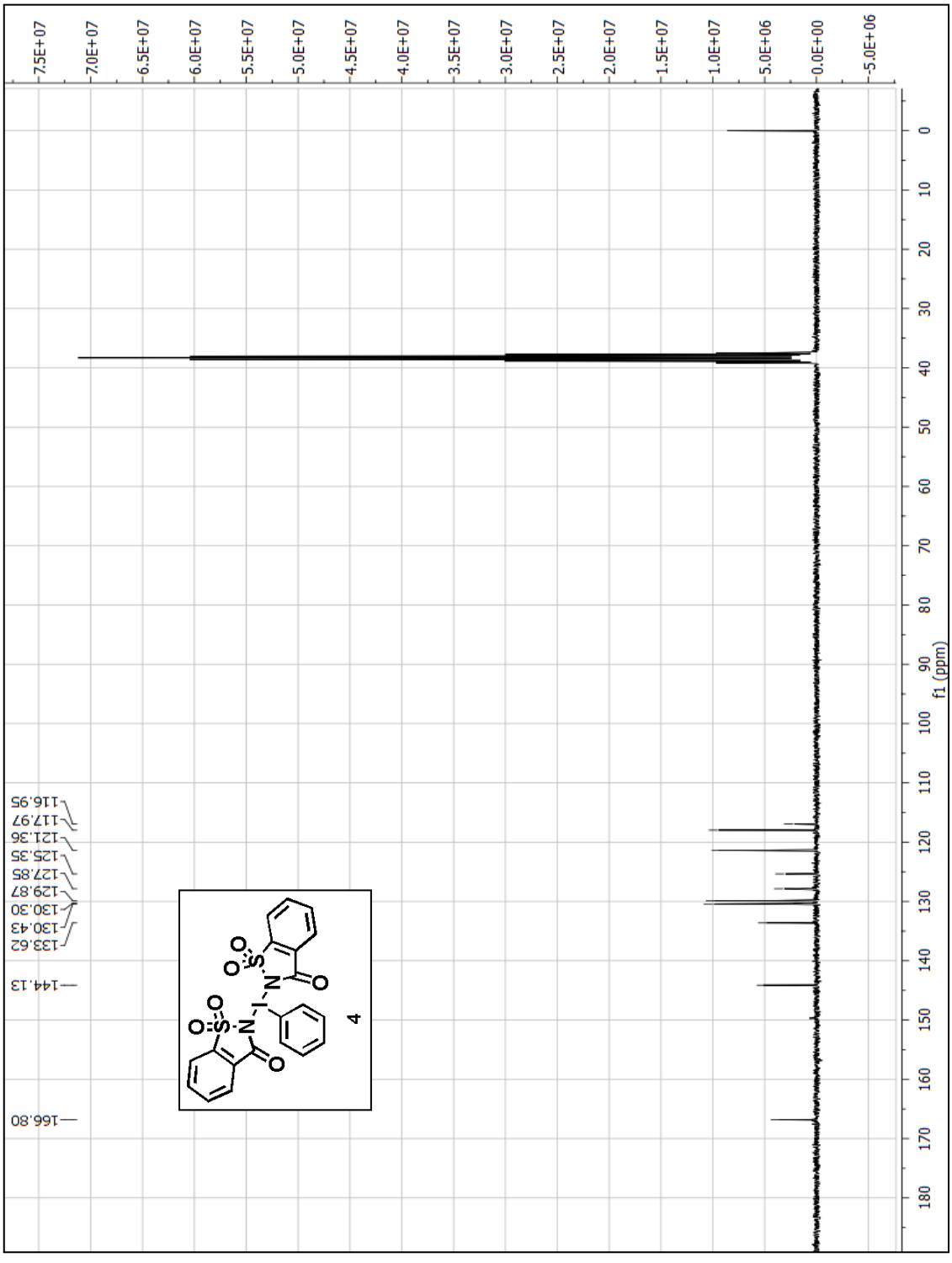
4

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 8.00 – 7.95 (m, 1H), 7.68 – 7.64 (m, 3H), 7.62 – 7.57 (m, 9H).

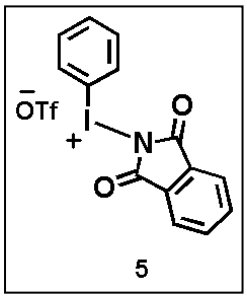
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 166.80, 144.13, 133.62, 130.43, 130.30, 129.87, 127.85, 125.35, 121.36, 117.97, 116.95.



Spectrum 1. ^{1}H NMR of **4**



Spectrum 1.1. ^{13}C NMR of **4**



5

Synthesis of Iodonium Triflate salt (5)

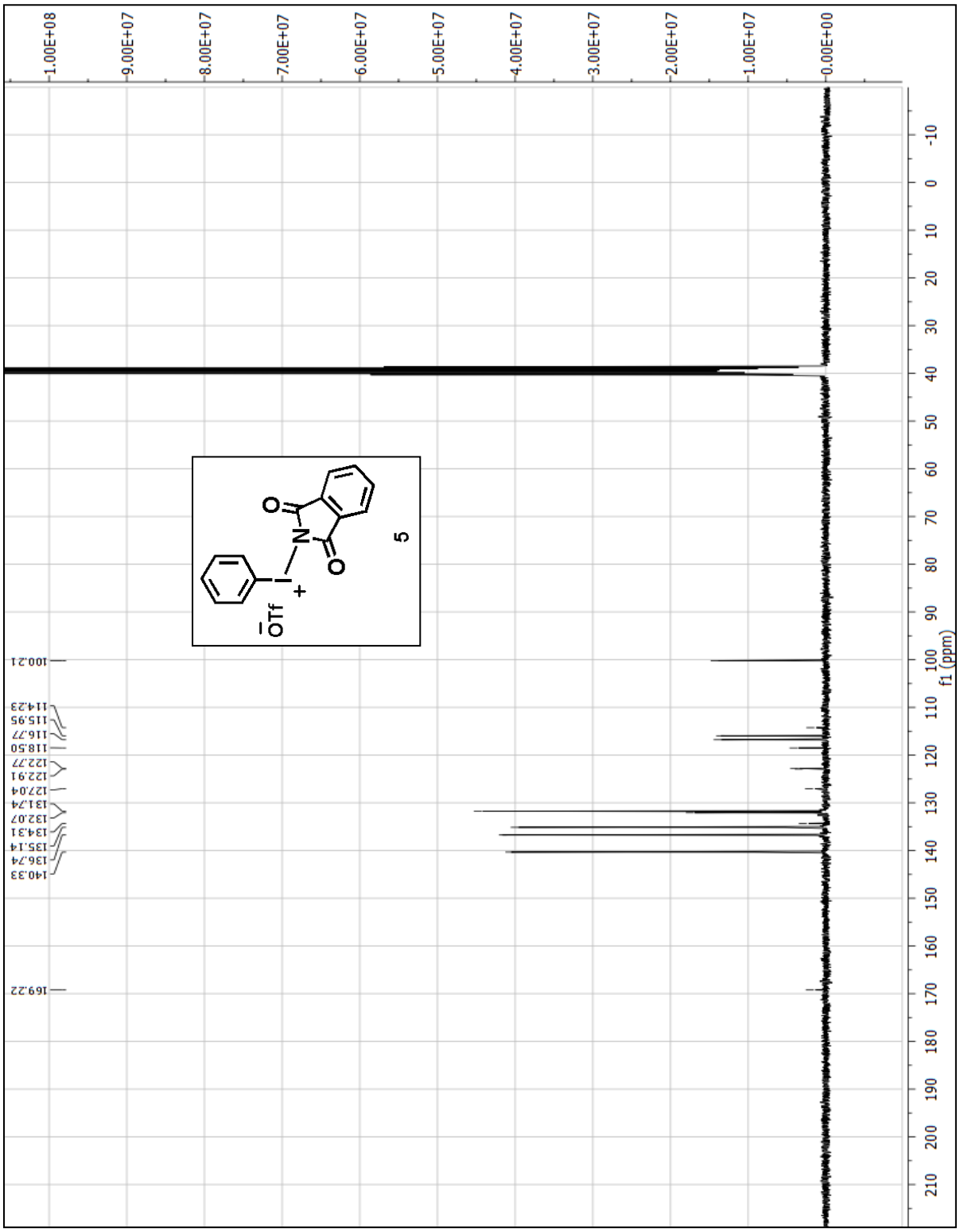
A mixture of *m*-chloroperoxybenzoic acid (0.25 g, 1.495 mmol) and iodobenzene (0.3 g, 1.495 mmol) were dissolved in 2 mL dichloromethane in a vial and stirred at room temperature for 5 minutes. Phathalimide (0.2 g, 1.359 mmol) was added to this vial and the vial was cooled to 0 ^{0}C. This was followed by dropwise addition of triflic acid (0.6 g, 4.078 mmol) to the reaction mixture to give a colored solution. The reaction mixture was later stirred for at room temperature for 30 mins and subsequently concentrated under vacuum. Diethyl ether (2 mL) was added to the vial and the mixture was stirred at room temperature for 10 mins to precipitate out an off-white solid. The solid was filtered off, washed with ether and dried under vacuum to obtain (0.42 g, 61 %) of **5**.

^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 8.24 (d, *J* = 7.8 Hz, 2H), 8.00 (d, *J* = 8.2 Hz, 2H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.67 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 2H)

^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 169.22, 140.33, 136.74, 135.14, 134.31, 132.07, 131.74, 127.04, 122.91, 122.77, 118.50, 116.77, 115.95, 114.23, 100.21

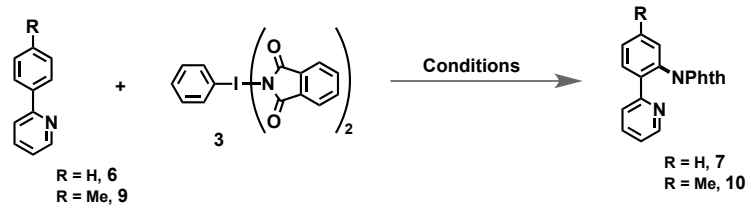
LRMS EI (m/z): [M+] calc'd for $C_{14}H_9INO_2$ $[M–TfO^-]^+$ 349.9672, 349.1 observed m/z.

Spectrum 2. ^{1}H NMR of **5**



Spectrum 2.1. ^{13}C NMR of Compound **5**

Initial Optimization Studies



Entry	R	Conditions	Yield 7/10 (%)
1	H	**3** (1 equiv), PIDA (2.5 equiv) , ACN, 145 ^{0}C (MW) , 3h	-
2	H	**3** (1 equiv), PIFA (2.5 equiv) , ACN , 145 ^{0}C (MW) , 3h	-
3	H	**3** (1 equiv), $Pd(OAc)_2$ (25 mol %) , ACN , 145 ^{0}C (MW) , 3h	13
4	H	**3** (1 equiv), $Cu(OTf)_2$ (25 mol %) , DCM, 70 ^{0}C , 5 h	9
5	H	**3** (2.5 equiv), $Cu(OTf)_2$ (25 mol %) , DCM, 70 ^{0}C , 48 h	63
6	H	**3** (1 equiv), $Cu(OTf)_2$ (1 equiv) , DCM, 70 ^{0}C , 48 h	80
7	H	**3** (2.5 equiv), $Cu(OTf)_2$ (1 equiv) , DCE, 70 ^{0}C , 48 h	87
8	Me	**3** (2.5 equiv), CuF_2 (1 equiv) , DCE, 80 ^{0}C , 48 h	5

9	Me	**3** (2.5 equiv), CuCl (1 equiv) , DCE, 80 ^{0}C , 48 h	6
10	Me	**3** (2.5 equiv), CuBr (1 equiv) , DCE, 80 ^{0}C , 48 h	9
11	Me	**3** (2.5 equiv), $CuBr_2$ (1 equiv) , DCE, 80 ^{0}C , 48 h	5
12	Me	**3** (2.5 equiv), $CuSO_4$ (1 equiv) , DCE, 80 ^{0}C , 48 h	5
13	Me	**3** (2.5 equiv), $Cu(NO_3)_2$ (1 equiv) , DCE, 80 ^{0}C , 48 h	5
14	Me	**3** (2.5 equiv), CuCN (1 equiv) , DCE, 80 ^{0}C , 48 h	5

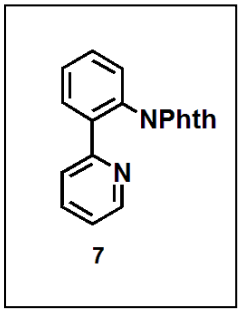
Table 1. Initial Optimization Studies

General procedure for Substrate Library

To a solution of the appropriate 2-arylpyridine (1 equiv) in 1,2-dichloroethane (4 mL) was added the appropriate iodane (2.5 equiv) and $Cu(OTf)_2$ (1 equiv). The reaction was stirred for the 48 h at 80 ^{o} C in an oil bath before dilution with DCM (30 mL) and washing with saturated sodium bicarbonate solution (30 mL). The aqueous phase was extracted further with DCM (25 mL) and the combined organic layers were dried over sodium sulphate and the excess solvent was removed under pressure. The crude residue was purified by flash column chromatography to obtain the pure aminated product.

This general procedure was followed for synthesis of **7, 10, 11, 12, 14, 15, 16, 17, 18, 19** and **20**

Synthesis of 2-(2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (7)



7

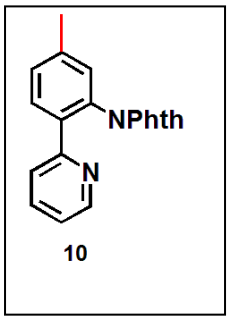
Substrate **6** was subjected to the general procedure. After purification by column chromatography, **7** was obtained (91 mg, 87%). The NMR matched with the one published in literature.[6]

***R*$_f$ -Value:** Hexane/Ethyl acetate (3:2 *v/v*) = 0.29.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 7.84 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.76 – 7.71 (m, 3H), 7.65 (td, *J* = 7.9, 2.1 Hz, 1H), 7.59-7.54 (m, 2H), 7.47 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.44-7.40 (m, 1H), 7.07 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H).

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{12}N_2O_2$ 300.0899, 300.11 observed m/z.

Synthesis of 2-(5-methyl-2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (10)



10

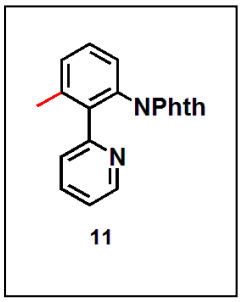
Substrate **9** was subjected to the general procedure. After purification by column chromatography, **10** was obtained (40.2 mg, 88 %). The NMR matched with the one published in literature.[6]

***R*$_f$ -Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.29.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (ddd, *J* = 4.8, 1.9, 1.0 Hz, 1H), 7.84 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.72 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.62 (td, *J* = 7.8, 1.7 Hz, 2H), 7.48 – 7.35 (m, 2H), 7.22 (d, *J* = 1.1 Hz, 1H), 7.03 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 2.46 (s, 3H).

LRMS EI (m/z): [M+] calc'd for $C_{20}H_{14}N_2O_2$ 314.1055, 314.19 observed m/z.

Synthesis of 2-(3-methyl-2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (11)



11

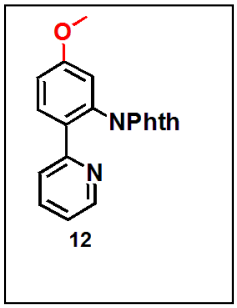
Substrate **9a** was subjected to the general procedure. After purification by column chromatography, **11** was obtained (13.1 mg, 40 %). The NMR matched with the one published in literature.[6]

***R*$_f$-Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.3.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (ddd, J = 4.8, 1.7, 1.0 Hz, 1H), 7.83-7.75 (m, 4H), 7.68 (td, J = 7.7, 1.8 Hz, 1H), 7.5 -7.45 (m, 2H), 7.37-7.33 (m, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.14 (ddd, J = 7.6, 4.9, 1.0 Hz, 1H), 2.06 (s, 3H)

LRMS EI (m/z): [M+] calc'd for $C_{20}H_{14}N_2O_2$ 314.1055, 314.19 observed m/z.

Synthesis of 2-(5-methoxy-2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (12)



12

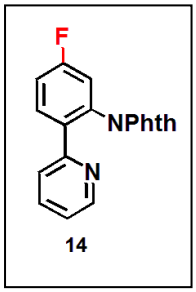
Substrate **9b** was subjected to the general procedure. After purification by column chromatography, **12** was obtained (22.1 mg, 50%). The NMR matched with the one published in literature.[6]

***R*$_f$-Value:** Hexane/Ethyl acetate (7:3*v/v*) = 0.24.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49 (d, *J* = 4.9 Hz, 1H), 8.33 – 8.29 (m, 1H), 8.04 – 7.67 (m, 4H), 7.60 – 7.48 (m, 2H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.05 – 6.99 (m, 2H), 3.82 (s, 3H).

LRMS EI (m/z): [M+] calc'd for $C_{20}H_{14}N_2O_3$ 330.1005, observed 330 m/z.

Synthesis of 2-(5-fluoro-2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (14)



14

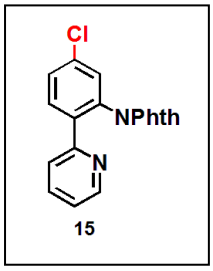
Substrate **9d** was subjected to the general procedure. After purification by column chromatography, **14** was obtained (16 mg, 37 %). The NMR matched with the one published in literature.[6]

***R_f*-Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.23

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 7.88 – 7.83 (m, 2H), 7.78 – 7.61 (m, 4 H), 7.44 (dt, *J* = 7.9, 1.1 Hz, 1H), 7.31 (dd, *J* = 8.3, 2.5 Hz, 1H), 7.17 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.08 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H).

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{11}FN_2O_2$ 318.0805, 318.01 observed m/z.

Synthesis of 2-(5-chloro-2-(pyridin-2-yl)phenyl)isoindoline-1,3-dione (15)



15

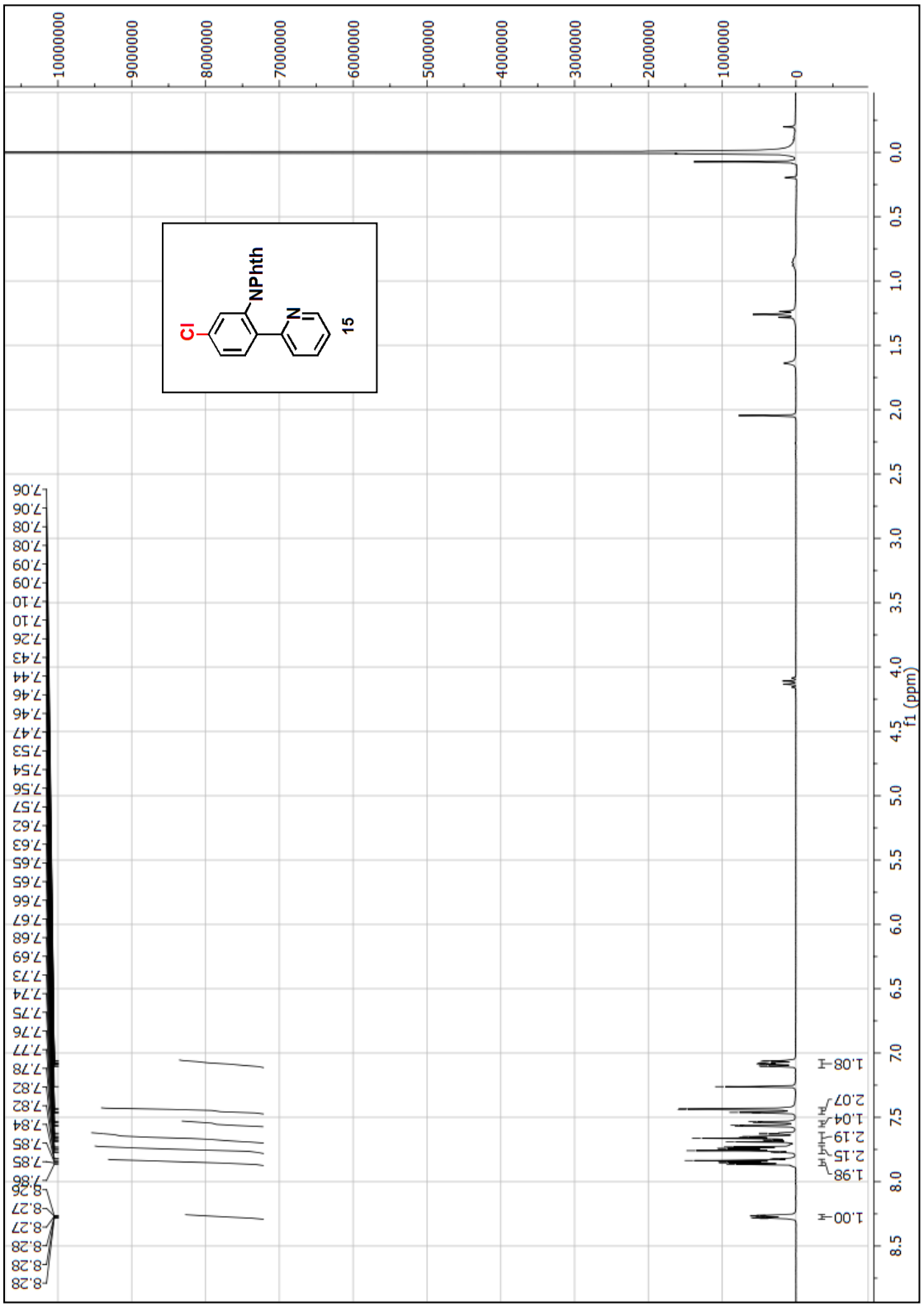
Substrate **9e** was subjected to the general procedure. After purification by column chromatography, **15** was obtained (106.5 mg, 41 %).

***R_f*-Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.26

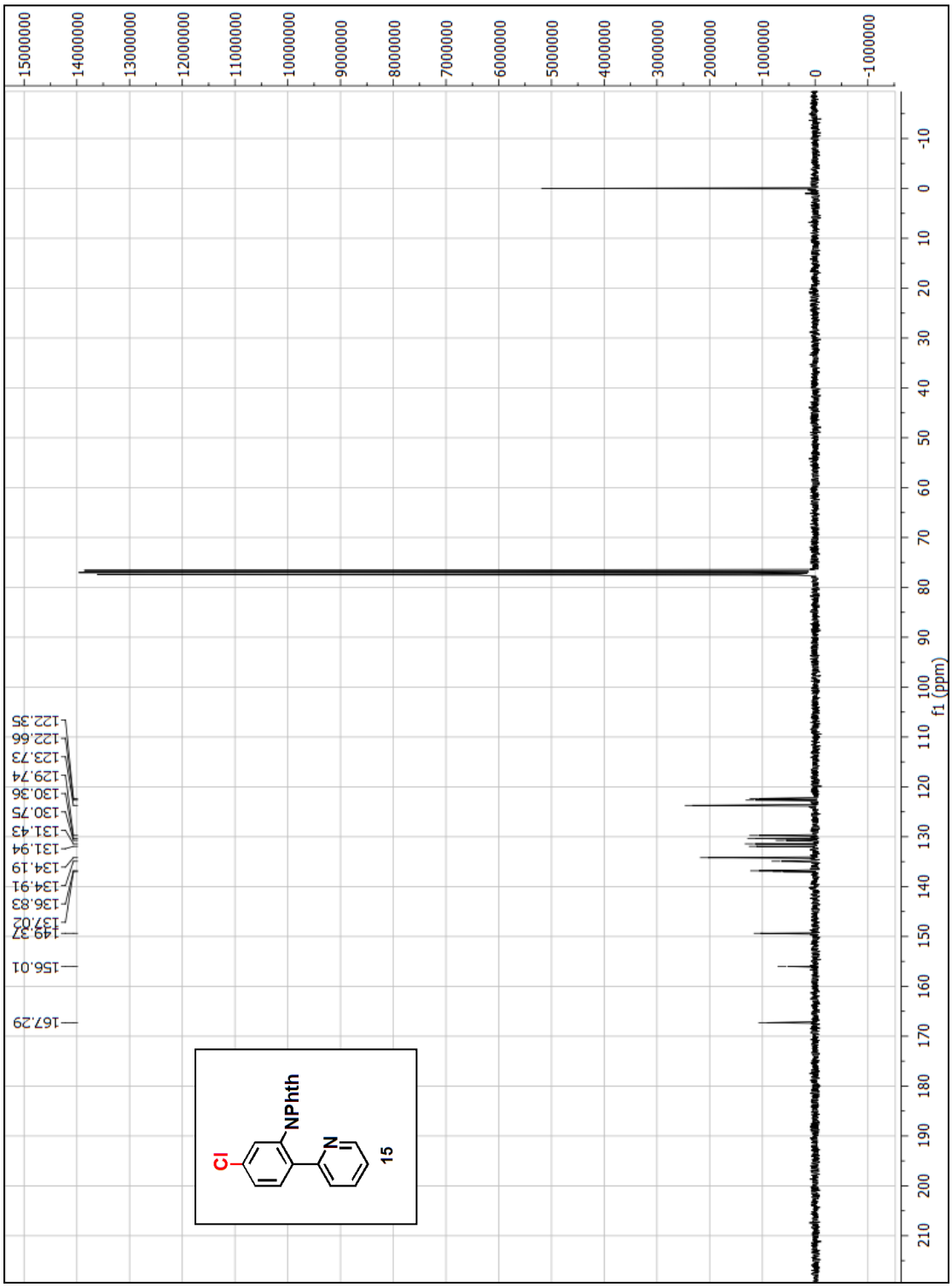
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (ddd, *J* = 4.8, 1.9, 0.9 Hz, 1H), 7.85 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.78 – 7.72 (m, 2H), 7.70 – 7.62 (m, 2H), 7.55 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.45 (dd, *J* = 7.3, 1.5 Hz, 2H), 7.08 (ddd, *J* = 7.7, 4.9, 1.2 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3, 156.0, 149.4, 137.0, 136.8, 134.9, 134.2, 131.9, 131.4, 130.8, 130.4, 129.7, 123.7, 122.7, 122.4.

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{11}ClN_2O_2$ 334.0509, 334.15 observed m/z.

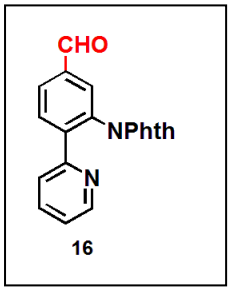


Spectrum 3. ^{1}H NMR of **15**



Spectrum 3.1. ^{13}C NMR of **15**

Synthesis of 3-(1,3-dioxoisoindolin-2-yl)-4-(pyridin-2-yl)benzaldehyde (16)



16

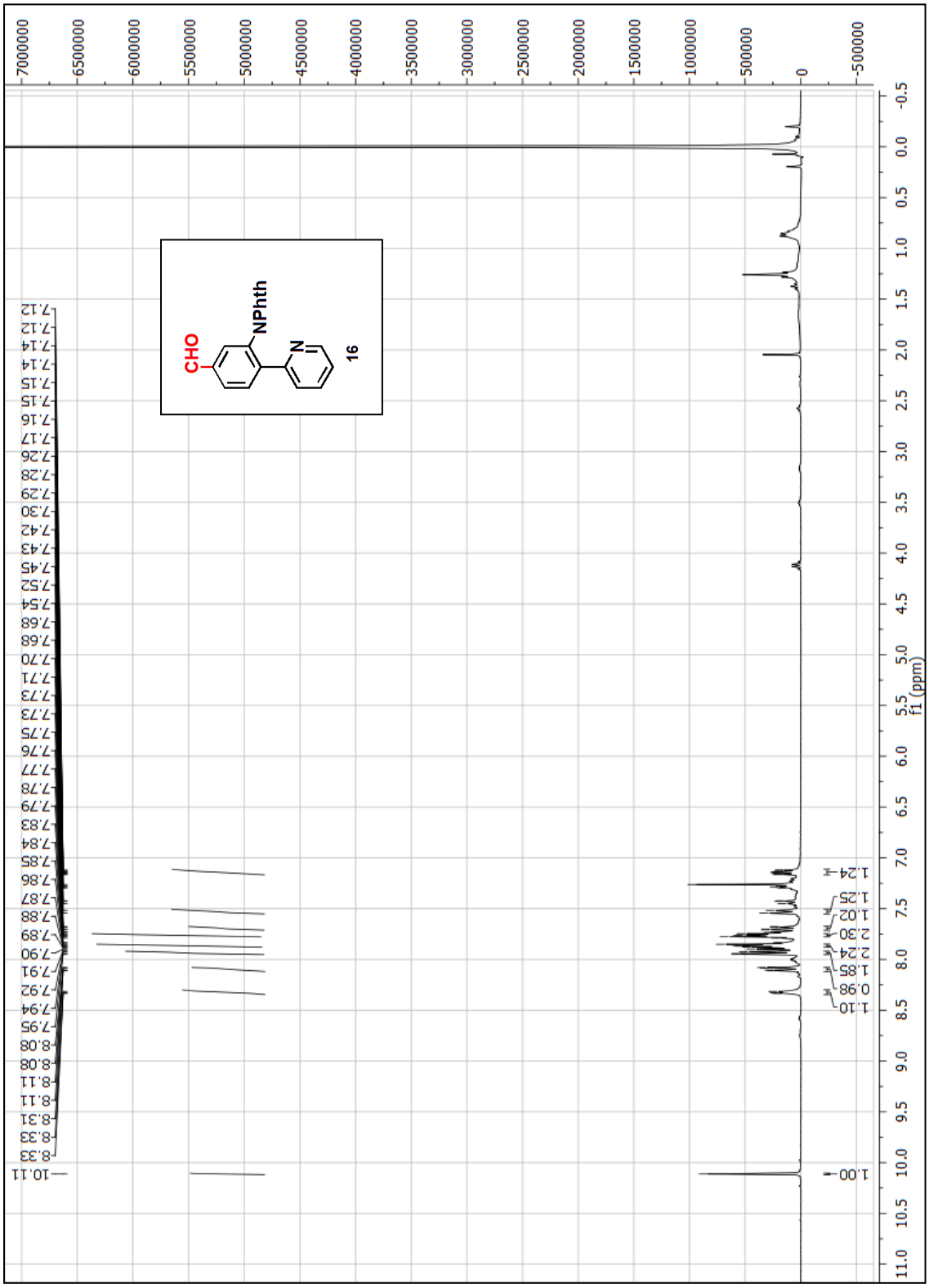
Substrate **9f** was subjected to the general procedure. After purification by column chromatography, **16** was obtained (69.3 mg, 40 %).

***R*$_f$ -Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.2

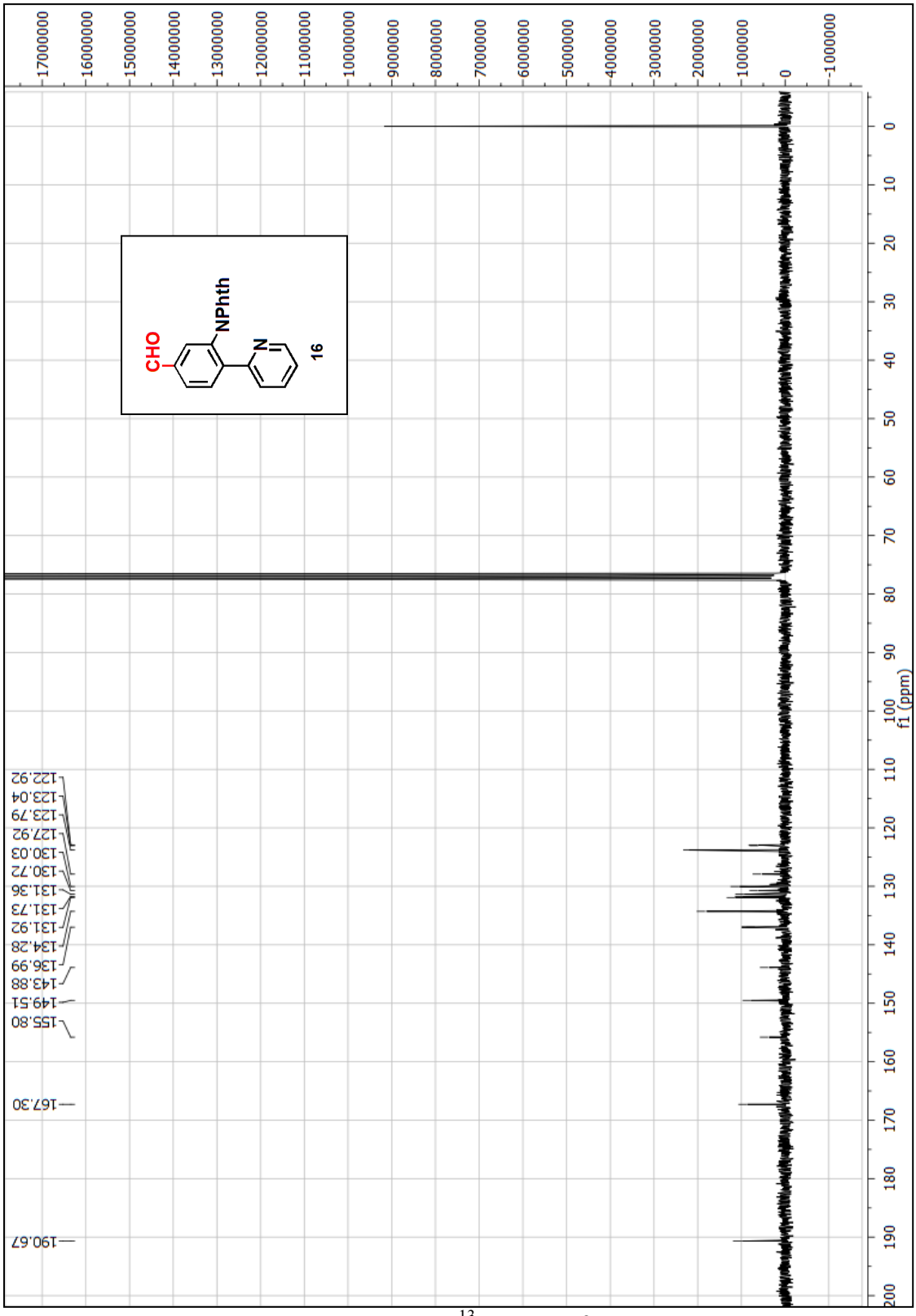
^{1}H NMR (300 MHz, $CDCl_3$): δ 10.11 (s, 1H), 8.35 – 8.30 (m, 1H), 8.10 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.95 – 7.92 (m, 2H), 7.87 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.76 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.69 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.14 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 190.7, 167.3, 155.8, 149.5, 143.9, 137.0, 134.3, 131.9, 131.7, 131.4, 130.7, 130.0, 127.9, 123.8, 123.0, 122.9.

LRMS EI (m/z): [M+] calc'd for $C_{20}H_{12}N_2O_3$ 328.0848, 328.04 observed m/z.

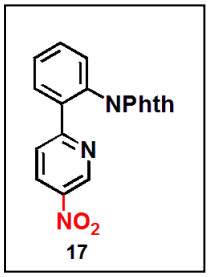


Spectrum 4. ^{1}H NMR of **16**



Spectrum 4.1. ^{13}C NMR of **16**

Synthesis of 2-(2-(5-nitropyridin-2-yl)phenyl)isoindoline-1,3-dione (17)



17

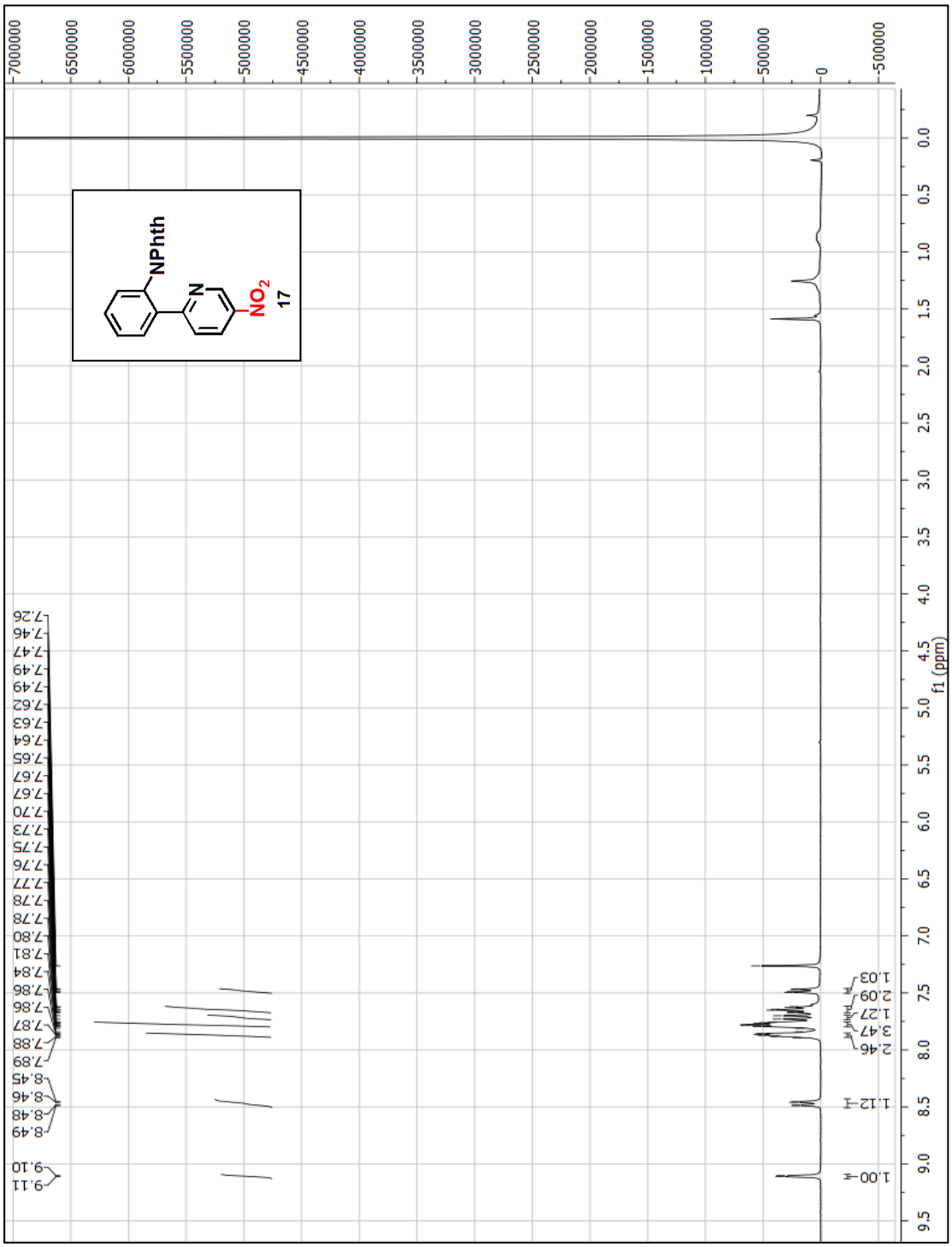
Substrate **9i** was subjected to the general procedure as described above. After purification by column chromatography, **17** was obtained (36.3 mg, 27%).

***R*$_f$ -Value:** Dichloromethane = 0.69

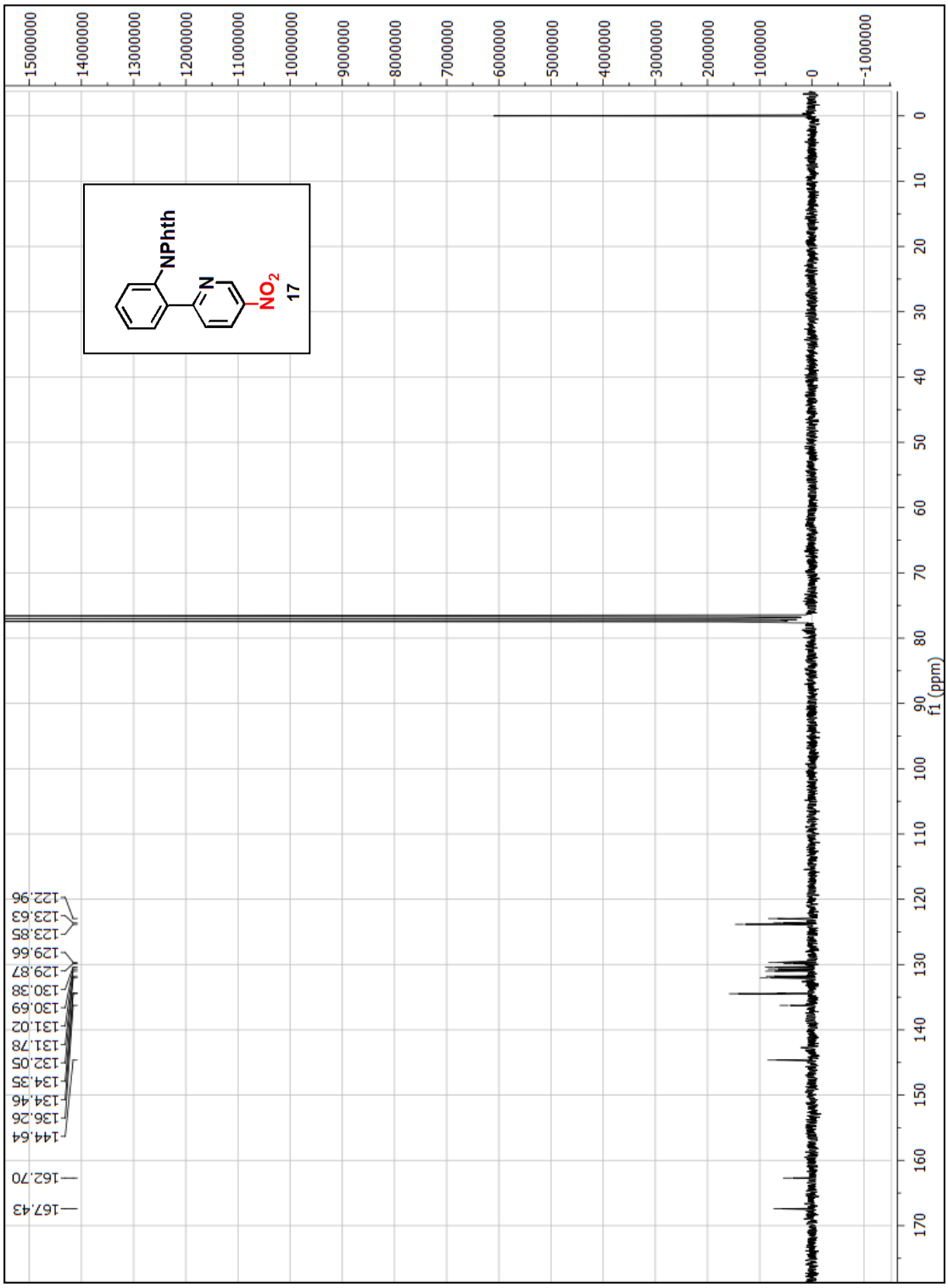
^{1}H NMR (300 MHz, $CDCl_3$): δ 9.11 (d, *J* = 2.7 Hz, 1H), 8.47 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.87 (dd, *J* = 5.4, 2.9 Hz, 2H), 7.80 – 7.76 (m, 3H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.65 (td, *J* = 6.7, 1.9 Hz, 2H), 7.48 (dd, *J* = 7.3, 1.9 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.4, 162.7, 144.6, 136.3, 134.5, 134.4, 132.05, 131.8, 131.0, 130.7, 130.4, 129.9, 129.7, 123.9, 123.6, 123.0.

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{11}N_3O_4$ 345.075, 345.02 observed m/z.

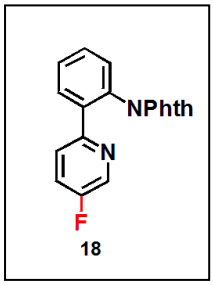


Spectrum 5. ^{1}H NMR of **17**



Spectrum 5.1. ^{13}C NMR of **17**

Synthesis of 2-(2-(5-fluoropyridin-2-yl)phenyl)isoindoline-1,3-dione (18)



18

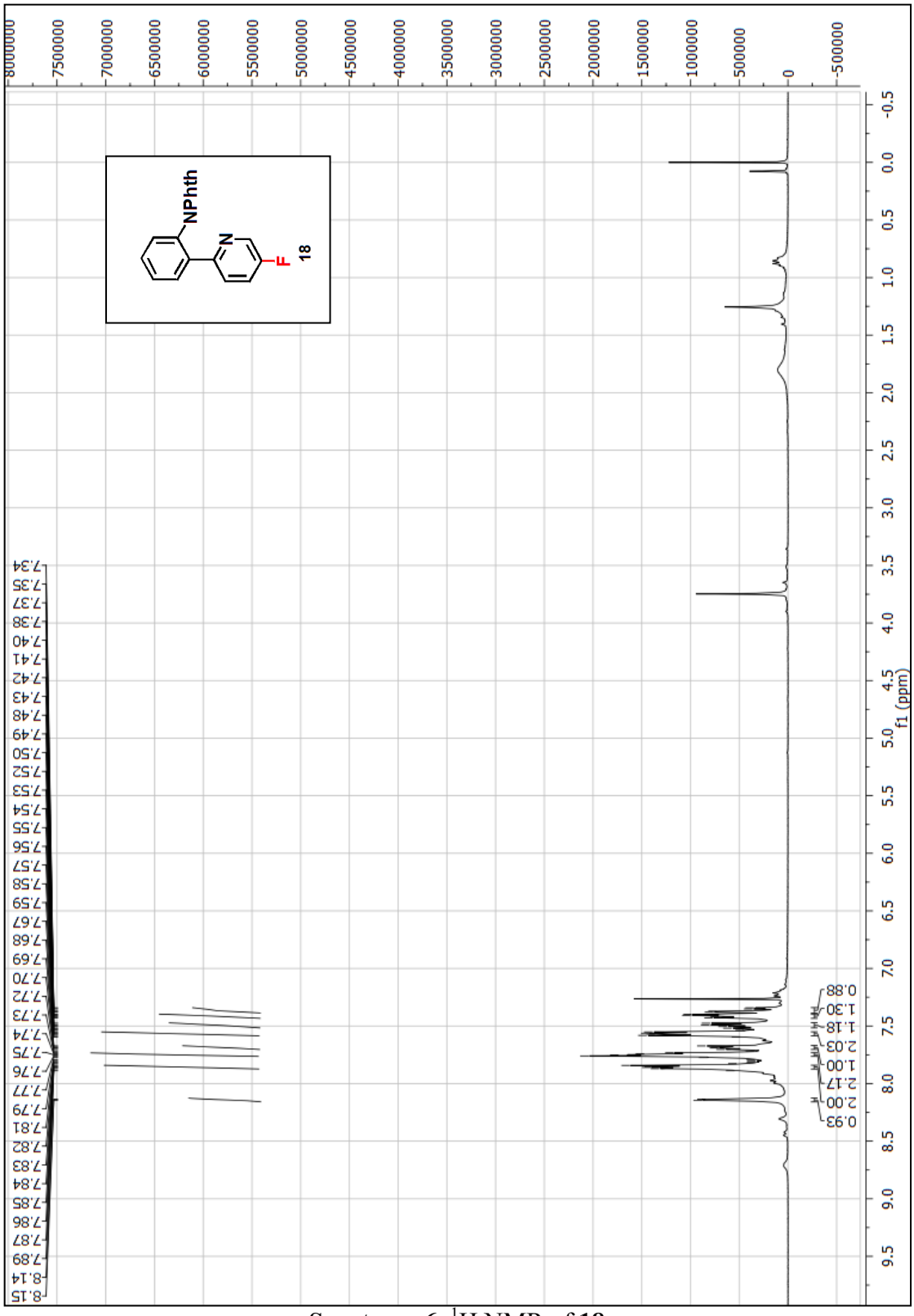
Substrate **9g** was subjected to the general procedure. After purification by column chromatography, **18** was obtained (152.1 mg, 83 %).

***R*$_f$ -Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.2.

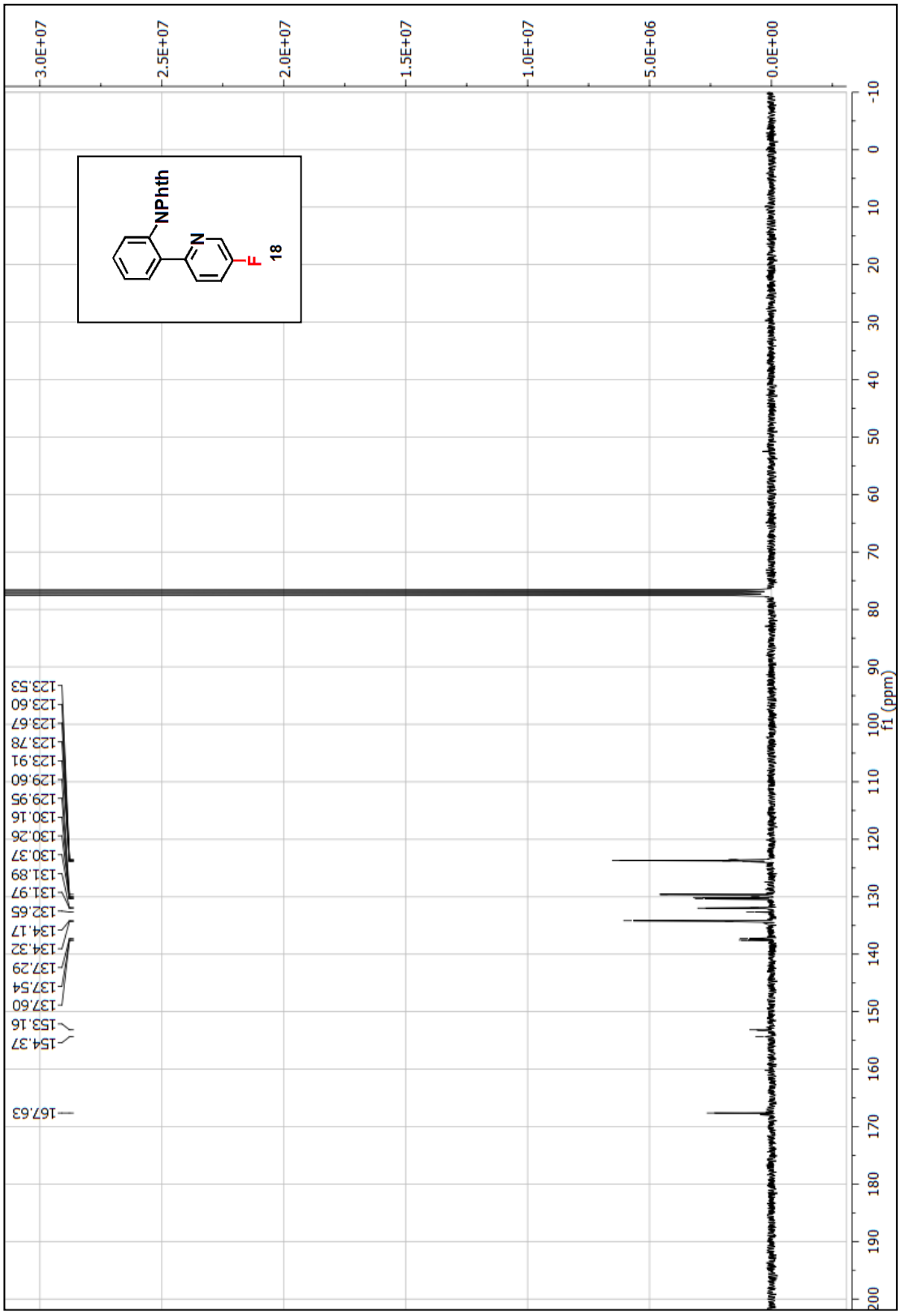
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, *J* = 2.8 Hz, 1H), 7.87 – 7.84 (m, 2H), 7.76 – 7.73 (m, 2H), 7.69 (dd, *J* = 5.7, 3.4 Hz, 1H), 7.57 (dd, *J* = 5.7, 3.4 Hz, 2H), 7.52 – 7.47 (m, 1H), 7.41 (dd, *J* = 6.0, 3.2 Hz, 1H), 7.36 (dd, *J* = 8.5, 2.8 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.6, 153.2 (d, $J_{C\text{-}F}$ = 4.3 Hz), 137.6 (d, $J_{C\text{-}F}$ = 4.2 Hz), 135.8 (d, $J_{C\text{-}F}$ = 223.7 Hz), 134.17, 132.7, 132.0, 130.3 (d, $J_{C\text{-}F}$ = 17.8 Hz), 129.6, 123.9 (br), 123.8, 123.7, 123.6, 123.5.

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{11}FN_2O_2$ 318.0805, 318.11 observed m/z.

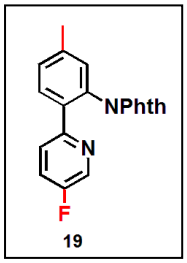


Spectrum 6. ^{1}H NMR of **18**



Spectrum 6.1. ^{13}C NMR of **18**

Synthesis of 2-(2-(5-fluoropyridin-2-yl)-5-methylphenyl)isoindoline-1,3-dione (19)



19

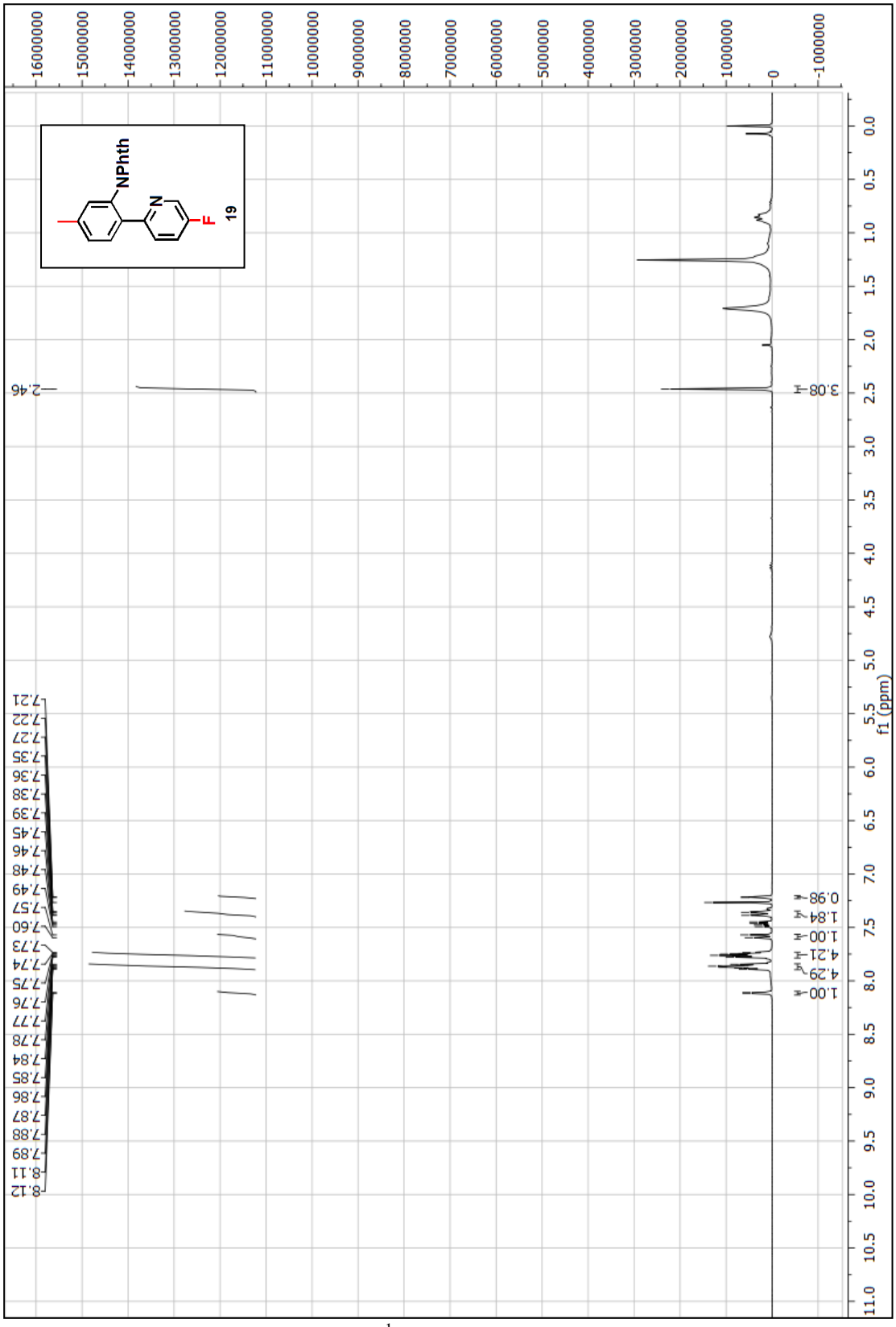
Substrate **9h** was subjected to the general procedure as described above. After purification by column chromatography, **19** was obtained (66.6 mg, 34 %).

***R_f*-Value:** Dichloromethane = 0.81.

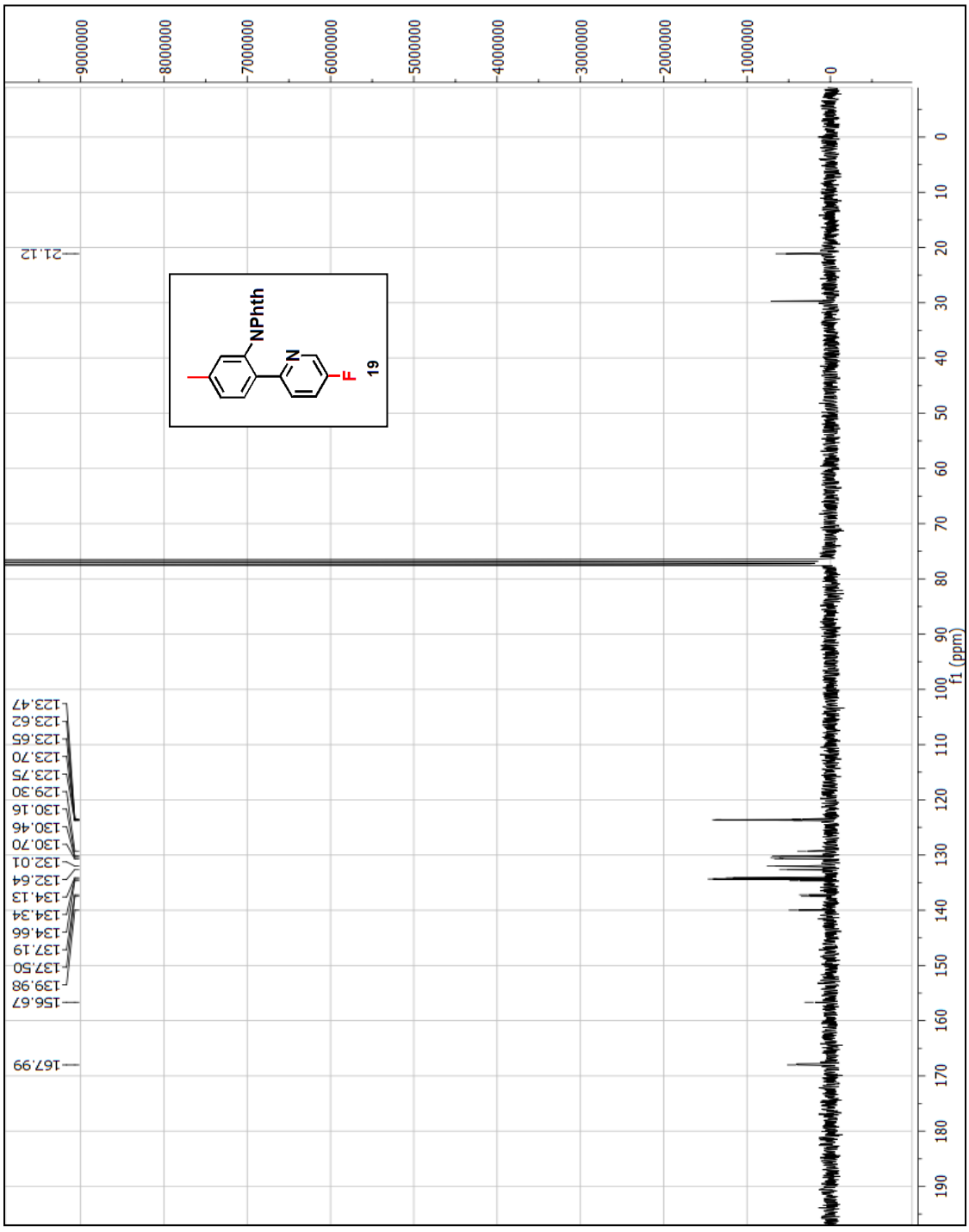
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (d, *J* = 2.9 Hz, 1H), 7.87 (td, *J* = 5.6, 3.0 Hz, 4H), 7.76 (td, *J* = 5.3, 3.0 Hz, 4H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.37 (dd, *J* = 8.7, 2.6 Hz, 2H), 7.22 (d, *J* = 1.8 Hz, 1H), 2.46 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 156.7, 138.6 (d, $J_{C\text{-}F}$ = 210.6 Hz), 137.5, 134.66, 134.34, 134.2 (d, $J_{C\text{-}F}$ = 16.2 Hz), 132.6, 132.0, 130.6 (d, $J_{C\text{-}F}$ = 17.3 Hz), 130.2, 129.3, 123.7, 123.6 (br), 21.1.

LRMS EI (m/z): [M+] calc'd for $C_{20}H_{13}FN_2O_2$ 332.0961, 332 observed m/z.

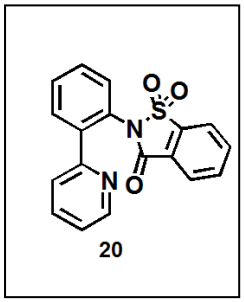


Spectrum 7. ^{1}H NMR of Compound **19**



Spectrum 7.1. ^{13}C NMR of **19**

Synthesis of 20



20

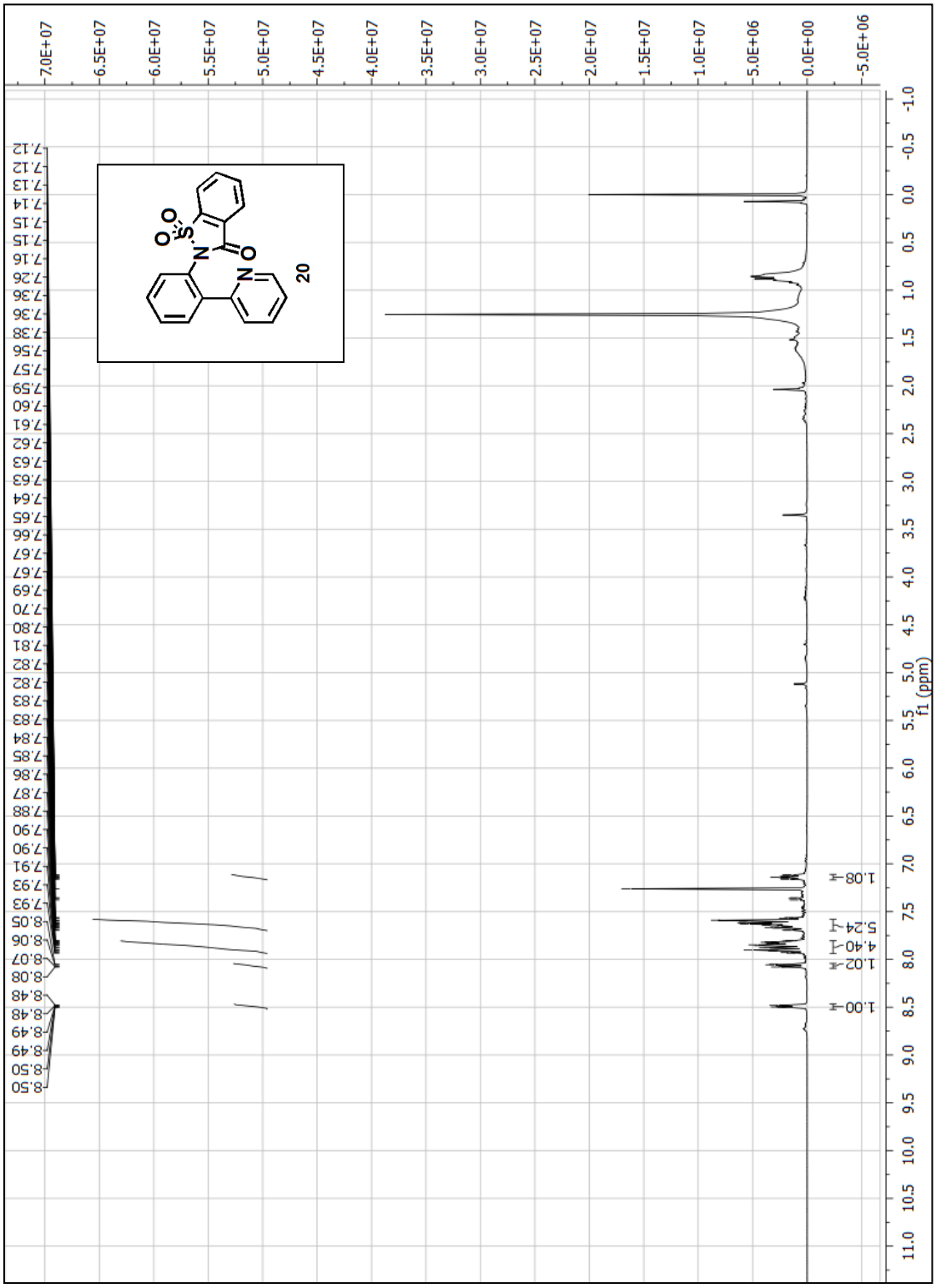
Substrate **4** was subjected to the general procedure as described above. After purification by column chromatography, **20** was obtained (10.5 mg, 88 %).

***R_f*-Value:** Hexane/Ethyl acetate (7:3 *v/v*) = 0.14.

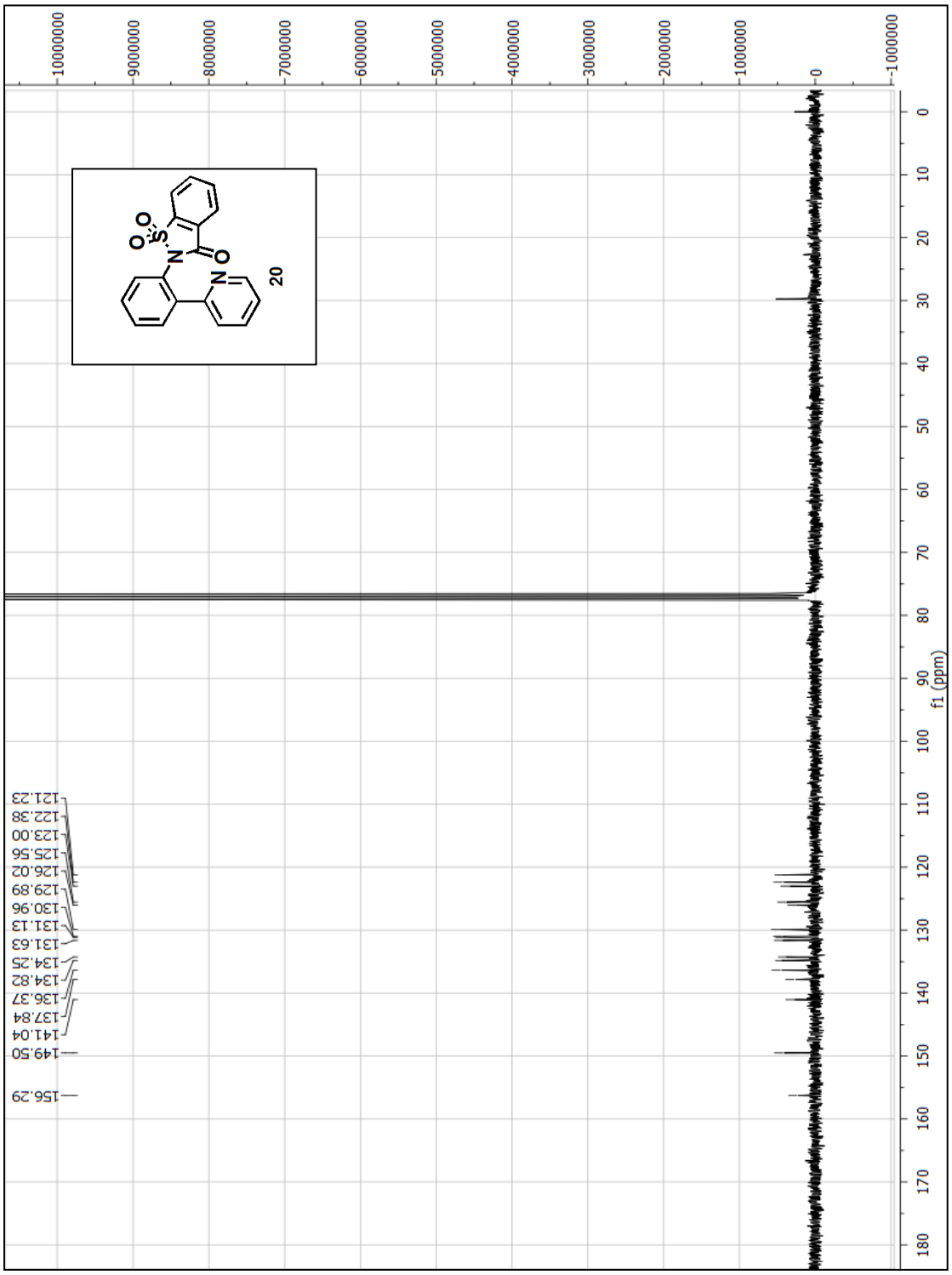
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49 (dt, *J* = 4.9, 1.4 Hz, 1H), 8.07 (dd, *J* = 6.8, 1.7 Hz, 1H), 7.94 – 7.81 (m, 4H), 7.70 – 7.58 (m, 5H), 7.14 (ddd, *J* = 6.7, 4.8, 2.1 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 156.3, 149.5, 141.0, 137.8, 136.4, 134.8, 134.3, 131.6, 131.1, 131.0, 129.9, 126.0, 125.6, 123.0, 122.4, 121.2.

LRMS EI (m/z): [M+] calc'd for $C_{19}H_{11}N_3O_4$ 336.0569, 336.11 observed m/z.

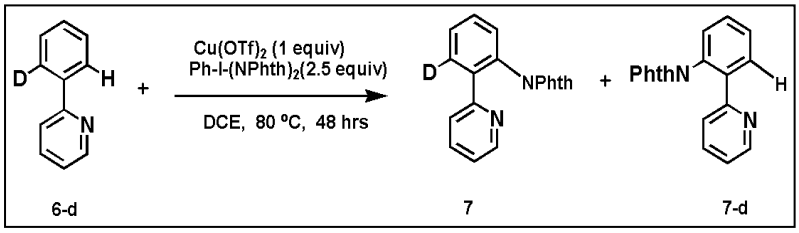


Spectrum 8. ^{1}H NMR of **20**



Spectrum 8.1. ^{13}C NMR of **20**

Kinetic Isotope Effect

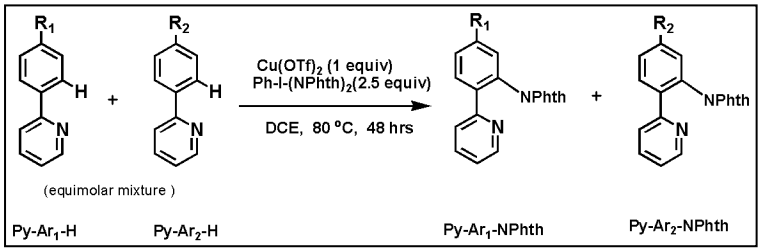


To a solution of **6-d** (0.0179 g, 0.114 mmol) in 1,2-dichloroethane (4 mL) was added the iodane **3** (0.142 g, 0.286 mmol) and $Cu(OTf)_2$ (0.041 g, 0.114 mmol). The reaction mixture was stirred for 48 h at 80 ^oC in an oil bath. The reaction was then cooled, and an aliquot was removed and analyzed by GC/MS.

GC/MS Conditions: J & W Scientific DB-1, capillary 25.0m x 200μm x 0.33μm, 1.3 mL/min, 40 ^oC, hold 0.50min, 12 ^oC/min to 320 ^oC, hold 6.0 min.

$\mathbf{k_H/k_D}$ = 1.15

Competition experiment

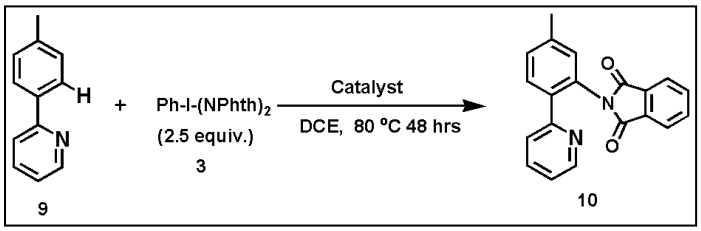


To a solution of equimolar amounts of Py-Ar_1-H (1equiv) and Py-Ar_2-H (1equiv) in 1,2-dichloroethane (4 mL) was added the iodane **3** (2.5 equiv) and $Cu(OTf)_2$ (1 equiv). The reaction was mixture was stirred for the 48 h at 80 ^oC in an oil bath. The reaction was then cooled, and an aliquot was removed and analyzed by GC/MS

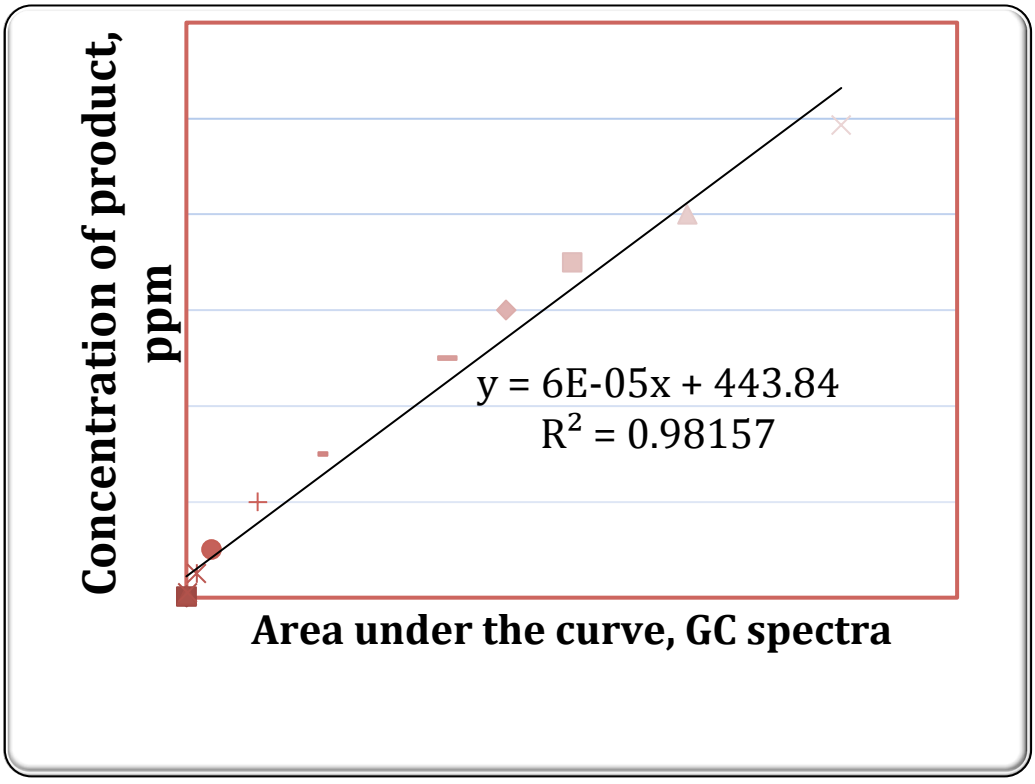
	Py-Ar_1-H **R_1**	**Py-Ar_2-H** **R_2**	**Py-Ar_1-NPhth (%)[a]**	**Py-Ar_2-NPhth (%)[a]**
1	H	Me	42	58
2	H	F	92	8
3	Me	F	94	6

[a] Mole fractions determined by GC/MS

Calibration Curve



The pure product **10** was isolated by column chromatography. Known Concentrations in ppm for (1-100% yield) were prepared in DCE. The plot of various concentrations against their area under the curve from GC-MS spectrum generates the calibration curve.



References

[1] C. Liu, W. Yang, *Chemical Communications* **2009**, 6267-6269.

[2] S.-H. Kim, R. D. Rieke, *Tetrahedron Letters* **2009**, *50*, 5329-5331.

[3] C. Liu, N. Han, X. Song, J. Qiu, *European Journal of Organic Chemistry* **2010**, *2010*, 5548-5551.

[4] X. Chen, X.-S. Hao, C. E. Goodhue, J.-Q. Yu, *Journal of the American Chemical Society* **2006**, *128*, 6790-6791.

[5] K. Moriyama, K. Ishida, H. Togo, *Organic Letters* **2012**, *14*, 946-949.

[6] S. Yu, B. Wan, X. Li, *Organic Letters* **2013**, *15*, 3706-3709.

MANUSCRIPT 3

Efforts Towards the Synthesis of a Molecular Probe Targeting the D_2 Receptor

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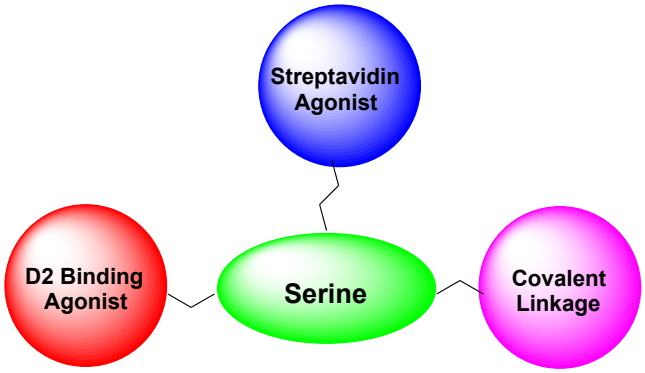
Manuscript 3

Efforts Towards the Synthesis of a Molecular Probe Targeting the D_2 Receptor



Abstract

Over the last several decades the dopamine receptors have been extensively targeted for drug development and design. Herein, a report focusing on the efforts made towards the development of a D_2 dopamine probe will be discussed. Additionally, a proposed synthetic pathway that should allow for the successful isolation of the D_2 receptor from lysed cellular mixtures will be detailed.

Introduction

The D_2 dopamine receptor is a membrane-bound protein that binds the neurotransmitter, dopamine, in the synaptic cleft. In addition to its normal functions that are associated with cognitive reward responses, the D_2 receptor is believed to be associated with a number of neurobehavioral disorders.[1] Degradation or inhibition of any of the D_1-D_5 receptor subunits of dopamine have been linked to Parkinson's disease, certain motor and hyperactivity disorders, schizophrenia, and substance abuse. As such, medicinal chemists have exhausted a significant amount of resources

attempting to construct dopamine agonists in order to develop a new class of pharmaceuticals, particularly with respect to the D_2 receptor family.[1] It is widely accepted that pharmaceuticals prescribed to treat such neurological disorders must share the common trait of inhibiting the D_2 receptor, but little is known with regards to the correlation between therapeutic benefit and the levels of D_2 agonist selectivity.[2] Due to the fact this target is a membrane bound receptor, a crystal structure elucidating it's characteristics has yet to be obtained thus making logical drug design difficult. In order to bridge this gap, mechanistic insight elucidating the therapeutic properties of D_2 selective agonists needs to be more thoroughly understood.[2]

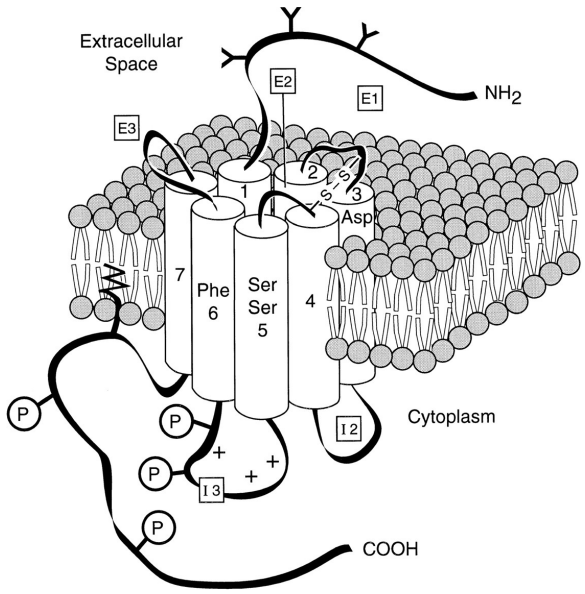


Figure 1: Dopamine Receptor Structure[3]

Dopamine is a membrane bound protein and is subdivided into five unique G protein-coupled receptors, D_1-D_5 (Figure 1).[3-4] Amongst these five subdivisions, each receptor is classified under two main families: D_1-like receptors and D_2-like receptors.[2] The D_2 receptor is the second most abundant dopamine receptor and is more highly concentrated in deeper regions of the mammalian forebrain such as

hypothalamus, amygdala, and hippocampus.[1,5] As a result, finding suitable ligands to serve as agonists that are capable of both crossing the blood-brain barrier and eliciting a biological response is often problematic. Extensive studies have been performed to elucidate general characteristics of D_2 receptor-selective ligands. Amongst the class of receptor-selective ligands are isoquinoline, 2-amino-1-phenyl-2,3-dihydroindene, aporphine and their derivatives.[1] However, molecules such as racolopride, *N*-methylspiperone, and IBZM have proven to be the most beneficial when trying to image the dopamine receptors as they are D_2 selective agonists (Figure 2). Through positron emission tomography and single-photon emission computer tomography the aforementioned molecules, once radio labeled, serve as excellent imaging probes due to their high binding affinity and sensitivity to competitive agonist inhibition.[1]

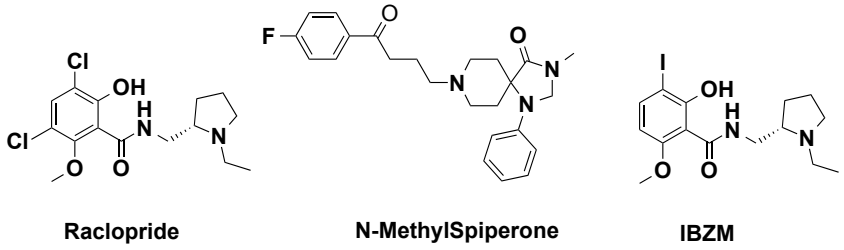
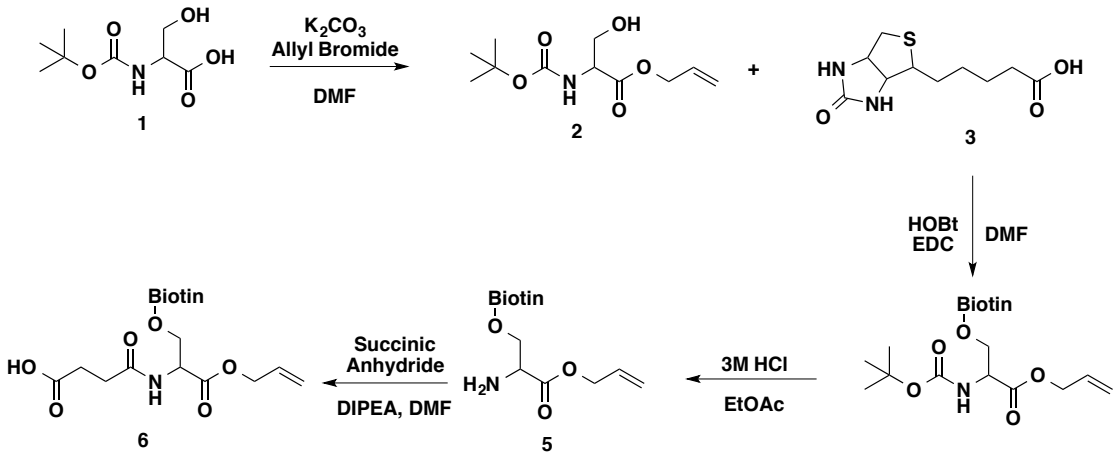


Figure 2: Imaging Ligands

Due to the high binding affinity of spiperone derivatives for the D_2 receptor, we set out to develop a chemical probe that possessed four key characteristics: (1) The ideal probe must contain an agonist that will elicit a biological response; (2) after binding, the probe must covalently tether the probe to the target receptor; (3) the probe must contain a separate functionality that will allow for isolation of the receptor

protein; and (4) a trifunctional scaffold must be identified that can incorporate the previously mentioned functionalities (Scheme 1).

Efforts Towards Proposed Probe Synthesis

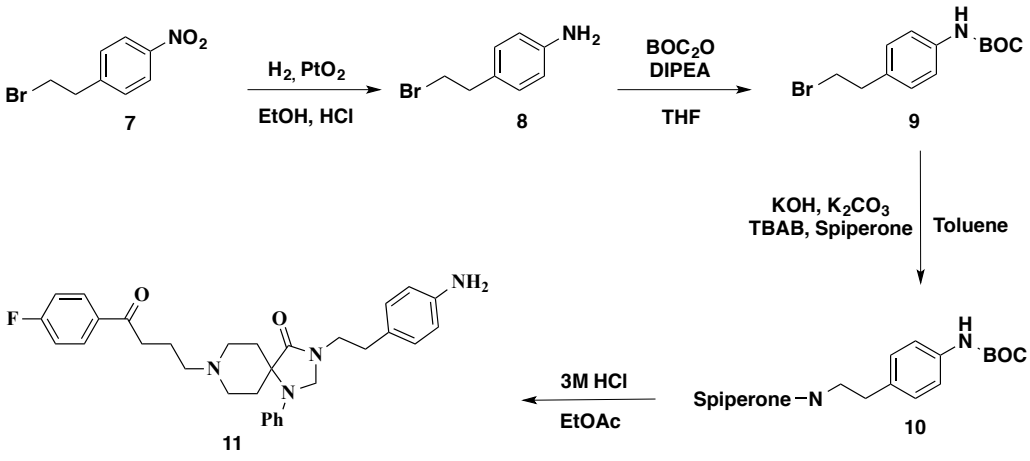


Scheme 1: Construction of Serine Scaffold

The investigation began with the identification of an amino acid scaffold that could incorporate the three functionalities. As a result, serine was ultimately deemed the most appropriate, as the hydroxyl residue provided an excellent pathway for biotin conjugation via an ester linkage. In order to couple Boc-(L)-Ser-OH (**1**) to biotin, the serine had to first be protected on the carboxyl terminus. By subjecting **1** so a solution of allylbromide and Na_2CO_3 in DMF, **2** was produced with an excellent yield (87%) and flash chromatography was unnecessary after extracting the product with EtOAc.[2] Boc-(L)-Ser-O(All) (**2**) was then dissolved in DMF and introduced to a flask containing *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), hydroxybenzotriazole (HOBt), diisopropylethylamine (DIPEA), and biotin (**3**). The mixture was allowed to stir uninterrupted for 24 h under a nitrogen atmosphere. Flash

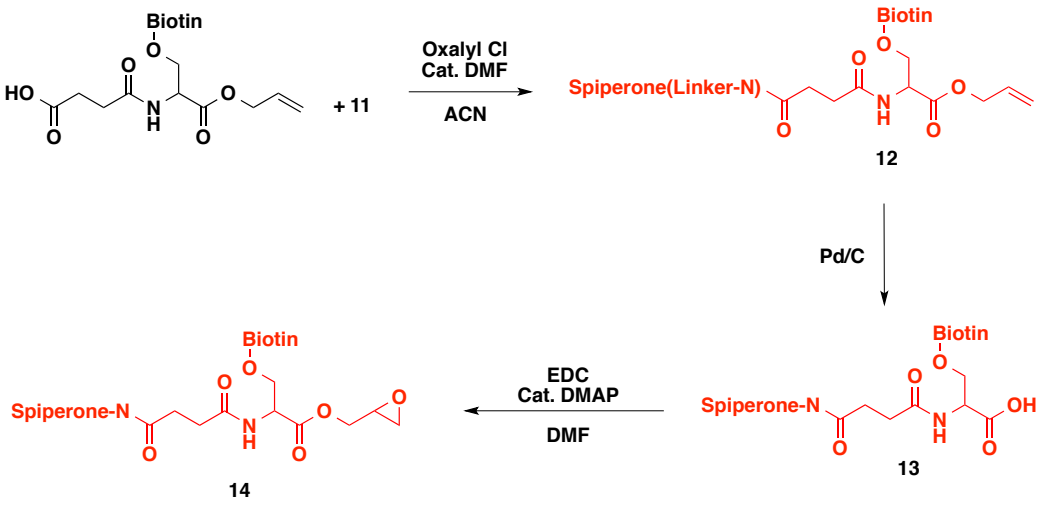
purification provided good yields of the biotin ester (**4**), albeit slightly impure. An inseparable mixture of **4** and unreacted biotin was observed. This mixture was carried on to the next step without further purification.

Boc deprotection commenced by dissolving **4** in EtOAc, cooling the solution to 0^{o}C, and adding conc. HCl drop wise to the reaction mixture. Deprotection was deemed completed when the evolution of CO_2 and ethylene gas was no longer observed. The biotin impurity present after the synthesis of **4** was removed by washing the reaction mixture containing **5** with water. The biotin impurity was extracted into EtOAc while the amine, **5**, remained in the aqueous layer. ^{13}C NMR indicated that **5** was epimerized as a result of the deprotection procedure, presumably at serine's chiral center, but the diastereomeric mixture was carried on without resolution. In order to add an additional site of functionalization, **5** was introduced to a stirred solution of succinic anhydride in order to afford **6.** The tethered carboxylic acid will serve as a site of attachment for the conjugated spiperone linker **11**, which was synthesized according to a known procedure (scheme 2).[3]



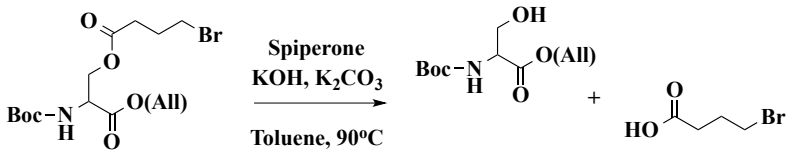
Scheme 2: Spiperone Alkylation

Proposed Probe Completion



Scheme 3: Proposed Synthesis of Target Molecule

Introduction of the spiperone moiety has proven to be the most challenging aspect of the proposed synthesis. Prior attempts to directly alkylate spiperone to the amino acid scaffold proved to be ineffective as conditions necessary to carryout the substitution reaction readily hydrolyzed the linker from the serine scaffold or resulted in the corresponding olefin due to the elimination of bromine (Scheme 4). As a result, efforts were directed towards the orthogonal construction of **11** so that the resulting aniline functionality may be coupled directly to **6**. However, aniline has proven to be too weak of a nucleophile and this step cannot be achieved through traditional peptide coupling procedures.



Scheme 4: Hydrolysis of Molecular Probe

As a result, the following proposed methodology should allow for the successful synthesis of **14** without the worry of degradation to the serine scaffold (scheme 3). To combat the poor nucleophilicity of aniline, the acid chloride analog of **6** will be synthesized from oxalyl chloride in order to afford an intermediate significantly more susceptible to nucleophilic attack. Once **12** has been isolated, removal of the allyl group on the carboxyl terminus of serine with stoichiometric Pd/C will give rise to **13**. Drawing inspiration from a 2003 communication by the Sames group this free carboxyl moiety allows for the introduction of an epoxide functional group, for covalent tethering. The necessity for covalent linkage is due to the likelihood of protein denaturation during probe manipulation. In order to effectively remove the dopamine receptor from the cell membrane, the lipids will need to be dissolved through the use of detergents. This process will potentially liberate spiperone from the D-2 receptor, as a conformational change will alter binding affinity, however, a covalent linkage will allow the probe to remain tethered to the target molecule The desired epoxide moiety can be added to **13** via esterification with glycidol, affording the target molecule, **14**.[7]

Conclusion

In summation, this manuscript details the efforts made towards the synthesis of a spiperone-based probe and a proposed synthetic route for its successful completion. Future work will focus on optimization and isolation of the target molecule with significant emphasis placed on the preservation of stereochemistry and synthesis of additional stereoisomers.

References

1) Ye, N.; Neumeyer, J.; Baldessarini, R.; Zuechu, Z.; Zhang, A. *Chem. Rev.* **2013**, 113, 123.
2) Bonci, A.; Woodward Hopf, F. *Neuron*. **2005**, *47*, 335.
3) Missale, C.; Nash, R.; Robinson, S.; Jaber, R.; Caron, M. *Physiological Reviews*, **1998**, *78*, 190.
4) Andersen, P.H.; Gingrich, J.A.; Bates, M.D.; Dearry, A.; Falardeau, P.; Senogles, S.E.; Caron, M.G. *Trends Pharmacol. Sci.* **1990**, *11*, 231.
5) Beaulieu, J.; Gainetdinov, R. *Pharmacol. Rev.* **2011**, *63*, 182.
6) Pattabiraman, V.; Stymiest, J.; Derksen, D.; Martin, N.; Verdes, J. *Org. Lett.* **2007**, *9,* 699.
7) Chen, G.; Heim, A.; Riether, D.; Yee, D.; Milgrom, Y.; Gawinowiza, M.; Sames, D. *J. Am. Chem. Soc.* **2003**, *125*, 8130.

Experimental Section

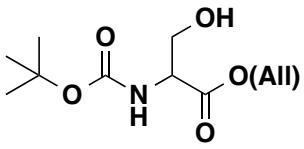
Reagents

Substrates were purchased from Sigma-Aldrich or Fisher Scientific and used as is. Flash chromatography was performed using a Biotage Isolera with SNAP Ultra silica cartridges.

Instrumentation

GC/MS analyses were performed on an Aglient Technologies 6890 GC system with a 5973 mass selective detector. NMR spectra were obtained using a Bruker Avance 300 MHz spectrometer and a Varian Inova 500 MHz spectrometer.

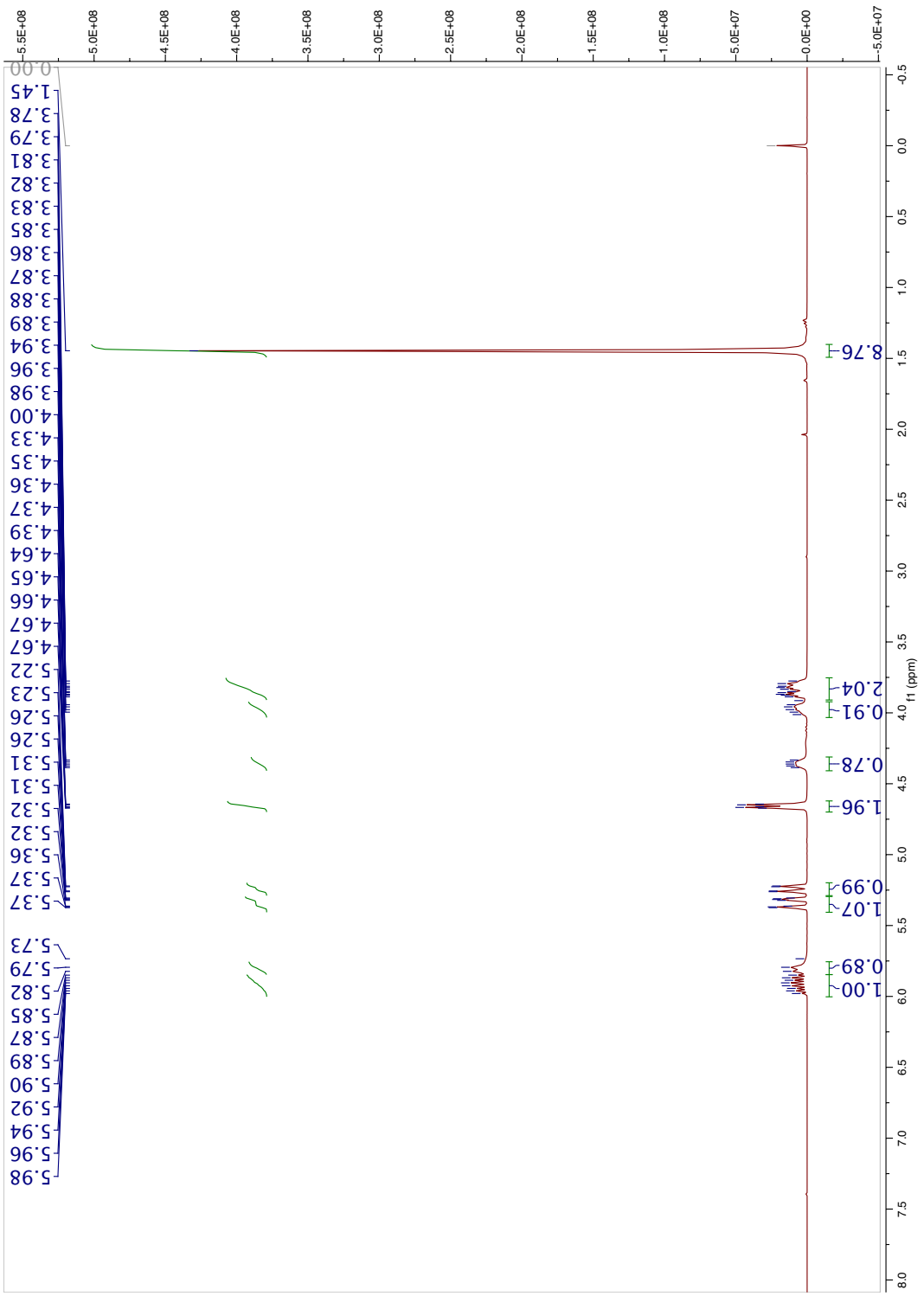
Synthesis of 2[1]



Boc-L-Ser (1.00g, 4.87 mmol) is dissolved in 25 mL of DMF. Na_2CO_3 (1.03g, 9.75 mmol) allyl bromide (0.46 mL, 5.37 mmol), and 0.7 mL of water is then added and stirred overnight. The reaction mixture is extracted into EtOAc and carried on to the next step without further purification (yield = 0.103 g, 89%).

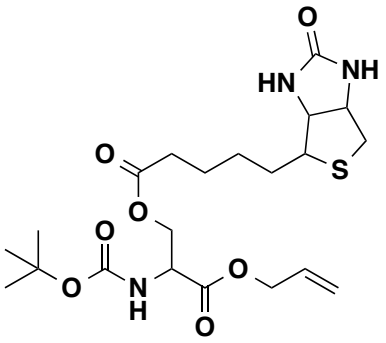
^{1}H NMR (300 MHz, Chloroform-*d*)

δ 5.91 (m, 1H), 5.81 (d, *J* = 8.6 Hz, 1H), 5.41 – 5.30 (m, 1H), 5.24 (dd, *J* = 10.5, 1.6 Hz, 1H), 4.70 – 4.62 (m, 2H), 4.36 (dt, *J* = 8.4, 3.8 Hz, 1H), 3.98 (dt, *J* = 10.4, 4.9 Hz, 1H), 3.91 – 3.75 (m, 2H), 1.45 (s, 9H).



Spectrum 1: ^{1}H NMR of **2**

Synthesis of 4



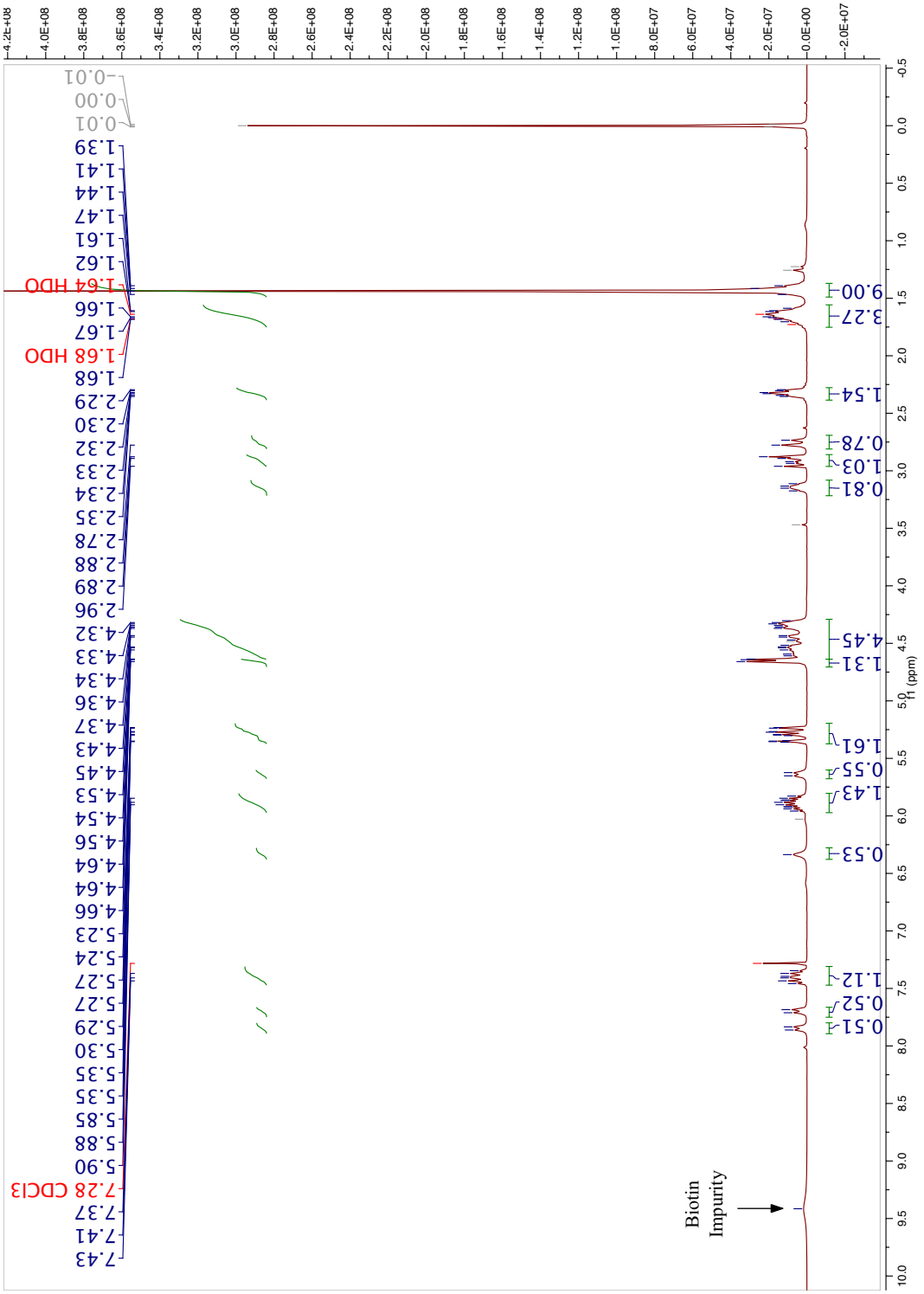
Biotin (93 mg, 0.381 mmol) EDC (80 mg, 0.419 mmol), HOBt (57 mg, 0.419 mmol) and DIPEA (0.165 mL, 0.953 mmol) were dissolved in 5 mL of DMF and stirred until dissolved. Boc-Ser-O(All) (94 mg, 0.381 mmol) was then added to the reaction mixture, placed under N2 and stirred for 24 h. The crude reaction mixture was extracted into EtOAc and was purified via flash chromatography in 1:9 MeOH/CH_2Cl_2 to afford **4** as a slightly impure oil (0.110, 89%).

^{1}H NMR (300 MHz, $CDCl_3$)

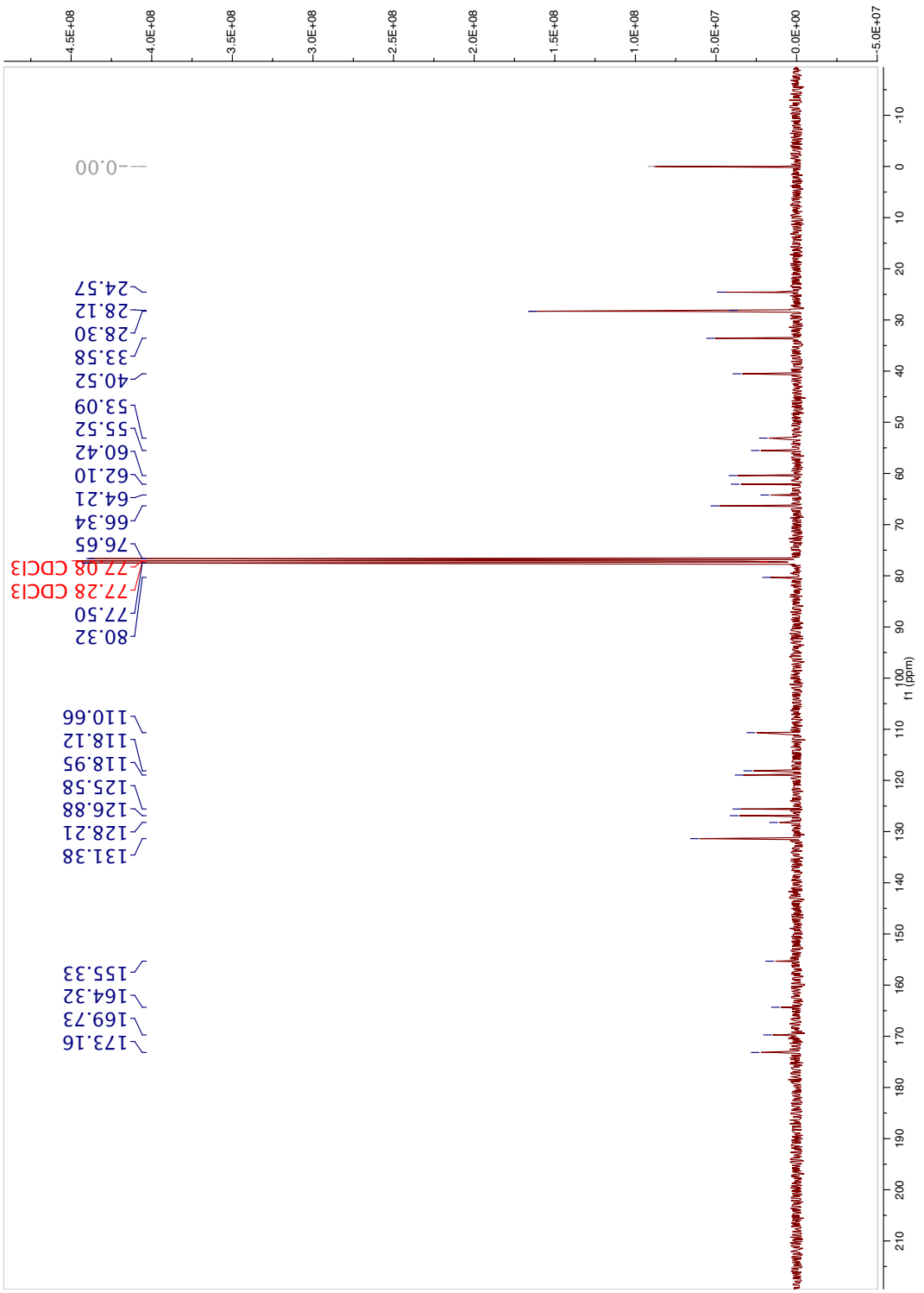
δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.40 (dt, *J* = 18.8, 7.1 Hz, 1H), 6.34 (s, 1H), 5.97 – 5.80 (m, 1H), 5.64 (d, *J* = 8.5 Hz, 1H), 5.37 – 5.20 (m, 2H), 4.65 (d, *J* = 5.0 Hz, 1H), 4.64 – 4.29 (m, 4H), 3.14 (q, *J* = 6.6 Hz, 1H), 2.96 – 2.86 (m, 1H), 2.76 (d, *J* = 12.9 Hz, 1H), 2.32 (td, *J* = 7.4, 2.7 Hz, 2H), 1.75 – 1.56 (m, 3H), 1.44 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$)

δ 173.16, 169.73, 164.32, 155.33, 131.38, 128.21, 126.88, 125.58, 118.95, 118.12, 110.66, 80.32, 77.50, 77.28, 77.08, 76.65, 66.34, 64.21, 62.10, 60.42, 55.52, 53.09, 40.52, 33.58, 28.30, 28.12, 24.57

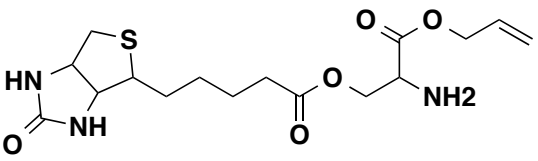


Spectra 2: ^{1}H NMR of **4**



Spectrum 2.1: ^{13}C NMR of **4**

Synthesis of 5



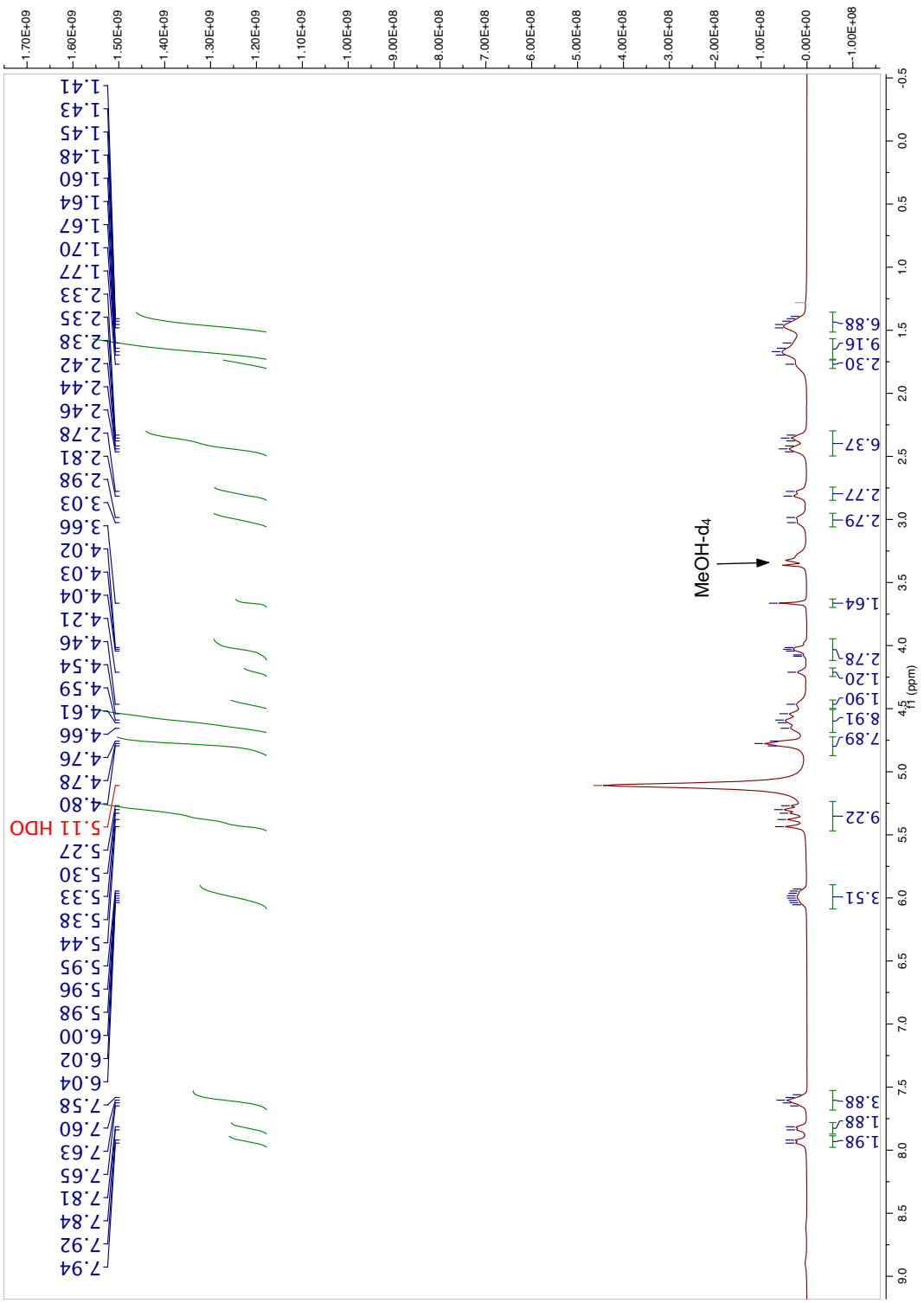
To a solution of **4** in EtOAc at 0°C, 3M HCl was added drop wise. The reaction was monitored by TLC until consumption of the starting material was observed. The reaction was quenched with water and the biotin impurity that could not be removed during the synthesis of **4** was extracted into EtOAc. **5** was present as a colorless oil in the aqueous layer. This protocol led to epimerization of the chiral center.

^{1}H NMR (300 MHz, Methanol-d_4)

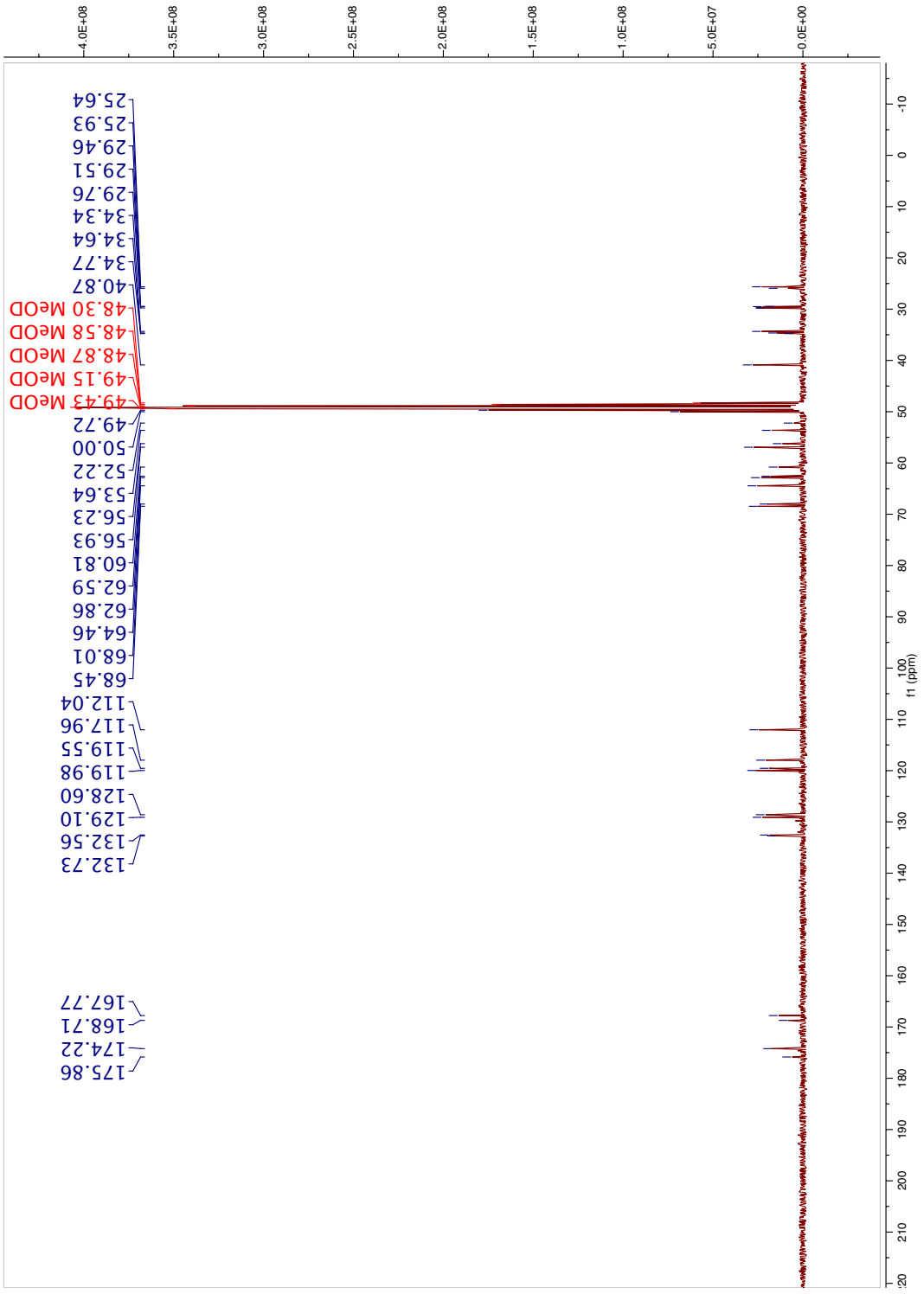
δ 7.93 (d, J = 7.3 Hz, 2H), 7.83 (d, J = 7.4 Hz, 2H), 7.60 (p, J = 7.0 Hz, 4H), 5.99 (ddt, J = 16.1, 10.2, 5.1 Hz, 4H), 5.47 – 5.24 (m, 9H), 4.78 (t, J = 5.7 Hz, 8H), 4.69 – 4.51 (m, 9H), 4.46 (s, 2H), 4.21 (s, 1H), 4.12 – 3.95 (m, 3H), 3.66 (s, 2H), 3.00 (d, J = 12.3 Hz, 3H), 2.80 (d, J = 11.2 Hz, 3H), 2.40 (dt, J = 25.9, 7.1 Hz, 6H), 1.77 (s, 2H), 1.73 – 1.57 (m, 9H), 1.44 (dd, J = 14.9, 6.9 Hz, 7H).

^{13}C NMR (75 MHz, MeOD)

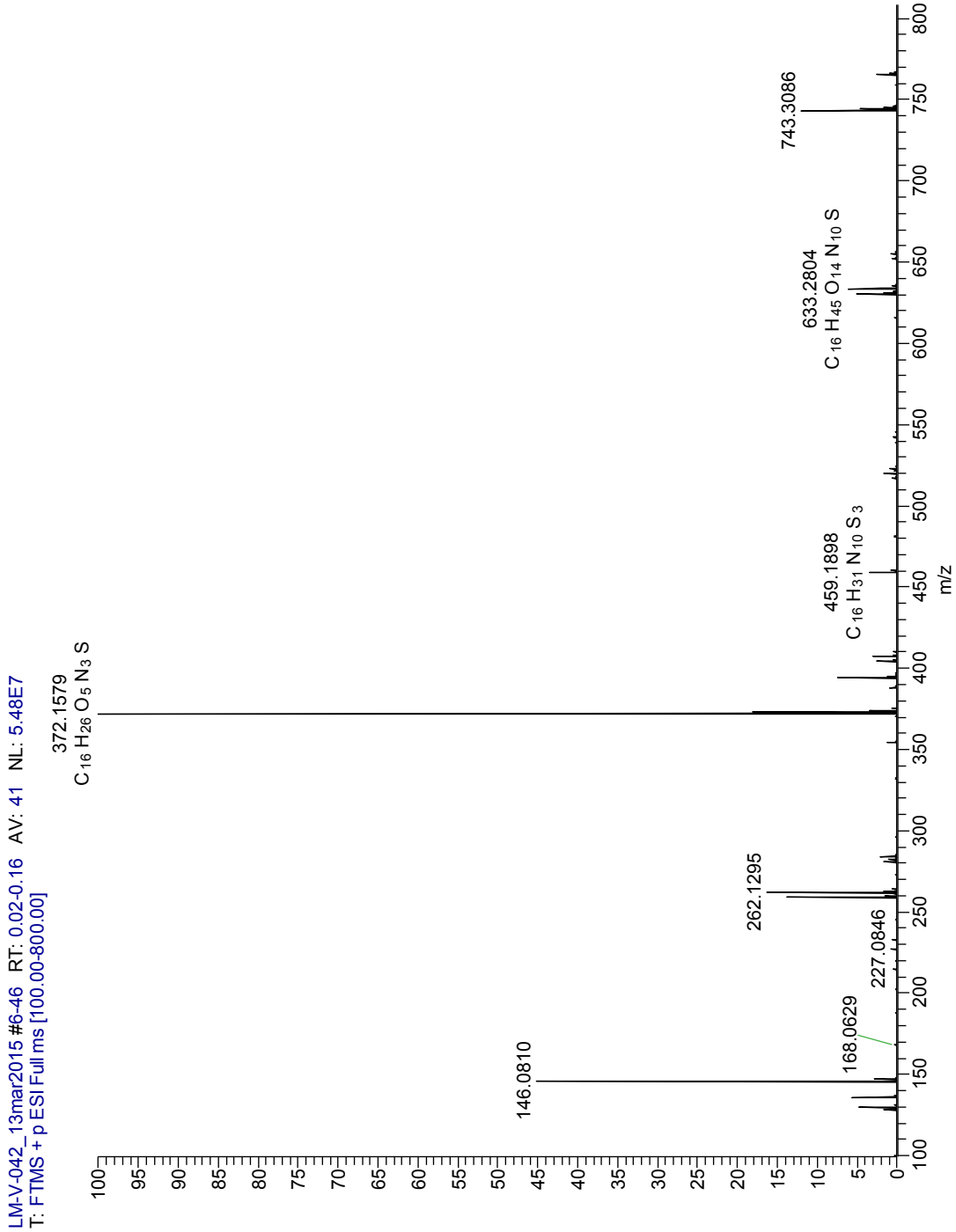
δ 175.86, 174.22, 168.71, 167.77, 132.73, 132.56, 129.10, 128.60, 119.98, 119.55, 117.96, 112.04, 68.45, 68.01, 64.46, 62.86, 62.59, 60.81, 56.93, 56.23, 53.64, 52.22, 50.00, 49.72, 49.43, 49.15, 48.87, 48.58, 48.30, 40.87, 34.77, 34.64, 34.34, 29.76, 29.51, 29.46, 25.93, 25.64.



Spectrum 3: ^{1}H NMR of **5**



Spectrum 3.1: ^{13}C NMR of **5**



Spectrum 3.2: Mass Spectrum of **5**

MANUSCRIPT 4

Accepted in the *Journal of Chemical Education*

Solution-Phase Synthesis of Dipeptides: A Capstone Project that Employs Key Techniques in an Organic Laboratory Course

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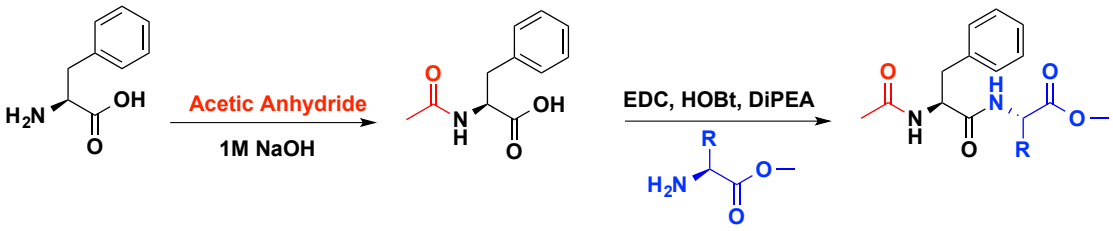
Manuscript 4

Solution-Phase Synthesis of Dipeptides: A Capstone Project that Employs Key Techniques in an Organic Laboratory Course



ABSTRACT

A contemporary approach to the synthesis and purification of several UV-active dipeptides has been developed for the sophomore-level organic laboratory. This experiment exposes students to the important technique of solution-phase peptide synthesis and allows the instructor to highlight the parallel between what they are accomplishing in the laboratory to the advancements being made in the pharmaceutical industry. By illustrating the importance of protecting group strategy and stereochemistry preservation, while also reinforcing the various separatory and purification techniques learned throughout the typical laboratory course, this experiment serves as an excellent candidate for a capstone demonstration of the

INTRODUCTION

The second-year organic laboratory course is responsible for training a multitude of students across a wide variety of fields. Students enrolled in this course are not just those with an interest in chemistry, but rather the bulk of the course's population is often composed of students majoring in other scientific disciplines such as biological

science, engineering, microbiology, or pharmaceutical sciences. As a result, the implementation of a curriculum that not only appeals to each student, but is also applicable to their chosen professions, is essential for maintaining an enthusiastic and engaged environment. Most students have been exposed to the topic of peptide coupling in their introductory life science courses and will continue to learn about the chemistry of amino acids, peptides and proteins as they progress toward their undergraduate science degrees. Several laboratory exercises about the synthesis of peptides have been described in this *Journal*, but, with two exception, they all rely on solid phase peptide synthesis techniques.[1-4] While this is a powerful technology, it does not necessarily employ the same skill set that is taught in a typical synthetic laboratory. Few examples of solution-phase peptide synthesis have been published in chemical education literature. The examples that do exist either employ harsh reagents and unsafe techniques (thionyl chloride, -45 °C, benzene extractions), utilize unnatural amino acids, or make use of procedures that do not reflect modern solution-phase peptide chemistry, particularly with respect to stereochemistry preservation.[5-9] In our opinion, the most straightforward method for solution-phase peptide synthesis that has been described as a laboratory exercise was published by 1989, but it involves the use of a rare coupling agent and chiral TLC.[8] Herein, we report a multi-step synthesis of dipeptides that has been performed by over 400 students over the past three years. The pedagogy of this laboratory exercise allows students to combine multiple techniques that they have learned throughout a typical second-year organic laboratory course.

PEDAGOGY

In the beginning of any organic laboratory course students are introduced to the basic techniques associated with organic synthesis. This typically includes techniques such as separatory extraction, pH manipulation, vacuum filtration, rotary evaporation etc. As they progress through the course the students are expected to become more confident with these skills. By the end of the one or two-semester laboratory course the students should be able to successfully synthesize, purify, and analyze moderately complex small molecules. The procedure detailed herein combines the skills students have learned throughout a typical organic laboratory course and concludes with the isolation and characterization of a bioorganic molecule made from starting materials that the students have developed themselves.

This experiment was carried out in the University of Rhode Island's second semester organic chemistry laboratory. The overarching goal of this experiment was to have the students perform a synthesis that utilized a majority of the skills and techniques covered throughout the semester. Students were graded based upon a predetermined rubric with substantial emphasis placed on the overall yield and purity of the desired product (See notes to the instructor for the complete rubric). Unfortunately, time typically does not allow for individual inspection of the flasks containing the student's product, and as a result, reported yields are graded based on the honor system. Purity is judged solely on the student's proton NMR where substantial points are deducted for non-solvent impurities.

This experiment has been conducted, and subsequently improved upon, over the course of six semesters. The average class size per semester is approximately 90

students. In the most recent semester yields ranging from 5% - 75% were reported for students who were successfully able to complete the experiment. It should be noted that each semester is typically met with several individuals who are unable to obtain product, however, in most cases it was determined that this was the result of an error made during the purification process (See notes to the instructor). In instances where product is not obtained the student is given a standard NMR of their target molecule and is awarded no credit for product yield and purity. Representative student spectra for each peptide can be found in the supporting information.

EXPERIMENTAL

The experiment began with the synthesis of *N*-acetyl-L-phenylalanine. Students were instructed to measure 0.20 g of (L)-phenylalanine and synthesize the N-protected amino acid according to a literature procedure.[1] After suspending L-phenylalanine in 1.0 M NaOH acetic anhydride is slowly, and carefully, added to the reaction vessel and allowed to stir uninterrupted for approximately 30 minutes. The product was extracted in ethyl acetate after acidifying the crude reaction mixture and was rotavapped to dryness. The reaction was very robust, producing yields >85%, with no evidence of unreacted starting material being observed after extraction. After the students had successfully isolated their product, the course instructor divided the class into three groups and each group was given a stock bottle containing a second amino acid that was protected at the carboxyl terminus as a methyl ester. These three amino acids were (L)-leucine methyl ester, (L)-valine methyl ester, and (L)-phenylalanine methyl ester. Different amino acids were chosen in order to add variability to the purification and analysis processes in addition to the overall cost

effectiveness of each reagent. This protocol should be amenable to any other naturally occurring amino acid, however, only the aforementioned reagents were tested. By completing the table below students determined the necessary amounts of each of the reagents needed in order to construct their dipeptide Table 1. All of the reagents shown in Table 1 were dissolved in 30 mL of DMF and were placed in the students' assigned lab drawers where they remained until their next scheduled lab period, approximately 48 hours later. (Note: Reactions were not stirred during this time.)

Table 1. Data Table For Dipeptide Synthesis

	Ac-Phe	R-OMe[a]	EDC[a]	HOBt	DIPEA
MW (g/mol)	207.22		191.70	135.12	129.25
d. (g/mL)	-	-	-	-	0.742
Amount (g)					
Mol					
Equiv.	1	1	1.1	1.1	3

[a] Weights correspond to the hydrochloride salt derivative

The second day of the experiment commenced by extracting the dipeptide solution into EtOAc, drying with magnesium sulfate, and removing the solvent under reduced pressure. The crude reaction mixture was dissolved in CH_2Cl_2 and TLC analysis was performed, using hexanes and ethyl acetate, in order to purify the reaction mixture by flash chromatography. Due to the presence of phenylalanine in the dipeptide, students usually had no issue detecting their product under a standard 254 nm UV-lamp. A

solution of ninhydrin was also prepared so students assigned non-UV active amino acids (i.e. (L)-leucine methyl ester, (L)-valine methyl ester) could co-spot their TLC plate and detect unreacted starting material.

After the correct solvent system was determined by trial and error, students prepared flash chromatography apparati resembling the image displayed in Figure 1. The benefits this flash chromatography system, in addition to instructions detailing how to assemble it, are reported in a prior communications.[2] The crude reaction mixture was dissolved in a minimal amount of CH_2Cl_2 and loaded onto a disposable silica column using a 1 mL syringe. The fractions containing the desired dipeptide were placed into a preweighed flask and concentrated under reduced pressure. The resulting powders were left to dry until the next scheduled lab period, when yields and samples for NMR analysis were obtained.

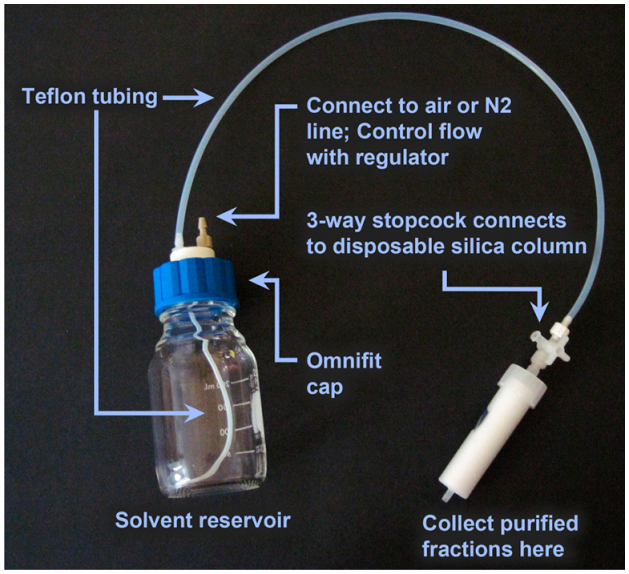


Figure 1. Flash Column Apparatus[10]

Over the course of three years an excess of 400 students have completed this lab. This experiment has served as the final evaluation of the skills student's have obtained

throughout a typical synthesis laboratory. When the dipeptide was appropriately isolated a typical yield within the 50% range was reported. The most common contaminants observed in the final NMRs were the student's respective methyl ester and residual solvent peaks. Due to the fact this experiment was serving as a final exam, communication with the instructor was limited as students were encouraged to perform the experiment as independently as possible. As a result, we hypothesize greater yields and purities may be achieved with more instructor interaction.

CONCLUSION

In summation, the experiment described herein serves as an excellent final assessment of the skills obtained in a second-year organic synthesis laboratory. Peptide coupling is a topic of significant importance and should appeal to a wide array of students across multiple disciplines while providing a hands-on opportunity to explore one of the more fundamental concepts covered in both introductory and advanced life science courses.

ASSOCIATED CONTENT

Student handouts, notes to the instructor, and standard NMR spectra for each dipeptide can be found via the Internet at http://pubs.acs.org

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ACKNOWLEDGMENT

This work was funded by the National Science Foundation (CAREER 0847222). The authors thank Mindy Levine for editorial assistance.

REFERENCES

1. Bockman, M. R.; Miedema, C. J.; Brennan, B. B. *J. Chem. Educ.* **2012**, *89*, 1470.
2. Neckers, D. C. *J. Chem. Educ.* **1975**, *52*, 695.
3. Taralp, A.; Turkseven, C.; Cakmak, A.; Cengel, O. *J. Chem. Educ.* **2002**, *79*, 87.
4. Qvit, N.; Barda, Y.; Gilon, C.; Shalev, D. *J. Chem. Educ.* **2007**, *84*, 1988.
5. Simmonds, R. J. *J. Chem. Educ.* **1987**, *64*, 966.
6. Menger, F. M.; Sorrells, J. *J. Chem. Educ.* **2009**, *86*, 859.
7. Lindeberg, G. *J. Chem. Educ.* **1987**, *64,* 1062.
8. Blatchly, R. A.; Allen, T. R.; Bergstro, D. T.; Yuki, S. *J. Chem. Educ.* **1989**, *66*, 965.
9. Young, E. P.; Campbell, A. *J. Chem. Ed.* **1982,** *59*, 701-701.
10. Naumiec, R. G.; Del Padre, N. A.; Hooper, M. M.; St. Germaine, A.; DeBoef, B. *J. Chem. Educ.* **2013,** *90,* 376.

SUPPORTING INFORMATION

Solution-Phase Synthesis of Dipeptides: A Capstone Project that Employs Key Techniques in an Organic Laboratory Course

Student Handout

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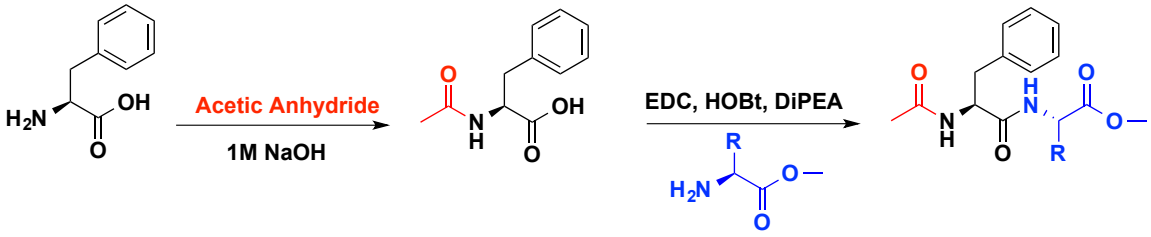
Materials and Methods

All reagents were purchased from either Fisher Scientific or Sigma Aldrich and were used without further purification. NMR spectra were obtained using a Bruker 300 MHz spectrometer in d_6-DMSO.

Solution-Phase Synthesis of Dipeptides: A Capstone Project that Employs Key Techniques in an Organic Laboratory Course



Throughout your organic chemistry tenure you have been taught the underlying principles necessary to construct simple organic molecules in the laboratory. In this lab you will see how the different branches of science can overlap as you strive to make one of life's most important structures, a peptide.

Thinking back to general biology you will recall that amino acids serve as the building blocks for life. Understanding how amino acid form peptides and proteins is pivotal for the development of vaccines and modern biologic drugs such as Enfuvirtide. The general features of an amino acid is a central carbon atom that is covalently bound to an amino group, a carboxyl group, a hydrogen atom, and an R-group; and with the exception of glycine, R is defined as any alkyl chain that may contain heteroatoms (Figure 1). It is also important to note that stereochemistry plays an important role in peptide synthesis. You should take note that the central carbon atom present in naturally occurring amino

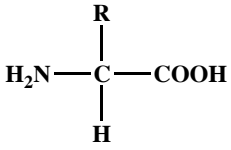


Figure 1: Generic amino acid structure

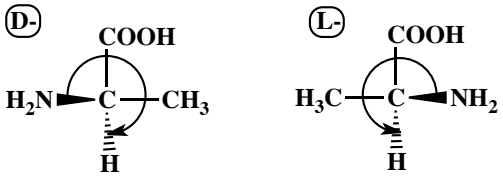


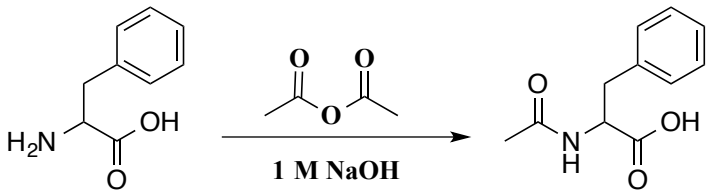
Figure 2: D- and L-Alaine configurations

acids, with the exception of glycine, is chiral. You should remember from your first-semester organic chemistry course that the terms *Rectus*, (R-), and *Sinister*, (S-), have been historically used to denote how a molecule rotates plane polarized light, where *Rectus* signifies clockwise rotation and *Sinister* signifies counter-clockwise rotation. In biological systems, the terms *Dexter,* (D-), and *Laevus*, (L-) are used, in replace of *Rectus* and *Sinister*, respectively, to represent a molecules chirality (Figure 2). Biological systems exist in a chiral environment, and all naturally occurring amino acids exist as the (L)-isomer.

How a molecule is oriented in space dramatically affects that molecules function. Speaking from a pharmaceutical standpoint, a drug's spatial configuration can be the difference between a cough remedy and a potentially lethal drug. With that in mind, chemists always seek to create enantiopure compounds. One methodology used to synthesize enantiopure peptides is to use carbodiimine coupling reagents. The function of a carbodiimine is to activate the carboxyl terminus of an amino acid, converting it into a leaving group for a subsequent nucleophilic attack from the free amino terminus of a second amino acid. You have previously learned that carboxylic acids can be converted into amides via an acid chloride intermediate, which can be synthesized using either thionyl chloride ($SOCl_2$) or oxalyl chloride [$(COCl)_2$]. Unfortunately, both of these reactions will partially racemize the chiral center of an amino acid. Consequently carbodiimides coupling agents such as dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide were developed.

The scheme at the beginning of this handout shows the amino terminus of phenylalanine and the carboxyl terminus of a second amino acid are protected; meaning, they are substituted with groups that render them chemically inactive to peptide coupling conditions. Protecting groups are used to deactivate one side of an amino acid in order to provide selective coupling. These protecting groups are usually easily removed in acid/base workups in order to generate even longer peptide chains once the primary coupling has completed. It should be easy to envision the concept of the amino protecting group being removed from your dipeptide so that it may attack the free carboxylic terminus of another amino acid. This would in essence create a tripeptide. Alternatively, removal of both protecting groups will result in a naturally occurring peptide.

Day 1 - Acyl Protection Of Phenylalanine:



Reference: Young, E. P.; Campbell, A. *J. Chem. Ed.* **1982**, *59*, 701-702.

Data Table: Have a completed copy of the following data table in your notebook prior to the beginning of lab. It is your responsibility to find the molecular weights and densities, if needed, before you arrive.

	Phenylalanine	Acetic Anhydride	1 M NaOH
Molecular Weight			-
Density	-	-	-
Amount			
mmol		-	-
Mole Ratio	1	-	-

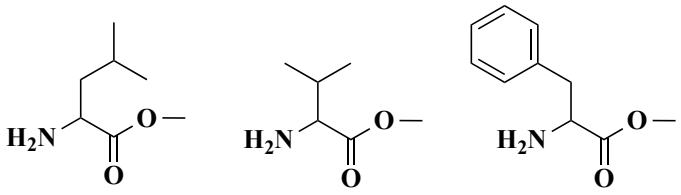
Procedure:

Take a 50 mL round bottom flask and charge it with 0.200 g of phenylalanine, and 10.00 mL of 1.00 M NaOH. Stir the contents of your flask until phenylalanine is fully dissolved then add 1.00 mL of acetic anhydride. Allow your flask to stir uninterrupted for 30 minutes. During this time you will observe a sudden increase in the solution's temperature. At the end of the 30 minutes, you will need to acidify your solution. Test your reaction with pH paper using a glass TLC capillary tube and acidify with 6.0 M HCl until you achieve a pH between 1 and 2. Be sure to do this very carefully and ensure proper mixing.

Once you have achieved the desired pH, you will notice crystals beginning to precipitate out of the solution; if not, cap the flask and shake the contents vigorously to coax the crystals out of the solution. Pour the contents of you vial into a separatory funnel and add an additional 15 mL of water; use the additional water to properly rinse out your vial. Extract your product into EtOAc by washing the aqueous solution three times with 20 mL portions of EtOAc. The acylated amino acid product should be soluble in organic solvents, so it should be found in the EtOAc layer. Combine all

three organic layers into an Erlenmeyer flask and, using magnesium sulfate, dry the EtOAc solution. Collect the mother liquor by vacuum filtration to remove the magnesium sulfate, and pour it into a dry, preweighed round bottom flask. Remove the solvent via rotary evaporation and weigh the residue in the flask to obtain a yield. Place a small amount of the solid into a vial to be used in the future for TLC analysis.

Synthesis Of The Dipeptide:



Leucine **Valine** **Phenylalanine**

Your instructor will give you a second amino acid that is already protected on the carboxyl terminus; this is the "R-OMe" section of your data table. Ask your instructor for the molecular weight of this amino acid. When you are determining your molar equivalents, you are going to use your newly synthesized *N*-acyl-L-phenylalanine as your limiting reagent. EDC is the peptide coupling agent and HOBt (1-hydroxybenzotriazole) is another activator with the purpose of further enhancing enantiopurity.

	Ac-Phe	R-OMe	EDC	HOBt	Diisopropylethylamine
MW					
Density	-	-	-	-	
Amount					
Mol					
Mole Ratio	1	1	1.1	1.1	3

Add 30 mL of DMF to the flask containing your *N*-acyl-L-phenylalanine. Add EDC, HOBt, diisoproplyethylamine, and your assigned amino acid to your reaction vessel and shake the contents of the flask vigorously until all reagents are fully dissolved. It is important that you use a glass septum for this step; rubber septa have a tendency to contaminate reactions with phthalates. Once fully dissolved, place your stoppered, round bottom flask in your drawers and allow them to sit until your next lab period.

Day 2 – Dipeptide Purification

EDC and HOBt are beneficial peptide coupling agents because they create water-soluble byproducts. You will be able to isolate the dipeptide and any unreacted amino acid starting material from DMF by performing an extraction. Using 75 mL of water, quench the reaction mixture and wash with 20 mL of EtOAc. Place the organic layer in a flask and wash the aqueous layer two more times using an additional 20 mL of EtOAc each time. Combine all three portions of EtOAc into your round bottom flask and remove the solvent under reduced pressure.

After rotary evaporation you will observe either a white power or a yellow oil in the flask. If oil is present it simply means that residual DMF remained in your organic layer. To remedy this, pour 10 mL of 1.0 M HCl into your flask; at which time a white precipitate should form. This precipitate contains your product and it should be soluble in organic solvents. Perform one final extraction using 20 mL of EtOAc, remove the solvent under reduced pressure, and discard the aqueous layer.

Your crude reaction mixture should now be a dry white powder. In order to perform TLC analysis, dissolve your product in 10 mL of CH_2Cl_2 and find an appropriate solvent system for flash purification by testing a variety of mixture of hexanes and EtOAc. You should observe 2 or 3 spots, and it will be up to you to determine which spot corresponds to your peptide. Start with a 1:1 of Hex:EtOAc (10 mL total) and observe where your spots travel. Remember to co-spot your TLC plate with your acylated amino acid, methylated amino acid, and your extracted product. Adjust your solvent system accordingly by increasing or decreasing your EtOAc concentration while remembering to keep the overall volume of the solvent system at 10 mL (i.e. if you decrease EtOAc by 1.0 mL be sure to add an additional 1.0 mL of hexanes). Once the appropriate solvent system has been determined (rf of desired product should be 0.3), show your instructor your TLC plate so he/she may confirm.

Transfer your solution into a pre-weighed round bottom and rotavap off the excess CH_2Cl_2. Once your round bottom containing the crude product has been dried, dissolve the product in a minimal amount of CH_2Cl_2 (remember to use as little solvent as possible for this step). Load your sample into your flash column (remembering to

saturate the column in the solvent system that you just determine by TLC prior to sample loading) and isolate your desired spot.

Combine all of the test tubes that contain your isolated dipeptide into a dry, preweighed round bottom flask and rotavap off the solvent. Allow your sample to dry overnight and prepare a sample for NMR analysis using deuterated DMSO during the next class.

HAZARDS

Acetic anhydride (CAS# 108-24-71) is flammable and can cause irritation of the lungs, skin and eyes. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, CAS# 1892-57-5) is hazardous in case of eye contact (irritant), of ingestion, of inhalation. 1-Hydroxybenzotriazole hydrate (HOBT, CAS#: 123333-53-9) is explosive when dry, risk of explosion if heated under confinement. Hydrochloric acid and sodium hydroxide solutions may cause skin irritation and eye damage upon contact. Solvent such a dimethylformamide (DMF, CAS# 68-12-2) is hazardous in case of skin or eye contact and by inhalation or ingestion. Common solvents for normal-phase flash chromatography, such as ethyl acetate [CAS# 141-78-6] and hexanes [CAS# 92112-69-1], are flammable. Appropriate personal protective equipment should be used at all times, and the reagents should only be handled in a well-ventilated fume hood. MSDS sheets are freely available from the vendor at http://www.sigmaaldrich.com.

Instructor Notes

Inst. 1

The addition of acetic anhydride to the solutions of (L)-phenylalanine in 1 M NaOH is noticeably exothermic. Ensure that students are not holding their flask during addition.

Inst. 2

When acidifying the reaction solution to a pH of 1-2, crystals will begin to crash out of solution. The crystals are pure *N*-acetyl-L-phenylalanine; however, much of the product still remains in solution. It is easiest to perform an extraction using EtOAc without isolating the crystals by filtration. The crystals will readily dissolve in the organic layer. The crystals can be coaxed out of solution with vigorous shaking if it does crystallization does not spontaneousl occur. This is a good indicator or whether or not the appropriate pH has been reached.

Inst. 3

If no crystals are observed, we have found that this typically is the result of over-acidification. This problem is easily remedied using the 1 M NaOH solution used to carryout the acylation procedure. Over-acidification resulted in severely reduced recovery of the acylated product after extraction, as it remained in the aqueous layer.

Inst. 4

If pH manipulation was performed correctly there was typically no evidence of unreacted (L)-phenylalanine present within the organic layer (TLC = 4% MeOH in CH_2Cl_2).

Inst. 5

N-Acetyl-L-phenylalanine will present as a yellow oil if not completely dried. 10 minutes under high vacuum typically allowed for white powder development. Continuing the experiment using the oil did not inhibit the reaction.

Inst. 6

When distributing the second amino acid be mindful as to whether or not it is the hydrochloride salt derivative. Let the students know the correct molecular weight to put into their data tables.

Inst. 7

EDC was chosen due to the extreme sensitivity some individuals experienced with DCC (i.e. skin irritation and severe rash). Also, DCC was found to still be present in the reaction after extraction, whereas no evidence of EDC was observed.

Inst. 8

Ninhydrin spray was necessary to elucidate (L)-valine methyl ester and (L)-leucine methyl ester.

Inst. 9

The dipeptides were liquid loaded onto the columns using CH_2Cl_2 because they often did not fully dissolve in the Hex:EtOAc mixture necessary to perform column chromatography. Liquid loading volume depends on column size but in general do not exceed 2 mL of CH_2Cl_2 when using a 10 g column. See reference 10 of the manuscript for details on the flash column apparatus system.

Inst. 10

Purification by adsorbing the crude reaction material onto silica provides the best separation. This method is slightly more costly, but may serve as a viable option for smaller classes. Biotage SNAP 10 g columns (Biotage HP-Sphere 25 *u*m) provide a means for loading dry silica on top of a disposable silica cartage that can then be attached to the flash chromatography apparatus described.

Inst. 11

Purification difficulties arose from overloading the flash columns. Using a standard 10 g column no more than 2 mL of CH_2Cl_2 should be used to load the crude reaction material onto the column. If not all of the material can be dissolved in 2 mL then run the column twice.

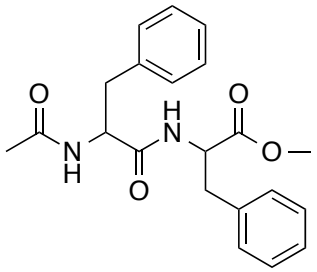
Inst. 12

It was found that students who did not obtain any product often did not perform the flash chromatography procedure correctly. Rather than spotting test tubes in order to determine if their product had successfully eluted from the column, several students simply stopped after 10 full test tubes had been collected (which was the outcome of a previous lab) and threw away their flash column.

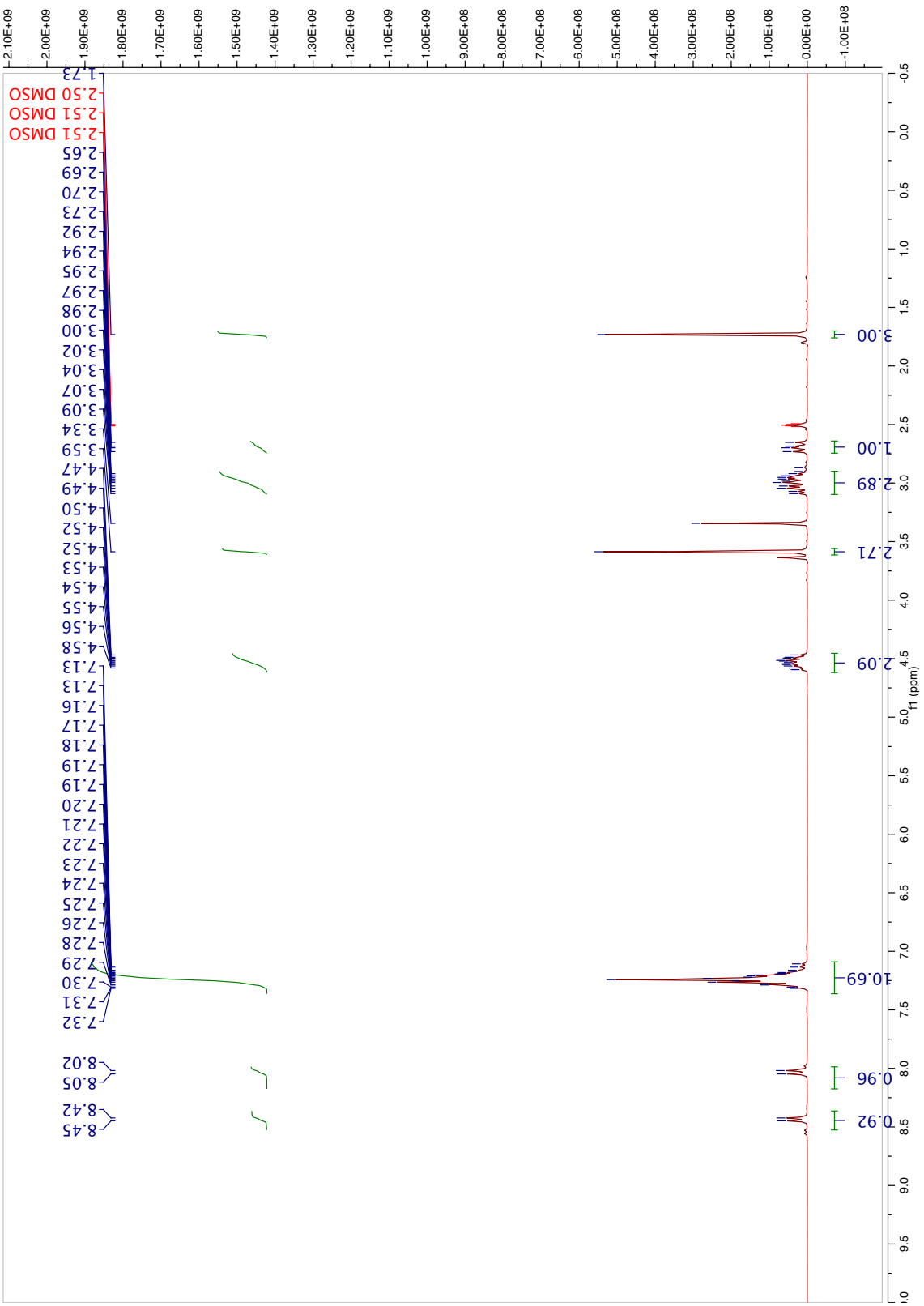
Inst. 13

Rotomers present in final NMR of valine containing product.

N-Ac-(L)-Phe-(L)-Phe-OMe

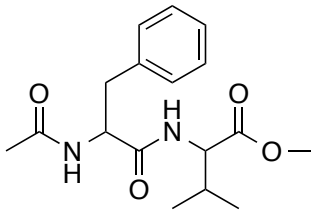


^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.11 – 7.32 (m, 5 H), 4.47 – 4.49 (m, 2H), 3.59 (s, 3H), 2.90 – 3.09 (m, 3H), 2.69 (q, *J* = 10 Hz, 1H), 1.73 (s, 3H).



Spectrum 1: Student ^{1}H NMR of *N*-Ac-(L)-Phe-(L)-Phe-OMe

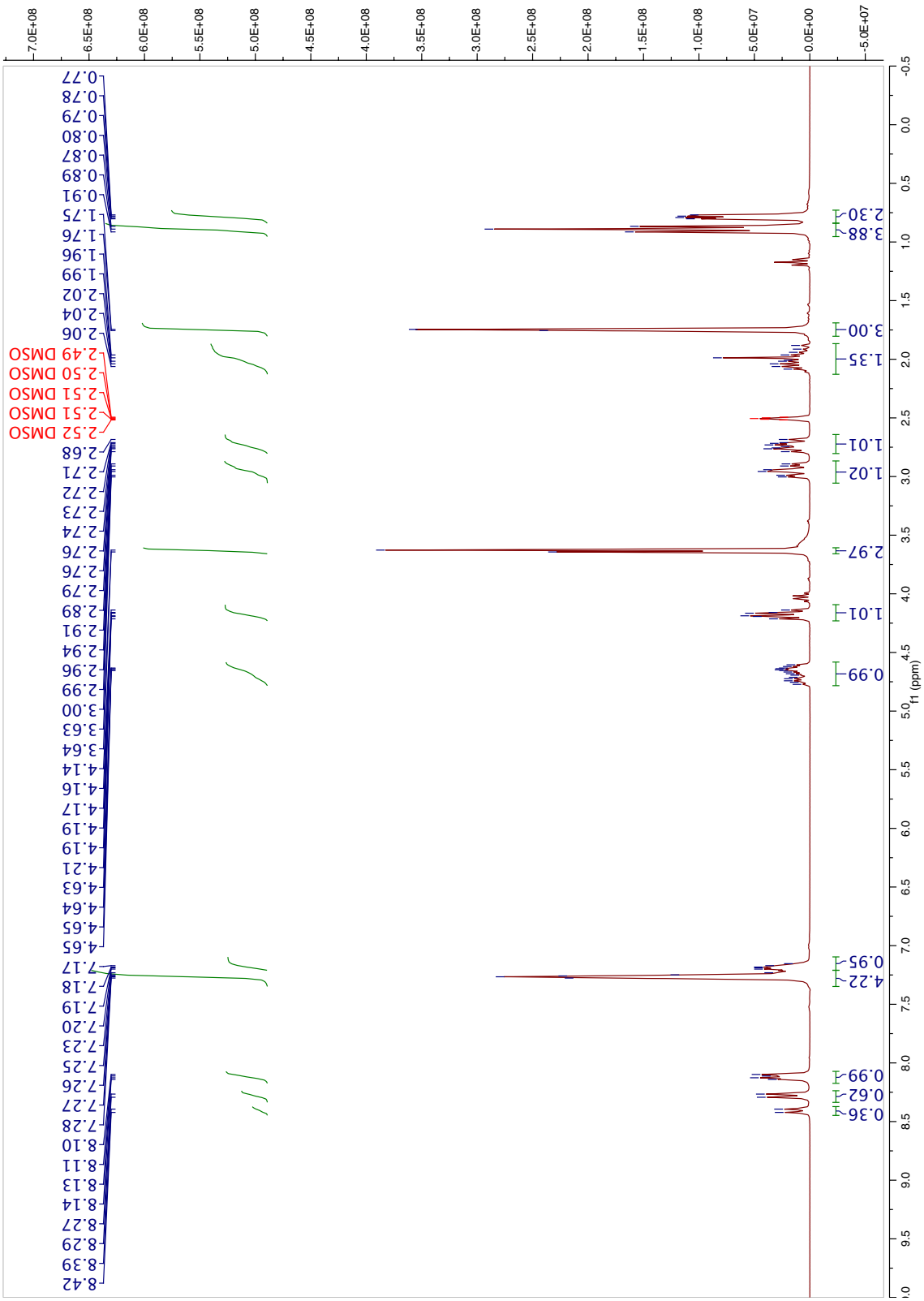
N-Ac-(L)-Phe-(L)-Val-OMe



^{1}H NMR (300 MHz, $CDCl_3$): δ 8.4 (d, J = 8.2 Hz, 1H), 8.28 (d, J= 8.2 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.15 – 7.28 (m, 5H), 4.61 – 4.77 (m, 1H), 4.14 – 4.21 (m, 1H), 3.64 (s, 3H) 3.63 (s, 3H), 2.95 (td, J = 14.9, 14.4, 4.8 Hz, 1H), 2.68 – 2.79 (m, 1H), 1.88 – 2.08 (m, 1H), 1.76 (s, 3H), 1.75 (s, 3H), 0.89 (t, *J* = 7.1 Hz, 4H), 0.79 (dd, *J* = 6.8, 3.2 Hz, 2H).

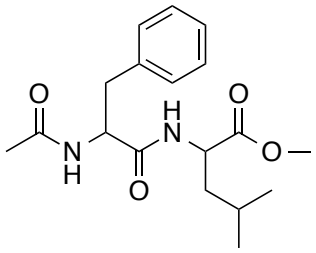
Rotomer Signals:

δ = 8.4 + 8.28 (1H), 3.64 + 3.63 (3H)

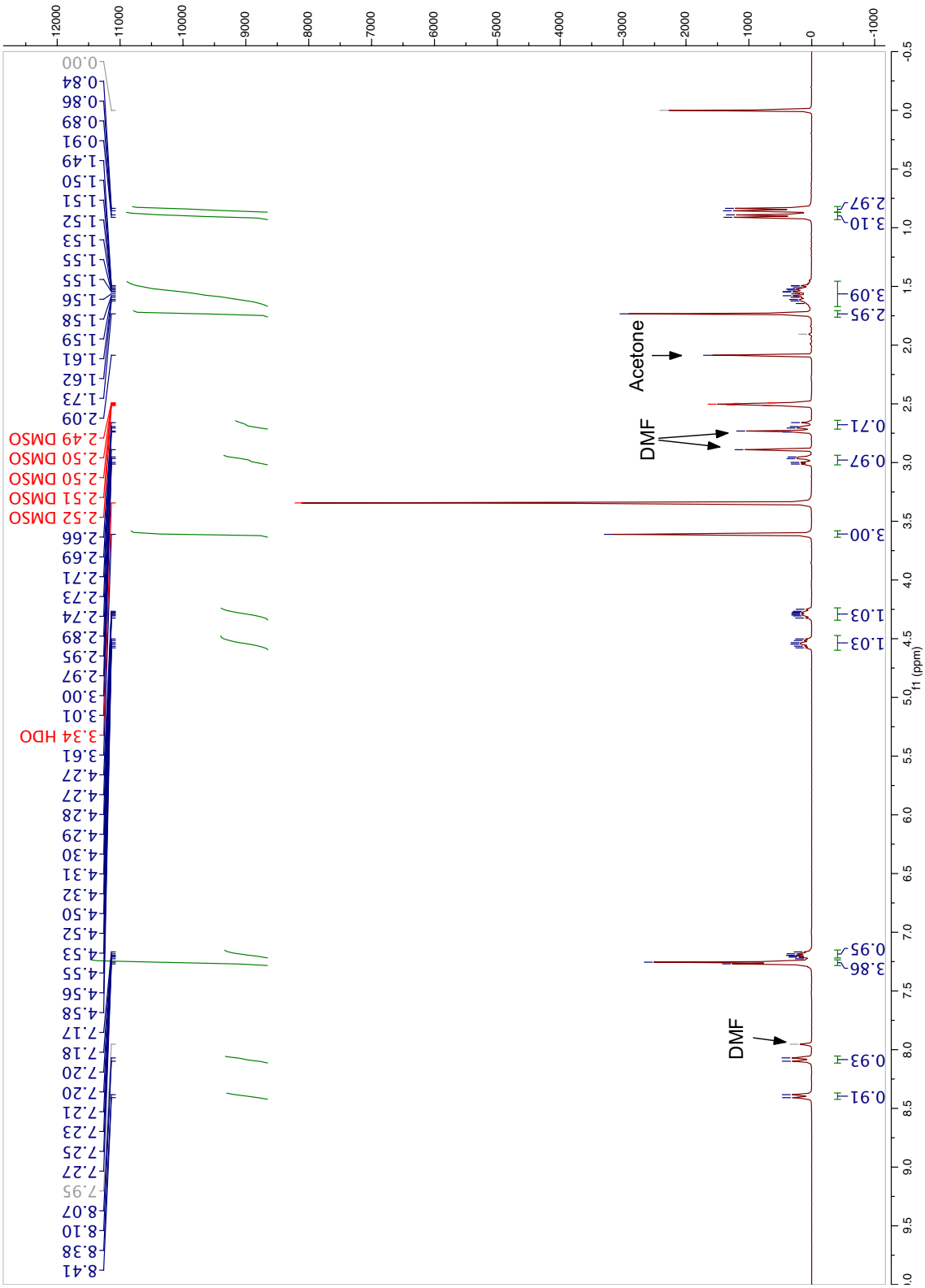


Spectrum 2: Student ^{1}H NMR of *N*-Ac-(L)-Phe-(L)-Val-OMe

N-Ac-(L)-Phe-(L)-Phe-OMe



^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.26 (d, *J* = 4.3 Hz, 4H), 7.17 – 7.27 (m, 5H), 4.54 (m, 1H), 4.29 (m, 1H), 3.61 (s, 3H), 2.98 (dd, *J* = 13.8, 4.2 Hz, 1H), 2.72 – 2.64 (m, 1H), 1.73 (s, 3H), 1.67 – 1.46 (m, 3H), 0.90 (d, *J* = 6.2 Hz, 3H), 0.85 (d, *J* = 6.2 Hz, 3H).



Spectrum 3: Student ^{1}H NMR of *N*-Ac-(L)-Phe-(L)-Leu-OMe

Hazards

Acetic anhydride (CAS# 108-24-71) is flammable and can cause irritation of the lungs, skin and eyes. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, CAS# 1892-57-5) is hazardous in case of eye contact (irritant), of ingestion, of inhalation. Some students may exhibit extreme skin sensitivity to carbodiimides and, as such, should be handeled with extreme care. 1-Hydroxybenzotriazole hydrate (HOBT, CAS#: 123333-53-9) is explosive when dry, risk of explosion if heated under confinement. Hydrochloric acid and sodium hydroxide solutions may cause skin irritation and eye damage upon contact. Solvent such a dimethylformamide (DMF, CAS# 68-12-2) is hazardous in case of skin or eye contact and by inhalation or ingestion. Common solvents for normal-phase flash chromatography, such as ethyl acetate [CAS# 141-78-6] and hexanes [CAS# 92112-69-1], are flammable and hexanes is a known neurotoxin. Dichloromethane [CAS# 75-09-2] is a skin and eye irritant in addition to being a known carcinogen. Dimethyl sulfoxide – d_6 [CAS# 2206-27-1] is a flammable liquid. Appropriate personal protective equipment should be used at all times, and the reagents should only be handled in a well-ventilated fume hood.

MSDS sheets are freely available from the vendor at http://www.sigmaaldrich.com.

Appendix I: Common Abbreviation and Symbols

AcOH	acetic acid
AgOAc	silver acetate
bs	broad singlet
BoNTA	botulinum nerurotoxic inhibitor A
BoNT	botulinum nerurotoxic inhibitor
n-BuLi	*n*-butyllithium
Bz	benzyl
$CDCl_3$	deuterated chloroform
$((CD_3)_2CO)$	deuterated acetone
CO_2Me	carboxylate group
$Cu(OAc)_2$	copper acetate
$Cu(OTf)_2$	copper triflate
^{13}C-NMR	carbon nuclear magnetic resonance
DMSO-d_6	deuterated dimethylsulfoxide
DMF	dimethylformamide
DMSO	dimethylsulfoxide

d	doublet
dd	doublet of doublets
EtOAc	ethylacetate
FG	functional group
GC/MS	gas chromatography/ mass spectrometry
^{1}H-NMR	proton nuclear magnetic resonance
HRMS ESI	high resolution mass spectrum electrospray ionization
LRMS EI	low resolution mass spectrum electron impact
MOM	methoxymethyl
Me	methyl
MW	microwave
MHz	megahertz
MeO	methoxy
m	multiplet
NaOH	sodiumhydroxide
NPhth	phthalimide
$Pd(OAc)_2$	palladium acetate

PivOH	pivalic acid
PhH	benzene
PhI	iodobenzene
R_f	retention factor
s	singlet
SEM	(trimethylsilyl)ethoxy methyl
SEMCl	(trimethylsilyl)ethoxy methyl chloride
t	triplet
TBAF	tetra-*n*-butylammoniumfluoride
TLC	thin layer chromatography
TMS	trimethylsilane
Tos	tosyl