NTNU
Norwegian University of
Science and Technology

Synthesis of fused benzene amphiphiles for antimicrobial evaluation

Kristian Njerve Myreng

Chemical Engineering and Biotechnology
Submission date: June 2017
Supervisor: Odd Reidar Gautun, IKJ

Norwegian University of Science and Technology
Department of Chemistry

Acknowledgements

This masters's thesis, titled *Synthesis of fused benzene amphiphiles for antimicrobial evaluation*, was conducted at the Department of Chemistry, Norwegian University of Science and Technology (NTNU) in Trondheim between January and June 2017. This thesis is a part of Thomas Alexander Bakka's PhD project titled *Synthesis of Novel Antibiotics Inspired by Marine Natural Products*. It is also a continuation of my own work, titled *Synthesis of Small Cationic Amphiphiles for Antimicrobial Screening*, performed as a part of TKJ4520 Organic Chemistry Specialization Project between August to December 2016.

First of all I would like to thank my supervisor, Associate Professor Odd Reidar Gautun for providing an exciting project and valuable guidance. Your theoretical and practical knowledge of organic chemistry are beyond my imagination. Without your assistance and guidance, this master's thesis wouldn't be possible. I will never forget your ability to discuss organic chemistry for hours, and then all of a sudden change the subject to either your dogs, or the junior football team you attended as a teenager.

Next I would like to thank PhD student Thomas Aleksander Bakka, who has functioned as a co-supervisor for both my Organic Chemistry Specialization Project and my master's thesis. The valuable skill, knowledge and guidance you have provided regarding the synthetic work presented in this thesis, as well as the endless amount of humour and jokes you bring to the table made both lab sessions and meetings one of the memories I will cherish for many years to come.

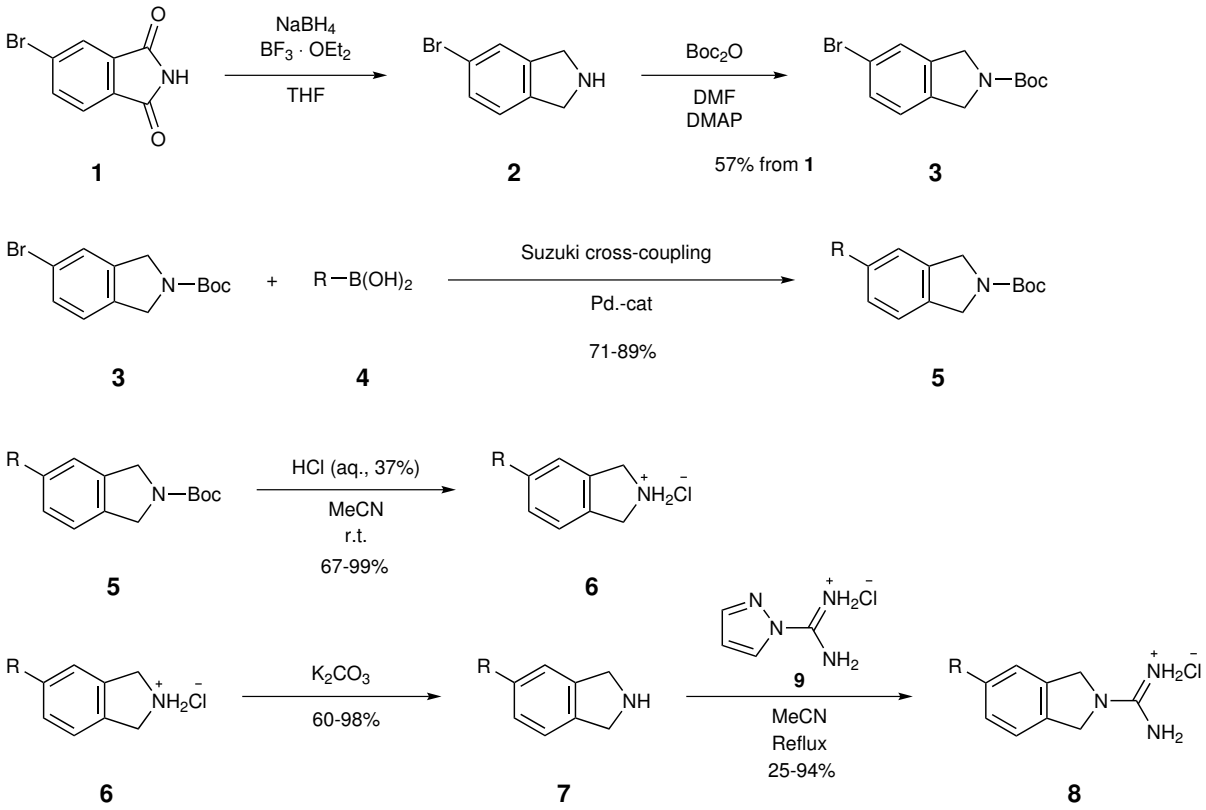
I would also like to thank my fellow MSc-candidate on this project, Daniel Lindberg. I have truly enjoyed our time time together, both our long sessions at the lab and our lunch breaks. Your ability to cheer me up even on the longest and heaviest days has been invaluable to me. Furthermore, I'm thankful for the friendship and scientific insight in other projects provided by the rest of the Gautun Research group, especially Hugo Fougner and Martin Furru Vold. The help and assistance provided by the technical staff at Department of Chemistry and NTNU has been crucial for this project. In particular I would like to mention engineer Roger Aarvik for speedy delivery of chemicals, Dr. Susana Villa Gonzales for analysis and knowledge of mass spectroscopy, engineer Julie Asmussen for help regarding HPLC and also analysis of mass spectra, and finally Torun Margareta Melø for guidance and pleasant conversations at the NMR-lab.

The support of my family, especially from my parents have been absolutely vital for my five years in Trondheim. Your faith in me has motivated me through even the hardest parts of the last five years. My time in Trondheim would not have been the same without my classmates, and in particular my flatmate Mohammed Mostafa Adnan. Your motivation, intelligence and humour never seizes to amaze me. Last bnot least, I would like to thank my girlfriend Solveig. Your love and support throughout the last two years has been beyond what I thought was possible. Without you this thesis would never have been completed.

Kristian Myreng
Trondheim, June 2017

Abstract

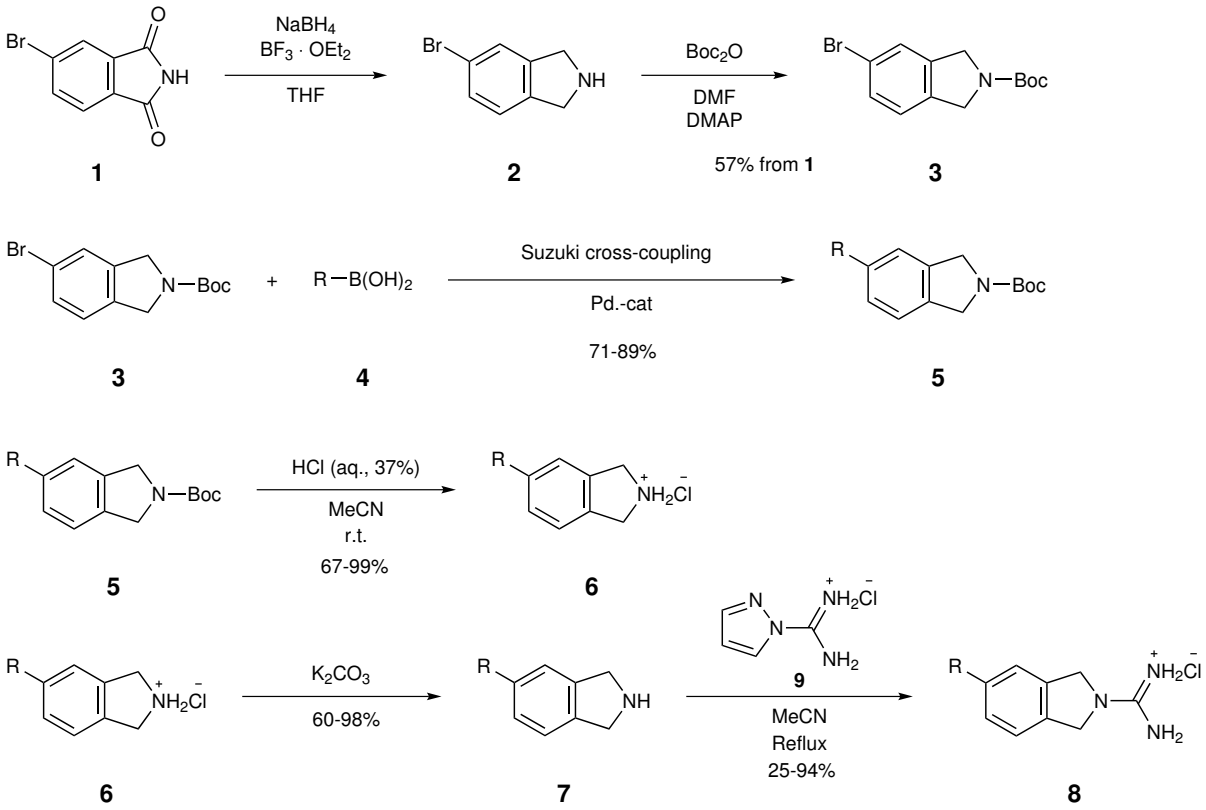
The goal of this project has been to synthesize small cationic amphiphiles with bulky aromatic substituents on a isoindoline scaffold, for antimicrobial screening, inspired by structures of marine natural products, with a big bulky lipophilic group linked to a cationic *N*-group. A total of 12 compounds were prepared for antimicrobial evaluation, 6 isoindoline HCl-salts and 6 guanidines. The guanidines showed impressive activity, but generally also high activity against human hepatic cells. An outline of the synthetic steps in this synthesis is given below in Scheme 0.1.



Scheme 0.1: An outline of the synthetic steps in this thesis.

Sammendrag

Hensikten med dette prosjektet har vært å syntetisere små kationiske amfifiler med forgrenede aromatiske substituenter på en isoindolinstruktur, for antimikrobiell screening, inspirert av strukturer funnet i marine naturprodukter, med en stor forgrenet lipofil gruppe koblet til en kationisk *N*-gruppe. Det ble fremstilt totalt 12 forbindelser for mikrobiell evaluering, 6 isoindolin salt og 6 guanidiner. Guanidinene viste imponerende aktivitet, men også en generell høy aktivitet mot humane leverceller. En disposisjon over de syntetiske trinnene i denne syntesen er gitt i Scheme 0.2.



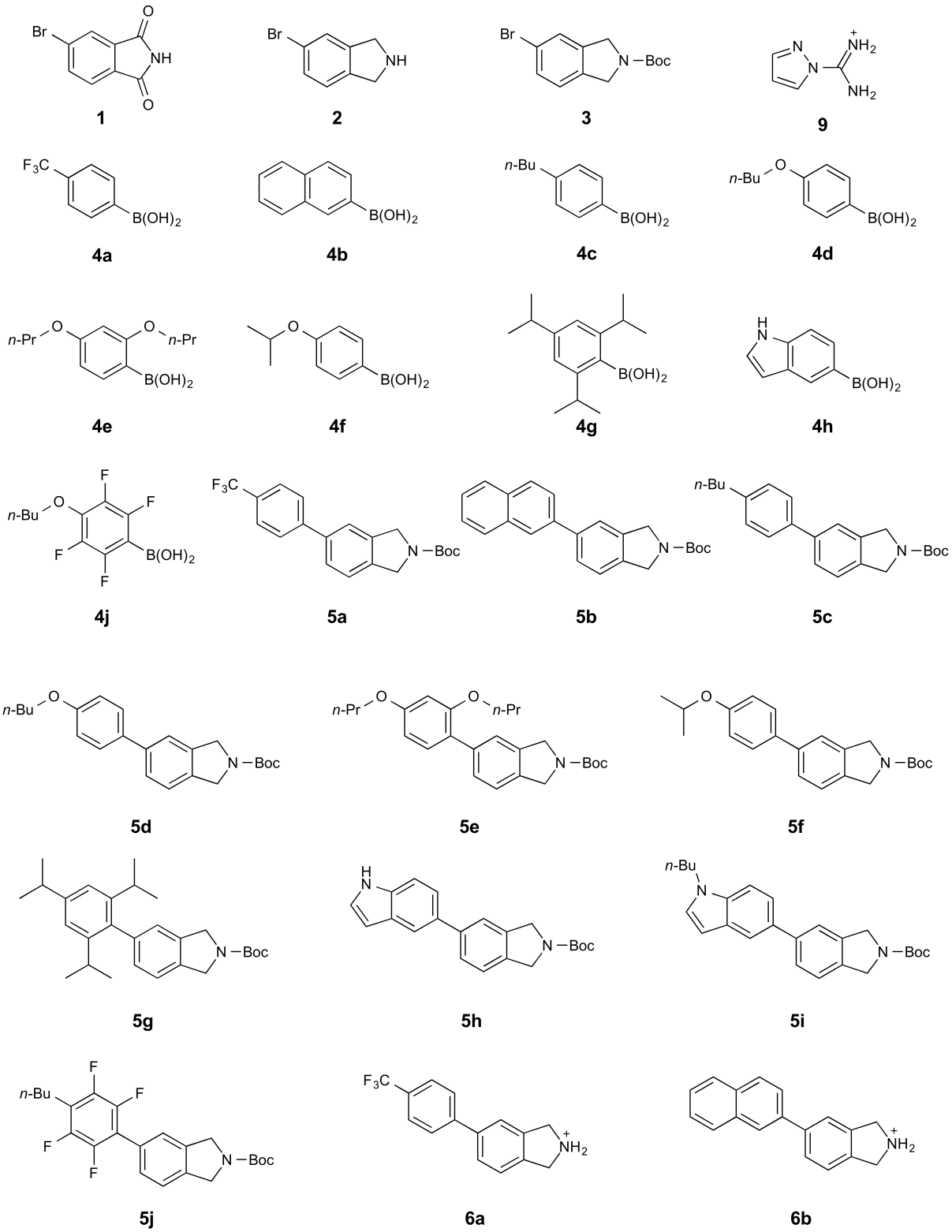
Scheme 0.2: Et overblikk over de syntetiske stegene brukt i denne oppgaven

Abbreviations and Symbols

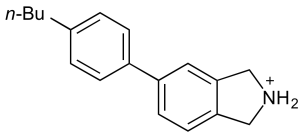
2D NMR	2-Dimensional NMR
AMP	Antimicrobial Peptide
APCI	Atmospheric Pressure Chemical Ionization
app	Apparent
aq.	Aqueous
Ar	Aryl
Ar-atm	Argon atmosphere
ASAP	Atmospheric Solids Analysis Probe
ATR	Attenuated total reflectance
Boc	*tert*-Butoxycarbonyl (protecting group for nitrogen)
br	Broad
cod	1,5-Cyclooctadiene
COSY	Correlation spectroscopy (H,H)
Cp	Cyclopentadienyl
Cp*	1,2,3,4,5-Pentamethylcyclopentadienyl
δ	Chemical Shift in NMR Spectroscopy
d	Doublet (NMR)
DCM	Dichloromethane
dd	Doublet of doublets (NMR)
DMAP	4-Dimethylaminopyridine
DMF	N,N-dimethyl formamide
DMSO	Dimethyl Sulfoxide
eq.	Equivalents
equiv.	Equivalents
ESI	Electron spray ionization
Et	Ethyl
h	hours
HMBC	Hetereonuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectroscopy
HSQC	Hetereonuclear Single Quantum Coherence
Hz	Frequency unit - defined as one cycle per second
IR	Infrared Radiation
J	Coupling constant used in NMR spectroscopy
m	Multiplet (NMR)
M	Molarity (mol/L)

m/z	Mass to Charge Ratio
mbar	Millibar (pressure unit)
Me	Methyl
MIDA	*N*-methyliminodiacetic acid
Mp.	Melting point
N_2-atm	Nitrogen atmosphere
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
ppm	Parts Per Million
q	Quartet (NMR)
qn	Quintet (NMR)
r.t.	Room Temperature
R_f	Retention Factor (TLC)
s	Singlet (NMR)
sat.	Saturated
sep	Septet (NMR)
sex	Sextet (NMR)
s.m.	Starting material
SPhos	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
t	Triplet (NMR)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatography
TMS	Trimethylsilyl/Tetramethylsilane
TOF	Time-of-Flight
UV	Ultraviolet

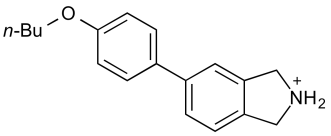
Numbered compounds



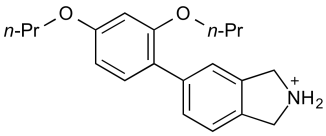
An overview of numbered compounds in this master thesis. For structures illustrated as a cations, the counter ion is Cl^-.



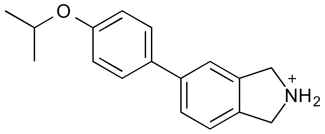
6c



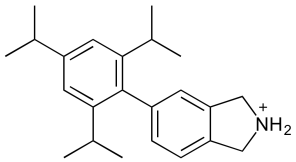
6d



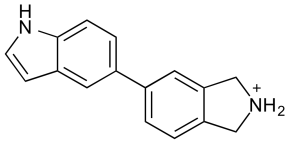
6e



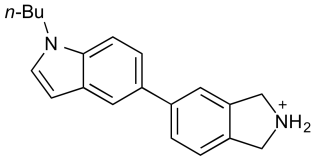
6f



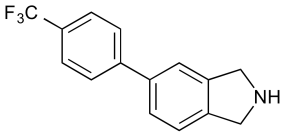
6g



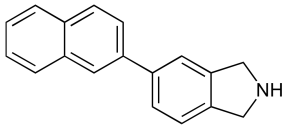
6h



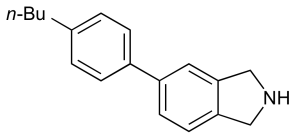
6h



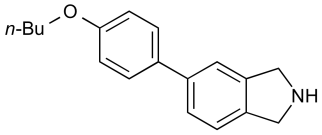
7a



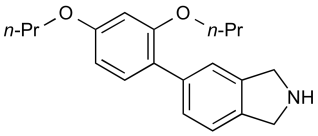
7b



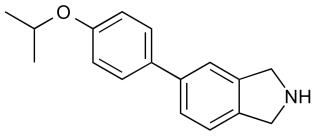
7c



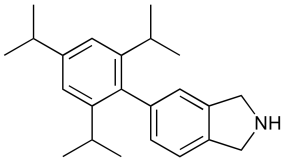
7d



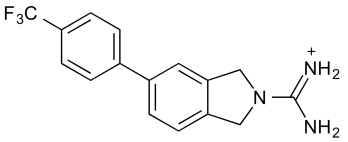
7e



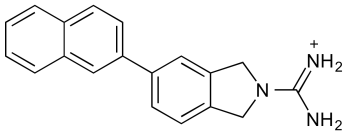
7f



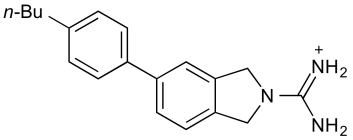
7g



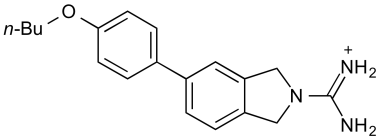
8a



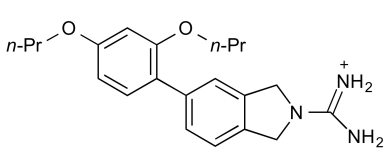
8b



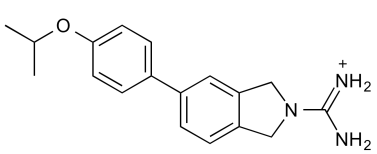
8c



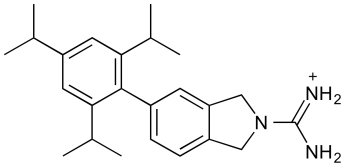
8d



8e



8f



8g

Table of Contents

1 Introduction

1.1 Background

Our society relies on a continuous development of new drugs, either as an improvement of existing and less efficient drugs, or even more importantly addressing clinical needs that are unmet today.[1] Natural products play a dominant role in the discovery of leads development of new drugs. It is estimated that almost 80% of the drugs prescribed are either natural products or derived from natural products.

Antibiotics, a class of drugs used to manage bacterial infections have since the discovery of penicillin by Alexander Fleming in 1928 saved millions of lives.[2,3] After the initial discovery followed the golden age of antibiotics, with more than 20 new and complex classes approved for human use developed within 3 decades.[4] Previously fatal infections are today manageable through various prescribed antibiotics, often being nothing more than a nuisance. Unfortunately, this may not be the case in the near future, as infectious bacteria are steadily developing resistance towards conventional treatment options.[5,6]

Antimicrobial resistance has been an obstacle in medical treatment of bacterial infections ever since first introduced for clinical use, but has not been of great concern until the last couple of decades, as the occurrence of antimicrobial increases rapidly. The initial flow of new classes of antibiotics seen after the initial discovery slowly died out, with only two new classes of antibiotics approved for human use in the last three decades.[7,8] Increased use of antibiotics in both agriculture and clinical settings, along with little development of new classes, are some of the leading causes to rapid emergence of global antimicrobial resistance.[9,10] Resistant infections are estimated to be the cause of around 700 000 deaths annually, a number expected to increase to a whooping 10 million deaths annually by 2050 if antimicrobial resistance is left unattended.[11] Therefore, development of new antibiotic classes is absolutely vital for our society.

1.2 Antimicrobial peptides

Most antibiotics today work by specific interactions with key sites in an intra- or extracellular environments.[12] Repeated exposure of antibiotics on these key sites stimulates the development of resistant mechanisms, usually undergoing a genetic change to lower its affinity towards the antibiotic.[13] An expanding field within antibiotic research is structures that work through less specific interactions, such as targeting non-specific sites in the intracellular environment, or interacting with the cell membrane.[14–16] A promising class of novel antimicrobial agents is antimicrobial peptides (AMP), first discovered in insects around 1980.[17] For more than 10^8 years AMPs have been a part of the innate immune response of a wide range of eukaryotes.[18] They are relatively small peptides of variable length, sequence and structure containing less than 100 amino acid residues. AMPs show amphipathic character, meaning they possess both a hydrophilic and a lipophilic moiety.[18] The antibiotic activity shown by AMPs are displayed due to their high net positive charge (aginine) while containing a large amount of bulky hydrophilic residues (tryptophan).[18,19] The antimicrobial activity is shown when folded into amphiphilic secondary structures, as this enables them to interact with the cell membrane. The membrane of Gram-negative

bacterial cells are made up of negatively charged phospholipids,[17] allowing the AMPs to interact with anionic bacterial cell through electrostatic interaction due to the AMPs cationic properties. Through various permeabilization mechanisms, the hydrophilic bulky residue is either bound to or penetrating the membrane. While conventional antibiotics interact with receptors on the cell membrane or inside the cell, AMPs interact directly with the cell membrane, potentially causing a rupture that kills the cell.[18] AMPs are not the only inspiration for creating new amphiphilic antimicrobials, as many amphiphilic antimicrobial natural products have also been isolated and characterized from marine environments, among them synoxazolidinone A,[20] hyrtioseragamine B,[21] and ianthelline,[22] shown in Figure 1.1. The simplest possible way of describing these compounds would be a hydrophobic group and a cationic nitrogen group attached to a linear or cyclic linker structure/scaffold. This principle has been has been explored by Strøm and co-workers with great success,[23] successfully preparing a library of amphiphilic aminobenzamides displaying high antimicrobial activity against resistant bacteria.

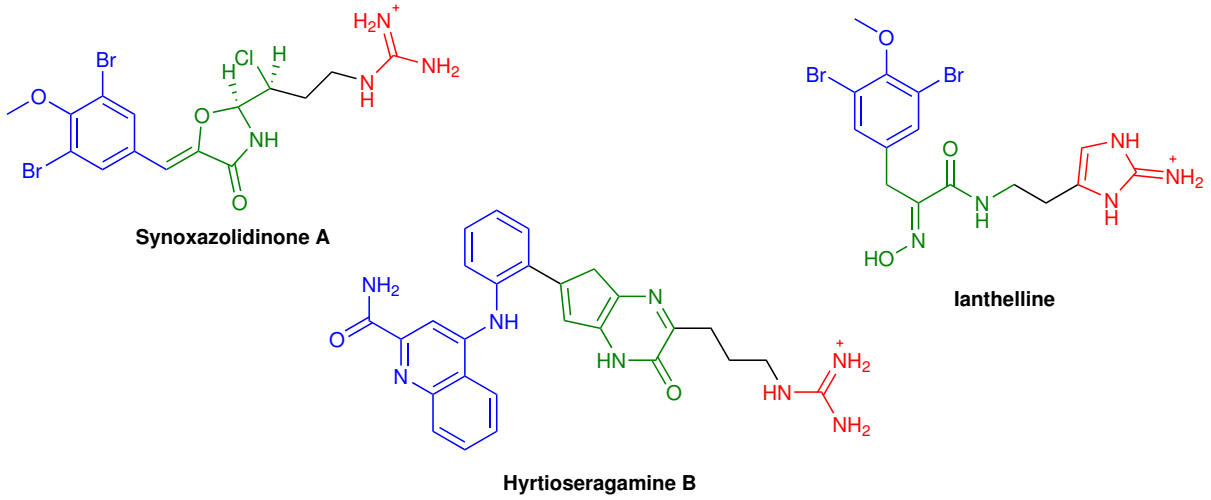


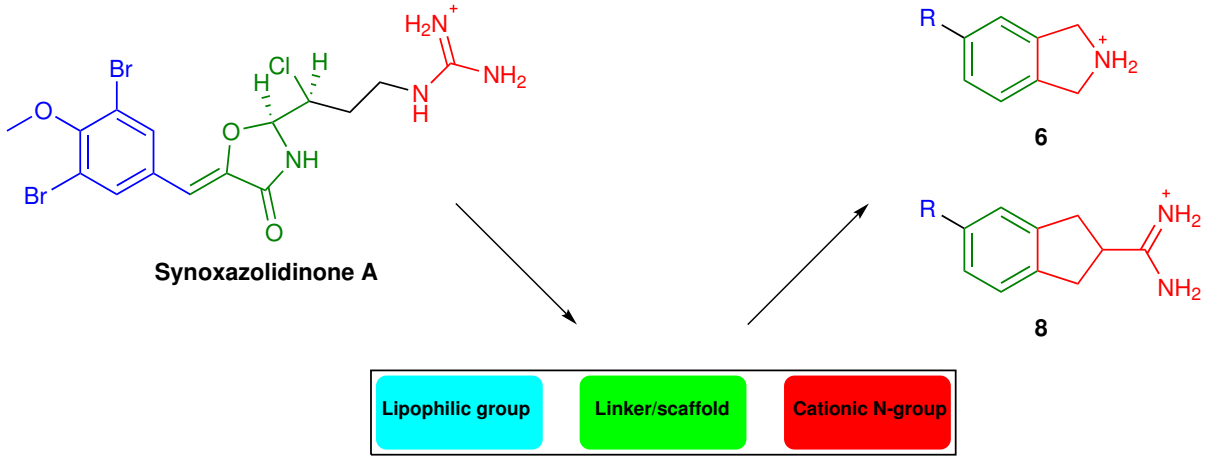
Figure 1.1: The marine natural products synoxazolidinone A,[20] hyrtioseragamine B,[21] and ianthelline,[22] in their charged state, with a lipophilic group and a cationic *N*-group connected through a cyclic linker or a central scaffold.

1.3 Strategy

The main objective and goal of the overall project is development of leads towards the discovery of new antimicrobial agents. The main focus will be synthesis of small cationic amphiphiles, a continuation of earlier work by Kristoffer L. Lea and Anton Brondz,[24,25] as well as a continuation of my own work.[26]

By simplifying marine natural product synoxazolidinone A,[20] to its simples form, it is possible to use isoindoline as a scaffhold for linking a variety bulky lipophilic groups, to either a cationic amine (**6**) or guanidine (**8**), as illustrated in Scheme 1.1

Lea prepared several isoindoline amines (**6**) that showed promising activities for different lipophilic groups, seen in Figure 1.1, but didn't successfully prepare guanidine **8** of sufficient purity for antimicrobial screening.[24]



Scheme 1.1: Model for target amphiphiles **6** and **8** derived from the marine natural product synoxazolidinone A.[20]

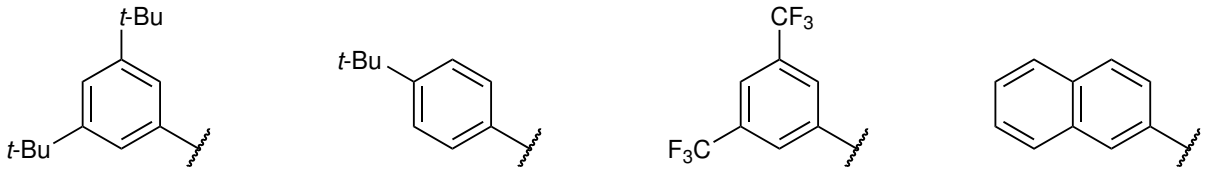
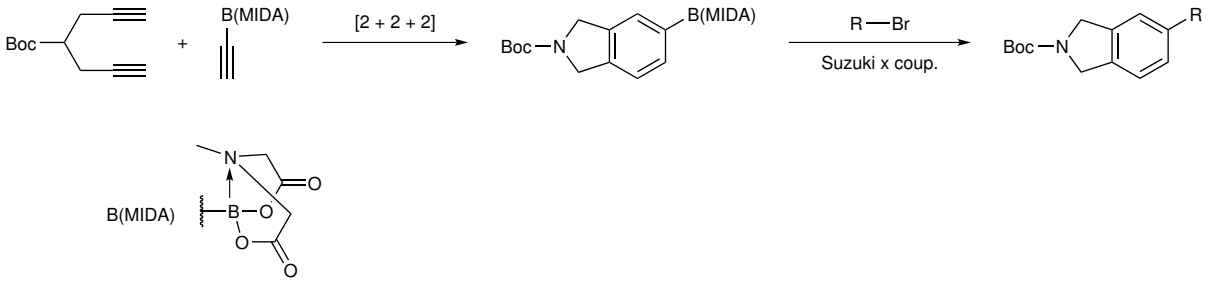


Figure 1.2: Bulky aromatic substituents for **6**.[24]

Lea and Brondz used transition metal catalyzed [2+2+2]-cyclotrimerization as a method of introducing different lipophilic groups to the isoindoline or fused pyridine skeleton.[24,25] The initial planned synthetic route for my Organic Specialization project,[26] shown in Scheme 1.2, was supposed to increase efficiency. One large scale [2+2+2]-cyclotrimerization of carbamate protected dipropargylamine and ethynyl MIDA boronate would allow for direct substitution with commercially available bromides through a Suzuki cross-coupling reaction, cutting the need for repeated [2+2+2]-cyclotrimerizations for each new substrate. Although this strategy looked promising on paper, issues with purification in the first step lead to the search of new synthetic pathways to reach targets **6** and **8**. The new

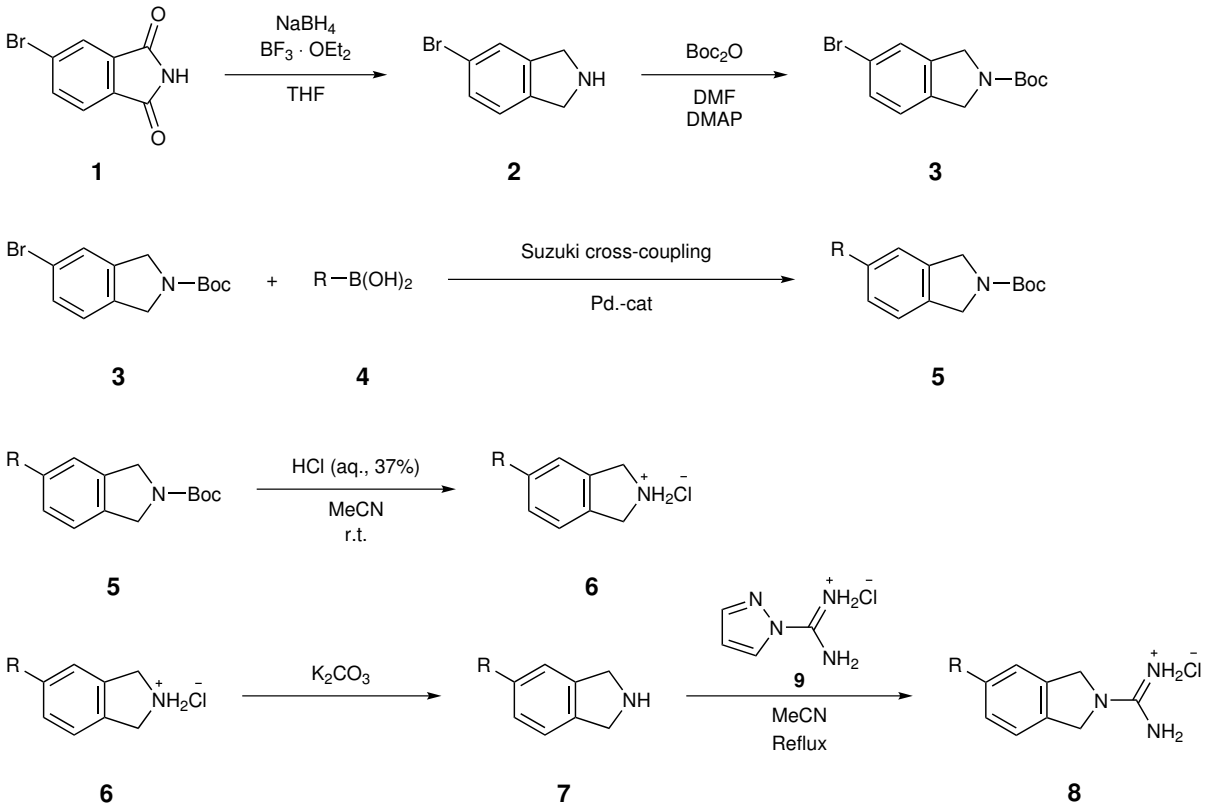


Scheme 1.2: The first two steps of the initial synthesis route to targets **6** and **8**

synthetic pathway, illustrated in Scheme 1.3 followed a two-step synthesis described by Patel and Barret.[27] Starting from commercially available 4-bromophthalimide (**1**), reduction by *insitu* diborane followed by carbamate protection was reported to yield *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**) in 64% from **1**. This method was successfully es-

tablished during the specialization project, isolating **3** in yields 35-51% from **1**, a valuable building block towards the target conpounds[26]

Suzuki cross-coupling reactions with **3** and commercially available boronic acids , yielding carbamate protected 5-substituted isoindline (**5**) seemed like a convenient and easy way to introduce a lipophilic group to the isoindoline scaffold. Deprotection of **5** with HCl would conveniently give isoindoline HCl-salt (**6**), one of the target compounds. Freebasing of **6** to **7**, along with a simplified method[28] for guanylation of free amine **7**should yield the targeted guanidine **8**.



Scheme 1.3: New synthetic route towards target isoindoline HCl-salt **6** and Guanidine **8**

The successfully prepared target compounds was planned sent to Marbio at UiT (The Arctic University of Norway) for testing. The sent compounds would be tested their antimicrobial activity against three gram-positive and two gram-negative bacteria; *Streptococcus agalacticae* (ATCC 12386), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212). Additionally, measuring the *in vitro* cytotoxicity against HepG2-cells (human hepatic cells) could give information about any adverse effects, in this case determining the hepatotoxic effects of the compounds.

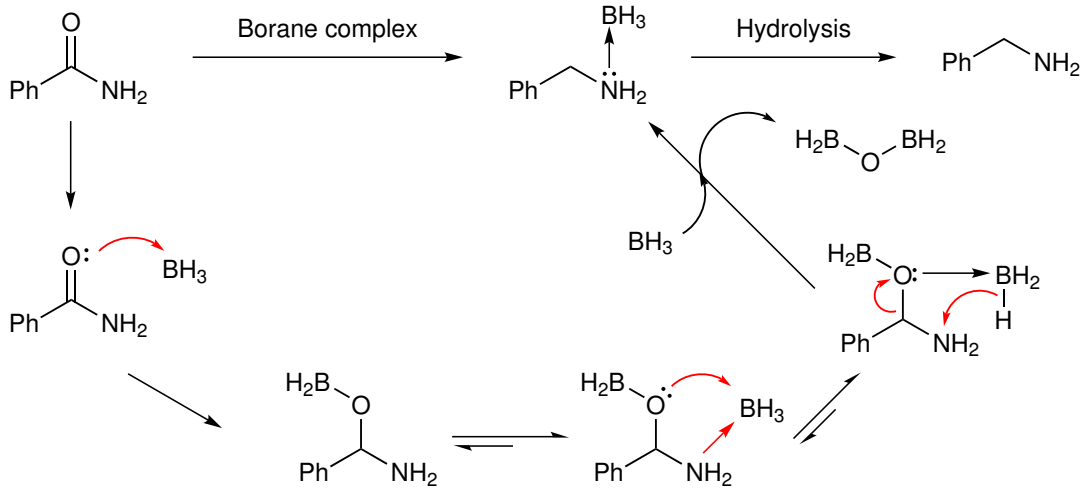
2 Theoretical background

This section will describe the theoretical principles behind the chemistry relevant for this master thesis. This includes a quick look at diborane as a reduction agent, a brief overview of transition metals and their uses in organic chemistry, the Suzuki cross-coupling reaction, carbamate protection/deprotection and guanylation.

2.1 Diborane as a reduction agent

Diborane, B_2H_6 is the simplest stable boron hydride. It is often represented as BH_3, which is the unstable monomeric form. The preparation and use of diborane as a reducing agent was first reported by Brown, Schlesinger and Burg in 1939.[29] Since then, boranes and metalhydrides as reducing agents has been explored widely. While metalhydride reagents are more commonly used, borane and some of its derivatives are still the preferred reagent for many applications.[30] One way of preparing diborane is by reaction of the Lewis acid $BF_3 \cdot OEt_2$ with LiH or $NaBH_4$ in an ethreal solvent.[31,32] Relevant for this thesis is the reductive properties of diborane towards phthalimides. Procedures for preparation of building block **3** has been reported, where the first step is reduction of 4-bromophthalimide (**1**) with diborane.[27,33] Both procedures utilize the reaction between $NaBH_4$ and $BF_3 \cdot OEt_2$ to generate diborane. By mixing the two components in a solution together with the phthalimide in THF, the generated diborane immediately reacts with the phthalimide.

Reduction of phthalimides with diborane is expected to happen very similarly to the mechanistic steps for reduction of an amide with diborane, due to structual similarities. A genereal mechanism for reduction of amides with diborane is illustrated in Scheme 2.1, reported by Burkhardt and Matos in 2006.[34] BH_3 is used instead of B_2H_6 for a simplicity. A total of 5 hydrides equivalents are used. Two of the hydrides are used to reduce the amide to amine, and the other three hydrides are utilized to form the amine borane complex.[34]



Scheme 2.1: General mechanism for reduction of amides with borane complexes.[34]

2.2 Transition metals in organic chemistry

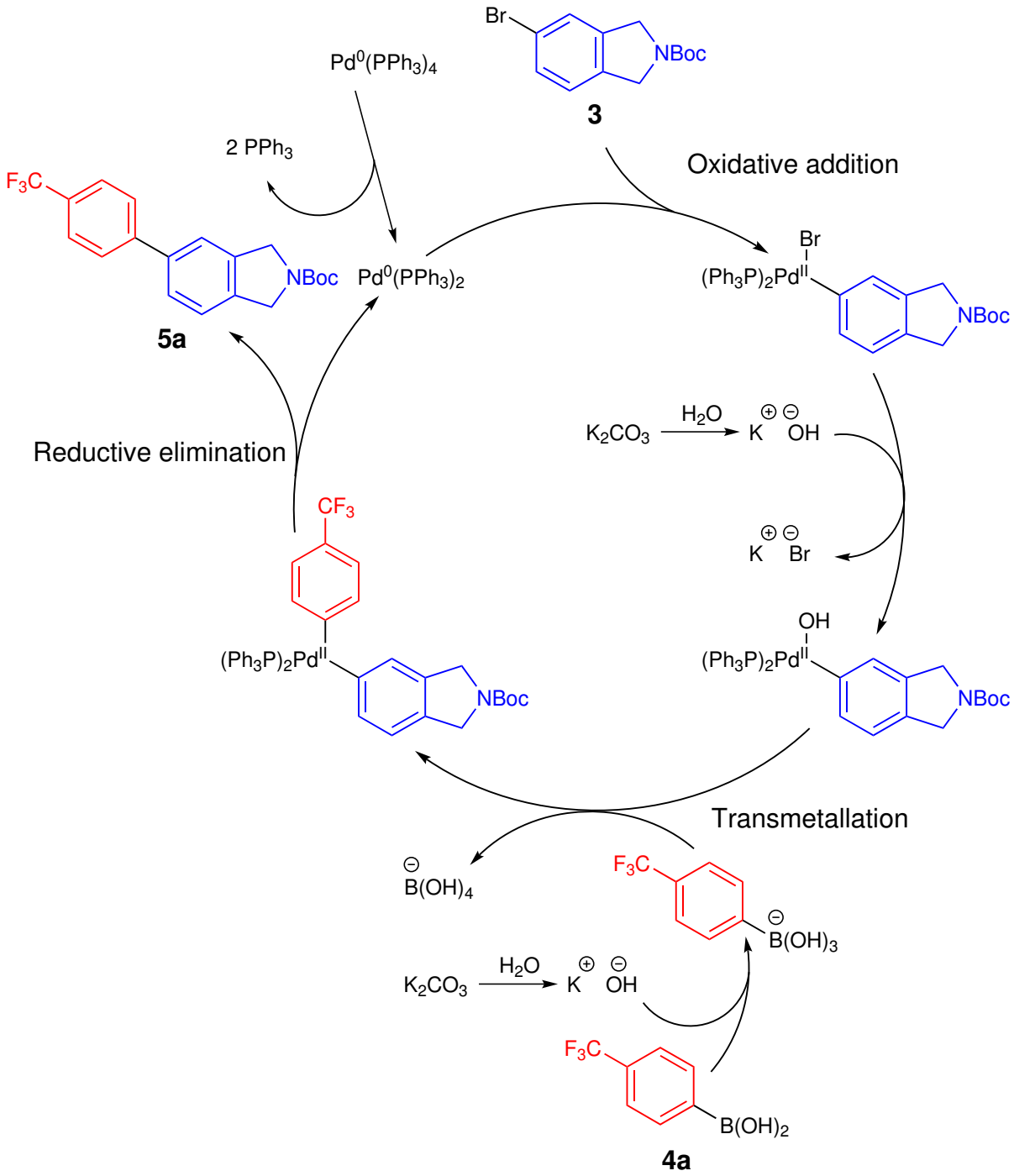
Transition metals are metals with incomplete *d*-orbitals located in the group 4-11 in the periodic table.[35a] The use of transition metals in organic synthesis allows for the formation of a wide range of compounds that would otherwise not be accessible or difficult to synthesize.[36] Transition metals coordinate with functional groups, altering the way compounds react in a wide number of ways, either by inverting the electrophilicity of species that normally undergo nucleophilic attack or by stabilizing intermediates that are too reactive under normal conditions. Transition metals make synthetic design more convenient, because these types of reactions are highly specific towards active sites on molecules. By complexation with organic ligands such as CO, CN^- and PPh_3, the metals can fill up their *d*-shells. It is common for reactions involving transition metal complexes to be catalytic processes, where mechanisms are described in terms of catalytic cycles, showing the role of the catalytic complex in the reaction and the regeneration.[35a]

2.2.1 Suzuki cross-coupling

The Suzuki cross-coupling reaction is among the most famous and well-known method for transition metal catalyzed carbon-carbon bond forming to date.[37] First published by Miyaura, Yamada, and Suzuki in 1979, the protocol was specific to cross-couple 1-alkenylboranes with 1-alkenyl or 1-alkenyl halides, catalyzed by the palladium complex tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$).[38] Since its inception there has been major advancements in Suzuki cross-coupling, allowing milder reaction conditions and expanding the substrate scope massively.[37,39] Several thousand publications,[37] a broad selection of catalyst systems and various classes of commercially available organoboron compounds clearly indicates the impact of Suzuki cross-coupling in organic synthesis. Akira Suzuki shared the 2010 Nobel Prize in Chemistry with Richard F. Heck and Ei-ichi Negishi, acknowledging their contribution to Pd-catalyzed cross-coupling in organic synthesis.[40]

Suzuki cross-coupling can be divided into three basic steps, oxidative addition, transmetallation, and reductive elimination. Both oxidative addition and transmetallation can be the rate determining step, depending on the reaction conditions.[35b] The mechanism of Suzuki cross-coupling has been widely studied and debated, with small variations depending on the reaction conditions.[37,41] For some organoboron compounds, such as the boronic acids, a base is needed for activation, believed to involve formation of the more reactive boronate anion in the transmetallation step.[35b] The mechanism is illustrated in Scheme 2.2, using the preparation of **5a** (Section 6.3.1) as an example.

Halide **3** reacts with the Pd^0-complex by oxidative addition, forming a Pd^{II} intermediate, which in most cases is the rate determining step.[37] This step is followed by transmetallation with the boronate anion of **4a**. The formed Pd^{II}-complex will in the final step undergo reductive elimination, leading to carbon-carbon bond formation (**5a**) and regeneration of the catalyst.



Scheme 2.2: Catalytic cycle of Suzuki cross-coupling showing the three basic steps: oxidative addition, transmetallation, and reductive elimination.[37,41] The preparation of **5a** (Section 6.3.1) is used as an example.

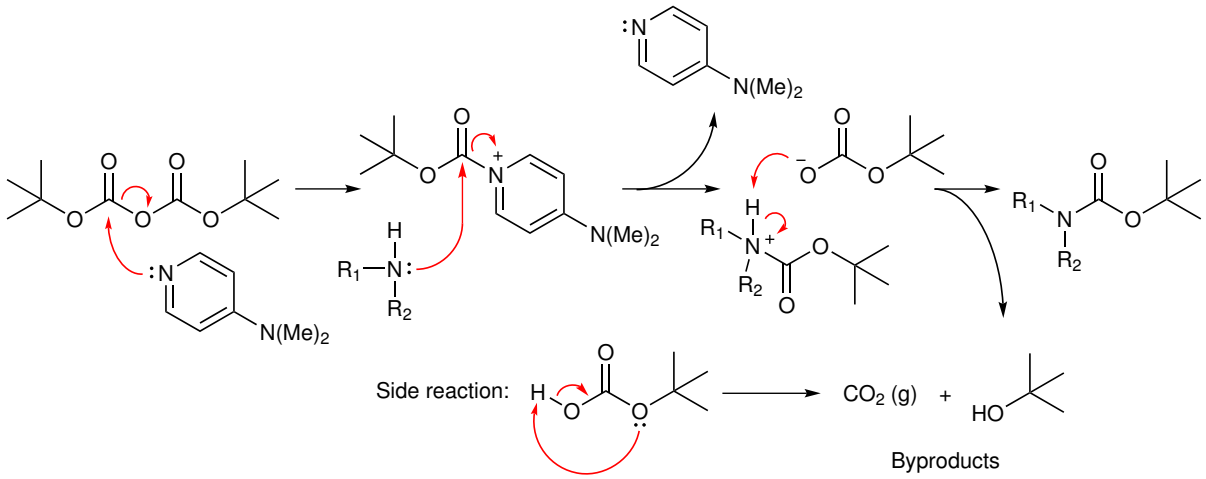
2.3 Protection and deprotection of the amino group

Amines are nucleophilic and easily oxidized, and sometimes also basic enough to deprotonate many organometallic reagents.[35c] The reactivity of the amino group can be greatly reduced if bonded to a protective group, allowing for reactions where amino groups usually interfere to take place. After the sensitive reaction step(s), the amine protection group can be removed, allowing for further reactions on the amino group.

A wide selection of amine protective groups with different properties are available.[42] The amine protective group used in this thesis, *tert*-butoxycarbonyl (Boc), belongs to a series of carbamates popularly used as protective groups for amines.[35c] The group offers stability under basic conditions, a necessity due to planned Suzuki cross-coupling, along with simple procedures for both protection[27,33] and deprotection.[35c 42]

2.3.1 Protection

One way of introducing the Boc group is reacting the amine with di-*tert*-butyl dicarbonate (Boc_2O) in presence of 4-dimethylaminopyridine (4-DMAP).[42,43] The mechanism for introduction of the Boc group is illustrated in Scheme 2.3.[43]

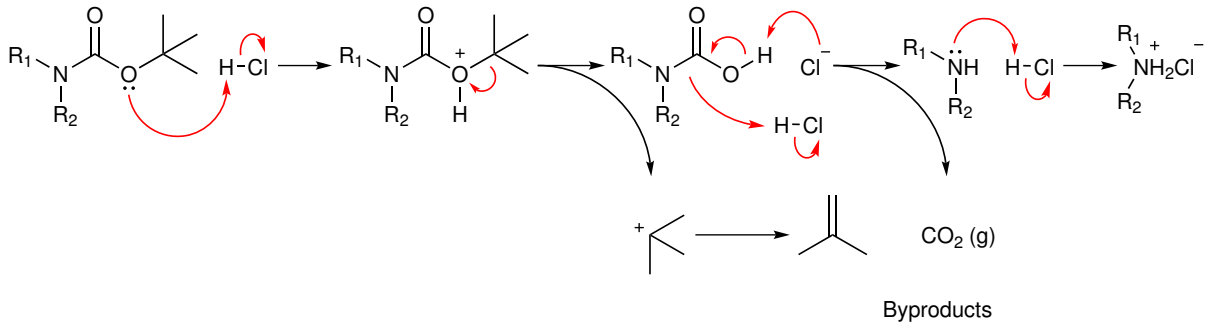


Scheme 2.3: Mechanism for introduction of the Boc protecting group to an amine with di-*tert*-butyl dicarbonate (Boc_2O), catalyzed by 4-DMAP.[43a]

4-DMAP attacks the carbonyl site of Boc_2O, resulting in Boc-pyridinium complex and *tert*-butyl carbonate.[43a] The amine then attacks the complex at the carbonyl site, releasing DMAP in the process. Finally, the earlier released *tert*-butyl carbonate picks up a proton, leaving the Boc-protected amine and *tert*-butyl hydrogen carbonate, the latter decomposing into *tert*-butanol and CO_2.

2.3.2 Deprotection

The Boc protective group is normally removed by through acid hydrolysis with a strong acid such as TFA or HCl.[35c] The mechanism for HCl deprotection of *N*-Boc is illustrated in Scheme 2.4.[43b]



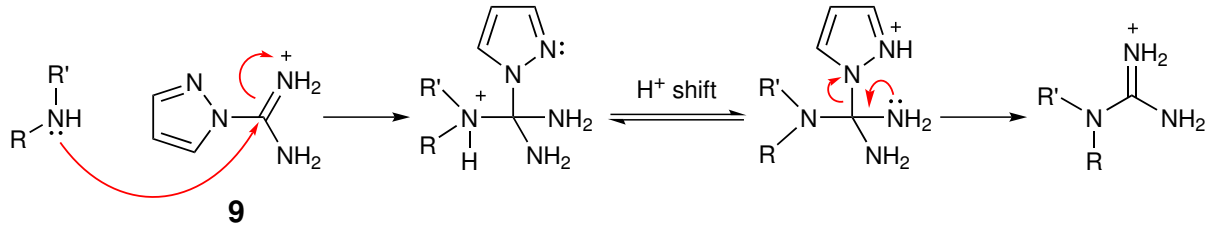
Scheme 2.4: Mechanism for removal of the Boc protecting group under strongly acidic conditions.[43b] Cleavage with HCl yields the deprotected amine as its HCl-salt.

The cleavage of the *tert*-butyl group is the driving force behind this reaction, forming a stable tertiary carbocation that subsequently eliminates into isobutylene.[43b] Deprotonation followed by the loss of CO_2 forms the neutral amine. HCl protonates the neutral amine, leaving the deprotected amine as a HCl-salt.

2.4 Guanylation

Guanidines can be prepared by reacting an amine with an electrophilic guanylation reagent, commonly based on amides or urea derivatives.[44] 1*H*-pyrazole carboxamidine HCl (**9**) is a widely used guanylation agent for amines.[44,45] Reported reaction conditions for the use of **9** involve the use of an inorganic or organic base in DMF as solvent.[44] Purication under these conditions can be troublesome due to high boiling point of solvent DMF and polar nature of guanidines.

A recent publication describes guanylation of amines with **9** under simpler reaction conditions.[28] Additive of base was found to be unnecessary, as **9** with a slight excess amine in MeCN at reflux was reported to yield the pure guanidine HCl-salt within a few hours. Eventual residual amine and by-product 1*H*-pyrazole are easily removed with little to no work-up. A proposed reaction mechanism for this reaction is illustrated in Scheme 2.5.



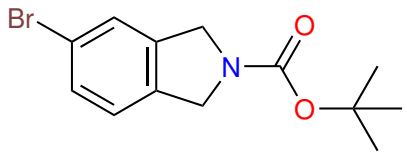
Scheme 2.5: Proposed mechanism for guanylation of an amine with 1*H*-pyrazole carboxamidine HCl (**9**). Counterions: Cl^-.

3 Results and discussion

This section presents and discusses the experimental results described in Section 6, along with the spectroscopic analysis found in Section 5. Additionally it will discuss the antimicrobial activity against 5 different bacteria, and cytotoxicity towards HepG2-cells for the successfully prepared target compounds sent to Marbio at UiT (The Arctic University of Norway) for testing.

3.1 Preparation of the building block 5-bromoisoindoline (3)

As mentioned in the introduction, the original planned synthetic route for preparation of the target isoindoline HCl-salts **6** and guanidines **8** didn't work out, a new synthetic route was developed and successfully established earlier in the project.[26] The new synthetic route followed a procedure described by Patel and Barrett,[27] where *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**), shown in Figure 3.1, was prepared in a two-step synthesis from commercially available 4-bromophthalimide (**1**). With reported total yields of 64% and 49% for the two steps,[27,33] the procedure seemed very promising, as **3** would be a good building block for introduction of different substituents to the isoindoline scaffold through Suzuki cross-coupling.



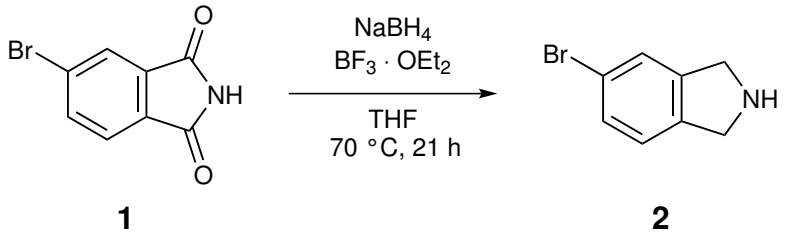
3

Figure 3.1: Structures of *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**).

3.1.1 Reduction with diborane

The experimental details and procedure for this reduction is described in Section 6.2.1. In the first step, illustrated in Scheme 3.1, phthalimide **1** (8.205 g) was mixed with $NaBH_4$ and Lewis acid $BF_3 \cdot OEt_2$ in THF. The hydride and Lewis acid were used to prepare diborane *in situ*,[30] because diborane is known to be unstable and highly reactive.[46] Due to electron-deficiency, diborane would react exothermically with oxygen if exposed to air. Acid/base extraction yielded a brown oil (5.648 g).

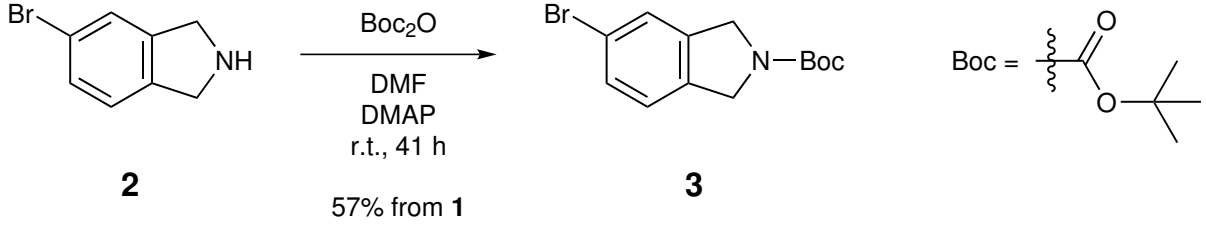
The ^{1}H NMR spectrum (Appendix A.1) of the brown oil was in accordance with reported data for **2**,[27] but also indicated a presence of impurities. From personal experience, purification of **2** by flash column chromatography can be very troublesome. After carbamate protection of **2**, purification should no longer be a problem. It was decided that further purification of the crude oil was unnecessary. As a consequence, yield was not calculated until after isolation of **3**.



Scheme 3.1: Reduction of 4-bromophthalimide (**1**) to 5-bromoisoindoline (**2**) utilizing diborane prepared *in situ* from $NaBH_4$ and the Lewis acid $BF_3 \cdot OEt_2$.

3.1.2 Carbamate protection

The experimental details and procedure for this reaction is described in Section 6.2.2. In the second step, illustrated in Scheme 3.2, utilized di-*tert*-butyl dicarbonate (Boc_2O) with a catalytic amount of 4-DMAP in DMF to introduce a *tert*-butoxycarbonyl (Boc) protecting group onto free amine **2**.[27] Purification by flash column chromatography yielded the carbamate protected amine in 57% over two steps.



Scheme 3.2: Carbamate protecton of 5-bromoisoindoline (**2**) with di-*tert*-butyl dicarbonate (Boc_2O), yielding *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**).

This two-step synthesis was also carried out earlier in the project, resulting in total yields 35-51%.[26] Reported yields for the same two-step synthesis from litterature were 64% and 49%.[27,33] An increase in yield compared to earlier attempts, as well as being close to the highest reported yield, 57% was a satisfying result. The yield could have been further increased by a few percent by repeated purification by flash chromatography, however, purification to increase these yield of **3** was not attempted.

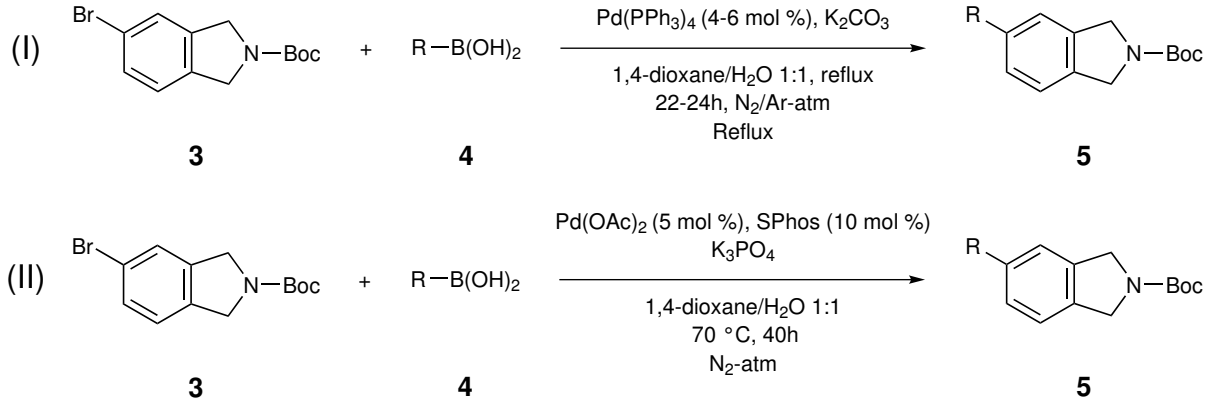
3.2 Preparation of 5-substituted isoindolines 5a-j

3.2.1 Suzuki cross-coupling

The experimental details and procedures for each reaction is described in Section 6.3. For analysis of spectroscopic data and elucidation of structures for isoindolines **5a**-**h**, see Section 5.2.1-5.2.8. Isoindolines **5a**-**h** and **5j** were prepared by a Suzuki cross-coupling between halide **3** and the appropriate boronic acids **4a**-**h** and **4j**. All preparations were first attempted prepared with $Pd(PPh_3)_4$ as catalyst, according to a procedure described by Hua *et al.*,[47] with modifications to the solvent system as described by Bugge *et al.*[48]. This procedure succesfully gave satisfactory pure **5a**-**5f** and **5h** in 71-89% yields. Isoindolines that were not successfully prepared by this method, were attempted prepared

using $Pd(OAc)_2$/SPhos, a more active catalyst system. Following a procedure described by Knapp, Gillis and Burke,[49] this catalyst system successfully gave **5g** (70% yield), but failed to prepare **5j** of satisfactory purity. The two employed methods for Suzuki cross-coupling, along with a summary of results for all attempted Suzuki cross-coupling reactions can be found in Table 3.1.

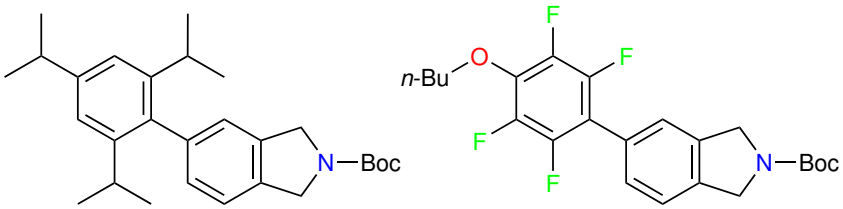
Table 3.1: Preparation of 5-substituted isoindolines from Suzuki cross-coupling reactions between **3** and boronic acids **4a-h** and **4j**. Entries marked with I were conducted according to the general procedure described on the first page of Section 6.3.[47,48] Entries marked with II were conducted according to the procedure described in Section 6.3.7 - Attempt 2.[49]



Entry #	**3** (mmol)	**4** (equiv.)	Proc.	R	Time (h)	Products	Yield[a] (mmol, %)
1a	1.88	**4a**, 1.17	I	4-CF_3-Ph	22	**5a**	1.46, 77%
1b	1.66	**4a**, 1.24	I	4-CF_3-Ph	22	**5a**	1.46, 78%
2	1.68	**4b**, 1.23	I	naphtalen-2-yl	24	**5b**	1.36, 81%
3a	1.68	**4c**, 1.17	I	4-*n*-BuPh	23	**5c**	1.20, 71%
3b	2.40	**4c**, 1.19	I	4-*n*-BuPh	21	**5c**	2.13, 89%
4	1.73	**4d**, 1.16	I	4-(*n*-BuO)Ph	22	**5d**	1.23, 71%
5	1.68	**4e**, 1.19	I	2,4-di-(*n*-PrO)Ph	24	**5e**	1.39, 83%
6	1.68	**4f**, 1.19	I	4-(*i*-PrO)Ph	24	**5f**	1.34, 80%
7a	1.68	**4g**, 1.19	I	2,4,6-tri-(*i*-Pr)Ph	25	**3**	-[b]
7b	2.00	**4g**, 1.20	II	2,4,6-tri-(*i*-Pr)Ph	40	**5g**	1.40, 70%
8	3.35	**4h**, 1.19	I	1*H*-indol-5-yl	23	**5h**	2.73, 81%
9a	1.68	**4j**, 1.31	I	4-(*n*-BuO)-(2,3,5,6-tetra-F)Ph	23	**3** + **5j**	-[c]
9b	1.68	**4j**, 1.19	I	4-(*n*-BuO)-(2,3,5,6-tetra-F)Ph	168	**3** + **5j**	-[c]
9c	1.38	**4j**, 1.19	II	4-(*n*-BuO)-(2,3,5,6-tetra-F)Ph	40	**3** + **5j**	-[c]

[a] Isolated yield after purification by flash column chromatography.
[b] Traces of **5g** was observed in the ^{1}H NMR spectrum (Appendix I.7).
[c] Unsuccessful isolation of **5j**. Molar ratios were estimated using ^{1}H NMR. Estimated ratios (**3** : **5j**):
Entry 9a: 3.8 : 1 (Appendix L.1).
Entry 9b: 1 : 6.9 (Appendix L.2).
Entry 9c: 2.7 : 1 (Appendix L.3).

A majority of attempted Suzuki cross-couplings were successful with the catalyst system employed in procedure I (Table 3.1[i], Entries 1-6 and 8). Yields for the seven successful cross-coupling reactions were consistant around 70-80%, comparable to those earlier reported by Hua *et al.*[47] While this protocol worked well for Entries 1-6 and 8, difficulties arose when attempting to prepare **5g** and **5j** (Entries 7a, 9a and 9b). The structures of isoindolines **5g** and **5j** are shown in Figure 3.2.



5g **5j**

Figure 3.2: Structures of isoindolines **5g** and **5j**.

The first attempt at preparing **5g** (Entry 7a, proc I) yielded only trace amounts of the desired cross-coupling product, and mainly unreacted starting materials were seen by ^{1}H NMR (Appendix I.7) The difficulties of cross-coupling **3** and **4g** may be due to sterical effects[50] from the isopropyl groups on the boronic acid (**4g**). Additionally, the possibility of a human error being responsible for the failed attempt cannot be ruled out, as this experiment was not duplicated. Due to limited amount of time, the second attempt (Entry 7b) followed procedure II, expected to be a more active catalyst system. The active catalyst system employed in the second attempt afforded **5g** in 70% yield, just below average yield for the successful cross-couplings with the original catalyst system.

The first attempt at preparing **5j** (Entry 9a) was carried out according to procedure I. This attempt was initially believed to be successful, as only one spot was observable in the TLC-chromatogram after flash column chromatography. Unfortunately, ^{1}H NMR-analysis indicated a mixture of **3** and **5j** (3.8 : 1, Appendix L.1). An additional attempt at purification by flash column chromatography with a more polar eluent system was unsuccessful. A second attempt (Entry 9b) was carried out according to procedure I.. ^{1}H NMR-analysis was used to determine conversion of **3** to **5j**, eventually stopping the reaction upon 100% conversion. Unfortunately the reaction rate seemed to rapidly decrease after passing 50% conversion (day 3 to 4). With a microscopic hope of achieving full conversion, the reaction mixture was kept at reflux for a total of 7 days (168 hours). No change in conversion was observed after day 5. While achieving a much higher conversion of **3** (**3** : **5j**, 1 : 6.9), Appendix L.2), attempted purification by flash column was still unsuccessful. A last attempt was made (Entry 9c), this time following procedure II. ^{1}H NMR-analysis after 40 hours indicated no big improvements to conversion rate of **3** (**3** : **5j**, 2.7 : 1, Appendix L.3)

Featuring four electron withdrawing fluorine substituents, the ring system of **4j** suffers from high electron-deficiency. This is unique for **4j** among the boronic acid emplyed in this project. A boronic acid very similar to **4j** is pentafluorophenylboronic acid. The structure of both is shown in Figure 3.3.

[i]Entries discussed below all refer to Table 3.1. This is valid for the rest of Section 3.2.

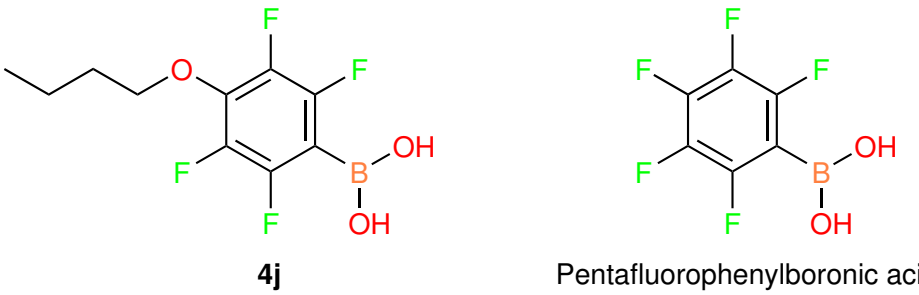
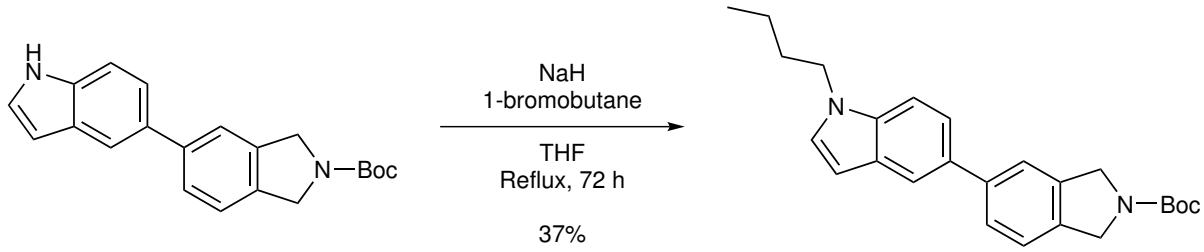


Figure 3.3: Structures of 4-butoxy-2,3,5,6-tetrafluorophenylboronic acid (**4j**) and pentafluorophenylboronic acid.

Pentafluorophenylboronic acid is known to be an inactive substrate under normal conditions for Suzuki cross-coupling.[51] Transmetalation of the highly electron-deficient C_6F_5 body to the Pd-center is believed to proceed only with difficulty.[52] To successfully employ pentafluorophenylboronic acid for Suzuki cross-coupling, special conditions like promotion by Ag_2O, DMF as solvent and/or extremely active catalyst systems such as $Pd_2(dba)_3/P(t\text{–}Bu)_3$ must be empoloyed.[52] While not as electron-deficient as pentafluorophenylboronic acid, it should be a reasonable assumption that the high electron-deficiency of **4j** explains the low reactivity in a Suzuki cross-coupling reaction with **3**.

3.2.2 Preparation of 5i by *N*-alkylation of 5h

Compound **5i** was prepared by *N*-alkylation of **5h** with 1-bromobutane, as shown in Scheme 3.3. While the original procedure described by Elkassih *et al.*,[53] was meant for *N*-alkylation of phenothiazine with 1-bromodecane, adapting the procedure to work for *N*-alkylation of **5h** with 1-bromobutane was expected to work well. The experimental details and procedure for preparation of **5c** is described in Section 6.3.9. For analysis of the spectroscopic data and elucidation of the structure of **5i**, see Section 5.2.9.



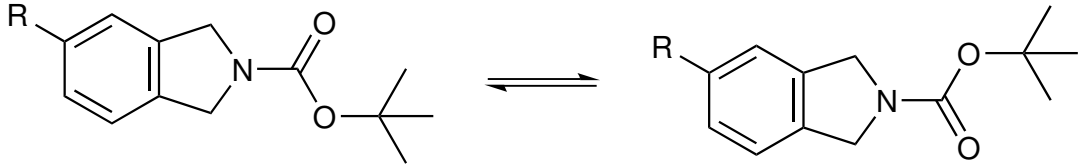
Scheme 3.3: N-alkylation of **5h** with NaH and 1-bromobutane in THF, to *tert*-Butyl 5-(1-butyl-1*H*-indol-5-yl)isoindoline-2-carboxylate (**5i**).

The *N*-alkylation of **5h** proceeded at a slower reaction rate than expected. After 48 hours at reflux, TLC-anlysis still indicated unreacted **5h** to be present, leading to addition of more 1-bromobutane, followed by additional 24 hours at reflux. TLC-analysis still indicated the presence of **5h** and as a result the reaction was stopped after a total of 72 hours. Purification by flash column chromatography yielded **5i** in 37%. The original

alkylation of phenothiazine reported a yield of 89% just by refluxing over night.[53] The low yield could be due to old/degraded NaH. The container was covered in corrosion, and the container has been opened outside of inert atmosphere on several occasions.

3.2.3 Observed rotamers

All compounds containing the *tert*-butoxycarbonyl (Boc) protecting group (**3** and **5a-5j**) featured additional signals both in their ^{1}H NMR and ^{13}C NMR spectra. Further investigation with 2D NMR techniques indicated two sets signals from the same proton/carbon on the isoindoline skeleton. Since this effect only was observed for compounds with carbamate protection, duplicate signals for these positions are likely due to two stable rotary confirmations of the carbamate protecting group, as illustrated in Scheme 3.4.



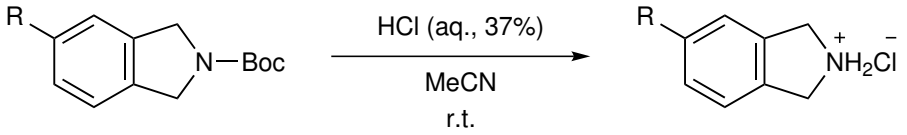
Scheme 3.4: Two hypothetical stable confirmations for the carbamate protecting group, leading to duplicate signals in both ^{1}H NMR and ^{13}C NMR spectra of **3** and **5a-j**.

The rotamers are observable in NMR spectra due to their slow interconversion at room temperature.[54] By increasing the temperature, the rate of interconversion may be increased, causing it to occur too rapidly for individual rotamers to be visible and thereby merging the signals. Temperature NMR experiments were not conducted.

3.3 Preparation of 5-substituted isoindoline HCl-salts 6a-i

The experimental details and procedures for each attempted deprotection is described in Section 6.4. For analysis of spectroscopic data and elucidation of structures for isoindoline HCl-salts **6a**-**g**, see Section 5.3.1-5.3.7. Cleavage of the carbamate protection groups was carried out using aqueous HCl (37%) in MeCN. The used procedure for both the reaction reaction conditions and work-up has been developed within the Gautun research group over the last couple of years. Carbamate protected isoindolines **5a**-**h** were dissolved in MeCN, followed by dropwise addition of 3-10 equivalents HCl (aq., 37%). The reation mixture was stirred at room temperature until TLC-analysis indicated full conversion. Work-up gave **6a**-**g** in 67-99% yields. Preparation and/or isolation of indole derivatives **6h** and **6i** was unsuccessful. An illustration of the employed method, as well as a summary of the successful carbamate deprotection reactions can be found in Table 3.2.

Table 3.2: Summary of the successful carbamate deprotections of isoindolines **5a**-**g** with HCl (aq., 37%) in MeCN, yielding the deprotected isoindolines **6a**-**g** as their HCl-salts.



5 **6**

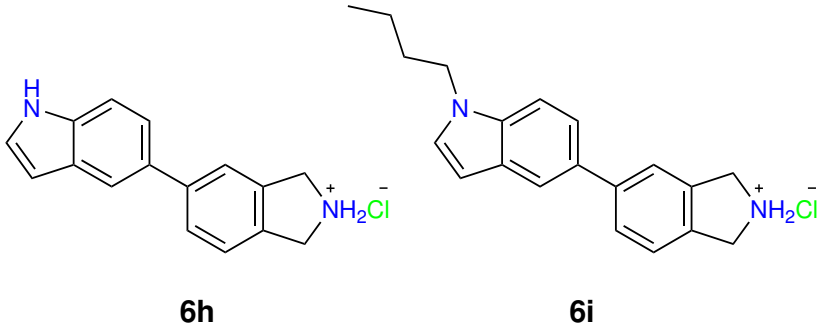
Entry #	Isoindoline **5**	HCl (aq., 37%) (equiv.)	R	Time (h)	Product **6**	Yield (mmol, %)
1	**5a**	5	4-CF_3-Ph	19	**6a**	0.407, 99%
2	**5b**	10	naphtalen-2-yl	19	**6b**	0.245, 79%
3	**5c**	5	4-*n*-BuPh	14	**6c**	0.403, 79%
4	**5d**	5	4-(*n*-BuO)Ph	16	**6d**	0.332, 87%
5	**5e**	5	2,4-di-(*n*-PrO)Ph	14	**6e**	0.486, 84%
6	**5f**	5	4-(*i*-PrO)Ph	19	**6f**	0.414, 67%
7	**5g**	3 + 5[a]	2,4,6-tri-(*i*-Pr)Ph	23	**6g**	0.497, 84%

[a] Additional 5 eq. of HCl (aq., 37%) was added after 21 hours to achieve full conversion of **5g**.

An unidentified by-product formed concurrently for all deprotections, showing up as broad signals in ^{1}H NMR spectra. The by-product, suspected to be acetamide, was removed through extensive drying at 60 °C under reduced pressure (0.5-2 mbar). Other impurities were removed through crystallisation with methanol and ether.

Preparation and isolation of **6f** was initially deemed successful from ^{1}H NMR analysis, but found to be unstable. The decomposition of **6f** is discussed in more detail together with decomposition of **8f** in Section 3.4.1.

In the case of indole isoindolines **5h** and **5i**, isolation of expected products **6h** and **6i**, illustrated in Figure 3.4, was not successful. The amino group of indoles were assumed to be less reactive than the isoindoline amino group, due to the nitrogen-lone pair being a part of the aromatic π-electron system.[55a] For this reason it was assumed that the second

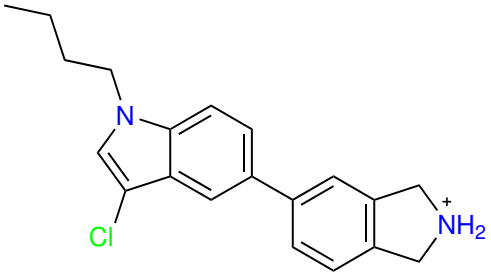


6h **6i**

Figure 3.4: Structures of expected deprotection products **6h** and **6i**.

amino group wouldn't react with HCl when deprotecting the isoindoline amino group. This was clearly not the case. For the mixture expected to contain **6h**, the ^{1}H NMR spectrum (Appendix T.1) indicated a mixture of products. MS-analysis (Appendix T.2) confirmed **6h** to be present in the product mixture, and it is suspected that excess HCl partially protonated both the indole and isoindoline.

Alkylation of the indole aminogroup did not help solve the problem. ^{1}H NMR-analysis (Appendix U.1) of the product mixture gave no answers, other than blurred and inconclusive spectra in both DMSO-d_6 and MeOD. MS-analysis indicated the presence of expected product **6i** (Appendix U.2), but also made for an interesting discovery. The mass spectrum featured a strong signal with a mass equivalent to the molecular formula of $C_{20}H_{22}ClN_2^+$ (Appendix U.3). This was surprising and unexpected, as the only way to relate $C_{20}H_{22}ClN_2^+$ to **6i** is replacing a proton with covalently bonded chlorine. Usually a source of "Cl^+" (e.g sulfuryl chloride) is necessary for chlorination of indoles.[55b] A likely structure of the chlorinated byproduct is illustrated in Figure 3.5, with chlorine substituted in the 3-position of the indole, as this position is the most susceptible for electrophillic aromatic substitution.[55b] However, this is purely speculative, and it is unknown how reaction conditions featuring HCl and MeCN at room temperature can lead to chlorination of **6i**. As mass spectra of **6h** did not indicate any similar relation, it is likely that the alkyl chain is an important factor, lowering the electron-deficiency in the ring system, thus promoting electrophilic aromatic substitution. Due to a limited time frame, no further investigation or experiments were conducted.



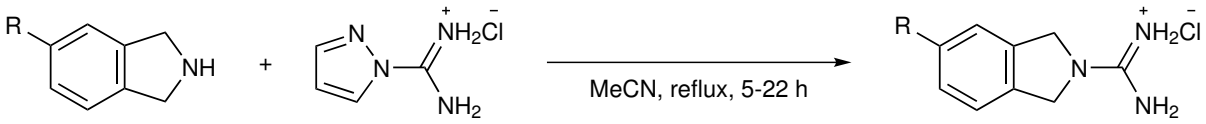
$C_{20}H_{22}ClN_2^+$

Figure 3.5: Proposed structure of M^+-ion belonging to chlorine byproduct after attempted deprotection of **5i**.

3.4 Preparation of guanidines 8a-g

The experimental details and procedures for the two steps is described in Section 6.5. For analysis of spectroscopic elucidation of structures for guanidines **8a-g**, see Section 5.4.1-5.4.7.Guanidines **8a-g** were successfully prepared in two steps from isoindoline HCl-salts **6a-g**. The first step a simple deprotonation of **6a-g** with K_2CO_3 (sat. aq), followed by extraction with EtOAc, giving the free amines **7a-c** and **7e-g** in 60-98% yield. The resulting isoindoline amines were guanylated with the elecrophilic guanylation reagent 1*H*-pyrazole carboxamidine HCl (**9**), according to a procedure described by Bakka and Gautun.[28] a simplification of the protocol originally reported by Bernatowicz *et al.*[45] The modified reaction conditions allowed for guanylation of **7** in MeCN at reflux, without any basic additive, simplifying the work-up process. A small excess of **7** ensured full conversion of **9**, simplifying work-up. Filtration and crystallisation yielded guanidines **8a-g** as their HCl-salts in 25-94%. It should be noted that free amine **7d** was seemingly unstable in air, and put under Ar-atm immediately after free-basing and taken directly guanylation with **9**. The reduced yield of 25% consequently had to be calculated from **6d**. The results of the guanylation reactions are summarized in Table 3.3.

Table 3.3: Summary of the successful guanylations of free amines **7a-g** with 1*H*-pyrazole carboxamidine HCl (**9**), yielding guanidines **8a-g** as their HCl-salts.



7 **9** **8**

Entry #	**7**	**9** (equiv.)	R	Time (h)	Product	Yield (%)
1	**7a**	0.94	4-CF_3-Ph	5	**8a**	80%
2	**7b**	0.95	naphtalen-2-yl	17	**8b**	48%
3	**7c**	0.89	4-*n*-BuPh	5	**8c**	73%
4[a]	**7d**	0.99[a]	4-(*n*-BuO)Ph	22	**8d**	25%[a]
5	**7e**	0.94	2,4-di-(*n*-PrO)Ph	6	**8e**	60%
6	**7f**	0.85	4-(*i*-PrO)Ph	6	**8f**	94%
7	**7g**	0.96	2,4,6-tri-(*i*-Pr)Ph	8	**8g**	67%

[a] Free amine **7d** was seemingly unstable in air, and put under Ar-atm and directly into guanylation. Thus equivalents of **9** and yield of **8d** were calculated from **6d**.

3.4.1 Decomposition of isopropoxy derivatives 6f and 8f

The preparation of isopropoxy derivatives **6f** and **8f** were according to ^{1}H NMR-analysis initially successful, with next to no impurities according to ^{1}H NMR (Appendix R.1 and AA.1). Surpisingly, HPLC analysis indicated purities of 77% and 39% for **6f** and **8f** 1 month later, far below the expected 95-99% (Appendix R.8 and R.8). The ^{1}H NMR spectra for both **6a** and **8a** had massive changes (Appendix R.9 and AA.9), confirming

both **6f** and **8f** be decomposing. It was theorized that the isopropyloxy ether possibly could be cleaved by acidic conditions leaving, further strengthened by the signals showing up around 11.0 ppm in both ^{1}H NMR spectra. Reanalysis with MS indicated (Appendix R.10 and AA.10) indicated the phenol derivatives of **6f** and **8f** to be present, confirming the theorized acidic cleavage of the isopropyloxy ether to be one of potentially several routes for decomposition of **6f** and **8f**.

3.5 Antimicrobial activity and cytotoxicity

A total of 12 different compounds were sent to Tromsø for antimicrobial evaluation, 6 isoindoline HCl-salts (**6a-e** and **6g**) and 6 guanidines (**8a-e** and **8g**). **6f** and **8f** were excluded due to their unstable nature. The compounds evaluated were evaluated for their antimicrobial activity against three gram-positive and two gram-negative bacteria; *Streptococcus agalacticae* (ATCC 12386), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212). Initially the activity was determined by subjecting all compounds to a single concentration assay with a concentration of 64 µg/mL. Compounds showing antimicrobial activity at this concentration were subjected to dose-response array, and the minimum inhibitory concentrations were determined. Additionally, measuring the *in vitro* cytotoxicity against HepG2-cells (human hepatic cells) could give information about any adverse effects, in this case determining the hepatotoxic effects of the compounds. Unfortunately, only 7 out of the 12 compounds were evaluated for antimicrobial acitivity before the deadline of this thesis. The minimal inibitory concentrations (MIC), along with the obtained EC_{50}-values for HepG2-cells for compounds **6a-d** and **8b-d** are listed in Table 3.4.

All tested compounds showed antimicrobial activity against the 5 different bacteria at 64 µg/mL, where **6d** tested compound to not show activity towards all 5 bacteria. It was also seen that a rather large lipophilic character was important to achieving high antimicrobial activity. The guanidine compounds (**8b-8d**) were as expected shown to be more potent than their HCl-salt counterparts (**6b-6d**, in most cases with a doubling of their potency.

When looking at the different lipophilic groups 4-*n*-butyl (**6c**, **8c**) showed the highest antimicrobial activity, with a higher potency than the other lipophilic groups both among the guanidines and HCl-salts. It is still worth noting that any value at 8 or lower is seen as very good activity when compared to previously tested compounds.

The HCl-salts **6** were in general more toxic than their guanidine counterparts, generally showing a 4-fold increase in potency against HepG2-cells. This was consistent with previous observations made in a preparation of a library of anti-microbial 1,2,3-triazoles conducted by Bakka,[56] where amines were shown to display higher toxicity than their guanidine counterparts. While the guanidines generally were less toxic, all of them still displayed EC_{50}-values below 10 µg/mL. All the tested compounds apart from **6a** displayed impressive activity towards the bacteria, but the high activity was unfortunately reflected by their high toxicity. The guanidine carrying one *n*-Bu-group (**8c**) displayed some of the highest activity among all amphiphiles based on the isoindoline scaffold that have been evaluated for antimicrobial activity.[56] While the activity was impressive, the closely

Table 3.4: Antimicrobial activity (minimal inhibitory activity (MIC) in µg/mL) and mammalian cytotoxicity (HepG2, EC_{50} in µg/mL) for compound **6a-d** and **8b-d**. No activity ≤64 µg/mL is indicated by a dash (-).

Entry	*E. faecalis*[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	HepG2[b] (EC_{50})
6a	64	16	32	32	64	2.7
6b	16	4	8	16	32	2.0
6c	8	2	4	8	32	1.3
6d	16	4	8	16	-	1.0
8b	8	2	2	4	8	7.8
8c	4	1	2	4	8	5.3
8d	8	2	4	4	16	7.1
Ref[c]	10	0.13	4	0.5	0.5	N.d.[d]

[a] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalacticae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[b] Evaluation of *in vitro* cytotoxicity.
[c] Reference: Gentamicin.
[d] N.d.: Not determined.

related isoindoline guanidine carrying a *t*-Bu-group, illustrated in Figure 3.6, displayed similar activity with only half the potency towards Hep2G-cells.[56]

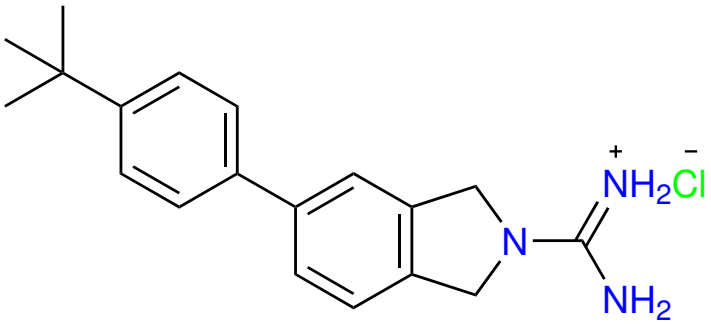
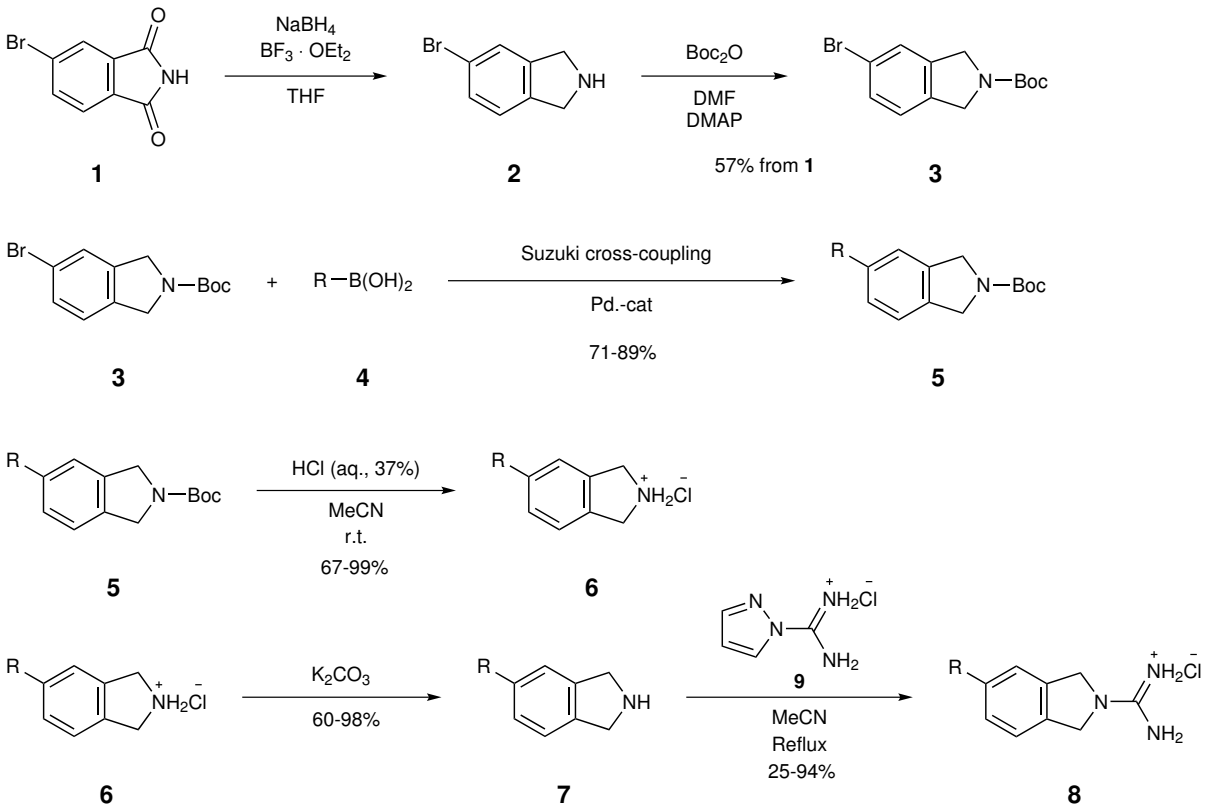


Figure 3.6: MIC: 2-8 µg/mL, HepG2 EC_{50}: 12 µg/mL

4 Conclusion and further work



Scheme 4.1: An outline of the synthetic steps in this thesis.

5 Spectroscopic analysis and characterization

5.1 General

New compounds were characterized using ^{1}H NMR and ^{13}C NMR, with a final confirmation from HRMS. Previously synthesized compounds were characterized by comparing the ^{1}H NMR spectra with previously reported data.

The chemical shifts of protons and carbons were assigned by the use of the two dimensional NMR techniques COSY, HSQC and HMBC.[57] The COSY (Correlated Spectroscopy) experiment gives a spectrum with with cross peaks for adjacent proton (^{1}H-^{1}H). Information from this spectrum can also be used to determine that a proton does not have any neighbours. HSQC (Heteronuclear Single Quantum Coherence) experiment gives the information of what protons and carbons are directly coupled, i.e the one-bond coupling. ($^{1}J_{\mathrm{CH}}$). If the position of either one is known, the other one can be found. HMBC (Heteronuclear Multiple Bond Coherence) experiment gives a spectrum similar to HSQC, but peaks indicate two- ($^{2}J_{\mathrm{CH}}$) and three-bond ($^{3}J_{\mathrm{CH}}$) couplings. This allows for determination of quaternary carbons by the peaks coupling to neighbouring protons. Additionally HMBC indirectly gives carbon-carbon bonds when used together with HSQC and/or COSY. The use of 2D NMR techniques are in most cases more than enough to fully assign shifts and elucidate the structure of an isolated compound. HRMS (High Resolution Mass Spectroscopy) was used to confirm the chemical composition of the isolated compounds, thus in many cases confirming the structure. A detailed walk-through for the structure elucidation of **5a** is given in Section 5.2.1. Other structures will be determined using similar methods, and will not be presented as detailed as **5a**.

Not all signals in the NMR spectra originate from the product structures. Usually they can be assigned to the impurities presented in Table 5.1.[58]

Table 5.1: Assignment of ^{1}H and ^{13}C shifts for common solvents as trace amounts.[58]

Solvent	Group and mult.	δ ^{1}H DMSO [ppm]	δ ^{1}H $CDCl_3$ [ppm]	δ ^{13}C DMSO [ppm]	δ ^{13}C CDCl3 [ppm]
Solvent		2.50	7.26	39.52	77.16
1,4-dioxane	CH_2, s	3.59	3.71	67.60	67.14
Acetone	CH_3, s	2.09	2.17	39.52	77.16
	CO, s	-	-	206.31	207.07
$CDCl_3$	CH, s	8.02	7.26	39.52	77.16
DCM	CH_2, s	5.63	5.30	54.84	53.52
DMF	CH, s	7.96	8.02	162.79	162.62
	CH_3, s	2.94	2.96	36.15	36.50
	CH_3, s	2.78	2.88	31.03	31.45
Et_2O	CH_3, t	1.01	1.21	15.78	15.20
	CH_2, q	3.41	3.48	66.12	66.91
EtOAc	CH_3CO, s	2.50	7.26	20.83	21.04
	CO -	-	-	170.96	171.36
	$\mathbf{CH_2}CH_3$, q	2.50	7.26	60.56	60.49
	$CH_2\mathbf{CH_3}$, t	2.50	7.26	14.50	14.19
H_2O	s	3.33	1.56	-	-
MeCN	CH_3, s	2.05	2.10	1.12	1.13
	CN, -	-	-	117.60	116.43
MeOH	CH_3, s	3.31	3.49	49.77	50.41
	OH, s	3.12	1.09	-	-
n-pentane	CH_3, t	0.86	0.88	14.08	14.08
	CH_2, m	1.27	1.27	22.98	22.38
	CH_2, m	1.27	1.27	34.83	34.16
THF	CH_3, m	1.79	1.85	26.16	25.62
	CH_2O, m	3.63	3.76	68.07	67.97

5.2 Carbamate protected compounds

5.2.1 *tert*-Butyl 5-(4-(trifluoromethyl)phenyl)isoindoline-2-carboxylate (5a)

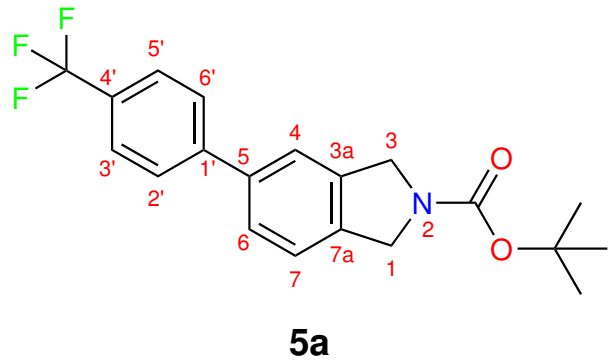
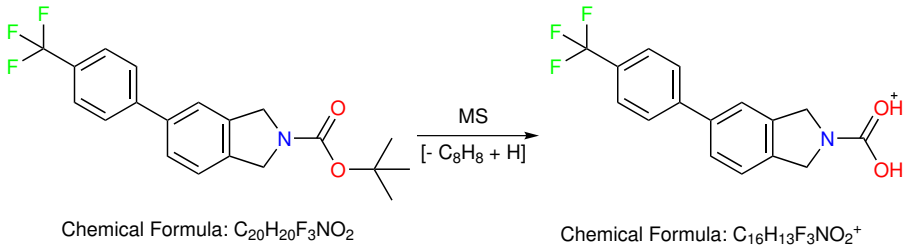


Figure 5.1: Structure of compound **5a** with numbered positions.

A detailed walk-through for the structure elucidation of **7a** will be presented here. As other structures were determined with very similar methods, these will not be presented with the same level of detail.

The HRMS spectrum in Appendix C.7 confirms the chemical formula of **5a**, but rather than the expected $[M]^+$ or $[M+H]^+$, the mass is found at $[M\text{-}C_8H_8+H]^+$, as illustrated in Scheme 5.1. This phenomenon is observable for all compounds in this thesis with the carbamate protective group. NMR data from Appendix C.1-C.5 will be used to assign the chemical shifts for the protons and carbons in Figure 5.1.



Scheme 5.1: Phenomenon observable for all compounds in this thesis with the carbamate protective group, here illustrated using **5a**.

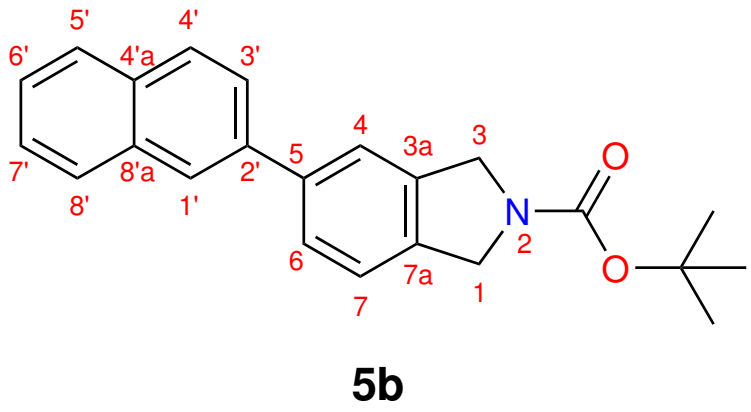
In the ^{1}H NMR spectrum (Appendix C.1), a large singlet at 1.53 ppm, with an integral of 9 is observed. This signal must originate from the 9 protons belonging to the carbamate protective group, while unnecessary, this can be confirmed by the HPLC specrum, where 1.53 shows no signs of long range coupling. HSQC gives us the carbon shift at 28.6 ppm, see Figure The next noticeable signal is the multiplet at 4.76-4.72 ppm, with an integral of 4. This must be the two CH_2 groups of positions 1 and 3. As for the two remaining carbons on the carbamate chain are quaternary with relativily unique shifts, the carbonyl can be assigned 154.5, and the last Cq belonging to Boc 79.9. Because of symmetry, the multiplet of 7.70-7.66 have to position 2′, 3′, 5′and 6′. HSQC give the respective carbon shifts 127.4 and 125.8. Only 2′and 6′should be able to see any other aromatic protons if

you exclude the multiplet they belong to themselves. Using the fact that ${}^{3}J_{\mathrm{CH}} > {}^{2}J_{\mathrm{CH}}$ for aromatic structures,[57] the rest of the structure falls out by itself. It is worth noting that J_{CF} coupling will split the signals of the CF_3 carbon and it's closest bonding carbons. CF_3 itself usually with a coupling constant of around 272 Hz, alpha-carbon around 32.2 Hz and around 3.8 Hz sometimes observable for the beta-carbon.

Table 5.2: Chemical shifts all protons and carbons assigned to their respective positions shown in Figure 5.1. Some positions feature double shifts due to rotamers. These signals are marked with an asterisk (*). Because of CF-coupling, some carbon signals are represented as quartets. These are marked with a q followed by the coupling constant J_{CF}.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)	m, J_{CF} (Hz)
1	4.76-4.72	m	-	52.1*, 51.8*	-
2	-	-	-	-	-
3	4.76-4.72	m	-	52.3*, 52.0*	-
3a	-	-	-	138.4*, 138.0*	-
4	7.50* and 7.44*	s and s	-	121.7*, 121.4*	-
5	-	-	-	144.5*, 144.4*	-
6	7.50*	s	-	126.7*, 126.6*	-
7	7.38*, 7.32*	d and d	7.8 and 8.4	123.4*, 123.1*	-
7a	-	-	-	137.5*, 137.0*	-
1′	-	-	-	139.3	-
2′and 6′	7.70-7.66	m	-	127.4	-
3′and 5′	7.70-7.66	m	-	125.8	-
4′	-	-	-	129.5	q, 32.2
CF_3	-	-	-	124.2	q, 272.7
Boc C=O	-	-	-	154.5	-
Boc Cq	-	-	-	79.9	-
Boc $(CH_3)_3$	1.53	s	-	28.6	-

5.2.2 *tert*-Butyl 5-(naphthalen-2-yl)isoindoline-2-carboxylate (5b)



5b

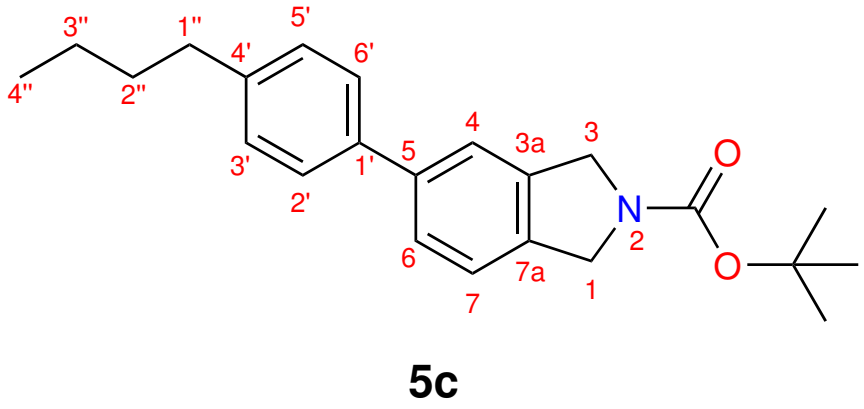
Figure 5.2: Structure of compound **5b** with numbered positions.

HRMS shown i Appendix D.7 confirmed the chemical formula of **5b**. The spectroscopic data in Appendix D.1-D.5 was used to assign the chemical shifts shown in Table 5.3. Rotamers are marked with an asterisk (*).

Table 5.3: Chemical shifts for protons and carbons assigned positions shown in Figure 5.2.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.78–4.71	m	-	52.1*, 51.9*
2	-	-	-	-
3	4.78–4.71	m	-	52.4*, 52.1*
3a	-	-	-	138.3*, 137.8*
4	7.63*, 7.56*	app s and s	-	121.8*, 121.5*
5	-	-	-	140.7*, 140.7*
6	7.62	app s	-	126.8*, 126.8*
7	7.39*, 7.33*	d and d	7.9 and 8.0	123.2*, 123.0*
7a	-	-	-	136.5*, 136.2*
1′	8.02	d	7.7	125.8
2′	-	-	-	138.2
3′	7.73–7.71	m	-	125.5
4′	7.92–7.86	m	-	128.5
4′a	-	-	-	132.6
5′	7.92–7.86	m	-	127.7
6′	7.49	qn	8.0	126.0
7′	7.49	qn	8.0	126.4
8′	7.92–7.86	m	-	128.2
8′a	-	-	-	133.7
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.8
Boc $(CH_3)_3$	1.54	s	-	28.6

5.2.3 *tert*-Butyl 5-(4-butylphenyl)isoindoline-2-carboxylate (5c)



5c

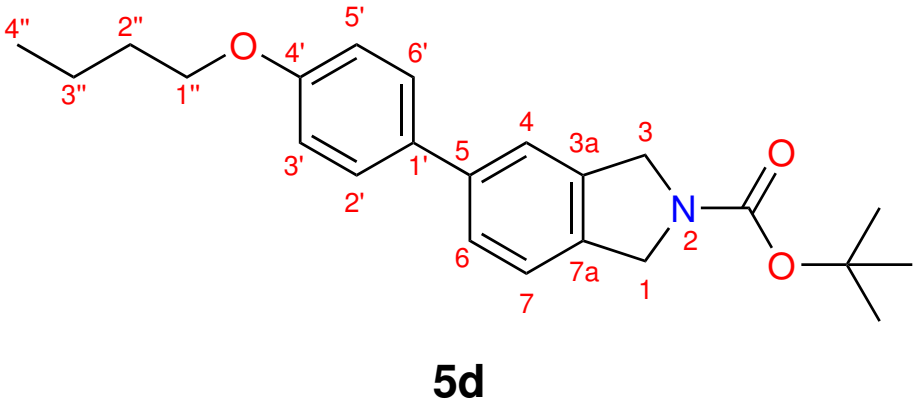
Figure 5.3: Structure of compound **5c** with numbered positions.

HRMS shown i Appendix E.7 confirmed the chemical formula of **5c**. The spectroscopic data in Appendix E.1-E.5 was used to assign the chemical shifts shown in Table 5.4. Rotamers are marked with an asterisk (*).

Table 5.4: Chemical shifts for protons and carbons assigned positions shown in Figure 5.3.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.74–4.68	m	-	52.1*, 51.8*
2	-	-	-	-
3	4.74–4.68	m	-	52.4*, 52.1*
3a	-	-	-	138.0*, 137.6*
4	7.49-7.47*, 7.42*	m and s	-	121.3*, 121.6*
5	-	-	-	140.7
6	7.49–7.47*	m	-	126.4*, 126.3*
7	7.32*, 7.27*	d and app d	7.9 and app 7.9	123.0*, 122.8*
7a	-	-	-	136.1*, 135.8*
1′	-	-	-	138.3*, 138.2*
2′and 6′	7.49–7.47*	m	-	127.0
3′and 5′	7.26–7.24	m	-	128.9
4′	-	-	-	142.2
1″	2.65	t	7.7	35.3
2″	1.63	app qn	app 7.5	33.7
3″	1.39	sex	7.7	22.4
4″	0.95	t	7.3	14.0
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.7
Boc $(CH_3)_3$	1.53	s	-	28.6

5.2.4 *tert*-Butyl 5-(4-butoxyphenyl)isoindoline-2-carboxylate (5d)



5d

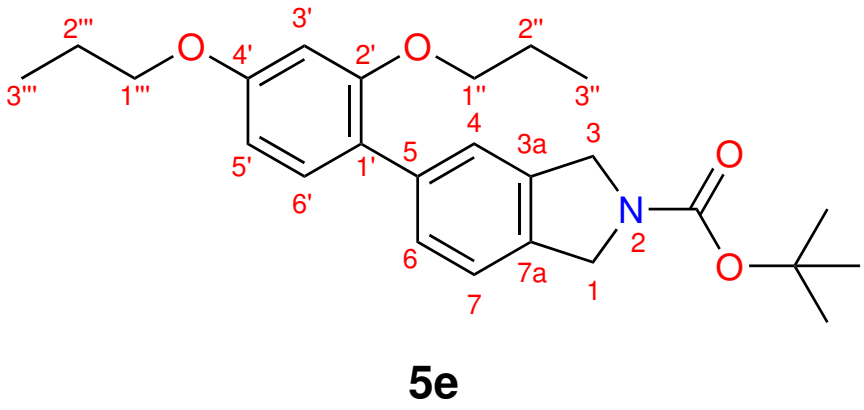
Figure 5.4: Structure of compound **5d** with numbered positions.

HRMS shown i Appendix F.7 confirmed the chemical formula of **5d**. The spectroscopic data in Appendix F.1-F.5 was used to assign the chemical shifts shown in Table 5.5. Rotamers are marked with an asterisk (*).

Table 5.5: Chemical shifts for protons and carbons assigned positions shown in Figure 5.4.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.73–4.67	m	-	52.1*, 51.8*
2	-	-	-	-
3	4.73–4.67	m	-	52.4*, 52.1*
3a	-	-	-	138.0*, 137.6*
4	7.44*, 7.38*	app s and s	-	121.0*, 120.8*
5	-	-	-	140.5
6	7.45	app d	7.9	126.1*, 126.0*
7	7.30*, 7.25*	d and app d	7.9 and 7.9	123.0*, 122.8*
7a	-	-	-	135.7*, 135.4*
1′			-	133.3*, 133.2*
2′and 6′	7.50–7.47	m	-	128.1
3′and 5′	6.96	d	7.5	114.8
4′	-	-	-	158.8
1″	4.00	t	6.5	67.8
2″	1.79	qn	6.5	31.4
3″	1.52	app sex	7.4	19.3
4″	0.99	t	7.4	13.9
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.7
Boc $(CH_3)_3$	1.53	s	-	28.6

5.2.5 *tert*-Butyl 5-(2,4-dipropoxyphenyl)isoindoline-2-carboxylate (5e)



5e

Figure 5.5: Structure of compound **5e** with numbered positions.

HRMS shown i Appendix G.7 confirmed the chemical formula of **5e**. The spectroscopic data in Appendix G.1-G.5 was used to assign the chemical shifts shown in Table 5.6. Rotamers are marked with an asterisk (*).

Table 5.6: Chemical shifts for protons and carbons assigned positions shown in Figure 5.5.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.71–4.67	-m	-	52.2*, 51.9*
2	-	-	-	-
3	4.71–4.67	m	-	52.4*, 52.1*
3a	-	-	-	137.0*, 136.6*
4	7.43–7.40*, 7.36*	m and s	-	123.8*, 123.6*
5	-	-	-	137.9*, 137.9*
6	7.43–7.40*	m	-	128.8*, 128.7*
7	7.26*, 7.22–7.20*	app d and m	8.1	122.1*, 121.9*
7a	-	-	-	135.2*, 134.9*
1′	-	-	-	123.2*, 123.1*
2′	-	-	-	156.9
3′	6.55–6.53	m	-	100.4*, 100.4*
4′	-	-	-	159.9
5′	6.55–6.53	m	-	105.4
6′	-	-	-	131.1*, 131.1*
1′′	0.97	td	7.4, 1.9	69.9*, 69.9*
2′′	1.77–1.71	m	-	22.5
3′′	3.91	t	6.4	10.7
1′′′	1.06	t	7.4	69.7
2′′′	1.83	sex	-	22.7
3′′′	3.95	t	6.6	10.6
Boc C=O	-	-	-	154.7
Boc Cq	-	-	-	79.6
Boc $(CH_3)_3$	1.53	s	-	28.6

5.2.6 *tert*-Butyl 5-(4-isopropoxyphenyl)isoindoline-2-carboxylate (5f)

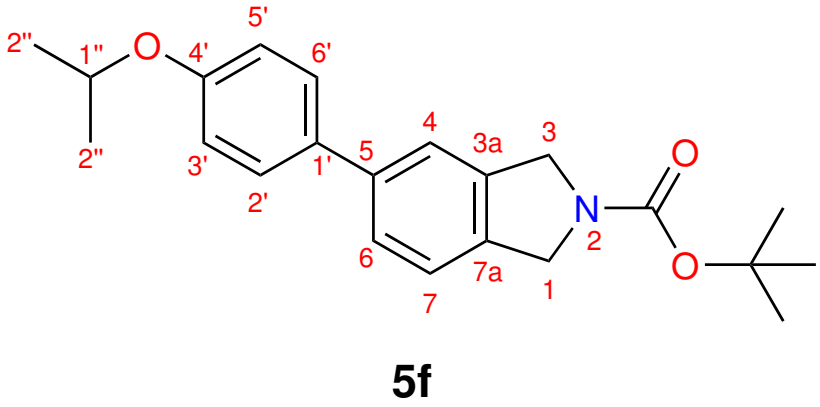


Figure 5.6: Structure of compound **5f** with numbered positions.

HRMS shown i Appendix H.7 confirmed the chemical formula of **5f**. The spectroscopic data in Appendix H.1-H.5 was used to assign the chemical shifts shown in Table 5.7. Rotamers are marked with an asterisk (*).

Table 5.7: Chemical shifts for protons and carbons assigned positions shown in Figure 5.6.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.74–4.68	m	-	52.1*, 51.9*
2	-	-	-	-
3	4.74–4.68	m	-	52.4*, 52.1*
3a	-	-	-	138.0*, 137.6*
4	7.51–7.47*, 7.42*	m and s	-	121.4*, 121.1*
5	-	-	-	140.7*, 140.7*
6	7.51–7.47*	m	-	126.4*, 126.3*
7	7.33–7.26*	m	-	123.0*, 122.8*
7a	-	-	-	136.1*, 135.8*
1′	-	-	-	138.5*, 138.4*
2′and 6′	7.51–7.47	m	-	127.1
3′and 5′	7.30	dd	8.2, 2.0	126.9
4′	-	-	-	148.2
1″	2.96	sep	6.9	33.8
2″	1.29	d	6.9	24.0
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.7
Boc $(CH_3)_3$	1.53	s	-	28.6

5.2.7 tert-Butyl 5-(2,4,6-triisopropylphenyl)isoindoline-2-carboxylate (5g)

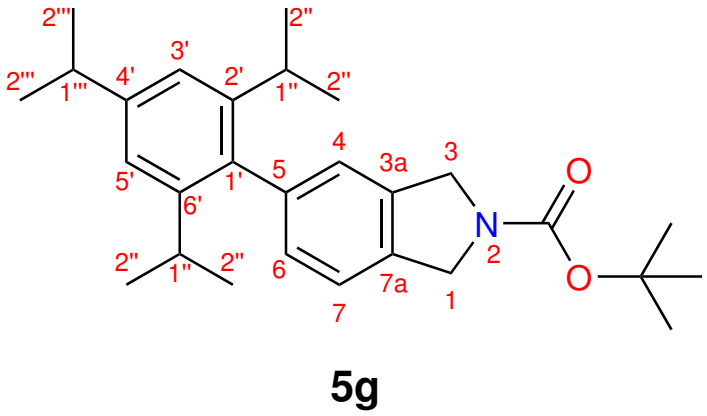


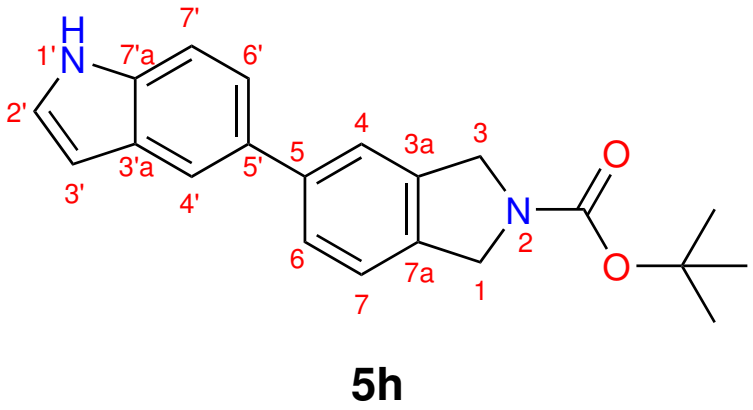
Figure 5.7: Structure of compound **5g** with numbered positions.

HRMS shown i Appendix I.6 confirmed the chemical formula of **5g**. The spectroscopic data in Appendix I.1-I.5 was used to assign the chemical shifts shown in Table 5.8. Rotamers are marked with an asterisk (*).

Table 5.8: Chemical shifts for protons and carbons assigned positions shown in Figure 5.7.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.76–4.70	m	-	52.3*, 52.0*
2	-	-	-	-
3	4.76–4.70	m	-	52.4*, 52.1*
3a	-	-	-	137.1*, 136.8*
4	7.08–7.02	m	-	123.9*, 123.7*
5	-	-	-	140.2*, 140.1*
6	7.08–7.02	m	-	129.0*, 128.9*
7	7.28*, 7.24*	d and d	7.6 and 7.9	122.3*, 122.0*
7a	-	-	-	135.5*, 135.2*
1′	-	-	-	136.6*, 136.6*
2′and 6′	-	-	-	146.6*, 146.5*
3′and 5′	7.08–7.02	m	-	120.5*, 120.5*
4′	-	-	-	148.0
1″	2.59–2.55	m	-	30.3
2″	1.07	d	6.8	24.2, 24.2
1‴	2.94	sep	6.7	34.3
2‴	1.30	d	6.7	24.1
Boc C=O	-	-	-	154.6*, 154.6*
Boc Cq	-	-	-	79.6
Boc $(CH_3)_3$	1.53	s	-	28.6

5.2.8 *tert*-Butyl 5-(1*H*-indol-5-yl)isoindoline-2-carboxylate (5h)



5h

Figure 5.8: Structure of compound **5h** with numbered positions.

HRMS shown i Appendix J.6 confirmed the chemical formula of **5h**. The spectroscopic data in Appendix J.1-J.5 was used to assign the chemical shifts shown in Table 5.9. Rotamers are marked with an asterisk (*).

Table 5.9: Chemical shifts for protons and carbons assigned positions shown in Figure 5.8.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.76–4.69	m	-	52.2*, 51.8*
2	-	-	-	-
3	4.76–4.69	m	-	52.4*, 52.1*
3a	-	-	-	137.9*, 137.4*
4	7.55*, 7.49*	app s and app s	-	121.7*, 121.4*
5	-	-	-	142.1*, 142.1*
6	7.55	app d	8.1	126.8*, 126.7*
7	7.33*, 7.28*	d and d	7.8 and 7.8	122.9*, 122.7*
7a	-	-	-	135.4*, 135.1*
1′	8.21	s	-	-
2′	7.25	app s	-	124.9
3′	6.61	s	-	103.0
3′a	-	-	-	128.4
4′	7.84	d	7.7	119.3
5′	-	-	-	133.2*, 133.1*
6′	7.47–7.41	m	-	121.9
7′	7.47–7.41	m	-	111.3
7′a	-	-	-	135.3
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.7
Boc $(CH_3)_3$	1.54	s	-	28.6

5.2.9 *tert*-Butyl 5-(1-butyl-1*H*-indol-5-yl)isoindoline-2-carboxylate (5i)

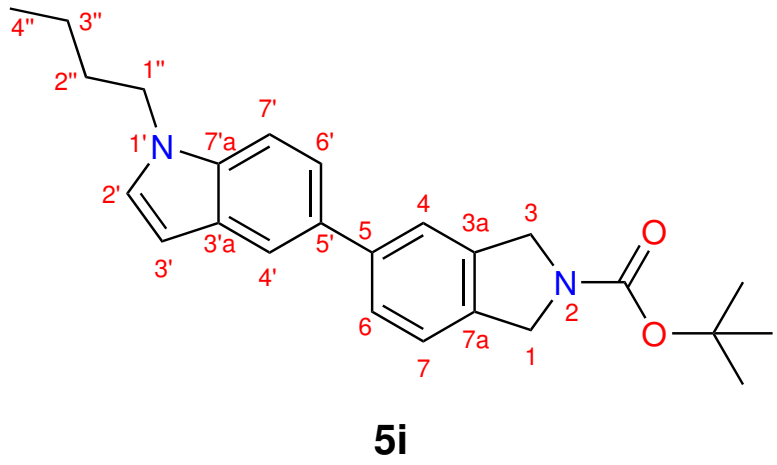


Figure 5.9: Structure of compound **5i** with numbered positions.

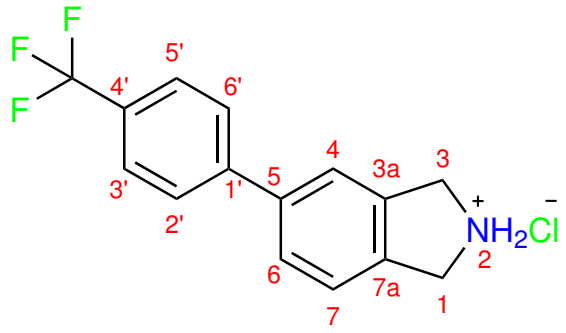
HRMS shown i Appendix K.6 confirmed the chemical formula of **5i**. The spectroscopic data in Appendix K.1-K.5 was used to assign the chemical shifts shown in Table 5.10. Rotamers are marked with an asterisk (*).

Table 5.10: Chemical shifts for protons and carbons assigned positions shown in Figure 5.9.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.76–4.69	m	-	52.2*, 51.8*
2	-	-	-	-
3	4.76–4.69	m	-	52.4*, 52.1*
3a	-	-	-	137.9*, 137.5
4	7.54*, 7.48*	app s and app s	-	121.6*, 121.4*
5	-	-	-	142.2*, 142.2*
6	7.55	app d	8.3	126.8*, 126.7*
7	7.33*, 7.27*	d and d	7.9 and 7.9	122.9*, 122.7*
7a	-	-	-	135.3*, 135.0*
1′	-	-	-	-
2′	7.13	t	2.5	129.1
3′	6.53	s	-	101.3
3′a	-	-	-	128.6
4′	7.81	d	7.4	119.5
5′	-	-	-	132.5*, 132.4*
6′	7.44–7.39	m	-	121.2
7′	7.44–7.39	m	-	109.7
7′a	-	-	-	135.6
1′′	4.15	t	7.1	46.3
2′′	1.85	qn	7.5	32.4
3′′	1.37	sex	7.5	20.2
4′′	0.95	t	7.4	13.7
Boc C=O	-	-	-	154.6
Boc Cq	-	-	-	79.6
Boc $(CH_3)_3$	1.53	s	-	28.6

5.3 Isoindoline HCl-salts

5.3.1 5-(4-(Trifluoromethyl)phenyl)isoindoline HCl (6a)



6a

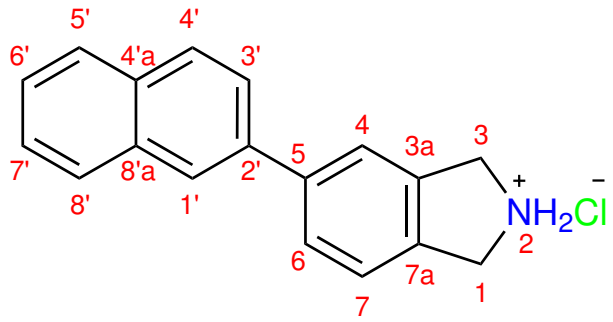
Figure 5.10: Structure of compound **6a** with numbered positions.

HRMS shown i Appendix M.7 confirmed the chemical formula of **6a**. The spectroscopic data in Appendix M.1-M.5 was used to assign the chemical shifts shown in Table 5.11.

Table 5.11: Chemical shifts for protons and carbons assigned positions shown in Figure 5.10. Because of CF-coupling, some carbon signals are represented as quartets. These are marked with a q followed by the coupling constant J_{CF}.

Position	δ_H (ppm)	Mult.	J (Hz)	δ_C (ppm)	Mult. J_{CF} (Hz)
1	4.56	s	-	49.8	-
2	9.96	br s	-	-	-
3	4.57	s	-	50.0	-
3a	-	-	-	136.3	-
4	7.78	s	-	121.6	-
5	-	-	-	143.6	-
6	7.74	d	7.9	127.3	-
7	7.54	d	8.0	123.6	-
7a	-	-	-	135.4	-
1′	-	-	-	140.4	-
2′and 6′	7.89	d	8.3	127.5	-
3′and 5′	7.84	d	8.3	125.9	q, 3.8
4′	-	-	-	128.1	q, 31.9
CF_3	-	-	-	124.3	q, 271.7

5.3.2 5-(Naphthalen-2-yl)isoindoline HCl (6b)



6b

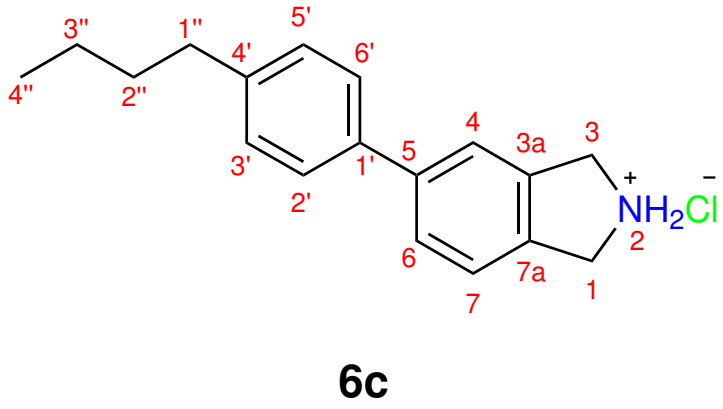
Figure 5.11: Structure of compound **6b** with numbered positions.

HRMS shown i Appendix N.7 confirmed the chemical formula of **6b**. The spectroscopic data in Appendix N.1-N.5 was used to assign the chemical shifts shown in Table 5.12.

Table 5.12: Chemical shifts protons and carbons assigned positions shown in Figure 5.11.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.57	s	-	49.9
2	9.84	br s	-	-
3	4.59	s	-	50.1
3a	-	-	-	136.1
4	7.86	app s	-	121.5
5	-	-	-	140.3
6	7.82	d	8.0	127.2
7	7.59–7.53	m	-	123.5
7a	-	-	-	134.4
1′	8.23	s	-	125.4
2′	-	-	-	136.9
3′	7.85	dd	8.6, 1.5	125.0
4′	8.03	d	8.6	128.6
4′a	-	-	-	132.3
5′	7.96	d	7.8	127.5
6′	7.59–7.53	m	-	126.3
7′	7.59–7.53	m	-	126.5
8′	8.00	d	7.7	128.2
8′a	-	-	-	133.3

5.3.3 5-(4-Butylphenyl)isoindoline HCl (6c)



6c

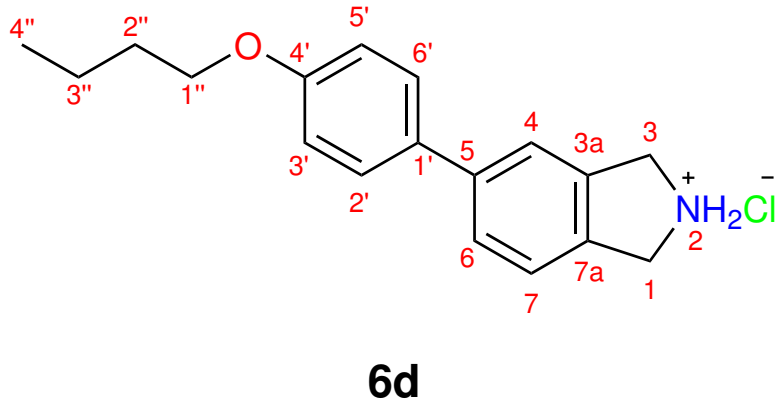
Figure 5.12: Structure of compound **6c** with numbered positions.

HRMS shown i Appendix O.7 confirmed the chemical formula of **6c**. The spectroscopic data in Appendix O.1-O.5 was used to assign the chemical shifts shown in Table 5.13.

Table 5.13: Chemical shifts for protons and carbons assigned positions shown in Figure 5.12.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.52	s	-	49.9
2	9.40	br s	-	-
3	4.53	s	-	50.0
3a	-	-	-	136.9
4	7.67	s	-	120.9
5	-	-	-	140.4
6	7.63	d	8.0	126.6
7	7.46	d	7.9	123.3
7a	-	-	-	136.1
1′	-	-	-	134.0
2′and 6′	7.57	d	8.1	126.6
3′and 5′	7.29	d	8.1	128.9
4′	-	-	-	141.9
1″	2.62	t	7.7	34.4
2″	1.58	qn	7.6	33.0
3″	1.33	sex	7.5	21.7
4″	0.91	t	7.4	13.8

5.3.4 5-(4-Butoxyphenyl)isoindoline HCl (6d)



6d

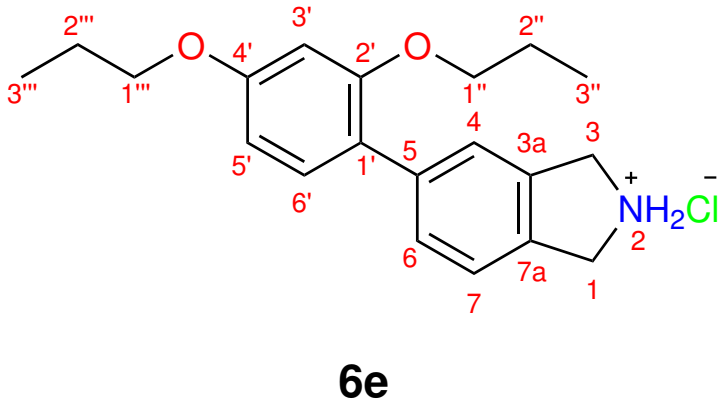
Figure 5.13: Structure of compound **6d** with numbered positions.

HRMS shown i Appendix P.7 confirmed the chemical formula of **6d**. The spectroscopic data in Appendix P.1-P.5 was used to assign the chemical shifts shown in Table 5.14.

Table 5.14: Chemical shifts all protons and carbons assigned positions shown in Figure 5.13.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.51	s	-	49.7
2	10.01	br s	-	-
3	4.53	s	-	49.9
3a	-	-	-	135.9
4	7.64	s	-	120.6
5	-	-	-	140.1
6	7.60–7.57	m	-	126.3
7	7.44	d	8.0	123.3
7a	-	-	-	133.4
1′	-	-	-	131.7
2′and 6′	7.60–7.57	m	-	127.8
3′and 5′	7.02	d	8.8	114.9
4′	-	-	-	158.5
1″	4.01	t	6.5	67.2
2″	1.71	qn	7.4	30.7
3″	1.45	sex	7.4	18.7
4″	0.94	t	7.4	13.7

5.3.5 5-(2,4-Dipropoxyphenyl)isoindoline HCl (6e)



6e

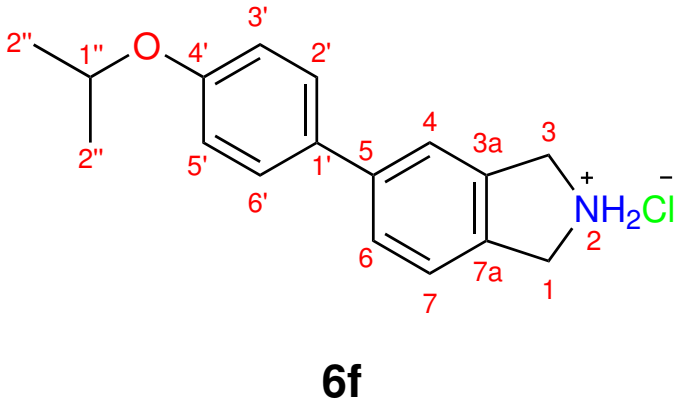
Figure 5.14: Structure of compound **6e** with numbered positions.

HRMS shown i Appendix Q.7 confirmed the chemical formula of **6e**. The spectroscopic data in Appendix Q.1-Q.5 was used to assign the chemical shifts shown in Table 5.15.

Table 5.15: Chemical shifts for protons and carbons assigned positions shown in Figure 5.14.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.50	app s	-	49.9
2	9.81	br s	-	-
3	4.51	app s	-	50.0
3a	-	-	-	134.9
4	7.45–7.43	m	-	123.3
5	-	-	-	138.3
6	7.45–7.43	m	-	129.2
7	7.39	d	7.9	122.2
7a	-	-	-	133.0
1′	-	-	-	121.7
2′	-	-	-	156.4
3′	6.63	d	2.2	100.1
4′	-	-	-	159.6
5′	6.59	dd	6.1, 2.3	105.9
6′	7.19	d	8.4	130.9
1″	3.94	t	6.3	69.3
2″	1.65	sex	7.1	22.0
3″	0.92	t	7.4	10.6
1‴	3.96	t	6.5	69.1
2‴	1.74	sex	7.1	22.1
3‴	0.99	t	7.4	10.4

5.3.6 5-(4-Isopropoxyphenyl)isoindoline HCl (6f)



6f

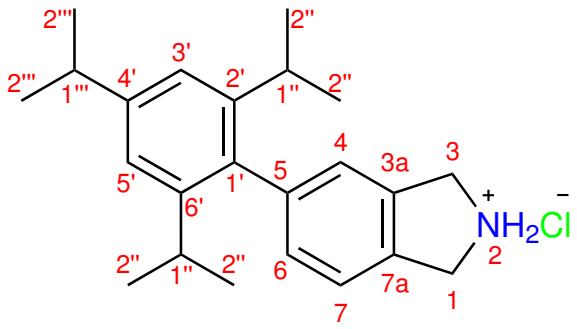
Figure 5.15: Structure of compound **6f** with numbered positions.

HRMS shown i Appendix R.7 confirmed the chemical formula of **6f**. The spectroscopic data in Appendix R.1-R.5 was used to assign the chemical shifts shown in Table 5.16.

Table 5.16: Chemical shifts for protons and carbons assigned positions shown in Figure 5.15.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.52	s	-	49.9
2	9.87	br s	-	-
3	4.54	s	-	50.0
3a	-	-	-	136.0
4	7.67	s	-	121.0
5	-	-	-	140.4
6	7.63	d	7.9	126.7
7	7.47	d	7.9	123.4
7a	-	-	-	134.0
1′	-	-	-	137.1
2′and 6′	7.58	d	8.2	126.7
3′and 5′	7.35	d	8.2	126.9
4′	-	-	-	148.0
1″	2.93	sep	6.9	33.1
2″	1.23	d	6.9	23.8

5.3.7 5-(2,4,6-Triisopropylphenyl)isoindoline HCl (6g)



6g

Figure 5.16: Structure of compound **6g** with numbered positions.

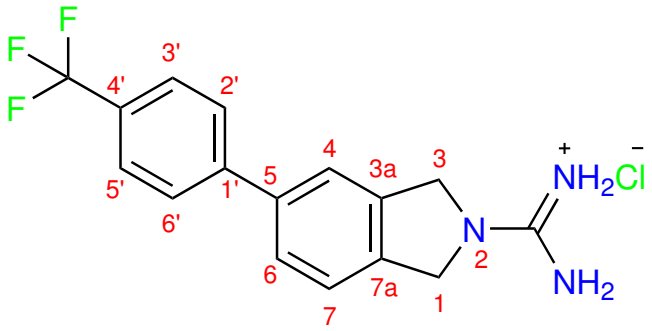
HRMS shown i Appendix S.6 confirmed the chemical formula of **6g**. The spectroscopic data in Appendix S.1-S.5 was used to assign the chemical shifts shown in Table 5.17.

Table 5.17: Chemical shifts for protons and carbons assigned positions shown in Figure 5.16.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.55	s	-	50.0
2	9.76	br s	-	-
3	4.57	s	-	50.1
3a	-	-	-	135.1
4	7.21	s	-	123.7
5	-	-	-	140.4
6	7.14	d	7.7	129.5
7	7.45	d	7.7	122.7
7a	-	-	-	133.6
1′	-	-	-	136.1
2′and 6′	-	-	-	145.8
3′and 5′	7.06	s	-	120.2
4′	-	-	-	147.8
1″	2.46	sep	6.9	29.8
2″	1.02	dd	11.5, 4.6	23.9
1‴	2.90	sep	6.9	33.6
2‴	1.24	d	7.0	24.0, 24.0

5.4 Guanidines

5.4.1 5-(4-(Trifluoromethyl)phenyl)isoindoline-2-carboximidamide HCl (8a)



8a

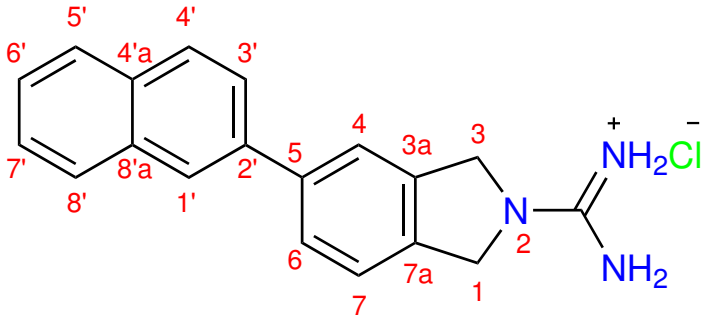
Figure 5.17: Structure of compound **8a** with numbered positions.

HRMS shown i Appendix V.7 confirmed the chemical formula of **8a**. The spectroscopic data in Appendix V.1-V.5 was used to assign the chemical shifts shown in Table 5.18. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.18: Chemical shifts all protons and carbons assigned positions shown in Figure 5.17. Because of CF-coupling, some carbon signals are represented as quartets. These are marked with a q followed by the coupling constant J_{CF}.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)	m, J_{CF} (Hz)
1	4.81	app s	-	52.7	-
2	-	-	-	-	-
3	4.82	app s	-	52.8	-
3a	-	-	-	136.4	-
4	7.75	s	-	121.4	-
5	-	-	-	143.7	-
6	7.73	d	8.0	126.8	-
7	7.25	d	8.0	123.4	-
7a	-	-	-	135.6	-
1′	-	-	-	138.4	-
2′and 6′	7.92	d	8.2	127.6	-
3′and 5′	7.83	d	8.2	125.8	q, 3.9
4′	-	-	-	128.0	q, 31.9
CF_3	-	-	-	124.3	q, 272.3
Guanyl	7.57	br s	-	155.0	-

5.4.2 5-(Naphthalen-2-yl)isoindoline-2-carboximidamide HCl (8b)



8b

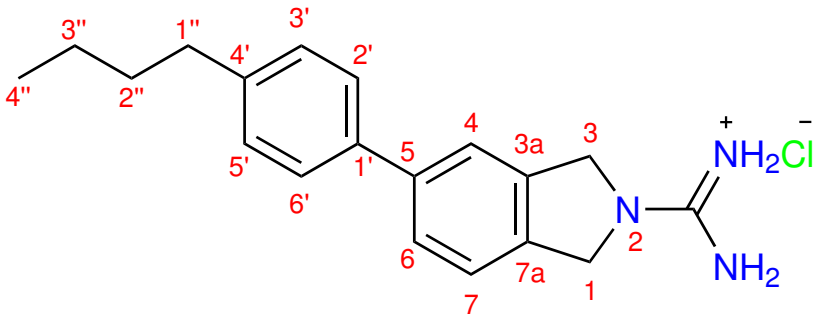
Figure 5.18: Structure of compound **8b** with numbered positions.

HRMS shown i Appendix W.7 confirmed the chemical formula of **8b**. The spectroscopic data in Appendix W.1-W.5 was used to assign the chemical shifts shown in Table 5.19. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.19: Chemical shifts for protons and carbons assigned to their respective positions shown in Figure 5.18.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.82	s	-	52.7
2	-	-	-	-
3	4.85	s	-	52.9
3a	-	-	-	136.2
4	7.82	app s	-	121.2
5	-	-	-	139.8
6	7.82	app d	8.0	126.8
7	7.56–7.51	m	-	123.3
7a	-	-	-	134.6
1′	8.26	s	-	125.4
2′	-	-	-	137.0
3′	7.87	dd	8.5, 1.8	125.1
4′	8.02	app d	8.6	128.5
4′a	-	-	-	132.3
5′	7.96	d	7.6	127.5
6′	7.56–7.51	m	-	126.2
7′	7.56–7.51	m	-	126.5
8′	8.00	app d	7.7	128.2
8′a	-	-	-	133.3
Guanyl	7.56–7.51	m	-	155.0

5.4.3 5-(4-Butylphenyl)isoindoline-2-carboximidamide HCl (8c)



8c

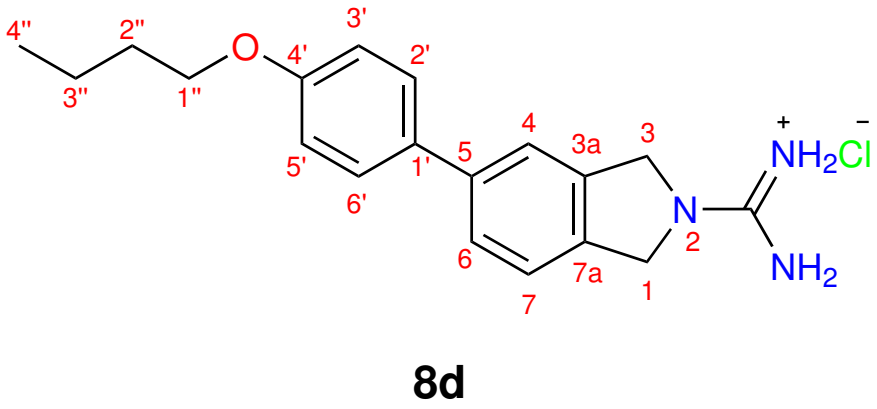
Figure 5.19: Structure of compound **8c** with numbered positions.

HRMS shown i Appendix X.7 confirmed the chemical formula of **8c**. The spectroscopic data in Appendix X.1-X.5 was used to assign the chemical shifts shown in Table 5.20. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.20: Chemical shifts for protons and carbons assigned positions shown in Figure 5.19.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.78	s	-	52.6
2	-	-	-	-
3	4.80	s	-	52.9
3a	-	-	-	137.1
4	7.63	app s	-	120.7
5	-	-	-	140.0
6	7.62	app d	8.0	126.3
7	7.44	d	8.4	123.1
7a	-	-	-	136.1
1′	-	-	-	134.1
2′and 6′	7.59	d	8.0	126.6
3′and 5′	7.29	d	8.0	128.9
4′	-	-	-	141.9
1″	2.62	t	7.6	34.4
2″	1.58	qn	7.6	33.1
3″	1.33	sex	7.5	21.7
4″	0.91	t	7.4	13.8
Guanyl	7.55	br s	-	155.0

5.4.4 5-(4-Butoxyphenyl)isoindoline-2-carboximidamide HCl (8d)



8d

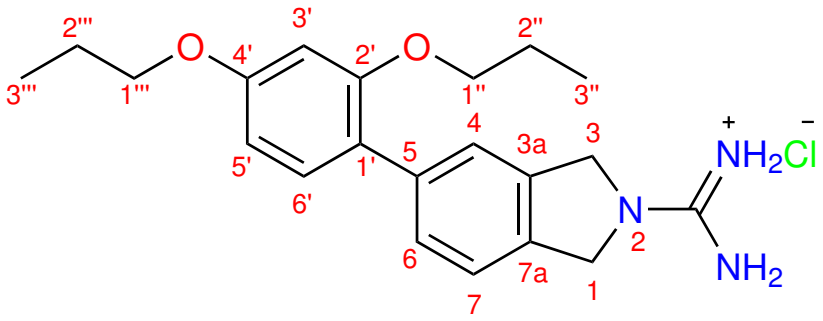
Figure 5.20: Structure of compound **8d** with numbered positions.

HRMS shown i Appendix Y.7 confirmed the chemical formula of **8d**. The spectroscopic data in Appendix Y.1-Y.5 was used to assign the chemical shifts shown in Table 5.21. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.21: Chemical shifts for protons and carbons assigned positions shown in Figure 5.20.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.77	s	-	52.7
2	-	-	-	-
3	4.79	s	-	52.9
3a	-	-	-	136.0
4	7.60–7.59	m	-	120.3
5	-	-	-	139.8
6	7.60–7.59	m	-	126.0
7	7.42	d	8.4	123.1
7a	-	-	-	133.6
1′	-	-	-	131.9
2′and 6′	7.60–7.59	m	-	127.9
3′and 5′	7.02	d	8.6	114.9
4′	-	-	-	158.5
1″	4.01	t	6.5	67.2
2″	1.72	p	6.8	30.7
3″	1.45	h	7.4	18.7
4″	0.95	t	7.4	13.7
Guanyl	7.47	br s	-	154.9

5.4.5 5-(2,4-Dipropoxyphenyl)isoindoline-2-carboximidamide HCl (8e)



8e

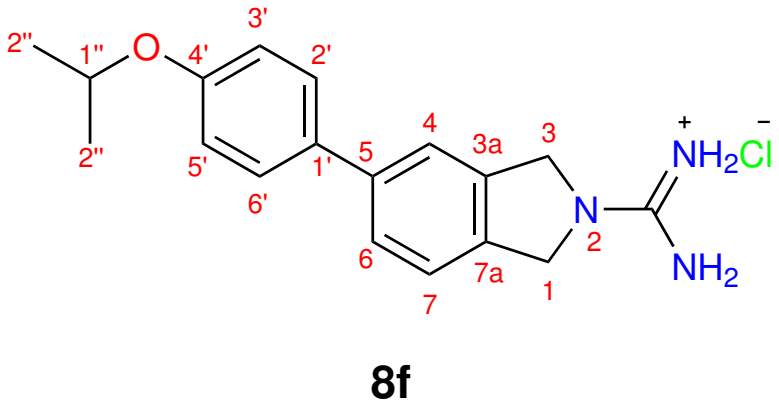
Figure 5.21: Structure of compound **8e** with numbered positions.

HRMS shown i Appendix Z.7 confirmed the chemical formula of **8e**. The spectroscopic data in Appendix Z.1-Z.5 was used to assign the chemical shifts shown in Table 5.22. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.22: Chemical shifts for protons and carbons assigned positions shown in Figure 5.21.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.77	s	-	53.2
2	-	-	-	-
3	4.77	s	-	53.4
3a	-	-	-	135.4
4	7.43	s	-	123.6
5	-	-	-	138.4
6	7.44	app d	8.3	129.3
7	7.37	d	8.3	122.6
7a	-	-	-	133.6
1′	-	-	-	122.3
2′	-	-	-	156.9
3′	6.63	d	2.1	100.5
4′	-	-	-	160.1
5′	6.60	dd	8.4, 2.3	106.4
6′	7.21	d	8.3	131.4
1′′	3.94	app t	6.3	69.8
2′′	1.65	sep	7.1	22.5
3′′	0.93	t	7.4	11.1
1′′′	3.97	app t	app 6.6	69.6
2′′′	1.74	sep	7.1	22.5
3′′′	0.99	t	7.4	10.9
Guanyl	7.48	br s	-	155.5

5.4.6 5-(4-Isopropoxyphenyl)isoindoline-2-carboximidamide HCl (8f)



8f

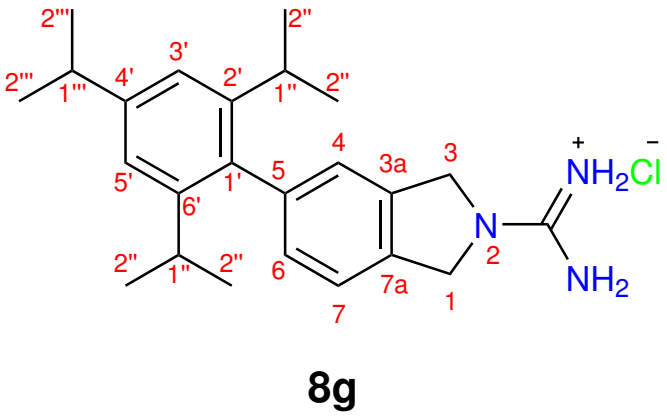
Figure 5.22: Structure of compound **8f** with numbered positions.

HRMS shown i Appendix AA.7 confirmed the chemical formula of **8f**. The spectroscopic data in Appendix AA.1-AA.5 was used to assign the chemical shifts shown in Table 5.23. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.23: Chemical shifts for protons and carbons assigned positions shown in Figure 5.22. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.78	app s	-	52.7
2	-	-	-	-
3	4.80	app s	-	52.9
3a	-	-	-	136.0
4	7.63–7.62	m	-	123.1
5	-	-	-	140.1
6	7.63–7.62	m	-	126.3
7	7.45	d	8.5	120.7
7a	-	-	-	134.1
1′	-	-	-	137.3
2′and 6′	7.60	d	8.2	126.7
3′and 5′	7.34	d	8.2	126.9
4′	-	-	-	147.9
1′′	2.94	sep	6.9	33.1
2′′	1.24	d	6.9	23.8
Guanyl	7.52	br s	-	155.0

5.4.7 5-(2,4,6-Triisopropylphenyl)isoindoline-2-carboximidamide HCl (8g)



8g

Figure 5.23: Structure of compound **8g** with numbered positions.

HRMS shown i Appendix AB.6 confirmed the chemical formula of **8g**. The spectroscopic data in Appendix AB.1-AB.5 was used to assign the chemical shifts shown in Table 5.24. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Table 5.24: Chemical shifts for protons and carbons assigned positions shown in Figure 5.23. The guanyl protons (NH_2) and the guanyl quartinary carbon are put on the same line, even though they are different positions.

Position	δ_H (ppm)	Multiplicity	J (Hz)	δ_C (ppm)
1	4.79	s	-	52.8
2	-	-	-	-
3	4.82	s	-	52.8
3a	-	-	-	135.2
4	7.19	s	-	123.4
5	-	-	-	140.0
6	7.14	d	7.7	129.1
7	7.43	d	7.7	122.4
7a	-	-	-	133.7
1′	-	-	-	136.2
2′and 6′	-	-	-	145.9
3′and 5′	7.06	s	-	120.2
4′	-	-	-	147.7
1″	2.49	sep	6.9	29.8
2″	1.03	dd	9.9, 2.9	23.9
1‴	2.90	sep	6.9	33.6
2‴	1.24	d	6.9	24.0, 24.0
Guanyl	7.48	br s	-	155.0

6 Experimental data

6.1 General information

With one exception, all chemicals were bought from Sigma-Aldrich or VWR International. The exception, 4-bromophthalimide (**3**), was bought from Tokyo Chemical Industri Co. Unless stated, no further purification of chemicals were carried out prior to use. Where use of degassed solvents is indicated, degassing was performed by bubbling helium through the solvent for 15 - 25 minutes.

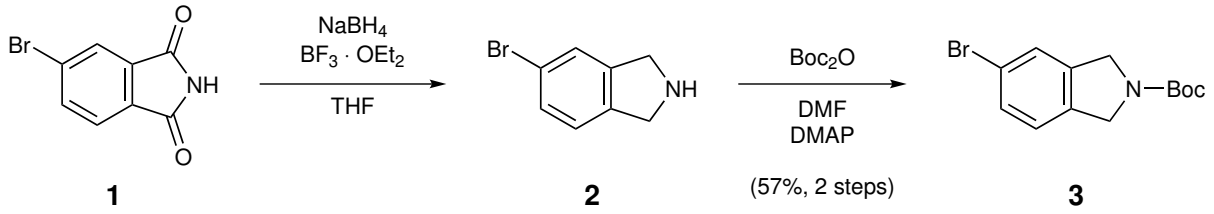
TLC-analysis was performed on Merck Silica Gel 60 F^{254} plates. Ultraviolet light at 312 nm or 365 nm was used for detection, together with chemical oxidation with phosphomolybdenic acid solution (12 g dissolved in EtOH (250 mL, 96%)). Flash column chromatography was performed with Silica gel (60 Å pore size, 230-400 mesh particle size) purchased from Fluka.

Melting points were determined on a Stuart SMP40 melting point apparatus without further corrections. NMR spectra were recorded on either a Bruker 600 MHz Avance III HD equipped with a 5 mm cryogenic CP-TCI z-gradient probe, or a Bruker 400 MHz Avance III HD equipped with a 5-mm SmartProbe z-gradient probe. The obtained spectras were analyzed in the software Bruker TopSpin 3.2 and Bruker TopSpin 3.5 pl6. Chemical shifts (δ) were reported in parts per million (ppm) and the integrals as number of protons ($^1_1$H). When using $CDCl_3$ with TMS, shift values for both proton and carbon are reported with reference to TMS (0.00). Reference values for other NMR-solvents are calibrated according to shifts presented by Gottlieb *et al.*[58] (^{1}H NMR: DMSO-d_6: 2.50, MeOD-d_4: 3.31; ^{13}C NMR: DMSO-d_6: 39.52, MeOD-d_4: 49.0). The signal patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), sex (sextet), sep (septet) and m (multiplet). The patterns can also be combined (e.g. dd means doublet of doublets). Coupling constants (J) are given in hertz (Hz). Some compounds (mainly those featuring carbamate protection) feature two stable rotamers, hence two signals from the same proton/carbon. These signals are marked with an asterisk (*). $^1_1$H and ^{13}C NMRC shifts for new compounds were assigned using 2D correlation techniques (COSY, HSQC and HMBC, see Section 5).

IR spectra were recorded using a Bruker Alpha FT-IR spectrometer with an ATR-module. Only the strongest/structurally most important peaks are listed as wavenumber (cm^{-1}), and are indicated as strong (s), medium (m) or weak (w). Accurate mass determination was performed on a Waters Synapt G2-S Q-TOF. Samples were ionized by the use of an ASAP (APCI) or ESI probe in positive mode. No chromatographic separation was used previous to the mass analysis. Calculated exact mass and spectrum processing was done by Waters Software Masslynx V4.1 SCN871. HPLC analyses were performed on an Agilent 1290 chromatograph equipped with a Zorbax Eclipse C18 5 µm (150 × 4.6 4.6 mm) column and a diode array detector (main detection region 214 nm). Reported purity for all samples are corrected by co-elution of a blank sample.

6.2 Preparation of building block 3

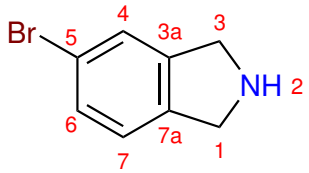
Preparation of *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**) was carried out over two steps according to a procedure described by Patel and Barrett.[27] The first step involved reducing 4-bromophthalimide (**1**) to 5-bromoisoindoline (**2**) using *in situ* preparation of diborane,[30] followed by carbamate protection with di-*tert*-butyl dicarbonate (Boc_2O), yielding **3**. The two reaction steps are illustrated in Scheme 6.1.



1 **2** **3**

Scheme 6.1: Illustration of the two reaction steps from 4-bromophthalimide (**1**) to *tert*-butyl 5-bromoisoindoline-2-carboxylate (**3**).

6.2.1 5-Bromoisoindoline (2)



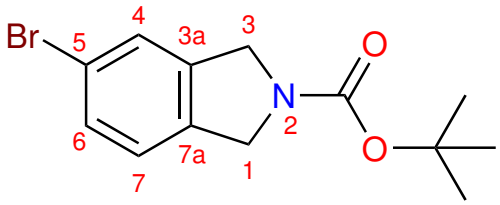
2

$NaBH_4$ (15 g, 396.5 mmol) was added to a suspension of 4-bromophthalimide (**1**, 8.205 g, 36.3 mmol) in THF (350 mL). The reaction mixture was cooled to −10 °C and $BF_3 \cdot OEt_2$ (52 mL, 421 mmol) was added slowly. Once the addition was completed, the reaction mixture was heated to 70 °C. After 21 hours, the reaction mixture was cooled to 0 °C, quenched slowly with H_2O (150 mL) and diluted with EtOAc (350 mL). The water phase was made alkaline to pH 10 using 6.0 M aq. NaOH. The organic layer was separated, washed with brine (3 × 200 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residual green oil was diluted with Et_2O (200 mL) and H_2O (200 mL) and the water phase acidified to pH 2 using 6.0 M aq. HCl. The aqueous layer was separated, made alkaline to pH 10 using 6.0 M aq. NaOH and extracted with EtOAc (3 × 100 mL). The organic phases were combined, washed with brine (3 × 200 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a brown oil (5.648 g) containing **2**. The obtained crude was used directly in the next step without further purification.

Spectroscopic data for **2**:
^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.39 (s, 1 H, H-6), 7.33 (d, 1 H, J = 7.9 Hz, H-4), 7.12 (d, 1 H, J = 8.0 Hz, H-7), 4.21 (s, 2 H, H-3), 4.18 (s, 2 H, H-1), 1.65 (br s, app 1 H, H-2).

The ^{1}H NMR spectrum was in accordance with reported data,[27] and is shown in Appendix A.1.

6.2.2 *tert*-butyl 5-bromoisoindoline-2-carboxylate (3)



3

Di-*tert*-butyl dicarbonate (Boc_2O, 8.1 g, 37.1 mmol) and a few crystals of DMAP was added to a suspension of crude 5-bromoisoindoline (**2**, 5.648 g) in DMF (100 mL). After 41 hours the reaction mixture was diluted with EtOAc (200 mL), washed with brine (3 × 150 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a dark brown oil (8.110 g). The residue was purified using flash column cromatography (1:9 EtOAc/*n*-pentane) to give **3** (6.034 g, 20.2 mmol, 57% from **1**) as a yellow solid.

Spectroscopic data for **3**:
R_f: 0.22 (1 : 4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.42* (s, 0.5 H, H-4), 7.40–7.38 (m, 1 H, H-6), 7.36* (s, 0.5 H, H-4), 7.14* (d, 0.5 H, J = 8.0 Hz, H-7), 7.09* (d, 0.5 H, J = 8.0 Hz, H-7), 4.67–4.59 (m, 4 H, H-1 and H-3), 1.51 (s, 9 H, Boc $(CH_3)_3$). HRMS (TOF ASAP+): m/z calcd for $C_9H_9NO_2Br$ $[M\text{-}C_8H_8+H]^+$: 241.9817; found: 241.9817.

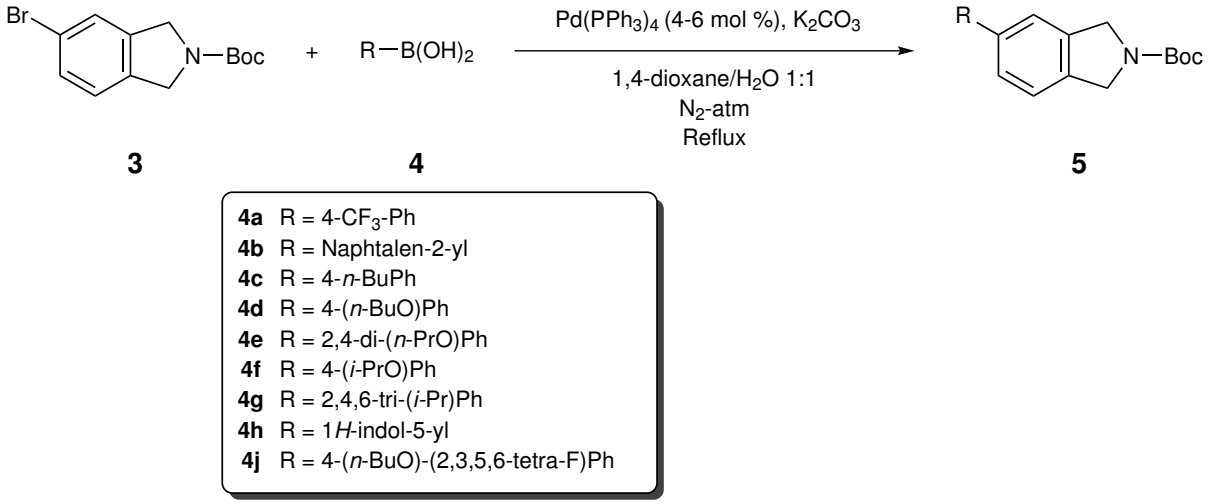
The ^{1}H NMR spectrum was in accordance with reported data,[27] and is shown in Appendix B.1. The MS spectrum can be seen in Appendix B.2.

*Rotamers.

6.3 Preparation of carbamate protected 5-substituted Isoindolines 5a-j

All but one of the carbamate protected 5-substituted isoindolines were prepared by Suzuki cross-coupling. The exception, **5i**, was prepared by *N*-alkylation of **5h**.

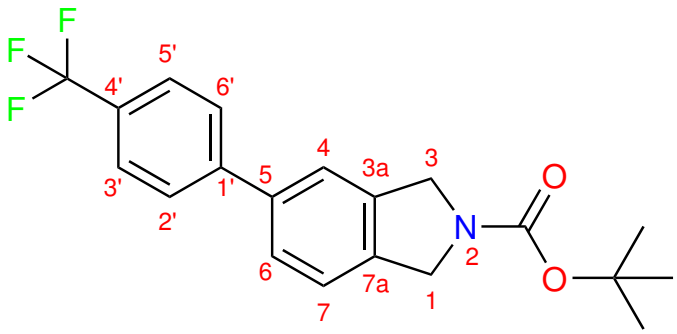
The general method for the Suzuki cross-couplings used a method described by Hua *et al.*,[47] with a few modifications to the solvent system as described by Bugge *et al.*[48] The method is illustrated in Scheme 6.2 and described below. Compound **5g** and **5j** were also attempted prepared with a more active catalyst system, following a procedure described by Knapp, Gillis and Burke.[49] The procedure is described in detail in the experimental section for **5g** (Section 6.3.7 - Attempt 2).



Scheme 6.2: Reaction scheme illustrating the general method used for Suzuki cross-coupling of halide **3** and boronic acid **4**.

In the general method used, a degassed solution of 1,4-dioxane/H_2O (1:1 v/v, 20-30 mL pr. mmol of **3**) was added to a mixture of the carbamate protected halide **3** (1 eq), the appropriate boronic acid (**4**, 1.15-1.25 eq), K_2CO_3 (1.5 eq) and $Pd(PPh_3)_4$ (4-6 mol%) under inert atmosphere (N_2 or Ar). The mixture was refluxed overnight, cooled to room temperature, quenched by addition of H_2O (~30 mL pr mmol of **3**), and extracted with DCM (3 × 50 mL). The combined organic phases were washed with H_2O (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified using flash column chromatography (EtOAc/*n*-pentane), affording 5-substituted isoindoline **5**.

6.3.1 *tert*-Butyl 5-(4-(trifluoromethyl)phenyl)isoindoline-2-carboxylate (5a)



5a

The title compound was prepared in two parallels following the general method for Suzuki cross-coupling, with carbamate protected halide **3** and 4-(trifluoromethyl)phenylboronic acid (**4a**). Ar-atm was used as inert atmosphere. Purification with flash column chromatography (1:4 and 1:9 EtOAc/*n*-pentane respectively) yielded **5a** as a white solid for both experiments. The specifics for each parallel can be seen in Table 6.1.

Table 6.1: Reaction conditions for the two Suzuki cross-coupling reactions between **3** and **4a**, yielding **5a**.

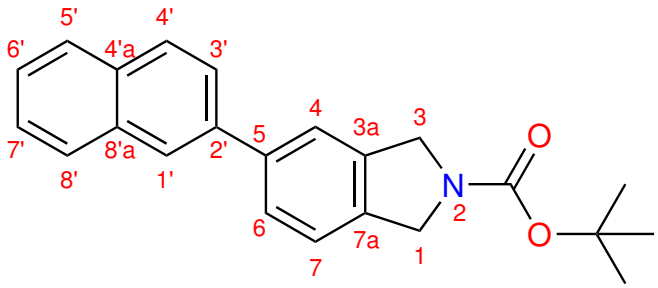
Exp.	Halide **3**	Boronic a. **4a**	K_2CO_3	$Pd(PPh_3)_4$	Diox : H_2O (1 : 1)	Time	Yield
#	[g, mmol]	[g, mmol]	[g, mmol]	[g, mmol]	[mL]	[h]	[g, mmol, %]
1	0.560, 1.88	0.420, 2.21	0.346, 2.50	0.100, 0.087	40	22	0.529, 1.46, 77%
2	0.496, 1.66	0.391, 2.06	0.392, 2.84	0.100, 0.087	40	22	0.474, 1.30, 78%

Spectroscopic and physical data for **5a**: Mp. 135-141 °C. R_f: 0.53 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.70–7.66 (m, 4 H, H-2′, H-3′, H-5′ and H-6′), 7.50* (s, 1.5 H, H-4 and H-6), 7.44 (s, 0.5 H, H-4), 7.38* (d, 0.5 H, J = 7.8 Hz, H-7), 7.32* (d, 0.5 H, J = 8.4 Hz, H-7), 4.76–4.72 (m, 4 H, H-1 and H-3), 1.53 (s, 9 H, Boc $(CH_3)_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.5 (Boc C=O), 144.5* (C-5), 144.4* (C-5), 139.3 (C-1′), 138.4* (C-3a), 138.0* (C-3a), 137.5* (C-7a), 137.0* (C-7a), 129.5 (q, $^2J_{CF}$ = 32.2 Hz, C-4′), 127.4 (2 C, C-2′ and C-6′), 126.7* (C-6), 126.6* (C-6), 125.8 (2 C, C-3′ and C-5′), 124.2 (q, $^1J_{CF}$ = 272.7 Hz, CF_3), 123.4* (C-7), 123.1* (C-7), 121.7* (C-4), 121.4* (C-4), 79.9 (Boc Cq), 52.3* (C-3), 52.1* (C-1), 52.0* (C-3), 51.8* (C-1), 28.6 (3 C, Boc $(CH_3)_3$). IR (ATR) (cm^{-1}): 2989 (w), 2937 (w), 2872 (w), 1738 (w), 1683 (m), 1613 (m), 1403 (m), 1325 (s), 1158 (m), 1111 (s), 1070 (m), 1012 (m), 876 (m), 849 (m), 814 (m), 771 (w), 712 (w). HRMS (TOF ASAP+): m/z calcd for $C_{16}H_{13}NOF_3$ $[M\text{-}C_8H_8+H]^+$: 308.0898; found: 308.0898.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5a** are shown in Appendix C.1-C.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.1.

*Rotamers.

6.3.2 *tert*-Butyl 5-(naphthalen-2-yl)isoindoline-2-carboxylate (5b)



5b

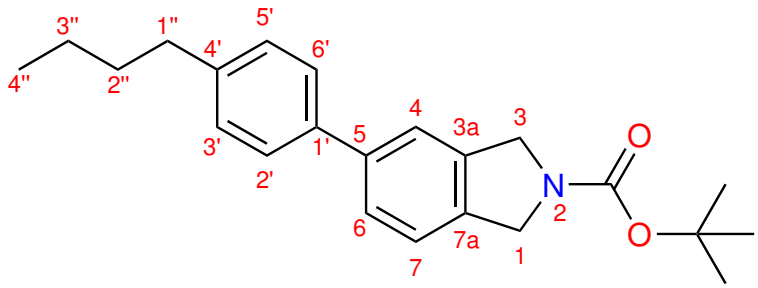
The title compound was prepared following the general method for Suzuki cross-coupling. Carbamate protected halide **3** (0.500 g, 1.68 mmol), 2-naphthylboronic acid (**4b**, 0.354 g, 2.06 mmol), K_2CO_3 (0.346 g, 2.50 mmol) and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (40 mL, 1:1). The reaction mixture was allowed to reflux for 24 hours. Purification by flash column chromatography (1:9 EtOAc/*n*-pentane) yielded **5b** (0.470 g, 1.36 mmol, 81%) as a blank solid.

Spectroscopic and physical data for **5b**: Mp. 116-118 °C. R_f: 0.56 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.02 (d, 1 H, J = 7.7 Hz, H-1′), 7.92–7.86 (m, 3 H, H-4′, H-5′ and H-8′), 7.73–7.71 (m, 1 H, H-3′), 7.63* (app s, 0.5 H, H-4), 7.62 (app s, 1 H, H-6), 7.56* (s, 0.5 H, H-4), 7.49 (qn, 2 H, J = 8.0 Hz, H-6′ and H-7′), 7.39* (d, 0.5 H, J = 7.9 Hz, H-7), 7.33* (d, 0.5 H, J = 8.0 Hz, H-7), 4.78–4.71 (m, 4 H, H-1 and H-3), 1.54 (s, 9 H, Boc $(CH_3)_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6 (Boc C=O), 140.7* (C-5), 140.7* (C-5), 138.3* (C-3a), 138.2 (C-2′), 137.8* (C-3a), 136.5* (C-7a), 136.2* (C-7a), 133.7 (C-8′a), 132.6 (C-4′a), 128.5 (C-4′), 128.2 (C-8′), 127.7 (C-5′), 126.8* (C-6), 126.8* (C-6), 126.4 (C-7′), 126.0 (C-6′), 125.8 (C-1′), 125.5 (C-3′), 123.2* (C-7), 123.0* (C-7), 121.8* (C-4), 121.5* (C-4), 79.8 (Boc Cq), 52.4* (C-3), 52.1* (C-1), 52.1* (C-3), 51.9* (C-1), 28.6 (3 C, Boc $(CH_3)_3$). IR (ATR) (cm^{-1}): 3054 (w), 2973 (w), 2918 (w), 2861 (w), 1692 (s), 1390 (s), 1363 (s), 1255 (m), 1167 (s), 1104 (s), 876 (m), 807 (s), 744 (s). HRMS (TOF ASAP+): m/z calcd for $C_{19}H_{16}NO_2$ $[M\text{-}C_8H_8+H]^+$: 290.1181; found: 290.1178.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5b** are shown in Appendix D.1-D.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.2.

*Rotamers.

6.3.3 *tert*-Butyl 5-(4-butylphenyl)isoindoline-2-carboxylate (5c)



5c

The title compound was prepared in two parallels following the general method for Suzuki cross-coupling, with carbamate protected halide **3** and 4-butylphenylboronic acid (**4c**). Ar- and N_2-atm were used as inert atmospheres for experiment 1 and 2 respectively. Purification was done with flash column chromatography (1:9 EtOAc/*n*-pentane). Both experiments yielded **5c** as an off-white solid. The specifics for each parallel can be seen in Table 6.2.

Table 6.2: Reaction conditions for the two Suzuki cross-coupling reactions between **3** and **4c**, yielding **5c**.

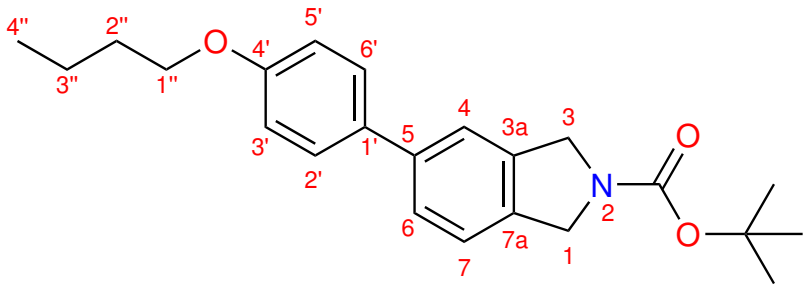
Exp.	Halide **3**	Boronic a. **4c**	K_2CO_3	$Pd(PPh_3)_4$	Diox : H_2O (1 : 1)	Time	Yield
#	[g, mmol]	[g, mmol]	[g, mmol]	[g, mmol]	[mL]	[h]	[g, mmol, %]
1	0.500, 1.68	0.350, 1.97	0.346, 2.5	0.100, 0.087	40	23	0.421, 1.20, 71%
2	0.715, 2.40	0.509, 2.86	0.553, 4.0	0.130, 0.112	50	21	0.747, 2.13, 89%

Spectroscopic and physical data for **5c**: Mp. 71-78 °C. R_f: 0.54 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.49–7.47* (m, 3.5 H, H-4, H-6, H-2′ and H-6′), 7.42* (s, 0.5 H, H-4), 7.32* (d, 0.5 H, J = 7.9 Hz, H-7), 7.27* (app d, 0.5 H, app J = 7.9 Hz, H-7), 7.26–7.24 (m, app 2 H, H-3′ and H-5′), 4.74–4.68 (m, 4 H, H-1 and H-3), 2.65 (t, 2 H, J = 7.7 Hz, H-1″), 1.63 (app qn, 2 H, app J = 7.5 Hz, H-2″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.39 (sex, 2 H, J = 7.7 Hz, H-3″), 0.95 (t, 3 H, J = 7.3 Hz, H-4″). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6 (Boc C=O), 142.2 (C-4′), 140.7 (C-5), 138.3* (C-1′), 138.2* (C-1′), 138.0* (C-3a), 137.6* (C-3a), 136.1* (C-7a), 135.8* (C-7a), 128.9 (2 C, C-3′ and C-5′), 127.0 (2 C, C-2′ and C-6′), 126.4* (C-6), 126.3* (C-6), 123.0* (C-7), 122.8* (C-7), 121.3* (C-4), 121.6* (C-4), 79.7 (Boc Cq), 52.4* (C-3), 52.1* (C-1), 52.1* (C-3), 51.8* (C-1), 35.3 (C-1″), 33.7 (C-2″), 28.6 (3 C, Boc $(CH_3)_3$), 22.4 (C-3″), 14.0 (C-4″). IR (ATR) (cm^{-1}): 2988 (m), 2950 (m), 2928 (m), 1738 (m), 1699 (s), 1474 (m), 1392 (s), 1366 (s), 1256 (m), 1165 (m), 1107 (s), 874 (m), 808 (s), 778 (m). HRMS (TOF ASAP+): m/z calcd for $C_{19}H_{22}NO_2$ $[M\text{-}C_8H_8+H]^+$: 296.1651; found: 296.1650.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5c** are shown in Appendix E.1-E.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.3.

*Rotamers.

6.3.4 *tert*-Butyl 5-(4-butoxyphenyl)isoindoline-2-carboxylate (5d)



5d

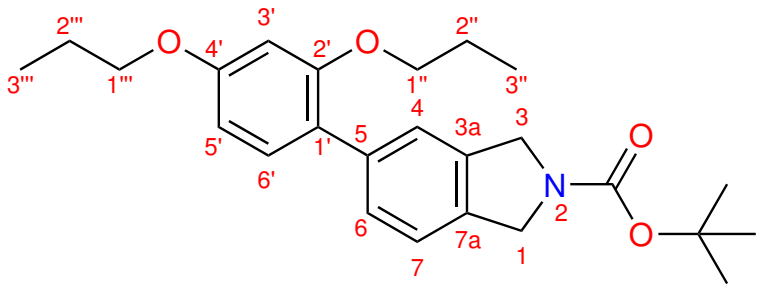
The title compound was prepared following the general method for Suzuki cross-coupling. Carbamate protected halide **3** (0.516 g, 1.73 mmol), 4-butoxyphenylboronic acid (**4d**, 0.388 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.50 mmol) and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (40 mL, 1:1). The reaction mixture was allowed to reflux for 22 hours. Purification by flash column cromatography (1:9 EtOAc/*n*-pentane) yielded **5d** (0.453 g, 1.23 mmol, 71%) as a blue solid.

Spectroscopic and physical data for **5d**: Mp. 121-123 °C. R_f: 0.50 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.50–7.47 (m, 2 H, H-2′ and H-6′), 7.45 (app d, 1 H, app J = 7.9 Hz, H-6), 7.44* (app s, 0.5 H, H-4), 7.38* (s, 0.5 H, H-4), 7.30* (d, 0.5 H, J = 7.9 Hz, H-7), 7.25* (app d, 0.5 H, app J = 7.9 Hz, H-7), 6.96 (d, 2 H, J = 7.5 Hz, H-3′ and H-5′), 4.73–4.67 (m, 4 H, H-1 and H-3), 4.00 (t, 2 H, J = 6.5 Hz, H-1″), 1.79 (qn, 2 H, J = 6.5 Hz, H-2″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.52 (app sex, 2 H, J = 7.4 Hz, H-3″), 0.99 (t, 3 H, J = 7.4 Hz, H-4″). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 158.8 (C-4′), 154.6 (Boc C=O), 140.5 (C-5), 138.0* (C-3a), 137.6* (C-3a), 135.7* (C-7a), 135.4* (C-7a), 133.3* (C-1′), 133.2* (C-1′), 128.1 (2 C, C-2′ and C-6′), 126.1* (C-6), 126.0* (C-6), 123.0* (C-7), 122.8* (C-7), 121.0* (C-4), 120.8* (C-4), 114.8 (2 C, C-3′ and C-5′), 79.7 (Boc Cq), 67.8 (C-1″), 52.4* (C-3), 52.1* (C-1), 52.1* (C-3), 51.8* (C-1), 31.4 (C-2″), 28.6 (3 C, Boc $(CH_3)_3$), 19.3 (C-3″), 13.9 (C-4″). IR (ATR) (cm^{-1}): 2957 (w), 2871 (w), 1684 (s), 1607 (w), 1519 (m), 1471 (m), 1400 (s), 1247 (s), 1180 (s), 1111 (s), 880 (m), 816 (s). HRMS (TOF ASAP+): m/z calcd for $C_{19}H_{22}NO_3$ $[M\text{-}C_8H_8+H]^+$: 312.1600; found: 312.1598.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5d** are shown in Appendix F.1-F.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.4.

*Rotamers.

6.3.5 *tert*-Butyl 5-(2,4-dipropoxyphenyl)isoindoline-2-carboxylate (5e)



5e

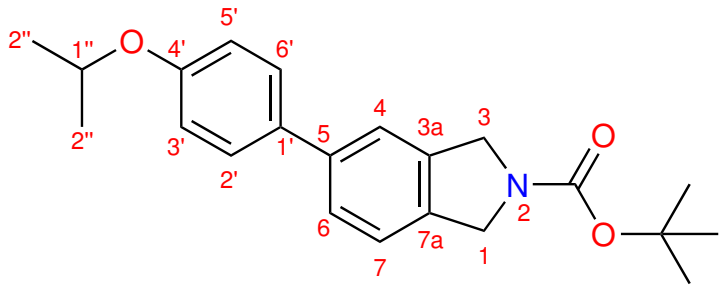
The title compound was prepared following the general method for Suzuki cross-coupling. Carbamate protected halide **3** (0.500 g, 1.68 mmol), 2,4-dipropoxyphenylboronic acid (**4e**, 0.476 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.50 mmol) and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (40 mL, 1:1). The reaction mixture was allowed to reflux for 24 hours. Purification by flash column cromatography (1:9 EtOAc/*n*-pentane) yielded **5e** (0.573 g, 1.39 mmol, 83%) as a blank wax.

Spectroscopic and physical data for **5e**:
R_f: 0.56 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.43–7.40* (m, 1.5 H, H-4 and H-6), 7.36* (s, 0.5 H, H-4), 7.26* (app d, app 0.5 H, app J = 8.1 Hz, H-7), 7.22–7.20* (m, 1.5 H, H-7 and H-6′), 6.55–6.53 (m, 2 H, H-3′ and H-5′), 4.71–4.67 (m, 4 H, H-1 and H-3), 3.95 (t, 2 H, J = 6.6 Hz, H-3‴), 3.91 (t, 2 H, J = 6.4 Hz, H-3″), 1.83 (sex, 2 H, J = 7.1 Hz, H-2‴), 1.77–1.71 (m, 2 H, H-2″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.06 (t, 3 H, J = 7.4 Hz, H-1‴), 0.97 (td, 3 H, J = 7.4, 1.9 Hz, H-1″). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 159.9 (C-4′), 156.9 (C-2′), 154.7 (Boc C=O), 137.9* (C-5), 137.9* (C-5), 137.0* (C-3a), 136.6* (C-3a), 135.2* (C-7a), 134.9* (C-7a), 131.1* (C-6′), 131.1* (C-6′), 128.8* (C-6), 128.7* (C-6), 123.8* (C-4), 123.6* (C-4), 123.2* (C-1′), 123.1* (C-1′), 122.1* (C-7), 121.9* (C-7), 105.4 (C-5′), 100.4* (C-3′), 100.4* (C-3′), 79.6 (Boc Cq), 69.9* (C-1″), 69.9* (C-1″), 69.7 (C-1‴), 52.4* (C-3), 52.2* (C-1), 52.1* (C-3), 51.9* (C-1), 28.6 (3 C, Boc $(CH_3)_3$), 22.7 (C-2‴), 22.5 (C-2″), 10.7 (C-3″), 10.6 (C-3‴). IR (ATR) (cm^{-1}): 2966 (w), 2933 (w), 2974 (w), 1696 (s), 1608 (m), 1572 (w), 1514 (m), 1471 (s), 1299 (m), 1278 (m), 1248 (m), 1175 (s), 1105 (s), 1066 (m), 1013 (m), 978 (w), 877 (m), 820 (m). HRMS (TOF ASAP+): m/z calcd for $C_{21}H_{26}NO_4$ $[M\text{-}C_8H_8+H]^+$: 356.1863; found: 356.1862.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5e** are shown in Appendix G.1-G.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.5.

*Rotamers.

6.3.6 *tert*-Butyl 5-(4-isopropoxyphenyl)isoindoline-2-carboxylate (5f)



5f

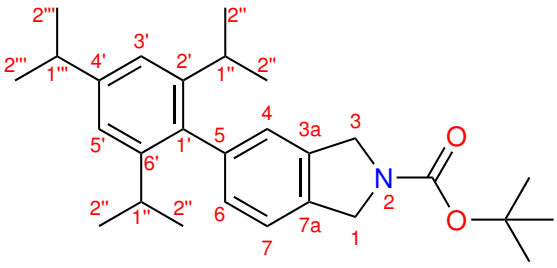
The title compound was prepared following the general method for Suzuki cross-coupling. Carbamate protected halide **3** (0.500 g, 1.68 mmol), 4-isopropoxyphenylboronic acid (**4f**, 0.360 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.50 mmol) and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (40 mL, 1:1). The reaction mixture was allowed to reflux for 25 hours. Purification by flash column chromatography (1:9 EtOAc/*n*-pentane) yielded **5f** (0.473 g, 1.34 mmol, 80%) as a white solid.

Spectroscopic and physical data for **5f**: Mp. 86-93 °C. R_f: 0.58 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.51–7.47* (m, 3.5 H, H-4, H-6, H-2′, and H-6′), 7.42* (s, 0.5 H, H-4), 7.33–7.26* (m, 3 H, H-7, H-3′ and H-5′), 4.74–4.68 (m, 4 H, J = Hz, H-1 and H-3), 2.96 (sep, 1 H, J = 6.9 Hz, H-1″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.29 (d, 6 H, J = 6.9 Hz, H-2″). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6 (Boc C=O), 148.2 (C-4′), 140.7* (C-5), 140.7* (C-5), 138.5* (C-1′), 138.4* (C-1′), 138.0* (C-3a), 137.6* (C-3a), 136.1* (C-7a), 135.8* (C-7a), 127.1 (2 C, C-2′ and C-6′), 126.9 (2 C, C-3′ and C-5′), 126.4* (C-6), 126.3* (C-6), 123.0* (C-7), 122.8* (C-7), 121.4* (C-4), 121.1* (C-4), 79.7 (Boc Cq), 52.4* (C-3), 52.1* (C-1), 52.1* (C-3), 51.9* (C-1), 33.8 (C-1″), 28.6 (3 C, Boc $(CH_3)_3$), 24.0 (2 C, C-2″). IR (ATR) (cm^{-1}): 2958 (w), 2865 (w), 1684 (s), 1402 (s), 1359 (m), 1171 (w), 1116 (m), 1055 (s), 878 (m), 845 (m), 816 (s), 772 (m). HRMS (TOF ASAP+): m/z calcd for $C_{18}H_{20}NO_2$ [M-C_8H_8O+H]$^+$: 282.1494; found: 282.1491.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **5f** are shown in Appendix H.1-H.7. For structure elucidation and assignment of chemical shifts, see Section 5.2.6.

*Rotamers.

6.3.7 tert-Butyl 5-(2,4,6-triisopropylphenyl)isoindoline-2-carboxylate (5g)



5g

Preparation of the title compund was attempted twice. The first attempt, following the general method for Suzuki cross-coupling failed. The successful attempt followed a procedure by Knapp, Gillis and Burke,[49] with modifications to the solvent system.

Attempt 1 - General method: Carbamate protected halide **3** (0.500 g, 1.68 mmol), 2,4,6-triisopropylphenylboronic acid (**4g**, 0.498 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.50 mmol) and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (40 mL, 1:1). The reaction mixture was allowed to reflux for 25 hours. ^{1}H NMR-analysis indicated traces desired product **5g** (see Appendix I.7).

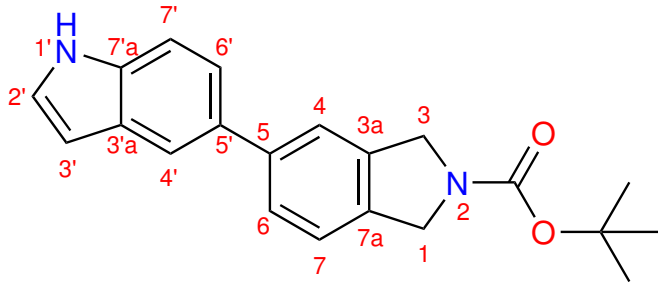
Attempt 2 - More active catalyst system: A degassed solution of 1,4-dioxane (30 mL) was added to a mixture of the carbamate protected halide **3** (0.596 g, 2.00 mmol), 2,4,6-triisopropylphenylboronic acid (**4g**, 0.596 g, 2.40 mmol), $Pd(OAc)_2$ (0.023 g, 0.10 mmol) and SPhos (0.082 g, 0.20 mmol) under N_2-atm. After stirring for 10 minutes, K_3PO_4 (1.698 g, 8.00 mmol) dissolved in H_2O (30 mL) was added to the reaction mixture. Upon completed addition, the mixture was heated to 70 °C for 40 hours, then worked up as described in the general method. Purification by flash column chromatography (1:9 EtOAc/*n*-pentane) yielded **5g** (0.589 g, 1.40 mmol, 70%) as a white solid.

Spectroscopic and physical data for **5g**:
R_f: 0.35 (1:9 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.28* (d, 0.5 H, J = 7.6 Hz, H-7), 7.24* (d, 0.5 H, J = 7.9 Hz, H-7), 7.08–7.02 (m, 4 H, H-4, H-6, H-3′ and H-5′), 4.76–4.70 (m, 4 H, H-1 and H-3), 2.94 (sep, 1 H, J = 6.7 Hz, H-1‴), 2.59–2.55 (m, 2 H, H-1″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.30 (d, 6 H, J = 6.7 Hz, H-2‴), 1.07 (d, 12 H, J = 6.8 Hz, H-2″), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6* (Boc C=O), 154.6* (Boc C=O), 148.0 (C-4′), 146.6* (C-2′ and C-6′), 146.5* (C-2′ and C-6′), 140.2* (C-5), 140.1* (C-5), 137.1* (C-3a), 136.8* (C-3a), 136.6* (C-1′), 136.6* (C-1′), 135.5* (C-7a), 135.2* (C-7a), 129.0* (C-6), 128.9* (C-6), 123.9* (C-4), 123.7* (C-4), 122.3* (C-7), 122.0* (C-7), 120.5* (2 C, C-3′ and C-5′), 120.5* (2 C, C-3′ and C-5′), 79.6 (Boc Cq), 52.4* (C-3), 52.3* (C-1), 52.1* (C-3), 52.0* (C-1), 34.3 (C-1‴), 30.3 (2 C, C-1″), 28.6 (3 C, Boc $(CH_3)_3$), 24.2 (2 C, C-2″), 24.2 (2 C, C-2″), 24.1 (2 C, C-2‴). HRMS (TOF ASAP+): m/z calcd for $C_{24}H_{32}NO_2$ $[M\text{-}C_8H_8+H]^+$: 366.2433; found: 366.2437.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra obtained for **5g** are shown in Appendix I.1-I.6. For structure elucidation and assignment of chemical shifts, see Section 5.2.7.

*Rotamers.

6.3.8 *tert*-Butyl 5-(1*H*-indol-5-yl)isoindoline-2-carboxylate (5h)



5h

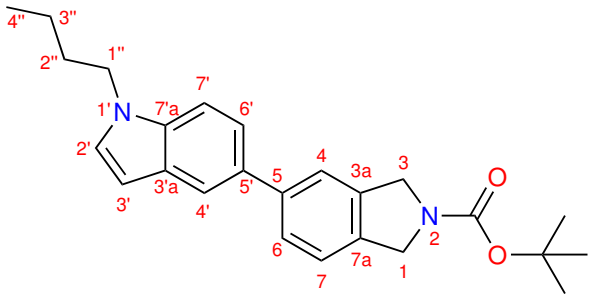
The title compound was prepared following the general method for Suzuki cross-coupling. Carbamate protected halide **3** (1.00 g, 3.35 mmol), 5-indolylboronic acid (**4h**, 0.644 g, 4.00 mmol), K_2CO_3 (0.692 g, 5.00 mmol) and $Pd(PPh_3)_4$ (0.200 g, 0.173 mmol) were mixed under N_2-atm, followed by addition of 1,4-dioxane/H_2O (60 mL, 1:1). The reaction mixture was allowed to reflux for 23 hours. Purification by flash column chromatography (3:7 EtOAc/*n*-pentane) yielded **5h** (0.912 g, 2.73 mmol, 81%) as an off-white solid.

Spectroscopic and physical data for **5h**:
R_f 0.14 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.21 (s, 1 H, H-1′), 7.84 (d, 1 H, J = 7.7 Hz, H-4′), 7.55 (app d, 1 H, J = 8.1 Hz, H-6), 7.55* (app s, 0.5 H, H-4), 7.49* (app s, 0.5 H, H-4), 7.47–7.41 (m, 2 H, H-6′ and H-7′), 7.33* (d, 0.5 H, J = 7.8 Hz, H-7), 7.28* (d, 0.5 H, J = 7.8 Hz, H-7), 7.25 (app s, app 1 H, H-2′), 6.61 (s, 1 H, H-3′), 4.76–4.69 (m, 4 H, H-1 and H-3), 1.54 (s, 9 H, Boc $(CH_3)_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6 (Boc C=O), 142.1* (C-5), 142.1* (C-5), 137.9* (C-3a), 137.4* (C-3a), 135.4* (C-7a), 135.3 (C-7′a), 135.1* (C-7a), 133.2* (C-5′), 133.1* (C-5′), 128.4 (C-3′a), 126.8* (C-6), 126.7* (C-6), 124.9 (C-2′), 122.9* (C-7), 122.7* (C-7), 121.9 (C-6′), 121.7* (C-4), 121.4* (C-4), 119.3 (C-4′), 111.3 (C-7′), 103.0 (C-3′), 79.7 (Boc Cq), 52.4* (C-3), 52.2* (C-1), 52.1* (C-3), 51.8* (C-1), 28.6 (3 C, Boc $(CH_3)_3$). HRMS (TOF ASAP+): m/z calcd for $C_{17}H_{15}N_2O_2$ $[M\text{-}C_8H_8+H]^+$: 279.1134; found: 279.1132.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra obtained for **5h** are shown in Appendix J.1-J.6. For structure elucidation and assignment of chemical shifts, see Section 5.2.8.

*Rotamers.

6.3.9 *tert*-Butyl 5-(1-butyl-1*H*-indol-5-yl)isoindoline-2-carboxylate (5i)



5i

The procedure for *N*-alkylation of **5h** to *tert*-butyl 5-(1-butyl-1*H*-indol-5-yl)isoindoline-2-carboxylate (**5i**) was adapted from a procedure by Elkassih *et al.*[53] The original procedure described *N*-alkylation of phenotiazine with 1-bromodecane.

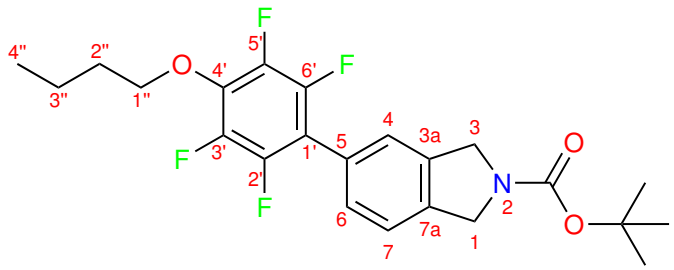
Compound **5h** (0.492 g, 1.47 mmol) and NaH (0.096 g, 4.00 mmol) was dissolved in dry THF (100 mL) under N_2-atm. When the evolution of hydrogen gas stopped, 1-bromobutane (0.548 g, 4.00 mmol) was added dropwise over a period of 30 minutes. The reaction mixture was heated at reflux for 48 hours. TLC-analysis (SiO_2, EtOAc/*n*-pentane 3:7, R_f = 0.40) still indicated the presence of unreacted **5h**. Additional 1-bromobutane (0.274 g, 2.00 mmol) was added slowly, and the reaction mixture was allowed to reflux for another 24 hours. TLC still showed traces of **5h** after 72 hours. The reaction mixture was cooled to r.t., and quenched by addition of NH_4Cl (aq., 50 mL, 5%). The reaction mixture was extracted with DCM (4 × 50 mL), washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:4 EtOAc/*n*-pentane) yielded **5i** (0.212 g, 0.54 mmol, 37%) as brown wax.

Spectroscopic and physical data for **5i**:
R_f: 0.47 (1:4 EtOAc/pentane). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.81 (d, 1 H, J = 7.4 Hz, H-4′), 7.55 (app d, 1 H, J = 8.3 Hz, H-6), 7.54* (app s, 0.5 H, H-4), 7.48* (app s, 0.5 H, H-4), 7.44–7.39 (m, 2 H, H-6′ and H-7′), 7.33* (d, 0.5 H, J = 7.9 Hz, H-7), 7.27* (d, 0.5 H, J = 7.9 Hz, H-7), 7.13 (t, 1 H, J = 2.5 Hz, H-2′), 6.53 (s, 1 H, H-3′), 4.76–4.69 (m, 4 H, H-1 and H-3), 4.15 (t, 2 H, J = 7.1 Hz, H-1″), 1.85 (qn, 2 H, J = 7.5 Hz, H-2″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.37 (sex, 2 H, J = 7.5 Hz, H-3″), 0.95 (t, 3 H, J = 7.4 Hz, H-4″). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 154.6 (Boc C=O), 142.2* (C-5), 142.2* (C-5), 137.9* (C-3a), 137.5 (C-3a), 135.6 (C-7′a), 135.3* (C-7a), 135.0* (C-7a), 132.5* (C-5′), 132.4* (C-5′), 129.1 (C-2′), 128.6 (C-3′a), 126.8* (C-6), 126.7* (C-6), 122.9* (C-7), 122.7* (C-7), 121.6* (C-4), 121.4* (C-4), 121.2 (C-6′), 119.5 (C-4′), 109.7 (C-7′), 101.3 (C-3′), 79.6 (Boc Cq), 52.4* (C-3), 52.2* (C-1), 52.1* (C-3), 51.8* (C-1), 46.3 (C-1″), 32.4 (C-2″), 28.6 (3 C, Boc $(CH_3)_3$), 20.2 (C-3″), 13.7 (C-4″). HRMS (TOF ASAP+): m/z calcd for $C_{21}H_{23}N_2O_2$ $[M\text{-}C_8H_8+H]^+$: 335.1760; found: 335.1761.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra obtained for **5i** are shown in Appendix K.1-K.6. For structure elucidation and assignment of chemical shifts, see Section 5.2.9.

*Rotamers.

6.3.10 *tert*-Butyl 5-(4-butoxy-2,3,5,6-tetrafluorophenyl)isoindoline-2-carboxylate (5j)



5j

Preparation of the title compund was attempted thrice with **3** and 4-butoxy-2,3,5,6-tetrafluorophenylboronic acid (**4j**). The two first attempts followed the general method for Suzuki cross-coupling, and the third attempt followed a modified version of a procedure described by Knapp, Gillis and Burke.[49] The procedure is described in detail in the experimental section for **5g** (Section 6.3.7 - Attempt 2).

None of the attempts resulted in full conversion of **3**. Attempted purification by flash column chromatography (1:9 and 1:19 EtOAc/*n*-pentane) had no effect for attempts 1 and 2, as ^{1}H NMR-analysis indicated the ratio of **3** to **5j** to be roughly the same. The specifics for each attempt can be seen in Table 6.3.

Table 6.3: Reaction conditions for the three attempts at preparing **5j**. All attempts were run in 1,4-dioxane/H_2O (1:1, 40 mL). Attempt 1 and 2 utilized $Pd(PPh_3)_4$ (5 mol %) as catalyst, and attempt 3 utilized $Pd(OAc)_2$ (5 mol %) and SPhos (10 mol %) as catalyst system. The ratio (molar) of **3** and **5** is estimated from ^{1}H NMR.

Exp. #	Halide **3** [g, mmol]	Boronic acid **4j** [g, mmol]	Base [g, mmol]	Time [h]	Yield [g]	Est. mol ratio **3 : 5j**
1	0.500, 1.68	0.532, 2.20	0.346, 2.50	23	0.375	3.8 : 1
2	0.500, 1.68	0.532, 2.00	0.346, 2.50	168	0.125	1 : 6.9
3	0.412, 1.38	0.441, 1.66	1.173, 5.53	40	0.716	2.7 : 1

Even though full conversion and/or purification was unsuccessful, the major product was confirmed to be **5j** using MS. Extraction of ^{1}H NMR data for **5j** was attempted, using ^{1}H NMR from attempt 2. (Appendix L.2.) The assigned positions are not confirmed by 2D-NMR techniques, but rather based on the NMR-signals from relatively similar compound **5d** (see Section 6.3.4 and Section 5.2.4).

Spectroscopic data for **5j**: ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.39–7.27 (m, 3 H, H-4, H-6 and H-7), 4.74–4.67 (m, 4 H, H-1 and H-3), 4.27 (t, 2 H, J = 6.5 Hz, H-1″), 1.79 (qn, 2 H, J = 6.5 Hz, H-2″), 1.53 (s, 9 H, Boc $(CH_3)_3$), 1.53 (app sex, 2 H, app J = 7.4 Hz, H-3″), 0.99 (t, 3 H, J = 7.4 Hz, H-4″). HRMS (TOF ESI+): m/z calcd for $C_{18}H_{18}NO_3F_4$ $[M\text{-}C_8H_8+H]^+$: 384.1223; found: 384.1222.

^{1}H NMR spectum for each attempt are shown in Appendix L.1-L.3. MS spectrum confirming **5j** to be present is shown in Appendix L.4.

6.4 Carbamate deprotection - Preparation of 6a-g

Carbamate deprotection with hydrochloric acid in MeCN was conducting according to a procedure developed within the Gautun research group. The method is illustrated in Scheme 6.3 and described below.

5

5a	R = 4-CF_3-Ph
5b	R = Naphtalen-2-yl
5c	R = 4-*n*-BuPh
5d	R = 4-(*n*-BuO)Ph
5e	R = 2,4-di-(*n*-PrO)Ph
5f	R = 4-(*i*-PrO)Ph
5g	R = 2,4,6-tri-(*i*-Pr)Ph
5h	R = 1*H*-indol-5-yl
5i	R = 1-butyl-1*H*-indol-5-yl

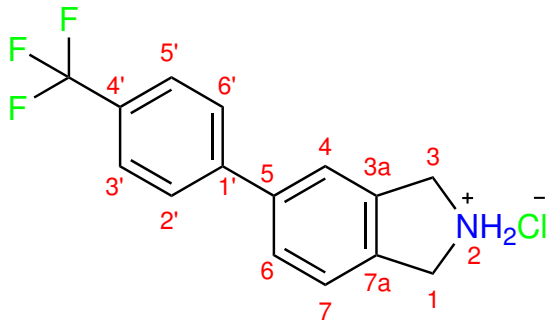
6

6a	R = 4-CF_3-Ph
6b	R = Naphtalen-2-yl
6c	R = 4-*n*-BuPh
6d	R = 4-(*n*-BuO)Ph
6e	R = 2,4-di-(*n*-PrO)Ph
6f	R = 4-(*i*-PrO)Ph
6g	R = 2,4,6-tri-(*i*-Pr)Ph

Scheme 6.3: Reaction scheme illustrating the general method used for carbamate deprotection of **5** with HCl (aq., 37%).

In the general method used for deprotection, the carbamate protected amine **5** was dissolved in MeCN (50-100 mL for every mmol protected amine), followed by dropwise addition of 3-10 eq. of HCl (aq., 37%). The reaction mixture was stirred at room temperature until TLC-analysis indicated full conversion. Evaporation of volatiles and additional drying (0.5 mbar, 60 °C) for two hours, followed by crystallisation from MeOH/Et_2O afforded the deprotected isoindoline **6** as its HCl-salt.

6.4.1 5-(4-(Trifluoromethyl)phenyl)isoindoline HCl (6a)



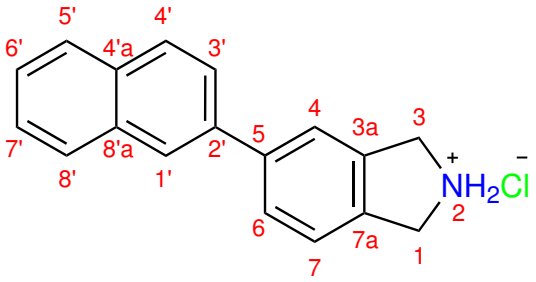
6a

The title compound was prepared from **5a** (0.149 g, 0.410 mmol) in MeCN (30 mL) with ~5 eq. HCl (aq., 37%, 0.17 mL, 2.04 mmol) for 19 hours, according to general method for carbamate deprotection. Work-up afforded **6a** (0.122 g, 0.407 mmol, 99%) as a grey solid.

Spectroscopic and physical data for **6a**:
Mp. >235 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.96 (br s, 2 H, H-2), 7.89 (d, 2 H, J = 8.3 Hz, H-2′ and H-6′), 7.84 (d, 2 H, J = 8.3 Hz, H-3′ and H-5′), 7.78 (s, 1 H, H-4), 7.74 (d, 1 H, J = 7.9 Hz, H-6), 7.54 (d, 1 H, J = 8.0 Hz, H-7), 4.57 (s, 2 H, H-3), 4.56 (s, 2 H, H-1). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 143.6 (C-5), 140.4 (C-1′), 136.3 (C-3a), 135.4 (C-7a), 128.1 (q, $^{2}J_{CF}$ = 31.9 Hz, C-4′), 127.5 (2 C, C-2′ and C-6′), 127.3 (C-6), 125.9 (q, 2 C, $^{3}J_{CF}$ = 3.8 Hz, C-3′ and C-5′), 124.3 (q, $^{1}J_{CF}$ = 271.7 Hz, CF_3), 123.6 (C-7), 121.6 (C-4), 50.0 (C-3), 49.8 (C-1). IR (ATR) (cm^{-1}): 2891 (w), 2701 (w), 2594 (w), 2502 (w), 1617 (w), 1603 (w), 1406 (w), 1324 (s), 1180 (m), 1160 (m), 1107 (s), 1070 (s), 818 (s). HRMS (TOF ASAP+): m/z calcd for $C_{15}H_{13}NF_3$ [M-Cl]$^{+}$: 264.1000; found: 264.1001. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 4.9 min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6a** are shown in Appendix M.1-M.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.1.

6.4.2 5-(Naphthalen-2-yl)isoindoline HCl (6b)



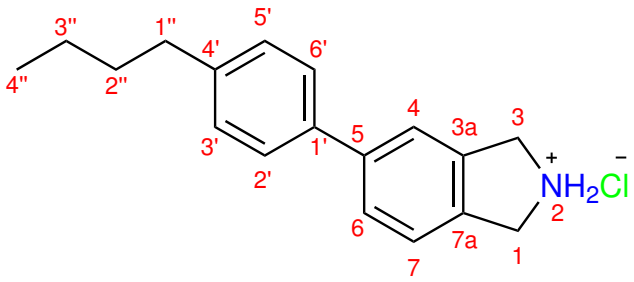
6b

The title compound was prepared from **5b** (0.107 g, 0.310 mmol) in MeCN (30 mL) with ~10 eq. HCl (aq., 37%, 0.26 mL, 3.12 mmol) for 19 hours, according to general method for carbamate deprotection. Work-up afforded **6b** (0.069 g, 0.245 mmol, 79%) as off-white crystals.

Spectroscopic and physical data for **6b**:
Mp. >175 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.84 (br s, 2 H, H-2), 8.23 (s, 1 H, H-1′), 8.03 (d, 1 H, J = 8.6 Hz, H-4′), 8.00 (d, 1 H, J = 7.7 Hz, H-8′), 7.96 (d, 1 H, J = 7.8 Hz, H-5′), 7.86 (app s, 1 H, H-4), 7.85 (dd, 1 H, J = 8.6, 1.5 Hz, H-3′), 7.82 (d, 1 H, J = 8.0 Hz, H-6), 7.59–7.53 (m, 3 H, H-7, H-6′ and H-7′), 4.59 (s, 2 H, H-3), 4.57 (s, 2 H, H-1). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 140.3 (C-5), 136.9 (C-2′), 136.1 (C-3a), 134.4 (C-7a), 133.3 (C-8′a), 132.3 (C-4′a), 128.6 (C-4′), 128.2 (C-8), 127.5 (C-5′), 127.2 (C-6), 126.5 (C-7′), 126.3 (C-6′), 125.4 (C-1′), 125.0 (C-3′), 123.5 (C-7), 121.5 (C-4), 50.1 (C-3), 49.9 (C-1). IR (ATR) (cm^{-1}): 2904 (m), 2706 (m), 2589 (m), 1595 (m), 1495 (m), 1409 (m), 1355 (m), 1271 (w), 884 (w), 862 (m), 813 (s), 755 (m). HRMS (TOF ASAP+): m/z calcd for $C_{18}H_{16}N$ [M-Cl]$^{+}$: 246.1283; found: 246.1278. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.8 min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6b** are shown in Appendix N.1-N.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.2.

6.4.3 5-(4-Butylphenyl)isoindoline HCl (6c)



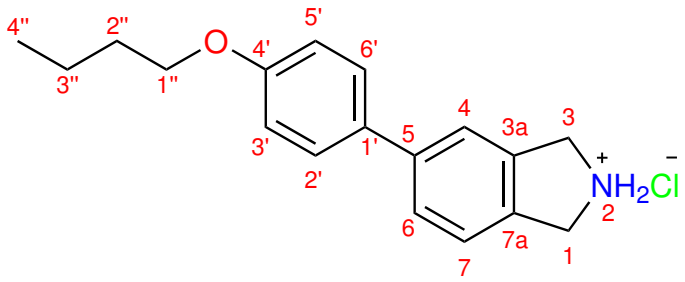
6c

The title compound was prepared from **5c** (0.179 g, 0.509 mmol) in MeCN (20 mL) with ~5 eq. HCl (aq., 37%, 0.21 mL, 2.52 mmol) for 14 hours, according to general method for carbamate deprotection. Work-up afforded **6c** (0.116 g, 0.403 mmol, 79%) as an off-white solid.

Spectroscopic and physical data for **6c**:
Mp. >200 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.40 (br s, 2 H, H-2), 7.67 (s, 1 H, H-4), 7.63 (d, 1 H, J = 8.0 Hz, H-6), 7.57 (d, 2 H, J = 8.1 Hz, H-2′ and H-6′), 7.46 (d, 1 H, J = 7.9 Hz, H-7), 7.29 (d, 2 H, J = 8.1 Hz, H-3′ and H-5′), 4.53 (s, 2 H, H-3), 4.52 (s, 2 H, H-1), 2.62 (t, 2 H, J = 7.7 Hz, H-1″), 1.58 (qn, 2 H, J = 7.6 Hz, H-2″), 1.33 (sex, 2 H, J = 7.5 Hz, H-3″), 0.91 (t, 3 H, J = 7.4 Hz, H-4″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 141.9 (C-4′), 140.4 (C-5), 136.9 (C-3a), 136.1 (C-7a), 134.0 (C-1′), 128.9 (2 C, C-3′ and C-5′), 126.6 (C-6), 126.6 (2 C, C-2′ and C-6′), 123.3 (C-7), 120.9 (C-4), 50.0 (C-3), 49.9 (C-1), 34.4 (C-1″), 33.0 (C-2″), 21.7 (C-3″), 13.8 (C-4″). IR (ATR) (cm^{-1}): 2956 (m), 2913 (s), 2694 (s), 2591 (s), 2495 (m), 2367 (w), 2258 (w), 1593 (m), 1489 (m), 1452 (m), 1427 (m), 1375 (m), 1356 (m), 1048 (w), 921 (w), 881 (m), 813 (s), 802 (s). HRMS (TOF ASAP+): m/z calcd for $C_{18}H_{22}N$ [M-Cl]$^{+}$: 252.1752; found: 252.1748. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 16.6 min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6c** are shown in Appendix O.1-O.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.3.

6.4.4 5-(4-Butoxyphenyl)isoindoline HCl (6d)



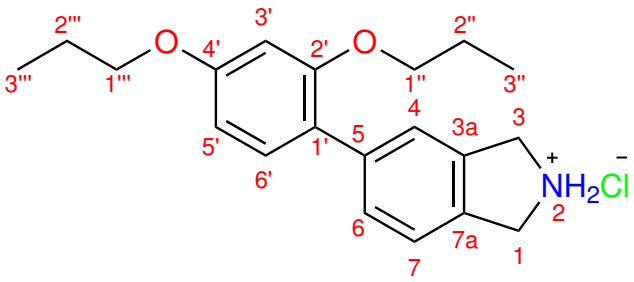
6d

The title compound was prepared from **5d** (0.140 g, 0.381 mmol) in MeCN (30 mL) with ~10 eq. HCl (aq., 37%, 0.32 mL, 3.84 mmol) for 16 hours, according to general method for carbamate deprotection. Work-up afforded **6d** (0.101 g, 0.332 mmol, 87%) as blank crystals.

Spectroscopic and physical data for **6d**:
Mp. 176-177 °C. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 10.01 (br s, 2 H, H-2), 7.64 (s, 1 H, H-4), 7.60–7.57 (m, 3 H, H-6, H-2′ and H-6′), 7.44 (d, 1 H, J = 8.0 Hz, H-7), 7.02 (d, 2 H, J = 8.8 Hz, H-3′ and H-5′)), 4.53 (s, 2 H, H-3), 4.51 (s, 2 H, H-1), 4.01 (t, 2 H, J = 6.5 Hz, H-1″), 1.71 (qn, 2 H, J = 7.4 Hz, H-2″), 1.45 (sex, 2 H, J = 7.4 Hz, H-3″), 0.94 (t, 3 H, J = 7.4 Hz, H-4″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 158.5 (C-4′), 140.1 (C-5), 135.9 (C-3a), 133.4 (C-7a), 131.7 (C-1′), 127.8 (2 C, C-2′ and C-6′), 126.3 (C-6), 123.3 (C-7), 120.6 (C-4), 114.9 (2 C, C-3′ and C-5′), 67.2 (C-1″), 49.9 (C-3), 49.7 (C-1), 30.7 (C-2″) 18.7 (C-3″), 13.7 (C-4″). IR (ATR) (cm^{-1}): 2930 (m), 2910, (m), 2727 (m), 2631 (m), 2605 (m), 1604 (m), 1519 (m), 1486 (m), 1466 (m), 1244 (s), 1179 (m), 1123 (m), 1058 (m), 971 (m), 885 (s). HRMS (TOF ASAP+): m/z calcd for $C_{18}H_{22}NO$ [M-Cl]$^{+}$: 268.1701; found: 268.1697. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 10.3 min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6d** are shown in Appendix P.1-P.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.4.

6.4.5 5-(2,4-Dipropoxyphenyl)isoindoline HCl (6e)



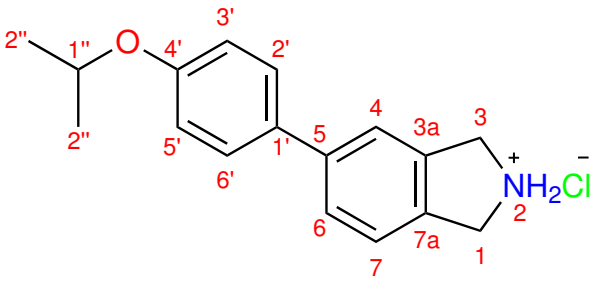
6e

The title compound was prepared from **5e** (0.238 g, 0.578 mmol) in MeCN (50 mL) with ~5 eq. HCl (aq., 37%, 0.25 mL, 3.00 mmol) for 14 hours, according to general method for carbamate deprotection. Work-up afforded **6e** (0.169 g, 0.486 mmol, 84%) as blank crystals.

Spectroscopic and physical data for **6e**:
^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.81 (br s, app 2 H, H-2), 7.45–7.43 (m, 2 H, H-4 and H-6), 7.39 (d, 1 H, J = 7.9 Hz, H-7), 7.19 (d, 1 H, J = 8.4 Hz, H-6′), 6.63 (d, 1 H, J = 2.2 Hz, H-3′), 6.59 (dd, 1 H, J = 6.1, 2.3 Hz, H-5′), 4.51 (app s, 2 H, H-33), 4.50 (app s, 2 H, H-1), 3.96 (app t, 2 H, J = 6.5 Hz, H-1‴), 3.94 (app t, 2 H, J = 6.3 Hz, H-1″), 1.74 (sex, 2 H, J = 7.1 Hz, H-2‴), 1.65 (sex, 2 H, J = 7.1 Hz, H-2″), 0.99 (t, 3 H, J = 7.4 Hz, H-3‴), 0.92 (t, 3 H, J = 7.4 Hz, H-3″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 159.6 (C-4′), 156.4 (C-2′), 138.3 (C-5), 134.9 (C-3a), 133.0 (C-7a), 130.9 (C-6′), 129.2 (C-6), 123.3 (C-4), 122.2 (C-7), 121.7 (C-1′), 105.9 (C-5′), 100.1 (C-3′), 69.3 (C-1″), 69.1 (C-1‴), 50.0 (C-3), 49.9 (C-1), 22.1 (C-2‴), 22.0 (C-2″), 10.6 (C-3″), 10.4 (C-3‴). IR (ATR) (cm^{-1}): 2962 (m), 2935 (m), 2875 (m), 2722 (m), 2582 (m), 1607 (s), 1514 (w), 1488 (m), 1466 (m), 1412 (m), 1298 (m), 1279 (s), 1244 (m), 1180 (s), 1128 (m), 1012 (m), 860 (w), 829 (m), 796 (m). HRMS (TOF ASAP+): m/z calcd for $C_{20}H_{26}NO_2$ [M-Cl]$^+$: 312.1964; found: 312.1959. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 16.5 min, 98% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6e** are shown in Appendix Q.1-Q.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.5.

6.4.6 5-(4-Isopropoxyphenyl)isoindoline HCl (6f)



6f

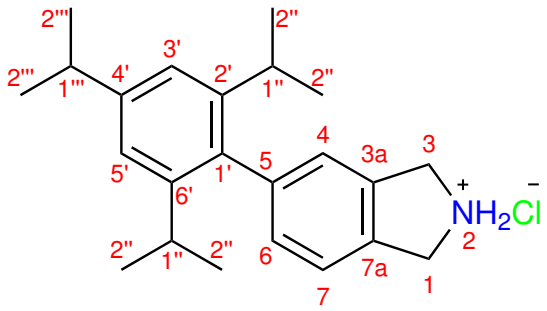
The title compound was prepared from **5f** (0.219 g, 0.620 mmol) in MeCN (50 mL) with ~5 eq. HCl (aq., 37%, 0.26 mL, 3.12 mmol) for 14 hours, according to general method for carbamate deprotection. Work-up afforded **6f** (0.120 g, 0.414 mmol, 67%) as blank crystals.

Spectroscopic and physical data for **6f**:
^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.87 (br s, 2 H, H-2), 7.67 (s, 1 H, H-4), 7.63 (d, 1 H, J = 7.9 Hz, H-6), 7.58 (d, 2 H, J = 8.2 Hz, H-2′ and H-6′), 7.47 (d, 1 H, J = 7.9 Hz, H-7), 7.35 (d, 2 H, J = 8.2 Hz, H-3′ and H-5′), 4.54 (s, 2 H, H-3), 4.52 (s, 2 H, H-1), 2.93 (sep, 1 H, J = 6.9 Hz, H-1″), 1.23 (d, 6 H, J = 6.9 Hz, H-2″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 148.0 (C-4′), 140.4 (C-5), 137.1 (C-1′), 136.0 (C-3a), 134.0 (C-7a), 126.9 (2 C, C-3′ and C-5′), 126.7 (2 C, C-2′ and C-6′), 126.7 (C-6), 123.4 (C-7), 121.0 (C-4), 50.0 (C-3), 49.9 (C-1), 33.1 (C-1″), 23.8 (2 C, C-2″). IR (ATR) (cm^{-1}): 2888 (m), 2809 (m),2696 (m), 2591 (m), 2497 (w), 2459 (w), 2368 (w), 2260 (w), 1594 (m), 1488 (m), 1425 (m), 1331 (m), 920 (m), 881 (w), 851 (s). HRMS (TOF ASAP+): m/z calcd for $C_{17}H_{20}NO$ [M-Cl]$^{+}$: 254.1545; found: 254.1540. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 8.5 min, 77% pure[ii].

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **6f** are shown in Appendix R.1-R.8. For structure elucidation and assignment of chemical shifts, see Section 5.3.6.

[ii]HPLC-analysis occurred more than one month after NMR-analysis. **6f** might initially have been pure, not stable over time. Decomposition is also the reason no melting point was measured.

6.4.7 5-(2,4,6-Triisopropylphenyl)isoindoline HCl (6g)



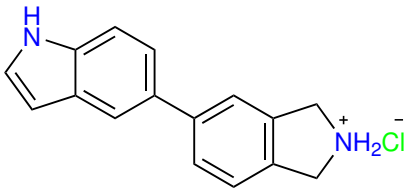
6g

The title compound was prepared from **5g** (0.250 g, 0.592 mmol) in MeCN (40 mL) with ~3 eq. HCl (aq., 37%, 0.15 mL, 1.80 mmol) for 21 hours, according to general method for carbamate deprotection. As TLC-analysis (SiO_2, EtOAc/*n-pentane* 1:9, R_f = 0.51) still showed unreacted **5g**, additional ~5 eq. HCl (0.25 mL, 3.00 mmol) was added. After 23 hours the no trace of **5g** could be observed. Work-up afforded **6g** (0.178 g, 0.497 mmol, 84%) as as a white solid.

Spectroscopic and physical data for **6g**:
^{1}H NMR (600 MHz, DMSO): δ (ppm) 9.76 (br s, 2 H, H-2), 7.45 (d, 1 H, J = 7.7 Hz, H-7), 7.21 (s, 1 H, H-4), 7.14 (d, 1 H, J = 7.7 Hz, H-6), 7.06 (s, 2 H, H-3′ and H-5′), 4.57 (s, 2 H, H-3), 4.55 (s, 2 H, H-1), 2.90 (sep, 1 H, J = 6.9 Hz, H-1‴), 2.46 (sep, 2 H, J = 6.9 Hz, H-1″), 1.24 (d, 6 H, J = 7.0 Hz, H-2‴), 1.02 (dd, 12 H, J = 11.5, 4.6 Hz, H-2″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 147.8 (C-4′), 145.8 (2 C, C-2′ and C-6′), 140.4 (C-5), 136.1 (C-1′), 135.1 (C-3a), 133.6 (C-7a), 129.5 (C-6), 123.7 (C-4), 122.7 (C-7), 120.2 (2 C, C-3′ and C-5′), 50.1 (C-3), 50.0 (C-1), 33.6 (C-1‴), 29.8 (2 C, C-1″), 24.0 (C-2‴), 24.0 (C-2‴), 23.9 (4 C, C-2″). HRMS (TOF ASAP+): m/z calcd for $C_{23}H_{32}N$ [M-Cl]$^+$: 322.2535; found: 322.2537. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 79.1 min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra along with HPLC chromatogram obtained for **6g** are shown in Appendix S.1-S.7. For structure elucidation and assignment of chemical shifts, see Section 5.3.7.

6.4.8 5-(1*H*-Indol-5-yl)isoindoline HCl (6h)



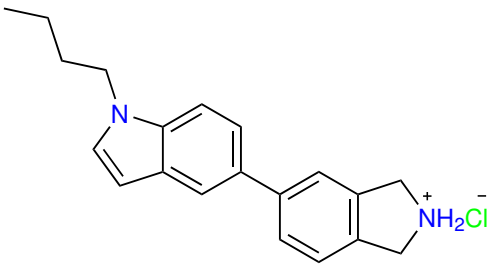
6h

The title compound was attempted prepared from **5h** (0.200 g, 0.598 mmol) in MeCN (20 mL) with ~5 eq. HCl (aq., 37%, 0.25 mL, 3.00 mmol) for 24 hours, according to general method for carbamate deprotection, resulting in a brown solid (0.151 g). Even though MS confirmed **6h** to be present in the product mixture, ^{1}H NMR-analysis indicated a mixture of products. Additional attempts of crystallisation with MeOH/Et_2O had no effect.

Spectroscopic data for **6h**:
HRMS (TOF ESI+): *m/z* calcd for $C_{16}H_{15}N_2$ [M-Cl]$^+$: 235.1235; found: 235.1233.

^{1}H NMR spectrum for the product mixture is shown in Appendix T.1. The MS spectrum confirming **6h** to be present is shown in Appendix T.2.

6.4.9 5-(1-Butyl-1*H*-indol-5-yl)isoindoline HCl (6i)



6i

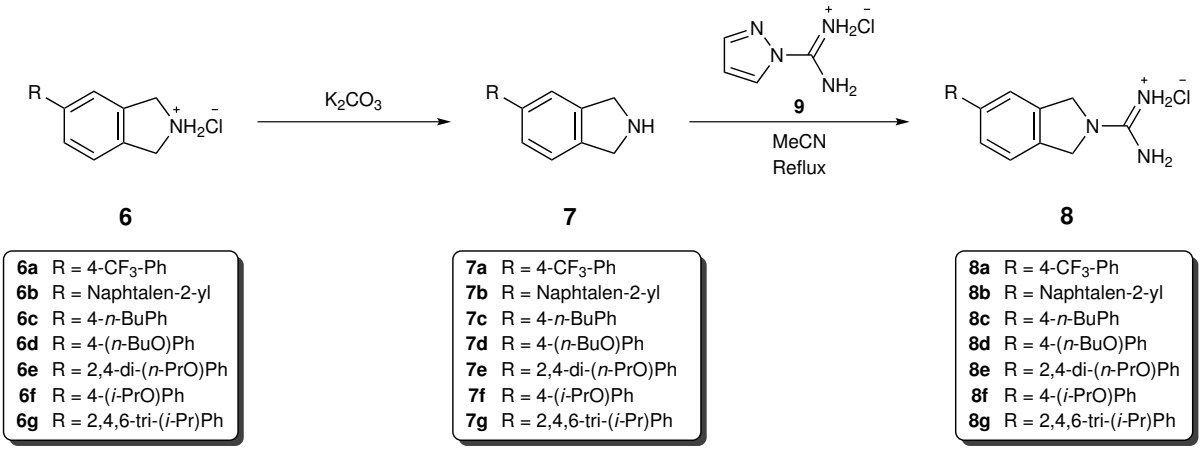
The title compound was attempted prepared from **5i** (0.150 g, 0.384 mmol) in MeCN (20 mL) with ~3 eq. HCl (aq., 37%, 0.10 mL, 1.20 mmol) for 24 hours, according to general method for carbamate deprotection, resulting in a tan solid (0.145 g). ^{1}H NMR-analysis was blurred and inconclusive. The product mixture seemed slightly soluble in both DMSO and MeOD but not soluble enough to produce any spectra of use. The clearest spectrum obtained for the mixture is shown in Appendix T.1 (600 MHz, MeOD). MS confirmed **6i** to be present, however additional attempts at purification had no noticeable effect.

Spectroscopic data for **6i**:
HRMS (TOF ESI+): *m/z* calcd for $C_{20}H_{23}N_2$ [M-Cl]$^+$: 291.1861; found: 291.1864.

The MS spectrum confirming **6i** to be present is shown in Appendix U.2.

6.5 Preparation of guanidines 8a-g

The general method for preparation of guanidines was a two step process. The first step prepared the free amine utilizing an aqueous saturated solution of K_2CO_3 followed by extraction. The second step utilized 1*H*-pyrazole carboxamidine HCl (**9**) as a guanylation agent, a method originally presented by Bernatowicz *et al.*[45] and recently modified by Bakka and Gautun.[28] Additional modifications were made to the work-up procedure. The general method is illustrated in Scheme 6.4 and described below.

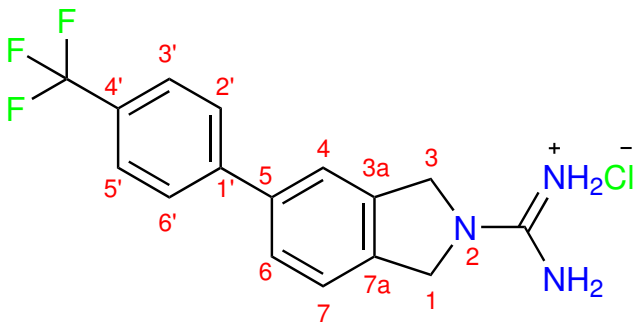


Scheme 6.4: Reaction scheme illustrating the general method used for synthesis of guanidine **8** from isoindoline HCl-salt **6** via free amine **7**.

In the general method used the isoindoline HCl-salt **6** (35-100 mg) was dissolved in an saturated aqueous solution of K_2CO_3 (15-40 mL) and extracted with EtOAc (3-5 × 25 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure, yielding the free amine **7**.

The free amine **7** was then dissolved in MeCN (3-6 mL), followed by addition of 0.85-0.99 eq. of 1*H*-pyrazole carboxamidine HCl (**9**). The reaction mixture was heated at reflux until TLC-analysis (SiO_2, Chloroform/Methanol/Ammonia 70:30:3) indicated complete disappearance of **9**. After cooling, excess solvent was removed by filtration and the precipitate was washed with cold MeCN (1-3 × 2-3 mL) and Et_2O (3-5 × 5 mL). The crude precipitate was then crystallized from MeOH/Et_2O, affording guanidine **8** as its HCl-salt.

6.5.1 5-(4-(Trifluoromethyl)phenyl)isoindoline-2-carboximidamide HCl (8a)



8a

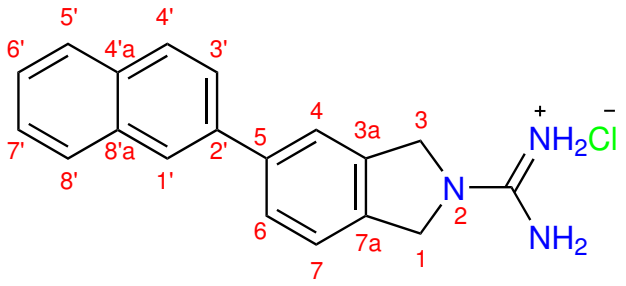
The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6a** (0.100 g, 0.334 mmol) with K_2CO_3 (sat. aq., 25 mL) afforded free amine **7a** (0.066 g, 0.251 mmol, 75%) as a blank oil.

Free amine **7a** (0.065 g, 0.247 mmol, 1.00 eq.), **9** (0.034 g, 0.232 mmol, ~0.94 eq.) and MeCN (5 mL) were refluxed for 5 hours. After cooling to room temperature, work-up afforded guanidine **8a** (0.064 g, 0.187 mmol, 80%) as a white solid.

Spectroscopic and physical data for **8a**:
Mp. >250 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.92 (d, 2 H, J = 8.2 Hz,H-2′ and H-6′), 7.83 (d, 2 H, J = 8.2 Hz, H-3′ and H-5′), 7.75 (s, 1 H, H-4), 7.73 (d, 1 H, J = 8.0 Hz, H-6), 7.57 (br s, 4 H, Guanyl), 7.25 (d, 1 H, J = 8.0 Hz, H-7), 4.82 (app s, 2 H, H-3), 4.81 (app s, 2 H, H-1). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 155.0 (Guanyl C_q), 143.7 (C-5), 138.4 (C-1′), 136.4 (C-3a), 135.6 (C-7a), 128.0 (q, $^2J_{CF}$ = 31.9 Hz, C-4′), 127.6 (2 C, C-2′ and C-6′), 126.8 (C-6), 125.8 (q, 2 C, $^3J_{CF}$ = 3.9 Hz, C-3′ and C-5′), 124.3 (q, $^1J_{CF}$ = 272.3 Hz, CF_3), 123.4 (C-7), 121.4 (C-4), 52.8 (C-3), 52.7 (C-1), IR (ATR) (cm^{-1}): 3390 (w), 3322 (w), 3132 (m), 1628 (s), 1457 (m), 1322 (s), 1171 (m), 1125 (s), 1069 (s), 1013 (m), 850 (m), 816 (s). HRMS (TOF ASAP+): m/z calcd for $C_{16}H_{15}N_3F_3$ [M-Cl]$^+$: 306.1218; found: 306.1219. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 8.5 min, 98% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **8a** are shown in Appendix V.1-V.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.1.

6.5.2 5-(Naphthalen-2-yl)isoindoline-2-carboximidamide HCl (8b)



8b

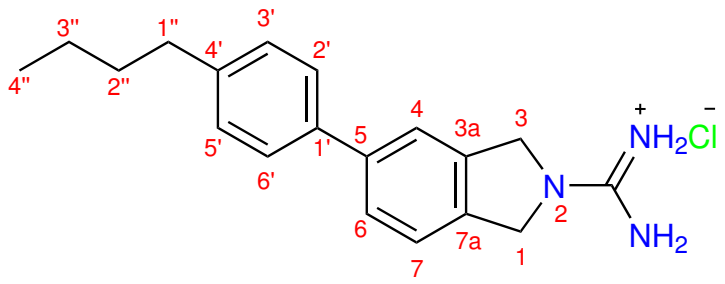
The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6b** (0.041 g, 0.146 mmol) with K_2CO_3 (sat. aq., 30 mL) afforded free amine **7b** (0.035 g, 0.143 mmol, 98%) as a yellow oil.

Free amine **7b** (0.035 g, 0.143 mmol, 1.00 eq.), **9** (0.020 g, 0.136 mmol, ~0.95 eq.) and MeCN (5 mL) were refluxed for 17 hours. After cooling to −18 °C for 2 hours, work-up afforded guanidine **8b** (0.021 g, 0.065 mmol, 48%) as orange crystals.

Spectroscopic and physical data for **6b**:
Mp. >175 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 8.26 (s, 1 H, H-1′), 8.02 (app d, 1 H, J = 8.6 Hz, H-4′), 8.00 (app d, 1 H, J = 7.7 Hz, H-8′), 7.96 (d, 1 H, J = 7.6 Hz, H-5′), 7.87 (dd, 1 H, J = 8.5, 1.8 Hz, H-3′), 7.82 (app s, 1 H, H-4), 7.82 (app d, 1 H, J = 8.0 Hz, H-6), 7.56–7.51 (m, 7 H, H-7, H-6′, H-7′ and Guanyl x4), 4.85 (s, 2 H, H-3), 4.82 (s, 2 H, H-1). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 155.0 (Guanyl C_q), 139.8 (C-5), 137.0 (C-2′), 136.2 (C-3a), 134.6 (C-7a), 133.3 (C-8′a), 132.3 (C-4′a), 128.5 (C-4′), 128.2 (C-8a), 127.5 (C-5′), 126.8 (C-6), 126.5 (C-7′), 126.2 (C-6′), 125.4(C-1′), 125.1 (C-3′), 123.3 (C-7), 121.2 (C-4), 52.9 (C-3), 52.7 (C-1). IR (ATR) (cm^{-1}): 3115 (m), 3018 (m), 2970 (m), 1738 (m), 1628 (s), 1576 (m), 1497 (m), 1448 (m), 1370 (s), 1217 (m), 1081 (w), 806 (s), 741 (m). HRMS (ESI+): m/z calcd for $C_{19}H_{18}N_3$ [M-Cl]$^+$: 288.1501; found: 288.1500. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 10.9 min, 95% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **8b** are shown in Appendix W.1-W.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.2.

6.5.3 5-(4-Butylphenyl)isoindoline-2-carboximidamide HCl (8c)



8c

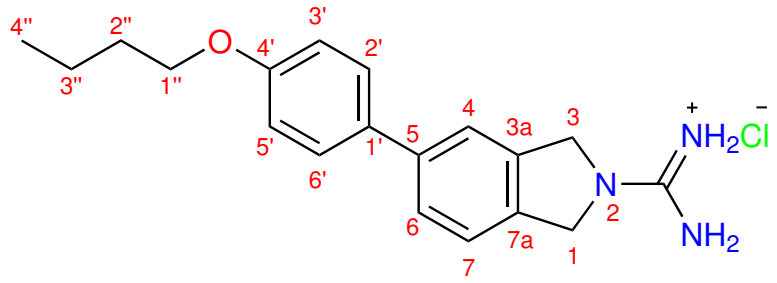
The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6c** (0.090 g, 0.313 mmol) with K_2CO_3 (sat. aq., 20 mL) afforded free amine **7c** (0.074 g, 0.294 mmol, 94%) as a yellow oil.

Free amine **7c** (0.073 g, 0.290 mmol, 1.00 eq.), **9** (0.038 g, 0.259 mmol, ~0.89 eq.) and MeCN (4 mL) were refluxed for 5 hours. After cooling to room temperature, work-up afforded guanidine **8c** (0.062 g, 0.188 mmol, 73%) as white crystals.

Spectroscopic and physical data for **8c**:
Mp. >245 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.63–7.62 (m, 2 H, H-4 and H-6), 7.59 (d, 2 H, J = 8.0 Hz, H-2′ and H-6′), 7.55 (br s, 4 H, Guanyl), 7.44 (d, 1 H, J = 8.4 Hz, H-7), 7.29 (d, 2 H, J = 8.0 Hz, H-3′ and H-5′), 4.80 (s, 2 H, H-3), 4.78 (s, 2 H, H-1), 2.62 (t, 2 H, J = 7.6 Hz, H-1″), 1.58 (qn, 2 H, J = 7.6 Hz, H-2″), 1.33 (sex, 2 H, J = 7.5 Hz, H-3″), 0.91 (t, 3 H, J = 7.4 Hz, H-4″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 155.0 (Guanyl C_q), 141.9 (C-4′), 140.0 (C-5), 137.1 (C-3a), 136.1 (C-7a), 134.1 (C-1′), 128.9 (2 C, C-3′ and C-5′), 126.6 (2 C, C-2′ and C-6′), 126.3 (C-6), 123.1 (C-7), 120.7 (C-4), 52.9 (C-3), 52.6 (C-1), 34.4 (C-1″), 33.1 (C-2″), 21.7 (C-3″), 13.8 (C-4″). IR (ATR) (cm^{-1}): 3319 (m), 3148 (m), 2956 (m), 2928 (m), 2858 (m), 2427 (w), 1635 (s), 1582 (m), 1493 (w), 1458 (m), 1370 (w), 1078 (w), 810 (m). HRMS (TOF ASAP+): m/z calcd for $C_{19}H_{24}N_3$ [M-Cl]$^+$: 294.1970; found: 294.1966. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 35.1 min, 98% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **8c** are shown in Appendix X.1-X.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.3.

6.5.4 5-(4-Butoxyphenyl)isoindoline-2-carboximidamide HCl (8d)



8d

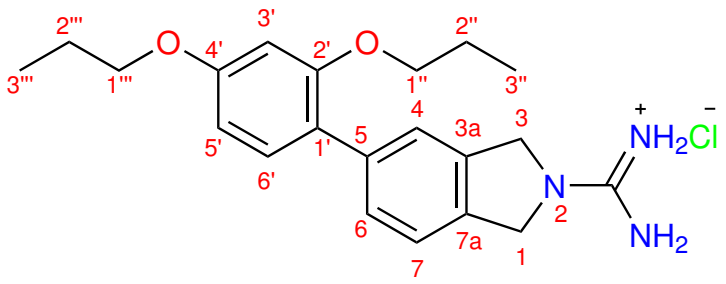
The title compound was prepared as described in the general method for guanylation; however the procedure required some modification because of problems with the first step. Isoindoline HCl-salt **6d** (0.101 g, 0.332 mmol) with K_2CO_3 (sat. aq., 40 mL) gave a tan wax (46 mg), which in a matter of minutes after evaporation of EtOAc turned to dark brown/black, and is assumed to be **7d** decomposing.

As **7d** seemed to be unstable, some modifications were made to the general procedure. Isoindoline HCl-salt **6d** (0.042 g, 0.138 mmol) was dissolved in K_2CO_3 (sat. aq., 15 mL), followed by addition of EtOAc (25 mL) and allowed to stir for 10 minutes. The organic phase was separated, and the water phase extracted with additional EtOAc (5 × 25 mL). The combined organic phases were dried over Na_2SO_4 and concentrated carefully under reduced pressure. The resulting white residue was immediately put under Ar-atm, followed by addition of **9** (0.020 g, 0.136 mmol) and MeCN (5 mL). After 22 hours at reflux, TLC-analysis (SiO_2, Chloroform/Methanol/Ammonia 70:30:3, $R_f = 0.83$) showed no trace of the dot assumed to belong to **7d**. The reaction mixture was cooled at −18 °C for 3 hours. excess solvent removed by filtration an the residue was washed with MeCN (~3 mL) and Et_2O (10-15 mL). Crystallisation with MeOH/H_2O afforded guanidine **8d** (0.012 mg, 0.035 mmol, 25% from **6d**) as a lightly red solid.

Spectroscopic and physical data for **8d**:
Mp. >240 °C (decomp.). ^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.60–7.59 (m, 4 H, H-4, H-6, H-2′ and H-6′), 7.47 (br s, 4 H, Guanyl), 7.42 (d, 1 H, $J = 8.4$ Hz, H-7), 7.02 (d, 2 H, $J = 8.6$ Hz, H-3′ and H-5′), 4.79 (s, 2 H, H-3), 4.77 (s, 2 H, H-1), 4.01 (t, 2 H, $J = 6.5$ Hz, H-1″), 1.72 (p, 2 H, $J = 6.8$ Hz, H-2″), 1.45 (h, 2 H, $J = 7.4$ Hz, H-3), 0.95 (t, 3 H, $J = 7.4$ Hz, H-4″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 158.5 (C-4′), 154.9 (Guanyl C_q), 139.8 (C-5), 136.0 (C-3a), 133.6 (C-7a), 131.9 (C-1′), 127.9 (C-2′ and C-6′), 126.0 (C-6), 123.1 (C-7), 120.3(C-4), 114.9 (C-3′ and C-5′), 67.2 (C-1″), 52.9 (C-3), 52.7 (C-1), 30.7 (C-2″) 18.7 (C-3″), 13.7 (C-4″). IR (ATR) (cm^{-1}): 3394 (m), 3326 (m), 3194 (w), 3129 (m), 2955 (w), 2934 (w), 2865 (w), 1625 (s), 1493 (s), 1460 (s), 1369 (m), 1247 (s), 1225 (s), 1181 (s), 1031 (m), 975 (m), 811 (s). HRMS (TOF ASAP+): m/z calcd for $C_{19}H_{24}N_3O$ [M-Cl]$^+$: 310.1919; found: 310.1916. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, $\lambda = 214$ nm): $t_R = 23.6$ min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **8d** are shown in Appendix Y.1-Y.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.4.

6.5.5 5-(2,4-Dipropoxyphenyl)isoindoline-2-carboximidamide HCl (8e)



8e

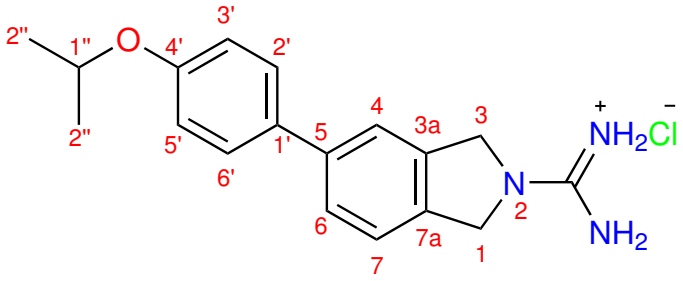
The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6e** (0.090 g, 0.259 mmol) with K_2CO_3 (sat. aq., 30 mL) afforded free amine **7e** (0.079 g, 0.254 mmol, 98%) as an yellow oil.

Free amine **7e** (0.079 g, 0.254 mmol, 1.00 eq.), **9** (0.035 g, 0.239 mmol, ~0.94 eq.) and MeCN (5 mL) were refluxed for 6 hours. After cooling to room temperature, work-up afforded guanidine **8e** (0.056 g, 0.144 mmol, 60%) as white crystals.

Spectroscopic and physical data for **8e**:
^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.48 (br s, 4 H, Guanyl), 7.44 (app d, 1 H, app J = 8.3 Hz, H-6), 7.43 (s, 1 H, H-4), 7.37 (d, 1 H, J = 8.3 Hz, H-7), 7.21 (d, 1 H, J = 8.3 Hz, H-6′), 6.63 (d, 1 H, J = 2.1 Hz, H-3′), 6.60 (dd, 1 H, J = 8.4, 2.3 Hz, H-5′), 4.77 (s, 4 H,H-1 and H-3), 3.97 (app t, 2 H, app J = 6.6 Hz, H-1‴), 3.94 (app t, 2 H, app J = 6.3 Hz, H-1″), 1.74 (sep, 2 H, J = 7.1 Hz, H-2‴), 1.65 (sep, 2 H, J = 7.1 Hz, H-2″), 0.99 (t, 3 H, J = 7.4 Hz, H-3‴), 0.93 (t, 3 H, J = 7.4 Hz, H-3″), ^{13}C NMR (150 MHz, DMSO): δ (ppm) 160.1 (C-4′), 156.9 (C-2′), 155.5 (Guanyl C_q), 138.4 (C-5), 135.4 (C-3a), 133.6 (C-7a), 131.4 (C-6′), 129.3 (C-6), 123.6 (C-4), 122.6 (C-7), 122.3 (C-1′), 106.4 (C-5′), 100.5 (C-3′), 69.8 (C-1″), 69.6 (C-1‴), 53.4 (C-3), 53.2 (C-1), 22.5 (C-2‴), 22.5 (C-2″), 11.1 (C-3″), 10.9 (C-3‴). IR (ATR) (cm^{-1}): 3309 (w), 3114 (m), 2963 (w), 2933 (w), 2872 (w), 1626 (s), 1607 (s), 1579 (s), 1462 (m), 1416 (m), 1282 (m), 1246 (m), 1180 (m), 1127 (m), 1047 (s), 1012 (m), 976 (m), 821 (m), 799 (m). HRMS (TOF ASAP+): m/z calcd for $C_{21}H_{28}N_3O_2$ [M-Cl]$^+$: 354.2182; found: 354.2176. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 31.2 min, 98% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra along with HPLC chromatogram obtained for **8e** are shown in Appendix Z.1-Z.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.5.

6.5.6 5-(4-Isopropoxyphenyl)isoindoline-2-carboximidamide HCl (8f)



8f

The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6f** (0.080 g, 0.276 mmol) with K_2CO_3 (sat. aq., 30 mL) afforded free amine **7f** (0.067 g, 0.264 mmol, 96%) as a yellow solid.

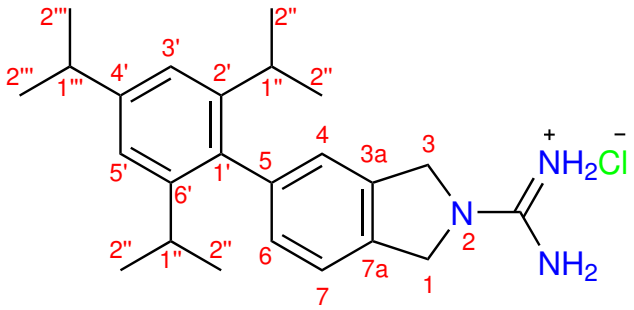
Free amine **7f** (0.065 g, 0.257 mmol, 1.00 eq.), **9** (0.032 g, 0.218 mmol, ~0.85 eq.) and MeCN (5 mL) were refluxed for 6 hours. After cooling to room temperature, work-up afforded guanidine **8f** (0.068 g, 0.205 mmol, 94%) as tan crystals.

Spectroscopic and physical data for **8f**:
^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.63–7.62 (m, 2 H, H-4 and H-6), 7.60 (d, 2 H, J = 8.2 Hz, H-2′ and H-6′), 7.52 (br s, 4 H, Guanyl), 7.45 (d, 1 H, J = 8.5 Hz, H-7), 7.34 (d, 2 H, J = 8.2 Hz, H-3′ and H-5′), 4.80 (app s, 2 H, H-3), 4.78 (app s, 2 H, H-1), 2.94 (sep, 1 H, J = 6.9 Hz, H-1″), 1.24 (d, 6 H, J = 6.9 Hz, H-2″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 155.0 (Guanyl C_q), 147.9 (C-4′), 140.1 (C-5), 137.3 (C-1′), 136.0 (C-3a), 134.1 (C-7a), 126.9 (2 C, C-3′ and C-5′), 126.7 (2 C, C-2′ and C-2′), 126.3 (C-6), 123.1 (C-4), 120.7 (C-7), 52.9 (C-3), 52.7 (C-1), 33.1 (C-1″), 23.8 (C-2″). IR (ATR) (cm^{-1}): 3314 (m), 3127 (m), 2956 (m), 2865 (w), 1622 (s), 1580 (s), 1455 (m), 1366 (m), 812 (s). HRMS (ESI+): m/z calcd for $C_{18}H_{22}N_3O$ [M-Cl]$^+$: 296.1763; found: 296.1761. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 17.1 min, 37% pure[iii].

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra along with HPLC chromatogram obtained for **8f** are shown in Appendix AA.1-AA.8. For structure elucidation and assignment of chemical shifts, see Section 5.4.6.

[iii]HPLC-analysis occurred more than one month after NMR-analysis. **8f** might initially have been pure, not stable over time. Decomposition is also the reason no melting point was measured.

6.5.7 5-(2,4,6-Triisopropylphenyl)isoindoline-2-carboximidamide HCl (8g)



8g

The title compound was prepared in two steps as described in the general method for guanylation. Isoindoline HCl-salt **6g** (0.090 g, 0.251 mmol) with K_2CO_3 (sat. aq., 20 mL) afforded free amine **7g** (0.077 g, 0.239 mmol, 95%) as a tan solid.

Free amine **7g** (0.073 g, 0.227 mmol, 1.00 eq.), **9** (0.032 g, 0.218 mmol, ~0.96 eq.) and MeCN (3 mL) were refluxed for 8 hours. After cooling to room temperature, work-up afforded guanidine **8g** (0.059 g, 0.147 mmol, 67%) as white crystals.

Spectroscopic and physical data for **8g**:
Mp. *Kommer*. ^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.48 (br s, 4 H, Guanyl), 7.43 (d, 1 H, $J = 7.7$ Hz, H-7), 7.19 (s, 1 H, H-4), 7.14 (d, 1 H, $J = 7.7$ Hz, H-6), 7.06 (s, 2 H, H-3′ and H-5′), 4.82 (s, 2 H, H-3), 4.79 (s, 2 H, H-1), 2.90 (sep, 1 H, $J = 6.9$ Hz, H-1‴), 2.49 (sep, 2 H, $J = 6.9$ Hz, H-1″), 1.24 (d, 6 H, $J = 6.9$ Hz, H-2‴), 1.03 (dd, 12 H, $J = 9.9, 2.9$ Hz, H-2″). ^{13}C NMR (150 MHz, DMSO): δ (ppm) 155.0 (Guanyl C_q), 147.7 (C-4′), 145.9 (2 C, C-2′ and C-6′), 140.0 (C-5), 136.2 (C-1′), 135.2 (C-3a), 133.7 (C-7a), 129.1 (C-6), 123.4 (C-4), 122.4 (C-7), 120.2 (2 C, C-3′ and C-5′), 52.8 (C-3), 52.8 (C-1), 33.6 (C-1‴), 29.8 (2 C, C-1″), 24.0 (C-2‴), 24.0 (C-2‴), 23.9 (4 C, C-2″). HRMS (ESI+): *m/z* calcd for $C_{24}H_{34}N_3$ $[M\text{-}Cl]^+$: 364.2753; found: 364.2754. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, $\lambda = 214$ nm): $t_R = 133.2$ min, 99% pure.

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and MS spectra along with HPLC chromatogram obtained for **8g** are shown in Appendix AB.1-AB.7. For structure elucidation and assignment of chemical shifts, see Section 5.4.7.

References

[1] Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311–335.

[2] So, A. D.; Gupta, N.; Cars, O. *BMJ* **2010**, *340*, c2071–c2071.

[3] Aminov, R. I. *Front. Microbiol.* **2010**, *1*, 134.

[4] Powers, J. *Clin. Microbiol. Infect.* **2004**, *10*, 23–31.

[5] Opar, A. *Nat. Rev. Drug. Disc.* **2007**, *6*, 943–944.

[6] Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. *Clin. Infect. Dis.* **2009**, *48*, 1–12.

[7] Slee, A.; Wuonola, M.; McRipley, R.; Zajac, I.; Zawada, M.; Bartholomew, P.; Gregory, W.; Forbes, M. *Antimicrob. Agents Chemother.* **1987**, *31*, 1791–1797.

[8] Tally, F. P.; DeBruin, M. F. *Antimicrob. Agents Chemother.* **2000**, *46*, 523–526.

[9] Howard, S. J.; Catchpole, M.; Watson, J.; Davies, S. C. *Lancet Infect. Dis.* **2013**, *13*, 1001–1003.

[10] Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A. K.; Wertheim, H. F.; Sumpradit, N.; Vlieghe, E.; Hara, G. L.; Gould, I. M.; Goossens, H. *Lancet Infect. Dis.* **2013**, *13*, 1057–1098.

[11] Review of animicrobial resistance. `https://amr-review.org/background.html`, Accessed 16th of June 2017.

[12] Kohanski, M. A.; Dwyer, D. J.; Collins, J. J. *Nat. Rev. Microbiol.* **2010**, *8*, 423–435.

[13] Lowy, F. D. *J. Clin. Invest.* **2003**, *111*, 1265–1273.

[14] Findlay, B.; Zhanel, G. G.; Schweizer, F. *Am. Soc. Microbiol.* **2010**, *54*, 4049–4058.

[15] Hickey, S. M.; Ashton, T. D.; Khosa, S. K.; Robson, R. N.; White, J. M.; Li, J.; Nation, R. L.; Heidi, Y. Y.; Elliott, A. G.; Butler, M. S. *Org. Biomol. Chem.* **2015**, *13*, 6225–6241.

[16] Haug, B. E.; Stensen, W.; Stiberg, T.; Svendsen, J. S. *J. Med. Chem.* **2004**, *47*, 4159–4162.

[17] Steiner, H.; Hultmark, D.; Engström, Å.; Bennich, H.; Boman, H. G. *Nature* **1981**, *292*, 246–248.

[18] Giuliani, A.; Pirri, G.; Nicoletto, S. *Open Life Sci.* **2007**, *2*, 1–33.

[19] Strøm, M. B.; Svendsen, J. S.; Rekdal, Ø. *J. Pept. Res.* **2000**, *56*, 265–274.

[20] Tadesse, M.; Strøm, M. B.; Svenson, J.; Jaspars, M.; Milne, B. F.; Tørfoss, V.; Andersen, J. H.; Hansen, E.; Stensvåg, K.; Haug, T. *Org. Lett.* **2010**, *12*, 4752–4755.

[21] Takahashi, Y.; Iinuma, Y.; Kubota, T.; Tsuda, M.; Sekiguchi, M.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org. Lett.* **2011**, *13*, 628–631.

[22] Hanssen, K. Ø.; Cervin, G.; Trepos, R.; Petitbois, J.; Haug, T.; Hansen, E.; Andersen, J. H.; Pavia, H.; Hellio, C.; Svenson, J. *J. Mar. Biotechnol.* **2014**, *16*, 684–694.

[23] Igumnova, E. M.; Mishchenko, E.; Haug, T.; Blencke, H.-M.; Sollid, J. U. E.; Fredheim, E. G. A.; Lauksund, S.; Stensvåg, K.; Strøm, M. B. *Bioorg. Med. Chem.* **2016**, *24*, 5884–5894.

[24] Lea, K. L. *Synthesis of Small Cationic Amphiphiles for Antimicrobial Screening. Transition Metal Catalyzed Benzene Synthesis and Subsequent Functionalization.* Master Thesis, NTNU, 2016.

[25] Brondz, A. *Synthesis of Small Cationic Amphiphiles for Antimicrobial Screening. Transition Metal Catalyzed Pyridine Synthesis and Subsequent Functionalization.* Master Thesis, NTNU, 2016.

[26] Myreng, K. *Synthesis of Small Cationic Amphiphiles for Antimicrobial Screening.* Organic Chemistry Specialization Project, NTNU, Autumn 2016.

[27] Patel, B. H.; Barrett, A. G. M. *J. Org. Chem.* **2012**, *77*, 11296–11301.

[28] Bakka, T. A.; Gautun, O. R. *Synth. Commun.* **2017**, *47*, 169–172.

[29] Brown, H. C.; Schlesinger, H.; Burg, A. B. *J. Am. Chem. Soc.* **1939**, *61*, 673–680.

[30] Smith, M. B. *Organic Synthesis*, 3rd ed.; Academic Press, 2011; pp 385–386.

[31] Schlesinger, H.; Brown, H. C.; Hoekstra, H. R.; Rapp, L. R. *J. Am. Chem. Soc.* **1953**, *75*, 199–204.

[32] Zweifel, G.; Nagase, K.; Brown, H. C. *J. Am. Chem. Soc.* **1962**, *84*, 190–195.

[33] Wang, Q. *et al. J. Med. Chem.* **2007**, *50*, 199–210.

[34] Burkhardt, E. R.; Matos, K. *Chem. Rev.* **2006**, *106*, 2617–2650.

[35] Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry: Part B: Reaction and Synthesis*, 5th ed.; Springer, 2007; pp a: 675, b: 739–748, c: 267–272.

[36] Heller, B.; Hapke, M. *Chem. Soc. Rev.* **2007**, *36*, 1085–1094.

[37] Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633–9695.

[38] Miyaura, N.; Yamada, K.; Suzuki, A. *Tetrahedron Lett.* **1979**, *20*, 3437–3440.

[39] Lennox, A. J. J.; Lloyd-Jones, G. C. *Chem. Soc. Rev.* **2014**, *43*, 412–443.

[40] *The Nobel Prize in Chemistry 2010.* `nobelprize.org`, Accessed 12th of June 2017.

[41] Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483.

[42] Greene, T. W.; Wuts, P. G. M. *Protective Groups In Organic Synthesis*, 2nd ed.; John Wiley and Sons, inc, 1991; pp 327–329.

[43] Bruckner, R. *Organic Mechanisms: Reactions, Stereochemistry and Synthesis*; Springer, 2010; pp a: 304–306, b: 344–346.

[44] Katritzky, A. R.; Rogovoy, B. V. *Arkivoc* **2005**, *4*, 49–87.

[45] Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497–2502.

[46] Zumdahl, S. S. *Chemical Principles*, 6th ed.; Cengage Learning, 2009; pp 897–898.

[47] Hua, Y.; Chang, S.; Huang, D.; Zhou, X.; Zhu, X.; Zhao, J.; Chen, T.; Wong, W.-Y.; Wong, W.-K. *Chem. Mat.* **2013**, *25*, 2146–2153.

[48] Bugge, S.; Buene, A. F.; Jurisch-Yaksi, N.; Moen, I. U.; Skjønsfjell, E. M.; Sundby, E.; Hoff, B. H. *Eur. J. Med. Chem.* **2016**, *107*, 255 – 274.

[49] Knapp, D. M.; Gillis, E. P.; Burke, M. D. *J. Am. Chem. Soc.* **2009**, *131*, 6961–6963, PMID: 19405470.

[50] Clayden, J.; Greeves, N.; Warren, S. *Organic Chemistry*, 2nd ed.; Oxford University Press, 2012; p 129.

[51] Thiemann, T.; Umeno, K.; Ohira, D.; Inohae, E.; Sawada, T.; Mataka, S. *New J. Chem.* **1999**, *23*, 1067–1070.

[52] Korenaga, T.; Kosaki, T.; Fukumura, R.; Ema, T.; Sakai, T. *Org. Lett.* **2005**, *7*, 4915–4917.

[53] Elkassih, S. A.; Sista, P.; Magurudeniya, H. D.; Papadimitratos, A.; Zakhidov, A. A.; Biewer, M. C.; Stefan, M. C. *Macromol. Chem. Phys.* **2013**, *214*, 572–577.

[54] Alazet, S.; Ollivier, K.; Billard, T. *Beilstein J. Org. Chem.* **2013**, *9*, 2354–2357.

[55] Gribble, G. W., Ed. *Heterocyclic Scaffolds II: Reactions and Applications of Indoles*; Springer, 2010; Vol. 26; pp a: 48–52, b: 55–61.

[56] Bakka, T. A. *Synthesis and Antimicrobial Evaluation of Small Molecule Amphiphiles Derived from Amphiphilic Antimicrobial Natural Procts*. Ph.D. thesis, Norwegian University of Science and Technology (NTNU), May 2017.

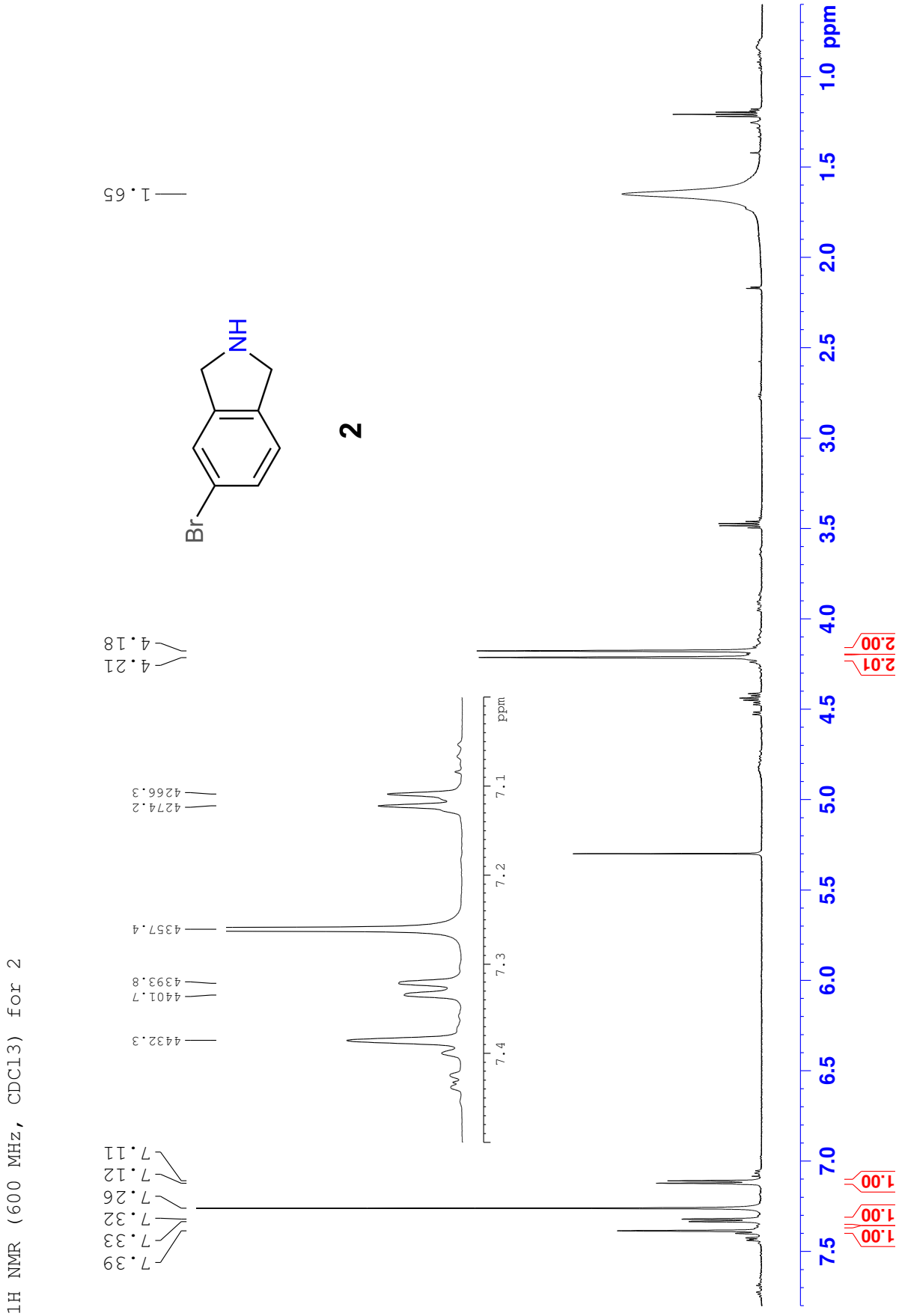
[57] Clayden, J.; Greeves, N.; Warren, S. *Basic One- and Two-Dimensional NMR Spectroscopy*, 5th ed.; Wiley, 2010.

[58] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512–7515.

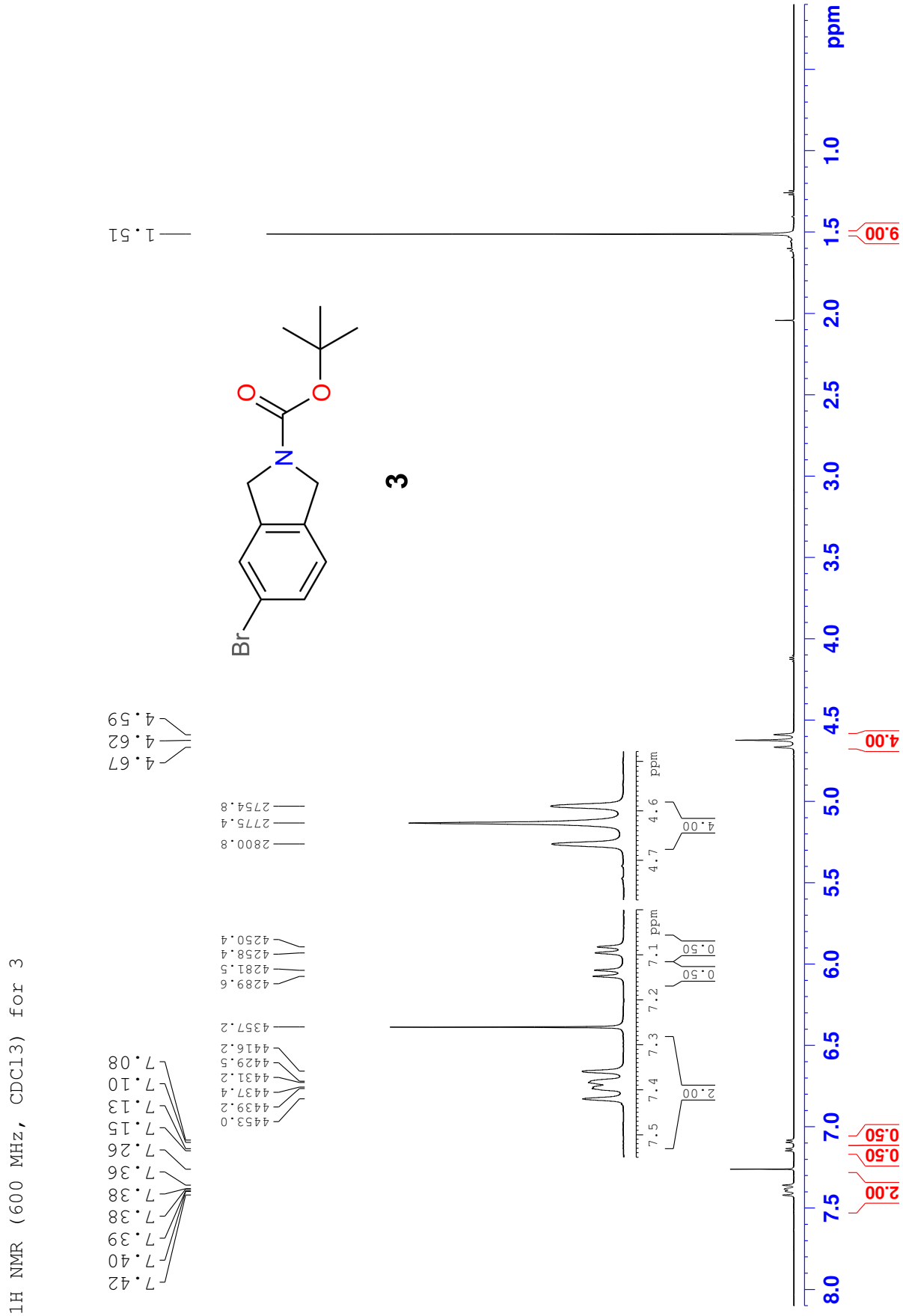
Appendix

The Appendix are organized after compound. For all new compounds, the appendices will be presented in the following order: ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, MS, and HPLC, followed by any extra appendices that might be relevant for that compound.

A.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 2



B.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 3



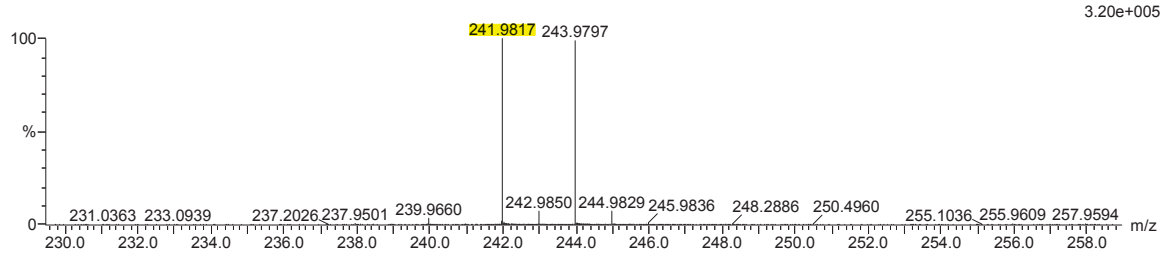
B.2 HRMS spectrum for 3

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
283 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10 O: 1-25 Br: 0-2

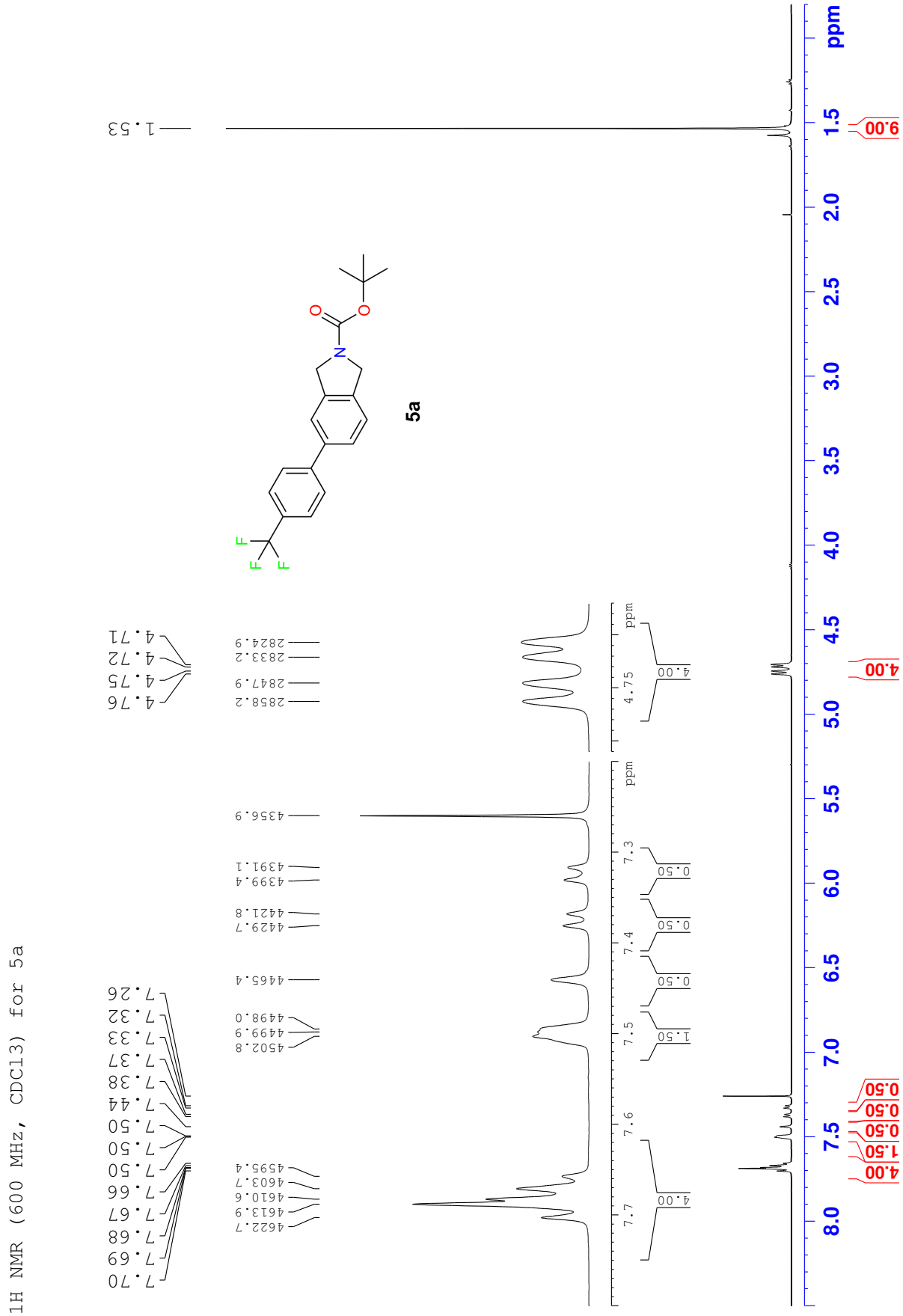
2017-160 8 (0.172) AM2 (Ar,35000.0,0.00,0.00); Cm (6:8)
1: TOF MS ASAP+



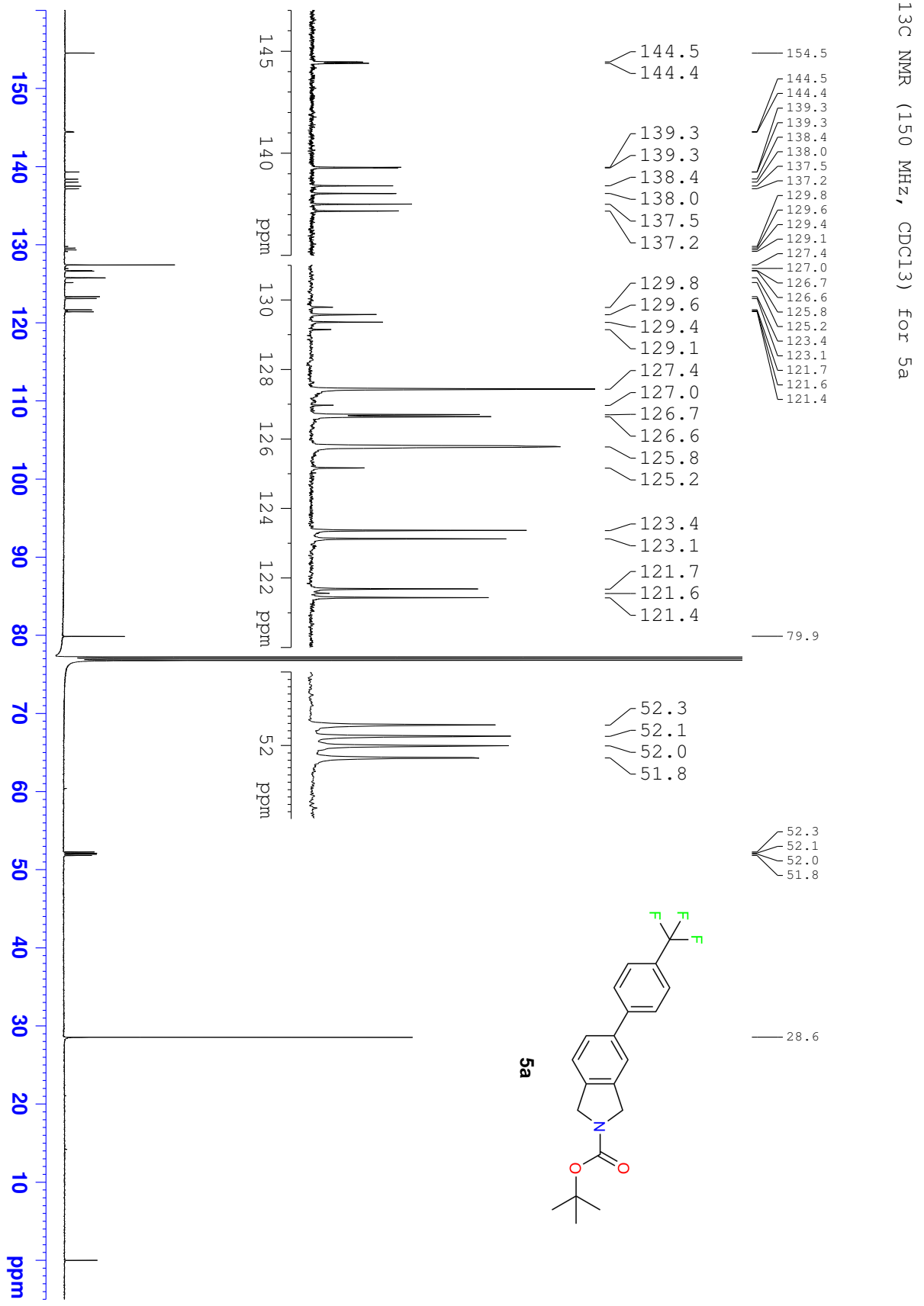
Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
241.9817	241.9817	0.0	0.0	5.5	1336.1	n/a	n/a	C9 H9 N O2 Br

ion observed [M-C4H8+H]

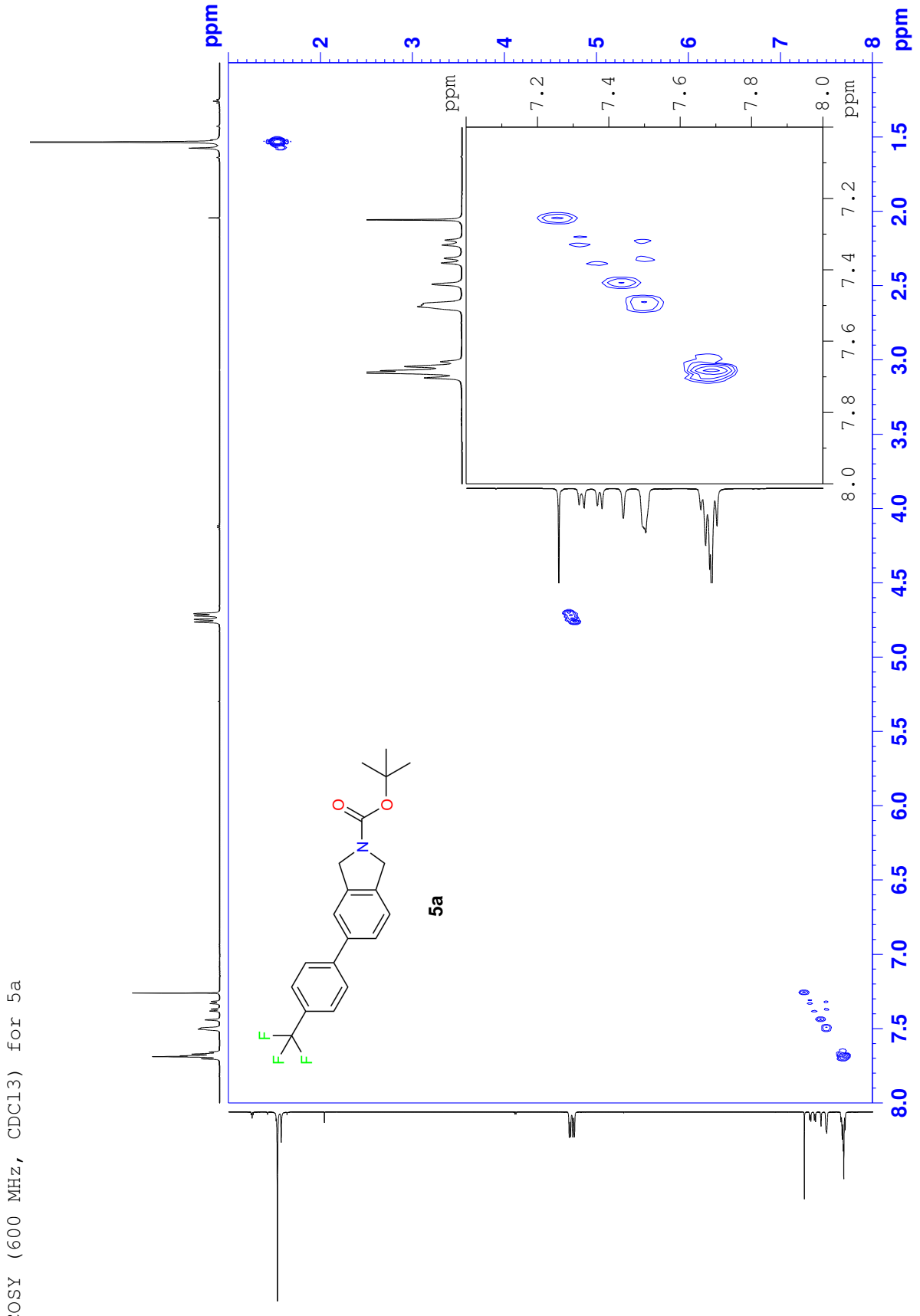
C.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5a



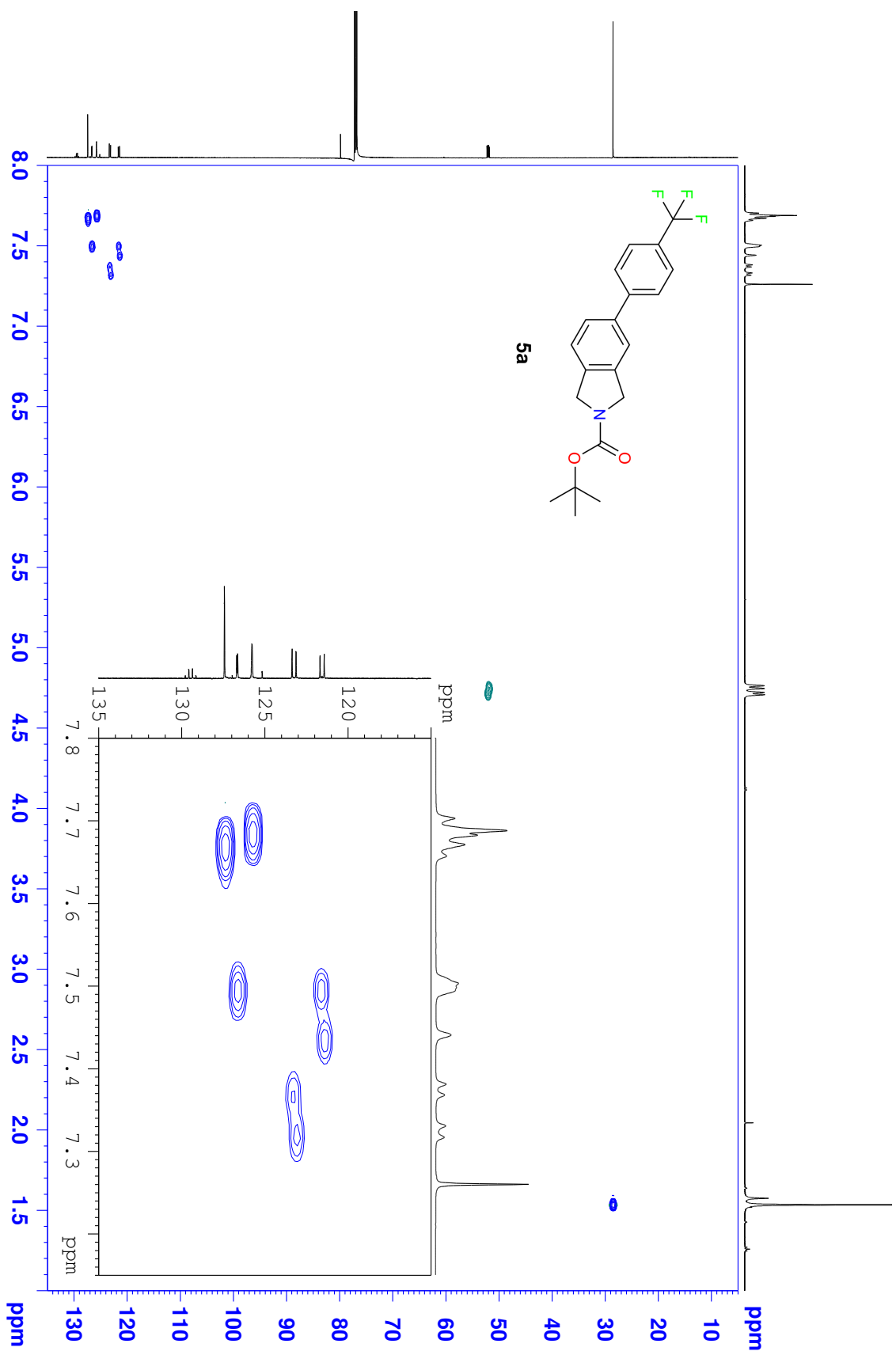
C.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5a



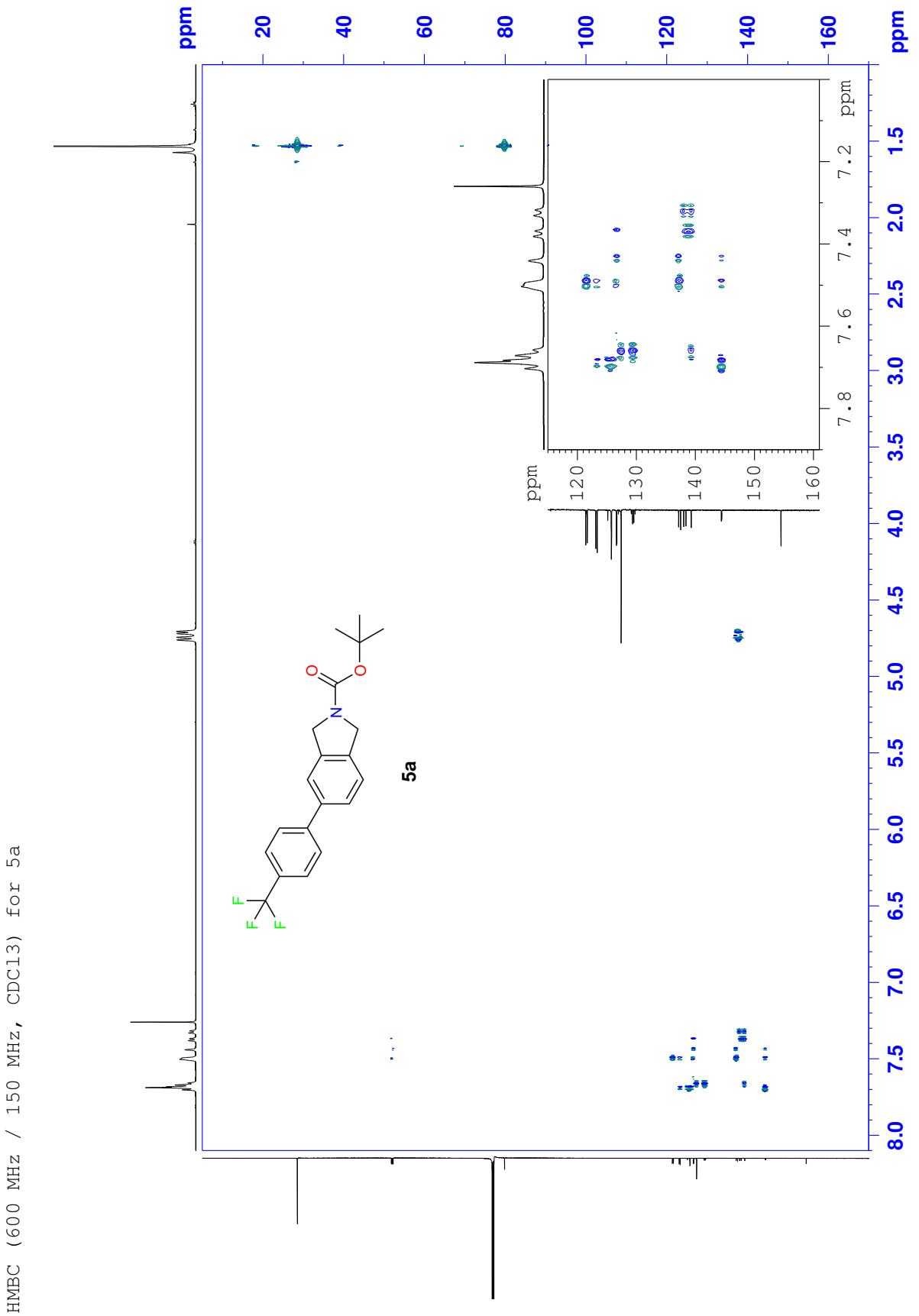
C.3 COSY (600 MHz, $CDCl_3$) spectrum for 5a



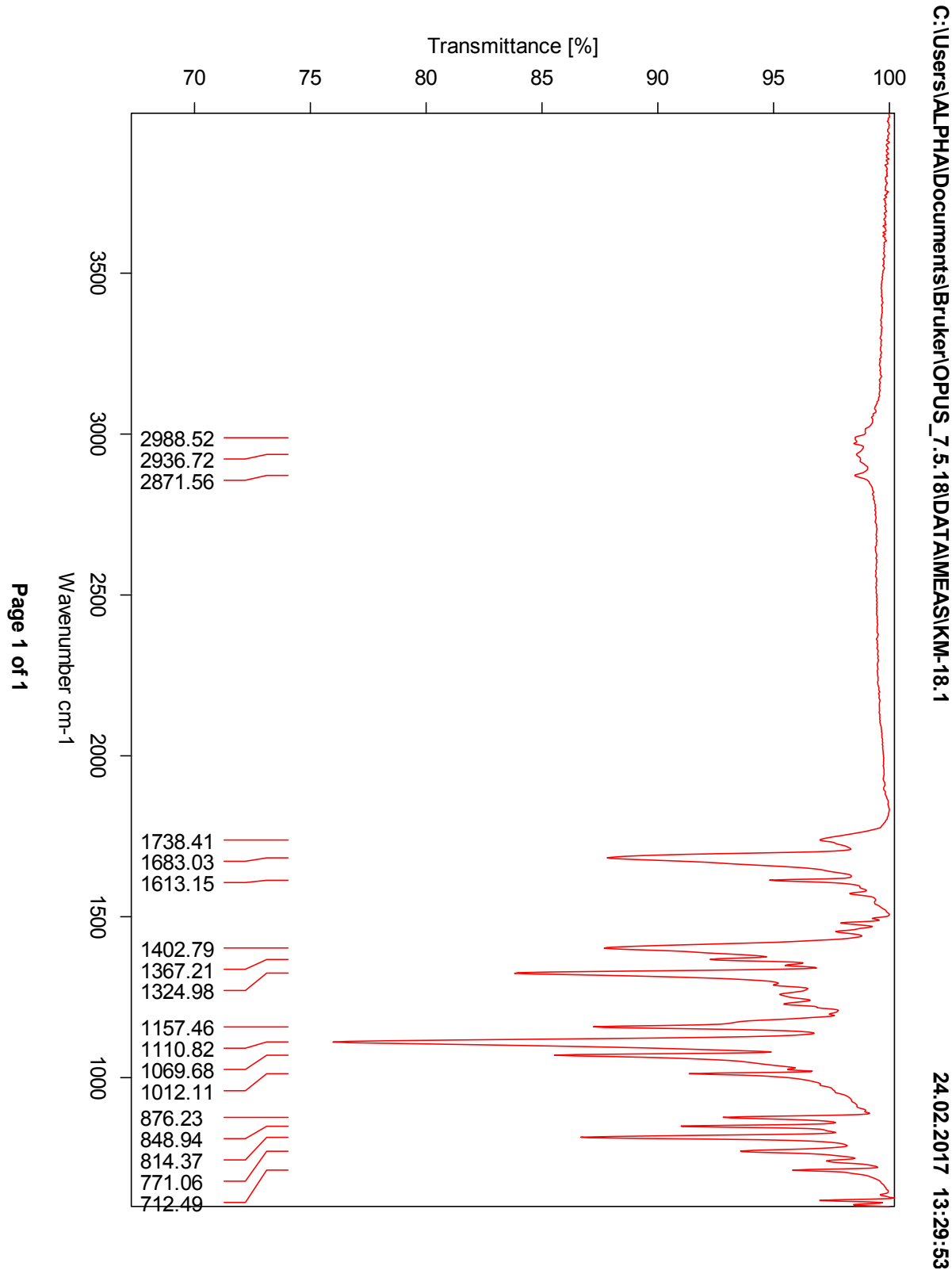
C.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5a



C.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5a



C.6 IR spectrum for 5a

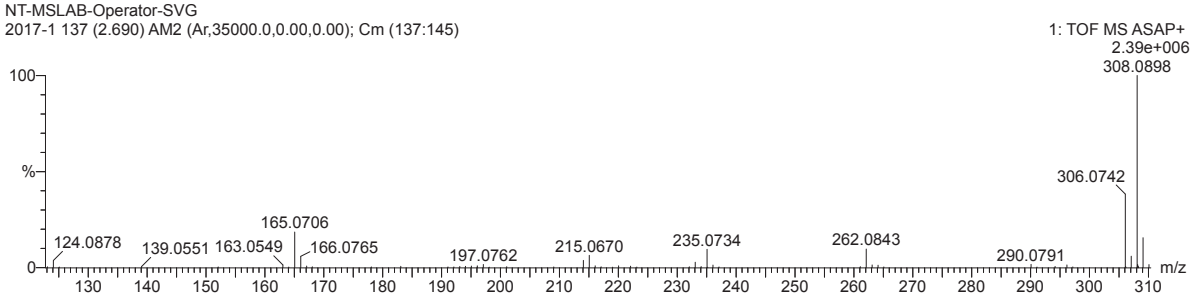


C.7 HRMS spectrum for 5a

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

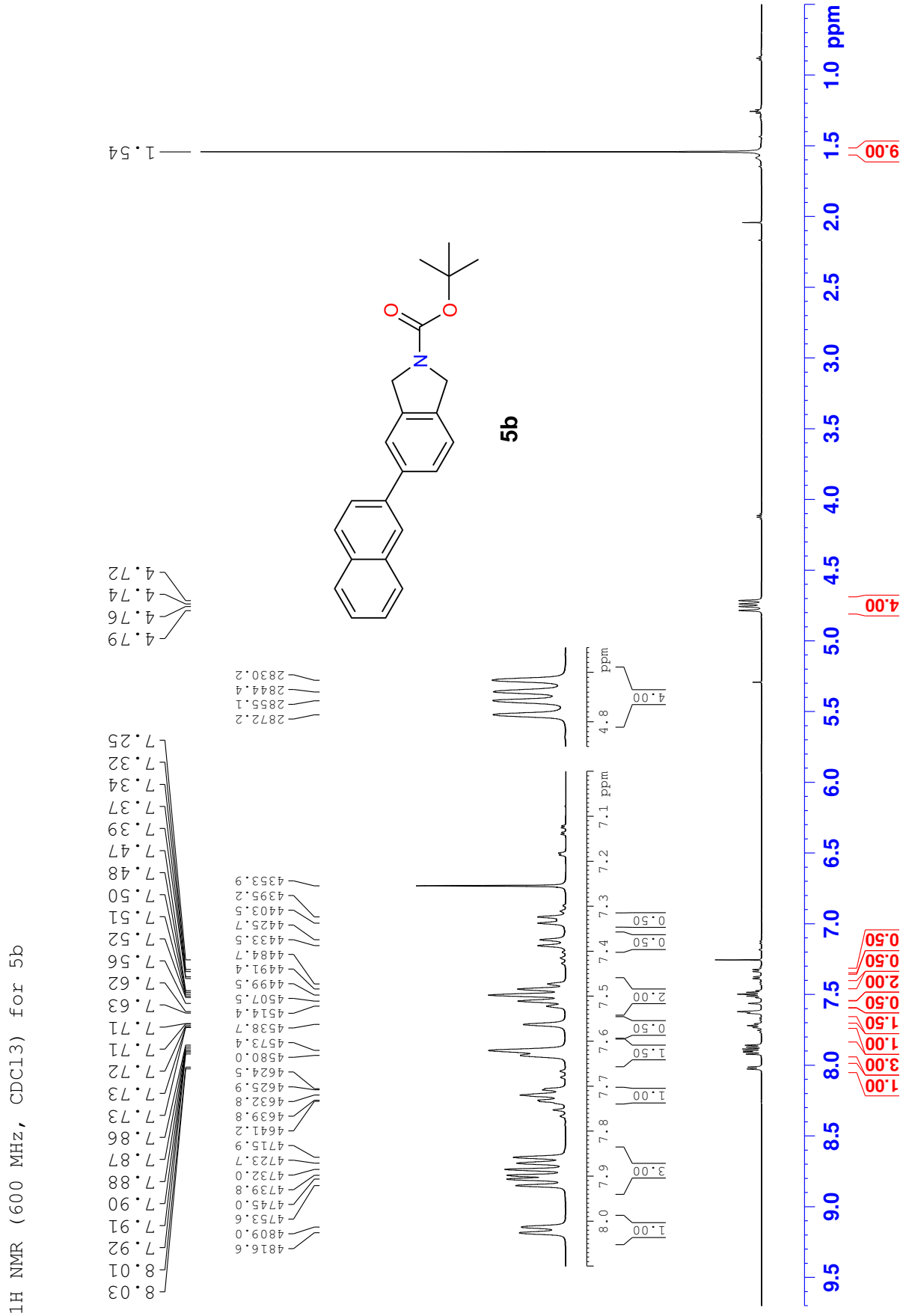
Monoisotopic Mass, Even Electron Ions
2519 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 0-1000 N: 0-20 O: 0-25 F: 0-6
NT-MSLAB-Operator-SVG
2017-1 137 (2.690) AM2 (Ar,35000.0,0.00,0.00); Cm (137:145)



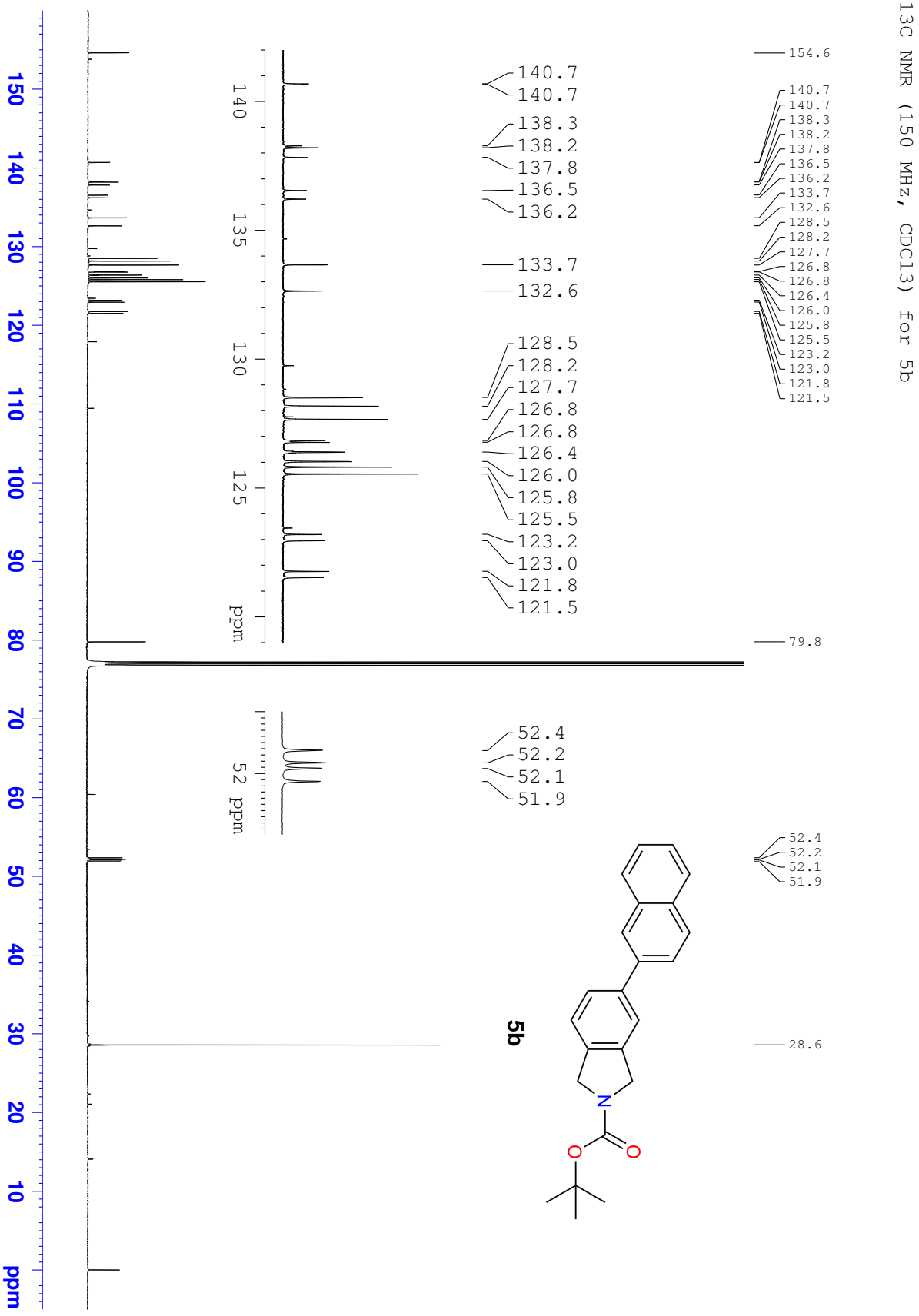
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
308.0898	308.0898	0.0	0.0	9.5	191.2	0.008	99.24	C16 H13 N O2 F3
	308.0896	0.2	0.6	13.5	196.2	5.007	0.67	C14 H10 N7 O2
	308.0894	0.4	1.3	4.5	198.2	6.972	0.09	C10 H15 N3 O7 F
	308.0894	0.4	1.3	2.5	202.7	11.509	0.00	C6 H11 N7 O2 F5
	308.0903	-0.5	-1.6	2.5	206.5	15.357	0.00	C H9 N13 O3 F3
	308.0892	0.6	1.9	6.5	204.9	13.680	0.00	C4 H8 N13 O2 F2

ION OBSERVED [M-C4H8+H]

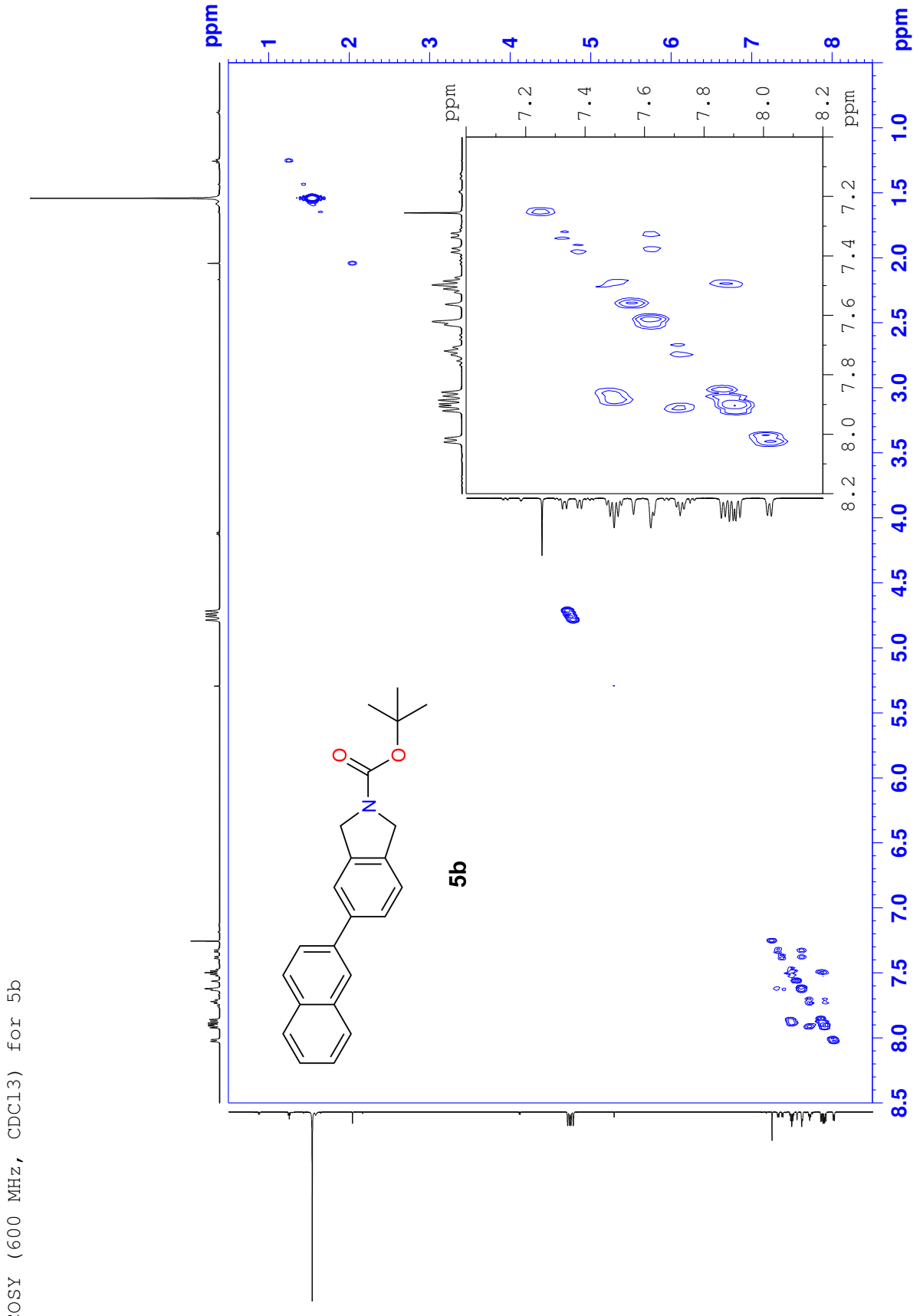
D.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5b



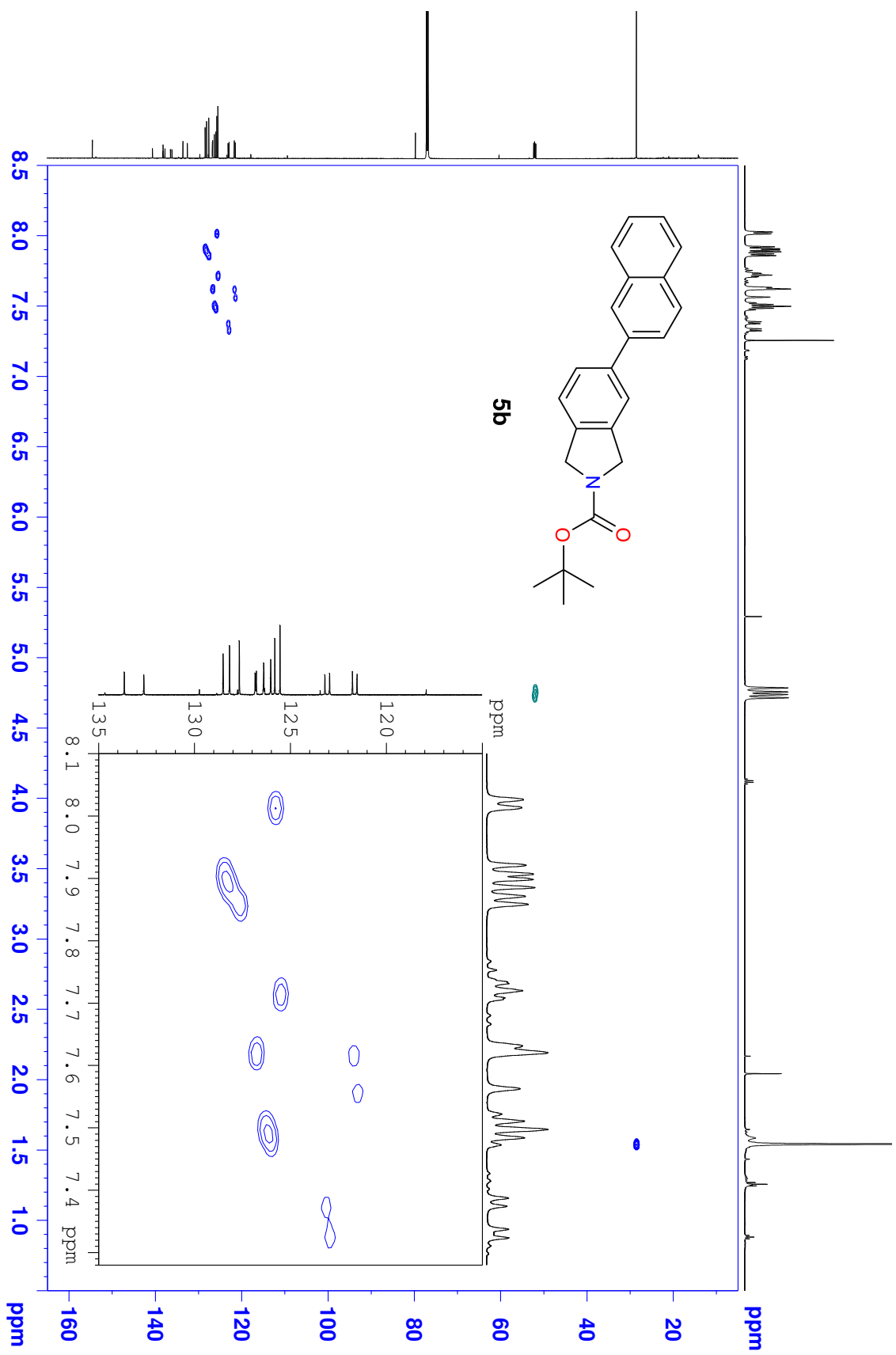
D.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5b



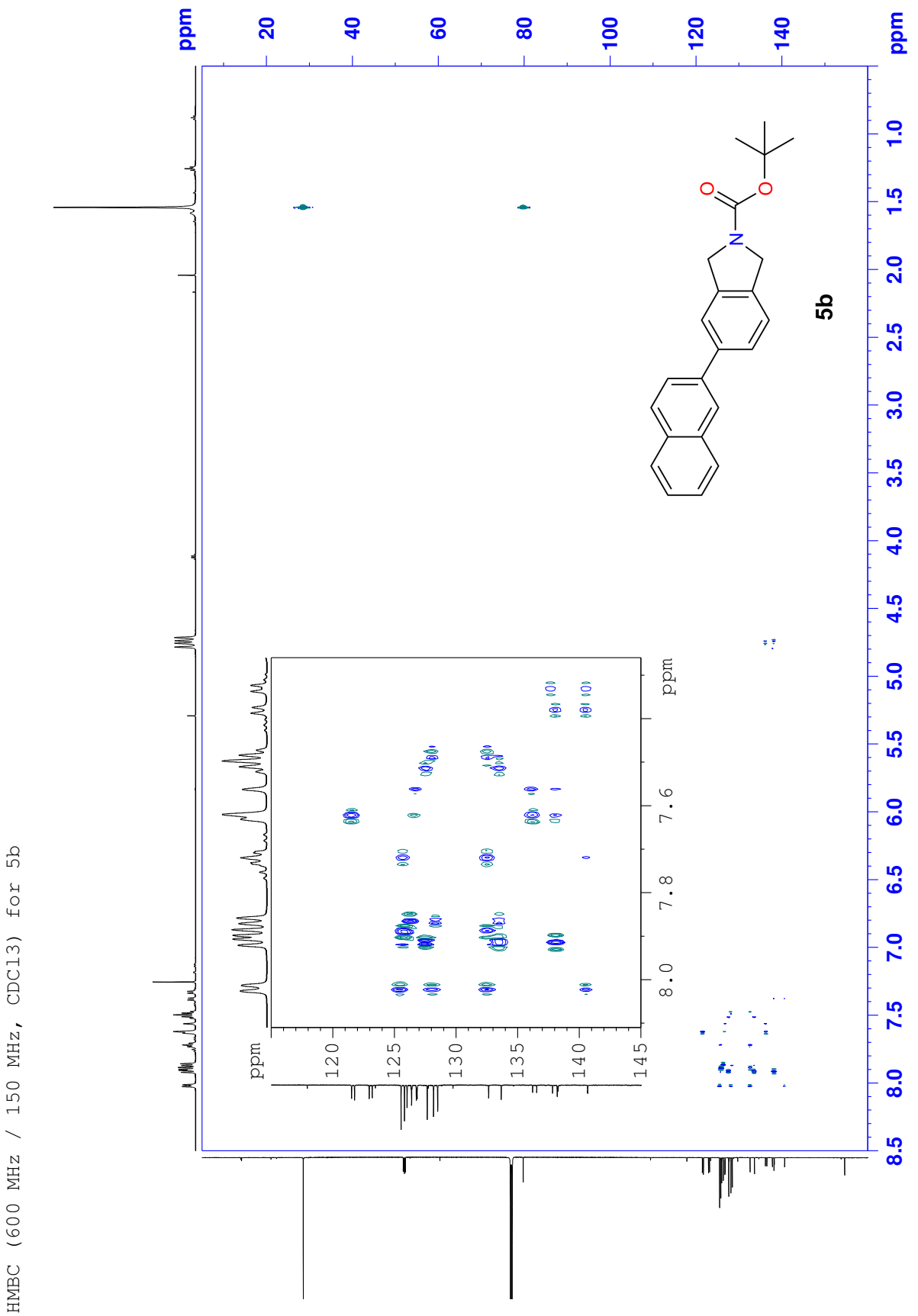
D.3 COSY (600 MHz, $CDCl_3$) spectrum for 5b



D.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5b

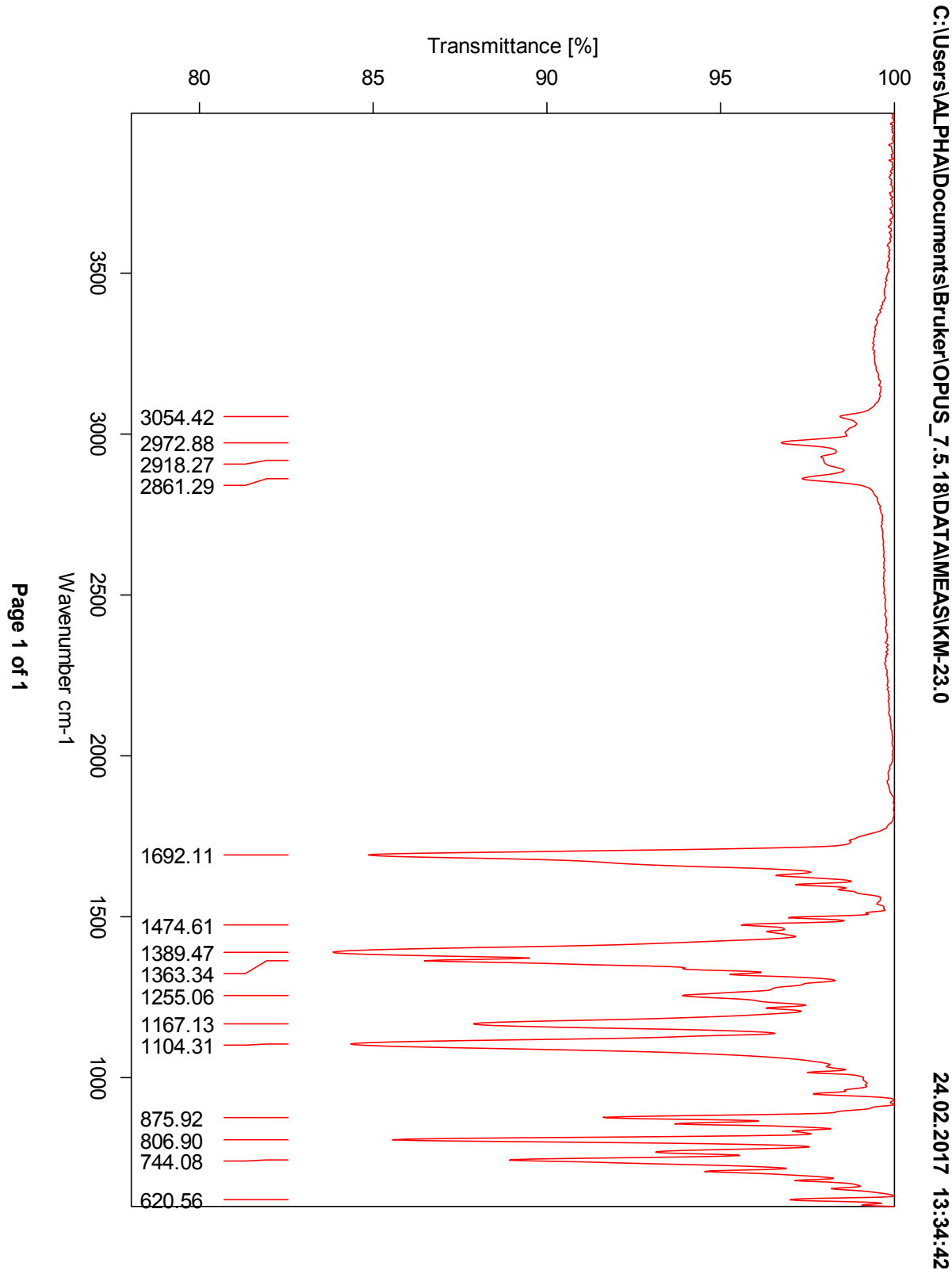


D.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5b



HMBC (600 MHz / 150 MHz, CDCl3) for 5b

D.6 IR spectrum for 5b

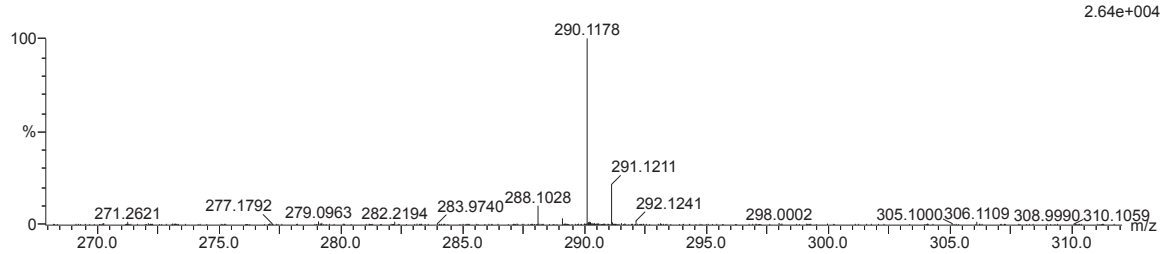


D.7 HRMS spectrum for 5b

Elemental Composition Report **Page 1**

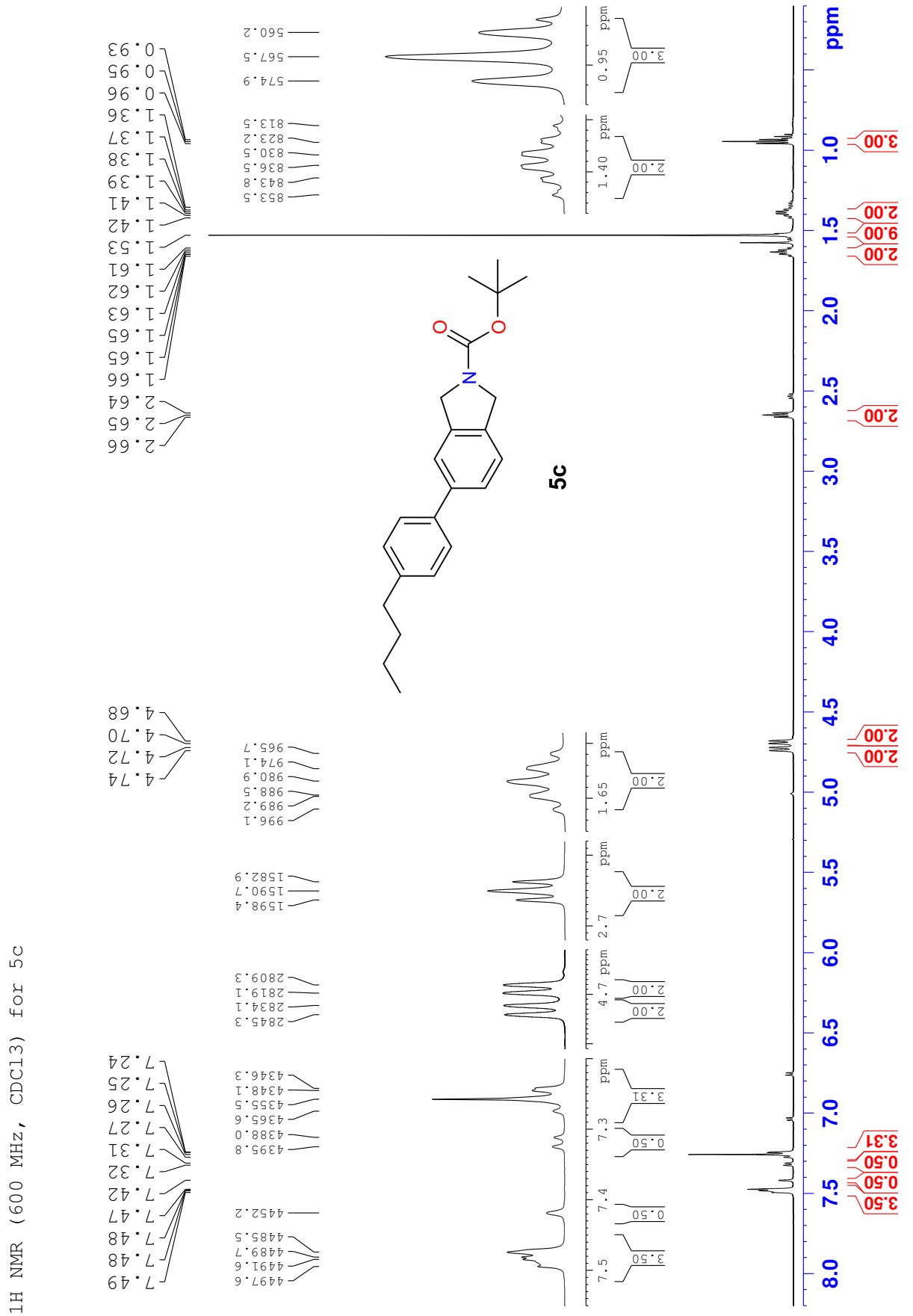
Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
297 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10 O: 1-25
2017-33 122 (2.397) AM2 (Ar,35000.0,0.00,0.00); Cm (117:129)
1: TOF MS ASAP+

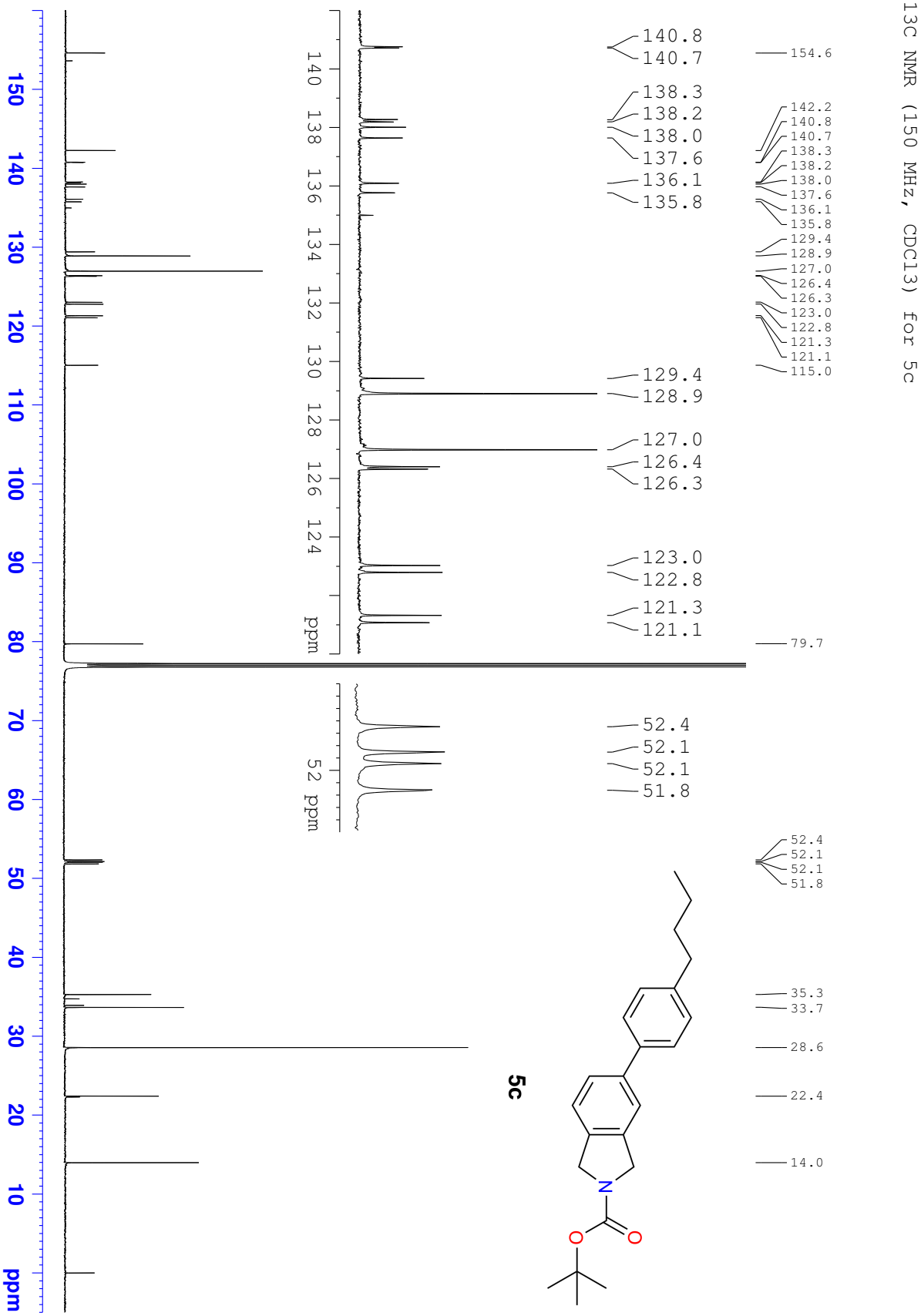


Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
290.1178	290.1181	-0.3	-1.0	12.5	429.6	0.000	100.00	C19 H16 N O2 ion observed [M-C4H8+H]
	290.1173	0.5	1.7	0.5	444.4	14.761	0.00	C3 H16 N9 O7

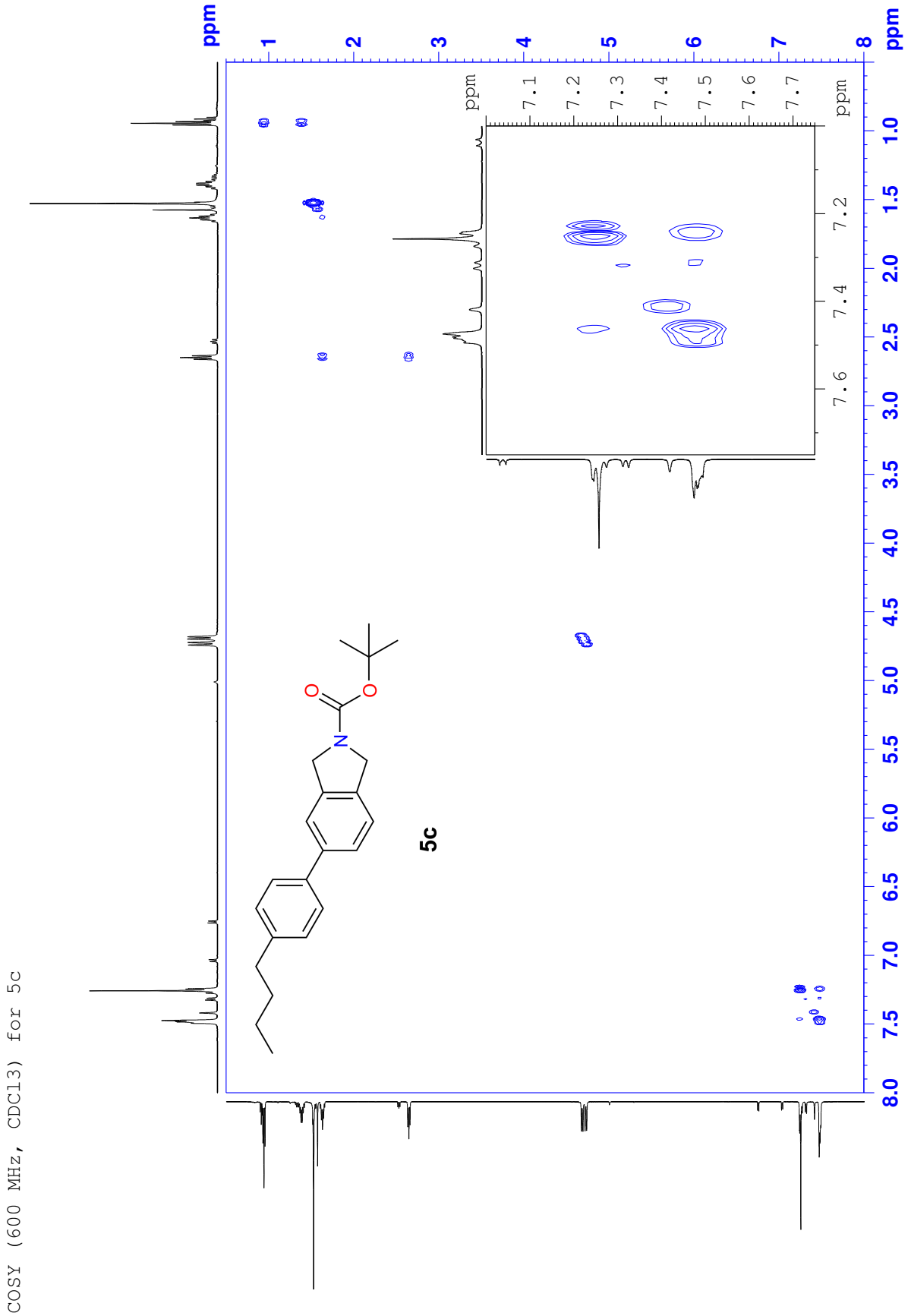
E.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5c



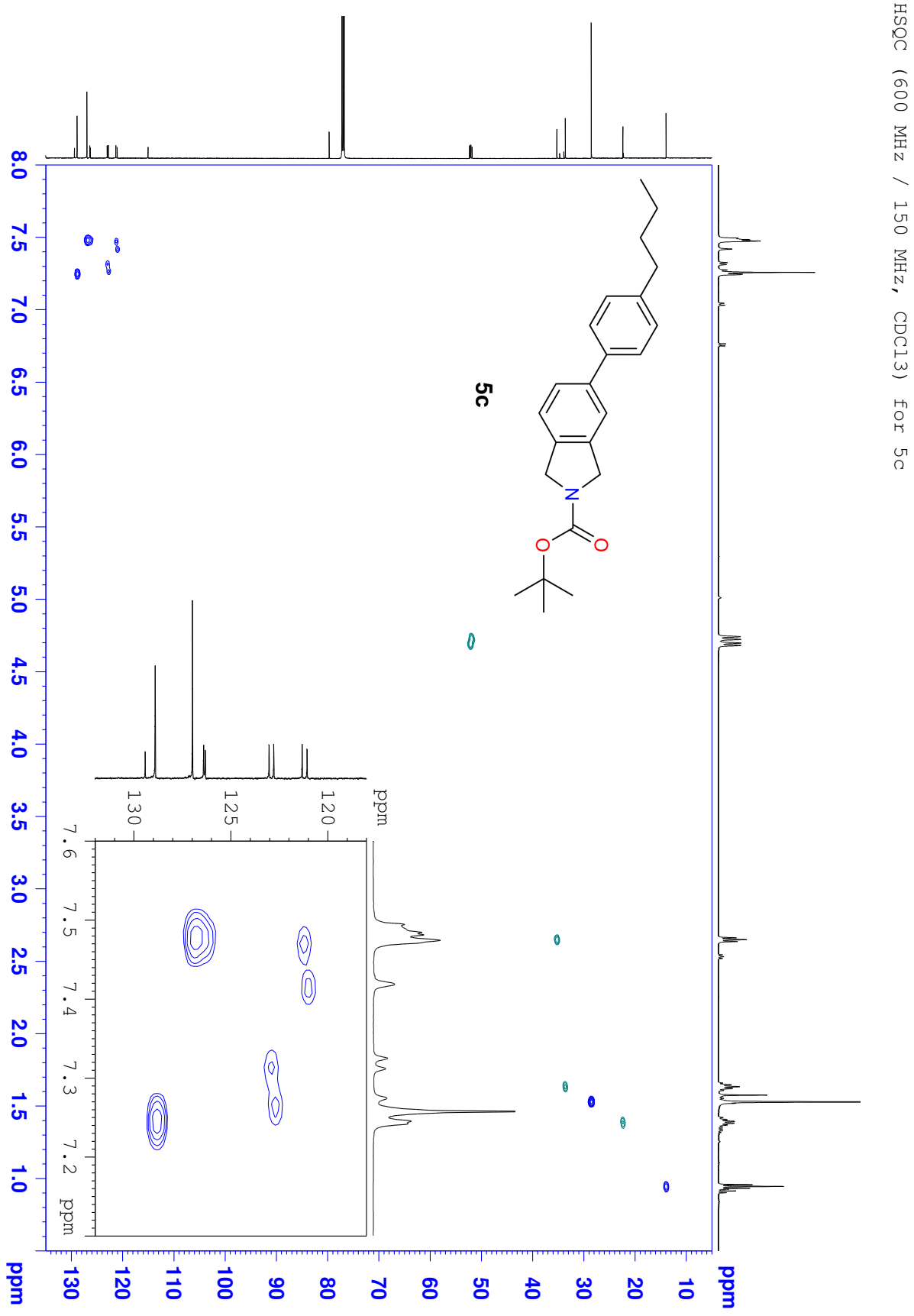
E.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5c



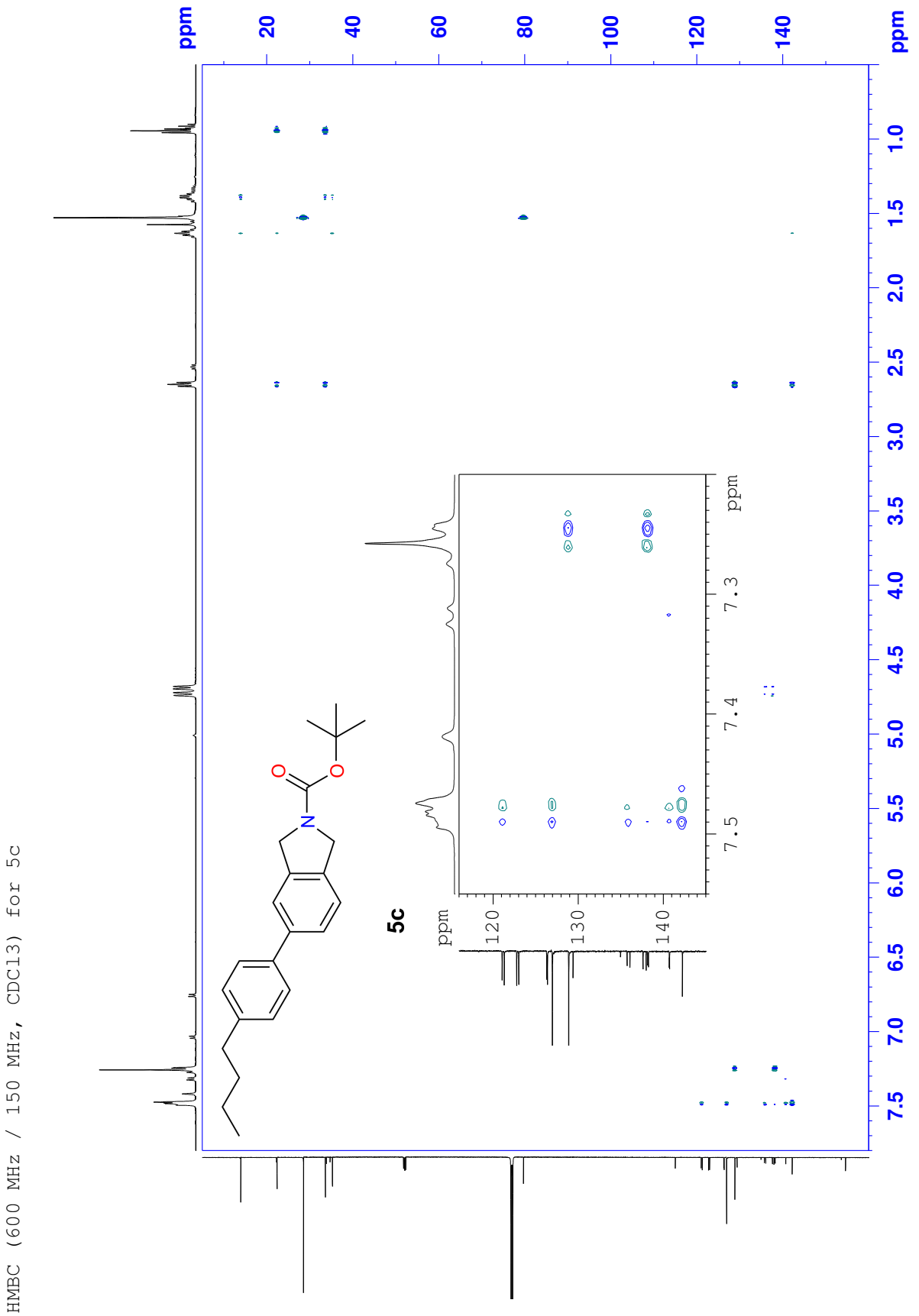
E.3 COSY (600 MHz, $CDCl_3$) spectrum for 5c



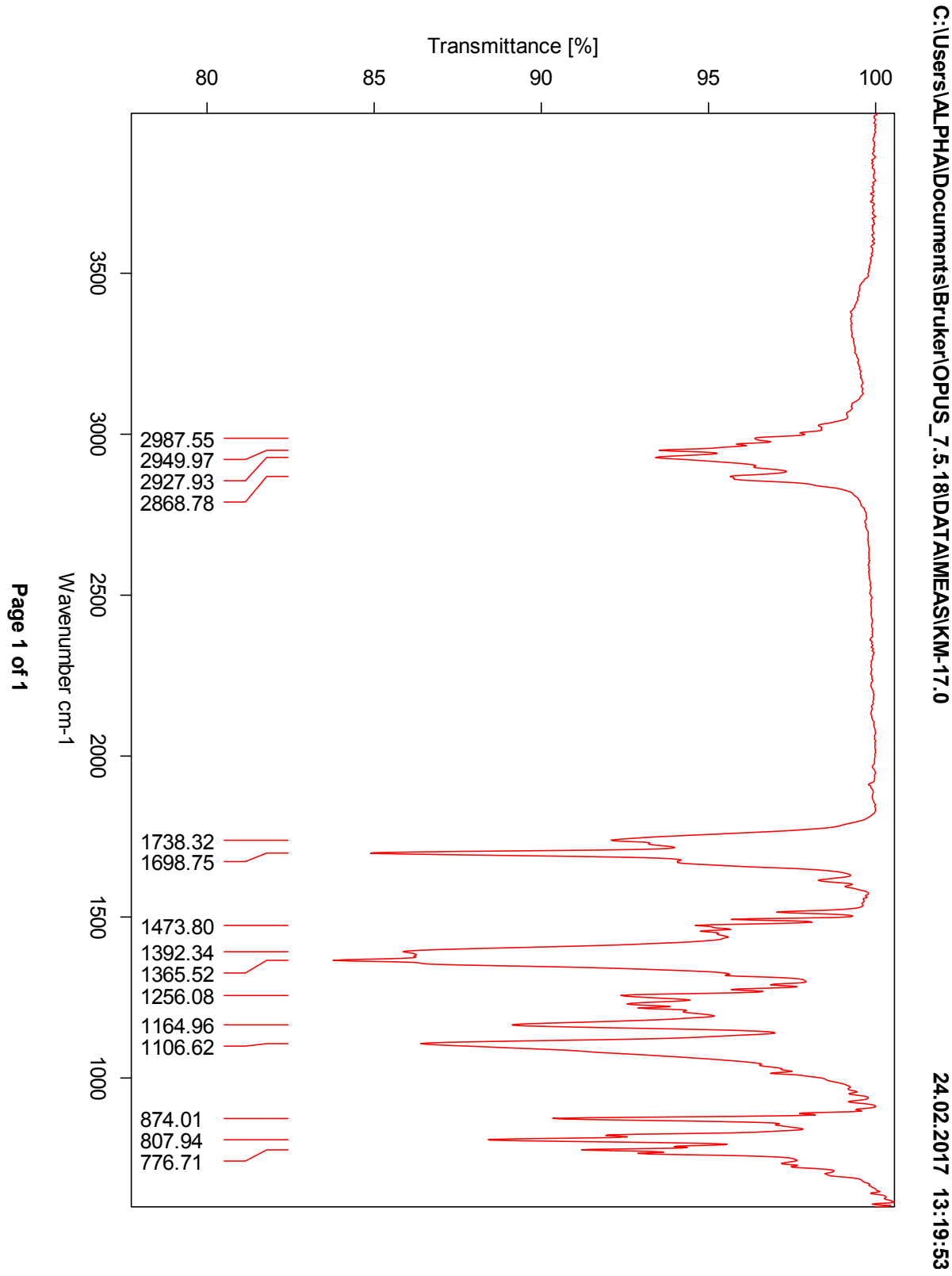
E.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5c



E.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5c



E.6 IR spectrum for 5c



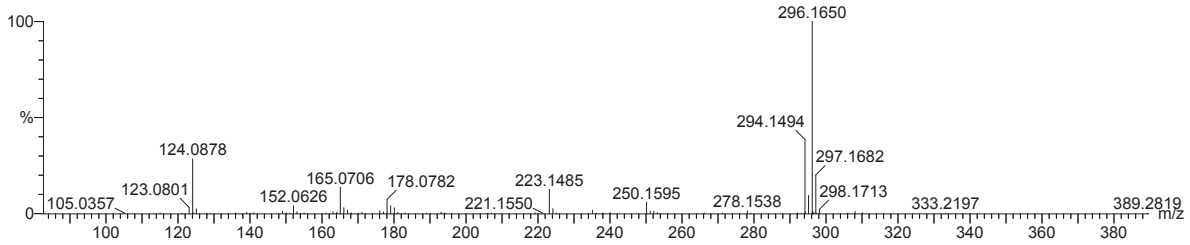
E.7 HRMS spectrum for 5c

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

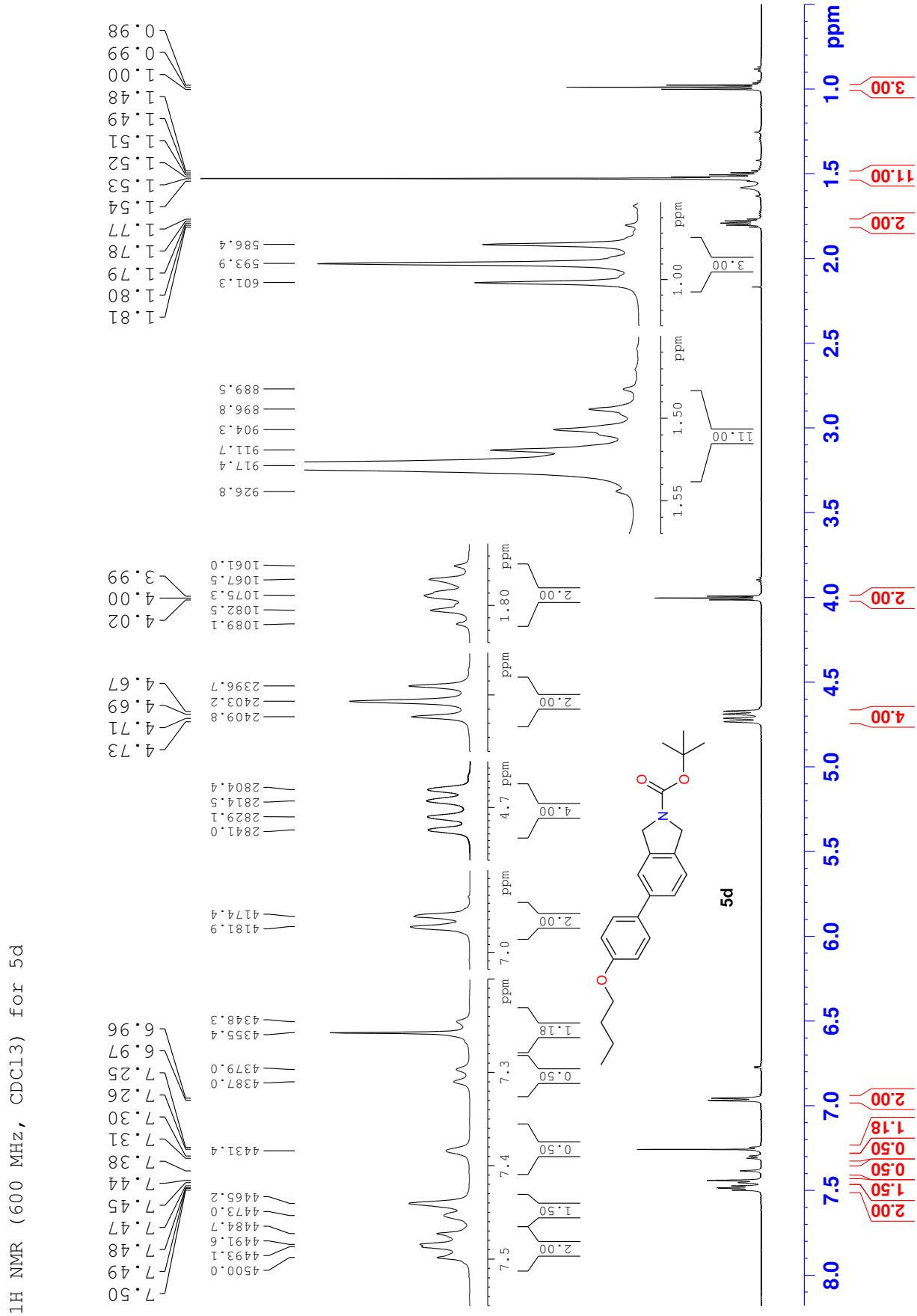
Monoisotopic Mass, Even Electron Ions
2218 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 0-1000 N: 0-20 O: 0-25 F: 0-6
NT-MSLAB-Operator-SVG
2017-2 127 (2.481) AM2 (Ar,35000.0,0.00,0.00); Cm (121:127)

1: TOF MS ASAP+
2.09e+005

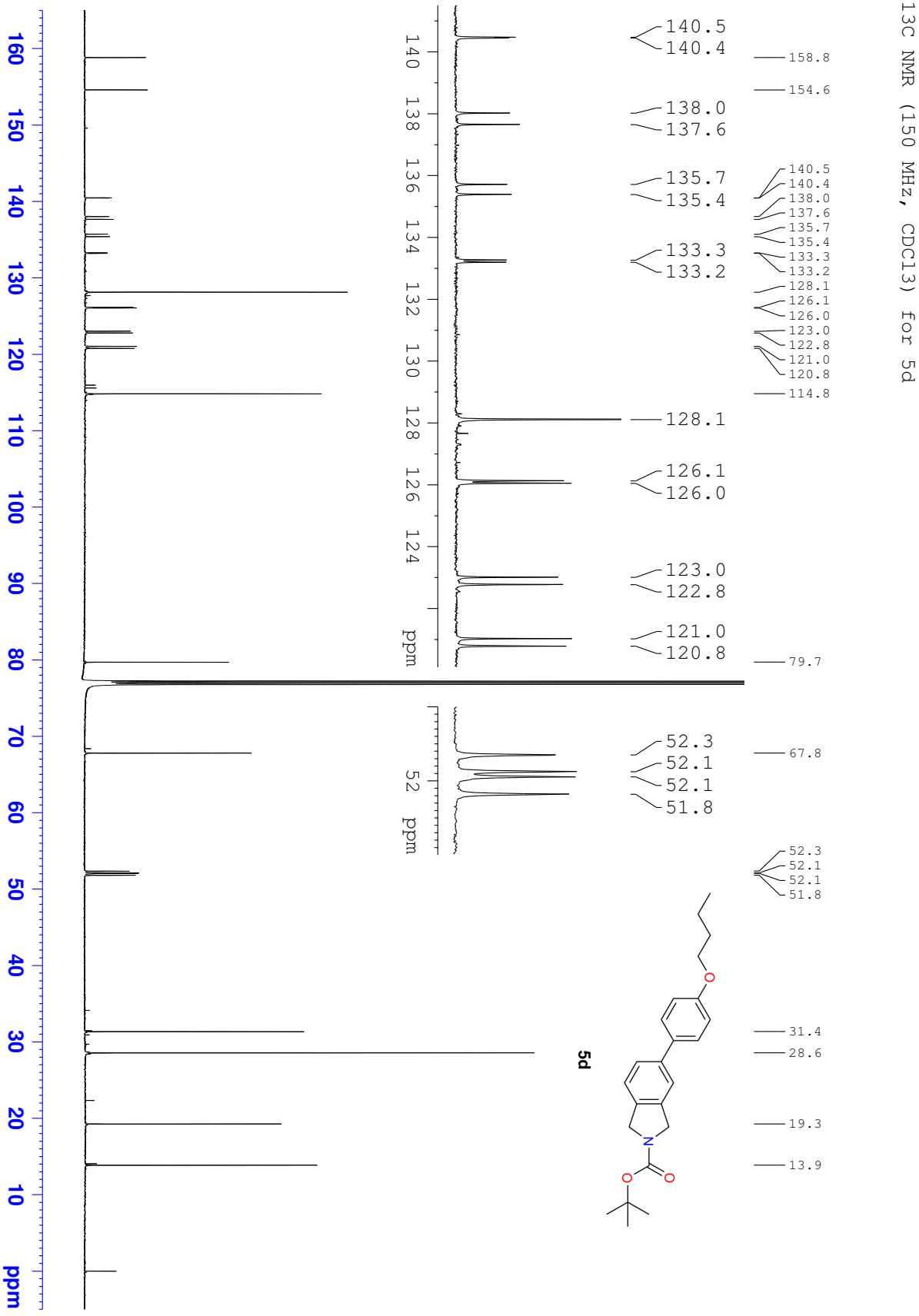


Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
296.1650	296.1651	-0.1	-0.3	9.5	189.6	0.000	99.98	C19 H22 N O2 Ion observed [M-C4H8+H]
	296.1649	0.1	0.3	-1.5	198.1	8.487	0.02	C11 H23 N O2 F5
	296.1647	0.3	1.0	2.5	202.8	13.213	0.00	C9 H20 N7 O2 F2
	296.1656	-0.6	-2.0	2.5	206.1	16.548	0.00	C4 H18 N13 O3

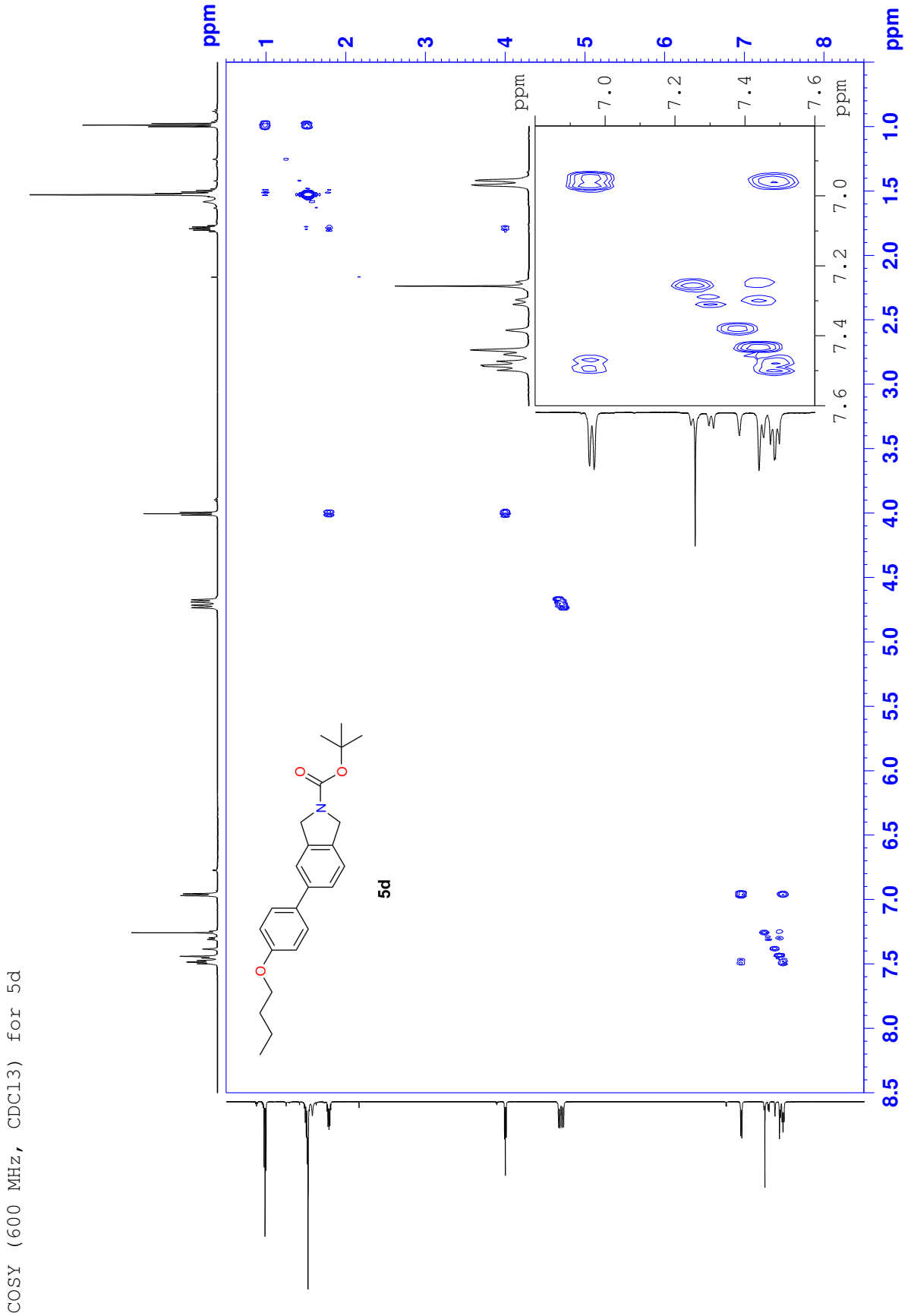
F.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5d



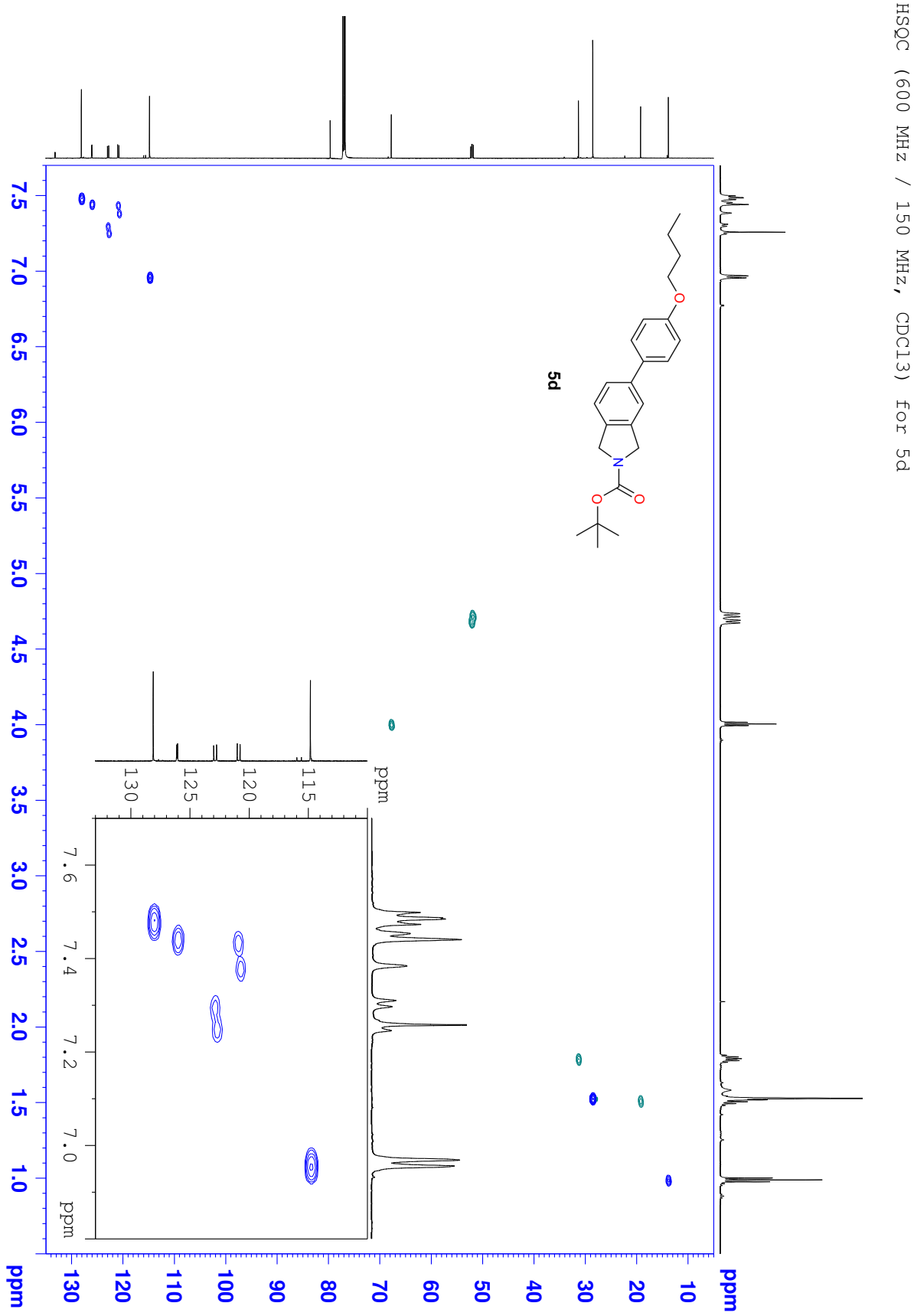
F.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5d



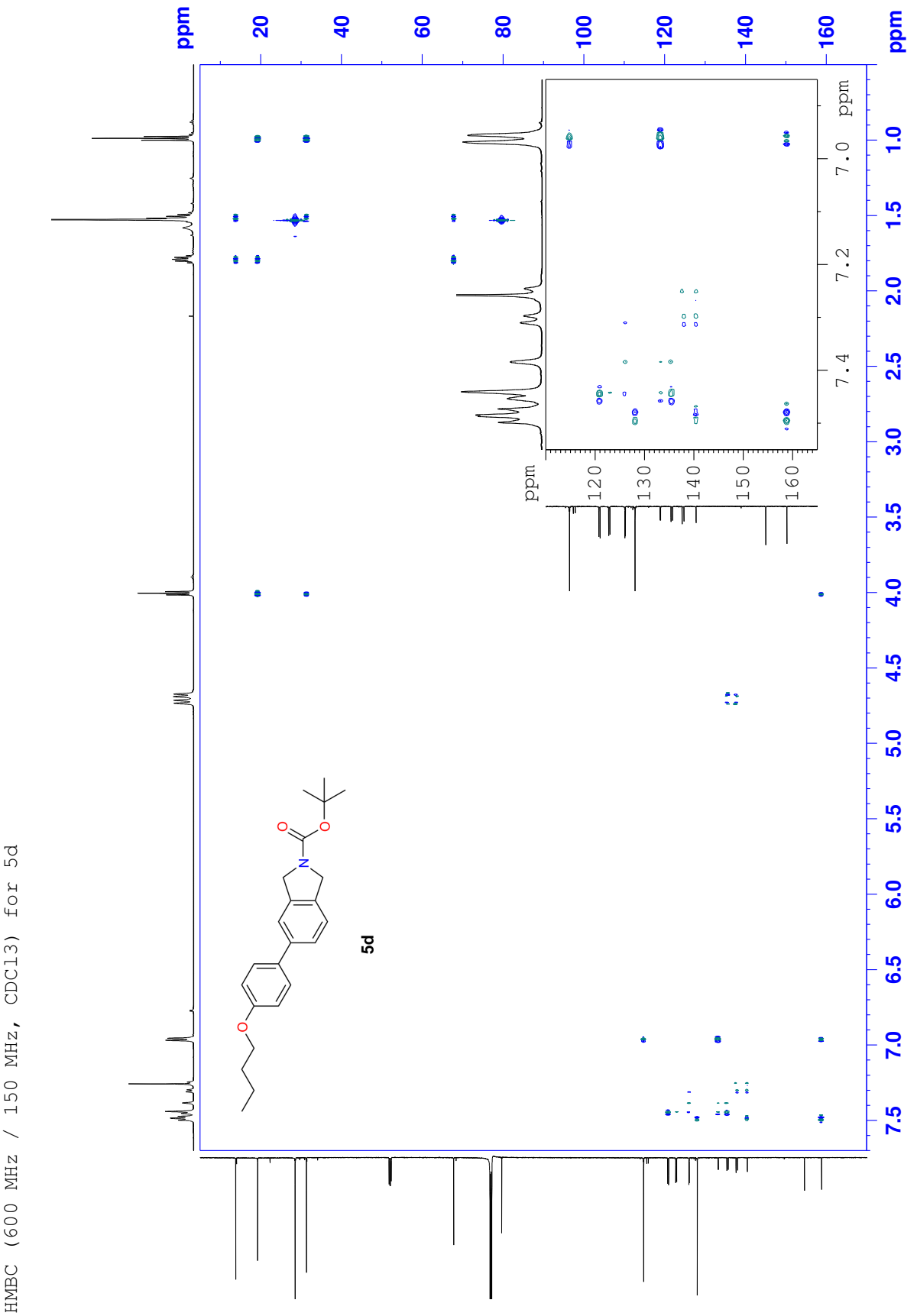
F.3 COSY (600 MHz, $CDCl_3$) spectrum for 5d



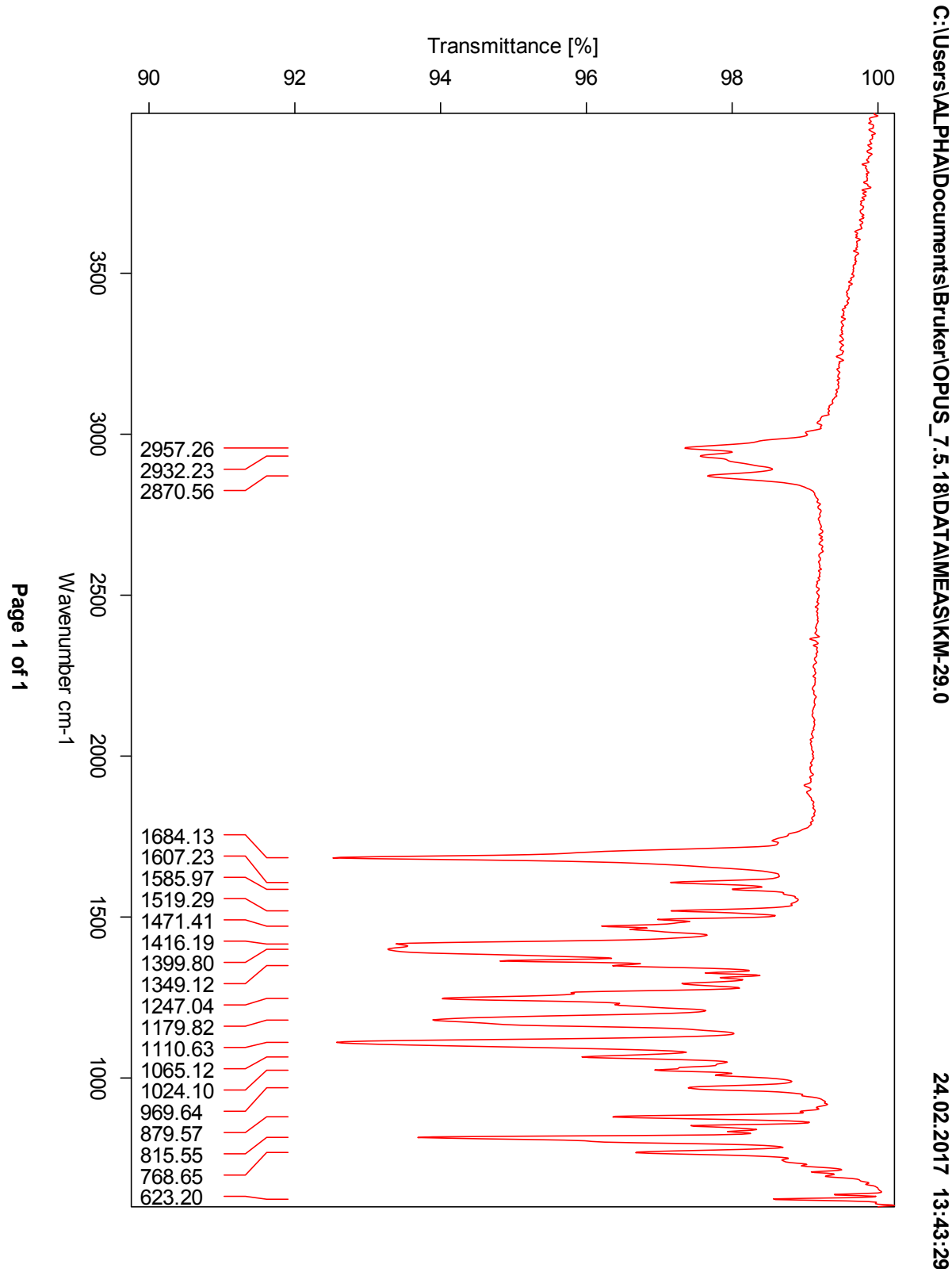
F.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5d



F.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5d



F.6 IR spectrum for 5d



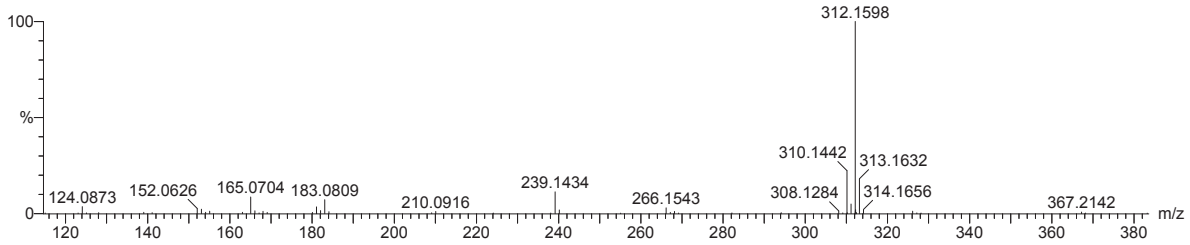
F.7 HRMS spectrum for 5d

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

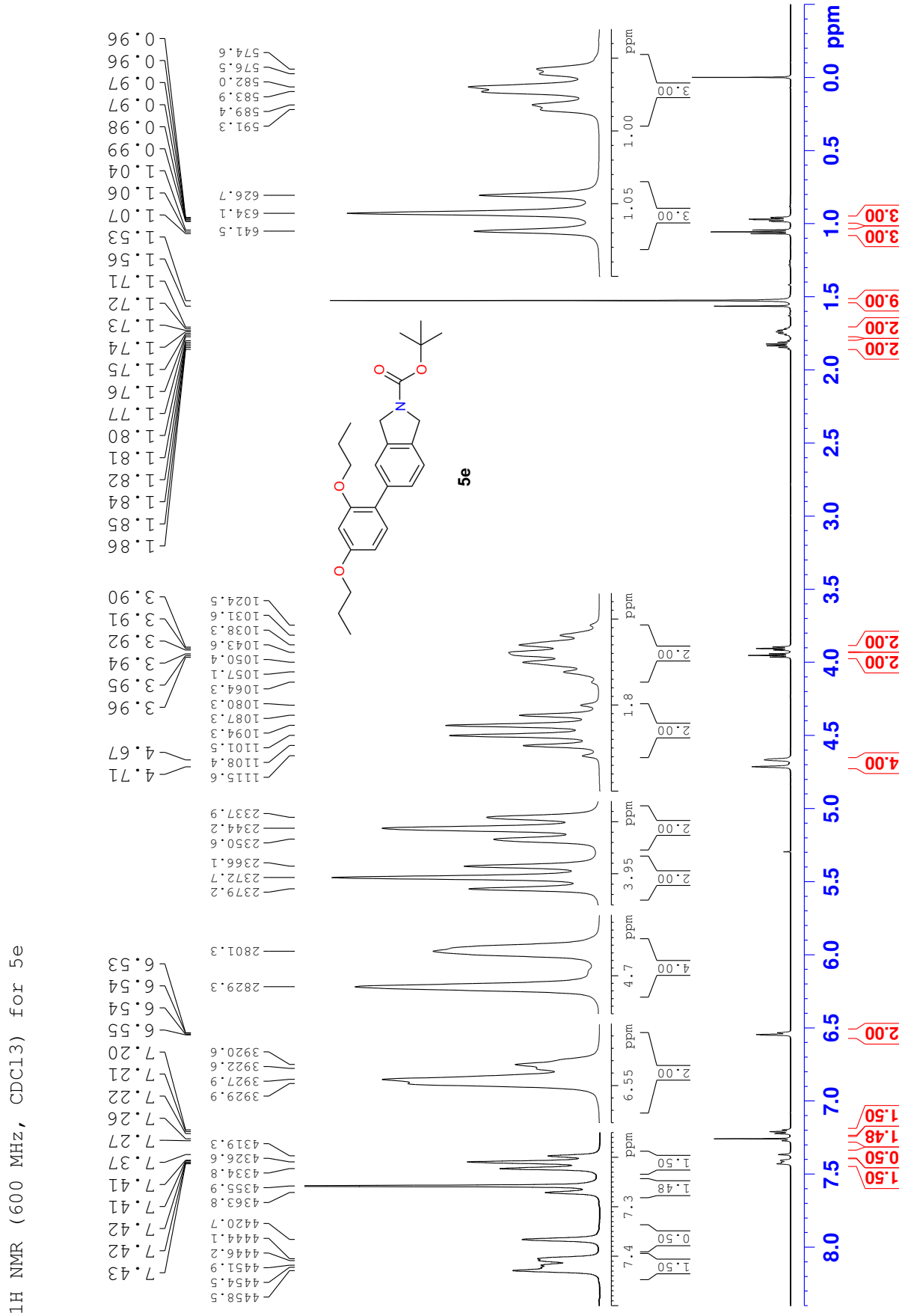
Monoisotopic Mass, Even Electron Ions
1404 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 2-500 H: 0-1000 N: 0-20 O: 0-25 S: 0-2
NT-MSLAB-Operator-SVG
2017-113 201 (3.930) AM2 (Ar,35000.0,0.00,0.00); Cm (195:213)

1: TOF MS ASAP+
6.47e+006

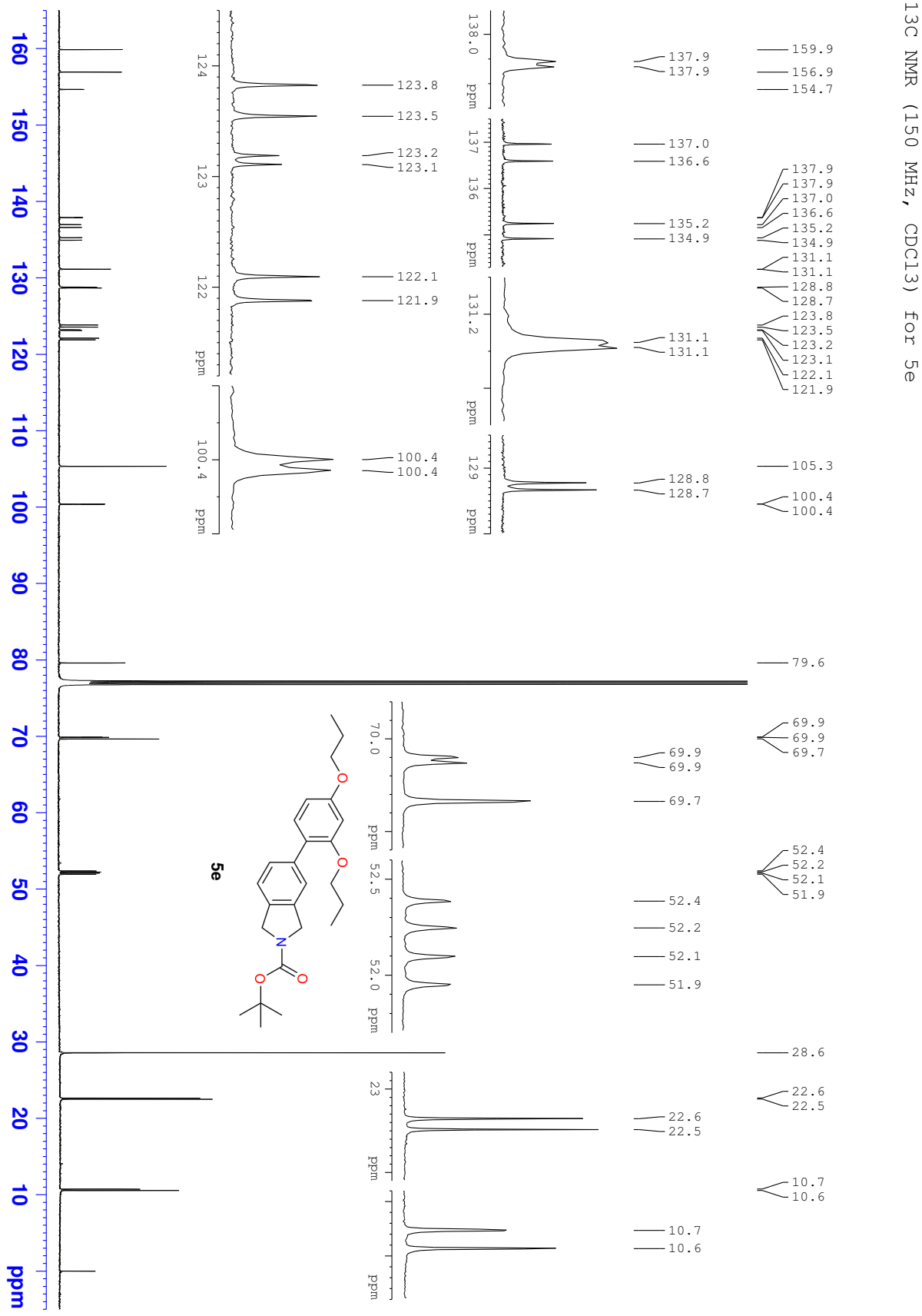


Minimum:				-1.5					
Maximum:		5.0	3.0	50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula	Ion Observed [M-C4H8+H]
312.1598	312.1600	-0.2	-0.6	9.5	233.7	0.000	100.00	C19 H22 N O3	
	312.1593	0.5	1.6	0.5	249.8	16.090	0.00	C11 H26 N3 O5 S	
	312.1605	-0.7	-2.2	2.5	244.8	11.085	0.00	C4 H18 N13 O4	
	312.1607	-0.9	-2.9	5.5	249.2	15.504	0.00	C12 H22 N7 O S	

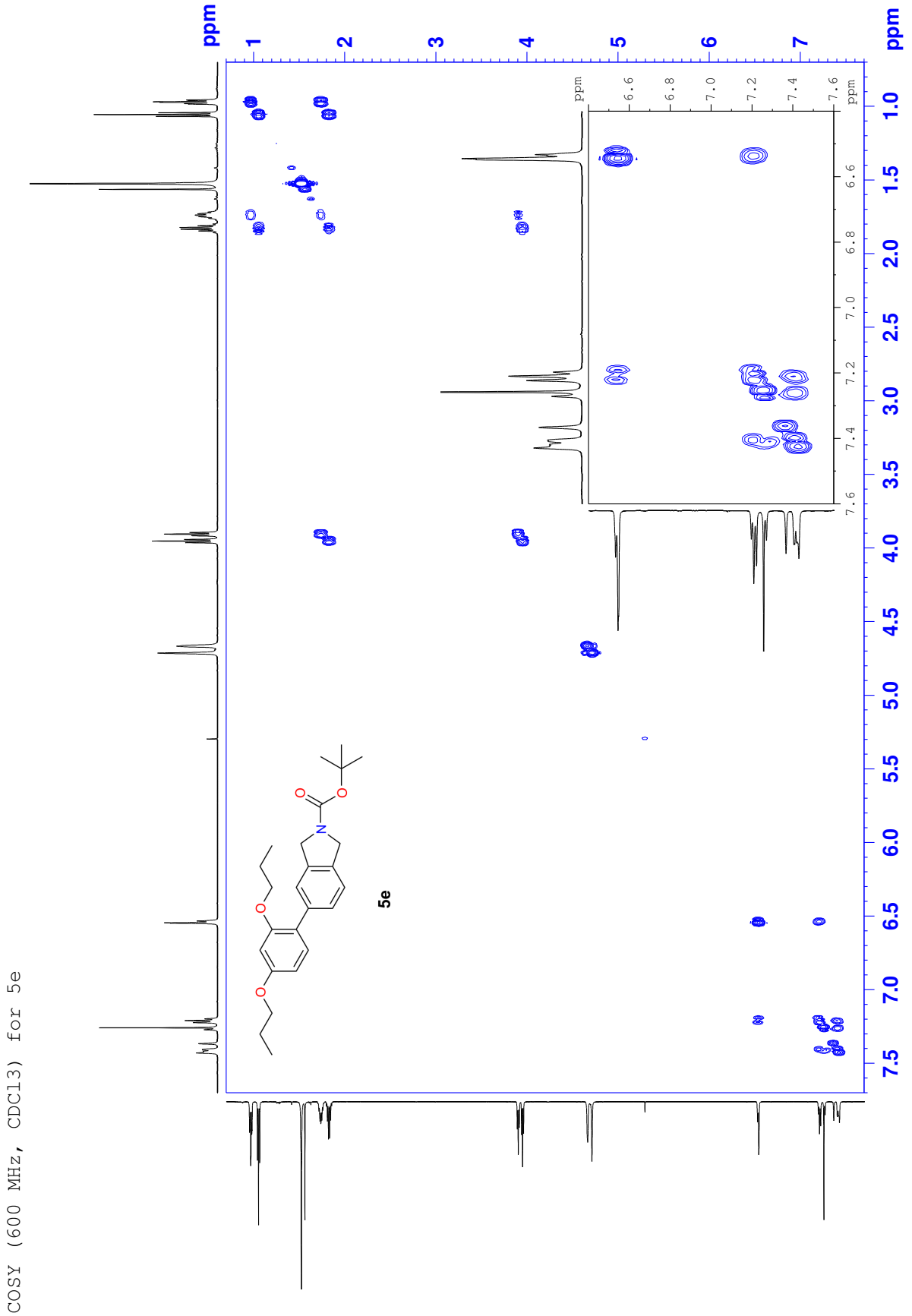
G.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5e



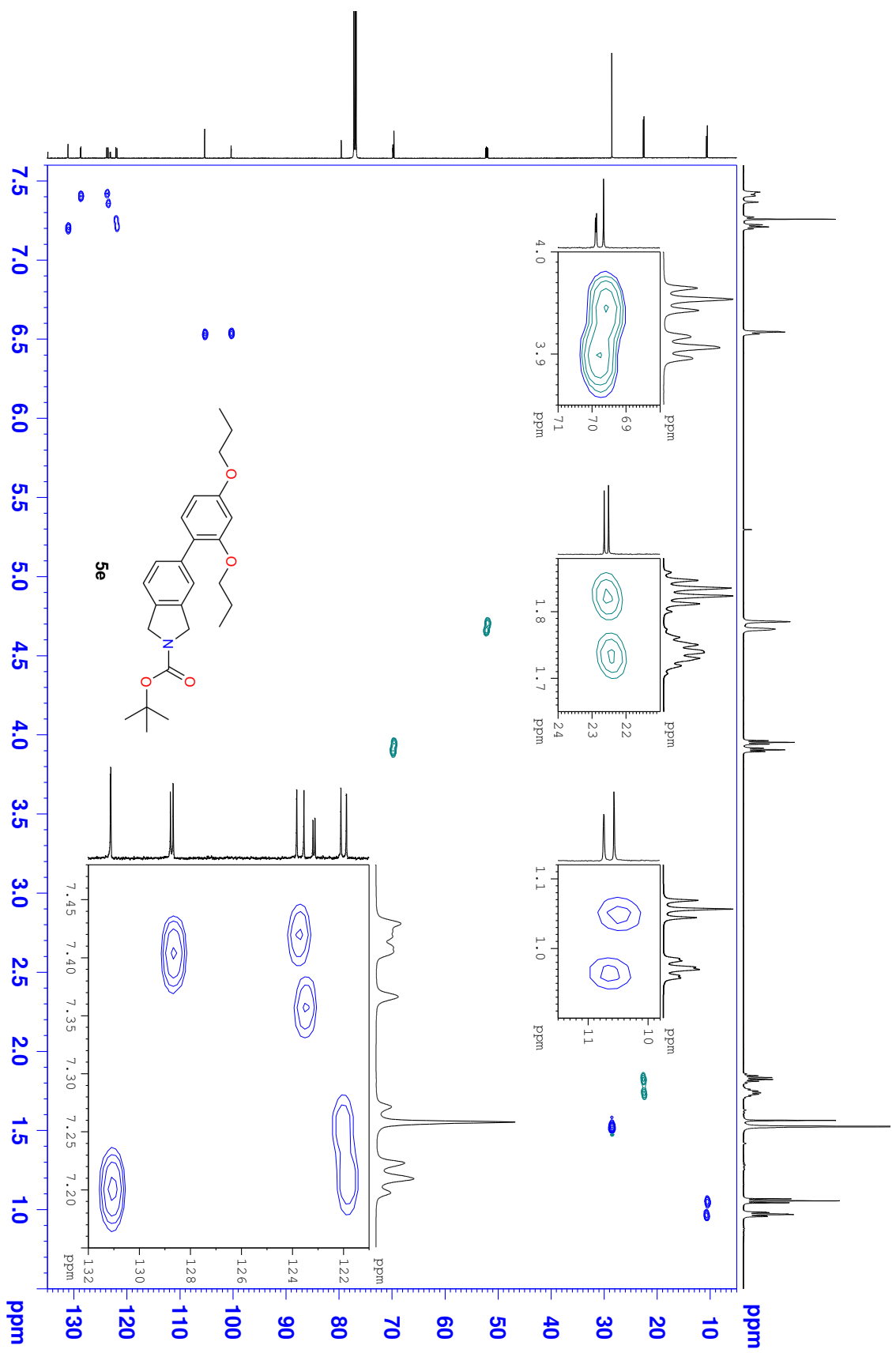
G.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5e



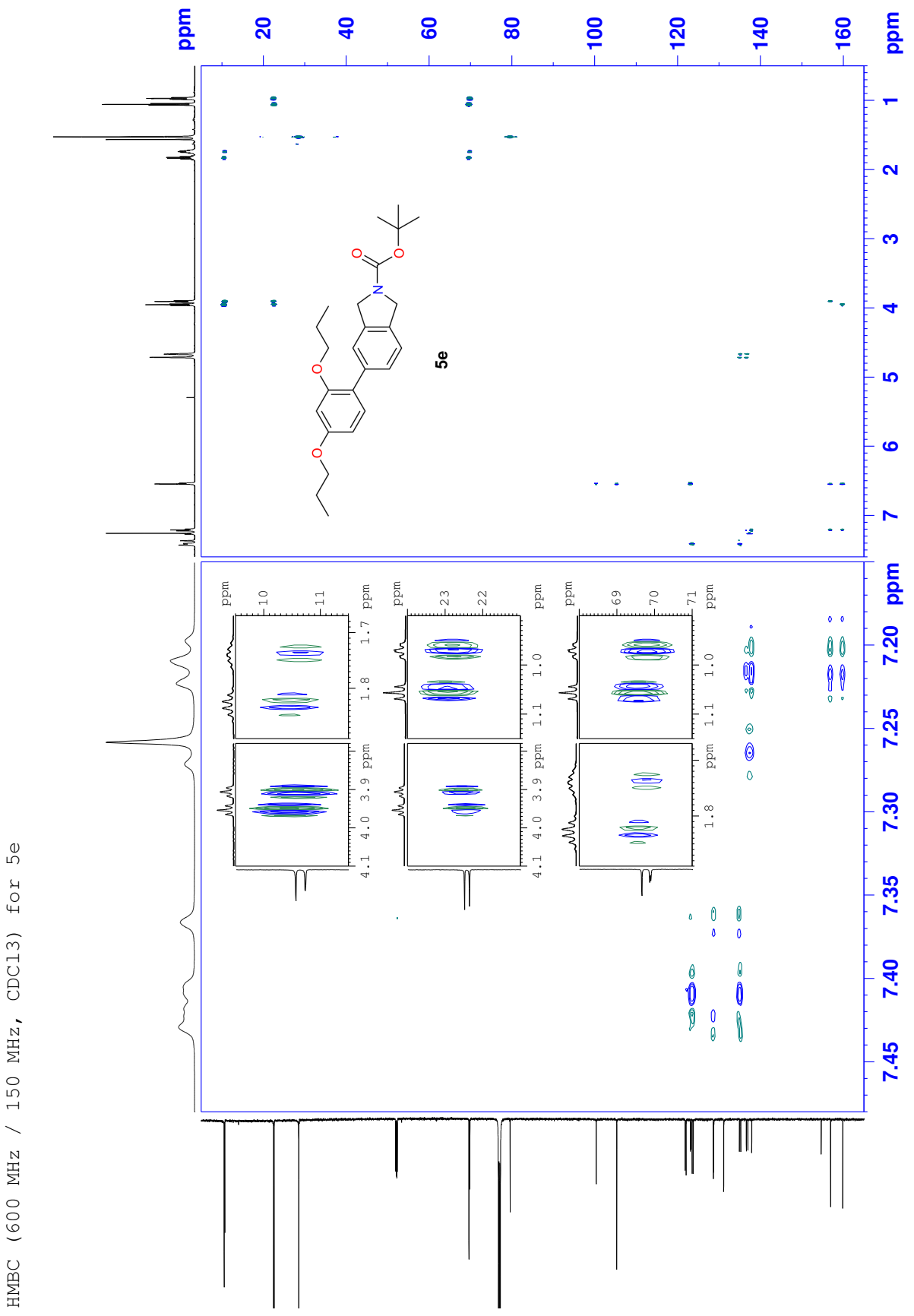
G.3 COSY (600 MHz, $CDCl_3$) spectrum for 5e



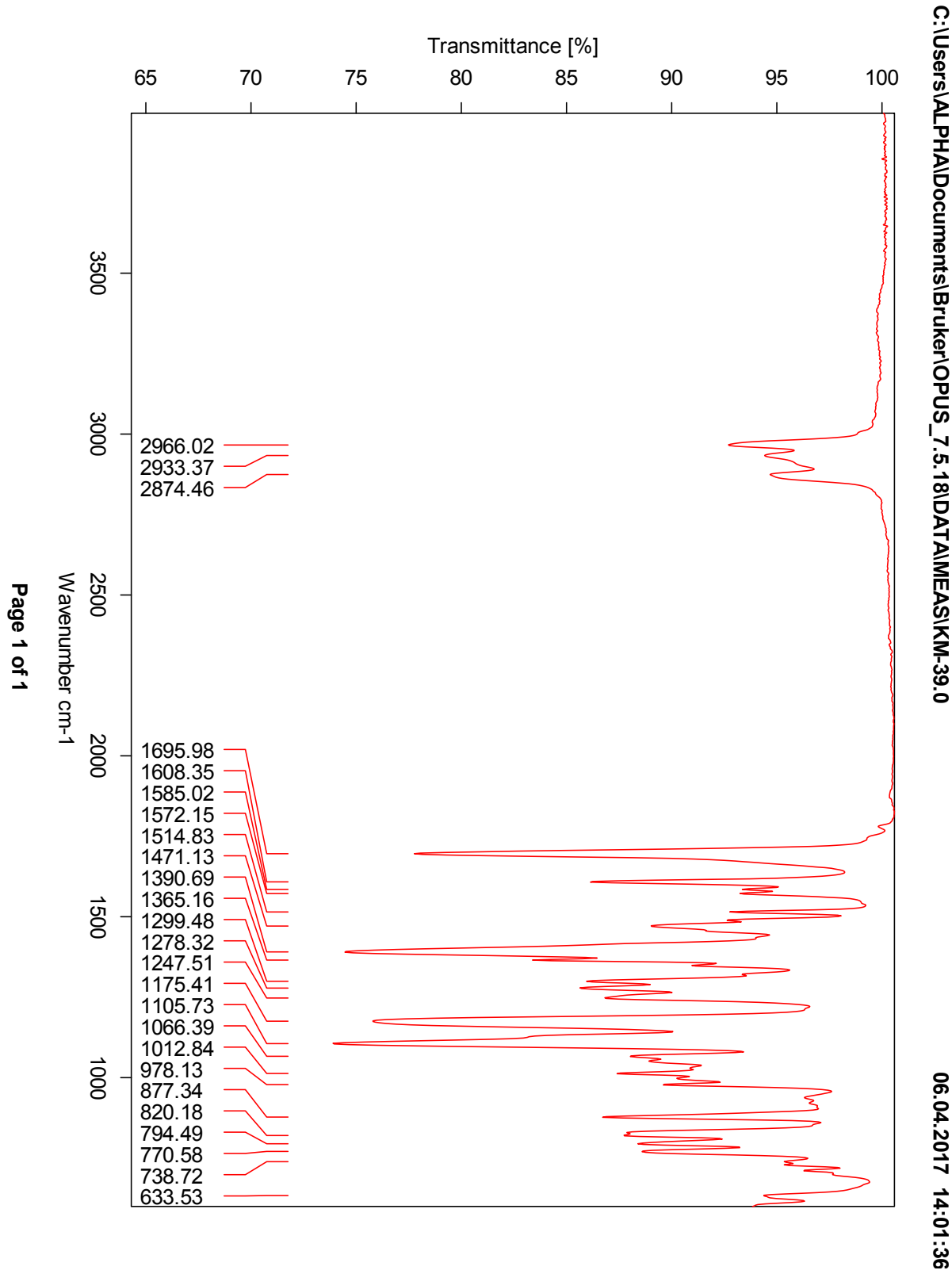
G.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5e



G.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5e



G.6 IR spectrum for 5e

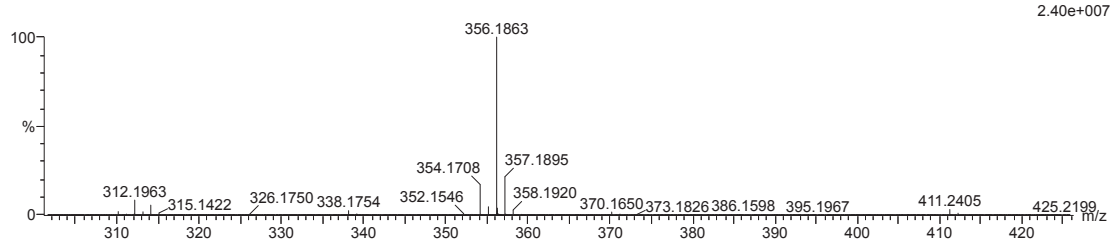


G.7 HRMS spectrum for 5e

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

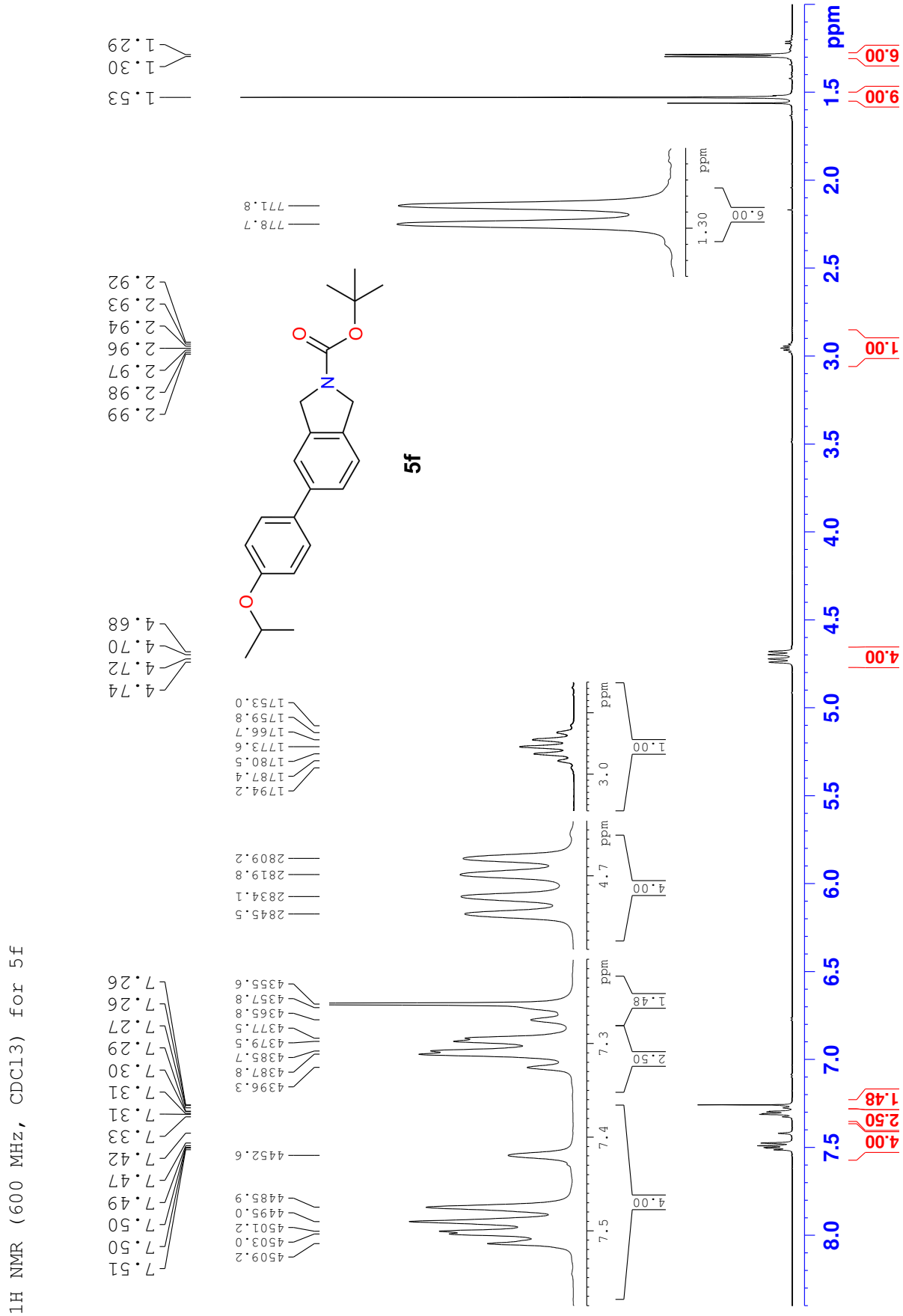
Monoisotopic Mass, Even Electron Ions
574 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 O: 0-25
2017-182 72 (1.412) AM2 (Ar,35000.0,0.00,0.00); Cm (68:72)
1: TOF MS ASAP+



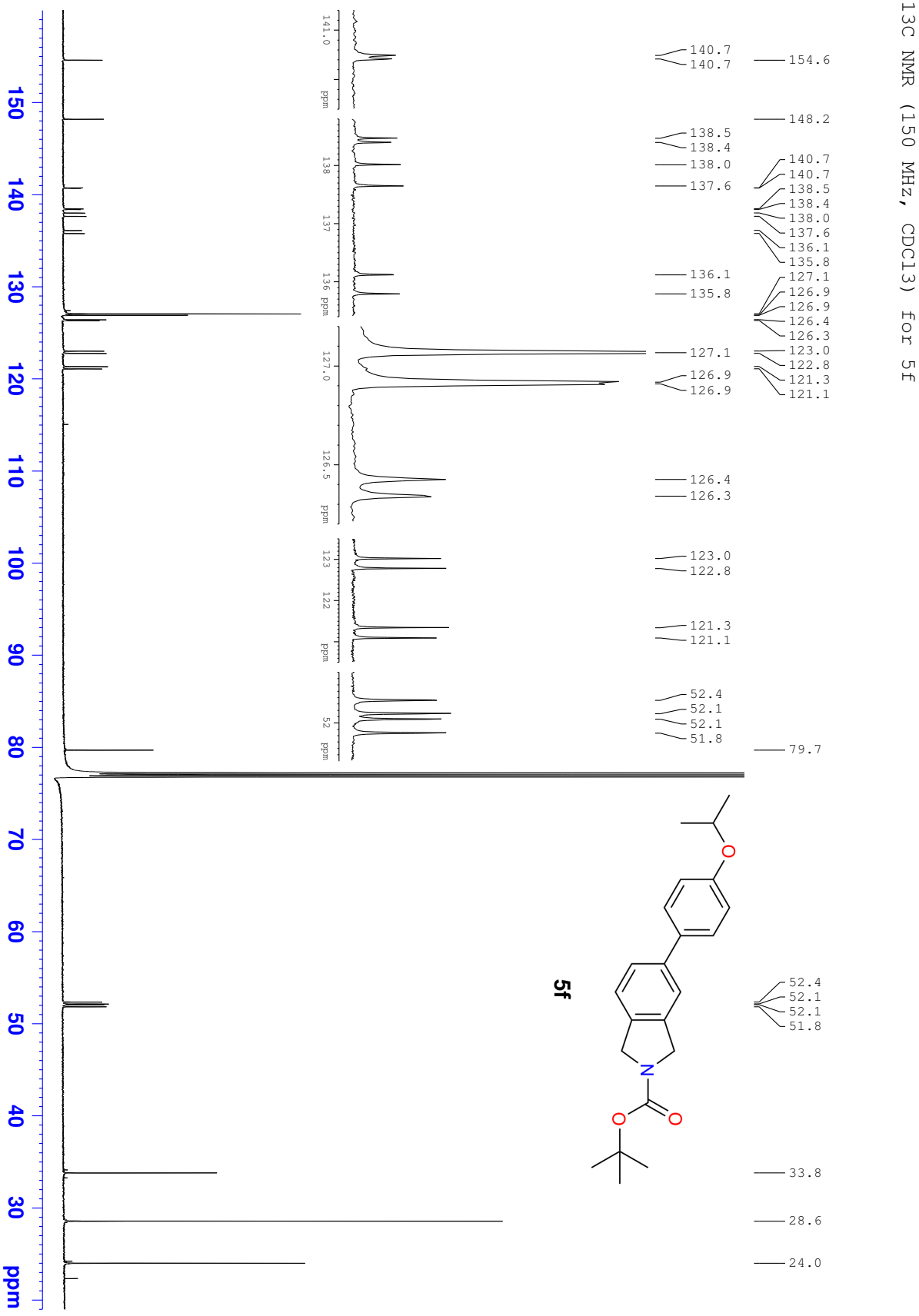
Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
356.1863	356.1862	0.1	0.3	9.5	1775.7	n/a	n/a	C21 H26 N O4

ion observed [M-C4H8+H]

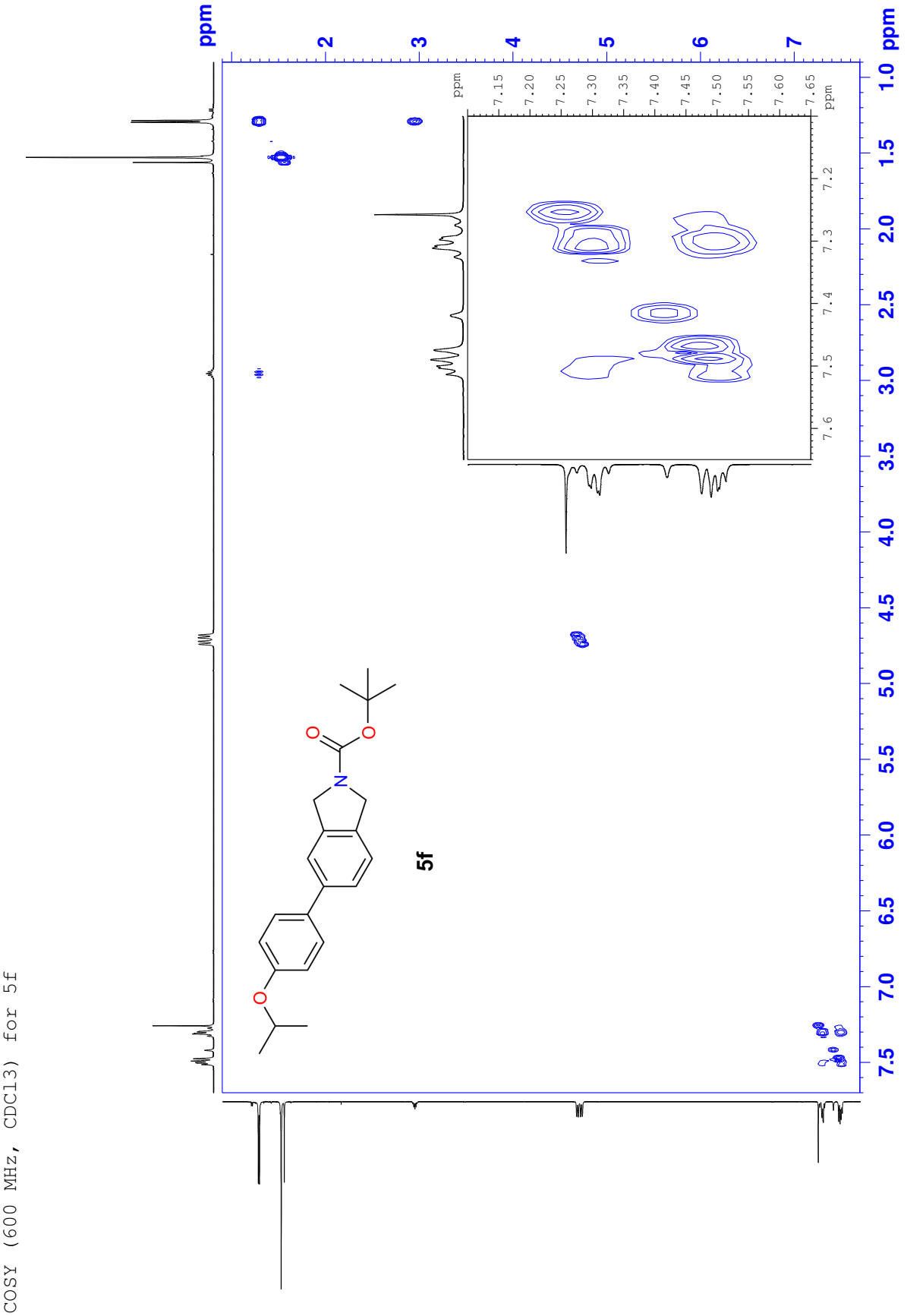
H.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5f



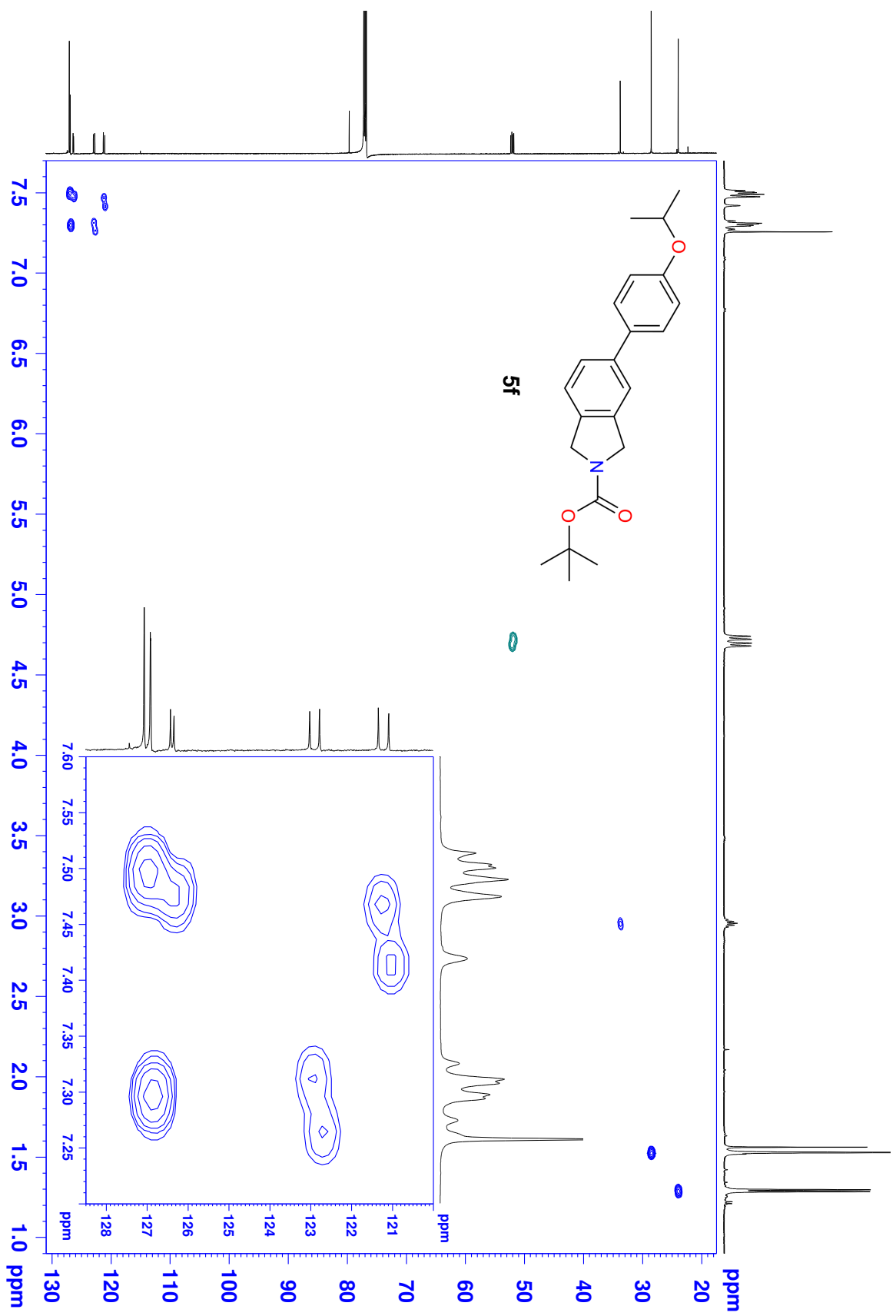
H.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5f



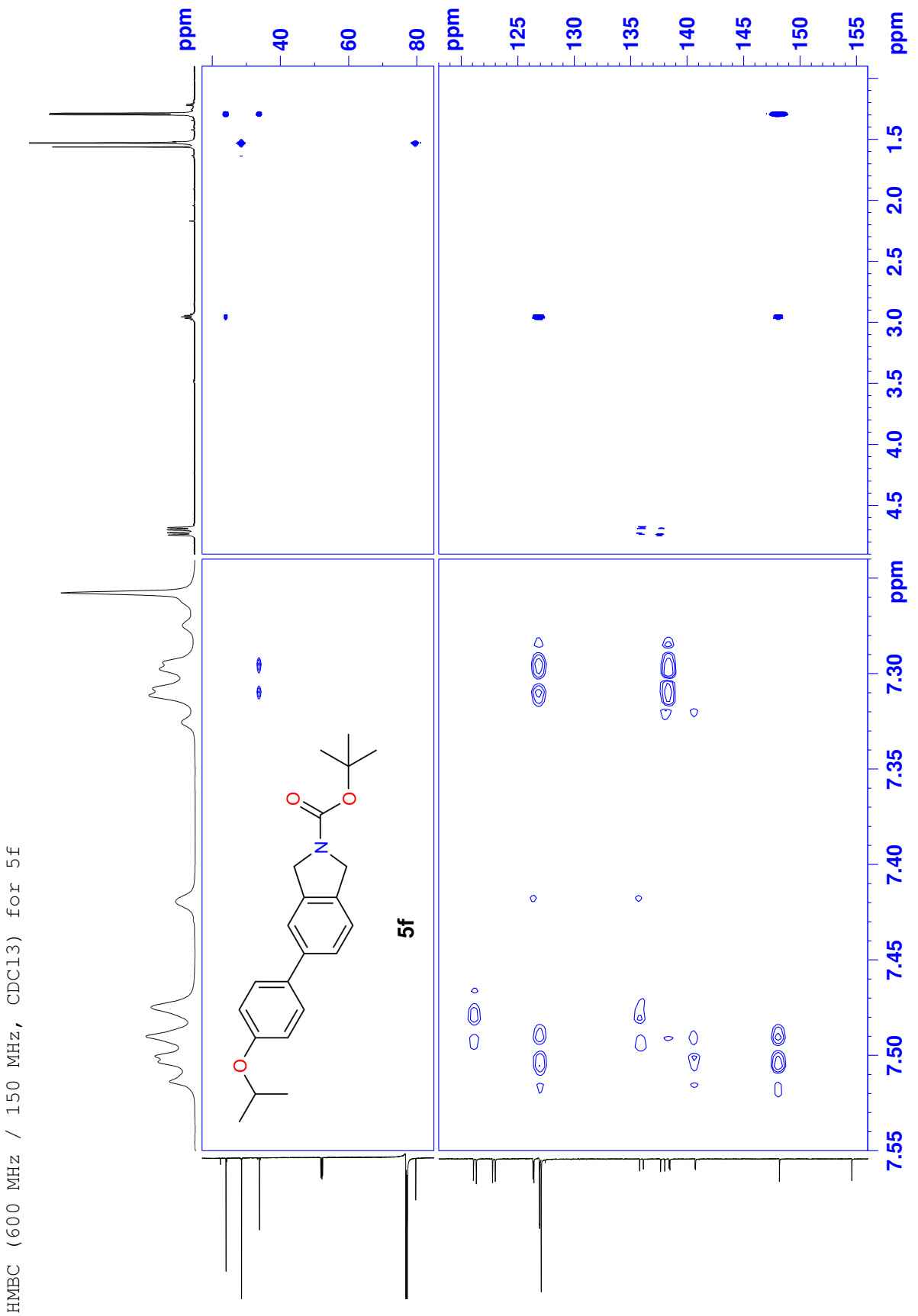
H.3 COSY (600 MHz, $CDCl_3$) spectrum for 5f



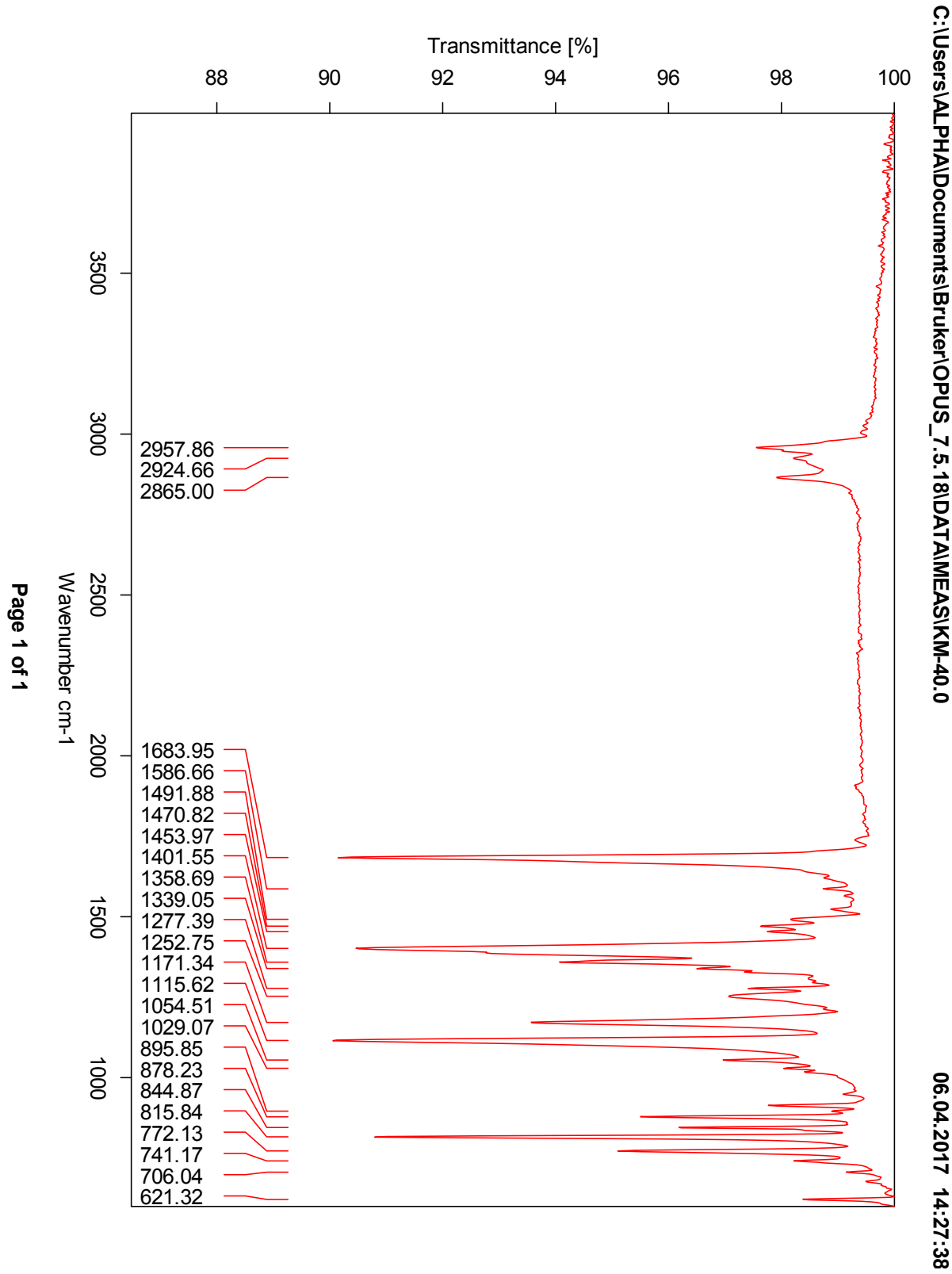
H.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5f



H.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5f



H.6 IR spectrum for 5f

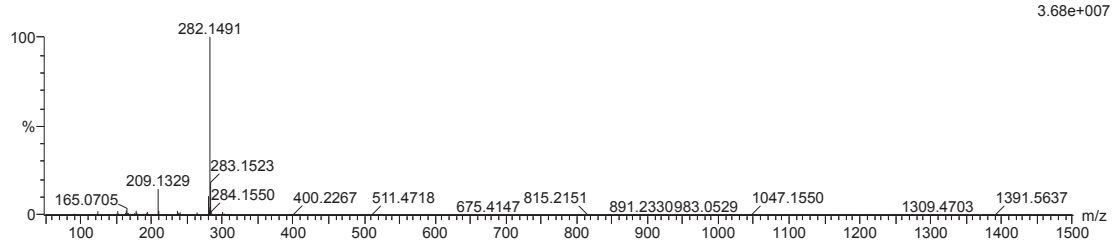


H.7 HRMS spectrum for 5f

Elemental Composition Report **Page 1**

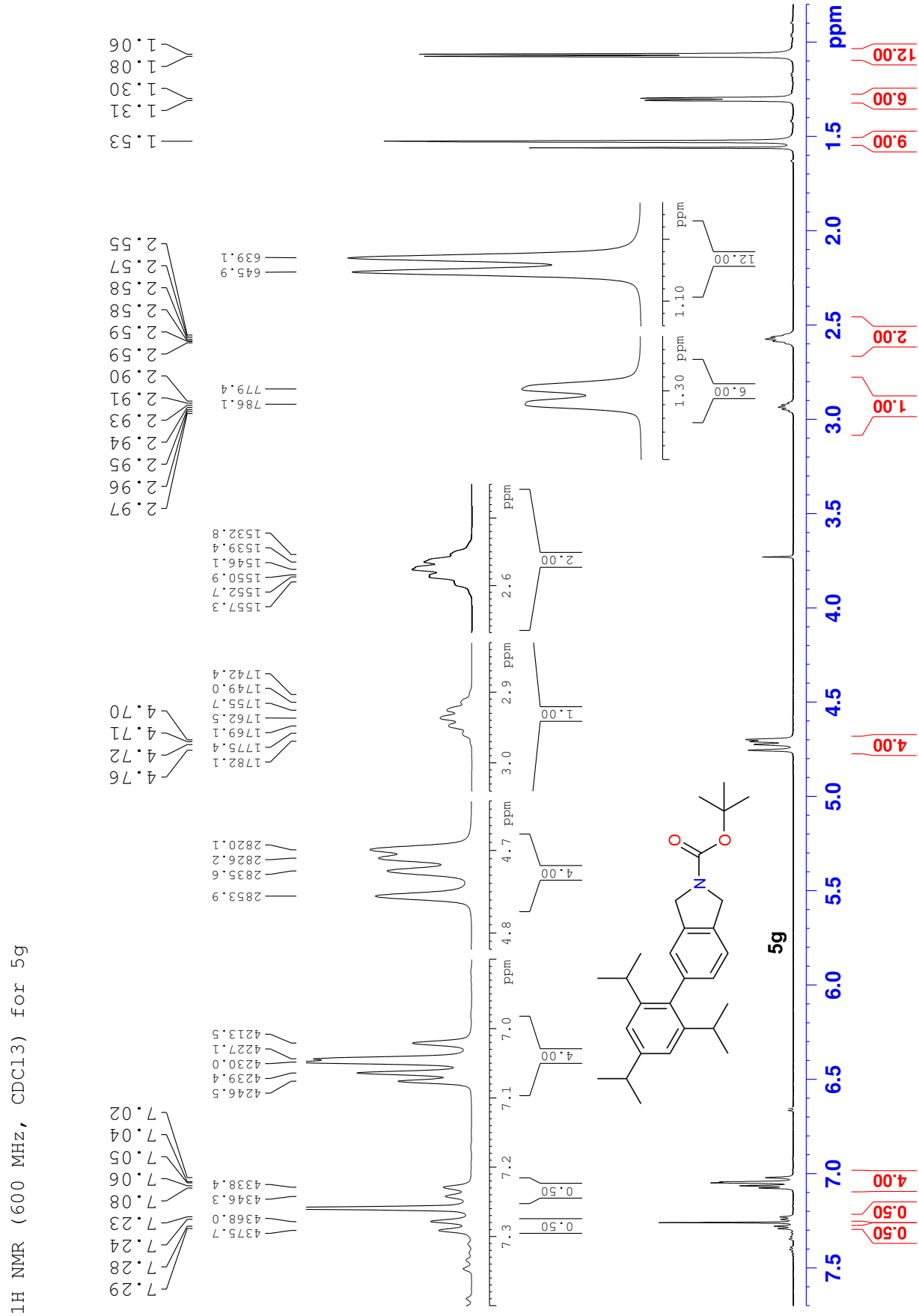
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
365 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 O: 0-25
2017-183 42 (0.846) AM2 (Ar,35000.0,0.00,0.00); Cm (37:44)
1: TOF MS ASAP+

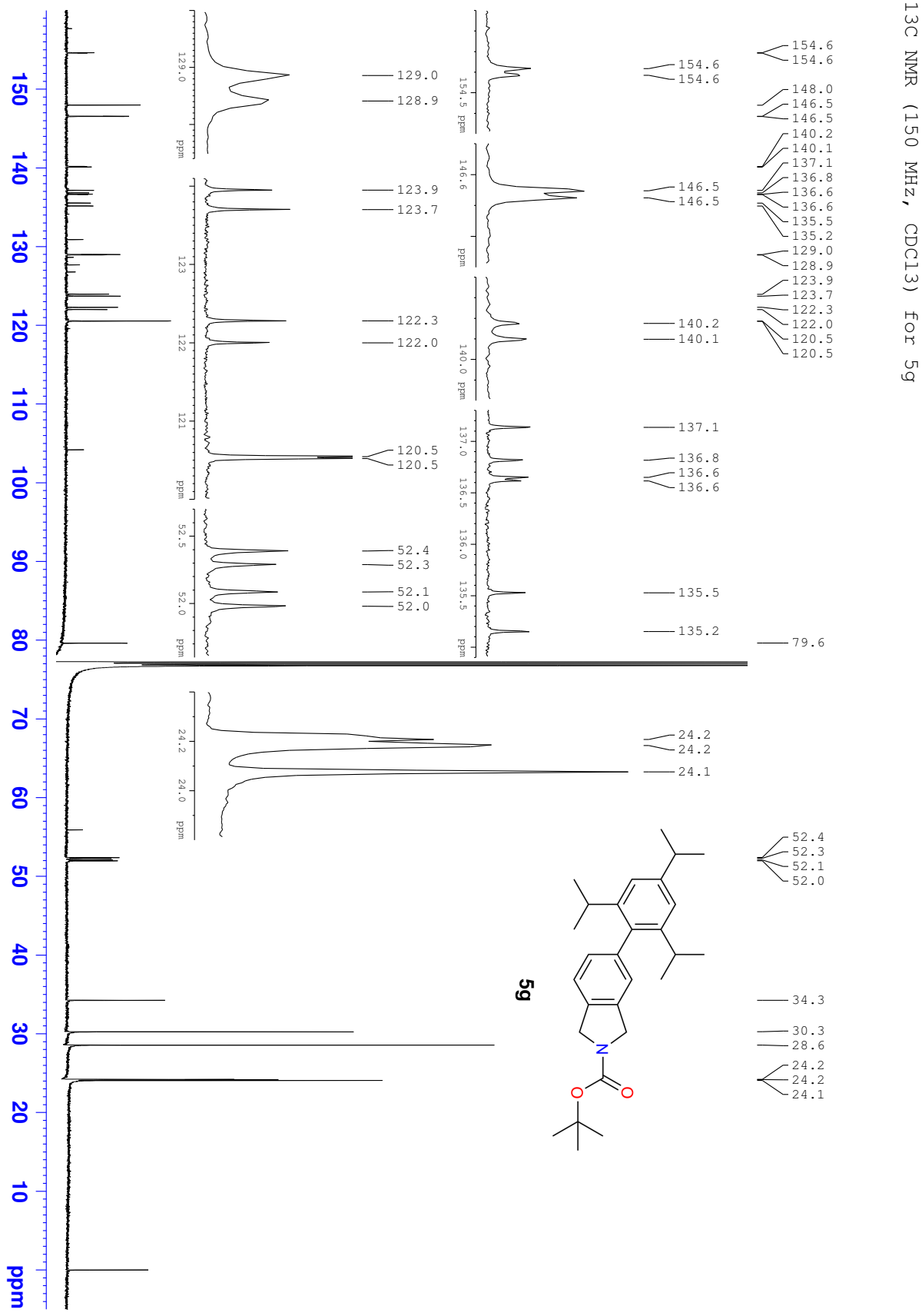


Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
282.1491	282.1494	-0.3	-1.1	9.5	2012.1	n/a	n/a	C18 H20 N O2 [M-C4H8O+H]

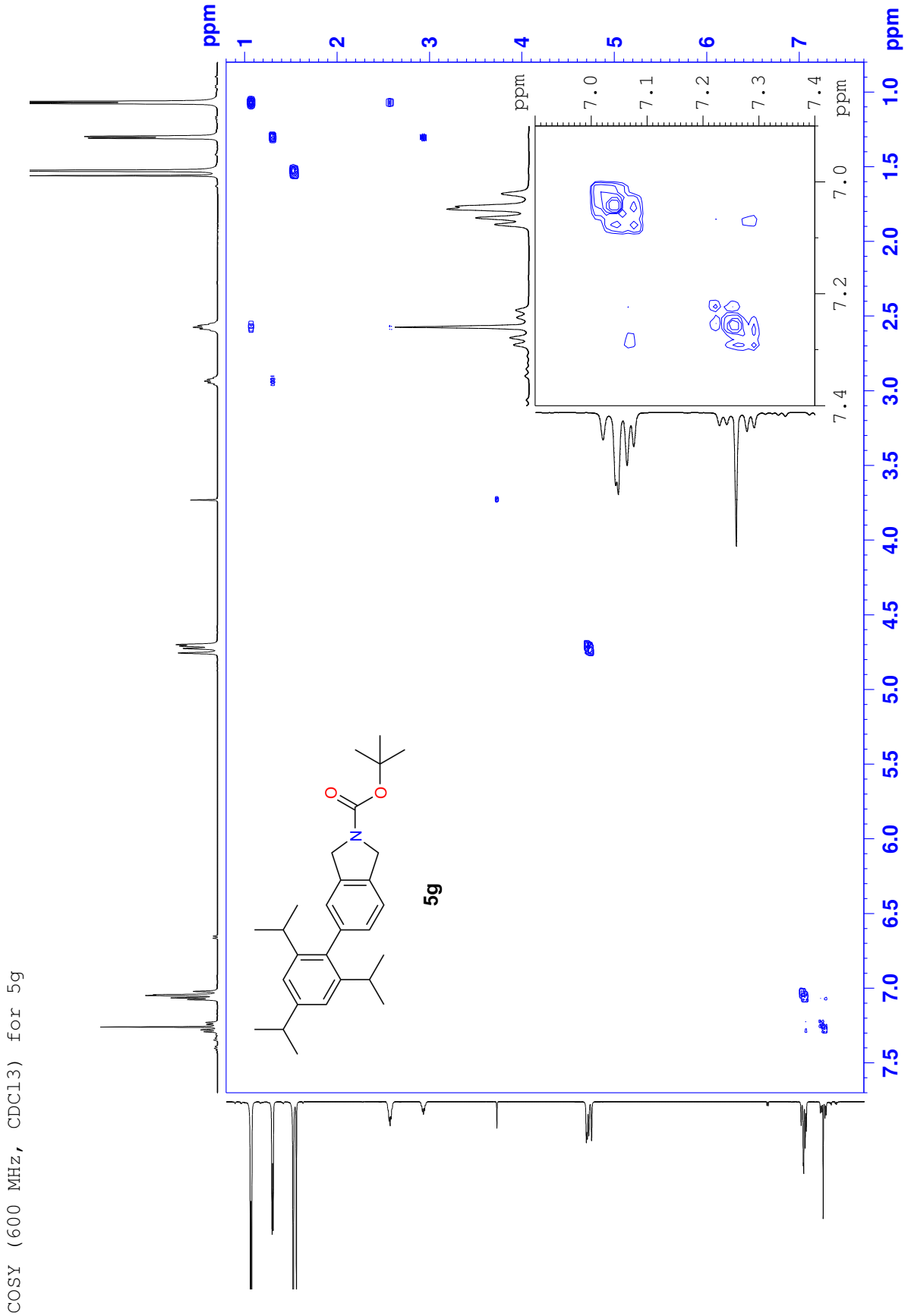
I.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5g



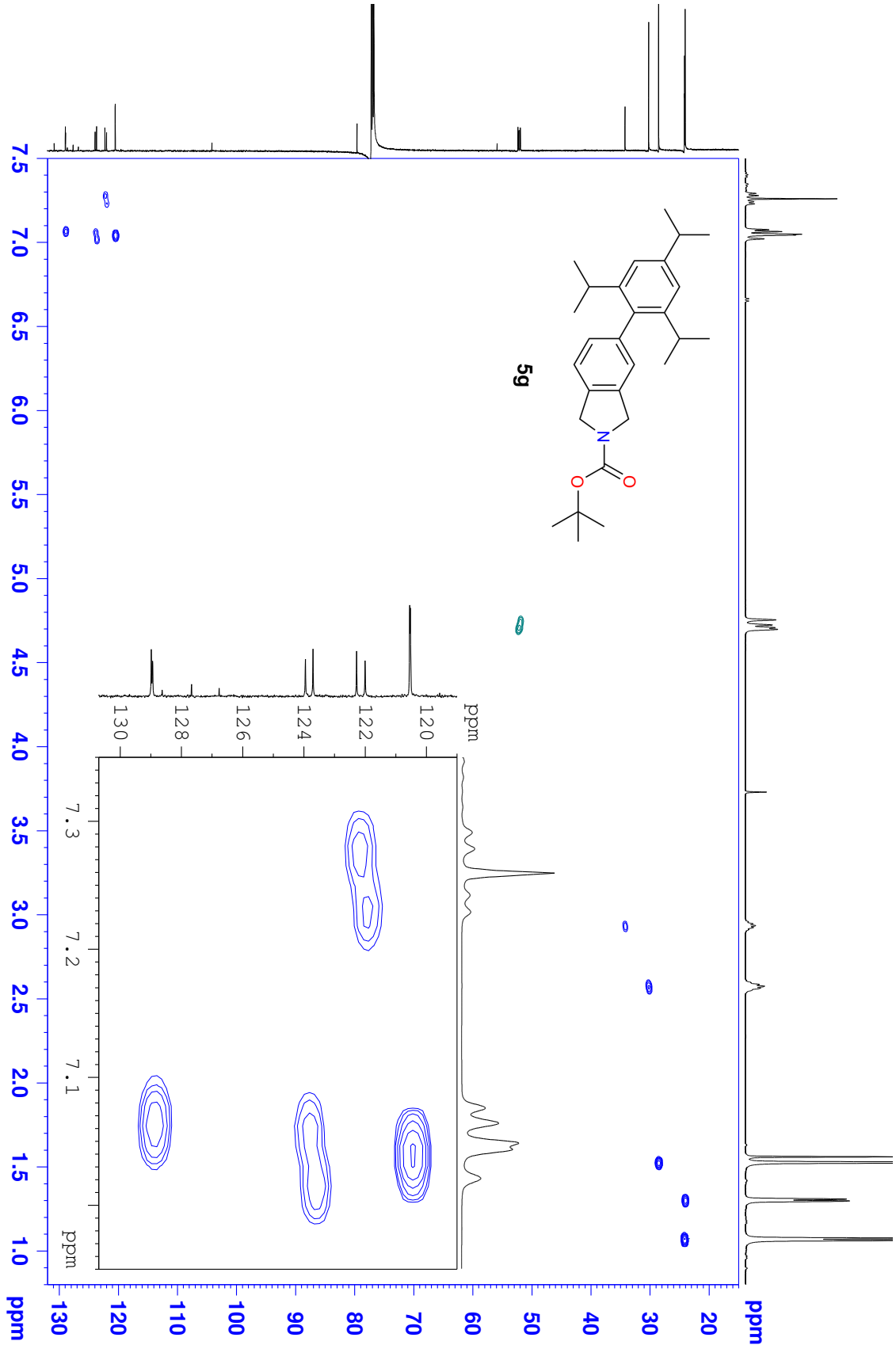
I.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5g



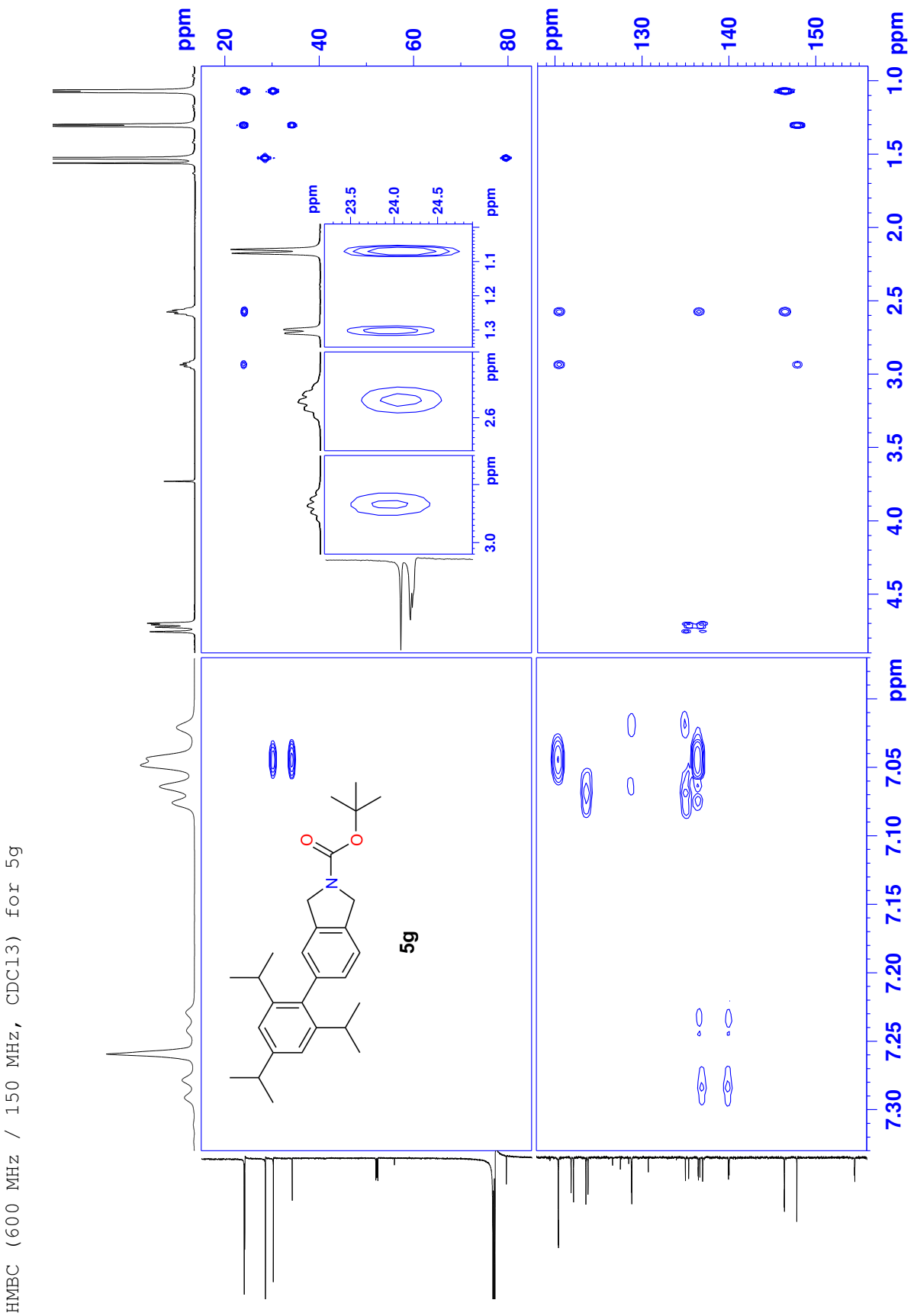
I.3 COSY (600 MHz, $CDCl_3$) spectrum for 5g



I.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5g



I.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5g

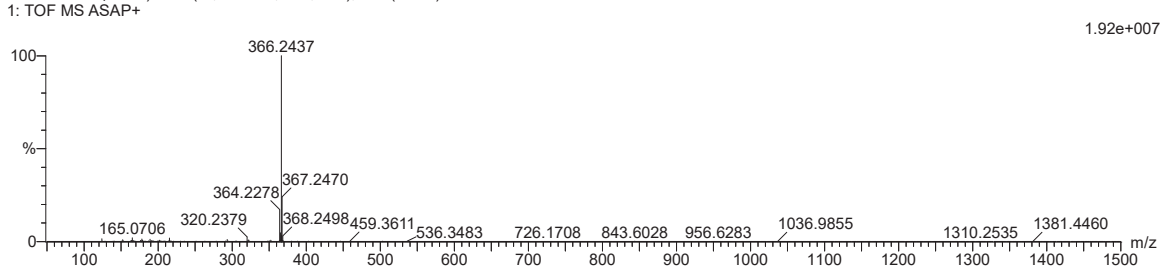


I.6 HRMS spectrum for 5g

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

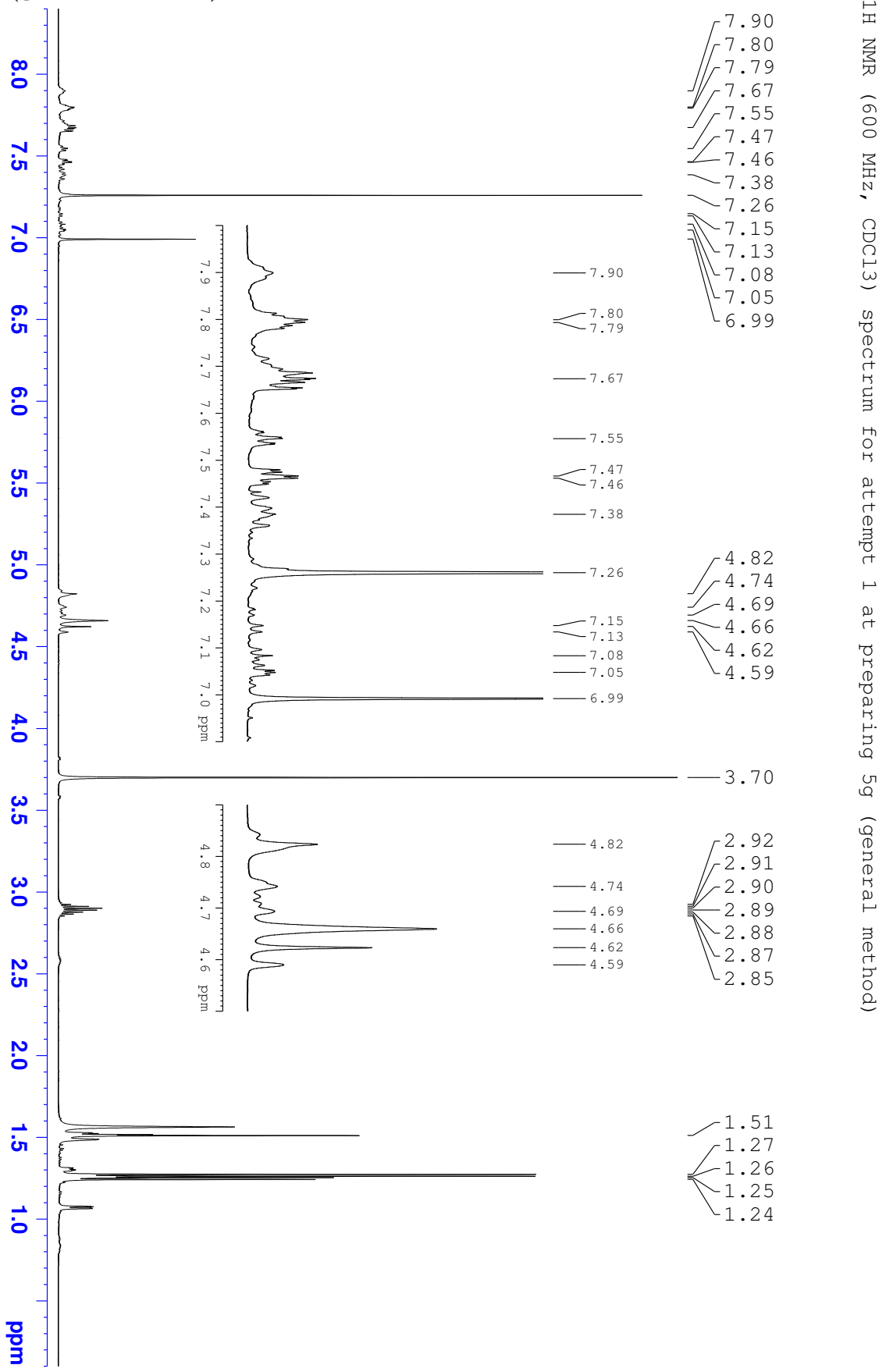
Monoisotopic Mass, Even Electron Ions
977 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200
2017-291 32 (0.637) AM2 (Ar,35000.0,0.00,0.00); Cm (27:32)
1: TOF MS ASAP+



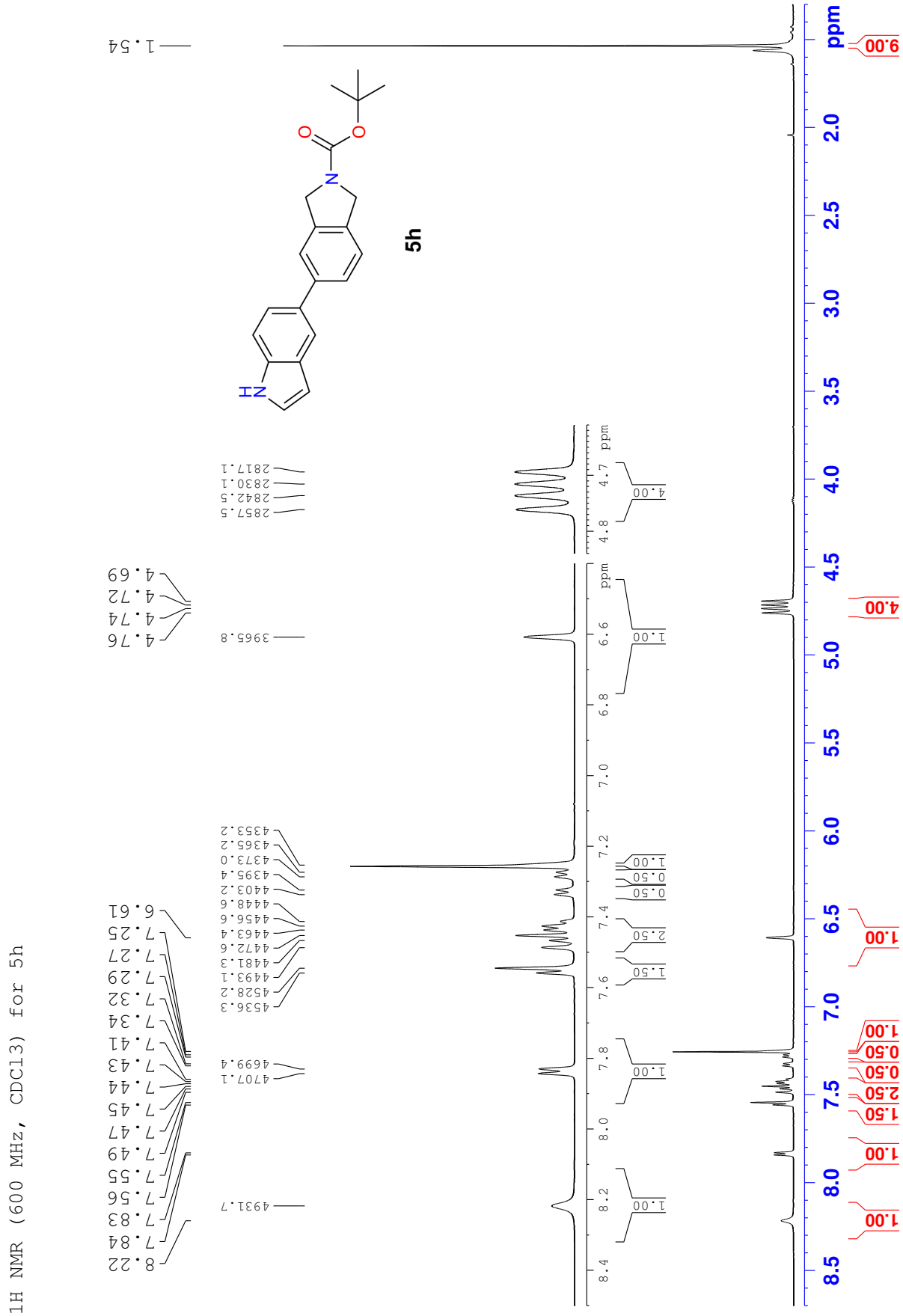
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
366.2437	366.2433	0.4	1.1	9.5	1662.2	0.000	100.00	C24 H32 N O2
	366.2438	-0.1	-0.3	2.5	1672.3	10.106	0.00	C9 H28 N13 O3

Ion Observed
[M-C4H8+H]+

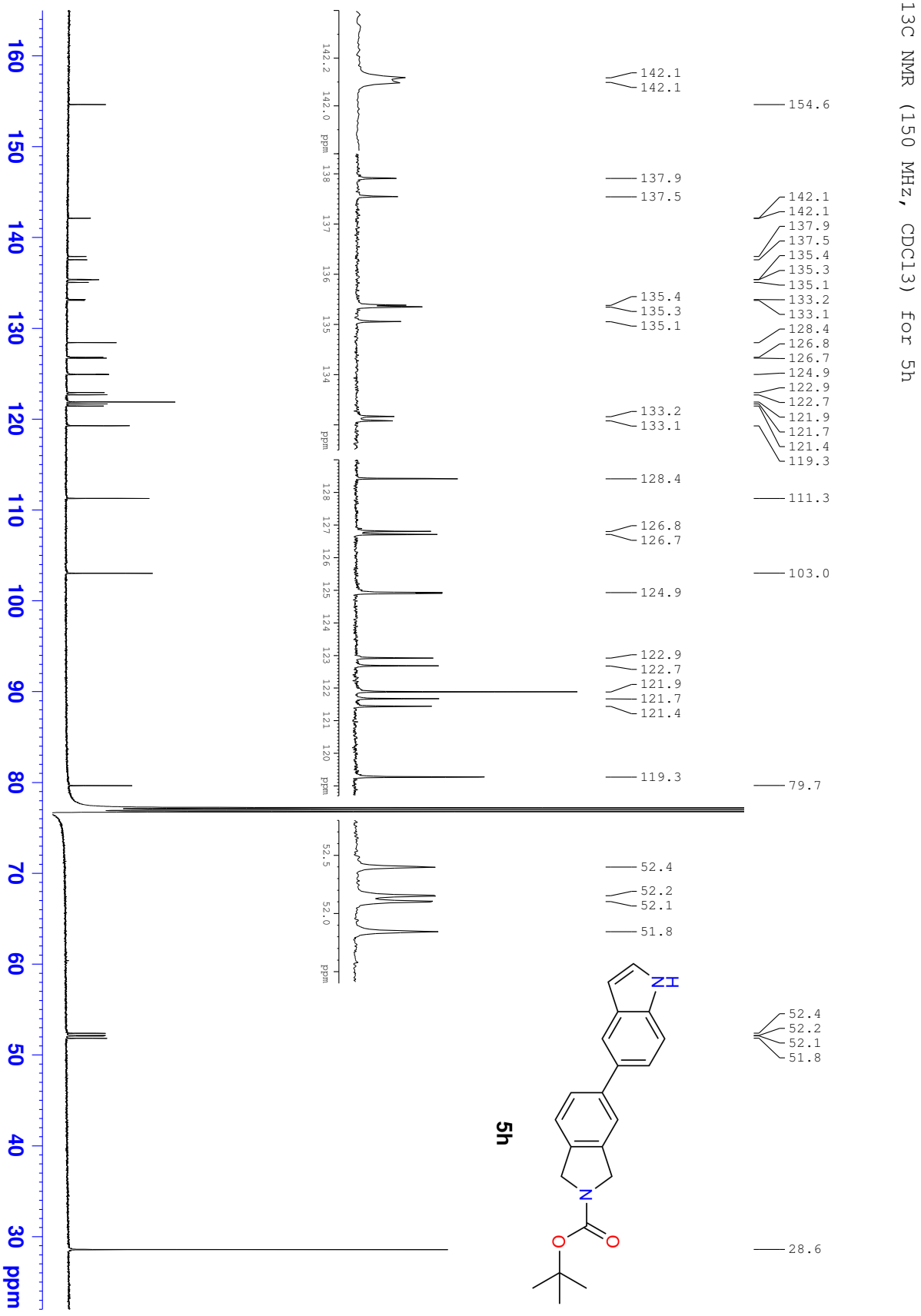
I.7 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for attempt 1 at preparing 5g (general method)



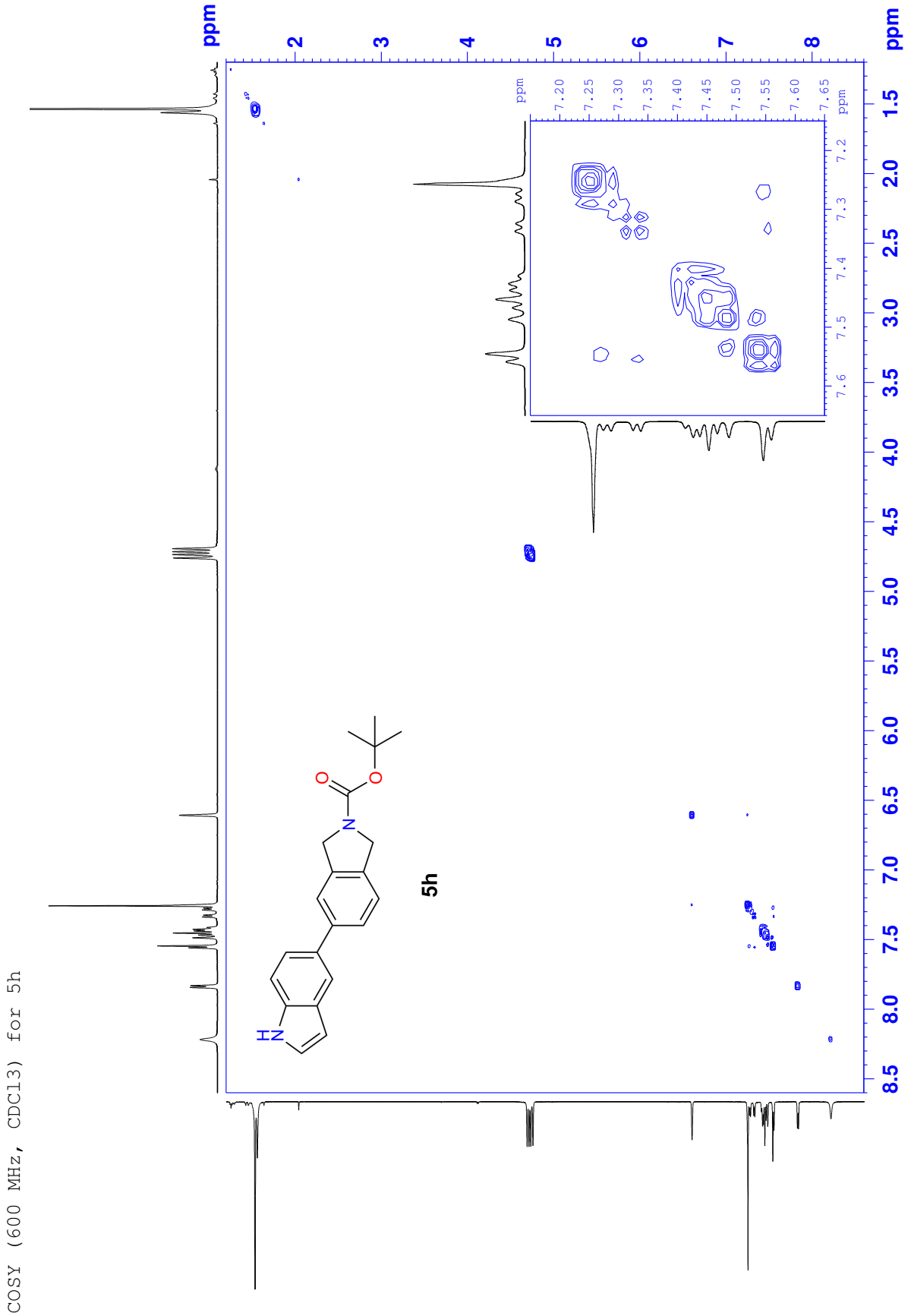
J.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5h



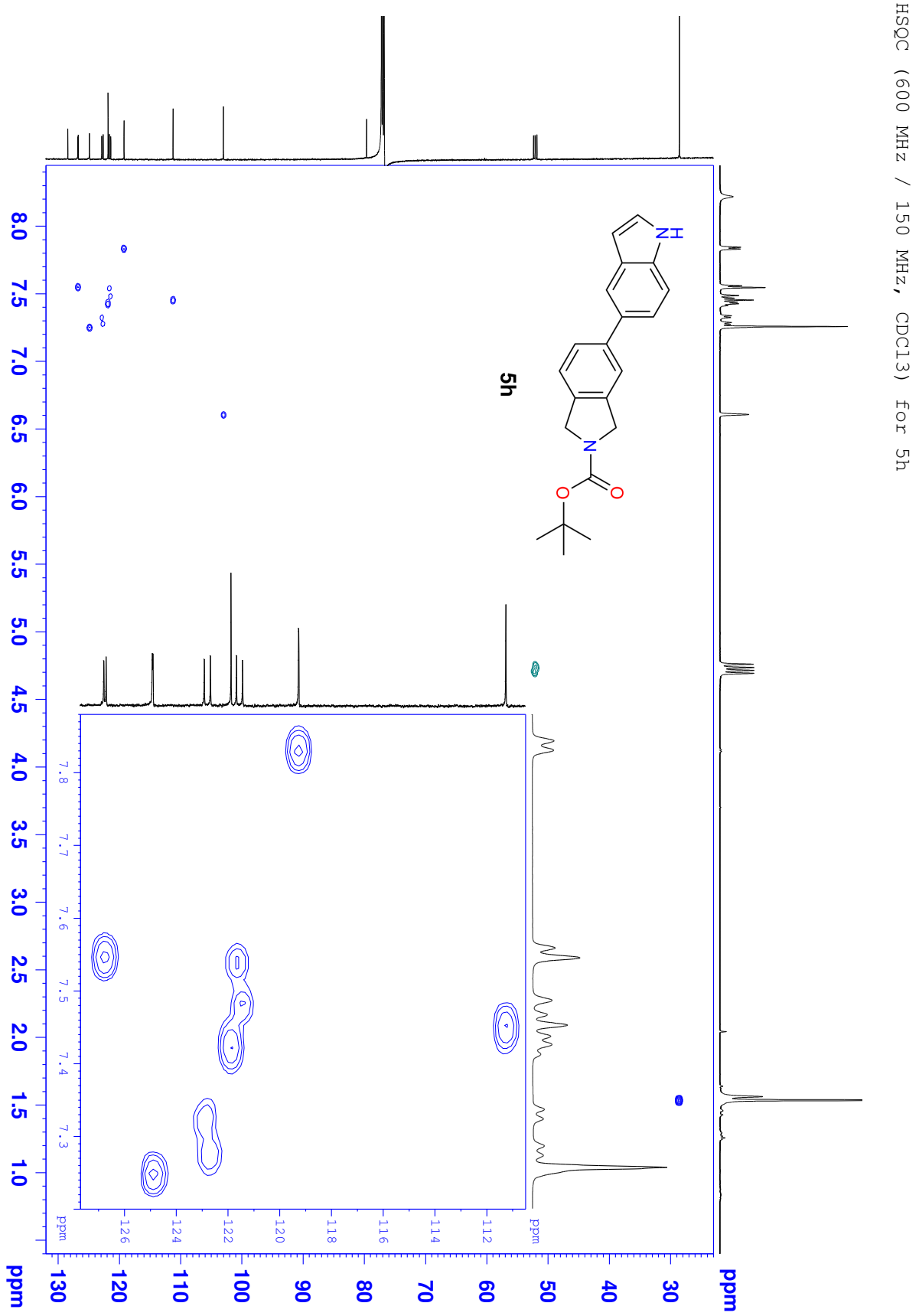
J.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5h



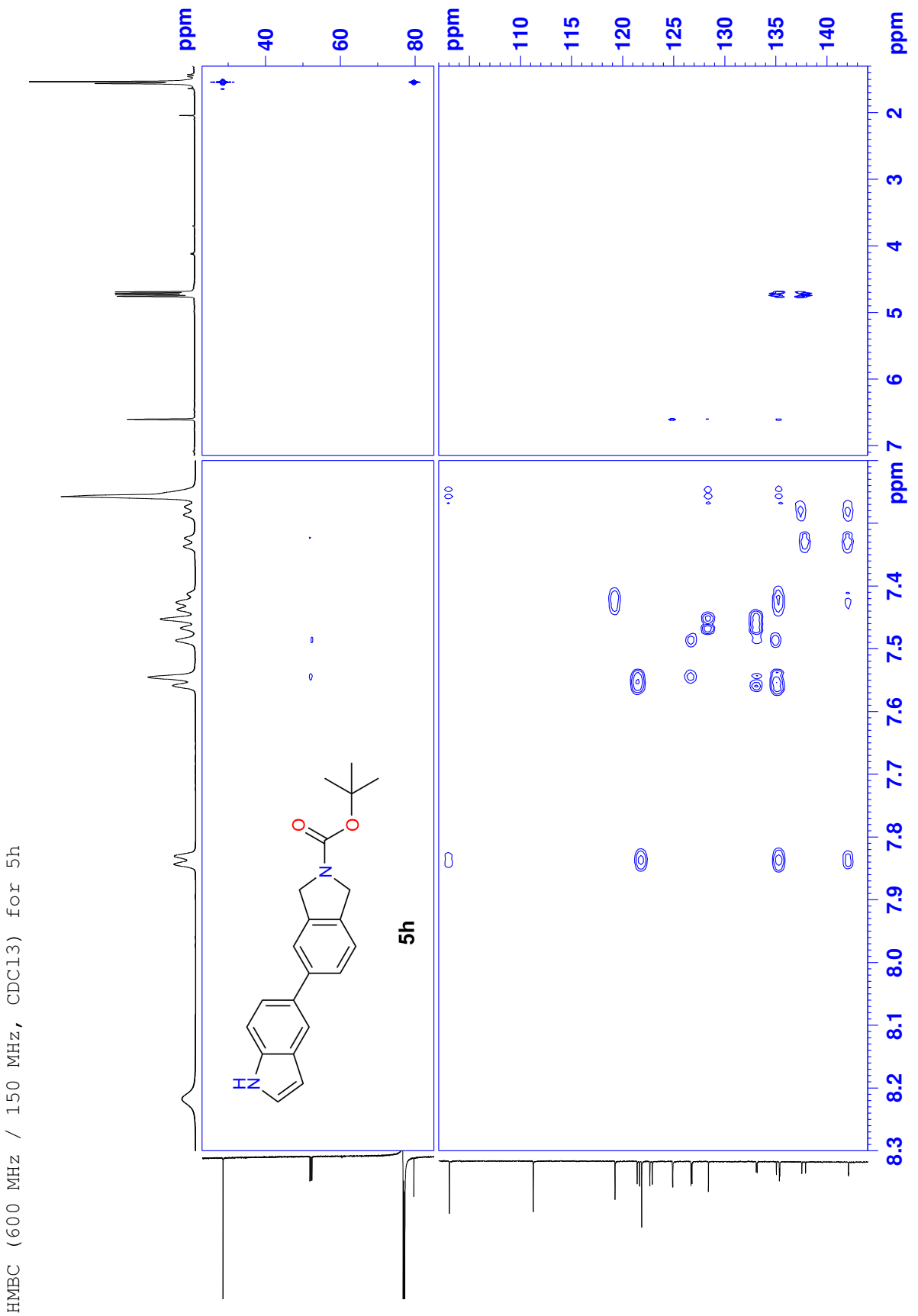
J.3 COSY (600 MHz, $CDCl_3$) spectrum for 5h



J.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5h



J.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5h

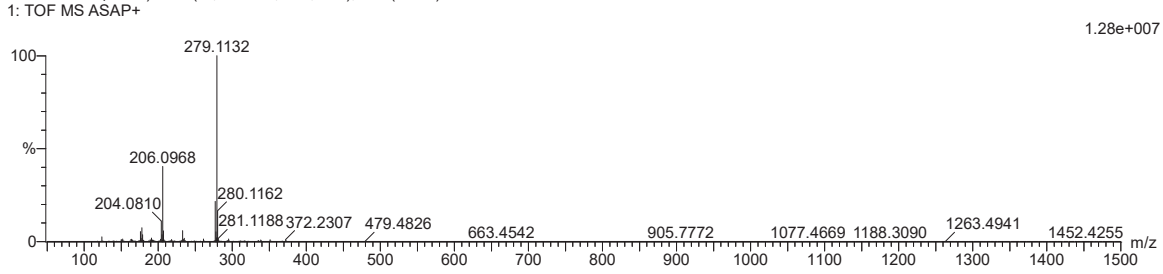


J.6 HRMS spectrum for 5h

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

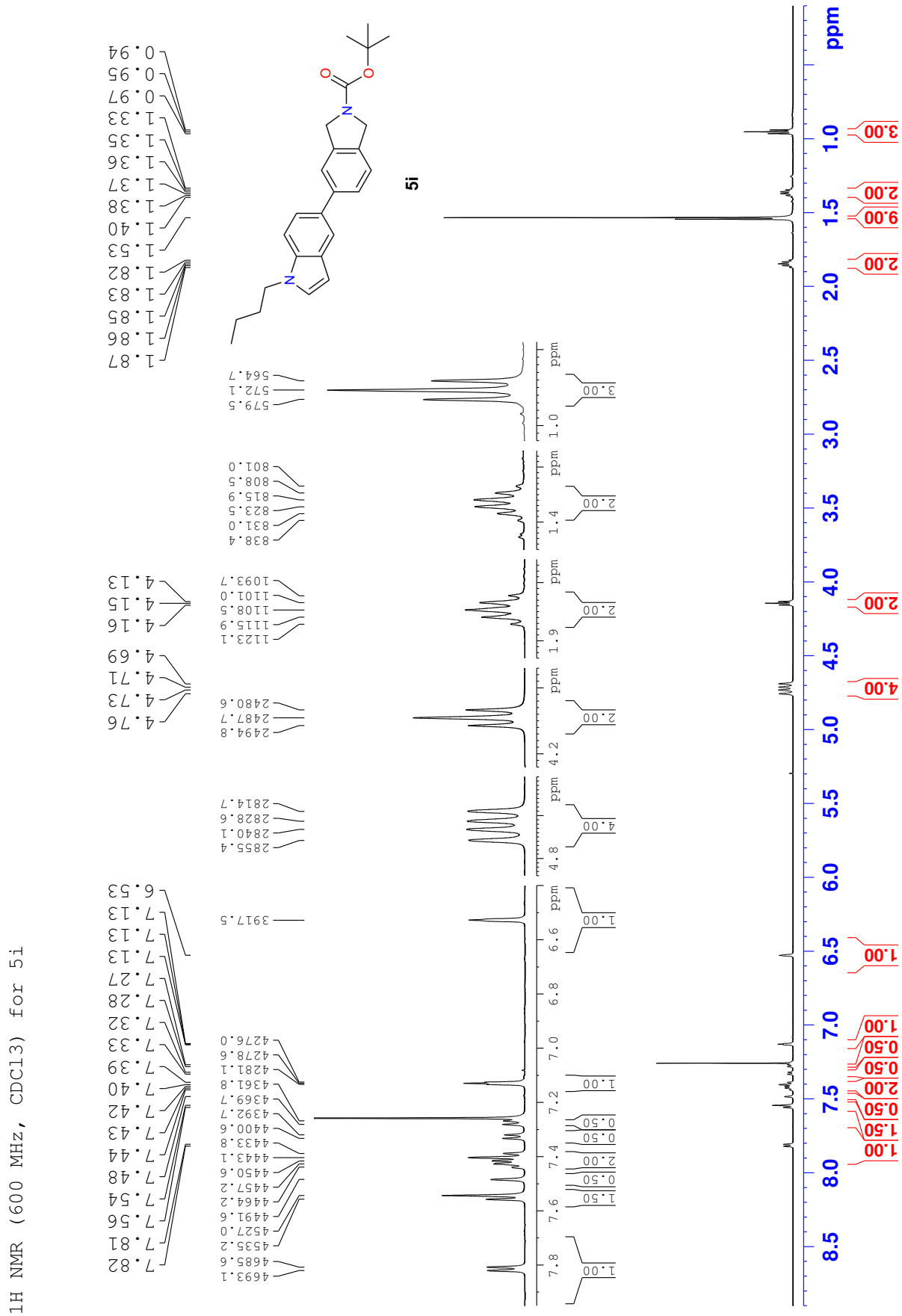
Monoisotopic Mass, Even Electron Ions
507 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200
2017-288 83 (1.638) AM2 (Ar,35000.0,0.00,0.00); Cm (76:86)
1: TOF MS ASAP+



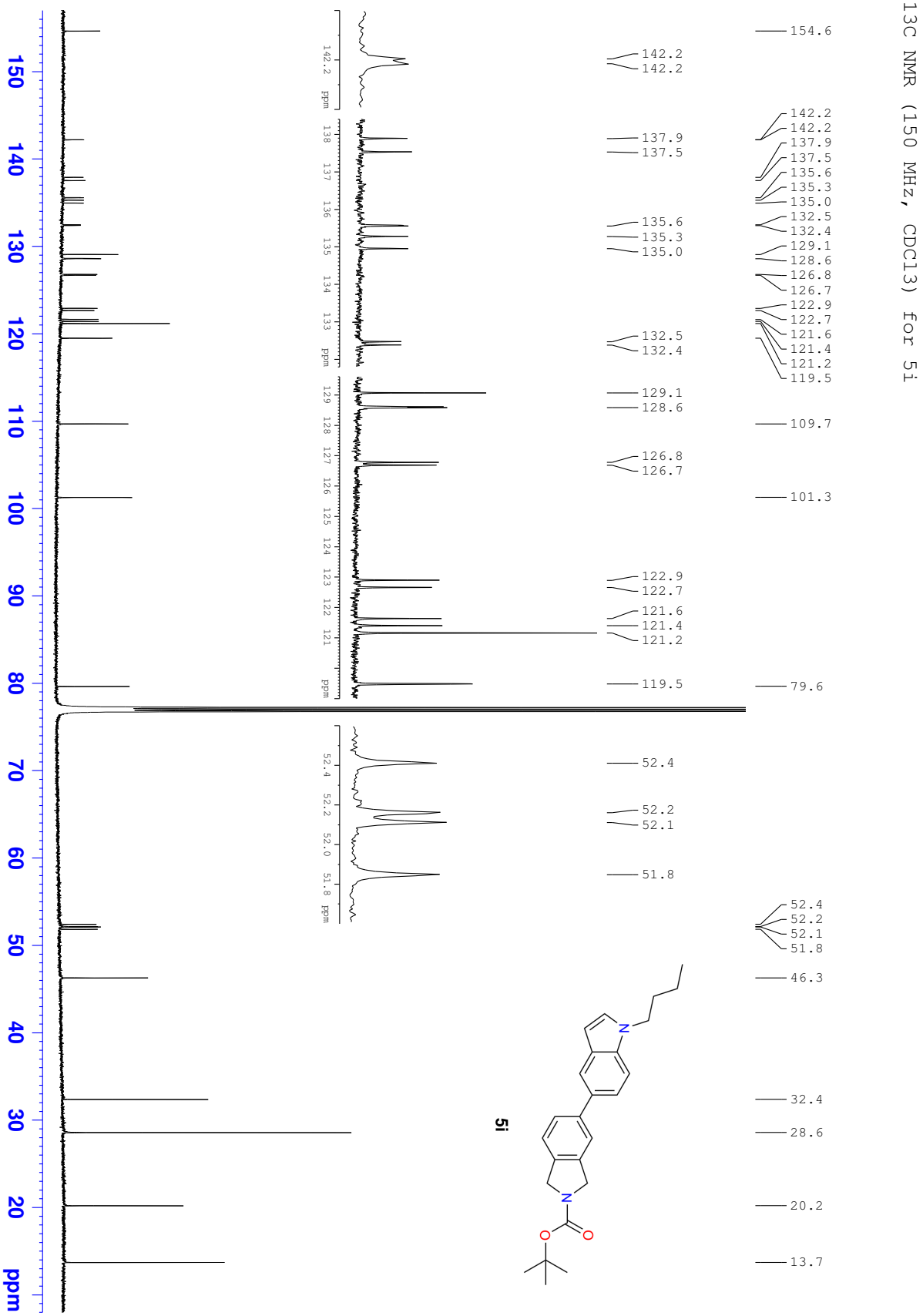
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
279.1132	279.1134	-0.2	-0.7	11.5	1712.8	n/a	n/a	C17 H15 N2 O2

Ion observed
[M-C4H8+H]+

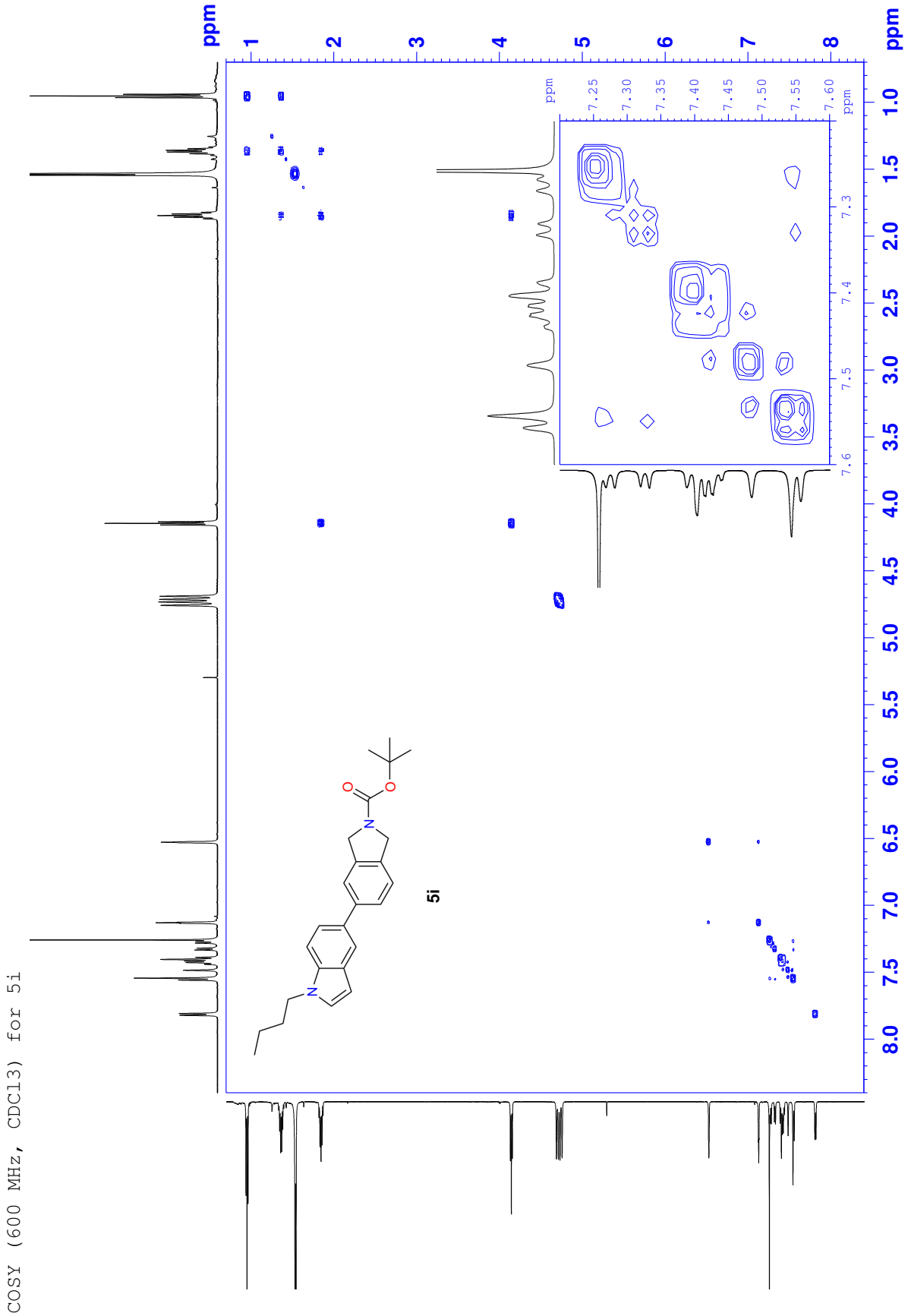
K.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for 5i



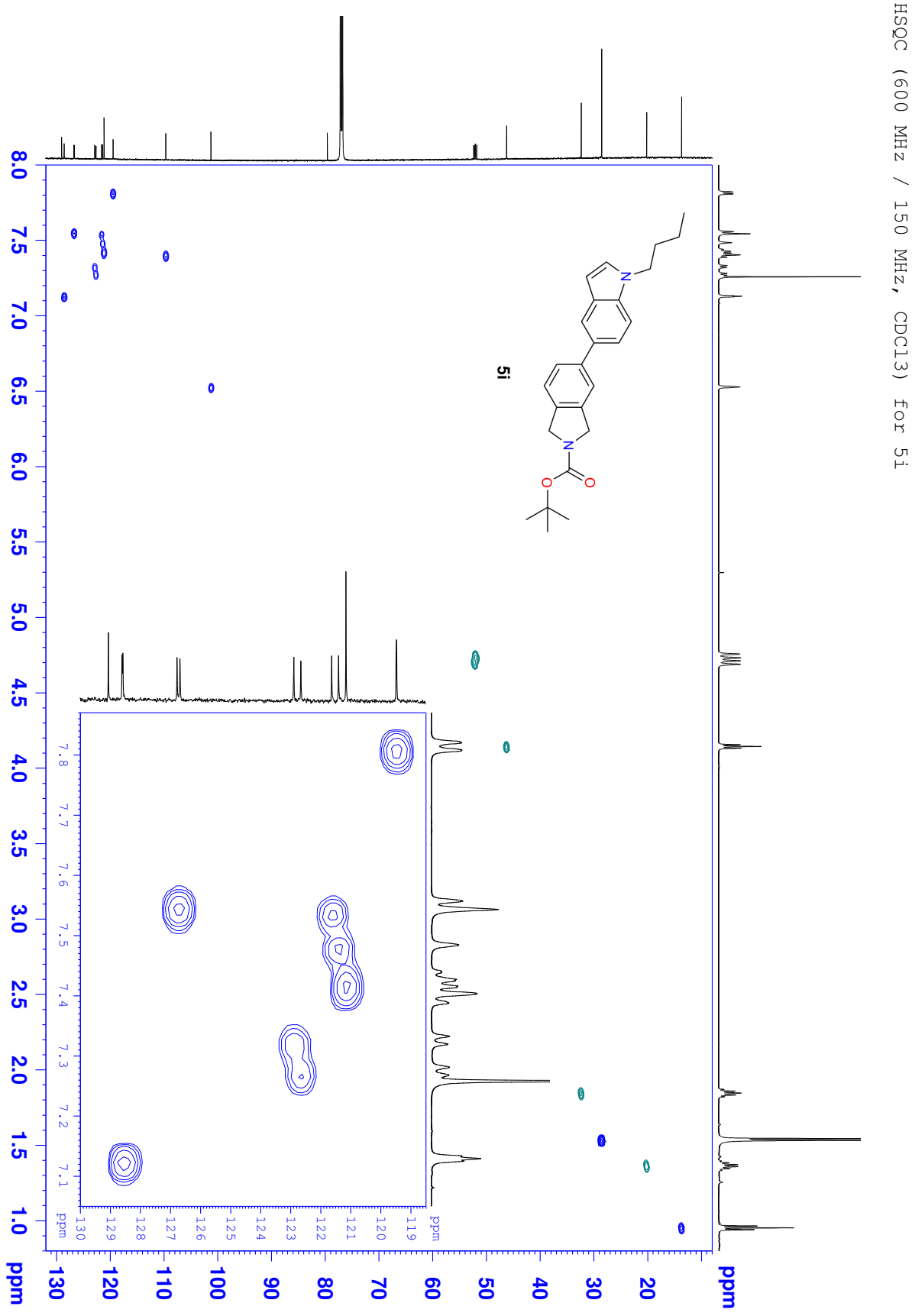
K.2 ^{13}C NMR (150 MHz, $CDCl_3$) spectrum for 5i



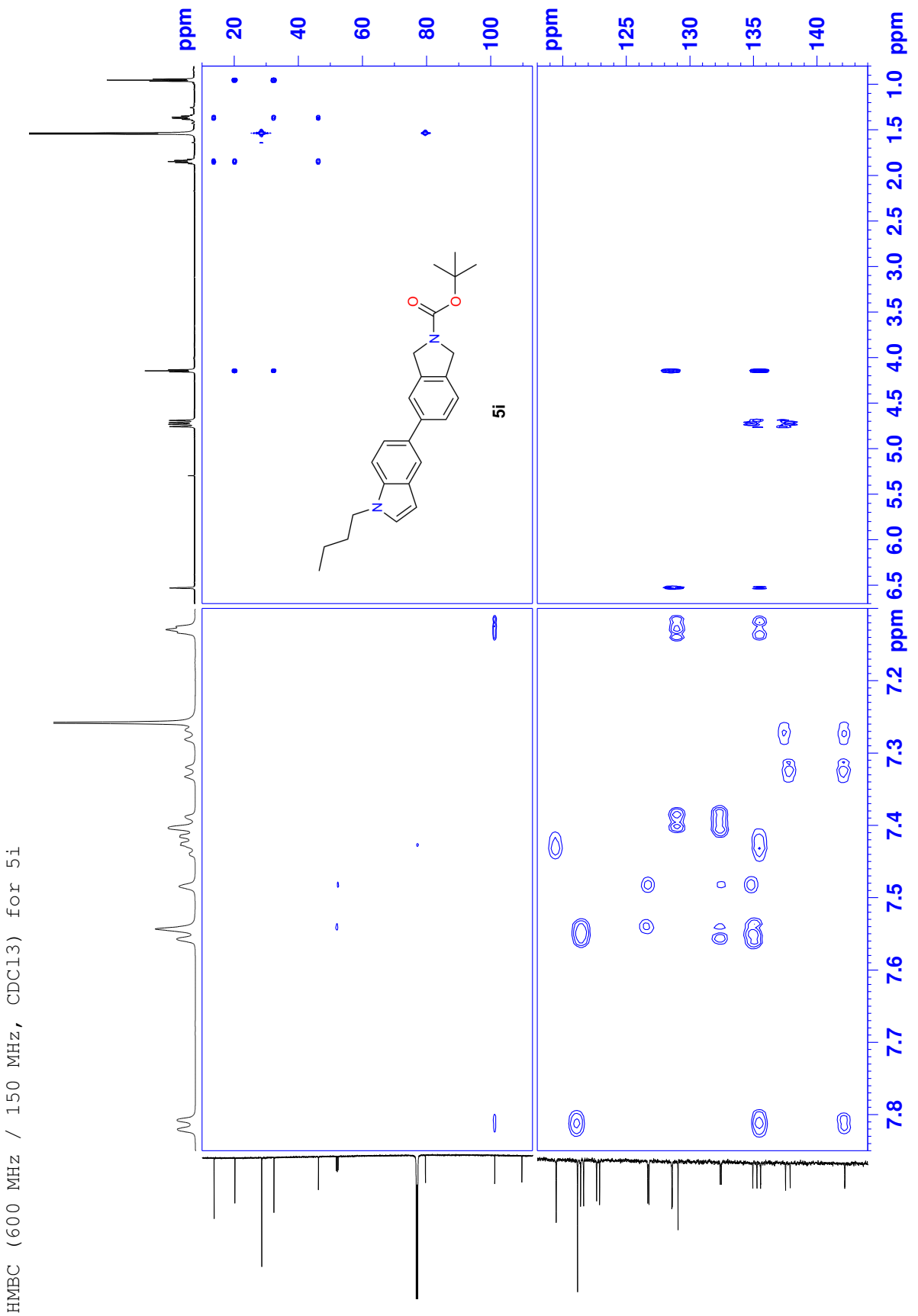
K.3 COSY (600 MHz, $CDCl_3$) spectrum for 5i



K.4 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5i



K.5 HMBC (600 MHz / 150 MHz, $CDCl_3$) spectrum for 5i

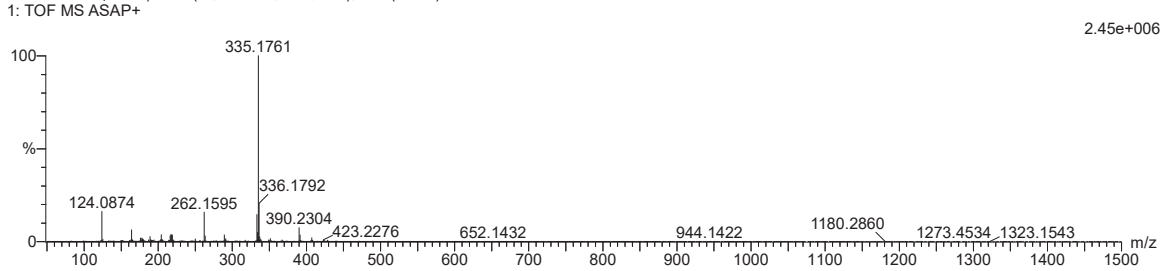


K.6 HRMS spectrum for 5i

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

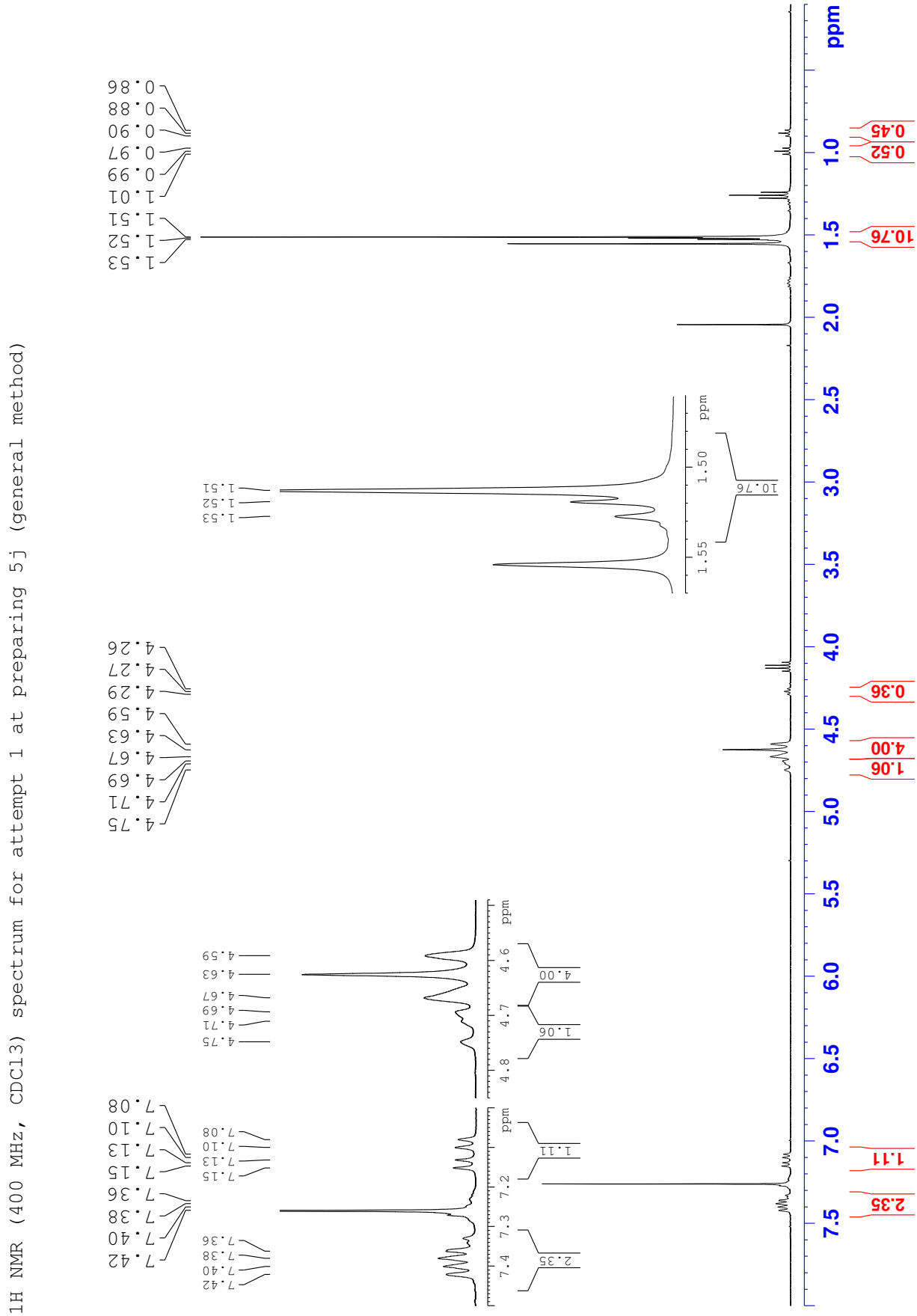
Monoisotopic Mass, Even Electron Ions
799 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200
2017-289 64 (1.257) AM2 (Ar,35000.0,0.00,0.00); Cm (56:64)
1: TOF MS ASAP+



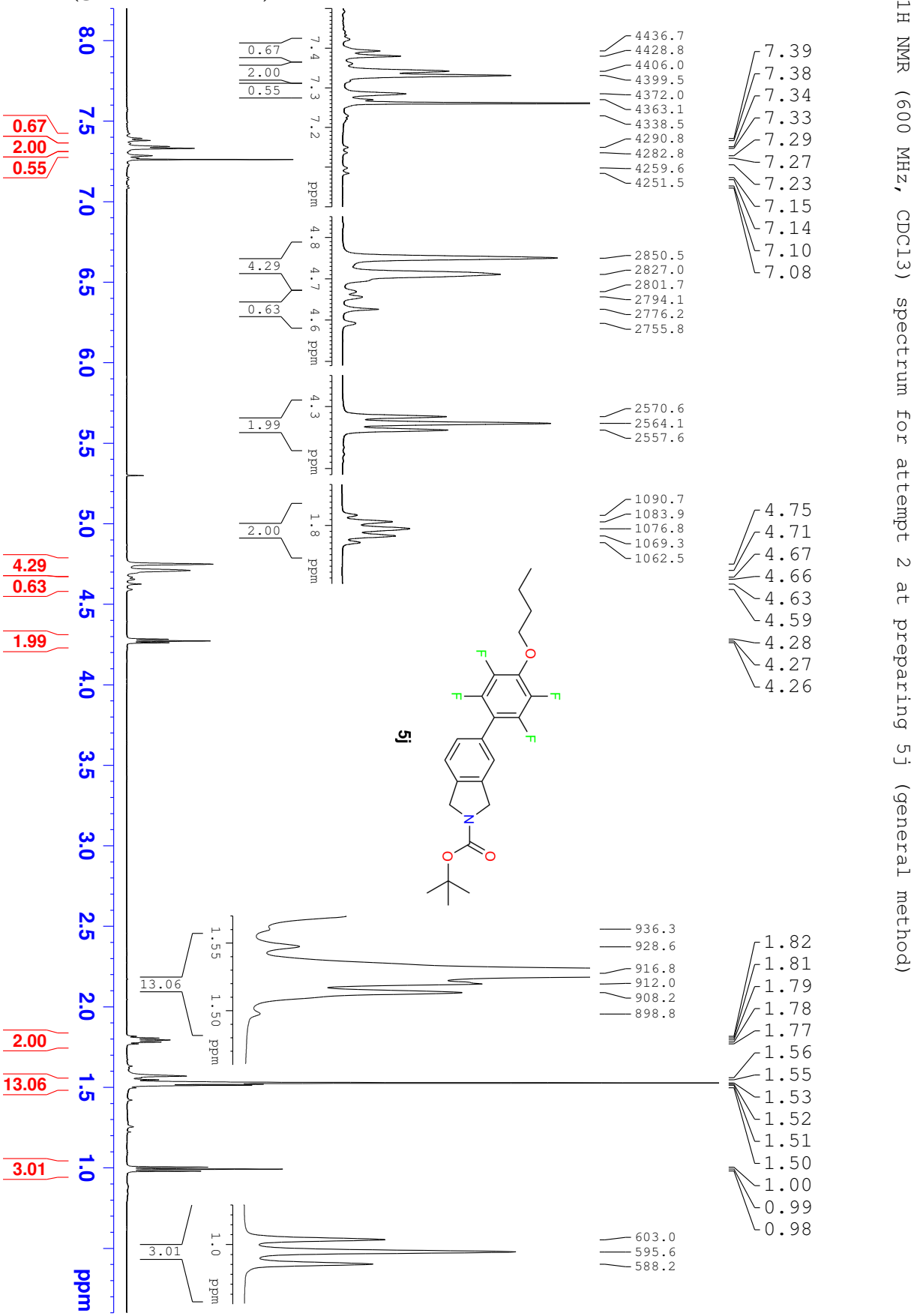
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
335.1761	335.1760	0.1	0.3	11.5	1316.3	0.000	100.00	C21 H23 N2 O2
	335.1765	-0.4	-1.2	4.5	1327.6	11.265	0.00	C6 H19 N14 O3

ion Observed [M-C4H8+H]

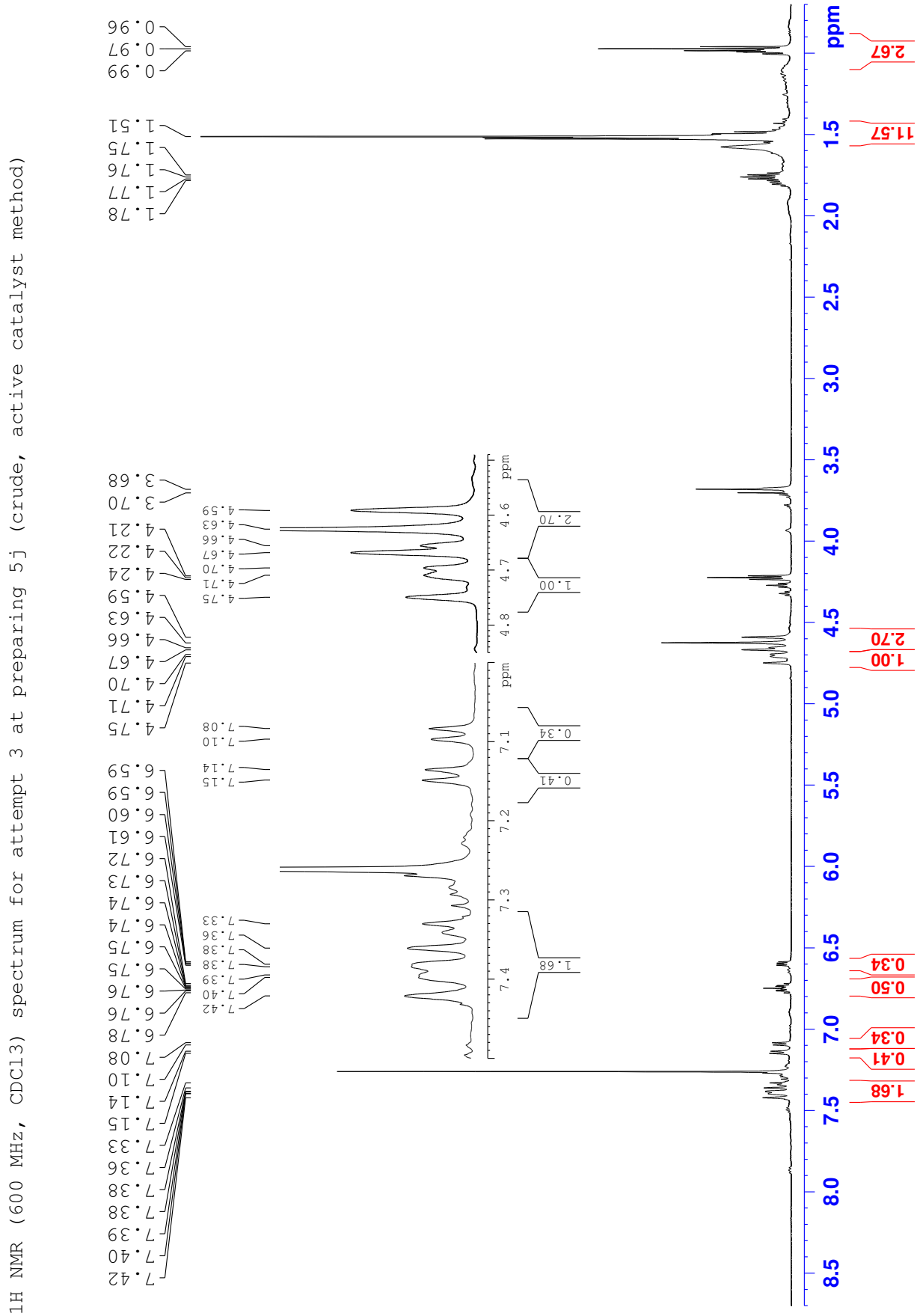
L.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for attempt 1 at preparing 5j (general method)



L.2 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for attempt 2 at preparing 5j (general method)



L.3 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for attempt 1 at preparing 5j (crude, active catalyst method)



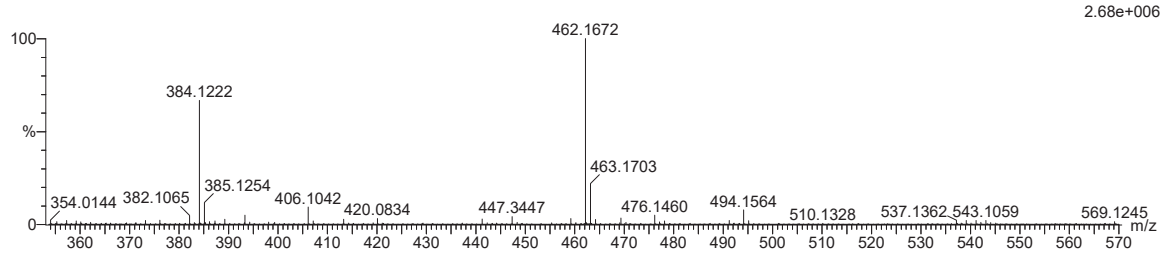
L.4 HRMS spectrum for 5j

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

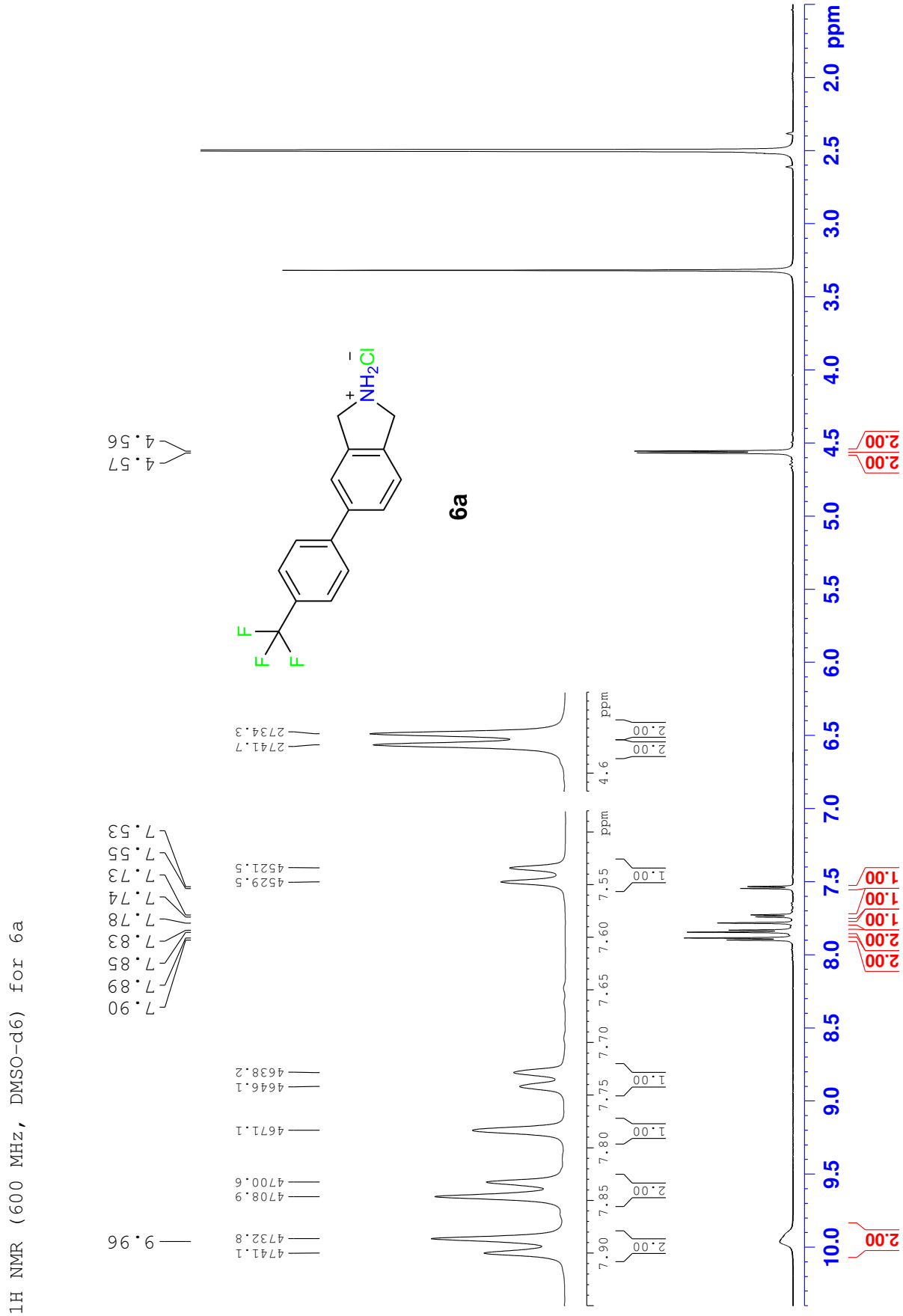
Monoisotopic Mass, Even Electron Ions
10085 formula(e) evaluated with 32 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1 F: 0-8

2017-303esi 149 (1.351) AM2 (Ar,35000.0,0.00,0.00); Cm (147:160)
1: TOF MS ES+

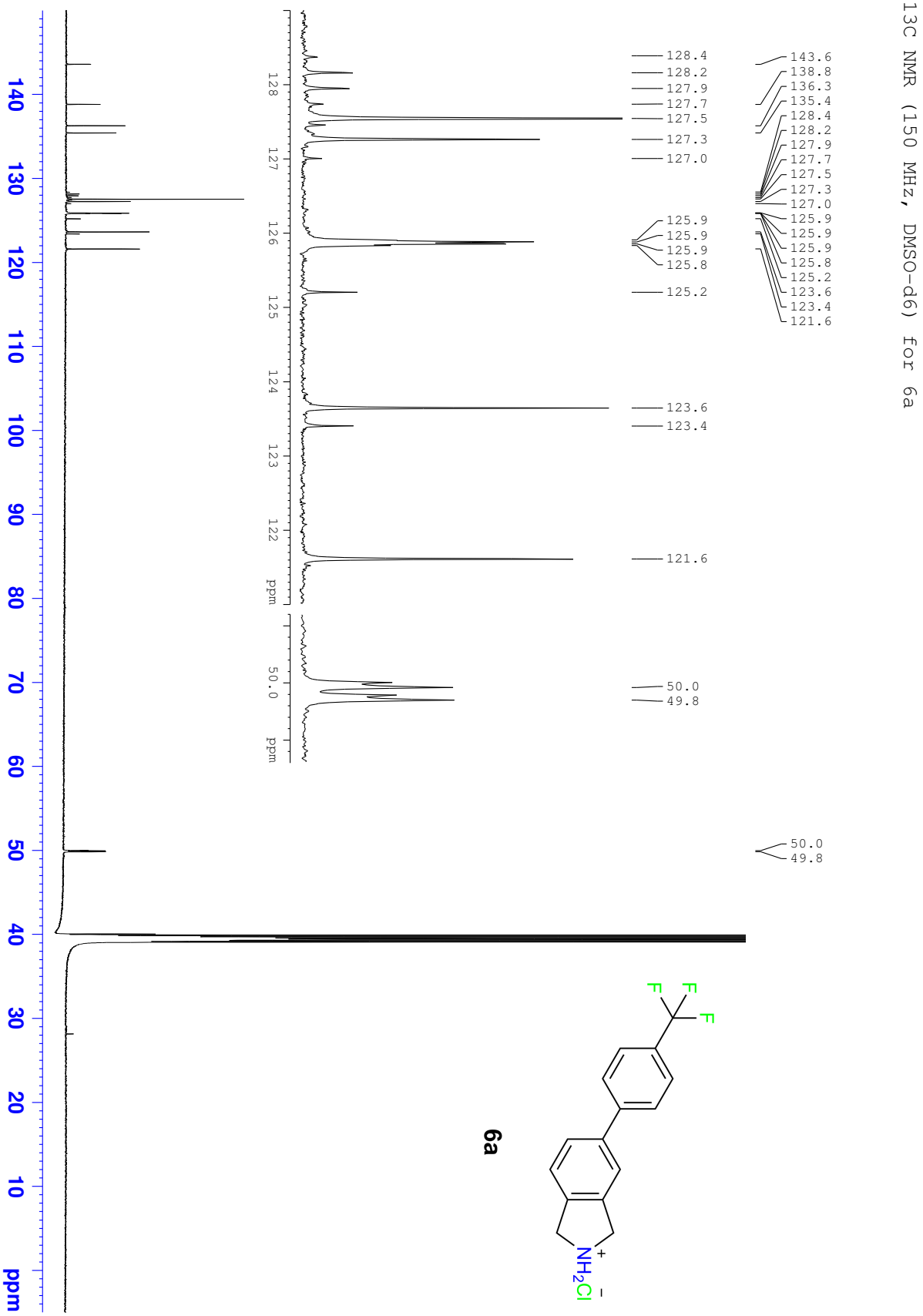


Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
384.1222	384.1224	-0.2	-0.5	7.5	1214.3	0.694	49.94	C15 H16 N5 Na F5
	384.1232	-1.0	-2.6	9.5	1214.4	0.869	41.92	C14 H16 N7 O4 F2
	384.1223	-0.1	-0.3	9.5	1216.7	3.114	4.44	C19 H18 N O3 F4
	384.1223	-0.1	-0.3	9.5	1217.5	3.966	1.89	C19 H20 N O5 Na F
	384.1218	0.4	1.0	4.5	1218.8	5.198	0.55	C13 H20 N3 O8 F2
	384.1221	0.1	0.3	11.5	1219.0	5.426	0.44	C13 H13 N11 Na F2
	384.1222	0.0	0.0	-1.5	1219.6	6.040	0.24	C11 H21 N O5 Na F6
	384.1220	0.2	0.5	13.5	1219.7	6.135	0.22	C17 H15 N7 O3 F
	384.1225	-0.3	-0.8	18.5	1220.2	6.611	0.13	C23 H15 N5 Na
	384.1230	-0.8	-2.1	0.5	1221.2	7.637	0.05	C10 H21 N3 O9 F3
	384.1211	1.1	2.9	13.5	1221.3	7.727	0.04	C22 H17 N O2 F3
	384.1212	1.0	2.6	13.5	1221.6	8.031	0.03	C22 H19 N O4 Na
	384.1230	-0.8	-2.1	0.5	1221.7	8.116	0.03	C10 H23 N3 O11 Na
	384.1233	-1.1	-2.9	7.5	1221.8	8.243	0.03	C10 H14 N11 O Na F3
	384.1219	0.3	0.8	2.5	1222.7	9.131	0.01	C9 H18 N7 O5 Na F3
	384.1227	-0.5	-1.3	4.5	1222.9	9.294	0.01	C8 H18 N9 O9
	384.1219	0.3	0.8	2.5	1223.2	9.607	0.01	C9 H16 N7 O3 F6
	384.1230	-0.8	-2.1	11.5	1223.9	10.370	0.00	C8 H11 N17 O Na
	384.1231	-0.9	-2.3	-1.5	1224.6	10.999	0.00	C6 H19 N7 O6 Na F4
	384.1217	0.5	1.3	6.5	1224.7	11.153	0.00	C7 H15 N13 O5 Na
	384.1230	-0.8	-2.1	-1.5	1224.9	11.322	0.00	C6 H17 N7 O4 F7
	384.1214	0.8	2.1	-0.5	1225.3	11.670	0.00	C7 H22 N5 O13
	384.1216	0.6	1.6	6.5	1225.4	11.849	0.00	C7 H13 N13 O3 F3
	384.1228	-0.6	-1.6	2.5	1226.7	13.148	0.00	C4 H16 N13 O6 Na F
	384.1214	0.8	2.1	10.5	1226.8	13.270	0.00	C5 H10 N19 O3
	384.1228	-0.6	-1.6	2.5	1227.2	13.577	0.00	C4 H14 N13 O4 F4
	384.1225	-0.3	-0.8	6.5	1228.7	15.143	0.00	C2 H11 N19 O4 F
	384.1220	0.2	0.5	0.5	1229.0	15.433	0.00	C5 H14 N11 Na F7
	384.1212	1.0	2.6	1.5	1229.9	16.300	0.00	C H15 N15 O8 F
	384.1217	0.5	1.3	4.5	1230.9	17.363	0.00	C3 H11 N17 Na F4
	384.1229	-0.7	-1.8	0.5	1231.7	18.103	0.00	H12 N17 O Na F5
	384.1215	0.7	1.8	8.5	1232.2	18.622	0.00	C H8 N23 Na F

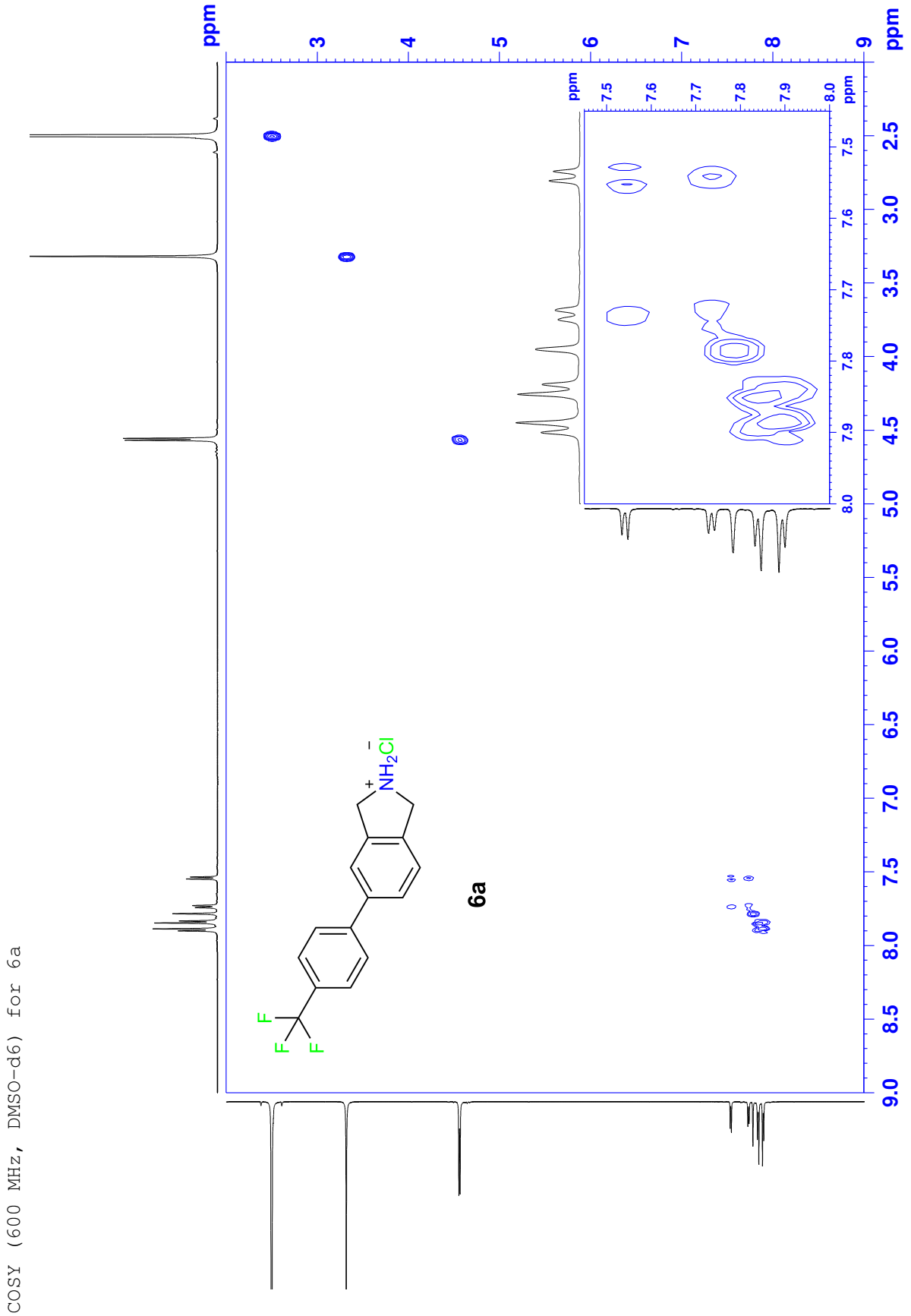
M.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6a



M.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6a

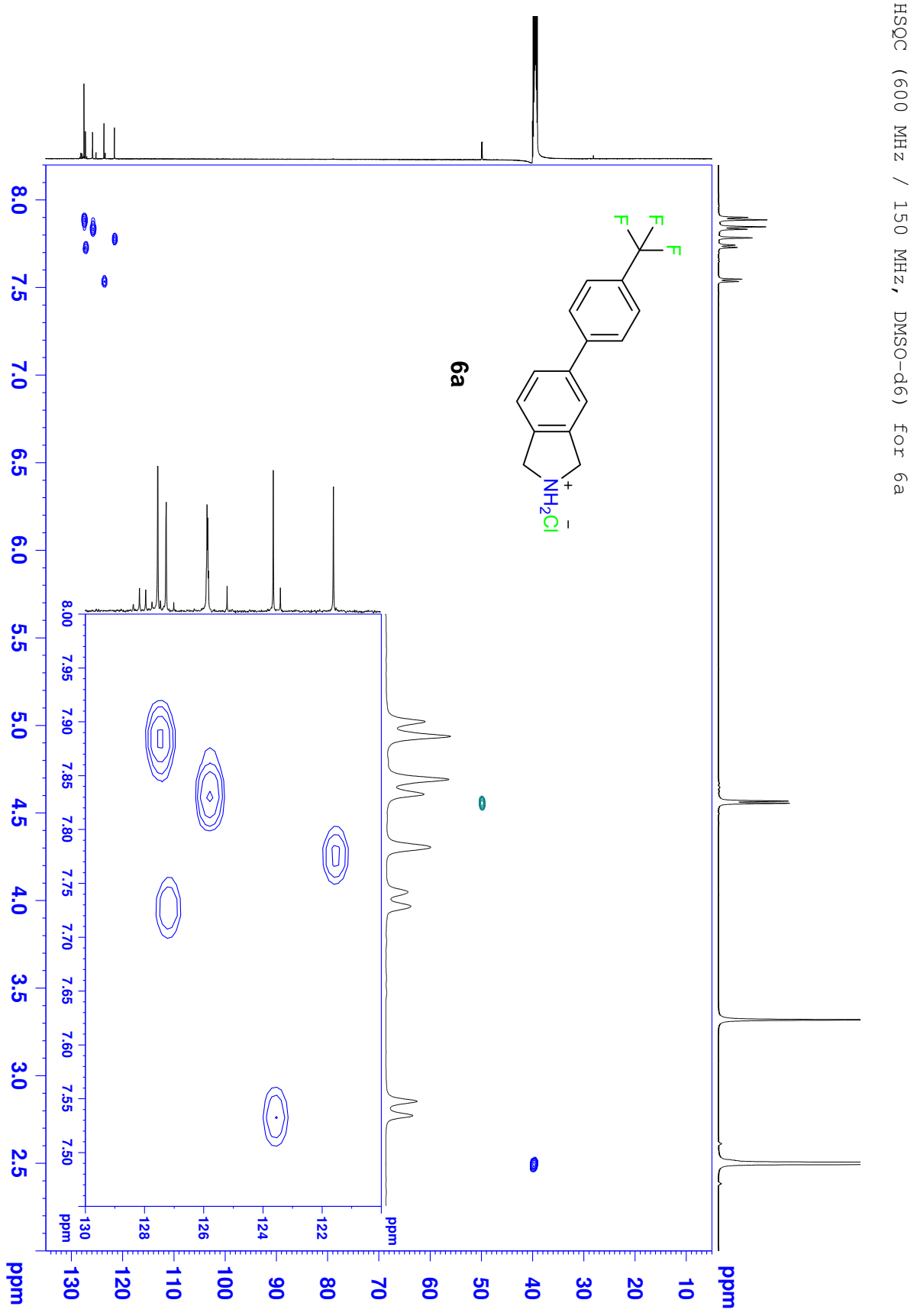


M.3 COSY (600 MHz, DMSO) spectrum for 6a

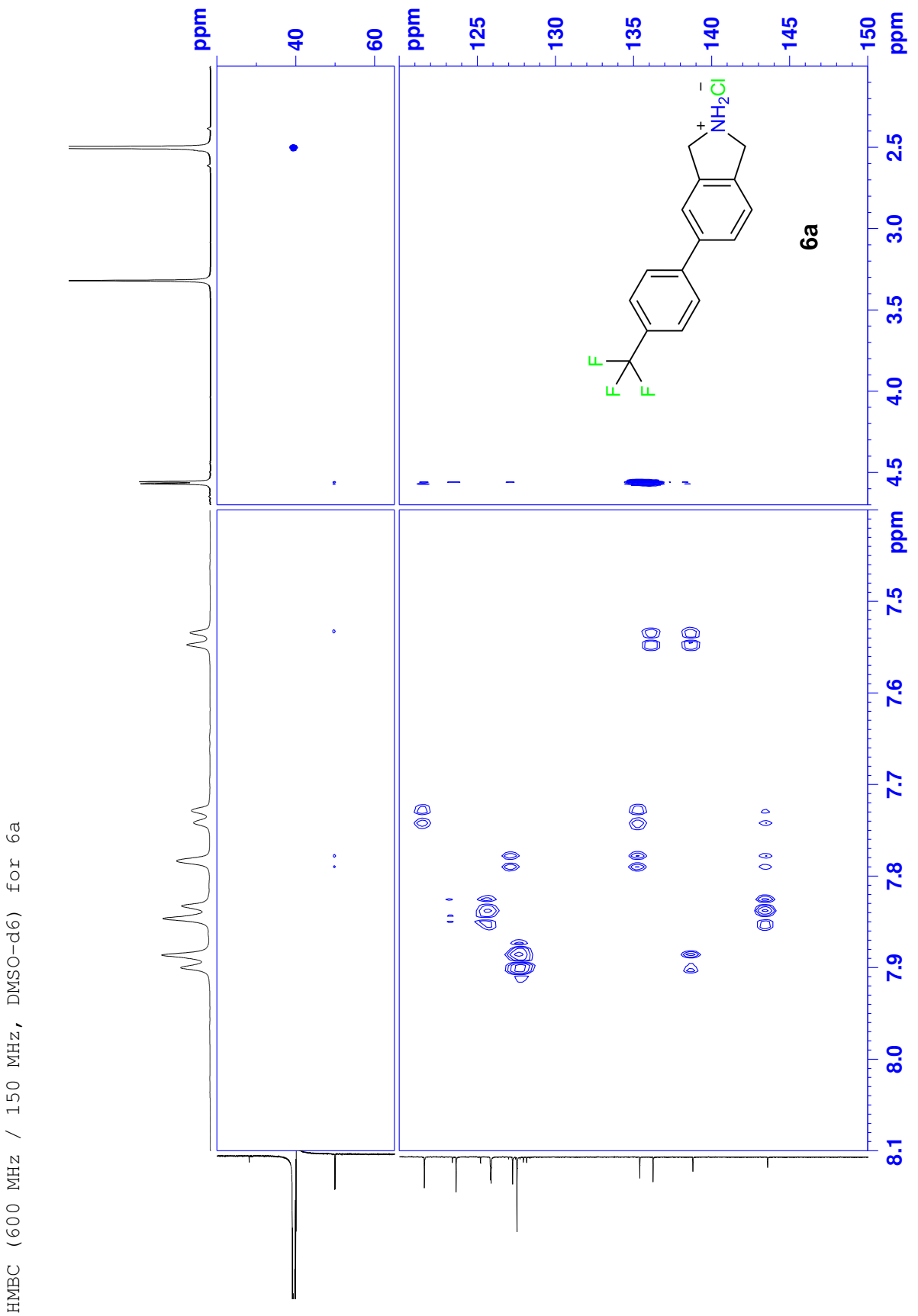


COSY (600 MHz, DMSO-d6) for 6a

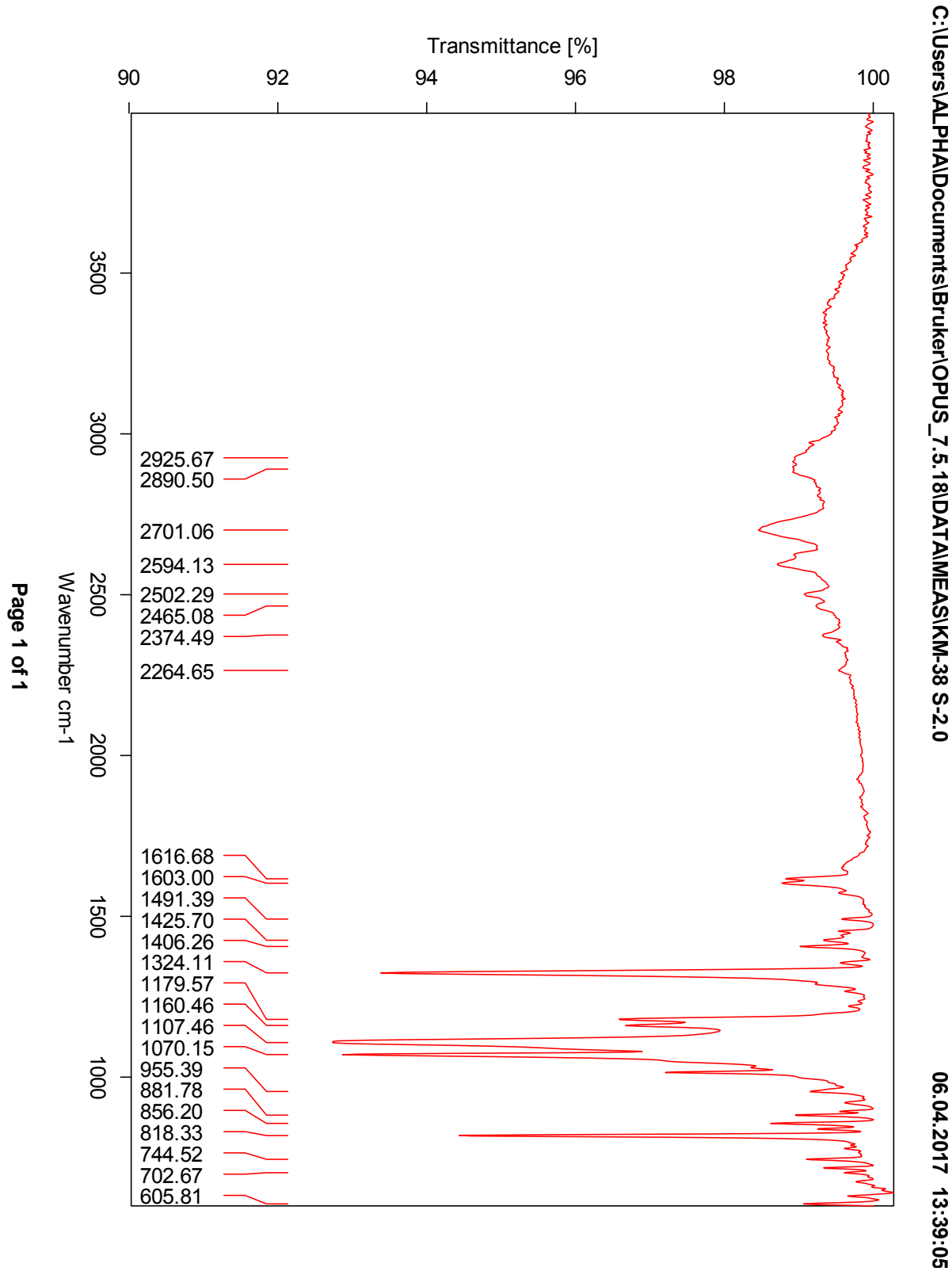
M.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6a



M.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6a



M.6 IR spectrum for 6a



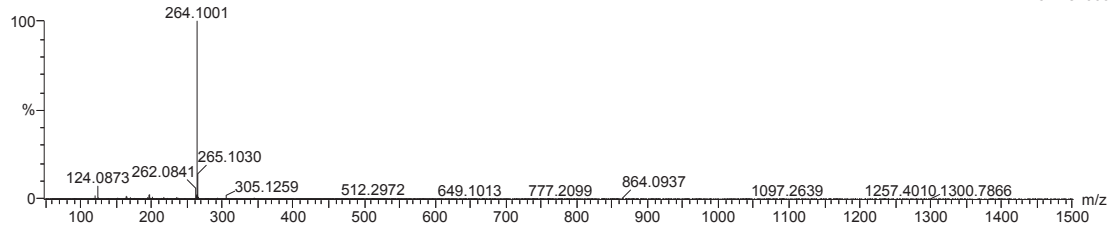
M.7 HRMS spectrum for 6a

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
162 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 F: 1-6
2017-180 15 (0.310) AM2 (Ar,35000.0,0.00,0.00); Cm (14:16)
1: TOF MS ASAP+

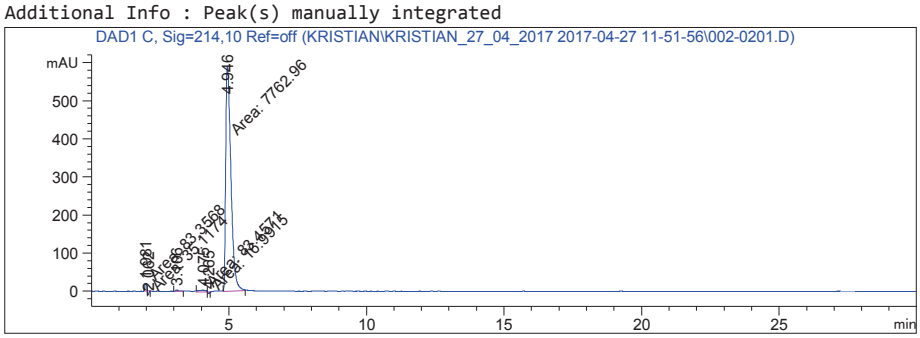
5.17e+006



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
264.1001	264.1000	0.1	0.4	8.5	1658.8	0.000	100.00	C15 H13 N F3 ion observed M+
	264.0996	0.5	1.9	1.5	1669.2	10.360	0.00	C5 H11 N7 F5

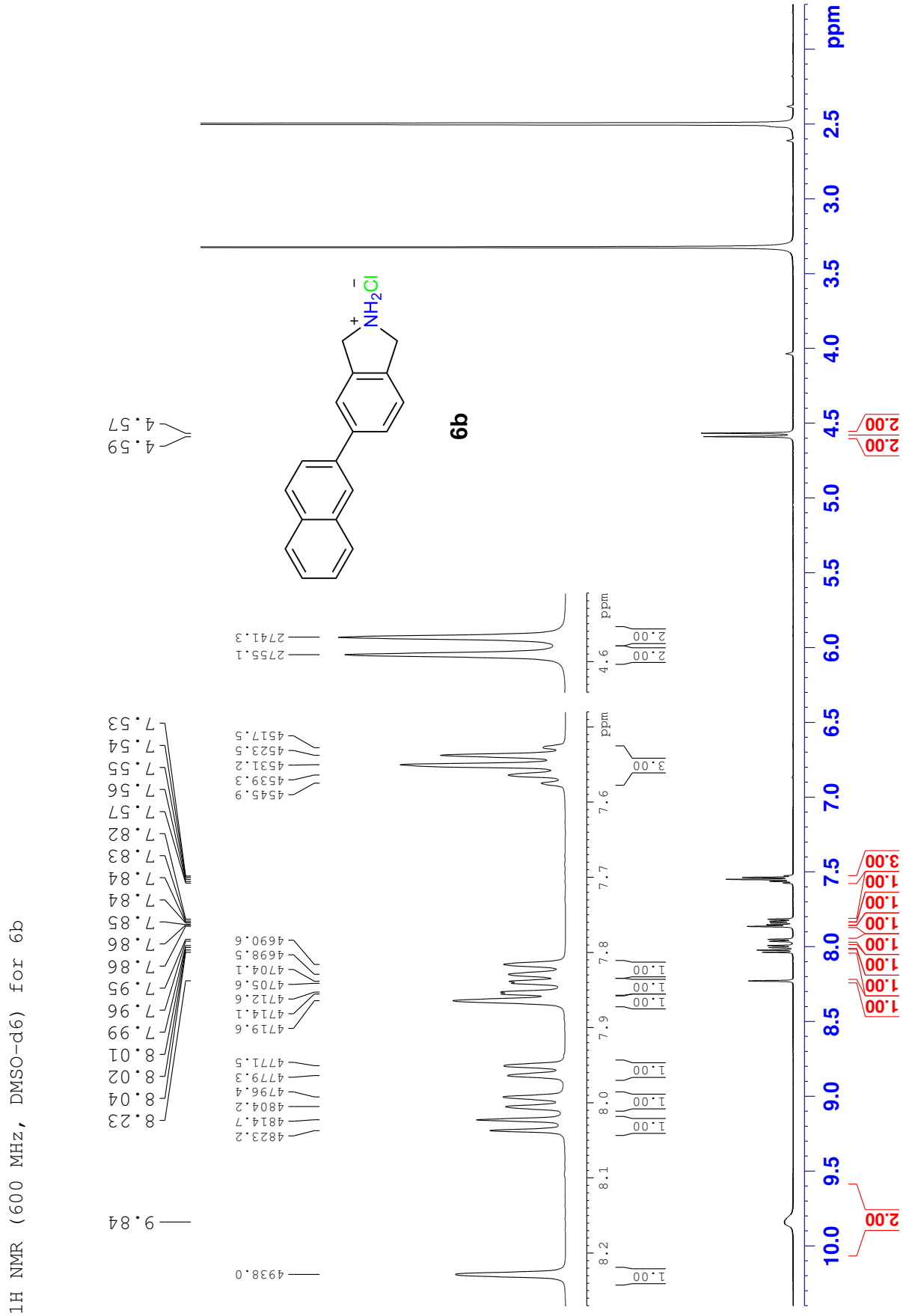
M.8 HPLC chromatogram for 6a

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\002-0201.D
Sample Name: KM-38 S-2

    ======================================================================
    Acq. Operator   : Kristian                      Seq. Line :   2
    Acq. Instrument : UPLC                          Location : Vial 2
    Injection Date  : 27.04.2017 12:03:42                Inj :   1
                                                   Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                      \C18PURITYSALT.M
    Last changed    : 27.04.2017 12:32:09 by Kristian
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\002-0201.
                      D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 27.04.2017 13:00:48 by Kristian M
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

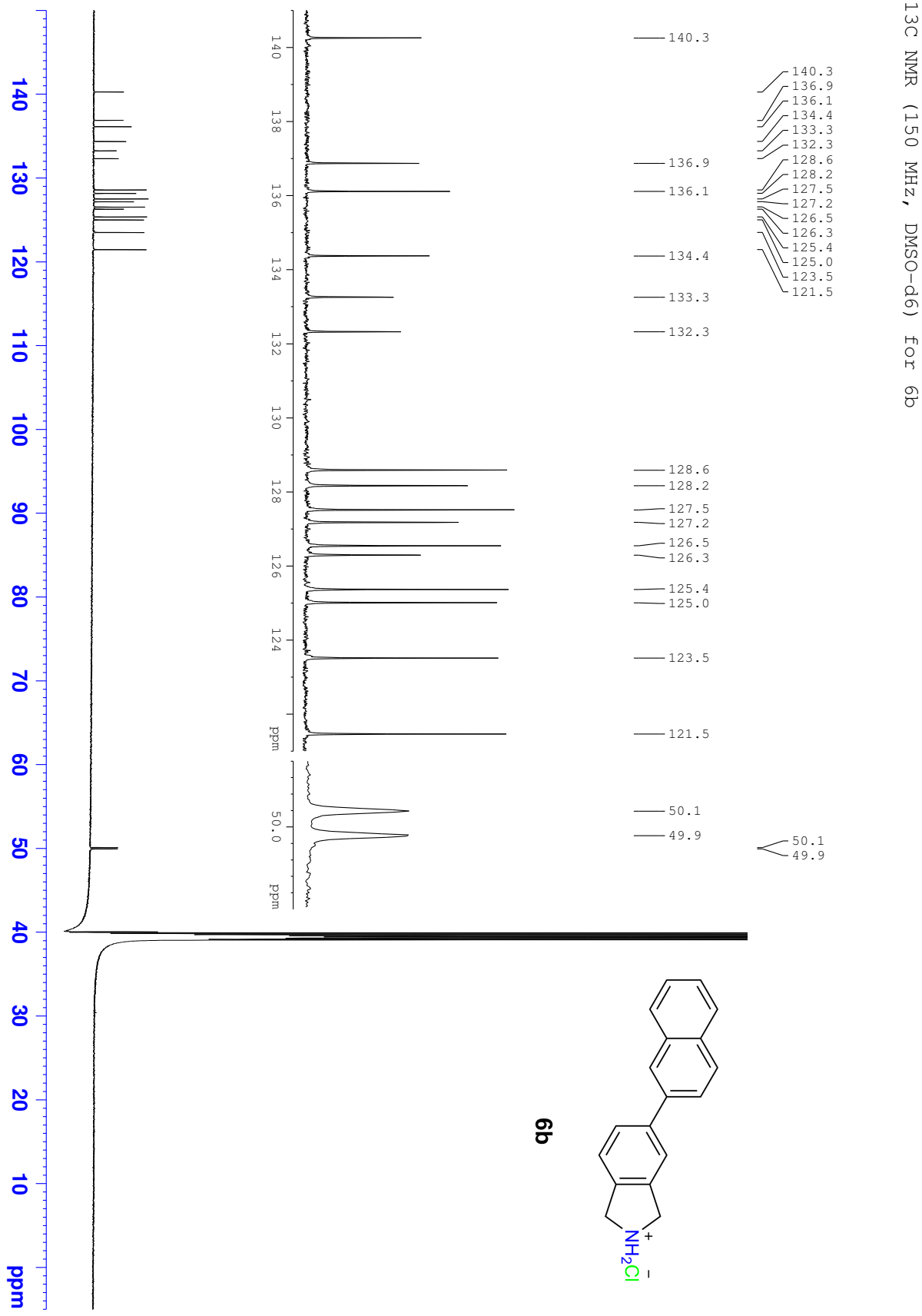



```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.981 MF    0.0675   83.35677   20.58140   1.0413
       2   2.062 MM N  0.0599   35.11737    9.77646   0.4387
       3   3.106 BB    0.1068   22.84236    3.21925   0.2854
       4   4.075 MM    0.3204   83.45708    4.34121   1.0426
       5   4.265 MP N  0.0699   16.99149    4.04962   0.2123
       6   4.946 MM    0.2188 7762.95654  591.42657  96.9797

    Totals :                   8004.72161  633.39452


    ======================================================================
                              *** End of Report ***

UPLC 27.04.2017 13:00:55 Kristian M                          Page   1 of 1
```

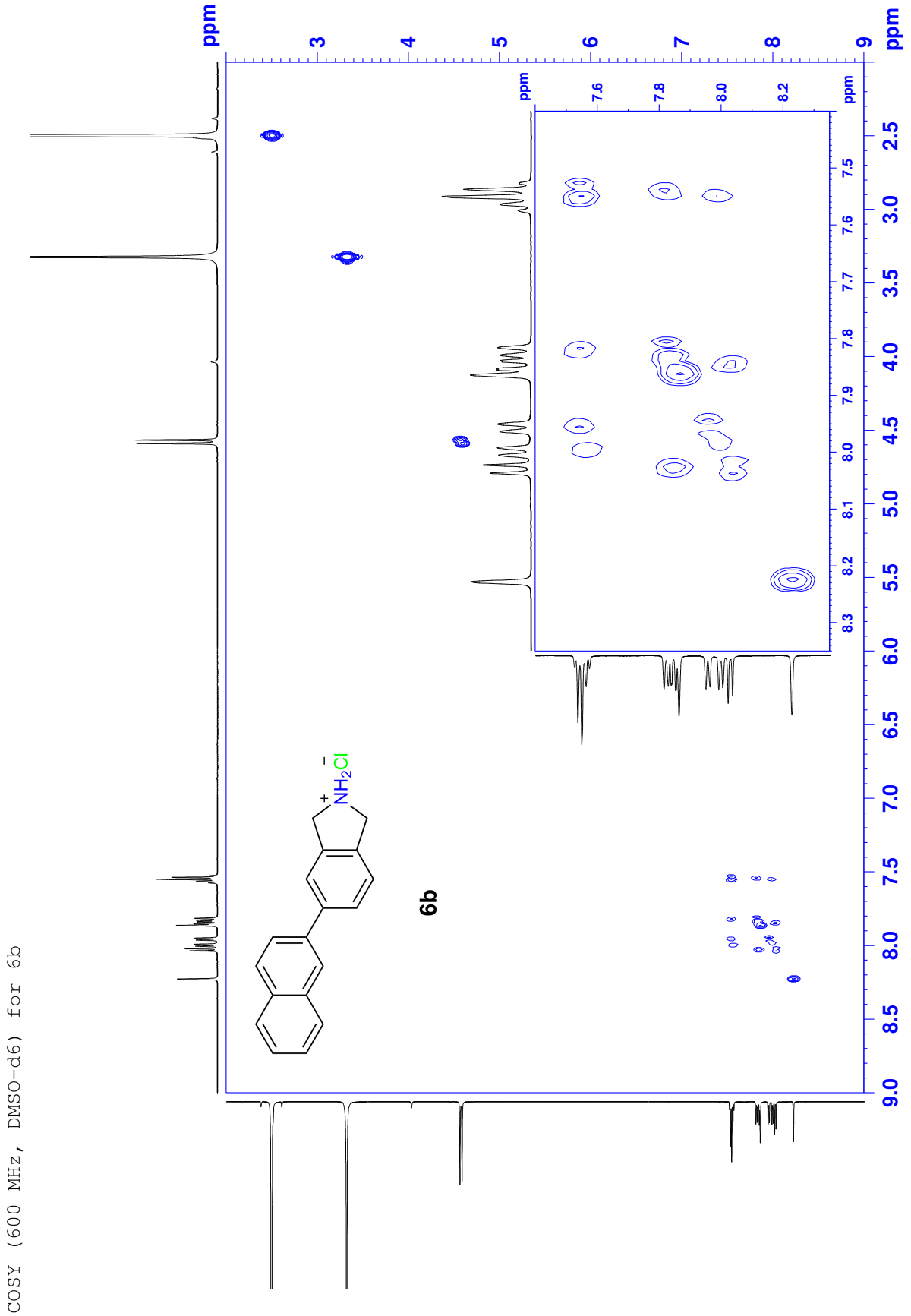
N.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6b



N.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6b

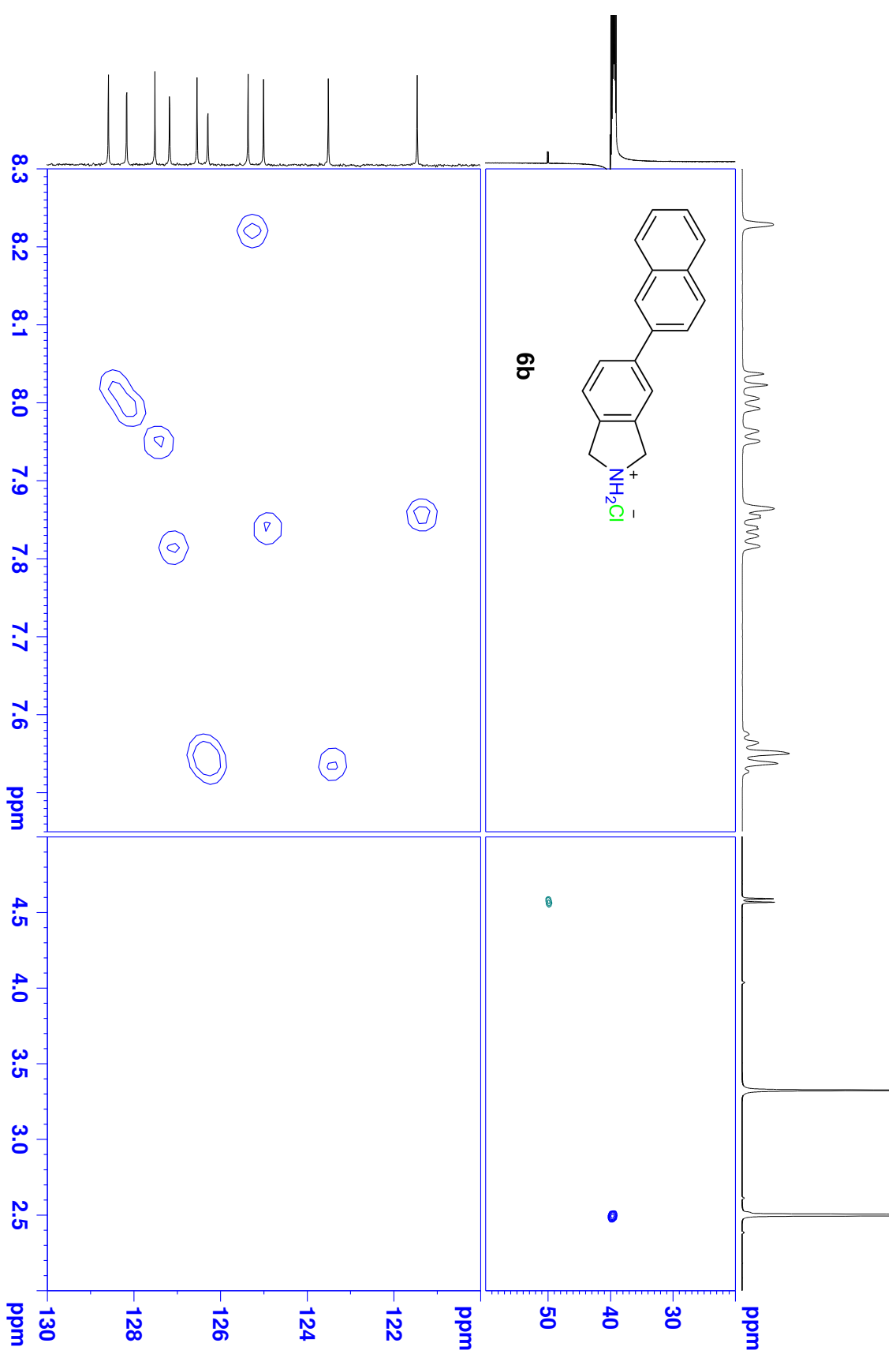


N.3 COSY (600 MHz, DMSO) spectrum for 6b

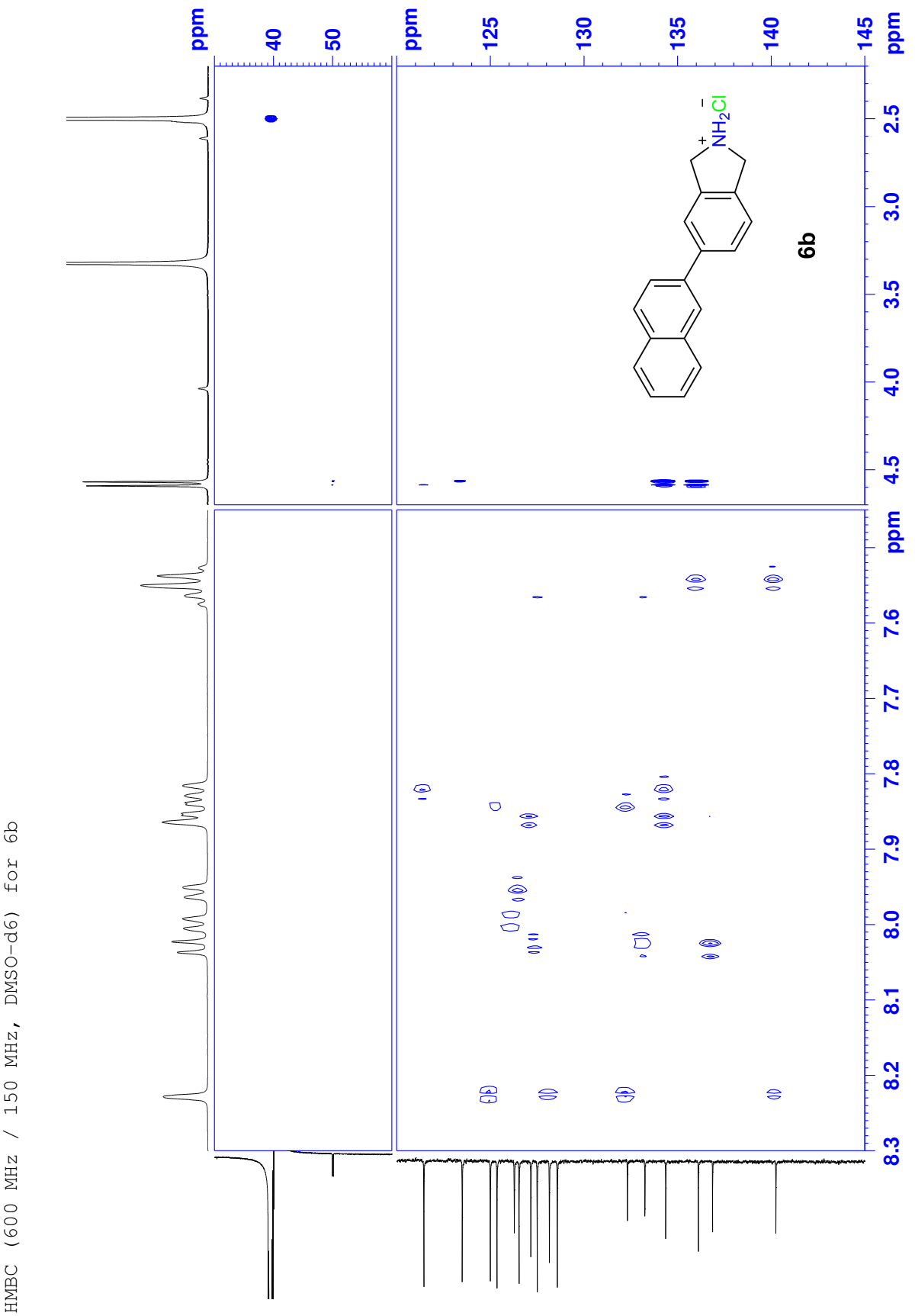


COSY (600 MHz, DMSO-d6) for 6b

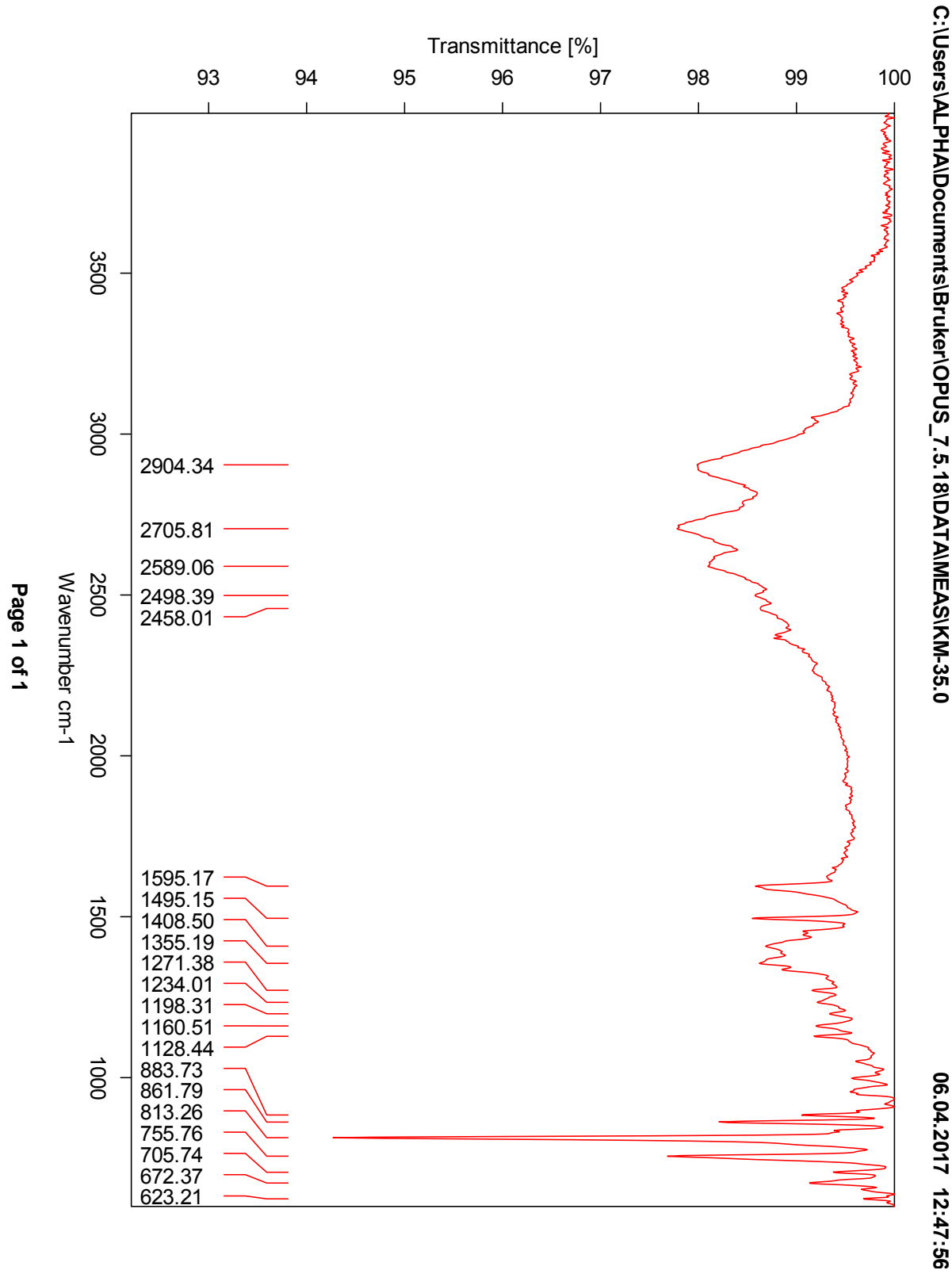
N.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6b



N.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6b



N.6 IR spectrum for 6b

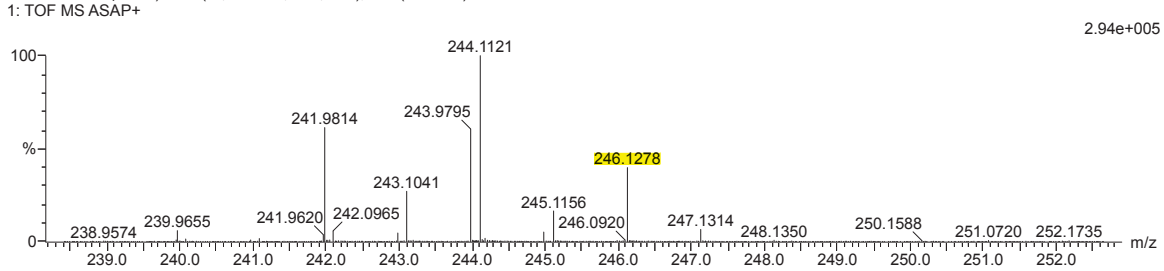


N.7 HRMS spectrum for 6b

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

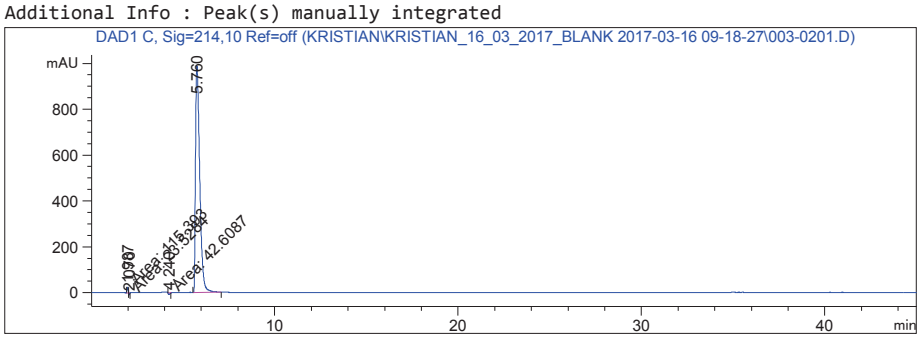
Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10
2017-161 206 (4.015) AM2 (Ar,35000.0,0.00,0.00); Cm (202:207)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
246.1278	246.1283	-0.5	-2.0	11.5	1097.5	n/a	n/a	C18 H16 N ion observed M+

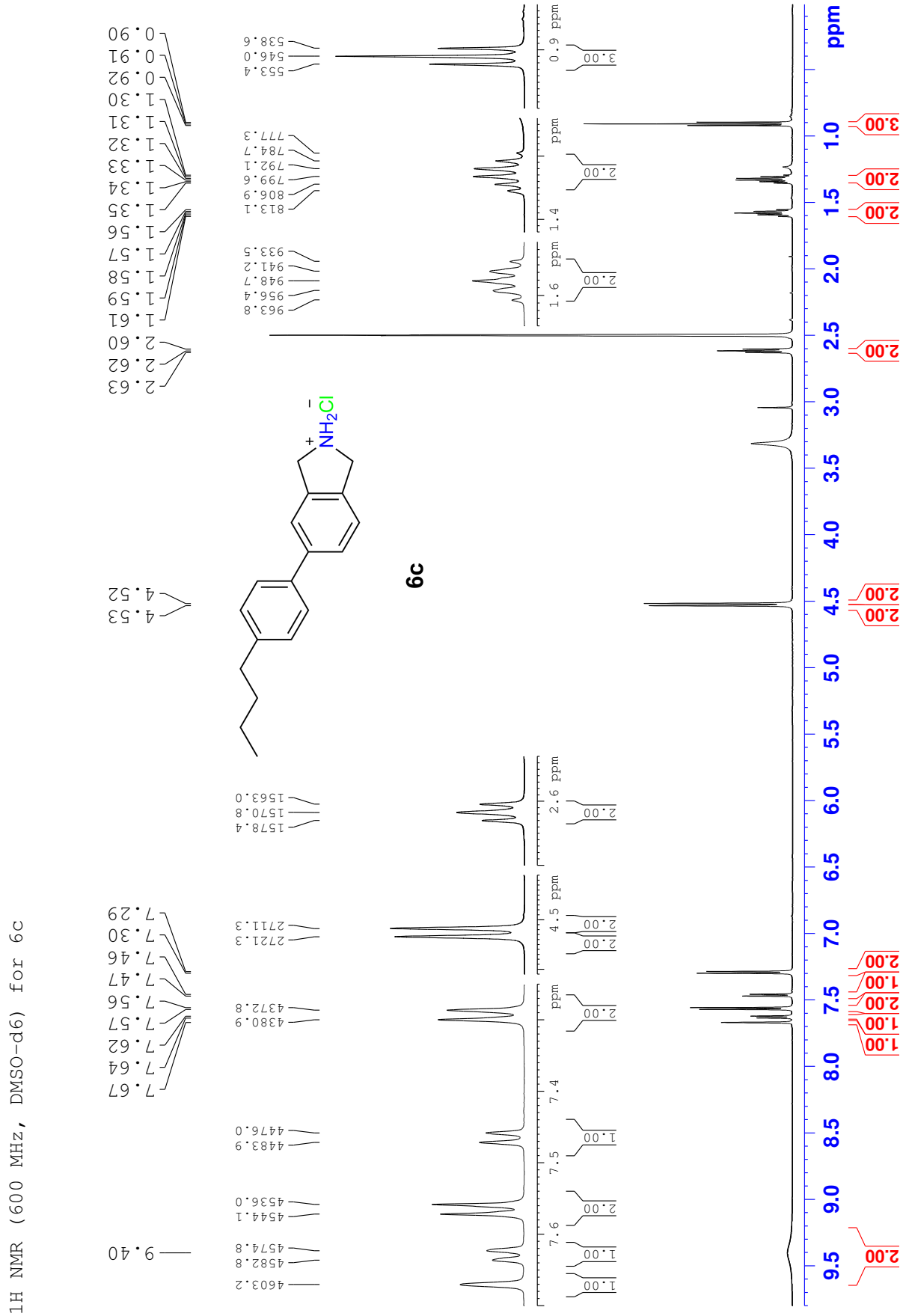
N.8 HPLC chromatogram for 6b

```
Data File C:\CHEM32\...A\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\003-0201.D
Sample Name: KM-35

    ======================================================================
    Acq. Operator   : Kristian M                    Seq. Line :   2
    Acq. Instrument : UPLC                          Location : Vial 3
    Injection Date  : 16.03.2017 10:05:13                Inj :   1
                                                   Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27
                      \C18PURITYSALT.M
    Last changed    : 16.03.2017 09:17:23 by Kristian M
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\003
                      -0201.D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 27.04.2017 12:22:24 by Kristian M
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

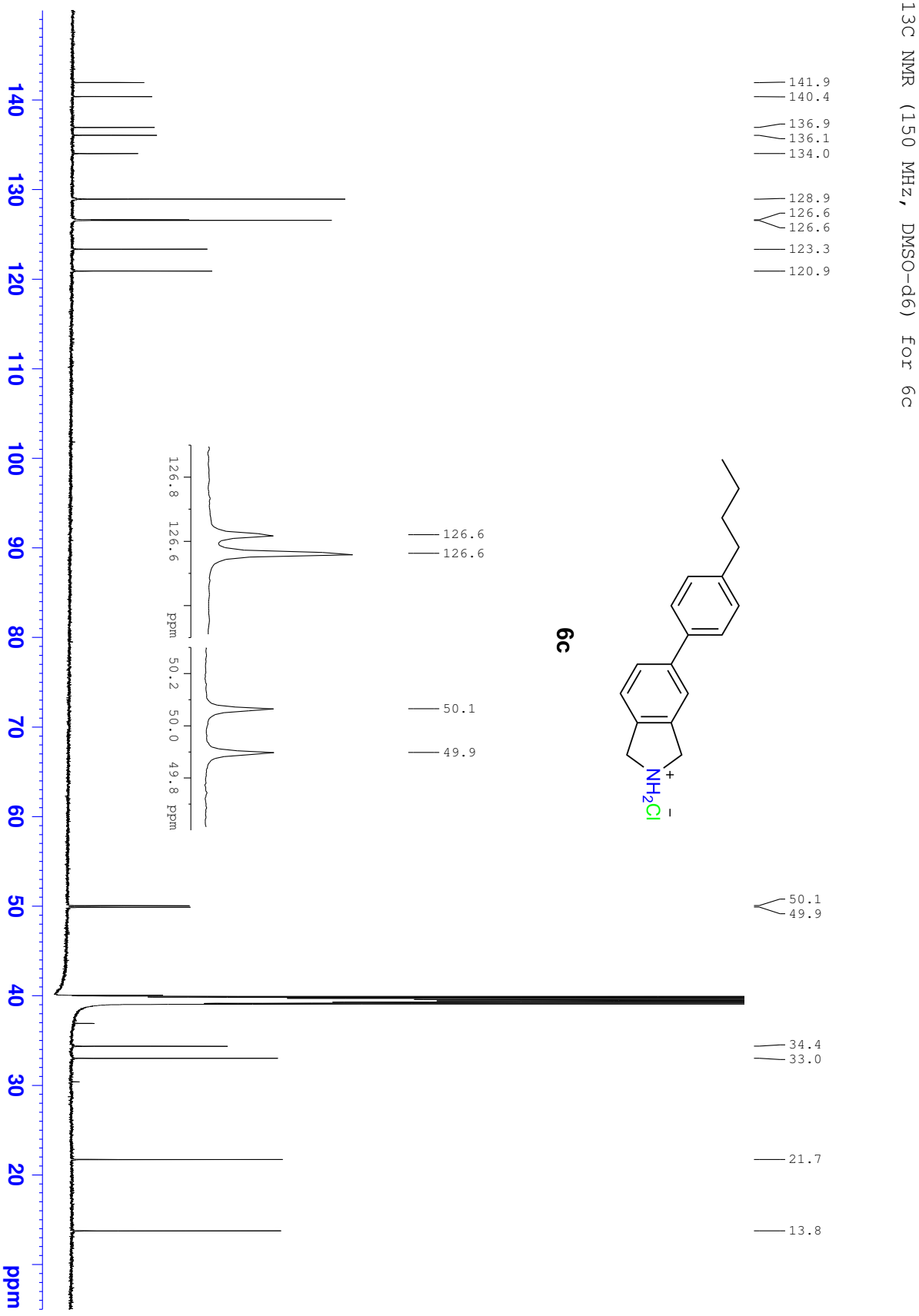



```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.987 MM    0.0756  115.39300   25.42356   0.7126
       2   2.070 MP N  0.0436   23.52836    8.99488   0.1453
       3   4.240 MM N  0.0950   42.60866    7.47844   0.2631
       4   5.760 BB    0.2512 1.60112e4   987.06079  98.8789

    Totals :                   1.61927e4  1028.95767


    ======================================================================
                            *** End of Report ***
```

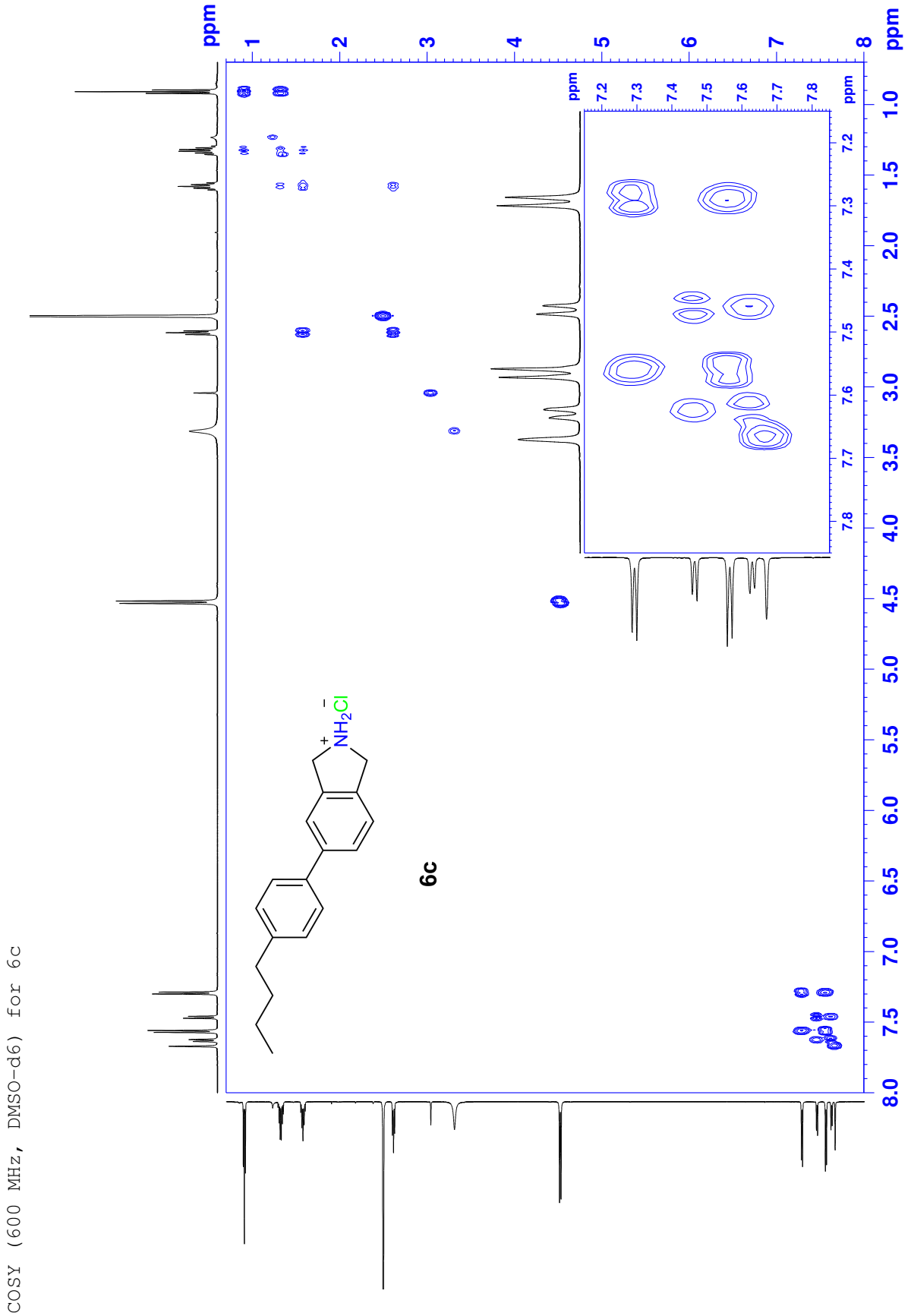
O.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6c



O.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6c

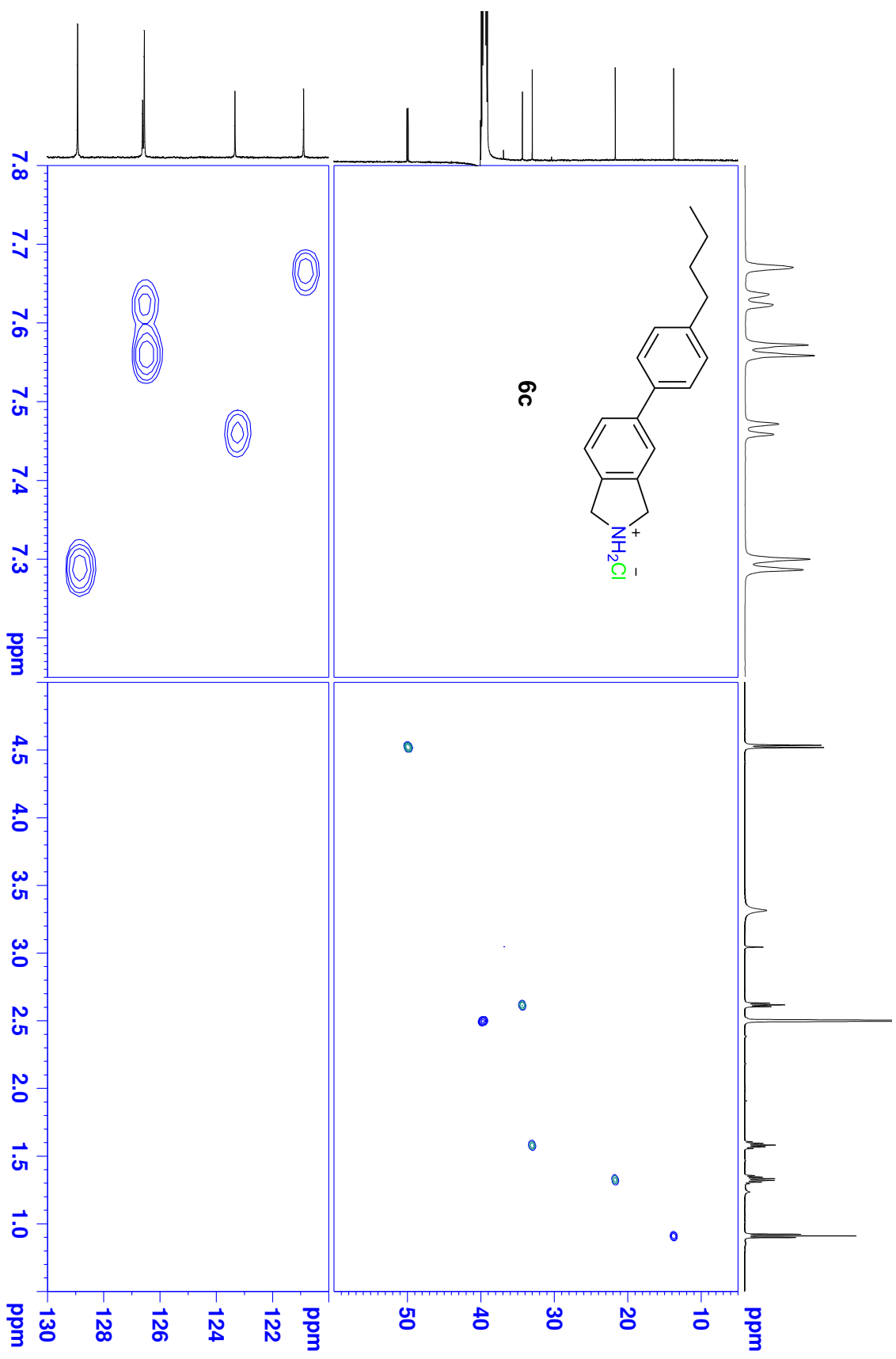


O.3 COSY (600 MHz, DMSO) spectrum for 6c

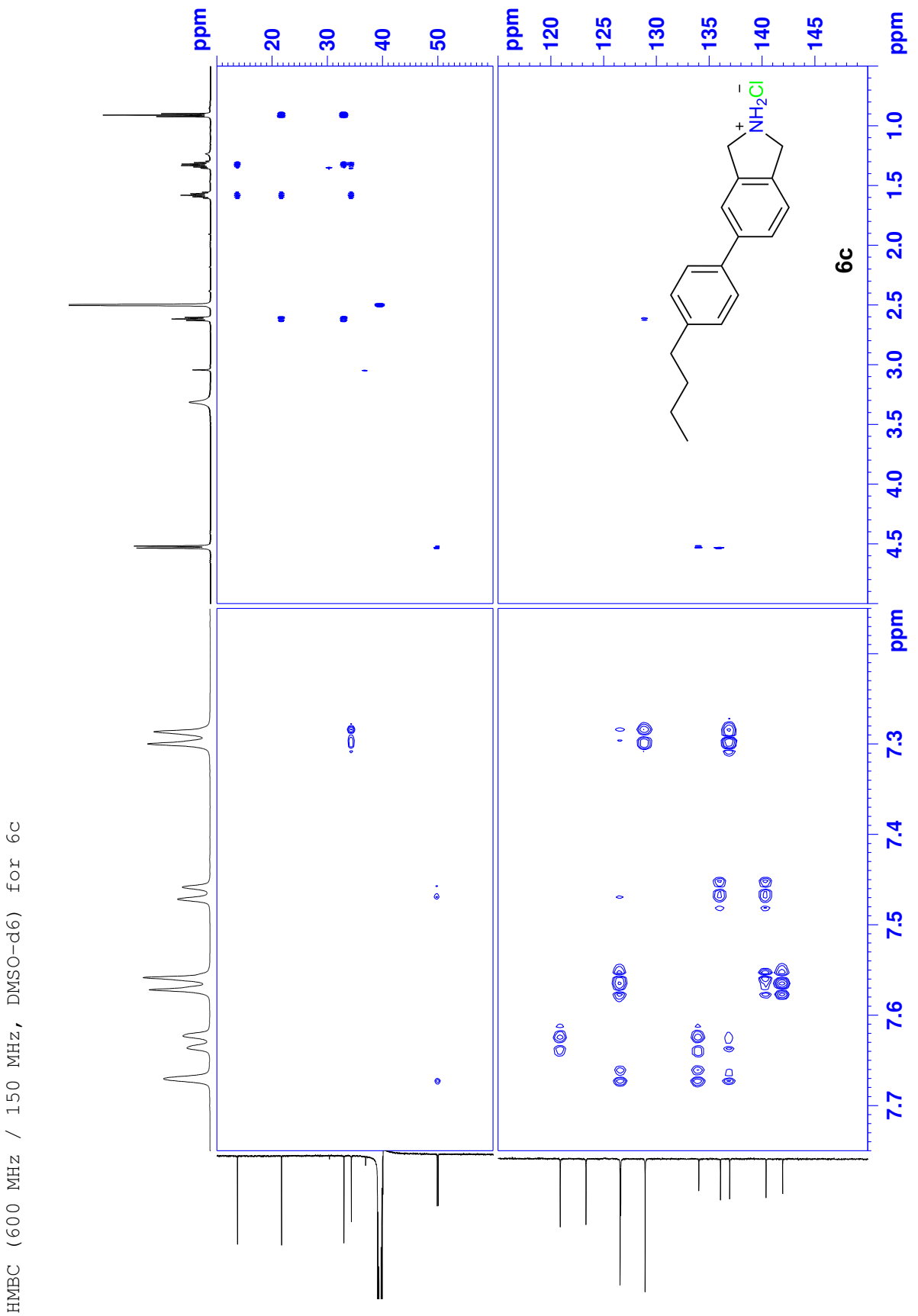


COSY (600 MHz, DMSO-d6) for 6c

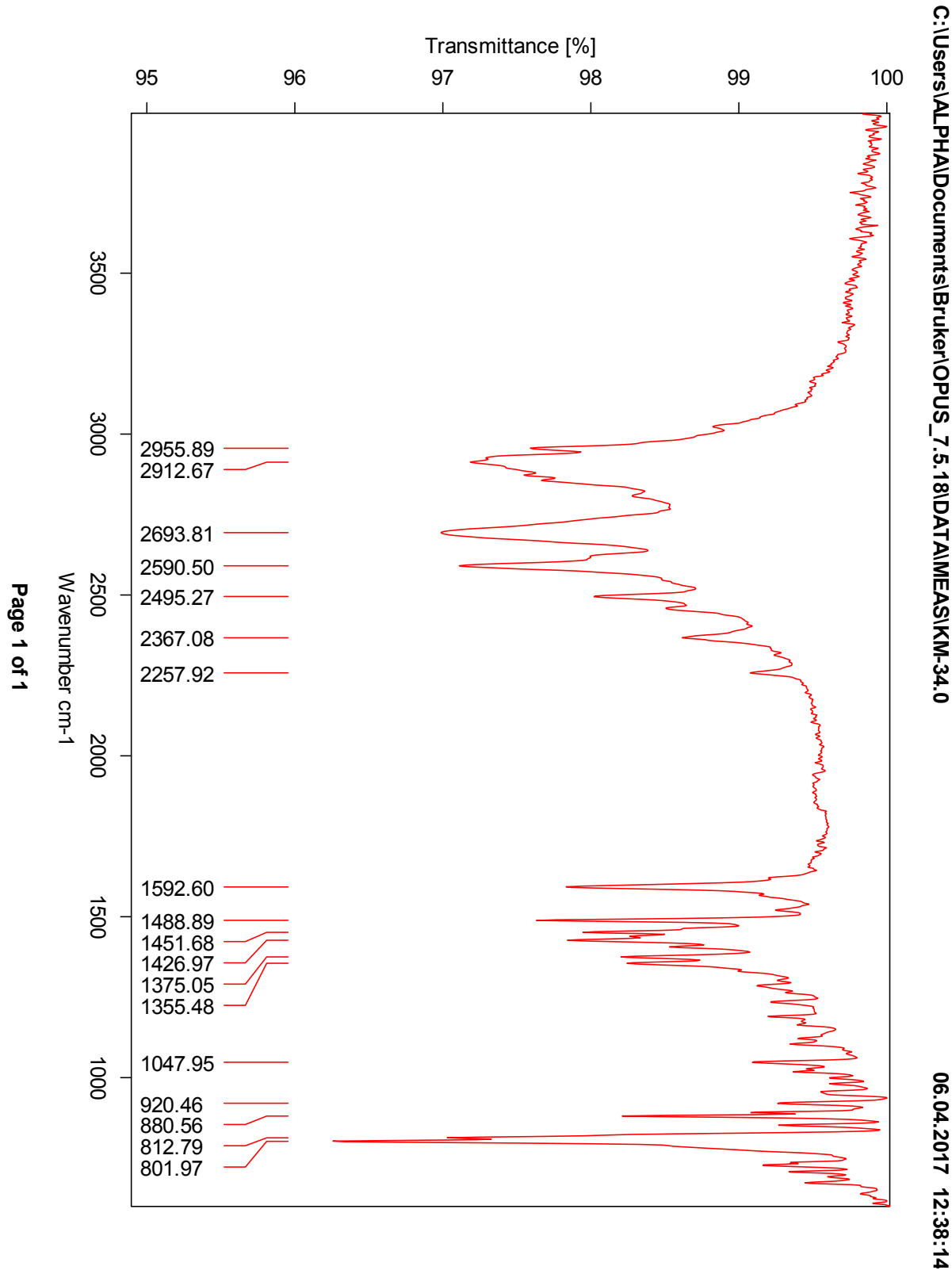
O.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6c



O.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6c



O.6 IR spectrum for 6c

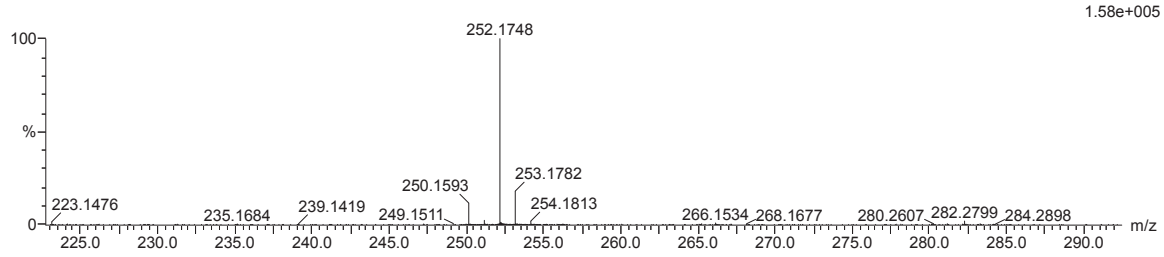


O.7 HRMS spectrum for 6c

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

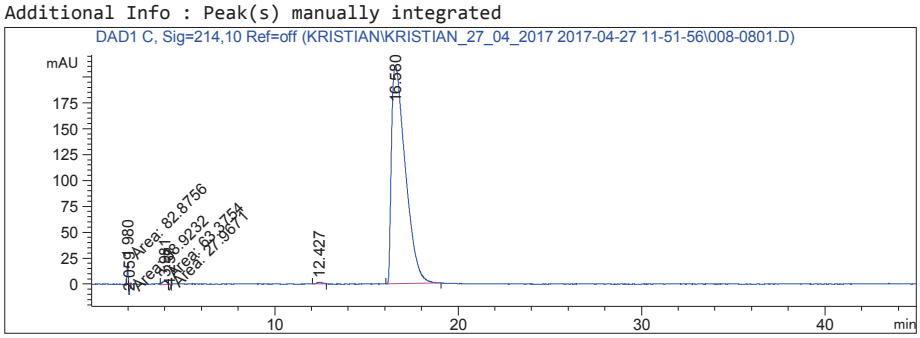
Monoisotopic Mass, Even Electron Ions
34 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10
2017-159 80 (1.567) AM2 (Ar,35000.0,0.00,0.00); Cm (80:83)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
252.1748	252.1752	-0.4	-1.6	8.5	928.5	n/a	n/a	C18 H22 N ion observed M+

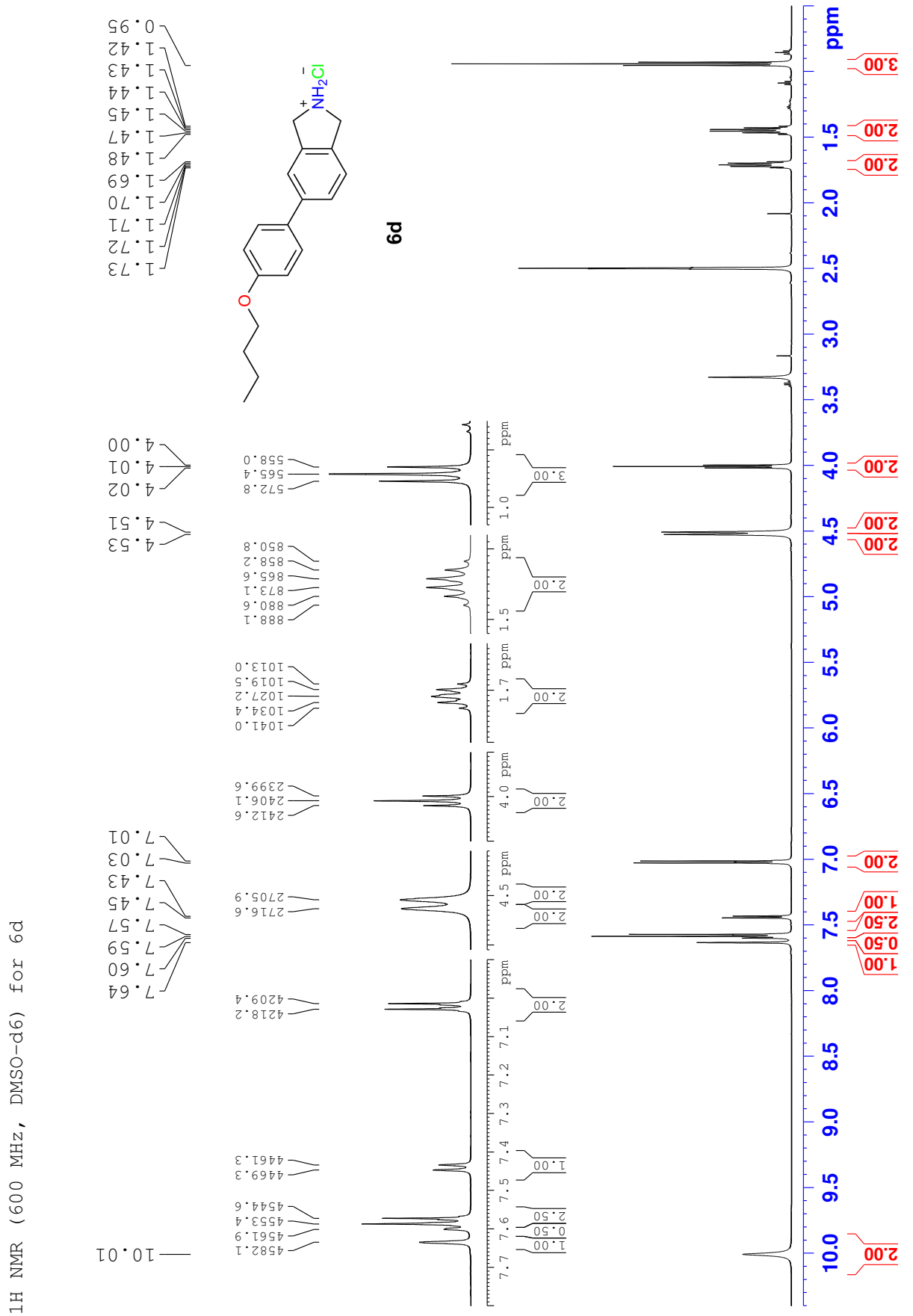
O.8 HPLC chromatogram for 6c

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\008-0801.D
Sample Name: KM-47 #2

======================================================================
Acq. Operator   : Kristian                      Seq. Line :   8
Acq. Instrument : UPLC                          Location : Vial 8
Injection Date  : 27.04.2017 15:03:41                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                  \C18PURITYSALT.M
Last changed    : 27.04.2017 13:33:07 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\008-0801.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 19:05:09 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

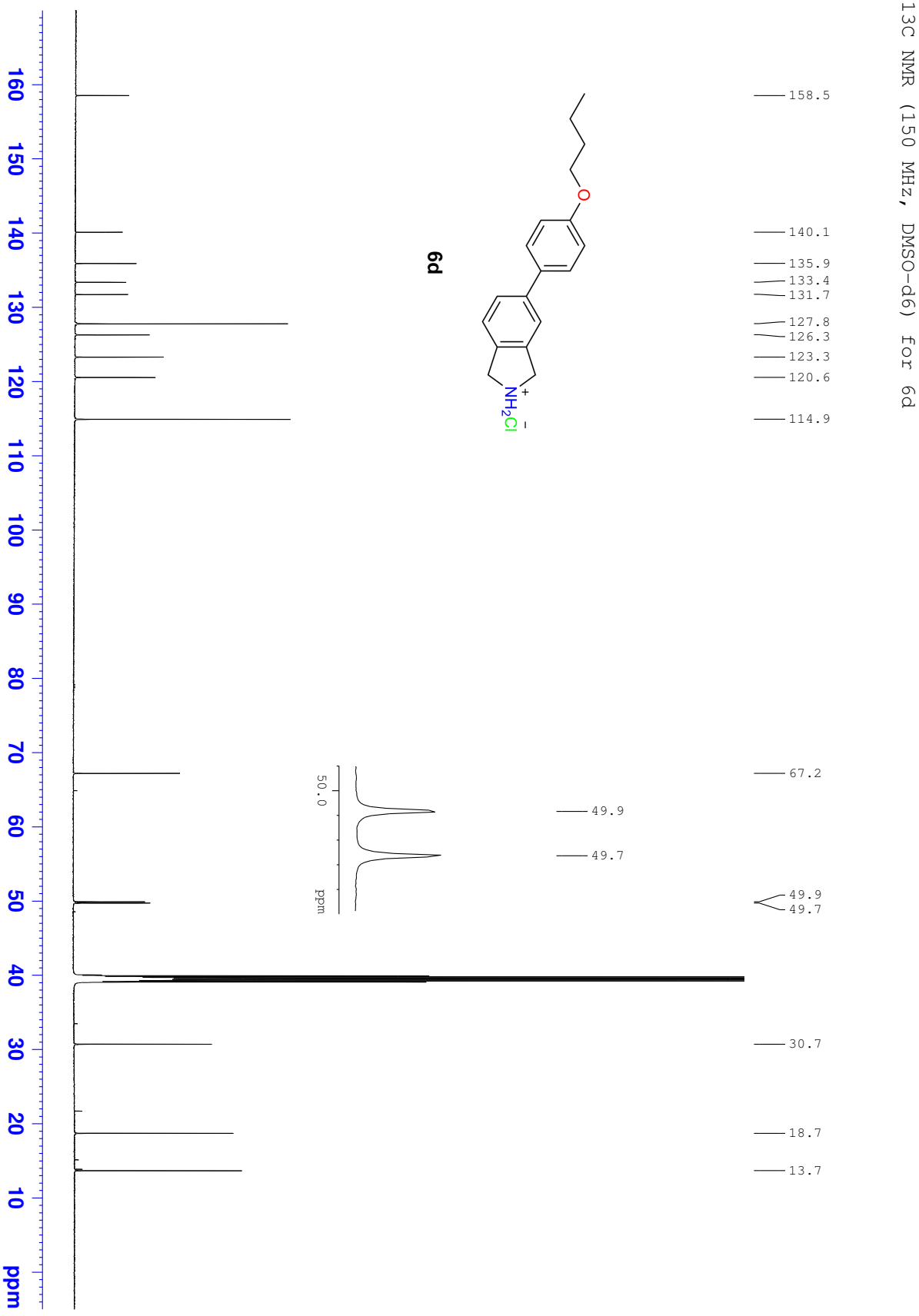



```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.980 MM    0.0665   82.87560   20.76117   0.7306
   2   2.059 MM N  0.0594   38.92320   10.91382   0.3431
   3   3.981 MM    0.3295   63.37542    3.20588   0.5587
   4   4.257 PM N  0.0883   27.96711    5.27744   0.2466
   5  12.427 BB    0.2605   31.10106    1.60231   0.2742
   6  16.580 BB    0.7896 1.10990e4   210.32925  97.8468

Totals :                  1.13432e4   252.08987


======================================================================
                        *** End of Report ***
```

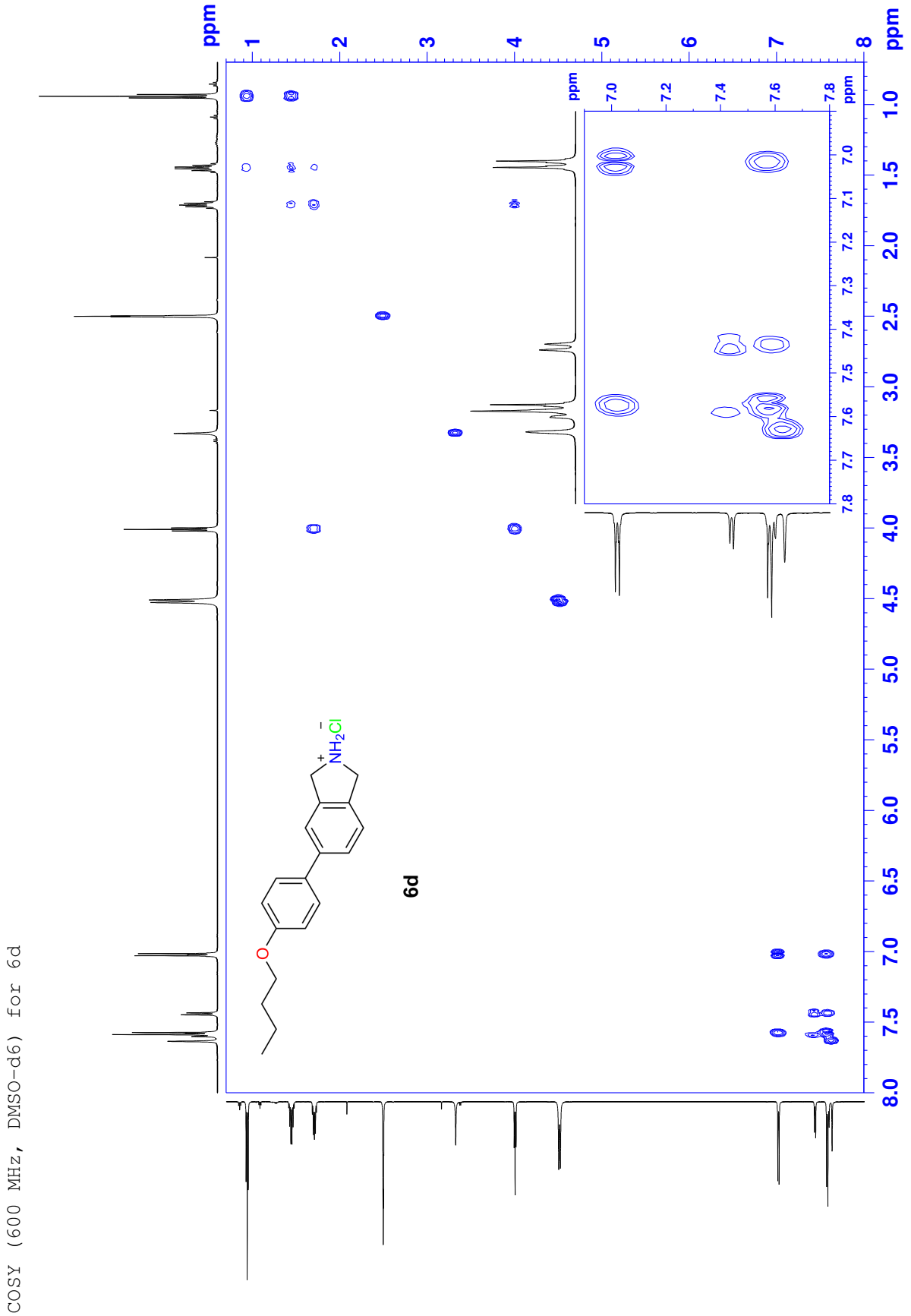
P.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6d



P.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6d

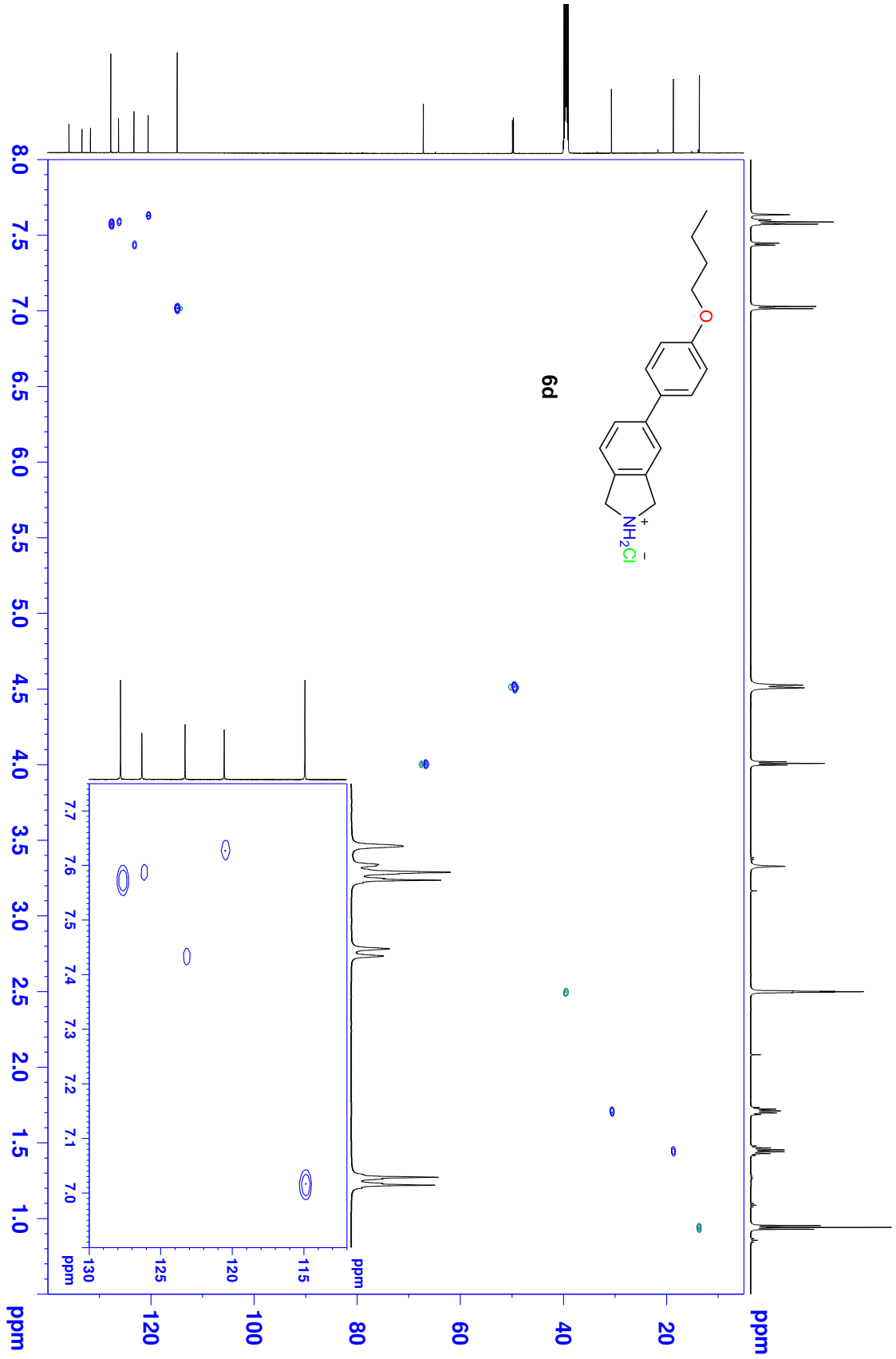


P.3 COSY (600 MHz, DMSO) spectrum for 6d

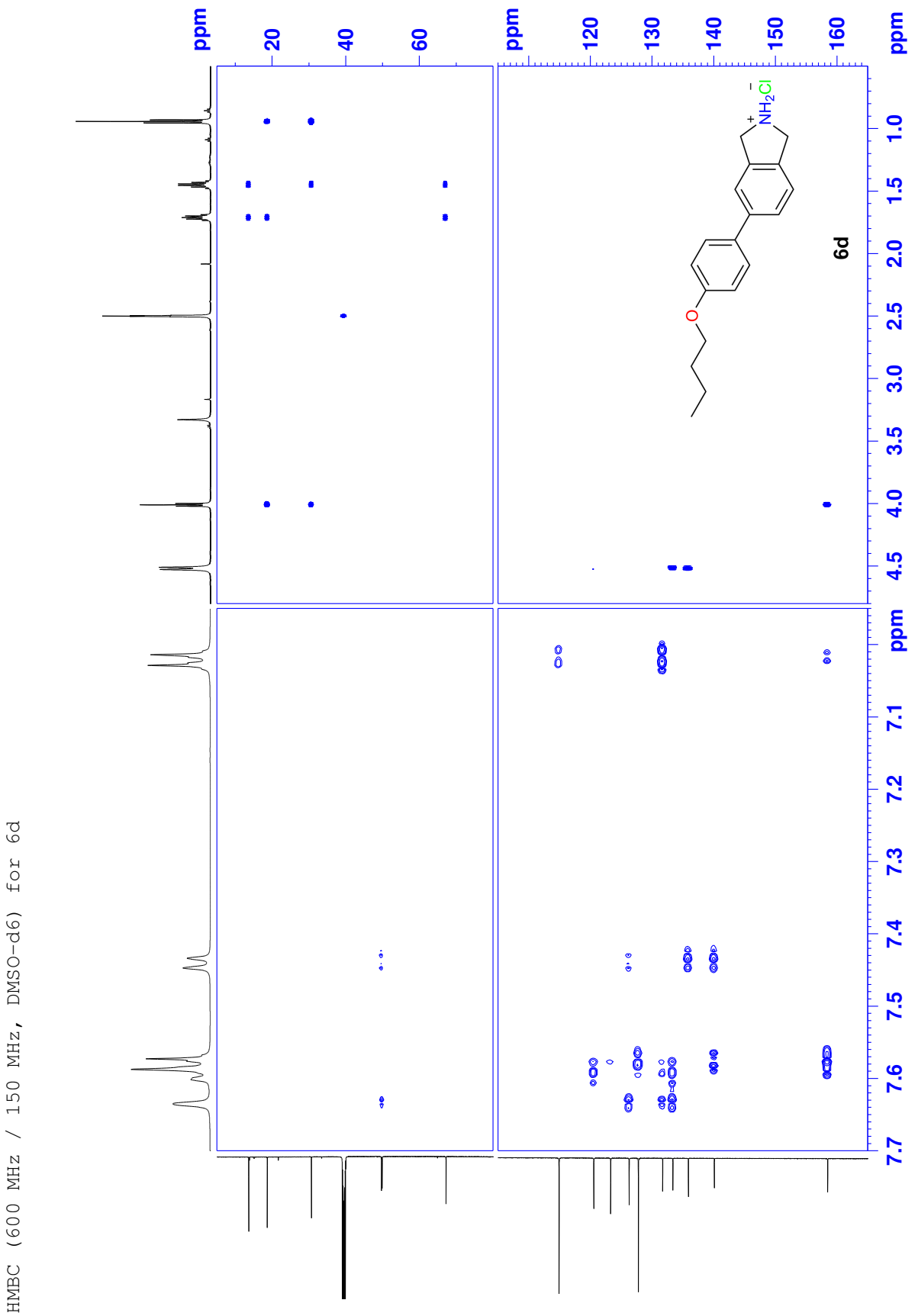


COSY (600 MHz, DMSO-d6) for 6d

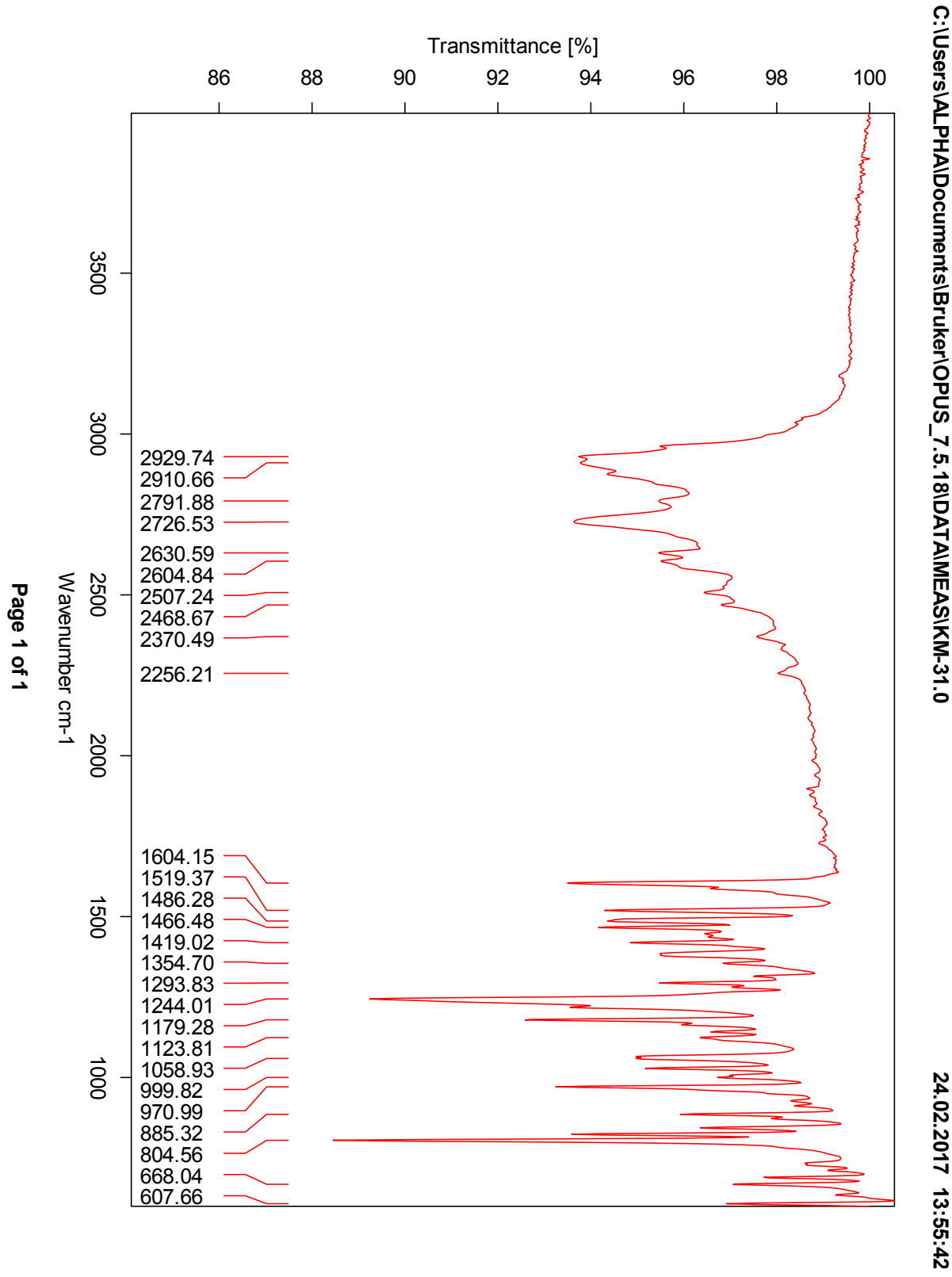
P.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6d



P.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6d



P.6 IR spectrum for 6d

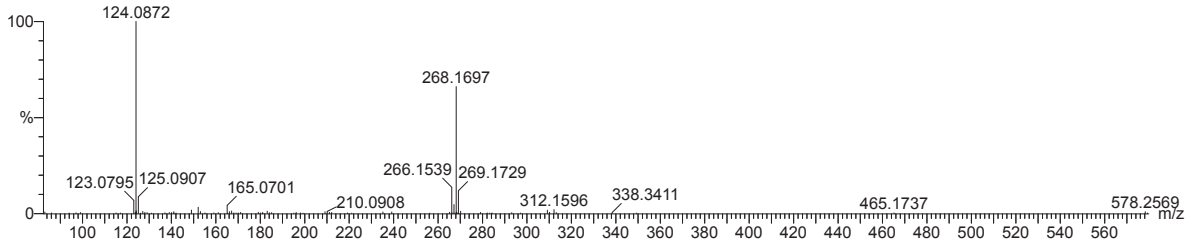


P.7 HRMS spectrum for 6d

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

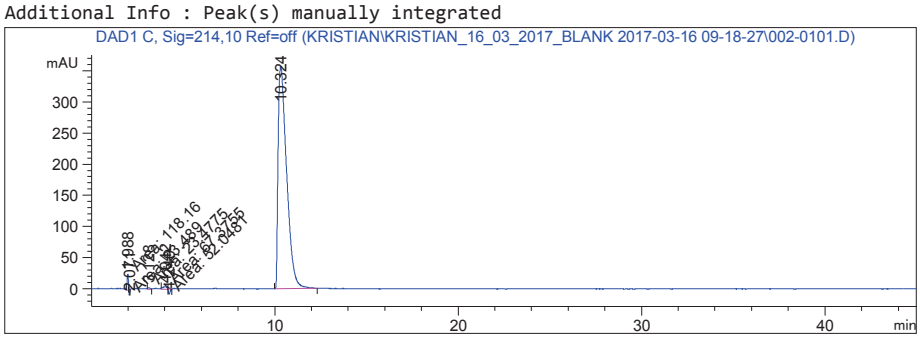
Monoisotopic Mass, Even Electron Ions
910 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 2-500 H: 0-1000 N: 0-20 O: 0-25 S: 0-2
NT-MSLAB-Operator-SVG
2017-114 158 (3.084) AM2 (Ar,35000.0,0.00,0.00); Cm (151:160) 1: TOF MS ASAP+ 1.78e+005



Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
268.1697	268.1695	0.2	0.7	-0.5	164.0	18.214	0.00	C10 H26 N3 O3 S
	268.1701	-0.4	-1.5	8.5	145.8	0.000	100.00	C18 H22 N O Ion observed [M+H]

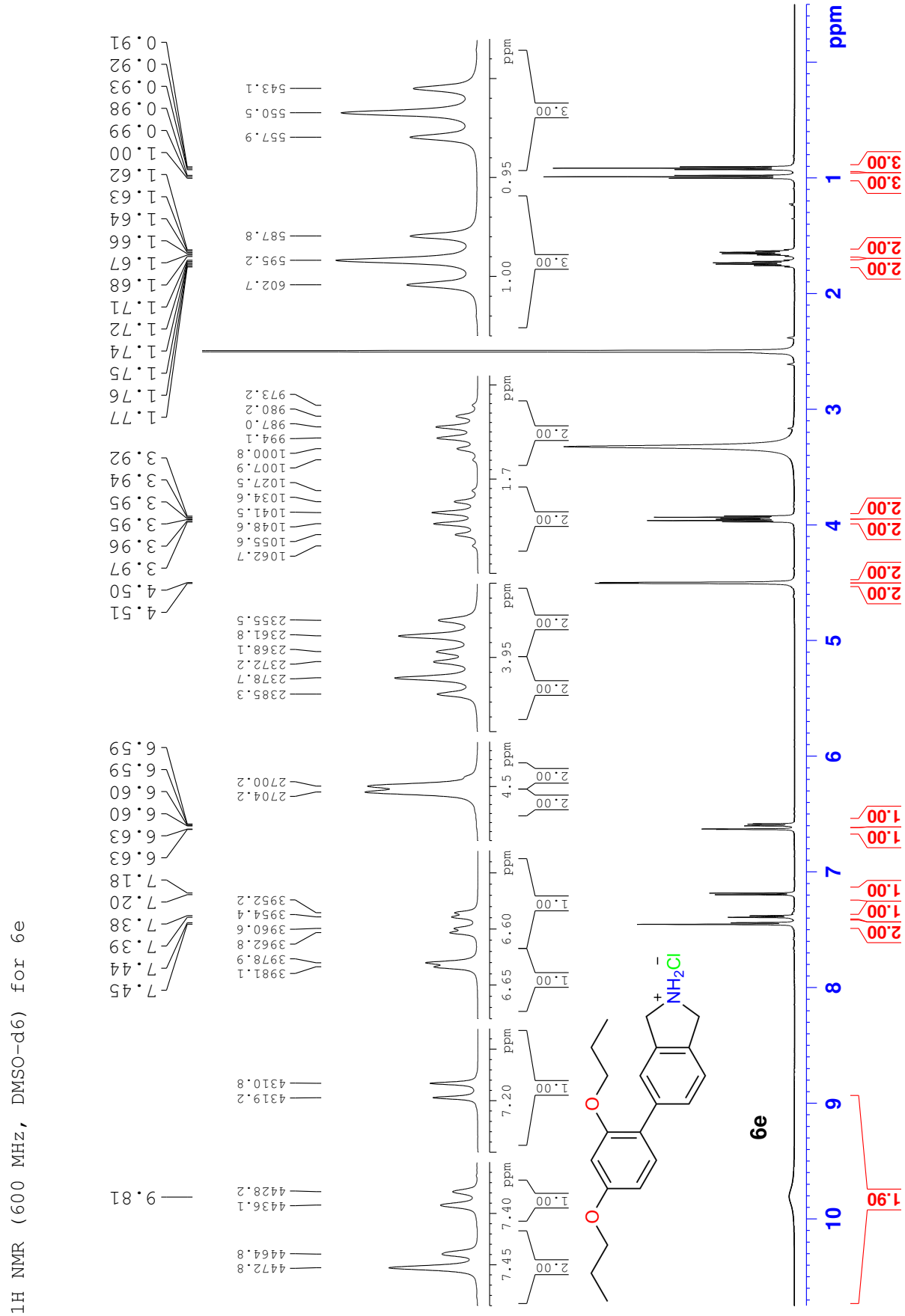
P.8 HPLC chromatogram for 6d

```
Data File C:\CHEM32\...A\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\002-0101.D
Sample Name: KM-31

======================================================================
Acq. Operator   : Kristian M                    Seq. Line :   1
Acq. Instrument : UPLC                          Location : Vial 2
Injection Date  : 16.03.2017 09:19:13                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27
                  \C18PURITYSALT.M
Last changed    : 16.03.2017 09:17:23 by Kristian M
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\002
                  -0101.D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 12:21:40 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

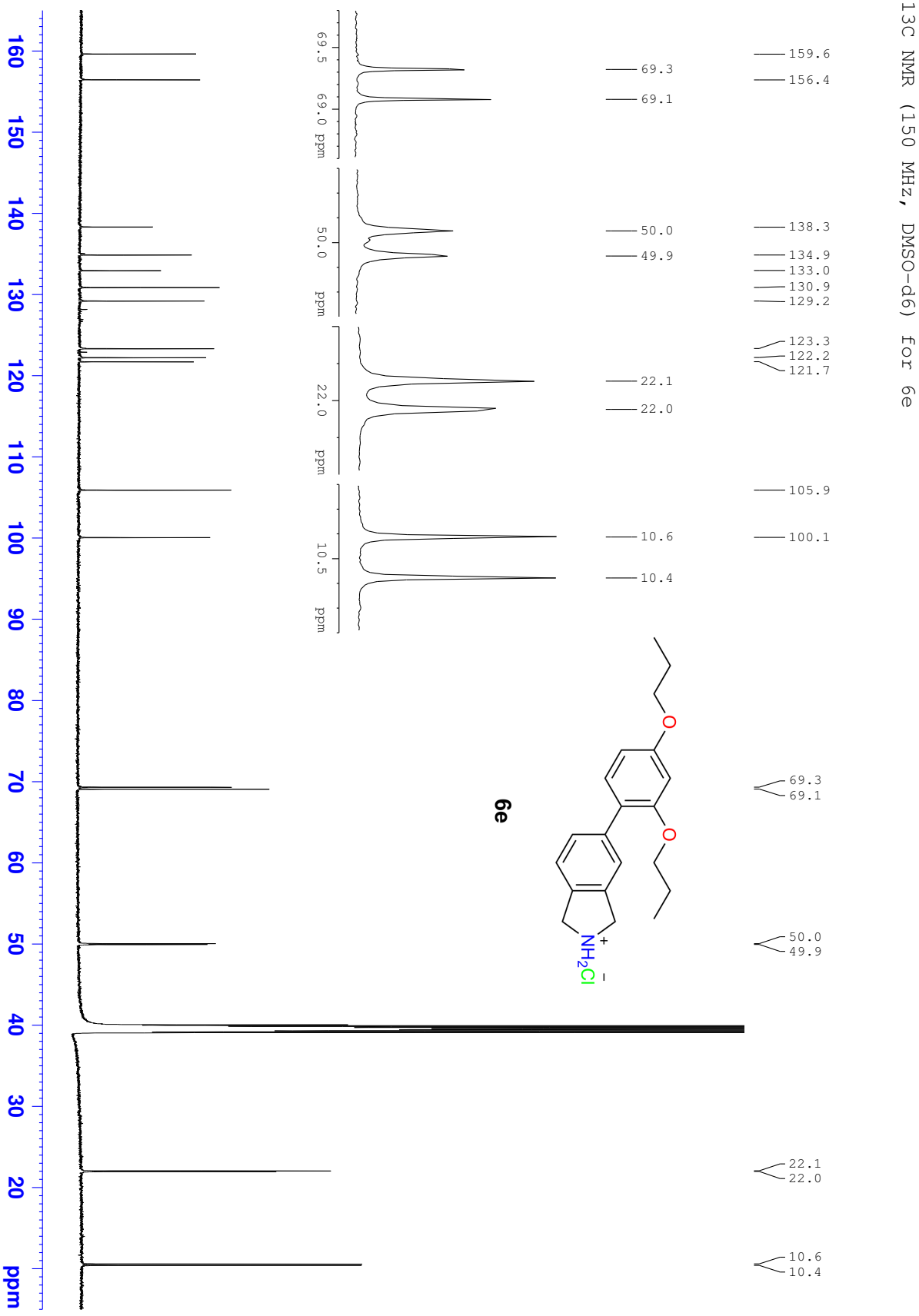



```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.988 MM    0.0773  118.15976   25.48305   1.0016
   2   2.071 MP N  0.0460   23.48899    8.51179   0.1991
   3   3.128 MM    0.1509   23.47748    2.59270   0.1990
   4   4.042 MM    0.2728   67.37554    4.11697   0.5711
   5   4.240 MM N  0.1011   52.04813    8.57691   0.4412
   6  10.324 BB    0.4955 1.15122e4   357.65146  97.5879

Totals :                  1.17967e4   406.93288


======================================================================
                        *** End of Report ***
```

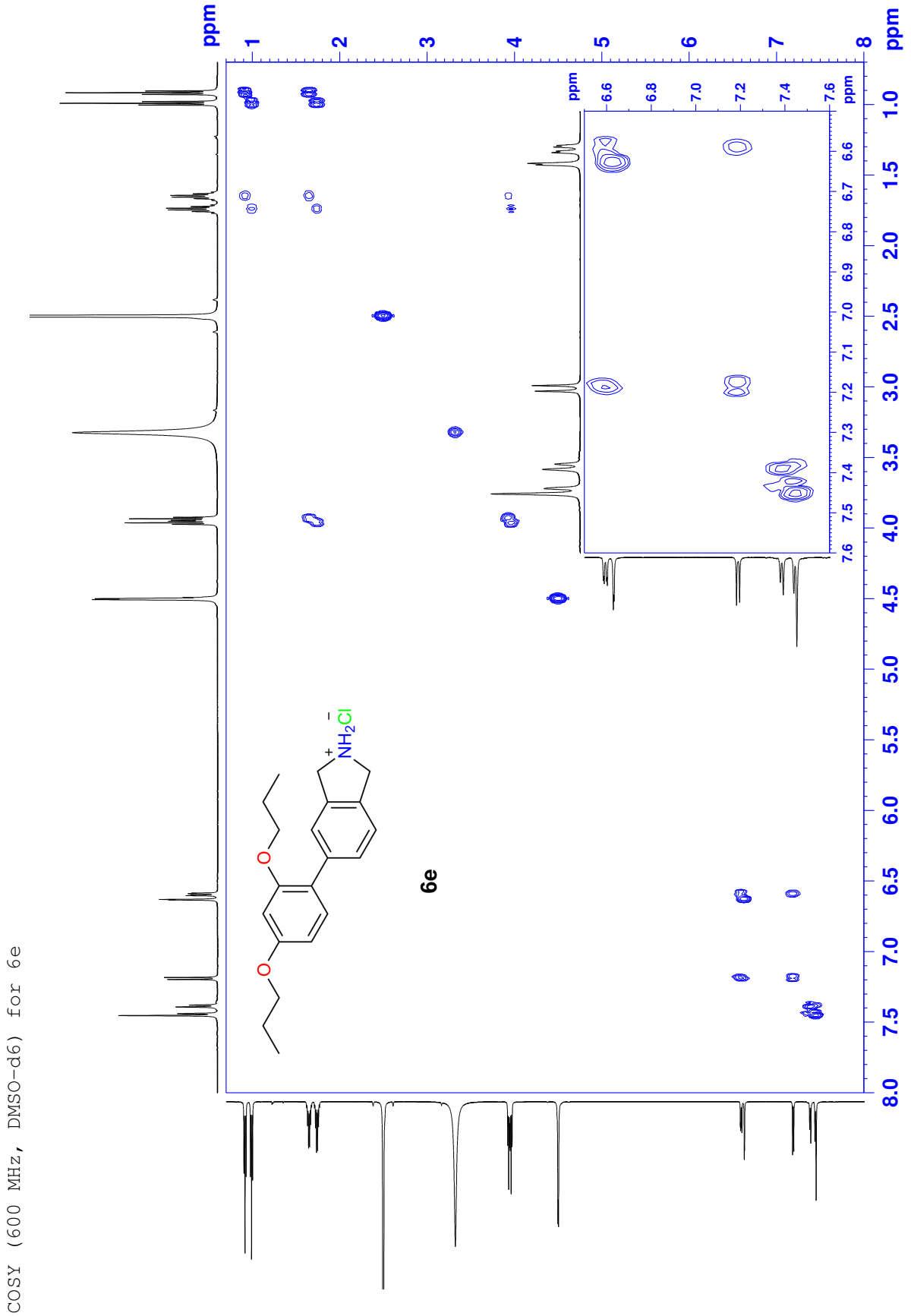
Q.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6e



Q.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6e

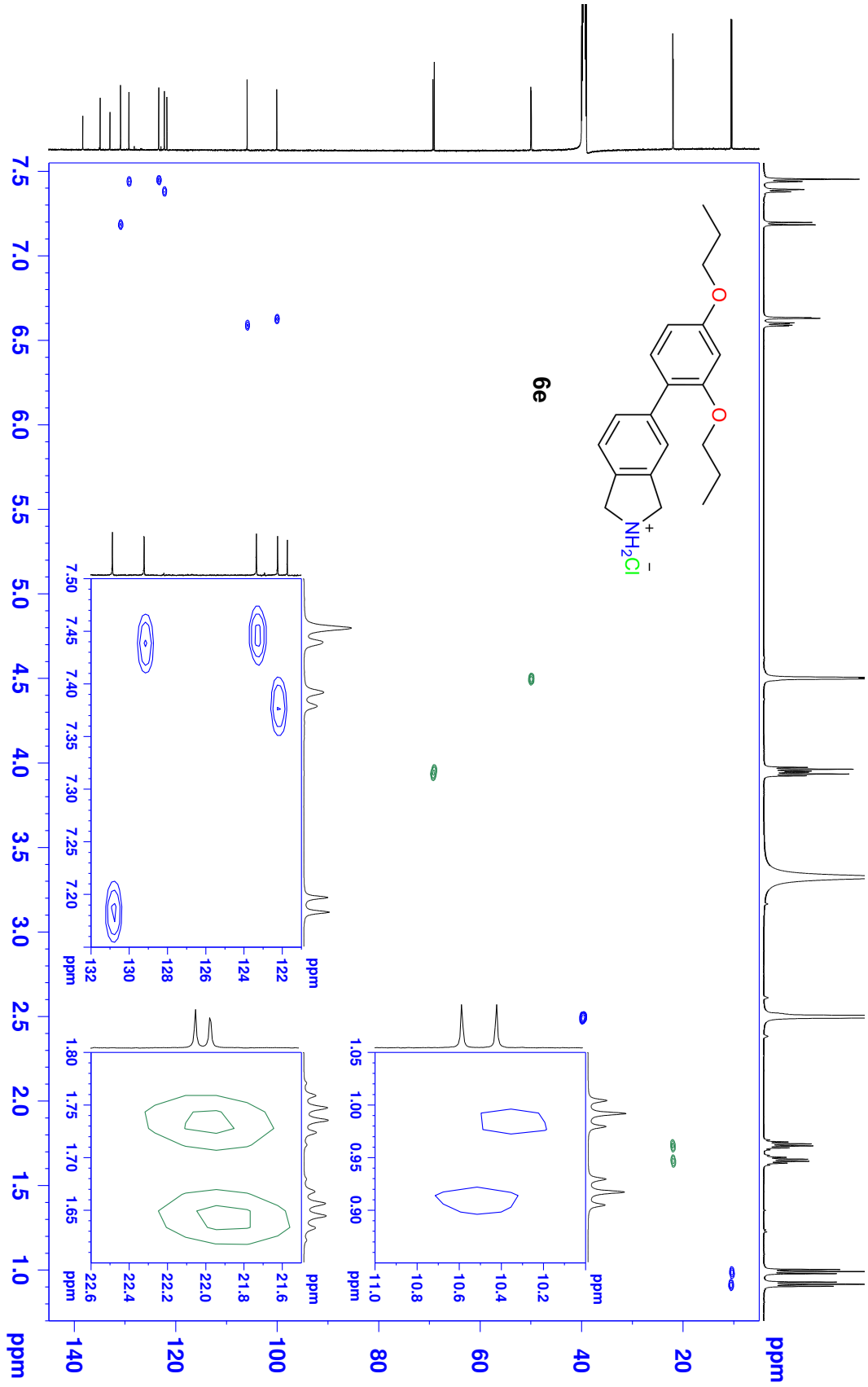


Q.3 COSY (600 MHz, DMSO) spectrum for 6e

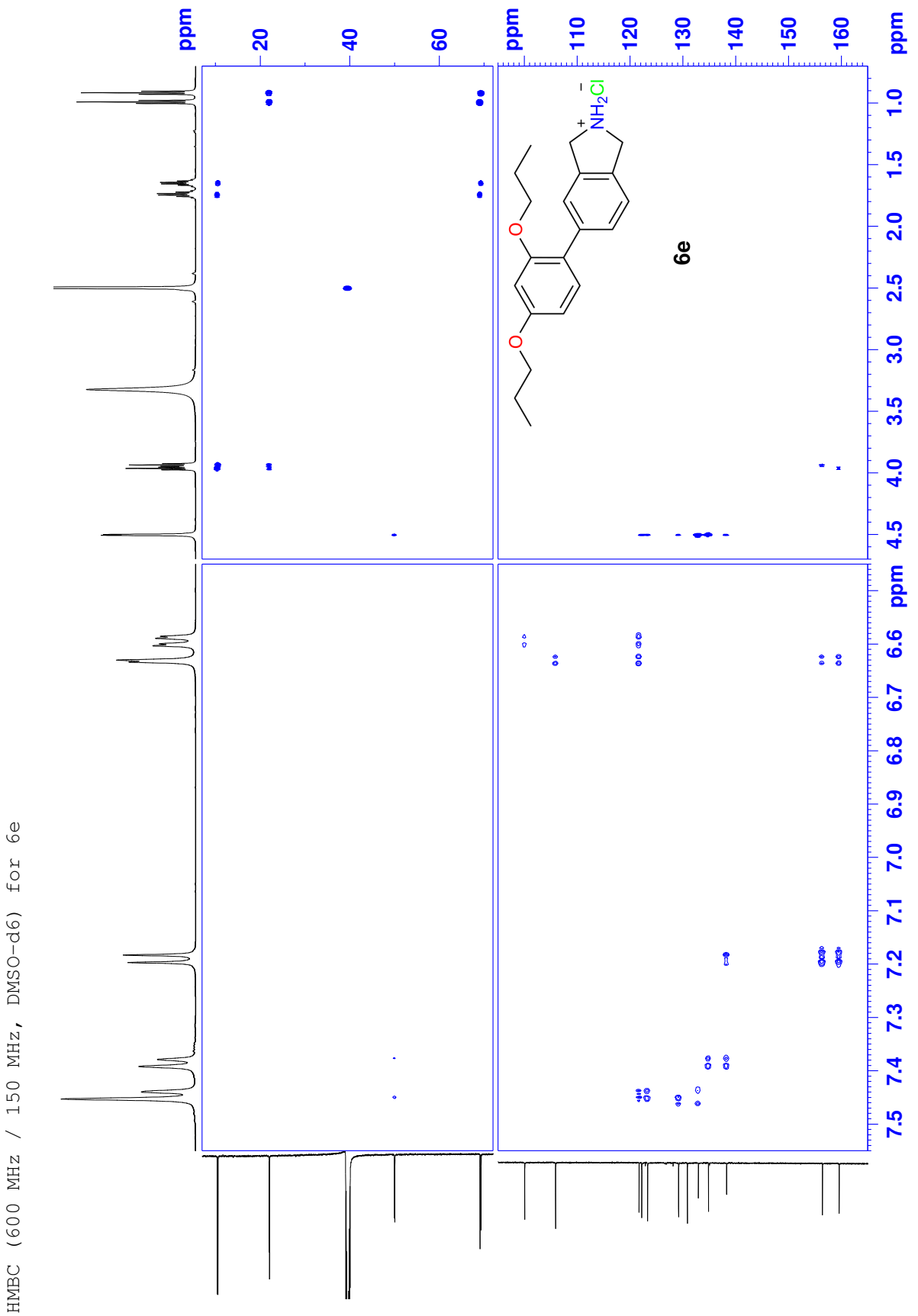


COSY (600 MHz, DMSO-d6) for 6e

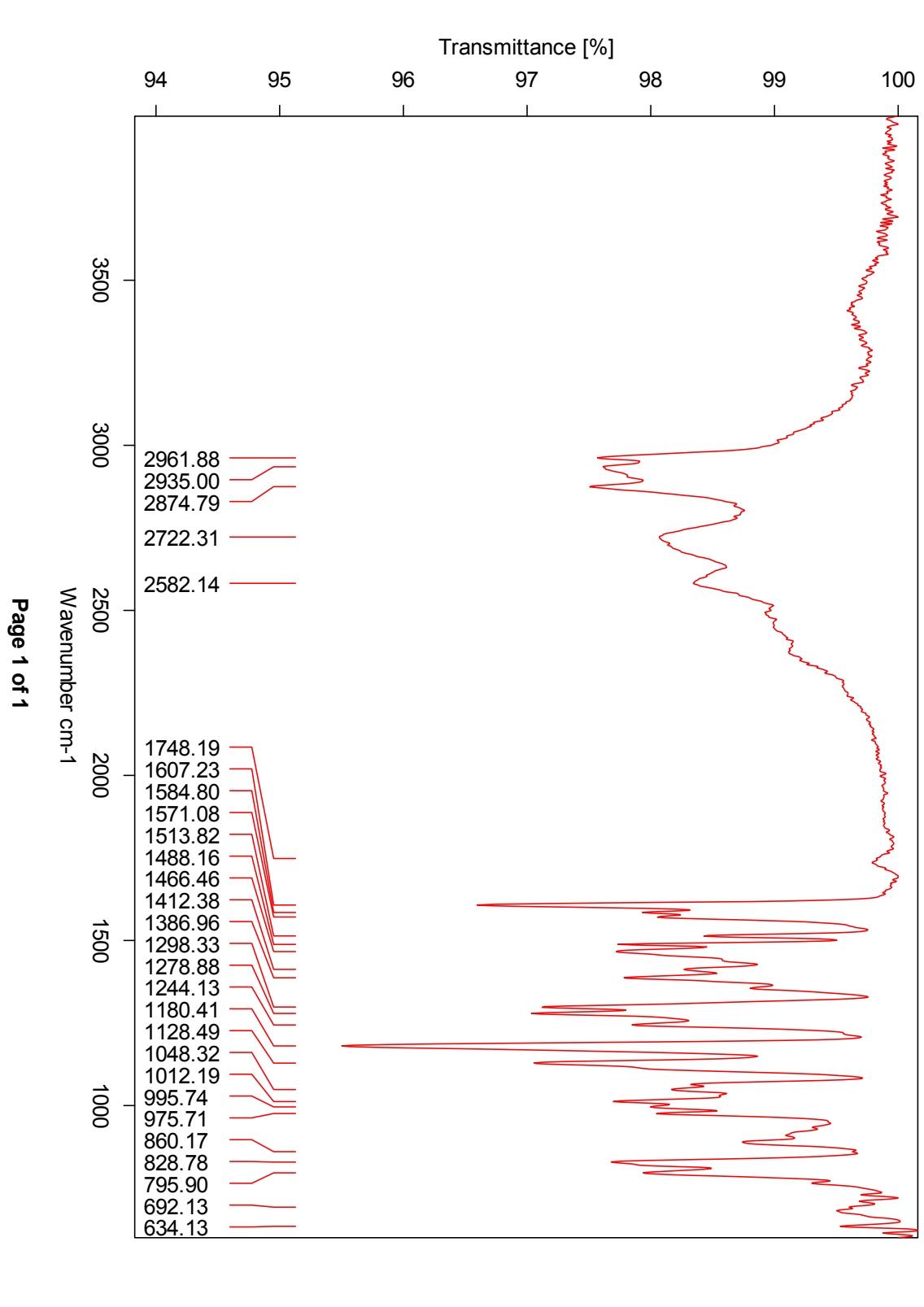
Q.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6e



Q.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6e



Q.6 IR spectrum for 6e

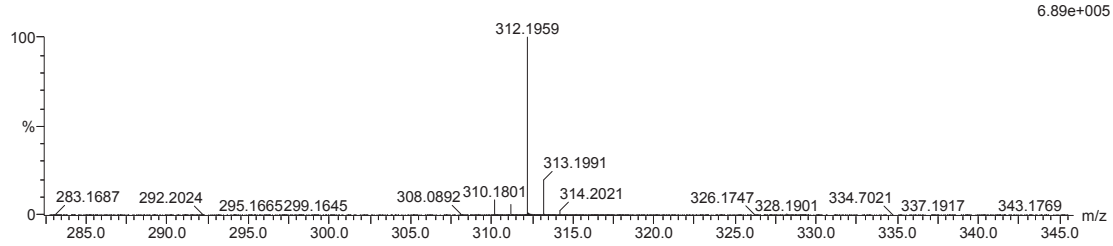


Q.7 HRMS spectrum for 6e

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

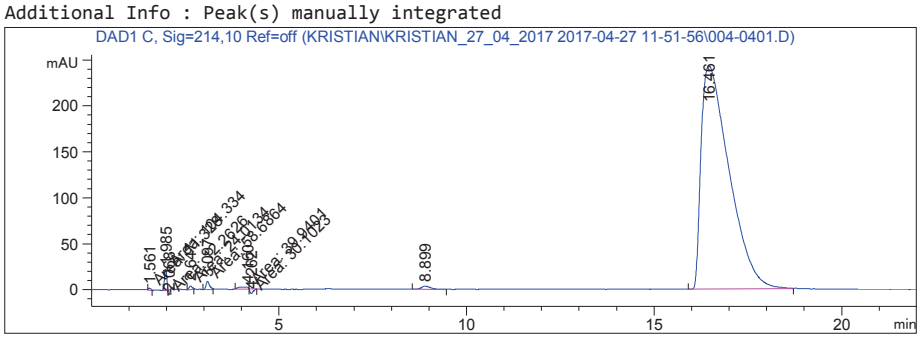
Monoisotopic Mass, Even Electron Ions
439 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 O: 0-25
2017-184 32 (0.637) AM2 (Ar,35000.0,0.00,0.00); Cm (30:32)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
312.1959	312.1964	-0.5	-1.6	8.5	1205.6	n/a	n/a	C20 H26 N O2 ion observed M+

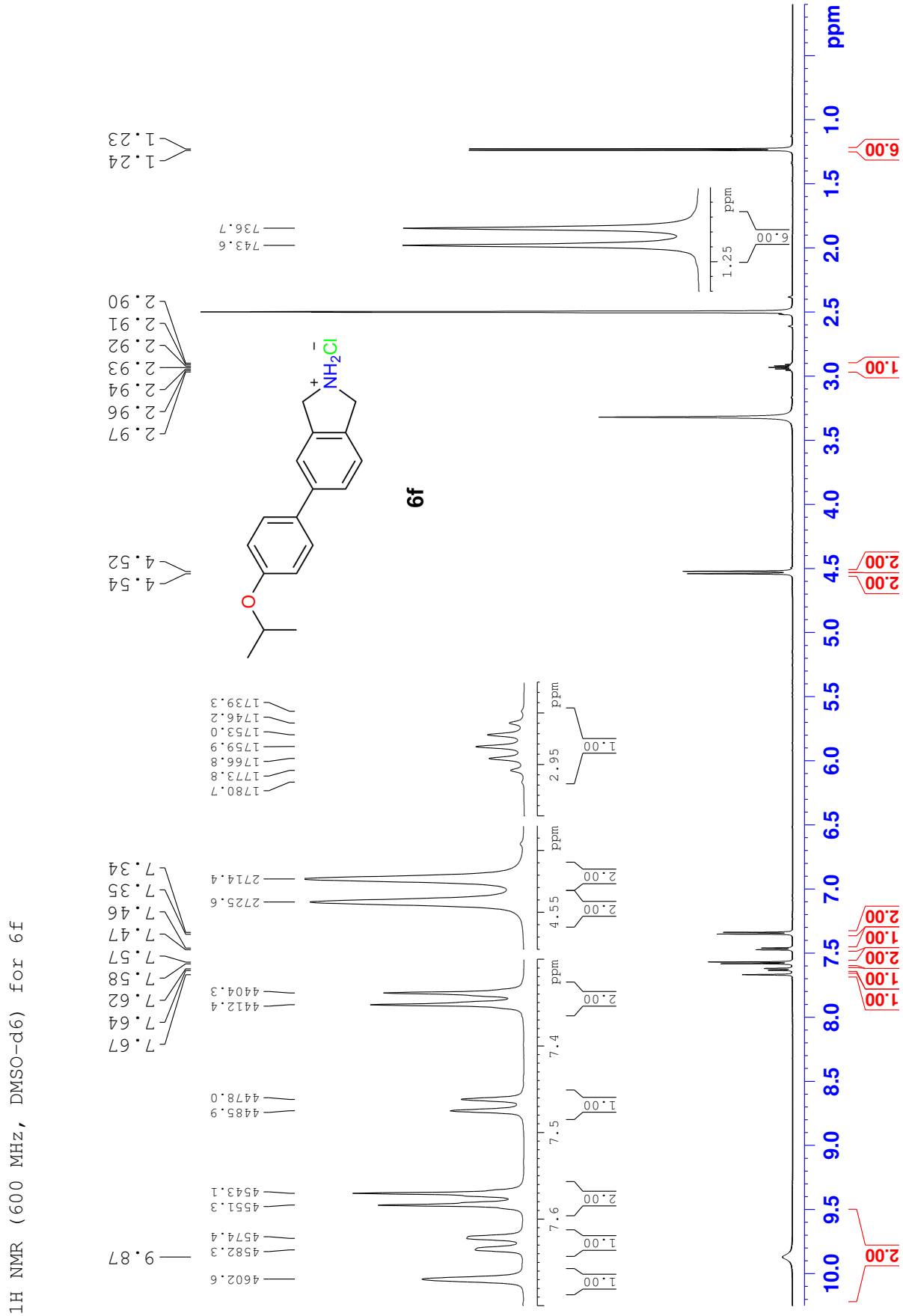
Q.8 HPLC chromatogram for 6e

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\004-0401.D
Sample Name: KM-41

======================================================================
Acq. Operator   : Kristian                      Seq. Line :   4
Acq. Instrument : UPLC                          Location : Vial 4
Injection Date  : 27.04.2017 12:55:42                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                  \C18PURITYSALT.M
Last changed    : 27.04.2017 13:16:02 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\004-0401.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 13:36:15 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

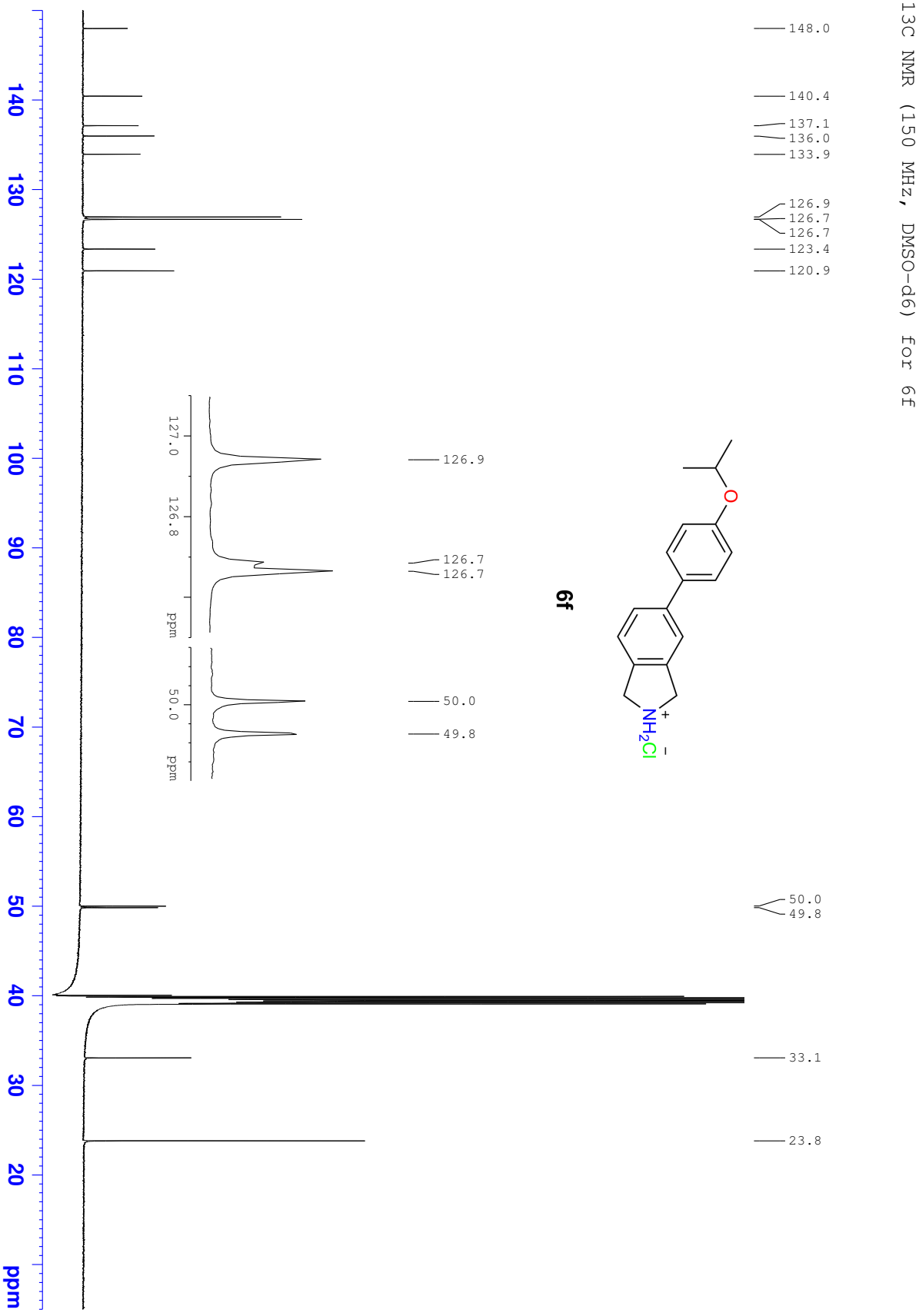



```
======================================================================
                          Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.561 MM    0.0822   11.32797    2.29591   0.0877
   2   1.985 MM    0.0748  104.33355   23.25313   0.8075
   3   2.068 MP N  0.0421   12.26260    4.85698   0.0949
   4   2.647 MM    0.1005   24.01343    3.98083   0.1859
   5   3.097 MF    0.1108   58.68644    8.82791   0.4542
   6   4.160 MM    0.2899   39.94006    2.29630   0.3091
   7   4.262 MM N  0.1037   30.10230    4.83849   0.2330
   8   8.899 BB    0.2678   61.97065    3.44656   0.4796
   9  16.461 BB    0.7786 1.25775e4   242.72241  97.3480

Totals :                  1.29202e4   296.51852


======================================================================
                          *** End of Report ***

UPLC 27.04.2017 13:36:18 Kristian M                          Page   1 of 1
```

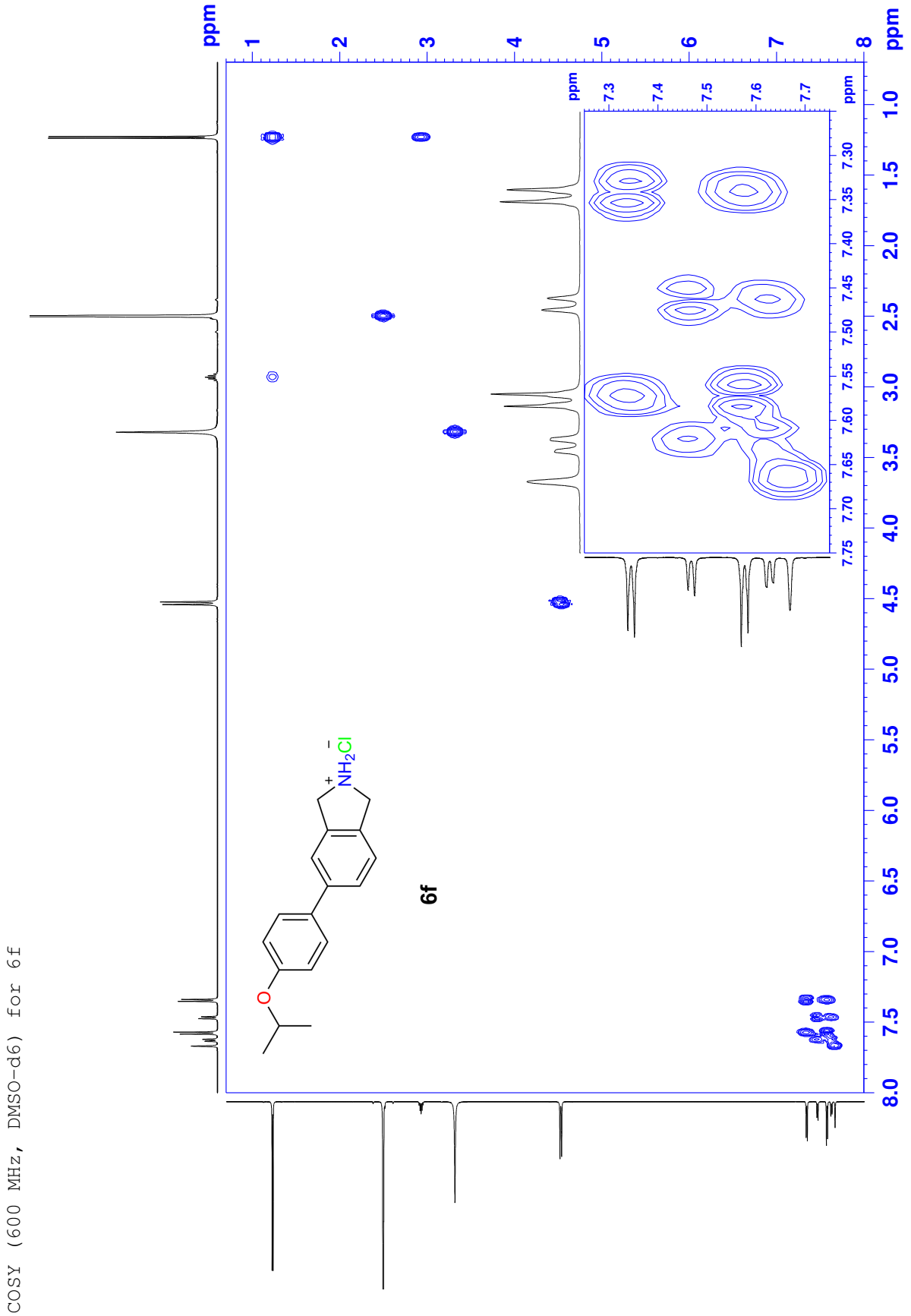
R.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6f



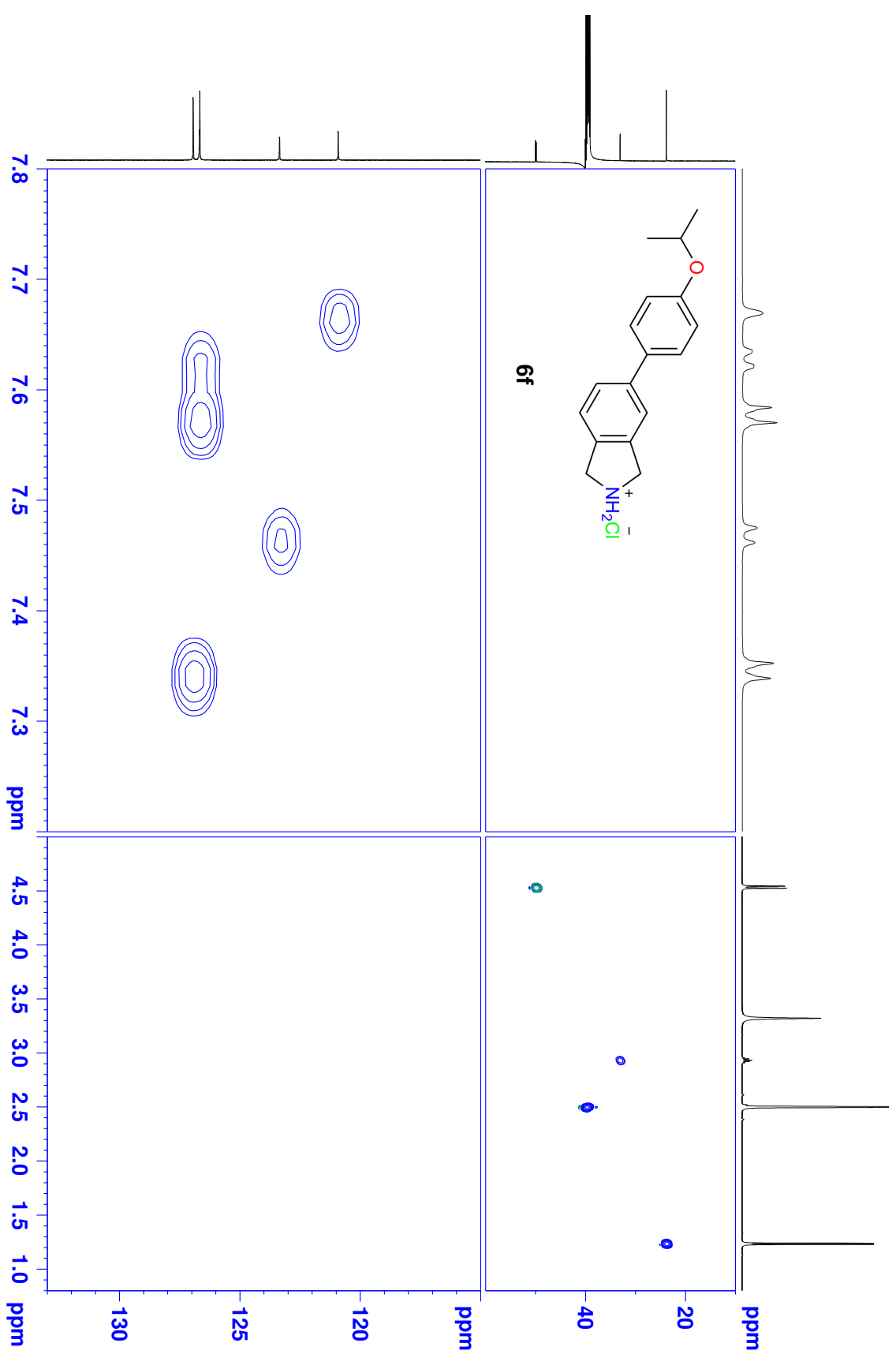
R.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6f



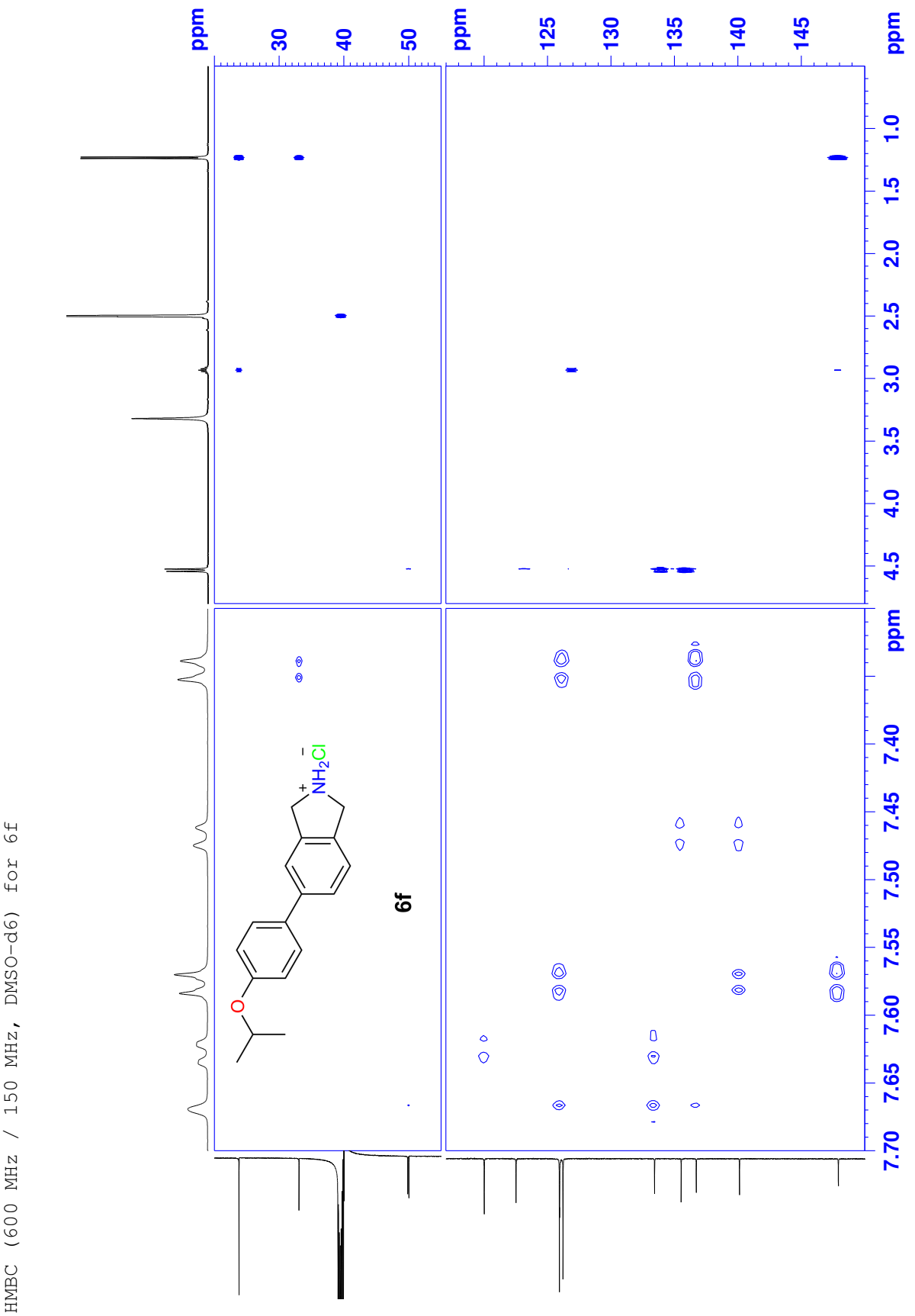
R.3 COSY (600 MHz, DMSO) spectrum for 6f



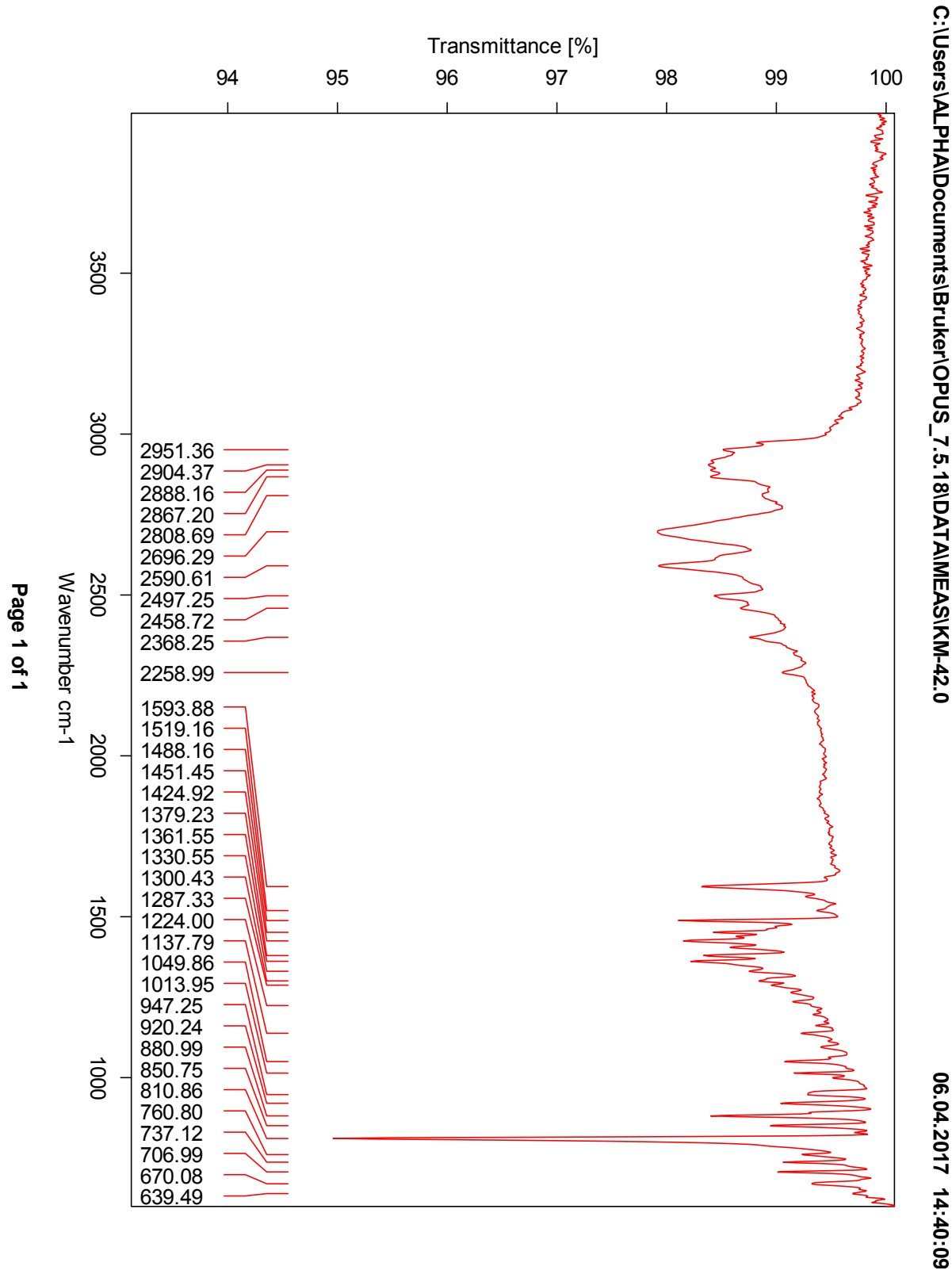
R.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6f



R.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6f



R.6 IR spectrum for 6f



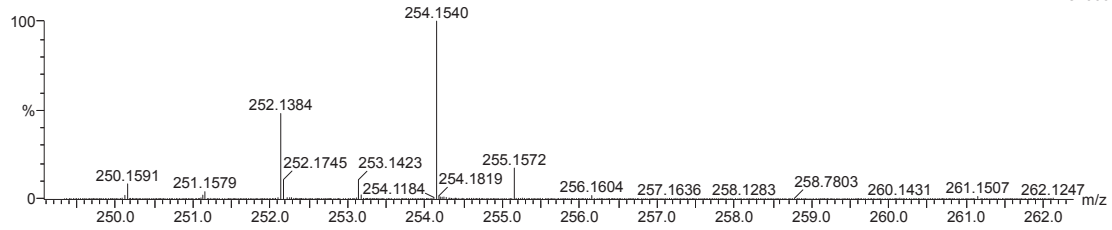
R.7 HRMS spectrum for 6f

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
298 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 O: 0-25
2017-185 101 (1.982) AM2 (Ar,35000.0,0.00,0.00); Cm (93:107)
1: TOF MS ASAP+

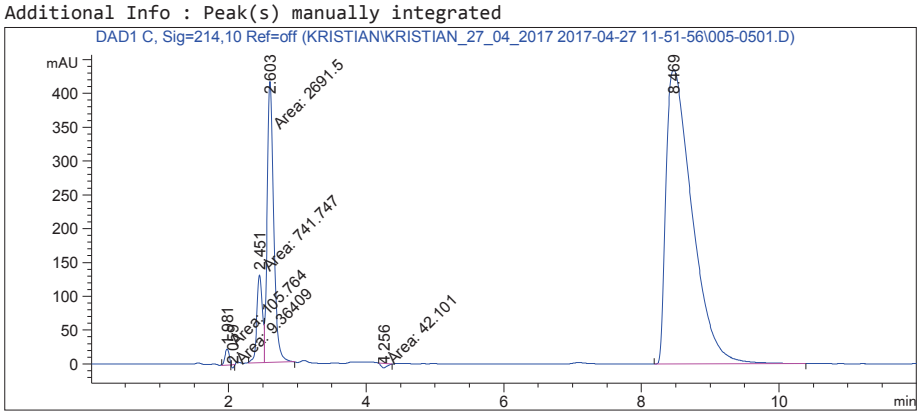
1.41e+006



Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
254.1540	254.1545	-0.5	-2.0	8.5	1699.3	n/a	n/a	C17 H20 N O ion observed M+

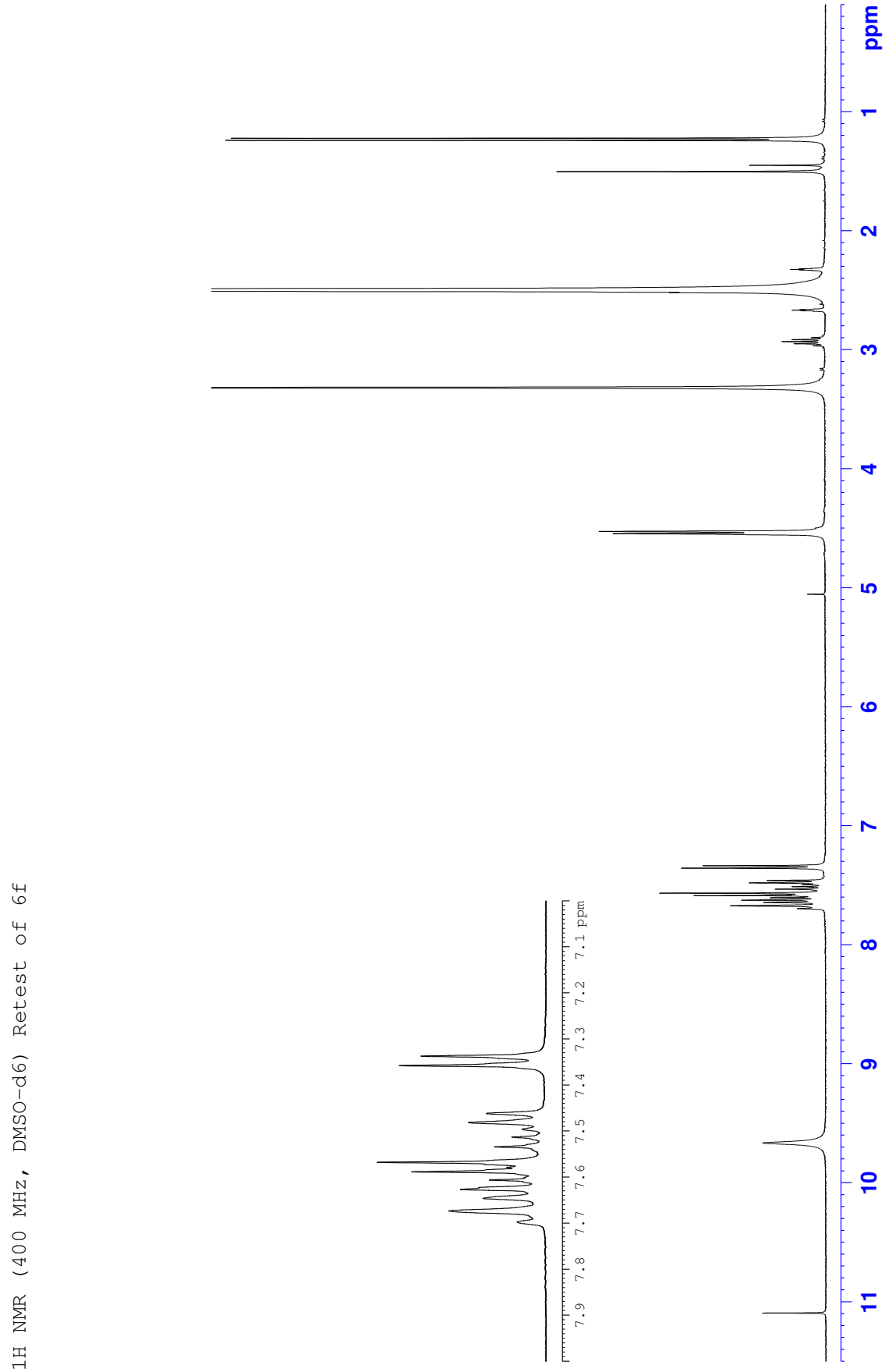
R.8 HPLC chromatogram for 6f

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\005-0501.D
Sample Name: KM-42 #2

======================================================================
Acq. Operator   : Kristian                      Seq. Line :   5
Acq. Instrument : UPLC                          Location : Vial 5
Injection Date  : 27.04.2017 13:18:42                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                  \C18PURITYSALT.M
Last changed    : 27.04.2017 13:29:09 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\005-0501.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 13:38:57 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.981 MM    0.0744  105.76404   23.69137   0.7035
   2   2.059 MM N  0.0330    9.36409    4.72942   0.0623
   3   2.451 MF    0.0953  741.74744  129.75235   4.9338
   4   2.603 FM    0.1076 2691.50024  416.96988  17.9028
   5   4.256 MM N  0.1047   42.10105    6.69937   0.2800
   6   8.469 BB    0.3987 1.14435e4   435.34644  76.1176

Totals :                  1.50340e4  1017.18883


======================================================================
                        *** End of Report ***

UPLC 27.04.2017 13:39:04 Kristian M                          Page   1 of 1
```

R.9 ^{1}H NMR (400 MHz, DMSO) retest for 6f

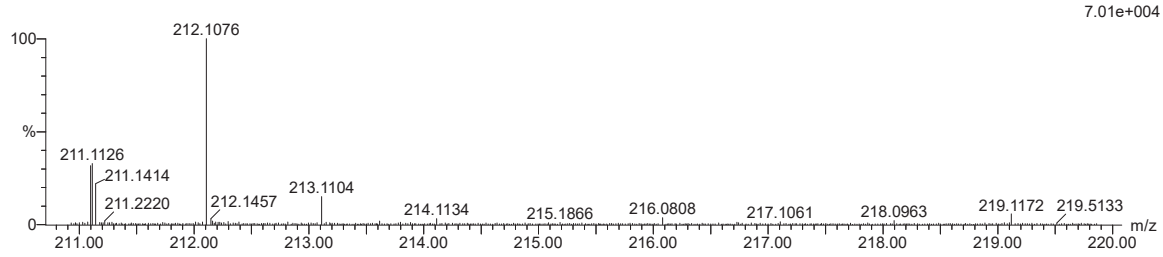


R.10 HRMS spectrum phenol derivative 6f

Elemental Composition Report **Page 1**

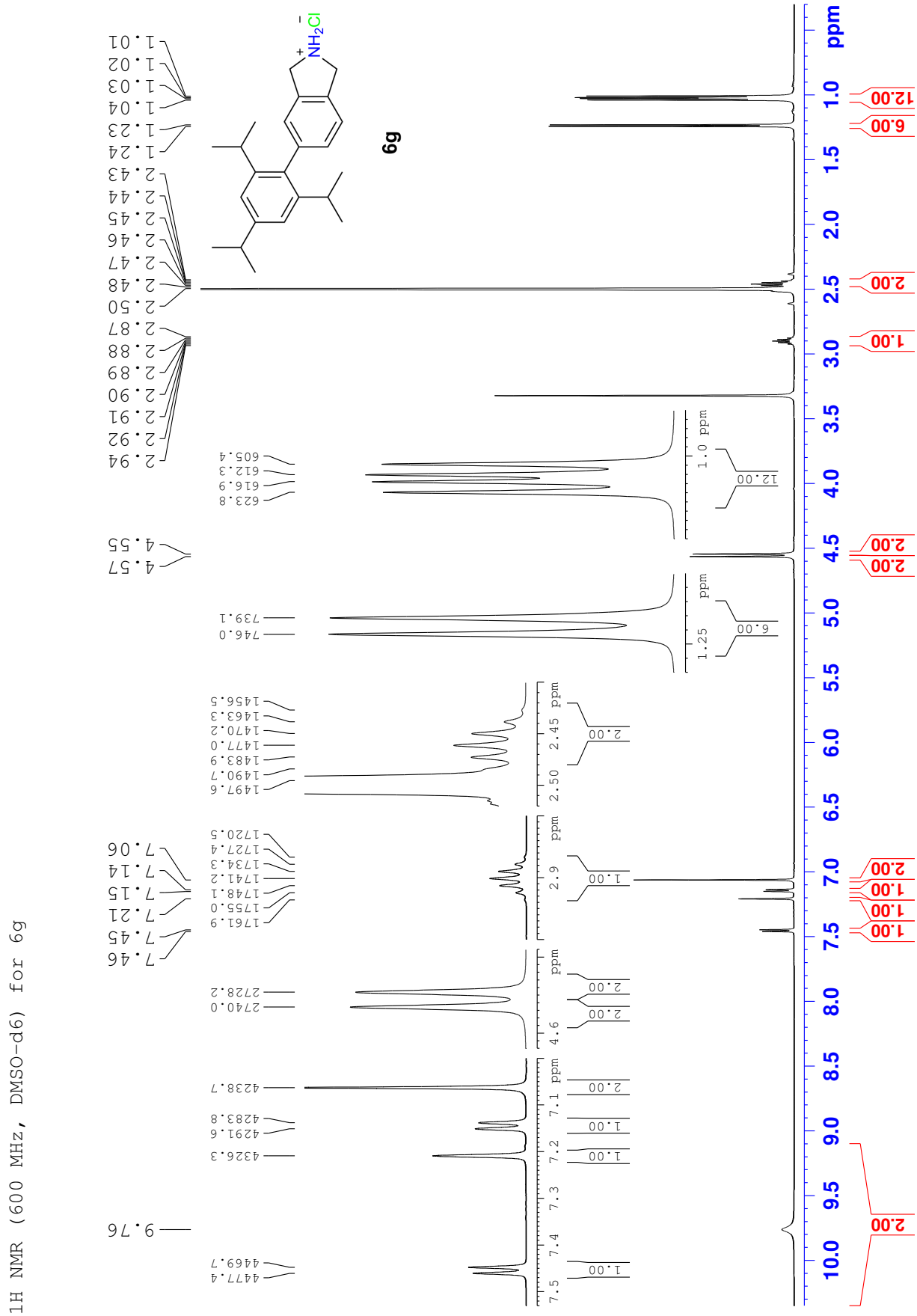
Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
962 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1 Cl: 0-8
2017-304esi 100 (0.909) AM2 (Ar,35000.0,0.00,0.00); Cm (94:102)
1: TOF MS ES+

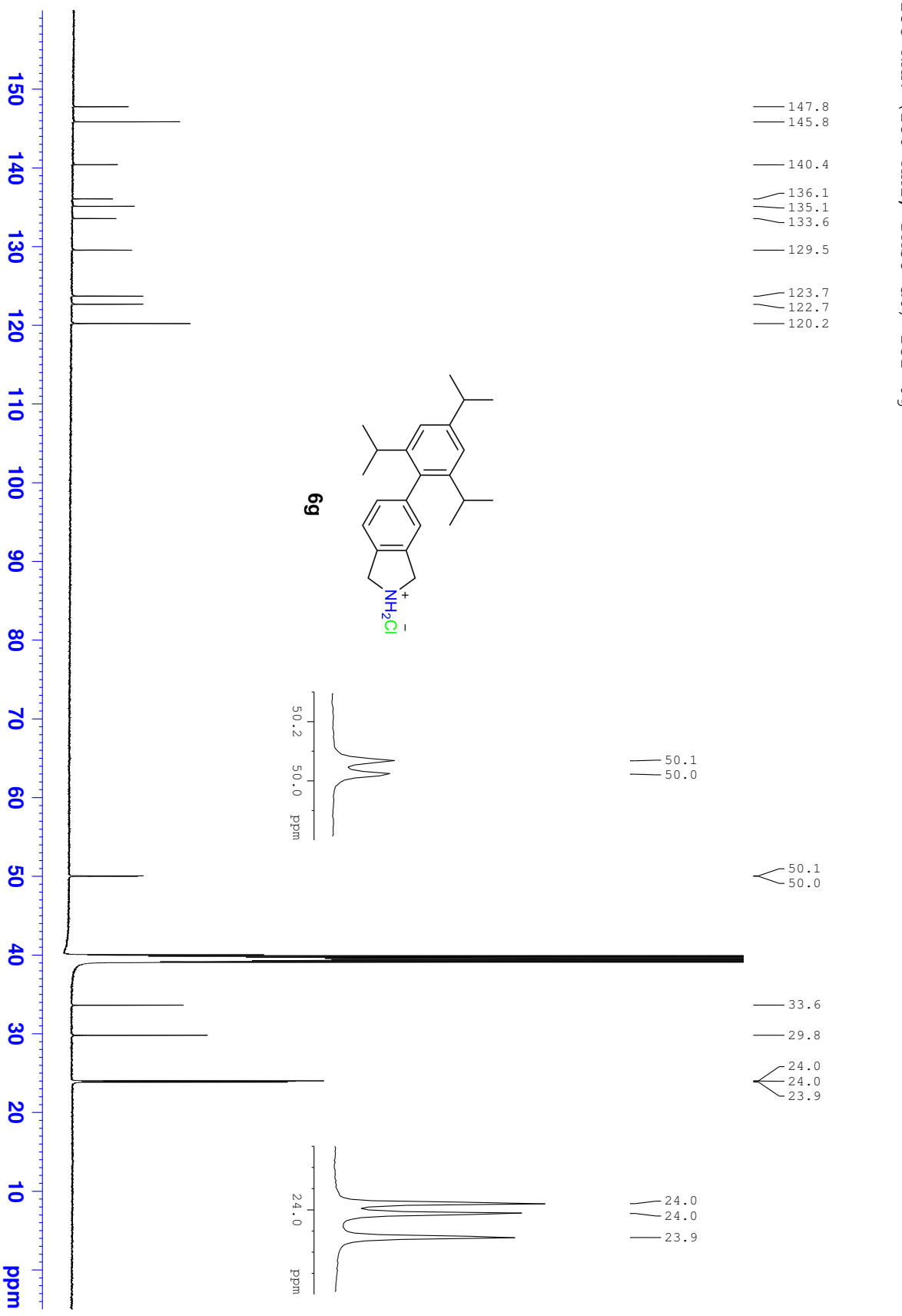


Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
212.1076	212.1075	0.1	0.5	8.5	1561.8	n/a	n/a	C14 H14 N O

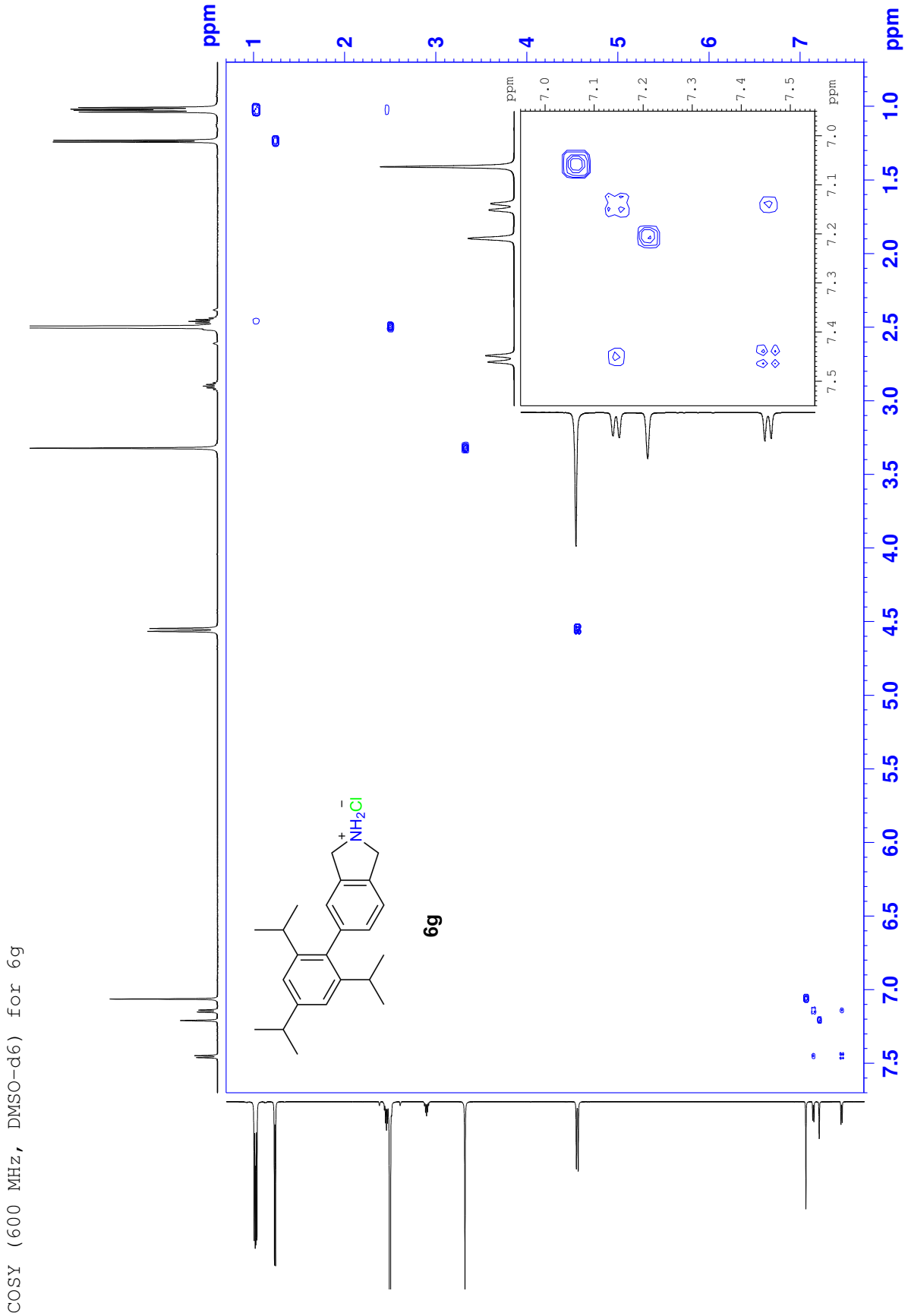
S.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 6g



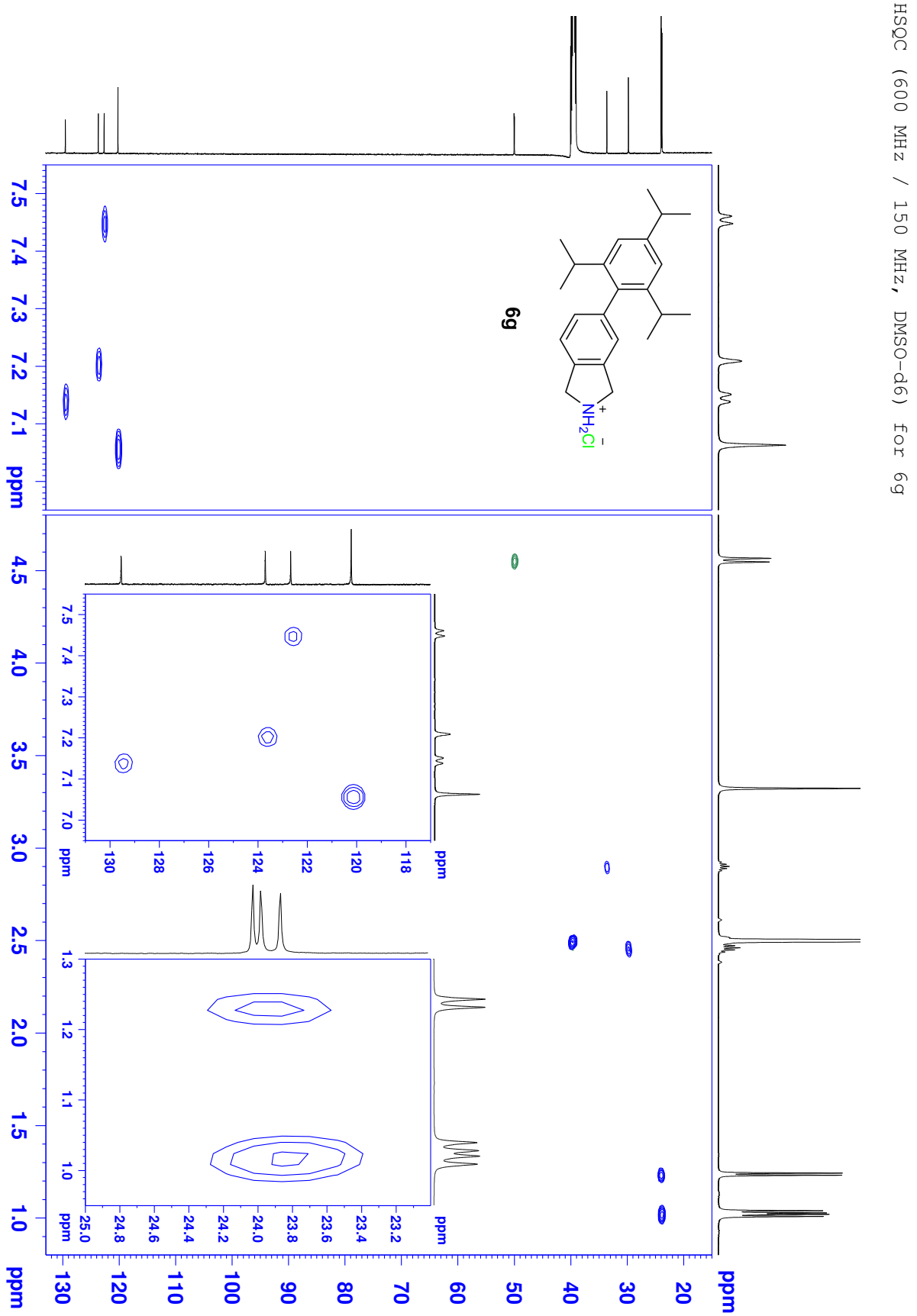
S.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 6g



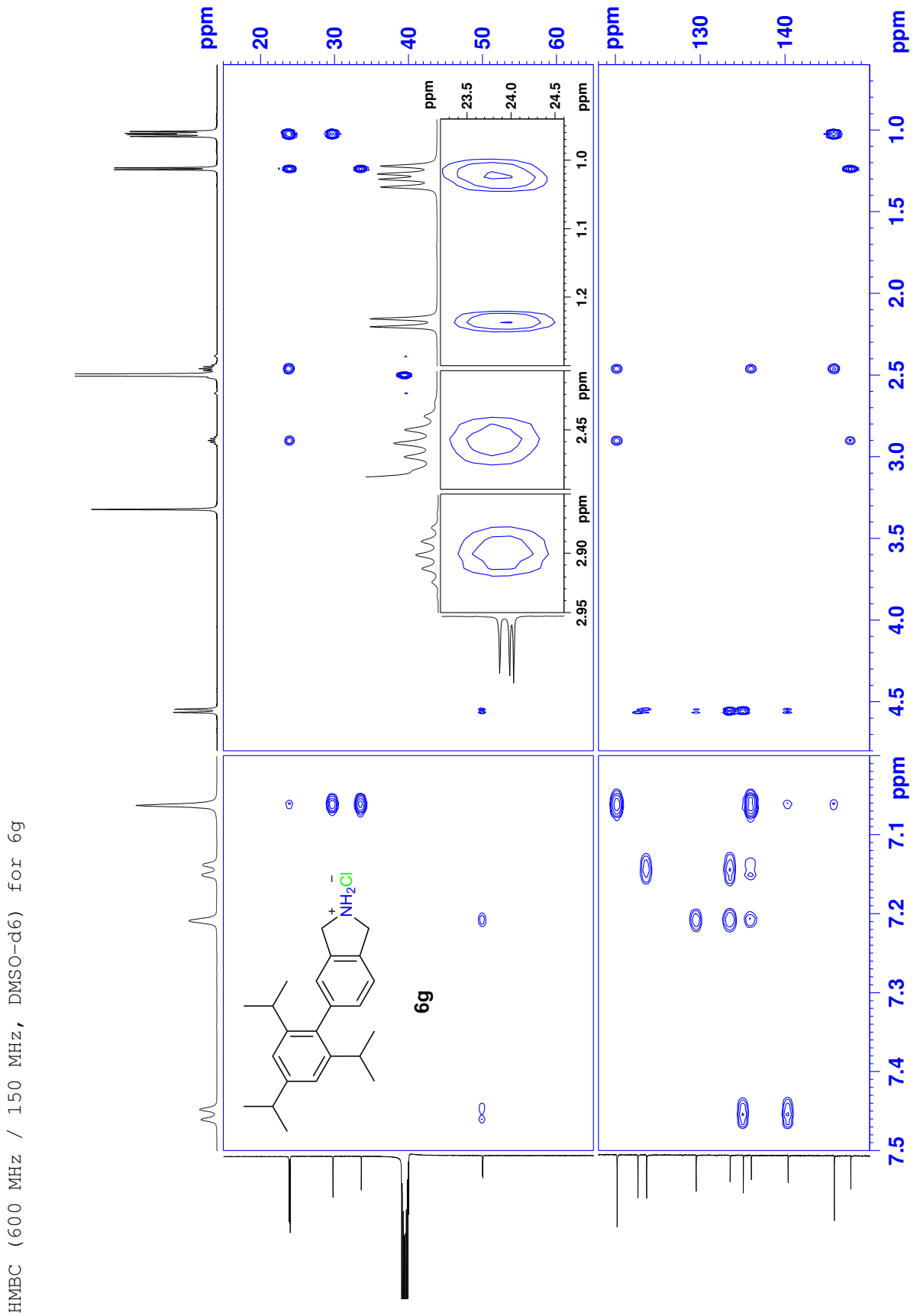
S.3 COSY (600 MHz, DMSO) spectrum for 6g



S.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 6g



S.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 6g

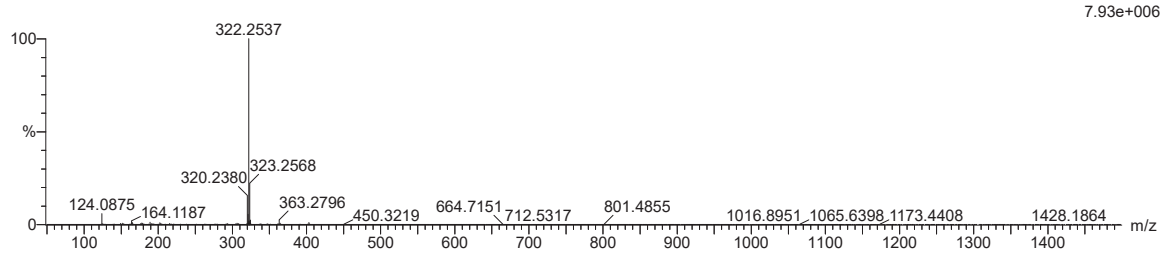


S.6 HRMS spectrum for 6g

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
701 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200
2017-292 25 (0.519) AM2 (Ar,35000.0,0.00,0.00); Cm (20:27)
1: TOF MS ASAP+



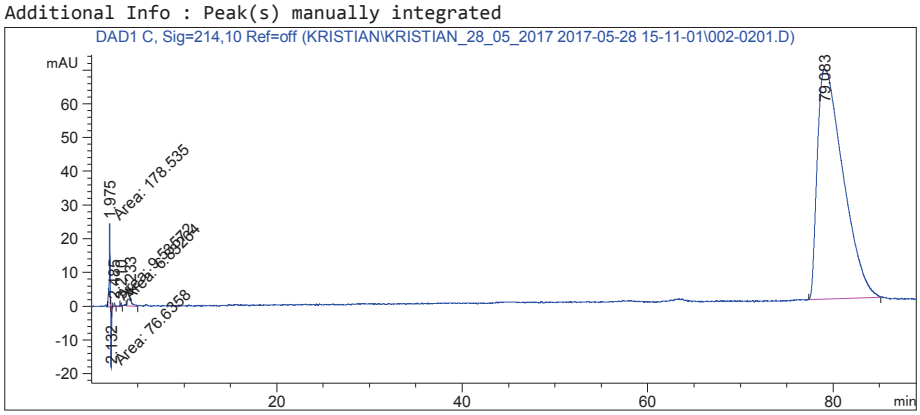
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
322.2537	322.2535	0.2	0.6	8.5	1589.8	0.000	100.00	C23 H32 N
	322.2540	-0.3	-0.9	1.5	1600.7	10.855	0.00	C8 H28 N13 O

Ion observed M+

S.7 HPLC chromatogram for 6g

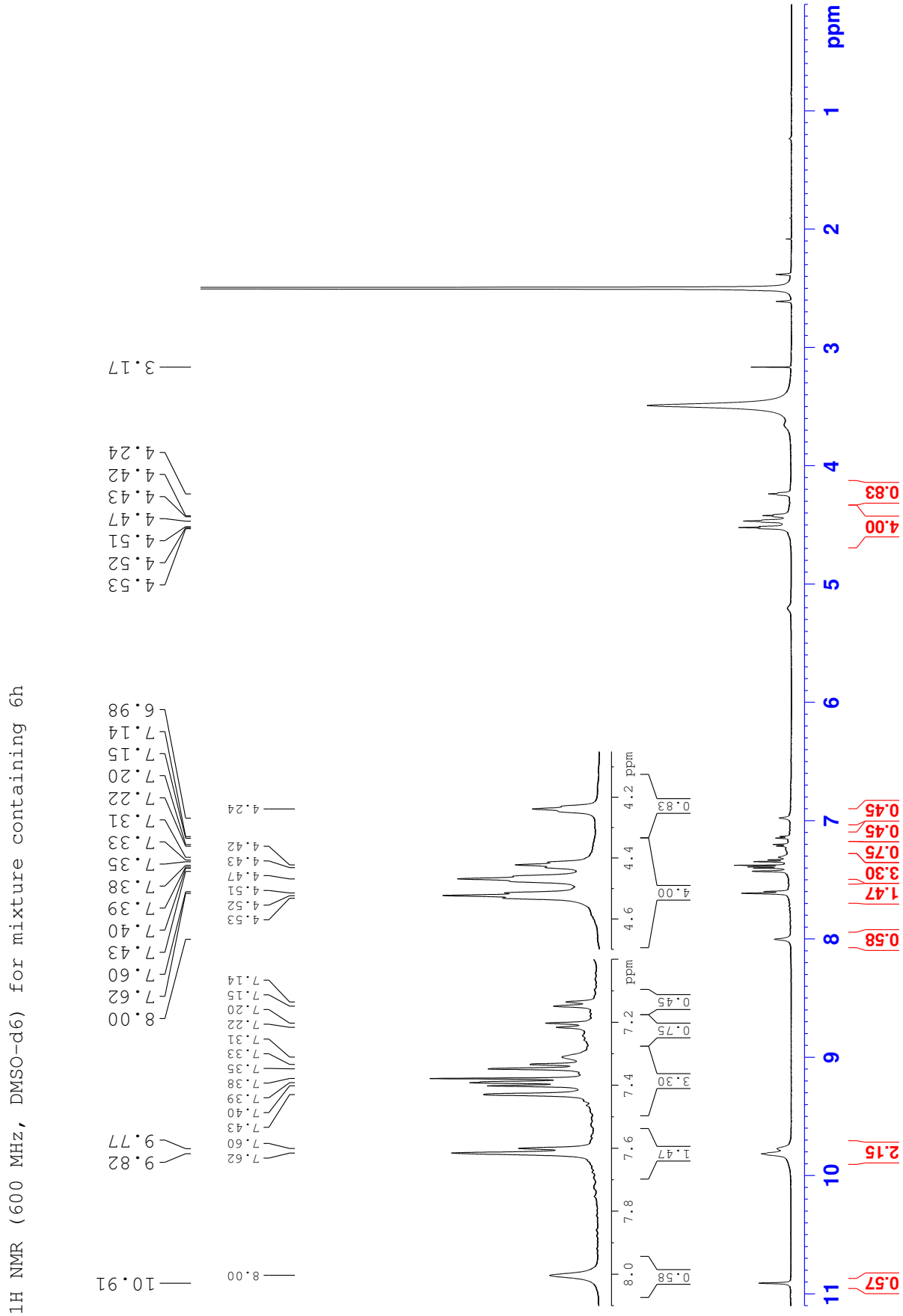
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01\002-0201.D
Sample Name: KM-58

```
=====================================================================
Acq. Operator   : Kristian                      Seq. Line :   2
Acq. Instrument : UPLC                          Location : Vial 2
Injection Date  : 28.05.2017 15:22:47                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01
                  \C18PURITYSALT.M
Last changed    : 28.05.2017 16:50:10 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01\002-0201.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 28.05.2017 16:57:16 by Kristian
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.975 MM    0.1197  178.53473   24.85040   1.3251
   2   2.132 MM N  0.0710   76.63580   17.99916   0.5688
   3   2.485 MM    0.1424    9.53572    1.11594   0.0708
   4   3.210 MM    0.1055    6.83264    1.07958   0.0507
   5   4.233 BB    0.4086   66.91183    2.01946   0.4966
   6  79.083 BB    2.5470 1.31351e4    68.01790  97.4880

Totals :                  1.34735e4   115.08244


=====================================================================
                        *** End of Report ***
```

T.1 ^{1}H NMR (600 MHz, DMSO) spectrum for mixture containing 6h

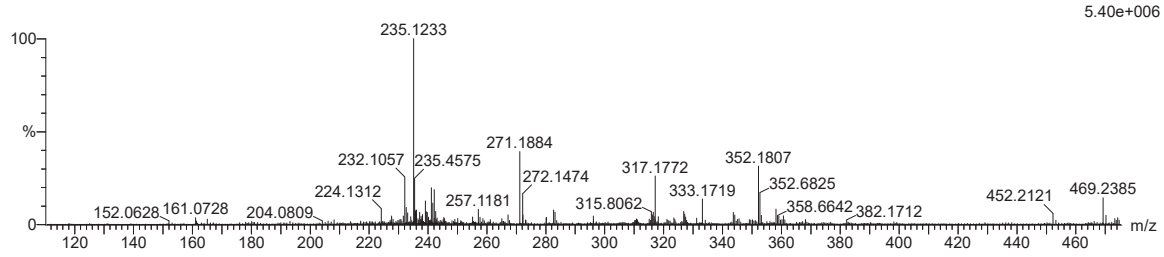


T.2 HRMS spectrum for 6h

Elemental Composition Report **Page 1**

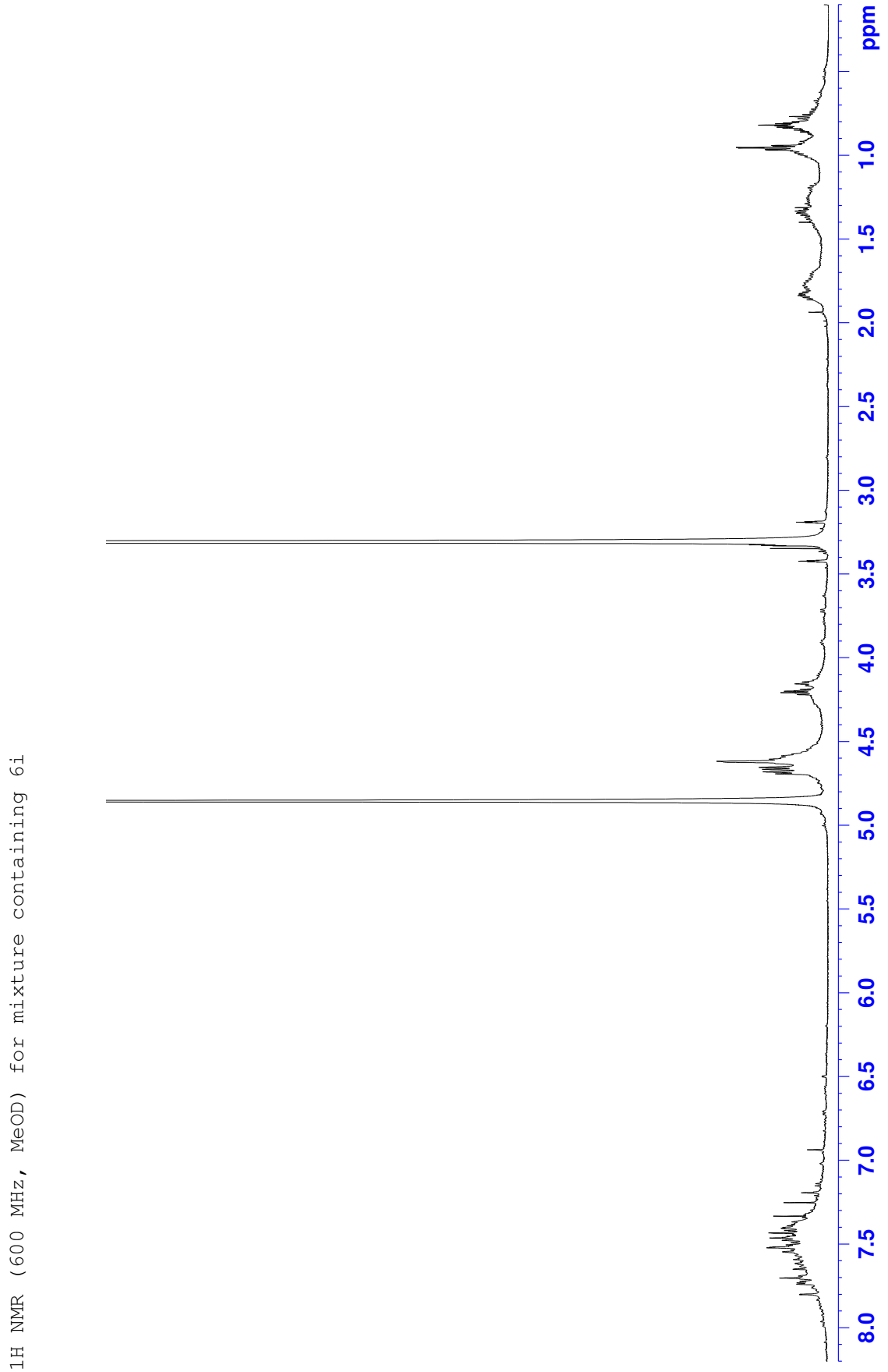
Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1342 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1 Cl: 0-8
2017-306esi 132 (1.194) AM2 (Ar,35000.0,0.00,0.00); Cm (123:133)
1: TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
235.1233	235.1235	-0.2	-0.9	10.5	1984.1	0.291	74.79	C16 H15 N2
	235.1229	0.4	1.7	2.5	1991.0	7.131	0.08	C13 H21 Na Cl
	235.1227	0.6	2.6	-1.5	1985.2	1.381	25.13	H15 N10 O5

U.1 ^{1}H NMR (600 MHz, MeOD) spectrum for mixture containing 6i (Solubility problems)

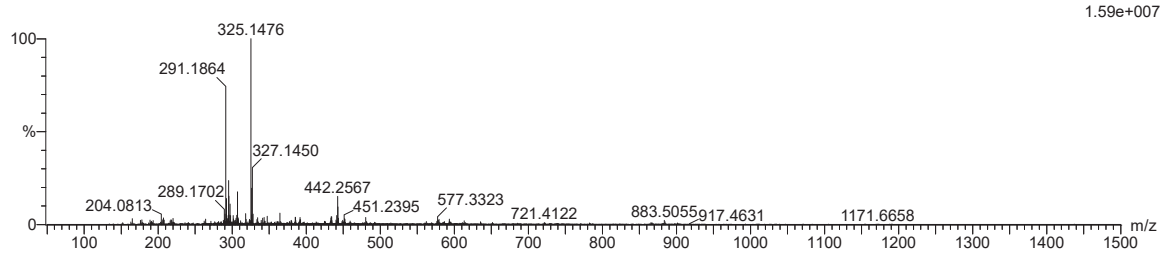


U.2 HRMS spectrum 6i

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1545 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Cl: 0-8
2017-290ESI 16 (0.165) AM2 (Ar,35000.0,0.00,0.00); Cm (9:30)
1: TOF MS ES+



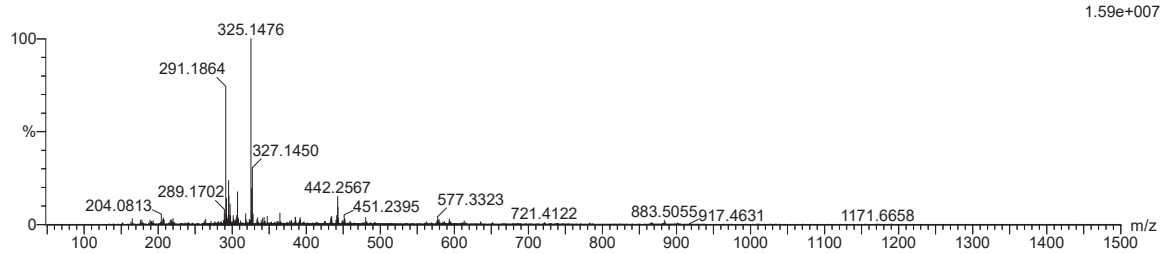
Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
291.1864	291.1866	-0.2	-0.7	3.5	1763.3	11.019	0.00	C5 H19 N14 O
	291.1861	0.3	1.0	10.5	1752.3	0.000	100.00	C20 H23 N2

U.3 HRMS spectrum 2 for product mixture (6i)

Elemental Composition Report **Page 1**

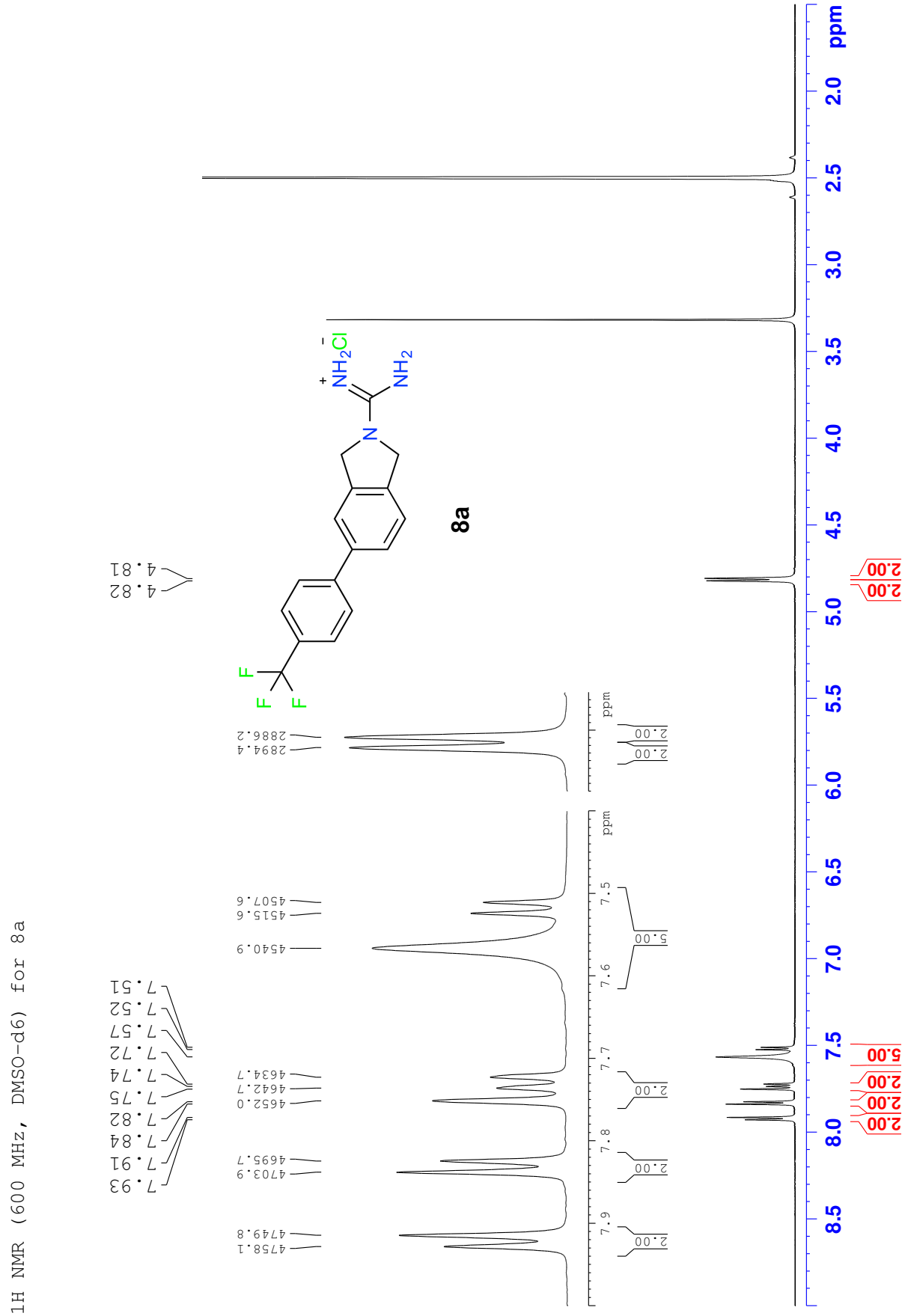
Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
2212 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Cl: 0-8
2017-290ESI 16 (0.165) AM2 (Ar,35000.0,0.00,0.00); Cm (9:30)
1: TOF MS ES+

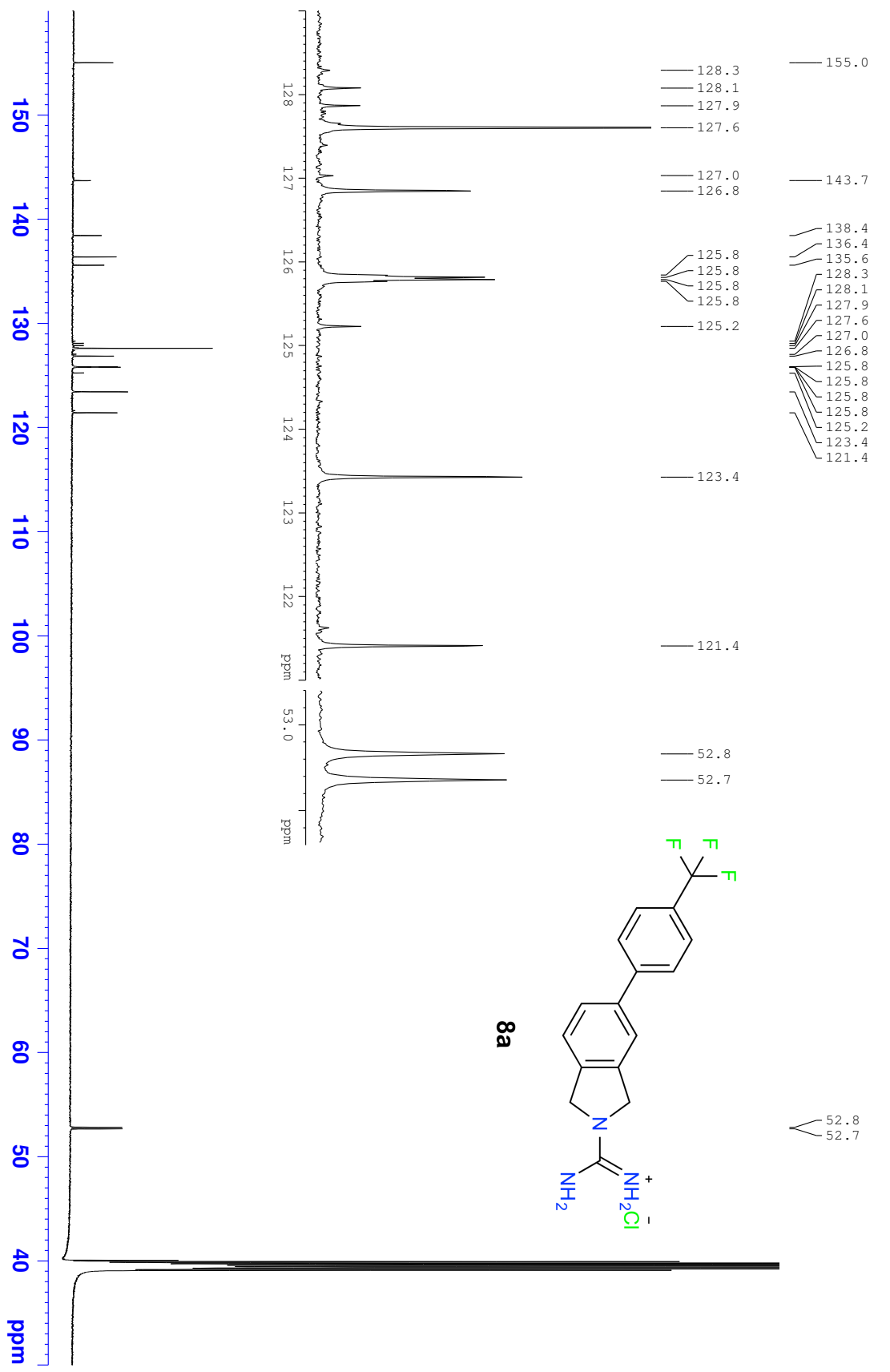


Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
325.1476	325.1477	-0.1	-0.3	3.5	1670.9	10.211	0.00	C5 H18 N14 O Cl
	325.1472	0.4	1.2	10.5	1660.7	0.000	100.00	C20 H22 N2 Cl
	325.1472	0.4	1.2	2.5	1679.1	18.414	0.00	C9 H21 N6 O7
	325.1485	-0.9	-2.8	7.5	1679.1	18.409	0.00	C10 H17 N10 O3

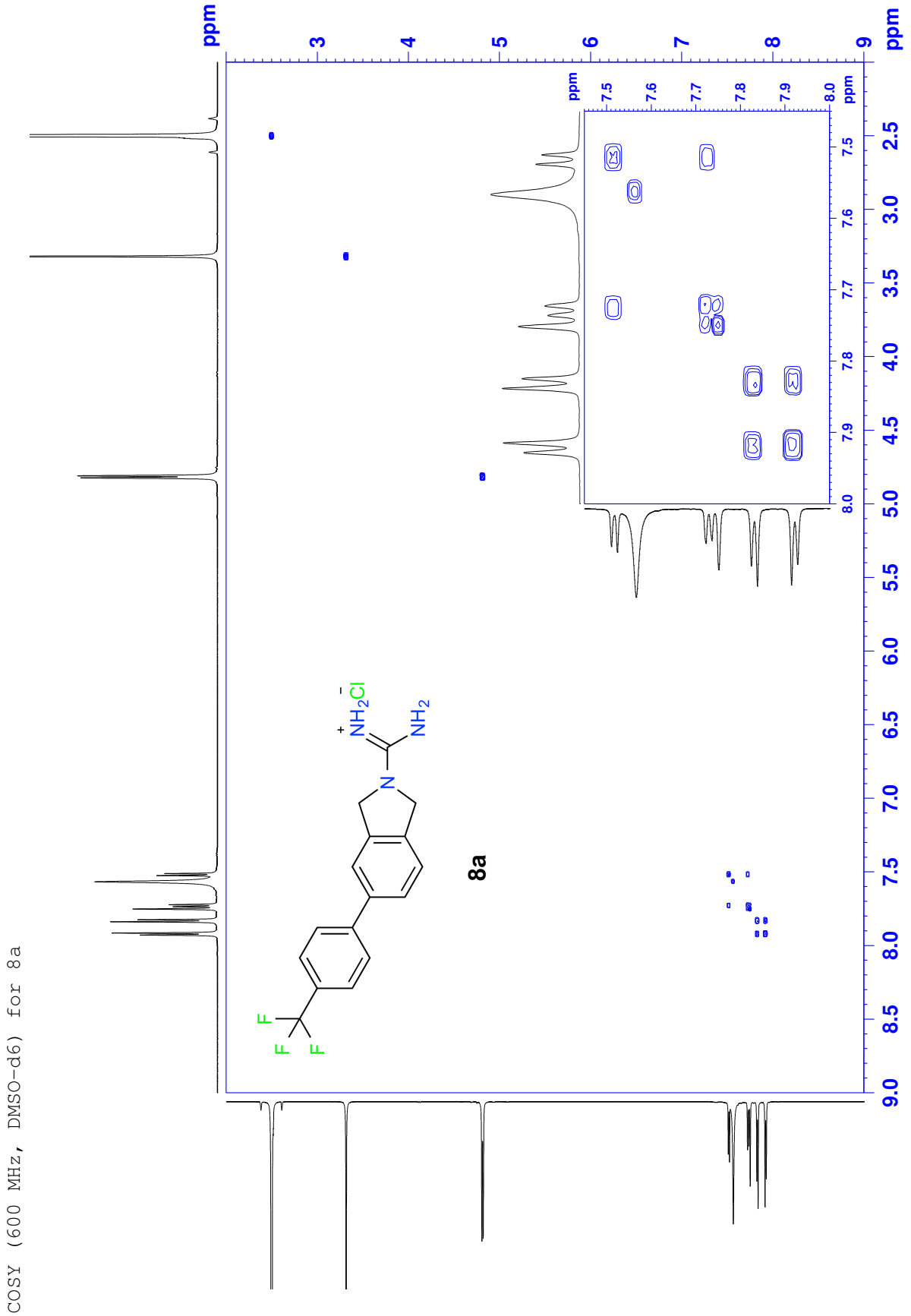
V.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8a



V.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8a

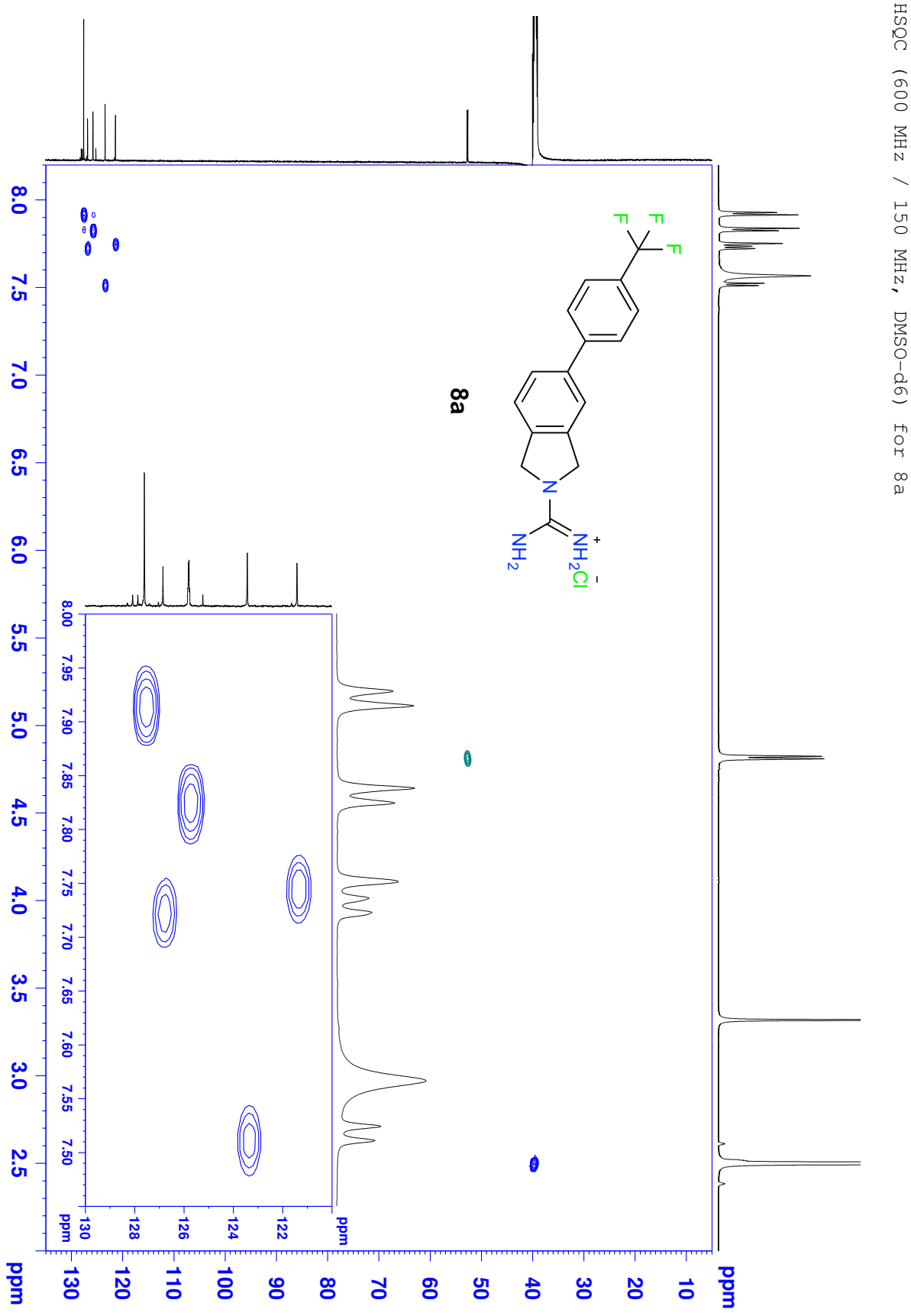


V.3 COSY (600 MHz, DMSO) spectrum for 8a

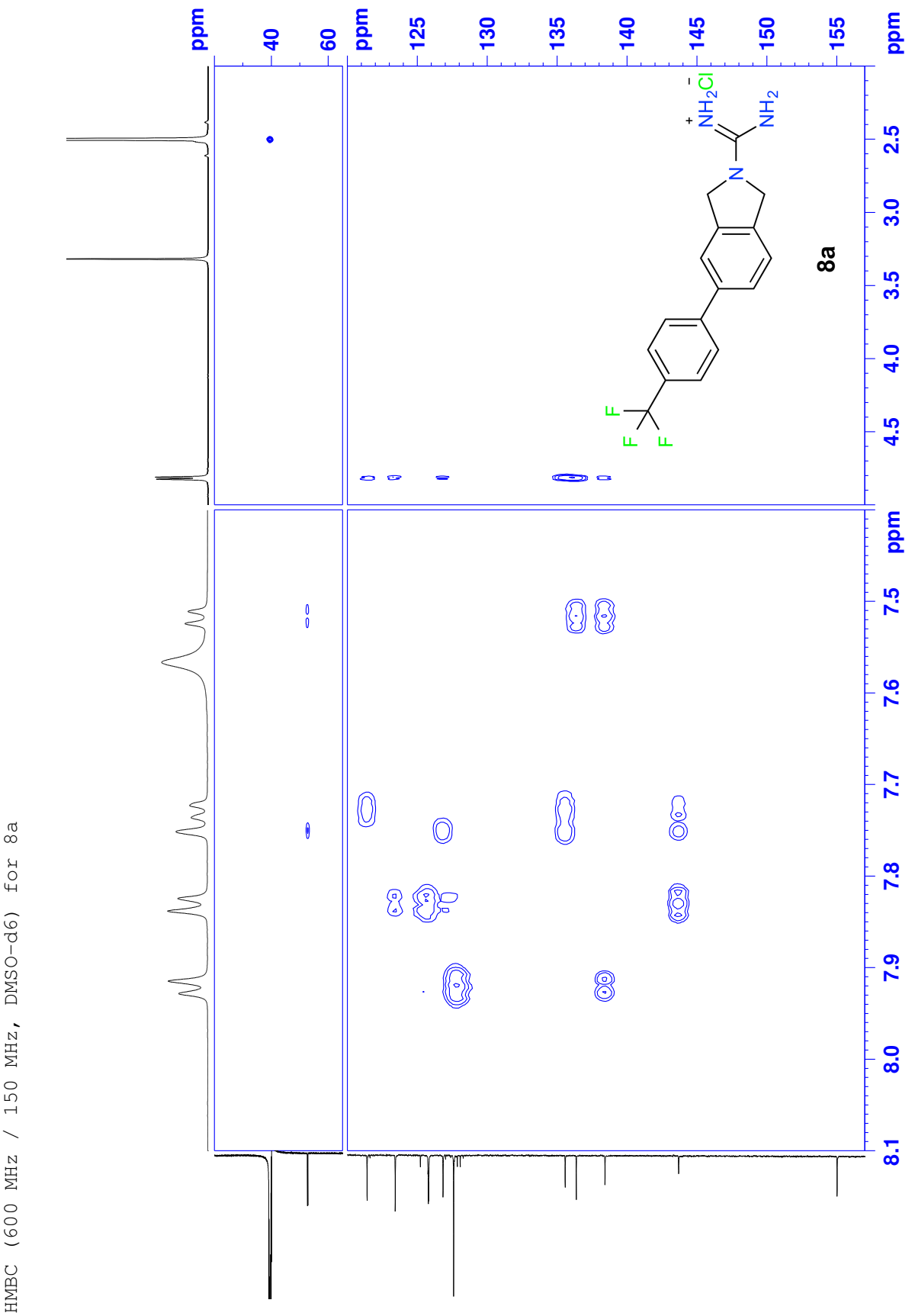


COSY (600 MHz, DMSO-d6) for 8a

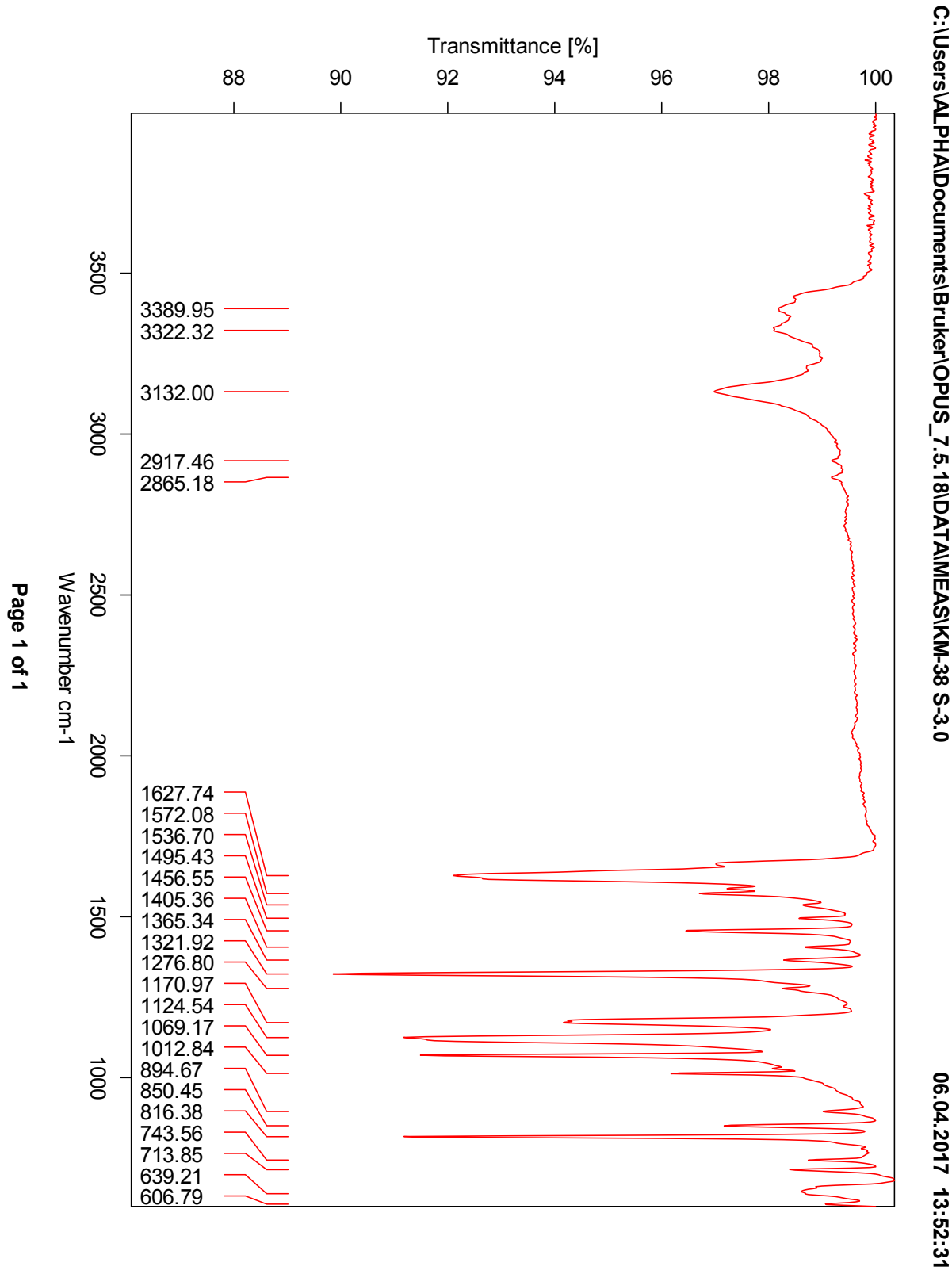
V.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8a



V.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8a



V.6 IR spectrum for 8a



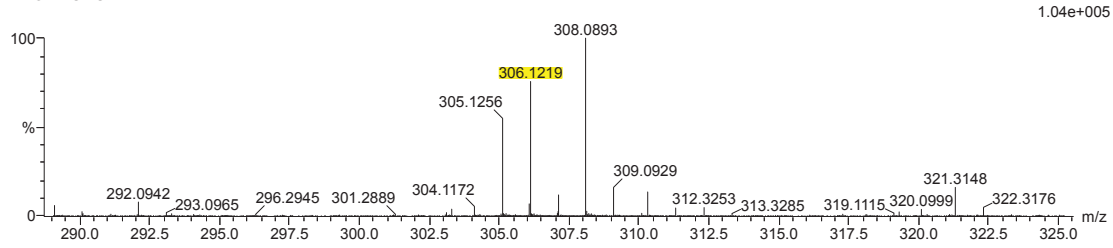
V.7 HRMS spectrum for 8a

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
195 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 F: 1-6

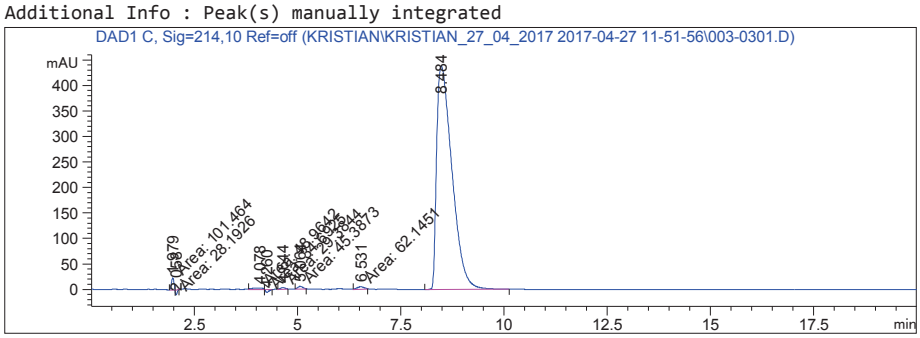
2017-181 137 (2.689) AM2 (Ar,35000.0,0.00,0.00); Cm (136:139)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
306.1219	306.1218	0.1	0.3	9.5	1054.5	0.002	99.78	C16 H15 N3 F3 ion observed M+
	306.1214	0.5	1.6	2.5	1060.6	6.114	0.22	C6 H13 N9 F5

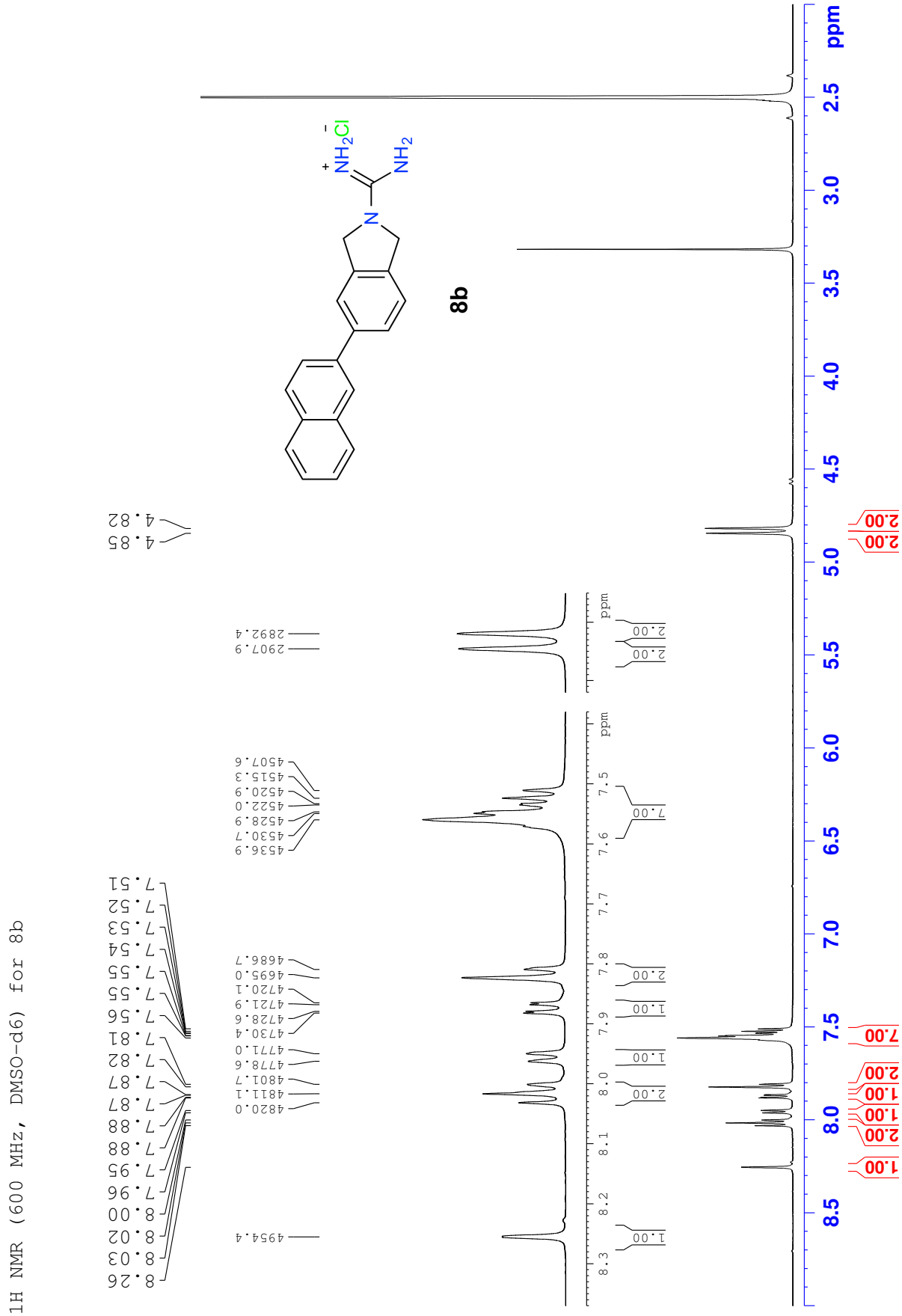
V.8 HPLC chromatogram for 8a

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\003-0301.D
Sample Name: KM-38 S-3

    ======================================================================
    Acq. Operator   : Kristian                       Seq. Line :   3
    Acq. Instrument : UPLC                           Location : Vial 3
    Injection Date  : 27.04.2017 12:34:42                 Inj :   1
                                                   Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                      \C18PURITYSALT.M
    Last changed    : 27.04.2017 12:53:28 by Kristian
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\003-0301.
                      D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 27.04.2017 13:04:09 by Kristian M
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

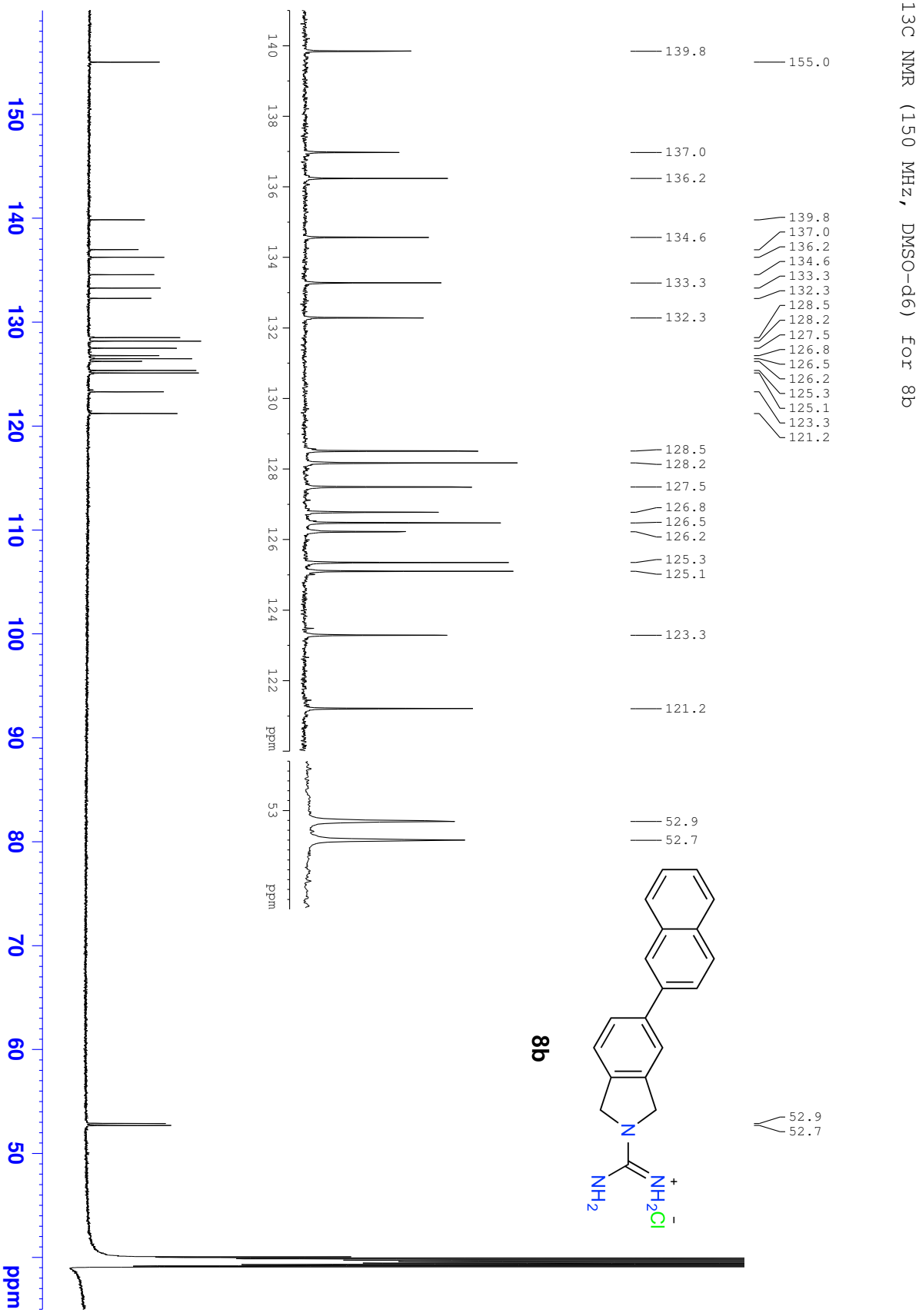



```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.979 MM    0.0717  101.46392   23.59270   0.8686
       2   2.058 MM N  0.0515   28.19256    9.11677   0.2413
       3   4.078 FM    0.2885   48.96418    2.82820   0.4192
       4   4.260 MM N  0.0984   34.69248    5.87569   0.2970
       5   4.644 MM    0.1426   29.38436    3.43325   0.2515
       6   5.064 MM    0.1457   45.38725    5.19264   0.3885
       7   6.531 MM    0.2061   62.14513    5.02559   0.5320
       8   8.484 BB    0.3956 1.13314e4   438.38815  97.0019

    Totals :                   1.16817e4   493.45299


    ======================================================================
                              *** End of Report ***

UPLC 27.04.2017 13:04:13 Kristian M                                Page   1 of 1
```

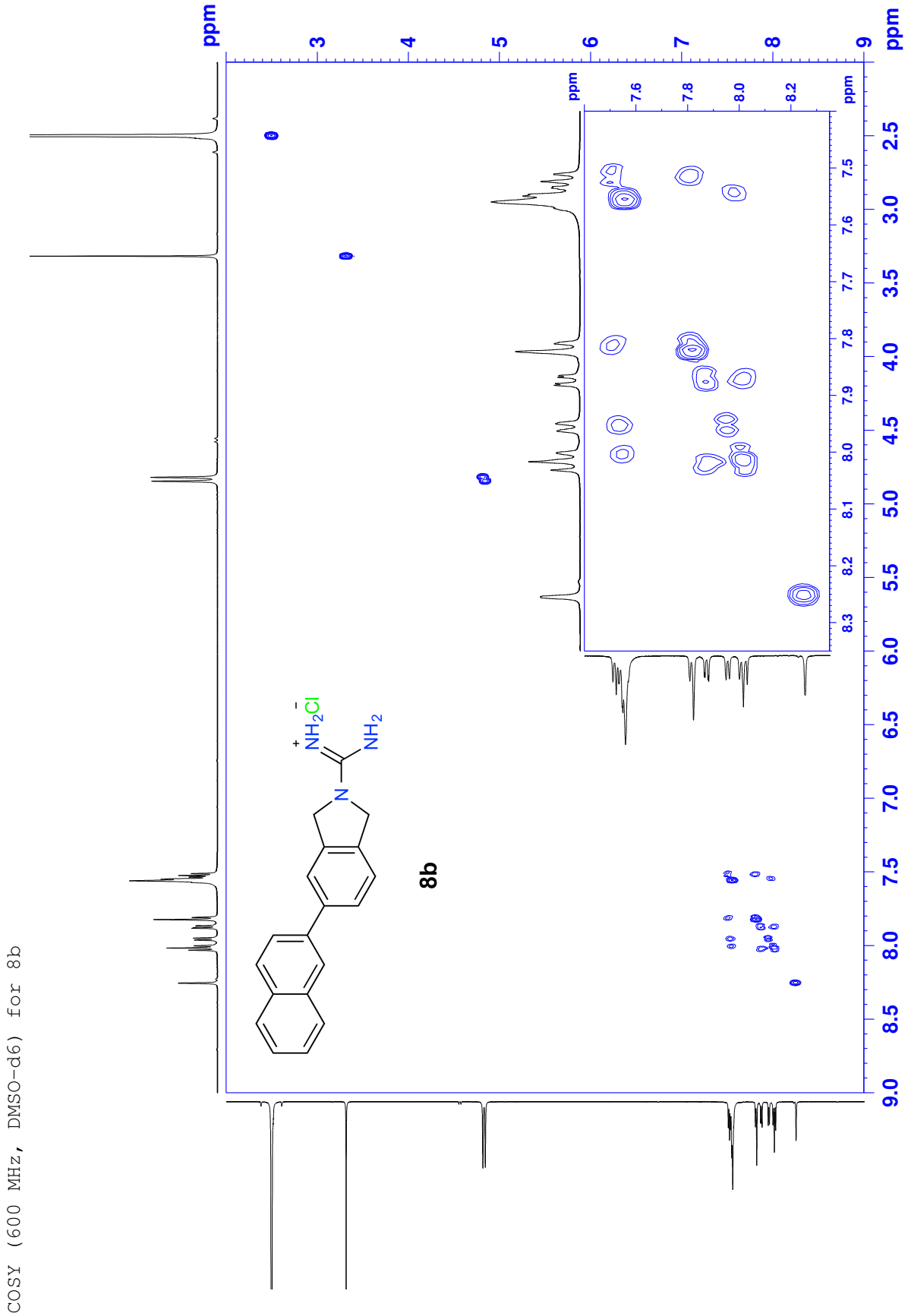
W.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8b



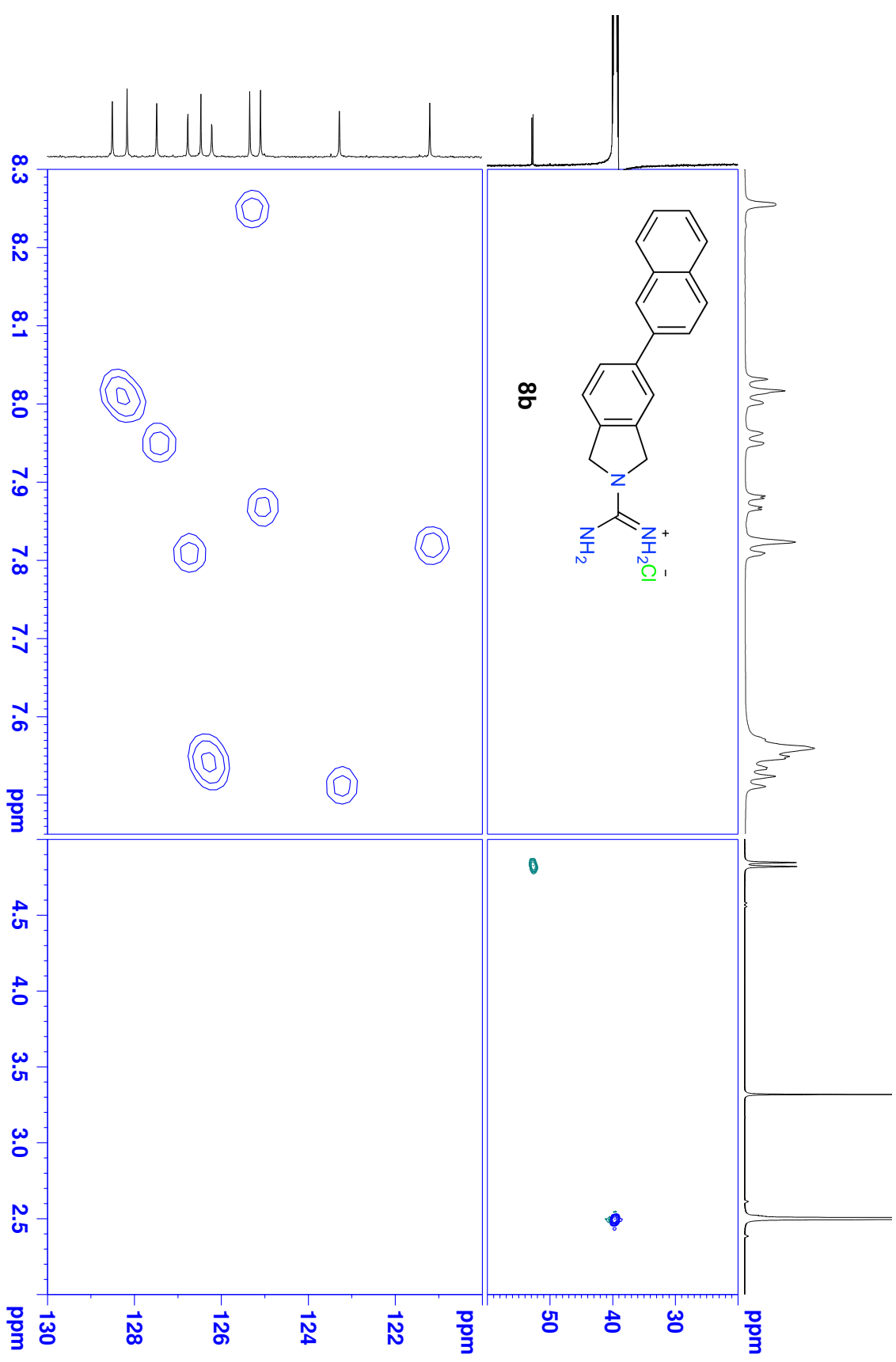
W.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8b



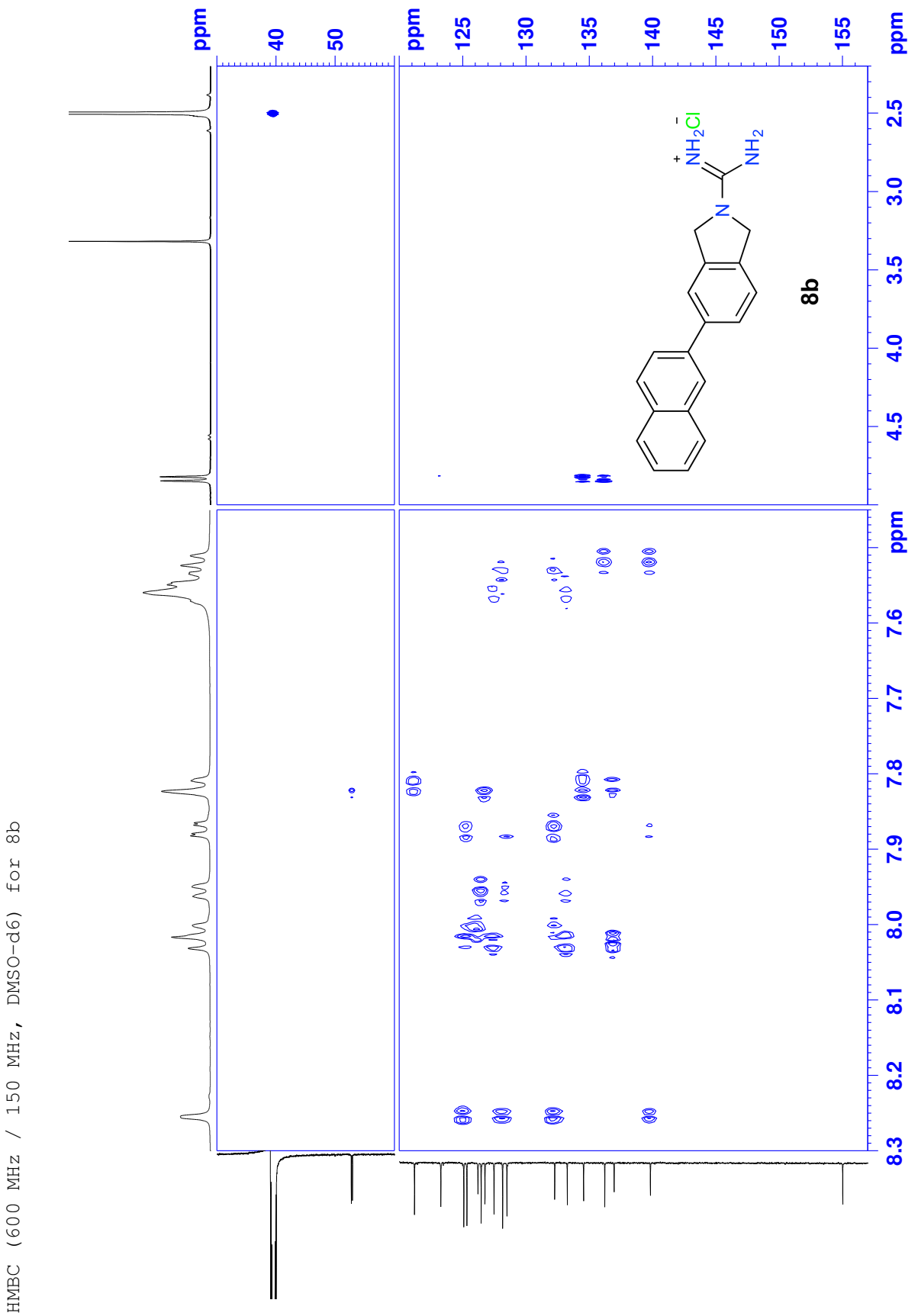
W.3 COSY (600 MHz, DMSO) spectrum for 8b



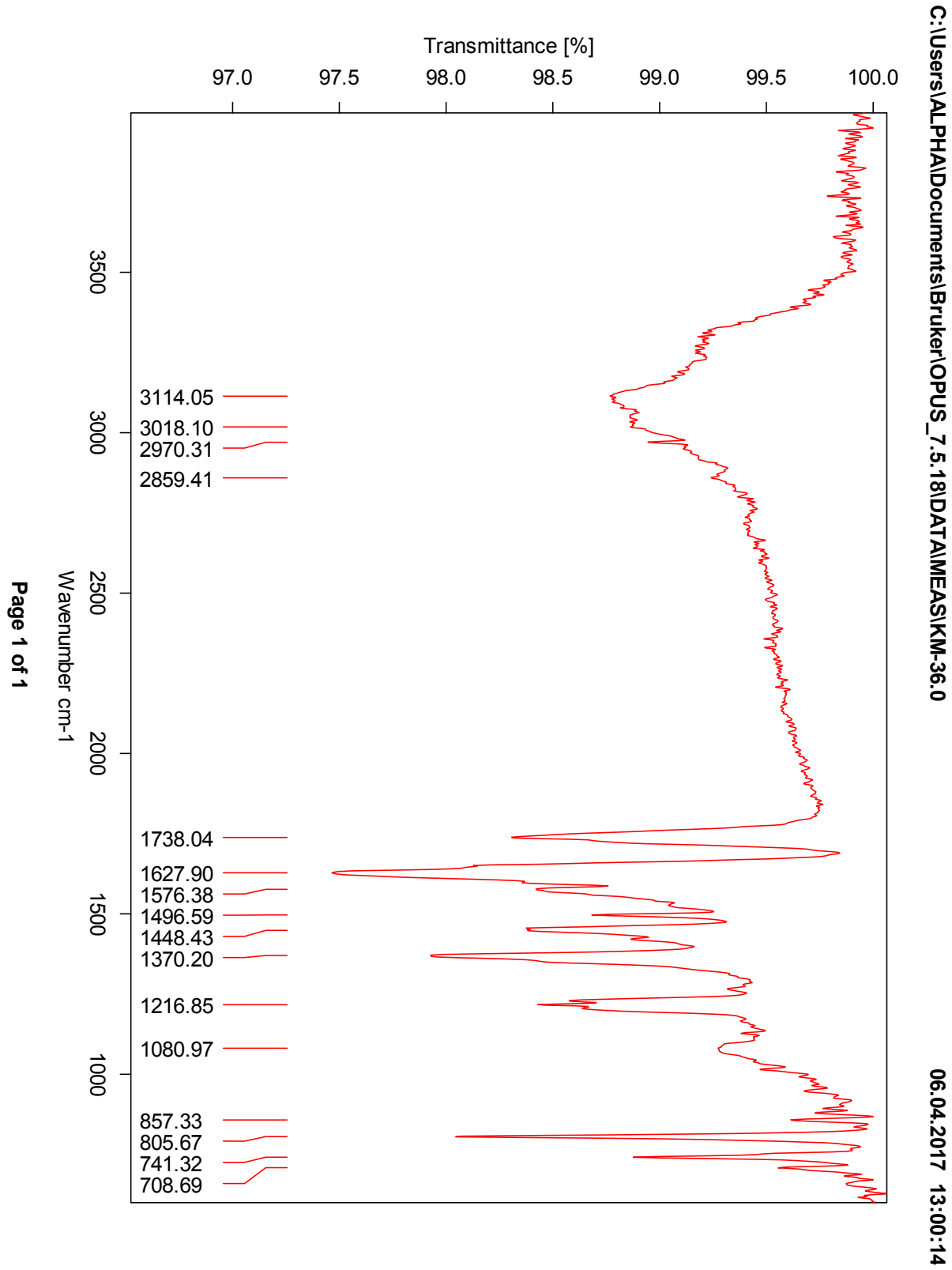
W.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8b



W.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8b



W.6 IR spectrum for 8b



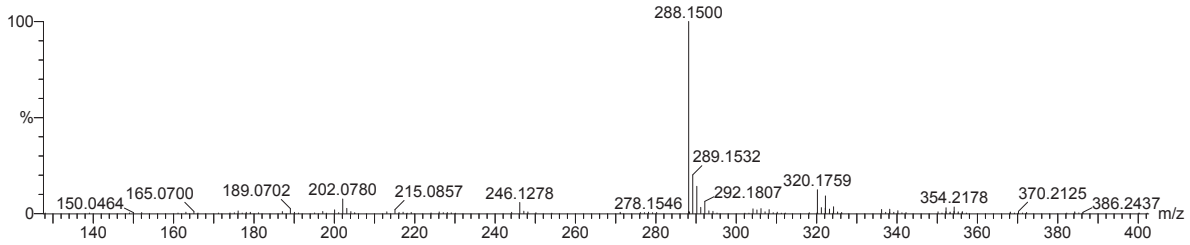
W.7 HRMS spectrum for 8b

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
493 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 2-40 H: 0-1000 N: 0-20 O: 0-25
NT-MSLAB-Operator-SVG
2017-189 60 (0.554) AM2 (Ar,35000.0,0.00,0.00); Cm (60:67)

1: TOF MS ES+
9.08e+005



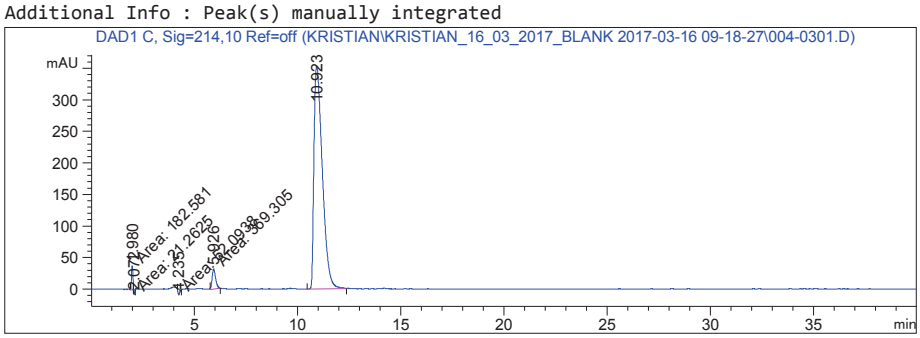
Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
288.1500	288.1501	-0.1	-0.3	12.5	193.0	0.064	93.84	C19 H18 N3
	288.1506	-0.6	-2.1	5.5	195.7	2.786	6.16	C4 H14 N15 O

Ion observed [M+H]

W.8 HPLC chromatogram for 8b

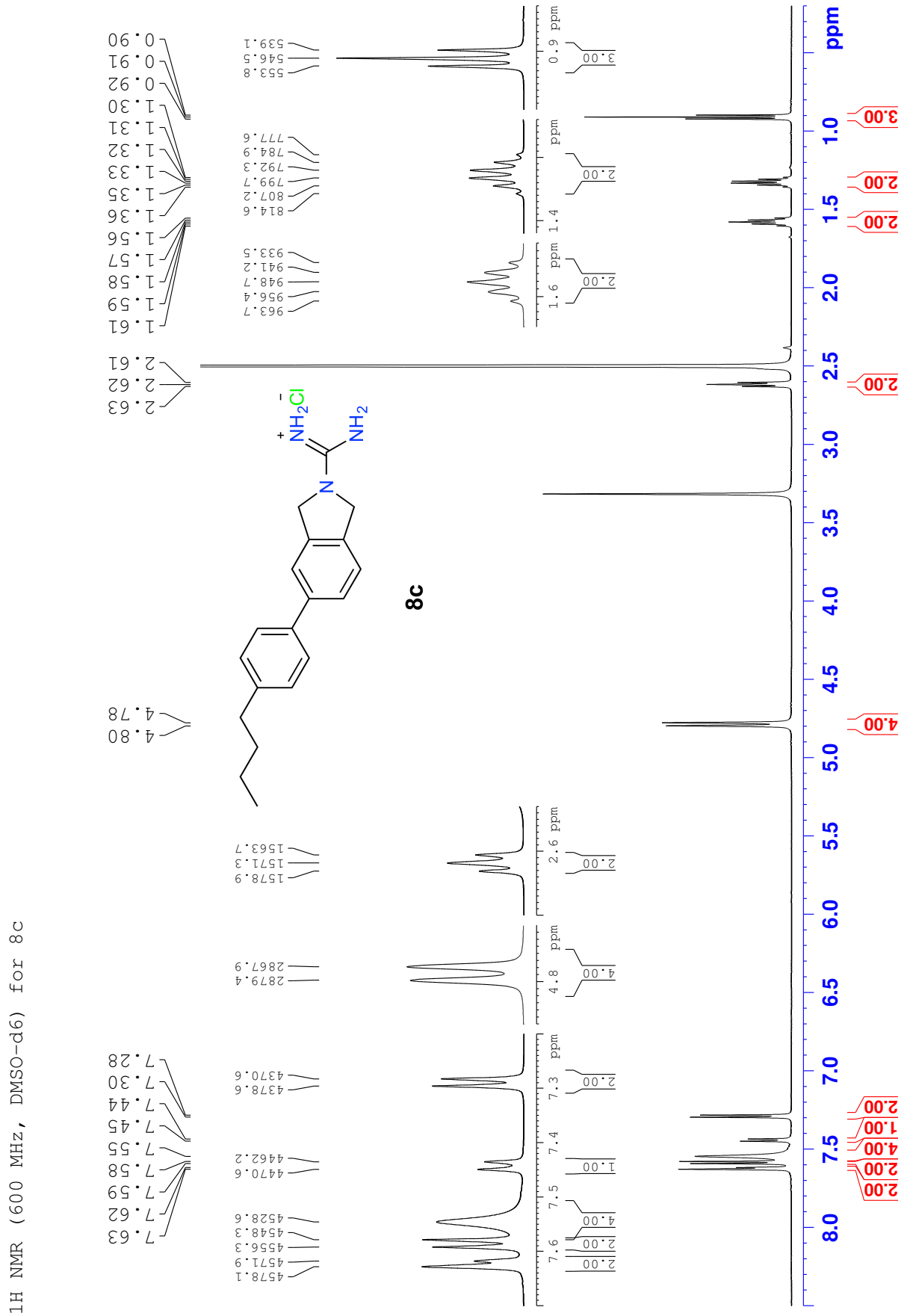
Data File C:\CHEM32\...A\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\004-0301.D
Sample Name: KM-36

```
======================================================================
Acq. Operator   : Kristian M                    Seq. Line :   3
Acq. Instrument : UPLC                          Location : Vial 4
Injection Date  : 16.03.2017 10:51:14                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27
                  \C18PURITYSALT.M
Last changed    : 16.03.2017 11:29:48 by Kristian M
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_16_03_2017_BLANK 2017-03-16 09-18-27\004
                  -0301.D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 12:23:17 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

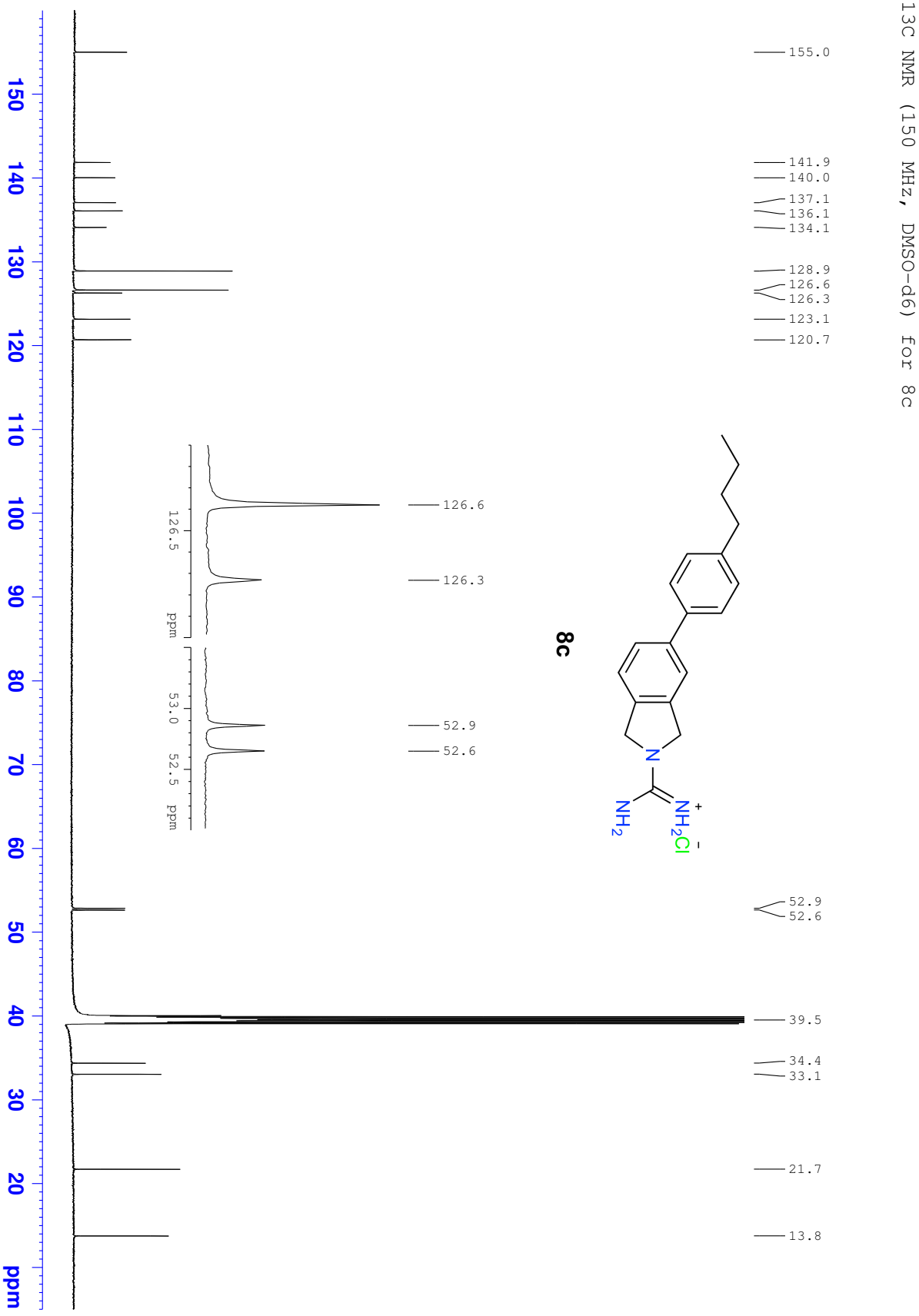



```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.980 MF    0.0809  182.58060   37.60683   1.7203
   2   2.072 MP N  0.0440   21.26252    8.05120   0.2003
   3   4.235 MM N  0.1026   52.09375    8.45871   0.4908
   4   5.926 MM    0.1938  369.30481   31.75330   3.4796
   5  10.923 BV    0.4420 9988.12500  353.15134  94.1089

Totals :                  1.06134e4   439.02137


======================================================================
                        *** End of Report ***
```

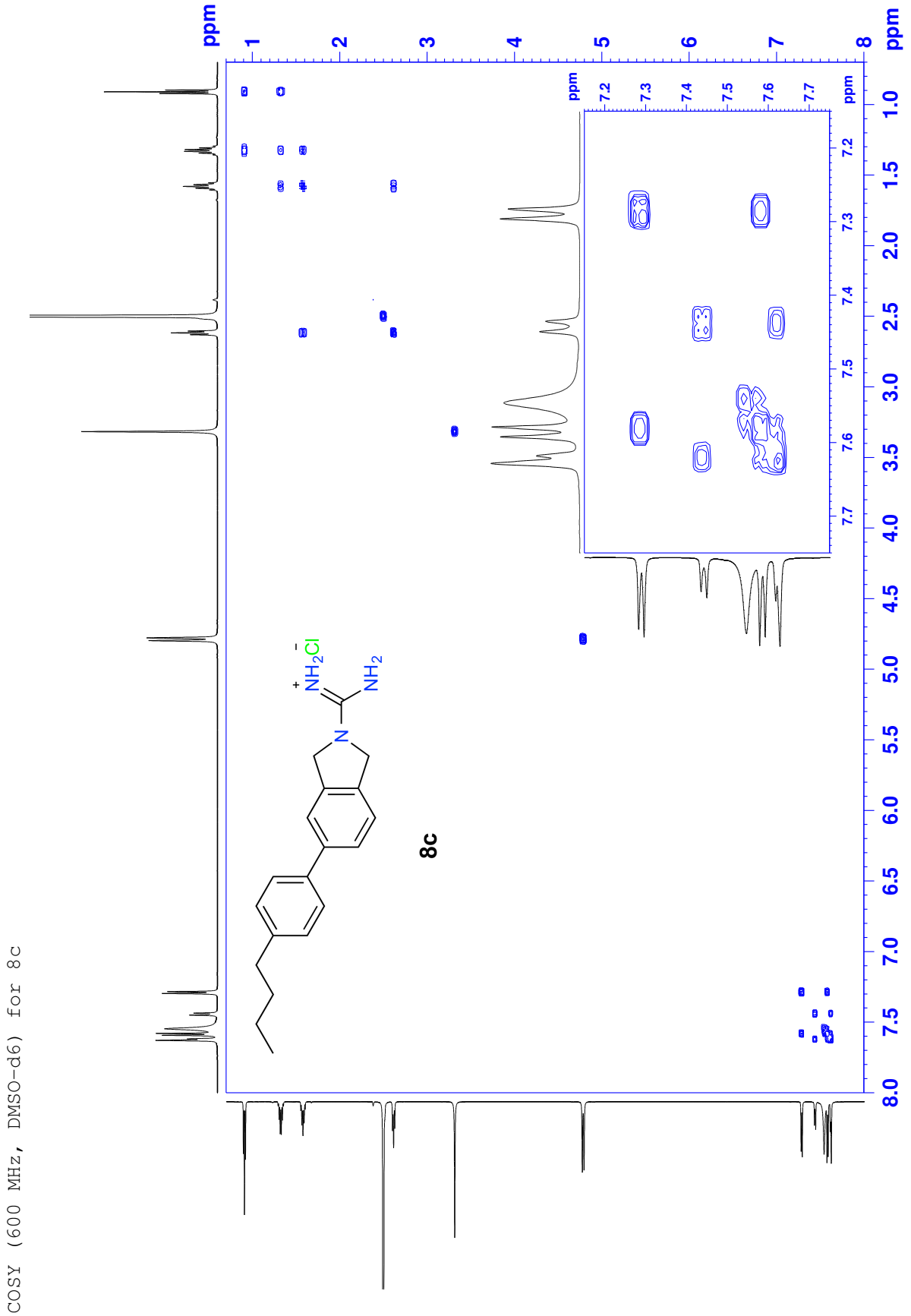
X.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8c



X.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8c

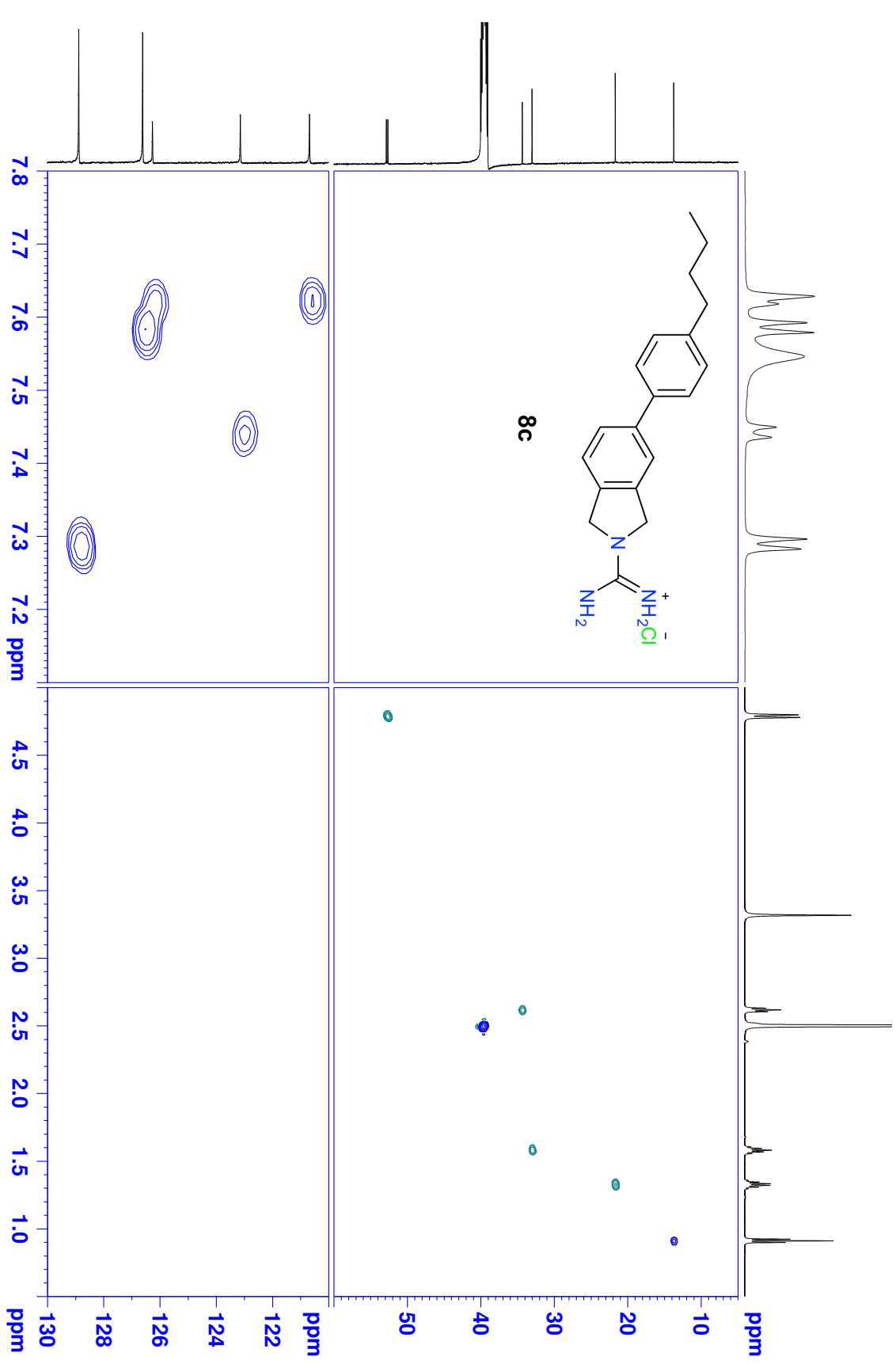


X.3 COSY (600 MHz, DMSO) spectrum for 8c

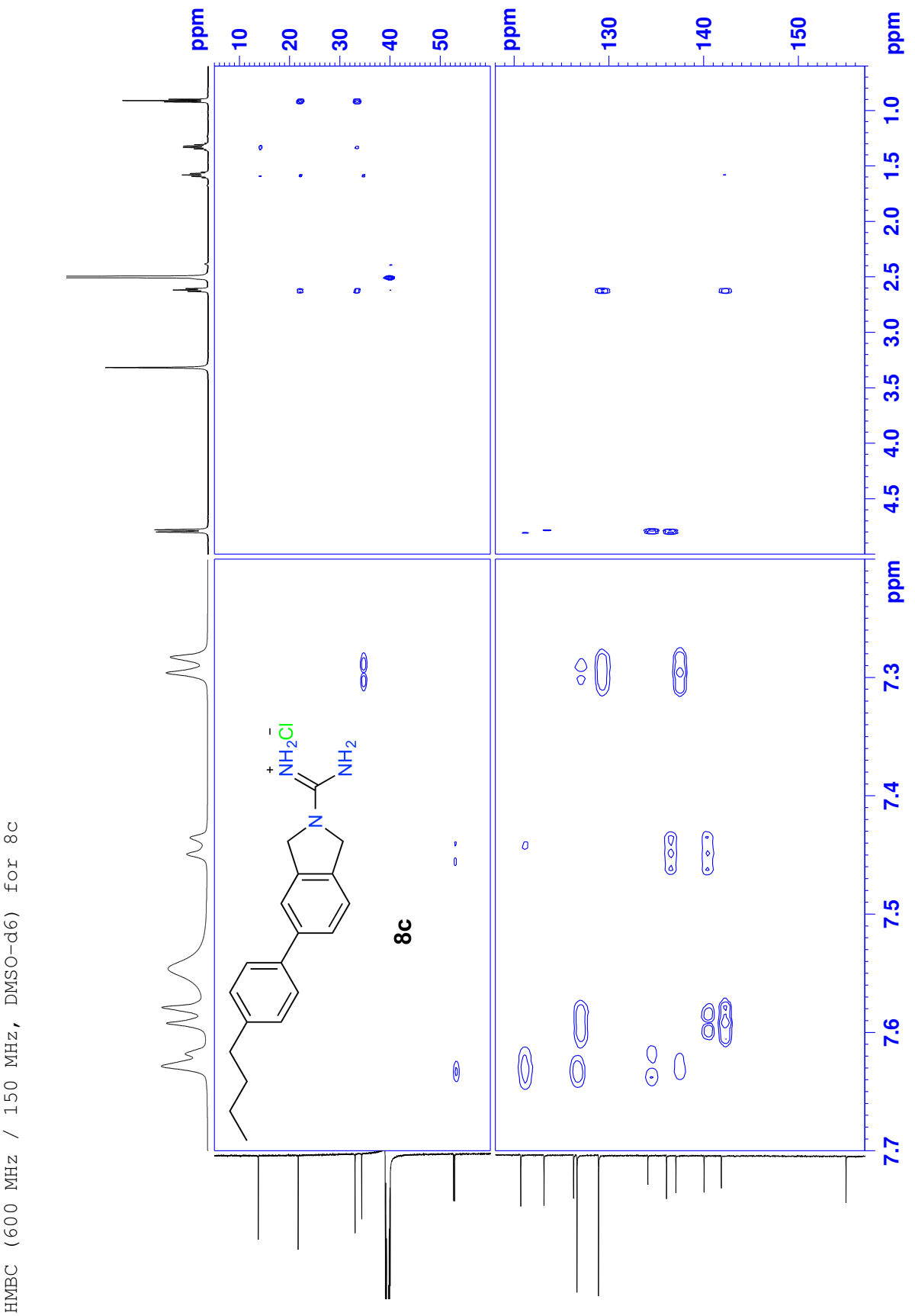


COSY (600 MHz, DMSO-d6) for 8c

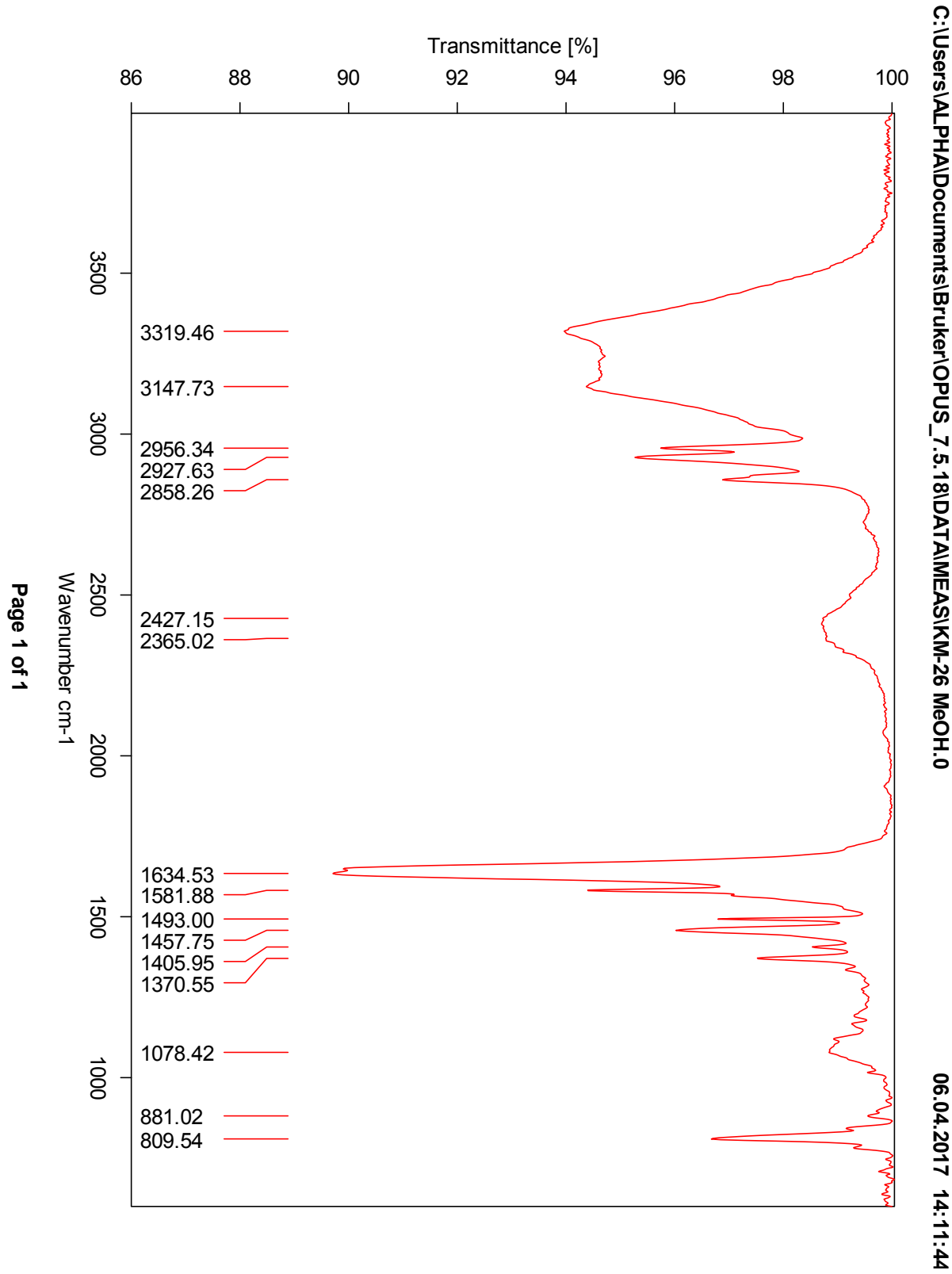
X.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8c



X.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8c



X.6 IR spectrum for 8c

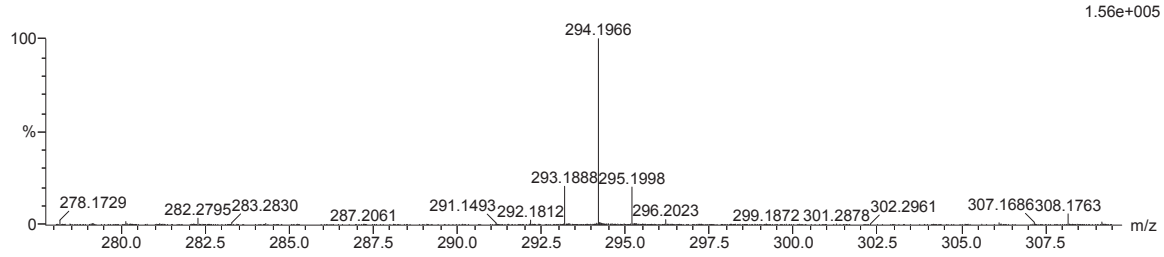


X.7 HRMS spectrum for 8c

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
39 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10
2017-157 166 (3.240) AM2 (Ar,35000.0,0.00,0.00); Cm (153:167)
1: TOF MS ASAP+

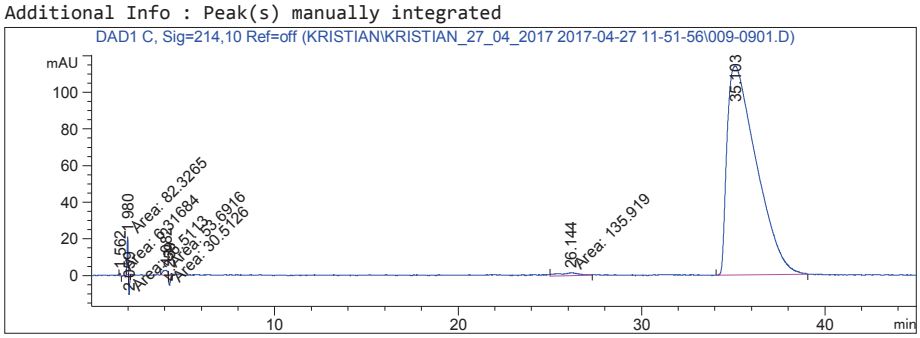


Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
294.1966	294.1970	-0.4	-1.4	9.5	925.5	n/a	n/a	C19 H24 N3

ion observed M+

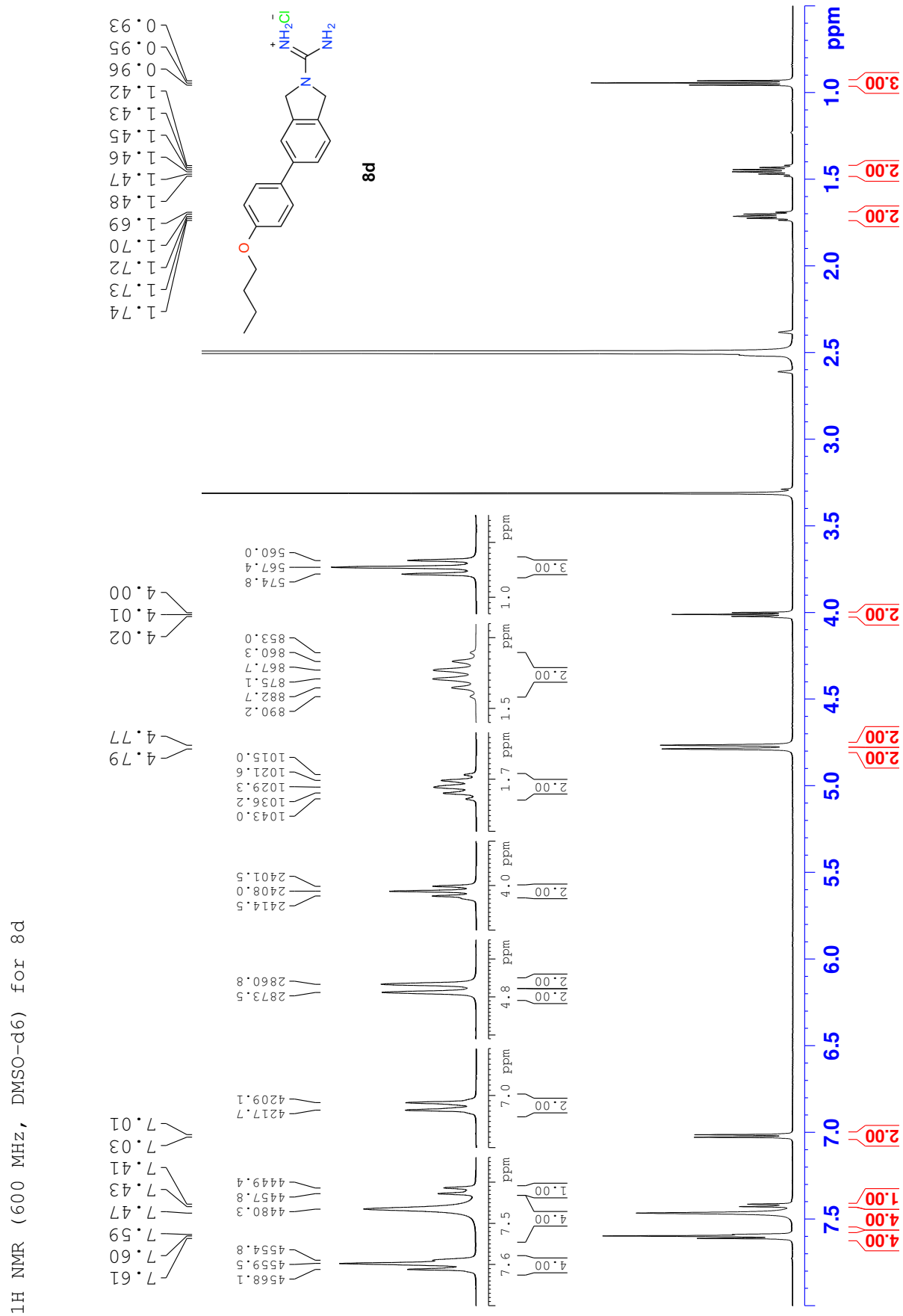
X.8 HPLC chromatogram for 8c

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\009-0901.D
Sample Name: KM-48 #2

======================================================================
Acq. Operator   : Kristian                      Seq. Line :   9
Acq. Instrument : UPLC                          Location : Vial 9
Injection Date  : 27.04.2017 15:49:39                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                  \C18PURITYSALT.M
Last changed    : 27.04.2017 13:33:07 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\009-0901.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 19:05:44 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

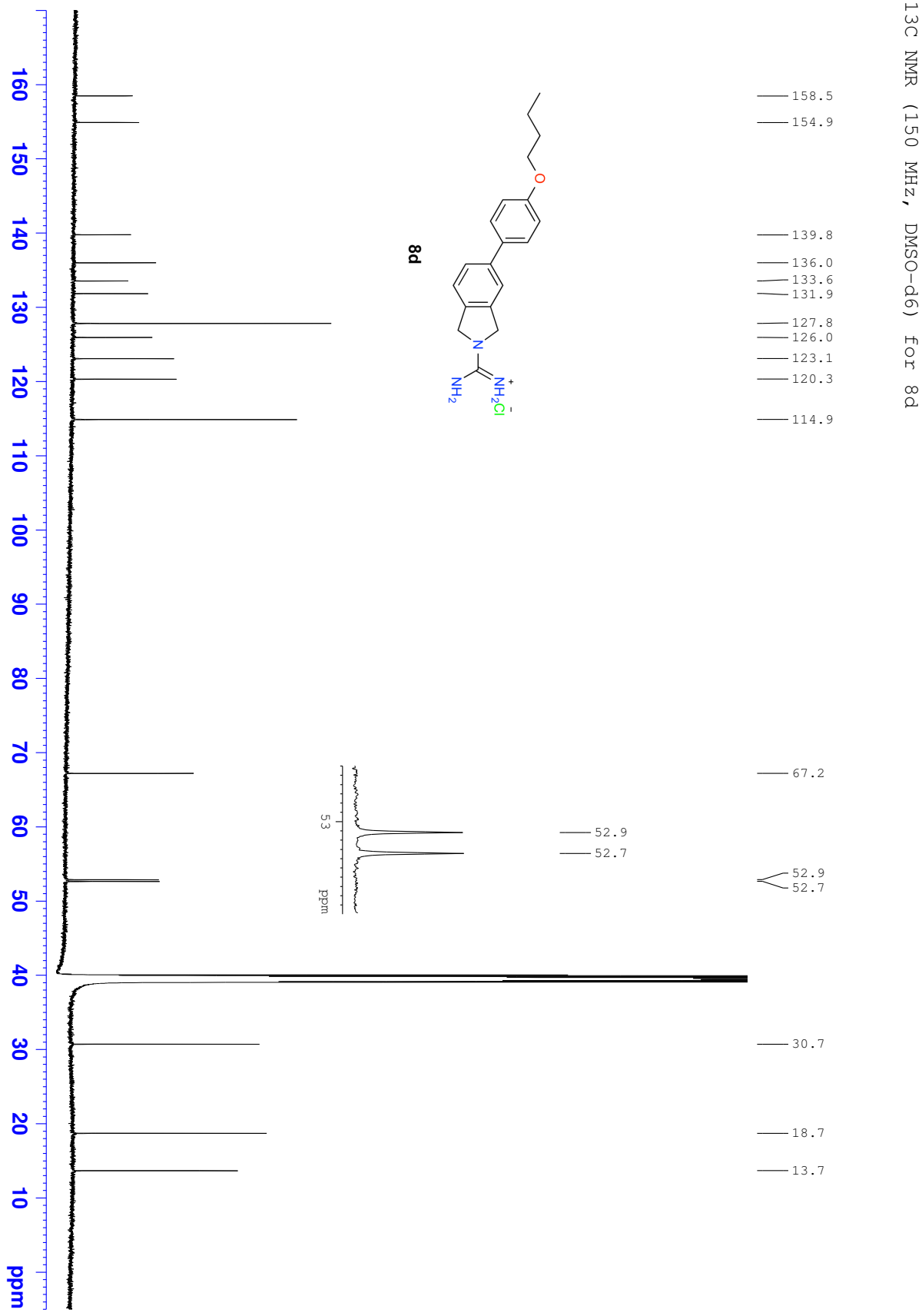



```
======================================================================
                          Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.562 MM    0.0753    6.31684    1.39841   0.0487
   2   1.980 MM    0.0662   82.32648   20.73595   0.6342
   3   2.059 MM N  0.0565   36.51129   10.76155   0.2813
   4   4.082 MM    0.2918   53.69156    3.06622   0.4136
   5   4.259 MM N  0.0983   30.51264    5.17246   0.2351
   6  26.144 MM    1.4383  135.91859    1.57496   1.0471
   7  35.103 BB    1.5016 1.26349e4   114.12705  97.3400

Totals :                  1.29802e4   156.83662


======================================================================
                          *** End of Report ***
```

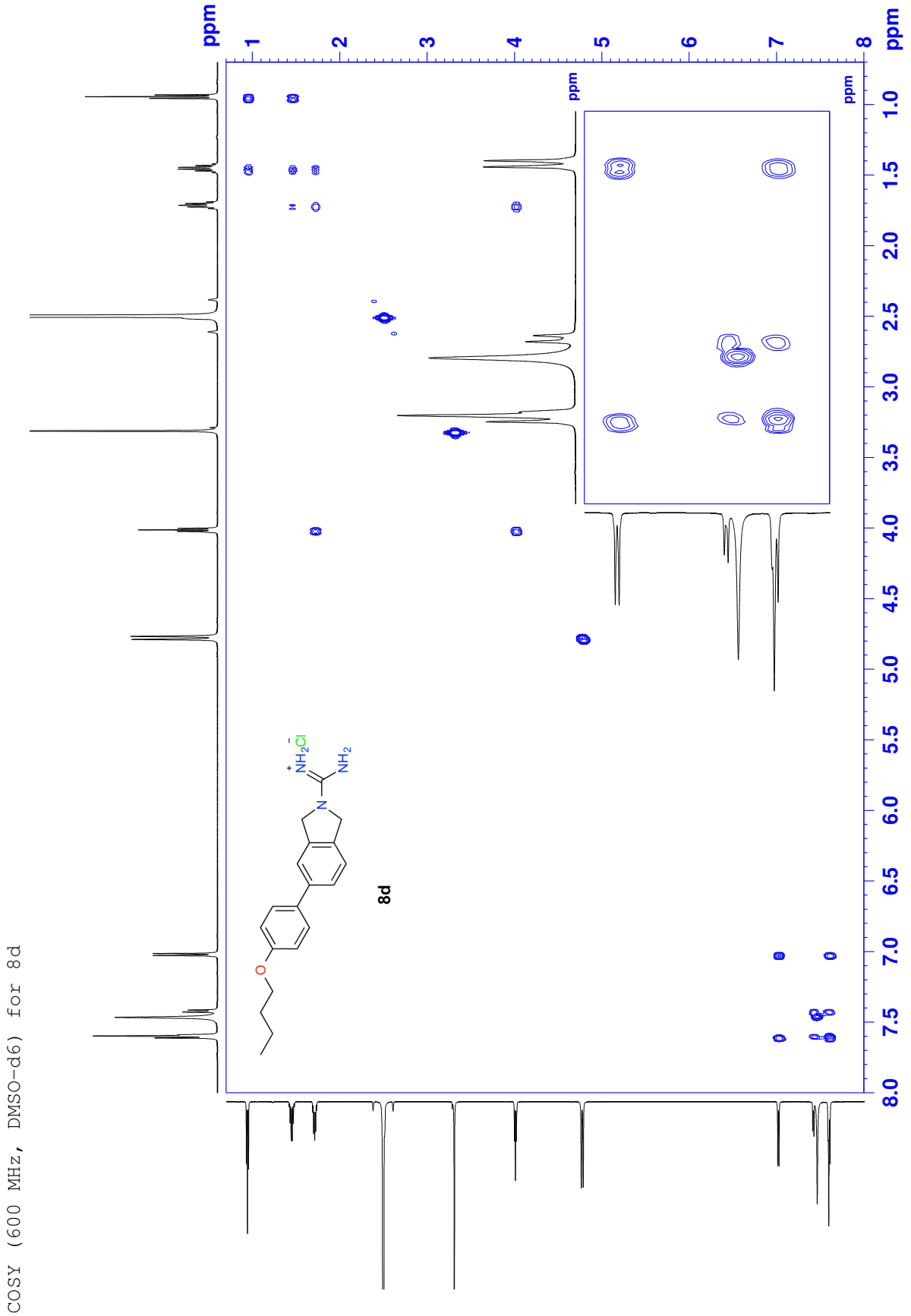
Y.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8d



Y.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8d

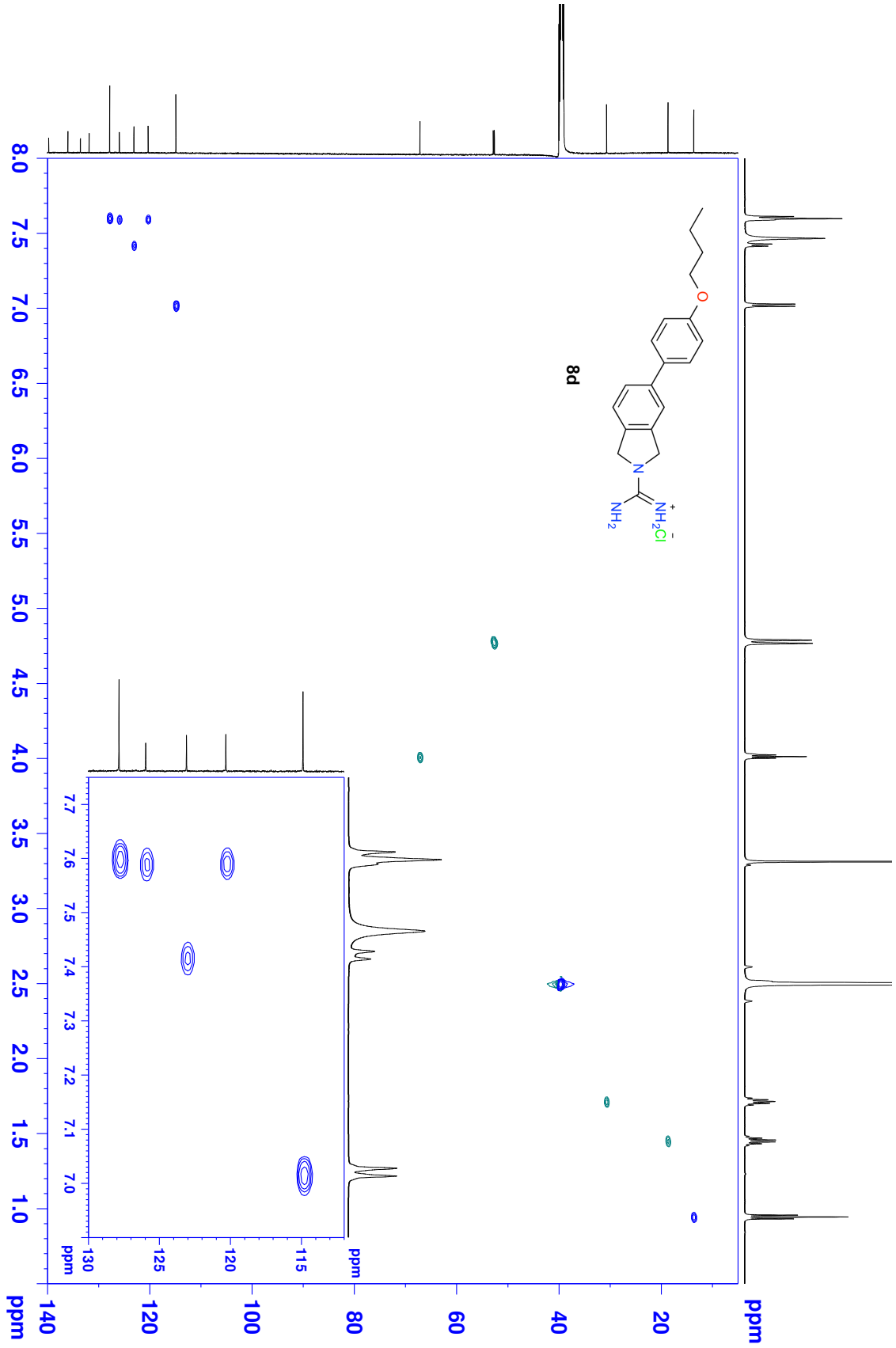


Y.3 COSY (600 MHz, DMSO) spectrum for 8d

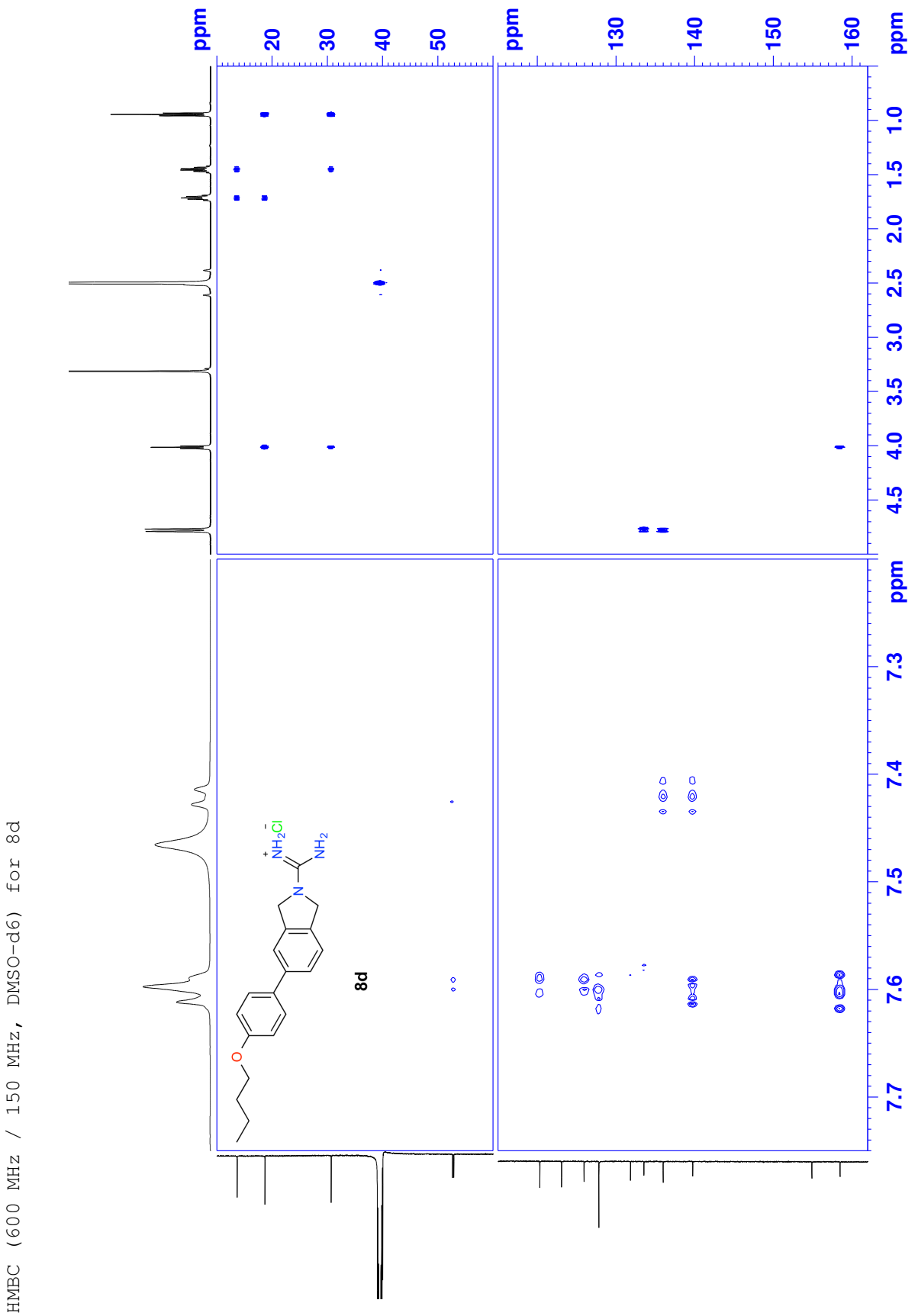


COSY (600 MHz, DMSO-d6) for 8d

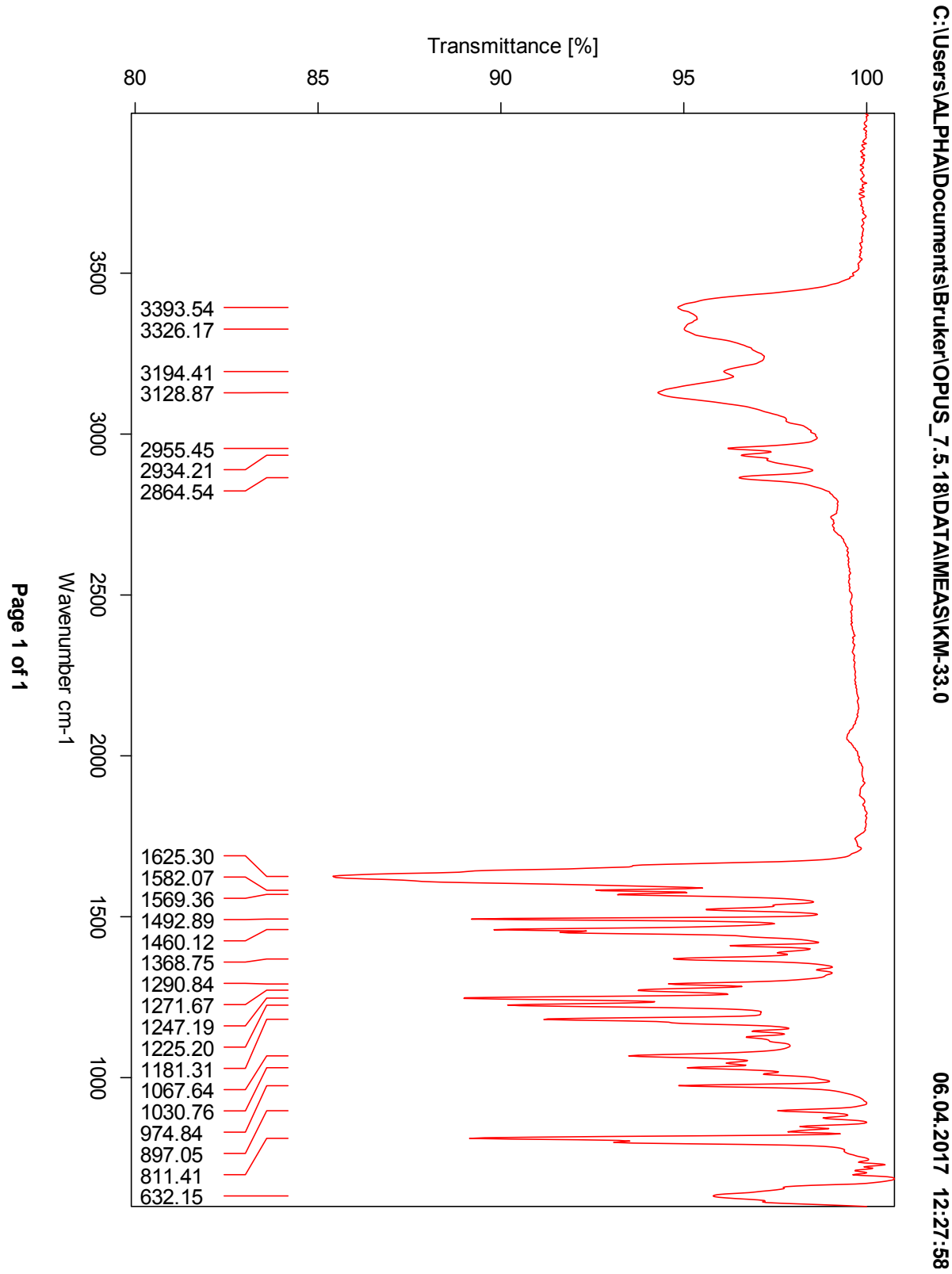
Y.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8d



Y.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8d



Y.6 IR spectrum for 8d

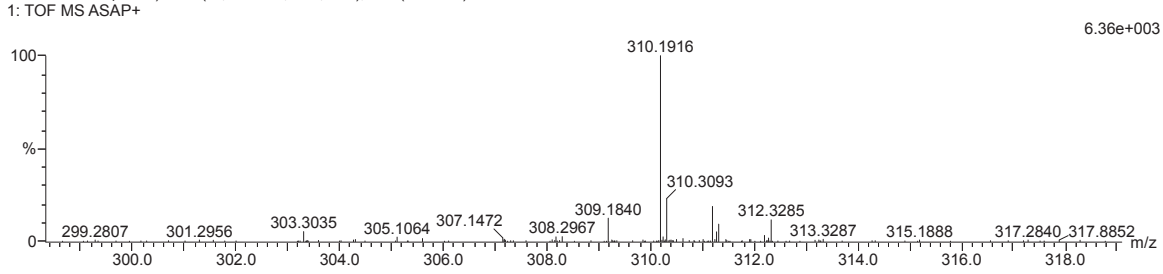


Y.7 HRMS spectrum for 8d

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

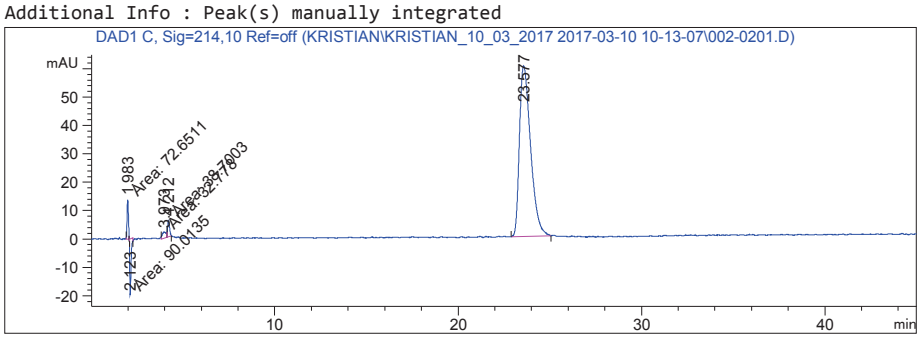
Monoisotopic Mass, Even Electron Ions
343 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 1-10 O: 1-25
2017-158 152 (2.963) AM2 (Ar,35000.0,0.00,0.00); Cm (145:152)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
310.1916	310.1919	-0.3	-1.0	9.5	323.8	n/a	n/a	C19 H24 N3 O ion observed M+

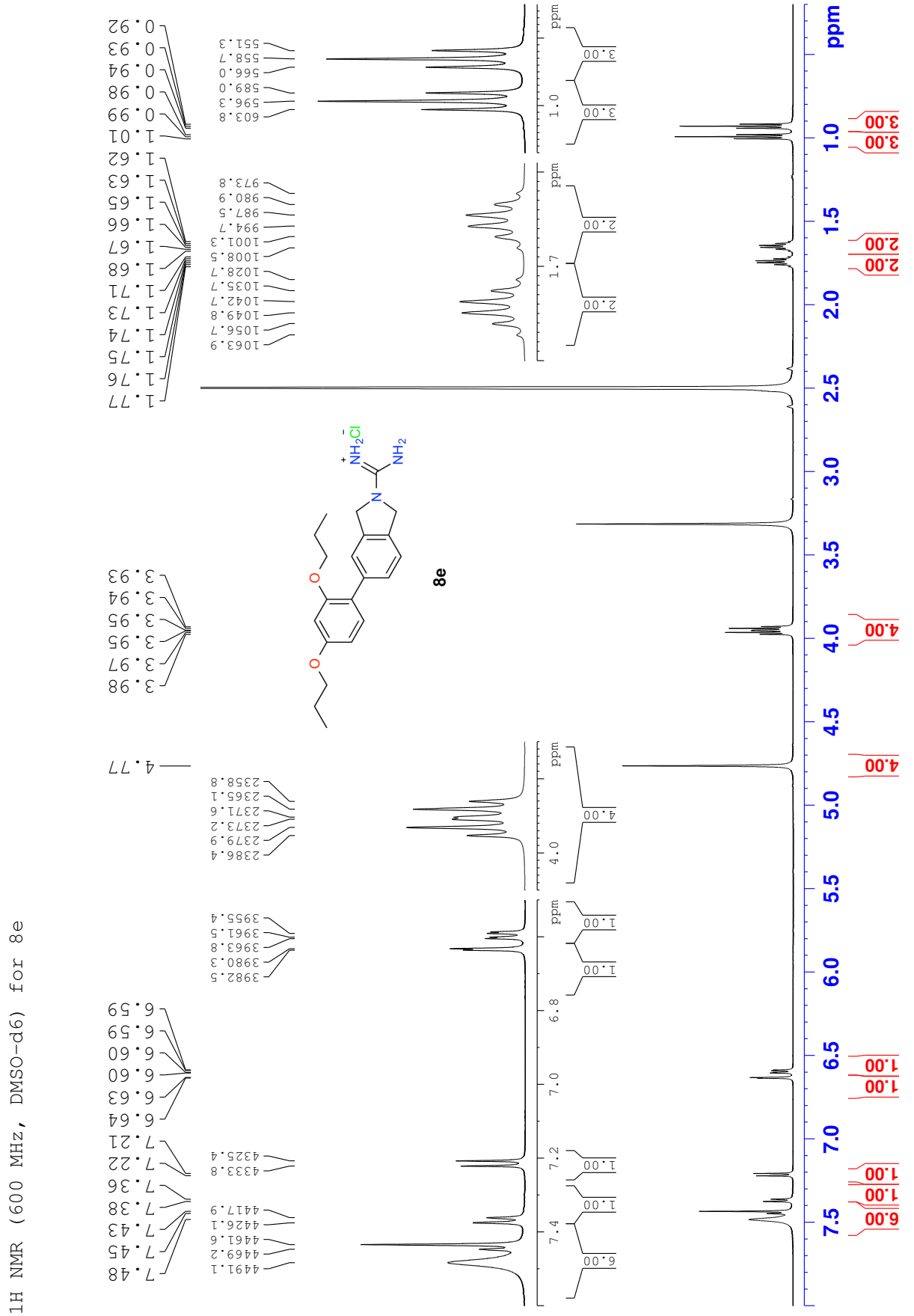
Y.8 HPLC chromatogram for 8d

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_10_03_2017 2017-03-10 10-13-07\002-0201.D
Sample Name: KM-33

    ======================================================================
    Acq. Operator   : Kristian M                    Seq. Line :   2
    Acq. Instrument : UPLC                          Location : Vial 2
    Injection Date  : 10.03.2017 10:59:53                Inj :   1
                                                   Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_10_03_2017 2017-03-10 10-13-07
                      \C18PURITYSALT.M
    Last changed    : 10.03.2017 10:48:35 by Kristian M
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_10_03_2017 2017-03-10 10-13-07\002-0201.
                      D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 27.04.2017 12:30:29 by Kristian M
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

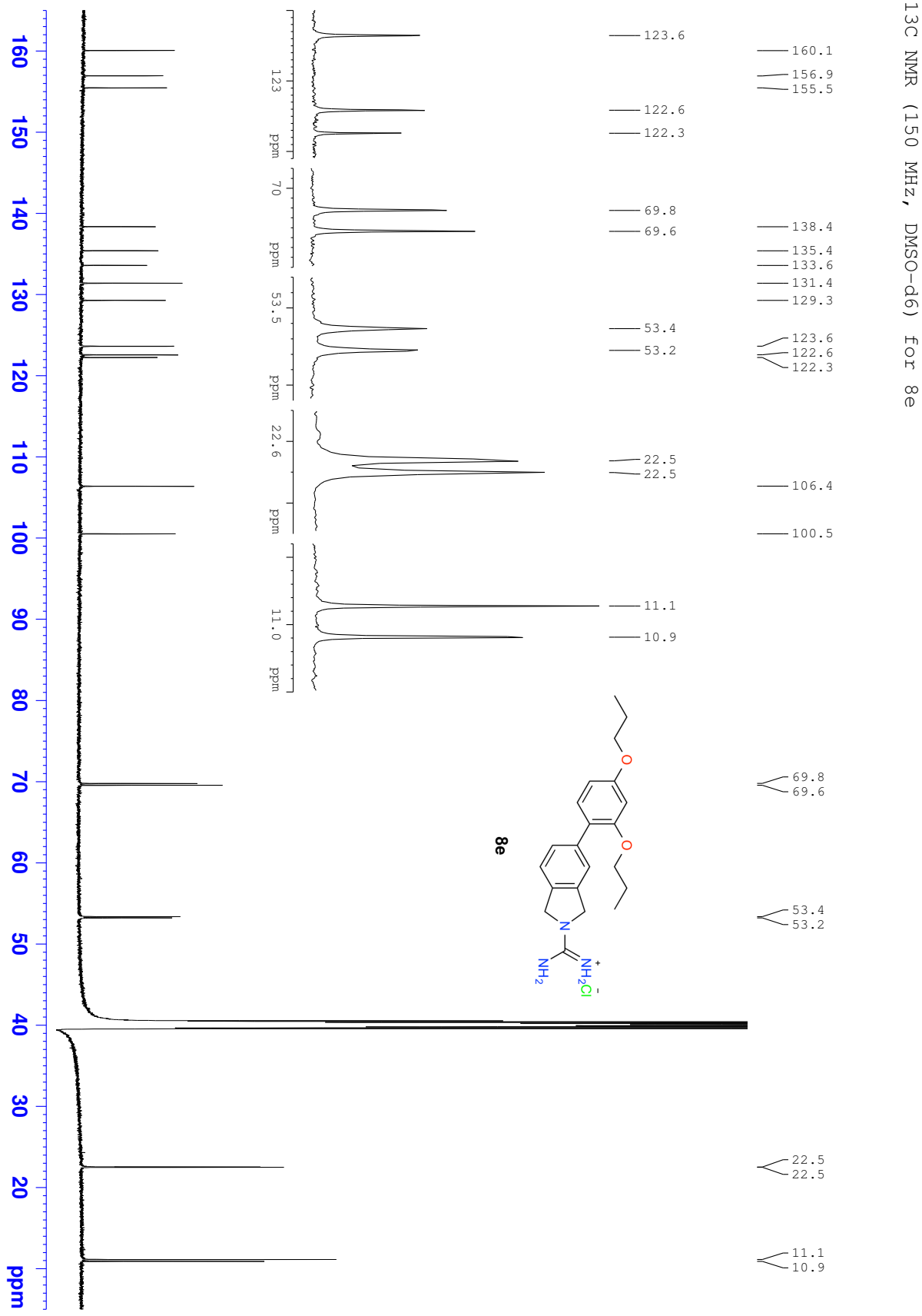



```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.983 MM    0.0876   72.65113   13.81558   2.6252
       2   2.123 MM N  0.0759   90.01349   19.76050   3.2525
       3   3.973 MF    0.2603   32.77798    2.09909   1.1844
       4   4.212 FM    0.1119   38.70035    5.76351   1.3984
       5  23.577 BB    0.6365 2533.34106   60.01245  91.5395

    Totals :                  2767.48401  101.45113


    ======================================================================
                              *** End of Report ***
```

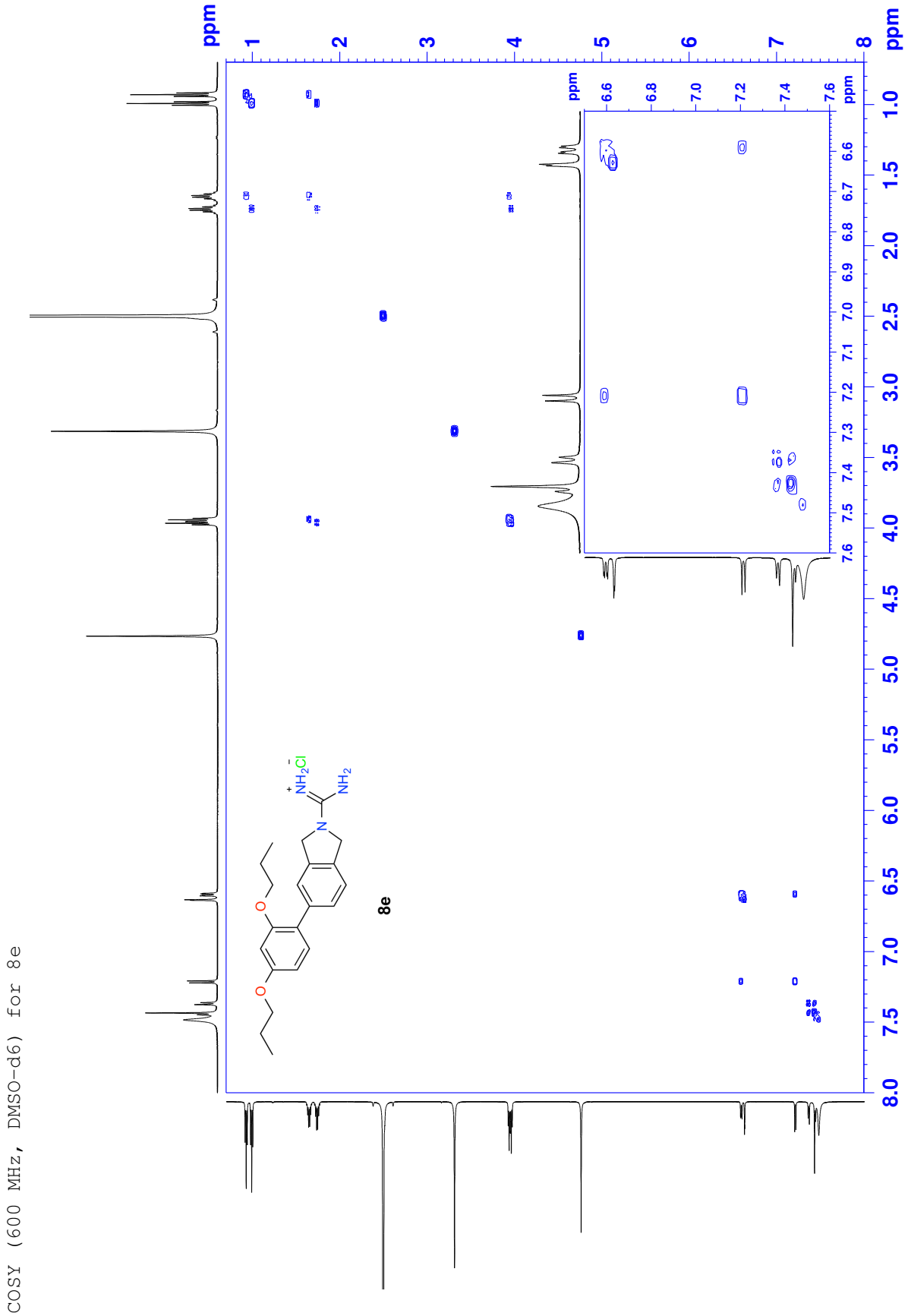
Z.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8e



Z.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8e

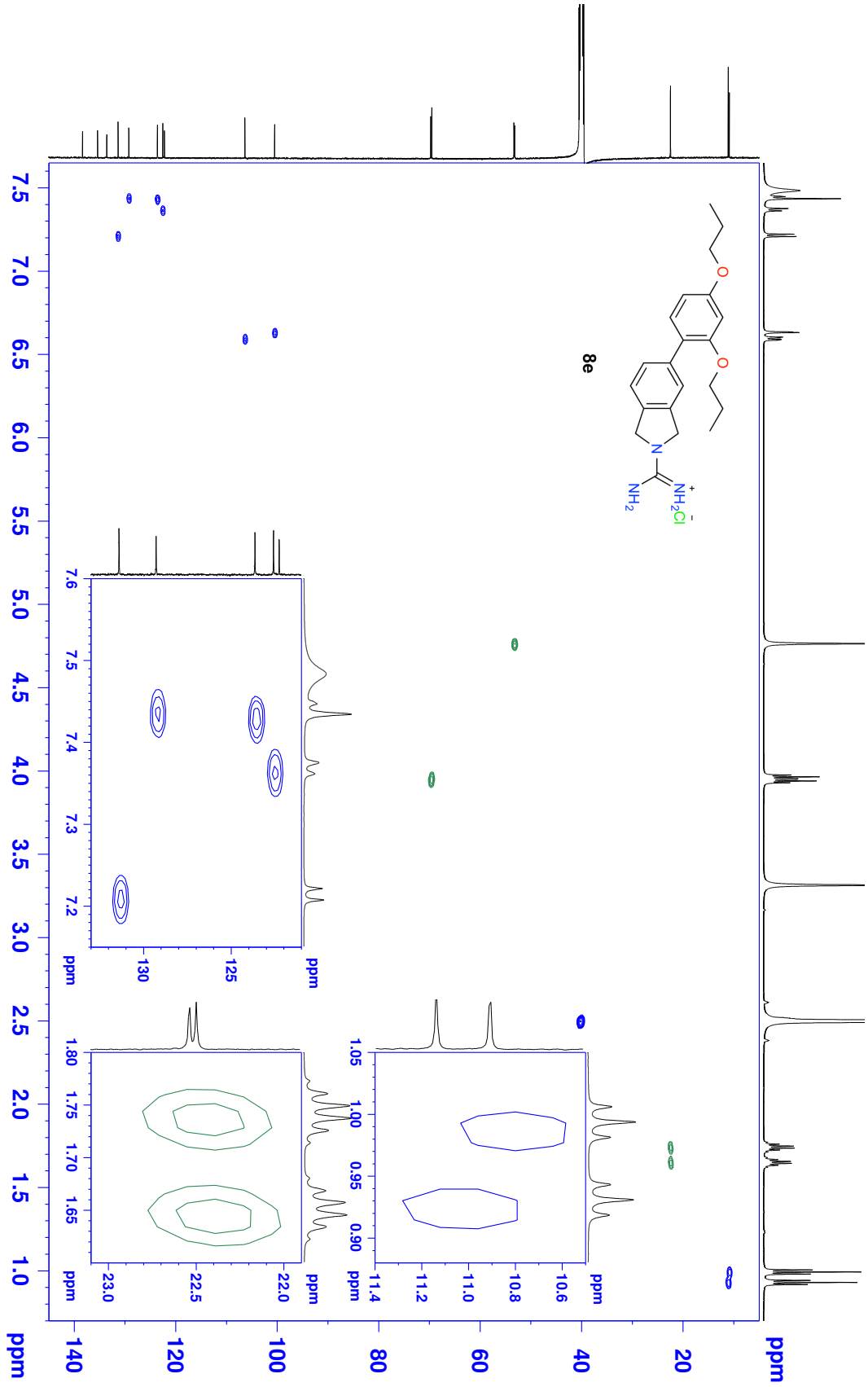


Z.3 COSY (600 MHz, DMSO) spectrum for 8e

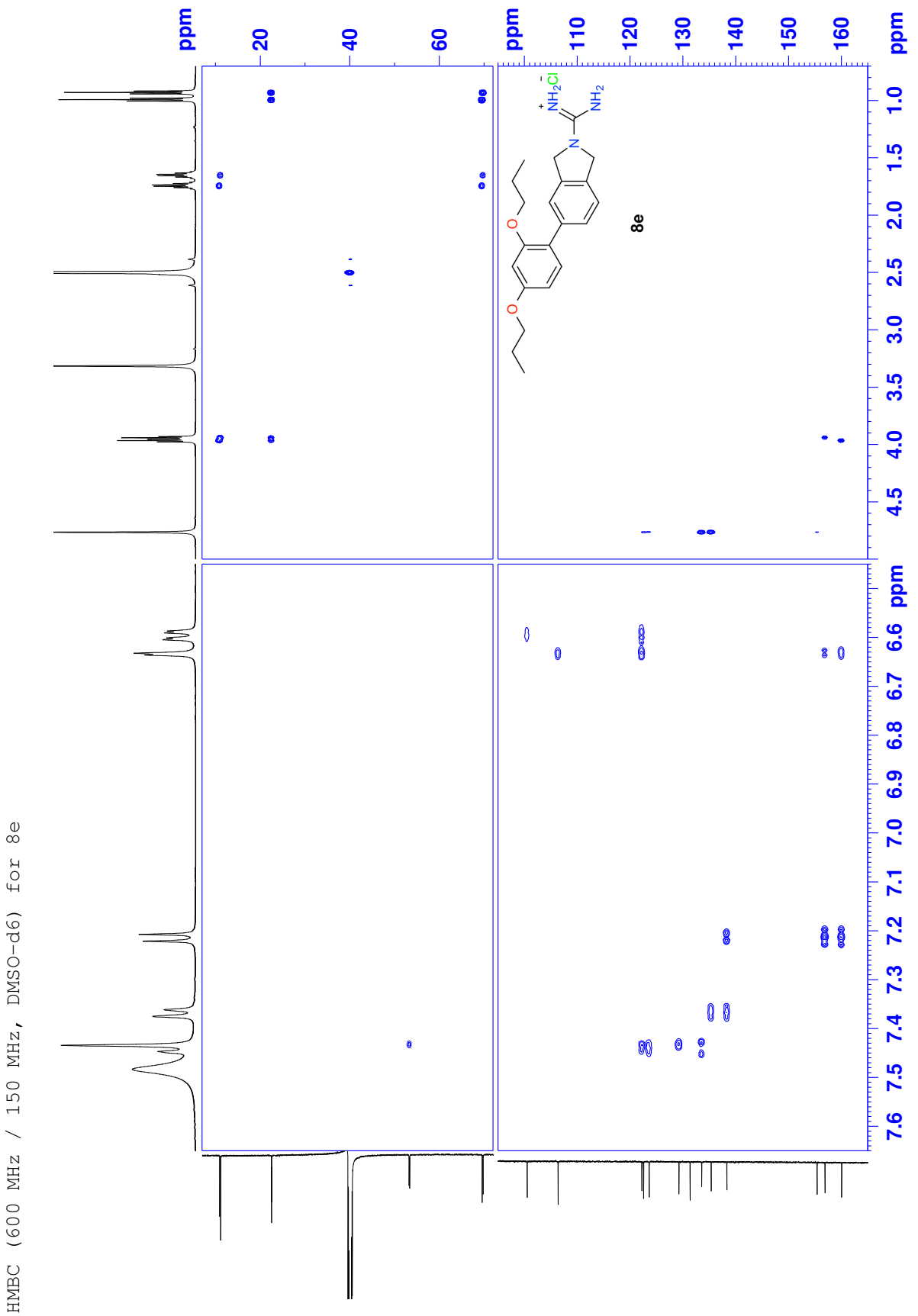


COSY (600 MHz, DMSO-d6) for 8e

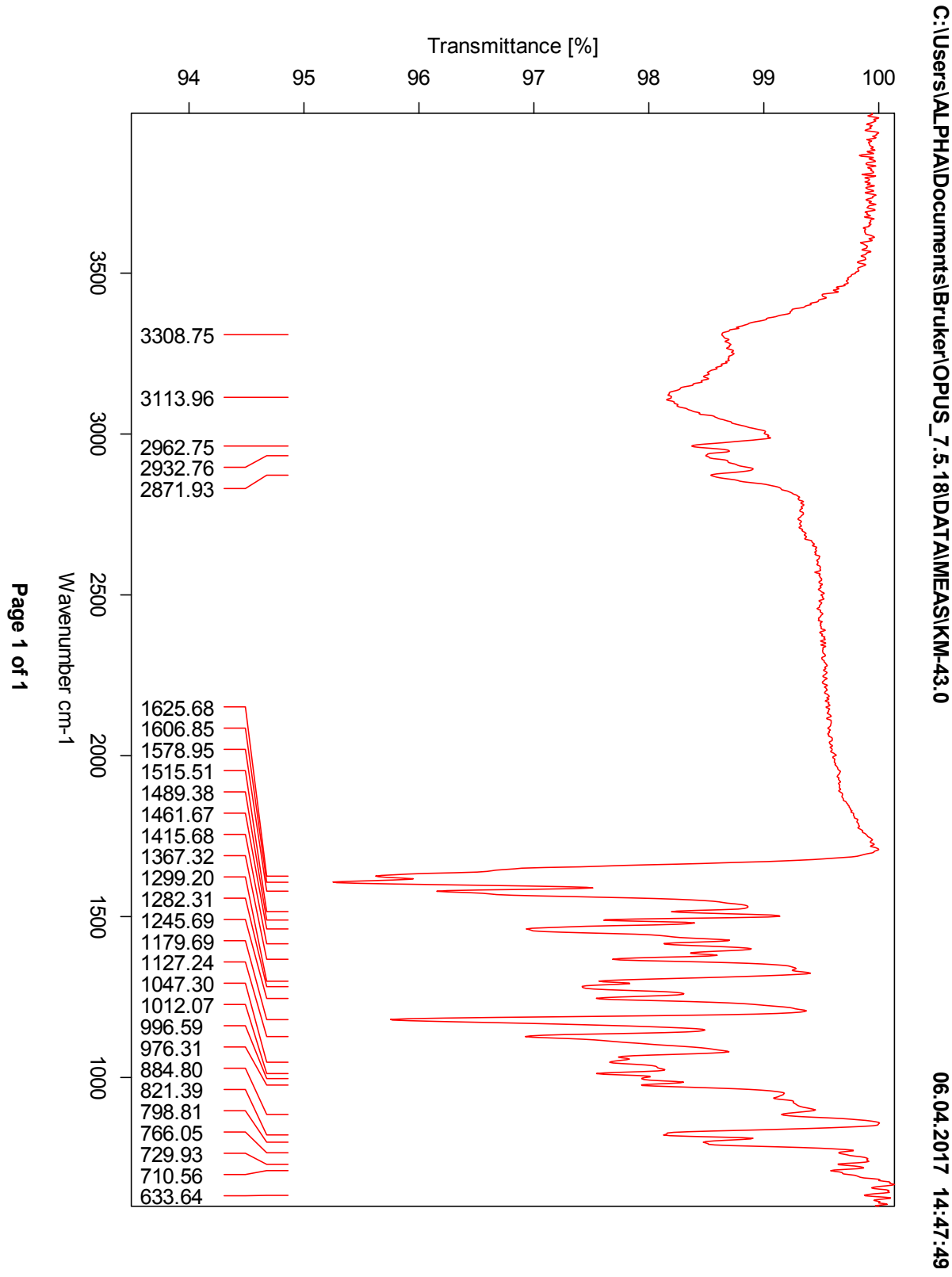
Z.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8e



Z.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8e



Z.6 IR spectrum for 8e

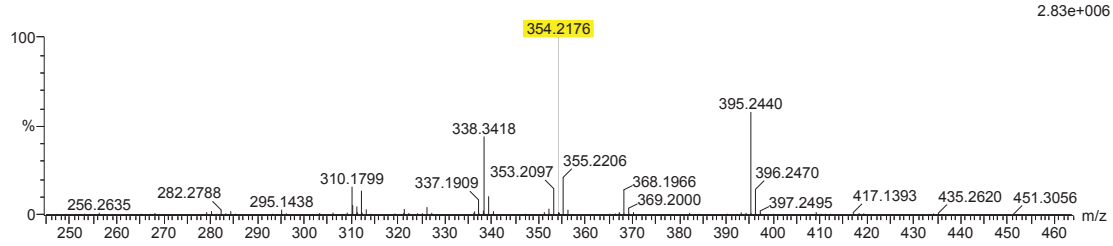


Z.7 HRMS spectrum for 8e

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

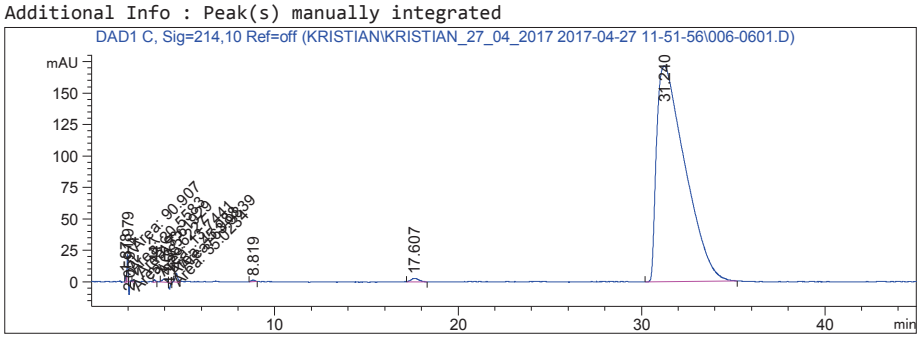
Monoisotopic Mass, Even Electron Ions
571 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-500 H: 1-1000 N: 0-10 O: 0-25
2017-186 130 (2.551) AM2 (Ar,35000.0,0.00,0.00); Cm (120:131)
1: TOF MS ASAP+



Minimum:				-1.5				
Maximum:		2.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
354.2176	354.2182	-0.6	-1.7	9.5	1264.0	n/a	n/a	C21 H28 N3 O2 ion observed M+

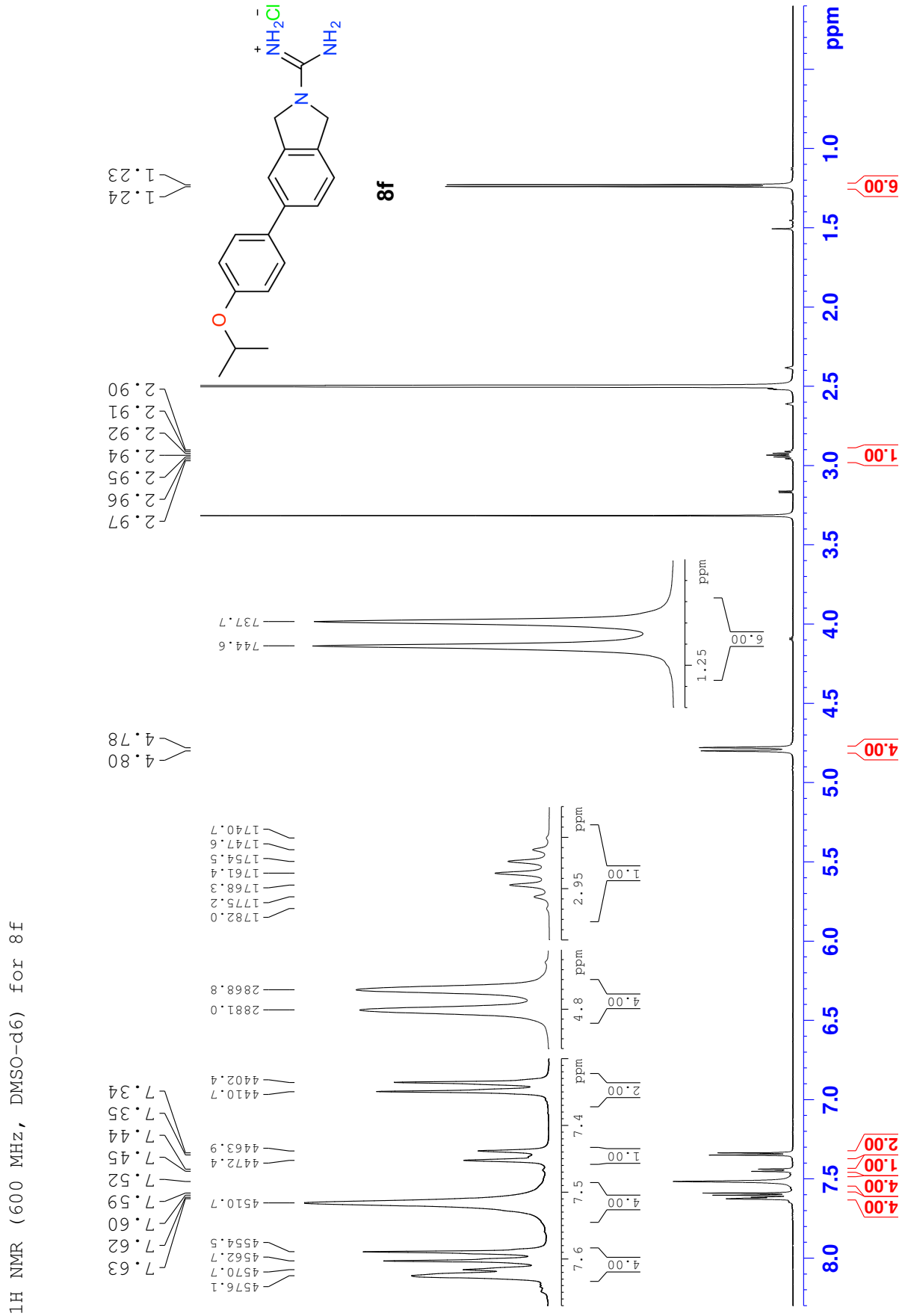
Z.8 HPLC chromatogram for 8e

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\006-0601.D
Sample Name: KM-43

======================================================================
Acq. Operator   : Kristian                      Seq. Line :   6
Acq. Instrument : UPLC                          Location : Vial 6
Injection Date  : 27.04.2017 13:31:42                Inj :   1
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                  \C18PURITYSALT.M
Last changed    : 27.04.2017 13:33:07 by Kristian
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\006-0601.
                  D\DA.M (C18PURITYSALT.M, From Data File)
Last changed    : 27.04.2017 19:01:32 by Kristian M
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```

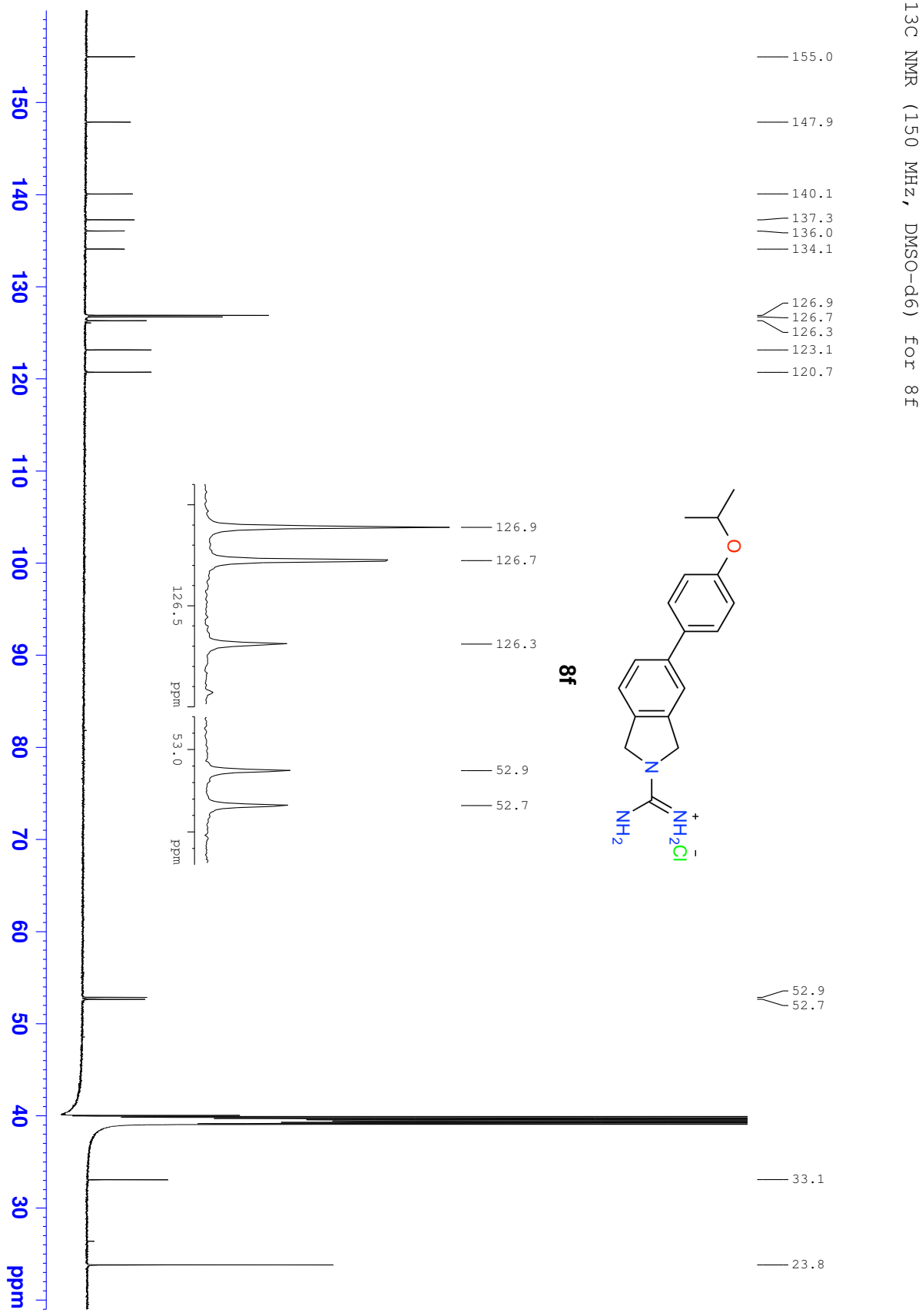



```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,10 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.878 MF    0.0718   20.55831    4.77395   0.1091
   2   1.979 FM    0.0692   90.90702   21.90943   0.4824
   3   2.059 PP N  0.0518   33.62271   10.81829   0.1784
   4   2.274 MM    0.0881    7.61929    1.44097   0.0404
   5   3.447 MM    0.1251   13.74412    1.83130   0.0729
   6   4.076 MM    0.2967   45.98799    2.58338   0.2440
   7   4.257 MM N  0.1026   35.02340    5.69011   0.1858
   8   4.633 MF    0.1588   63.98392    6.71692   0.3395
   9   8.819 BB    0.2339   17.47565    1.24421   0.0927
  10  17.607 BB    0.4008   78.23629    2.64726   0.4151
  11  31.240 BB    1.5376 1.84382e4   171.59807  97.8395

Totals :                  1.88454e4   231.25388
```

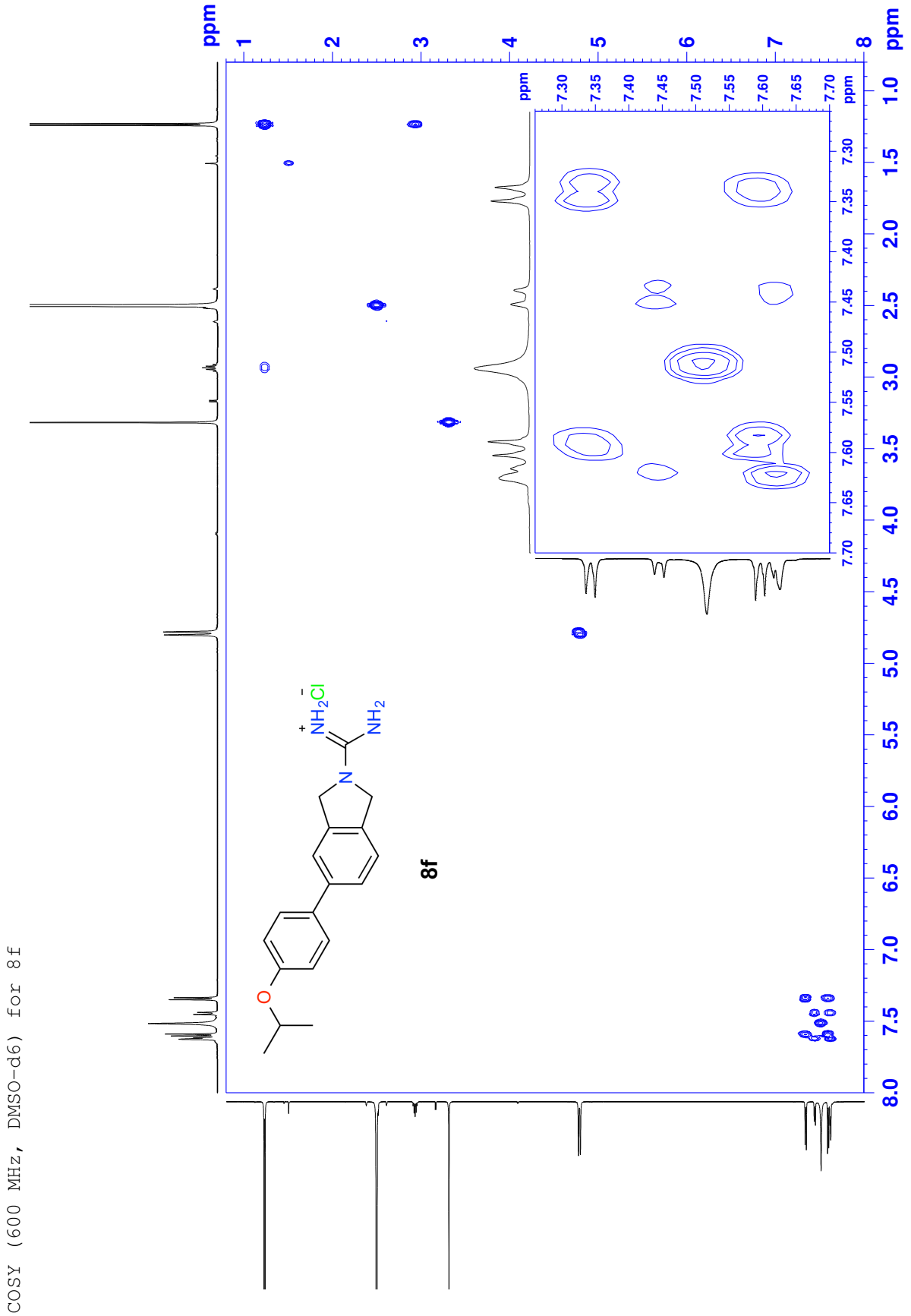
AA.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8f



AA.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8f

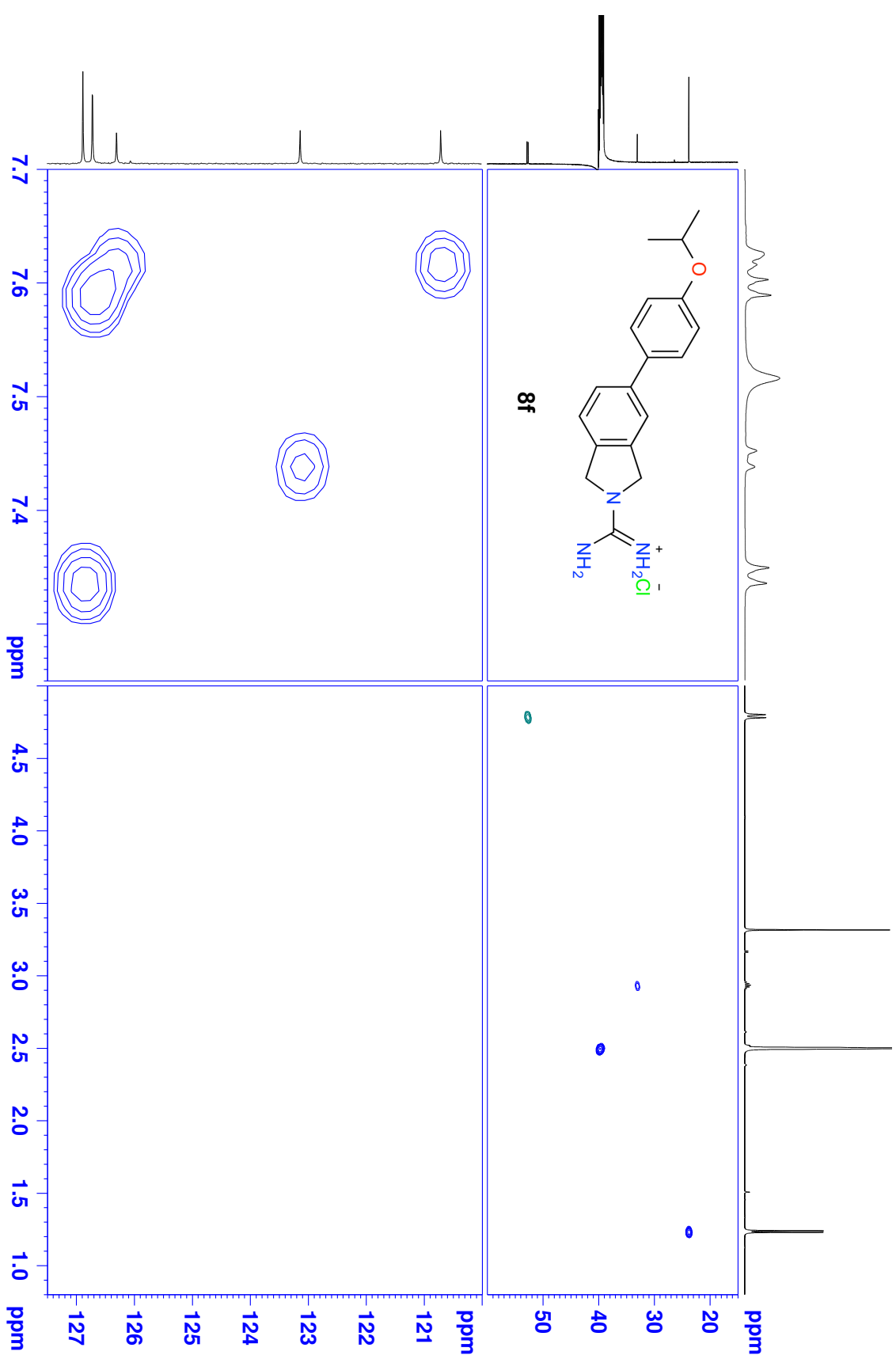


AA.3 COSY (600 MHz, DMSO) spectrum for 8f

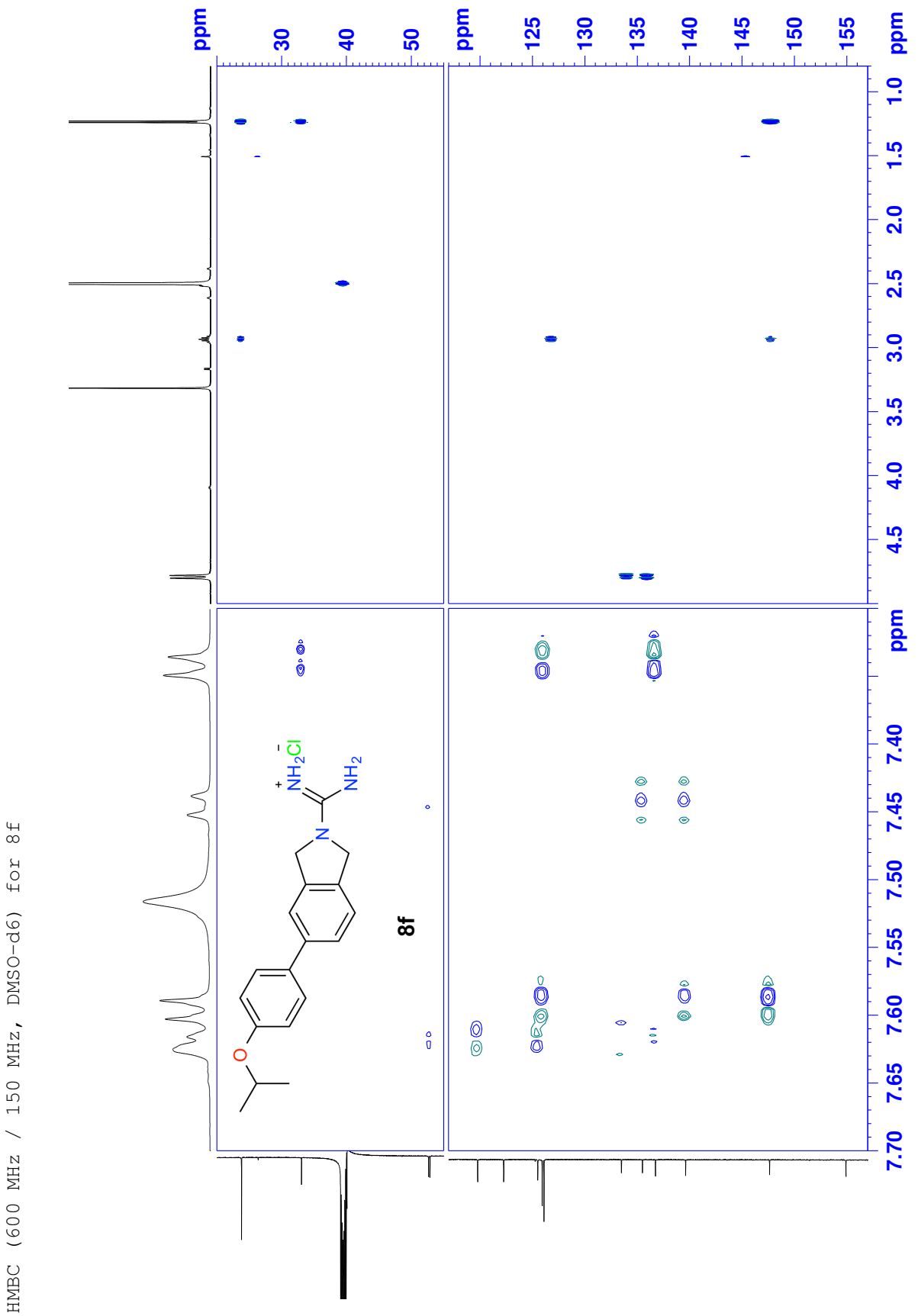


COSY (600 MHz, DMSO-d6) for 8f

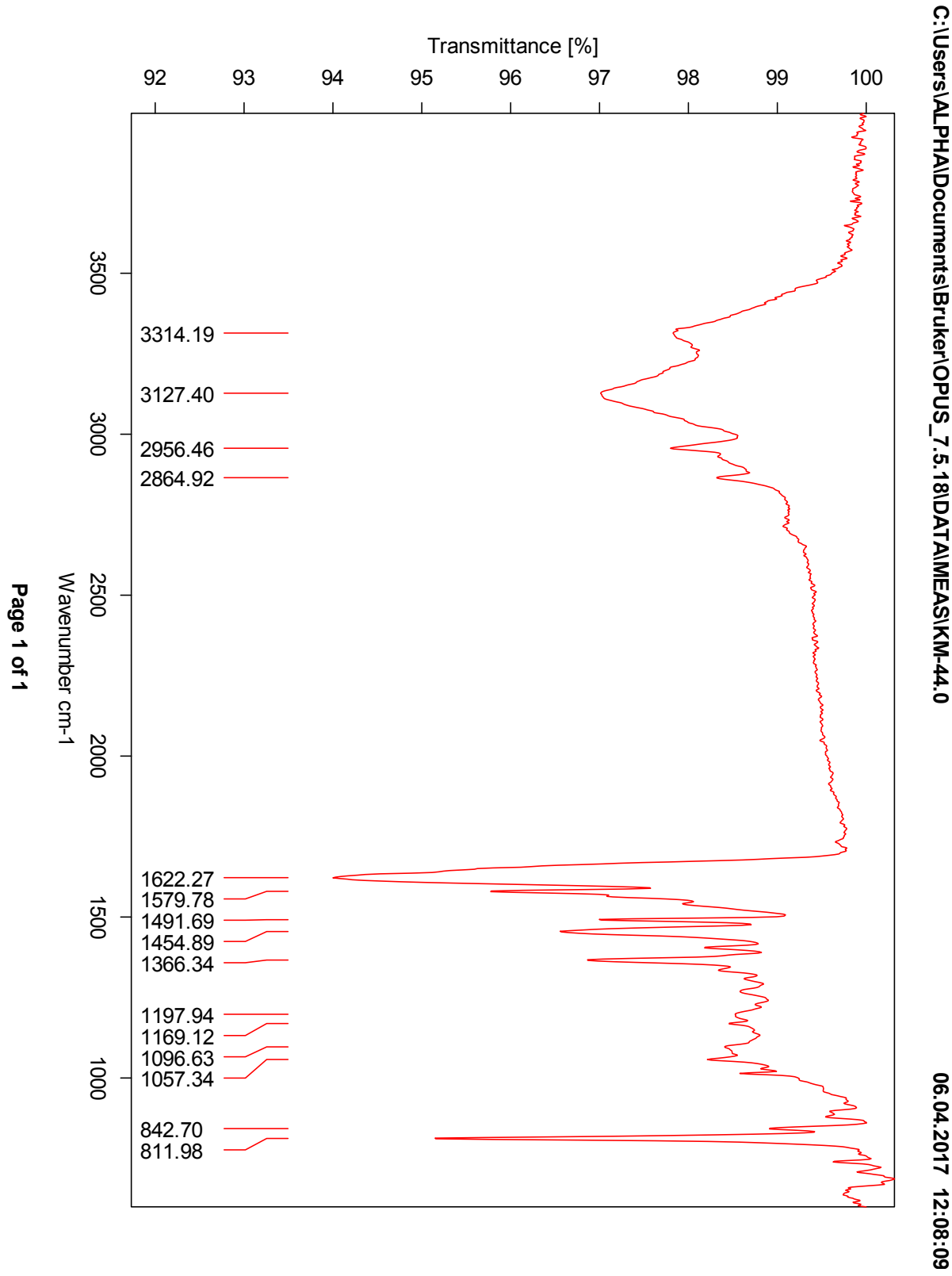
AA.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8f



AA.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8f



AA.6 IR spectrum for 8f



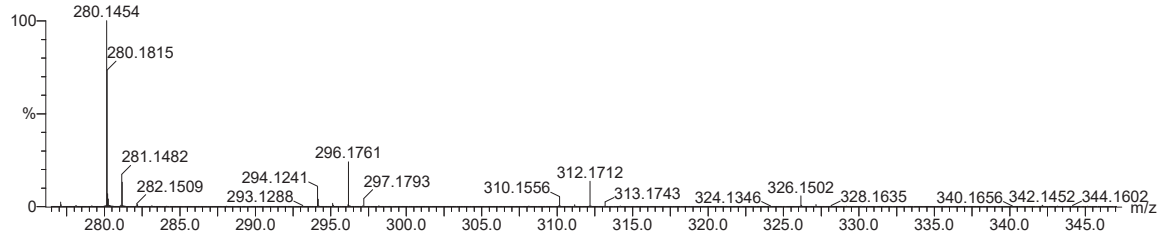
AA.7 HRMS spectrum for 8f

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
2836 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1 Cl: 0-8
2017-305esi 112 (1.023) AM2 (Ar,35000.0,0.00,0.00); Cm (96:113)
1: TOF MS ES+

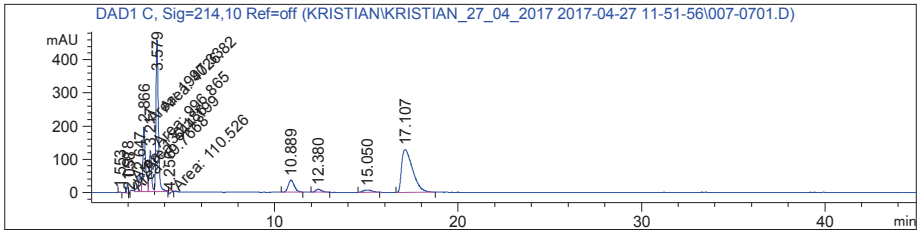
4.38e+007



Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
296.1761	296.1763	-0.2	-0.7	9.5	1731.7	0.000	100.00	C18 H22 N3 O
	296.1757	0.4	1.4	1.5	1753.7	22.091	0.00	C15 H28 N O Na Cl
	296.1768	-0.7	-2.4	2.5	1743.8	12.166	0.00	C3 H18 N15 O2

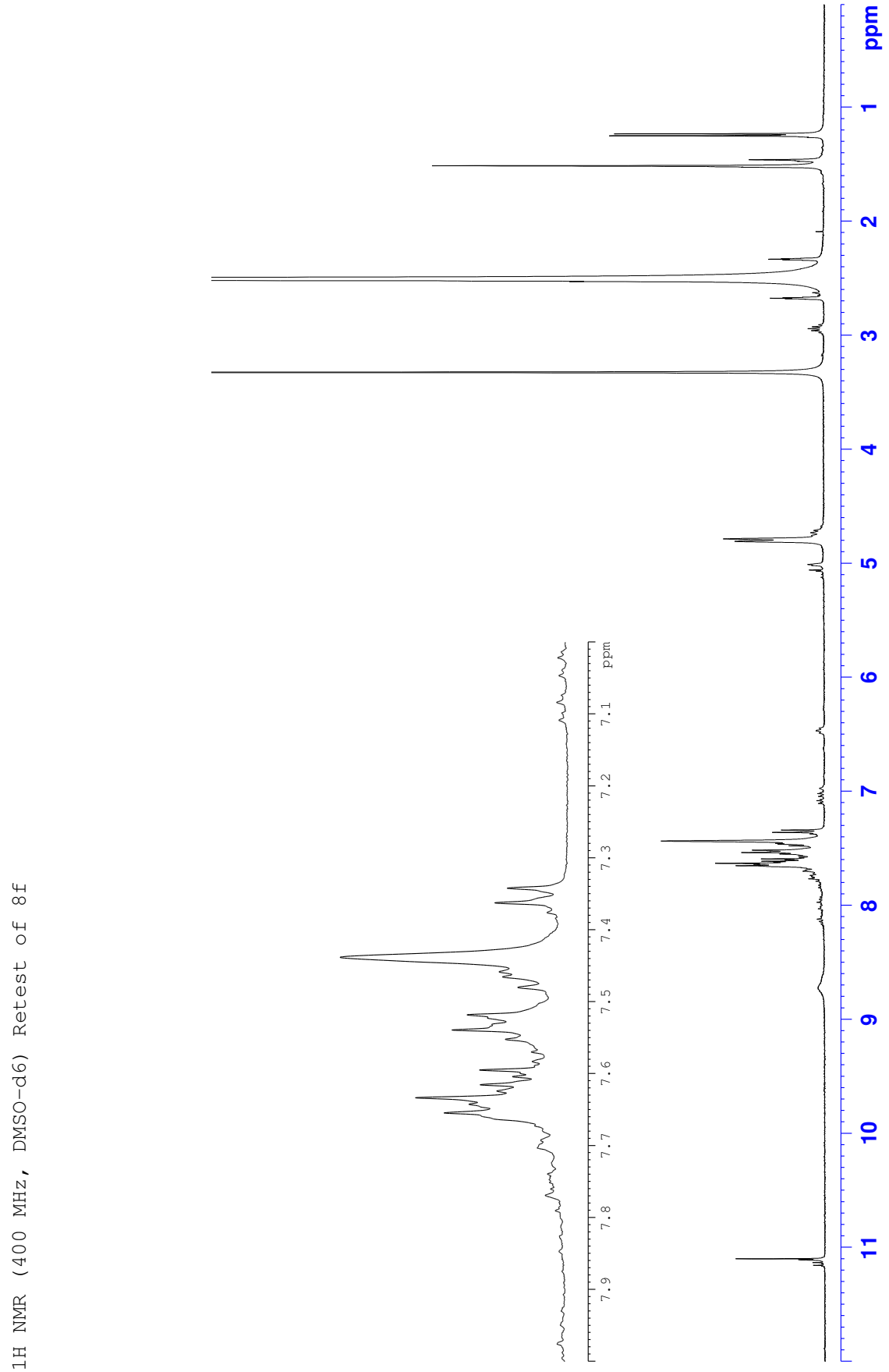
AA.8 HPLC chromatogram for 8f

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\007-0701.D
Sample Name: KM-44 #2
    ======================================================================
    Acq. Operator   : Kristian                       Seq. Line :   7
    Acq. Instrument : UPLC                           Location : Vial 7
    Injection Date  : 27.04.2017 14:17:41                 Inj :   1
                                                   Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56
                      \C18PURITYSALT.M
    Last changed    : 27.04.2017 13:33:07 by Kristian
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_27_04_2017 2017-04-27 11-51-56\007-0701.
                      D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 27.04.2017 19:03:49 by Kristian M
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
    ======================================================================
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.553 BB    0.0791    7.14394    1.40994   0.0495
       2   1.978 MM    0.0794  130.18578   27.31110   0.9012
       3   2.058 MP N  0.0429   19.76680    7.68801   0.1368
       4   2.647 MF    0.1745  544.19891   51.98820   3.7674
       5   2.866 MF    0.1702 1997.33337  195.63892  13.8270
       6   3.217 MF    0.1354  996.86499  122.70609   6.9010
       7   3.579 FM    0.1461 4026.81714  459.35022  27.8766
       8   4.256 MM N  0.1685  110.52583   10.93135   0.7651
       9  10.889 BB    0.3588  825.60205   36.09624   5.7154
      10  12.380 BB    0.3511  204.69038    8.87369   1.4170
      11  15.050 BB    0.4865  236.04997    7.12493   1.6341
      12  17.107 BB    0.6466 5345.94971  128.70755  37.0087

    Totals :                  1.44451e4  1057.82624


    ======================================================================

UPLC 27.04.2017 19:04:14 Kristian M                                  Page   1 of 1
```

AA.9 ^{1}H NMR (400 MHz, DMSO) retest for 8f



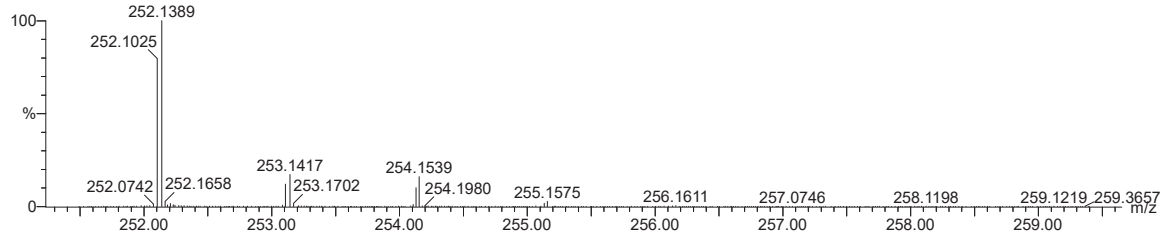
AA.10 HRMS spectrum phenol derivative 8f

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

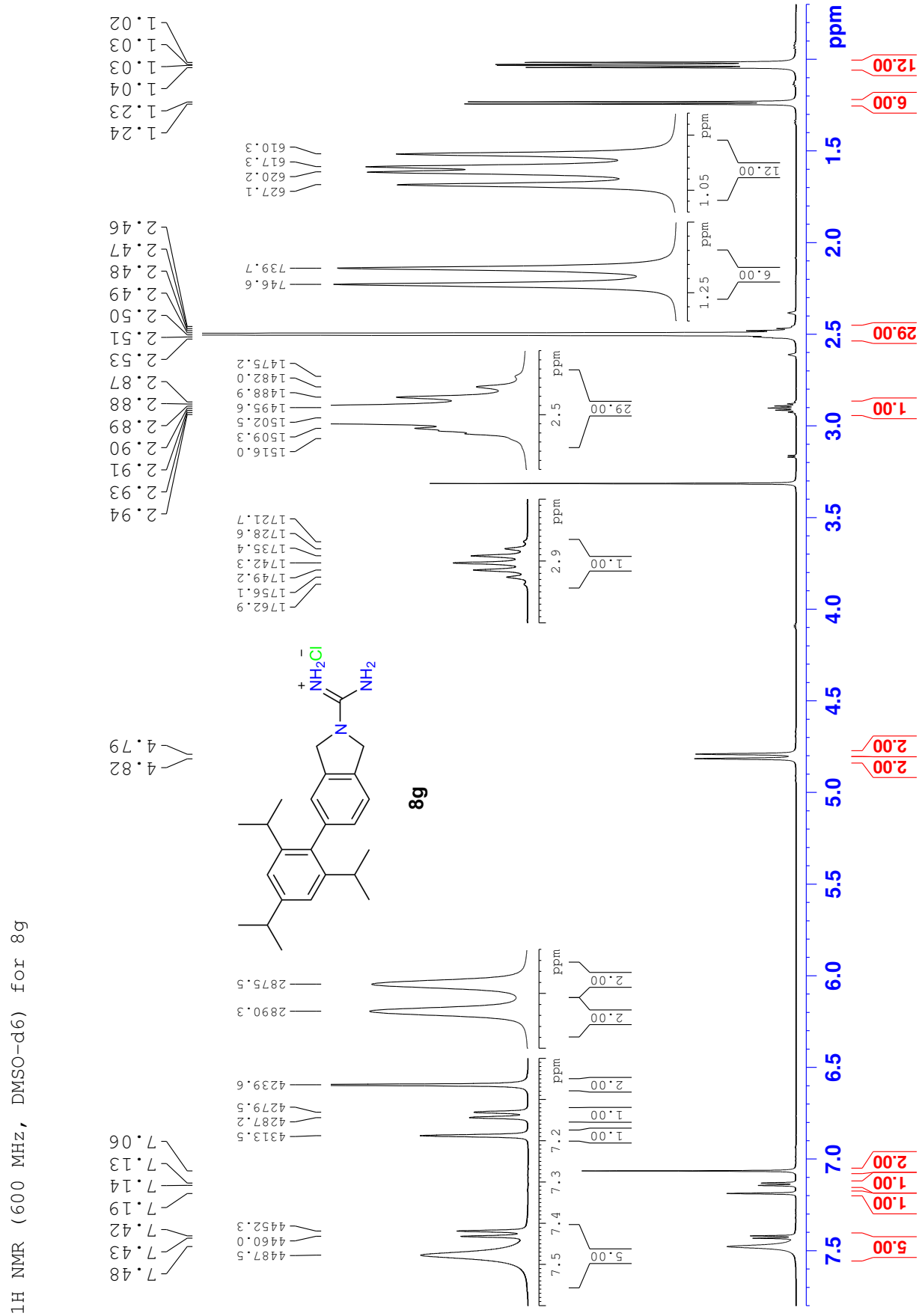
Monoisotopic Mass, Even Electron Ions
1722 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1 Cl: 0-8
2017-305esi 112 (1.023) AM2 (Ar,35000.0,0.00,0.00); Cm (96:113)
1: TOF MS ES+

3.43e+006

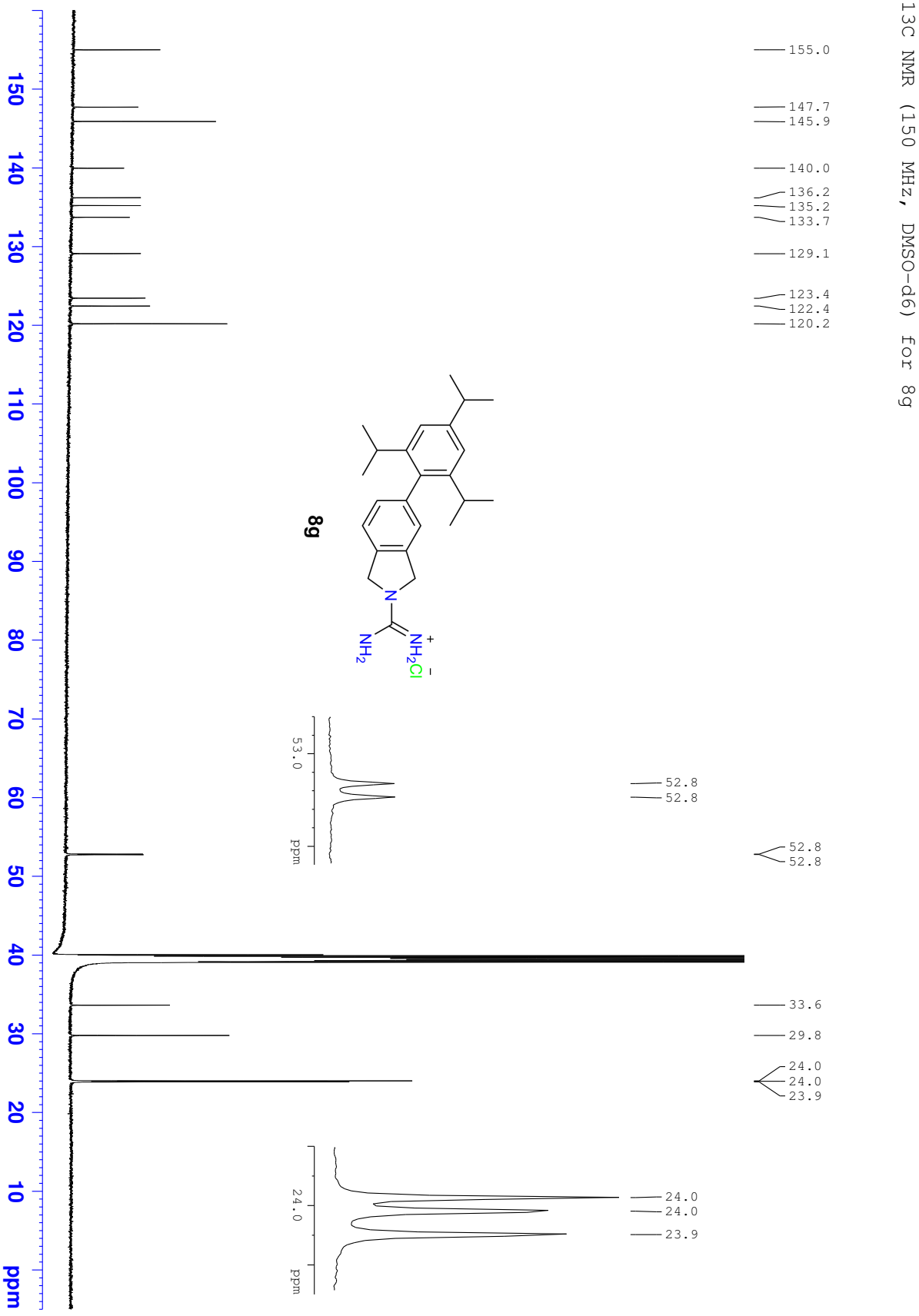


Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
254.1291	254.1293	-0.2	-0.8	9.5	1746.5	0.000	100.00	C15 H16 N3 O
	254.1288	0.3	1.2	1.5	1770.9	24.456	0.00	C12 H22 N O Na Cl
	254.1298	-0.7	-2.8	2.5	1763.8	17.298	0.00	H12 N15 O2

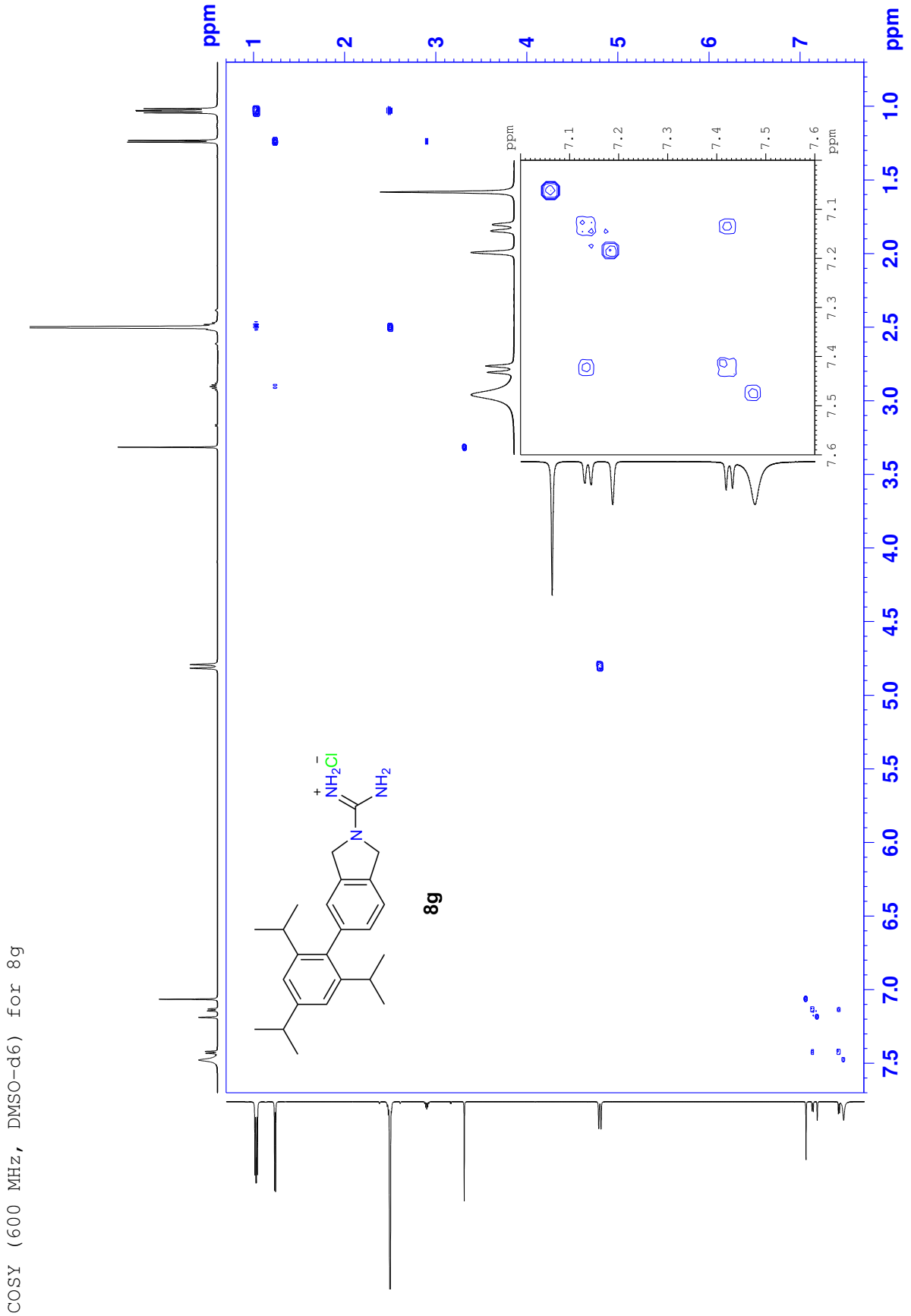
AB.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8g



AB.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8g

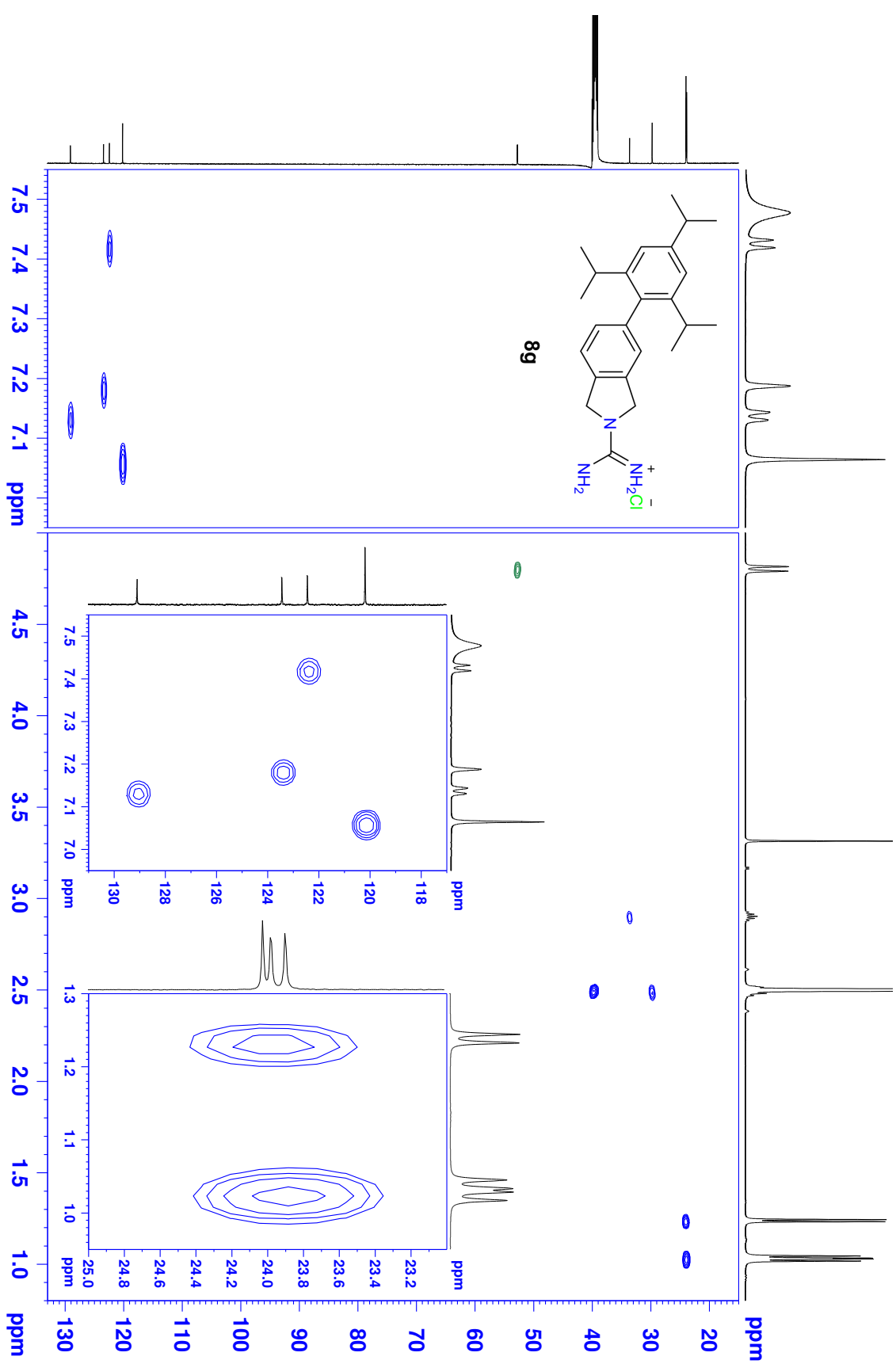


AB.3 COSY (600 MHz, DMSO) spectrum for 8g

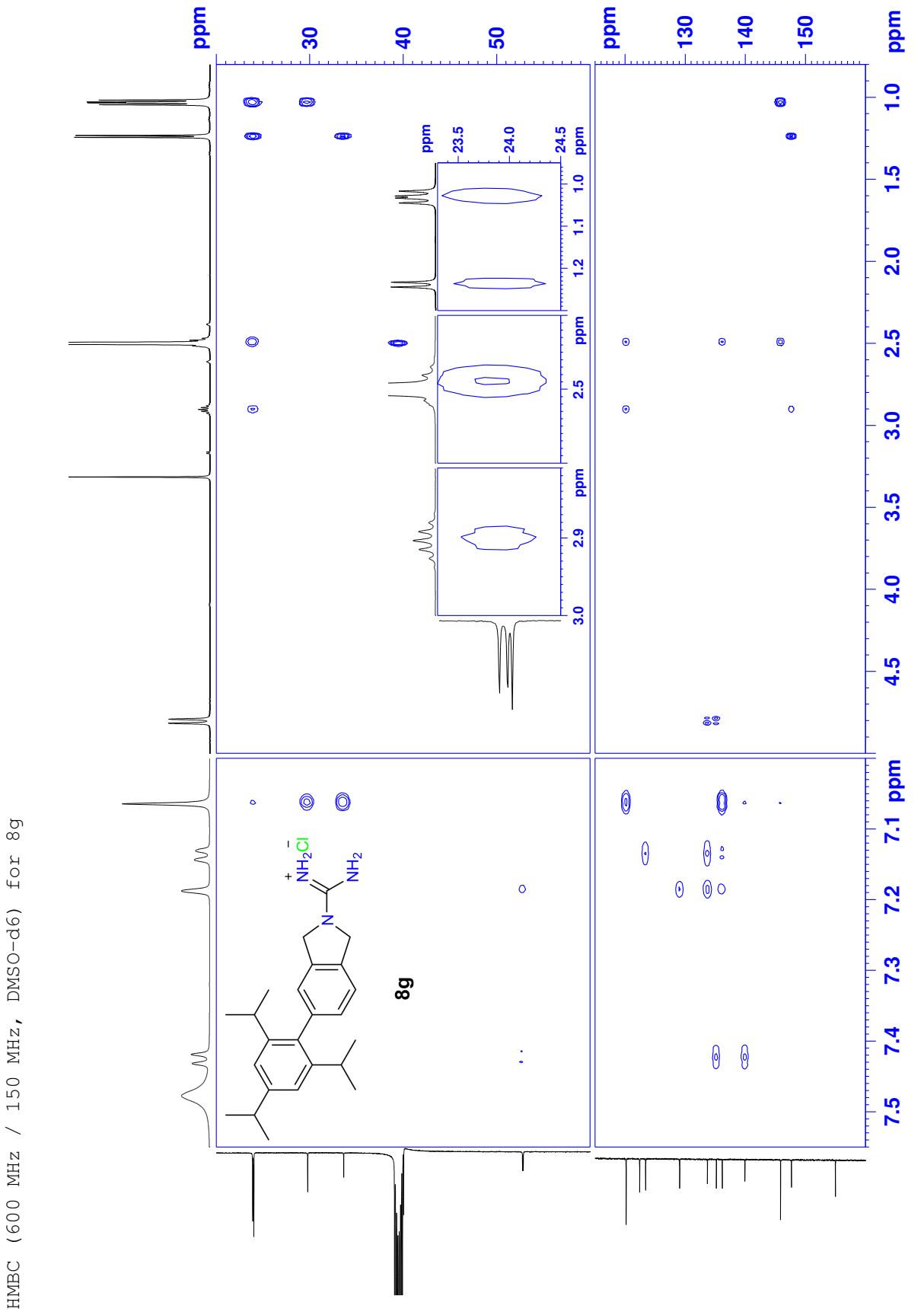


COSY (600 MHz, DMSO-d6) for 8g

AB.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 8g



AB.5 HMBC (600 MHz / 150 MHz, DMSO) spectrum for 8g

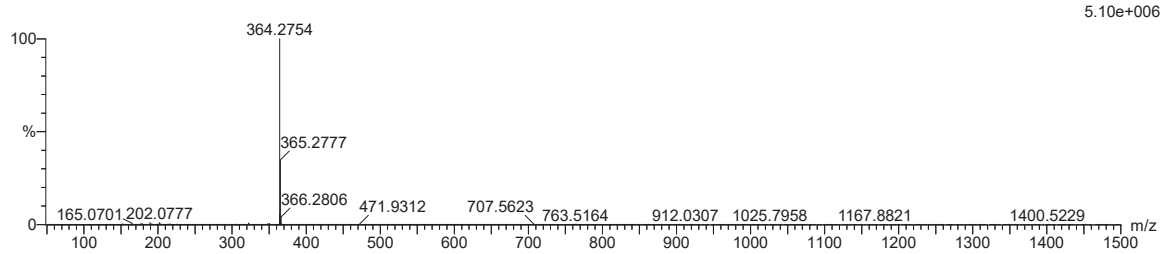


AB.6 HRMS spectrum for 8g

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
960 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-200 H: 0-1000 N: 0-200 O: 0-200
2017-293ESI 37 (0.345) AM2 (Ar,35000.0,0.00,0.00)
1: TOF MS ES+

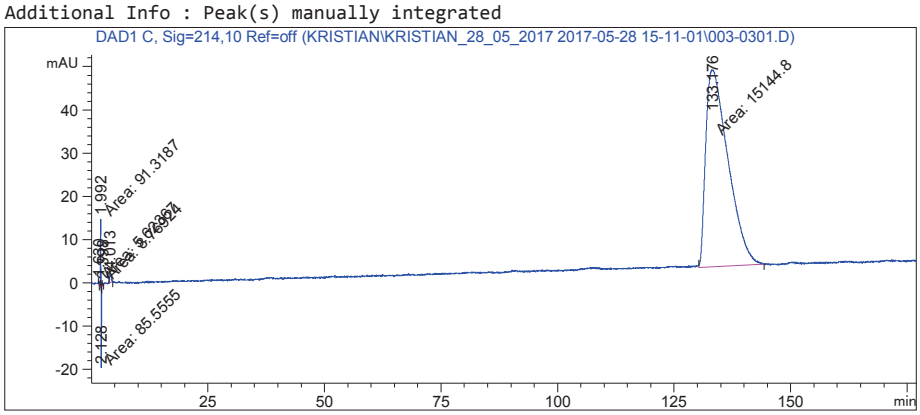


Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
364.2754	364.2753	0.1	0.3	9.5	1465.9	0.011	98.94	C24 H34 N3
	364.2758	-0.4	-1.1	2.5	1473.8	7.917	0.04	C9 H30 N15 O
	364.2785	-3.1	-8.5	1.5	1470.5	4.579	1.03	C13 H34 N9 O3

ion observed [M+H]

AB.7 HPLC chromatogram for 8g

```
Data File C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01\003-0301.D
Sample Name: KM-59

    ======================================================================
    Acq. Operator   : Kristian                       Seq. Line :   3
    Acq. Instrument : UPLC                           Location : Vial 3
    Injection Date  : 28.05.2017 16:52:47                 Inj :   1
                                                    Inj Volume : 2.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01
                      \C18PURITYSALT.M
    Last changed    : 28.05.2017 19:48:21 by Kristian
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\KRISTIAN\KRISTIAN_28_05_2017 2017-05-28 15-11-01\003-0301.
                      D\DA.M (C18PURITYSALT.M, From Data File)
    Last changed    : 28.05.2017 19:54:46 by Kristian
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 C, Sig=214,10 Ref=off

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.630 MM    0.1313    5.62367 7.14005e-1   0.0366
       2   1.992 MM    0.0959   91.31868   15.86816   0.5937
       3   2.128 MM N  0.0737   85.55548   19.35160   0.5562
       4   2.398 MM    0.2064    8.76924 7.07956e-1   0.0570
       5   4.013 BB    0.3137   45.10150    2.10719   0.2932
       6 133.176 MM    5.5351 1.51448e4    45.60271  98.4633

    Totals :                   1.53812e4    84.35162


    ======================================================================
                            *** End of Report ***

UPLC 28.05.2017 19:54:52 Kristian                                Page   1 of 1
```