

Kristine Øya

Synthesis of Cationic 2,3-dihydro-1H-indene Amphiphiles for Antimicrobial Evaluation

Master's thesis in Chemical Engineering and Biotechnology

Supervisor: Odd Reidar Gautun

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Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

 **NTNU**
Norwegian University of
Science and Technology

Acknowledgments

This masters' thesis, titled *Synthesis of cationic 2,3-dihydro-1H-indene amphiphiles for antimicrobial evaluation*, was conducted at the Department of Chemistry, Norwegian University of Science and Technology (NTNU) in Trondheim between January and June 2019. This thesis is a continuation of D. Lindberg's and S. Valderhaug's master projects, as well as my own work, titled *Synthesis of fused benzene amphiphiles for antimicrobial evaluation*, performed as a part of the Organic Chemistry Specialisation Project TKJ4520 between August to December 2018.

First and foremost, I would like to thank my supervisor, associated professor Odd Reidar Gautun, for providing an immensely interesting project, all challenges included, and for your continued guidance and support. I have learned so much during this last year, and your vast knowledge and willingness to teach is truly inspiring. I would also like to extend my thanks and gratitude to Roger Aarvik, Susana Villa Gonzales, Julie Asmussen, Torun Margareta Melø, Nebojsa Simic and Kristian Myreng for help with chemicals, spectroscopy, training and questions.

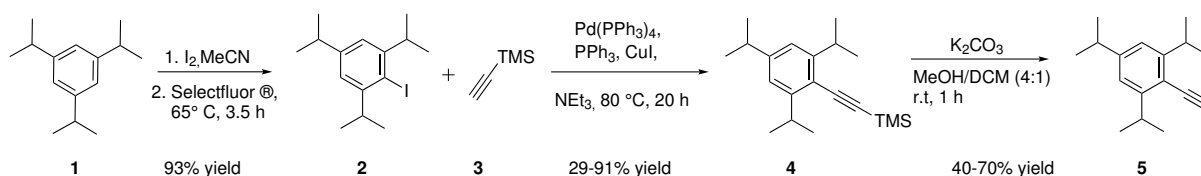
Next, I would like to thank my fellow M. Sc candidates Lise Pernille Løberg, Daniel Fridberg Moen and Jorge Alberto Rodas Zavala for creating a fun and helpful atmosphere in the lab. I will look back at our countless hours of banter, cinnamonbuns and discussions with great fondness.

I would also like to mention my closest friends Frida P. Danmo and Solveig Valderhaug. The last six years would not have been the same without you. Lastly, I would like to thank my family for their encouragement and support throughout my entire life, and my beloved Eskild R. Mageli, for your continued love and support over the last 6 years. Without you, I would not be the person I am today.

Abstract

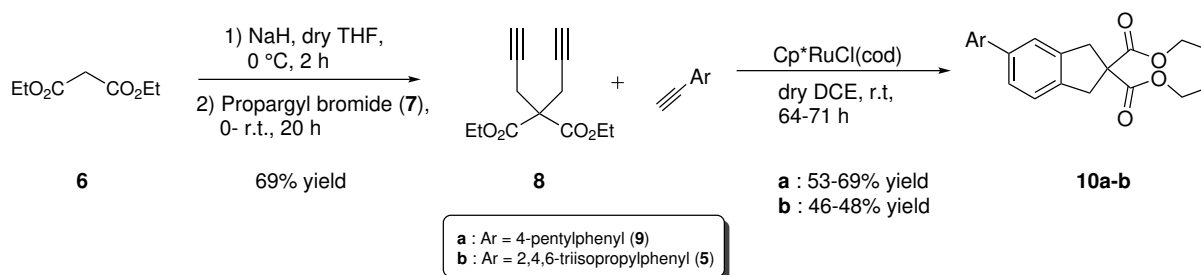
The scope of this master thesis has been to synthesise fused benzene amphiphiles with one or more cationic N-groups. If the degree of purity is sufficient (>95%), the products will be sent to Tromsø (Uit) for antimicrobial evaluation.

Target molecule **19b** and an amine salt **20a** (Scheme 0.4) and target molecule **23b** (Scheme 0.5) was prepared utilising an already established synthetic route. The initial step was synthesis of monoalkyne **5** through iodination of **2**, followed by a Sonogashira coupling and the subsequent removal of the TMS-protecting group (Scheme 0.1). The first step proceeded with an excellent yield (93%). Both the Sonogashira coupling and the deprotection of the TMS-group were performed twice, where the first experiment yielded unpure **5** in low yields (40%), and the second experiment gave pure **5** in fair yields (70%).



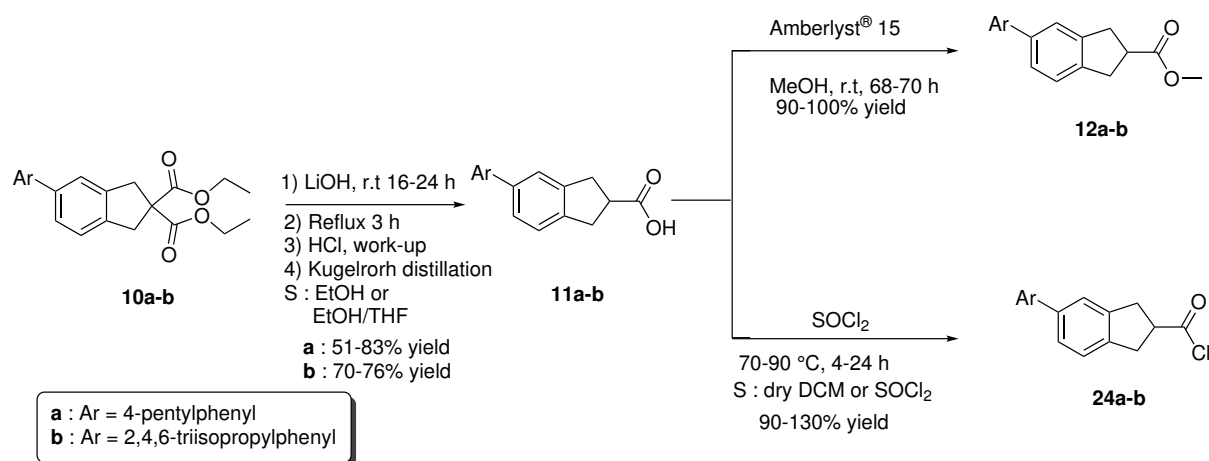
Scheme 0.1: The synthesis of monoalkyne **5**.

The next two steps included the synthesis of **8** from diethyl malonate (**6**) and a [2+2+2] cycloaddition reaction to form the fused benzene linker **10a-b** (Scheme 0.2). The first reaction was performed in a large scale (15 g) with a good yield (69%). The [2+2+2] cycloaddition reaction was performed with the air and moisture sensitive Cp^{*}RuCl(cod) catalyst, and yielded the diesters **10a-b** in fair to good yields (46-69%).



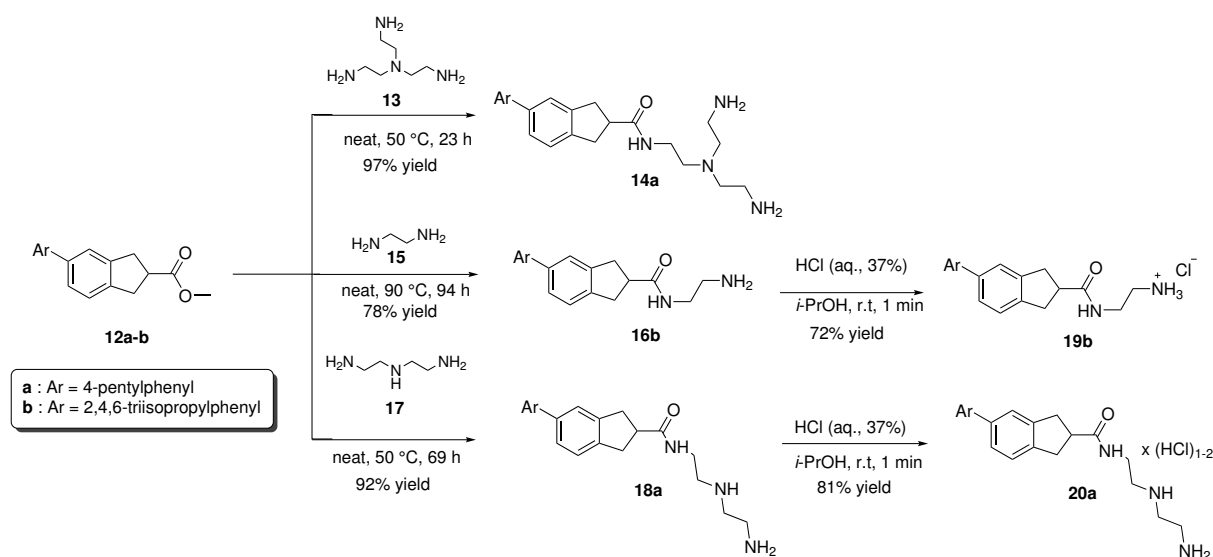
Scheme 0.2: The synthesis of diesters **10a-b**.

Next, **10a-b** were hydrolysed to the acids **11a-b**, and then transformed to methyl esters **12a-b** and acid chlorides **24a-b** in good to excellent yields (Scheme 0.3).



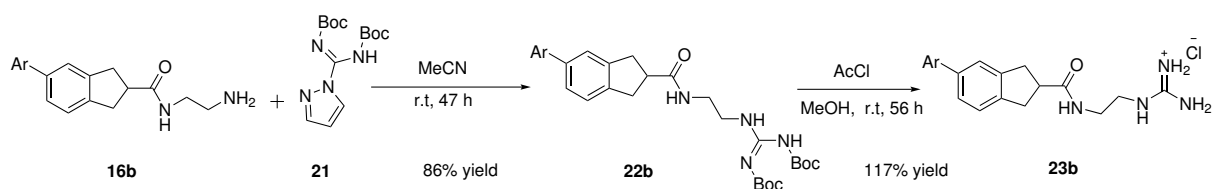
Scheme 0.3: The synthesis of methyl esters **12a-b** and acid chlorides **24a-b**.

The amidoamines **14a**, **16b** and **18a** were synthesised from methyl esters **12a-b** following the same general procedure (Scheme 0.4). Target molecule **19b** and the amine salt **20a** was then prepared using HCl (aq., 37%). This was done in low to good yields and with sufficient purity for antimicrobial testing (HPLC_{19b}: 96%, HPLC_{20a}: >99%).



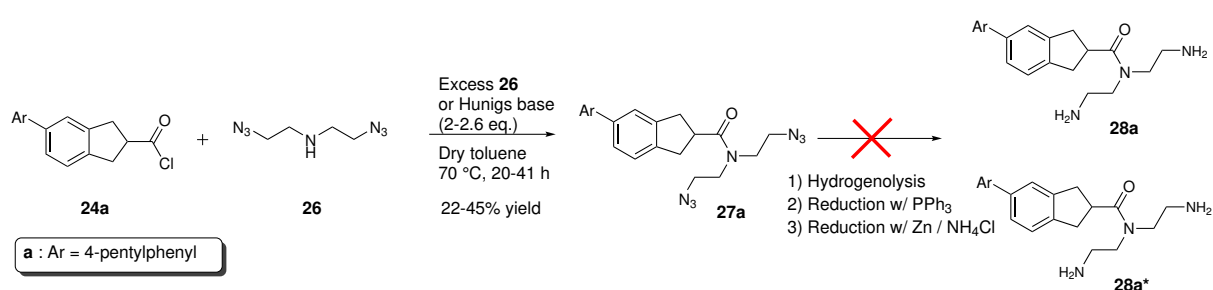
Scheme 0.4: The synthesis of target molecule **19b** and amine salt **20a**.

Guanidine **23b** was synthesised from amidoamine **16b** over two steps with good yield but insufficient purity (HPLC_{23b}: 94.6%)(Scheme 0.5). As there were some difficulties removing MeOH from the product, further work-up would be required to determine precise yields.



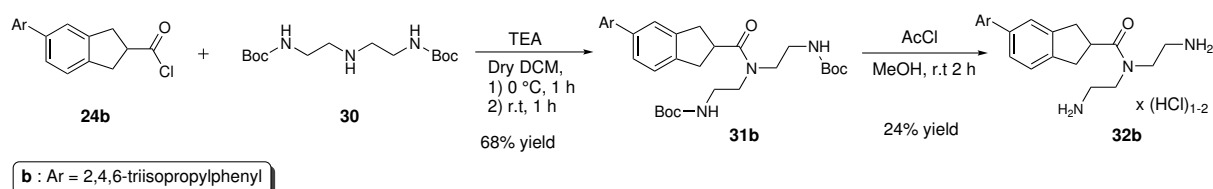
Scheme 0.5: The synthesis of target molecule **23b**.

Branched amidoamine **28a** was attempted synthesised through a bis-azide intermediate (Scheme 0.6). The synthesis of **27a** was performed three times in poor to low yields (22-45%). The reduction of **27a** to **28a** was attempted through several methods, but the results were either complex product mixtures or an amidoamine byproduct, later identified as amine salt **20a**.



Scheme 0.6: Synthesis of **27a** and the failed attempts of synthesising **28a** and its corresponding amine salt **28a***.

Another method of synthesising branched amides like **28a** was also explored (Scheme 0.7). This method yielded **32b** in poor yields (24%) and with insufficient purity (HPLC_{32b}:75%). The main difficulties were the purification of the Boc-protected intermediate **31b**, where the ¹H NMR spectrum indicated the presence of impurities, even after attempting purification by flash column chromatography twice. Due to time limitations, priority was given to determine whether or not the reaction would yield the intended product **32b** instead of attempting to obtain **31b** with a higher degree of purity.

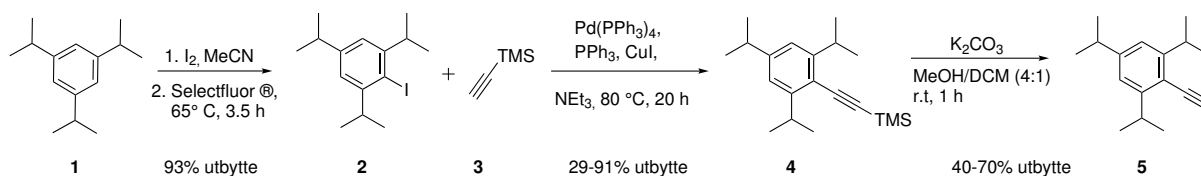


Scheme 0.7: Synthesis of target compound **32b**.

Sammendrag

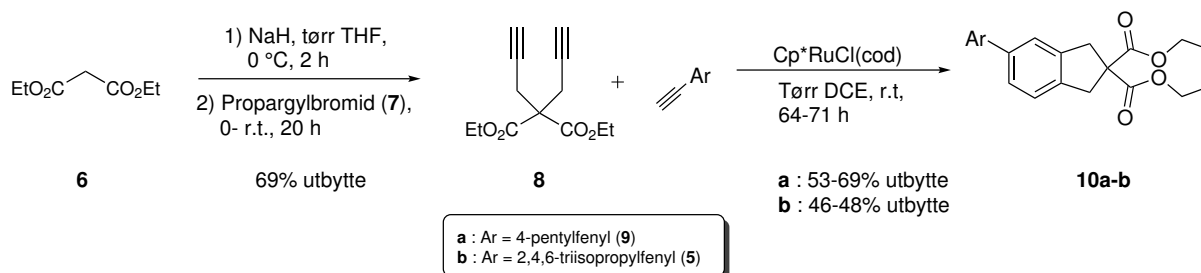
Formålet med denne masteroppgaven har vært å syntetisere inden koblede amfipatiske stoffer med ulike kationiske grupper. De nye stoffene kan sendes til biologisk testing, hvis høy nok renhetsgrad oppnås (>95%).

Målmolekyl **19b** og aminsaltet **20a** (Scheme 0.11), samt målmolekylet **23b** (Scheme 0.12) ble syntetisert med å følge en allerede utprøvd synteserute (Scheme 0.8). Først ble monoalkynen **5** syntetisert fra **2**, og deretter fulgte en Sonogashira kryss-kobling og fjerning av TMS-beskyttelsesgruppen. Syntesen av **3** ble gjennomført med bra utbytte (93%). Syntesen av **4** og **5** ble gjennomført to ganger, hvor det første eksperimentet gav uren **5** med lavt utbytte (40%), og det andre eksperimentet gav ren **5** med bra utbytte (70%).



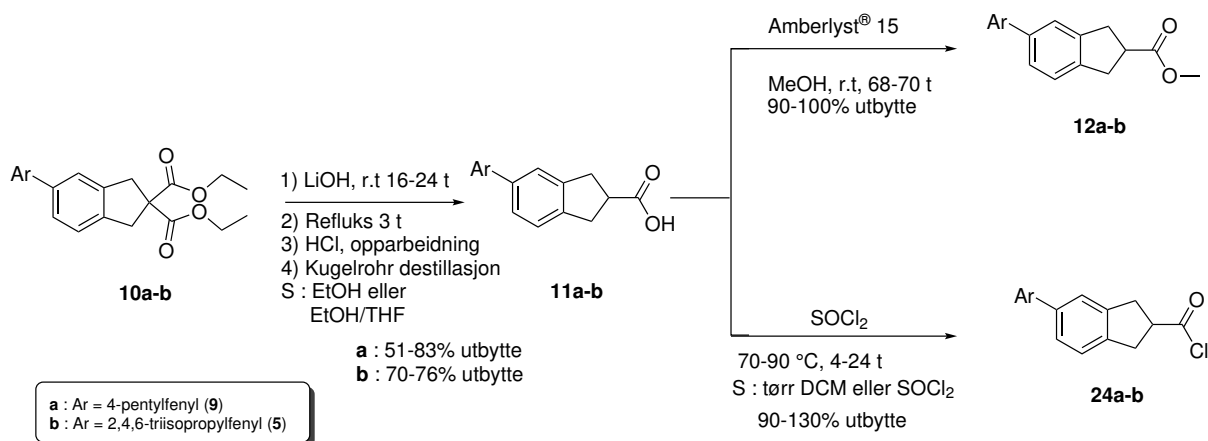
Scheme 0.8: Syntese av monoalkyne **5**.

De neste to stegene i synteseruten var syntesen av dialkynforbindelse **8** fra diethylmalonat, og en [2+2+2] sykloaddisjonsreaksjon for å danne inden koblingsstrukturen (**10a-b**) (Scheme 0.9). Syntesen av **8** ble gjennomført i stor skala (15 g) og med bra utbytte (69%). Diesterne **10a-b** ble syntetisert via en [2+2+2] sykloaddisjon mellom monoalkynene **5** eller **9** og dialkyn **8**. [2+2+2] sykloaddisjonsreaksjonen ble katalysert av Cp^{*}RuCl(cod), som er både luft- og fuktsensitiv. Dette kan ha bidratt til de noe varierende utbyttene.



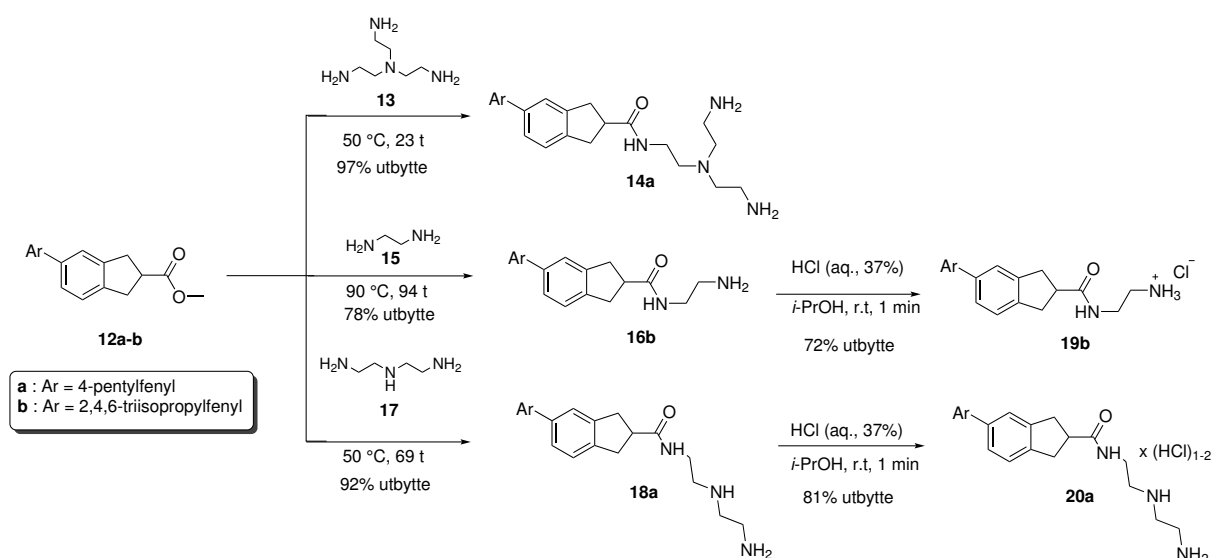
Scheme 0.9: Syntese av diesterne **10a-b**.

Diesterne **10a-b** ble hydrolysert til disyrer og så dekarboxylert til monosyrene **11a-b** (Scheme 0.10). Deretter ble syrene omdannet til metylestere **12a-b** og syreklorider **24a-b** for å øke reaktiviteten.



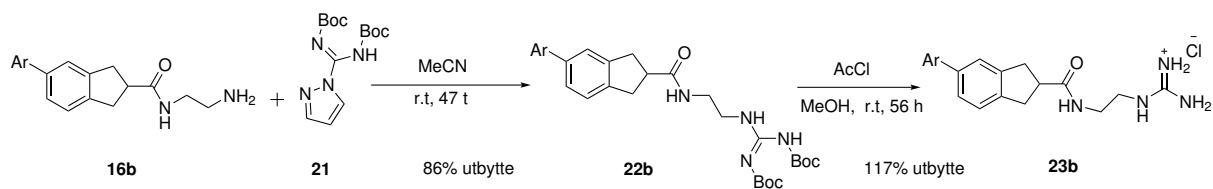
Scheme 0.10: Syntese av metylesterne **12a-b** og syrekloridene **24a-b**.

Amidoaminene **14a**, **16b** og **18a** ble syntetisert fra metylesterne **12a-b** ved bruk av samme generelle prosedyre (Scheme 0.11). Deretter bleaminsaltene **19b** og **20a** syntetisert med gode utbytter (72-81%) og høy renhetsgrad (HPLC_{19b}: 96%, HPLC_{20a}: 100%).



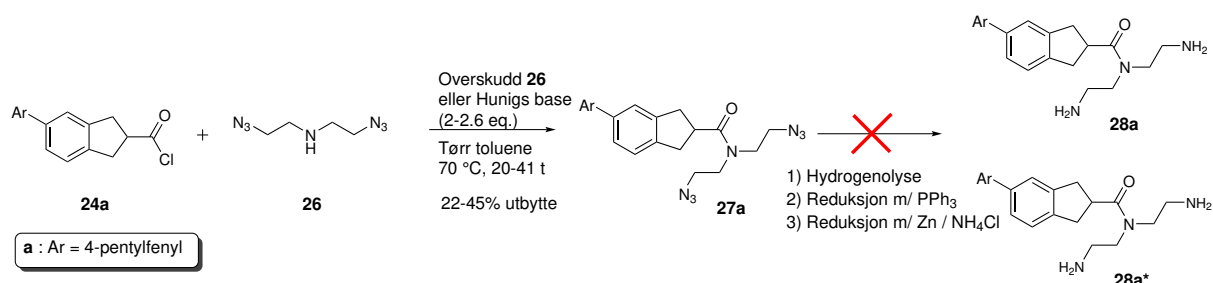
Scheme 0.11: Syntese av amidoaminene **14a**, **16b** og **18a**, samt syntese av HCl-saltene **19a** og **20a**.

Guanidin **23b** ble syntetisert fra amidoamin **16b** over to steg og med gode utbytter (Scheme 0.12), men med en utilstrekkelig grad av renhet (94.6%). Videre arbeid burde fokusere på å optimalisere opparbeidningen.



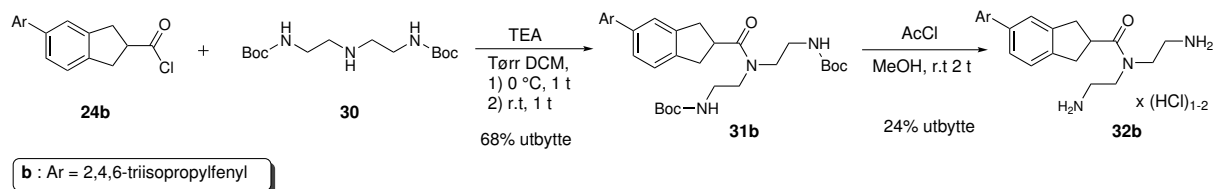
Scheme 0.12: Syntese av målstruktur **23b**.

Forgrenede amidoamin **28a** ble forsøkt syntetisert fra syreklorid **24a** og amidobisazid **26**, med påfølgende reduksjon av nøkkelintermediatet **27** (Scheme 0.13). Syntesen av **27a** ble gjennomført tre ganger med lave utbytter (22–45%). Hydrolyse av **27a** ble forøkt med tre forskjellige metoder, hvor ingen gav det ønskede produktet (**28a/28a***).



Scheme 0.13: Syntese av nøkkelintermediatet **27a** og påfølgende forsøk på danne bisamin produktene **28a** og **28a***.

En annen metode for å syntetisere forgrenede amidoaminer ble også forsøkt (Scheme 0.14). Denne metoden gav bisaminsaltet **32b** i lavt utbytte (24%) og i en utilstrekkelig grad av renhet (HPLC_{32b}: 75%). Hovedutfordringen lå i opparbeidingen av **31b**, hvor ¹H NMR spektra viste tegn til urenheter selv etter to runder med kolonnekromatografi. Grunnet tidsbegrensninger ble det bestemt at det skulle prioriteres å finne ut om denne metoden ville gi det ønskede produktet, istedenfor videre opparbeiding av **31b**.



Scheme 0.14: Syntese av målstruktur **32b**.

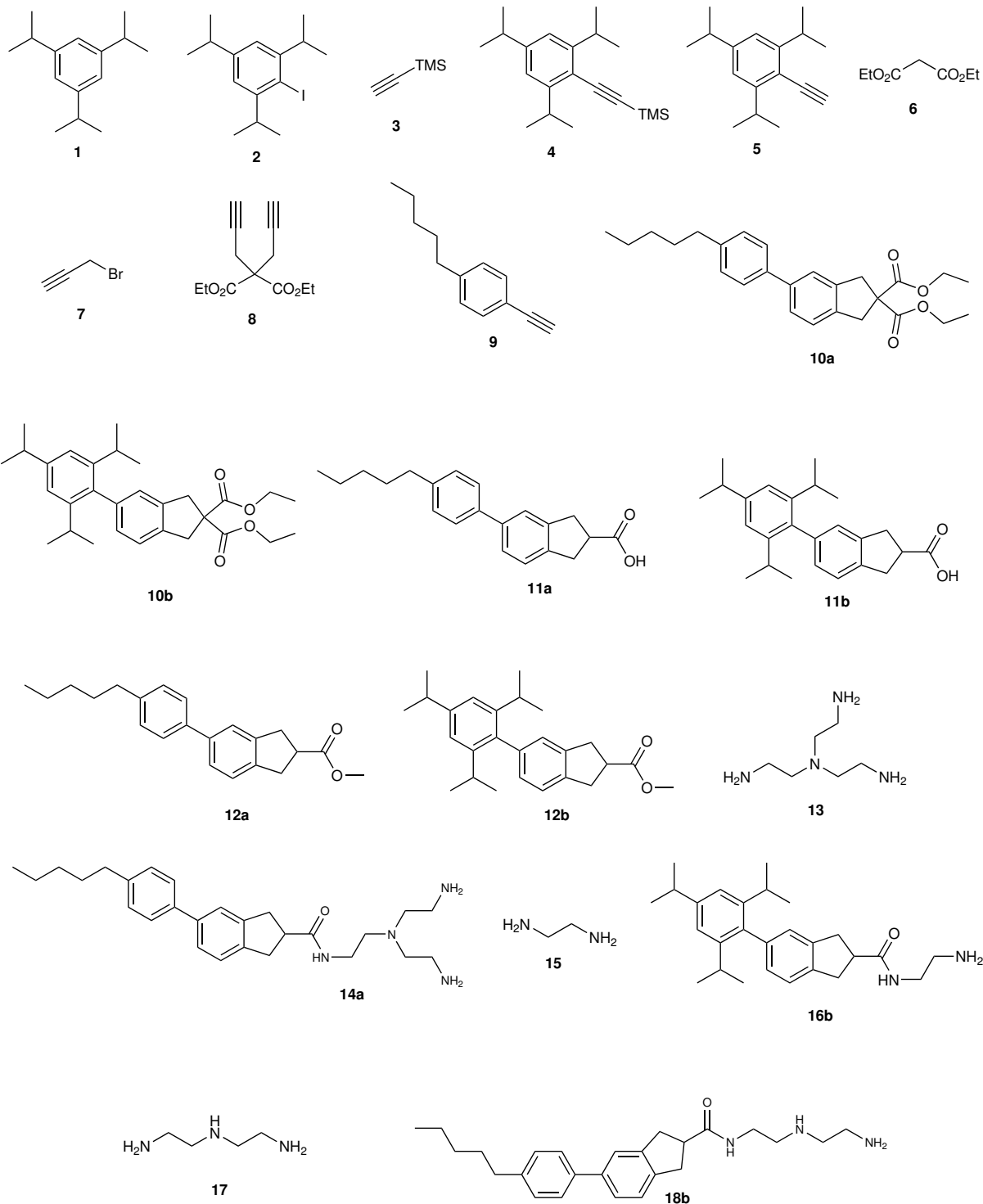
Abbreviations and Symbols

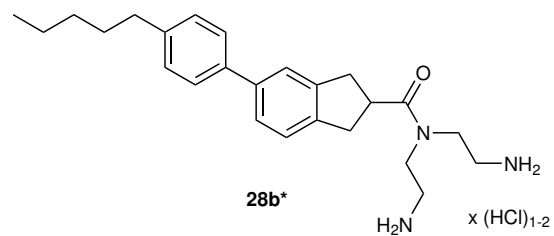
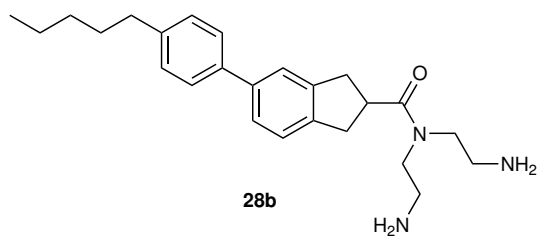
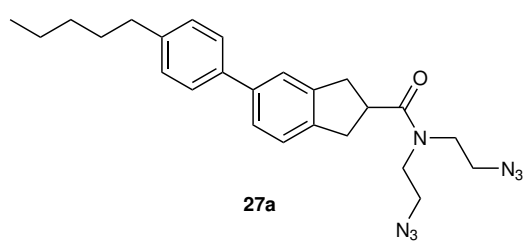
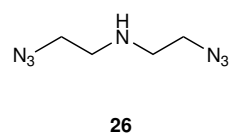
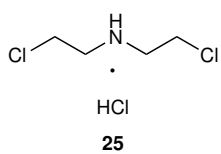
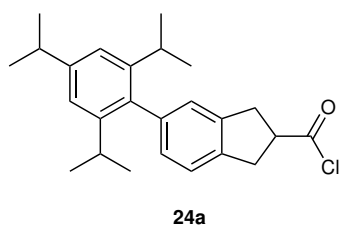
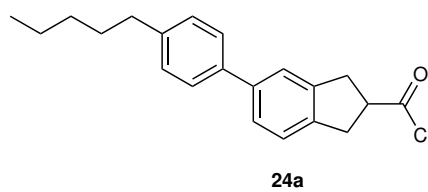
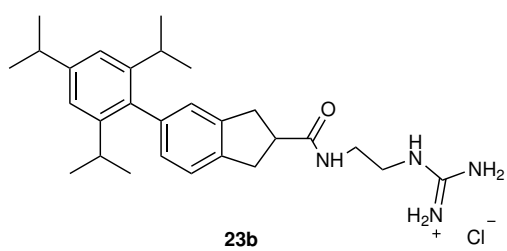
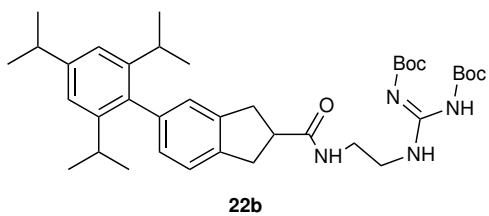
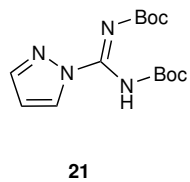
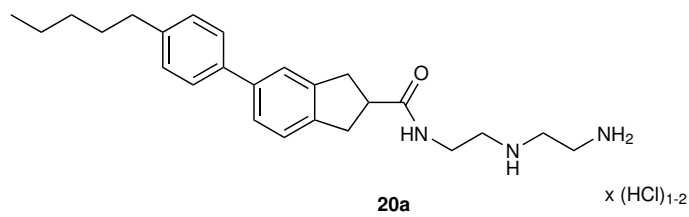
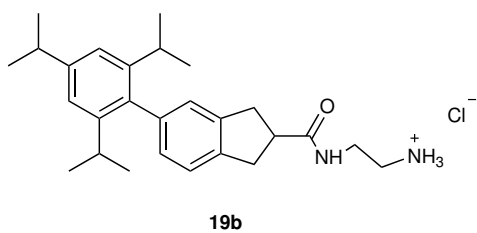
1D NMR	1-Dimensional NMR
2D NMR	2-Dimensional NMR
AMP	Antimicrobial Peptide
approx.	Approximately
aq.	Aqueous
Ar	Aryl
Boc	<i>tert</i> -Butoxycarbonyl (protecting group for nitrogen)
C _q	Quarternary carbon (NMR)
°C	Degrees celsius
br	Broad
cod	1,5-Cyclooctadiene
COSY	Correlation spectroscopy (H,H)
Cp*	1,2,3,4,5-Pentamethylcyclopentadienyl
δ	Chemical shift in NMR-spectroscopy [ppm]
d	Doublet (NMR)
DCE	1,2-Dichloroethane
DCM	Dichloromethane
dd	Doublet of doublets (NMR)
DMF	N,N-dimethyl formamide
DMSO	Dimethyl Sulfoxide
dt	Doublet of triplets (NMR)
EC ₅₀	Half maximal effective concentration
Et	Ethyl
eq.	Equivalents
equiv.	Equivalents
ESI	Electron spray ionization
h	Hours
HGT	Horizontal gene transfer
HMBC	Hetereonuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectroscopy
HSQC	Hetereonuclear Single Quantum Coherence
Hep2G-cells	Human liver cancer cell line
Hz	Frequency unit - defined as one cycle per second
IR	Infrared radiation (spectroscopy)
m	Multiplet (NMR)
M	Molar concentration
mbar	Millibar (pressure unit)
Me	Methyl
MIC	Minimum inhibitory concentration
mmol	Millimol
Mp.	Melting point
N ₂ -atm	Nitrogen atmosphere

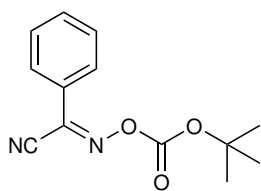
NMR	Nuclear Magnetic Resonance
Nu	Nucleophile
Ph	Phenyl
ppm	Parts Per Million
q	Quartet (NMR)
quint.	Quintet (NMR)
r.t.	Room temperature
R_f	Retention factor (TLC)
S_N2	Nucleophile bimolecular substitution
s	Singlet (NMR)
sat.	Saturated
sept.	Septet (NMR)
s.m.	Starting material
t	Triplet (NMR)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl/Tetramethylsilane
UV	Ultra violet

Numbered compounds

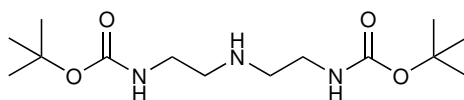
Compounds synthesised or utilised in this master project



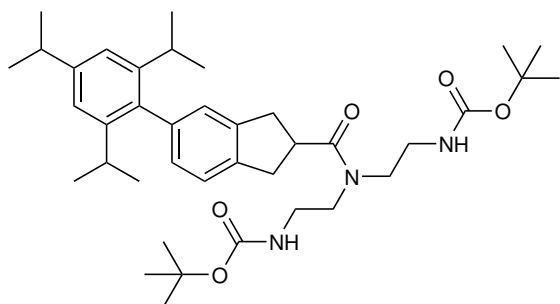




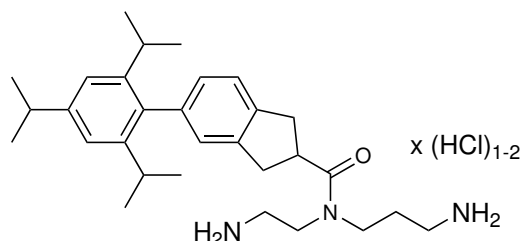
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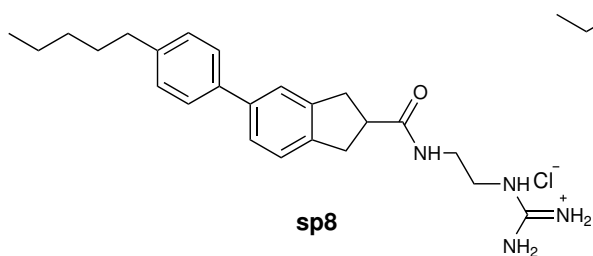


31b

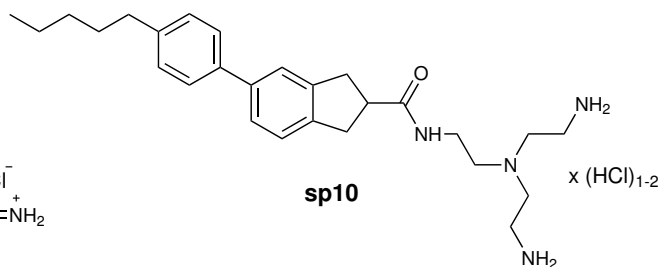


32b

Other compounds mentioned in the Introduction

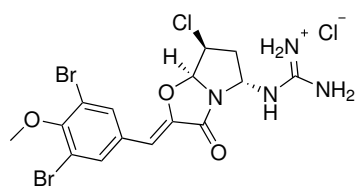


sp8

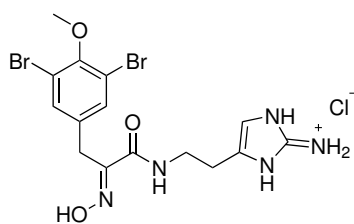


sp10

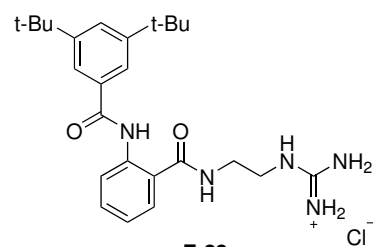
Other compounds mentioned in the Theory



Synoxazolidinone A



lanthelline



E-23

Other compounds mentioned in Further Work

R =

- a** : 4-pentyl-
- b** : 2,4,5-triisopropyl-
- c** : 3,5-di-*t*-Bu
- d** : 2,4,6-tri-Me
- e** : 4-*t*-Bu

R* =

- a*** : Me-
- b*** : Et-
- c*** : Ph-CH₂-
- d*** : C₃H₆-CH₂-

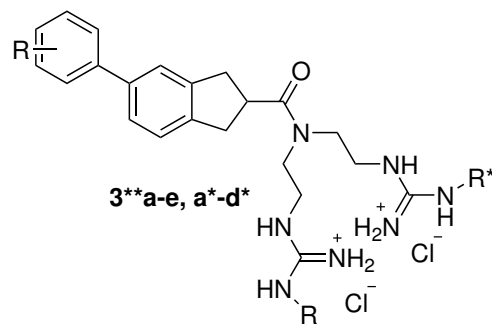
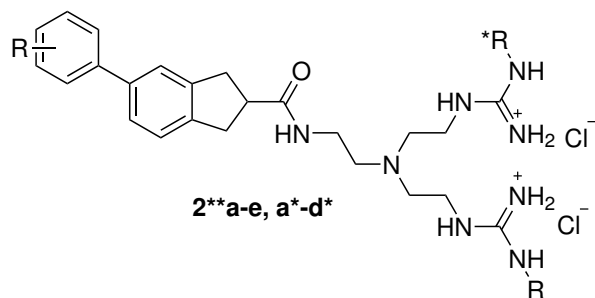
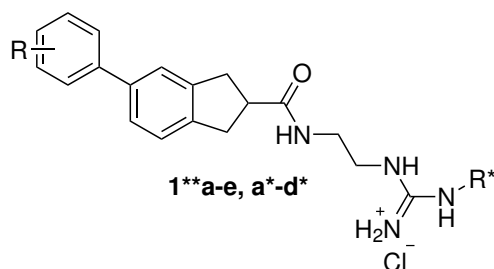
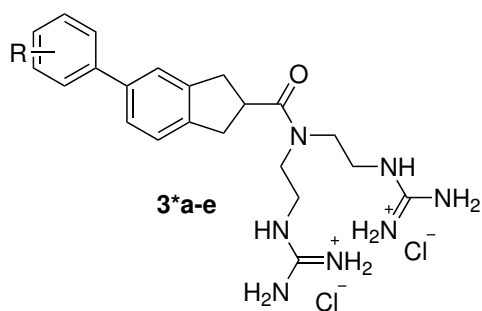
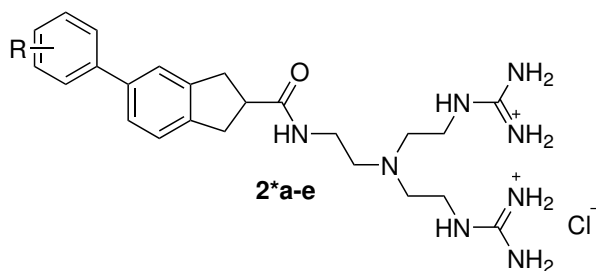
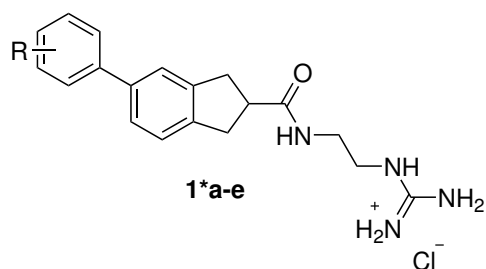


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1 Introduction and Objective

1.1 Motivation and Background

Since the discovery of Penicillin in 1928 by A. Fleming,¹ antibiotics have revolutionised modern medicine, and made complicated procedures such as transplants, neonatal care, cancer treatments and more possible.² Per definition, an antibiotic is a substance or compound which exhibits antimicrobial properties, meaning they either kill the bacteria or inhibit their growth.³ Despite the discovery of several new antibiotics between 1940 and now,⁴ antibiotic resistance has become an ever-increasing problem, and has been deemed one of the greatest threats towards human health.^{5,6} It is estimated that 700 000 people die annually by causes related to antibiotic resistance. If current trends continue, this number will rise to 10 million people per year by 2050, making antimicrobial resistance a more common cause of death than cancer.⁷

Antibiotic resistance is, however, not a new phenomenon, and Fleming spoke about the problem as early as in 1945.⁸ Antibiotic resistance is a natural phenomenon where, through evolution, bacteria becomes resistant towards an antimicrobial substance.⁹ The process is, in its core, nothing more than a means of survival for the bacteria,⁸ as the widespread use of antibiotics exert an evolutionary pressure to adapt or go extinct. The process can occur through random mutations, expression of a previously latent resistance gene or the bacteria may acquire the resistant genes from non-relatives through horizontal gene transfer (HGT). Any combination of the three is also feasible, as none of them are mutually exclusive.^{8,9} Bacteria may also become resistant towards multiple types of antibiotics through the same mechanisms, and thus become multiresistant.⁹ These newly resistant bacteria may spread or go extinct like any other species, but do gain an evolutionary advantage when antimicrobial treatment fails, leaving only the resistant strain behind.¹⁰ This ensures that the new strain becomes the dominant version, making the need for new ways to treat the infections urgent.^{5,9,10}

Although antibiotic resistance is alarming in itself, the real problem lies in the speed at which bacteria are becoming resistant.^{5,10} Due to the constant overuse of antibacterial substances for treatment of both humans and animals,⁵ an environment where the bacteria have a constant exposure to antibiotics is created. Adding in the rapid cell division of bacteria and rate of mutation, bacteria is becoming resistant faster than the researchers are finding new cures.¹¹ While a multitude of different antibiotics have been developed, clinical trials are expensive and heavily regulated.¹² Couple this with the industries' tendencies to invest in safe and already established medicines used in treating chronic conditions as a way of securing income, less funding goes toward the expensive and often fruitless development of new drugs.¹¹ Thus, infections due to resistant and multiresistant bacteria is becoming an increasing problem and these infections usually leads to longer and more expensive hospital stays and a higher death toll.^{5,7}

1.2 Target molecules and Strategy

This master project is a part of the antibiotics research group at the Norwegian University of Science and Technology (NTNU), and is a continuation of previous work done by M. Sc. Daniel Lindberg¹³ and M. Sc. Solveig Valderhaug.¹⁴ The main objective is to synthesis new indane linked cationic amphiphiles, which will later be tested for antimicrobial activity and cytotoxicity at the University of Tromsø (UiT). During this master project four novel compounds were synthesised (**19b**, **20a**, **23b** and **32b**), and of them two (**19b** and **20a**) were of sufficient purity to enable them for biological testing. In a preceding specialisation project (TKJ4520)¹⁵ two additional novel compounds were synthesised (**sp8** and **sp10**) and found to be of an acceptable degree of purity. The results of the biological evaluation will not be determined within the time limits of this master project, and so the main focus will be on the total synthesis of the target compounds.

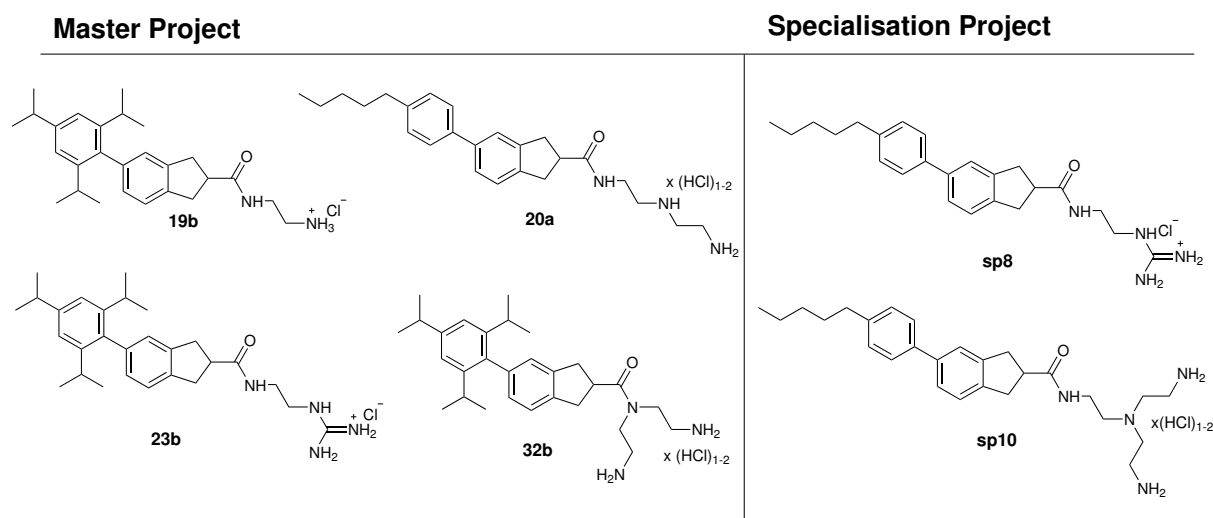
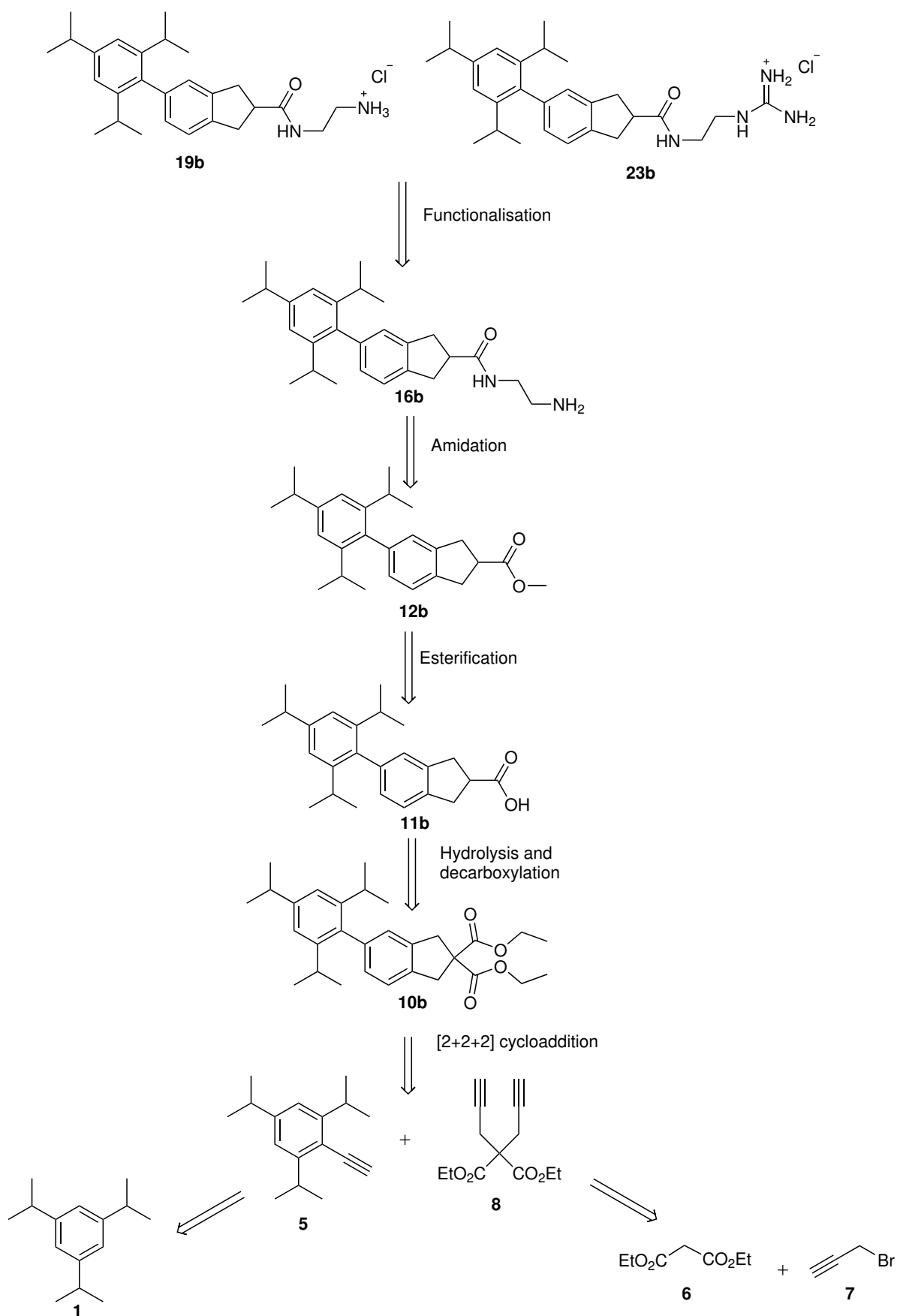


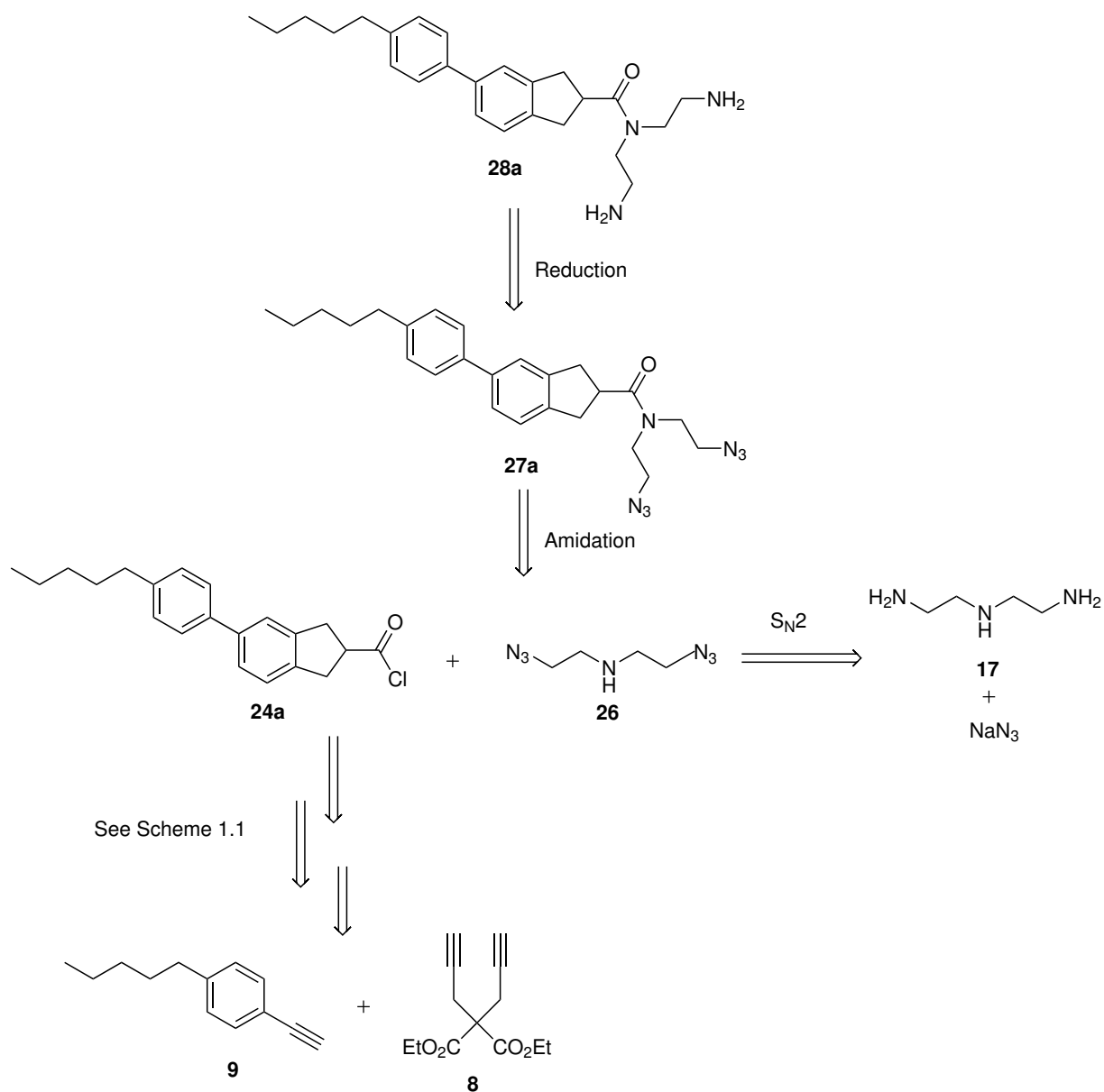
Figure 1.1: The structure of the target molecules **19b**, **23b**, **32b** and amine salt **20a**, as well as previously synthesised guanidine **sp8** and amine salt **sp10**.

As mentioned, this master project is a continuation of previous work done within the research group, and due to this, large parts of the synthetic paths were already established. The general synthesis of **19b**, **23b** and by extension also **20a** is resented in Scheme 1.1.



Scheme 1.1: Proposed synthesis of the target molecules **19b** and **23b**. Amine salt **20a** will be synthesised using a similar strategy, but with **9** instead of **5** as a starting material.

Branched amidoamine **28a** represented a new type of target molecule within the research group, and had as a result no established synthetic route. The start of the synthesis would proceed in the same manner as suggested for the other target molecules. Then, instead of an esterification, transformation into an acid chloride was suggested. From here, the acid chloride would be reacted with a bisazide compound, and then hydrolysed into its amine counterpart. See Scheme 1.2 for the proposed synthetic route.



Scheme 1.2: Proposed synthesis of the target molecule **28a**. From **28a** different functionalizations may be attempted, i.e. preparation of HCl-salts or a bis-guanidine compound.

This route (Scheme 1.2) was subjected to some changes during the project, as several problems were encountered and new strategies had to be explored.

2 Theoretical background

In this section a brief introduction to the biology behind the target compounds and their precursors will be given, together with a short introduction to the chemical reactions and principals utilised in this master project.

2.1 Biological Background

Since the 1940s researchers have been looking for molecules with promising antibacterial properties in nature.^{16–18} Based on the general structure of marine natural products Synoxazolidone A and Ianthelline isolated by researchers at UiT^{19,20} and the aminobenzamides (E-23) prepared by Igumnova *et al.*²¹ and the antimicrobial activity of these compounds, a large library of cationic amphiphilic indenes have been synthesised by the Gautun research group at The Norwegian University for Science and Technology (NTNU). The general structure of these products can be separated into three distinct parts, a **hydrophobic part**, a **linker/ scaffold** and a **hydrophilic/cationic part**. See Figure 2.1 for a comparison between the marine natural products Synoxazolidone A and Ianthelline and compound **23b**.

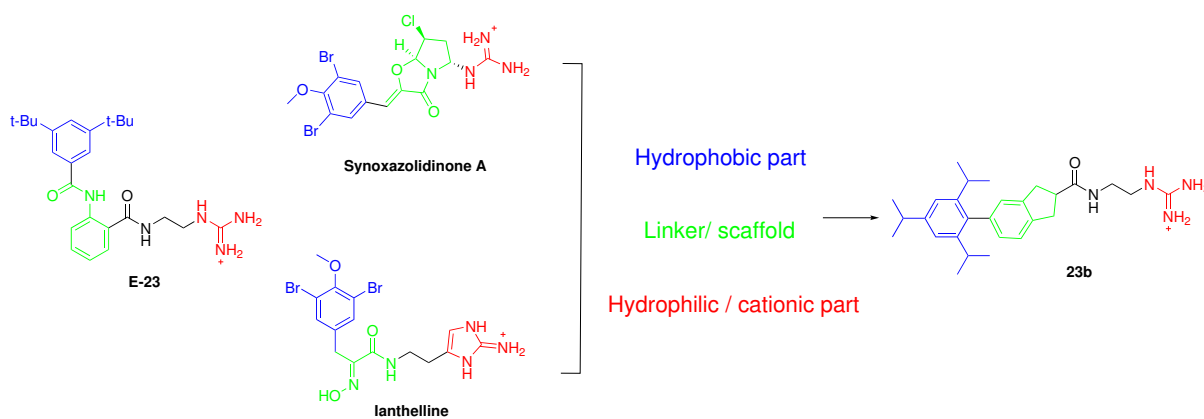
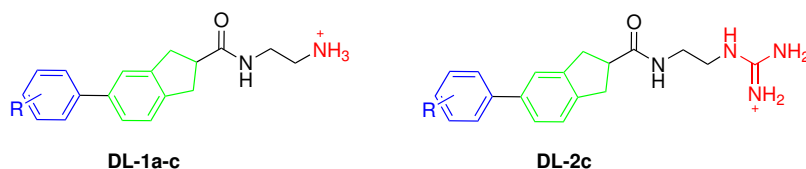


Figure 2.1: Synoxazolidone A, Ianthelline, E-23 and 23b.

As can be seen in Figure 2.1, target compound **23b** contains the same general parts as the marine natural product. These structural principles apply to all the previously synthesised target molecules in this research group. The idea is that by introducing cationic groups and mimicking the structural design of natural compounds already known to exhibit antimicrobial properties, new molecules with antimicrobial properties may be discovered.²² This approach has previously yielded promising results, and the results from some of these evaluations is displayed in Table 2.1.

Table 2.1: Minimal Inhibitory Concentrations (MIC)- and Half maximal effective concentration (EC₅₀)-values in $\mu\text{g}/\text{ml}$ for the tested indene compounds **DL-1a-c** and **DL-2c** made by Daniel Lindberg.¹³ The counter ion is Cl⁻. I = Inactive, N.d. = No data.

a : R = 3,5-di-CF₃
b : R = 4-n-C₅H₁₁
c : R = 4-*t*-Bu



	<i>E. faecalis</i> (MIC)+	<i>S. aureus</i> (MIC)+	<i>Strep B.</i> (MIC)+	<i>E. Coli</i> (MIC)-	<i>P. aerugin</i> (MIC)-	Hep 2G <50% survival
DL-1a	8	8	4	8	16	8
DL-1b	4	4	2	4	I ^{a)}	8
DL-1c	8	16	4	8	16	8
DL-2c	4	4	2	4	8	16
Ref*	10	0.13	4	0.5	0.5	N.d.

*Gentamicin

As can be seen in Table 2.1, the novel compounds were tested against the Gram-positive *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalacticae* (ATCC 12386), and Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). While no definitive conclusions can be drawn from such a small number of results, some interesting observations can be made. The evaluation showed that compound **DL-2c** displayed broad spectrum antimicrobial activities against both Gram-positive and Gram-negative bacteria (MIC: 2-8 $\mu\text{g}/\text{mL}$), making it one of the most potent compounds so far.¹³ Another observation is that **DL-2c** displays a EC₅₀-value that is twice the value of the other compounds (EC₅₀ = 16 $\mu\text{g}/\text{ml}$). **DL-2c** is a guanidinium compound, while the others are primary amine salts. The only other compound with similar values is **DL-1b**, which has a EC₅₀ of 8 $\mu\text{g}/\text{ml}$, in addition to being inactive against the Gram-negative *P. aerugin*. A combination of the two, **sp8** (Figure 1.1), was synthesised during the specialisation project,¹⁵ and is waiting to be tested.

Although the antibacterial properties of **DL-2c** were promising, and the toxicity towards human hepatic cells (Hep2G-cells) was lower than for the rest of the tested compounds, the toxicity was still too high to be ignored. It was evident that some tuning of the activity was in order. Thus, the primary goal of this master project is to retain the high activity towards bacteria, but with a ten-fold lower toxicity towards human cells. The current strategy with this type of fused benzene indene amphiphiles is to introduce multiple cationic groups. This lowers the overall lipophilicity of the molecules, making them less likely to interact with the lipophilic surface of the eucaryotic cells. Introduction of multiple cationic groups will also introduce more areas of the molecule which can electrostatically interact with the bacterial cell wall. These combined effects may

achieve the wanted activity-toxicity profile, and from Table 2.1 the guanidine group seems like a promising cationic group. Thus molecules with one or more guanidine groups are of particular interest.

This type of antimicrobial agents are thought to work through membrane disruption mechanisms, similar to those of native antimicrobial peptides (AMP).²³ AMPs are a part of the primary immune systems of most eucaryotes,^{24,25} and may either be produced continuously or only when injury or infection occurs.²² They are amphipathic molecules of variable length (6-100 amino acids), sequence and structure, which give rise to a wide range of activity towards microorganisms such as bacteria, viruses, fungi etc.^{22,24-26} They carry an overall positive charge, ranging from +2 to +9,²⁶ due to an excess of arginine and lysine residues.²⁷

To ensure the AMPs does not attack their own host, they require a high degree of selectivity in favour of i.e. the bacterial cell membranes.²³ Their amphipathic and positively charged structure allows them to interact with negatively charged phospholipids on the surface of the bacterial membrane.^{22,28} This eventually leads to membrane disruption and cell lysis (cell death). The exact mechanisms for the membrane disruptions are not known, however, there are four common models describing the interactions, see Figure 2.2.^{23,29}

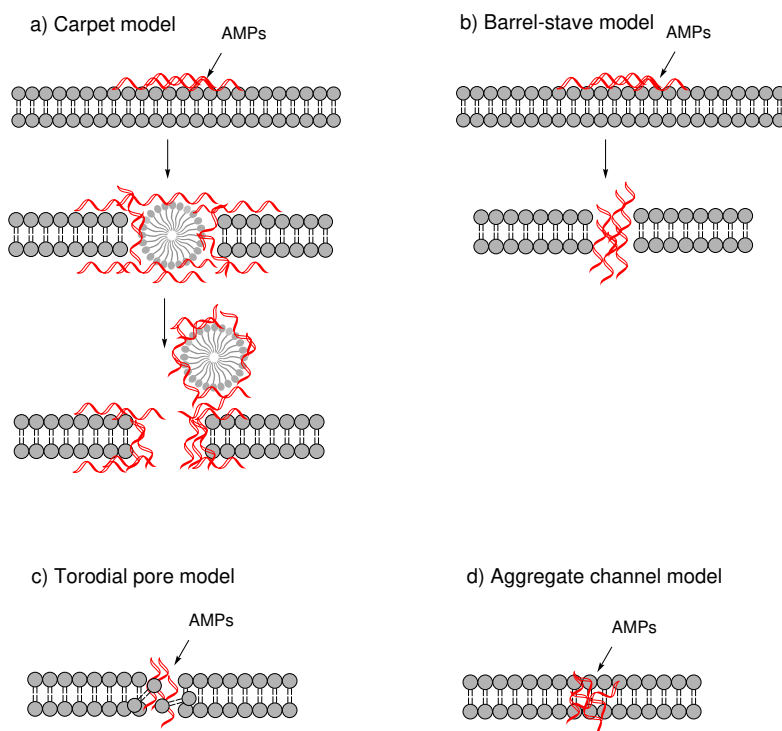


Figure 2.2: The four membrane disruption theories. Top left: a) the carpet model,²⁹ Top right: b) the barrel-stave model,²⁹ Bottom left: c) the torodial pore model,²³ Bottom right: d) the aggregate channel model.²³

The carpet model ((a) in Figure 2.2) describes the interaction as a membrane destruction/solubilization mechanism.²⁹ In the initial steps the peptides bind to the surface of the membrane. When a threshold concentration of peptides is reached, the AMPs

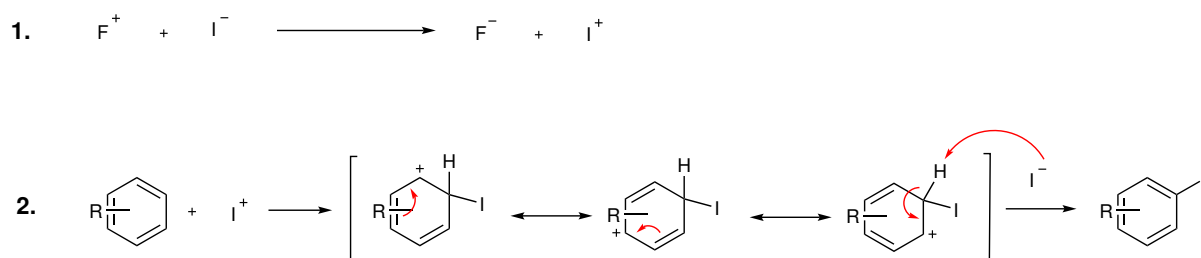
will start to permeate the membrane, and the membrane will eventually be destroyed. In the barrel stave model ((b) in Figure 2.2), the interactions are described as a trans-membrane pore formation mechanism.²⁹ The peptides will bind to the surface of the membrane like in the carpet model, but will then form bundles, and start to penetrate the membrane surface. When this occurs, the hydrophobic parts of the AMPs will interact with the lipid core of the membrane, creating pores. As the concentration of peptides increase, the pores grow in size and numbers. This will in time destroy the integrity of the membrane, causing cell lysis.²⁹ In the torodial model ((c) in Figure 2.2), the mechanism is similar to the barrel-stave model, but it involves both the AMPs and the membrane lipids in the formation of the pores.²³ In the aggregate channel model ((d) in Figure 2.2), it is proposed that the AMPs may also have different targets than just the bacterial membrane itself. This was postulated as a possible explanation to activities not explained by the three previously mentioned models, and indicates that the possibility for intracellular targets in the other models should not be disregarded. In the aggregation channel model the AMPs coordinate to and insert themselves into the membrane, where they cluster together to form aggregates. These clusters can pass through the membrane in its entirety, and enter the intracellular space. Here they can attack specific targets and cause cell lysis through other means than just membrane disruption.²³

2.2 Applied Chemistry

In this chapter, theory regarding the different reactions utilised in this master project will be presented.

2.2.1 Iodination

Halogenated aromatic compounds are important chemical entities in organic chemistry, and participates in a number of different chemical reactions.^{30a} One such reaction type is metal-catalysed cross-coupling, which is one of the most useful tools a chemist have to create new carbon-carbon bonds.³¹ The iodo aryls are more reactive than their bromo or chloro equivalents, and thus are more susceptible for further functionalisation. As such, several methods exists for the synthesis of iodo aryls, such as halogenation by electrophilic aromatic substitution or by way of aryl diazonium salts.³⁰ Another method, utilised in this master project, where the iodo aryl is prepared with molecular I_2 and SelectFluor[®] is also relatively well known.^{32,33} This transformation is a two-step synthesis.³⁴ First iodine is converted to an electrophile through reaction with SelectFluor[®], and then an electrophilic aromatic substitution occurs. See Scheme 2.1 for the reaction mechanism.



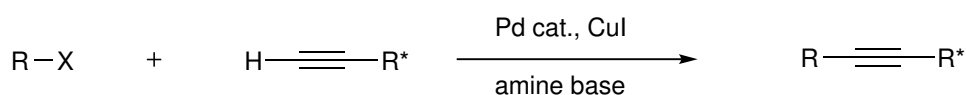
Scheme 2.1: The mechanism for the synthesis of the iodo aryl, including both the conversion from I^- to I^+ with SelectFluor[®], and the electrophilic aromatic substitution reaction.³⁰

2.2.2 Sonogashira cross-coupling

As mentioned, metal-catalysed cross-coupling reactions are one of the most important tools a chemist may utilise when creating new carbon-carbon bonds. One of the most significant advances in this field was the discovery of palladium as catalyst.^{31,35a}

Palladium is by now regarded as one of the most versatile and useful metals in organic chemistry, and is one of the most common catalysts in metal-catalyzed cross-coupling.^{35a} Some well-known cross-coupling reactions utilising Pd as catalyst include Negishi, Stille, Suzuki and Sonogashira cross-coupling reactions.^{35b-d}

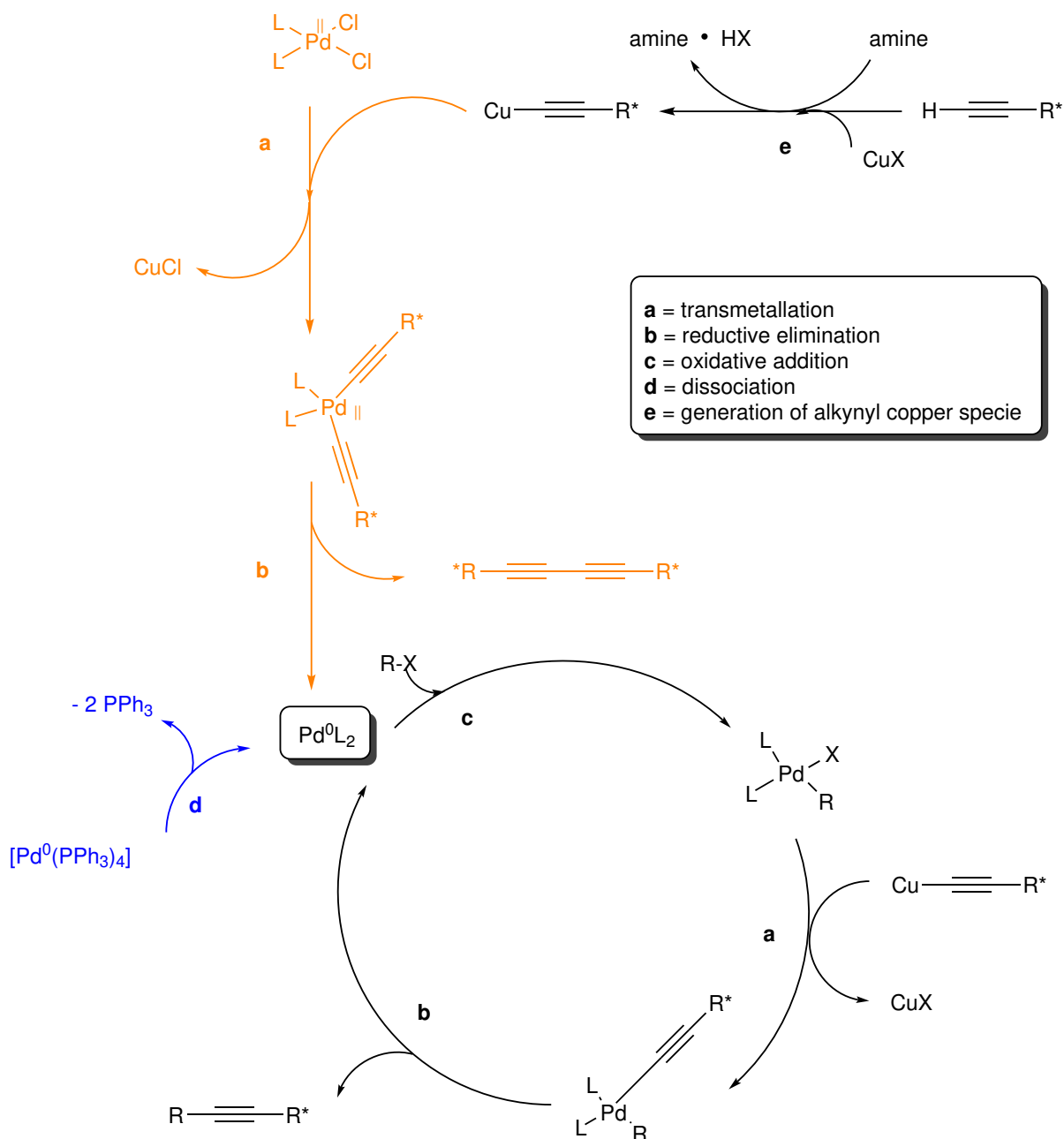
Sonogashira cross-coupling is currently one of the most utilised cross-coupling reactions. This is due to its mild conditions, high tolerance for functional groups and the simple starting materials.³¹ The general reaction equation is presented in Scheme 2.2.



<p>R = aryl, vinyl X = I, Br, OTf R* = aryl, alkenyl, alkyl, SiR₃</p>
--

Scheme 2.2: General reaction conditions for a Sonogashira reaction.³¹

The reaction involves an aryl/vinyl halide and a terminal acetylene, together with a Pd-catalyst, an amine base, and CuX as a co-catalyst. The reaction proceeds in three general steps (transmetallation, reductive elimination and oxidative addition), but the exact mechanism is not known, as some studies suggests a more complex mechanism than the one originally proposed by Sonogashira *et al.*^{31,35g} The most common catalyst are [Pd(PPh₃)₂Cl₂] and [Pd(PPh₃)₄].³¹ A mechanism similar to the one proposed by Sonogashira *et al.*³⁶ is presented in Scheme 2.3.³¹



Scheme 2.3: General reaction mechanism for the Sonogashira reaction.³¹

The mechanism starts with the activation of the catalyst, and formation of the active catalytic species. The exact nature of this species is as mentioned still under debate, but the classic model uses the coordinatively unsaturated 14-electron $[\text{Pd}^0(\text{PPh}_3)_2]$ complex. This complex is thought to form by **dissociation of two (PPh_3) from $[\text{Pd}(\text{PPh}_3)_4]$** or by **a three-step process** if $[\text{Pd}(\text{PPh}_3)_2\text{Cl}_2]$ is used as the catalyst. The steps include a reaction between the amine base, CuX and the terminal alkyne (**e**), followed by a transmetalation step (**a**), and lastly reductive elimination with the generation of the active catalytic species and the bis-alkyne by-product (**b**). Both pathways are illustrated in Scheme 2.3. After generation of the catalytic active species, oxidative addition with the aryl/alkyl halide occurs (**c**). The next step is transmetalation with the copper alkynyl (**a**), followed by a reductive elimination (**b**) which yields the cross-coupled product

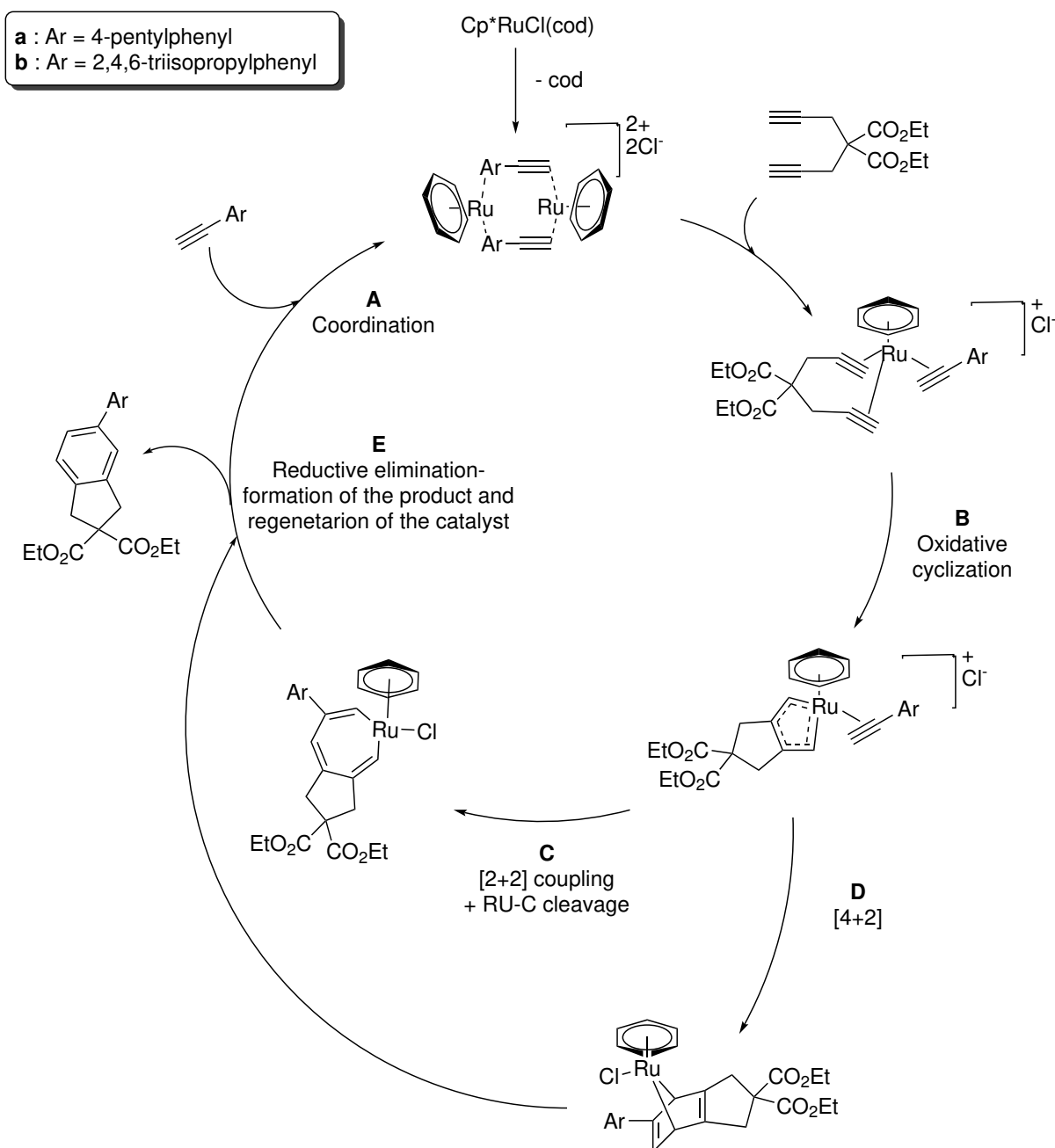
and also regenerates the active catalyst. In this master project, $[\text{Pd}(\text{PPh}_3)_4]$ was used as the catalyst.

2.2.3 [2+2+2] cycloaddition

Cycloaddition reactions are deemed to be one of the most important classes of reactions when it comes to the simultaneous formation of several bonds in one reaction step.³⁷ Transition metal catalyzed [2+2+2] cycloaddition is an elegant and efficient way to synthesise complex carbo- and heterocycles in one step under mild conditions.³⁷ The resulting formation of multiple new C-C bonds in one step enables the synthesis of more complex systems from relatively simple substrates, and thus this method is widely used to synthesise substituted aromatic ring-systems selectively and efficiently.³⁷⁻³⁹

One major advantage is the mild reaction conditions, which makes it possible to introduce sensitive substituents without having to use protective groups. This shortens the synthetic paths, as the reactions required to add on and remove the Protection groups are no longer necessary.

Cyclotrimerisation is catalysed by a large variety of organometallic complexes, where transition metals play an important role.⁴⁰ $\text{Cp}^*\text{RuCl}(\text{cod})$ is a commercially available catalyst used for [2+2+2] cycloaddition reactions. As a transition metal, Ru may have several possible oxidation states, and possesses the ability to act as a transition metal catalyst. $\text{Cp}^*\text{RuCl}(\text{cod})$ as been shown to be an efficient and seletcive catalyst in cyclotrimerisation involving monoalkynes and dialkynes to form fused ring-systems.^{38,39,41} A proposed reaction mechanism by Yamamoto *et al.*⁴¹ for the [2+2+2] cycloaddition is illustrated in Scheme 2.4.

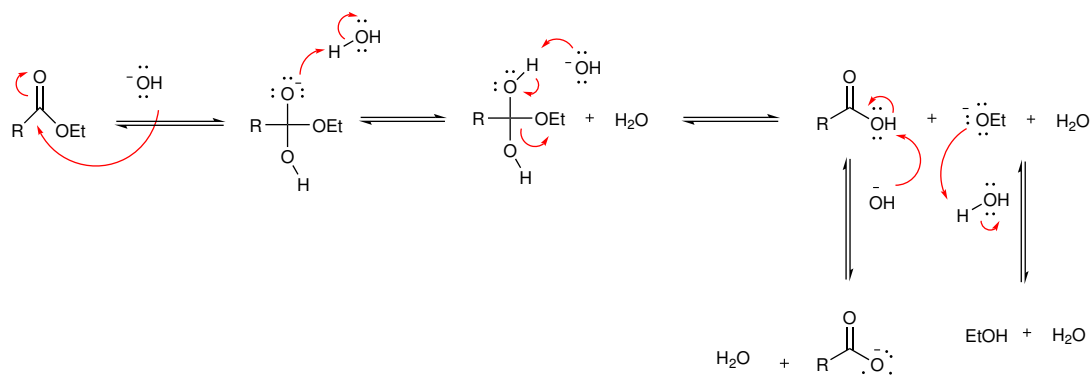


Scheme 2.4: The proposed mechanism for the ruthenium catalyzed [2+2+2+] cycloaddition.⁴¹

The first step (**A**) involves coordinating the monoalkyne to the Ru-complex. The second step (**B**) is oxidative cyclisation with the dialkyne and formation of the cyclic product. The next step is an insertion step, which can either be end-on coordinated (**C**) or side-coordinated (**D**).⁴² The last step (**E**) is the reductive elimination step, where the product is formed and released, and the catalyst is regenerated.

2.2.4 Hydrolysis and decarboxylation

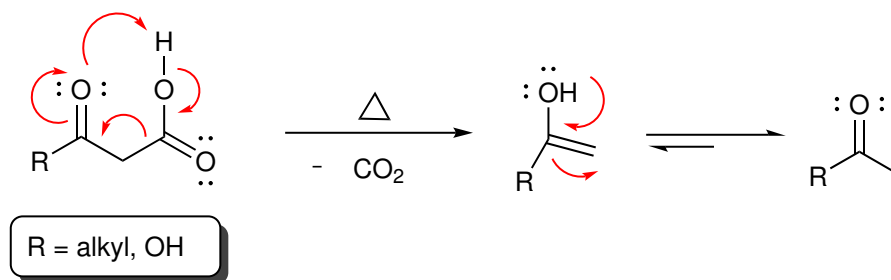
Hydrolysis of esters is one of the most studied and utilised reactions in organic chemistry.^{43a} The reaction can be both acid and base catalysed.^{44a} During this master project, base catalysed hydrolysis was utilised, and the general mechanism is illustrated in Scheme 2.5.^{30b}



Scheme 2.5: Example of base-catalysed hydrolysis of an ester.^{30b}

The reaction involves a nucleophilic attack by the hydroxy-ion, followed by elimination of the alkoxide-group with regeneration of the carbonyl.

The relevant compound in this synthesis is a diester, and after transformation to a diacid, it is desirable to remove one of the acid groups by decarboxylation. The reaction mechanism is illustrated in Scheme 2.6.^{45b}

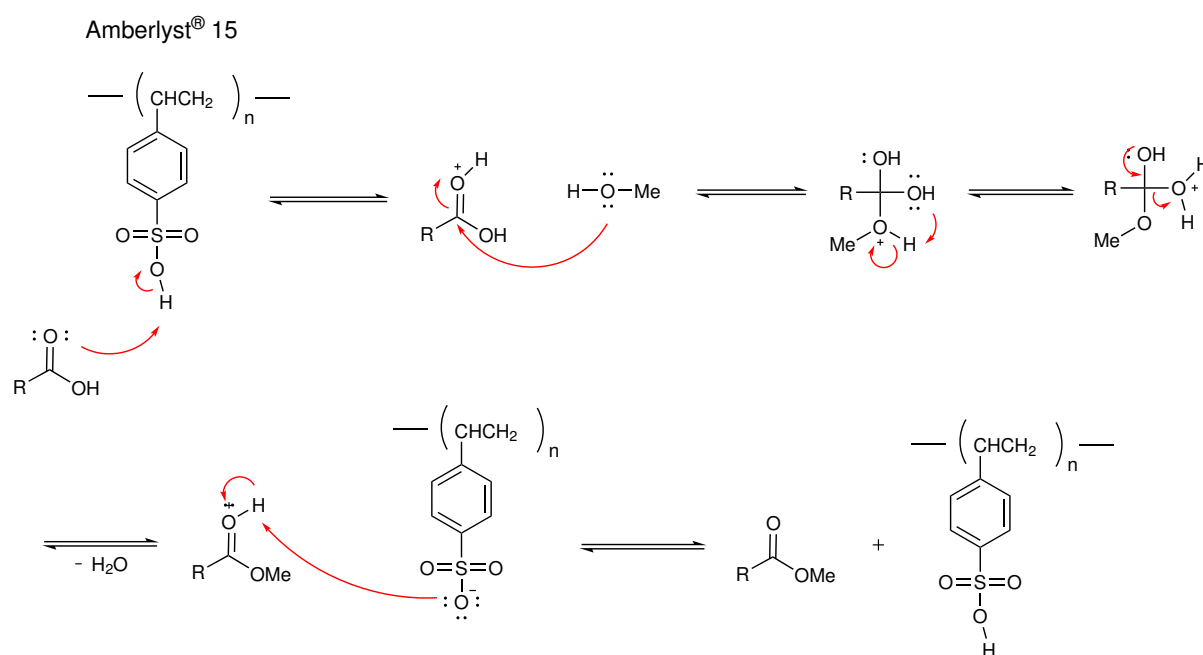


Scheme 2.6: Example of decarboxylation of an acid.^{45b}

This reaction requires heating, and the result in this particular case is a mono-acid.

2.2.5 Esterification

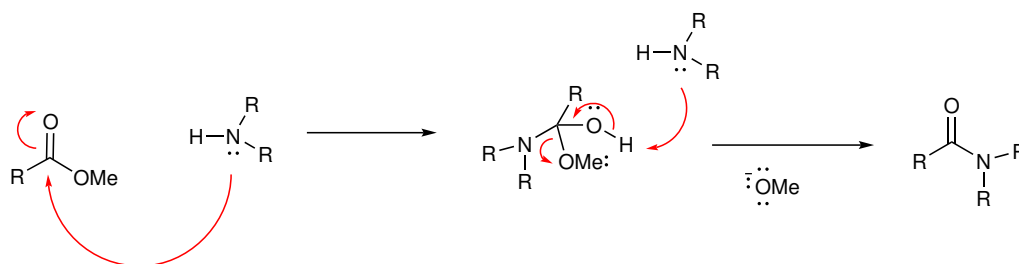
Transformation of an acid to an ester with the use of Amberlyst[®] 15 ion exchanger catalyst is a frequently used method.⁴⁶ The Amberlyst[®] 15 contains highly acidic sulfuric protons, which acts as Brønsted acid, and donates a proton.⁴⁶ The mechanism is thus similar to that of a Fischer esterification.^{13, 45c, 46} Mixed together with methanol this catalyst gives methyl esters in high yields under mild conditions.⁴⁷ The reaction mechanism is illustrated in Scheme 2.7.^{45d}



Scheme 2.7: The mechanism of esterification by the use of Amberlyst[®] 15 ion exchanger catalyst and methanol.^{45d}

2.2.6 Amidation

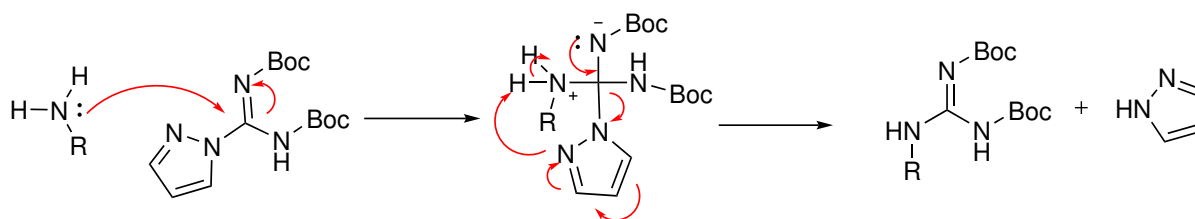
Esters will react with amines to form amides.^{44b} These reactions can be performed with or without solvents. Amines are excellent nucleophiles, which makes them ideal reagents in substitution reactions. They will react with acyl chlorides, acid anhydrides, esters, carboxylic acids and carboxylate salts to form amides.^{44b} The mechanism for a general amidation is illustrated in Scheme 2.8.^{45e}



Scheme 2.8: The general mechanism of an amidation reaction.^{45e}

2.2.7 Guanylation

Guanidines are an important group of compounds which possesses great biochemical and pharmaceutical potential.⁴⁸ Guanidines can be prepared by reacting a primary amine with a guanylation reagent. There exists several known classes of guanylating agents, such as thioureas/isothioureas, carbodiimides, cyanamides, pyrazole-1-carboximidamides etc.⁴⁸ Because of their great importance in pharmaceutical chemistry, a lot of research have been done in the last 30 years to discover new guanylating agents and improve upon already existing procedures.⁴⁸⁻⁵⁰ A proposed mechanism of the guanylation of a primary amine is illustrated in Scheme 2.9.⁵¹



Scheme 2.9: A proposed mechanism for the guanylation reaction.⁵¹

The amine is acting as a nucleophile and attacks the electrophilic carbon of the guanylating agent. This results in the formation of a tetrahedral intermediate, which through elimination of pyrazole becomes the final guanylated product.

2.2.8 Protection Groups

Protection groups often play a key role in multistep synthesis.⁵² A protection group is often used when a compound contains a functional group which is not stable under the reaction conditions necessary in the next reaction step.^{44c} The functional groups may either decompose or react (either directly with other reactants or indirectly by activating parts of the molecule and thus encourage unwanted reactions), which will lead to undesired products, low yields etc. Good examples are amino and hydroxy groups attached to a benzene ring. These are strongly activating groups, and as such causes the benzene ring to be more reactive. This may lead to a higher degree of substitution, and also activate the ring towards oxidation.^{44d}

There exists a myriad of different protection groups, and there are in general three important aspects of choosing the right one:⁵²

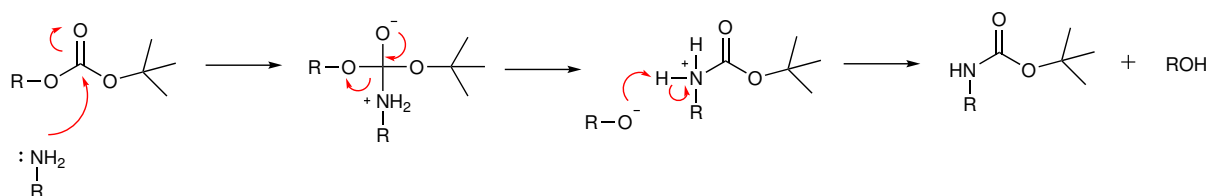
1. Which functional group needs Protection.
2. The reaction conditions under which the protective group needs to be stable.
3. The conditions which can be tolerated for the removal of the Protection group.

Some common protection groups and their targets are displayed in Table 2.2.

Table 2.2: A short list over some common protecting groups, their structure, name, abbreviation and which functional group they are used to protect.⁵²

Structure	Name	Abbreviation	Target	Removal
	Acetate	Ac	Hydroxy-group	Acetal hydrolysis
	Trimethylsilyl	TMS	Hydroxy-group	Hydrolysis
	Carbobenzyloxy	Cbz	Amines	Hydrogenolysis
	<i>t</i> -Butoxycarbonyl	Boc	Amines	Acid
	dioxolane formation	-	Carbonyl	Alkaline hydrolysis
	<i>t</i> -butyl esters	<i>t</i> -Bu	Carboxylic Acids	Acetal hydrolysis

In this master project the Boc group was used to protect primary amines and guanylation agents. A suggested mechanism for the Protection of a primary amine with a general Boc-reagent is illustrated in Scheme 2.10.^{43b}

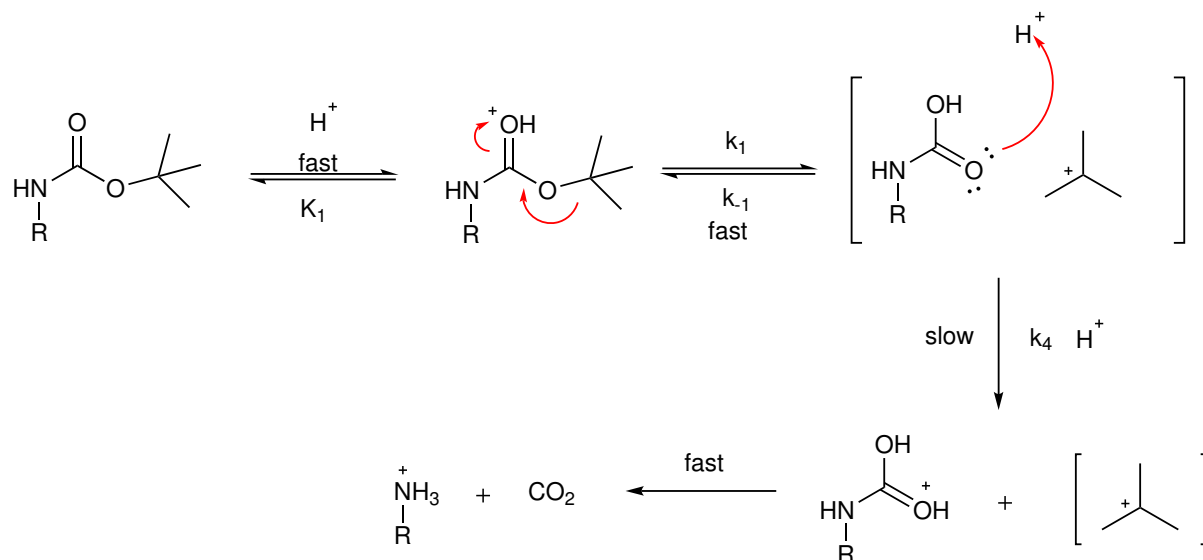


Scheme 2.10: The general mechanism for protecting a primary amine with a Boc-Protection group.^{43b}

The reaction follows the general principles of aminolysis of esters. It includes a nucleophilic attack from the amine on the carbonyl-carbon, followed by expulsion of the leaving group from the tetrahedral intermediate.^{43b}

The cleavage of the Boc-group during the deprotection is usually acid catalysed.^{43b,53} The long accepted mechanism for the cleavage of the Boc-group⁵³ includes a fast pre-equilibrium protonation of the Boc-group, followed by the fragmentation of the protonated intermediate to yield a carbamic acid. This fragmentation is the rate limiting step. The next step is a decarboxylation yielding CO₂ and the protonated amine.^{53,54} In 2010, Ashworth *et al.* proposed two new mechanisms based on kinetic studies.⁵⁴ Differentiation between the "old" and the two new mechanisms were not possible at

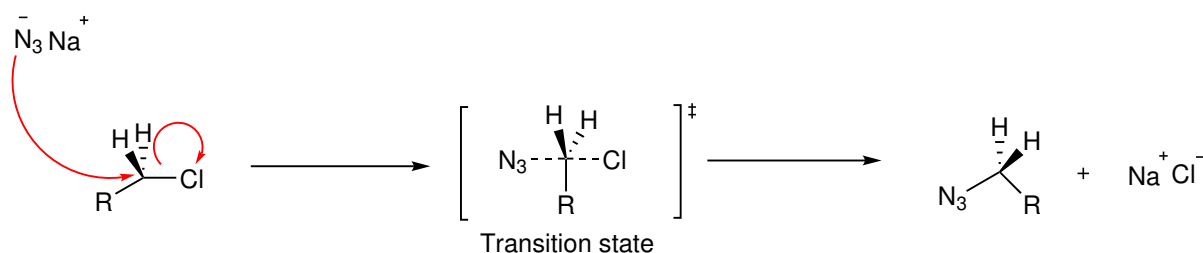
the time, since all three were based on the experimentally described rate law. However, one of the mechanisms was deemed more likely than the two others, due to the fact that it does not require the formation of intermediate species that have yet to be experimentally observed. This mechanism is illustrated in Scheme 2.11.



Scheme 2.11: A new mechanism for the cleavage of Boc-protection group suggested by Ashworth *et al.*⁵⁴

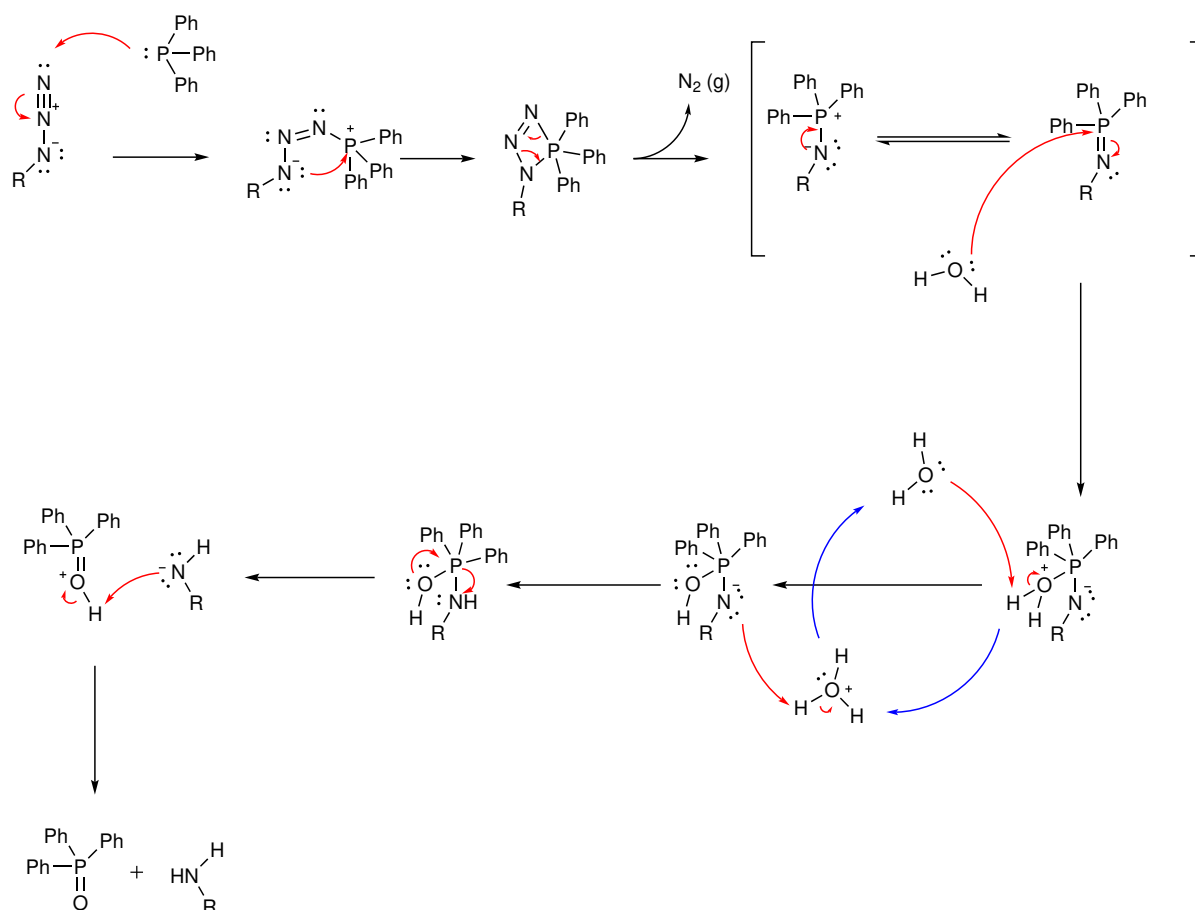
2.2.9 Azides

Since their discovery in 1864, organic azides have become widely used intermediates in organic chemistry.⁵⁵ They are useful intermediates as they can be reduced to amines or undergo cycloaddition reactions.⁵⁶ Alkylazides are often introduced by nucleophilic substitution reactions, using NaN_3 and a halide compound.^{52b} These reactions follow a standard $\text{S}_{\text{N}}2$ -reaction mechanism, as illustrated in Scheme 2.12.^{44e}



Scheme 2.12: An illustration of the formation of an azide compound by nucleophilic substitution reaction.^{44e}

Reduction of azides can be done in a myriad of ways, but one reaction utilised in this master thesis is the Staudinger reduction.⁵⁷ This reduction involves PPh_3 and water, and its mechanism is illustrated in Scheme 2.13.



Scheme 2.13: An illustration of the reduction of an azide using the Staudinger reaction.^{57,58}

The mechanism starts with phosphorus attacking the end-nitrogen and thus forming a phosphoazide intermediate. This intermediate then undergoes a rearrangement to a N-P ylide and simultaneously releases a molecule of N₂-gas. A water molecule will then attack the P-atom, and after a couple of proton transfer steps, the final product is a primary amine and triphenylphosphine oxide.

Azides are high-energy molecules, and many are potentially explosive.⁵⁵ Bräse *et al.*⁵⁵ states that for organic azides to be manipulative and non-explosive, the number of nitrogen atoms must not exceed that of carbon, and that

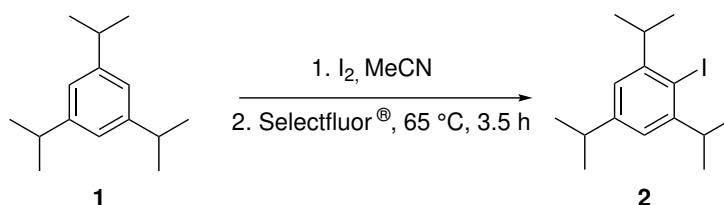
$$\frac{N_C + N_O}{N_N} \geq 3 \quad (2.1)$$

where N equals the number of atoms. While low-weight molecules with a composition which ignores one or both of these rules have been synthesised, and in practice have been deemed non-reactive, special care should always be taken when working with potentially explosive compounds.⁵⁵

3 Results and Discussion

This section will describe and discuss the results of the reactions performed in this master project. Topics covered in this section include preparation of the aryl iodide **2** and dialkyne **8**, Sonogashira coupling, [2+2+2] cycloaddition, hydrolysis and decarboxylation, esterification with Amberlyst[®] 15, amidation, preparation of azides, Boc-protected amines as well as *N*-functionalization of different amidoamines. Most of the synthetic paths have already been tested during the specialisation project,¹⁵ or previously by D. Lindberg¹³ and S. Valderhaug.¹⁴ Exceptions include the preparation of compounds **27a**, **31b** and **32b**.

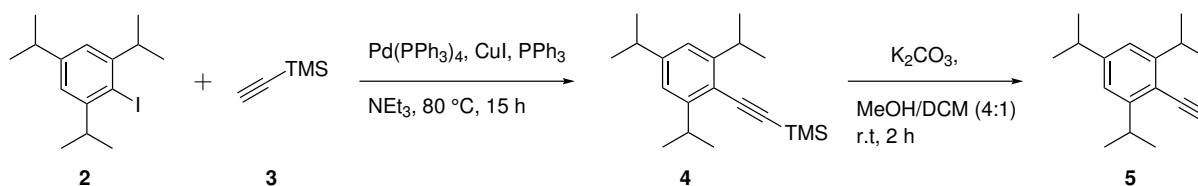
3.1 Preparation of aryl iodide **2**



Scheme 3.1: Reaction equation and conditions for the preparation of **2**.

The aryl iodide **2** was successfully prepared once in a large (15 g) scale with an excellent yield of 93%. Previously reported yields are 92%³⁴ and 102%.¹⁴ Detailed experimental procedure can be found in Chapter 6.2.1, while the reaction mechanism is described in Chapter 2.2.1.

3.2 Preparation of monoalkyne **5**



Scheme 3.2: Reaction equation and conditions for the preparation of **5**.

The synthesis of **5** is a two-step synthesis. Initially, TMS-intermediate **4** was successfully synthesised through a Sonogashira coupling, using iodo aryl **2** as a substrate and **3** as a reagent. The next step was the cleavage of the TMS-group from intermediate **4** to yield the desired product **5**. A detailed experimental description for the synthesis of **4** and **5** can be found in Chapter 6.2.2 and 6.2.3 respectively. Reaction mechanism for the Sonogashira reaction is described in Chapter 2.2.2. The first step was performed twice with varied results. See Table 3.1 for a summary of the reaction details and subsequent

yields.

Table 3.1: Experimental data for the preparation of compound 4

Entry	2 (g)	Pd(PPh ₃) ₄ (ml)	CuI (g)	PPh ₃ (g)	3 (ml)	4 (%)
1	4.07	0.73	0.09	0.12	2.5	29*
2	4.00	0.70	0.08	0.12	2.52	91

*Yield calculated from ¹H NMR spectrum (Appendix B.2).

As can be seen from Table 3.1, the first entry had a very poor yield of just 30%. Previous reported yields for this reaction were in the 97-99% region.¹⁴ One explanation for this poor yield might be the use of a mixture of old and new [Pd(PPh₃)₄]. The old catalyst accounted for roughly 50% of the total catalyst used in this entry. The catalyst did not have the characteristic bright yellow colour normally associated with [Pd(PPh₃)₄],³¹ but rather a dark yellow-orange colour. This might indicate that the catalyst have been exposed to temperatures higher than recommended (0 °C), too much air exposure, or both. This might have lead to a decreased reactivity.³¹ The total mass of assumed product in this reaction was 1.66 g, which was roughly 45 w% yield. However, the ¹H NMR spectrum (Appendix B.1) showed that this was not pure product, but rather a mixture of the product and something else. Calculations from the ¹H NMR spectrum indicated that the product stands for approximately two-thirds of the sample, thus bringing the actual yield down to 29%. The other compound was suspected to be unreacted 2, and comparing the two ¹H NMR spectra further strengthened the suspicions. See Figure 3.1 for an excerpt of a particularly interesting part of the ¹H NMR spectra for both compounds.

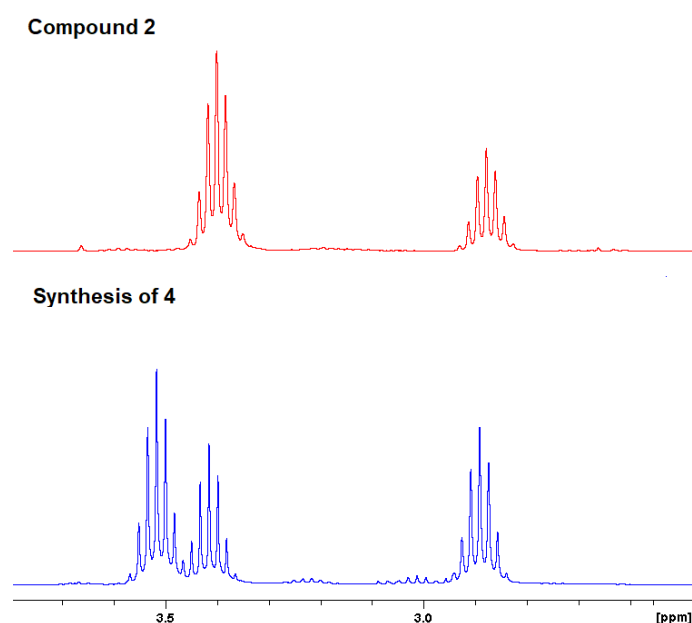


Figure 3.1: An excerpt the ¹H NMR spectra of compound 2 (top) (Appendix A.1) and the product mixture of synthesis of 4 (bottom) (B.2).

As can be seen from Figure 3.1, there is an extra signal (δ_{H} 3.42 ppm) in the spectrum for the product mixture that overlaps with the signals present in the ^1H NMR spectrum for compound **2**. No additional signals were observed, but the signal for the aromatic proton displayed some slight broadening and the signal for the Me-groups also displayed some signs of duplication, indicating the presence of a compound with almost identical shifts.

The second entry (Table 3.1) was performed using the same procedure as last time, but this time fresh $[\text{Pd}(\text{PPh}_3)_4]$ catalyst was used. This reaction afforded **4** in 91% yield, which is closer to the reported yields of 97-91%.¹⁴ This strengthens the suspicion that the old catalyst was at least partly to blame for the poor yield in the previous entry. Other reasons may include air and water contamination, as insufficient degassing is a probable cause for poor reaction yields.

The next step in this synthesis was the removal of the TMS-group. See Table 3.2 for a summary of the reaction details.

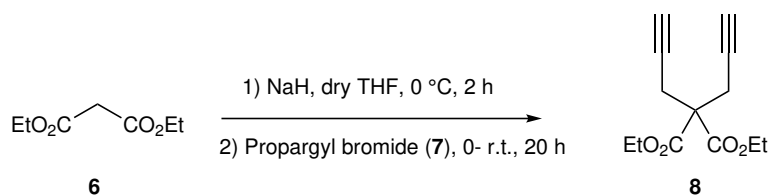
Table 3.2: Summary of reaction data for the preparation of compound **5**.

Entry	4 (g)	Time (h)	5 (%)
1	1.66	1.5 + 2.5	40*
2	2.97	1 + 1	70

*Yield calculated from ^1H NMR spectrum (Appendix D.1).

The two entries in Table 3.2 were reacted in parallel for the next reaction steps. This particular reaction was repeated twice per entry, as the the ^1H NMR spectra indicated incomplete reaction the first time. This was clear from the lingering presence of the TMS-signals. The first entry reached close to full conversion after 4 hours total reaction time, while the second entry reached full conversion after two hours total reaction time, yielding 1.6 g pure product (70%). In addition to the remaining TMS-intermediate **4**, the ^1H NMR spectrum of the first entry showed that there were two compounds present in the sample, in near 60/40 ratio. HRMS analysis confirmed **2** as the other compound. The ^1H NMR spectrum and the HRMS report can be found in Appendix D. As **2** and **5** turned out to be difficult to separate, it was decided that this product batch was not to be used in further reactions, as the catalyst in the next [2+2+2] cycloaddition reaction is very sensitive to impurities.

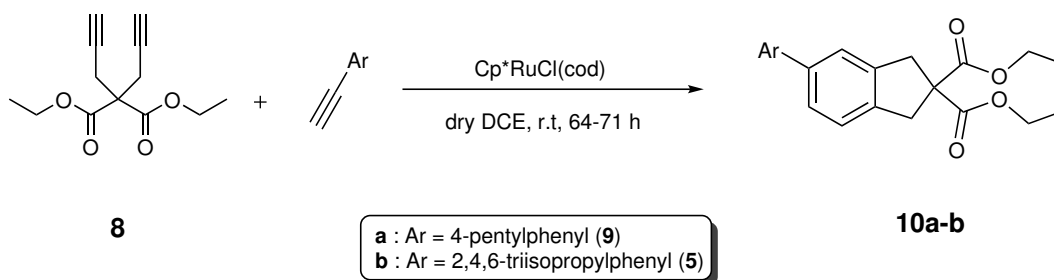
3.3 Synthesis of the diyne 8



Scheme 3.3: Synthesis of diyne **8**.

The synthesis of the diyne **8** was performed as described by Mandal *et al.*⁵⁹ A detailed experimental procedure can be found in Chapter 6.3. This reaction was performed once in a large scale (10 g), with a fair yield of 69%. Reported yields for this reaction lie in the 70-75% region.^{13,59}

3.4 [2+2+2] cycloaddition



Scheme 3.4: Reaction equation for the [2+2+2] cycloadditions.

The [2+2+2] cycloaddition of monoalkynes **5** and **9** with dialkyne **8** was successfully carried out several times. A detailed experimental procedure for the synthesis of **10a-b** can be found in Chapter 6.4 and the results are summarised in Table 3.3.

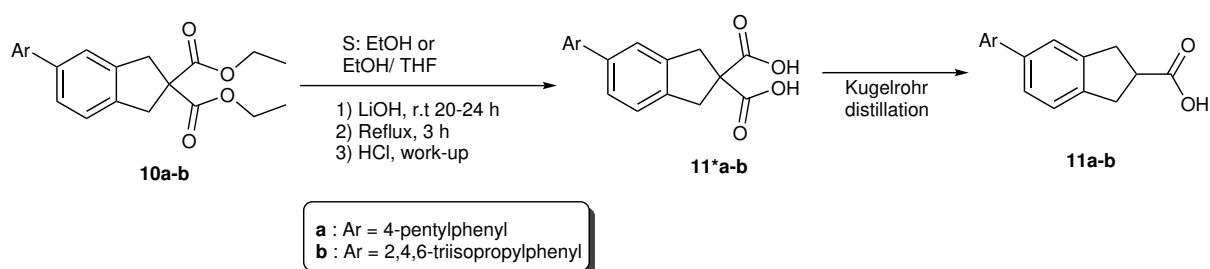
Table 3.3: The results from the [2+2+2] cycloaddition reactions.

Entry	8 (g)	Monoalkyne (1.5 eq.)	Time (h)	Yield (%)
1	1.92	9	65	10a : 53
2	1.53	9	71	10a : 69
3	0.5	5	70	10b : 46
4	0.87	5	64	10b : 48

Reported yields for the synthesis of **10a** are 54-67%,¹⁵ while for **10b** the yields are 36-73%.¹⁴ Similar reactions performed by Yamamoto *et al.*³⁸ reported yields in the 74-94%

region. The catalyst Cp*RuCl(cod) is very sensitive to water, air and other contaminations, which might explain the relative diversity in the reported yields. Even small amounts of any contamination may severely impact the efficiency of the catalyst.³¹ While both the reactants were dissolved in DCE and then degassed with He (g) in the synthesis of **10b** (Table 3.3, entry 3 and 4), only the pure solvent was degassed in the synthesis of **10a** (Table 3.3, entry 1 and 2). While the yields for this reaction were consistently better than for the synthesis of **10b**, the yield might be further improved if degassing of the dissolved reactants, rather than just the solvent alone, were to be included in the procedure.

3.5 Hydrolysis and decarboxylation of the diesters **10a** and **10b**



Scheme 3.5: Hydrolysis and decarboxylation of **10a-b** to yield **11a-b**.

The hydrolysis and subsequent decarboxylation of **10a-b** were performed a total of four times with varying yields. Detailed experimental procedures for both compounds can be found in Chapter 6.5 and the results can be seen in Table 3.4.

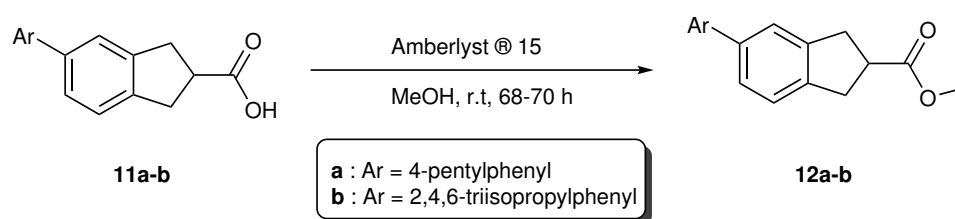
Table 3.4: The results from the hydrolysis and decarboxylation reactions.

Entry	10a-b	(g)	Yield (%)
1	10a	1.5	11a: 83
2	10a	1.78	11a: 51
3	10b	0.47	11b: 70
4	10b	0.81	11b: 76

Flynn and Beight reported a yield of 71% for similar compounds.⁶⁰ Within the research group reported yields for **11a** are 42-77%¹⁵ and for **11b** 72%.¹⁴ For the synthesis of **11a** it was noted that **10a** was not very soluble in EtOH, requiring a rather large amount of solvent (100 ml for 1.78 g) as well as extensive stirring prior to addition of LiOH (aq., 1M). This might have elongated the reaction times. It is worth noting here that **10a** is an oil at room temperature, and only crystallises after being kept in the refrigerator. It might be worth attempting to start this reaction while **10a** is still an oil, to see if this requires less solvent and perhaps also shortens the reaction time. An other suggestion is to run this reaction in a similar solvent system as the reaction with **11b** (EtOH/THF), to see if this increases the solubility.

In these reactions the intermediates **11*a-b** were never purified or analysed. **11a*** has as a dark brown colour, while **11*b** has a lighter more beige appearance. Both intermediates are solids. Upon melting both solids turn into a black viscous liquid, and thermal decomposition yields both **11a** and **11b** as white solids. The melting point of **11a** match that of previously reported melting points,¹⁵ while the melting point of **11b** was considerably higher than previously reported values (96-100 vs 170.0-170.5 °C). As the ¹H NMR spectra of the compounds does not display any particular differences (it is worth mentioning that one sample was dissolved in CDCl₃ and the other in DMSO), the reasons for this rather large difference is hard to obtain. The only conceivable difference was that **11b** is described as a dark brown solid, instead of the white solid afforded in this reaction. The darker colour might indicate the presence of impurities, but is not visible in the ¹H NMR spectrum (Appendix I.1).

3.6 Esterification with Amberlyst® 15



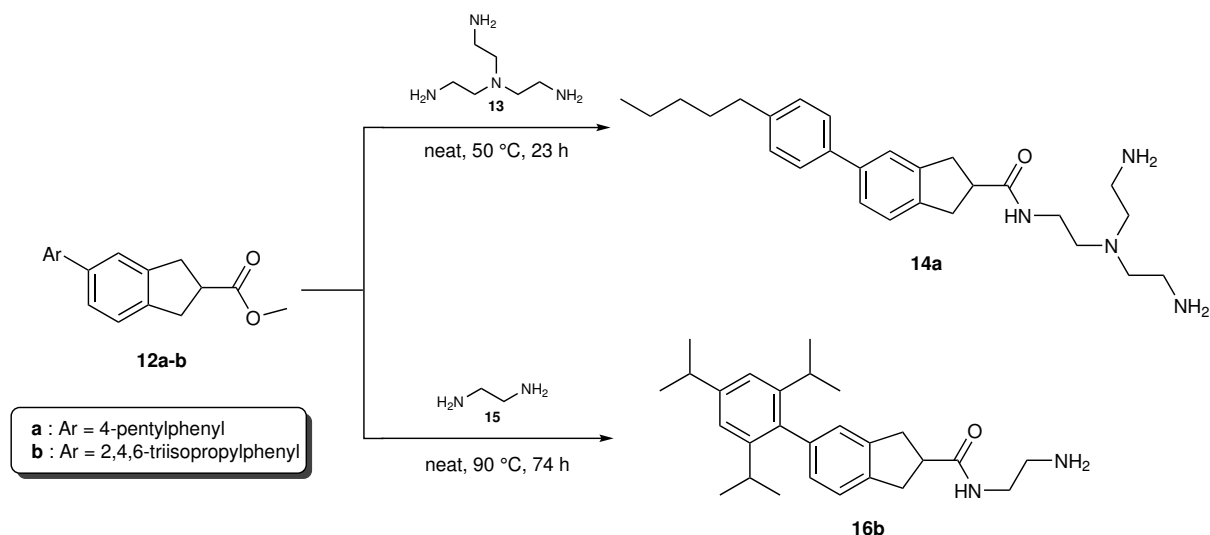
Scheme 3.6: Esterification of **11a-b** to **12a-b**.

The esterification of **11a-b** to yield **12a-b** was performed several times in good to excellent yields following the procedure described by Petrini *et al.*⁴⁷ See Table 3.5 for a summary of the results. A detailed experimental procedure for the synthesis of both esters can be found in Chapter 6.7.

Table 3.5: The results from the esterification reactions.

Entry	Acid	11a-b (g)	12a-b (%)
1	11a	0.94	>99
2	11b	0.11	90
3	11b	0.15	97

Petrini *et al.*⁴⁷ states that this esterification method (using Amberlyst® 15 and methanol) should proceed with quantitative yields. The yields obtained in this reaction were somewhat lower, but still excellent. The only real drawback with this reaction is the long reaction time (68-70 h), but with such excellent yields, mild reaction conditions and simple method of purification, no other alternatives were explored.



Scheme 3.7: The amidation of **12a-b** to yield amidoamines **14a** and **16b**.

3.7 Amidation

The amidation of esters **12a** and **12b** were performed with different amines according to the procedure described by Jasiński *et al.*,⁶¹ to yield amidoamines **14a** and **16b**. A detailed experimental procedure for the synthesis of **14a** and **16b** can be found in Chapter 6.8 and a summary of the reaction results can be seen in Table 3.6.

Table 3.6: The results from the amidation of **12a-b** to **14a** and **16b**.

Entry	Ester	Amine	Yield (%)
1	12a	13	14a : 97
2	12b	15	16b : 78

The synthesis of **14a** (Table 3.6, entry 1) was performed once in excellent yield (97%). The reaction was performed with excess **13** as a solvent, and after complete reaction the excess **13** was removed with kugelrohr distillation (0.03 mbar, 105-110 °C). This afforded **14a** as a golden oil, which is in accordance with previous work done within the research group.^{13,15} This is the only purification step performed in this synthesis, as ¹H NMR analysis indicated that all the methyl ester **12a** had reacted and no other impurities were visible (Appendix L.1).

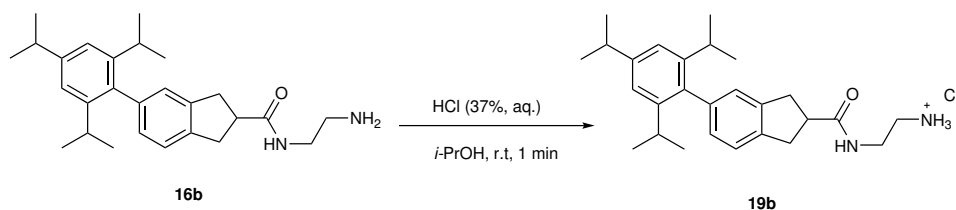
The preparation of amidoamine **16b** (Table 3.6, entry 2) was performed once with a good yield of 78%. Purification of this reaction was done in two steps. Firstly, most of the excess **15** was removed *in vacuo*. Next, the crude was dissolved in EtOAc and washed with water repeatedly in an attempt to wash away any remaining amine. ¹H NMR confirmed that all the **15** was removed (Appendix M.1). This method works fairly well, but as other similar reactions proceeded with near quantitative yields following the same procedure,⁶¹ it is fair to assume that some of the product may be lost in the washing process. Removal of excess **15** through kugelrohr distillation should be explored as an alternative method of purification, to see if this is actually the case.

No melting point have been reported for **16b**, as it has previously been described as a beige wax.¹⁴

3.8 N-functionalization of amidoamine 16b

Amidoamine **16b** was further functionalised by transformation into the HCl-salt **19b** and to the guanidine compound **23b**.

3.8.1 Preparation of the HCl-salt 19b



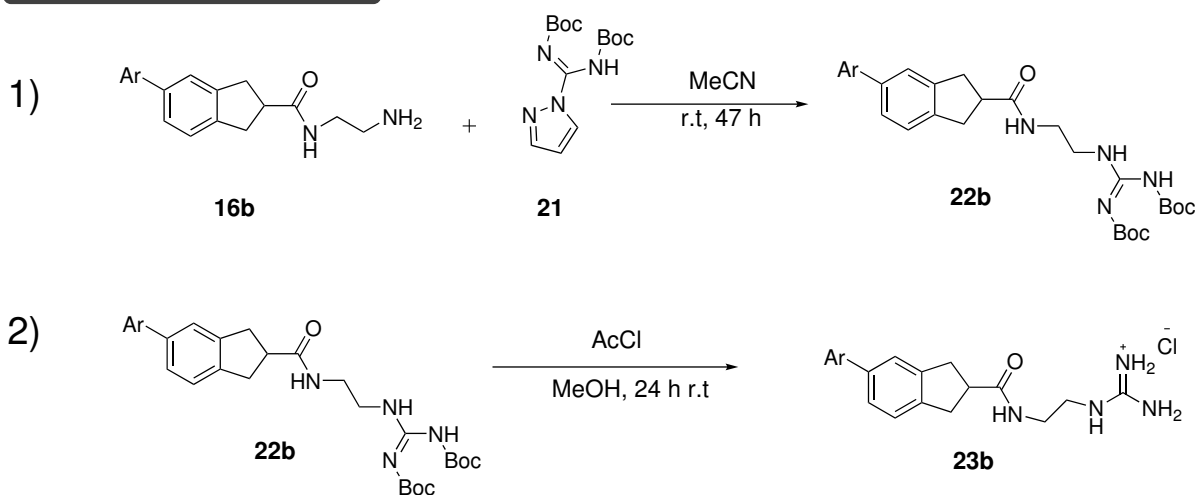
Scheme 3.8: Preparation of the HCl-salt **19b**.

The preparation of HCl-salt **19b** for antimicrobial evaluation was performed once with a fair yield (72%), according to the procedure described by Bakka *et al.*^{23,49} This salt have previously been synthesised within the research group,¹⁴ but sufficient purity (>95 %) was not achieved. A detailed experimental procedure can be found in Chapter 6.9.1.

The amine salt was recrystallised by dissolving in MeOH and Et₂O and leaving it in the freezer (-18 °C) for five days. This is not optimal recrystallisation conditions as it takes a very long for the crystals to grow. However, as more and more solid appeared every day and time was not an issue at this point, it was decided to wait and see if one could achieve a higher yield than previously reported (15-37%).¹⁴ The resulting white solid were analysed by HPLC chromatography (MeOH/ H₂O 80:20, + 0.1% TFA, 1 ml/min, λ = 214 nm, T_R = 6.8 min) and was found to be 96% pure. The HPLC report can be found in Appendix O.3. This is above the stipulated 95% threshold, and so the compound may be sent to biological testing. The HPLC analysis did uncover two additional peaks with a retention time of T_R = 3.09 min and T_R = 7.81 min. As they are not clearly visible in the ¹H NMR spectrum (Appendix O.1), it is impossible to say what they are. This compound have previously been synthesised within the research group by S. Valderhaug,¹⁴ and the HPLC analysis then also had similar peaks.¹⁴ This may indicate that the impurities are not coincidental, but rather a direct result of one of the stages in the synthesis. As no HPLC analysis is done before the final product, it was difficult to determine the origin of the impurities.

3.8.2 Preparation of the guanidine 23b

b : Ar = 2,4,6-triisopropylphenyl



Scheme 3.9: Preparation of the guanidine **23b**.

This synthesis is a two-step procedure, where the first step attaches a Boc-protected guanidyl group to the primary amine group, and the next step removes the protecting groups. The first step is described by Drake *et al.*⁶² and the next by Hickey *et al.*⁶³ A detailed experimental procedure can be found in Chapter 6.11.

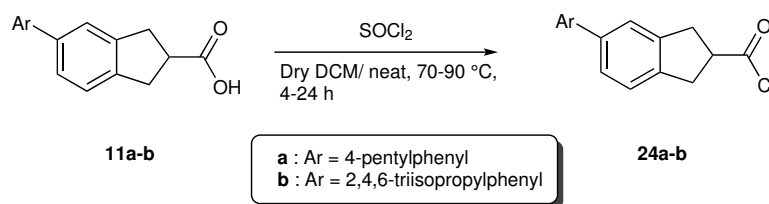
The first step proceeded with an apparent yield of 91%. The NMR analysis indicated that some EtOAc was still present in the sample. This was supported by peaks corresponding to EtOAc⁶⁴ being prominent in both ¹H NMR (δ_{H} 1.99, 4.03 and 1.17), ¹³C NMR (δ_{C} 20.7, 170.3 and 59.8) and the 2D-spectra (COSY, HSQC, HMBC) (Appendix Q.1-Q.8). The solvent proved difficult to remove, as **22b** solidified into a transparent glass under concentration. Some areas of the glass would bubble up and solidify in brittle bubble-like shapes, and those were the only areas where it was possible to obtain a sample for NMR analysis. These areas probably contained more EtOAc than the rest of the material. Another indication of this was obtained during the melting point analysis. As the temperature reached 98 °C, the material went from a brittle white solid to what appeared like a transparent liquid. After retrieving the sample after complete analysis, it was discovered that the sample was not liquid at all, but rather a transparent solid, much like the rest of the material. This might indicate either decomposition or that the material started to melt which allowed the remaining EtOAc to evaporate, leaving only the glass-like material behind. The transparent glass was highly difficult to take a sample of, due to its hardness, and thus a new melting point analysis was not attempted, due to having used all the brittle crystals for other types of analysis. The product was attempted dried on the vacuum line (r.t, 0.02 mbar) for 48 hours without success, as NMR-analysis still confirmed the presence of EtOAc. The calculated yield from the ¹H NMR spectra was 86% (0.129 g, 0.19 mmol).

The next reaction (from **22b** to **23b**, Scheme 3.9) proceeded with a crude yield of 117%.

This time, MeOH was the only evident impurity. The purification method used to purify this product was to repeatedly co-evaporate with 10 ml portions of MeOH as described by the literature.⁶³ Unfortunately, the MeOH proved difficult to remove. Even after 2 hours on the rotary-evaporator (40 °C, 6 mbar), co-evaporating with *i*-PrOH and later 24 hours on the vacuum line (r.t, 0.019 mbar), MeOH was still present in the sample. The off-white guanidine **23b** turned dark brown at 198 °C, and the brown solid melted at 260 °C. As this was a target compound, a sample was tested with HPLC to determine its purity (Appendix R.10). The analysis (80:20 MeOH/ H₂O + 0.1% TFA, 1 ml/min, λ = 214 nm, T_R = 7.1 min) came back as 94.6% pure, which is below the threshold of 95%.

Both the HPLC-analysis of **19b** and **23** were ran with the same eluent systems, and are therefore comparable. They appear to have the same two impurities, with a T_R = 3.09/7.8 and 3.15/ 8.0 min respectively. This indicates that the impurities are not from these functionalization reactions, but rather from an earlier reaction. As no HPLC analysis was performed on any of the earlier compounds, it is difficult to determine the origin of the impurities.

3.9 Preparation of the acid chlorides **24a** and **24b**



Scheme 3.10: Preparation of the acid chlorides **24a-b**.

The acid chlorides **24a** and **24b** were synthesised several times over the course of this master project. The yields ranged from 91-130%. A detailed experimental procedure for the synthesis of both compounds can be found in Chapter 6.12, and a summary of the reaction results can be seen in Table 3.7.

Table 3.7: Summary of the results from the synthesis of the acid chlorides **24a-b**.

Entry	Acid	Yield (%)
1	11a	24a: 91
2	11a	24a: 98
3	11a	24a: >99
4	11b	24b: 130

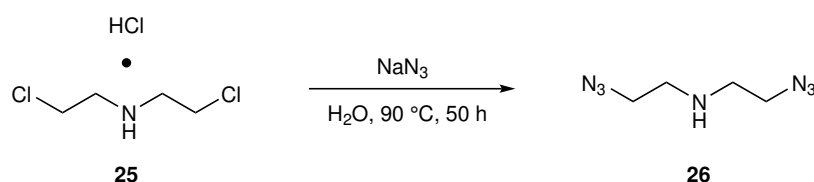
The acid chlorides were used immediately, without any further purification other than concentrating *in vacuo*. The reactions with **11a** (Table 3.7, entry 1-3) were performed in a mixture of SOCl₂ and dry DCM, as opposed to pure SOCl₂. The reasoning behind

this was that after being stored in the refrigerator, the acid presented as a very hard solid, which proved difficult to break apart. As the acid is very soluble in DCM, it was decided to first dissolve it in dry DCM and then add the SOCl_2 to make the acid more accessible. The only difference in the three entries were the reaction times, where the first reaction (entry 1) had a reaction time of 4 hours, while the reaction was left stirring overnight in the other two entries (2 and 3).

As can be seen from Table 3.7, the synthesis of **24b** (Table 3.7, entry 4) proceeded with a 130% yield. This is most likely due to either leftover SOCl_2 or a weighing error. As SOCl_2 is not visible on the ^1H NMR spectrum, and no ^{13}C NMR analysis was performed, it is hard to tell whether or not this is the cause of the excess weight. The product presented as a dark red coloured oil, with no visible signs of any residual **11b** (white crystals). As both acid chlorides were assumed to be highly reactive, full characterisation was not attempted, due to long analysis times. ^1H NMR and ^{13}C NMR and IR spectra were obtained for both compounds, and can be found in Appendix S and T respectively. Both ^1H NMR spectra indicated that small amounts of **11a-b** were present in the samples. This may either be unreacted starting material or the result of the acid chlorides reacting with water present in the sample, and thus reverting back to the corresponding acids. IR analysis of the freshly made acid chlorides **24a-b** lacked the broad signal usually accompanied with a carboxylic acid signal,^{65f} which is present in the IR spectrum of the carboxylic acids **11a-b**. This supports the assumption that the carboxylic acid was fully converted into its acid chloride counterpart at the end of the reactions.

The formation of acid chlorides is supposed to be a rather fast reaction, with common reaction times in the 0.5-6 hours range.^{66,67} However, as the acid chlorides were to be used immediately after preparation, it was found convenient to start the reaction the day before and let it react overnight, thus being able to start the next reaction step early in the morning instead of early/late afternoon, which had its advantages. It was also discovered that a higher yield was obtained, at least for the synthesis of **24a**, which is always preferable.

3.10 Preparation of the bisazide reagent 26



Scheme 3.11: Preparation of the bisazide 26.

The bisazide reagent **26** was synthesised once over the course of this master project, strictly following the procedure described by Chen *et al.*⁶⁸ A detailed experimental procedure can be found in Chapter 6.13.1.

The reaction proceeded with a yield of 61%, which is lower than the yield reported by the literature⁶⁸ (88%). Azides are high energy molecules,⁵⁵ and thus extreme caution was executed when handling both the finished product and during the synthesis. No heat or gas development was observed during this synthesis, and **26** presents as a transparent oil. As mentioned in Chapter 2.2.9, there are certain guidelines to determine the relative stability of azides. Specifically, the number of nitrogen should not exceed the number of other comparable atoms, such as carbon and oxygen, and the relationship between them should not exceed the limitations of equation 2.1. That is, $(N_C + N_O / N_N) \geq 3$. For **26**, the ratio is 0.57, which is not ideal. There are almost twice as many nitrogen atoms as carbon atoms, and so this molecule appears to be rather unstable. But as this molecule had been synthesised and isolated before without incident,⁶⁸ it was deemed relatively safe, as long as nothing was changed from the described procedure. This meant no scaling, either up or down, and all work was conducted behind a blast shield.

Other molecules with similar C/N-ratios have been previously synthesised^{69,70} and isolated. See Figure 3.2 for two such structures. Azidotetrazole **A** (88% nitrogen) was synthesised by Hammerl *et al.*,⁶⁹ while diazidomethane **B** (85% nitrogen) was prepared by Hassner *et al.*⁷⁰

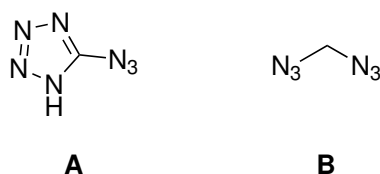
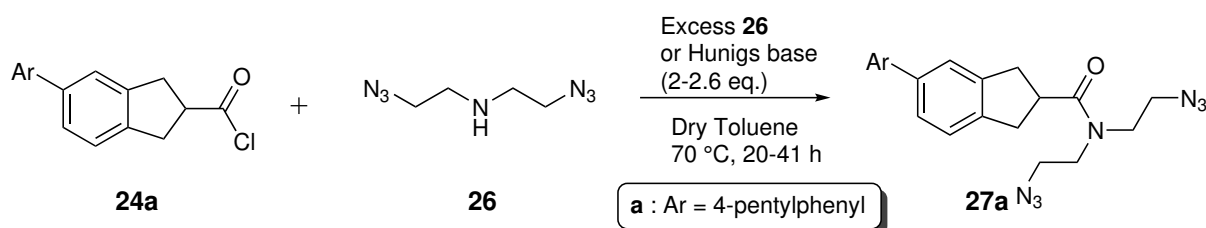


Figure 3.2: Two examples of previously synthesised low-weight molecules with a high percentage of nitrogen.

Although these and several other low-weight azides have been synthesised⁵⁵ without incident, special care should always be taken when working with high energy molecules. Even azides which in practice are deemed non-reactive, may decompose under particular and unexplained circumstances,⁵⁵ and should be treated as such.

3.11 Preparation of amido bisazide **27a**



Scheme 3.12: Preparation of the amido bisazide **27a**.

This reaction was performed thrice with low and variable yields (22-45%). The reaction was performed as described by Singh *et al.*,⁶⁷ and a detailed experimental procedure

can be found in Chapter 6.14. A summary of the reaction results can be seen in Table 3.8.

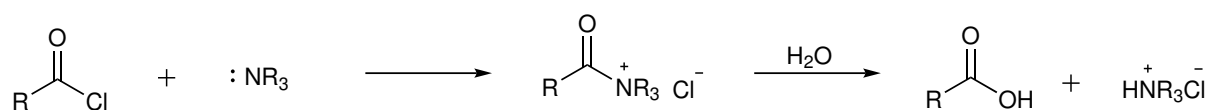
Table 3.8: Results of the preparation of amido bisazide **27a**.

Entry	24a (g)	26 (eq.)	Hünigs base (eq.)	27a (%)
1	0.19	2.0	-	45
2	0.49	1.2	2.0	35
3	0.13	1.4	2.6	22

Singh *et al.*⁶⁷ reported a yield of 54% by using this method on similar compounds, which is a better yield than achieved here. This reaction was attempted using two different co-bases, different reaction times as well as different extraction methods.

The first entry in Table 3.8 yielded the best results, achieving **27a** in 45% yield. In this reaction, excess **26** was used as a base. The reaction time was 20 hours, which was also the shortest reaction time for this reaction. The extraction was performed in a basic environment. Overall this method yielded the best results, but might be improved by increasing the reaction time.

In the second and third entry, Hünigs base, or *N,N*-diisopropylethylamine was used as a co-base, in 2.0-2.6 equivalents. This yielded much poorer results (22-35%), even when increasing the reaction time from 21 to 45 hours. In the 2nd entry, a large amount of the acid **11a** (confirmed by ¹H NMR spectroscopy, 124 mg) was recovered during the extraction, and the yield was only 35% after purification. This reaction is difficult to follow on TLC, as the acid and amine spots have an R_f value of 0 and does not move. Is it thus challenging to determine when all the starting material is converted, as the amine is used in excess. In the third entry, it was attempted to double the reaction time to see if this would increase the yield of **27a**. It did not, and instead the yield of **27a** was only 22%. This lead to the belief that some other reaction(s) might take place, and one such possibility is illustrated in Scheme 3.13.⁷¹



Scheme 3.13: Possible by-reaction between a tertiary amine and an acid chloride⁷¹

Acid chlorides may react with tertiary amines, here Hünigs base, by a nucleophilic addition-elimination reaction. The acylammonium chloride intermediate will prevent the wanted reaction with the bis azide reagent, and may help explain the poor yields, when comparing to the reaction from entry 1. This intermediate is not stable in the presence of water or hydroxylic solvents, which can explain the rather large amounts of acid present in the water phase after extraction. This is especially true in entry 2, where approximately 100 mg of **11a** was recovered.

Before purification, the crude in entry 3 weighed 173 mg (93%), but after purification by flash column chromatography, only 41 mg (22%) was obtained. As the excess amine

should have been removed during the extraction, and thus not affected the crude yield, the column was flushed with different solvents to see if any by-products could be isolated. An eluent of (10% EtOAc in DCM) was used first, and this resulted in the discovery of two more compounds/compound mixtures, named **BP3a.1** (20 mg) and **BP3a.2** (4 mg). While **BP3a.1** presented as a transparent oil with a weak yellow tint, **BP3a.2** was an off-white solid. Their different NMR spectra can be found in Appendix W and X respectively.

There was enough of **BP3a.1** to do a full characterisation by NMR spectroscopy. Unfortunately, this seemed to be a complex mixture of different compounds, rather than only one product, which made the characterisation difficult. However, some information could be obtained. The ^1H NMR spectrum showed that several of the signals overlap with that of the acid chloride **24a**, suggesting some type of by-product with the indene scaffold and 4-pentylphenyl part intact. The ^{13}C NMR spectrum displayed signals with shifts of δ_{C} 196.9 and 196.6 ppm, suggesting aldehydes or ketones,^{65c} however, no $^1\text{J}_{\text{C-H}}$ coupling was observed between these carbons and any hydrogen atoms, thus eliminating the aldehydes as a possibility. The ^{13}C NMR also contained three peaks at δ_{C} 175.1, 175.4 and 175.6 ppm which suggests alkenes, carboxylic acids, esters, anhydrides or amides.^{65c} These carbons does not display any coupling in the HSQC spectrum, but does display $^2\text{J}_{\text{C-H}} / ^3\text{J}_{\text{C-H}}$ coupling to different proton signals in the HMBC spectrum. In the end, the mixture proved too complex to characterise, and no useful information regarding possible by-product(s) were obtained.

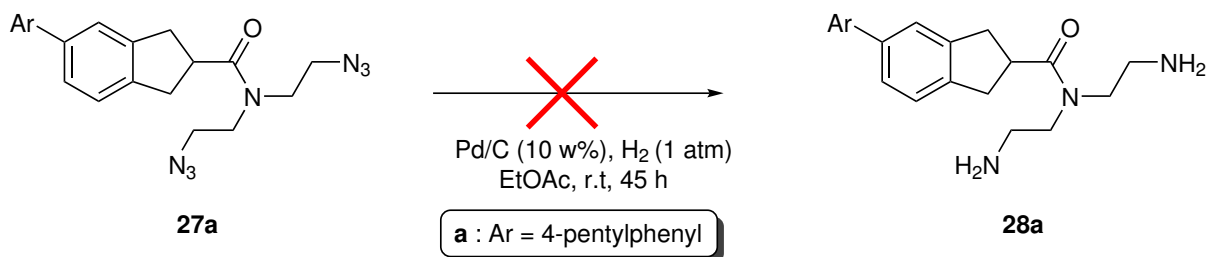
Very little of **BP3a.2** was recovered, approximately 4 mg, just enough to get one ^1H NMR analysis (Appendix X.1). This did not provide enough information to be able to determine structure of the by-product, but some information could be obtained. As with **BP3a.1**, several of the peaks in the ^1H NMR spectrum overlap with that of the acid chloride **24a**, suggesting that it is some form of by-product with the indene scaffold and 4-pentylphenyl part intact. Other than that, the spectrum does not give any more clues as to what this by-product may look like.

From this, one can draw two conclusions regarding future use of this reaction: *One*: longer reaction times could be beneficial, and should be investigated, and *two*: No additional co-base should be utilised to avoid possible by-reactions and by-products, and instead use 2.1-2.1 eq. of **26**.

3.12 Attempted reduction of bisazide **27a** to yield the bisamine **28a**

The reduction of **27a** to yield **28a** was attempted using three different methods: hydrogenolysis^{35g}, reduction with PPh_3 ⁷² and reduction with zinc.⁷³ As none of these methods yielded the wanted product, it was decided to abandon this particular path, and instead try to find another way of achieving the branched amido bisamine structure.

3.12.1 Attempted hydrogenolysis



Scheme 3.14: Attempted reduction of the bisazide **27a** to yield **28a**.

The reduction of **27a** by hydrogenolysis was attempted once following the general procedure described in the literature.^{35g} A detailed experimental procedure can be found in Chapter 6.15.1.

The reaction was followed by TLC analysis, and stopped after 45 hours, when all the starting material seemed to have reacted. After work-up the product presented as a transparent oil, with a yield of 0.034 g (if pure, this would have been 42%). The ¹H NMR spectrum (Appendix Y.1) proved difficult to interpret, as it was clear that the sample was not pure. While all the signals from the indene scaffold and the 4-pentylphenyl group are present, so are a myriad of other signals, especially in the 0.8-4.0 ppm region. See Figure 3.3 for an excerpt of the ¹H NMR spectrum (Appendix Y.1).

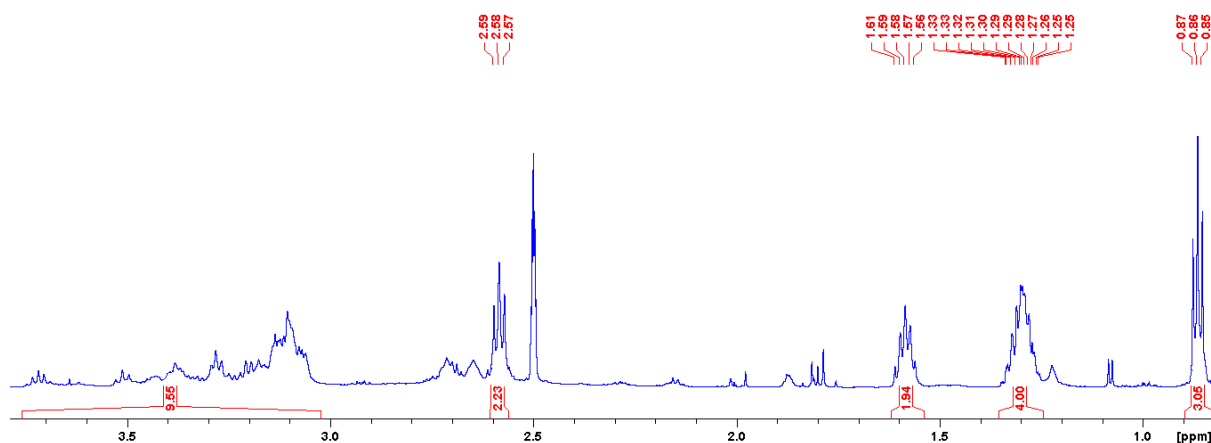


Figure 3.3: Excerpt from the 0.8-3.8 ppm region of the ¹H NMR spectrum after the attempted hydrogenolysis of **27a**.

The signals in Figure 3.3, which are marked by their shift value, are the ones confirmed through full characterisation to belong to the pentyl chain. The multiplets in the area of 3.0-3.8 ppm, integrate to 9.55 H, which is the same amounts of protons one would expect from the structure of **27a** (5 protons on the fused five membered ring + 4 from the two ethyl arms). However, when looking at the HSQC spectrum (Appendix Y.4), see Figure 3.4, it became clear that it was not that easy. Even the familiar peaks had

multiple carbon couplings, and the 3.05-3.8 ppm area had a significantly larger than expected number of couplings.

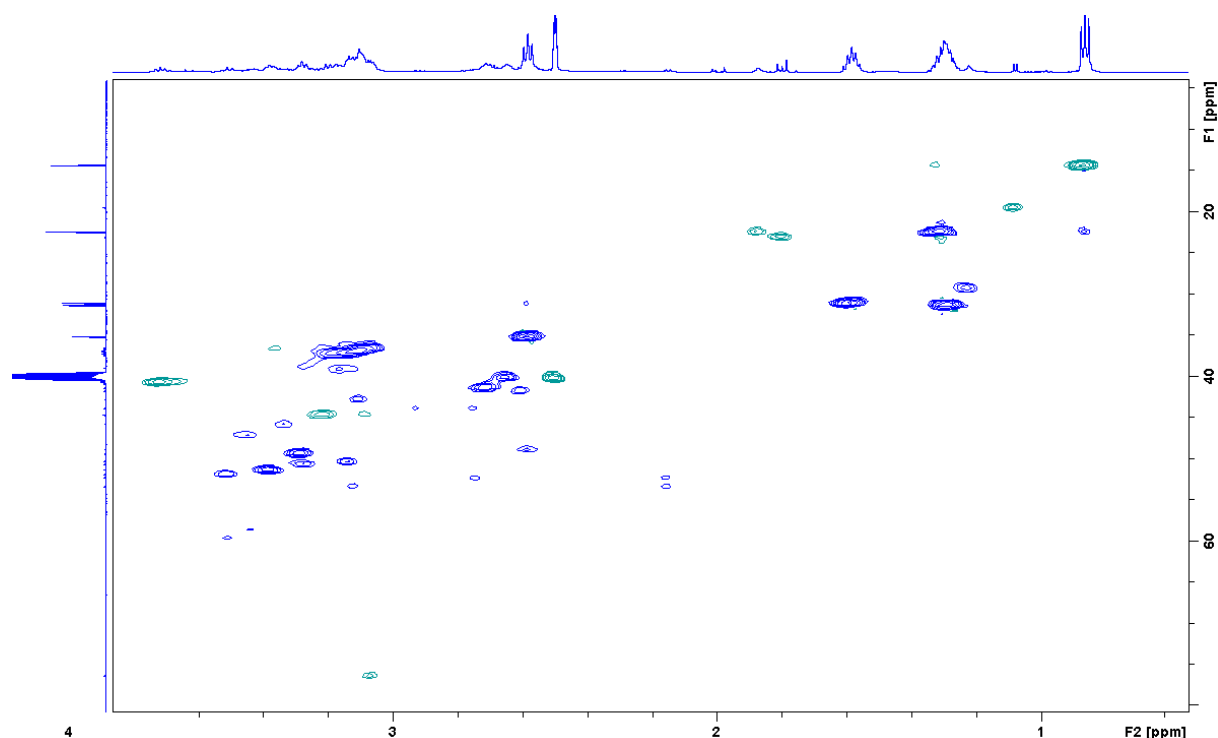


Figure 3.4: Excerpt from the 0.8-3.8 ppm region of the HSQC spectrum after the attempted hydrogenolysis of **27a**.

The aromatic region had few extra signals, the most noticeable being a signal at δ_{H} 7.97, with no J_{CH} couplings in the HSQC spectrum and $^2J_{\text{C-H}} / ^3J_{\text{C-H}}$ coupling to a carbonyl carbon in the HMBC spectrum. A high δ_{H} shift and coupling to a carbonyl carbon might indicate an amide compound.^{65a-c}

Full characterisation was attempted using ^1H NMR, ^{13}C NMR, COSY, HSQC and HMBC spectra, but no structure or structures were determined. All the spectra can be found in Appendix Y. The ^{13}C NMR spectrum (Appendix Y.2) did contain five different signals at δ_{C} 174.4, 174.5, 174.6, 174.8, 174.9 ppm, which indicates that at least five different carbonyl groups were present in the mixture. There are also three peaks at δ_{C} 169.4, 169.6 and 169.7 ppm, which could be alkenes, heteroaromatics, anhydrides, amines or oximes. None of these signals had any J_{CH} couplings in the HSQC spectrum, but several $^2J_{\text{C-H}} / ^3J_{\text{C-H}}$ couplings in the HMBC-spectrum. Due to overlapping of signals, it was challenging to determine accurately which carbon coupled to which proton signal. See Figure 3.5 for an excerpt of the HMBC spectrum. The complete spectra can be found in Appendix Y.5.

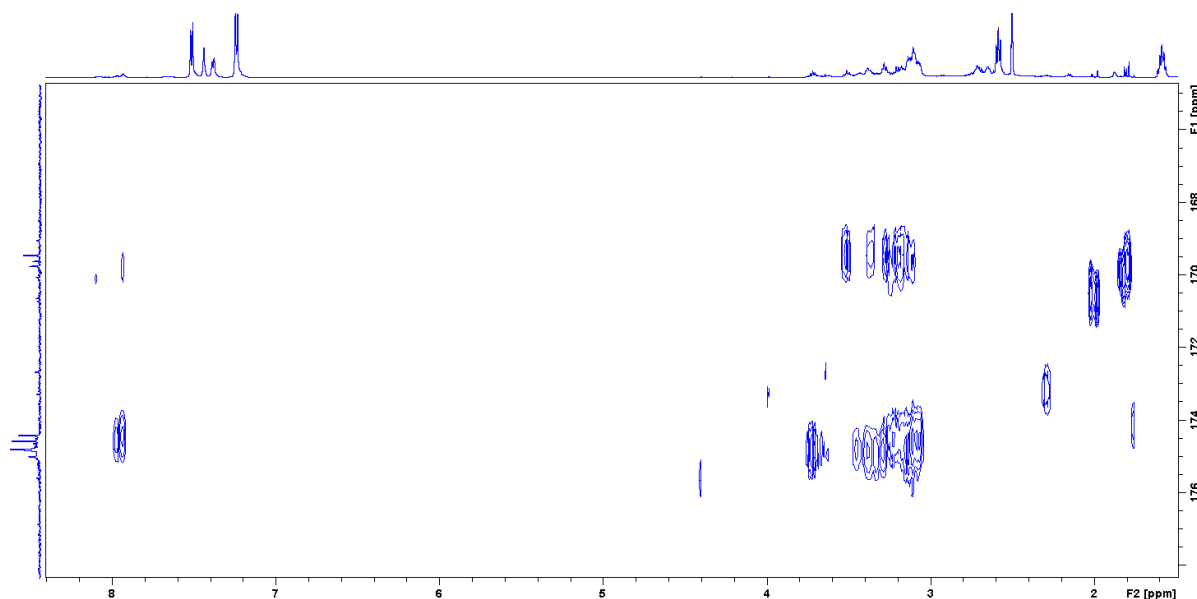
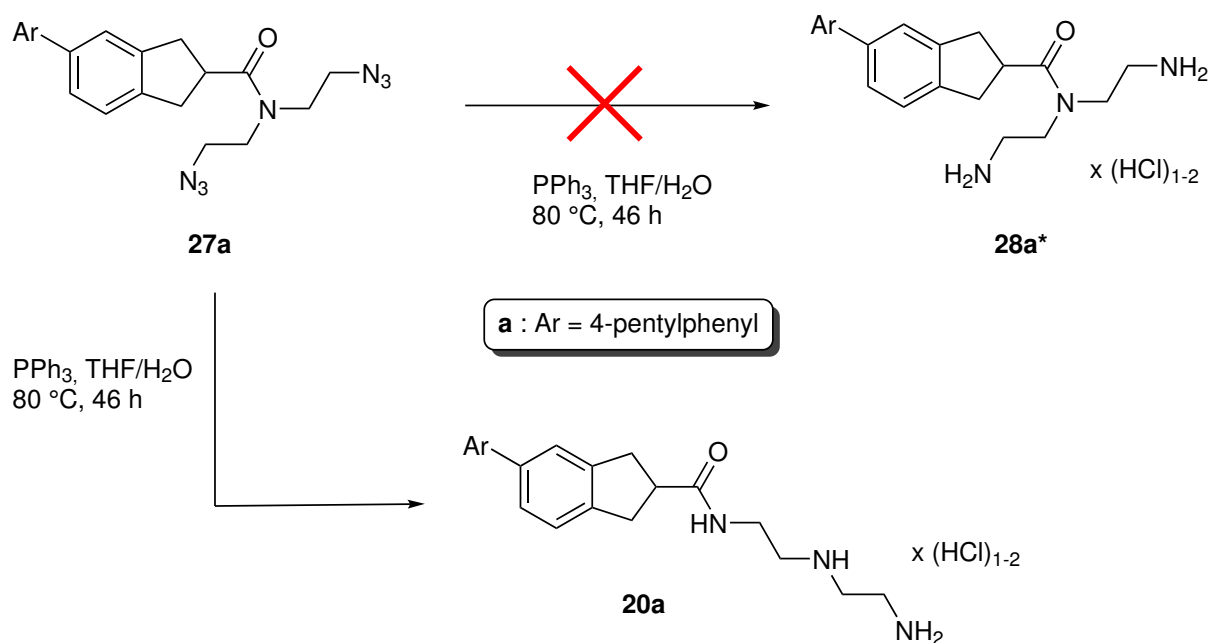


Figure 3.5: Excerpt from the 160-180 ppm region of the HMBC spectrum after the attempted hydrogenolysis of **27a**.

3.12.2 Attempted reduction of **27a** with PPh_3



Scheme 3.15: Attempted reduction of the bis-azide **27a** to yield **28a**.

The reduction of **27a** with PPh_3 was attempted once following the general procedure described by Pal *et al.*⁷² with small deviations. A detailed experimental procedure can be found in Chapter 6.15.2.

After 24 hours reaction time more solvent was added, as the TLC analysis indicated

that the reaction had somewhat halted and no progress had been made in the last hours. Considering the fact that water is an important part of the reaction mechanism, see Chapter 2.2.9, one reason for the low reaction rate might have been too little water, and so some additional solvent mixture was added. After a total of 46 hours reaction time, the reaction was stopped and the work-up procedure was performed as described,⁷² by first dissolving the crude in EtOAc (30ml) and extracting with HCl (aq.,1M, 30 ml). The organic phase was also washed with pure water, before the combined water and acid phases were concentrated *in vacuo*. The yield at this point was estimated to be 102%, and it was suspected that not all of the by-product, triphenylphosphine oxide, had been washed away. ¹H NMR analysis confirmed this (Appendix Z.1), but also indicated that this was not the only issue. Tackling one problem at a time, the work-up procedure was repeated in an attempt to remove the last of the triphenylphosphine oxide. ¹H NMR analysis indicated that this did not work as intended (Appendix Z.2), as the next sample also contained the by-product. See Figure 3.6 for an excerpt of the relevant area of the ¹H NMR spectrum. While the amount of PPh₃O did seem to decrease after the 2nd extraction, showing that the work-up procedure does work, there was still a significant amount of triphenylphosphine oxide left in the sample. Other solvents should perhaps be considered to investigate if i.e. DCM would be more efficient.

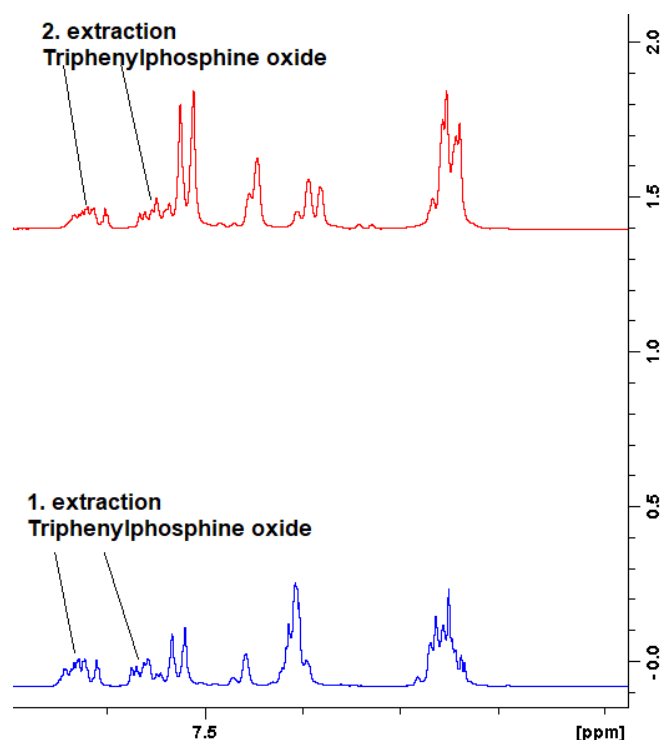
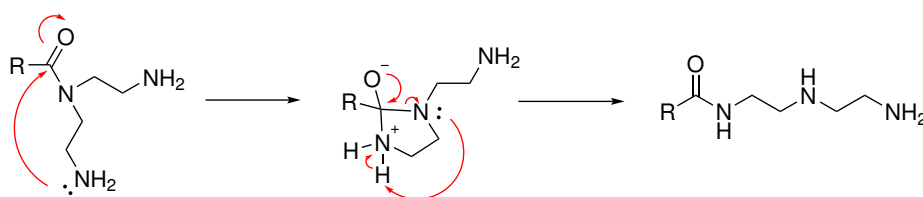


Figure 3.6: Excerpt from the ¹H NMR spectra illustrating the effect of a 2nd extraction. Bottom: after the first work-up, Top: after repeating the work-up procedure.

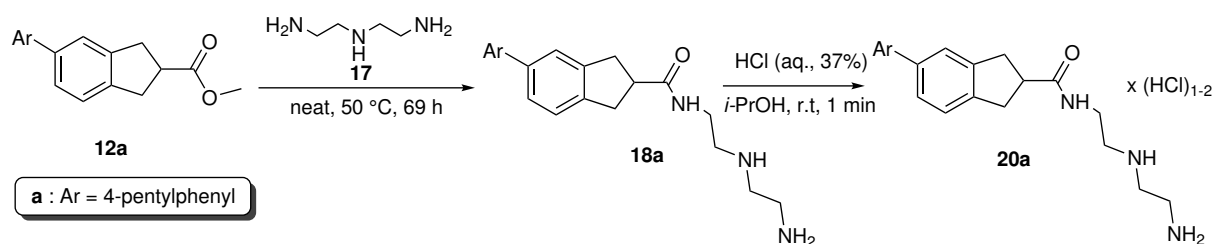
During the second extraction, a white solid precipitated, and did not seem soluble in either the organic or the water phase. It was filtered off, dried and analysed by ¹H NMR spectroscopy. At first this was believed to be the product, but the ¹H NMR analysis (Appendix AA.1) suggested something else. Among the more interesting signals was a signal at δ_H 8.3 ppm (t, 1 H, $J = 5.3$ Hz), with no J_{CH} couplings in the HSQC

spectrum (Appendix AA.4), but with $^2J_{\text{CH}}/^3J_{\text{CH}}$ coupling to a carbonyl carbon in the HMBC spectrum (AA.5). This is in accordance with an amide-group^{65a-d}, and indicated that the white crystals were not the intended product. A sample was sent to HRMS analysis (Appendix AA.6), which stated that the molecule had the same molecular weight as **28a**. At this point possible by-products were discussed, and a possible rearrangement was postulated,⁵¹ see Scheme 3.16. This compound should have the same mass as **28a**, and also give a similar ^1H NMR spectrum, in addition to explain the amide-peak at δ_{H} 8.3 ppm. It is worth noting here that the final form of this compound would be a HCl-salt, as part of the work-up is acidic extraction.



Scheme 3.16: Postulated rearrangement of amido bis amine **28a** to a new amide.⁵¹

To test the hypothesis that this indeed was the main product of the reaction, the suggested compound was synthesised by following the general procedure described by Jasiński *et al.*⁶¹ A detailed experimental procedure can be found in Chapter 6.8.3 for the first step, and in Chapter 6.9.2 for the second step.



Scheme 3.17: Synthesis of amidoamine **18a** and HCl-salt **20a**.

HRMS analysis of **20a** (Appendix P.7) confirmed the molecular weight to be $[\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}][\text{M}-\text{Cl}]^+ = 394.2854$, which is close to the value found for the postulated by-product ($[\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}][\text{M}-\text{Cl}]^+ = 394.2855$). A full spectroscopic analysis of **20a** was performed, including ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra (Appendix P). The ^1H NMR and ^{13}C NMR spectra of the unknown white crystals and **20a** were compared, and found to be a match. See Figure 3.7 for the comparison of the ^1H NMR spectra and Figure 3.8 for a comparison of the ^{13}C NMR spectra. All the spectra can be found in Appendix, P for **20a** and AA for the isolated by-product.

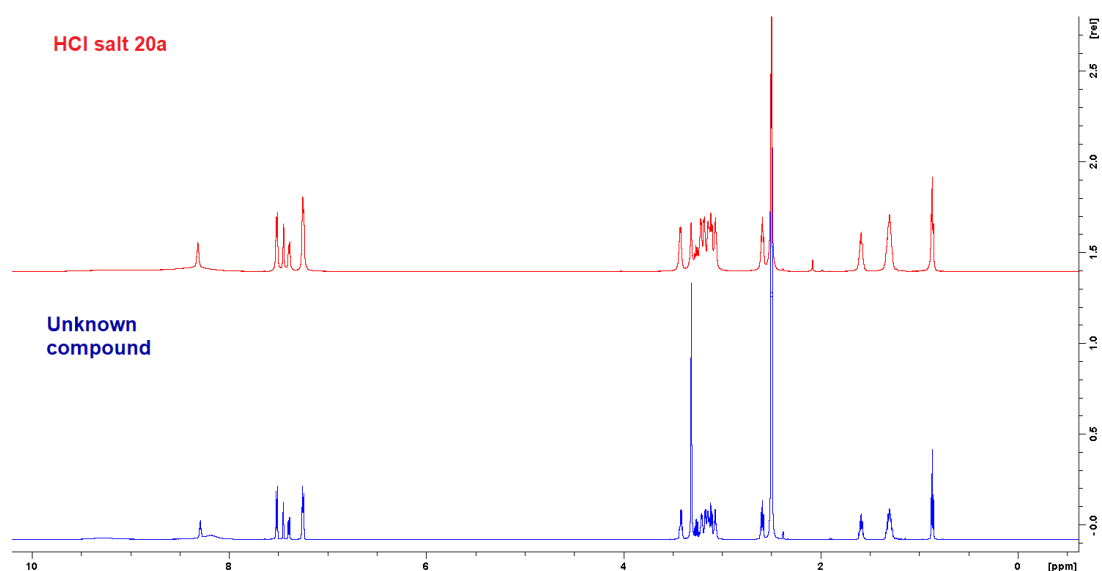


Figure 3.7: Comparison of the ^1H NMR spectra of the unknown compound and the synthesised HCl salt **20a**.

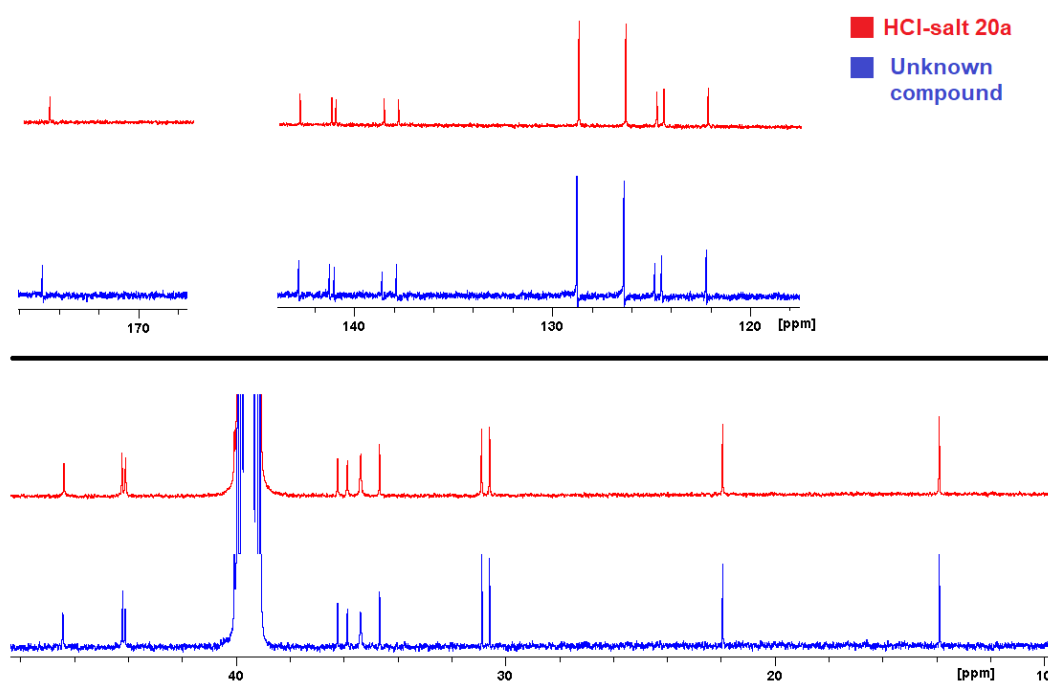
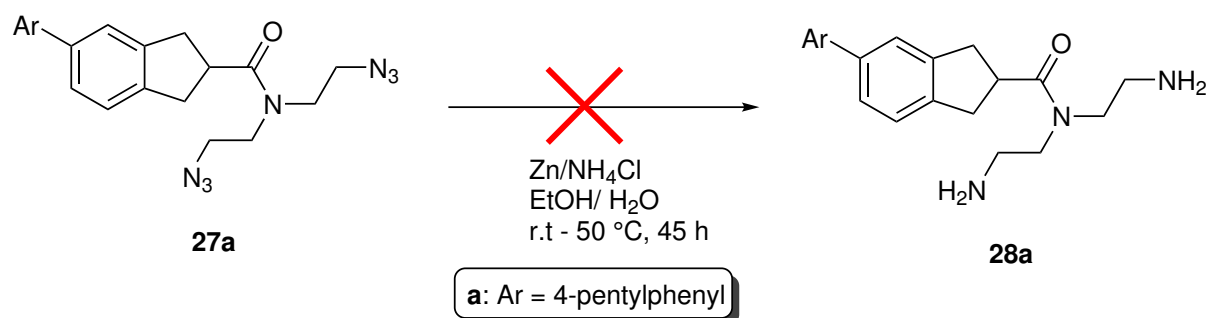


Figure 3.8: Comparison of the ^{13}C NMR spectra of the unknown compound and the synthesised HCl-salt **20a**.

As can be seen from Figure 3.7 and Figure 3.8, the spectra appears to be a match, and the unknown compound was confirmed to be HCl-salt **20a** through spectral elucidation, using the methods described in Chapter 5. This reaction did thus not yield the intended product in any quantitative yield, but instead a isomeric amidoamine. Although **20a** was not a target molecule, it still follows the same structural formula described in Chapter 2.1, and such may be biologically active. HPLC analysis of the syn-

thesised compound **20a** indicated a purity of >99% (Appendix P.9), and it may therefore be sent to biological evaluation.

3.12.3 Attempted reduction of **27a** with zinc



Scheme 3.18: Attempted reduction of the bis-azide **27a** to yield **28a**.

The reduction of **27a** with Zn and NH₄Cl was attempted once following the general procedure described by Lin *et al.*⁷³ A detailed experimental procedure can be found in Chapter 6.15.3.

The reaction was followed by TLC analysis (EtOAc) and after 18 hours, more Zn-powder was added, as it looked like a lot of powder was either stuck to the magnet or on the sides of the flask. After six hours, it looked like the reaction had progressed with the appearance of a spot with a R_f-value = 0, which is appropriate for amines. It was decided to elevate the temperature to 50 °C, and leave it overnight. Complete solvent loss was experienced during the night. Nonetheless, the work-up procedure was performed, and the crude was analysed by ¹H NMR spectroscopy (Appendix AB.1). The spectrum indicated that no reaction had taken place. See Figure 3.9 for a comparison between the starting material and the sample.

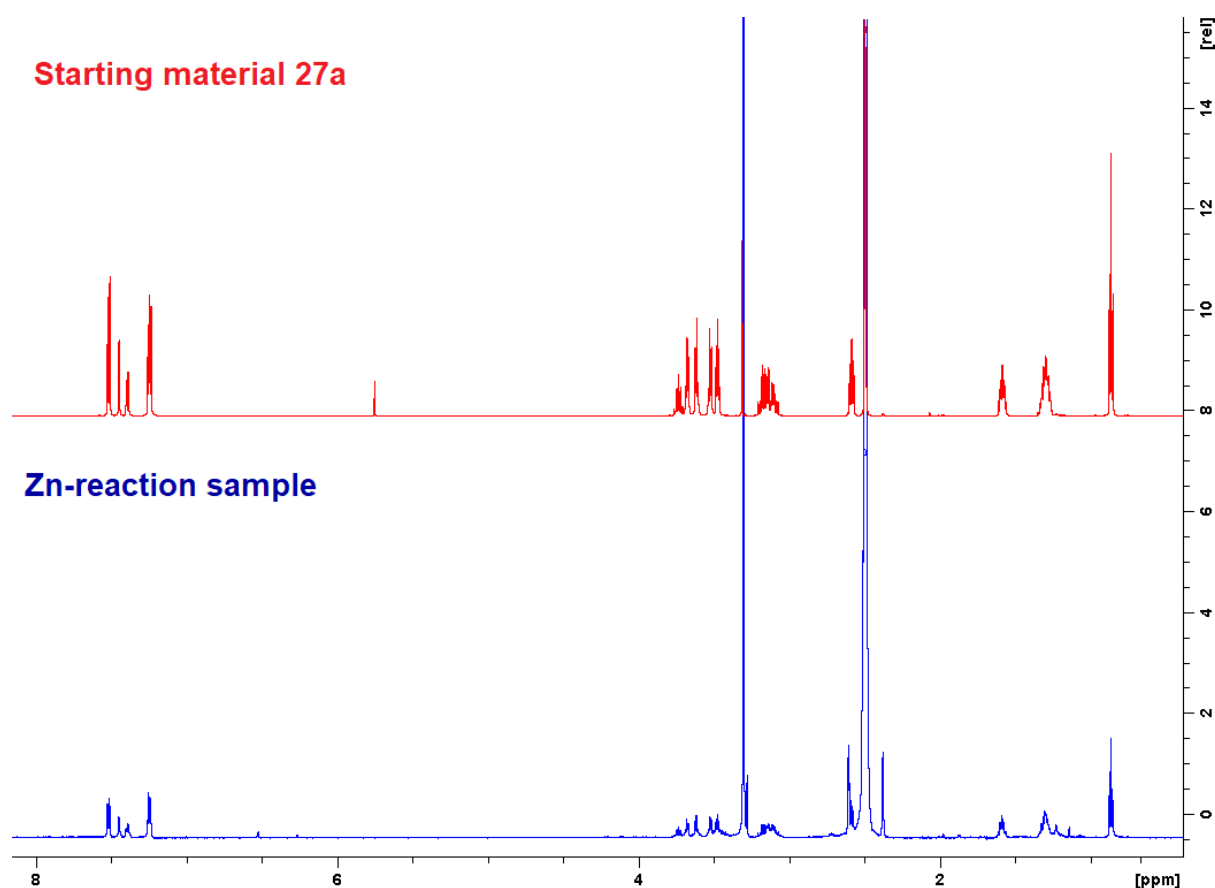
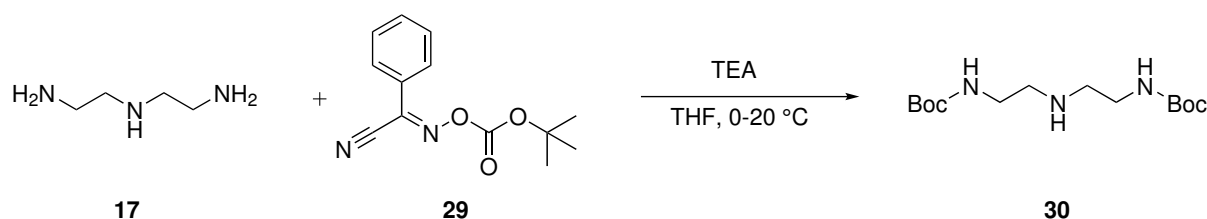


Figure 3.9: Comparison between ¹H NMR spectra of the starting material 27a (top), and the reaction mixture (bottom).

3.13 Synthesis of Boc-protected 30



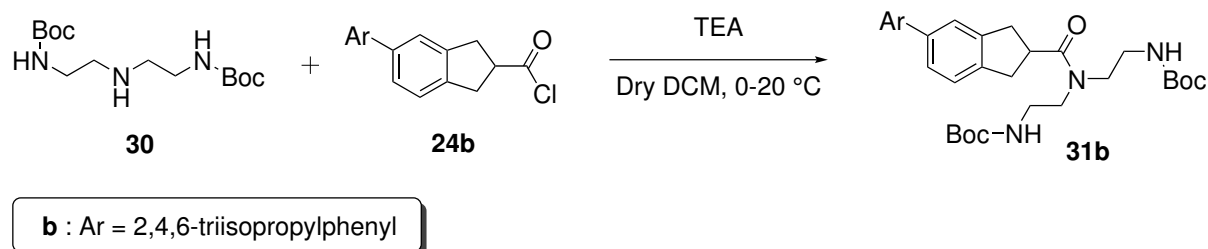
Scheme 3.19: The synthesis of Boc-protected amine 30.

In a last attempt of synthesising the amido bisamine compounds, it was decided to try a different approach. The Boc-protected amine was synthesised in a medium scale (5 g), following a procedure described by Raines.⁷⁴ A detailed experimental procedure can be found in Chapter 6.16.1.

This reaction proceeded with a yield of 70%, which is lower than shown in the literature⁷⁴ (88%). The reaction proceeded without difficulties, however, the product presented as a thick viscous liquid, which was very difficult to dry completely of solvent,

especially MeOH. Even after 48 hours on the vacuum line (r.t, 0.019 mbar), MeOH was visible in the ^1H NMR spectrum (Appendix AC.1).

3.14 Synthesis of Boc-protected amido bisamine 31

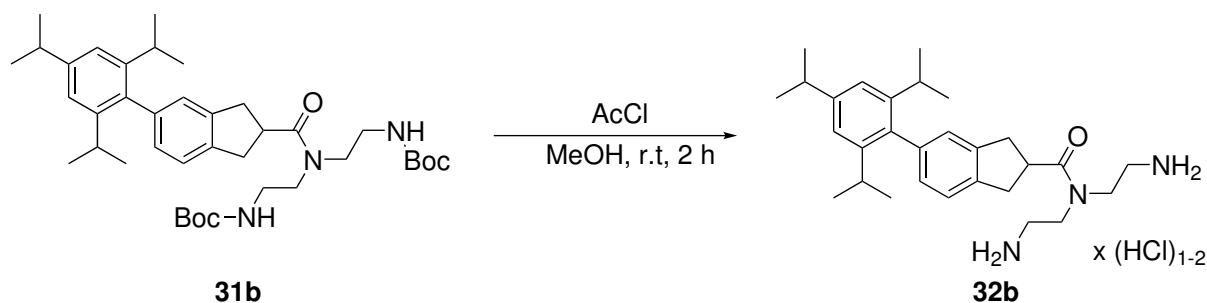


Scheme 3.20: The synthesis of Boc-protected amido bisamine **31**.

The Boc-protected intermediate **31** was synthesised once with a yield of 68%, which is somewhat lower than described for a similar compound in the literature⁷⁴ (82%). A detailed experimental procedure can be found in Chapter 6.16.2.

Due to convenience, the reaction was left stirring overnight at room temperature. Due to either a faulty septum or disrupted nitrogen flow, total solvent loss was experienced during the night. As the reaction should have been finished the prior evening, this was not believed to be of major importance for the outcome of the reaction. Purification was done by column chromatography (EtOAc/n-pentane, 1:1), but the ^1H NMR spectrum indicated the presence of impurities, and so it was decided to repeat the purification, but with a new eluent system. Several combinations of EtOAc and n-pentane were tested, but pure EtOAc gave the best separation. However, even after a second attempt at purification the ^1H NMR spectra displayed an extra signal at δ_{H} 1.47 ppm and a couple of the known peaks had to high integrals suggesting overlap with something else. Due to time limitations, it was decided to continue with the present degree of purity. Priority was given to explore if this reaction path would yield the wanted product, or if the same rearrangement would occur. All ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR spectra and HRMS report can be found in Appendix AD.

3.15 Synthesis of amido bisamine salt 32



Scheme 3.21: The synthesis of the bisamine-salt **32**.

The amido bisamine-salt was prepared once by following the general procedure described by Hickey *et al.*⁶³ A detailed experimental procedure can be found in Chapter 6.17.

The reaction proceeded without incidents and was complete after only two hours. ¹H NMR spectroscopy (Appendix AD.1) revealed that although the compound was not pure, the reaction had worked as intended. No amide-proton peak was visible in the spectrum, indicating that the rearrangement from a tertiary to a secondary amide did not occur. The crystals were attempted recrystallised using many different solvent systems, and two general methods. **Method 1:** dissolved partially in a solvent, heated until completely dissolved, cooled to room temperature and then transferred to the freezer (-19 °C). **Method 2:** Dissolved completely in a small amount of good solvent, added a poor solvent and put in freezer. The amount of poor solvent may vary, and more of the poor solvent may be added over time to facilitate further precipitation. See Table 3.9 for a summary.

Table 3.9: Summary of solvent, recrystallisation method and time spent in freezer for the recrystallisation of **32b**.

Attempt	Solvent system	Method	Time in freezer h (-19 °C)
1	MeCN	1	<i>Insoluble</i>
2	MeOH/ MeCN	2	48
3	H ₂ O / MeCN	2	12
4	EtOH	1	24
5	EtOH	1	48

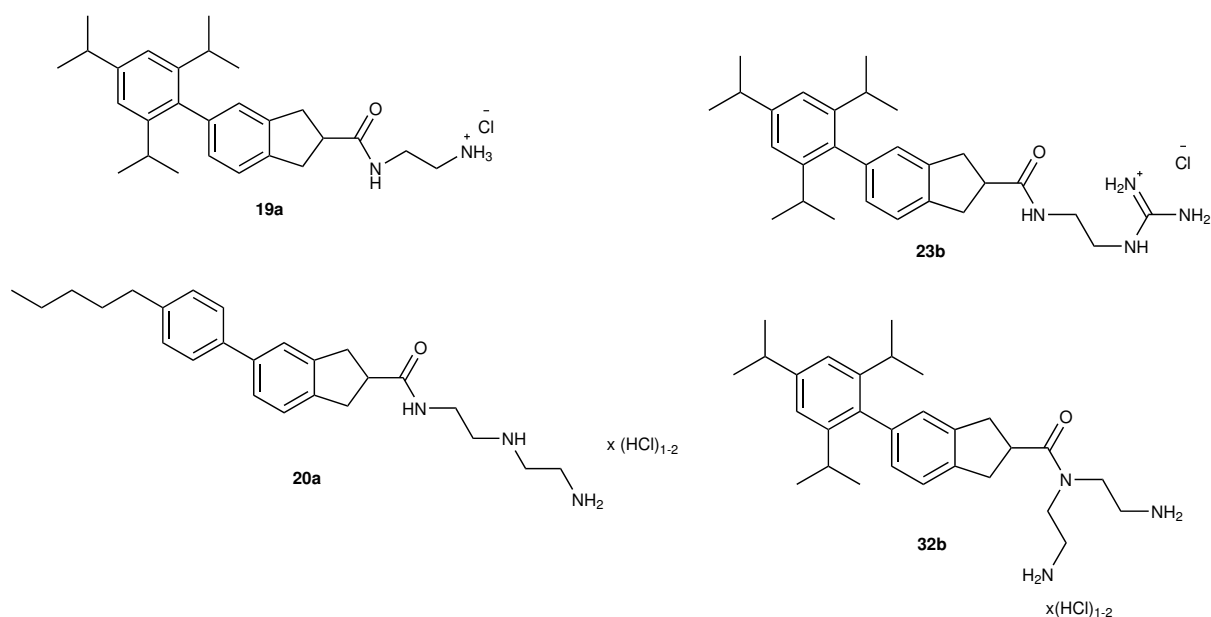
The fourth attempt using pure EtOH gave the best results (Table 3.9, entry 4). Using method 1, the solution was first placed in the refrigerator (5 °C) for 24 hours and then out in the freezer (-19 °C) for another 24 hours. This was to try to facilitate a slower precipitation rate, as the crystals tended to be very fine upon precipitation. After 24 hours in the freezer, the solution was filtered off and the resulting yield was 8 mg. As this was barely enough to complete all the required analysis, the filtrate from Table 3.9 entry 4

was concentrated *in vacuo*, dissolved in a smaller amount of EtOH, and yet again put in the freezer. 48 hours later another 12 mg of white solid was filtered off, bringing the total yield up to 20 mg (Table 3.9 entry 4 + entry 5). ^1H NMR spectroscopy (Appendix AE.1) revealed the amine salt to be purer than before, but still not completely pure. HPLC analysis gave an estimated purity of 75% (Appendix AE.10), which is far from the required degree of purity of 95%. However, the structure of **32b** was confirmed by spectroscopic elucidation, and so a method for synthesising this types of compounds have been established. Work remains to be done regarding work-up procedures and further purification.

4 Conclusion and Further work

4.1 Conclusion

Throughout this master project, three target molecules **19b**, **23b** and **32b** were synthesised. Those are one amine salt **19b**, one guanidine **23b** and one bisamine salt **32b**. Of these three only **19b** was measured to be sufficiently pure (>95%) to enable biological testing, while the others had varying degrees of purity (94.6 and 75% respectively). An additional amine salt **20a**, was also prepared for different purposes. As the general structure of this compound fits the criteria presented in Chapter 2.1, and HPLC analysis determined the purity to be acceptable (>99%), this amine salt may also be evaluated for antibacterial properties. Most of the synthetic routes had previously been established by D. Lindberg and S. Valderhaug, exceptions being the synthesis of the tertiary amides **31** and **32b**. Several attempts to synthesise such compounds were made, and will require a more detailed conclusion.



Scheme 4.1: The synthesised target compounds, **19b**, **20a**, **23b** and **32b**.

The monoalkyne compound **5** was prepared following literature methods, but with modifications as described by S. Valderhaug. Some difficulties were encountered with the removal of the TMS-group, but this should be remedied by using a different base. The dialkyne **8** was prepared once in good yields (69%) without any incidents.

The [2+2+2] cycloaddition reactions were performed on two different series and in accordance with the literature, with fair to good yields (49-59%). Modifications to further improve the reaction have been presented, but as the catalyst is very sensitive to air, moisture and other contaminations, yields are often varying.

In preparation for further functionalisation the esters **12a-b** and the acid chlorides **24a-b** were prepared. The esterification with Amberlyst[®] 15 in MeOH is an established method of synthesising esters from carboxylic acids in near quantitative yields. The synthesis of the acid chlorides is assumed to be completed in a matter of hours, but is often left overnight for convenience.

The amidation of both **12a-b** and **24a-b** occurred without problems, but other options for removal of excess **14a** have been discussed. Preparation of the HCl-salts of amidoamines **16b** and **18a** was performed using HCl (aq., 37%) in *i*-PrOH. Purification of these salts proved difficult, as they are both charged and amphiphilic and not particularly crystalline. Being charged makes them unsuitable for purification by flash column chromatography and being amphiphilic causes solubility issues. The most utilised method of purification is currently recrystallisation, as this should produce crystals of high purity. Currently there is no described recrystallisation method that works for all the compounds, and in general it becomes a matter of trial and error. If recrystallisation does not yield a sufficient degree of purity, preparative HPLC should be considered.

The same issues are encountered when purifying guanidines. The guanidine compound **23b** was synthesised utilising a guanidylating agent where the only work-up described was to coevaporate repeatedly with MeOH. This method was not as effective as described, and further work-up methods should be evaluated. The synthesis of bisazide intermediate **27a** was conducted over two steps. The first step was the synthesis of the bisazide reagent **26** from **17** and NaN₃. This compound is assumed to be explosive, and all work was conducted behind a blast shield and with utmost caution. No deviations from the described protocol was attempted. The next step was the synthesis of **27a** from **24a** and **26**. This was performed three times with varying yields and work-up procedures. The method affording the highest yield used excess **26** as a base, and thus avoided suspected by-reactions and by-products caused by the presence of the tertiary amine base used in the other entries.

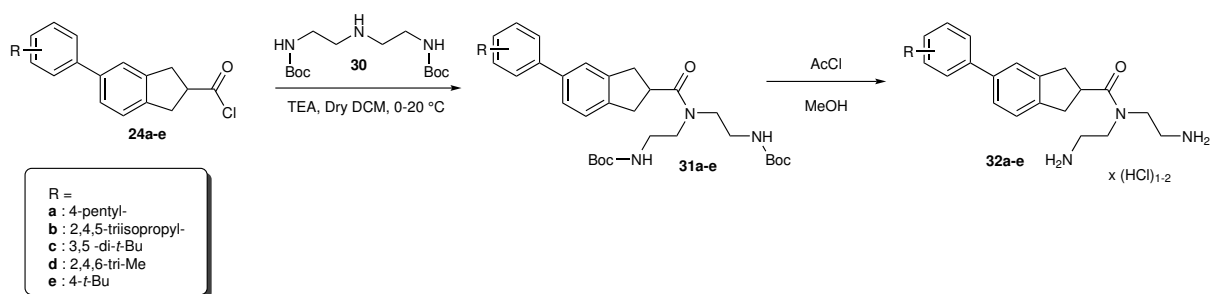
Amido bisamine **28a** was attempted synthesised through three different methods, none of which yielded **28a** as the main product. Hydrogenolysis yielded a complex mixture of compounds, with no evidence indicating that the wanted product had been formed. Reduction with PPh₃ yielded an unknown main product, which was confirmed to be amine salt **20a** through NMR spectroscopy. Reduction with Zn and NH₄Cl did not work at all, as ¹H NMR spectroscopy indicated that no reaction had taken place.

An entirely different approach to synthesise the wanted branched amides (**28a**, **31b**, **32b**) was attempted next, starting with the synthesis of Boc-protected amine **30**. The

reaction proceeded without problems, but removal of the solvents from the product proved difficult. Next, the Boc-protected amido bisamine **31b** was synthesised. The product was attempted purified by flash column chromatography twice, but contaminations were still visible in the ^1H NMR spectrum. Due to time limitations, priority was given to investigate whether or not the reaction path would yield **32b**, and the contaminated product was used in the next step. The deprotection of **31b** to yield **32b** proceeded without complications, but HPLC analysis indicated that the product was only 75% pure after recrystallisation. This is most likely due to impure starting material, and alternative methods of purification of **31b** should be considered.

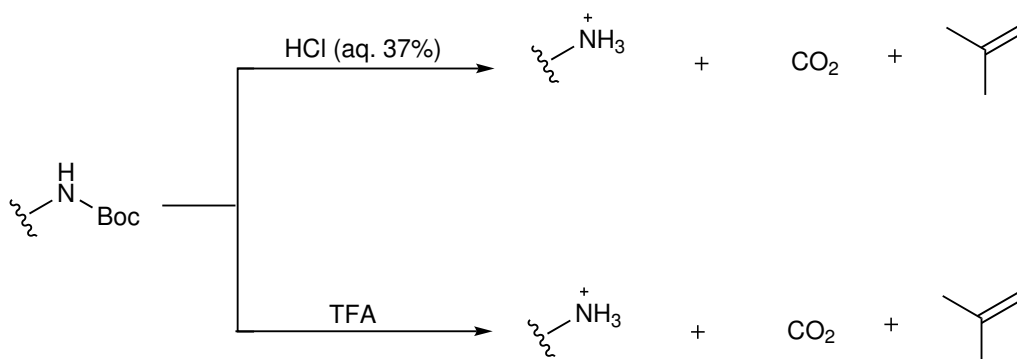
4.2 Further Work

A synthetic path for synthesising tertiary amido bisamine compounds have been established, see Scheme 4.2. This enables series with this type of amido bisamine groups to be synthesised. Series with aryl groups with previously good results in regards to biological testing should be synthesised.



Scheme 4.2: General reaction path for the synthesis of branched amido bisamine compounds.

Although a reaction path has been established, none of the work-up procedures yielded product with a satisfactory degree of purity. Work remains to be done both for the purification of the Boc-protected amido bisamine **31a-e** and the bisamine salt **32a-e**. In regards to the removal of the Boc-groups, several methods currently exists, and should be explored in an attempt to optimise the reaction. Two of the most common methods are illustrated in Scheme 4.3. One methods utilises a strong acid like HCl (aq. 35%)^{52a, 53} and another utilises a weaker acid like TFA.^{75,76}



Scheme 4.3: Two common methods for removal of Boc-groups.^{52a, 53,75,76}

Work also remains on the synthesis of guanylated compounds, especially the bis-substituted guanidines. See Figure 4.1 for three different types of amidoamines where complete series of guanylated compounds have yet to be synthesised.

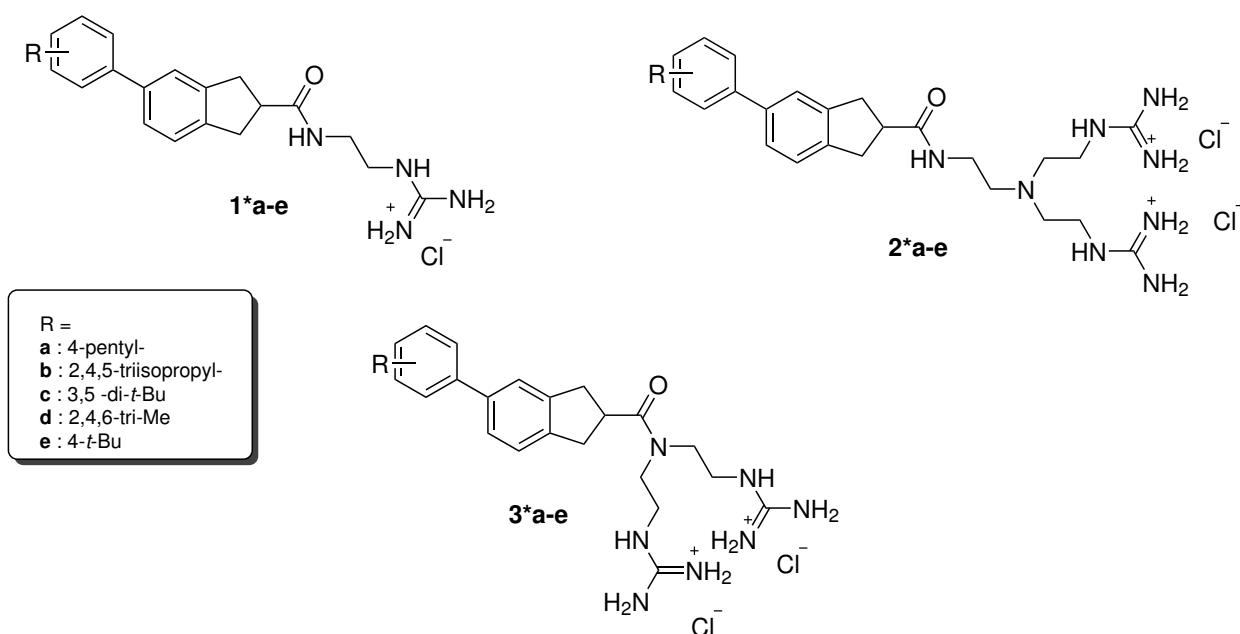


Figure 4.1: Three different types of amidoamines with either one or two guanidine groups.

The synthetic path for for guanidine **1*a-e** have already been established, and this series if the most complete of the three. Among other things, guanidine **23b** will need to be resynthesised, as sufficient purity was not accomplished. For bisguanidines **2*a-e** and **3*a-e**, the synthetic paths have yet to be established. In this master project guanylating agent **21** was utilised, but there exists a multitude of guanylating reagents,^{48,50} and different methods should be tested to determine the best path.

Another interesting approach is to synthesise substituted guanidines. This type of compounds has shown a high antibacterial activity against both Gram-positive and Gram-negative bacterial strains, with MIC-values ranging between 1-8 $\mu\text{g/ml}$.⁷⁷ The

modification of the guanidine group by *N*-methylation, *N*-alkylation and *N*-acetylation has proved to be useful in fine-tuning the reactivity of the group to target specific receptors.⁷⁸ The so-far promising results of these substituted guanidines make them a highly interesting subject, and attempting to synthesise substituted guanidines (**1**a-e**, **a*-d***, **2**a-e**, **a*-d*** and **3**a-e**, **a*-d***, see Figure 4.2) and using them as the cationic part of the already established structural motif of this research group, should make for interesting projects.

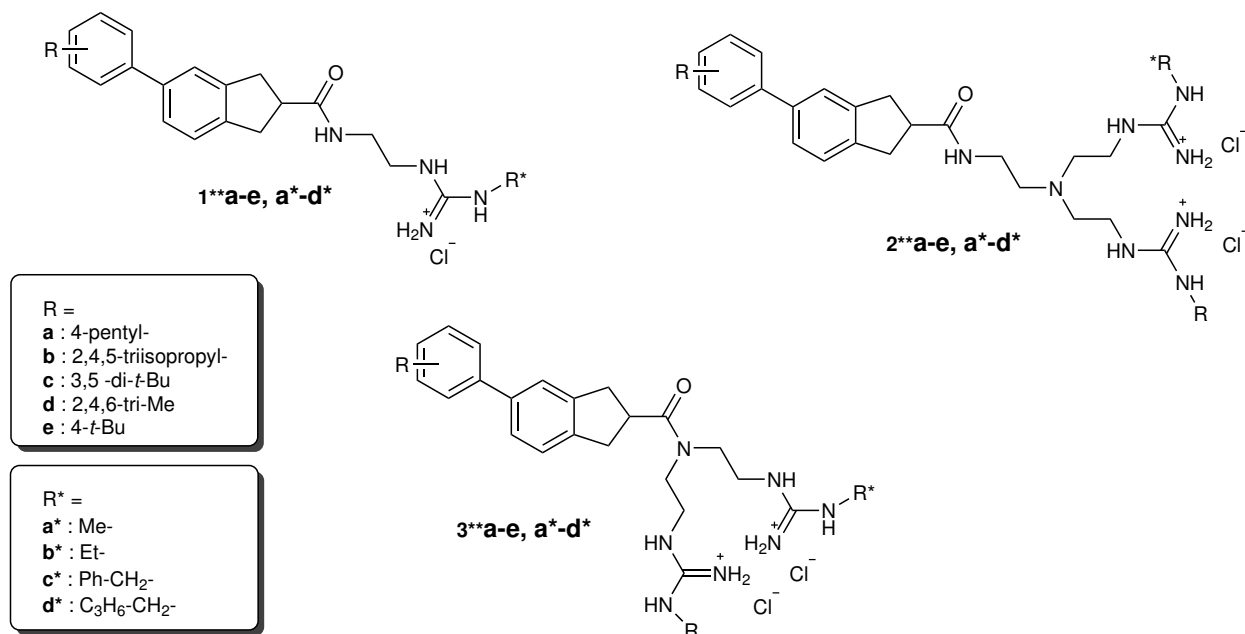


Figure 4.2: New types of substituted guanidine compounds.

5 Spectroscopic Analysis and Characterisation

5.1 General Information

Previously synthesised compounds that have already been published were simply characterised by comparing the ^1H NMR spectra with the reported data. New compounds were characterised by using ^1H and ^{13}C NMR analysis, in addition to IR and HRMS. The chemical shifts of the protons were assigned using 2D-NMR techniques. This include COSY, HSQC and HMBC. The COSY-spectra gives information of vicinal protons. The method will produce spectra with cross-peaks indicating which protons are adjacent to each other. The HSQC technique gives a spectrum where cross-peaks indicates which protons are attached to specific carbon atoms in a molecule. This can also be used to determine quaternary carbons, as these will have no protons attached to them. The HMBC technique gives a spectrum with cross-peaks for two- ($^2\text{J}_{\text{CH}}$), three- ($^3\text{J}_{\text{CH}}$) and infrequently four-bond ($^4\text{J}_{\text{CH}}$) couplings. This gives information about which carbon and protons are close to each other, but not connected. This is useful when determining the structure of a molecule, as one can determine which carbons and protons should be placed in the same vicinity. IR spectroscopy was used to confirm the presence of different functional groups by looking for characteristic absorption bands. HRMS analysis was used to accurately determine and confirming the mass of the molecules. All the spectra can be found in Appendix A-AE.

5.2 Elucidating Structures and Assigning Chemical Shifts

This section will give a detailed walk-through of the spectral elucidation of amido bisazide **27a**. The other new compounds were all elucidated using the same techniques and methods, but their elucidation will not be presented in the same detail. A summary of all their spectroscopic data and subsequent assignment of shifts and positions can be found in Chapter 5.4-5.10.

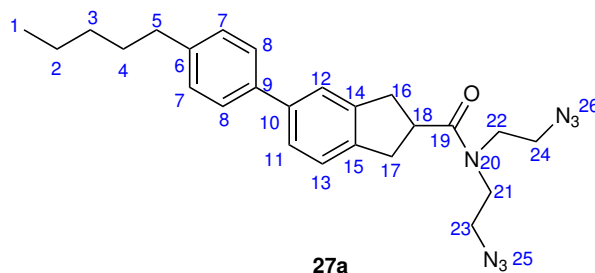


Figure 5.1: Amido-bisazide **27a** with numbered positions.

First of the molecular formula was confirmed by HRMS analysis to be $C_{25}H_{32}N_7O^+$ ($[M+H]$). This is in accordance with the molecular structure of **27a**. The report can be found in Appendix V.7. IR spectroscopy indicated that a carbonyl group^{65a} and a azide group⁷⁹ is present in the molecule by displaying the appropriate signals (1641 cm^{-1} and 2091 cm^{-1} respectively). The spectrum can be found in Appendix V.6.

First, all positions with protons will be assigned and then all the C_q will be placed using the HMBC spectrum. Looking at the structure of **27a** (Figure 5.1) position 1 seems to be the best starting point, as it is a unique position in the molecule (the only methyl group), and thus should be relatively easy to recognise. By looking at the ^1H NMR spectrum (Appendix V.1), illustrated in Figure 5.2, the rightmost signal at δ_H 0.87 ppm (t, 3 H, $J = 7.0$) seems like the best candidate. The low δ_H shift and a J -value of 7.0 Hz is appropriate for a terminal methyl group.^{65b} The splitting pattern (a triplet) is consistent with a group having two vicinal protons. The HSQC-spectrum was then used to determine $^1J_{C-H}$ coupling (Appendix V.4). An excerpt from the HSQC-spectrum of **27a** can be found in Figure 5.2.

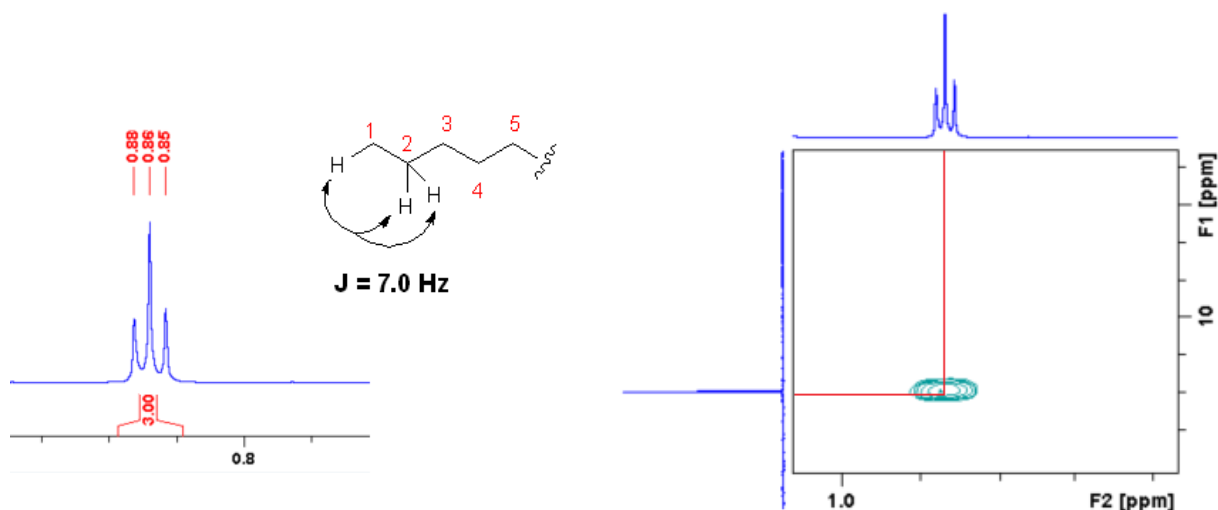


Figure 5.2: To the left: Excerpt from the ^1H NMR spectrum of **27a**. To the right: Excerpt from the HSQC spectrum of **27a**.

As seen in Figure 5.2, the protons at δ_{H} 0.87 couple to the carbon at δ_{C} 13.9. This δ_{C} value is consistent with a terminal methyl group carbon.^{65c} The positions in the remaining part of the alkyl chain were determined next. The HSQC spectrum was used to determine the $^1\text{J}_{\text{C-H}}$ coupling, the COSY spectrum (Appendix V.3) was used to determine which protons couple with each other and the HMBC spectrum (Appendix V.5) was used for confirmation and when the information from the HSQC/COSY spectra did provided too little information. See Figure 5.3 for an illustration of the interpretation of the relevant area of the HSQC spectrum.

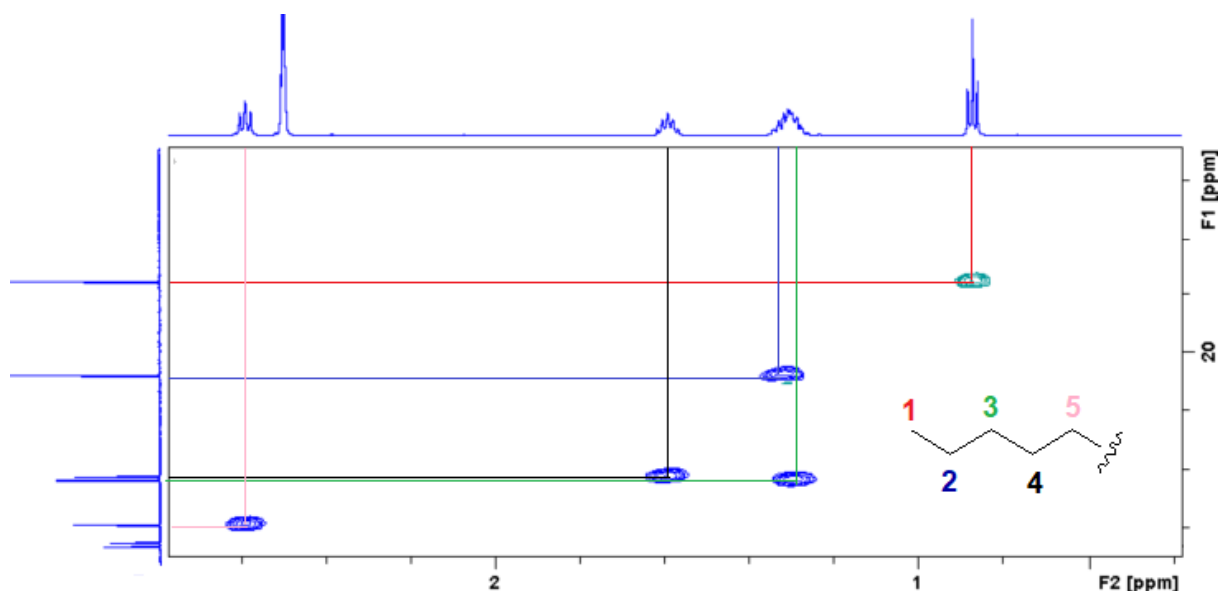


Figure 5.3: Excerpt from the HSQC spectrum of **27a**.

As seen in Figure 5.3 the proton signal at δ_{H} 1.27-1.35 (m, 4 H) couples to two different carbons. The cross-peaks are blue, indicating a CH_2 -type carbon. As the proton signal

have an integral of 4 H, it is safe to assume that this signal stems from two different CH₂- groups. The leftmost group in the signal couples to the carbon with a shift at δ_C 21.9 ppm and the rightmost group couples to the carbon with a shift of δ_C 30.9 ppm. The other protons signals only couples with one carbon each. Next, the COSY spectrum was then used to determine which protons are vicinal to each other, and thus assign positions using the already determined position 1 as a starting point. See Figure 5.4 for a cut-out of the relevant area of the COSY spectrum.

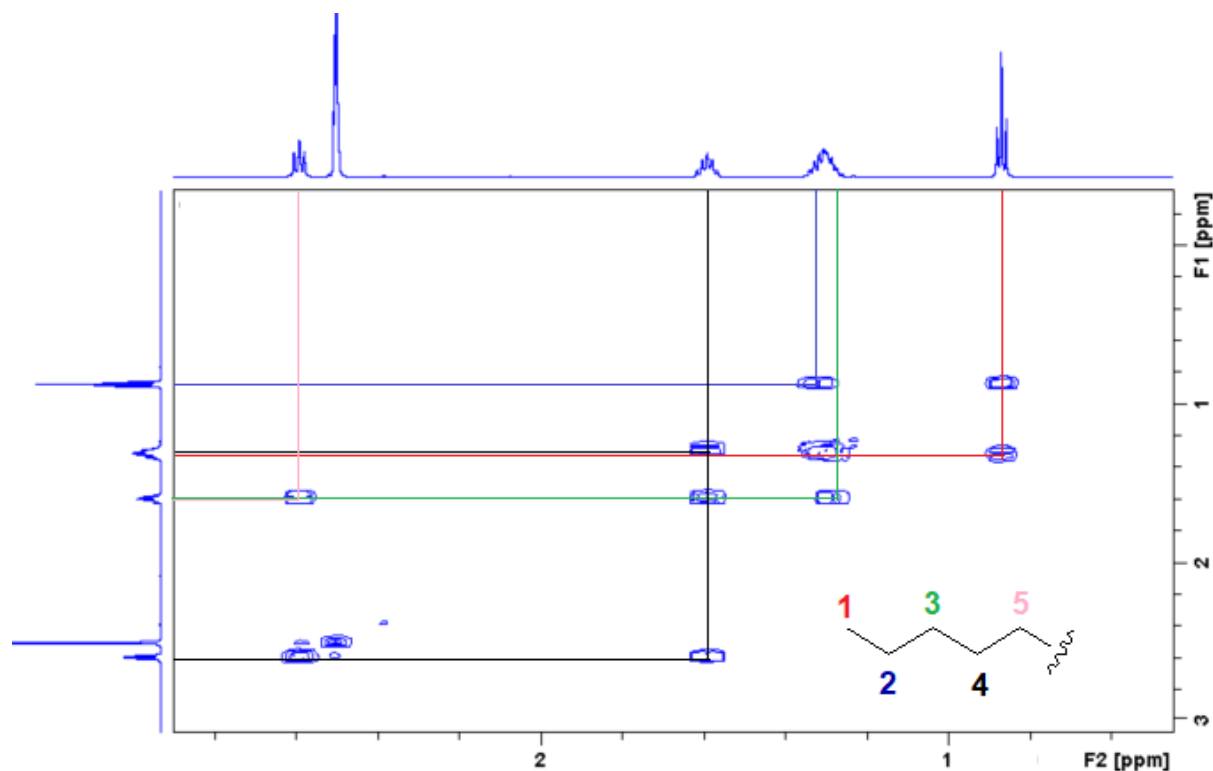


Figure 5.4: An excerpt from the relevant area of the COSY spectrum of **27a** showing vicinal protons at the alkyl chain.

Starting with the rightmost signal again, one can observe that these protons only couple to the signal at δ_H 1.27-2.35 (m, 4 H). This signal stems from two CH₂-groups, and looking at the spectra, this signal couples both to the signal at δ_H 0.87 (t, 3 H, $J = 7.0$ Hz) and at δ_H 1.59 (p, 2 H, $J = 7.5$ Hz). Upon closer inspection, one can see that the two cross-peaks in the spectrum are not directly above each other, indicating that the two CH₂-groups couple to different protons. One of the groups couples to the signal at δ_H 0.87 (the leftmost cross peak), and the other group to the signal at δ_H 1.59 (the rightmost cross peak), thus making it likely that these are the protons in position 2 and 3 in the molecule respectively.

Following this line of thinking, the protons giving rise to the signal at 1.59 (p, 2 H, $J = 7.5$ Hz) should be in position 4, as it couples with the protons deemed to be in position 3. It also couples with the signal at δ_H 2.59 (t, 2 H, $J = 7.6$ Hz). This signal has a splitting pattern indicating two vicinal protons (a triplet), and also a somewhat higher shift than the rest. Given this information it is likely that this signal belong to the protons in position 5. Viewing the entire COSY spectrum reveals that these protons

also couple with protons in the aromatic region δ_{H} 7.25 (dd, 3 H, $J = 7.3$ Hz, $J = 8.1$ Hz), further solidifying this claim. See Table 5.1 for a summary of the assigned shifts and positions on the alkyl side chain.

Table 5.1: Summary of the assigned positions, chemical shifts, multiplicity and coupling constants for the protons/carbons positioned on the alkyl-side chain of **27a**.

Position	δ_{H} (ppm)	Multiplicity	J (Hz)	δ_{C} (ppm)
1	0.97	t	7.0	13.9
2	1.27-1.35	m	-	22.0
3	1.27-1.35	m	-	30.9
4	1.59	quint.	7.5	30.6
5	2.59	t	7.6	34.7

The positions of the indene scaffold and the other aromatic protons were assigned next. Looking at the ^1H NMR spectrum, see Figure 5.5, the signal at δ_{H} 3.74 (p, 1 H, $J = 8.4$ Hz) is the only non-aromatic signal which integrates to 1 H. There is only one such proton in the entire molecule, which is the proton in position **18**. The splitting pattern (a quintet) also indicates that this proton has 4 vicinal protons,^{65d} which is in accordance with the structure of **27a**.

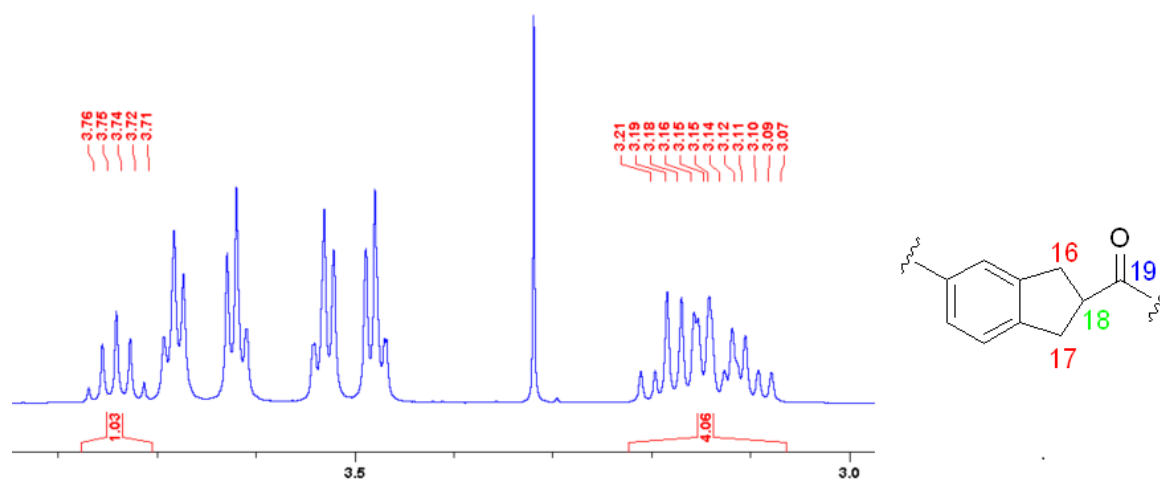


Figure 5.5: Excerpt from the 3-4 ppm region of the ^1H NMR spectrum of **27a**.

The COSY spectrum, See Figure 5.6, reveals that this particular signal only couples with the signals at δ_{H} 3.08-3.21 (m, 4 H), which stems from 4 protons making it likely that these are the protons in position **16** and **17**.

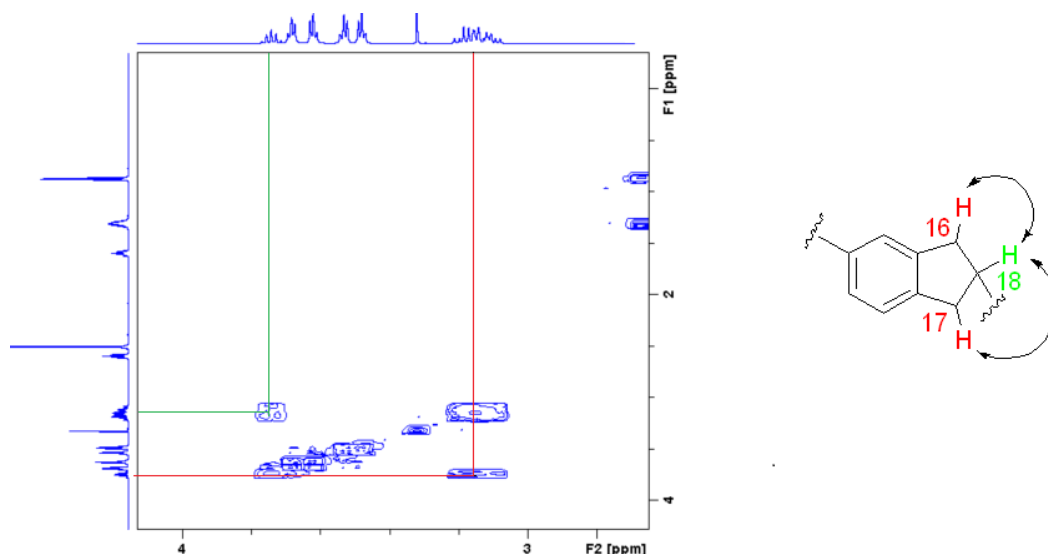


Figure 5.6: Excerpt from the 3-4 ppm region of the COSY spectrum of **27a**.

If the proton signal at δ_{H} 3.74 is the one in position 18, there should be $^2\text{J}_{\text{C-H}}$ coupling between this proton and the carbon in position 19 in the HMBC spectrum. See Figure 5.7 for an excerpt from both the ^{13}C NMR spectra and the HMBC spectra of **27a**. This is a carbonyl carbon, and should be easily identified in the ^{13}C NMR spectrum due to its characteristically high shift.^{65c} The ^{13}C NMR spectrum only displays one such high peak, at δ_{C} 175.0 ppm. Now, looking at the HMBC spectrum in the relevant area, one can observe $^2\text{J}_{\text{C-H}} / ^3\text{J}_{\text{C-H}}$ coupling between the carbonyl carbon and the protons at δ_{H} 3.74 and 3.08-3.21 ppm.

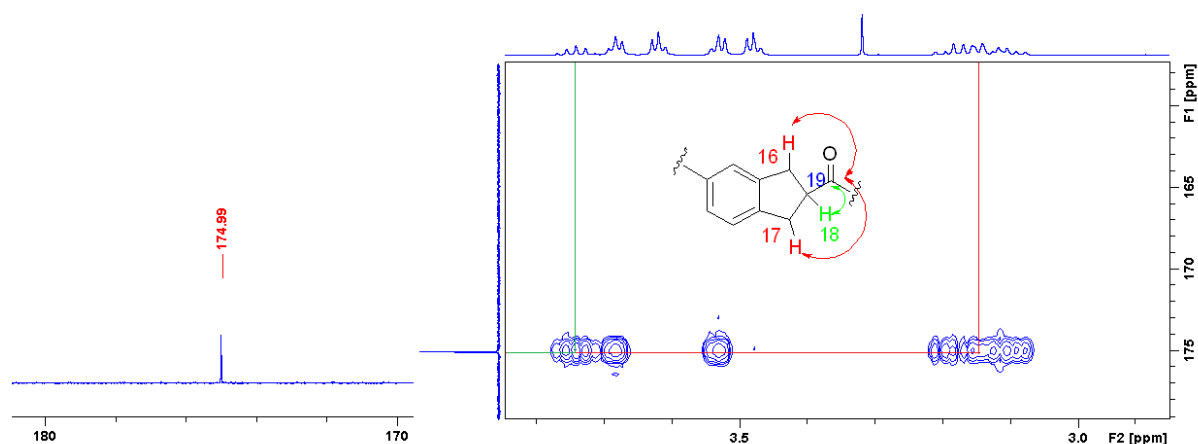


Figure 5.7: Left: Excerpt from the ^{13}C NMR spectrum of **27a**. Right: Excerpt from the HMBC spectrum of **27a**.

For aliphatic coupling, $^2\text{J}_{\text{C-H}}$ signals are stronger than $^3\text{J}_{\text{C-H}}$ signals.⁸⁰ This can be observed in Figure 5.7, as the cross peak for the would-be $^2\text{J}_{\text{C-H}}$ coupling between δ_{C} 175.5 and the protons in position 18 (δ_{H} 3.74) are stronger than the would-be $^3\text{J}_{\text{C-H}}$ coupling between δ_{C} 175.0 and positions 16 and 17 (δ_{H} 3.08-3.21). The HSQC spectrum displays two green cross-peaks coupling with this signal (δ_{C} 36.6 and 36.2 ppm), indicating that

it stems from two CH₂-groups. Looking at the full COSY spectrum (Appendix V.3), one can observe coupling between these protons and two aromatic signals, namely the ones in position δ_{H} 7.4 and 7.25 ppm. The only close enough aromatic positions are 12 and 13. To distinguish which CH₂-group belong in which position, these positions needs to be assigned first.

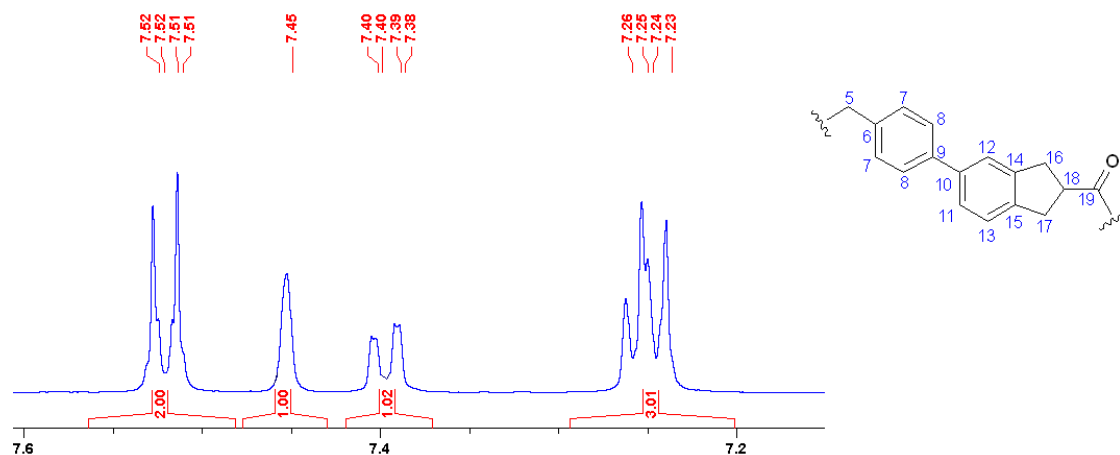


Figure 5.8: Left: Excerpt from the aromatic region of the ¹H NMR spectrum of 27a, Right: Structure of 27a in the relevant positions.

Looking at Figure 5.8 one can note that all the signals displays an apparent doublet splitting pattern indicating a neighbouring proton, except the signal at δ_{H} 7.45 (s, 1 H), which is a singlet. This suggests that this aromatic proton has no vicinal protons. There is only one such proton in this molecule, the one in position 12. Using the HSQC spectrum, ¹J_{C-H} coupling to the carbon at δ_{C} 124.9 ppm was observed. The rightmost aromatic signal δ_{H} 7.25 (dd, 3 H, J = 7.3 Hz, 8.3 Hz) contains three protons which couples to only two carbon signals. As there are no aromatic CH₂-carbons, this must mean that two of the carbons are identical, thus they have the same chemical environment.^{65e}

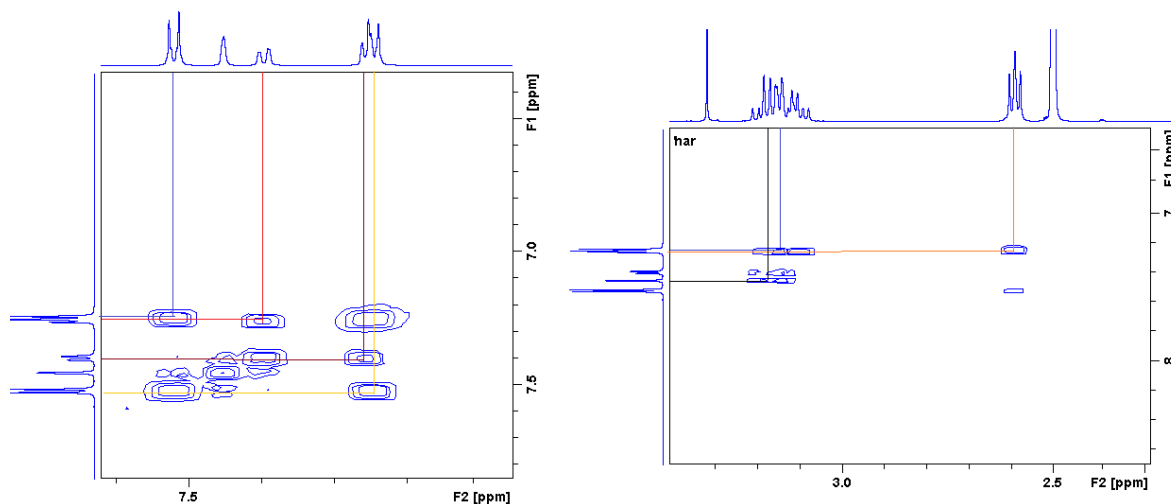


Figure 5.9: Left: Excerpt from the aromatic region of the COSY spectrum of **27a**. Right: Excerpt from the 2.5-3.6 ppm region of the COSY spectrum of **27**.

From the COSY spectrum, see Figure 5.9, one can observe that these protons couple to the signals at δ_{H} 7.52, 7.70, 3.08-3.21 and 2.59 ppm. As there is only one aromatic position where coupling to the protons at δ_{H} 2.59 is possible, and so this signal needs to stem from the protons at position 7. The two 7 positions are also chemically equivalent, which makes it possible that these two positions share one carbon signal at δ_{C} 128.8 ppm. The last proton in this signal must then be the one coupling to the other signals.

One of these signals are the protons at δ_{H} 3.08-3.21 ppm. These protons have previously been determined to be the protons in position 16 and 17. By observing that one of the CH_2 -groups couples with the signal at δ_{H} 7.4 ppm (position 12) in the COSY spectrum (Figure 5.9), it is fair to assume that one should be able to detect $^2\text{J}_{\text{C-H}} / ^3\text{J}_{\text{C-H}}$ coupling between the C_{16} and the proton in position 12. From Figure 5.10 one can observe that this is indeed the case. Coupling between the δ_{C} 36.2 ppm carbon and the protons at δ_{H} 7.25 ppm is also observed.

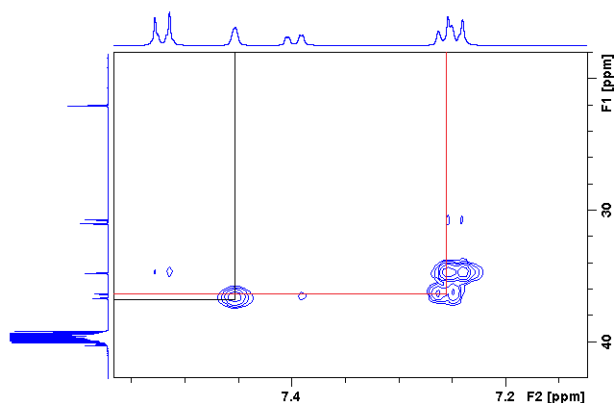


Figure 5.10: Excerpt from the 7.1-7.7 ppm region of the HMBC spectrum of **27a**.

Thus it can be determined with relative certainty that the carbon at δ_{C} 36.5 ppm may be placed in position 16 and that the carbon at δ_{C} 36.2 ppm then must be in position

17. As the last proton at δ_{H} 7.25 also couples with these protons, it must be in position 13. The signal at δ_{H} 7.52 (d, 2 H, $J = 8.2$ Hz) ppm couples with the protons at δ_{H} 7.4 in the COSY spectrum, and nothing else. This places them in either position 8 or 11. As the signal integrates to 2 protons, but only displays one $^1\text{J}_{\text{C-H}}$ coupling in the HSQC spectrum, the same arguments regarding chemical equivalency can be made for these protons as for the protons in position 7. This places the protons in position 8.

The last aromatic proton signal has a chemical shift of δ_{H} 7.4 (d, 1 H, $J = 8.2$) and couples with the protons at δ_{H} 7.25 (13), but nothing else. As position 8 is already taken, this must mean that these protons belong in position 11. See Table 5.2 for a summary of the assigned aromatic and indene liker protons.

Table 5.2: Summary of the assigned positions, chemical shifts, multiplicity and coupling constants for the indene linker and other aromatic protons/ carbons of **27a**.

Position	δ_{H} (ppm)	Multiplicity	J (Hz)	δ_{C} (ppm)
7	7.25	app. d	8.1	128.8
8	7.52	d	8.2	126.4
11	7.4	d	8.2	122.2
12	7.45	s	-	124.9
13	7.25	app. d	7.3	124.4

The last remaining protons in the ^1H NMR spectrum without an assigned position is the protons at δ_{H} 3.53, 3.46, 3.61 and 3.68 ppm. See Figure 5.11, for excerpt from the ^1H NMR and HSQC spectra.

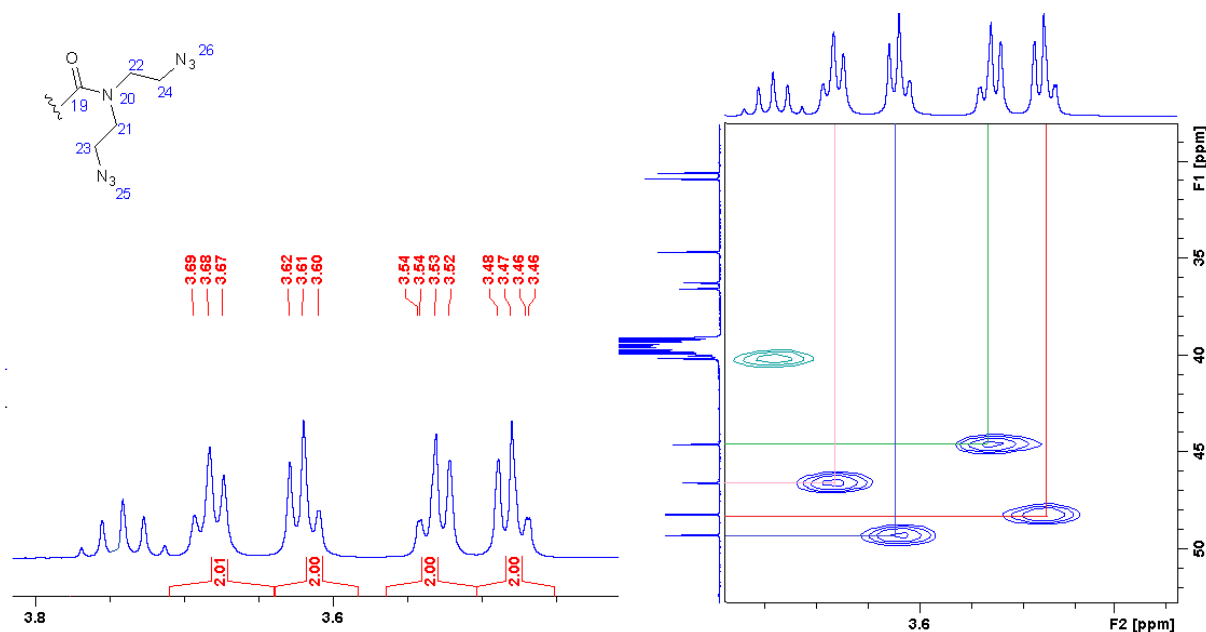


Figure 5.11: Left: excerpt from the ^1H NMR spectrum of **27a**, Right: excerpt from the HSQC spectrum of **27a**.

The only available positions with protons are positions 21-24. The four signals all integrate to 2 protons and couple with one carbon each (δ_{C} 44.6, 48.2, 49.3, 46.6, re-

spectably). The COSY spectrum, see Figure 5.12, indicates that they couple with each other in groups of two (δ_{H} 3.48/3.53 and δ_{H} 3.62/3.74) but nothing else.

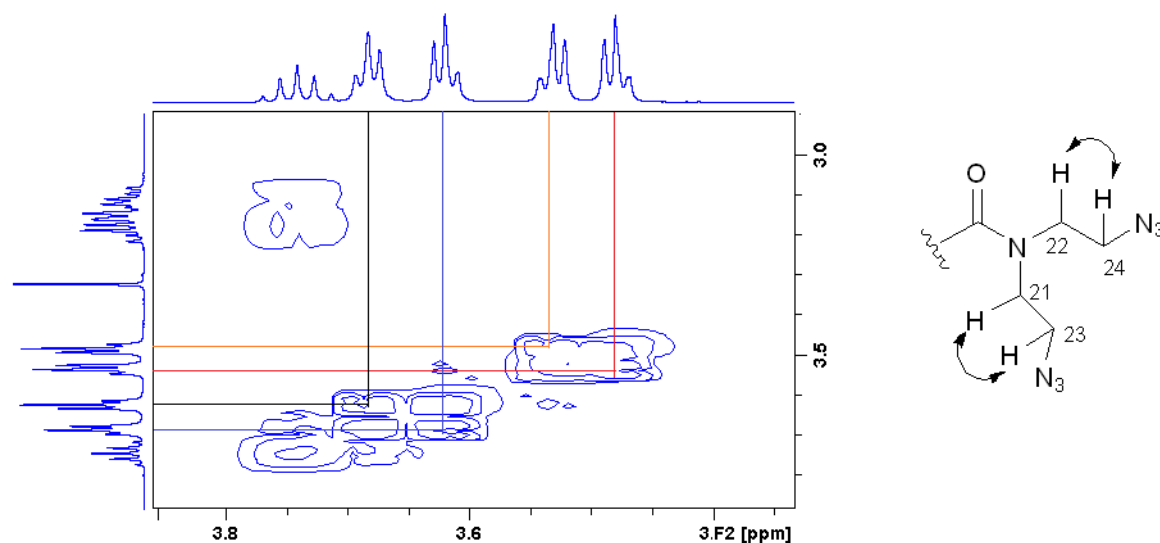


Figure 5.12: An excerpt from the 3.5-3.8 ppm region of the COSY spectrum of **27a** showing coupling between the different proton signals.

As there is no proton in positions 20 or 26, thus the COSY spectrum cannot help distinguish which protons are in which positions.

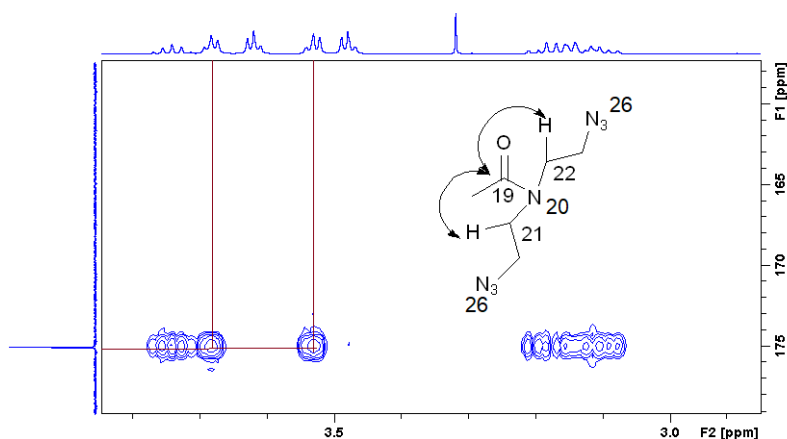


Figure 5.13: An excerpt from the 3-3.8 ppm region of the HMBC spectrum of **27a** displaying the $^2\text{J}_{\text{C-H}}$ / and $^3\text{J}_{\text{C-H}}$ couplings between the carbonyl carbon at δ_{C} 174.9 ppm and the protons with δ_{H} 3.53 and 3.74.

In Figure 5.13, one can notice that, there is $^3\text{J}_{\text{C-H}}$ coupling between the carbonyl carbon and two of the signals, namely δ_{H} 3.53 and 3.74. This suggests that these are the protons closest to the carbonyl carbon (position 21 and 22). From the COSY spectrum, see Figure 5.12 information on vicinal protons were gained, and so it can be determined that if the protons with a shift of δ_{H} 3.53 are in position 21 and the protons at δ_{H}

3.74 are in position 22, the protons at δ_{H} 3.48 and δ_{H} 3.62 are in position 23 and 24 respectively. However, it is impossible to tell with the currently available information, which protons are positioned at which "ethylene-arm". The δ_{H} 3.48/3.53 pair might be in positions 21/23, but they might also be in positions 22/24.

This will be a reoccurring theme in other synthesised molecules (**22b**, **23b**, **31b** and **32b**) where there exists two different chains originating from the same place.

All the tertiary carbons were identified by the absence of cross-peaks in the HSQC spectrum, and placed through $^2\text{J}_{\text{C-H}}$ / $^3\text{J}_{\text{C-H}}$ couplings in the HMBC spectrum. One example is the carbons in position 14 and 15. They have no directly attached protons, and thus should have no cross-peaks in the HSQC spectrum. The carbon at position 14 should have $^2\text{J}_{\text{C-H}}$ coupling with the protons at positions 12 and 16, while the carbon in position 15 should have $^2\text{J}_{\text{C-H}}$ coupling with the protons at in position 13 and 17. Looking at the HSQC and HMBC spectra, see Figure 5.14 and Figure 5.15, one can observe that the two ^{13}C NMR signals at 140.9 and 142.7 fits these criteria. None of them have cross-peaks connecting them to any protons in the HSQC spectrum, and in the HMBC spectrum δ_{C} 140.9 couples with the protons at δ_{H} 7.4 and 3.08-3.21, while δ_{C} 142.7 couples with signals at 7.25 and 3.08-3.21 ppm. This places them in positions 14 and 15 respectively.

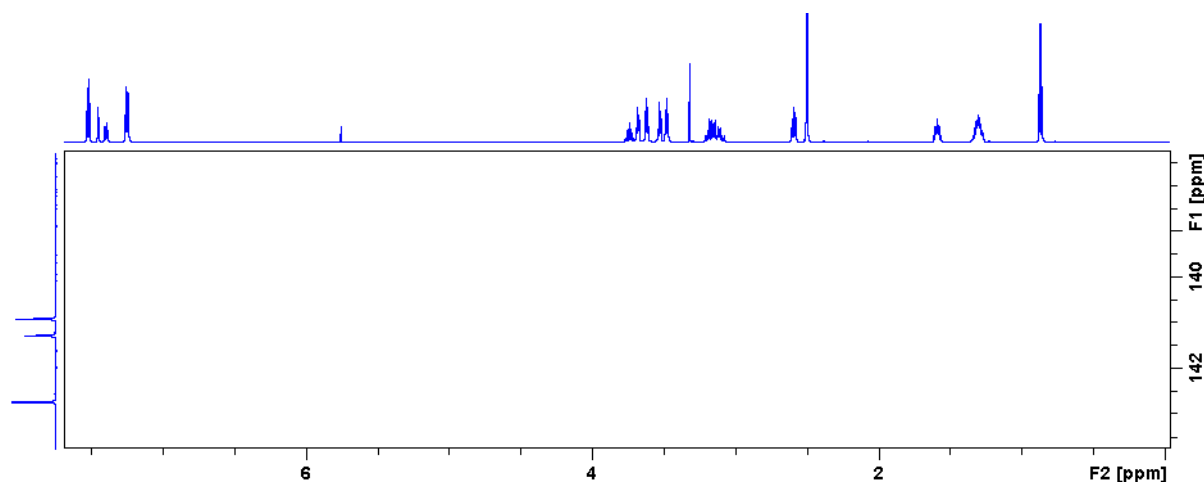


Figure 5.14: An excerpt from the HSQC spectrum of **27a** showing that the two carbons with shifts δ_{C} 140.9 and 142.7 ppm displays no $^1\text{J}_{\text{C-H}}$ coupling with any protons.

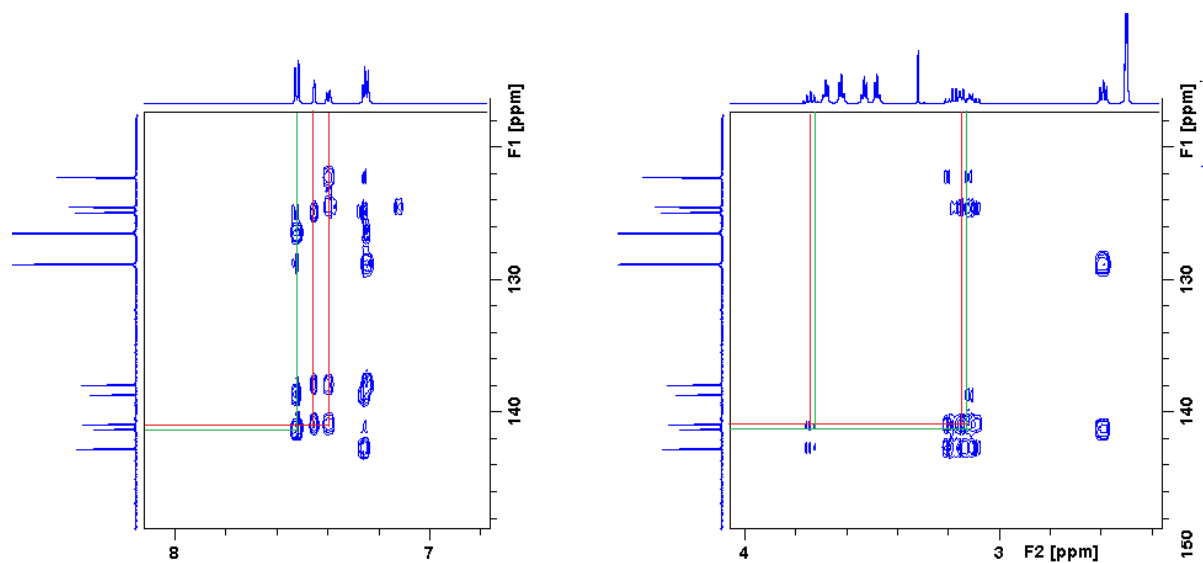


Figure 5.15: An excerpt from the 2.5-4 ppm and 6.8-8 ppm region of the HMBC spectrum of **27a** displaying the $^2J_{C-H}$ / and $^3J_{C-H}$ couplings between the two carbons at δ_C 142.6 and 140.8 ppm an different proton signals.

The rest of the tertiary carbons were placed using similar arguments. This concludes the elucidation of **27a** as an example, and the assignments are summarised in Table 5.7.

5.3 Special Cases

5.3.1 Hydrogen on Heteroatoms

Protons which are directly bonded to either oxygen, nitrogen or sulfur atoms differ from those bonded to carbon in that they are exchangeable and subjectable to hydrogen bonding.^{65g} This may affect their chemical shifts and the peaks appearance, and the change is dependant on temperature, concentration, solvent effects and rate of exchange.^{65g}

For protons directly bonded to nitrogen atoms, two factors are of major importance; the rate of exchange and the electric quadruple moment of the ^{14}N nucleus.^{65g} As the ^{14}N nucleus have a spin quantum number of 1, one would expect the proton attached to it and a vicinal proton to display a triplet pattern^{65d, g}. However, this might not always be the case, and the signals position and shape is as mentioned subject to change depending on i.e the rate of exchange. The rate of exchange may be rapid, intermediate or slow (relative to other signals), and these rates will have unique effects on the appearance of the spectra.

An example of slow to intermediate exchange rate is displayed by the guanidine group protons in **23b**. They appear as a broad signal along the baseline beneath the signals of the aromatic protons. When integrating the entire area, the result is nine protons. After eliminating the five aromatic protons, one is left with the four protons from the guanidine group. See Figure 5.16 for a excerpt from the ^1H NMR spectrum of **23b**. The original spectrum can be found in Appendix R.1.

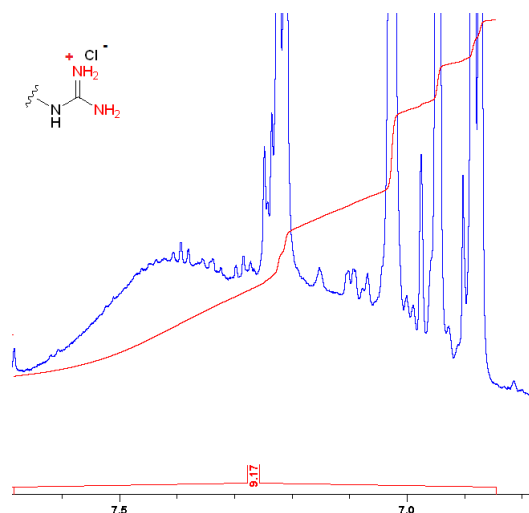
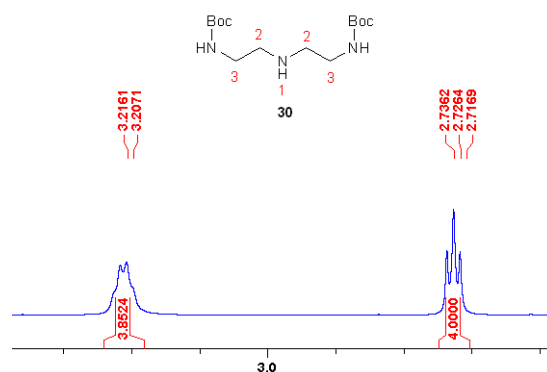


Figure 5.16: The H-N protons of the guanidine group in **23b** appearing as a broad peak blending in the baseline.

Another example of relative exchange rates can be observed in the ^1H NMR spectrum of **30** (Appendix AC.1), see Scheme 5.1 for an excerpt from the spectrum.



Scheme 5.1: Excerpt from the ^1H NMR spectrum of **30**, showing two different splitting patterns for two CH_2 -groups with the same amount of neighbours (3).

In Scheme 5.1 one can observe that the two signals stemming from the two CH_2 -groups in positions 2 and 3 display different splitting patterns in spite of having the same number of neighbours (3). This is due to the relative exchange rates of the H-N protons, where rapid and intermediate exchange rates make it so that no coupling between these protons and any vicinal protons is observed.^{65g} The splitting pattern of any vicinal protons would appear as though the H-N proton was not there at all, causing a decrease in the splitting pattern (i.e. a triplet instead of a quartet). This can be observed in Scheme 5.1, as the leftmost signal appears as an apparent quartet, while the rightmost signal is a clear triplet. Both H-N signals appear as singlets, see Figure 5.17, but vary in shape. The signal at δ_{H} 3.21 ppm are the protons in position 3, and these are the protons which appear to be coupled, indicating that the amide protons have a slower rate of exchange than the amine proton,^{65g} and thus they appear different.

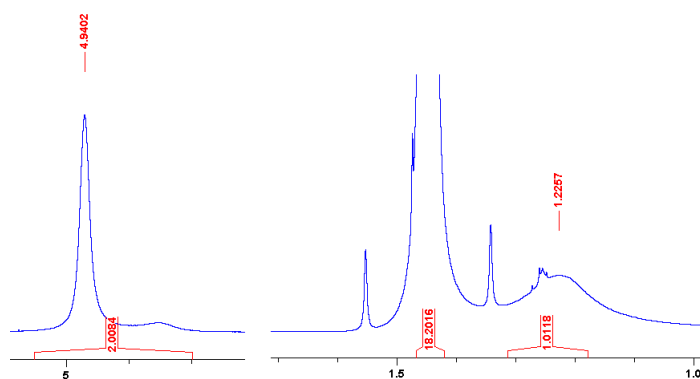


Figure 5.17: An excerpt from the ^1H NMR spectrum showing the H-N protons of Boc-protected amine **30**.

5.3.2 *i*-Pr groups in 22b, 23b, 31b, 32b

The expected splitting pattern for the *i*-Pr protons in position 1, 3, 7 and 9 would be a doublet (1 vicinal proton), however, this is not the case **22b**, **23b**, **31b** and **32b** with (Figure 5.18).

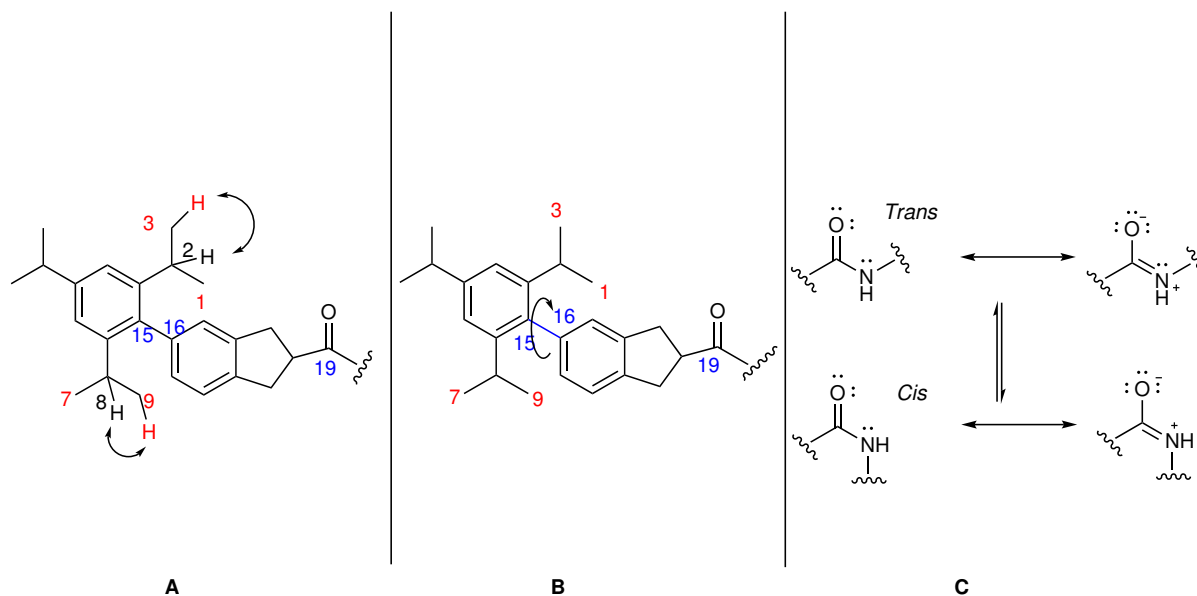


Figure 5.18: **A:** The $^1J_{C-H}$ coupling between the protons in position 1/ 3 and 7/ 9 with the protons in position 2 and 8 respectively. **B:** Restricted rotation of the C15-C16 bond. **C:** *Cis/Trans* configuration of the amide bond in position 19-20.

This might be due to restricted rotation about the C15-C16 bond (Figure 5.18, **B**), which can give rise to rotamers, present in different ratios. *Cis/trans*- configuration of over the amide bond in position 19/ 20 or other amide bonds (Boc) present in the molecule might also contribute (Figure 5.18, **C**), as one can observe the splitting pattern of the methyl groups in the *i*-Pr groups change depending on what the carbonyl carbon is attached to. See Figure 5.19 for an comparison of the between the 1H NMR spectra of **22b**, **23b**, **31b** and **32b**.

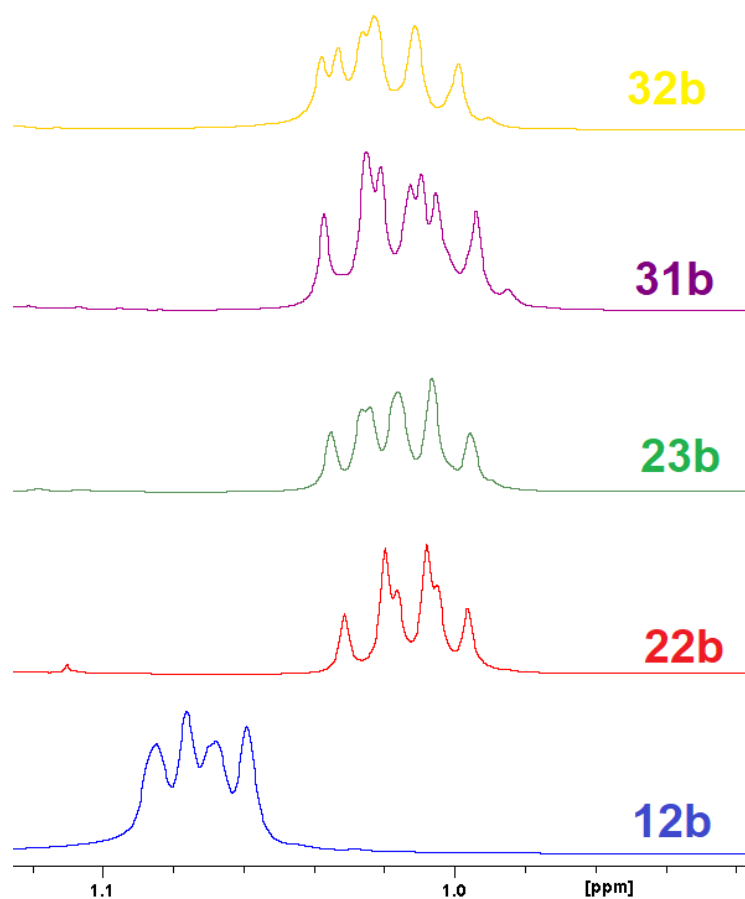
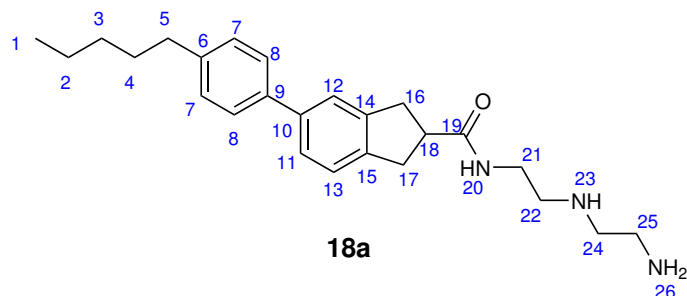


Figure 5.19: The different splitting patterns for the protons in position 1, 3, 7 and 9 in **22b**, **23b**, **31b** and **32b**.

5.3.3 Solvent Peaks

As the ^1H NMR spectrum gives a total overview of all the compounds present in a sample, the identification of potential impurities is important. Fulmer *et al.*⁶⁴ wrote an article listing chemical shifts of impurities stemming from common laboratory solvents and gases in deuterated solvents. This list was used extensively during this master project to separate between actual by-products and solvent residues.

5.4 Structural elucidation of *N*-(2-((2-aminoethyl)amino)ethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (18a)

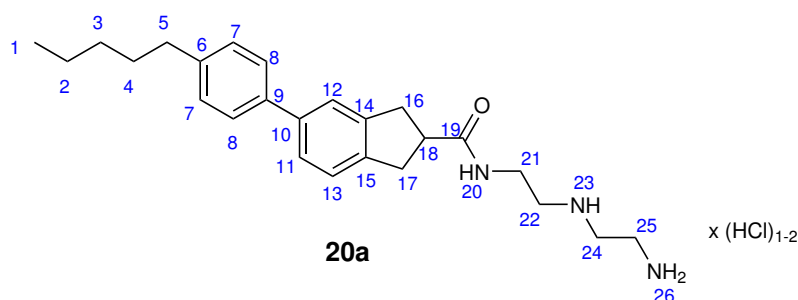


HRMS analysis confirmed the molecular formula $C_{25}H_{35}N_3O$, see Appendix N.7. The IR spectrum displayed peaks characteristic for amine, amide and carbonyl groups^{65f}, and can be found in Appendix N.6. As described in Chapter 5.3.1, the protons in position 23 and 26 appear as a broad singlet (s (br)) which disappear in the base line of the 1H NMR spectrum. The signal integrates to approx. 3 H, but shows no correlation in the COSY spectrum. This might be due to the rate of exchange of the H-N protons, as described in Chapter 5.3.1. 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR spectra, as well as the HRMS report can be found in Appendix N.

Table 5.3: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **18a**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.87	t	3 H	7.0	13.9
2	1.29-1.35	m	2 H	-	21.9
3	1.29-1.35	m	2 H	-	30.9
4	1.59	p	2 H	7.5	30.6
5	2.26-2.61	m	2 H	-	34.7
6	-	-	-	-	141.7
7	7.24	app. d	2 H	8.2	128.7
8	7.51	d	2 H	8.2	126.4
9	-	-	-	-	138.5
10	-	-	-	-	137.9
11	3.37	d	1 H	7.8	124.8
12	7.43	s	1 H	-	122.2
13	7.24	app. d	1 H	8.2	124.4
14	-	-	-	-	141.2
15	-	-	-	-	143.0
16	3.05-3.09	m	2 H	-	36.4
17	3.05-3.09	m	2 H	-	36.1
18	3.18-3.24	m	1	-	44.1
19	-	-	-	-	174.0
20	7.91	t	1 H	5.5	-
21	3.14-3.18	m	2 H	-	40.1
22	2.56-2.61	m	2 H	-	41.5/48.6
23	1.62-1.96	s (br)	1 H	-	-
24	2.56-2.61	m	2 H	-	41.5/48.6
25	2.48-2.51	m	2 H	-	52.2
26	1.62-1.96	s (br)	2 H	-	-

5.5 Structural elucidation of 2-((2-(5-(4-pentylphenyl)-2,3-dihydro-1H-indene-2-carboxamido) ethyl)amino) - ethan-1-aminium chloride ((20a))

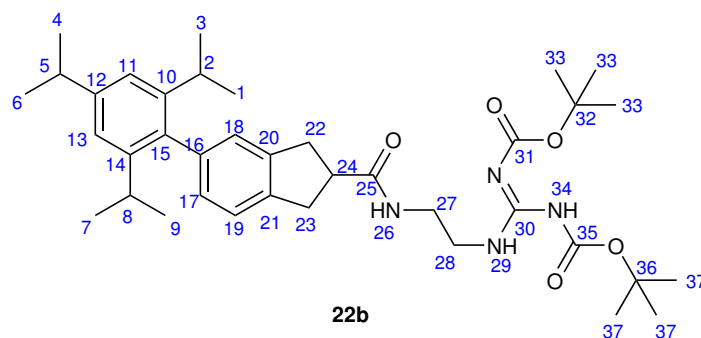


HRMS analysis confirmed the molecular formula $C_{25}H_{36}N_3O$, see Appendix P.7. The IR spectrum displayed peaks characteristic for a primary amine salt and a carbonyl group^{65f}, and can be found in Appendix P.6. As described in Chapter 5.3.1, the protons in position 23 and 26 appears as a broad signal which disappears in the baseline in the aromatic region of the 1H NMR spectrum of HCl-salt of **20a**. The signal integrates to approx. 4 H, and overlaps with the amide proton signal. Despite this, it is possible to determine that these protons show no $^1J_{H-H}$ correlations in the COSY spectrum. This might be due to the rate of exchange of the H-N protons, as described in Chapter 5.3.1. 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as HRMS report and HPLC chromatogram can be found in Appendix P.

Table 5.4: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **20a**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.87	app.t	3 H	6.8	13.9
2	1.24-1.64	m	2 H	-	21.9
3	1.24-1.64	m	2 H	-	30.9
4	1.54-1.64	m	2 H	-	30.6
5	2.59	t	2 H	7.4	34.7
6	-	-	-	-	141.2
7	7.24	app. d	2 H	7.5	128.8
8	7.51	d	2 H	7.8	126.4
9	-	-	-	-	138.6
10	-	-	-	-	137.9
11	7.38	d	1 H	7.5, 124.8	
12	7.44	s	1 H	-	122.2
13	7.24	app. d	1 H	7.5	124.5
14	-	-	-	-	141.0
15	-	-	-	-	142.8
16	3.01-3.29	m	2 H	-	36.2
17	3.01-3.29	m	2 H	-	35.9
18	3.01-3.29	m	1 H	-	44.2
19	-	-	-	-	174.8
20	8.3	app. t	1 H	4.3	-
21	3.38-3.47	m	2 H	-	35.3
22	3.01-3.29	m	2 H	-	46.4
23	7.7-9.87	br	1/2 H	-	-
24	3.01-3.29	m	2 H	-	35.4/44.1
25	3.01-3.29	m	2 H	-	35.4/44.1
26	7.7-9.87	br	3/2 H	-	-

5.6 Structural elucidation of Bis-Boc(amino(2-(5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxamido) ethyl)amino))guanidine (22b)

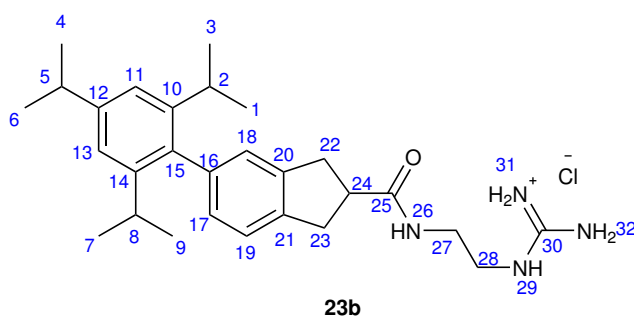


HRMS analysis confirmed the molecular formula $C_{38}H_{56}N_4O_5$, see Appendix Q.8. The IR spectrum displayed peaks characteristic for amides and two carbonyl groups^{65f}, and can be found in Appendix Q.7. Some difficulties were observed when elucidating the structure of **22b**. Some of the challenge was addressed in Chapter 5.3.2), however, there were also other challenges. Firstly, the $^1J_{H-H}$ correlations on the *i*-Pr groups were challenging to determine accurately. In addition to the challenges portrayed in Chapter 5.3.2) Positions 1, 3, 7 and 9 differ from positions 4 and 6, but the correlation peaks were too wide to attach specific carbons to specific protons. By running a selective HSQC experiment, with the F1 range set to 22-26 ppm, it should be possible to assign different carbons to the different proton signals. The same problems occurred when determining positions 10 and 14. The cross peak in the HMBC is so broad that it covers both carbon signals, making it very challenging to determine which carbon is positioned where. The same goes for the protons and carbons in positions 33 and 37. As with the two indistinguishable ethylene arms in amido-bisamine **27a**, it is difficult to determine which protons/carbons are in position 32/36 and positions 33/37. It is only possible to determine the *t*-Bu group pairs (δ_H 1.38 / δ_C 78.6 and δ_H 1.46 / δ_C 83.3) but not if they're in position 32/33 or position 36/37. 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as the HRMS report can be found in Appendix Q.

Table 5.5: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **22b**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.99-1.03	m	3 H	-	23.9
2	2.47-2.51/ 2.51-2.57	m	1 H	-	29.6
3	0.99-1.03	m	3 H	-	23.9
4	1.22	d	3 H	6.9	24.0
5	2.88	sept.	1 H	6.9	33.6
6	1.22	d	3 H	6.9	24.0
7	0.99-1.03	m	3 H	-	23.9
8	2.47-2.51/ 2.51-2.57	m	1 H	-	29.6
9	0.99-1.03	m	3 H	-	23.9
10	-	-	-	-	145.9/146.0
11	7.023/7.027	app. s	1 H	-	120.0
12	-	-	-	-	147.2
13	7.023/7.027	app. s	1 H	-	120.0
14	-	-	-	-	145.9/146.0
15	-	-	-	-	137.0
16	-	-	-	-	138.3
17	6.86	d	1 H	7.5	127.5
18	6.91	s	1 H	-	124.8
19	7.19	d	1 H	7.6	123.7
20	-	-	-	-	140.3
21	-	-	-	-	141.9
22	3.06-3.11	m	2 H	-	36.4
23	3.06-3.11	m	2 H	-	36.0
24	3.19-3.29	m	1 H	-	44.2
25	-	-	-	-	174.4
24	8.1	t	1 H	5.5	-
27	3.19-3.29	m	2 H	-	38.1
28	3.39-3.43	m	2 H	-	40.1
29	8.41	t	1 H	5.8	-
30	-	-	-	-	163.1
31	-	-	-	-	155.7
32	-	-	-	-	78.1/ 82.8
33	1.38/1.46	s	9 H	-	27.9/27.6
34	11.49	s	1 H	-	-
35	-	-	-	-	151.9
36	-	-	-	-	78.1/ 82.8
37	1.38/1.46	s	9	-	27.9/27.6

5.7 Structural elucidation of amino((2-(5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxamido)ethyl) amino)methaniminium chloride (23b)



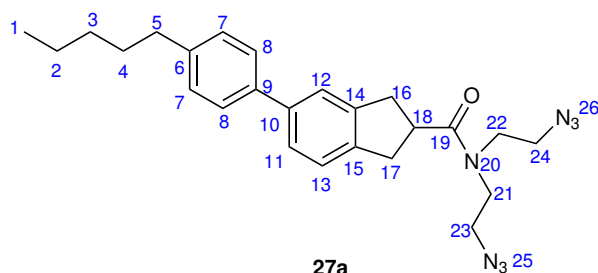
HRMS analysis confirmed the molecular formula $C_{28}H_{41}N_4O$, (see Appendix R.8). The IR spectrum displayed peaks characteristic for amide, amine salts, imine and carbonyl group,^{65,79f} and can be found in Appendix R.7.

The same difficulties regarding positions 1, 3, 7, 9, 10 and 14 mentioned in Chapters 5.3.2 and 5.5 were encountered with this compound. The solutions is also the same. As mentioned in Chapter 5.3.1, the guanidine protons were observed as a broad peak appearing near the baseline in the aromatic region. They are reported in Table 5.6 as δ_H 6.84-7.68 (br, 4 H). 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as HRMS report and HPLC chromatogram can be found in Appendix R.

Table 5.6: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **23b**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.99-1.03	m	3 H	-	23.9
2	2.47-2.56	m	1 H	-	29.7
3	0.99-1.03	m	3 H	-	23.9
4	1.22	d	3 H	6.9	24.0
5	2.88	app. sept.	1 H	8.3	33.6
6	1.22	d	3 H	6.9	24.0
7	0.99-1.03	m	3 H	-	23.9
8	2.47-2.56	m	1 H	-	29.7
9	0.99-1.03	m	3 H	-	23.9
10	-	-	-	-	145.9/146.0
11	7.02	s	1 H	-	120.0
12	-	-	-	-	147.2
13	7.02	s	1 H	-	120.0
14	-	-	-	-	145.9/146.0
15	-	-	-	-	137.0
16	-	-	-	-	138.3
17	6.87	d	1 H	7.6	127.5
18	6.94	s	1 H	-	124.9
19	7.22	d	1 H	7.6	123.7
20	-	-	-	-	140.3
21	-	-	-	-	141.8
22	3.09-3.14	m	2 H	-	36.3
23	3.09-3.14	m	2 H	-	36.1
24	3.23-3.31	m	1 H	-	43.9
25	-	-	-	-	174.9
26	8.25	s	1 H	-	-
27	3.23-3.31	m	2 H	-	38.1
28	3.23-3.31	m	2 H	-	40.4
29	7.71	s	1 H	-	-
30	-	-	-	-	157.2
31	6.84-7.68	br	2 H	-	-
32	6.84-7.68	br	2 H	-	-

5.8 *N,N*-bis(2-azidoethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (27a)



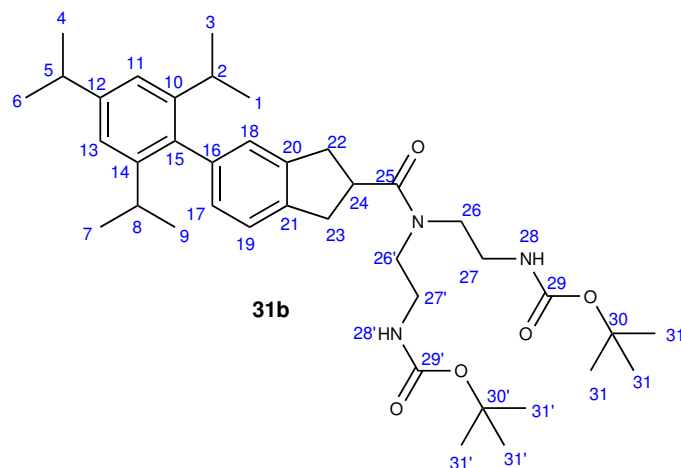
HRMS analysis confirmed the molecular formula $C_{25}H_{32}N_7O$, (see Appendix V.7). The IR spectrum displayed peaks characteristic for azide and carbonyl groups^{65,79f}, and can be found in Appendix V.6.

As mentioned in Chapter 5.2 it was challenging to determine which CH_2 -pairs are positioned on which ethylene arm. The δ_H 3.48/3.53 pair might be in positions 21/23, with δ_H 3.74/3.62 in position 22/24, or vice versa. With the current available information, this cannot be determined with any confidence. 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as HRMS report and HPLC chromatogram can be found in Appendix V.

Table 5.7: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **27a**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.87	t	3 H	7.1	13.9
2	1.27-1.35	m	2 H	-	22.0
3	1.27-1.35	m	2 H	-	30.9
4	1.59	p	2 H	7.5	30.6
5	2.59	t	2 H	7.6	34.7
6	-	-	-	-	141.2
7	7.25	d	2 H	8.1	128.8
8	7.52	app. d	2 H	8.2	126.4
9	-	-	-	-	138.6
10	-	-	-	-	137.9
11	7.4	app. d	1 H	8.2	122.2
12	7.45	s	1 H	-	124.9
13	7.25	d	1 H	7.3	124.4
14	-	-	-	-	140.9
15	-	-	-	-	142.6
16	3.08-3.21	m	2 H	-	36.6
17	3.08-3.21	m	2 H	-	36.3
18	3.74	p	1 H	8.38	40.2
19	-	-	-	-	175.0
20	-	-	-	-	Nitrogen
21	3.53 / 3.74	m	2 H	-	44.6/46.6
22	3.53 / 3.74	m	2 H	-	44.6/46.6
23	3.48 / 3.62	m	2 H	-	48.2/49.3
24	3.48 / 3.62	m	2 H	-	48.2/49.3
25	-	-	-	-	Nitrogen
26	-	-	-	-	Nitrogen

5.9 Structural elucidation of di-tert-butyl (((5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1*H*-indene-2-carbonyl) azanediyl)bis(ethane-2,1-diyl)) dicarbamate (31b)



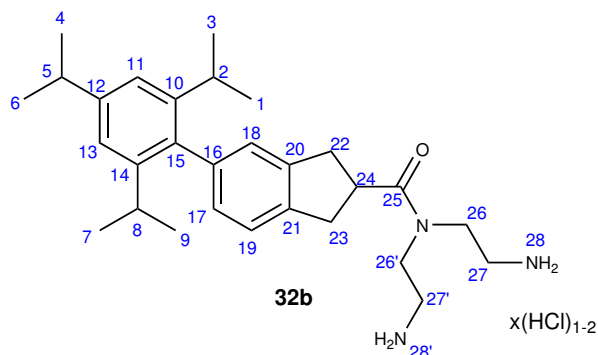
HRMS analysis confirmed the molecular formula $C_{39}H_{59}N_3O_5$, see Appendix AD.8). The IR spectrum displayed peaks characteristic for amide, esters and carbonyl groups^{65f}, and can be found in Appendix AD.7.

The same previously mentioned problems regarding positions 1,3,7,9, 10 and 14 also apply with this compound. In addition the positions 26-31 and 26'-31' share the same conditions as mentioned in Chapter 5.2, where all the groups of proton/carbon can be determined, but not if they belong in the 26-31 positions or the 26'-31' positions. 1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as HRMS report can be found in Appendix AD.

Table 5.8: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **31b**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.99-1.03	m	3 H	-	23.9
2	2.45-2.62	m	1 H	-	29.6/29.7
3	0.99-1.03	m	3 H	-	23.9
4	1.22-1.24	d	3 H	6.9	24.0
5	3.03-3.2	app. sept	1 H	10.3	33.6
6	1.22-1.24	d	3 H	6.9	24.0
7	0.99-1.03	m	3 H	-	23.9
8	2.45-2.62	m	1 H	-	29.6/29.7
9	0.99-1.03	m	3 H	-	23.9
10	-	-	-	-	145.9/146.0
11	7.03	s	1 H	-	119.9/120.0
12	-	-	-	-	147.2
13	7.03	s	1 H	5.9	119.9/120.0
14	-	-	-	-	145.9/146.0
15	-	-	-	-	137.0
16	-	-	-	-	138.3
17	6.87-6.9	m	1 H	-	127.5
18	6.92	s	1 H	-	124.8
19	7.18-7.23	m	1 H	-	123.6
20	-	-	-	-	140.1
21	-	-	-	-	141.8
22	3.03-3.2	m	2 H	-	36.7
23	3.03-3.2	m	2 H	-	36.6
24	3.59-3.69	1 H	-	40.1	
25	m	-	-	-	174.5
26	3.29-3.33/ 3.36-3.5	m	2 H	-	45.5/47.3
26'	3.29-3.33/ 3.36-3.5	m	2 H	-	45.5/47.3
27	3.03-3.2	m	2 H	-	37.9/38.7
27'	3.03-3.2	m	2 H	-	37.9/38.7
28	6.83/7.0	t	1 H	5.4/5.9	-
28'	6.83/7.0	t	1 H	5.4/5.9	-
29	-	-	-	-	155.6
29'	-	-	-	-	155.6
30	-	-	-	-	77.6/77.8
30'	-	-	-	-	77.6/77.8
31	1.33/1.37	s	9 H	-	28.1/28.2
31'	1.33/1.37	s	9 H	-	28.1/28.2

5.10 Structural elucidation of 2,2'-((5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carbonyl)-azanediyl)bis(ethan-1-aminium chloride) (32b)



HRMS analysis confirmed the molecular formula C₂₉H₄₄N₃O, see Appendix AE.8). The IR spectrum displayed peaks characteristic for amide, amine salts and carbonyl groups^{65f}, and can be found in Appendix AE.7.

The same previously mentioned problems regarding positions 1,3,7,9, 10 and 14 also apply with this compound. In addition the positions 26-28 and 26'-28' share the same conditions as mentioned in Chapter 5.2, where all the groups of proton/carbon can be determined, but not if they belong in the 26-28 positions or the 26'-28' positions. ¹H NMR, ¹³C NMR, COSY, HSQC, HMBC and IR spectra, as well as HRMS report and HPLC chromatogram can be found in Appendix AE.

Table 5.9: Assignment of ^1H (600 MHz, DMSO- d_6) and ^{13}C (150 MHz, DMSO- d_6) NMR shifts, multiplicity, integrals and coupling constants (Hz) for **32b**.

Position	δ_{H} [ppm]	Multiplicity	Integral	J [Hz]	δ_{C} [ppm]
1	0.99-1.03	m	3 H	-	23.9
2	2.45-59	m	1 H	-	29.7
3	0.99-1.03	m	3 H	-	23.9
4	1.22	d	3 H	6.9	24.0
5	2.88	app. sept	1 H	6.9	33.6
6	1.22	d	3 H	6.9	24.0
7	0.99-1.03	m	3 H	-	23.9
8	2.45-2.59	m	1 H	-	29.7
9	0.99-1.03	m	3 H	-	23.9
10	-	-	-	-	145.9/146.0
11	7.03	s	1 H	-	119.9/120.0
12	-	-	-	-	147.3
13	7.03	s	1 H	-	119.9/120.0
14	-	-	-	-	145.9/146.0
15	-	-	-	-	136.9
16	-	-	-	-	138.4
17	6.89	d	1 H	7.5	127.6
18	6.96	s	1 H	-	124.9
19	7.21-7.28	m	1 H	-	123.7
20	-	-	-	-	140.0
21	-	-	-	-	141.6
22	3.17-3.24	m	2 H	-	36.6
23	3.17-3.24	m	2 H	-	36.4
24	3.71-3.81	m	1 H	-	40.2
25	-	-	-	-	175.8
26	3.53-3.63/ 3.71-3.81	2 H	m	-	43.4/44.9
26'	3.53-3.63/ 3.71-3.81	2 H	m	-	43.4/44.9
27	2.97/3.06	s (br)	2 H	-	37.2/37.4
27'	2.97/3.06	s (br)	2 H	-	37.2/37.4
28	8.04/8.25	s	2/3 H	-	-
28'	8.04/8.25	s	2/3 H	-	-

6 Experimental

6.1 General information

All chemicals used were bought from Sigma-Aldrich and VWR Chemicals, and were used without further purification. The exception was Amberlyst[®] 15, which was washed thoroughly with MeOH before use. Air and/or moisture sensitive reactions were performed under nitrogen atmosphere, with dried reagents and solvents. DCM used in sensitive reactions were purified in and collected from a MBraun-SPS-800 membrane filtration system. Anhydrous DCE was purchased from Sigma Aldrich and used as is. A Vacuum Atmosphere Company (VAC) HE-493 glove box was used for handling the air and moisture sensitive catalyst Cp^{*}RuCl(cod). Degassing of solvents with helium were performed for 20-30 minutes when necessary.

TLC-analysis were performed on Merck silica gel 60 F₂₅₄ plates, using a Vilber Lourmat CN-6 UV instrument, with UV set to 312 nm for detection. To further visualise the results, chemical oxidation with phosphomolybdenic acid solution (12 g phosphomolybdenic acid in 250 mL EtOH (96%)) was also used. Column chromatography was performed with Silica gel (60 Å pore size, 200-400 mesh particle size) purchased from VWR Chemicals.

Purity assessment was performed with a Agilent Technologies Infinity 1260 HPLC binary LC system with an autosampler and a Zorbax Eclipse XDB-C18 5µ(150 x 4.6 mm) column. Detection was performed with a diode array detector (214 nm) and recorded chromatograms were processed in Agilent ChemStation LC software program.

Melting points were determined with a Gallenkamp FUSE F1A melting point apparatus. ¹H NMR, ¹³C NMR as well as 2D experiments were performed on either a 400 MHz Bruker Avance III HD NMR spectrometer from Nanaobay electronics with a smartprobe 5mm probehead (1H, 15N, 31P, 13C, 19F, 11B) or a 600 MHz Ultrashielded Bruker Avance III HD NMR spectrometer with a CryoProbe 5 mm (¹H, ¹³C, ¹³N) with Z gradients. The spectra generated were analysed with TopSpin 4.0.6 software. The chemical shifts (δ) are given in ppm and the integrals as number of protons (H) per signal. When CDCl₃ was used as a solvent with TMS, both the TMS-shifts for both protons and carbons were set to 0.00. When DMSO-d₆ was used, the shifts were calibrated according to the shifts presented in Fulmer et al⁶⁴ (¹H NMR: 2.50, ¹³C NMR: 39.52). The signal patterns are given as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septett), m (multiplet), br (broad). The coupling constant, J, is reported in Hz.

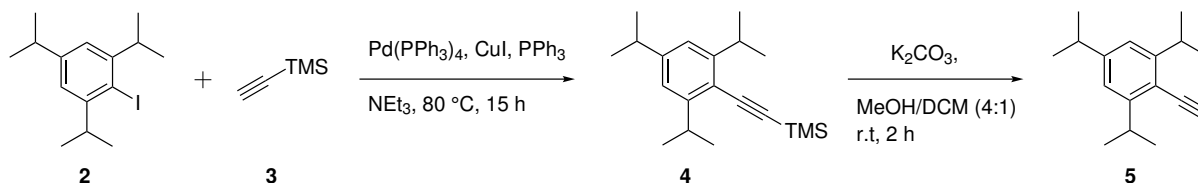
The IR-spectra were recorded on a Bruker ALPHA ECO-ATR instrument and processed in OPUS v.25 software program. The spectra were interpreted using IR absorption tables. The signals are indicated as s (strong), m (medium), w (weak), br (broad), sh (sharp).

Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters TM. Samples were ionized by the use of ASAP

probe (APCI) or ESI probe. No chromatographic separation was used previous to the mass analysis. Calculated exact mass and spectra processing was done by Waters TM Software Masslynx V4.1 SCN871.

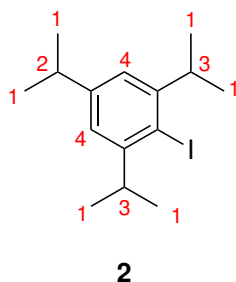
6.2 Preparation of monoalkyne reagent 5

Monoalkyne **5** was synthesised following the procedure described by Cresswell *et al.*³⁴ and Zhang *et al.*⁸¹ Iodo-aryl **2** was prepared once in a large scale (15 g), while TMS-intermediate **4** and monoalkyne **5** were prepared twice on a smaller scale. Experimental procedure, work-up, characterisation and yields are described under each experiment. See Scheme 6.1 for the reaction path.



Scheme 6.1: Synthesis of the monoalkynereagent **5**. Original articles are by Cresswell *et al.*³⁴ (synthesis of **2**) and Zhang *et al.*⁸¹ (synthesis of **4** and **5**).

6.2.1 Synthesis of 2-iodo-1,3,5-triisopropylbenzene (**2**)



SelectFluor[®] (13.3 g, 0.75 eq.) was added to a mixture of **1** (12.1 ml, 50 mmol, 1 eq.), I₂ (9.54, 0.75 eq.) in MeCN (500 ml). The mixture was heated to 65 °C and stirred for 4 h. Excess solvent was removed in vacuo, and the crude product was titrated with Et₂O (3 x 100 mL). Na₂SO₃ (100 ml, sat., aq.) was added to the filtrate, and the layers were separated. The aqueous phase was then extracted with Et₂O (2 x 50 ml), and the combined organic phases were dried with anhydrous MgSO₄ and concentrated in vacuo. The crude product was purified with a silica plug (1:19 EtOAc/n-pentane), which afforded **2** as a golden liquid. The spectrum was in accordance with the literature,³⁴ and can be found in Appendix A. Yield: 93% (15.3 g, 46 mmol). Spectroscopic data for **2**: ¹H NMR (400 MHz, CDCl₃): δ 1.23 (dd, 18 H, J = 6.8 Hz, 6.9 Hz, H-1), 2.86 (sept., 1 H, J = 6.9 Hz, H-2), 3.39 (sept., 2 H, J = 6.8 Hz, H-3), 6.94 (s, 2 H, H-4).

6.2.2 Synthesis of trimethyl((2,4,6-triisopropylphenyl)ethynyl)silane (**4**)

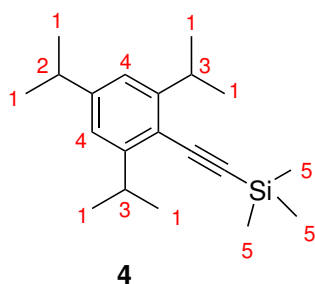


Table 6.1: Experimental data for the preparation of compound **4**.

Entry	2 (g)	Pd(PPh ₃) ₄ (g)	CuI (g)	PPh ₃ (g)	3 (ml)	Yield (%)
1	4.07	0.73	0.09	0.12	2.5	29*
2	4.00	0.70	0.08	0.12	2.52	91

* Yield calculated from the ¹H NMR spectra (Appendix B.2)

The NEt₃ (80 ml) and **3** (2.52 ml, 0.02 mol) was mixed and degassed with He-gas for approx. 30 min. The catalyst Pd(PPh₃)₄ (0.70 g, 0.6 mmol), PPh₃ (0.12 g, 0.48 mmol), CuI (0.08 g, 4.2 mmol), and **2** (4.00 g, 0.01 mol) was weighed out and placed under N₂-atmosphere. After the degassing, the TEA and **3** mix was added to the reaction mix by a cannula and the rx-mixture was heated to 80 °C. The reaction was monitored with TLC-analysis and stopped after 14.5 h. The excess solvents were removed in vacuo, and the crude was filtered through a thin pad of Celite[®] using EtOAc as solvent. The crude was then purified by flash column chromatography (DCM/n-pentane (2:1)), yielding **4** as a dark yellow oil. The resulting ¹H NMR spectra was matched to the literature data⁸¹ and can be found in Appendix B. Yield: 91% (3.3 g, 11 mmol). Spectroscopic data for **4**: ¹H-NMR (400 MHz, CDCl₃): δ0.24 (s, 9 H, H-5), 1.24 (dd, 18 H, J = 6.9 Hz, J = 6.9 Hz, H-1), 2.94 (sept., 1 H, J = 6.9 Hz, H-2), 3.57 (sept., 2 H, J = 6.9 Hz, H-3), 7.02 (s, 2 H, H-4).

6.2.3 Synthesis of 2-ethynyl-1,3,5-triisopropylbenzene (5)

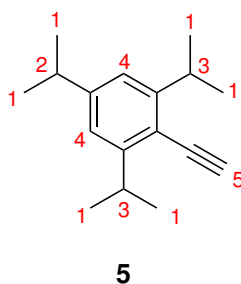


Table 6.2: Experimental data for the preparation of compound 5.

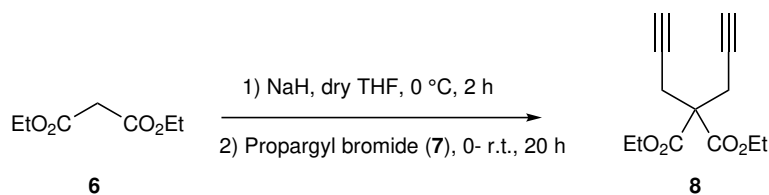
Entry	4 (g)	K ₂ CO ₃ (g)	Time (h)	Yield (%)
1	1.66	1.8	1	40*
2	2.97	2.3 + 0.55	1 + 1	70

* Yield calculated from the ¹H NMR spectrum (Appendix D.1)

Compound 4 was mixed with K₂CO₃ (2.3 g, 16.6 mmol) and dissolved in MeOH/DCM (100 ml, 4:1) and stirred for 1 h at room temperature. The reaction mixture was then diluted with DCM (100 ml) and washed with NH₄Cl (aq., sat.) (200 ml x 3) and water (100 ml), dried over MgSO₄ and concentrated in vacuo. ¹H NMR of the resulting pale yellow oil indicated incomplete reaction (5:4 = 80:20). The product mixture was then added to K₂CO₃ (0.55 g, 3.97 mmol) and dissolved in MeOH/DCM (50 ml, 4:1) and stirred for 1 h at room temperature. The work-up followed the same procedure as earlier, with the same amounts of solvents. The resulting product presented as a pale yellow oil. The ¹H NMR spectra indicated full conversion and was matched to the literature.⁸¹ The ¹H NMR spectrum can be found in Appendix C Yield: 65 % (1.56 g, 6.9 mmol). Spectroscopic data for 5: ¹H-NMR (400 MHz, CDCl₃): δ1.26 (dd, 18H, J = 6.8 Hz, J = 6.9 Hz, H-1), 2.88 (sept., 1 H, J = 6.9 Hz, H-2), 3.4 (s, 1 H, H-5) 3.53 (sept., 2 H, J = 6.9 Hz, H-3), 6.9 (s, 2 H, H-4)

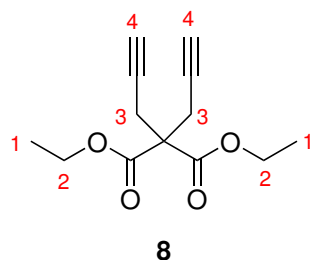
6.3 Synthesis of the terminal diyne (8)

The synthesis of the desired product 8 was performed once as described by D. Lindberg¹³ and Mandal *et al.*⁵⁹ Experimental procedure, work-up, characterisation and yields are described under each experiment. See Scheme 6.2 for the reaction equation.



Scheme 6.2: The reaction conditions for the synthesis of **8**.

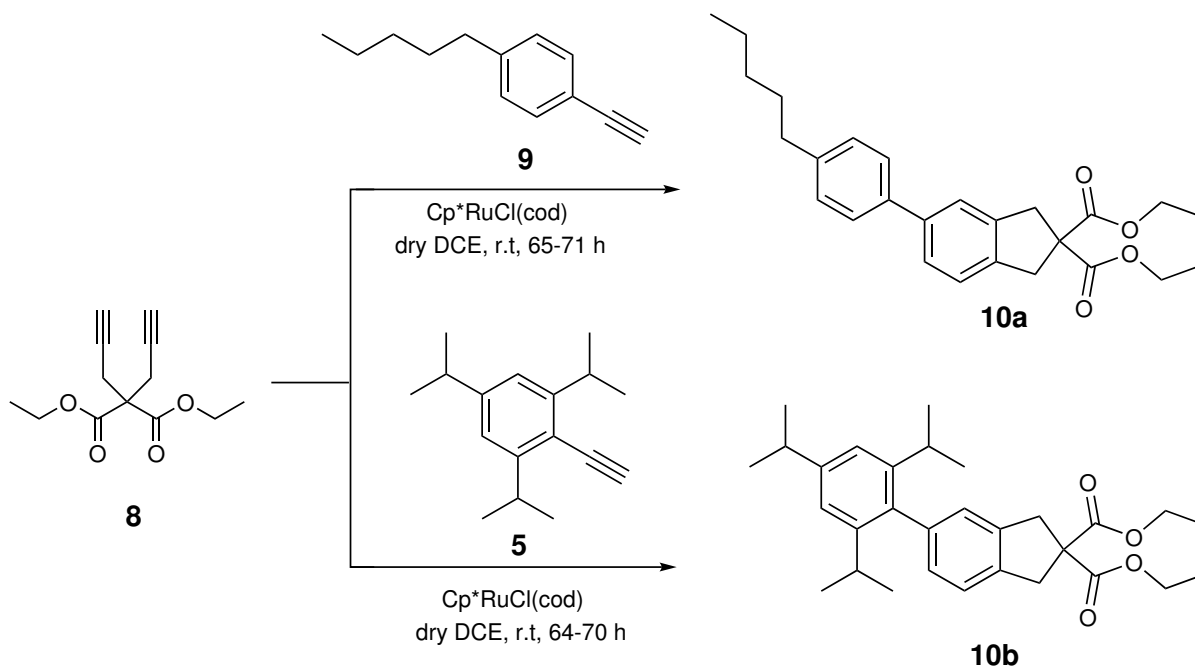
6.3.1 Synthesis of diethyl 2,2-di(prop-2-yn-1-yl)malonate (**8**)



The diester **6** (7.51 mL, 49.4 mmol, 1 eq.) was added dropwise over the course of 1 h to a stirred cooled suspension of NaH (3.54 g, 0.15 mol, 3 eq.) in dry THF (120 mL). Heat development and gas evolution were observed. The solution was stirred vigorously at 0 °C for 2 h. White precipitate was observed after 1 h. Cooled **7** (80% in toluene, 14.8 mL, 0.17 mol, 3.3 eq.) was added dropwise, and the now pale yellow reaction mixture was allowed to reach room temperature. The reaction was left stirring at r.t for 20 h, when TLC-analysis (10% EtOAc in n-pentane) indicated that all the starting material had reacted. The reaction mixture was now a pale beige color. The reaction was stopped by adding NH₄Cl (aq., sat., 200 mL) and water (100 mL) to the reaction mixture. The aqueous phase was extracted with EtOAc (20 mL x 4), the combined organic phases were dried over MgSO₄, and the solvent removed in vacuo. The bright orange crude product was distilled using vacuum distillation (b.p.: 1.6 mbar/108-110 °C). This gave **8** as clear liquid, which crystallised to a hard transparent solid upon cooling to r.t. The ¹H NMR data and melting point was matched to the literature.¹³ The ¹H NMR spectrum can be found in Appendix E.1. Yield: 69% (8.03 g, 34 mmol). Melting point: 45-46 °C. Spectroscopic data for **8**: ¹H NMR (400 MHz, CDCl₃): δ 1.26 (t, 6 H, J = 7.1 Hz, H-1), 2.02 (t, 2 H, J = 2.6 Hz, H-4), 2.99 (d, 4 H, J = 2.6 Hz, H-3), 4.23 (q, 4 H, J = 7.1 Hz, H-2).

6.4 [2+2+2] Cycloaddition

The diesters **10a** and **10b** were synthesised as described by the literature.^{38,39} See 2.2.3 for the reaction mechanism. Experimental procedure, work-up, characterisation and yields are described under each experiment. See Scheme 6.3 for the reaction equation.



Scheme 6.3: Reaction equation for the synthesis of the diesters **10a** and **10b**.

6.4.1 Synthesis of diethyl 5-(4-pentylphenyl)-1,3-dihydro-2*H*-indene-2,2-dicarboxylate (**10a**)

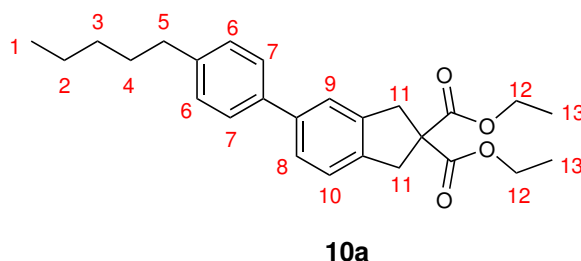


Table 6.3: Summary of the amounts of each chemical used in each experiment.

Entry	8 (g)	Catalyst (g)	9 (ml)	Dry DCE (mL)	Time (h)	10a (%)
1	1.92	0.157	2.4	15 + 25	65	53
2	1.53	0.125	1.89	15 + 25	71	69

$\text{Cp}^*\text{RuCl}(\text{cod})$ (0.13 g, 0.33 mmol) was weighed out in a glovebox to a 100 mL round bottom flask equipped with a septum. The catalyst was then placed under nitrogen atmosphere and dissolved in dry, degassed (He gas, 20 min) DCE (15 mL). The monoalkyne **9** (1.90 mL, 9.76 mmol) was then added using a dry syringe. The diyne **8** (1.53 g, 6.5 mmol) was dissolved in dry, degassed (He gas, 20 min) DCE (25 mL) and added dropwise to the reaction mixture over the course of 40 min. The reaction was then left stirring for 71 h at room temperature. The solvent was removed in vacuo, and

the crude product was purified with flash column chromatography. Excess **9** was removed using pure pentane as mobile phase, and then the polarity was increased with EtOAc (5% EtOAc/n-pentane). All fractions containing the product were collected and the solvent removed in vacuo. This gave **10a** as a dark brown oil that crystallises to a dark brown solid in the refrigerator. The ^1H NMR spectrum was matched to the literature,¹³ and can be found in Appendix F. Yield: 69%. Spectroscopic data for **10a**: ^1H NMR (400 MHz, CDCl_3): δ 0.9 (t, 3 H, J = 6.9 Hz, H-1), 1.27 (t, 6 H, J = 7.1 Hz, H-13), 1.34 (m, 4 H, H-2, H-3), 1.61-1.69 (m, 2 H, H-4), 2.63 (t, 2 H, J =15.5 Hz, H-5), 3.63 (d, 4 H, J = 7.7 Hz, H-11), 4.22 (q, 4 H, J = 7.1, H-12), 7.21-7.24 (m, 3 H, H-6, H-10), 7.37 (s, 1 H, H-9), 7.39 (s, 1H, H-8), 7.46 (d, 2 H, J = 8.1, H-7).

6.4.2 Synthesis of diethyl 5-(2,4,6-triisopropylphenyl)-1,3-dihydro-2H-indene-2,2-dicarboxylate (**10b**)

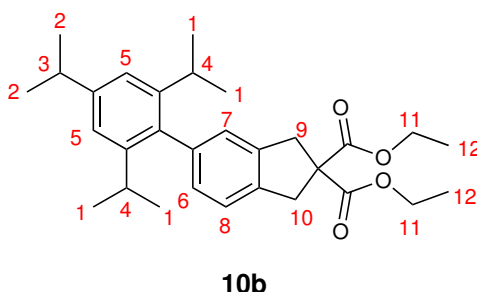


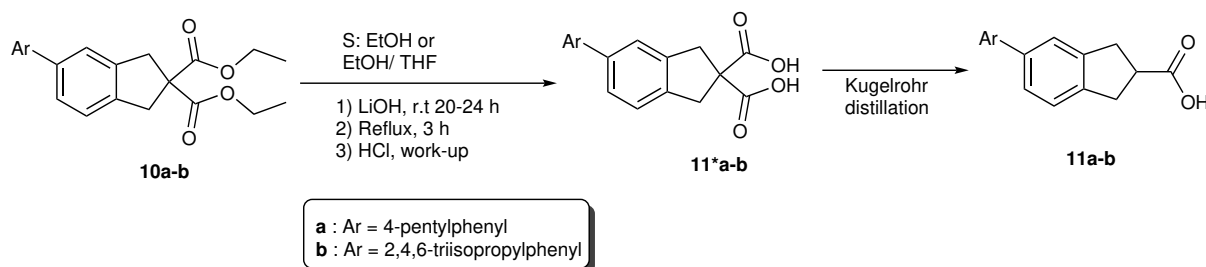
Table 6.4: Summary of the amounts of each chemical used in each experiment.

Entry	8 (g)	Catalyst (g)	5 (g)	Dry DCE (mL)	Time (h)	10b (%)
1	0.5	0.05	0.73	15 + 10	70	46
2	0.87	0.09	1.3	30 + 20	64	48

$\text{Cp}^*\text{RuCl}(\text{cod})$ (0.09 g, 0.23 mmol) was weighed out in a glovebox to a 100 mL round bottom flask equipped with a septum. The catalyst was then placed under nitrogen atmosphere. Monoalkyne **5** (1.3 g, 5.5 mmol) was dissolved in dry DCE (20 ml) and degassed (He, 20 min). The degassed monoalkyne was then added to the catalyst using a dry syringe. The diyne **8** (0.87 g, 3.7 mmol) was dissolved in dry DCE (30 mL) and added dropwise to the reaction mixture over the course of 10 min. The reaction was then left stirring for 64 h at room temperature. The solvent was removed in vacuo, and the crude product was purified with flash column chromatography (1:19 EtOAc/ n-pentane). All fractions containing the product were collected and the solvent removed in vacuo. This gave **10b** as an off-white solid. The ^1H NMR spectrum was matched to the literature,¹⁴ and can be found in Appendix G. Yield: 48% (0.813 g, 1.8 mmol). Spectroscopic data for **10b**: ^1H NMR (400 MHz, CDCl_3): δ 1.04-1.07 (m, 12 H, H-1), 1.24-1.30 (m, 12 H, H-2, H-12), 2.58 (sept., 2 H, J = 6.8 Hz, H-4), 2.92 (sept., 1 H, J = 6.9 Hz, H-3), 3.62 (s, 2 H, H-9), 3.65 (s, 2 H, H-10), 4.23 (q, 4 H, J = 7.1 Hz, H-11), 6.95 (app. d, 2 H, H-6, H-7), 7.03 (s, 2 H, H-5), 7.18 (d, 1 H, J = 7.5 Hz, H-8)

6.5 Hydrolysis and decarboxylation

The hydrolysis and subsequent decarboxylation of the diesters **10a-b** to obtain the monoacids **11a-b** was performed as described by Flynn and Beight.⁶⁰ See Chapter 2.2.4 for the reaction mechanism. Experimental procedure, work-up, characterisation and yields are described under each experiment. See Scheme 6.4 for the reaction equation.



Scheme 6.4: Reaction equation for the hydrolysis and decarboxylation of the diesters **10a-b** to acids **11a-b**.

6.5.1 Synthesis of 5-(4-pentylphenyl)-2,3-dihydro-1H-indene-2-carboxylic acid (**11a**)

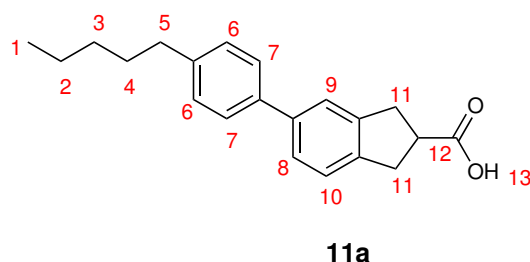


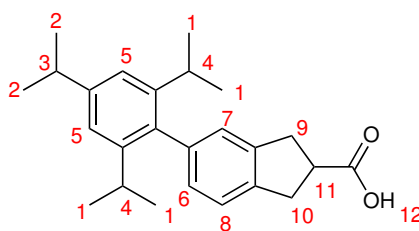
Table 6.5: Experimental data for the hydrolysis and decarboxylation of diester **10a**.

Entry	10a (g)	LiOH (aq., 1 M, mL)	HCl (aq., 6 M, mL)	Reaction time (h) (Stirring at r.t + reflux)	11a (%)
1	1.50	11	70	24 + 3	83
2	1.78	13.1	100	22 + 3	51

The compound **10a** (1.5 g, 3.7 mmol) was dissolved in EtOH (70 mL). A solution of 1M LiOH (aq.) (11 mL, 3 eq) was added to the reaction mixture, and the reaction was left stirring for 24.5 h. The reaction mixture was then heated to reflux at 95 °C, and stirred vigorously at this temperature for 3 h. The solution was cooled to room temperature, and EtOAc (100 mL) was added. The organic phase was washed with HCl (aq., 6 M, 70 mL), and dried over MgSO₄. The solvent was removed in vacuo, and the diacid **11a*** was decarboxylated with reduced pressure distillation (b.p.: 4.2 x 10⁻² mbar/180- 250 °C). The diacid **11b*** was never isolated and decarboxylated without any purification or spectroscopic analysis. This gave **11a** as a white solid. The ¹H NMR spectrum and melting point was matched to the literature,¹³ and the ¹H NMR spectrum can be

found in Appendix H. Yield: 83% (0.94 g, 3.05 mmol). Melting point: 121.2-122 °C. The ¹H NMR spectrum were compared to the literature¹³ and the spectra can be found in appendix Spectroscopic data for **11a**: ¹H NMR (400 MHz, DMSO-d₆): δ0.86 (t, 3 H, J= 6.9 Hz, H-1), 1.24-1.37 (m, 4 H, H-2, H-3), 1.55-1.62 (m, 2 H, H-4), 2.59 (t, 2 H, J=15.52 Hz, H-5), 3.10-3.22 (m, 4 H, H-11), 3.26-3.32 (m, 1 H, H-13), 7.24-7.28 (m, 3 H, H-6, H-10), 7.39 (d, 1 H, J = 7.9 Hz, H-8), 7.46 (s, 1 H, H-9), 7.51 (d, 2 H, J= 9.2, H-7), 12.3 (s, 1 H, H-13).

6.6 Synthesis of 5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxylic acid (**11b**)



11b

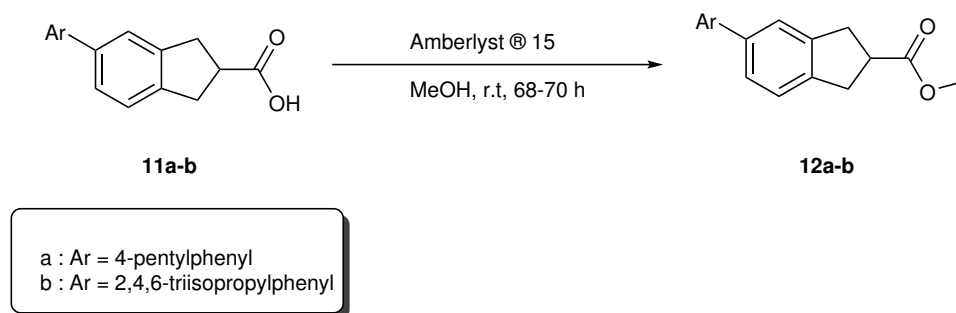
Table 6.6: Experimental data for the hydrolysis and decarboxylation of diester **10b**.

Entry	10b (g)	LiOH (aq., 1 M, mL)	HCl (aq., 6 M, mL)	Reaction time (h) (Stirring at r.t + reflux)	Yield (%)
1	0.47	3.03	50	20 + 3	70
2	0.813	5.3	100	18 + 3	76

The compound **10b** (0.813 g, 1.75 mmol) was dissolved in a mixture of EtOH/THF (15 + 20 ml). A solution of 1M LiOH (aq.) (3.03 mL, 3 eq) was added to the reaction mixture, and the reaction was left stirring for 18 h. The reaction mixture was then heated to reflux at 95 °C, and stirred vigorously at this temperature for 3 h. The solution was cooled to room temperature, and EtOAc (100 mL) was added. The organic phase was washed with HCl (aq., 6 M, 70 mL), and dried over MgSO₄. The solvent was removed in vacuo, and the diacid **11b*** was decarboxylated with reduced pressure distillation (b.p.: 4.2 x 10⁻² mbar/180- 250 °C). The diacid **11b*** was never isolated and decarboxylated without any purification or spectroscopic analysis. This gave **11b** as a white solid. The ¹H NMR spectrum was matched to the literature,¹⁴ and the ¹H NMR spectrum can be found in Appendix I. Yield: 76% (0.485 g, 1.33 mmol). Melting point: 170.0 °C. Spectroscopic data for **11b**: ¹H NMR (600 MHz, DMSO-d₆): δ0.99-1.03 (m, 12 H, H-1), 1.22 (d, 6 H, J = 6.9 Hz, H-2), 2. 48-2.55 (m, 2 H, H-4), 2.88 (sept, 1 H, J = 6.9 Hz, H-3), 3.11-3.23 (m, 4 H, H-9, H-10), 3.34 (app. pent, 1 H, H-11), 6.88 (d, 1 H, J = 7.6 Hz, H-8), 6.96 (s, 1 H, H-7), 7.02 (s, 2 H, H-5), 7.23 (d, 1 H, J = 7.6 Hz, H-6)

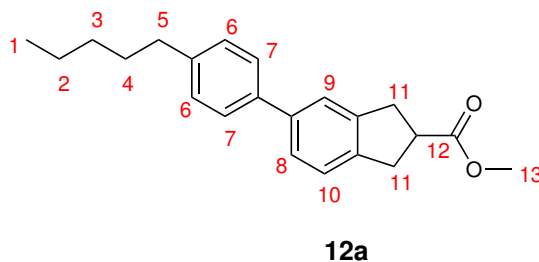
6.7 Esterification

Compounds **12a-b** were synthesised from **11a-b** for further functionalization. The reactions were performed as described by Petrini *et al.*⁴⁷ Reaction mechanism can be found in Chapter 2.2.4 and reaction equation is presented in Scheme 6.5. Yields, characterisation data and work-up procedure for the products are presented under each experiment.



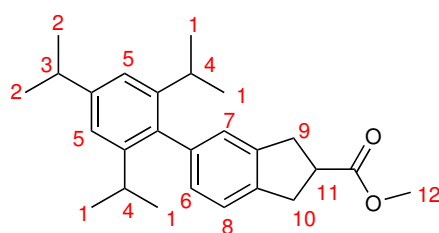
Scheme 6.5: Synthesis of esters **12a** and **12b**.

6.7.1 Synthesis of methyl 5-(4-pentylphenyl)-2,3-dihydro-1H-indene-2-carboxylate (**12a**)



The Amberlyst[®] 15 (13.2 g) was washed with MeOH, and stored in MeOH until use. **11a** (0.94 mg, 3.05 mmol) was dissolved in MeOH (70 ml) and the Amberlyst[®] 15 was added. The reaction was left with continuous stirring at room temperature for 70 h. The reaction mixture was then filtrated, before the solvent was removed in vacuo. The crude product was then dissolved in EtOAc, and purified with a silica plug (EtOAc) to get rid off Amberlyst[®] 15 residue. Lastly, the EtOAc was removed in vacuo. This gave **12a** as a transparent oil that crystallises in the refrigerator. The ¹H NMR spectrum was matched to the literature¹³ and can be found in Appendix J. Yield: >99% (0.98 g, 3.03 mmol). Spectroscopic data for **12a**: ¹H NMR (400 MHz, CDCl₃): δ0.9 (t, 3 H, J= 8.1 Hz, H-1), 1.32-1.36 (m, 4 H, H-2, H-3), 1.60-1.68 (m, 2 H, H-4), 2.63 (t, 2 H, J = 15.5 Hz, H-5), 3.20-3.43 (m, 5 H, H-11, H-12), 3.73 (s, 3 H, H-13), 7.21-7.26 (m, 3 H, H-6, H-10), 7.38 (d, 1 H, J = 7.8 Hz, H-8), 7.41 (s, 1 H, H-9), 7.47 (d, 2 H, J = 8.1 Hz, H-7).

6.7.2 Synthesis of methyl 5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxylate (**12b**)



12b

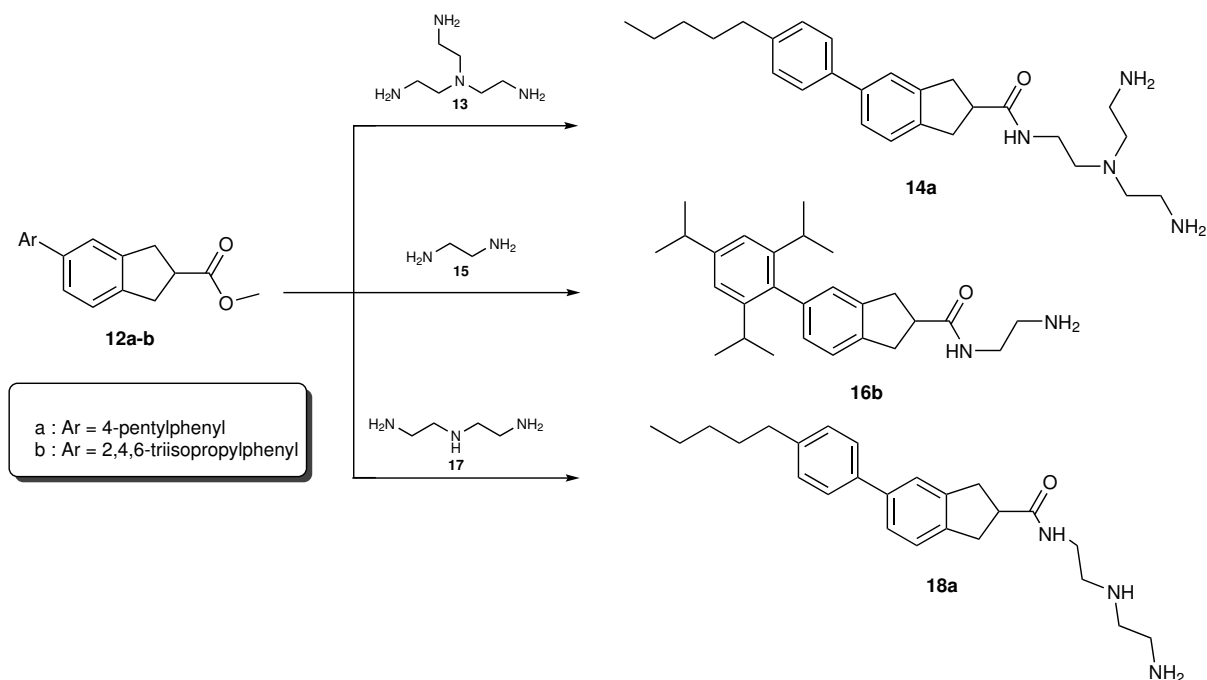
Table 6.7: Experimental data for the esterification of **11b**.

Entry	11b (g)	Amberlyst [®] 15 (g)	Time (h)	Yield (%)
1	0.11	4	69	> 90
1	0.15	4	70	97

The Amberlyst[®] 15 (4 g) was washed with MeOH, and stored in MeOH until use. **11b** (0.15 g, 0.4 mmol) was dissolved in MeOH (6 ml) and the Amberlyst[®] 15 was added. The reaction was left with continuous stirring at room temperature for 70 h. The reaction mixture was then filtrated, before the solvent was removed in vacuo. The crude product was then dissolved in EtOAc, and purified with a silica plug (EtOAc) to get rid off Amberlyst[®] 15 residue. Lastly, the EtOAc was removed in vacuo. This gave **12b** as white solid. The ¹H NMR spectrum was matched to the literature¹⁴ and can be found in Appendix K. Yield: 67 % (0.15 g, 0.38 mmol). Spectroscopic data for **12b**: ¹H-NMR (400 MHz, CDCl₃): δ 1.05-1.08 (m, 12 H, H-1), 1.30 (d, 6 H, J = 6.9 Hz, H-2), 2.53-2.67 (m, 2 H, H-4), 2.93 (appt. sept, 1 H, J = 6.9 Hz, H-3), 3.20-3.35 (m, 4 H, H-9, H-10), 3.37-3.47 (m, 1 H, H-11), 3.75 (s, 3 H, H-12), 6.95 (d, 1 H, J = 7.5 Hz, H-8), 6.98 (s, 1 H, H-7), 7.03 (s, 2 H, H-5), 7.20 (d, 1 H, J = 7.5 Hz, H-6)

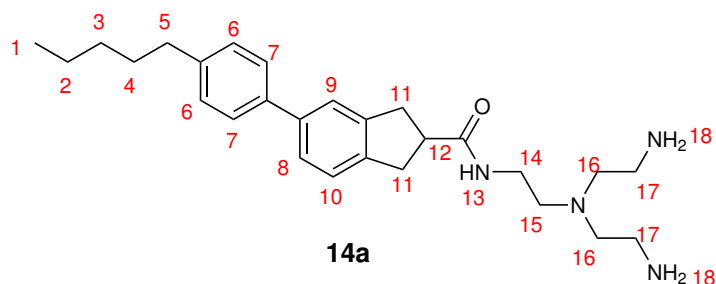
6.8 Further functionalisation of the esters **12a** and **12b**

Further functionalization of the esters **12a-b** was executed by synthesising the amidoamines **14a**, **16b** and **18a**. The reactions were performed as described in the literature,⁶¹ and the reaction equations are presented below in Scheme 6.6. Yields, characterisation data and work-up procedure for the products are presented under each experiment.



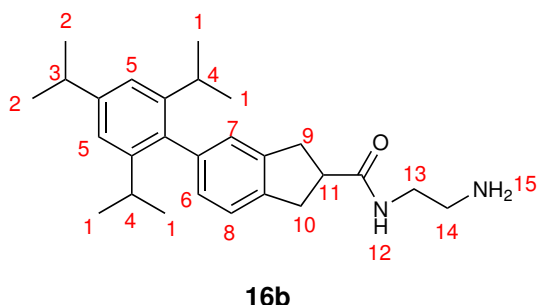
Scheme 6.6: The different amidations reactions and their corresponding products.

6.8.1 Synthesis of *N*-(2-(bis(2-aminoethyl)amino)ethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (**14a**)



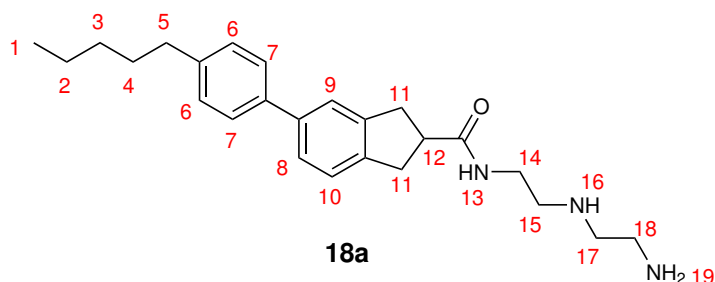
The compound **12a** (0.1 g, 0.32 mmol) was dissolved in **13** (7.2 mL, 0.048 mol, 150 eq). The reaction was then stirred at 50 °C for 23 h. The reaction was monitored with TLC-analysis (EtOAc), and stopped when all the starting material was gone. Excess **13** was removed using kugelrohr distillation (0.03 mbar, 105-110 °C). This gave **14a** as a golden oil. The ^1H NMR spectrum was matched to the literature¹⁵ and can be found in Appendix L. Yield: 97% (0.16 g, 0.31 mmol). Spectroscopic data for **14a**: ^1H NMR (600 MHz, DMSO- d_6): δ 0.86 (t, 3 H, J = 7.0 Hz, H-1), 1.24-1.34 (m, 4 H, H-2, H-3), 1.55-1.62 (m, 2 H, H-4), 2.36-2.60 (m, 14 H, H-5, H-15, H-16, H-17, H-18), 3.04-3.26 (m, br, 9 H, H-11, H-12, H-14, H-18), 7.24 (d, 3 H, J = 8.1 Hz H-6, H-10), 7.38 (d, 1 H, J = 7.7 Hz, H-9), 7.43 (s, 1 H, H-8), 7.51 (d, 2 H, J = 8.1 Hz, H-7), 7.9-8.1 (t, 1 H, J = 5.5 Hz, H-13)

6.8.2 Synthesis of *N*-(2-aminoethyl)-5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (**16b**)



The ester **12b** (0.22 g, 0.6 mmol) was dissolved in **15** (5.0 ml, 0.06 mol, 100 eq.) and left stirring at 90 °C for 74 h. Excess **15** was removed in vacuo. The crude was then dissolved in EtOAc (70 ml) and washed with water (50 ml x 2). The organic phase was dried with MgSO₄ and concentrated in vacuo. This afforded **16b** as an off-white solid. The ¹H NMR spectrum and melting point were matched with the literature,¹⁴ and can be found in Appendix M. Mp: 162-164 °C. Yield: 78 % (0.19 g, 0.46 mmol). The Spectroscopic data for **16b**: ¹H-NMR (600 MHz, CDCl₃): δ 1.05-1.08 (m, 12 H, H-1), 1.30 (d, 6 H, J = 6.9 Hz, H-2), 1.78 (s (br), 2 H, H-15), 2.57-2.65 (m, 2 H, H-4), 2.87 (t, 2 H, J = 6.4 Hz, H-13), 2.92 (appt. sept, 1 H, J = 6.9 Hz, H-3), 3.17-3.32 (m, 6 H, H-9, H-10, H-14), 3.63-3.38 (q, 1 H, J = 5.6 Hz, H-11), 6.94 (d, 1 H, J = 7.5 Hz, H-8), 6.98 (s, 1 H, H-7), 7.03 (s, 2 H, H-5), 7.19 (d, 1 H, J = 7.6 Hz, H-6), 7.91 (t, 1 H, J = 5.5 Hz, H-12)

6.8.3 Synthesis of *N*-(2-((2-aminoethyl)amino)ethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (**18a**)

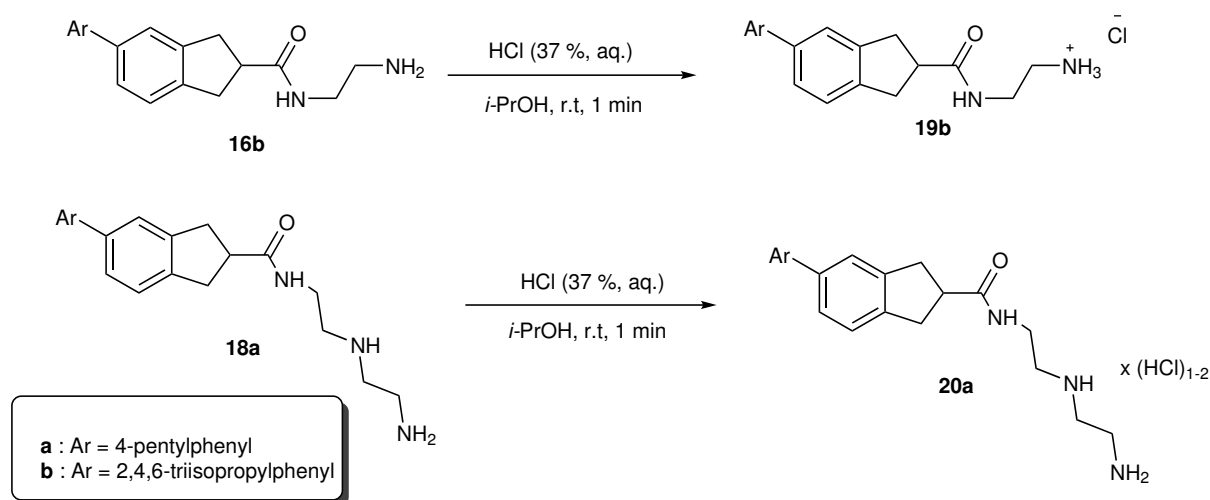


The ester **12a** (0.1 g, 0.31 mmol) was dissolved in **17** (6 ml, 0.05 mol, 161 eq.) and heated to 50 °C under N₂ atmosphere. The reaction was followed by TLC (EtOAc) and stopped after 69 h. The amine **17** was removed by Kugelrohr distillation (50-70 °C, 0.03 mbar), followed by co-evaporation with *i*-PrOH (5 ml). This yielded **18a** as an off-white wax. Yield: 92 % (0.11 g, 0.03 mmol). Spectroscopic data for **18a**: ¹H NMR (600 MHz, DMSO-*d*₆): δ 0.87 (t, 3 H, J = 7.0 Hz, H-1), 1.26-1.35 (m, 4 H, H-2, H-3), 1.56-1.61 (m, 2 H, H-4), 1.61-1.96 (s (br), 3 H, H-23, H-26), 2.48-2.51 (m, 2 H, H-25), 2.56-2.61 (m, 6 H, H-5, H-22, H-24), 3.05-3.09 (m, 4 H, H-16, H-17), 3.14-3.18 (m, 2 H, H-21), 3.18-3.24 (m, 1H, H-18), 7.4 (d, 3 H, J = 8.2 Hz, H-7, H-13), 7.37 (d, 1 H, J = 7.7 Hz, H-11), 7.43 (s, 1 H, H-12), 7.51 (d, 2 H, J = 8.2 Hz, H-8), 7.9-8.1 (t, 1 H, J = 5.5 Hz, H-13). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 13.9 (C-1), 21.9 (C-2), 30.9 (C-3), 30.6 (C-4), 34.7 (C-5), 36.1 (C-17),

36.4 (C-16), 40.1 (C-21), 41.5 (C-22/ C-24), 44.1 (C-18), 48.6 (C-22/ C-24), 52.2 (C-25), 122.2 (C-12), 124.4 (C-13), 124.8 (C-11), 126.4 (C-8), 128.7 (C-7), 137.9 (C-10), 138.5 (C-9), 141.2 (C-14), 143.0 (C-15), 174.0 (C-19). IR (neat, cm^{-1}): 3315 (m), 3026 (w), 2953 (m), 2925 (s), 2870 (m), 2851 (m), 1908 (w), 1635 (s), 1539 (s), 1484 (s), 1455 (m), 1378 (m), 1305 (m), 1255 (m), 1227 (m), 1187 (m), 1126 (m), 1083 (m), 1009 (m), 885 (m), 801 (s), 712 (m), 671 (m), 591 (w), 516 (w), 463 (w), 421 (w). HRMS (TOF ASAP +): m/z calcd for $\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}[\text{M}]^+$: 398.2858; found 394.2857. The ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, MS and IR spectra can be found in Appendix N.

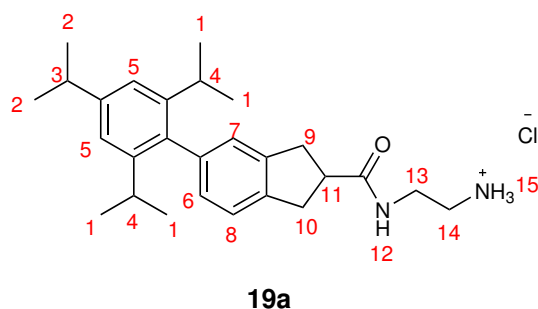
6.9 Synthesis of the HCl-salts 19b and 20a

The HCl-salts of the amidoamine compounds **16b** and **18a** were synthesised for antimicrobial testing. The reactions were performed as described by Bakka *et al.*²³ with small deviations. The reaction equation is presented in Scheme 6.7. Yields, characterisation data and work-up procedure for the products are given under for each experiment.



Scheme 6.7: Synthesis of the HCl-salts **19b** and **20a**.

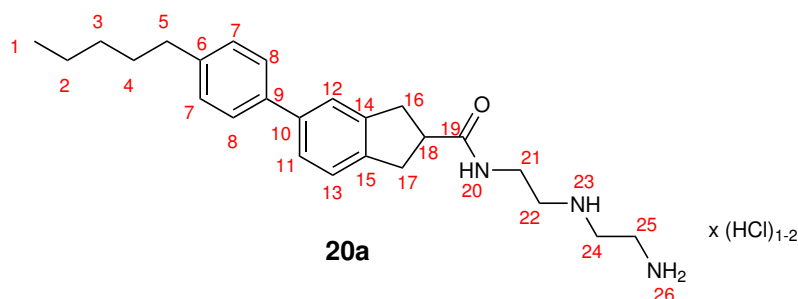
6.9.1 Synthesis of *N*-(2-aminoethyl)-5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1Hindene-2-carboxamide hydrochloride (**19b**)



The amine **16b** (0.023 g, 0.56 mmol) was dissolved in *i*-PrOH (3 ml) and HCl (0.1 ml, 37%, aq.) was added. The reaction was stirred for 1 minute at room temperature, and

then the solvent were removed in vacuo. The crude product was dissolved in MeOH (0.3 ml) and 2 ml Et₂O was added dropwise. The solution was left in the freezer for 5 days. The resulting crystals were filtered off and washed with MeCN (0 °C, 15 ml). This yielded **19b** as a white solid. T_{Decomp.}: 198.2 °C, Mp: 260 °C. Yield: 72% (0.018 g, 0.04 mmol). Spectroscopic data for **19b**: ¹H-NMR (600 MHz, DMSO-d₆): δ0.99-1.03 (m, 12 H, H-1), 1.22 (d, 6 H, J = 6.9 Hz, H-2), 2.50-2.56 (m, 2 H, H-4), 2.86-2.9 (m, 3 H, H-3, H-14), 3.11-3.15 (m, 4 H, H-16, H-17), 3.25-3.28 (m, 1 H, J = H-11), 3.34 (q, 2 H, J 6.0 Hz, H-13), 6.88 (d, 1 H, J = 7.5 Hz, H-6), 6.95 (s, 1 H, H-7), 7.02 (s, 2 H, H-5), 7.22 (d, 1 H, J = 7.6 Hz, H-8), 7.91 (s, 3 H, H-15), 8.24 (t, 1 H, J = 5.1 Hz, H-12). HPLC (MeOH/H₂O 80:20, + 0.1% TFA, 1 ml/min, λ= 214 nm): t_R = 6.88 min, 96% pure. The ¹H NMR spectrum and the HPLC chromatogram can be found in Appendix O.

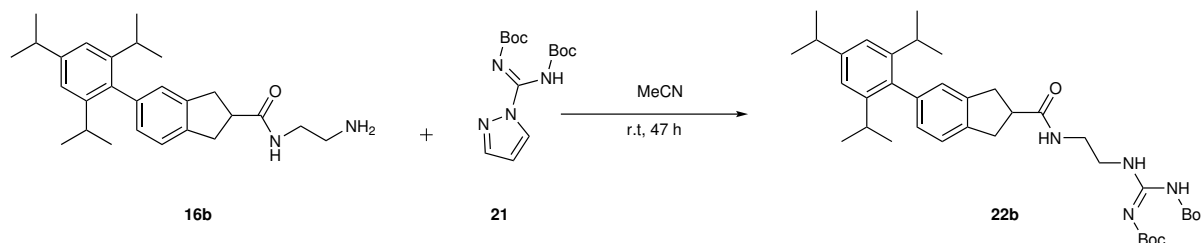
6.9.2 Synthesis of *N*-(2-((2-aminoethyl)amino)ethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide hydrochloride (**20a**)



The amine **18a** (0.049 g, 0.13 mmol) was dissolved in *i*-PrOH (4.5 ml) and HCl (aq., 37%, 0.2) was added. The reaction was stirred for 1 minute at room temperature, and then the solvent were removed in vacuo. The crude product was recrystallised in EtOH, filtered off and washed with ice cold MeCN (5 ml) and *i*-PrOH (5 ml). This yielded **20a** as an off-white hard wax. Yield: 81% (0.035 g, 0.1 mmol). Spectroscopic data for **20a**: ¹H NMR (600 MHz, DMSO-d₆): δ0.86 (app. t, 3 H, J = 6.7 Hz, H-1), 1.24-1.37 (m, 4 H, H-2, H-3), 1.56-1.64 (m, 2 H, H-4), 2.59 (t, 2 H, J= 7.4 Hz, H-5), 3.06-3.28 (m, 11 H, H-16, H-17, H-18, H-22, H-24, H-25), 3.38-3.47 (app. q., 2 H, J = 5.8 Hz, H-21), 7.2-7.29 (m, 3 H, H-7, H-13), 7.39 (d, 1 H, J = 7.5 Hz, H-11), 7.44 (s, 1 H, H-12), 7.51 (d, 2 H, J = 7.8 Hz, H-8), 8.3 (app. t, 1 H, J = 4.2 Hz, H-20), 7.7-9.87 (br, 4 H, H-23, H-26). ¹³C NMR (150 MHz, DMSO-d₆): δ13.9 (C-1), 21.9 (C-2), 30.6 (C-4), 30.9 (C-3), 34.7 (C-5), 35.4 (C-21, C-24, C-25), 35.9 (C-17), 36.2 (C-16), 44.1 (C-24/ C-25), 44.2 (C-18), 46.4 (C-22), 122.2 (C-12), 124.5 (C-13), 124.8 (C-11), 126.4 (C-8), 128.8 (C-7), 137.9 (C-10), 138.6 (C-9), 141.0 (C-14), 141.2 (C-6), 142.8 (C-15), 174.8 (C-19). IR (neat, cm⁻¹): 3306 (m), 2991(br), 2925 (br), 2872 (br), 2852 (br), 2735 (br), 2701 (m), 2561 (w), 2460 (w), 2430 (w), 2387 (w), 1651 (s), 1611 (w), 1530 (s), 1487 (w), 1471 (m), 1446 (w), 1398 (w), 1379 (w), 1365 (w), 1347 (w), 1303 (w), 1273 (w), 1256 (m), 1236 (m), 1185 (w), 1122 (w), 1086 (w), 1069 (w), 1041 (w), 995 (m), 950 (m), 940 (w), 885 (w), 846 (w), 801 (s), 766 (w), 712 (w), 678 (s), 608 (w), 592 (w), 535 (w), 522 (w), 512 (w), 459 (w), 420 (w). HRMS (TOF ASAP +): *m/z* calculated for C₂₅H₃₆N₃O [M-Cl]⁺: 394.2858; found: 394.2854. HPLC (MeOH/H₂O,70:30 + 0.1% TFA, 1 ml/min, λ= 214 nm): t_R = 10.6 min, >99% pure. The ¹H NMR, ¹³C NMR, COSY, HSQC, HMBC and IR spectra, as well as MS report and HPLC chromatogram can be found in Appendix P.

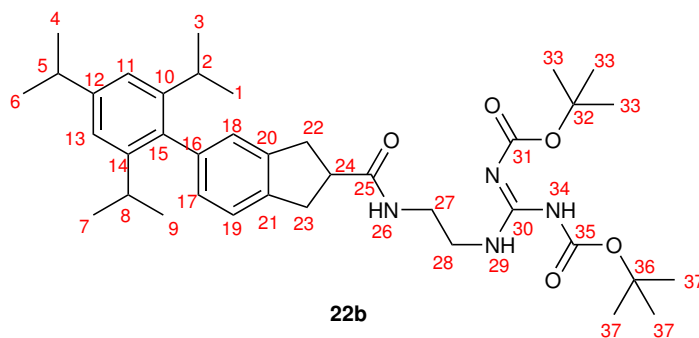
6.10 Further functionalization of 16b to its guanylated version (23b)

The amine **16b** was further functionalised by reacting with a guanylyating agent. The reaction was performed as described by the literature.⁶² The reaction equation is illustrated in Scheme 6.8, and yields, characterisation and spectroscopy is presented below.



Scheme 6.8: Guanylation of **16b**.

6.10.1 Synthesis of bis-Boc(amino((2-(5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxamido) ethyl)amino))guanidine **22b**

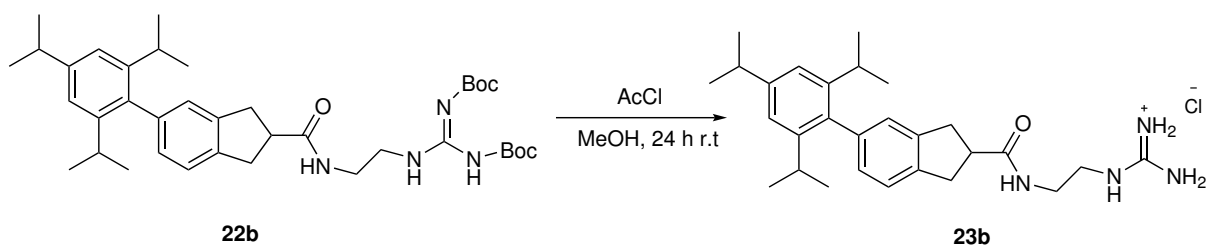


To a stirred solution of the guanylyating reagent **21** (0.07 g, 0.23 mmol, 1 eq.) in MeCN (2 ml) at room temperature, a solution of the amine **16b** (0.11 g, 0.26 mmol, 1.1 eq.) dissolved in MeCN (7 ml) was added. The reaction was stirred at r.t. and followed with TLC-analysis (EtOAc). After 47 h the reaction was stopped, and the solvent removed in vacuo. The crude was then purified by flash column chromatography (silica, 40% EtOAc in n-pentane). This yielded **22b** as an transparent glass, with some more crystalline areas. ¹H NMR analysis indicated that EtOAc was still present in the sample (Appendix Q.1). Yield_{Calculated}: 86% (0.129 g, 0.19 mmol). mp: 97.7 °C. Spectroscopic data for **22b**: ¹H NMR (600 MHz, DMSO-d₆): δ0.99-1.03 (m, 12 H, H-1, H-3, H-7, H-9), 1.22 (d, 6H, J = 6.9 Hz, H-4, H-6), 1.38 (s, 3 H, H-34/H-37), 1.46 (s, 3 H, H-34/H-37), 2.47-2.57 (m, 2 H, H-2, H-8), 2.88 (sept., 1 H, J = 6.9 Hz, H-5), 3.06-3.11 (m, 2 H, 4 H, H-22, H-23), 3.19-3.29 (m, 3 H, H-24, H-27), 3.39-3.43 (m, 2 H, H-28), 6.86 (d, 1 H, J = 7.5 Hz, H-17), 6.91 (s, 1 H, H-18), 7.023 (app. s, 1 H, H-11), 7.027 (app. s, 1 H, H-13), 7.19 (d, 1 H, J = 7.6 Hz, H-19), 8.1 (t, 1H, J = 5.5, H-24), 8.41 (t, 1 H, J = 5.8 Hz, H-29), 11.49 (s, 1H, 34). ¹³C NMR (150 MHz, DMSO-d₆): δ23.9 (C-1, C-3, C-7, C-9), 24.0 (C-4, C-6), 27.6 (C-33/ C-37), 27.9 (C-33/ C-37), 29.7 (C-2, C-8), 33.6 (C-5), 36.0 (C-23), 36.4 (C-22), 38.1 (C-27), 40.1 (C-28), 44.2 (C-24), 78.1 (C-32/C-36), 82.8 (C-32/C-36), 120.0 (C-11, C-13), 123.7 (C-19), 124.8 (C-18), 127.5 (C-17), 137.0 (C-15), 138.3 (C-16), 140.3 (C-20), 141.9 (C-21), 145.9 (C-10/C-14), 146.0 (C-10/C-14), 147.2 (C-12), 151.9 (C-35), 155.7 (C-31), 163.1

(C-30), 174.4 (C-25). IR (neat, cm^{-1}): 3315 (w), 3065 (w), 2959 (w), 2928 (w), 2868 (w), 1773 (w), 1722 (w), 1640 (m), 1613 (m), 1567 (w), 1465 (w), 1412 (m), 1392 (w), 1330 (m), 1300 (m), 1277 (m), 1248 (m), 1228 (m), 1154 (m), 1132 (s), 1049 (s), 940 (w), 909 (w), 876 (m), 827 (w), 810 (m), 767 (m), 724 (w), 696 (w), 648 (w), 586 (m), 559 (m), 538 (w), 511 (w), 464 (w), 434 (m), 422 (m). HRMS (TOF ASAP +): m/z calculated for $\text{C}_{38}\text{H}_{56}\text{N}_4\text{O}_5$ $[\text{M}-\text{Cl}]^+$: 649.4329; found: 3649.4328. HPLC (MeOH/ H_2O , + 0.1% TFA, 1 ml/min, $\lambda = 214$ nm): $t_{\text{R}} = 10.6$ min, >99% pure. The ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as the MS report can be found in Appendix Q.

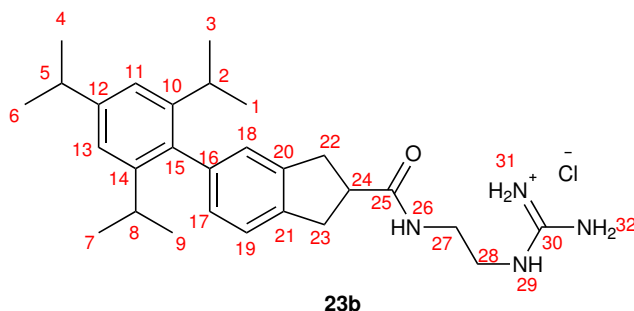
6.11 Deprotection of 22b to the guanydyl product 23b

The deprotection of **22b** to **23b** was performed according to literature.⁶³ The reaction equation is presented in Scheme 6.9. Yields, characterisation and spectroscopy is presented below.



Scheme 6.9: The reaction equation illustrating the deprotection of **22b** to **23b**.

6.11.1 Synthesis of amino((2-(5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carboxamido)ethyl)amino)methaniminium chloride 23b

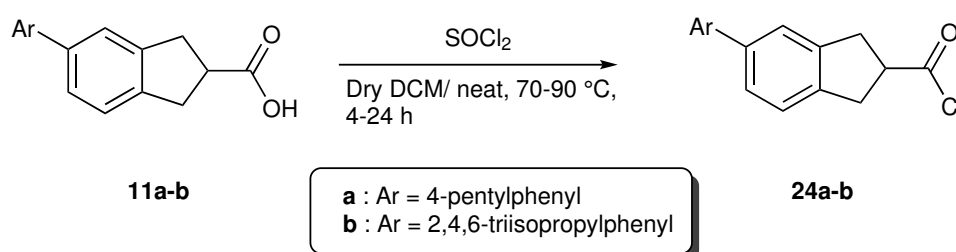


Compound **22b** (0.12 g, 0.19 mmol) was dissolved in MeOH (1.2 ml) and AcCl (0.33 ml, 4.6 mmol) was added dropwise. The reaction was left stirring at r.t for 56 h, monitored by TLC-analysis (EtOAc). The crude was purified by repeatedly co-evaporating with MeOH (6 x 12 ml). This yielded **23b** as a white solid. $T^{\text{Color change}}$: 198 °C, M.p: 260 °C. Yield: 117% (0.104 g, 0.21 mmol). Spectroscopic data for **23b**: ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 0.99-1.03 (m, 12 H, H-1, H-3, H-7, H-9), 1.22 (d, 6H, $J = 6.9$ Hz, H-4, H-6), 2.47-2.56 (m, 2 H, H-2, H-8), 2.88 (sept., 1 H, $J = 8.3$ Hz, H-5), 3.09-3.14 (m, 2 H, 4 H, H-22, H-23), 3.23-3.31 (m, 5 H, H-24, H-27, H-28), 6.87 (d, 1 H, $J = 7.6$ Hz, H-17), 6.94 (s, 1 H, H-18), 7.02 (s, 2 H, H-11, H-13), 7.22 (d, 1 H, $J = 7.6$ Hz, H-19), 7.71 (s, 1H,

H-29), .6.84-7.68 (br, 4 H, H-31, H-32) ^{13}C NMR (150 MHz, DMSO- d_6): δ 23.9 (C-1, C-3, C-7, C-9), 24.0 (C-4, C-6), 29.7 (C-2, C-8), 33.6 (C-5), 36.1 (C-23), 36.3 (C-22), 38.1 (C-27), 40.1 (C-28), 43.9 (C-24), 120.0 (C-11, C-13), 123.7 (C-19), 124.9 (C-18), 127.5 (C-17), 137.0 (C-15), 138.3 (C-16), 140.3 (C-20), 141.8 (C-21), 145.9 (C-10/C-14), 146.0 (C-10/C-14), 147.2 (C-12), 157.2 (C-30), 174.9 (C-25). IR (neat, cm^{-1}): 3327 (br), 3195 (br), 2958 (s), 2866 (m), 1775 (m), 1614 (s), 1554 (m), 1464 (m), 1380 (m), 1361 (m), 1333 (m), 1316 (m), 1295 (m), 1446 (m), 1209 (m), 1168 (m), 1123 (m), 1100 (m), 1055 (m), 1033 (m), 876 (m), 648 (m), 560 (m), 538 (m), 513 (m), 489 (m), 452 (m), 437 (m), 428 (m). HRMS (TOF ASAP +): m/z calculated for $\text{C}_{28}\text{H}_{41}\text{N}_4\text{O}$ $[\text{M}-\text{Cl}]^+$: 449.3280; found: 3649.4279. HPLC (MeOH/ H_2O , 80:20 +0.1 % TFA, 1 ml/min, $\lambda = 214$ nm): $t_{\text{R}} = 7.1$ min, 94.5% pure. The ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as the MS report can be found in Appendix Q.

6.12 Synthesis of the acid chlorides 24a-b

The acid chlorides **24a-b** were synthesised from **11a-b** for further functionalization, by following the procedure described by Singh *et al.*⁶⁷ with some minor deviations. The reaction equation is presented in Scheme 6.10. Yields, characterisation data and work-up procedure for the products are presented under each experiment.



Scheme 6.10: Synthesis of acid chlorides **24a-b**.

6.12.1 Synthesis of 5-(4-pentylphenyl)-2,3-dihydro-1H-indene-2-carbonyl chloride (24a)

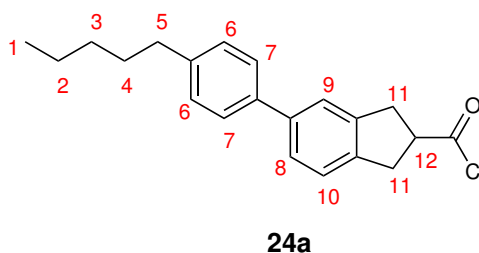


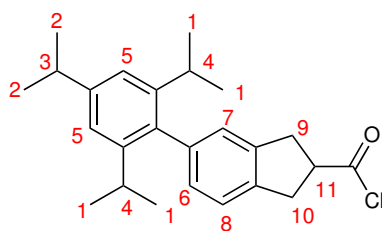
Table 6.8: Experimental data for the preparation of the acid chloride **24a**.

Entry	11a (g)	SOCl ₂ (ml)	dry DCM (ml)	Time (h)	24a (%)
1	0.202	0.14	2	4	91
2	0.468	0.33	3.5	24	98
3	0.124	0.116	1	24	>99

The acid **11a** (0.02 g, 0.7 mmol) was dissolved in dry DCM (2 ml). SOCl₂ (0.14 ml, 0.2 mmol, 3 eq.) was added carefully and the reaction mixture was heated to reflux and stirred for 4 h under N₂-atmosphere. After complete reaction excess SOCl₂ and solvent were removed in vacuo. This yielded **24a** as a dark brown oil. Yield: 91% (0.19 g, 0.28 mmol). Spectroscopic data for **24a**: ¹H-NMR (400 MHz, CDCl₃): δ0.86 (t, 3H, J = 7.0 Hz), 1.24-1.37 (m, 4H), 1.55-1.62 (quint., 2 H, J = 14.4 Hz), 2.58 (t, 2 H, J = 14.8 Hz), 3.08-3.34 (m, 5 H), 7.23-7.28 (m, 3 H), 7.39 (d, 2H, J = 7.8 Hz), 7.47 (s, 2 H), 7.51 (d, 2 H, J = 8.1 Hz). ¹³C NMR (150 MHz, DMSO-d₆): δ13.9, 22.0, 30.6, 30.9, 34.7, 35.3, 35.7, 42.9, 122.3, 124.6, 124.9, 126.4, 128.8, 137.9, 138.7, 140.7, 141.3, 142.5, 176.2. IR (neat, cm⁻¹): 3021 (m), 2956 (s), 2869 (s), 2854 (s), 2771 (w), 2684 (w), 2654 (w), 1904 (w), 1788 (s), 1696 (s), 1680 (w), 1580 (w), 1559 (w), 1521 (w), 1904 (s), 1465 (s), 1449 (s), 1399 (m), 1380 (w), 1348 (m), 1336 (m), 1304 (m), 1276 (m), 1260 (m), 1233 (w), 1184 (w), 1162 (w), 1119 (w), 1080 (s), 1053 (s), 1028 (s), 1010 (s), 956 (m), 945 (w), 924 (m), 889 (m), 854 (s), 799 (s), 776 (s), 725 (m), 697 (m), 677 (w), 641 (w), 608 (m), 587 (w), 576 (w), 52 (s), 424 (s). HRMS (TOF ASAP +): *m/z* calculated for C₂₁H₂₂ClO [M]⁺: 326.1437; found: 326.1441.

*Due to an error, the peak labels in the IR spectra were not printed. The values listed are manually placed, and some deviance from the actual values is to be expected.¹H NMR, ¹³C NMR, IR spectra as well as MS report can be found in Appendix S.

6.12.2 Synthesis of 5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carbonyl chloride (**24b**)

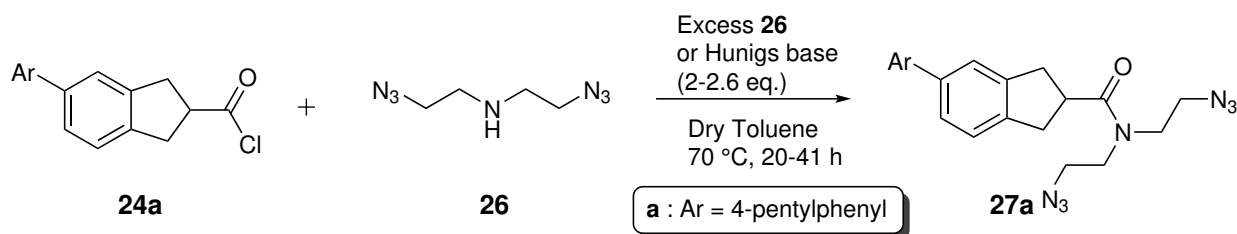
**24b**

The acid **11b** (0.19 g, 0.52 mmol) was dissolved in SOCl₂ (5 ml) and heated to 70 °C. The reaction was left stirring for 18 h. Excess SOCl₂ was removed in vacuo. This yielded **24b** as a dark red oil. Yield: 0.26 g (130%). Spectroscopic data for **24b**: ¹H-NMR (400 MHz, DMSO-d₆): δ0.98-1.02 (m, 12 H), 1.22 (dd, 6 H, J = 6.9 Hz, J = 6.7), 2.44-2.55 (m, 2 H), 2.88 (sept., 1 H, J = 6.8 Hz), 3.11-3.28 (m, 4 H), 3.30-3.37 (m, 1 H), 6.87-6.90 (m, 1 H), 6.96 (s, 1H), 7.02 (s, 2 H), 7.22-7.26 (m, 1H). ¹³C NMR (150 MHz, DMSO-d₆): δ23.9, 24.0, 29.7, 33.7, 35.5, 35.7, 42.6, 120.1, 123.8, 125.0, 127.6, 137.0, 138.4, 139.9, 141.5, 145.9,

146.0, 147.3, 176.2. IR (neat, cm^{-1}): 2948 (s), 2927 (m), 2867 (m), 1790 (s), 1733 (w), 1703 (w), 1643 (w), 1608 (w), 1568 (w), 1495 (w), 1461 (m), 1415 (w), 1382 (m), 1361 (m), 1319 (w), 1232 (m), 1171 (m), 1103 (w), 1056 (m), 1015 (m), 956 (w), 939 (w), 922 (w), 876 (m), 856 (m), 829 (m), 813 (m), 779 (m), 731 (m), 650 (w), 329 (w), 600 (w), 553 (w), 540 (w), 482 (w), 437 (w). HRMS (TOF ASAP +): m/z calculated for $\text{C}_{25}\text{H}_{30}\text{ClO}$ $[\text{M}]^+$: 382.2063; found: 382.2061. ^1H NMR, ^{13}C NMR, IR spectra as well as MS report can be found in Appendix T.

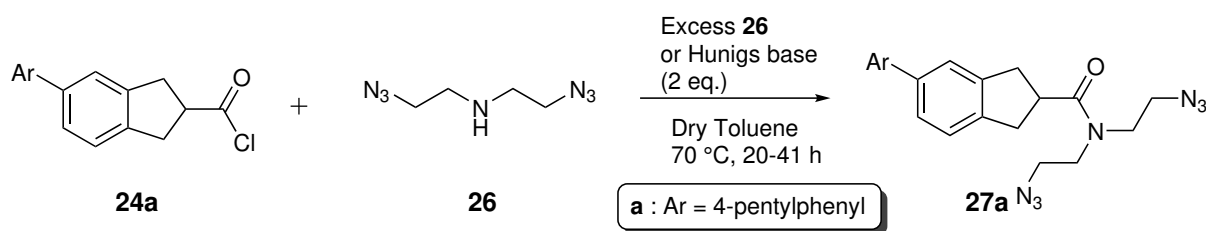
6.13 Synthesis of the bisazide intermediate 27a

The synthesis of the bisazide intermediate **27a** is a two step synthesis, starting with the synthesis of the amine bisazide **26**. This reaction was performed once in accordance with the literature.⁶⁸ The reaction equation is presented in Scheme 6.11, while the yield, characterisation and work-up procedure is presented in Chapter 6.13.1.



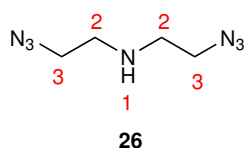
Scheme 6.11: Synthesis of the bisazide reagent **26**.

Compound **27a** was synthesised three times following the procedure described by Singh *et al.*⁶⁷ with small deviations. The reaction equation is presented in Scheme 6.12. Yields, characterisation data and work-up procedure for the products are presented under each experiment in Chapter 6.13.1 and Chapter 6.14 respectively.



Scheme 6.12: Reaction equation illustrating the synthesis of the bisazide intermediate **27a**.

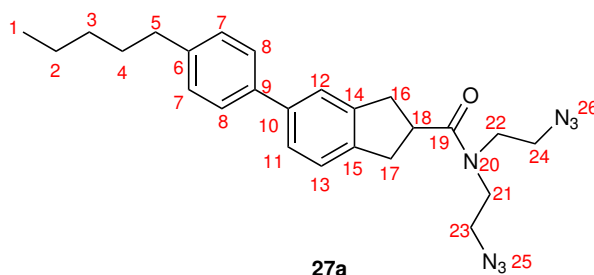
6.13.1 Synthesis of bis(2-azidoethyl)amine (**26**)



As the bisazide **26** is assumed to be explosive, all the work was conducted behind a blast shield, and the synthesis was conducted exactly like described by Chen *et al.*⁶⁸

To a stirred solution of NaN₃ (2.74 g, 42 mmol) in deioniticed water (30 ml), **25** (3.07g, 17.2 mmol) was added. After stirring for 2 h at 90 °C, another portion of NaN₃ (2.73, 42 mmol) was added slowly. The reaction mixture was the stirred at 90 °C for 48 h. After cooling to r.t. the pH of the solution was adjusted to approx. 10 with NaOH (12 ml, 1 M, aq.). The aqueous solution was extracted with EtOAc (4 x 30 ml), and the combined organic phases was dried with anhydrous MgSO₄, and the solvents were removed in vacuo. The crude product was then distilled under reduced pressure (0.032 x 10⁻² mbar, 55-60°C), yielding **26** as a transparent oil (Appendix U.1). Yield: 61% (1.64 g, 11 mmol). Spectroscopic data for **26**: ¹H-NMR (400 MHz, CDCl₃): δ1.43 (s, 1 H), 2.8-2.83 (m, 4 H), 3.41-3.43 (t, 4 H, J = 11.4 Hz)

6.14 Synthesis of *N,N*-bis(2-azidoethyl)-5-(4-pentylphenyl)-2,3-dihydro-1*H*-indene-2-carboxamide (**27a**)



This bisazide compound **27a** was synthesised three times in low yields. The general method used is described by Singh *et al.*⁶⁷ The first entry used excess **26** as a cobase, while the other entries used *N,N*-diisopropylethylamine as a cobase. Both the extraction methods and reaction times were varied in the different entries, in an attempt to optimise the reaction. See table for experimental data.

First attempt

The acid chloride **24a** (0.19 g, 0.57 mmol) was dissolved in dry toluene (5 ml), and added slowly to a cooled (icebath) solution of **26** (0.088 g, 0.57 mmol) in dry toluene (4 ml) over 10 minutes. The mixture was then heated to approx. 70 °C, and stirred for 1 h. At this time another equivalent of **26** (0.096 g, 0.62 mmol) was added to the reaction mixture, and the reaction was left stirring overnight. After approx 20 h the reaction was stopped and the solvent was removed in vacuo. The crude was dissolved in DCM (30 ml) and washed with base (NaOH, 1M aq.) and water (50 ml). The organic phase was dried over MgSO₄ and concentrated in vacuo. Purification was done by flash column chromatography (20% DCM/EtOAc), yielding **27a** as a transparent oil. Yield: 45% (0.11 g, 0.26 mmol).

Second attempt

The acid chloride **24a** (0.49 g, 1.5 mmol) was dissolved in dry toluene (8 ml), and added slowly to a cooled (icebath) solution of **26** (0.281 g, 1.8 mmol, 1.2 eq.) in dry toluene (5 ml) over 10 minutes. *N,N*-diisopropylethylamine (0.52 ml, 2 eq.) was added directly after. The mixture was then heated to approx. 70 °C, and stirred for 21 h. After complete reaction the solvent was removed in vacuo. The crude was dissolved in DCM (30 ml) and washed with acid (HCl, 1M aq., 30 ml) and water (50 ml). The organic phase was then washed with base (NaOH, 1M, 4 ml) and water (50 ml). The organic phase was dried over MgSO₄ and concentrated in vacuo. Purification was done by flash column chromatography (DCM), yielding **27a** as a transparent oil. Yield: 35% (0.23 g, 0.52 mmol).

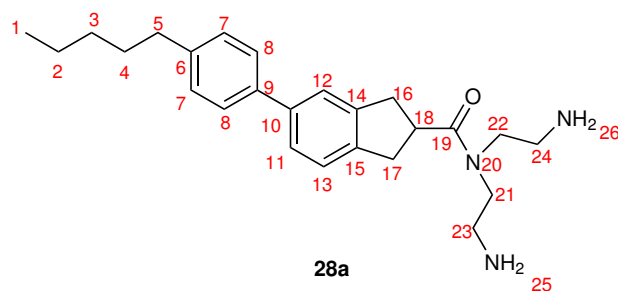
Third attempt

The acid chloride **24a** (0.13 g, 0.4 mmol) was dissolved in dry toluene (3 ml), and added slowly to a cooled (icebath) solution of **26** (0.087 g, 0.56 mmol, 1.4 eq.) in dry toluene (2 ml) over 10 minutes. *N,N*-diisopropylethylamine (0.14 ml, 2 eq.) was added directly after. The mixture was then heated to approx. 70 °C, and stirred for 45 h. After complete reaction the solvent was removed in vacuo. The crude was dissolved in EtOAc (30 ml) and washed with acid (aq., 1M HCl, 35 ml) and water (5 x 50 ml, until pH in the organic solution was approx. 4-5.). The water phase was then extracted with DCM (3 x 50 ml). The combined organic phases were dried over MgSO₄ and concentrated in vacuo. Purification was done by flash column chromatography (n-pentane/EtOAc (4:1)), yielding **27a** as a transparent oil with a pale yellow tint. Yield: 22% (0.04 g, 0.09 mmol).

Spectroscopic data for **27a**: ¹H-NMR (400 MHz, DMSO-d₆): δ0.87 (m, 2 H, H-1), 1.27-1.35 (m, 4 H, H-2, H-3), 1.59 (quint., 2 H, J = 7.5 Hz, H-4), 2.59 (t, 2 H, J = 7.6 Hz, H-5), 3.08-3.21 (m, 4 H, H-16, H-17), 3.48 (m, 2 H, H-23/H-24), 3.53 (m, 2 H, H-21/H-22), 3.62 (m, 2 H, H-21/H-22), 3.74 (m, 2 H, H-23/H-24), 7.25 (dd, 3 H, J₇ = 8.1, J₁₃ = 7.3, H-7, H-13), 7.4 (d, 1 H, J = 8.2, H-11), 7.45 (s, 1 H, H-12), 7.52 (d, 2 H, J = 8.2 Hz, H-8). ¹³C NMR (150 MHz, DMSO-d₆): δ13.9 (C-1), 22.0 (C-2), 30.6 (C-3), 30.9 (C-4), 34.7 (C-5), 36.3 (C-17), 36.6 (C-16), 40.2 (C-18), 44.6 (C-21/C-22), 46.6 (C-21/C-22), 48.2 (C-23/C-24), 49.3 (C-23/C-24), 122.2 (C-11), 124.4 (C-13), 124.9 (C-12), 126.4 (C-8), 128.8 (C-7), 137.9 (C-10), 138.6 (C-9), 140.9 (C-14), 141.2 (C-6), 142.7 (C-15), 175.0 (C-19). IR (neat, cm⁻¹): 3022 (m), 2926 (m), 2855 (m), 2091 (s), 1640 (s), 1518 (m), 1486 (m), 1441 (m), 1416 (w), 1368 (w), 1347 (m), 1280 (m), 1204 (m), 1184 (m), 1097 (m), 1045 (m), 1006 (w), 916 (w), 888 (w), 845 (w), 815 (m), 801 (m), 736 (w), 639 (w), 603 (w), 580 (w), 554 (m), 526 (w), 423 (w). HRMS (TOF ASAP +): *m/z* calculated for C₂₅H₃₂N₇O [M]⁺: 446.2668; found: 446.2664. ¹H NMR, ¹³C NMR, IR spectra as well as MS report can be found in Appendix V.

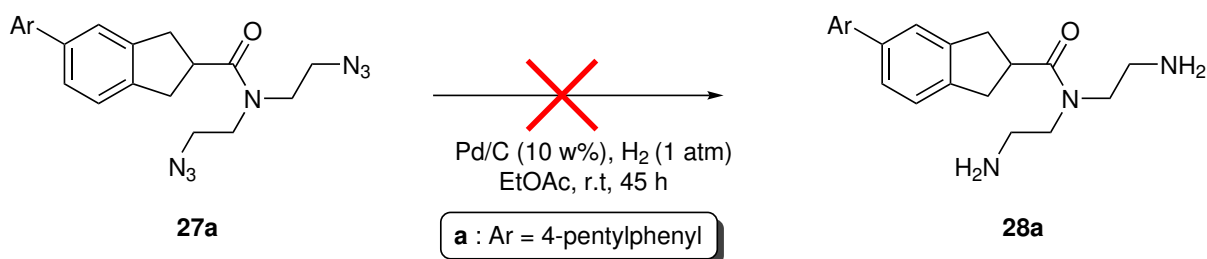
6.15 Attempted reduction of **27** to yield the bisamine compound **28**

The reduction of **27a** to yield **28a** was attempted using three different methods as presented below.



6.15.1 Hydrogenolysis (*failed*)

The reduction of **27a** to **28a** was attempted using Pd/C and hydrogen gas (1 atm).^{35g} See Scheme 6.13 for the reaction equation.

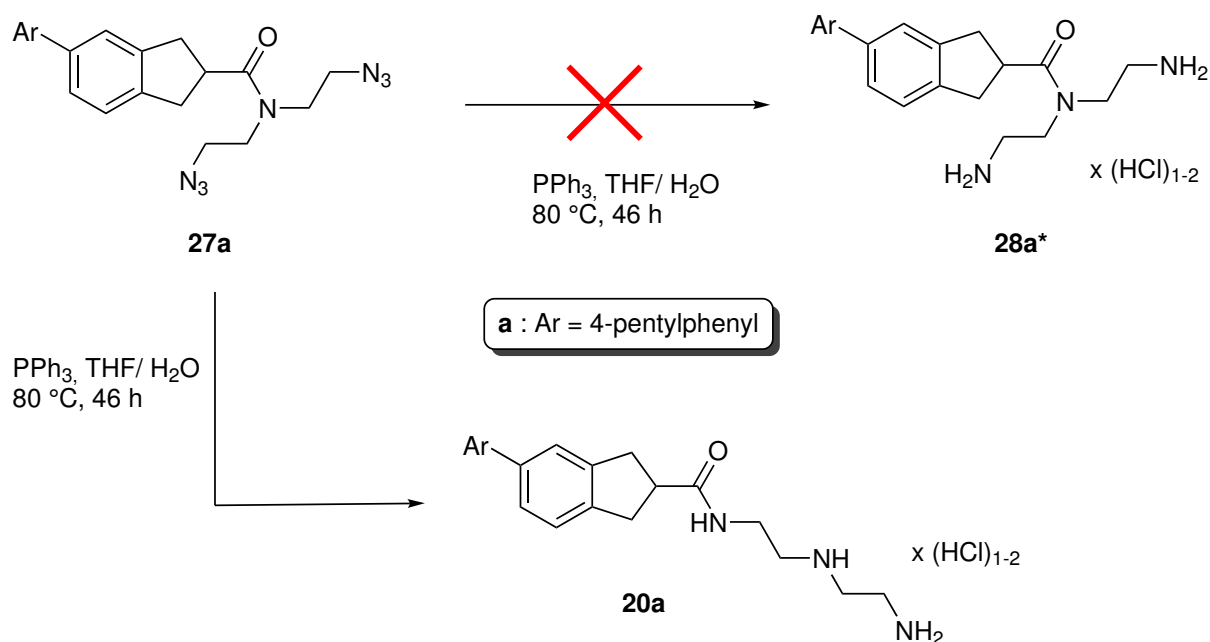


Scheme 6.13: Reaction conditions for the attempted hydrogenolysis of **27a** to **28a**.

The bisazide **27a** (0.09 g, 0.2 mmol) was mixed with Pd/C (10 w%, 0.023 g) and dissolved in EtOAc (5 ml). The reaction mixture was placed under H₂-atmosphere (1 atm). After 45 h the reaction was stopped, and the solvent was removed in vacuo. The crude product was purified by filtration through a thin layer of silica (EtOAc) to remove the catalyst. This yielded a transparent oil (0.034 g), but the ¹H NMR analysis showed that the sample contained other unknown compounds. No attempts at further purification were attempted. The ¹H NMR and ¹³C NMR spectra can be found in Appendix Y.

6.15.2 Reduction with PPh₃ (*failed*)

The reduction of **27a** to yield **28a** was attempted using PPh₃ as described by Pal *et al.*⁷² See Scheme 6.14 for the reaction equation.

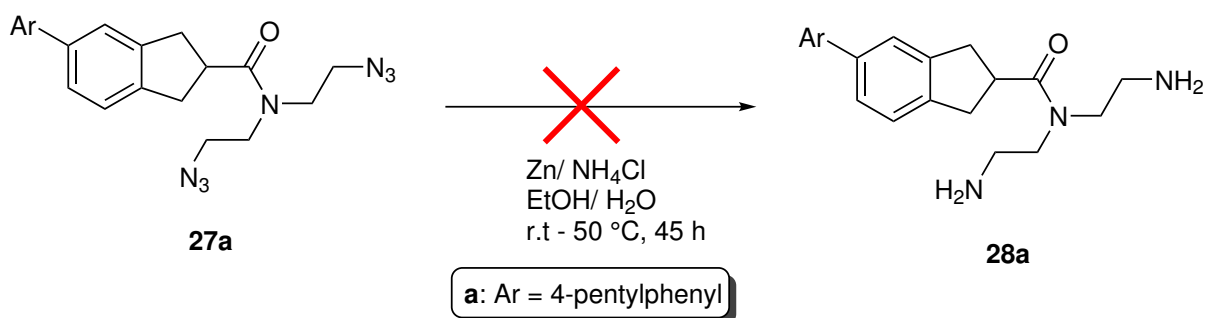


Scheme 6.14: Attempted reduction of the bisazide **27a** to **28a** using PPh_3 .

PPh_3 (0.81 g, 0.31 mmol, 6 eq.) was dissolved in a solution of the bisazide **27a** (0.23 g, 5.1 mmol, 1 eq.) in $\text{THF}/\text{H}_2\text{O}$ (11 ml, 10:1). The reaction mixture was heated to $80\text{ }^\circ\text{C}$ and followed with TLC-analysis (EtOAc). After 24 h more solvent mixture was added ($\text{THF}:\text{H}_2\text{O}$, 2:0.2 ml). After a total of 46 h the reaction was stopped, and the solvents were removed in vacuo. The crude was then dissolved in EtOAc (30 ml) and extracted with HCl (aq., 1M, 30 ml). An off white solid precipitated during extraction, and was isolated and concentrated separately. The organic phase was then washed with water (3 x 30 ml), and the combined water phases were concentrated in vacuo. This yielded an off-white solid, 102% (0.244 g). ^1H NMR analysis indicated that this was a product mix, containing PPh_3O and a mixture of possible product and by-products. No further purification was attempted. The ^1H NMR spectrum can be found in Appendix Z. ^1H NMR analysis of the white precipitate indicated an amide-compound. This amide product was later matched to amidoamine salt **20a**. The ^1H NMR and ^{13}C NMR spectra can be found in Appendix AA, and for other spectroscopic details see Chapters 6.9.2 and 5.5.

6.15.3 Reduction with Zn and NH_4Cl (failed)

The reduction of **27a** to yield **28a** was attempted using a $\text{Zn}/\text{NH}_4\text{Cl}$ combination as described by Lin *et al.*⁷³ See Scheme 6.15 for the reaction equation.

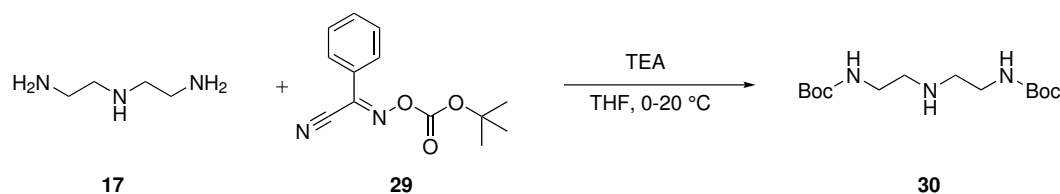


Scheme 6.15: Attempted reduction of the bisazide **27a** to **28a** using Zn and NH_4Cl .

The bisazide **27a** (0.045 g, 0.1 mmol) was dissolved in EtOH/ H_2O (2 ml, 3:1), and NH_4Cl (0.025 g, 0.5 mmol, 5.7 eq.) was added. The mixture was stirred for 1 minute, and then the Zn-powder (0.018 g, 0.27 mmol, 2.6 eq.) was added. The reaction mixture was stirred vigorously at r.t for 18 h. At this point more Zn-powder (0.012 g, 0.018 mmol, 1.8 eq.) was added. The reaction was left stirring at r.t for another 6 h. The temperature was then increased to 50 °C and the reaction was left stirring for 19 h. The reaction mixture was then diluted with EtOAc (5 ml) and NH_3 (aq, 0.2 ml) was added. The mixture was filtered, and the filtrate was washed with brine (3 x 5 ml). The organic phase was then dried over MgSO_4 and concentrated in vacuo. The crude was analysed by ^1H NMR spectroscopy, which revealed that the desired product had not been formed, and the sample contained mostly starting material. The ^1H NMR spectrum can be found in Appendix AB.

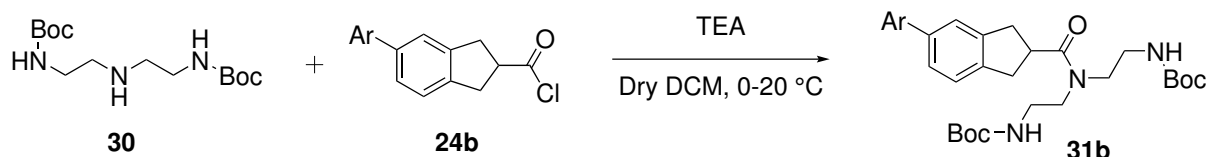
6.16 Synthesis of the Boc-protected bisamine intermediate (**31**)

The synthesis of the Boc-protected bisamine intermediate **31** is a two step synthesis, starting with the synthesis of the Boc-protected amine reagent **30**. This reaction was performed once in accordance with the literature.⁷⁴ The reaction equation is presented in Scheme 6.16, while the yield, characterisation and work-up procedure is presented in Chapter 6.16.1.



Scheme 6.16: Boc protecting bisamine **17** to obtain **30**.

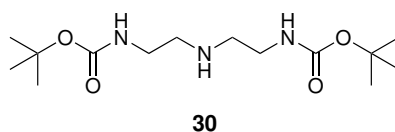
Compound **31** was synthesised once following the general procedure described by Raines.⁷⁴ The reaction equation is presented in Scheme 6.12. Yield, characterisation data and work-up procedure for the product is presented in Chapter 6.16.2.



b : Ar = 2,4,6-triisopropylphenyl

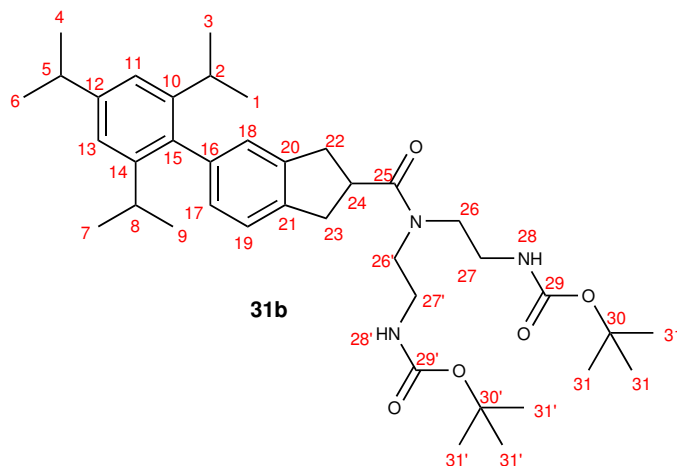
Scheme 6.17: Synthesis of the Boc-protected bisamine **31b**.

6.16.1 Synthesis of di-tert-butyl (azanediylbis(ethane-2,1-diyl)) dicarbamate (**30**)



The amine **17** (2.1 ml, 0.019 mol) and TEA (8.1 ml) was dissolved in THF (100 ml), put under N₂-atm and cooled to 0 °C in an ice bath. A solution of the Boc-reagent **29** (9.56 g, 0.04 mol) in THF (40 ml) was added dropwise over the course of 30 min. The reaction was then stirred at 0 °C for 1h. Then the ice bath was removed, and the reaction mixture was stirred at r.t. for 1 h. After the reaction was complete with TLC-analysis (EtOAc), the solvent was removed i vacuo. The crude was dissolved in DCM (50 ml), washed with 5% w/v NaOH solution (3 x 50 ml). The organic phase was dried over MgSO₄ and concentrated in vacuo. The crude product was then purified with FCC (silica, 10% v/v MeOH in DCM, 1% ammonium hydroxide). This yielded **30** as a transparent oil with a weak yellow tint. Yield: 87% (5.1 g, 0.017 mol). Spectroscopic data for **30**: ¹H-NMR (600 MHz, CDCl₃): δ 1.23 (s, 1 H), 1.44 (s, 18 H), 2.73 (t, 4 H, J = 5.8 Hz), 2.21 (app. q, 4 H, J = 5.1 Hz), 4.94 (s (br), 2 H). The ¹H NMR spectrum can be found in Appendix AC.

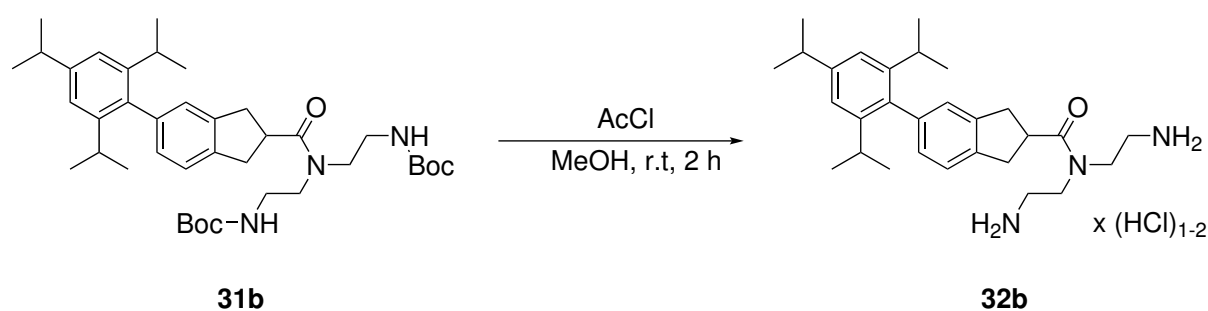
6.16.2 Synthesis of di-tert-butyl (((5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carbonyl) azanediyl)bis(ethane-2,1-diyl))dicarbamate (**31b**)



The Boc-protected amine **30** (0.124 g, 0.4 mmol) was dissolved in dry DCM (4 ml) and cooled to 0 °C in an ice bath. TEA (0.3 ml) and the acid chloride **24b** (0.18 g, 0.5 mmol) was added and the reaction was stirred at 0 °C for 1 h. After 1 h the ice bath was removed and the stirring was continued at r.t for 17 h. The solvents were removed in vacuo, and the crude was purified twice by flash column chromatography (1. silica, EtOAc/n-pentane(1:1), 2. silica, EtOAc). This yielded **31** as brown-tinted oil/ glass. Yield: 54% (0.143 g, 0.22 mmol). Mp: 80-82 °C. Spectroscopic data for **31b**: ¹H-NMR (600 MHz, DMSO-d₆): δ0.99-1.03 (m, 12 H, H-1, H-3, H-7, H-9), 1.23 (dd, 6 H, J = 6.9 Hz, H-4, H-6), 2.45-2.62 (m, 2 H, H-2, H-8), 2.88 (app. sept, 1 H, J = 10.3, H-5), 3.03-3.2 (m, 8 H, H-22, H-23, H-27, H-27'), 3.29-3.33 (m, 2 H, H-26/H-26'), 3.36-3.5 (m, 2 H, H-26/H-26'), 3.59-3.69 (m, 1 H, H-24), 6.82 (t, 1 H, J = 5.4 Hz, H-28/H-28'), 6.87-6.9 (m, 1H, H-17), 6.92 (s, 1 H, H-18), 7.0 (t, 1 H, J = 5.9 Hz, H-28/H-28'), 7.18-7.23 (m, 1 H, H-19). ¹³C NMR (150 MHz, DMSO-d₆): δ23.9 (C-1, C-3, C-7, C-9), 24.0 (C-4, C-6), 28.1 (C-31/ C-31'), 28.2 (C-31/ C-31'), 29.6 (C-2/ C-8), 29.7 (C-2/ C-8), 33.6 (C-5), 36.5 (C-23), 36.7 (C-22), 37.9 (C-27/ C-27'), 38.7 (C-27/ C-27'), 40.1 (C-24), 45.5 (C-26/ C-26'), 47.3 (C-26/ C-26'), 77.6 (C-30/ C-30'), 77.8 (C-30/ C-30'), 119.9 (C-11/ C-13), 120.0 (C-11/ C-13), 123.6 (C-19), 124.8 (C-18), 127.5 (C-17), 137.0 (C-15), 138.3 (C-16), 140.1 (C-20), 141.8 (C-21), 145.9 (C-10/ C-14), 146.0 (C-10/ C-14), 147.2 (C-12), 155.6 (C-29, C-29'), 174.5 (C-25). IR (neat, cm⁻¹): 3334 (br), 2960 (m), 2930 (m), 2868 (w), 1695 (s), 1631 (s), 1514 (s), 1454 (s), 1390 (m), 1364 (s), 1341 (m), 1320 (m), 1268 (m), 1247 (s), 1166 (s), 1106 (s), 1070 (m), 1042 (m), 984 (m), 964 (m), 940 (m), 875 (m), 829 (w), 814 (w), 781 (w), 758 (w), 725 (w), 649 (m), 632 (m), 494 (w), 431 (w). HRMS (TOF ASAP +): *m/z* calculated for C₃₉H₅₉N₃O₅ [M+Na]⁺: 672.4352; found: 672.4360. ¹H NMR, ¹³C NMR, IR spectra as well as MS report can be found in Appendix AD.

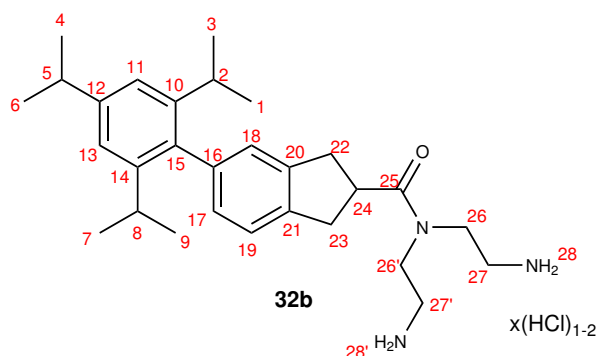
6.17 Deprotection of 31b to yield the amine salt 32b

The deprotection of **31b** was performed as according to literature.⁶³ The reaction equation is presented in Scheme 6.18, and yields, characterisation and spectroscopic data are presented below.



Scheme 6.18: Deprotecting of **31b** to yield **32b**.

6.17.1 Synthesis of 2,2'-((5-(2,4,6-triisopropylphenyl)-2,3-dihydro-1H-indene-2-carbonyl)-azanediyl)bis(ethan-1-aminium chloride) (**32b**)



The Boc-protected bisamine (0.12 g, 0.18 mmol) was dissolved in MeOH (1.5 ml), and the AcCl (0.4 ml, 0.55 mmol, 30 eq.) was added slowly. The reaction was followed by TLC-analysis (EtOAc) and stopped after 2 h. The crude was purified by coevaporation with MeOH (10 x 10 ml). ^1H NMR analysis indicated that the compound was not pure, and it was therefore recrystallised in EtOH (freezer, 50 h). This yielded **32b** as an off-white solid. Yield: 24% (20 mg, 0.04 mmol). Mp: 263-266 °C. Spectroscopic data for **32b**: ^1H -NMR (600 MHz, DMSO- d_6): δ 0.99-1.03 (m, 12 H, H-1, H-3, H-7, H-9), 1.22 (d, 6 H, J = 6.9 Hz, H-4, H-6), 2.45-2.59 (m, 2 H, H-2, H-8), 2.88 (app. sept, 1 H, J = 6.9 Hz, H-5), 2.97 (s, 2 H, H-27/ H-27'), 3.06 (s, 2 H, H-27/ H-27'), 3.17-3.24 (m, 4 H, H-22, H-23), 3.56-3.3.59 (m, 2 H, H-26/H-26'), 3.72-3.77 (m, 3 H, H-19,H-26/H-26'), 6.89 (d, 1 H, J = 7.5 Hz, H-17), 6.96 (s, 1 H, H-18), 7.03 (s, 2 H, H-11, H-13), 7.21-7.28 (m, 1 H, H-19), 8.04 (s, 3 H, H-28/H-28'), 8.25 (s, 3 H, H-28/H-28'). ^{13}C NMR (150 MHz, DMSO- d_6): δ 23.9 (C-1, C-3, C-7, C-9), 24.0 (C-4, C-6), 29.7 (C-2, C-8), 33.6 (C-5), 36.4 (C-23), 36.6 (C-22), 37.2 (C-27/C-27'), 37.4 (C-27/C-27'), 40.2 (C-24), 43.4 (C-26/ C-26'), 44.9 (C-26/ C-26'), 119.9 (C-11/ C-13), 120.0 (C-11/ C-13), 123.7 (C-19), 124.9 (C-18), 127.6 (C-17), 136.9 (C-15), 138.4 (C-16), 140.0 (C-20), 141.6 (C-21), 145.9 (C-10/ C-14), 146.0 (C-10/ C-14), 147.3 (C-12), 175.8 (C-25). IR (neat, cm^{-1}): 2957 (s), 2925 (s), 2866 (s), 1630 (s), 1608 (s), 1464 (s), 1427 (s), 1381 (m), 1361 (s), 1316 (m), 1259 (m), 1153 (s), 1107 (m), 1054 (m), 1007 (m), 940 (m), 875 (m), 830 (m), 814 (m). HRMS (TOF ASAP +): m/z calculated for $\text{C}_{29}\text{H}_{44}\text{N}_3\text{O}$ [M] $^+$: 450.3477; found: 450.3477. HPLC (MeOH/ H_2O , 70:30 + 0.1% TFA, 1 ml/min, λ = 214 nm): t_{R} = 10.5 min, 75% pure. The ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC and IR spectra, as well as MS and HPLC reports can be found in Appendix AE.

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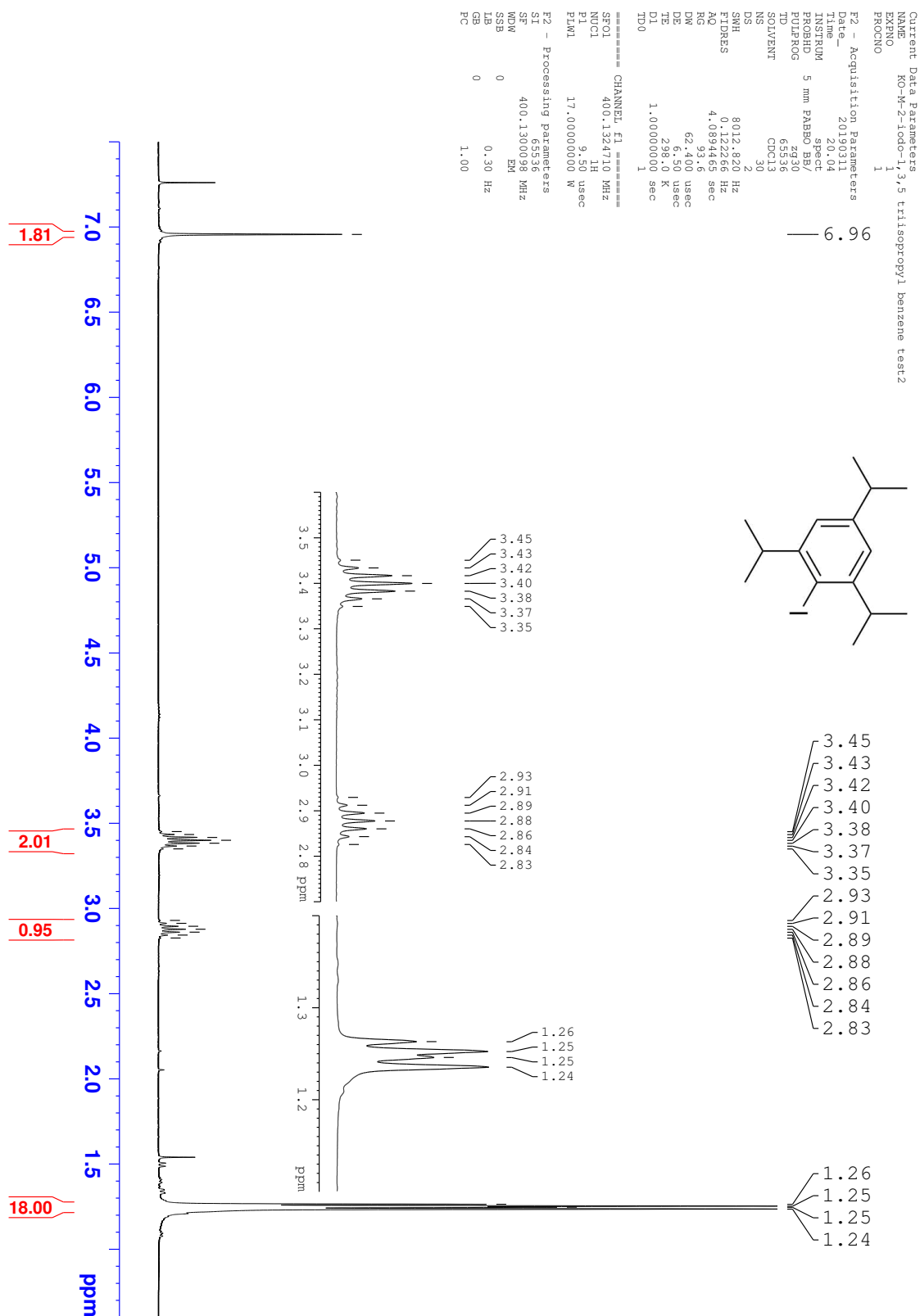
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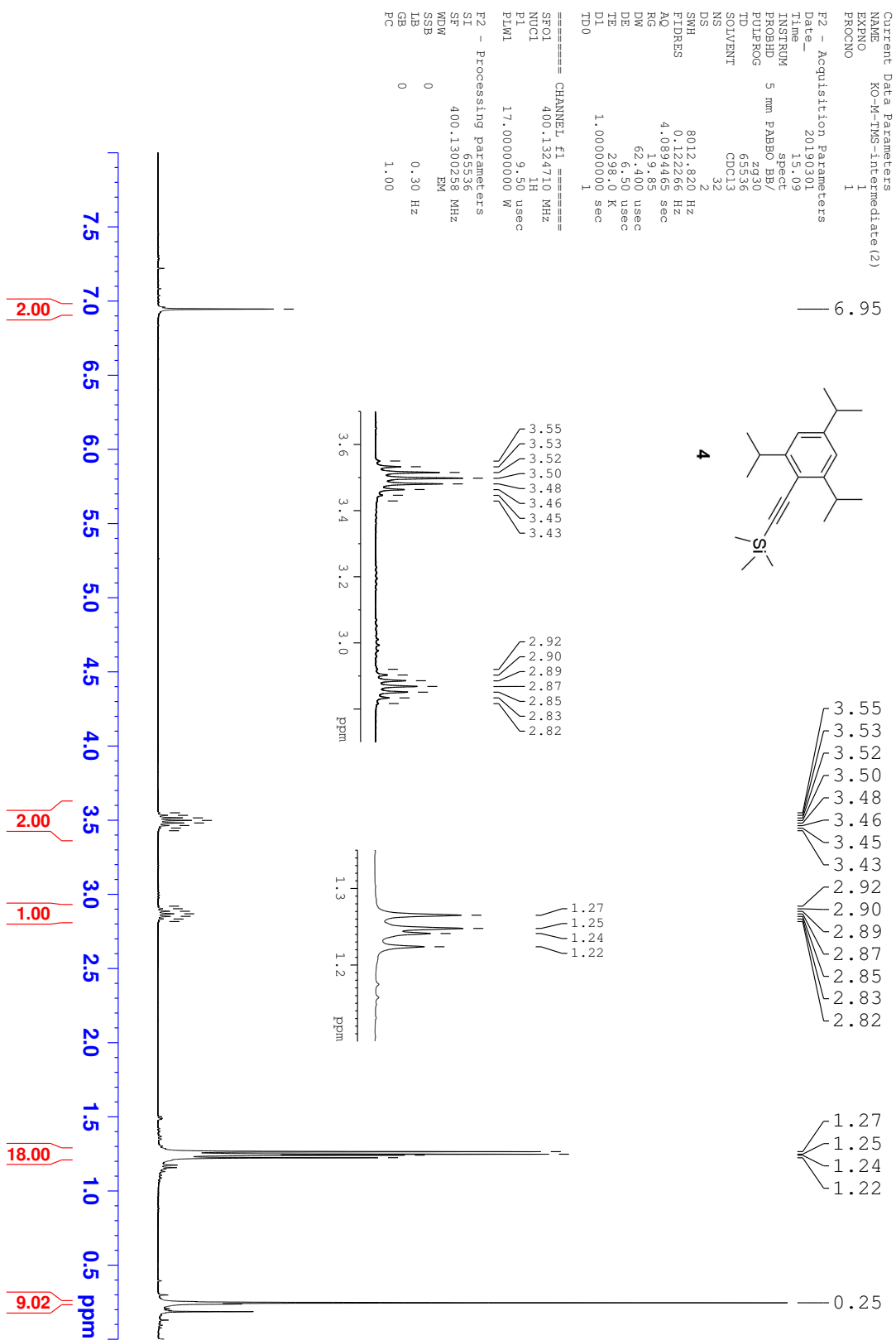
Appendix

The Appendix is organised after the numbering of the prepared compounds. It includes ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS for each new compound, additional HPLC chromatograms for the target compounds and ^1H NMR spectrum for previously prepared compounds.

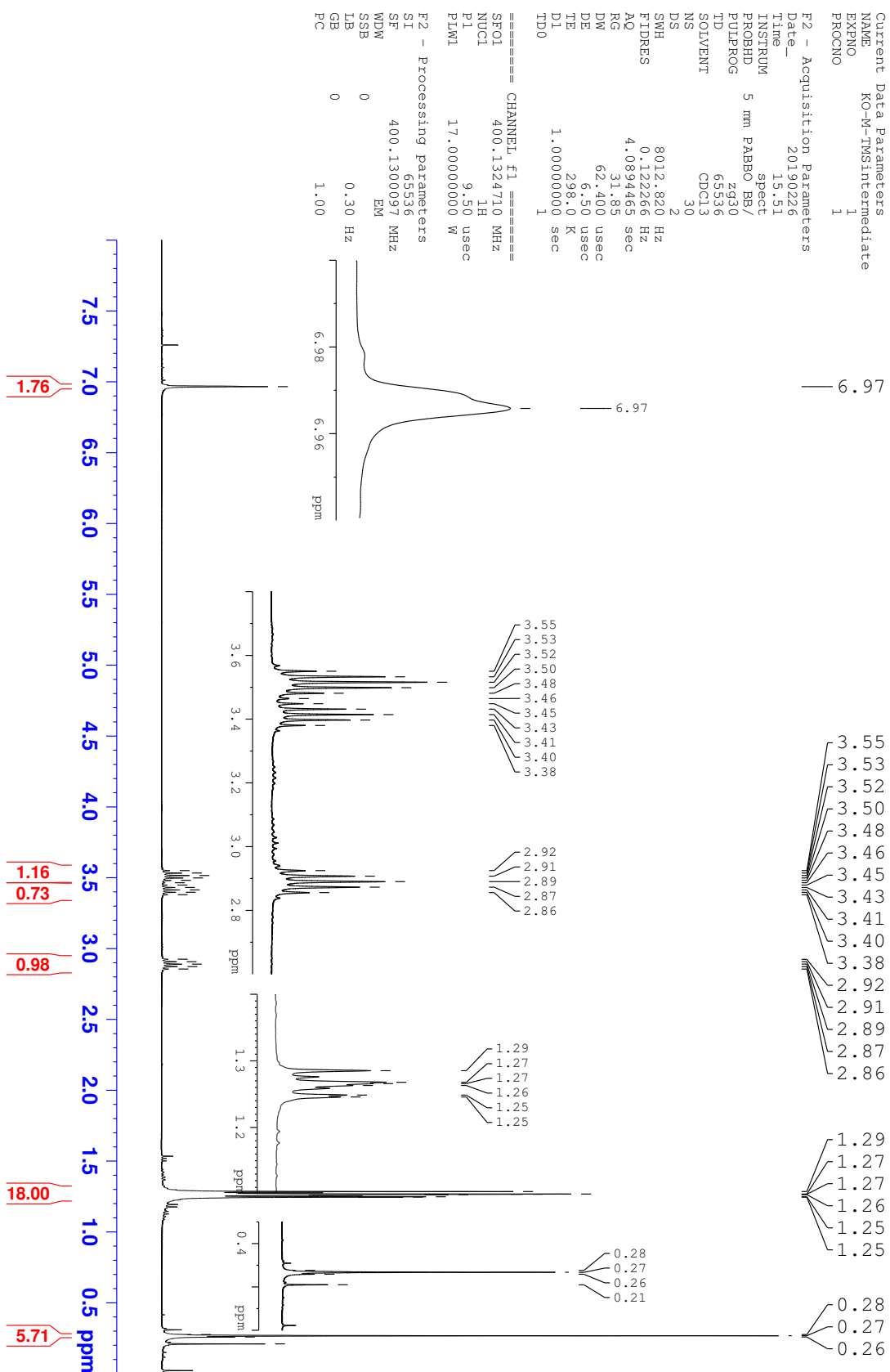
A.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 2



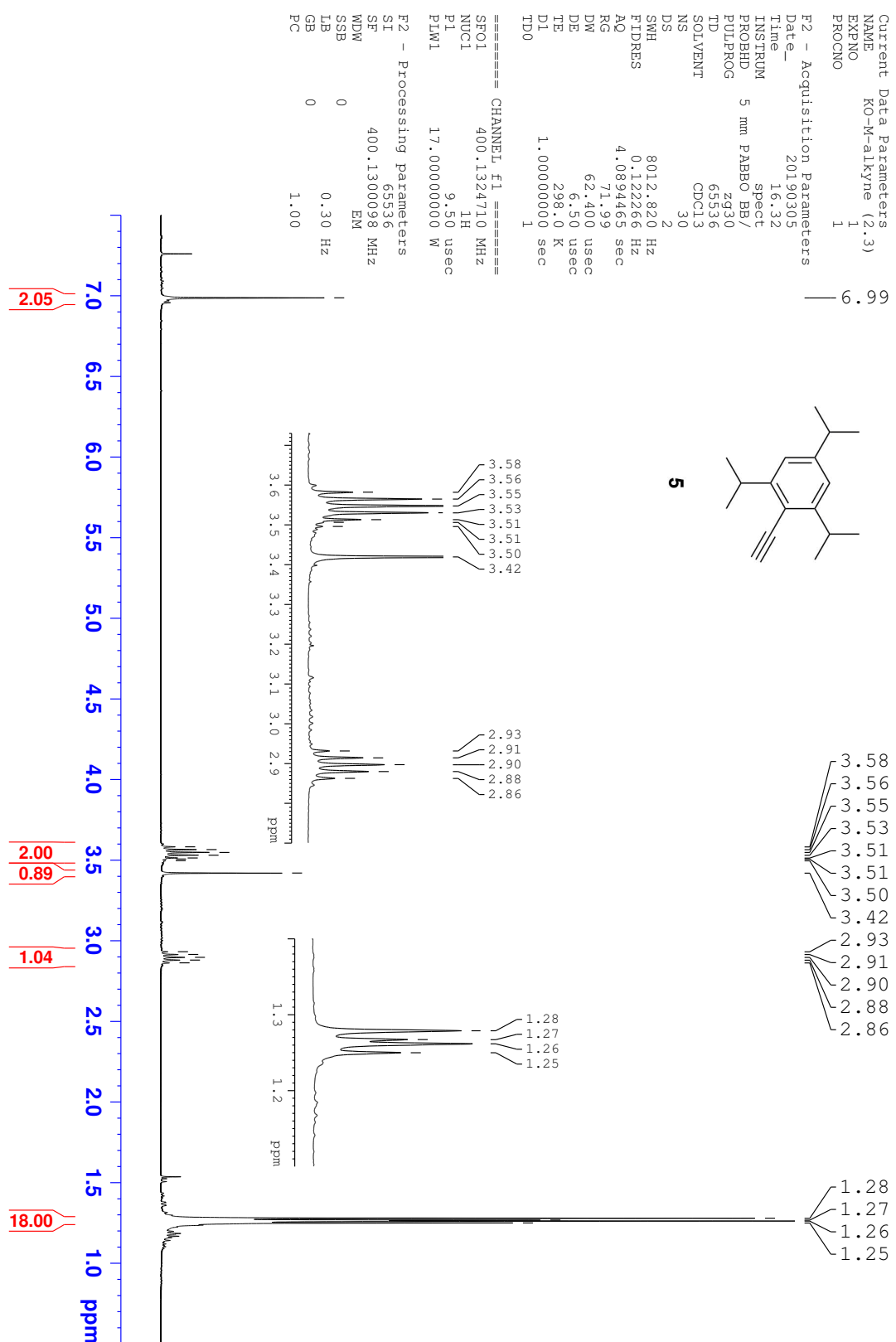
B.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for pure 4



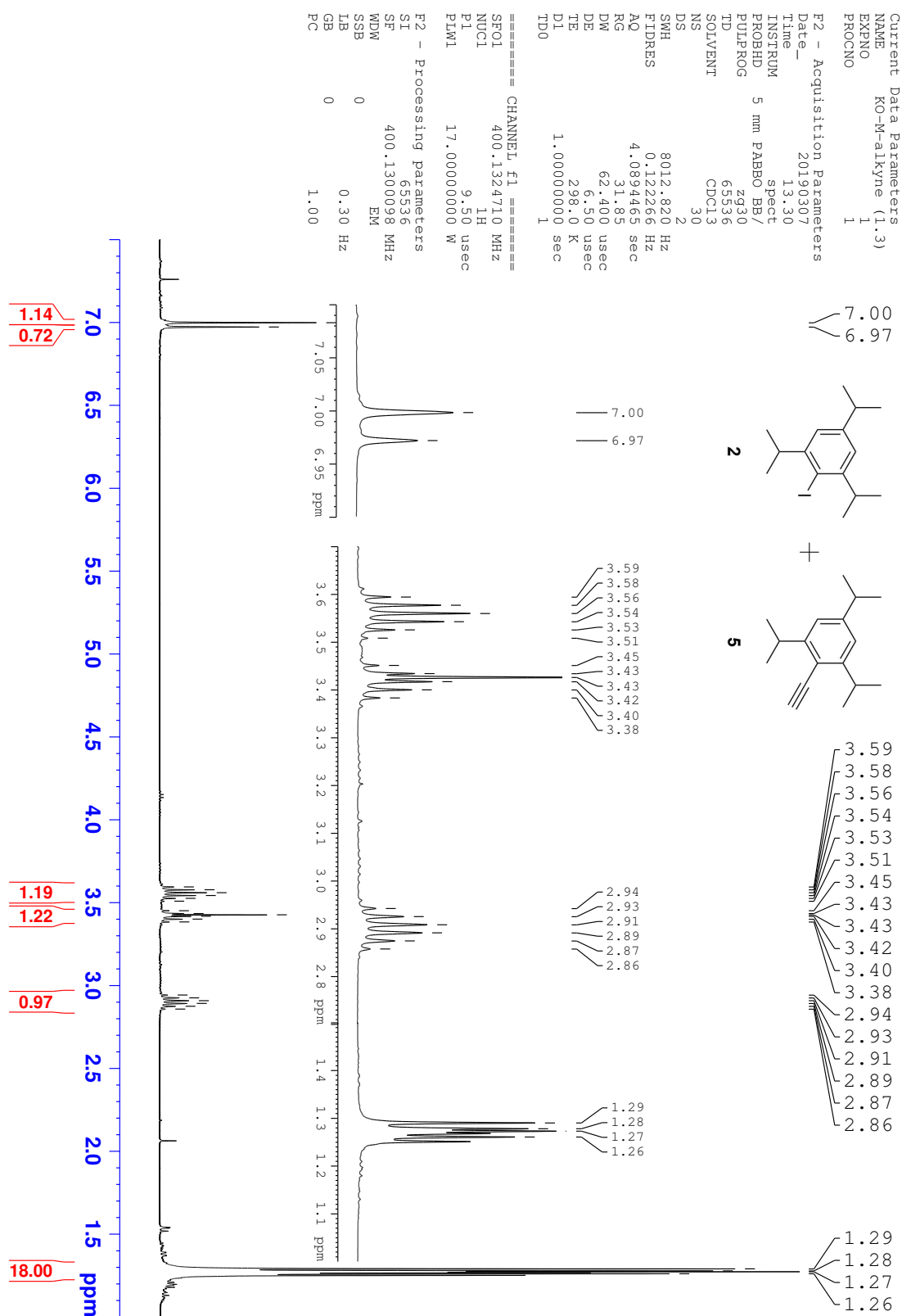
B.2 ¹H NMR (400 MHz, CDCl₃) Spectrum for unpure 4



C.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 5



D.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for product mixture of 2 and 5



D.2 HRMS Report for product mixture confirming 2 as the second compound.

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -2.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions

660 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

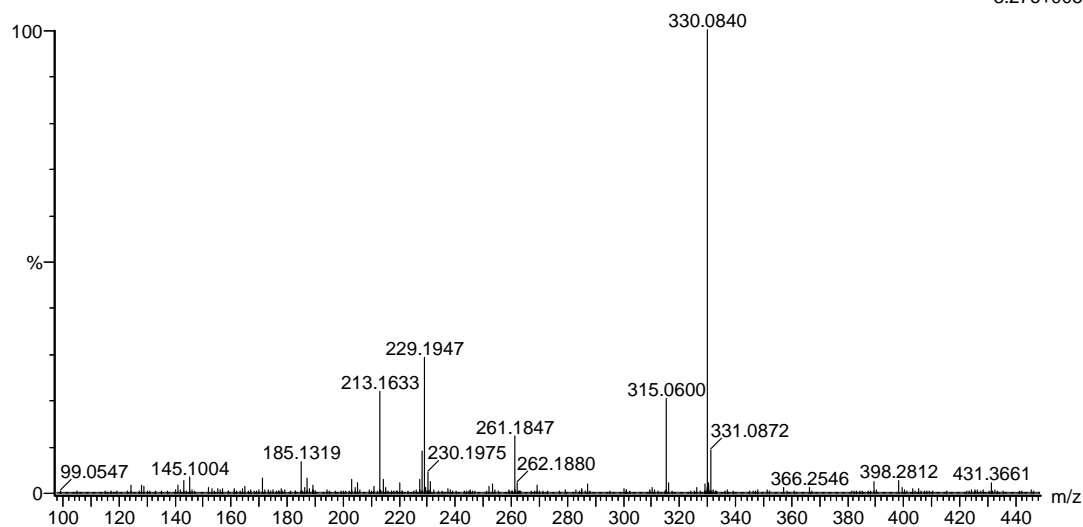
Elements Used:

C: 0-100 H: 0-150 N: 0-10 O: 0-10 I: 0-2

2019-169 25 (0.519) AM2 (Ar,35000.0,0.00,0.00); Cm (23:30)

1: TOF MS ASAP+

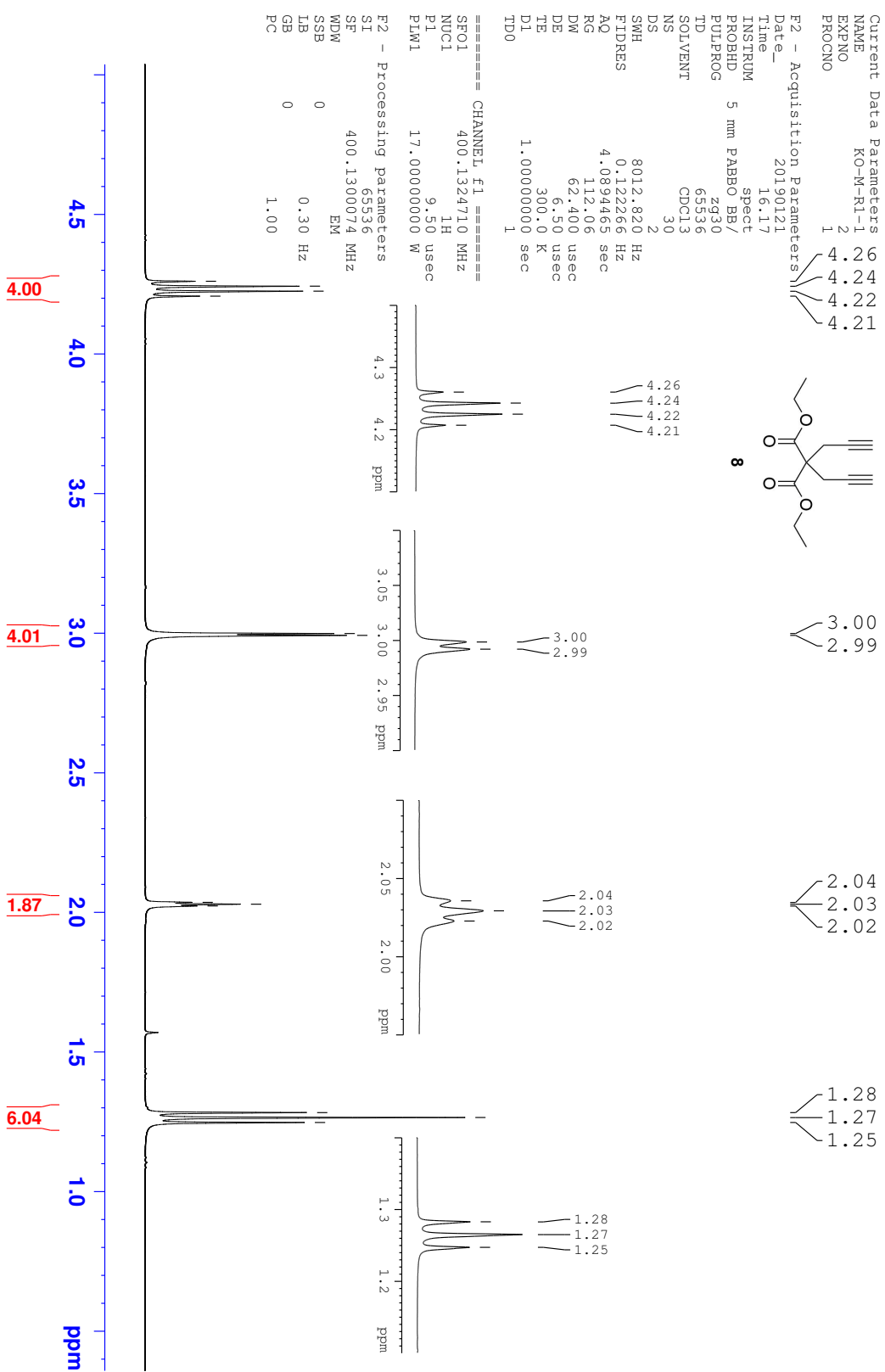
3.27e+005



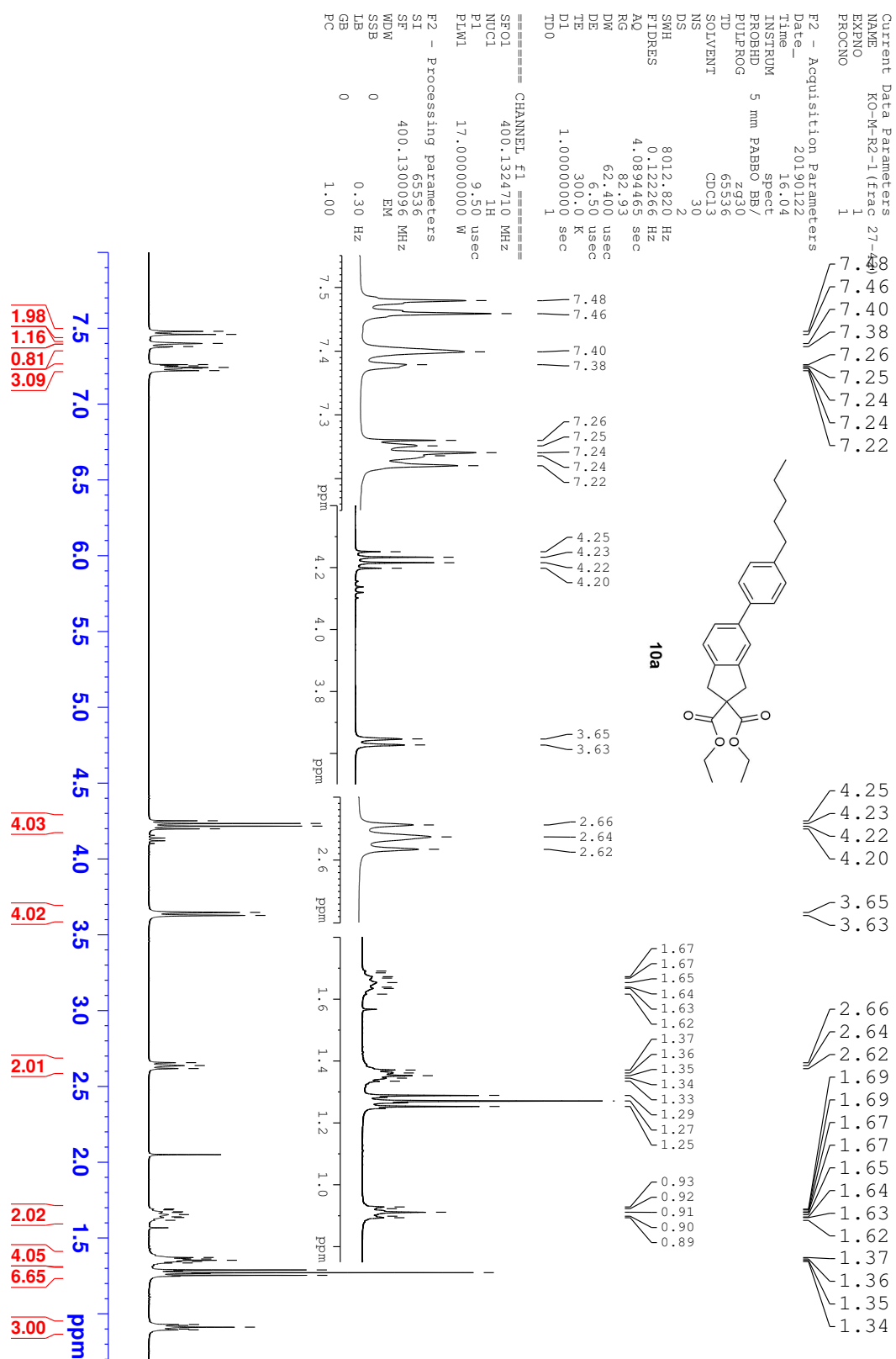
Minimum: -2.0
Maximum: 5.0 2.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
330.0840	330.0844	-0.4	-1.2	4.0	969.8	n/a	n/a	C15 H23 I

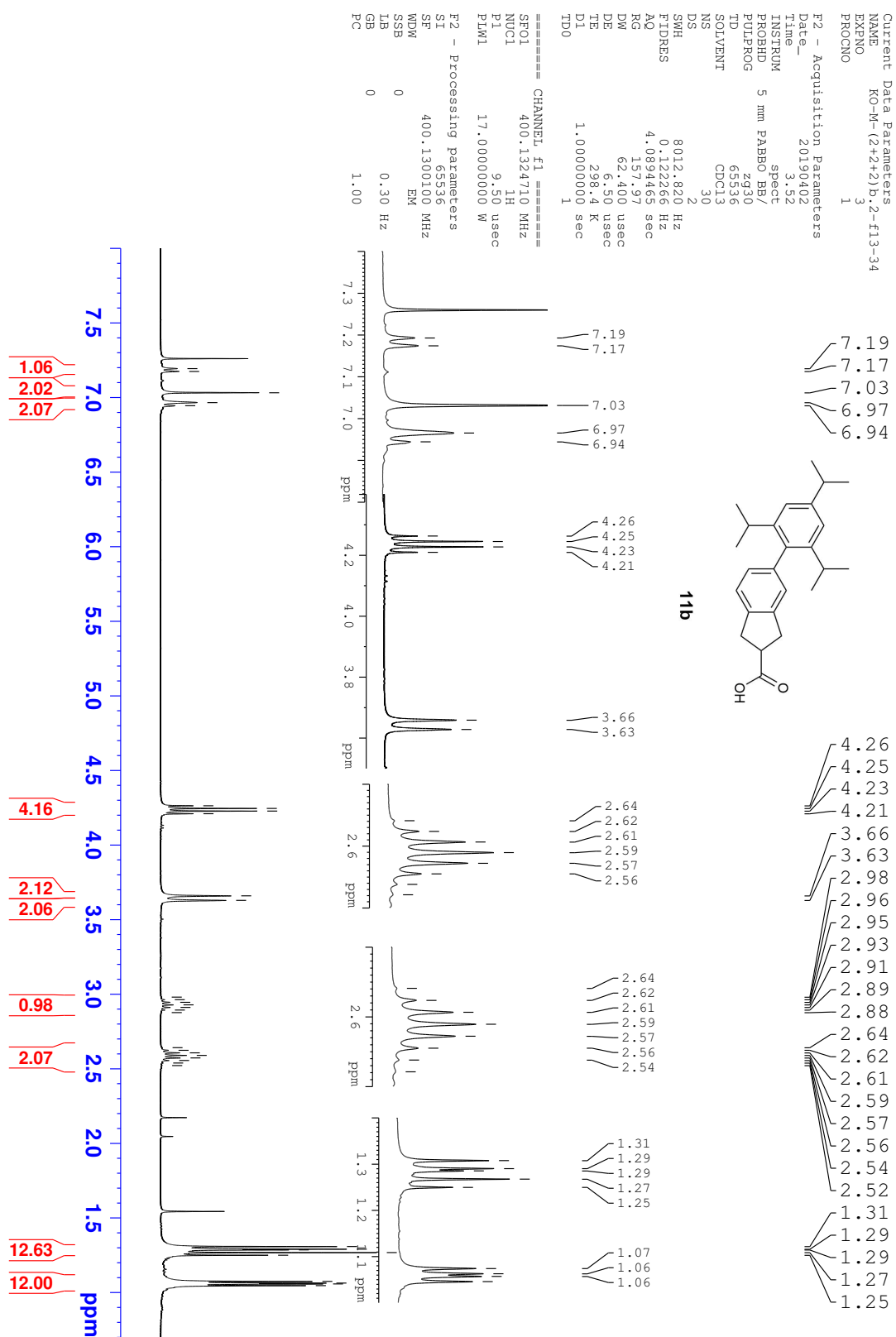
E.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 8



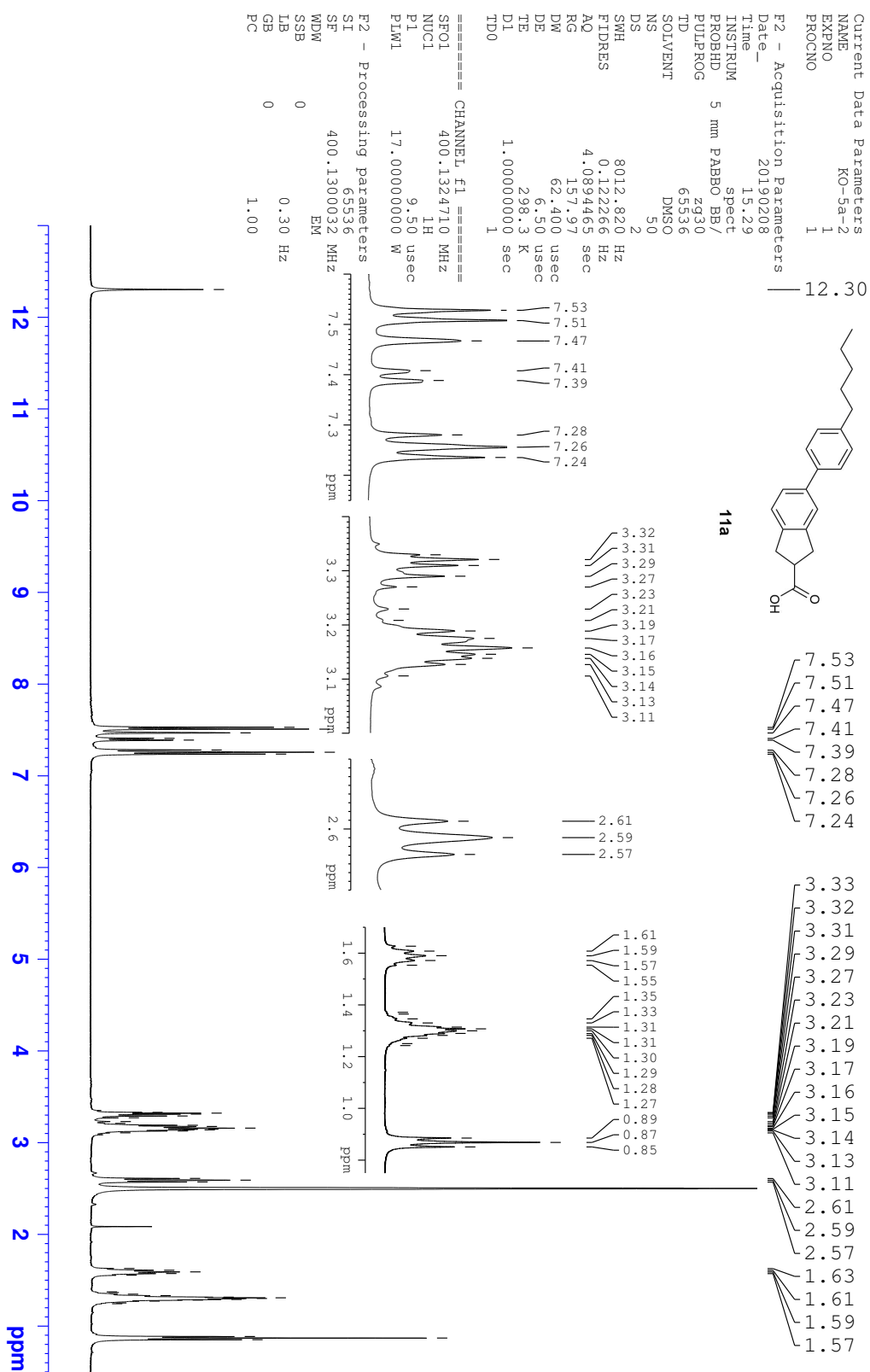
F1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 10a



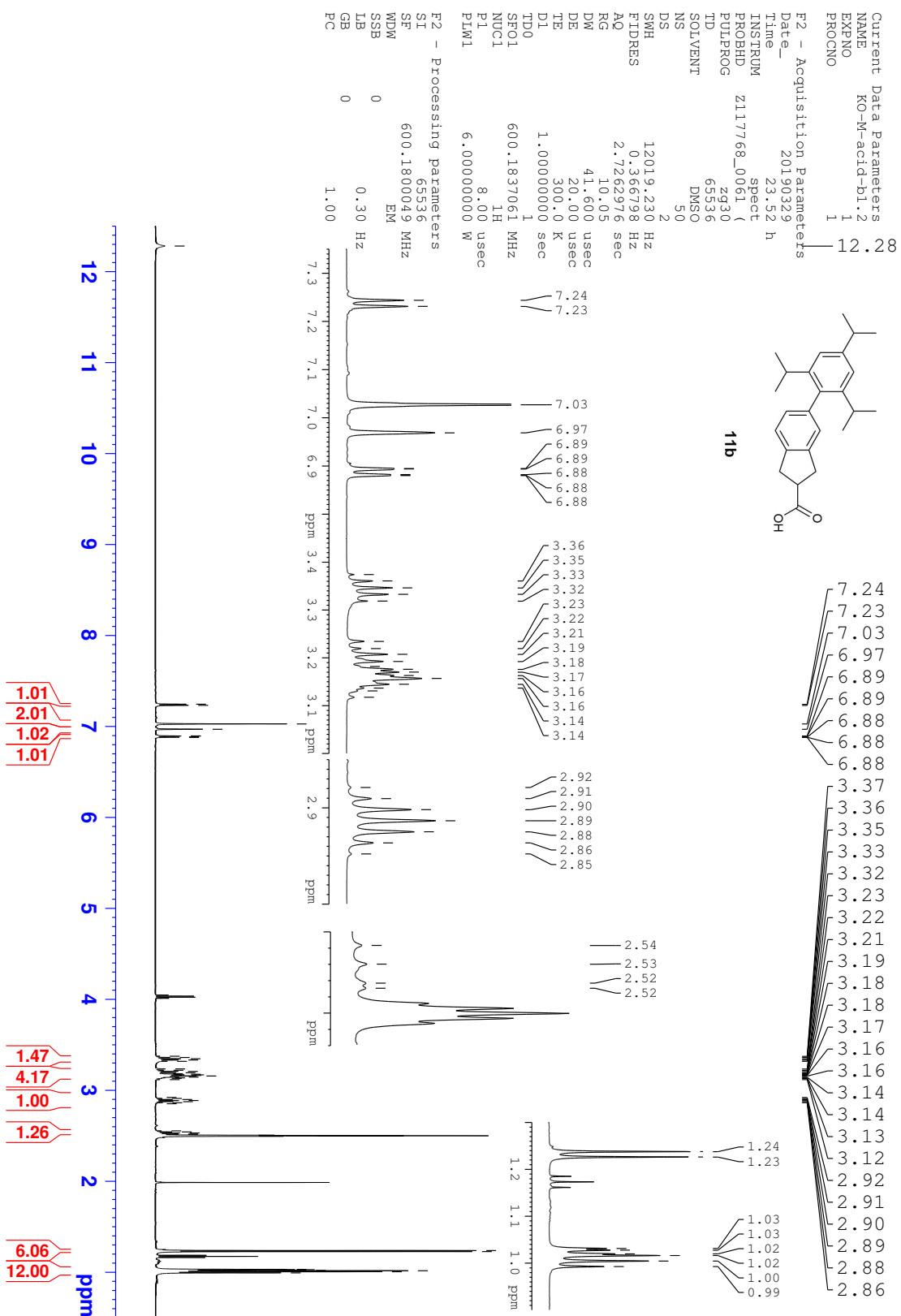
G.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 10b



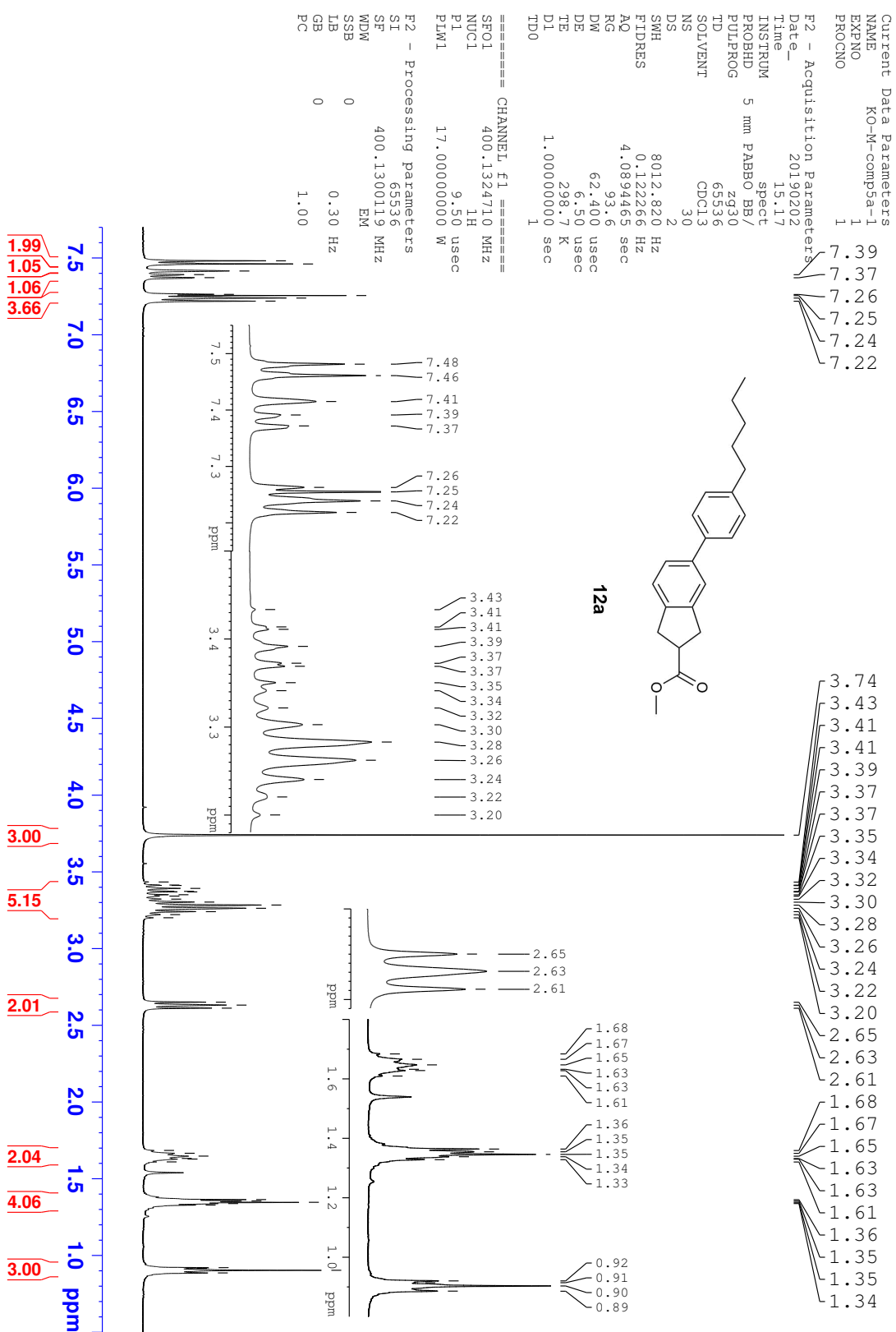
H.1 ¹H NMR (400 MHz, DMSO-d₆) Spectrum for 11a



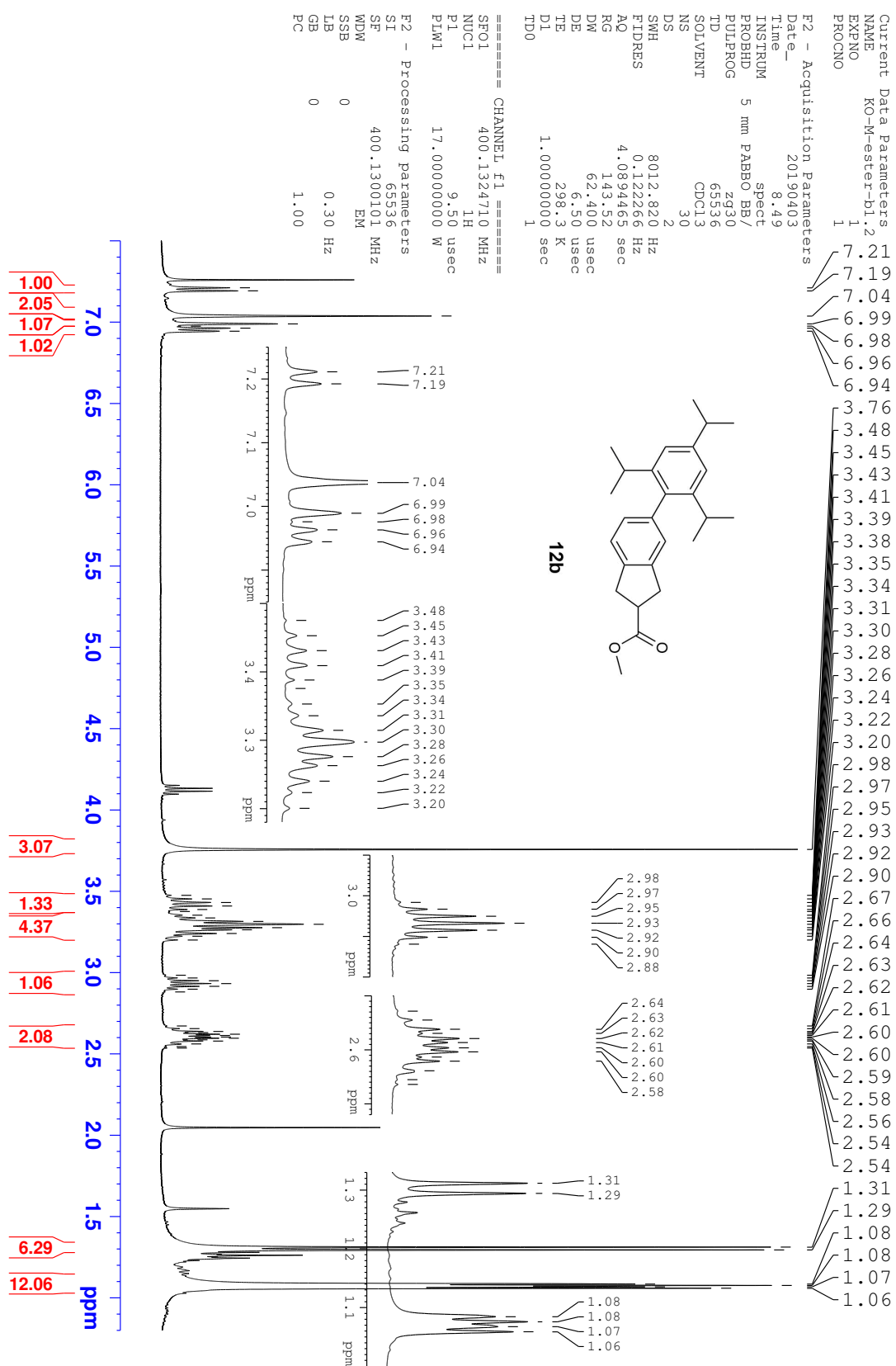
I.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 11b



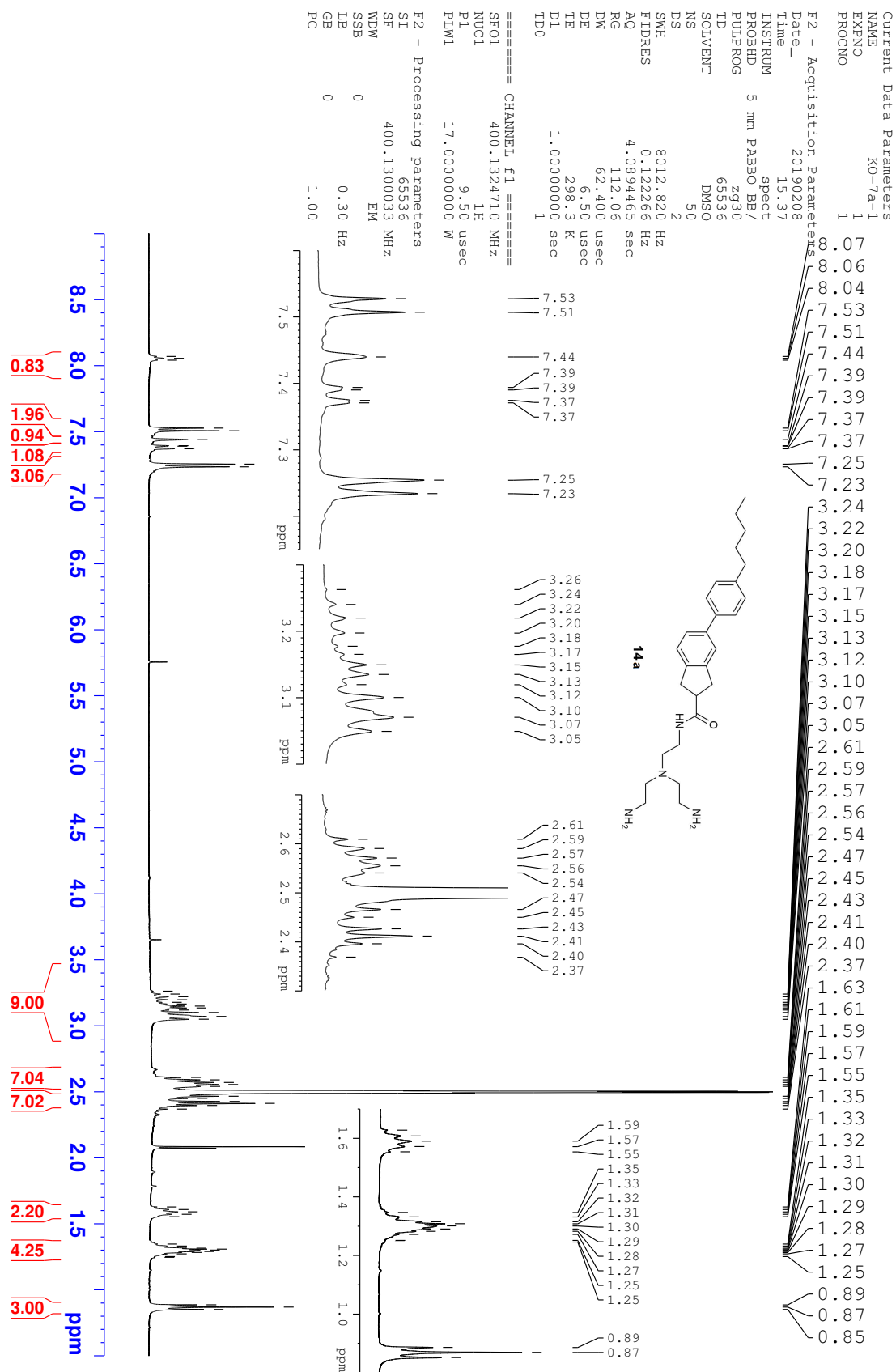
J.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 12a



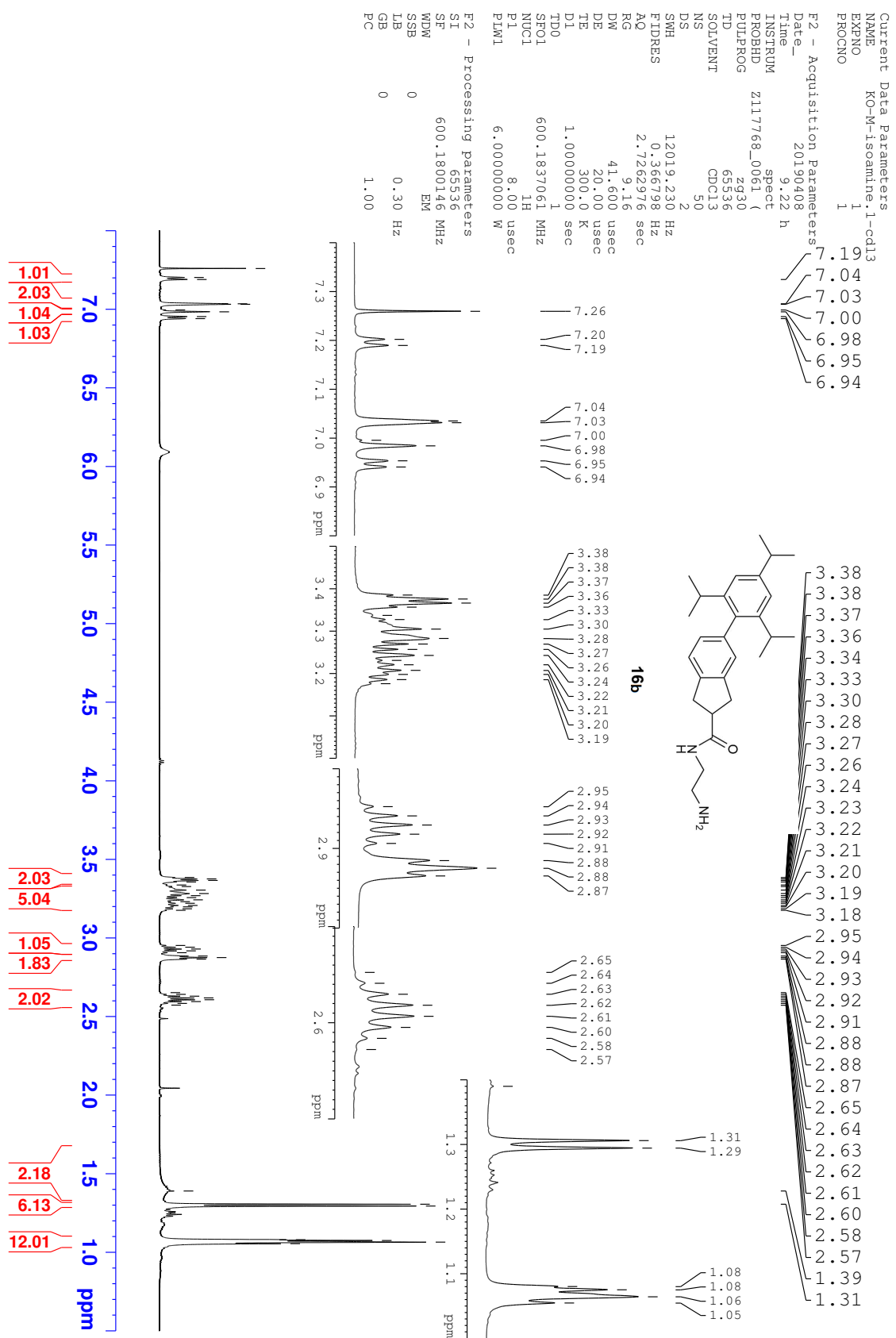
K.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 12b



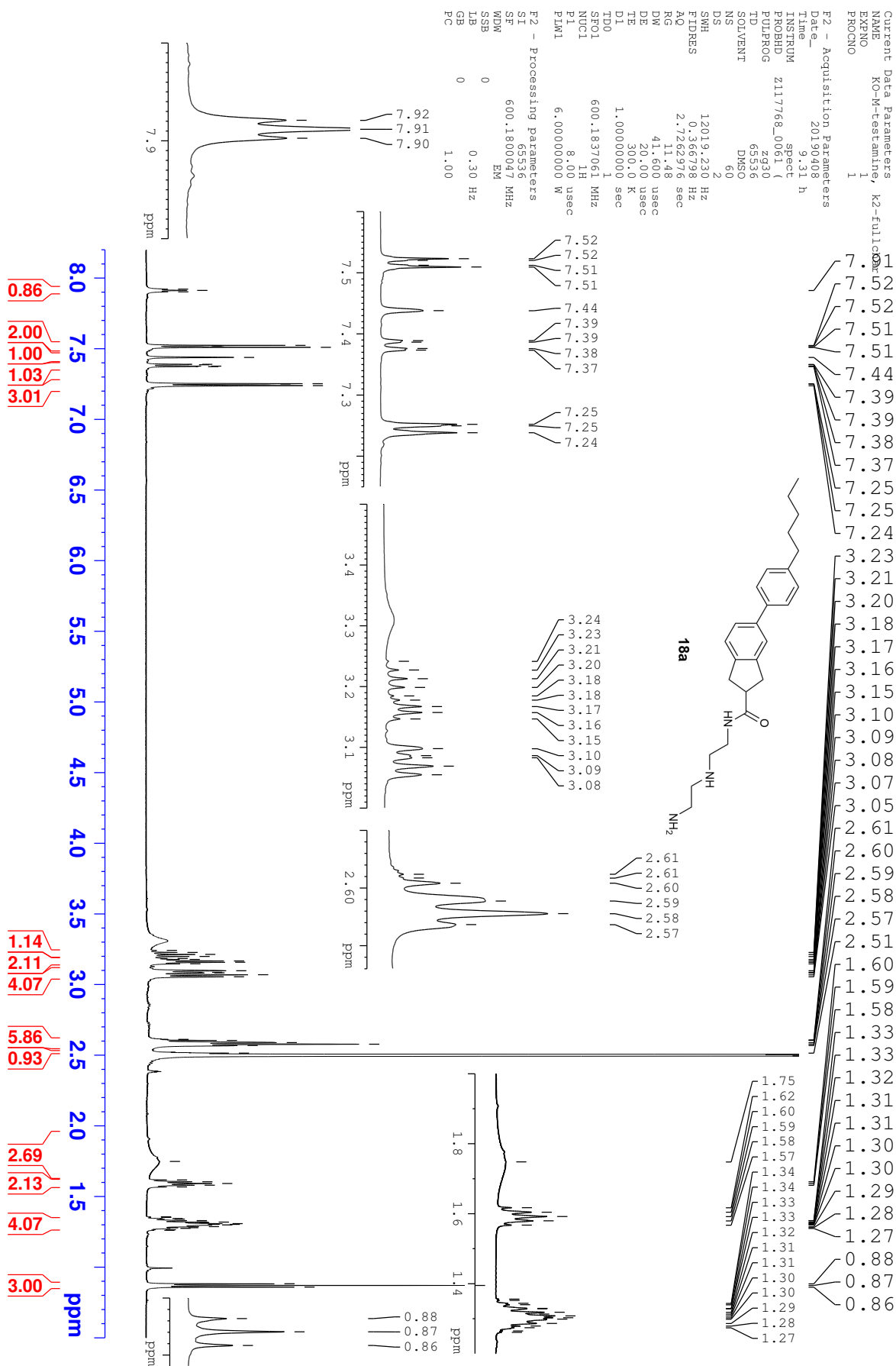
L.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 14a



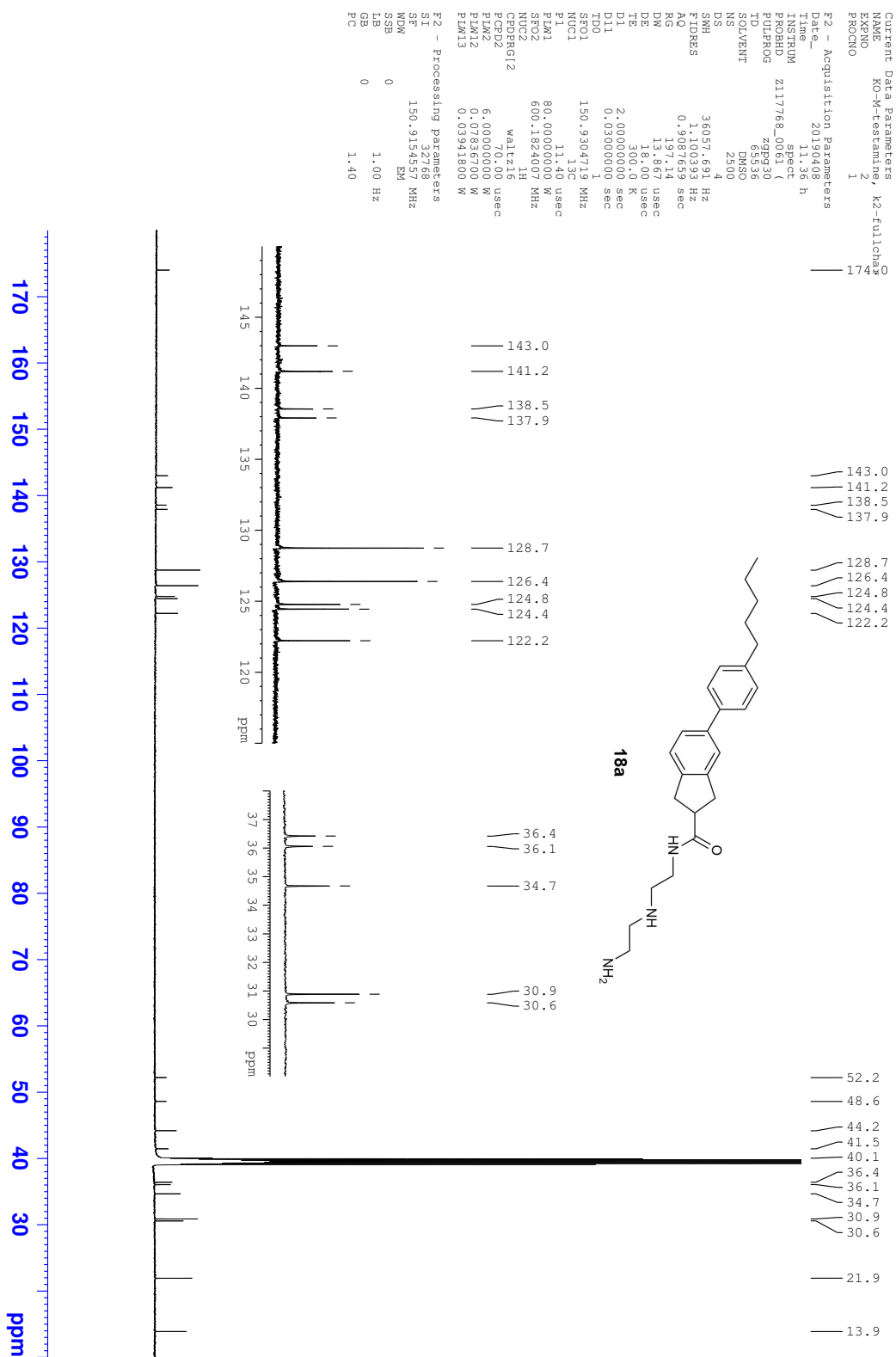
M.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 16b



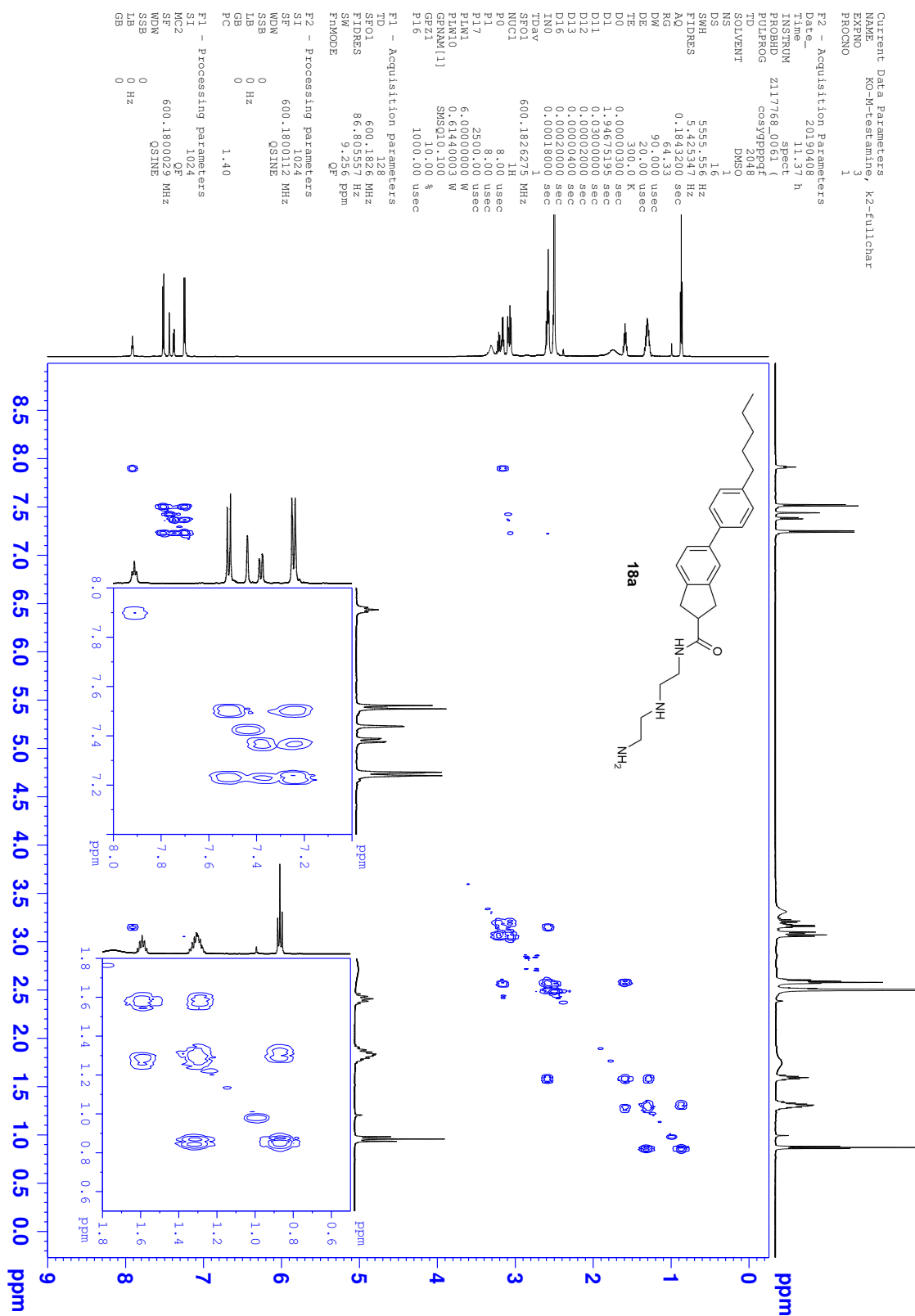
N.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 18a



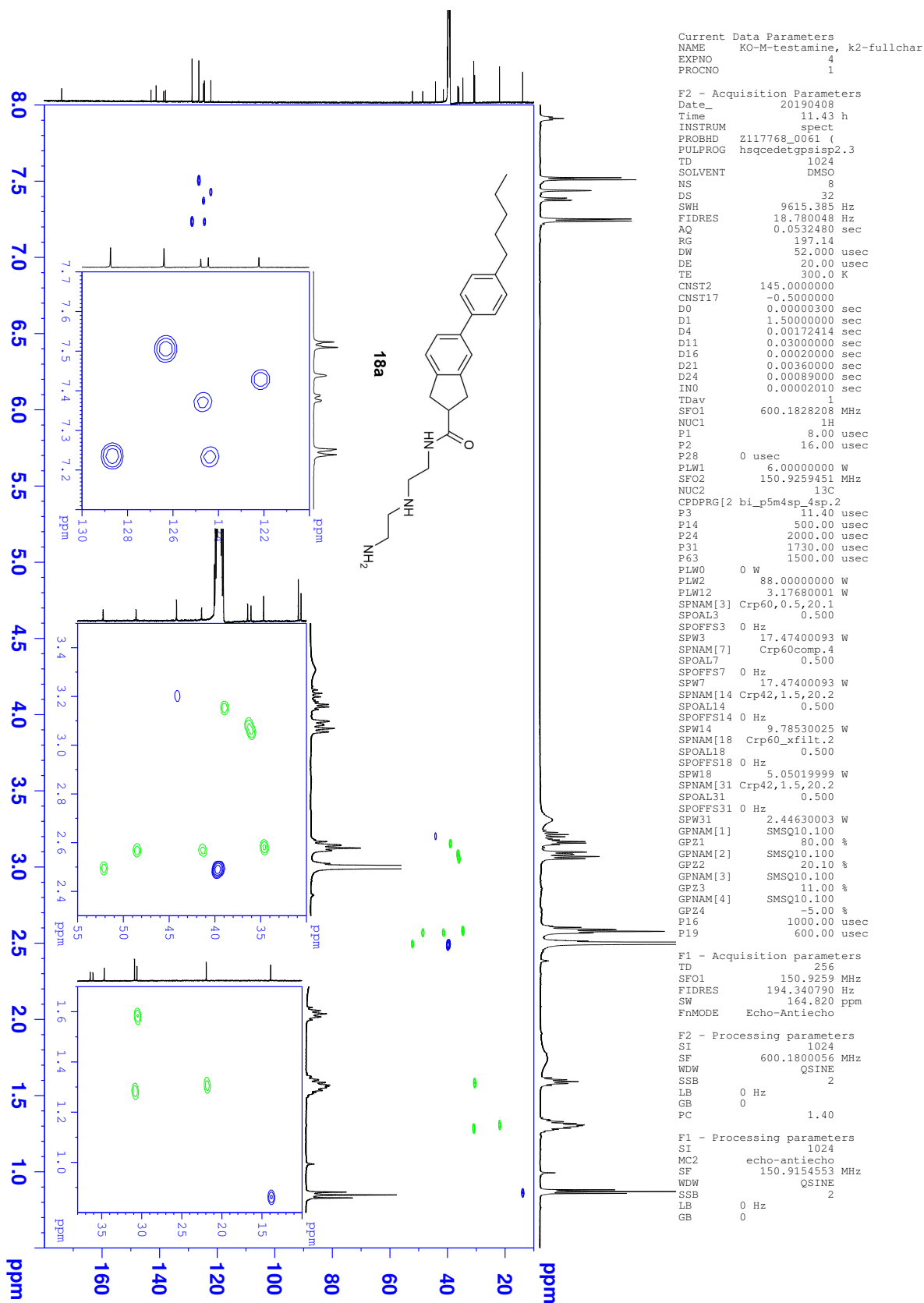
N.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 18a



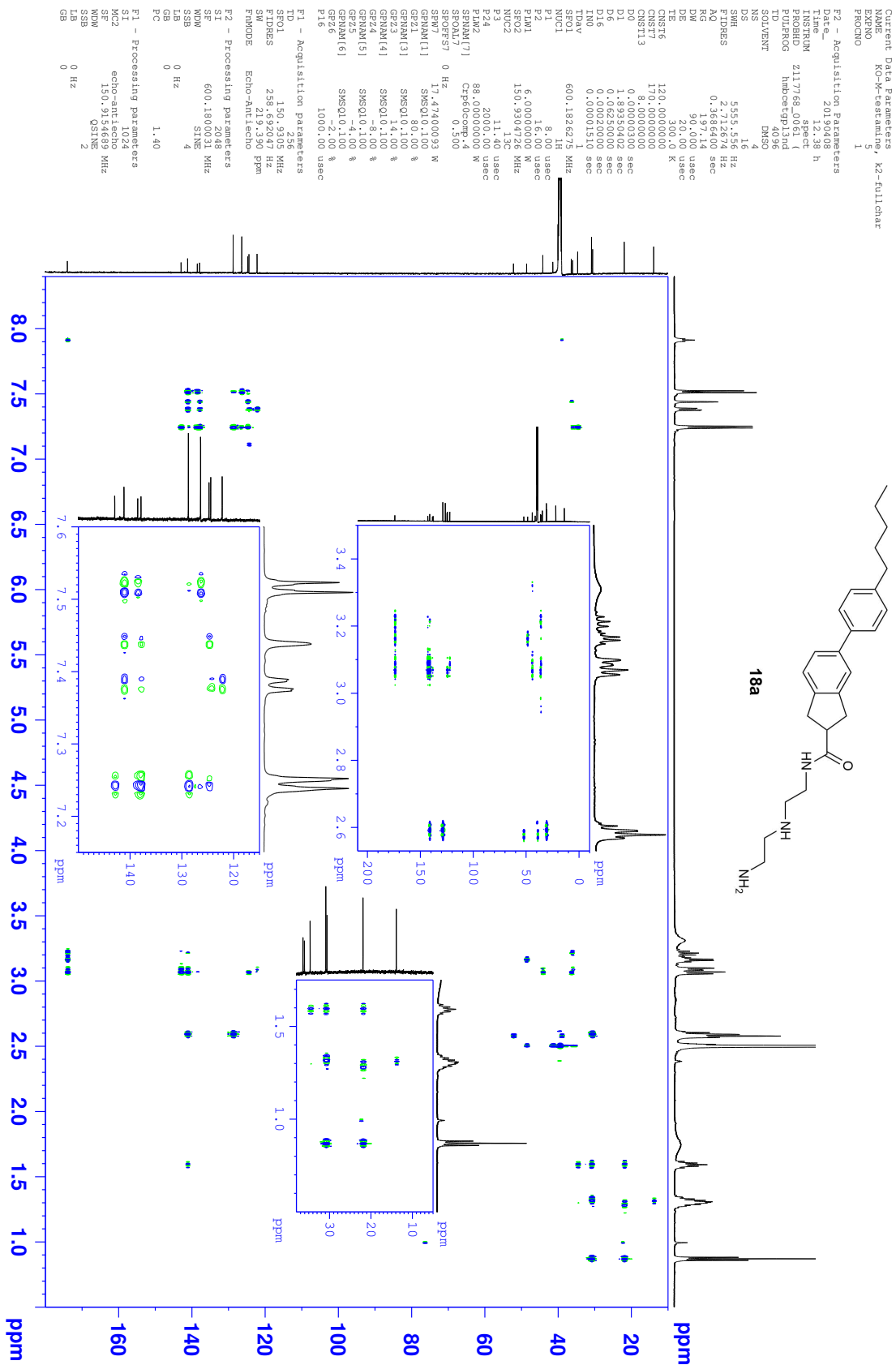
N.3 COSY (600 MHz, DMSO-d₆) Spectrum for 18a



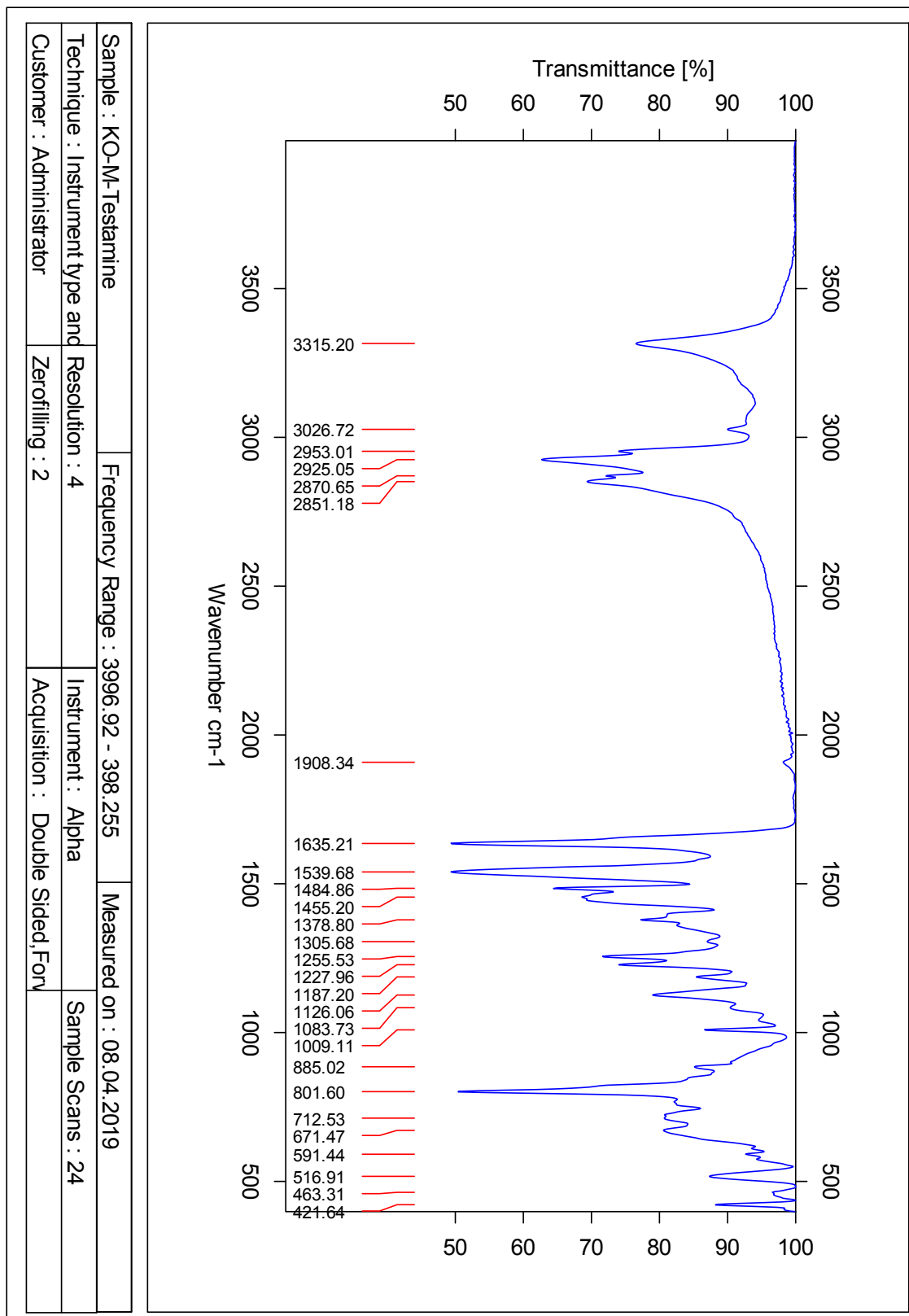
N.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 18a



N.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 18a



N.6 IR Spectrum for 18a



N.7 HRMS Report for 18a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1487 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

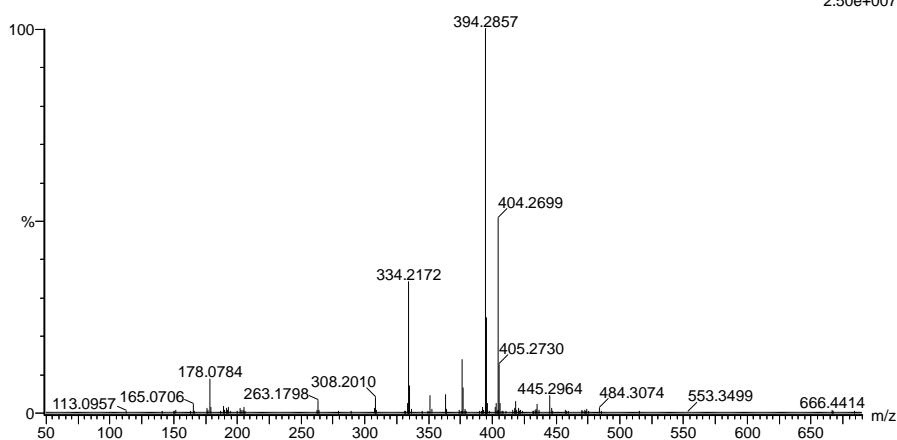
Elements Used:

C: 0-100 H: 0-150 N: 0-5 O: 0-10 Na: 0-1

2019-296 247 (4.806) AM2 (Ar,35000.0,0.00,0.00); Cm (246:258)

1: TOF MS ASAP+

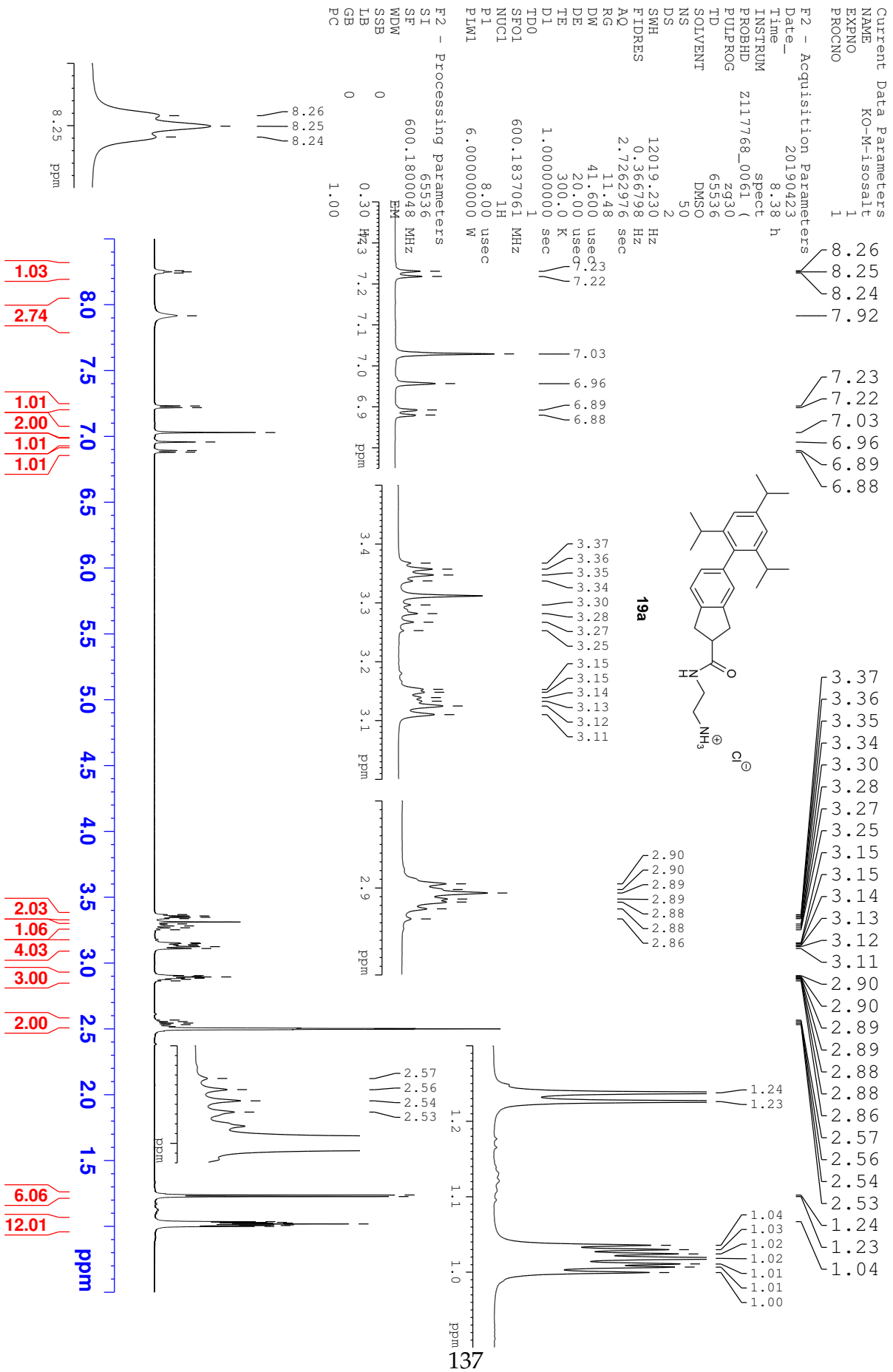
2.50e+007



Minimum: -50.0
Maximum: 5.0 2.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
394.2857	394.2858	-0.1	-0.3	9.5	1527.0	0.002	99.84	C25 H36 N3 O
	394.2853	0.4	1.0	-6.5	1533.4	6.414	0.16	C11 H41 N5 O8 Na

O.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 19b



O.2 HPLC chromatogram for 19b

Data File C:\CHEM32\1\DATA\KRISTINEØYAKO-M-ISOAMINESALT.D

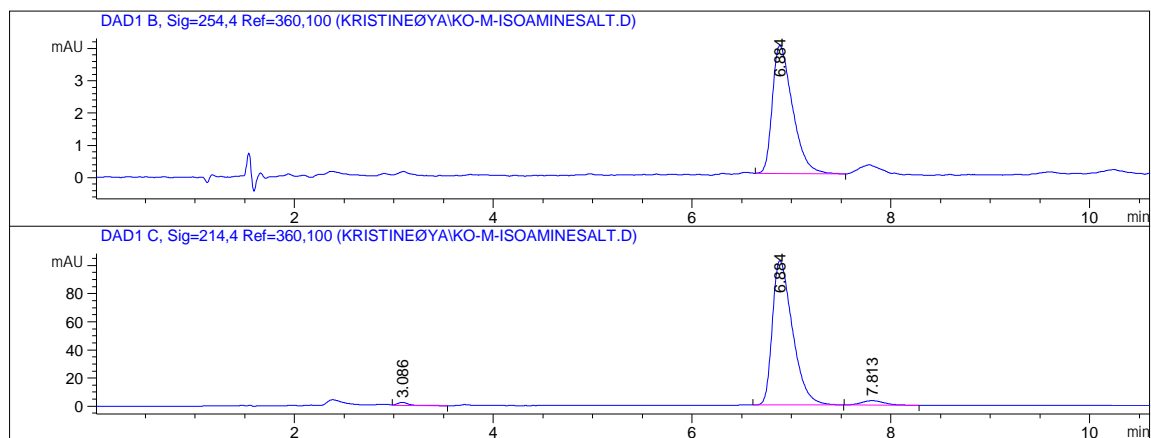
Sample Name: KO-M-isoaminesalt

```

=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 2
Injection Date  : 10.05.2019 12:32:12
                                                    Inj Volume : 2.000 µl
Acq. Method    : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed   : 10.05.2019 12:30:35 by Kristine
                (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed   : 07.05.2019 14:57:04 by Jorge
                (modified after loading)
Method Info    : Renhetsanalyse Sondre

Sample Info    : 80:20 MeOH/ H2O + 0.1 % TFA, 1 ml/min
  
```

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 B, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.884	BB	0.2100	55.70650	3.96172	100.0000

Totals : 55.70650 3.96172

O.3 HPLC chromatogram for 19b

Data File C:\CHEM32\1\DATA\KRI STI NEØYA\K0-M-I SOAMI NESALT.D

Sample Name: K0-M-i soami nesalt

```
=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 2
Injection Date  : 10.05.2019 12:32:12
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 12:30:35 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NI CO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 80:20 MeOH/ H2O + 0.1 % TFA, 1 ml/min
```

Additional Info : Peak(s) manually integrated

```
=====
Signal 2: DAD1 C, Sig=214,4 Ref=360,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.086	VB	0.1204	18.84107	2.32748	1.2504
2	6.884	BB	0.2138	1439.06152	102.49727	95.5025
3	7.813	BB	0.2478	48.92807	3.10492	3.2471

Totals : 1506.83067 107.92967

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

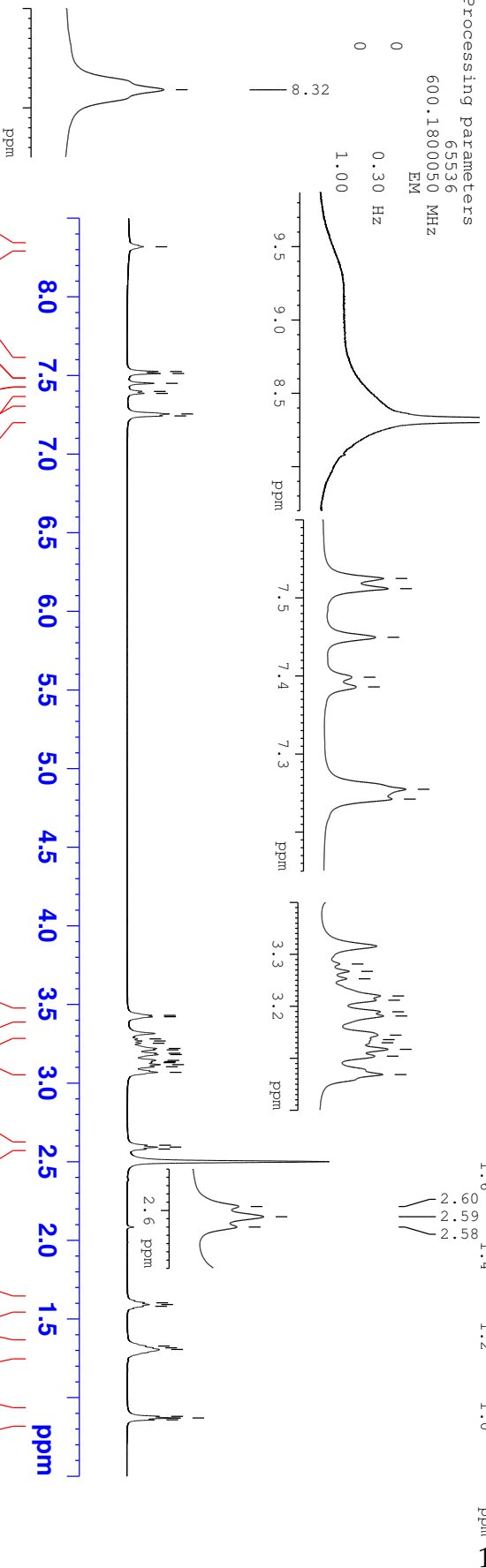
P.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 20a

Current Data Parameters
 NAME KO-M-testaminesalt
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20190428
 Time 12.20 h

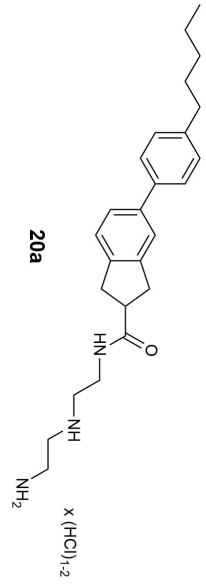
INSTRUM spect
 PROBHD zq30
 PULPROG zgpg30
 TD 65536
 SOLVENT DMSO
 NS 50
 DS 2
 SWH 12019.230 Hz
 FIDRES 0.366798 Hz
 AQ 2.7262976 sec
 RG 11.48
 DW 41.600 usec
 DE 20.00 usec
 TE 300.0 K
 D1 1.00000000 sec
 TD0 1
 SFO1 600.1837061 MHz
 NUC1 1H
 P1 8.00 usec
 PL1 6.00000000 W

F2 - Processing parameters
 SI 65536
 SF 600.1800050 MHz
 WDW EM
 SSB 0
 LB 0
 GB 0
 PC 0



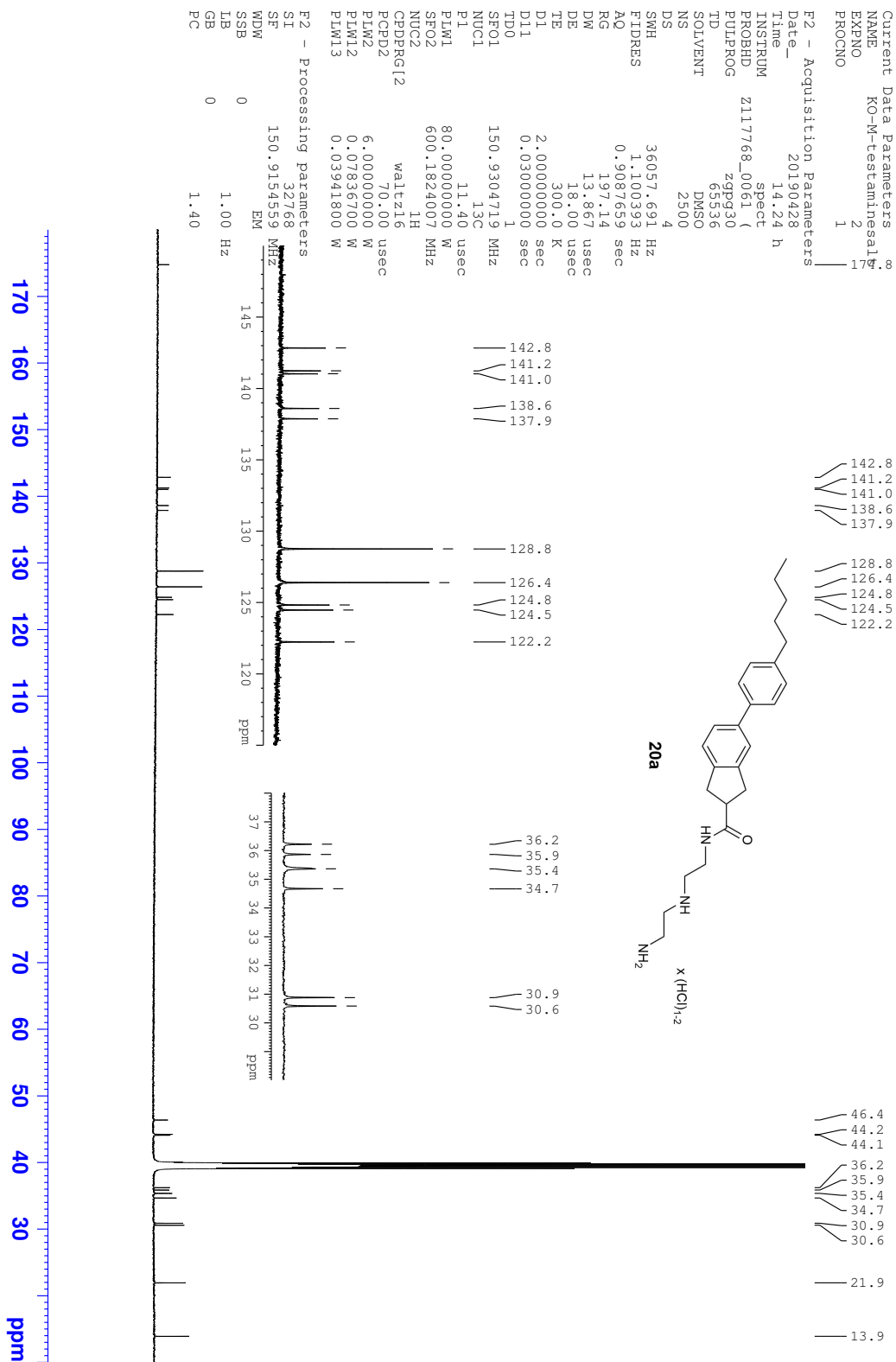
Integration values (red boxes):
 1.09, 1.98, 1.03, 1.06, 3.01, 2.03, 11.05, 2.01, 2.02, 4.04, 3.00

Peak list (ppm):
 7.52, 7.51, 7.45, 7.40, 7.39, 7.25, 7.24

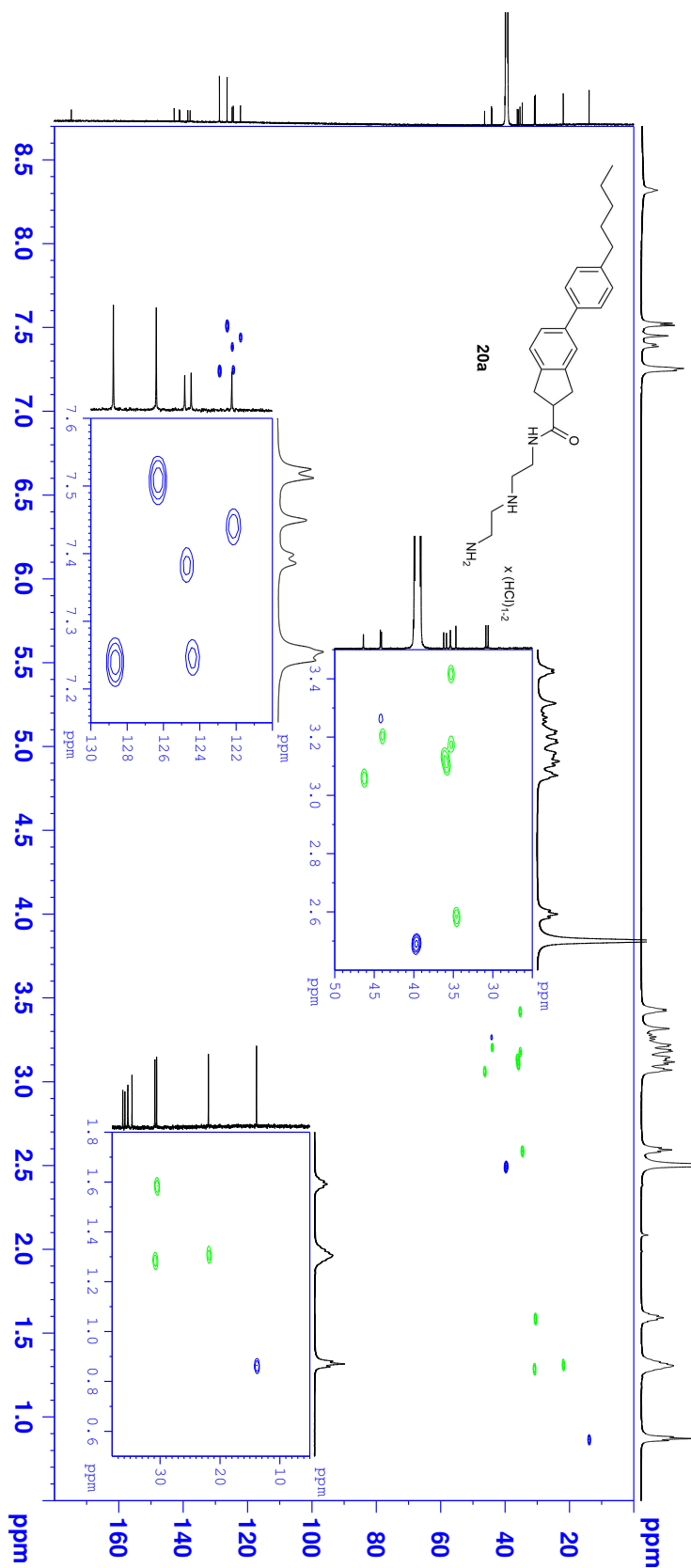


Peak list (ppm):
 3.43, 3.42, 3.28, 3.27, 3.25, 3.22, 3.21, 3.19, 3.18, 3.18, 3.14, 3.14, 3.13, 3.12, 3.10, 3.07, 1.60, 1.59, 1.58, 1.33, 1.32, 1.31, 1.60, 1.59, 1.58, 1.33, 1.32, 1.31, 0.88, 0.87, 0.86

P.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 20a



P.3 COSY (600 MHz, DMSO-d₆) Spectrum for 20a



```

Current Data Parameters
NAME      KO-M-testaminesalt
EXPNO    4
PROCNO   1

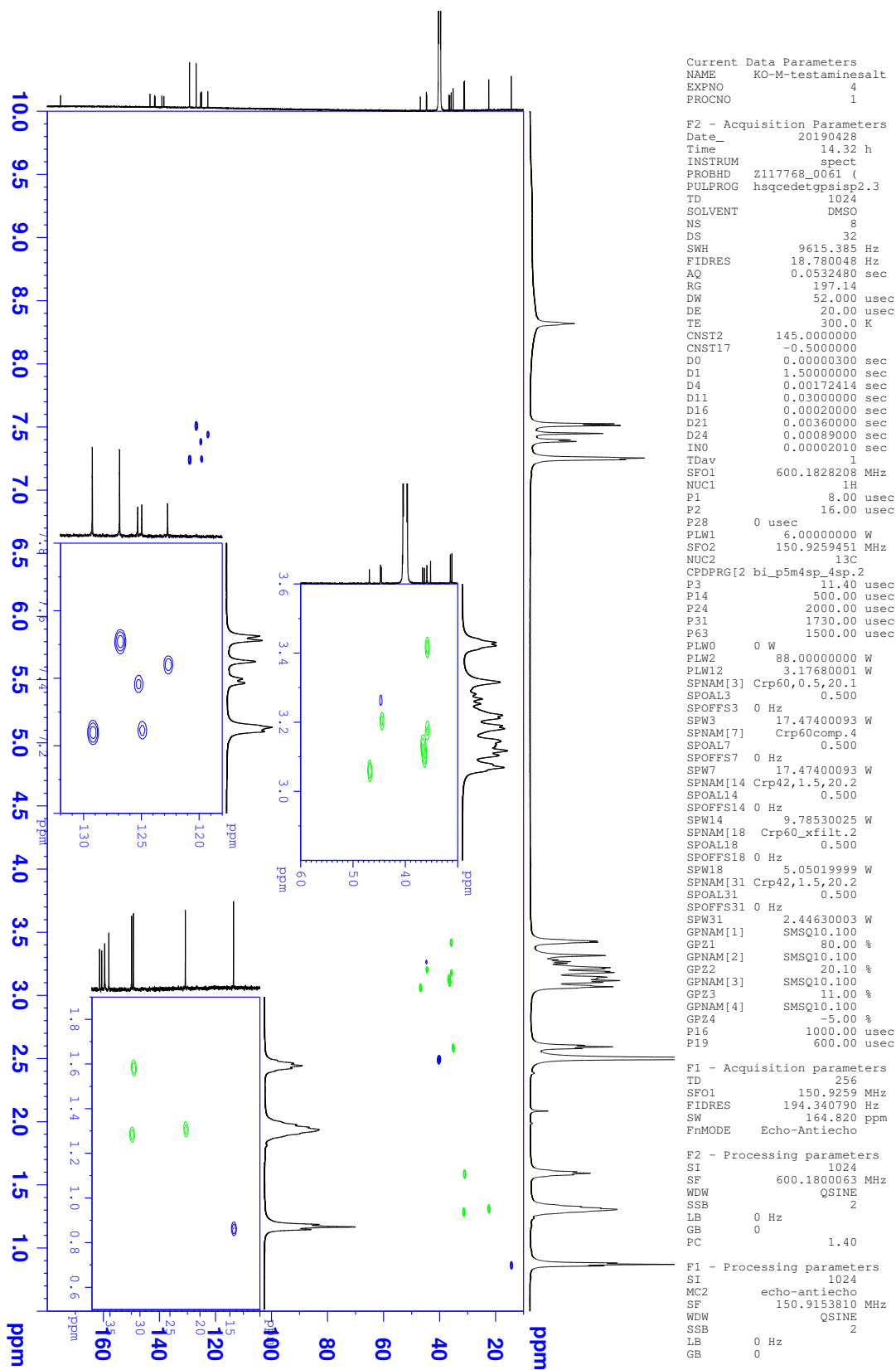
F2 - Acquisition Parameters
Date_    20190428
Time     14.32 h
INSTRUM  spect
PROBHD   Z117768_0061 (
PULPROG  hsqcetgpsi2p2.3
TD       1024
SOLVENT  DMSO
NS       8
DS       32
SWH      9615.385 Hz
FIDRES   18.780048 Hz
AQ       0.0532480 sec
RG       197.14
DW       52.000 usec
DE       20.00 usec
TE       300.0 K
CNST2    145.0000000
CNST17   -0.5000000
D0       0.00000300 sec
D1       1.50000000 sec
D4       0.00172414 sec
D11      0.03000000 sec
D16      0.00020000 sec
D21      0.00360000 sec
D24      0.00089000 sec
INO      0.00002010 sec
TDav     1
SFO1     600.1828208 MHz
NUC1     1H
P1       8.00 usec
P2       16.00 usec
P28      0 usec
PLW1     6.00000000 W
SFO2     150.9259451 MHz
NUC2     13C
CPDPRG[2] bi_p5m4sp_4sp_2
P3       1.40 usec
P14      500.00 usec
P24      2000.00 usec
P31      1730.00 usec
P63      1500.00 usec
PLW0     0 W
PLW2     88.00000000 W
PLW12    3.17680001 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3   0.500
SPOFFS3  0 Hz
SPW3     17.47400093 W
SPNAM[7] Crp60comp.4
SPOAL7   0.500
SPOFFS7  0 Hz
SPW7     17.47400093 W
SPNAM[14] Crp42,1.5,20.2
SPOAL14  0.500
SPOFFS14 0 Hz
SPW14    9.78530025 W
SPNAM[18] Crp60_xfil1.2
SPOAL18  0.500
SPOFFS18 0 Hz
SPW18    5.05019999 W
SPNAM[31] Crp42,1.5,20.2
SPOAL31  0.500
SPOFFS31 0 Hz
SPW31    2.44630003 W
GPNAM[1] SMSQ10.100
GPZ1     80.00 %
GPNAM[2] SMSQ10.100
GPZ2     20.10 %
GPNAM[3] SMSQ10.100
GPZ3     11.00 %
GPNAM[4] SMSQ10.100
GPZ4     -5.00 %
P16     1000.00 usec
P19     600.00 usec

F1 - Acquisition parameters
TD       256
SFO1     150.9259 MHz
FIDRES   194.340790 Hz
SW       164.820 ppm
FnMODE   Echo-Antiecho

F2 - Processing parameters
SI       1024
SF       600.1800062 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
PC       1.40

F1 - Processing parameters
SI       1024
MC2      echo-antiecho
SF       150.9154599 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
    
```

P4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 20a



P.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 20a

Current Data Parameters
 NAME KO-M-testaminalsalt
 EXPNO 5
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 2015.09.08
 Time 15.26 h
 INSTRUM spect
 PROBDH 2117168.0061 ((

PULPROG hmcetp13rd
 TD 4096
 SFO1 600.131368 MHz
 SOLVENT DMSO
 NS 4
 DS 16
 SWH 6756.757 Hz
 FIDRES 3.299198 Hz
 AQ 0.3031040 sec
 RG 327
 DE 1.40 usec
 TE 300.0 K

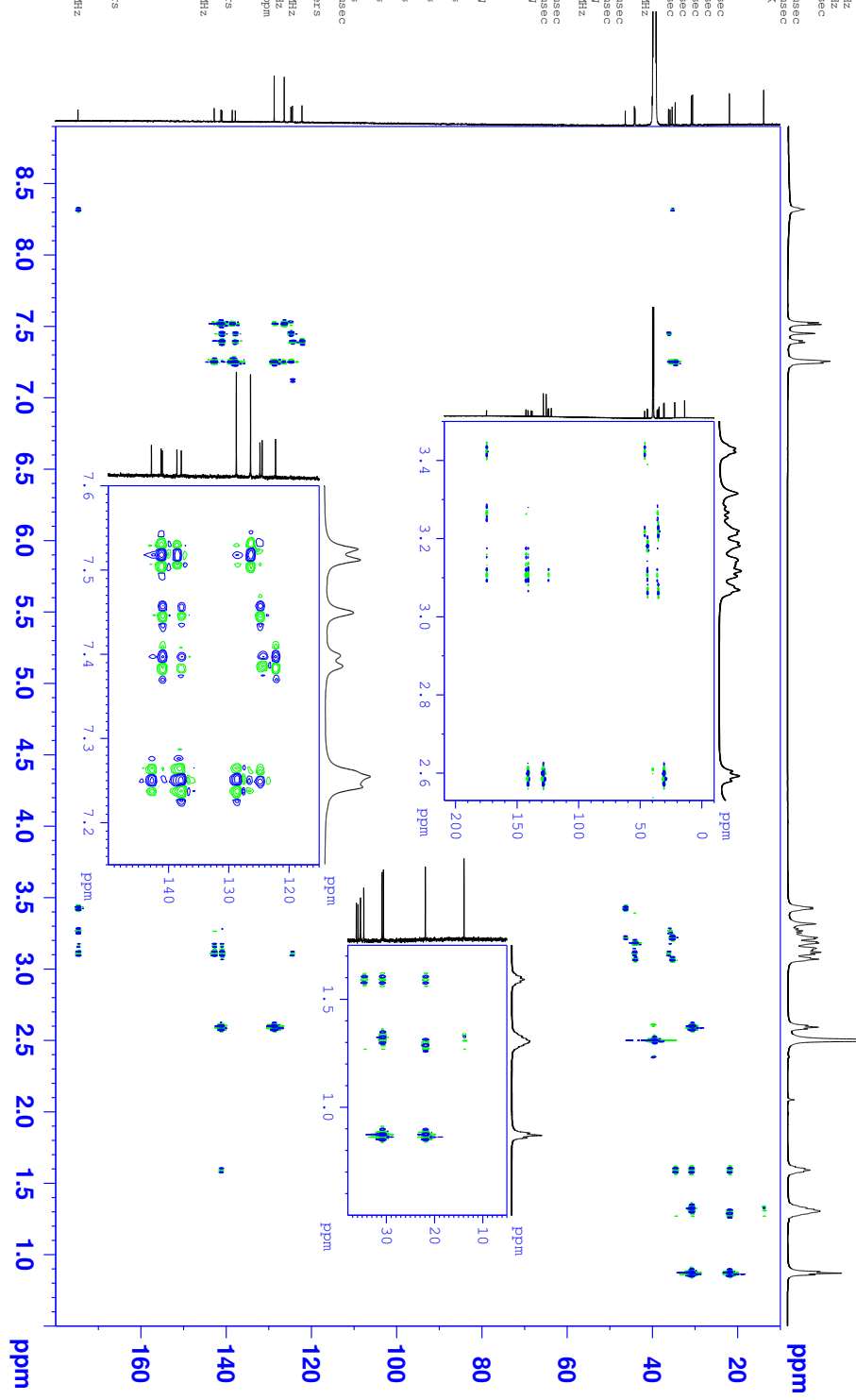
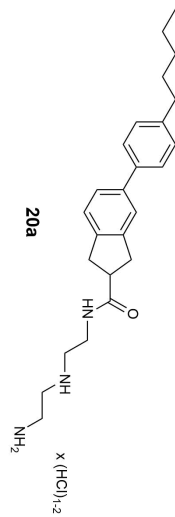
CNSTR6 120.000000
 CNSTR7 170.000000
 CNSTR8 8.000000
 CNSTR9 0.000000
 D1 0.000000 sec
 D1 1.95904005 sec
 D6 0.06250000 sec
 D16 0.00020000 sec
 INO 0.00001510 sec
 TD0AV 1
 SFO2 600.1831968 MHz
 NUC1 1H
 P1 8.00 usec
 P2 16.00 usec

PL0 6.00000000 W
 SFO1 150.9304726 MHz
 BPC2 11.40
 P24 2000.00 usec
 PL0 88.00000000 W
 SFO2 600.1831968 MHz
 SFO3 0.500
 SFO4 17.4740093 W
 GPNAM11 SMSQ10.100
 GP21 80.00 %
 GPNAM13 SMSQ10.100
 GP23 14.00 %
 GPNAM14 SMSQ10.100 %
 GPNAM15 SMSQ10.100
 GP25 -4.00 %
 GPNAM16 SMSQ10.100
 GP26 -2.00 %
 PL6 1000.00 usec

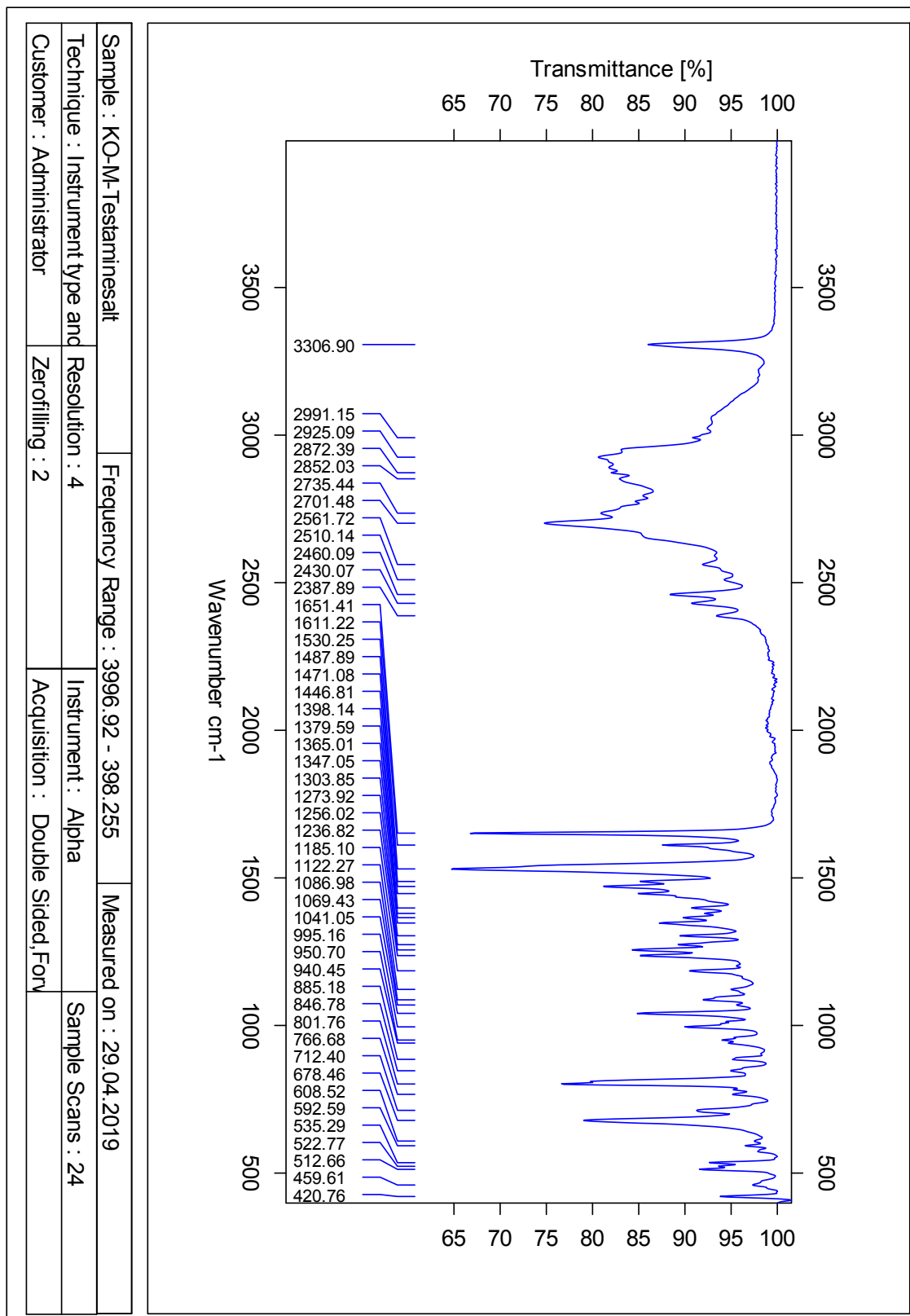
F1 - Acquisition parameters
 TD 256
 SFO1 150.9305 MHz
 FIDRES 258.692047 Hz
 SW 219.390 ppm
 FREQ0 Echo-AntiEcho

F2 - Processing parameters
 SI 2048
 SF 600.1800061 MHz
 WDW SINE
 SSB 4
 GB 0 Hz
 PC 1.40

F1 - Processing parameters
 SI 1024
 SF 150.931579 MHz
 WDW COSTINE
 SSB 2
 GB 0 Hz



P.6 IR Spectrum for 18a



P.7 HRMS Report for 18a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -2.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1234 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

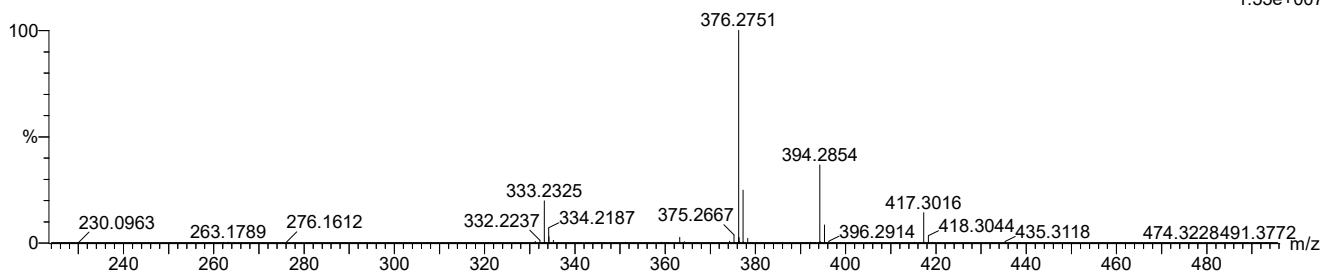
Elements Used:

C: 0-500 H: 0-1000 N: 0-5 O: 0-5 Na: 0-1 S: 0-3

2019-375_2 217 (4.240) AM2 (Ar,35000.0,0.00,0.00); Cm (209:217)

1: TOF MS ASAP+

1.55e+007



Minimum: -2.0
Maximum: 5.0 2.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
394.2854	394.2852	0.2	0.5	0.5	1245.8	12.056	0.00	C17 H40 N5 O3 S
	394.2858	-0.4	-1.0	9.5	1233.7	0.000	100.00	C25 H36 N3 O

P.8 HPLC chromatogram for 20a

Data File C:\CHEM32\1\DATA\KRISTINEØYA\20190507_TESTAMIESALT7030.D

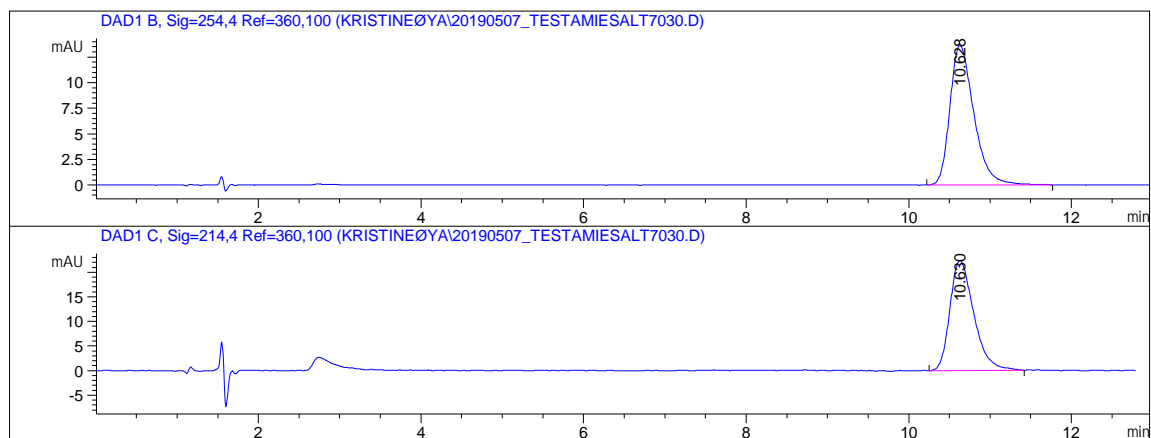
Sample Name: KO-M-testamiesalt

```

=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                      Location : Vial 9
Injection Date  : 07.05.2019 17:15:27
                                           Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 17:03:37 by Li se
                 (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                 (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : MeOH/H2O 70:30 + 0.1%TFA in H2O, 1mL/min
    
```

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 B, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.628	BB	0.3155	279.63715	13.62197	100.0000

Totals : 279.63715 13.62197

P.9 HPLC chromatogram for 20a

Data File C:\CHEM32\1\DATA\KRI STI NEØYA\20190507_TESTAMI ESALT7030.D

Sample Name: KO-M-testami nesal t

```
=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                      Location : Vial 9
Injection Date  : 07.05.2019 17:15:27      Inj Volume : 2.000 µl
Acq. Method    : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed   : 07.05.2019 17:03:37 by Li se
                (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NI CO-KORT.M
Last changed   : 07.05.2019 14:57:04 by Jorge
                (modified after loading)
Method Info    : Renhetsanalyse Sondre
Sample Info    : MeOH/H2O 70:30 + 0.1%TFA in H2O, 1mL/min
Additional Info : Peak(s) manually integrated
=====
```

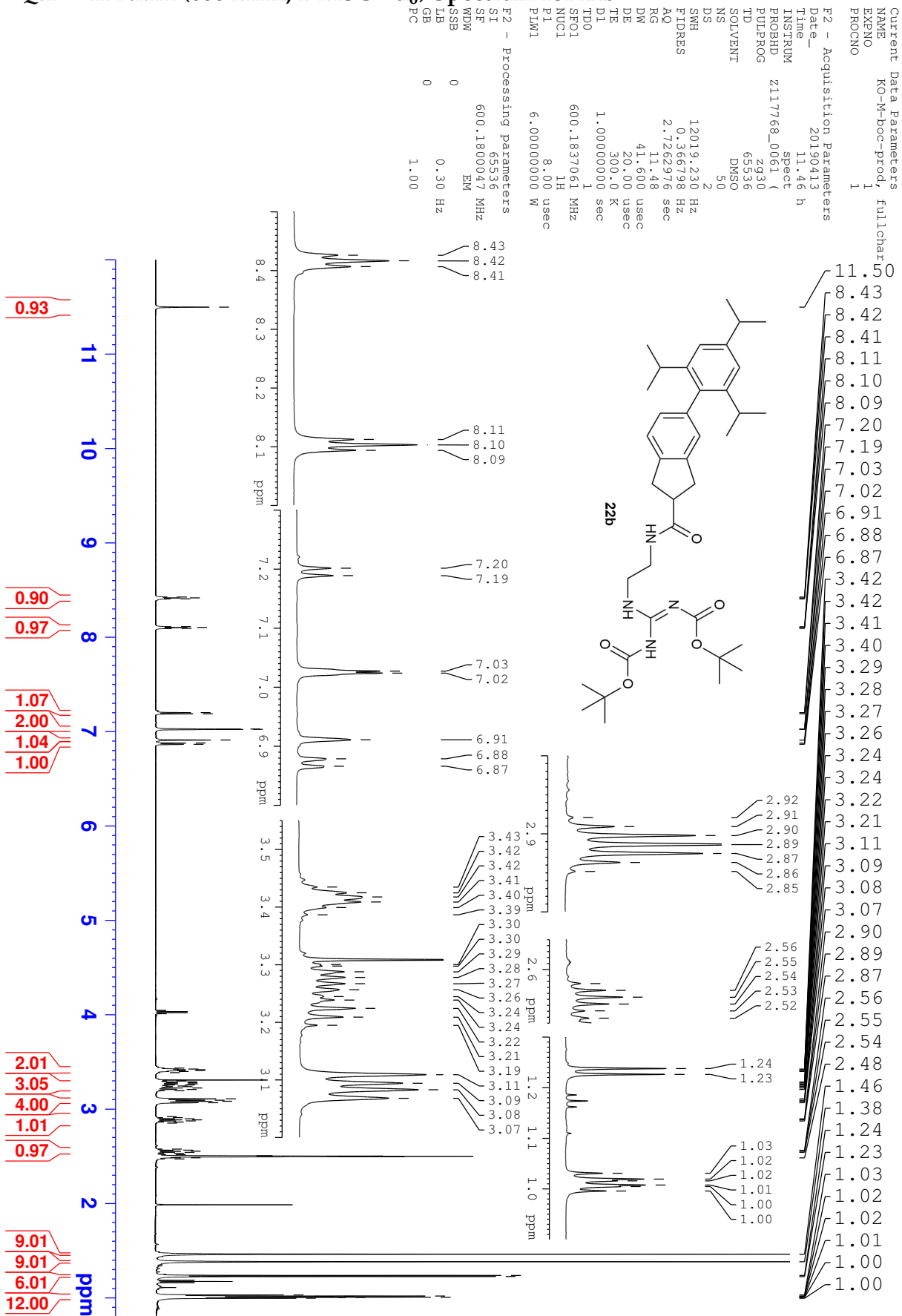
Signal 2: DAD1 C, Sig=214,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.630	BB	0.3087	457.18213	22.34796	100.0000

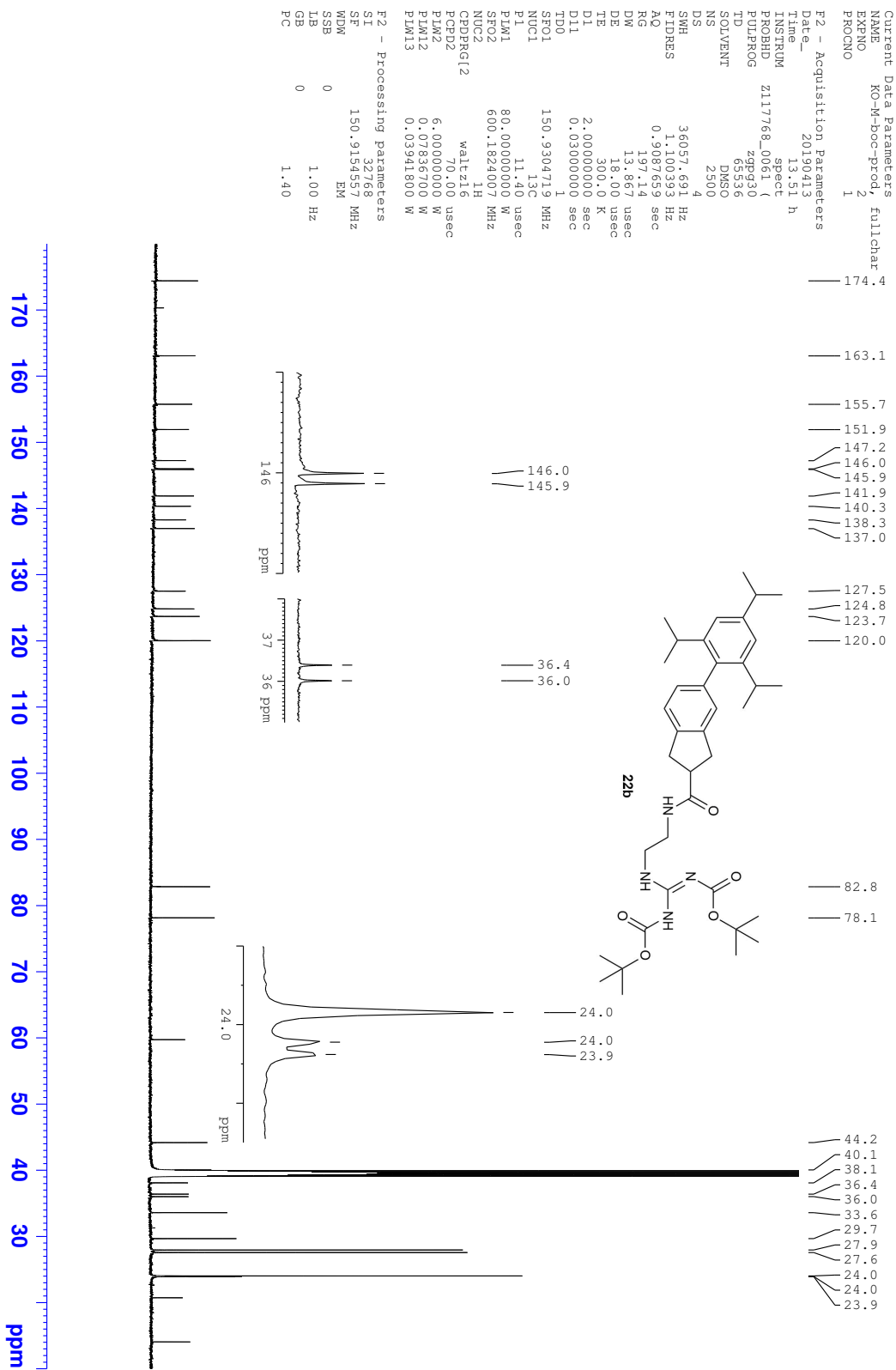
Totals : 457.18213 22.34796

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

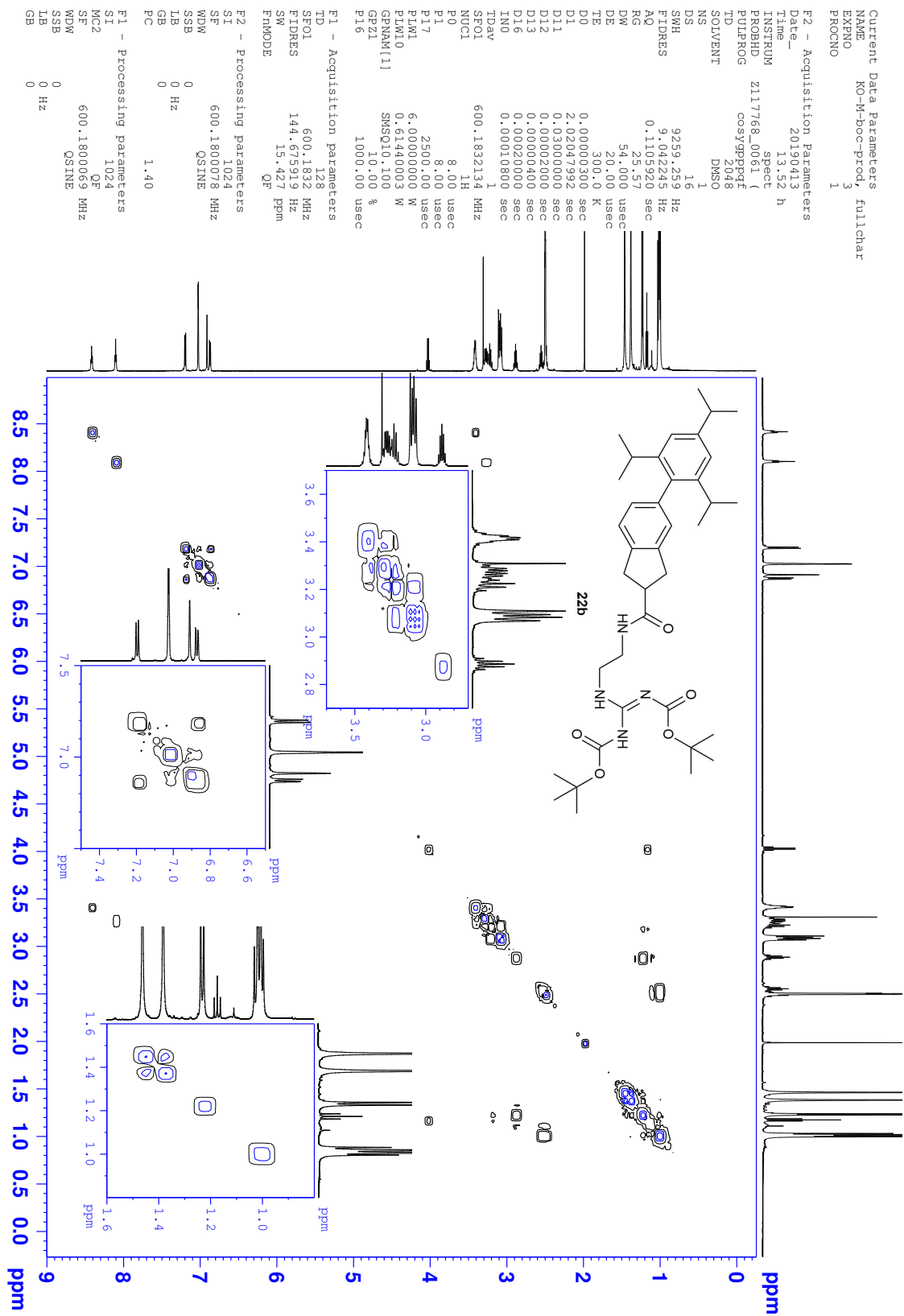

Q.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 22b



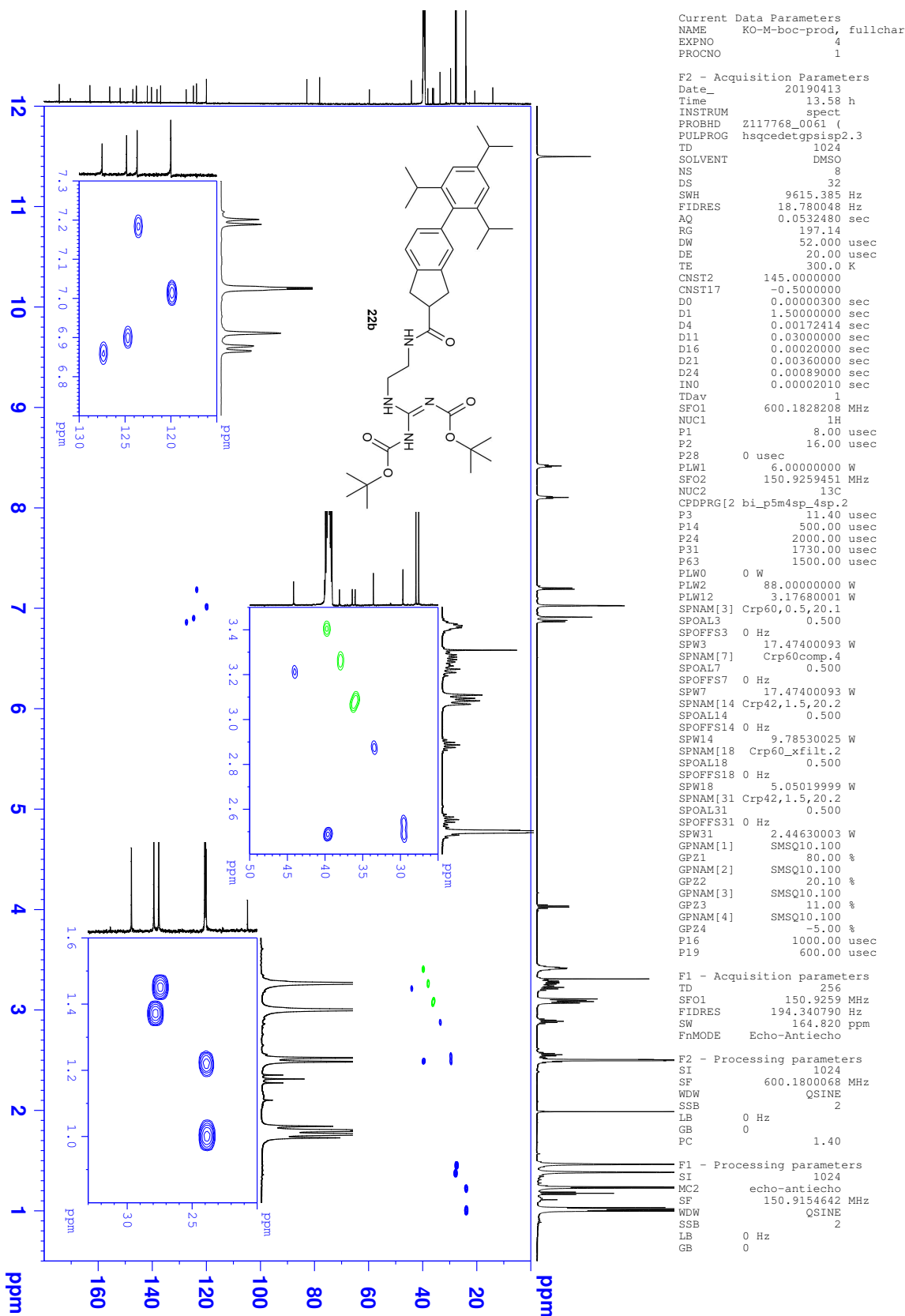
Q.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 22b



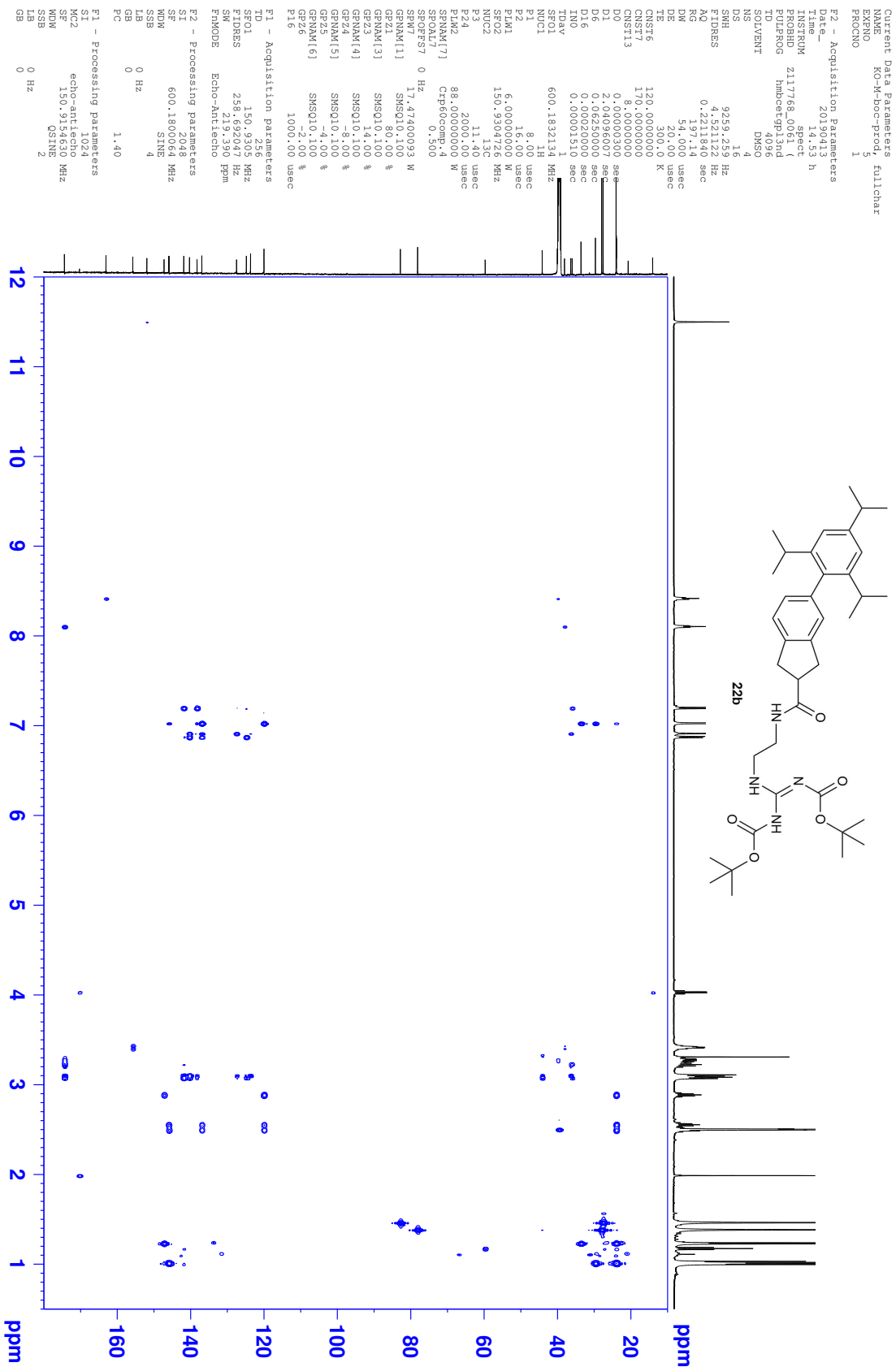
Q.3 COSY (600 MHz, DMSO-d₆) Spectrum for 22b



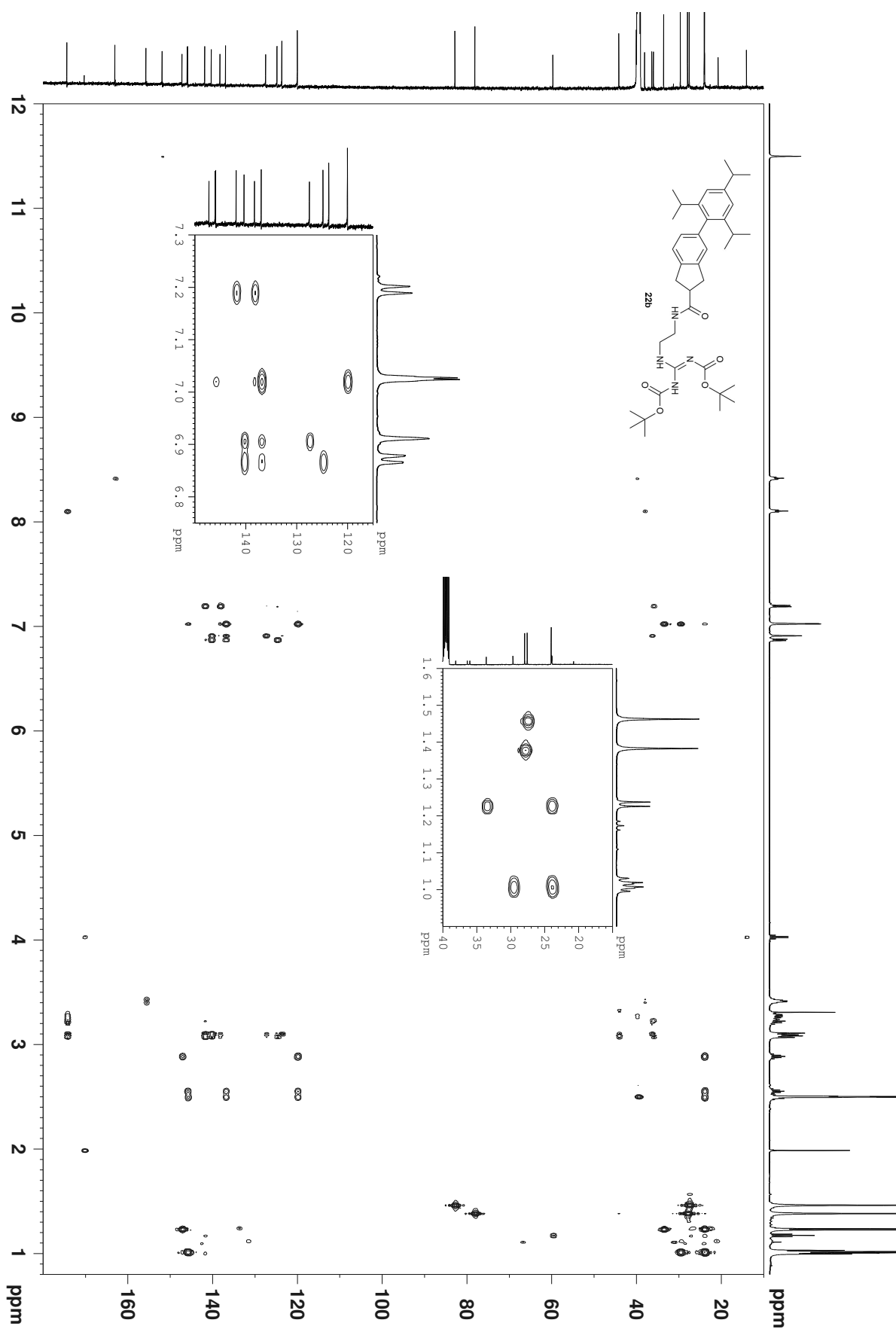
Q.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 22b



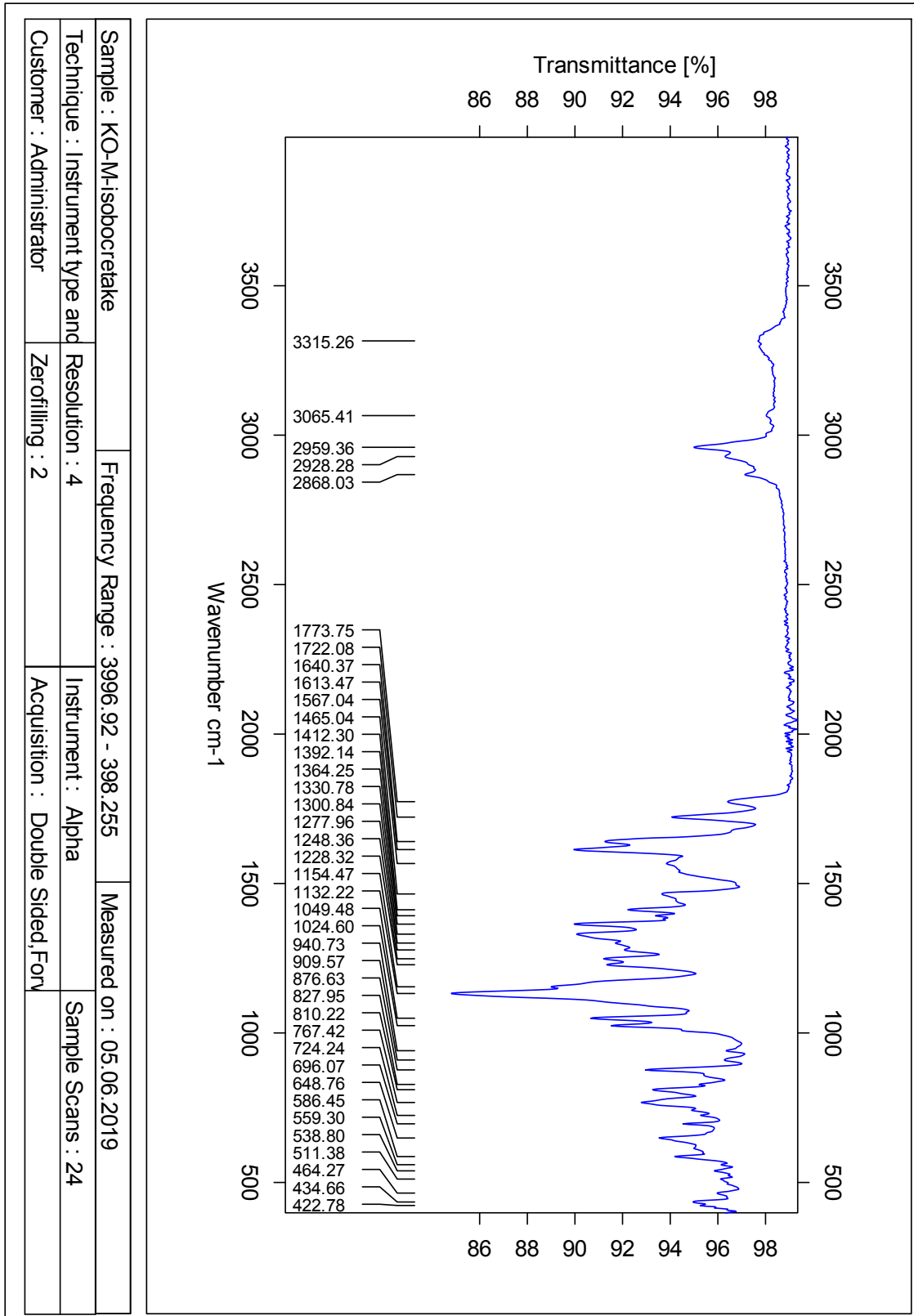
Q.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 22b



Q.6 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 22b (Large)



Q.7 IR Spectrum for 22b



Q.8 HRMS Report for 22b

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1551 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

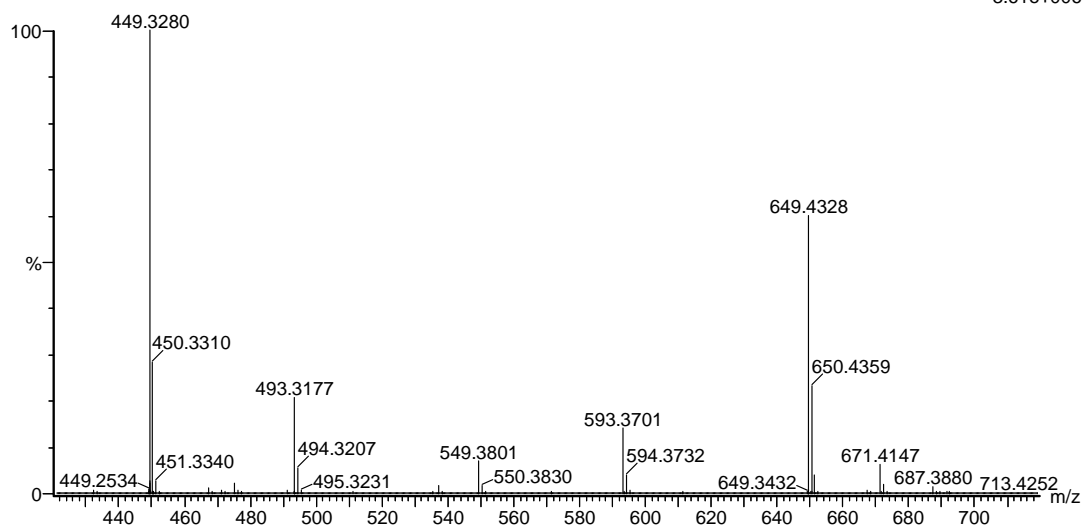
Elements Used:

C: 2-100 H: 0-150 N: 0-10 O: 0-10

svg_20190423_2019_340 35 (0.653) AM2 (Ar,35000.0,0.00,0.00); Cm (35:37)

1: TOF MS ES+

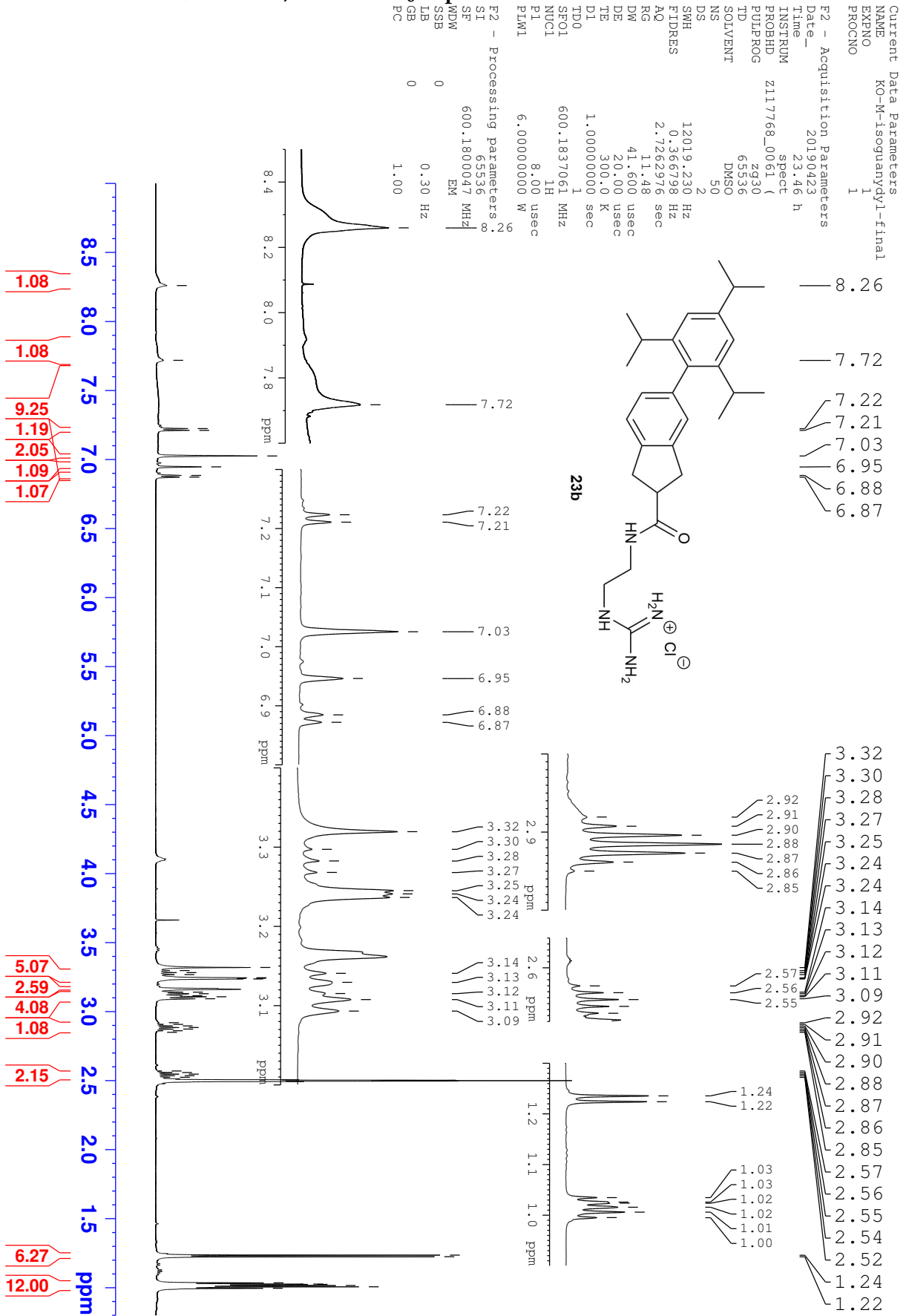
3.61e+006



Minimum: -50.0
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
649.4328	649.4329	-0.1	-0.2	12.5	799.1	0.317	72.86	C38 H57 N4 O5
	649.4316	1.2	1.8	7.5	800.0	1.304	27.14	C37 H61 O9

R.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 23b

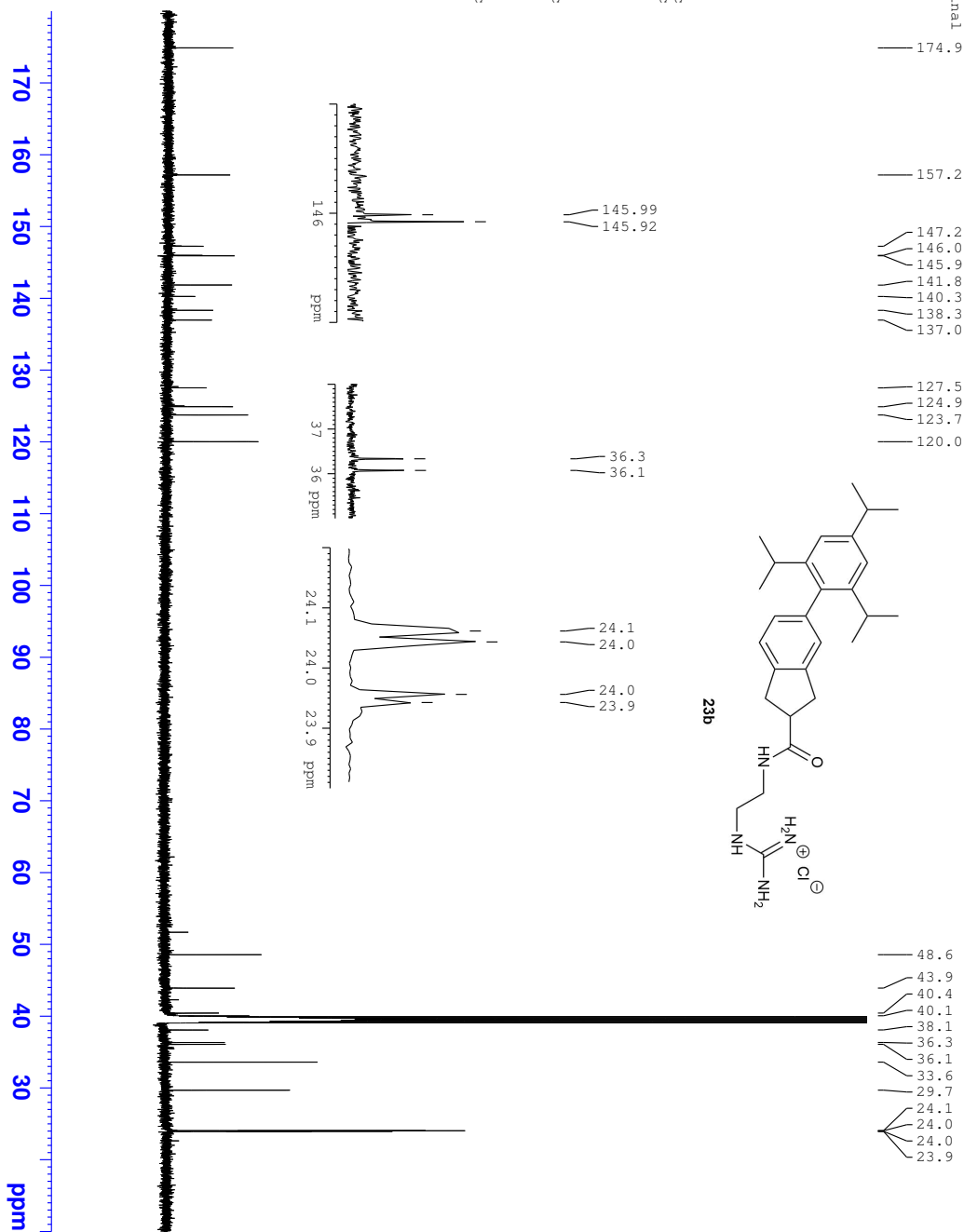


R.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 23b

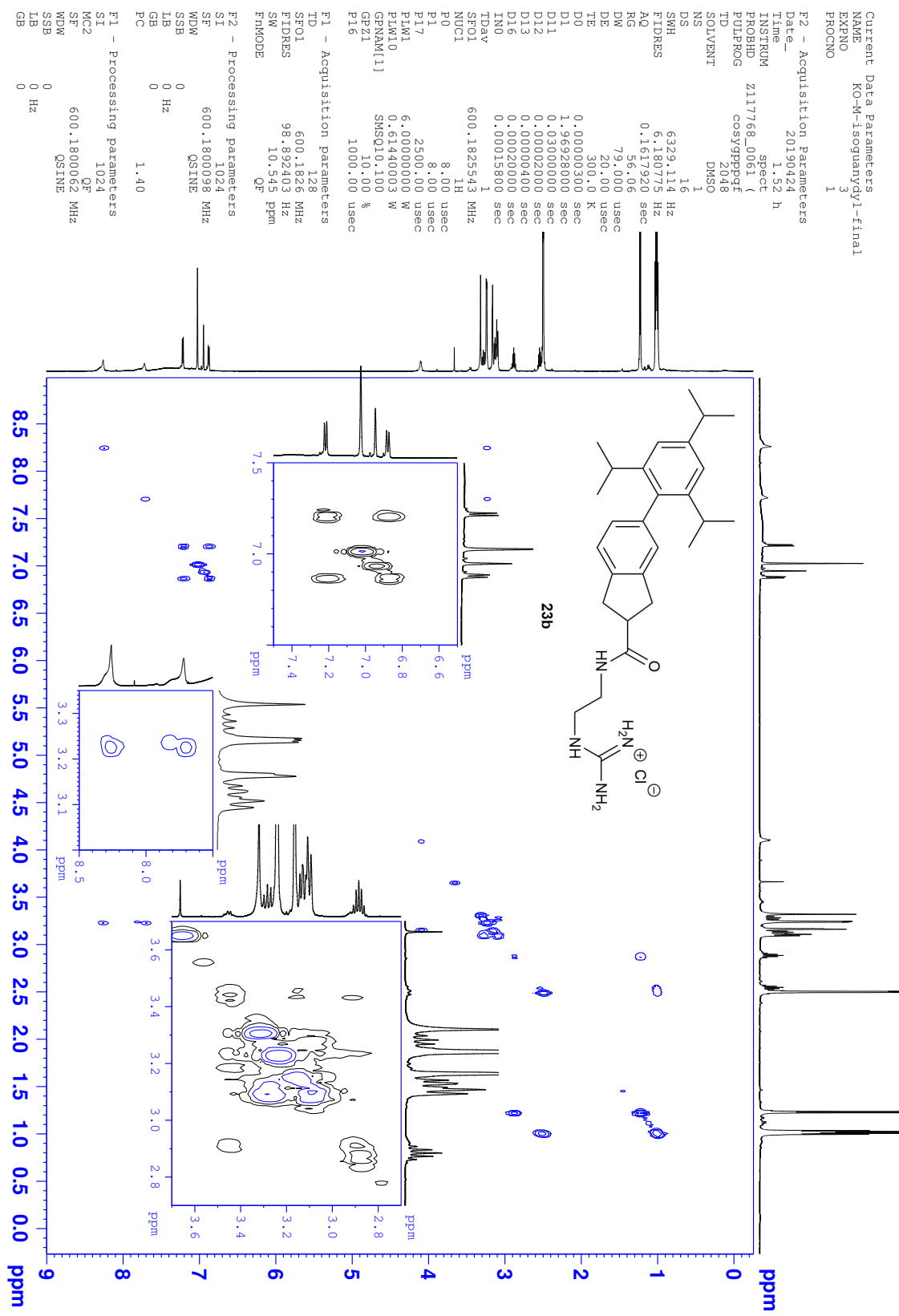
Current Data Parameters
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 EXPTNO 2
 PROCNO 1

F2 - Acquisition Parameters
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 INSTRUM spect
 PROBHD zgpg30
 PULPROG zgpg30
 TD 65536
 SOLVENT DMSO
 NS 2500
 DS 4
 SWH 36057.691 Hz
 FIDRES 1.100393 Hz
 AQ 0.9087659 sec
 RG 197.14
 DW 13.867 usec
 DE 18.00 usec
 TE 300.0 K
 D1 2.0000000 sec
 D11 0.0300000 sec
 TDO 1
 SFO1 150.9304719 MHz
 NUC1 ¹³C
 P1 11.40 usec
 PL1 80.0000000 W
 SFO2 600.1824007 MHz
 NUC2 ¹H
 CPDPRG12 waltz16
 PCPD2 70.00 usec
 PLM2 6.0000000 W
 PLM12 0.07836700 W
 PLM13 0.03941800 W

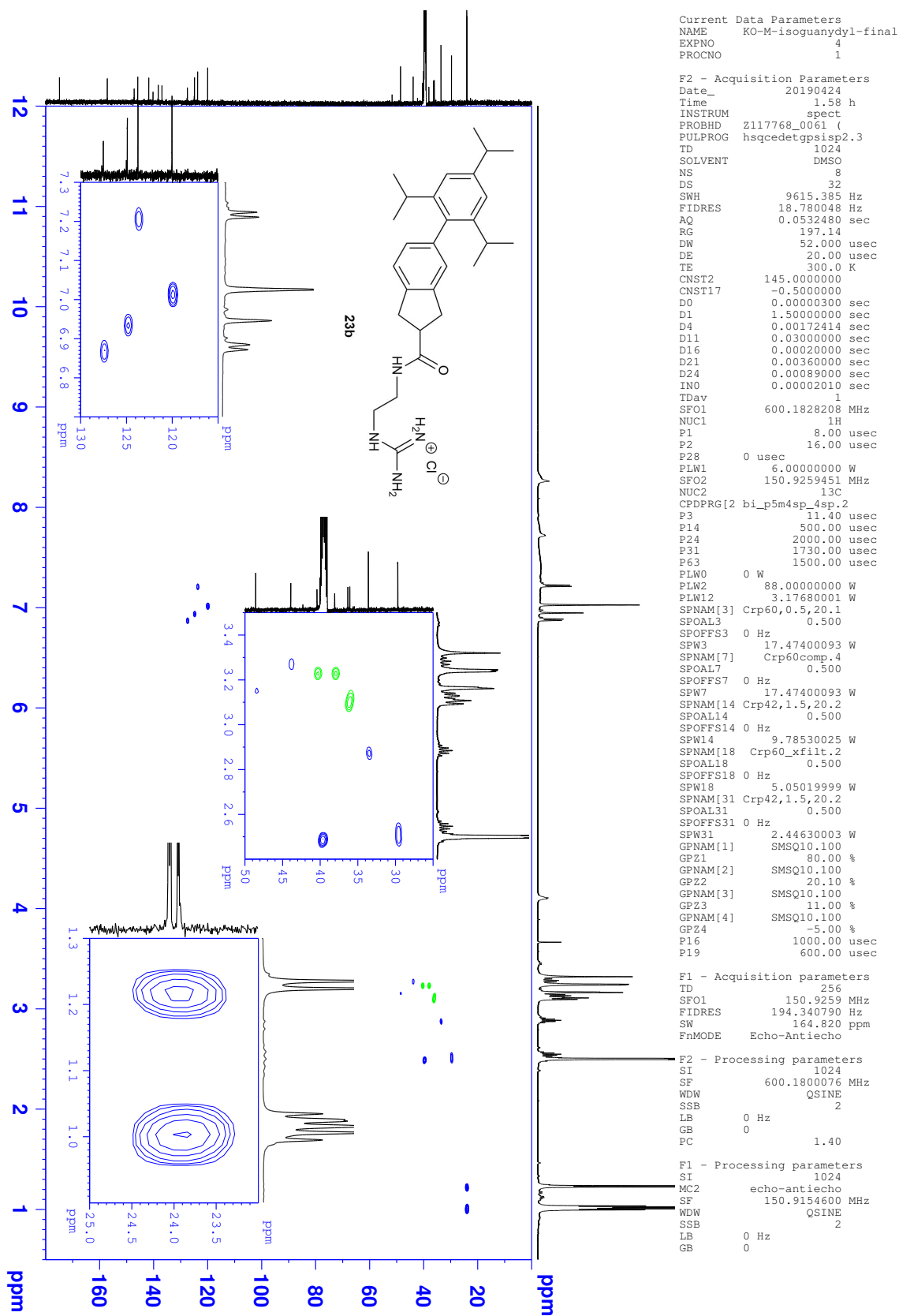
F2 - Processing parameters
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 SF 150.9154547 MHz
 WDW EM
 SSB 0
 IB -0.20 Hz
 GB 0
 PC 1.40



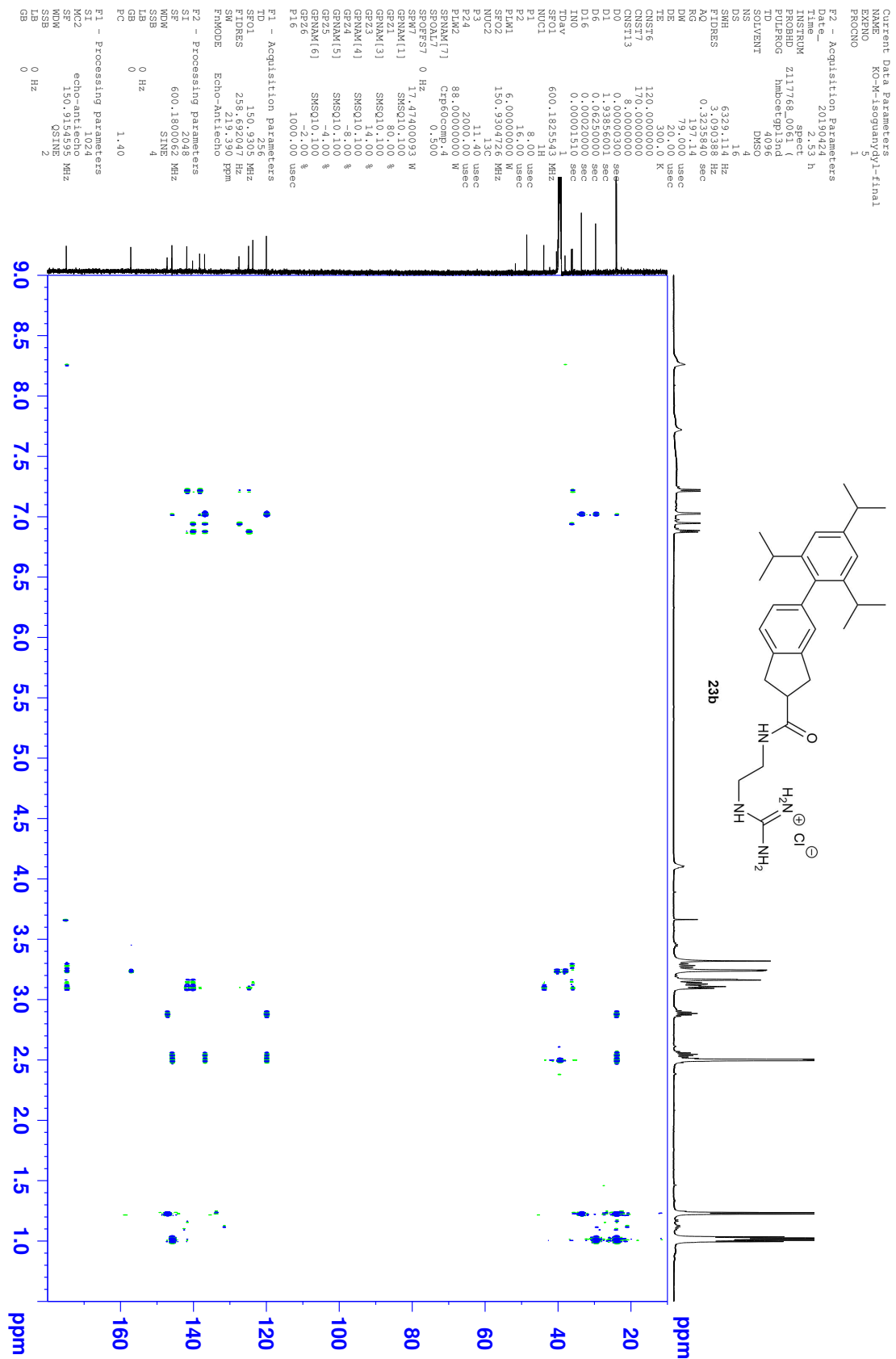
R.3 COSY (600 MHz, DMSO-d₆) Spectrum for 23b



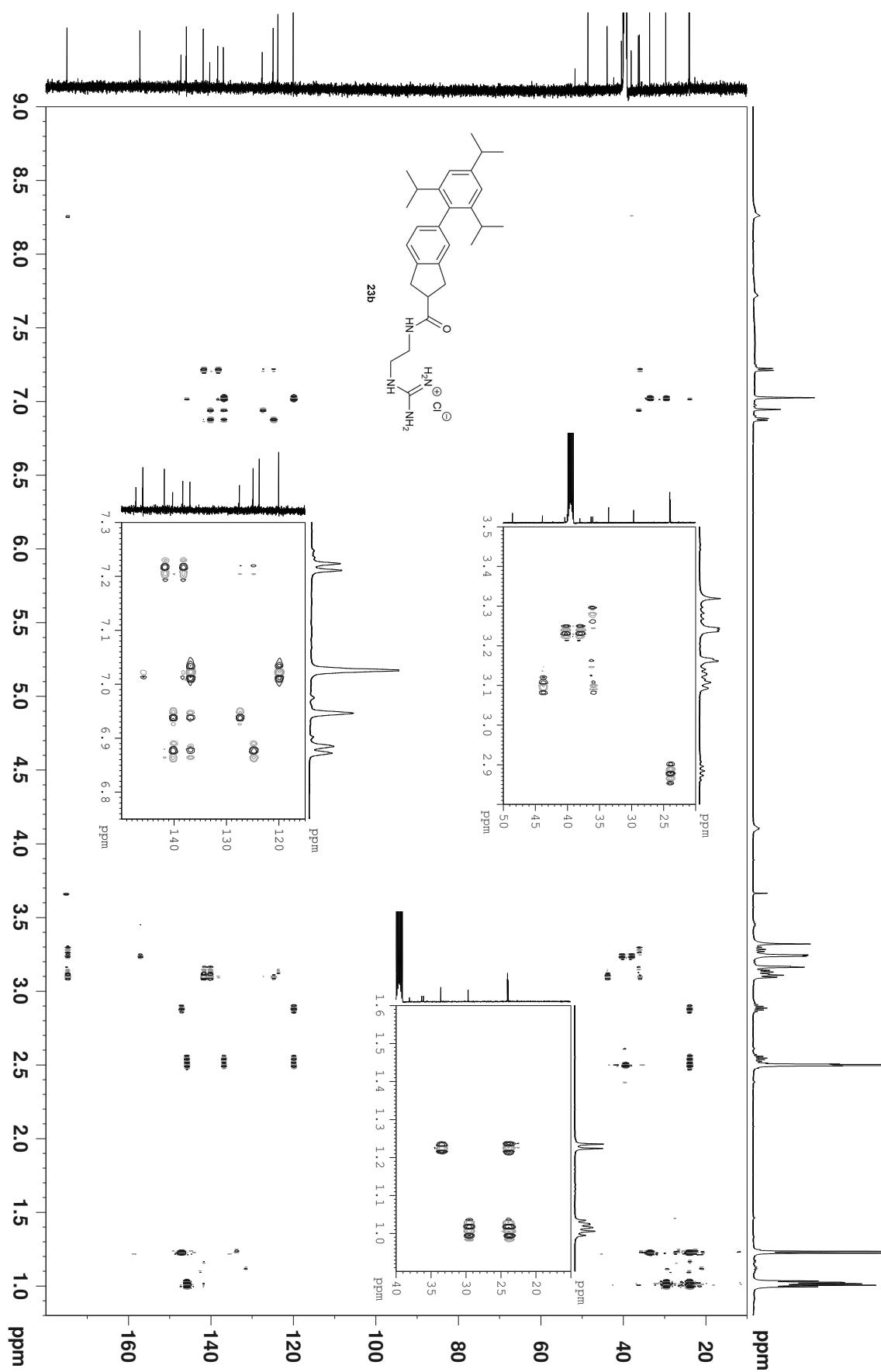
R.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 23b



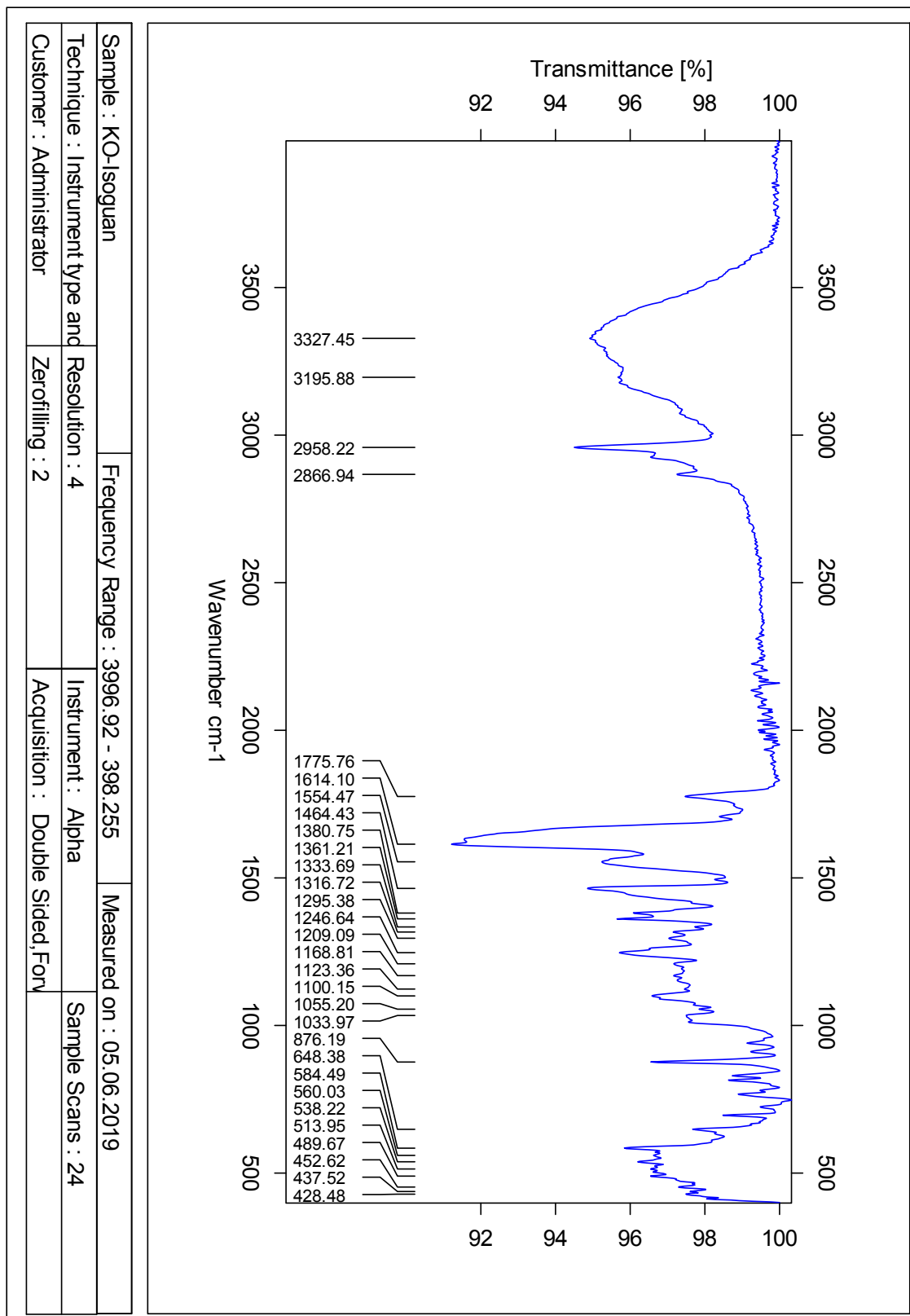
R.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 23b



R.6 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 23b (Large)



R.7 IR Spectrum for 23b



R.8 HRMS Report for 23b

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1439 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

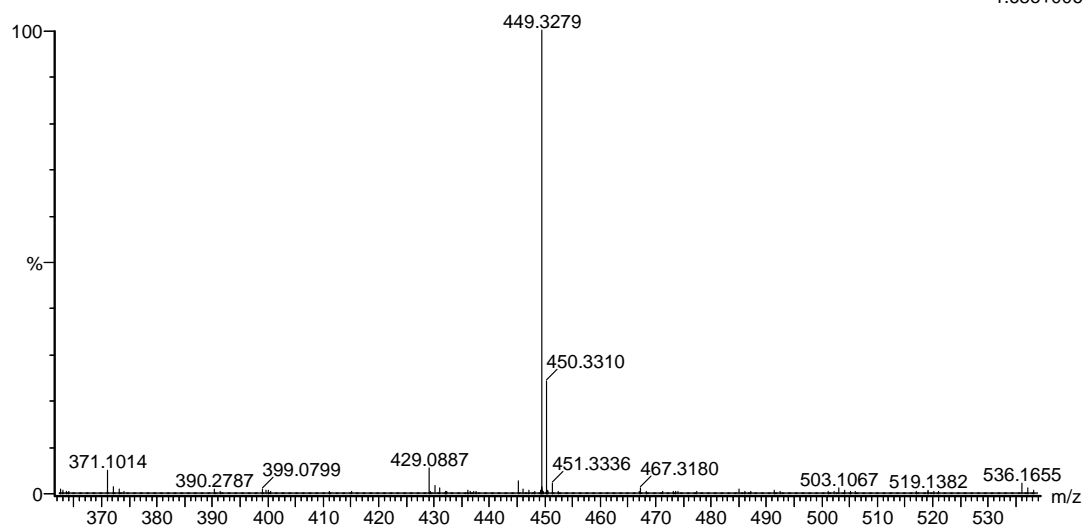
Elements Used:

C: 0-100 H: 0-150 N: 0-10 O: 0-10

svg_20190423_2019_354 37 (0.699) AM2 (Ar,35000.0,0.00,0.00); Cm (37:44)

1: TOF MS ES+

1.68e+006



Minimum: -50.0
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
449.3279	449.3280	-0.1	-0.2	10.5	1041.2	n/a	n/a	C28 H41 N4 O

R.9 HPLC chromatogram for 23b

Data File C:\CHEM32\1\DATA\KRISTINEØYAKO-M-ISOGUANIDYL.D

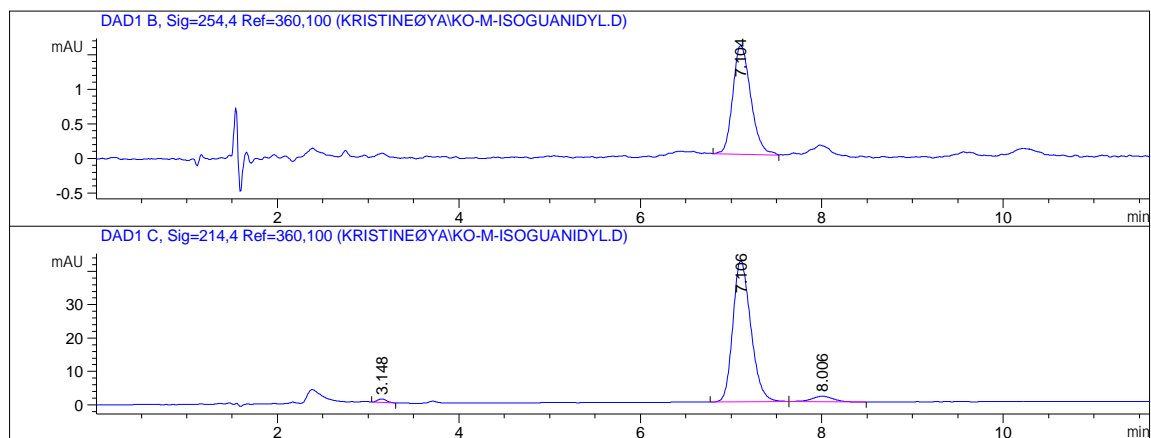
Sample Name: KO-M-i soguani dyl

```

=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 3
Injection Date  : 10.05.2019 12:18:47
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 12:16:53 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 80:20 MeOH/ H2O + 0.1 % TFA, 1 ml/min
  
```

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 B, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.104	BB	0.2124	21.60015	1.57070	100.0000

Totals : 21.60015 1.57070

R.10 HPLC chromatogram for 23b

Data File C:\CHEM32\1\DATA\KRI STI NEØYA\K0-M-I SOGUANI DYL. D

Sample Name: K0-M-i soguani dyl

```
=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 3
Injection Date  : 10.05.2019 12:18:47
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 12:16:53 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NI CO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 80:20 MeOH/ H2O + 0.1 % TFA, 1 ml/min
=====
```

Additional Info : Peak(s) manually integrated

Signal 2: DAD1 C, Sig=214,4 Ref=360,100

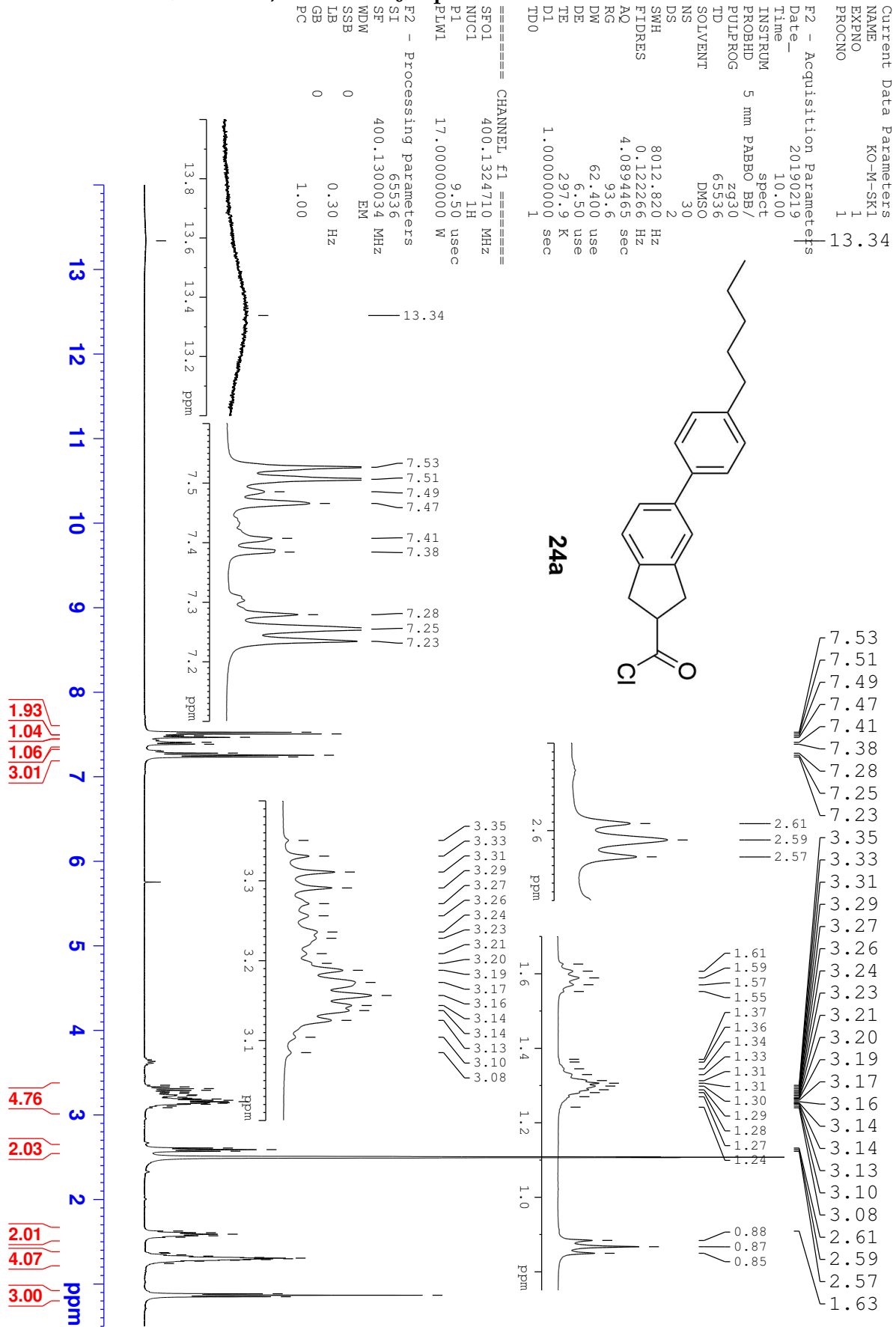
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.148	BB	0.1078	7.08313	1.03524	1.1547
2	7.106	BB	0.2140	580.24481	42.29860	94.5896
3	8.006	BB	0.2400	26.10626	1.63784	4.2558

Totals : 613.43420 44.97168

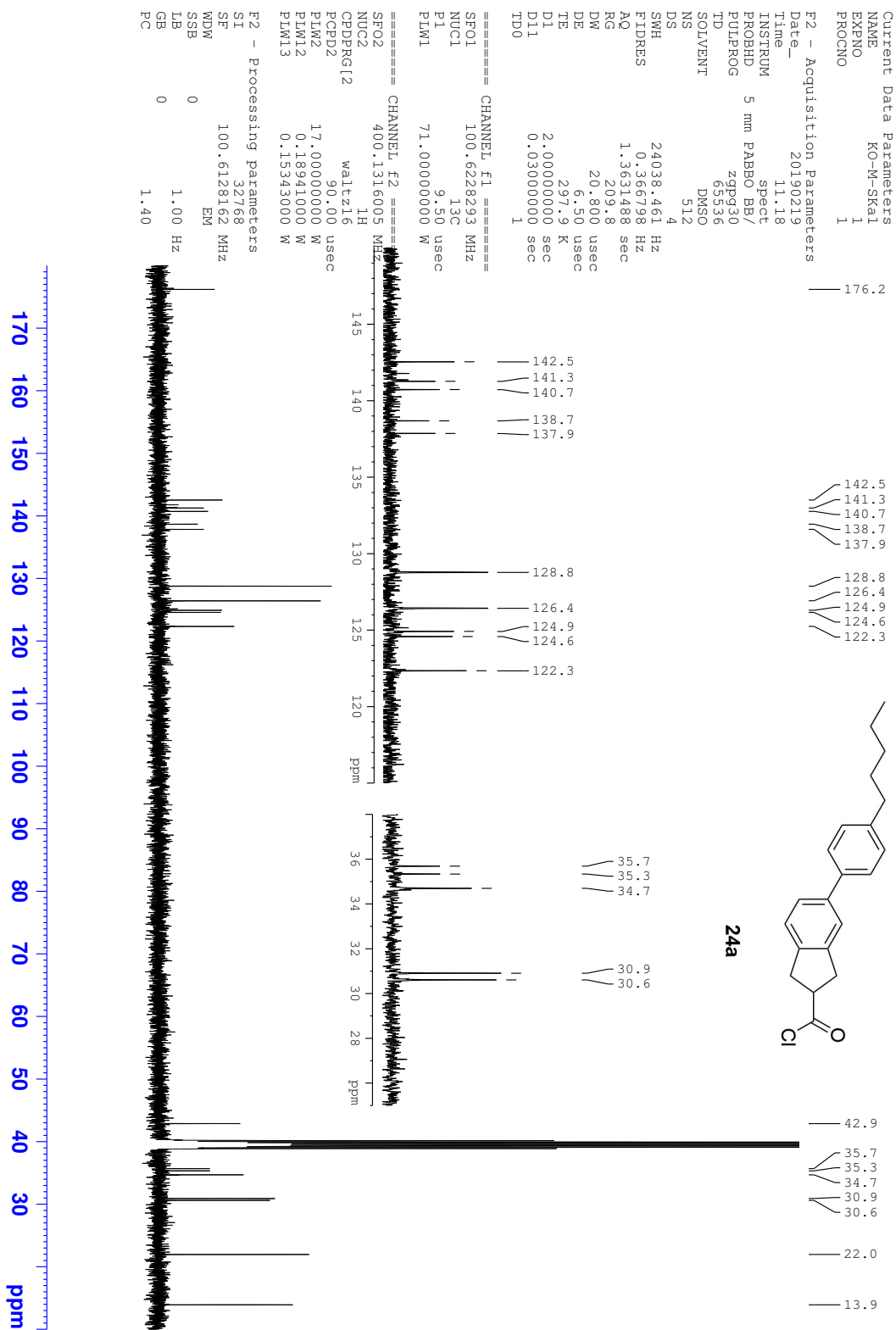
=====

*** End of Report ***

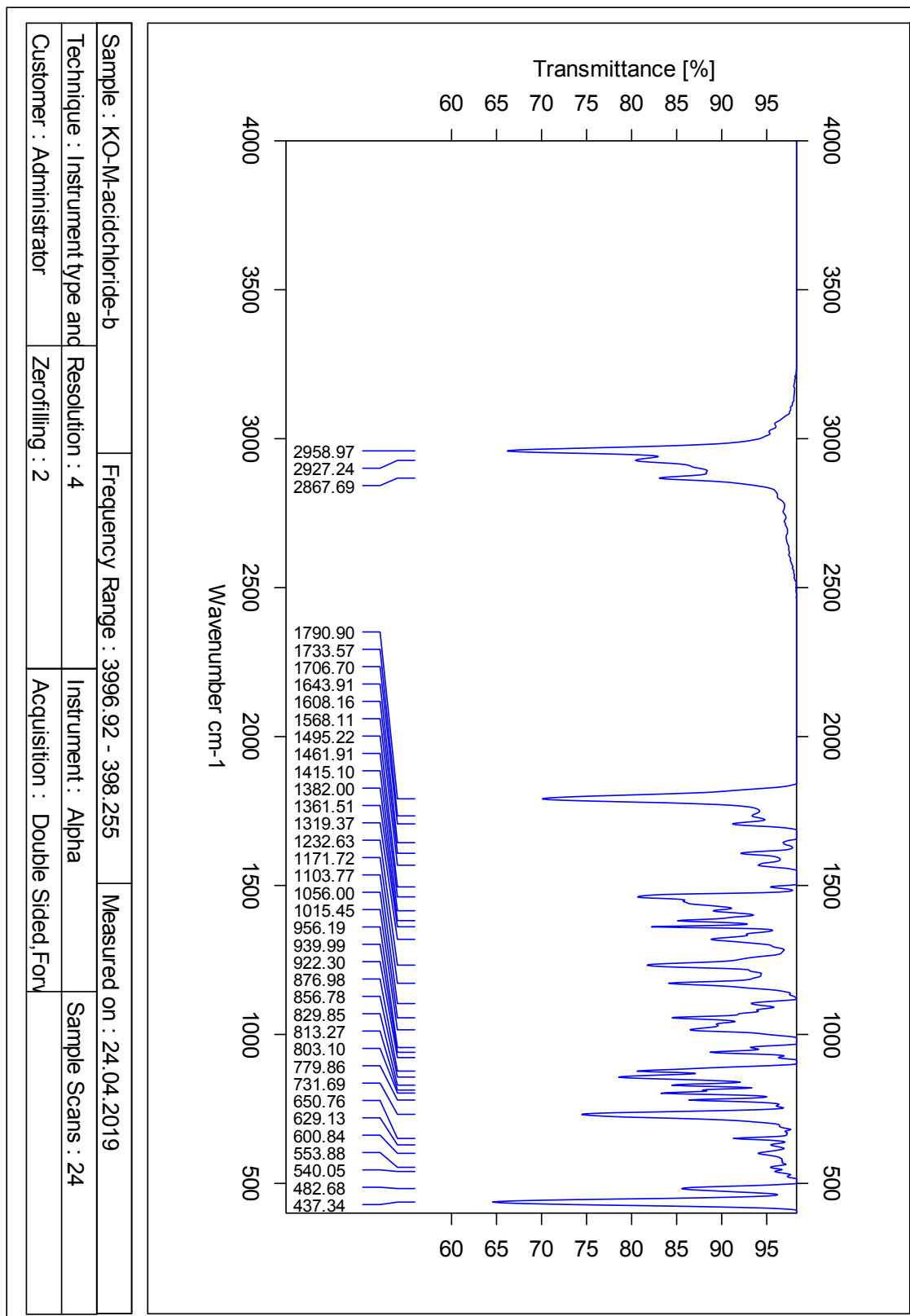
S.1 ¹H NMR (400 MHz, DMSO-d₆) Spectrum for 24a



S.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 24a



S.3 IR Spectrum for 24a



S.4 HRMS Report for 24a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -2.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions

116 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

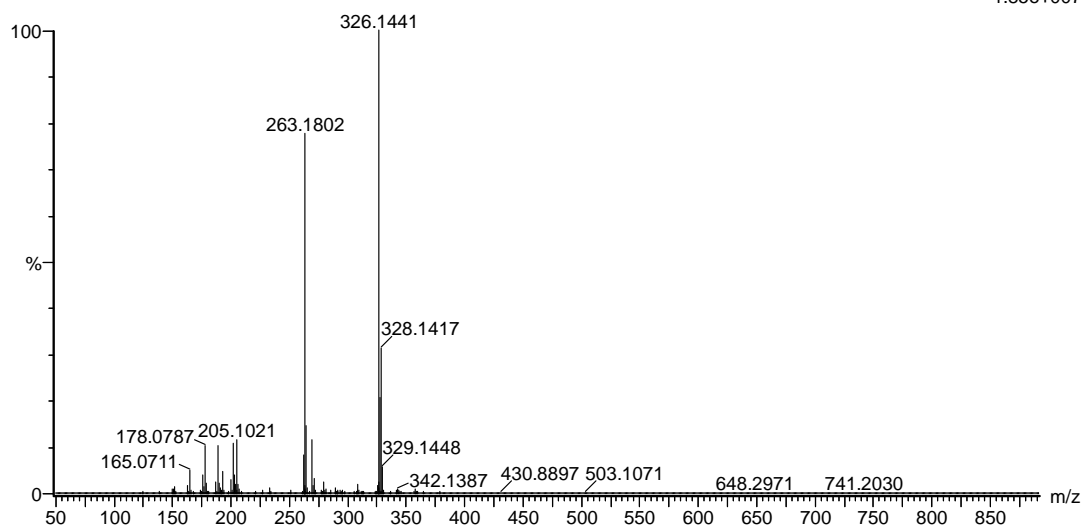
Elements Used:

C: 0-100 H: 0-150 O: 0-10 Cl: 0-2

2019-131 101 (1.981) AM2 (Ar,35000.0,0.00,0.00); Cm (99:105)

1: TOF MS ASAP+

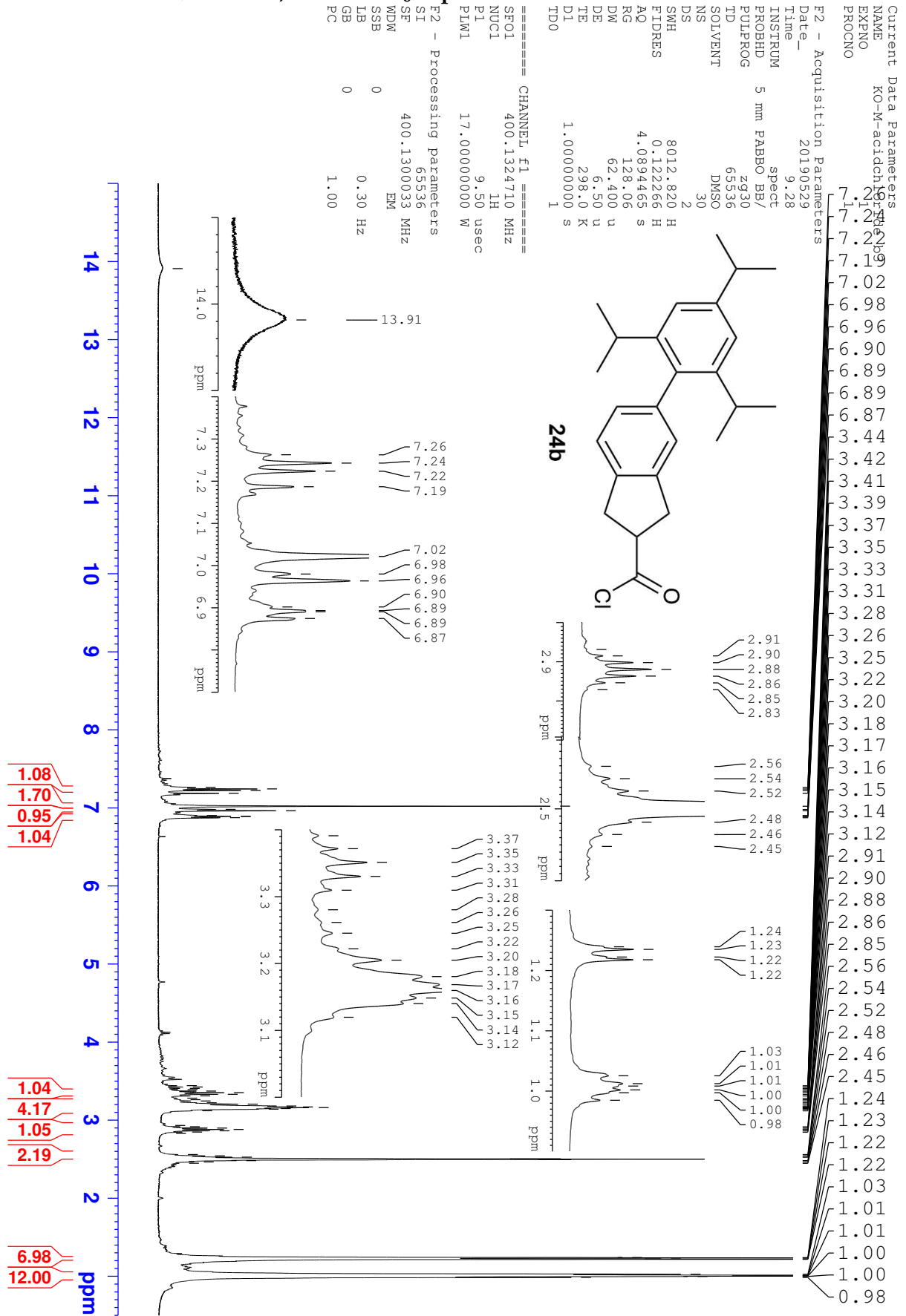
1.85e+007



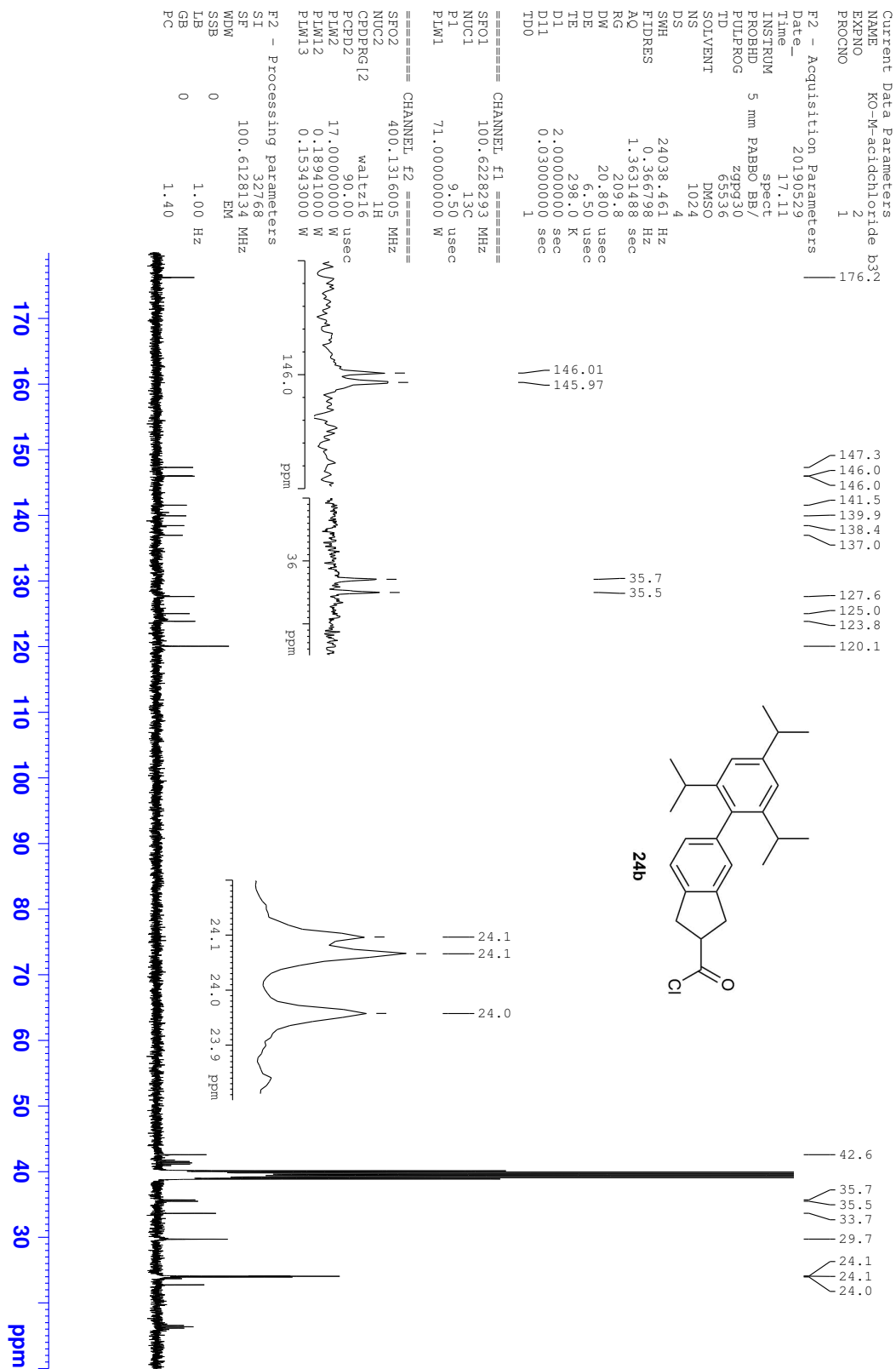
Minimum: -2.0
Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
326.1441	326.1437	0.4	1.2	10.0	1574.4	n/a	n/a	C21 H23 O Cl

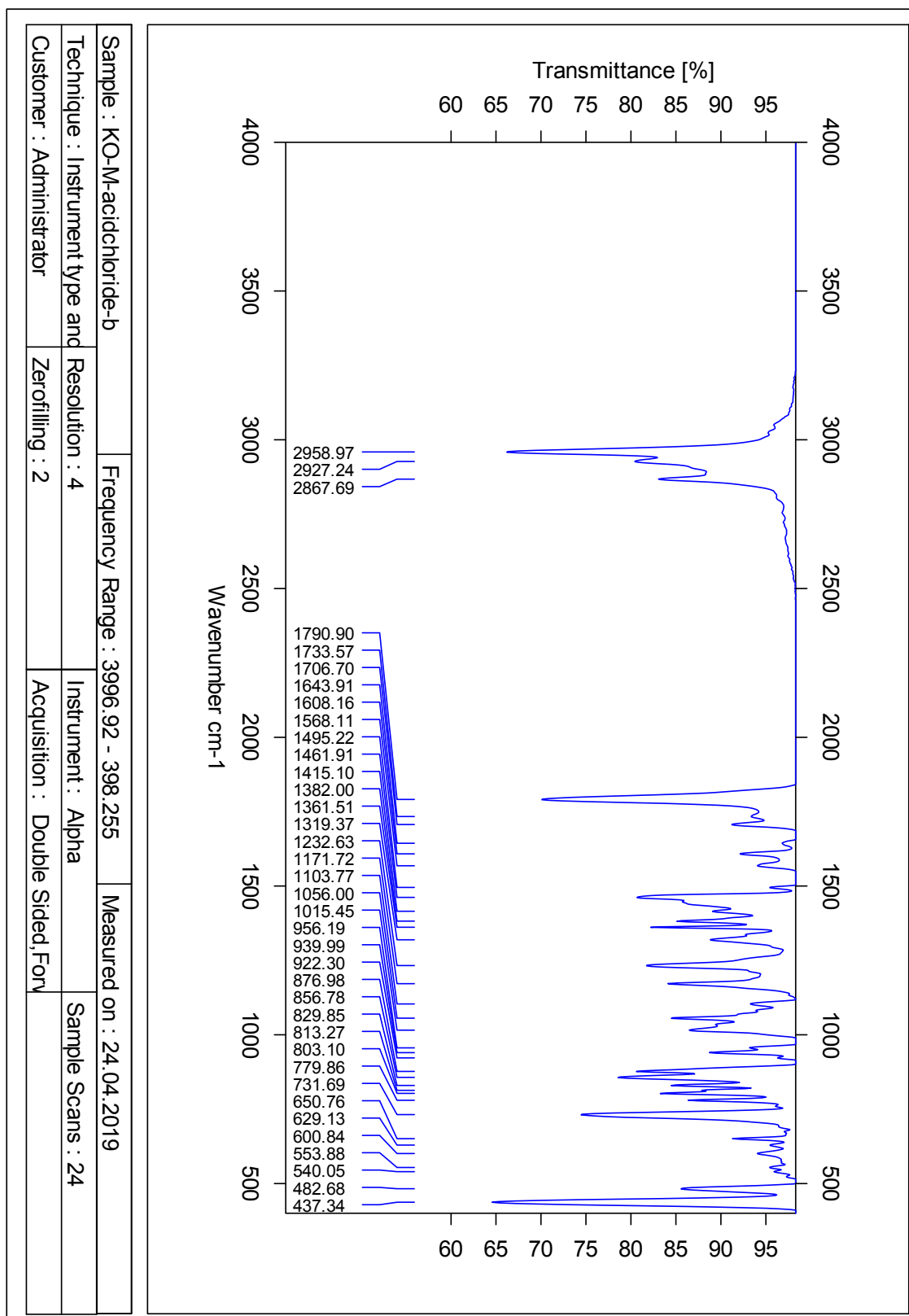
T.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 24b



T.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 24b



T.3 IR Spectrum for 24b



T.4 HRMS Report for 24b

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions

363 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

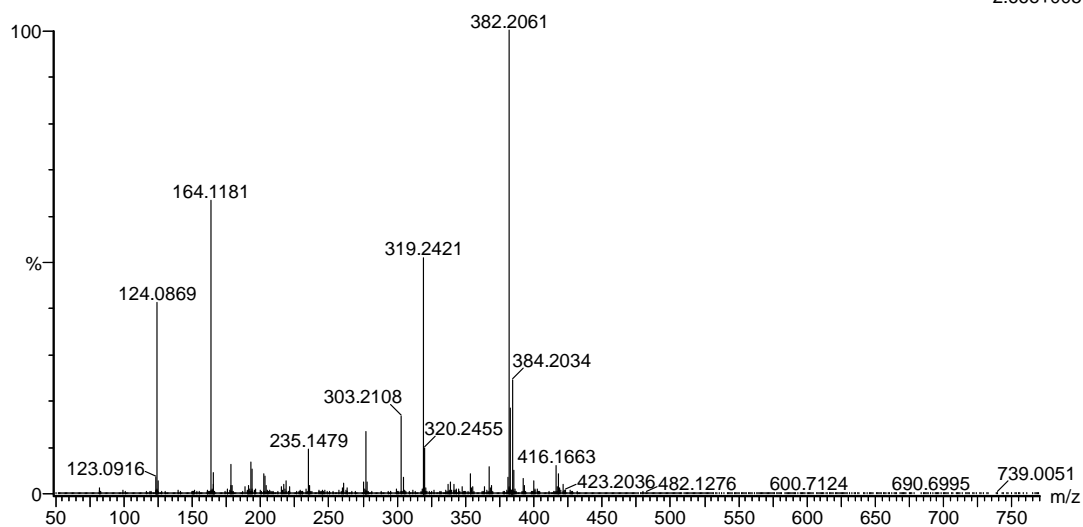
Elements Used:

C: 0-100 H: 0-150 O: 0-10 Cl: 0-2

2019-358 15 (0.310) AM2 (Ar,35000.0,0.00,0.00); Cm (11:16)

1: TOF MS ASAP+

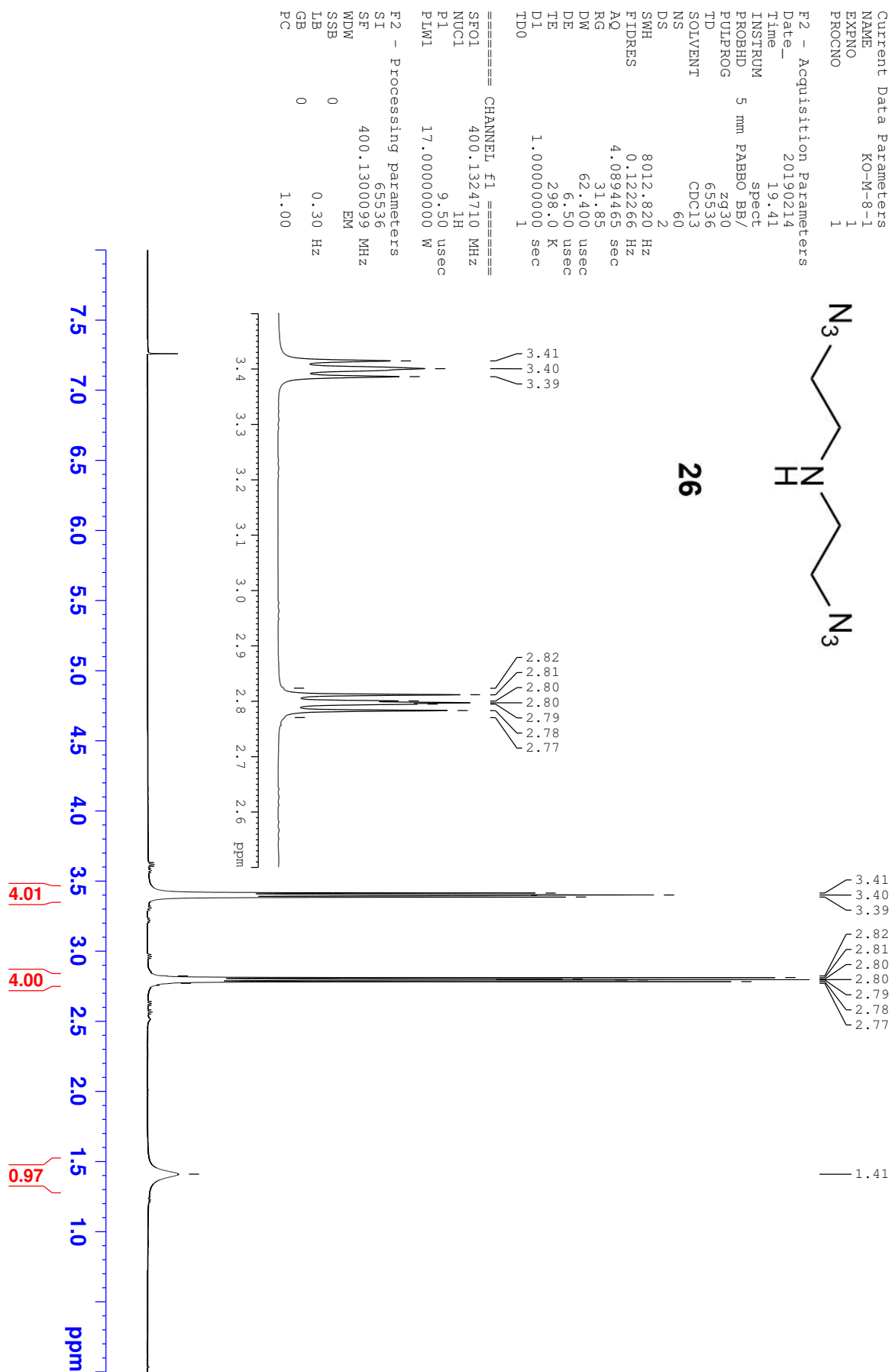
2.66e+005



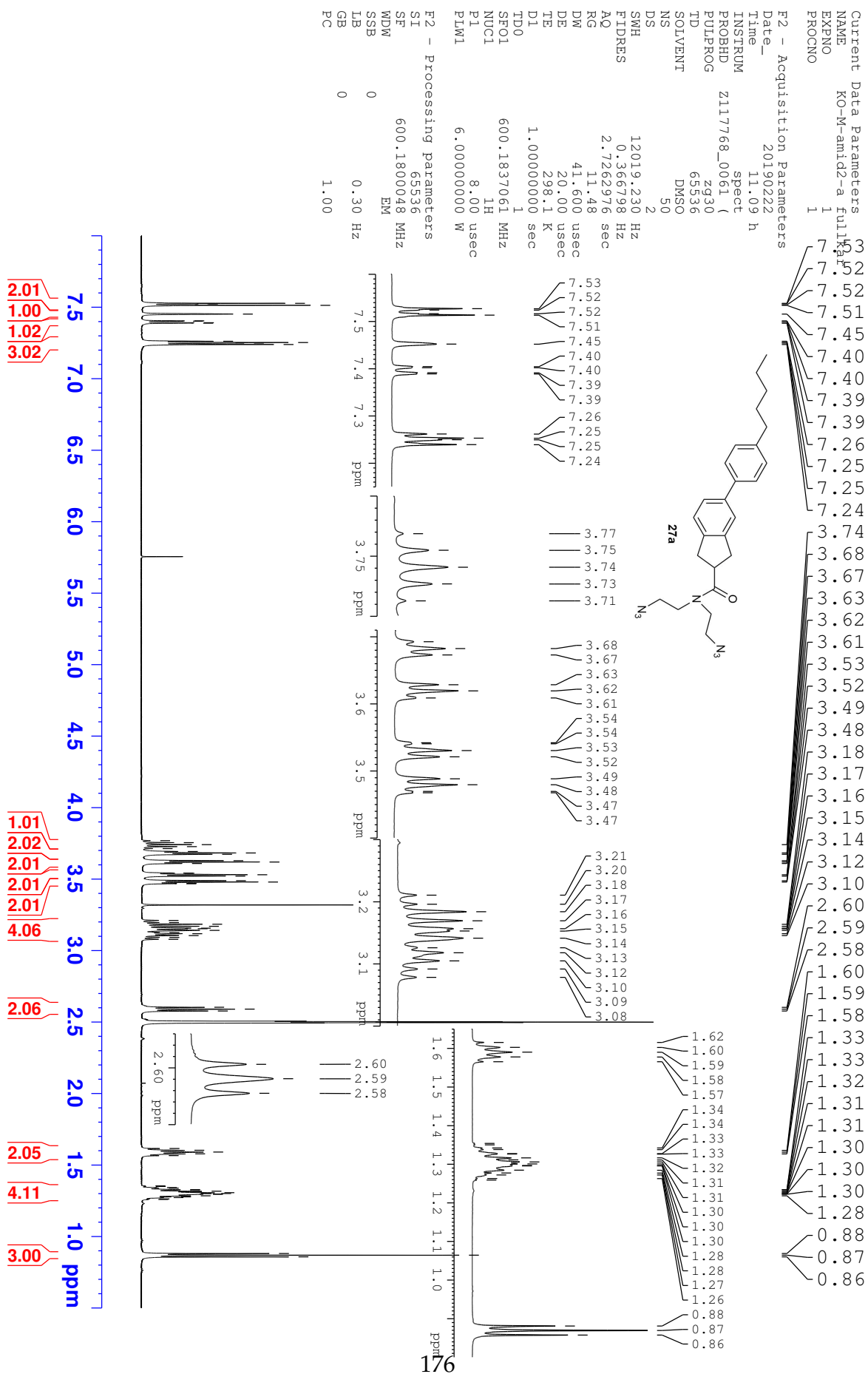
Minimum: -50.0
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
382.2061	382.2063	-0.2	-0.5	10.0	938.0	n/a	n/a	C25 H31 O Cl

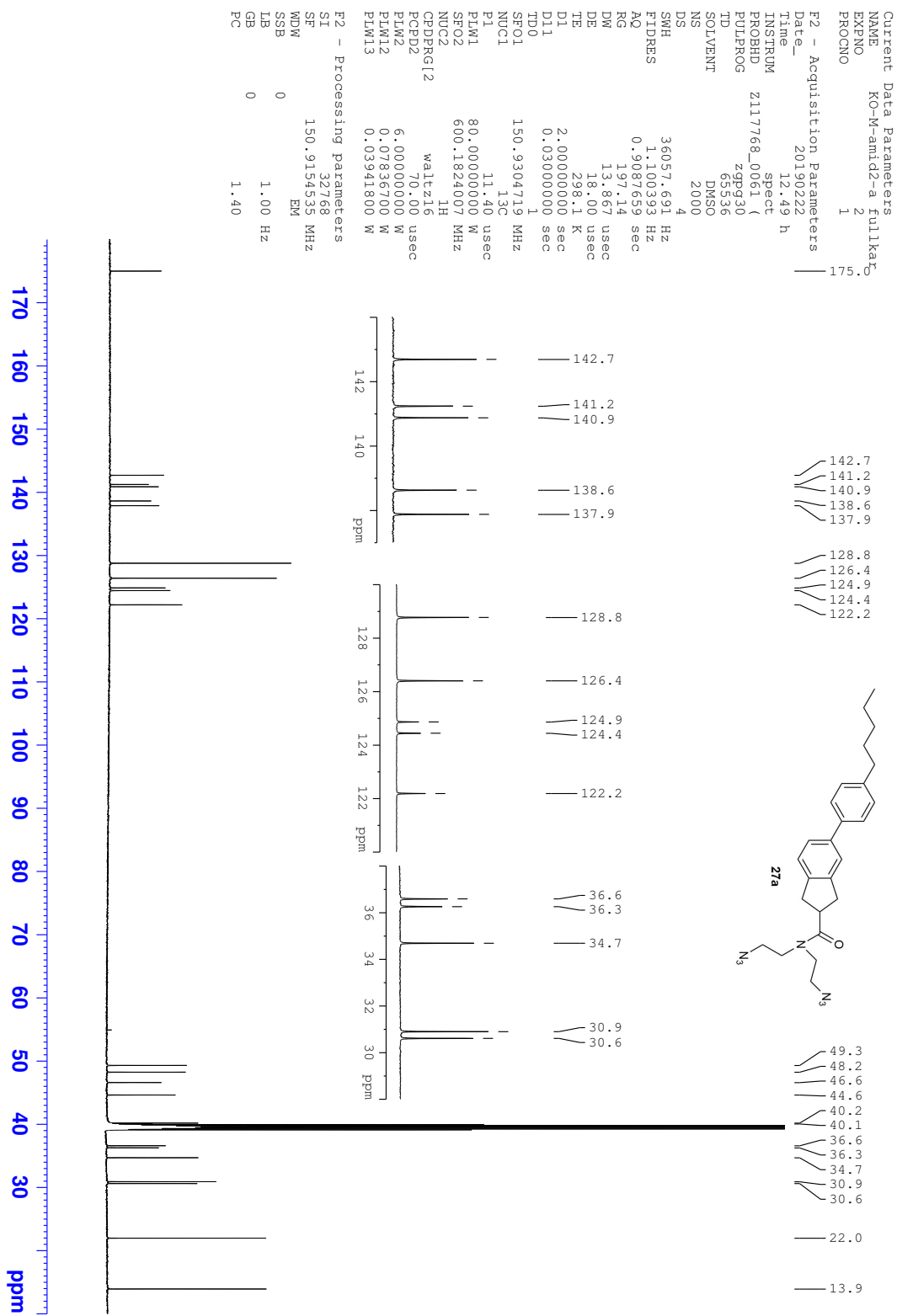
U.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 26



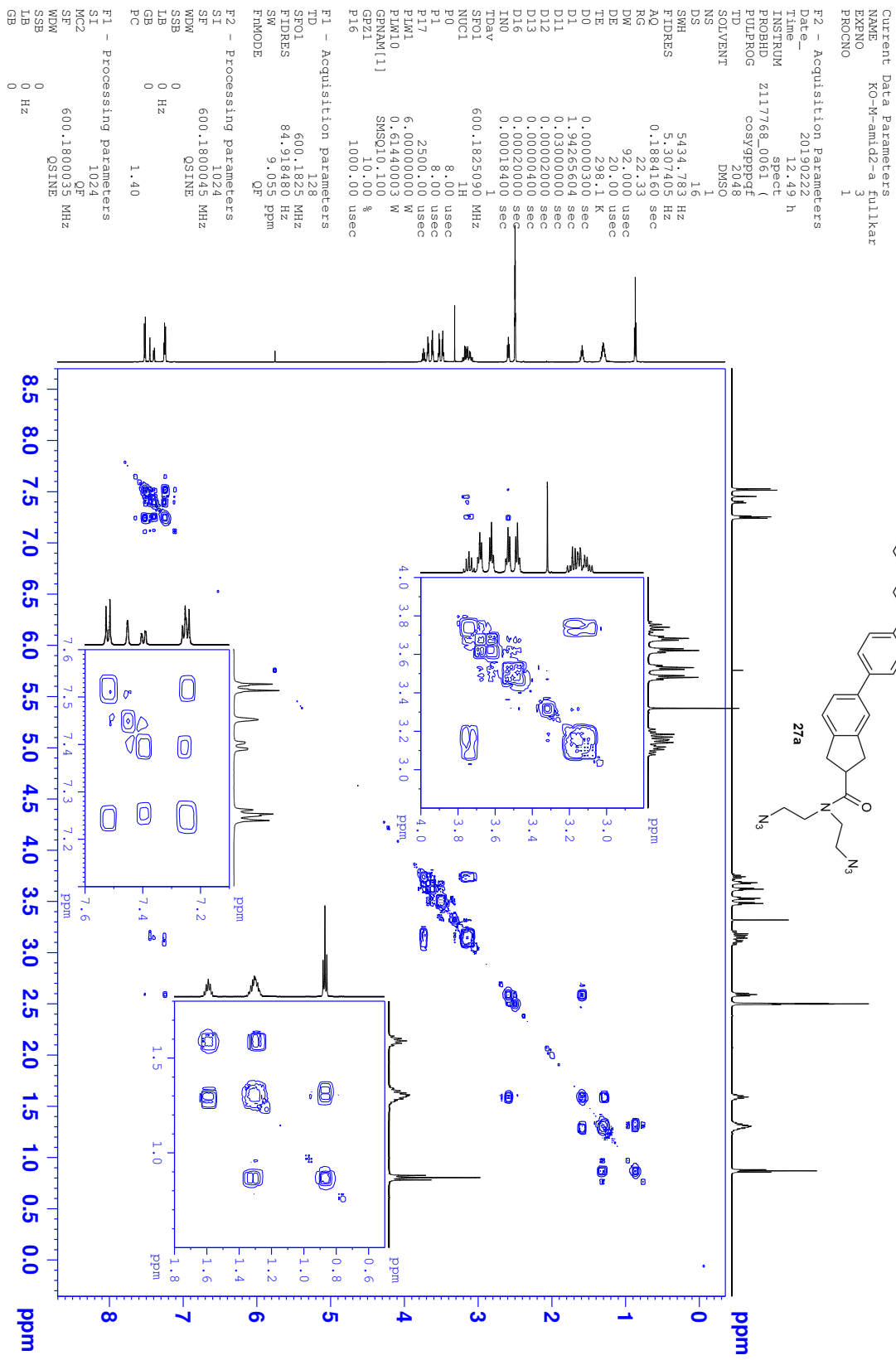
V.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 27a



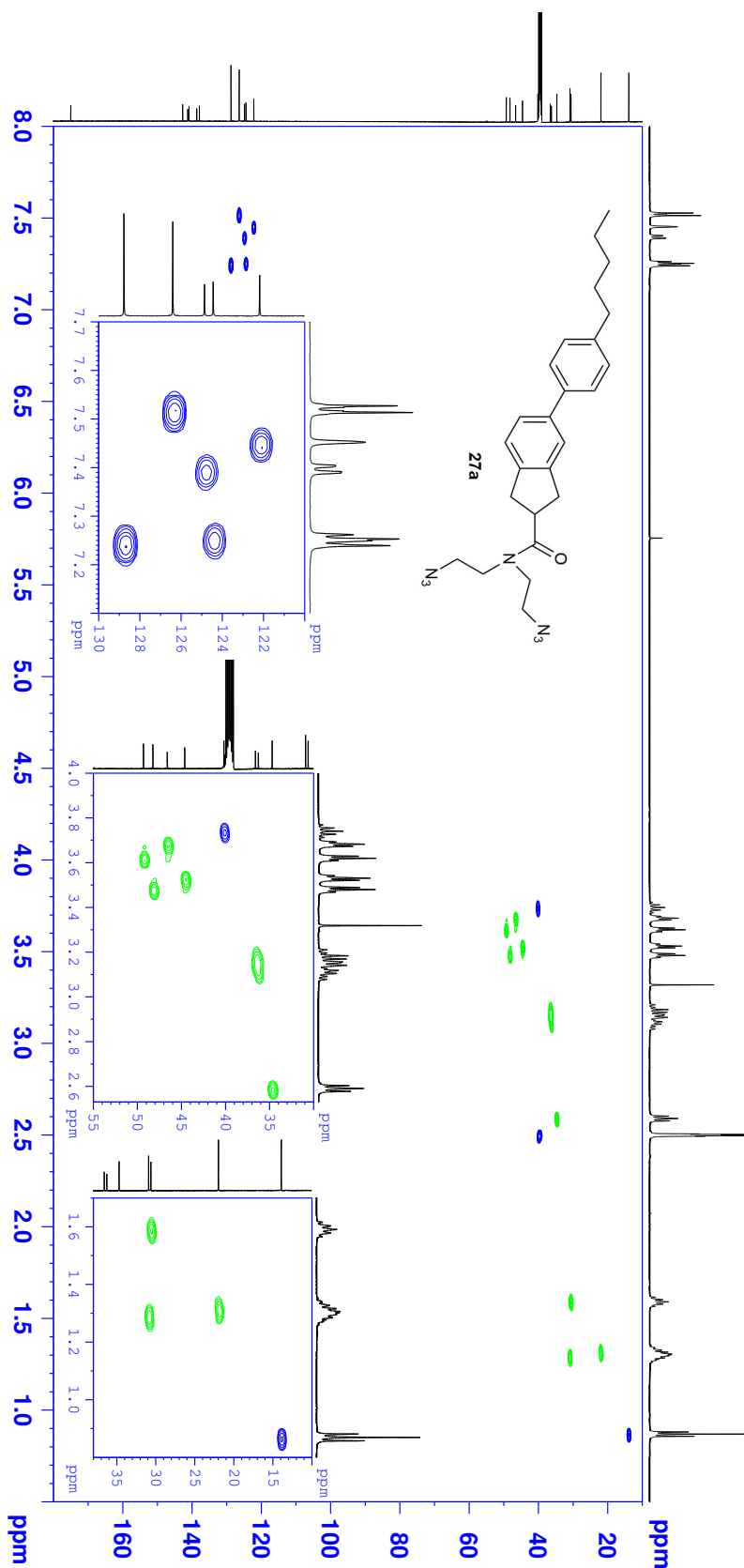
V.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 27a



V.3 COSY (600 MHz, DMSO-d₆) Spectrum for 27a



V.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 27a



```

Current Data Parameters
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EXPNO    4
PROCNO   1

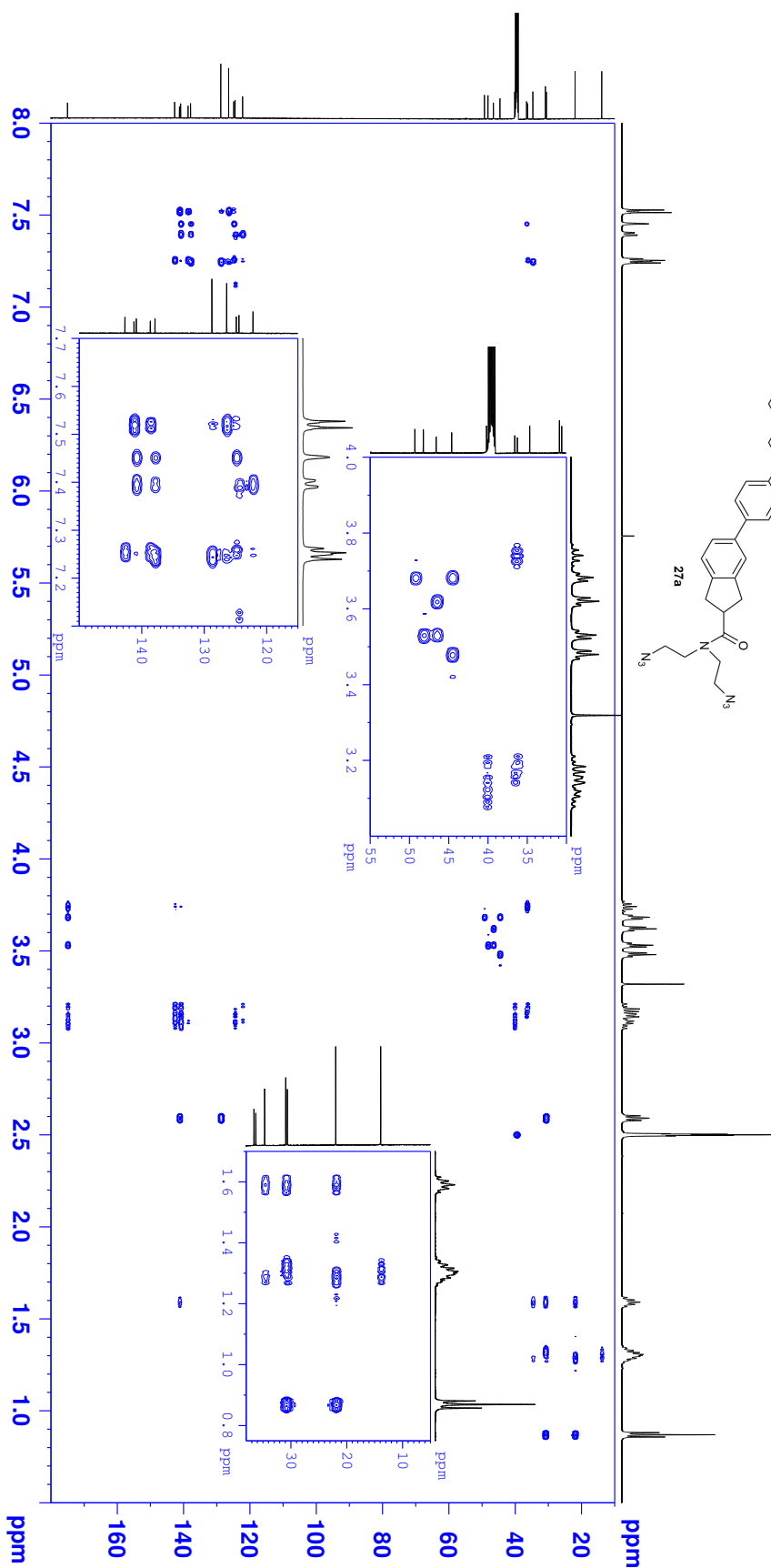
F2 - Acquisition Parameters
Date_    20190222
Time     12.56 h
INSTRUM  spect
PROBHD   Z117768_0061 (
PULPROG  hsqcetedgpsisp2.3
TD       1024
SOLVENT  DMSO
NS       8
DS       32
SWH      9615.385 Hz
FIDRES   18.780048 Hz
AQ       0.0532480 sec
RG       197.14
DW       52.0000 sec
DE       20.00 usec
TE       298.1 K
CNST2    145.0000000
CNST17   -0.5000000
D0       0.00000300 sec
D1       1.50000000 sec
D4       0.00172414 sec
D11      0.03000000 sec
D16      0.00020000 sec
D21      0.00360000 sec
D24      0.00089000 sec
IN0      0.00002010 sec
TDav     1
SF01     600.1828208 MHz
NUC1     1H
P1       8.00 usec
P2       16.00 usec
P28      0 usec
PLW1     6.00000000 W
SF02     150.9259451 MHz
NUC2     13C
CPDPRG2  bi_p5m4sp_4sp.2
P3       11.40 usec
P14      500.00 usec
P24      2000.00 usec
P31      1730.00 usec
P63      1500.00 usec
PLW0     0 W
PLW2     88.00000000 W
PLW12    3.17680001 W
SPNAM[3] Crp60,0.5,20.1
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SPOFFS3  0 Hz
SPW3     17.47400093 W
SPNAM[7] Crp60comp.4
SPOAL7   0.500
SPOFFS7  0 Hz
SPW7     17.47400093 W
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SPOFFS14 0 Hz
SPW14    9.78530025 W
SPNAM[18] Crp60_xfilt.2
SPOAL18  0.500
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SPW18    5.05019999 W
SPNAM[31] Crp42,1.5,20.2
SPOAL31  0.500
SPOFFS31 0 Hz
SPW31    2.44630003 W
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GPZ1     80.00 %
GPNAM[2] SMSQ10.100
GPZ2     20.10 %
GPNAM[3] SMSQ10.100
GPZ3     11.00 %
GPNAM[4] SMSQ10.100
GPZ4     -5.00 %
P16      1000.00 usec
P19      600.00 usec

F1 - Acquisition parameters
TD       256
SF01     150.9259 MHz
FIDRES   194.340790 Hz
SW       164.820 ppm
FnMODE   Echo-Antiecho

F2 - Processing parameters
SI       1024
SF       600.1800039 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
PC       1.40

F1 - Processing parameters
SI       1024
MC2      echo-antiecho
SF       150.9154547 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
    
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V.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 27a



Current Data Parameters
 NAME KO-M-amid2-a fullkar
 EXPNO 5
 PROCNO 1

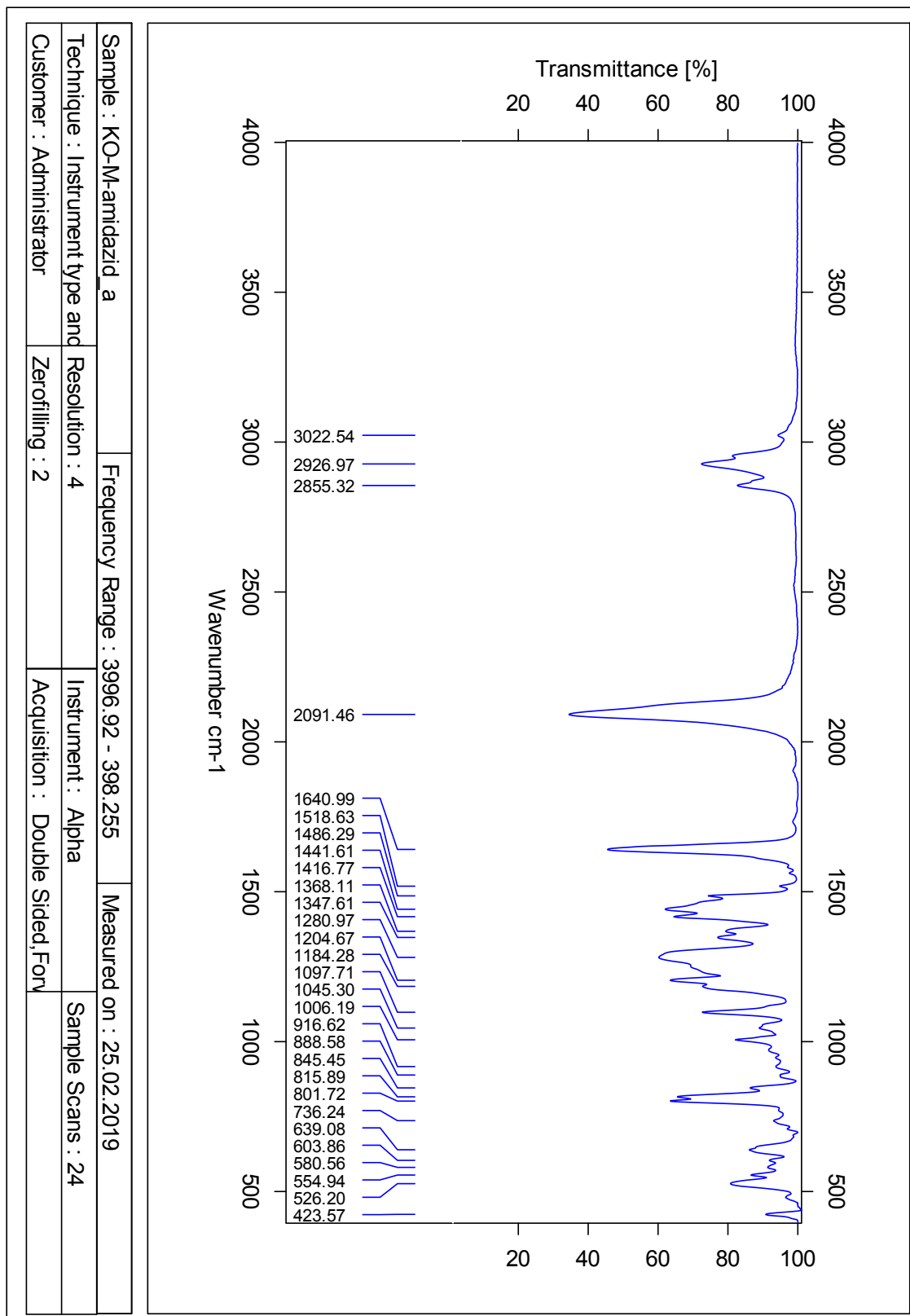
F2 - Acquisition Parameters
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 Time_ 13.50 h
 INSTRUM spect
 PROBHD Z117768_0061 (
 PULPROG hmbcetgp13nd
 TD 4096
 SOLVENT DMSO
 NS 4
 DS 16
 SWH 5434.783 Hz
 FIDRES 2.653702 Hz
 AQ 0.3768320 sec
 RG 197.14
 DW 92.000 usec
 DE 20.00 usec
 TE 298.1 K
 CNST6 120.0000000
 CNST7 170.0000000
 CNST13 8.0000000
 D0 0.00000300 sec
 D1 1.88531196 sec
 D6 0.06250000 sec
 D16 0.00020000 sec
 IN0 0.00001510 sec
 TDav 1
 SFO1 600.1825090 MHz
 NUC1 1H
 P1 8.00 usec
 P2 16.00 usec
 PLW1 6.00000000 W
 SFO2 150.9304726 MHz
 NUC2 13C
 P3 11.40 usec
 P24 2000.00 usec
 PLW2 88.00000000 W
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 SPOAL7 0.500
 SPOFFS7 0 Hz
 SPW7 17.47400093 W
 GPNAM[1] SMSQ10.100
 GPZ1 80.00 %
 GPNAM[3] SMSQ10.100
 GPZ3 14.00 %
 GPNAM[4] SMSQ10.100
 GPZ4 -8.00 %
 GPNAM[5] SMSQ10.100
 GPZ5 -4.00 %
 GPNAM[6] SMSQ10.100
 GPZ6 -2.00 %
 P16 1000.00 usec

F1 - Acquisition parameters
 TD 256
 SFO1 150.9305 MHz
 FIDRES 258.692047 Hz
 SW 219.390 ppm
 FnMODE Echo-Antiecho

F2 - Processing parameters
 SI 2048
 SF 600.1800050 MHz
 WDW SINE
 SSB 4
 LB 0 Hz
 GB 0
 PC 1.40

F1 - Processing parameters
 SI 1024
 MC2 echo-antiecho
 SF 150.9154582 MHz
 WDW QSINE
 SSB 2
 LB 0 Hz
 GB 0

V.6 IR Spectrum for 27a



V.7 HRMS Report for 27a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -2.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

355 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

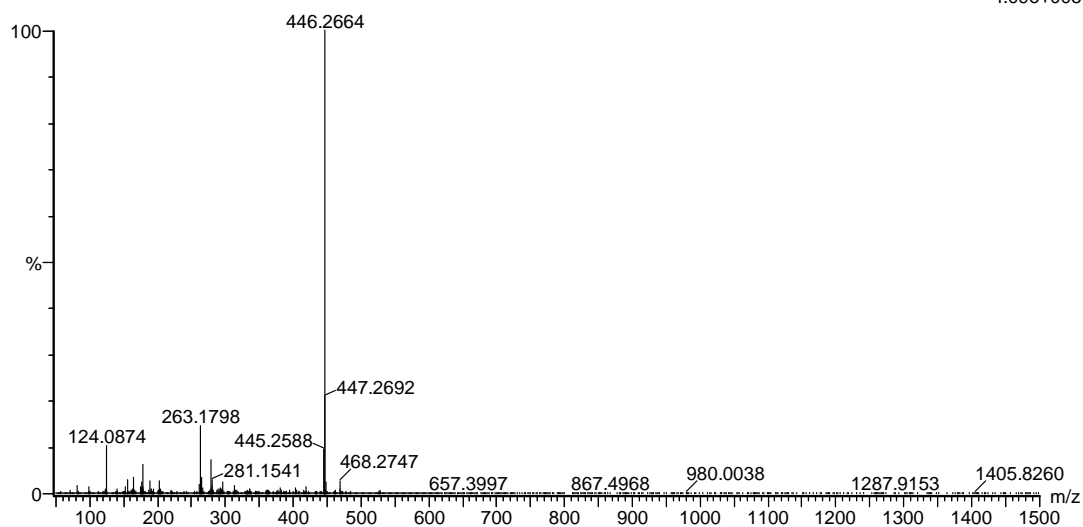
Elements Used:

C: 0-100 H: 0-150 N: 0-10 O: 0-5

2019-130 96 (1.878) AM2 (Ar,35000.0,0.00,0.00); Cm (93:96)

1: TOF MS ASAP+

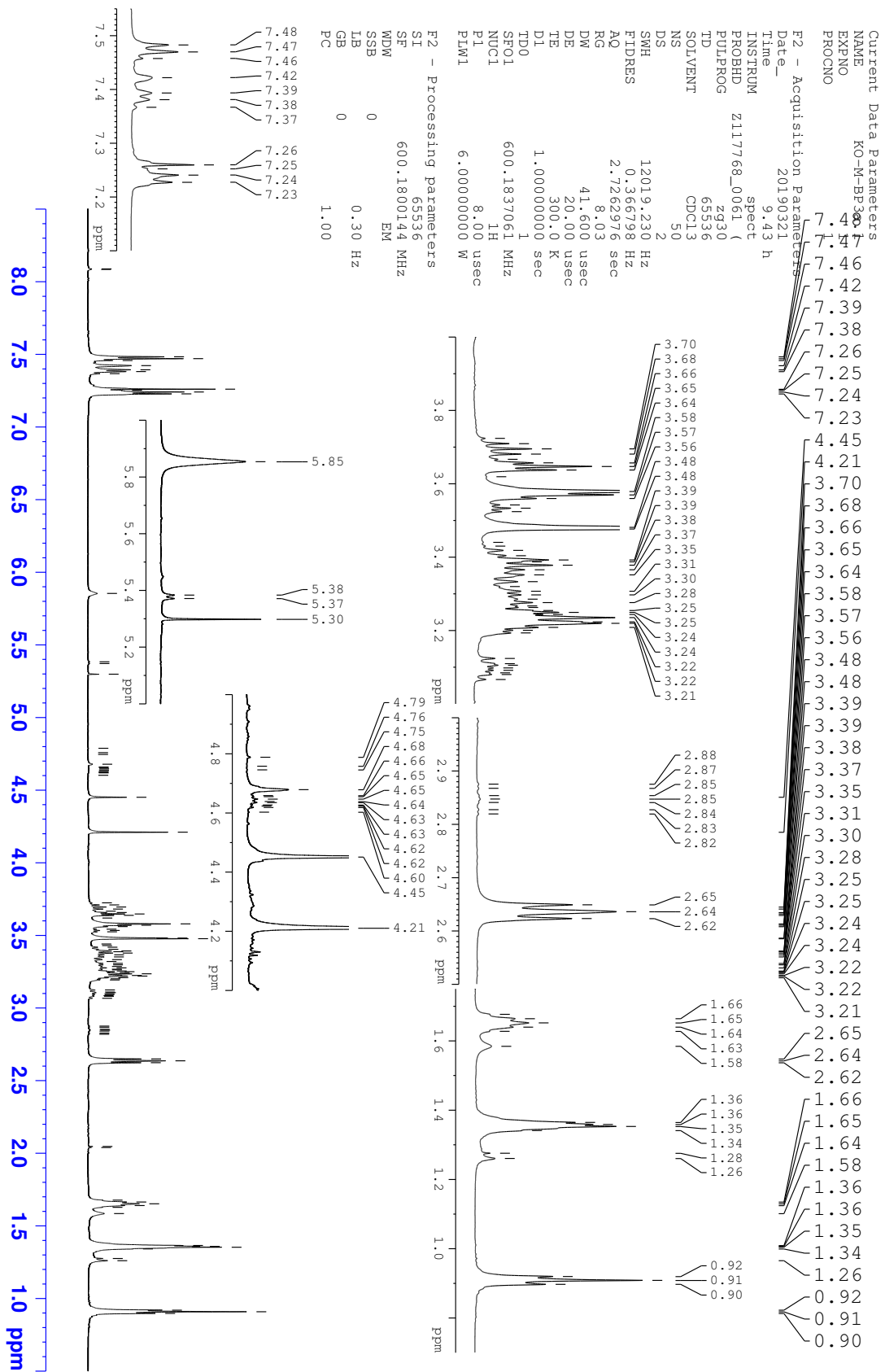
4.09e+005



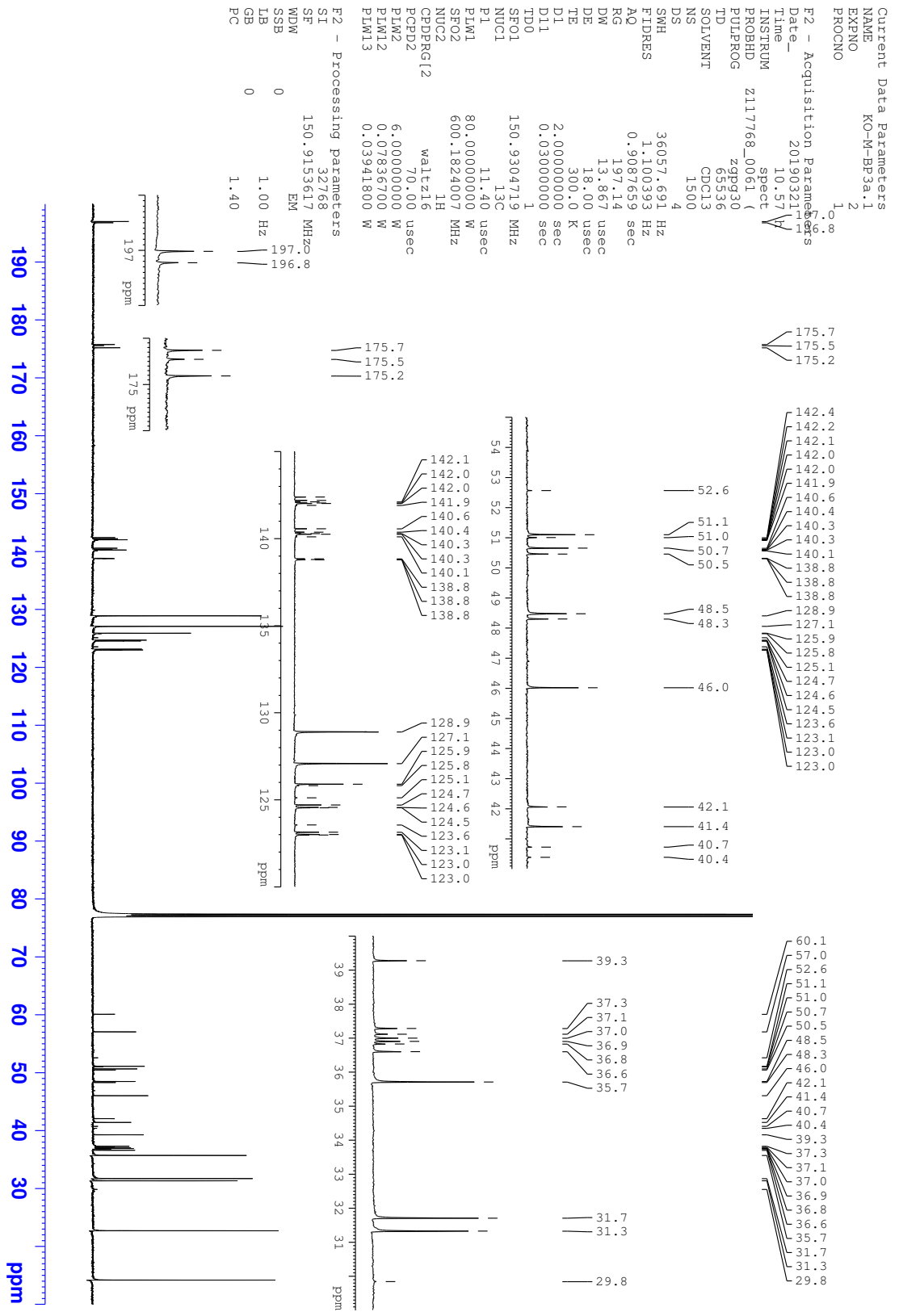
Minimum: -2.0
Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
446.2664	446.2668	-0.4	-0.9	13.5	840.6	0.661	51.64	C25 H32 N7 O
	446.2655	0.9	2.0	8.5	840.7	0.727	48.36	C24 H36 N3 O5

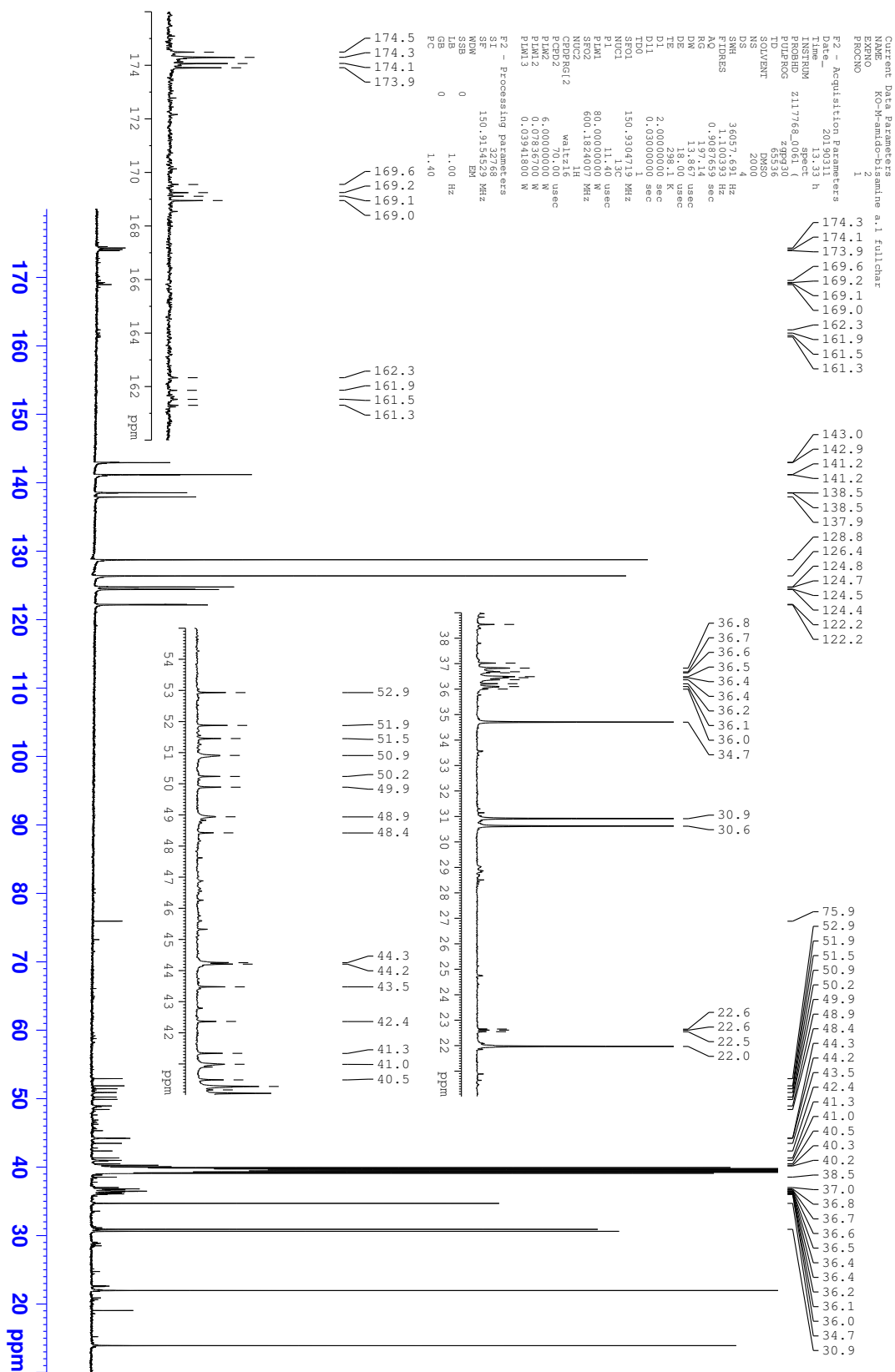
W.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for BP3a.1



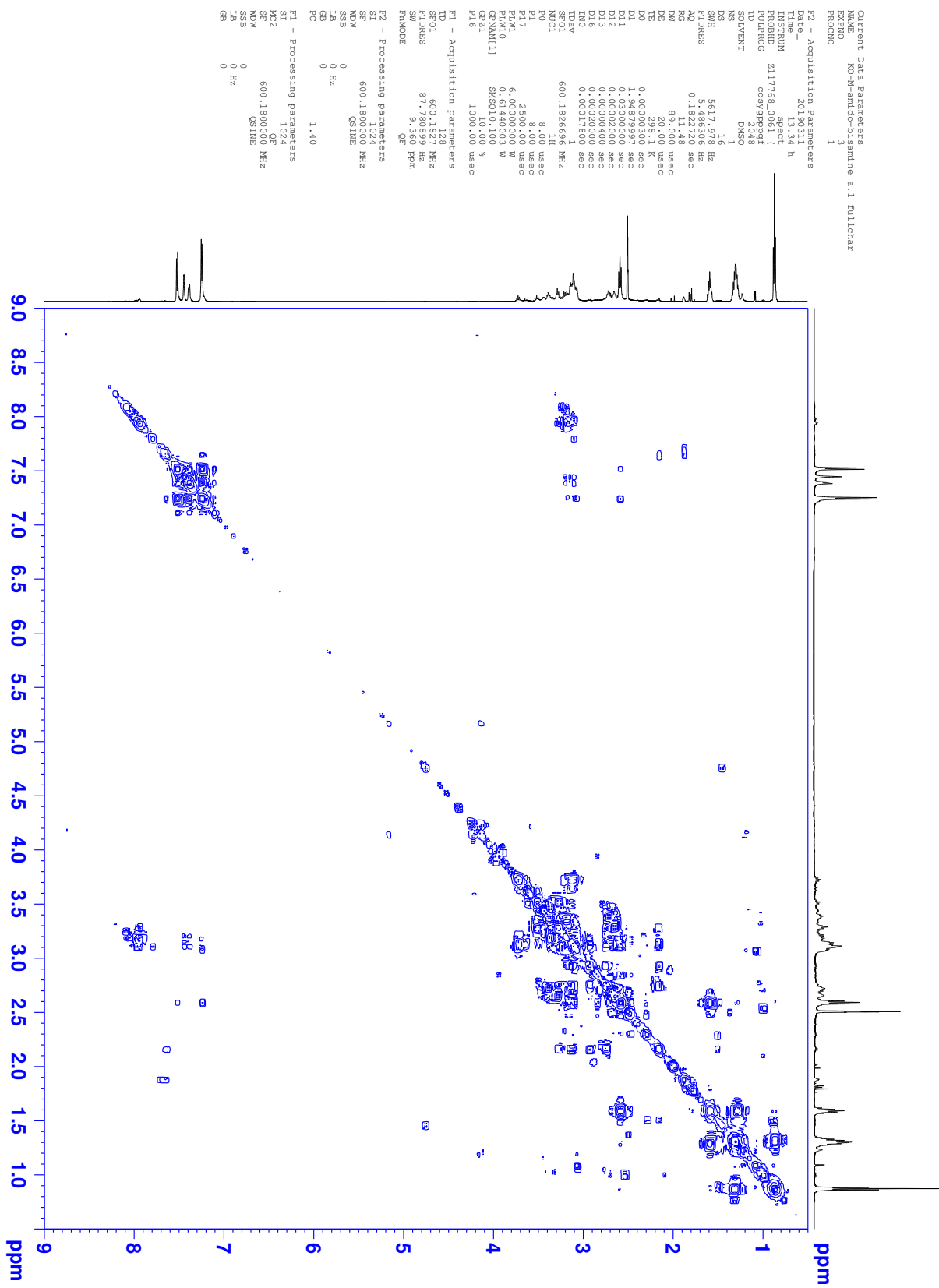
W.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for BP3a.1



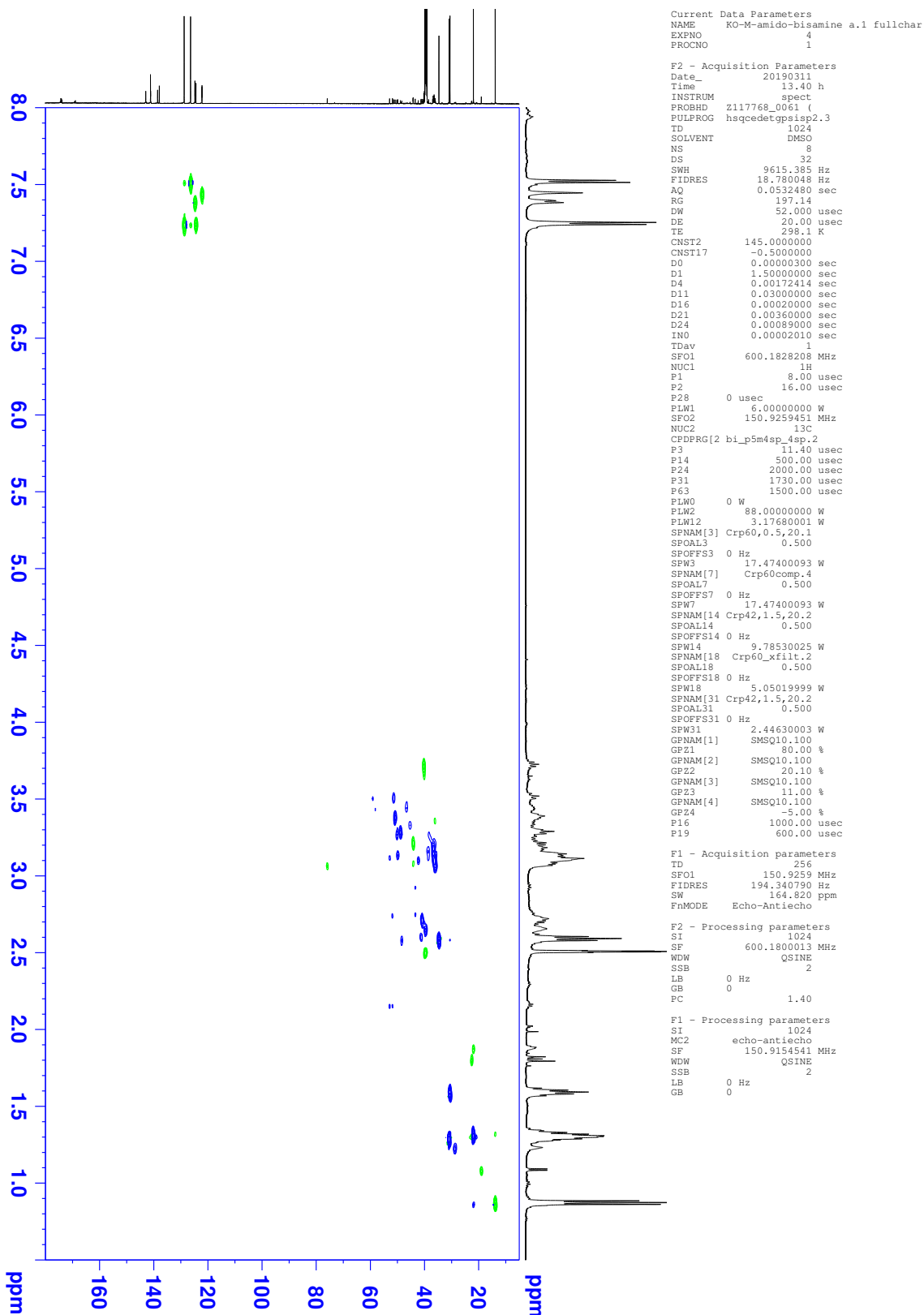
Y.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for the attempted hydrogenolysis reaction



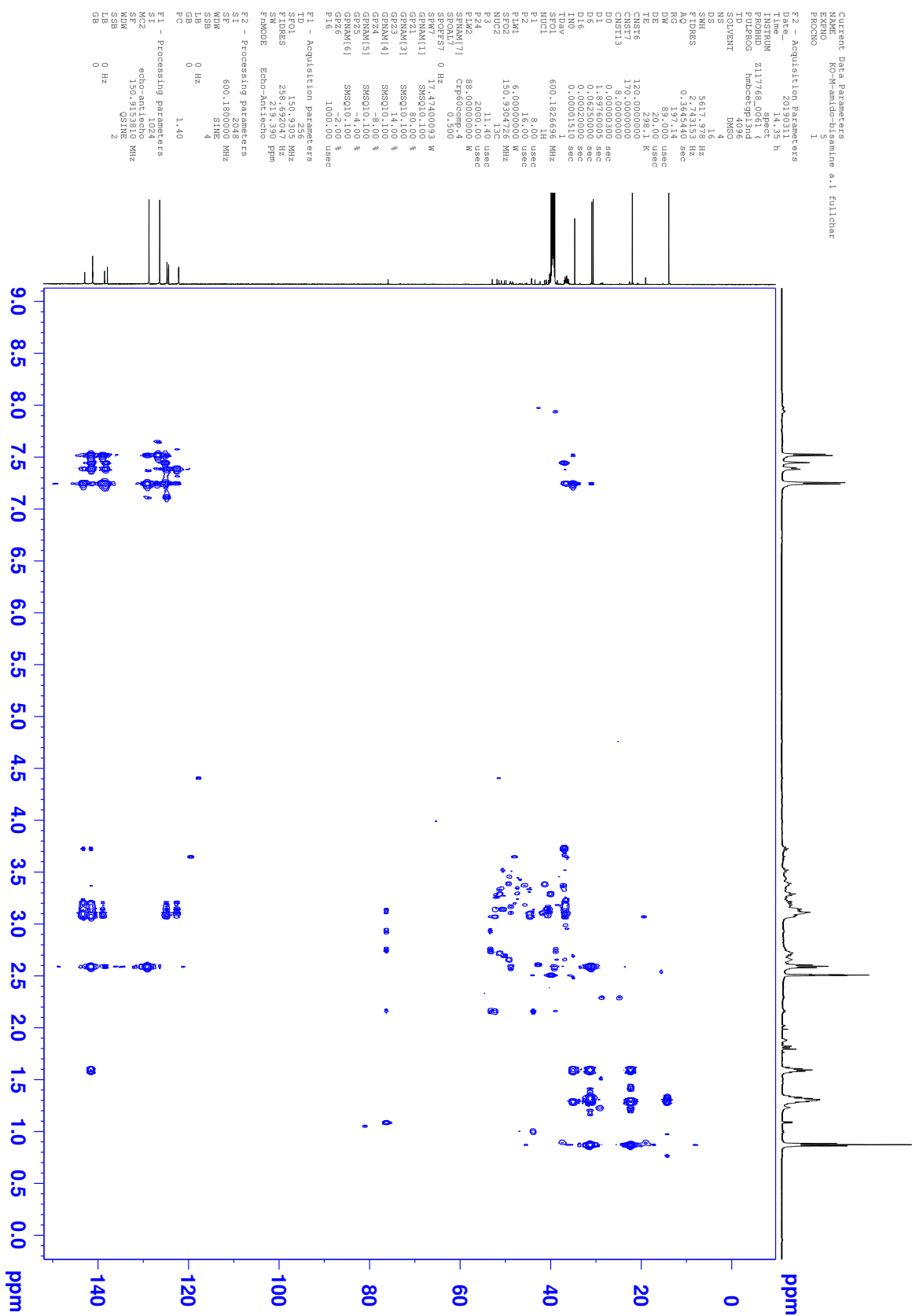
Y.3 COSY (600 MHz, DMSO-d₆) Spectrum for the attempted hydrogenolysis reaction



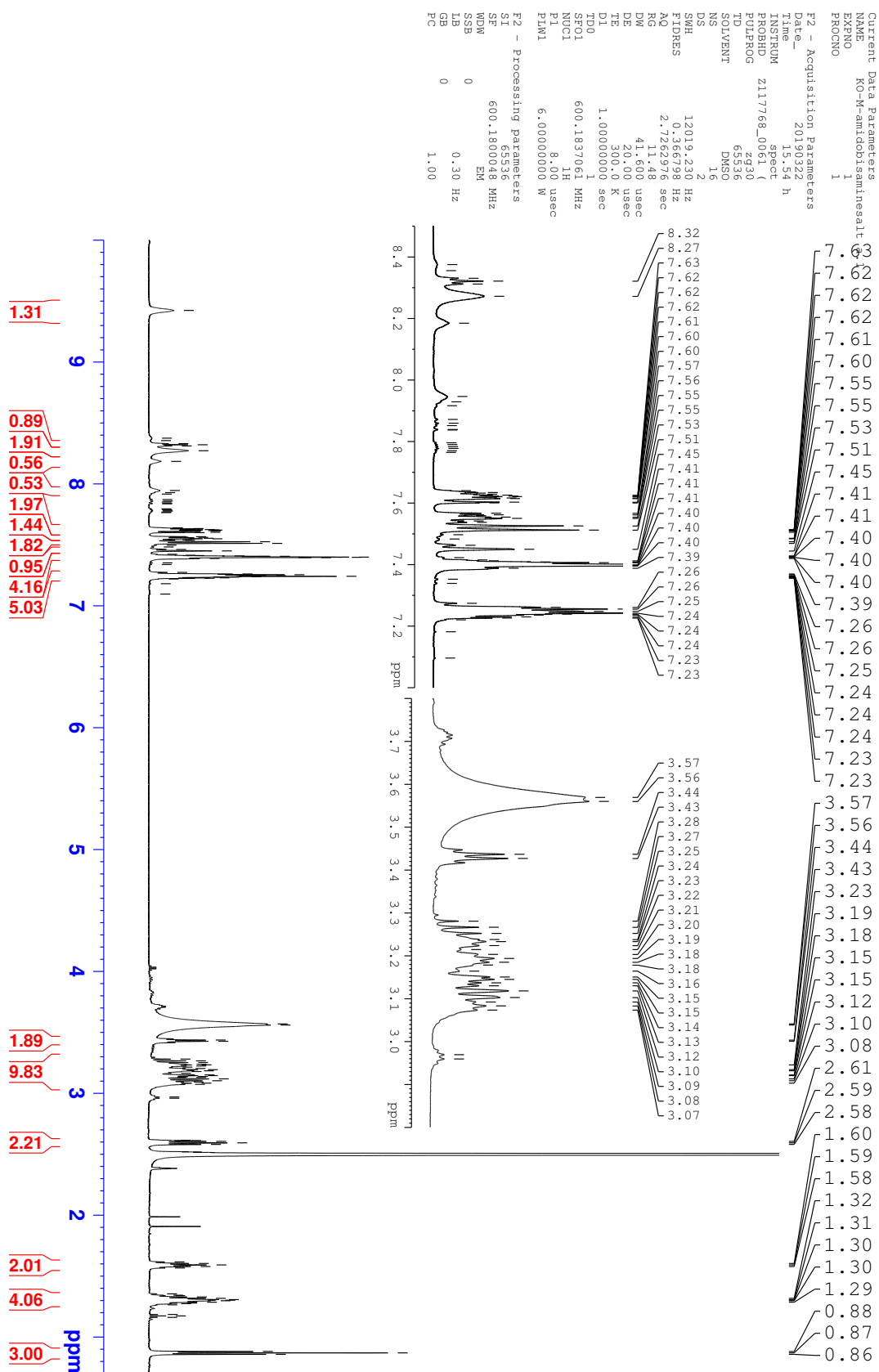
Y.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for the attempted hydrogenolysis reaction



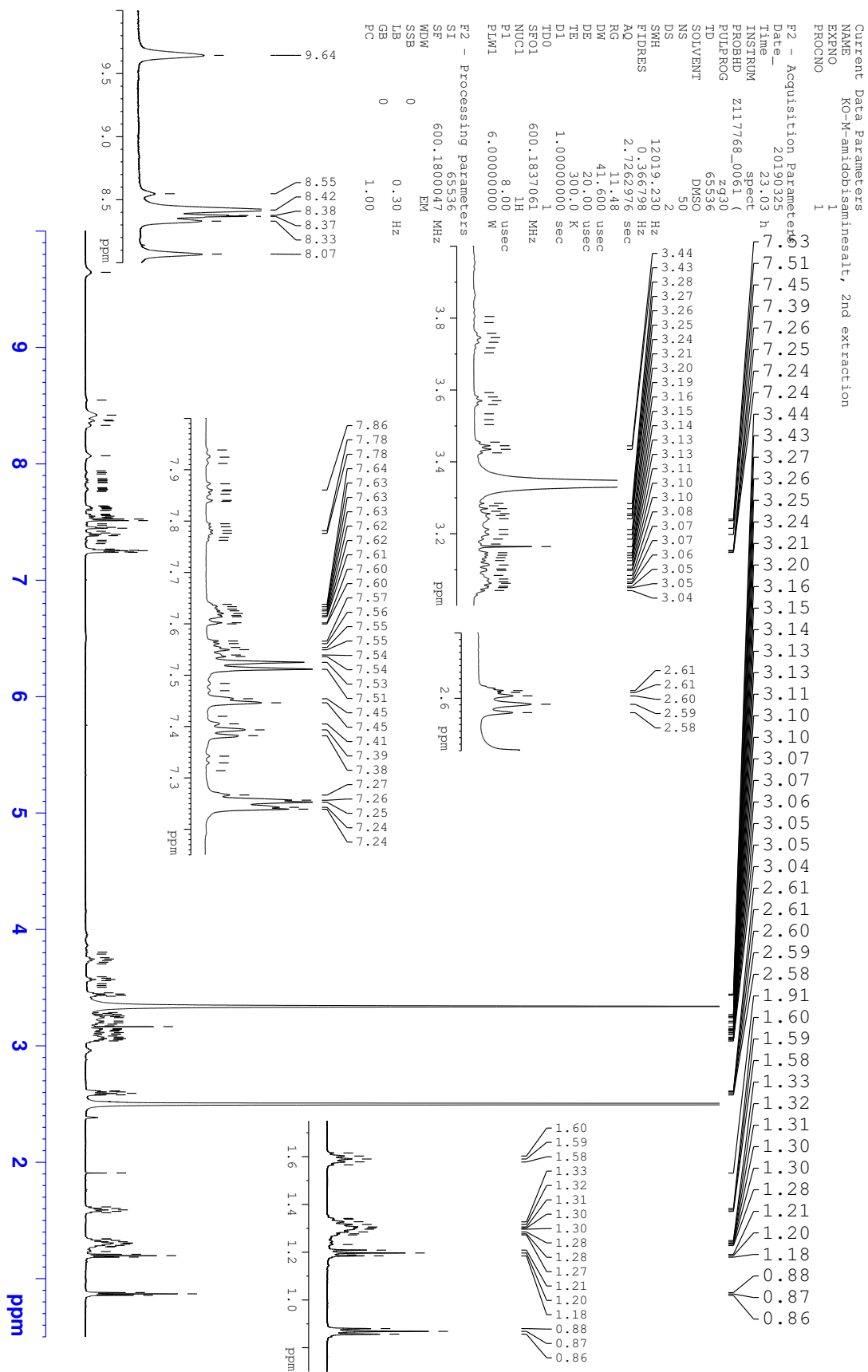
Y.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for the attempted hydrogenolysis reaction



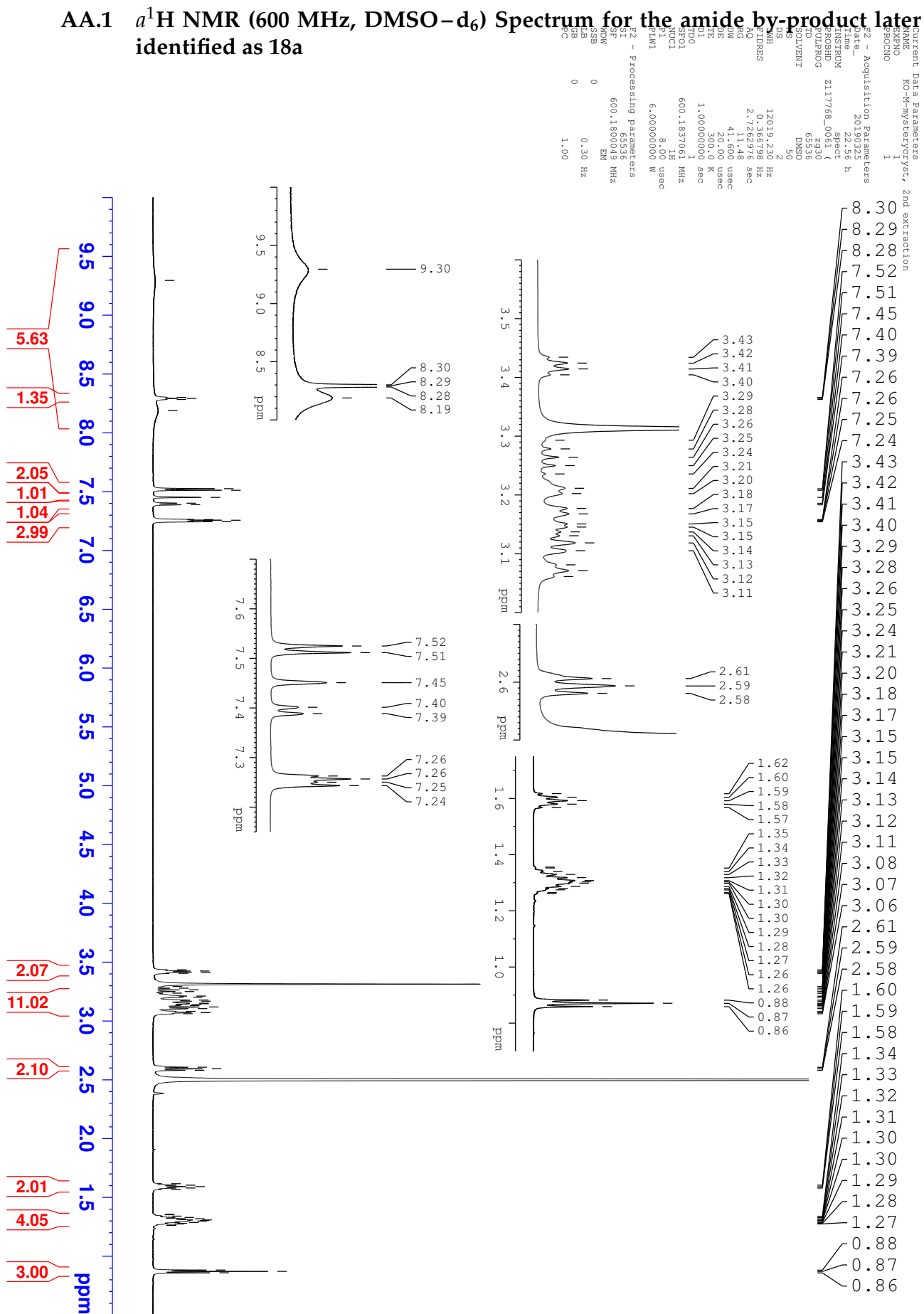
Z.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for the attempted reduction with PPh₃ after the 1st work-up



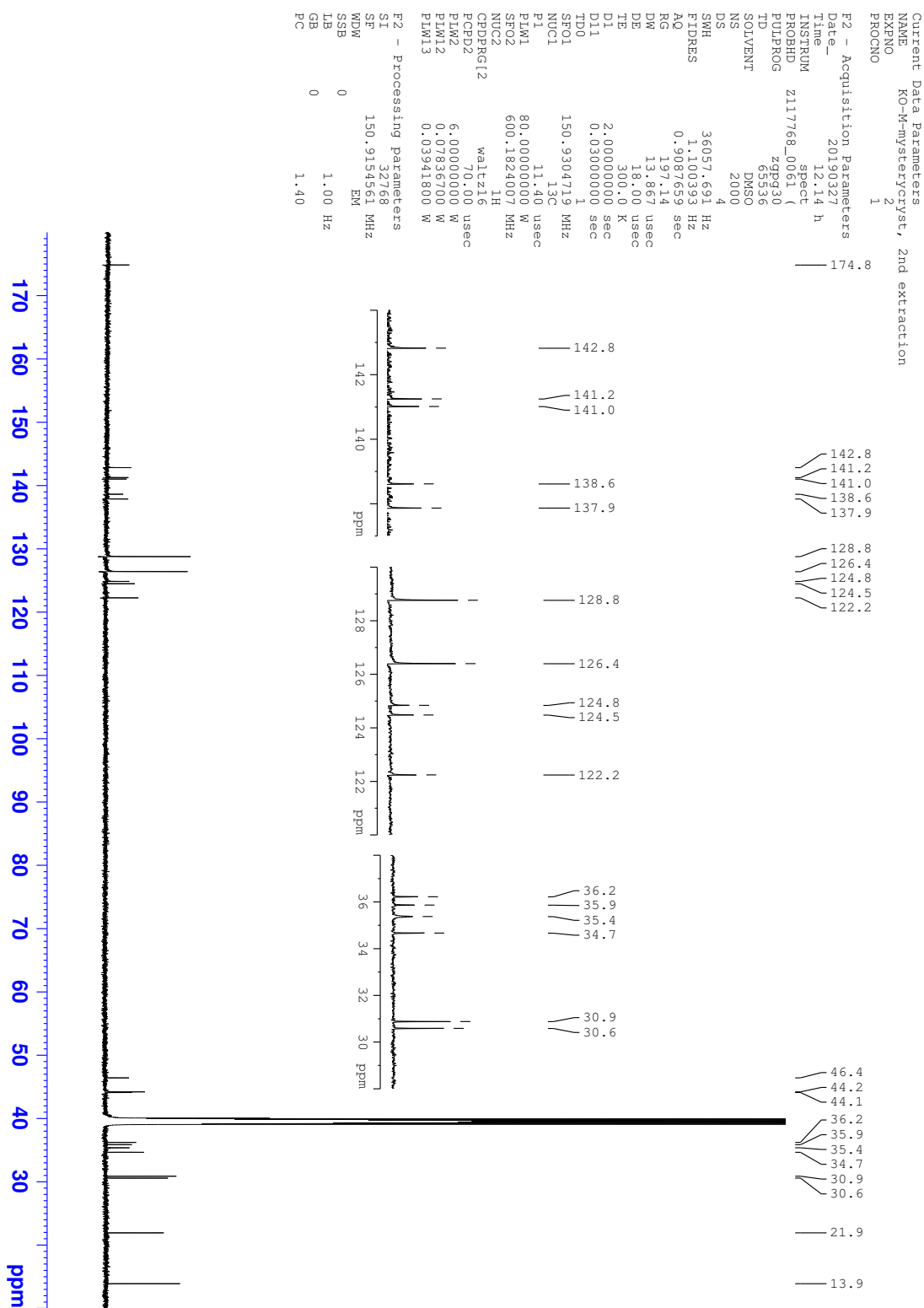
Z.2 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for the attempted reduction with PPh₃ after the 2nd work-up



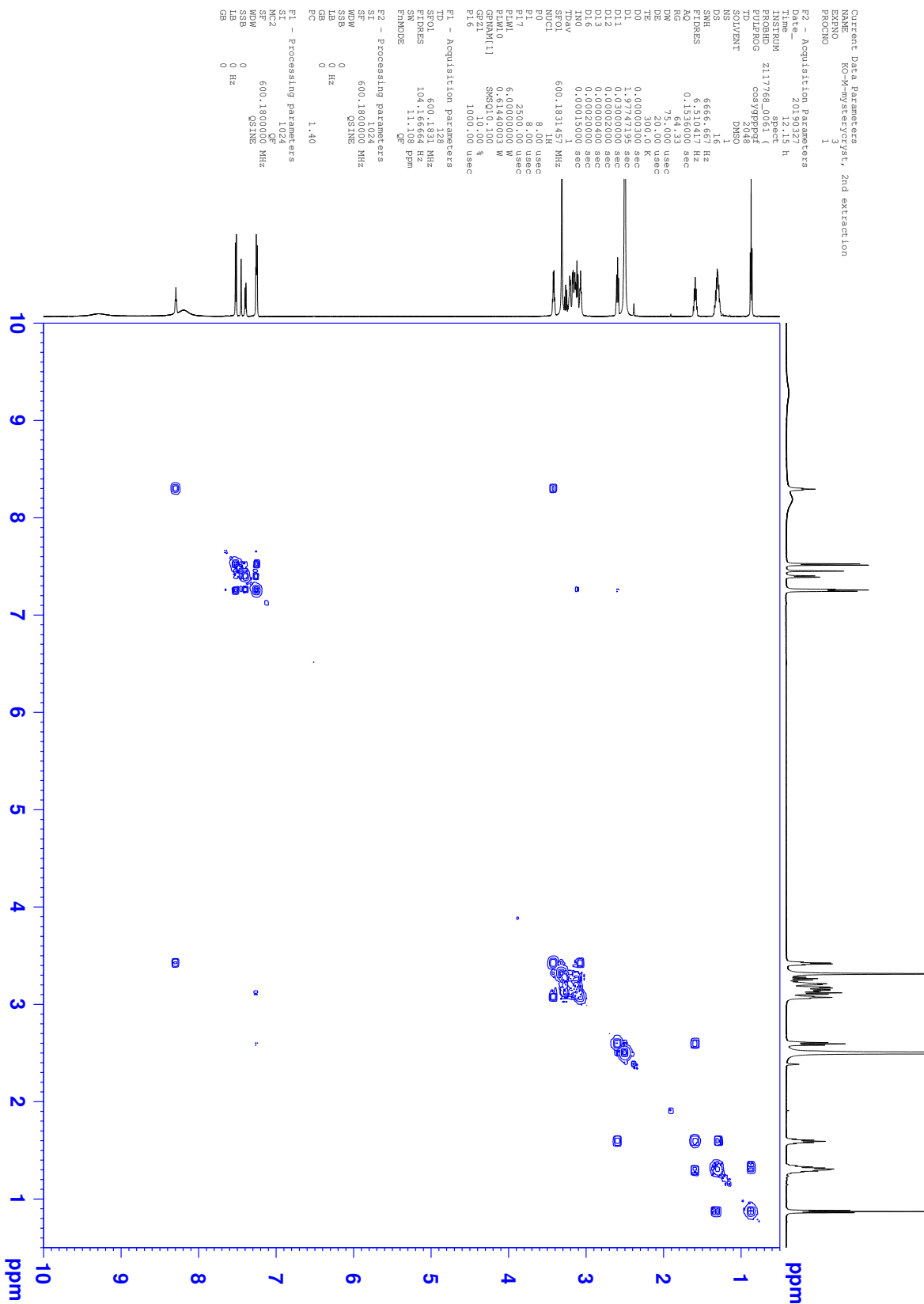
AA.1 $a^1\text{H}$ NMR (600 MHz, DMSO- d_6) Spectrum for the amide by-product later identified as 18a



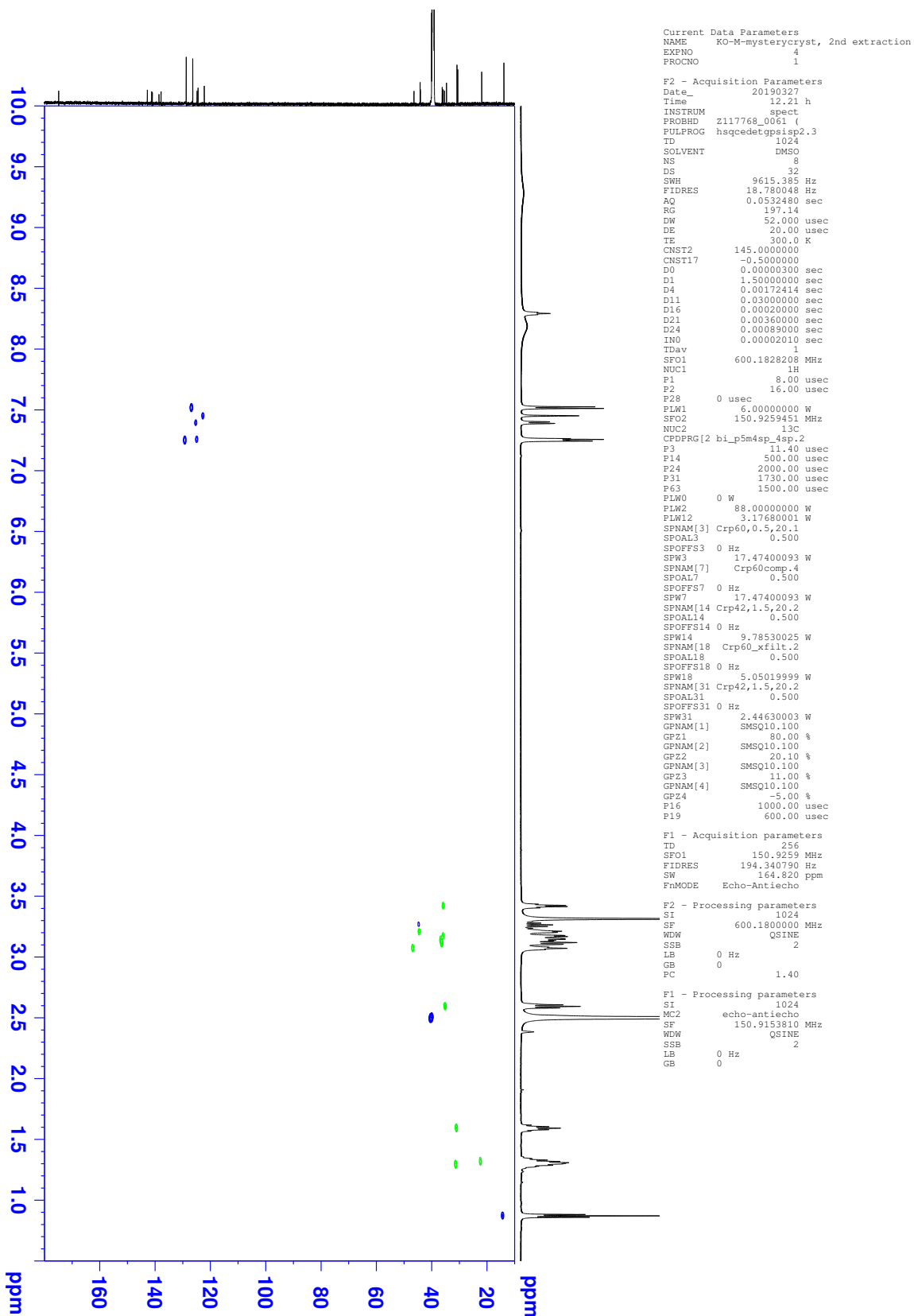
AA.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for the amide-byproduct later identified as 18a



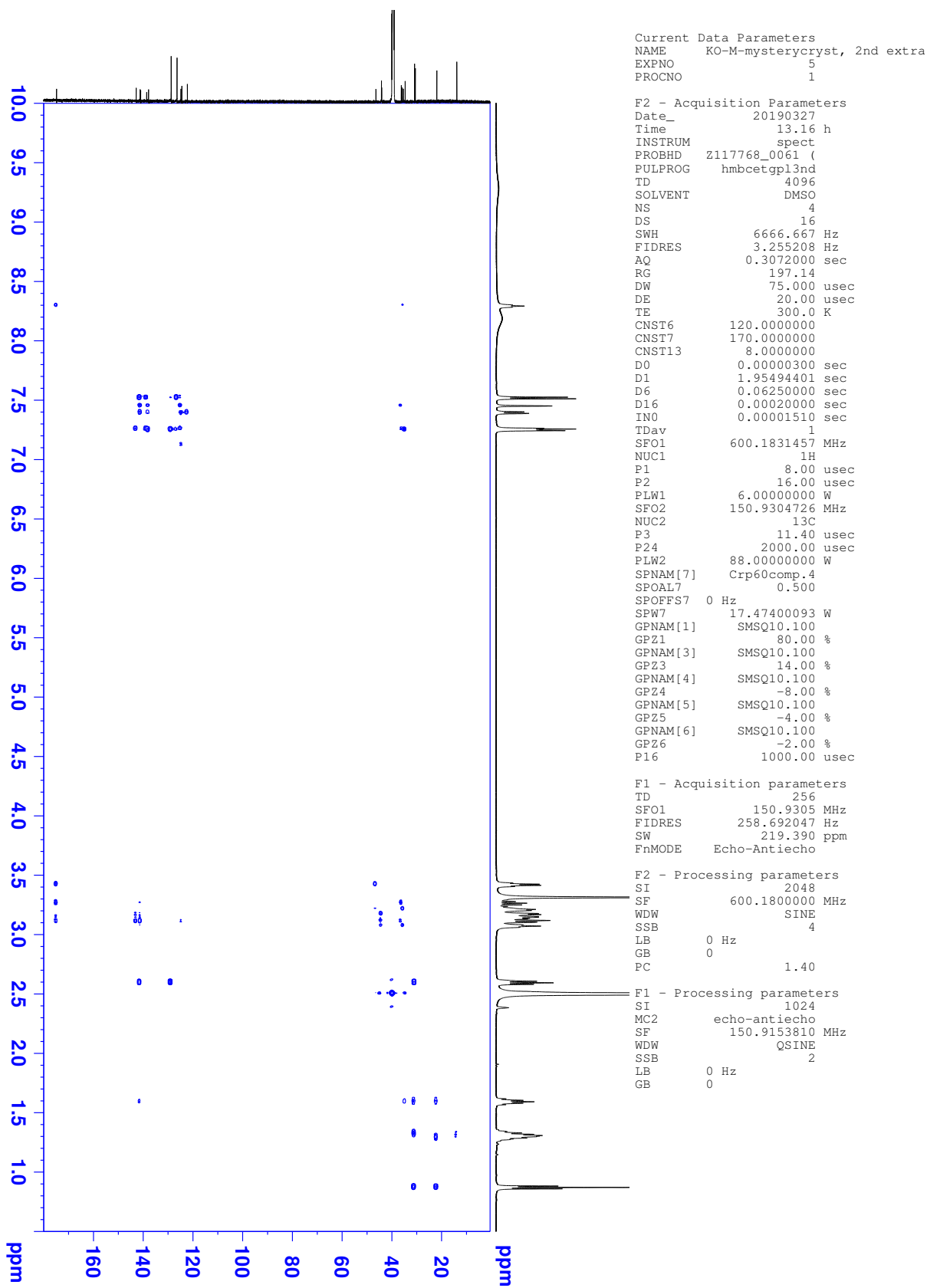
AA.3 COSY (600 MHz, DMSO-d₆) Spectrum for the amide-byproduct later identified as 18a



AA.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for the amide-byproduct later identified as 18a



AA.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for the amide-byproduct later identified as 18a



AA.6 HRMS Spectrum for the amide-byproduct later identified as 18a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

429 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

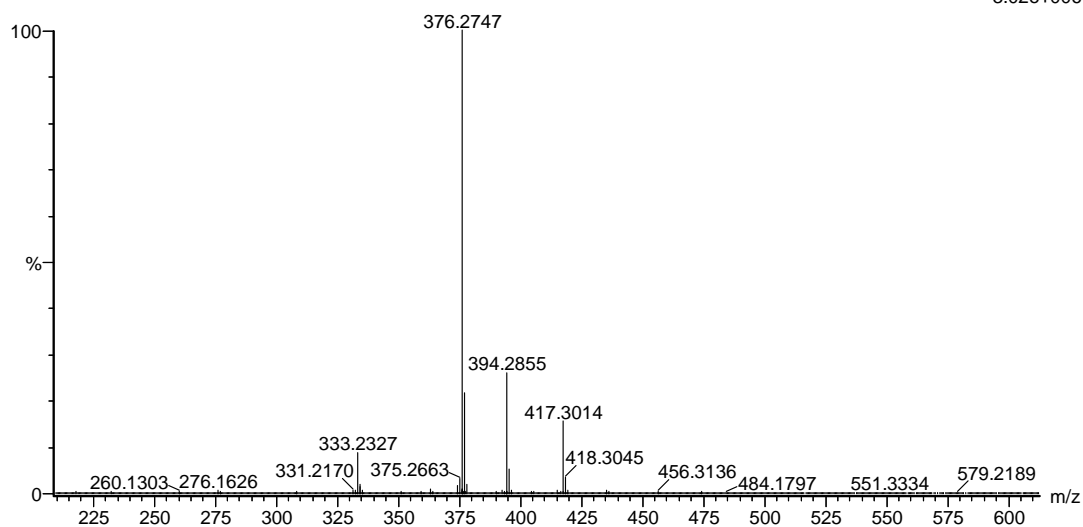
Elements Used:

C: 0-100 H: 0-150 N: 0-5 O: 0-5

2019-240 219 (4.275) AM2 (Ar,35000.0,0.00,0.00); Cm (213:220)

1: TOF MS ASAP+

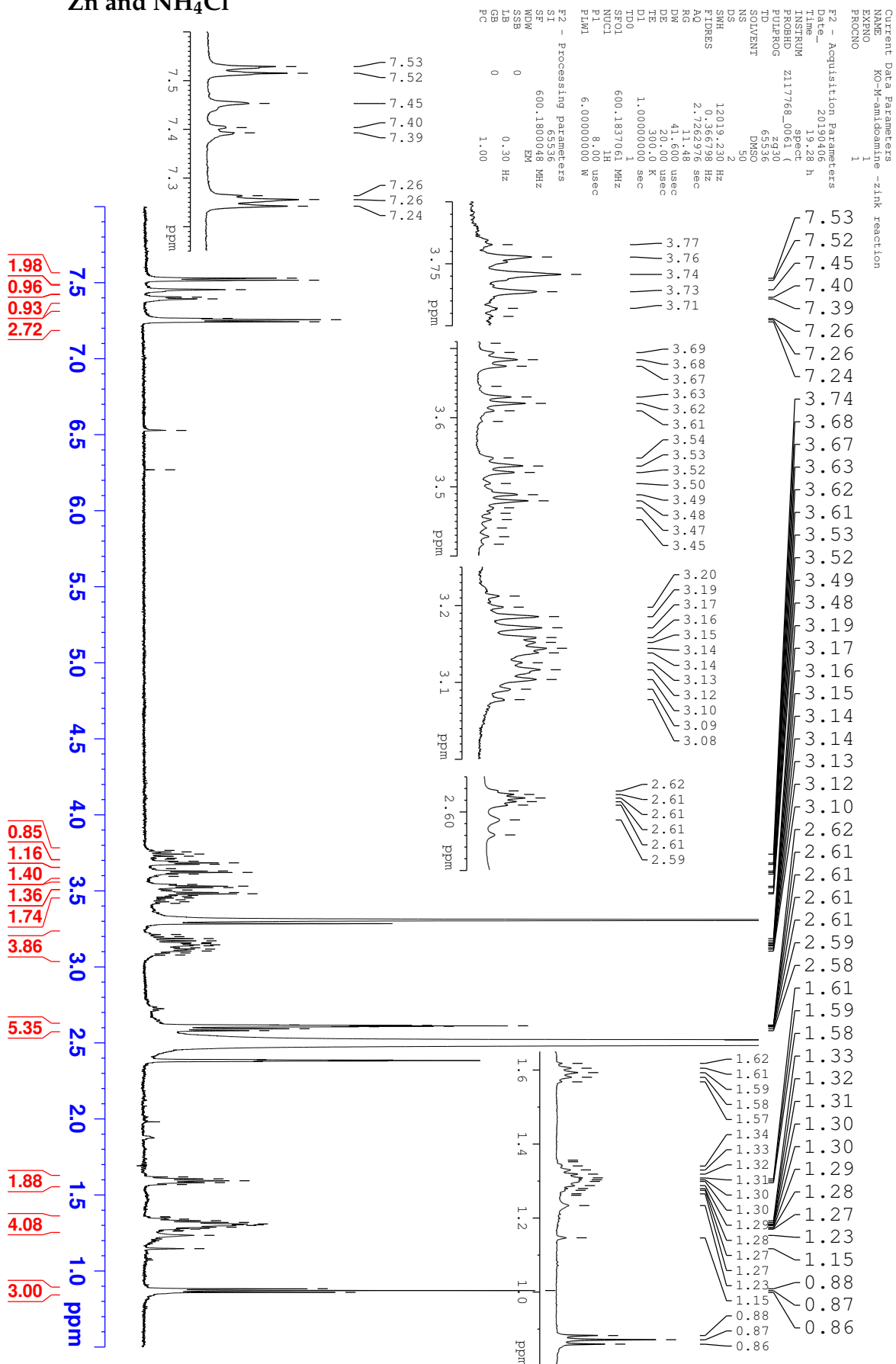
3.02e+006



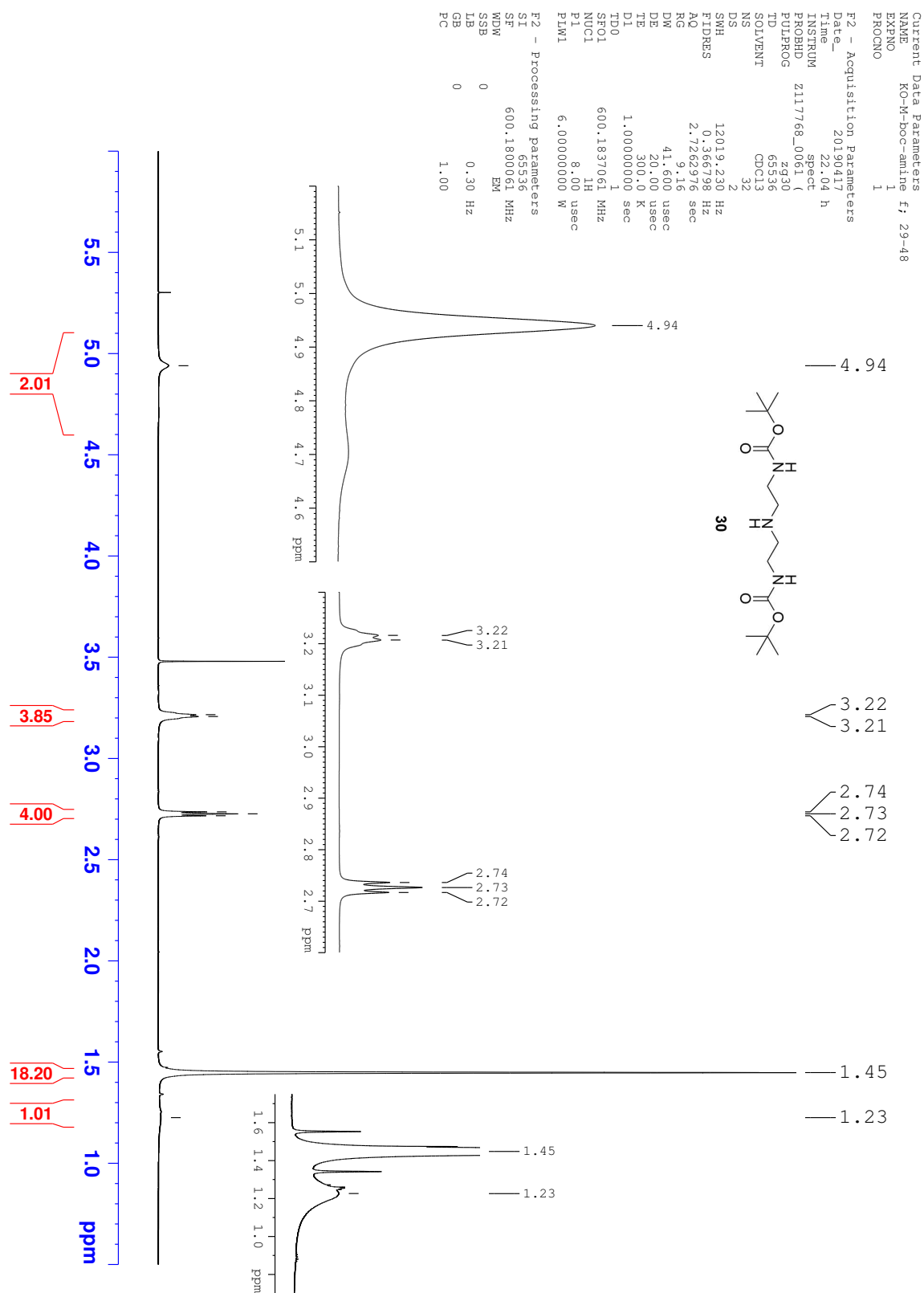
Minimum: -50.0
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
394.2855	394.2858	-0.3	-0.8	9.5	889.6	n/a	n/a	C25 H36 N3 O

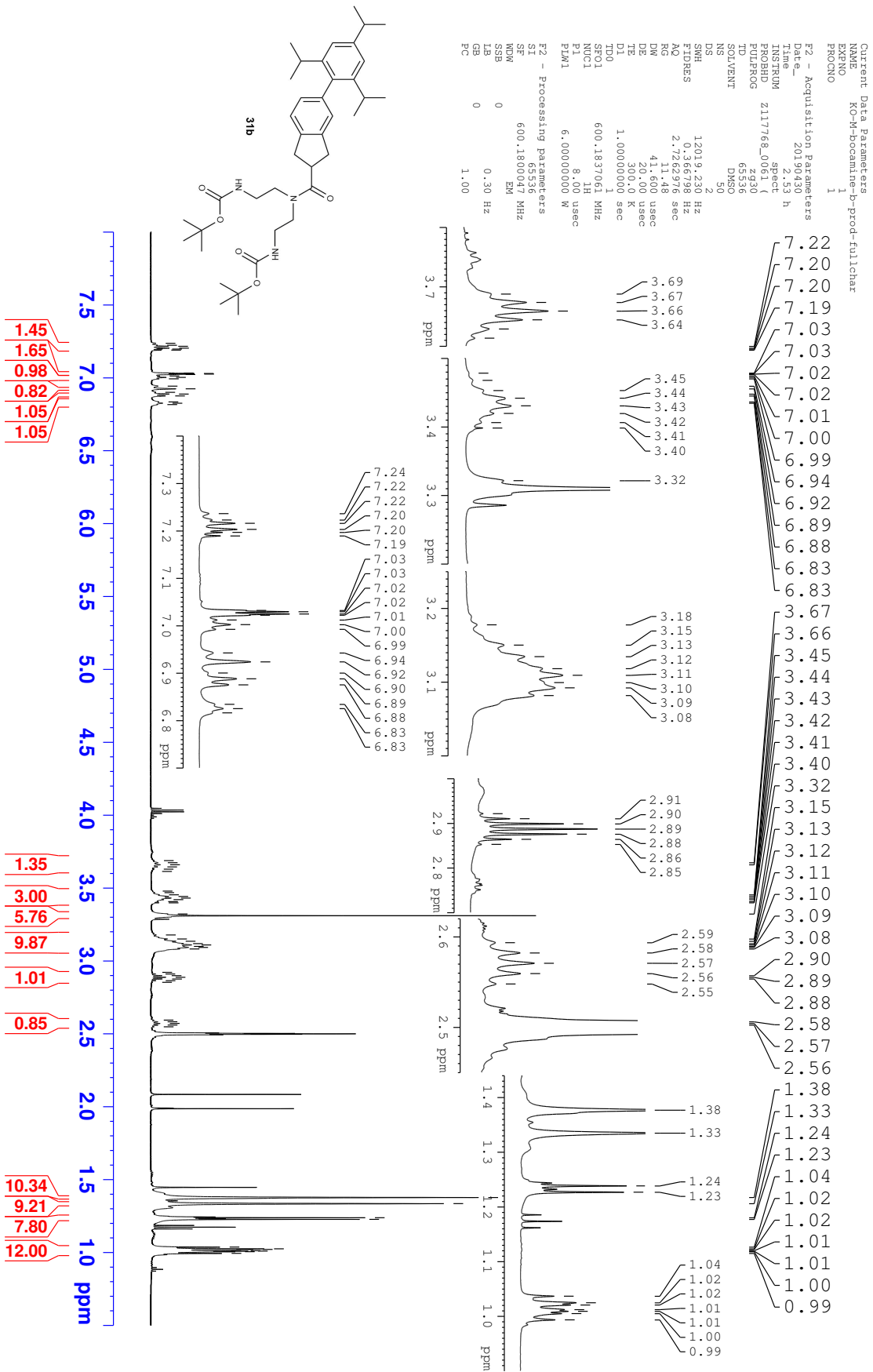
AB.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for the attempted reduction with Zn and NH₄Cl



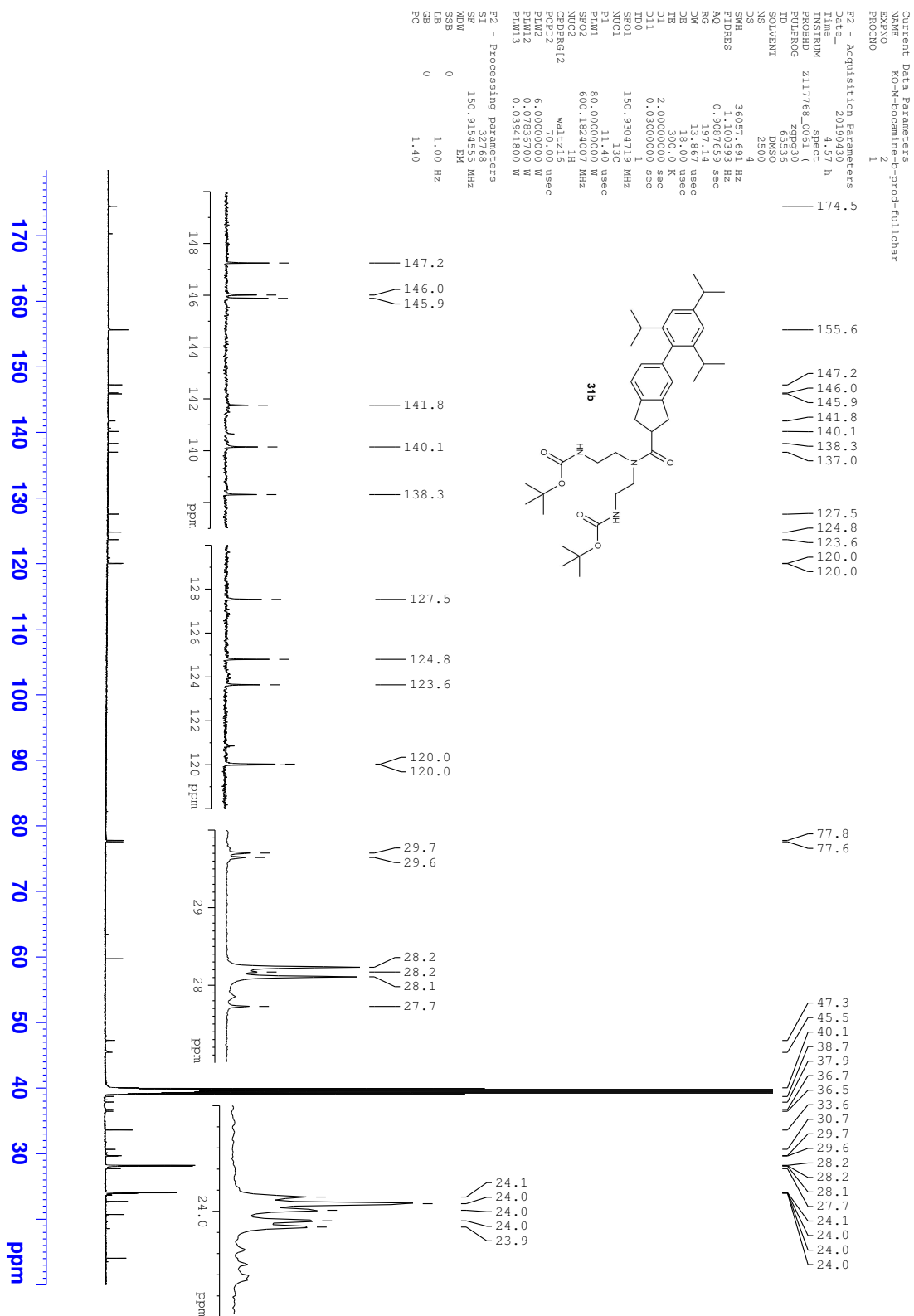
AC.1 ¹H NMR (400 MHz, CDCl₃) Spectrum for 30



AD.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 31b



AD.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 31b



AD.3 COSY (600 MHz, DMSO-d₆) Spectrum for 31b

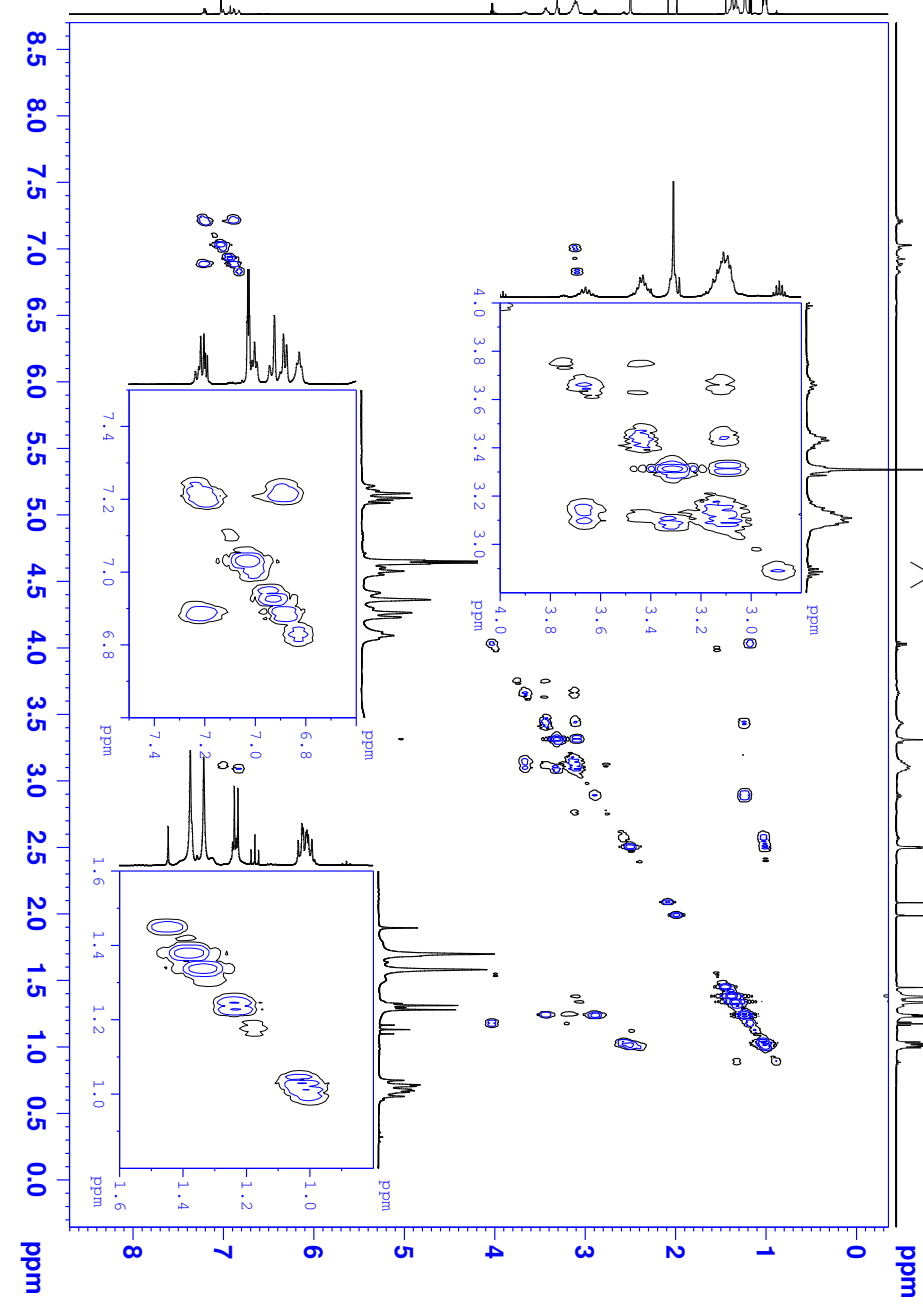
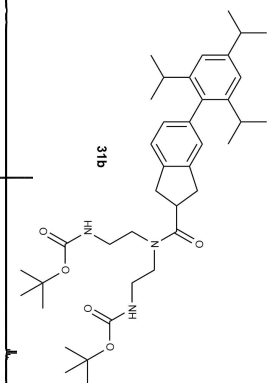
Current Data Parameters
 NAME KO-M-bocamine-b-prod-fullchar
 EXPNO 3
 F2ACQNO 1

F2 - Acquisition Parameters
 Date_ 20190430
 Time 4:58 h
 INSTRUM spect
 PROBHD Z117768-0061 (cosy)
 PULPROG zgpg30
 SOLVENT DMSO
 NS 16
 DS 1
 SWH 5319.149 Hz
 FIDRES 5.194481 Hz
 AQ 0.1925120 sec
 RG 924.53
 TE 300.0 K
 DE 20.00 usec
 TE 300.0 K
 DO 0.00000300 sec
 D1 1.93856001 sec
 D11 0.03000000 sec
 D12 0.00020000 sec
 D13 0.00020000 sec
 D14 0.00020000 sec
 INO 0.00018800 sec
 TDAV 1
 SFO1 600.1823862 MHz
 NUC1 1H
 P0 8.00 usec
 F1 8.00 usec
 F17 250.00 usec
 PLMI 6.00000000 W
 PLMI0 0.63440003 W
 GPNM1 [1] SMSQ10.100
 GPZ1 10.00 %
 P16 1000.00 usec

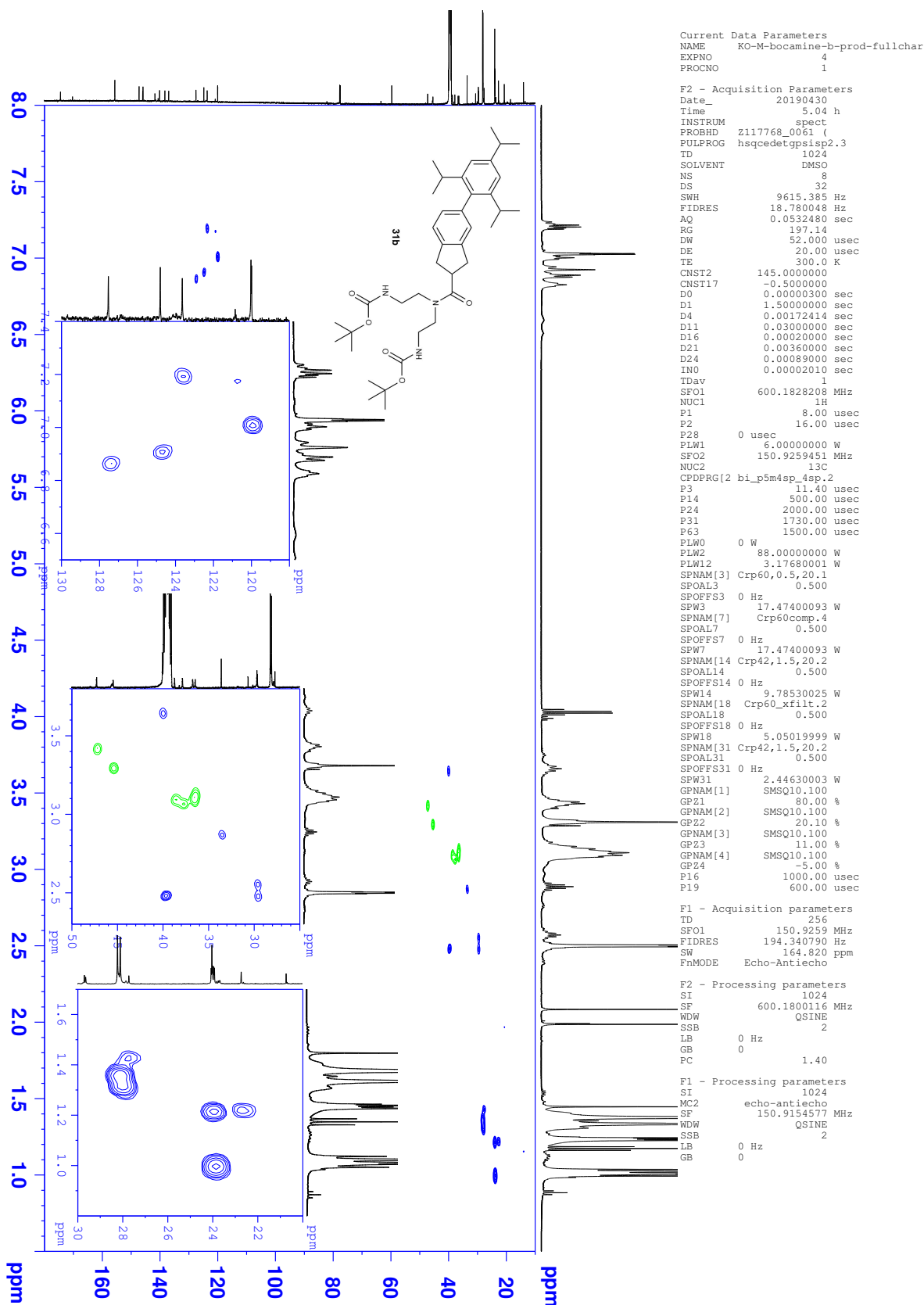
F1 - Acquisition Parameters
 TD 498
 SFO1 600.1824 MHz
 FIDRES 83.111702 Hz
 SW 8.863 ppm
 FMODE QF

F2 - Processing parameters
 SF 600.1800000 MHz
 WDW OSINE
 SSB 0 Hz
 LB 0 Hz
 GB 0
 PC 1.40

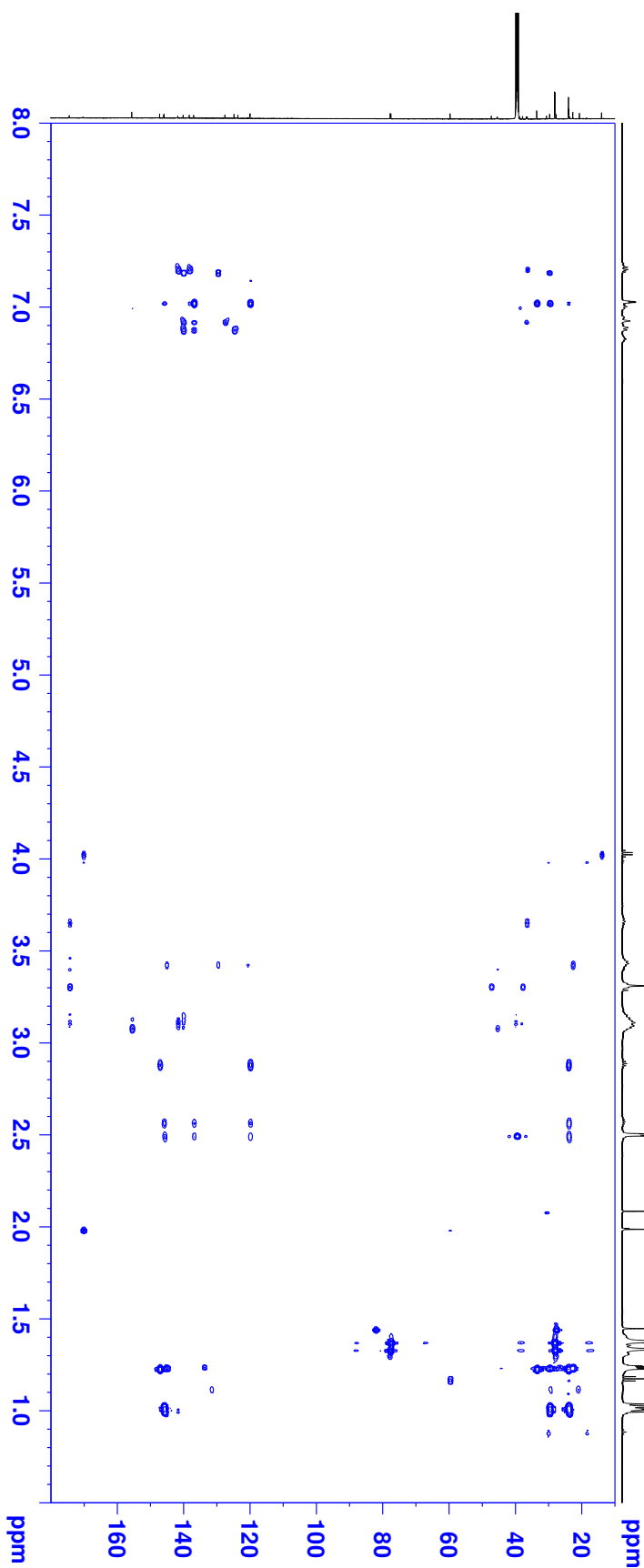
F1 - Processing parameters
 SI 1024
 MC2 QF
 SF 600.1800000 MHz
 WDW OSINE
 SSB 0 Hz
 LB 0
 GB 0



AD.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 31b



AD.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 31b



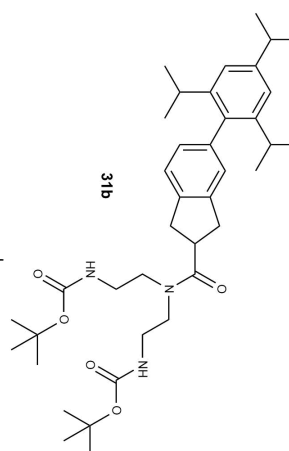
Current Data Parameters
 NAME KO-M-bocamine-b-prod-fullchar
 EXPNO 5
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20190430
 Time 5.59 h
 INSTRUM spect
 PROBHD Z117768_0061 (
 PULPROG hmbcetgp13nd
 TD 4096
 SOLVENT DMSO
 NS 4
 DS 16
 SWH 5319.149 Hz
 FIDRES 2.597241 Hz
 AQ 0.3850240 sec
 RG 197.14
 DW 94.000 usec
 DE 20.00 usec
 TE 300.0 K
 CNST6 120.0000000
 CNST7 170.0000000
 CNST13 8.0000000
 D0 0.00000300 sec
 D1 1.87712002 sec
 D6 0.06250000 sec
 D16 0.00020000 sec
 IN0 0.00001510 sec
 TdAv 1
 SF01 600.1823862 MHz
 NUC1 1H
 P1 8.00 usec
 P2 16.00 usec
 PLW1 6.00000000 W
 SF02 150.9304726 MHz
 NUC2 13C
 P3 11.40 usec
 P24 2000.00 usec
 PLW2 88.00000000 W
 SPNAM[7] Crp60comp.4
 SPOAL7 0.500
 SPOFFS7 0 Hz
 SPW7 17.47400093 W
 GPAM[1] SMSQ10.100
 GPZ1 80.00 %
 GPAM[3] SMSQ10.100
 GPZ3 14.00 %
 GPAM[4] SMSQ10.100
 GPZ4 -8.00 %
 GPAM[5] SMSQ10.100
 GPZ5 -4.00 %
 GPAM[6] SMSQ10.100
 GPZ6 -2.00 %
 P16 1000.00 usec

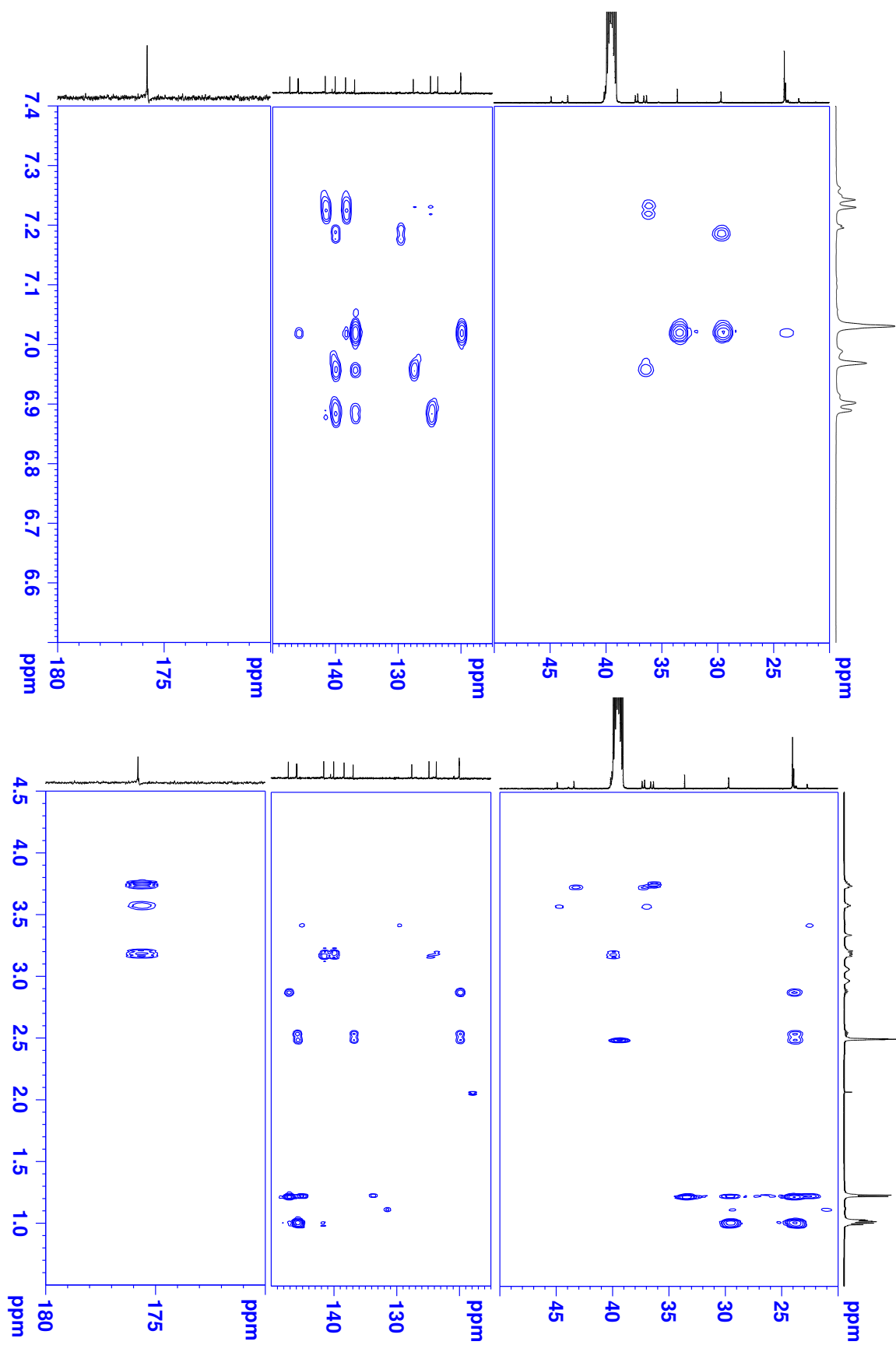
F1 - Acquisition parameters
 TD 256
 SF01 150.9305 MHz
 FIDRES 258.692047 Hz
 SW 219.390 ppm
 FnmODE Echo-Antiecho

F2 - Processing parameters
 SI 2048
 SF 600.1800084 MHz
 WDW SINE
 SSB 4
 LB 0 Hz
 GB 0
 PC 1.40

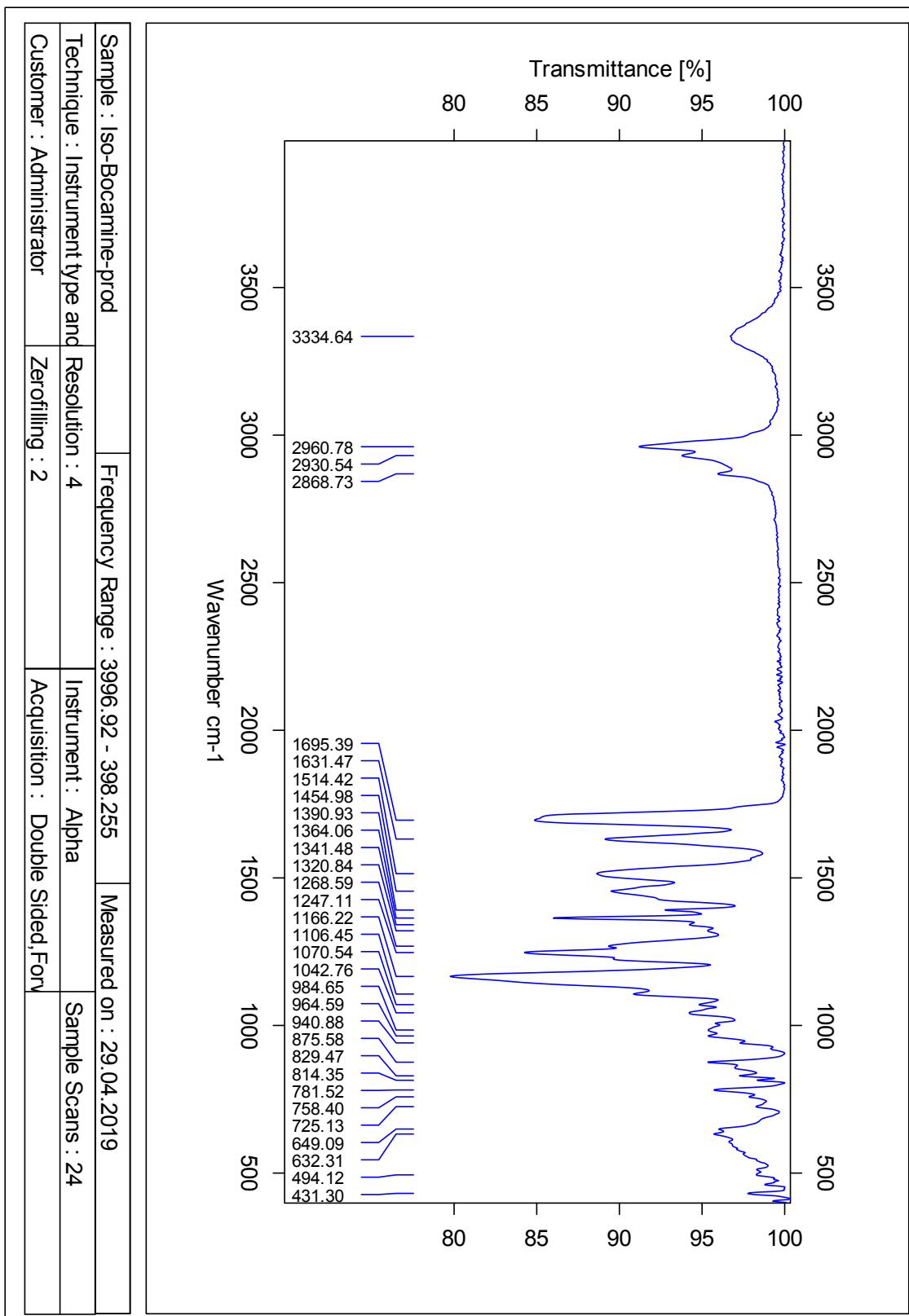
F1 - Processing parameters
 SI 1024
 MC2 echo-antiecho
 SF 150.9154674 MHz
 WDW QSINE
 SSB 2
 LB 0 Hz
 GB 0



AD.6 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 31b



AD.7 IR Spectrum for 31b



AD.8 HRMS Report for 27a

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -50.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

2064 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

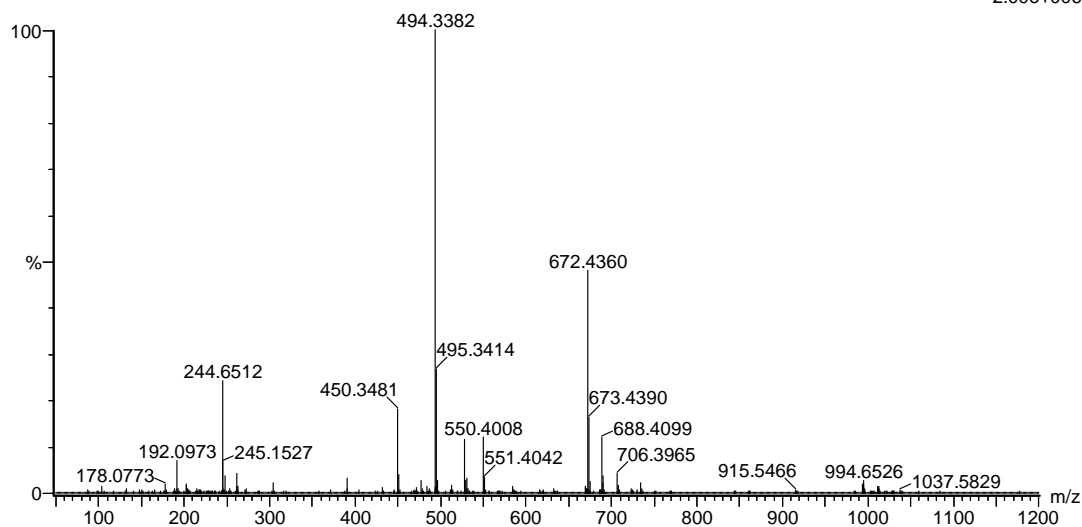
Elements Used:

C: 0-100 H: 0-150 N: 0-8 O: 0-8 Na: 0-1

2019-374 33 (0.619) AM2 (Ar,35000.0,0.00,0.00); Cm (30:37)

1: TOF MS ES+

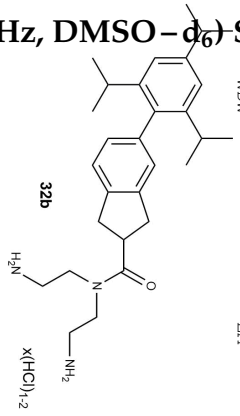
2.00e+006



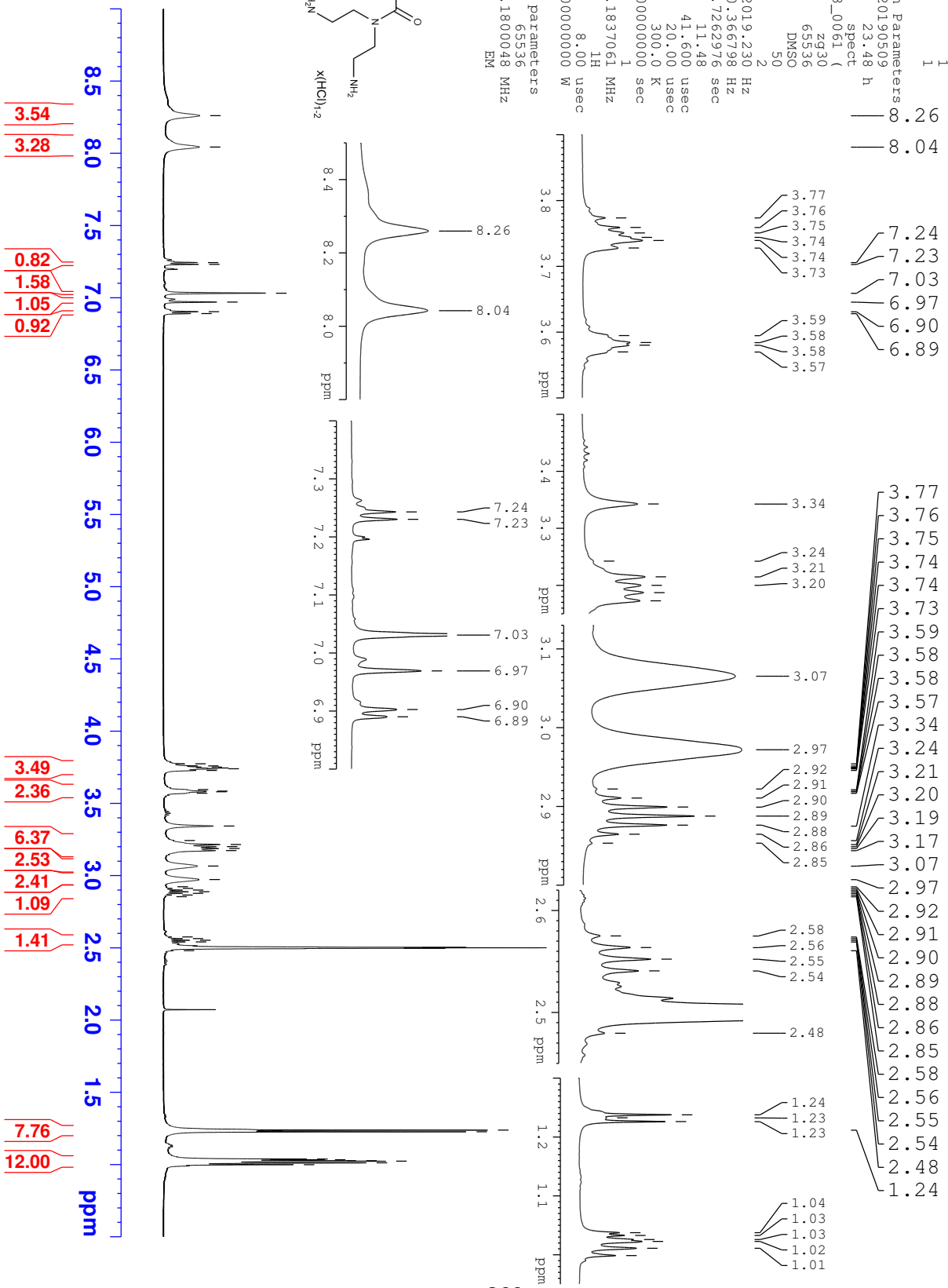
Minimum: -50.0
Maximum: 5.0 2.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
672.4360	672.4366	-0.6	-0.9	16.5	750.9	0.910	40.25	C40 H55 N7 O Na
	672.4352	0.8	1.2	11.5	750.5	0.515	59.75	C39 H59 N3 O5 Na

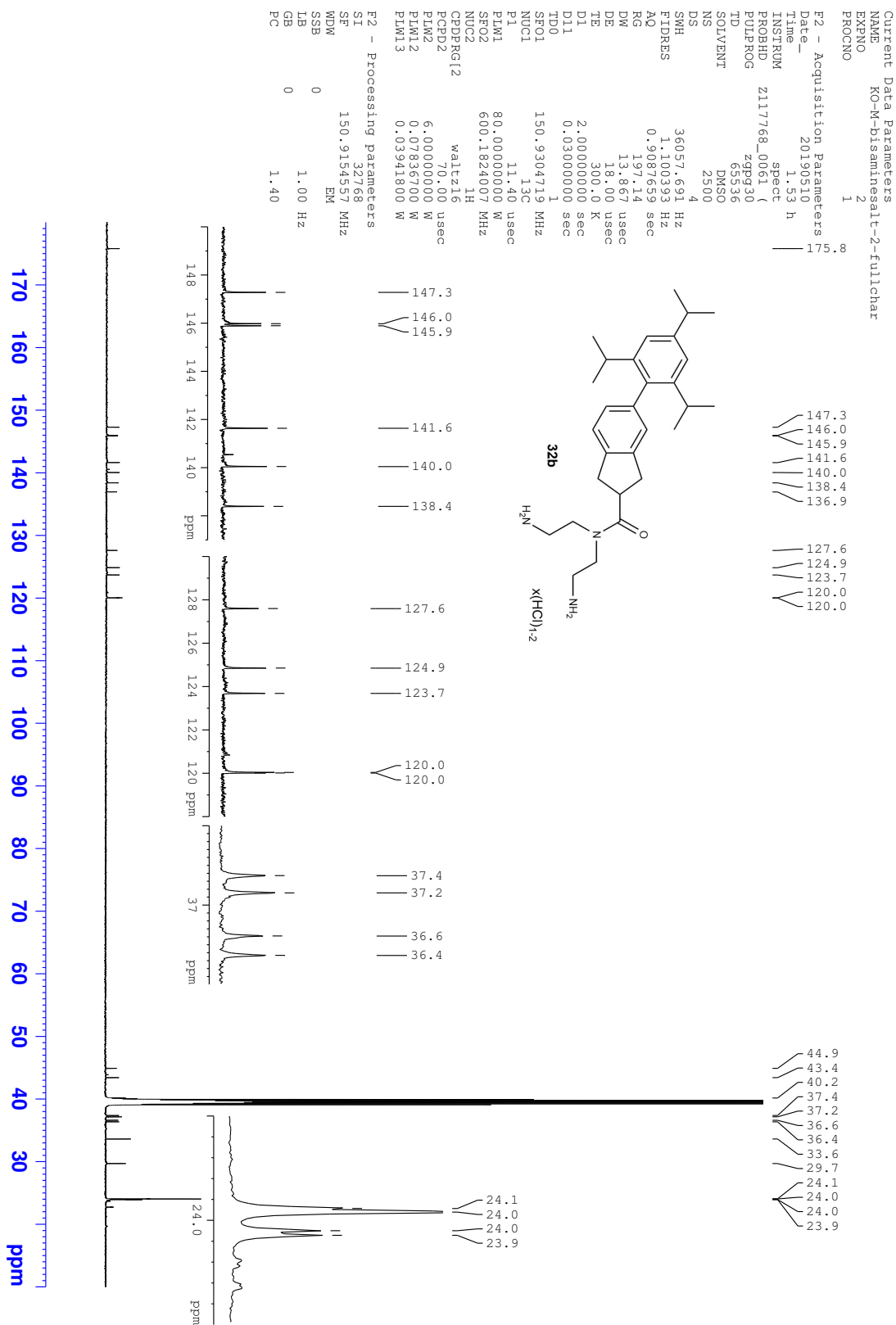
AE.1 ¹H NMR (600 MHz, DMSO-d₆) Spectrum for 32b



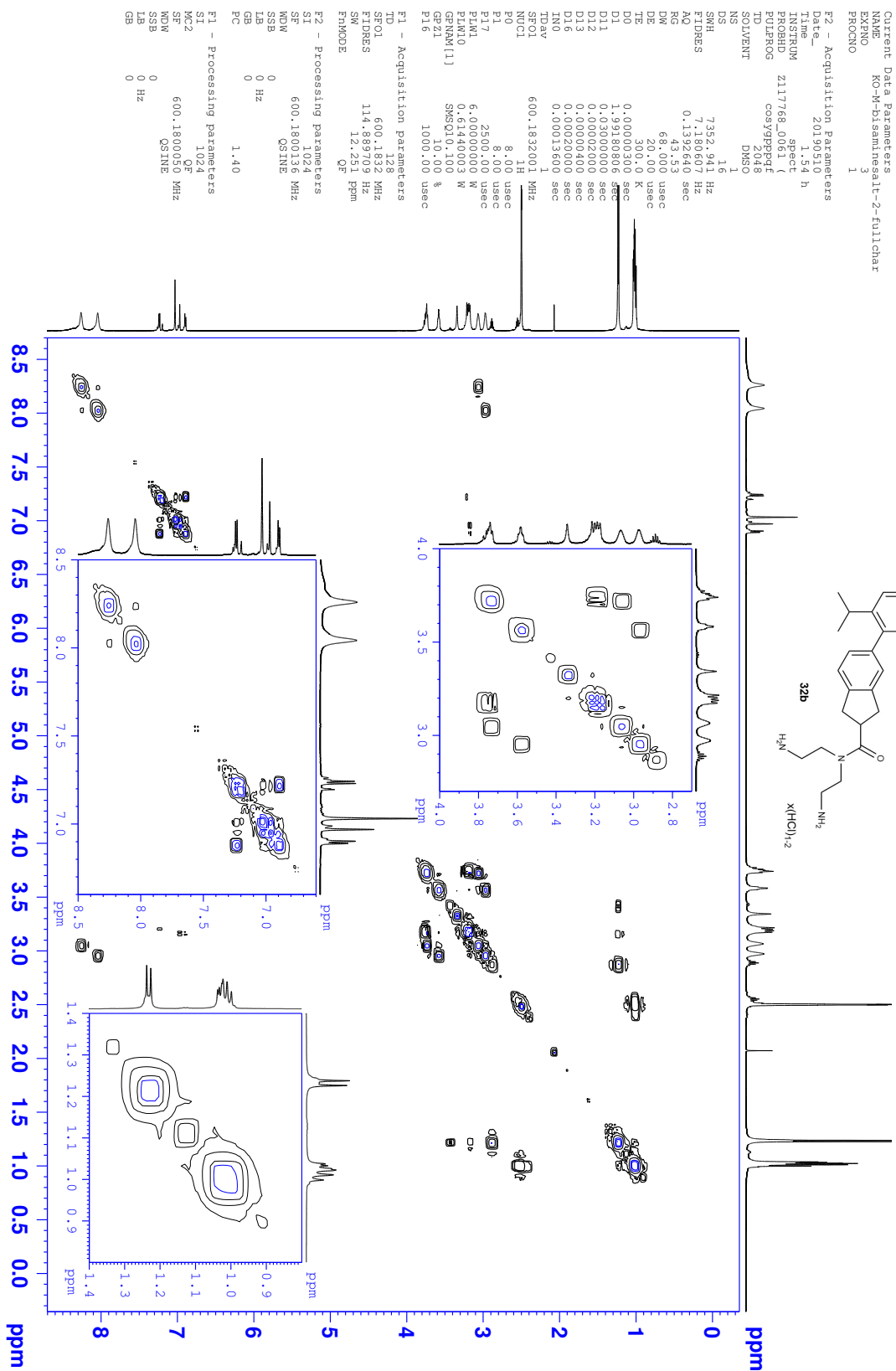
Current Data Parameters
 NAME KO-M-bisammonesalt-2-fullchar
 EXPNNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20190509
 Time 23.48 h
 INSTRUM spect
 PROBHD zg30
 PULPROG 65536
 TD 65536
 SOLVENT DMSO
 NS 50
 DS 2
 SWH 12019.230 Hz
 FIDRES 0.366798 Hz
 AQ 2.7262976 sec
 RG 11.48
 DW 41.600 usec
 DE 20.00 usec
 TE 300.0 K
 D1 1.00000000 sec
 SFO1 600.1837061 MHz
 NUC1 1H
 P1 8.00 usec
 PL1 6.00000000 W
 F2 - Processing parameters
 SI 65536
 SF 600.1800048 MHz
 WDM EM



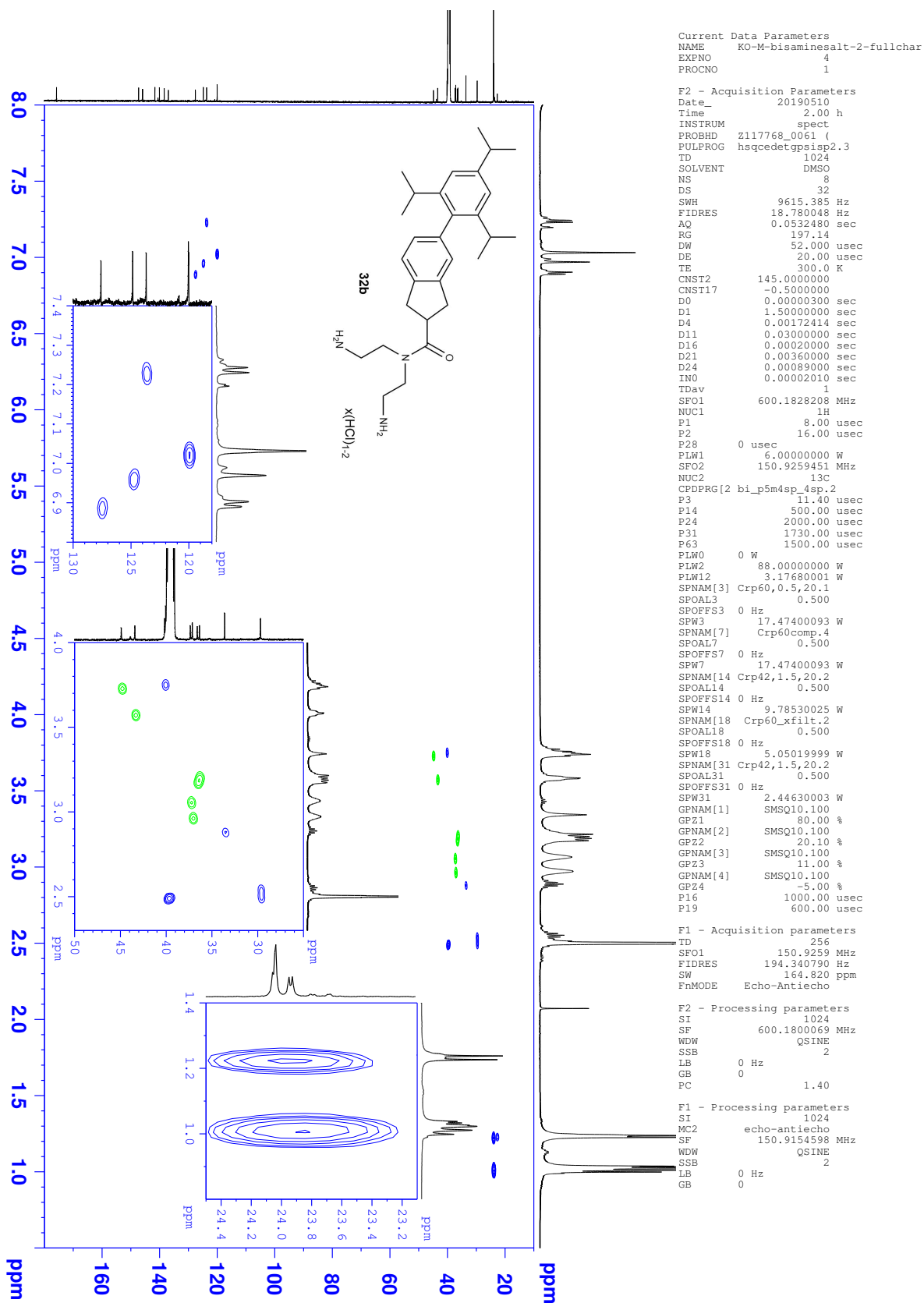
AE.2 ¹³C NMR (150 MHz, DMSO-d₆) Spectrum for 32b



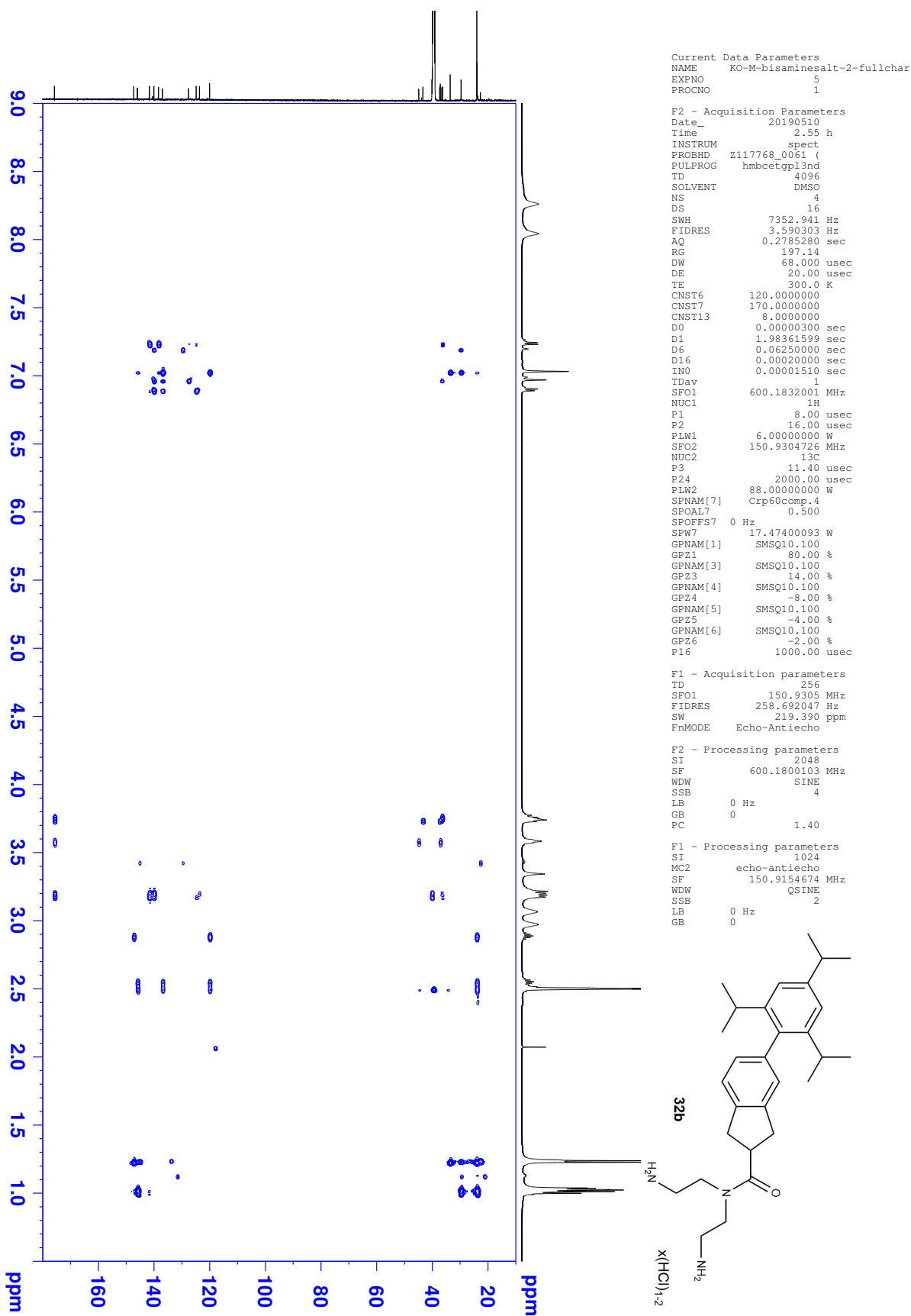
AE.3 COSY (600 MHz, DMSO-d₆) Spectrum for 32b



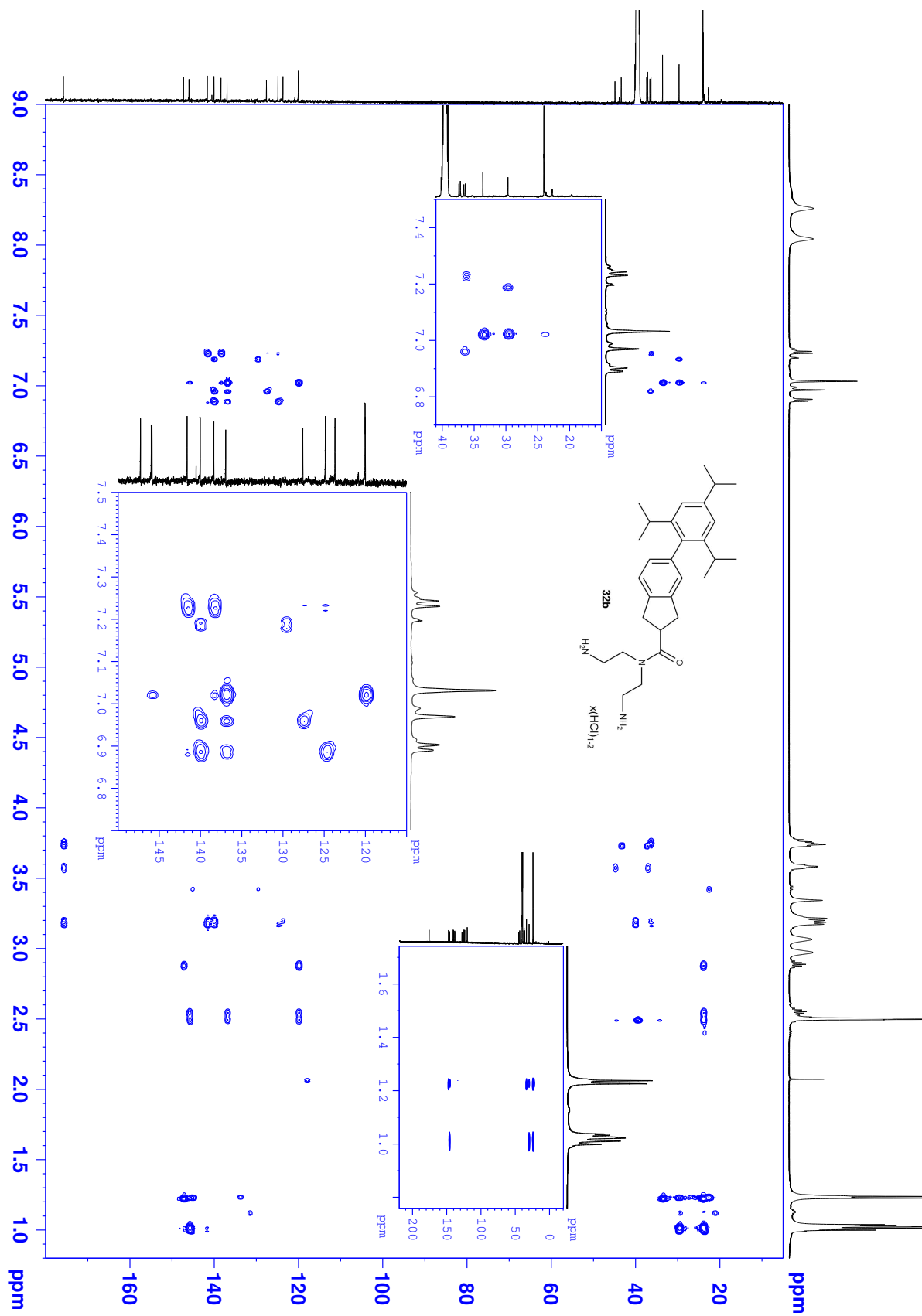
AE.4 HSQC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 32b



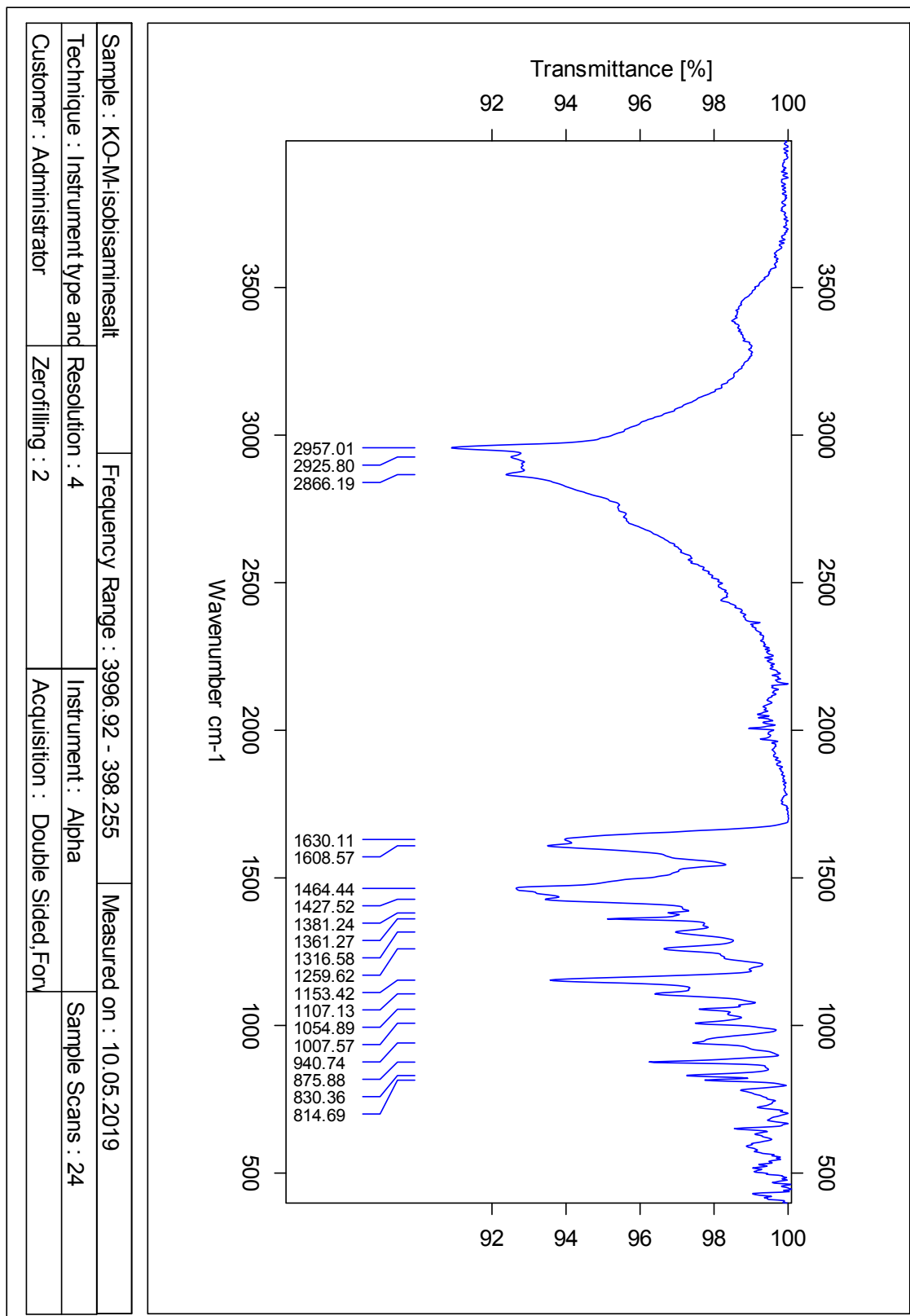
AE.5 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 32b



AE.6 HMBC (600 MHz / 150 MHz, DMSO-d₆) Spectrum for 32b



AE.7 IR Spectrum for 32b



AE.8 HRMS Report for 32b

Elemental Composition Report

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -2.0, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1768 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

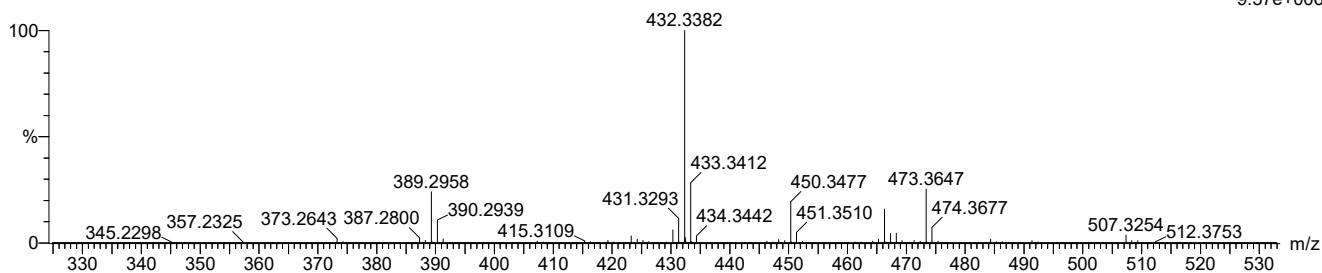
Elements Used:

C: 0-500 H: 0-1000 N: 0-10 O: 0-20 Na: 0-1

2019-535_UKJENT 177 (3.465) AM2 (Ar,35000.0,0.00,0.00); Cm (170:179)

1: TOF MS ASAP+

9.57e+006



Minimum: -2.0
Maximum: 5.0 2.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
450.3477	450.3484	-0.7	-1.6	9.5	1136.9	n/a	n/a	C29 H44 N3 O

AE.9 HPLC chromatogram for 32b

Data File C:\CHEM32\1\DATA\KRISTINEØYAKO-M-ISOBISAMINESALT.D

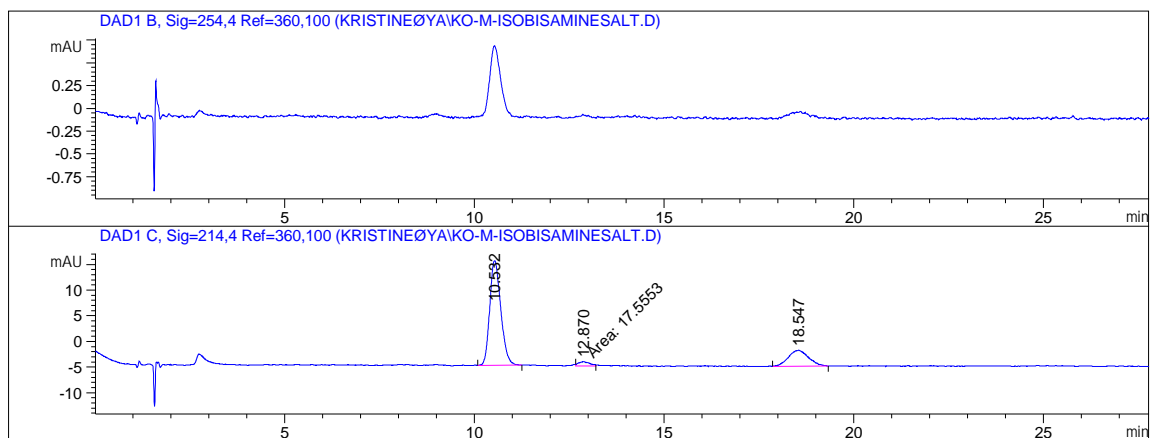
Sample Name: KO-M-i sobi sami nesalt

```

=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 4
Injection Date  : 10.05.2019 14:54:39              Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 14:51:59 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 70:30 MeOH/ H2O + 0.1 % TFA, 1 ml/min
    
```

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 B, Sig=254,4 Ref=360,100

Signal 2: DAD1 C, Sig=214,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.532	BB	0.3165	413.70490	20.41128	75.6860
2	12.870	MM	0.3469	17.55532	8.43484e-1	3.2117
3	18.547	BB	0.4520	115.34672	3.11528	21.1023

AE.10 HPLC chromatogram for 32b

Data File C:\CHEM32\1\DATA\KRI STI NEØYA\K0-M-I SOBI SAMI NESALT.D

Sample Name: K0-M-i sobi sami nesalt

```
=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 4
Injection Date  : 10.05.2019 14:54:39
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 14:51:59 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NI CO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 70:30 MeOH/ H2O + 0.1 % TFA, 1 ml/min
```

Additional Info : Peak(s) manually integrated

```
=====
```

```
Totals :                546.60693    24.37004
```

```
=====
```

*** End of Report ***

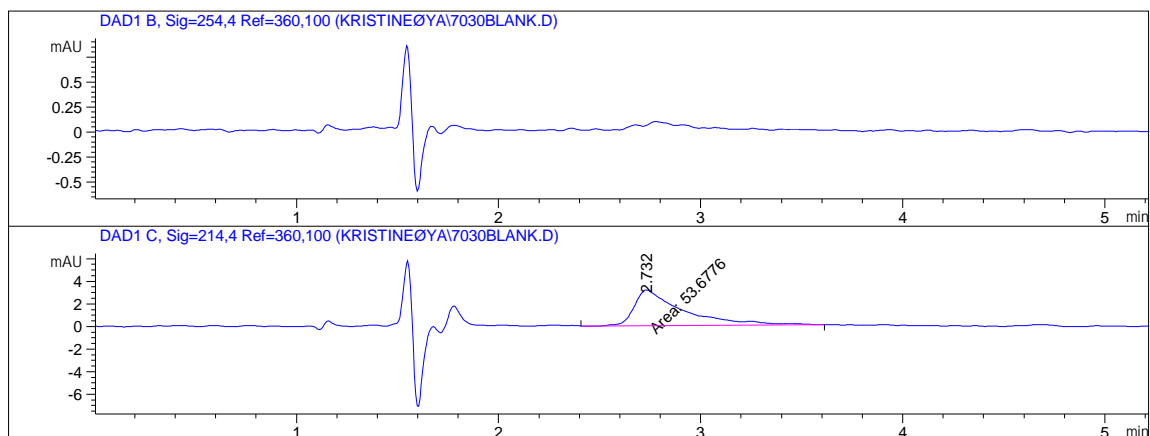
AF.1 HPLC chromatogram for MeOH in (70:30) MeOH/H₂O + 0.1% TFA

Data File C:\CHEM32\1\DATA\KRISTINEØYA\7030BLANK.D

Sample Name: 7030Blank

=====
Acq. Operator : Kristine
Acq. Instrument : UPLC Location : Vial 1
Injection Date : 10.05.2019 15:53:51 Inj Volume : 2.000 µl
Acq. Method : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed : 10.05.2019 15:52:27 by Kristine
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed : 07.05.2019 14:57:04 by Jorge
(modified after loading)
Method Info : Renhetsanalyse Sondre
Sample Info : 70:30 MeOH/ H2O + 0.1 % TFA, 1 ml/min

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 B, Sig=254,4 Ref=360,100

Signal 2: DAD1 C, Sig=214,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.732	MM	0.2818	53.67757	3.17450	100.0000

Totals : 53.67757 3.17450

AF.2 HPLC chromatogram for MeOH in (70:30) MeOH/H₂O + 0.1% TFA

Data File C:\CHEM32\1\DATA\KRI STI NEØYA\7030BLANK.D

Sample Name: 7030Blank

```
=====
Acq. Operator   : Kristine
Acq. Instrument : UPLC                               Location : Vial 1
Injection Date  : 10.05.2019 15:53:51
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 10.05.2019 15:52:27 by Kristine
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : 70:30 MeOH/ H2O + 0.1 % TFA, 1 ml/min
```

Additional Info : Peak(s) manually integrated

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

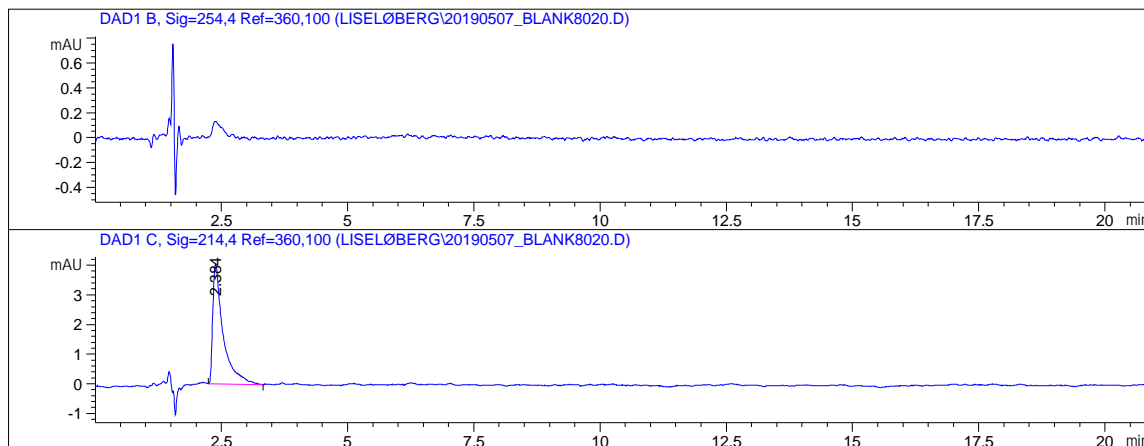

AF.3 HPLC chromatogram for MeOH in (80:20) MeOH/H₂O + 0.1% TFA

Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_BLANK8020.D

Sample Name: Blank

```

=====
Acq. Operator   : Lise
Acq. Instrument : UPLC                      Location : Vial 1
Injection Date  : 07.05.2019 15:37:53      Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 15:36:45 by Lise (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge (modified after loading)
Method Info     : Renhetsanalyse Sondre
Sample Info     : H2O/MeOH 80:20 + 0.1%TFA in H2O, 1mL/min
=====
    
```



Area Percent Report

```

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

Signal 1: DAD1 B, Sig=254,4 Ref=360,100

Signal 2: DAD1 C, Sig=214,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.384	BB	0.2023	58.29042	4.04042	100.0000

Totals : 58.29042 4.04042

AF.4 HPLC chromatogram for MeOH in (80:20) MeOH/H₂O + 0.1% TFA

Data File C:\CHEM32\1\DATA\LI SELØBERG\20190507_BLANK8020.D

Sample Name: Blank

```
=====
Acq. Operator   : Lise
Acq. Instrument : UPLC                               Location : Vial 1
Injection Date  : 07.05.2019 15:37:53
                                                    Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 15:36:45 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\MARCUSDB\SONDRE-R2-NICO-KORT.M
Last changed    : 07.05.2019 14:57:04 by Jorge
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : H2O/MeOH 80:20 + 0.1%TFA in H2O, 1mL/min
=====
```

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

