Lise Pernille Løberg

Synthesis of New Amphiphilic 1,2,3-Triazoles for Antimicrobial Evaluation

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Odd Reidar Gautun
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NTNU
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Faculty of Natural Sciences
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This master's thesis, titled *Synthesis of New Amphiphilic 1,2,3-Triazoles for Antimicrobial Evaluation*, was performed at the Department of Chemistry at NTNU Trondheim in collaboration with my supervisor, Associate Professor Odd Reidar Gautun.

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Lise Pernille Løberg
Trondheim, June 2019

Abstract

This master's thesis was a continuation of the study of amphiphilic 1,2,3-triazoles as possible candidates as novel antimicrobial agents. The aim has been to synthesise such amphiphiles with both one and multiple cationic *N*-groups. The pure enough target amphiphiles will be tested for antimicrobial activity against both Gram-positive and Gram-negative bacteria.

All the target molecules were synthesised from the key intermediate triazole methyl esters **4** (Scheme 0.2). The corresponding anilines **2** were the starting material for the preparation of **4**. Triazole **4a** (Ar = 4-*tert*-butylphenyl), **4b** (Ar = 2,4,6-triisopropylphenyl) and **4c** (Ar = 2,4,6-trimethylphenyl) had been prepared earlier in this project. The azidation of **2d** was performed three times and afforded **3d** in 61-78% yields, see Scheme 0.1. The following coupling of **3d** with methyl propiolate in a copper(I)-catalysed alkyne-azide cycloaddition, successfully afforded **4d**. The reaction gave 4-5% of a byproduct. NMR analysis, including NOE-experiments, confirmed this to be the 1,5-regioisomer **4'd**.
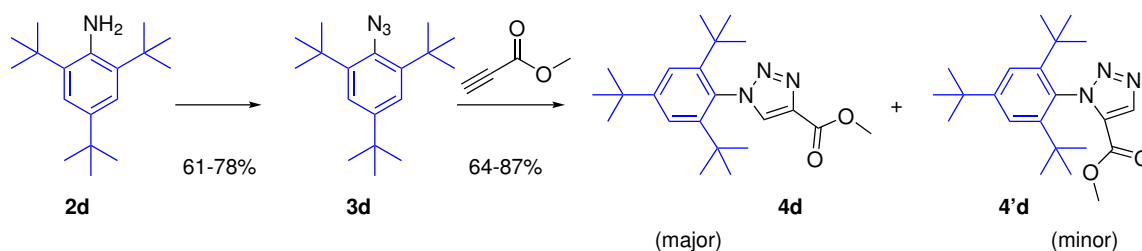
Scheme 0.1: Synthetic steps towards the 1,2,3-triazole methyl ester **4d** including the yields.

Amidation of **4c** and **4d** with ethylenediamine, afforded the corresponding amines **7** (Scheme 0.2). The following protonation of **7c** and **7d** with HCl (37% aq.) successfully yielded the target molecules **7***c* and **7***d* with a HPLC purity of $> 99\%$. Both these reactions were done in varying yields. Target guanidines **8a** and **8c** were prepared in 15-19% yields from the corresponding amines **7** in a reaction with 1*H*-pyrazole-1-carboxamidine hydrochloride. The HPLC purities were 98% and 99% respectively.
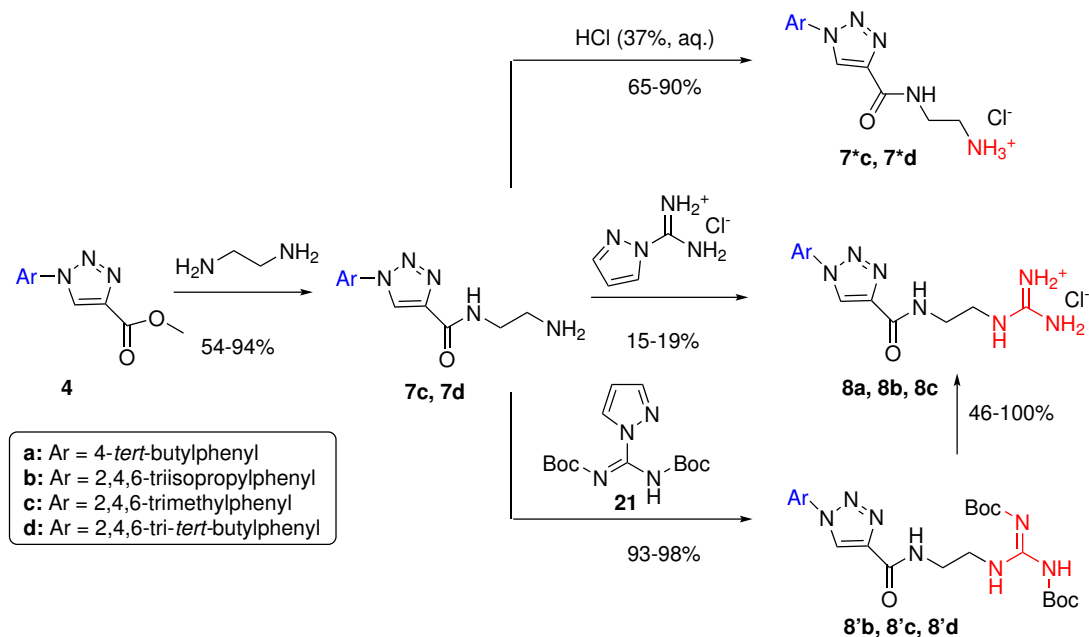
Scheme 0.2: Synthetic steps towards the target molecules **7*** and **8** including the yields.

In an attempt of finding a better synthetic route towards **8**, the Boc-protected **8'b-d** were successfully prepared in excellent yields, see Scheme 0.2. The following deprotection using AcCl in MeOH gave **8b** and **8c** in 46-100% yields with a HPLC purity of 99% and > 99% respectively.

The branched amines **5** were prepared from the respective triazoles **4** using tris(2-aminoethyl)-amine, see Scheme 0.3. Protonation of **5b** and **5c** afforded the branched ammonium salts **5*b** and **5*c** in 25-91% yields and a HPLC purity of > 99%. An attempt of preparing **14a** using 1*H*-pyrazole-1-carboxamidine hydrochloride and the base TEA afforded a product mixture of **14a** and **14'a** (Scheme 0.3).
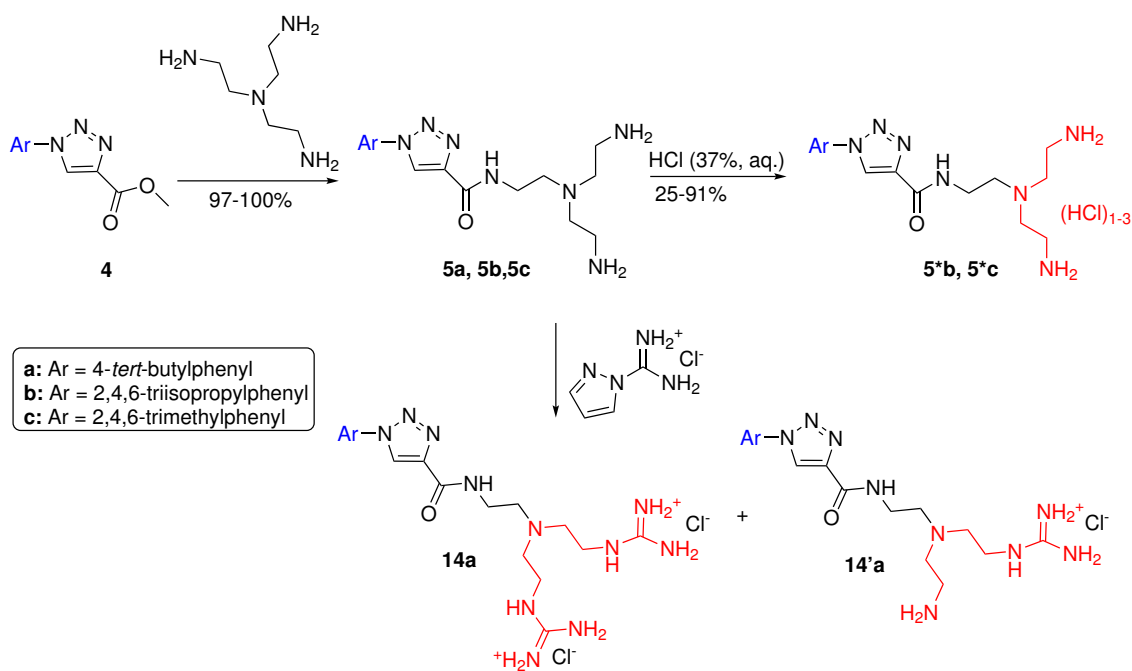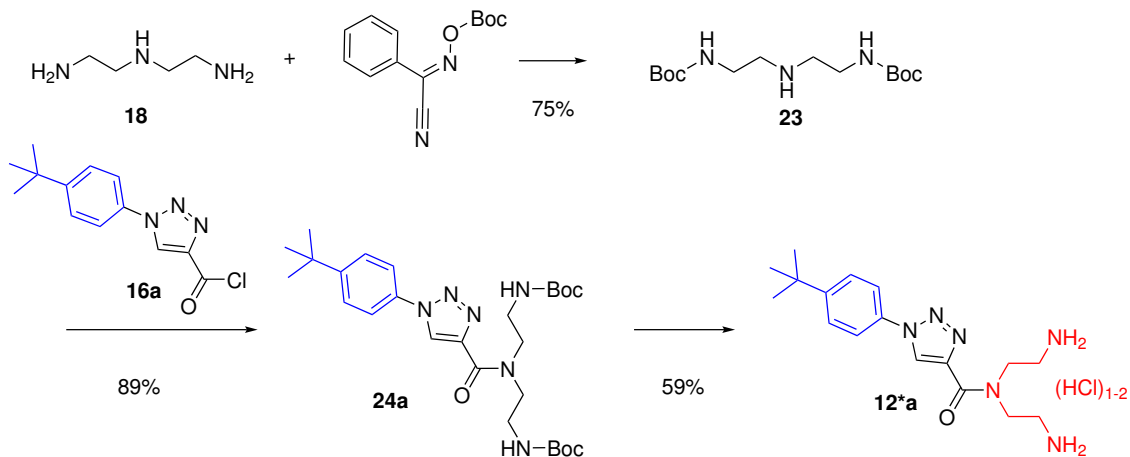
Scheme 0.3: Synthetic steps towards target molecule **5*** and **14a** including the yields.

An optimisation study towards the branched bisazide **11a** resulted in **11a** successfully prepared in 65-86% yields from **16a** and **10**, see Scheme 0.4.
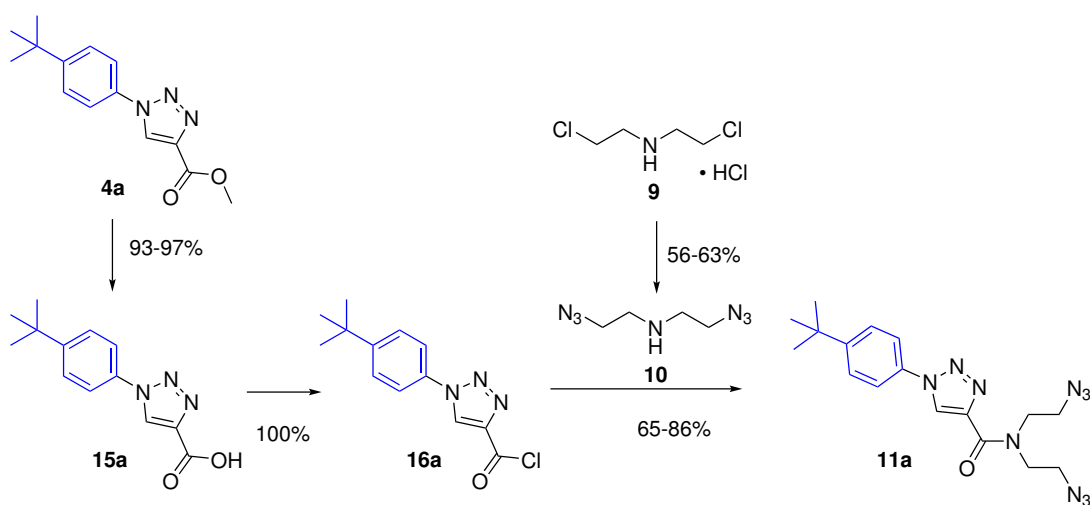
Scheme 0.4: Synthetic steps towards the branched bisazide **11a** including the yields.

Several attempts were made to reduce **11a** to **12a** or **12*a**, but this formed an unwanted isomer, possibly due to a rearrangement reaction taking place. After considerable attempts of preparing **12*a**, the target amphiphile was successfully synthesised in a three steps procedure via the Boc-protected amide **23** in 39% overall yield, see Scheme 0.5. The HPLC purity was 98%. A synthetic route towards **12*a** was established, but the route remains to be tested for amphiphiles similar to **12*a**.


Scheme 0.5: Synthetic steps towards **12*a** including the yields for the successful preparation of the target amphiphile.

Sammendrag

Denne masteroppgaven var en fortsettelse av studiet av amfifile 1,2,3-triazoler som mulige kandidater til nye antibiotikaforbindelser. Formålet har vært å fremstille slike amfifiler både med en og flere kationiske nitrogengrupper. Målmolekylene som ble rene nok skal sendes til testing for å måle antimikrobiell aktivitet mot både Gram-positive og Gram-negative bakterier.

Målmolekylene ble fremstilt fra triazol metyl esterne **4** (Skjema 0.2). Anilinene **2** var startmaterialet for tillagingen av **4**. Triazol **4a** (Ar= 4-*tert*-butylfenyl), **4b** (Ar = 2,4,6-triisopropylfenyl) and **4c** (Ar = 2,4,6-trimetylfenyl) ble laget tidligere i prosjektet. Azideringen av **2d** ble gjort tre ganger og ga **3d** i 61-78 % utbytte, se Skjema 0.1. Videre kunne **3d** kobles med metyl propiolat i en kobber(I)-katalysert alkyne-azide sykloaddisjon. Reaksjonen var vellykket og ga **4d**, men i tillegg 4-5 % av et biprodukt. NMR analyser, inkludert NOE-forsøk, bekreftet at dette var 1,5-regioisomeren **4'd**.
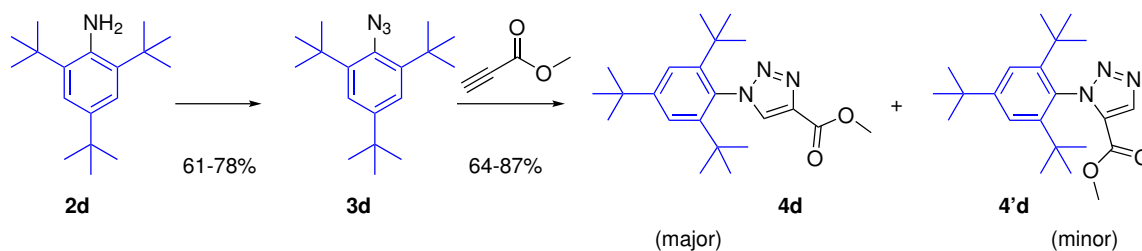
Skjema 0.1: Syntesetrinn for fremstillingen av 1,2,3-triazol metyl ester **4d** inkludert utbytter.

Amidering av **4c** og **4d** med etylendiamine, ga aminene **7c** og **7d** (Skjema 0.2). Den påfølgende protoneringen med HCl (37 % aq.) ga målmolekylene **7*c** og **7*d** med en HPLC-renhet på > 99 %. Utbyttene for begge disse reaksjonene var varierende. Guanidinene **8a** og **8c** ble fremstilt i 15-19 % utbytte fra aminene **7a** og **7c**, i reaksjon med 1*H*-pyrazole-1-carboxamidine hydrochloride. HPLC-renheten var henholdsvis 98 % og 99 %.
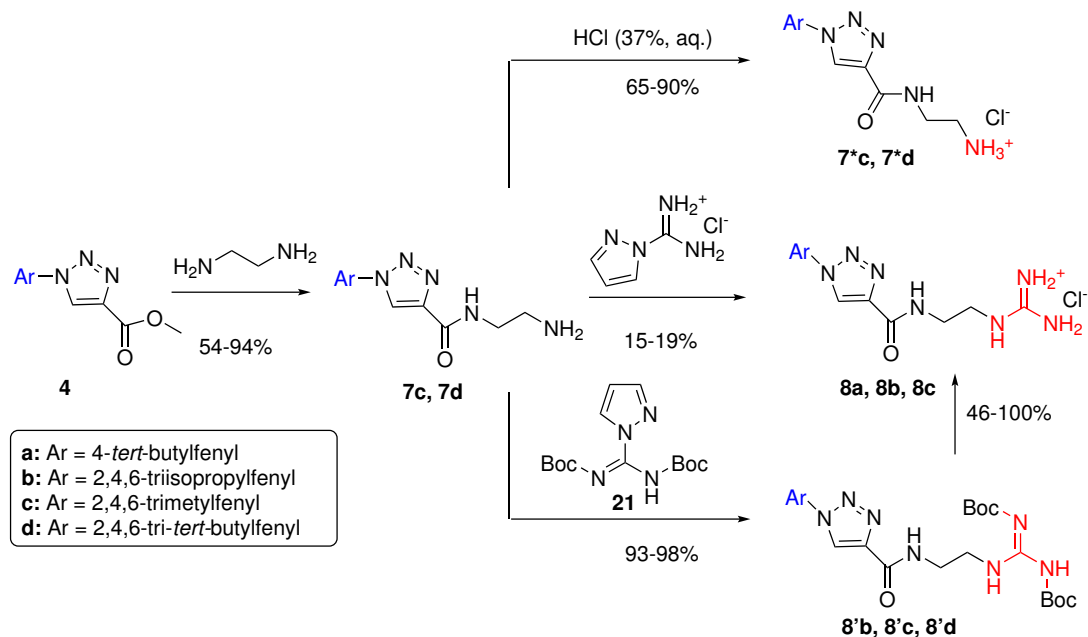
Skjema 0.2: Syntesetrinn for fremstillingen av **7*** og **8** inkludert utbytter.

I et forsøk på å optimalisere synteseruten for **8**, ble **8'b-d** laget i gode utbytter (93-98 %), se Skjema 0.2. Den påfølgende avbeskyttelsen ved bruk av AcCl i metanol, ga **8b** og **8c** i 46-100 % utbytte og med HPLC-renheter på henholdsvis 99 % og > 99 %.

De forgrenede aminene **5** ble fremstilt fra de respektive triazolene **4**, se Skjema 0.3. Protonering av **5b** og **5c** ga **5*****b** og **5*****c** i 25-91 % utbytte, og begge hadde HPLC-renhet på > 99 %. Et forsøk på å lage **14a** ved å bruke 1*H*-pyrazole-1-carboxamidine hydrochloride og basen trietylamin, ga en produktblanding av **14a** og **14'a**, se Skjema 0.3.
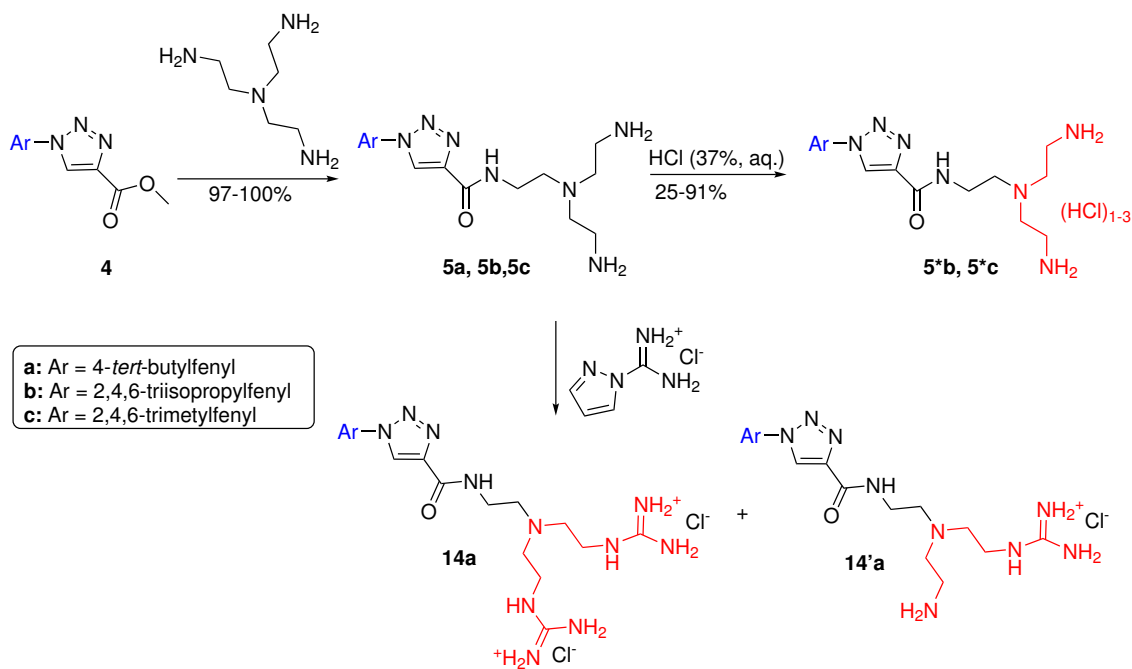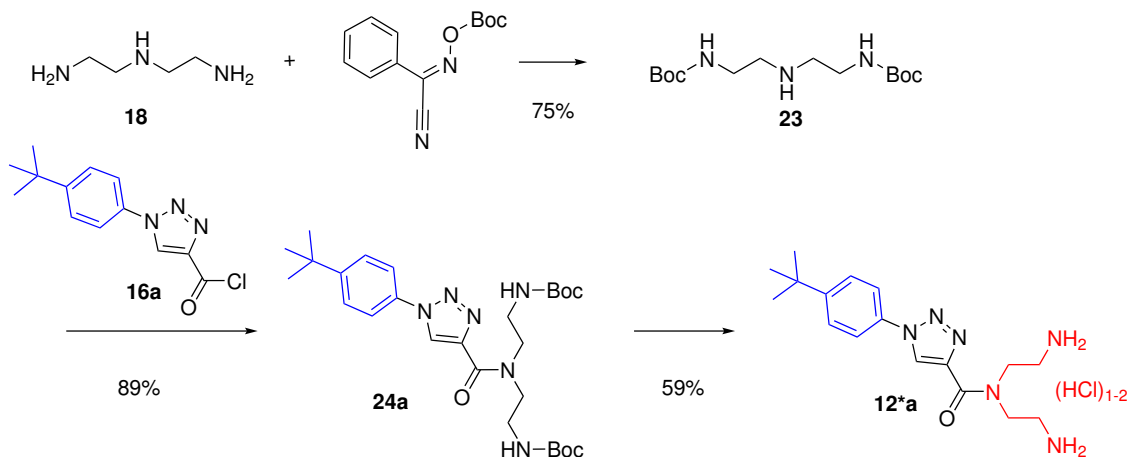
Skjema 0.3: Syntesetrinn for fremstillingen av **5*** og **14a** inkludert utbytter.

En optimaliseringsstudie for bisazid **11a** resulterte i en vellykket fremstilling av **11a** i 65-86 % utbytte fra **16a** og **10**, se Skjema 0.4.
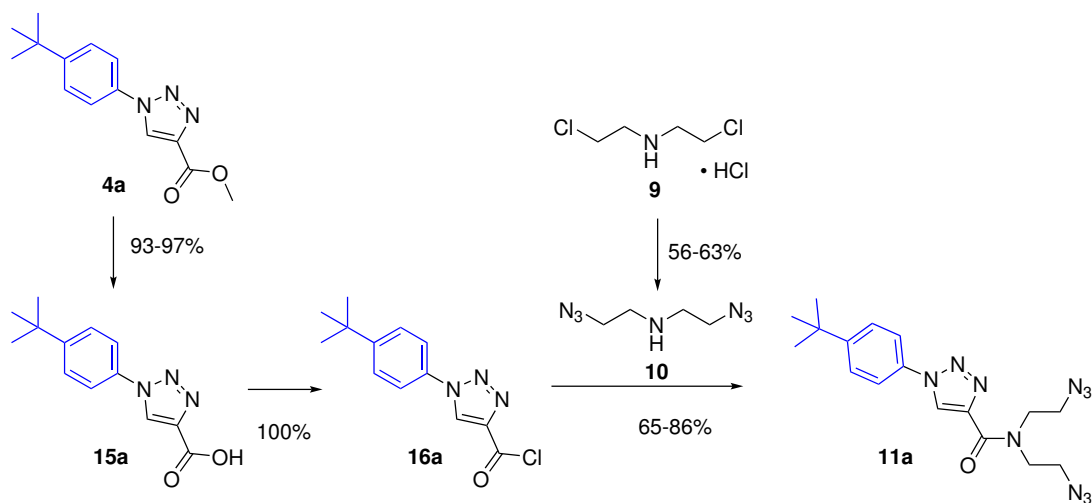
Skjema 0.4: Syntesetrinn for fremstillingen av **11a** inkludert utbytter.

Det ble gjort flere forsøk på å redusere **11a** til **12a** eller **12*a**, men en uønsket isomer ble dannet. Dette skyldes trolig at det skjer en omleiringsreaksjon. Etter mange forsøk på å fremstille **12*a**, ble målforbindelsen vellykket fremstilt i tre trinn via det Boc-beskyttede amidet **23** i 39 % utbytte (fra **18**), se Skjema 0.5. HPLC-renheten var 98 %. En synteserute mot **12*a** er etablert, og ruten kan videre testes for andre lignende amfifiler.
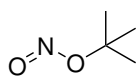

Skjema 0.5: Syntesetrinn for fremstillingen av **12*a** inkludert utbytter.

Abbreviations and Symbols

2D	Two dimensional
AMP	Antimicrobial Peptide
APCI	Atmospheric Pressure Chemical Ionization
app	Apparent
aq.	Aqueous
Ar	Aryl
ASAP	Atmospheric Solids Analysis Probe
atm	Atmosphere
ATR	Attenuated total reflectance
br	Broad
calcd	Calculated
COSY	Correlation spectroscopy (H,H)
CuAAC	Copper(I)-Catalysed Azide-Alkyne Cycloaddition
d	Doublet (NMR)
DAD	Diode Array Detector
DCM	Dichloromethane
decomp.	Decompose
DMF	*N,N*-Dimethyl formamide
DMSO	Dimethyl Sulfoxide
EC$_{50}$	Half maximal effective concentration
equiv	Equivalents
ESI	Electron spray ionization
Et	Ethyl
FT	Fourier Transform
h	Hours
HMBC	Hetereonuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectroscopy
HSQC	Hetereonuclear Single Quantum Coherence
Hz	Frequency unit - defined as one cycle per second
IR	Infrared radiation (spectroscopy)
J	Coupling constant used in NMR-spectroscopy
mbar	Millibar (pressure unit)

m	Multiplet (NMR)
M	Molarity (mol/L)
Me	Methyl
MeCN	Acetonitrile
Min	Minutes
MIC	Minimal Inhibitor Concentration
mmol	Millimol
Mp.	Melting point
m/z	Mass to Charge Ratio
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
Nu	Nucleophile
Ph	Phenyl
ppm	Parts Per Million
q	Quartet (NMR)
r.t.	Room temperature
ref.	Reference
$\mathbf{R}_f$	Retention factor (TLC)
s	Singlet (NMR)
sept	Septet (NMR)
t	Triplet (NMR)
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TOF	Time-of-Flight
$\mathbf{t}_R$	Retention time
UV	Ultra violet
°C	Degrees celsius
δ	Chemical shift in NMR-spectroscopy [ppm]

Numbered Compounds
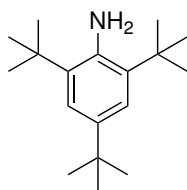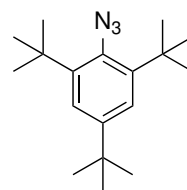
1   **1'**   **2d**   **3d**

4a   **4b**   **4c**

4d   **4'd**

5a   **5'a**

5b   **5'b**

5c   **5'c**

XIII

5*a

5*b

5*c

(HCl)$_{1-3}$

6

7'c

7'd

7a

7b

7c

7d

7*a

7*b

7*c

7*d

8a

8b

XIV

8c

8d

8'a

8'b

8'c

8'd

9

10

11a

12a

12*a (HCl)$_{1-2}$

13

14a

14'a

XV

15a

15c

16a

17

18

19

20a

21

22a

22*a

$(HCl)_{1-2}$ or $(CH_3CO_2H)_{1-2}$

23

24a

25

26

XVI

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1 Introduction

1.1 Background

The introduction of antibiotics revolutionised modern medicine and have saved millions of lives.[1] Infections like pneumonia used to have high mortality rate, but can now easily be treated.[2] Today, one of the major constituents in any basic health system is the ability to treat bacterial infections with antibiotics.

The modern era of antibiotics started with Sir Alexander Flemming's discovery of penicillin in 1928,[3] and Gerhard Domagk's discovery of the first sulfonamide in 1935.[4] This was followed by 30 years of great attention in developing new and more complex classes of antibiotics.[5,6] More than 20 novel classes, defined as antibiotics with a new mode of action, were introduced to the marked. However, this evolution within medicine was replaced with a decline in the emergence of new classes. Only two novel types of antibiotics have entered the market in the last three decades: oxazolidinones in year 2000 and lipopeptides in 2003.[6]

Already in the late 1930s, very soon after the introduction of antibiotics, the first evidence of antimicrobial resistance was reported.[7] Bacteria develop resistance through evolution and this is naturally occurring.[8] However, in the recent years it has become a threat to human health.[7,8] This is caused by overuse and misuse of antibiotics, combined with this reduced effort in developing new classes of antimicrobial agents. Today, resistance in bacteria occurs faster than the emergence of new types of antibiotics. Disturbingly, resistance has developed to all clinically used antibiotics.[9] It is essential to develop novel efficient antibiotics.

Most antibiotics used today target specific intracellular sites in the bacteria.[10] With this high target specificity, resistance is allowed to develop, often through mutations. A promising agent for fighting the resistant bacteria is antimicrobial peptides (AMP). AMPs work through a less specific mechanism, targeting the bacterial cell membrane directly.[10,11] It is unlikely that AMPs will be affected by antimicrobial resistance. To destroy the AMPs, the bacteria will have to change their cell membrane. Developing resistance against the AMPs will probably be too demanding for the microbes. This makes the AMPs an interesting class of novel antimicrobial agents for further investigation.

1.2 Objective

The aim of this master's thesis is to prepare amphiphilic 1,2,3-triazoles with both one and multiple cationic hydrophilic *N*-groups. The background for the thesis is the work done by Thomas A. Bakka in his PhD.[12] Based on a pharmacophore model of marine natural product peptidomimetics,[13–15] Bakka prepared a series of amphiphilic 1,2,3-triazoles with **B10b** as the most potent one.[12] This amphiphile showed antimicrobial activities against both Gram-positive and Gram-negative bacteria. However, **B10b** was too toxic towards eukaryotic cells. The working theory is to introduce multiple cationic hydrophilic groups, see Figure 1.1. The overall lipophilicity will be lower with several cationic groups, and give more groups to interact electrostatically with the cell membrane of the bacteria.[12] Hopefully, this characteristic will lower the toxicity against eukaryotic cells while the antimicrobial activity is retained. In addition, it is interesting to see how a more compact structure will affect the activity.

B10b

MIC (*S.aureus*): 4 µg/mL
EC$_{50}$ (HepG2): 17.7 µg/mL
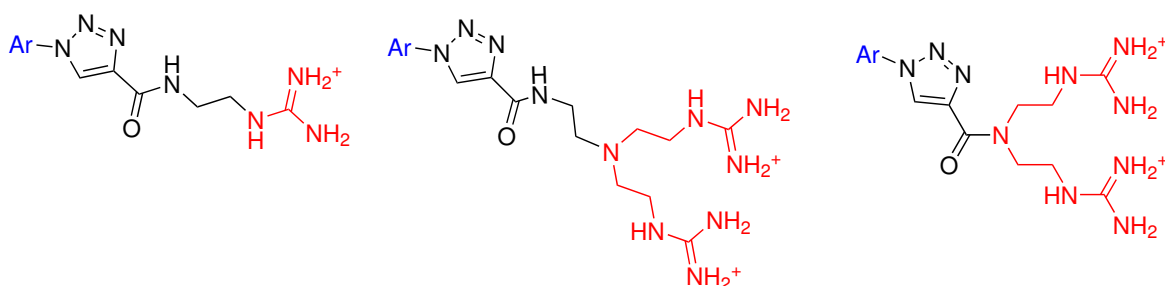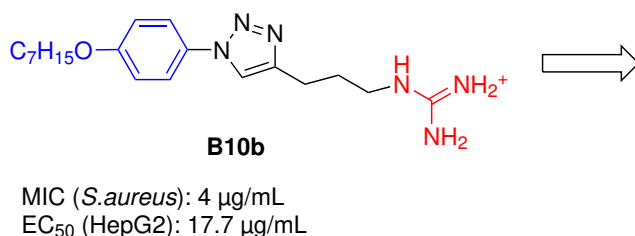
Figure 1.1: Possible optimisation of **B10b**, in addition to values for the minimal inhibitor concentration (MIC) and half maximal effective concentration (EC$_{50}$) for **B10**. Counter ion is Cl$^-$.

This thesis is also a continuation of M. Sc. Maren Grøndahl's work,[16] in addition to the work done in the specialisation project prior to this master thesis.[17]

2 Theory

This section will give an overview of the biology and chemistry relevant for the project. This include an introduction to antimicrobial peptides, a retrosynthetic analysis of the target molecules and a presentation of the most important chemistry used in the thesis.

2.1 Antimicrobial Peptides and Peptide Mimics

In most living organisms, antimicrobial peptides (AMPs) are an important part of the innate immune response.[18] These peptides can be active against a variety of organisms, like parasites, bacteria and fungi.[19] They are small peptides with an overall positive charge (+2 to +9), a significant hydrophobic character (>30%) and different length and structure.[19,20] One main characteristic of the AMPs is the ability to form secondary structures, a capability that originates from their amphiphilic nature. This makes it possible for the AMPs to interact with anionic phospholipids on the surface of the bacterial cell membrane, thereby causing cell lysis.[21]

The fact that many eukaryotic cells produce AMPs makes it important that the peptides display a high selectivity towards the cell membrane of the bacteria.[18,20] This selectivity derives from the different charge of the eukaryotic and microbial cell membranes. The cationic AMPs can interact with the negatively charged cell membrane of the bacteria. In contrast, the cell membrane of eukaryotes consist of zwitter-ionic phospholipids.

There are four main models describing the disrupting interaction of the AMPs, see Figure 2.1. The "carpet" model suggests the AMPs bind to the surface of the cell membrane forming clusters.[20] This causes curvature strain, micellisation and disruption of the membrane. According to the "barrel-stave" model, a small number of peptides are inserted perpendicularly and by that form pores in the cell membrane. The "toroidal pore" model is similar to the "barrel-stave" model, but in this model both peptides and membrane lipids are involved in the pore formation. The "aggregate channel" model postulates that the AMPs after coordination to the cell membrane, insert themselves into the cell membrane and form aggregates. The clusters can then pass the membrane and attack intracellular targets.
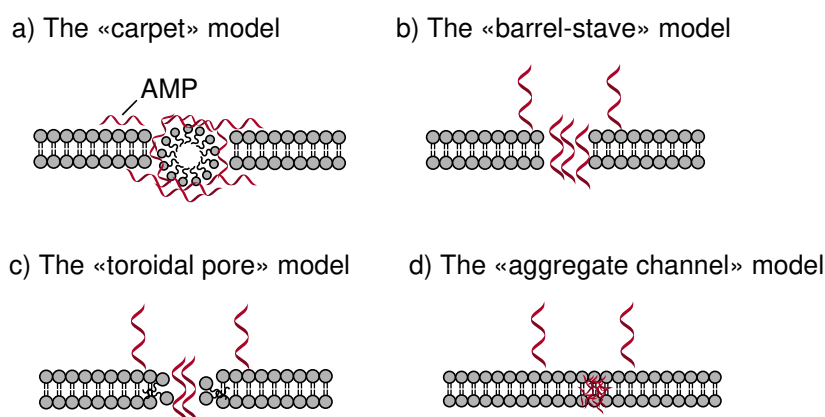
Figure 2.1: Models describing the disrupting interactions of the AMPs: a) the "carpet" model, b) the "barrel-stave" model, c) the "toroidal pore" model and d) the "aggregate channel" model.[20]

Even though antimicrobial peptides possess high antimicrobial activity, only a few AMPs are used as antimicrobial agents.[22] This is due to challenges like low bio-availability and poor metabolic stability, in addition to high manufacturing costs. These drawbacks are partly due to the size of the peptides.[23] A proposed solution to the obstacles regarding the AMPs are antimicrobial peptide mimics, smaller molecules designed to mimic the antimicrobial properties of the AMPs.

Amphiphilic antimicrobial marine products are also a motivation for developing such novel antimicrobial agents. Two examples are Synoxazolidone A[13] and Ianthelline[14] (shown in Figure 2.2), which have been isolated and characterised from marine environments. Simplified, the structure of these compounds consists of a hydrophobic part, a linker scaffold and a cationic nitrogen group.
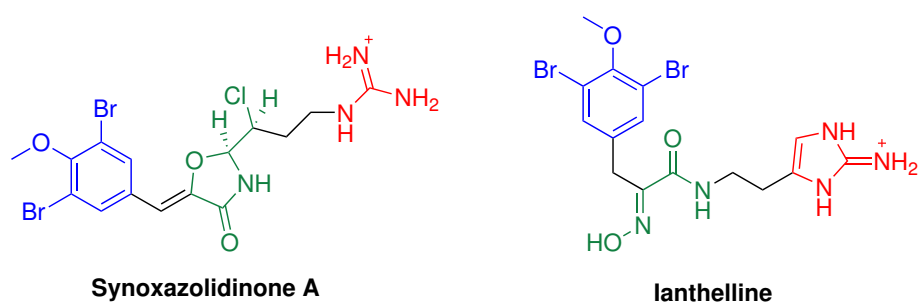
Figure 2.2: The structure of the marine amphiphiles Synoxazolidone A and Ianthelline in their charged state, consisting of a hydrophobic part, a linker scaffold and a cationic nitrogen group.[13,14]

Based on this antimicrobial inspiration, the research group of Strøm at the Arctic University of Norway (UiT) has prepared amphiphilic aminobenzamides showing high antimicrobial activity.[15] By testing, they confirmed a membranolytic mode of action similar to small AMPs.

Previous work in the Gautun research group based on these principals, has resulted in a series of different amphiphilic 1,2,3-triazoles.[12,24] A selection of these amphiphiles are shown in Table 2.1. The amphiphiles are tested for antimicrobial activity against Gram-positive *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus agalacticae* (ATCC 12386) and Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). In addition, the toxicity towards the human liver cell Hep G2 was tested.

The results presented in Table 2.1 indicate some important trends regarding the structure of the amphiphilic triazoles. A comparison between the MIC-values of ammonium salts **21** and the corresponding guanidines **22** shows that the guanidines give a higher antimicrobial potency. In addition, the guanidines **22f** and **22e** have a lower toxicity against human liver cells than **21f** and **21e**.

The only structural difference between **21/22c** and **21/22e** is the benzylic methylene group. Removing the methylene group led to a 2- to 4-fold increase in the activity towards the different bacteria, indicating that a restricted rotational freedom is preferred.[24] The amphiphile **28e** is the only one with two cationic nitrogen groups. Comparing the MIC-values of **21e** and **28e**, multiple cationic groups seem to reduce the toxicity against human cells.

As these results indicate that the 1,2,3-triazole directly substituted with an aromatic group leads to antimicrobial potency, this was chosen as the "scaffold" for all the target molecules in this thesis. From literature, 1,2,3-triazoles with a wide variety of substituents have shown antimicrobial activity.[25] This makes the motivation for preparing 1,2,3-triazoles even higher.

The main challenge is to establish a good enough activity-cytotoxicity profile. A motivation is the introduction of multiple cationic *N*-groups based on the results of **28e**. Since guanidines also showed promising results, guanylation of branched amines is hopefully affording an even better effect.

Table 2.1: MIC- and EC$_{50}$-values in μg/mL for amphiphiles prepared by Bakka.[12] The counter ion is Cl$^-$.
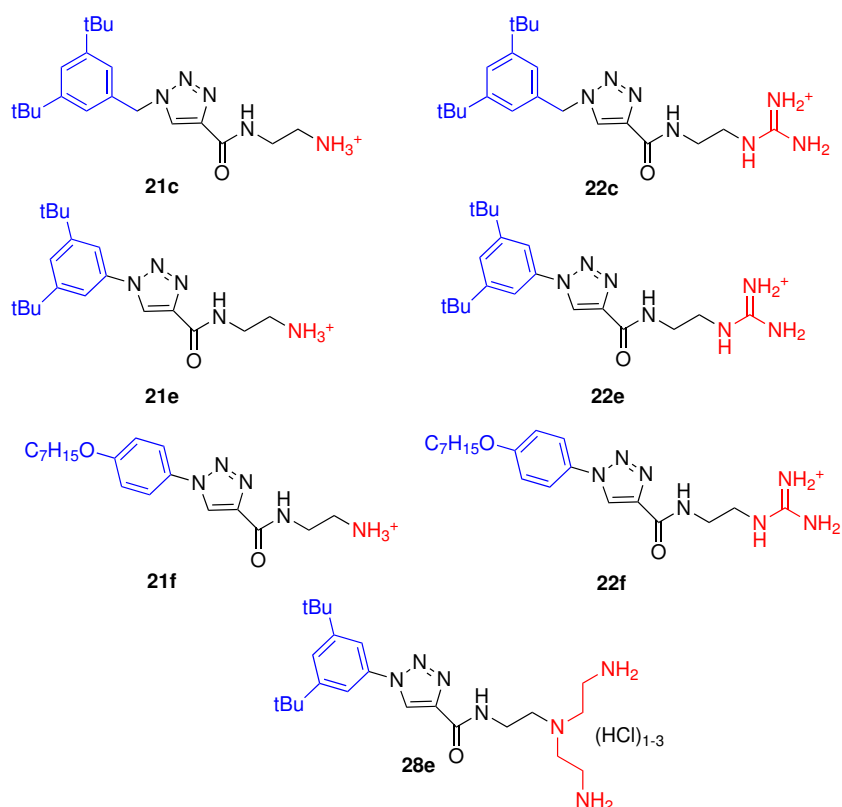
	E. faecalis[a] (MIC)+	*S. aureus*[a] (MIC)+	*StreptB*[a] (MIC)+	*E. coli*[a] (MIC)-	*P aerugin*[a] (MIC)-	Hep G2[b] <50% survival
21f	I[c]	4	8	I[c]	16	3.5
22f	16	4	8	8	8	23.8
21c	I[c]	64	64	32	64	n.d.[d]
22c	64	32	16	64	32	n.d.[d]
21e	32	16	16	16	32	8.0
22e	16	8	8	16	16	31.3
28e	16	16	8	16	4	32-64[e]
Ref.[f]	10	0.13	4	0.5	0.5	n.d.[d]

[a] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalacticae* (ATCC 12386), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853).
[b] Human liver cell.
[c] No activity $\leq 64\,\mu$g/mL.
[d] n.d.: Not determined.
[e] No activity at 32 μg/mL, 2% cell-survival at 64%.
[f] Ref.: gentamicin.

2.2 Retrosynthetic Analysis

This section includes the retrosynthetic analysis of all the target compounds. Only the planned retrosynthetic strategies will be included. Changes that were made to the plan during the project are discussed in Section 3.

The retrosynthetic analysis of target molecules **7**[*] and **8** are shown in Scheme 2.1.
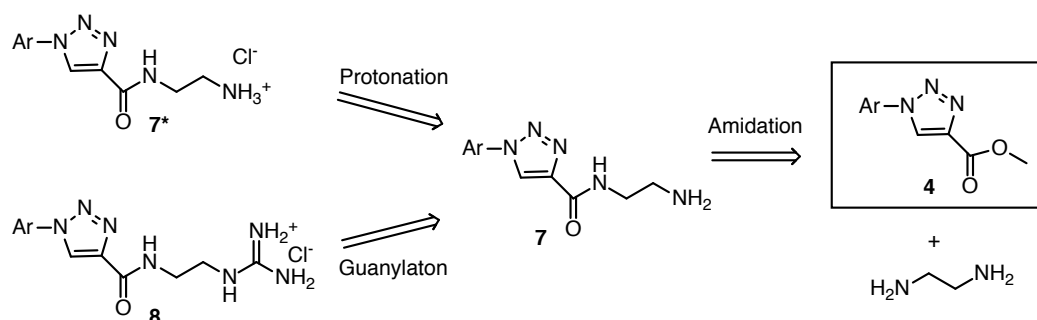
Scheme 2.1: Retrosynthesis of target molecules **7**[*] and **8**.

As shown in Scheme 2.1, ammonium salt **7**[*] can be prepared by protonation of **7**. Guanylation of **7** affords the target **8**. This can be done using the guanylation reagent 1*H*-pyrazole car-boxamidine hydrochloride, which has previously been done successfully within the research group.[12,16] Amine **7** can be retrosynthetically cleaved at the amide bond. This disconnection makes amidation of triazole **4** with ethylendiamine a possible step towards **7**.

The retrosynthesis of target **5**[*] and **14** are shown in Scheme 2.2. It is similar to monobranched **7**[*] and **8**. In order to introduce the branched amine part, tris(2-aminoethyl)amine can be utilised as amidation reagent instead of ethylenediamine.
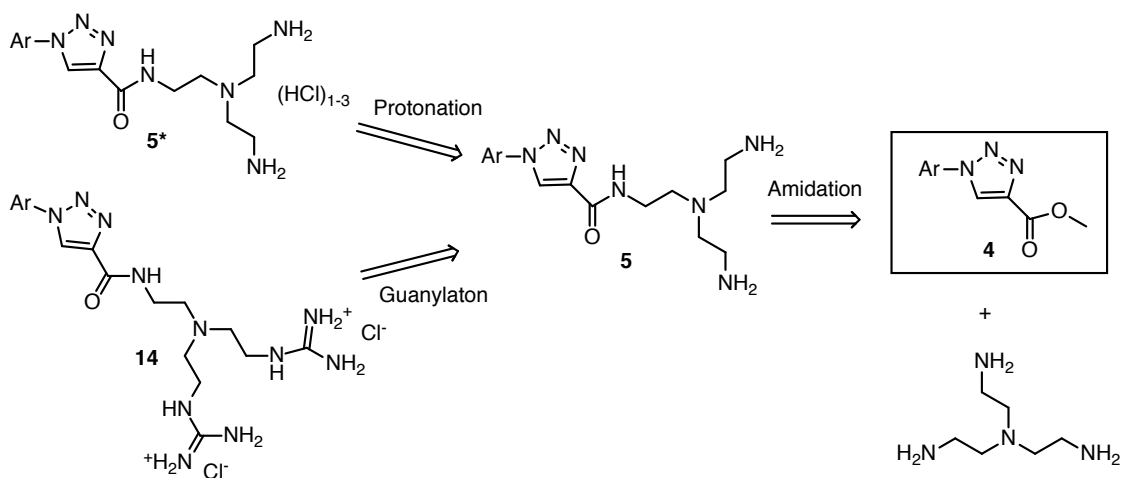
Scheme 2.2: Retrosynthesis of target molecules **5**[*] and **14**.

The more compact target molecule **12**∗ might also be available from **4**, see Scheme 2.3. Protonation of **12** gives the target **12**∗. The bisamine functionality might be introduced by reduction of the corresponding branched bisazide **11**. A retrosynthetical disconnection at the amide bond, makes amidation of **4** with **10** a possible key step in the synthesis. Bisazide **10** may be prepared from the commercial available **9** in a substitution reaction.
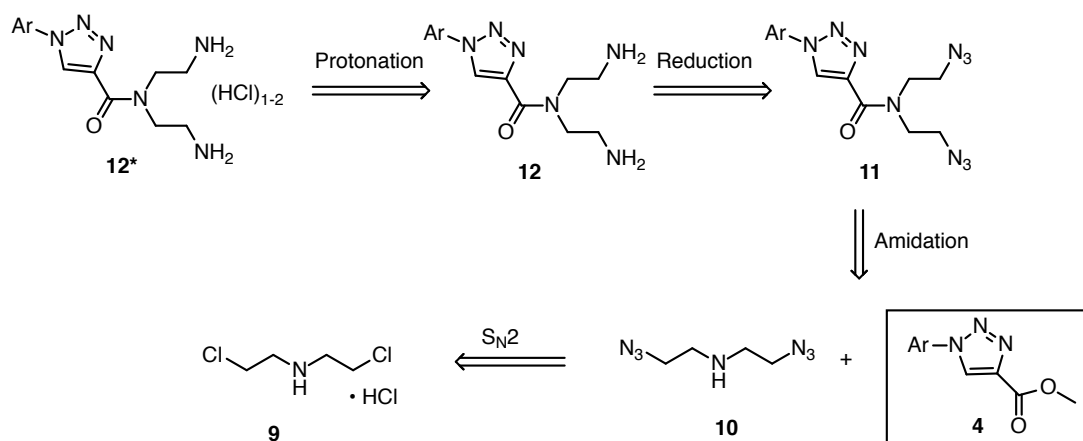
Scheme 2.3: Retrosynthesis of target molecule **12**∗.

The 1,2,3-triazole methyl ester **4** is a key intermediate for all the target molecules. A retrosynthetic analysis of **4** is shown in Scheme 2.4.
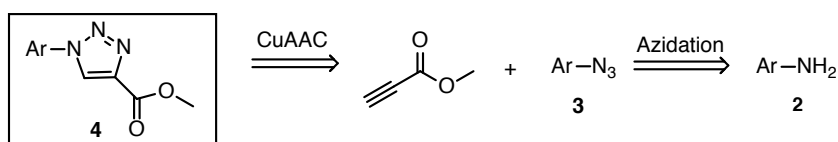
Scheme 2.4: Retrosynthesis of key intermediate **4**.

As shown in Scheme 2.4 the desired triazole is the 1,4-regioisomer. A well-established method for the preparation of only this regioisomer,[26,27] is the CuAAC reaction between azide **3** and methyl propiolate. Azidation of the corresponding aniline **2** gives the aromatic azide **3**.

2.3 Applied Chemistry

2.3.1 Azides

The synthesis of organic azides was first carried out by Peter Grieβ in 1864.[28,29] These azides have proven to be important intermediates in organic synthesis.

Aryl azides

One method for forming aryl azides is diazotisation of the corresponding anilines.[29] Following treatment of the diazonium ion intermediate with an azide yields the aryl azides. Usually the diazotisation takes place by addition of nitrous acid to the aniline followed by elimination of water.[30,31c] The nitrous acid is first generated *in situ* from nitrite salt in acidic environment at about 0 °C. However, diazonium ions can also be generated in organic solvents with anhydrous condition.[30,32] Alkyl nitrites like *tert*-butyl nitrite have shown to be a good source for diazonium ions in such conditions. The mechanism for formation of the disazonium ion is not known. It is postulated that *tert*-butyl nitrite reacts in a radical mechanism,[33–35] but also that the nitrosation gives a protonated nitrosoamine followed by dehydration affording the diazonium ion.[36] The reaction is shown in Scheme 2.5. The anion is not reported in the literature, thus it was assumed this was OH$^-$ according to stoichiometry. This based on the fact that the byproduct *tert*-butanol have been reported.[35]
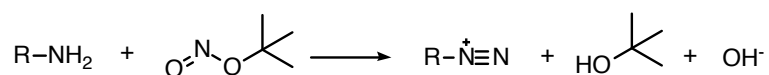
Scheme 2.5: The reaction for forming diazonium ion using *tert*-butyl nitrite as reagent.

When *tert*-butyl nitrite is used as diazotisation agent, the only byproduct is the nontoxic *tert*-butanol, making it a green reagent.[35]

The next step is the reaction between the diazonium and azide ions. This gives the aryl azide and releases N_2(g).[29] Both alkali azides and trimethylsilyl azide can be the source of azide ions.[28] The latter one can be used when the reaction takes place in non-aqueous conditions. Whether the reaction goes through a concerted [3+2] mechanism or occurs stepwise have been discussed.[28,37] Based on ^{1}H NMR and ^{15}N NMR analysis, Butler *et al.* have reported that the azide ion attacks the diazonium β nitrogen atom yielding pentazene intermediates.[37] They identified three isomeric aryl pentazenes, the (*Z,E*), (*E,E*) and (*E,Z*) isomers. The (*Z,E*)-pentazene formed the aryl azide directly, while the (*E,Z*) gave the pentazole intermediate. Their research supported the stepwise theory.
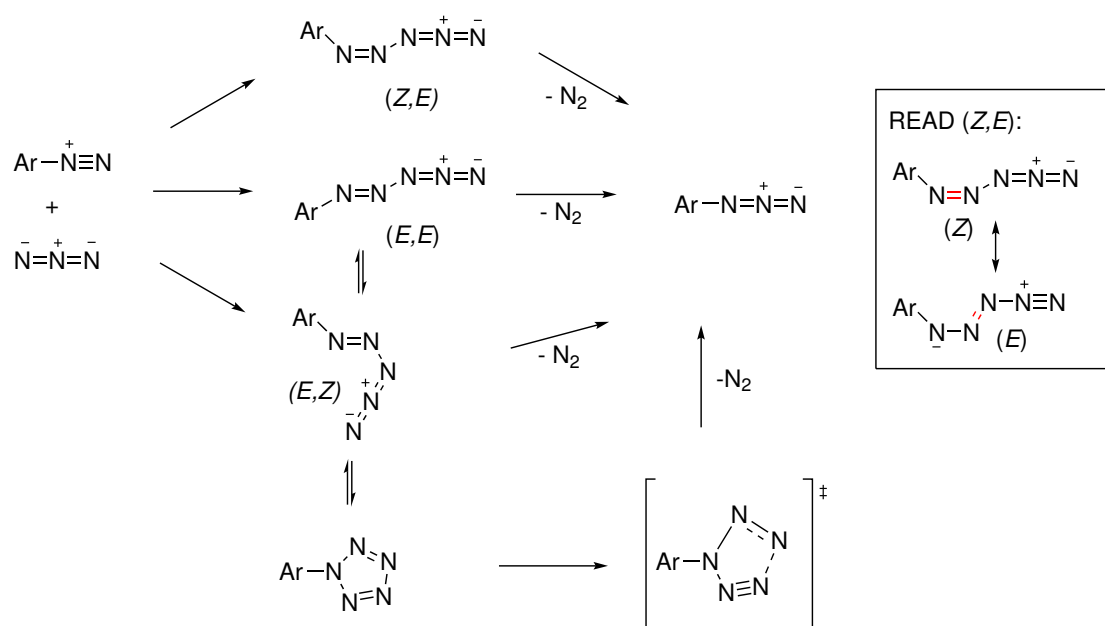
Scheme 2.6: Proposed mechanism with pentazene and pentazole intermediates in the preparation of aryl azides from the corresponding aryl diazonium ions.[37]

Nucleophilic substitution reactions with azides

Azides are good nucleophiles and can be used in nucleophilic substitution reactions,[31b, 38] for example for forming alkyl azides. Alkali metal azides like sodium azide, are often used in these reactions together with alkyl halides or compounds with another good leaving group like sulfonates. One example of a reaction like this performed in this thesis, is the preparation of bisazide **10** in a substitution reaction between **9** and sodium azide, see Scheme 2.7.
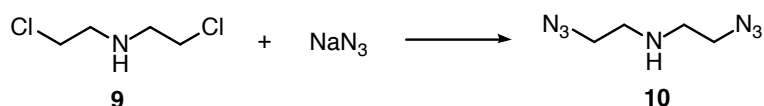
Scheme 2.7: Example of a substitution reaction performed in this theses.

The product, the alkyl azide, is no longer nucleophilic and the azide therefore reacts only once with the alkyl halide.[31b]

Reduction of Azides

The reduction of azides to the corresponding amines can be a useful reaction in organic synthesis due to the often easy preparation of the azides.[39] There are a lot of different methods and reagents available for this purpose, like hydrogenolysis on Pd/C, reduction using zink and ammonium chloride, borhydrides[40] and reduction using triphenylphosphine[41] (Staudinger reaction).

The Staudinger reaction was one of the reactions utilised in this thesis. A proposed mechanism for this reaction is shown in Scheme 2.8.[31d] The first step is an attack by triphenylphosphine on the azide. The negatively charged nitrogen attacks the phosphine and a phosphinimine is formed through a four-membered cyclic transition state. Hydrolysis of the phosphinimine in the presence of water gives the amine and the stable by-product phosphine oxide.
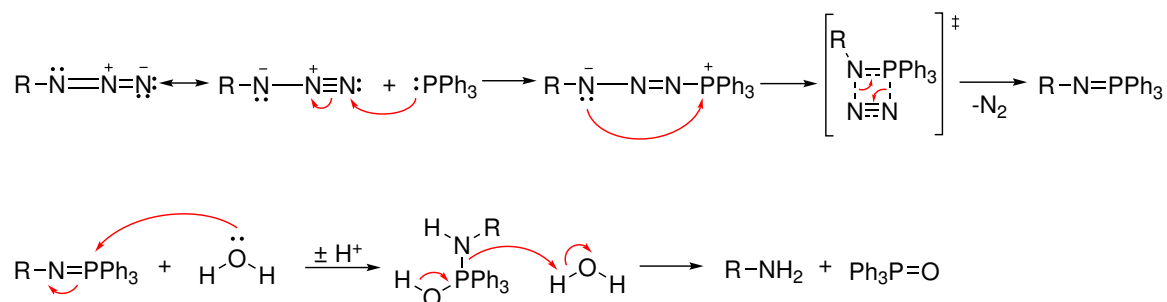
Scheme 2.8: Proposed mechanism for the Staudinger reaction for reduction of an azide to the corresponding amine.[31d]

Caution

Organic azides are energy rich molecules and potentially explosive, and should be handled with caution.[28,42] The C/N ratio, the ratio between the total number of carbon atoms in the azide and the total number of nitrogen atoms, should not be less than one. The "rule of six" can also be utilised, meaning that six carbons should be present per energetically functional group like azides. Especially at elevated temperatures the azides can become heat and shock sensitive, so special care must be taken when heating azides.[43]

2.3.2 1,2,3-Triazoles

The thermal Huisgen 1,3-dipolar cycloaddition was for a long time the standard method for synthesising 1,2,3-triazoles (1, Scheme 2.9).[44] This method gives a mixture of the 1,4- and 1,5-substituted triazoles and usually requires high temperatures.[44,45] In 2001 the groups of Sharpless[26] and Meldal[27] independently presented the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) (2, Scheme 2.9), as a solution to these drawbacks. CuAAC is categorised as a "click" reaction and can proceed under mild condition with simple work-up. The method produces only the 1,4-regioisomer and is faster than the Huisgen reaction.
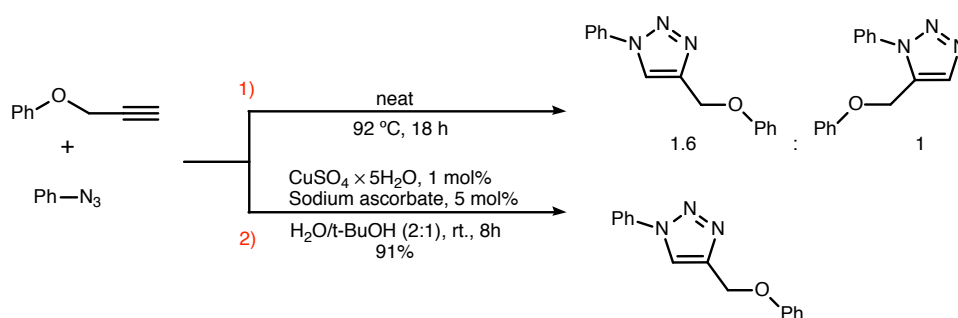
Scheme 2.9: Comparison of the thermal Huisgen 1,3-dipolar cycloaddition (1) and CuAAC (2).[26,27,44]

The complicated properties of copper has made it difficult to establish the reaction mechanism for CuAAC. Based on several studies, a mechanistic cycle has been proposed by Worrell *et al.*,[46] see Scheme 2.10. The reaction begins with the reduction of copper(II) to copper(I) by sodium ascorbate, and copper(I)-coordination to the alkyne π-system (I). This is followed by deprotonation of the alkyne and formation of copper-acetylide (II). The π-bonded copper coordinates to the azide and performs a nucleophilic attack which forms a covalent bond (III $\rightarrow$ IV). This leads to a ring closure and removal of one copper (IV $\rightarrow$ V). The final step is the substitution of the last copper with a proton (V $\rightarrow$ VI).
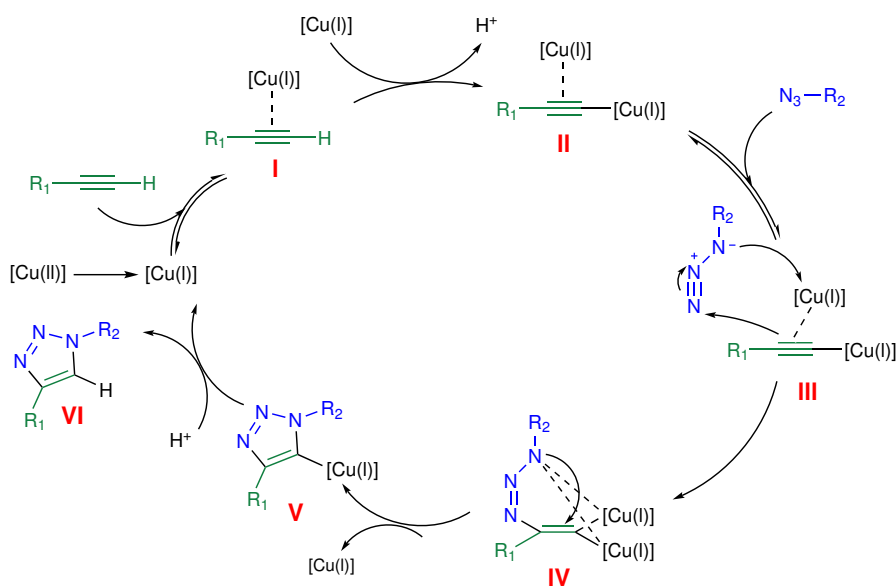
Scheme 2.10: Proposed reaction mechanism for CuAAC: I: Coordination of Cu(I) to the alkyne π-system, I → II: deprotonation of the alkyne, II → III: nucleophilic attack, IV → V: ring closure, V → VI: Product formation.[46]

In addition to being available from "click" chemistry synthesis, 1,2,3-triazoles have shown interesting properties regarding usage in medicinal chemistry.[47] Structurally they are able to mimic amide bonds, making them possible bioisosteres for peptides. Their size, planarity, dipole moment and capability of forming hydrogen bonds are comparable to the peptide bonds.[47,48] The 1,4-substituted 1,2,3-triazole mimics *trans*-amide while the 1,5-substituted triazole is comparable to the *cis*-amide, as shown in Figure 2.3. The 1,2,3-triazoles are also more stable against protolytic and metabolic degradation than peptides.[47,49] As mentioned, a variety of 1,2,3-triazoles have shown antimicrobial activity.[25] This makes compounds with this functionality attractive for usage in antimicrobial peptide mimics.

trans-Peptide bond   [1,4]-1,2,3-Triazole   *cis*-Peptide bond   [1,5]-1,2,3-Triazole
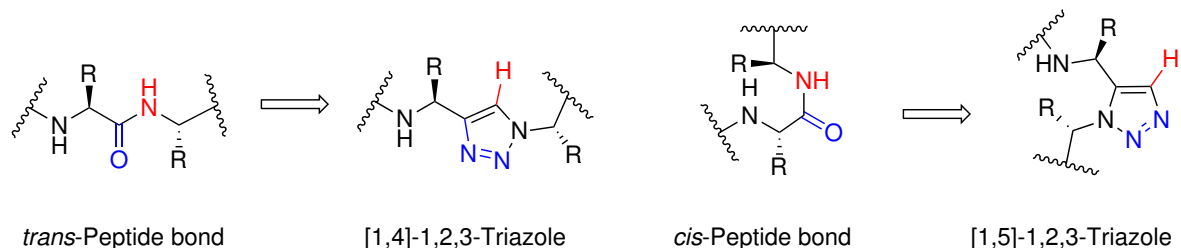
Figure 2.3: Illustration of the similarities between 1,2,3-triazoles and amide bonds. Hydrogen-bond acceptors are shown in blue and hydrogen-bond donors are shown in red.[47]

2.3.3 Hydrolysis of Esters

Hydrolysis of esters is a well studied and utilised reaction within carbonyl chemistry.[50] The reaction can be both acid- and base-catalysed, and gives the corresponding carboxylic acid or carboxylate anion. In acid-catalysed hydrolysis, protonation of the carbonyl oxygen makes the carbonyl group more electrophilic and prone for nucleophilic attack by water. In the base-catalysed reaction the more reactive nuchleophile OH^- is used instead of water.[31a] Unlike the acid-catalysed reaction, hydrolysis under basic conditions is irreversible. This is because the formed carboxylate anions are stable under the reaction conditions.[50] A mechanism for the base-catalysed ester hydrolysis is shown in Scheme 2.11.[50]
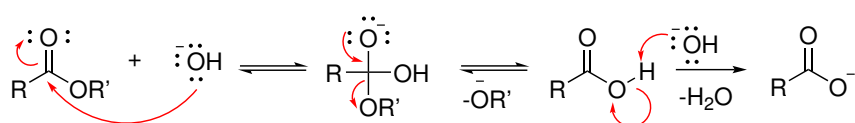
Scheme 2.11: Proposed mechanism for the base-catalysed hydrolysis of an ester.[50]

The first step is a nucleophilic attack on the carbonyl group of the ester.[50] Next the tetrahedral intermediate dissociates with elimination of an alkoxide ion and the carbonyl is regenerated. Proton transfer gives the carboxylate anion in the last and irreversible step. To form the corresponding acid, acidic work-up is necessary.

2.3.4 Guanylation

Guanidines are molecules with the general formula $R_1\text{-}N\text{=}C(NR_2R_3)(NR_4R_5)$, and are important organic molecules due to their wide range of properties.[51,52] These molecules are both found in nature and can be synthesised using different methods. Due to the resonance stabilisation of the conjugate acid, guanidines are categorised as superbases.[53] They have therefore been used in many based-catalysed organic reactions. Guanidines have also shown to be important in medicinal chemistry. Synthetic guanidines have shown antibacterial activity,[12,54] the ability to be used as cardiovascular drugs,[51] anti-influenza agents,[55] etc. A commonly used reagent for guanylation is 1*H*-pyrazole carboxamidine hydrochloride. The use of this reagent was first presented by Bernatowicz *et al.* in 1992 for use in peptide synthesis.[56,57] The most common conditions for guanylation of free amines are the amine and 1*H*-pyrazole carboxamidine hydrochloride in equimolar amounts, a base and DMF as the solvent. This method often resulted in trouble with the work-up, and purification with the use of chromatography or other techniques were necessary. A new method established within the Gautun research group involves

the use of MeCN as solvent, and the amine and 1*H*-pyrazole carboxamidine hydrochloride in a ratio of 1:0.9 - 1:0.99.[57] The guanylation reagent can be difficult to remove, which makes a small excess of the amine beneficial. A proposed mechanism for the guanylation of a primary amine with 1*H*-pyrazole carboxamidine hydrochloride is given in Scheme 2.12.[58] The amine act as a nucleophile and is added to the guanidyl carbon forming a tetrahedral intermediate. A hydrogen shift and elimination of the 1*H*-pyrazole ring, result in the final monosubstituted guanidine salt.
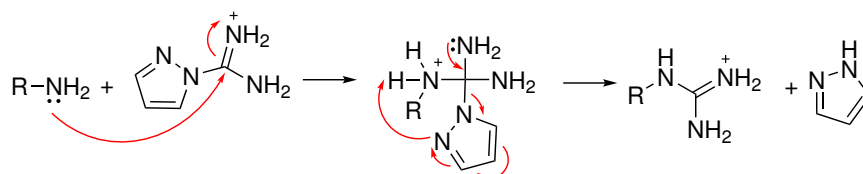
Scheme 2.12: Proposed mechanism for the guanylation of a primary amine with 1*H*-pyrazole carbox-amide hydrochloride. Counter ion: Cl⁻.[58]

In addition, *t*-Boc protected guanylation reagents are widely used for the preparation of guanidines, see Figure 2.4.[59] By using these type of reagents, purification by chromatography methods becomes easier.
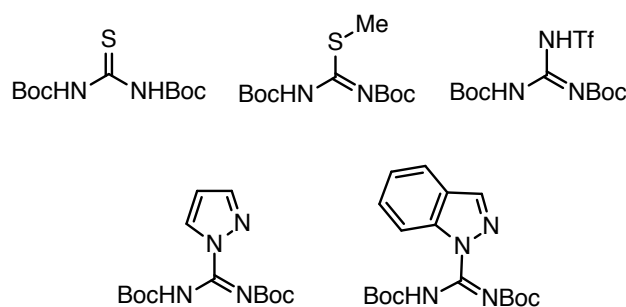
Figure 2.4: *t*-Boc protected guaylation reagents.[59]

2.3.5 Protection and Deprotection of Amino Groups

The use of protecting groups is important in organic synthesis, specially in multistep synthesis. These groups have a passive role in the synthesis and are used to get less reactive groups to react in the presence of more reactive groups.[60] Three main considerations are decisive when choosing protecting groups: which reaction conditions the group has to be stable under, the nature of the functional group that need protection and which conditions that are required for the removal of the protecting group.

It can be necessary to reduce the nucleophilic character of amines.[60] Protecting groups can then be utilised. Acylation with the use of carbamates is a often used method for this purpose. Two of the most used carbamates are *t*-butoxycarbonyl (*t*-Boc) and benzyloxycarbonyl (Cbz), see Figure 2.5 for the structures.
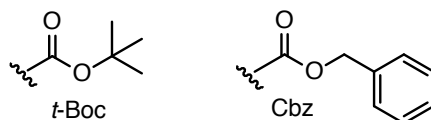
Figure 2.5: Structure of *t*-butoxycarbonyl (*t*-Boc) and benzyloxycarbonyl (Cbz), two of the most used carbamates for protection of amine groups.

In this thesis, 2-(Boc-oxyimino)-2-phenylacetonitrile was used to introduce *t*-Boc. Based on the general mechanism for aminolysis of esters,[61] and the fact that the byproduct 2-hydroxyimino-2-phenylacetonitrile has been observed,[62] a proposed mechanism is shown in Scheme 2.13. In the first step the nucleophilic amine attacks the carbonyl site of the Boc-reagent forming a tetrahedral intermediate.[61] This is followed by removal of the alkoxy group, regeneration of the carbonyl and deprotonation.
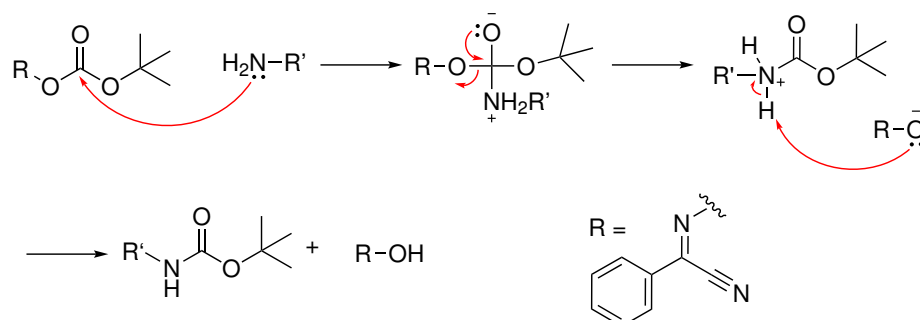
Scheme 2.13: Proposed mechanism for the introduction of the *t*-Boc protecting group to an amine with the use of 2-(Boc-oxyimino)-2-phenylacetonitrile as Boc-reagent.[61,62]

The *t*-Boc protecting group is normally removed through acid-hydrolysis. Either a large excess of an acid like trifluoroacetic acid (TFA) or a smaller amount of a stronger acid like HCl can be used.[63] When HCl is used, Ashworth *et al.* found the reaction rate for the deprotection to be second-order upon the concentration of HCl. A possible reaction mechanism is shown in Scheme 2.14.
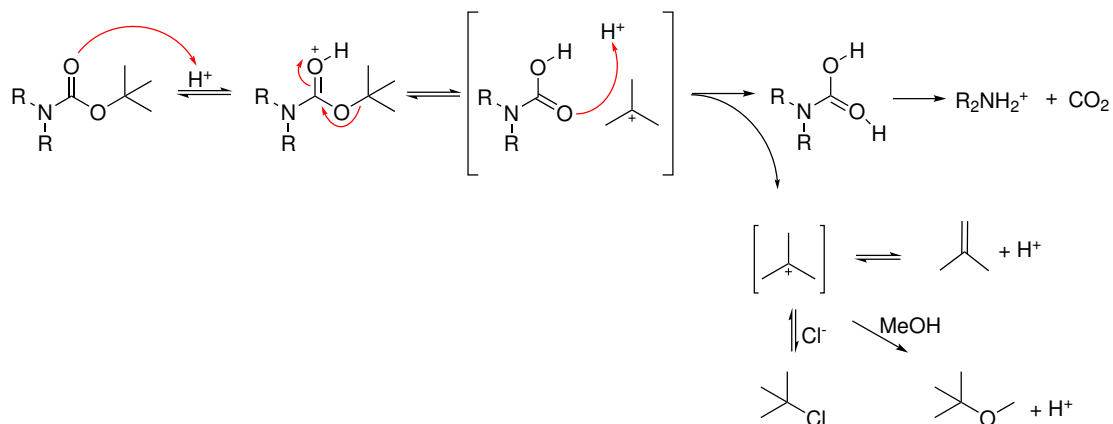
Scheme 2.14: Proposed mechanism for acid-catalysed deprotection of a *t*-Boc protected amine. [63]

The first step of the deprotection is a protonation of the carbonyl oxygen. [63] This is followed by a reversible fragmentation affording a molecular-ion pair. A second prototonation within the molecular-ion pair gives the protonated carbamic acid, in addition to the *tert*-butyl cation. Decarboxylation yields the desired ammonium salt. The *tert*-butyl cation can react with Cl⁻ or the alcohol used as solvent, or give the alken in an elimination reaction.

3 Results and Discussion

First in this section, the synthesis of the key intermediate 1,2,3-triazole methyl ester **4d** will be presented (Section 3.1). The second part covers the preparation of the target compounds **7**[*] and **8** (Figure 3.1), with a focus on developing a new synthetic route towards guanidines **8** (Section 3.2). The following part consists of the synthesis of the branched ammonium salts **5**[*] and the attempted synthesis of bisguanidine **14a** (Figure 3.1), where two cationic *N*-functionalities have been introduced (Section 3.3). The last section includes the many approaches that were made to establish a synthetic route towards target bisammonium salt **12**[*]**a** (Section 3.4).

a: Ar = 4-*tert*-butylphenyl
b: Ar = 2,4,6-triisopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-tri-*tert*-butylphenyl
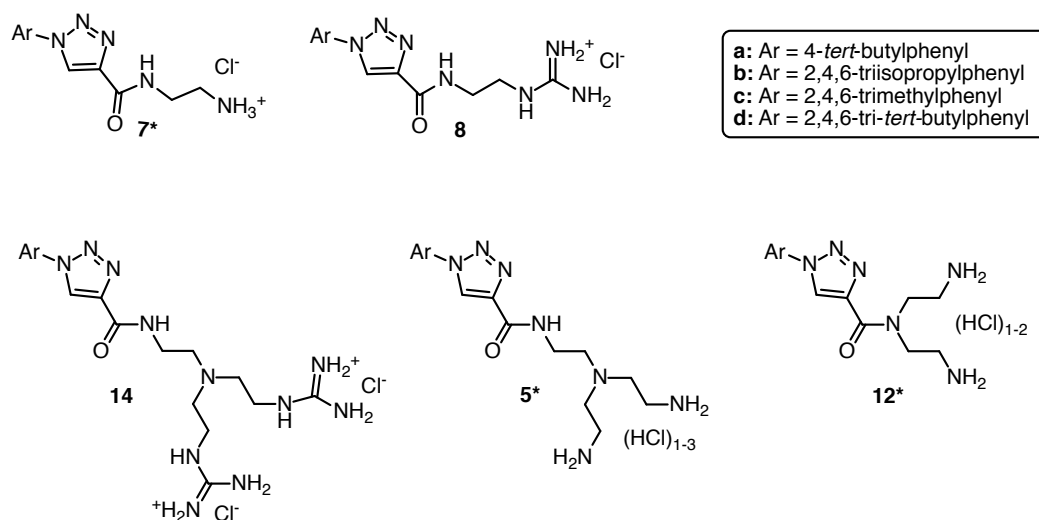
Figure 3.1: Structure of target molecules **5**[*], **7**[*], **8**, **12**[*] and **14**.

3.1 Preparation of Key Substrate 1,2,3-Triazole Methyl Ester 4d

The 1,2,3-triazole methyl esters **4** are the key intermediate for all of the target compounds, and can be *N*-functionalised to yield the potentially biologically active amphiphiles. Triazole ester **4a** (Ar = 4-*tert*-butylphenyl), **4b** (Ar = 2,4,6-triisopropylphenyl) and **4c** (Ar = 2,4,6-trimethylphenyl) have been prepared earlier in this project,[16,17] thus only the synthesis of **4d** will be included, see Scheme 3.1. Azidation of the corresponding aniline **2d** afforded azide **3d**. A copper(I)-catalysed azide-alkyne cycloaddition between **3d** and methyl propiolate yielded **4d**.
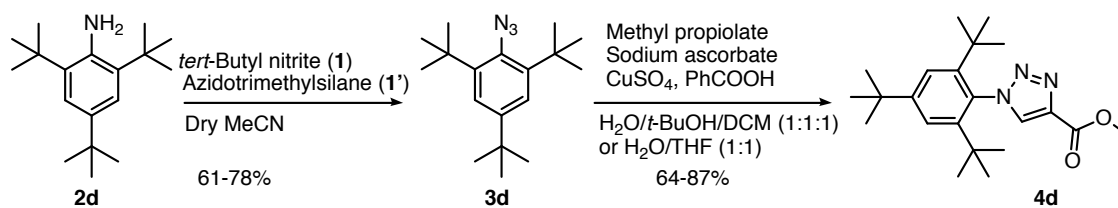
Scheme 3.1: The synthetic route towards 1,2,3-triazole methyl ester **4d**.

3.1.1 Synthesis of Azide 3d

Previous work in the Gautun research group have prepared azides from the corresponding anilines using sodium azide and sodium nitrite in aqueous solution.[16,17] It is observed that azidation of sterically hindered anilines in aqueous conditions, gives a significant amount of the corresponding phenol in addition to the azide.[17] The azidation of **2d** was therefore performed in anhydrous conditions with the use of dry MeCN, *tert*-butyl nitrite (**1**) and azidotrimethylsilane (**1'**). Azide **3d** was synthesised three times following a procedure described by Ching *et al.*[64] The experimental procedure is presented in Section 6.2, and the reaction conditions and results are summarised in Table 3.1.

Table 3.1: Reaction conditions and results for the synthesis of **3d**.

Entry	**2d** [g]	**1** [a] [equiv]	Time 0 °C [min] [b] Time r.t. [h] [b]	**1** [c] [equiv]	Time 0 °C [min] [d] Time r.t. [h] [d]	**3d** [g, %]
1	0.41	1.5	20 95	1.4	120 24	0.28, 63
2	0.41	2.9	15 45	1.5	0 89	0.27, 61
3	2.05	1.5	10 46	1.4	0 45	1.77, 78

[a] First portion of **1**.
[b] Time before the second portion of **1** was added.
[c] Second portion of **1**.
[d] Time after the second portion of **1** was added.

According to the literature procedure,[64] stirring overnight at room temperature with the same reaction conditions as used in entry 1 (Table 3.1), gave **3d** in 75% yield. However, ^{1}H NMR analysis of the reaction mixture from entry 1 after 94 h at r.t., showed 85% unreacted **2d** (Appendix A.2). Another portion of both **1** (1.4 equiv) and **1'** (1.1 equiv) were therefore added. This afforded a yellow precipitate. After adding extra reagents, the reaction mixture was stirred for additional 120 minutes at 0 °C. This because gass formation and foaming were observed when trying to heat the mixture to r.t. Some of the precipitate dissolved after 10 minutes at r.t. After stirring for additional 24 h, ^{1}H NMR analysis, see Appendix A.3, showed only 10% of **2d** so the reaction was stopped. Work-up and purification by column chromatography afforded **3d** in 63% yield as a white crystalline solid.

Since the extra portion added of **1** and **1'** seemed to increase the conversion, it was tried to double the amount of **1** added in the first portion in entry 2 (Table 3.1). In previous successful preparations of azides **3**, the reaction mixture was stirred a while forming the diazonium ion before the azidation agent was added.[17] After the addition of **1** in entry 2, the mixture was therefore stirred at r.t. for 2.5 h before **1'** was added. However, ^{1}H NMR analysis after 24 h did not show any product formation. The mixture was stirred for additional 21 h, before a second portion of **1** and **1'** were added. Again the new portion of reagents afforded formation of a thick precipitate. Further stirring for 89 h, work-up and purification by column chromatography afforded **3d** in 61% yield.

For entry 3 the same method as entry 1 was used, but the reaction was performed in larger scale (2 g). In addition, the second portion of reagents were added after a shorter reaction time, see Table 3.1. Again, precipitation and foaming were observed when adding the second portion.

Baranton and Belanger have studied the *in situ* reaction between amines and *tert*-butyl nitrite in anhydrous conditions.[65] They observed that the formation of diazonium ions are much slower compared to the same reaction in aqueous media. Based on this, they propose that a coupling reaction take place between the formed diazonium cation and the amine. This contributes to a longer reaction time. The same coupling reaction is previously described by Wistar and Bartlett.[66]

This observation, in combination with **2d** being a sterically hindered molecule, can explain the long reaction time for the azidation. The reason why it was not possible to reproduce the result from the literature procedure is not known.

3.1.2 Synthesis of Triazole Methyl Ester 4d

The preparation of **4d** was performed as described by Bakka *et al.* with modifications.[24] The experimental procedure is presented in Section 6.3. The reaction conditions and results are summarised in Table 3.2.

Table 3.2: Reaction conditions and results for the synthesis of **4d**.
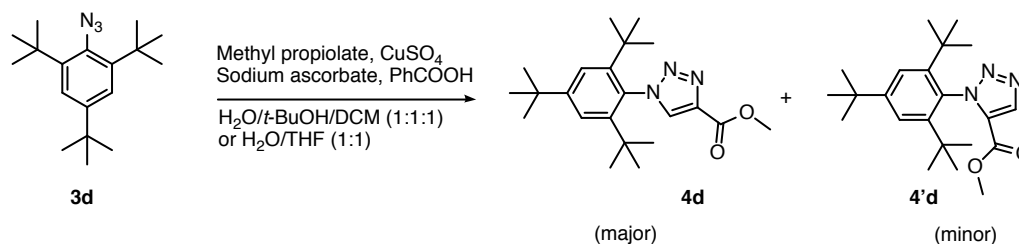
Entry	3d [g]	Methyl propiolate [equiv]	Solvent	Temp. [°C]	Time [h]	4d : 4'd[a]	4d + 4'd [g, %][b]
1	0.25	0.95	H_2O/t-BuOH/DCM	r.t.	126	96 : 4	0.06, 64
2	0.26	2 + 2	H_2O/THF	50	48	95 : 5	0.25, 74
3	1.56	2 + 2	H_2O/THF	50	70	95 : 5	1.73, 87

[a] Ratio determined by ^{1}H NMR analysis of the crude, see Appendix B.1-B.3.
[b] Yield calculated based on all the fractions from the column consisting of **4d**, **4'd** or a mixture of both of them.

Due to **3d** being insoluble in t-BuOH/H_2O, the solvent mixture previously used for the "click reaction", azide **3d** was dissolved in DCM and added to the rest of the reagents in t-BuOH/H_2O. A mixture of THF/H_2O was used as the solvent for entry 2 and 3 (Table 3.2) without problems.

Even after stirring for 126 h at room temperature, TLC analysis indicated that both **3d** and methyl propiolate was present for entry 1 (Table 3.2). The temperature was therefore increased to 50 °C for entry 2 and 3. In addition, a total of 4 equivalents of methyl propiolate were added in two portions. Starting material **3d** was still present after stirring for 48 h (entry 2) and 70 h (entry 3), determined from ^{1}H NMR analysis. Nevertheless, the reaction was stopped and purification by column chromatography afforded the product in 74-87% yields. It can be seen from the results (Table 3.2) that the yield increased with the reaction time. The long reaction time is probably due to steric hindrance in **3d**.

The ^{1}H NMR spectra of the crude products (Appendix B.1-B.3), showed presence of a byproduct. Even though the CuAAC reaction is known to give only the 1,4-regioisomer of the triazole,[26,27] it was suspected from ^{1}H NMR analysis that the byproduct was the 1,5-regioisomer **4'd** (Table 3.2). The IR spectrum of the mixture, see Appendix B.10, shows two strong peaks

at 1743 and 1730 cm^{-1}, which coincides with the formation of two different carbonyl groups. From column chromatography and recrystallisation, both **4d** and **4'd** were isolated. This made it possible to characterise them separately. The MS spectra, see Appendix B.12 and C.8, confirmed that the two compounds have the same mass. Full characterisation by NMR, including NOE-experiments, supports the theory about both regioisomers being formed.

The NOESY spectrum for the main product, see Figure 3.2, shows coupling between H-4 and H-8 indicating that this was the 1,4-regioisomer **4d**. The NOESY spectrum of the byproduct (Figure 3.3), does not show this coupling, but shows a coupling between H-1' and H-8'. This supports the theory about this being the 1,5-regioisomer **4'd**. Presumably, the steric hindrance in the molecule, in combination to long reaction time, may be the reason for **3d** resulting in the formation of both regioisomers.
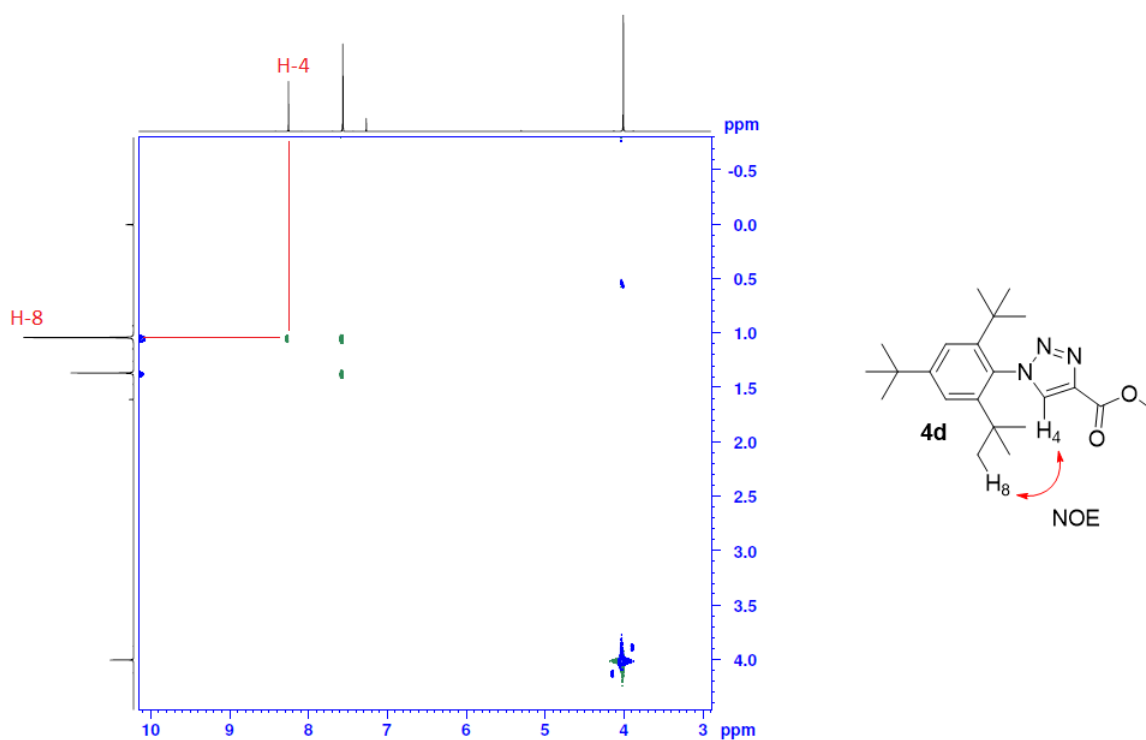
Figure 3.2: A part of the NOESY spectrum, see Appendix B.9, for the main product indicating that it is the 1,4-regioisomer **4d**.
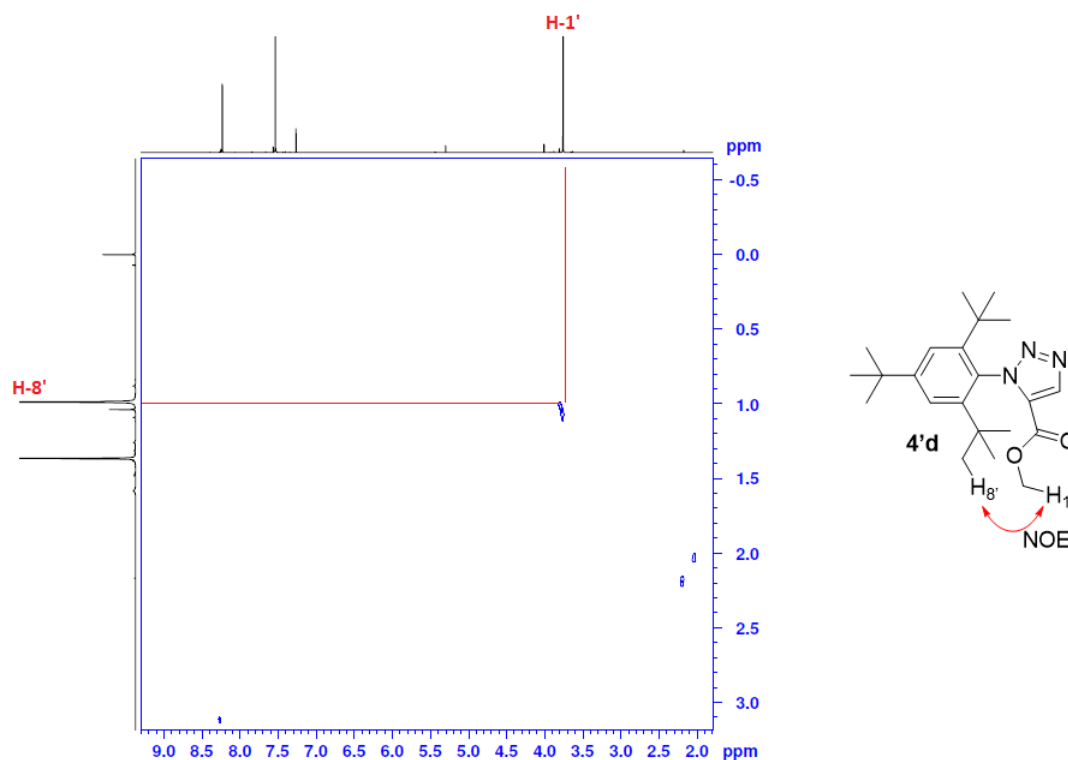
Figure 3.3: A part of the NOESY spectrum, see Appendix C.6, for the byproduct suspected to be the 1,5-regioisomer **4'd**. The NOE coupling between H-1' and H-8' supports this theory.

3.2   Preparation of Target Compounds 7*and 8

After the 1,2,3-triazole scaffold **4** had been prepared, the next step was the synthesis of the monobranched target compounds **7*** and **8**, see Scheme 3.2. The aim of the project is to prepare compounds that are to be tested for antimicrobial activity. A requirement for biological testing is a HPLC purity of 95% or higher.[12] The main focus have therefore been the purity of the compounds, and not the yields. Earlier in this project,[17] attempts of preparing the guanidine **8c** have afforded a product mixture. One goal has therefore been to establish a more expedient synthetic route towards the target guanidines **8**. This include preparation of **8** from both amine **7** and the Boc-protected guanidine **8'** (Section 3.2.3).
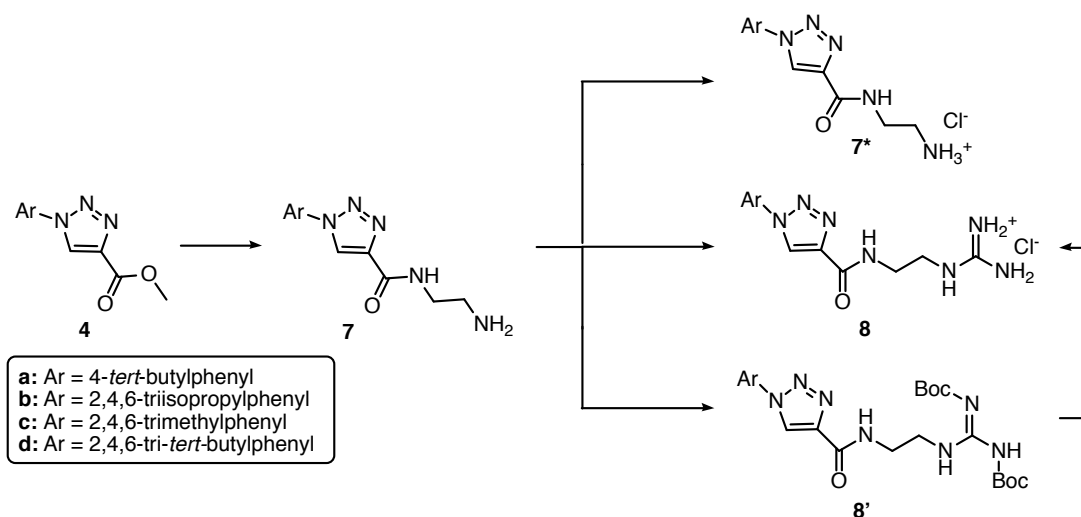
Scheme 3.2: Synthesis of target molecules **7*** and **8** with *N*-functionalisation of **4**.

3.2.1   Synthesis of Ammonium Salts 7*

The ammonium salts **7*a** (Ar = 4-*tert*-butylphenyl), **7*b** (Ar = 2,4,6-triisopropylphenyl) and **7*c** (Ar = 2,4,6-trimethylphenyl) have been prepared pure enough for antimicrobial testing earlier in this project (Scheme 3.2).[16,17] Target compounds **7*c** and **7*d** (Ar = 2,4,6-tri-*tert*-butylphenyl) were synthesised following a two steps procedure developed within the research group.[12] The purpose of synthesising **7*c** was to purify **7c** prior to the synthesis of **8c**.

First, triazole ester **4** was *N*-functionalised with ethylenediamine (EDA) affording **7**. The reaction conditions and results are summarised in Table 3.3. A detailed experimental procedure is presented in Section 6.4.

Table 3.3: Reaction conditions and results for the synthesis of **7**.

c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-tri-*tert*-butylphenyl
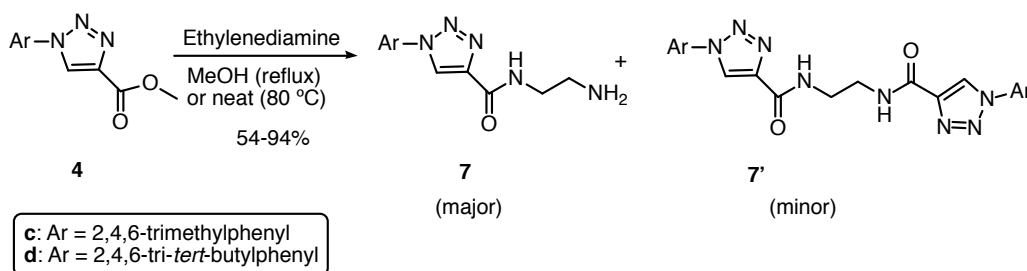
Entry	Substrate [g]	EDA [equiv]	MeOH [mL]	Time [h]	7 : 7'	Yield 7 [g, %]
1	**4c** (0.50)	15	10.5	1	97.4 : 2.6 [a]	0.50, 94
2	**4c** (2.10)	90	-	1	99.0 : 1.0 [a]	1.80, 76
3	**4d** (0.11)	100	-	2.5	- [b]	0.06, 54
4	**4d** (0.59)	100	-	5	- [b]	0.54, 86

[a] Ratio determined by ^{1}H NMR assuming δ_H 8.82 ppm corresponds to **7'c**, see Appendix I.1 and I.2.

[b] The dimer **7'd** was not observed by MS analysis.

Amine **7c** was first prepared using 15 equivalents of EDA and MeOH as solvent (entry 1, Table 3.3). ^{1}H NMR analysis showed an extra peak at δ_H 8.82 ppm. The same impurity has been observed in this reaction before.[16,17] It was suspected to be the dimer **7'c**, and the presence of **7'c** was confirmed by MS analysis, see Appendix I.4. From ^{1}H NMR analysis, the percentage of the dimer were calculated to be 2.6%. According to statistics, increasing the amount of EDA will decrease the probability of forming dimers. The next amidation of **4c** (entry 2, Table 3.3) was therefore performed in neat EDA (90 equiv). This reduced the amount of the dimer **7'c** to 1.0%. Amine **7c** was to be used for the preparation of target molecule **8c**, and the purity was therefore essential. Even though the ^{1}H NMR spectra contained only trace impurities, it was decided to purify **7c** by making the corresponding HCl-salt. In addition, the product from the second synthesis was purified by recrystallisation in toluene to removed the weak yellow colour of the crude.

Amine **7d** was prepared twice in neat EDA (100 equiv, entry 3 and 4, Table 3.3). The ^{1}H NMR spectrum from the first preparation, see Appendix J.1, showed presence of unidentified impurities. Instead of purifying this product it was decided to repeat the amidation of **4d** in larger scale (entry 4, Table 3.3) and purify that product instead. However, the product from the second synthesis did not have the same impurities. The ^{1}H NMR spectrum, shown in Appendix J.2, showed only trace impurities and purification was not attempted. Unlike **7c**, the dimer **7'd** was not observed by MS analysis. The structure of **7d** was determined by one- and two-dimensional NMR, see Section 5. Detailed experimental data and corresponding spectral

data are given in Section 6.4.

The following protonations of **7c** and **7d** with 10-11 equivalents of HCl (37% aq.) were successful and gave the target molecules **7***c** and **7***d**, see Scheme 3.3.

c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-tri-*tert*-butylphenyl
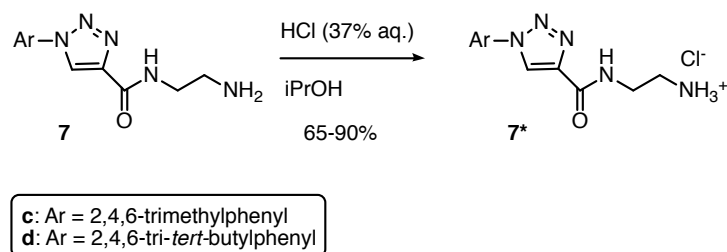
Scheme 3.3: Synthesis of ammonium salts **7***.

Protonation of **7c** was successfully done twice. Purification by washing with MeCN or MeOH afforded **7***c** in 90% and 65% yields respectively. Since Grøndahl has prepared **7***c** pure enough for antimicrobial testing,[16] the purpose of the protonation was, as mentioned, to purify amine **7c** prior to the synthesis of **8c**, see Section 3.2.3. The ^{1}H NMR spectra of **7***c**, shown in Appendix K.1 and K.2, indicated pure products and were in accordance with reported spectra.[16] HPLC (XDB-C18, MeOH/H_2O: 50/50 + 0.1% TFA in the water) showed a purity of $> 99\%$, see Appendix K.3.

For the protonation of **7d**, recrystallisation of the crude product in iPrOH afforded **7***d** in 69% yield, and a HPLC purity of $> 99\%$, see Appendix L.9. With a HPLC purity over 95% and a ^{1}H NMR spectrum indicating pure product (Appendix L.1), ammonium salt **7***d** will be submitted for antimicrobial testing at a later point. The structure of **7***d** was determined by one- and two-dimensional NMR, see Section 5. Detailed experimental procedure is presented in Section 6.5.

3.2.2 Synthesis of the Boc-protected Guanidines 8'

The Boc-protected guanidines were prepared following a general procedure described by Drake *et al.*[67] The experimental procedure is presented in Section 6.6 and the reaction is given in Scheme 3.4. The purpose of synthesising **8'** was later to remove the Boc-groups to give target compounds **8**. The Boc-protected guanidine **8'a** (Ar = 4-*tert*-butylphenyl) was not synthesised since **8a** has been prepared pure enough for antimicrobial testing before.[16]
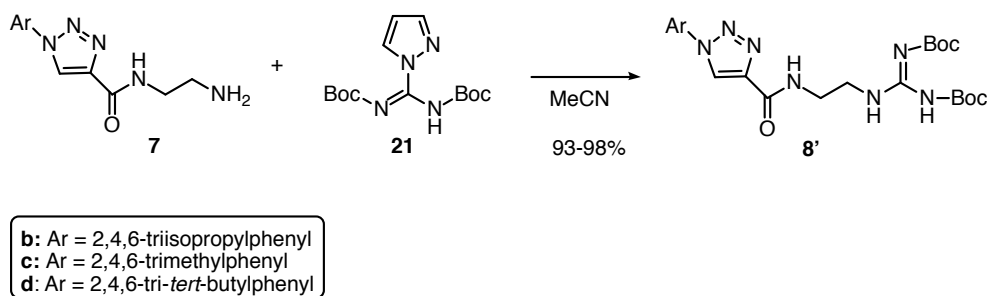
Scheme 3.4: Synthesis of Boc-protected Guanidines 8'.

The Boc-protected guanidines **8'b-d** were prepared in excellent yields (93-98%) after purification by column chromatography (40% EtOAc in DCM). Compound **8'c** (Ar = 2,4,6-trimethylphenyl) was prepared twice. Two columns were used for the purification in the first synthesis. This because the first column (40% EtOAc in *n*-pentane) resulted in overlapping fractions of **8'c** and the byproduct pyrazole. A white precipitate was formed after 5-10 minutes both in the synthesis of **8'b** (Ar = 2,4,6-triisopropylphenyl) and **8'd** (Ar = 2,4,6-tri-*tert*-butylphenyl). This made it difficult to maintain the stirring, and additional MeCN was therefore added. The precipitates were presumably the desired products, meaning this were fast reactions. TLC analysis indicated full conversion after 30 minutes for the synthesis of **8'b**. The R_f-values of **8'd** and the Boc-reagent **21** were almost the same, making it difficult to monitor the reaction. After 1.5 h the reaction was stopped and ^{1}H NMR analysis confirmed full conversion. Precipitation was not observed in the preparations of **8'c**, but the reactions were finished after 2-3 h at room temperature.

The ^{1}H NMR-analysis of **8'b-d**, shown in Appendix P.1, Q.1 and R.1, indicated pure products. The structure of **8'b-d** were determined by one- and two-dimensional NMR, see Section 5.

3.2.3   Synthesis of Guanidines 8

The first attempt of synthesising **8c** was carried out in two steps, see Scheme 3.5. The starting material was ammonium salt **7*c** (HPLC purity > 99%, see Appendix K.3). Deprotonation using K_2CO_3 afforded the neutral **7c**. In the second step, the guanylation reagent 1*H*-pyrazole-1-carboxamide hydrochloride was used according to a procedure described by Bakka and Gautun.[57]
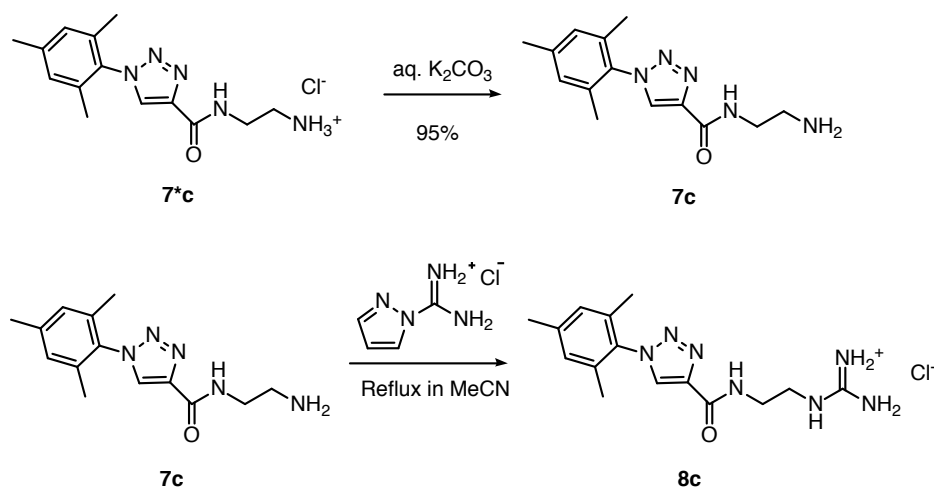
Scheme 3.5: Two steps synthesis of **8c** from **7*c**.

^{1}H NMR analysis of the crude product confirmed **8c**, but also indicated formation of a byprod-
uct. HPLC (XDB-C18, MeOH/H$_2$O: 50/50 + 0.1% in the water) showed two compounds
present in a 72 : 28 ratio, see Figure 3.4 and Appendix O.7.
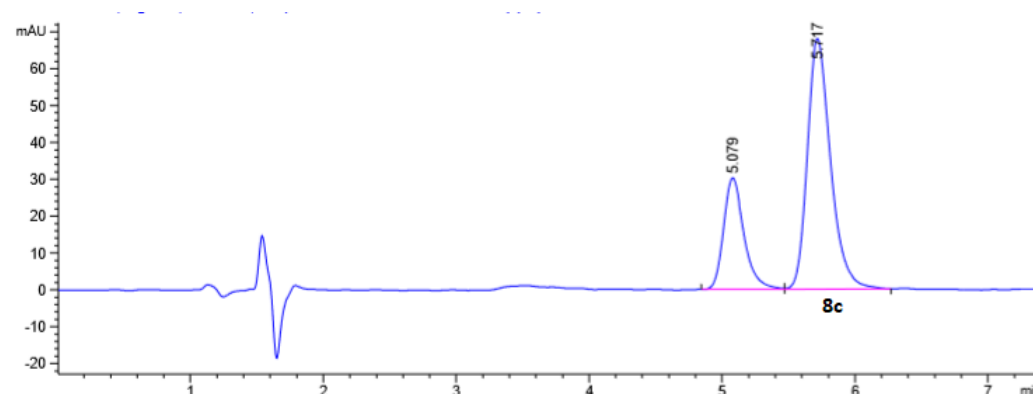
Figure 3.4: HPLC chromatogram (XDB-C18, MeOH/H2O: 50/50 + 0.1% TFA in the water, 1.0
mL/min, λ = 214 nm) of **8c** (t$_r$ = 5.7 min) with an impurity (t$_r$ = 5.1 min), see Appendix O.7.

Previously attempted preparation of **8c** has resulted in formation of the ammonium salt **7*c** in
mixture with **8c**.[17] Coeluation of **7*c** and **8c** confirmed the byproduct to be **7*c**, see Figure 3.5
and Appendix O.8.
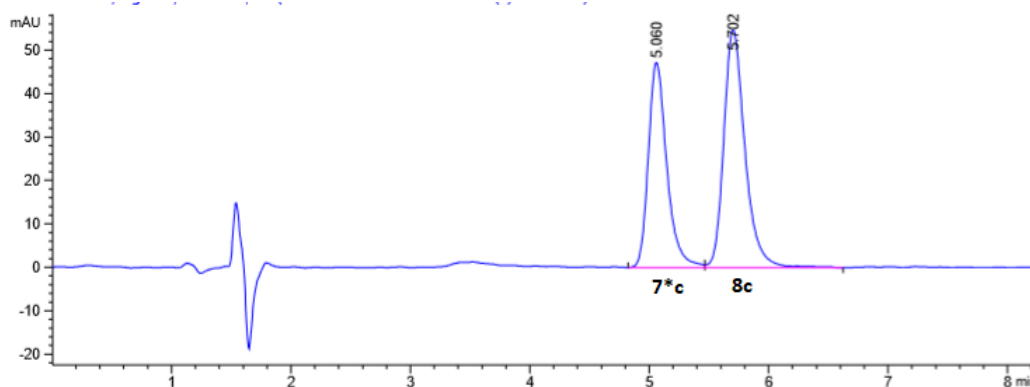
Figure 3.5: HPLC chromatogram (XDB-C18, MeOH/H2O: 50/50 + 0.1% TFA in the water, 1.0 mL/min, $\lambda = 214$ nm) of **8c** ($t_r = 5.7$ min) and **7***c** ($t_r = 5.1$ min), see Appendix O.8.

Guanidine **8a** (Ar = 4-*tert*-butylphenyl) was synthesised in order to compare the reaction with the preparation of **8c** (Ar = 2,4,6-trimethylphenyl), in addition to see if **7***a** (Ar = 4-*tert*-butylphenyl) was formed as well. Starting from **7a** (Ar = 4-*tert*-butylphenyl), guanidine **8a** was prepared following the procedure from a previous synthesis of **8a** in the research group.[16] To remove some of the weak yellow colour, amine **7a** was first washed with H_2O. The off-white **7a** was refluxed in MeCN with 0.98 equivalents of 1*H*-pyrazole-1-carboxamide hydrochloride for 23 h. Purification of crude **8a** by crystallisation from MeOH/Et$_2$O and washing with Et$_2$O afforded **8a** (34 mg, 15%) with a HPLC purity of 98%. However, the ^{1}H NMR spectrum of crude **8a** indicates formation of **7***a** in a 6 : 94 mixture with **8a**, see Appendix M.1.[16] It seems to be an equilibrium between the formation of **8** and **7*** under these reaction conditions. However, the percentage of **7***a** was much lower than **7***c**. In addition it was easier to separate **8a** from **7***a**. Different physical properties of the products make it difficult to predict the outcome of the reactions and how successful the purification is.

Using a new 1*H*-pyrazole-1-carboxamide hydrochloride reagent and copying the reaction conditions described for **8a**, the synthesis of **8c** was repeated, now starting with the neutral **7c**. The reaction mixture was allowed to reflux for 23 h. The partly cooled mixture was filtered and the filtrate was concentrated under reduced pressure, unlike the first synthesis where the precipitate was used. The filtrate did not contain **7***c**, like the precipitate did, so purification by crystallisation from MeOH/Et$_2$O and washing with Et$_2$O successfully yielded **8c** (48 mg, 19%) with a HPLC purity of 99%, see Appendix O.9.

Even with a HPLC purity of **8c** high enough for antimicrobial testing, this method is not good enough. The reaction conditions produce a mixture of **8** and **7*** in different ratios, and a general purification method has not been developed. Another method was therefore tested. Trying to reduce the possibility of forming the ammonium salt **7***c**, preparation of **8c** using the base TEA

and excess of the guanylation reagent was attempted, see Scheme 3.6. The plan was to remove the excess of the reagents with preparative HPLC. Since preparative HPLC was to be used, the reaction was performed directly from the neutral amine **7**.
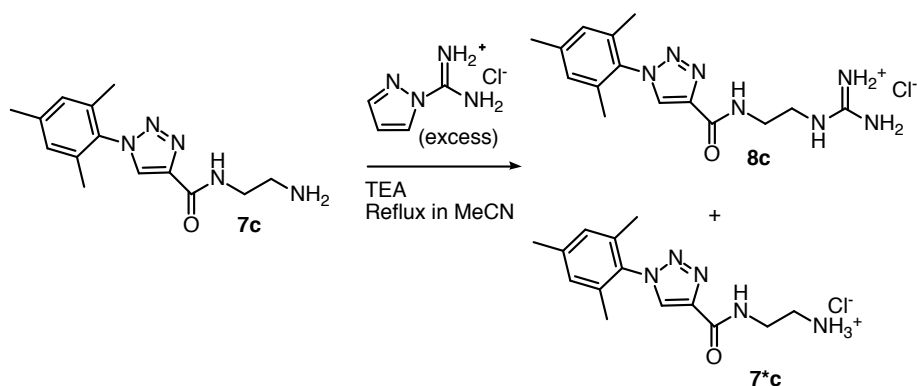
Scheme 3.6: Synthesis of **8c** using excess guanylation reagent and the base TEA.

A total of 4 equivalents of TEA, excess 1*H*-pyrazole-1-carboxamide hydrochloride (2 equiv) and refluxing for 66 h afforded a 94 : 6 mixture of **8c** and **7*c** determined from HPLC, see Appendix O.10. As anticipated, the percentage **7*c** was greatly reduced compared to the reaction without TEA. In an attempt of reacting some **7*c** to **8c**, the crude was refluxed for additional 29 h in MeCN with an extra portion of TEA (5 equiv) and the guanylation reagent (0.6 equiv). However, HPLC analysis showed a large number of unknown impurities, see Appendix O.11, and purification was therefore not attempted.

Another approach towards a better synthetic route for guanidines **8** was tested, this time starting with the Boc-protected guanidines **8'**, see Scheme 3.7.

b: Ar = 2,4,6-isopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
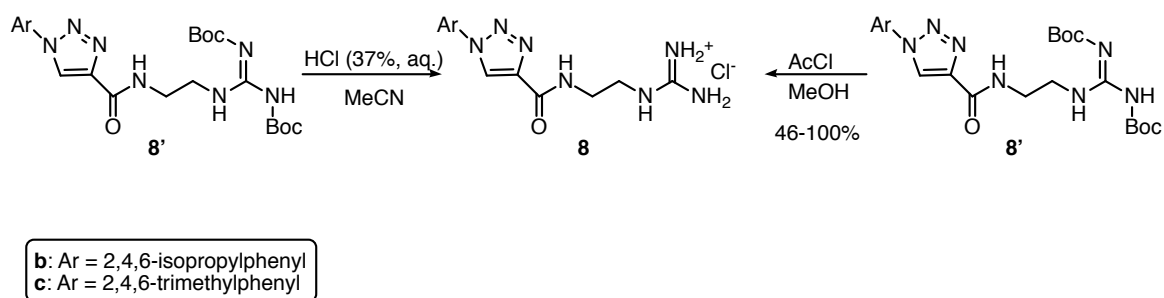
Scheme 3.7: Deprotection of the Boc-protected Guanidines **8'**.

Deprotection of **8'c** was first attempted following a procedure described by Bakka with modifications.[12] HCl (37% aq., 10 equiv) was added dropwise to a solution of **8'c** in MeCN and stirred at r.t. for 18 h. Additional HCl (37% aq., 5 equiv) was added and the stirring continued

for 5.5 h. From the ^{1}H NMR spectrum of the crude it was suspected that some of the intermediate with one Boc-group was still present. The crude was therefore dissolved in MeCN and reacted with HCl (37% aq., 5 equiv) for additional 17 h at 50 °C. Seen from the ^{1}H NMR spectrum, these reaction conditions gave a byproduct. Using this method for Boc-deprotection has previously resulted in formation of a byproduct that could be removed with Kügelrohr distillation.[68] Kügelrohr distillation at 50-60 °C (0.02-0.04 mbar) for 7 h, gave **8c** with a HPLC purity of 99%. However, impurities can still be seen from the ^{1}H NMR spectrum, see Appendix O.4. The broad signal in the aromatic area should have an integral of 7H, but the total integral of this area is 9H. These impurities could be remainings from the byproduct since the ^{1}H NMR of the byproduct, see Appendix O.5, partly overlap with **8c** in the aromatic area. Repeating the distillation could have removed some of the impurity, however this was not done.

Another method for deprotection was tested, following a general procedure described by Hickey *et al.* with the use of AcCl and MeOH.[69] This method was used for Boc-cleavage of both **8'b** and **8'c**. Addition of AcCl to the solution of the Boc-protected guanidines in MeOH generated some heat. For the deprotection of **8'c**, the reaction mixture was stirred for 24 h at room temperature before work-up by coevaporation with MeOH was done. From ^{1}H NMR analysis small impurities was observed, see Appendix O.6. Purification by recrystallisation in toluene and MeCN, crystallisation from MeOH/Et$_2$O, MeOH/MeCN and washing with MeCN was attempted, but not successful in removing the impurities.

For the deprotection of **8'b** a reaction time of 48 h was used based on TLC analysis. ^{1}H NMR analysis after work-up showed almost the same impurities as for **8c**. Based on this, it was again suspected that the byproduct was some unreacted intermediate with only one Boc-group removed. The crude of **8c** and a fraction of the crude of **8b** were therefore reacted with 10 equivalents of AcCl in MeOH for additional 24 h. The ^{1}H NMR spectra of the products after work-up (Appendix O.6 and Appendix N.1), showed only trace impurities. The HPLC purity **8b** and **8c** were > 99% and 99% respectively, see Appendix N.9 and O.15.

According to the literature procedure,[69] the work-up was coevaporation with MeOH. Both **8b** and **8c** became a glassy solid when MeOH was removed under reduced pressure. This made it difficult to remove all the solvent. Due to this, additional coevaporation using a 1 : 1 mixture of MeOH/MeCN (10 mL) was done 4-5 times for each of the guanidines. When this mixture was used, MeOH evaporated of first making the guanidines precipitate as a white solid instead, which were easier to get free from solvent.

The byproduct present in the crude products of **8'** seem to be the intermediate with only one Boc-group removed. Either this means that the first Boc-group is easier to remove than the

second one, or it is due to the fact that acetyl chloride can be consumed in reaction with MeOH (Section 2.3.5). If this procedure is to be used for future Boc-deprotections, it seems to be useful to add an extra portion of AcCl during the reaction. Other, possibly more efficient, methods for removal of the Boc-protection group should also be tested. However, this was not done in this project due to time restrictions.

3.3 Preparation of Target Compounds 5* and 14a

Introducing two cationic nitrogen functionalities decrease the overall lipophilicity of the molecule. The working theory is that this will contribute to a decrease in the toxicity of the amphiphile. The preparation of branched ammonium salts **5*** and bisguanidines **14** are therefore essential, see Scheme 3.8. As mentioned, the purity of the target molecules has been the most important factor, and not the yields. Target compounds **5*b** and **5*c** were synthesised in two steps following a procedure described by Bakka with modifications.[12] Ammonium salt **5*a** has been prepared earlier in this project.[17] Bisguanidine **14a** was attempted synthesised in two steps.
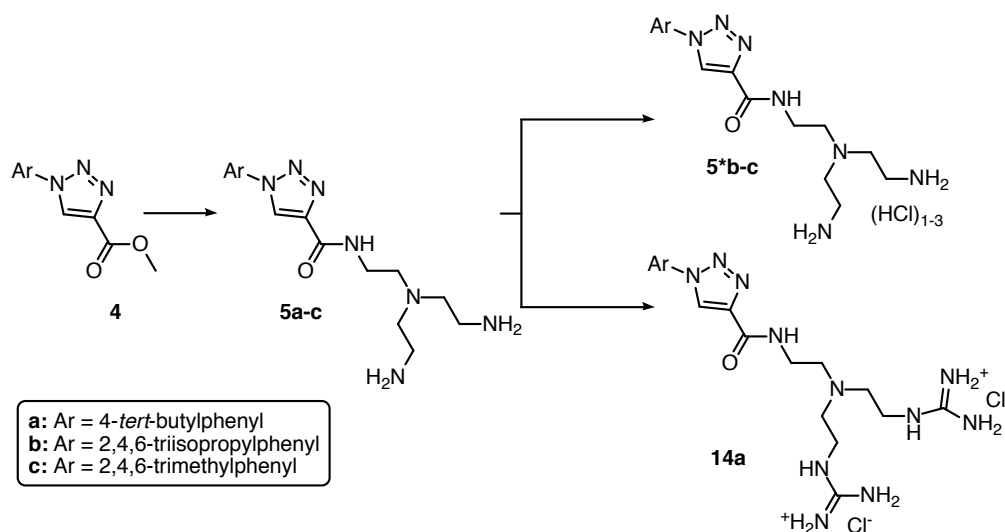
Scheme 3.8: Synthesis of branched ammonium salt **5*** and bisguanidine **14a**.

The first step towards target amphiphiles **5*** and **14** was *N*-functionalisation of the respective triazole esters **4** with tris(2-aminoethyl)amine (**6**) affording the branched amines **5**. Preparation of **5** earlier in this project showed formation of the dimer **5'** (Table 3.4) in an inseparable mixture with **5**.[17] Since neat conditions with 150 equivalents of **6** reduced the percentage of the dimer by 70%, the same conditions were used for the synthesis of **5**. The reaction conditions and results are summarised in Table 3.4.

Table 3.4: Reaction conditions and results for the synthesis of **5**.

a: Ar = 4-*tert*-butylphenyl
b: Ar = 2,4,6-triisopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
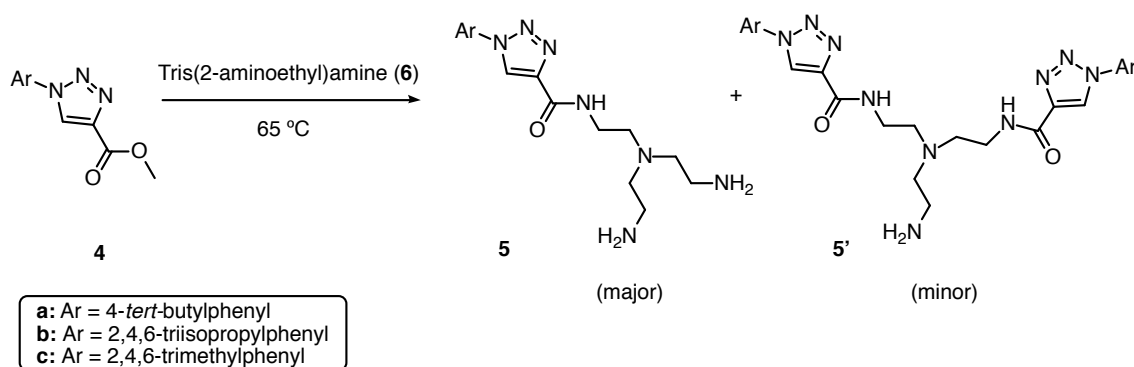
Entry	Substrate [g]	Time [min]	Crude [g]	5 : 5'[a]
1	**4a**: 0.09	60	0.14	99.6 : 0.4
2	**4b**: 0.10	80	0.15	99.5 : 0.5
3	**4c**: 0.10	75	0.16	99.4 : 0.6
4	**4c**: 0.20	60	0.29	99.6 : 0.4

[a] Ratio determined by ^{1}H NMR analysis assuming δ 9.17 ppm corresponds to **5'a**, δ 7.19 ppm corresponds to **5'b** and δ 7.06 ppm corresponds to **5'c**. See Appendix D.1, E.1, F.1 and F.2.

MS analysis confirmed the presence of the dimers **5'**, see Appendix D.3, E.8 and F.4. From ^{1}H NMR analysis, the percentages were found to be only 0.4-0.6% (Table 3.4). The crude of **5** was therefore used without further purification after removal of excess **6** with Kügelrohr distillation.

The ^{1}H NMR spectra for **5a** and **5c** were in accordance with reported spectra.[17] The structure of amine **5b** was determined by one- and two-dimensional NMR, see Section 5. Detailed experimental procedure is presented in Section 6.9. The structure of the dimer **5'b** was not possible to elucidate because all signals with the exception of two, overlapped with the signals for **5b**, in addition to having low intensity.

3.3.1 Synthesis of Branched Ammonium Salts 5*

Protonation of **5b** and **5c** with HCl (37% aq., 25 equiv) afforded the branched ammonium salts **5*b** and **5*c** in 25% and 91% yields respectively, see Scheme 3.9. The lower yield of **5*b** is probably due to washing with MeOH which **5*b** is partly soluble in.
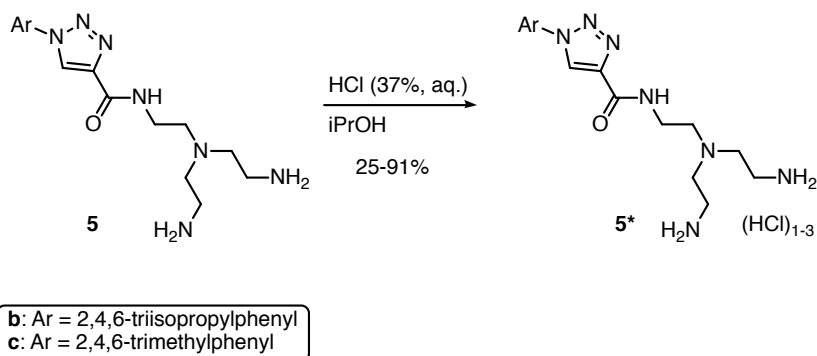
Scheme 3.9: Synthesis of ammonium salts **5**[*].

Up to three of the amine groups of the ammonium salts **5**[*] can be protonated. Only the mono-protonated salts were found by MS analysis for both **5**[*]**b** and **5**[*]**c**, see Appendix G.8 and H.8. This corresponded with the ^{1}H NMR analysis where the shifts of the amine groups integrated to 5.

The ^{1}H NMR spectra of **5**[*]**b** and **5**[*]**c** showed several broad peaks and the structures were difficult to elucidate, see Figure 3.6 (bottom) for a part of the ^{1}H NMR spectrum of **5**[*]**b**. Based on previous success with conducting the NMR experiment at 90 °C, this was done.[17,70] The result was narrower peaks with more separation, making it possible to elucidate the structures, see Figure 3.6 (top). Dynamic processes of the amide CN bond or hydrogen bonding, are probably the reason for this broadening.[71b] The amide bond has partly double bond character resulting in hindered rotation in the molecule. If the temperature is raised, the rotation barrier decreases, thus resulting in less broadening of the peaks.
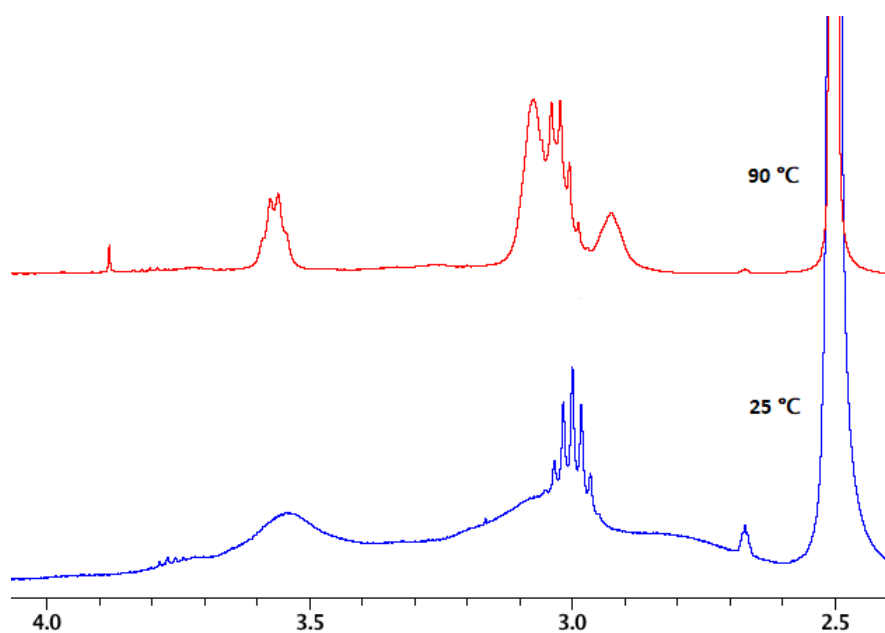
Figure 3.6: Part of the ^{1}H NMR (400, DMSO-d_6) spectrum of **5***b at room temperature (bottom) and at 90°C (top) for comparison of the broadening of the signals, see Appendix G.1 and G.2.

^{1}H NMR analysis of both **5***b and **5***c, see Appendix G.2 and H.2, indicated pure products, and the HPLC purities were > 99%, see Appendix G.9 and H.9. The target amphiphiles thus have a purity high enough to be submitted for antimicrobial testing.

The structures of **5***b and **5***c were determined by one- and two- dimensional NMR, see Section 5. Detailed experimental procedure is presented in Section 6.10.

3.3.2 Attempted Synthesis of Bisguanidine 14a

Bisguanidine **14a** was attempted synthesised from **5a** following a procedure described by Bakka *et al.* with modifications,[24] see Scheme 3.10.
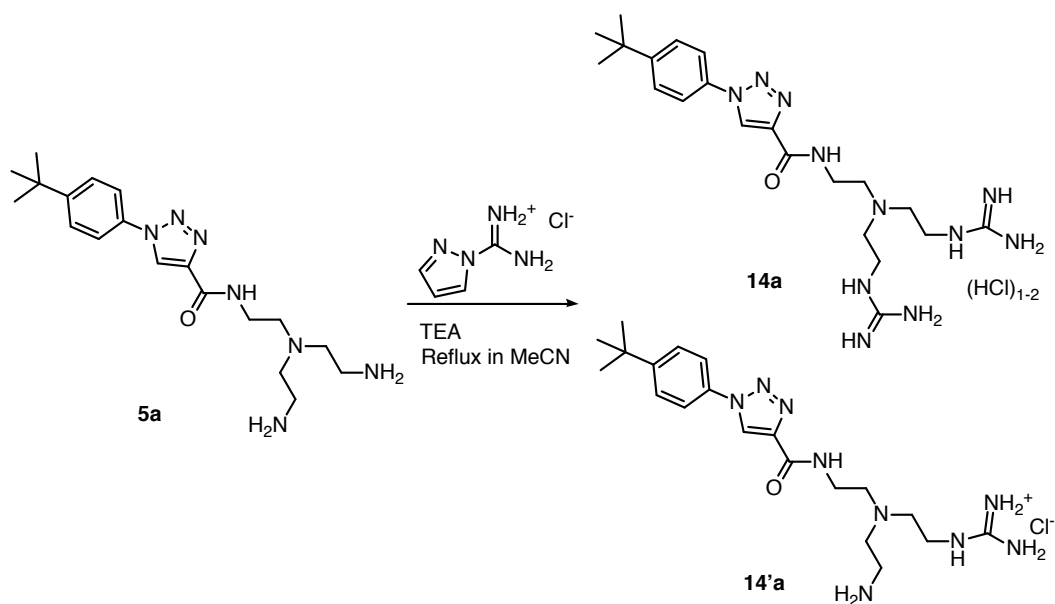
Scheme 3.10: Attempted synthesis of bisguanidine **14a**.

Refluxing **5a** with 1*H*-pyrazole-1-carboxamide hydrochloride (2.2 equiv) in MeCN for 23 h, and washing with MeCN and Et$_2$O afforded a mixture of the monoprotonated **14a**, the diprotonated **14a** and **14'a** (Scheme 3.10), confirmed by MS analysis (Appendix V.2-V.4). An attempt of reacting **14'a** to **14a** was made. The crude of **14a** was dissolved in MeCN, and guanylation reagent (2 equiv) and TEA (6 equiv) were added. The refluxing continued for 26 h. H$_2$O was added due to solubility problems of the crude of **14a** in MeCN. The ^{1}H NMR analysis of the crude shows presence of unidentified impurities. HPLC analysis showed three products in a 5 : 85 : 10 mixture, in addition to excess TEA, see Appendix V.5.

This route towards **14** is not optimal and other synthetic routes should be tested. One possibility could be to use a Boc-protected guanylation reagent as for **8'**. Due to time restrictions, attempts of purifying **14a** or testing other procedures for synthesising **14a** were not done.

3.4 Preparation of Target Compound 12*a

In addition to introduce two cationic nitrogen functionalities in the molecule, it is interesting to see how a more compact system that reduce the flexibility of the molecule, will affect the antimicrobial potency. The preparation of target molecules **12*** is therefore important.

Several approaches were made to develop a reaction path towards **12*a**. The different attempts and results will be presented in Section 3.4.1, 3.4.2 and 3.4.3.

3.4.1 Attempted Synthesis of 12*a from the Branched Bisazide 11a

The first approach at synthesising **12*a** was through the bisazide **11a**, followed by an attempt of reducing the azide groups to amine groups. The plan was to synthesise bisazide **10** and prepare the branched bisazide **11a** by amidation of the triazole ester **4a** with **10**, see Scheme 3.11.
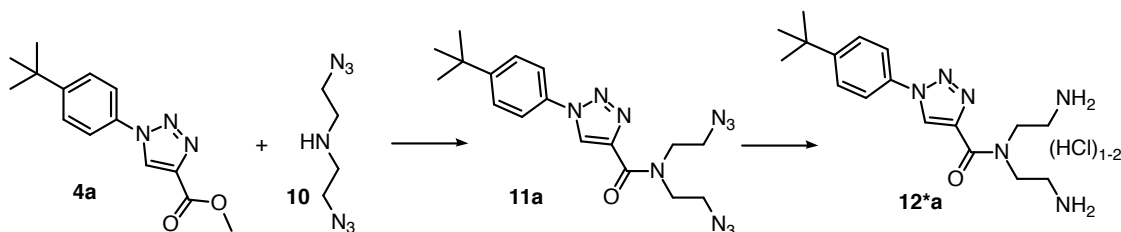
Scheme 3.11: First approach at synthesising **12*a** via **11a**.

First, bisazide **10** was prepared following a procedure by Chen *et al.*,[72] see Scheme 3.12.

Scheme 3.12: Synthesis of bisazide **10**.

Azide **10** has a C/N value less than one, but has previously been successfully prepared.[72] It was therefore decided to try synthesising **10** using the exact same amounts and conditions as the literature procedure. In addition, precautions were taken, for example by using a transparent safety shield. The preparations of **10** were successful and gave **10** as a colourless liquid in 56-63% yields. The ^{1}H NMR spectrum of **10** was in accordance with reported spectra,[43] and is shown in Appendix S.1.
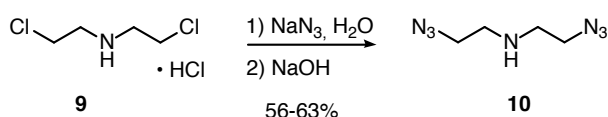
The branched bisazide **11a** was first attempted prepared from the triazole methyl ester **4a** using two different methods, see Scheme 3.13.[24,72] For method 2 (Scheme 3.13), the base K_2CO_3 was added after refluxing for 22-28 h, because TLC analysis indicated no conversion. Even after a total of 3-5 days, two of the reactions showed no conversion, and the last one (using K_2CO_3 and MeOH), showed formation of an unknown product, see Appendix T.1-T.3.
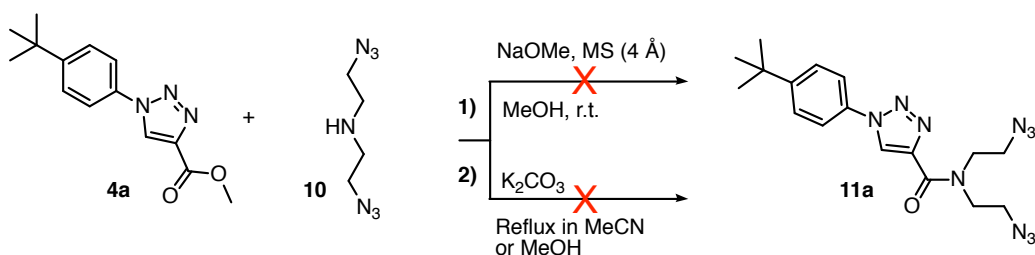
Scheme 3.13: Attempted preparations of **11a** from triazole methyl ester **4a** and **10**.

The unsuccessful reaction between **4a** and **10** may be due to poor reactivity of **4a**. Since acid chlorides are more reactive than the corresponding esters,[73] it was decided to try making acid chloride **16a** instead, and react it with **10**.

The first step towards the acid chlorides **16** was hydrolysis of triazole methyl esters **4** to the corresponding carboxylic acids **15**. The hydrolysis of **4a** and **4c** were performed following a general procedure described by Flynn and Beight,[74] see Scheme 3.14. Acidic work-up afforded the carboxylic acid.

a: Ar = 4-*tert*-butylphenyl
c: Ar = 2,4,6-trimethylphenyl
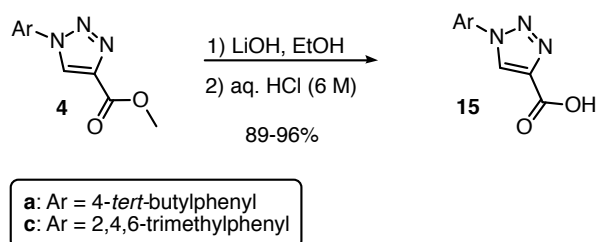
Scheme 3.14: Hydrolysis of triazole methyl ester **4** to the corresponding carboxylic acid **15**.

The hydrolysis of **4a** and **4c** afforded the respective carboxylic acids as white solids in very good to excellent yields (89-96%). The reactions were fast and full conversions were observed by TLC analysis after 60-120 minutes at r.t. The ^{1}H NMR spectra indicated pure products, see Appendix W.1 and X.1. Both **15a** and **15c** are previously synthesised,[75,76] but not described. The structures of **15a** and **15c** were therefore determined by one- and two-dimensional NMR, see Section 5. Detailed experimental procedure and corresponding spectral data are given in Section 6.13.

When **15a** had been prepared, the next step was the synthesis of **16a**, followed by reacting **16a** with **10**. This was successfully done following a general procedure described by Singh *et al.* with modifications,[77] see Scheme 3.15.
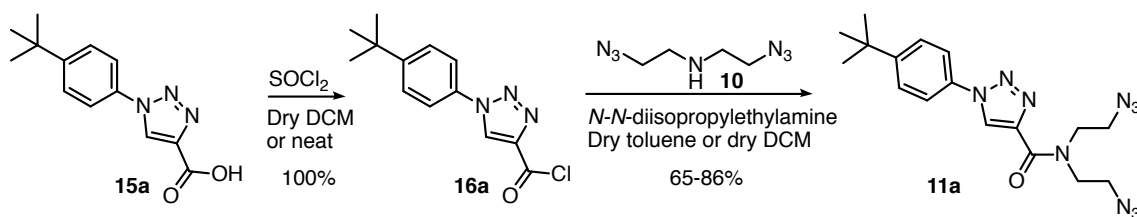
Scheme 3.15: Two steps synthesis of **11a** from the carboxylic acid **15a**.

For the first synthesis of **16a**, DCM was used as solvent with only 3 equivalents SOCl$_2$. Due to poor solubility of **15a** in DCM, additional SOCl$_2$ (30 equiv) was added after 45 minutes. A total reaction time of 4 h and reflux in DCM, gave **16a** in quantitative yield. Since **15a** had higher solubility in SOCl$_2$, neat SOCl$_2$ (22 equiv) and 70 °C was used for the rest of the preparations of **16a**. Again, a reaction time of 4 h afforded **16a** as a white oily solid in quantitative yields.

It was difficult to monitor the reaction due to the instability of **16a**, causing it to hydrolyse back to **15a** on the silica plates used for TLC. However, the IR spectrum (see Appendix Y.3) of the product after 4 h reaction time, indicated full conversion. The broadening of the IR spectrum caused by the presence of an acid group, was no longer present. It was therefore concluded that the reaction was finished. Due to the instability of **16a**, only ^{1}H NMR and ^{13}C NMR spectra were recorded of **16a** in addition to the IR spectrum, see Appendix Y.1-Y.3. The crude of **16a** was used without further purification.

The following reaction between **16a** and **10** was first performed using dry toluene as solvent (entry 1, Table 3.5). Because of poor solubility of **16a** in toluene, dry DCM was used in the two following preparations of **11a** (entry 2 and 3, Table 3.5). The fact that **16a** was soluble in DCM was another confirmation that no **15a** was present. This because **15a** was insoluble in DCM. The reaction between **16a** and **10** successfully gave **11a** as an off-white solid in 65-86% yields after purification by column chromatography. The reaction conditions and results are summarised in Table 3.5.

Table 3.5: Reaction conditions and results for the synthesis of **11a** in two steps from **15a**.

| Entry	**15a** [g]	Crude of **16a** [g]	Time step 2 [h]	Yield **11a** [g, %] [a]
1	0.21	0.25	17	0.21, 65
2	0.50	0.66	47	0.52, 70
3	0.48	0.55	64	0.71, 86

[a] Yield calculated over two steps from **15a**.

N,N-Diisopropylethylamine (Hünig's base) was used in the synthesis of **11a** to neutralise the HCl formed. Bisazide **10** could have been used as well, but to avoid using the double amount of **10** that was synthesised only in a limited amount, another base was used.

The yield of **11a** increased with the reaction time used, see Table 3.5. Toluene and 70 °C were used in entry 1 (Table 3.5) and reflux in DCM for entry 2 and 3. Entry 1 is therefore not directly comparable to entry 2 and 3. Nevertheless, an increase of 16% can be seen when increasing the reaction time with 17 h from entry 2 to 3. This indicates a slow reaction. If time restrictions do not make it inexpedient, this reaction should go for 2-3 days.

With neat conditions for the preparation of **16a** and dry DCM as solvent for the synthesis of **11a**, the reaction conditions towards **11a** was concluded optimised. Only **11a** was prepared in order to optimise a reaction path towards target molecule **12*****a** (Scheme 3.16) before testing for other aryl groups. The structure of **11a** was determined by one- and two-dimensional NMR, see Section 5. Detailed experimental data and corresponding spectral data are given in Section 6.14.2.

With **11a** successfully prepared, the next step towards target molecule **12*****a** was the reduction of the azide groups to amine groups. Three different methods were attempted, none of them successful, see Scheme 3.16.
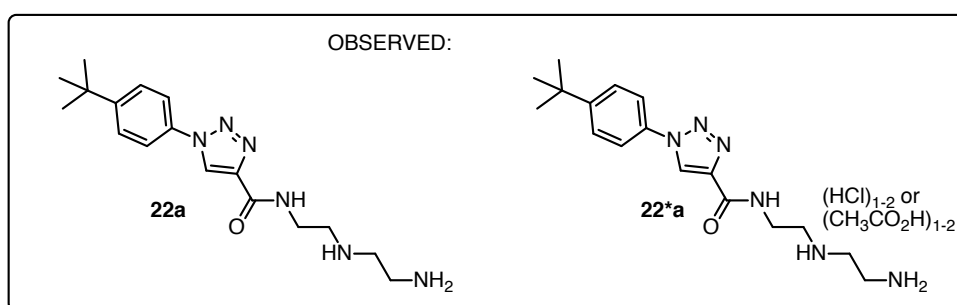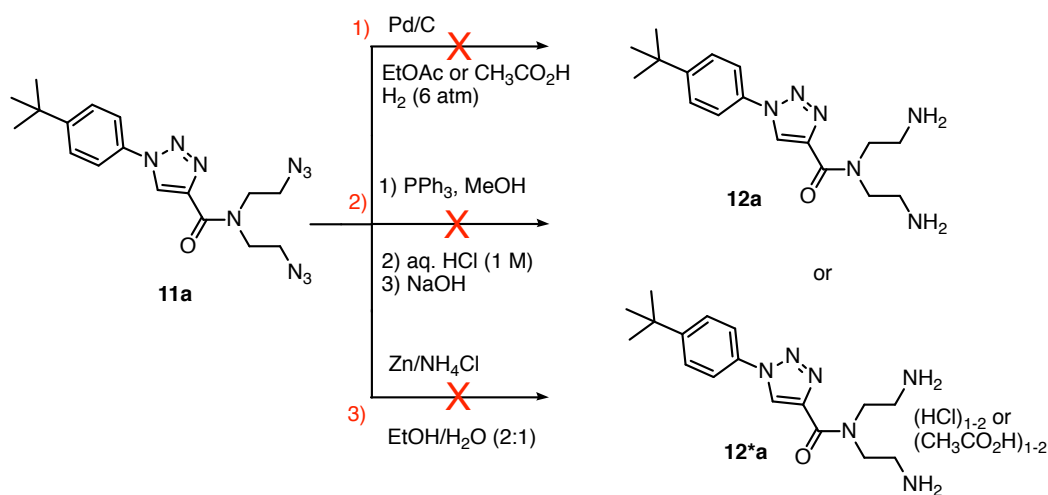
Scheme 3.16: The attempted reductions of **11a**, including the structures of **22a** and **22*a** that seemed to be the products formed in the reduction reactions.

The reduction of **11a** was first attempted with hydrogenolysis on a 10% Pd/C catalyst with EtOAc as solvent (reaction 1, Scheme 3.16). After 21 h, TLC analysis indicated full conversion. ^{1}H NMR analysis of the product, see Appendix AF.1, shows two products being present in a 2:1 ratio based on the signals in the aromatic area. However none of these products seemed to be **12a**. An indication for having wrong product, was the presence of triplets with shifts approximately at δ_H 8.5 ppm. These triplets correspond to protons observed on secondary amide groups of **7**, **7***, **8**, **5** and **5***. The bisamine **12a** has a tertiary amide group and should not have a triplet signal at δ_H 8.5 ppm.

Following a general method described by Pal *et al.* with modification,[41] reduction with PPh$_3$ in dry MeOH was attempted (reaction 2, Scheme 3.16). H$_2$O and aqueous HCl (1 M) were added to react the phosphinimine intermediate to the amine. Work-up afforded a 3:1 mixture of the same two products as the reduction on 10% Pd/C yielded, see Appendix AF.3 for the ^{1}H NMR spectrum. A fraction of this product mixture was reacted to the corresponding HCl-salt by addition of HCl (37% aq., 20 equiv) to the crude product in iPrOH. NMR analysis of this product after recrystallisation in MeOH, see Appendix AF.4-AF.8, indicated that **22*a** had been

formed instead of **12***a*. In addition, MS analysis (Appendix AF.9) confirmed that the obtained product had the same mass as **12***a*, which **22***a* has.

The ammonium salt **22***a* was synthesised to confirm that this was the product formed in the reductions of **11a**. This was done from the triazole methyl ester **4a** in two steps, see Scheme 3.17.
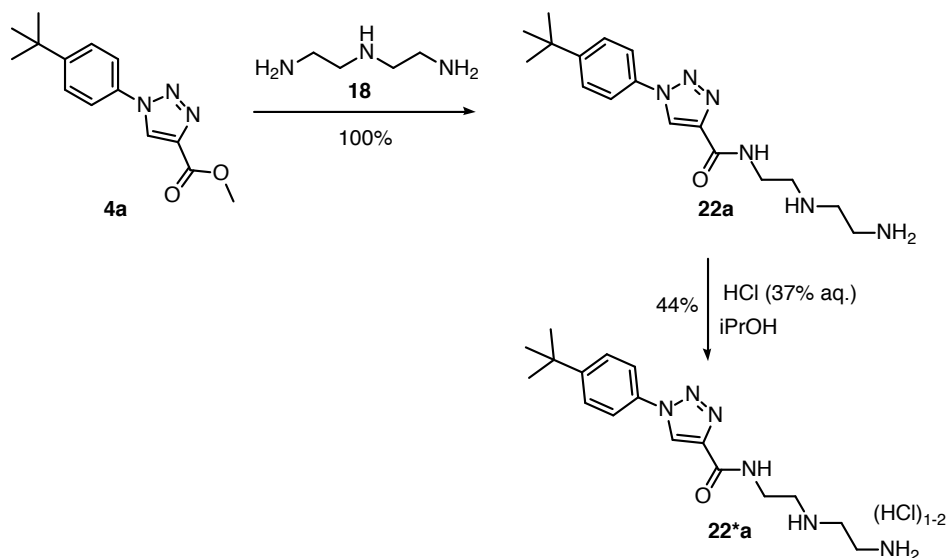
Scheme 3.17: Synthesis of ammmonium salt **22***a*.

Based on previous success with amidation of triazole esters **4** in neat solutions of both ethylene diamine and tris(2-aminoethyl)amine, neat **18** (150 equiv) was used in the synthesis of **22a**. Excess **18** was removed with Kügelrohr distillation affording **22a** in quantitative yields. The ^{1}H NMR spectrum of **22a**, shown in Appendix AB.1, indicated pure product thus the crude was used without purification. The following protonation of **22a** was successful and afforded **22***a* in 44% yield after washing with EtOH (96% aq.). ^{1}H NMR analysis of **22***a* indicated pure product, see Appendix AC.1. The HPLC purity of **22***a* was > 99%, see Appendix AC.9. This means **22***a* can be submitted for antimicrobial testing, even though this was not the purpose of synthesising the compound. The structures of **22a** and **22***a* were determined by one- and two-dimensional NMR, see Section 5. Detailed experimental procedure and corresponding spectral data are given in Section 6.18.

The preparation of **22a** and **22***a* confirmed this compounds to be the main products from the attempted reductions of **11a**. Rearrangement of the bisamine can be a possible explanation for the formation of **22a**. A proposed mechanism for this rearrangement is shown in Scheme 3.18.[58]
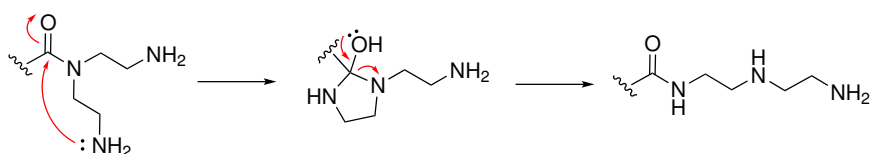
Scheme 3.18: A proposed mechanism for rearrangement of the bisamine forming the wrong isomer.[58]

Expecting acidic conditions would give the desired product, the reduction on Pd/C (10%) was repeated using acetic acid as solvent (reaction 1, Scheme 3.16). Protonation of the carbonyl oxygen makes the carbonyl group more electrophilic which is not preferred in order to avoid the formation of **22a**. Since protonation of the amine groups at the same time blocks their nucleophilic character, this may give **12a**. Work-up and purification by crystallisation from MeOH/Et$_2$O again afforded the wrong isomer. From ^{1}H NMR analysis, the signals of the product are somewhat shifted compared to both **22a** and **22*a**, see Appendix AF.10. A possible explanation for this can be the difference in counter ion when using acetic acid compared to hydrochloric acid.

One last method for reducing **11a** was tested (reaction 3, Scheme 3.16). Zn and NH$_4$Cl were used following a general procedure described by Lin *et al.*[39] After 17 h of reflux, a white precipitate was observed. EtOAc was added, unsuccessfully trying to dissolve it. From TLC analysis, the reaction was not complete after a total of 22 h at reflux. Extra Zn (1.3 equiv) was therefore added before the reaction mixture was refluxed for additional 25 h. Work-up afforded what again seemed to be **22a** from ^{1}H NMR analysis, see Appendix AF.11. To test if a shorter reaction time could give another result, the reaction was repeated with only 30 minutes at reflux. However, ^{1}H NMR analysis (Appendix AF.12), indicated that **22a** had been formed in a mixture with another product which did not seem to be **12a**.

Both basic and acidic conditions had now been tested, in addition to variations in the reaction time. The reduction of **11a** was concluded to be unsuccessful, affording the wrong isomer or product mixture of what seemed to be **22a** or **22*a** as main products.

3.4.2 Attempted Synthesis of 12*a using the Schiff base 19

The unsuccessful reduction of **11a** made it necessary to test another approach towards **12*a**. This was done following a general three steps procedure described by Arthi *et al.*,[78] see Scheeme 3.19.
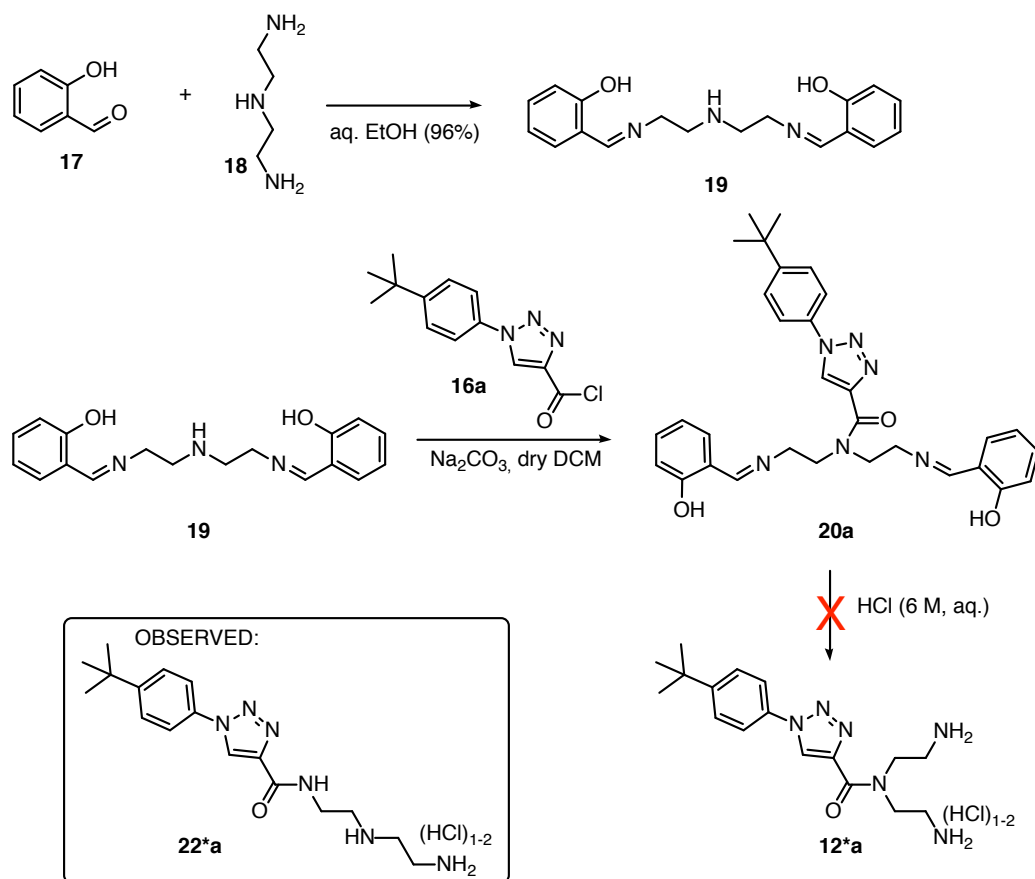
**Scheme 3.19:** Attempted synthesis of **12*a** using the Schiff base **19**.

Amidation of **16a** with **18** would have given the wrong isomer, see Scheme 3.17. Schiff base **19** was therefore prepared. According to the literature procedure,[78] the reaction mixture was to be stirred for 2 h at room temperature, followed by 6 h at reflux. However, starting material was still present after these 8 hours, seen from TLC analysis. The reaction mixture was therefore allowed to reflux for a total of 22 h. This gave the crude **19** as a yellow oil in quantitative yield. The ^{1}H NMR spectrum, see Appendix Z.1, was in accordance with reported data.[78]

Without purification, the crude product of **19** was reacted with **16a** affording **20a**. The ^{1}H NMR spectrum, shown in Appendix AA.1, was difficult to interpret, but MS analyses confirmed the presence of **20a**, see Appendix AA.2. Without purification, the crude **20a** was dissolved in HCl (6 M, aq.) and refluxed for 4 h. According to the procedure,[78] addition of EtOH was suppose

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to give a precipitate when used for other compounds. However, precipitation did not occur so solvent were removed under reduced pressure. ^{1}H NMR analysis again indicated formation of **22*a** instead of **12*a**, see Appendix AF.13.

3.4.3 Synthesis of 12*a from the Boc-protected Amine 23

The last, and now successful, attempt at synthesising **12*a** was a three steps procedure via the Boc-protected amine **23**. The reactions are given in Scheme 3.20.
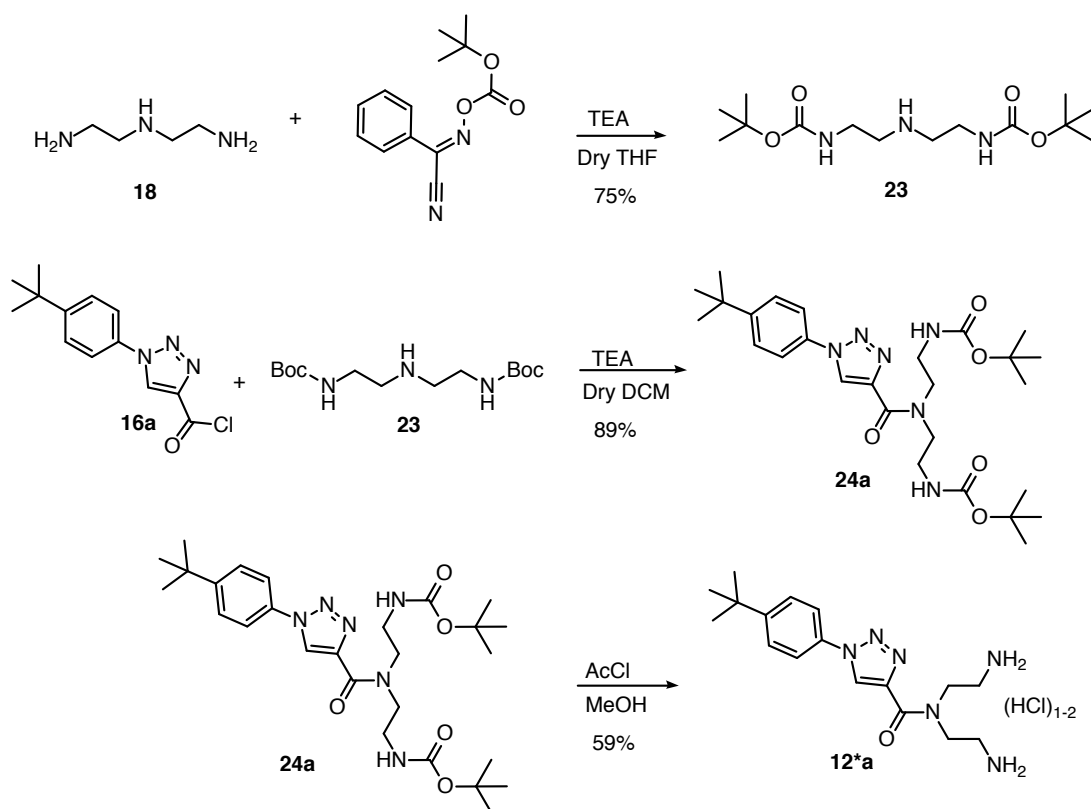
Scheme 3.20: Synthesis of **12*a**.

The Boc-protected amine **23** was successfully prepared following a general procedure described by Raines and Lukesh.[79] Purification by column chromatography (MeOH/DCM/NH$_4$OH: 1 : 9: 0.1) afforded **23** in 75% yield as a colourless oil. Because of the viscosity of the product, it was difficult to remove all the solvent. The ^{1}H NMR spectrum of **23** was in accordance with reported data for **23**,[79] and is shown in Appendix AD.1.

The acid chloride **16a** was prepared in neat SOCl$_2$ at 70 °C for 4 h, as previously discussed (Section 3.4.1). The following amidation of **23** with **16a**, with stirring at 0 °C for 1 h and at room temperature for 19 h, successfully afforded **24a** in 89% yield as a white crystalline solid.

TEA was used to neutralise HCl formed in the reaction. The ^{1}H NMR analysis indicated pure product and is shown in Appendix AE.1. The MS analysis, see Appendix AE.7, however shows extra peaks with higher mass than **24a**. Since the ^{1}H NMR analysis indicated pure product, it was not possible to explain these extra signals. After further reaction of **24a**, MS analysis showed that these signals were no longer present.

The final step towards target molecule **12*a** was the Boc-deprotection of **24a**. This was done following a general procedure described by Hickey *et al.*[69] Using 30 equivalents of AcCl in MeOH and stirring at r.t. for 19 h, successfully afforded the crude product of **12*a**. Purification by recrystallisation in EtOH (96% aq.) and washing with EtOH (96% aq.) afforded **12*a** as white crystals in 59% yield (39% overall yield from **18**). ^{1}H NMR analysis, see Appendix U.1, indicated pure product, and the HPLC purity was 98%, see Appendix U.9. The purity of **12*a** is therefore high enough for the product to be tested for antimicrobial activity at a later point.

The structures of **24** and **12*a** were determined by one- and two-dimensional NMR, see Section 5. Detailed experimental data and corresponding spectral data are given in Section 6.17.

A successful route towards **12*a** is established. The route remains to be tested for other bisamine salts similar to **12*a**. However, this was not done due to time restrictions.

4 Conclusion and Further Work

4.1 Conclusion

This thesis has covered the preparation of target amphiphilic 1,2,3-triazoles with both one and multiple cationic *N*-groups. The work includes the preparation of target ammonium salts **7*c**, **7*d**, **5*b** and **5*c**, in addition to an optimisation study towards guanidines **8**. The thesis has also covered considerable attempts of preparing bisammonium salt **12*a**. The latter resulted in a successful synthetic route towards this target amphiphile.

The azidation of **2d** to **3d** was a slow reaction (4-5 days) and it was necessary to add an extra portion of the reagents in order to achieve considerable conversion. The steric hindrance in **2d** is probably the reason for the challenges in the preparation. The following coupling between **3d** and methyl propiolate took 2-5 days and resulted in 4-5% of a byproduct. NMR analysis, including NOE-experiments, indicated that the byproduct was the 1,5-regioisomer **4'd**.

Both the preparation of amines **7** and the following protonation was successful and yielded the target molecules **7*c** and **7*d** with a HPLC purity of > 99%. The first synthesis of **7c** gave 2.6% of the dimer **7'c** when MeOH was used as solvent. By using neat ethylenediamine (90-100 equiv), the percentage **7'c** was minimised and the dimer **7'd** was not formed.

The preparation of guanidines **8** from amines **7** and 1*H*-pyrazole-1-carboxamide hydrochloride, formed different amounts of the corresponding ammonium salt **7*** in mixture with **8**. Different physical properties of the products make it difficult to predict the outcome of the reactions and how successful the purification is. However, purification by crystallisation afforded **8a** and **8c**, in 15-19% yields and a HPLC-purity of 98% and 99% respectively. Trying to reduce the possibility of forming **7*c**, guanidine **8c** was attempted synthesised using the base triethylamine and an excess of the guanylation reagent. The percentage of **7*c** was reduced from 28% to 6%, but further reaction afforded a large amount of unidentified impurities seen from HPLC.

In an attempt of finding a better synthetic route towards **8**, the Boc-protected **8'** were prepared without problems in 93-98% yields. The first attempt of deprotecting **8'c** using HCl (37% aq.) in MeCN afforded a byproduct that was difficult to remove. AcCl in MeOH was used for the next deprotection. With the addition of an extra portion AcCl, this successfully afforded **8b** and **8c** (46-100% yields), with HPLC purities of > 99% and 99% respectively, and only trace impurities seen from the ¹H NMR analysis.

The preparation of the branched amines **5** and the following protonation successfully afforded

5*b and **5***c in varying yields (25-91%) and a HPLC purity of $> 99\%$. Bisguanidine **14a** was attempted synthesised from **5a** using the guanylation reagent 1*H*-pyrazole-1-carboxamide hydrochloride in excess, and the base TEA. This resulted in a product mixture of **14a** and **14'a** with only one guanidine group.

Bisazide **10** was prepared in 56-63% yields without problems. Three attempts were made to synthesise the branched bisazide **11a** from the triazole methyl ester **4a** and **10**. However, this was not successful. Two of the reactions gave no conversion, and the last one formed an unidentified product. The more reactive acid chloride **16a** was therefore prepared from hydrolysis of **4a** via **15a**. Optimisation of this reaction showed **15a** in neat $SOCl_2$ at 70 °C to be the preferred conditions. The following reaction between **16a** and **10** was successful and afforded **11a** in 65-86% yields. The favoured solvent was dry DCM. The reaction was slow, and if time restrictions do not make it inexpedient, it should go for 2-3 days.

The reduction of the branched bisazide **11a** was attempted with different methods: hydrogenolysis on 10% Pd/C with both EtOAc and acetic acid as solvent, reduction using Zn and NH_4Cl and a Staudinger reaction with PPh_3. All these reductions afforded **22a** or **22***a instead of **12a/12***a. This was possibly due to a rearrangement reaction taking place under these reaction conditions. A new method was tested using the Schiff base **19** in a three steps procedure. NMR analysis again confirmed the formation of **22***a. The last attempt of preparing **12***a was successful. A three steps procedure via the Boc-protected amide **23** gave the target amphiphile **12***a in 39% overall yield, and a HPLC purity of 98%. A synthetic route towards **12***a was established, and the route remains to be tested for ammmonium salts similar to **12***a.

4.2   Further Work

The synthesis of the Boc-protected guanidines **8'** showed to be effective. However, other methods for the deprotection should be tested in order to further optimise the preparation of the guanidines **8**. Two widely used conditions are TFA in DCM,[52,80,81] and aq. HCl in dioxane.[82]

If the byproduct pyrazole prove to give problems when using *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine (**21**), other Boc-protected guanylation reagents could be tested. There are several other reagents available, like *N,N'*-bis(Boc)-S-methylisothiourea, *N,N'*-bis(Boc)-*N''*-triflylguanidine, *N,N'*-bis(Boc)-1*H*-pyrazole-1-carboxamidine and *N,N'*-bis(Boc)benzotriazole-carboxamidine.[59]

The branched ammonium salts **5*** have been successfully prepared with different aryl groups. Further work should include testing other methods for the preparation of bisguanidines **14**. An

important route is the study of other guanylation reagents. The Boc-protected reagent **21** used for the guanylation of **7**, or one of the other reagents mentioned above, could be tested, see Scheme 4.1.

A synthetic route towards target molecule **12**∗**a** was established. This route need to be tested for other aryl groups. From previous work, guanidines have shown to be prior to the corresponding ammonium salts both regarding the antimicrobial activity and the toxicity towards human cells.[12] A successful preparation of bisamine salts **12**∗ should therefore be followed by the preparation of the corresponding guanidines **13**, see Scheme 4.1, possibly with the use of a Boc-protected guanylation reagent as recommended for further synthesis of **14**.
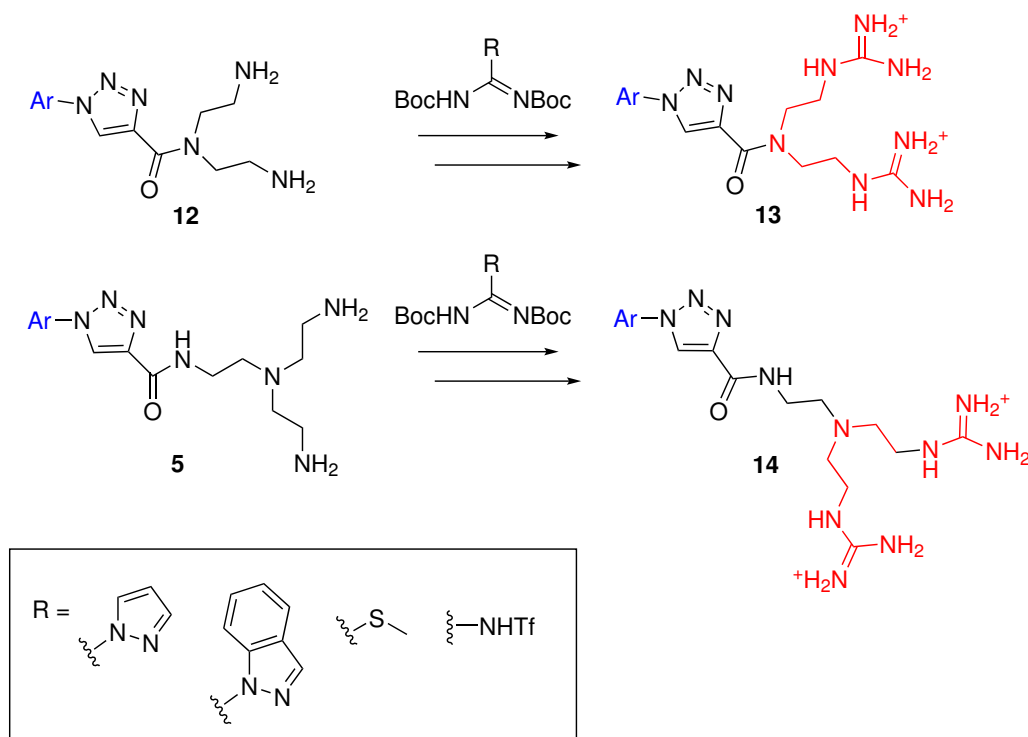
Scheme 4.1: Possible preparation of **13** and **14** using a Boc-protected guanylation reagent. Counterion: Cl⁻. Ar = aromatic group.

Another reaction path worth noticing is the preparation of guanidines substituted with alkyl groups like cyclopropylmethyl and methyl, see Scheme 4.2. Such guanidines have shown antimicrobial activity,[83] and may be interesting to test for both mono- and bisfunctional guanidines.
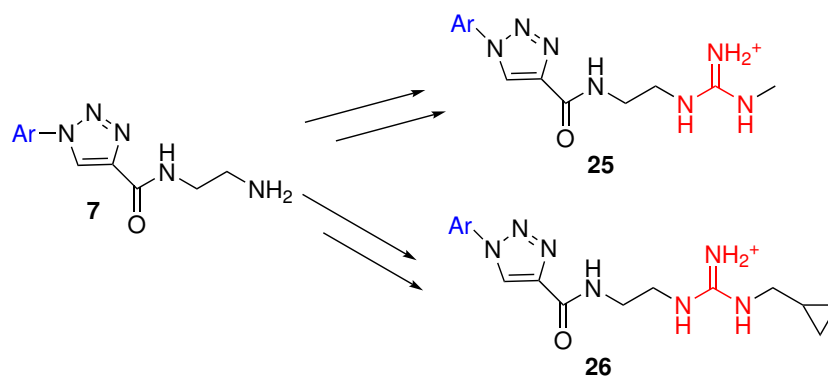
Scheme 4.2: Possible guanidines **25** and **26** substituted with cyclopropylmethyl and methyl. Ar = aromatic group.

Guanidines have shown to be more challenging to prepare than the corresponding ammonium salts. This does not coincide with guandines showing, as mentioned, superior antimicrobial potency. An effective synthetic route towards guanidines would therefore be valuable. The study will be an important step in providing insight into the design and search for novel antimicrobial agents.

5 Spectroscopic Analysis and Characterisation

5.1 General

This section covers the spectroscopic characterisation of new compounds prepared during this master's thesis, in addition to **15a** and **15c** which are not described in literature. By using ^{1}H and ^{13}C NMR, in addition to the two dimensional techniques COSY, HSQC, HMBC and NOESY, the chemical shifts of protons (δ_H) and carbons (δ_C) were assigned. The use of 2D methods enabled the assignment of chemical shifts to the corresponding atoms. The COSY spectrum gave information about which hydrogen atoms were adjacent to one another. HSQC shows the short-range ^{1}H-^{13}C correlation over one bond length, and provided information about which proton were attached to which carbon. HMBC showed the ^{1}H-^{13}C correlation over two, three or four bond lengths, thus the signals of the quaternary carbons could be assigned. The NOESY spectra showed the NOE coupling in **4d** and **4'd**.

HRMS was used to confirm the mass and composition of the compound, and by that the chemical formula. A further prove of the elucidated structure was given by characteristic absorption pattern in the IR spectrum.

All spectra contained signals from the deuterated solvent used for the NMR analysis. Some spectra also showed signal remainings from solvents used in the synthesis. All these signals were identified with the use of litterature from Fulmer *et al.*,[84] and will not be discussed further.

A detailed structural elucidation will be given for **11a**. The remaining compounds will be characterised using a similar methology, but will not be presented in the same level of detail. Since all the target molecules have hydrogens on nitrogen groups, a discussion about hydrogens on heteroatoms is included, see Section 5.3.

5.2 *N,N*-Bis(2-azidoethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (11a)

The HRMS analysis, shown in Appendix T.10, confirms the predicted chemical formula of **11a**: $C_{17}H_{23}N_{10}O$ [M+H]$^+$ (calcd.: 383.2056; found: 383.2054).

The presence of azide groups is consistent with the IR spectrum, see Appendix T.9, which shows a strong peak at 2099 cm^{-1}.
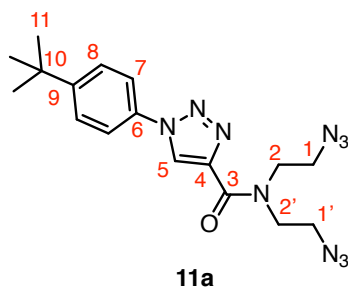
Figure 5.1: Structure of **11a** with assigned positions.

The ^{1}H NMR spectrum shown in Appendix T.4 displays a singlet-signal at δ_H 1.33 ppm with an intensity of 9. This singlet must derive from nine equivalent protons without adjacent hydrogens. Thus this signal was assigned to H-11, seen from the structure of **11a**. The HSQC spectrum (see Appendix T.7), shows that H-11 couples to, and by that are attached to, the carbons at δ_C 30.9 ppm, see Figure 5.2.
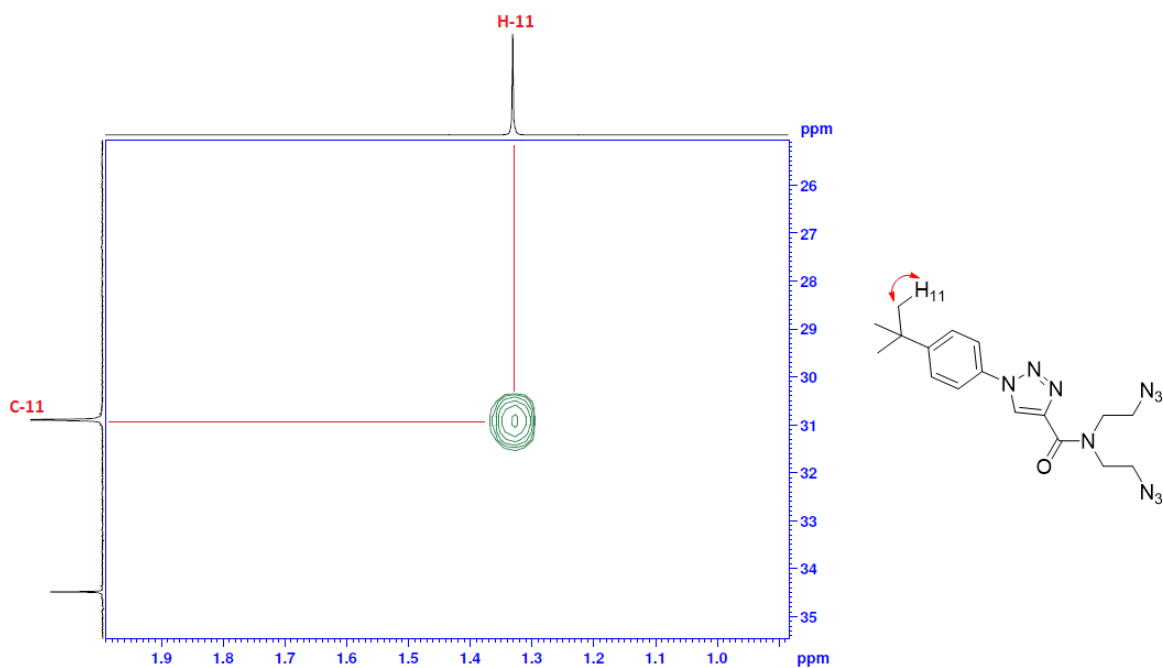
Figure 5.2: Part of the HSQC spectrum of **11a** used to assign δ_C at position 11, see Appendix T.7

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The quarternary carbon at δ_C 34.5 ppm was assigned using HMBC, see Appendix T.8 and Figure 5.3. Seen from HMBC, δ_H 1.33 ppm couples to δ_C 34.5 ppm. Since the other quarternary carbons near δ_H 1.33 ppm should have a chemical shift value corresponding to the aromatic area,[71c] δ_C 34.5 ppm was assigned to C-10.
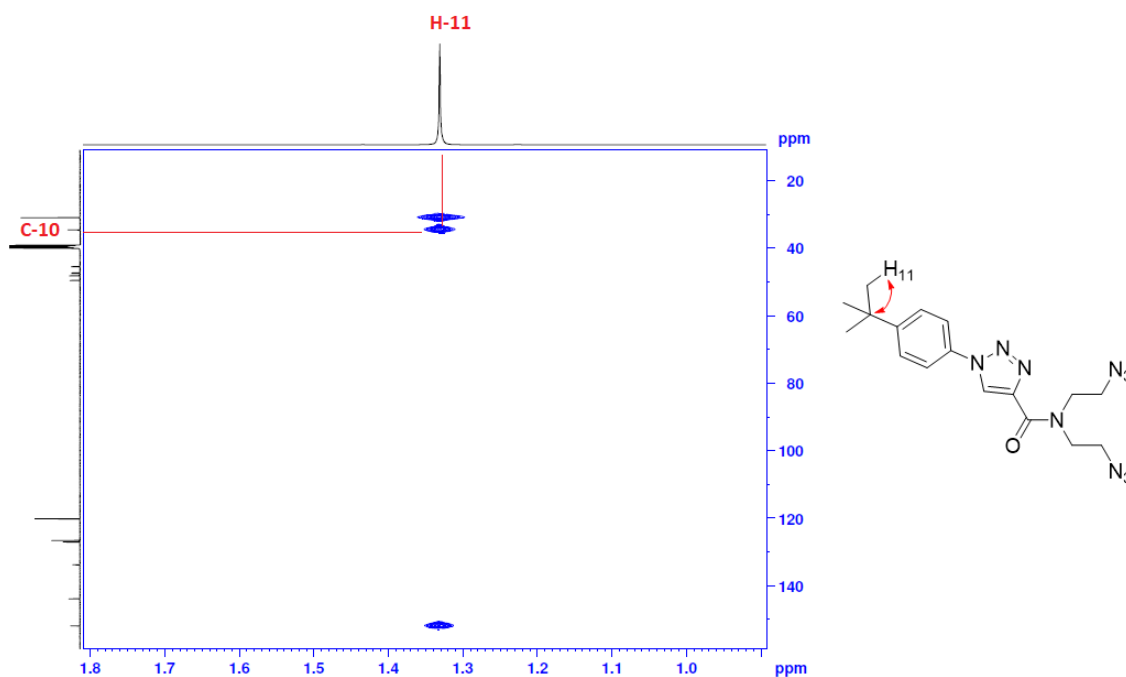
Figure 5.3: Part of the HMBC spectrum of **11a** used to assign the quarternary carbon at δ_C 34.5 ppm, see Appendix T.8. The segment display coupling between H-11 and C-10.

Two multiplets with shifts 7.91-7.87 ppm and 7.64-7.60 ppm can be observed in the ^{1}H NMR spectrum. Using HSQC in the same way as above, these hydrogens were found to be attached to δ_C 120.2 and δ_C 126.6 ppm respectively. With a chemical shift value corresponding to the aromatic area,[71c] and an integral of 2, these signals must belong to H-7 and H-8. From HMBC δ_H 7.64-7.60 ppm couples to C-10 and C-11, thus this signal was assigned to H-8 and δ_H 7.91-7.87 ppm was assigned to H-7.

The two quarternary carbons in the phenyl substituent were assigned using HMBC as above. H-11 couples to δ_C 151.9 ppm, thus δ_C 151.9 ppm was assigned to C-9. Both H-7 and H-8 couple to δ_C 133.8 ppm, and the signal was therefore assigned to C-6.

A singlet signal with intensity of one can be seen in the ^{1}H NMR spectrum. This signal must derive from a proton with no equivalent or adjacent protons. Thus this signal was assigned to H-5, seen from the structure of **11a**.

The two last quarternary carbon signals are δ_C 143.9 and 161.0 ppm, which have to be the two carbons in position 3 and 4. HMBC shows that H-5 couples to δ_C 143.9 ppm. This signal was therefore assigned to C-4. The remaining signal at 161.0 ppm, which corresponds to a typical value of a carbonyl,[71c] was assigned to C-3.

54

The next step was assignment of the shifts in the bisazide part of the molecule. Four triplets are observed in the ^{1}H NMR spectrum. These signals must derive from H-1, H-1', H-2 and H-2'. Which carbons these protons were attached to were found from HSQC as above. From HMBC, δ_H 4.17 and 3.71 ppm couple to δ_C 161.0 ppm (C-3). These two signals were therefore assigned to H-2 and H-2'. It was not possible to further specify which of the protons were H-2 and which were H-2'. The two remaining triplets were assigned to H-1 and H-1'. From COSY, see Appendix T.6 and Figure 5.4, δ_H 4.17 ppm couples to, and thereby is the neighbour to δ_H 3.67 ppm. In the same way δ_H 3.71 ppm is next to δ_H 3.60 ppm.
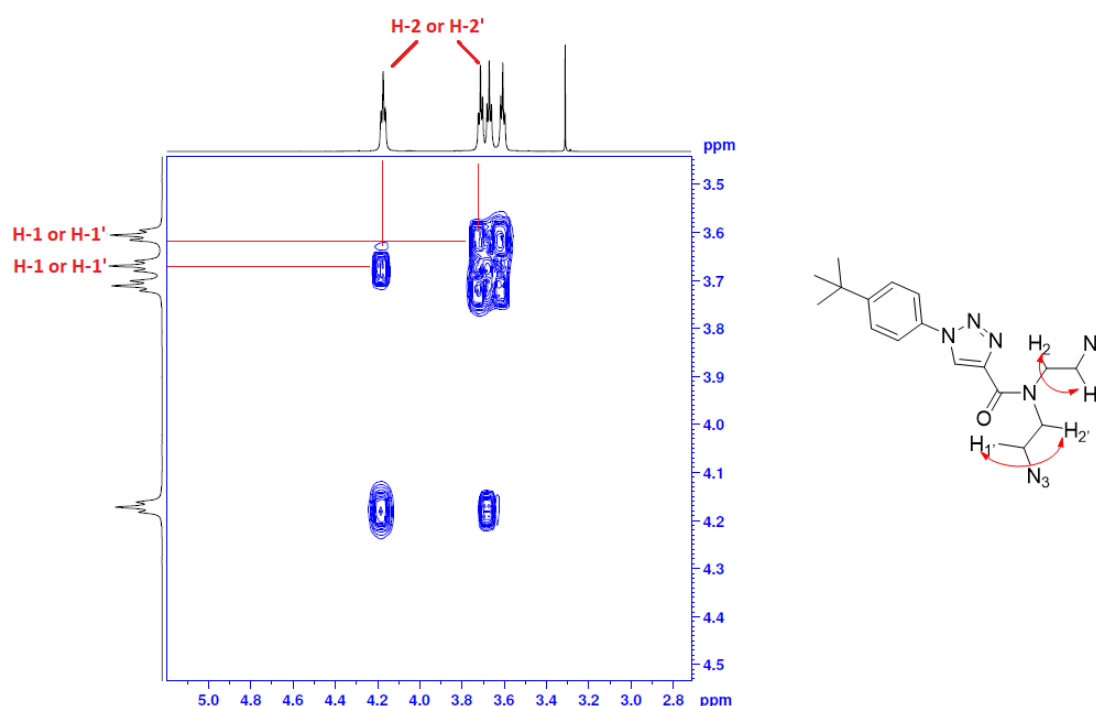
Figure 5.4: A part of the COSY spectrum (Appendix T.6) of **11a** used to show coupling between δ_H 4.17 and δ_H 3.67 ppm, in addition to coupling between δ_H 3.71 and δ_H 3.60 ppm. It was not possible to further specify which of the protons of δ_H 4.17 and δ_H 3.71 ppm were H-2 and H-2', and thus not which of the two other signals were H-1 and H-1'.

This completes the characterisation of **11a**, with all proton and carbon signals assigned. The chemical shifts, multiplicities, integrals and coupling constants are given in Table 5.1.

Table 5.1: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **11a**. The positions are illustrated in Figure 5.1.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1 or 1'	3.67	t	2H	6.2	49.5
1 or 1'	3.60	t	2H	6.0	48.1
2 or 2'	4.17	t	2H	6.1	47.3
2 or 2'	3.71	t	2H	6.2	45.4
3	-	-	-	-	161.0
4	-	-	-	-	143.9
5	9.29	s	1H	-	127.0
6	-	-	-	-	133.8
7	7.91-7.87	m	2H	-	120.2
8	7.64-7.61	m	2H	-	126.6
9	-	-	-	-	151.9
10	-	-	-	-	34.5
11	1.33	s	9H	-	30.9

5.3   Protons on Heteroatoms

Protons bonded to heteroatoms like nitrogen and oxygen can be labile and therefore exchangeable.[71b] Due to this they can differ from protons bonded to carbons. Depending on the exchange rate, the signals from protons on nitrogen and the protons on the neighbouring carbon atoms will have different appearance in the ^{1}H NMR spectrum. As an example, two different exchange rates can be observed for NH in the ^{1}H NMR spectrum for **23**, see Appendix AD.1. Both the NH-shifts appear as a broad singlet. The NH group that integrates to two couples to one of the CH$_2$-groups so this groups appear as a quartet. This means the exchange rate on the NMR timescale is slow,[71b] see Figure 5.5. The spin states of the nitrogen nucleus have, in this case, an intermediate lifetime. The protons on the nitrogens can therefore interact with the three spin states on N, and coupling of the NH proton to the neighbouring protons can take place.
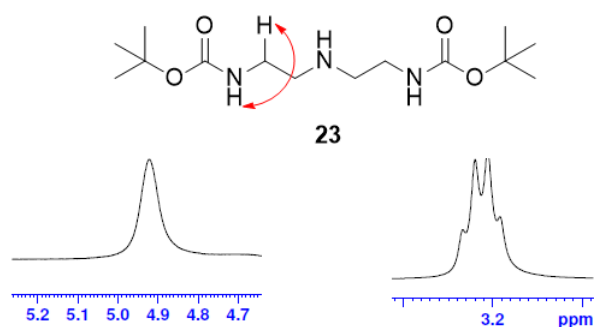
Figure 5.5: Segments from the ^{1}H NMR spectrum of **23**, see Appendix AD.1, showing a broad singlet signal assigned to the the two symmetrical NH-groups. The quartet indicates coupling between these NH and the neighbouring protons, meaning the NH exchange rate is slow.[71b]

For the other NH-group integrating to one, no coupling between NH and the neighbouring protons is observed, and the neighbouring CH_2-group appear as a triplet, see Figure 5.6. The NH exchange rate is faster on the NMR timescale compared to the other NH-groups. The spin states of the proton on N are averaged out and no coupling to neighbouring protons are observed.
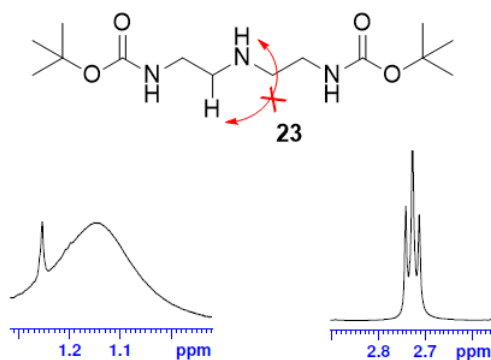
Figure 5.6: Segments from the ^{1}H NMR spectrum of **23**, see Appendix AD.1, showing a broad singlet signal assigned to the NH-group that integrate to one. The triplet indicates no coupling between this NH and the neighbouring protons, meaning the NH exchange rate is faster than for the other NH-groups.[71b]

5.4 Methyl 1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (4d)

The chemical shifts in Table 5.2 were assigned from the spectroscopic data for **4d**, see Appendix B.4-B.12. The experimental procedure is given in Section 6.3.

The HRMS analysis, shown in Appendix B.12, confirms the predicted chemical formula of **4d**: $C_{22}H_{34}N_3O_2$ [M+H]$^+$ (calcd.: 372.2651; found: 372.2651). The presence of an ester group is consistent with the IR spectrum, see Appendix B.11, which shows a strong peak at 1723 cm^{-1}.

NOE-coupling between H-4 and H-8 confirmed the 1,4-regioisomer. See discussion in Section 3.1.2 and the NOESY spectrum in Appendix B.9.

4d
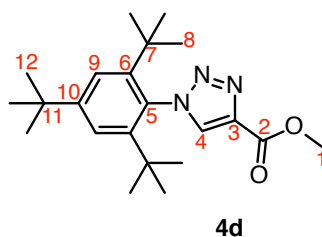
Figure 5.7: Structure of **4d** with assigned positions.

Table 5.2: Assigned ^{1}H (600 MHz, CDCl$_3$) and ^{13}C (150 MHz, CDCl$_3$) NMR shifts, multiplicity and integrals for **4d**. The positions are illustrated in Figure 5.7.

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1	4.00	s	3H	52.3
2	-	-	-	161.3
3	-	-	-	138.7
4	8.25	-	1H	134.4
5	-	-	-	130.9
6	-	-	-	147.4
7	-	-	-	36.8
8	1.04	s	18H	32.2
9	7.56	s	2H	123.8
10	-	-	-	152.6
11	-	-	-	35.5
12	1.36	s	9H	31.3

5.5 Methyl 1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-5-carboxylate (4'd)

The chemical shifts in Table 5.3 were assigned from the spectroscopic data for **4'd**, see Appendix C.1-C.8. The experimental procedure is given in Section 6.3.

The HRMS analysis, shown in Appendix C.8, confirms the predicted chemical formula of **4'd**: $C_{22}H_{34}N_3O_2$ [M+H]$^+$ (calcd.: 372.2650; found: 372.2651).

The presence of an ester group is consistent with the IR spectrum, see Appendix C.7, which shows a strong peak at 1743 cm^{-1}.

NOE-coupling between H-1' and H-8' confirmed the 1,5-regioisomer. See discussion in Section 3.1.2 and the NOESY spectrum in Appendix C.6.
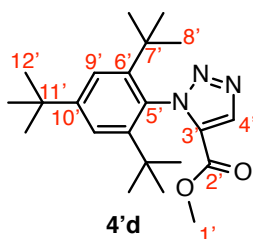
Figure 5.8: Structure of **4'd** with assigned positions.

Table 5.3: Assigned ^{1}H (600 MHz, CDCl$_3$) and ^{13}C (150 MHz, CDCl$_3$) NMR shifts, multiplicity and integrals for **4'd**. The positions are illustrated in Figure 5.8.

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1'	3.76	s	3H	52.3
2'	-	-	-	158.5
3'	-	-	-	134.4
4'	8.23	-	1H	137.2
5'	-	-	-	130.4
6'	-	-	-	146.3
7'	-	-	-	37.1
8'	0.99	s	18H	32.0
9'	7.53	s	2H	124.0
10'	-	-	-	151.7
11'	-	-	-	35.1
12'	1.37	s	9H	31.3

5.6 *N*-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (5b) and *N*,*N'*-(((2-aminoethyl)azanediyl) bis(ethane-2,1-diyl))bis(1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide) (5'b)
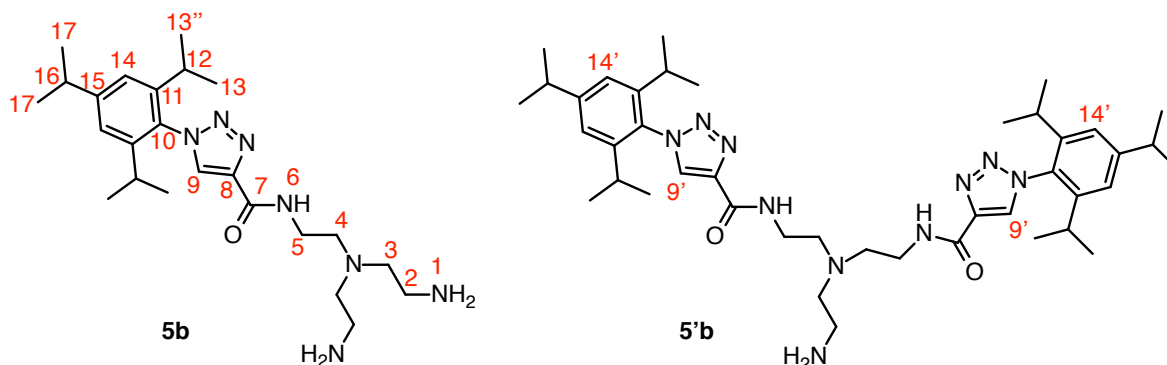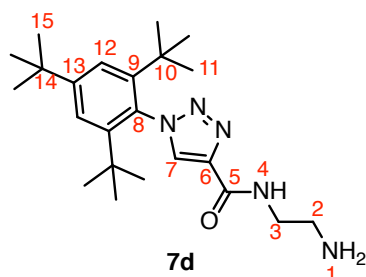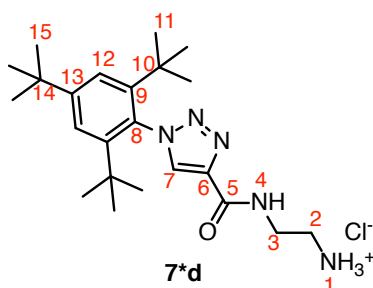

Figure 5.9: Structure of **5b** and **5'b** with assigned positions.

The chemical shifts in Table 5.4 were assigned from the spectroscopic data for **5b**, see Appendix E.1-E.7. The experimental procedure is given in Section 6.9.

The HRMS analysis, shown in Appendix E.7, confirms the predicted chemical formula of **5b**: $C_{24}H_{42}N_7O$ [M+H]$^+$ (calcd.: 444.3451; found: 444.3451). In addition, the HRMS results confirmed presence of the dimer **5'b**: $C_{42}H_{65}N_{10}O_2$ [M+H]$^+$ (calcd.: 741.5292; found: 741.5282), see Appendix E.8.

The presence of an amide group is consistent with the IR spectrum, see Appendix E.6, which shows a strong peak at 1657 cm^{-1}.

The ^{1}H NMR spectrum for **5b**, see Appendix E.1, displayed two apparent singlet-signals (δ_H 9.16 and 1.31 ppm) assumed to come from the dimer **5'b**. Because the shifts were almost the same as the shifts for H-9 and H-14 in **5b**, δ_H 8.90 ppm was assumed to be H-9'and δ_H 7.19 ppm was assumed to belong to H-14'. However, due to overlapping signals with **5b** and the low percentage of **5'b** (0.5%) in the mixture, further structure elucidation of **5'b** was not possible.

The ^{1}H NMR spectrum displayed two different doublet-signals for H-13 and H-13", see Table 5.4. This is presumably due to restricted rotation in **5b**.

Table 5.4: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **5b**. The positions are illustrated in Figure 5.9

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	2.00-1.30	br s	4H	-	-
2	2.59	t	4H	6.2	39.8
3	2.46	t	4H	6.2	57.6
4	2.61	t	2H	6.9	53.4
5	3.36	t	2H	6.6	37.0
6	8.59	br s	1H	-	-
7	-	-	-	-	159.4
8	-	-	-	-	142.9
9	8.88	s	1H	-	129.3
10	-	-	-	-	128.8
11	-	-	-	-	145.0
12	2.04	sept	2H	6.8	28.1
13 or 13"	1.08	d	6H	6.9	23.7
13 or 13"	1.11	d	6H	6.9	23.5
14	7.25	s	2H	-	121.6
15	-	-	-	-	151.3
16	3.00	sept	1H	6.9	33.7
17	1.26	d	6H	6.9	23.8

5.7 *N*-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (5*b)
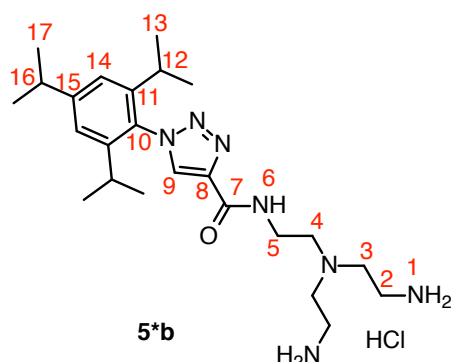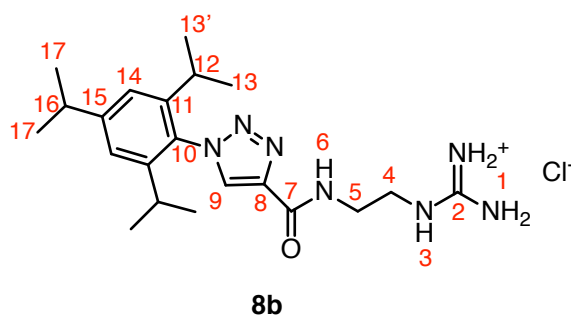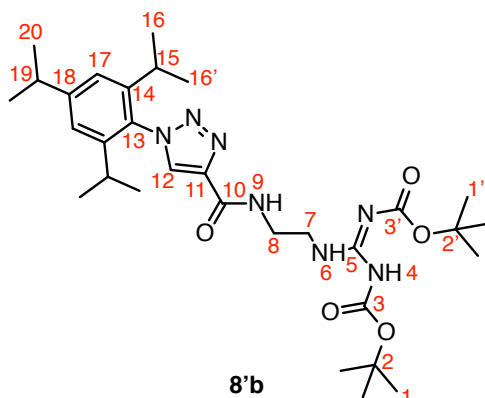

Figure 5.10: Structure of **5*b** with assigned positions.

The chemical shifts in Table 5.5 were assigned from the spectroscopic data for **5*b**, see Appendix G.2-G.8. To overcome the broadening of the peaks presumably due to dynamic processes, the NMR analysis were performed at 90 °C. It was not possible to distinguish between position 2 and 3 from NMR analysis. This because no long range coupling could be seen for these protons in the HMBC spectrum (Appendix G.6).

Up to three of the amino groups of **5*b** can be protonated.The HRMS analysis, shown in Appendix G.8 confirmed the chemical formula of only the monoprotonated salt: $C_{24}H_{42}N_7O$ [M-Cl]$^+$ (calcd.: 444.3451; found: 444.3449). This is consistent with the ^{1}H NMR analysis (Appendix G.2), where the shift of the amino groups integrated to 5.

The presence of an amide group is consistent with the IR spectrum, see Appendix G.7, which shows a peak at 1673 cm^{-1}. The experimental procedure is given in Section 6.10.

Table 5.5: Assigned ^{1}H (400 MHz, 90 °C, DMSO-d_6) and ^{13}C (100 MHz, 90 °C, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **5*b**. The positions are illustrated in Figure 5.10.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	8.22	br s	5H	-	-
2 or 3	3.13-3.05	m	4H	-	35.3
2 or 3	3.05-2.97 (4 of 5H)	m	4H	-	50.5
4	2.97-2.85	m	2H	-	52.2
5	3.61-3.51	m	2H	-	35.3
6	8.63-8.44	m	1H	-	-
7	-	-	-	-	159.5
8	-	-	-	-	142.3
9	8.82	s	1H	-	130.2
10	-	-	-	-	128.8
11	-	-	-	-	144.7
12	2.14	sept	2H	6.8	27.7
13	1.18-1.01	m	12H	-	23.2
14	7.23	s	2H	-	121.2
15	-	-	-	-	150.9
16	3.05-2.97 (1 of 5H)	m	1H	-	33.2
17	1.29	d	6H	6.9	23.0

5.8 *N*-(2-(Bis(2-aminoethyl)amino)ethyl)-1-mesityl-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (5*c)
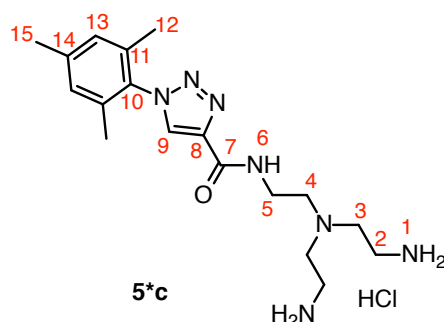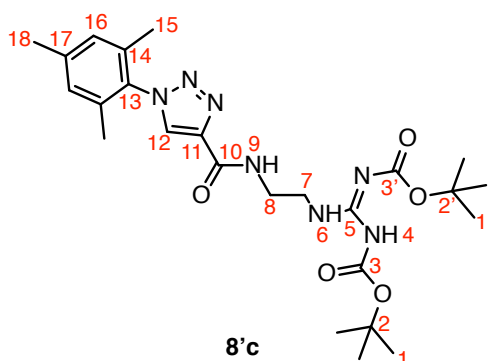

Figure 5.11: Structure of **5*c** with assigned positions.

The chemical shifts in Table 5.6 were assigned from the spectroscopic data for **5*c**, see Appendix H.2-H.8. To overcome the broadening of the peaks presumably due to dynamic processes, the NMR analysis were performed at 90 °C. As for **5*b** it was not possible to distinguish between position 2 and 3 from NMR analysis.

Up to three of the amino groups of **5*c** can be protonated. The HRMS analysis, shown in Appendix H.8, confirmed the chemical formula of only the monoprotonated salt: $C_{18}H_{30}N_7O$ [M-Cl]$^+$ (calcd.: 360.2512; found: 360.2513). This is consistent with the ^{1}H NMR analysis (Appendix H.2), where the shift of the amino groups integrated to 5.

The presence of an amide group is consistent with the IR spectrum, see Appendix H.7, which shows a peak at 1656 cm^{-1}. The experimental procedure is given in Section 6.10.

Table 5.6: Assigned ^{1}H (400 MHz, 90 °C, DMSO-d_6) and ^{13}C (100 MHz, 90 °C, DMSO-d_6) NMR shifts, multiplicity and integrals for **5*c**. The positions are illustrated in Figure 5.11.

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1	8.22	br s	5H	-
2	3.14-2.98 (4 of 8H)	m	4H	50.6 or 35.4
3	3.14-2.98 (4 of 8H)	m	4H	50.6 or 35.4
4	2.98-2.87	m	2H	52.3
5	3.61-3.52	m	2H	35.4
6	8.66-8.56	m	1H	-
7	-	-	-	159.6
8	-	-	-	142.4
9	8.75	s	1H	127.8
10	-	-	-	134.0
11	-	-	-	132.6
12	1.92	s	6H	16.3
13	7.10	s	2H	128.5
14	-	-	-	139.4
15	2.35	s	3H	20.1

## 5.9 *N*-(2-Aminoethyl)-1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (7d)

Figure 5.12: Structure of **7d** with assigned positions.

The chemical shifts in Table 5.7 were assigned from the spectroscopic data for **7d**, see Appendix J.2-J.8. The experimental procedure is given in Section 6.4.

The HRMS analysis, shown in Appendix J.8, confirms the predicted chemical formula of **7d**: $C_{23}H_{38}N_5O$ $[M+H]^+$ (calcd.: 400.3076; found: 400.3075).

The presence of an amide group is consistent with the IR spectrum, see Appendix J.7, which shows a strong peak at 1653 cm^{-1}.

Table 5.7: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **7d**. The positions are illustrated in Figure 5.12.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	1.48	br s	2H	-	-
2	2.71	t	2H	6.6	41.2
3	3.28	q	2H	6.4	42.1
4	8.55	t	1H	5.7	-
5	-	-	-	-	159.5
6	-	-	-	-	142.1
7	8.99	s	1H	-	133.1
8	-	-	-	-	131.3
9	-	-	-	-	146.8
10	-	-	-	-	36.4
11	0.97	s	18H	-	31.7
12	7.56	s	2H	-	123.4
13	-	-	-	-	151.6
14	-	-	-	-	34.9
15	1.34	s	9H	-	31.0

## 5.10 *N*-(2-aminoethyl)-1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (7*d)

Figure 5.13: Structure of **7*d** with assigned positions.

The chemical shifts in Table 5.8 were assigned from the spectroscopic data for **7*d**, see Appendix L.1-L.7. The experimental procedure is given in Section 6.5.

The HRMS analysis, shown in Appendix L.7, confirms the predicted chemical formula of **7*d**: $C_{23}H_{38}N_5O$ [M-Cl]$^+$ (calcd.: 400.3076; found: 400.3075).

The presence of an amide group is consistent with the IR spectrum, see Appendix L.6, which shows a strong peak at 1660 cm^{-1}.

Table 5.8: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **7*****d**. The positions are illustrated in Figure 5.13.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	8.00	br s	3H	-	-
2	3.02	t	2H	6.4	38.5
3	3.56	q	2H	6.4	36.6
4	8.88	t	1H	5.7	-
5	-	-	-	-	160.1
6	-	-	-	-	141.7
7	9.09	s	1H	-	146.8
8	-	-	-	-	131.2
9	-	-	-	-	146.8
10	-	-	-	-	36.4
11	0.98	s	18H	-	31.7
12	7.57	s	2H	-	123.4
13	-	-	-	-	151.7
14	-	-	-	-	34.9
15	1.34	s	9H	-	31.0

5.11 *N*-(2-Guanidinoethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (8b)
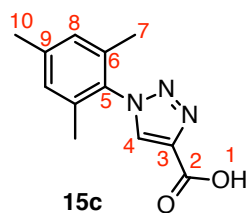

Figure 5.14: Structure of **8b** with assigned positions.

The chemical shifts in Table 5.9 were assigned from the spectroscopic data for **8b**, see Appendix N.1-N.7. The ^{1}H NMR spectrum (Appendix N.1), displayed two different doublet-signals for H-13 and H-13', see Table 5.9. This is presumably due to restricted rotation in **8b**.

The HRMS analysis, shown in Appendix N.7, confirms the predicted chemical formula of **8b**: $C_{21}H_{34}N_7O$ [M-Cl]$^+$ (calcd.: 400.2825; found: 400.2822).

The presence of an amide group is consistent with the IR spectrum, see Appendix N.6, which shows a strong peak at 1650 cm^{-1}.

The experimental procedure is given in Section 6.8.

Table 5.9: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **8b**. The positions are illustrated in Figure 5.14.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	7.81-6.75	br s	4H	-	-
2	-	-	-	-	157.2
3	7.85	t	1H	5.7	-
4	3.38-3.34	m	2H	-	40.3
5	3.44-3.40	m	2H	-	37.9
6	8.81	t	1H	5.8	-
7	-	-	-	-	160.0
8	-	-	-	-	142.5
9	8.98	s	1H	-	130.5
10	-	-	-	-	129.6
11	-	-	-	-	145.0
12	2.03	sept	2H	6.8	28.1
13 or 13'	1.11	d	6H	6.9	23.5
13 or 13'	1.07	d	6H	6.9	23.7
14	7.25	s	2H	-	121.7
15	-	-	-	-	151.4
16	3.00	sept	1H	6.8	33.8
17	1.26	d	6H	6.9	23.8

5.12 *N,N'*-DiBoc-protected *N*-(2-guanidinoethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (8'b)
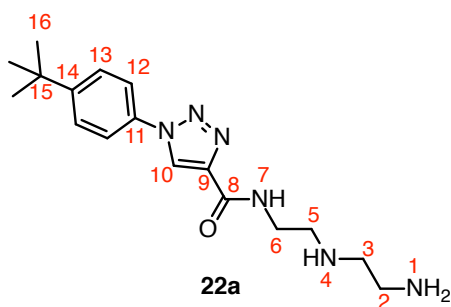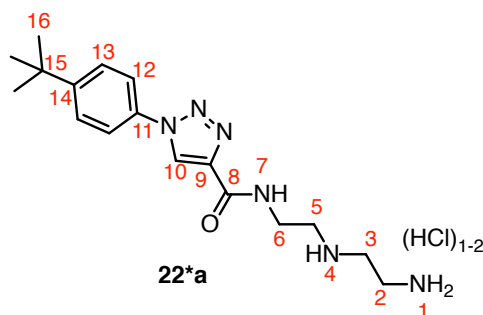

Figure 5.15: Structure of **8'b** with assigned positions.

The chemical shifts in Table 5.10 were assigned from the spectroscopic data for **8'b**, see Appendix P.1-P.7. From NMR analysis it was not possible to distinguish between position 1 and 1', nor 2 and 2'. However, from the HMBC spectrum (Appendix P.5) it can be seen that δ_C 82.8 and 27.6 ppm are next to each other, and that δ_C 78.2 and 28.0 ppm are neighbouring carbons. This reasoning also applies to **8'c** and **8'd**, see Section 5.13 and 5.14. The carbon shifts for position 1/1' and 2/2'were the same for all these three Boc-protected guanidines.

The ^{1}H NMR spectrum (Appendix P.1), displayed two different doublet-signals for H-16 and H-16', see Table 5.10. This is presumably due to restricted rotation in **8'b**.

The HRMS analysis, shown in Appendix P.7, confirms the predicted chemical formula of **8'b**: $C_{31}H_{50}N_7O_5$ [M+H]$^+$ (calcd.: 600.3873; found: 600.3869).

The presence of carbonyl groups is consistent with the IR spectrum, see Appendix P.6, which shows peaks at 1721, 1637 and 1614 cm^{-1}. The experimental procedure is given in Section 6.6.

Table 5.10: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **8'b**. The positions are illustrated in Figure 5.15.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1 or 1'	1.46	s	9H	-	27.6
1 or 1'	1.40	s	9H	-	28.0
2 or 2'	-	-	-	-	82.8
2 or 2'	-	-	-	-	78.2
3	-	-	-	-	151.8
3'	-	-	-	-	155.7
4	11.52	s	1H	-	-
5	-	-	-	-	163.1
6	8.52	t	1H	5.7	-
7	3.55	q	2H	5.8	39.8
8	3.46	q	2H	5.8	38.5
9	8.81	t	1H	5.4	-
10	-	-	-	-	159.9
11	-	-	-	-	142.7
12	8.89	s	1H	-	129.4
13	-	-	-	-	130.5
14	-	-	-	-	145.0
15	2.04	sept	2H	7.0	28.1
16 or 16'	1.10	d	6H	6.8	23.5
16 or 16'	1.06	d	6H	6.8	23.7
17	7.25	s	2H	-	121.6
18	-	-	-	-	151.3
19	3.00	sept	1H	6.8	33.7
20	1.26	d	6H	6.8	23.8

5.13 *N,N'*-DiBoc-protected *N*-(2-guanidinoethyl)-1-mesityl-1*H*-1,2,3-triazole-4-carboxamide (8'c)
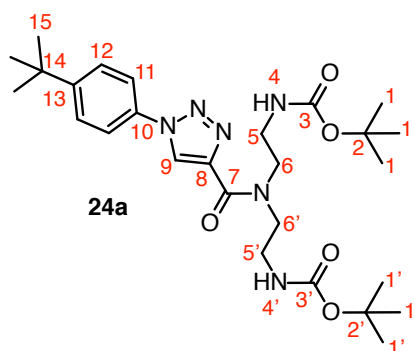

Figure 5.16: Structure of **8'c** with assigned positions.

The chemical shifts in Table 5.11 were assigned from the spectroscopic data for **8'c**, see Appendix Q.1-Q.7. See Section 5.12 for the discussion of position 1, 1', 2 and 2'.

The HRMS analysis, shown in Appendix Q.7, confirms the predicted chemical formula of **8'c**: $C_{25}H_{38}N_7O_5$ [M+H]$^+$ (calcd.: 516.2934; found: 516.2931).

The presence of carbonyl groups is consistent with the IR spectrum, see Appendix Q.6, which shows peaks at 1722, 1637 and 1614 cm^{-1}. The experimental procedure is given in Section 6.6.

Table 5.11: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **8'c**. The positions are illustrated in Figure 5.16.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1 or 1'	1.45	s	9H	-	27.6
1 or 1'	1.40	s	9H	-	28.0
2 or 2'	-	-	-	-	82.8
2 or 2'	-	-	-	-	78.2
3	-	-	-	-	151.8
3'	-	-	-	-	155.6
4	11.51	s	1H	-	-
5	-	-	-	-	163.1
6	8.50	t	1H	5.9	-
7	3.54	q	2H	5.5	40.0
8	3.46	q	2H	5.5	38.4
9	8.79	t	1H	5.3	-
10	-	-	-	-	159.9
11	-	-	-	-	142.8
12	8.78	s	1H	-	128.4
13	-	-	-	-	134.4
14	-	-	-	-	133.0
15	1.89	s	6H	-	16.7
16	7.11	s	2H	-	128.9
17	-	-	-	-	139.8
18	2.33	s	3H	-	20.6

5.14 *N,N'*-DiBoc-protected 1-(2,4,6-tri-*tert*-butylphenyl)-*N*-(2-guanidinoethyl)-1*H*-1,2,3-triazole-4-carboxamide (8'd)
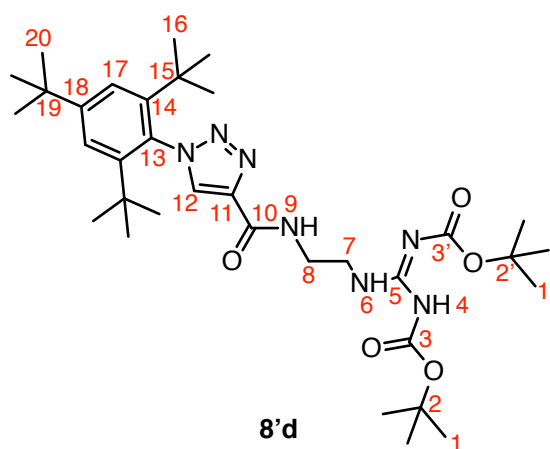
Figure 5.17: Structure of **8'd** with assigned positions.

The chemical shifts in Table 5.12 were assigned from the spectroscopic data for **8'd**, see Appendix R.1-R.7. See Section 5.12 for the discussion of position 1, 1', 2 and 2'.

The HRMS analysis, shown in Appendix R.7, confirms the predicted chemical formula of **8'd**: $C_{34}H_{56}N_7O_5$ [M+H]$^+$ (calcd.: 642.4343; found: 642.4344).

The presence of carbonyl groups is consistent with the IR spectrum, see Appendix R.6, which shows peaks at 1723, 1640 and 1618 cm^{-1}. The experimental procedure is given in Section 6.6.

Table 5.12: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **8'd**. The positions are illustrated in Figure 5.17.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1 or 1'	1.46	s	9H	-	27.6
1 or 1'	1.40	s	9H	-	28.0
2 or 2'	-	-	-	-	82.8
2 or 2'	-	-	-	-	78.2
3	-	-	-	-	151.8
3'	-	-	-	-	155.7
4	11.52	s	1H	-	-
5	-	-	-	-	163.1
6	8.52	t	1H	5.3	-
7	3.55	q	2H	5.7	39.5
8	3.45	q	2H	5.4	38.5
9	8.80	t	1H	5.3	-
10	-	-	-	-	159.8
11	-	-	-	-	141.9
12	8.99	s	1H	-	131.2
13	-	-	-	-	133.2
14	-	-	-	-	146.7
15	-	-	-	-	36.3
16	0.97	s	18H	-	31.7
17	7.56	s	2H	-	123.4
18	-	-	-	-	151.7
19	-	-	-	-	34.9
20	1.34	s	9H	-	31.0

5.15   *N,N*-Bis(2-aminoethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (12*a)
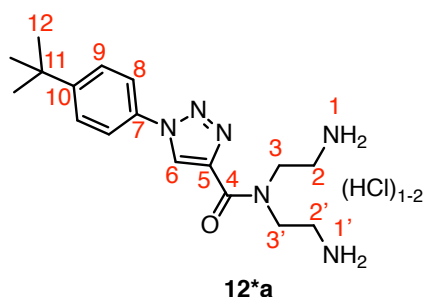
Figure 5.18: Structure of **12*a** with assigned positions.

The chemical shifts in Table 5.13 were assigned from the spectroscopic data for **12*a**, see Appendix U.1-U.7. It was not possible to distinguish between position 2, 2', 3 and 3' by NMR analysis. No long range coupling can be seen for the protons at these positions. From the COSY spectrum (Appendix U.3), it can be seen that δ_H 4.06 ppm couples to δ_H 3.22 ppm, meaning these two signals are hydrogen 2 and 3 or 2' and 3'. In the same way δ_H 3.78 ppm and δ_H 3.10 ppm are neighbouring hydrogens.

The HRMS analysis, shown in Appendix U.7, confirmed only the monoprotonated salt: $C_{17}H_{27}N_6O$ [M-Cl]$^+$ (calcd.: 331.2246; found: 331.2248). However, the amine groups integrate to 6 in the ^{1}H NMR spectrum indicating presence of the diprotonated salt.

The presence of an amide group is consistent with the IR spectrum, see Appendix U.6, which shows a strong peak at 1606 cm^{-1}.

The experimental procedure is given in Section 6.17.

Table 5.13: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, and integrals for **12*****a**. The positions are illustrated in Figure 5.18

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1 and 1'	8.28	br s	6H	-
2/2'/3/3'	4.06	app s	2H	45.9
2/2'/3/3'	3.78	app s	2H	43.8
2/2'/3/3'	3.22	app s	2H	37.4
2/2'/3/3'	3.10	app s	2H	36.7
4	-	-	-	161.9
5	-	-	-	143.5
6	9.29	s	1H	126.9
7	-	-	-	133.8
8	7.95-7.87	m	2H	120.2
9	7.67-7.60	m	2H	126.7
10	-	-	-	152.0
11	-	-	-	34.6
12	1.33	s	9H	31.0

5.16   1-(4-(*tert*-Butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (15a)
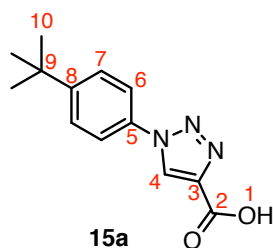
Figure 5.19: Structure of **15a** with assigned positions.

The chemical shifts in Table 5.14 were assigned from the spectroscopic data for **15a**, see Appendix W.1-W.7. Carboxylic acid **15a** has previously been synthesised,[75] but is not described.

The HRMS analysis, shown in Appendix W.7, confirms the predicted chemical formula of **15a**: $C_{13}H_{16}N_3O_2$ $[M+H]^+$ (calcd.: 246.1243; found: 246.1238).

The presence of an acid group is consistent with the IR spectrum, see Appendix W.6, which shows a broad peak at 3127 cm^{-1} and a strong peak at 1692 cm^{-1}. The experimental procedure is given in Section 6.13.

Table 5.14: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity and integrals for **15a**. The positions are illustrated in Figure 5.19.

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1	13.28	br s	1H	-
2	-	-	-	161.6
3	-	-	-	140.6
4	9.35	s	1H	127.0
5	-	-	-	133.9
6	7.63-7.61	m	2H	126.6
7	7.89-7.86	m	2H	120.2
8	-	-	-	151.9
9	-	-	-	34.6
10	1.33	s	9H	31.0

5.17   1-Mesityl-1*H*-1,2,3-triazole-4-carboxylic acid (15c)

Figure 5.20: Structure of **15c** with assigned positions.

The chemical shifts in Table 5.15 were assigned from the spectroscopic data for **15c**, see Appendix X.1-X.7. Carboxylic acid **15c** has previously been synthesised,[76] but is not described.

The HRMS analysis, shown in Appendix X.7, confirms the predicted chemical formula of **15c**: $C_{12}H_{14}N_3O_2$ [M+H]$^+$ (calcd.: 232.1086; found: 232.1086).

The presence of an acid group is consistent with the IR spectrum, see Appendix W.6, which shows a broad peak at 3149 cm^{-1} and a strong peak at 1686 cm^{-1}. The experimental procedure is given in Section 6.13.

Table 5.15: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity and integrals for **15c**. The positions are illustrated in Figure 5.20.

Position	δ_H [ppm]	Multiplicity	Integral	δ_C [ppm]
1	13.28	br s	1H	-
2	-	-	-	162.1
3	-	-	-	140.4
4	8.96	s	1H	131.4
5	-	-	-	133.3
6	-	-	-	134.9
7	1.89	s	6H	17.3
8	7.11	s	2H	129.4
9	-	-	-	140.4
10	2.33	s	3H	21.1

5.18   *N*-(2-((2-Aminoethyl)amino)ethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (22a)

Figure 5.21: Structure of **22a** with assigned positions.

The chemical shifts in Table 5.16 were assigned from the spectroscopic data for **22a**, see Appendix AB.1-AB.7. The experimental procedure is given in Section 6.18.

The HRMS analysis, shown in Appendix AB.7, confirms the predicted chemical formula of **22a**: $C_{17}H_{27}N_6O$ [M+H]$^+$ (calcd.: 331.2246; found: 331.2242).

The presence of an amide group is consistent with the IR spectrum, see Appendix AB.6, which shows a peak at 1645 cm^{-1}.

Table 5.16: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **22a**. The positions are illustrated in Figure 5.21.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	1.58 (2 of 3H)	br s	2H	-	-
2	2.62-2.56	m	2H	-	41.5
3	2.55-2.51	m	2H	-	52.2
4	1.58 (1 of 3H)	br s	1H	-	-
5	2.73-2.65	m	2H	-	48.4
6	3.37	q	2H	6.3	126.6
7	8.53	t	1H	5.6	-
8	-	-	-	-	159.4
9	-	-	-	-	143.7
10	9.22	s	1H	-	124.4
11	-	-	-	-	134.0
12	7.89-7.85	m	2H	-	120.1
13	7.63-7.59	m	2H	-	126.6
14	-	-	-	-	151.8
15	-	-	-	-	34.5
16	1.33	s	9H	-	31.0

5.19 *N*-(2-((2-Aminoethyl)amino)ethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (22*a)

Figure 5.22: Structure of **22*a** with assigned positions.

The chemical shifts in Table 5.17 were assigned from the spectroscopic data for **22*a**, see Appendix AC.1-AC.7.

The HRMS analysis, shown in Appendix AC.7, confirms the predicted chemical formula of only the monoprotonated salt: $C_{17}H_{27}N_6O$ $[M-Cl]^+$ (calcd.: 331.2246; found: 331.2240). This is not consistent with the ^{1}H NMR results which shows one amino group integrating to almost 2 and the other one integrated to 3, see Appendix AC.1. The fact that the ^{1}H NMR spectrum shows two signals with shifts corresponding to protonated amine groups indicates that the diprotonated salt is present.

The presence of an amide group is consistent with the IR spectrum, see Appendix AC.6, which shows a peak at 1660 cm^{-1}. The experimental procedure is given in Section 6.18.

Table 5.17: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **22***a. The positions are illustrated in Figure 5.22.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1	8.43	br s	3H	-	-
2 or 3	3.27	t	2H	6.0	44.1
2 or 3	3.22-3.14 (2 of 4H)	m	2H	-	35.2 or 46.2
4	9.53	br s	2H	-	-
5	3.22-3.14 (2 of 4H)	m	2H	-	35.2 or 46.2
6	3.65	q	2H	6.0	35.1
7	8.89	t	1H	5.7	-
8	-	-	-	-	160.0
9	-	-	-	-	143.2
10	9.34	s	1H	-	124.7
11	-	-	-	-	133.9
12	7.91-7.84	m	2H	-	120.1
13	7.65-7.59	m	2H	-	126.7
14	-	-	-	-	151.9
15	-	-	-	-	34.5
16	1.33	s	9H	-	31.0

5.20 Di-*tert*-butyl (((1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carbonyl) azanediyl)bis(ethane-2,1-diyl))dicarbamate (24a)

Figure 5.23: Structure of **24a** with assigned positions.

The chemical shifts in Table 5.18 were assigned from the spectroscopic data for **24a**, see Appendix AE.1-AE.7. Two multiplet signals with integrals of 1.7H (δ_H 6.99-6.87 ppm) and 0.3H (δ_H 6.64-6.48 ppm) were observed in the ^{1}H NMR spectrum (Appendix AE.1). From COSY (Appendix AE.3), both of these signals couple to the multiplet at δ_H 3.23-3.13 ppm. They were therefore assigned to H-4 and H-4'. The appearance of these signals is probably due to a dynamic process. This could have been confirmed by performing the ^{1}H NMR analysis at different temperatures. It was not possible to distinguish between the position 1/1', 2/2', 3/3', 5/5' or 6/6' from NMR analysis.

The HRMS analysis, shown in Appendix AE.7, confirms the predicted chemical formula of **24a**: $C_{27}H_{42}N_6O_5Na$ [M+Na]$^+$ (calcd.: 553.3114; found: 553.3119).

The presence of carbonyl groups is consistent with the IR spectrum, see Appendix AE.6, which shows strong peaks at 1694 and 1614 cm^{-1}. The experimental procedure is given in Section 6.17.

Table 5.18: Assigned ^{1}H (600 MHz, DMSO-d_6) and ^{13}C (150 MHz, DMSO-d_6) NMR shifts, multiplicity, integrals and coupling constants for **24a**. The positions are illustrated in Figure 5.23.

Position	δ_H [ppm]	Multiplicity	Integral	J [Hz]	δ_C [ppm]
1 or 1'	1.37	s	9H	-	28.2
1 or 1'	1.31	s	9H	-	28.1
2	-	-	-	-	77.6
2'	-	-	-	-	77.6
3	-	-	-	-	155.6 or 155.5
3'	-	-	-	-	155.6 or 155.5
4/4'	6.99-6.87/6.64-6.48	m	2H	-	-
5 or 5'	3.23-3.13 (2 of 4H)	m	2H	-	39.2
5 or 5'	3.23-3.13 (2 of 4H)	m	2H	-	37.8
6 or 6'	3.91-3.82	m	2H	-	48.3
6 or 6'	3.48	t	2H	6.4	46.5
7	-	-	-	-	160.9
8	-	-	-	-	144.0
9	9.16	s	1H	-	126.0
10	-	-	-	-	124.4
11	7.65-7.59	m	2H	-	126.6
12	7.92-7.59	m	2H	-	120.0
13	-	-	-	-	151.7
14	-	-	-	-	34.5
15	1.33	s	9H	-	31.0

6 Experimental

6.1 General

All chemicals used in this master's thesis were bought from Sigma-Aldrich and VWR International, except for methyl 1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3- triazole-4-carboxylate (**4a**) made by M. Sc. Maren Grøndahl,[16] and methyl 1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (**4b**), methyl 1-(2,4,6-trimethylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (**4c**) and *N*-(2-aminoethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**7b**), made in the specialisation project prior to this master thesis.[17] All chemicals were used without further purification. The air sensitive reactions were performed under nitrogen atmosphere.

Melting points were determined using a Gallenkamp FUSE F1A melting point apparatus and are uncorrected. TLC-analysis were performed on Merck Silica Gel 60 F_{254} plates. Ultraviolet light at 312 nm, chemical oxidation with phosphomolybdic acid solution (12 g in EtOH (250 mL, 96%)) and a permanganate solution (1.5 g $KMnO_4$ + 10 g K_2CO_3 + NaOH (2.5 mL, 5%, aq.) + H_2O (150 mL)) were used for detection. Silica gel (60 Å pore size, 230-400 mesh particle size) purchased from VWR International, was used for flash column chromatography. For HPLC analysis, an Agilent Technologies Infinity 1290 chromatograph with an Eclipsed XDB-C18 5 μm (150 × 4.6 mm) column, was used. Detection was done with a diode array detector (DAD). All analysis were performed with $MeOH/H_2O$ and 0.1% TFA in the water as eluent, 2 μl injection volume and 1 mL/min flow rate. Elution of a blank sample was used to correct the reported purity for all the products, see Appendix AG.1-AG.4.

NMR analysis were performed on either a Bruker 400 MHz Avance III HD equipped with a 5 mm SmartProbe z-gradient probe, or a Bruker 600 MHz Avance III HD equipped with a 5 mm cryogenic CP-TCI z-gradient probe. The recorded spectra were analysed in Bruker TopSpin 3.5 pl7 software. Chemical shifts (δ) are reported in parts per million (ppm) and the integrals as number of protons per signal. When $CDCl_3$ with TMS was used as NMR-solvent, the chemical shifts for both proton and carbon are reported with reference to TMS (0.00). When using DMSO-d_6 as solvent the shifts are calibrated using reference value 2.50 (^{1}H NMR) and 39.52 (^{13}C NMR).[84] The signal patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), m (multiplet) and/or br (broad). The coupling constant (J) are given in hertz (Hz). For the new compounds, ^{1}H and ^{13}C NMR shifts were assigned using 2D correlation spectroscopy techniques (COSY, HSQC, HMBC).

Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S"

Q-TOF instrument from Water TM. Samples were ionised 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. IR spectra were recorded using a Bruker Alpha FT-IR spectrometer with an ATR-module. The spectra were analysed in Opus 7.5. Only the strongest and structurally most important peaks are listed as with wavenumbers (cm^{-1}), and are indicated as strong (s), m (medium), weak (w) and/or broad (br).

6.1.1   Caution

Azides are potentially explosive and should be handled with caution.[42]

6.2 Synthesis of 2-azido-1,3,5-tri-*tert*-butylbenzene (3d)

Azide **3d** was prepared three times as described by Ching *et al.* with modifications.[64] The reaction conditions and results are given in Table 6.1. Aniline **2d** (1 equiv) was dissolved in dry MeCN (2.5 mL/mmol **2d**). The solution was cooled to 0 °C before *tert*-butyl nitrite (**1**) (1.5-2.9 equiv) was added dropwise. For entry 1 and 3 (Table 6.1), azidotrimethylsilane (**1'**) (1.2 equiv) was added dropwise right after the addition of **1**. For entry 2 (Table 6.1), the reaction mixture was stirred for 40 min at 0 °C and 2.5 h at r.t., before the solution was cooled to 0 °C and **1'** (2.3 equiv) was added dropwise. The mixture was stirred for 10-20 min at 0 °C and 45-95 h at r.t., before additional **1** (1.4-1.5 equiv) and **1'** (1.1 equiv) were added dropwise at 0 °C. This afforded a precipitate. After stirring for additional 0-120 minutes at 0 °C and for 24-89 h at r.t., solvent was removed under reduced pressure. Purification by column chromatography (7:3 cyclohexane/DCM) afforded **3d** as a white crystalline solid in 61-78% yields.

Table 6.1: Reaction conditions and results for the synthesis of **3d**.

Entry	2d [g, mmol]	1 [equiv][a]	Time 0 °C [min][b] Time r.t. [h][b]	1 [equiv][c]	Time 0 °C [min][d] Time r.t. [h][d]	3d [g, mmol, %]
1	0.41, 1.57	1.5	20 95	1.4	120 24	0.28, 0.98, 63
2	0.41, 1.57	2.9	15 45	1.5	0 89	0.27, 0.95, 61
3	2.05, 7.85	1.5	10 46	1.4	0 45	1.77, 6.14, 78

[a] First portion of **1**.
[b] Time before second portion of **1** was added.
[c] Second portion of **1**.
[d] Time after second portion of **1** was added.

Data for **3d**: Mp. 124.6-126.0 °C. R_f: 0.64 (7:3 cyclohexane/DCM). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.34 (s, 2H, H-5), 1.48 (s, 18H, H-4), 1.31 (s, 9H, H-8). IR (ATR): 2985 (m), 2907 (w), 2872 (w), 2130 (s), 2103 (m), 1595 (w), 1478 (w), 1454 (w), 1422 (m), 1392 (w), 1362 (m), 1304 (m), 1266 (m), 1244 (w), 1220 (w), 1078 (w), 927 (w), 881 (w), 809 (w), 739 (s), 652 (w), 554 (w) cm^{-1}. The ^{1}H NMR analysis corresponded with reported data for **3d**.[64] The

6.3 Synthesis of Methyl 1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (4d) and Methyl 1-(2,4,6-tri-*tert*-butylphenyl)-1*H*-1,2,3-triazole-5-carboxylate (4'd)

The synthesis of **4d** was carried out as described by Bakka *et al.* with modifications.[24] The reaction conditions and results are given in Table 6.2. To a suspension of methyl propiolate (0.95-2 equiv), $CuSO_4 \cdot 5H_2O$ (0.05 mL/mmol **3d**, 1 M in H_2O, 5 mol%), sodium ascorbate (0.05 mL/mmol **3d**, 2 M in H_2O, 10 mol%) and benzoic acid (11.5 mg/mmol **3d**, 0.1 equiv) in H_2O/*t*-BuOH or H_2O (see Table 6.2) was added **3d** (1 equiv) in DCM or THF (see Table 6.2). For entry 2 and 3 (Table 6.2), additional methyl propiolate (2 equiv) was added after 29 h (entry 2) or 25 h (entry 3). After stirring for a total of 48-126 h, the reaction mixture was cooled to r.t. before solvent were removed under reduced pressure. The crude product was dissolved in H_2O (22 mL/mmol **3d**) and extracted with DCM (4 × 22 mL/mmol **3d**). The organic phase was washed with H_2O (1-3 × 22 mL/mmol **3d**), dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. Purification by column chromatography (gradient: 0-10% EtOAc in DCM (entry 1 and 2) and DCM (entry 3)) afforded a mixture of **4d** and **4'd** as a white solid in 64-87% yields.

Table 6.2: Reaction conditions and results for the synthesis of **4d**.
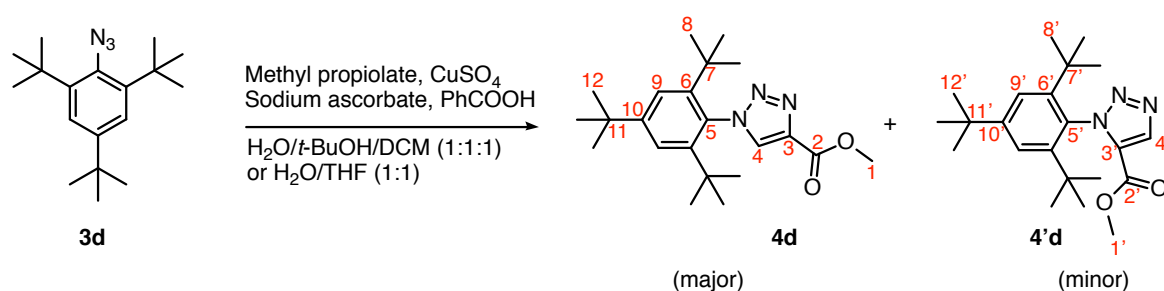
Entry	3d [g, mmol]	Methyl propiolate [equiv]	Solvent	Temp. [°C]	Time [h]	4d+4d' [g, %]
1	0.25, 0.86	0.95	H_2O/*t*-BuOH/DCM[a]	r.t.	126	0.06, 64
2	0.26, 0.90	2 + 2	H_2O/THF[b]	50	48	0.25, 74
3	1.56, 5.4	2 + 2	H_2O/THF[c]	50	70	1.73, 87

[a] H_2O (0.67 mL), *t*-BuOH (0.67 mL), DCM (0.67 mL)
[b] H_2O (0.9 mL), THF (0.9 mL)
[c] H_2O (5.4 mL), THF (5.4 mL)

For entry 2 (Table 6.2) the mixture of **4d** and **4'd** was crystallised from DCM/*n*-pentane. This afforded **4d** (0.13 g, 0.35 mmol, 39%) as white crystals. Data for **4d**: Mp. 189.2-191.4 °C. R_f: 0.10 (DCM). ^{1}H NMR (600 MHz, CDCl$_3$): δ 8.25 (s, 1H, H-4), 7.56 (s, 2H, H-9), 4.00 (s, 3H, H-1), 1.36 (s, 9H, H-12), 1.04 (s, 18H, H-8). ^{13}C NMR (150 MHz, CDCl$_3$): δ 161.3 (C-2), 152.6 (C-10), 147.4 (C-6), 138.7 (C-3), 134.4 (C-4), 130.9 (C-5), 123.8 (C-9), 52.3 (C-1), 36.8 (C-7), 35.3 (C-11), 32.2 (C-8), 31.3 (C-12). IR (ATR): 3120 (w), 2955 (s), 2907 (m), 2871 (w), 1723 (s), 1597 (w), 1537 (w), 1468 (w), 1433 (m), 1394 (w), 1362 (m), 1334 (w), 1242 (s), 1220 (s), 1153 (m), 1138 (m), 1031 (s), 859 (w), 805 (w), 780 (m), 728 (w), 651 (w) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{22}$H$_{34}$N$_3$O$_2$ [M+H]$^+$: 372.2651; found: 372.2651.

For entry 3 (Table 6.2) the column afforded a fraction consisting of pure **4'd** (7 mg, 0.02 mmol) as a white solid. The other fractions from the column contained both **4d** and **4'd**. Crystallisation of these fractions from DCM/*n*-pentane, afforded **4d** (1.01 g, 2.7 mmol, 50% from **3d**) as white crystals. Data for **4d** was in accordance with entry 2. Data for **4'd**: R_f: 0.21 (DCM). ^{1}H NMR (600 MHz, CDCl$_3$): δ 8.23 (s, 1H, H-4'), 7.53 (s, 2H, H-9'), 3.76 (s, 3H, H-1'), 1.37 (s, 9H, H-12'), 0.99 (s, 18H, H-8'). ^{13}C NMR (150 MHz, CDCl$_3$): δ 158.5 (C-2'), 151.7 (C-10'), 146.3 (C-6'), 137.2 (C-4'), 134.4 (C-3'), 130.4 (C-5'), 124.0 (C-9'), 52.3 (C-1'), 37.1 (C-8'), 35.1 (C-11'), 32.0 (C-8'), 31.3 (C-12'). IR (ATR): 2962 (s), 2910 (w), 2872 (w), 1743 (s), 1598 (w), 1528 (w), 1462 (w), 1449 (w), 1364 (m), 1309 (m), 1280 (m), 1243 (m), 1243 (w), 1198 (m), 1172 (m), 1119 (m), 1080 (m), 1018 (w), 972 (w), 881 (w), 806 (w), 774 (w) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{22}$H$_{34}$N$_3$O$_2$ [M+H]$^+$: 372.2651; found: 372.2650.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, NOESY, IR, and MS spectra obtained for **4d** and **4'd** are shown in Appendix B.4-C.8. For structure elucidation and assignment of chemical shifts, see Section 5.

6.4 Synthesis of Amines 7

c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-*tert*-butylphenyl
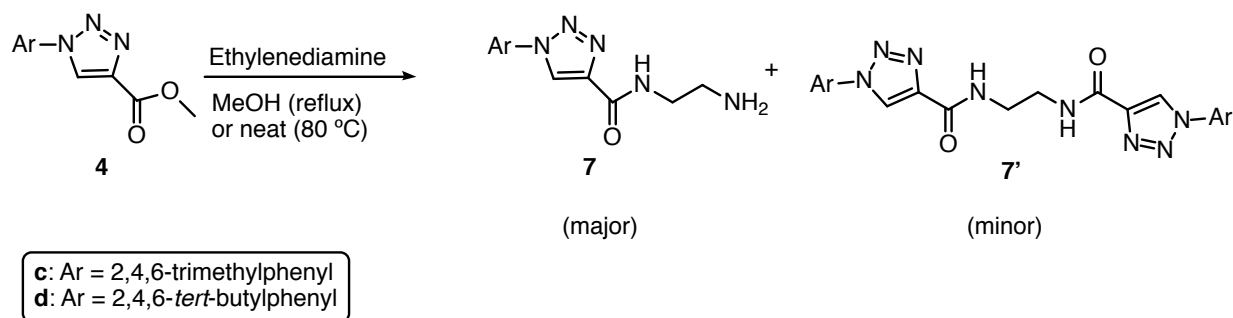
6.4.1 General Procedure A for Preparation of Amines 7

Amines **7** were prepared as described by Bakka *et al.*[24] Triazole ester **4** (1 equiv) and ethylene-diamine (EDA) (15-100 equiv) were either stirred at 80 °C or refluxed in MeOH (5 mL/mmol **4**) for 1-5 h. The volatiles were removed under reduced pressure, before the crude was dissolved in DCM or EtOAc and washed with H_2O. The organic phase was dried over $MgSO_4$ and filtered, before solvent was removed under reduced pressure. This afforded **7** in 54-94% yields.

N-(2-Aminoethyl)-1-mesityl-1*H*-1,2,3-triazole-4-carboxamide (7c) and
N,N'-(ethane-1,2-diyl)bis(1-mesityl-1*H*-1,2,3-triazole-4-carboxamide) (7'c)
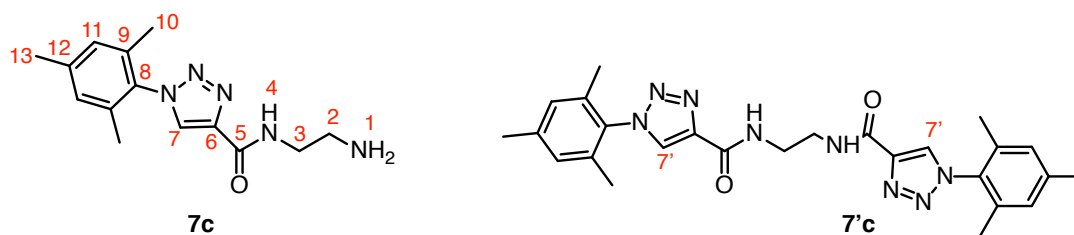
Amine **7c** was prepared twice. Following the general procedure A yielded an inseparable mixture of **7c** and **7'c** as white or off-white crystals. The reaction conditions and results are given in Table 6.3. For entry 1 (Table 6.3), the crude was dissolved in DCM (130 mL) and washed with H_2O (4 × 80 mL). For entry 2 (Table 6.3), the crude was dissolved in DCM (200 mL) and washed with H_2O (8 × 150 mL), followed by recrystallisation in toluene (10 mL).

Table 6.3: Reaction conditions and results for the synthesis of **7c**.

Entry	**4c** [g, mmol]	EDA [equiv]	MeOH [mL]	Time [h]	**7c : 7'c** [a]	Yield **7c** [g, mmol, %][b]
1	0.50, 2.02	15	10.5	1	97.4 : 2.6	0.50, 1.9, 94
2	2.10, 8.6	90	-	1	99.0 : 1.0	1.80, 6.5, 76

[a] Ratio determined by ^{1}H NMR assuming δ 8.82 ppm corresponds to **7'c**, see Appendix I.1 and I.2.

[b] Yield calculated based on the content of **7c** in the mixture determined from ^{1}H NMR analysis.

Data for the mixture: Mp. 138.6-140.5 °C. Data for **7c**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, 1H, H-7), 8.55 (t, J = 5.7 Hz, 1H, H-4), 7.11 (s, 2H, H-11), 3.28 (q, J = 6.4 Hz, 2H, H-3), 2.70 (t, J = 6.6 Hz, 2H, H-2), 2.33 (s, 3H, H-13), 1.89 (s, 6H, H-10), 1.44 (br s, 2H, H-1). HRMS (TOF ES+) m/z calcd for $C_{14}H_{20}N_5O$ [M+H]$^+$: 274.1668; found: 274.1670. Data for **7'c**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (s, assumed to be H-7'). HRMS (TOF ES+) m/z calcd for $C_{26}H_{31}N_8O_2$ [M+H]$^+$: 487.2570; found: 487.2577. ^{1}H NMR analysis corresponded with reported data for **7c**.[16]

The other peaks of **7'c** overlapped with peaks from **7c**, in addition to having low intensity, and further assignment was not possible. The ^{1}H NMR and MS spectra are shown in Appendix I.1-I.3.

N-(2-Aminoethyl)-1-(2,4,6-tri-*tert*-butylphenyl)-1H-1,2,3-triazole-4-carboxamide (7d)
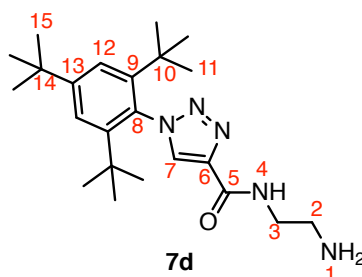
Amine **7d** was prepared twice following general procedure A. This afforded **7d** as a white solid. The dimer **7'd** was not observed. The reaction conditions and results are given in Table 6.4. For entry 1 (Table 6.4) the crude was dissolved in EtOAc (30 mL) and washed with H_2O (4 × 30 mL). For entry 2 (Table 6.4) the crude was dissolved in DCM (110 mL) and washed with H_2O (4 × 65 mL).

Table 6.4: Reaction conditions and results for the synthesis of **7d**.

Entry	**4d** [g, mmol]	EDA [equiv]	Time [h]	Yield **7d** [g, mmol, %]
1	0.11, 0.28	100	2.5	0.06, 0.15, 54
2	0.59, 1.6	100	5	0.54, 1.36, 86

Data for **7d**: Mp. 194.9-195.7 °C (decomp.). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.99 (s, 1H, H-7), 8.55 (t, J = 5.7 Hz, 1H, H-4), 7.56 (s, 2H, H-12), 3.28 (q, J = 6.5 Hz, 2H, H-3), 2.71 (t, J = 6.6 Hz, 2H, H-2), 1.48 (br s, 2H, H-1), 1.34 (s, 9H, H-15), 0.97 (s, 18H, H-11). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.5 (C-5), 151.6 (C-13), 146.8 (C-9), 142.1 (C-6), 133.1 (C-7), 131.3 (C-8), 123.4 (C-12), 42.1 (C-3), 41.2 (C-2), 36.4 (C-10), 34.9 (C-14), 31.7 (C-11), 31.0 (C-15). IR (ATR): 3338 (w, br), 3111 (w), 2960 (m), 2870 (w), 1653 (s), 1597 (w), 1570 (s), 1504 (w), 1463 (m), 1447 (m), 1395 (w), 1363 (m), 1271 (m), 1241 (m), 1219 (w), 1035 (w), 879 (w), 773 (w), 735 (w) cm^{-1}. HRMS (TOF ASAP+) m/z calcd for $C_{23}H_{38}N_5O$ [M+H]$^+$: 400.3076; found: 400.3075.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **7d** are shown in Appendix J.1-J.8. For structure elucidation and assignment of chemical shifts, see Section 5.

6.5 Synthesis of Ammonium Salts 7*

Ammonium salts **7*** were prepared following a general procedure described by Bakka with modifications.[12]
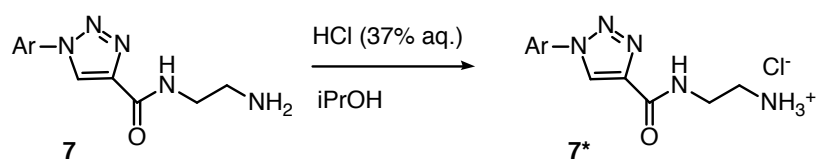
c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-tri-*tert*-butylphenyl

95

N-(2-Aminoethyl)-1-mesityl-1H-1,2,3-triazole-4-carboxamide hydrochloride (7*c)

7*c
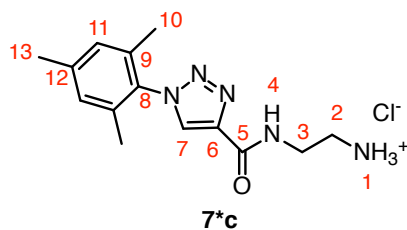
Amine **7c** (0.23 g, 0.84 mmol) was partially dissolved in iPrOH (20 mL). The mixture was heated to dissolve all of **7c**, before the mixture was filtered. Addition of HCl (0.80 mL, 9.6 mmol, 37% aq.) to the filtrate yielded a white precipitate. The reaction mixture was stirred for 2 minutes, before solvents were concentrated *in vacou*. The crude (0.25 g) was washed with MeCN (1.5 mL) affording the title compound **7*c** (0.24 g, 0.75 mmol, 90%) as white crystals.

The procedure was repeated with **7c** (0.48 g, 1.75 mmol), iPrOH (50 mL) and HCl (1.5 mL, 18 mmol, 37% aq.) affording a white crude (0.53 g). Washing with MeOH (10 mL) yielded **7*c** (0.35 g, 1.1 mmol, 65%) as a white solid.

Data for **7*c**: Mp. 275.6-276.7 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H, H-7), 8.87 (t, J = 5.9 Hz, 1H, H-4) 8.01 (s, 3H, H-1), 7.12 (s, 2H, H-11), 3.56 (q, J = 6.0 Hz, 2H, H-3), 3.06-2.95 (m, 2H, H-2), 2.34 (s, 3H, H-13), 1.90 (s, 6H, H-10). HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t$_R$ = 5.1 min, > 99% pure.

^{1}H NMR analysis corresponded with reported data for **7*c**.[16] The ^{1}H NMR spectra and HPLC chromatogram are shown in Appendix K.1-K.3.

N-(2-Aminoethyl)-1-(2,4,6-tri-*tert*-butylphenyl)-1H-1,2,3-triazole-4-carboxamide hydrochloride (7*d)

7*d
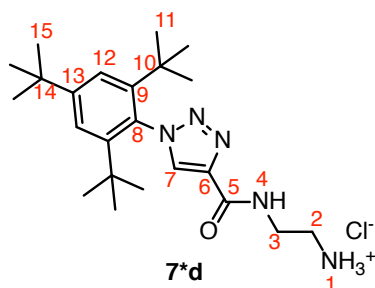
Amine **7d** (0.15 g, 0.38 mmol) was dissolved in iPrOH (8 mL) and HCl (0.32 mL, 3.8 mmol, 37% aq.) was added. The reaction mixture was stirred for 2 minutes before solvents were removed under reduced pressure. Recrystallisation in iPrOH afforded **7*d** (0.12 g, 0.26 mmol,

69%) as a white solid. Data for **7***d*: Mp. 292-294 °C (decomp.). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H, H-7), 8.88 (t, J = 5.7 Hz, 1H, H-4), 8.00 (s br, 3H, H-1), 7.57 (s, 2H, H-12), 3.56 (q, J = 6.4 Hz, 2H, H-3), 3.02 (t, J = 6.4 Hz, 2H, H-2), 1.34 (s, 9H, H-15), 0.98 (s, 18H, H-11). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.1 (C-5), 151.7 (C-13), 146.8 (C-9), 141.7 (C-6), 133.4 (C-7), 131.2 (C-8), 123.4 (C-12), 38.5 (C-2), 36.6 (C-3), 36.4 (C-10), 34.9 (C-14), 31.7 (C-11), 31.0 (C-15). IR (ATR): 3408 (w), 3105 (w), 2957 (m), 2873 (m), 1660 (s), 1598 (w), 1572 (s), 1503 (m), 1464 (m), 1447 (w), 1363 (w), 1330 (w), 1265 (w), 1243 (m), 1218 (w), 1034 (m), 878 (w), 839 (w), 776 (w), 559 (w, br) cm^{-1}. HRMS (TOF ES+) *m/z* calcd for $C_{23}H_{38}N_5O$ [M-Cl]$^+$: 400.3076; found: 400.3074. HPLC: (MeOH/H$_2$O: 80/20 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 3.6 min, > 99% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra, in addition to the HPLC chromatogram obtained for **7***d* are shown in Appendix L.1-L.9. For structure elucidation and assignment of chemical shifts, see Section 5.

6.6   Synthesis of Boc-Protected Guanidines 8'

The Boc-protected guanidines **8'** were prepared following a general procedure described by Drake *et al.*[67]
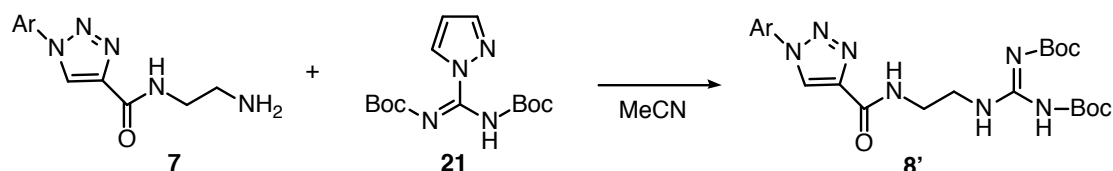
b: Ar = 2,4,6-triisopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
d: Ar = 2,4,6-tri-*tert*-butylphenyl

6.6.1   General Procedure B for Preparation of Boc-protected Guanidines 8'

To a stirred solution of *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine (**21**) (1 equiv) in MeCN (6.8-7.6 mL/mmol **21**), was added amine **7** (1.2 equiv). The reaction mixture was stirred at r.t. for 30-120 minutes before solvent was removed under reduced pressure. Purification by column chromatography (40% EtOAc in DCM) afforded **8'** as a white crystalline solid in 93-98% yields.

N,N'-DiBoc-protected N-(2-guanidinoethyl)-1-(2,4,6-triisopropylphenyl)-1H-1,2,3-triazole-4-carboxamide (8'b)

8'b
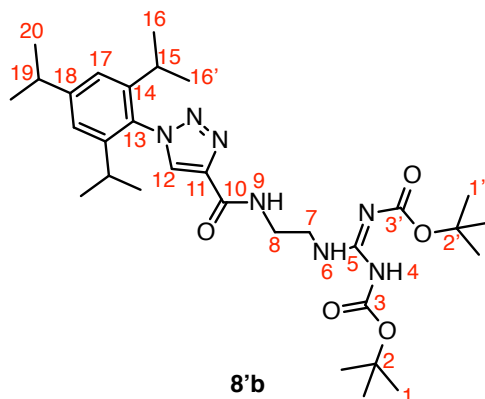
Following the general procedure B with **7b** (0.17 g, 0.47 mmol), **21** (0.12 g, 0.39 mmol) and 30 minutes reaction time afforded **8'b** (0.22 g, 0.37 mmol, 95%) as a white crystalline solid. After 5 minutes, additional MeCN (2 mL) was added due to precipitation that made it difficult to maintain the stirring. Data for **8'b**: Mp. > 210 °C (decomp.). R_f: 0.56 (40% EtOAc in DCM). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.52 (s, 1H, H-4), 8.89 (s, 1H, H-12), 8.81 (t, $J =$ 5.4 Hz, 1H, H-9), 8.52 (t, $J =$ 5.7 Hz, 1H, H-6), 7.25 (s, 2H, H-17), 3.55 (q, $J =$ 5.8 Hz, 2H, H-7), 3.46 (t, $J =$ 5.4 Hz, 2H, H-8), 3.00 (sept, $J =$ 7.0 Hz, 1H, H-19), 2.04 (sept, $J =$ 6.8 Hz, 2H, H-15), 1.46 (s, 9H, H-1 or H-1'), 1.40 (s, 9H, H-1 or H-1'), 1.26 (d, $J =$ 6.8 Hz, 6H, H-20), 1.10 (d, $J =$ 6.8 Hz, 6H, H-16 or H-16'), 1.06 (d, $J =$ 6.8 Hz, 6H, H-16 or H-16'). ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.1 (C-5), 159.9 (C-10), 155.7 (C-3'), 151.8 (C-3), 151.3 (C-18), 145.0 (C-14), 142.7 (C-11), 130.5 (C-13), 129.4 (C-12), 121.6 (C-17), 82.8 (C-2 or C-2'), 78.2 (C-2 og C-2'), 39.8 (C-7), 38.5 (C-8), 33.7 (C-19), 28.1 (C-15), 28.0 (C-1 or C-1'), 27.6 (C-1 or C-1'), 23.8 (C-20), 23.7 (C-16 or C-16'), 23.5 (C-16 og C-16'). IR (ATR): 3327 (w), 2964 (w), 2932 (w), 1721 (w), 1637 (m), 1614 (m), 1568 (m), 1460 (w), 1389 (m), 1363 (m), 1228 (w), 1155 (m), 1133 (s), 1049 (w), 1029 (w), 878 (w), 810 (w), 771 (w), 736 (w) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{31}$H$_{50}$N$_7$O$_5$ [M+H]$^+$: 600.3873; found: 600.3869.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **8'b** are shown in Appendix P.1-P.7. For structure elucidation and assignment of chemical shifts, see Section 5.

_N,N'_-DiBoc-protected _N_-(2-guanidinoethyl)-1-mesityl-1_H_-1,2,3-triazole-4-carboxamide (8'c)

8'c
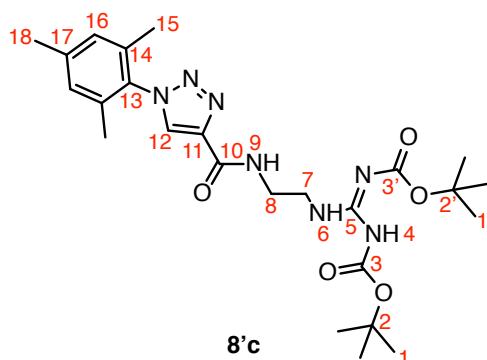
The Boc-protected guanidine **8'c** was prepared twice as a white crystalline solid, following the general procedure B. The reaction conditions and results are given in Table 6.5. In the first entry (Table 6.5) the crude was purified by two columns (40% EtOAc/_n_-pentane and 40% EtOAc/DCM) because the first column resulted in overlapping fractions of **8'c** and the byproduct pyrazole.

Table 6.5: Reaction conditions and results for the synthesis of **8'c**.

Entry	**7c** [g, mmol]	**21** [g, mmol]	MeCN [mL]	Time [h]	Yield **8'c** [g, mmol, %]
1	0.15, 0.53	0.14, 0.44	3	2	0.21, 0.41, 93
2	0.23, 0.91	0.23, 0.74	5	3	0.38, 0.73, 98

Data for **8'c**: Mp. 101.8-103.6 °C. R$_f$: 0.52 (40% EtOAc in DCM). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.51 (s, 1H, H-4), 8.79 (t, J = 5.3 Hz, 1H, H-9), 8.78 (s, 1H, H-12), 8.50 (t, J = 5.9 Hz, 1H, H-6), 7.11 (s, 2H, H-16), 3.54 (q, J = 5.5 Hz, 2H, H-7), 3.46 (q, J = 5.5 Hz, 2H, H-8), 2.33 (s, 3H, H-18), 1.89 (s, 6H, H-15), 1.45 (s, 9H, H-1 or H-1'), 1.40 (s, 9H, H-1 or H-1'). ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.1 (C-5), 159.9 (C-10), 155.6 (C-3'), 151.8 (C-3), 142.8 (C-11), 139.8 (C-17), 134.4 (C-13), 133.0 (C-14), 128.9 (C-16), 128.4 (C-12), 82.8 (C-2 or C-2'), 78.2 (C-2 or C-2'), 40.0 (C-7), 38.4 (C-8), 28.0 (C-1 or C-1'), 27.6 (C-1 or C-1'), 20.6 (C-18), 16.7 (C-15). IR (ATR): 3328 (w), 2964 (w), 2931 (w), 1722 (m), 1637 (s), 1614 (s), 1569 (s), 1490 (w), 1411 (m), 1365 (m), 1327 (s), 1156 (m), 1134 (s), 1049 (m), 1030 (m), 853 (w), 810 (w), 772 (w), 700 (w) cm^{-1}. HRMS (TOF ASAP+) _m/z_ calcd for C$_{25}$H$_{38}$N$_7$O$_5$ [M+H]$^+$: 516.2934; found: 516.2931.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **8'c** are shown in Appendix Q.1-Q.7. For structure elucidation and assignment of chemical shifts, see Section 5.

N,N'-DiBoc-protected 1-(2,4,6-tri-*tert*-butylphenyl)-N-(2-guanidinoethyl)-1*H*-1,2,3-triazole-4-carboxamide (8'd)

8'd
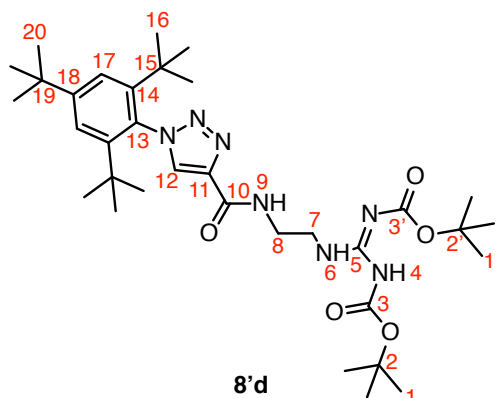
Following the general procedure B with **7d** (0.15 g, 0.38 mmol), **21** (0.10 g, 0.32 mmol) and 1.5 h, afforded **8'd** (0.20 g, 0.31 mmol, 97%) as a white crystalline solid. After 10 minutes, additional MeCN (2 mL) was added due to precipitation that made it difficult to maintain the stirring. Data for **8'd**: Mp. > 250 °C (decomp.). R_f: 0.60 (40% EtOAc in DCM). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.52 (s, 1H, H-4), 8.99 (s, 1H, H-12), 8.80 (t, *J* = 5.3 Hz, 1H, H-9), 8.52 (t, *J* = 5.8 Hz, 1H, H-6), 7.56 (s, 2H, H-17), 3.55 (q, *J* = 5.7 Hz, 2H, H-7), 3.45 (t, *J* = 5.4 Hz, 2H, H-8), 1.46 (s, 9H, H-1 or H-1'), 1.40 (s, 9H, H-1 or H-1'), 1.34 (s, 9H, H-20), 0.97 (s, 18H, H-16). ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.1 (C-5), 159.8 (C-10), 155.7 (C-3'), 151.8 (C-3), 151.7 (C-18), 146.7 (C-14), 141.9 (C-11), 133.2 (C-13), 131.2 (C-12), 123.4 (C-17), 82.8 (C-2 or C-2'), 78.2 (C-2 og C-2'), 39.5 (C-7), 38.5 (C-8), 36.3 (C-15), 34.9 (C-19), 31.7 (C-16), 31.0 (C-20), 28.0 (C-1 or C-1'), 27.6 (C-1 or C-1'). IR (ATR): 3330 (w), 2966 (w), 2873 (w), 1723 (w), 1640 (s), 1618 (s), 1572 (m), 1507 (w), 1414 (m), 1365 (m), 1330 (m), 1274 (w), 1229 (w), 1157 (m), 1136 (s), 1050 (w), 1028 (w), 988 (w), 880 (w) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for $C_{34}H_{56}N_7O_5$ [M+H]$^+$: 642.4343; found: 642.4344.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **8'd** are shown in Appendix R.1-R.7. For structure elucidation and assignment of chemical shifts, see Section 5.

6.7 Synthesis of Guanidines 8 from Amines 7

a: Ar = 4-*tert*-butylphenyl
c: Ar = 2,4,6-trimethylphenyl

a TEA was used only in the synthesis of **8c** (method 2).
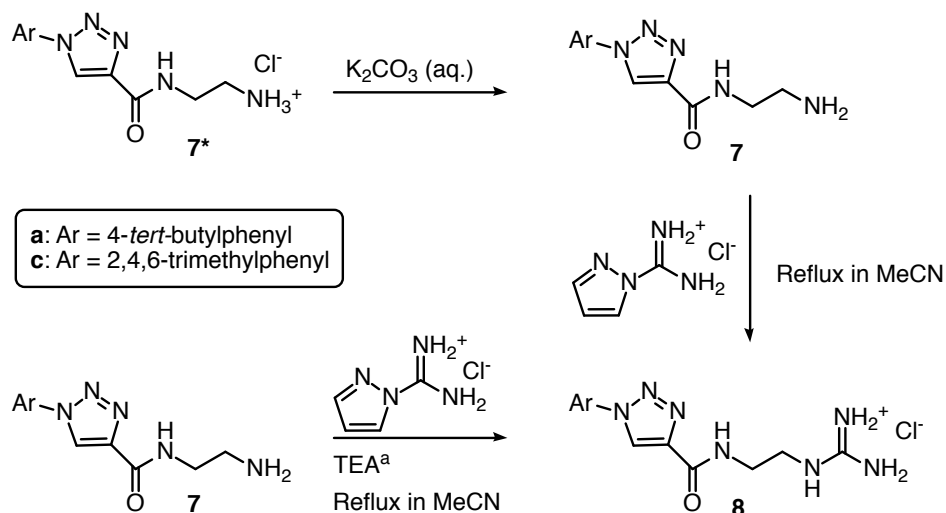
1-(4-(*tert*-Butyl)phenyl)-*N*-(2-guanidinoethyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (8a)

8a
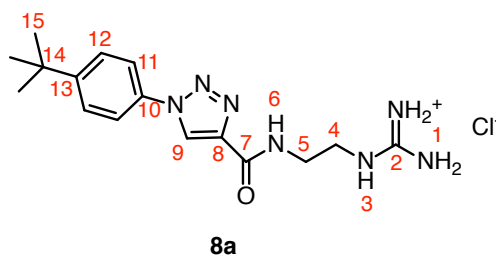
Amine **7a** (0.21 g, 0.73 mmol) was washed with H_2O (2 × 10 mL) and dried under reduced pressure. This afforded **7a** (0.19 g, 0.66 mmol, 1 equiv) as an off-white powder. Following a general procedure described by Bakka and Gautun,[57] amine **7a** was dissolved in MeCN (12 mL) and 1*H*-pyrazole-1-carboxamide hydrochloride (92 mg, 0.63 mmol, 0.98 equiv) was added. The reaction mixture was refluxed for 23 h before it was cooled to r.t. and solvent was removed under reduced pressure. The crude product was partly dissolved in MeOH (20 mL) and filtered. The filtrate was crystallised with Et_2O, filtered and washed with Et_2O (16 mL). This afforded **8a** (34 mg, 0.10 mmol, 15%) as a white solid. Data for **8a**: Mp. 282.7-284.5 °C (decomp.). ^1H NMR (600 MHz, DMSO-d_6): δ 9.28 (s, 1H, H-9), 8.74 (t, *J* = 5.9 Hz, 1H, H-6), 7.90-7.86 (m, 2H, H-11), 7.83-6.50 (m, 4H, H-1), 7.65-7.60 (m, 2H, H-12), 7.52 (t, *J* = 5.8 Hz, 1H, H-3), 3.45 (q, *J* = 6.0 Hz, 2H, H-5), 3.34 (q, *J* = 6.0 Hz, 2H, H-4), 1.33 (s, 9H, H-15). HRMS (TOF ASAP+) *m/z* calcd for $C_{16}H_{24}N_7O$ [M-Cl]$^+$: 330.2042; found: 330.2043.

HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 17.9 min, 98% pure.

The ^{1}H NMR analysis corresponded with reported data for **8a**.[16] The ^{1}H NMR and MS spectra, in addition to the HPLC chromatogram are shown in Appendix M.2-M.3.

N-(2-Guanidinoethyl)-1-mesityl-1*H*-1,2,3-triazole-4-carboxamide (8c)

8c
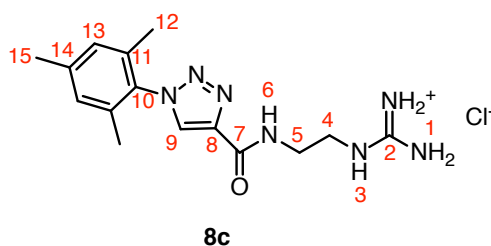
Guanidine **8c** was first prepared in two steps according to a procedure described by Bakka and Gautun with modifications (method 1),[57] and then following a procedure described by Bakka (method 2).[24]

Method 1

Ammonium salt **7*c** (0.14 g, 0.46 mmol) was dissolved in an aqueous saturated solution of K$_2$CO$_3$ (41 mL) and extracted with DCM (4 × 40 mL) and EtOAc (4 × 40 mL). The combined organic phases were dried over MgSO$_4$, filtered and solvents were removed under reduced pressure. This yielded the neutral **7c** (0.12 g, 0.44 mmol, 95%). Amine **7c** (93 mg, 0.34 mmol, 1 equiv) was dissolved in MeCN (6.2 mL), and 1*H*-pyrazole-1-carboxamide hydrochloride (47 mg, 0.32 mmol, 0.95 equiv) was added. The reaction mixture was refluxed for 4 h, cooled to r.t. and filtered, before the solvent was removed from the off-white precipitate under reduced pressure. This afforded a 72:28 mixture (determined from HPLC) of **8c** and **7*c** as an off-white solid (85 mg). The ^{1}H NMR spectrum, in addition to the HPLC chromatogram are shown in Appendix O.1 and O.7.

Method 1 was repeated with modifications, starting with the neutral amine **7c**. Amine **7c** (0.21 g, 0.76 mmol, 1 equiv) was dissolved in MeCN (12 mL), and 1*H*-pyrazole-1-carboxamide hydrochloride (0.11 g, 0.75 mmol, 0.99 equiv) was added. The reaction mixture was refluxed for 23 h. The partly cooled reaction mixture was filtered and the filtrate was concentrated under reduced pressure. Crystallisation from MeOH/Et$_2$O and washing with Et$_2$O (3 × 2 mL) yielded **8c** (48 mg, 0.14 mmol, 19%) as white crystals. Data for **8c**: Mp. > 180 °C (decomp.). ^{1}H NMR (600 MHz, DMSO-*d$_6$*): δ 8.85 (s, 1H, H-9), 8.78 (t, *J* = 5.9 Hz, 1H, H-6), 7.71-7.68 (br s, 4H, H-1), 7.58 (t, *J* = 5.8 Hz, 1H, H-3), 7.12 (s, 2H, H-13), 3.44 (q, *J* = 5.8 Hz, 2H, H-5), 3.34 (q,

J = 5.8 Hz, 2H, H-4), 2.34 (s, 3H, H-15), 1.89 (s, 6H, H-12). HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1.0 mL/min, λ = 214 nm): t$_R$ = 5.9 min, 99% pure.

The ^{1}H NMR spectrum was in accordance to reported data for **8c**.[16] The ^{1}H NMR spectrum and the HPLC chromatogram are shown in Appendix O.1 and O.9.

Method 2

Amine **7c** (0.45 g, 1.63 mmol) was dissolved in MeCN (25 mL), and 1*H*-pyrazole-1-carboxamide hydrochloride (0.33 g, 2.28 mmol) was added. The reaction mixture was refluxed for 20 h before triethylamine (0.5 mL) was added. After refluxing for additional 22 h, an extra portion of 1*H*-pyrazole-1-carboxamide hydrochloride (0.14 g, 0.96 mmol) and triethylamine (0.38 mL) were added, and the refluxing continued for 24 h. The solution was cooled to r.t. before solvent was removed under reduced pressure. This afforded a 94:6 mixture (determined from HPLC, see Appendix O.10) of **8c** and **7*c** as a yellow oil. The crude was dissolved in MeCN (22 mL), and 1*H*-pyrazole-1-carboxamide hydrochloride (0.14 g, 0.96 mmol) and triethylamine (1.4 mL) were added. The reaction mixture was refluxed for 29 h before solvent was removed under reduced pressure. This afforded the crude of **8c** (0.92 g) as a yellow oil. Purification was not attempted due to the large number of impurities indicated from the HPLC analysis, see Appendix O.11. The ^{1}H NMR spectrum of the crude is shown in Appendix O.3.

6.8 Synthesis of Guanidine 8 from 8'

Guanidines **8b** and **8c** were prepared from the corresponding Boc-protected **8'**. For the synthesis of **8c** two different methods were used.
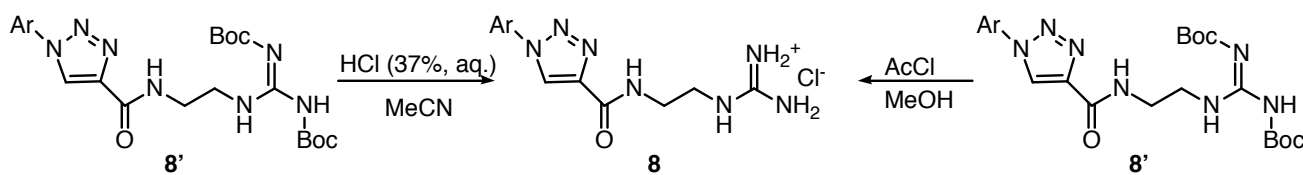
b: Ar = 2,4,6-isopropylphenyl
c: Ar = 2,4,6-trimethylphenyl

N-(2-Guanidinoethyl)-1-(2,4,6-triisopropylphenyl)-1H-1,2,3-triazole-4-carboxamide hydrochloride (8b)

8b
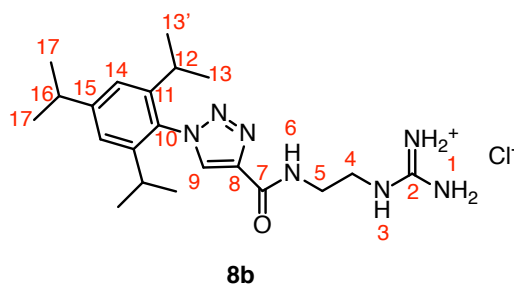
Following a general procedure described by Hickey *et al.*[69], AcCl (0.52 mL, 25 equiv) was added to a stirred solution of **8'b** (0.18 g, 0.29 mmol, 1 equiv) in MeOH (3.2 mL). The reaction mixture was stirred for 21 h at r.t., before solvent were removed under reduced pressure. Coevaporation with MeOH (5 × 10 mL) and MeOH/MeCN (5 × 10 mL, 1:1) yielded crude **8b** (0.13 g). A fraction (57%) of crude **8b** (76 mg) was dissolved in MeOH (1 mL) and AcCl (0.1 mL, 10 equiv) was added. The reaction mixture was stirred for 24 h before solvent were removed under reduced pressure. Coevaporation with MeOH (4 × 10 mL) and MeOH/MeCN (4 × 10 mL, 1:1) afforded **8b** (73 mg, 0.17 mmol, 100%) as a white solid. Data for **8b**: Mp. > 200 °C (decomp.). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.98 (s, 1H, H-9), 8.81 (t, J = 5.8 Hz, 1H, H-6), 7.85 (t, J = 5.7 Hz, 1H, H-3), 7.81-6.75 (br s, 4H, H-1), 7.25 (s, 2H, H-14), 3.44-3.40 (m, 2H, H-5), 3.38-3.34 (m, 2H, H-4), 3.00 (sept, J = 6.8 Hz, 1H, H-16), 2.03 (sept, J = 6.8 Hz, 2H, H-12), 1.26 (d, J = 6.9 Hz, 6H, H-17), 1.11 (d, J = 6.9 Hz, 6H, H-13 or H-13'), 1.07 (d, J = 6.9 Hz, 6H, H-13 or H-13'). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.0 (C-7), 157.2 (C-2), 151.4 (C-15), 145.0 (C-11), 142.5 (C-8), 130.5 (C-9), 129.6 (C-10), 121.7 (C-14), 40.3 (C-4), 37.9 (C-5), 33.8 (C-16), 28.1 (C-12), 23.8 (C-17), 23.7 (C-13 or C-13'), 23.5 (C-13 or C-13'). IR (ATR): 3345 (m, br), 3186 (m), 2962 (m), 2931 (w), 2971 (w), 1650 (s), 1584 (m), 1481 (w), 1461 (w), 1384 (w), 1365 (w), 1274 (w), 1199 (w), 1174 (w), 1124 (w), 1099 (w), 1070 (w), 877 (w), 851 (w) cm^{-1}. HRMS (TOF ES+) *m/z* calcd for $C_{21}H_{34}N_7O$ [M-Cl]$^+$: 400.2825; found: 400.2822. HPLC: (MeOH/H$_2$O: 70/30 + 0.1% TFA in the water, 1.0 mL/min, λ = 214 nm): t$_R$ = 6.0 min, > 99% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra, in addition to the HPLC chromatogram obtained for **8b** are shown in Appendix N.1-N.9. For structure elucidation and assignment of chemical shifts, see Section 5.

N-(2-Guanidinoethyl)-1-mesityl-1H-1,2,3-triazole-4-carboxamide hydrochloride (8c)

8c
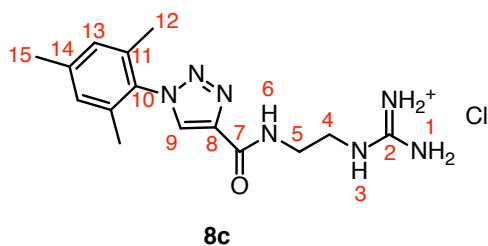
The Boc-deprotection of **8'c** was first attempted following a method descirbed by Bakka with modifications.[12] HCl (0.2 mL, 37% aq., 10 equiv) was added dropwise to the solution of **8'c** (0.11 g, 0.22 mmol, 1 equiv) in MeCN (12 mL). The reaction mixture was stirred at r.t. for 18 h before additional HCl (0.1 mL, 37% aq., 5 equiv) was added. After stirring for additional 5.5 h, solvent was removed under reduced pressure. The crude was dissolved in MeCN (12 mL) and HCl (0.1 mL, 37% aq., 5 equiv) was added before the reaction mixture was stirred for additional 17 h at 50 °C. Removal of solvent under reduced pressure and Kügelrohr distillation (0.02-0.04 mbar, 50-60 °C, 7 h) yielded crude **8c** (85 mg) as a white solid. Crystallisation from MeOH/Et$_2$O was attempted. HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1.0 mL/min, λ = 214 nm): t_R = 5.9 min, > 99% pure. The ^{1}H NMR analysis showed small impurities, see Appendix O.4.

The second method for removal of the Boc-protecting group was carried out following a general method described by Hickey *et al.*[69] AcCl (0.5 mL, 25 equiv) was added to a stirred solution of **8'c** (0.14 g, 0.28 mmol, 1 equiv) in MeOH (2.3 mL). The reaction mixture was stirred at r.t. for 24 h. Removal of solvent under reduced pressure and coevaporation with MeOH (8 × 10 mL) afforded the crude product of **8c** (0.12 g) as a white glassy solid. ^{1}H NMR analysis showed formation of a byproduct. Purification by recrystallisation in toluene and MeCN, crystallisation from MeOH/Et$_2$O and MeOH/MeCN, and washing with MeCN (6 mL) was attempted, but not successful. It was suspected that the byproduct was some unreacted intermediate with only one of the Boc-group removed. Due to this, the crude (50 mg) was dissolved in MeOH (0.8 mL) and additional AcCl (0.1 mL) added. Stirring at r.t. for 24 h, removal of solvent under reduced pressure and coevaporation with MeOH (3 × 10 mL) and MeOH/MeCN (4× 10 mL, 1:1), afforded **8c** (0.04 g, 0.13 mmol, 46%) as a white solid. HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1.0 mL/min, λ = 214 nm): t_R = 5.9 min, 99% pure.

For both methods used for the deprotection, data for **8c** were in accordance with reported data for **8c**,[16] and data given in Section 6.7. The ^{1}H NMR spectra and HPLC chromatograms are shown in Appendix O.4-O.15.

6.9 Synthesis of Branched Amines 5

The synthesis of amines **5** was carried out as described by Bakka with modifications.[12]

a: Ar = 4-*tert*-butylphenyl
b: Ar = 2,4,6-triisopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
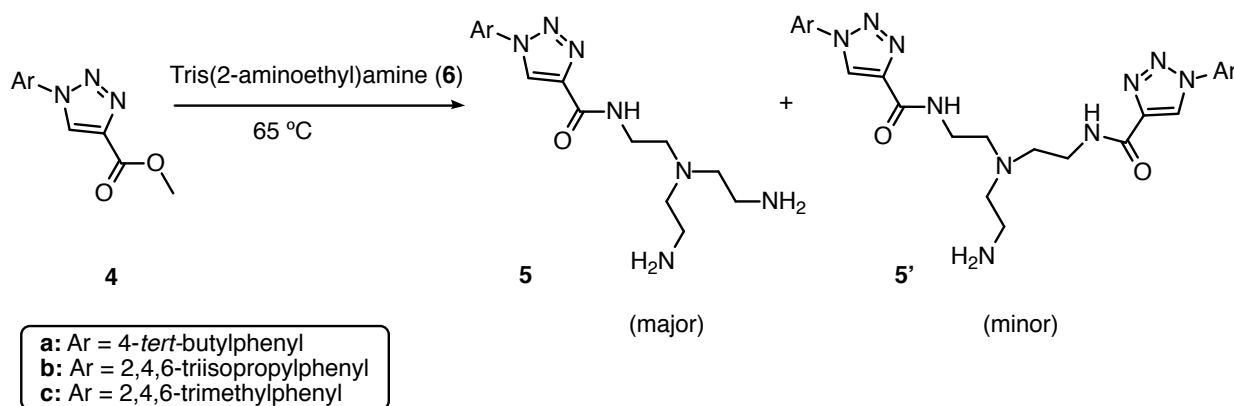
6.9.1 General Procedure C for Preparation of Amines 5

Triazole ester **4** (1 equiv) was dissolved in tris(2-aminoethyl)amine (**6**) (150 equiv) and the mixture was stirred at 65 °C for 60-80 minutes. Excess **6** was removed with Kügelrohr distillation (0.03-0.06 mbar, 90-110 °C, 2-8 h). This afforded the crude **5** in an inseparable mixture with **5'**.

***N*-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (5a) and *N,N'*-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide) (5'a)**
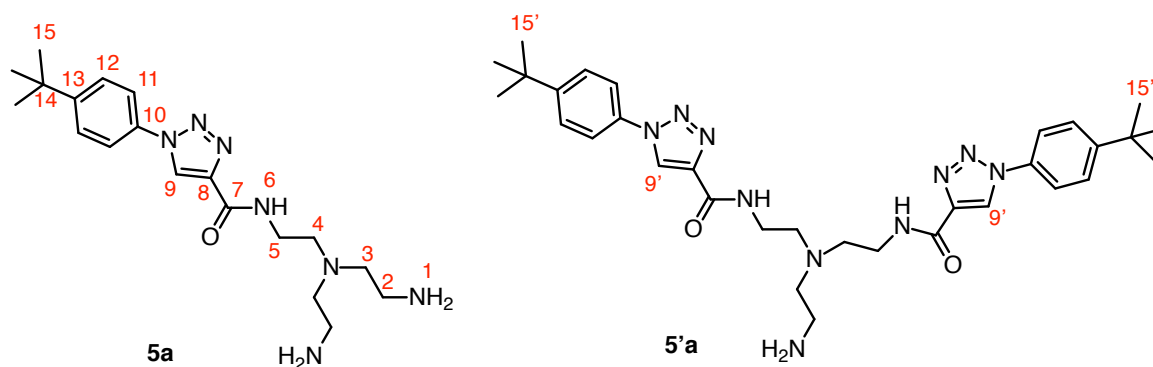
Following the general procedure C, with **4a** (0.09 g, 0.35 mmol), **6** (7.9 mL, 150 equiv) and 60 minutes afforded an 99.6 : 0.4 inseparable mixture (determined from ^{1}H NMR, see Appendix D.1) of **5a** and **5'a** as an orange wax (**5a**: 0.13 g, 0.34 mmol, 97%).

Data for **5a**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.22 (s, 1H, H-9), 8.60 (s, 1H, H-6), 7.90-7.85 (m, 2H, H-12), 7.64-7.59 (m, 2H, H-11), 3.36 (t, $J = 6.3$ Hz, 2H, H-5), 2.60 (t, $J = 6.6$ Hz, 2H, H-4), 2.57 (t, $J = 6.0$ Hz, 4H, H-2), 2.45 (t, $J = 6.3$ Hz, 4H, H-3), 2.2-1.4 (br s, shows 3.4H, H-1), 1.33 (s, 9H, H-15). Data for **5'a** ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.17 (s, assumed to be H-9'), 1.32 (s, assumed to be H-15'). The other signals for **5'a** overlapped with signals from **5a** and further assignment was not possible. The ^{1}H NMR spectrum was in accordance to reported data for **5a** and **5'a**,[17] and is shown in Appendix D.1.

N-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (5b) and *N*,*N*'-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide) (5'b)
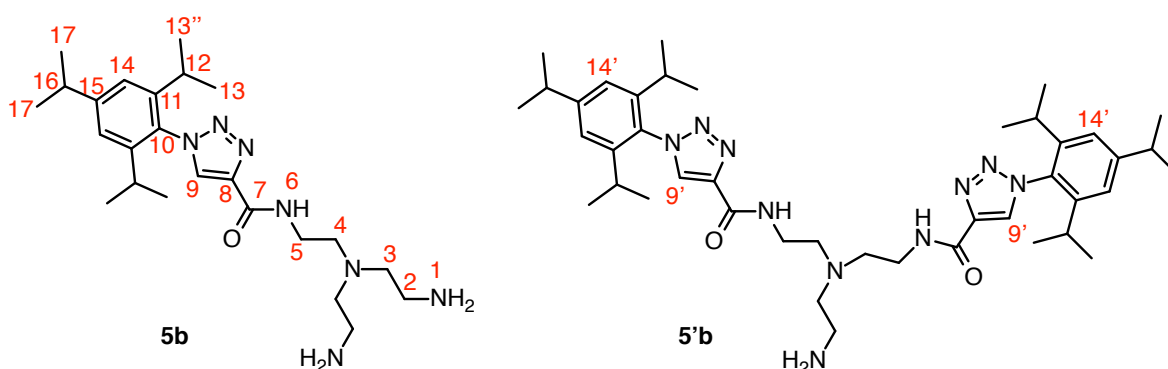
Following general procedure C, with **4b** (0.10 g, 0.31 mmol), **6** (7.0 mL, 150 equiv), addition of MeOH (1.5 mL) and 80 minutes afforded an 99.5 : 0.5 inseparable mixture (determined from ^{1}H NMR, see Appendix N.1) of **5b** and **5'b** as an orange wax (**5b**: 0.14 g, 0.32 mmol, quant.).

Data for the mixture: IR (ATR): 3310 (w, br), 3111 (w), 2961 (s), 2930 (m), 2870 (m) 2818 (w), 1657 (s), 1570 (s), 1504 (m) 1384 (m), 1364 (m), 1033 (m), 878 (w), 771 (w), 733 (w) cm^{-1}. Data for **5b**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.88 (s, 1H, H-9), 8.59 (br s, 1H, H-6), 7.25 (s, 2H, H-14), 3.36 (t, $J = 6.6$ Hz, 2H, H-5), 3.00 (sept, $J = 6.9$ Hz, 1H, H-16), 2.61 (t, $J = 6.9$ Hz, 2H, H-4), 2.59 (t, $J = 6.2$ Hz, 4H, H-2), 2.46 (t, $J = 6.2$ Hz, 4H, H-3), 2.04 (sept, $J = 6.8$ Hz, 2H, H-12), 2.0-1.3 (br s, 4H, H-1), 1.26 (d, $J = 6.9$ Hz, 6H, H-17), 1.11 (d, $J = 6.9$ Hz, 12H, H-13 or H-13"), 1.08 (d, $J = 6.9$ Hz, 12H, H-13 or H-13"). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.4 (C-7), 151.3 (C-15), 145.0 (C-11), 142.9 (C-8), 130.5 (C-10), 129.3 (C-9), 121.6 (C-14), 57.6 (C-3), 53.4 (C-4), 39.8 (C-2), 37.0 (C-5), 33.7 (C-16), 28.1 (C-12), 23.8 (C-17), 23.7 (C-13 or C-13"), 23.5 (C-13 or C-13"). HRMS (TOF ASAP+) *m/z* calcd for C$_{24}$H$_{42}$N$_7$O [M+H]$^+$: 444.3451; found: 444.3451. Data for **5'b**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.90 (s, assumed to be H-9'), 7.19 (s, assumed to be H-14'). HRMS (TOF ASAP+) *m/z* calcd for C$_{42}$H$_{65}$N$_{10}$O$_2$ [M+H]$^+$: 741.5292; found: 741.5282.

The other signals for **5'b** overlapped with signals from **5b**, in addition to having low intensity, so further assignment of shift for **5'b** was not possible. The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **5b** and **5'b** are shown in Appendix E.1-E.8. For structure elucidation and assignment of chemical shifts, see Section 5.

_N_-(2-(Bis(2-aminoethyl)amino)ethyl)-1-mesityl-1_H_-1,2,3-triazole-4-carboxamide (5c) and _N,N'_-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(1-mesityl-1_H_-1,2,3-triazole-4-carboxamide) (5'c)
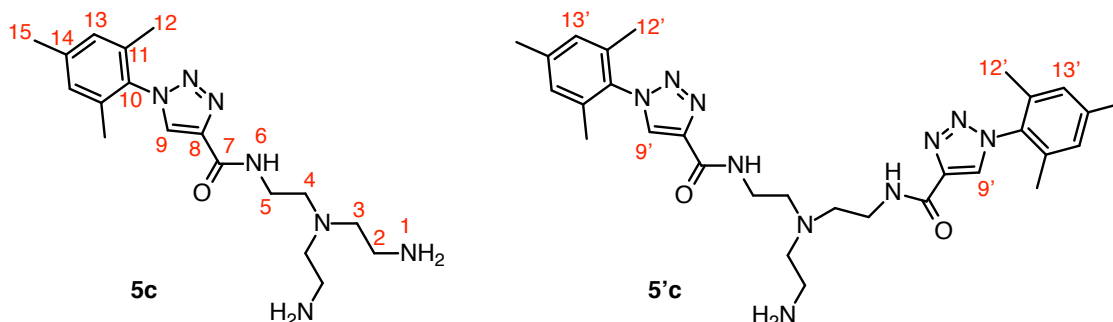
Amine **5c** was prepared twice. The reaction conditions and results are given in Table 6.6. Following the general procedure C yielded an inseparable mixture of **5c** and **5'c** as a orange wax.

Table 6.6: Reaction conditions and results for the synthesis of **5c**.

Entry	**4c** [g, mmol]	**6** [equiv]	Time [min]	Crude [g]	**5c : 5'c** [a]	Yield **5c** [g, %] [b]
1	0.10, 0.42	150	75	0.16	99.4 : 0.6	0.15, 98
2	0.20, 0.82	150	60	0.29	99.6 : 0.4	0.29, 99

[a] Ratio determined by ^{1}H NMR assuming δ 7.06 ppm corresponds to **5'c**, see Appendix F.1 and F.1.
[b] Yield **5c** calculated based on the content of **5c** in the mixture.

Data for **5c**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H, H-9), 8.60 (s, 1H, H-6), 7.11 (s, 2H, H-13), 3.40-3.31 (m, 2H, H-5), 2.60 (t, J = 6.8 Hz, 2H, H-4), 2.58 (t, J = 6.0 Hz, 4H, H-2), 2.45 (t, J = 6.0 Hz, 4H, H-3), 2.33 (s, 3H, H-15), 1.89 (s, 6H, H-12), 1.49 (br s, shows 3.4H, H-1). HRMS (TOF ASAP+) m/z calcd for $C_{18}H_{30}N_7O$ [M+H]$^+$: 360.2512; found: 360.2513. Data for **5'c**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, assumed to be H-9'), 7.06 (s, assumed to be H-13') 1.87 (s, assumed to be H-12'). HRMS (TOF ASAP+) m/z calcd for $C_{30}H_{41}N_{10}O_2$ [M+H]$^+$: 573.3414; found: 573.3409.

The other signals for **5'c** overlapped with signals from **5c**, in addition to having low intensity,

so further assignment of shifts for **5'c** was not possible. Data for **5c** and **5'c** was in accordance to reported data, [17] and the ^{1}H NMR and MS spectra are shown in Appendix F.1-F.4.

6.10 Synthesis of Branched Ammonium Salts 5*

The branched ammonium salts **5*** were prepared as described by Bakka. [12] The yields are cal-culated from the monoprotonated **5*** indicated from the ^{1}H NMR and MS analysis.

b: Ar = 2,4,6-triisopropylphenyl
c: Ar = 2,4,6-trimethylphenyl
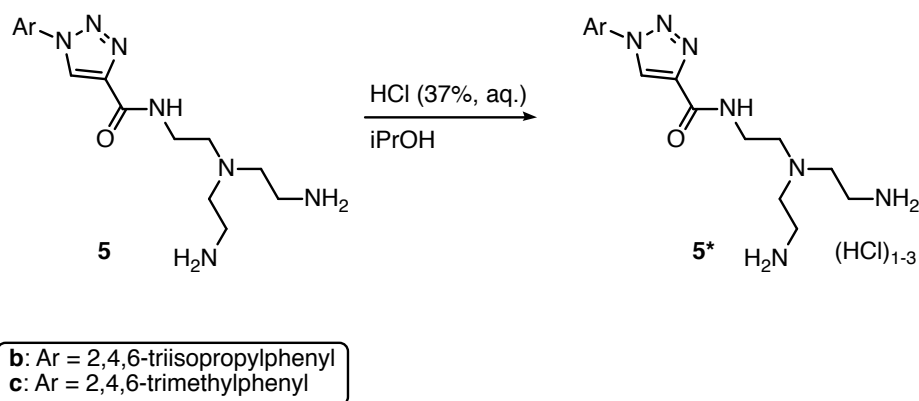
6.10.1 General Procedure D for Preparation of Branched Ammonium Salts 5*

Amine **5** (1 equiv) was dissolved iPrOH (33 mL/mmol **5**) and HCl (25 equiv, 37% aq.) was added. Stirring for 2 minutes and removal of solvents under reduced pressure, gave the crude product as a light brown solid. Purification afforded **5*** in 25-91% yields.

***N*-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(2,4,6-triisopropylphenyl)-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (5*b)**
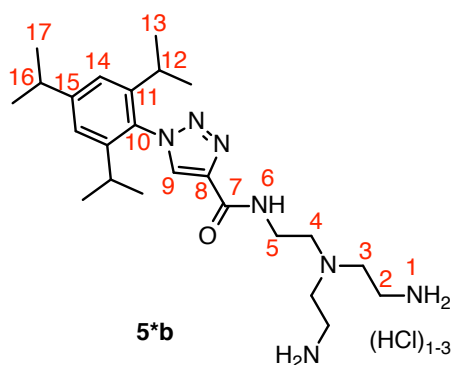
Following the general procedure D with **5b** (0.11 g, 0.25 mmol) and washing the crude product with MeOH (11 mL) afforded **5*b** (0.03 g, 0.06 mmol, 25%) as an off-white solid. Data for

5*b: Mp. > 240 °C (decomp.). ^{1}H NMR (400 MHz, 90 °C, DMSO-d_6): δ 8.82 (s, 1H, H-9), 8.63-8.55 (m, 1H, H-6), 8.22 (br s, 5H, H-1), 7.23 (s, 2H, H-14), 3.61-3.51 (m, 2H, H-5), 3.13-3.05 (m, 4H, H-2 or H-3), 3.05-2.97 (m, 5H, H-16 and H-2 or H-3), 2.97-2.85 (m, 2H, H-4), 2.14 (sept, J = 6.8 Hz, 2H, H-12), 1.29 (d, J = 6.9 Hz, 6H, H-17), 1.18-1.01 (m, 12H, H-13). ^{13}C NMR (100 MHz, 90 °C, DMSO-d_6): δ 159.5 (C-7), 150.9 (C-15), 144.7 (C-11), 142.3 (C-8), 130.2 (C-9), 128.8 (C-10), 121.2 (C-14), 52.2 (C-4), 50.5 (C-2 or C-3), 35.8 (C-2 or C-3), 35.3 (C-5), 33.2 (C-16), 27.7 (C-12), 23.2 (C-13), 23.0 (C-17). IR (ATR): 3396 (w), 3077 (w, br), 2961 (m), 1673 (s), 1569 (s), 1471 (s), 1262 (w), 1196 (w), 1057 (m), 880 (m), 765 (m), 623 (w, br) cm^{-1}. HRMS (TOF ES+) m/z calcd for $C_{24}H_{42}N_7O$ [M-Cl]$^+$: 444.3451; found: 444.3449. HPLC: (MeOH/H$_2$O: 60/40 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 10.1 min, > 99% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra, in addition to the HPLC chromatogram obtained for **5*b** are shown in Appendix G.2-G.9. For structure elucidation and assignment of chemical shifts, see Section 5.

N-(2-(Bis(2-aminoethyl)amino)ethyl)-1-mesityl-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (5*c)

5*c
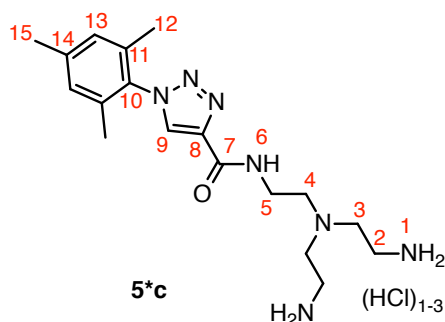
Following the general procedure D with **5c** (0.12 g, 0.33 mmol) and washing the crude product with EtOH (2 × 2 mL) afforded **5*c** (0.12 g, 0.30 mmol, 91%) as a light brown solid. Data for **5*c**: Mp. > 200 °C (decomp.). ^{1}H NMR (400 MHz, 90 °C, DMSO-d_6): δ 8.75 (s, 1H, H-9), 8.66-8.56 (m, 1H, H-6), 8.25 (br s, 5H, H-1), 7.10 (s, 2H, H-13), 3.61-3.53 (m, 2H, H-5), 3.14-2.98 (m, 8H, H-2 and H-3), 2.98-2.87 (m, 2H, H-4), 2.35 (s, 3H, H-15), 1.92 (s, 6H, H-12). ^{13}C NMR (100 MHz, 90 °C, DMSO-d_6): δ 159.6 (C-7), 142.4 (C-8), 139.4 (C-14), 134.0 (C-10), 132.6 (C-11), 128.5 (C-13), 127.8 (C-9), 52.3 (C-4), 50.6 (C-2 or C-3), 35.8 (C-2 or C-3), 35.4 (C-5), 20.1 (C-15), 16.3 (C-12). IR (ATR): 2979 (w), 1656 (s), 1566 (s), 1490 (s), 1266 (w), 1068 (m), 1033 (m), 851 (s), 770 (w), 630 (w), 536 (w) cm^{-1}. HRMS (TOF ASAP+) m/z calcd for $C_{18}H_{30}N_7O$ [M-Cl]$^+$: 360.2512; found: 360.2513. HPLC: (MeOH/H$_2$O: 40/60 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 9.8 min, > 99% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra, in addition to the HPLC chromatogram obtained for 5^***c** are shown in Appendix H.2-H.9. For structure elucidation and assignment of chemical shifts, see Section 5.

6.11 Attempted Synthesis of Branched Bisguanidine 14a

Bisguanidine **14a** was attempted prepared as described by Bakka with modification.[24]
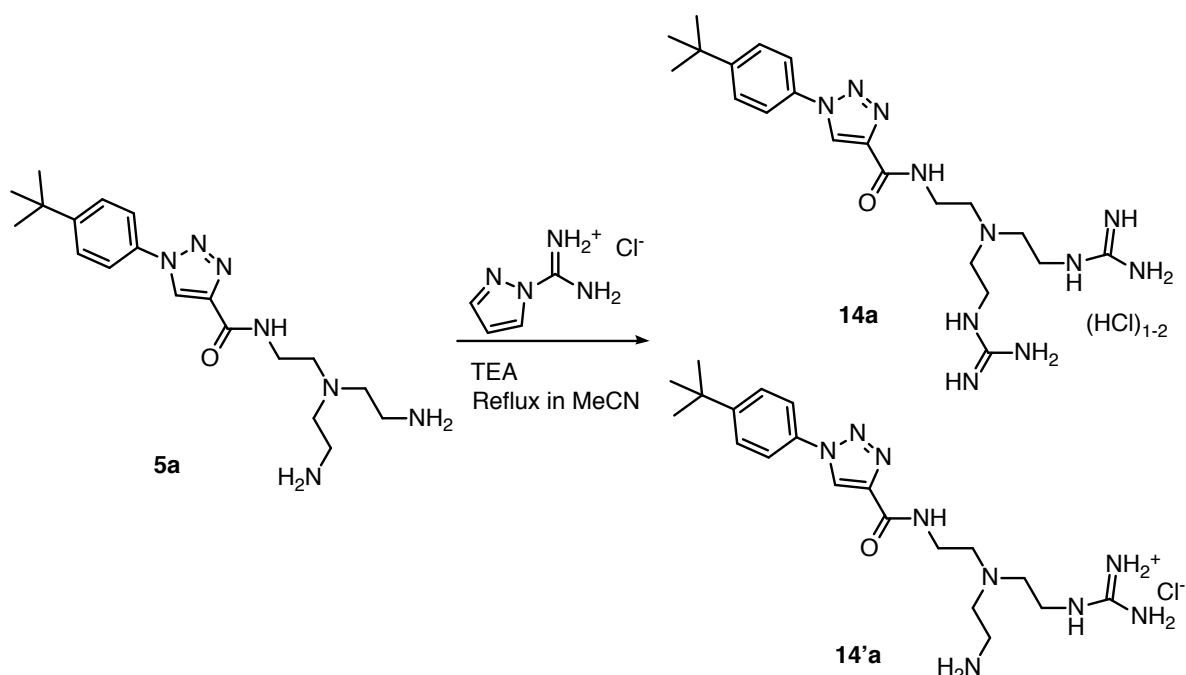
The branched amine **5a** (88 mg, 0.24 mmol) was dissolved in MeCN (8.8 mL) and 1H-pyrazole-1-carboxamide hydrochloride (67 mg, 0.46 mmol, 1.9 equiv) was added. The reaction mixture was refluxed for 3 h before additional 1H-pyrazole-1-carboxamide hydrochloride (5.8 mg, 0.04 mmol, 0.2 equiv) was added. After further refluxing for 20 h, the solution was cooled to r.t. and filtered. The brown precipitation was washed with MeCN (5 mL) and Et$_2$O (5 mL), before it was dissolved in MeOH, fitered and concentrated under reduced pressure. MS analysis confirmed the presence of both the mono- and diprotonated **14a**, in addition to **14'a**, see Appendix V.2-V.4. Trying to react **14'a** to **14a**, the brown solid crude (73 mg) was dissolved in MeCN (10 mL), and TEA (0.2 mL, 6 equiv) and 1H-pyrazole-1-carboxamide hydrochloride (73 mg, 0.5 mmol, 2 equiv) were added. After refluxing for 30 min, H$_2$O (0.4 mL) was added due to solubility problems. The refluxing continued for 26 h, before solvents were removed under reduced pressure. This afforded a brown oil (0.17 g). ^{1}H NMR shows unidentified impurities,

see Appendix V.1. HPLC analysis shows three compounds present in an 5:85:10 mixture, in addition to excess TEA, see Appendix V.5.

6.12   Synthesis of Bis(2-azidoethyl)amine (10)
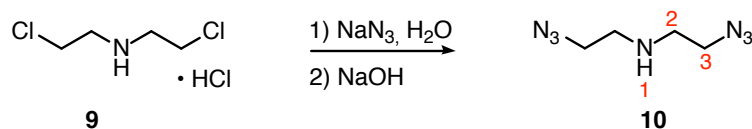
Azides are potentially explosive and a transparent safety shield was used when handling **10**.

The title compound **10** was prepared as described by Chen *et al.*[72] Bis(2-chloroethyl)amine hydrochloride (**9**, 3.00 g, 16.8 mmol) was added to a stirred solution of NaN_3 (2.70 g, 41.5 mmol) in deionised H_2O (30 mL). After stirring for 2 h at 90 °C, another portion of NaN_3 (2.71 g, 41.7 mmol) was added. The reaction mixture was stirred for 48 h at 90 °C, before it was cooled to room temperature. The pH in the solution was adjusted to approximately 10 with aqueous NaOH (1 M, 11-12 mL) and the mixture was extracted with EtOAc (4 × 30 mL). The organic phase was washed with H_2O (5 mL), dried over $MgSO_4$ and filtered, before the solvent was removed under reduced pressure. This yielded a brown crude (1.86 g). Kügelrohr distillation (0.036-0.037 mbar, 62 °C) afforded **10** (1.47 g, 9.49 mmol, 56%) as a colourless liquid. Data for **10**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.53-3.35 (m, 4H, H-3), 2.85-2.80 (m, 4H, H-2), 1.41 (br s, 1H, H-1). IR (ATR): 3322 (w, br), 2932 (w), 2834 (w), 2086 (s), 1444 (w), 1338 (w), 1263 (m), 1137 (w), 915 (w), 736 (w), 639 (w), 555 (w) cm^{-1}.

The procedure was repeated with the same amounts. Extraction with EtOAc (8 × 30 mL) yielded a brown crude. Kügelrohr distillation (0.022 mbar, 60 °C) afforded **10** (1.63 g, 10.5 mmol, 63%) as a colourless liquid.

The ^{1}H NMR spectrum corresponded with reported spectra.[43] The ^{1}H NMR and IR spectra are shown in Appendix S.1-S.2.

6.13 Synthesis of Carboxylic Acids 15

The carboxylic acids **15** were prepared following a general procedure described by Flynn and Beight.[74]

a: Ar = 4-*tert*-butylphenyl
c: Ar = 2,4,6-trimethylphenyl
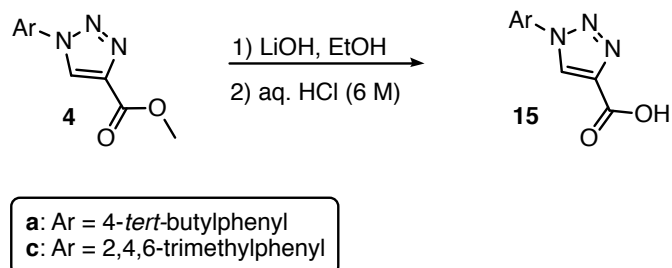
6.13.1 General Procedure E for Preparation of Carboxylic Acid 15

Triazole ester **4** (1 equiv) was dissolved in EtOH (10.5-10.9 mL/mmol **4**, 96% aq.) and a solution of aqueous LiOH (1 M, 1.5 equiv) was added. The reaction mixture was stirred at r.t. for 60-120 minutes before solvent was removed under reduced pressure. The reaction mixture was dissolved in EtOAc (20-58 mL/mmol **4**) and washed with an aqueous solution of HCl (13 mL/mmol **4**, 6 M), before the organic phase was washed with H_2O (13 mL/mmol **4**) and dried over $MgSO_4$. Filtration and removal of the solvent under reduced pressure afforded **15** in 89-96% yield.

1-(4-(*tert*-Butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (15a)
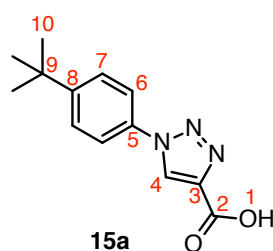
Following the general procedure E with **4a** (0.50 g, 1.93 mmol) and 60 minutes, but adding 3 equivalents LiOH and washing with HCl (48 mL, 6 M, aq.), yielded **15a** (0.44 g, 1.79 mmol, 93%) as a white solid. Data for **15a**: Mp. 158.3-159.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.28 (br s, 1H, H-1), 9.35 (s, 1H, H-4), 7.90-7.86 (m, 2H, H-7), 7.64-7.59 (m, 2H, H-6), 1.33 (s, 9H, H-10). ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.6 (C-2), 151.9 (C-8), 140.6 (C-3), 133.9 (C-5), 127.0 (C-4), 126.6 (C-6), 120.2 (C-7), 34.6 (C-9), 31.0 (C-10). IR (ATR): 3127 (w), 2958 (w), 1692 (s), 1549 (m), 1533 (m), 1516 (m), 1395 (m), 1273 (m), 1247 (s), 909 (m),

736 (s), 572 (m), 549 (s) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{13}$H$_{16}$N$_3$O$_2$ [M+H]$^+$: 246.1243; found: 246.1238.

The general procedure E was repeated with **4a** (4.23 g, 16.3 mmol) and 120 minutes. This afforded **15a** (3.85, 15.7 mmol, 96%) as an off-white solid.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **15a** are shown in Appendix W.1-W.7. For structure elucidation and assignment of chemical shifts, see Section 5.

1-Mesityl-1*H*-1,2,3-triazole-4-carboxylic acid (15c)
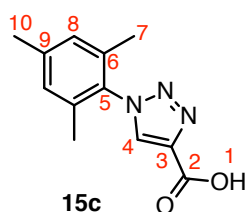
Following the general procedure E with **4c** (1.04 g, 4.22 mmol) and 75 minutes, afforded **15c** (0.86 g, 3.72 mmol, 89%) as a white solid. Data for **15c**: Mp. 170.3-171.0 °C. ^{1}H NMR (600 MHz, DMSO-*d$_6$*): δ 13.23 (br s, 1H, H-1), 8.96 (s, 1H, H-4), 7.11 (s, 2H, H-8), 2.33 (s, 3H, H-10), 1.89 (s, 6H, H-7). ^{13}C NMR (150 MHz, DMSO-*d$_6$*): δ 162.1 (C-2), 140.4 (C-9 and C-3), 134.9 (C-6), 133.3 (C-5), 131.4 (C-4), 129.4 (C-8), 21.1 (C-10), 17.3 (C-7). IR (ATR): 3149 (w), 2954 (w), 1686 (s), 1537 (m), 1410 (m), 1242 (s), 1190 (m), 1035 (s), 859 (m), 844 (m), 769 (m), 584 (m), 548 (m) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{12}$H$_{14}$N$_3$O$_2$ [M+H]$^+$: 232.1086; found: 232.1089.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **15c** are shown in Appendix X.1-X.7. For structure elucidation and assignment of chemical shifts, see Section 5.

6.14 Synthesis of *N,N*-Bis(2-azidoethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (11a)

Bisazide **11a** was first attempted synthesised from triazole methyl ester **4a** (Section 6.14.1). This was however unsuccessful. Using the more reactive acid chloride **16a** successfully afforded **11a**, see Section 6.14.2.

6.14.1 Attempted Synthesis of 11a from Triazole Methyl Ester 4a

Bisazide **11a** was attempted synthesised from **4a** following a modified procedure by Chen *et al.* [72] (method 1) and a procedure described by Bakka *et al.* with modifications (method 2). [24]
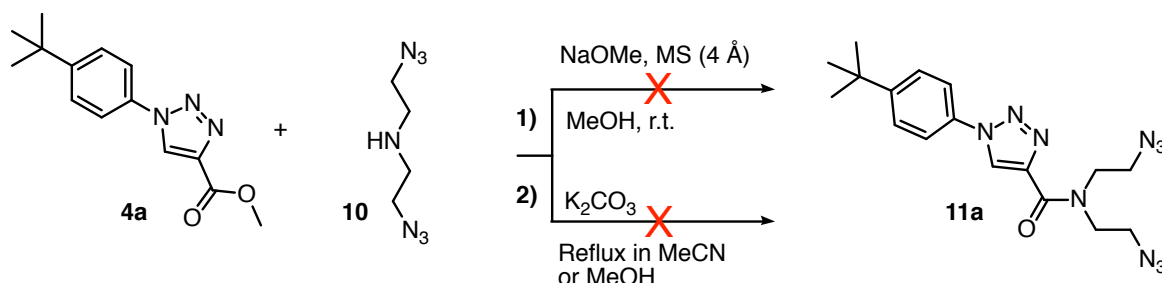
Method 1

A suspension of **4a** (0.11 g, 0.42 mmol, 1 equiv), **10** (0.19 g, 1.22 mmol, 3 equiv) (Section 6.12), NaOMe (0.02 g, 1 equiv), molecular sieves (0.4 g, activated, 4 Å) and MeOH (3 mL) was stirred under nitrogen atmosphere for 144 h at r.t. ^{1}H NMR analysis (see Appendix T.1) showed no conversion and work-up was not attempted.

Method 2

Compound **11a** was attempted synthesised twice following this method. The reaction conditions are given in Table 6.7. A solution of **4a** (1 equiv) in MeOH or MeCN was added to **10** (1 equiv) (Section 6.12) and heated to reflux for 22-28 h before K_2CO_3 (1 equiv) was added. The reaction mixture was again refluxed for 63-65 h. For entry 1 (Table 6.7), the reaction mixture was dissolved in DCM (30 mL) and washed with H_2O (2 × 30 mL) and HCl (30 mL, 1 M, aq.). The organic phase was dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. This afforded a white solid crude (15 mg) of an unidentified product, see Appendix T.2 for the ^{1}H NMR spectrum. For entry 2 (Table 6.7) work-up was not attempted since ^{1}H NMR analysis showed no conversion, see Appendix T.3.

Table 6.7: Reaction conditions for the attempted synthesis of **11a**.

Entry	**4a** [g, mmol]	Solvent
1	0.18, 0.69	MeOH (3 mL)
2	0.17, 0.66	MeCN (1.7 mL)

6.14.2 Preparation of 11a from Acid Chloride 16a

Bisazide **11a** was prepared in two steps from carboxylic acid **15a** following a general procedure described by Sing *et al.* with modifications.[77]
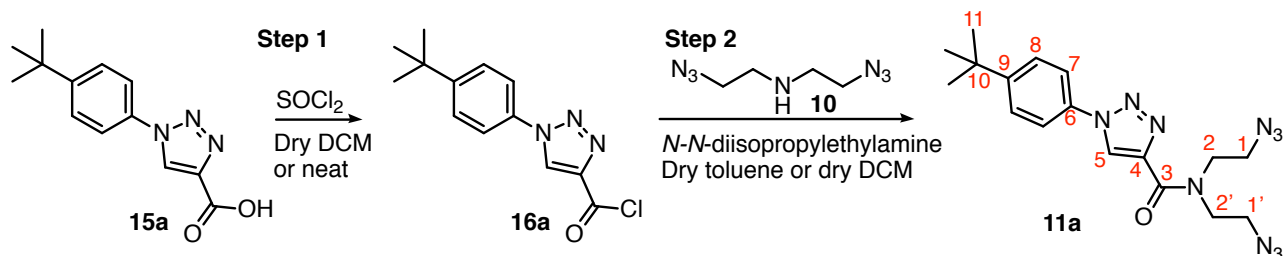
Method 1

Step 1: Carboxylic acid **15a** (0.21 g, 0.86 mmol, 1 equiv) (Section 6.13.1) was partly dissolved in dry DCM (5 mL) before $SOCl_2$ (0.19 mL, 2.57 mmol, 3 equiv) was added. The reaction mixture was refluxed for 45 minutes before additional $SOCl_2$ (2 mL, 27.6 mmol) was added due to poor solubility of **15a** in DCM. The solution was refluxed for 3 h more before solvents were removed under reduced pressure. This yielded the crude product of **16a** as a white oily solid (0.25 g, quant.).

Step 2: The crude of **16a** was partly dissolved in dry toluene (8 mL) and added over 10 minutes to a solution of **10** (0.16 g, 1.03 mmol, 1.2 equiv) (Section 6.12) in dry toluene (4 mL) cooled on an ice-bath. *N,N*-Diisopropylethylamine (0.4 mL, 2.6 equiv) was added and the reaction mixture was stirred at 70 °C for 17 h, before it was cooled to r.t. and solvent was removed under reduced pressure. The crude was dissolved in DCM (30 mL/mmol **15a**) and washed with HCl (30 mL/mmol **15a**, 1 M, aq.) and H_2O (2 × 30 mL/mmol **15a**). The organic phase was dried over $MgSO_4$ and filtered, before solvent was removed *in vacuo*. Purification by column chromatography (40% EtOAc in *n*-pentane) afforded **11a** (0.21 g, 0.55 mmol, 65%) as a yellow solid.

Method 2

Due to the solubility problems observed using method 1, this modified method was used to synthesise **11a** twice. The reaction conditions and results are given in Table 6.8.

Step 1: Carboxylic acid **15a** (1 equiv) (Section 6.13.1) was dissolved in $SOCl_2$ (2.5 mL/mmol **15a**) and stirred at 70 °C for 4 h under nitrogen atmosphere, before solvent was removed under reduced pressure. This yielded the crude product of **16a** as a white solid.

Step 2: A solution of **10** (1.1 equiv) (Section 6.12) dissolved in dry DCM (2.5 mL/mmol **15a)** was added over 10 minutes to a solution of the crude of **16a** in dry DCM (2.5 mL/mmol **15a**) at

0 °C. *N,N*-Diisopropylethylamine (2 equiv) was added and the reaction mixture was refluxed for 47-64 h. Work-up and purification as described in method 1 (step 2), afforded **11a** as an off-white solid in 70-86% yields.

Table 6.8: Reaction conditions and results for the synthesis of **11a**.

Entry	**15a** [g, mmol]	Crude of **16a** [g]	Time step 2 [h]	Yield **11a** [g, mmol, %][a]
1	0.50, 2.04	0.66	47	0.52, 1.35, 70
2	0.48, 1.97	0.55	64	0.71, 1.86, 86

[a] Yield calculated over two steps from **15a**.

Data for **16a**: ^{1}H NMR (400 MHz, CDCl$_3$): 8.59 (s, 1H), 7.70-7.66 (m, 2H), 7.61-7.57 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 159.3, 153.8, 143.4, 133.4, 127.0, 120.7, 35.0, 31.2 . IR (ATR): 3129 (w), 2961 (w), 2902 (w), 2867 (w), 1755 (s), 1512 (m), 1473 (w), 1463 (w), 1410 (w), 1362 (w), 1267 (w), 1210 (m), 1198 (m), 1171 (m), 1121 (w), 1108 (w), 997 (m), 987 (m), 832 (s), 715 (m), 680 (w), 555 (m), 492 (m), 437 (w) cm^{-1}. Data for **11a**: Mp. 76.1-77.7 °C. R$_f$: 0.38 (40% EtOAc inn *n*-pentane). ^{1}H NMR (600 MHz, DMSO-*d$_6$*): δ 9.29 (s, 1H, H-5), 7.91-7.87 (m, 2H, H-7), 7.64-7.60 (m, 2H, H-8), 4.17 (t, *J* = 6.1 Hz, 2H, H-2 or H-2'), 3.71 (t, *J* = 6.2 Hz, 2H, H-2 or H-2'), 3.67 (t, *J* = 6.2 Hz, 2H, H-1 or H-1'), 3.60 (t, *J* = 6.0 Hz, 2H, H-1 or H-1'), 1.33 (s, 9H, H-11). ^{13}C NMR (150 MHz, DMSO-*d$_6$*): δ 161.0 (C-3), 151.9 (C-9), 143.9 (C-4), 133.8 (C-6), 127.0 (C-5), 126.6 (C-8), 120.2 (C-7), 49.5 (C-1 or C-1'), 48.1 (C-1 or C-1'), 47.3 (C-2 or C-2'), 45.4 (C-2 or C-2'), 34.5 (C-10), 30.9 (C-11). IR (ATR): 3120 (w), 2932 (w), 2099 (s), 1622 (m), 1537 (w), 1523 (w), 1404 (w), 1265 (w, br), 1039 (W), 837 (w), 760 (w), 556 (w) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for C$_{17}$H$_{23}$N$_{10}$O [M+H]$^+$: 383.2056; found: 383.2054.

The ^{1}H NMR, ^{13}C NMR and IR spectra obtained for **16a** are shown in Appendix Y.1-Y.3. The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **11a** are shown in Appendix T.4-T.10. For structure elucidation and assignment of chemical shifts for **11a**, see Section 5.

6.15 Attempted Synthesis of 12a and/or 12*a from Bisazide 11a

The reduction of the branched bisazide **11a** was attempted using three different methods, none of them successful.

Method 1:
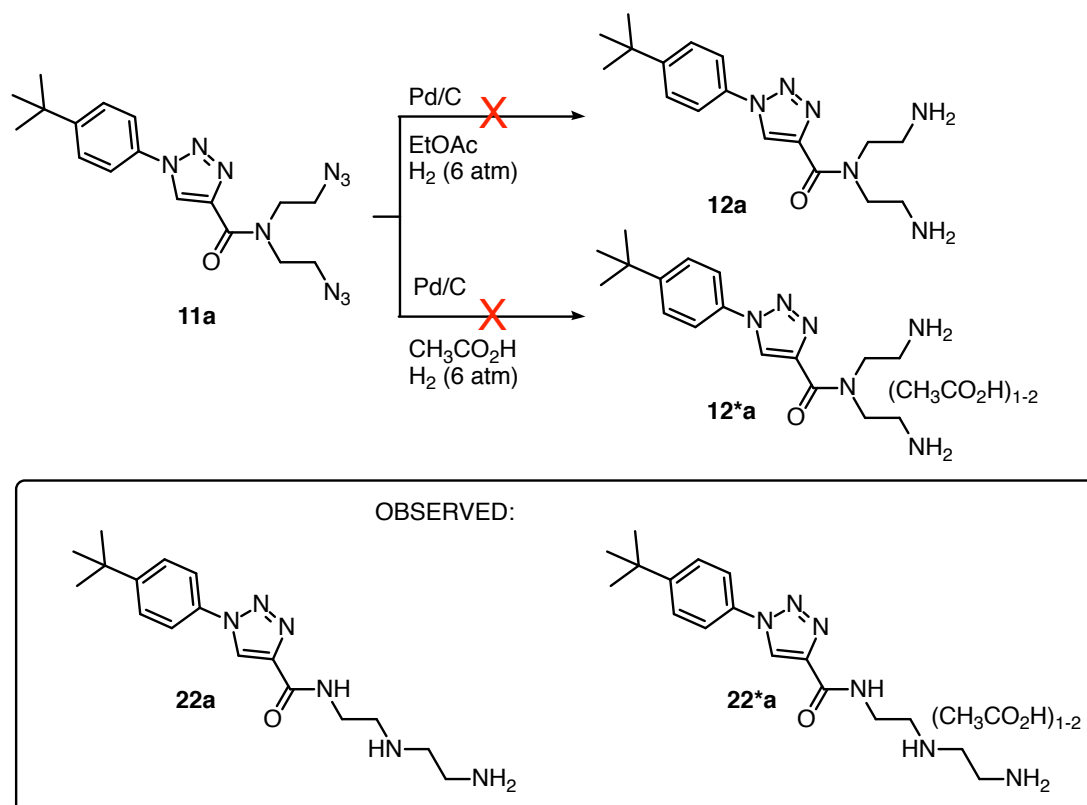
Pd/C (10%, 31 mg) was added to a solution of **11a** (0.17 g, 0.61 mmol) dissolved in EtOAc (5 mL), and **11a** was hydrogenated at 6 atm H_2 with vigorous stirring for 22 h. The catalyst was removed by filtration through a plug of celite (EtOAc) and the solvent concentrated under reduced pressure. This yielded a white wax (0.14 g) assumed to be **22a** and another unidentified product in a 2:1 mixture (determined from ^{1}H NMR analysis). HRMS (TOF ASAP+) *m/z* calcd for $C_{17}H_{27}N_6O$ [M+H]$^+$: 331.2246; found: 331.2245. The ^{1}H NMR and MS spectra are shown in Appendix AF.1 and AF.2, and spectroscopic data for **22a** are given in Section 5.18.

The method was repeated with **11a** (0.11 g, 0.29 mmol), Pd/C (10%, 29 mg) and acetic acid (3 mL) as solvent. This afforded the crude (0.17 g) as a light brown solid. Crystallisation from MeOH/Et$_2$O afforded an off-white solid (24 mg), assumed to be **22*a** and another unidentified product in a 84:16 mixture. The ^{1}H NMR spectrum is shown in Appendix AF.10.

Method 2
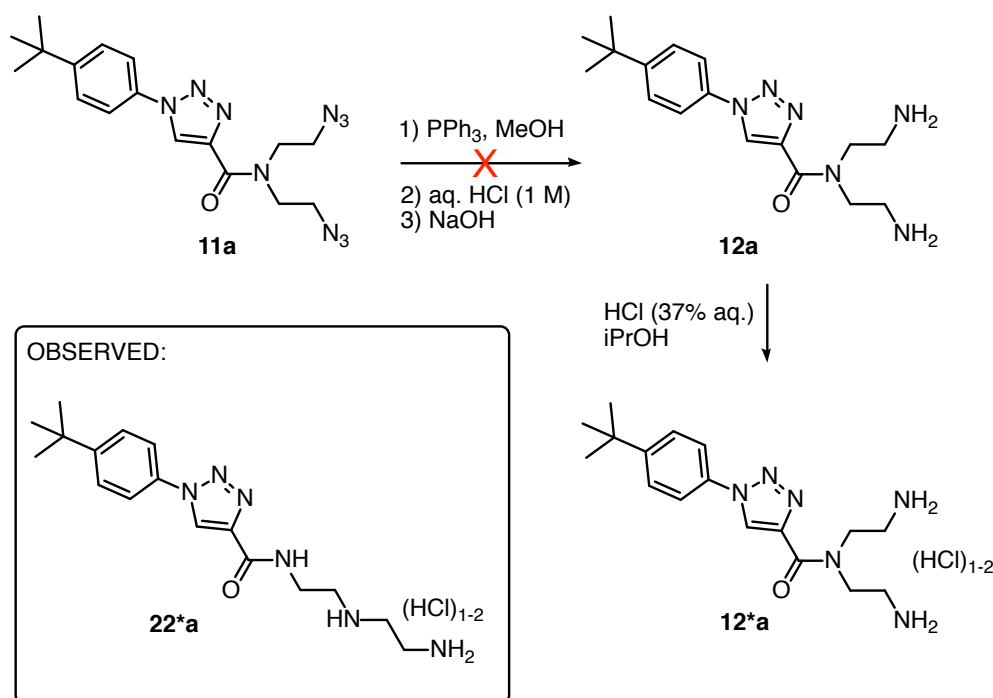
Following a general procedure described by Pal *et al.* with modifications,[41] the bisazide **11a** (0.25 g, 0.66 mmol, 1 equiv) was partly dissolved in dry MeOH (5 mL) and PPh₃ (0.87 g, 3.3 mmol, 5 equiv) in dry MeOH (10 mL) was added. The reaction mixture was refluxed for 2 h before solvent was removed under reduced pressure. The intermediate was partly dissolved in H₂O (20 mL) and stirred for 15 min before HCl (1.7 mL, 37% aq.) was added, and the stirring continued for 2 min. HCl (20 mL, 1 M, aq.) was added and the solution was washed with DCM (50 mL). The organic phase was extracted with HCl (30 mL, 1 M, aq.). The combined acidic phase was washed with EtOAc (5 × 40 mL), before NaOH (3 M) was added to a pH of about 10 and extracted with EtOAc (6 × 50 mL). The combined organic phases were dried over MgSO₄ and filtered, before solvent was removed under reduced pressure. This afforded the crude (0.16 g) as an off-white wax assumed to be **22a** and another unidentified product in a 3:1 mixture, see Appendix AF.3 and spectroscopic data for **22a** in Section 5.18.

A fraction of the crude (0.13 g) was dissolved in iPrOH (10 mL) and filtered. HCl (0.6 mL, 37% aq.) was added, the reaction mixture was stirred for 2 minutes and solvent were removed under reduced pressure. Recrystallisation in MeOH (11 mL) afforded an off-white solid (18 mg) assumed to be **22***a*. See Appendix AF.4-AF.8 for the ¹H NMR, ¹³C NMR, COSY, HSQC and HMBC spectra, and Section 5.19 for spectroscopic data for **22***a*.

Method 3
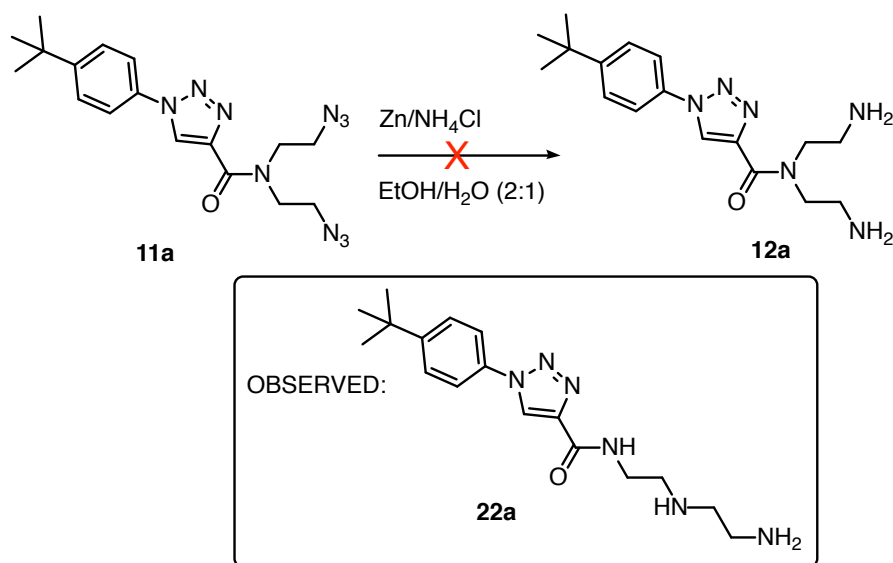
The reduction of **11a** was attempted twice following a general procedure described by Lin *et al.* with modifications.[39] The reaction conditions and results are given i Table 6.9. To a solution of bisazide **11a** (1 equiv) and NH_4Cl (4.6 equiv) in EtOH (5.9 mL/mmol **11a**, 96% aq.) and H_2O (2.1 mL/mmol **11a**), was added zinc powder (2.6 equiv). After the reaction mixture had refluxed for 0.5-47 h and cooled to r.t., EtOAc (8 mL) and NH_3 (0.25-1.0 mL, aq.) were added. The solution was filtered and the filtrate was washed with brine (7 mL). The organic phase was dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. This yielded a white solid assumed to be **22a** in addition to some unidentified byproducts.

For entry 1 (Table 6.9), EtOAc (1 mL) was added after 19 h, unsuccessfully trying to dissolve a white precipitate formed. Unreacted **11a** was observed from TLC analysis, so additional zink powder (1.3 equiv) was added after 22 h.

Table 6.9: Reaction conditions and results for the reduction of **11a** (method 3).

Entry	**11a** [g, mmol]	EtOH/H_2O [mL]	aq. NH_3 [mL]	Time [h]	Crude [g]
1	0.13, 0.34	2.7	0.25	47	0.11
2	0.11, 0.28	2.7	1.0	0.5	0.04

The ^{1}H NMR spectra are given in Appendix AF.11 and AF.12, and spectroscopic data for **22a** are shown in Section 5.18.

6.16 Attempted Synthesis of 12*a using Schiff Base 19

The unsuccessful reduction of **11a** made it necessary to test another approach. Ammonium salt **12*a** was therefore attempted synthesised following a three steps procedure described by Arthi *et al.*[78]

Step 1
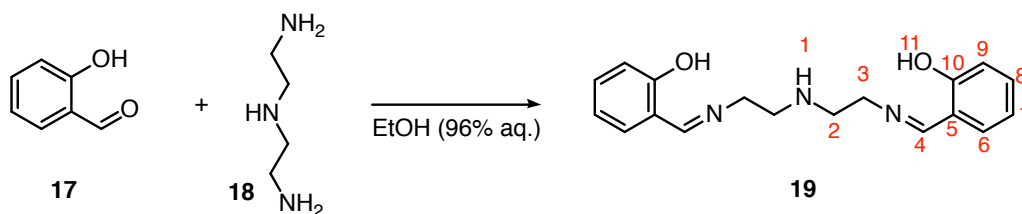
A solution of **18** (1.08 mL, 10 mmol, 1 equiv) in EtOH (20 mL, 96% aq.) was added dropwise to a stirred solution of **17** (2.1 mL, 20 mmol, 2 equiv) in EtOH (20 mL, 96% aq.) under nitrogen atmosphere. The reaction mixture was stirred at r.t. for 2 h and refluxed for 22 h, before solvent was removed under reduced pressure. This afforded the crude product of **19** (3.11 g, 10 mmol, 100%) as a yellow oil, which was used without further purification. Data for **19**: 13.56 (s, 2H, H-11), 8.51 (s, 2H, H-4), 7.45-7.21 (m, 4H, H-aromatic), 6.91-6.74 (m, 4H, H-aromatic), 3.65 (br s, 4H, H-3), 2.85 (br s, 4H, H-2), 1.81 (br s, 1H, H-1). HRMS (TOF ES+) *m/z* calcd for $C_{18}H_{22}N_3O_2$ [M+H]$^+$: 312.1712; found: 312.1708. The ^{1}H NMR spectrum corresponded with reported spectra,[85] and is shown in Appendix Z.1. The MS spectrum is given in Appendix Z.2.

Step 2
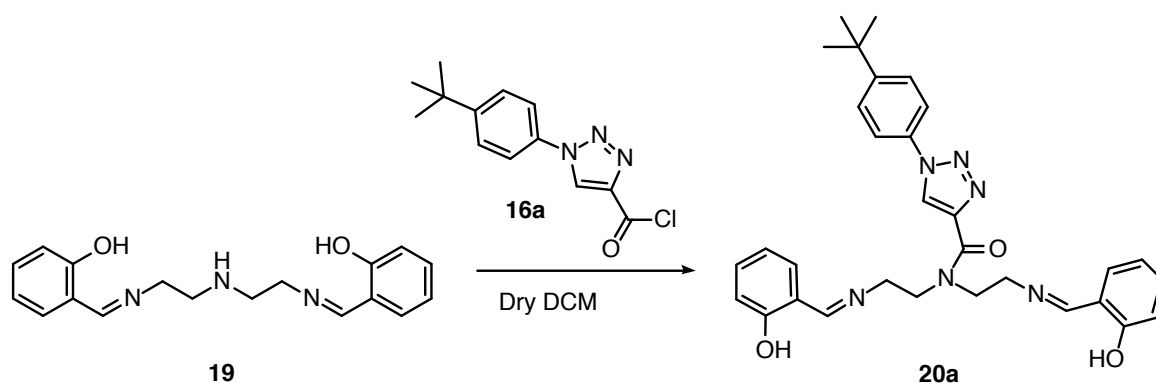
Acid chloride **16a** (0.25, 0.93 mmol, quant.) was prepared as described in Section 6.14.2 (step

1, method 2) using **15a** (0.23 g, 0.93 mmol), SOCl$_2$ (2.5 mL) and 4 h. Na$_2$CO$_3$ (0.13 g, 1.2 mmol) was added to a stirred solution of the crude **19** (0.30 g, 0.96 mmol) in dry DCM (4 mL) under nitrogen, before a solution of crude **16a** (0.25 g, 0.93 mmol) in dry DCM (5 mL) was added. The reaction mixture was refluxed for 21 h before additional dry DCM (7 mL) was added due to loss of solvent. After refluxing for another 24 h, the solution was filtered and the filtrate was concentrated under reduced pressure. This yielded the crude of **20a** (72 mg) as a yellow solid. The crude was used without further purifications. HRMS (TOF ES+) *m/z* calcd for C$_{31}$H$_{35}$N$_6$O$_3$ [M+H]$^+$: 539.2771; found: 539.2775. The ^{1}H NMR and MS spectra are given in Appendix AA.1 and AA.2.

Step 3
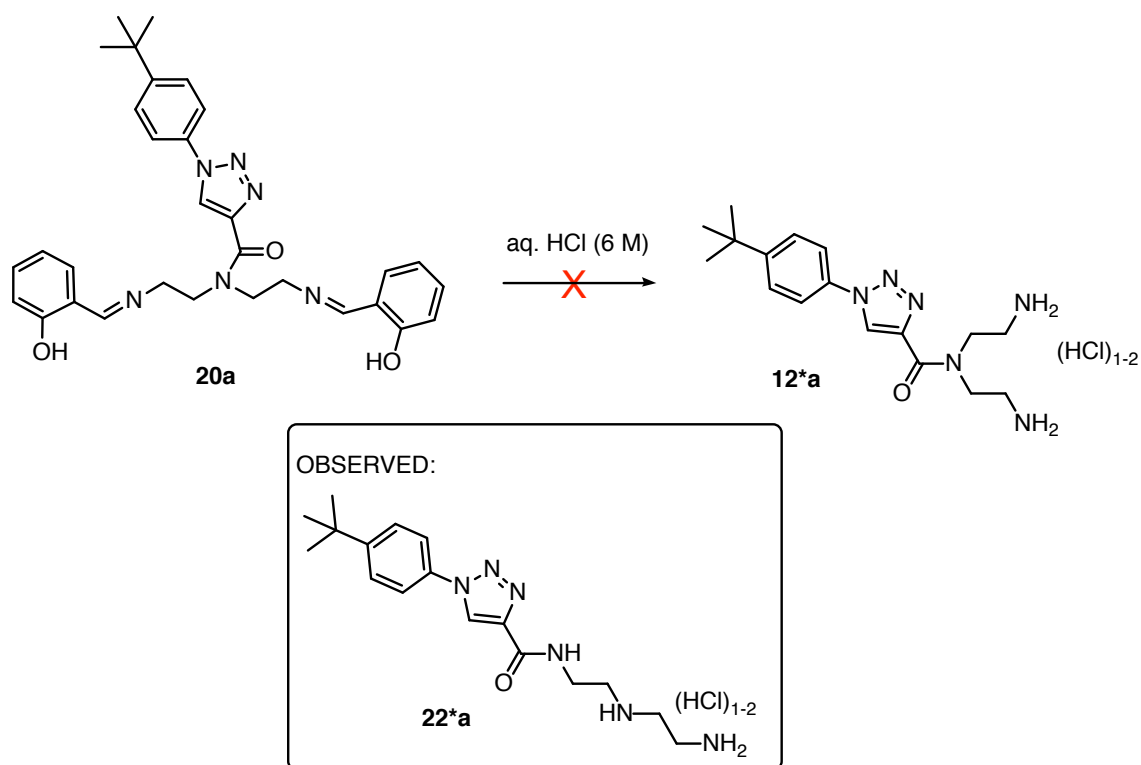
The crude product of **20a** (62 mg) was dissolved in HCl (2 mL, 6 M, aq.) and refluxed for 4 h. The reaction mixture was cooled to r.t. and filtered. Precipitation was attempted with EtOH (96% aq.), but not successful. Solvent were removed under reduced pressure affording **22*a** (5.2 mg). See Appendix AF.13 for the ^{1}H NMR spectrum and Section 5.19 for spectroscopic data for **22*a**.

6.17 Synthesis of 12*a via the Boc-protected Amine 23 and Amide 24

Ammonium salt **12*a** was successfully prepared in three steps via the Boc-protected amine **23** and the amide **24**.

Bis(2-*tert*-butyloxycarbonylaminoethyl)amine (23)
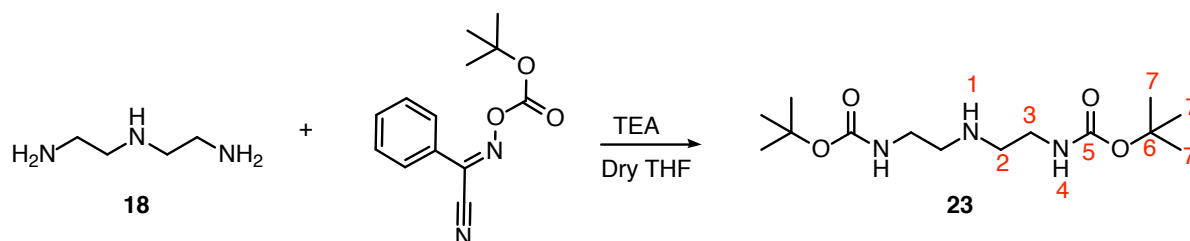
The Boc-protected amine **23** was prepared following a procedure described by Raines and Lukesh.[79] Diethylenetriamine (**18**) (2.1 mL, 0.02 mol, 1 equiv) and triethylamine (8.1 mL, 0.06 mol, 3 equiv) were dissolved in dry THF (100 mL) under nitrogen atmosphere. The solution was cooled to 0 °C before a solution of 2-(Boc-oxyimino)-2-phenylacetonitrile (9.56 g, 0.04 mol, 2 equiv) in dry THF (40 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, followed by 2 h at r.t., before solvent was removed under reduced pressure. The crude was dissolved in DCM (200 mL) and washed with an aqueous solution of NaOH (5 × 50 mL, 5% w/v). The organic phase was dried over $MgSO_4$, filtered and the solvent was concentrated under reduced pressure. Purification by column chromatography (MeOH/DCM/NH_4OH 1 : 9 : 0.1) afforded **23** (4.55 g, 0.015 mol, 75%) as a colourless oil. Data for **23**: R_f: 0.23 (MeOH/DCM/NH_4OH 1 : 9 : 0.1). ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.92 (br s, 2H, H-4), 3.21 (q, *J* = 5.5 Hz, 4H, H-3), 2.73 (t, *J* = 5.7 Hz, 4H, H-2), 1.45 (s, 18H, H-7), 1.15 (br s, 1H, H-1). HRMS (TOF ES+) *m/z* calcd for $C_{14}H_{30}N_3O_4$ [M+H]$^+$: 304.2236; found: 304.2231.

The ^{1}H NMR spectrum was in accordance to reported spectra.[79] The ^{1}H NMR and MS spectra are shown in Appendix AD.1 and AD.2.

Di-*tert*-butyl (((1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carbonyl)azanediyl)bis(ethane-2,1-diyl))dicarbamate (24a)

The amidation of **23** was performed according to a general procedure described Raines and Lukesh.[79]
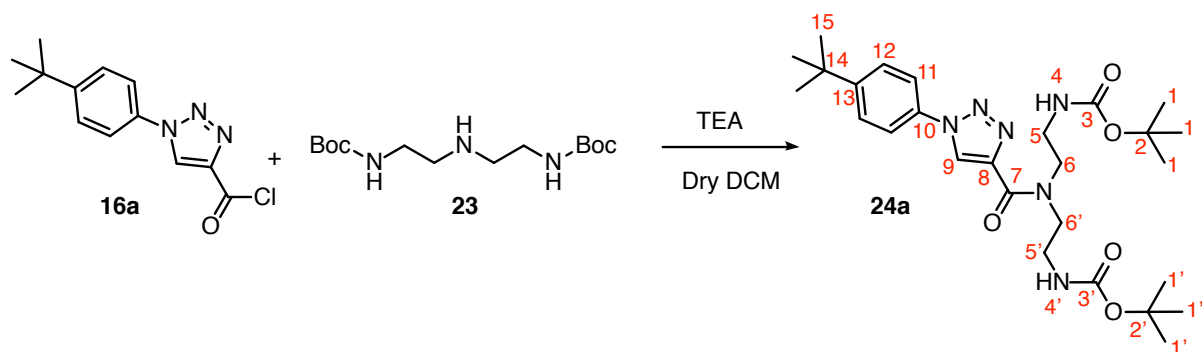
Acid chloride **16a** was first prepared as described in Section 6.14.2 (step 1, method 2) using **15a** (0.21 g, 0.85 mmol) and $SOCl_2$ (2.5 mL). The crude product of **16a** (0.22 g, 0.83 mmol, 1.2 equiv) was dissolved in dry DCM (3 mL) and added to a solution of **23** (0.21 g, 0.70 mmol, 1 equiv) and TEA (0.5 mL, 5 equiv) in dry DCM (4 mL) on an ice-bath. The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 19 h, before solvent was removed under reduced pressure. Purification by column chromatography (50% EtOAc in *n*-pentane) afforded **24a** (0.33 g, 0.62 mmol, 89%) as a white crystalline solid. Data for **24a**: Mp. 71-73 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.16 (s, 1H, H-9), 7.92-7.85 (m, 2H, H-12), 7.65-7.59 (m, 2H, H-11), 6.99-6.87 (m, shows 1.7H, H-4 and H-4'), 6.64-6.48 (m, shows 0.3H, H-4 and H-4'), 3.91-3.82 (m, 2H, H-6 or H-6'), 3.48 (t, *J* = 6.4 Hz, 2H, H-6 or H-6'), 3.23-3.13 (m, 4H, H-5 and H-5'), 1.37 (s, 9H, H-1 or H-1'), 1.33 (s, 9H, H-15), 1.31 (s, 9H, H-1 or H-1'). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.9 (C-7), 155.6 (C-3 or C-3'), 155.5 (C-3 or C-3'), 151.7 (C-13), 144.0 (C-8), 133.9 (C-10), 126.6 (C-11), 126.0 (C-9), 120.0 (C-12), 77.6 (C-2 and C-2'), 48.3 (C-6 or C-6'), 46.5 (C-6 or C-6'), 37.8 (C-5 or C-5'), 39.2 (C-5 or C-5'), 34.5 (C-14), 31.0 (C-15), 28.2 (C-1 or C-1'), 28.1 (C-1 or C-1'). IR (ATR): 3334 (w), 2966 (w), 2870 (w), 1694 (s), 1614 (s), 1519 (s), 1475 (w), 1454 (w), 1391 (m), 1364 (s), 1266 (m), 1246 (s), 1165 (s), 1073 (w), 1039 (m), 991 (w), 837 (m), 780 (w), 760 (w), 736 (w), 700 (w), 558 (w) cm^{-1}. HRMS (TOF ES+) *m/z* calcd for $C_{27}H_{42}N_6O_5Na$ [M+Na]$^+$: 553.3114; found: 553.3119.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, and MS spectra obtained for **24a** are shown in Appendix AE.1-AE.7. For structure elucidation and assignment of chemical shifts, see Section 5.

N,N-Bis(2-aminoethyl)-1-(4-(*tert*-butyl)phenyl)-1H-1,2,3-triazole-4-carboxamide hydrochloride (12*a)
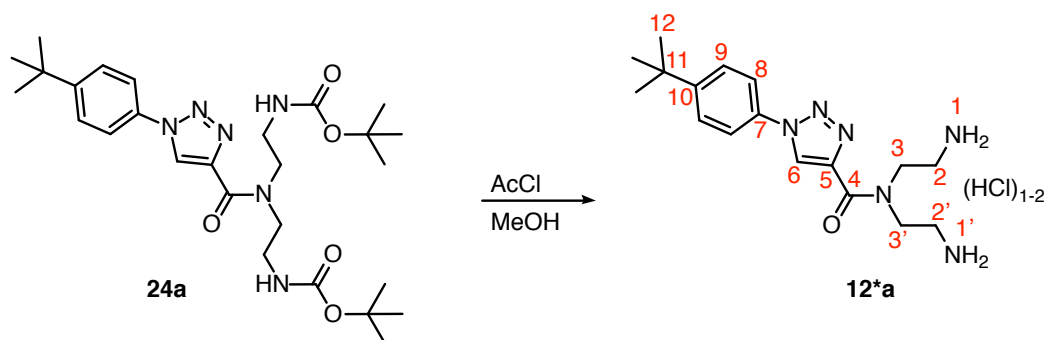
Following a general procedure described by Hickey *et al.*,[69] AcCl (0.61 mL, 30 equiv) was added to a solution of **24a** (0.15 g, 0.29 mmol, 1 equiv) in MeOH (2.3 mL), generating some heat. The reaction mixture was stirred for 2.5 h at r.t. Solvent was removed under reduced pressure, followed by coevaporation with MeOH (4 × 10 mL). Recrystallisation in EtOH (2.3 mL) and washing the precipitate with cold EtOH (2 × 3 mL) afforded **12*a** (64 mg, 0.17 mmol, 59%) as white crystals. Data for **12*a**: Mp. 121.3-123.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.29 (s, 1H, H-6), 8.28 (br s, 6H, H-1 and H-1'), 7.95-7.87 (m, 2H, H-8), 7.67-7.60 (m, 2H, H-9), 4.06 (app s, 2H, H-2/H-2'/H-3/H-3'), 3.78 (app s, 2H, H-2/H-2'/H-3/H-3'), 3.22 (app s, 2H, H-2/H-2'/H-3/H-3'), 3.10 (app s, 2H, H-2/H-2'/H-3/H-3'), 1.33 (s, 9H, H-12). ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.9 (C-4), 152.0 (C-10), 143.5 (C-5), 133.8 (C-7), 126.9 (C-6), 126.7 (C-9), 120.2 (C-8), 45.9 (C-2/C-2'/C-3/C-3'), 43.8 (C-2/C-2'/C-3/C-3'), 37.4 (C-2/C-2'/C-3/C-3'), 36.7 (C-2/C-2'/C-3/C-3'), 34.6 (C-11), 31.0 (C-12). IR (ATR): 2990 (m), 2924 (m), 2771 (w), 1606 (s), 1555 (m), 1522 (w), 1506 (m), 1478 (m), 1428 (m), 1379 (w), 1364 (w), 1278 (w), 1239 (w), 1226 (w), 1164 (w), 1137 (w), 1041 (s), 957 (w), 832 (s), 753 (m), 737 (w), 559 (w), 524 (w), 414 (w) cm^{-1}. HRMS (TOF ES+) *m/z* calcd for $C_{17}H_{27}N_6O$ [M-Cl]$^+$: 331.2246; found: 331.2248. HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 5.3 min, 98% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra, in addition to the HPLC chromatogram obtained for **12*a** are shown in Appendix U.1-U.9. For structure elucidation and assignment of chemical shifts, see Section 5.

6.18 Synthesis of Amine 22a and Ammonium Salt 22*a

N-(2-((2-Aminoethyl)amino)ethyl)-1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (22a)
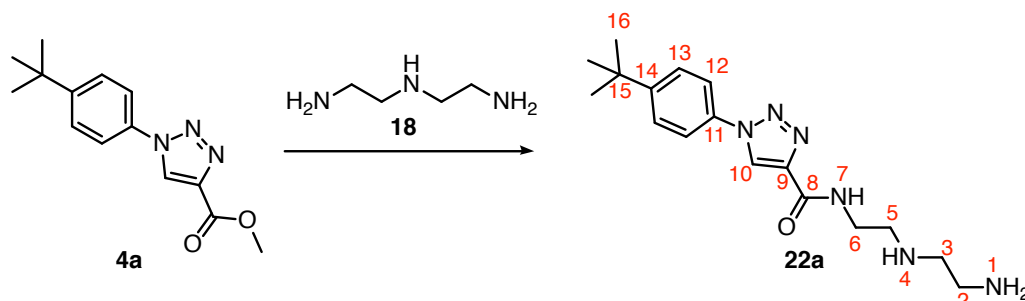
Triazole ester **4a** (0.11 g, 0.42 mmol, 1 equiv) was dissolved in **18** (6.6 mL, 150 equiv) and the reaction mixture was stirred at 50 °C for 75 minutes. Excess **18** was removed with Kügelrohr distillation (0.05-0.08 mbar, 60-70 °C, 8 h) and by coevaporation with iPrOH (3 × 10 mL). This afforded **22a** as an off-white solid (0.14 g, 0.42 mmol, 100%). Data for **22a**: Mp. 120.1-122.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.22 (s, 1H, H-10), 8.53 (t, J = 5.6 Hz, 1H, H-7), 7.89-7.85 (m, 2H, H-12), 7.63-7.59 (m, 2H, H-13), 3.37 (q, J = 6.3 Hz, 2H, H-6), 2.73-2.65 (m, 2H, H-5), 2.62-2.56 (m, 2H, H-2), 2.55-2.51 (m, 2H, H-3), 1.58 (br s, 3H, H-1, H-4), 1.33 (s, 9H, H-16). ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.4 (C-8), 151.8 (C-14), 143.7 (C-9), 134.0 (C-11), 126.6 (C-13), 124.4 (C-10), 120.1 (C-12), 52.2 (C-3), 48.4 (C-5), 41.5 (C-2), 38.7 (C-6), 34.5 (C-15), 31.0 (C-16). IR (ATR): 3323 (w), 3125 (w), 2959 (w), 2867 (w), 1645 (s), 1568 (s), 1502 (m), 1462 (w), 1439 (w), 1364 (w), 1267 (m), 1035 (m), 991 (w), 832 (s), 731 (m), 686 (w), 557 (m) cm^{-1}. HRMS (TOF ASAP+) *m/z* calcd for $C_{17}H_{27}N_6O$ [M+H]$^+$: 331.2246; found: 331.2242.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra obtained for **22a** are shown in Appendix AB.1-AB.7. For structure elucidation and assignment of chemical shifts, see Section 5.

_N_-(2-((2-Aminoethyl)amino)ethyl)-1-(4-(_tert_-butyl)phenyl)-1_H_-1,2,3-triazole-4-carboxamide hydrochloride (22*a)
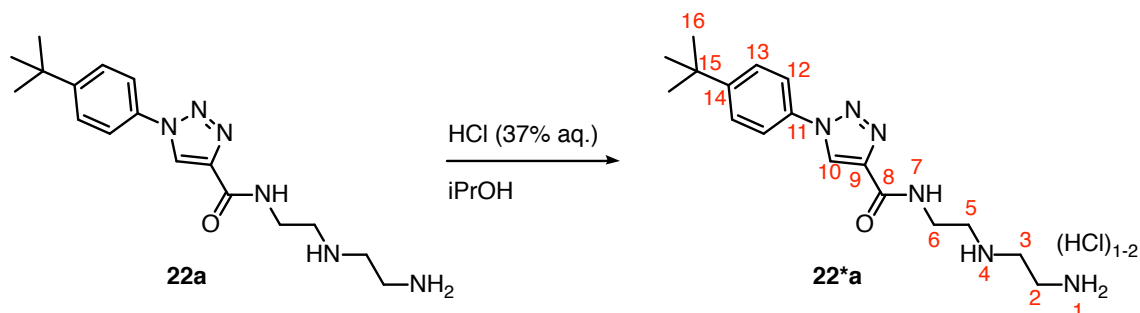
The synthesis of **22*a** was performed following a procedure described by Bakka.[12] Amine **22a** (0.092 g, 0.28 mmol, 1 equiv) was dissolved in iPrOH (8 mL) and filtered. HCl (0.47 mL, 20 equiv, 37% aq.) was added to the filtrate and the reaction mixture was stirred for 2 minutes. Solvent was removed under reduced pressure affording the crude of **22a** (0.093 g). Washing the crude with EtOH (7 mL, 96% aq.) yielded **22*a** (0.045 g, 0.12 mmol, 44%) as a light brown solid. Data for **22*a**: Mp. > 250 (decomp.) °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.53 (br s, 2H, H-4), 9.39 (s, 1H, H-10), 8.89 (t, _J_ = 5.7 Hz, 1H, H-7), 8.43 (br s, 3H, H-1), 7.91-7.84 (m, 2H, H-12), 7.65-7.59 (m, 2H, H-13), 3.65 (q, _J_ = 6.0 Hz, 2H, H-6), 3.27 (t, _J_ = 6.0 Hz, 2H, H-2 or H-3), 3.22-3.14 (m, 4H, H-5 and H-2 or H-3), 1.33 (s, 9H, H-16). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.0 (C-8), 151.9 (C-14), 143.2 (C-9), 133.9 (C-11), 126.7 (C-13), 124.7 (C-10), 120.1 (C-12), 46.2 (C-2, C-3 or C-5), 44.1 (C-2, C-3 or C-5), 35.2 (C-2, C-3 or C-5), 35.1 (C-6), 34.5 (C-15), 31.0 (C-16). IR (ATR): 3321 (w), 2952 (w), 2903 (w), 2786 (w), 2688 (w), 2462 (w), 1660 (m), 1568 (s), 1503 (m), 1464 (m), 1438 (w), 1268 (m), 1245 (w), 1175 (w), 1032 (m), 991 (w), 833 (s), 764 (w), 674 (w), 561 (w), 549 (w) cm^{-1}. HRMS (TOF ES+) _m/z_ calcd for $C_{17}H_{27}N_6O$ [M-Cl]$^+$: 331.2246; found: 331.2240. HPLC: (MeOH/H$_2$O: 50/50 + 0.1% TFA in the water, 1 mL/min, λ = 214 nm): t_R = 9.7 min, > 99% pure.

The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR and MS spectra, in addition to the HPLC chromatogram obtained for **22*a** are shown in Appendix AC.1-AC.9. For structure elucidation and assignment of chemical shifts, see Section 5.

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Appendix

The Appendix is organised after compound number. For all new compounds ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, IR, MS, and for some compounds HPLC, will be given. For previously prepared compounds, the ^{1}H NMR spectrum and in some cases other relevant appendices, will be presented. The HPLC chromatograms of blank samples with the different compositions of the eluent used, are given the last appendices.

A.1 ^{1}H NMR (400 MHz, CDCl$_3$) spectrum for 3d

A.2 ¹H NMR (400 MHz, CDCl₃) spectrum for the reaction mixture for the first synthesis of 3d after 95 h

A.3 ^{1}H NMR (400 MHz, CDCl$_3$) spectrum for the reaction mixture for the first synthesis of 3d 24 hours after the second addition of reagents
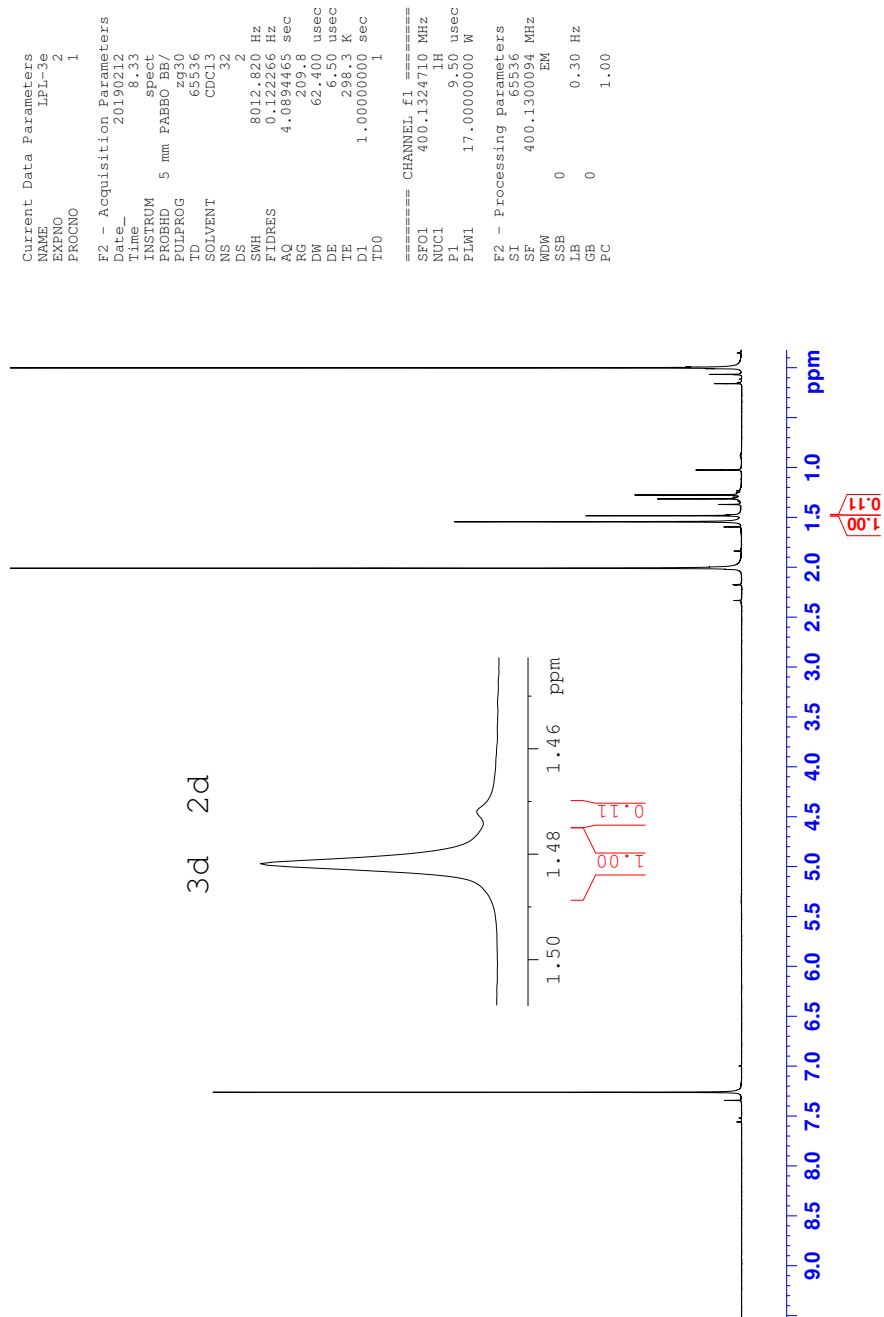
A.4 ^{1}H NMR (400 MHz, CDCl$_3$) spectrum for the reaction mixture for the second synthesis of 3d after 24 hours reaction time showing no conversion

A.5 IR spectrum for 3d

B.1 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for the crude product of 4d from the first synthesis

Current Data Parameters
NAME   LPI-4e(1)600
EXPNO   1
PROCNO   1
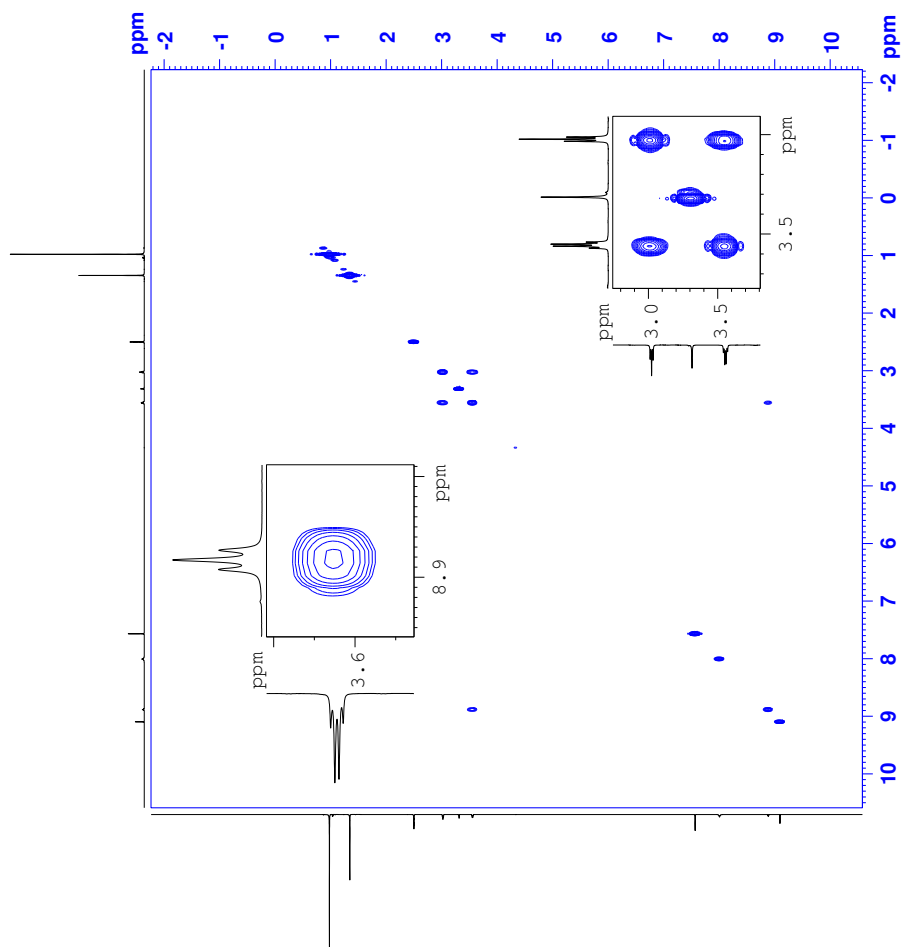
F2 - Acquisition Parameters
Date_   20190225
Time   13.24 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zg30
TD   65536
SOLVENT   CDCl3
NS   32
DS   2
SWH   12019.230 Hz
FIDRES   0.366798 Hz
AQ   2.7262976 sec
RG   11.48
DW   41.600 usec
DE   20.00 usec
TE   298.1 K
D1   1.00000000 sec
TD0   1
SFO1   600.1837061 MHz
NUC1   1H
P1   8.00 usec
PLW1   6.00000000 W
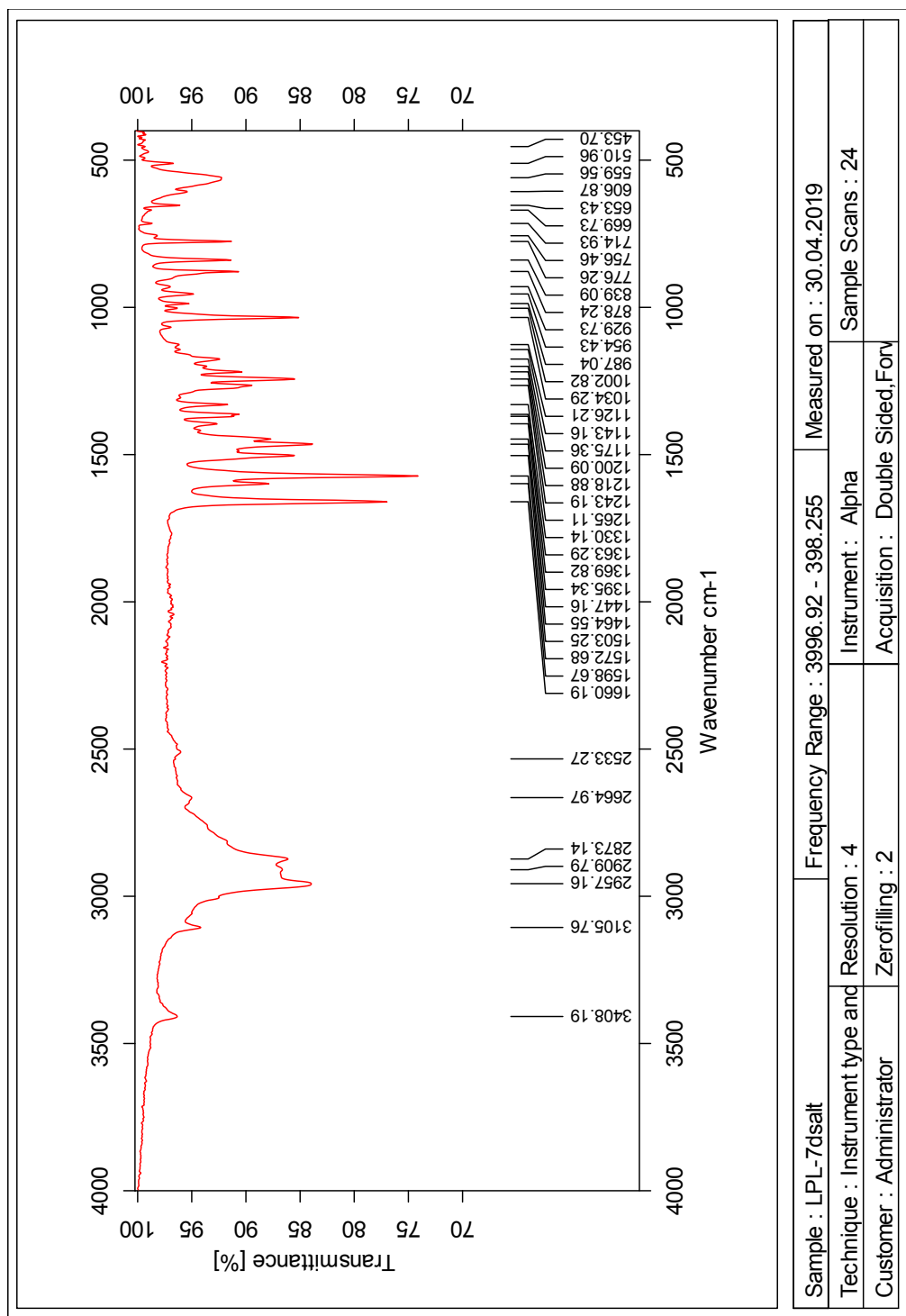
F2 - Processing parameters
SI   65536
SF   600.1800128 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

vii

B.2 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for the crude product of 4d from the second synthesis

Current Data Parameters
NAME   LPL-4e(2)600
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20190228
Time   6.24 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zg30
TD   65536
SOLVENT   CDCl3
NS   32
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   298.1 K
D1   1.00000000 sec
TD0   1
SFO1   600.1837061 MHz
NUC1   1H
P1   8.00 usec
PLW1   6.00000000 W

F2 - Processing parameters
SI   65536
SF   600.1800147 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

1.04

1.36

3.76

4.01

7.54
7.56

8.25

7.54

7.56

0.100

1.989

17.99

9.05

3.00

0.09
0.10
1.99

0.97

ppm

B.3 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for the crude product of 4d from the third synthesis

B.4 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for 4d

B.5 ^{13}C NMR (150 MHz, CDCl$_3$) spectrum for 4d

B.6 COSY (600 MHz, CDCl₃) spectrum for 4d

B.8   HMBC (600 MHz / 150 MHz, CDCl₃) spectrum for 4d

B.9   NOESY (600 MHz, CDCl₃) spectrum for 4d

B.10 IR spectrum for the mixture of 4d and 4'd
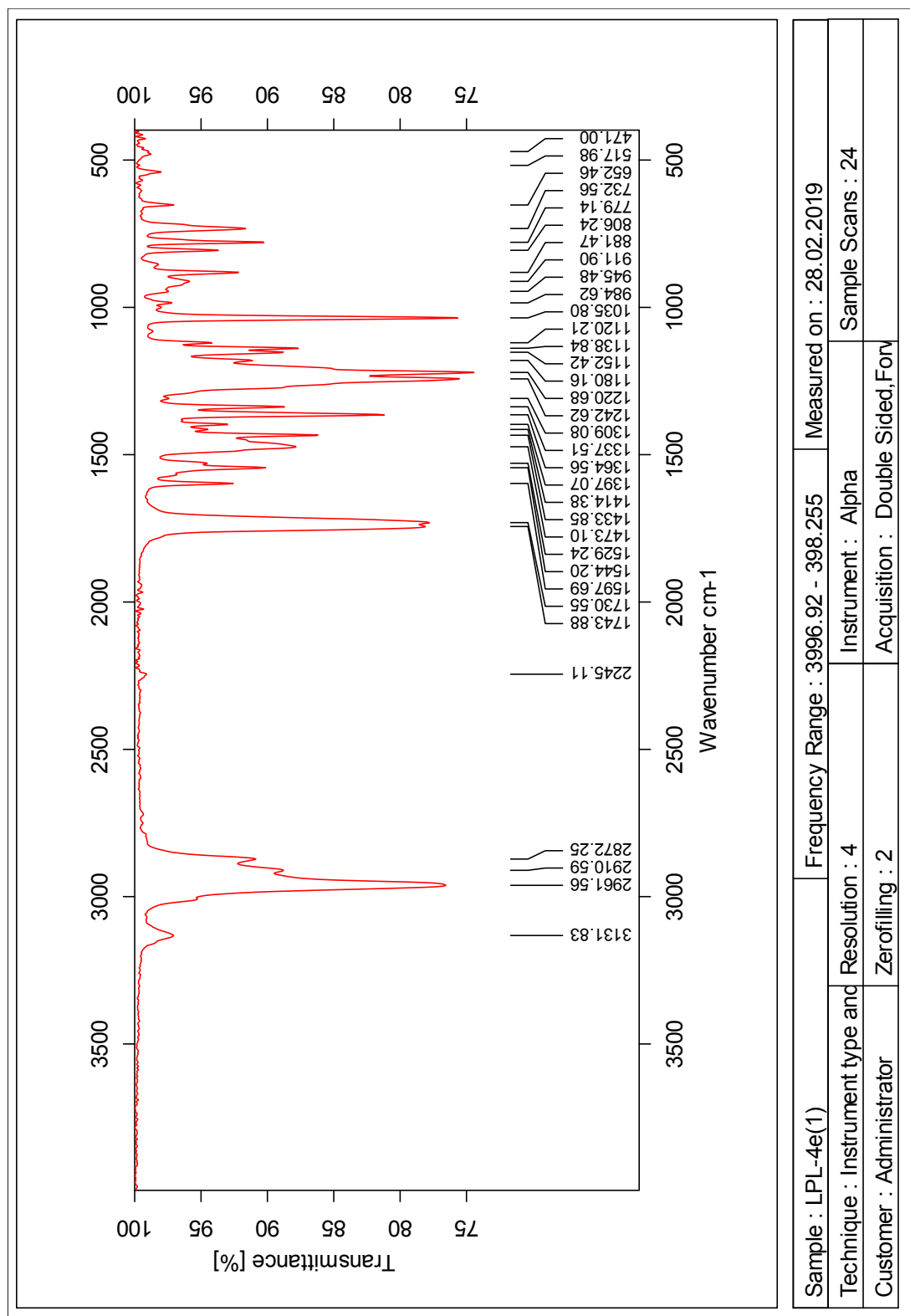
B.11   IR spectrum for 4d
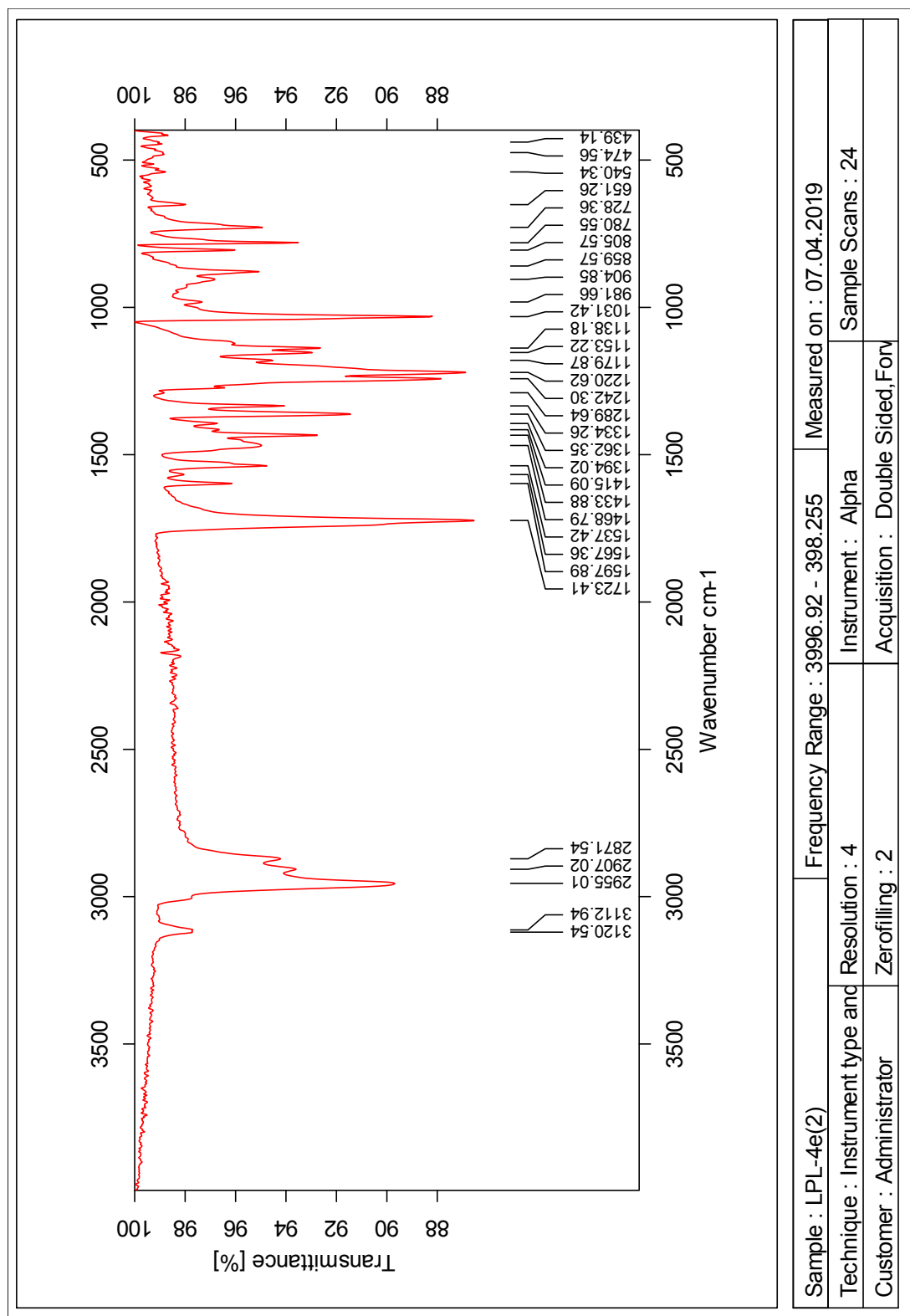
B.12 HRMS spectrum for 4d

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
1930 formula(e) evaluated with 3 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   S: 0-4
2019-144 94 (1.843) AM2 (Ar,35000.0,0.00,0.00); Cm (79:103)
1: TOF MS ASAP+
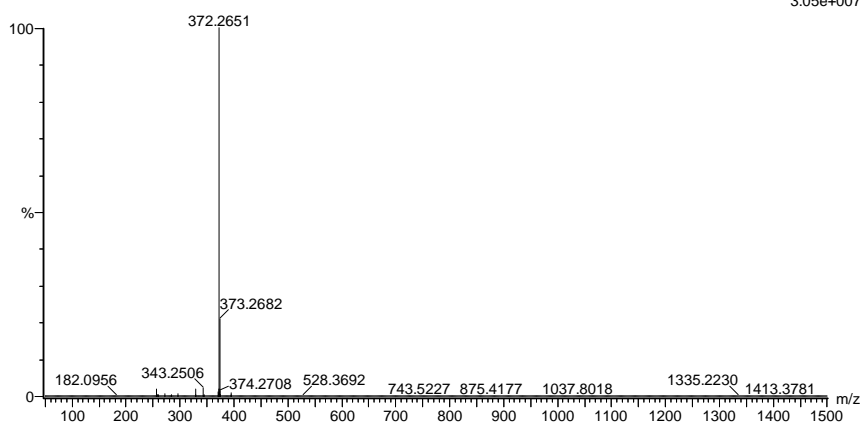
| Minimum: | | | -2.0 | | | |
| Maximum: | | 5.0 | 2.0 | 50.0 | | |

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
372.2651	372.2651	0.0	0.0	7.5	1547.6	0.000	100.00	C22 H34 N3 O2
	372.2645	0.6	1.6	-1.5	1561.3	13.730	0.00	C14 H38 N5 O4 S
	372.2658	-0.7	-1.9	3.5	1560.9	13.300	0.00	C15 H34 N9 S

xviii

C.1 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for 4'd

C.2 ^{13}C NMR (150 MHz, CDCl$_3$) spectrum for 4'd
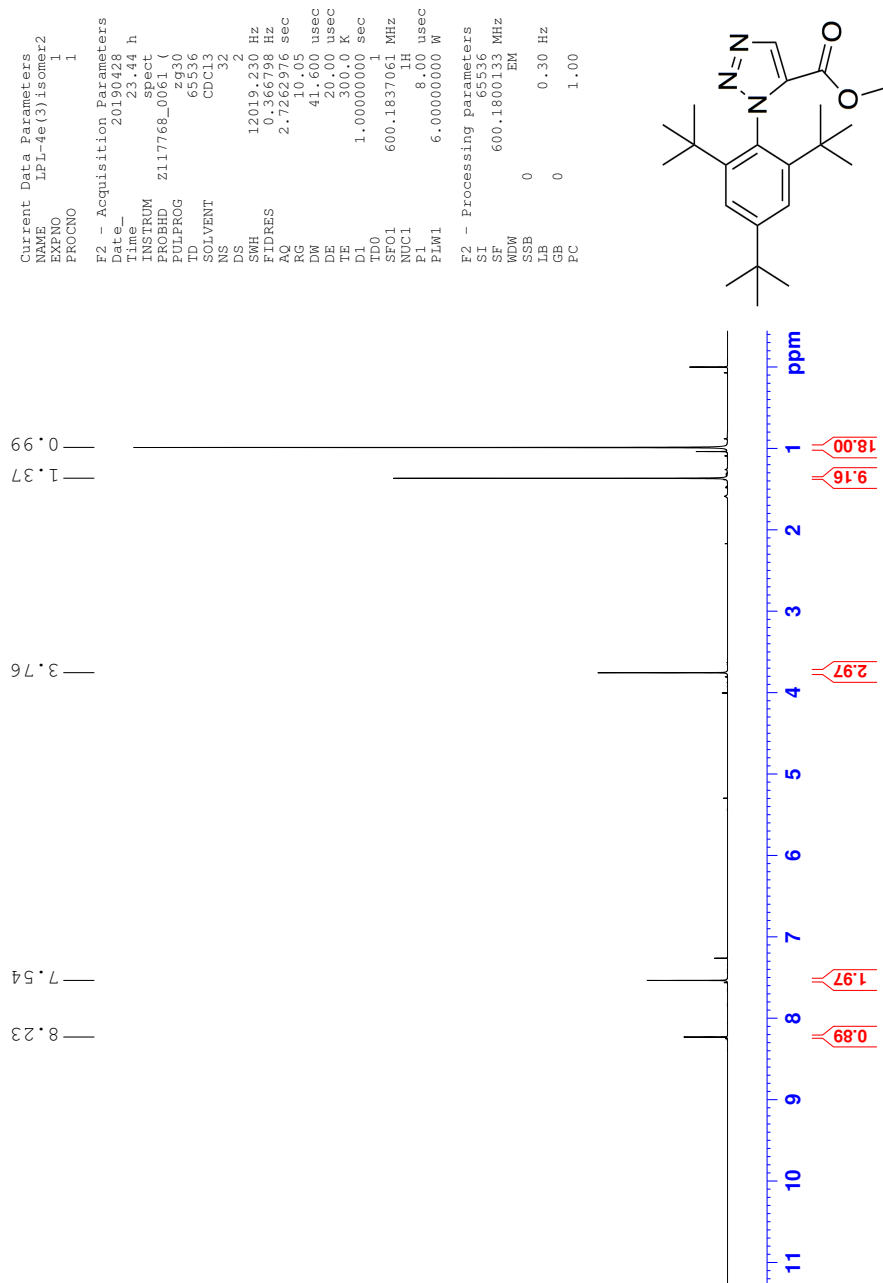
C.3 COSY (600 MHz, CDCl₃) spectrum for 4'd
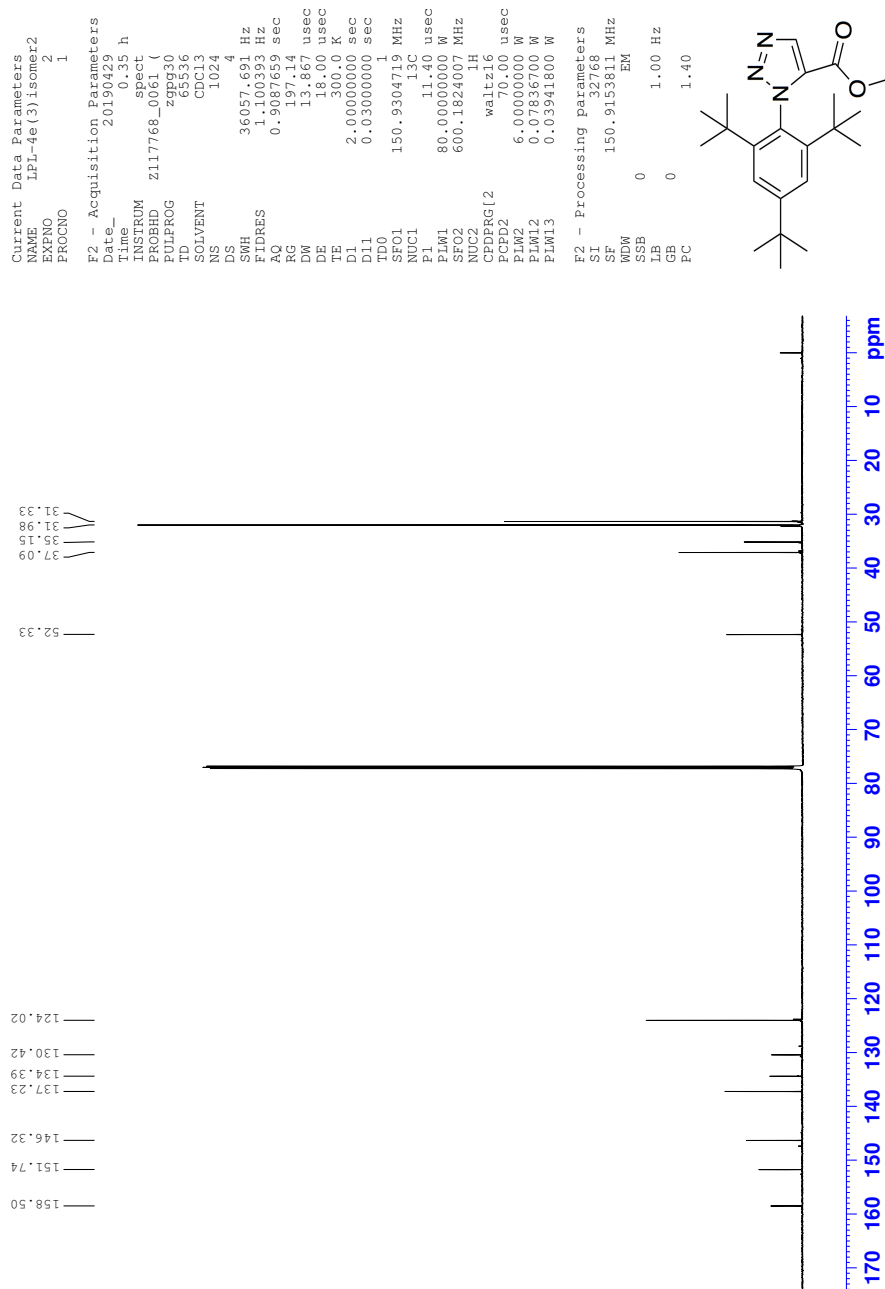
C.4 HSQC (600 MHz / 150 MHz, CDCl₃) spectrum for 4'd

C.5   HMBC (600 MHz / 150 MHz, CDCl₃) spectrum for 4'd
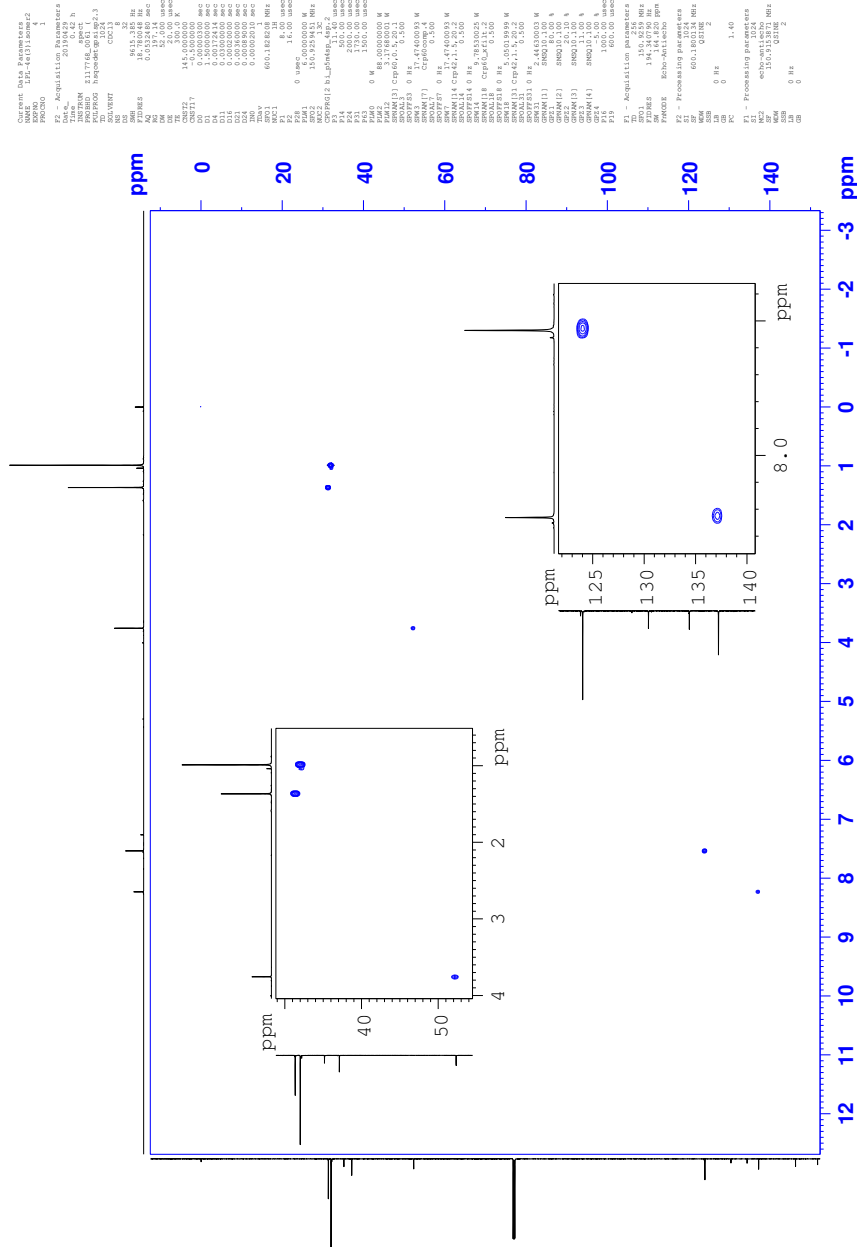
C.6 NOESY (600 MHz, CDCl₃) spectrum for 4'd
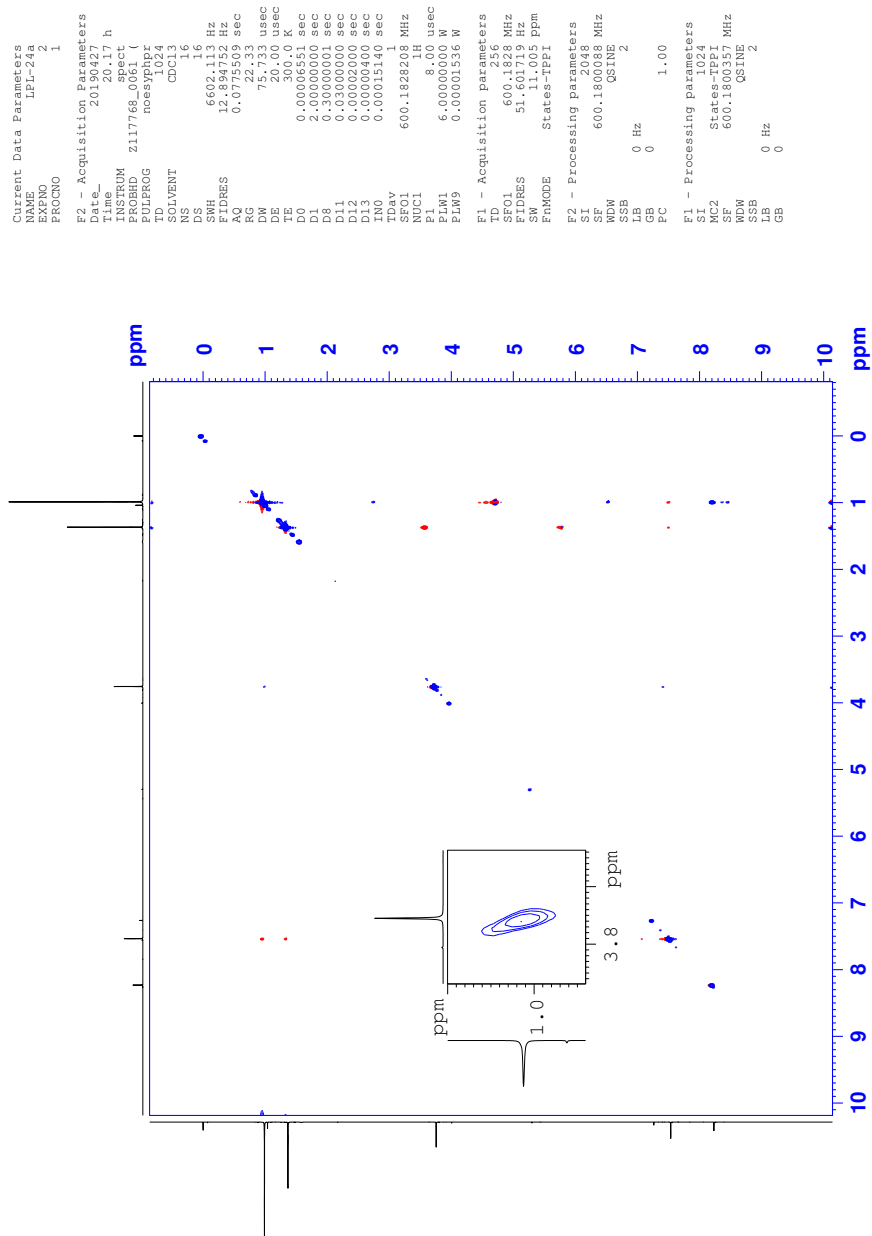
C.7 IR spectrum for 4'd
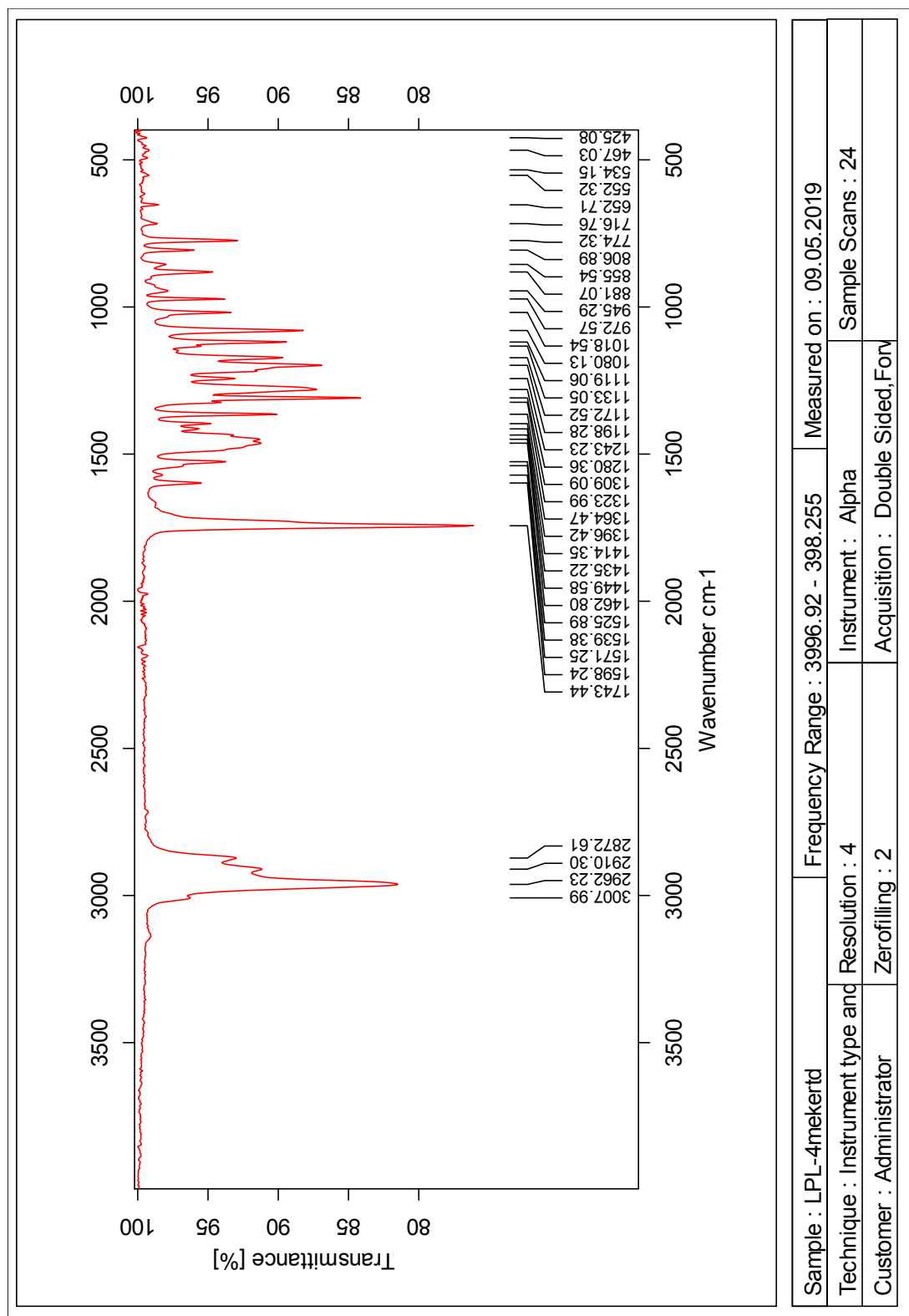
C.8 HRMS spectrum for 4d

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
1308 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
2019-528 44 (0.878) AM2 (Ar,35000.0,0.00,0.00); Cm (44:49)
1: TOF MS ASAP+
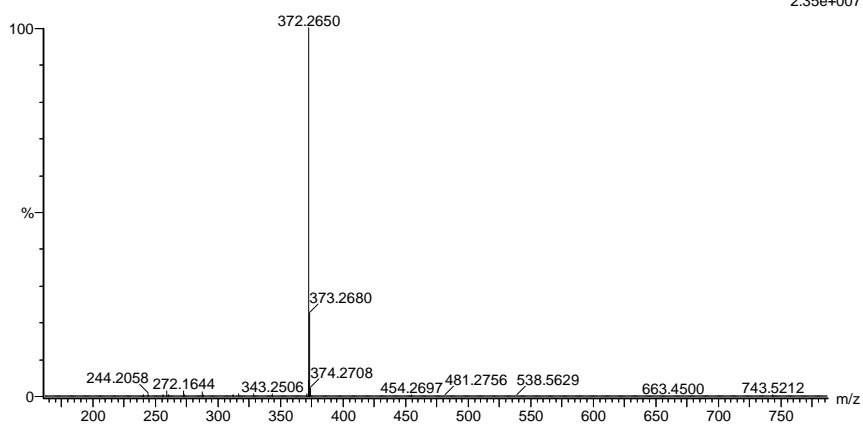
Minimum:			-50.0					
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
372.2650	372.2651	-0.1	-0.3	7.5	1436.5	n/a	n/a	C22 H34 N3 O2

D.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 5a

D.2 HRMS spectrum for 5a

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
1660 formula(e) evaluated with 4 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   S: 0-3
2019-52 181 (3.531) AM2 (Ar,35000.0,0.00,0.00); Cm (177:181)
1: TOF MS ASAP+
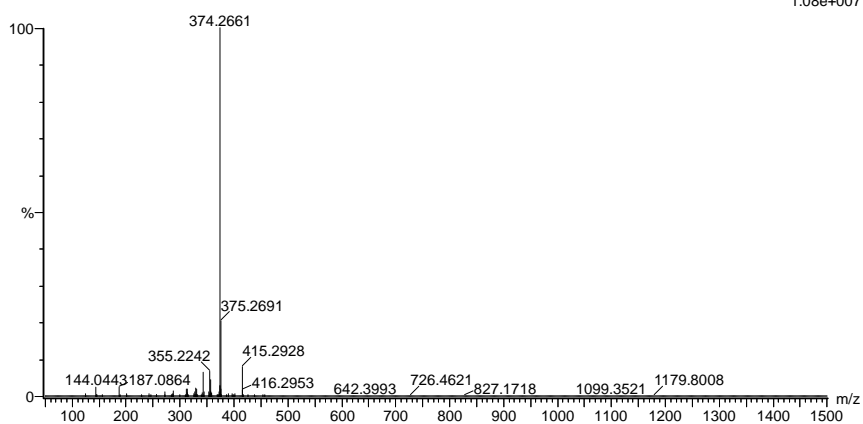
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-2.0				
Maximum:		5.0	5.0	50.0				
374.2661	374.2668	-0.7	-1.9	7.5	1435.4	0.635	53.00	C19 H32 N7 O
	374.2655	0.6	1.6	2.5	1435.5	0.755	47.00	C18 H36 N3 O5
	374.2662	-0.1	-0.3	-1.5	1450.2	15.435	0.00	C11 H36 N9 O3 S
	374.2664	-0.3	-0.8	1.5	1452.6	17.869	0.00	C19 H40 N3 S2

D.3 HRMS spectrum for 5'a

Single Mass Analysis
Tolerance = 1.0 PPM   /   DBE: min = -2.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
1358 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100   H: 0-500   N: 0-10   O: 0-20
2018-512 202 (3.947) AM2 (Ar,35000.0,0.00,0.00); Cm (202:213)
1: TOF MS ASAP+
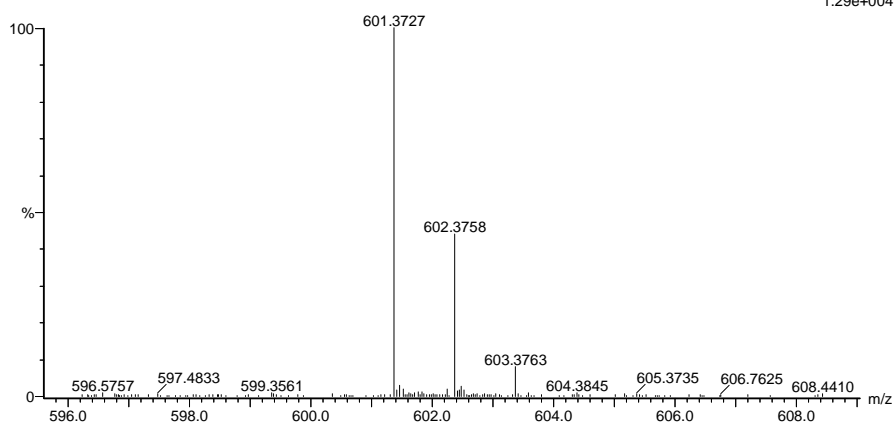

```
Minimum:                        -2.0
Maximum:               5.0    1.0    50.0

Mass       Calc. Mass  mDa    PPM    DBE    i-FIT   Norm    Conf(%) Formula

601.3727   601.3727    0.0    0.0    15.5   320.5   n/a     n/a     C32 H45 N10 O2
```

E.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 5b
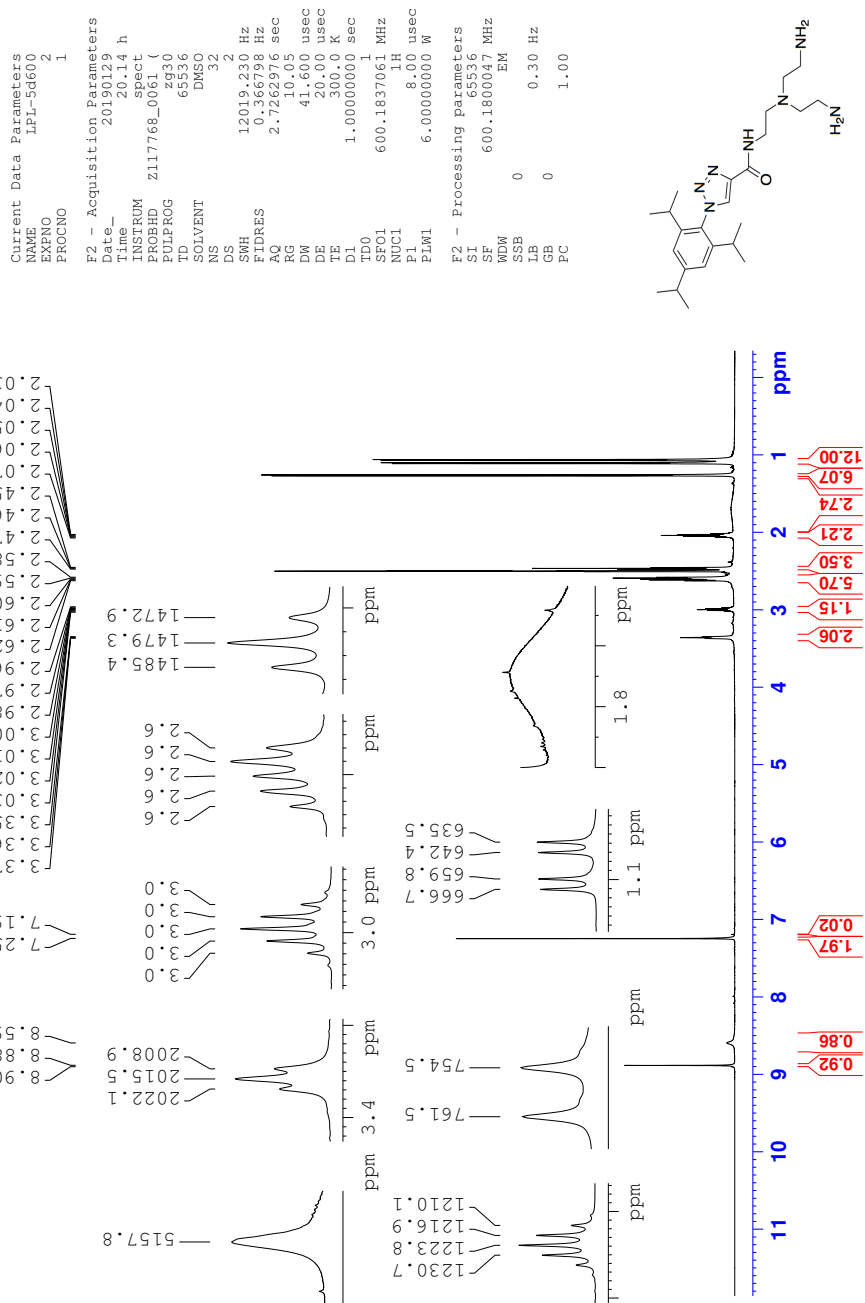
E.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 5b

E.3 COSY (600 MHz, DMSO) spectrum for 5b

E.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 5b

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

E.6 IR spectrum for the mixture of 5b and 5'b

Sample : LPL-5d	Frequency Range : 3996.92 - 398.255	Measured on : 30.01.2019
Technique : Instrument type and	Resolution : 4	Instrument : Alpha	Sample Scans : 24
Customer : Administrator	Zerofilling : 2	Acquisition : Double Sided,Forw

E.7 HRMS spectrum for 5b
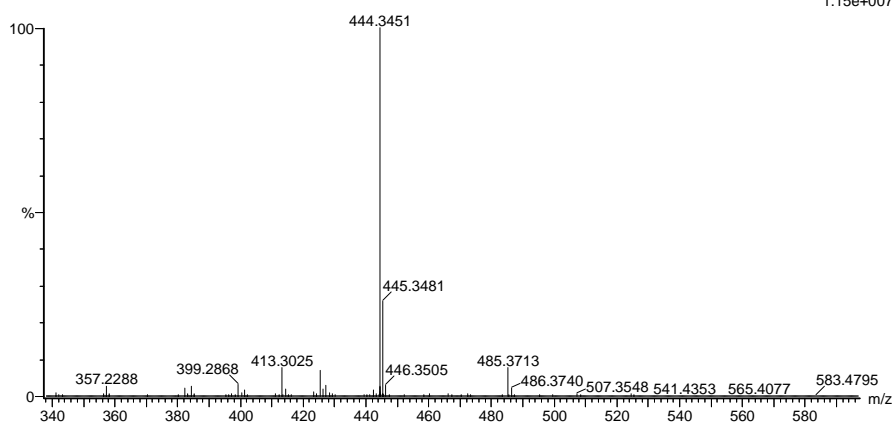
```
Minimum:                          -2.0
Maximum:             5.0    2.0    50.0

Mass       Calc. Mass  mDa    PPM    DBE    i-FIT   Norm   Conf(%) Formula

444.3451   444.3451    0.0    0.0    7.5    1230.2  n/a    n/a     C24 H42 N7 O
```

E.8   HRMS spectrum for 5'b

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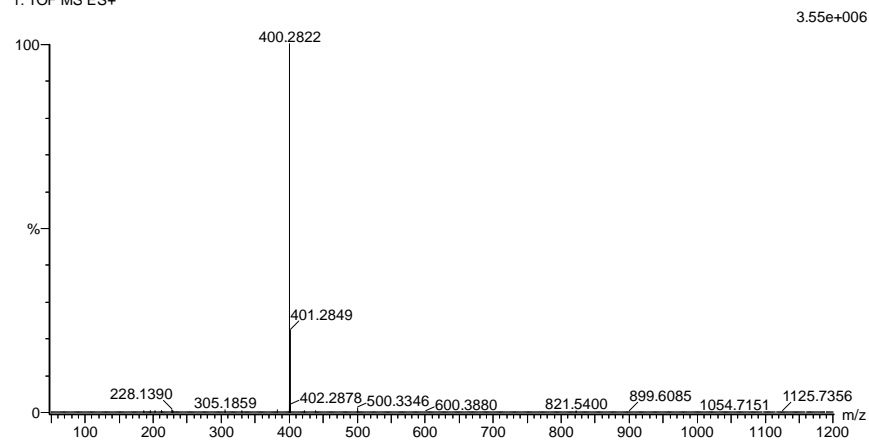Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1030 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: 0-10
2019-32re 265 (5.170) AM2 (Ar,35000.0,0.00,0.00); Cm (259:268)
1: TOF MS ASAP+

1.28e+004
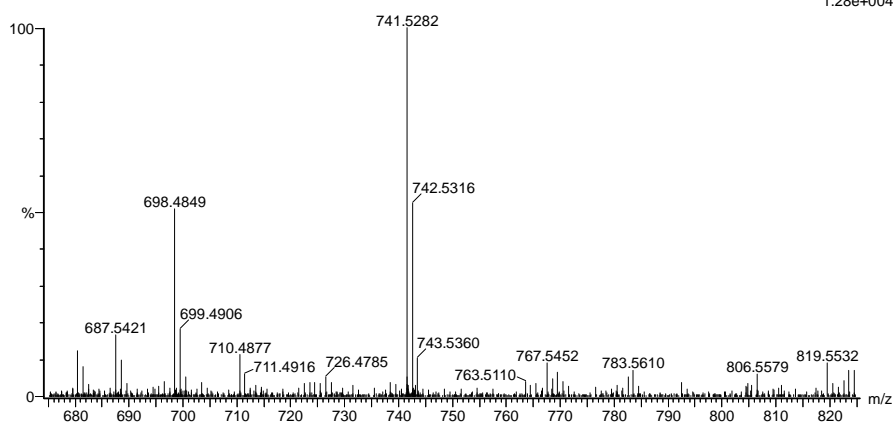

```
Minimum:                        -2.0
Maximum:              5.0    2.0   50.0

Mass       Calc. Mass   mDa    PPM    DBE    i-FIT    Norm   Conf(%) Formula

741.5282   741.5292    -1.0   -1.3   15.5   400.4    0.379  68.47   C42 H65 N10 O2
           741.5279     0.3    0.4   10.5   401.2    1.154  31.53   C41 H69 N6 O6
```

F.1 ^{1}H NMR (400 MHz, DMSO) spectrum for the first synthesis of 5c
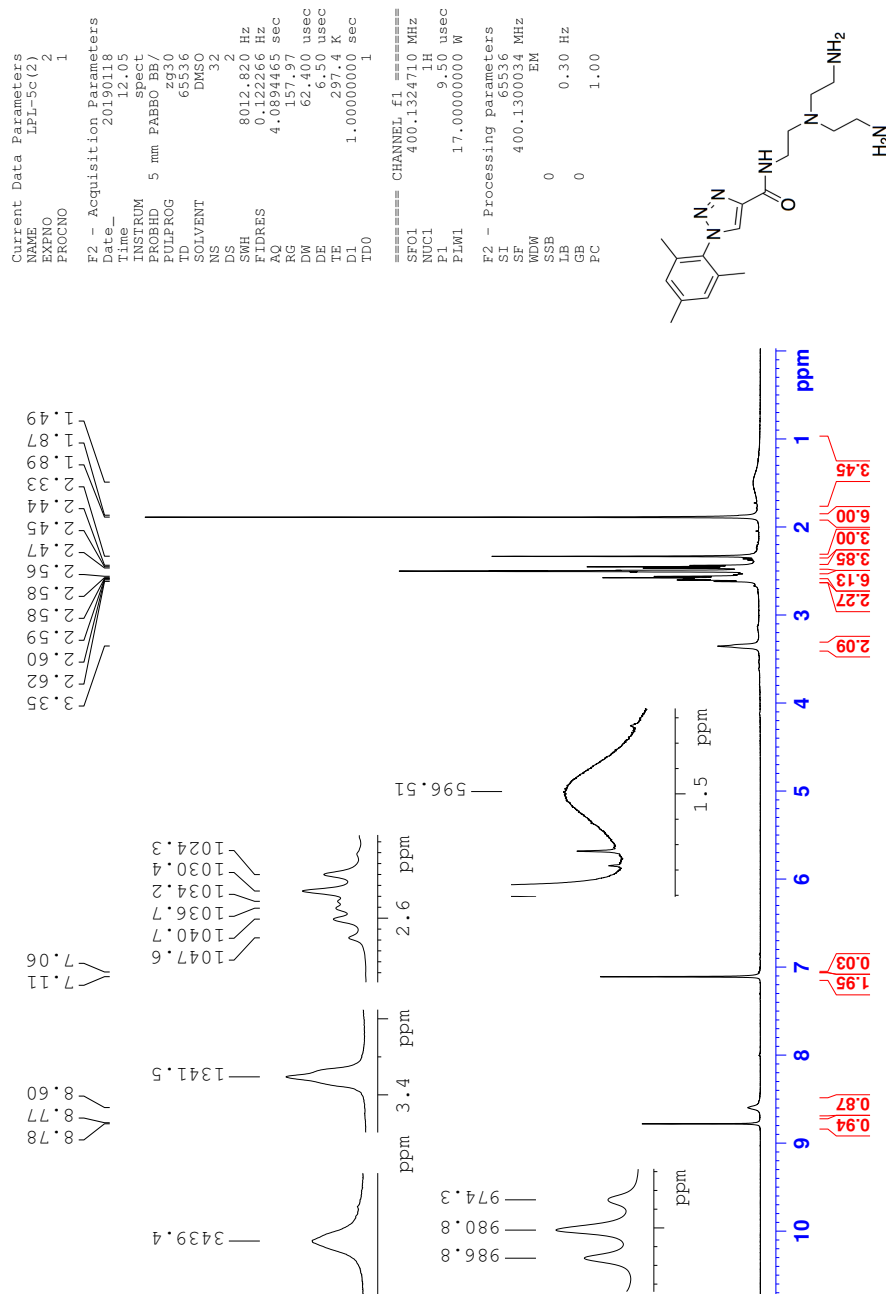
F.2 ^{1}H NMR (400 MHz, DMSO) spectrum for the second synthesis of 5c

F.3 HRMS spectrum for 5c

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
468 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
2019-33re 178 (3.482) AM2 (Ar,35000.0,0.00,0.00); Cm (172:182)
1: TOF MS ASAP+

1.19e+007
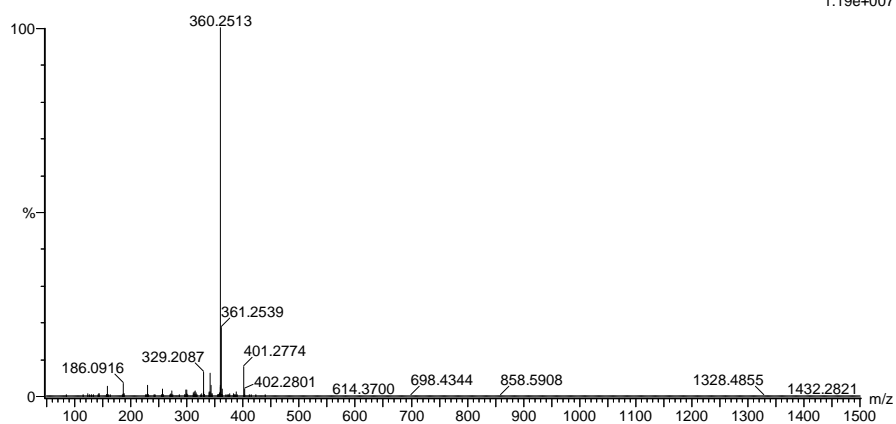

```
Minimum:                        -2.0
Maximum:            5.0    2.0   50.0

Mass       Calc. Mass  mDa    PPM    DBE    i-FIT   Norm   Conf(%) Formula

360.2513   360.2512    0.1    0.3    7.5    1462.5  n/a    n/a     C18 H30 N7 O
```

xl

F.4   HRMS spectrum for 5'c
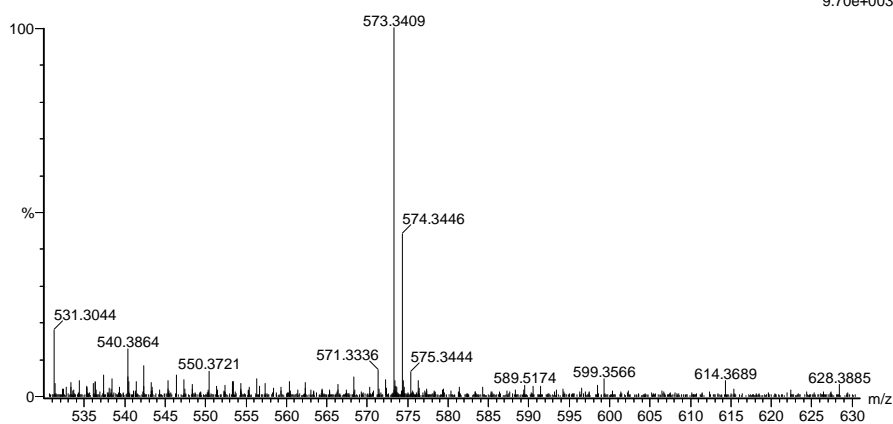
```
Minimum:                              -2.0
Maximum:                   5.0    2.0   50.0

Mass        Calc. Mass   mDa    PPM    DBE    i-FIT    Norm    Conf(%) Formula

573.3409    573.3414    -0.5   -0.9   15.5   384.9    0.273   76.12   C30 H41 N10 O2
            573.3401     0.8    1.4   10.5   386.1    1.432   23.88   C29 H45 N6 O6
```

xli

G.1 ¹H NMR (400 MHz, DMSO, 25°C) spectrum for 5*b

G.2 ^{1}H NMR (400 MHz, DMSO, 90°C) spectrum for 5*b

G.3 ^{13}C NMR (100 MHz, DMSO, 90°C) spectrum for 5*b

G.4 COSY (400 MHz, DMSO, 90°C) spectrum for 5*b

G.6   HMBC (400 MHz / 100 MHz, DMSO, 90°C) spectrum for 5*b

G.7 IR spectrum for 5*b

G.8 HRMS spectrum for 5*b

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
589 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
2019-63 16 (0.165) AM2 (Ar,35000.0,0.00,0.00); Cm (16:18)
1: TOF MS ES+

5.14e+005
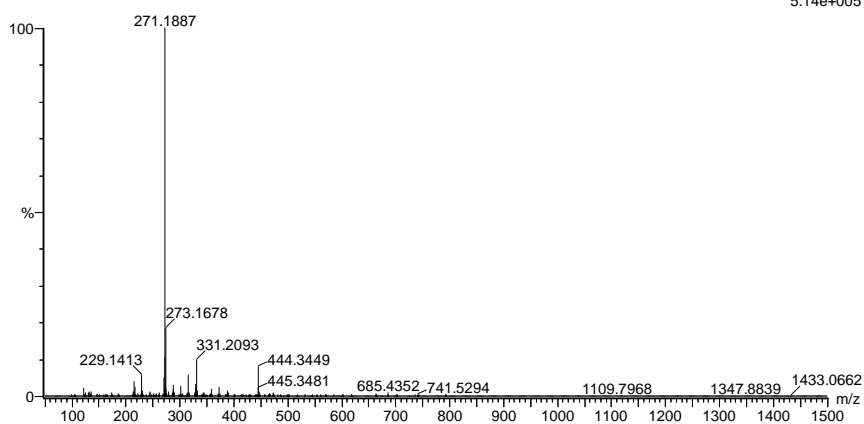
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
					Minimum:			-2.0
			Maximum:	5.0	2.0			50.0
444.3449	444.3451	-0.2	-0.5	7.5	603.6	n/a	n/a	C24 H42 N7 O

xlix

G.9 HPLC chromatogram for 5*b

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-LPL5DSALT.D
Sample Name: LPL5dsalt
====================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                         Location : Vial 2
 Injection Date  : 20.02.2019 12:28:23
                                                Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 20.02.2019 12:23:58 by Lise
                   (modified after loading)
 Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
 Last changed    : 20.02.2019 15:10:28 by Edvard
                   (modified after loading)
 Method Info     : Renhetsanalyse Sondre

 Sample Info     : Isocratic 60/40 MeOH/H2O with 0.1% TFA in H2O, 1mL/min

 Additional Info : Peak(s) manually integrated
====================================================================
```
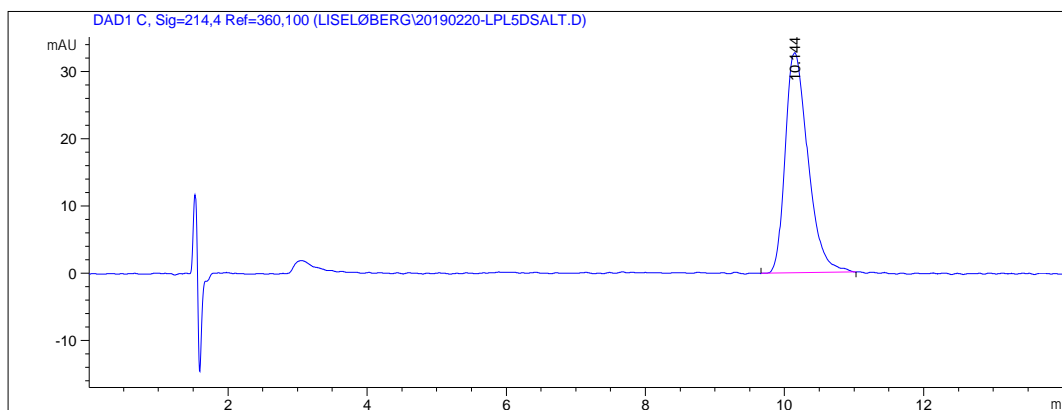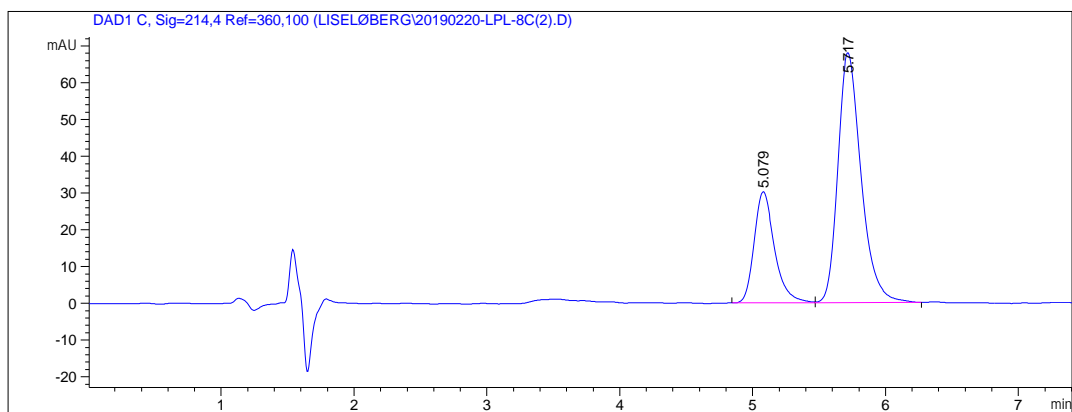


```
====================================================================
                      Area Percent Report
====================================================================

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


 Signal 1: DAD1 C, Sig=214,4 Ref=360,100

 Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
   1  10.144 BB   0.3493  745.44031   32.77837 100.0000

 Totals :                  745.44031   32.77837


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

UPLC 20.02.2019 15:49:48 Edvard   Page   1 of 1

1

H.1 ^{1}H NMR (400 MHz, DMSO, 25°C) spectrum for 5*c

H.3 ^{13}C NMR (100 MHz, DMSO, 90°C) spectrum for 5*c

H.4 COSY (400 MHz, DMSO, 90°C) spectrum for 5*c

H.5 HSQC (400 MHz / 100 MHz, DMSO, 90°C) spectrum for 5*c

H.7 IR spectrum for 5*c

H.8 HRMS spectrum for 5*c

Minimum:				-2.0			
Maximum:		5.0	2.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%) Formula
360.2513	360.2512	0.1	0.3	7.5	1154.9	n/a	n/a C18 H30 N7 O

H.9 HPLC chromatogram for 5*c

```
=====================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                           Location : Vial 1
Injection Date  : 20.02.2019 15:04:04
                                          Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 20.02.2019 15:02:37 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
Last changed    : 20.02.2019 15:10:28 by Edvard
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : Isocratic 40/60 MeOH/H2O with 0.1% TFA in H2O, 1mL/min

Additional Info : Peak(s) manually integrated
=====================================================================
```
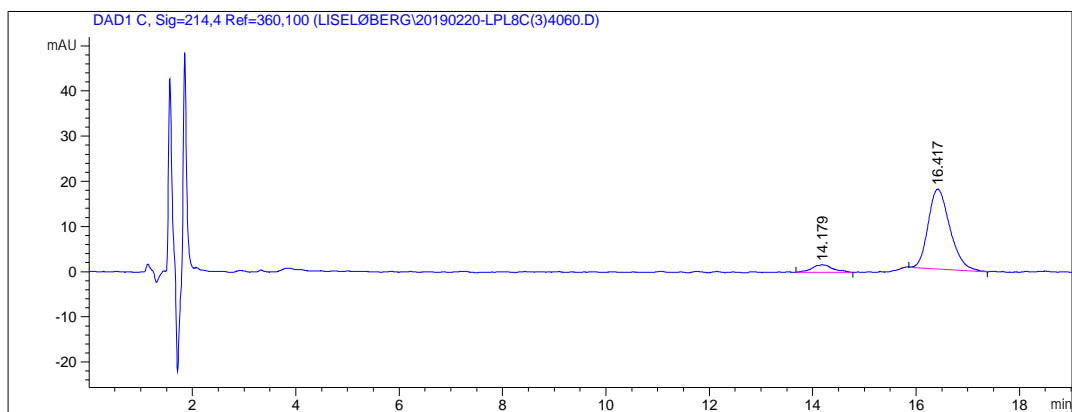

DAD1 C, Sig=214,4 Ref=360,100 (LISELØBERG\20190220-LPL-5CSALT4060.D)
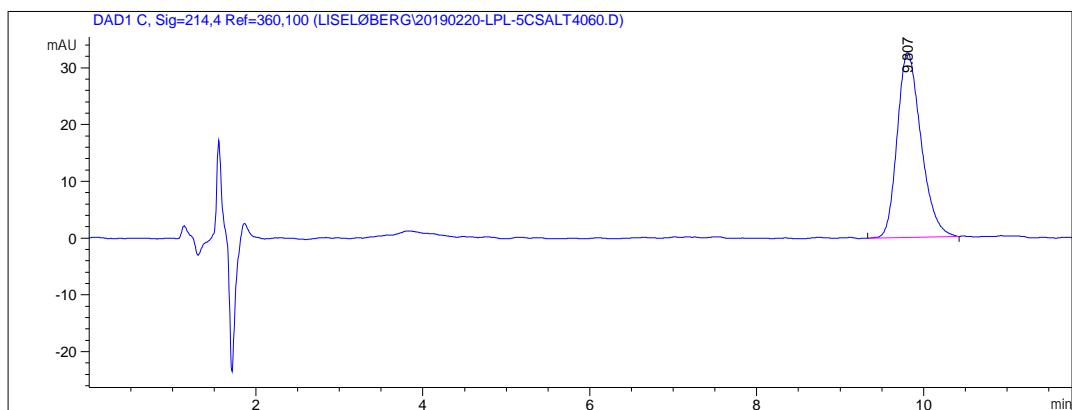
```
=====================================================================
                  Area Percent Report
=====================================================================

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


Signal 1: DAD1 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
 #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   9.807 BB   0.3053  645.24359   32.54375 100.0000

Totals :                 645.24359   32.54375


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

I.1 ^{1}H NMR (400 MHz, DMSO) spectrum from the first synthesis of 7c

I.2 ^{1}H NMR (400 MHz, DMSO) spectrum from the second synthesis of 7c

I.3 HRMS spectrum for 7c

```
Minimum:                    -2.0
Maximum:             5.0    5.0    50.0

Mass        Calc. Mass  mDa   PPM   DBE    i-FIT    Norm   Conf(%)  Formula
274.1670    274.1668    0.2   0.7   7.5    1417.9   n/a    n/a      C14 H20 N5 O
```

I.4 HRMS spectrum for 7'c

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
1157 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100   H: 0-150   N: 0-8   O: 0-10   I: 0-2

2019_26 204 (1.834) AM2 (Ar,35000.0,0.00,0.00); Cm (201:206)
1: TOF MS ES+
1.35e+007
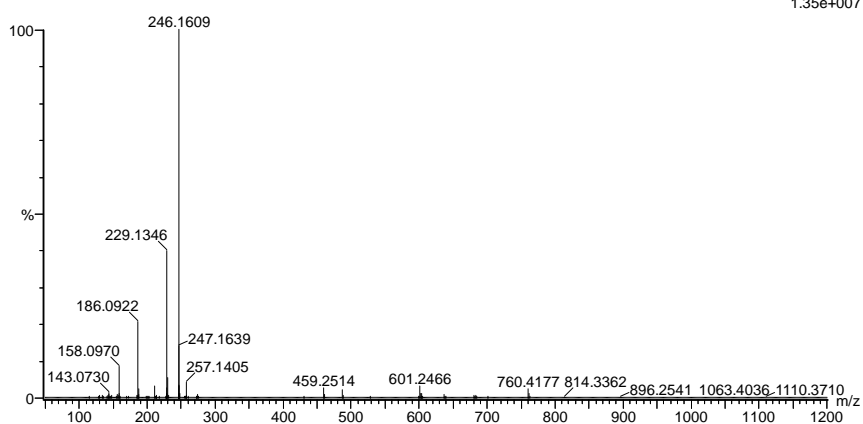
Minimum:				-2.0				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
487.2577	487.2570	0.7	1.4	15.5	905.4	0.286	75.11	C26 H31 N8 O2
	487.2597	-2.0	-4.1	14.5	911.4	6.307	0.18	C30 H35 N2 O4
	487.2557	2.0	4.1	10.5	906.5	1.398	24.70	C25 H35 N4 O6

J.1 ^{1}H NMR (600 MHz, DMSO) spectrum for the first synthesis of 7d

J.3 ^{13}C NMR (150 MHz, DMSO) spectrum for 7d

J.4   COSY (600 MHz, DMSO) spectrum for 7d
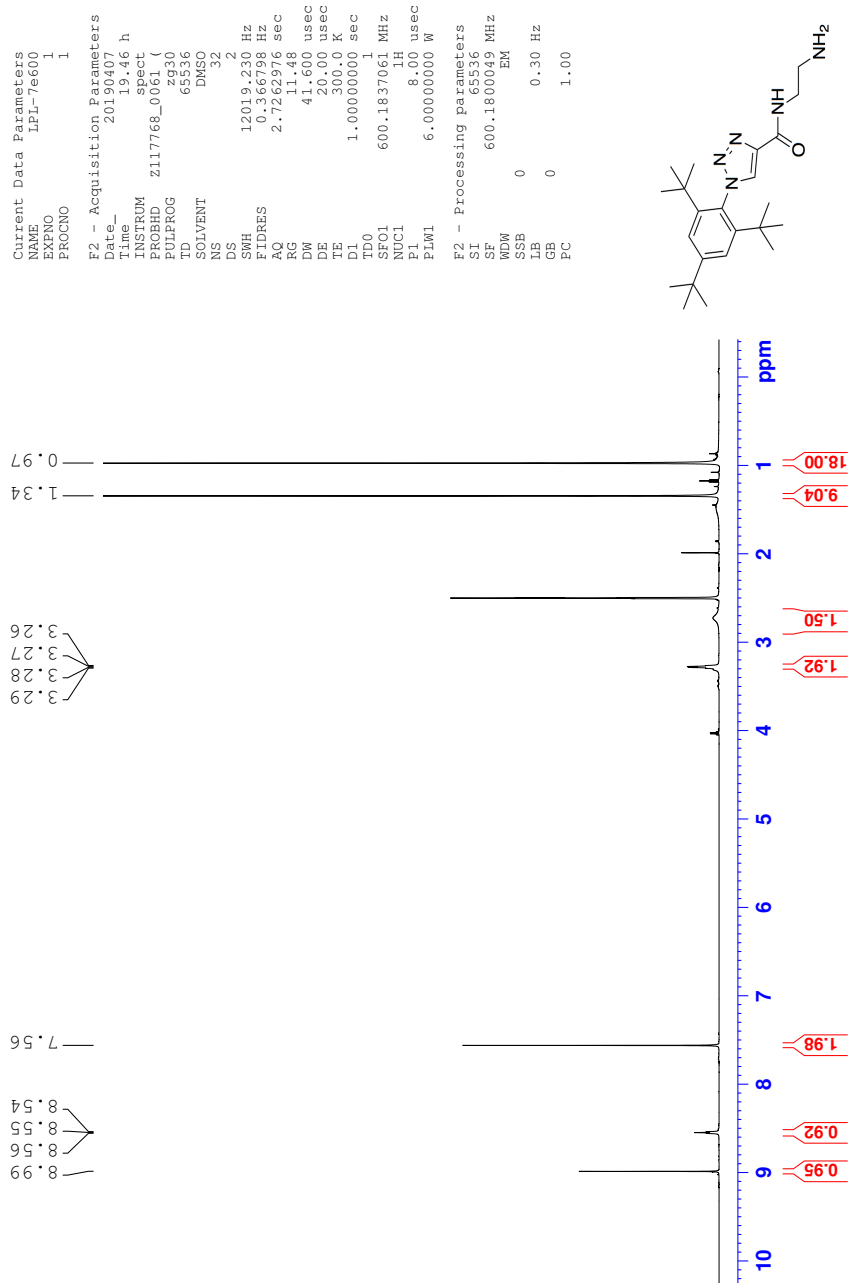
J.5 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 7d
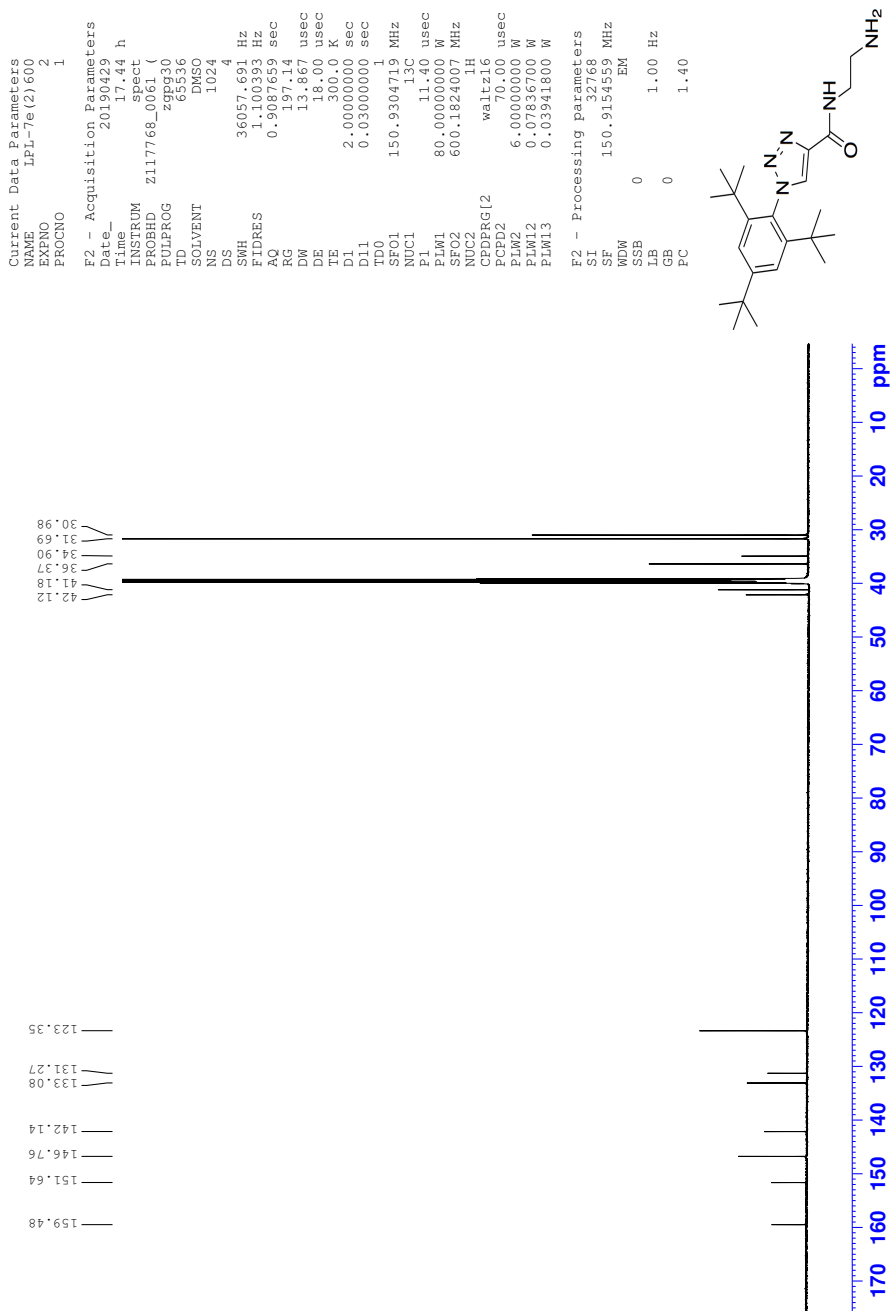
J.6   HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 7d
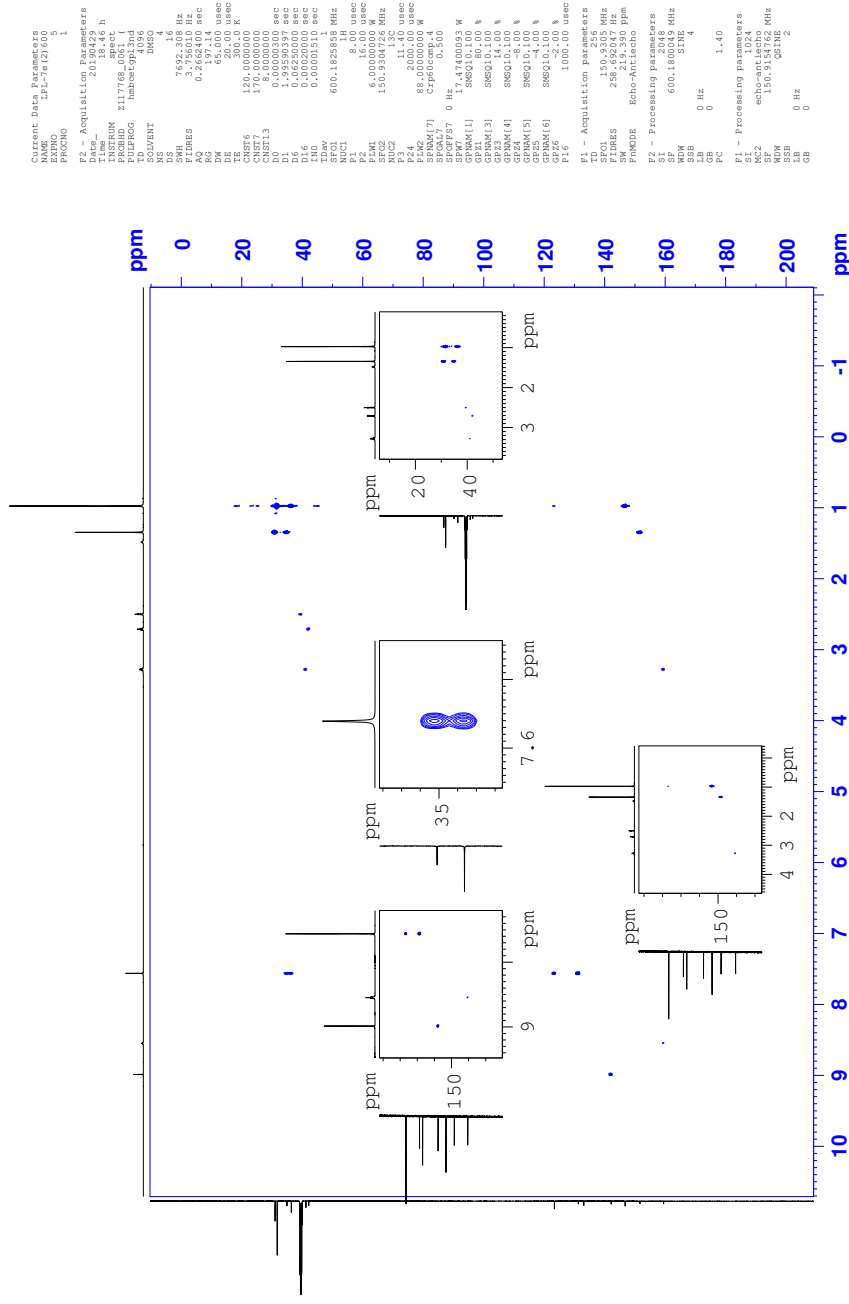
J.7   IR spectrum for 7d
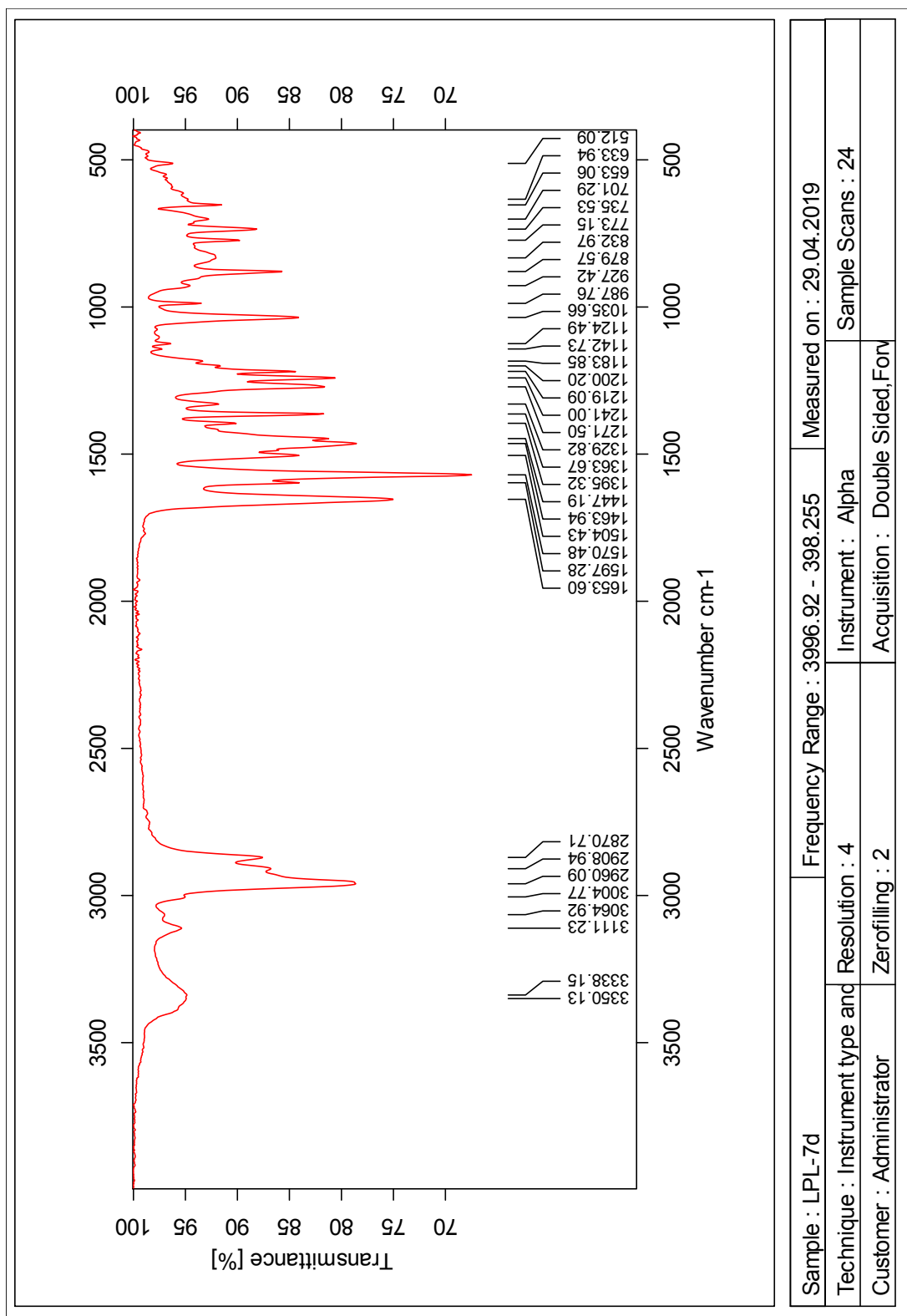
J.8 HRMS spectrum for 7d

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
932 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: 0-8   O: 0-8
2019-375 77 (1.517) AM2 (Ar,35000.0,0.00,0.00); Cm (73:77)
1: TOF MS ASAP+

1.33e+006
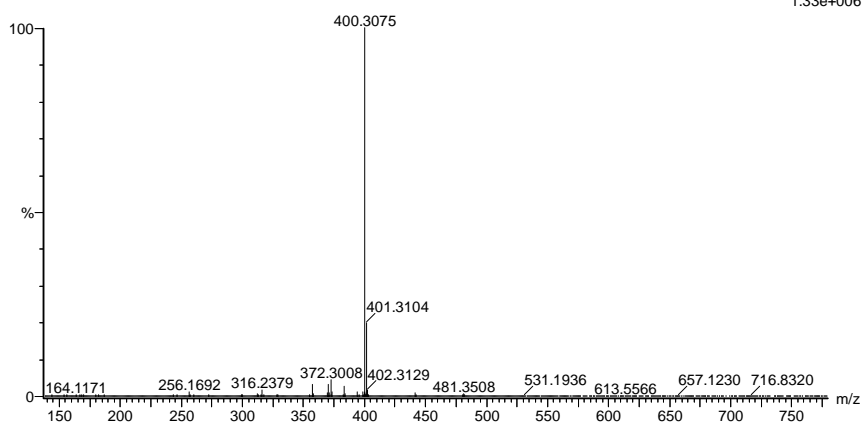

```
Minimum:                        -50.0
Maximum:              5.0   2.0   50.0

Mass       Calc. Mass  mDa   PPM   DBE    i-FIT   Norm   Conf(%)  Formula

400.3075   400.3076   -0.1  -0.2   7.5    1060.6  n/a    n/a      C23 H38 N5 O
```

K.2 ^{1}H NMR (400 MHz, DMSO) spectrum for the secocond synthesis of 7*c
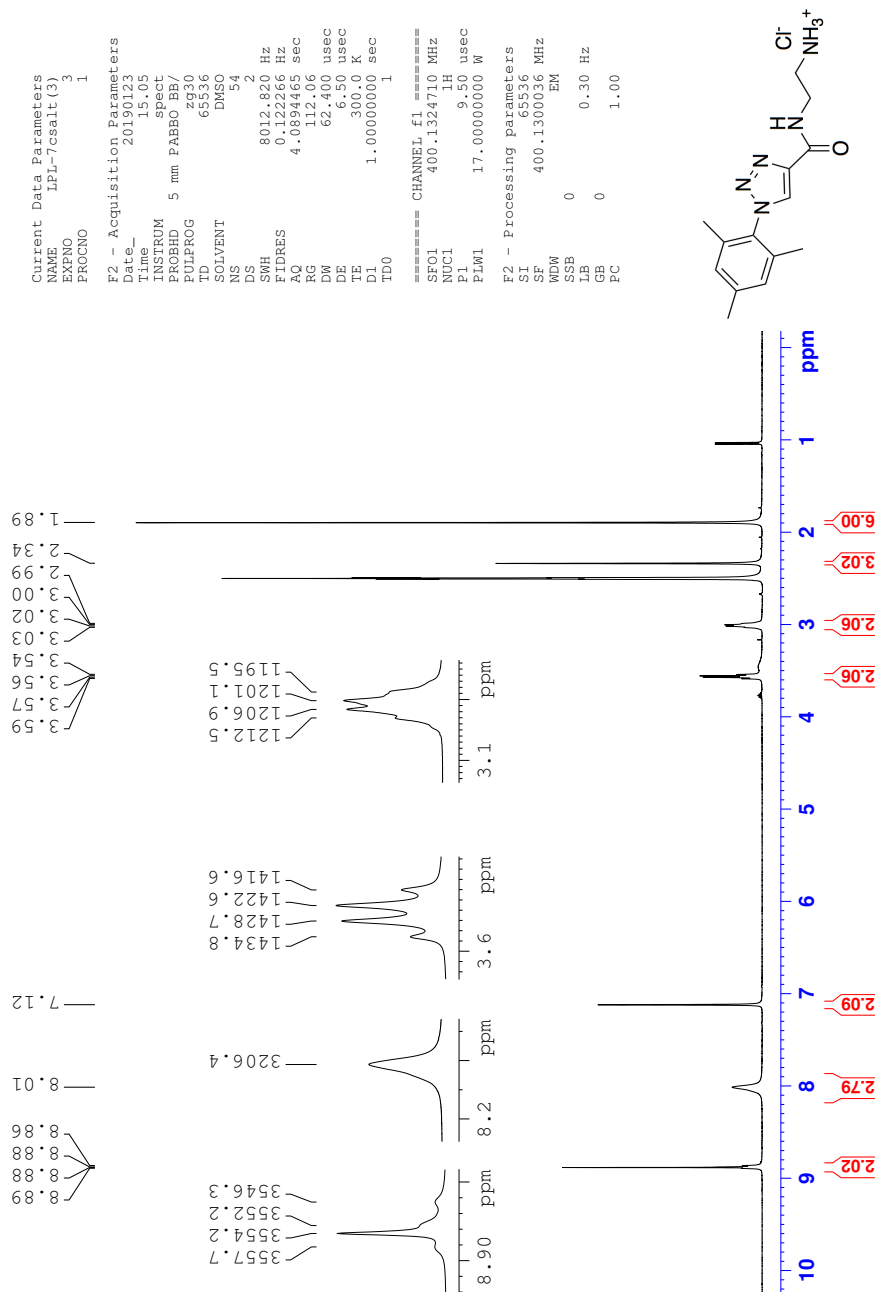
K.3 HPLC chromatogram for 7*c

Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-LPL-7CSALT.D
Sample Name: LPL-7csalt
==
```
Acq. Operator   : Lise
Acq. Instrument : UPLC                            Location : Vial 5
Injection Date  : 20.02.2019 13:26:28
                                                 Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 20.02.2019 13:24:53 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
Last changed    : 20.02.2019 15:10:28 by Edvard
                  (modified after loading)
Method Info     : Renhetsanalyse Sondre

Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1% TFA in H2O, 1mL/min

Additional Info : Peak(s) manually integrated
```
==

DAD1 C, Sig=214,4 Ref=360,100 (LISELØBERG\20190220-LPL-7CSALT.D)

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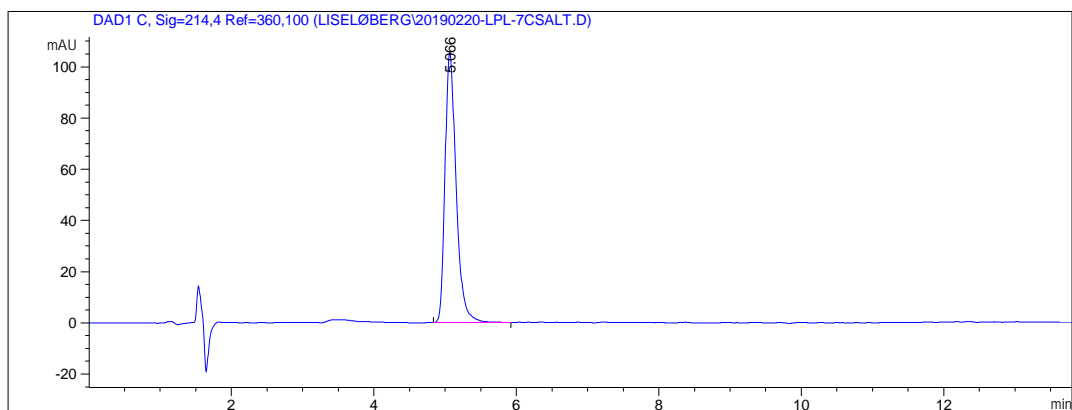
```
Sorted By           :      Signal
Multiplier          :      1.0000
Dilution            :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   5.066 BB    0.1667 1145.48987  105.51291 100.0000

Totals :                 1145.48987  105.51291
```

==
                         *** End of Report ***

L.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 7*d

L.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 7*d

L.3 COSY (600 MHz, DMSO) spectrum for 7*d

L.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 7*d

L.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 7*d

L.6   IR spectrum for 7*d

L.7   HRMS spectrum for 7*d

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-50.0				
Maximum:		5.0	2.0	50.0				
400.3074	400.3076	-0.2	-0.5	7.5	1179.4	0.002	99.85	C23 H38 N5 O
	400.3071	0.3	0.7	-8.5	1185.9	6.490	0.15	C9 H43 N7 O8 Na

L.8 HPLC chromatogram for 7*d

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_LPL7ESALT.D
Sample Name: LPL-7esalt
=====================================================================
  Acq. Operator   : Lise
  Acq. Instrument : UPLC                          Location : Vial 7
  Injection Date  : 07.05.2019 14:17:28
                                                  Inj Volume : 2.000 µl
  Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
  Last changed    : 07.05.2019 14:15:47 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     : H2O/MeOH 80:20 + 0.1%TFA in H2O, 1mL/min

  Additional Info : Peak(s) manually integrated
=====================================================================
```
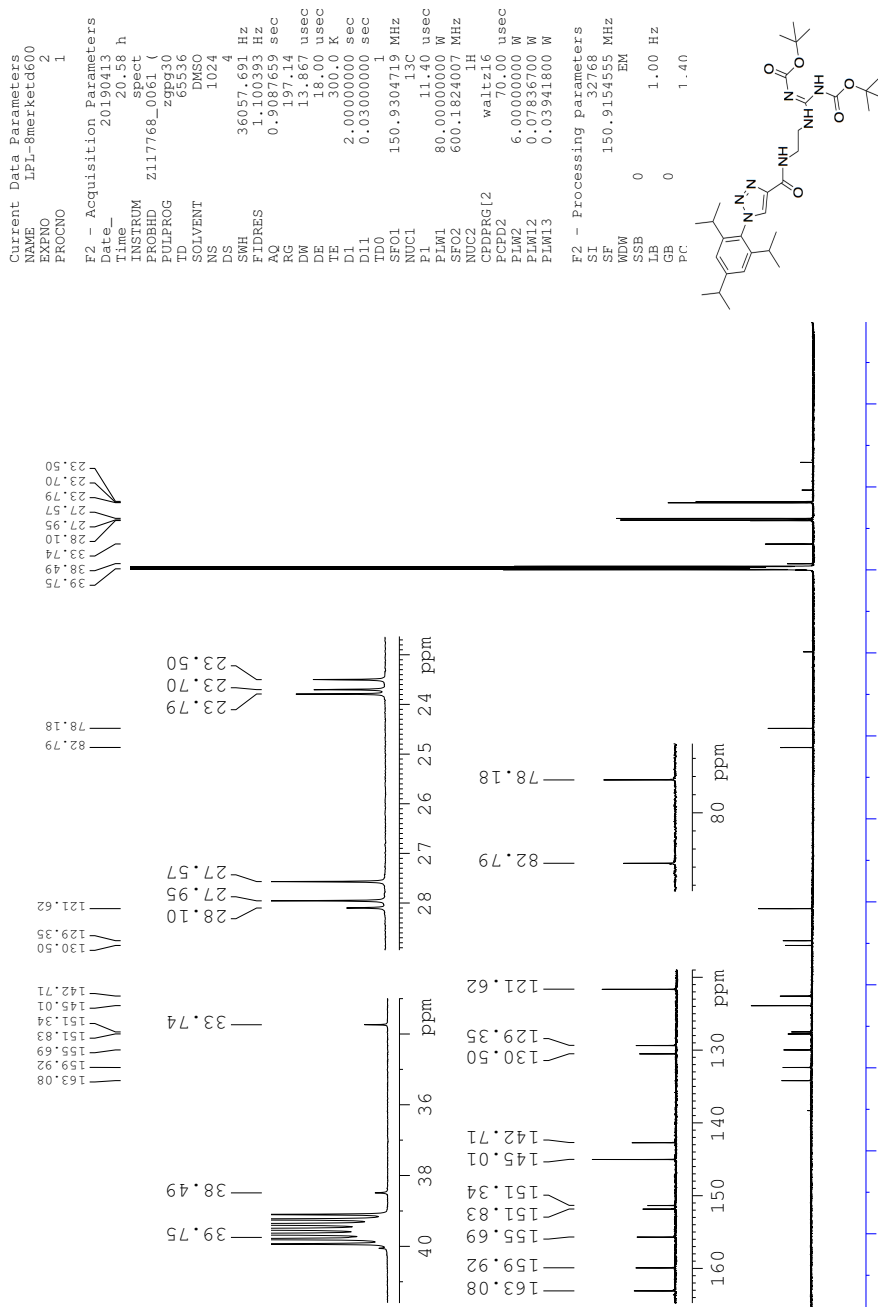


```
=====================================================================
                      Area Percent Report
=====================================================================

  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 Type  Width     Area       Height     Area
   #   [min]        [min]   [mAU*s]      [mAU]        %
  ----|-------|----|-------|----------|----------|--------|
   1   3.597 BB   0.1164   23.60251    3.11604 100.0000

  Totals :                 23.60251    3.11604
```

L.9   HPLC chromatogram for 7*d

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_LPL7ESALT.D
Sample Name: LPL-7esalt
====================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                          Location : Vial 7
Injection Date  : 07.05.2019 14:17:28
                                                Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 14:15:47 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     : H2O/MeOH 80:20 + 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   3.597 BB   0.1145  679.25708   89.53406 100.0000

Totals :                 679.25708   89.53406


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

M.1 ^{1}H NMR (400 MHz, DMSO) spectrum for the crude of 8a

Current Data Parameters
NAME   LPI-8a
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20190320
Time_   12.49
INSTRUM   spect
PROBHD   5 mm PABBO BB/
PULPROG   zg30
TD   65536
SOLVENT   DMSO
NS   16
DS   2
SWH   8012.820 Hz
FIDRES   0.122266 Hz
AQ   4.0894465 sec
RG   209.8
DW   62.400 usec
DE   6.50 usec
TE   297.4 K
D1   1.00000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   400.1324710 MHz
NUC1   1H
P1   9.50 usec
PLW1   17.00000000 W

F2 - Processing parameters
SI   65536
SF   400.1300033 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

1.33

2.99
3.00
3.02
3.34
3.36
3.42
3.44
3.45
3.47

7.56
7.57
7.58
7.60
7.61
7.63
7.63
7.87
7.88
7.89
7.89
8.74
8.76
8.77
9.29

0.11
1.69

1194.4
1200.7
1206.8

ppm

0.11

3.1 3.2 3.3 3.4

1337.7
1343.5
1369.6
1375.4
1381.3

1.69

lxxxiv

M.2 ^{1}H NMR (600 MHz, DMSO) spectrum for 8a

Current Data Parameters
NAME   LPL-8a600
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20190321
Time   0.01 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zg30
TD   65536
SOLVENT   DMSO
NS   32
DS   2
SWH   12019.230 Hz
FIDRES   0.366798 Hz
AQ   2.7262976 sec
RG   41.600
DW   20.00 usec
DE   6.50 usec
TE   300.0 K
D1   1.00000000 sec
TD0   1
SFO1   600.1837061 MHz
NUC1   1H
P1   8.00 usec
PLW1   6.00000000 W

F2 - Processing parameters
SI   65536
SF   600.1800049 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

1.33

3.32
3.33
3.34
3.35
3.43
3.44
3.45
3.46

7.51
7.52
7.53
7.61
7.62
7.62
7.63
7.63
7.63
7.87
7.87
7.88
7.88
7.89
7.89
8.73
8.74
8.75
9.28

4508.2
4513.8
4519.4

4570.6
4572.6
4577.3
4579.4

4725.3
4727.4
4732.0
4734.1

5239.6
5245.5
5251.3

1993.4
1999.8
2005.8
2011.7

2060.1
2066.1
2072.1
2078.1

3.35 ppm
3.45 ppm
7.9 ppm
7.5   7.0   ppm
8.75 ppm
7.0   ppm

9.00
2.43
2.00
1.00
2.06
2.01
8.55
0.97
0.97

13 12 11 10   9   8   7   6   5   4   3   2   1 ppm

M.3 HPLC chromatogram for 8a

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190326_LPL8A.D
Sample Name: LPL8a
```

```
========================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                           Location : Vial 2
Injection Date  : 26.03.2019 14:02:22
                                                 Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 26.03.2019 14:00:28 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\ED-MASTER\H2O SVAK 2.M
Last changed    : 26.03.2019 14:03:25 by ED
                  (modified after loading)
Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1%TFA in H2O, 1mL/min
```

```
Additional Info : Peak(s) manually integrated
```


DAD1 C, Sig=214,4 Ref=360,100 (LISELØBERG\20190326_LPL8A.D)
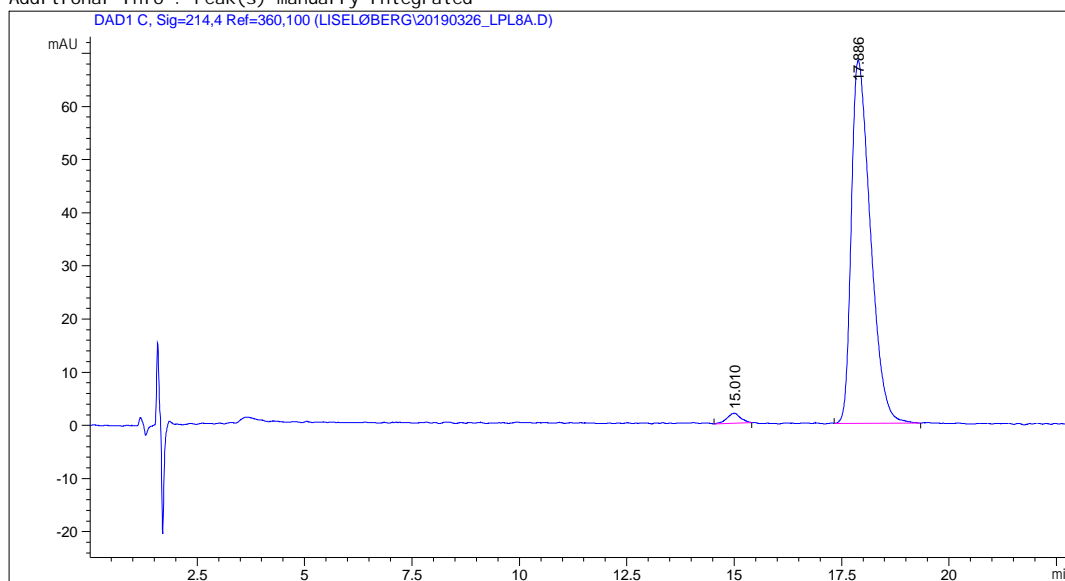
```
========================================================================
                        Area Percent Report
========================================================================

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


Signal 1: DAD1 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
  1  15.010 BB    0.2828    42.09561   1.88318    1.9532
  2  17.886 BB    0.4788  2113.08032  68.32137   98.0468

Totals :                 2155.17593   70.20455
```
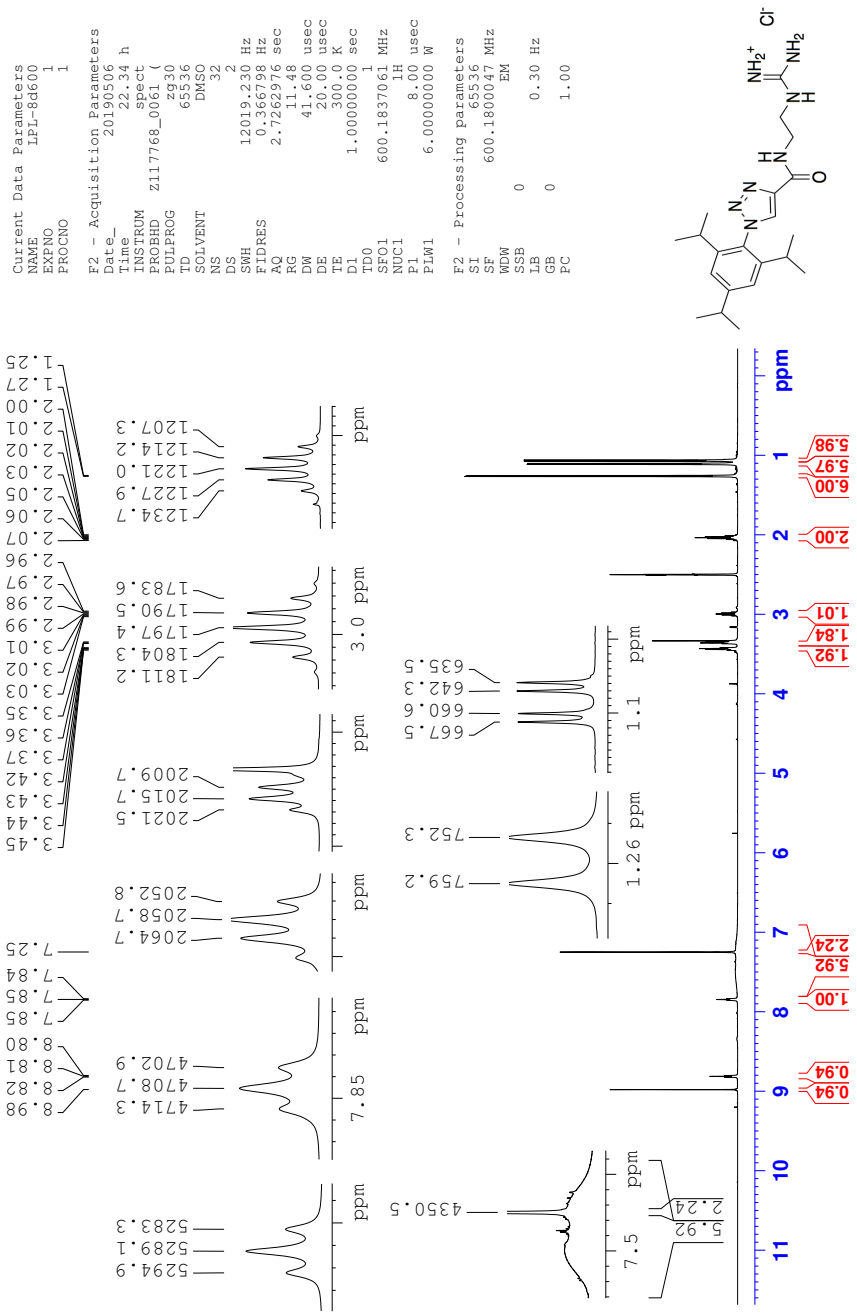
N.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 8b

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

N.6   IR spectrum for 8b

N.7 HRMS spectrum for 8b

3.55e+006


```
Minimum:                      -50.0
Maximum:             5.0   3.0   50.0

Mass       Calc. Mass  mDa   PPM   DBE   i-FIT   Norm   Conf(%)  Formula

400.2822   400.2825   -0.3  -0.7   8.5  1109.9  0.137  87.18    C21 H34 N7 O
           400.2819    0.3   0.7  -7.5  1116.5  6.790  0.11     C7 H39 N9 O8 Na
           400.2828   -0.6  -1.5   4.5  1113.2  3.486  3.06     C23 H39 N O3 Na
           400.2811    1.1   2.7   3.5  1112.1  2.339  9.65     C20 H38 N3 O5
```

xciii

N.8 HPLC chromatogram for 8b

Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8D7030(2).D
Sample Name: LPL-8d
===
 Acq. Operator   : Lise
 Acq. Instrument : UPLC   Location : Vial 4
 Injection Date : 07.05.2019 16:33:42
                                                  Inj Volume : 2.000 µl
 Acq. Method   : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed   : 07.05.2019 16:32:14 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   : 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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
 ----|-------|----|-------|----------|----------|--------|
  1   5.989 BB   0.1750   30.58376   2.68252 100.0000

 Totals :   30.58376   2.68252

UPLC 10.05.2019 14:23:25 Jorge   Page   1 of 2

xciv

N.9 HPLC chromatogram for 8b

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8D7030(2).D
Sample Name: LPL-8d
===================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                          Location : Vial 4
Injection Date  : 07.05.2019 16:33:42
                                                Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 16:32:14 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     : 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   5.989 BB   0.1772  544.76318   47.70761 100.0000

Totals :                 544.76318   47.70761


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

O.1 ^{1}H NMR (400 MHz, DMSO) spectrum for the first synthesis of 8c

O.3 ^{1}H NMR (400 MHz, DMSO) spectrum for the crude from the third synthesis of 8c

O.4 ^{1}H NMR (600 MHz, DMSO) spectrum for the fourth synthesis of 8c

O.5 ^{1}H NMR (600 MHz, DMSO) spectrum for the byproduct formed in the fourth synthesis of 8c

1.78
1.90

4.98

6.92
7.27
7.36
7.44

9.00
0.40

11.20

7.07

c
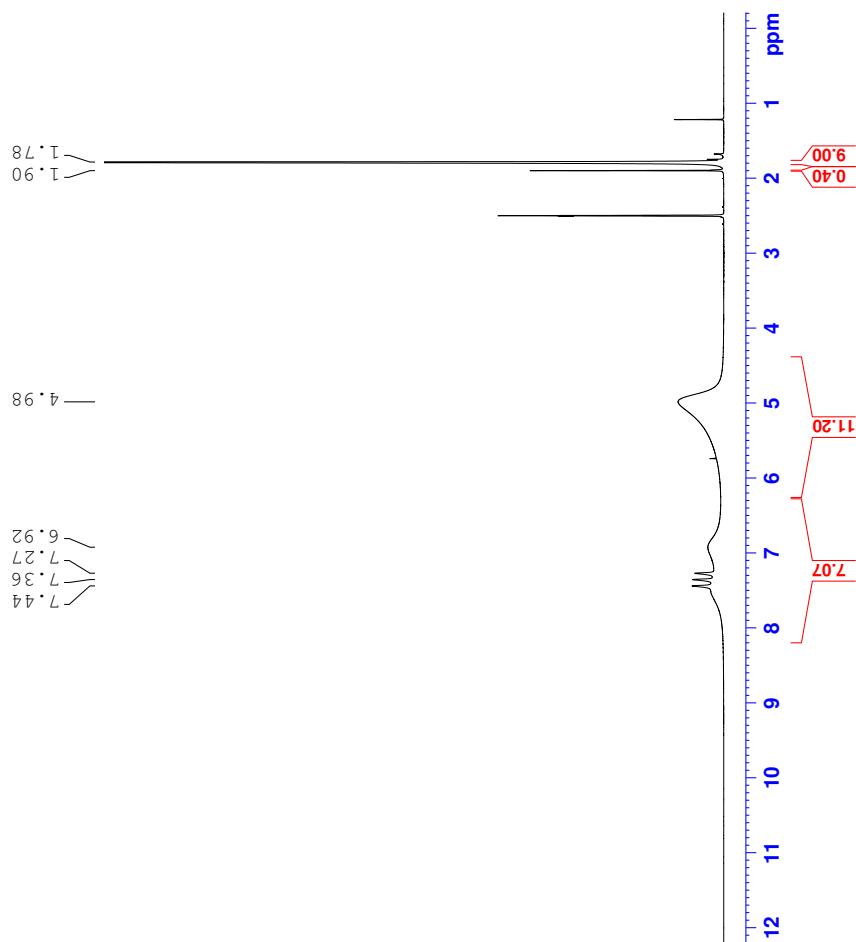
O.6 ^{1}H NMR (600 MHz, DMSO) spectrum for the fifth synthesis of 8c
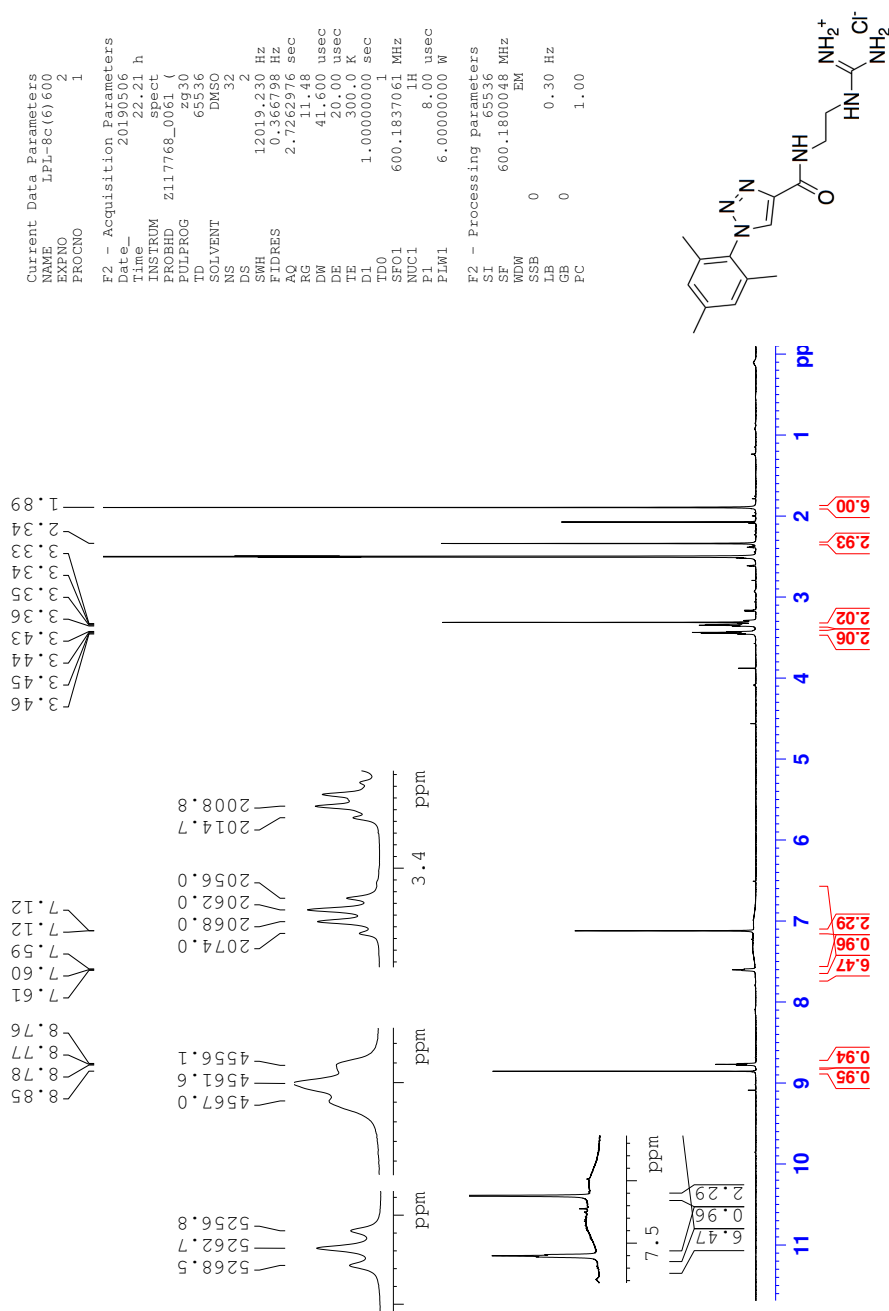
O.7 HPLC chromatogram for 8c from the first synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-LPL-8C(2).D
Sample Name: LPL-8c(2)
====================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                          Location : Vial 4
 Injection Date  : 20.02.2019 13:17:15
                                                 Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 20.02.2019 13:15:54 by Lise
                   (modified after loading)
 Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
 Last changed    : 20.02.2019 15:10:28 by Edvard
                   (modified after loading)
 Method Info     : Renhetsanalyse Sondre

 Sample Info     : Isocratic 50/50 MeOH/H2O with 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 C, Sig=214,4 Ref=360,100

 Peak RetTime Type  Width     Area       Height     Area
   #   [min]        [min]   [mAU*s]      [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
   1   5.079 BV    0.1624  322.50293   30.23416   28.0356
   2   5.717 VB    0.1860  827.82983   68.00027   71.9644

 Totals :                  1150.33276  98.23443


====================================================================
```

UPLC 20.02.2019 15:12:34 Edvard   Page   1 of 1

O.8 HPLC chromatogram for the first synthesis of 8c after addition of 7*c

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-LPL8C(2)7CSALT.D
Sample Name: LPL-8c(2)7csalt
=====================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                        Location : Vial 4
 Injection Date  : 20.02.2019 13:52:13
                                               Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 20.02.2019 13:49:52 by Lise
                   (modified after loading)
 Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
 Last changed    : 20.02.2019 15:10:28 by Edvard
                   (modified after loading)
 Method Info     : Renhetsanalyse Sondre

 Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1% TFA in H2O, 1mL/min

 Additional Info : Peak(s) manually integrated
=====================================================================
```
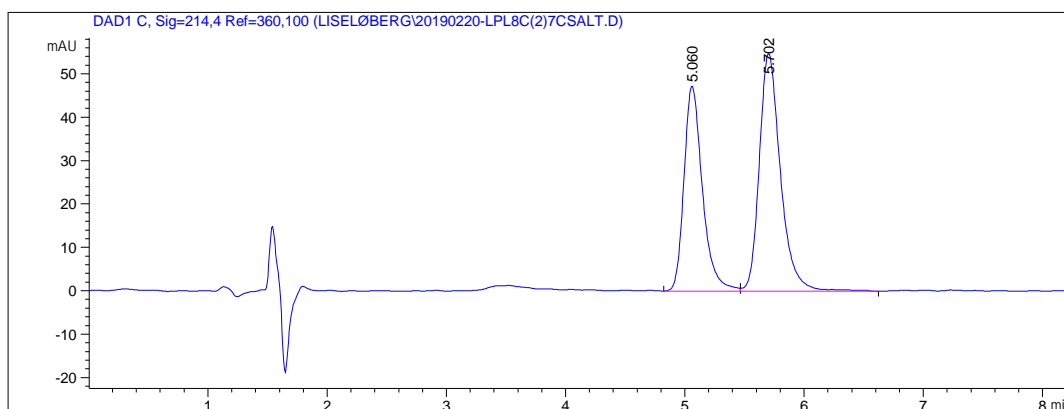


```
=====================================================================
                        Area Percent Report
=====================================================================

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


 Signal 1: DAD1 C, Sig=214,4 Ref=360,100

 Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
 ----|-------|----|-------|----------|----------|--------|
   1   5.060 BV    0.1617  509.19849   47.26824  42.9106
   2   5.702 VB    0.1864  677.45135   54.74327  57.0894

 Totals :                 1186.64984  102.01151


=====================================================================
```

UPLC 20.02.2019 15:10:30 Edvard   Page   1 of 1

O.9 HPLC chromatogram for 8c from the second synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190326_LPL8C(4).D
Sample Name: LPL8c
```

```
=====================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                           Location : Vial 3
Injection Date  : 26.03.2019 13:48:13
                                                 Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 26.03.2019 12:59:32 by Gruppe9
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\ED-MASTER\H2O SVAK 2.M
Last changed    : 26.03.2019 14:03:25 by ED
                  (modified after loading)
Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1%TFA in H2O, 1mL/min

Additional Info : Peak(s) manually integrated
```
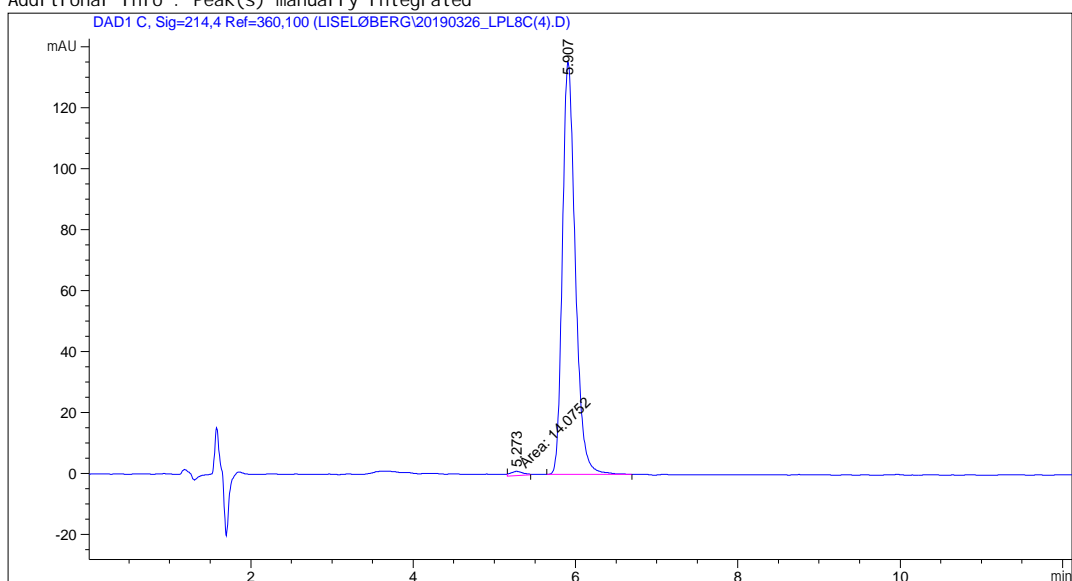


```
=====================================================================
                      Area Percent Report
=====================================================================

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


Signal 1: DAD1 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area        Height     Area
  #   [min]        [min]    [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   5.273 MM   0.1740    14.07519    1.34848   0.9345
  2   5.907 BB   0.1687  1492.10266  135.22052  99.0655

Totals :                 1506.17786  136.56900
```

O.10 HPLC chromatogram of the crude for the third synthesis of 8c

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-LPL8C(3)4060.D
Sample Name: LPL-8c(3)
=====================================================================
  Acq. Operator   : Lise
  Acq. Instrument : UPLC                          Location : Vial 6
  Injection Date  : 20.02.2019 14:43:25
                                                  Inj Volume : 2.000 µl
  Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
  Last changed    : 20.02.2019 14:27:43 by Lise
                    (modified after loading)
  Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
  Last changed    : 20.02.2019 15:10:28 by Edvard
                    (modified after loading)
  Method Info     : Renhetsanalyse Sondre

  Sample Info     : Isocratic 40/60 MeOH/H2O with 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 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
 #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1  14.179 BB   0.3543    48.02504   1.67611   8.5494
  2  16.417 BB   0.4435   513.71100  17.65149  91.4506

Totals :                  561.73603  19.32760


=====================================================================
```

UPLC 20.02.2019 15:55:30 Edvard   Page   1 of 1

O.11 HPLC chromatogram of the crude for the third synthesis of 8c after further reaction

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20181203_LPL8C1.D
Sample Name: LPL8c
====================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                          Location : Vial 2
 Injection Date  : 12.03.2019 12:40:39
                                                 Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 12.03.2019 12:18:32 by Pia
                   (modified after loading)
 Analysis Method : C:\CHEM32\1\METHODS\PIATRAPP\PC ACETOPHENONE.M
 Last changed    : 07.02.2019 16:00:39 by Edvard
 Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1%TFA in H2O, 1mL/min

 Additional Info : Peak(s) manually integrated
====================================================================
```
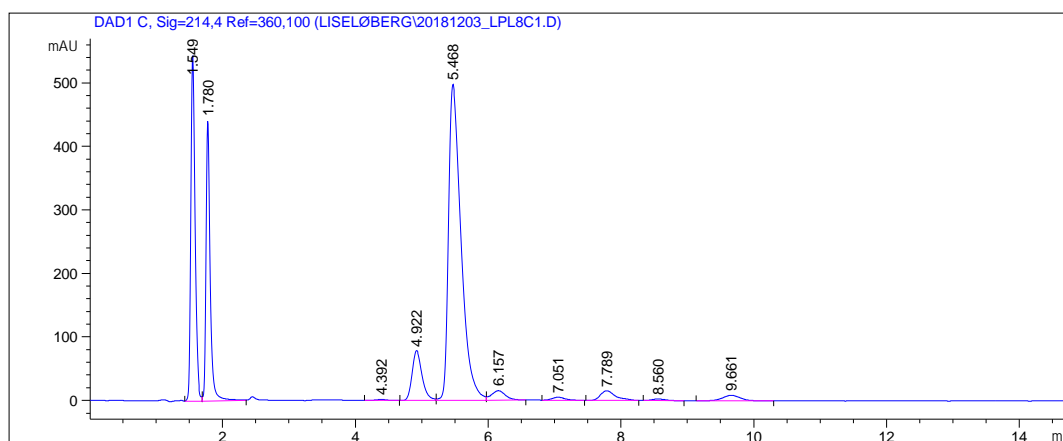


```
====================================================================
                      Area Percent Report
====================================================================

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


 Signal 1: DAD1 C, Sig=214,4 Ref=360,100

 Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]    [mAU*s]     [mAU]         %
 ----|-------|----|-------|----------|----------|--------|
   1   1.549 BV    0.0678 2422.67090  545.59009  19.3581
   2   1.780 VV    0.0641 1899.75671  442.61365  15.1798
   3   4.392 BB    0.1712   18.46771    1.66803   0.1476
   4   4.922 BV    0.1631  831.44788   78.82059   6.6436
   5   5.468 VV    0.2029 6607.98340  497.59692  52.8005
   6   6.157 VB    0.2201  220.81830   14.97041   1.7644
   7   7.051 BB    0.2120   68.58807    4.87789   0.5480
   8   7.789 BB    0.2384  243.96043   15.26775   1.9493
   9   8.560 BB    0.2317   36.30714    2.46697   0.2901
```

O.12 HPLC chromatogram for 8c for the fourth synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8C(5).D
Sample Name: LPL-8c(5)
=====================================================================
  Acq. Operator   : Lise
  Acq. Instrument : UPLC                       Location : Vial 2
  Injection Date  : 07.05.2019 10:13:51
                                               Inj Volume : 2.000 µl
  Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
  Last changed    : 07.05.2019 10:12:37 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     : H20/MeOH 50:50 + 0.1%TFA in H20, 1mL/min

  Additional Info : Peak(s) manually integrated
=====================================================================
```
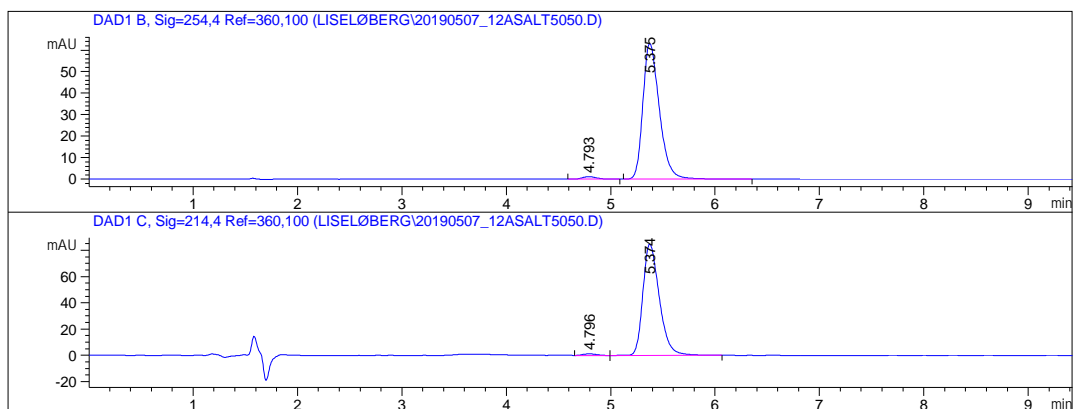


```
=====================================================================
                      Area Percent Report
=====================================================================

  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 Type  Width     Area      Height     Area
   #   [min]        [min]   [mAU*s]     [mAU]        %
  ----|-------|----|-------|----------|----------|--------|
   1   5.953 BB   0.1672   77.47211    7.10695 100.0000

  Totals :                 77.47211    7.10695
```

O.13 HPLC chromatogram for 8c for the fourth synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8C(5).D
Sample Name: LPL-8c(5)
==================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                           Location : Vial 2
Injection Date  : 07.05.2019 10:13:51
                                            Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 10:12:37 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 50:50 + 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 Type  Width     Area      Height     Area
 #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
 1   5.953 BB   0.1675  981.38672   89.83286 100.0000

Totals :                981.38672   89.83286


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

O.14 HPLC chromatogram for 8c for the fifth synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8C(6).D
Sample Name: LPL-8c(6)
======================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                        Location : Vial 3
 Injection Date  : 07.05.2019 10:00:17
                                               Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 07.05.2019 09:05:13 by ED
                   (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 50:50 + 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 Type  Width     Area       Height     Area
  #   [min]        [min]    [mAU*s]      [mAU]       %
 ----|-------|----|-------|----------|----------|--------|
   1   5.953 BB   0.1697  114.67876   10.47769  100.0000

 Totals :                 114.67876   10.47769
```

O.15   HPLC chromatogram for 8c for the fifth synthesis

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_8C(6).D
Sample Name: LPL-8c(6)
==================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                            Location : Vial 3
Injection Date  : 07.05.2019 10:00:17
                                              Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 09:05:13 by ED
                  (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 50:50 + 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   2.358 MM   0.1043   10.18204    1.62681   0.6967
  2   5.953 BB   0.1701 1451.19556  132.17917  99.3033

Totals :                 1461.37760  133.80598


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

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

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

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

Wavenumber cm-1

Transmittance [%]

3327.14

3105.65
3063.65

2964.32
2932.32
2871.93

1721.72
1637.25
1614.22
1568.60
1508.17
1479.64
1460.21
1411.98
1389.24
1363.51
1325.49
1283.45
1250.09
1228.65
1155.84
1133.39
1049.32
1029.93
989.77
945.18
878.44
838.56
810.06
771.77
736.03
700.92
653.42
637.74
621.41
463.97
437.86
421.69

Sample : LPL-8merketd	Frequency Range : 3996.92 - 398.255	Measured on : 13.04.2019
Technique : Instrument type and	Resolution : 4	Instrument : Alpha	Sample Scans : 24
Customer : Administrator	Zerofilling : 2	Acquisition : Double Sided,Forw

P.7   HRMS spectrum for 8'b

Minimum:				-50.0			
Maximum:		5.0	2.0	50.0			

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
600.3869	600.3860	0.9	1.5	5.5	420.9	0.176	83.88	C30 H54 N3 O9
	600.3873	-0.4	-0.7	10.5	422.6	1.893	15.06	C31 H50 N7 O5
	600.3860	0.9	1.5	13.5	425.8	5.088	0.62	C37 H51 11B N O5
	600.3874	-0.5	-0.8	18.5	426.9	6.152	0.21	C38 H47 11B N5 O
	600.3875	-0.6	-1.0	13.5	427.6	6.851	0.11	C39 H54 N O2 S
	600.3867	0.2	0.3	9.5	428.0	7.253	0.07	C30 H51 11B N7 O3 S
	600.3867	0.2	0.3	1.5	428.4	7.679	0.05	C23 H54 N9 O7 S
	600.3869	0.0	0.0	12.5	431.7	11.029	0.00	C38 H55 11B N S2
	600.3869	0.0	0.0	4.5	432.1	11.403	0.00	C31 H58 N3 O4 S2
	600.3861	0.8	1.3	0.5	432.8	12.056	0.00	C22 H55 11B N9 O5 S2
	600.3876	-0.7	-1.2	0.5	434.3	13.598	0.00	C24 H58 N9 O2 S3
	600.3862	0.7	1.2	3.5	434.4	13.714	0.00	C30 H59 11B N3 O2 S3
	600.3881	-1.2	-2.0	-9.5	434.7	13.940	0.00	C18 H63 11B N5 O9 S3
	600.3862	0.7	1.2	-4.5	434.7	14.030	0.00	C23 H62 N5 O6 S3

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

Q.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 8'c
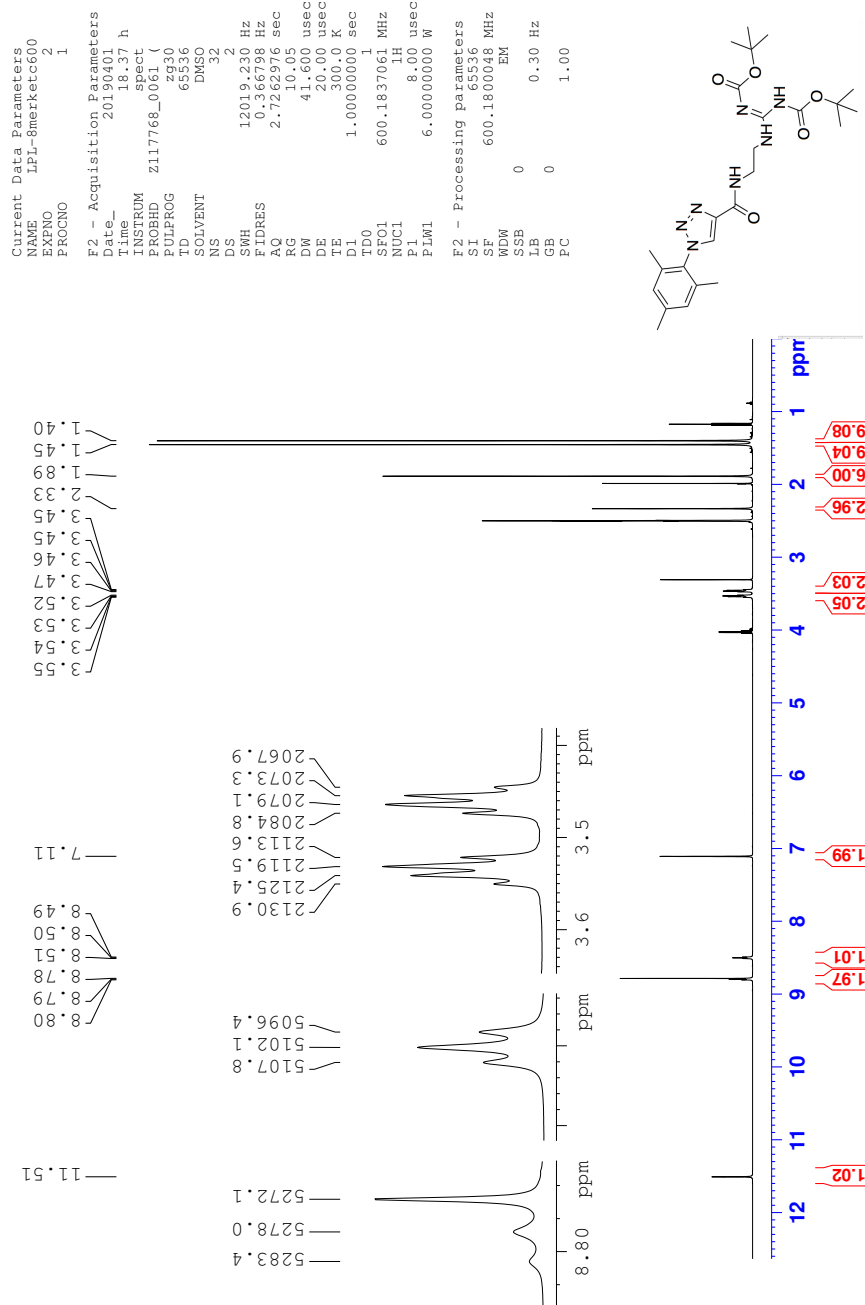
Q.3 COSY (600 MHz, DMSO) spectrum for 8'c

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

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

Q.6 IR spectrum for 8'c
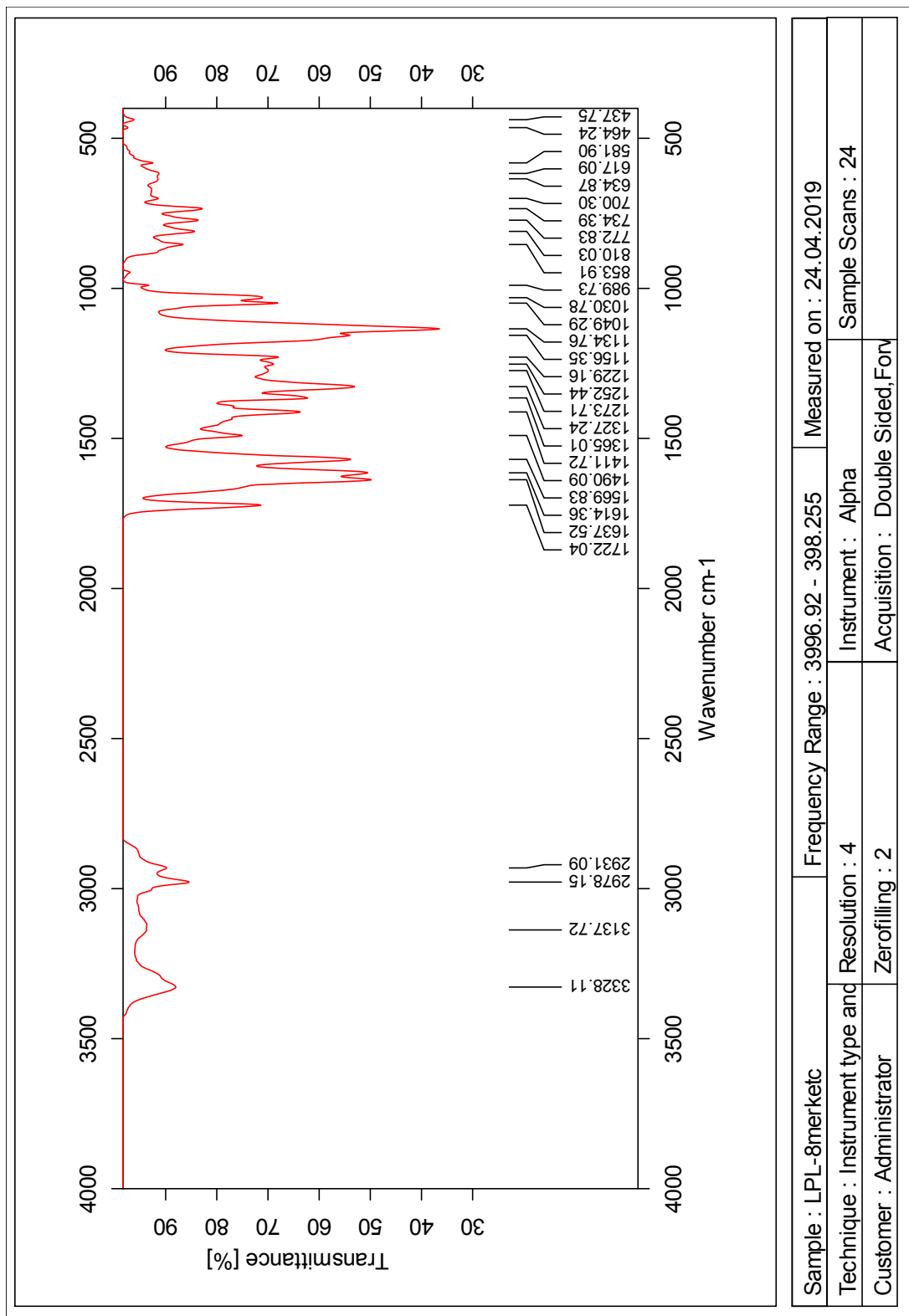
Q.7 HRMS spectrum for 8'c

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, Even Electron Ions
1499 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: 0-10
2019-357 104 (2.033) AM2 (Ar,35000.0,0.00,0.00); Cm (96:104)
1: TOF MS ASAP+

1.25e+005

Minimum:				-50.0			
Maximum:		5.0	3.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
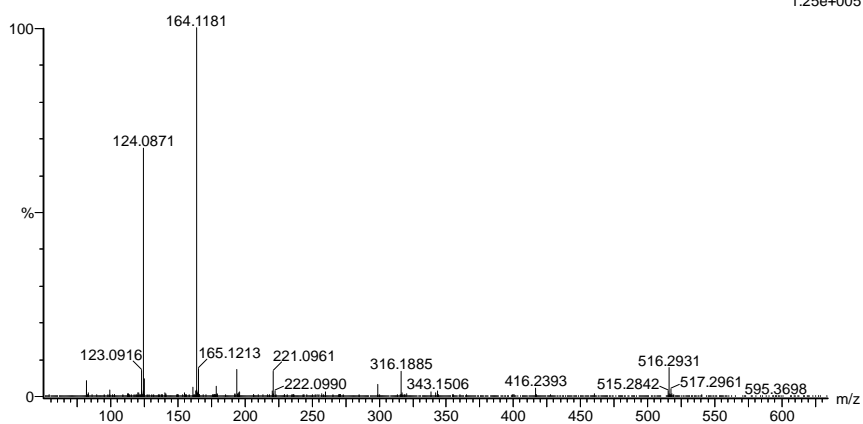
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
516.2931	516.2934	-0.3	-0.6	10.5	289.9	2.415	8.93	C25 H38 N7 O5
	516.2921	1.0	1.9	5.5	287.6	0.094	91.07	C24 H42 N3 O9

cxxiv

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

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

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

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

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

R.6 IR spectrum for 8'd

Sample : LPL-8merketd | Frequency Range : 3996.92 - 398.255 | Measured on : 22.05.2019
Technique : Instrument type and | Resolution : 4 | Instrument : Alpha | Sample Scans : 24
Customer : Default | Zerofilling : 2 | Acquisition : Double Sided,Forv

R.7   HRMS spectrum for 8'd

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
3087 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: 0-10   Na: 0-1
2019-419 97 (1.914) AM2 (Ar,35000.0,0.00,0.00); Cm (96:99)
1: TOF MS ASAP+
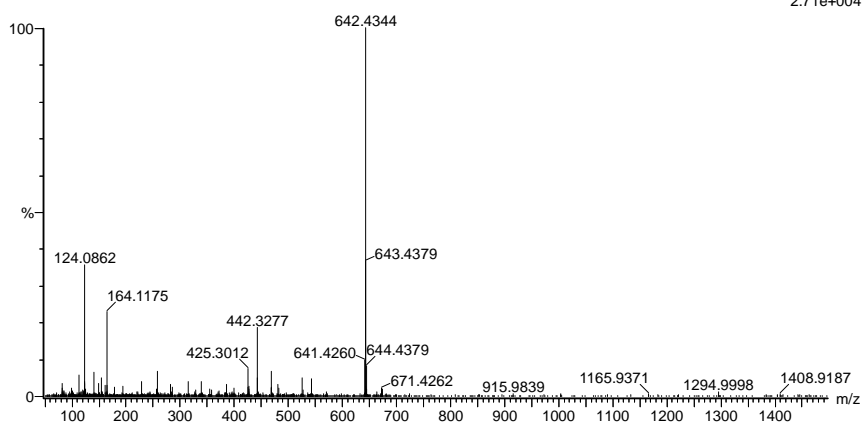
Minimum:				-50.0			
Maximum:		5.0	2.0	50.0			

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
642.4344	642.4343	0.1	0.2	10.5	392.0	0.244	78.35	C34 H56 N7 O5
	642.4346	-0.2	-0.3	6.5	393.2	1.530	21.65	C36 H61 N O7 Na

S.1 ^{1}H NMR (600 MHz, CDCl$_3$) spectrum for 10
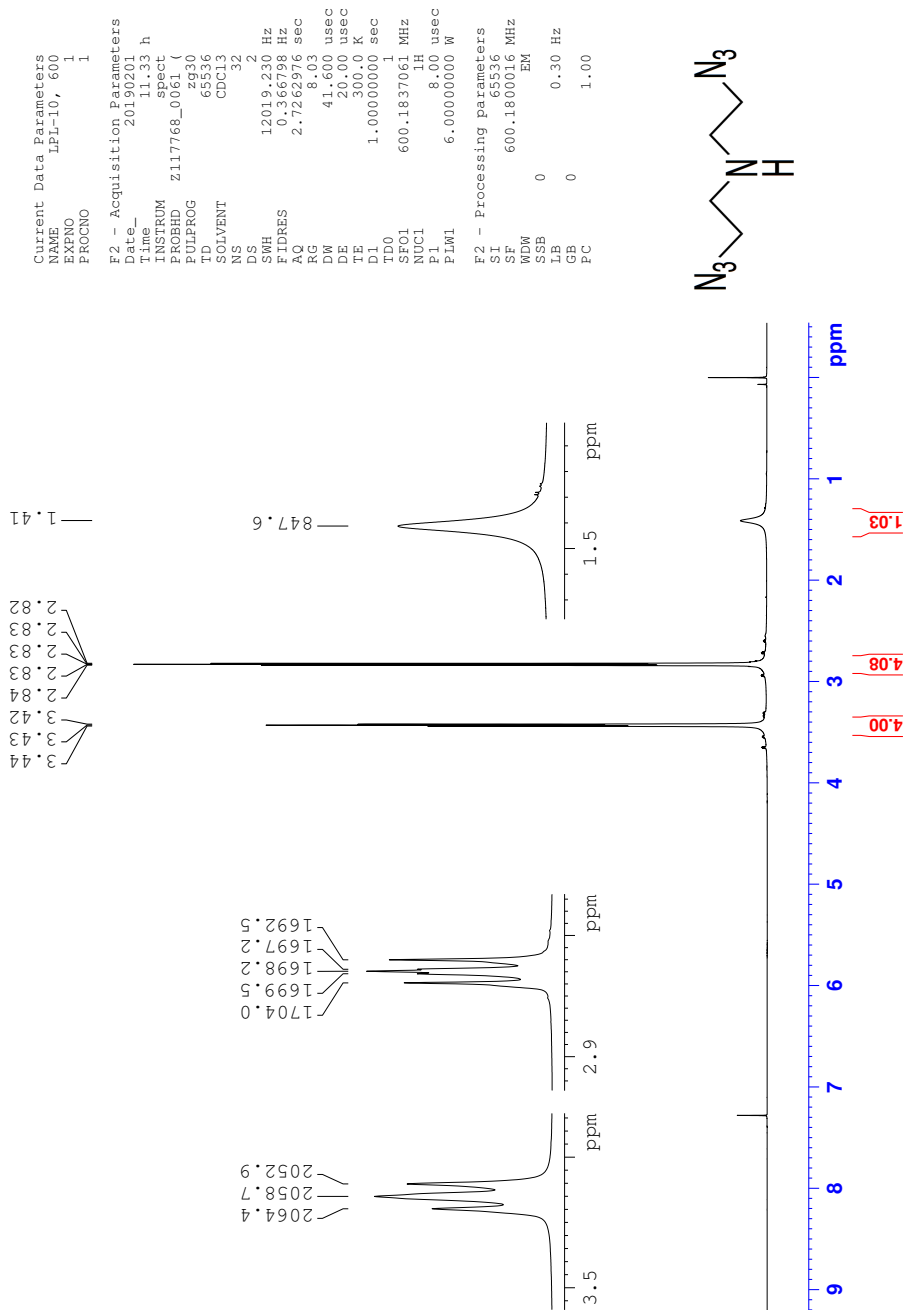
S.2 IR spectrum for 10
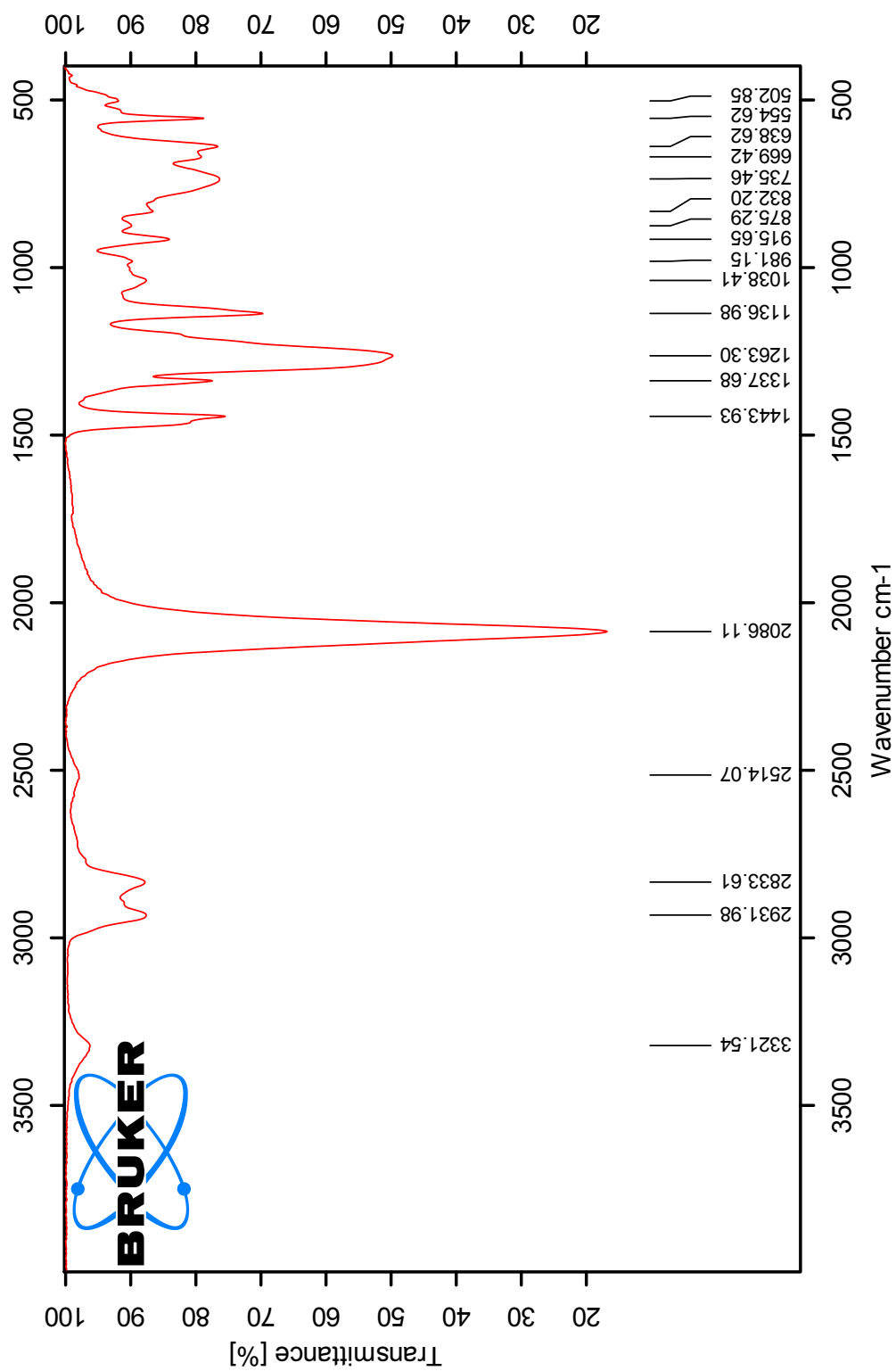
Current Data Parameters
NAME   LPl-11a(3)
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20190211
Time_   10.24
INSTRUM   spect
PROBHD   5 mm PABBO BB/
PULPROG   zg30
TD   65536
SOLVENT   CDCl3
NS   54
DS   2
SWH   8012.820 Hz
FIDRES   0.122266 Hz
AQ   4.0894465 sec
RG   209.8
DW   62.400 usec
DE   6.50 usec
TE   298.3 K
D1   1.00000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   400.1324710 MHz
NUC1   1H
P1   9.50 usec
PLW1   17.00000000 W

F2 - Processing parameters
SI   65536
SF   400.1300000 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

1.40

2.84
2.86
2.87
3.45
3.46
3.47
4.03

7.58
7.60
7.68
7.71
8.50

ppm 0 1 2 3 4 5 6 7 8 9 10 11 12 13
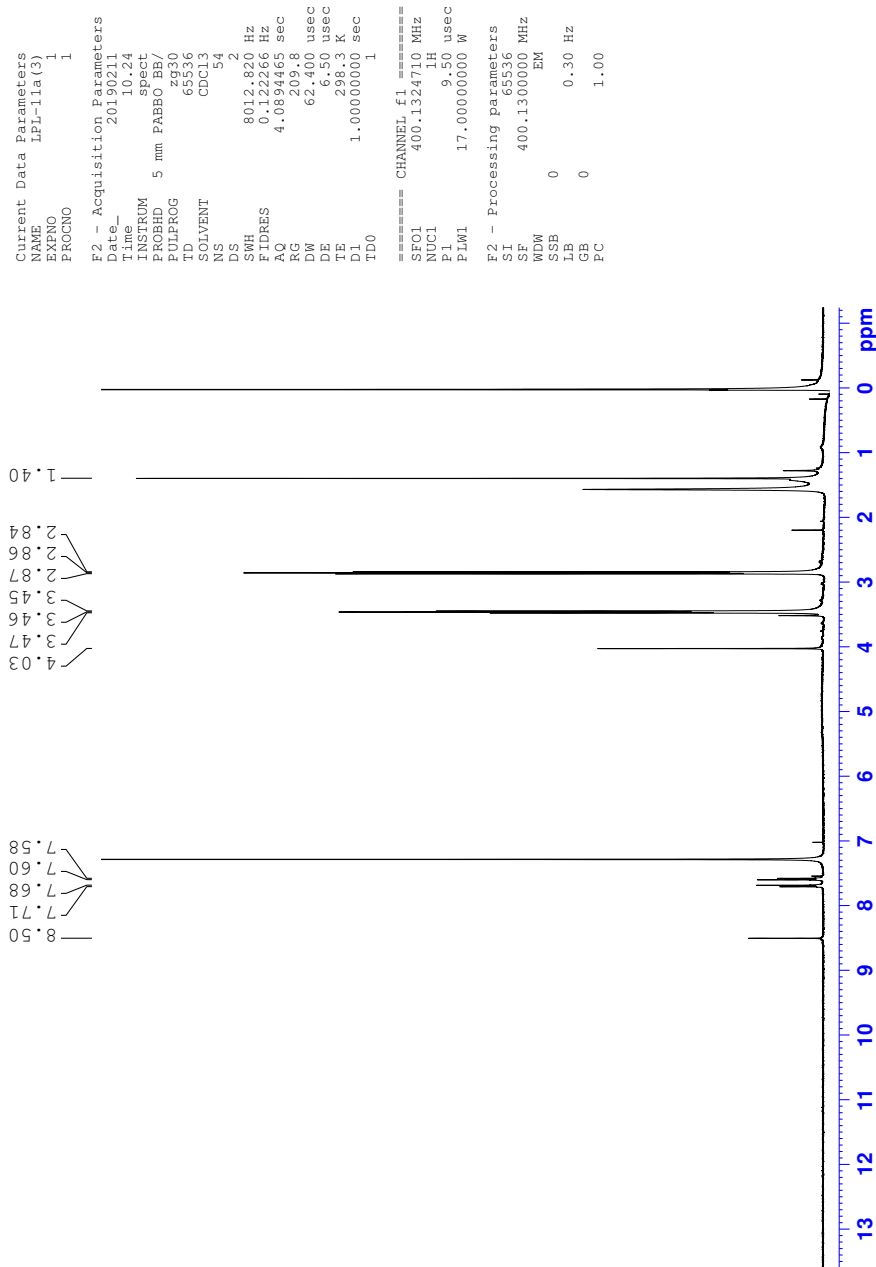
T.3 ^{1}**H NMR (400 MHz, CDCl$_3$) spectrum for the third attempted synthesis of 11a**
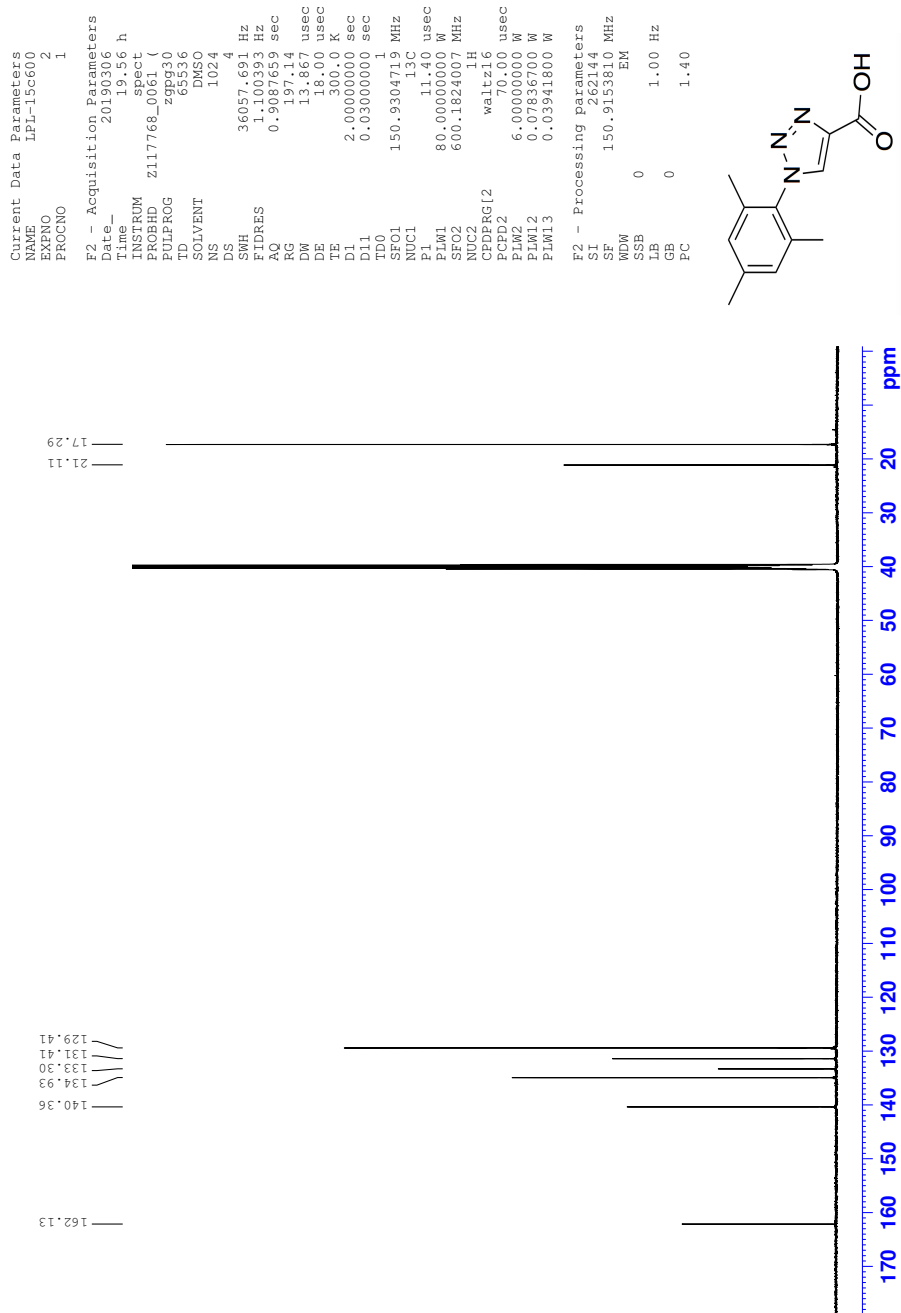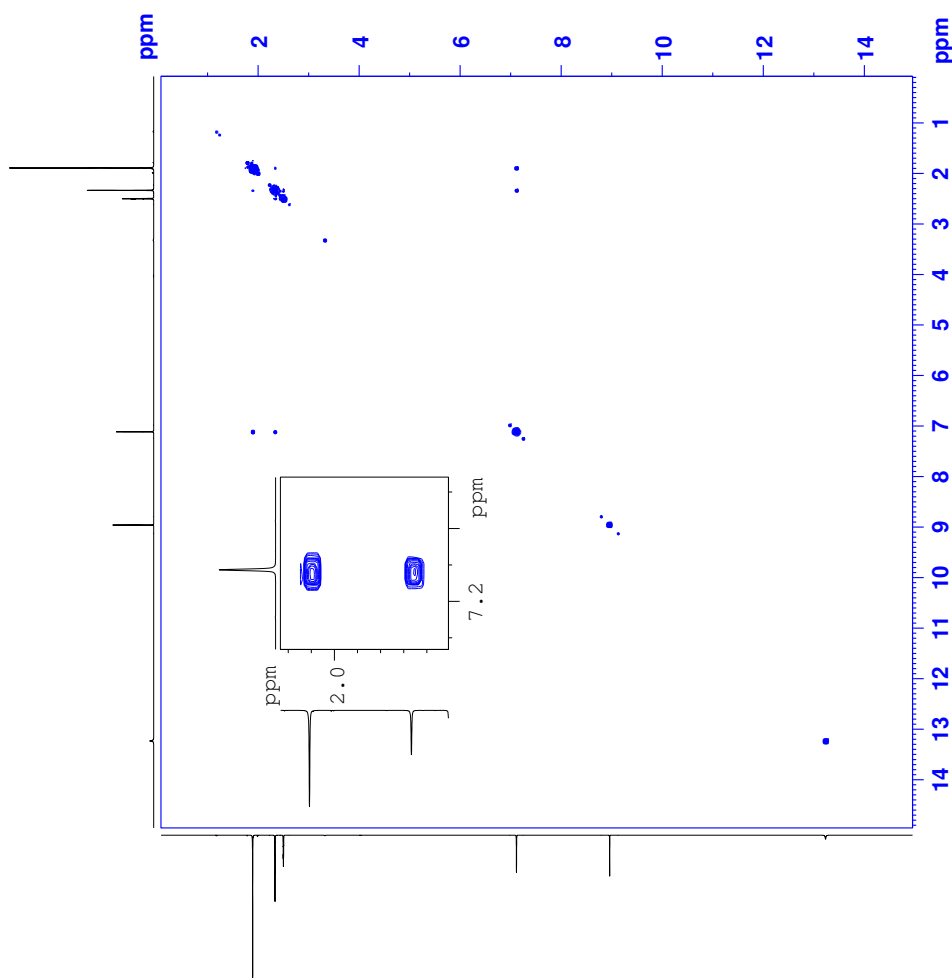
Current Data Parameters
NAME   LPL-11a(2)400
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20190204
Time   9.02
INSTRUM   spect
PROBHD   5 mm PABBO BB/
PULPROG   zg30
TD   65536
SOLVENT   CDCl3
NS   54
DS   2
SWH   8012.820 Hz
FIDRES   0.122266 Hz
AQ   4.0894465 sec
RG   209.8
DW   62.400 usec
DE   6.50 usec
TE   298.7 K
D1   1.00000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   400.1324710 MHz
NUC1   1H
P1   9.50 usec
PLW1   17.00000000 W

F2 - Processing parameters
SI   65536
SF   400.1300000 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

1.40
2.86
3.45
3.46
3.47
4.03
7.58
7.60
7.68
7.71
8.50
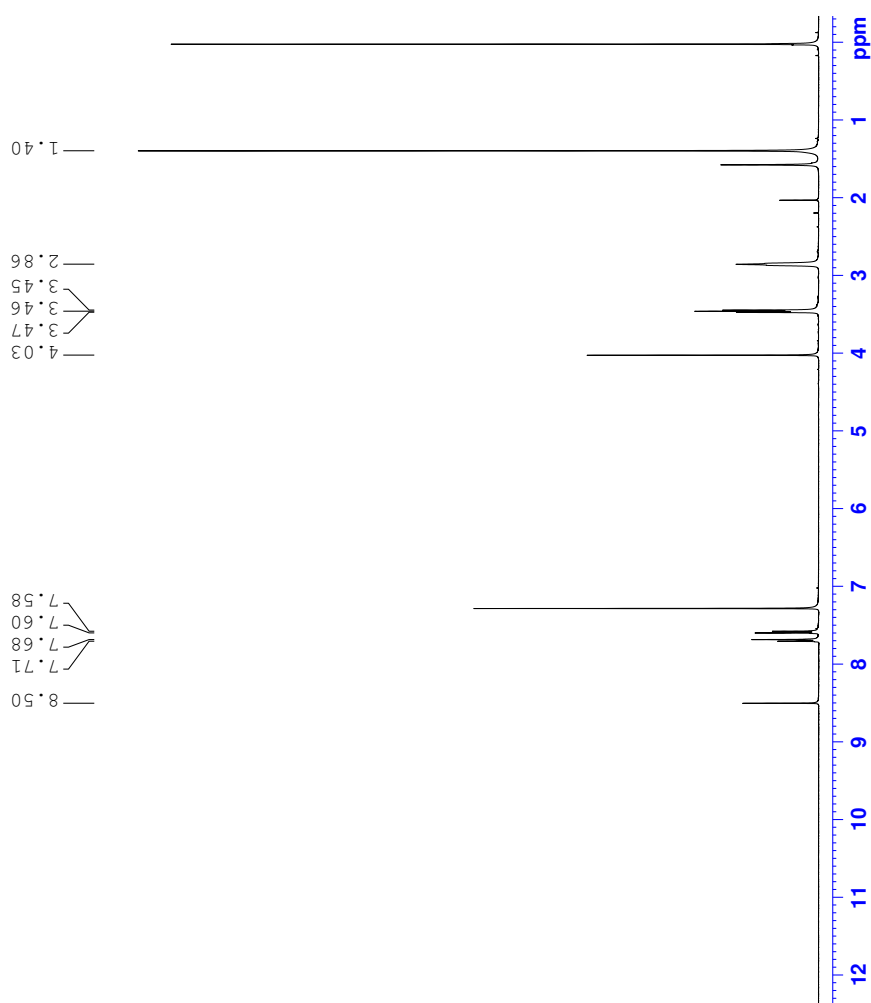
T.4 ^{1}H NMR (600 MHz, DMSO) spectrum for 11a
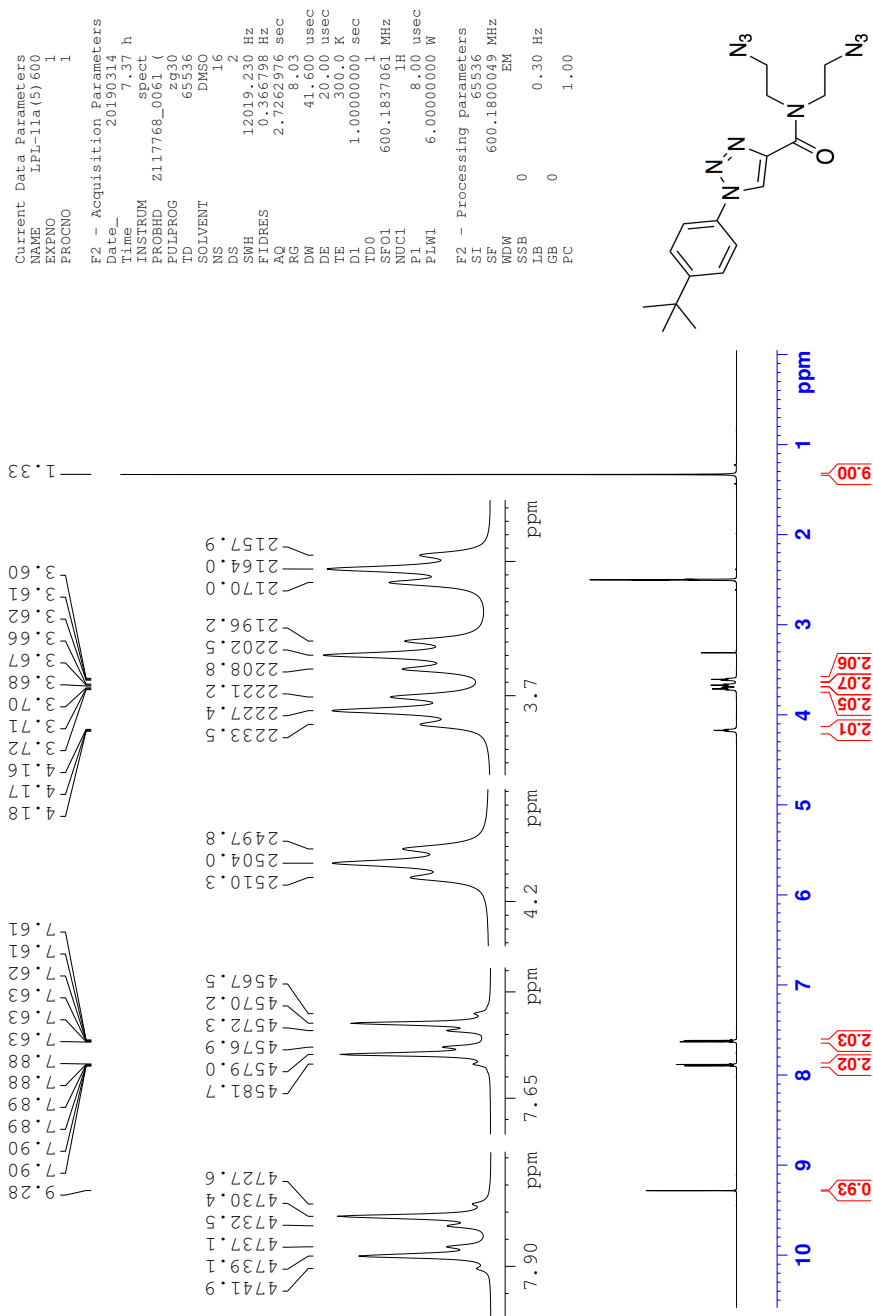
T.5 ^{13}**C NMR (150 MHz, DMSO) spectrum for 11a**

T.6 COSY (600 MHz, DMSO) spectrum for 11a

cxl

T.8 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 11a

T.9 IR spectrum for 11a
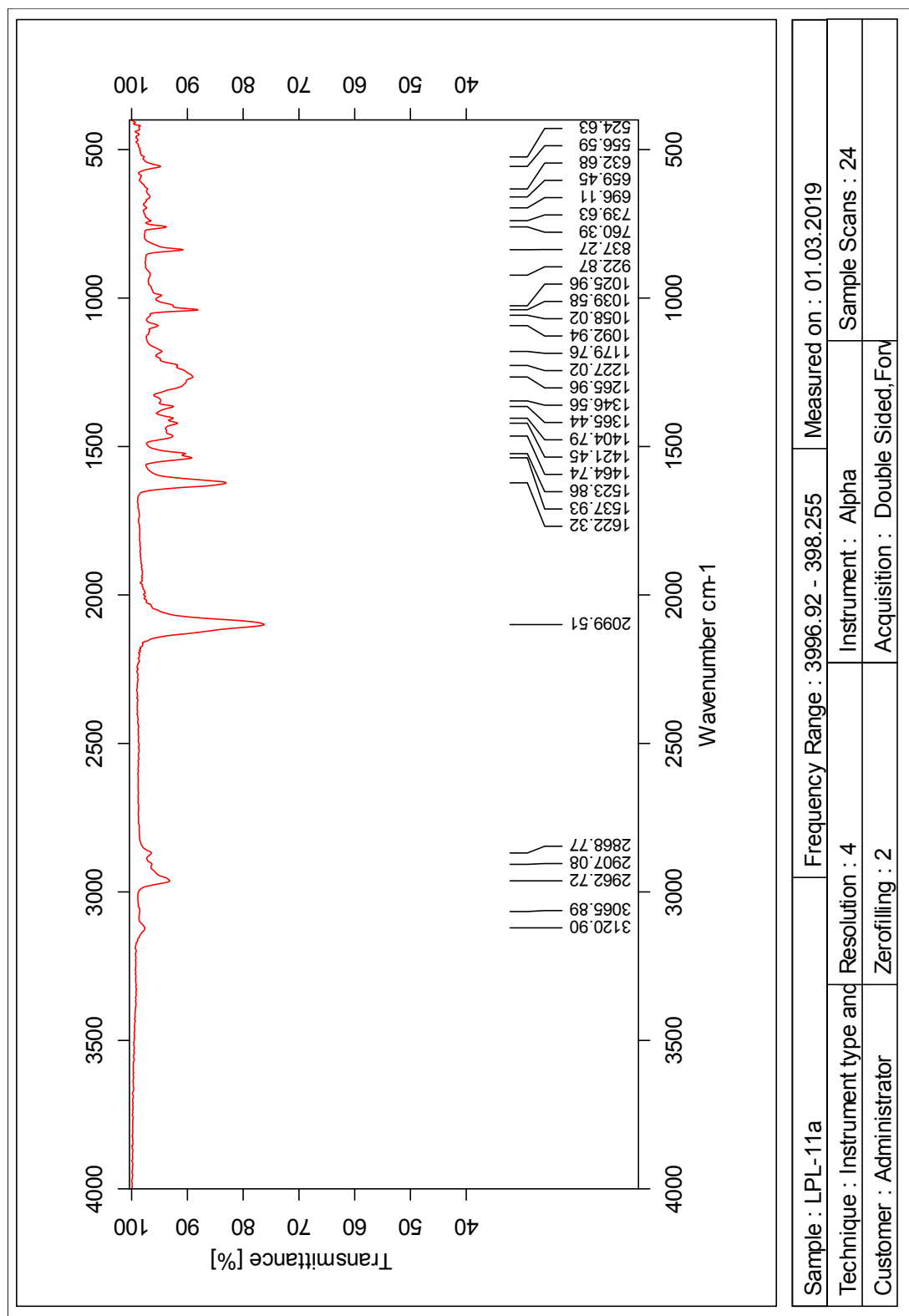
Frequency Range : 3996.92 - 398.255 | Measured on : 01.03.2019

Technique : Instrument type and | Resolution : 4 | Instrument : Alpha | Sample Scans : 24

Customer : Administrator | Zerofilling : 2 | Acquisition : Double Sided,Forv

T.10 HRMS spectrum for 11a

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
2089 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: 0-10   S: 0-4
2019-146 104 (2.033) AM2 (Ar,35000.0,0.00,0.00); Cm (78:106)
1: TOF MS ASAP+

1.05e+007
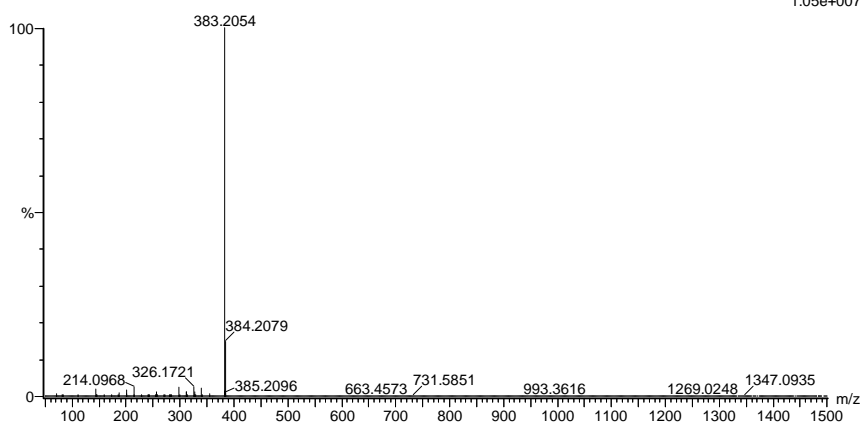
Minimum:				-2.0			
Maximum:		5.0	2.0	50.0			

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
383.2054	383.2052	0.2	0.5	5.5	1315.0	13.870	0.00	C17 H31 N6 S2
	383.2056	-0.2	-0.5	11.5	1301.1	0.000	100.00	C17 H23 N10 O

U.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 12*a

U.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 12*a

U.3 COSY (600 MHz, DMSO) spectrum for 12*a

U.4   HSQC (600 MHz / 150 MHz, DMSO) spectrum for 12*a

U.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 12*a

U.6 IR spectrum for 12*a

Sample : LPL-12asalt		Frequency Range : 3996.92 - 398.255	Measured on : 22.03.2019	
Technique : Instrument type and	Resolution : 4		Instrument : Alpha	Sample Scans : 24
Customer : Default	Zerofilling : 2		Acquisition : Double Sided,For	

U.7 HRMS spectrum for 12*a

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
1455 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   Au: 0-3

2019-418_RERUN_2 34 (0.636) AM2 (Ar,35000.0,0.00,0.00); Cm (34:38)
1: TOF MS ES+
6.25e+006
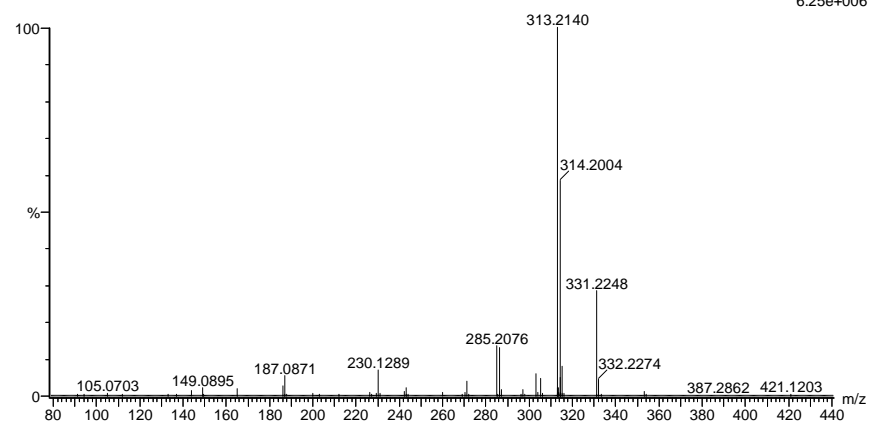
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-50.0				
Maximum:		5.0	2.0	50.0				
331.2248	331.2246	0.2	0.6	7.5	1249.8	n/a	n/a	C17 H27 N6 O

cl

U.8   HPLC chromatogram for 12*a

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_12ASALT5050.D
Sample Name: LPL-12asalt
======================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                          Location : Vial 5
 Injection Date  : 07.05.2019 19:22:48
                                                 Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 07.05.2019 19:07:14 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     : MeOH/H2O 50:50 + 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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
 ----|-------|----|-------|----------|----------|--------|
   1   4.793 BB   0.1425   10.83450    1.18712   1.5436
   2   5.375 BB   0.1678  691.05090   63.08109  98.4564

 Totals :                 701.88541   64.26821
```

cli

U.9   HPLC chromatogram for 12*a

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_12ASALT5050.D
Sample Name: LPL-12asalt
===================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                              Location : Vial 5
Injection Date  : 07.05.2019 19:22:48
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 19:07:14 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     : MeOH/H2O 50:50 + 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   4.796 BB    0.1363    10.67321    1.24166   1.1389
  2   5.374 BB    0.1677   926.49884   84.67239  98.8611

Totals :                   937.17205   85.91405


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

V.1 ^{1}H NMR (600 MHz, DMSO) spectrum for the crude of 14a
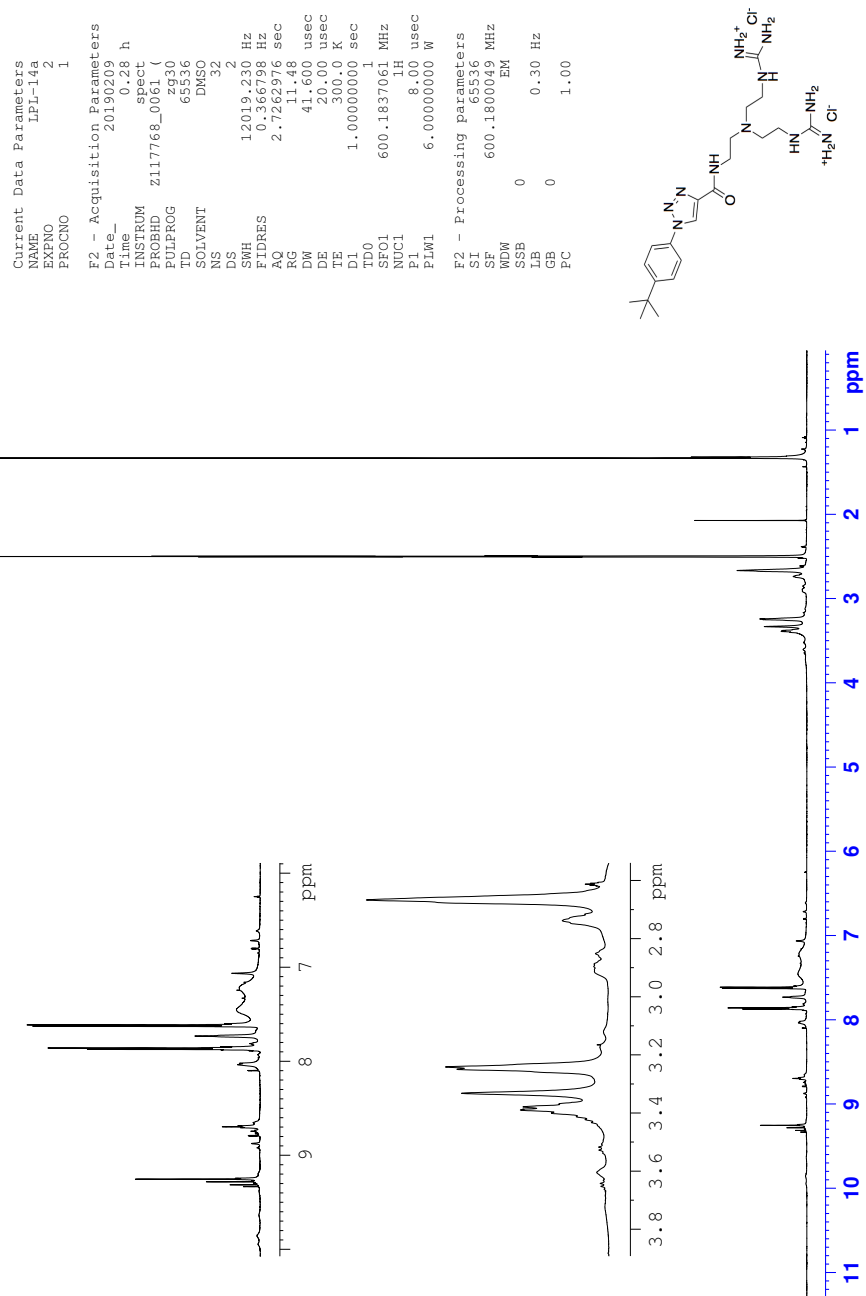
V.2 HRMS spectrum for the monocharged 14a

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
848 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500   H: 0-1000   N: 0-15   O: 0-10
svg_20190211_86 40 (0.750) AM2 (Ar,35000.0,0.00,0.00); Cm (40:41)
1: TOF MS ES+
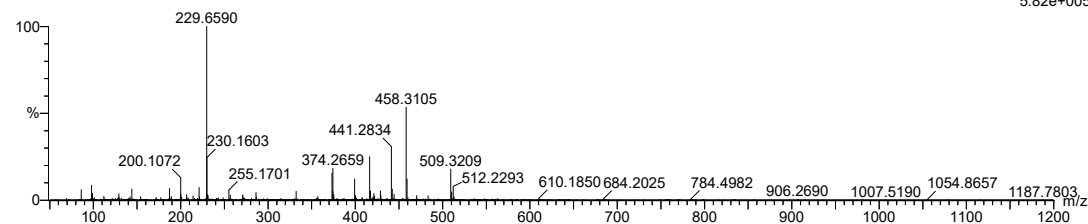
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-2.0				
Maximum:		5.0	5.0	50.0				
458.3105	458.3104	0.1	0.2	9.5	821.6	0.779	45.90	C21 H36 N11 O
	458.3118	-1.3	-2.8	3.5	824.9	4.061	1.72	C24 H44 N O7
	458.3091	1.4	3.1	4.5	821.5	0.647	52.37	C20 H40 N7 O5

V.3 HRMS spectrum for the bischarged 14a

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
865 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500   H: 0-1000   N: 0-15   O: 0-10
svg_20190211_86 40 (0.750) AM2 (Ar,35000.0,0.00,0.00); Cm (40:41)
1: TOF MS ES+

5.82e+005

```
100─           229.6590
                                              458.3105
 %─
                                        441.2834
            200.1072  230.1603      374.2659      509.3209
                        255.1701              512.2293  610.1850 684.2025   784.4982  906.2690   1007.5190  1054.8657  1187.7803
   0                                                                                                                              m/z
      100      200      300      400      500      600      700      800      900     1000     1100     1200
```
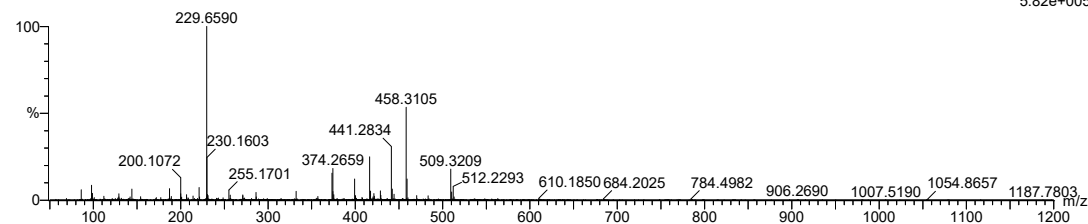
Minimum: -2.0
Maximum: 5.0   5.0   50.0

Mass   Calc. Mass   mDa   PPM   DBE   i-FIT   Norm   Conf(%) Formula
459.3180   459.3183   -0.3   -0.7   9.0   794.7   1.252 28.58   C21 H37 N11 O
            459.3169   1.1   2.4   4.0   793.9   0.450 63.75   C20 H41 N7 O5
            459.3196   -1.6   -3.5   3.0   796.0   2.568 7.67   C24 H45 N O7

clv

V.4 HRMS spectrum for 14'a

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
759 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: 0-15   O: 0-10
svg_20190211_86 40 (0.750) AM2 (Ar,35000.0,0.00,0.00); Cm (40:41)
1: TOF MS ES+

5.82e+005
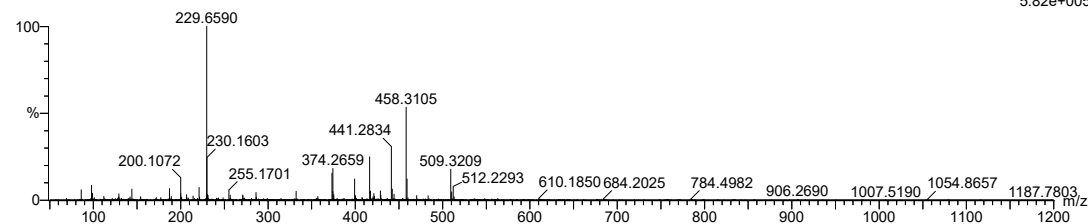

```
Minimum:                        -2.0
Maximum:             5.0   5.0   50.0

Mass       Calc. Mass  mDa   PPM   DBE   i-FIT   Norm   Conf(%)  Formula
416.2885   416.2886    -0.1  -0.2  8.5   828.2   0.555  57.40    C20 H34 N9 O
           416.2873    1.2   2.9   3.5   828.5   0.853  42.60    C19 H38 N5 O5
```

V.5 HPLC chromatogram for the crude of 14a after reaction with the base TEA

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20181203_LPL14A.D
Sample Name: LPL-14a
```

```
========================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                         Location : Vial 3
Injection Date  : 12.03.2019 13:12:11
                                               Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 12.03.2019 13:11:00 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 20.05.2019 09:43:48 by Kristine
                  (modified after loading)
Sample Info     : Isocratic 50/50 MeOH/H2O with 0.1%TFA in H2O, 1mL/min

Additional Info : Peak(s) manually integrated
```
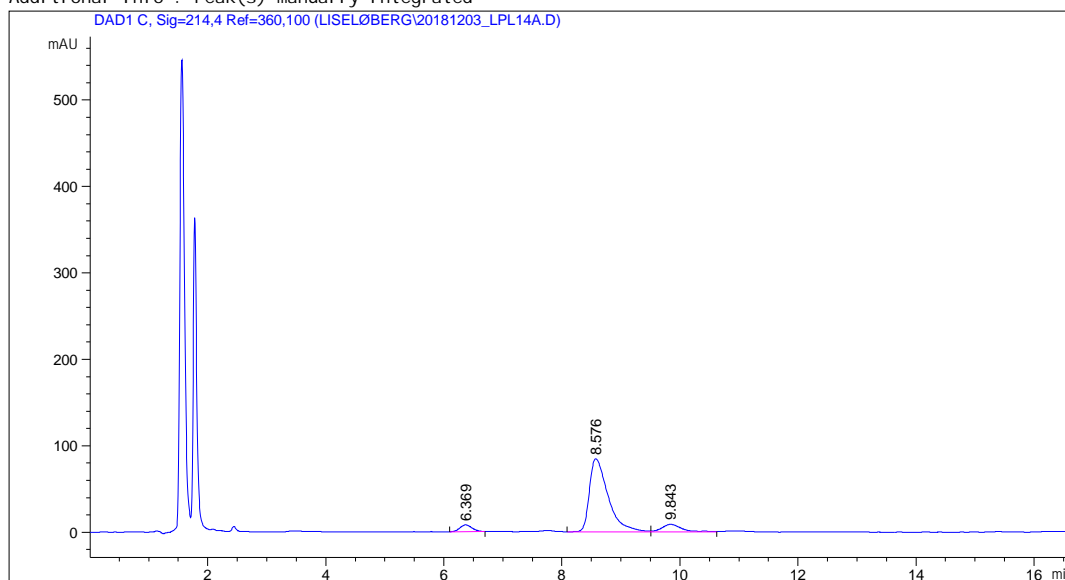


```
========================================================================
                       Area Percent Report
========================================================================

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


Signal 1: DAD1 C, Sig=214,4 Ref=360,100

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   6.369 BB   0.2220   111.48672    7.83749    5.1043
  2   8.576 BV   0.3326  1861.49976   84.64682   85.2268
  3   9.843 VV   0.3648   211.18640    8.77346    9.6689
```

W.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 15a

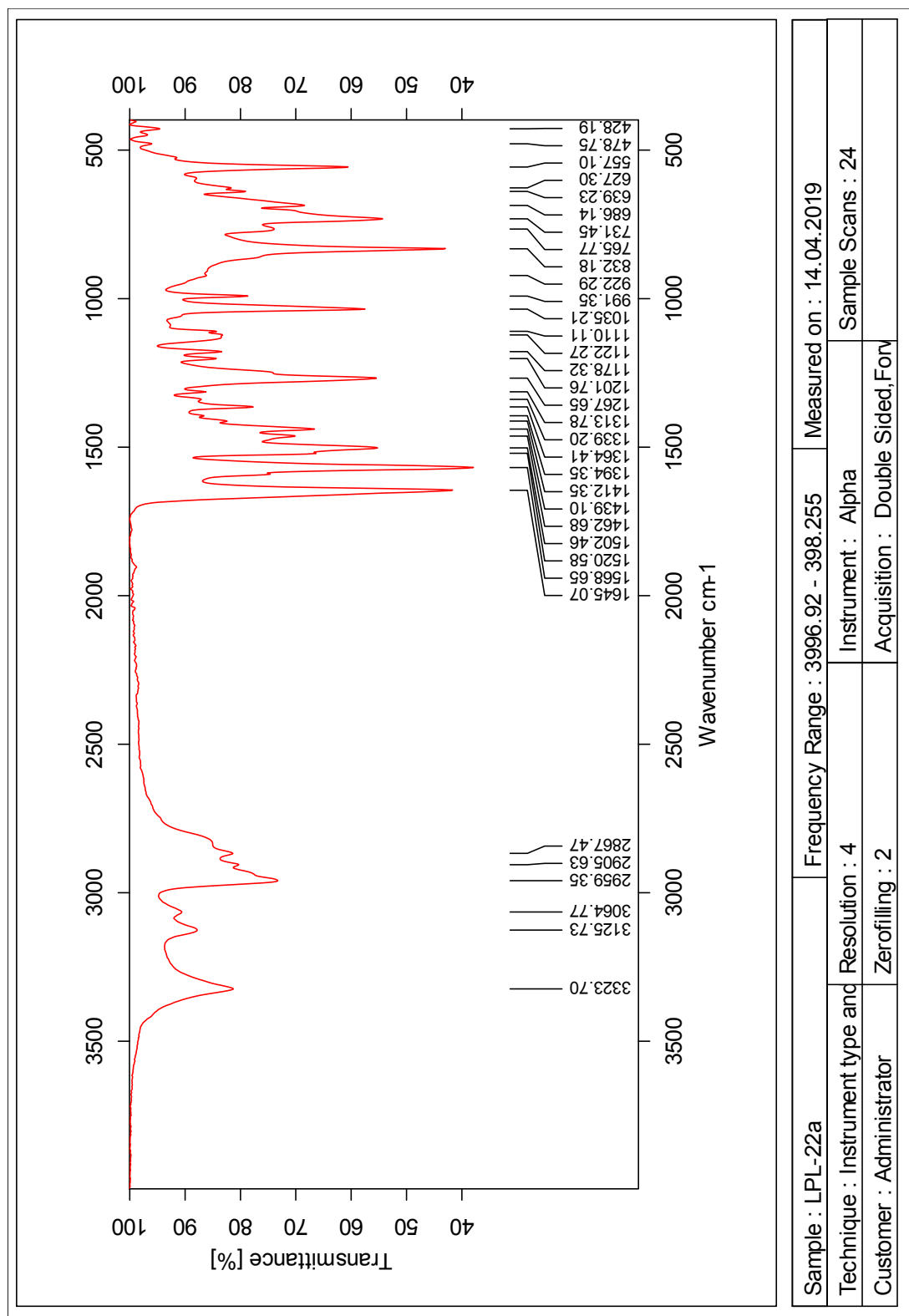# W.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 15a

Current Data Parameters
NAME   LPL-15a600
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20190223
Time   5.36 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   1024
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   298.1 K
D1   2.00000000 sec
D11   0.03000000 sec
TD0   1
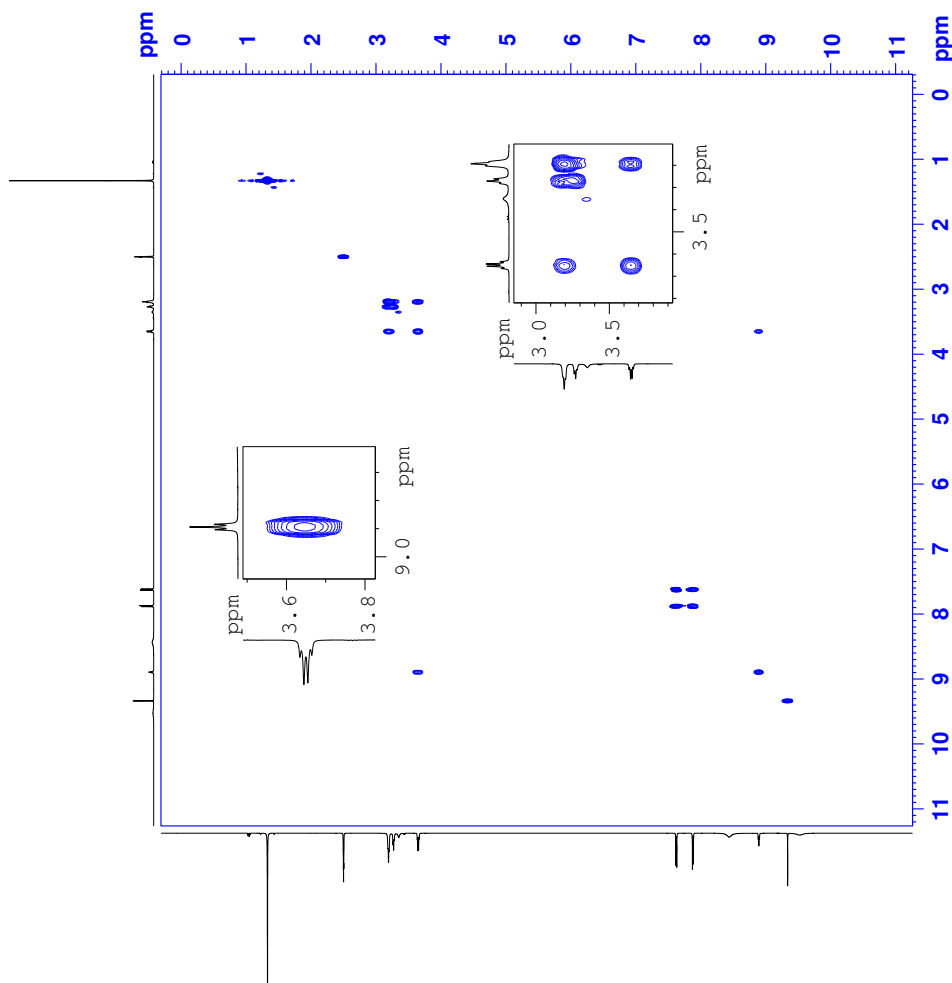SFO1   150.9304719 MHz
NUC1   13C
P1   11.40 usec
PLW1   80.00000000 W
SFO2   600.1824007 MHz
NUC2   1H
CPDPRG[2   waltz16
PCPD2   70.00 usec
PLW2   6.00000000 W
PLW12   0.07836700 W
PLW13   0.03941800 W

F2 - Processing parameters
SI   32768
SF   150.9154526 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

W.3 COSY (600 MHz, DMSO) spectrum for 15a

W.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 15a

W.6 IR spectrum for 15a

Peaks (Wavenumber cm-1): 412.49, 435.17, 456.17, 470.33, 483.73, 549.13, 572.35, 594.41, 699.88, 736.29, 767.17, 781.03, 838.30, 858.58, 909.99, 987.41, 1109.02, 1162.30, 1169.71, 1197.64, 1247.01, 1273.88, 1313.08, 1330.37, 1347.32, 1361.74, 1369.05, 1395.57, 1420.54, 1452.31, 1462.67, 1479.37, 1516.38, 1533.17, 1549.79, 1692.15, 2568.20, 2639.36, 2870.12, 2907.65, 2958.70, 3127.36

Sample : LPI-15a(2)		Frequency Range : 3996.92 - 398.255	Measured on : 13.03.2019
Technique : Instrument type and	Resolution : 4	Instrument : Alpha	Sample Scans : 24
Customer : Administrator	Zerofilling : 2	Acquisition : Double Sided,For|	

clxiii

W.7   HRMS spectrum for 15a

Single Mass Analysis
Tolerance = 12.0 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
179 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: 0-10
2019-145POS 64 (1.257) AM2 (Ar,35000.0,0.00,0.00); Cm (61:64)
1: TOF MS ASAP+
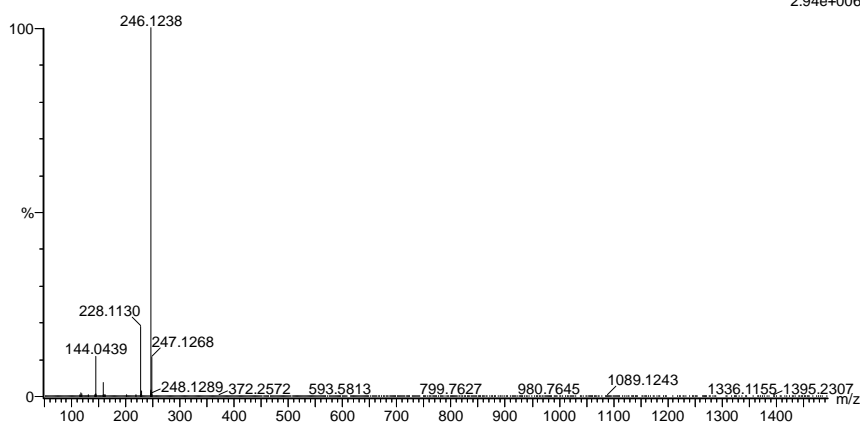
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-2.0				
Maximum:		5.0	12.0	50.0				
246.1238	246.1243	-0.5	-2.0	7.5	1508.6	n/a	n/a	C13 H16 N3 O2

clxiv

X.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 15c

Current Data Parameters
NAME   LPl-15c600
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20190306
Time   19.56 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   1024
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   2.00000000 sec
D11   0.03000000 sec
TD0   1
SFO1   150.9304719 MHz
NUC1   13C
P1   11.40 usec
PLW1   80.00000000 W
SFO2   600.1824007 MHz
NUC2   1H
CPDPRG[2   waltz16
PCPD2   70.00 usec
PLW2   6.00000000 W
PLW12   0.07836700 W
PLW13   0.03941800 W

F2 - Processing parameters
SI   262144
SF   150.9153810 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

17.29
21.11

129.41
131.41
133.30
134.93
140.36

162.13

ppm 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170

X.3 COSY (600 MHz, DMSO) spectrum for 15c

X.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 15c

X.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 15c

X.6   IR spectrum for 15c

X.7 HRMS spectrum for 15c
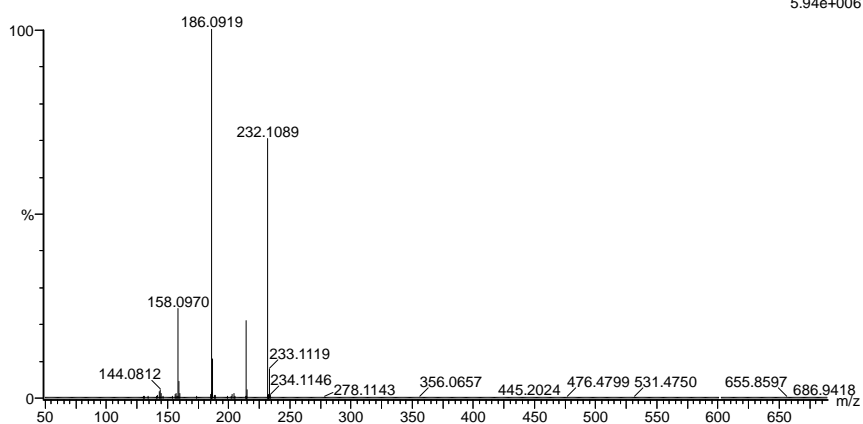
```
Minimum:                        -2.0
Maximum:              5.0   2.0   50.0

Mass      Calc. Mass  mDa   PPM   DBE   i-FIT   Norm   Conf(%) Formula

232.1089  232.1086    0.3   1.3   7.5   1650.8  n/a    n/a     C12 H14 N3 O2
```

Y.1 ^{1}H NMR (400 MHz, CDCl$_3$) spectrum for 16a
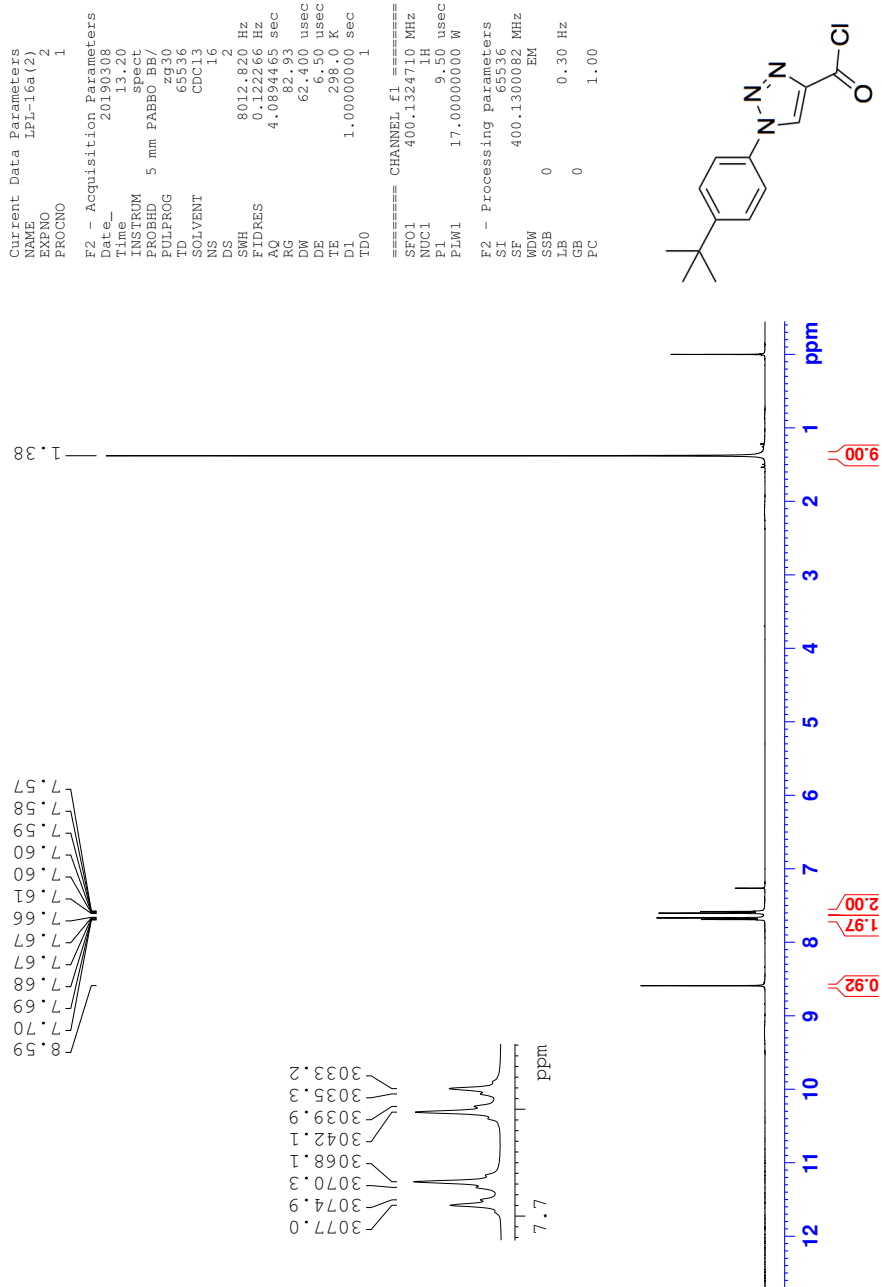
Y.2 ^{13}C NMR (100 MHz, CDCl$_3$) spectrum for 16a

Current Data Parameters
NAME   LPL-16a(2)
EXPNO   3
PROCNO   1

F2 - Acquisition Parameters
Date_   20190308
Time   17.08
INSTRUM   spect
PROBHD   5 mm PABBO BB/
PULPROG   zgpg36
TD   65536
SOLVENT   CDCl3
NS   1024
DS   4
SWH   24038.461 Hz
FIDRES   0.366798 Hz
AQ   1.3631488 sec
RG   209.8
DW   20.800 usec
DE   6.50 usec
TE   298.0 K
D1   2.00000000 sec
D11   0.03000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   100.6228293 MHz
NUC1   13C
P1   9.50 usec
PLW1   71.00000000 W

======== CHANNEL f2 ========
SFO2   400.1316605 MHz
NUC2   1H
CPDPRG[2   waltz16
PCPD2   90.00 usec
PLW2   17.00000000 W
PLW12   0.18941000 W
PLW13   0.15343000 W

F2 - Processing parameters
SI   32768
SF   100.6127697 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40
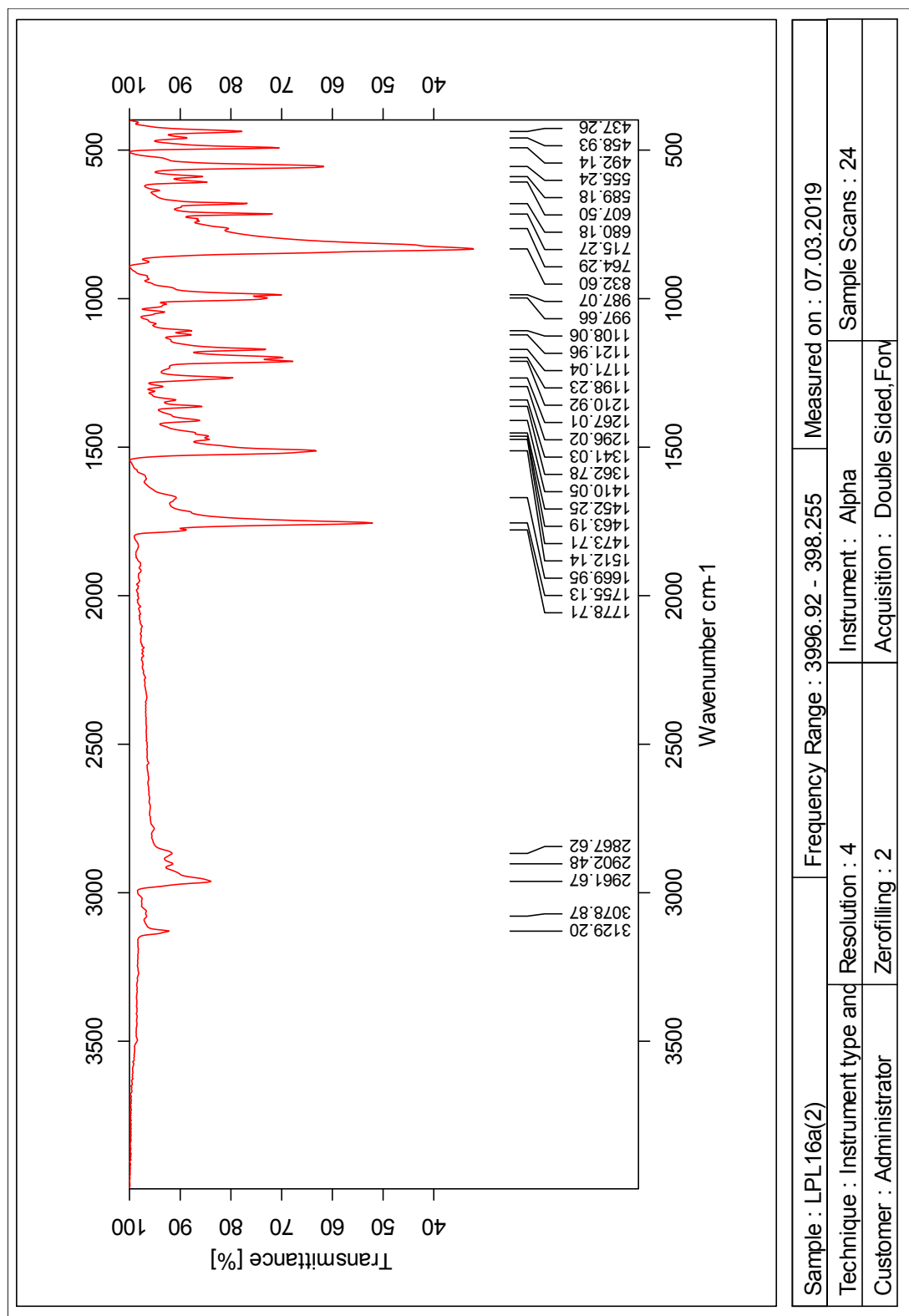
31.20
34.96
120.65
127.03
133.40
143.36
153.78
159.26

200   180   160   140   120   100   80   60   40   20   0   ppm

clxxiii

Y.3   IR spectrum for 16a

Z.1 ^{1}H NMR (400 MHz, DMSO) spectrum for 19

Z.2 HRMS spectrum for 19
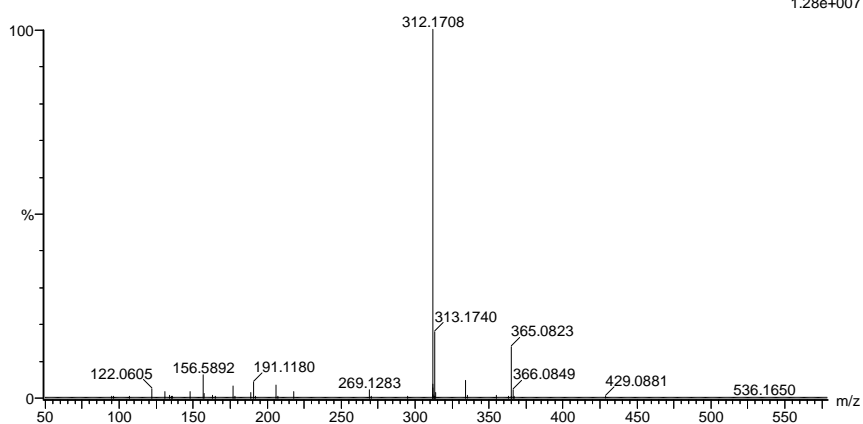
```
Minimum:                        -50.0
Maximum:                5.0     2.0      50.0

Mass        Calc. Mass  mDa    PPM     DBE     i-FIT   Norm    Conf(%) Formula

312.1708    312.1712    -0.4   -1.3    9.5     1582.1  n/a     n/a     C18 H22 N3 O2
```

AA.1 ^{1}H NMR (400 MHz, DMSO) spectrum for the crude of 20a

AA.2   HRMS spectrum for 20a

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
1431 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500   H: 0-1000   N: 0-10   O: 0-10   Na: 0-1
2019_301_fia 36 (0.412) AM2 (Ar,35000.0,0.00,0.00); Cm (32:36)
1: TOF MS ES+
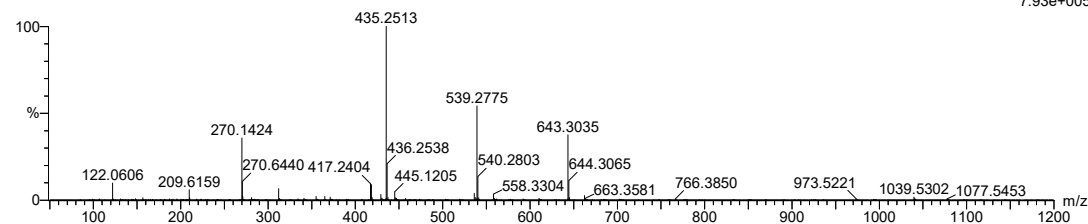

```
Minimum:                        -2.0
Maximum:             5.0   2.0   50.0

Mass        Calc. Mass  mDa   PPM   DBE   i-FIT   Norm   Conf(%)  Formula
539.2775    539.2773    0.2   0.4   13.5  759.3   3.912  2.00     C33 H40 O5 Na
            539.2771    0.4   0.7   17.5  757.4   1.996  13.59    C31 H35 N6 O3
            539.2765    1.0   1.9   1.5   755.6   0.170  84.41    C17 H40 N8 O10 Na
```

AB.1 ^{1}H NMR (600 MHz, DMSO) spectrum for 22a

AB.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 22a

AB.3 COSY (600 MHz, DMSO) spectrum for 22a

AB.4   HSQC (600 MHz / 150 MHz, DMSO) spectrum for 22a

AB.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 22a

AB.6   IR spectrum for 22a

AB.7   HRMS spectrum for 22a

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
3757 formula(e) evaluated with 3 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   S: 0-3
2019-343 177 (3.465) AM2 (Ar,35000.0,0.00,0.00); Cm (172:179)
1: TOF MS ASAP+

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
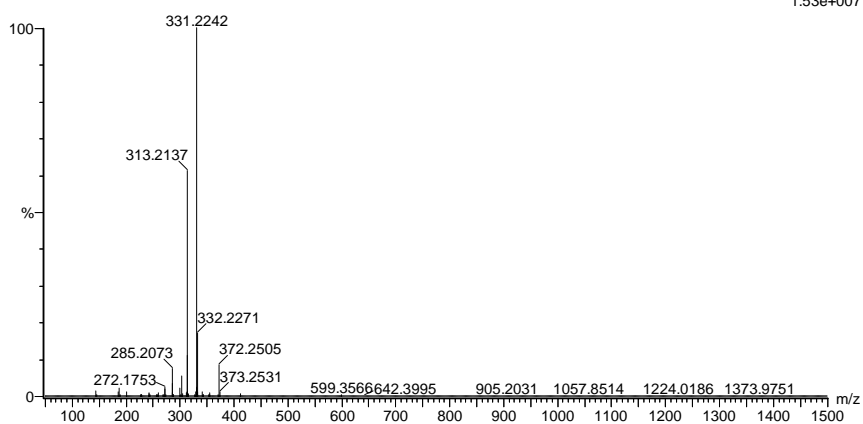
Minimum: -50.0
Maximum: 5.0   2.0   50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
331.2242	331.2242	0.0	0.0	1.5	1520.6	16.288	0.00	C17 H35 N2 S2
	331.2240	0.2	0.6	-1.5	1518.6	14.317	0.00	C9 H31 N8 O3 S
	331.2246	-0.4	-1.2	7.5	1504.3	0.000	100.00	C17 H27 N6 O

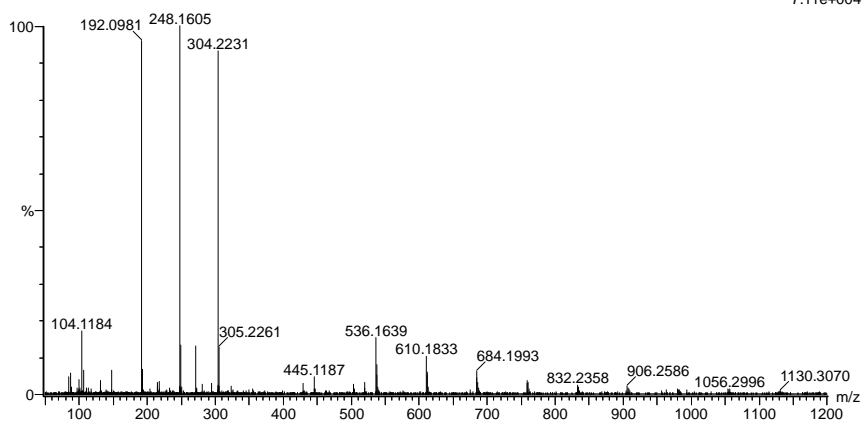# AC.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 22*a
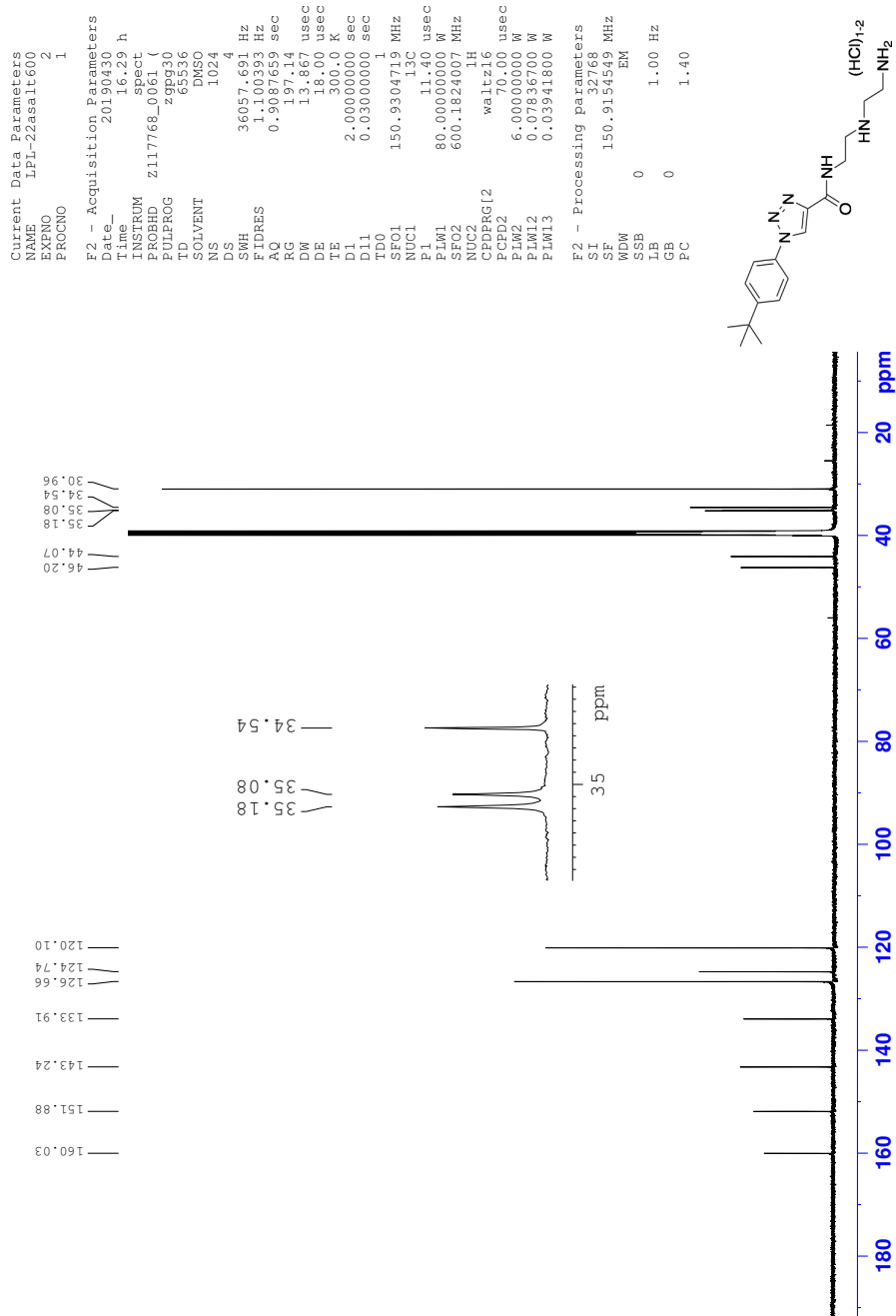
Current Data Parameters
NAME   LPL-22asalt600
EXPNO   2
PROCNO   1

F2 - Acquisition Parameters
Date_   20190430
Time   16.29 h
INSTRUM   spect
PROBHD   Z117768_0061 (
PULPROG   zgpg30
TD   65536
SOLVENT   DMSO
NS   1024
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   2.00000000 sec
D11   0.03000000 sec
TD0   1
SFO1   150.9304719 MHz
NUC1   13C
P1   11.40 usec
PLW1   80.00000000 W
SFO2   600.1824007 MHz
NUC2   1H
CPDPRG[2   waltz16
CPCD2   70.00 usec
PLW2   6.00000000 W
PLW12   0.07836700 W
PLW13   0.03941800 W

F2 - Processing parameters
SI   32768
SF   150.9154549 MHz
WDW   EM
SSB   0
LB   1.00 Hz
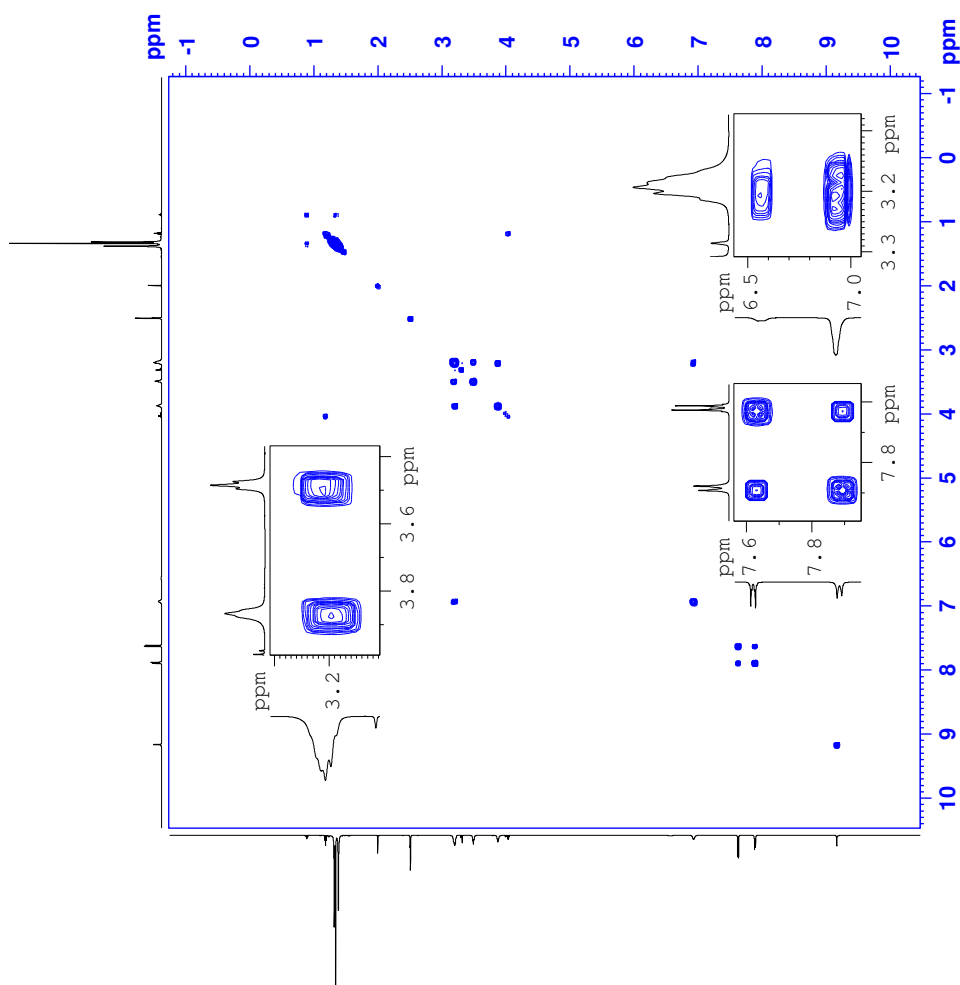GB   0
PC   1.40

clxxxvii

AC.3 COSY (600 MHz, DMSO) spectrum for 22*a

AC.4 HSQC (600 MHz / 150 MHz, DMSO) spectrum for 22*a

AC.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 22*a

AC.6   IR spectrum for 22*a

Sample : LPL-22asalt

Technique : Instrument type and Resolution : 4

Customer : Administrator

Frequency Range : 3996.92 - 398.255

Zerofilling : 2

Instrument : Alpha

Acquisition : Double Sided,For

Measured on : 09.05.2019

Sample Scans : 24

AC.7 HRMS spectrum for 22*a

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
	Minimum:			-50.0				
	Maximum:	5.0	2.0	50.0				
331.2240	331.2241	-0.1	-0.3	-8.5	1276.5	4.769	0.85	C3 H32 N8 O8 Na
	331.2246	-0.6	-1.8	7.5	1271.7	0.009	99.15	C17 H27 N6 O

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_22ASALT5050.D
Sample Name: LPL-22asalt
===================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                         Location : Vial 6
 Injection Date  : 07.05.2019 19:33:44
                                                Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 07.05.2019 19:32:30 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     : MeOH/H2O 50:50 + 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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
   1   9.703 BB   0.2800  645.81854   35.54591 100.0000

 Totals :                 645.81854   35.54591
```

AC.9 HPLC chromatogram for 22*a

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190507_22ASALT5050.D
Sample Name: LPL-22asalt
====================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                        Location : Vial 6
Injection Date  : 07.05.2019 19:33:44
                                              Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 07.05.2019 19:32:30 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     : MeOH/H2O 50:50 + 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 Type  Width     Area      Height     Area
 #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   9.703 BB   0.2777  948.43042   52.27547 100.0000

Totals :                 948.43042   52.27547


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

AD.1 ^{1}H NMR (400 MHz, CDCl$_3$) spectrum for 23

AD.2   HRMS spectrum for 23

```
Minimum:                          -50.0
Maximum:              5.0    2.0    50.0

Mass       Calc. Mass   mDa    PPM    DBE    i-FIT   Norm   Conf(%) Formula

304.2231   304.2230    0.1    0.3   -7.5   680.0   15.018 0.00    C6 H34 N5 O6 S
           304.2236   -0.5   -1.6   1.5    664.9   0.000  100.00  C14 H30 N3 O4
           304.2225    0.6    2.0   -13.5  684.4   19.410 0.00    C6 H42 N O5 S3
```

cxcvi

AE.1 ¹H NMR (600 MHz, DMSO) spectrum for 24a

AE.2 ^{13}C NMR (150 MHz, DMSO) spectrum for 24a

AE.3 COSY (600 MHz, DMSO) spectrum for 24a

AE.4   HSQC (600 MHz / 150 MHz, DMSO) spectrum for 24a

AE.5 HMBC (600 MHz/ 150 MHz, DMSO) spectrum for 24a

AE.6   IR spectrum for 24a

Sample : LPL-24a		Frequency Range : 3996.92 - 398.255	Measured on : 09.05.2019	
Technique : Instrument type and	Resolution : 4	Instrument : Alpha		Sample Scans : 24
Customer : Administrator	Zerofilling : 2	Acquisition : Double Sided,Forw		

AE.7 HRMS spectrum for 24a

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
3035 formula(e) evaluated with 3 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   Na: 0-1
2019-364 28 (0.533) AM2 (Ar,35000.0,0.00,0.00); Cm (28)
1: TOF MS ES+
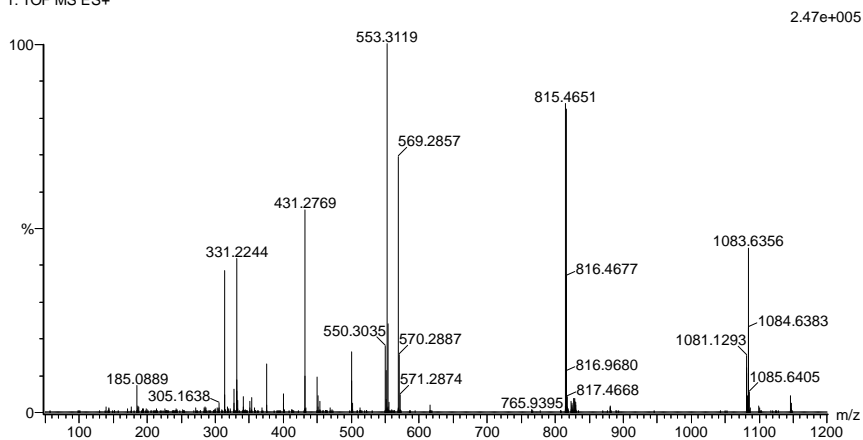

```
Minimum:                        -50.0
Maximum:            5.0    2.0    50.0

Mass       Calc. Mass  mDa    PPM    DBE    i-FIT   Norm    Conf(%)  Formula

553.3119   553.3114    0.5    0.9    9.5    619.4   0.351   70.37    C27 H42 N6 O5
                                                                     Na
           553.3125    -0.6   -1.1   7.5    621.3   2.301   10.02    C28 H45 N2 O9
           553.3128    -0.9   -1.6   14.5   620.7   1.629   19.61    C28 H38 N10 O
                                                                     Na
```

AF.1 ^{1}H NMR (400 MHz, DMSO) spectrum for the first attempted synthesis of 12a
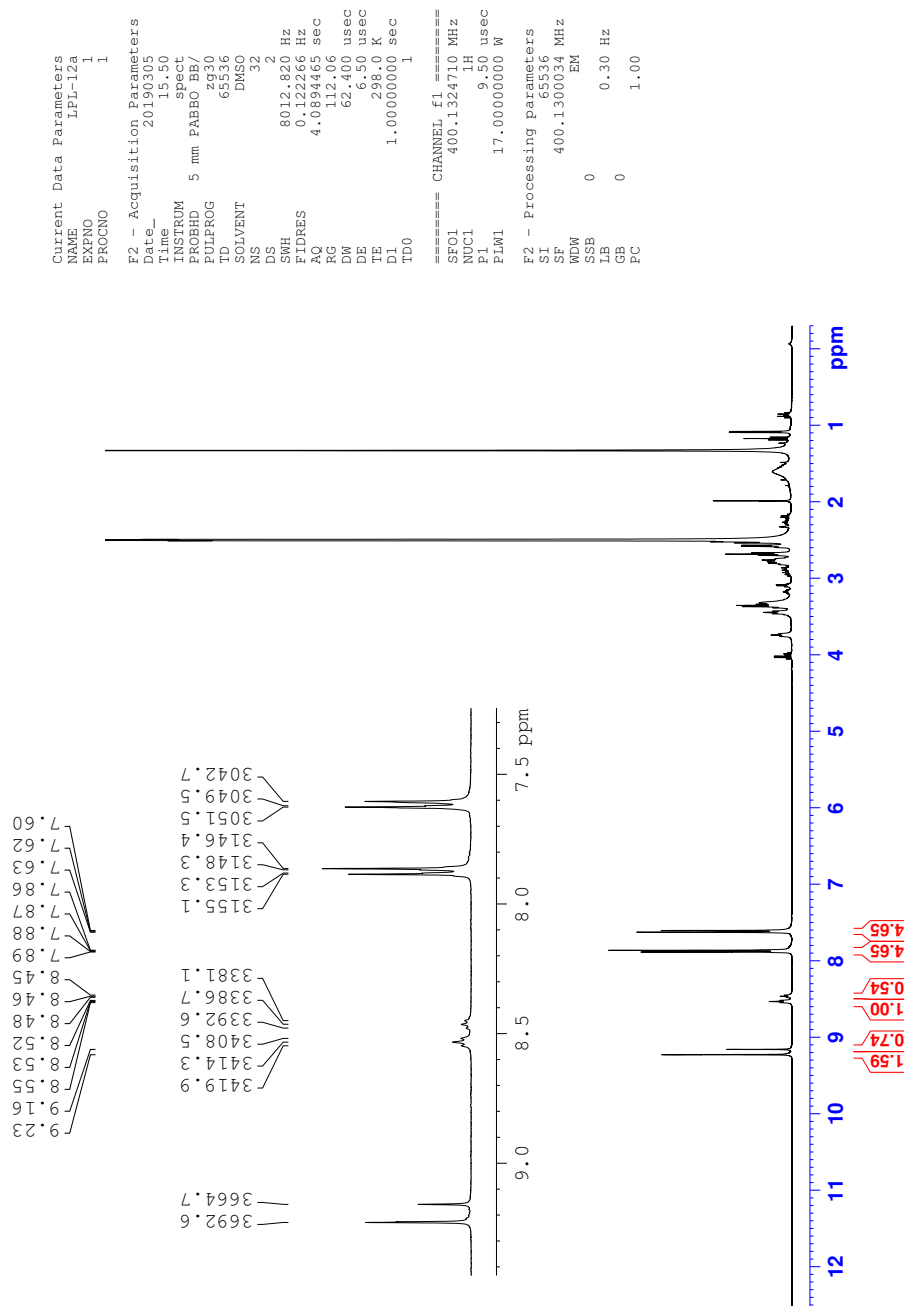
AF.2 HRMS spectrum for the first attempted synthesis of 12a

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
823 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: 0-10   Na: 0-1
2019-162 112 (2.188) AM2 (Ar,35000.0,0.00,0.00); Cm (105:112)
1: TOF MS ASAP+

8.67e+006
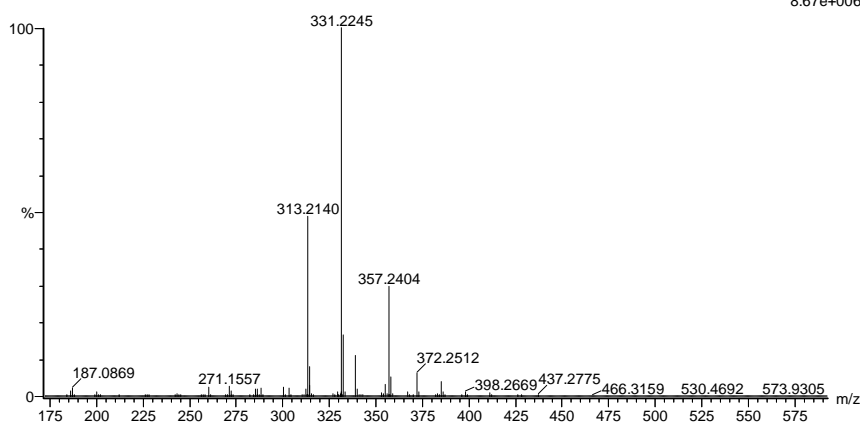
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-2.0				
Maximum:		5.0	2.0	50.0				
331.2245	331.2246	-0.1	-0.3	7.5	1540.6	0.014	98.64	C17 H27 N6 O
	331.2249	-0.4	-1.2	3.5	1544.9	4.298	1.36	C19 H32 O3 Na

ccv

AF.3 ^{1}H NMR (400 MHz, DMSO) spectrum for the second attempted synthesis of 12a

AF.5 ^{13}C NMR (150 MHz, DMSO) spectrum for the attempted synthesis of 12*a after Staudinger reaction

IKJ

AF.9 HRMS spectrum for the attempted synthesis of 12*a after Staudinger reaction

Elemental Composition Report

Page 1

Single Mass Analysis
Tolerance = 3.0 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
516 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
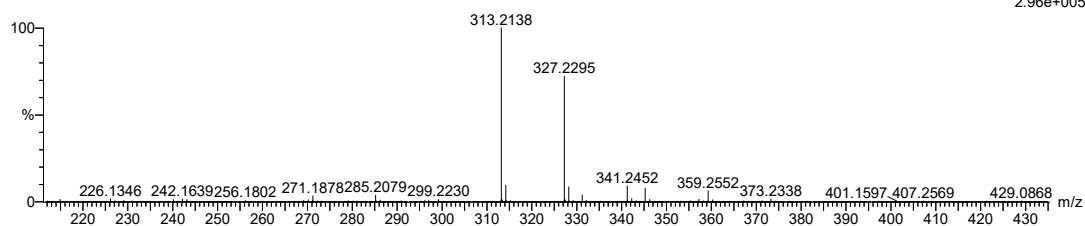Elements Used:
C: 0-500   H: 0-1000   N: 0-10   O: 0-5   Na: 0-1

svg_20190321_2019_221 32 (0.602) AM2 (Ar,35000.0,0.00,0.00); Cm (32:36)
1: TOF MS ES+

2.96e+005


```
Minimum:                      -2.0
Maximum:            5.0   3.0   50.0

Mass       Calc. Mass  mDa   PPM   DBE   i-FIT   Norm   Conf(%)  Formula
331.2240   331.2246   -0.6  -1.8  7.5   574.7   3.073  4.63     C17 H27 N6 O
           331.2233    0.7   2.1  2.5   571.7   0.069  93.34    C16 H31 N2 O5
           331.2249   -0.9  -2.7  3.5   575.5   3.896  2.03     C19 H32 O3 Na
```

ccxii

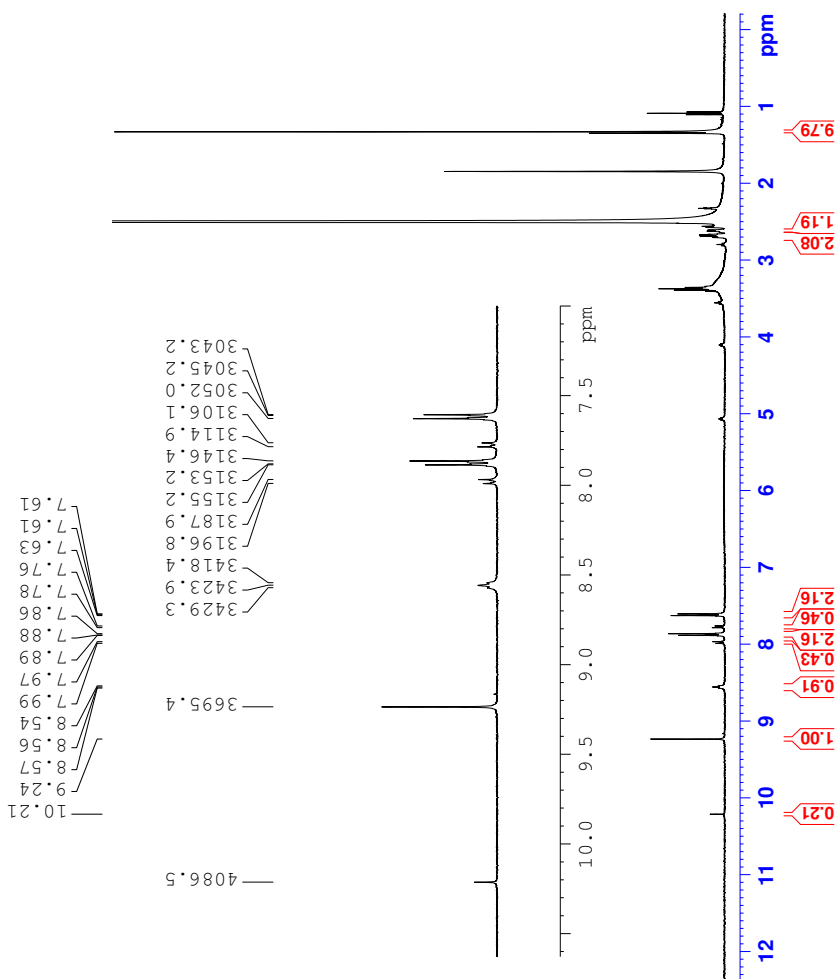# AF.10 ^{1}H NMR (400 MHz, DMSO) spectrum for the third attempted synthesis of 12a

AF.12 ^{1}H NMR (400 MHz, DMSO) spectrum for the fifth attempted synthesis of 12a

AG.1 HPLC chromatogram for the blank sample with MeOH/H$_2$O: 50/50 as eluent.

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190326_BLANK5050.D
Sample Name: Blank

=====================================================================
Acq. Operator   : Lise
Acq. Instrument : UPLC                          Location : Vial 1
Injection Date  : 26.03.2019 14:26:39
                                                Inj Volume : 2.000 µl
Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
Last changed    : 26.03.2019 14:25:28 by Lise
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\ED-MASTER\H2O SVAK 2.M
Last changed    : 26.03.2019 14:03:25 by ED
                  (modified after loading)
Sample Info     : Isocratic 50/50 MeOH/H2O with 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

No peaks found

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

AG.2 HPLC chromatogram for the blank sample with MeOH/H₂O: 60/40 as eluent.

```
Data File C:\CHEM32\1\DATA\LISELØBERG\20190220-BLANK6040.D
Sample Name: Blank 6040
=====================================================================
 Acq. Operator   : Lise
 Acq. Instrument : UPLC                            Location : Vial 3
 Injection Date  : 20.02.2019 12:18:33
                                             Inj Volume : 2.000 µl
 Acq. Method     : C:\CHEM32\1\METHODS\ODD\C18PURITYSALT_6_4.M
 Last changed    : 20.02.2019 12:16:25 by Lise
                   (modified after loading)
 Analysis Method : C:\CHEM32\1\METHODS\SONDRE_PHD\SONDRE-R2-NICO.M
 Last changed    : 20.02.2019 15:10:28 by Edvard
                   (modified after loading)
 Method Info     : Renhetsanalyse Sondre

 Sample Info     : Isocratic 60/40 MeOH/H2O with 0.1% TFA in H2O, 1mL/min

 Additional Info : Peak(s) manually integrated
=====================================================================
```
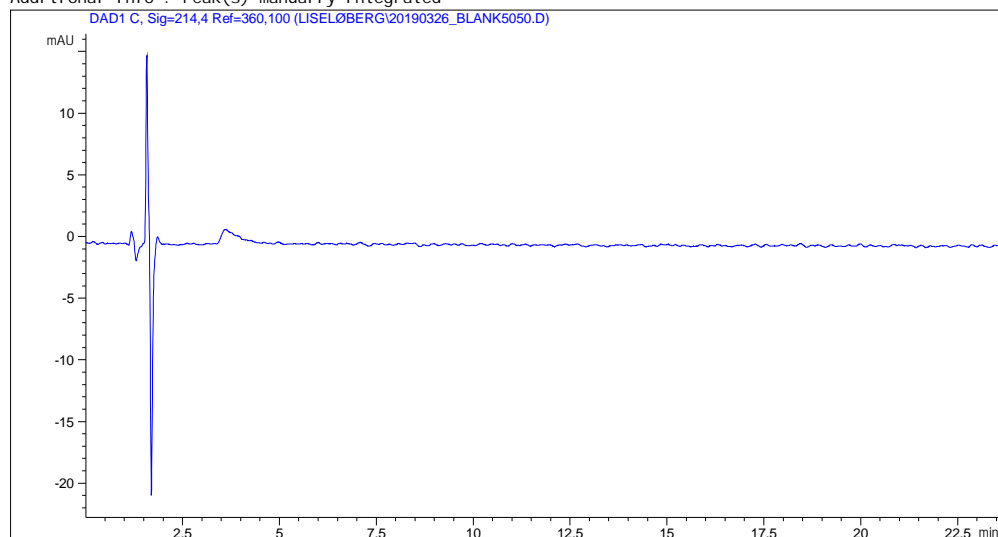

DAD1 C, Sig=214,4 Ref=360,100 (LISELØBERG\20190220-BLANK6040.D)

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

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

 No peaks found

=================================================================
                    *** End of Report ***
```
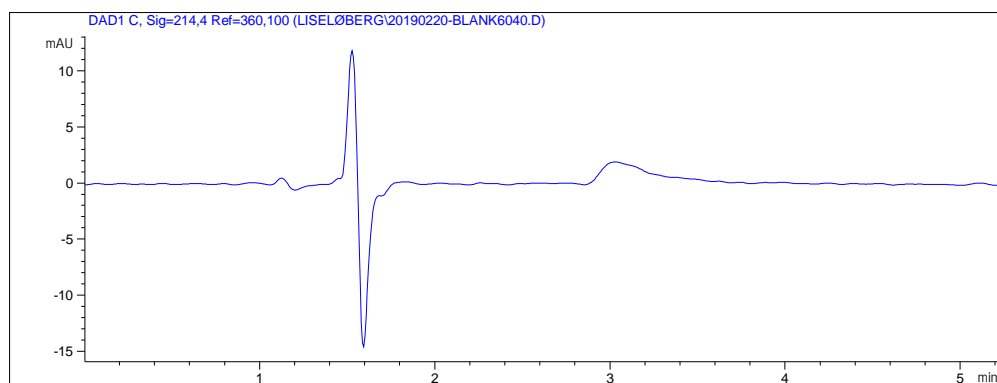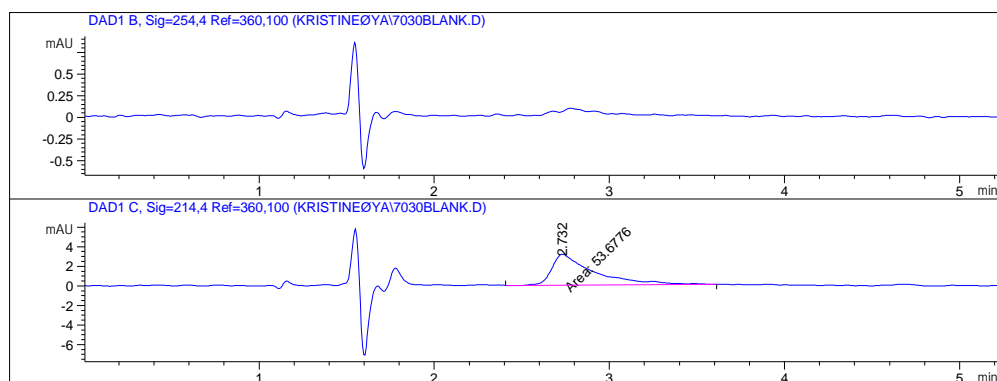
AG.3 HPLC chromatogram for the blank sample with MeOH/H₂O: 70/30 as eluent.

```
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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
 ----|-------|----|-------|----------|----------|--------|
   1   2.732 MM   0.2818   53.67757    3.17450 100.0000

 Totals :                  53.67757    3.17450
```

UPLC 10.05.2019 16:00:43 Jorge   Page   1 of 2

AG.4 HPLC chromatogram for the blank sample with MeOH/H₂O: 80/20 as eluent.

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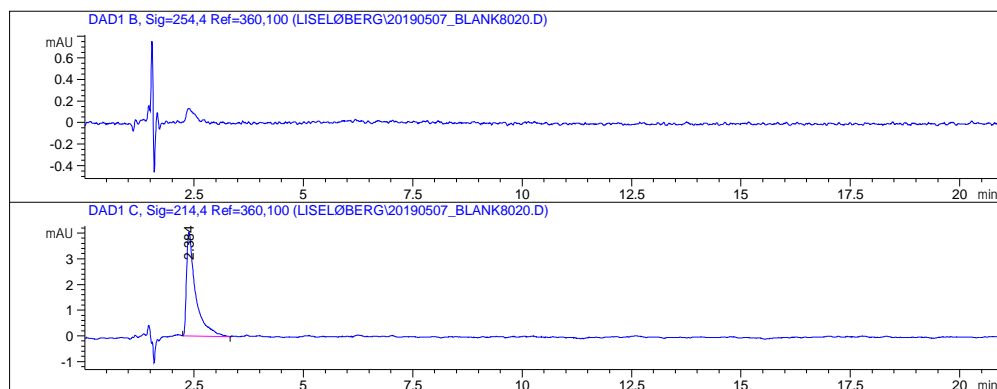
Sorted By   :   Signal
Multiplier   :   1.0000
Dilution   :   1.0000
Use Multiplier & Dilution Factor with ISTDs

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

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

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

Totals :   58.29042   4.04042

UPLC 10.05.2019 14:22:40 Jorge   Page   1 of 2

ccxx

Lise Pernille Løberg

NTNU
Norwegian University of
Science and Technology