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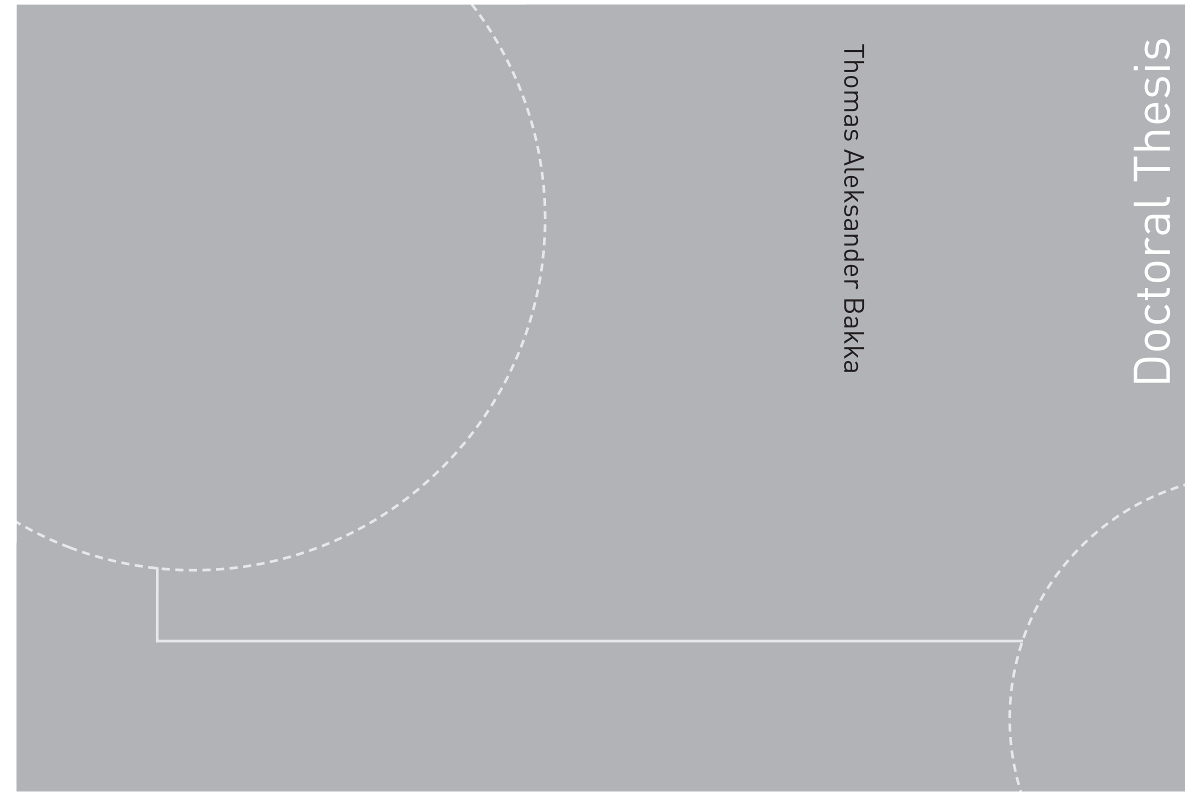
Thomas Aleksander Bakka

Synthesis and Antimicrobial Evaluation of Small Molecule Amphiphiles Derived from Amphiphilic Antimicrobial Natural Products



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Department of Chemistry

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Thesis for the degree of Philosophiae Doctor

Trondheim, May 2017

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Faculty of Natural Sciences
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The presented work has been conducted at the Department of Chemistry, Norwegian University of Science and Technology (NTNU) from August 2013 to May 2017. The funding was provided by the the faculty of natural sciences at NTNU. The work was done under the supervision of Associate Professor Odd Reidar Gautun at NTNU and Professor Morten Bøhmer Strøm at UiT - The Arctic University of Norway.

First of all I would like to thank Associate Professor Odd Reidar Gautun for being a fantastic supervisor through my PhD-work at NTNU. Your extensive theoretical and practical knowledge of organic chemistry will never cease to amaze me. I am also grateful for the freedom you have given me in order for me to sculpt my own project (within a reasonable framework). I will never forget our many meetings that usually starts with a scientific discussion, but slips over to discussions concerning politics or dogs in a matter of minutes.

Next I would like extend my gratitude to co-supervisor Morten Bøhmer Strøm at UIT - The arctic university of Norway for providing valuable knowledge regarding the biological evaluations in this project. Without you, we would truly be stumbling in the dark, and the project would not have become the same without you. In this regard one particular memory comes to mind; after our first round of biological testing Odd and I were not even able to read the excel-file with the data without your help. I am also grateful for the hard work you put in during writing of our article manuscripts and for the rigorous proofreading of my thesis.

Furthermore, I would like to thank my fellow PhD-students at the department of chemistry for providing a good working environment. I would particularly like to mention Mel Siah for proofreading my thesis. And Karsten Kirste

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This work could not have been completed without the love and unconditional support of my family. And to my parents; knowing that you believe in me no matter what, has been vital for spurring me on through hardest days of this project. Last but not the least, I would like to thank my soon-to-be wife Ingvild for her endless love and support throughout the last couple of years. Coming home every day after work to you and Svela have kept me sane through this time of insanity, I could not have done this without you.

Thomas A. Bakka
Trondheim, May 2017

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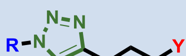
ABSTRACT

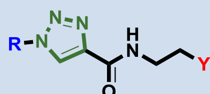
Minimal model for antimicrobial natural product amphiphiles:

Lipophilic group — **Linker/ scaffold** — **Cationic N-group**

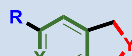
Synthesis:



42 derivatives



38 derivatives



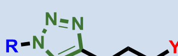
20 derivatives

R = Aromate or alkyl group
X = N or CH
Y = Cationic nitrogen group

Triazoles
Isoindolines
Fused pyridines
N-functionalization

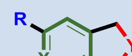
E. faecalis
S. aureus
S. agalacticae
E. coli
P. aeruginosa

Activity:



9 derivatives

17 derivatives



15 derivatives

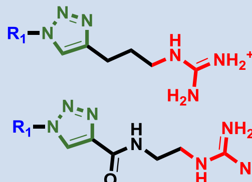
R = Aromate
X = N or CH
Y = Cationic nitrogen group

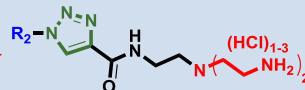
Selection:

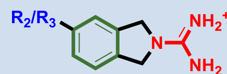
- High activity
- Low toxicity
- High selectivity

Current leads:

R_1 = 4-($C_7H_{15}O$)Ph
R_2 = 3,5-di-*t*-BuPh
R_3 = 4-*t*-BuPh







Development of antimicrobial agents that work through novel mechanisms is of importance for combating the steadily increasing proliferation of resistant bacteria. Infections caused by resistant bacteria have become an increasing global problem, where clinicians in the worst case scenarios are left without treatment options against severe bacterial infections. If this trend is left unchecked, the modern society will return to the medicinal dark-ages before the antibiotic era, where bacterial infections were often untreatable life-threatening ailments. Investigations into new antimicrobials are therefore not only important, but vital for the continuation of the current *status quo* in medicine.

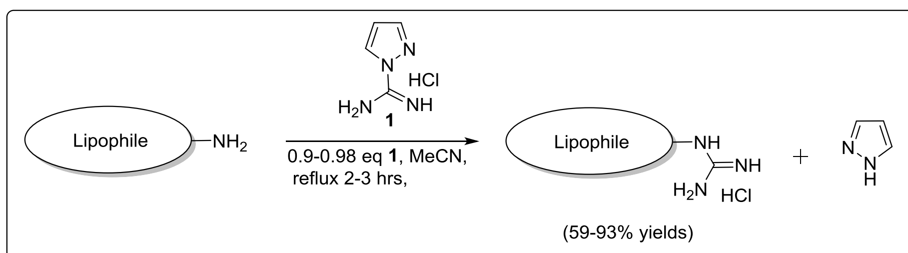
This project has focused on preparation of novel amphiphilic antimicrobials based on a model developed from antimicrobial peptides and marine antimicrobial natural products. The aim was to create a library of cationic amphiphiles for biological evaluation. The current compound library has now reached over 100 compounds, consisting mostly of 1,2,3-triazoles in addition to around 20 compounds based on isoindoline and dihydro pyrrolopyridine. The synthetic workhorses in this project have been the copper-catalyzed azide-alkyne cycloaddition (CuAAC) and transition metal catalyzed [2+2+2] cycloaddition reactions.

After scaffold synthesis and *N*-functionalization, the target amphiphiles were evaluated against five strains of clinically important bacteria: *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus agalacticae*, *Pseudomonas aeruginosa*, and *Escheria coli*. In addition to antimicrobial evaluations, the most active target compounds in the antimicrobial assays were evaluated for mammalian toxicity against HepG2-cells (human hepatic cells). All biological testing was performed at Marbio at UiT - The arctic university of Norway.

The biological evaluations were used to evaluate the relative potencies and toxicities of different functional groups. Some functional groups have therefore become more prevalent in the later substrates and future work, whereas some functional groups have been excluded due to low antimicrobial potency or high level of cytotoxicity against HepG2. On basis of these evaluations, the "current lead" compounds in the library were chosen. The compounds were coined "current leads", as they still have some selectivity issues that needs to be addressed in order to make them more suitable as lead structures.

GRAPHICAL ABSTRACTS

Paper I:



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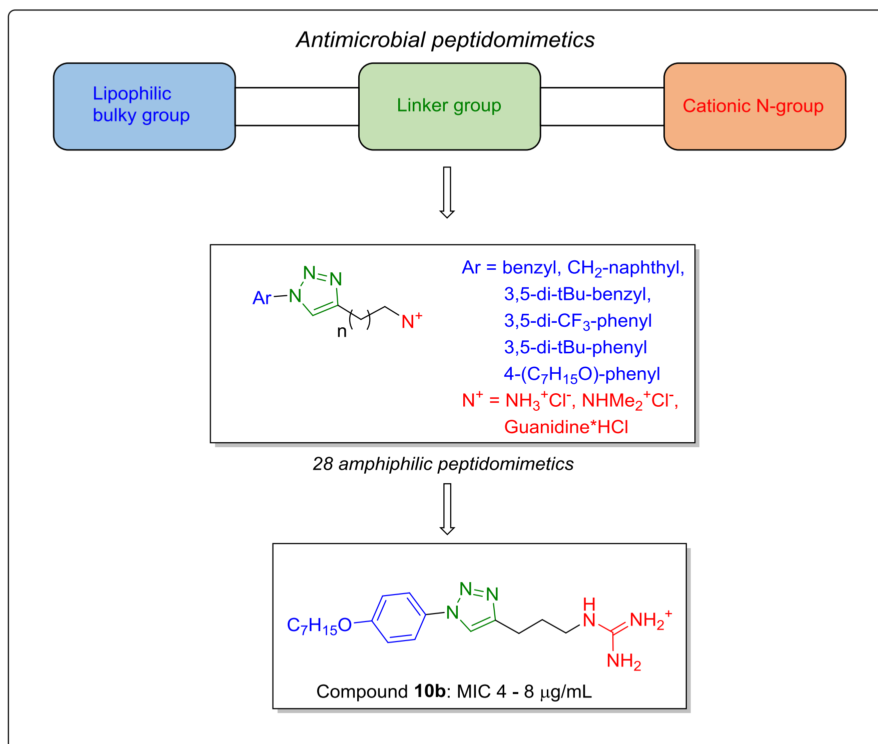

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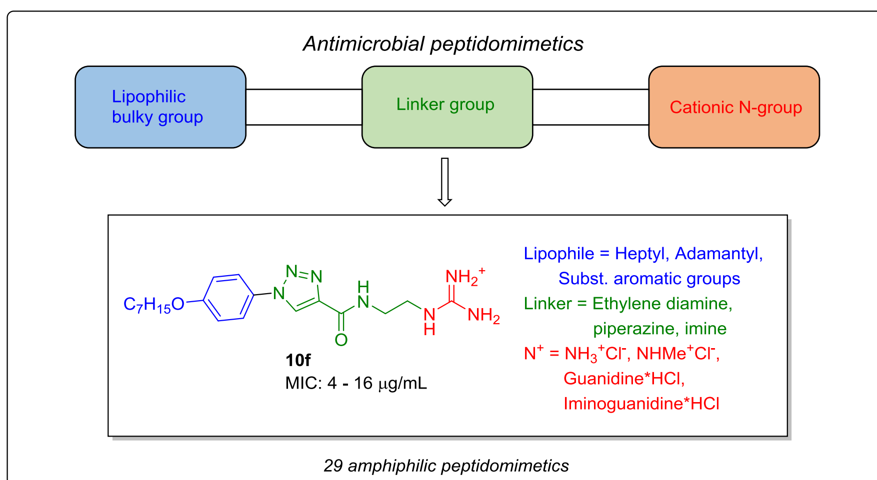

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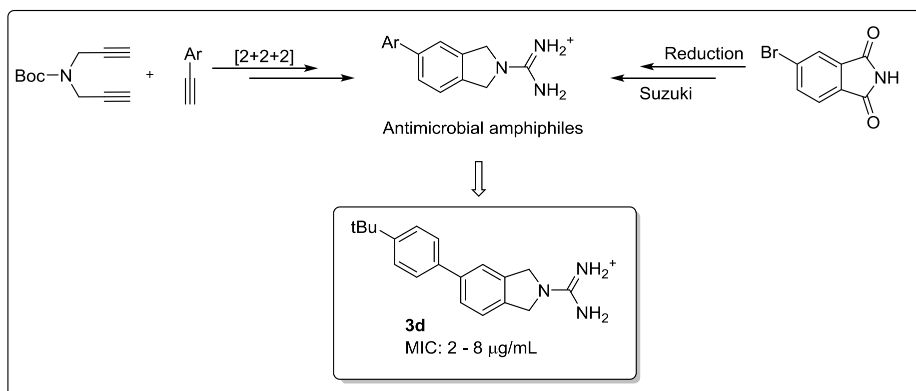


List of Publications

Paper I

"Simple generalized reaction conditions for the conversion of primary aliphatic amines to surfactant-like guanidine salts with 1H-pyrazole carboxamidine hydrochloride"
Bakka, Thomas A.; Gautun, Odd R.
Synthetic Communications, **2017**, Volume 47, 169-172.

Paper II

"Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles"
Bakka, Thomas A.; Strøm, Morten B.; Andersen, Jeanette H.; Gautun, Odd R.
Bioorganic and Medicinal Chemistry Letters, **2017**, Volume 27, 1119-1123.

Paper III

"Methyl propiolate and 3-butynone: starting points for synthesis of amphiphilic 1,2,3-triazole peptidomimetics for antimicrobial evaluation"
Bakka, Thomas A.; Strøm, Morten B.; Andersen, Jeanette H.; Gautun, Odd R.
Bioorganic and Medicinal Chemistry, **2017**, Accepted manuscript.

Paper IV

"Synthesis and antimicrobial evaluation of fused pyridine
and isoindoline amphiphiles"
Bakka, Thomas A.; Myreng, Kristian N.; Lea, Kristoffer L.; Brondz, Anton C; Strøm, Morten B.; Andersen, Jeanette H.; Gautun, Odd R.
Manuscript.

ABBREVIATIONS

AMP	Antimicrobial peptide
aq	Aqueous
Ar	Aromatic group
ASAP	Atmospheric solids analysis probe
ATR	Attenuated total reflectance
Bn	Benzylic
CO-ADD	Community for Open Antimicrobial Drug Discovery
cod	1,5-Cyclooctadiene
Cp	Cyclopentadienyl
Cp*	Pentamethylcyclopentadienyl
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIPEA	*N*,*N*-Diisopropylethylamine
4-DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMB	Dimethyoxybenzyl
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
EAS	Eletrophilic aromatic substitution
EC_{50}	Half maximal effective concentration
E. faecalis	*Enterococcus faecalis*
E. coli	*Escheria coli*
equiv	Equivalents
ESBL	Extended spectrum β-lactamase
ESI	Electron spray ionization
Est.	Estimated
FBDD	Fragment-based drug design.
FCC	Flash column chromatography
FDA	US Food and Drug Administration
GBS	Group B streptococcus
GI-system	Gastro-intestinal system
h	Hours
HPLC	High performance liquid chromatography

HRMS	High resolution mass spectrometry
HTS	High-throughput screening
IC_{50}	Half maximal inhibitory concentration
IR	Infrared spectroscopy
LPS	Lipopolysaccharide
MCR-1	Mobilized colistin resistance gene
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
mp	Melting point
MS	Molecular sieves
MRSA	Methicillin-resistant *Staphylococcus aureus*
MW	Microwave heating
NAS	Nucleophilic aromatic substitution
NMR	Nuclear magnetic resonance spectroscopy
P. aeruginosa	*Pseudomonas aeruginosa*
PBP	Penicillin binding protein
Ph	Phenyl
PSA	Polar surface area
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
rt	Room temperature
RuAAC	Ruthenium-catalyzed azide-alkyne cycloaddition
SAR	Structure-activity relationship
S. aureus	*Staphylococcus aureus*
SMAMP	Synthetic mimic of antimicrobial peptides
S. agalacticae	*Streptococcus agalacticae*
TBAC	Tetrabutylammonium chloride
TLC	Thin-layer chromatography
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THIQ	Tetrahydroisoquinoline
t_R	Retention time
VRE	Vancomycin-resistant *enterococci*
VRSA	Vancomycin-resistant *Staphylococcus aureus*

PREFACE

Objectives

This doctoral thesis is written as a collection of articles, and consists of two published scientific peer-reviewed papers, one accepted manuscript, and one manuscript prepared for submission (found as appendices after the references in this thesis). This project has been presented in its entirety through this thesis and the articles/manuscripts. Unpublished experimental work is presented in their respective chapters. The main focus of this project was to investigate and evaluate new possible scaffolds for antimicrobial amphiphiles, based on antimicrobial peptides (AMPs) and marine antimicrobial natural products. The goal was to develop a library of low molecular weight cationic amphiphiles and investigate their abilities to inhibit bacterial growth. The biological evaluations of the current compound library are presented in Chapter 2 and Paper/manuscripts II, III, and IV.

In addition to the main objective, the development of synthetic methodologies for efficient and versatile synthesis of the target amphiphiles was important. This was done to ensure efficient and versatile synthesis of the current compound library, and to establish the synthetic groundwork for future work in this project. The synthetic strategies and methods are presented in Chapter 2 and Papers/manuscripts I-IV.

Chapter 1 covers common classes of antibiotics, antimicrobial resistance, and membrane active antimicrobials. Also, some important physicochemical principles and testing of cytotoxicity are presented, followed by an introduction of the different bacteria targeted in the project. The last sections in Chapter 1 cover synthesis and medicinal applications of 1,2,3-triazoles, isoindolines, and fused pyridines (dihydro pyrrolopyridines). The copper-catalyzed azide-alkyne cycload-

dition and [2+2+2] cycloaddition reactions are particularly highlighted for the preparation of 1,2,3-triazoles and dihydro pyrrolopyridines/isoindolines respectively. Chapter 2 covers the development of the current compound library based on the scaffolds and the scaffold-chemistry presented in Chapter 1. Chapter 2 is split into sections with regard to the different papers/manuscripts associated to this dissertation:

- **Section 2.1** covers the preparation and biological evaluations of the aliphatic amino 1,2,3-triazoles presented in paper II.
- **Section 2.2** covers the preparation and biological evaluations of the amido 1,2,3-triazoles presented in paper III.
- **Section 2.3** covers the preparation and biological evaluations of dihydro pyrrolopyridine and isoindoline amphiphiles paper IV.

Section 2.4 shows comparison of the most promising structures from the different papers/manuscripts with regards to antimicrobial potencies and cytotoxicity. Chapter 3 offers a summary and concluding remarks, and suggests further work on the most promising structures from Chapter 2. Finally, Chapter 4 covers the experimental data for the unpublished work.

Contributions

The author of this thesis has prepared and characterized 84 target amphiphiles for biological evaluations. Additionally, synthesis development and evaluation of biological data have been important tasks. The following people are acknowledged for their contribution to the synthetic work: MSc Anton Brondz for preparation of 4 target compounds and their precursors in Paper IV; MSc Kristoffer Lea for preparation of 5 target compounds and their precursors in Paper IV; MSc-candidate Kristian Njerve Myreng for preparation of 7 target compounds and their precursors in Paper IV; Biological assays have been performed by Marbio (UiT - The arctic university of Norway) led by Professor Jeanette H. Andersen. Professor Morten B. Strøm is acknowledged for his extensive contribution to the evaluation of biological data and optimization strategies as a co-supervisor in this project.

CHAPTER 1

INTRODUCTION

Antibiotics have been one of the cornerstones of modern medicine ever since the discovery of the first systemic antimicrobials.[1,2] Due to the lack of efficient systemic therapeutics at the time, infectious diseases were one of the leading causes of death in the pre-antibiotic era.[3] The discoveries of the therapeutic potential of sulfonamides[4] (1935) and β-lactams[5] (1928) were therefore scientific events that changed the world. Most of the common bacterial infections then became managable with "over the counter"-antibiotics, and went from being deadly threats to often being nothing more than a nuisance. After the initial antibiotics followed a period of three decades with intense development of different and more complex classes of antibiotics. This led to more than 20 classes of antibiotics being introduced for human use in the period between 1930 and 1962.[6–8] However, after the initial flow of novel compound classes coming through the antibiotic pipeline up to the late 1960's, the steady flow of development became a slow dribble.[6–8] The development of novel classes of antibiotics has become so slow, that in the last three decades only two new classes of antibiotics have emerged: the oxazolidinones (linezolid[9,10]) and the cyclic lipopeptides (daptomycin[11,12]).[13]

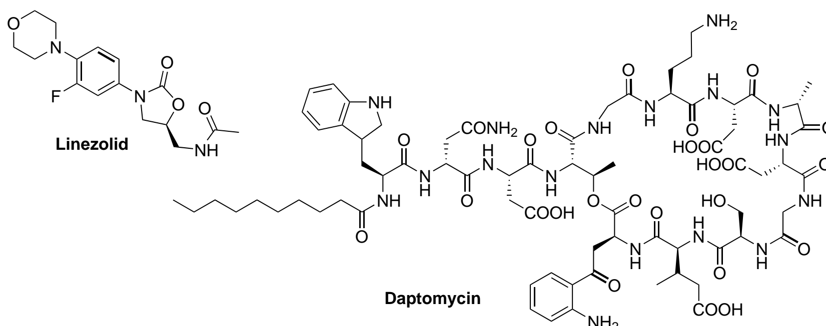


Figure 1.1. The oxazolidinone antibiotic linezolid and the cyclic lipopeptide daptomycin.

Increased consumption of antibiotics in agriculture and clinical settings, combined with recycling of old antibiotic classes, are some of the leading causes for the rapid global emergence of antimicrobial resistance.[14–19] It is estimated that resistant infections lead to around 700,000 deaths globally every year, which by itself is frightening.[20] Moreover, the estimates predict an increase to a staggering 10 million deaths by 2050, if the development of antimicrobial resistance is left unattended.[20] It is therefore imperative to develop more efficient novel antibiotics to overcome this global threat.

This chapter will give a short introduction to antibiotics and antimicrobial resistance, and a more specific introduction concerning membrane-active antimicrobials. Then follows a short introduction of some important medicinal chemistry concepts and bacteria targeted in this project. The final sections in this chapter give an introduction to the medicinal applications and chemistry of the chosen scaffolds in the structure library.

1.1 Classes of Antibiotics and Mechanisms of Action

Modern antibiotics are based on a range of different structures, most of which were developed in the golden age of antibiotics (1930 - 1962).[6–8] Fig. 1.2 shows a distribution of different antibiotics in 323 hospitals in the US in 2010, where the data collected were hospital discharges with at least one day of treatment. The figure also shows the year of which the particular antibiotics were discovered or taken into clinical use. The general structures of penicillin, cephalosporin, and fluoroquinolones show the year of discovery, whereas the specific examples vancomycin, streptomycin, and erythromycin show the year of clinical application.[21] The antibiotics making up over 75% of the total antibiotics in Fig. 1.2, belong to compound classes that were discovered more than 50 years ago. Despite the age of these compound classes, they are still extensively targeted in antibiotic research, as many antibiotics coming through the pipeline every year are modifications of these structure classes.[22] Modifications are carried out in order to optimize pharmacological properties and negate adverse effects,[23] in addition to making them efficacious against resistant bacteria. Activity against resistant bacteria is becoming increasingly important, since many antibiotics become gradually less effective due to proliferation of resistant pathogens.[24]

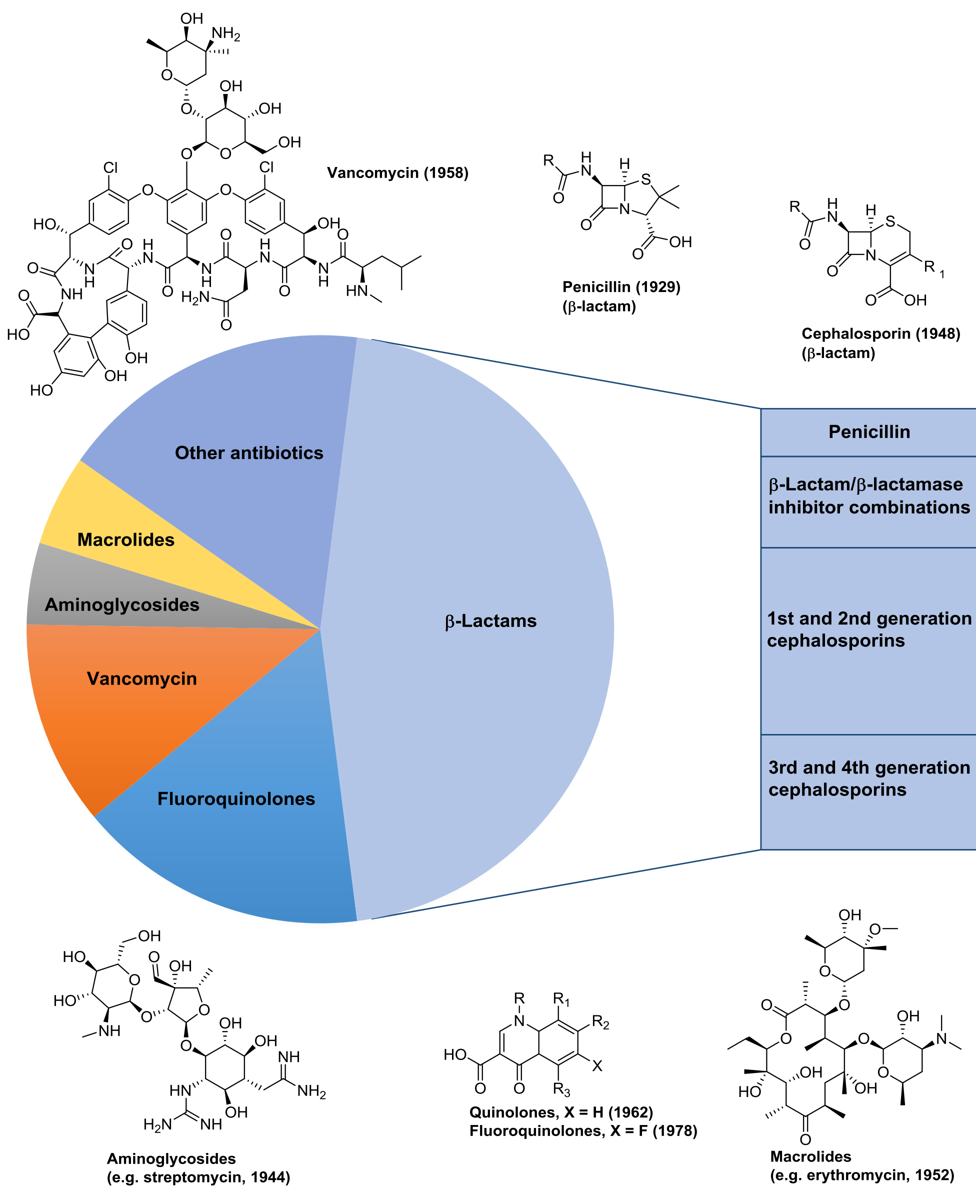


Figure 1.2. Distribution of antibiotics in hospital care from 323 hospitals in the US in 2010 (data gathered by Truven Health MarketScan Hospital Drug Database).[21]

1.1.1 β-Lactams (penicillins, cephalosporins, and carbapenems)

Antibiotics based on the β-lactam scaffold constitute the largest portion of important antibiotics to human health.[21,25] The β-lactams can be further divided into different classes, with the most utilized structures being penicillins, cephalosporins, and carbapenems (shown in Fig. 1.3). The penicillin class was discovered by Fleming in 1928,[5] which in turn started what has become known as the antibiotic era. This was followed by the discovery of the antibiotic effects of cephalosporins by Brotzu in 1948.[26] He isolated cultures of *Cephalosporium acremonium* from a sewer in Sardinia, and analysis showed that this bacteria produced substances with antimicrobial properties. The last of the three classes, the carbapenems, were developed at Merck and Co. and were approved for use in the US in 1985.[27] They were developed to combat the emergence of β-lactamase-expressing bacteria in the late 1960s, since they were less prone to hydrolysis and sustained their activity towards many bacteria resistant to other β-lactams at the time.[28] Today the carbapenems are still used to treat infections caused by β-lactamase-expressing bacteria (e.g. *Enterobacteriaceae*), as they are enzymatically stable towards many β-lactamases.[29]

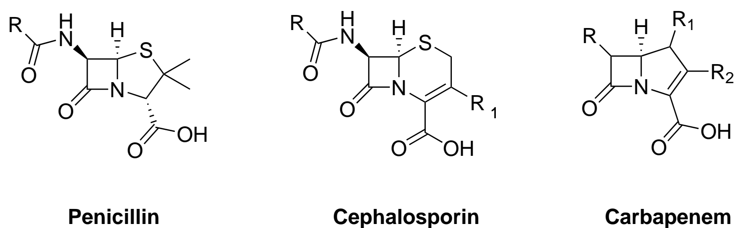


Figure 1.3. Different β-lactam antibiotics.

β-Lactam antibiotics get their antibiotic properties from inhibiting the function of D-Ala-D-Ala carboxypeptidase, also known as penicillin binding proteins (PBPs).[30,31] The PBPs are responsible for synthesis of peptidoglycans, an important constituent of the bacterial cell wall. Inhibition of cell wall synthesis will lead to growth inhibition by stopping bacterial division, as insufficient peptidoglycan synthesis will cause the bacteria to shed the cell wall and fail to divide. β-Lactams also trigger autolytic events in the bacteria from the build-up of peptidoglycan precursors that signal hydrolases to break down existing peptidoglycan.[31]

The main resistance mechanism against β-lactams is the expression of β-lactamases in the bacteria.[28] These enzymes hydrolyze the β-lactam ring of the antibiotic and renders it inactive for binding to PBP, which leads to no antibiotic effect. All the β-lactamase classes are able to hydrolyze some penicillins and cephalosporins, but only a few are active enough to hydrolyze carbapenems.[32] However, occurence of bacteria expressing enzymes capable of hydrolyzing carbapenems is rapidly escalating, and "extended spectrum β-lactamases" (ESBLs) are considered to be an increasingly critical clinical problem.[24] Aside from expression of β-lactamases, other resistance mechanisms against β-lactams involve expression of efflux pumps,[33] changes in PBP,[34] and loss of membrane porins.[35]

1.1.2 Vancomycin and Glycopeptide Antibiotics

Vancomycin (shown in Fig. 1.2) is a glycopeptide natural product antibiotic that was first isolated from a soil sample collected in Borneo in 1953.[36] Due to the growing concerns regarding β-lactam resistance, its approval as a clinical drug was fast-tracked by the US Food and Drug Administration (FDA) in 1958.[37] Despite the fast FDA approval, the importance of vancomycin was diminished throughout the 1950s and -60s, mostly due to some studies showing adverse effects like nephrotoxicity from the use of vancomycin, in addition to development of the new potentially safer methicillins. However due to the emergence of methicillin-resistant bacteria in the 1980s and new studies on more refined vancomycin showing none of the nephrotoxic activities found in the 1950s, vancomycin became an important treatment alternative to combat methicillin-resistant *S. aureus* (MRSA).[36,38] Around the same time as vancomycin got its second wind, the teicoplanins were approved for clinical applications.[37] The teicoplanins are semi-synthetic glycopeptides, with efficacies against Gram-positive bacteria comparable to those of vancomycin.[39]

Vancomycin and other glycopeptide antibiotics target the same process in bacteria as β-lactam antibiotics; the cell wall synthesis machinery.[40] The glycopeptides, however, inhibit the cell wall synthesis in a different way than the β-lactams, by hindering cross-linking of new residues to the cell wall. This is done by non-covalent association to the amino acids involved in the transglycosylation reaction (cross-linking), and blocking them from reaching the active site in

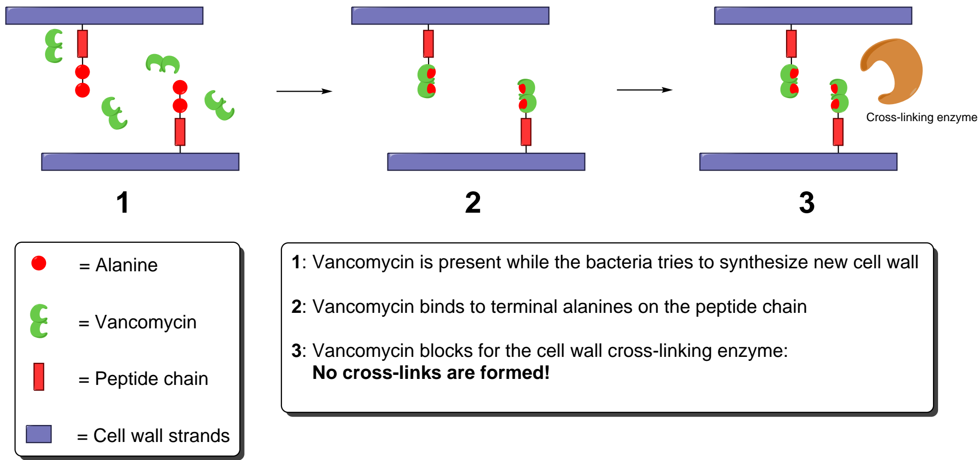


Figure 1.4. The main mode of action of vancomycin.[40]

the enzyme.[40] This can be seen in Fig. 1.4, where the mechanism is shown over three frames. The first being the presence of vancomycin in the area where cell wall synthesis takes place, followed by binding of vancomycin to the two alanines on the end of the peptide attached to the cell wall. Leading to the third step in the mechanism: blocking the peptide strands from reaching the active site in the cross linking enzyme, and failure to synthesize the cell wall.

Resistance against glycopeptide antibiotics emerged in the mid 1980s,[41] and has since then steadily escalated. Especially vancomycin-resistant enterococci (VRE) have become a significant problem in clinical settings, as it is already intrinsically resistant to a range of common antibiotics.[42,43] There are also some bacteria that display intrinsic resistance towards vancomycin.[44] The most common mechanism of resistance is modification of the "D-alanine-D-alanine"-site where vancomycin binds and inhibits cross-linking. The most effective modification is substitution of one alanine with a lactate, whereas substitution with a serine only causes moderate lowering of vancomycin affinity.[43]

1.1.3 Quinolones and Fluoroquinolones

The quinolones are a class of broad spectrum antibiotics that was discovered in the early 1960s, as an impurity in the production of quinine.[45] Nalidixic acid was the first quinolone to be introduced as an antibiotic in 1962, for the treatment of urinary tract infections. The quinolones were given a lot of attention due to their large therapeutic potential, as they were potent over a broad spectrum, had good bio-availability, and displayed a low incidence of side-effects.[45] The large scientific and clinical interests have led to synthesis and evaluation of more than 10,000 different quinolones. Nonetheless, only 2% of this massive number have entered clinical trials and about 20 substrates have been launched into the market.[46] The most important substrates in clinical settings today are the fluoroquinolones, where the quinolone core is carrying a fluorine atom in the 6-position. This change from the original quinolone structure was investigated in the late 1970s (i.e. norfloxacin), and led to enhanced affinity for the enzymes and lowered minimum inhibitory concentrations (MIC) by a significant factor against both Gram-positive and Gram-negative bacteria.[47,48] Continued research on fluoroquinolines eventually led to ciprofloxacin, the first quinolone to display noteworthy activity outside the urinary tract.[49] Ciprofloxacin remains to this day one of the most commonly prescribed antibiotics.[50]

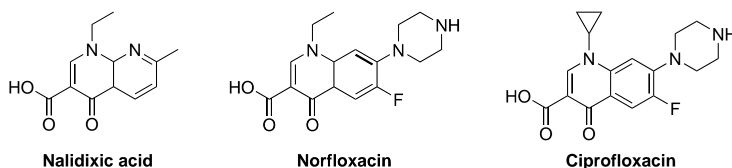


Figure 1.5. Examples of quinolone and fluoroquinolone antibiotics.

The main mode of action of the quinolones comes from their ability to turn gyrase and topoisomerase enzymes into cellular toxins.[51–53] These enzymes are encoded by most bacteria, and are important for nucleic acid processes like unwinding deoxyribonucleic acid (DNA) and removing knots and tangles from the bacterial chromosome. In order to fulfill their purpose in the bacteria, these enzymes are able to generate double-stranded breaks in the bacterial DNA. This ability to cleave DNA is vital for the quinolone mode of action, as quinolone molecules interacts with the enzyme after the strand break and are inserted as non-covalent

intercelators at both the cleaved scissible bonds. While the quinolone molecules are inserted in the complex, the ligation of the DNA-strands is blocked, leading to increased concentration of the cleavage complexes shown in Fig. 1.6. When these stabilized complexes encounter other DNA machinery like replication forks or transcription complexes, they are converted to permanent chromosomal breaks. If sufficient amounts of these breaks are made, the DNA-repairing systems will not be able to fix them fast enough and bacterial cell death occurs.

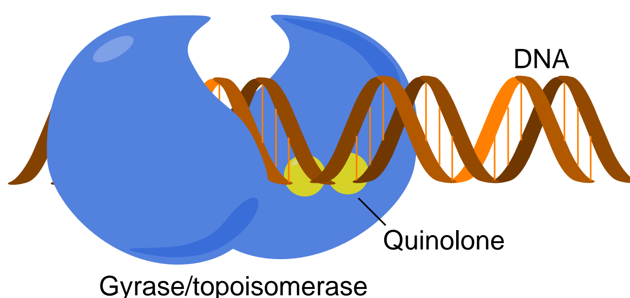


Figure 1.6. The stabilized enzyme cleavage complex with two quinolone molecules inserted in the scissible bonds, blocking re-ligation.[51–53]

As quinolones are among the most prescribed antibiotics,[50] resistance has become common and widespread all over the world.[54] A survey conducted in 2003 on clinical isolates of enteric bacteria in the US showed that more than 10% of the isolates were resistant to ciprofloxacin.[55] The resistance mechansisms against quinolones can be divided into three groups; target-mediated quinolone resistance, plasmid-mediated quinolone resistance, and chromosome-mediated quinolone resistance.[50] Target-mediated quinolone resistance happens through modifications of the target enzyme, where mutations of the binding pocket leads to lowerered quinolone binding affinity.[50] Plasmid-mediated quinolone resistance happens through the expression of plasmid-encoded proteins. Expression of these proteins can lead to: lower gyrase-/topoisomerase-binding to DNA,[56] hindering of quinolone from entering cleavage complexes,[50] acylation of quinolone,[57] or lowering of the quinolone concentration through efflux pumps.[58] Lastly, chromosome-mediated quinolone resistance can happen through chromosomal down-regulation of porins and up-regulation of efflux pumps,[50,59] which lowers the intracellular quinolone concentration. Unlike bacterial resistance against β-lactams, resis-

tance mechanisms against quinolone are not high-level mechanisms. Instead there are many low-level mechanisms that added together lead to high-level survival in quinolone-containing environments.[54]

1.1.4 Aminoglycosides

The antibiotic effects of aminoglycosides were discovered in 1944 (streptomycin, shown in Fig. 1.2), in a targeted search for antibacterial substances in the wake of the success of penicillin.[60] In the years following the discovery of streptomycin, many aminoglycosides, both naturally isolated and semi-synthetic derivatives, were launched into the market.[61] A large part of the interest in the aminoglycosides came from their ability to treat Gram-negative infections, as they are considered somewhat harder to target with antibiotics.[62] Aminoglycosides like streptomycin (1944) and gentamicin (1963) are therefore still highly relevant therapeutics in the treatment of infections inflicted by Gram-negative bacteria. Streptomycin is used in the treatment of tuberculosis (*M. tuberculosis*), and gentamicin (shown in Fig. 1.7) is one of the main aminoglycosides used in the treatment of major sepsis.[61]

The mechanism of action of aminoglycoside antibiotics was initially thought to be only the inhibition of the 30S ribosomal subunit, which is responsible for ribosomal ribonucleic acid (rRNA) translation in bacterial protein synthesis.[61] The aminoglycoside is transported into the intracellular environment in separate processes, where the initial step is association to anionic functions on the cellular membrane followed by energy-dependent transport across the cytoplasmic membrane.[63] The glycoside then binds with high affinity to the 30S ribosomal subunit, which in turn leads to faults and inhibition of protein synthesis.[61] Although this mode of action does not completely explain the antibiotic properties of the aminoglycosides, the fact that they exhibit extracellular antimicrobial effects cannot be explained by protein synthesis inhibiton, as it is an intracellular process.[64] Thus, it was found that the initial binding of the cationic aminoglycoside led to disruption of the packing order of lipopolysaccharides (LPS) in the outer membrane, an important part of the cell envolope in Gram-negative bacteria. This disruption led to formation of pores and holes, ultimately leading to cell lysis.[64]

Several resistance mechanisms against aminoglycosides have been discovered and characterized.[65] There are some bacteria that display intrinsic resistance to small doses of aminoglycosides, but are susceptible to higher treatment concentrations. This observed resistance may be partially due to production of inactivating enzymes (acyltransferases, phosphotransferases, and nucleotidyltransferases).[66] Another important resistance mechanism to aminoglycosides is the expression of efflux pumps, which reduces the amount of intracellular aminoglycoside. Many *Pseudomonas* bacteria are highly resistant to aminoglycosides largely from their ability to efficiently pump it out of the cell.[67,68] A third mechanism for resistance to aminoglycosides, is methylation of rRNA within the target site (30S).[69] Methylation at certain places in the 30S subunit will weaken the aminoglycoside affinity for binding, hence reducing drug efficacy.

1.1.5 Macrolides and Tetracyclines

One of the last classes of antibiotics to be specifically introduced in this section is the macrocylic lactone natural products called macrolides (erythromycin shown in Fig. 1.2). The macrolide pikromycin was discovered by Brockmann in 1950, and was the first antimicrobial macrolide to isolated and characterized.[70] The first macrolide applied in a clinical setting on the other hand was Erythromycin A, which was mainly used to treat respiratory, skin, and soft tissue infections.[71] The macrolides are efficacious mainly against Gram-positive bacteria, and work by blocking the protein synthesis machinery. The mode of action is therefore somewhat analogous to the one of the aminoglycosides. The macrolides however target the 23S ribosomal rRNA in the ribosomal 50S-subunit,[71] whereas the aminoglycosides target the 30S ribosomal subunit. The most common resistance mechanisms to macrolides are expression of methylases and efflux pumps. The methylases methylates a specific adenine residue in the rRNA, which in turn leads to blocking of the macrolide when it attempts to bind.[72] Other resistance mechanisms to macrolides are other mutations to rRNA, mutations to ribosomal proteins, and various forms of enzymatic macrolide inactivation (e.g. esterases, phosphotransferases, and glycosyltransferases).[73]

The last group of antibiotics to be introduced in this section are the tetracycline antibiotics (Fig. 1.7). The tetracyclines are not shown in Fig. 1.2, but they are still an important class of antibiotics, both in agriculture and human

medicine.[74] Tetracyclines were introduced as antibiotics in 1948, and have since then been an extensively used compound class in treatment of both Gram-positive and Gram-negative infections.[75] The mode of action for the tetracyclines is reversible binding to the 30S ribosomal subunit. Thus, inhibiting protein synthesis in a similar way to the aminoglycosides presented earlier in this section. Due to its extensive use, resistance to tetracycline is not uncommon. A study conducted in 2003 showed that 11% of the oral microflora of 20 people in the UK was resistant to tetracycline.[76] Common resistance mechanisms to tetracyclines involve expression of efflux proteins, enzymatic inactivation of tetracycline, and expression of ribosomal protection proteins.[75] Ribosomal protection proteins are cytoplasmic proteins that protects the ribosome from tetracycline by dislodging bound tetracyclin from the ribosome, leading to an increased drug dissociation constant.[77]

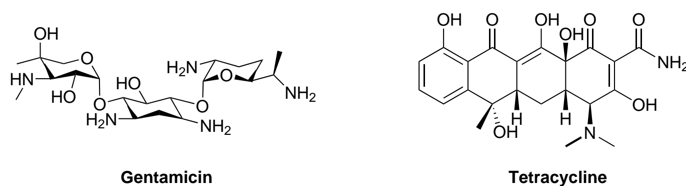


Figure 1.7. Structure of gentamicin and tetracycline.

1.2 Membrane-Active Antimicrobials

When compared to the number of antibiotics targeting other vital, often intracellular, targets in bacteria (cell wall synthesis, protein synthesis, and the replication machinery), there are only a few antibiotics used in clincal settings that specifically target the bacterial cell membrane as their main mode of action.[21] This low number may be attributed to adverse effects and narrow therapeutic windows that are associated with some membranolytic antibiotics on the market (e.g. colistin).[78–80] Development of new antibiotics have additionally had a tendency to follow known tracks, where modifications of existing compound classes are developed instead of novel drug-discovery.[81] This tendency is still highly visible when looking at new drugs coming through the antibiotic pipeline as of 2016, where the majority of antibiotics in clinical trials are derivatives of old compound classes.[22] However, due to the problems arising from antimicrobial resistance,[20,82–84] the concept of membrane-active antibiotics have been suggested as a possible way to combat multi-resistant bacteria.[85–87] Current membrane-active antibiotics on the market (most known: colistin and daptomycin) are mostly used in complicated cases of resistant infections, which is largely due to their high activity against resistant strains of bacteria and their sometimes substantial adverse effects (e.g. nephrotoxicity).[78–80,88–90] Moreover, these antibiotics cannot sustain the pressure of being last-resort drugs on the market for long, as the number of reported cases of colistin and daptomycin resistance is increasing.[91–94] The clinical interest for these compounds to treat resistant bacteria have also rekindled the interest for development of new membrane-active antimicrobials. The following sections will cover the types and mechanisms of existing membrane-active antibiotics, the concept of antimicrobial peptides and peptide mimics, and some membrane-active antimicrobials in development and clinical trials.

1.2.1 Important Commercial Membrane-Active Antibiotics

Polymyxins Against Gram-Negative Bacteria

The first antibiotics of the polymyxin class of membrane-active antibiotics were isolated from *B. polymyxa* in 1947.[95] The polymyxins are a class of polycationic peptide antibiotics with high activity against most Gram-negative bacteria, in addition many resistant Gram-negative pathogens.[96,97] Out of the five naturally occuring polymyxins, polymyxin B and E (colistin) found their way to

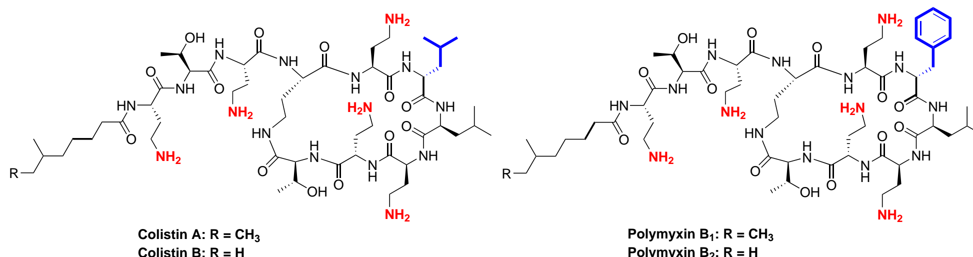


Figure 1.8. Structure of the main constituents in colistin (colistin A and colistin B) and polymyxin B (polymyxin B_1 and polymyxin B_2). The main difference between the two being the leucine and phenylalanine amino acids displayed in blue. Amine groups contributing to the positive character important for the mode of action are displayed in red.

clinical applications for the treatment of infections caused by Gram-negative bacteria, and both of them are used as mixtures of over 30 compounds when applied in clinical treatments. The two main components in the two drugs are shown in Fig. 1.8.

The use of polymyxin antibiotics declined in the 1970s, and they were largely not applied in clinical settings until the mid 1990s.[90] This is partially attributed to the adverse effects patients experienced when being treated with polymyxin antibiotics.[78,98,99] Even when using the less toxic prodrug colistine methane sulfonate, several problematic adverse effects were observed.[99] The other reason for the abandonment of polymyxins as a treatment option, was the development of potentially safer alternatives to treat the same infections (e.g. aminoglycosides).[100] Polymyxins have nonetheless resurfaced as last-resort antibiotics the later years, due to the rapid emergence of multidrug-resistant (MDR) Gram-negative bacteria (e.g. *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*). However, due to their long absence from clinical medicine, one of the large problems concerning the use of polymyxin B and colistin today is finding appropriate dosing regimens.[100] Finding the correct dose is important, as giving a sub-optimal dose may lead either to proliferation of resistant bacteria (low doses) or to unneccesary adverse effects (high doses).[100]

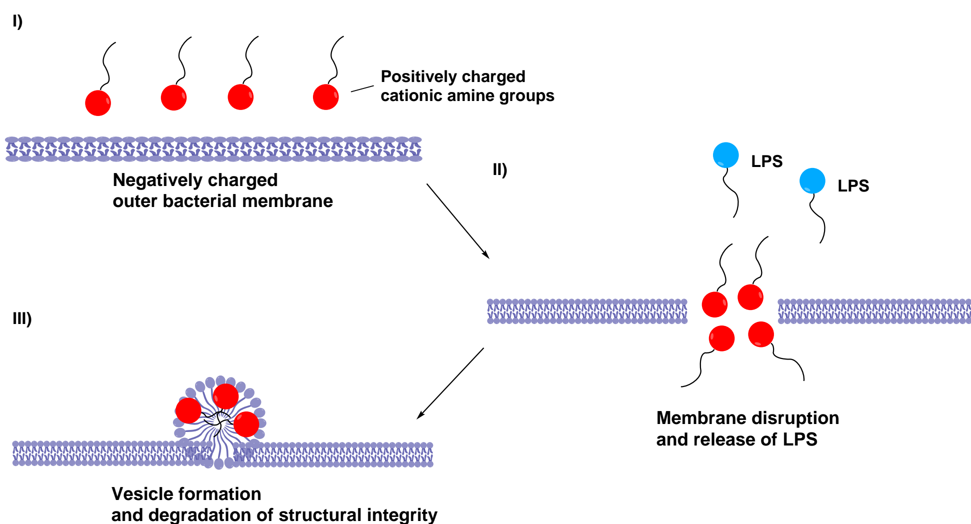


Figure 1.9. Polymyxin mode of action: initial coordination and displacement of divalent cations on the outer membrane (I) is followed by pore formation and leakage of LPS (II), which in turn is followed by formation of membrane vesicles, loss of structural functions, and cell lysis (III).[90,101–103]

The main mode of action of the polymyxins is to disrupt the bacterial cell membrane.[104] This is done through electrostatic interactions between the positively charged amine groups on the polymyxin and the negatively charged outer membrane on the bacteria.[90,101–103] These interactions cause polymyxin to displace divalent cations (Mg^{2+} and Ca^{2+}) on the membrane surface, and disrupt its structural integrity. This disruption leads to formation of vesicles, release of LPS, and leakage of proteins from the bacteria (as displayed in Fig 1.9). The mechanism is nonspecific and not dependent on bacterial metabolics,[105] it will therefore kill active and dormant bacteria at the same relentless pace.[90]

Polymyxin resistance has not been common in clinical isolates, but due to the increased use to combat resistant infections there has been an increased prevalence of resistance.[106] And since polymyxins often are last-resort solutions it is critical that they work, as there are few other options for treatment of Gram-negative bacteria if polymyxins fail.[106] It has recently been shown that bacteria not only develop resistance to colistin through chromosomal mutations, but also through horizontal gene transfer of the mobilized colistin resistance

(MCR-1) gene.[107] Bacteria expressing this gene have recently been isolated from both humans and livestock. Moreover, recent observations show that genes encoding for ESBLs can be colocated on the same plasmid as the one carrying the MCR-1 gene,[108] meaning that there can be a higher prevalence of colistin-resistance among ESBL-expressing bacteria. The primary resistance mechanism against polymyxins is post-translational modification of the LPS outer membrane.[109–111] This is done through expression of enzymes that add 4-amino-4-deoxy-L-arabinose, phosphoethanolamine, or galactosamine to the LPS core, which in turn reduces the negative charge of the LPS-layer. This charge reduction results in reduced binding affinity to the surface, and lowered translocation of polymyxins through the outer membrane. Even though enzymatic modification of the LPS-core is the most common resistance mechanism, complete loss of the LPS-component lipid A has been observed in some isolates.[91]

Daptomycin Against Gram-Positive Bacteria

Daptomycin (Fig. 1.10) is an antibiotic for the treatment of infections caused by Gram-positive bacteria, that also acts through a membrane-disrupting mechanism.[112] It is a more recently developed antibiotic compared to the polymyxins, and was developed by Eli Lilly in the late 1980s. However, due to the discovery of adverse effects at high doses, the development of daptomycin was discontinued after phase II clinical trials.[113] This was, nonetheless, not the end of daptomycin, as Cubist pharmaceuticals bought the rights to daptomycin in 1999 and continued its clinical development.[11] Daptomycin was FDA approved in 2003 and is now used as an antibiotic against complicated skin infections caused by Gram-positive bacteria.[89]

The mechanism displayed by daptomycin is not seen for any other antibiotics.[89,114] Its unique mode of action, as shown in Fig. 1.10, involves calcium-dependent binding to the bacterial membrane, followed by formation of channels in the membrane. These channels allows for transport of ions, leading to efflux of K^+ and cell depolarization. The depolarization leads to inhibition of multiple vital systems and death of the bacteria. One interesting note for daptomycin is that unlike the polymyxins, it does not rupture the bacteria but leaves the structural integrity of the membrane intact. This can be beneficial for treating infections caused by toxin-producing bacteria, such as toxic shock syndrome.[114]

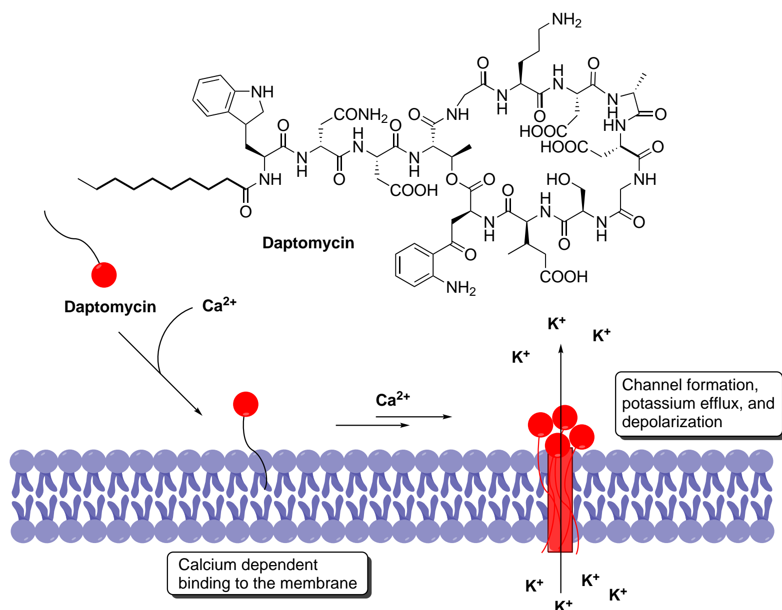


Figure 1.10. Daptomycin main mode of action: calcium-dependent binding to the membrane surface, followed by clustering and potassium efflux channel formation, which ultimately leads to depolarization and death of the bacteria.[89,114]

Even though the majority of Gram-positive bacteria remains susceptible to daptomycin, there have been reported cases of daptomycin resistance.[115] Since daptomycin is mainly used to treat infections resistant to other antibiotics, widespread resistance to daptomycin would be highly worrisome.[116] Two main mechanisms for daptomycin resistance have been proposed: the repulsion and diversion mechanisms.[116] The repulsion mechanism is similar to the mechanism for resistance to polymyxins, as the bacteria modifies the cytoplasmic membrane and reduces the net negative charge on the surface. This in turn leads to lowered affinity for binding of daptomycin on the surface, examples of modifications can be insertion of lysines in the membrane. The diversion mechanism takes place through expression of cardiolipin microdomains on the membrane, which has a high affinity for daptomycin. Binding daptomycin here leaves less daptomycin to be bound on the membrane, leading to lower concentrations of membrane-bound daptomycin. The lowered concentration of membrane-bound daptomycin increases likelihood of cell survival, as daptomycin requires a certain concentration on the membrane in order to induce depolarization.

1.2.2 Antimicrobial Peptides and Peptide Mimics

Between the introduction of important membrane-active antibiotics available on the market and promising substrates under development, this section will serve as an intermission to introduce important concepts utilized in current research on membrane-interacting antimicrobials. The fact that the only membrane-targeting antibiotic currently in US clinical trials (brilacidin) is a defensin mimic,[22] shows the importance of antimicrobial peptides in recent antibiotic research. Additionally, the membrane-active antibiotics presented in the previous section (polymyxins and daptomycin) can be broadly classified as antimicrobial peptides.

Structure and Classification of Antimicrobial Peptides

Antimicrobial peptides (AMPs) are part of the primary immune response in most living organisms and this evolutionarily conserved mechanism is mobilized as a first response by the immune system against invading pathogens.[117,118] Due to their prevalence in most living organisms (from prokaryotes to large mammals), there is a large structural diversity of naturally occuring AMPs. Due to their interesting biological activities, several thousand synthetic AMPs have also been created and added to the diversity of this type of peptides.[119] So in order to describe AMPs in broad strokes, most AMPs would be covered by these characteristics:[117,118]

- **Medium to small in size:** Less than 100 amino acid residues (usually between 12 and 45).
- **Overall positive charge:** Overall charge between +2 and +9 (from e.g. lysine and arginine).
- **Substantial hydrophobic character:** $\geq$30% hydrophobic residues (e.g. tryptophan).

Due to their overall positive charge and large hydrophobic character, AMPs are able to fold into amphiphilic secondary structures. This can take place spontanously or upon interaction with cell membranes, and is assumed to be important for their antimicrobial properties. The amphiphilic secondary structures of AMPs interact with the membranes of both Gram-positive and Gram-negative bacteria, and through several mechanisms lead to bacteriostatic or bactericidal

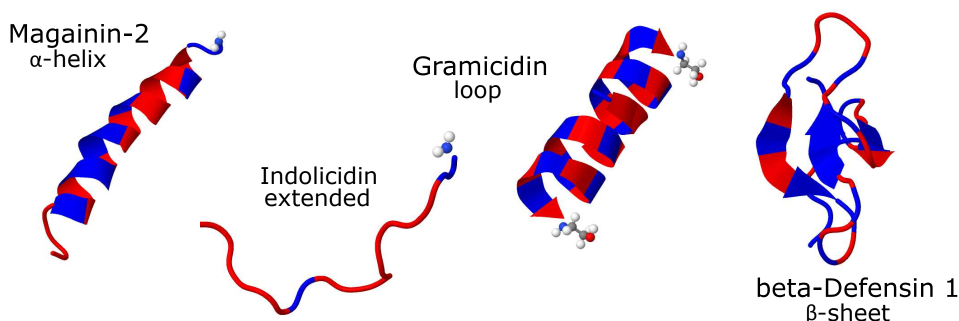


Figure 1.11. Four main structural classes of AMPs: α-helix (magainin-2), β-sheet (human β-defensin 1), loop (gramicidin A), and extended peptide (indolicidin). The structures were downloaded from the RSCB Protein Data Bank (PDB) (http//www.pdb.org/).[123]

effects.[120] The secondary structure of AMPs is not only important for them to exhibit their antibacterial activities, it also serves as a tool for further classification.[121] The AMPs are divided into classes based on their secondary structures: β-sheets, α-helices, loops, and extended peptides, where β-sheets and α-helices are most common for AMPs isolated from natural sources.[118] The different AMP-classes are shown in Fig. 1.11, where they are exemplified by magainin-2 (α-helix), β-defensin 1 (β-sheet), gramicidin A (loop), and indolicidin (extended peptide).[122]

- **Magainin-2**[124,125] is a 23-residue AMP isolated from the African clawed frog in 1987, that forms amphiphilic helices when associated to a cytoplasmic membrane. This AMP exhibits high antimicrobial activity against bacteria, in addition to little hemolytic activity against eukaryotic cells.

- **Human β-defensin 1**[126,127] is a 36-residue AMP that is produced in epithelial cells, particularly in kidney epithelial cells. All defensins form β-sheet secondary structures, the main difference between α- and β-defensins is the placement of cysteine bridges in the primary structure. In addition to antimicrobial effects, defensins have important immunomodulatory functions.

- **Gramicidin A**[128–130] is a looped AMP with two 16-residue secondary structures connected together to form a amphiphilic structure. It is highly hydrophobic with the majority of the amino acids contributing to the hydrophobic face of the amphiphile. When gramicidin A interacts with a membrane, it forms small channels that are selective for translocation of small cations. This, in turn, leads to depolarization of the membrane (similarly to daptomycin) and ultimately cell lysis. Gramicidin A has been used as a topical treatment similarly to some polymyxins, but due to high hemolytic activity it is unsuitable as a systemic drug.
- **Indolicidin**[131] is a 13-residue tryptophan-rich AMP isolated from bovine neutrophils. Despite its small size, it displays high broad spectrum activity against Gram-positive and Gram-negative bacteria. Unlike many other membrane-active structures, indolicidin does not induce lysis of the bacteria. It is postulated that it uses its membrane affinity to pass the membrane and attack targets in the cytoplasm (e.g. macromolecular synthesis).

Antimicrobial Peptide Mode of Action

The classical mode of membrane disruption by AMPs is derived from their amphiphilic character and positive charge.[117] Many AMPs are produced by eukaryotic organisms, they therefore need to display a high degree of selectivity for bacterial cell membranes, as low selectivity between bacteria and host cells can lead to adverse effects such as high hemolytic activity (as seen for gramicidin A).[130] Luckily, the selectivity of AMPs exploits the difference in membrane surface charge of eukaryotic and prokaryotic cells.[132] Most eukaryotic cell membranes have zwitter-ionic phospholipid head groups pointing outwards on the membrane surface, whereas bacterial membranes often carry an overall negative charge from negatively charged membrane species. This makes the AMP able to selectively target bacteria on the basis of electrostatic interactions between the positive charge of the AMP and the negatively charged teichoic acids (Gram-positive bacteria) or LPS (Gram-negative bacteria). After the initial electrostatic coordination and uptake of the AMP into the bacterial envelope, there exist several proposed models of how the AMP exhibits its bacteriostatic or bactericidal properties, and there is no consensus on what model or mechanism AMPs acts through.[117] The four most common models describing the membrane disrupting interactions are therefore listed here:

- **The "carpet" model**[133] (1 in Fig. 1.12) is a fairly diffuse model suggesting initial binding of AMP monomers to the membrane surface, leading to clustering of monomers on the membrane surface. This clustering imposes significant curvature and strain on the membrane, eventually causing membrane disruption.

- **The "barrel-stave" model**[133,134] (2 in Fig. 1.12) suggests that the AMP inserts into the membrane perpendicularly and through aggregation form a barrel-like pore in the membrane. The monomer inserts into the membrane through hydrophobic and hydrophilic eletrostatic interactions, where the hydrophobic part of the AMP faces into the nonpolar tail-groups of the membrane lipids. When the number of inserted peptides reaches a certain amount, a self-aggregation process is initiated. This self-aggregation leads to insertion of more peptides, reaching deeper into the membrane untill a pore is formed through the membrane.

- **The "toroidal pore" model**[135,136] (3 in Fig. 1.12) is somewhat different from the "barrel-stave" model, and involves both AMP and membrane lipids in the formation of the membrane pore. This model is often supported in newer studies, where it often is favored over the "barrel-stave" model. The origin of the model came from studies of magainin 2, where they observed flipping of membrane lipids in the pore formation process. The postulated model from this observation stated that the membrane lipids and AMP monomers form well-defined pores, where the lipid head groups point towards the center of the pore and AMP lines the hole in the membrane.

- **The "aggregate channel" model**[137,138] (4 in Fig. 1.12) was introduced as a possible explanation to antibacterial activities not explainable by the previous three models, as it has been shown that depolarization alone does not explain the AMPs antibacterial activities. It was hypothesized that AMPs also have intracellular targets for killing bacteria, in addition to the membrane activity. The "aggregate channel" model postulates that the AMPs coordinate to and insert into the membrane, followed by clustering into aggregates with varying sizes and structures. These clusters can pass through the membrane, enter the intracellular space and attack specific intracellular targets.

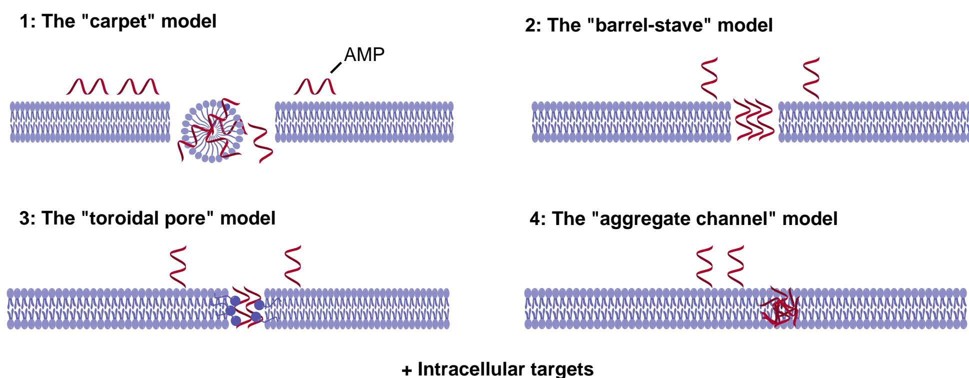


Figure 1.12. The four major membrane interaction models for AMPs: The "carpet",[133] "barrel-stave",[134] "toroidal",[135,136] and "aggregate channel" model.[137,138]

As previously mentioned, not all antimicrobial effects of AMPs can be explained by their membrane-disrupting abilities.[117,139] Even so, they still use their bacterial cell membrane affinity to differentiate between host and target cells, as well as traverse the membrane to reach their intracellular targets. Many of these intracellular targets are similar to those of classical antibiotics on the market, such as cell wall synthesis inhibition[140] and inhibition of nucleic acid[141] and protein synthesis.[142,143] AMPs have also been shown to induce programmed cell death by changing intracellular potassium concentrations (similarly to daptomycin).[144]

Clinical Potential of Antimicrobial Peptides

AMPs can be of significance for development of new antibiotics, as they often display selective antimicrobial activity and other biological effects (e.g. immunomodulatory effects).[126,127,145] AMPs have also been shown to be active against resistant bacteria, and it is assumed to be difficult for bacteria to develop resistance against AMPs, since the AMPs are not hindered by resistance mechanisms used against other antibiotics.[145,146] The mechanisms of resistance against AMPs are similar to the mechanisms utilized against polymyxins (Gram-negative) and daptomycin (Gram-positive).[147] On the basis of the beneficial characteristics of AMPs, being able to harness the full clinical potential of AMPs would be a significant weapon for combating antimicrobial resistance.

There are, on the other hand, some challenges related to the potential use of AMPs in clinical medicine. One of the largest obstacles is the complicated production routes associated with AMPs, as this makes them expensive compounds to prepare in a large scale.[145] Many AMPs also suffer from poor pharmacokinetic properties such as poor bio-availability and low systemic stability. If a high dose of the drug is needed for it to reach its target site in sufficient concentration, it increases the chance of adverse effects close to the site of application.[117] Moreover, AMPs have also been shown to induce subtle toxicities arising from their ability to translocate into cells, such as induction of apoptosis and mast-cell degranulation.[148] Because of these issues related to systemic use, most of the research on AMPs as antibiotics has revolved around utilizing their potential through topical applications.[117] Several AMPs have nevertheless entered clinical trials both for topical and potential systemic use, due to their high potency against resistant bacteria.[121] AMPs have also become a starting point for development of antimicrobial peptide mimics, which are suggested as possible workarounds for the drawbacks of the native AMPs.

Antimicrobial Peptide Mimics

Antimicrobial peptide mimics are structures intended to emulate the favorable antimicrobial properties of AMPs. And peptide mimics have been suggested as possible solutions to the drawbacks often obstructing clinical usefulness of AMPs.[149–152] This workaround is made possible from the way AMPs gets their antimicrobial activitiy, as the activity is assumed to be unrelated to the primary structure of the AMP. The antimicrobial activity instead comes from the physicochemical properties of the secondary structure of the AMPs. This in turn allows for large structural freedom in the synthesis of AMP-mimicking molecules, as they only need to emulate the physicochemical properties of the AMPs and not the primary sequence. Some of the most classical backbone strategies for building synthetic AMP-mimics are shown in Fig. 1.13.[153] The structural freedom is however not only restricted to the backbone of the compound, which allows for a multitude of different moieties contributing to structural diversity.

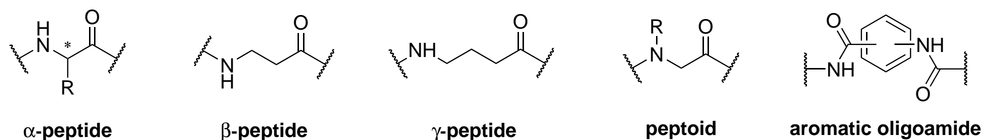


Figure 1.13. Some of the most common backbones for antimicrobial peptide mimics.[153]

The peptide mimics that closest resemble the native AMPs in backbone structure, are the ones built from peptide residues. They can be regular amino acids (α-peptides) or amino acids with one or two additional spacer carbons (β-peptides and γ-peptides). Out of these structures, the most studied compounds are β-peptides based on β-amino acids.[153] One common trait for β-peptides is that they commonly form helical secondary structures, although other conformations have been observed (like antiparallel hairpins and sheet structures). This is shown by Gellman and co-workers, who synthesized a 17-mer β-peptide with two repeating units (one hydrophilic and one lipophilic) inspired from the structure of magainin 2.[154] Not surprisingly, the synthetic β-peptide adopted a helical structure that exhibited antimicrobial effects similar to those of magainin 2. The β-peptide was also found to cause less hemolysis than magainin 2, giving it a higher selectivity for bacteria compared to human cells. It is also shown that a helical structure is not necessary, as long as the hydrophobic and hydrophilic residues are located on either side of what would be the helical axis.[87] This has been utilized to develop the antibiotic brilacidin, which is currently in phase II clinical trials for the US market.[155,156] This arylamide based compound (shown in Fig. 1.14) exhibited antibacterial effects comparable to those of daptomycin in a comparison study for treatment of Gram-positive skin infections.[156]

Going back to the β-peptides, Hansen *et al.* developed a library of disubstituted $\beta^{2,2}$-amino acids coupled with arginine at the *C*-terminus,[157] and based on a minimal pharmacophore model derived from lactoferricin B by the group of Svendsen.[158–164] These structures were found to be highly potent antimicrobial agents exhibiting a high selectivity towards bacteria. This initial library was then refined to yield a focused library of potent and selective peptide mimics with potential for oral administration (example shown in Fig. 1.14).[165,166] The pharmacophore model based on lactoferricin B was originally utilized by the group of Svendsen to develop a series of peptide mimics consisting of two

or three amino acid residues.[167] They increased the lipophilicity of the structures using non-genetically encoded amino acids (e.g. substituted tryptophan residues), which led to highly active amphiphiles with good selectivities. The most promising of these compounds LTX-109 (shown in Fig. 1.14) was taken to phase II clinical trials as a topically applied antibiotic against resistant bacteria.[168] Utilizing a similar dipeptide approach, Teng *et al.* have recently developed a library of highly potent antimicrobial amphiphiles, where the most active compound (shown in Fig. 1.14) displayed high potency against resistant Gram-positive bacteria.[169] Furthermore, Ghosh *et al.* have shown that even simpler small molecule amphiphiles can display high antimicrobial activities.[170] Their library of compounds was based on functionalization of a single lysine amino acid, that achieved high antimicrobial potency against resistant bacteria when functionalized with an alkyl chain and an aromatic group (shown in Fig 1.14).

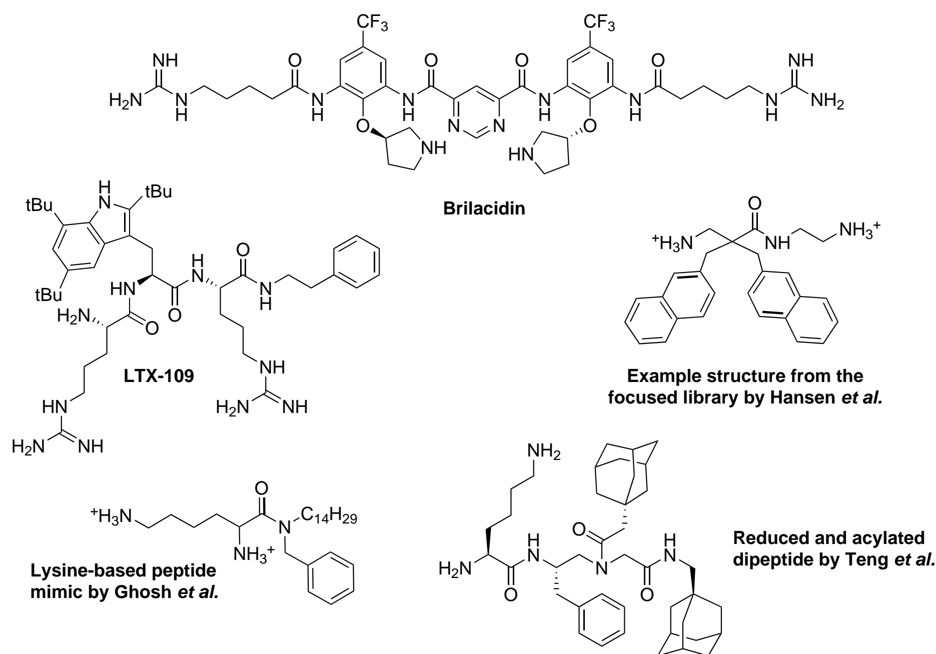


Figure 1.14. Brilacidin by Cellceutix,[155,156,171] LTX-109 by Lytix Biopharma,[167,168] an example structure from the library of compounds by Hansen *et al.* with potential for oral administration,[165,166] a lysine based peptide mimic by Ghosh *et al.*,[170] and a reduced and acylated dipeptide by Teng *et al.*[169] Ionizable groups are reproduced as depicted in their respective publications (charged/neutral).

AMPs and peptide mimics are, however, not the only inspiration for creating new amphiphilic antimicrobials, as many amphiphilic antimicrobial natural products have also been isolated and characterized from marine environments (e.g. ianthelline,[172] synoxazolidinone A[173] and hyrtioseragamine B,[174] shown in Fig 1.15).[175–179] One interesting feature for some of these marine antimicrobials is their simple structure, at least compared to many of the antimicrobial peptide mimics previously presented. The simplest model for the peptide mimics in Fig. 1.14 utilized two hydrophilic and two hydrophobic moieties to induce the wanted antimicrobial effects. The marine antimicrobials in Fig. 1.15, on the other hand, utilize only one hydrophobic group and one hydrophilic group to yield antimicrobial activity. The simplest possible model for these compounds can be expressed as: a hydrophobic group and a cationic nitrogen group attached to a linear or cyclic linker structure/scaffold (as shown in Fig. 1.15). This motif has been explored by Strøm and co-workers, who have prepared a library of amphiphilic aminobenzamides based on this model from marine antimicrobials.[180] These compounds were shown to display high antimicrobial activity against both antibiotic-susceptible and resistant bacteria. These amphiphiles were also subjected to membrane disruption studies, in order to confirm a membrane-targeting mode of action. Membrane disruption was confirmed for these compounds, but they were not able conclude whether the membrane disruption was the only mechanism or if there were any intracellular secondary targets observed.

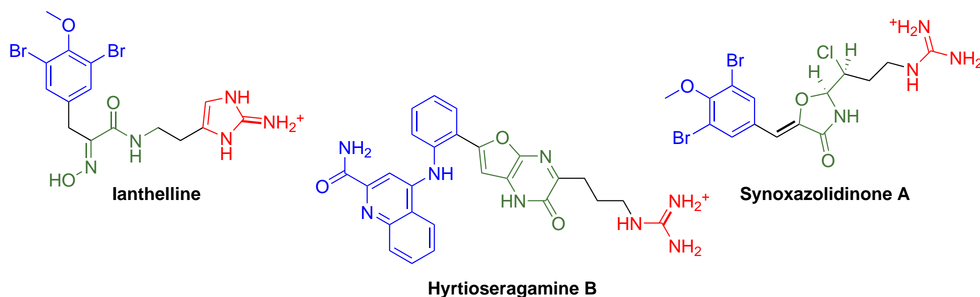


Figure 1.15. The marine natural products ianthelline,[172] synoxazolidinone A,[173] and hyrtioseragamine B (shown in their charged state),[174] with a lipophilic group and a cationic *N*-group connected through a linker or central scaffold.

1.3 Lipophilicity, Cationic Groups, and Cytotoxicity

1.3.1 Lipophilicity

Antimicrobial amphiphiles derived from AMPs and marine natural product antimicrobials are assumed to be dependent on some lipophilic character in order to induce the wanted antimicrobial properties.[168,180] Being able to predict the lipophilic character of a target structure may therefore provide useful information concerning the lipophilic-/hydrophilic-balance. A balanced lipophilic character is also an important factor for appropriate absorption of drugs in the body and translocation of drugs across membranes.[181,182] It has also been shown that high lipophilicity may invoke non-specific cytotoxic interactions. These kind of toxicities usually takes place if logP is above 3 and the polar surface area (PSA) is below 75 Å.[183,184] Knowledge of the lipophilic character is therefore important in order to make the correct trade-off for achieving the best physicochemical properties and high activity, while still affording a good toxicity profile.

The lipophilicity can be expressed as the partitioning between octanol and water, giving a partition coefficient (logP).[185] For determining the partition coefficients for compounds with ionizable groups, water is exchanged for buffer at a given pH (7.4 for physiological conditions), which then gives the distribution coefficient logD. Furthermore, as partition coefficients often are calculated* and not empirically determined, values from reversed-phase HPLC may offer some experimental insight for the determination of relative lipophilicities within a compound series. Retention times (t_R) from C18-HPLC have been used to determine relative lipophilicities in a compound library, as well as displaying an activity trend for observed antimicrobial potency.[157]

1.3.2 Cationic Groups of Lysine and Arginine

Cationic amphiphilic antimicrobials often get their positive charge from amine and guanidine groups, in AMPs this is achieved by having multiple lysine and arginine residues in the primary structure (Fig. 1.16).[117]

*When distribution coefficients are calculated they are given the prefix "C": ClogP and ClogD.

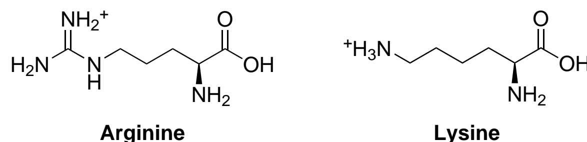


Figure 1.16. The genetically encoded amino acids arginine and lysine.

The cationic group in arginine is the highly basic guanidine group. The guanidinium ion in arginine has a pKa of 13.8, meaning the equilibrium will be pushed towards the ionized form at physiological pH (7.4).[186] The high basicity of guanidine comes from the efficient resonance stabilization of the guanidinium ion. Furthermore, the structure of the guanidine group can allow for strong electrostatic interactions and bidentate hydrogen-bonding with appropriate hydrogen-bond acceptors. These characteristics are thought to be the reason behind the efficient translocation of arginine-rich peptides across cellular membranes.[187,188] The group of Wender have furthermore shown that methylation or dimethylation of the guanidine groups on an Arg_8-species reduced the cellular uptake by 80% and 95% respectively.[187,188] They therefore hypotesized that the efficient formation of ion pairs between the guanidine groups and the membrane components led to the effective translocation.

The amino acid lysine is structurally similar to arginine, but instead of the guanidinium functional group on the end of the chain, lysine has a primary amine. The ammonium group has a pKa of 10.52, meaning this will also mostly be charged at physiological pH, however not to the same extent as guanidine.[189] The primary ammonium group on lysine is also a hydrogen-bond donor, but assumed to form less efficient hydrogen-bonds and ion pairs compared to guanidine. Less efficient formation of ion pairs between ammonium groups and cell membrane components was hypothesized to be the reason for ornithine-oligomers failing to display the same affinity as Arg_8 for translocation of cell membranes.[187] Lysines are, on the other hand, important for the snorkeling effects of some proteins with transmembrane sections.[190] The lysine/arginine-snorkeling is when the hydrophobic part of a protein segment burrows into the nonpolar part of a membrane, and the polar and flexible lysine/arginine-chain points like a snorkel out towards the polar membrane head groups and the aqueous surroundings.

1.3.3 Cytotoxicity Evaluation

In every initial drug discovery assessment, evaluation of human cell toxicity is important, as there is no point in developing a lead drug that displays a very narrow therapeutic window. There is usually also a trade-off between adverse effects (i.e. toxicity) and activity in development of drug leads. The main characteristic of AMPs other amphiphilic antimicrobials is their ability to interact with bacterial cell membranes. The initial cytotoxicity evaluations should, therefore, include measurements of lysis due to membrane interactions with eurkaryotic cells. A common assay for studies of such toxicity in similar drug investigations is the hemolysis assay.[154,155,157,167,180] This assay offers information regarding the drugs ability to lyse red blood cells at different concentrations, giving a toxicity profile and a selectivity index for the drug candidate. Another assay often used in drug discovery is testing against human liver cells (HepG2-cells), which is used to assess potential hepatotoxic effects of a drug candidate.[191] HepG2-cells were originally gathered from the liver of a caucasian male suffering from liver cancer, and they have epithelial morphology and are not tumorigenic.[192] The HepG2-assay has become a popular assay to include in early drug discovery, as liver toxicity is an important reason for why drugs fail in clinical trials.[191] An optimal drug target in this regard should display no effect in the HepG2-assay at 50 times the IC_{50}, EC_{50}, or MIC.

1.4 Target Bacteria

The bacteria used in the evaluation of the library of amphiphiles were chosen to offer broad-spectrum testing (Gram-positive and Gram-negative bacteria) against opportunistic pathogens that often create problems in clinical care, both through antibiotic-susceptible infections and resistant infections (with focus on the latter).[83] This section will also provide a small introduction to the differences in cell envelope composition of Gram-positive and Gram-negative bacteria.

1.4.1 Gram-positive Bacteria

Gram-positive bacteria is a classification encompassing bacteria that gives a positive result in the Gram staining test. The method invented by Christian Gram in 1884 differentiates between bacteria that has a thick peptidoglycan cell

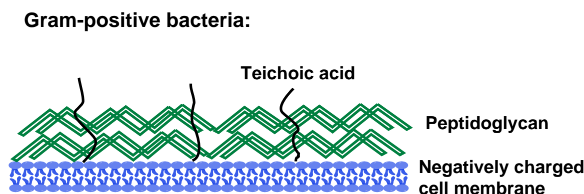


Figure 1.17. The basis of the Gram-positive bacteria cell envelope.[193,194]

wall (Gram-positive), and bacteria with an outer membrane and a thinner peptidoglycan layer (Gram-negative).[193] Peptidoglycan consists of repeating units of N-acetyl glucosamine and N-acetyl muramic acid and, when cross-linked, forms a rigid skeleton that is important for bacterial structural integrity. The reason for the thick peptidoglycan layer in Gram-positive bacteria, is that they lack the protective LPS outer membrane that is found in Gram-negative bacteria. The peptidoglycan layer therefore needs to be thicker in order to withstand turgor pressure and to protect the bacteria from their surroundings. In the cell wall there are also found anionic polymers called teichoic acids that provide further structural integrity and is involved in cell growth regulation. The basis of the cell envelope of Gram-positive bacteria is shown in Fig. 1.17.

The bacterial cytoplasmic membrane, which is found under the peptidoglycan layer, carries a net negative charge from the membrane phospholipid head groups. The phospholipid head groups in bacteria are, unlike the zwitter-ionic lipids in eukaryotic plasma membranes, negatively charged groups such as phosphatidylglycerol, cardiolipin, or phosphatidylserine.[117,147,194] This net negative charge is why cationic amphiphilic antimicrobials have a larger affinity for bacteria over that of mammalian cells. In Gram-positive bacteria it is under some debate whether the anionic teichoic acids assist cationic antimicrobials in reaching the membrane, or hinder them from reaching the membrane by binding up the drug before it reaches the target site.[195]

Staphylococcus aureus

S. aureus is a Gram-positive bacteria that is held responsible for the highest fraction of hospital-acquired bacterial infections.[196] This bacteria in the *Staphylococci* genus may cause anything from mild skin and soft tissue infections to

life-threatening conditions, like infective endocarditis. It is also a leading cause for bacteremia, which in turn can lead to sepsis or even septic shock.[197] *S. aureus* is also a bacteria that colonizes humans, and a large portion of the human population are carriers of this particular bacteria.[198] *S. aureus* is perhaps most known in its methicillin-resistant version, the infamous methicillin-resistant *Staphylococcus aureus* (MRSA). Accounts of infections caused by MRSA have been rapidly increasing the last couple of decades. Just in 10 years (1990-2000), the portion of bacteremia caused by MRSA increased from 1-2% to 40% (in England and Wales).[199] Infections caused by MRSA have traditionally been treated sucessfully with vancomycin, which is a solution that may be coming to an end.[200] The numbers of vancomycin-resistant *Staphylococcus aureus* (VRSA) have, like other resistant bacteria, proliferated the last decades.[201] Additionally, it is shown that *S. aureus* can survive on livestock and pets, and in that way provide an additional source for spreading of the bacteria.[202,203] *S. aureus* is therefore an important target bacteria for development of new antimicrobials. The importance of finding new antimcrobials with efficacy towards *S. aureus* is evident from the prevalence of bacteria in hospital settings, the severity of the infections, and the increasing number of infections caused by resistant strains (MRSA and VRSA).

Enterococcus faecalis

E. faecalis is an opportunistic Gram-positive bacteria in the *Enterococcus* genus that inhabits the gastrointestinal system (GI-system) of humans and other mammals.[204] This bacteria and other enterococci are among the leading causes of hospital-acquired infections, second only to *Staphylococci* bacteria.[196] *E. faecalis* can cause life-threatening infections in humans, most notably endocarditis and meningitis.[204] In addition to the severity of the infections caused by the bacteria, the prevalence of resistant enterococci is increasing. Many enterococci are intrinsically resistant to β-lactams and only moderately susceptible to aminoglycosides. Additionally, the number of reported cases of acquired vancomycin-resistance is increasing.[205] The aquired resistance of enterococci is thought to be connected to its natural habitat, as the bacteria are able to reside in the GI-tract for extended amounts of time.[206] Enterococci residing in the GI-tract of hospitalized patients can then act as reservoirs for spreading antibiotic resistance determinants. The severity of the infections caused by this bacteria and its increasing prevalence of resistance, makes it an important target for antimicrobial research. Being able to selectively kill *E. faecalis* with novel antimicrobials will therefore

be important for combating so-called hospital-infections in the future.

Streptococcus agalacticae

S. agalacticae, also known as group B streptococcus (GBS), is a Gram-positive bacteria that colonizes the GI-system in many adults.[207] In most cases, carriers of this bacteria are asymptomatic and do not display any sign of disease. However, as *S. agalacticae* is classified as an opportunistic pathogen, it can also cause severe life-threatening infections in the carrier.[207,208] In addition to causing a range of infections in adults (e.g. endocarditis and meningitis), GBS is also the most common cause of neonatal infections.[209] The mother carries GBS in the lower reproductory tract, which then is transmitted to the infant during birth.[210] Neonatal infections are highly problematic due to the high mortality rates for infants contracting these infections.[211,212] Identification of pregnant carriers and suitable antibiotic treatment before and during labor is therefore important for diminishing the numbers of neonatal infections. The antibiotic of choice for treatment of GBS is usually penicillins, but macrolides, clindamycin, and vancomycin are frequently used in cases of β-lactam allergies.[212] And as with most other pathogens, resistant strains of GBS is occurring more frequently than before.[213] The most problematic resistance seems to be towards clindamycin and the macrolides, but there are also reported cases of vancomycin-resistant GBS.[214] Penicillins, on the other hand, seems to remain efficacious for the time being, but increasing resistance towards the main treatment options in allergic patients is a significant problem. Finding novel antimicrobials with efficacy against GBS and providing alternative treatment options is therefore important for the management of infections caused by GBS.

1.4.2 Gram-negative Bacteria

Gram-negative bacteria does not stain in the Gram staining test, due to their different envelope composition compared to the peptidoglycan-rich Gram-positive bacteria. The cell envelope of Gram-negative bacteria consists of three main parts; the outer membrane, the peptidyglycan cell wall, and the cytoplasmic membrane.[193] The outer membrane is one of the factors that make Gram-negative bacteria less susceptible to antibiotics, as this LPS-layer is a very effective barricade.[62,193] Underneath the outer LPS-layer there is a thin layer of peptidoglycan attached to the outer membrane, that has the same structural functions as in

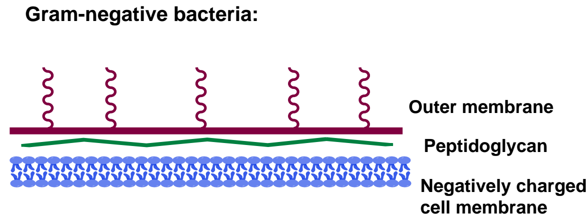


Figure 1.18. The basis of the Gram-Negative bacteria cell envelope.[193,194]

Gram-positive bacteria. However, due to the outer membrane, the peptidoglycan layer in Gram-negative bacteria is a lot thinner than in Gram-positive bacteria. And then follows, after a small compartment called the periplasmic space, the inner membrane which is enriched in negatively charged phospholipids similarly to Gram-positive bacteria. The basis of the cell envelope of Gram-negative bacteria is shown in Fig. 1.18.

The net negative charge from the LPS-layer and the cytoplasmic membrane makes cationic antimicrobials able to target Gram-negative bacteria similarly to Gram-positive bacteria. In order to traverse the LPS-layer, cationic antimicrobials (e.g. AMPs) displaces cations that stabilizes LPS, which leads to destabilization and the antimicrobial may pass the outer membrane.[117] This term is coined "self-promoted uptake" for AMPs.[117]

Pseudomonas aeruginosa

P. aeruginosa is a common Gram-negative bacteria that often causes infections in hospital settings, where the more serious infections often occur together with pre-existing conditions like cystic fibrosis.[215] This opportunistic pathogen is classified as one of the "ESKAPE"-pathogens, which is a group of bacteria thought to pose considerable threat to humans in the form of resistant infections.[83] The severity arises both from the type of infections caused, and the fact that these bacteria have a high degree of intrinsic resistance or rapidly develop resistance to common antibiotics.[216] *P. aeruginosa* has been shown to display an extensive regimen of resistance towards commonly used antibiotics, and there have even been reported cases of pan-resistant strains of *P. aeruginosa*.[217] Many of the most common resistance mechanisms are associated with the expression of ESBLs, biofilm generation, and increased expression of efflux pumps.[216,218,219]

These mechanisms, in addition to the many minor and more specific mechanisms, have made *P. aeruginosa* into a hard bacteria to fight in a clinical setting. Due to the many resistance mechanisms, it shows widespread resistance to β-lactams, quinolones, aminoglycosides, and even colistin in some particularly serious cases.[216,220] Infections caused by Gram-negative bacteria not treatable with polymyxin antibiotics provide few alternative treatment options, and may be very hard to treat. The lack of clinical alternatives to colistin is partially due to Gram-negative bacteria being hard to kill, as they have the LPS-layer and often extensive efflux mechanisms to protect them against antibiotics.[218] Being able to kill *P. aeruginosa* with new antimicrobials is therefore becoming more and more important, as resistance to many conventional antibiotics is becoming a global issue.[83,221]

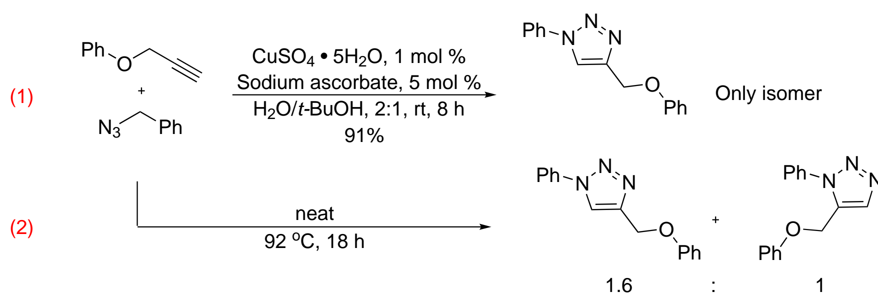
Escheria coli

E. coli is a Gram-negative bacteria that is part of the normal intestinal flora in all mammals, and contributes to keeping a healthy organism and in the production of vitamin K_2.[222] It has also been shown that *E. coli* helps the organism it resides in by hindering the colonization of pathogenic bacteria, and in this way preventing infection.[223,224] Even though most *E. coli* strains are harmless and even beneficial, there are occurences of pathogenic strains.[225] Pathogenic *E. coli* is most often related to cases of food poisoning, where antibiotic treatment is not always necessary. *E. coli* does however come into play as a factor in development of resistant bacteria, as it has been shown to participate in horizontal gene transfer of resistance genes.[226] The transfer of resistance genes, combined with the fact that *E. coli* colonizes the GI-tract of all humans could be problematic. Many systemic antibiotics today are administered orally, and will have some contact with the GI-system. *E. coli* thereby gets exposed to antibiotics used for other purposes, develops resistance and is able to pass that resistance onto other bacteria. Research on new antimicrobials should therefore keep an eye on what happens to *E. coli* when it is exposed to new drugs, even though the drug is intended for other purposes. Also, as *E. coli* helps keeping the GI-system clear of pathogenic bacteria, developing antimicrobials with lowered susceptibility towards *E. coli* may also be beneficial when aiming for oral drug administration.

1.5 1,2,3-Triazoles in Medicinal Chemistry

Ever since the establishment of the "click" chemistry concept in 2001, a large number of papers have been published on the synthesis of 1,2,3-triazole heterocycles.[227] The most important reaction is the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) (1, Scheme 1.1), as it yields the target 1,4-triazole in high yields using simple conditions and workup. Using a copper(I) catalyst in this fashion was presented concurrently by the research groups of Sharpless[228] and Meldal[229] in 2001. These publications revolutionized the application potential of 1,2,3-triazole heterocycles by making it a utilizable functionality. Prior to the introduction of "click" chemistry, 1,2,3-triazoles were usually synthesized through a thermal Huisgen 1,3-dipolar cycloaddition (2, Scheme 1.1).[230] This reaction requires longer reaction times at elevated temperatures and give product mixtures of the 1,4- and 1,5-substituted product. Going from this synthetic regime, to running a reaction in a water mixture for short amounts of time that gives complete regioselectivity is simply astonishing. This greatly improved regimen for synthesis of triazoles has made the triazole scaffold an important tool for synthesis, selective modifications, and couplings.[231–238]

Scheme 1.1. Comparison of CuAAC (1) and classic thermal Huisgen cycloaddition (2).[228]

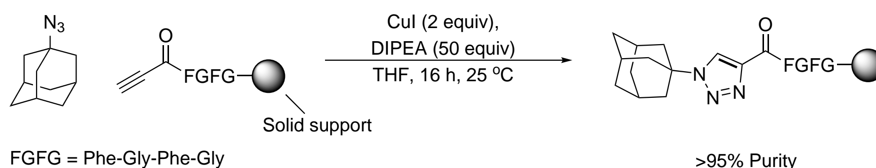


1.5.1 Preparation of 1,2,3-triazoles

The interest in azide-alkyne cycloadditions was rejuvenated by the work presented separately by Sharpless and Meldal.[228,229] The conditions presented by Sharpless are shown in Scheme 1.1, whereas the conditions applied by Meldal in their synthesis is shown in Scheme 1.2. From the two schemes it can be seen

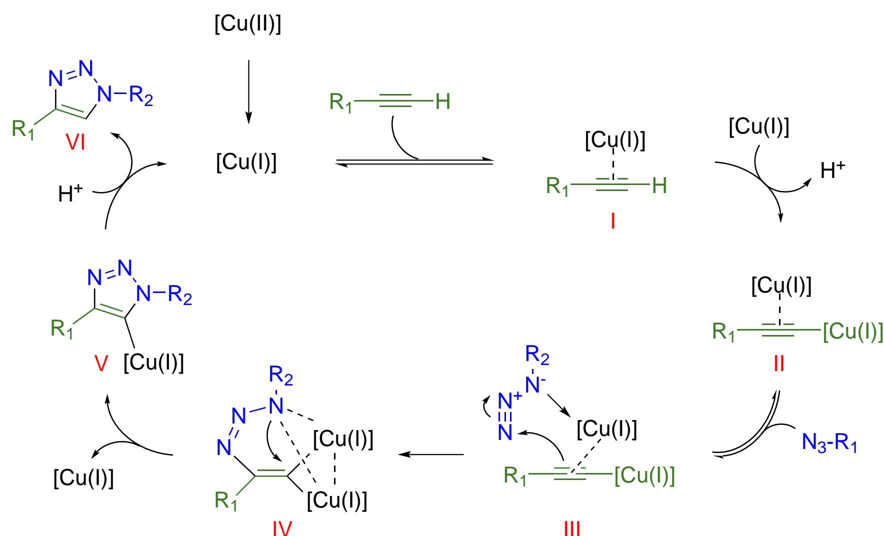
that they used different methods for introducing copper(I) to the reaction. Meldal added a copper(I)-salt directly to the reaction, and Sharpless used a Cu(II) pre-catalyst that was reduced *in situ* by sodium ascorbate. The $CuSO_4$-method by Sharpless has become the most widely applied for synthesis of 1,2,3-triazoles (out of 39157 reactions found in Reaxys; 22862 utilized $CuSO_4$ and 6933 utilized CuI),[239] as it is performed in a water mixture with no addition of base. The base addition is not neccessary as the water acts both as a solvent and a base in the reaction, where a water molecule abstracts the remaining proton when copper is bound to the alkyne (Scheme 1.3).

Scheme 1.2. Example from the reactions presented by Meldal, where an alkynepeptide on solid support was reacted with azidoadamantane in the presence of DIPEA and CuI in THF.[229]



Despite the extensive use of this reaction type, the mechanism for formation of triazoles through CuAAC has remained somewhat elusive. This is mostly due to the tricky characteristics of copper, as copper has a tendency to both disproportionate and aggregate in solution.[240,241] These characteristics make it difficult to keep track of the copper species and their respective effective concentrations. However, on the background of reported studies[242–244] and by using several analytical tools, Worrell *et al.* have proposed a mechanistic cycle (Scheme 1.3) on the background of their observations.[245] For the $CuSO_4$-procedure the reaction starts with reduction of Cu(II) to Cu(I) by sodium ascorbate, followed by copper coordination to the π-system of the alkyne (I). This activation of the alkyne promotes the formation of a copper acetilyde with a π-bound copper atom (II). The π-bound copper then coordinates to the organic azide, which in turn leads to a nucleophilic attack by the β-carbon on the azide in the 3-position and formation of the first covalent bond (III). This initial bond formation is then followed by ring closure and expulsion of one copper atom (IV) and a final substitution of the last copper with a proton (V), leading to product formation (VI) and regeneration of the catalyst.

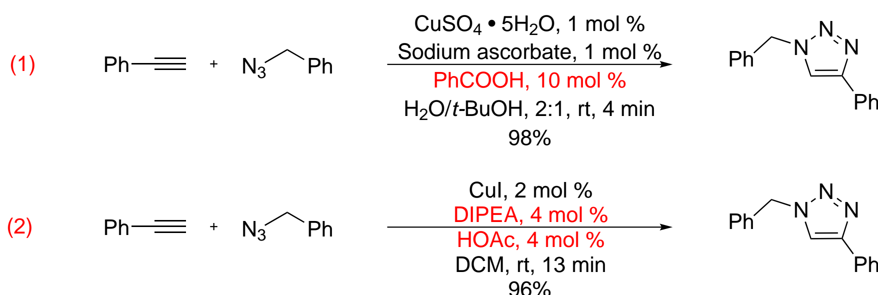
Scheme 1.3. I: π-Binding of copper, II: formation of the copper acetilyde, III: coordination to azide and nucleophilic attack, IV: ring closure, V: substitution of copper with a proton, and VI: product formation and catalyst regeneration.[245]



In the wake of the rigorous investigation on the CuAAC mechanism, some interesting observations were made. First of which, it was seen that presence of an amine additive increased the speed for formation of the copper acetilydes (II). This came as no surprise, as several procedures for preparation of copper acetilydes are performed in aqueous ammonia and the fact that Meldal's procedure involved DIPEA.[229,246,247] The rate enhancement was assumed to come from increased rate in the deprotonation step (I to II) and the ability of the amine to act as a ligand to break up stable copper(I)-aggregates. However, the basic conditions created by the amine slows the conversion considerably, as it slows down the protonation step to form the final product (V to VI).[228] The final protonation step (V to VI) was also shown to proceed poorly with H_2O or an alkyne as the proton source, but proceeded very quickly in an acidic environment created by addition of HOAc.[244] The group of Hu then did a study on the effect of different acids on the CuAAC reaction, and discovered that benzoic acid was an excellent promoter for CuAAC-synthesis of 1,2,3-triazoles (1, Scheme 1.4).[248] They showed that the deprotonation and cycloaddition steps accepted an acidic environment and that

a considerable rate enchancement was observed for the protonation step. Why exactly benzoic acid gave the best rate enhancement was not completely clear, but the bidentate binding to copper is assumed to be of importance. They also observed that non-carboxylic acids and strongly chelating carboxylic acids were not suitable for the reaction. The same group has also published a procedure where they utilized both acid and base in order to enhance the rates of both deprotonation and protonation, in addition to breaking up inactive copper-aggregates that form during the reaction (2, Scheme 1.4).[249]

Scheme 1.4. I: Acid promoted CuAAC, II: Acid-base promoted CuAAC.[248,249]

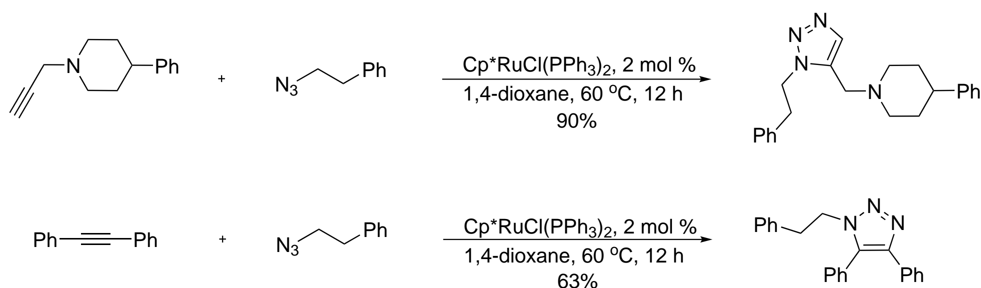


This introduction to the preparation of 1,2,3-triazoles has focused mostly on the CuAAC method for providing 1,4-substituted 1,2,3-triazoles, as these are widely more applied than their 1,5-substituted counterparts (39157 hits for 1,4-substitution vs 2117 for 1,5-substitution in Reaxys).[239] However, there also exist methods for selectively obtaining the 1,5-substituted 1,2,3-triazole through metal catalysis. The perhaps most known method for inducing the 1,5-substitution pattern is the ruthenium-catalysed cycloaddition presented by Fokin (RuAAC) (Scheme 1.5).[250] Additionally, unlike CuAAC the RuAAC-procedure can also be performed with internal alkynes to provide fully substituted 1,2,3-triazoles (Scheme 1.5).

1.5.2 Applications of 1,2,3-Triazoles in Medicincal Chemistry

Ever since the presentation of "click" chemistry and CuAAC in 2001, 1,2,3-triazoles have become popular functionalities in medicinal chemistry.[251–253]

Scheme 1.5. RuAAC for synthesis of 1,5-substituted 1,2,3-triazoles and fully substituted 1,2,3-triazoles.[250]



In addition to their versatile synthesis, 1,2,3-triazoles have some additional features making them interesting for medicinal chemistry applications. The 1,2,3-triazoles are shown to display similar structural properties to amide bonds, making them candidates for bioisosteric replacements in peptides.[237,254,255] The 1,4-substituted 1,2,3-triazole mimics a *trans*-amide bond and the 1,5-substituted 1,2,3-triazole mimics a *cis*-amide bond, as shown in Fig. 1.19. The ability to mimic peptide bonds arises from the similar size, degree of planarity, hydrogen-bonding abilities, and dipole moment of the triazole compared to the amide bond. In addition to being bioisosteric replacements for amides, the 1,2,3-triazoles have been shown to be stable towards proteolytic degradation.[256,257] Substituting a proteolytically labile peptide bond in a drug candidate with a triazole may then increase the *in vivo* stability and give a better metabolic profile.

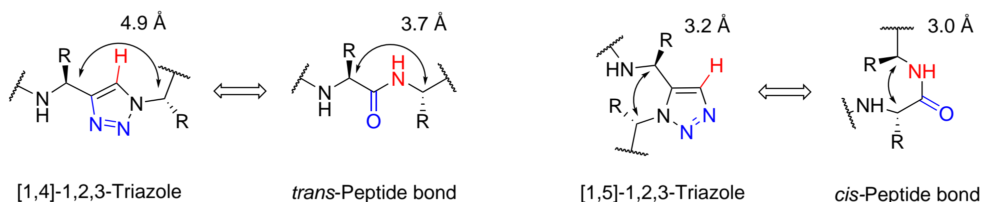


Figure 1.19. Bioisosteric properties of 1,4- and 1,5-substituted 1,2,3-triazoles as peptide bond mimics, hydrogen-bond donors are shown in red and hydrogen-bond acceptors in blue.[255]

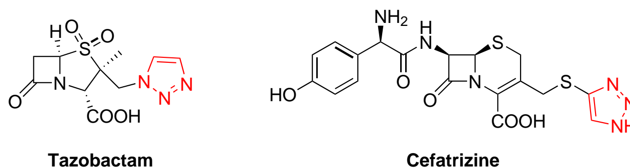


Figure 1.20. Structure of the β-lactamase inhibitor tazobactam[258,260] and the broad-spectrum cephalosporin cefatrizine.[259]

Due to their stability, ability to interact with biological systems similarly to amide bonds (e.g. hydrogen-bonding), and accessible synthesis, there are many examples of 1,2,3-triazoles in drug candidates and in structure-activity relationship (SAR) libraries.[233,251,253,254] Most of the examples utilize the 1,2,3-triazole for connecting biologically active fragments, but there are also some examples using the triazole itself as a structurally important group for biological activity. The β-lactamase inhibitor tazobactam[258] and the broad-spectrum cephalosporin cefatrizine[259] (shown in Fig. 1.20) are examples of drugs utilizing the 1,2,3-triazole ring as a part of the biologically active motif.

Most triazole synthesis in drug discovery today is nonetheless applied in order to link biologically important fragments together. This concept is often utilized in high-throughput screening (HTS) and fragment-based drug design (FBDD), with some examples shown in Fig. 1.21.[251,253,254] Triazoles in HTS are most often used to increase the diversity of a compound library by affording versatile conditions through the CuAAC reaction. This is done by making a range of alkynes and azides and coupling them together in different combinations, and creating large libraries for screening. The utilization of triazoles in FBDD is somewhat analogous to the strategy from HTS, however the approach towards finding drug candidates is slightly different. The concept of FBDD is based on screening for structural fragments that contribute to favorable ligand-protein binding, whereupon combination of promising fragments will afford a high-affinity drug candidate.[251] The different fragments may then be equipped with synthons for 1,2,3-triazole synthesis, in order to provide the combined fragment products through the CuAAC reaction. The process of linking fragments together can also be performed *in situ*, where the fragments are allowed to template the target before they are coupled together. This form of FBDD is called dynamic template-assisted drug discovery.[251]

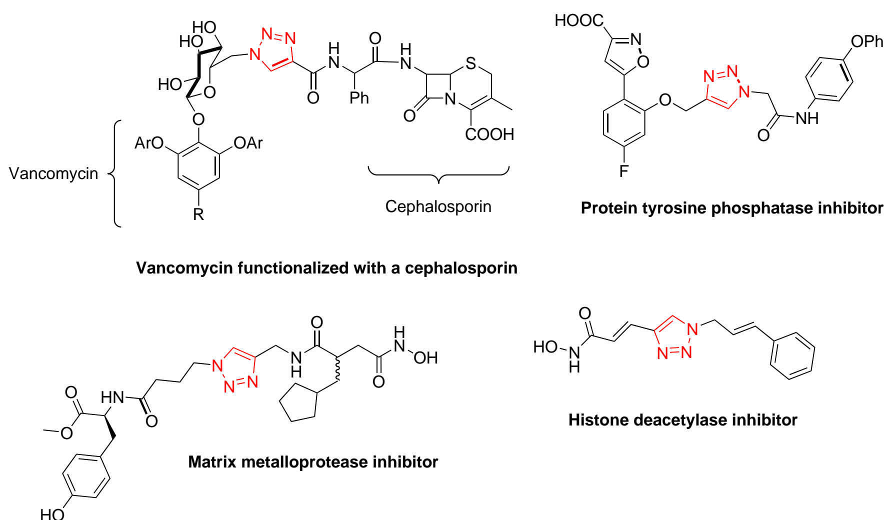


Figure 1.21. Examples of 1,2,3-triazoles as fragment linkers in drug discovery: vancomycin functionalized with a cephalosporin,[261] a protein tyrosine phosphatase inhibitor,[262] a matrix metalloprotease inhibitor,[263] and a histone deacetylase inhibitor.[264]

As a final note in this section, 1,2,3-triazoles have also been extensively used in bioconjugation chemistry. Bioconjugation is the concept of forming stable and covalent links between molecules, where at least one of the coupling partners is a biomolecule.[265] The reason for using triazole chemistry in the field of bioconjugation can be partially attributed to the advantages described above, but also the fact that both coupling partners in an alkyne-azide coupling are bioorthogonal functional groups.[266] The concept of bioorthogonal functional groups, and the chemistry between them, was presented by the group of Bertozzi, and involves using functional groups in couplings that does not interfere with biological systems. In that way one can perform selective chemical transformations in living systems. CuAAC has been successfully used to selectively modify virus particles, nucleic acid, and proteins in tissue lysates.[267] One drawback of using the CuAAC method in living systems is the cytotoxic properties of accumulated copper in the organism. The group of Bertozzi has therefore developed a copper-free strain-promoted coupling reaction shown in Fig. 1.22, which successfully has been used in living systems.[266,268]

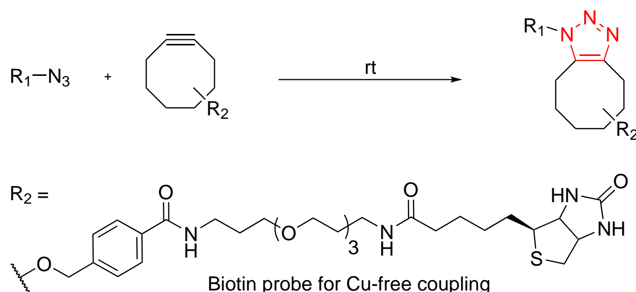


Figure 1.22. The copper-free azide-alkyne cycloaddition reaction presented by Bertozzi, and an example of a biotin-functionalized cyclooctyne probe.[266]

1.6 Isoindolines and Dihydro Pyrrolopyridines in Medicinal Chemistry

This final section of the introduction will give a brief introduction to isoindolines and fused pyridines (dihydro pyrrolopyridines), as well as how to prepare them using [2+2+2] cycloaddition reactions. The isoindolines and dihydro pyrrolopyridines are often seen used as functional groups in medicinal chemistry, and they can be found in natural products. One example of the isoindoline skeleton in a natural product is the alkaloid ±-chilenamine, which is shown in Fig. 1.23 together with some drug candidates utilizing isoindolines and dihydro pyrrolopyridines.[269–272]

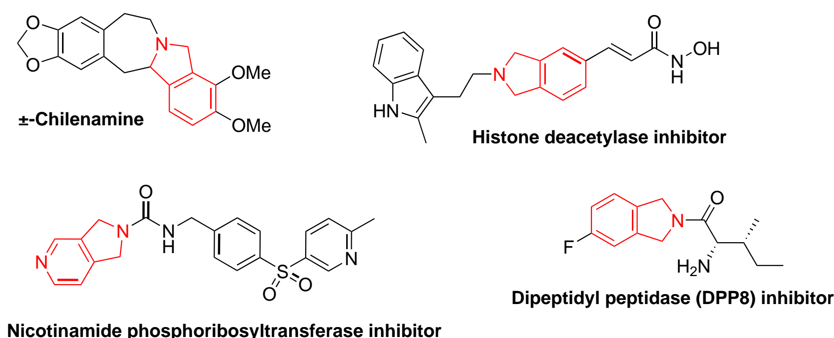


Figure 1.23. The natural alkaloid ±-chilenamine,[269] a histone deacetylase inhibitor,[270] a dipeptidyl peptidase (DPP8) inhibitor,[271] and a nicotinamide phosphoribosyltransferase inhibitor.[272]

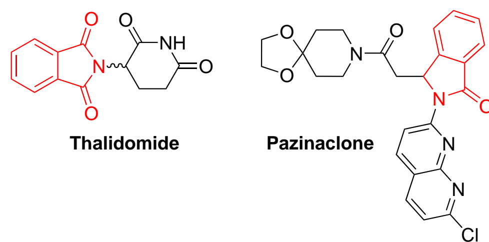


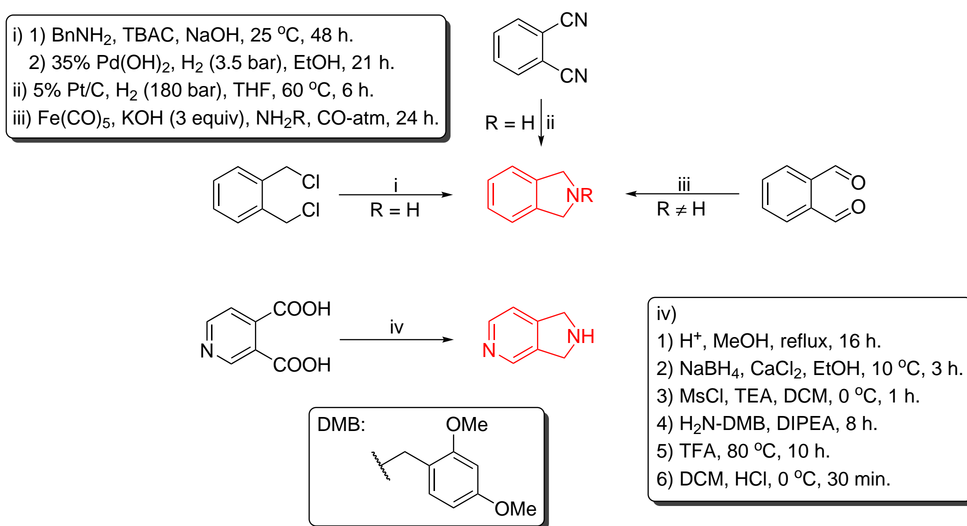
Figure 1.24. Thalidomide and pazinaclone.[277,279]

The oxidized versions of isoindolines; the phthalimides (1,3-dihydro-2*H*-isoindole-1,3-dione) and the isoindolinones (1,3-dihydro-2H-isoindole-1-one), are also functionalities found in drugs and drug candidates.[273–276] Most known to the public is perhaps the infamous phthalimide-based drug thalidomide (shown in Fig. 1.24), that was marketed to alleviate morning sickness during pregnancy.[277] But, in addition to the desired effects, thalidomide also caused phocomelia (where infants were born with limb malformations) and fetal/neonatal deaths, as a result from the teratogenic effects of the drug. Thalidomide is today used in treatment of multiple myeloma.[278] Lastly, an example of a drug utilizing the isoindolinone skeleton is the sedative/anxiolytic drug pazinaclone (shown in Fig. 1.24).[279]

1.6.1 Preparation of Isoindolines and Dihydro Pyrrolopyridines

Isoindolines can be prepared from several starting materials as shown in Scheme 1.6. Firstly, 1,2-bischloromethyl benzene can be doubly aminated under basic conditions to give isoindoline upon *N*-deprotection.[280] Furthermore, phthalonitrile can be subjected to pretty harsh hydrogenation conditions to afford isoindoline,[281] and lastly, phthalaldehyde can be reacted with a primary amine in the presence of $FeH(CO)_4$ (made *in situ* from $Fe(CO)_5$) to give the *N*-substituted isoindoline product.[282] For the synthesis of dihydro pyrrolopyridine, a pyridine dicarboxylic acid can be turned into dihydro pyrrolopyridine over six steps.[283]

Scheme 1.6. Synthetic procedures for preparation of isoindoline and dihydro pyrrolopyridine.[280–283]

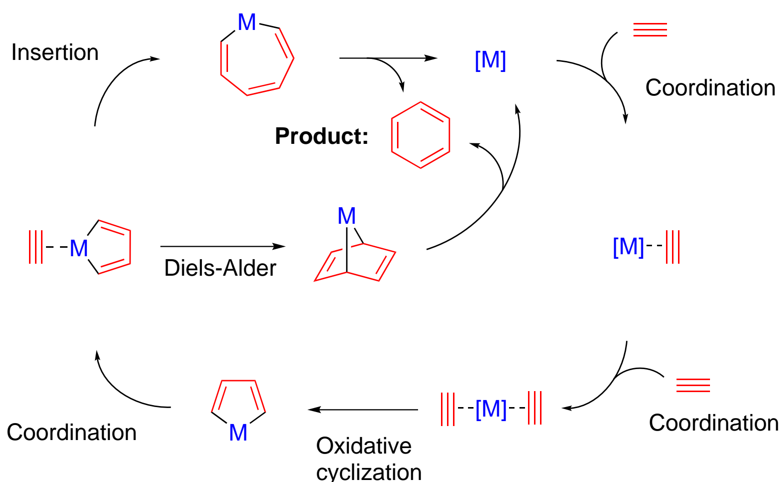


Transition Metal-Catalyzed [2+2+2] Cycloaddition Reactions

Due to their bicyclic nature, isoindoles and dihydro pyrrolopyridine with interesting substitution profiles can also be prepared through transition metal catalyzed [2+2+2] cycloaddition reactions. Alkyne cyclotrimerization was discovered by Reppe *et al.* in 1948, when they isolated benzene as a by-product from an attempt to prepare cyclooctatetraene from acetylene using a Ni-catalyst.[284] Ever since then, [2+2+2] cycloaddition has become an ever expanding reaction type that now encompasses a range of substrates and transition metal catalysts (Co, Rh, Ru, Ir, Pd, and Ni).[285–288] Additionally, cyclotrimerization has also become an important tool for providing aromatic substitution patterns that are not readily obtainable from nucleophilic aromatic substitution (NAS) or electrophilic aromatic susbstitution (EAS), as the aromatic electronic effects does not come into play untill after the synthesis of the ring system.

The exact mechanism of how alkynes trimerize in the presence of a catalyst is still under some discussion, but the most commonly accepted mechanism is displayed in Scheme 1.7.[285,287,289] The reaction is initiated by coordination of two alkynes to the metal catalyst, which then is followed by an oxidative cyclization

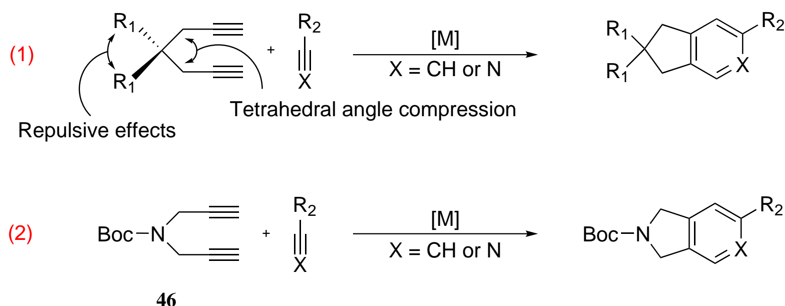
Scheme 1.7. The most agreed upon reaction mechanism for transition metal catalyzed cyclotrimerization.[285,287,289] Firstly, the two alkynes are consequtively coordinated to the metal, before formation of a five-ring metallacycle. The metal then coordinates to the final alkyne which then is incorporated either through an insertion type reaction or a Diels-Alder-like cycloaddition. The metal is then reductively eliminated from the cycle and the product is formed together with regeneration of the metal catalyst.



to form a metallacycle. The metallacycle formation is also assumed to be the rate determining step for the catalytic cycle. The metal then coordinates to the third and final alkyne, which then is either inserted to make a seven-ring metallacycle or undergoes a Diels-Alder-like cycloaddition. The Diels-Alder type of addition of the alkyne is favored when the alkyne is electron deficient and the metallacycle has an electron-rich cyclopentadiene character. The last step of the cycle is the removal of the metal through reductive elimination, giving the trimerized product (in this case benzene) and the regenerated catalyst.

One of the major drawbacks for cyclotrimerization reactions is the formation of regioisomers, leading to complex product mixtures.[285,287–289] Just by doing a selftrimerization with an unsymmetrical alkyne there are two possible regioisomers. Imagine then the complexity of utilizing three different alkynes in this type of cyclization. Fortunately, today there exist both catalysts and methods for obtaining the desired selectivity through fine-tuning of the reaction conditions. Additionally, a popular method for reducing the number of possible regioismers

Scheme 1.8. 1) A tether inducing the Thorpe-Ingold effect through tetrahedral angle compression.[291] 2) *N*-Boc protected alkynylated propargylamine tether (**46**) in cyclotrimerization with an alkyne or a nitrile.[292–295]



is to connect two alkynes through some kind of tether, forcing the orientation of these two alkynes in the reaction. It is also important to choose the correct tether for the reaction, as there are secondary effects coming into play besides the locked orientation of the alkynes. By using a tether that forms a five-ring in the bicyclic product, one can achieve an entropic bonus over using a same type of tether forming a six-ring.[290] The use of sterically crowded tethers is also popular, as it forces the alkynes closer in order to form the metallacycle.[291] A tether with a quaternary center will also induce a tetrahedral angle-compressing effect on the alkynes and force them closer to each other, this effect is commonly known as the Thorpe-Ingold effect. Hence, using a tether as shown in reaction 1 in Scheme 1.8 can lead to enhancement of reactivity, in addition to helping minimize by-product formation, and leading to the wanted product in good yields.

One tether that has been used for cycloaddition and utilizes both the concepts presented above is based on an alkylated propargylamine (**46**), Scheme 1.8.[292–295] The tether **46** is prepared from carbamate protection of propargylamine followed by *N*-alkylation with propargyl bromide.[295,296] The tetrahedral angle-compression in this tether comes from the lone pair on nitrogen and the carbamate protecting group. As can be seen from reaction 2 in Scheme 1.8, the bis-alkyne **46** can then be turned into an isoindoline or a dihydro pyrrolopyridine through [2+2+2] cycloaddition with an alkyne or nitrile respectively.[297]

CHAPTER 2

DEVELOPMENT OF CATIONIC AMPHIPHILIC ANTIMICROBIALS

This chapter is divided into four main sections, where three parts focus on different parts of the prepared compound library and the last part compares the best compounds. The first section (Section 2.1) is based on Paper II, and presents the development of antimicrobial amphiphiles based on aliphatic amino 1,2,3-triazoles.[298] Following the first section is the work presented in Paper III (Section 2.2), where a different approach to creating amphiphiles based on amido 1,2,3-triazoles and related compounds was investigated.[299] The third section (Section 2.3) shows the work presented in Paper IV, and covers the development and antimicrobial evaluation of fused pyridine (dihydro pyrrolopyridines) and isoindoline amphiphiles.[297] The last section (Section 2.4) compares the most promising compounds from Papers II, III, and IV with regards to their biological activities. Paper I is not presented in a separate section, as it is a method paper describing a procedure that has been applied in all the subsequent publications II-IV.[300]

All the target compounds presented in the different papers and this chapter were based on the principle of amphiphilic antimicrobials presented in Section 1.2.2. The material presented in the introduction concerning AMPs, synthetic peptide mimics, and marine antimicrobial natural products, have given a non-specific model for antimicrobial amphiphiles. The model and the target structures presented in the papers and subsequent sections is rationalized in Fig. 2.1, and show the antimicrobial amphiphilic natural products presented in Fig. 1.15 rationalized into a general structure. The amphiphilic model structure is divided into three parts: a lipophilic part often carrying an aromatic group, a linker or core skeleton, and a hydrophilic end carrying a cationic nitrogen. In both Papers II and III the linker was a 1,2,3-triazole, where the chains leading to the cationic

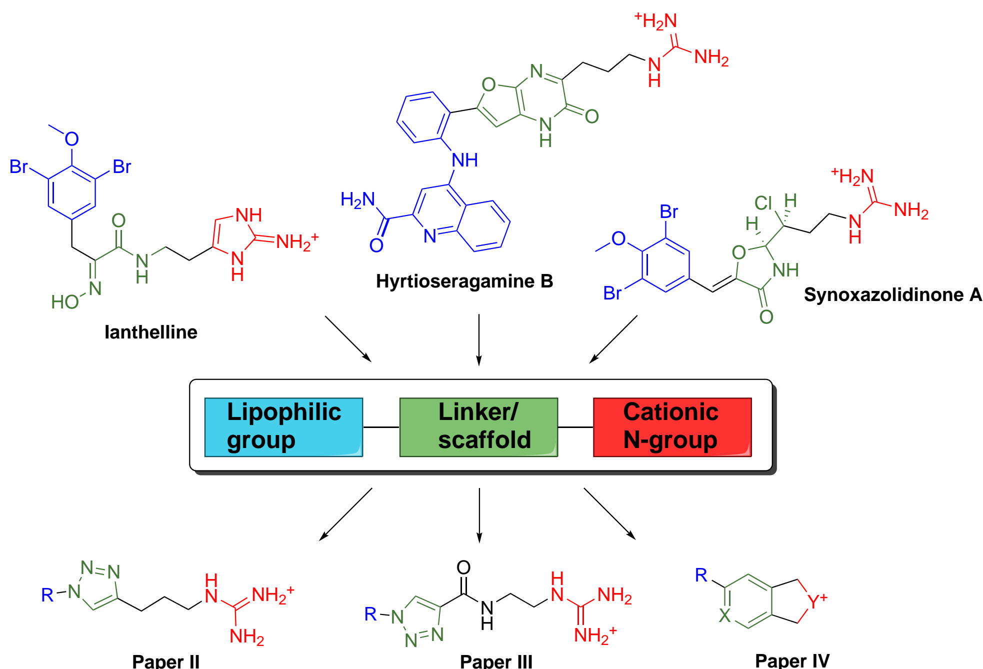


Figure 2.1. Model for target amphiphiles derived from the marine natural product antimicrobials ianthelline,[172] hyrtioseragamine B,[174] and synoxazolidinone A.[173]

group were different in the two series (as seen from Fig 2.1). In Paper IV, the skeleton was a fused pyridine (X = N, Y = NH_2^+) or an isoindoline (X = CH, Y = NH_2^+ or guanidine) functionalized with a cationic group that was part of the bicyclic core structure.

2.0.1 General Information Regarding Biological Testing

After preparation and confirmation of purity by HPLC (>95%), the target amphiphiles were submitted to MarBio (UiT - The arctic university of Norway) for biological evaluations. The sections in Papers II, III, and IV concerning biological evaluations are divided into separate subsections concerning antimicrobial activity and cytotoxicity against human cell lines. The assays were performed by first doing single-concentration evaluations (50 or 64 μg/mL), followed by dose-response assays to quantitatively determine the activity level. The antimicrobial evaluations of the prepared amphiphiles were performed against the bacteria introduced in Section 1.4; *Staphylococcus aureus* (ATCC 25923), *Enterococcus*

faecalis (ATCC 29212), *Streptococcus agalacticae* (ATCC 12386), *Pseudomonas aeruginosa* (ATCC 27853), and *Escheria coli* (ATCC 25922).

As this was a pursuit of possible leads for future drugs, it was also important to assess the toxicity of the compounds displaying interesting antimicrobial activities (see Section 1.3.3). The most potent antimicrobial compounds from the different series were therefore evaluated against HepG2-cells (ATCC HB-8065) in order to evaluate their *in vitro* cytotoxicity against human liver cells. Dose-response curves plotted using non-linear regression were used to find the HepG2 EC_{50}-values for the target amphiphiles.

2.0.2 General Information Regarding Calculated Properties and Dose-Response Curves for HepG2

All physicochemical properties discussed for compounds in this dissertation were calculated using the "MarvinSketch 16.11.7"-software from ChemAxon, this includes partition coefficients (ClogP/ClogD), polar surface area (PSA), and pKa-estimates.[301] The dose-response curves from the data obtained in the HepG2-assays were plotted through a four-variable normalized non-linear regression using the "GraphPad Prism 7.02"-software from GraphPad Software.[302]

2.1 Paper II: Synthesis and Antimicrobial Evaluation of Amphiphiles Based on Aliphatic Amino 1,2,3-Triazoles

This section will present the preparation and antimicrobial evaluations of 42 1,2,3-triazole amphiphiles. The synthesis of 28 target molecules and their intermediates is presented in the supporting information of Paper II, whereas the experimental data for the remaining 14 compounds and their intermediates is presented in Sections 4.1.1 - 4.1.4 and 4.1.8. Experimental data for the azides **11g** and **11j** can be found in the experimental section of Paper III. Additionally, the general experimental information is found in the supporting information of Paper II. Information regarding the experimental procedures for the biological assays is also found in the supporting information of Paper II (HepG2 experimental details are found in the experimental part of Paper III). In order to provide a simple overview and allow for easier compound referencing, all the 42 target structures are presented in Fig. 2.2 with a number for the thesis as well as their corresponding compound number from paper II (in parantheses). The numbering was conducted in such a way that the different lipophilic groups were denoted with the same letter throughout the series of 1,2,3-triazoles (e.g. **a**: R = Ph). Therefore, somewhat strange numbering events may occur, especially in the synthesis section (e.g. bromoheptane = **9j**). It was done in order to attach one label to each lipophilic group throughout the series. It should be noted that the numbering method in Paper II is different from the one in Section 2.1, where numbers denoted the lipophilic groups and sub letters the cationic *N*-groups.

The 1,2,3-triazole linker was chosen on the background of the simple synthesis through the CuAAC reaction, and favorable biological properties depicted in Section 1.5.2. The objective was then to create amphiphiles following the antimicrobial model presented in Fig. 2.1. The 24 first amphiphiles prepared in this library (**1a** - **6d**), were also the first compounds prepared in the overall project. These structures were used to develop the synthetic protocols and to carry out the initial antimicrobial investigations. Some of the amphiphiles in this series (**1a** - **6d**) therefore have functional groups that were not utilized in later substrates (e.g. R = Ph, Fig. 2.2), due to the lack of antimicrobial activity. Additionally, the later compounds prepared in this series were prepared concurrently to most of the target structures presented in Section 2.2.

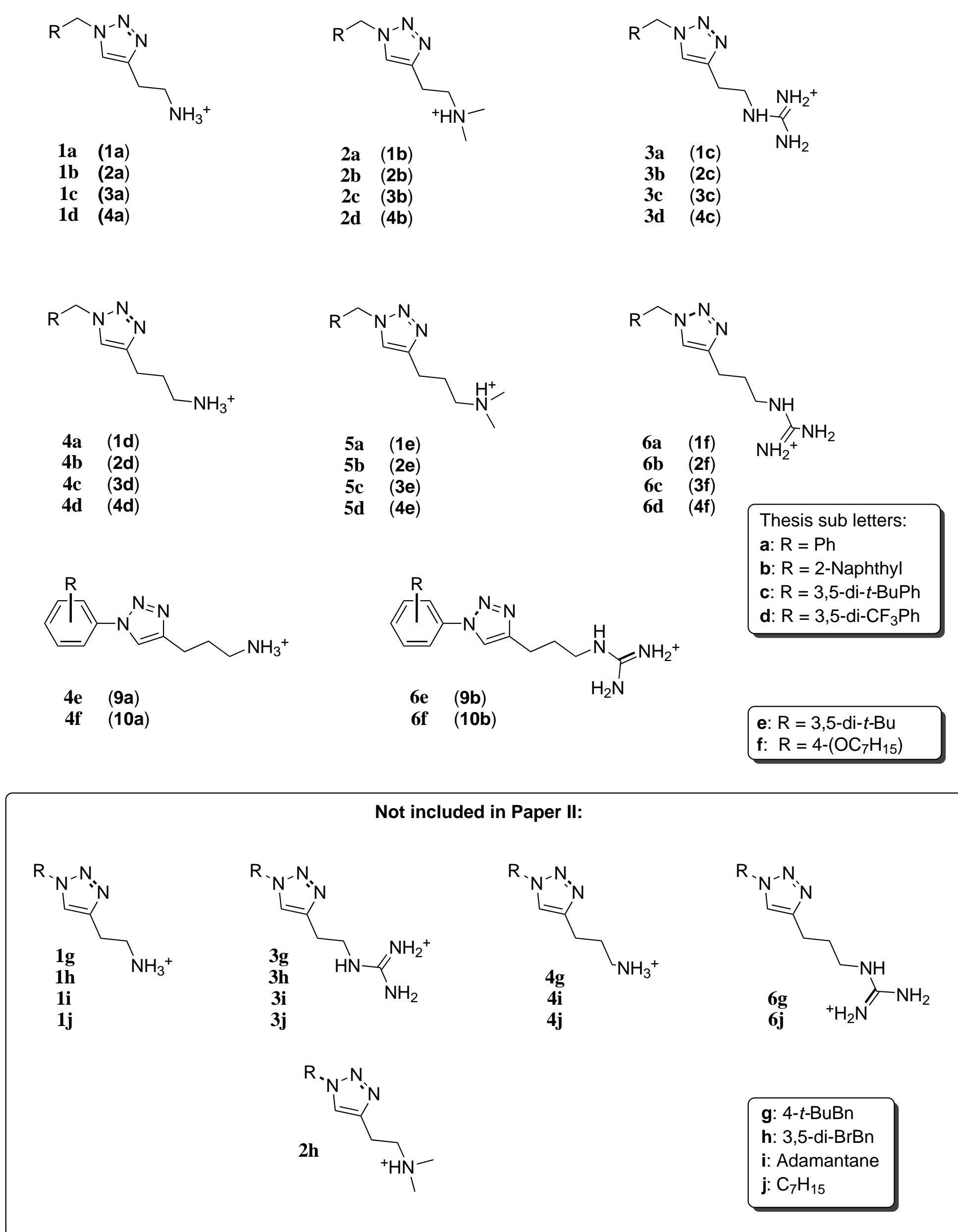
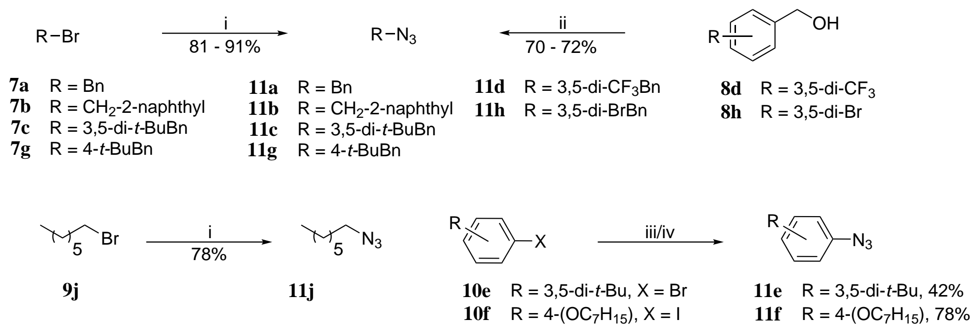


Figure 2.2. The 42 target structures presented in this section, the numbers in parantheses are the numbers used in Paper II. Counterion: Cl^{-}.

Scheme 2.1. i) NaN_3, 19 - 24 h and conditions: acetone/water (4:1) at rt for **11a**,[303] DMSO at 45 °C for **11b**,[304] acetone at reflux for **11c**, and DMF at 45 °C for **11g** and **11j**.[305] ii) DPPA, DBU, 0 °C - rt, 22 - 28 h.[306] iii) For **11e**: NaN_3, CuI (10 mol %), L-proline (30 mol %), NaOH (30 mol %), EtOH/H_2O 7:3, 95 °C, 23 h.[307] iv) For **11f**: NaN_3, CuI (10 mol %), L-proline (20 mol %), NaOH (20 mol %), DMSO, 60 °C, 14 h.[307]

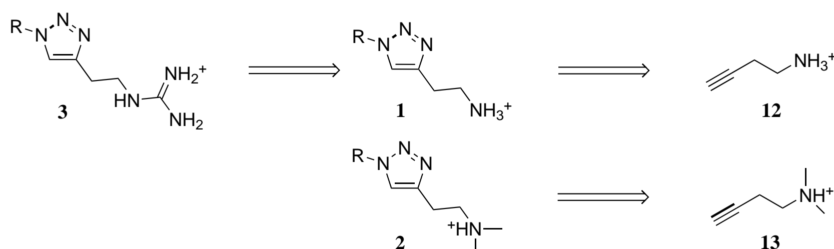


2.1.1 Synthesis

In order to prepare the 1,2,3-triazole amphiphiles displayed in Fig. 2.2, the appropriate coupling partners for the CuAAC reactions had to be prepared. It was decided to have the lipophilic group carry the azide and the cationic moiety carry the alkyne. The azides **11** were then prepared through established procedures from commercial starting materials, as displayed in Scheme 2.1. The benzylic azides (**11a** - **11d**, **11g**, and **11h**) were prepared from their corresponding benzyl bromides (**7a** - **7c** and **7g**) or alcohols (**8d** and **8h**),[303–306] heptylazide (**11j**) was prepared from bromoheptane (**9j**) similarly to the benzylic azides, and azidoadamantane (**11i**) was commercially available. The phenylic azides **11e** and **11f** were prepared through a procedure described by Zhu *et al.*, where the azides were prepared from the corresponding bromo- (**10e**) or iodo-benzenes (**10f**) through a copper(I)- and proline-catalyzed transformation.[307] It should be noted that **10f** was prepared from iodophenol through *O*-alkylation using **9j** and K_2CO_3 in DMF (69% yield).[308]

The plan was initially to couple the azides **11** with the alkynes **12** and **13** through CuAAC, and in that way prepare the target compounds directly from the triazole synthesis (Scheme 2.2). The azides had already been prepared in one or two steps, and the alkyne amines **12** and **13** could be prepared in two or

Scheme 2.2. Initial strategy for creating the target structures **1**, **2** and **3**. The alkynes **12** and **13** could be prepared from 3-butynol through mesylation, azidation, and reduction (**12**) or mesylation and amination (**13**).



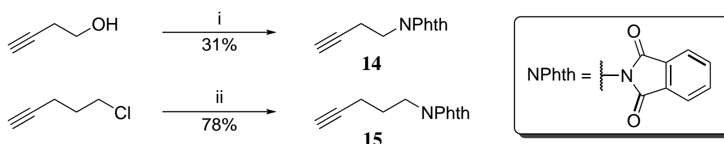
three steps from 3-butynol. There were unfortunately observed some drawbacks when attempting CuAAC with these substrates. Firstly, **12** and **13** proved to be somewhat difficult to prepare, as the reduction and amination were low-yielding and the purifications of **12** and **13** were challenging. Additionally, attempts at coupling **12** and **13** with **11** to give the target 1,2,3-triazoles gave slow converting reactions, incomplete conversion, and purification issues.

It was concluded, from these initial observations, that *N*-protection would provide easier synthesis and less challenging purification of intermediates. The phthalimide group was then chosen as the preferred protecting group, as the phthalimide-protected alkyne amines **14** and **15** were available from commercially available reagents in one step as displayed in Scheme 2.3. The protected alkyne **14** was obtained from doing a Mitsunobu reaction with 3-butynol,[309] whereas **15*** was obtained from a Gabriel reaction with 5-chloropentyne.[310] In addition to the simple one-step preparation of **14** and **15**, removal of the phthalimide group is usually fairly straightforward, where the amine is liberated through a hydrazinolysis.

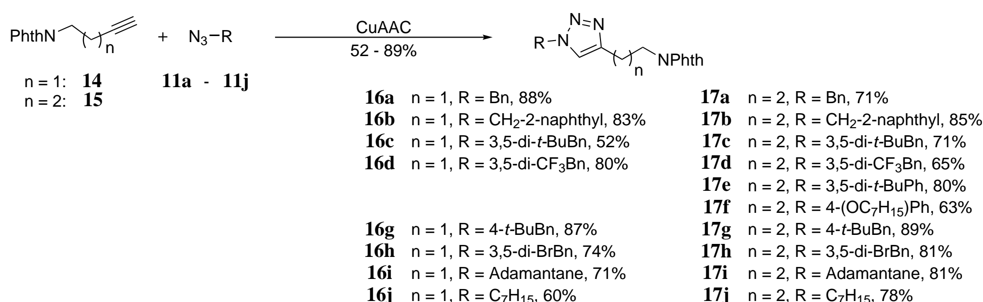
After preparation of the azides **11a - 11j** (**11i** was not prepared as it was commercially available) and the alkynes **14** and **15**, the corresponding 1,2,3-triazoles **16a - 17j** (except for **16e** and **16f**) were prepared in moderate to good yields as shown in Scheme 2.4. All of the 1,2,3-triazoles were prepared using the acid-promoted CuAAC conditions by Shao *et al.* presented in Section 1.5.1.[248] One of the larger benefits of the CuAAC reaction was the simple work-up where, in

*The protected alkyne **15** have also been periodically available from Sigma-Aldrich.

Scheme 2.3. i) Phthalimide, PPh_3, DEAD, toluene/MeOH, rt, 2 h.[309] ii) Potassium phthalate, NaI (cat.), DMF, 100 °C, 12 h.[310]



Scheme 2.4. CuAAC: $CuSO_4 \cdot 5\,H_2O$ (1-5 mol %), sodium ascorbate (2-10 mol %), PhCOOH (10 mol %), H_2O/*t*-BuOH (2:1), 10 min - 50 h.[248]



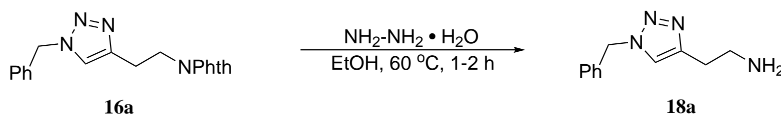
many cases, no chromatographic purification was necessary to obtain the pure products. For the synthesis of **16a - 17j**, only a handful of the reactions were chromatographically purified (**16c**, **17b**, **17c**, and **17e**), the remaining crudes were purified using crystallization in DCM/pentane or washing with H_2O and pentane. Using crystallization techniques in the work-up may also explain some of the more moderate yields, as a slightly higher compound loss can be expected compared to purification with flash column chromatography (FCC).

N-Deprotection of Phthalimido 1,2,3-Triazoles 16 and 17

The most common conditions for removal of the phthalimide protecting group are using hydrazine hydrate in EtOH or MeOH.* Some screening reactions were therefore conducted on **16a** using hydrazine hydrate in EtOH. The triazole **16a** underwent conversion into the neutral **18a** (according to TLC) typically in 1-2 hours at 60 °C, as shown in Scheme 2.5. However, even though the reaction

*23569 out of 25529 entries in Reaxys used either EtOH or MeOH.[311]

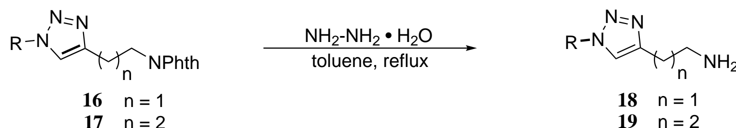
Scheme 2.5. Common reaction conditions for deprotection of phthalimide-protected amines using hydrazine hydrate in EtOH.



seemingly gave full conversion, purification of **18a** proved to be challenging. It had been assumed that **18a** could be obtained from evaporation and extraction under basic conditions, but attempts at extraction gave only small amounts of highly impure **18a**. Other workup techniques were then explored, but neither filtration nor various "solid-phase extraction"-resins gave **18a** in higher purities.

In an attempt to circumvent the purification issue, a less polar solvent was used in the reaction. This was done based on a patent showing that filtration of the reaction mixture gave the deprotected pure amine as the filtrate when using toluene as solvent.[312] The conditions were therefore changed to the ones shown in Table 2.1, where EtOH at 60 °C was swapped for toluene at reflux. These conditions gave **18a** in good yield and sufficient purity after filtration and purification with FCC. However, most of the remaining amino 1,2,3-triazoles **18** and **19** were sufficiently pure after evaporation of the filtrate and did not require purification with FCC. Some amines that needed additional workup were **19g**, **19h**, and **19i**. Out of these, only **19i** (entry 17) was purified with FCC analogously to **18a** (entry 1). The amine **19g** (entry 15) was taken to the next step as a mixture of **17g** and **19g** without further purification, whereas the impure **19h** was not taken further. Some observations were also made concerning the reaction times and amounts of hydrazine added in some cases. It was observed that the reaction was particularly slow for the least polar compounds with the longest chain (n = 2), the most extreme example being **19f** (entry 14). The deprotection of **17f** to form **19f** (entry 14) was run for 75 h with periodically added hydrazine up to 40 equiv. Even though the reaction mixture was kept at reflux for an extended amount of time, it resulted in high yield (98%) and little observed impurities. Why these reactions were slower to afford complete conversion was not further investigated. The deprotections gave 16 pure (determined by ^{1}H NMR) aliphatic amino 1,2,3-triazoles (two were purified using FCC), and **19g** that was a mixture of **19g** and **17g** (entry 15, Table 2.1).

Table 2.1. Hydrazinolysis of phthalimide-protected 1,2,3-triazoles **16** and **17** to form the aliphatic amino 1,2,3-triazoles **18** and **19**.[312]



Entry	16/17	n	R	NH_2-NH_2 [equiv]	Time [h]	18/19	Yield [%]
1	**16a**	1	Bn	2	1.5	**18a**	53[a]
2	**16b**	1	CH_2-2-naphthyl	4	23	**18b**	74
3	**16c**	1	3,5-di-*t*-BuBn	5	3.5	**18c**	91
4	**16d**	1	3,5-di-CF_3Bn	6	3	**18d**	91
5	**16g**	1	4-*t*-BuBn	7	7	**18g**	93
6	**16h**	1	3,5-di-BrBn	5	2	**18h**	85
7	**16i**	1	Adamantane	6	8	**18i**	83
8	**16j**	1	C_7H_{15}	6	5	**18j**	98
9	**17a**	2	Bn	5	2	**19a**	77
10	**17b**	2	CH_2-2-naphthyl	5	22	**19b**	80
11	**17c**	2	3,5-di-*t*-BuBn	9	8	**19c**	66[b]
12	**17d**	2	3,5-di-CF_3Bn	10	6.5	**19d**	87
13	**17e**	2	3,5-di-*t*-BuPh	15	24[c]	**19e**	89
14	**17f**	2	4-(C_7H_{15}O)Ph	40	75	**19f**	98
15	**17g**	2	4-*t*-BuBn	20	50	**19g**	65[d]
16	**17h**	2	3,5-di-BrBn	-	-	**19h**	-[e]
17	**17i**	2	Adamantane	5	9	**19i**	65[a]
18	**17j**	2	C_7H_{15}	7	9	**19j**	32

[a] FCC: **18a**: $CHCl_3$/MeOH/NH_4OH 70:30:3, **19i**: $CHCl_3$/MeOH/TEA 80:20:1.
[b] Done in two steps: 1) 7.5 equiv for 4.5 h. 2) 1.5 equiv for 4.5 h.
[c] + stirred for 44 h at rt.
[d] Not pure, est. 50 wt % from ^{1}H NMR.
[e] Highly impure after workup, not taken further.

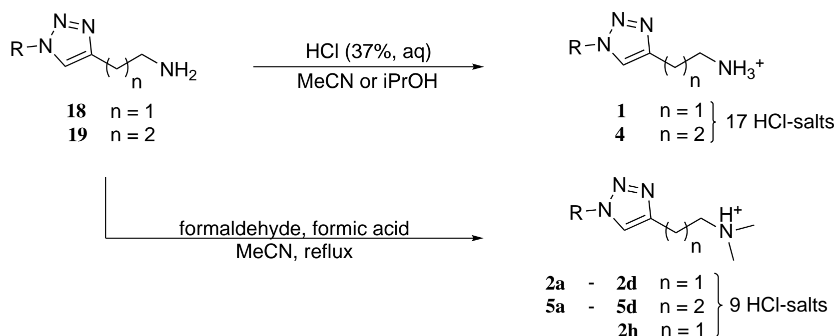
N-Functionalization of Aliphatic Amino 1,2,3-Triazoles 18 and 19

The final step for preparing the target amphiphiles displayed in Fig. 2.2, was to introduce the wanted cationic *N*-groups on the hydrophilic end. The three different target cationic groups in this series were primary amine salts (**1** and **4**), tertiary dimethylamine salts (**2** and **5**), and guanidine salts (**3** and **6**). The HCl-salts **1** and **4** were prepared from treatment of **18** and **19** with HCl (37%, aq) in *i*-PrOH or MeCN* as seen in Table 2.2. The tertiary amine HCl-salts **2** and

*One HCl-salt was not prepared according to these conditions: **1b** was prepared using HCl (2M, Et_2O) in DCM.

5 were obtained from Eschweiler-Clarke reductive amination of **18** and **19** with formaldehyde and formic acid in MeCN or water at reflux, and followed by acidic work-up as shown in Table 2.2.[313,314]

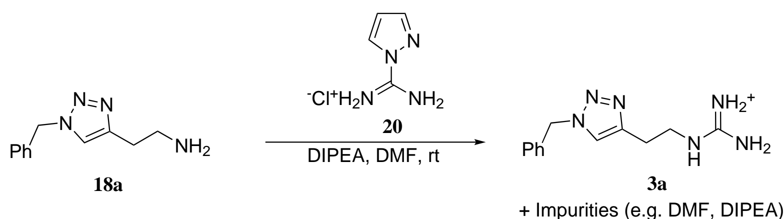
Table 2.2. Preparation of **1**, **2**, **4**, and **5** from **18** and **19**.[313,314] Counterion: Cl^-.



Entry	**18/19**	n	R	**1/4**	Yield **1/4** [%]	**2/5**	Yield **2/5** [%]
1	**18a**	1	Bn	**1a**	20[a]	**2a**	76[b,c]
2	**18b**	1	CH_2-2-naphthyl	**1b**	51[d]	**2b**	40[e]
3	**18c**	1	3,5-di-*t*-BuBn	**1c**	43	**2c**	55
4	**18d**	1	3,5-di-CF_3Bn	**1d**	86	**2d**	67
5	**18g**	1	4-*t*-BuBn	**1g**	100	-	-
6	**18h**	1	3,5-di-BrBn	**1h**	55	**2h**	67
7	**18i**	1	Adamantane	**1i**	100	-	-
8	**18j**	1	C_7H_{15}	**1j**	100	-	-
9	**19a**	2	Bn	**4a**	100	**5a**	53[b,c]
10	**19b**	2	CH_2-2-naphthyl	**4b**	100	**5b**	56[b]
11	**19c**	2	3,5-di-*t*-BuBn	**4c**	36	**5c**	40[f]
12	**19d**	2	3,5-di-CF_3Bn	**4d**	89	**5d**	81
13	**19e**	2	3,5-di-*t*-BuPh	**4e**	100	-	-
14	**19f**	2	4-(C_7H_{15}O)Ph	**4f**	100	-	-
15	**19g**	2	4-*t*-BuBn	**4g**	71	-	-
16	**19i**	2	Adamantane	**4i**	100	-	-
17	**19j**	2	C_7H_{15}	**4j**	100	-	-

[a] Recrystallized from MeCN with a small additive of water (1-3 drops).
[b] Purified with FCC: $CHCl_3$/MeOH/NH_4OH 70:30:3.
[c] Solvent: H_2O.
[d] Prepared from HCl in Et_2O (2 M).
[e] Purified twice with FCC:
1) $CHCl_3$/MeOH/NH_4OH 70:30:3. 2) $CHCl_3$/MeOH/NH_4OH 95:5:1.
[f] Purified with Dowex 50 WX8 ion-exchange resin.

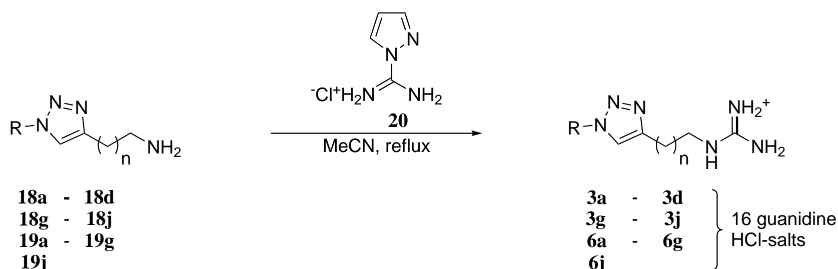
Scheme 2.6. Guanylation using **20** and DIPEA in DMF at rt.[315]



The amphiphiles with a guanidine cationic group (**3** and **6**), shown in Fig. 2.2, were prepared using the electrophilic guanylation reagent 1*H*-carboxamidine hydrochloride (**20**). Bernatowicz *et al.* has shown that **20** can be used to convert various amines into their corresponding guanidium salts in high yields.[315] One of the methods for introducing the guanidyl moiety on an amine presented in this paper, was to add an amine to **20** and *N,N*-diisopropylethylamine (DIPEA) in DMF at room temperature (Scheme 2.6). Applying these conditions in an attempt to prepare **3a** gave full conversion, but removal of impurities like residual base and DMF from the crude **3a** proved to be challenging.

In order to avoid the issues when using DIPEA and DMF, attempts were made to run the guanylation in acetonitrile at reflux without addition of DIPEA. Using acetonitrile at reflux gave full conversion into the guanidines in shorter time, in addition to providing the pure products through simple filtration or crystallization. These observations concerning the reaction conditions became the basis of Paper I in this thesis.[300] The final 16 of the 42 target compounds in Fig. 2.2 were then prepared according to Table 2.3. The yields ranged from very poor (8%, entry 1) to very good (91%, entry 8), where the varying yields may be attributed to their difference in physical properties. More specifically, low yields were likely due to loss of product during workup, as all reactions achieved full conversion (from TLC) before they were stopped and little formation of byproducts was observed. Furthermore, as can be seen from Table 2.3, the guanylation product **6i** was not sucessfully prepared from **19i**. Multiple attempts were made to introduce the guanidinium functionality on **19i**, but ^{1}H NMR analysis of the worked up reactions always showed multiple unidentified byproducts in addition to incomplete conversion. The reason behind the unsucsessful preparation of **6i** was not further investigated, and this target structure was not prepared.

Table 2.3. Preparation of **3/6** from **18/19** and **20** in MeCN at reflux.[300,315] Counterion: Cl^-.



Entry	**18/19**	n	R	**20** [equiv]	Time [h]	**3/6**	Yield [%]
1	**18a**	1	Bn	0.9	4	**3a**	8
2	**18b**	1	CH_2-2-naphthyl	0.9	20	**3b**	44
3	**18c**	1	3,5-di-*t*-BuBn	0.9	1.5	**3c**	63
4	**18d**	1	3,5-di-CF_3Bn	0.9	2	**3d**	53
5	**18g**	1	4-*t*-BuBn	1.0	2	**3g**	40
6	**18h**	1	3,5-di-BrBn	0.9	2	**3h**	57
7	**18i**	1	Adamantane	1.0	5	**3i**	48
8	**18j**	1	C_7H_{15}	0.98	4	**3j**	91
9	**19a**	2	Bn	0.9	2	**6a**	51
10	**19b**	2	CH_2-2-naphthyl	1.0	19	**6b**	76
11	**19c**	2	3,5-di-*t*-BuBn	0.9	3.5	**6c**	63
12	**19d**	2	3,5-di-CF_3Bn	0.9	4.5	**6d**	57
13	**19e**	2	3,5-di-*t*-BuPh	0.95	1.5	**6e**	67
14	**19f**	2	4-(C_7H_{15}O)Ph	0.98	2	**6f**	30
15	**19g**	2	4-*t*-BuBn	0.92	4	**6g**	29
16	**19i**	2	Adamantane	0.99	29	**6i**	-[a]
17	**19j**	2	C_7H_{15}	1.0	5	**6j**	50

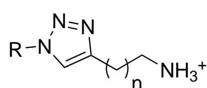
[a] Incomplete conversion and formation of unidentified byproducts.

2.1.2 Biological Evaluation of Target Amphiphiles

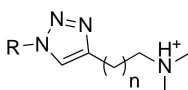
Antimicrobial Activity

The amphiphiles exhibiting any antimicrobial activity (≤64 μg/mL) and their corresponding MIC-values are shown in Table 2.4, together with the HepG2 EC_{50}-values for **4e**, **4f**, **6e**, and **6f**.

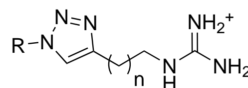
Table 2.4. Minimum inhibitory concentrations (MIC-values) and EC_{50}-values in μg/mL. Counterion: Cl^-.



1c n = 1, R = 3,5-di-*t*-BuBn
4e n = 2, R = 3,5-di-*t*-BuPh
4f n = 2, R = 4-(OC_7H_{15})Ph



5c n = 2, R = 3,5-di-*t*-BuBn



3c n = 1, R = 3,5-di-*t*-BuBn
6c n = 2, R = 3,5-di-*t*-BuBn
6e n = 2, R = 3,5-di-*t*-BuPh
6f n = 2, R = 4-(OC_7H_{15})Ph
6g n = 2, R = 4-*t*-BuBn

	Antimicrobial activities [MIC]					HepG2[b]
	E. faecalis[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	[EC_{50}]
1c	-[c]	50	40	40	50	n.d.[d]
3c	40	20	10	50	40	n.d.
5c	-	40	50	-	-	n.d.
6c	40	10	10	40	40	n.d.
6g	-	64	64	-	-	n.d.
4e	32	16	16	16	32	8.7
4f	16	16	8	8	16	11.7
6e	16	4	4	16	16	18.7
6f	8	4	4	8	8	17.7
Ref.[e]	10	0.13	4	0.5	0.5	n.d.

[a] *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *S. agalacticae* (ATCC 12386), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).
[b] EC_{50} determined from Fig. 2.4.
[c] "-": No activity ≤64 μg/mL.
[d] n.d.: Not determined.
[e] Ref.: gentamicin.

The initial project strategy was to attach the lipophilic aromate in a benzylic fashion to the 1,2,3-triazole ring. The amphiphiles carrying a benzylic functionality (**a**-**d**, **g**, and **h**), were therefore prepared prior to the ones carrying a non-aromatic group (**i** and **j**) or a phenylic substituent (**e** and **f**). The most potent

compound of the benzylic species was found to be the 3,5-di-*t*-Bu–benzyl substituted **6c** with MIC-values ranging between 10 μg/mL and 40 μg/mL against the five test bacteria. All of the five structures with benzylic lipophiles displaying any activity below 64 μg/mL, were carrying the bulky *t*-Bu group either in the 3,5- or the 4-position (**1c**, **3c**, **5c**, **6c**, and **6g**). Amphiphiles with two *t*-Bu groups (**1c**, **3c**, **5c**, and **6c**) were more potent than the amphiphiles with one *t*-Bu group (**6g**). Furthermore, testing of the benzylic structures also showed that the guanidines (**3** and **6**) were more potent than the primary amine HCl-salts (**1** and **4**) and dimethylamine HCl-salts (**2** and **5**).

The inital observations corresponded well with the work presented by Igumnova *et al.*,[180] where they found that aminobenzamide amphiphiles carrying 3,5-*t*-Bu-phenyl and guanidine groups gave high antimicrobial activity. This also correlated well with previous work done by the separate groups of Strøm and Svendsen, where they have shown that large lipophilic bulk and guanidine groups are important for achieving high antimicrobial activity.[157,167,168] However, even though **6c** was the most potent of the benzylic amphiphiles, the potential for optimization was evident when comparing the MIC-values to those of the reference antibiotic gentamicin in the assays.

In an attempt at improving the potency of **6c**, the 3,5-di-*t*-Bu–phenyl ring was attached directly to the 1,2,3-triazole (**6e**). Removal of the benzylic methylene group led to a large increase in antimicrobial efficacy, as seen in Table 2.4 where **6e** was more than twice as potent as **6c**. The increase in antimicrobial activity could be due to steric and rotational effects, as removal of the benzylic methylene would give a more rigid structure. The compounds were assumed to be more rigid due to removal of a rotational bond and increased repulsion between the ortho-protons and the 1,2,3-triazole ring, thus giving a more rigid and "twisted"-like conformation. Removal of the benzylic methylene also give possibility for conjugation between the phenyl and 1,2,3-triazole ring. The possibility for conjugation and having a more restricted conformation was also shown to be beneficial for the aminobenzamides prepared by Igumnova *et al.*[180]

In addition to removal of the benzylic methylene on the amphiphile, several modifications of the lipophilic group were investigated. Substituting the benzylic aromate with a box-like adamantyl structure (**i**) or a linear heptyl chain (**j**) led

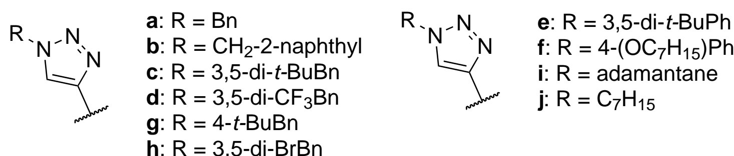


Figure 2.3. Benzylic and non-benzylic lipophilic groups utilized in this series.

to complete loss of antimicrobial activity, as none of the amphiphiles carrying these groups displayed activity ≤ 64 μg/mL. Retaining the phenyl ring and modifying the aliphatic groups attached to the phenyl, on the other hand, proved to be a more promising modification. Where insertion of a 4-heptyloxy group on the phenyl ring instead of the 3,5-di-*t*-Bu-groups led to a 2-fold increase in potency for **4f** against four of the test bacteria compared to **4e**. The 4-heptyloxy was chosen as it gave similar molecular weight and aliphatic contribution compared to the 3,5-di-*t*-Bu-. The guanidines **6** also showed improved activity when equipped with this group, where the 4-heptyloxy-phenyl guanidine **6f** displayed a 2-fold increase in potency against *E. faecalis*, *E. coli*, and *P. aeruginosa* compared to the 3,5-di-*t*-Bu–phenyl guanidine **6e**. The increased activity of **6f** against Gram-negative bacteria was particularly interesting, as they generally are harder to kill due to their LPS outer layer and tendency to express efflux pumps (see Section 1.4.2).

The increase in antimicrobial activity for the 4-heptyloxy-phenyl **4f** and **6f** could possibly be explained by how deep into the membrane the amphiphiles were able to penetrate. The activity could also be explained by increased amphiphilicity of the structures, as this may enhance the interaction with the bacterial cell membrane.[316] Other groups have also had success in using alkyl chains in their amphiphiles for achieving high antimicrobial activities. Zhang *et al.* used two octyl chains in their antimicrobial tartaric acids and Ghosh *et al.* showed that a tetradecyl chain led to high antimicrobial activity for their target compounds.[170,316] Why the amphiphiles equipped solely with a heptyl chain (**j**) did not afford any antimicrobial activity could be due to too little lipophilic contribution of the heptyl group, as the compunds by Zhang *et al.* and Ghosh *et al.* either had several chains or longer chains and a phenyl ring substituent. This was supported by the increase in activity for the 4-heptyloxy-phenyl compounds, and the ClogD-value of **6j** (ClogD = −0.34) compared to that of **6f** (ClogD = 1.16).

In an attempt to rationalize the observed antimicrobial effects on the background of physicochemical properties, the distribution coefficients (ClogD) were calculated for **4e**, **4f**, **6e**, and **6f**, and are shown in Table 2.5.

Table 2.5. ClogD and retention times from C18-HPLC for **4e**, **6e**, **4f**, and **6f**.

	4e	**4f**	**6e**	**6f**
ClogD (pH = 7.40)	2.00	1.41	1.75	1.16
t_R (min)[a]	22.9	20.4	28.0	34.6

[a] C18-HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm).

The ClogD-values shown in Table 2.5 were somewhat surprising, as the more potent compounds were assumed to have a higher lipophilic character. For the four amphiphiles in Table 2.5, **6f** was calculated to be the least lipophilic even though it was the most potent compound in the entire series. The confusing ClogD-values were then compared to retention times from C18-HPLC, which can act as an empiric measure of lipophilicity. When looking at the retention times from Table 2.5, the most potent amphiphile **6f** had the highest HPLC retention time and was therefore the empirically most lipophilic compound of the four. The use of HPLC to measure relative lipophilicity has been reported by Hansen *et al.*, who used HPLC to measure relative lipophilic character for a library of $\beta^{2,2}$-amino acid peptide mimics.[157] Being able to use HPLC to indicate activity-levels of the compounds was useful, as analytical HPLC was run prior to biological testing to confirm their purity. Lastly, the HPLC analyses of the compounds were performed with an acidic additive (0.1% TFA), meaning that the amphiphiles will be mostly in their charged state during the elution. The ClogD-values, on the other hand, take into account the equilibrium of ionic vs. neutral compound at pH = 7.4, which may be the reason behind the conflicting values. Additionally, this information could also indicate that the lipophilicity of the charged amphiphile was important for the antimicrobial activity, and in that way point towards an amphiphilic mechanism of action.

The improved potency of the guanidines **6e** and **6f** over the amines **4e** and **4f** may, however, not only come from difference in relative lipophilic character. The guanidinium group allows for more extensive hydrogen-bonding with appropriate hydrogen-bond acceptors, affording more efficient association with membranes than ammonium groups. This was discussed in Section 1.3.2, where the

group of Wender have shown that guanidinium groups have a higher affinity for membrane components than primary amine groups.[187,188] This could explain the increased potency of **6e** and **6f**, as the guanidine group may offer stronger electrostatic interactions with the bacterial cell membrane. This efficient ionpair-binding may promote the interaction of the lipophile with the lipophilic membrane areas, and leading to disruption. This rationale could also explain why the dimethylamines **2** and **5** gave little activity in the antimicrobial assay, as they are less capable of hydrogen-bonding than the primary amines and guanidines, which was also shown for methylated guanidines by the group of Wender.[187]

Cytotoxicity

The amphiphiles **4e**, **4f**, **6e**, and **6f** were evaluated for cytotoxicity against HepG2-cells and the EC_{50}-values shown in Table 2.4 were determined from the dose-response curves in Fig. 2.4.

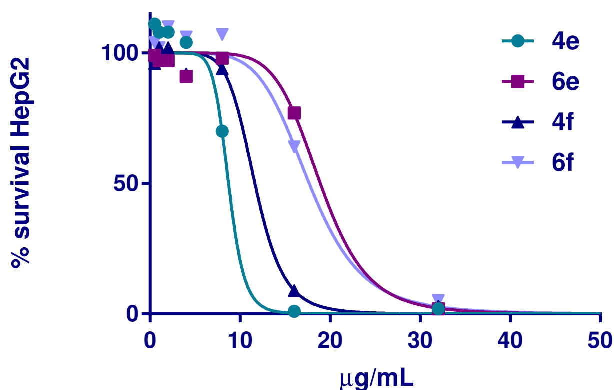


Figure 2.4. Dose-response curves from non-linear curve regression for *in vitro* cytotoxicity against HepG2-cells.

From the EC_{50}-values shown in Table 2.4 it was observed that the two guanidines **6** were less toxic, and had EC_{50}-values approximately double that of their corresponding amines **4**. This trend corresponded with the calculated ClogD-values from Table 2.5, as compounds with high lipophilic character more often give rise to non-specific toxic interactions (see Section 1.3.1). The observed data

may also be linked to the difference in basicity for the amines and the guanidines, as the amines are less basic* (see Section 1.3.2) and probably more prone to exist in an equilibrium than the guanidines.[317,318] This would also correspond well with the observed difference between calculated ClogD and retention times from HPLC, as the acid additive in HPLC forces the equilibrium towards the charged state and the retention of the fully charged compounds were measured. This may then be interpreted as the lipophilic character of the neutral compound being connected to the observed toxicity. If the higher toxic character of the amines is related to the equilibrium of charged vs. neutral compound, there is a possibility that the neutral amines may be able to traverse the membrane and have secondary targets inside the cells. The observed activity against HepG2-cells may also be explained by effects similar to the ones seen for lysine/arginine-snorkeling in proteins (discussed in Section 1.3.2).[190] The aliphatic chain on **4** and **6** may allow for similar behaviour of the cationic groups, leading to the lipophilic part of the molecule being allowed to interact with the lipophilic portion of the HepG2 cell membrane.

2.1.3 Conclusion

This chapter describes the synthesis of 42 cationic amphiphiles based on aliphatic amino 1,2,3-triazoles prepared from CuAAC between **14** or **15** and the lipophilic azides **11**. The 42 target compounds were obtained from N-deprotection of the CuAAC-products **16** and **17**, followed by appropriate N-functionalization. All the target compounds were prepared from commercially available reagents in two to five steps where all the final products were shown to be of sufficient purity for biological evaluation (>95% in HPLC). The 42 amphiphiles were then screened for antimicrobial activity against five different strains of bacteria (two Gram-negative and three Gram-positive) and tested for cytotoxic properties against human HepG2-cells.

The most active compound **6f**, shown in Fig. 2.5, displayed broad-spectrum antimicrobial activities with MIC-values ≤ 8 μg/mL against all five test bacteria. The activity was highest against the Gram-positive *S. aureus* and *S. agalacticae* (4 μg/mL), and lower against Gram-positive *E. faecalis*, and the two Gram-

*Protonated amines were calculated to have a pKa of 10.0 and the protonated guanidines a pKa of 12.1.

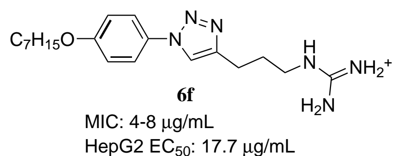


Figure 2.5. Most promising amphiphile from Section 2.1. Counterion: Cl^-.

negative *P. aeruginosa* and *E. coli* (8 μg/mL). In the cytotoxicity assay (HepG2) it was shown that **6f** displayed an EC_{50}-value against HepG2-cells two to four times higher than the MIC-values from the antimicrobial assays. It will then be important in the preparation of future compounds in this library to lower the toxicity of **6f**, whilst retaining the high antimicrobial activities in order to widen the therapeutic window.

2.2 Paper III: Synthesis and Antimicrobial Evaluation of Amphiphilic Amido 1,2,3-Triazoles

This section will present the synthesis and antimicrobial evaluations of 38 1,2,3-triazole amphiphiles. The synthesis of 29 target molecules and their intermediates is presented in Paper III, whereas experimental data for the remaining 9 compounds and their intermediates is presented in Section 4.1.1 and Sections 4.1.5 - 4.1.7.[299] The general experimental information is presented in the experimental section of Paper III, together with the experimental procedures for the biological assays. Similarly to Section 2.1, Fig. 2.6 shows the 38 target structures for this section with a number for the thesis as well as their corresponding compound number from Paper III. The number labels are a continuation of the previous section, and letter sub-labels were used so that each lipophilic group is assigned the same letter throughout both series of 1,2,3-triazoles.

Similarly to Section 2.1, the 1,2,3-triazole linker was chosen on the background of the its straightforward synthesis through CuAAC (Section 1.5.1), and the favorable biological properties described in Section 1.5.2. The objective was to prepare amphiphiles following the antimicrobial model presented in Fig. 2.1 in Section 2. As stated in Section 2.1, many of the target structures in this section were developed concurrently to some of the later target structures in Paper II. Lastly, Martin Furru Vold and Kristine Olsen Strandheim are acknowledged for their contribution to this section with the synthesis of **33l**, **33m**, and their intermediates.

2.2.1 Synthesis

The first step towards preparation of 34 of the 38 target amphiphiles shown in Fig. 2.6, was to couple azides **11c** and **11e** - **11m** with methyl propiolate (**29**), in order to obtain the corresponding 1,2,3-triazole methyl esters **30**. The azides **11c** and **11e** - **11j** had already been prepared and utilized in the synthesis of the aliphatic amino 1,2,3-triazoles in Section 2.1, whereas **11k**, **11l**, and **11m** had to be prepared prior to CuAAC. Azide **11k** was prepared analogously to **11f** in 59% yield over two steps, and the two fluorinated azides **11l** and **11m** were prepared from their corresponding benzyl bromides similarly to **11g** in 65% and 84% yields (conditions shown in Scheme 2.1 in Section 2.1.1).[305,307,308,319]

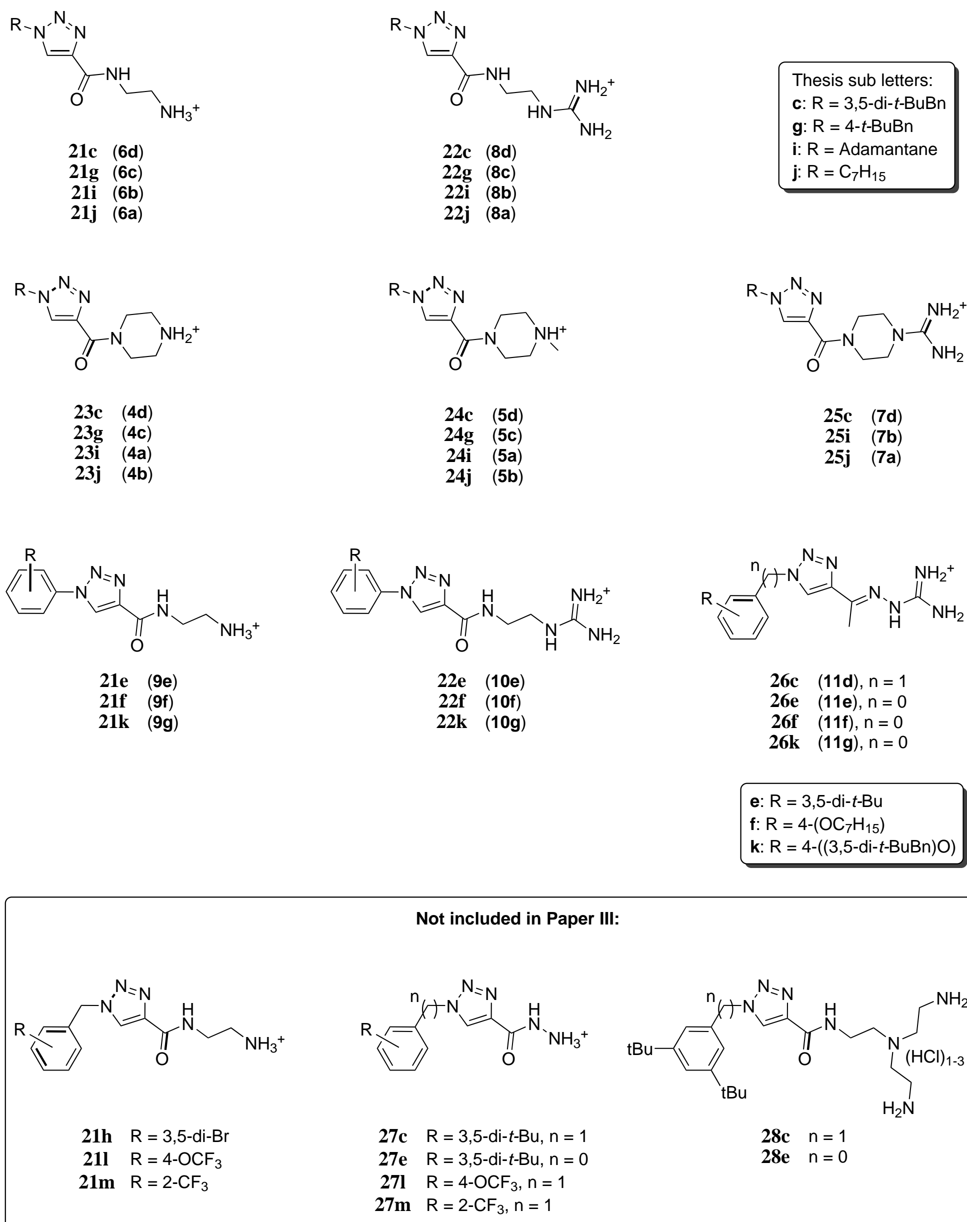


Figure 2.6. The 38 target structures presented in this section; the numbers in parantheses are the numbers used in Paper III. Counterion: Cl^- (CF_3COO^- for **26e** and **26f**).

tBu, O, N_3, tBu — **11k** 59%

F_3CO, N_3 — **11l** 65%

CF_3, N_3 — **11m** 84%

Figure 2.7. Azides prepared for this series of 1,2,3-triazoles (not utilized in Section 2.1).[305,307,308,319]

Scheme 2.7. CuAAC: $CuSO_4 \cdot 5\,H_2O$ (5 mol %), sodium ascorbate (10 mol %), PhCOOH (10 mol %), H_2O/*t*-BuOH (2:1), 17 - 28 h.[248]

R−N_3 + (29) —CuAAC→

11c
11e - **11m**

29

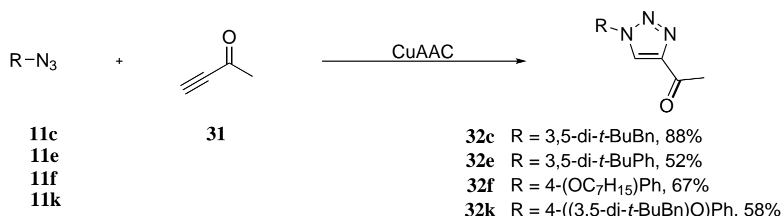
30c R = 3,5-di-*t*-BuBn, 71%
30e R = 3,5-di-*t*-BuPh, 85%
30f R = 4-(OC_7H_{15})Ph, 84%
30g R = 4-*t*-BuBn, 66%
30h R = 3,5-di-BrBn, 58%
30i R = Adamantane, 55%
30j R = C_7H_{15}, 89%
30k R = 4-((3,5-di-*t*-BuBn)O)Ph, 95%
30l R = 4-(OCF_3)Bn, 84%
30m R = 2-CF_3Bn, 83%

The azides **11c** and **11e - 11m** were then coupled with methyl propiolate (**29**) using the CuAAC procedure described by Shao *et al.*,[248] giving the ten 1,2,3-triazole methyl esters **30** shown in Scheme 2.7. Nine of the prepared esters **30** were purified through crystallization from DCM/pentane, whereas **30k** was purified with FCC (DCM - DCM/EtOAc 9:1).

Out of the 38 target amphiphiles in Fig. 2.6, 34 could be prepared from the ten 1,2,3-triazole methyl esters **30** prepared in Scheme 2.7. The remaining four target structures (**26**) in Fig. 2.6 had an iminoguanidine cationic group, which could be prepared from the reaction between a methyl ketone and aminoguanidine hydrochloride.*

*The iminoguanidines were somewhat challenging to prepare in sufficient purity, and only the lipophiles that were assumed to be most potent were used for the preparation of **32**.

Scheme 2.8. CuAAC for **32c**: $CuSO_4 \cdot 5H_2O$ (5 mol %), sodium ascorbate (10 mol %), PhCOOH (10 mol %), H_2O/*t*-BuOH (2:1), 18 h.[248] CuAAC for **32e**, **32f**, and **32k**: **31** (2-3 equiv), $CuSO_4 \cdot 5H_2O$ (5 mol %), sodium ascorbate (10 mol %), PhCOOH (10 mol %), DCM/H_2O/*t*-BuOH (1:1:1), 44-70 h.[320]

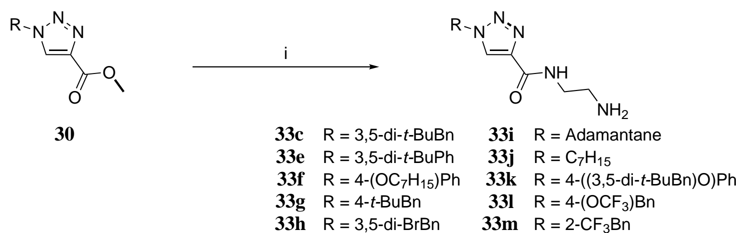


The 1,2,3-triazole methyl ketones **32c**, **32e**, **32f**, and **32k** were then prepared from 3-butynone (**31**) and the corresponding azides **11c**, **11e**, **11f** and **11k** using the CuAAC conditions shown in Scheme 2.8. Conditions for preparation of **32c** were the same as for **30** prepared in Scheme 2.7, but some modification was found to be necessary for successful preparation of **32e**, **32f**, and **32k**. The conditions reported by Shao *et al.* gave slow and incomplete conversion into **32e**, **32f**, and **32k**.[248] The alkyne **31** also seemed to be unstable over time under the reaction conditions used and formed unidentified byproducts. The conversion was enhanced by reducing the solvent polarity and by adding additional **31** throughout the reaction. Thus, using DCM/*t*-BuOH/H_2O (1:1:1), together with additional **31** (added in batches) gave **32e**, **32f**, and **32k** in acceptable yields (52-67%) after purification with FCC (DCM).[320]

Amide Bond Formation

The 1,2,3-triazole methyl esters **30** prepared in the previous section were functionalized into amides, in order to introduce the hydrophilic amino part of the target structures. Hence, the amines **33** (Table 2.6), **34** (Scheme 2.9), **35** (Table 2.8), **37** (Table 2.8), and **38** (Scheme 2.10) were prepared in one step from **30** and the diamines: ethylene diamine, piperazine (**36a**), *N*-methylpiperazine (**36b**), tris(2-aminoethyl)amine, and hydrazine. These amines could then be turned into their corresponding HCl-salts or *N*-functionalized with a guanidine group, in that way providing 34 of the 38 target amphiphiles in Fig. 2.6 in one or two steps from **30**.

Table 2.6. i) Ethylene diamine (15 equiv), MeOH, rt - reflux, 18 - 28 h (92 h for **33m**).[321]



Entry	**30**	Time [h]	Temp. [°C]	**33**	Yield [%]
1	**30c**	18	50	**33c**	100
2	**30e**	20	rt	**33e**	90
3	**30f**	20	65	**33f**	92
4	**30g**	24	50	**33g**	100
5	**30h**	24	rt	**33h**	92[a]
6	**30i**	17	65	**33i**	100
7	**30j**	17	65	**33j**	100
8	**30k**	28	65	**33k**	96
9	**30l**	22	rt	**33l**	100
10	**30m**	92[b]	rt	**33m**	100

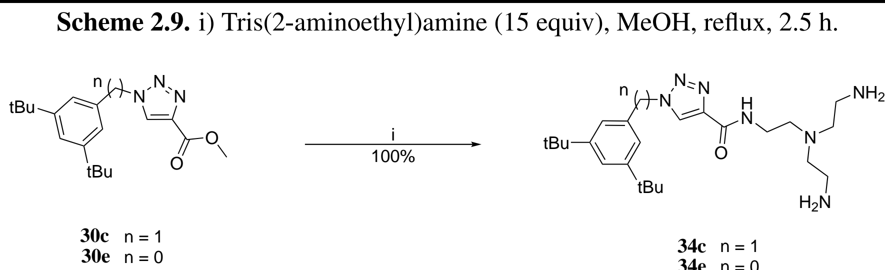
[a] Filtered before evaporation to remove unidentified solid byproducts.
[b] Left for 92 h, probably 100% conversion earlier.

The 10 methyl esters **30** were reacted with a large excess of ethylene diamine in MeOH at rt to reflux, affording **33** in good yields after evaporation under reduced pressure (Table 2.6).[321] Many of the methyl esters **30** were poorly soluble in the reaction mixture at room temperature. The temperature was therefore elevated for these substrates in order to provide homogeneous reaction mixtures (entries 1, 3, 4, and 6-8 in Table 2.6).

The conditions shown in Scheme 2.9, where ethylene diamine was substituted with tris(2-aminoethyl)amine, were used to prepare **34c** and **34e**[*] in short time and quantitative yields. The higher boiling point of tris(2-aminoethyl)amine (114 °C at 15 mmHg) compared to that of ethylene diamine (118 °C at 760 mmHg) made it necessary to use a one-step distillation (110 °C at 3 mbar) in order to remove excess amine in the work-up, instead of regular evaporation under reduced pressure (40 °C at 1-3 mbar).

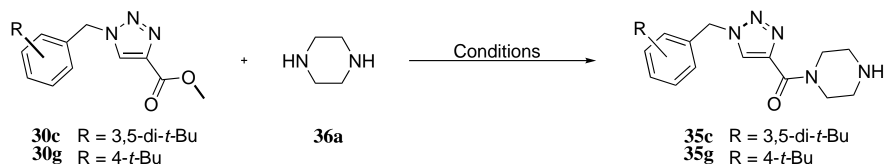
[*]Only two examples of **34** were prepared, as these compounds were made towards the end of the project.

Scheme 2.9. i) Tris(2-aminoethyl)amine (15 equiv), MeOH, reflux, 2.5 h.



Attempting to utilize the conditions shown in Table 2.6 in the preparation of **35**, by substituting ethylene diamine with piperazine (**36a**), showed piperazine to be less reactive than both ethylene diamine and tris(2-aminoethyl)amine. Unlike the preparation of **33** and **34**, the reaction to form **35c** from **30c** did not offer full conversion even after 64 h at 50 °C (entry 1, Table 2.7). Some modifications were therefore carried out in order to enhance the conversion into the target **35**.

Table 2.7. Amidation of **30c** and **30g** with piperazine (**36a**) to form **35c** and **35g**.



Entry	30	36a [equiv]	Conditions	Conv.[a] [%]
1	**30c**	15	MeOH, 50 °C, 64 h	90
2	**30c**	1.5	MeOH, MW (200 W), 1.5 h	26
3	**30c**	1.5	Imidazole, DBU, MeOH, 50 °C, 5.5 h	22
4	**30c**	1.5	Imidazole, DBU, MeOH, MW (200 W), 1.5 h	31
5	**30c**	1.5	Imidazole, DBU, MeOH, 95 °C,[b] 5 h	100[c]
6	**30g**	1.5	1,2,4-Triazole,[d] DBU,[d] MeOH, reflux, 23 h	25[e]
7	**30g**	1.5	1,2,4-Triazole,[d] DBU,[d] MeCN, reflux, 23 h	38
8	**30g**	1.5	1,2,4-Triazole,[d] DBU,[d] MeOH, 95 °C,[b] 1 h	22
9	**30g**	3	Xylenes, reflux, 24 h	0
10	**30g**	3	NaOMe, MS (4 Å), N_2-atm, rt, 23 h	90

[a] Conversion from ^{1}H NMR analysis of crude reaction mixtures.
[b] Pressure tube.
[c] **30c** gone and **35c** formed together with unidentified byproducts.
[d] 0.2 equiv.
[e] Major product formed was not **35g**.

Using microwave heating (MW) instead of oil bath heating was the first attempt at enhancing the conversion into **35**. Microwave heating is known to increase conversion rates compared to conventional heating sources, as it provides faster and more uniform heating of the reaction mixture.[322] Using microwave heating had a positive impact on the conversion as seen for entries 2 and 4 in Table 2.7, but there was still room for optimization.

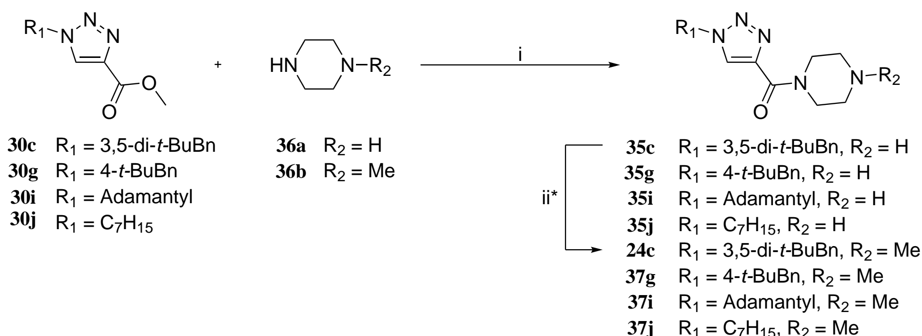
Attempts at changing the solvent were then carried out to improve the conversion between **30** and **36a**, as the polar protic MeOH may compete with **36a** in the reaction with **30**. Using an aprotic polar solvent like MeCN led to somewhat increased conversion compared to the same reaction with MeOH, as seen in entries 3 and 4 in Table 2.7. Furthermore, the use of xylenes as solvent in amidation reactions had been used successfully by the author of this thesis to prepare amides in a different project. A significant reduction in solvent polarity did unfortunately not afford any conversion, as only **30** and **36a** was seen after 24 h at reflux (entry 9, Table 2.7).

When neither microwave heating nor change of solvent afforded satisfying results, some other options were explored. Attempting to use stochiometric amounts of imidazole and DBU as acylation reagents provided faster conversion compared to using only heating, as can be seen from entries 3-5 in Table 2.7. Using a pressure tube and heating the reaction to 95 °C in MeOH (entry 5) afforded full conversion, but ^{1}H NMR analysis of the crude reaction mixture showed unidentified byproducts to be the major products. However, work published by Yang *et al.* has shown 1,2,4-triazole to be a good acylation catalyst for similar reactions, stoichiometric imidazole and DBU was then swapped for catalytic amounts of 1,2,4-triazole and DBU (entries 6-8, Table 2.7).[323] Using these conditions afforded slower conversion into **35**, but afforded less byproduct formation (seen from crude ^{1}H NMR).

A last attempt at improving the conversion was based on a method by Ohshima *et al.* for amidation of esters, using catalytic amounts of sodium methoxide at room temperature.[324] They have shown that 10 mol % of NaOMe, together with an additive like trifluoromethylphenol in toluene, afforded a system for efficient peptide coupling, using methyl esters as the carbonyl source. This system was modified, by using stoichiometric NaOMe, molecular sieves (MS), and no triflu-

oromethanol, and tested on the amidation reaction to form **35g** as can be seen from entry 10 in Table 2.7. After 23 h of stirring at room temperature, ^{1}H NMR showed the product **35g** in 90% conversion and **30g** as the only impurity.

Table 2.8. i) **36** (3 equiv), NaOMe (1 equiv), MS (4 Å), N_2-atm, rt, 43 - 68 h (for **35**) and 63 - 115 h (for **37**).[324] ii) HCOOH (20 equiv), HCHO (20 equiv), MeCN, reflux, 1.5 h.[313,314]

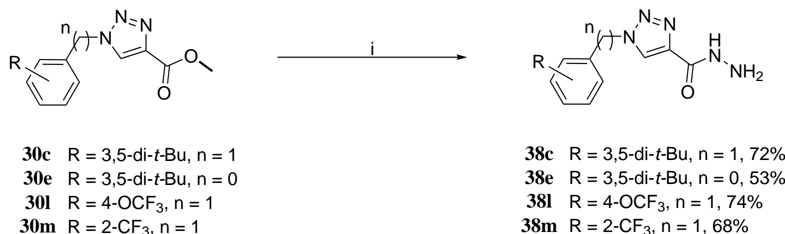


Entry	30	Time [h]	R_2	35/37	Yield [%]
1	**30c**	43	H	**35c**	69
2	**30g**	44	H	**35g**	58
3	**30i**	68	H	**35i**	74
4	**30j**	43	H	**35j**	54
5	**30c**	-	Me	**37c**	-[a,b]
6	**30g**	63	Me	**37g**	39
7	**30i**	115	Me	**37i**	53
8	**30j**	76	Me	**37j**	51

[a] Prepared from **35c** instead.
[b] Isolated as the salt **24c** (not the free amine **37c**) in 81% yield.

The modified conditions described by Ohshima *et al.* for the amidation of **30** with **36** (entry 10, Table 2.7) were subsequently utilized to prepare **35** and **37** as shown in Table 2.8. From Table 2.8 it can also be seen that **37c** was not prepared from the amidation of **30c** with **36b**. Multiple attempts at preparing **37c** from **30c** and **36b** was attempted, but none afforded the target product **37c**. Why this reaction failed was not investigated, and **37c** was not prepared using this method. The salt **24c** was instead directly prepared from a reductive amination of **35c** followed by acidic work-up.[313,314]

Scheme 2.10. i) Hydrazine hydrate (2 equiv), EtOH, reflux, 25 h.[325]



30c	R = 3,5-di-*t*-Bu, n = 1	**38c**	R = 3,5-di-*t*-Bu, n = 1, 72%
30e	R = 3,5-di-*t*-Bu, n = 0	**38e**	R = 3,5-di-*t*-Bu, n = 0, 53%
30l	R = 4-OCF_3, n = 1	**38l**	R = 4-OCF_3, n = 1, 74%
30m	R = 2-CF_3, n = 1	**38m**	R = 2-CF_3, n = 1, 68%

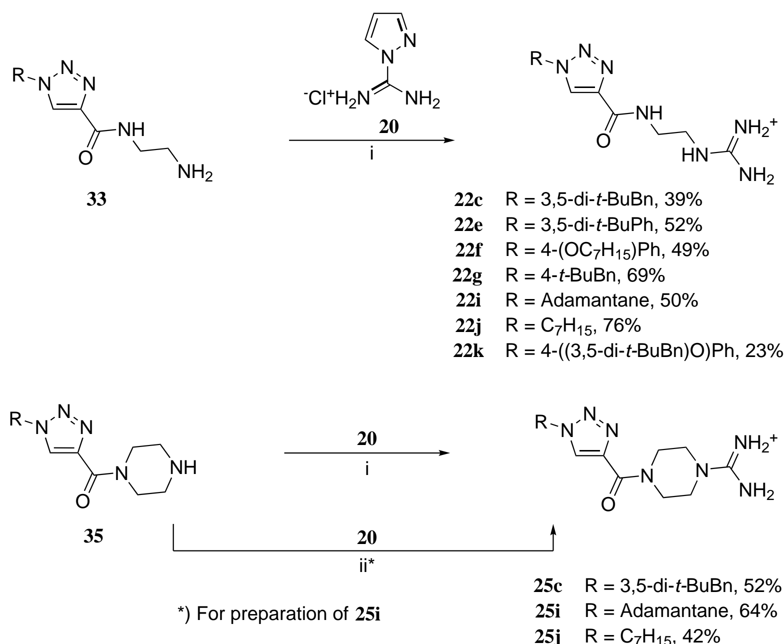
The final part of the amidation section shows the synthesis of 1,2,3-triazole hydrazides **38** from **30** and hydrazine hydrate. The hydrazides **38** were prepared according to Scheme 2.10, where the methyl esters **30** were refluxed with hydrazine hydrate in EtOH.[325] After complete conversion, the hydrazide **38** was isolated by cooling the reaction mixture and removing the filtrate after complete precipitation of **38**. However, as the hydrazide salts **27** performed poorly in the biological assays compared to their amine (**21**) and guanidine (**22**) counterparts, only four versions of **38** were prepared.

N-Functionalization

The amines prepared in the previous section (**33**, **34**, **35**, **37**, and **38**) were turned into their corresponding HCl-salts from treatment with HCl in an appropriate solvent, affording the 24 HCl-salts **21**, **23**, **24**, **27**, and **28** (1-3 x HCl) shown in Fig. 2.6.

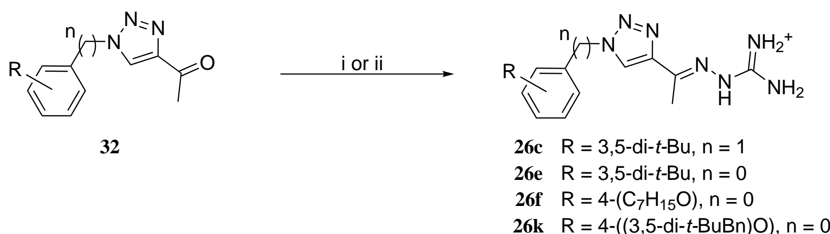
In order to introduce a guanidine function to the amines available for functionalization, the method described in Paper I and Section 2.1.1 was used to prepare the guanidine amphiphiles shown in Scheme 2.11. Nine of the ten guanidines shown in Fig. 2.6 were prepared by refluxing **33** or **35** with **20** in MeCN. The piperazinyl guanidine **25i** was not prepared according to this protocol, as the reaction was slow and did not give full conversion. The lack of conversion was assumed to be due to solubility issues. The guanidine **25i** was therefore prepared using DMF at room temperature, as in the original procedure described by Bernatowicz *et al.*[315]

Scheme 2.11. i) **20**, MeCN, reflux, 4 - 60 h.[300] ii) **20**, DMF, rt, 97 h.[315] Guanidine **22k** was prepared through a combination of the conditions i and ii (for details, see the experimental section of Paper III). Counterion: Cl^-.



The four iminoguanidines **26** in Fig. 2.6 were first prepared from **32** using a modified procedure described by Mohammad *et al.*, as shown in Table 2.9 (entries 1-4).[326] The methyl ketones **32**, aminoguanidine hydrochloride, and catalytic amounts of LiCl in EtOH were heated to reflux or 90 °C in a pressure tube (entries 1-4, Table 2.9). These conditions gave slow and incomplete conversion (75% conversion into **26c** after 51 h, entry 1), making work-up and purification more challenging. In an attempt to enhance the conversion, catalytic LiCl was substituted with an excess of aqueous HCl as can be seen in entries 5-7 in Table 2.9.[327] These somewhat harsher conditions gave full conversion into **26** in shorter time, but analysis of the crude products of **26f** and **26k** (entry 6 and 7) showed formation of multiple byproducts. Thus, **26c** was prepared using HCl (entry 5), whereas **26e**, **26f**, and **26k** were prepared using catalytic amounts of LiCl (entries 2-4).

Table 2.9. i) Aminoguanidine hydrochloride, LiCl (cat.), EtOH, 90 °C, sealed tube, 25 - 72 h.[326] ii) Aminoguanidine hydrochloride, HCl (excess), EtOH, 90 °C, sealed tube, 21 - 22 h.[327] Counterion for **26c** and **26k**: Cl^-, counterion for **26e** and **26f**: CF_3COO^-.



Entry	32	Cond. i/ii	Time [h]	26	Conv. [%][a]	Yield [%]
1	**32c**	i	51	**26c**	75	9
2	**32e**	i	48	**26e**	-[b]	46
3	**32f**	i	51	**26f**	-[b]	31
4	**32k**	i[c]	25	**26k**	63	14
5	**32c**	ii	22	**26c**	100	58
6	**32f**	ii	21	**26f**	100	-[d]
7	**32k**	ii	21	**26k**	100	-[d]

[a] Conversion from ^{1}H NMR analysis of crude or crude samples.
[b] Crudes not analyzed.
[c] Run at reflux in normal flask.
[d] Not worked up due to byproduct formation.

One interesting observation made during the preparation of **26**, was the appearance of two sets of signals in both NMR and HPLC analysis of the products. The signal sets in ^{1}H NMR were of identical compositions, but had different shifts, which could indicate some sort of isomerism. This assumption was further strengthened when elute from analytical HPLC was analyzed with MS, showing that the two peaks in the chromatogram had the same molecular weight. Furthermore, treatment of NMR samples with HCl affected the ratios of the different signal sets. The different peaks were then assumed to be E- or Z-isomers of **26** (1:9 - 4:6 ratios from ^{1}H NMR).

2.2.2 Biological Evaluation of Target Amphiphiles

Antimicrobial Activity

The amphiphiles exhibiting antimicrobial activity and their corresponding MIC-values are shown in Table 2.10, together with the HepG2 EC_{50}-values for the 11 most promising target structures.

The top 19 compounds in Fig. 2.6 (**21** - **25**, with R-groups: **c**, **g**, **i**, **j**) were prepared and tested prior to the remaining 19. Out of these compounds four displayed any antimicrobial activities and are shown above the horizontal line in Table 2.10. These results were used as a rationale for optimization and led to preparation of the latter 19 amphiphiles (similarly to Section 2.1.2), and gave the active compounds below the horizontal line in Table 2.10. From the initial investigations it was found that the 3,5-di-*t*-Bu-group (**c**) was the more efficient lipophilic group, ethylene diamine (**21** and **22**) was a more efficient linker than piperazine (**23** and **25**), and guanidine (**22** and **25**) was most efficient as cationic group. These observations also corresponded to the observations made from the first set of targets in Section 2.1 and the work of the separate groups of Strøm and Svendsen.[157,167,168] The MIC-values of these compounds (**21c**, **22c**, **23c**, and **25c**) were however somewhat disappointing, and it was clear that there was room for optimization of the antimicrobial activity.

Based on the initial results, some structural changes were investigated in an effort to increase the antimicrobial potency past that of **22c**:

- **Removal of the benzylic methylene.** As was also discussed in Section 2.1.2, the benzylic methylene group between the phenyl ring and the 1,2,3-triazole allows for a more freely rotating structure. Removal of this methylene would hopefully lead to some repulsion between the ortho-protons and the 1,2,3-triazole, and further, in addition to removing a rotable bond, reducing the rotational freedom and giving the molecule a twisted conformation. Removing the benzylic methylene could also allow for conjugation between the aromate and the 1,2,3-triazole.

- **Substituting the 3,5-di-*t*-Bu-group with an alkyl ether chain.** The initial screening and the evaluations performed in Section 2.1.2 showed that a heptyl chain alone, without an aromatic ring, did not lead to any

Table 2.10. Minimum inhibitory concentrations (MIC-values) and EC_{50}-values in μg/mL. Counterion: Cl^- (**26e** and **26f**: CF_3COO^-).

21c **22c** **23c** **25c**

21e **21f** **21k**

22e **22f** **22k**

26c n = 1
26e n = 0
26f n = 0
26k n = 0

27e

28c n = 1
28e n = 0

c: R = 3,5-di-*t*-Bu, n = 1
e: R = 3,5-di-*t*-Bu, n = 0
f: R = 4-(OC_7H_{15})
k: R = 4-((3,5-di-*t*-BuBn)O)

	Antimicrobial activities [MIC]					HepG2[b]
	E. faecalis[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	[EC_{50}]
21c	-[c]	64	64	32	64	n.d.[d]
22c	64	32	16	64	32	n.d.
23c	-	64	64	64	-	n.d.
25c	-	64	32	-	64	n.d.
21e	32	16	16	16	32	8.0
21f	-	4	8	-	16	3.5
21k	8	-	2	-	16	2.9
22e	16	8	8	16	16	31.3
22f	16	4	8	8	8	23.8
22k	16	8	4	-	-	16.2
26c	8	4	4	8	8	2.3
26e	4	4	2	4	16	2.6
26f	32	-	8	16	64	2.0
26k	64	-	0.5	-	-	1.9
27e	64	-	32	-	-	n.d.
28c	64	8	-	64	8	n.d.
28e	16	16	8	16	4	32-64[e]
Ref.[f]	10	0.13	4	0.5	0.5	n.d.

[a] *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *S. agalacticae* (ATCC 12386), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).
[b] EC_{50} determined from Fig. 2.8.
[c] "-": No activity $\leq$64 μg/mL.
[d] n.d.: Not determined.
[e] Unable to fit dose-response from data: no activity at 32 μg/mL, 2% cell-survival at 64 μg/mL (not shown in Fig. 2.8).
[f] Ref.: gentamicin.

antimicrobial effects. However, substituting the 3,5-di-*t*-Bu-group on the aromate with a heptyl ether could give more potent amphiphiles, since the heptyl ether chain may penetrate deeper into the cell membrane or provide increased amphiphilic character (discussed in Section 2.1.2). This was also based on the heptyl ether chain giving similar molecular weight and aliphatic contribution as the 3,5-*t*-Bu-group.

- **Insertion of an additional aromate.** In their studies of antimicrobial $\beta^{2,2}$-amino acids, Hansen *et al.* used two aromatic rings in their target amphiphiles that gave high antimicrobial activity.[157] Introducing a 3,5-di-*t*-Bu-benzyl ether on the phenyl group analogously to the heptyl ether chain could therefore yield amphiphiles with increased antimicrobial potency.
- **Further evaluation of cationic *N*-groups.** Testing other cationic *N*-groups on efficacious lipophiles could either confirm the superiority of the guanidines or give more potent cationic groups. Through the preparation of a library of antimicrobial thiazoles, Mohammad *et al.* have shown that thiazole imines formed from a thiazole methyl ketone and aminoguanidine hydrochloride were highly potent against resistant Gram-positive bacteria (MRSA and VRSA).[326] Furthermore, investigating the effect of introducing several nitrogen groups could also be of interest, as many peptide mimics with high antimicrobial activity had more than one ionizable nitrogen group.[156,157,168–170] Lastly, a small screen using hydrazides as functional groups instead of ethylene diamine could be of interest for activity trend analysis, as it is less basic than both amines and guanidines.[317,318]

The structural modifications were carried out as shown in Section 2.2.1 and the active structures from the modifications are shown below the horizontal line in Table 2.10. Removal of the benzylic methylene group (**e**) led to a 2- to 4-fold increase in antimicrobial potency for the 3,5-di-*t*-Bu-phenyl derivatives **21e**, **22e**, and **28e**, compared to their 3,5-di-*t*-Bu–benzylic counterparts **21c**, **22c**, and **28c** (except for **28e** against *S. aureus*). The increase in potency from removing the benzylic methylene group was also seen for the iminoguanidines **26**, where the overall activity of **26e** was seen to be higher than for the 3,5-di-*t*-Bu–benzylic **26c**.

Introduction of a heptyl ether chain in the 4-position on the phenyl ring (**21f**, **22f**, and **26f**) led to greatly increased potency compared to the amphiphiles equipped with just a heptyl chain (**21j** - **25j**), affording MIC-values at 4 μg/mL

against *S. aureus* (**21f** and **22f**, Table 2.10). When comparing the activity of the heptyloxy-phenyl substituted **21f**, **22f**, and **26f** with the activity of the 3,5-di-*t*-Bu–phenyl derivatives **21e**, **22e**, and **26e**, the guanidine **22f** was seen to offer the overall best improvement in antimicrobial activity. As the guanidine **22f** was seen to be 2-fold more potent against *S. aureus*, *E. coli*, and *P. aeruginosa* than the 3,5-di-*t*-Bu–phenyl guanidine **22e**. The 4-heptyloxy-phenyl substituted amine **21f** also offered increased potency compared to the 3,5-di-*t*-Bu–phenyl derivative **21e**, where it was 2- to 4-fold more potent against the three strains *S. aureus*, *S. agalacticae*, and *P. aeruginosa*. However, unlike the guanidine, the amine **21f** did not retain its activity against all five bacteria, as the observed MIC-values against *E. faecalis* and *E. coli* were observed to be >64 μg/mL, thus showing some strain variation. The iminoguanidines **26** behaved somewhat differently to the amines **21** and guanidines **22**, where the bulky 3,5-di-*t*-Bu–phenyl substituted **26e** was seen to be more potent than the 4-heptyloxy-phenyl substituted iminoguanidine **26f**.

The amphiphiles carrying the 3,5-di-*t*-Bu–benzyl ether in the 4-position (**21k**, **22k**, and **26k**) displayed particularly high potency against *S. agalacticae*, where the iminoguanidine **26k** was found to be the most potent compound with an impressive MIC-value of 0.5 μg/mL. The activity of **26k** against *S. agalacticae* made it 8-fold more potent against the bacteria compared to the reference antibiotic gentamicin (MIC 4 μg/mL). Furthermore, the 3,5-di-*t*-Bu–benzyl ether iminoguanidine **26k** also displayed lowered efficacy against the other four test bacteria, giving high selectivity (>128) against *S. agalacticae* compared to the remaining four bacteria. The same trend was also seen for the amine **21k** and the guanidine **22k**, where they both were highly potent against *S. agalacticae* (MIC 2 and 4 μg/mL) and showed lowered potency against several of the other test bacteria. The amphiphiles carrying the 3,5-di-*t*-Bu–benzyl ether (**21k**, **22k**, and **26k**) were estimated to be the most lipophilic compounds in the series, the profound selectivity could mean that *S. agalacticae* is more sensitive towards amphiphiles with a higher lipophilic ratio than the other four bacteria.

Introduction of the iminoguanidine group (**26**) showed that this functional group led to amphiphiles with high antimicrobial activity. The iminoguanidine together with the 3,5-di-*t*-Bu-group (**26c** and **26e**) led to the most notable increase in potency by being 2- to 8-fold more potent compared the corresponding

guanidines **22c** and **22e** (only exception: **22e** and **26e** both had MIC-values of 16 μg/mL against *P. aeruginosa*). This increase in potency led to **26c** and **26e** giving the highest broad-spectrum activity of the compounds shown in Table 2.10 with MIC-values <10 μg/mL (except for **26e** against *P. aeruginosa*, MIC 16 μg/mL). Furthermore, as mentioned in the previous paragraph, introducing the iminoguanidine together with the 4-heptyloxy-phenyl group (**26f**) did not lead to increased antimicrobial potency compared to the guanidine **22f**. Hence, the 4-heptyloxy-phenyl derivative **26f** was inferior to both the iminoguanidine **26e** and the guanidine **22f**, thereby displaying the opposite activity trend of the other active amphiphiles.

Substituting the cationic group to a hydrazide (**27**) led to a reduction in antimicrobial potency. The compounds proving often to give high antimicrobial activity were amphiphiles with the basic guanidine group (**22**), which is more basic than the primary amine group (**21**).[317,318] The basicity may be important for the equilibrium between charged and non-charged state of the compounds under physiological conditions, as the cationic character of the hydrophile is assumed to be of importance both for amphiphilicity and coordination to bacterial membranes (as discussed in Section 1.3.2 and 2.1.2). Hence, the highly basic guanidines (**22**) may exist primarily in their charged state at physiological pH (7.4), whereas the amines (**21**) and hydrazides (**27**) may exist more in an equilibrium between the charged and non-charged states. This could in turn lead to higher effective concentrations of the amphiphile at the site of action and increased potency. The lack of antimicrobial efficacy for the less basic hydrazides could support this rationale.

Introduction of additional amine groups (**28**) on the amphiphiles also led to some interesting activities. Most notably, introduction of the tris(2-aminoethyl)-amine functionality (**28c** and **28e**) led to a 4-fold increase in potency against *P. aeruginosa* compared to the 3,5-di-*t*-Bu–phenyl substituted guanidines **22c** and **22e**. Except for a 4-fold increase in potency for **28c** against *S. aureus*, the potency of **28c** and **28e** against the remaining four bacteria were, on the other hand, similar or lower compared to the guanidines **22c** and **22e**. The increase in potency against *P. aeruginosa* was interesting, as being able to specifically target bacteria (like **26k** against *S. agalacticae*) was also of interest for further studies in this project. It should also be noted that the charge of the HCl-salts

of **28** was not specifically known and could potentially range between +1 to +3. MS analyses of **28** showed the singly charged species and NMR analyses were difficult to interpret for the salts as the signals broadened significantly. However, the ^{1}H NMR spectra of **28c** in DMSO showed a broad signal in the aromatic region integrating to 5-6 protons, which may indicate a doubly charged species (2x NH_3^+).

The general tendency for the different cationic groups followed the observations made in Section 2.1.2, where the basic guanidinium functional group (**22** and **26**) gave the overall best antimicrobial efficiency, compared to the amines (**21**) and hydrazides (**27**). The more basic guanidine may then be assumed to give stronger electrostatic bonding to the bacterial cell membrane following the principles discussed in Sections 1.3.2 and 2.1.2. Additionally, the lack of antimicrobial activity for the *N*-methylpiperazines **24** could also support the need for efficient hydrogen-bonding. These piperazines are, similarly to the dimethylamines **2** and **5** in Section 2.1, assumed to be less capable of forming hydrogen-bonds compared to the other more active species. Lastly, these principles could also explain the increased antimicrobial activity of **28**, as it has several nitrogen-species available for association to the bacterial membrane.

Cytotoxicity

The 11 most promising amphiphiles from the antimicrobial assays were evaluated for *in vitro* cytotoxicity against HepG2-cells, the EC_{50}-values shown in Table 2.10 were determined from the dose-response curves in Fig. 2.8. It should be noted that the dose-response curve for **28e** was not plotted in Fig. 2.8 due to insufficient data in the EC_{50}-area of the curve.

The trend regarding the toxicity of the 11 amphiphiles against HepG2-cells corresponded with the data recorded for the four most potent compounds from Section 2.1, where the guanidines **22** were seen to offer lower HepG2 toxicity compared to the corresponding amines **21**. The guanidine carrying the 3,5-di-*t*-Bu-group (**22e**) was the least toxic of the three tested **22** and displayed an EC_{50}-value of 31.3 μg/mL, and gave an approximate 2- to 4-fold selectivity towards bacteria compared to HepG2-cells. The 4-heptyloxy-phenyl substituted guanidine **22f** was more toxic, but was also more potent against four of the bacteria, giving a 1.5- to 6-fold preference for bacteria when comparing the EC_{50}-value

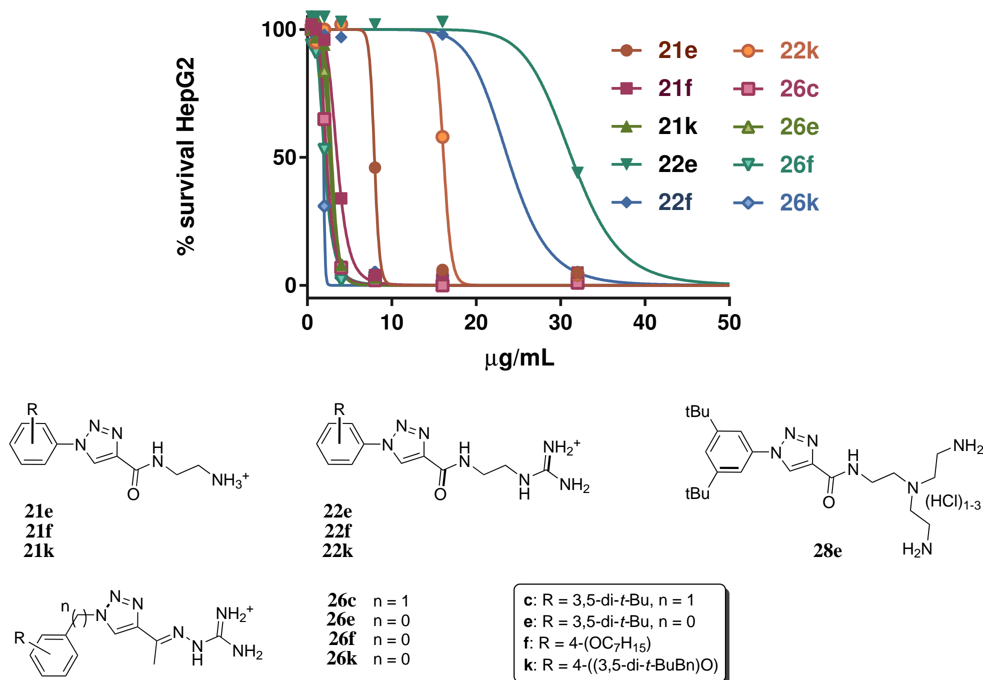


Figure 2.8. Dose-response curves from non-linear curve regression for *in vitro* cytotoxicity against HepG2-cells and the different structures tested in the assay (dose-response curve for **28e** was not plotted due to insufficient data in the EC_{50}-area). Counterion: Cl^- (**26e** and **26f**: CF_3COO^-).

against HepG2-cells and the obtained MIC-values. For the guanidine carrying an additional phenyl ring (**22k**), there was only observed some selectivity for *S. agalacticae*.

The structural similarity of **22e** to some of the amphiphilic aminobenzamides described by Igumnova *et al.* was also reflected in the biological activities.[180] The 1,2,3-triazole **22e** was however less potent and more toxic than the most similar benzamide from their study (**A3** in Fig. 2.9), which could possibly be explained by **A3** having a larger spatial separation of the cationic group and the lipophilic group. As a similar trend was also seen from their study, where increasing the length of the amphiphile with one methylene group (**A3** vs. **A4**), led to the same level of antimicrobial activity and a reduction in hemolytic activity. A possible improvement for future generations of compounds could then be to increase the

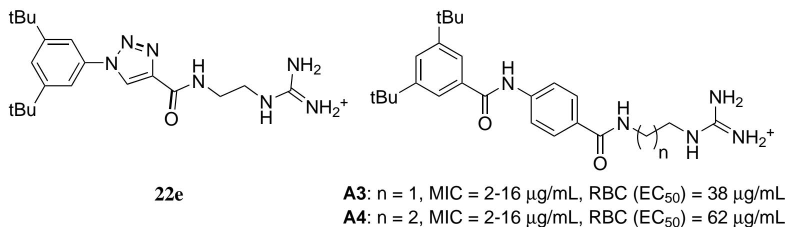


Figure 2.9. Most similar benzamides by Igumnova *et al.* (**A3** and **A4**) and **22e**.[180] Counterion: Cl^-.

length of the target compounds, as this seems to have a positive effect on the selectivity (**A3** vs. **A4** and **22f** vs. **22e**). Furthermore, elongation of the scaffold, instead of either end of the amphiphiles, may possibly further promote selectivity. This may also be an option for reducing the toxicity that was observed for the 4-heptyloxy-phenyl substituted structures (**f**) and the compounds carrying an additional benzene ring (**k**).

The iminoguanidines **26** were seen to display high toxicity with EC_{50}-values ≤ 2.6 μg/mL, meaning they were even more toxic than the amines **21** (EC_{50} ≤ 8 μg/mL). The antimicrobial thiazoles reported by Mohammad *et al.* did not display toxicity towards HeLa-cells <11 μg/mL, giving a selectivity factor above 20 for Gram-positive bacteria.[326] While, on the other hand, the iminoguanidines **26** prepared in this project were generally more toxic to HepG2-cells than active against bacteria, yielding selectivity factors below 1. What caused the 1,2,3-triazole-based **26** to be less active and more toxic than the thiazoles was not obvious from these initial investigations. One possibility could be the lipophilicity of the scaffold, the thiazole moiety used in the work by Mohammad *et al.* was calculated to be more lipophilic (ClogP = 0.76) than the 1,2,3-triazole moiety used in **26** (ClogP = -0.50). Thus, as the thiazole contributes more to the overall lipophilicity than the 1,2,3-triaozole, less lipophilic contribution was needed from the substituents on the lipophilic side of the motif. This, more even distribution of lipophilic character across the scaffold, could then be beneficial for reducing toxicity. Regardless of the high antimicrobial activities, the high toxicity of the iminoguanidines made them unfit for further development. The same conclusion was drawn for the primary amines **21e**, **21f**, and **21k**, as they also generally displayed EC_{50}-values below the MIC-values from the antimicrobial assays. Pos-

sible reasons for the increased toxicity of **21** followed the same rationale as for **1** and **4** discussed in Section 2.1.2.

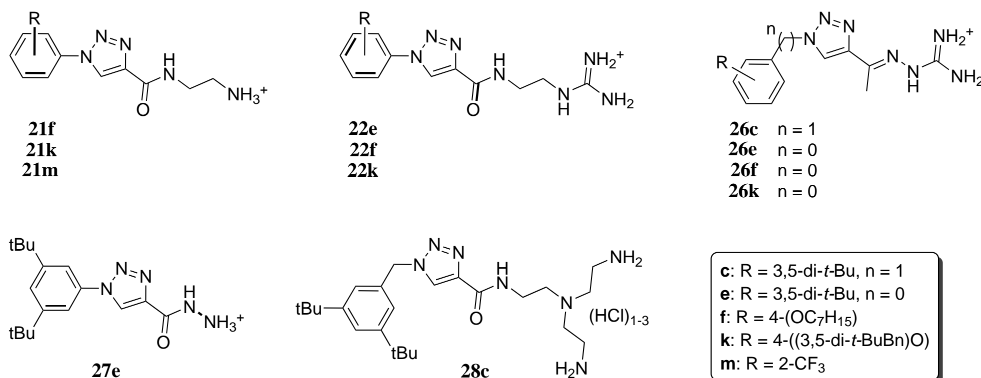
One interesting observation in the toxicity evaluation was the low observed toxicity of **28e**, where the EC_{50}-value was seen to be somewhere between 32 and 64 μg/mL. The amine **28e** was then the least toxic compound out of the 11 different amphiphiles in Table 2.10 against HepG2-cells. In addition to the low toxicity, the activity against *P. aeruginosa* was among the highest recorded in this library with a MIC-value of 4 μg/mL. The high potency and low cytotoxicity led to a selectivity factor 8-16 for *P. aeruginosa* over mammalian cells. The lower toxicity may be due to the increased hydrophilic character from insertion of additional nitrogen groups, as the ClogD for **28e** was calculated to be -1.22 (in comparison; **21e**, ClogD = 1.68). The additional amino groups will also render the compound less probable to exist in its neutral form at physiological pH, compared to the monofunctionalized amines **21**. The high selective activity and lower toxicity of **28e** could possibly be further improved by guanylating the amine groups, following the rationale from Section 2.1.2 and the antimicrobial evaluation in this section.

Biofilm Inhibition

The amphiphiles in Fig. 2.6 were evaluated for possible inhibition of *S. epidermis* biofilm formation, where 37 out of the 38 amphiphiles in Fig. 2.6 were tested in the single-concentration assay (50 μg/mL) and 12 of these were taken into dose-response evaluation. The results from the dose-response assays of the 12 amphiphiles are shown in Table 2.11 and it should be noted that **21c**, **22c**, **23c**, and **25c** showed activities <50 μg/mL in the single-concentration assays, but were not taken into dose-response testing.

Most of these amphiphiles displayed biofilm inhibiting effects with MIC-values ≤ 8 μg/mL (except for **21m** and **27e**), with the most potent amphiphile being the guanidine **22k** with a MIC-value of 2 μg/mL. It was, however, difficult to judge from these results whether the observed effect came from biofilm inhibition or from general antimicrobial activity, as the biofilm inhibition values mostly correlated with the best MIC-values from the antimicrobial assays shown in Table 2.10. Deviations from this trend were observed; amongst others for **21m** with a MIC-value of 16 (μg/mL) in the biofilm inhibition assay and MIC-values >64 (μg/mL) in the antibacterial assays. This may point towards a more specific

Table 2.11. MIC-values (μg/mL) for inhibition of *S. epidermis* biofilm formation. Counterion: Cl^- (**26e** and **26f**: CF_3COO^-).



Compound	MIC[a] [μg/mL]	Compound	MIC[a] [μg/mL]
21f	4	**26c**	4
21k	8	**26e**	4
21m	16	**26f**	8
22e	4	**26k**	4
22f	4	**27e**	32
22k	2	**28c**	8

[a] Inhibition of *S. epidermis* biofilms.

biofilm inhibiting mode of action for **21m** compared to many of the other amphiphiles. The amphiphiles carrying the additional aromate (**k**) were also prone to be more active in the biofilm inhibition assays than in the antimicrobial assays (with the exception of against *S. agalacticae*). However, due to the high potency against *S. agalacticae*, the biofilm inhibition MIC-values displayed by **21k**, **22k**, and **26k** may also have come from less specific antimicrobial interactions.

2.2.3 Conclusion

This section describes the synthesis of 38 cationic amphiphiles based on the CuAAC-products **30** and **32**. These key substrates were functionalized with appropriate *N*-groups through either amidation and *N*-functionalization (**30**) or iminoguanylation with aminoguanidine hydrochloride (**32**). Which in turn afforded the 38 target amphiphiles from commercially available reagents in two to five steps, where all the final products were of sufficient purity for biological

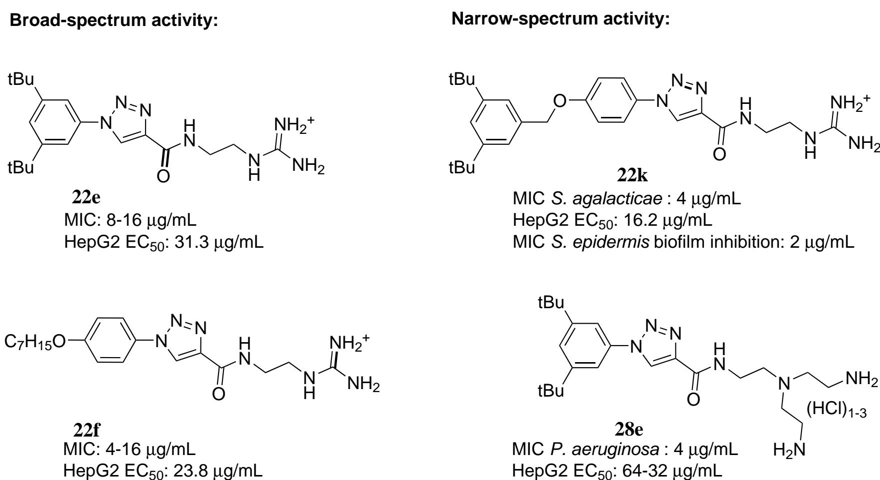


Figure 2.10. The four most promising amphiphiles from the series presented in Section 2.2. Counterion: Cl^-.

evaluation (>95% in HPLC). The 38 amphiphiles were tested for antimicrobial activity against three Gram-positive and two Gram-negative bacteria, inhibition of *S. epidermis* biofilms, and cytotoxic properties against HepG2-cells.

The four most promising structures for further optimization are shown in Fig. 2.10. The guanidines **22e** and **22f** were assessed to be the best compromise between activity and toxicity and will therefore be important for developing future compounds with broad-spectrum antimicrobial effects. Development of targets for more narrow spectrum activities can be based on **28e** against *P. aeruginosa* and **22k** against *S. agalacticae*. The amine **28e** was particularly interesting with regard to the relatively low toxicity against HepG2-cells. The selective activity of **22k** against *S. agalacticae* could also be interesting for development of antibiotics for preventing neonatal infections, as these are severe infections often caused by *S. agalacticae*.[209] In addition to the selective activity against *S. agalacticae* in the antimicrobial assays, **22k** was also observed to be highly active in the biofilm inhibition assay with a MIC-value of 2 μg/mL for inhibition of *S. epidermis* biofilm formation.

2.3 Paper IV: Synthesis and Antimicrobial Evaluation of Isoindoline and Fused Pyridine Amphiphiles

This section will present the preparation and antimicrobial evaluation of 20 fused pyridine (dihydro pyrrolopyridines) and isoindoline amphiphiles.[297] All experimental details for preparation of the 20 target amphiphiles and their intermediates can be found in the experimental section of Paper IV. The general experimental information is also presented in Paper IV, together with the experimental procedures for the biological assays. Similarly to the two previous sections, Fig. 2.11 shows the 20 target structures for this section with a number for the thesis as well as their corresponding compound number from Paper IV. The number labels are a continuation of the previous sections, but new letter sub-labels for the lipophilic groups are assigned to the isoindolines and fused pyridines both separately from each other and from the previous lipophile numbering for the 1,2,3-triazoles (starting from **a**).

The dihydro pyrrolopyridines and isoindolines were chosen as scaffolds for antimicrobial amphiphiles, since they could be prepared from transition metal catalyzed [2+2+2] cycloaddition. This reaction type is previously investigated by current and past members of the research group, and applied in methodologies for total synthesis.[328,329] It was therefore of interest to use this synthetic knowledge to prepare amphiphiles following the model presented in Fig. 2.1, in order to expand the compound library in this SAR-project. In addition to the preparation of dihydro pyrrolopyridines and isoindolines via [2+2+2] cycloaddition, a focused set of seven target isoindolines was prepared via Suzuki cross-coupling reactions.

Lastly, MSc Kristoffer Larsen Lea, MSc Anton Brondz, and master student Kristian Njerve Myreng are acknowledged for their extensive contribution to this section through the preparation of 16 target amphiphiles. Kristoffer Larsen Lea and Anton Brondz prepared **39a** - **39d**, **40a** - **40e**, and their intermediates, and Kristian Njerve Myreng prepared **40g** - **40j**, **41g** - **41i**, and their intermediates.

2.3.1 Synthesis of Amphiphiles via [2+2+2] Cycloaddition Reactions

This part of the project originally started with the desire to prepare tetrahydronaphthalenes and tetrahydroisoquinolines with one lipophilic group and two

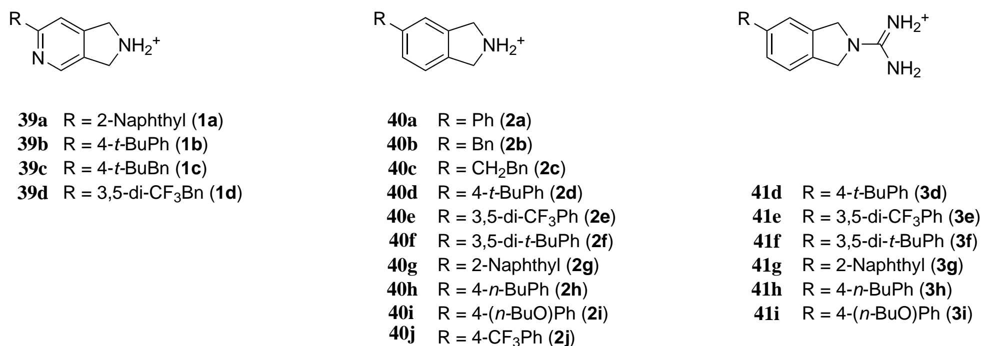


Figure 2.11. The 20 target structures presented in this section; the numbers in parantheses are the numbers used in Paper IV. Counterion: Cl^-.

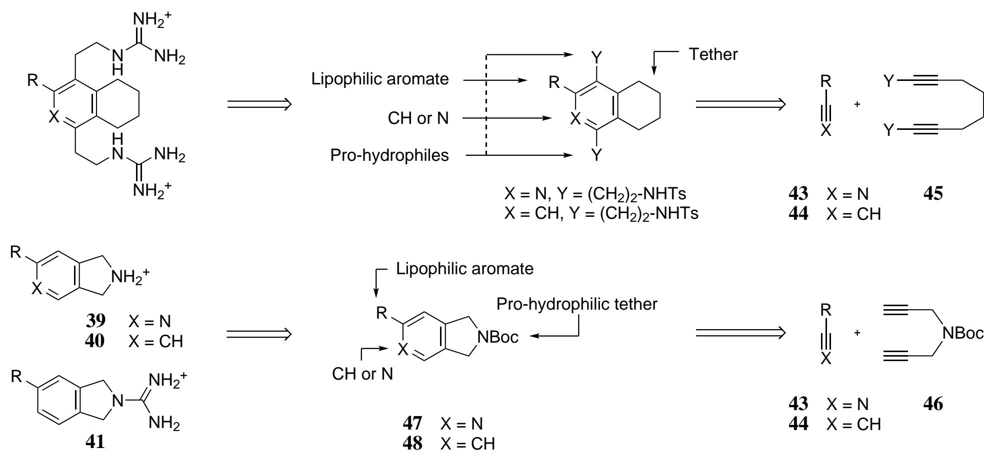
hydrophilic cationic functionalities. These structures could be obtained from cycloaddition of the substituted diyne **45** with nitriles **43** and alkynes **44**, as shown in Scheme 2.12. The diyne substrate was, however, found to be sensitive towards structural modifications of **43**, and also afforded little to no conversion when **44** was used in the reaction.* The more complex diyne was therefore replaced by a simpler diyne **46**, as shown in the bottom half of Scheme 2.12. The diyne **46** is also a more studied substrate in [2+2+2] cycloadditions (discussed in Section 1.6.1).[292–295] This system for [2+2+2] cycloaddition was shown to allow for more modification of **43** and **44** without critical depression of the yields (Scheme 2.13).

After re-focusing the strategy towards the synthesis of dihydro pyrrolopyridines (**39**) and isoindolines (**40**), the cobalt-catalyst $CpCo(CO)_2$[287,330,331] was chosen for cycloaddition between **46** and **43**, and the ruthenium-catalyst Cp*RuCl(cod)[332,333] for the cycloaddition between **46** and **44**. The carbamate-protected cycloaddition products were then prepared from **46** and **43**/**44** according to the conditions displayed in Scheme 2.13, affording **47a** - **47d** and **48a** - **48f** in 36 - 78% yields. Scheme 2.13 also shows the conditions for preparation of **46** in two steps from propargylamine.[295,296]

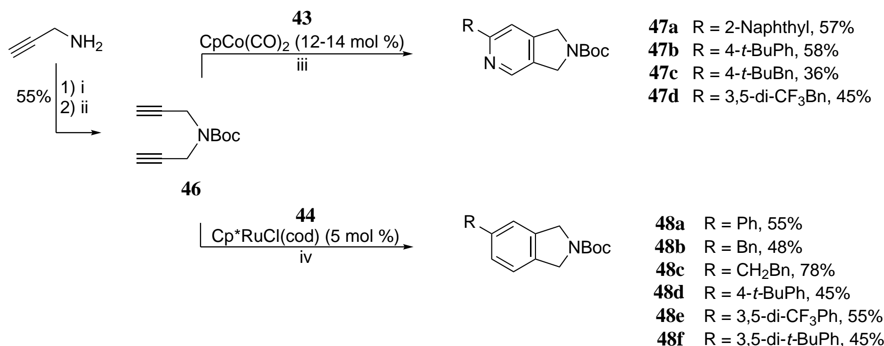
The four dihydro pyrrolopyridines **47a** - **47d** were prepared in 36-58% yields, the highest yielding reaction being the cycloaddition with 4-(*tert*-butyl)benzonitrile (**43b**). It has been previously reported that nitriles conjugated to arenes or sim-

*Experimental details found in Section 4.1.9.

Scheme 2.12. Retrosynthetic analysis of the two synthetic routes for amphiphiles from [2+2+2] cycloaddition reactions. The bottom route was chosen for synthesis as it proved to be the more versatile method of the two.

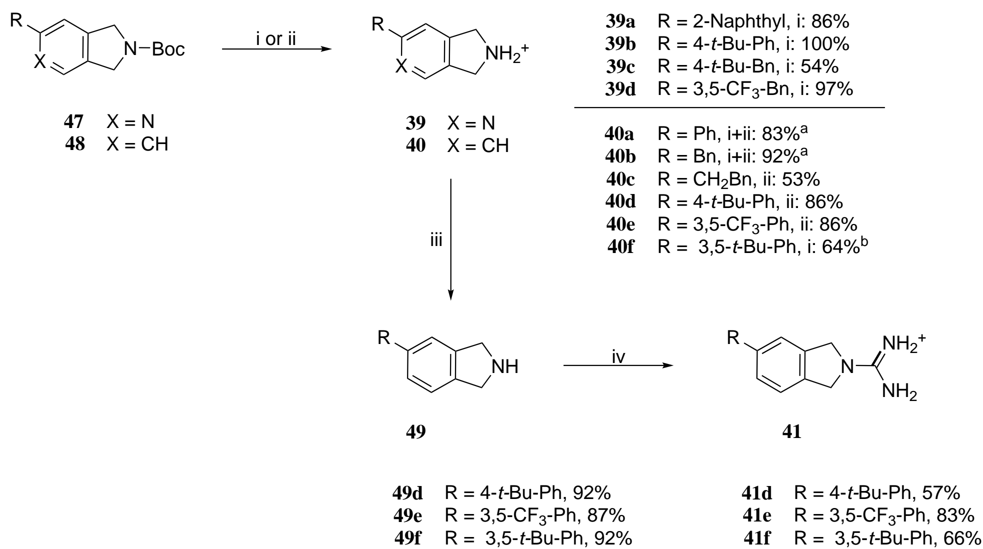


Scheme 2.13. i) Boc_2O, DCM, 0 °C - rt, Ar-atm, 2 h. ii) Propargyl bromide, NaH, THF, rt, 25 h.[295,296] iii) 1,4-dioxane, Ar-atm, *hv* (two halogen lamps, 400 W, 118 nm, 50 Hz), 22 - 48 h.[287,330,331] iv) DCE, Ar-atm, rt, 18 - 32 h.[332,333]



ilar systems perform better in cycloadditions with this Co-catalyst.[331] This corresponded with the observation made during the synthesis of **47**, as the benzylic nitrile **43c** gave lower yield in the cycloaddition (36%) compared to the phenylic nitrile **43b** (58%). Furthermore, the six carbamate-protected isoindolines **48a** - **48f** were prepared in 45-78% yields, the highest yielding reaction being the cycloaddition of **46** with but-3-ynylbenzene (**44c**) to give **48c**. The five benzylic and

Scheme 2.14. i) HCl (37%, aq), MeCN, rt, 23 - 48 h. ii) HCl (2 M, Et_2O), Et_2O or DCM, rt, 24 - 71 h. iii) K_2CO_3(sat. aq)/solvent. iv) **20** (0.9 - 1.0 equiv), MeCN, reflux, 3 - 31 h.[300] a) Conditions i followed by ii to give full conversion. b) Also isolated as its TFA-salt from TFA in DCM in 75% yield. Counterion for **39/40** and **41**: Cl^- (+ CF_3COO^- for **40f**).



phenylic alkynes afforded yields around 50%, whereas **44c** gave the cycloaddition product in close to 80% yield. This may indicate some steric interactions affecting the reaction yields, and it has been shown that this Ru-catalyst often offer sterically favored cycloaddition products due to interactions with the bulky Cp*-ligand.[332,333]

Deprotection and *N*-Guanylation of Cycloaddition Products

In order to provide the target amphiphile HCl-salts **39** and **40**, the carbamate protecting group on **47** and **48** had to be cleaved. The cycloaddition products **47a** - **47d** and **48a** - **48f** were therefore subjected to deprotection under acidic conditions using either etheric HCl (2 M) or aqueous HCl (37%) as shown in Scheme 2.14. The deprotection conditions gave the ten HCl-salts **39a** - **39d** and **40a** - **40f** in 53-100% yields.

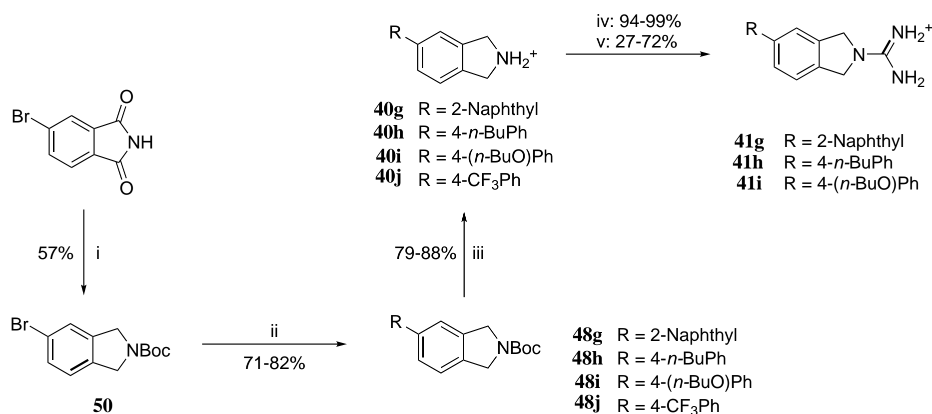
As a final part of this section, the three isoindolines **40d - 40f** were free-based using K_2CO_3(sat. aq)/solvent and *N*-guanylated, shown in Scheme 2.14, using the conditions presented in Paper I and earlier sections (2.1.1 and 2.2.1).[300] The guanylation with 1*H*-carboxamidine hydrochloride (**20**) in refluxing MeCN afforded the guanidine amphiphiles **41d - 41f** in 57-83% yields. One interesting observation made during the work-up of the guanylated isoindolines **41d - 41f**, was that the products appeared to be poorly soluble in MeCN both at reflux and at room temperature. Hence, a simple filtration of the cooled down reaction mixture gave the pure products in decent to good yields (57-83%).

2.3.2 Synthesis of Isoindoline Amphiphiles via Suzuki Cross-Coupling

The seven isoindoline amphiphiles not prepared via [2+2+2] cycloaddition reactions (**40g - 40j** and **41g - 41i**) were prepared via Suzuki cross-coupling between **50** and appropriate boronic acids ($R\text{-}B(OH)_2$), as shown in Scheme 2.15.[334,335] The key compound **50** was obtained from reduction and *N*-protection of 5-bromophthalimide in 57% yield over two steps.[336] Cross-coupling with aromatic boronic acids afforded the pro-amphiphilic isoindole carbamates **48g - 48j** in 71-82% yields.

After preparation of **48g - 48j** through Suzuki cross-coupling, the carbamate group was cleaved by treatment with HCl in MeCN giving the four amphiphilic HCl-salts **40g - 40j**. The three isoindoline HCl-salts **40g - 40i** were then free-based using K_2CO_3 (sat. aq)/EtOAc before being reacted with 1*H*-pyrazole carboxamidine hydrochloride (**20**) in MeCN at reflux, analogously to the previous sections (2.1 and 2.2) and the deprotected [2+2+2] cycloaddition products.[300] The reaction with **20** afforded the three guanidine salts **41g - 41i** in 27-72% yields. It should be noted that the free amine of **40i** was unstable in air, and was placed under argon after free-basing and used directly in the guanylation reaction to form **41i** in 27% yield. The apparent low stability of the neutral version of **40i** may also explain the lowered yield observed in this reaction.

Scheme 2.15. i) 1) $NaBH_4$, $BF_3 \cdot Et_2O$, THF, 70 °C, 18 h. 2) Boc_2O, 4-DMAP, DMF, rt, 36 h.[336] ii) R-B$(OH)_2$, $Pd(PPh_3)_4$ (7 mol %), K_2CO_3, 1,4-dioxane/H_2O 1:1, Ar-atm, reflux, 22 h.[334,335] iii) HCl (37%, aq), MeCN, rt, 23-48 h. iv) K_2CO_3 (sat. aq)/EtOAc. v) **20**, MeCN, reflux, 3-31 h.[300,315] Counterion for **40** and **41**: Cl^-.



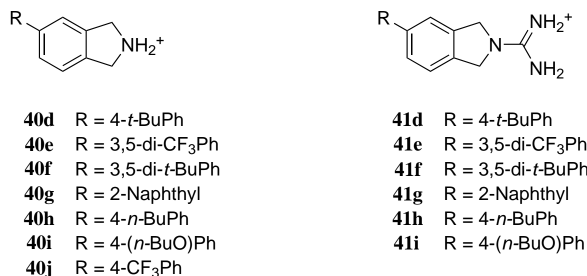
2.3.3 Biological Evaluation of Target Amphiphiles

Antimicrobial Activity

The amphiphiles exhibiting antimicrobial activity $\leq$64 μg/mL and their corresponding MIC-values are shown in Table 2.10, together with the EC_{50}-values from testing of *in vitro* cytotoxicity against HepG2-cells. It should be noted that **39a** and **39c** displayed a MIC-value of 64 μg/mL against *S. aureus*, but were not included in Table 2.12.

The activity trend seen in Section 2.1 and 2.2 was also observed in this series of amphiphiles. The amphiphiles with large lipophilic character was seen to display high antimicrobial activity, and the structures with low lipophilic contribution (**40a - 40c**) displayed no antimicrobial activity in the tested range ($\leq$64 μg/mL). The lack of activity was also seen for the four dihydro pyrrolopyridines **39**, which all displayed little to no activity. The lowered activity of **39** could possibly be explained by the increased polar character of the core fused ring structure, as the additional nitrogen probably increases the polarity of the dihydro pyrrolopyridine structure compared to the isoindoline ring. ClogD-values (pH = 7.4) for the dihydro pyrrolopyridine and isoindoline cores supported this ratio-

Table 2.12. Minimum inhibitory concentrations (MIC-values) and EC_{50}-values in μg/mL. Counterion: Cl^-.



	Antimicrobial activities [MIC]					HepG2[b]
	E. faecalis[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	[EC_{50}]
40d	32	8	16	32	64	1.3
40e	32	16	16	16	-[c]	6.1
40f	4	4	1	8	32	2.0
40g	16	4	8	16	32	2.0
40h	8	2	4	8	32	1.3
40i	16	4	8	16	-	1.0
40j	64	16	32	32	64	2.7
41d	4	2	4	4	8	12
41e	32	16	16	16	32	>64
41f	-	-	4	16	64	64
41g	8	2	2	4	8	7.8
41h	4	1	2	4	8	5.3
41i	8	2	4	4	16	7.1
Ref.[d]	10	0.13	4	0.5	0.5	n.d.[e]

[a] *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *S. agalacticae* (ATCC 12386), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).
[b] EC_{50} determined from Fig. 2.12.
[c] -: No activity $\leq$64 μg/mL.
[d] Ref.: gentamicin.
[e] n.d.: not determined.

nale, as **39** (R = H, ClogD: -0.88) was shown to be more polar than **40** (R = H, ClogD: -0.20).

Introduction of the guanidine group to the isoindolines (**41**) had a positive impact on the antimicrobial activity, also following the trend from the previous sections (2.1 and 2.2). The three guanidines **41g**, **41h**, and **41i** displayed a 2- to 4-fold improved potency over the corresponding amines (**40g - 40i**), whereas

the 4-*t*-Bu-phenyl guanidine **41d** displayed an impressive 4- to 8-fold improvement of potency compared to the amine **40d**. There were, on the other hand, also a couple of guanidines (**41e** and **41f**) that did not display the same notable improvement in potency when compared to the corresponding amines (**40e** and **40f**). The 3,5-CF_3-phenyl guanidine **41e** showed improved potency against *P. aeruginosa*, but the efficacies against the other four bacteria were the same as for **40e**. The highly lipophilic 3,5-di-*t*-Bu–phenyl substituted isoindoline guanidine **41f** even showed a decline in potency compared to the amine **40f**, but this observation may not have been related to antimicrobial activity. The amine **40f** was calculated to have a ClogD of 4.48 and a calculated PSA of 12.03, indicating a very high lipophilic character. The observed MIC-values of **40f** in the antimicrobial assays may therefore have come from non-specific cytotoxic interactions, as such toxicities are more prone to take place when the logD is >3 and the PSA <75 Å.[183,184] Introduction of the guanidine (**41f**) then led to a somewhat lowered lipophilic character (ClogD = 3.11 and PSA = 53.11), and lowered potency was observed. There is also a possibility that highly lipophilic compounds precipitate in the assays and create erroneous test results.

The lipophilic groups found to offer the highest activity in the antimicrobial assays were the 4-butyl-phenyl species (4-*t*-Bu, 4-*n*-Bu, and 4-*n*-BuO) and the 2-naphthalene group, as they mostly showed MIC-values <10 μg/mL. The MIC-values for **41d**, **41g**, **41h**, and **41i** against Gram-negative bacteria were identical, with 4 μg/mL against *E. coli* and 8 μg/mL against *P. aeruginosa* (except for **41i**: MIC 16 μg/mL). There was on the other hand observed a small difference in potency against the Gram-positive bacteria, where the *n*-Bu-phenyl derivative **41h** was found to be the most potent of the four structures with MIC-values ranging between 1-4 μg/mL. The other three compounds had very comparable activity levels, where the only differences were a 2-fold increase in potency of the 4-*t*-Bu-phenyl substituted **41d** against *E. faecalis* and a 2-fold increase in potency of the naphthalene isoindoline **41g** against *S. agalacticae*.

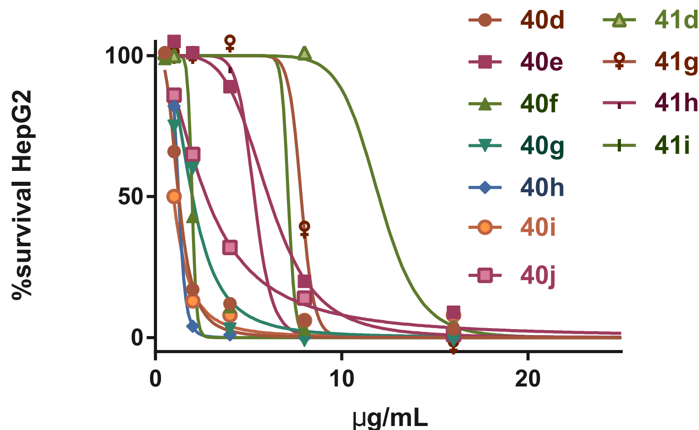


Figure 2.12. Dose-response curves from non-linear curve regression for *in vitro* cytotoxicity against HepG2-cells. Curves for **41e** and **41f** are not shown.

Cytotoxicity

The 13 isoindoline amphiphiles displaying interesting antimicrobial activity were evaluated for cytotoxicity against HepG2-cells, and the EC_{50}-values shown in Table 2.12 were determined from the dose-response curves* in Fig. 2.12.

The guanidines **41** were found to be less toxic than the amines **40**, which corresponded well with the toxicity data obtained for the 1,2,3-triazoles in Sections 2.1.2 and 2.2.2. Most of the amines **40** were, on the other hand, seen to display EC_{50}-values <3 μg/mL against HepG2-cells (only exception: **40e**, EC_{50} 6.1 μg/mL), affording virtually no selectivity between bacteria and mammalian cells. The least toxic guanidines were seen to be **41d**, **41e**, and **41f**, as neither of these three showed EC_{50}-values <10 μg/mL unlike the remaining compounds in the series. The isoindoline guanidine with the 4-*t*-Bu-group (**41d**) was the least toxic isoindoline showing broad-spectrum activity with an EC_{50}-value of 12 μg/mL. Interestingly, due to the high antimicrobial activity, the guanidine **41d** displayed a selectivity that was highly similar to the one seen for the 1,2,3-triazole guanidine **22f**.

The two isoindolines that displayed the best toxicity profile in the HepG2-assay were the 3,5-CF_3-phenyl guanidine **41e** and the 3,5-di-*t*-Bu–phenyl guani-

*EC_{50}-value for **41e** was found from raw data, as 64 μg/mL afforded 53% cell survival.

dine **41f**. The low toxicity of **41e** may be attributed to a generally low level of biological activity, as also the MIC-values for **41e** were moderate compared to the other isoindoline guanidines in Table 2.12. The toxicity of **41f** on the other hand deviated from the common observations made regarding lipophilic character and toxicity, by displaying a 5-fold lowered toxicity compared to that of the 4-*t*-Bu-phenyl variant (**41d**). The reason for the lowered toxicity of **41f** compared to the other active isoindolines is unknown, as it was somewhat counterintuitive to what was expected when compared to other compounds. Due to the high lipophilic bulk of **41f** (ClogD = 3.11 at pH = 7.4), it was assumed to display higher toxicity than the less lipophilic **41d**. Since compounds with high overall lipophilicity often display nonspecific toxic interactions (discussed in the start of this section and in Section 1.3.1).[183,184] One possible explanation to the observed toxicity could be that **41f** precipitated out at high concentrations during the assays, lowering the effective concentration of the compound and led to the observed EC_{50}-value.

The high toxicity of the amines **40** in the HepG2-assay could, as previously discussed, be attributed to the higher lipophilic character and lower polar surface area of **40** compared to the guanidines **41**. The difference in basicity may also be a contributing factor, as the amines are assumed to be less basic than the guanidines (as discussed in Section 2.1.2).[317,318]

2.3.4 Conclusion

This section describes the synthesis of 20 dihydro pyrrolopyridine and isoindoline amphiphiles for antimicrobial evaluations. The four carbamate-protected dihydro pyrrolopyridines **47a** - **47d** and the six carbamate-protected isoindolines **48a** - **48f** were prepared through [2+2+2] cycloaddition between **46** and **43**/**44** using a cobalt- ($CpCo(CO)_2$) or ruthenium-catalyst (Cp*RuCl(cod)) in 36-78% yields. The remaining four carbamate-protected **48g** - **48j** were obtained through Suzuki cross-coupling of **50** with aromatic boronic acids. The carbamate group was then cleaved and a selected set of isoindolines were *N*-guanylated, affording the 20 target amphiphiles shown in Fig. 2.11.

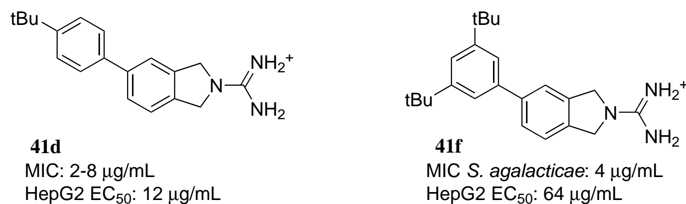


Figure 2.13. The two most promising amphiphiles from Section 2.3. Counterion: Cl^-.

The two most promising structures from the compounds presented in Section 2.3 are shown in Fig. 2.13, together with some key biological data. The highly active isoindoline guanidine **41d** was assessed to be the most promising compound for broad-spectrum antimicrobial activity. This 4-*t*-Bu-phenyl guanidine displayed low MIC-values between 2-4 μg/mL against Gram-positive bacteria and 4-8 μg/mL against Gram-negative bacteria. The other highlighted structure from this set was the isoindoline **41f**, as it displayed high selective activity against *S. agalacticae* (MIC 4 μg/mL). The somewhat lowered toxicity and high selective activity of **41f** gave a 16-fold increased potency against *S. agalacticae* over mammalian cells (HepG2). The high activity against *S. agalacticae* observed for **41f** followed the trend seen from Section 2.2, where more lipophilic substrates were shown to display hightened potency against *S. agalacticae*.

2.4 Comparison of Best Candidates in the Library

As a final section in Chapter 2, the best candidates from Section 2.1 - 2.3 will be compared on the basis of their antimicrobial activity and level of toxicity. The best candidates taken from the conclusions of the different sections (**22f** and **28e** chosen from Fig. 2.10) and the respective papers are shown in Table 2.13, and were selected as the current best candidates in the project on the basis of the following criteria:

- **Antimicrobial potency** (broad- or narrow-spectrum activity).
- **Toxicity** (highly toxic compounds eliminated).
- **Selectivity** (antimicrobial efficacy compared to toxic effects).

The amphiphiles **6f**, **22f**, and **41d** displayed high broad-spectrum antimicrobial activity and similar selectivity factors. Where the aliphatic 1,2,3-triazole **6f** was more potent against Gram-positive bacteria than the more polar amido 1,2,3-triazole **22f**, and the isoindoline **41d** surpassed the activity of both the triazoles against *E. faecalis*, *S. aureus*, and *E. coli* (Table 2.13). The three amphiphiles **6f**, **22f**, and **41d** were also seen to display increased potency against Gram-negative bacteria compared to the most promising aminobenzamide **E23** (Fig. 2.14) by Igumnova *et al.*[180] Where all three were 2-fold more potent against *P. aeruginosa* and the isoindoline guanidine **41d** additionally was seen to be 2-fold more potent against *E. coli* compared to **E23**. The increased potency seen against Gram-negative bacteria for **6f**, **22f**, and **41d** was, however, not observed against Gram-positive bacteria, where **E23** had a MIC-value of 1 μg/mL against *S. aureus* and **6f**, **22f**, and **41d** were 2- to 4-fold less potent.

The three amphiphiles **6f**, **22f**, and **41d** also showed increased or comparable antimicrobial potency compared to the marine antimicrobial ianthelline (**52**, Fig. 2.14), shown in Table 2.13. The 1,2,3-triazoles **6f** and **22f** matched or surpassed the activity of the natural product **52** against all bacteria except against *S. aureus*. They were also 2.5-fold more potent against *S. aureus* than synoxazolidinone A (**53**, Fig. 2.14). The isoindoline **41d** on the other hand, displayed a 2- to 5-fold increased potency against three bacteria compared to the natural product ianthelline (**52**), and matched the activity against *S. aureus* and *P. aeruginosa*.

Table 2.13. Best candidates from Section 2.1 - 2.3, and data for antimicrobial amphiphiles from published literature. Counterion: Cl^-.

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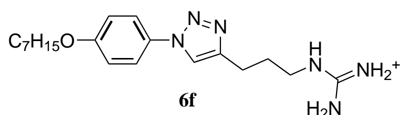

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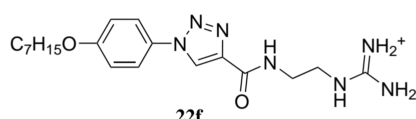

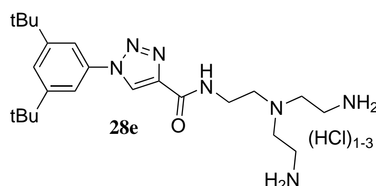

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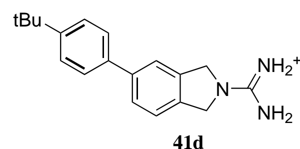
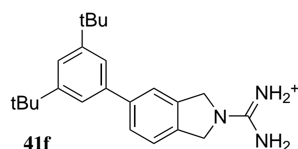




	Antimicrobial activities [MIC]					HepG2[b]
	E. faecalis[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	[EC_{50}]
6f	8	4	4	8	8	17.7
22f	16	4	8	8	8	23.8
28e	16	16	8	16	4	32-64
41d	4	2	4	4	8	12
41f	-[b]	-	4	16	64	64
Ref.[c]	10	0.13	4	0.5	0.5	n.d.[d]
E23[180]	n.r.[e]	1	n.r.	8	16	37[f]
52[172]	22.5	2.5	7.5	7.5	7,5	>100[g]
53[173]	n.r.	10	n.r.	n.r.	n.r.	n.r.
54[170]	n.r.	3.1	n.r.	3.1	2.8	50[f]
55[169]	1.56	n.r.	n.r.	3.12	6.25	85[f]
51[168]	n.r.	2	n.r.	3	8	175[f]

[a] *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *S. agalacticae* (ATCC 12386), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).
[b] "-": No activity at or below 64 μg/mL.
[c] Ref.: gentamicin.
[d] n.d.: Not determined.
[e] n.r.: Not reported.
[f] Hemolytic activity: HC_{50}.
[g] MRC5 EC_{50}.

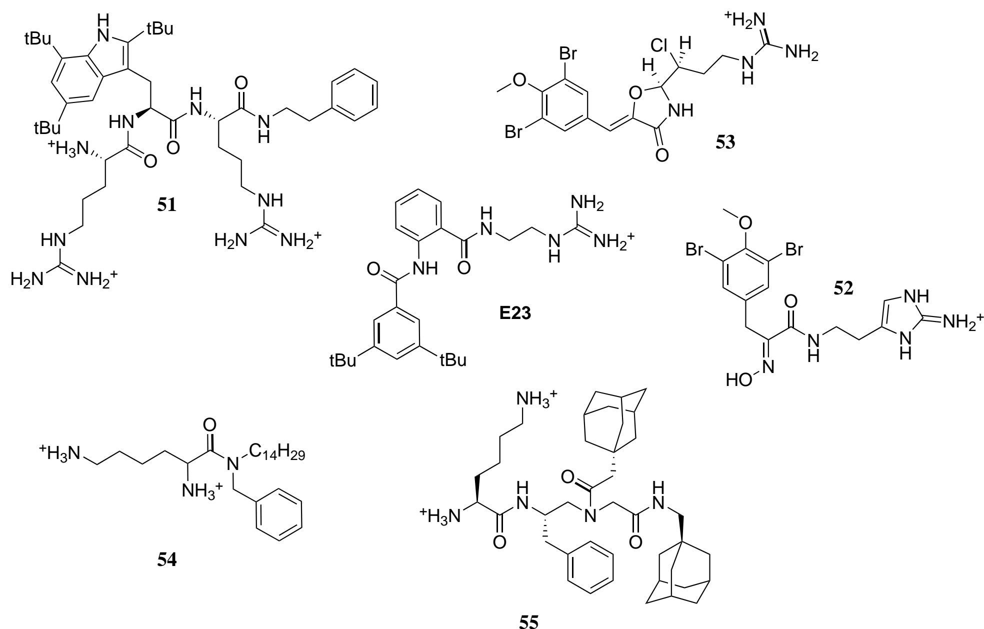


Figure 2.14. LTX-109 by the group of Svendsen (**51**),[168] **E23** by Igumnova *et al.*,[180] ianthelline (**52**),[172] synoxazolidinone A (**53**),[173] **54** by Ghosh *et al.*,[170] and **55** by Teng *et al.*[169] All structures shown in their charged state.

The isoindoline guanidine **41d** was furthermore seen to be more potent against *S. aureus* than the lysine derivative **54** (Fig. 2.14) presented by Ghosh *et al.*[170] It should also be noted that, in addition to the high potency of **41d** against *S. aureus*, the MIC-values of **6f**, **22f**, and **41d** against *P. aeruginosa* (6.25 μg/mL) were just above that of Teng's reduced acylated amide **55** (Fig. 2.14), and the MIC-value of **41d** against *E. coli* was just above that of **54** and **55** (4 vs. 3.1 μg/mL).[169,170] The general lower level of activity of **6f**, **22f**, and **41d** compared to the lead compounds of Teng *et al.* (**55**) and Ghosh *et al.* (**54**) was somewhat expected due to the lower degree of functionalization and complexity of the two 1,2,3-triazoles and the isoindoline guanidine (**6f**, **22f**, and **41d**).

The most promising isoindoline **41d** was more potent in the antimicrobial assays than the 1,2,3-triazoles **6f** and **22f**, but also more toxic against HepG2-cells. The aliphatic 1,2,3-triazole **6f** was in turn more toxic than the amido 1,2,3-triazole **22f**, but similarly to **41d**, it was also more potent in the antimicrobial

assays. The differences in potency and toxicity could possibly be attributed difference in lipophilicity, as estimated by the ClogD-values for **6f**, **22f**, and **41d** (shown in Table 2.14). Thus, **22f** was calculated to be less lipophilic than **6f**, which furthermore had a lower ClogD than the isoindoline **41d**. The elevated toxicities of **6f**, **22f**, and **41d** were also clear when compared to other amphiphilic antimicrobials. Both the marine antimicrobial ianthelline (**52**, MRC5 EC_{50} >100 μg/mL) and Igumnova's most promising aminobenzamide **E23** (RBC EC_{50} 37 μg/mL) displayed reduced toxicity compared to **6f**, **22f**, and **41d**.[172,180] Additionally, the antibiotic used as positive reference in the assays (gentamicin), showes toxic activity against kidney epithelial cells at 1 mM concentration. This is a substantially higher toxic threshold than the threshold observed for these three amphiphiles.[337]

Table 2.14. Selectivity factors and some calculated physicochemical properties for the best candidates from the three papers (II, III, and IV).

Compound	**6f**	**22f**	**28e**	**41d**	**41f**
Selectivity factors[a]	2.2 - 4.4	1.5 - 6.0	2.0 - 16.0[b]	1.5 - 6.0	1.0 - 16.0
Avg. selectivity factor	3.1	3.3	-	3.3	7[c]
pKa	12.1	11.9	9.67	11.8	11.5
ClogD (pH = 7.4)	1.16	0.10	−1.22	1.56	3.11

[a] HepG2 (EC_{50}) divided by antimicrobial MIC-values.
[b] Due to uncertain EC_{50}-value.
[b] Only active against three out of five bacteria.

The somewhat poor selectivity of **6f**, **22f**, and **41d** (average selectivity factor: 3.1 for **6f** and 3.3 for **22f** and **41d**, Table 2.14) could also possibly be explained by the presence of electron-rich aromatic rings, as these are more prone to be oxidized in phase I metabolism than more electron-poor species.[338] Compounds with electron-poor aromatic rings and the equivalent lipophilic contribution should therefore be prepared to see if this could explain the observed toxicity. However, as Hep2G-cells have been shown to give poor representations of hepatic metabolism, the toxic effects may not come from site-specific toxicity at all.[339] The enzymes usually responsible for most of the metabolism discussed above are the cytochrome P450 enzymes, and work presented by Gerets *et al.* shows that HepG2-cells display little cytochrome P450 activity when treated with inducers.[339] Chasing specific mechanisms responsible for the observed toxicity based on the HepG2-assay may therefore prove to be a red herring. The

working hypothesis is then considered to be that the compounds induced nonspecific lipophilic toxicity (lysis) in the assay.[183,184]

One of the more interesting structures in the library aside from the three discussed above, was the amido 1,2,3-triazole functionalized with tris(2-aminoethyl)amine (**28e**). This amphiphile displayed high antimicrobial potency against *P. aeruginosa* and had a relatively high EC_{50}-value (between 32 and 64 μg/mL) in the HepG2-assay, giving a selectivity factor between 8 and 16 towards *P. aeruginosa* (Table 2.14). The amine salt **28e** was more potent against *P. aeruginosa* (Table 2.13) than both **55** by Teng *et al.* and LTX-109 (**51**) by the group of Svendsen.[168,169] Compared to the work by Ghosh *et al.*, the potency was lower for **28e** compared to their best structure (**54**) against *P. aeruginosa*.[170] The toxicity profile of **28e** against HepG2 was, on the other hand, also less optimal when compared to the activity of these three compounds (**51**, **54**, and **55**) against red blood cells, as shown in Table 2.13.

Unfortunately, only two target compounds of **28** were prepared during the project, so only the benzylic and phenylic 3,5-di-*t*-Bu-groups have been evaluated together with this cationic nitrogen group. This functionality must therefore be investigated further due to the interesting biological effects seen for **28e**. Additionally, it is of interest to see the effect of one or two guanidine functional groups on these compounds and see if the structures accept higher lipophilic contributions without becoming too toxic. This rationale is based on the apparent low lipophilic character of **28e** (ClogD -1.22) compared to the other lead compounds in Table 2.13, as the low toxicity seen for **28e** corresponded well with the calculated distribution coefficient. The lowered toxicity from adding an additional nitrogen group on the hydrophilic side could possibly also be used to reduce the toxicity of the most promising compounds with only one lipophilic- and hydrophilic group (e.g. **6f** and **22f**).

The last amphiphile shown in Table 2.13, the 3,5-di-*t*-Bu–phenyl guanidine **41f**, showed high selective potency against *S. agalacticae*, making it two-fold more potent against this bacteria compared to ianthelline (**52**). It was particularly interesting due to the seemingly low activity against HepG2-cells with an EC_{50}-value of 64 μg/mL. The high potency and low toxicity led to a selectivity factor of 16 for **41f** against *S. agalacticae* (Table 2.14), making it into one of the

more selective compounds in the library. The high lipophilicity of **41f** together with the high selective potency against *S. agalacticae*, corresponded well with the tendency seen for the more lipophilic structures (e.g. **22k**) in Section 2.2.2. These compounds also displayed high potency against *S. agalacticae*. The low toxicity of **41f** on the other hand, contradicted the common tendency for the compounds in the library, as more lipophilic compounds usually displayed higher toxicity against HepG2-cells. It is therefore a possibility that something went awry in the dose-response assay (e.g. precipitation, as discussed in Section 2.3.3), which led to the observed activity.

2.4.1 Biological Activity Against other Targets

In a pursuit of obtaining more data for promising compounds in the library, a selected set of structures were submitted to the "Community for Open Antimicrobial Drug Discovery" (CO-ADD)*. This Australia-based organization provided free screening against MRSA, *E. coli*, multidrug-resistant *Klebsiella pneumoniae*, *P. aeruginosa*, and *Acinetobacter baumannii*, in addition to the two species of fungi *Candida albicans* and *Cryptococcus neoformans*. The two 4-heptyloxyphenyl triazoles **6f** and **22f** were among the 11 compounds submitted to CO-ADD, and preliminary results from the single-concentration assays gave some interesting data. The initial assays showed that **6f** and **22f** were active in all seven assays at 32 μg/mL, including both MRSA and multidrug-resistant *K. pneumonia*. Depending on the results from dose-response assays, this could be an indication that the amphiphiles in the library giving activity against antibiotic-susceptible bacteria also give efficacy against resistant strains. Unfortunately, the dose-respsonse evaluations of these compounds were not completed in time to be included in this dissertation.

*Funded by the Wellcome Trust (UK) and the University of Queensland (Australia).

CHAPTER 3

CONCLUSION AND FURTHER WORK

3.1 Summary

The main focus of the work conducted in this thesis has been to establish synthetic routes for synthesis of amphiphilic target compounds based on a model for small molecule amphiphilic antimicrobials. The goal was to prepare a high number of target structures for biological evaluation, based on different scaffolds and synthetic methodologies. Scaffold synthesis of 1,2,3-triazoles, isoindolines, and fused pyridines (dihydro pyrrolopyridines) were important, in addition to development of versatile *N*-functionalization routines in order to provide target structures in high purity.

Paper I[300] has not been given its own section in the thesis. It has however been a very important tool for providing most of the target guanidine amphiphiles in the project. This method paper describes the efficient preparation of guanidines using a known reagent in refluxing acetonitrile. The target guanidine amphiphiles were obtained using these conditions in high purity with little work-up.

Paper II[298] describes the synthesis and biological evaluation of 28 cationic amphiphiles based on aliphatic amino 1,2,3-triazoles. The synthetic strategy presented in the paper is based mainly on subjecting phthalimide-protected alkynes to CuAAC, followed by deprotection, *N*-functionalization by guanylation and reductive amination. The most promising structure was found to be **6f** with a heptyl ether chain attached to the lipophilic benzene ring and a cationic guanidinium functionality as the cationic group.

Paper III[299] was a further investigation of the 1,2,3-triazole ring as a scaffold for low molecular weight amphiphilic antimicrobials. The 29 target compounds were prepared from CuAAC between an azide and methyl propiolate or 3-butynone, followed by subsequent amidation or iminoguanylation. In addition to screening different lipophiles and hydrophiles, this paper also investigated the effect of increasing the linker rigidity of on the hydrophilic side of the 1,2,3-triazole skeleton. The most promising amphiphile for further development was **22f**, which in turn was similar to the most promising structure (**6f**) from the series assessed in Paper II.

Paper IV[297] describes the synthesis and biological evaluation of 20 dihydro pyrrolopyridine and isoindoline amphiphiles. Of these 20 amphiphiles, 13 were prepared through a route utilizing transition metal catalyzed [2+2+2] cycloaddition using a cobalt- and a ruthenium-catalyst. The remaining seven isoindolines were prepared through a route utilizing Suzuki cross-coupling of carbamate protected 5-bromoisoindoline with aromatic boronic acids. After synthesis of the core aromate, the pro-amphiphiles were deprotected and functionalized in the *N*-position, affording 20 target amphiphiles for antimicrobial evaluation. The most promising amphiphile from biological evaluation of this part of the library was found to be the isoindoline **41d** carrying a 4-*t*-Bu-group and a guanidine cationic group. The antimicrobial potency was found to be higher than the potency of the most promising 1,2,3-triazoles, however the increased cytotoxicity afforded an average selectivity factor for **41d** similar to that found for **22f**.

In addition to the target compounds presented in the papers, an additional 23 amphiphiles have been prepared and evaluated, and are presented in the sections together with the target structures from the corresponding papers. Of the structures not included in the papers/manuscripts, **28e** was shown to be particularly interesting with regards to the observed high potency against *P. aeruginosa* and the lowered toxicity against HepG2-cells.

3.2 Conclusion

Evaluation of the compound library so far has shown 41 compounds within the different classes with antimicrobial activities in the tested range. Out of these, 36 amphiphiles were considered unfit due to:

- **Low antimicrobial activity:** A portion of the compounds displayed only moderate antimicrobial activity, and were therefore not evaluated further.
- **Cytotoxicty:** Most of the biologically active amines and iminoguanidines displayed high cytotoxic activity against HepG2-cells.
- **Selectivity:** Poor selectivity between human cells and bacterial cells was also an issue for many of the compounds displaying high toxicity.

After filtering out inactive and unacceptably toxic amphiphiles, the selection consisting of the five compounds shown below were chosen as the lead structures for further research in this compound library.

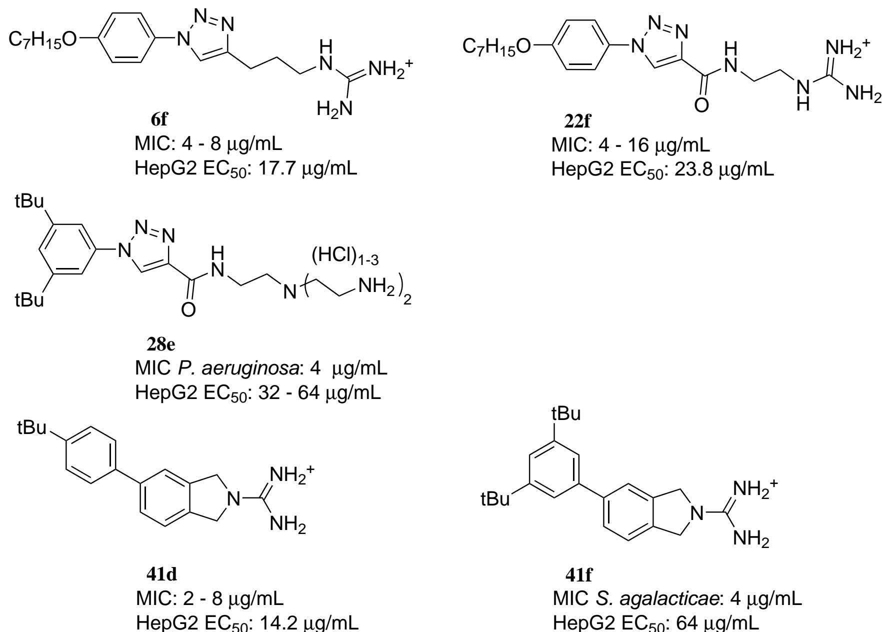


Figure 3.1. Current lead structures. Counterion: Cl^-.

3.3 Further Work

Based on the structures of the compounds chosen as current lead structures in the library, there exist multiple ways to possibly enhance the activities of the structures and address the recurring toxicity issues that have haunted the most active compounds in the library. The 1,2,3-triazoles carrying one cationic group and one lipophile (**6f** and **22f**) may not be suited for drug development due to their poor selectivity. However, as the synthetic routes have been established, they can be used to gain a perspective of the relative potencies of different functional groups.

The further optimization of the 1,2,3-triazole compounds should focus on optimization of their antimicrobial activity and reduction of their toxicity. One possible way to do this is through further development of **28e**, who displayed high antimicrobial activity against *P. aeruginosa* and lowered toxicity compared to the other active 1,2,3-triazoles. The first attempts at optimization should be to install one or two guanidine groups on the amine groups of **28e** (as shown in Fig. 3.2), in order to see if this increases the antimicrobial potency and reduces possible toxic effects. Furthermore, expanding this compound series with more lipophilic groups would also be beneficial for the overall progress of the project.

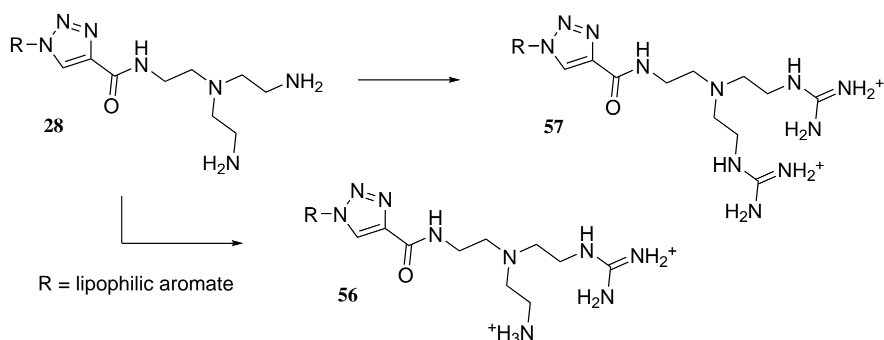


Figure 3.2. Guanylation of **28** to **56** or **57**.

One way to reduce the toxicity of the amphiphiles could be to increase the number of lipophilic groups on the scaffold. Many of the heavily lipophilic groups in this project contribute with electron donating effects to the aromatic group

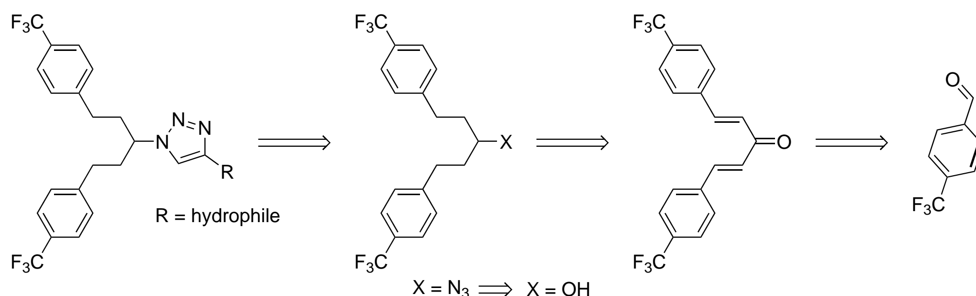


Figure 3.3. Strategy for preparation of 1,2,3-triazole amphiphiles with two lipophilic groups from an aromatic aldehyde.

they are attached to, which also increases the reactivity of the ring. Having electron rich aromatic systems in biological systems is known to increase toxicity, with hepatic toxicity as a particular concern.[338] Thus, by having two lipophilic groups on the 1,2,3-triazole it could be possible to have the same net lipophilic bulk with assumed less toxic lipophiles. A strategy for achieving this substitution pattern is shown in Fig. 3.3, where the lipophilic azide is prepared from an aromatic aldehyde through an Aldol condensation followed by reduction and azidation.

Improvement of the pharmacological properties of the isoindoline-based amphiphiles (**41d** and **41f**) could employ some of the same strategies presented for the 1,2,3-triazoles. Increasing the polar surface area, in addition to introduction of two hydrophilic groups analogously to the triazoles above, may therefore prove to be beneficial for the selectivity. One way to prepare the amphiphiles **58** utilizing this strategy is amidation of indane esters **59** as shown in Fig. 3.4 (1). The indane **59** may be prepared through [2+2+2] cycloaddition between an di-alkylated malonate and aromatic alkynes. Subsequent decarboxylation and esterification will then yield **59**. A different strategy for increasing the hydrophilic character of the isoindolines could also be to couple the most potent isoindolines with polar amino acids (e.g. arginine), as also seen in Fig. 3.4 (2). This strategy can be applied using the already prepared isoindolines **49**, since it can be peptide-coupled to a protected arginine. The target amphiphile **60** will then be obtained from N-deprotection of the peptide-coupling product.

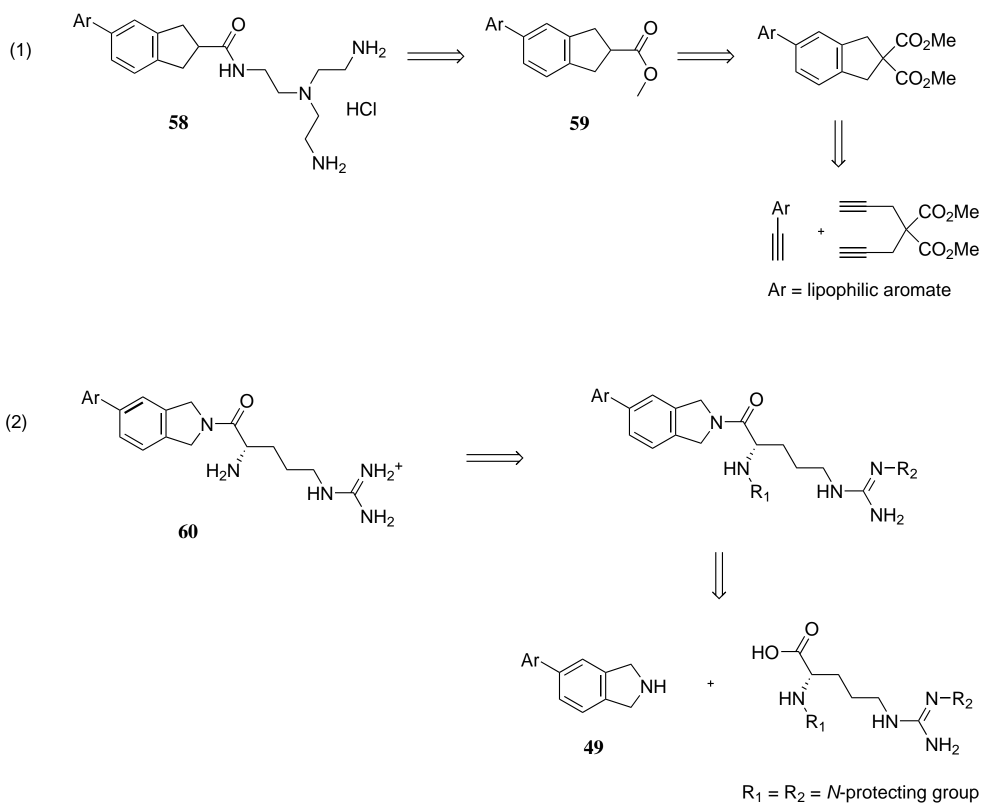


Figure 3.4. 1) Strategy for preparation of amphiphiles similar to isoindolines, with a larger hydrophilic portion. 2) Strategy for coupling already prepared isoindolines to polar cationic amino acids.

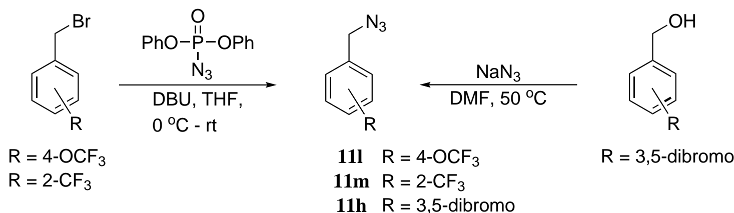
CHAPTER 4

EXPERIMENTAL

4.1 Chemistry

This chapter covers experimental data regarding compounds not published in any of the papers. The general experimental and analytical methods are the same as described in Paper III.[299]

4.1.1 Synthesis of Benzylic Azides 11



1-(Azidomethyl)-4-(trifluoromethoxy)benzene (11l)

The preparation of **11l** was carried out according to a published method,[305,319] by Martin Furru Vold as part of a student project. A suspension of 1-(bromomethyl)-4-(trifluoromethoxy)benzene (4.62 g, 18.1 mmol) and NaN_3 (1.79 g, 27.5 mmol) in DMF (50 mL) was heated to 50 °C for 2.5 h. The cooled reaction mixture was then added H_2O (25 mL) and extracted with Et_2O (3 x 20 mL). The combined organic phases were washed with H_2O (20 mL) and brine (20 mL), before it was dried over $MgSO_4$. Evaporation under reduced pressure afforded **11l** as a pale yellow oil (2.57 g, 11.8 mmol, 65%). ^{1}H NMR analysis corresponded with previously reported data for **11l**.[340] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 (d, 2H, J = 8.5 Hz), 7.27 (d, 2H, J = 8.2 Hz), 4.40 (s, 2H).

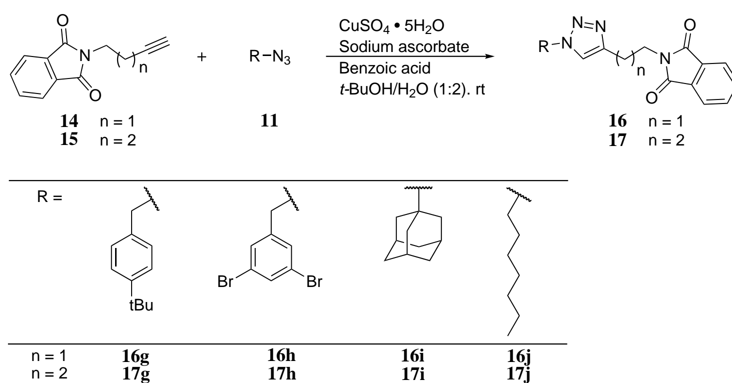
1-(Azidomethyl)-2-(trifluoromethyl)benzene (11m)

The preparation of **11m** was carried out as described for **11l** with 2-(trifluoromethyl)-benzylbromide (5.40 g, 22.6 mmol) by Kristine Olsen Strandheim, as part of a student project. Affording **11m** as a clear oil (3.83 g, 19.0 mmol, 84%). ^{1}H NMR analysis corresponded with previously reported data for **11m**.[341] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, 1H, J = 7.8 Hz), 7.62 - 7.53 (m, 2H), 7.44 (t, 1H, J = 7.2 Hz), 4.57 (s, 2H).

1-(Azidomethyl)-3,5-dibromobenzene (11h)

The preparation of **11h** was carried out according to a procedure described by Sharma *et al.*,[306] where (3,5-dibromophenyl)methanol (1.00 g, 3.76 mmol) in THF (20 mL) was cooled down to 0 °C before DPPA (0.89 mL, 4.14 mmol) and DBU (0.64 mL, 4.14 mmol) were added dropwise. The reaction mixture was then allowed to reach rt and stirred for 28 h. Evaporation and purification of the residue with flash column chromatography (pentane), afforded **11h** as a clear oil (0.78 g, 2.70 mmol, 72%). ^{1}H NMR analyses corresponded with previously reported data for **11h**.[342] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 - 7.63 (m, 1H), 7.41 - 7.40 (m, 2H), 4.32 (s, 2H).

4.1.2 Synthesis of Phthalimido Triazoles 16 and 17



General Procedure for Synthesis of 16 and 17

The synthesis was carried out according to a procedure described by Shao *et al.*[248] Where **14** or **15** (1.0 equiv) was added azide **11** (1.05 equiv), $CuSO_4 \cdot H_2O$

(5 mol %, 1 M in H_2O), sodium ascorbate (10 mol %, 2 M in H_2O), benzoic acid (10% mol), and *t*-BuOH/H_2O (2:1, 2 mL/mmol alkyne). The suspension was then stirred for 5 - 50 h at rt before H_2O (10 mL/mmol alkyne) was added, extracted with DCM (3 x 10 mL/mmol alkyne), dried over $MgSO_4$, and partially evaporated. Crystallization of the partially evaporated mixture with pentane afforded **16** or **17** as solids in 60 - 89% yields.

2-(2-(1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (16g)

The general procedure with **14** (0.200 g, 1.00 mmol) and **11g** (0.200 g, 1.05 mmol) at rt for 16 h afforded **16g** as a white solid (0.341 g, 0.88 mmol, 87%, mp 140.0 - 141.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 - 7.78 (m, 2H, Phth), 7.73 - 7.67 (m, 2H, Phth), 7.39 - 7.34 (m, 2H, Ph-3 and Ph-5), 7.31 (s, 1H, triazole-5), 7.18 - 7.13 (m, 2H, Ph-2 and Ph-6), 5.45 (s, 2H, Bn), 3.99 (t, 2H, J = 7.5 Hz, N-CH_2), 3.11 (t, 2H, J = 7.5 Hz, CH_2), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1 (C=O), 151.7 (Ph-4), 144.6 (triazole-4), 133.9 (Phth), 132.1 (Phth), 131.8 (Ph-1), 127.7 (Ph-2 and Ph-6), 126.0 (Ph-3 and Ph-5), 123.3 (Phth), 121.3 (triazole-5), 53.8 (Bn), 37.5 (N-CH_2), 34.6 (Cq-*t*-Bu), 31.3 (*t*-Bu), 24.9 (CH_2). IR (ATR): 2947 (w), 1709 (s), 1398 (m), 1102 (m), 1049 (m), 999 (m), 714 (s), 697 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 388.1893 (calcd. $C_{23}H_{24}N_4O_2$, 388.1899 $[M^*]^+$).

2-(2-(1-(3,5-Dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (16h)

The general procedure with **14** (0.254 g, 1.28 mmol) and **11h** (0.390 g, 1.34 mmol) for 24 h at rt and a different workup (filtration of the reaction mixture and washing the precipitate with water before crystallization), afforded **16h** as a white solid (0.459 g, 0.94 mmol, 74%, mp 161.9 - 164.4 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 - 7.78 (m, 2H), 7.74 - 7.67 (m, 2H), 7.64 (t, 1H, J = 1.7 Hz), 7.41 (s, 1H), 7.31 (d, 2H, J = 1.5 Hz), 5.42 (s, 2H), 4.01 (t, 2H, J = 7.1 Hz), 3.15 (t, 2H, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 145.3, 138.5, 134.4, 134.0, 132.0, 129.5, 123.6, 123.3, 121.6, 52.6, 37.3, 24.9. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 1710 (s), 1397 (m), 1050 (w), 989 (w), 862 (w), 738 (m), 714 (s), 706 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 488.9561 (calcd. $C_{19}H_{15}Br_2N_4O_2$, 488.9562 $[M+H]^+$).

2-(2-(1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (16i)

The general procedure with **14** (0.195 g, 0.98 mmol) and **11i** (0.200 g, 1.13 mmol) for 18 h at rt afforded **16i** as a white solid (0.263 g, 0.70 mmol, 71%, mp 168.4 - 170.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 - 7.80 (m, 2H), 7.73 - 7.68 (m, 2H), 7.48 (s, 1H), 4.01 (t, 2H, J = 7.4 Hz), 3.14 (t, 2H, J = 7.5 Hz), 2.28 - 2.17 (m, 9H, Ada), 1.84 - 1.73 (m, 6H, Ada). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 143.2, 133.9, 132.1, 123.2, 117.8, 59.3, 43.0, 37.5, 35.9, 29.4, 25.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 2908 (w), 1708 (s), 1393 (m), 1382 (m), 1010 (w), 714 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 377.1973 (calcd. $C_{22}H_{25}N_4O_2$, 377.1978 $[M+H]^+$).

2-(2-(1-Heptyl-1*H*-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (16j)

The general procedure with **14** (0.450 g, 2.26 mmol) and **11j** (0.335 g, 2.37 mmol) for 16 h at rt afforded **16j** as a white solid (0.463 g, 1.36 mmol, 60%, mp 117.8 - 119.2 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 - 7.80 (m, 2H), 7.72 - 7.68 (m, 2H), 7.39 (s, 1H), 4.31 (t, 2H, J = 7.2 Hz), 4.02 (t, 2H, J = 7.2 Hz), 3.15 (t, 2H, J = 7.5 Hz), 1.85 (p, 2H, J = 7.2 Hz), 1.33 - 1.20 (m, 8H), 0.88 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 144.2, 133.9, 132.1, 123.3, 121.1, 50.3, 37.5, 31.6, 30.3, 28.7, 26.4, 24.9, 22.5, 14.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 2922 (w), 1712 (s), 1397 (m), 1366 (m), 992 (m), 868 (w), 719 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 341.1975 (calcd. $C_{19}H_{25}N_4O_2$, 341.1978 $[M+H]^+$).

2-(3-(1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (17g)

The general procedure with **15** (0.175 g, 0.82 mmol) and **11g** (0.190 g, 0.90 mmol) for 50 h at rt afforded **17g** as a white solid (0.293 g, 0.73 mmol, 89%, mp 114.0 - 116.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 - 7.80 (m, 2H), 7.74 - 7.68 (m, 2H), 7.40 - 7.36 (m, 2H), 7.31 (s, 1H), 7.21 - 7.17 (m, 2H), 5.45 (s, 2H), 3.74 (t, 2H, J = 6.9 Hz), 2.75 (t, 2H, J = 7.6 Hz), 2.06 (p, 2H, J = 7.0 Hz), 1.31 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.4, 151.7, 147.2, 133.9, 132.1, 131.9, 127.8, 126.0, 123.2, 120.9, 53.7, 37.3, 34.6, 31.3, 28.2, 23.1. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 2961 (w), 1712 (s), 1393 (s), 1354 (m), 1026 (m), 771 (m), 719 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 405.2055 (calcd. $C_{24}H_{26}N_4O_2$, 402.2056 $[M^*]^+$).

2-(3-(1-(3,5-Dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (17h)

The general procedure with **15** (0.346 g, 1.62 mmol) and **11h** (0.500 g, 1.70 mmol) for 21 h at rt and a different workup (filtration of the reaction mixture after water addition, then dissolved in DCM, dried and evaporated), afforded **17h** as a white solid (0.662 g, 1.32 mmol, 81%, mp 162.4 - 164.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 - 7.82 (m, 2H), 7.75 - 7.70 (m, 2H), 7.64 (t, 1H, J = 1.8 Hz), 7.42 (s, 1H), 7.33 (d, 2H, J = 1.8 Hz), 5.43 (s, 2H), 3.75 (t, 2H, J = 6.6 Hz), 2.78 (t, 2H, J = 7.3 Hz), 2.09 (p, 2H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.4, 147.7, 138.6, 134.4, 134.0, 132.1, 129.6, 123.6, 123.2, 121.2, 52.6, 37.2, 28.0, 23.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 1698 (s), 1395 (s), 1360 (w), 719 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 502.9716 (calcd. $C_{20}H_{17}Br_2N_4O_2$, 502.9718 $[M+H]^+$).

2-(3-(1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (17i)

The general procedure with **15** (0.300 g, 1.41 mmol) and **11i** (0.262 g, 1.48 mmol) for 27 h at rt and addition of additional 0.03 equiv **11i** after 24 h, afforded **17i** as an off-white solid (0.445 g, 1.14 mmol, 81%, mp 145.8 - 149.7 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 - 7.82 (m, 2H), 7.75 - 7.68 (m, 2H), 7.50 (bs, 1H), 3.76 (t, 2H, J = 6.8 Hz), 2.78 (t, 2H, J = 7.5 Hz), 2.28 - 2.19 (m, 9H), 2.09 (p, 2H, J = 7.2 Hz), 1.84 - 1.73 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.4, 147.3, 133.9, 132.1, 123.2, 118.5, 59.2, 43.0, 37.4, 36.0, 29.5, 28.2, 23.2. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 2909 (w), 1704 (s), 1396 (s), 1357 (w), 713 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 390.2056 (calcd. $C_{23}H_{26}N_4O_2$, 390.2056 $[M^*]^+$).

2-(3-(1-Heptyl-1*H*-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (17j)

The general procedure with **15** (0.300 g, 1.41 mmol) and **11j** (0.213 g, 1.48 mmol) for 5 h at rt afforded **17j** as an off-white solid (0.390 g, 1.10 mmol, 78%, mp 63.8 - 65.8 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 - 7.80 (m, 2H), 7.77 - 7.68 (m, 2H), 7.42 (s, 1H), 4.30 (t, 2H, J = 6.7 Hz), 3.75 (t, 2H, J = 7.1 Hz), 2.78 (t, 2H, J = 7.4 Hz), 2.09 (p, 2H, J = 6.7 Hz), 1.88 (p, 2H, J = 6.4 Hz), 1.38 - 1.19 (m, 8H), 0.92 - 0.83 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.5, 146.7, 134.0, 132.1, 123.2, 120.8, 50.3, 37.3, 31.6, 30.3, 28.7, 28.2, 26.5, 23.0, 22.5, 14.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **16g**. IR (ATR): 2933 (w), 1698 (s), 1400 (m), 1366 (m), 1018 (m), 891 (w), 717 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 355.2130 (calcd. $C_{20}H_{27}N_4O_2$, 355.2134 $[M+H]^+$).

4.1.3 Synthesis of Amino Triazoles 18/19 and Their HCl-salts 1/4

Hydrazine hydrate, Toluene, reflux; HCl (37%, aq)

16 n = 1 / **17** n = 2 → **18** n = 1 / **19** n = 2 → **1** n = 1 / **4** n = 2

R =	tBu	Br Br		
n = 1	**18g**	**18h**	**18i**	**18j**
n = 2	**19g**	-	**19i**	**19j**
n = 1	**1g**	**1h**	**1i**	**1j**
n = 2	**4g**	-	**4i**	**4j**

General Procedure for Synthesis of 18/19 and Their HCl-salts 1/4

The synthesis was carried out according to a known procedure,[312,343,344] to which **16** or **17** in toluene (10 mL/mmol **16** or **17**) was added hydrazine hydrate (64 - 65%, 5 equiv) and refluxed for 2 - 48 h, with additional hydrazine hydrate added throughout the reaction (64-65%, 1-2 equiv). The warm reaction mixture was then filtered and evaporated, affording **18** or **19** in 32 - 98% yields. The free amines **18** and **19** were then turned into their corresponding HCl-salts with addition of HCl (0.1 - 0.5 mL, 37% aq) to the amine (25 - 80 mg) in MeCN (2-5 mL). Evaporation of volatiles afforded **1** and **4** as white solids in 55 - 100% yield.

2-(1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (18g) and 2-(1-(4-(*tert*-butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-aminium chloride (1g)

The general procedure with **16g** (0.328 g, 0.84 mmol), 7 h reflux, and additional hydrazine hydrate (0.100 mL) added after 5 h (total: 0.414 mL, 5.61 mmol), afforded **18g** as a slightly yellow oil (0.212 g, 0.82 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 - 7.37 (m, 2H, Ph), 7.26 (s, 1H, triazole-5), 7.22 - 7.19 (m, 2H, Ph), 5.46 (s, 2H, Bn), 3.01 (t, 2H, J = 6.5 Hz, N-CH_2), 2.82 (t, 2H, J = 6.6 Hz, CH_2), 1.31 (s, 9H, t-Bu).
The general procedure for converting **18g** (50 mg, 0.19 mmol) into its HCl-salt afforded **1g** as a white solid (55 mg, 0.19 mmol, quant., mp 188.9 - 191.0 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 6.2 min,

99% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.15 (bs, 3H, NH_3^+), 8.05 (s, 1H, triazole-5), 7.40 - 7.36 (m, 2H, Ph), 7.27 - 7.22 (m, 2H, Ph), 5.51 (s, 2H, Bn), 3.10 - 3.03 (m, 2H, N-CH_2), 2.96 (t, 2H, J = 7.2 Hz, CH_2), 1.25 (s, 9H, t-Bu). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 150.6 (Ph-4), 142.9 (triazole-4), 133.1 (Ph-1), 127.8 (Ph-3 and Ph-5), 125.5 (Ph-2 and Ph-6), 122.9 (triazole-5), 52.5 (Bn), 38.1 (N-CH_2), 34.3 (Cq-t-Bu), 31.1 (t-Bu), 23.3 (CH_2). IR (ATR): 2962 (m), 1606 (m), 1150 (m), 1059 (s), 700 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 259.1921 (calcd. $C_{15}H_{23}N_4$, 259.1921 [M-Cl]$^+$).

2-(1-(3,5-Dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (18h) and 2-(1-(3,5-dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-aminium chloride (1h)

The general procedure with **16h** (0.460 g, 0.94 mmol), hydrazine hydrate (0.350 mL, 4.69 mmol, 64-65%), and 2 h reflux, afforded **18h** as a light yellow oil (0.286 g, 0.80 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (s, 1H), 7.37 - 7.31 (m, 3H), 5.44 (s, 2H), 3.04 (t, 2H, J = 6.4 Hz), 2.85 (t, 2H, J = 6.4 Hz).
The general procedure for converting **18h** (80 mg, 0.22 mmol) into its HCl-salt with HCl (0.5 mL, 6.1 mmol, 37% aq) in MeCN (1 mL), followed by filtration and drying afforded **1h** as a white solid (48 mg, 0.12 mmol, 55%, mp 194.7 - 201.5 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.3 min, 97% pure. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 7.98 (s, 1H), 7.73 (s, 1H), 7.53 (s, 2H), 5.60 (s, 2H), 3.28 (t, 2H, J = 7.1 Hz), 3.08 (t, 2H, J = 7.3 Hz). ^{13}C NMR (100 MHz, MeOD-d_4) δ: 144.9, 141.0, 135.3, 131.4, 124.7, 124.5, 53.6, 40.1, 24.5. ^{1}H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2899 (m), 2371 (m), 1900 (m), 1560 (s), 1427 (s), 859 (s), 743 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 358.9509 (calcd. $C_{11}H_{13}Br_2N_4$, 358.9507 [M-Cl]$^+$).

2-(1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (18i) and 2-(1-(adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)ethan-1-aminium chloride (1i)

The general procedure with **16i** (0.494 g, 1.31 mmol), 8 h reflux, and additional hydrazine hydrate (0.1 mL) added after 6 h (total: 0.590 mL, 7.9 mmol, 64-65%), afforded **18i** as a lightly yellow oil (0.268 g, 1.09 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (s, 1H), 3.03 (t, 2H, J = 6.7 Hz), 2.84 (t, 2H, J = 6.7 Hz), 2.28 - 2.20 (m, 9H), 1.84 - 1.75 (m, 6H).
The general procedure for converting **18i** (50 mg, 0.20 mmol) into its HCl-salt with HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **1i** as a white

solid (57 mg, 0.20 mmol, quant., mp >176 °C decomp.). HPLC ($MeOH/H_2O$, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 4.7 min, 95% pure. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.10 (s, 1H), 8.08 (bs, 3H), 3.13 - 3.05 (m, 2H), 2.95 (t, 2H, J = 7.5 Hz), 2.21 - 2.13 (m, 9H), 1.77 - 1.70 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.9, 119.4, 58.8, 42.3, 38.2, 35.3, 28.9, 23.4. ^1H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2911 (s), 2887 (s), 2849 (m), 1914 (w), 1513 (m), 1457 (m), 1328 (s), 1172 (s), 1101 (s), 1044 (s), 1016 (s), 903 (s), 685 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 247.1921 (calcd. $C_{14}H_{23}N_4$, 247.1923 $[M-Cl]^+$).

2-(1-Heptyl-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (18j) and 2-(1-heptyl-1*H*-1,2,3-triazol-4-yl)ethan-1-aminium chloride (1j)

The general procedure with **16j** (0.529 g, 1.55 mmol), 5 h reflux, and additional hydrazine hydrate (0.12 mL) added after 4 h (total: 0.700 mL, 9.38 mmol, 64-65%), afforded **18j** as an off-white wax (0.319 g, 1.52 mmol, 98%). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.33 (s, 1H), 4.31 (t, 2H, J = 7.0 Hz), 3.04 (t, 2H, J = 6.5 Hz), 2.85 (t, 2H, J = 6.5 Hz), 1.95 - 1.83 (m, 2H), 1.40 - 1.21 (m, 8H), 0.92 - 0.83 (m, 3H).
The general procedure for converting **18j** (45 mg, 0.21 mmol) into its HCl-salt with HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **1j** as a white solid (53 mg, 0.21 mmol, quant., mp 150.3 - 153.3 °C). HPLC ($MeOH/H_2O$, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.0 min, 96% pure. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.16 (bs, 3H), 8.01 (s, 1H), 4.31 (t, 2H, J = 7.5 Hz), 3.11 - 3.03 (m, 2H), 2.96 (t, 2H, J = 7.5 Hz), 1.79 (p, 2H, J = 7.4Hz), 1.33 - 1.19 (m, 8H), 0.86 (t, 3H, J = 7.2Hz). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 142.4, 122.6, 49.3, 38.2, 31.1, 29.7, 28.0, 25.8, 23.3, 22.0, 13.9. ^1H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2952 (m), 2919 (s), 2851 (m), 2368 (m), 1885 (m), 1495 (s), 1467 (m), 1437 (m), 1154 (s), 1057 (s), 1022 (s), 961 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 211.1919 (calcd. $C_{11}H_{23}N_4$, 211.1923 $[M-Cl]^+$).

3-(1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)propan-1-amine (19g) and 3-(1-(4-(*tert*-butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)propan-1-aminium chloride (4g)

The general procedure with **17g** (0.114 g, 0.28 mmol), hydrazine hydrate added throughout the reaction (total: 0.42 mL, 5.66 mmol, 64-65%), and 50 h reflux, afforded **19g** as a yellow oil in mixture with **17g** and toluene (98 mg, 50% wt. est. from ^1H NMR, 0.18 mmol, 65%). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.42 - 7.39 (m, 2H), 7.28 (s, 1H), 7.23 - 7.21 (m, 2H), 5.47 (s, 2H), 8.81 - 2.73 (m, 4H), 1.83 (p, 2H,

J = 7.4 Hz), 1.33 (s, 9H).
The general procedure was used for converting **19g** (0.100 g, 0.18 mmol, 50% wt.) into its HCl-salt with HCl (0.300 mL, 3.66 mmol, 37% aq) in MeCN (3 mL), where the suspension after HCl-addition was heated up and filtered. The filtrate was then evaporated, and careful washing of the evaporated filtrate afforded **4g** as a lightly yellow solid (40 mg, 0.13 mmol, 71%, mp 216.0 - 219.2 °C). HPLC ($MeOH/H_2O$, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 6.7 min, 98% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.12 (bs, 3H), 7.96 (s, 1H), 7.40 - 7.35 (m, 2H), 7.25 - 7.20 (m, 2H), 5.50 (s, 2H), 2.84 - 2.76 (m, 2H), 2.69 (t, 2H, J = 7.6 Hz), 1.89 (p, 2H, J = 7.6 Hz), 1.25 (s, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 150.6, 145.9, 133.2, 127.7, 125.5, 122.3, 52.5, 38.3, 34.3, 31.1, 26.7, 22.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2964 (s), 2859 (s), 1617 (s), 1523 (m), 1150 (m), 1016 (s), 832 (s), 686 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 273.2079 (calcd. $C_{16}H_{25}N_4$, 273.2076 $[M-Cl]^+$).

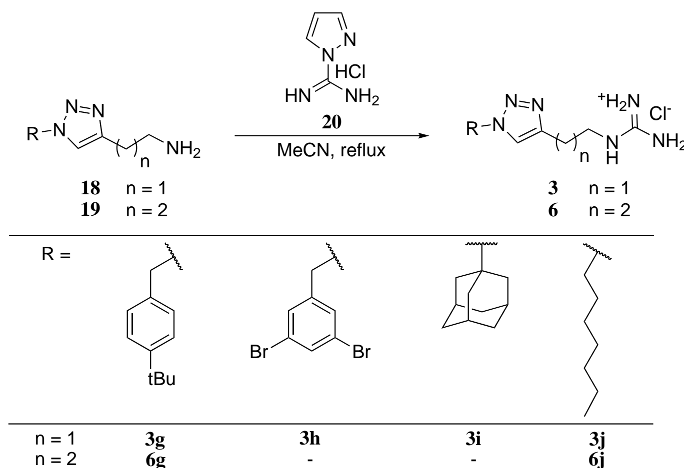
3-(1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-amine (19i) and 3-(1-(adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-aminium chloride (4i)

The general procedure with **17i** (0.447 g, 1.13 mmol), hydrazine hydrate (0.422 mL, 5.67 mmol), and 9 h reflux, followed by purification of the crude with flash column chromatography ($CHCl_3$/MeOH/TEA 80:20:1) afforded **19i** as an orange oil (0.193 g, 0.74 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (s, 1H), 4.10 (bs, 2H), 2.91 (t, 2H, J = 7.0), 2.81 (t, 2H, J = 7.0), 2.28 - 2.18 (m, 9H), 1.97 (p, 2H, J = 7.0), 1.84 - 1.73 (m, 6H).
The general procedure for converting **19i** (25 mg, 0.10 mmol) into its HCl-salt with HCl (0.1 mL, 1.22 mmol, 37%) in MeCN (0.5 mL), afforded **4i** as a white wax (28 mg, 0.10 mmol, quant.). HPLC ($MeOH/H_2O$, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.1 min, 97% pure. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 8.58 (s, 1H), 3.05 (t, 2H, J = 8.1 Hz), 2.98 (t, 2H, J = 7.5 Hz), 2.35 - 2.27 (m, 9H), 2.11 (t, 2H, J = 8.1Hz), 1.93 - 1.80 (m, 6H). ^{13}C NMR (100 MHz, MeOD-d_4) δ: 144.5, 124.0, 65.2, 43.5, 40.0, 36.7, 31.2, 27.4, 22.1. ^{1}H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2906 (s), 2848 (s), 1608 (w), 1454 (m), 1303 (w), 1149 (s), 1016 (s), 840 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 261.2080 (calcd. $C_{15}H_{25}N_4$, 261.2079 $[M-Cl]^+$).

3-(1-Heptyl-1*H*-1,2,3-triazol-4-yl)propan-1-amine (19j) and 3-(1-heptyl-1*H*-1,2,3-triazol-4-yl)propan-1-aminium chloride (4j)

The procedure for **19i** with **17j** (0.370 g, 1.04 mmol), hydrazine hydrate (1.167 mL, 15.67 mmol, 64-65%, added at the start, after 3 h, and 6 h of reflux), and 8 h reflux, afforded **19j** as a white wax (76 mg, 0.34 mmol, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 (s, 1H), 4.30 (t, 2H, J = 7.2 Hz), 2.78 (t, 2H, J = 6.7 Hz), 1.93 - 1.78 (m, 6H), 1.36 - 1.19 (m, 8H), 0.91 - 0.84 (m, 3H).
The general procedure for converting **19j** (26 mg, 0.12 mmol) into its HCl-salt with HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **4j** as a white solid (30 mg, 0.12 mmol, quant., mp >122 °C decomp.). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.4 min, 97% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.01 (bs, 3H), 7.90 (s, 1H), 4.29 (t, 2H, J = 7.1 Hz), 2.85 - 2.78 (m, 2H), 2.69 (t, 2H, J = 7.7 Hz), 1.89 (p, 2H, J = 7.7 Hz), 1.78 (p, 2H, J = 7.1 Hz), 1.32 - 1.16 (m, 8H), 0.85 (t, 3H, J = 7.1 Hz). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 145.4, 121.9, 49.2, 38.3, 31.1, 29.7, 28.0, 26.8, 25.8, 22.0, 21.96, 13.9. ^{1}H and ^{13}C NMR signals are assigned similarly to **18g** and **1g**. IR (ATR): 2918 (s), 1616 (m), 1465 (m), 1146 (m), 1122 (s), 970 (s), 725 (m), 682 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 225.2076 (calcd. $C_{12}H_{25}N_4$, 225.2079 $[M-Cl]^+$).

4.1.4 Synthesis of Guanidinium Triazoles 3 and 6



General Procedure for Synthesis of 3 and 6

The synthesis was carried out according to a modified[300] procedure described by Bernatowicz *et al.*,[315] where **20** (0.9 - 1.0 equiv) was added to **18** or **19** in MeCN (10 mL/mmol **18** or **19**) and refluxed for 2 - 6 h. Various crystallization routines afforded **3** and **6** in 28 - 91% yields.

Amino((2-(1-(4-(*tert*-butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)ethyl)amino)-methaniminium chloride (3g)

The general procedure with **18g** (0.051 g, 0.197 mmol), **20** (29 mg, 0.197 mmol), and 2 h reflux, was followed by crystallization of the reaction mixture by cooling it to 5 °C. The isolated precipitate was then washed with Et_2O and dried under reduced pressure, affording **3g** as an off-white wax (26 mg, 0.08 mmol, 40%). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 6.8 min, 95% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.98 (s, 1H, triazole-5), 7.21 (s, 1H, NH), 7.42 - 7.35 (m, 2H, Ph), 7.29 - 7.19 (m, 2H, Ph), 5.52 (s, 2H, Bn), 3.40 (q, 2H, J = 5.4 Hz, NH-CH_2), 2.83 (t, 2H, J = 7.4 Hz, CH_2), 1.25 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 156.9 (guanidine), 150.6 (Ph-4), 143.8 (triazole-4), 133.1 (Ph-1), 127.7 (Ph-3 and Ph-5), 125.5 (Ph-2 and Ph-6), 122.8 (triazole-5), 52.4 (Bn), 40.2 (NH-CH_2), 34.3 (Cq-*t*-Bu), 31.0 (*t*-Bu), 25.0 (CH_2). IR (ATR): 3135 (m), 2962 (m), 1645 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 301.2141 (calcd. $C_{16}H_{25}N_6$, 301.2141 $[M-Cl]^+$).

Amino((2-(1-(3,5-dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)ethyl)amino)-methaniminium chloride (3h)

The general procedure with **18h** (0.100 g, 0.28 mmol), **20** (36 mg, 0.25 mmol), and 2 h reflux, was followed by crystallization of the reaction mixture by cooling it to 5 °C. The precipitated crude was crystallized in MeOH/Et_2O, washed with DCM and dried under reduced pressure, affording **3h** as an off-white solid (70 mg, 0.16 mmol, 57%, mp 185.2 - 186.8 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.6 min, 95% pure. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 7.92 (s, 1H), 7.72 (s, 1H), 7.50 (s, 2H), 5.58 (s, 2H), 3.51 (t, 2H, J = 7.1 Hz), 2.99 (t, 2H, J = 6.8 Hz). ^{13}C NMR (100 MHz, MeOD-d_4) δ: 158.8, 146.0, 141.2, 135.3, 131.3, 124.5, 124.47, 53.5, 42.0, 26.2. ^{1}H and ^{13}C NMR signals are assigned similarly to **3g**. IR (ATR): 3057 (w), 1660 (s), 1423 (m), 1163 (w), 1066 (w), 841

(m), 730 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 400.9721 (calcd. $C_{12}H_{15}Br_2N_6$, 400.9719 $[M-Cl]^+$).

((2-(1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)ethyl)amino)-methaniminium chloride (3i)

The general procedure with **18i** (50 mg, 0.20 mmol), **20** (29 mg, 0.20 mmol), and 5 h reflux, was followed by crystallization of the reaction mixture by cooling it to 5 °C. The precipitated crude was washed with Et_2O and DCM, before it was recrystallized in MeCN/H_2O (99:1) and warm filtered. Affording **3i** as a white solid (32 mg, 0.10 mmol, 48%, mp 100.1 - 106.0 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.1 min, 96% pure. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.06 (s, 1H), 7.76 (t, 1H, J = 5.2 Hz), 3.43 (q, 2H, J = 6.9 Hz), 2.83 (t, 2H, J = 6.9 Hz), 2.20 - 2.12 (m, 9H), 1.77 - 1.70 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 157.0, 142.8, 119.3, 58.7, 42.3, 40.3, 35.3, 28.9, 25.1. ^1H and ^{13}C NMR signals are assigned similarly to **3g**. IR (ATR): 3324 (m), 3122 (m), 2906 (s), 1686 (m), 1647 (s), 1624 (s), 1064 (m), 1012 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 289.2139 (calcd. $C_{15}H_{25}N_6$, 289.2141 $[M-Cl]^+$).

Amino((2-(1-heptyl-1*H*-1,2,3-triazol-4-yl)ethyl)amino)methaniminium chloride (3j)

The general procedure with **18j** (53 mg, 0.25 mmol), **20** (36 mg, 0.246 mmol), and 4 h reflux, was followed by partial evaporation, filtration and washing of the precipitate with Et_2O. Affording **3j** as a yellow wax (63 mg, 0.22 mmol, 91%). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.4 min, 97% pure. ^1H NMR (600 MHz, DMSO-d_6) δ: 7.92 (s, 1H), 7.54 (t, 1H, J = 5.6 Hz), 4.31 (t, 2H, J = 7.1 Hz), 3.42 (q, 2H, J = 5.7 Hz), 2.84 (t, 2H, J = 6.9 Hz), 1.78 (p, 2H, J = 7.6 Hz), 1.32 - 1.18 (m, 8H), 0.85 (t, 3H, J = 7.4 Hz). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 156.8, 143.3, 122.5, 49.2, 40.3, 31.1, 29.7, 28.0, 25.8, 24.9, 22.0, 13.9. ^1H and ^{13}C NMR signals are assigned similarly to **3g**. IR (ATR): 3392 (m), 3163 (m), 1677 (s), 1644 (s), 1626 (s), 1466 (w), 1223 (w), 1060 (w), 1040 (w), 654 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 253.2139 (calcd. $C_{12}H_{25}N_6$, 289.2141 $[M-Cl]^+$).

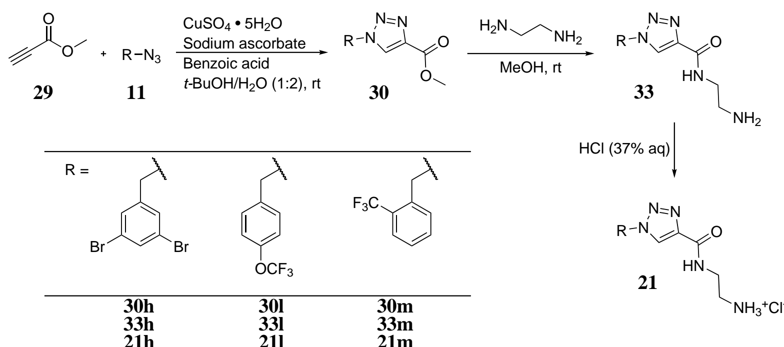
Amino((3-(1-(4-(*tert*-butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)propyl)amino)-methaniminium chloride (6g)

The general procedure with **19g** (0.070 g, 0.26 mmol), **20** (36 mg, 0.24 mmol), and 4 h reflux, was followed by crystallization of the reaction mixture with Et_2O. The precipitated crude was then recrystallized in MeCN and dried, affording **6g** as a white solid (25 mg, 0.07 mmol, 29%, mp 166.1 - 169.0 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 7.8 min, 95% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 7.75 (s, 1H), 7.44 - 7.39 (m, 2H), 7.28 - 7.23 (m, 2H), 5.52 (s, 2H), 3.23 (t, 2H, J = 7.2 Hz), 2.75 (t, 2H, J = 7.7 Hz), 1.93 (p, 2H, J = 6.9 Hz), 1.30 (s, 9H). ^{13}C NMR (150 MHz, MeOD-d_4) δ: 158.9, 153.0, 148.3, 134.0, 129.1, 127.1, 123.5, 54.8, 41.9, 35.6, 31.8, 29.7, 23.3. ^{1}H and ^{13}C NMR signals are assigned similarly to **3g**. IR (ATR): 3291 (w), 3122 (w), 2953 (w), 1673 (s), 1641 (s), 1615 (s), 781 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 315.2294 (calcd. $C_{17}H_{27}N_6$, 315.2297 [M-Cl]$^+$).

Amino((3-(1-heptyl-1*H*-1,2,3-triazol-4-yl)propyl)amino)methaniminium chloride (6j)

The general procedure with **19j** (48 mg, 0.21 mmol), **20** (31 mg, 0.21 mmol), and 5 h reflux, was followed by crystallization of the reaction mixture by cooling it to 5 °C. The isolated precipitate was then washed with Et_2O and DCM, and dried under reduced pressure. Affording **6j** as an off-white solid (32 mg, 0.11 mmol, 50%, mp 114.9 - 116.3 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 6.0 min, 96% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 7.79 (s, 1H), 4.37 (t, 2H, J = 7.2 Hz), 3.25 (t, 2H, J = 7.2 Hz), 2.77 (t, 2H, J = 8.0 Hz), 1.95 (p, 2H, J = 7.1 Hz), 1.89 (p, 2H, J = 7.3 Hz), 1.39 - 1.24 (m, 8H), 0.90 (t, 3H, J = 7.1 Hz). ^{13}C NMR (150 MHz, MeOD-d_4) δ: 158.9, 147.9, 123.5, 51.5, 41.9, 32.9, 31.4, 29.9, 29.8, 27.6, 23.7, 23.3, 14.5. ^{1}H and ^{13}C NMR signals are assigned similarly to **3g**. IR (ATR): 3333 (w), 3128 (w), 2930 (w), 1677 (s), 1642 (s), 1605 (w), 1473 (w), 1060 (w), 791 (w), 684 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 267.2297 (calcd. $C_{13}H_{27}N_6$, 267.2297 [M-Cl]$^+$).

4.1.5 Synthesis of 1,2,3-Triazole Methyl Esters 30, amido 1,2,3-triazoles 33, and their HCl-salts 21



General Procedure for Synthesis of 30

The general procedure for synthesis of **16** and **17** shown in 4.1.2 afforded **30** in 58 - 84% yields.

Methyl 1-(3,5-dibromobenzyl)-1*H*-1,2,3-triazole-4-carboxylate (30h)

The general procedure with **11h** (0.200 g, 0.69 mmol) and **29** (55 mg, 0.66 mmol) for 28 h at rt afforded **30h** as a white solid (0.143 g, 0.38 mmol, 58%, mp 145.7 - 150.2 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H, triazole-5), 7.69 (t, 1H, J = 1.6 Hz, Ph-4), 7.36 (d, 2H, J = 1.8 Hz, Ph-2 and Ph-6), 5.53 (s, 2H, Bn), 3.96 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.9 (CO), 140.8 (triazole-4), 137.3 (Ph-1), 135.0 (Ph-4), 129.8 (Ph-2 and Ph-6), 127.5 (triazole-5), 123.9 (Ph-3 and Ph-5), 53.0 (Bn), 52.4 (Me). IR (ATR): 2103 (w), 1716 (s), 1585 (m), 1556 (m), 1529 (w), 1554 (m), 1425 (m), 1371 (s), 1046 (s), 860 (m), 738 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 373.9138 (calcd. $C_{11}H_{10}Br_2N_3O_2$, 373.9140 $[M+H]^+$).

Methyl 1-(4-(trifluoromethoxy)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (30l)

This compound was prepared by Martin Furru Vold, as part of a student project. The general procedure with **11l** (2.48 g, 11.4 mmol) and **29** (1.05 mL, 11.8 mmol) for 24 h at rt afforded **30l** as a yellow solid (2.88 g, 9.60 mmol, 84%, mp 121.3 - 123.9 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.02 (s, 1H), 7.36 - 7.32 (m, 2H), 7.26 - 7.23 (m, 2H), 5.60 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 161.0,

149.7 (m), 140.6, 132.4, 129.8, 127.3, 121.7, 120.3 (q, $J_{C\text{-}F}$ = 258 Hz), 53.6, 52.3. ^{1}H and ^{13}C NMR signals are assigned similarly to **30h**. IR (ATR): 3124 (w), 2957 (w), 1727 (s), 1252-1152 cm^{-1}. HRMS (APCI/ASAP, m/z): 302.0751 (calcd. $C_{12}H_{11}F_3N_3O_2$, 302.0753 [M+H]$^+$).

Methyl 1-(2-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carboxylate (30m)

This compound was prepared by Kristine Olsen Strandheim, as part of a student project. The general procedure with **11m** (3.79 g, 18.8 mmol) and **29** (1.52 g, 18.0 mmol) for 22 h at rt afforded **30m** as an off-white solid (4.27 g, 15.0 mmol, 83%, mp 86.9 - 87.4 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.75 (d, 1H, J = 7.8), 7.57 (t, 1H, J = 7.5 Hz), 7.51 (t, 1H, J = 7.7 Hz), 7.29 (d, 1H, J = 7.7 Hz), 5.79 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 161.0, 140.5, 132.9, 132.1, 130.7, 129.3, 128.3 (q, $J_{\text{C-F}}$ = 30.5 Hz), 127.8, 126.5 (q, J = 5.5 Hz), 124.0 (q, $J_{C\text{-}F}$ = 274), 52.6, 50.3 (CH2). ^{1}H and ^{13}C NMR signals are assigned similarly to **30h**. IR (ATR): 3112 (w), 1725 (m), 1538 (w), 1429 (w), 1319 (m), 1247 (m), 1106 (m), 1049 (m), 764 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 286.0803 (calcd. $C_{12}H_{11}F_3N_3O_2$, 286.0803 [M+H]$^+$).

General Procedure for Synthesis of 33 and 21

The synthesis was carried out according to a procedure by Boutureira *et al.*,[321] where **30** in MeOH (10 mL/mmol **30**) was added ethylene diamine (15 equiv) and stirred at rt for 22 - 90 h. Affording **33** in 92 - 100% yields. The free amines **33** were turned into their HCl-salts according to the procedure described in section 4.1.3, affording **21** in quantitative yields.

***N*-(2-Aminoethyl)-1-(3,5-dibromobenzyl)-1*H*-1,2,3-triazole-4-carboxamide (33h) and 2-(1-(3,5-dibromobenzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (21h)**

The general procedure with **30h** (0.137 g, 0.36 mmol) and ethylene diamine (0.37 mL, 5.48 mmol) for 24 h at rt (with filtration of the reaction mixture before evaporation) afforded **33h** as a white solid (0.135 g, 0.34 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (s, 1H, triazole-5), 7.68 (t, 1H, J = 1.6 Hz, Ph), 7.48 (bs, 1H, NH), 7.35 (d, 2H, J = 1.6 Hz, Ph), 5.50 (s, 2H, Bn), 3.51 (q, 2H, J = 5.9 Hz, NH-CH_2), 2.95 (bs, 2H, CH_2).

The general procedure for converting **33h** (56 mg, 0.14 mmol) into its HCl-salt with HCl (0.200 mL, 2.44 mmol, 37% aq) in MeCN (3 mL) afforded **21h** as a lightly yellow solid (61 mg, 0.14 mmol, quant., mp 267.4 - 270.1 °C). HPLC (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 15.9 min, 98% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 8.50 (s, 1H, triazole-5), 7.74 (t, 1H, J = 1.7 Hz, Ph-4), 7.52 (d, 2H, J = 1.7 Hz, Ph-2 and Ph-6), 5.66 (s, 2H, Bn), 3.68 (t, 2H, J = 6.0 Hz, NH-CH_2), 3.16 (t, 2H, J = 6.0 Hz, CH_2). ^{13}C NMR (150 MHz, MeOD-d_4) δ: 163.6 (CO), 144.2 (triazole-4), 140.9 (Ph-1), 135.4 (Ph-4), 131.4 (Ph-2 and Ph-6), 128.0 (triazole-5), 124.5 (Ph-3 and Ph-5), 53.7 (Bn), 41.2 (CH_2), 38.2 (NH-CH_2). IR (ATR): 2902 (m), 1661 (s), 1578 (s), 1558 (m), 1250 (w), 1048 (m), 865 (m), 842 (m), 757 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 401.9561 (calcd. $C_{12}H_{14}Br_2N_5O$, 401.9565 [M-Cl]$^+$).

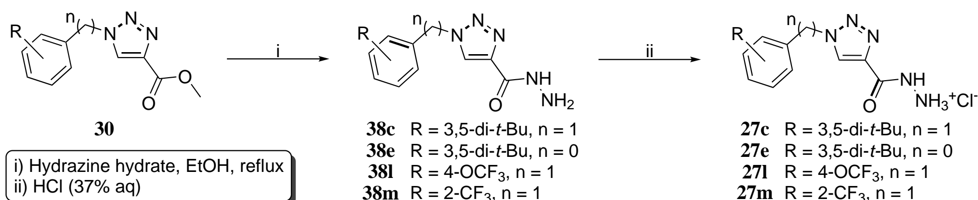
***N*-(2-Aminoethyl)-1-(4-(trifluoromethoxy)benzyl)-1*H*-1,2,3-triazole-4-carboxamide (33l) and 2-(1-(4-(trifluoromethoxy)benzyl)-1*H*-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (21l)**

This compound was prepared by Martin Furru Vold, as part of a student project. The general procedure with **30l** (0.250 g, 0.83 mmol) and ethylene diamine (0.83 mL, 12.5 mmol) for 22 h afforded **33l** as an off-white solid (0.273 g, 0.83 mmol, quant.). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.47 (bs, 1H), 7.34 - 7.30 (m, 2H), 7.26 - 7.22 (m, 2H), 5.57 (s, 2H), 3.50 (q, 2H, J = 6.3 Hz), 2.93 (t, 2H, J = 5.7 Hz).
The general procedure for converting **33l** (50 mg, 0.15 mmol) into its HCl-salt using HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **21l** as a white solid (55 mg, 0.15 mmol, quant., mp >270 °C decomp.). HPLC (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 8.8 min, 98% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 8.45 (s, 1H), 7.47 (app d, 2H, J = 8.2 Hz), 7.30 (app d, 2H, J = 8.2 Hz), 5.71 (s, 2H), 3.67 (t, 2H, J = 6.0 Hz), 3.16 (t, 2H, J = 6.0 Hz). ^{13}C NMR (150 MHz, MeOD-d_4) δ: 163.7, 150.8, 144.1, 135.9, 131.3, 127.7, 122.7, 122.0 (q, $J_{C\text{-}F}$ = 254.9 Hz), 54.3, 41.2, 38.1. ^{1}H and ^{13}C NMR signals are assigned similarly to **33h** and **21h**. IR (ATR): 3341 (w), 2883 (bs), 1656 (m), 1572 (m), 1508 (m), 1299 (m), 1103 (s), 843 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 330.1178 (calcd. $C_{13}H_{15}F_3N_5O_2$, 330.1178 [M-Cl]$^+$).

N-(2-Aminoethyl)-1-(2-(trifluoromethyl)benzyl)-1H-1,2,3-triazole-4-carboxamide (33m) and 2-(1-(2-(trifluoromethyl)benzyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (21m)

This compound was prepared by Kristine Olsen Strandheim, as part of a student project. The general procedure with **30m** (0.400 g, 1.40 mmol) and ethylene diamine (1.40 mL, 21.0 mmol) for 92 h afforded **33m** as a white solid (0.438 g, 1.40 mmol, quant., mp 213 - 217 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.98 (s, 1H), 7.75 (d, 1H, J = 7.7 Hz), 7.55 (t, 1H, J = 7.5 Hz), 7.52 - 7.44 (m, 2H), 7.23 (d, 1H, J = 7.7 Hz), 5.77 (s, 2H), 3.50 (q, 2H, J = 6.0 Hz), 2.93 (t, 2H, J = 6.1 Hz).
The general procedure for converting **33m** (50 mg, 0.16 mmol) into its HCl-salt using HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **21m** as a white solid (56 mg, 0.16 mmol, quant., mp 213 - 217 °C). HPLC (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.6 min, 98% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 8.36 (s, 1H), 7.81 (app d, 1H, J = 7.8 Hz), 7.66 (app t, 1H, J = 7.8), 7.58 (app t, 1H, J = 7.8), 7.33 (app d, 1H, J = 7.8 Hz), 5.88 (s, 2H), 3.68 (t, 2H, J = 6.1 Hz), 3.16 (t, 2H, J = 5.8 Hz). ^{13}C NMR (150 MHz, MeOD-d_4) δ: 163.7, 143.9, 134.2 (2xC), 132.2, 130.5, 129.4 (q, $J_{C\text{-}F}$ = 31.2 Hz), 128.1, 127.7 (q, $J_{C\text{-}F}$ = 5.6 Hz), 125.8 (q, $J_{C\text{-}F}$ = 273.1 Hz), 51.9, 41.2, 38.2. ^{1}H and ^{13}C NMR signals are assigned similarly to **33h** and **21h**. IR (ATR): 3355 (w), 2961 (bs), 1654 (m), 1575 (s), 1509 (m), 1312 (s), 1239 (w), 1076 (m), 1167 (s), 1039 (s), 768 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 314.1228 (calcd. $C_{13}H_{15}F_3N_5O_2$, 314.1229 [M-Cl]$^+$).

4.1.6 Synthesis of 1,2,3-Triazole Hydrazides 38 and their HCl-salts 27



General Procedure for Synthesis of 38

The preparation of **38** was carried out according to a procedure described by Cunha *et al*,[345] where **30** and hydrazine hydrate (2 equiv, 64-65% in H_2O) were refluxed in EtOH (5 mL/mmol **30**) for 25 h. The reaction mixture was then cooled

down to 5 °C and filtered. The isolated precipitate was then washed with small amounts of EtOH and dried under reduced pressure, affording **38** in 53 - 74% yields. The free amines **38** were turned into their HCl-salts by adding HCl (0.1 mL/25 mg **38**, 37%, aq) to a suspension of **38** in MeCN (1 mL/10 mg **38**). Filtration and drying of the precipitate afforded **27** in 60 - 68% yields.

1-(3,5-Di-*tert*-butylbenzyl)-1*H*-1,2,3-triazole-4-carbohydrazide (38c) and 1-(3,5-di-*tert*-butylbenzyl)-1*H*-1,2,3-triazole-4-carbohydrazide hydrochloride (27c)

The general procedure with **30c** (0.250 g, 0.76 mmol) and hydrazine hydrate (0.11 mL, 1.52 mmol, 64-65%) afforded **38c** as a white solid (0.181 g, 0.55 mmol, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.69 (bs, 1H, NH), 8.61 (s, 1H, triazole-5), 7.36 (t, 1H, J = 1.5 Hz, Ph), 7.19 (d, 2H, J = 1.7 Hz, Ph), 5.60 (s, 2H, Bn), 4.44 (bs, 2H, NH_2), 1.25 (s, 18H, *t*-Bu).
The general procedure for converting **38c** (33 mg, 0.10 mmol) into its HCl-salt using HCl (0.300 mL, 3.66 mmol, 37% aq) in MeCN (3 mL) afforded **27c** as a white solid (22 mg, 0.06 mmol, 60%, mp 233 - 238 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 35.5 min, 96% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.66 (bs, 1H, NH), 10.50 (bs, 3H, NH_3^+) 8.96 (s, 1H, triazole-5), 7.37 (t, 1H, J = 1.7 Hz, Ph), 7.21 (d, 2H, J = 1.7 Hz, Ph), 5.66 (s, 2H, Bn), 1.26 (s, 18H, *t*-Bu). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 160.0 (C=O), 150.9 (Ph-3 and Ph-5), 139.6 (triazole-4), 134.6 (Ph-1), 127.8 (triazole-5), 122.2 (Ph-2 and Ph-6), 122.0 (Ph-4), 53.8 (Bn), 34.5 (Cq-*t*-Bu), 31.1 (*t*-Bu). IR (ATR): 2957 (w), 2666 (bw), 1669 (s), 1599 (m), 1203 (s), 767 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 330.2288 (calcd. $C_{18}H_{28}N_5O$, 330.2294 [M-Cl]$^+$).

1-(3,5-Di-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (38e) and 1-(3,5-di-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carbohydrazide hydrochloride (27e)

The general procedure with **30e** (0.250 g, 0.79 mmol) and hydrazine hydrate (0.12 mL, 1.59 mmol, 64-65%) afforded **38e** as a white solid (0.133 g, 0.42 mmol, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.81 (bs, 1H), 9.34 (s, 1H), 7.73 (d, 2H, J = 1.7 Hz), 7.53 (t, 1H, J = 1.7 Hz), 4.53 (bs, 2H), 1.35 (s, 18H).
The general procedure for converting **38e** (30 mg, 0.095 mmol) into its HCl-salt using HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (2 mL) afforded **27e** as a white solid (21 mg, 0.06 mmol, 63%, mp 181 - 186 °C). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 41.0 min, 98% pure. ^{1}H NMR (400

MHz, DMSO-d_6) δ: 9.60 (s, 1H), 7.75 (d, 2H, J = 1.5 Hz), 7.56 (t, 1H, J = 1.8 Hz), 1.35 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.9, 152.6, 140.5, 135.9, 126.3, 123.0, 115.1, 35.0, 31.0. ^{1}H and ^{13}C NMR signals are assigned similarly to **38c** and **27c**. IR (ATR): 2956 (w), 1695 (m), 1607 (s), 1482 (m), 1363 (w), 1249 (w), 1035 (s), 875 (m), 707 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 316.2133 (calcd. $C_{17}H_{26}N_5O$, 316.2137 [M-Cl]$^+$).

1-(4-(Trifluoromethoxy)benzyl)-1*H*-1,2,3-triazole-4-carbohydrazide (38l) and 1-(4-(trifluoromethoxy)benzyl)-1*H*-1,2,3-triazole-4-carbohydrazide hydrochloride (27l)

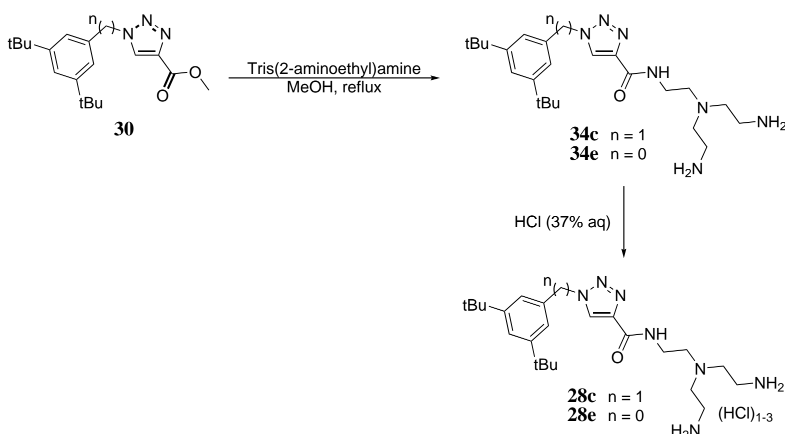
The general procedure with **30l** (0.250 g, 0.83 mmol) and hydrazine hydrate (0.12 mL, 1.66 mmol, 64-65%) afforded **38l** as white crystals (0.191 g, 0.61 mmol, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.69 (bs, 1H), 8.62 (s, 1H), 7.50 - 7.44 (m, 2H), 7.41 - 7.35 (m, 2H), 5.69 (s, 2H), 4.44 (d, 2H, J = 3.8 Hz).
The general procedure for converting **38l** (33 mg, 0.11 mmol) into its HCl-salt using HCl (0.200 mL, 2.44 mmol, 37% aq) in MeCN (3 mL) afforded **27l** as a white solid (22 mg, 0.07 mmol, 60%, mp 240 - 247 °C). HPLC (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 8.9 min, 98% pure. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.65 (bs, 1H), 10.49 (bs, 3H), 8.96 (s, 1H), 7.51 (app d, 2H, J = 8.8 Hz), 7.40 (app d, 2H, J = 8.2 Hz), 5.75 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 158.9, 148.2, 139.7, 134.8, 130.2, 128.1, 121.4, 120.0 (q, $J_{C\text{-}F}$ = 256.7 Hz), 52.3. ^{1}H and ^{13}C NMR signals are assigned similarly to **38c** and **27c**. IR (ATR): 2623 (w), 1665 (m), 1584 (m), 1488 (m), 1261 (s), 1177 (s), 1037 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 302.0864 (calcd. $C_{11}H_{11}F_3N_5O_2$, 314.1229 [M-Cl]$^+$).

1-(2-(Trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carbohydrazide (38m) and 1-(2-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazole-4-carbohydrazide hydrochloride (27m)

The general procedure with **30m** (0.250 g, 0.88 mmol) and hydrazine hydrate (0.13 mL, 1.75 mmol, 64-65%) afforded **38m** as white crystals (0.171 g, 0.60 mmol, 68%) ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (bs, 1H), 8.56 (s, 1H), 7.82 (d, 1H, J = 7.9 Hz), 7.70 (t, 1H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.9 Hz), 7.22 (d, 1H, J = 7.9 Hz), 5.84 (s, 2H), 4.45 (bs, 2H).
The general procedure for converting **38m** (30 mg, 0.11 mmol) into its HCl-salt using HCl (0.100 mL, 1.22 mmol, 37% aq) in MeCN (3 mL) afforded **27m** as a white solid (23 mg, 0.07 mmol, 68%, mp 223 - 226 °C) HPLC (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.9 min, 98% pure. ^{1}H NMR (600

MHz, DMSO-d_6) δ: 11.62 (bs, 1H), 10.40 (bs, 3H), 8.88 (s, 1H), 7.83 (d, 1H, J = 7.3 Hz), 7.72 (t, 1H, J = 7.3 Hz), 7.62 (t, 1H, J = 7.3 Hz), 7.29 (d, 1H, J = 7.8 Hz), 5.90 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 158.8, 139.6, 133.3, 132.8, 130.7, 129.2, 128.5, 126.7 (q, $J_{\text{C-F}}$ = 30.7 Hz), 126.4 (q, $J_{\text{C-F}}$ = 5.6 Hz), 124.1 (q, $J_{\text{C-F}}$ = 274.0 Hz), 50.1. ^{1}H and ^{13}C NMR signals are assigned similarly to **38c** and **27c**. IR (ATR): 2614 (w), 1668 (m), 1584 (m), 1486 (m), 1311 (s), 1182 (s), 1110 (s), 1037 (s), 771 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 286.0911 (calcd. $C_{11}H_{11}F_3N_5O$, 286.0916 [M-Cl]$^+$).

4.1.7 Synthesis of Triazole Triamine Amides 34 and their HCl-salts 28



N-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(3,5-di-*tert*-butylbenzyl)-1H-1,2,3-triazole-4-carboxamide (34c) and N-(2-(bis(2-aminoethyl)amino)ethyl)-1-(3,5-di-*tert*-butylbenzyl)-1H-1,2,3-triazole-4-carboxamide hydrochloride (28c)

The title compound **34c** was prepared according to a procedure by Wang *et al.*,[346] where tris(2-aminoethyl)amine (0.34 mL, 2.27 mmol) was added to a suspension of **30c** (50 mg, 0.15 mmol) in MeOH (9 mL), followed by 2.5 h of reflux. The reaction mixture was then evaporated to remove MeOH before excess tris-(aminoethyl)amine was removed in a kügelrohr-distillation (3 mmbar, 110 °C), affording **34c** as a white solid (67 mg, 0.15 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (bs, 1H, NH), 7.91 (s, 1H, triazole-5), 7.43 (t, 1H, J = 1.6 Hz, Ph-4), 7.12 (d, 2H, J = 1.6 Hz, Ph-2 and Ph-6), 5.50 (s, 2H, Bn), 3.51 (q, 2H, J = 5.8 Hz,

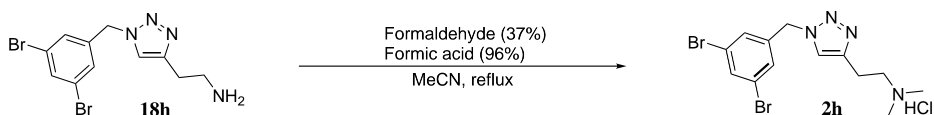
CONNH-CH_2), 2.78 (t, 4H, J = 6.3 Hz, 2x NH_2-CH_2), 2.69 (t, 2H, J = 5.8 Hz, N-CH_2), 2.58 (t, 4H, J = 5.8 Hz, 2x N-CH_2), 1.30 (s, 18H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 160.3 (C=O), 152.0 (Ph-3 and Ph-5), 143.7 (triazole-4), 132.8 (Ph-1), 125.1 (triaozle-5), 123.2 (Ph-4), 122.8 (Ph-2 and Ph-6), 56.4 (N-CH_2), 55.2 (Bn), 53.9 (N-CH_2), 39.4 (NH_2-CH_2), 37.4 (CONH-CH_2), 34.9 (Cq-*t*-Bu), 31.4 (*t*-Bu).
The free amine **34c** (35 mg, 0.07 mmol) dissolved in MeCN (3 mL) was added HCl (0.2 mL, 37%, aq.) and evaporated. Crystallization from MeOH/MeCN afforded **28c** as a white solid (17 mg, 0.03 mmol, 44%, mp 240 - 246 °C) HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 13.0 min, 97% pure. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.69 (s, 1H), 8.62 (s, 1H), 7.90 (bs, 5-6H), 7.37 (t, 1H, J = 1.7 Hz), 7.22 (d, 2H, J = 1.8 Hz), 5.60 (s, 2H), 3.39 (bs, 2H), 3.00 - 2.56 (m, 10H), 1.26 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.9, 150.9, 142.9, 134.8, 126.5, 122.3, 121.9, 53.7, 52.3, 50.9, 36.7 (broad), 34.5, 31.2. ^{1}H and ^{13}C NMR signals have been assigned for the neutral **34c**. IR (ATR): 3008 (s), 1652 (m), 1573 (s), 1456 (s), 1362 (m), 1247 (m), 1046 (w) cm^{-1}. HRMS (ESI, m/z): 444.3459 (calcd. $C_{24}H_{42}N_7O$, 444.3451 $[M-Cl]^+$, singly charged).

_N_-(2-(Bis(2-aminoethyl)amino)ethyl)-1-(3,5-di-_tert_-butylphenyl)-1_H_-1,2,3-triazole-4-carboxamide (34e) and _N_-(2-(bis(2-aminoethyl)amino)ethyl)-1-(3,5-di-_tert_-butylphenyl)-1_H_-1,2,3-triazole-4-carboxamide hydrochloride (28e)

The title compound **34e** was prepared from the procedure for synthesis of **34c** using **30e** (0.100 g, 0.32 mmol) and tris(2-aminoethyl)amine (0.47 mL, 3.17 mmol), affording **34e** as a white solid (0.136 g, 0.32 mmol, quant.). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.50 (s, 1H, triazole-5), 8.20 (bs, 1H, NH), 7.53 (s, 3H, Ph), 3.58 (q, 2H, J = 6.0 Hz, CONH-CH_2), 2.81 (t, 4H, J = 6.0 Hz, 2x NH_2-CH_2), 2.74 (t, 2H, J = 6.0 Hz, N-CH_2), 2.61 (t, 4H, J = 5.6 Hz, 2x N-CH_2), 1.37 (s, 18H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 160.0 (C=O), 153.1 (Ph-3 and Ph-5), 143.9 (triazole-4), 136.3 (Ph-1), 123.6 (Ph-4), 123.5 (triazole-5), 115.4 (Ph-2 and Ph-6), 57.3 (N-CH_2), 53.6 (N-CH_2), 39.9 (NH_2-CH_2), 37.3 (CONH-CH_2), 35.2 (Cq-*t*-Bu), 31.3 (*t*-Bu).
The free amine **34c** (25 mg, 0.06 mmol) was dissolved in MeCN (3 mL), filtered and added HCl (0.1 mL, 37%, aq.). Filtration and drying of the precipitate afforded **28e** as a white solid (27 mg, 0.06 mmol, 92%, mp 245 - 248 °C). HPLC (MeOH/H_2O, 3:1 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 3.5 min, 97% pure. ^{1}H NMR (600 MHz, MeOD-d_4) δ: 9.02 (s, 1H), 7.68 (d, 2H, J = 1.6 Hz), 7.63 (app t, 1H, J = 1.4 Hz), 3.71 (s, 2H), 3.46 - 2.99 (m, 10H), 1.40 (s, 18H). ^{13}C NMR (150

MHz, MeOD-d_4) δ: 163.5, 154.6, 144.3, 137.9, 126.1, 124.9, 116.6, 55.3, 52.8, 37.4 (broad), 36.3, 31.8. ^{1}H and ^{13}C NMR signals have been assigned for the neutral **34e**. IR (ATR): 3382 (bs), 2954 (s), 1573 (s), 1505 (m), 1480 (m), 1363 (w), 1295 (w), 1249 (w), 1029 (w). HRMS (ESI, m/z): 430.3293 (calcd. $C_{23}H_{40}N_7O$, $[M\text{-}Cl]^+$, singly charged).

4.1.8 2-(1-(3,5-Dibromobenzyl)-1*H*-1,2,3-triazol-4-yl)-*N,N*-dimethyl-ethan-1-aminium chloride (2h)



The title compound **2h** was prepared through an Eschweiler-Clarke reductive amination,[313,314] where **18h** (0.10 g, 0.27 mmol), formic acid (0.10 mL, 2.54 mmol, 96%), and formaldehyde (0.20 mL, 2.69 mmol, 37%) were refluxed in MeCN (2 mL) for 2 h. The reaction mixture was then cooled to rt, added HCl (0.50 mL, 37%, aq), and evaporated. The crude residue was then washed with DCM and MeCN before it was dried, affording **2h** as an off white solid (78 mg, 0.18 mmol, 67%, mp >154 °C decomp.). HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL/min, λ = 214 nm): t_R = 5.1 min, 96% pure. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 8.02 (s, 1H, triazole-5), 7.72 (s, 1H, Ph-4), 7.52 (s, 2H, Ph-2 and Ph-6), 5.60 (s, 2H, Bn), 3.50 (t, 2H, J = 7.6 Hz, NH-CH_2), 3.22 (t, 2H, J = 7.6 Hz, CH_2), 2.96 (s, 6H, 2x Me). ^{13}C NMR (100 MHz, MeOD-d_4) δ: 144.3 (triazole-4), 141.6 (Ph-1), 135.3 (Ph-4), 131.4 (Ph-2 and Ph-6), 124.9 (Ph-3 and Ph-5), 124.5 (triazole-5), 57.9 (NH-CH_2), 53.6 (Bn), 43.8 (Me), 22.0 (CH_2). IR (ATR): 3410 (w), 2466 (m), 1889 (m), 1556 (s), 1425 (s), 967 (m), 856 (m), 743 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 386.9815 (calcd. $C_{13}H_{17}Br_2N_4$, 386.9815 $[M\text{-}Cl]^+$).

4.1.9 Conditions for Tetrahydronaphthalenes and Tetrahydroisoquinolines Shown in Scheme 2.12

Synthesis of *N,N′*-((3-Benzyl-5,6,7,8-tetrahydroisoquinoline-1,4-diyl) bis(ethane-2,1-diyl))bis(4-methylbenzenesulfonamide)

The title compound was prepared as a mixture of the procedures described by Geny *et al.*, Vollhardt *et al.*, and Boñaga *et al.* with modifications.[287,330,331] Where **45** (0.200 g, 0.40 mmol) was dissolved in dry degassed 1,4-dioxane (15 mL) under Ar, followed by addition of 2-phenylacetonitrile (97 μL, 0.84 mmol) and $CpCo(CO)_2$ (7.4 μL, 0.055 mmol). The reaction mixture was then irradiated by two halogen lamps (400 W, 118 nm, 50 Hz) for 44 h before it was evaporated under reduced pressure and affording the title compound as a brown oil (0.200 g, 0.32 mmol, 81%) after purification with FCC (EtOAc/pentane 4:6). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.63 (dd, 4H, J = 8.4, 8.2 Hz, Ts-2 and Ts-6), 7.24 (s, 2H, Ts), 7.12 - 7.22 (m, 5H, Ph), 7.06 (d, 2H, J = 7.2 Hz, Ts), 6.23 (bs, 1H, NH), 4.76 (bs, 1H, NH), 4.05 (s, 2H, Bn), 3.33 (t, 2H, J = 5.4 Hz, NH-CH_2), 2.89 - 2.72 (m, 6H, NH-CH_2 + 2x NH-CH_2-CH_2), 2.61 - 2.51 (m, 2H, THIQ*-5a), 2.49 - 2.43 (m, 2H, THIQ-5), 2.40 (s, 3H, Me), 2.35 (s, 3H, Me), 1.76 - 1.64 (m, 4H, THIQ-6 and THIQ-7). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.3 (THIQ-1), 153.9 (THIQ-3), 146.2 (THIQ-4a), 145.5 (THIQ-8a), 143.5 (Ts), 142.9 (Ts), 139.7 (Ph-1), 137.4 (Ts), 137.0 (Ts), 129.5 (2x Ph), 128.6 (2x CPh, 2x Ts), 128.5 (2x Ts), 128.0 (THIQ-4), 127.0 (2x Ts), 126.9 (2x Ts), 126.3 (Ph), 42.0 (NH-CH_2), 41.3 (Bn), 40.9 (Ts), 32.0 (CH_2), 29.0 (CH_2), 26.6 (THIQ-5), 25.5 (THIQ-8), 22.1/21.9 (THIQ-6 and THIQ-7), 21.5 (2x Me). IR (ATR): 3272 (w, NH), 2925 (w), 1597 (w), 1566 (w), 1419 (w), 1321 (m), 1153 (s), 1091 (m), cm^{-1}. HRMS (TOF ASAP, m/z): 618.2460 (calcd. $C_{34}H_{40}N_3O_4S_2$, 618.2460 $[M+H]^+$).

Attempted synthesis of *N,N′*-((2-phenyl-5,6,7,8-tetrahydronaphthalene-1,4-diyl)bis(ethane-2,1-diyl))bis(4-methylbenzenesulfonamide)

The title compound was attempted prepared using a Rh-catalyzed procedure described by Tanaka *et al.*[347] Where $[Rh(cod)_2BF_4]$ (19.7 mg, 0.05 mmol) and BINAP (25.1 mg, 0.04 mmol) was added dry degassed DCM (5 mL), stirred for 5 minutes, and put under H_2 (1 atm.). The flask was then evaporated to dryness before being redissolved in DCM (5 mL), added **45** (0.370 g, 0.74 mmol) and **44a**

*THIQ: tetrahydroisoquinoline.

(0.830 mL, 7.51 mmol), and stirred for 21 h at rt. Evaporation of the reaction mixture under reduced pressure and separation with FCC (Et_2O/pentane 1:39), afforded a fraction that contained what could be interpreted as the product, however in miniscule amounts.

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Appended papers

Paper I

Paper II

Paper III

Paper IV

PAPER I

Simple generalized reaction conditions for the conversion of primary aliphatic amines to surfactant-like guanidine salts with 1*H*-pyrazole carboxamidine hydrochloride

Is not included due to copyright

PAPER II

Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles

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Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles

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ABSTRACT

A library of 28 small cationic 1,4-substituted 1,2,3-triazoles was prepared for studies of antimicrobial activity. The structures addressed the pharmacophore model of small antimicrobial peptides and an amphipathic motif found in marine antimicrobials. Eight compounds showed promising antimicrobial activity, of which the most potent compound **10b** displayed minimum inhibitory concentrations of 4–8 μg/mL against *Streptococcus agalactiae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. The simple syntheses and low degree of functionalization make these 1,4-substituted 1,2,3-triazoles interesting for further optimizations.

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Antimicrobial resistance to conventional antibiotic treatment is rapidly increasing, combined with lackluster efforts to develop novel classes of antibiotics by major pharmaceutical companies.[1–3] Infections caused by multi resistant bacteria is therefore one of the fastest growing medical threats to modern society.[4] Disturbingly, resistant bacteria have existed since the discovery of the first antibiotics. In recent years the race between growing resistance and progress of new antibiotics has intensified in favor of the bacteria. Unfortunately, no antibiotic has yet passed clinical trials for which there has not been reported cases of resistance.[5,6]

Most antibiotics applied today work through specific interactions with key intra- and extra-cellular targets in bacteria, and in a highly specific manner.[7] Due to the high target specificity, uncritical use of antibiotics easily selects for mutated bacteria to proliferate. A well known mechanism of resistance is expression of beta-lactamases that metabolizes beta-lactam based antibiotics.[8] An expanding field within antibiotic research in academia focuses on structures working through less specific mechanisms, like interactions with the bacterial cell membrane and non-specific interactions with intracellular targets.[9–12] The interest in these mechanisms of action comes from antimicrobial peptides (AMPs), that are important constituents of innate immunity in most living organisms. AMPs have a net positive charge (+2 to +9), consist of 12–50 residues, and fold into secondary structures with bactericidal properties.[13] These amphipathic structures, having a positively charged hydrophilic face and a lipophilic face, interact with anionic phospholipids on the surface of bacterial cell membranes. This is followed by membrane permeabilization by the lipophilic residues, leading to cell membrane disruption and ultimately cell lysis.[14,15]

Even though AMPs are considered to be highly active therapeutic compounds, there are some major issues in utilizing them on a large scale. Important drawbacks include low oral bio-availability, low metabolic stability, high manufacturer costs, and lack of patient-friendly administration methods aside from topical treatments.[16] Due to these obstacles, only a small number of antimicrobial agents utilized today are AMPs.[17] A way to circumvent the practical challenges associated with AMPs is to make smaller peptides and scaffold-based peptidomimetics that maintain the antimicrobial activity, but have improved pharmacokinetic properties. This has been demonstrated by Strøm et al., who have synthesized small beta-peptidomimetic structures (MW < 650) with high activity against a variety of resistant bacteria and with potential for *per oral* administration.[18,19] Recently, the group of Strøm[20] has reported a series of small cationic aminobenzamides (example shown as **E23** in Fig. 1) that mimic amphipathic structures found in marine antimicrobials such as synoxazolidinone A[21] and ianthelline[22] and display a membranolytic effect resembling many AMPs. The focus of this work was to further develop such amphipathic structures addressing both small AMPs and marine antimicrobials, and optimize these for antimicrobial activity. The di-functionalized 1,2,3-triazole was chosen as the core

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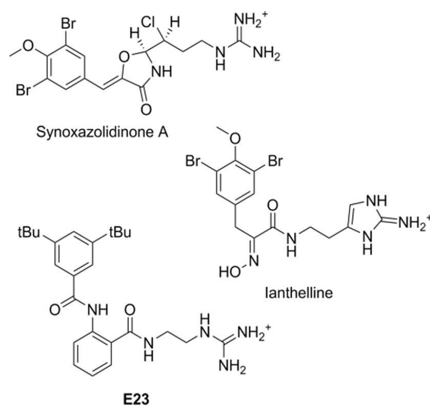
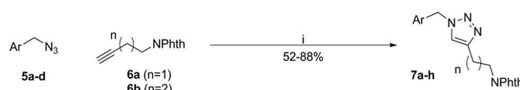


Fig. 1. Synoxazolidone A[21] (MRSA (MIC); 10 μg/mL), Ianthelline[22] (MRSA (MIC); 20 μg/mL), and **E23**; a natural product mimic by Strøm et al.[20] (MRSA (MIC); 4 μg/mL).

scaffold, due to the known biochemical properties of this type of structures.[23,24] Of importance was that triazoles are bioisosteres of amide bonds, which are more stable against proteolytic degradation than amides in AMPs.[25–27] The study included initial synthesis of 24 compounds to investigate the effects of varying between four lipophilic groups and three cationic groups, and including chain length variations. These results were followed up by synthesis of four optimized compounds based on the results from the initial series of di-functionalized 1,2,3-triazoles.

In order to synthesize a collection of disubstituted 1,2,3-triazole amphiphiles with the desired lipophilic- and cationic hydrophilic functionalities, the "click" chemistry protocol developed by Sharpless[28] and Meldal[29] was chosen. By using different catalysts for the "click" chemistry step, 1,2,3-triazoles with different substitution patterns can be prepared, i.e., 1,4-substitution when using copper (I) and 1,5-substitution when using ruthenium(II)-catalysis.[30] The



Scheme 1. (i) $CuSO_4 \cdot 5H_2O$ (5 mol %), Sodium ascorbate (10 mol%), Benzoic acid (10 mol%), tBuOH:H_2O (1:2), rt, 10 min – over night (Ar; **5a** = Ph, **5b** = naphthyl, **5c** = 3,5-di-*t*-Bu-Ph and **5d** = 3,5-CF_3-Ph).

1,4-substitution pattern was chosen here, due to the fact that the copper(I) catalysts used in these reactions are water insensitive (unlike their ruthenium counterparts), excluding the need for working under inert conditions. Thus, the first target compounds given in Fig. 2 (**1a–4f**) were prepared in order to screen the effects of different lipophilic aromatic groups and hydrophilic cationic nitrogen groups.

The "click" chemistry protocol requires two coupling partners carrying an azide and a terminal alkyne. It was found most convenient to insert the azide on the lipophilic moiety and the terminal alkyne on the nitrogen carrying functionality. The azides (**5a-d**, shown in Scheme 1) were synthesized from the respective commercially available bromides and alcohols, by well-established reactions (details are shown in the supporting information).[31–34] The alkynes carrying a handle for N-functionalization, were prepared from 3-butyn-1-ol and 1-chloropent-4-yne respectively, under Mitsunobu- or Finkelstein modified Gabriel-conditions (details shown in the supporting information).[35,36] This yielded **6a** and **6b** (shown in Scheme 1) with the same masked N-functionality and a difference of one methylene group in the carbon chain.

The alkynes (**6a** and **6b**) and azides (**5a-d**) were then combined to form [1,4]-1,2,3-triazoles (**7a-h**) using copper catalyzed "click"-chemistry conditions as shown in Scheme 1.[37] Thus, by using four different azides and two lipophiles, eight different "core" 1,2,3-triazoles ready for N-functionalization (**7a-h**) were prepared (see Scheme 1).

Three different cationic groups were evaluated; a primary amine (**a** and **d**), a tertiary amine (**b** and **e**) and a guanidine group (**c** and **f**) as shown in Scheme 2. The interest for the primary amine and the guanidine came from the functionalities found in AMPs,

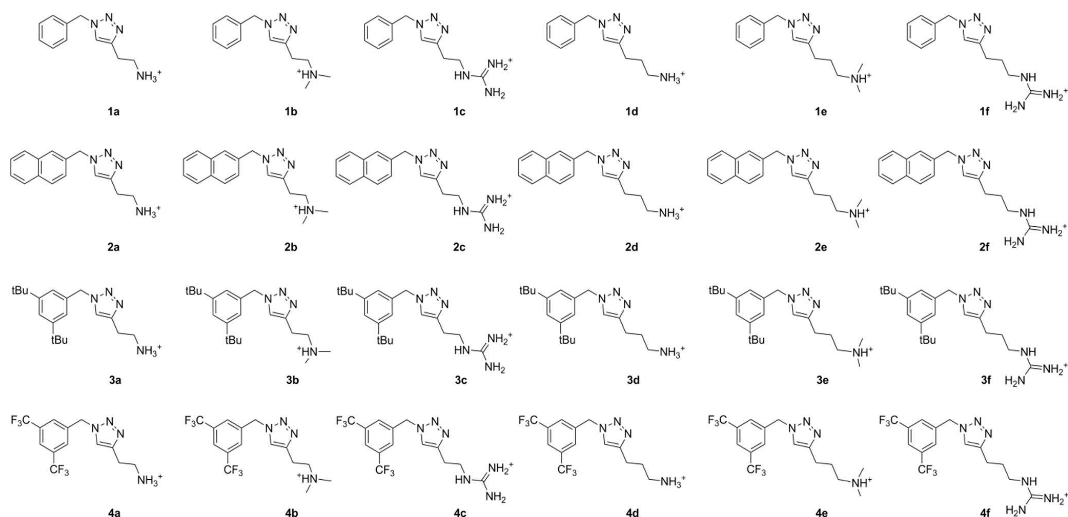
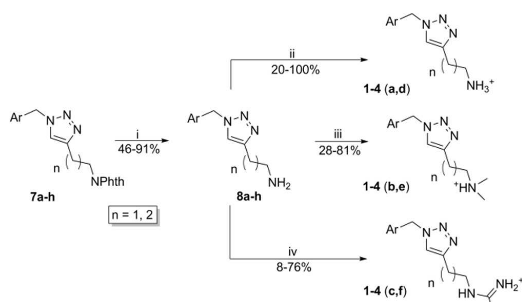


Fig. 2. Initial target [1,4]-1,2,3-triazoles **1a–4f** to be screened for antimicrobial effects. Counter-ion: Cl^-.



Scheme 2. (i) Hydrazine hydrate, toluene, reflux, (ii) HCl (conc. aq. or 2 M in Et_2O), iPrOH, MeCN or DCM, (iii) Formaldehyde, formic acid, MeCN, reflux, 1 h, acidic work up and (iv) 1H-pyrazole carboxamidine hydrochloride, MeCN, reflux 2–4 h.

were lysine and arginine residues contribute these groups to the amphiphile, thus taking a vital part in induction of antimicrobial activity.[13] A tertiary amine was expected to be a steric and electronic mid-point between the two naturally occurring cationic groups. In order to increase the steric bulk, without introducing additional nitrogens and resonance possibilities, the tertiary dimethylamino group was chosen as a mid-point between primary amines and guanidines. The eight protected 1,2,3-triazoles were deprotected using hydrazine hydrate according to a protocol developed by Gabriel.[38–40] The primary amines (**8a**–**h**) were subsequently functionalized in order to introduce the chosen functionality, and the primary amine HCl-salts **1**–**4** (**a**, **d**) (Scheme 2) were obtained by treatment with hydrochloric acid. The Eschweiler-Clarke reductive amination was utilized to create the tertiary amines **1**–**4** (**b**, **e**) (Scheme 2),[41,42] and an electrophilic guanidine reagent to create the guanidines **1**–**4** (**c**, **f**) (Scheme 2).[43] Performing the given transformations on all eight protected triazoles yielded the 24 different compounds depicted in Fig. 2 (**1a-4f**), in sufficient purity (>95% HPLC) for biological evaluation.

The 24 amphiphilic triazoles **1a-4f** were tested against three gram-positive and two gram-negative bacterial strains. In addition, all 24 compounds were subjected to toxicity studies against human fibroblasts (MRC-5). No activity was detected below 50 µg/mL, indicating low toxicity of the structures towards this type of human cells. Four of the 24 structures (**3a**, **3c**, **3e** and **3f**) showed promising activities against several cell lines. These were subjected to dilution assays in order to determine the minimum inhibitory concentrations (MICs) against the chosen bacteria. The MIC-values for the active compounds are shown in Table 1.

All the active compounds (**3a**, **3c**, **3e** and **3f**) contained the heavily hindered and non-polar 3,5-di-*tert*-butyl-phenyl functionality. This indicated that a bulky and non-polar lipophilic contribution was important for the activities in these structures. Furthermore, the guanidine hydrochloride functionality appeared to be related to the observed activities. As they (**3c** and **3f**, Fig. 2) were more potent than the tertiary dimethyl- and primary amines (**3a** and **3e**, Fig. 2), with the exception of **3a** against *E. coli*. The difference in activity was most pronounced against the gram-positive *S. aureus* and *Streptococcus* gr. B (*S. agalacticae*) bacteria, where a 2- to 4-fold increase in activity was observed for the guanidine compared to the other two cationic nitrogen groups.

So far, we have determined which lipophilic and cationic group that most likely promoted the highest activity against the five strains of bacteria tested. The third varying factor in the series of amphipathic 1,2,3-triazoles tested was the two or three carbon chain of the hydrophilic end of the triazole ring. A small increase in efficacy was observed for the longer **3f** compared to **3c** against *S. aureus* and *E. coli*. Furthermore, **3f** showed the overall highest activity against the gram-positive *S. aureus* and *S. agalacticae* (10 µg/mL), while there was a 4-fold decrease in the activity against gram-positive *E. faecalis* and the gram-negative *E. coli* and *P. aeruginosa* (40 µg/mL). Lowered activity against gram-negative compared to gram-positive bacteria is commonly observed, due to different outer membrane compositions.[44] However, it was surprising that the activity against *E. faecalis* was in the range of the gram-negative strains. In addition to the antimicrobial effects, some biofilm inhibition was observed in single concentration assays of these structures. The amphiphiles **2f** and **3** (except **3d**) showed biofilm inhibition at 50 µg/mL.

It was assumed from the pharmacological model[18–20] that a rather large lipophilic contribution would be important for achieving the desired antimicrobial effects. In order to rationalize our findings we attempted to use calculated pKa adjusted partition coefficients (ClogD) as an indicator for lipophilicity. The ClogD values were calculated (using the Marvinsketch software[45]) at physiological pH (pH = 7.40), showing the guanidines (**c** and **f**, Fig. 2) to be mostly protonated and the primary (**a** and **d**, Fig. 2) and tertiary amines (**b** and **e**, Fig. 2) to exist in more partitioned equilibria. However, when plotted against the values from the antimicrobial MIC-assays, no apparent connection was found between the ClogD and MIC-values. On the other hand, plotting all structures according to their retention times (Rt) from C18-HPLC as shown in Fig. 3, gave a more accurate picture of the effective lipophilic contributions. As the HPLC analyses were performed with an acid additive (0.1% TFA) in order to inhibit peak broadening, all of the compounds were assumed to exist mainly in their positively charged state. This indicated that the lipophilic nature of the charged structures is an important parameter for biological activity; e.g. **3f** is more active than **3e**, even though the calculated ClogD (displayed in Fig. 3) of **3e** is nearly the double of the one for **3f**. The fact that the Rts may be used as a rough indicator of antimicrobial activity may prove useful when targeting new potential candidates for optimization.

Compound **3f** from the initial screening and dose response assessments showed the highest antimicrobial activities, with MICs ranging from 10 to 40 µg/mL. In order to optimize the activities towards the target bacteria, a small and focused set of compounds was prepared based on the structure of **3f**. The first change was inspired by the planar benzamide peptide mimics

Table 1
Antimicrobial activity (MIC in µg/mL) for the 1,2,3-triazoles that showed any activity in the antibacterial assays. The "–"-sign in the table indicates no activity in the assay at the highest tested concentration (50 µg/mL).

Entry	3a	3c	3e	3f	Ref.[a]
E. faecalis[b]	–	40	–	40	10
S. aureus[b]	40	20	40	10	0.13
S. agalacticae[b]	40	10	50	10	4
E. coli[b]	40	50	–	40	0.5
P. aeruginosa[b]	50	40	–	40	0.5

[a] Ref.: Gentamicin.
[b] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalacticae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).

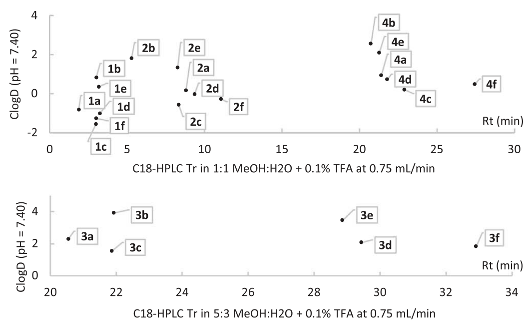
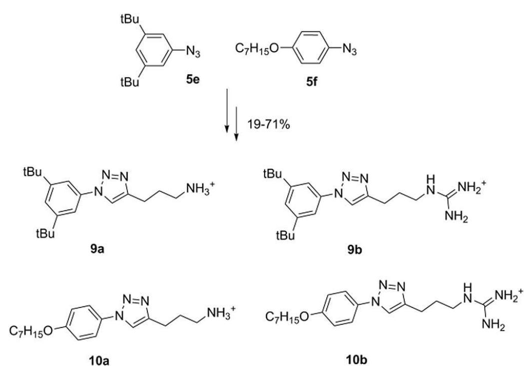


Fig. 3. Top plot: C18-Rt (MeOH/water, 1:1 + 0.1% TFA) for **1a-f**, **2a-f** and **4a-f** plotted against calculated ClogD at pH 7.40 (using the MarvinSketch suite). Bottom plot: C18-Rt (MeOH/water, 5:3 + 0.1% TFA) for **3a-f** plotted against calculated ClogD at pH 7.40 (using the MarvinSketch suite).



Scheme 3. Improved structures **9a-b** and **10a-b** based on **3f**, synthesized in three steps from **5e** and **5f** utilizing the chemistry displayed in Schemes 1 and 2. Counter ion: Cl^-.

presented by Strøm et al.,[20] where they achieved <10 µg/mL against the same bacteria. By using an aromatic azide instead of a benzylic azide in the "click"-coupling, the obtained amphiphiles would be more planar and rigid compared to **1a-4f** (Fig. 2). This was expected since the benzylic methylene group create an angle between the triazole ring and the lipophilic aromatic group and give more rotational freedom, which might be disfavourable for antibacterial activity. A second modification of **3f** in addition to removing the benzylic methylene group, was to change the structure of the lipophilic group. By going from the bulky *t*-Bu-functions to a linear alkyl chain with similar surface area and molecular weight, we hoped to mimic the membrane snorkeling effect of lysine and arginine rich proteins.[46] Thus, the four amphiphiles shown in Scheme 3 (**9a-b** and **10a-b**) were prepared for further studies.

The synthesis of **9a-b** and **10a-b** from the azides **5e** and **5f** were performed according to the methods presented in Schemes 1 and 2 with total yields ranging from 19 to 71% over three steps. The azides (**5e** and **5f**) were synthesized from commercially available iodophenol and 3,5-*t*-Bu-bromobenzene using a copper catalyzed synthesis presented by Zhu et al.[47] (experimental details are found in the supplementary information). The four triazole amphiphiles were subjected to the same bacterial strains as the initial 24 amphiphiles. The obtained MIC values are displayed in Table 2 together with the reference antibiotic gentamicin.

Removal of the benzylic methylene group and introduction of a more rigid and planar structure with possibility for conjugation lead to an approximate two-fold increase in activity against all the tested bacteria (**9b** compared to **3f**), and MIC-values as low as 4 µg/mL against *S. aureus* and *S. agalactiae*. Again, the guanidine hydrochloride (**9b** and **10b**) proved to be the most active hydrophile (compared to NH_3^+), as it led to a two-fold increase in activity against all bacteria (except for *E. coli*) compared to the ammonium hydrochlorides (**9a** and **10a**). Substituting the bulky 3,5-*t*-Bu group with a heptyl ether chain (**10a** and **10b**) led to a further two-fold increase in the activity against the gram-negative strains and the gram-positive *E. faecalis*. This may in turn be attributed to the heptyl chain's (**10b**) ability to penetrate deeper into the membrane compared to the *t*-Bu groups in **9b**. However, the exact mechanism of action for these compounds has not been investigated yet. It should also be noted that the activity of **10b** surpassed that of Gentamicin against *E. faecalis* and matched the activity against *S. agalactiae*.

As for the initial 24 amphiphiles, there was no evident correlation between the MIC-values and calculated CLogD. The most active compound (**10b**) had the lowest calculated CLogD of the four structures (Table 2). However, the retention times from C18-HPLC showed a better correlation, where the most active structure had the highest retention time on the C18-column (Table 2).

We have successfully synthesized 28 low molecular weight cationic triazole-based amphiphiles with different lipophilic and hydrophilic functionalities, and screened for antimicrobial effects against *S. agalactiae*, *S. aureus*, *P. aeruginosa*, *E. coli*, and *E. faecalis*. The most potent compound in our library (**10b**) displayed MIC-values between 4 and 8 µg/mL, which either matched or surpassed the activity of the marine natural product peptide mimics Synoxazolidone A and Ianthelline. The activity of **10b** also matched the activity against gram-negative bacteria for the benzamides presented by Strøm et al.[20] Thus, bioisosteres of amide bonds can be

Table 2
Antimicrobial activity (MIC in µg/mL) for the improved amphiphilic triazoles based on **3f**, calculated ClogD (pH = 7.4) and reverse phase HPLC retention times (min in 5:3 MeOH:H_2O + 0.1% TFA).

Entry	9a	9b	10a	10b	Ref.[a]
E. faecalis[b]	32	16	16	8	10
S. aureus[b]	16	4	16	4	0.13
S. agalactiae[b]	16	4	8	4	4
E. coli[b]	16	16	8	8	0.5
P. aeruginosa[b]	32	16	16	8	0.5
ClogD (calc.)[c]	2.00	1.75	1.41	1.16	–
RT (C18-HPLC)[d]	22.9	28.0	20.4	34.6	–

[a] Ref.: Gentamicin.
[b] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalactiae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[c] Calculated at pH = 7.40 using the MarvinSketch suite.
[d] In 5:3 MeOH:H_2O + 0.1% TFA with 0.75 mL/min.

applied without compromising the activity of the substrates. This provides a higher degree of structural freedom when choosing substrates for this type of activity-driven library design of antimicrobial scaffolds. We believe our findings may serve as basis for further investigations into artificial peptide mimics.

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A. Supplementary material

Supporting information containing all experimental procedures (for both synthesis and biological assays) and full characterization of novel compounds is electronically available through the publisher's website. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.01.092.

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Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles

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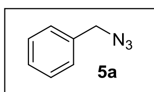
Supplementary information

Experimental methods for syntheses

Chemicals were purchased from Sigma Aldrich and used without further purification. All reactions sensitive to air or moisture were performed under nitrogen atmosphere with dried solvents and reagents. Melting points were determined on a Buchi 535 apparatus and are uncorrected. TLC was performed on Merck silica gel 60 F254 plates, using UV light at 312 nm and a 5% solution of molybdophosphoric acid in 96% EtOH for detection. Column chromatography was performed with Silica gel (pore size 60 A, 230 - 400 mesh particle size) from Fluka. NMR spectra were recorded on a Bruker 600 MHz Avance III HD or a Bruker 400 MHz Avance III HD instrument. HPLC analyses were performed on an Agilent 1290 chromatograph equipped with a Zorbax Eclipse C18 5 µm (150 x 4.6 mm) column and a diode array detector (main detection region 214 nm). IR analyses were performed on a Thermo Nicolet Nexus FT-IR spectrometer equipped with a Smart Endurance reflection cell. Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters™. Samples were ionized by the use of ASAP probe (APCI) or ESI probe. Chemical shifts (δ) are reported in parts per million. Where $CDCl_3$ has been used, shift values for proton are reported with reference to TMS (0.00) via the lock signal of the solvent. Reference values for other NMR-solvents are taken from Fulmer *et al*[1] (1H NMR: DMSO-d6: 2.49, MeOD-d4: 3.31; 13C NMR: DMSO-d6: 39.5, CD_3Cl: 77.0, MeOD-d4: 49.15). Signal patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) or bs (broad singlet). 1H and 13C NMR signals were assigned by 2D correlation techniques (COSY, HSQC, HMBC). IR spectra were recorded from a Thermo Nicolet FT-IR NEXUS instrument, and only the strongest/structurally most important peaks are listed as either weak (w), medium (m) or strong (s) (cm^{-1}).

Benzyl azide (**5a**):



The title compound was synthesized according to a procedure by Feringa.[2] Benzyl bromide (10.00 g, 58.5 mmol) was added to a suspension of NaN_3 (5.70 g, 88 mmol, 1.5 eq) in a acetone:water-mixture (4:1, 375 mL) and stirred for 24 hours at room temperature. The mixture was then added DCM (300 mL) and the aqueous layer was extracted with DCM (3x100 mL), before the combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. Benzyl azide (**5a**) was obtained as a colorless clear oil in 81% yield (6.83 g, 47.4 mmol). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[2]

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.36-7.26 (m, 5H, **Ph**), 4.28 (s, 2H, **CH_2**) ppm.

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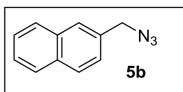
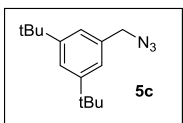
2-Azidomethylnaphthalene (**5b**):



The title compound was synthesized according to a procedure by Ceroni.[3] 2-Bromomethylnaphthalene (4.08 g, 18.5 mmol) and NaN_3 (9.01 g, 138.8 mmol, 7.5 eq) was stirred in DMSO (70 mL) under nitrogen at 45 °C for 22 hours. The reaction mixture was extracted with DCM (3 x 150 mL). The combined organic extracts were then washed with water (3 x 100 mL) before it was dried over $MgSO_4$ and evaporated under reduced pressure. Purification with flash column chromatography (1:1 pentane:Et_2O) afforded **5b** as a white solid (2.87 g, 15.7 mmol, 85% yield). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[3]

^{1}H NMR (400 MHz, *CDCl₃*): 7.88-7.78 (m, 4H, **naphth**), 7.52-7.42 (m, 3H, **naphth**), 4.51 (s, 2H, **CH_2**) ppm.

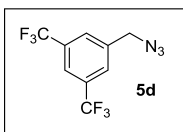
3,5-Di-tert-butylbenzylazide (**5c**):



The title compound was synthesized according to a procedure by Feringa.[2] 3,5-Di-tBu-benzylazide (1.50 g, 5.3 mmol) and NaN_3 (2.58 g, 39.7 mmol, 7.5 eq) was added to acetone (45 mL) and refluxed for 22 hrs. The reaction mixture was cooled to room temperature, added H_2O (25 mL) and extracted with DCM (3 x 20 mL). The combined organic phase was then dried over $MgSO_4$, filtered and evaporated under reduced pressure, affording **5c** as a lightly yellow oil (1.18 g, 4.8 mmol, 91% yield). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[4] Synthesis is similar to the one reported for **5a**[2] except for the usage of anhydrous acetone.

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.40 (s, 1H, **H_{Ar}-4**), 7.13 (d, *J* = 1.3 Hz, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 4.32 (s, 2H, **CH_2**), 1.33 (s, 18H, 2x **tBu**) ppm.

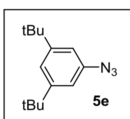
3,5-Di-trifluoromethylbenzylazide (**5d**):



The title compound was synthesized according to a procedure by Hamilton.[5] 3,5-Trifluoromethylphenylmethanol (2.50 g, 10.2 mmol) in THF (50 mL) was cooled to 0 °C, and added DBU (1.68 mL, 11.3 mmol, 1.1 eq) and DPPA (3.10 g, 11.3 mmol, 1.1 eq). The reaction was then allowed to reach room temperature and stirred for 22 hrs. The crude mixture was then evaporated to dryness and purified using flash column chromatography (100% pentane), affording **5d** as a lightly colored oil (2.04 g, 7.2 mmol, 70%). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[6]

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.86 (s, 1H, **H_{Ar}-4**), 7.79 (s, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 4.55 (s, 2H, **CH_2**) ppm.

1-Azido-3,5-di-tert-butylbenzene (**5e**):

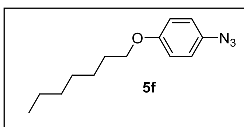


Was performed according to a procedure by Zhu *et al.*[7] Where 1-bromo-3,5-di-tert-butylbenzene (2.50 g, 9.30 mmol), CuI (0.177 g, 0.93 mmol), NaN_3 (1.21 g, 18.57 mmol), L-proline (0.321 g, 2.74 mmol) and NaOH (0.11 g, 2.79 mmol) were added to EtOH:H_2O (7:3) and heated to 95 °C in a sealed tube for 23 hours. The reaction mixture was then added water (30 mL) and extracted with EtOAc (3 x 30 mL). Drying over $MgSO_4$ and evaporation yielded a yellow oil, which then was purified using flash column chromatography

(pentane), affording **5e** as a colorless oil (0.89 g, 3.87 mmol, 42 %). ^{1}H NMR spectra coincided with previously reported data.[8]

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.20 (t, 1H, *J* = 1.5 Hz, **H$_{Ph}$-4**), 6.86 (d, 2H, *J* = 1.6 Hz, **H$_{Ph}$-2** and **H$_{Ph}$-6**), 1.31 (s, 18H, 2x **tBu**).

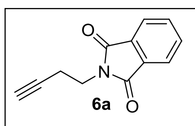
1-Azido-4-(heptyloxy)benzene (**5f**):



1-(Heptyloxy)-4-iodobenzene was synthesized using heptyl bromide and K_2CO_3 in DMF at rt, as reported by Ban *et al*[9] in 69% yield. The spectra coincided with previously reported data.[10] This aromatic iodide was turned into its corresponding azide (**5f**) using a procedure by Zhu *et al*.[7] Where 1-(Heptyloxy)-4-iodobenzene (1.50 g, 4.71 mmol), CuI (0.09 g, 0.47 mmol), NaN_3 (0.37 g, 5.66 mmol), L-proline (0.11 g, 0.94 mmol) and NaOH (0.04 g, 0.94 mmol) were added to DMSO and heated to 60 °C in a sealed tube for 14 hours. After which the mixture was added water (35 mL), extracted with EtOAc (3 x 40 mL), and dried over $MgSO_4$. Evaporation under reduced pressure yielded a brown oil, which then was purified using flash column chromatography (pentane), affording **5f** as a yellow oil (0.86 g, 3.67 mmol, 78%).

^{1}H NMR (400 MHz, *CDCl$_3$*): 6.96 – 9.91 (m, 2H, **H$_{Ph}$-3** and **H$_{Ph}$-5**), 6.90 – 6.85 (m, 2H, **H$_{Ph}$-2** and **H$_{Ph}$-6**), 3.92 (t, 2H, *J* = 6.8 Hz, N$_3$-**CH$_2$**), 1.77 (p, 2H, *J* = 7.3 Hz, N$_3$-CH$_2$-**CH$_2$**), 1.49 – 1.23 (m, 8H, 4x **CH$_2$**), 0.92 – 0.85 (m, 3H, **CH$_3$**). ^{13}C NMR (100 MHz, *CDCl$_3$*): 156.6 (**C$_{Ph}$-4**), 132.1 (**C$_{Ph}$-1**), 120.0 (**C$_{Ph}$-3** and **C$_{Ph}$-5**), 115.7 (**C$_{Ph}$-2** and **C$_{Ph}$-6**), 68.4 (**CH$_2$**), 31.8 (**CH$_2$**), 29.3 (**CH$_2$**), 29.1 (**CH$_2$**), 26.0 (**CH$_2$**), 22.6 (**CH$_2$**), 14.1 (**CH$_3$**). IR: 2927 (w), 2857 (w), 2105 (s), 1503 (s), 1470 (w), 1280 (m), 1239 (s), 822 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 233.1531 (Calcd. $C_{13}H_{19}N_3O$, 233.1528, [M]$^-$).

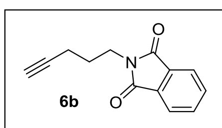
4-Phthal-butyne (**6a**):



The title compound was synthesized according to a procedure by Panolil.[11] 3-Butynol (9.53 g, 136 mmol), PPh_3 (35.70 g, 136 mmol) and phthalimide (20.00 g, 136 mmol) was added to toluene (200 mL) and cooled to -5 °C. DEAD-solution (64.40 mL, 141 mmol, 40% in toluene) was added so that the temperature was kept between 15 °C and 25 °C. The mixture was allowed to reach room temperature before MeOH (120 mL) was added, followed by 60 minutes of stirring. The mixture was then filtered and the precipitate was washed with cool MeOH (3 x 15 mL) and dried, affording **6a** as a white solid (8.40 g, 40.6 mmol, 31%). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[11]

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.89 – 7.83 (m, 2H, **Pht**), 7.77 – 7.69 (m, 2H, **Pht**), 3.89 (t, *J* = 7.2 Hz, 2H, N-**CH$_2$**), 2.62 (td, J_{aa} = 2.5 Hz, J_{ab} = 7.1 Hz, 2H, **CH$_2$**), 1.96 (t, *J* = 2.6, 1H, **Alkyne-H**) ppm.

5-Phthal-pentyne (**6b**):



The title compound was synthesized according to a procedure by Dehaen.[12] 4-Chloropentyne (5.00 g, 48.8 mmol), potassium phthalate (10.84 g, 58.5 mmol, 1.2 eq) and NaI (10 mg) was added to DMF (50 mL) and heated to 100 °C for 12 hours. The reaction mixture was then poured into water (500 mL), the formed precipitate was then filtered off, washed with water and dried. The crude product was then recrystallized in EtOH (abs.), evaporated and purified with flash column chromatography (20% EtOAc in pentane), affording **6b** as a white solid

(8.10 g, 38.1 mmol, 78%). ^{1}H NMR data was in accordance with previously reported spectra for this compound.[12]

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 – 7.82 (m, 2H, **Pht**), 7.74 – 7.69 (m, 2H, **Pht**), 3.80 (t, *J* = 7.0 Hz, 2H, N-**CH$_2$**), 2.27 (td, J_{aa} = 2.7 Hz, J_{ab} = 7.1 Hz, 2H, **Alkyne-CH$_2$**), 1.93 (p, *J* = 7.1 Hz, 2H, CH$_2$-**CH$_2$**-CH$_2$), 1.92 (t, *J* = 2.7 Hz, 1H, **Alkyne-H**) ppm.

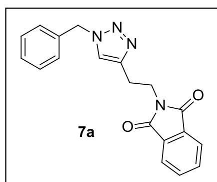
General procedure[13] for "Click"-coupling of azides (5a-f) and protected alkynes (6a-b), affording the protected triazoles (7a-j):

Alkyne (**6a-b**) (1 eq), $CuSO_4$ x $5H_2O$ (1-5 mol %, 1M in water), sodium ascorbate (2-10 mol %, 2M in water) and benzoic acid (0.1 eq) was added to a water:tBuOH-mixture (2:1, 2 mL/mmol alkyne), before the chosen azide (**5a-f**) (1,05 eq) was added and the mixture was stirred for a given time (10 min – 18 hours) at room temperature. The mixture was worked up in one of the following ways:

Procedure A: The mixture was added water (5-10 mL/mmol) and extracted with DCM (10-20 mL/mmol), before the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was either triturated with pentane as in *procedure B* or purified on a flash column using a EtOAc:DCM-mixture (1:3) yielding the triazole (**7a-j**) as a solid.

Procedure B: The mixture was added water (5-10 mL/mmol), filtered and washed with water (15 mL/mmol). The precipitate was then dissolved in an appropriate amount of DCM and dried over $MgSO_4$ before it was partially evaporated under reduced pressure. The saturated DCM-solution was then triturated and washed with pentane, yielding the triazole (**7a-j**) as a solid.

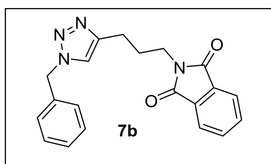
2-(2-(1-Benzyl-1H-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (**7a**):



Alkyne **6a** (0.60 g, 3.0 mmol) was reacted with azide **5a** (0.47 g, 3.2 mmol) using; 1% $CuSO_4$, 2% sodium ascorbate, water:tBuOH (2:1, 3 mL), for 10 min at room temperature. The reaction was worked up using *procedure A* with chromatographic separation, yielding **7a** as a white solid (0.88 g, 2.6 mmol, 88%, MP 143.8 – 145.3 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.84 – 7.78 (m, 2H, **Pht**), 7.72 – 7.66 (m, 2H, **Pht**), 7.38 – 7.30 (m, 4H, **H$_{Ar}$-3**, **H$_{Ar}$-4**, **H$_{Ar}$-5** and **H$_{triazole}$-5**), 7.23 – 7.18 (m, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.48 (s, 2H, **H$_{Bn}$**), 3.99 (t, *J* = 7.4 Hz, 2H, **Pht-CH$_2$**), 3.12 (t, *J* = 7.4 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.1 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 144.7 (**C$_{triazole}$4**), 134.8 (**C$_{Ar}$-1**), 133.9 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.0 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 129.0 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 128.6 (**C$_{Ar}$-4**), 127.9 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 123.3 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 121.4 (**C$_{triazole}$5**), 54.0 (**C$_{Bn}$**), 37.4 (N-**CH$_2$**-CH$_2$), 24.9 (N-CH$_2$-**CH$_2$**) ppm. IR: 2359 (w), 2342 (w), 1711 (s), 1400 (m), 1380 (m), 1101 (w), 1051 (w), 996 (w), 870 (w), 714 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 333.1349 (Calcd. $C_{19}H_{17}N_4O_2$, 333.1352, $[M+H]^+$).

2-(3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7b**):

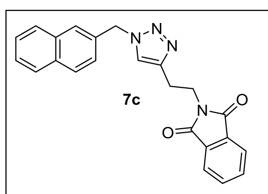


Alkyne **6b** (1.00 g, 4.6 mmol) was reacted with azide **5a** (0.68 g, 4.8 mmol) using; 1% $CuSO_4$ and 2% sodium ascorbate in water:tBuOH (2:1, 4.5 mL) for 10 min at room temperature. The reaction was worked up using *procedure A* with trituration, affording **7b** as a white solid (1.11 g, 3.2 mmol, 71%, MP 105.5 – 106.6 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.85 – 7.80 (m, 2H, **Pht**), 7.73 – 7.68 (m, 2H, **Pht**), 7.39 – 7.31 (m, 4H, **H$_{Ar}$-3**, **H$_{Ar}$-4**, **H$_{Ar}$-5** and **H$_{triazole}$-5**), 7.27 – 7.23 (m, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.48 (s, 2H, **H$_{Bn}$**), 3.73 (t, *J* = 7.3 Hz, 2H, N-**CH$_2$**), 2.75 (t, *J* = 7.3 Hz, 2H, **Triazole-CH$_2$**), 2.06 (t, *J* = 7.4 Hz,

2H, CH_2-$\mathbf{CH_2}$-CH_2) ppm. ^{13}C NMR (100 MHz, *CDCl₃*): ^{13}C NMR (100 MHz, *CDCl₃*): 168.4 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 147.4 (**C$_{triazole}$4**), 134.9 (**C$_{Ar}$-1**), 134.0 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.1 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 129.1 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 128.6 (**C$_{Ar}$-4**), 128.0 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 123.2 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 121.0 (**C$_{triazole}$5**), 54.0 (**C$_{Bn}$**), 37.3 (**Pht-CH$_2$**), 28.4 (N-CH_2-$\mathbf{CH_2}$), 23.1 (**Triazole-CH$_2$**) ppm. IR: 1703 (s), 1400 (m), 719 (s), 710 (m), 695 (w) cm^{-1}. HRMS (APCI/ASAP, m/z): 347.1508 (Calcd. $C_{20}H_{19}N_4O_2$, 347.1508, $[M+H]^+$).

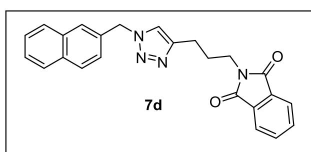
2-(2-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (**7c**):



Alkyne **6a** (1.00 g, 5.0 mmol) was reacted with azide **5b** (0.97 g, 5.3 mmol) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 4.5 mL) for 10 min at room temperature. The reaction was worked up using *procedure A* and washed with pentane, affording **7c** as a white solid (1.59 g, 4.2 mmol, 83%, MP 152.2 – 153.7 °C).

^{1}H NMR (400 MHz, *CDCl₃*): 7.86 – 7.79 (m, 3H, **H$_{Ar}$-7**, **H$_{Ar}$-8** and **H$_{Ar}$-4**), 7.76 – 7.70 (m, 2H, **Pht**), 7.68 (s, 1H, **H$_{Ar}$-1**), 7.66 – 7.61 (m, 2H, **Pht**), 7.53 – 7.48 (m, 2H, **H$_{Ar}$-5** and **H$_{Ar}$-6**), 7.34 (s, 1H, **H$_{Triazole}$-5**), 7.30 (dd, J_{aa} = 1.8 Hz, J_{ab} = 8.7 Hz, 1H, **H$_{Ar}$-3**), 5.63 (s, 2H, **H$_{Bn}$**), 3.98 (t, J = 7.2 Hz, 2H, **Pht-CH$_2$**), 3.12 (t, J = 7.2 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, *CDCl₃*): 168.1 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 144.2 (**C$_{triazole}$4**, assigned from HMBC), 133.9 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 133.2 (**C$_{Ar}$-4a**), 133.1 (**C$_{Ar}$-8a**), 132.2 (**C$_{Ar}$-2**), 132.0 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 129.1 (**C$_{Ar}$-4**), 128.0 (**C$_{Ar}$-7**), 127.8 (**C$_{Ar}$-8**), 127.2 (**C$_{Ar}$-1**), 126.7 (**C$_{Ar}$-6**), 126.6 (**C$_{Ar}$-5**), 125.2 (**C$_{Ar}$-3**), 123.2 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 121.4 (**C$_{triazole}$5**, assigned from HMBC), 54.3 (**C$_{Bn}$**), 37.4 (**Pht-CH$_2$**), 24.9 (**Triazole-CH$_2$**) ppm. IR: 1707 (s), 1396 (s), 1365 (m), 996 (m), 868 (w) 789 (w), 719 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 383.1503 (Calcd. $C_{23}H_{19}N_4O_2$, 383.1508, $[M+H]^+$).

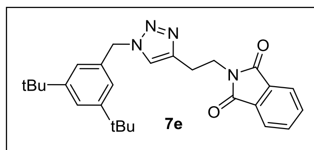
2-(3-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7d**):



Alkyne **6b** (0.53 g, 2.5 mmol) was reacted with azide **5b** (0.50 g, 2.7 mmol, 1.1 eq) using; 5% $CuSO_4$ and 10% sodium ascorbate (added in two portions) in water:tBuOH (2:1, 4.5 mL) for 18 hours at room temperature. The reaction was worked up using *procedure A* with chromatographic purification, affording **7d** as a sticky white solid (0.84 g, 2.1 mmol, 85%, MP 105.8 – 106.8 °C).

^{1}H NMR (400 MHz, *CDCl₃*): 7.85 – 7.78 (m, 5H, **Pht**, **H$_{Ar}$-7**, **H$_{Ar}$-8** and **H$_{Ar}$-4**), 7.72 (s, 1H, **H$_{Ar}$-1**), 7.71 – 7.67 (m, 2H, **Pht**), 7.52 – 7.47 (m, 2H, **H$_{Ar}$-5** and **H$_{Ar}$-6**), 7.41 (bs, 1H, **H$_{Triazole}$-5**), 7.34 (dd, J_{aa} = 1.5 Hz, J_{ab} = 8.3 Hz, 1H, **H$_{Ar}$-3**), 5.65 (s, 2H, **H$_{Bn}$**), 3.73 (t, J = 6.6 Hz, 2H, **Pht-CH$_2$**), 2.75 (t, J = 6.3 Hz, 2H, **Triazole-CH$_2$**), 2.10 – 2.03 (m, 2H, CH_2-$\mathbf{CH_2}$-CH_2) ppm. ^{13}C NMR (100 MHz, *CDCl₃*): 168.4 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 147.7 (**C$_{triazole}$4**), 133.9 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 133.2 (**C$_{Ar}$-4a**), 133.1 (**C$_{Ar}$-8a**), 132.2 (**C$_{Ar}$-2**), 132.1 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 129.1 (**C$_{Ar}$-4**), 128.0 (**C$_{Ar}$-7**), 127.8 (**C$_{Ar}$-8**), 127.2 (**C$_{Ar}$-1**), 126.7 (**C$_{Ar}$-6**), 126.6 (**C$_{Ar}$-5**), 125.3 (**C$_{Ar}$-3**), 123.2 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 121.4 (**C$_{triazole}$5**, assigned from HMBC), 54.3 (**C$_{Bn}$**), 37.3 (**Pht-CH$_2$**), 28.1 (CH_2-$\mathbf{CH_2}$-CH_2), 23.1 (**Triazole-CH$_2$**) ppm. IR: 1702 (s), 1396 (m), 1363 (m), 1020 (m), 788 (m), 717 (s), 710 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 397.1659 (Calcd. $C_{24}H_{21}N_4O_2$, 397.1665, $[M+H]^+$).

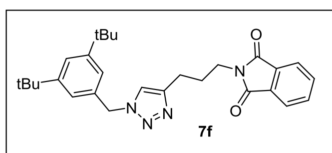
2-(2-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (**7e**):



Alkyne **6a** (0.49 g, 2.5 mmol) was reacted with azide **5c** (0.64 g, 2.6 mmol, 1.05 eq) using; 1% $CuSO_4$ and 2% sodium ascorbate in water:tBuOH (2:1, 6 mL) for 24 hours at room temperature. The reaction was worked up using *procedure A* with chromatographic purification, affording **7e** as a white solid (0.58 g, 1.3 mmol, 52%, MP 141.0 – 141.6 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.83 – 7.80 (m, 2H, **Pht**), 7.71 – 7.68 (m, 2H, **Pht**), 7.41 (t, *J* = 2.0 Hz, 1H, **H$_{Ar}$-4**), 7.33 (s, 1H, **H$_{Triazole}$-5**), 7.09 (d, *J* = 2.0 Hz, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.46 (s, 2H, **H$_{Bn}$**), 3.99 (t, *J* = 7.6 Hz, 2H, **Pht-CH$_2$**), 3.12 (t, *J* = 7.3 Hz, 2H, **Triazole-CH$_2$**), 1.30 (s, 18H, **tBu**) ppm. ^{13}C NMR (100 MHz, *CDCl$_3$*): 167.9 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 151.8 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 145.1 (**C$_{triazole}$4**, confirmed by HMBC), 133.9 (**C$_{Ar}$-1**), 133.8 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.1 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 123.3 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 122.7 (**C$_{Ar}$-4**), 122.4 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 121.1 (**C$_{triazole}$5**), 54.7 (**C$_{Bn}$**), 37.5 (**Pht-CH$_2$**), 34.9 (**C$_q$-tBu**), 31.4 (**tBu**), 24.9 (**Triazole-CH$_2$**) ppm. IR: 2952 (w), 1775 (w), 1707 (s), 1434 (w), 1405 (w), 1248 (w), 1100 (w), 1055 (w), 992 (w), 869 (w), 713 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 444.2526 (Calcd. $C_{27}H_{32}N_4O_2$, 444.2525, $[M]^+$).

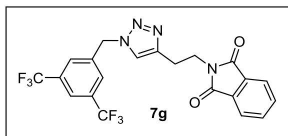
2-(3-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7f**):



Alkyne **6b** (0.50 g, 2.3 mmol) was reacted with azide **5c** (0.60 g, 2.4 mmol) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 4.5 mL) for 1 hour at room temperature. The reaction was worked up using *procedure B* with chromatographic purification, affording **7f** as a white solid (0.76 g, 1.6 mmol, 71%, MP 130.5 – 131.0 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.86 – 7.80 (m, 2H, **Pht**), 7.74 – 7.68 (m, 2H, **Pht**), 7.40 (t, *J* = 1.7 Hz, 1H, **H$_{Ar}$-4**), 7.34 (s, 1H, **H$_{Triazole}$-5**), 7.09 (d, *J* = 1.7 Hz, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.46 (s, 2H, **H$_{Bn}$**), 3.74 (t, *J* = 6.9 Hz, 2H, **Pht-CH$_2$**), 2.75 (t, *J* = 7.7 Hz, 2H, **Triazole-CH$_2$**), 2.06 (p, *J* = 7.1 Hz, 2H, CH$_2$-**CH$_2$**-CH$_2$), 1.30 (s, 18H, **tBu**) ppm. ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.4 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 151.7 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 146.8 (**C$_{triazole}$4**, confirmed by HMBC), 134.0 (**C$_{Ar}$-1**), 133.9 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.1 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 123.2 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 122.7 (**C$_{Ar}$-4**), 122.3 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 120.7 (**C$_{triazole}$5**, confirmed by HMBC), 54.7 (**C$_{Bn}$**), 37.3 (**Pht-CH$_2$**), 34.9 (**C$_q$-tBu**), 31.4 (**tBu**), 28.2 (CH$_2$-**CH$_2$**-CH$_2$), 23.1 (**Triazole-CH$_2$**) ppm. IR: 2964 (w), 1767 (w), 1707 (s), 1442 (w), 1396 (m), 1351 (w), 1052 (w), 1030 (w), 873 (w), 864 (w), 723 (s), 713 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 459.2754 (Calcd. $C_{28}H_{35}N_4O_2$, 459.2760, $[M+H]^+$).

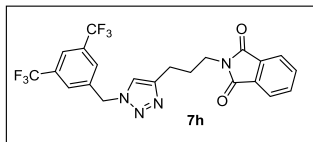
2-(2-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)ethyl)isoindoline-1,3-dione (**7g**):



Alkyne **6a** (0.54 g, 2.7 mmol) was reacted with azide **5d** (0.80 g, 2.8 mmol, 1.05 eq) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 4.5 mL) for 18 hours at room temperature. The reaction was worked up using *procedure B*, affording **7e** as a white solid (1.01 g, 2.2 mmol, 80%, MP 167.5 – 168.6 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 (s, 1H, **H$_{Ar}$-4**), 7.82 – 7.76 (m, 2H, **Pht**), 7.72 – 7.66 (m, 4H, **Pht**, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 7.47 (s, 1H, **H$_{Triazole}$-5**), 5.62 (s, 2H, **H$_{Bn}$**), 4.01 (t, *J* = 6.9 Hz, 2H, **Pht-CH$_2$**), 3.16 (t, *J* = 6.9 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.2 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 145.5 (**C$_{triazole}$4**), 137.4 (**C$_{Ar}$-1**), 134.0 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.6 (q, *J* = 35.9 Hz, **C$_{Ar}$-3** and **C$_{Ar}$-5**), 132.0 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 128.0 (m, **C$_{Ar}$-2** and **C$_{Ar}$-6**), 123.3 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 122.9 (q, *J* = 274 Hz, **CF$_3$**), 122.7 (m, **C$_{Ar}$-4**), 121.7 (**C$_{triazole}$5**), 52.8 (**C$_{Bn}$**), 37.3 (**Pht-CH$_2$**), 24.9 (**Triazole-CH$_2$**) ppm. IR: 1773 (w), 1712 (s), 1399 (m), 1372 (m), 1275 (s), 1177 (m), 1139 (s), 1119 (s), 1104 (s), 994 (m), 900 (w), 719 (s), 683 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 469.1096 (Calcd. $C_{21}H_{15}N_4O_2F_6$, 469.1099, $[M+H]^+$).

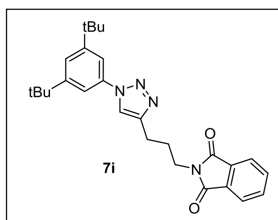
2-(3-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7h**):



Alkyne **6b** (0.53 g, 2.5 mmol) was reacted with azide **5d** (0.70 g, 2.6 mmol, 1.05 eq) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 4.5 mL) for 2 hours at room temperature. The reaction was worked up using *procedure B*, affording **7e** as a white solid (0.77 g, 1.6 mmol, 65%, MP 117.2 – 118.3 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 (s, 1H, **H$_{Ar}$-4**), 7.85 – 7.81 (m, 2H, **Pht**), 7.74 – 7.69 (m, 4H, **Pht**, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 7.49 (s, 1H, **H$_{Triazole}$-5**), 5.63 (s, 2H, **H$_{Bn}$**), 3.73 (t, *J* = 6.8 Hz, 2H, **Pht-CH$_2$**), 2.79 (t, *J* = 7.3 Hz, 2H, **Triazole-CH$_2$**), 2.10 (p, *J* = 7.3 Hz, 2H, CH$_2$-**CH$_2$**-CH$_2$) ppm. ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.5 (**C$_{Pht}$-1** and **C$_{Pht}$-3**), 147.9 (**C$_{triazole}$4**), 137.5 (**C$_{Ar}$-1**), 134.0 (**C$_{Pht}$-5** and **C$_{Pht}$-6**), 132.6 (q, *J* = 33.6 Hz, **C$_{Ar}$-3** and **C$_{Ar}$-5**), 132.1 (**C$_{Pht}$-8** and **C$_{Pht}$-9**), 128.0 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 123.2 (**C$_{Pht}$-4** and **C$_{Pht}$-7**), 122.9 (q, *J* = 274 Hz, **CF$_3$**), 122.7 (**C$_{Ar}$-4**), 121.4 (**C$_{triazole}$5**), 52.8 (**C$_{Bn}$**), 37.0 (**Pht-CH$_2$**), 28.0 (**Triazole-CH$_2$**), 22.9 (CH$_2$-**CH$_2$**-CH$_2$) ppm. IR: 3068 (w), 1714 (s), 1400 (m), 1381 (m), 1366 (m), 1350 (m), 1277 (s), 1173 (s), 1117 (s), 1032 (s), 903 (m), 718 (s), 681 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 483.1254 (Calcd. $C_{22}H_{17}N_4O_2F_6$, 483.1256, [M+H]$^+$).

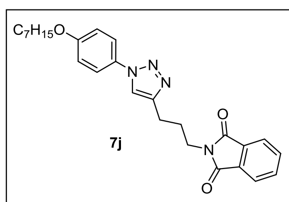
2-(3-(1-(3,5-Di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7i**):



Alkyne **6b** (0.26 g, 1.23 mmol) was reacted with azide **5e** (0.30 g, 1.29 mmol, 1.05 eq) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 3 mL) for 18 hours at room temperature. The reaction was worked up using *procedure A* with chromatographic purification, affording **7i** as a white solid (0.46 g, 0.53 mmol, 80%, MP 134.1 – 135.2 °C).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 – 7.82 (m, 3H, **Pht** + **H$_{Triazole}$-5**), 7.74 - 7.69 (m, 2H, **Pht**), 7.51 – 7.49 (m, 2H, **H$_{Ph}$-2** and **H$_{Ph}$-6**), 7.48 (t, 1H, *J* = 1.7 Hz, **H$_{Ph}$-4**), 3.82 (t, 2H, *J* = 6.8 Hz, **Pht-CH$_2$**), 2.88 (t, 2H, *J* = 7.9 Hz, **Triazole-CH$_2$**), 2.17 (p, 2H, 6.7 Hz, Pht-CH$_2$-**CH$_2$**), 1.37 (s, 18H, 2x **tBu**). ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.5 (**C$_{Pht}$-1** + **C$_{Pht}$-3**), 152.7 (**C$_{Ph}$-3** + **C$_{Ph}$-5**), 147.3 (**C$_{Triazole}$-4**), 136.9 (**C$_{Ph}$-1**), 134.0 (**C$_{Pht}$-5** + **C$_{Pht}$-6**), 132.1 (**C$_{Pht}$-8** + **C$_{Pht}$-9**), 123.2 (**C$_{Pht}$-4** + **C$_{Pht}$-7**), 122.7 (**C$_{Ph}$-4**), 119.8 (**C$_{Triazole}$-5**), 115.4 (**C$_{Ph}$-2** + **C$_{Ph}$-6**), 37.4 (Pht-**CH$_2$**), 35.2 (tBu-**C$_q$**), 31.5 (**tBu**), 28.2 (Pht-CH$_2$-**CH$_2$**), 23.1 (Triazole-**CH$_2$**). IR: 2955 (w), 1708 (s), 1595 (w), 1394 (m), 1362 (m), 1248 (w), 1043 (m), 718 (s), 707 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 444.2521 (Calcd. $C_{27}H_{32}N_4O_2$, 444.2525, [M*]$^+$).

2-(3-(1-(4-(Heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)propyl)isoindoline-1,3-dione (**7j**):



Alkyne **6b** (0.16 g, 0.74 mmol) was reacted with azide **5f** (0.18 g, 0.77 mmol, 1.05 eq) using; 5% $CuSO_4$ and 10% sodium ascorbate in water:tBuOH (2:1, 1.5 mL) for 20 minutes at room temperature. The reaction was worked up using *procedure A* with trituration, affording **7j** as an off-white solid (0.21 g, 0.46 mmol, 63%, MP 135.2 – 136.4 °C).

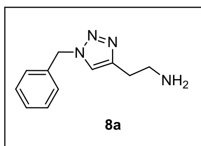
^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 – 7.83 (m, 2H, **Pht**), 7.80 (s, 1H, **H$_{triazole}$-5**), 7.74 – 7.69 (m, 2H, **Pht**), 7.61 – 7.56 (m, 2H, **H$_{Ph}$-3** and **H$_{Ph}$-5**), 7.01 – 6.96 (m, 2H, **H$_{Ph}$-2** and **H$_{Ph}$-6**), 4.00 (t, 2H, *J* = 6.2 Hz, O-**CH$_2$**), 3.80 (t, 2H, *J* = 6.9 Hz, **Pht-CH$_2$**), 2.85 (t, 2H, *J* = 7.5 Hz, **Triazole-CH$_2$**), 2.16 (p, 2H, *J* = 7.5 Hz, Pht-CH$_2$-**CH$_2$**), 1.81 (p, 2H, *J* = 8.0 Hz, O-CH$_2$-**CH$_2$**), 1.47 (p, 2H, *J* = 8.0 Hz, **CH$_2$**), 1.42 – 1.28 (m, 6H, 3x **CH$_2$**), 0.90 (t, 3H, *J* = 6.5 Hz, **CH$_3$**). ^{13}C NMR (100 MHz, *CDCl$_3$*): 168.5 (**C$_{Pht}$-1** + **C$_{Pht}$-3**), 159.2 (**C$_{Ph}$-4**), 147.3 (**C$_{Triazole}$-4**), 134.0 (**C$_{Pht}$-5** + **C$_{Pht}$-6**), 132.1 (**C$_{Pht}$-8** + **C$_{Pht}$-9**), 130.5 (**C$_{Ph}$-1**), 123.2 (**C$_{Pht}$-4** + **C$_{Pht}$-7**), 122.0 (**C$_{Ph}$-3** + **C$_{Ph}$-5**), 119.6 (**C$_{Triazole}$-5**), 115.2 (**C$_{Ph}$-2** + **C$_{Ph}$-6**), 68.5 (O-**CH$_2$**), 37.3

(Pht-**CH₂**), 31.8 (**CH₂**), 29.2 (O-CH₂-**CH₂**), 29.0 (**CH₂**), 28.1 (Pht-CH₂-**CH₂**), 26.0 (**CH₂**), 23.0 (Triazole-**CH₂**), 22.6 (**CH₂**), 14.1 (**CH₃**). IR: 2930 (w), 1699 (s), 1523 (m), 1402 (w), 1259 (w), 1206 (m), 832 (w), 718 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 447.2394 (Calcd. $C_{26}H_{31}N_4O_3$, 447.2396, $[M+H]^+$).

General procedure[14-16] for deprotection of protected triazoles (7a-j) to primary amines (8a-j):

Phthalimide protected triazole (**7a-h**) in toluene was added hydrazine hydrate (2-10 eq, 64-65%), and refluxed until disappearance of **7a-h** from TLC. The reaction mixture was then warm filtered and evaporated, affording the free amine (**8a-h**) in 46-90% yield. ^{1}H NMR was used to confirm full conversion of the free amine and the structure was then fully elucidated for the corresponding HCl-salt (**1-4 a,d**) prior to biological screening.

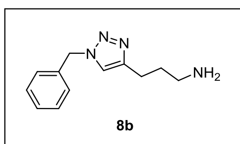
2-(1-Benzyl-1H-1,2,3-triazol-4-yl)ethan-1-amine (**8a**):



Triazole **7a** (2.00 g, 6.0 mmol) was reacted with hydrazine hydrate (0.89 mL, 11.9 mmol, 2 eq, 64-65%) in toluene (20 mL) for 1.5 hours at reflux. Filtration and chromatographic purification (70:30:3 $CHCl_3$:MeOH:NH_4OH) afforded **8a** as a yellow oil (0.64 g, 3.2 mmol, 53%).

^{1}H NMR (400 MHz, *CDCl₃*): 7.41 – 7.32 (m, 3H, **Ph**), 7.29 – 7.23 (m, 3H, **Ph** and **H$_{Triazole}$-5**), 5.49 (s, 2H, **H$_{Bn}$**), 3.05 – 2.94 (m, 2H, NH₂-**CH₂**), 2.81 (t, *J* = 7.4 Hz, 2H, **Triazole-CH₂**) ppm. HRMS of **8a***HCl (**1a**) showed the same molecular ion (203.1297 $[M-Cl]^+$) as a previously reported synthesis of **8a** by Novartis (from LC-MS).[17]

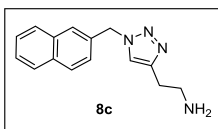
3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propan-1-amine (**8b**):



Triazole **7b** (0.30 g, 0.8 mmol) was reacted with hydrazine hydrate (0.32 mL, 4.2 mmol, 5 eq, 64-65%) in toluene (4 mL) for 2.25 hours at reflux. Work-up afforded **8b** as a white solid (0.14 g, 0.65 mmol, 77%).

^{1}H NMR (400 MHz, *CDCl₃*): 7.41 – 7.33 (m, 3H, **Ph**), 7.28 – 7.23 (m, 2H, **Ph**), 7.20 (s, 1H, **H$_{Triazole}$-5**), 5.49 (s, 2H, **H$_{Bn}$**), 2.78 – 2.69 (2x t, J_1 = 7.7 Hz, J_2 = 7.2 Hz, 4H, **Triazole-CH₂** and **CH₂**-NH₂), 1.80 (p, *J* = 7.5 Hz, 2H, CH₂-**CH₂**-CH₂) ppm.

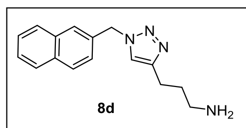
2-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)ethan-1-amine (**8c**):



Triazole **7c** (1.00 g, 2.6 mmol) was reacted with hydrazine hydrate (0.77 mL, 10.4 mmol, 4 eq, in two portions, 64-65%) in toluene (10 mL) for 23 hours reaction at reflux. The mixture was warm filtered, evaporated, dissvolved in DCM, filtered through celite and, evaporated. Affording **8c** as a white solid (0.48 g, 1.9 mmol, 74%).

^{1}H NMR (400 MHz, *CDCl₃*): 7.87 – 7.80 (m, 3H, **Napht**), 7.75 (s, 1H, **H$_{Triazole}$-5**), 7.54 – 7.49 (m, 2H, **Napht**), 7.35 (dd, J_{aa} = 1.7 Hz, J_{ab} = 8.5 Hz, 1H, **Napht**), 7.28 (s, 1H, **Napht**), 5.66 (s, 2H, **H$_{Bn}$**), 3.01 (t, *J* = 6.6 Hz, 2H, **CH₂**-NH₂), 2.82 (t, *J* = 6.6 Hz, 2H, **Triazole-CH₂**) ppm.

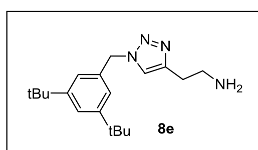
3-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)propan-1-amine (**8d**):



Triazole **7d** (0.82 g, 2.1 mmol) was reacted with hydrazine hydrate (0.77 mL, 10.4 mmol, 5 eq, 64-65%) in toluene (10 mL) for 22 hours at reflux. Work-up afforded **8b** as a white solid (0.45 g, 1.7 mmol, 80%).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.86 – 7.80 (m, 3H, **Napht**), 7.73 (s, 1H, **H$_{Triazole}$-5**), 7.53 – 7.49 (m, 2H, **Napht**), 7.34 (dd, J_{aa} = 1.7 Hz, J_{ab} = 8.5 Hz, 1H, **Napht**), 7.28 (s, 1H, **Napht**), 5.65 (s, 2H, **H$_{Bn}$**), 2.76 – 2.70 (2x t, J_1 = 7.5 Hz, J_1 = 7.0 Hz, 4H, **Triazole-CH$_2$** and **CH$_2$**-NH$_2$), 1.79 (p, J = 7.4 Hz, 2H, CH$_2$-**CH$_2$**-CH$_2$) ppm.

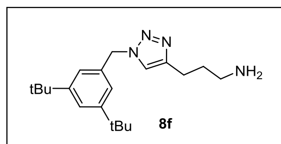
2-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethan-1-amine (**8e**):



Triazole **7e** (1.50 g, 3.4 mmol) was reacted with hydrazine hydrate (1.51 mL, 20.3 mmol, 4 eq + 1 eq after 2 hours, 64-65%) in toluene (15 mL) for 3.5 hours at reflux. Work-up afforded **8e** as a clear oil (0.96 g, 3.1 mmol, 91%).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.42 – 7.40 (m, 1H, **H$_{Ar}$-4**), 7.28 (s, 1H, **H$_{Triazole}$-5**), 7.09 (d, J = 1.8 Hz, 2H, 2x **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.48 (s, 2H, **H$_{Bn}$**), 3.01 (t, J = 6.8 Hz, 2H, **CH$_2$**-NH$_2$), 2.82 (t, J = 6.5 Hz, 2H, **Triazole-CH$_2$**), 1.28 (s, 18H, 2x **tBu**) ppm.

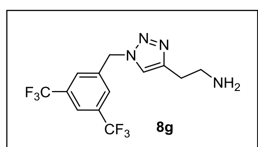
3-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)propan-1-amine (**8f**):



Triazole **7f** (0.76 g, 1.6 mmol) was reacted with hydrazine hydrate (0.92 mL, 12.4 mmol, 5 eq + 2.5 eq after 4 hours, 64-65%) in toluene (10 mL) for 4.5 hours at reflux. Followed by a second reaction in hydrazine hydrate (0.2 mL) and toluene (5 mL) for 3.5 hours at reflux afforded **8f** as a slightly yellow oil after work-up (0.36 g, 1.1 mmol, 66%).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.42 – 7.39 (m, 1H, **H$_{Ar}$-4**), 7.21 (s, 1H, **H$_{Triazole}$-5**), 7.07 (d, J = 1.8 Hz, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.47 (s, 2H, **H$_{Bn}$**), 2.79 – 2.69 (m, 4H, **Triazole-CH$_2$** and **CH$_2$**-NH$_2$), 1.80 (p, J = 7.2 Hz, 2H, CH$_2$-**CH$_2$**-CH$_2$), 1.30 (s, 18H, 2x **tBu**) ppm.

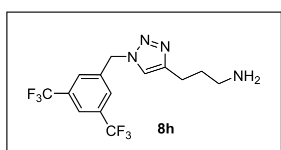
2-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)ethan-1-amine (**8g**):



Triazole **7g** (1.00 g, 2.1 mmol) was reacted with hydrazine hydrate (0.99 mL, 13.4 mmol, 5 eq + 1.25 eq after 2 hours, 64-65%) in toluene (13 mL) for 3 hours at reflux. Work-up afforded **8g** as a slightly yellow oil (0.66 g, 1.9 mmol, 91%).

^{1}H NMR (400 MHz, *CDCl$_3$*): 7.87 (s, 1H, **H$_{Ar}$-4**), 7.72 (s, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 7.40 (s, 1H, **H$_{Triazole}$-5**), 5.64 (s, 2H, **H$_{Bn}$**), 3.05 (t, J = 6.5 Hz, 2H, **CH$_2$**-NH$_2$), 2.87 (t, J = 6.5 Hz, 2H, **Triazole-CH$_2$**) ppm.

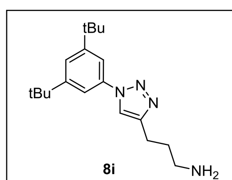
3-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)propan-1-amine (**8h**):



Triazole **7h** (0.77 g, 1.6 mmol) was reacted with hydrazine hydrate (1.19 mL, 16.0 mmol, 5 eq + 2.5 eq after 4 hours + 2.5 eq after 6 hours, 64-65%) in toluene (10 mL) for 6.5 hours at reflux. Work-up afforded **8h** as a clear oil (0.50 g, 1.4 mmol, 87%).

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.87 (s, 1H, **H_{Ar}-4**), 7.70 (s, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 7.32 (s, 1H, **$H_{Triazole}$-5**), 5.63 (s, 2H, **H_{Bn}**), 2.83 – 2.72 (m, 4H, **Triazole-CH_2** and **CH_2**-NH_2), 1.83 (p, *J* = 6.7 Hz, 2H, CH_2-**CH_2**-CH_2) ppm.

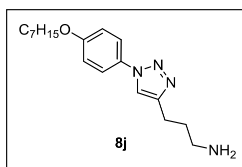
3-(1-(3,5-Di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)propan-1-amine (**8i**):



Triazole **7i** (0.44 g, 0.99 mmol) was reacted with hydrazine hydrate (0.74 mL, 9.88 mmol, 5 eq + 5 eq after 3 hours, 64-65%) in toluene (15 mL) for 4 hours at reflux. The mixture was then let cool to room temperature and stirred for 44 hours. Addition of additional hydrazine hydrate (0.37 mL, 4.94 mmol, 5 eq) and reflux for 19 hours followed by work-up afforded **8i** as a clear oil (0.28 g, 0.88 mmol, 89%).

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.72 (s, 1H, **$H_{triazole}$-5**), 7.49 (s, 2H, **H_{Ph}-2** and **H_{Ph}-6**), 7.48 (s, 1H, **H_{Ph}-4**), 2.87 (t, 2H, *J* = 7.6 Hz, **Triazole-CH_2**), 2.81 (t, 2H, *J* = 7.1 Hz, **CH_2**-NH_2), 1.91 (p, 2H, *J* = 7.2 Hz, **CH_2**-CH_2-NH_2), 1.36 (s, 18H, 2x **tBu**).

3-(1-(4-(Heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)propan-1-amine (**8j**):



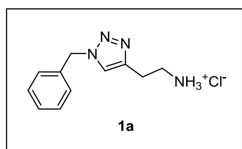
Triazole **7j** (0.20 g, 0.44 mmol) was reacted with hydrazine hydrate (1.31 mL, 17.6 mmol, 40 eq, 64-65%, added in 5 eq increments over 73 hours) in toluene (15 mL) for 75 hours at reflux. Work-up afforded **8j** as an off-white solid (0.14 g, 0.43 mmol, 98%).

^{1}H NMR (400 MHz, *$CDCl_3$*): 7.64 (s, 1H, **$H_{triazole}$-5**), 7.61 – 7.57 (m, 2H, **H_{Ph}-3** and **H_{Ph}-5**), 7.02 – 6.97 (m, 2H, **H_{Ph}-2** and **H_{Ph}-6**), 4.00 (t, 2H, *J* = 6.5 Hz, **CH_2**-O), 2.88 – 2.78 (m, 4H, **Triazole-CH_2** + **CH_2**-NH_2), 1.93 – 1.76 (m, 4H, **CH_2**-CH_2-O + **CH_2**-CH_2-NH_2), 1.54 – 1.24 (m, 12H, 4x **CH_2** + H_2O from $CDCl_3$), 0.92 – 0.87 (m, 3H, **CH_3**).

Creating HCl-salts:

Amine **8a-h** (50-100 mg) was added to MeCN, iPrOH or DCM (1-3 mL) and added an excess of HCl (0.1-0.4 mL/50 mg **8**, 37%), followed by filtration (if precipitation occurred) or evaporation (if precipitation did not occur) and washing with small amounts of nonpolar solvent (ether/pentane/DCM) followed by drying.

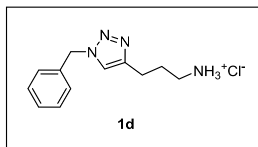
2-(1-Benzyl-1H-1,2,3-triazol-4-yl)ethan-1-aminium chloride (**1a**):



Amine **8a** (50 mg, 0.25 mmol) was added to iPrOH (2mL) with excess HCl (0.1 mL, 37%) and recrystallized in MeCN (3 mL) with a small additive of water (1-3 dr.), affording final product **1a** as a white powder (12 mg, 0.05 mmol, 20%, MP 212.5 – 214.1 ºC).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 1.9 min, 214 nm, 95% pure. ^{1}H NMR (400 MHz, *d_4*-MeOD): 7.87 (s, 1H, **$H_{Triazole}$-5**), 7.40 – 7.31 (m, 5H, **Ph**), 5.58 (s, 2H, **H_{Bn}**), 3.27 (t, *J* = 7.4 Hz, 2H, NH_3^+-**CH_2**), 3.04 (t, *J* = 7.4 Hz, 2H, **Triazole-CH_2**) ppm. ^{13}C NMR (100 MHz, *d_4*-MeOD): 144.5 (**$C_{Triazole}$-4**), 136.7 (**C_{Ar}-1**), 130.0 (**C_{Ar}**), 129.7 (**C_{Ar}**), 129.2 (**C_{Ar}**), 124.1 (**$C_{Triazole}$-5**), 55.0 (**C_{Bn}**), 40.1 (**CH_2**-NH_3^+), 24.4 (**Triazole-CH_2**) ppm. IR: 2955 (bw), 2906 (bw), 1596 (w), 1492 (w), 1454 (w), 1154 (w), 1052 (m), 949 (m), 860 (w), 761 (m), 717 (s), 696 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 203.1295 (Calcd. $C_{11}H_{15}N_4$, 203.1297, $[M-Cl]^+$).

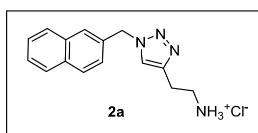
3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**1d**):



Amine **8b** (50 mg, 0.23 mmol) was added to iPrOH (2 mL) with excess HCl (0.1 mL, 37%) and evaporated, affording **1d** as a white solid (MP 149.9 – 150.5 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 3.0 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d$_4$*-MeOD): 8.22 (s, 1H, **H$_{Triazole}$-5**), 7.43 – 7.36 (m, 5H, **Ph**), 5.70 (s, 2H, **H$_{Bn}$**), 3.00 (t, *J* = 7.9 Hz, 2H, NH_3^+-**CH$_2$**), 2.91 (t, *J* = 7.3 Hz, 2H, **Triazole-CH$_2$**), 2.05 (p, *J* = 7.9 Hz, 2H, CH_2-**CH$_2$**-CH_2) ppm. ^{13}C NMR (100 MHz, *d$_4$*-MeOD): 146.0 (**C$_{Triazole}$-4**), 135.4 (**C$_{Ar}$-1**), 130.2 (**C$_{Ar}$**), 130.18 (**C$_{Ar}$**), 129.7 (**C$_{Ar}$**), 125.9 (**C$_{Triazole}$-5**), 56.5 (**C$_{Bn}$**), 39.9 (**CH$_2$**-NH_3^+), 27.5 (CH_2-**CH$_2$**-CH_2), 22.3 (**Triazole-CH$_2$**) ppm. IR: 3012 (bw), 2305 (bw), 1898 (w), 1589 (w), 1456 (s), 1472 (w), 1435 (w), 1340 (w), 1148 (s), 1025 (m), 987 (m), 941 (s), 784 (s), 729 (s), 698 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 217.1452 (Calcd. $C_{12}H_{17}N_4$, 217.1453, [M-Cl]$^+$).

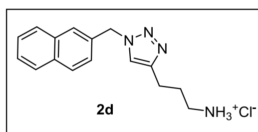
2-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium chloride (**2a**):



Amine **8c** (50 mg, 0.20 mmol) was dissolved in DCM (2 mL) and added HCl (2.5 mL, 2 M in Et_2O), the precipitate was filtered off and dried, affording **2a** as a white solid (29 mg, 0.1 mmol, 51%, MP 160.0 – 162.1 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 8.8 min, 214 nm, 98% pure. ^{1}H NMR (400 MHz, *d$_4$*-MeOD): 8.13 (s, 1H, **H$_{Triazole}$-5**), 7.91 (s, 1H, **Napht**), 7.90 – 7.85 (m, 3H, **Napht**), 7.53 – 7.50 (m, 3H, **Napht**), 7.47 (dd, J_{aa} = 1.6 Hz, J_{ab} = 8.6 Hz, 1H, **Napht**), 5.82 (s, 2H, **H$_{Bn}$**), 3.28 (t, *J* = 7.1 Hz, 2H, **CH$_2$**-NH_3^+), 3.12 (t, *J* = 7.1 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, *d$_4$*-MeOD): 143.8 (**C$_{Triazole}$-4**), 134.8, 134.7, 133.3, 130.0, 129.1, 129.0, 128.8, 127.8, 127.7, 126.7, 125.5 (**C$_{Triazole}$-5**), 55.9 (**C$_{Bn}$**), 39.7 (**CH$_2$**-NH_3^+), 24.0 (**Triazole-CH$_2$**) ppm (unassigned peaks stem from the naphthyl ring in a similar pattern as that of **7c**). IR: 2962 (bs), 2920 (s), 2359 (s), 2341 (m), 1598 (w), 1489 (m), 1474 (w), 1152 (w), 1135 (w), 1050 (s), 1022 (m), 947 (w), 832 (m), 783 (s), 755 (s), 729 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 253.1452 (Calcd. $C_{15}H_{17}N_4$, 253.1453, [M-Cl]$^+$).

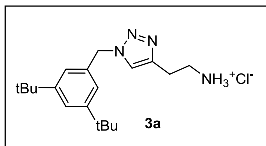
3-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**2d**):



Amine **8d** (50 mg, 0.19 mmol) was dissolved in iPrOH (2 mL), added HCl (0.1 mL, 37%) and dried, affording amine **2d** as a white solid (MP 200.3 – 202.5 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 9.4 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d$_4$*-MeOD): 8.31 (s, 1H, **H$_{Triazole}$-5**), 7.96 (s, 1H, **Napht**), 7.95 – 7.83 (m, 3H, **Napht**), 7.59 – 7.56 (m, 2H, **Napht**), 7.49 (dd, J_{aa} = 1.6 Hz, J_{ab} = 8.7 Hz, 1H, **Napht**), 5.89 (s, 2H, **H$_{Bn}$**), 3.00 (t, *J* = 7.4 Hz, 2H, **CH$_2$**-NH_3^+), 2.94 (t, *J* = 7.4 Hz, 2H, **Triazole-CH$_2$**), 2.05 (p, *J* = 7.4 Hz, 2H, CH_2-**CH$_2$**-CH_2) ppm. ^{13}C NMR (100 MHz, *d$_4$*-MeOD): 145.9 (**C$_{Triazole}$-4**), 134.9, 134.7, 132.4, 130.2, 129.4, 129.1, 128.8, 128.0, 127.9, 126.6, 126.2 (**C$_{Triazole}$-5**), 56.9 (**C$_{Bn}$**), 39.9 (**CH$_2$**-NH_3^+), 27.4 (CH_2-**CH$_2$**-CH_2), 22.2 (**Triazole-CH$_2$**) ppm (unassigned peaks stem from the naphthyl ring in a similar pattern as that of **7c**). IR: 2900 (bw), 1910 (w), 1601 (w), 1509 (w), 815 (s), 749 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 267.1607 (Calcd. $C_{15}H_{17}N_4$, 267.1610, [M-ClH]$^+$).

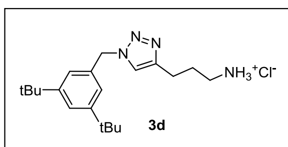
2-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium chloride (**3a**):



Amine **8e** (100 mg, 0.32 mmol) was dissolved in iPrOH (3 mL), added excess of HCl (0.75 mL, 37%, aq), dried and washed with MeCN (5 mL), affording **3a** as a white solid (48 mg, 0.14 mmol, 43%, MP 188.3 – 190.1 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 20.5 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 7.91 (s, 1H, **H$_{Triazole}$-5**), 7.44 (t, *J* = 1.4 Hz, 1H, **H$_{Ar}$-4**), 7.23 (d, *J* = 1.7 Hz, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.57 (s, 2H, **H$_{Bn}$**), 3.26 (t, *J* = 7.2 Hz, 2H, **CH$_2$**-NH_3^+), 3.07 (t, *J* = 7.5 Hz, 2H, **Triazole-CH$_2$**), 1.30 (s, 18H, 2x **tBu**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 152.9 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 144.3 (**C$_{Triazole}$-4**), 135.7 (**C$_{Ar}$-1**), 124.3 (**C$_{Triazole}$-5**), 123.8 (**C$_{Ar}$-4**), 123.6 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 55.8 (**C$_{Bn}$**), 40.0 (**CH$_2$**-NH_3^+), 35.7 (**C$_q$-tBu**), 31.8 (**tBu**), 24.3 (**Triazole-CH$_2$**) ppm. IR: 2963 (s), 2867 (s), 2647 (w), 2543 (w), 2476 (w), 1602 (s), 1512 (s), 1466 (m), 1362 (s), 1062 (s), 789 (s), 776 (s), 667 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 315.2548 (Calcd. $C_{19}H_{31}N_4$, 315.2549, [M-ClH]$^+$).

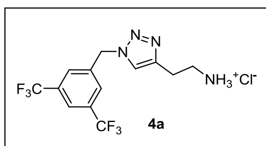
3-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**3d**):



Amine **8f** (50 mg, 0.15 mmol) was dissolved in iPrOH (3 mL), added an excess of HCl (0.3 mL, 37%, aq), dried and washed with MeCN (3 mL) and DCM (3 mL), affording **3d** as a white solid (20 mg, 0.05 mmol, 36%, MP 218.4 – 219.2 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 29.4 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 7.77 (s, 1H, **H$_{Triazole}$-5**), 7.43 (s, 1H, **H$_{Ar}$-4**), 7.18 (s, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.54 (s, 2H, **H$_{Bn}$**), 2.96 (t, *J* = 7.6 Hz, 2H, **CH$_2$**-NH_3^+), 2.80 (t, *J* = 7.6 Hz, 2H, **Triazole-CH$_2$**) 1.99 (p, *J* = 7.2 Hz, 2H, CH_2-**CH$_2$**-CH_2), 1.30 (s, 18H, 2x **tBu**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 152.9 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 147.6 (**C$_{Triazole}$-4**), 136.1 (**C$_{Ar}$-1**), 123.6 (**C$_{Triazole}$-5**), 123.4 (**C$_{Ar}$-4**), 123.3 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 55.5 (**C$_{Bn}$**), 40.2 (**CH$_2$**-NH_3^+), 35.7 (**C$_q$-tBu**), 31.8 (**tBu**), 28.3 (CH_2-**CH$_2$**-CH_2), 23.2 (**Triazole-CH$_2$**) ppm. IR: 2961 (s), 2853 (s), 2750 (w), 2715 (w), 2669 (w), 2608 (w), 1601 (w), 1475 (m), 1361 (s), 1223 (s), 1132 (s), 1063 (s), 1039 (m), 1016 (m), 874 (s), 827 (s), 802 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 329.2703 (Calcd. $C_{20}H_{33}N_4$, 329.2705, [M-Cl]$^+$).

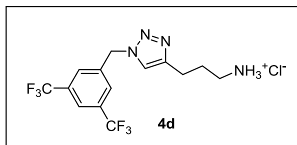
2-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium chloride (**4a**):



Amine **8g** (100 mg, 0.30 mmol) was dissolved in MeCN (1 mL), added excess of HCl (0.5 mL, 37%, aq), dried and washed with DCM (3 mL), affording **4a** as a white solid (95 mg, 0.25 mmol, 86%, MP 169.5 – 173.0 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 21.4 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 8.25 (s, 1H, **H$_{Triazole}$-5**), 8.04 (s, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 8.00 (s, 1H, **H$_{Ar}$-4**), 5.88 (s, 2H, **H$_{Bn}$**), 3.31 (t, *J* = 8.2 Hz, 2H, **CH$_2$**-NH_3^+), 3.16 (t, *J* = 8.2 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 144.1 (**C$_{Triazole}$-4**), 139.3 (**C$_{Ar}$-1**), 133.4 (q, *J* = 32 Hz, **C$_{Ar}$-3** and **C$_{Ar}$-5**), 130.4 (m, **C$_{Ar}$-2** and **C$_{Ar}$-6**), 125.8 (**C$_{Triazole}$-5**), 124.6 (q, *J* = 272 Hz, **CF$_3$**), 123.6 (m, **C$_{Ar}$-4**), 54.2 (**C$_{Bn}$**), 39.7 (**CH$_2$**-NH_3^+), 24.0 (**Triazole-CH$_2$**) ppm. IR: 1378 (w), 1279 (s), 1184 (m), 1136 (w), 1113 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 339.1044 (Calcd. $C_{13}H_{13}N_4F_6$, 339.1044, [M-Cl]$^+$).

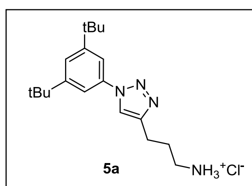
3-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**4d**):



Amine **8h** (100 mg, 0.28) was dissolved in MeCN (1 mL), added excess of HCl (0.5 mL, 37%, aq), dried and washed with DCM (3 mL) and MeCN (3 mL), affording **4d** as a white solid (98 mg, 0.25 mmol, 89%, MP 181.1 – 182.3 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 21.8 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d_4*-MeOD): 8.45 (s, 1H, **$H_{Triazole}$-5**), 8.10 (s, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 8.03 (s, 1H, **H_{Ar}-4**), 5.96 (s, 2H, **H_{Bn}**), 3.03 (t, *J* = 7.3 Hz, 2H, **CH_2**-NH_3^+), 2.96 (t, *J* = 7.9 Hz, 2H, **Triazole-CH_2**), 2.09 (p, *J* = 7.9 Hz, 2H, CH_2-**CH_2**-CH_2) ppm. ^{13}C NMR (100 MHz, *d_4*-MeOD): 146.1 (**$C_{Triazole}$-4**), 138.3 (**C_{Ar}-1**), 133.5 (q, *J* = 34 Hz, **C_{Ar}-3** and **C_{Ar}-5**), 130.8 (m, **C_{Ar}-2** and **C_{Ar}-6**), 126.7 (**$C_{Triazole}$-5**), 124.5 (q, *J* = 274 Hz, **CF_3**), 123.9 (m, **C_{Ar}-4**), 55.1 (**C_{Bn}**), 39.9 (**CH_2**-NH_3^+), 27.4 (CH_2-**CH_2**-CH_2), 22.2 (**Triazole-CH_2**) ppm. IR: 2903 (bw), 1917 (w), 1373 (w), 1279 (s), 1169 (s), 1114 (s), 1060 (m), 1014 (w), 889 (m), 703 (s), 683 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 353.1199 (Calcd. $C_{14}H_{15}N_4F_6$, 353.1201, [M-Cl]$^+$).

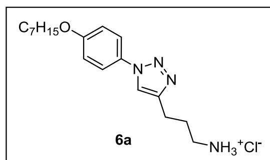
3-(1-(3,5-Di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**9a**):



Amine **8i** (32 mg, 0.10 mmol) was dissolved in MeCN (3 mL) and added HCl (0.1 mL, 37%, aq). Evaporation under reduced pressure afforded **5a** as a white solid (36 mg, 0.10 mmol, quant., MP 196.2 – 198.8 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 22.9 min, 214 nm, 98% pure. ^{1}H NMR (600 MHz, *d6-DMSO*): 8.71 (s, 1H, **$H_{triazole}$-5**), 8.04 (bs, 3H, **NH_3^+**), 7.66 – 7.64 (m, 2H, **H_{Ph}-2** and **H_{Ph}-6**), 7.50 (s, 1H, **H_{Ph}-4**), 2.93 – 2.86 (m, 2H, **CH_2**-NH_3^+), 2.81 (t, 2H, *J* = 7.5 Hz, **Triazole-CH_2**), 2.00 (p, 2H, *J* = 7.2 Hz, **CH_2**-CH_2-NH_3^+), 1.35 (s, 18H, 2x **tBu**). ^{13}C NMR (150 MHz, *d6-DMSO*): 152.8 (**C_{Ph}-3** and **C_{Ph}-5**), 147.1 (**$C_{Triazole}$-4**), 137.0 (**C_{Ph}-1**), 122.5 (**C_{Ph}-4**), 121.3 (**$C_{Triazole}$-5**), 114.9 (**C_{Ph}-2** and **C_{Ph}-6**), 38.8 (**CH_2**-NH_3^+), 35.5 (**C_q-tBu**), 31.5 (**tBu**), 27.1 (**CH_2**-CH_2-NH_3^+), 22.5 (Triazole-**CH_2**). IR: 2958 (s), 2317 (m), 1877 (w), 1593 (s), 1439 (s), 1106 (m), 1024(m), 874 (s), 704 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 315.2548 (Calcd. $C_{19}H_{31}N_4$, 315.2549, [M+H]$^+$).

3-(1-(4-(Heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**10a**):



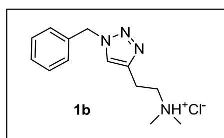
Amine **8j** (25 mg, 0.08 mmol) was dissolved in MeCN (3 mL), filtered and added HCl (0.1 mL, 37%, aq). Evaporation under reduced pressure afforded **6a** as a slightly yellow wax (28 mg, 0.08 mmol, quant.)

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): min, 214 nm, 98% pure. ^{1}H NMR (400 MHz, *d6-DMSO*): 8.52 (s, 1H, **$H_{triazole}$-5**), 7.95 (bs, 3H, **NH_3^+**), 7.79 – 7.74 (m, 2H, **H_{Ph}-3** and **H_{Ph}-5**), 7.15 – 7.09 (m, 2H, **H_{Ph}-2** and **H_{Ph}-6**), 4.03 (t, 2H, *J* = 6.6 Hz, **CH_2**-O), 2.95 – 2.85 (m, 2H, **CH2**-NH_3^+), 2.79 (t, 2H, *J* = 7.2 Hz, **Triazole-CH_2**), 1.97 (p, 2H, *J* = 7.2 Hz, **CH2**-CH_2-NH_3^+), 1.74 (p, 2H, *J* = 7.2 Hz, **CH_2**-CH_2-O), 1.47 – 1.23 (m, 8H, 4x **CH_2**), 0.91 – 0.84 (m, 3H, **CH_3**). ^{13}C NMR (100 MHz, *d6-DMSO*): 159.0 (**C_{Ph}-4**), 147.1 (**$C_{Triazole}$-4**), 130.5 (**C_{Ph}-1**), 121.9 (**C_{Ph}-3** and **C_{Ph}-5**), 120.9 (**$C_{Triazole}$-5**), 115.8 (**C_{Ph}-2** and **C_{Ph}-6**), 68.4 (**CH_2**-O), 38.7 (**CH2**-NH_3^+), 31.7 (**CH_2**), 29.1 (**CH_2**-CH_2-O), 28.9 (**CH_2**), 27.0 (**CH2**-CH_2-NH_3^+), 25.9 (**CH_2**), 22.5 (**Triazole-CH_2** + **CH_2**), 14.4 (**CH_3**). IR: 2920 (m), 2854 (w), 1518 (s), 1248 (s), 1222 (m), 1044 (s), 830 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 317.2339 (Calcd. $C_{18}H_{29}N_4O$, 317.2341, [M]$^+$).

General procedure[18,19] for Eschweiler-Clarke reductive amination:

Amine **8a-h** was added to MeCN (or H_2O), HCOOH (5-20 eq), HCHO 5-20 eq) and refluxed for 1-3 hours. The mixture was then added an excess of HCl (0.5 mL, 37%, aq.) and evaporated to dryness. Washing, recrystallization or chromatographic separation yielded the tertiary amine HCl-salts (**1-4 b,e**).

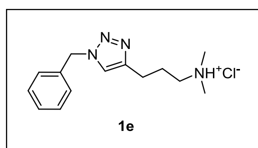
2-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N,N-dimethylethan-1-aminium chloride (**1b**):



Amine **8a** (0.07 g, 0.35 mmol) was added H_2O (1 mL), formic acid (0.1 mL, 96%, 7 eq) and formaldehyde (0.2 mL, 37%, 7 eq) followed by 1 hour reflux. The mixture was added HCl (2 mL, 2 M, Et_2O) and washed with MeCN (5 mL). The evaporated mixture was then purified using flash column chromatography (70:30:3 $CHCl_3$:MeOH:NH_4OH). The eluted amine was added etheric HCl (2 mL, 2 M) and evaporated, affording **1b** as a white wax (70 mg, 0.26 mmol, 76%).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 3.0 min, 214 nm, 99% pure. ^1H NMR (400 MHz, d_4-MeOD): 8.14 (s, 1H, $\mathbf{H_{Triazole}}$**-5**), 7.43 – 7.35 (m, 5H, **Ph**), 5.66 (s, 2H, $\mathbf{H_{Bn}}$), 3.51 (t, *J* = 7.7 Hz, 2H, $NH(CH_3)_2^+$-$\mathbf{CH_2}$), 3.25 (t, *J* = 7.7 Hz, 2H, **Triazole-**$\mathbf{CH_2}$), 2.96 (s, 6H, 2x **Me**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 143.0 ($\mathbf{C_{Triazole}}$**-4**), 135.8 ($\mathbf{C_{Ar}}$**-1**), 130.1, 130.0, 129.5, 125.6 ($\mathbf{C_{Triazole}}$**-5**), 57.3 ($NH(CH_3)_2^+$-$\mathbf{CH_2}$), 55.9 ($\mathbf{C_{Bn}}$), 43.6 (**Me**), 21.4 (**Triazole-**$\mathbf{CH_2}$) ppm. IR: 3383 (bs), 2959 (bw), 2674 (bs), 1459 (s), 1053 (s), 966 (w), 714 (s), 706 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 231.1610 (Calcd. $C_{13}H_{19}N_4$, 231.1610, $[M-Cl]^+$).

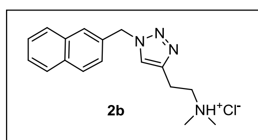
3-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N,N-dimethylpropan-1-aminium chloride (**1e**):



Amine **8b** (0.10 g, 0.46 mmol) was added H_2O (1 mL), formic acid (0.1 mL, 96%, 5 eq) and formaldehyde (0.2 mL, 37%, 6 eq) followed by 1 hour reflux. The mixture was added HCl (2 mL, 37%, aq.) and evaporated. The crude residue was purified using flash column chromatography (70:30:3 $CHCl_3$:MeOH:NH_4OH) and the elute was added HCl (3 mL, 2 M, in Et_2O) and evaporated, affording **1e** as a white wax (0.07 g, 0.24 mmol, 53%).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 3.2 min, 214 nm, 99% pure. ^1H NMR (400 MHz, d_4-MeOD): 8.24 (s, 1H, $\mathbf{H_{Triazole}}$**-5**), 7.47 – 7.35 (m, 5H, **Ph**), 5.70 (s, 2H, $\mathbf{H_{Bn}}$), 3.25 – 3.18 (m, 2H, $\mathbf{CH_2}$-$NH(CH_3)_2^+$), 2.93 – 2.86 (m, 8H, **Triazole-**$\mathbf{CH_2}$ + 2x **Me**), 2.14 (p, *J* = 7.5 Hz, 2H, CH_2-$\mathbf{CH_2}$-CH_2) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 145.7 ($\mathbf{C_{Triazole}}$**-4**), 135.3 ($\mathbf{C_{Ar}}$**-1**), 130.3, 130.2, 129.7, 126.0 ($\mathbf{C_{Triazole}}$**-5**), 58.0 ($NH(CH_3)_2^+$-$\mathbf{CH_2}$), 56.6 ($\mathbf{C_{Bn}}$), 43.5 (**Me**), 24.6 (CH_2-$\mathbf{CH_2}$-CH_2), 22.2 (**Triazole-**$\mathbf{CH_2}$) ppm. IR: 3373 (bs), 2964 (bw), 2678 (bm), 2474 (w), 1631 (w), 1456 (s), 1334 (w), 1155 (m), 1055 (s), 1028 (w), 967 (s), 714 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 245.1769 (Calcd. $C_{14}H_{21}N_4$, 245.1766, $[M-Cl]^+$).

N,N-Dimethyl-2-(1-(naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)ethan-1-aminium chloride (**2b**):

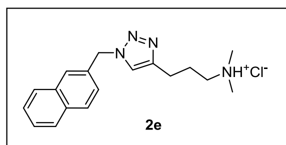


Amine **8c** (0.10 g, 0.36 mmol) was added MeCN (2 mL), formic acid (0.1 mL, 96%, 7 eq) and formaldehyde (0.2 mL, 37%, 7 eq) followed by 1.5 hours reflux. The mixture was evaporated and purified twice with flash column chromatography (#1: 70:30:3 $CHCl_3$:MeOH:NH_4OH, #2: 95:5:1 $CHCl_3$:MeOH:NH_4OH). The evaporated elute was dissolved in iPrOH (2

mL), added HCl (0.5 mL, 37%, aq.) and evaporated, affording **2b** as a white solid (48 mg, 0.14 mmol, 40%, MP 156.7 – 158.2 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 5.3 min, 214 nm, 98% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 7.98 (s, 1H, **$H_{Triazole}$-5**), 7.90 – 7.83 (m, 4H, **Napht**), 7.55 – 7.48 (m, 2H, **Napht**), 7.44 (dd, J_{aa} = 1.8 Hz, J_{ab} = 8.5 Hz, 1H, **Napht**), 5.76 (s, 2H, **H_{Bn}**), 3.48 (t, J = 7.9 Hz, 2H, **CH_2**-$NH(CH_3)_2^+$), 3.18 (t, J = 7.4 Hz, 2H, **Triazole-CH_2**), 2.94 (s, 6H, 2x **Me**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 143.9 (**$C_{Triazole}$-4**), 134.8, 134.7, 133.8, 130.0, 129.0, 128.8, 128.7, 127.73, 127.71, 126.6, 124.5 (**$C_{Triazole}$-5**), 57.8 ($NH(CH_3)_2^+$-**CH_2**), 55.4 (**C_{Bn}**), 43.6 (**Me**), 21.7 (**Triazole-CH_2**) ppm (unassigned peaks belong to the naphtyl ring and can be assigned after the pattern for **7c**). IR: 3016 (w), 2959 (w), 2697 (m), 1477 (m), 1455 (m), 1300 (m), 1196 (m), 1056 (m), 969 (s), 866 (s), 836 (s), 746 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 281.1762 (Calcd. $C_{17}H_{21}N_4$, 281.1766, $[M-Cl]^+$).

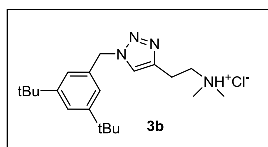
N,N-Dimethyl-3-(1-(naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)propan-1-aminium chloride (**2e**):



Amine **8d** (0.10 g, 0.36 mmol) was added MeCN (2 mL), formic acid (0.1 mL, 96%, 7 eq) and formaldehyde (0.2 mL, 37%, 7 eq) followed by 2 hours reflux. The mixture was passed through a pipette with $CaCl_2$, added iPrOH (2 mL) and HCl (0.15 mL, 37%, aq.) before it was dried. The crude product was purified with flash column chromatography (70:30:3 $CHCl_3$:MeOH:NH_4OH). The evaporated elute was then added iPrOH (2 mL), HCl (0.1 mL, 37%, aq.) and evaporated, affording **2e** as a white solid (0.07 g, 0.21 mmol, 56%, MP 159.6 – 162.1 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 8.3 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 7.90 – 7.82 (m, 5H, **Napht** + **$H_{Triazole}$-5**), 7.54 – 7.49 (m, 2H, **Napht**), 7.42 (dd, J_{aa} = 1.7 Hz, J_{ab} = 8.5 Hz, 1H, **Napht**), 5.74 (s, 2H, **H_{Bn}**), 3.21 – 3.14 (m, 2H, **CH_2**-$NH(CH_3)_2^+$), 2.87 (s, 6H, 2x **Me**), 2.80 (t, J = 7.5 Hz, 2H, **Triazole-CH_2**), 2.13 – 2.03 (m, 2H, CH_2-**CH_2**-CH_2) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 147.4 (**$C_{Triazole}$-4**), 134.8, 134.6, 134.1, 129.9, 129.0, 128.8, 128.5, 127.7, 127.67, 126.5, 123.8 (**$C_{Triazole}$-5**), 58.4 ($NH(CH_3)_2^+$-**CH_2**), 55.2 (**C_{Bn}**), 43.5 (**Me**), 25.2 (CH_2-**CH_2**-CH_2), 23.1 (**Triazole-CH_2**) ppm (unassigned peaks belong to the naphtyl ring and can be assigned after the pattern for **7d**). IR: 3381 (bw), 3136 (w), 2572 (w), 2466 (w), 1491 (w), 1473 (w), 1456 (w), 1438 (m), 1423 (m), 1204 (m), 1161 (m), 1118 (m), 1054 (s), 974 (s), 838 (m), 822 (s), 808 (s), 782 (s), 762 (s), 736 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 295.1920 (Calcd. $C_{18}H_{23}N_4$, 295.1923, $[M-Cl]^+$).

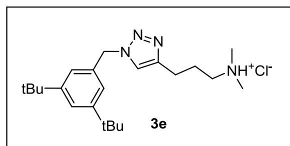
2-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)-N,N-dimethylethan-1-aminium chloride (**3b**):



Amine **8e** (0.10 g, 0.29 mmol) was added MeCN (2 mL), formic acid (0.1 mL, 96%, 9 eq) and formaldehyde (0.2 mL, 37%, 9 eq) followed by 4 hours reflux. The mixture was added HCl (1 mL, 37%, aq.) and evaporated, affording **3b** as a white solid (0.06 g, 0.16 mmol, 55%, MP 195.1 – 198.4 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 21.9 min, 214 nm, 98% pure. ^{1}H NMR (400 MHz, d_4-MeOD): 7.89 (s, 1H, **$H_{Triazole}$-5**), 7.44 (t, J = 1.9 Hz, 1H, **H_{Ar}-4**), 7.23 (d, J = 1.5 Hz, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 5.56 (s, 2H, **H_{Bn}**), 3.48 (t, J = 8.0 Hz, 2H, **CH_2**-$NH(CH_3)_2^+$), 3.17 (t, J = 7.5 Hz, 2H, **Triazole-CH_2**), 2.95 (s, 6H, 2x **Me**) 1.30 (s, 18H, 2x **tBu**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 153.0 (**C_{Ar}-3** and **C_{Ar}-5**), 144.1 (**$C_{Triazole}$-4**), 136.0 (**C_{Ar}-1**), 124.2 (**$C_{Triazole}$-5**), 123.8 (**C_{Ar}-4**), 123.7 (**C_{Ar}-2** and **C_{Ar}-6**), 58.0 ($NH(CH_3)_2^+$-**CH_2**), 55.8 (**C_{Bn}**), 43.7 (**Me**), 35.9 (**C_q-tBu**), 31.9 (**tBu**), 22.0 (**Triazole-CH_2**) ppm. IR: 3951 (s), 2546 (bs), 2449 (bs), 1599 (m), 1468 (s), 1362 (s), 1248 (m), 1213 (s), 1121 (s), 1057 (s), 967 (s), 883 (s), 793 (s), 707 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 343.2858 (Calcd. $C_{21}H_{35}N_4$, 343.2856, $[M-Cl]^+$).

3-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)-N,N-dimethylpropan-1-aminium chloride (**3e**):

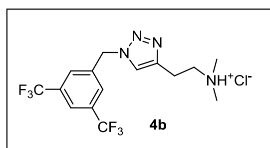


Amine **8f** (0.08 g, 0.24 mmol) was added MeCN (2 mL), formic acid (0.1 mL, 96%, 11 eq) and formaldehyde (0.2 mL, 37%, 11 eq) followed by 1.5 hours reflux. The mixture was applied to Dowex 50 WX8, washed with MeCN/MeOH (5 mL of each) and eluted with 2M HCl (37%, aq) in MeOH. The evaporated elute afforded **3e** as a sticky wax (0.04 g, 0.09 mmol, 40%).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 28.5 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d*$_4$-MeOD)*: 7.81 (s, 1H, **H$_{Triazole}$-5**), 7.45 – 7.42 (m, 1H, **H$_{Ar}$-4**), 7.22 – 7.17 (m, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.50 (s, 2H, **H$_{Bn}$**), 3.21 – 3.16 (m, 2H, **CH$_2$**-NH(CH$_3$)$_2^+$), 2.88 (s, 6H, 2x **Me**), 2.80 (t, *J* = 7.2 Hz, 2H, **Triazole-CH$_2$**) 2.11 – 2.04 (m, 2H, CH$_2$-**CH$_2$**-CH$_2$), 1.30 (s, 18H, 2x **tBu**) ppm. ^{13}C NMR (100 MHz, *d*$_4$-MeOD)*: 153.1 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 146.1 (**C$_{Triazole}$-4**, found from HMBC), 135.5 (**C$_{Ar}$-1**), 124.0 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 123.9 (**C$_{Ar}$-4**), 123.6 (**C$_{Triazole}$-5**, found from HMBC), 58.4 (NH(CH$_3$)$_2^+$-**CH$_2$**), 56.4 (**C$_{Bn}$**), 43.8 (**Me**), 35.9 (**C$_q$-tBu**), 31.9 (**tBu**), 25.2 (CH$_2$-**CH$_2$**-CH$_2$), 22.9 (**Triazole-CH$_2$**) ppm. IR: 3385 (bs), 2956 (s), 1708 (s), 1601 (s), 1477 (s), 1394 (m), 1363 (s), 1249 (m), 1202 (w), 1053 (m), 967 (w), 874 (w), 781 (w), 723 (m), 711 (s) cm^{-1}. HRMS HRMS (APCI/ASAP, m/z): 357.3015 (Calcd. $C_{22}H_{37}N_4$, 357.3013, [M-Cl]$^+$).

*) Some minor peaks have emerged in storage between synthesis/testing and full characterization.

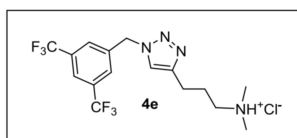
2-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)-N,N-dimethylethan-1-aminium chloride (**4b**):



Amine **8g** (0.10 g, 0.30 mmol) was added MeCN (2 mL), formic acid (0.1 mL, 96%, 9 eq) and formaldehyde (0.2 mL, 37%, 9 eq) followed by 2.5 hours reflux. The mixture was added HCl (1 mL, 37%, aq.) and evaporated, affording **4b** as a white solid (80 mg, 0.20 mmol, 67%, MP 175.2 – 177.0 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 20.7 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d*$_4$-MeOD): 8.11 (s, 1H, **H$_{Triazole}$-5**), 8.00 (s, 3H, **H$_{Ar}$-2**, **H$_{Ar}$-4** and **H$_{Ar}$-6**), 5.83 (s, 2H, **H$_{Bn}$**), 3.51 (t, *J* = 7.7 Hz, 2H, **CH$_2$**-NH(CH$_3$)$_2^+$), 3.22 (t, *J* = 7.3 Hz, 2H, **Triazole-CH$_2$**), 2.96 (s, 6H, 2x **Me**) ppm. ^{13}C NMR (100 MHz, *d*$_4$-MeOD): 144.3 (**C$_{Triazole}$-4**), 140.0 (**C$_{Ar}$-1**), 133.5 (q, *J* = 34 Hz, **C$_{Ar}$-3** and **C$_{Ar}$-5**), 130.2 (m, **C$_{Ar}$-2** and **C$_{Ar}$-6**), 125.2 (**C$_{Triazole}$-5**), 124.7 (d, *J* = 271 Hz, 2x **CF$_3$**), 123.6 (**C$_{Ar}$-4**), 57.8 (NH(CH$_3$)$_2^+$-**CH$_2$**), 53.9 (**C$_{Bn}$**), 43.8 (**Me**), 21.9 (**Triazole-CH$_2$**) ppm. IR: 2596 (w), 1902 (w), 1461 (w), 1378 (w), 1360 (w), 1278 (s), 1187 (m), 1167 (m), 1125 (s), 1056 (w), 947 (w), 908 (w), 841 (w), 781 (w), 682 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 367.1360 (Calcd. $C_{15}H_{17}N_4F_6$, 367.1357, [M-Cl]$^+$).

3-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)-N,N-dimethylpropan-1-aminium chloride (**4e**):



Amine **8h** (0.10 g, 0.27 mmol), was added MeCN (2 mL), formic acid (0.1 mL, 96%, 9 eq) and formaldehyde (0.2 mL, 37%, 10 eq) followed by 3.5 hours reflux. The mixture was added HCl (0.5 mL, 37%, aq.) and evaporated, affording **4e** as a white waxy solid (91 mg, 0.22 mmol, 81%).

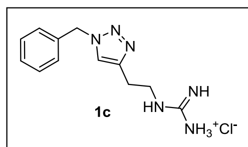
HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 21.3 min, 214 nm, 96% pure. ^{1}H NMR (400 MHz, *d*$_4$-MeOD): 8.03 (s, 1H, **H$_{Triazole}$-5**), 7.98 (s, 1H, **H$_{Ar}$-4**), 7.97 (s, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.82 (s, 2H, **H$_{Bn}$**), 3.24 – 3.17 (m, 2H, **CH$_2$**-NH(CH$_3$)$_2^+$), 2.96 (s, 6H, 2x **Me**), 2.85 (t, *J* = 7.7 Hz, 2H, **Triazole-CH$_2$**), 2.17 – 2.07 (m, 2H, CH$_2$-**CH$_2$**-CH$_2$) ppm. ^{13}C NMR (100 MHz, *d*$_4$-MeOD): 147.7 (**C$_{Triazole}$-4**), 140.1 (**C$_{Ar}$-1**), 133.5 (q, *J* = 33.5 Hz, **C$_{Ar}$-3** and **C$_{Ar}$-5**), 130.1 (m, **C$_{Ar}$-2** and **C$_{Ar}$-6**), 124.7 (q, *J* = 269 Hz,

2x $\mathbf{CF_3}$), 124.6 ($\mathbf{C_{Triazole}}$**-5**), 123.6 (m, $\mathbf{C_{Ar}}$**-4**), 58.5 ($NH(CH_3)_2^+$-$\mathbf{CH_2}$), 53.8 ($\mathbf{C_{Bn}}$), 43.6 (**Me**), 25.3 (CH_2-$\mathbf{CH_2}$-CH_2), 23.1 (**Triazole-CH₂**) ppm. IR: 3384 (bw), 1470 (w), 1376 (w), 1359 (w), 1277 (s), 1170 (s), 1120 (s), 1056 (w), 891 (m), 706 (m), 683 (s), 661 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 381.1516 (Calcd. $C_{16}H_{19}N_4F_6$, 381.1508, $[M-Cl]^+$).

General procedure[20,21] for electrophilic guanylation of amines:

Amines **8a-h** was added to MeCN, 1*H*-pyrazole carboxamidine hydrochloride (0.95 - 1.00 eq) and refluxed for 1-3 hours (except for **2c** and **2f** where the reaction time was extended to 19-20 hours.) The mixture was either filtered and the precipitate washed with MeCN and DCM or added Et_2O to induce precipitation followed by washing with nonpolar solvents, to afford the guanylated compounds **1-4 c,f** in 8-76% yields.

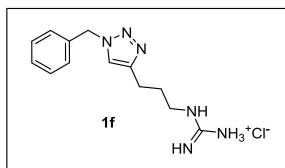
1-(2-(1-Benzyl-1H-1,2,3-triazol-4-yl)ethyl)guanidinium chloride (**1c**):



Amine **8a** (0.13 g, 0.53 mmol) was added to MeCN (3 mL) and 1*H*-pyrazole carboxamidine hydrochloride (0.07 g, 0.48 mmol, 0.9 eq) and refluxed for 4 hours. After cooling to room temperature the precipitate was washed with MeCN (3 mL), affording **1c** as a white powder (12 mg, 0.04 mmol, 8%, MP 66.2 – 67.9 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 3.0 min, 214 nm, 95% pure. ^1H NMR (400 MHz, d_4-MeOD): 7.83 (s, 1H, $\mathbf{H_{Triazole}}$**-5**), 7.42 – 7.28 (m, 5H, **Ph**), 5.58 (s, 2H, $\mathbf{H_{Bn}}$), 3.49 (t, *J* = 7.1 Hz, 2H, $\mathbf{CH_2}$-NH), 2.96 (t, *J* = 7.1 Hz, 2H, **Triazole-CH₂**) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 158.8 ($\mathbf{C_{guanidine}}$), 145.8 ($\mathbf{C_{Triazole}}$**-4**), 136.9 ($\mathbf{C_{Ar}}$**-1**), 130.2 (**Ph**), 129.8 (**Ph**), 129.3 (**Ph**), 124.2 ($\mathbf{C_{Triazole}}$**-5**), 55.1 ($\mathbf{C_{Bn}}$), 42.0 ($\mathbf{CH_2}$-NH), 26.2 (**Triazole-CH₂**) ppm. IR: 3323 (bs), 3141 (bs), 2359 (w), 2341 (w), 1667 (s), 1650 (s), 1456 (w), 1220 (w), 1129 (w), 1060 (w) cm^{-1}. HRMS (APCI/ASAP, m/z): 245.1514 (Calcd. $C_{12}H_{17}N_6$, 245.1515, $[M-Cl]^+$).

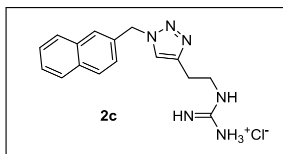
1-(3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propyl)guanidinium chloride (**1f**):



Amine **8b** (0.14 g, 0.62 mmol) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (0.08 g, 0.56 mmol, 0.9 eq) and refluxed for 2 hours. The reaction mixture was evaporated and triturated with MeOH and Et_2O (5 mL and 50 mL). Followed by washing of the precipitated oil with large amounts of Et_2O (150 mL), which upon drying afforded **1f** as a clear wax (0.94 g, 0.32 mmol, 51%).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 3.0 min, 214 nm, 97% pure. ^1H NMR (400 MHz, d_4-MeOD): 7.76 (s, 1H, $\mathbf{H_{Triazole}}$**-5**), 7.43 – 7.26 (m, 5H, **Ph**), 5.56 (s, 2H, $\mathbf{H_{Bn}}$), 3.23 (t, *J* = 7.3 Hz, 2H, $\mathbf{CH_2}$-NH), 2.76 (t, *J* = 7.3 Hz, 2H, **Triazole-CH₂**), 1.93 (p, *J* = 7.0 Hz, 2H, CH_2-$\underline{\mathbf{CH_2}}$-$CH_2$) ppm. ^{13}C NMR (100 MHz, d_4-MeOD): 159.0 ($\mathbf{C_{guanidine}}$, determined from HMBC), 146.8 ($\mathbf{C_{Triazole}}$**-4**), 135.4 ($\mathbf{C_{Ar}}$**-1**), 128.6 (**Ph**), 128.2 (**Ph**), 127.7 (**Ph**), 122.0 ($\mathbf{C_{Triazole}}$**-5**), 53.5 ($\mathbf{C_{Bn}}$), 40.3 ($\mathbf{CH_2}$-NH), 28.1 (CH_2-$\mathbf{CH_2}$-CH_2), 21.7 (**Triazole-CH₂**) ppm. IR: 3327 (bm), 3141 (bm), 1646 (s), 1455 (m), 1217 (w), 1174 (w), 1133 (w), 1059 (w) cm^{-1}. HRMS (APCI/ASAP, m/z): 259.1674 (Calcd. $C_{13}H_{19}N_6$, 259.1671, $[M-Cl]^+$).

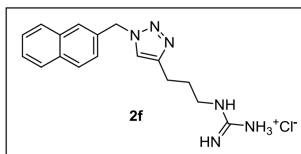
1-(2-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)ethyl)guanidinium chloride (**2c**):



Amine **8c** (75 mg, 0.29 mmol) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (39 mg, 0.27 mmol, 0.9 eq) and refluxed for 20 hours. After complete reaction on TLC, the mixture was added MeCN (3 mL) and filtered. The precipitate was washed with MeCN (2x 25 mL) and dried, affording **2c** as an off-white solid (43 mg, 0.13 mmol, 44%, MP 106.2 – 107.3 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 8.331 min, 214 nm, 96% pure. ^{1}H NMR (400 MHz, *d$_4$*-MeOD): 7.93 – 7.74 (m, 5H, **Napht** and **H$_{Triazole}$-5**), 7.54 – 7.48 (m, 2H, **Napht**), 7.42 (dd, J_{aa} = 1.6 Hz, J_{ab} = 8.6 Hz, 1H, **Napht**), 5.74 (s, 2H, **H$_{Bn}$**), 3.49 (t, *J* = 7.1 Hz, 2H, **CH$_2$**-NH), 2.96 (t, *J* = 6.7 Hz, 2H, **Triazole-CH$_2$**) ppm. ^{13}C NMR (100 MHz, *d$_4$*-MeOD): 158.9 (**C$_{guanidine}$**, determined from HMBC), 145.8 (**C$_{Triazole}$-4**), 134.9, 134.8, 134.3, 130.1, 129.1, 128.9, 128.6, 127.84, 127.82, 126.7, 124.3 (**C$_{Triazole}$-5**), 55.3 (**C$_{Bn}$**), 42.0 (**CH$_2$**-NH), 26.2 (**Triazole-CH$_2$**) ppm (unassigned peaks belong to the naphtyl ring and can be assigned after the pattern for **7c**). IR: 3392 (bm), 3143 (bm), 1675 (s), 1646 (s), 1626 (s), 1559 (w), 1465 (w), 1220 (w), 1062 (m), 1040 (w), 864 (w), 822 (w), 782 (s), 764 (s), 654 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 295.1671 (Calcd. $C_{16}H_{19}N_6$, 295.1671, [M-Cl]$^+$).

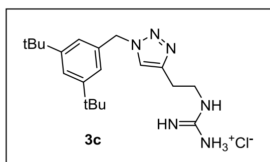
1-(3-(1-(Naphthalen-2-ylmethyl)-1H-1,2,3-triazol-4-yl)propyl)guanidinium chloride (**2f**):



Amine **8d** (0.10 g, 0.38 mmol) was added to MeCN (3 mL) and 1*H*-pyrazole carboxamidine hydrochloride (55 mg, 0.38 mmol, 1.0 eq) and refluxed for 19 hours. The reaction was cooled to room temperature and filtered. The precipitate was washed with MeCN (3x 10 mL) and dried, affording **2f** as a white solid (0.98 g, 0.29 mmol, 76%, MP 162.2 – 164.2 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 11.1 min, 214 nm, 98% pure. ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.91 – 7.83 (m, 5H, **Napht** and **H$_{Triazole}$-5**), 7.55 – 7.50 (m, 2H, **Napht**), 7.40 (dd, 1H, *J* = 1.6, 8.4 Hz, **Napht**), 5.72 (s, 2H, **H$_{Bn}$**), 3.22 (t, 2H, *J* = 7.1 Hz, **CH$_2$**-NH-), 2.76 (t, 2H, *J* = 7.5 Hz, **Triazole-CH$_2$**), 1.93 (p, 2H, *J* = 7.3 Hz, CH$_2$-**CH$_2$**-CH$_2$) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 158.7 **C$_{guanidine}$**), 148.2 (**C$_{Triazole}$-4**), 134.8, 134.6, 134.2, 129.9, 129.0, 128.8, 128.4, 127.7, 127.6, 126.5, 123.6 (**C$_{Triazole}$-5**), 55.1 (**C$_{Bn}$**), 41.7 (**CH$_2$**-NH-), 29.5 (CH$_2$-**CH$_2$**-CH$_2$), 23.1 (**Triazole-CH$_2$**) ppm (unassigned peaks belong to the naphtyl ring and can be assigned after the pattern for **7d**). IR: 3451 (bs), 3134 (bs), 2951 (m), 1680 (s), 1648 (s), 1617 (s), 1466 (s), 1342 (s), 1060 (s), 794 (s), 783 (s), 769 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 309.1830 (Calcd. $C_{17}H_{21}N_6$, 309.1822, [M-Cl]$^+$).

1-(2-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethyl)guanidinium chloride (**3c**):

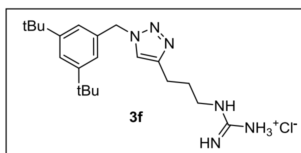


Amine **8e** (0.10 g, 0.29 mmol, 90%) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (38 mg, 0.26 mmol, 0.9 eq) and refluxed for 1.5 hours. The reaction was cooled to room temperature and filtered. The precipitate was washed with MeCN (5 mL) and dried, affording **3c** as a white solid (71 mg, 0.18 mmol, 63%, MP 221.0 – 222.4 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 21.9 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.82 (s, 1H, **H$_{Triazole}$-5**), 7.45 (s, 1H, **H$_{Ar}$-4**), 7.22 (s+s, 2H, **H$_{Ar}$-2** and **H$_{Ar}$-6**), 5.56 (s, 2H, **H$_{Bn}$**), 3.51 (t, 2H, *J* = 6.7 Hz, **CH$_2$**-NH-), 2.98 (t, 2H, *J* = 6.7 Hz, **Triazole-CH$_2$**), 1.32 (s, 18H, 2x **tBu**) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 158.6 (**C$_{Guanidine}$**, found from HMBC), 152.9 (**C$_{Ar}$-3** and **C$_{Ar}$-5**), 145.6 (**C$_{Triazole}$-4**), 136.0 (**C$_{Ar}$-1**), 124.0 (**C$_{Triazole}$-5**), 123.6 (**C$_{Ar}$-4**), 123.5 (**C$_{Ar}$-2** and **C$_{Ar}$-6**), 55.5 (**C$_{Bn}$**), 41.9 (**CH$_2$**-NH-), 35.7 (**C$_q$-tBu**), 31.8 (**tBu**), 26.0 (**Triazole-CH$_2$**) ppm. IR: 3396 (bs), 3233 (bs), 3110

(bs), 1684 (s), 1637 (s), 1621 (s), 1600 (s), 1475 (s), 1364 (s), 882 (s), 814 (s), 781 (s), 711 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 357.2767 (Calcd. $C_{20}H_{33}N_6$, 357.2761, $[M-Cl]^+$).

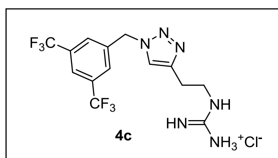
1-(3-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)propyl)guanidinium chloride (**3f**):



Amine **8f** (78 mg, 0.23 mmol, 95%) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (30 mg, 0.20 mmol, 0.9 eq) and refluxed for 3.5 hours. The reaction was cooled to room temperature and filtered. The precipitate was washed with MeCN/DCM (5 mL of each) and dried, affording **3f** as a white solid (58 mg, 0.14 mmol, 63%, MP 219.0 – 220.1 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 32.915 min, 214 nm, 99% pure. ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.75 (s, 1H, **$H_{Triazole}$-5**), 7.43 (s, 1H, **H_{Ar}-4**), 7.17 (s, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 5.54 (s, 2H, **H_{Bn}**), 3.22 (t, 2H, *J* = 7.3 Hz, **CH_2**-NH-), 2.76 (t, 2H, *J* = 7.3 Hz, **Triazole-CH_2**), 1.93 (p, 2H, *J* = 7.3 Hz, CH_2-**CH_2**-CH_2) 1.32 (s, 18H, 2 x **tBu**) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 158.6 (**$C_{Guanidine}$**), 152.8 (**C_{Ar}-3** and **C_{Ar}-5**), 148.2 (**$C_{Triazole}$-4**), 136.3 (**C_{Ar}-1**), 123.5 (**C_{Ar}-4**), 123.4 (**C_{Ar}-2** and **C_{Ar}-6**), 123.3 (**$C_{Triazole}$-5**), 55.4 (**C_{Bn}**), 41.7 (**CH_2**-NH-), 35.7 (**C_q-tBu**), 31.8 (**tBu**), 29.6 (CH_2-**CH_2**-CH_2), 23.1 (**Triazole-CH_2**) ppm. IR: 3328 (m), 3110 (bs), 2949 (s), 1677 (s), 1639 (s), 1601 (s), 1476 (s), 1361 (s), 1060 (s), 854 (s), 791 (s), 709 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 371.2924 (Calcd. $C_{21}H_{35}N_6$, 371.2918, $[M-Cl]^+$).

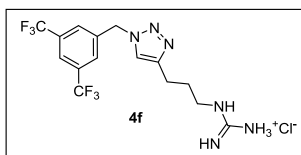
1-(2-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)ethyl)guanidinium chloride (**4c**):



Amine **8g** (0.10 g, 0.30 mmol) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (39 mg, 0.27 mmol, 0.9 eq) and refluxed for 2 hours. The reaction mixture was evaporated to dryness and washed with Et_2O (150 mL) and DCM (3 x 5 mL). Upon drying, the polar precipitate afforded **4c** as a white solid (65 mg, 0.16 mmol, 53 %, MP 134.5 – 136.4 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 22.9 min, 214 nm, 97% pure. ^{1}H NMR (400 MHz, *d_4*-MeOD): 7.98 (s, 1H, **$H_{Triazole}$-5**), 7.97 (s, 1H, **H_{Ar}-4**), 7.96 (s, 2H, **H_{Ar}-2** and **H_{Ar}-6**), 5.80 (s, 2H, **H_{Bn}**), 3.52 (t, *J* = 6.8 Hz, 2H, **CH_2**-NH), 2.99 (t, *J* = 6.8 Hz, 2H, **Triazole-CH_2**) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.6 (**$C_{Guanidine}$**, determined from HMBC), 146.1 (**$C_{Triazole}$-4**), 140.3 (**C_{Ar}-1**), 133.5 (q, *J* = 34.3 Hz, **C_{Ar}-3** and **C_{Ar}-5**), 130.0 (m, **C_{Ar}-2** and **C_{Ar}-6**), 124.8 (d(q), *J* = 272 Hz, 2x **CF_3**), 124.6 (m, **C_{Ar}-4**), 123.0 (**$C_{Triazole}$-5**) 53.6 (**C_{Bn}**), 42.0 (**CH_2**-NH-), 26.1 (**Triazole-CH_2**) ppm. IR: 3145 (bw), 1659 (m), 1615 (w), 1381 (w), 1357 (w), 1278 (s), 1169 (s), 1125 (s), 703 (w), 680 (s), 664 (m) cm^{-1}. HRMS (APCI/ASAP, m/z): 381.1262 (Calcd. $C_{14}H_{15}N_6F_6$, 381.1262, $[M-Cl]^+$).

1-(3-(1-(3,5-Bis(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)propyl)guanidinium chloride (**4f**):

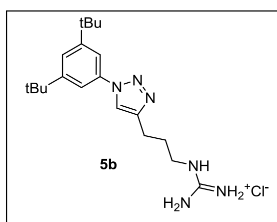


Amine **8g** (0.10 g, 0.27 mmol) was added to MeCN (2 mL) and 1*H*-pyrazole carboxamidine hydrochloride (36 mg, 0.24 mmol, 0.9 eq) and refluxed for 4.5 hours. After cooling to room temperature, the mixture was partially evaporated and filtered. The precipitate was washed with DCM (3 x 5 mL) and dried, affording **4f** as a white solid (66 mg, 0.15 mmol, 57%, MP 146.0 – 146.8 °C).

HPLC (C18 Zorbax Eclipse, 1:1 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 27.5 min, 214 nm, 95% pure. ^{1}H NMR (400 MHz, *d_4*-MeOD): 7.97 (s, 1H, **$H_{Triazole}$-5**), 7.94 – 7.91 (m, 3H, **H_{Ar}-2**, **H_{Ar}-4** and **H_{Ar}-6**), 5.90 (s,

2H, **H_{Bn}**), 3.25 (t, *J* = 7.1 Hz, 2H, **CH_2**-NH), 2.80 (t, *J* = 7.7 Hz, 2H, **Triazole-CH_2**), 1.96 (p, *J* = 7.1 Hz, 2H, CH_2-**CH_2**-CH_2) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 158.9 (**$C_{Guanidine}$**), 148.7 (**$C_{Triazole}$-4**), 140.4 (**C_{Ar}-1**), 133.4 (q, *J* = 32.8 Hz, **C_{Ar}-3** and **C_{Ar}-5**), 129.9 (m, **C_{Ar}-2** and **C_{Ar}-6**), 124.7 (d, *J* = 273 Hz, 2x **CF_3**), 124.1 (**$C_{Triazole}$-5**), 123.5 (m, **C_{Ar}-4**), 53.6 (**C_{Bn}**), 41.9 (**CH_2**-NH), 29.7 (CH_2-**CH_2**-CH_2), 23.3 (**Triazole-CH_2**) ppm. IR: 3338 (bw), 3136 (bw), 1675 (m), 1645 (m), 1614 (m), 1279 (s), 1164 (s), 1128 (s), 1145 (w), 1109 (m), 1056 (m), 907 (w), 872 (w), 680 (s), 584 (s), 579 (s), 571 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 395.1416 (Calcd. $C_{15}H_{17}N_6F_6$, 395.1416, $[M-Cl]^+$).

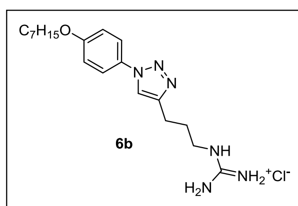
Amino((3-(1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)propyl)amino)methaniminium chloride (**9b**):



Amine **8i** (40 mg, 0.12 mmol) was added to MeCN (3 mL) and 1*H*-pyrazole carboxamidine hydrochloride (17 mg, 0.115 mmol, 0.95 eq) and refluxed for 1.5 hours. The reaction mixture was evaporated and washed with pentane (3x 50 mL) and Et_2O (3x 10 mL). Drying under reduced pressure afforded **5b** as a white solid (32 mg, 0.08 mmol, 67%, MP 86 – 100 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 28.0 min, 214 nm, 95% pure. ^{1}H NMR (400 MHz, *d6-DMSO*): 8.67 (s, 1H, **$H_{triazole}$-5**), 7.72 (t, 1H, *J* = 6.1 Hz, **NH**), 7.65 (d, 2H, *J* = 1.7 Hz, **H_{Ph}-2** and **H_{Ph}-6**), 7.50 (t, 1H, *J* = 1.7 Hz, **H_{Ph}-4**), 3.22 (q, 2H, *J* = 5.3 Hz, guan-**CH_2**), 2.77 (t, 2H, *J* = 7.7 Hz, **Triazole-CH_2**), 1.90 (p, 2H, *J* = 7.1 Hz, guan-CH_2-**CH_2**) 1.35 (s, 18H, 2x **tBu**). ^{13}C NMR (100 MHz, *d6-DMSO*): 157.3 (**C_{guan}**), 152.8 (**C_{Ph}-3** and **C_{Ph}-5**), 147.4 (**$C_{Triazole}$-4**), 137.0 (**C_{Ph}-1**), 122.5 (**C_{Ph}-4**), 121.2 (**$C_{Triazole}$-5**), 114.9 (**C_{Ph}-2** and **C_{Ph}-6**), 40.5 (**CH_2**-guan) 35.4 (**C_q-tBu**), 31.5 (**tBu**), 28.6 (**CH2**-CH_2-guan), 22.6 (**Triazole-CH_2**). IR: 3140 (w), 2959 (m), 1648 (s), 1609 (s), 1595 (s), 1453 (m), 1363 (m), 1248 (w), 1045 (w), 874 (w), 706 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 357.2766 (Calcd. $C_{20}H_{33}N_6$, 357.2767, $[M]^+$).

Amino((3-(1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)propyl)amino)methaniminium chloride (**10b**)



Amine **8j** (40 mg, 0.126 mmol) was added to MeCN (3 mL) and 1*H*-pyrazole carboxamidine hydrochloride (18 mg, 0.124 mmol, 0.98 eq) and refluxed for 2 hours. The reaction mixture was then filtered and evaporated, before it was washed with MeCN (3x 3 mL) and Et_2O (3x 10 mL). The off-white wax was then left in MeCN (7 mL) at 5 °C for 48 hours, removal of the supernatant and drying afforded **6b** as a white solid (15 mg, 0.038 mmol, 30%, MP: 230.7 – 235.5 °C).

HPLC (C18 Zorbax Eclipse, 3:5 H_2O:MeOH + 0.1% TFA, 0.75 mL/min): 31.5 min, 214 nm, 95% pure. ^{1}H NMR (400 MHz, *d6-DMSO*): 8.49 (s, 1H, **$H_{triazole}$-5**), 7.77 – 7.74 (m, 2H, **H_{Ph}-3** and **H_{Ph}-5**), 7.59 (t, 1H, J = 4.9 Hz, NH-**CH_2**), 7.14 – 7.10 (m, 2H, **H_{Ph}-2** and **H_{Ph}-6**), 4.04 (t, 2H, *J* = 6.5 Hz, O-**CH_2**), 3.21 (q, 2H, *J* = 6.3 Hz, guan-**CH_2**), 2.74 (t, 2H, *J* = 7.6 Hz, **Triazole-CH_2**) 1.88 (p, 2H, *J* = 7.0 Hz, guan-CH_2-**CH_2**), 1.74 (p, 2H, *J* = 7.9 Hz, **CH_2**-CH_2-O), 1.43 (p, 2H, *J* = 7.6 Hz, **CH_2**-CH_2-CH_2-O), 1.38 – 1.25 (m, 6H, 3x **CH_2**), 0.90 – 0.85 (m, 3H, **CH_3**). ^{13}C NMR (100 MHz, *d6-DMSO*): 159.0 (**C_{Ph}-4**), 157.2 (**C_{guan}**), 147.3 (**$C_{Triazole}$-4**), 130.5 (**C_{Ph}-1**), 121.9 (**C_{Ph}-3** and **C_{Ph}-5**), 120.8 (**$C_{Triazole}$-5**), 115.8 (**C_{Ph}-2** and **C_{Ph}-6**), 68.4 (**CH_2**-O), 40.6 (**CH_2**-guan), 31.7 (**CH_2**), 29.1 (**CH_2**-CH_2-O), 28.9 (**CH_2**), 28.6 (guan-CH_2-**CH_2**), 25.9 (**CH_2**), 22.6 (**Triazole-CH_2**), 22.5 (**CH_2**), 14.4 (**CH_3**). IR: 2856 (w), 1650 (m), 1612 (w), 1517 (s), 1473 (w), 1249 (s), 1044 (s), 989 (w), 831 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 359.2558 (Calcd. $C_{19}H_{31}N_6O$, 359.2559, $[M]^+$).

Experimental methods for biological assays

Inhibition of bacterial growth

The antibacterial activity was tested on 5 different strains; *E.faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *Streptococcus agalactiae* group B (ATCC 12386). Growth medium with sterile MilliQ H2O was used as a negative control while sterile MilliQ H2O and bacteria suspension was used as a positive control. Bacteria were transferred from a blood plate to growth medium (MH-bullion (VL787693 717, Merck) for *E. coli*, *P. aeruginosa* and *S. aureus* and BHI-bullion (CM1135, OXOID) for *E. faecalis* and *S. agalactiae* gr. B) and incubated at 37°C overnight. The following day a part of the bacteria suspension was transferred to fresh medium and cultivated in a shaker incubator at 37°C for 1,5 h (*E. coli, E. faecalis* and *Streptococcus* gr. B) or 2,5 h (*S. aureus* and *P. aeruginosa*). The bacteria suspension was then diluted 1:100 in medium and added all wells in a 96-well microtiter plate (Nunc 167008), followed by test compounds in duplicates. The plates were incubated at 37ºC overnight before growth was controlled visually and photometrical at 600 nm. The total reaction volume was 100 µL.

Inhibition of biofilm formation

S. epidermidis was used to assess the effect of the test compounds on biofilm formation. Growth media: tryptic soy broth (TS; Merck, Darmstadt, Germany). An overnight culture of *S. epidermidis* grown in TS was diluted with fresh TS containing 1 % glucose (1:100). Aliquots of 50 µL were transferred to a 96-well microtiter plate, and 50 µL of test compounds, dissolved in water at ranging concentrations, was added. After overnight incubation at 37 °C, the bacterial suspension was carefully discarded and the wells washed with water. The plate was dried and the biofilm fixed by incubation for 1 h at 55 °C before the surface attached cells were stained with 100 µL of 0.1 % crystal violet for 5 min. The crystal violet solution was removed and the plate once more washed with water and dried at 55 °C for 1 h. After adding 70 µL of 70 % ethanol, the plate was incubated at room temperature for 10 min. Biofilm formation was observed by visual inspection of the plates. The MIC was defined as the lowest concentration where no biofilm formation was visible. A *S. epidermidis* suspension, diluted with 50 µL of water, was used as a positive control, and 50 µL Staphylococcus haemolyticus suspension with 50 µL of water was employed as a negative control. A mixture of 50 µL water and 50 µL TS was used as assay control.

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Paper III

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Methyl propiolate and 3-butynone: Starting points for synthesis of amphiphilic 1,2,3-triazole peptidomimetics for antimicrobial evaluation

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ABSTRACT

A library of 29 small 1,4-substituted 1,2,3-triazoles was prepared for studies of antimicrobial activity. The pharmacophore model investigated with these substrates was based on small peptidomimetics of antimicrobial peptides and antimicrobials isolated from marine organisms from sub-arctic regions. Using methyl 1,2,3-triazole-carboxylates and 1,2,3-triazole methyl ketones prepared through "click" chemistry we were able to synthesize the different cationic amphiphiles through three steps or less. Several structural modifications to the lipophilic side and hydrophilic sides of the amphiphiles were investigated and compared with regards to antimicrobial activity and cytotoxicity in particular. The most promising amphiphile **10f** displayed minimum inhibitory concentrations (MICs) between 4–16 μg/mL against Gram-positive *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. The decent level of antimicrobial activity and biofilm inhibition, short synthesis, and accessible reagents, makes this type of amphiphilic mimics interesting leads for further development.

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1. Introduction

The ability to treat bacterial infections with antibiotics is one of the major constituents in any basic health care system.[1–3] However, increased consumption of antibiotics, both through agriculture and health services is causing rapid proliferation of resistant bacteria.[3] Combined with reduced focus on development of novel antibiotics, has made antimicrobial resistance one of the fastest growing threats to human health.[2,4] It is estimated that 700,000 people die each year due to events related to antimicrobial resistance.[3] Moreover, if resistance is allowed to develop without countermeasures, as many as 10 million people may die annually by the year 2050. This means that deaths related to antimicrobial resistance will surpass the number of deaths caused by cancer.[3]

Some of the first antibiotics were natural products and many important antibiotics today are based on natural or semi-synthetic compounds.[5] Antimicrobial natural products are found in animals and plants, and have through evolution evolved in eukaryotes living in a world inhabited by potential pathogenic prokaryotes. The ability to prevent and overcome infections has always been important for survival.[6] Natural product antimicrobials therefore form an important starting platform when searching for novel antimicrobial scaffolds. One natural product class of particular interest is antimicrobial peptides (AMPs).[7] These are small peptides between 12–50 residues that take part in the primary immune response system of all living organisms. AMPs have an overall net positive charge (+2 to +9) and fold into amphiphilic secondary structures with one lipophilic face and one hydrophilic face. These amphiphilic secondary structures interact more or less selectively with bacterial cell membranes, and through various mechanisms of membrane disruption processes cause inhibition of growth or lysis of the bacteria. Several mechanisms of action are suggested for the membranolytic effect of AMPs and for an excellent review on the topic see Giuliani et al.[7] Even though AMPs are highly active against bacteria, there may be some drawbacks to their use as drugs. Most of these problems are related to poor pharmacokinetic properties,[8] such as low bioavailability, low metabolic stability and lack of patient-friendly administration routes. This, in addition to high manufacturing costs, makes AMPs less desirable for clinical development. There are however some AMP-based drugs in clinical use today, but they are usually based on topical use, since pharmacokinetic issues make them unfit for systemic use.[9]

AMPs may however provide a starting point for investigations of smaller drugs with improved pharmacokinetic properties. It has been shown by the research group of Svendsen et al. that

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AMP-like oligopeptides consisting solely of arginine and tryptophan have similar antimicrobial activities as the native AMPs.[10] This work eventually led to the synthetic antimicrobial peptidomimetic LTX 109, which is currently undergoing clinical trials. Furthermore, the research group of Strøm et al. have synthesized a library of tri-functionalized $\beta^{2,2}$-amino acid derivatives based on AMPs without compromising the activity against resistant bacteria.[11,12] Assuming that these structures display membranolytic effects similar to that of AMPs, Strøm et al. also developed a library of antimicrobial aminobenzamide amphiphiles[13] (**E23**, Fig. 1) mimicking the structures of the antimicrobial marine antimicrobials synoxazolidinone A[14] and ianthelline.[15]

Based on the pharmacophore model of small AMPs and marine peptide mimics we have created a library of 1,2,3-triazole amphiphiles based on the structural motifs shown in Fig. 1. The nature of the lipophilic part and the rigidity at the hydrophilic cationic nitrogen functionalities were varied as shown in Fig. 2. The initial library was followed by optimization of activity by a more focused set of compounds shown in Fig. 3. The 1,2,3-triazole was chosen as a link between the lipophilic and hydrophilic side due to the simple synthesis[16,17] and accessible starting materials. Furthermore, triazoles are bioisosteres of amide bonds and stable against proteolytic degradation.[18–20] The library presented in this publication was prepared in parallel to a similar library of amphiphiles based on 1,2,3-triazole phthalimides.[21]

2. Results and discussion

2.1. Synthesis of the initial library

The initial 19 compounds evaluated for antimicrobial activity in this study (Fig. 2) were prepared according to Scheme 1. The chosen core molecules for this library of amphiphilic amido 1,2,3-triazoles were the methyl 1,2,3-triazole carboxylates **3a-d**. These carboxylates were obtained from copper catalyzed "click" chemistry between the organic azides **1a-d** and methyl propiolate (**2**), using a method[22] based on the established procedures by Sharpless[16] and Meldal[17] as displayed in Scheme 1. The ester group on **3** was then amidated with either piperazine (**n4**: R_2 = H), *N*-methyl-piperazine (**n5**: R_2 = Me),[23] or ethylene diamine (EDA) (**n6**).[24] Preparation of the piperazine amides **n4** and **n5** were performed with stoichiometric NaOMe in addition to piperazine under dry conditions in order to give the desired amides **n4** and **n5** in sufficient to good yields (39–74%). The reactions with *N*-methylpiperazine for preparation of **n5** afforded lower yields than the synthesis of **n4**, and generally required substantially longer reaction times (63–115 h for **n5** compared to 24–68 h for **n4**). Several attempts at preparing **n5d** through this route failed, and **5d** was eventually managed prepared through a reductive amination of formaldehyde in acetic acid from **n4d**.[25,26] The amides **n6a-d** were obtained through addition of a large excess of EDA (typically 15 equiv) in MeOH and heating from room temperature to reflux.[24] The neutral (**n**) *C*-carbamoyl-1,2,3-triazole amines (**n4**, **n5**, and **n6**) were then turned into their corresponding HCl-salts **4**, **5**, and **6** using aqueous HCl in MeCN. The guanidinium salts (**7** and **8**) were prepared by reacting **n4** and **n6** with the electrophilic guanylation reagent 1*H*-pyrazole carboxamidine hydrochloride in refluxing MeCN.[27,28] All structures of **n4** and **n6** underwent guanylation into **7a**, **7d**, and **8a-d** in moderate to good yields (39–76%) except for conversion of **n4b** and **n4c**. Concerning **n4b** solubility issues probably inhibited the conversion. Thus, **7b** was prepared using DMF at room temperature instead of MeCN at reflux.[27] This gave **7b** in 64% yield after 93 h at room temperature. The underlying cause for the unsuccessful preparation of **7c** was not further investigated.

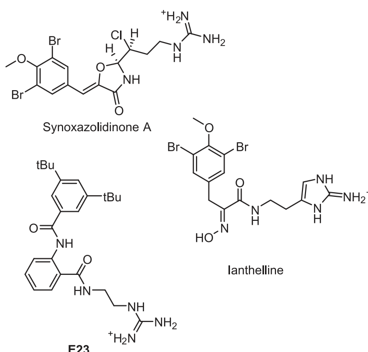

Fig. 1. Synoxazolidone A[14] (methicillin-resistant *S. aureus* MIC: 10 μg/mL), ianthelline[15] (methicillin-resistant *S. aureus* MIC: 20 μg/mL), and **E23**: example of aminobenzamide peptidomimetic[13] based on marine natural products (methicillin-resistant *S. aureus* MIC: 4 μg/mL).

2.2. Antimicrobial activity of the initial library

The 19 amphiphiles (**4a-8d**, Fig. 2) were tested for antimicrobial activity against Gram-positive *Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus agalacticae,* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. The minimum inhibitory concentrations (MIC) are shown in Table 1 together with the MIC value for the reference antibiotic gentamicin.

The only amphiphiles from Fig. 2 displaying antimicrobial activity were **4d**, **6d**, **7d**, and **8d** (MIC 16–64 μg/mL), and all contained the bulky 3,5-di-*t*-Bu-benzyl group as the lipophile. No antimicrobial activity was observed for the other amphiphilies in Fig. 2. These observations were in line with results presented by Strøm et al.,[12] in which the most potent compounds contained the same 3,5-di-*t*-Bu-benzyl group. The other lipophiles introduced in this initial library were an attempt to either reduce the amount of lipophilic bulk (4-*t*-Bu-benzyl, **c**) or change the structure of the lipophilic contribution with an aliphatic heptyl chain (**a**) or an adamantyl box-like structure (**b**). The heptyl chain (**a**) was also inspired by the successful use of alkyl chains in antimicrobial peptide mimics by Ghosh et al.,[29] whereas introduction of the adamantyl group (**b**) was done to investigate the effects of increasing the three-dimensional bulk of the lipophile. However, this initial screening showed that the presence of an aromatic ring on the lipophile and a large lipophilic contribution was important for achieving antimicrobial activity. The differences in lipophilicity related to antimicrobial activity was supported by measuring C18-HPLC retention times (Rts), in which **4a-8a**, **4b-8b**, and **4c-8c** all had Rts below 10 min, while the active amphiphiles **4d-8d** had Rts of approx. 30 min (results shown in experimental section).

For the active amphiphiles, highest overall antimicrobial potency was observed for the guanidine derivative **8d** with a guanylated EDA link. Both the guanylated piperazine **7d** and the guanidine **8d** showed higher antimicrobial activity than their corresponding piperazine **4d** and EDA **6d** derivatives, except against *E. coli* where the EDA **6d** was most potent. The *N*-methyl-piperazine derivative **5d** was inactive within the concentration range tested. The results indicated that having a piperazine group (**4d** and **5d**) was less beneficial for antimicrobial activity compared to a cationic EDA group (**6d**), guanylated piperazine (**7d**), or guanylated EDA group (**8d**). However, the low activity of the *N*-methyl-piperazine derivative **5d** could also be attributed to increased steric hindrance around the cationic nitrogen. In conclusion, high-

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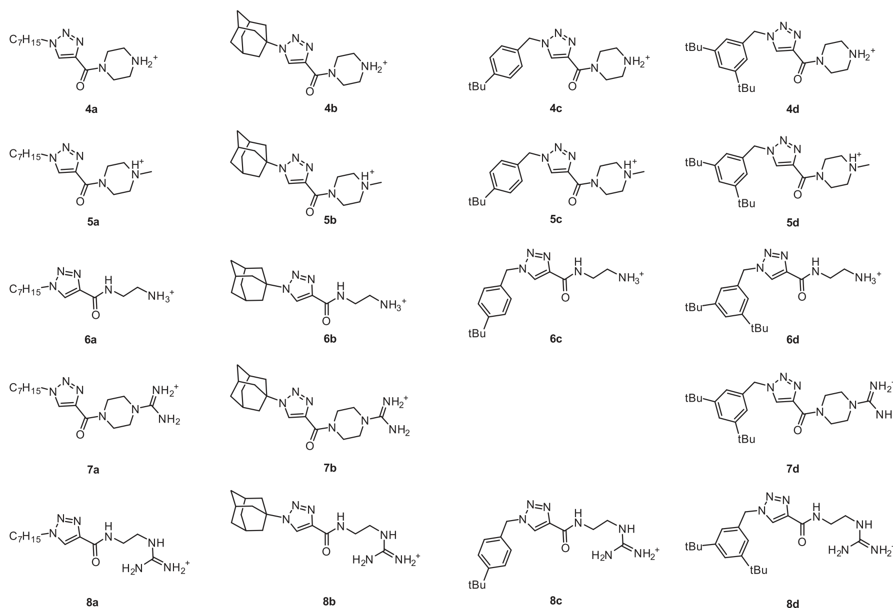

Fig. 2. Compounds **4a-8d** synthesized and evaluated for antimicrobial activity. Amphiphile **7c** was not prepared, as discussed in the synthesis section. Counter ion: Cl^-.

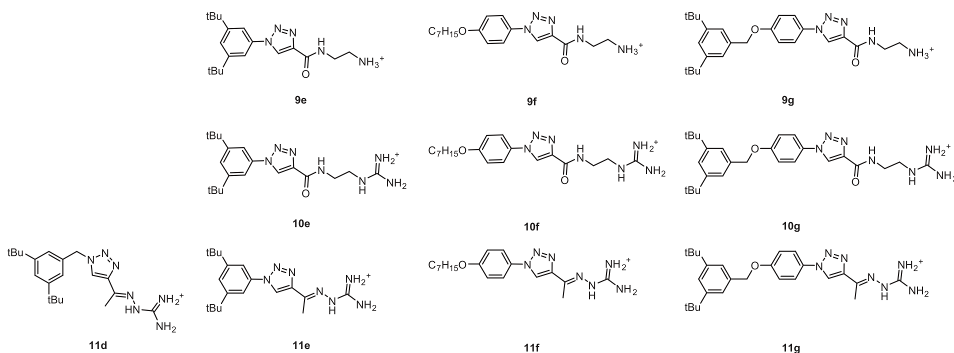

Fig. 3. Improved structures **9e-11g** based on **8d**, for antimicrobial evaluation. Counter ion: Cl^- (CF_3COO^- for **11e** and **11f**, from preparative HPLC).

est antimicrobial activity was observed for the amphiphile **8d** prepared with the 3,5-*t*-Bu-benzyl group (**d**), the ethylene diamine chain (EDA) and a cationic guanidine hydrochloride group.

*2.3. Design and synthesis of a focused library based on **8d***

The cationic amphiphile **8d** was the most potent structure of all the amphiphiles shown in Fig. 2 and active against all five bacteria tested (Table 1). However, the MIC-values for **8d** were somewhat disappointing as the level of activity was not close to that of the reference antibiotic gentamicin. Thus, a more focused library based on **8d** was prepared. Several changes to the structure of **8d** were included in the synthesis of the optimized structures **9e-11g** shown in Fig. 3. In all amphiphiles except **11d**, the benzylic methylene group on the lipophile was removed to give a more rigid system between the phenyl group and the 1,2,3-triazole ring. Repulsion between the *ortho*-protons of the phenyl group and the 1,2,3-triazole ring was thought to reduce rotational freedom and give the resulting molecules a "twisted" conformation. This kind of *rigidification* was also inspired by the aminobenzamides

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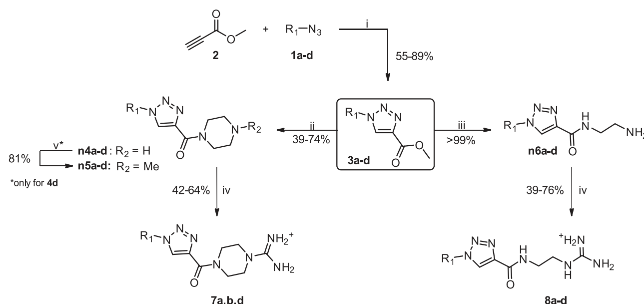

Scheme 1. i) **1a-d** (1–1.05 equiv), $CuSO_4 \times 5H_2O$ (5% mol), sodium ascorbate (10% mol), benzoic acid (10% mol), *t*-BuOH:H_2O (1:2), rt, 10–18 h. ii) Piperazine (R_2 = H) or 1-methyl piperazine (R_2 = Me) (3 equiv), NaOMe (1 equiv), MS 4 Å, MeOH, rt, 24–115 h. iii) EDA (15 equiv), MeOH, rt – reflux, 18 h. iv) 1*H*-Pyrazole carboxamidine hydrochloride (0.9–1.0 equiv), MeCN, reflux, 3–18 h. v) for **4d**: HCHO (approx. 20 equiv), HCOOH (approx. 20 equiv), MeCN, reflux, 1.5 h. (R_1; **a** = heptyl, **b** = adamantyl, **c** = 4-*t*-Bu-benzyl, and **d** = 3,5-*t*-Bu-benzyl). Counter ions for charged species: Cl^-. Free amine versions of the HCl-salts were given the prefix "**n**" for neutral, in order to distinguish them from their ionic versions. Amphiphile **7c** was not successfully prepared using these reaction conditions.

Table 1
Antimicrobial activity (MIC in µg/mL) for the 1,2,3-triazoles in Fig. 2 that were antimicrobially active at ≤64 µg/mL.

Entry	*E. faecalis*[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]
4d	–[b]	64	64	64	–
6d	–	64	64	32	64
7d	–	64	32	–	64
8d	64	32	16	64	32
Ref.[c]	10	0.13	4	0.5	0.5

[a] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalactiae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[b] The "–"-sign in the table indicates no antimicrobial activity at or below 64 µg/mL.
[c] Ref. Gentamicin.

prepared by Strøm et al. (**E23**, Fig. 1).[13] The first resulting amphiphiles (**9e-11e**) all contained a 3,5-di-*t*-Bu-phenyl group instead of the 3,5-di-*t*-Bu-benzyl group in **8d**.

The second improvement, was to introduce a linear ether chain instead of the 3,5-di-*t*-Bu-groups on the aromatic ring (**9f-11f**). The initial screening indicated that having a heptyl chain alone (**4a-8a**, Fig. 2) was not sufficient for achieving antimicrobial activity. However, an alkyl ether chain combined with a phenyl group might be beneficial for antimicrobial efficacy. Conclusively, a heptyl ether chain was introduced in the 4-position on the benzene ring giving **9f-11f**. This was also based on the heptyl ether group providing comparable aliphatic contribution as two *tert*-Butyl groups and also differing marginally in molecular weight.

The last change on the lipophilic side of **8d** was to introduce an additional aromatic ring to increase the lipophilicity of the amphiphiles (**9g-11g**). The $\beta^{2,2}$-amino acid derivatives prepared by Strøm et al.[11,12] all contained two aromatic lipophilic groups in order to mimic the functionality of two lipophilic amino acids (e.g. tryptophan). Thus, a 3,5-di-*t*-Bu-benzyl ether group was introduced in the 4-position of the benzene ring (**9g-11g**), analogously to the placement of the heptyl ether chain in **9f-11f**.

At the cationic end an iminoguanidine group (**11d-g**) was introduced in addition to compounds with a primary EDA amine and guanidine functionality. The iminoguanidine functional group is reported to improve antimicrobial effects against resistant strains of Gram-positive bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus*.[30] The iminoguanidine group was also introduced in **8d** and thereby resulting in the analogue **11d**.

The amphiphiles **9e-g** and **10e-g** were obtained in two or three steps (21–76%) from azides **1e-g** and methyl propiolate (**2**) similarly to the synthesis of **6a-d** and **8a-d** as shown in Scheme 1. The azides **1e-g** were prepared utilizing a copper catalyzed procedure by Zhu et al.[31] Compound **1e** was prepared directly from commercially available 3,5-di-*t*-Bu-bromobenzene, whereas **1f-g** were prepared with one extra step from iodophenol. Furthermore, the methyl ketone analogues of the methyl-1,2,3-triazole carboxylates **13d-g** were prepared from 3-butynone (**12**) and the azides **1d-g**. Compound **13d** was prepared using the "click" chemistry conditions shown in Scheme 1, while **13e-g** were obtained through a modified procedure[32] as shown in Scheme 2. DCM was added in addition to *t*-BuOH and water to reduce the polarity of the solvent, which seemed to enhance the conversion. Also, the amount of added 3-butynone (**12**) was increased since it seemed to be unstable over time under the current reaction conditions.

The amphiphilic iminoguanidines **11d-g** were attempted prepared from **13d-g** and aminoguanidine hydrochloride according to a LiCl-catalyzed method presented by Seleem et al.[30] However, these conditions gave rather slow conversion of **13** to **11**, and not even elevation of the temperature to 90 °C in a pressure tube for 51 h gave full conversion to **11d-g**. In an attempt to furnish full conversion, catalytic amounts of LiCl was replaced with an excess of aqueous HCl[33] (6 equiv). This improved the reaction considerably and gave full conversion of **13d** to **11d** within 22 h, with minimal formation of byproducts. However, when attempting the same conditions for synthesis of **11e-g**, multiple additional signals appeared in the ^{1}H NMR spectra after workup, indicating formation of various unidentified byproducts. Iminoguanidines **11e-g** were instead prepared through the LiCl-catalyzed procedure, which also provided easier purification using HPLC or crystallization. The final purification of **11e** and **11f** was achieved with preparative C18-HPLC while **11g** was purified through crystallization (giving a poor

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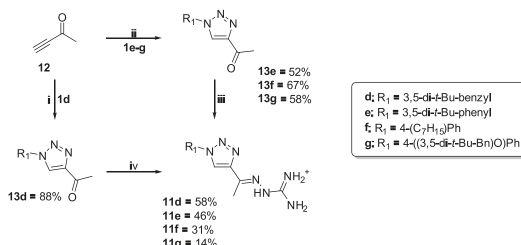

Scheme 2. Synthesis of improved structures **11d-g** based on **12**: i) **1d** (1 equiv), $CuSO_4 \times 5H_2O$ (5 mol %), Na-ascorbate (10 mol %), benzoic acid (10 mol %), t-BuOH/H_2O (1:2), rt, 18 h. ii) **12** (2–3 equiv), **1e-g** (1 equiv), $CuSO_4 \times 5H_2O$ (5 mol %), Na-ascorbate (10 mol %), benzoic acid (10 mol %), t-BuOH/H_2O/DCM (1:1:1), rt, 44–70 h. iii) Aminoguanidine hydrochloride (1.2–1.3 equiv), LiCl (0.3–0.7 equiv), EtOH, reflux–90 °C (pressure tube), 25–54 h. iv) Aminoguanidine hydrochloride (1.2 equiv), HCl (37% aq, 6 equiv), EtOH, 90 °C (pressure tube), 22 h.

isolated yield). ^{1}H NMR spectra of **11d-g** and HPLC analysis displayed a mixture of two compounds, both of which were confirmed to have molecular weight corresponding to the wanted products after MS analysis of analytical HPLC elute. Thus, the final products of **11d-g** were assumed to consist of a mixture of *E*- and *Z*-isomers of the imine. No separation was attempted and the antimicrobial evaluation was performed on the mixtures of **11d-g** (1:9–4:6 isomeric ratios as determined from ^{1}H NMR spectra of **11d-g**).

2.4. Antimicrobial activity and cytotoxicity of the improved structures *9e-11g*

All of the 10 amphiphiles in Fig. 3 (**9e-11g**) were tested for antimicrobial activity against the same panel of bacteria as the 19 initial compounds (Fig. 2), in addition to investigation of inhibition of *Staphylococcus epidermis* biofilm formation. The amphiphiles were also tested for cytotoxic properties against human hepatic cells in the HepG2-assay in order to investigate the selectivity of the structures for bacteria. All the data from these assays are displayed in Table 2.

2.5. Evaluation of antimicrobial activities of *9e-11g*

Removal of the benzylic methylene group on the lipophile led immediately to an increase in antimicrobial activity, and the 3,5-di-*t*-Bu-phenyl derivatives **9e** and **10e** were 2- to 4-fold more potent against the five test bacteria than their previous 3,5-di-*t*-Bu-benzyl counterparts **6d** and **8d**. Linking the 3,5-di-*t*-Bu-phenyl group directly to the 1,2,3-triazole in **9e-11e** and restricting rotational freedom of the lipophile was thereby shown beneficial for antimicrobial activity. Improved antimicrobial activity by removing the benzylic methylene group was also seen for the iminoguanidines, in which **11e** with a 3,5-di-*t*-Bu-phenyl was overall more potent than the more flexible **11d** with a 3,5-di-*t*-Bu-benzyl group.

Introducing a heptyl ether chain together with the benzene ring in **9f-11f** also improved antimicrobial activity compared to the previous inactive compounds **4a-8a** with only a heptyl chain (Fig. 2). This series of compounds showed that having an aromatic phenyl group as part of the lipophile together with the heptyl ether chain was clearly of importance for achieving high antimicrobial activity. Antimicrobial activity of the amine **9f** and guanidine **10f** with a 4-heptyloxy-phenyl lipophile was also improved against certain test bacteria compared to the corresponding amine **9e** and guanidine **10e** with the 3,5-di-*t*-Bu-phenyl lipophile. The improvement in antimicrobial potency by changing lipophile was most obvious for **10f** (4-heptyloxy-phenyl) compared to **10e** (3,5-di-*t*-Bu-phenyl), where a twofold increase in antimicrobial activity was observed for **10f** against three strains of bacteria (*S. aureus*, *E. coli*, and *P. aeruginosa*). The improved activity of **10f** against the Gram-negative bacteria was particularly fascinating since

Table 2
Antimicrobial activity (MIC in μg/mL), actitivity against HepG2 cells (EC_{50} in μg/mL), inhibition of *S. epidermis* biofilms (MIC in μg/mL), and the selectivity index (SI) for amphiphiles **9e-11g**.

Entry	*E. faecalis*[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	HepG2 (EC_{50})[b]	SI[c]	*S. epidermis*[d]
9e	32	16	16	16	32	8.0	0.50	–
9f	–[e]	4	8	–	16	3.5	0.44	4
9g	8	–	2	–	16	2.9	1.44	8
10e	16	8	8	16	16	31.3	3.91	4
10f	16	4	8	8	8	23.8	2.97	4
10g	16	8	4	–	–	16.2	4.04	2
11d	8	4	4	8	8	2.3	0.57	4
11e	4	4	2	4	16	2.6	1.32	4
11f	32	–	8	16	64	2.0	0.25	8
11g	64	–	0.5	–	–	1.9	3.86	4
Ref.[f]	10	0.13	4	0.5	0.5	N.d.[g]	N.d.	N.d.

[a] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalacticae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[b] EC_{50}-value shown, not MIC.
[c] SI; selectivity index (EC_{50} HepG2 / MIC S. agalacticae).
[d] *S. epidermis* biofilm inhibition.
[e] The "–"-sign in the table indicates no activity at or below 64 μg/mL.
[f] Ref. gentamicin.
[g] N.d. not determined.

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Gram-negative bacteria are considered as more difficult targets.[34] When comparing the amines **9e** and **9f**, introduction of the 4-heptyloxy-phenyl-group in **9f** gave a two- to fourfold increase in antimicrobial activity (4–16 μg/mL) against *S. aureus*, *S. agalactiae*, and *P. aeruginosa* compared to the 3,5-di-*t*-Bu-phenyl in **9e**. However, we also observed reduced antimicrobial activity for **9f** (4-heptyloxy-phenyl) against *E. faecalis* and *E. coli* (>64 μg/mL) compared to **9e** (3,5-di-*t*-Bu-phenyl), showing strain variation against the present amphiphiles. For the iminoguanidines a different tendency was observed, in which **11e** (3,5-di-*t*-Bu-phenyl) was overall more potent than **11f** (4-heptyloxy-phenyl) against all test bacteria.

Introduction of an additional phenyl group in addition to the 3,5-di-*t*-Bu benzyl group in **9g-11g** improved the antimicrobial activity further, and especially against *S. agalactiae*. The most potent amphiphile (**11g**) displayed a MIC-value of 0.5 μg/mL, which was eight times lower than the MIC-value of gentamicin against *S. agalactiae*. The profound selectivity and high antimicrobial potency of **11g** against *S. agalactiae* could be of interest for developing antibiotics for prevention of neo-natal infections, since *S. agalactiae* is one of the leading causes of infections in newborns.[35] It should also be noted that **10g** displayed high potency against the Gram-positive bacteria (4–16 μg/mL), but no activity against the Gram-negative strains. Compound **9g** was highly potent against *S. agalactiae* (MIC 2 μg/mL), but displayed otherwise only antimicrobial activity against *E. faecalis* and *P. aeruginosa* (MIC 8–16 μg/mL).

The iminoguanidine group was the most efficient cationic group in the library, and resulting in **11d** and **11e** being the most potent amphiphiles with broad-spectrum activity and MIC-values ≤10 μg/mL against all five bacteria (only exception: **11e** MIC 16 μg/mL against *P. aeruginosa*). The high potency of the iminoguanidine compounds was particularly pronounced for **11d**, which had a 3,5-di-*t*-Bu-benzyl lipophile as in **8d** from the first series of compounds (Fig. 2). The MIC-values of the iminoguanidine **11d** were however 4- to 8-fold improved compared to the guanidine **8d**. We also observed a 2- to 4-fold improvement in antimicrobial activity for iminoguanidine **11e** compared to the corresponding guanidine **10e**, except for against *P. aeruginosa* where they both had MIC-values of 16 μg/mL.

The amphiphiles **10e** and **10f** with a cationic guanidine group were in general more potent than the similar amine derivatives **9e** and **9f**. However, an exception to the superiority of the guanidines was observed for the amine **9g** that was more potent than the guanidine **10g** against the three strains *E. faecalis*, *S. agalactiae*, and *P. aeruginosa*. Against *S. agalactiae* the amine **9g** was the second most potent compound prepared, displaying a MIC-value of 2 μg/mL.

Following the increased antimicrobial activity, the *in vitro* toxicity of the compounds also increased. The *in vitro* toxicity was determined against HepG2 cells and dose–response curves are shown in Fig. 4. The EC_{50}-values determined from the generated dose–response curves are shown in Table 2. The selectivity index (SI) in Table 2 was furthermore calculated from the EC_{50}-values against HepG2 cells divided by the MIC-value against *S. agalactiae*, and showed that the structures displayed rather poor selectivity with exception of **10e-g** and **11g**. The guanidines **10e-g** were least toxic and the only amphiphiles displaying EC_{50}-values against HepG2 above 16 μg/mL. The highest SI achieved for the 10 amphiphiles in Table 2 was 4.04 for **10g**, meaning that the MIC-value against *S. agalactiae* was 4 times lower than the EC_{50}-value against HepG2. The cytotoxicity was particularly pronounced for the iminoguanidines **11d-g**, where all amphiphiles displayed an EC_{50}-value ≤2.6 μg/mL. The high toxicity may be attributed to the relatively high overall lipophilicity of the iminoguanidines **11d-g**, as

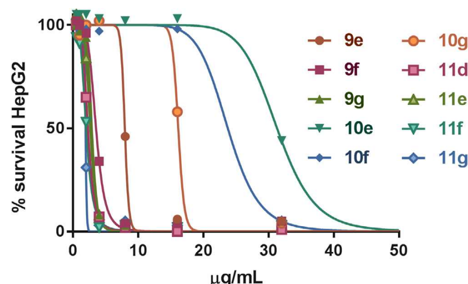

Fig. 4. Anti-proliferative activities of **9e-11g** against human hepatic cells (HepG2) after 24 h of incubation. Graphs were plotted using a four-variable slope normalized nonlinear regression according to least squares fit (using the GraphPad Prism 7.02 software).[36]

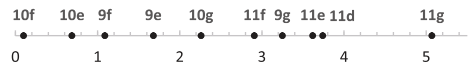

Fig. 5. Calculated ClogD at pH = 7.4 for amphiphiles **9e-11g**. Calculated using MarvinSketch 16.11.7 from ChemAxon.[36]

their calculated ClogD-values (pH = 7.40)[36] shown in Fig. 5 were generally higher than for the corresponding amines (**9e-g**) and guanidines (**10e-g**).

Greene et al. have reported that compounds with a ClogP exceeding 3 are more likely to be active against human cells in <10 μM concentrations.[37] Thus, the toxicity observed for **11d-g** may be due to nonspecific toxic interactions arising from a too large lipophilic bulk. However, this does not completely explain why **11f** (ClogD = 2.91) was among the most toxic compound of the series (EC_{50} = 2.0 μg/mL). It was also observed that the guanidines **10e-g** were notably less toxic than their amine **9e-g** and iminoguanidine **11d-g** counterparts. The guanidine group thereby remains the main cationic group of choice for future target compounds. The lowered toxicity of the guanidines **10e-g** compared to the iminoguanidines **11d-g** also corresponded well with the calculated ClogD-values shown in Fig. 5.

2.6. Biofilm inhibiting activities of 9e–11g

The 10 amphiphiles in Fig. 3 were also investigated for biofilm inhibiting effects against *S. epidermis*, and the obtained MIC-values are shown in Table 2. All of the amphiphiles displayed good biofilm inhibiting effects (2–8 μg/mL), with the exception of **9e** (MIC > 64 μg/mL). The compound displaying the highest activity for inhibition of biofilms was **10g** with a MIC-value of 2 μg/mL. This was remarkable considering that **10g** was among the least toxic amphiphiles tested with an EC_{50}-value of 16.2 μg/mL against HepG2 cells. If the biofilm inhibition were due to general toxicity, one would expect the most toxic structures to display highest activity towards biofilm inhibition. Thus, the biofilm inhibition may arise from more specific inhibition mechanisms. However, as the amphiphiles have not been tested in an antimicrobial assay against *S. epidermis*, the observed values from biofilm inhibition assays may be caused by general antimicrobial properties and not specific biofilm-targeting mechanisms.

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3. Conclusion

This study describes the synthesis of a library of 29 novel low molecular weight amphiphilic 1,2,3-triazoles. The library was prepared using the "click" chemistry products **3** and **13** as key intermediates, followed by functionalization leading to various cationic nitrogen hydrophiles, i. e. primary amines, tertiary amines, guanidines, and iminoguanidines. The 1,2,3-triazole amphiphiles were then assessed for antimicrobial activities against three Gram-positive and two Gram-negative bacteria, in addition to their ability to inhibit *S. epidermis* biofilm formation. The *in vitro* toxicities against human hepatic cells (HepG2) were also measured for the ten most active structures. The amphiphiles **10e** and **10f** displayed the most promising broad-spectrum antimicrobial activities, with MIC-values <16 μg/mL against all five test bacteria. It should also be noted that the guanidine amphiphile **10g** was shown to display selective activity against the Gram-positive bacteria and with MIC-values of 4–16 μg/mL. Furthermore, the amphiphiles with the iminoguanidine cationic group (**11d-g**) displayed increased potency compared to the corresponding guanidines (**10e-g**) in the antimicrobial assays, but this also led to enhanced toxicity in the HepG2-assay. The iminoguanidines **11d-g** therefore gave lower bacterial selectivity (except for **11g** against *S. agalalcticae*) compared to the guanidine amphiphiles **10e-g**. The guanidine **10f** was 2.5 times more potent against *S. aureus* than synoxazolidinone A whereas **10e** was comparable to the marine natural product (8 μg/mL vs. 10 μg/mL). Furthermore, it was shown that structures functionalized with an additional phenyl ring displayed more selective activity, particularly against *S. agalacticae*. The overall most potent structure **11g** against *S. agalacticae* – displayed a MIC-value of 0.5 μg/mL, which was 8 times lower than the reference antibiotic gentamicin. The presented structures also displayed promising activity towards biofilm inhibition, where **10g** was the most potent compound against *S. epidermis* biofilm formation with a MIC-value of 2 μg/mL. Based on broad-spectrum activity against all five test strains and good antibiofilm activity, **10f** was one of the most promising compounds prepared and with second lowest toxicity against HepG2 cells. Further studies on this type of amphiphilic 1,2,3-triazoles will revolve around further reducing HepG2 toxicity, while retaining a high antimicrobial activity.

4. Experimental

4.1. General information

Chemicals were purchased from Sigma Aldrich and used without further purification. All reactions sensitive to air or moisture were performed under nitrogen atmosphere with dried solvents and reagents. Melting points were determined on a Buchi 535 apparatus and are uncorrected. TLC was performed on Merck silica gel 60 F254 plates, using UV light at 312 nm and a 5% solution of molybdophosphoric acid in 96% EtOH for detection. Column chromatography was performed with Silica gel (pore size 60 A, 230–400 mesh particle size) from Fluka. HPLC analyses were performed on an Agilent 1290 chromatograph equipped with a Zorbax Eclipse C18 5 μm (150 × 4.6 mm) column and a diode array detector (main detection region 214 nm). Preparative HPLC purifications were performed on an Agilent 1260 Infinity equipped with a Zorbax XDB-C18 5 μm (150 × 21.2 mm) and a diode array detector (main detection region 214 nm). NMR spectra were recorded on a Bruker 600 MHz Avance III HD or a Bruker 400 MHz Avance III HD instrument. Chemical shifts (δ) are reported in parts per million. Where $CDCl_3$ has been used, shift values for proton are reported with reference to TMS (0.00) via the lock signal of the solvent. Reference values for other NMR-solvents are taken from Fulmer et al.[38] (^{1}H NMR: DMSO-d_6: 2.49, MeOD-*d4*: 3.31; ^{13}C NMR: DMSO-d_6: 39.5, $CDCl_3$: 77.0, MeOD-d4: 49.15). Signal patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) or bs (broad singlet). ^{1}H and ^{13}C NMR signals were assigned by 2D correlation techniques (COSY, HSQC, HMBC). IR spectra were recorded from a Thermo Nicolet FT-IR NEXUS instrument (only the strongest/structurally most important peaks are listed as either weak (w), medium (m) or strong (s) (cm^{-1})). Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters™. Samples were ionized by the use of ASAP probe (APCI) or ESI probe.

4.2. 1-Azidoheptane (*1a*)

The title compound **1a** was prepared according to a published procedure.[39,40] A mixture of 1-bromoheptane (5.00 g, 27.9 mmol) and NaN_3 (2.72 g, 41.9 mmol) in DMF (50 mL) was heated to 50 °C for 19 h. The suspension was then added DCM (80 mL) and washed with water (3 × 100 mL), before it was dried over $MgSO_4$ and evaporated. Yielding **1a** as a lightly yellow oil (3.06 g, 21.7 mmol, 78%). ^{1}H NMR analyses corresponded with previously reported spectra for **1a**.[41] ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.25 (t, 2H, *J* = 6.9 Hz, azide-CH_2), 1.60 (p, 2H, *J* = 7.4 Hz, CH_2), 1.41–1.25 (m, 8H, 4× CH_2), 0.92–0.86 (m, 3H, CH_3).

4.3. 1-(Azidomethyl)-4-(tert-butyl)benzene (*1c*)

The title compound **1c** was prepared according to the procedure for **1a** from 1-(bromomethyl)-4-(*tert*-butyl)benzene (3.00 g, 13.2 mmol), affording **1c** as a yellow oil (2.08 g, 11.0 mmol, 83%). ^{1}H NMR analyses corresponded with previously reported spectra for **1c**.[42] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.37 (m, 2H, Ph), 7.27–7.22 (m, 2H, Ph), 4.29 (s, 2H, CH_2), 1.32 (s, 9H, *t*-Bu).

4.4. 1-(Azidomethyl)-3,5-di-tert-butylbenzene (*1d*)

The title compound **1d** was prepared according to the procedure for **1a** from 1-(bromomethyl)-3,5-di-(*tert*-butyl)benzene (0.80 g, 2.82 mmol), affording **1d** as a clear oil (0.620 g, 2.53 mmol, 90%). ^{1}H NMR analyses corresponded with previously reported spectra for **1d**.[43] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (s, 1H, H_{Ph}-4), 7.13 (d, *J* = 1.3 Hz, 2H, H_{Ph}-2 and H_{Ph}-6), 4.32 (s, 2H, CH_2), 1.33 (s, 18H, 2× *t*-Bu) ppm.

4.5. 1-Azido-3,5-di-tert-butylbenzene (*1e*)

The title compound **1e** was prepared according to a procedure described by Zhu et al.[31] Where 1-bromo-3,5-di-*tert*-butylbenzene (2.50 g, 9.30 mmol), CuI (0.177 g, 0.93 mmol), NaN_3 (1.21 g, 18.57 mmol), l-proline (0.321 g, 2.74 mmol) and NaOH (0.11 g, 2.79 mmol) were added to EtOH:H_2O (7:3, 20 mL) and heated to 95 °C in a sealed tube for 23 h. The reaction mixture was then added water (30 mL) and extracted with EtOAc (3 × 30 mL). Drying over $MgSO_4$ and evaporation under reduced pressure yielded a yellow oil, which then was purified using flash column chromatography (pentane), affording **1e** as a colorless oil (0.725 g, 3.13 mmol, 34%). ^{1}H NMR spectra coincided with previously reported data.[44] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (t, 1H, *J* = 1.5 Hz, H_{Ph}-4), 6.86 (d, 2H, *J* = 1.6 Hz, H_{Ph}-2 and H_{Ph}-6), 1.31 (s, 18H, 2× *t*-Bu).

4.6. 1-Azido-4-(heptyloxy)benzene (*1f*)

The iodo-precursor (1-(heptyloxy)-4-iodobenzene) to **1f** was prepared using 4-iodophenol (2.00 g, 9.09 mmol), heptyl bromide (1.57 mL, 10.00 mmol), and K_2CO_3 (1.62 g, 11.7 mmol) in DMF (12 mL) at rt, as reported by Ban et al.[45] in 69% yield (1.99 g,

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6.24 mmol). The spectra coincided with previously reported data.[46] This aromatic iodide was turned into its corresponding azide (**1f**) using a procedure described by Zhu et al.[31] Where 1-(Heptyloxy)-4-iodobenzene (1.50 g, 4.71 mmol), CuI (0.09 g, 0.47 mmol), NaN_3 (0.37 g, 5.66 mmol), l-proline (0.11 g, 0.94 mmol) and NaOH (0.04 g, 0.94 mmol) were added to DMSO (10 mL mL) and heated to 60 °C in a sealed tube for 14 h. After which the mixture was added water (35 mL), extracted with EtOAc (3 × 40 mL), and dried over $MgSO_4$. Evaporation under reduced pressure yielded a brown oil, which then was purified using flash column chromatography (pentane), affording **1f** as a yellow oil (0.856 g, 3.67 mmol, 78%). ^1H NMR (400 MHz, $CDCl_3$): δ 6.96–6.91 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 6.90–6.85 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 3.92 (t, 2H, *J* = 6.8 Hz, O—CH_2), 1.77 (p, 2H, *J* = 7.3 Hz, O—CH_2-CH_2), 1.49–1.23 (m, 8H, 4× CH_2), 0.92–0.85 (m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6 (C_{Ph}-4), 132.1 (C_{Ph}-1), 120.0 (C_{Ph}-3 and C_{Ph}-5), 115.7 (C_{Ph}-2 and C_{Ph}-6), 68.4 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 26.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3). IR: 2927 (w), 2857 (w), 2105 (s), 1503 (s), 1470 (w), 1280 (m), 1239 (s), 822 (s) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 233.1531 (Calcd. $C_{13}H_{19}N_3O$, 233.1528, $[M]^-$).

4.7. 1-((4-Azidophenoxy)methyl)-3,5-di-tert-butylbenzene (**1g**)

The iodo-precursor (1,3-di-*tert*-butyl-5-((4-iodophenoxy) methyl)benzene) to **1g** was prepared according to the procedure described for the iodo-precursor of **1f**, using 4-iodophenol (1.00 g, 4.55 mmol), 1-(bromomethyl)-3,5-di-*t*-Bu-benzene (1.17 g, 4.13 mmol), and K_2CO_3 (0.74 g, 5.37 mmol).[45] This afforded 1,3-di-*tert*-butyl-5-((4-iodophenoxy)methyl)benzene as a white solid (1.55 g, 3.67 mmol, 89%, mp 147.1–148.2 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 7.60–7.53 (m, 2H, H_{Phenox}-2 and -6), 7.40 (t, 1H, *J* = 1.8 Hz, H_{Ph}-4), 7.23–7.28 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 6.82–6.74 (m, 2H, H_{Phenox}-3 and -5), 4.98 (s, 2H, CH_2), 1.33 (s, 18H, 2× *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9 (C_{Phenox}-1), 151.2 (C_{Ph}-3 and C_{Ph}-5), 138.2 (C_{Phenox}-2 and -6), 135.4 (C_{Ph}-1), 122.4 (C_{Ph}-4), 122.1 (C_{Ph}-2 and C_{Ph}-6), 117.3 (C_{Phenox}-3 and -5), 82.9 (C_{Phenox}-1), 71.0 (C_{Bn}), 34.9 (C_q-*t*Bu), 31.5 (*t*Bu). IR: 2958 (w), 1585 (w), 1485 (m), 1232 (s), 1006 (m), 895 (m), 831 (m), 803 (w), 714 (w), 681 (w) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 421.1022 (Calcd. $C_{21}H_{26}OI$, 421.1028, $[M-H]^-$). 1,3-Di-*tert*-butyl-5-((4-iodophenoxy)methyl) benzene (0.40 g, 0.947 mmol) was turned into its corresponding azide **1g** using the procedure decribed for preparation of **1f** with CuI (18 mg, 0.095 mmol), NaN_3 (0.074 g, 1.137 mmol), L-proline (22 mg, 0.189 mmol), and NaOH (7.6 mg, 0.189 mmol). This afforded **1g** as a yellow solid (0.210 g, 0.62 mmol, 66%, mp 119.0–120.2 °C) after purification with flash column chromatography (pentane). ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 (t, 1H, *J* = 1.4 Hz, H_{Ph}-4), 7.26 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 7.02–6.93 (m, 4H, H_{Phenox}), 5.00 (s, 2H, CH_2), 1.33 (s, 18H, 2× *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.5 (C_{phenox}-1), 151.1 (C_{Ph}-3 and C_{Ph}-5), 132.5 (C_{phenox}-4), 122.3 (C_{Ph}-4), 122.1 (C_{Ph}-2 and C_{Ph}-6), 120.0 (C_{phenox}-3 and -5), 116.2 (C_{phenox}-2 and -6), 71.3 (CH_2), 34.5 (C_q-*t*Bu), 31.5 (*t*Bu). IR: 2961 (w), 2112 (s), 2079 (w), 1504 (s), 1307 (s), 1011 (w) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 308.2015 (Calcd. $C_{21}H_{26}NO$, 308.2014, $[M-N_2-H]^-$). pH = 3,5-di-*tert*-butylbenzyl.

4.7.1. Method A, "Click" reactions with methyl propiolate: Synthesis of methyl 1-heptyl-1H-1,2,3-triazole-4-carboxylate (**3a**)

The title compound **3a** was prepared according to a general procedure described by Shao et al.[22] Where a suspension of methyl propiolate (**2**) (0.57 g, 6.74 mmol), $CuSO_4·5H_2O$ (0.34 mL, 1 M in H_2O, 5 mol %), sodium ascorbate (0.34 mL, 2 M in H_2O, 10 mol %) and benzoic acid (82 mg, 10 mol %) in H_2O/*t*-BuOH (9 mL, 2:1) was added **1a** (1.00 g, 7.08 mmol) and stirred for 23 h at room temperature. The suspension was then added H_2O (20 mL), filtered and the precipitate washed with H_2O. Before being dissolved in DCM (30 mL), dried over $MgSO_4$ and partially evaporated. Crystallization with pentane afforded **3a** as a lightly yellow solid (1.35 g, 5.59 mmol, 89%, mp 80.2–81.6 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H, $H_{triazole}$-5), 4.41 (t, 2H, *J* = 7.2 Hz, triazole-CH_2), 3.96 (s, 3H, OMe), 1.93 (p, 2H, *J* = 7.0 Hz, triazole-CH_2-CH_2), 1.39–1.21 (m, 8H, 4× CH_2), 0.88 (t, 3H, *J* = 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.3 ($C_{C=O}$, from HMBC), 140.0 ($C_{triazole}$-4, from HMBC), 127.2 ($C_{triazole}$-5), 52.2 (OMe), 50.7 (triazole-CH_2), 31.5 (CH_2), 30.1 (triazole-CH_2-CH_2), 28.6 (CH_2), 26.3 (CH_2), 22.5 (CH_2), 14.0 (CH_3). IR: 3123 (w), 2953 (w), 2915 (w), 2850 (w), 1728 (s), 1542 (m), 1239 (s), 1048 (m), 1019 (m), 777 (m) cm^{-1}. 226.1553 (Calcd. $C_{11}H_{20}N_3O_2$, 226.1556, $[M+H]^+$).

4.7.2. Methyl 1-(adamantan-1-yl)-1H-1,2,3-triazole-4-carboxylate (**3b**)

The title compound **3b** was prepared according to Method A from **2** (0.14 mL, 1.61 mmol) and azidoadamantane (0.30 g, 1.69 mmol), affording **3b** as a lightly yellow solid (0.231 g, 0.88 mmol, 55%, mp 110.1–111.7 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H, $H_{triazole}$-5), 3.95 (s, 3H, OMe), 2.33–2.22 (m, 9H, H_{Ada}-CH/CH_2), 1.86–1.75 (m, 6H, 3× H_{Ada}-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.6 ($C_{C=O}$), 139.1 ($C_{triazole}$-4, from HMBC), 124.2 ($C_{triazole}$-5), 60.5 (C_q-Ada), 52.1 (OMe), 42.9, 35.8, 29.4. IR: 2928 (w), 2894 (w), 1731 (s), 1366 (m), 1205 (s), 1037 (s), 781 (s) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 262.1553 (Calcd. $C_{14}H_{20}N_3O_2$, 262.1556, $[M+H]^+$).

4.7.3. Methyl 1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carboxylate (**3c**)

The title compound **3c** was prepared according to Method A from **2** (0.571 g, 6.79 mmol) and **1c** (1.50 g, 7.13 mmol), affording **3c** as a light blue solid (1.23 g, 4.50 mmol, 66%). ^1H NMR analyses corresponded with previously reported spectra for **3c**.[47] ^1H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H, $H_{triazole}$-5), 7.42 (d, 2H, *J* = 8.4 Hz, H_{Ph}), 7.23 (d, 2H, *J* = 7.8 Hz, H_{Ph}), 5.54 (s, 2H, CH_2), 3.93 (s, 3H, OMe), 1.32 (s, 9H, *t*-Bu).

4.7.4. Methyl 1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carboxylate (**3d**)

The title compound **3d** was prepared according to Method A from **2** (0.294 g, 3.49 mmol) and **1d** (1.00 g, 3.67 mmol), affording **3d** as a white solid (0.818 g, 2.48 mmol, 71%, mp 172.8–174.4 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H, $H_{triazole}$-5), 7.44 (t, 1H, *J* = 1.8 Hz, H_{Ph}-4), 7.12 (d, 2H, *J* = 1.8 Hz, H_{Ph}-2 and H_{Ph}-6), 5.55 (s, 2H, CH_2), 3.94 (s, 3H, OMe), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.2 ($C_{C=O}$), 152.1 (C_{Ph}-3 and C_{Ph}-5), 140.2 ($C_{triazole}$-4), 132.7 (C_{Ph}-1), 127.3 ($C_{triazole}$-5), 123.3 (C_{Ph}-4), 122.6 (C_{Ph}-2 and C_{Ph}-6), 55.2 (C_{Bn}), 52.2 (OMe), 34.9 (C_q-*t*-Bu), 31.4 (*t*-Bu). IR: 2957 (w), 1713 (s), 1540 (m), 1234 (s), 1045 (s), 1017 (m), 782 (m) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 330.2179 (Calcd. $C_{19}H_{28}N_3O_2$, 330.2182, $[M+H]^+$).

4.7.5. Methyl 1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazole-4-carboxylate (**3e**)

The title compound **3e** was prepared according to Method A from **2** (0.182 g, 2.16 mmol) and **1e** (0.50 g, 2.16 mmol), with a different workup: after complete conversion (17 h), the suspension was added H_2O (25 mL) and extracted with DCM (3 × 25 mL). The organic phase was then dried over $MgSO_4$ and partially evaporated, before it was crystallized with pentane affording **3e** as white solid (0.580 g, 1.84 mmol, 85%, mp 105.1–107.1 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.51 (s, 1H, $H_{triazole}$-5), 7.55 (t, 1H, *J* = 1.7 Hz, H_{Ph}-4), 7.53 (d, 2H, *J* = 1.7 Hz, H_{Ph}-2 and H_{Ph}-6), 4.01 (s, 3H, OMe), 1.38 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.3 ($C_{C=O}$), 153.2 (C_{Ph}-3 and C_{Ph}-5), 140.3 ($C_{triazole}$-4), 136.1 (C_{Ph}-1), 126.0 ($C_{triazole}$-5), 123.8 (C_{Ph}-4), 115.6 (C_{Ph}-2 and C_{Ph}-6),

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52.4 (OMe), 35.2 (C_q-*t*-Bu), 31.3 (*t*-Bu). IR: 2952 (w), 1746 (s), 1533 (m), 1361 (s), 1211 (s), 1182 (w), 1146 (s), 1035 (s), 879 (m), 770 (s), 709 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 316.2019 (Calcd. $C_{18}H_{26}N_3O_2$, 316.2025, $[M+H]^+$).

*4.7.6. Methyl 1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazole-4-carboxylate (**3f**)*

The title compound **3f** was prepared according to the procedure described for **3e** using **2** (0.119 g, 1.41 mmol) and **1f** (0.30 g, 1.29 mmol), affording **3f** as an off-white solid (0.341 g, 1.07 mmol, 84%, mp 120.1–121.5 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H, $H_{triazole}$-5), 7.66–7.60 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.06–7.00 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.04–3.98 (m, 5H, OMe + O—CH_2), 1.87–1.76 (m, 2H, O—CH_2-**CH_2**), 1.53–1.26 (m, 8H, 4× CH_2), 0.93–0.87 (m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.2 ($C_{C=C}$), 160.0 (C_{Ph}-4), 140.4 ($C_{triazole}$-4), 129.5, 125.6 ($C_{triazole}$-5), 122.4 (C_{Ph}-3 and C_{Ph}-5), 115.5 (C_{Ph}-2 and C_{Ph}-6), 68.6 (O-CH_2), 52.3 (OMe), 31.8 (CH_2), 29.1 (O-CH_2-**CH_2**), 29.0 (CH_2), 25.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3). IR: 2926 (w), 1711 (s), 1540 (w), 1520 (m), 1269 (m), 1252 (s), 1135 (s), 831 (s), 775 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 318.1812 (Calcd. $C_{17}H_{24}N_3O_3$, 318.1818, $[M+H]^+$).

*4.7.7. Methyl 1-(4-((3,5-di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazole-4-carboxylate (**3g**)*

The title compound **3g** was prepared according to the procedure described for **3e** using **2** (53 mg, 0.63 mmol) and **1g** (0.20 g, 0.57 mmol), followed by purification with flash column chromatography (DCM – 10% EtOAc in DCM). Affording **3g** as a white solid (0.227 g, 0.54 mmol, 95%, mp 199.3–201.1 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H, $H_{triazole}$-5), 7.69–7.62 (m, 2H, H_{phenox}-3 and -5), 7.43 (t, 1H, *J* = 1.8 Hz, H_{Ph}-4), 7.29 (d, 2H, *J* = 1.8 Hz, H_{Ph}-2 and H_{Ph}-6), 7.17–7.12 (m, 2H, H_{phenox}-2 and -6), 5.10 (s, 2H, H_{Bn}), 4.00 (s, 3H, OMe), 1.35 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.3 ($C_{C=O}$), 159.9 (C_{phenox}-4), 151.4 (C_{Ph}-3 and C_{Ph}-5), 140.5 ($C_{triazole}$-4), 135.2 (C_{Ph}-1), 129.9 (C_{phenox}-1), 125.7 ($C_{triazole}$-5), 122.7 (C_{Ph}-4), 122.6 (C_{phenox}-3 and -5), 122.2 (C_{Ph}-2 and C_{Ph}-6), 116.0 (C_{phenox}-2 and -6), 71.5 (OMe), 52.5 (C_{Bn}), 35.0 (C_q-*t*-Bu), 31.6 (*t*-Bu). IR: 2959 (w), 1729 (s), 1518 (s), 1237 (s), 1152 (m), 1042 (s), 1006 (s), 881 (w), 847 (m), 778 (w), 695 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 422.2436 (Calcd. $C_{25}H_{32}N_3O_3$, 422.2444, $[M+H]^+$). pH = 3,5-di-*tert*-butylbenzyl.

*4.7.8. 1-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethan-1-one (**13d**)*

The title compound **13d** was prepared according to the procedure described for **3e** using 3-butynone (**12**) (0.139 g, 1.94 mmol) and **1d** (0.50, 1.94 mmol), affording **13d** as an off-white solid (0.531 g, 1.69 mmol, 88%, mp 145.0–146.8 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H, $H_{triazole}$-5), 7.45 (t, 1H, *J* = 1.8 Hz, H_{Ph}-4), 7.13 (d, 2H, *J* = 1.8 Hz, H_{Ph}-2 and H_{Ph}-6), 5.53 (s, 2H, H_{Bn}), 2.68 (s, 3H, CH_3), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.0 ($C_{C=O}$), 152.1 (C_{Ph}-3 and C_{Ph}-5), 148.3 ($C_{triazole}$-4), 132.7 (C_{Ph}-1), 125.2 ($C_{triazole}$-5), 123.3 (C_{Ph}-4), 122.7 (C_{Ph}-2 and C_{Ph}-6), 55.2 (C_{Bn}), 34.9 (C_q-*t*-Bu), 31.4 (*t*-Bu), 27.1 (CH_3). IR: 2953 (w), 1684 (s), 1528 (m), 1360 (m), 1238 (w), 1200 (s), 1045 (m), 756 (s), 676 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 314.2227 (Calcd. $C_{19}H_{28}N_3O$, 314.2232, $[M+H]^+$).

*4.7.9. Method B, "Click" reactions with 3-butynone (12): Synthesis of 1-(1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)ethan-1-one (**13e**)*

The title compound **13e** was prepared according to a published procedure,[32] where a suspension of 3-butynone (**12**) (68 mg, 1.00 mmol), $CuSO_4{\cdot}5H_2O$ (17 μL, 1 M in H_2O, 5 mol %), sodium ascorbate (17 μL mL, 2 M in H_2O, 10 mol %) and benzoic acid (3 mg, 10 mol %) in H_2O/*t*-BuOH/DCM (1.5 mL, 1:1:1) was added **1e** (77 mg, 0.33 mmol) and stirred for 45 h at room temperature (with addition of additional 2 eq of **12** after 6 h). The reaction mixture was then added H_2O (10 mL) and extracted with DCM (3 × 15 mL), before the organic phase was dried over $MgSO_4$ and evaporated. Purification with flash column chromatography (DCM) afforded **13e** as a white solid (52 mg, 0.17 mmol, 52%, mp 140.5–145.3 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H, $H_{triazole}$-5), 7.56–7.54 (m, 1H, H_{Ph}-4), 7.54–7.52 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 2.77 (s, 3H, CH_3), 1.38 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.1 ($C_{C=O}$), 153.2 (C_{Ph}-3 and C_{Ph}-5), 148.4 ($C_{triazole}$-4), 136.1 (C_{Ph}-1), 123.8 (C_{Ph}-4), 123.7 ($C_{triazole}$-5), 115.6 (C_{Ph}-2 and C_{Ph}-6), 35.2 (C_q-*t*-Bu), 31.3 (*t*-Bu), 27.3 (CH_3). IR: 2958 (w), 1683 (s), 1532 (m), 1236 (m), 1028 (w), 990 (w), 878 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 300.2070 (Calcd. $C_{18}H_{26}N_3O$, 300.2070, $[M+H]^+$).

*4.7.10. 1-(1-(4-(Heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)ethan-1-one (**13f**)*

The title compound **13f** was prepared according to Method B from **12** (0.123 g, 1.80 mmol) and **1f** (0.20 g, 0.86 mmol). Affording **3f** as a white solid (0.172 g, 0.57 mmol, 67%, mp 112.7–115.5 °C) after purification with flash column chromatography (DCM). ^1H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, $H_{triazole}$-5), 7.66–7.59 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.06–7.00 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.02 (t, 2H, *J* = 6.9 Hz, O-CH_2), 2.75 (s, 3H, ketone-CH_3), 1.82 (p, 2H, *J* = 7.3 Hz, O-CH_2-**CH_2**), 1.53–1.27 (m, 8H, 4× CH_2), 0.94–0.86 (m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.0 ($C_{C=O}$), 160.0 (C_{Ph}-4), 148.4 ($C_{triazole}$-4), 129.5 (C_{Ph}-1), 123.3 ($C_{triazole}$-5), 122.4 (C_{Ph}-3 and C_{Ph}-5), 115.5 (C_{Ph}-2 and C_{Ph}-6), 68.6 (O-CH_2), 31.8 (CH_2), 29.1 (O-CH_2-**CH_2**), 29.0 (CH_2), 27.3 (ketone-CH_3), 26.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3). IR: 3131 (w), 2923 (w), 1682 (s), 1516 (s), 1253 (s), 1241 (s), 1171 (m), 823 (s), 678 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 302.1863 (Calcd. $C_{17}H_{24}N_3O_2$, 302.1869, $[M+H]^+$).

*4.7.11. 1-(1-(4-((3,5-Di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazol-4-yl)ethan-1-one (**13g**)*

The title compound **13g** was prepared according Method B in two reactions. Firstly with **12** (30 mg, 0.41 mmol) and **1g** (0.15 g, 0.41 mmol) for 24 h then followed by addition of **12** (60 mg, 0.83 mmol) to the extracted crude (0.26 g, **1g/13g** 3:1) followed by stirring for 47 h at room temperature. Purification with flash column chromatography (DCM) afforded **13g** as a white solid (98 mg, 0.24 mmol, 58%, mp 179.4–181.1 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H, $H_{triazole}$-5), 7.69–7.63 (m, 2H, H_{phenox}-3 and -5), 7.44 (t, 1H, *J* = 1.7 Hz, H_{Ph}-4), 7.28 (d, 2H, *J* = 2.0 Hz, H_{Ph}-3 and H_{Ph}-5), 7.18–7.12 (m, 2H, H_{phenox}-2 and -6), 5.10 (s, 2H, H_{Bn}), 2.75 (s, 3H, ketone-CH_3), 1.35 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.9 ($C_{C=O}$), 159.8 (C_{phenox}-4), 151.3 (C_{Ph}-3 and C_{Ph}-5), 148.4 ($C_{triazole}$-4), 135.1 (C_{Ph}-1), 123.3 ($C_{triazole}$-5), 122.5 (C_{Ph}-4), 122.4 (C_{phenox}-3 and C_{phenox} -5), 122.1 (C_{Ph}-2 and C_{Ph}-6), 116.0 (C_{phenox}-2 and C_{phenox} -6), 71.4 (C_{Bn}), 34.9 (C_q-*t*-Bu), 31.5 (*t*-Bu), 27.3 (ketone-CH_3). IR: 2955 (w), 1693 (s), 1517 (s), 1248 (s), 985 (m), 882 (w), 829 (s), 696 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 406.2490 (Calcd. $C_{25}H_{32}N_3O_2$, 406.2495, $[M+H]^+$). pH = 3,5-di-*tert*-butylbenzyl.

*4.7.12. Method C, piperazine amidation reactions: Synthesis of (1-heptyl-1H-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4a) and 4-(1-heptyl-1H-1,2,3-triazole-4-carbonyl)piperazin-1-ium chloride (**4a**)*

The title compound **n4a** was prepared according to a general procedure described by Oshima et al.[23] with some modifications. Where a suspension of **3a** (0.30 g, 1.33 mmol), piperazine (0.344 g, 3.99 mmol), NaOMe (0.07 g, 1.33 mmol), mol. sieves (0.5–1.0 g, activated, 4 Å) and MeOH (6 mL) was stirred under N_2-atmosphere for 43 h. After completed stirring, the reaction mixture was evaporated and dissolved in DCM before it was filtered through Celite. Subsequent purification with flash column chro-

matography (SiO_2 pre-deactivated with 1% TEA in eluent, eluent: $CHCl_3$/MeOH 95:5) afforded **n4a** as a white solid (0.201 g, 0.72 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H, $H_{triazole}$-5), 4.37 (t, 2H, J = 7.1 Hz, triazole-CH_2), 4.30 (t, 2H, J = 4.8 Hz, H_{Pip}-2 and H_{Pip}-6), 3.77 (t, 2H, J = 5.0 Hz, H_{Pip}-2 and H_{Pip}-6), 3.01–2.94 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.92 (t, 2H, J = 7.0 Hz, CH_2), 1.41–1.20 (m, 8H, 4× CH_2), 0.91–0.84 (m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9 ($C_{C=O}$), 144.4 ($C_{triazole}$-4), 128.0 ($C_{triazole}$-5), 50.5 (CH_2), 48.0 (C_{Pip}-2 or C_{Pip}-6), 46.7 (C_{Pip}-3 and C_{Pip}-5), 46.0 (C_{Pip}-3 and C_{Pip}-5), 43.8 (C_{Pip}-2 or C_{Pip}-6), 31.5 (CH_2), 30.1 (CH_2), 28.6 (CH_2), 26.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3). The free amine **n4a** was then turned into its HCl-salt, by mixing **n4a** (40 mg, 0.14 mmol) in MeCN (3 mL) and adding HCl (0.1 mL, 1.22 mmol, 37%, aq.). The suspension was evaporated, washed with MeCN (3 × 1 mL) and dried, affording **4a** as a white solid (27 mg, 0.09 mmol, 60%, mp 228.8–230.7 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.1 min, 99% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.46 (s, 1H, $H_{triazole}$-5), 4.61–4.39 (m, 4H, H_{Pip}-2 and H_{Pip}-6 + CH_2), 4.01 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.40–3.35 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.95 (p, 2H, J = 7.3 Hz, CH_2), 1.44–1.25 (m, 8H, 4× CH_2), 0.92 (t, 3H, J = 6.9 Hz, CH_3). IR: 2931 (w), 2730 (w), 1625 (s), 1594 (w), 1429 (m), 1248 (m), 1049 (m), 988 (m), 759 (m) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 280.2139 (Calcd. $C_{14}H_{26}N_5O$, 280.2137, $[M-Cl]^+$).

*4.7.13. (1-(Adamantan-1-yl)-1H-1,2,3-triazol-4-yl)(piperazin-1-yl) methanone (**n4b**) and 4-(1-(adamantan-1-yl)-1H-1,2,3-triazole-4-carbonyl)piperazin-1-ium chloride (**4b**)*

The title compound **n4b** was prepared according Method C from **3b** (0.23 g, 0.88 mmol) and piperazine (0.227 g, 2.64 mmol), with 68 h reaction time at room temperature. Affording **n4b** as a white solid (0.205 g, 0.65 mmol, 74%) after purification with flash column chromatography ($CHCl_3$/MeOH 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H, $H_{triazole}$-5), 4.31 (s, 2H, H_{Pip}-2 and H_{Pip}-6), 3.76 (s, 2H, H_{Pip}-2 and H_{Pip}-6), 2.98–2.93 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 2.31–2.21 (m, 9H, H_{Ada}-CH/CH_2), 1.86–1.75 (m, 6H, H_{Ada}-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.3 ($C_{C=O}$), 143.5 ($C_{triazole}$-4), 125.0 ($C_{triazole}$-5), 60.1 (C_q-ada), 48.1 (C_{Pip}-2 or C_{Pip}-6), 46.7 (C_{Pip}-3 and C_{Pip}-5), 46.1 (C_{Pip}-3 and C_{Pip}-5), 43.8 (C_{Pip}-2 or C_{Pip}-6), 42.8 (C_{Ada}), 35.8 (C_{Ada}), 29.4 (C_{Ada}). The free amine **n4b** was then turned into its HCl-salt, by adding HCl (5 mL, 10 mmol, 2 M in Et_2O) to a solution of **n4b** (30 mg, 0.09 mmol) followed by filtration. Drying afforded **4b** as a white solid (33 mg, 0.09 mmol, 99%, mp > 280 °C decomp.). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 4.8 min, 99% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.49 (s, 1H, $H_{triazole}$-5), 4.48 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 4.01 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.40–3.33 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 2.33–2.23 (m, 9H, H_{Ada}-CH/CH_2), 1.91–1.79 (m, 6H, H_{Ada}-CH_2). ^{13}C NMR (100 MHz, *d4*-MeOD, rotamers*): δ 162.5 ($C_{C=O}$), 143.1 ($C_{triazole}$-4), 126.9 ($C_{triazole}$-5), 62.0 (C_q-Ada), 44.9 (bs, C_{Pip}), 43.9*/43.8* (C_{Ada}), 40.8 (bs, C_{Pip}) 36.9*/36.9* (C_{Ada}), 31.1 (C_{Ada}). IR: 3376 (bw), 2912 (m), 1606 (s), 1547 (m), 1451 (m), 1423 (m), 1250 (m), 1235 (w), 1013 (m), 756 (m) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 316.2135 (Calcd. $C_{17}H_{26}N_5O$, 316.2137, $[M-Cl]^+$).

*4.7.14. (1-(4-(tert-Butyl)benzyl)-1H-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4c) and 4-(1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carbonyl)piperazin-1-ium chloride (**4c**)*

The title compound **n4c** was prepared according to Method C from **3c** (0.10 g, 0.37 mmol) and piperazine (0.095 g, 1.10 mmol), with 44 h reaction time at room temperature. Affording **n4c** as a clear oily solid (69 mg, 0.21 mmol, 58%) after purification with flash column chromatography ($CHCl_3$/MeOH 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H, $H_{triazole}$-5), 7.45–7.36 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.29–7.19 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.50 (s, 2H, H_{Bn}), 4.29 (t, 2H, J = 4.9 Hz, H_{Pip}-2 and H_{Pip}-6), 3.74 (t, 2H, J = 5.0 Hz, H_{Pip}-2 and H_{Pip}-6), 2.99–2.91 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.8 ($C_{C=O}$), 152.4 (C_{Ph}-4), 144.6 ($C_{triazole}$-4), 130.7 (C_{Ph}-1), 128.3 (C_{Ph}-3 and C_{Ph}-5), 128.2 ($C_{triazole}$-5), 126.2 (C_{Ph}-2 and C_{Ph}-6), 54.1 (C_{Bn}), 47.9 (C_{Pip}-2 or C_{Pip}-6), 46.6 (C_{Pip}-3 and C_{Pip}-5), 46.0 (C_{Pip}-3 and C_{Pip}-5), 43.7 (C_{Pip}-2 and C_{Pip}-6), 34.7 (C_q-*t*-Bu), 31.2 (*t*-Bu). The free amine **n4c** was turned into its HCl-salt using the procedure for **4a** with **n4c** (51 mg, 0.13 mmol), affording **4c** as a white solid (42 mg, 0.12 mmol, 74%, mp > 190 °C decomp.). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 6.5 min, 96% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.42 (s, 1H, $H_{triazole}$-5), 7.50–7.41 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.36–7.29 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.63 (s, 2H, H_{Bn}), 4.49 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 4.00 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.39–3.34 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.32 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 153.3 (C_{Ph}-4), 144.2 ($C_{triazole}$-4, from HMBC), 133.5 (C_{Ph}-1), 130.1 ($C_{triazole}$-5), 129.3 (C_{Ph}-3 and C_{Ph}-5), 127.2 (C_{Ph}-2 and C_{Ph}-6), 55.0 (C_{Bn}), 44.8 (C_{Pip}, from HSQC), 40.7 (C_{Pip}, from HSQC), 35.6 (Cq-*t*-Bu), 31.8 (*t*-Bu). IR: 2951 (w), 2730 (w), 1616 (s), 1593 (w), 1540 (w), 1431 (m), 1248 (s), 1049 (s), 990 (s), 757 (s) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 328.2137 (Calcd. $C_{18}H_{26}N_5O$, 328.2137, $[M-Cl]^+$).

*4.7.15. (1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4d) and 4-(1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carbonyl)piperazin-1-ium chloride (**4d**)*

The title compound **n4d** was prepared according to Method C from **3d** (0.25 g, 0.76 mmol) and piperazine (0.196 g, 2.28 mmol), affording **n4d** as an off-white solid (0.199 g, 0.52 mmol, 69%) after purification with flash column chromatography ($CHCl_3$/MeOH 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H, $H_{triazole}$-5), 7.44 (s, 2H, H_{Ph}-4), 7.15 (d, 2H, J = 1.2 Hz, H_{Ph}-2 and H_{Ph}-6), 5.50 (s, 2H, H_{Bn}), 4.28 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.74 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 2.99–2.92 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9 ($C_{C=O}$), 152.0 (C_{Ph}-3 and C_{Ph}-5), 144.5 ($C_{triazole}$-4), 132.8 (C_{Ph}-1), 128.1 ($C_{triazole}$-5), 123.2 (C_{Ph}-4), 122.8 (C_{Ph}-2 and C_{Ph}-6), 55.1 (C_{Bn}), 48.0 (C_{Pip}-2 or C_{Pip}-6), 46.7 (C_{Pip}-3 and C_{Pip}-5), 46.0 (C_{Pip}-3 and C_{Pip}-5), 43.7 (C_{Pip}-2 or C_{Pip}-6), 34.9 (C_q-*t*-Bu), 31.4 (*t*-Bu). The free amine **n4d** was turned into its HCl-salt by adding an excess of HCl (5 mL, 10 mmol, 2 M in Et_2O) to **n4d** (30 mg, 0.08 mmol) in DCM (3 mL). Drying afforded **4d** as a white solid (30 mg, 0.07 mmol, 91%, mp 221.2–226.6 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 30.2 min, 99% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.44 (s, 1H, $H_{triazole}$-5), 7.47 (s, 1H, H_{Ph}-4), 7.27 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 5.64 (s, 2H, H_{Bn}), 4.52 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 4.00 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.56 (bs, 4H, H_{Pip}-3 and H_{Pip}-5), 1.32 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 162.1 ($C_{C=O}$), 153.1 (C_{Ph}-3 and C_{Ph}-5), 144.1 ($C_{triazole}$-4), 135.7 (C_{Ph}-1), 130.2 ($C_{triazole}$-5), 124.0 (C_{Ph}-4), 123.8 (C_{Ph}-2 and C_{Ph}-6), 55.8 (C_{Bn}), 45.0 (bs, C_{Pip}), 40.8 (bs, C_{Pip}) 35.9 (Cq-*t*-Bu), 31.9 (*t*-Bu). IR: 2953 (w), 1605 (s), 1443 (m), 1248 (s), 1053 (m), 994 (s), 758 (s) cm^{-1}. HRMS (APCI/ASAP, *m*/*z*): 384.2759 (Calcd. $C_{22}H_{34}N_5O$, 384.2763, $[M-Cl]^+$).

*4.7.16. (1-Heptyl-1H-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl) methanone (**n5a**) and 4-(1-heptyl-1H-1,2,3-triazole-4-carbonyl)-1-methylpiperazin-1-ium chloride (**5a**)*

The title compound **n5a** was prepared according to Method C from **3a** (0.15 g, 0.67 mmol) and 1-methylpiperazine (0.20 g, 2.00 mmol), with 76 h reaction time at room temperature. Affording **n5a** as a white solid (99 mg, 0.34 mmol, 51%) after purification with flash column chromatography ($CHCl_3$/MeOH 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H, $H_{triazole}$-5), 4.33–4.28 (m, 4H, CH_2+H_{Pip}-2 and H_{Pip}-6), 3.77 (t, 2H, J = 5.0 Hz, H_{Pip}-2 and H_{Pip}-6), 2.54–2.47 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 2.33 (s, 3H, N—CH_3) 1.92 (p, 2H, J = 7.0 Hz, CH_2), 1.40–1.21 (m, 8H, 4× CH_2), 0.91–0.84 (m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9 ($C_{C=O}$), 144.4 ($C_{triazole}$-

4), 128.1 ($C_{triazole}$-5), 55.6 (C_{Pip}-3 and C_{Pipe}-5), 54.8 (C_{Pip}-3 and C_{Pip}-5), 50.2 (CH_2), 46.6 (C_{Pip}-2 or C_{Pip}-6), 46.1 (N—CH_3), 42.6 (C_{Pip}-2 or C_{Pip}-6), 31.5 (CH_2), 30.1 (CH_2), 28.6 (CH_2), 26.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3). The free amine **n5a** was turned into its HCl-salt by adding HCl (0.15 mL, 1.83 mmol, 37%, aq) to **n5a** (40 mg, 0.136 mmol) dissolved in MeCN (4 mL), which upon drying afforded **5a** as a white solid (43 mg, 0.130 mmol, 96%, mp 189.9–191.5 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.0 min, 97% pure. ^{1}H NMR (400 MHz, *d4*-MeOD, rotamers*): δ 8.57*/8.47* (s, 1H, $H_{triazole}$-5), 5.46 (bs, 1H, Pip), 4.82 (bs, 1H, Pip), 4.48 (t, 2H, J = 6.9 Hz, CH_2), 3.81–3.48 (m, 4H, Pip), 3.26 (bs, 2H, Pip), 3.04*/2.99* (s, 3H, N—CH_3), 1.98–1.90 (m, 2H, CH_2), 1.43–1.25 (m, 8H, 4× CH_2), 0.96–0.87 (m, 3H, CH_3). ^{13}C NMR (100 MHz, *d4*-MeOD, rotamers*): δ 162.0 ($C_{C=O}$), 143.7 ($C_{triazole}$-4), 130.3*/129.7* ($C_{triazole}$-5), 54.5 (bs, C_{Pip}), 51.8*/51.7* (CH_2), 51.3 (C_{Pip}), 43.9*/43.8* (N—CH_3), 42.2 (C_{Pip}), 32.9*/32.9* (CH_2), 31.3*/31.3* (CH_2), 29.9*/29.8* (CH_2), 27.5*/27.5* (CH_2), 23.7 (CH_2), 14.5*/14.5* (Me). IR: 2919 (w), 1625 (s), 1539 (w), 1425 (m), 1246 (s), 1049 (m), 974 (s), 759 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 294.2293 (Calcd. $C_{15}H_{28}N_5O$, 294.2294, $[M-Cl]^+$).

*4.7.17. (1-(Adamantan-1-yl)-1H-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)methanone (n5b) and 4-(1-(adamantan-1-yl)-1H-1,2,3-triazole-4-carbonyl)-1-methylpiperazin-1-ium chloride (**5b**)*

The title compound **n5b** was prepared according to Method C from **3b** (0.15 g, 0.57 mmol) and 1-methylpiperazine (0.172 g, 1.72 mmol), with 115 h at room temperature. Affording **n5b** as a white wax (99 mg, 0.30 mmol, 53%) after purification with flash column chromatography ($CHCl_3$/MeOH 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H, $H_{triazole}$-5), 4.37 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.81 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 2.51 (bs, 4H, H_{Pip}-3 and H_{Pip}-5), 2.34 (s, 3H, N-CH_3), 2.31–2.19 (m, 9H, H_{Ada}-CH/CH_2), 1.86–1.74 (m, 6H, H_{Ada}-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.2 ($C_{C=O}$), 143.5 ($C_{triazole}$-4), 125.0 ($C_{triazole}$-5), 60.1 (C_q-Ada), 55.6 (C_{Pip}-3 and C_{Pip}-5), 54.9 (C_{Pip}-3 and C_{Pip}-5), 46.6 (C_{Pip}-2 or C_{Pip}-6), 46.0 (N—CH_3), 42.9 (C_{Ada}), 42.6 (C_{Pip}-2 or C_{Pip}-6), 35.8 (C_{Ada}), 29.4 (C_{Ada}). The free amine **n5b** was turned into its HCl-salt according to the procedure for **5a** with **n5b** (35 mg, 0.11 mmol) and HCl (0.10 mL, 1.22 mmol, 37% aq), affording **5b** as a white solid (39 mg, 0.11 mmol, quant., mp 250.3–253.9 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 4.8 min, 98% pure. ^{1}H NMR (400 MHz, *d4*-MeOD, rotamers*): δ 8.64*/8.52* (s, 1H, $H_{triazole}$-5), 5.43 (bs, 1H, Pip), 4.80 (bs, 1H, Pip), 3.92–3.45 (m, 4H, Pip), 3.26 (bs, 2H, Pip), 3.04*/2.99* (s, 3H, N—CH_3), 2.35–2.25 (m, 9H, Ada), 1.93–1.81 (m, 6H, Ada). ^{13}C NMR (100 MHz, *d4*-MeOD, rotamers*): δ 162.3 ($C_{C=O}$), 143.1 ($C_{triazole}$-4), 127.1 ($C_{triazole}$-5), 62.2*/62.0* (C_q-Ada), 54.5 (bs, C_{Pip}), 51.3 (C_{Pip}), 43.9 (C_{Ada}), 43.8*/43.8* (N-CH_3), 42.2 (C_{Pip}), 36.9*/36.9* (C_{Ada}), 31.1 (C_{Ada}). IR: 2909 (m), 1620 (s), 1539 (w), 1450 (m), 1422 (m), 1244 (m), 979 (m), 756 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 330.2294 (Calcd. $C_{18}H_{28}N_5O$, 330.2294, $[M-Cl]^+$).

*4.7.18. (1-(4-(tert-Butyl)benzyl)-1H-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)methanone (n5c) and 4-(1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carbonyl)-1-methylpiperazin-1-ium chloride (**5c**)*

The title compound **n5c** was prepared according to Method C from **3c** (0.15 g, 0.55 mmol) and 1-methylpiperazine (0.165 g, 1.65 mmol), with 63 h reaction time at room temperature. Affording **n5c** as an off-white solid (75 mg, 0.21 mmol, 39%) together with 3% of **3c** (from ^{1}H NMR) after purification with flash column chromatography (SiO_2 pre-deactivated with 1% TEA in eluent, eluent: $CHCl_3$/MeOH 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H, $H_{triazole}$-5), 7.43–7.38 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.26–7.22 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.50 (s, 2H, H_{Bn}), 4.32 (t, 2H, J = 4.9 Hz, H_{Pip}-2 and H_{Pip}-6), 3.77 (t, 2H, J = 4.9 Hz, H_{Pip}-2 and H_{Pip}-6), 2.52–2.45 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 2.32 (s, 3H, N-CH_3), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.8 ($C_{C=O}$), 152.4 (C_{Ph}-4, from HMBC), 144.6 ($C_{triazole}$-4), 130.7 (C_{Ph}-1, from HMBC), 128.3 (C_{Ph}-3 and C_{Ph}-5), 128.2 ($C_{triazole}$-5), 126.2 (C_{Ph}-2 and C_{Ph}-6), 55.5 (C_{Pip}-3 and C_{Pip}-5), 54.8 (C_{Pip}-3 and C_{Pip}-5), 54.1 (C_{Bn}), 46.5 (C_{Pip}-2 or C_{Pip}-6), 46.0 (N-CH_3), 42.6 (C_{Pip}-2 or C_{Pip}-6), 34.7 (C_q-*t*-Bu), 31.2 (*t*-Bu). The free amine **n5c** was turned into its HCl-salt according to the procedure described for **4a** with **n5c** (63 mg, 0.18 mmol) and HCl (0.25 mL, 3.04 mmol, 37% aq), affording **5c** as a white solid (43 mg, 0.11 mmol, 60%, mp 227.5–231.5 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 6.3 min, 97% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.43 (s, 1H, $H_{triazole}$-5), 7.47–7.42 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.35–7.31 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.63 (s, 2H, H_{Bn}), 5.45 (bs, 1H, Pip), 4.82 (bs, 1H, Pip), 3.90–3.40 (m, 4H, Pip), 3.26 (bs, 2H, Pip), 2.96 (s, 3H, N-CH_3), 1.33 (s, 9H, *t*-Bu). IR: 2955 (w), 2434 (w), 1624 (s), 1540 (w), 1427 (w), 1247 (s), 1049 (s), 976 (s), 757 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 342.2292 (Calcd. $C_{19}H_{28}N_5O$, 342.2294, $[M-Cl]^+$).

*4.7.19. 4-(1-(3,5-Di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carbonyl)-1-methylpiperazin-1-ium chloride (**5d**)*

The title compound **5d** was prepared through an Eschweiler-Clarke reductive amination,[25,26] where **n4d** (50 mg, 0.13 mmol), formaldehyde (0.20 mL, 37% aq, 2.69 mmol) and formic acid (0.10 mL, 96% aq, 2.50 mmol) were refluxed in MeCN (2 mL) for 1.5 h. After cooling to room temperature, HCl (0.25 mL, 3.04 mmol, 37%, aq.) was added and the reaction mixture was evaporated. The crude was then crystallized from DCM and Et_2O, affording **5d** as a white solid (46 mg, 0.11 mmol, 81%, mp 150.4–155.8 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 29.3 min, 97% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.45 (s, 1H, $H_{triazole}$-5), 7.47 (s, 1H, H_{Ph}-4), 7.27 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 5.64 (s, 2H, H_{Bn}), 5.49 (bs, 1H, H_{Pip}), 4.74 (bs, 1H, H_{Pip}), 3.62 (bs, 3H, H_{Pip}), 3.28 (bs, 3H, H_{Pip}), 2.97 (s, 3H, N—CH_3), 1.32 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 161.9 ($C_{C=O}$), 153.1 (C_{Ph}-3 and C_{Ph}-5), 144.1 ($C_{triazole}$-4), 135.7 (C_{Ph}-1), 130.2 ($C_{triazole}$-4), 124.0 (C_{Ph}-4), 124.0 (C_{Ph}-2 and C_{Ph}-6), 67.0, 55.8 (C_{Bn}), 54.6 (broad, C_{Pip}-3 and C_{Pip}-5), 45.1 (broad, C_{Pip}-2 or C_{Pip}-6), 43.8 (N-CH_3), 41.1 (broad, C_{Pip}-2 or C_{Pip}-6), 35.9 (C_q-*t*-Bu), 31.9 (*t*-Bu), 15.6. IR: 2956 (w), 1634 (s), 1425 (m), 1240 (s), 1052 (s), 975 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 398.2916 (Calcd. $C_{23}H_{36}N_5O$, 398.2920, $[M-Cl]^+$).

*4.7.20. Method D, ethylene diamine amidation reactions: Synthesis of N-(2-aminoethyl)-1-heptyl-1H-1,2,3-triazole-4-carboxamide (n6a) and 2-(1-heptyl-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**6a**)*

The title compound **n6a** was prepared using a general procedure described by Davis et al.,[24] where **3a** (0.15 g, 0.67 mmol) and ethylene diamine (0.67 mL, 10 mmol) was heated to reflux in MeOH (3 mL) for 17 h. Evaporation of volatiles afforded **n6a** as a light green solid (0.170 g, 0.67 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H, $H_{triazole}$-5), 7.46 (bs, 1H, NH), 4.39 (t, 2H, J = 7.2 Hz, CH_2), 3.51 (q, 2H, J = 6.6 Hz, CH_2), 2.94 (bs, 2H, CH_2), 1.92 (p, 2H, J = 6.9 Hz, CH_2), 1.40–1.21 (m, 8H, 4× CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3). The free amine **n6a** was turned into its HCl-salt by adding HCl (0.5 mL, 6.1 mmol, 37%, aq) to **n6a** (74 mg, 0.29 mmol) in MeCN (4 mL). The evaporated crude salt was then crystallized from EtOH, washed with MeCN (3 × 2 mL) and dried, affording **6a** as an off-white solid (23 mg, 0.08 mmol, 27%, mp 144.0–146.2 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.1 min, 99% pure. ^{1}H NMR (600 MHz, *d4*-MeOD):

δ 8.42 (s, 1H, $H_{triazole}$-5), 4.48 (t, 2H, J = 6.9 Hz, CH_2), 3.70 (t, 2H, J = 5.8 Hz, CH_2), 3.19 (t, 2H, J = 5.7 Hz, CH_2), 1.94 (p, 2H, J = 7.4 Hz, CH_2), 1.41–1.26 (m, 8H, 4× CH_2), 0.91 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 164.0 ($C_{C=O}$), 143.6 ($C_{triazole}$-4), 127.5 ($C_{triazole}$-5), 51.7 (triazole-CH_2), 41.2 (CH_2), 38.2 (CH_2), 32.9 (CH_2), 31.4 (CH_2), 29.9 (CH_2), 27.5 (CH_2), 23.7 (CH_2), 14.5 (CH_3). IR: 3280 (w), 1919 (m), 2855 (w), 1653 (s), 1570 (s), 1522 (m), 1509 (w), 1466 (w), 1256 (m), 1233 (m), 1169 (s), 1047 (s), 848 (s), 716 (m), 688 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 254.1980 (Calcd. $C_{12}H_{24}N_5O$, 254.1981, $[M-Cl]^+$).

*4.7.21. 1-(Adamantan-1-yl)-N-(2-aminoethyl)-1H-1,2,3-triazole-4-carboxamide (**n6b**) and 2-(1-(adamantan-1-yl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**6b**)*

The title compound **n6b** was prepared according to Method D with **3b** (70 mg, 0.27 mmol) and ethylene diamine (0.338 g, 5.63 mmol), affording **n6b** as a white solid (82 mg*, 0.27 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H, $H_{triazole}$-5), 7.45 (bs, 1H, NH), 3.51 (q, 2H, J = 6.1 Hz, CH_2), 2.93 (t, 2H, J = 6.1 Hz, CH_2), 2.32–2.19 (m, 9H, H_{Ada}-CH/CH_2), 1.86–1.73 (m, 6H, H_{Ada}-CH_2). The free amine **6b** was turned into its HCl-salt by adding HCl (0.25 mL, 3.04 mmol, 37%, aq) to **n6a** (25 mg, 0.09 mmol) in MeCN (4 mL). The evaporated crude salt was then washed with MeCN (3 × 2 mL) and DCM (3 × 2 mL) and dried, affording **6b** as a white solid (31 mg**, 0.09 mmol, quant., mp > 195 °C decomp.). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.0 min, 98%. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.49 (s, 1H, $H_{triazole}$-5), 3.70 (t, 2H, J = 5.8 Hz, CH_2), 2.93 (t, 2H, J = 5.6 Hz, CH_2), 2.35–2.25 (m, 9H, H_{Ada}-CH/CH_2), 1.94–1.81 (m, 6H, H_{Ada}-CH_2). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 164.3 ($C_{C=O}$, from HMBC), 143.1 ($C_{triazole}$-4, from HMBC), 124.2 ($C_{triazole}$-5), 61.9 (C_q-Ada), 44.0 (C_{Ada}), 41.1 (CH_2), 38.1 (CH_2), 37.0 (C_{Ada}), 31.1 (C_{Ada}). IR: 3349 (m), 2910 (s), 1556 (s), 1578 (s), 1510 (m), 1259 (w), 1237 (w), 1169 (w), 1035 (w), 1022 (w), 848 (m), 690 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 290.1980 (Calcd. $C_{15}H_{24}N_5O$, 290.1981, $[M-Cl]^+$).

*Theoretic 100% yield = 78 mg: 4 mg from unidentified byproducts and residual ethylene diamine.

**The additional 3 mg were mostly water and solvent residues.

*4.7.22. N-(2-Aminoethyl)-1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carboxamide (**n6c**) and 2-(1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**6c**)*

The title compound was prepared according to Method D with **3c** (0.20 g, 0.73 mmol) and ethylene diamine (0.66 g, 10.98 mmol), for 24 h at 50 °C. Affording **n6c** as a white solid (0.220 g, 0.73 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 1H, $H_{triazole}$-5), 7.48–7.36 (m, 3H, H_{Ph}-2 and H_{Ph}-6 + NH), 7.25–7.19 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.51 (s, 2H, H_{Bn}), 3.49 (q, 2H, J = 6.0 Hz, CH_2), 2.92 (t, 2H, J = 5.7 Hz, CH_2), 1.31 (s, 9H, *t*-Bu). The free amine **n6c** was turned into its HCl-salt by adding HCl (0.75 mL, 9.12 mmol, 37%, aq) to **n6c** (75 mg, 0.25 mmol) in *i*-PrOH (5 mL). The evaporated crude salt was then crystallized in DCM and washed with MeCN (3 × 2 mL) and DCM (3 × 2 mL) and dried, affording **6c** as a white solid (50 mg, 0.15 mmol, 59%, mp 218.0–220.4 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 6.7 min, 99% pure. ^{1}H NMR (600 MHz, *d4*-MeOD): δ 8.39 (s, 1H, $H_{triazole}$-5), 7.47–7.42 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.34–7.28 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.63 (s, 2H, H_{Bn}), 3.68 (t, 2H, J = 6.0 Hz, CH_2), 3.17 (t, 2H, J = 5.7 Hz, CH_2), 1.32 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 163.7 ($C_{C=O}$), 153.2 (C_{Ph}-4), 143.9 ($C_{triazole}$-4), 133.6 (C_{Ph}-1), 129.2 (C_{Ph}-3 and C_{Ph}-5), 127.5 ($C_{triazole}$-5), 127.2 (C_{Ph}-2 and C_{Ph}-6), 55.0 (C_{Bn}), 41.1 (CH_2), 38.1 (CH_2), 35.6 (C_q-*t*-Bu), 31.8 (*t*-Bu). IR: 2955 (w), 2904 (s), 1658 (s), 1571 (s), 1507 (s), 1236 (m), 1033 (m), 844 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 302.1980 (Calcd. $C_{16}H_{24}N_5O$, 302.1981, $[M-Cl]^+$).

*4.7.23. N-(2-Aminoethyl)-1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carboxamide (n6d) and 2-(1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**6d**)*

The title compound **n6d** was prepared according Method D with **3d** (0.05 g, 0.15 mmol) and ethylene diamine (0.136 g, 2.28 mmol), for 18 h at 50 °C. Affording **n6d** as a white solid (54 mg, 0.15 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H, $H_{triazole}$-5), 7.50–7.41 (m, 2H, H_{Ph}-4 + NH), 7.12 (d, 2H, J = 1.6 Hz, H_{Ph}-2 and H_{Ph}-6), 5.51 (s, 2H, H_{Bn}), 3.49 (q, 2H, J = 6.2 Hz, CH_2), 2.92 (t, 2H, J = 6.2 Hz, CH_2), 1.30 (s, 18H, 2× *t*-Bu). The free amine **n6d** was turned into its HCl-salt by adding HCl (0.25 mL, 3.04 mmol, 37%, aq) to **n6d** (37 mg, 0.10 mmol) in *i*-PrOH (3 mL). Evaporation of volatiles afforded **6d** as an off-white solid (38 mg, 0.097 mmol, 93%, mp 228.7–231.2 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm) 31.5 min, 97% pure. ^{1}H NMR (400 MHz, *d6*-DMSO): δ 8.76–8.70 (m, 2H, $H_{triazole}$-5 + NH), 8.00 (bs, 3H, NH_3^+), 7.37 (t, 1H, J = 1.9 Hz, H_{Ph}-4), 7.23 (d, 2H, J = 1.7 Hz, H_{Ph}-2 and H_{Ph}-6), 5.62 (s, 2H, H_{Bn}), 3.51 (q, 2H, J = 5.8 Hz, CH_2), 2.96 (q, 2H, J = 5.8 Hz, CH_2), 1.26 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d6*-DMSO): δ 160.3 ($C_{C=O}$), 150.9 (C_{Ph}-3 and C_{Ph}-5), 142.7 ($C_{triazole}$-4), 134.8 (C_{Ph}-1), 126.6 ($C_{triazole}$-5), 122.3 (C_{Ph}-2 and C_{Ph}-6), 121.9 (C_{Ph}-4), 53.7 (C_{Bn}), 38.6 (CH_2), 36.5 (imp.), 36.3 (CH_2), 34.5 (C_q-*t*-Bu), 31.2 (*t*-Bu). IR: 2953 (m), 2903 (w), 1666 (s), 1572 (s), 1503 (m), 1362 (m), 1248 (m), 1047 (m), 1031 (m), 878 (w), 837 (m), 713 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 358.2608 (Calcd. $C_{20}H_{32}N_5O$, 358.2607, $[M-Cl]^+$).

*4.8. Amino(4-(1-heptyl-1H-1,2,3-triazole-4-carbonyl)piperazin-1-yl)methaniminium chloride (**7a**)*

The title compound **7a** was prepared according to a general and modified[28] procedure by Bernatowicz.[27] Where **n4a** (0.10 g, 0.36 mmol) and *1H*-pyrazole carboxamidine hydrochloride (50 mg, 0.34 mmol) were refluxed in MeCN (5 mL) for 3.5 h. Then, the reaction mixture was cooled down, and the formed precipitate was filtered off and washed with MeCN and DCM. The crude was then recrystallized twice in MeCN and dried, affording **7a** as white crystals (51 mg, 0.14 mmol, 42%, mp 170.7–173.0 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.4 min, 96% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.42 (s, 1H, $H_{triazole}$-5), 4.46 (t, 2H, J = 6.4 Hz, CH_2), 4.35 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.87 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.67–3.60 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.92 (p, 2H, J = 7.4 Hz, CH_2), 1.42–1.23 (m, 8H, 4× CH_2), 0.90 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 162.5 ($C_{C=O}$), 158.6 (C_{Guan}), 144.0 ($C_{triazole}$-4), 129.9 ($C_{triazole}$-5), 51.7 (CH_2), 46.9 (C_{Pip}-2 or C_{Pip}-6), 46.1 (C_{Pip}-3 and C_{Pip}-5), 43.0 (C_{Pip}-2 or C_{Pip}-6), 32.9 (CH_2), 31.4 (CH_2), 29.9 (CH_2), 27.5 (CH_2), 23.7 (CH_2), 14.5 (CH_3). IR: 3310 (w), 3122 (w), 1649 (w), 1598 (s), 1247 (m), 1229 (w), 1052 (w), 988 (s), 762 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 322.2354 (Calcd. $C_{15}H_{28}N_7O$, 322.2355, $[M-Cl]^+$).

*4.9. (4-(1-(Adamantan-1-yl)-1H-1,2,3-triazole-4-carbonyl)piperazin-1-yl)(amino)methaniminium chloride (**7b**)*

The title compound **7b** was prepared according to a procedure described by Bernatowicz et al.[27] Where **n4b** (25 mg, 0.08 mmol) and *1H*-pyrazole carboxamidine hydrochloride (12 mg, 0.08 mmol) were stirred in DMF (2 mL) for 97 h at room temperature. The crude product was precipitated from the mixture with Et_2O and filtered. The crude precipitate was crystallized from MeOH and Et_2O and washed with DCM (3 × 2 mL) before it was dried, affording **7b** as a white solid (20 mg, 0.05 mmol, 64%, mp > 175 °C decomp.). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.0 min, 95% pure. ^{1}H NMR (400 MHz, *d4*-MeOD): δ 8.47 (s, 1H, $H_{triazole}$-5), 4.35 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.89 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.67–3.60 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 2.33–2.24 (m, 9H,

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H_{Ada}-CH/CH_2), 1.91–1.80 (m, 6H, H_{Ada}-CH_2). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 162.7 ($C_{C=O}$), 158.6 (C_{Guan}), 143.5 ($C_{triazole}$-4), 126.7 ($C_{triazole}$-5), 62.0 (C_q-Ada), 46.9 (broad, C_{Pip}-2 or C_{Pip}-6), 46.1 (broad, C_{Pip}-3 and C_{Pip}-5), 44.0 (C_{Ada}), 43.0 (broad, C_{Pip}-2 or C_{Pip}-6), 37.0 (C_{Ada}), 31.1 (C_{Ada}). IR: 3323 (w), 3154 (w), 1658 (m), 1597 (s), 1529 (m), 1441 (m), 1239 (s), 1017 (m), 990 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 358.2353 (Calcd. $C_{18}H_{28}N_7O$, 358.2355, $[M-Cl]^+$).

*4.10. Amino(4-(1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carbonyl)piperazin-1-yl)methaniminium chloride (**7d**)*

The title compound **7d** was prepared according to the conditions described for **7a** from **n4d** (0.05 g, 0.13 mmol) and *1H*-pyrazole carboxamidine hydrochloride (19 mg, 0.128 mmol), with 22 h reflux. Filtration of the cooled reaction mixture and washing the precipitate with MeCN (3 × 3 mL), DCM (3 × 2 mL) and Et_2O (3 × 5 mL) followed by drying, afforded **7d** as a white solid (31 mg, 0.067 mmol, 52%, mp 230.1–232.2 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 33.1 min, 94% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.39 (s, 1H, $H_{triazole}$-5), 7.45 (s, 1H, H_{Ph}-4), 7.25 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 5.62 (s, 2H, H_{Bn}), 4.35 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.85 (bs, 2H, H_{Pip}-2 and H_{Pip}-6), 3.67–3.58 (m, 4H, H_{Pip}-3 and H_{Pip}-5), 1.31 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 162.4 ($C_{C=O}$), 158.6 (C_{Guan}), 153.1 (C_{Ph}-3 and C_{Ph}-5), 144.4 ($C_{triazole}$-4), 135.7 (C_{Ph}-1), 129.9 ($C_{triazole}$-5), 124.0 (C_{Ph}-4), 123.8 (C_{Ph}-2 and C_{Ph}-6), 55.8 (C_{Bn}), 46.9 (broad, C_{Pip}-2 or C_{Pip}-6), 46.1 (broad, C_{Pip}-3 and C_{Pip}-5), 43.0 (broad, C_{Pip}-2 or C_{Pip}-6), 35.9 (C_q-*t*-Bu), 31.9 (*t*-Bu). IR: 2954 (w), 1668 (w), 1588 (s), 1549 (m), 1433 (m), 1244 (m), 1055 (w), 992 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 426.2978 (Calcd. $C_{23}H_{36}N_7O$, 426.2981, $[M-Cl]^+$).

*4.11. Amino((2-(1-heptyl-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)methaniminium chloride (**8a**)*

The title compound **8a** was prepared according to the conditions described for **7a** from **n6a** (0.10 g, 0.39 mmol) and *1H*-pyrazole carboxamidine hydrochloride (0.052 g, 0.36 mmol), with 4 h reflux. Filtration upon cooling and careful washing of the precipitate with MeCN (3 × 3 mL) afforded **8a** as a red solid (89 mg, 0.27 mmol, 76%, mp 144.0–146.2 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.6 min, 96% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.39 (s, 1H, $H_{triazole}$-5), 4.45 (t, 2H, *J* = 7.1 Hz, CH_2), 3.58 (t, 2H, *J* = 6.0 Hz, CH_2), 3.42 (t, 2H, *J* = 6.2 Hz, CH_2), 1.92 (p, 2H, *J* = 6.9 Hz, CH_2), 1.41–1.22 (m, 8H, 4× CH_2), 0.89 (t, 3H, *J* = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 163.6 ($C_{C=O}$), 159.1 (C_{Guan}), 143.7 ($C_{triazole}$-4), 127.4 ($C_{triazole}$-5), 51.7 (triazole-CH_2), 42.2 (CH_2), 39.4 (CH_2), 32.9 (CH_2), 31.4 (CH_2), 29.9 (CH_2), 27.5 (CH_2), 23.7 (CH_2), 14.5 (CH_3). IR: 3350 (w), 3108 (w), 2921 (w), 1651 (s), 1629 (s), 1575 (s), 1504 (w), 1450 (w), 1225 (m), 1048 (m), 774 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 296.2197 (Calcd. $C_{13}H_{26}N_7O$, 296.2199, $[M-Cl]^+$).

*4.12. ((2-(1-(Adamantan-1-yl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)(amino)methaniminium chloride (**8b**)*

The title compound **8b** was prepared according to the procedure described for **8a** from **n6b** (0.047 g, 0.16 mmol) and *1H*-pyrazole carboxamidine hydrochloride (24 mg, 0.16 mmol). Affording **8b** as a red solid (30 mg, 0.08 mmol, 50%, mp 180.0–186.8 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 5.1 min, 95% pure. ^1H NMR (400 MHz, *d6*-DMSO): δ 8.68 (s, 1H, $H_{triazole}$-5), 8.59 (t, 1H, *J* = 6.0 Hz, NH), 7.57 (t, 1H, *J* = 6.0 Hz, NH), 3.45–3.37 (m, 2H, CH_2), 3.34–3.27 (m, 5H, CH_2+H_2O from *d6*-DMSO), 2.21 (bs, 9H, H_{Ada}-CH/CH_2), 1.75 (bs, 6H, H_{Ada}-CH_2). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 163.7 ($C_{C=O}$), 159.1 (C_{Guan}), 143.0 ($C_{triazole}$-4), 124.1 ($C_{triazole}$-5), 61.9 (C_q-Ada), 44.0 (C_{Ada}), 42.2 (CH_2), 39.4 (CH_2), 37.0 (C_{Ada}), 31.1 (C_{Ada}). IR: 3358 (w), 3110 (w), 1653 (s), 1628 (s), 1573 (m), 1498 (w), 1049 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 332.2198 (Calcd. $C_{16}H_{26}N_7O$, 332.2199, $[M-Cl]^+$).

*4.13. Amino((2-(1-(4-(tert-butyl)benzyl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)methaniminium chloride (**8c**)*

The title compound **8c** was prepared according to the conditions described for **7a** from **n6c** (0.073 g, 0.24 mmol) and *1H*-pyrazole carboxamidine hydrochloride (34 mg, 0.23 mmol), where filtration of the cooled reaction mixture and careful washing with MeCN (3 × 2 mL) afforded **8c** as a red solid (60 mg, 0.16 mmol, 69%, mp 131.2–136.1 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 7.1 min, 95% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.35 (s, 1H, $H_{triazole}$-5), 7.45–7.38 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.32–7.25 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 5.60 (s, 2H, H_{Bn}), 3.56 (t, 2H, *J* = 5.9 Hz, CH_2), 3.41 (t, 2H, *J* = 5.9 Hz, CH_2), 1.30 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 163.4 ($C_{C=O}$), 159.1 (C_{Guan}), 153.2 (C_{Ph}-4), 144.0 ($C_{triazole}$-4), 133.5 (C_{Ph}-1), 129.2 (C_{Ph}-3 and C_{Ph}-5), 127.3 (C_{Ph}-2 and C_{Ph}-6), 127.2 ($C_{triazole}$-5), 55.0 (C_{Bn}), 41.2 (CH_2), 39.3 (CH_2), 35.6 (C_q-*t*-Bu), 31.8 (*t*-Bu). IR: 3352 (w), 3112 (w), 2959 (w), 1652 (s), 1631 (s), 1573 (s), 1253 (w), 1230 (w), 1044 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 344.2199 (Calcd. $C_{17}H_{26}N_7O$, 344.2199, $[M-Cl]^+$).

*4.14. Amino((2-(1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)methaniminium chloride (**8d**)*

The title compound **8d** was prepared according to the protocol shown for **8c** from **n6d** (0.103 g, 0.29 mmol) and *1H*-pyrazole carboxamidine hydrochloride (40 mg, 0.27 mmol), where the cooled reaction mixture was evaporated and crystallized with MeCN and Et_2O. The crude was then recrystallized in MeCN affording **8d** as a red solid (47 mg, 0.11 mmol, 39%, mp 205.9–209.8 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 33.7 min, 97% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.36 (s, 1H, $H_{Triazole}$-5), 7.44 (s, 1H, H_{Ph}-4), 7.23 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 5.62 (s, 2H, H_{Bn}), 3.57 (t, 2H, *J* = 5.6 Hz, CH_2), 3.41 (t, 2H, *J* = 6.0 Hz, CH_2), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): δ 163.4 ($C_{C=O}$), 159.1 (C_{Guan}), 153.2 (C_{Ph}-3 and C_{Ph}-5), 143.9 ($C_{triazole}$-4), 135.7 (C_{Ph}-1), 127.3 ($C_{triazole}$-5), 123.9 (C_{Ph}-4), 123.7 (C_{Ph}-2 and C_{Ph}-6), 55.8 (C_{Bn}), 42.2 (CH_2), 39.3 (CH_2), 35.9 (C_q-*t*-Bu), 31.9 (*t*-Bu). IR: 2960 (w), 1679 (w), 1656 (s), 1641 (s), 1574 (s), 1223 (m), 1061 (w), 848 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 400.2821 (Calcd. $C_{21}H_{34}N_7O$, 400.2821, $[M-Cl]^+$).

*4.15. N-(2-Aminoethyl)-1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazole-4-carboxamide (**n9e**) and 2-(1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**9e**)*

The title compound **n9e** was prepared according to Method D from **3e** (0.20 g, 0.63 mmol) and ethylene diamine (0.57 g, 9.51 mmol), for 20 h at room temperature. Affording **n9e** as a white solid (0.196 g, 0.57 mmol, 90%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H, $H_{triazole}$-5), 7.57–7.47 (m, 4H, H_{Ph}+NH), 3.56 (q, 2H, *J* = 6.2 Hz, CH_2), 2.98 (t, 2H, *J* = 5.5 Hz, CH_2), 1.38 (s, 18H, 2× *t*-Bu). The free amine **n9e** was turned into its HCl-salt by adding HCl (0.10 mL, 1.22 mmol, 37%, aq.) to **n9e** (30 mg, 0.09 mmol) in MeCN (2 mL). Evaporation of volatiles afforded **9e** as a white solid (37 mg, 0.09 mmol, quant., mp 261.1–267.5 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 37.7 min, 98% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.96 (s, 1H, $H_{triazole}$-5), 7.68 (bs, 2H, H_{Ph}-2 and H_{Ph}-6), 7.63 (bs, 1H, H_{Ph}-4), 3.73 (t, 2H, *J* = 5.7 Hz, CH_2), 3.21 (t, 2H, *J* = 5.5 Hz, CH_2), 1.39 (s, 18H, 2× *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): 163.7 ($C_{C=O}$), 154.6 (C_{Ph}-3 and C_{Ph}-5),

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144.3 ($C_{triazole}$-4), 137.9 (C_{Ph}-1), 126.0 ($C_{triazole}$-5), 124.8 (C_{Ph}-4), 116.6 (C_{Ph}-2 and C_{Ph}-6), 41.2 (CH_2), 38.2 (CH_2), 36.3 (C_q-*t*-Bu), 31.8 (*t*-Bu). IR: 3284 (w), 2957 (w), 1665 (s), 1580 (s), 1490 (s), 1169 (m), 1051 (m), 875 (m), 703 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 344.2449 (Calcd. $C_{19}H_{30}N_5O$, 344.2450, $[M-Cl]^+$).

*4.16. N-(2-Aminoethyl)-1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazole-4-carboxamide (**n9f**) and 2-(1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**9f**)*

The title compound **n9f** was prepared according to Method D from **3f** (0.10 g, 0.32 mmol) and ethylene diamine (0.28 g, 4.73 mmol), with 20 h at reflux. re-evaporation of the crude from DCM afforded **n9f** as a white solid (0.100 g, 0.29 mmol, 92%). ^1H NMR (400 MHz, *d6*-DMSO): δ 9.15 (s, 1H, $H_{triazole}$-5), 8.53 (t, 1H, $J = 6.1$ Hz, NH), 7.88–7.82 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.17–7.10 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.05 (t, 2H, $J = 6.5$ Hz, O—CH_2), 3.29 (q, 2H, $J = 6.5$ Hz, CH_2), 2.70 (t, 2H, $J = 6.5$ Hz, CH_2), 1.79–1.70 (m, 2H, CH_2), 1.48–1.24 (m, 8H, 4× CH_2), 0.91–0.85 (m, 3H, CH_3). The free amine **n9f** was turned into its HCl-salt by adding HCl (0.15 mL, 1.83 mmol, 37% aq) to **n9f** (30 mg, 0.09 mmol) in MeCN (3 mL). Evaporation of volatiles, washing with MeCN (3 × 2 mL) and drying afforded **9f** as an off white solid (27 mg, 0.07 mmol, 81%, mp 240.0–246.0 °C). HPLC (C18, 1:3 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 7.3 min, 95% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.83 (s, 1H, $H_{triazole}$-5), 7.76 (d, 2H, $J = 7.9$ Hz, H_{Ph}-3 and H_{Ph}-5), 7.12 (d, 2H, $J = 8.6$ Hz, H_{Ph}-2 and H_{Ph}-6), 4.06 (t, 2H, $J = 6.5$ Hz, O—CH_2), 3.71 (t, 2H, $J = 5.6$ Hz, CH_2), 3.20 (t, 2H, $J = 6.1$ Hz, CH_2), 1.82 (p, 2H, $J = 7.0$ Hz, CH_2), 1.56–1.28 (m, 8H, 4× CH_2), 0.95–0.88 (m, 3H, CH_3). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 163.7 ($C_{C=O}$), 161.6 (C_{Ph}-4), 144.2 ($C_{triazole}$-4), 131.3 (C_{Ph}-1), 125.7 ($C_{triazole}$-5), 123.6 (C_{Ph}-3 and C_{Ph}-5), 116.7 (C_{Ph}-2 and C_{Ph}-6), 69.7 (O—CH_2), 41.2 (CH_2), 38.2 (CH_2), 33.1 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 27.3 (CH_2), 23.8 (CH_2), 14.6 (CH_3). IR: 2914 (w), 1659 (m), 1599 (m), 1578 (m), 1515 (s), 1250 (s), 1215 (m), 1180 (w), 1166 (m), 1054 (m), 1037 (m), 987 (w), 831 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 346.2242 (Calcd. $C_{18}H_{28}N_5O_2$, 346.2243, $[M-Cl]^+$).

*4.17. N-(2-Aminoethyl)-1-(4-((3,5-di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazole-4-carboxamide (**n9g**) and 2-(1-(4-((3,5-di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazole-4-carboxamido)ethan-1-aminium chloride (**9g**)*

The title compound **n9g** was prepared according to Method D from **3g** (0.10 g, 0.24 mmol) and ethylene diamine (0.21 g, 3.56 mmol), with 28 h at reflux. Affording **n9g** as a white solid (0.103 g, 0.23 mmol, 96%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, $H_{triazole}$-5), 7.68–7.61 (m, 2H, H_{phenox}-3 and -5), 7.52 (t, 1H, $J = 6.0$ Hz, NH), 7.43 (t, 1H, $J = 1.9$ Hz, H_{Ph}-4), 7.29 (d, 2H, $J = 1.6$ Hz, H_{Ph}-2 and H_{Ph}-6), 7.17–7.11 (m, 2H, H_{phenox}-2 and -6), 5.09 (s, 2H, H_{Bn}), 3.55 (q, 2H, $J = 6.0$ Hz, CH_2), 2.97 (t, 2H, 6.0 Hz, CH_2), 1.35 (s, 18H, 2× *t*-Bu). The free amine **n9g** was turned into its HCl-salt by adding HCl (0.10 mL, 1.22 mmol, 37% aq) to a filtered solution of **n9g** (20 mg, 0.044 mmol) in THF (3 mL). Evaporation of volatiles, washing with THF (3 × 2 mL) and drying afforded **9g** as a white solid (18 mg, 0.037 mmol, 83%, mp 250.0–254.5 °C). HPLC (C18, 1:3 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 23.3 min, 97% pure. ^1H NMR (400 MHz, *d4*-MeOD): δ 8.84 (s, 1H, $H_{triazole}$-5), 7.78 (d, 2H, $J = 8.4$ Hz, H_{phenox}-3 and -5), 7.43 (s, 1H, H_{Ph}-4), 7.31 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 7.22 (d, 2H, $J = 8.4$ Hz, H_{phenox}-2 and -6), 5.16 (s, 2H, H_{Bn}), 3.71 (t, 2H, $J = 5.7$ Hz, CH_2), 3.20 (t, 2H, $J = 5.4$ Hz, CH_2), 1.33 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 163.7 ($C_{C=O}$), 161.3 ($C_{phenoxyl}$-4), 152.4 (C_{Ph}-3 and C_{Ph}-5), 144.3 ($C_{triazole}$-4), 137.4 (C_{Ph}-1), 131.5 (C_{phenox}-1), 125.7 ($C_{triazole}$-5), 123.6 (C_{phenox}-3 and -5), 123.2 (C_{Ph}-4), 123.19 (C_{Ph}-2 and C_{Ph}-6), 117.2 (C_{phenox}-2 and -6), 72.3 (C_{Bn}), 41.2 (CH_2), 38.2 (CH_2), 35.9 (C_q-*t*-Bu), 32.0 (*t*-Bu). IR: 2957 (w), 1662 (m), 1602 (m), 1581 (m), 1517 (s), 1248 (m), 1167 (m), 1054 (m), 873 (w), 823 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 450.2869 (Calcd. $C_{26}H_{36}N_5O_2$, 450.2869, $[M-Cl]^+$). pH = 3,5-di-*tert*-butylbenzyl.

*4.18. Amino((2-(1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)methaniminium chloride (**10e**)*

The title compound **10e** was prepared according to the conditions shown for **7a** from **n9e** (0.05 g, 0.15 mmol) and *1H*-pyrazole carboxamidine hydrochloride (21 mg, 0.15 mmol), with 21 h reflux. The cooled reaction mixture was evaporated, dissolved in MeOH (1–2 mL), filtered and crystallized with Et_2O. Washing of the formed precipitate with MeCN (2 × 2 mL) and Et_2O (3 × 10 mL) followed by drying, afforded **10e** as a pink solid (32 mg, 0.076 mmol, 52%, mp 272–276 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 42.3 min, 95% pure. ^1H NMR (600 MHz, *d4*-MeOD): δ 8.95 (s, 1H, $H_{triazole}$-5), 7.67 (d, 2H, $J = 1.7$ Hz, H_{Ph}-2 and H_{Ph}-6), 7.63 (t, 1H, $J = 1.7$ Hz, H_{Ph}-4), 3.62 (t, 2H, $J = 6.2$ Hz, NH-CH_2), 3.46 (t, 2H, $J = 6.2$ Hz, guanidine-CH_2), 1.40 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 163.3 ($C_{C=O}$), 159.1 (C_{Guan}), 154.6 (C_{Ph}-3 and C_{Ph}-5), 144.4 ($C_{triazole}$-4), 137.9 (C_{Ph}-1), 125.9 ($C_{triazole}$-5), 124.8 (C_{Ph}-4), 116.6 (C_{Ph}-2 and C_{Ph}-6), 42.2 (guanidine-CH_2), 39.5 (NH-CH_2), 36.3 (C_q-*t*-Bu), 31.8 (*t*-Bu). IR: 3287 (w), 3139 (w), 2954 (w), 1657 (m), 1620 (s), 1578 (s), 876 (w), 851 (w), 705 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 386.2668 (Calcd. $C_{20}H_{32}N_7O$, 386.2668, $[M-Cl]^+$).

*4.19. Amino((2-(1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino)methaniminium chloride (**10f**)*

The title compound **10f** was prepared according to a modified[28] general procedure described by Bernatowicz et al.[27] Where **n9f** (40 mg, 0.12 mmol) and *1H*-pyrazole carboxamidine hydrochloride (26 mg, 0.07 mmol) was refluxed for 20 h, added triethylamine (50 mg, 0.49 mmol) refluxed for 20 h, added *1H*-pyrazole carboxamidine hydrochloride (10 mg, 0.17 mmol) and triethylamine (20 mg, 0.20 mmol) followed by a third round of reflux for 20 h. The cooled reaction mixture was evaporated, and the crude was washed with MeCN (3 × 2 mL) and H_2O (3 × 1 mL) before it was dried. The dried crude was dissolved in MeOH and filtered, evaporation afforded **10f** as a red solid (24 mg, 0.057 mmol, 49%, mp 196.0–200.4 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 33.7 min, 95% pure. ^1H NMR (400 MHz, *d6*-DMSO): δ 9.21 (s, 1H, $H_{triazole}$-5), 8.74 (t, 1H, $J = 5.5$ Hz, NH), 7.89–7.83 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.53 (t, 1H, $J = 5.5$ Hz, NH), 7.17–7.10 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.05 (t, 2H, $J = 7.4$ Hz, O—CH_2), 3.45 (q, 2H, $J = 5.8$ Hz, CH_2), 3.37–3.29 (m, CH_2+H_2O from DMSO), 1.75 (p, 2H, $J = 8.2$ Hz, CH_2), 1.48–1.22 (m, 8H, 4× CH_2), 0.92–0.85 (m, 3H, CH_3). ^{13}C NMR (150 MHz, *d6*-DMSO): δ 159.9 ($C_{C=O}$), 159.1 (C_{Ph}-4), 157.0 (C_{Guan}), 143.3 ($C_{triazole}$-4), 129.5 (C_{Ph}-1), 124.6 ($C_{triazole}$-5), 122.1 (C_{Ph}-3 and C_{Ph}-5), 115.4 (C_{Ph}-2 and C_{Ph}-6), 68.0 (O—CH_2), 40.3 (CH_2), 37.8 (CH_2), 31.2 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 25.4 (CH_2), 22.0 (CH_2), 13.9 (CH_3). IR: 3346 (w), 3098 (w), 2923 (w), 1658 (s), 1630 (s), 1572 (s), 1518 (s), 1500 (s), 1242 (s), 1036 (m), 826 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 388.2458 (Calcd. $C_{19}H_{30}N_7O_2$, 388.2461, $[M-Cl]^+$).

*4.20. Amino((2-(1-(4-((3,5-di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazole-4-carboxamido)ethyl)amino) methaniminium chloride (**10g**)*

The title compound **10g** was prepared according to the procedure shown for **10f** from **n9g** (44 mg, 0.098 mmol) and *1H*-pyrazole carboxamidine hydrochloride (16 mg + 5 mg + 5 mg, total: 26 mg, 0.177 mmol), with DMF at room temperature for the third round of stirring. Affording **10g** as a red solid (12 mg, 0.023 mmol,

23%, mp 259.6–261.3 °C). HPLC (C18, 3:7 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 40.2 min, 96% pure. ^{1}H NMR (600 MHz, *d4*-MeOD): δ 8.82 (s, 1H, $H_{triazole}$-5), 7.80–7.75 (m, 2H, H_{phenox}-3 and -5), 7.43 (t, 1H, *J* = 1.7 Hz, H_{Ph}-4), 7.31 (d, 2H, *J* = 1.5 Hz, H_{Ph}-2 and H_{Ph}-6), 7.23–7.19 (m, 2H, H_{phenox}-2 and -6), 5.15 (s, 2H, H_{Bn}), 3.61 (t, 2H, *J* = 6.5 Hz, CH_2), 3.45 (t, 2H, *J* = 6.1 Hz, CH_2), 1.33 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD): δ 163.3 ($C_{C=O}$), 161.3 (C_{Guan}), 159.1 (C_{phenox}-4), 152.4 (C_{Ph}-3 and C_{Ph}-5), 144.3 ($C_{triazole}$-4), 137.4 (C_{Ph}-1), 131.5 (C_{phenox}-1), 125.6 ($C_{triazole}$-5), 123.6 (C_{phenox}-3 and -5), 123.3 (C_{Ph}-4), 123.2 (C_{Ph}-2 and C_{Ph}-6), 117.2 (C_{phenox}-2 and -6), 72.3 (C_{Bn}), 42.3 (CH_2), 39.4 (CH_2), 35.9 (C_q-*t*-Bu), 32.0 (*t*-Bu). IR: 2952 (w), 1660 (s), 1574 (s), 1506 (s), 1245 (s), 829 (s) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 492.3090 (Calcd. $C_{27}H_{38}N_7O_2$, 492.3087, [M−Cl]$^+$). pH = 3,5-di-*tert*-butylbenzyl.

4.21. Amino(2-(1-(1-(3,5-di-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)ethylidene)hydrazinyl)methaniminium chloride (11d)

The title compound **11d** was prepared according to a general procedure described by Hu-Ri et al.[33] Where **13d** (40 mg, 0.13 mmol), aminoguanidine hydrochloride (20 mg, 0.18 mmol) and HCl (75 mg, 0.76 mmol, 37% aq) in EtOH (2 mL, abs.) was heated to 90 °C in a sealed tube for 22 h. The cooled reaction mixture was then evaporated, washed with H_2O (2 × 2 mL) and dried, affording **11d** as a white solid isomer mixture (30 mg, 0.07 mmol, 58%, 4:6 isomer ratio from ^{1}H NMR, mp 253.6–259.0 °C). HPLC (C18, 3:7 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 11.2 min (major isomer), 13.0 min (minor isomer), 97% pure (both). IR: 3314 (bm), 2953 (m), 1674 (m), 1599 (s), 1362 (w), 1224 (w), 1047 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 370.2713 (Calcd. $C_{20}H_{32}N_7$, 370.2713, [M−Cl]$^+$). ^{1}H NMR (600 MHz, *d4*-MeOD, major isomer): δ 8.47 (s, 1H, $H_{triazole}$-5), 7.46–7.43 (m, 1H, H_{Ph}-4), 7.22 (d, 2H, *J* = 1.7 Hz, H_{Ph}-2 and H_{Ph}-6), 5.62 (s, 2H, H_{Bn}), 2.38 (s, 3H, imine-CH_3), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD, major isomer): δ 157.9 (C_{Guan}), 153.1 (C_{Ph}-3 and C_{Ph}-5), 147.6 ($C_{triazole}$-4), 147.0 (C_{imine}), 135.9 (C_{Ph}-1), 127.5 ($C_{triazole}$-5), 123.9 (C_{Ph}-4), 123.5 (C_{Ph}-2 and C_{Ph}-6), 55.9 (H_{Bn}), 35.9 (C_q-*t*-Bu), 31.9 (*t*-Bu), 14.3 (imine-CH_3). ^{1}H NMR (600 MHz, *d4*-MeOD, minor isomer): δ 8.53 (s, 1H, $H_{triazole}$-5), 7.46–7.43 (m, 1H, H_{Ph}-4), 7.26 (d, 2H, *J* = 1.7 Hz, H_{Ph}-2 and H_{Ph}-6), 5.68 (s, 2H, H_{Bn}), 2.35 (s, 3H, imine-CH_3), 1.30 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD, minor isomer): δ 157.5 (C_{Guan}), 153.1 (C_{Ph}-3 and C_{Ph}-5), 144.8 ($C_{triazole}$-4), 141.0 (C_{imine}), 135.6 (C_{Ph}-1), 125.4 ($C_{triazole}$-5), 124.0 (C_{Ph}-4), 123.7 (C_{Ph}-2 and C_{Ph}-6), 55.9 (H_{Bn}), 35.9 (C_q-*t*-Bu), 31.9 (*t*-Bu), 21.6 (imine-CH_3).

4.22. Amino(2-(1-(1-(3,5-di-tert-butylphenyl)-1H-1,2,3-triazol-4-yl)ethylidene)hydrazinyl)methaniminium trifluoroacetate (11e)

The title compound **11e** was prepared according to a procedure described by Mohammad et al.[30] Where **13e** (35 mg, 0.116 mmol), aminoguanidine hydrochloride (16 mg, 0.14 mmol) and LiCl (2 mg, 0.05 mmol) in EtOH (2 mL, abs.) were heated to 90 °C in a sealed tube for 48 h. After which, the reaction mixture was evaporated and purified with preparative C18-HPLC (80:20 MeOH/H_2O + 0.1% TFA, 20 mL min, Rt: 2.8–4.0 min), affording **11e** as an off-white solid isomer mixture (25 mg, 0.053 mmol, 46%, 3:7 isomer ratio from ^{1}H NMR, mp 106.0–110.1 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 59.2 min (both isomers), 98% pure (both). IR: 2961 (w), 1681 (m), 1606 (m), 1592 (m), 1200 (m), 1182 (m), 1135 (s), 1045 (w), 800 (w), 703 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 356.2560 (Calcd. $C_{19}H_{30}N_7$, 356.2560, [M−TFA]$^+$). ^{1}H NMR (600 MHz, *d4*-MeOD, major isomer): δ 9.07 (s, 1H, $H_{triazole}$-5), 7.74 (s, 1H, H_{Ph}-4), 7.70 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 2.47 (s, 3H, imine-CH_3), 1.41 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD, major isomer): δ 162.5 (bs, TFA), 158.0 (C_{Guan}), 154.6 (C_{Ph}-3 and C_{Ph}-5), 148.0 ($C_{triazole}$-4), 147.0 (C_{imine}), 138.0 (C_{Ph}-1), 123.6 ($C_{triazole}$-5), 116.8 (C_{Ph}-4), 116.4 (C_{Ph}-2 and C_{Ph}-6), 36.3 (C_q-*t*-Bu), 31.8 (*t*-Bu), 30.9 (TFA), 14.0 (imine-CH_3). ^{1}H NMR (600 MHz, *d4*-MeOD, minor isomer): δ 9.11 (s, 1H, $H_{triazole}$-5), 7.65 (s, 1H, H_{Ph}-4), 7.63 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 2.47 (s, 3H, imine-CH_3), 1.41 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD, minor isomer): δ 162.5 (bs, TFA), 157.6 (C_{Guan}), 154.7 (C_{Ph}-3 and C_{Ph}-5), 145.1 ($C_{triazole}$-4), 141.1 (C_{imine}), 137.7 (C_{Ph}-1), 126.2 ($C_{triazole}$-5), 125.1 (C_{Ph}-4), 124.7 (C_{Ph}-2 and C_{Ph}-6), 36.32 (C_q-*t*-Bu), 31.8 (*t*-Bu), 30.9 (TFA), 21.7 (imine-CH_3).

4.23. Amino(2-(1-(1-(4-(heptyloxy)phenyl)-1H-1,2,3-triazol-4-yl)ethylidene)hydrazinyl)methaniminium trifluoroacetate (11f)

The title compound **11f** was prepared according to the procedure shown for **11e** from **13f** (0.05 g, 0.166 mmol) and aminoguanidine hydrochloride (22 mg, 0.20 mmol), affording **11f** as an off-white solid isomer mixture (24 mg, 0.051 mmol, 31%, 1:9 isomer ratio from ^{1}H NMR, mp 139.7–144.3 °C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 68.1 min (major), 65.4 min (minor), 95% pure (both). IR: 2927 (w), 1672 (m), 1606 (s), 1517 (m), 1262 (w), 1198 (m), 1169 (m), 1131 (s), 825 (m), 797 (m), 720 (w) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 358.2354 (Calcd. $C_{18}H_{28}N_7O$, 358.2355, [M−TFA]$^+$). ^{1}H NMR (600 MHz, *d4*-MeOD, major isomer): δ 8.93 (s, 1H, $H_{triazole}$-5), 7.78–7.72 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.16–7.07 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.05 (t, 2H, *J* = 6.4 Hz, O—CH_2), 1.81 (p, 2H, *J* = 7.9 Hz, CH_2), 1.50 (p, 2H, *J* = 7.6 Hz, CH_2), 1.44–1.31 (m, 6H, 3 × CH_2), 0.95–0.89 (m, 3H, CH_3). ^{13}C NMR (150 MHz, *d4*-MeOD, major isomer): δ 163.0 (bs, TFA), 161.5 (C_{Guan}), 157.9 (C_{Ph}-4), 147.9 ($C_{triazole}$-4), 146.9 (C_{imine}), 131.4 (C_{Ph}-1), 123.4 (C_{Ph}-3 and C_{Ph}-5), 123.3 ($C_{triazole}$-5), 116.65 (C_{Ph}-2 and C_{Ph}-6), 69.7 (O—CH_2), 33.1 (CH_2), 30.9 (TFA), 30.5 (CH_2), 30.3 (CH_2), 27.3 (CH_2), 23.8 (CH_2), 14.6 (CH_3), 14.1 (imine-CH_3). ^{1}H NMR (600 MHz, *d4*-MeOD, minor isomer): δ 8.95 (s, 1H, $H_{triazole}$-5), 7.82–7.78 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.14–7.10 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 4.05 (t, 2H, *J* = 6.4 Hz, O—CH_2), 1.81 (p, 2H, *J* = 7.9 Hz, CH_2), 1.50 (p, 2H, *J* = 7.6 Hz, CH_2), 1.44–1.31 (m, 6H, 3× CH_2), 0.95–0.89 (m, 3H, CH_3).

4.24. Amino(2-(1-(1-(4-((3,5-di-tert-butylbenzyl)oxy)phenyl)-1H-1,2,3-triazol-4-yl)ethylidene)hydrazinyl)methaniminium chloride (11g)

The title compound **11g** was prepared according to the procedure for **11e** from **13g** (0.04 g, 0.099 mmol) and aminoguanidine hydrochloride (13 mg, 0.12 mmol), for 25 h at reflux. The crude product was crystallized from the partially evaporated reaction mixture with H_2O. The crude was in turn crystallized from THF and EtOAc, affording **11g** as a white solid (7 mg, 0.014 mmol, 14%, 1:9 isomer ratio from ^{1}H NMR, mp 230–232 °C). HPLC (C18, 1:4 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 11.2 min (major), 10.5 (minor) min, 98% pure (both). IR: 2958 (w), 1672 (m), 1622 (m), 1595 (s), 1542 (s), 1253 (s), 1035 (m), 830 (m) cm^{-1}. HRMS (APCI/ASAP, *m/z*): 462.2978 (Calcd. $C_{26}H_{36}N_7O$, 462.2981, [M−Cl$^+$). ^{1}H NMR (600 MHz, *d6*-DMSO, major isomer): δ 10.94 (s, 1H, NH), 9.29 (s, 1H, $H_{triazole}$-5), 7.83–7.79 (m, 2H, H_{phenox}-3 and -5), 7.39 (t, 1H, *J* = 1.8 Hz, H_{Ph}-4), 7.33–7.26 (m, 4H, H_{phenox}-2 and -6 + H_{Ph}-2 and H_{Ph}-6), 5.17 (s, 2H, H_{Bn}), 2.42 (s, 3H, CH_3), 1.31 (s, 18H, 2× *t*-Bu). ^{13}C NMR (150 MHz, *d6*-DMSO, major isomer): δ 158.9 (C_{phenox}-4), 150.5 (C_{Ph}-3 and C_{Ph}-5), 146.8 ($C_{triazole}$-4, from HMBC), 146.0 (C_{imine}, from HMBC) 135.6 (C_{Ph}-1), 129.8 (C_{phenox}-1), 122.2 (C_{Ph}-2 and C_{Ph}-6), 121.8 (C_{phenox}-3 and -5), 121.6 (C_{Ph}-4 + $C_{triazole}$-5), 115.9 (C_{phenox}-2 and -6), 70.5 (C_{Bn}), 34.5 (C_q-*t*-Bu), 31.3 (*t*-Bu), 13.6 (imine-CH_3). ^{1}H NMR (400 MHz, *d6*-DMSO, minor isomer): δ 9.38 (s, 1H, $H_{triazole}$-5), 7.94–7.89 (m, 2H, H_{phenox}-3 and -5), 7.33–7.26 (m, 4H, H_{phenox}-2 and -6 + H_{Ph}-2 and

H_{Ph}-6), 5.18 (s, 2H, H_{Bn}), 2.41 (s, 3H, CH_3), 1.31 (s, 18H, 2× *t*-Bu). pH = 3,5-di-*tert*-butylbenzyl.

4.25. Inhibition of bacterial growth

Growth medium with MilliQ H_2O was used as a negative control, while sterile MilliQ H_2O and bacteria suspension was used as a positive control. Bacteria were transferred from a blood plate to growth medium (MH-bullion, VL787693 717, Merck) for *E. coli*, P. aeruginosa and S. aureus and BHI-bullion (CM1135, OXOID) for E. faecalis and S. agalactiae gr. B and incubated at 37°C overnight. The following day part of the bacteria suspension was transferred to fresh medium and cultivated in a shaker incubator at 37°C for 1.5 h (E. coli, E. faecalis and Streptococcus gr. B) or 2.5 h (S. aureus and P. aeruginosa). The bacteria suspension was then diluted 1:100 in medium and added to all wells on a 96-well microtiter plate (Nunc 167008), followed by sample aliquotes (and Gentamicin as a reference antibiotic) in duplicates. The plates were incubated at 37°C overnight before growth was controlled visually and photometrically at 600 nm.

4.26. Inhibition of biofilm formation

S. epidermidis was used to assess the effect of the test compounds on biofilm formation. Growth media: tryptic soy broth (TS; Merck, Darmstadt, Germany). An overnight culture of *S. epidermidis* grown in TS was diluted with fresh TS containing 1% glucose (1:100). Aliquots of 50 μL were transferred to a 96-well microtiter plate, and 50 μL of test compounds, dissolved in water at ranging concentrations, was added. After overnight incubation at 37 °C, the bacterial suspension was carefully discarded and the wells washed with water. The plate was dried and the biofilm fixed by incubation for 1 h at 55 °C before the surface attached cells were stained with 100 μL of 0.1% crystal violet for 5 min. The crystal violet solution was removed and the plate once more washed with water and dried at 55 °C for 1 h. After adding 70 μL of 70% ethanol, the plate was incubated at room temperature for 10 min. Biofilm formation was observed by visual inspection of the plates. The MIC was defined as the lowest concentration where no biofilm formation was visible. A *S. epidermidis* suspension, diluted with 50 μL of water, was used as a positive control, and 50 μL Staphylococcus haemolyticus suspension with 50 μL of water was employed as a negative control. A mixture of 50 μL water and 50 μL TS was used as assay control.

4.27. Cytotoxicity to HepG2-cells

Cytotoxicity of the test compounds was evaluated after 24 h exposure in human hepatocellular liver carcinoma (HepG2, ATCC HB-8065™) cells. HepG2 were grown overnight (20,000 cells/well), and then incubated with test compound (range of concentrations) diluted in MEM Earle's supplemented with gentamycin (10 μg/mL), non-essential amino acids (1%), sodium pyruvate (1 mM), L-alanyl-L-glutamine (2 mM), but without FBS (total volume was 100 μl) for 24 h. Ten μL of CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI, USA) was added and plates were then further incubated for 1 h. Absorbance was measured at 485 nm in a DTX 880 Multimode Detector. Results were calculated as % survival compared to negative (assay media) and positive (Triton X-100; Sigma-Aldrich) controls.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.07.060.

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PAPER IV

Synthesis and antimicrobial evaluation of fused pyridine and isoindoline amphiphiles

Synthesis and antimicrobial evaluation of fused pyridine and isoindoline amphiphiles

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ABSTRACT

A library of 20 low molecular weight amphiphiles based on fused pyridines and isoindolines was prepared for studies of antimicrobial activities. The amphiphiles were designed after a motif from marine antimicrobial natural products and previous work on antimicrobial 1,2,3-triazole amphiphiles. The core motifs were prepared through transition metal catalyzed [2+2+2] cycloaddition or Suzuki cross-coupling, followed by functionalization and antimicrobial evaluation. The most promising amphiphile **3d** displayed minimum inhibitory concentrations of 2-8 µg/mL against *Streptococcus agalacticae, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. The low structural complexity and high antimicrobial activity makes these structures interesting for further studies and optimization.

1. Introduction

The ability to cure bacterial infections with systemic antimicrobials, have since the discovery of the first antibiotics been crucial to the development of modern health care.[1,2] As previously fatal conditions have become managable through prescription of «over the counter»-drugs. However, these utopian treatment conditions may be coming close to an end.[3-5] The first cases of antimicrobial resistance were documented shortly after the development of the first antibiotics, and has since then been an ever-increasing obstacle in clinical management of infectious diseases. The concerns regarding antimicrobial resistance has increased during the last couple of decades, as the occurence of bacterial resistance has also been rapidly increasing.[6] The heavily increasing prevalence of resistant infections has been allowed to develop seemingly uninhibited, as the numbers are currently being propelled at an alarming rate. The reason for the explosive development of resistant infections are due to reduced development of novel antibiotics combined with increased use of antimicrobials both in agriculture and clinical settings.[7-10] Development of novel antimicrobials is therefore of crucial importance to modern medicince.

The research group of Strøm at the university of Tromsø have explored an antimicrobial motif based on antimicrobial marine peptide mimics isolated from the Barents sea (synoxazolidinone A and ianthelline shown in Fig. 1).[11,12] They have utilized knowledge from their previous work on antimicrobial peptide mimics[13-16] and this motif to prepare a library of cationic amphipathic benzamides (**E23**) with high antimicrobial potencies against resistant bacteria.[17] These peptide mimics are thought to work through membrane disruption mechanisms similar to those of native antimicrobial peptides (AMPs). AMPs are relatively small peptides with an overall positive charge (+2 to +9) that is part of the primary immune

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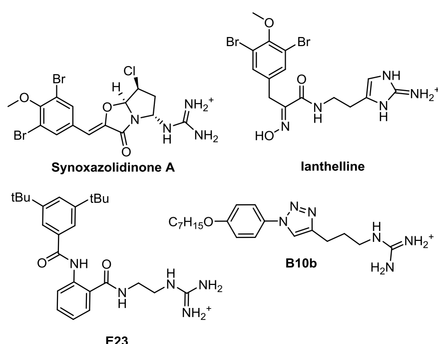
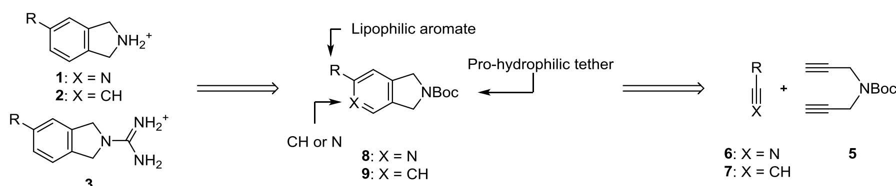


Fig. 1 Synoxazolidone A[12] (methicillin-resistant *S. aureus*, MIC: 10 µg/mL), ianthelline[13] (methicillin-resistant *S. aureus*, MIC: 20 µg/mL), **E23**[18] (methicillin-resistant *S. aureus*, MIC: 2.1 µg/mL), and **B10b**[21] (*S. aureus*, MIC: 4 µg/mL).

response in most eukaryotes.[18,19] These peptides consist of a balance of lipophilic and hydrophilic residues, making them able to fold into amphipathic secondary structures. These amphipathic secondary structures exhibit antimicrobial activity through membrane disruption mechanisms and interactions with intracellular targets.

Based on the structures and activities of synoxazolidone A,[11] ianthelline[12] and the aminobenzamides by Igumonva *et al.*,[17] we have also previously prepared a library of cationic amphiphilic 1,2,3-triazoles.[20] The most potent amphiphile **B10b** displayed broad spectrum antimicrobial activities against both Gram-positive and Gram-negative bacteria (MIC: 4-8 µg/mL). The potency of this 1,2,3-triazole amphile matched or surpassed the potencies of the marine natural products synoxazolidinone A and ianthelline against four strains of antibiotic-susceptible bacteria.

In an effort to further expand our library of low molecular weight antimicrobial amphiphiles, fused pyridines (dihydro pyrrolopyridines) and isoindolines obtainable from [2+2+2] cycloaddition reactions were investigated. Transition metal catalyzed [2+2+2] cycloaddition was chosen as the preferred synthesis route, as this particular methodology readily offers fused ring systems such as the dihydro pyrrolopyridines and the isoindolines (Scheme 1). In addition to giving the wanted fused ring scaffolds through cyclization, it is also an extensively studied and established reaction type for synthesis of aromatic rings (for extensive publications and reviews; Vollhardt *et al.*,[21-23] Yamamoto *et al.*,[24-27] Bönnemann *et al.*,[28,29] Tanaka *et al.*,[30,31] and more[32-34]). For the scaffold synthesis of the fused rings



Scheme 1 Preparation of amphiphilic dihydro pyrrolopyridines and isoindolines through [2+2+2] cycloaddition of the tethered diyne **5** with a nitrile (**6**) or alkyne (**7**) respectively.

(dihydro pyrrolopyridines and isoindolines) we chose to use the carbamate protected tethered alkyne **5**, as this has been reported as a functioning substrate for [2+2+2] cycloaddition reactions together with a variety of substrates and metal catalysts.[35-42] The strategy, as displayed in Scheme 1, was to perform [2+2+2] cycloaddition between **5** and nitriles **6a-d** to give the carbamate protected fused pyridines **8a-d**, and cycloaddition of **5** and alkynes **7a-f** would afford the carbamate protected isoindolines **9a-f**. Subsequent deprotection and *N*-functionalization would then give the target amphiphiles **1a-3i** shown in Fig. 2 in two or three steps.

Initial antimicrobial evaluations of the amphiphiles prepared through [2+2+2] cycloaddition reactions showed that isoindolines generally exhibited higher antimicrobial activity than the dihydro pyrrolopyridines. In an attempt to simplify preparation of amphipathic isoindolines, efforts were made to find a more suitable preparation route in order to avoid the sometimes low-yielding [2+2+2] cycloadditions. The work presented herein therefore also includes the synthesis of amphiphilic isoindolines through Suzuki cross-coupling reactions. After scaffold synthesis or cross-coupling and appropriate *N*-functionalization, this small library of 20 amphiphilic dihydro pyrrolopyridines and isoindolines (**1a-3i**, shown in Fig. 2) was then evaluated for antimicrobial activities against the three

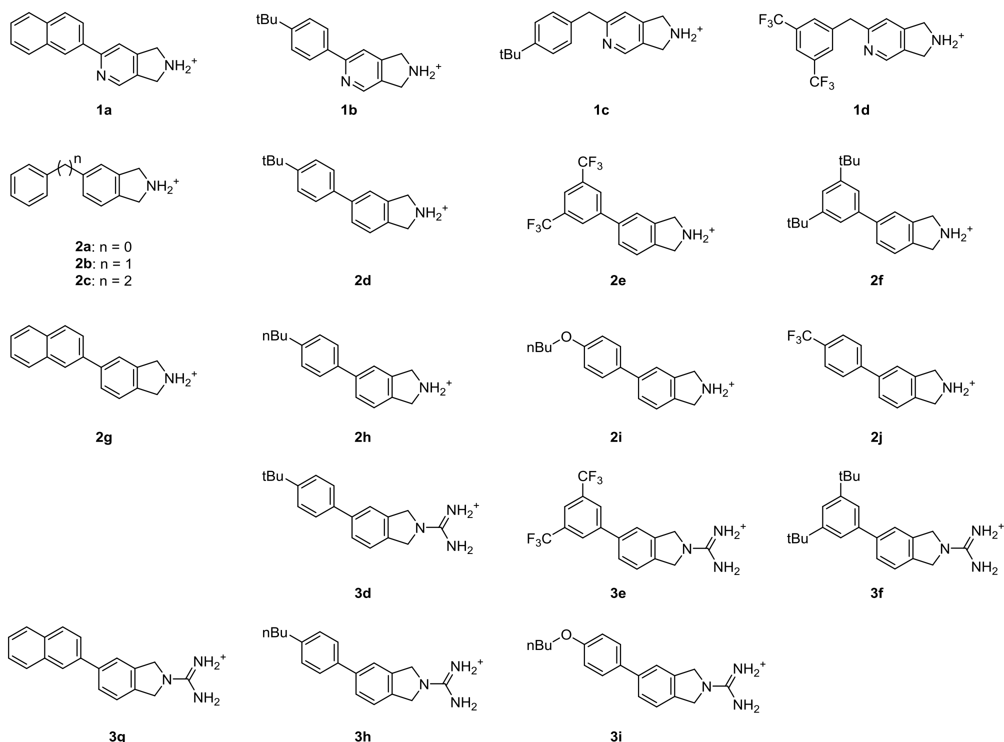


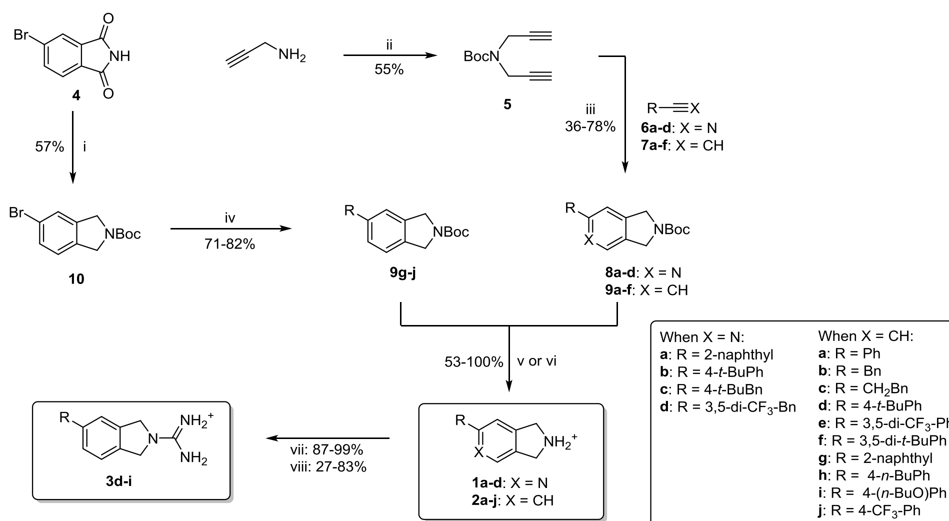
Fig. 2 Series of dihydro pyrrolopyridine- and isoindoline-based peptide mimics prepared and investigated for antimicrobial activity. Counterion: Cl^-.

Gram-positive *Enterococcus faecalis, Staphylococcus aureus,* and *Streptococcus agalacticae* and the two Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. The amphiphiles displaying antimicrobial activity were also tested against human heptic cells (HepG2), in order to assess mammalian cytotoxicity and to afford a scope concerning selectivity. In this series of antimicrobial amphiphiles, the impact on antimicrobial potency of the scaffolds and variations of the lipophilic aromatic groups were investigated, in addition to the relative potencies of amine and guanidine cationic groups.

2. Results and discussion

2.1 Synthesis of target amphiphiles (1a-3i)

The library of amphiphiles **1a-3i** shown in Fig. 2 was prepared through the two synthetic routes shown in Scheme 2, where **1a-d**, **2a-f**, and **3d-f** were prepared from transition metal catalyzed [2+2+2] cycloaddition[21,24,25,43,44] and the remaining isoindoline amphiphiles **2g-j** and **3g-i** were prepared from Suzuki cross-coupling of arylboronic acids and carbamate protected 5-bromoisoindoline (**10**).[45-47] The key starting material **5** was prepared from propargylamine in two steps through carbamate protection and *N*-alkylation with propargyl bromide in 55%,[39,48] whereas the key substrate **10** was prepared in two steps from 5-bromophthalimide (**4**) through a reduction of the amido groups with $NaBH_4$ and BF_3-etherate followed by carbamate protection affording **10** in 57% yield.[45]

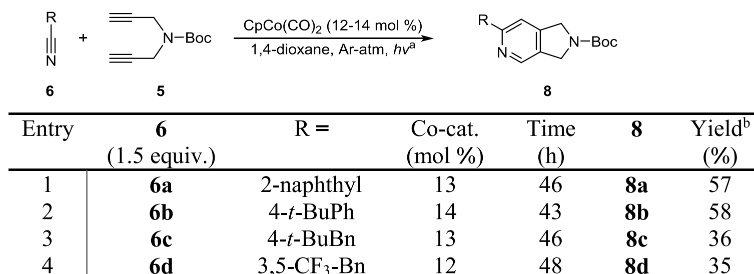


Scheme 2 Preparation of the target library: i) 1) $NaBH_4$, BF_3 x OEt_2, THF, 70 °C, 21 h. 2) Boc_2O, 4-DMAP, DMF, rt, 41 h. ii) 1) Boc_2O, DCM, 0 °C - rt, Ar-atm, 2 h. 2) Propargyl bromide, NaH, THF, rt, 25 h. iii) X = N: $CpCo(CO)_2$ (12-14 mol %), 1,4-dioxane, Ar-atm, *hv* (two halogen lamps, 400 W, 118 nm, 50 Hz), 43-48 h. X = CH: Cp*RuCl(cod) (5 mol %), DCE, Ar-atm, rt, 18-32 h. iv) R-$B(OH)_2$ (**11g-j**), $Pd(PPh_3)_4$ (7 mol %), K_2CO_3, 1,4-dioxane/H_2O 1:1, Ar-atm, reflux, 22-24 h. v) HCl (2M, Et_2O), DCM or Et_2O, rt, 22-48 h (used for **3c-e**). vi) HCl (37%, aq), MeCN, rt, 14-48 h (used for **1a-d** and **2f-j**). Solvent for **2f**: THF. **2a-b** were prepared through a combination of v and vi. The TFA-salt of **2f** was also prepared from TFA in DCM over 48 h at rt (75%). vii) K_2CO_3 (sat. aq)/EtOAc for free-basing of **2d-i** to form **12d-i**. viii) *1H*-Pyrazole carboxamidine hydrochloride (**13**, 0.9-1.0 equiv), MeCN, reflux, 5-31 h. Counterions for **1**, **2**, and **3**: Cl^- (and TFA^- for **2f**).

Fused pyridines and isoindolines from [2+2+2] cycloaddition reactions

The fused pyridines **8a-d** were prepared from **6a-d** and **5** in 36-58% yields using the cobalt-catalyst $CpCo(CO)_2$ under visible-light irradiation, as seen in Table 1.[21,43,44] Where the nitriles conjugated to an arene system (entry 2: **6b**, 58%) seemed to offer better isolated yields in the cycloaddition reaction compared to the benzylic nitriles (entry 3: **6c**, 36%). Some improved reactivity of conjugated nitriles have also been reported by Boñaga *et al.*, who observed this trend for [2+2+2] cycloaddition reactions utilizing the same Co-catalyst.[44]

Table 1 Cobalt catalyzed cyclotrimerization of **6a-d** and **5** to pyridines **8a-d**.

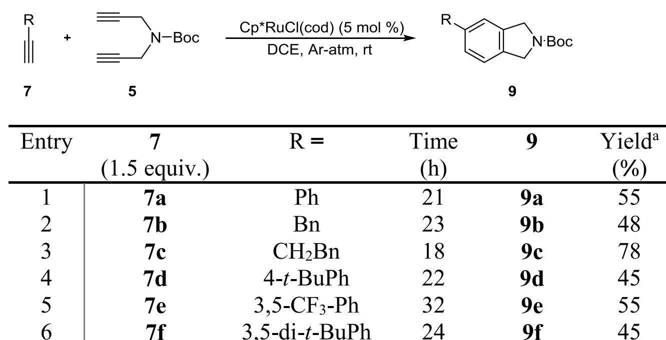


Entry	6 (1.5 equiv.)	R =	Co-cat. (mol %)	Time (h)	8	Yield[b] (%)
1	**6a**	2-naphthyl	13	46	**8a**	57
2	**6b**	4-*t*-BuPh	14	43	**8b**	58
3	**6c**	4-*t*-BuBn	13	46	**8c**	36
4	**6d**	3,5-CF_3-Bn	12	48	**8d**	35

[a] *hν*: irradiation with two halogen lamps (400 W, 118 nm, 50 Hz)
[b] Isolated yields after flash column chromatography.

The isoindolines **9a-f** were prepared from **7a-f** and **5** in 45-78% yields using the ruthenium-catalyst Cp*RuCl(cod) at room temperature, as seen in Table 2.[24,25] The yields from the isoindoline synthesis were seen to be similar to the moderate yields observed in the synthesis of **8a-d** seen in Table 1, with the exception being synthesis of **9c** (78%). The decent yield seen for this reaction may be explained by steric factors, as the alkyne **7c** was the least sterically hindered substrate in this set of [2+2+2] cycloadditions. It has also been reported Yamamoto *et al.* that this particular Ru-catalyst offers sterically favorable products in [2+2+2] cycloaddition, due to the steric demand of the large and bulky pentamethylcyclopentadiene ligand.[24,25]

Table 2 Ruthenium catalyzed cyclotrimerization of **7a-f** and **5** to isoindolines **9a-f**.



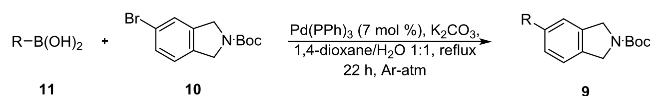
Entry	7 (1.5 equiv.)	R =	Time (h)	9	Yield[a] (%)
1	**7a**	Ph	21	**9a**	55
2	**7b**	Bn	23	**9b**	48
3	**7c**	CH_2Bn	18	**9c**	78
4	**7d**	4-*t*-BuPh	22	**9d**	45
5	**7e**	3,5-CF_3-Ph	32	**9e**	55
6	**7f**	3,5-di-*t*-BuPh	24	**9f**	45

[a] Isolated yields after flash column chromatography.

Substituted Isoindolines from Suzuki cross-coupling reactions

The Suzuki cross-coupling, using a modified[47] version of the procedure presented by Hua *et al.*,[46] between **10** and commericially available boronic acids (**11g-j**) gave the carbamate protected isoindolines **9g-j** in 71-82% yields (as seen in Table 3). The benefit of the Suzuki cross-coupling was the increased yields compared to those of shown in Tables 1 and 2, however the drawbacks of this route were that 5-bromopthalimide was fairly expensive and the synthesis did not offer preparation of dihydro pyrrolopyridines analogously to the [2+2+2] cycloaddition reactions.

Table 3 Suzuki cross-coupling of **11g-j** and **10** to isoindolines **9g-j**.



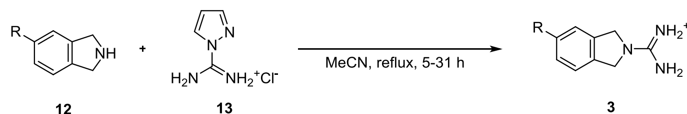
Entry	**11**	Equiv. **11**	R =	**9**	Yield[a] (%)
1	**11g**	1.19	2-naphthyl	**9g**	82
2	**11h**	1.17	4-*n*-BuPh	**9h**	72
3	**11i**	1.16	4-(*n*-BuO)Ph	**9i**	71
4	**11j**	1.18	4-CF_3-Ph	**9j**	77

[a)] Isolated yields after flash column chromatography.

Deprotection and *N*-functionalization

Cleavage of the carbamate protection groups was carried out using either etheric HCl (2M) in Et_2O (**2c-e**) or DCM in 53-86% yields, or aqueous HCl (37%) in MeCN (**1a-d** and **2f-j**) in 53-100% yields.[49] It should be noted that **2a** and **2b** were prepared using a combination of the two methods "v" and "vi" from Scheme 2 in 83% and 92% yields. For the deprotections carried out in MeCN, an unidentified byproduct was sometimes formed concurrently with the product, and showed up as broad signals in the aromatic region in ^{1}H NMR spectra of the crude products. This byproduct was conveniently removed from the formed HCl-salts through kügelrohr-distillation under reduced pressure (0.5 - 3 mbar at 60 °C). A selected set of the HCl-salts (**2d-i**) was then free-based using standard conditions (sat. K_2CO_3/solvent), giving the free amines **12d-i** in 87-99% yield. The isoindoline amines **12d-i** were then guanylated using the electrophilic guanylation reagent 1*H*-pyrazole carboxamidine hydrochloride (**13**) as seen in Table 4. This reagent has been reported by Bernatowicz *et al.* to guanylate a range of amines under various conditions, common conditions for this reaction being DMF at room temperature with an organic base additive.[50] A modified version of this procedure has been developed, as it was observed that a range of amines converted to their corresponding guanidine salts in MeCN at reflux with no basic additive.[51] Being able to use just **13** and an amine (**12**) simplified the work-up, which sometimes may be difficult for this type of compounds. Additionally, the conversion was often observed to be faster when using MeCN at reflux instead of DMF at rt. The guanidines **3d-i** were then prepared in 27-83% yields from using the conditions in Scheme 2. It should be noted that **12i** was seemingly unstable in air, and was put under argon atmosphere after free-basing and taken directly into the guanylation reaction, giving **3i** in with a reduced yield (27%).

Table 3 Guanylation between free-based amines **12d-i** and 1*H*-pyrazole carboxamidine hydrochloride (**13**) to form the guanidine salts **3d-i**. Counterion: Cl^-.



Entry	12	13 (equiv.)	R =	Time (h)	3	Yield (%)
1	**12d**	0.98	4-*t*-BuPh	31	**3d**	57
2	**12e**	1.00	3,5-CF_3-Ph	8	**3e**	83
3	**12f**	1.00	3,5-di-*t*-BuPh	5	**3f**	66
4	**12g**	0.97	2-naphthyl	17	**3g**	47
5	**12h**	0.89	4-*n*-BuPh	5	**3h**	72
6	**12i**	1.06	4-(*n*-BuO)Ph	6	**3i**	27[a]

[a)] Free amine **12i** unstable in air, put under Ar-atm and taken directly into guanylation.

2.2 Antimicrobial activity and cytotoxicity of 1a-3h

The 20 amphiphiles (**1a-3i**, Fig. 2) were evaluated for antimicrobial activity against three gram-positive and 2 gram-negative strains of bacteria; *Streptococcus agalacticae* (ATCC 12386), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Enterococcus faecalis* (ATCC 29212). In order to determine initial activity, all the structures were subjected to a single-concentration assay at 64 µg/mL. Any compounds displaying activity below this threshold was then subjected to dose-response assay, and the minimum inhibitory concentrations (MIC) were determined and are shown in Table 1. Additionally, to give some information regarding possible adverse effects, the *in vitro* cytotoxicity against HepG2-cells (human hepatic cells) was measured. This assay determined the possible hepatotoxic effects of the compounds, and the results from these assays are shown in Fig. 3 and the obtained EC_{50}-values are displayed in Table 1 together with the antimicrobial activities.

Evaluation of antimicrobial activities

There was observed a large difference in activity based on the different scaffolds, as most of the isoindolines (**2**/**3**) were highly active in the biological assays whereas the dihydro pyrrolopyridines (**1**) displayed little to no biological activity in the tested range. This may be caused by the increased polarity of the dihydro pyrrolopyridine core, as inserting more heteroatoms in a ring increases the polarity of the aromate. The increased polarity may also have assisted with charge distribution and in that way negating

Table 2 Antimicrobial activity (MIC in µg/mL) and mammalian cytotoxicity (HepG2, EC_{50} in µg/mL) for the biologically active compounds in the series. The "-"-sign in the table indicates no activity ≤64 µg/mL.

Entry	*E. faecalis*[a]	*S. aureus*[a]	*S. agalacticae*[a]	*E. coli*[a]	*P. aeruginosa*[a]	HepG2[b] (EC_{50})
1a	-	64	-	-	-	n.d.[c]
1c	-	64	-	-	-	n.d.
2d	32	8	16	32	64	1.3
2e	32	16	16	16	-	6.1
2f	4	4	1	8	32	2.0
2g	16	4	8	16	32	2.0
2h	8	2	4	8	32	1.3
2i	16	4	8	16	-	1.0
2j	64	16	32	32	64	2.7
3d	4	2	4	4	8	12
3e	32	16	16	16	32	>64
3f	-	-	4	16	64	64[d]
3g	8	2	2	4	8	7.8
3h	4	1	2	4	8	5.3
3i	8	2	4	4	16	7.1
Ref.[e]	10	0.13	4	0.5	0.5	n.d.[e]

[a] *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), *S. agalacticae* (ATCC 12386), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[b] Evaluation of *in vitro* cytotoxicity.
[c] n.d.: not determined.
[d] Approximated from a single value: 53% cell survival at 64 µg/mL.
[e] Ref.: Gentamicin.

the amphiphilic character of the structure. Aside from the observation concerning the scaffold, it was also seen that a rather large lipophilic character was important for achieving high antimicrobial activities of the isoindolines (**2**/**3**).

The isoindoline guanidines **3** were shown to be more potent in the antimicrobial assays than their amine HCl-salt counterparts (**2**). This increase of potency was most evident for the isoindolines equipped with a 4-*t*-Bu-phenyl group (**2d**/**3d**), where there was observed a 4- to 8-fold increase in potency for the guanidine **3d** compared to that of the amine **2d**. Increase in potency when introducing a guanidine was also observed for the lipophilic groups **g**, **h**, and **I**, giving a 2- to 4-fold increase in antimicrobial potency for the guanidines (**3g**, **3h**, and **3i**) over the amines (**2g**, **2h**, and **2i**). It should also be noted that the isoindolines equipped with 3,5-di-CF3-groups (**e**) and 3,5-di-*t*-Bu-groups (**f**) did not offer increased potency similarly to the other targets when equipped with a guanidine function (**3e** and **3f**). The guanidine **3e** showed increased potency against *P. aeruginosa* compared to **2e**, the other MIC-values on the other hand remained the same for the two structures. Attempting to functionalize **2f** with a guanidine (**3f**) led to reduced antimicrobial potency against all five bacteria. This reduction in potency

seen for **3f** may be attributed to the heavily lipophilic character of **2f**, as very lipophilic substrates are known to induce non-specific cell toxicity. The observed activities of **2f** may therefore come from general toxicity, and reducing the lipophilic character by introducing a guanidine function (**3f**) led to the observed reduction in activity.

Concerning the different lipophilic groups, the 4-butyl phenyl (**3d**, **3h**, and **3i**) and the 2-napthalene group (**3g**) was found to offer the highest antimicrobial potency, as they mostly displayed <10 µg/mL MIC-values against both Gram-positive and Gram-negative bacteria. The four isoindoline guanidines (**3d**, **3g**, **3h**, and **3i**) displayed the same high level of activity against *E. coli* (4 µg/mL) and *P. aeruginosa* (8 µg/mL, except **3i**: 16 µg/mL). Against the Gram-positive bacteria however, the isoindoline guanidine **3h** was seen to display the highest potency, with impressive MIC-values ranging from 1-4 µg/mL. The remaining three structures (**3d**, **3g**, and **3i**) had a very comparable level of activity which was lower than for **3h**, where **3d** was 2-fold more potent than **3g**/**3i** against *E. faecalis* and **3g** was 2-fold more potent than **3d**/**3i** against *S. agalacticae*.

Cytotoxicity

The 13 amphiphiles displaying interesting antimicrobial activity were evaluated for cytotoxicity against mammalian HepG2-cells, and the EC_{50}-values shown in Table 2 were determined from the dose-response curves shown in Fig. 3.

The guanidines **3** were found to be least toxic, but most of the isoindoline guanidines **3** still displayed EC_{50}-values below 10 µg/mL against HepG2-cells (except for **3d-f**). The isoindoline guanidine carrying one *t*-Bu-group (**3d**) was shown to give the best selectivity of the compounds, with a 1.5- to 6-fold preference for bacteria. The high toxicity may however indicate less specific modes of action for the antimicrobial activity, instead of the membrane targeting mechanism that is sought after for amphiphilic peptide mimics.

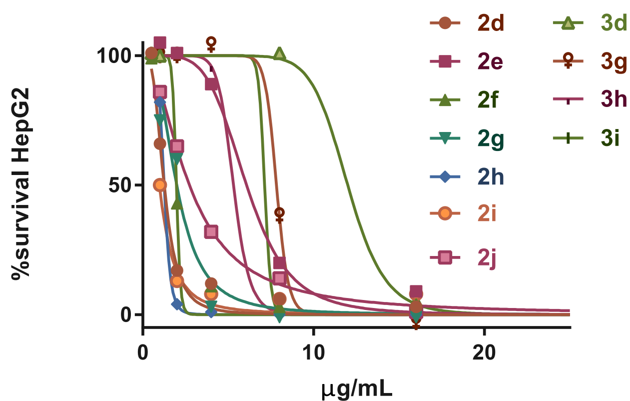


Figure 3 Dose-response curves for the amphiphiles taken into the HepG2-assay. Curves were not generated for: **3e** as EC_{50} >64 µg/mL, **3f** as 64 µg/mL displayed a 53% cell survival.

The isoindoline amines **2** were shown to display extensive toxicity towards human hepatic cells in this assay, and most of the amines **2** were shown to have EC_{50}-values below 3 µg/mL (except for **2e** at 6.1 µg/mL). The toxicity of the amines corresponded well with observations made in the preparation of a library of antimicrobial 1,2,3-triazoles currently undergoing publishing, where 1,2,3-triazole amine amphiphiles were shown to display higher toxicity than their corresponding guanidines.[52] These observations may be correlated to the increased lipophilicity and lowered polar surface area of **2** compared to **3**.[53,54] Additionally, as the guandines **3** are considered to be more basic molecules than the amines, they will also most likely be more prone to exist in their charged state under physiological conditions (pH = 7.4).[55,56]

The two isoindolines displaying the lowest toxicity in the HepG2-assay, were **3e** and **3f** with EC_{50}-values ranging higher than 64 µg/mL. The isoindoline **3e** displayed moderate antimicrobial potency, the low toxicity may therefore possibly be attributed to a generally low level of biological activity. The toxicity of **3f** on the other hand deviated from the common observations made regarding lipophilic character and toxicity, by displaying a 5-fold lowering in toxicity compared to that of **3d**. What caused **3f** to be less toxic than other active isoindolines is not known, as it was counterintuitive to what was expected.

Selectivity

The most selective amphiphile that showed broad-spectrum antimicrobial activity was the guanidine **3d** with a 1.5- to 6-fold selectivity towards bacteria compared to against mammalian cells. It should also be noted that the heavily lipophilic **3f** displayed highly selective antimicrobial activity against *S. agalacticae* with a MIC-value of 4 µg/mL and an EC_{50}-value of 64 µg/mL, giving a 16-fold selectivity towards *S. agalacticae* compared to human cells. Many of the other target amphiphiles in this library also displayed extensive activity in the antimicrobial assays, this high activity was unfortunately also reflected in the cytotoxicity assay (HepG2). The guanidines **3**, however, generally gave some measure of selectivity towards some of the bacteria. All of the amines **2**, on the other hand, were deemed unsuitable on background of their EC_{50}-values (HepG2) most often being lower than their observed MIC-values against bacteria.

3. Conclusion

We have successfully prepared a library of low molecular weight dihydro pyrrolopyridine and isoindoline amphiphiles for antimicrobial evaluation. The target compounds **1a**-**3i** were available from the important carbamate protected intermediates **8a**-**d** and **9a**-**j**, which in turn were prepared through [2+2+2] cycloaddition reactions (**8a**-**d** and **9a**-**f**) or Suzuki cross-coupling reactions (**9g**-**j**). The scope of the antimicrobial study was to evaluate the relative potencies of the two different scaffolds, in addition to evaluation of two different cationic nitrogen groups and 12 different lipophilic moieties. The 20 prepared amphiphiles were evaluated against three Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *S. agalacticae*), two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), and assessed for mammalian toxicity through the HepG2-assay. The most promising compound from the biological evaluation was the isoindoline guanidine **3d**, which showed high antimicrobial potency with MIC-values between 2-8 µg/mL. The antimicrobial potency of **3d** surpassed that of both ianthelline and synoxazolidinone A (except for ianthelline against *P. aeruginosa*, MIC: 7.5 µg/mL) and were 2-fold more potent against Gram-negative

bacteria than the most promising compound (**E23**) by Igumnova *et al*.[11,12,17] The low molecular complexity and high antimicrobial potency makes these isoindoline amphiphiles interesting for further investigations.

4. Experimental

General information

Chemicals were purchased from Sigma Aldrich and used without further purification. All reactions sensitive to air or moisture were performed under nitrogen atmosphere with dried solvents and reagents. Melting points were determined on a Buchi 535 apparatus and are uncorrected. TLC was performed on Merck silica gel 60 F_{254} plates, using UV light at 312 nm and a 5% solution of molybdophosphoric acid in 96% EtOH for detection. Column chromatography was performed with Silica gel (pore size 60 A, 230 - 400 mesh particle size) from Fluka. HPLC analyses were performed on an Agilent 1290 chromatograph equipped with a Zorbax Eclipse C18 5 µm (150 x 4.6 mm) column and a diode array detector (main detection region 214 nm). NMR spectra were recorded on a Bruker 600 MHz Avance III HD or a Bruker 400 MHz Avance III HD instrument. Chemical shifts (δ) are reported in parts per million. Where $CDCl_3$ has been used, shift values for proton are reported with reference to TMS (0.00) via the lock signal of the solvent. Reference values for other NMR-solvents are taken from Fulmer *et al.*[57] (^{1}H NMR: DMSO-d6: 2.49, MeOD-d4: 3.31; ^{13}C NMR: DMSO-d6: 39.5, $CDCl_3$: 77.0, MeOD-d4: 49.15). Signal patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), sex (sextet), h (heptet), m (multiplet), or br. s (broad singlet). ^{1}H and ^{13}C NMR signals were assigned by 2D correlation techniques (COSY, HSQC, HMBC). IR spectra were recorded from a Thermo Nicolet FT-IR NEXUS instrument or a Bruker Alpha FT-IR, and only the strongest/structurally most important peaks are listed as either weak (w), medium (m) or strong (s) (cm^{-1}). Accurate mass determination in positive and negative mode was performed on a “Synapt G2-S” Q-TOF instrument from Waters™. Samples were ionized by the use of ASAP probe (APCI) or ESI probe.

***N*-Boc-propargylamine.** The title compounds was prepared according to a procedure described by Molander *et al*.[48] Where di-*tert*-butyl dicarbonate (17.5 g, 80.0 mmol) in DCM (60 mL) was added propargylamine (5.12 mL, 80.0 mmol) at 0 °C under Ar-atm. The mixture was then stirred for 2 hours at room temperature before it was evaporated under reduced pressure, affording *N*-Boc-propargylamine as a yellow oil (12.3 g, 79.2 mmol, 99%). Spectroscopic data were in accordance with reported results.[48] ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.69 (br. s, 1H), 3.92 (br. s, 2H), 2.22 (app t, 1H, J = 2.5 Hz), 1.46 (s, 9H).

***tert*-Butyl di(prop-2-yn-1-yl)carbamate (5).** The title compound was prepared according to a procedure described by Liang *et al*.[39] Where *N*-Boc-propargylamine (3.99 g, 25.7 mmol) was dissolved in dry THF (60 mL) and added to NaH (0.93 g, 38.7 mmol) in dry THF (10 mL) dropwise at rt under Ar. The suspension was stirred for 2 hours. Propargyl bromide (4.43 mL, 41.1 mmol, 80% in toluene) was added dropwise to the suspension and the mixture was stirred at rt for 25 hours. The reaction mixture was concentrated under reduced pressure, quenched with NH_4Cl (sat., 100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with water (3 × 30 mL) and brine (30 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (n-pentane/EtOAc, 9:1), affording **5** as a brown oil (2.80 g, 14.5 mmol, 56%). Spectroscopic data were in accordance with reported results.[39] ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.17 (br. s, 4H, 2x CH_2), 2.23 (t, 2H, J = 2.5 Hz, 2x alkyne-H), 1.48 (s, 9H, *t*-Bu).

Method A: Synthesis of *tert*-butyl 6-(naphthalen-2-yl)-1,3-dihydro-2*H*-pyrrolo[3,4-c]pyridine-2-carboxylate (8a). Co-catalyzed cyclotrimerization was performed as a mixture of procecures described by Geny et al.,[43] Vollhardt,[21] and Bõnaga et al.,[44] with modifications. Where a flask containing **5** (0.15 g, 0.78 mmol) and dry, degassed 1,4-dioxane (15 mL) was added **6a** (0.18 mL, 1.16 mmol) and $CpCo(CO)_2$ (14 µL, 0.10 mmol) under Ar-atm. After complete addition, the reaction was irradiated by two halogen lamps (400 W, 118 nm, 50 Hz) for 46 hours. Concentration and purification with flash column chromatography (pentane/EtOAc, 7:3) afforded **8a** as a light yellow solid (0.153 g, 0.44 mmol, 57%). R_f (pentane/EtOAc, 7:3) 0.33. Mp: 83.0 – 90.7 °C. IR (ATR): 2970 (w), 2360 (w), 1683 (s), 1399 (m), 1254 (m), 1166 (m), 1115 (s) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.66 (d, 1H, *J* = 15.4 Hz, H_{Pyr}-4), 8.46 (d, 1H, *J* = 5.3 Hz, H_{Naph}-5), 8.12 (t, 1H, *J* = 7.5 Hz, H_{Naph}-4), 7.94 (d, 2H, *J* = 8.9 Hz, H_{Naph}-1 and H_{Naph}-3), 7.90 – 7.85 (m, 1H, H_{Naph}-6), 7.78 (d, 1H, *J* = 26.4 Hz, H_{Pyr}-7), 7.59 – 7.44 (m, 2H, H_{Naph}-7 and H_{Naph}-8), 4.83 – 4.70 (m, 4H, H_{Pyr}-1 and H_{Pyr}-3), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 156.5 (C_{Pyr}-6), 154.4* (C=O), 154.3* (C=O), 147.8* (C_{Pyr}-3a), 147.5* (C_{Pyr}-3a), 144.3* (C_{Pyr}-4), 144.1* (C_{Pyr}-4), 136.5* (Naph), 136.4* (Naph), 133.6 (Naph), 133.5 (Naph), 132.4* (C_{Pyr}-7a), 132.0* (C_{Pyr}-7a), 128.7 (Naph), 128.5 (Naph), 127.7 (Naph), 126.6 (Naph), 126.4 (Naph), 126.3 (Naph), 124.6 (Naph), 115.0* (C_{Pyr}-7), 114.8* (C_{Pyr}-7), 80.2 (Cq-*t*-Bu), 52.1* (C_{Pyr}-1/C_{Pyr}-3), 51.8* (C_{Pyr}-1/C_{Pyr}-3), 50.2* (C_{Pyr}-1/C_{Pyr}-3), 50.0* (C_{Pyr}-1/C_{Pyr}-3), 28.5 (*t*-Bu). HRMS (ESI+) *m/z* calcd. for $C_{22}H_{23}N_2O_2$ $[M+H]^+$: 347.1760; found: 347.1762. Spectral data for **8b**-**d** are assigned similarly to **8a**.

***tert*-Butyl 6-(4-(*tert*-butyl)phenyl)-1,3-dihydro-2*H*-pyrrolo[3,4-c]pyridine-2-carboxylate (8b).** Method A with **5** (1.008 g, 5.22 mmol), **6b** (1.30 mL, 7.67 mmol) and $CpCo(CO)_2$ (100 µL, 0.72 mmol) for 43 hours, afforded **8b** as a pale yellow solid (1.068 g, 3.03 mmol, 58%) after purification with flash column chromatography (pentane/EtOAc, 7:3). Mp 109.3 – 116.3 °C. R_f (pentane/EtOAc, 7:3) 0.38. IR (ATR): 2963 (w), 2359 (m), 2341 (w), 1697 (s), 1613 (w), 1477 (w), 1391 (s), 1252 (w), 1168 (m), 1109 (s), 1013 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.59 (d, 1H, *J* = 16.0 Hz, H_{Pyr}-4), 7.94 – 7.85 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.62 (d, 1H, *J* = 22.9 Hz, H_{Pyr}-7), 7.50 (d, 2H, *J* = 8.2 Hz, H_{Ph}-3 and H_{Ph}-5), 4.80 – 4.68 (m, 4H, H_{Pyr}-1 and H_{Pyr}-3), 1.53 (s, 9H, *t*-Bu), 1.36 (s, 9H, Carbamate *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 156.6, 154.4*, 154.3*, 152.2, 147.7*, 147.3*, 144.2*, 143.9*, 136.4*, 136.3*, 131.9*, 131.5*, 126.6, 125.8, 114.5*, 114.3*, 80.2, 52.0*, 51.8*, 50.2*, 49.9*, 34.7, 31.3, 28.5. HRMS (TOF ASAP+) *m/z* calcd. for $C_{22}H_{29}N_2O_2$ $[M+H]^+$: 353.2229; found: 353.2224.

***tert*-Butyl 6-(4-(*tert*-butyl)benzyl)-1,3-dihydro-2*H*-pyrrolo[3,4-c]pyridine-2-carboxylate (8c).** Method A with **5** (0.15 g, 0.78 mmol), **2c** (210 µL, 1.15 mmol), and $CpCo(CO)_2$ (14 µL, 0.10 mmol) for 46 hours, afforded **8c** as a brown oil (0.102 g, 0.28 mmol, 36%) after purification with flash column chromatography (pentane/EtOAc, 2:8). R_f (pentane/EtOAc, 2:8) 0.56. IR (neat): 2963 (w), 2866 (w), 1697 (s), 1617 (w), 1391 (s), 1253 (m), 1165 (s), 1109 (s), 1019 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.47 (d, 1H, *J* = 17.0 Hz, H_{Pyr}-4), 7.34 (d, 2H, *J* = 7.3 Hz, H_{Ph}-3 and H_{Ph}-5), 7.21 (t, 2H, *J* = 7.1 Hz, H_{Ph}-2 and H_{Ph}-6), 7.03 (d, 1H, *J* = 32.5 Hz, H_{Pyr}-7), 4.65 (d, 2H, *J* = 13.2 Hz, H_{Pyr}-3), 4.60 (d, 2H, *J* = 16.9 Hz, H_{Pyr}-1), 4.15 (s, 2H, H_{Bn}), 1.50 (d, *J* = 4.5 Hz, 9H, carbamate *t*-Bu), 1.32 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 160.1, 154.3, 149.3, 147.5*, 147.2*, 143.8*, 143.6*, 136.3, 131.3*, 130.9*, 128.7, 125.6, 117.4*, 117.1*, 80.1, 51.9*, 51.6*, 50.1*, 49.8*, 44.0, 34.4, 31.4, 28.5. HRMS (TOF ASAP+) *m/z* calcd. for $C_{23}H_{31}N_2O_2$ $[M+H]^+$: 367.2386; found: 367.2379.

***tert*-Butyl 6-(3,5-bis(trifluoromethyl)benzyl)-1,3-dihydro-2*H*-pyrrolo[3,4-c]pyridine-2-carboxylate (8d).** Method A with **5** (0.156 g, 0.83 mmol), **6d** (210 µL, 1.15 mmol), and $CpCo(CO)_2$ (14 µL, 0.10 mmol) for 48 hours, afforded **8d** as a brown oil (0.130 g, 0.29 mmol, 35%) after purification with flash column

chromatography (pentane/EtOAc, 1:1). R_f (pentane/EtOAc, 1:1) 0.42. IR (ATR): 2980 (w), 1782 (w), 1697 (m), 1621 (w), 1456 (w), 1372 (m), 1275 (s), 1165 (s), 1124 (s) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (d, 1H, J = 17.4 Hz, H_{Pyr}-4), 7.74 (s, 1H, H_{Ph}-4), 7.72 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 7.09 (d, 1H, J = 36.4 Hz, H_{Pyr}-7), 4.68 (t, 4H, J = 15.7 Hz, H_{Pyr}-1 and H_{Pyr}-3), 4.27 (s, 2H, H_{Bn}), 1.51 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 157.5, 154.3*, 154.2*, 148.1*, 147.7*, 144.5*, 144.3*, 141.9, 132.3*, 131.9*, 131.8 (q, J_{CF} = 33.3 Hz), 129.3 – 129.1 (m), 123.3 (q, J_{CF} = 279.9 Hz), 120.6 (p, J_{CF} = 3.7 Hz), 117.6*, 117.3*, 80.3, 51.9*, 51.6*, 50.1*, 49.9*, 43.9, 28.5. HRMS (TOF ASAP+) *m/z* calcd. for $C_{21}H_{21}N_2O_2F_6$ $[M+H]^+$: 447.1507; found: 447.1503.

Method B: synthesis of *tert*-butyl 5-phenylisoindoline-2-carboxylate (9a). Ru-catalyzed cyclotrimerization as described by Yamamoto et al.,[24,25] with slight modifications. Where a flask containing Cp*Ru(cod)Cl (20 mg, 0.05 mmol) was added a solution of **5** (0.193 g, 1.00 mmol, in 3 mL dry, degassed DCE), **7a** (0.165 mL, 1.50 mmol) and dry, degassed DCE (2 mL) followed by stirring for 21 hours under Ar-atm at room temperature. Concentration and purification by flash column chromatography (EtOAc/pentane 1:9) afforded **9a** as a brown solid (0.162 g, 0.55 mmol, 55%). Spectroscopic data were in accordance with published data.[37] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 – 7.56 (m, 2H, H_{Ph}-2 and H_{Ph}-6), 7.51 – 7.49 (m, 1H, H_{Iso}-7), 7.46 – 7.42 (m, 3H, H_{Ph}-3, H_{Ph}-5 and H_{Iso}-4), 7.35 (t, 1H, J = 7.5 Hz, H_{Ph}-4), 7.30 (d, 1H, J = 8.3 Hz, H_{Iso}-6), 4.75 (d, 2H, J = 8.4 Hz, H_{Iso}-3), 4.71 (d, 2H, J = 6.8 Hz, H_{Iso}-1), 1.53 (s, 9H, *t*-Bu).

***tert*-Butyl 5-benzylisoindoline-2-carboxylate (9b).** Method B with **5** (0.194 g, 1.00 mmol), **7b** (187 µL, 1.50 mmol) and Cp*Ru(cod)Cl (19.2 mg, 0.05 mmol) for 23 hours, afforded **9b** as a yellow solid (0.149 g, 0.50 mmol, 48% yield) after purification with flash column chromatography (EtOAc/pentane, 1:9). Mp 97.7 – 100.9 °C. R_f (EtOAc/pentane, 1:9) 0.29. IR (ATR): 2972, 2927 (m), 2860 (m), 1683 (s), 1401 (s), 1171 (s), 1105 (s) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, rotamers*): δ 7.31 – 7.27 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.22 – 7.17 (m, 3H, H_{Ph}-2, H_{Ph}-4 and H_{Ph}-6), 7.14 – 7.09 (m, 2H, H_{Iso}-6 and H_{Iso}-7), 7.02 (br. s, 1H, H_{Iso}-4), 4.64 (s, 2H, H_{Iso}-3), 4.60 (s, 2H, H_{Iso}-1), 3.98 (s, 2H, H_{Bn}), 1.51* (s, 9H, *t*-Bu), 1.50* (s, 9H, *t*-Bu). ^{13}C (NMR, 100 MHz, $CDCl_3$, rotamers*): δ 154.6 (C=O), 141.0 (C_{Iso}-5), 140.6 (C_{Ph}-1), 137.7 (C_{Iso}-3a and C_{Iso}-7a), 128.9 (C_{Ph}-4), 128.5 (C_{Ph}-3 and C_{Ph}-5), 128.1 (C_{Iso}-6), 126.2 (C_{Ph}-2 and C_{Ph}-6), 123.2* (C_{Iso}-7), 123.0*(C_{Iso}-7), 122.8* (C_{Iso}-4), 122.5* (C_{Iso}-4), 79.6 (Cq-*t*-Bu), 52.3* (C_{Iso}-3), 52.1* (C_{Iso}-3), 52.0* (C_{Iso}-1), 51.8* (C_{Iso}-1), 41.8 (C_{Bn}), 28.6 (*t*-Bu). HRMS (TOF ASAP+) *m/z* calcd. for $C_{16}H_{16}NO_2$ $[M-C_4H_7]^+$: 254.1181; found: 254.1186. Spectral data for **9c**-**f** are assigned similarly to **9b**.

***tert*-Butyl 5-phenethylisoindoline-2-carboxylate (9c).** Method B with **5** (0.197 g, 1.02 mmol), **7c** (211 µL, 1.50 mmol), and Cp*Ru(cod)Cl (19.8 mg, 0.052 mmol) for 18 hours, afforded **9c** as a yellow solid (257 mg, 0.79 mmol, 78%) after purification with flash column chromatography (EtOAc/pentane, 1:9). Mp 76.3 – 80.2 °C. R_f (EtOAc/pentane, 1:9) 0.39. IR (ATR): 2979 (m), 2913 (m), 2855 (m), 1686 (s), 1494 (w), 1471 (w), 1449 (w), 1398 (s), 1171(s), 1106 (s) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 – 7.26 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.21 – 7.16 (m, 3H, H_{Ph}-2, H_{Ph}-4 and H_{Ph}-6), 7.13 – 7.06 (m, 2H, H_{Iso}-6 and H_{Iso}-7), 7.02 (s, 1H, H_{Iso}-4), 4.65 (s, 2H, H_{Iso}-1), 4.61 (br. s, 2H, H_{Iso}-3), 2.94 – 2.89 (m, 4H, CH_2-CH_2), 1.52 (s, 9H, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 141.6, 141.1, 137.5, 137.2, 134.6, 128.5, 128.4, 127.7*, 127.6*, 126.0, 122.8*, 122.6*, 122.5*, 122.4*, 79.6, 52.2*, 52.1*, 51.9*, 51.8*, 38.1, 37.8, 28.6. HRMS (TOF ASAP+) *m/z* calcd. for $C_{17}H_{18}NO_2$ $[M-C_4H_7]^+$: 268.1338; found: 268.1335.

***tert*-Butyl 5-(4-(tert-butyl)phenyl)isoindoline-2-carboxylate (9d).** Method B with **5** (0.193 g, 1.00 mmol), **7d** (271 µL, 1.50 mmol), and Cp*Ru(cod)Cl (20.3 mg, 0.053 mmol) for 22 hours, afforded **9d** as a beige solid (159 mg, 0.45 mmol, 45%) after being purified with flash column chromatography twice (EtOAc/pentane, 1:9). Mp 148.3 – 158.0 °C. R_f (EtOAc/pentane, 1:9) 0.32. IR (ATR): 2957 (m), 2858 (m),

1686 (s), 1466 (w), 1397(s), 1173 (s), 1115 (s) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 – 7.46 (m, 5H, H_{Ph}-2, H_{Ph}-3, H_{Ph}-5, H_{Ph}-6 and H_{Iso}-7), 7.43 (br. s, 1H, H_{Iso}-4), 7.33 (dd, 1H, *J* = 7.9, 30.6 Hz, H_{Iso}-6), 4.74 (d, 2H, *J* = 11.8 Hz, H_{Iso}-3), 4.70 (d, 2H, *J* = 11.5, H_{Iso}-1), 1.53 (s, 9H, carbamate *t*-Bu), 1.36 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, $CDCl_3$, rotamers*): δ 154.6, 150.4, 140.6, 138.0, 137.7, 136.2*, 135.8*, 126.8, 126.4*, 126.3*, 125.8, 123.0*, 122.8*, 121.3*, 121.1*, 79.7, 52.4*, 52.2*, 52.1*, 51.8*, 34.6, 31.4, 28.6. HRMS (TOF ASAP+) *m/z* calcd. for $C_{19}H_{22}NO_2$ $[M-C_4H_7]^+$: 296.1651; found: 296.1652.

***tert*-Butyl 5-(3,5-bis(trifluoromethyl)phenyl)isoindoline-2-carboxylate (9e).** Method B with **5** (0.187 g, 0.97 mmol), **7e** (257 µL, 1.45 mmol), and Cp*Ru(cod)Cl (19.7 mg, 0.052 mmol) for 32 hours, afforded **9e** as a brown solid (230 mg, 0.53 mmol, 55%) after purification with flash column chromatography (EtOAc/pentane, 1:9). Mp 113.2 – 126.3 °C. R_f (EtOAc/pentane, 1:19) 0.33. IR (ATR): 2970 (m), 1691 (s), 1400 (s), 1373 (s), 1277 (s), 1109 (s), 1047 (s) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, 2H, *J* = 5.9 Hz, H_{Ph}-2 and H_{Ph}-6), 7.86 (s, 1H, H_{Ph}-4), 7.52 – 7.51 (m, 1H, H_{Iso}-7), 7.46 (s, 1H, H_{Iso}-4), 7.42 (dd, 1H, *J* = 7.9, *J* = 30.5 Hz, H_{Iso}-6), 4.78 (d, 2H, *J* = 13.3 Hz, H_{Iso}-3), 4.74 (d, 2H, *J* = 8.9 Hz, H_{Iso}-1), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 154.5, 143.1*, 143.0*, 138.8, 138.4*, 138.3*, 137.8*, 137.7*, 132.2 (q, J_{CF} = 33.0 Hz), 127.2, 126.7*, 126.6*, 123.7*, 123.4*, 123.3 (q, J_{CF} = 272.6 Hz), 121.7*, 121.4*, 121.0 (p, J_{CF} = 4.0 Hz) , 80.0, 52.2*, 52.1*, 52.0*, 51.8*, 28.5. HRMS (TOF ASAP+) *m/z* calcd. for $C_{17}H_{12}NO_2F_6$ $[M-C_4H_7]^+$: 376.0772; found: 376.0771.

***tert*-Butyl 5-(3,5-di-tert-butylphenyl)isoindoline-2-carboxylate (9f).** Method B with **5** (0.25 g, 1.29 mmol), **7f** (0.416 g, 1.94 mmol), and Cp*Ru(cod)Cl (21.5 mg, 0.058 mmol) for 24 hours, afforded **9f** as a white solid (235 mg, 0.58 mmol, 45%) after purification with flash column chromatography (Et_2O/pentane, 1:9). Mp 155.2 – 158.0 °C. R_f (Et_2O/pentane, 1:9) 0.18. IR (ATR): 2962 (m), 1700 (s), 1393 (s), 1364 (m), 1171 (m), 1109 (s), 873 (m), 712 (m) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 – 7.42 (m, 3H, H_{Ph}-4, H_{Iso}-6 and H_{Iso}-7), 7.39 (t, 2H, *J* = 1.34 Hz, H_{Ph}-2 and H_{Ph}-6), 7.31 (dd, 1H, *J* = 8.1 Hz, *J* = 20.2 Hz, H_{Iso}-4), 4.78 – 4.67 (m, 4H, H_{Iso}-1 and H_{Iso}-3), 1.53 (s, 9H, carbamate *t*-Bu), 1.38 (s, 18H, 2x *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, rotamers*): δ 154.6, 151.2, 142.1*, 142.0*, 140.4, 138.0*, 137.6*, 136.1*, 135.8*, 126.8, 122.9*, 122.7*, 121.8, 121.7, 121.5, 79.7, 52.4*, 52.2*, 52.1*, 51.8*, 35.0, 31.5, 28.6. HRMS (TOF ASAP+) *m/z* calcd. for $C_{23}H_{30}NO_2$ $[M-C_4H_7]^+$: 352.2280; found: 352.2277.

***tert*-Butyl 5-bromoisoindoline-2-carboxylate (10).** The title compound **10** was prepared according to a procedure by Patel *et al.*[45] Where a suspension of 5-bromophthalimide (**4**, 8.05 g, 35.6 mmol) in THF (350 mL) was added $NaBH_4$ (15 g, 396.5 mmol), before BF_3 x OEt_2 (52 mL, 421 mmol) was added slowly at -10 °C. Upon completed addition, the mixture was heated to 70 °C for 21 hours before it was cooled to 0 °C and carefully quenched with H_2O (150 mL). The aqueous suspension was then diluted with EtOAc (350 mL) and the pH was adjusted to 10 using NaOH (6 M, aq), before the organic phase was washed with brine (3 x 200 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The green crude oil was then added Et_2O (200 mL) and H_2O (200 mL), before the pH was adjusted to 2 using HCl (6 M, aq). The separated aqueous phase was then made alkaline (pH= 10) using NaOH (6 M, aq) before it was extracted with EtOAc (3 x 100 mL) and the combined organic phases were washed with brine (3 x 200 mL). Drying over $MgSO_4$, filtration, and evaporation under reduced pressure afforded 5-bromoisoindoline as a brown oil. The crude 5-bromoisoindoline in DMF (100 mL) was added Boc_2O (8.10 g, 37.1 mmol) and catalytic amounts of 4-DMAP (2-3 crystals) before it was stirred for 41 hours at room temperature. The reaction mixture was then diluted with EtOAc (200 mL) and washed with brine (3 x 150 mL), before it was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude was purified with flash column chromatography (EtOAc/pentane, 1:9), affording **10** as a yellow solid (6.03 g, 20.2 mmol,

57%). ^{1}H NMR analyses were in accordance with published data. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.42 (s, 0.5H, rotamer, H_{Iso}-4), 7.40 – 7.38 (m, 1H, H_{Iso}-6), 7.36 (s, 0.5H, rotamer, H_{Iso}-4), 7.14 (d, 0.5H, rotamer, *J* = 8.0 Hz, H_{Iso}-7), 7.09 (d, 0.5H, rotamer, *J* = 8.0 Hz, H_{Iso}-7), 4.67 – 4.59 (m, 4H, H_{Iso}-1 and H_{Iso}-3), 1.51 (s, 9H, *t*-Bu).

Method C: synthesis of *tert*-butyl 5-(naphthalen-2-yl)isoindoline-2-carboxylate (9g). Suzuki cross-coupling using a procedure described by Hua *et al*.[46] with modifications by Bugge *et al*.[47] Where a mixture of carbamate protected bromide **10** (0.500 g, 1.68 mmol), **11g** (0.354 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.53 mmol), and $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol) was added degassed 1,4-dioxane/H_2O (40 mL, 1:1) under Ar-atm. before it was refluxed for 24 hours. Upon cooling to room temperature, the reaction mixture was added H_2O (50 mL) and extracted with DCM (3 x 50 mL). The combined organic phases were washed with H_2O (2 x 50 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification of the crude with flash column chromatography (EtOAc/pentane, 1:9) afforded **9g** as a white solid (0.470 g, 1.36 mmol, 82%). Mp 116-118 °C. IR (ATR): 3054 (w), 2973 (w), 2918 (w), 2861 (w), 1692 (s), 1390 (s), 1363 (s), 1255 (m), 1167 (s), 1104 (s), 876 (m), 807 (s), 744 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.02 (d, 1H, *J* = 7.7 Hz, Naph), 7.92 – 7.86 (m, 3H, Naph), 7.73 – 7.71 (m, 1H, Naph), 7.63 (app d, 1H, *J* = 8.0 Hz, H_{Iso}-6), 7.59 (app d, 1H, *J* = 7.9 Hz, H_{Iso}-4), 7.49 (p, 2H, *J* = 8.0 Hz, Naph), 7.39 (d, 0.5H, *J* = 7.9 Hz, rotamer H_{Iso}-7), 7.33 (d, 0.5H, *J* = 8.0 Hz, rotamer H_{Iso}-7), 4.78 – 4.71 (m, 4h, H_{Iso}-1 and H_{Iso}-3), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 150 MHz, rotamers*): δ 154.6, 140.7*, 138.3*, 138.2*, 137.8*, 136.5*, 136.2*, 133.7, 132.6, 128.5, 128.2, 127.7, 126.8*, 126.8*, 126.4, 126.0, 125.8, 125.5, 123.2*, 123.0*, 121.8*, 121.5*, 79.8, 52.4*, 52.1*, 52.1*, 51.9*, 28.6. HRMS (TOF ASAP+) m/z calcd. for $C_{19}H_{16}NO_2$ $[M-C_8H_7]^+$: 290.1181; found: 290.1178.

***tert*-Butyl 5-(4-butylphenyl)isoindoline-2-carboxylate (9h).** Method C with **10** (0.500 g, 1.68 mmol), **11h** (0.350 g, 1.97 mmol), K_2CO_3 (0.346 g, 2.53 mmol), $Pd(PPh_3)_4$ (0.100 g, 2.53 mmol), and 23 hours at reflux, afforded **9h** as an off-white solid (0.421 g, 1.20 mmol, 72%) after purification with flash column chromatography (EtOAc/pentane, 1:9). MP 75 – 78 °C. IR (ATR): 2988 (m), 2950 (m), 2928 (m), 1738 (m), 1699 (s), 1474 (m), 1392 (s), 1366 (s), 1256 (m), 1165 (m), 1107 (s), 874 (m), 808 (s), 777 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.49-7.47 (m, 3H, H_{Ar}-2, H_{Ar}-6 and H_{iso}- 6), 7.45 (app d, 1H, *J* = 33.4 Hz, H_{iso}-4), 7.32 (d, 0.5H, *J* = 7.9 Hz, rotamer, H_{iso}-7), 7.27 (app d, 0.5H, app *J* = 7.9 Hz, rotamer, H_{iso}-7), 7.26-7.24 (m, 2H, H_{Ar}-3 and H_{Ar}-5), 4.74-4.68 (m, 4H, H_{iso}-1 and H_{iso}-3), 2.65 (t, 2H, *J* = 7.7 Hz, CH_2), 1.63 (p, 2H, *J* = 6.9 Hz, CH_2), 1.53 (s, 9H, *t*-Bu), 1.39 (sex, 2H, *J* = 7.2 Hz, CH_2), 0.95 (t, 3H, *J* = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz, rotamers*): δ 154.6 (C=O), 142.2 (C_{Ar}-4), 140.7 (C_{iso}-5), 138.3* (C_{Ar}-1), 138.2* (C_{Ar}-1), 138.0* (C_{iso}-3a), 137.6* (C_{iso}-3a), 136.1* (C_{iso}-7a), 135.8* (C_{iso}-7a), 128.9 (C_{Ar}-3 and C_{Ar}-5), 127.0 (C_{Ar}-2 and C_{Ar}-6), 126.4* (C_{Iso}-6), 126.3* (C_{Iso}-6), 123.0* (C_{iso}-7), 122.8* (C_{iso}- 7), 121.3* (C_{iso}-4), 121.6* (C_{iso}-4), 79.7 (Cq-*t*-Bu), 52.4* (C_{iso}-3), 52.1* (C_{iso}-3), 52.1* (C_{iso}-1), 51.8*(C_{iso}-1), 35.3, (CH_2) 33.7 (CH_2), 28.6 (*t*-Bu), 22.4 (CH_2), 14.0 (CH_3). HRMS (TOF ASAP+) m/z calcd. for $C_{19}H_{22}NO_2$ $[M-C_8H_7]^+$: 296.1651; found: 296.1650. Spectral data for **9g** and **9i**-**j** are assigned similarly to **9h**.

***tert*-Butyl 5-(4-butoxyphenyl)isoindoline-2-carboxylate (9i).** Method C with **10** (0.516 g, 1.73 mmol), **11i** (0.388 g, 2.00 mmol), K_2CO_3 (0.346 g, 2.53 mmol), $Pd(PPh_3)_4$ (0.100 g, 2.53 mmol), and 22 hours at reflux, afforded **9i** as a blue solid (0.453 g, 1.23 mmol, 71%) after purification with flash column chromatography (EtOAc/pentane, 1:9). Mp 121 - 123 °C. IR (ATR): 2957 (w), 2871 (w), 1684 (s), 1607 (w), 1519 (m), 1471 (m), 1400 (s), 1247 (s), 1180 (s), 1111 (s), 880 (m), 816 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.50-7.47 (m, 2H, H_{Ar}-2 and H_{Ar}-6), 7.44 (app. d, 1H, app *J* = 7.9 Hz, H_{iso}-6), 7.41 (app d, 1H, app *J* = 17.0

Hz, H_{iso}-4), 7.30 (d, 0.5H, *J* = 7.9 Hz, rotamer, H_{iso}-7), 7.25 (app d, 0.5H, app *J* = 7.9 Hz, rotamer, H_{iso}-7), 6.96 (d, 2H, *J* = 7.5 Hz, H_{Ar}-3 and H_{Ar}-5), 4.73- 4.67 (m, 4H, H_{iso}-1 and H_{iso}-3), 4.00 (t, 2H, *J* = 6.5 Hz, OCH_2), 1.79 (p, 2H, *J* = 6.5 Hz, CH_2), 1.53 (s, 9H, *t*-Bu), 1.52 (app sex, 2H, *J* = 7.4 Hz, CH_2), 0.99 (t, 3H, *J* = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz, rotamers*): *δ* 158.8, 154.6, 140.5, 138.0*, 137.6*, 135.7*, 135.4*, 133.3*, 133.2*, 128.1, 126.1*, 126.0, 123.0*, 122.8*, 121.0*, 120.8, 114.8, 79.7, 67.8, 52.4*, 52.1*, 52.1*, 51.8*, 31.4, 28.6, 19.3, 13.9. HRMS (TOF ASAP+) m/z calcd. for $C_{19}H_{22}NO_3$ $[M-C_8H_7]^+$: 312.1600; found: 312.1598.

***tert*-Butyl 5-(4-(trifluoromethyl)phenyl)isoindoline-2-carboxylate (9j).** Method C with **10** (0.560 g, 1.88 mmol), **11j** (0.420, 2.21 mmol), K_2CO_3 (0.346 g, 2.53 mmol), $Pd(PPh_3)_4$ (0.100 g, 0.087 mmol), and 22 hours at reflux, afforded **9j** as a pink solid (0.529 g, 1.46 mmol, 77%) after purification with flash column chromatography (EtOAc/pentane, 1:4). Mp 135 – 141 °C. IR (ATR): 2989 (w), 2937 (w), 2872 (w), 1738 (w), 1683 (m), 1613 (m), 1403 (m), 1367 (m), 1325 (s), 1158 (m), 1111 (s), 1070 (m), 1012 (m), 876 (m), 849 (m), 814 (m), 771 (w), 713 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): *δ* 7.70 - 7.66 (m, 4H, H_{Ph}), 7.50 (s, 1H, H_{Iso}-6), 7.47 (app d, 1H, *J* = 34.4 Hz, H_{Iso}-4), 7.38 (d, 0.5H, *J* = 7.8 Hz, H_{Iso}-7), 7.32 (d, 0.5H, *J* = 8.4 Hz, H_{Iso}-7), 4.76 – 4.72 (m, 4H, H_{Iso}-1 and H_{Iso}-3), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 150 MHz, rotamers*): *δ* 154.5, 144.5*, 144.4*, 139.3, 138.4*, 138.0*, 137.5*, 137.0*, 129.5 (q, J_{CF} = 32.2 Hz), 127.4, 126.7*, 126.6*, 125.8, 124.2 (q, J_{CF} = 272.7 Hz), 123.4*, 123.1*, 121.7*, 121.4*, 79.9, 52.3*, 52.1*, 52.0*, 51.8*, 28.6. HRMS (TOF ASAP+) m/z calcd. for $C_{16}H_{13}NOF_3$ $[M-C_8H_7]^+$: 308.0898; found: 308.0898.

6-(Naphthalen-2-yl)-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-2-ium chloride (1a). The carbamate protected **8a** (0.101 g, 0.29 mmol) in MeCN (10 mL) was added HCl (44 µL, 0.54 mmol, 37% aq) in two portions and stirred for 45 hours at room temperature. Evaporation under reduced pressure and purification with flash column chromatography ($CHCl_3$/MeOH 7:3) afforded **1a** as a white solid (0.071 g, 0.25 mmol, 86%). Mp 209.0 – 215.0 °C. R_f (CH_3Cl/MeOH, 7:3) 0.64. IR (ATR): 2668 (w), 1617 (w), 1605 (m), 1393 (w), 1276 (w), 1138 (w) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): *δ* 9.77 (br. s, 2H, NH_2^+), 8.76 (s, 1H, H_{Pyr}-4), 8.66 (d, 1H, *J* = 1.1 Hz, H_{Naph}-5), 8.25 (dd, 1H, *J* = 8.6, *J* = 1.9 Hz, H_{Naph}-4), 8.21 (s, 1H, H_{Pyr}-7), 8.07 – 8.02 (m, 2H, H_{Naph}-1 and H_{Naph}-3), 7.97 (t, 1H, *J* = 4.9 Hz, H_{Naph}-6), 7.60 – 7.56 (m, 2H, H_{Naph}-7 and H_{Naph}-8), 4.63 (s, 4H, H_{Pyr}-1 and H_{Pyr}-3). ^{13}C NMR (150 MHz, DMSO-d_6): *δ* 155.7 (C_{Pyr}-6), 146.5 (C_{Pyr}-3a), 144.3 (C_{Pyr}-4), 135.6 (Naph), 133.4 (Naph), 133.1 (Naph), 130.8 (C_{Pyr}-7a), 128.7 (Naph), 128.5 (Naph), 127.6 (Naph), 126.7 (2x Naph), 126.0 (Naph), 124.7 (Naph), 115.0 (C_{Pyr}-7), 50.1 (C_{Pyr}-1/ C_{Pyr}-3), 48.7 (C_{Pyr}-1/ C_{Pyr}-3). HRMS (TOF ASAP+) *m/z* calcd. for $C_{17}H_{15}N_2$ $[M-Cl]^+$: 247.1230; found: 247.1227. HPLC: (MeOH/H_2O, 1:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 9.3 min, 92% pure. Spectral data for **1b**-**d** are assigned similarly to **1a**.

Method D: synthesis of 6-(4-(*tert*-Butyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-2-ium chloride (1b). Carbamate deprotection using aqueous HCl in MeCN. Where **8b** (0.497 g, 1.41 mmol) in MeCN (30 mL) was added HCl (0.142 mL, 1.73 mmol, 37% aq) and stirred for 23 hours at room temperature. The evaporated reaction mixture was washed with DCM and filtered, affording **1b** as a grey solid (0.408 g, 1.41 mmol, 100%) after drying. Mp >212 °C (decomp.). R_f (CH_3Cl/MeOH/NH_3 (25% aq), 70:30:3) 0.58. IR (ATR): 2949 (w), 2698 (w), 1603 (m), 1415 (m), 1348 (w), 1271 (w), 1206 (w), 1200 (w), 1111 (w), 1014 (w) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): *δ* 9.96 (br. s, 2H, NH_2^+), 8.71 (s, 1H, H_{Pyr}-4), 8.04 (s, 1H, H_{Pyr}-7), 8.01 (d, 2H, *J* = 8.5 Hz, H_{Ph}-2 and H_{Ph}-6), 7.55 (d, 2H, *J* = 8.5 Hz, H_{Ph}-3 and H_{Ph}-5), 4.64 – 4.59 (m, 4H, H_{Pyr}-1 and H_{Pyr}-3), 1.33 (s, 9H, *t*Bu). ^{13}C NMR (150 MHz, DMSO-d_6): *δ* 155.5, 152.3, 146.8, 143.7, 135.0, 130.4, 126.5, 125.8, 114.7, 50.5, 48.5, 34.5, 31.2. HRMS (TOF ASAP+) *m/z* calcd. for $C_{17}H_{21}N_2$ $[M-Cl]^+$: 253.1699; found: 253.1700. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 4.8 min, 97% pure.

6-(4-(*tert*-Butyl)benzyl)-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-2-ium chloride (1c). Method D with **8c** (0.333, 0.91 mmol), HCl (0.135 mL, 1.64 mmol, 37% aq), and 45 hours of stirring afforded **1c** as a grey solid (0.147 g, 0.49 mmol, 54%). Mp >225 °C (decomp.). R_f ($CH_3Cl/MeOH/NH_3$ (25% aq), 70:30:3) 0.71. IR (ATR): 3055 (m), 3030 (m), 2959 (m), 2872 (m), 2687 (m), 2620 (m), 2573 (m), 2509 (m), 2268 (br. m), 2045 (s), 1962 (m), 1647 (w), 1619 (s), 1582 (m), 1514 (m), 1464 (s), 1415 (s), 1362 (m), 1320 (s), 1268 (m), 1179 (s), 1109 (m), 1020 (w) cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 10.27 (br. s, 2H, NH_2^+), 8.71 (s, 1H, H_{Pyr}-4), 7.69 (s, 1H, H_{Pyr}-7), 7.35 (d, 2H, J = 8.3 Hz, H_{Ph}-3 and H_{Ph}-5), 7.28 (d, 2H, J = 8.2 Hz, H_{Ph}-2 and H_{Ph}-6), 4.61 (d, 4H, J = 18.6 Hz, H_{Pyr}-1 and H_{Pyr}-3), 4.28 (s, 2H, H_{Bn}), 1.25 (s, 9H, *t*Bu). ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.7, 151.3, 149.3, 139.6, 134.7, 131.8, 128.7, 125.6, 119.7, 50.2, 48.3, 40.1, 34.2, 31.2. HRMS (TOF ASAP+) *m/z* calcd. for $C_{18}H_{23}N_2$ [M-Cl]$^+$: 267.1861; found: 268.1860. HPLC: ($MeOH/H_2O$, 1:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 21.5 min, 99% pure.

6-(3,5-Bis(trifluoromethyl)benzyl)-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-2-ium chloride (1d). Method D with **8d** (0.056 g, 0.125 mmol), HCl (10 µL, 0.12 mmol, 37% aq), and 45 hours of stirring afforded **1d** as a grey solid (46.5 mg, 0.122 mmol, 97%). Mp >217 °C (decomp.). R_f ($CH_3Cl/MeOH/NH_3$ (25% aq), 70:30:3) 0.66. IR (ATR): 3386 (w), 3101 (w), 2576 (w), 2306 (w), 1648 (w), 1617 (m), 1535 (w), 1466 (w), 1378 (m), 1277 (s), 1185 (s), 1138 (s), 1118 (s), 1108 (s), 1051 (w), 1008 (w) cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 9.74 (br. s, 2H, NH_2^+), 8.58 (s, 1H, H_{Pyr}-4), 8.03 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 7.97 (s, 1H, H_{Ph}-4), 7.50 (s, 1H, H_{Pyr}-7), 4.57 – 4.50 (m, 4H, H_{Pyr}-1 and H_{Pyr}-3), 4.39 (s, 2H, H_{Bn}). ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.3, 148.9, 142.1, 131.1, 130.5, 130.3 (3x C), 124.3 (q, J_{CF} = 272.9 Hz), 122.5, 120.6, 118.8, 50.0, 48.4, 40.8. HRMS (TOF ASAP+) *m/z* calcd. for $C_{16}H_{13}N_2F_6$ [M-Cl]$^+$: 347.0983; found: 347.0978. HPLC: ($MeOH/H_2O$, 1:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 34.7 min, 99% pure.

5-phenylisoindolin-2-ium chloride (2a). The carbamate protected **9a** (0.304 g, 1.03 mmol) was added to HCl (10 mL, 20 mmol, 2M in Et_2O) and stirred for 22 hours at room temperature, before addition of more HCl (0.174 mL, 2.09 mmol, 37% aq) and two hours additional stirring. The reaction mixture was then filtered and the precipitate washed with Et_2O, affording **2a** as a grey solid (0.198 g, 0.85 mmol, 83%) upon drying. Mp 204 – 206 °C. R_f ($CHCl_3/MeOH/NH_3$ (25% aq), 70:30:3) 0.29. Spectroscopic data were in accordance with reported spectra.[49] ^1H NMR (600 MHz, DMSO): δ 9.83 (br. s, 2H, NH_2^+), 7.70 (br. s, 1H, H_{Iso}-4), 7.67 – 7.65 (m, 3H, H_{Iso}-7, H_{Ph}-2 and H_{Ph}-6), 7.50 – 7.47 (m, 3H, H_{Iso}-6, H_{Ph}-3 and H_{Ph}-5), 7.40 – 7.38 (m, 1H, H_{Ph}-4), 4.55 (d, 4H, J = 9.4 Hz, H_{Iso}-1 and H_{Iso}-3). HPLC: ($MeOH/H_2O$ 1:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 7.0 min, 99% pure.

5-Benzylisoindolin-2-ium chloride (2b). The carbamate protected **9b** (0.469 g, 1.52 mmol) was added to HCl (13.5 mL, 27 mmol, 2M in Et_2O) and stirred for 22 hours at room temperature, before addition of more HCl (2.25 mL, 27.5 mmol, 37% aq) and 22 hours additional stirring. The reaction mixture was then filtered, whereupon the filtrate was added more HCl (2.00 mL, 24.4 mmol, 37% aq) and stirred for 27 hours at room temperature. A second filtration and combination of the precipitates afforded **2b** as a grey solid (0.343 g, 1.40 mmol, 92%). Mp 240 – 244 °C. R_f ($CHCl_3/MeOH/NH_3$ (25% aq), 70:30:3) 0.61. IR (ATR): 2877 (m), 2684 (m), 2578 (m), 2477 (m), 1583 (m), 1490 (m), 1453 (m), 1427 (m), 1343 (s) cm^{-1}. ^1H NMR (600 MHz, DMSO): δ 9.68 (br. s, 2H, NH_2^+), 7.32 – 7.27 (m, 3H H_{Iso}-7, H_{Ph}-3 and H_{Ph}-5), 7.25 – 7.21 (m, 4H, H_{Iso}-4, H_{Ph}-2, H_{Ph}-6 and H_{Iso}-6), 7.20 – 7.17 (m, 1H, H_{Ph}-4), 4.44 (br. s, 4H, H_{Iso}-1 and H_{Iso}-3), 3.96 (s, 2H, H_{Bn}). ^{13}C NMR (150 MHz, DMSO): δ 141.7 (C_{Iso}-5), 141.0 (C_{Ph}-1), 135.4 (C_{Iso}-4a), 132.7 (C_{Iso}-7a), 128.8 (C_{Iso}-6), 128.7 (C_{Ph}-2 and C_{Ph}-6), 128.5 (C_{Ph}-3 and C_{Ph}-5), 126.1 (C_{Ph}-4), 123.0 (C_{Iso}-4), 122.9 (C_{Iso}-7), 50.0 (C_{Iso}-1 and C_{Iso}-3), 40.8 (C_{Bn}). HRMS (TOF ASAP+) *m/z* calcd. for $C_{15}H_{16}N$ [M-Cl]$^+$: 210.1283; found: 210.1279.

HPLC: (MeOH/H_2O, 1 : 1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 9.8 min, 98% pure. Spectral data for **2c**-**f** are assigned similarly to **2b**.

Method E: synthesis of 5-phenethylisoindolin-2-ium chloride (2c). Carbamate deprotection adapted from a procedure by Young *et al.*[49] The carbamate protected **9c** (0.386 g, 1.19 mmol) was added to HCl (9 mL, 18 mmol, 2 M in Et_2O) and stirred for 22 hours at room temperature. The reaction mixture was then filtered and the precipitate washed with Et_2O, affording **2c** as a grey solid (0.163 g, 0.63 mmol, 53%) upon drying. Mp 260 – 262 °C. IR (ATR): 2894 (m), 2714 (m), 2615 (m), 2493 (m), 1581 (m), 1496 (m), 1425 (m), 1366 (s) cm^{-1}. ^1H NMR (600 MHz, DMSO): δ 9.78 (br. s, 2H, NH_2^+) 7.30 – 7.26 (m, 4H, H_{Iso}-6, H_{Iso}-7, H_{Ph}-2 and H_{Ph}-6), 7.23 – 7.21 (m, 3H, H_{Iso}-4, H_{Ph}-3 and H_{Ph}-5), 7.19 – 7.16 (m, 1H, H_{Ph}-4), 4.45 (br. s, 4H, H_{Iso}-1 and H_{Iso}-3), 2.91 – 2.87 (m, 4H, 2x H_{Bn}). ^{13}C (150 MHz, DMSO): δ 141.9, 141.2, 135.1, 132.5, 128.5, 128.3 (2x C), 128.2 (2x C), 125.9, 122.7 (2x C), 49.9, 49.8, 37.0, 36.7. HRMS (TOF ASAP+) *m/z* calcd. for $C_{16}H_{18}N$ $[M-Cl]^+$: 224.1439; found: 224.1435. HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 5.9 min, >99% pure.

5-(4-(*tert*-Butyl)phenyl)isoindolin-2-ium chloride (2d). Method E with **9d** (0.210 g, 0.60 mmol) and HCl (4.5 mL, 9 mmol, 2 M in Et_2O) for 48 hours afforded **2d** as a dark grey solid (0.149 g, 0.52 mmol, 86%). Mp 246 – 260 °C. IR (ATR): 2958 (m), 2733 (m), 1636 (w), 1488 (m), 1390 (m), 1361 (w), 1268 (w), 1111 (w) cm^{-1}. ^1H NMR (600 MHz, DMSO): δ 10.02 (br. s, 2H, NH_2^+), 7.67 (br. s, 1H, H_{Iso}-4), 7.64 (d, 1H, J = 7.9 Hz, H_{Iso}-7), 7.60 – 7.58 (m, 2H, H_{Ph}-3 and H_{Ph}-5), 7.50 – 7.46 (m, 3H, H_{Iso}-6, H_{Ph}-2 and H_{Ph}-6), 4.55 – 4.51 (m, 4H, H_{Iso}-1 and H_{Iso}-3), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz, DMSO): δ 150.2, 140.8, 137.2, 136.0, 134.0, 126.7, 126.4 (2x C), 125.8 (2x C), 123.4, 120.9, 49.9, 49.8, 34.3, 31.1 (3x C). HRMS (TOF ASAP+) *m/z* calcd. for $C_{18}H_{22}N$ $[M-Cl]^+$: 252.1752; found: 252.1749. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 16.9 min, 99% pure.

5-(3,5-Bis(trifluoromethyl)phenyl)isoindolin-2-ium chloride (2e). Method E with **9e** (0.396, 0.92) and HCl (9.5 mL, 19 mmol, 2 M in Et_2O) for 27 hours afforded **2e** as a white solid (0.291 g, 0.79 mmol, 86%). Mp 226 – 232 °C). R_f ($CHCl_3$/MeOH/NH_3 (25% aq), 70:30:3) 0.67. IR (ATR): 2893 (w), 2685 (w), 2587 (w), 1378 (m), 1280 (s) cm^{-1}. ^1H NMR (600 MHz, DMSO): δ 9.99 (br. s, 2H, NH_2^+), 8.34 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 8.12 (s, 1H, H_{Ph}-4), 7.93 (s, 1H, H_{Iso}-4), 7.89 (d, 1H, J = 8.0 Hz, H_{Iso}-6), 7.57 (d, 1H, J = 8.0 Hz, H_{Iso}-7), 4.57 (s, 4H, H_{Iso}-1 and H_{Iso}-3). ^{13}C NMR (150 MHz, DMSO): δ 142.1, 137.1, 136.4, 136.0, 131.3 (2x C), 127.6, 127.4 (2x C), 123.3 (q, J_{CF} = 272.9 Hz, 2x C), 123.7, 122.1, 121.2, 49.9 (2x C). HRMS (TOF ASAP+) *m/z* calcd. for $C_{16}H_{12}NF_6$ $[M-Cl]^+$: 332.0874; found: 332.0871. HPLC (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 15.2 min, 99% pure.

5-(3,5-Di-*tert*-butylphenyl)isoindolin-2-ium chloride (2f). The carbamate protected **9f** (33 mg, 0.081 mmol) was added to THF (8 mL) and HCl (0.072 mL, 0.96 mmol, 37% aq), after which HCl was added to a total of 0.54 mL (90 equiv, 37% aq) over 48 hours. The reaction mixture was then concentrated, and crystallization from MeOH/Et_2O afforded **2f** as an off-white solid (18 mg, 0.052 mmol, 64%). Mp 193 – 202 °C. IR (ATR): 2868 (s), 1597 (w), 1362 (m), 1248 (m), 876 (s), 711 (s) cm^{-1}. ^1H NMR (400 MHz, DMSO): δ 9.69 (br. s, 2H, NH_2^+), 7.70 (s, 1H, H_{Iso}-4), 7.67 – 7.62 (m, 1H, H_{Iso}-6), 7.49 (d, 1H, J = 7.8 Hz, H_{Iso}-7), 7.43 (s, 3H, Ph), 4.56 (d, 4H, J = 9.4 Hz, H_{Iso}-1 and H_{Iso}-3), 1.35 (s, 18H, 2x *t*-Bu). ^{13}C NMR (100 MHz, DMSO): δ 151.4 (2x C), 139.6, 136.4, 134.3, 127.7, 123.7, 121.9, 121.8, 121.5 (3x C), 50.6, 50.4, 35.1 (2x C), 31.7 (6x C). HRMS (TOF ASAP+) *m/z* calcd. for $C_{22}H_{30}N$ $[M-Cl]^+$: 308.2378; found: 308.2376. HPLC (MeOH/H_2O, 3:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 12.3 min, 98% pure.

5-(3,5-di-*tert*-butylphenyl)isoindolin-2-ium 2,2,2-trifluoroacetate (2f, TFA$^-$ counterion). The protected amine **9f** (90 mg, 0.22 mmol) was added to DCM (5 mL) and TFA (0.17 mL, 2.2 mmol) and stirred for 48 hours. Partial evaporation and crystallization with Et_2O afforded the TFA salt of **2f** as a white solid (69.8 mg, 0.17 mmol, 75%). ^{1}H NMR data were in accordance with the spectra recorded for **2f** (Cl^- counterion). ^{1}H NMR (400 MHz, DMSO): *δ* 9.41 (br. s, 2H, NH_2^+), 7.70 (s, 1H, H_{Iso}-4), 7.68 – 7.64 (m, 1H, H_{Iso}-6), 7.49 (d, 1H, *J* = 8.2 Hz, H_{Iso}-7), 7.42 (s, 3H, Ph), 4.57 (d, 4H, *J* = 8.7 Hz, H_{Iso}-1 and H_{Iso}-3), 1.35 (s, 18H, 2x *t*-Bu).

Method F: synthesis of 5-(naphthalen-2-yl)isoindolin-2-ium chloride (2g). The carbamate protected **9g** (0.107 g, 0.31 mmol) was added MeCN (30 mL) and HCl (0.258 mL, 3.10 mmol, 37% aq), followed by stirring for 19 hours at room temperature. Evaporation of volatiles and drying for two hours under vacuum (0.5 mbar, 60 °C), followed by crystallization from MeOH/Et_2O afforded **2g** as an off-white solid (69 mg, 0.25 mmol, 79%). Mp >175 °C (decomp.). ^{1}H NMR (DMSO-*d6*, 600 MHz): *δ* 9.84 (br. s, 2H, NH_2^+), 8.23 (s, 1H, Naph), 8.03 (d, 1H, *J* = 8.6 Hz, Naph), 8.00 (d, 1H, *J* = 7.7 Hz, Naph), 7.96 (d, 1H, *J* = 7.8 Hz, Naph), 7.86 (s, 1H, H_{Iso}-4), 7.85 (dd, 1H, *J* = 8.6, 1.5 Hz, Naph), 7.82 (d, 1H, *J* = 8.0 Hz, H_{Iso}-6), 7.59 – 7.53 (m, 3H, H_{Iso}-7 and 2x Naph), 4.59 (s, 2H, H_{Iso}-3), 4.57 (s, 2H, H_{Iso}-1). ^{13}C NMR (DMSO-*d6*, 150 MHz): *δ* 140.3, 136.9, 136.1, 134.4, 133.3, 132.3, 128.6, 128.2, 127.5, 127.2, 126.5, 126.3, 125.4, 125.0, 123.5, 121.5, 50.1, 49.9. IR(ATR): 2904 (m), 2706 (m), 2589 (m), 2498 (m), 2458 (w), 1595 (m), 1495 (m), 1409 (m), 1355 (m), 1271 (w), 1234 (w), 1198 (w), 1161 (w), 1128 (w), 884 (w), 862 (m), 813 (s), 776 (m), 706 (w), 672 (w), 623 (w) cm^{-1}. HRMS (TOF ASAP+) m/z calcd. for $C_{18}H_{16}N$ [M-Cl]$^+$: 246.1283; found: 246.1278. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 5.8 min, 99% pure.

5-(4-Butylphenyl)isoindolin-2-ium chloride (2h). Method F with **9h** (0.179 g, 0.509 mmol) and HCl (0.21 mL, 2.55 mmol, 37% aq) for 14 hours at room temperature afforded **2h** as an off-white solid (0.116 g, 0.403 mmol, 79%). Mp >200 °C (decomp.). ^{1}H NMR (DMSO-*d6*, 600 MHz): *δ* 9.40 (br. s, 2H, NH_2^+), 7.67 (s, 1H, H_{Iso}-4), 7.63 (d, 1H, *J* = 8.0 Hz, H_{Iso}-6), 7.57 (d, 2H, *J* = 8.1 Hz, H_{Ar}-2 and H_{Ar}-6), 7.46 (d, 1H, *J* = 7.9 Hz, H_{Iso}-7), 7.29 (d, 2H, *J* = 8.1 Hz, H_{Ar}-3 and H_{Ar}-5), 4.53 (s, 2H, H_{Iso}-3), 4.52 (s, 2H, H_{Iso}-1), 2.62 (t, 2H, *J* = 7.7 Hz, CH_2), 1.58 (p, 2H, *J* = 7.6 Hz, CH_2), 1.33 (sex, 2H, *J* = 7.5 Hz, CH_2), 0.91 (t, 3H, *J* = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-*d6*, 150 MHz): *δ* 141.9 (C_{Ar}-4), 140.4 (C_{Iso}-5), 136.9 (C_{Iso}-3a), 136.1 (C_{Iso}-7a), 134.0 (C_{Ar}-1), 128.9 (C_{Ar}-3 and C_{Ar}-5), 126.6 (C_{Iso}-6), 126.6 (C_{Ar}-2 and C_{Ar}-6), 123.3 (C_{Iso}-7), 120.9 (C_{Iso}-4), 50.0 (C_{Iso}-3), 49.9 (C_{Iso}-1), 34.4 (CH_2), 33.0 (CH_2), 21.7 (CH_2), 13.8 (CH_3). IR(ATR): 2956 (m), 2913 (s), 2694 (s), 2591 (s), 2495 (m), 2367 (w), 2258 (w), 1593 (m), 1489 (m), 1452 (m), 1427 (m), 1375 (m), 1356 (m), 1048 (w), 921 (w), 881 (m), 813 (s), 802 (s) cm^{-1}. HRMS (TOF ASAP+) m/z calcd. for $C_{18}H_{22}N$ [M-Cl]$^+$: 252.1748; found: 252.1748. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 18.3 min, 99% pure. Spectral data for **2g** and **2i**-**j** are assigned similarly to **2h**.

5-(4-Butoxyphenyl)isoindolin-2-ium chloride (2i). Method F with **9i** (0.140 g, 0.38 mmol) and HCl (0.318 mL, 3.81 mmol, 37% aq) for 16 hours at room temperature afforded **2i** as a transparent solid (0.101 g, 0.332 mmol, 87%). Mp 176 – 177 °C. ^{1}H NMR (DMSO-*d6*, 600 MHz): *δ* 10.01 (br. s, 2H, NH_2^+), 7.64 (s, 1H, H_{Iso}-4), 7.60 – 7.59 (m, 1H, H_{Iso}-6), 7.58 (app d, 2H, *J* = 8.0 Hz, H_{Ar}-2 and H_{Ar}-6), 7.44 (d, 1H, *J* = 8.0 Hz, H_{Iso}-7), 7.02 (d, 2H, *J* = 8.8 Hz, H_{Ar}-3 and H_{Ar}-5), 4.53 (s, 2H, H_{Iso}-3), 4.51 (s, 2H, H_{Iso}-1), 4.01 (t, 2H, *J* = 6.5 Hz, OCH_2), 1.71 (p, 2H, *J* = 7.4 Hz, CH_2), 1.45 (sex, 2H, *J* = 7.4 Hz, CH_2), 0.94 (t, 3H, *J* = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-*d6*, 150 MHz): *δ* 158.5, 140.1, 135.9, 133.4, 131.7, 127.8, 126.3, 123.3, 120.6, 114.9, 67.2, 49.9, 49.7, 30.7, 18.7, 13.7. IR (ATR): 2930 (m), 2727 (m), 2631 (m), 2507 (w), 1604 (m), 1519 (m), 1244 (s), 1179 (m), 1124 (m), 1059 (m), 885 (s) cm^{-1}. HRMS (TOF ASAP+) m/z calcd. for $C_{18}H_{22}NO$ [M-Cl]$^+$: 268.1701; found: 268.1697. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 10.3 min, 98% pure.

5-(4-(Trifluoromethyl)phenyl)isoindolin-2-ium chloride (2j). Method F with **9j** (26 mg, 0.072 mmol) and HCl (30 µL, 0.36 mmol, 37% aq) for 16 hours at room temperature afforded **2j** as an off-white solid (19 mg, 0.064 mmol, 88%). Mp >235 °C (decomp.). ^{1}H NMR (DMSO-*d6*, 600 MHz): δ 9.96 (br s, 2H, NH_2^+), 7.89 (d, 2H, J = 8.3 Hz, H_{Ar}-2 and H_{Ar}-6), 7.84 (d, 2H, J = 8.3, H_{Ar}-3 and H_{Ar}-5), 7.78 (s, 1H, H_{Iso}-4), 7.74 (d, 1H, J = 7.9 Hz, H_{Iso}-6), 7.54 (d, 1H, J = 8.0 Hz, H_{Iso}-7), 4.57 (s, 2H, H_{Iso}-3), 4.56 (s, 2H, H_{Iso}-1). ^{13}C NMR (DMSO-*d6*, 150 MHz): δ 143.6, 140.4, 136.3, 135.4, 128.1 (q, J_{CF} = 31.9), 127.5, 127.3, 125.9 (q, J_{CF} = 3.8 Hz), 124.3 (q, J_{CF} = 271.7 Hz), 123.6, 121.6, 50.0, 49.8. IR (ATR): 2926 (w), 2891 (w), 2701 (w), 2594 (w), 2502 (w), 2465 (w), 2374 (w), 2265 (w), 1617 (w), 1603 (w), 1491 (w), 1324 (s), 1180 (m), 1161 (m), 1108 (s), 1070 (s), 818 (s) cm^{-1}. HRMS (TOF ASAP+) m/z calcd. for $C_{15}H_{13}NF_3$ [M-Cl]$^+$: 264.0997; found: 264.1000. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 4.9 min, 98% pure.

Method G: synthesis of amino(5-(4-(*tert*-butyl)phenyl)isoindolin-2-yl)methaniminium chloride (3d) via 5-(4-(*tert*-butyl)phenyl)isoindoline (12d). Modified reaction conditions to the procedure presented by Bernatowicz *et al*.[50,51] Where the salt **2d** (0.100 g, 0.35 mmol) was added to K_2CO_3 (15 mL, sat. aq) and extracted with EtOAc (3 x 25 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure, affording the free amine **12d** (80 mg, 0.32 mmol, 92%). The free amine **12d** (30 mg, 0.119 mmol) and **13** (17 mg, 0.117 mmol) was added to MeCN (3 mL) and refluxed for 31 hours. Filtration of the reaction mixture and washing the precipitate with MeCN (3 x 2 mL) afforded **3d** as a grey solid (22 mg, 0.067 mmol, 57%) upon drying. Mp 299 – 303 °C. IR (ATR): 3322 (w), 3140 (w), 2957 (w), 1623 (s), 1580 (m), 1458 (m), 1368 (m), 813 (s) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d6): δ 7.66 – 7.59 (m, 4H, H_{Iso}-4, H_{Iso}-7, H_{Ph}-3 and H_{Ph}-5), 7.52 – 7.41 (s, 7H, H_{Iso}-6, Guan, H_{Ph}-2 and H_{Ph}-6), 4.80 (d, 4H, J = 8.5 Hz, H_{Iso}-1 and H_{Iso}-3), 1.33 (s, 9H, tBu). ^{13}C NMR (150 MHz, DMSO): δ 155.3 (Guan), 150.6 (C_{Ph}-4), 140.5 (C_{Ph}-1), 137.3 (C_{Iso}-5), 136.5 (C_{Iso}-7a, from HMBC), 134.6 (C_{Iso}-4a), 126.9 (C_{Ph}-3 and C_{Ph}-5), 126.8 (C_{Iso}-7, from HMBC), 126.2 (C_{Ph}-2 and C_{Ph}-6), 123.6 (C_{Iso}-6), 121.2 (C_{Iso}-4), 53.4 (C_{Iso}-1), 53.2 (C_{Iso}-3), 34.7 (C_q-tBu), 31.6 (*t*Bu). HRMS (TOF ASAP+) *m/z* calcd. for $C_{19}H_{23}N_3$ [M-HCl]$^{*+}$: 293.1892; found: 293.1888. HPLC: (MeOH/H_2O 1:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 6.1 min, 99% pure. Spectral data for **3e** and **3f** are assigned similarly to **3d**.

Amino(5-(3,5-bis(trifluoromethyl)phenyl)isoindolin-2-yl)methaniminium chloride (3e) via 5-(3,5-bis(trifluoromethyl)phenyl)isoindoline (12e). Method G with **2e** (0.150 g, 0.41 mmol) afforded **12e** as a dark solid (0.118 g, 0.36 mmol, 87%). The amine **12e** (40 mg, 0.121 mmol), **13** (18 mg, 0.121 mmol), and 8 hours reflux according to method G afforded **3e** as an off-white solid (41 mg, 0.100 mmol, 83%). Mp 278 - 284 °C. IR (ATR): 3454 (w), 3329 (w), 3143 (w), 1628 (s), 1377 (m), 1279 (s), 1168 (m), 1129 (s), 1051 (m), 683 (s) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d6): δ 8.38 (s, 2H, H_{Ph}-2 and H_{Ph}-6), 8.13 (s, 1H, H_{Ph}-4), 7.94 (s, 1H, H_{Iso}-4), 7.90 – 7.85 (m, 1H, H_{Iso}-6), 7.60 – 7.47 (m, 5H, H_{Iso}-7 and Guan), 4.83 (s, 4H, H_{Iso}-1 and H_{Iso}-3). ^{13}C NMR (150 MHz, DMSO): δ 155.0, 142.3, 136.8 (2x C), 136.5, 136.2, 130.9, 127.5 (2x C), 127.2, 123.3 (q, J_{CF} = 267.4 Hz, 2x C), 123.5, 122.4, 121.9, 52.8 (2x C). HRMS (TOF ASAP+) *m/z* calcd. for $C_{17}H_{13}N_3F_6$ [M-HCl]$^+$: 374.1092; found: 374.1086. HPLC (MeOH/H_2O, 3:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 5.0 min, 95% pure.

Amino(5-(3,5-di-*tert*-butylphenyl)isoindolin-2-yl)methaniminium chloride (3f) via 5-(3,5-di-*tert*-butylphenyl)isoindoline (12f). Method G with the TFA-salt of **2f** (68 mg, 0.16 mmol) afforded **12f** as a brown oil (46 mg, 0.150 mmol, 92%), which was reacted directly with **13** (22 mg, 0.150 mmol) for 5 hours at reflux, affording **3f** as a lightly purple solid (38 mg, 0.098 mmol, 66%). Mp 283 - 290 °C. IR (ATR): 3339 (w), 3144 (w), 2966 (w), 1635 (s), 1462 (w), 1363 (w), 869 (w), 829 (w) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-

d6): δ 7.66 – 7.59 (m, 2H, H_{Iso}-4 and H_{Iso}-6), 7.52 (s, 4H, Guan), 7.48 – 7.44 (m, 1H, H_{Iso}-7), 7.42 (s, 3H, Ph), 4.80 (d, 4H, J = 8.0 Hz, H_{Iso}-1 and H_{Iso}-3), 1.35 (s, 18H, 2x *t*Bu). ^{3}C NMR (100 MHz, DMSO): δ 155.5, 151.4 (2x C), 141.8, 136.4, 134.5, 127.3, 123.5, 121.8, 121.7, 121.6 (3x C), 53.3, 53.1, 35.1 (2x C), 31.8 (6x C). HRMS (TOF ASAP+) *m/z* calcd. for $C_{23}H_{32}N_3$ [M-Cl]$^+$: 350.2596; found: 350.2589. HPLC (MeOH/H_2O, 3:1 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 18.1 min, 97% pure.

Method H: synthesis of amino(5-(naphthalen-2-yl)isoindolin-2-yl)methaniminium chloride (3g) via 5-(naphthalen-2-yl)isoindoline (12g). Modified reaction conditions to the procedure presented by Bernatowicz *et al*.[50,51] Where the salt **2g** (41 mg, 0.144 mmol) was added to K_2CO_3 (30 mL, sat. aq) and extracted with EtOAc (5 x 25 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure, affording the free amine **12g** (35 mg, 0.143 mmol, 99%). The free amine **12g** (35 mg, 0.143 mmol) was added **13** (20.2 mg, 0.138 mmol) and MeCN (3 mL), before it was refluxed for 17 hours. The cooled reaction mixture was then filtered, and the precipitate was washed with Et_2O (5 x 5 mL) and MeCN (3 x 1 mL). The crude precipitate was then crystallized from MeOH/Et_2O affording **3g** as an orange solid (21 mg, 0.065 mmol, 47%). Mp 180 – 185 °C. IR (ATR) 3114 (m), 3018 (m), 2970 (m), 1738 (m), 1628 (s), 1576 (m), 1497 (m), 1448 (m), 1370 (s), 1217 (m), 1081 (w), 857 (w), 806 (s), 741 (m), 708 (w) cm^{-1}. ^{1}H NMR (DMSO-*d6*, 600 MHz): δ 8.26 (s, 1H, Naph), 8.02 (app d, 1H, J = 8.6 Hz, Naph), 8.00 (app d, 1H, J = 7.7 Hz, Naph), 7.96 (d, 1H, J = 7.6 Hz, Naph), 7.87 (dd, 1H, J = 8.5, 1.8 Hz, Naph), 7.82 (s, 1H, H_{Iso}-4), 7.82 (app d, 1H, J = 8.0 Hz, H_{Iso}-6), 7.56 – 7.51 (m, 7H, H_{Iso}-7 and 2x Naph and 4x H_{Guan}), 4.85 (s, 2H, H_{Iso}-3), 4.82 (s, 2H, H_{Iso}-1). ^{13}C NMR (DMSO-*d6*, 150 MHz): δ 154.9, 139.8, 137.0, 136.2, 134.6, 133.3, 132.3, 128.5, 128.2, 127.5, 126.8, 126.5, 126.2, 125.4, 125.1, 123.3, 121.2, 52.9, 52.7. HRMS (ESI+) m/z calcd. for $C_{19}H_{18}N_3$ [M-Cl]$^+$: 288.1500; found: 288.1501. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 10.9 min, 96% pure.

Amino(5-(4-butylphenyl)isoindolin-2-yl)methaniminium chloride (3h) via 5-(4-butylphenyl)isoindoline (12h). Method C with **2h** (90 mg, 0.31 mmol) afforded **12h** (74 mg, 0.294 mmol, 94%), which then was added **13** (38 mg, 0.261 mmol) and MeCN (4 mL). Reflux for 5 hours and work-up afforded **3h** as a white solid (62 mg, 0.188 mmol, 72%). Mp >245 °C (decomp.). IR (ATR): 3320 (m), 3148 (m), 2956 (m), 2928 (m), 2858 (m), 2427 (w), 1635 (s), 1582 (m), 1493 (m), 1458 (m), 1406 (w), 1371 (m), 1078 (w), 810 (m) cm^{-1}. ^{1}H NMR (DMSO-*d6*, 600 MHz): δ 7.63 (s, 1H, H_{Iso}-4), 7.62 (app d, 1H, J = 8.0 Hz, H_{Iso}-6), 7.59 (d, 2H, J = 8.0 Hz, H_{Ar}-2 and H_{Ar}-6), 7.55 (br. s, 4H, guan), 7.44 (d, 1H, J = 8.4 Hz, H_{Iso}-7), 7.29 (d, 2H, J = 8.0 Hz, H_{Ar}-3 and H_{Ar}-5), 4.80 (s, 2H, H_{Iso}-3), 4.78 (s, 2H, H_{Iso}-1), 2.62 (t, 2H, J = 7.6 Hz, CH_2), 1.58 (p, 2H, J = 7.6 Hz, CH_2), 1.33 (sex, 2H, J = 7.5 Hz, CH_2), 0.91 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-*d6*, 150 MHz): δ 155.0 (C_{Guan}), 141.9 (C_{Ar}-4), 140.0 (C_{Iso}-5), 137.1 (C_{Iso}-3a), 136.1 (C_{Iso}-7a), 134.1 (C_{Ar}-1), 128.9 (C_{Ar}-3 and C_{Ar}-5), 126.6 (C_{Ar}-2 and C_{Ar}-2) 126.3 (C_{Iso}-6), 123.1 (C_{Iso}-7), 120.7 (C_{Iso}-4), 52.9 (C_{Iso}-3), 52.6 (C_{Iso}-1), 34.4 (CH_2), 33.1 (CH_2), 21.7 (CH_2), 13.8 (CH_3). HRMS (TOF ASAP+) m/z calcd. for $C_{19}H_{24}N_3$ [M-Cl]$^+$: 294.1970; found: 294.1966. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 39.1 min, 98% pure. Spectral data for **3g** and **3i** are assigned similarly to **3h**.

Amino(5-(4-butoxyphenyl)isoindolin-2-yl)methaniminium chloride (3i) via 5-(4-butoxyphenyl)-isoindoline (12i). Method C with **2i** (40 mg, 0.132 mmol) afforded **12i** which then was added directly to **13** (20.6 mg, 0.140 mmol) and MeCN (3 mL) under argon. Reflux for 6 hours and work-up afforded **3i** as a lightly red solid (12 mg, 0.035 mmol, 27% from **2i**). Mp >240 °C (decomp.). IR (ATR): 3394 (m), 3326 (m), 3194 (w), 3129 (m), 2956 (w), 2934 (w), 2865 (w), 1625 (s), 1582 (m), 1569 (m), 1493 (s), 1460 (s), 1369 (m), 1291 (m), 1272 (m), 1247 (s), 1225 (s), 1181 (s), 1031 (m), 975 (m), 897 (w), 811 (s), 632 (m) cm^{-1}. ^{1}H NMR (DMSO-*d6*, 600 MHz): δ 7.60 – 7.59 (m, 4H, H_{Iso}-4 + H_{Iso}-6 + H_{Ar}-2 + H_{Ar}-6), 7.47 (br. s, 4H, H_{Guan}),

7.42 (d, 1H, J = 8.4 Hz, H_{Iso}-7), 7.02 (d, 2H, J = 8.6 Hz, H_{Ar}-3 and H_{Ar}-5), 4.79 (s, 2H, H_{Iso}-3), 4.77 (s, 2H, H_{Iso}-1), 4.01 (t, 2H, J = 6.5 Hz, OCH_2), 1.72 (p, 2H, J = 6.8 Hz, CH_2), 1.45 (sex, 2H, J = 7.4 Hz, CH_2), 0.95 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-*d6*, 150 MHz): δ 158.5, 154.9, 139.8, 136.0, 133.6, 131.9, 127.9, 126.0, 123.1, 120.3, 114.9, 67.2, 52.9, 52.7, 30.7, 18.7, 13.7. HRMS (TOF ASAP+) m/z calcd. for $C_{19}H_{24}N_3O$ [M-Cl]$^+$: 310.1919; found: 319.1916. HPLC: (MeOH/H_2O, 5:3 + 0.1% TFA, 0.75 mL min^{-1}, 214 nm): t_R = 23.6 min, 99% pure.

Inhibition of bacterial growth

Growth medium with MilliQ H_2O was used as a negative control, while sterile MilliQ H_2O and bacteria suspension was used as a positive control. Bacteria were transferred from a blood plate to growth medium (MH-bullion, VL787693 717, Merck) for *E. coli*, *P. aeruginosa* and *S. aureus* and BHI-bullion (CM1135, OXOID) for *E. faecalis* and *S. agalactiae* gr. B and incubated at 37°C overnight. The following day part of the bacteria suspension was transferred to fresh medium and cultivated in a shaker incubator at 37°C for 1.5 h (*E. coli, E. faecalis* and *Streptococcus* gr. B) or 2.5 h (*S. aureus* and *P. aeruginosa*). The bacteria suspension was then diluted 1:100 in medium and added to all wells on a 96-well microtiter plate (Nunc 167008), followed by sample aliquotes (and Gentamicin as a reference antibiotic) in duplicates. The plates were incubated at 37ºC overnight before growth was controlled visually and photometrically at 600 nm.

Inhibition of biofilm formation

S. epidermidis was used to assess the effect of the test compounds on biofilm formation. Growth media: tryptic soy broth (TS; Merck, Darmstadt, Germany). An overnight culture of *S. epidermidis* grown in TS was diluted with fresh TS containing 1% glucose (1:100). Aliquots of 50 µL were transferred to a 96-well microtiter plate, and 50 µL of test compounds, dissolved in water at ranging concentrations, was added. After overnight incubation at 37 °C, the bacterial suspension was carefully discarded and the wells washed with water. The plate was dried and the biofilm fixed by incubation for 1 h at 55 °C before the surface attached cells were stained with 100 µL of 0.1% crystal violet for 5 min. The crystal violet solution was removed and the plate once more washed with water and dried at 55 °C for 1 h. After adding 70 µL of 70% ethanol, the plate was incubated at room temperature for 10 min. Biofilm formation was observed by visual inspection of the plates. The MIC was defined as the lowest concentration where no biofilm formation was visible. A *S. epidermidis* suspension, diluted with 50 µL of water, was used as a positive control, and 50 µL Staphylococcus haemolyticus suspension with 50 µL of water was employed as a negative control. A mixture of 50 µL water and 50 µL TS was used as assay control.

Cytotoxicity to HepG2-cells

Cytotoxicity was evaluated after 24 h exposure in human hepatocellular liver carcinoma (HepG2, ATCC HB-8065™) cells, and 20,000 HepG2 cells were seeded per well. HepG2 were grown overnight, and then incubated with test compound (range of concentrations) diluted in MEM Earle's supplemented with gentamycin (10 µg/mL), non-essential amino acids (1%), sodium pyruvate (1 mM), L-alanyl-L-glutamine (2 mM), but without FBS (total volume was 100 µl). Ten µL of CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI, USA) was added and plates were then further incubated for 1 h.

Absorbance was measured at 485 nm in a DTX 880 Multimode Detector. Results were calculated as % survival compared to negative (assay media) and positive (Triton X-100; Sigma-Aldrich) controls.

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