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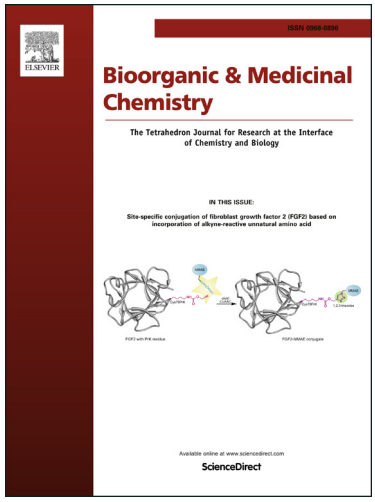

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Methyl propiolate and 3-butynone: starting points for synthesis of amphiphilic 1,2,3-triazole peptidomimetics for antimicrobial evaluation

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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 lipopohilic 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 agalacticae*, 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.

Keywords: Antibacterial; Click chemistry; Marine natural product mimics; 1,2,3-Triazoles

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

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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 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

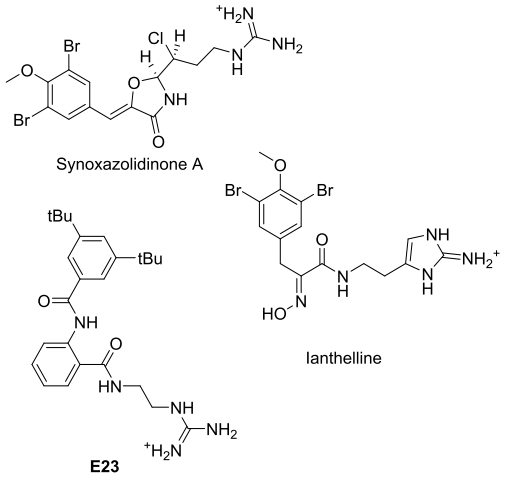

Figure 1. Synoxazolidone A[14] (methicillin-resistant S. aureus MIC: 10 µg/mL), ianthelline[15] (methicillin-resistant S. aureus MIC: 20 µg/mL), and **E23**: example of aminobenzamide peptidomimetic[13] based on marine antimicrobials synoxazolidinone A[14] and ianthelline.[15]

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

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presented in this publication was prepared in parallel to a similar library of amphiphiles based on 1,2,3-triazole phthalimides.[21]

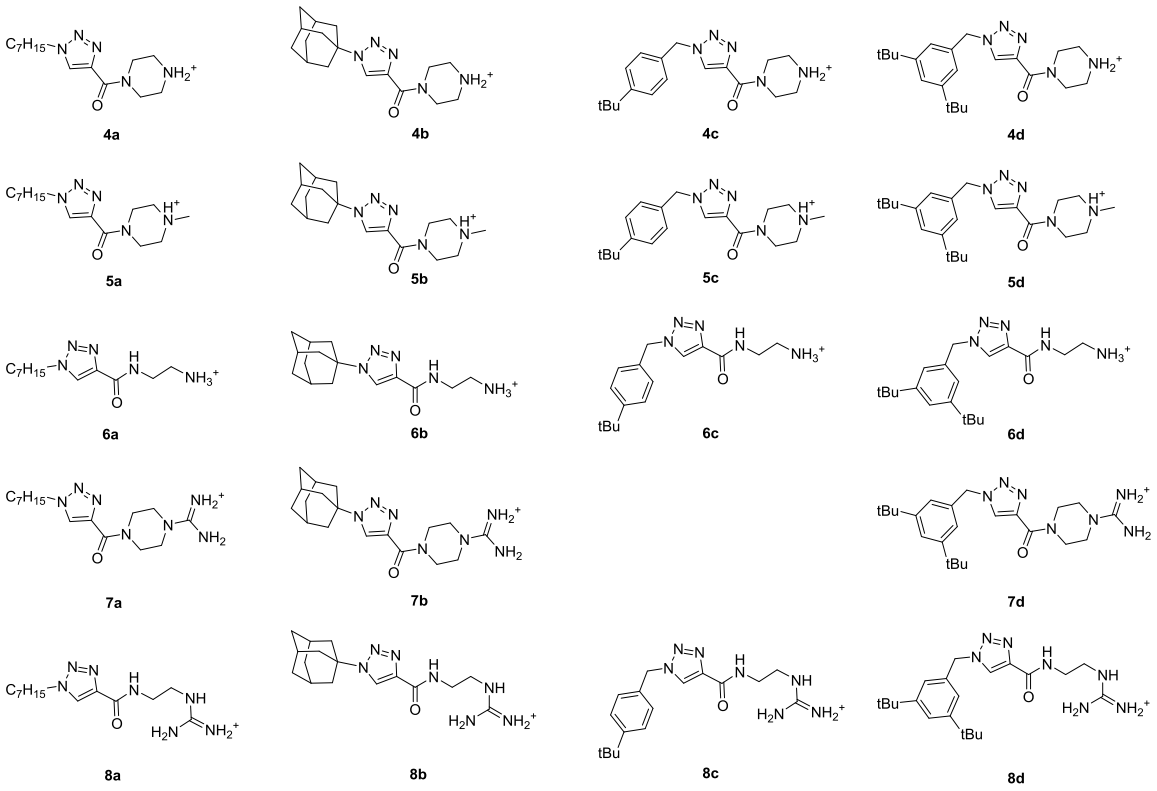

Figure 2. Compounds **4a-8d** synthesized and evaluated for antimicrobial activity. Amphiphile **7c** was not prepared, as discussed in the synthesis section. Counter ion: Cl^-.

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**

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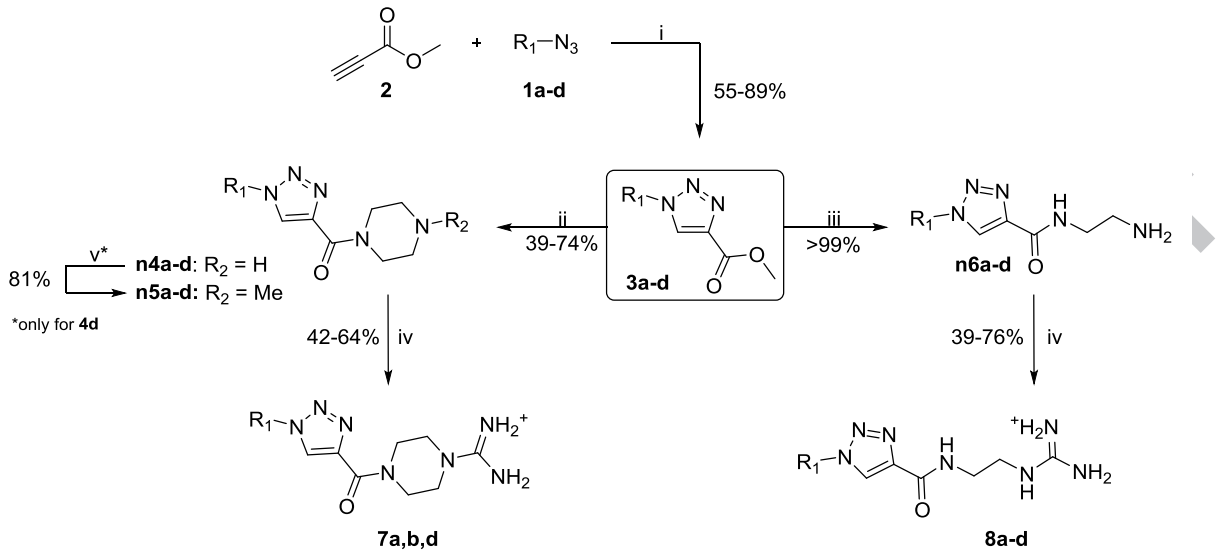

Scheme 1. i) **1a-d** (1 – 1.05 equiv), $CuSO_4$ x $5H_2O$ (5% mol), sodium ascorbate (10% mol), benzoic acid (10% mol), *t*-BuOH:H_2O (1:2), rt, 10-18 h. ii) Piperazine (R_2 = H) or 1-methyl piperazine (R_2 = Me) (3 equiv), NaOMe (1 equiv), MS 4 Å, MeOH, rt, 24 - 115 h. iii) EDA (15 equiv), MeOH, rt - 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.

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.

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

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. agalacticae* (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.

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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, highest 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 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.

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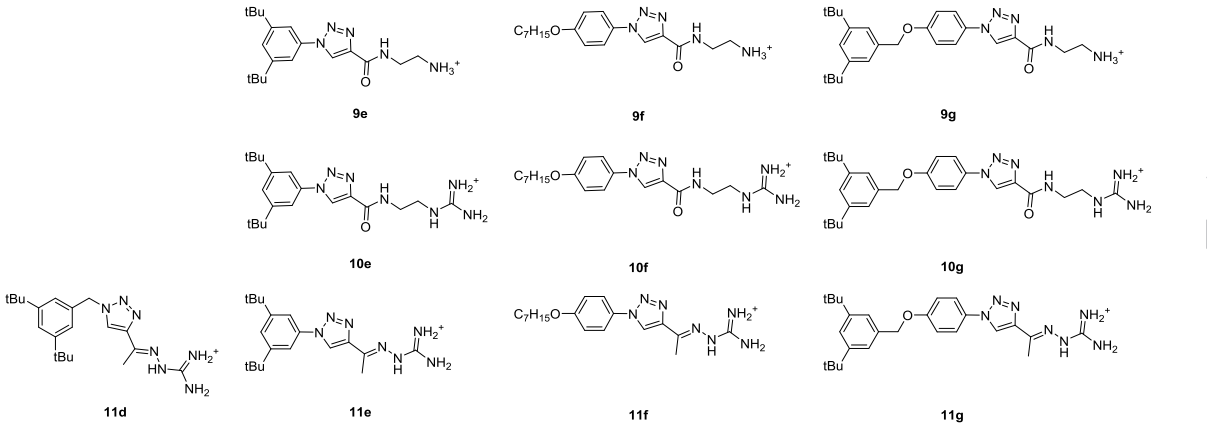

Figure 3. Improved structures **9e-11g** based on **8d**, for antimicrobial evaluation. Counter ion: Cl^- (CF_3COO^- for **11e** and **11f**, from preparative HPLC).

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] Compund **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.

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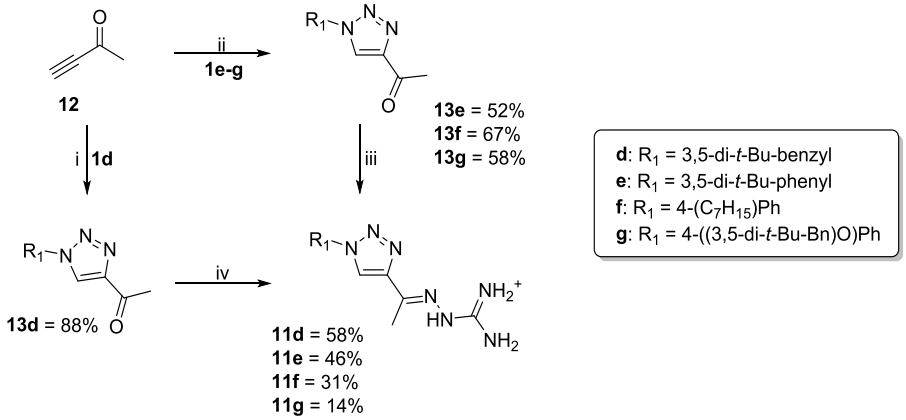

Scheme 2. Synthesis of improved structures **11d-g** based on **12**: i) **1d** (1 equiv), $CuSO_4$ x $5H_2O$ (5 mol %), Na-ascorbate (10 mol %), benzoic acid (10 mol %), t-BuOH/H_2O (1:2), rt, 18 h. ii) **12** (2-3 equiv), **1e**-**g** (1 equiv), $CuSO_4$ x $5H_2O$ (5 mol %), Na-ascorbate (10 mol %), 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 –

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 ^{o}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 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**.

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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

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.

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 two-fold 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 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 four-fold increase in antimicrobial activity (4-16 μg/mL) against *S. aureus*, *S. agalacticae*, 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

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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. agalacticae*. 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. agalacticae*. The profound selectivity and high antimicrobial potency of **11g** against *S. agalacticae* could be of interest for developing antibiotics for prevention of neonatal infections, since *S. agalacticae* 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. agalacticae* (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. agalacticae*, and *P. aeruginosa*. Against *S. agalacticae* 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. agalacticae*, 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. agalacticae* was 4 times lower than the EC_{50}-value against HepG2. The cytotoxicity was particularly pronounced for the

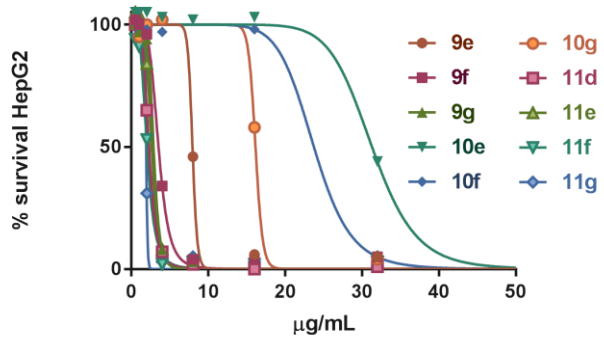

Figure 4. Anti-proliferative activities of **9e-11g** against human hepatic cells (HepG2) after 24 h of incubation. Graphs were plotted using a four-variable slope normalized nonlinear regression

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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 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.

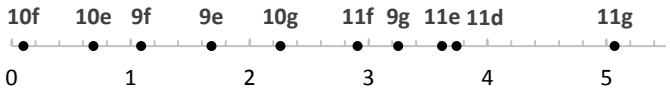

Figure 5. Calculated ClogD at pH = 7.4 for amphiphiles **9e-11g**. Calculated using MarvinSketch 16.11.7 from

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.

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

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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, whilst 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 x 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 x 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-*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), 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 ^{o}C for 19 hours. The suspension was then added DCM (80 mL) and washed with water (3 x 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

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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, 4x 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, 2x *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 ^{o}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 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, 2x *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, 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) and heated to 60 ^{o}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 **1f** as a yellow oil (0.856 g, 3.67 mmol, 78%). ^{1}H 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, 4x 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 ^{o}C). ^{1}H 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, 2x *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, $[M-H]^-$).

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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). ^{1}H 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, 2x *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, $[M\text{-}N_2\text{-}H]^-$). Ph = 3,5-di-*tert*-butylbenzyl.

4.8.1. 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), $CuSO_4$ x $5H_2O$ (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 hours 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). ^{1}H 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, 4x 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, $[M+H]^+$).

4.8.2. Methyl 1-(adamantan-1-yl)-1*H*-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). ^{1}H 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, 3x 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, $[M+H]^+$).

4.8.3. Methyl 1-(4-(*tert*-butyl)benzyl)-1*H*-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%). ^{1}H NMR analyses corresponded with previously reported spectra for **3c**.[47] ^{1}H 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).

4.8.4. Methyl 1-(3,5-di-*tert*-butylbenzyl)-1*H*-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). ^{1}H 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, 2x *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, $[M+H]^+$).

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4.8.5. Methyl 1-(3,5-di-*tert*-butylphenyl)-1*H*-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 hours), the suspension was added H_2O (25 mL) and extracted with DCM (3 x 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). ^{1}H 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, 2x *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), 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, $[M+H]^+$).

4.8.6. Methyl 1-(4-(heptyloxy)phenyl)-1*H*-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). ^{1}H 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, 4x 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, $[M+H]^+$).

4.8.7. Methyl 1-(4-((3,5-di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-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). ^{1}H 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, 2x *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.

4.8.8. 1-(1-(3,5-Di-*tert*-butylbenzyl)-1*H*-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). ^{1}H 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, 2x *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, $[M+H]^+$).

4.9.1. Method B, "Click" reactions with 3-butynone (12): synthesis of 1-(1-(3,5-di-*tert*-butylphenyl)-1*H*-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), $CuSO_4$ x $5H_2O$ (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 hours at room temperature (with addition of additional 2 eq of **12** after 6 hours). The reaction mixture was then added H_2O (10 mL) and extracted with DCM (3x 15 mL), before the organic phase

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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). ^{1}H 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, 2x *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, $[M+H]^+$).

4.9.2. 1-(1-(4-(Heptyloxy)phenyl)-1*H*-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). ^{1}H 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, 4x 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, $[M+H]^+$).

4.9.3. 1-(1-(4-((3,5-Di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-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 hours 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 hours 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). ^{1}H 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, 2x *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.

4.10.1. Method C, Piperazine amidation reactions: synthesis of (1-heptyl-1*H*-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4a) and 4-(1-heptyl-1*H*-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 hours. After completed stirring, the reaction mixture was evaporated and dissolved in DCM before it was filtered through celite. Subsequent purification with flash column chromatography (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, 4x 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 x 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

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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, 4x 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, $[M\text{-}Cl]^+$).

4.10.2. (1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4b) and 4-(1-(adamantan-1-yl)-1*H*-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 hours 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, 2M 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 ^{o}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, $[M\text{-}Cl]^+$).

4.10.3. (1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4c) and 4-(1-(4-(*tert*-butyl)benzyl)-1*H*-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 hours 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 ^{o}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, $[M\text{-}Cl]^+$).

4.10.4. (1-(3,5-Di-*tert*-butylbenzyl)-1*H*-1,2,3-triazol-4-yl)(piperazin-1-yl)methanone (n4d) and 4-(1-(3,5-di-*tert*-butylbenzyl)-1*H*-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,

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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, 2x *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, 2x *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, $[M\text{-}Cl]^+$).

4.10.5. (1-Heptyl-1*H*-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)methanone (n5a) and 4-(1-heptyl-1*H*-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 hours 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, 4x 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, 4x 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, $[M\text{-}Cl]^+$).

4.10.6. (1-(Adamantan-1-yl)-1*H*-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)methanone (n5b) and 4-(1-(adamantan-1-yl)-1*H*-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 hours 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 –

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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]$^+$).

4.10.7. (1-(4-(*tert*-Butyl)benzyl)-1*H*-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)methanone (n5c) and 4-(1-(4-(*tert*-butyl)benzyl)-1*H*-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 hours 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]$^+$).

4.10.8. 4-(1-(3,5-Di-*tert*-butylbenzyl)-1*H*-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 hours. 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, 2x *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]$^+$).

4.11.1. Method D, Ethylene diamine amidation reactions: synthesis of *N*-(2-aminoethyl)-1-heptyl-1*H*-1,2,3-triazole-4-carboxamide (n6a) and 2-(1-heptyl-1*H*-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

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to reflux in MeOH (3 mL) for 17 hours. 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, 4x 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 x 2 mL) and dried, affording **6a** as an off-white solid (23 mg, 0.08 mmol, 27%, mp 144.0 – 146.2 ^{o}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, 4x 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, $[M\text{-}Cl]^+$).

4.11.2. 1-(Adamantan-1-yl)-*N*-(2-aminoethyl)-1*H*-1,2,3-triazole-4-carboxamide (n6b) and 2-(1-(adamantan-1-yl)-1*H*-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 x 2 mL) and DCM (3 x 2 mL) and dried, affording **6b** as a white solid (31 mg******, 0.09 mmol, quant., mp >195 ^{o}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, $[M\text{-}Cl]^+$).
*****) Theoretic 100% yield = 78 mg: 4 mg from unidentified byproducts and residual ethylene diamine.
******) The additional 3 mg were mostly water and solvent residues.

4.11.3. *N*-(2-Aminoethyl)-1-(4-(*tert*-butyl)benzyl)-1*H*-1,2,3-triazole-4-carboxamide (n6c) and 2-(1-(4-(*tert*-butyl)benzyl)-1*H*-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 hours at 50 ^{o}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 x 2 mL) and DCM (3 x 2 mL) and dried, affording **6c** as a white solid (50 mg, 0.15 mmol, 59 %, mp 218.0 – 220.4 ^{o}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),

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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, $[M\text{-}Cl]^+$).

4.11.4. *N*-(2-Aminoethyl)-1-(3,5-di-*tert*-butylbenzyl)-1*H*-1,2,3-triazole-4-carboxamide (n6d) and 2-(1-(3,5-di-*tert*-butylbenzyl)-1*H*-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 hours 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, 2x *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, 2x *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, $[M\text{-}Cl]^+$).

4.12. Amino(4-(1-heptyl-1*H*-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 hours. 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, 4x 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, $[M\text{-}Cl]^+$).

4.13. (4-(1-(Adamantan-1-yl)-1*H*-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 hours 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 x 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, 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:

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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, $[M\text{-}Cl]^+$).

4.14. Amino(4-(1-(3,5-di-*tert*-butylbenzyl)-1*H*-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 hours reflux. Filtration of the cooled reaction mixture and washing the precipitate with MeCN (3 x 3 mL), DCM (3 x 2 mL) and Et_2O (3 x 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. ^{1}H 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, 2x *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, $[M\text{-}Cl]^+$).

4.15. Amino((2-(1-heptyl-1*H*-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 hours reflux. Filtration upon cooling and careful washing of the precipitate with MeCN (3 x 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. ^{1}H 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, 4x 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, $[M\text{-}Cl]^+$).

4.16. ((2-(1-(Adamantan-1-yl)-1*H*-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. ^{1}H 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, $[M\text{-}Cl]^+$).

4.17. Amino((2-(1-(4-(*tert*-butyl)benzyl)-1*H*-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 x 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. ^{1}H 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 –

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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]$^+$).

4.18. Amino((2-(1-(3,5-di-*tert*-butylbenzyl)-1*H*-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. ^{1}H 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, 2x *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]$^+$).

4.19. *N*-(2-Aminoethyl)-1-(3,5-di-*tert*-butylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (n9e) and 2-(1-(3,5-di-*tert*-butylphenyl)-1*H*-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 hours at room temperature. Affording **n9e** as a white solid (0.196 g, 0.57 mmol, 90%). ^{1}H 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, 2x *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. ^{1}H 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, 2x *t*-Bu). ^{13}C NMR (100 MHz, *d4*-MeOD): 163.7 ($C_{C=O}$), 154.6 (C_{Ph}-3 and C_{Ph}-5), 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]$^+$).

4.20. *N*-(2-Aminoethyl)-1-(4-(heptyloxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (n9f) and 2-(1-(4-(heptyloxy)phenyl)-1*H*-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 hours at reflux. Re-evaporation of the crude from DCM afforded **n9f** as a white solid (0.100 g, 0.29 mmol, 92%). ^{1}H 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, 4x 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 x 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,

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0.75 mL/min, 214 nm): 7.3 min, 95% pure. ^{1}H 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, 4x 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, $[M\text{-}Cl]^+$).

4.21. *N*-(2-Aminoethyl)-1-(4-((3,5-di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-1,2,3-triazole-4-carboxamide (n9g) and 2-(1-(4-((3,5-di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-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 hours at reflux. Affording **n9g** as a white solid (0.103 g, 0.23 mmol, 96%). ^{1}H 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, 2x *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 x 2 mL) and drying afforded **9g** as a white solid (18 mg, 0.037 mmol, 83%, mp 250.0 – 254.5 ^{o}C). HPLC (C18, 1:3 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 23.3 min, 97% pure. ^{1}H 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, 2x *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, $[M\text{-}Cl]^+$). Ph = 3,5-di-*tert*-butylbenzyl.

4.22. Amino((2-(1-(3,5-di-*tert*-butylphenyl)-1*H*-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 hours 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 x 2 mL) and Et_2O (3 x 10 mL) followed by drying, afforded **10e** as a pink solid (32 mg, 0.076 mmol, 52%, mp 272 - 276 ^{o}C). HPLC (C18, 3:5 H_2O/MeOH + 0.1% TFA, 0.75 mL/min, 214 nm): 42.3 min, 95% pure. ^{1}H 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, 2x *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, $[M\text{-}Cl]^+$).

4.23. Amino((2-(1-(4-(heptyloxy)phenyl)-1*H*-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,

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0.12 mmol) and *1H*-pyrazole carboxamidine hydrochloride (26 mg, 0.07 mmol) was refluxed for 20 hours, added triethylamine (50 mg, 0.49 mmol) refluxed for 20 hours, 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 hours. The cooled reaction mixture was evaporated, and the crude was washed with MeCN (3 x 2 mL) and H_2O (3 x 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. ^{1}H 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, 4x 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]$^+$).

4.24. Amino((2-(1-(4-((3,5-di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-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, 2x *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.

4.25. Amino(2-(1-(1-(3,5-di-*tert*-butylbenzyl)-1*H*-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 hours. The cooled reaction mixture was then evaporated, washed with H_2O (2 x 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, 2x *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, 2x *t*-Bu). ^{13}C NMR (150 MHz, *d4*-MeOD, minor isomer): δ 157.5 (C_{Guan}), 153.1 (C_{Ph}-3 and C_{Ph}-

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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).

4.26. Amino(2-(1-(1-(3,5-di-*tert*-butylphenyl)-1*H*-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 hours. 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, $[M\text{-}TFA]^+$). ^{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, 2x *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, 2x *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).

4.27. Amino(2-(1-(1-(4-(heptyloxy)phenyl)-1*H*-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, $[M\text{-}TFA]^+$). ^{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 x 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, 3x CH_2), 0.95 – 0.89 (m, 3H, CH_3).

4.28. Amino(2-(1-(1-(4-((3,5-di-*tert*-butylbenzyl)oxy)phenyl)-1*H*-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 hours 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

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(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, 2x *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, 2x *t*-Bu). Ph = 3,5-di-*tert*-butylbenzyl.

4.29. 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.

4.30. 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.

4.31. Cytotoxicity to HepG2-cells

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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 hours. 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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Supporting information

Supporting information containing full characterization (^{1}H- and ^{13}C-NMR spectra) of novel compounds is electronically available through the publisher's website.

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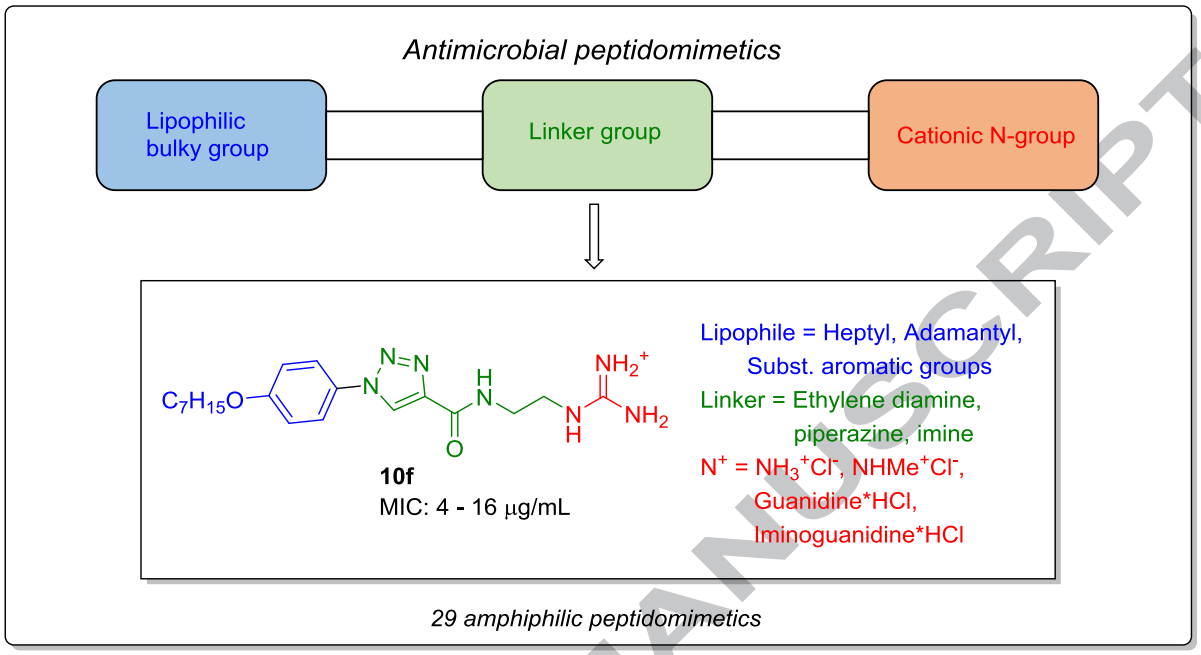
Graphical abstract



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