The synthesis and biological evaluation of a library of autoinducer-antibiotic conjugates

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This dissertation is submitted for the degree of Doctor of Philosophy
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1 Declaration

This dissertation describes work carried out in the Department of Chemistry, University of Cambridge under the supervision of Professor David Spring, and in the Department of Biochemistry, University of Cambridge under the supervision of Dr Martin Welch. This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution, except those parts which were included in my CPGS dissertation. The dissertation does not exceed the word limit specified by the Physics and Chemistry Degree Committee.

Lois Overvoorde
7th of September 2018
2 Abstract

Microbial resistance to antibiotics is a serious global health threat, and the discovery of new, safe and effective antibiotics is required urgently. A new class of antibiotics, namely sideophore-antibiotic conjugates, has shown promise in initial studies. Siderophores are used by bacteria for iron uptake, and so attaching antibiotics to them allows the antibiotic to be carried across cell membranes. This study investigated conjugates designed using a similar approach, but using bacterial autoinducers instead of siderophores. Autoinducers are required for coordination of bacterial behaviours and are involved in the control of swarming, virulence factor production and biofilm formation.

The quorum sensing molecules produced by *Pseudomonas aeruginosa* were chosen for investigation as *P. aeruginosa* is a significant human pathogen which displays high resistance to many antibiotics and uses quorum sensing to coordinate its group behaviours. Ciprofloxacin and trimethoprim were chosen as the antibiotic partners. Ciprofloxacin is commonly used against *P. aeruginosa* but resistance to it is developing, whereas *P. aeruginosa* is inherently resistant to trimethoprim. It was hypothesised that the autoinducers would aid retention of the antibiotics in cells, hence increasing or restoring activity.

An initial library was synthesised in two halves which were coupled together using a copper(I)-catalysed azide-alkyne cycloaddition. The autoinducers were functionalised with azide groups and the antibiotics (specifically ciprofloxacin and trimethoprim) were functionalised with alkynes. Two cleavable alkynyl ciprofloxacin derivatives were also included.

A second set of compounds, namely homoserine lactone analogue-ciprofloxacin conjugates were then synthesised, building on the one known report of a conjugate of a quorum sensing modulator and an antibiotic.

The most active conjugate found was a cleavable conjugate of homocysteine thiolactone (a homoserine lactone analogue) and ciprofloxacin. This compound showed enhanced antibacterial activity against *P. aeruginosa* compared to ciprofloxacin, and *P. aeruginosa* may develop less resistance towards it.
3 Acknowledgements

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4 Nomenclature

\( m \) Mass
\( v \) Volume
\( J \) Coupling constant in Hz
\( m/z \) Mass to charge ratio in Daltons
\( R_f \) Retention factor
\( Ac \) Acetate
\( AIP \) Autoinducing peptide
\( aq. \) Aqueous
\( atm \) Atmosphere(s)
\( BHL \) Butyryl homoserine lactone = C4-HSL
\( Boc \) tert-Butyloxycarbonyl
\( Bu \) Butyl
\( Cip \) Ciprofloxacin
\( conc. \) Concentrated
\( COSY \) Correlation spectroscopy
\( d \) Day(s)
\( Da \) Daltons
\( DBU \) 1,8-Diazabicyclo[5.4.0]undec-7-ene
\( DIPEA \) N,N-Diisopropylethylamine
\( DMAP \) 4-Dimethylaminopyridine
\( DMF \) Dimethylformamide
\( DMP \) Dess-Martin periodinane
\( DMSO \) Dimethylsulfoxide
\( EDC \) 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
\( eq. \) Equivalents
\( ESI \) Electrospray ionization
\( Et \) Ethyl
\( FT \) Fourier transform
\( h \) Hour(s)
\( HCTL \) Homocysteine thiolactone
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>HHQ</td>
<td>2-Heptylquinolin-4(1H)-one</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple-bond correlation spectroscopy</td>
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<tr>
<td>HMQC</td>
<td>Heteronuclear multiple-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>HOBr</td>
<td>1-Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectroscopy</td>
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<tr>
<td>HSL</td>
<td>Homoserine lactone</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography mass spectroscopy</td>
</tr>
<tr>
<td>LCT</td>
<td>Liquid chromatography time-of-flight</td>
</tr>
<tr>
<td>lit.</td>
<td>Literature value</td>
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<tr>
<td>M</td>
<td>Molar</td>
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<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>mol</td>
<td>Mole(s)</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl</td>
</tr>
<tr>
<td>NMP</td>
<td>N-Methyl-2-pyrrolidone</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OdDHL</td>
<td>N-(3-Oxododecanoyl)-homoserine lactone = 3-oxo-C_{12}-HSL</td>
</tr>
<tr>
<td>P.E.</td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>PAI-1</td>
<td><em>Pseudomonas</em> autoinducer 1 = 3-oxo-C_{12}-HSL</td>
</tr>
<tr>
<td>PAI-2</td>
<td><em>Pseudomonas</em> autoinducer 2 = C_{4}-HSL</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Palladium on carbon</td>
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<tr>
<td>PQS</td>
<td>Pseudomonas Quinolone Signal</td>
</tr>
<tr>
<td>Q-TOF</td>
<td>Quadrupole time-of-flight</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Second(s)</td>
</tr>
</tbody>
</table>
SAM  $S$-adenosyl-$l$-methionine
SAR  Structure activity relationship
sat.  Saturated
SD  Standard deviation
spp. Species
TBAF Tetrabutylammonium fluoride
TBDMS tert-Butyldimethylsilyl
TEA Triethylamine
Tf Trifluoromethanesulfonyl
TFA Trifluoroacetic acid
THF Tetrahydrofuran
THPTA Tris(3-hydroxypropyltriazolylmethyl)amine
TLC Thin layer chromatography
TMS Trimethylsilyl
Ts $para$-Toluenesulfonyl
UV Ultraviolet
5 Introduction

5.1 Antibiotic resistance

Antibiotics add, on average, twenty years to a person’s life.\(^1\) However, antibiotic resistance is increasing alarmingly and is now recognised as a major threat to global health.\(^1,2\) Antibiotic discovery had its heyday in the 1940s to 60s, which saw the discovery of many new classes of antibiotic. Since then, the rate of discovery of new classes has slowed, and resistance to existing treatments has increased.

The story of how Alexander Fleming discovered penicillin by accidentally allowing a Petri dish containing \textit{Staphylococcus aureus} to become contaminated with \textit{Penicillium} mould whilst he was on holiday in Suffolk\(^1\) is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,\(^3\) but it was not until 1943 that the drug was mass produced thanks to the research of Ernst Chain and Howard Florey. Unfortunately, bacterial resistance to penicillin was being found in hospitals by the late 1940s.\(^4,5\) This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see Table 1) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories:\(^1,6\)

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of \(\beta\)-lactam antibiotics such as penicillin by \(\beta\)-lactamase enzymes.

2. The bacterium may produce a membrane, cell wall or biofilm which does not allow the drug to pass through. For example, biofilm formation may allow bacterial resistance to antibiotics to increase 1000-fold compared with bacteria in suspension culture.\(^7\)

3. The bacterium may pump antibacterial molecules out of its cell membrane using efflux pumps, for example the MexAB and MexXY pumps used by \textit{Pseudomonas aeruginosa}.\(^8\)

4. Mutations may cause the target of the antibacterial molecule to alter such that the molecule no longer effectively binds the target, for example the alteration of penicillin binding proteins which are involved in the final stages of peptidoglycan biosynthesis in the cell walls of MRSA and other penicillin-resistant bacteria.\(^9\)

5. The bacterium may switch to using a metabolic pathway which does not involve the target of the antibacterial molecule, for example sulfonamide resistance may be achieved by taking in folic acid from the environment rather than synthesising it using \textit{para}-aminobenzoic acid - a process which is blocked by sulfonamides.\(^10\)
Table 1: A timeline of when various antibiotics were first introduced and when resistance to them first appeared.11–16 *Resistance was seen during a compassionate-use program before the drug was widely released.15

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Introduction</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>1930s</td>
<td>1940s</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1943</td>
<td>1946</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1943</td>
<td>1959</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1947</td>
<td>1959</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1953</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1988</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1988</td>
</tr>
<tr>
<td>Methicillin</td>
<td>1960</td>
<td>1961</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1961</td>
<td>1973</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1962</td>
<td>1972</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1960s</td>
<td>late 1960s</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1987</td>
<td>1988</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2000</td>
<td>1997*</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2003</td>
<td>2005</td>
</tr>
</tbody>
</table>

The current pipeline of new antibiotics is widely thought to be worryingly inadequate.17–19 Significant changes in how we use the antibiotics we already have, as well as investments in the discovery of new ones, are required. Antibiotics currently in late-stage clinical trials nearly all rely on non-novel mechanisms of action,17 and so it is almost inevitable that resistance to them will develop quickly, as it has done for their predecessors.

There is therefore increasing interest in treatments which would not easily provoke the development of resistance.20 These treatments often target bacterial virulence rather than killing bacteria outright, hence decreasing selection pressure for resistance.11 One obvious target is toxin production, for example, an LpxC inhibitor was shown to prevent lethal Acinetobacter baumannii infection in mice, despite being inactive against the bacterium in vitro.21 This was due to inhibition of lipopolysaccharide shedding, and hence reduced inflammation in the host. Co-ordination of virulence has also been targeted, for example, analogues of P. aeruginosa homoserine lactone (HSL) autoinducers (see 5.3.1) inhibit the production of virulence factors and increase the survival time of mice in a lethal P. aeruginosa lung infection model.11

A second strategy in novel antibiotic discovery is to enhance or restore activity of a known antibiotic by lessening or avoiding a resistance mechanism. For example, antibiotics are often excluded from cells due to membrane impermeability or efflux. This may be overcome by attaching the antibiotic ‘warhead’ to a molecule which the cell imports. The most well known example of this strategy is antibody-drug conjugates22 in the treatment of cancer, but progress has also made against bacteria. In particular, siderophore-antibiotic conjugates (see 5.2) have been investigated in the hope of hijacking bacterial uptake mechanisms to import antibiotics,23 and the autoinducer-antibiotic conjugates in this study may gain activity by avoiding efflux pumps (see 5.3). These conjugates may have competing mechanisms of action: either the antibiotic accumulates in the cell to a greater extent and acts by its usual mechanism, or an important bacterial system must be disrupted to avoid accumulation of the antibiotic, hence leading to decreased fitness and/or loss of virulence.

5.2 Siderophore-antibiotic conjugates

Siderophore-antibiotic conjugates have been receiving attention in recent years as a way to enhance the uptake of known antibiotics.23 This section will discuss the role of siderophores, sideromycins (natural siderophore-antibiotic conjugates), and the synthetic siderophore-antibiotic conjugates inspired by them. Many of the ob-
servations made about these molecules could be relevant to the autoinducer-antibiotic conjugates synthesised in this study.

5.2.1 Siderophores

Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of ‘iron mining’. Soluble iron is often scarce but it is crucial for many cellular processes including respiration and DNA synthesis. Siderophores are synthesised by the microorganisms and secreted into the extracellular environment where they bind to Fe$^{3+}$, often with exceptionally high affinities. The iron-bound siderophores are then brought back into the cell by active transport and the iron is released, either by reduction of the Fe$^{3+}$ to Fe$^{2+}$ or by enzymatic degradation of the siderophore. Siderophores have a wide range of structures (see Figure 2 and Figure 1), possibly so one species can avoid its siderophores being taken up by another species.

![Pyoverdine PaA](image)

![Pyochelin](image)

Figure 1: Iron-siderophore complexes: pyoverdine PaA $^{7,26,27}$ ($P. aeruginosa$, PAO1 strain) and pyochelin $^{8,28,29}$ ($P. aeruginosa$). Note that pyochelin 8 is a tetradentate ligand, hence the iron ion has two sites which can bind other ligands.
Figure 2: Iron-siderophore complexes: Deferoxamine B 1^{26} (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid 2^{30} (*Rhodotorula pilimanae*), fusarinine C 3^{31} (*Fusarium roseum*), enterobactin 4^{26} (*Escherichia coli* and enteric bacteria), ferrichrome 5^{32} (*Ustilago sphaerogena*, *U. maydis*, *Aspergillus niger*, *A. quadricintus*, *A. daricaulis* and *Penicillium resticulosum*), yersiniabactin 6^{26} (*Yersinia pestis*).
5.2.2 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins\textsuperscript{23} (see Figure 3). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds.

For example, albomycin 9 (see Figure 3) is a sideromycin produced by \textit{Actinomyces subtropicus} and \textit{Streptomyces griseus}\textsuperscript{33,34} which has been used to treat infections caused by various bacteria including \textit{Yersinia enterocolitica} and \textit{Streptococcus pneumoniae} in mice and humans.\textsuperscript{35,36} Albomycin 9 contains a siderophore coupled to a nuceloside antibiotic via a peptide linker. The siderophore section is structurally similar to ferrichrome 5 (see Figure 2), a siderophore produced by various fungi, but also taken up by bacteria including \textit{Escherichia coli}, \textit{Salmonella typhimurium} and \textit{P. aeruginosa}.\textsuperscript{32,37} It has been shown that because of the structural similarity to ferrichrome 5, \textit{E. coli} will also take up albomycin 9.\textsuperscript{33} The linker is hydrolysed in the cytoplasm of the \textit{E. coli}, releasing the active nuceloside antibiotic. This leads to 500-fold concentration of the antibiotic within the \textit{E. coli} cells, enough to have significant effect on growth.

The success of albomycin\textsuperscript{35} and other sideromycins such as salmycin A\textsuperscript{24,38,39} and ferrimycin A\textsuperscript{140,41} has served as encouragement to many researchers to explore synthetic siderophore-antibiotic conjugates, which will be discussed in the next section.
Figure 3: Iron sideromycin complexes: Albomycin 9 [24, 42] (Actinomyces subtropicus and Streptomyces griseus), salmycin A [24, 38, 39] (Streptomyces violaceus) and ferrimycin [24] (Streptomyces griseoflavus).

5.2.3 Synthetic siderophore-antibiotic conjugates

Sideromycins served as inspiration for the design, synthesis and biological evaluation of a wide range of synthetic siderophore-antibiotic conjugates. Antibiotics used include $\beta$-lactams, nucleosides, glycopeptides and macrolides. Sideromycin-fluoroquinolone conjugates have also been studied by several groups, including conjugates with linkers which can be cleaved in a similar manner to albomycin. Some of these showed comparable activity to the parent antibiotic, but it is not clear whether attachment of the siderophore improved uptake or whether the conjugates acted as classical prodrugs.

$\beta$-lactam-sideromycin conjugates have been more widely investigated and show good activity in vitro. However, resistance can evolve by loss of the TonB transporter or of the relevant siderophore receptor, e.g. Cir and Fiu for catecholate siderophores or FluA for hydroxamate siderophores. Recently a conjugate (Ent-Amp 12, see Figure 4) of enterobactin and ampicillin joined using a copper(I)-catalyzed azide-alkyne cycloaddition has been shown to have increased activity against pathogenic E. coli when compared to native ampicillin. Other
work has focused on monocyclic $\beta$-lactams, for example pirazmonam 13 and U-78608 14, which show high potency against Gram-negative bacteria including $P. \ aeruginosaa^{53,54}$ Monocyclic $\beta$-lactams are generally fairly stable to $\beta$-lactamase activity, which is an advantage compared with many bicyclic $\beta$-lactams.

Three siderophore-antibiotic conjugates are reported as being in clinical trials: 55 MC-1 15, 56 BAL30072 16, 23 (see Figure 4) and cefiderocol 17. 57, 58

MC-1 15 is reported as being ‘in clinical phases of development’, 55 but no reports of studies in humans could be found. However, experiments in mice have been promising. 56 BAL30072 16 is a siderophore-$\beta$-lactam conjugate which showed initial promise as it is a poor substrate for $\beta$-lactamases, and resistance due to loss of transport proteins is infrequent. 23 However, it is unclear whether it will progress further in trials as it causes liver toxicity. 59 Cefiderocol 17 is a cephalosporin-catechol conjugate in phase 1 trials. Recent results indicate that ‘single and 35 multiple intravenous doses of cefiderocol at up to $2000 \text{ mg}$ were well tolerated in healthy 36 subjects’. 58

These examples show that siderophore-antibiotic conjugates are a promising strategy to deliver antibiotics across bacterial membranes, but it is worth noting that conjugation to a siderophore may lead to loss of activity, or resistance may be acquired by loss of transport proteins. Encouragingly though, albomycin 9-resistant mutants have been shown to be less virulent, 36 indicating that bacteria may lose out either by susceptibility to the antibiotic or by loss of fitness due to decreased iron transport.

Building on these positive examples, it is hoped that the strategy of conjugating a molecule which is important for virulence 60 with an antibiotic can be extended to conjugates of autoinducers and antibiotics in a similar ‘Trojan horse’ approach.
Figure 4: Examples of siderophore-antibiotic conjugates: Ent-Amp 12,52 pirazmonam 13,53,54 U-78608 14,53,54 MC-1 15,56 BAL30072 1623 and cefiderocol 17.57,58
5.3 Autoinducer-antibiotic conjugates

This study extends the conjugation strategy discussed above by creating autoinducer-antibiotic conjugates. It was hypothesised that attaching an autoinducer to a known antibiotic could lead to increased cellular retention of the antibiotic, and could potentially restore function against resistant strains. This is thought to be the first large study of autoinducer-antibiotic conjugates, with only one such molecule having been reported previously. This section begins by introducing the concept of quorum sensing, followed by discussion of the autoinducers and antibiotics used in this study and the mechanisms of their efflux from *P. aeruginosa* cells, and how these mechanisms could be exploited by conjugates.

5.3.1 Quorum sensing

A quorum is defined as ‘A fixed minimum number of members of an assembly or society that must be present at any of its meetings to make the proceedings of that meeting valid.’ A similar concept is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum concentration of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors, swarming and biofilm formation. It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium. The process by which bacteria determine the concentration of similar bacteria in their vicinity, and act on that information, is known as quorum sensing.

Quorum sensing has been observed in many species of bacteria, including *Vibrio fischeri*, *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *S. aureus*, *V. harveyi*, *Escherichia coli*, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica*, *Aeromonas spp.* and *Acinetobacter spp.* Many of these bacteria are significant causes of disease and death in humans, for example, in a typical year in the U.S. *P. aeruginosa* causes 6,700 multidrug-resistant infections and 440 deaths, methicillin-resistant *S. aureus* causes 80,500 severe infections and 11,300 deaths and non-typhoidal *Salmonella* causes 1.2 million illnesses, 23,000 hospitalisations and 450 deaths.

5.3.1.1 *Vibrio fischeri*

The first example of quorum sensing was discovered in *V. fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid, *Euprymna scolopes* (see Figure 5). This bacterium receives amino acids from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself.

If a low population of *V. fischeri* were present in the photophore, the light that the bacteria could produce would be insufficient to provide counterillumination. Therefore, the bacteria conserve resources by not producing light. However, if there is a high population of *V. fischeri* it is useful for them all to produce light, as this incentives the squid to provide them with nutrients.
V. fischeri uses the LuxR-LuxI system to sense cell density. This system is seen as a paradigm of quorum sensing, and a simplified explanation of it is presented to show typical features of such a system (see Figure 6).

V. fischeri senses cell concentration by the detection of 3-oxo-C6-HSL \( 18^{76} \) (see Figure 7), a freely diffusible\(^{77} \) molecule which is synthesised by LuxI\(^{78,79} \) and secreted by all V. fischeri cells\(^{80} \) at a low basal level.\(^{63} \) When
the bacterial population density, and hence the concentration of 3-oxo-C$_6$-HSL 18, reaches a threshold, 3-oxo-C$_6$-HSL 18 binds to LuxR,\textsuperscript{81–83} a receptor which is also synthesised at a low basal level.

![Figure 7: 3-oxo-C$_6$-HSL 18.](image)

The LuxR complex binds to the lux operator, upregulating production of LuxI and hence 3-oxo-C$_6$-HSL 18, and luciferase enzymes and hence blue-green light.\textsuperscript{84–86} Production of more 3-oxo-C$_6$-HSL 18 enables a positive feedback loop, reinforcing the effect of high population density on 3-oxo-C$_6$-HSL 18 concentration and hence light production. This is the reason that 3-oxo-C$_6$-HSL 18 is known as an autoinducer.

The system also contains a negatively feedback loop to avoid excessive expression of proteins: at high concentrations of 3-oxo-C$_6$-HSL 18 production of LuxR is inhibited.\textsuperscript{87} Such balancing effects, as well as interactions with other quorum sensing and metabolic systems, are seen in many bacteria.\textsuperscript{63,88}

5.3.1.2 \textit{Pseudomonas aeruginosa}

Another well-studied example of quorum sensing is in \textit{P. aeruginosa}.\textsuperscript{88–90} \textit{P. aeruginosa} is a Gram-negative opportunistic pathogen which typically infects immunocompromised individuals such as those with cystic fibrosis, neutropenia and AIDS. It can infect the pulmonary and urinary tracts as well being the most frequent cause of burn wound infections and the most frequent coloniser of medical devices such as catheters.\textsuperscript{91} Multidrug-resistant \textit{P. aeruginosa} is classified as a ‘serious threat’ by the United States Centers for Disease Control and Prevention\textsuperscript{2} and carbapenem-resistant \textit{P. aeruginosa} is classified as ‘priority 1: critical’ by the World Health Organisation.\textsuperscript{18}

\textit{P. aeruginosa} has a low susceptibility to many antibiotics and readily acquires antibiotic resistance by mutation or horizontal gene transfer.\textsuperscript{92} It is difficult for antibiotics to cross into cells due to low cell membrane permeability,\textsuperscript{93} and they are pumped out again by its multiple chromosomally encoded multidrug efflux pumps.\textsuperscript{8} \textit{P. aeruginosa} also forms biofilms: colonies of microbes held together and protected by a matrix containing polysaccharides, proteins and DNA. Bacteria are usually studied in the planktonic state, where they are present as free-floating cells which are often more metabolically active. However, the study of bacteria in biofilms is important due to their importance in chronic infections and the colonisation of implanted medical devices.\textsuperscript{7,94} \textit{P. aeruginosa} biofilms are more resistant to many drugs including ciprofloxacin 24 and trimethoprim 25 compared with planktonic cells.\textsuperscript{95,96} This high level of antibiotic resistance makes \textit{P. aeruginosa} an important target for drug discovery.

Quorum sensing in \textit{P. aeruginosa} involves a complex interplay of five signalling molecules (see Figure 8) and various proteins (see Figure 9).\textsuperscript{88–90} These can be broken down into three main, interacting systems: Las, Rhl and Pqs.
In the Las system, LasI\textsuperscript{97} synthesises the 3-oxo-C\textsubscript{12}-HSL \textbf{20}\textsuperscript{98} autoinducer. 3-oxo-C\textsubscript{12}-HSL \textbf{20} binds LasR,\textsuperscript{99} and this complex upregulates the production of LasI\textsuperscript{100} (thus causing autoinduction) as well as alkaline protease,\textsuperscript{101} elastase,\textsuperscript{101} exotoxin A,\textsuperscript{101} HCN\textsuperscript{102} and LasA protease.\textsuperscript{103} The LasR complex is also important in late-stage biofilm formation,\textsuperscript{98} and upregulates the Rhl\textsuperscript{104} and Pqs systems.\textsuperscript{105,106}

In the Rhl system, RhlI\textsuperscript{107} synthesises the C\textsubscript{4}-HSL \textbf{19}\textsuperscript{108} autoinducer. C\textsubscript{4}-HSL \textbf{19} binds RhlR,\textsuperscript{109} and this complex upregulates the production of RhlI\textsuperscript{100} (again causing autoinduction), alkaline protease,\textsuperscript{107} elastase,\textsuperscript{107} haemolysin,\textsuperscript{110} HCN,\textsuperscript{102,110} LasA protease,\textsuperscript{107} LecA,\textsuperscript{111} pyocyanin\textsuperscript{107,110} and rhamnolipids.\textsuperscript{107} The RhlR complex also downregulates the Pqs system.\textsuperscript{106,112} The Rhl system is controlled by both the Las and Pqs systems, as production of both RhlR and RhlI is upregulated by the LasR complex\textsuperscript{104} and production of both RhlR is upregulated by the PqsR complex.\textsuperscript{113}

In the Pqs system, the main autoinducer, PQS \textbf{22},\textsuperscript{114} is synthesised by multiple enzymes: PhnAB,\textsuperscript{115} PqsA, PqsBC, PqsD\textsuperscript{116,117} and PqsE\textsuperscript{118,119} produce the precursor HHQ \textbf{21}, and PqsH converts HHQ \textbf{21} to PQS \textbf{22}. PQS \textbf{22}\textsuperscript{106} or HHQ \textbf{21} binds PqsR,\textsuperscript{120} and either complex can upregulate the synthesis of HHQ \textbf{21} causing autoinduction. The PqsR-PQS complex upregulates the production of chitinase,\textsuperscript{121} elastase,\textsuperscript{114} HCN,\textsuperscript{121} LecA,\textsuperscript{122} pyocyanin\textsuperscript{105,123} and pyoverdine,\textsuperscript{123} as well as increasing biofilm production\textsuperscript{122} and vesicle formation.\textsuperscript{124} The PqsR-PQS complex also upregulates production of RhlR, so the Pqs system has control over the Rhl system.\textsuperscript{113} The Pqs system is controlled by both the Las and Rhl systems, as production of PqsR\textsuperscript{106} and PqsH\textsuperscript{105} is upregulated by the LasR complex and production of PqsA, PqsBC, PqsD, PqsE\textsuperscript{112} and PqsR\textsuperscript{106} is downregulated by the RhlR complex.
In addition to the above systems, AI-2 (see Figure 8), an interspecies signalling molecule, is known to increase biofilm production and virulence in \textit{P. aeruginosa} \cite{125}. This is thought to be achieved by interaction with the Las and Rhl systems, but the exact mechanism is not known.

In summary, \textit{P. aeruginosa} uses the autoinducers shown in Figure 8 as part of three interacting quorum sensing systems to coordinate virulence and biofilm production, and this makes these autoinducers interesting therapeutic targets.

### 5.3.2 Autoinducers

Quorum sensing has been successfully targeted using many different modulators \cite{89,128} but this study takes a slightly different approach. Inspired by the success of various siderophore-antibiotic conjugates (see 5.2.3),
a library of autoinducer-antibiotic conjugates was synthesised to test the hypothesis that the importance of autoinducers in harmful cellular behaviours could lead to increased activity of the conjugates (see 5.3).

The *P. aeruginosa* autoinducers (see Figure 8) were chosen for use in this study as *P. aeruginosa* is a significant human pathogen which shows high antibiotic resistance and utilises quorum sensing to coordinate pathogenic behaviours (see 5.3.1.2). Specifically, C$_4$-HSL 19, HHQ 21 and PQS 22 derivatives were chosen as they were considered to be the most synthetically tractable.

### 5.3.3 Autoinducer efflux

Autoinducers must be exported from the cell in order to be used for intercellular communication, and the five known *P. aeruginosa* autoinducers are exported by various different transport mechanisms. The mechanism is not well known for HHQ 21 or AI-2 23, but it is know that PQS 22 is exported in vesicles. C$_4$-HSL 19 passively diffuses in and out of cells, and 3-oxo-C$_{12}$-HSL 20 is taken up passively, accumulates in the cell membrane and is actively pumped out by efflux pumps. The difference in transport mechanism for C$_4$-HSL 19 and 3-oxo-C$_{12}$-HSL 20 is thought to be largely due to chain length rather than the 3-oxo modification, as a shorter-chain version, 3-oxo-C$_6$-HSL 18 has been shown to be freely diffusible through *V. fischeri* membranes.

3-oxo-C$_{12}$-HSL 20 is exported primarily via the MexAB-OprM efflux system. The increased removal of 3-oxo-C$_{12}$-HSL 20 from the cell by upregulation of the MexAB-OprM system leads to decreased production of additional 3-oxo-C$_{12}$-HSL 20 (as the positive feedback loop is disrupted, see 5.3.1.2), and hence decreased production of pyocyanin, elastase and casein protease. It is expected that MexAB-OprM upregulation would also disrupt biofilm formation as a decrease in 3-oxo-C$_{12}$-HSL 20 levels would disrupt Las-mediated quorum sensing, but no direct studies of this could be found.

### 5.3.4 Antibiotics

Ciprofloxacin 24 and trimethoprim 25 (see Figure 10) were chosen as the antibiotic sides of the conjugates.

Ciprofloxacin 24 is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections including *P. aeruginosa*. Ciprofloxacin 24 inhibits DNA replication by binding to DNA gyrase and topoisomerase IV.

Trimethoprim 25 (see Figure 10) is a dihydrofolate reductase inhibitor used primarily to treat bladder infections. It is active against several significant human pathogens including *Streptococcus pneumoniae* and *Haemophilus influenzae*, but not against *P. aeruginosa*. It was primarily chosen in this study as it was considered easy to functionalise, but also to test the feasibility of creating antibiotic activity against *P. aeruginosa*.

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**Figure 10:** The antibiotics used in this section.
5.3.5 Antibiotic efflux

Ciprofloxacin 24 enters *P. aeruginosa* by diffusion, but is pumped out by efflux pumps. In the planktonic state several efflux pumps are known to pump out ciprofloxacin 24, including MexAB–OprM, MexCD–OprJ, MexEF–OprN, MexXY–OprM, MexJK–OprM and MexVW–OprM. However, in biofilms only MexEF–OprN has an effect.

Trimethoprim 25 is mainly exported by the MexAB–OprM, MexCD–OprJ and MexEF–OprN multidrug efflux systems in the planktonic state. It is not known which pumps are used to export trimethoprim 25 from biofilms, but biofilms do show increased resistance to it.

5.3.6 Conjugate efflux and antibiotic action

There are two synergistic mechanisms by which the conjugates could disrupt *P. aeruginosa* growth:

1. *P. aeruginosa* could develop resistance to an autoinducer-antibiotic conjugate by upregulation of the autoinducer’s export mechanism, but this would also lead to increased export of the native autoinducer, thus disrupting the quorum sensing system and hence biofilm formation and virulence. For HSL conjugates this would mean upregulation of the MexAB-OprM pump, as this is the pump used for export of 3-oxo-C12-HSL 20. For PQS conjugates this would mean upregulation of vesicle formation.

2. The autoinducer section could make the conjugate a poor substrate for the antibiotic section’s usual efflux mechanism, leading to accumulation of the conjugate within cells and hence increased antibacterial activity. For autoinducer-ciprofloxacin conjugates acting on planktonic *P. aeruginosa* this would mean the conjugate being a poor substrate of the various efflux pumps listed in the previous section. For autoinducer-ciprofloxacin conjugates acting on biofilms this would mean the conjugate being a poor substrate of MexEF–OprN (the sole exporter of ciprofloxacin 24 in biofilms and not an exporter of HSLs or PQS). This mechanism could in principal work for trimethoprim 25 as well, but it is not known which pumps are active against this antibiotic in biofilms.

It is worth noting that for either of these mechanisms we are primarily interested in the autoinducer’s interaction with its import/export mechanism, rather than its receptor in the quorum sensing system. However, binding to receptors could help retention within the cell, so either way it is important that both the autoinducer and antibiotic sides of the conjugates closely resemble the unconjugated molecules. With this in mind, an initial library was designed using a copper(I)-catalysed azide-alkyne cycloaddition reaction to join each combination of autoinducer and antibiotic together, using relatively long linkers in order to stop one side interfering with the binding of the other to its target protein.

5.3.7 Cleavable linkers

As part of the library, a set of cleavable HSL-Cip triazole conjugates was synthesised in collaboration with Professor Eddy Sotelo. These were based on the cleavable pyochelin–norfloxacin conjugates synthesised by Rivault *et al.* (see Figure 11). It was envisaged that the linker would be stable under the extracellular assay conditions, but would be cleaved upon entry into the cell by intracellular esterases. It was hoped that the attached HSLs would improve retention of the conjugate in cells.
The properties of similar linkers (see Figure 12, R = Me) were studied by Gogate et al., who found that they were stable for more than 3 years under optimal conditions.\textsuperscript{146} The hydrolysis of a secondary amine prodrug is dependent on ester hydrolysis rate, therefore the cleavage rate can be tuned by changing the R group between the ester and amide.\textsuperscript{147} The $N$-(acetoxyethoxycarbonyl) (R = Me) linkers have been shown to be cleaved by esterases at an enhanced rate compared to buffer, and thus show promise in prodrugs.\textsuperscript{148} It was therefore hoped that they will allow intracellular release of the ciprofloxacin\textsuperscript{24} payload from the conjugates in this study. Both the $N$-(acetoxymethoxycarbonyl) (R = H) and $N$-(acetoxyethoxycarbonyl) (R = Me) were used, to investigate whether differences in cleavage rate could tune activity.

5.3.8 Homoserine lactone analogue-ciprofloxacin conjugates

Following on from the library of compounds based on \textit{P. aeruginosa} autoinducers, a series of conjugates based on analogues of HSL were planned. This strategy was inspired by a paper\textsuperscript{61} and patent\textsuperscript{149} by Ganguly \textit{et al.}, who synthesised and characterised a conjugate \textbf{154} of methyl ciprofloxacin \textbf{151} with homocysteine thiolactone (see Figure 13). Homocysteine thiolactone is an analogue of HSL with the ring oxygen replaced by sulfur, and has been used as the head group in several other known quorum sensing modulators.\textsuperscript{80,150–156}
As part of their characterisation of the HCTL-CipMe conjugate 154, Ganguly et al. found the minimum inhibitory concentration (MIC) of the conjugate in *P. aeruginosa* under standard planktonic conditions. The MIC was found to be ten times higher for the conjugate vs. ciprofloxacin 24 (50 vs. 5 µm), indicating that the conjugate was less effective than ciprofloxacin 24 under planktonic conditions.

Ganguly et al. then investigated the effect of the conjugate on biofilms. The conjugate 154 and ciprofloxacin 24 were first added to dilute *P. aeruginosa* liquid culture at 25 µm. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin 24, but did grow in the presence of the conjugate 154. They then incubated cultures for 24 h, to allow biofilms to grow, before adding the compounds. In contrast, they found that the conjugate 154 disrupted the biofilm more effectively than ciprofloxacin 24. When the biofilm was grown for 48 or 72 hours the conjugate had similarly disruptive effects, whereas ciprofloxacin 24 ‘did not show any significant antibacterial activity’.

These results are exciting as they hint that an autoinducer conjugate might be able to combat an established *P. aeruginosa* infection more effectively than the unmodified antibiotic. Ganguly et al. suggest that their conjugate 154 is more effective than ciprofloxacin 24 in penetrating biofilms, and/or better at avoiding being pumped out by multidrug efflux pumps. They posit that this could be due to the thiolactone head, as they also showed that unconjugated C4-HCTL 28 (see Figure 14) has ‘either enhanced uptake or functional activity’ when compared with C4-HSL 19.

It is possible that the conjugate 154 has higher activity against biofilms when compared with ciprofloxacin 24 because the conjugate 154 avoids being pumped out by multidrug efflux pumps, or selects for the survival of mutants with upregulated efflux pumps, and hence disrupts quorum sensing systems (see 5.3.6).

While one might expect the conjugate 154 to behave like C4-HSL 19, and hence passively diffuse in and out of cells, it is possible that its transport more closely resembles that of 3-oxo-C12-HSL 20. 3-oxo-C12-HSL 20’s accumulation in membranes and interaction with efflux pumps is thought to be based primarily on tail chain length (see 5.3.3), and the ciprofloxacin half of the conjugate 154 could be seen as a long tail, especially as the carboxylic acid is methylated and hence less polar.

Figure 14: C4-HSL 19 and C4-HCTL 28. Note that Ganguly et al. tested the *S* enantiomer of C4-HCTL 28, but used a racemic mixture in their HCTL-CipMe conjugate.

While the results found by Ganguly et al. show promise, they only test one conjugate, and do not include controls to show that the HCTL group specifically is necessary for the enhanced effect. It was therefore decided...
to build on this work by synthesising a series of ciprofloxacin conjugates with head groups taken from known quorum sensing modulators,\textsuperscript{128,157} a selection of which are described in Table 2.

<table>
<thead>
<tr>
<th>Head group</th>
<th>Partial agonist and antagonist against LasR.\textsuperscript{154} Shown to increase biofilm formation in \textit{P. aeruginosa}.\textsuperscript{61}</th>
<th>Strong agonist against LasR, with comparable activity to the native ligand.\textsuperscript{151,152,154,158}</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Head group" /></td>
<td><img src="image2" alt="Head group" /></td>
<td><img src="image3" alt="Head group" /></td>
</tr>
<tr>
<td>Partial agonist against LasR.\textsuperscript{157}</td>
<td>Strong antagonist against LasR.\textsuperscript{157}</td>
<td></td>
</tr>
<tr>
<td><img src="image4" alt="Head group" /></td>
<td><img src="image5" alt="Head group" /></td>
<td></td>
</tr>
<tr>
<td>Poor agonist and antagonist against RhlR.\textsuperscript{159,160}</td>
<td>Strong antagonist against LasR.\textsuperscript{159}</td>
<td></td>
</tr>
<tr>
<td><img src="image6" alt="Head group" /></td>
<td><img src="image7" alt="Head group" /></td>
<td></td>
</tr>
<tr>
<td>Strong agonist against RhlR.\textsuperscript{159} \textit{SS} enantiomer is more potent.\textsuperscript{160}</td>
<td>Partial agonist against LasR.\textsuperscript{159}</td>
<td></td>
</tr>
<tr>
<td><img src="image8" alt="Head group" /></td>
<td><img src="image9" alt="Head group" /></td>
<td></td>
</tr>
<tr>
<td>Strong agonist against RhlR.\textsuperscript{159} \textit{SS} enantiomer is more potent, with comparable activity to the native ligand.\textsuperscript{160}</td>
<td>Strong agonist against LasR.\textsuperscript{152,159} \textit{SS} enantiomer is more potent, with comparable activity to the native ligand.\textsuperscript{160}</td>
<td></td>
</tr>
<tr>
<td><img src="image10" alt="Head group" /></td>
<td><img src="image11" alt="Head group" /></td>
<td></td>
</tr>
<tr>
<td>Strong agonist against RhlR.\textsuperscript{159} \textit{SS} enantiomer is more potent.\textsuperscript{160}</td>
<td>Partial antagonist against LasR.\textsuperscript{159} Shown to reduce biofilm formation in \textit{P. aeruginosa}.\textsuperscript{159}</td>
<td></td>
</tr>
<tr>
<td><img src="image12" alt="Head group" /></td>
<td><img src="image13" alt="Head group" /></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Activities of quorum sensing modulators containing the head groups used in this study.

6 Project aims and summary

The aim of this project is to produce and test a library of autoinducer-antibiotic conjugates with the goal of producing conjugates with greater potency than the parent antibiotics. The work is divided into two main sections. Section 7 focuses on conjugates of three \textit{P. aeruginosa} autoinducers (see Figure 8) with ciprofloxacin \textsuperscript{24} and trimethoprim \textsuperscript{25} (see Figure 10) joined using a copper(I)-catalyzed azide-alkyne cycloaddition. Section 8 focuses on conjugates of HSL analogues with ciprofloxacin \textsuperscript{24} (see 5.3.8) joined either using a copper(I)-catalyzed azide-alkyne cycloaddition or an \textit{SN}2 reaction or peptide coupling.
7 Results and discussion: autoinducer-antibiotic conjugates

7.1 Overview

The first part of this project was focused on producing a library of autoinducer-antibiotic conjugates. *P. aeruginosa* autoinducers were used, in particular C₄-HSL 19, HHQ 21 and PQS 22 (see Figure 8). Azido derivatives of these compounds were coupled to alkynyl derivatives of antibiotics, specifically ciprofloxacin 24 and trimethoprim 25 (see Figure 10), using a copper(I)-catalysed azide-alkyne cycloaddition. The compounds were then tested for antibiotic and anti-biofilm activity against *P. aeruginosa*. The decisions on where to attach the azide or alkyne handles to the chosen molecules are discussed below.

7.1.1 Azido autoinducer derivatives

The structure-activity relationships in HHQ 21 and PQS 22 have been previously studied, and it was shown various substitutions on the benzene ring could be made without significantly decreasing activity. The 6-azido derivatives (see Figure 15) were chosen for this study as routes to them have previously been found.

![Figure 15: The azido derivatives of HHQ 21 and PQS 22: 38 and 49.](image)

Alteration of the lactone group of HSL derivatives is known to significantly decrease activity, especially where the number of H-bond donors or acceptors is altered. Acyl tail length is known to play an important role in affinity, so three derivatives of C₄-HSL 19 were synthesised: N3-C₂-HSL 55, N3-C₄-HSL 58 and N3-C₆-HSL 61 (see Figure 16).

![Figure 16: The azido derivatives of C₄-HSL 19: 55, 58 and 61.](image)

7.1.2 Alkynyl antibiotic derivatives

The structure-activity relationships for ciprofloxacin 24 have been investigated and modifications at the cyclopropane and piperazine groups were found not to cause loss of activity. It was decided an alkyne tail would be added onto the free NH of the piperazine ring, as this position is more synthetically accessible. Alkynyl ciprofloxacin derivative 68 (see Figure 17) was synthesised in this study (see 7.3.1), and two cleavable alkynyl ciprofloxacin derivatives 90 and 91 were synthesised by Professor Eddy Sotelo and combined with the azido HSL derivatives described above to create cleavable conjugates (see 7.4.3).
Figure 17: The alkynyl ciprofloxacin derivatives 68, 90 and 91.

The choice to of alkyne tail attachment point on trimethoprin 25 (see Figure 18) is based on the use of that same point in a fluorogenic trimethoprim tag synthesised by Jing et al.\textsuperscript{167}

Figure 18: The alkynyl trimethoprim derivative 71.

7.1.3 Synthesis of the conjugates

A copper(I)-catalysed azide-alkyne cycloaddition\textsuperscript{144,145} was used to join each combination of autoinducer and antibiotic together (see Scheme 1).

Scheme 1: The construction of the triazole-linked autoinducer-antibiotic conjugate library using a copper(I)-catalysed azide-alkyne cycloaddition.

7.2 Synthesis of the azido autoinducer derivatives

7.2.1 Synthesis of 6-N$_3$-HHQ 38

The synthesis of 6-N$_3$-HHQ 38 is shown in Scheme 2 and follows a route devised by Baker.\textsuperscript{164} Octanoyl chloride 32 was converted to $\beta$-ketoester 33 via a Meldrum’s acid adduct.\textsuperscript{168,169} The $\beta$-ketoester 33 was condensed with N-Boc-para-phenylenediamine 35 to form enamine 36. The disappointing yield of this step was in part due to the reaction proceeding to an equilibrium state rather than to completion, and hence not all of the starting material being consumed; starting materials can be recycled to improve the yield. Alternatively, Baker later found a higher-yielding reaction using a ZrCl$_4$ catalyst.\textsuperscript{164}

The enamine 36 was cyclised with polyphosphoric acid to form amino-HHQ 37 in good yield. The amine group of amino-HHQ 37 was converted to a diazo group by reaction with NaNO$_2$ and HCl, followed by displacement with NaN$_3$ to form the final azido-HHQ product 38.\textsuperscript{170}
Scheme 2: The synthesis of 38. a) i) Pyridine, CH$_2$Cl$_2$, 0 °C. ii) MeOH, reflux, 66% over two steps. b) MeOH, reflux, 19%. c) Polyphosphoric acid, 120 °C, 72%. d) i) NaNO$_2$, HCl, water, 0 °C. ii) NaN$_3$, water, r.t., 41%.

7.2.2 Synthesis of 6-N$_3$-PQS 49

The synthesis of 6-N$_3$-PQS 49 is shown in Scheme 3, and also follows a route devised by Baker.$^{164}$ The Weinreb amide 43$^{89}$ was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide 40 to form 1-chlorononan-2-one 44 following a procedure described by Hodgkinson et al.$^{171}$

The synthesis of PQS 22 described by Hodgkinson et al.$^{171}$ used a microwave reaction of 1-chlorononan-2-one 44 with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid 45 in the place of anthranilic acid in this microwave reaction, so that the nitro group could then be converted to an azide group via an amine. However, the microwave-catalysed reaction failed when 5-nitroanthranilic acid 45 was used.$^{164}$ Therefore, a two step process was employed instead.

5-Nitroanthranilic acid 45 was heated with K$_2$CO$_3$ to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one 44 to form the ester 46 by SN$_2$ displacement of the chlorine atom in a procedure adapted from Hlaváč et al.$^{172}$ Cyclisation with polyphosphoric acid produced nitro-PQS 47 cleanly.$^{172,173}$

Conditions for the reduction of the nitro group were then compared (see Table 3). Baker initially used Zn and HCl, however this gave a yield over 100% suggesting coordination of Zn to the amino-PQS 48 (this product was taken through and purified after the next step). She also attempted reduction with Pd/C and H$_2$ or ammonium formate, but no reaction was observed.

Further conditions were tested in this work in order to obtain a clean sample of amino-PQS 48. An initial test of reduction with SnCl$_2$ produced no detectable product by LCMS. Catalytic hydrogenation using harsher conditions was then attempted, and it was determined that increasing the pressure to 3 atm using a Paar hydrogenator causes full conversion in 4 h using Pd/C and H$_2$. Good yields (80%) were also achieved using PtO$_2$ as a catalyst, with the advantage that the reaction proceeds more quickly, and at atmospheric pressure and temperature.$^{174}$

Finally, amino-PQS 48 was converted to azido-PQS 49 by reaction with NaNO$_2$ and HCl to form diazo-PQS, followed by displacement of the diazo group using NaN$_3$ to give the azido-PQS 49.$^{170}$ The yield of this reaction was rather disappointing (28%), and is probably due to loss of product in the supernatant following precipitation.$^{164}$
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2$, Pd/C, 1 atm, r.t., 18 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>$\text{NH}_4\text{HCO}_2$, Pd/C, 1 atm, r.t., 18 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>Zn, HCl (aq), r.t., 5 min h</td>
<td>Product 48 + Zn, assumed quantitative yield</td>
</tr>
<tr>
<td>$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, MeOH, r.t., 18 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>$\text{H}_2$, Pd/C, MeOH, 3 atm, r.t., 4 h.</td>
<td>Product 48, &gt;99% yield</td>
</tr>
<tr>
<td>$\text{H}_2$, PtO$_2$, MeOH, 1 atm, r.t., 45 min</td>
<td>Product 48, 80% yield</td>
</tr>
</tbody>
</table>

Table 3: Conditions attempted for the synthesis of 48. Rows 1-3 were carried out by Baker,$^{164}$ rows 4-6 were carried out as part of this study.

Scheme 3: The synthesis of 49. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) $\text{N}_3\text{O}-\text{dimethylhydroxyl amine hydrochloride}$, $\text{K}_2\text{CO}_3$, toluene, water, - 5 $^\circ$C to r.t., 30 min, 71%. c) THF, 0 $^\circ$C to r.t., 15 h, 96%. d) 45, $\text{K}_2\text{CO}_3$, DMF, 90 $^\circ$C, 1 h, then 44, r.t., 18 h, >99%. e) Polyphosphoric acid, 90 $^\circ$C, 5.5 h, 40%. f) $\text{H}_2$, PtO$_2$, MeOH, 1 atm, r.t., 45 min, 80%. g) i) NaNO$_2$, HCl, water, 0 $^\circ$C, 50 min. ii) NaN$_3$, water, r.t., 4 h, 28% over two steps.
7.2.3 Synthesis of the azido C₄-HSL derivatives 55, 58 and 61

N₃-C₂-HSL 55 (the azido derivative of C₄-HSL with a C₂ chain, see Scheme 4) has previously been prepared by Stacy et al. Their synthesis was followed, starting with the cyclisation of l-methionine 50 using bromoacetic acid to form the HSL HBr salt 52. The disappointing yield can be attributed to difficulties in precipitating the final product. The HSL HBr salt 52 was then converted by a biphasic one-pot process to N₃-C₂-HSL 55 using bromoacetyl bromide 53 and NaN₃.

Scheme 4: The synthesis of 55. a) Bromoacetic acid, i-PrOH:water:AcOH (5:5:2), r.t., 18 h, 41%. b) NaN₃, NaHCO₃, water/CH₂Cl₂, r.t., 18 h, 41%.

It was hoped that this procedure could also be used to produce the C₄ and C₆ derivatives, however, attempts to convert HSL 50 to N₃-C₄-HSL 58 using 4-bromobutyryl chloride 56 produced a complex mixture of products. This is likely to be because the S₂N₂ reaction in which the azide anion displaces bromine is slower for the C₄ derivative as the bromine atom being displaced is no longer adjacent to a carbonyl group. In addition, the longer chain length allows intramolecular cyclisation of the bromide with the secondary amide. The conversion was therefore carried out as a two-step process, where a bromoacyl chain was initially installed, followed by the S₂N₂ reaction with NaN₃ (see Scheme 5).

Reaction of the HSL HBr salt 52 with 4-bromobutyryl chloride 56 or 6-bromohexanoyl chloride 59 produced Br-C₄-HSL 57 or Br-C₆-HSL 60 respectively, in good yields. Heating with NaN₃ in DMF converted Br-C₆-HSL 60 to N₃-C₆-HSL 61. Similar conditions were used by Dr Bin Yu, a visiting PhD student in the Spring group, to convert the bromo-C₄ derivative 57 to the azido-C₄ derivative 58, and this compound was kindly donated to complete the set. Yields for the S₂N₂ reaction could probably be improved by decreasing the temperature (see Scheme 5).

Scheme 5: The synthesis of 58 and 61. a) Bromoacetic acid, i-PrOH:water:AcOH (5:5:2), r.t, 18 h, 41%. b) NaN₃, NaHCO₃, water/CH₂Cl₂, r.t., 18 h, 57: 80%, 60: 66%. c) NaN₃, DMF, 100 °C, 5 h, 61: 27% (donated by Dr Bin Yu), 61: 56%.
7.3 Synthesis of the alkynyl antibiotic derivatives

7.3.1 Synthesis of the alkynyl ciprofloxacin derivative 68

The retrosynthesis of ciprofloxacin derivative 68 is shown in Scheme 6. The disconnection to an alkynyl piperazine 68 and a commercially available ciprofloxacin precursor 67 was chosen based on a study by Renau et al., who found this route to be “...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide.”\textsuperscript{166,175}

It was envisaged that the alkynyl piperazine 68 could be prepared from mono-Boc-protected piperazine 64 and hex-5-ynal 63 using conditions similar to those used by Renau et al.\textsuperscript{166}

Unlike the aldehydes and ketones used by Renau et al.,\textsuperscript{166} hex-5-ynal 63 is not commercially available and so it was hoped that this could be prepared by oxidation of hex-5-ynol 62.

Scheme 6: The retrosynthesis of 68.

The synthesis of ciprofloxacin derivative 68 is shown in Scheme 7. Hex-5-ynal 63 was prepared by pyridinium chlorochromate oxidation of hex-5-ynol 62 in good yield according to the procedure described by Kocsis et al.\textsuperscript{176}

Renau et al.\textsuperscript{166} used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal 63 and 1-Boc-piperazine 64. However, it was decided to attempt this transformation using the less toxic sodium triacetoxylborohydride following a procedure reported by Abdel-Magid et al.\textsuperscript{177} This reaction yielded compound 65 in excellent yield, which was deprotected using TFA using the procedure described by Renau et al.\textsuperscript{166} to give the alkynyl piperazine 66 quantitatively.

The alkynyl piperazine 66 was refluxed in acetonitrile with the ciprofloxacin precursor 67 according to the procedure described by Renau et al.,\textsuperscript{166} however the reaction did not proceed. Addition of 2 eq. of TEA did not lead to reaction, however it was found that refluxing in neat TEA led to conversion to the final ciprofloxacin derivative 68.

With a small sample of the final product in hand, less harsh conditions were sought for a larger-scale version of the final reaction. Microwave irradiation at 115 °C was used, following a procedure by Reddy et al.\textsuperscript{178} DMSO and NMP were tested as solvents, with or without the addition of TEA. The reactions were monitored using
LCMS, and NMP without TEA was found to give the highest conversion.

Work-up of this reaction proved challenging, with an unknown dark brown viscous liquid being formed which was difficult to separate from the white solid product. A pure sample was obtained by recrystallisation from EtOAc, but the yield was poor (12%). The reaction was observed to stall after a certain point, while still having some of the ciprofloxacin precursor 67 present. The alkynyl piperazine 66 was not observed by TLC despite having been added in two-fold excess, suggesting that it degraded to a by-product before having chance to react.

Further attempts to refine this reaction might involve lower temperatures, higher ratios of the alkynyl piperazine 66 or improvement of the purification, e.g. by finding better precipitation conditions or by using reverse-phase chromatography. A Buchwald-Hartwig coupling or Ullmann reaction could also be attempted, but, as seen later, coordination of ciprofloxacin 24 to Cu can hinder catalysis.

![Diagram](image.png)

Scheme 7: The synthesis of 68. a) Pyridinium chlorochromate, CH$_2$Cl$_2$, r.t., 5 h, 72%. b) NaBH$_3$(AcO)$_3$, 1,2-dichloroethane, r.t., 10.5 h, 99%. c) TFA, r.t., 1 h, >99%. d) NMP, microwave, 115 °C 24 h, 12%.

7.3.2 Synthesis of the alkynyl trimethoprim derivative 71

The synthesis of trimethoprim derivative 71 is shown in Scheme 8. Trimethoprim 25 was selectively deprotected using HBr (aq.) using a procedure described by Jing et al.\textsuperscript{167} to form 69. A slightly longer reaction time (40 min vs 20 min) probably led to the yield being somewhat lower than that obtained by Jing et al.. The main impurity was asymmetrically di-demethylated trimethoprim, which could be identified by the presence of two aryl peaks at 6.41 (d, J=2.0 Hz, 1 H) and 6.34 (d, J=2.0 Hz, 1 H) and a corresponding methyl peak at 3.82 (s, 3 H) in the crude NMR.

The alkynyl trimethoprim derivative 71 was synthesised from the demethylated trimethoprim 69 and 6-chloro-1-hexyne 70 using a Cs$_2$CO$_3$-catalysed S$_N$2 reaction similar to that used by Jing et al.\textsuperscript{167}
Scheme 8: The synthesis of 71. a) HBr (aq.), 100 °C, 40 min, 43%. b) Cs₂CO₃, DMF, 70 °C, 7 h, 25%.

7.4  Synthesis of the triazole-linked autoinducer-antibiotic conjugates

7.4.1  Optimisation of the click reaction

Test reactions using N₃-C₂-HSL 55 and the alkynyl ciprofloxacin derivative 68 were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see Table 4 and Scheme 9). Stirring at r.t. had no effect even with an extended reaction time. Heating to 50 °C did lead to slow formation of the product, but a mixture of the 1,4 72 and 1,5 73 isomers was observed in an approximately 4:1 ratio by LCMS (see Figure 20). It is possible that the Cu(I) catalyst was not involved in this reaction because it had been oxidised, and hence the mixture of products was formed by an uncatalysed cycloaddition. Such reactions are known to produce a mixture of products.¹⁴⁵

Use of the ligand tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) 74 (see Figure 19) led to some conversion at room temperature (seen by LCMS), however the reaction stopped before completion, probably due to oxidation of the Cu(I) catalytic species. When degassed solvent and an argon atmosphere were used the reaction proceeded to completion at room temperature in around 3 h.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuSO₄·5H₂O, sodium ascorbate, water, t-BuOH, air, r.t., 7 d.</td>
<td>No reaction</td>
</tr>
<tr>
<td>CuSO₄·5H₂O, sodium ascorbate, water, t-BuOH, air, 50 °C, 5 d.</td>
<td>1,3-Triazole product 72 and 1,5 triazole impurity 73 4:1</td>
</tr>
<tr>
<td>CuSO₄·5H₂O, sodium ascorbate, THPTA 74, water, t-BuOH, air, r.t., 3 h.</td>
<td>1,3-Triazole product 72 and starting materials 55 and 68 5:4:4</td>
</tr>
<tr>
<td>CuSO₄·5H₂O, sodium ascorbate, THPTA 74, water, t-BuOH, Ar, r.t., 3 h.</td>
<td>1,3-Triazole product 72, 30% yield</td>
</tr>
</tbody>
</table>

Table 4: Conditions attempted for the synthesis of 72 (see Scheme 9).
Scheme 9: Synthesis of 72. For conditions see Table 4.

Figure 19: Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (THPTA) 74.

Figure 20: 1,4 (left) and 1,5 (right) triazoles.

7.4.2 Synthesis of the autoinducer-ciprofloxacin and autoinducer-trimethoprim triazole conjugates

Once conditions had been found for the click reaction, the synthesis of other conjugates was attempted. Two additional azides were kindly donated by members of the Spring group: the azido derivative of 3-oxo-C12-HSL 75 was synthesised by Ryan Howard, a master’s student under my supervision179 and the tail azide derivative of PQS 76 was synthesised by Dr Ysobel Baker164 (see Figure 21).
Figure 21: Further azido autoinducer derivatives synthesised by Howard\textsuperscript{179} 75 and Baker\textsuperscript{164} 76.

Synthesis of the conjugates proved more difficult than expected, for several reasons. Firstly some compounds did not dissolve in the reaction solvent (50% water/t-BuOH) requiring addition of co-solvents such as CH\textsubscript{2}Cl\textsubscript{2}. Secondly, some compounds were unstable: HSL derivatives hydrolysed upon attempted preparative HPLC purification and the 3-oxo-C\textsubscript{12}-HSL conjugates degraded during the reaction. Finally, the reaction was highly air-sensitive which led to stalling. The most reliable procedure was determined over the course of several reactions, and is shown in 9.25.

Despite the unforeseen difficulties in synthesis of the conjugates enough material was successfully prepared for biological testing. The results of the reactions are shown in Table 5, Table 6, Table 7 and Table 8. It was intended that the failed reactions would be repeated, but as preliminary biological testing (see 7.5) proved unpromising it was decided that attention should be focused elsewhere.

Scheme 10: General scheme for the click reaction, where R\textsubscript{1}-N\textsubscript{3} is an azido autoinducer derivative and R\textsubscript{2}= is an alkynyl antibiotic derivative a)CuSO\textsubscript{4}, sodium ascorbate, THPTA, water, t-BuOH.
<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Product</th>
<th>Outcome</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 and 68</td>
<td><img src="image1" alt="Image of Product" /></td>
<td>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO₂, 0-20% MeOH/CH₂Cl₂).</td>
<td>30%</td>
</tr>
<tr>
<td>58 and 68</td>
<td><img src="image2" alt="Image of Product" /></td>
<td>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO₂, 0-20% MeOH/CH₂Cl₂).</td>
<td>47%</td>
</tr>
<tr>
<td>61 and 68</td>
<td><img src="image3" alt="Image of Product" /></td>
<td>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO₂, 0-20% MeOH/CH₂Cl₂).</td>
<td>38%</td>
</tr>
<tr>
<td>75 and 68</td>
<td><img src="image4" alt="Image of Product" /></td>
<td>X Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO₂, 20% MeOH/CH₂Cl₂).</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Click reactions attempted.
<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Product</th>
<th>Outcome</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 and 68</td>
<td><img src="image1" alt="Structure" /></td>
<td>✔️ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.</td>
<td>27%</td>
</tr>
<tr>
<td>49 and 68</td>
<td><img src="image2" alt="Structure" /></td>
<td>✗ Reaction did not go to completion (approx. 6:1 starting material 68 to product 81 seen by LCMS). Attempted purification by prep. HPLC but unsuccessful.</td>
<td></td>
</tr>
<tr>
<td>76 and 68</td>
<td><img src="image3" alt="Structure" /></td>
<td>✗ No reaction seen by LCMS.</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Click reactions attempted.
<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Product</th>
<th>Outcome</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 and 71</td>
<td><img src="image1.png" alt="Image" /></td>
<td>× Reaction complete by LCMS in 2 h, but lactone hydrolysed on prep. HPLC column.</td>
<td></td>
</tr>
<tr>
<td>58 and 71</td>
<td><img src="image2.png" alt="Image" /></td>
<td>✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO₂, 20% MeOH/CH₂Cl₂).</td>
<td>17%</td>
</tr>
<tr>
<td>61 and 71</td>
<td><img src="image3.png" alt="Image" /></td>
<td>✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO₂, 20% MeOH/CH₂Cl₂).</td>
<td>27%</td>
</tr>
<tr>
<td>75 and 71</td>
<td><img src="image4.png" alt="Image" /></td>
<td>× Degraded during reaction.</td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Click reactions attempted.
<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Product</th>
<th>Outcome</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 and 71</td>
<td><img src="image" alt="Product 87" /></td>
<td>✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.</td>
<td>41%</td>
</tr>
<tr>
<td>49 and 71</td>
<td><img src="image" alt="Product 88" /></td>
<td>✗ Reaction did not go to completion (approx. 10:1 starting material 71 to product 88 seen by LCMS). Attempted purification by prep. HPLC but unsuccessful.</td>
<td></td>
</tr>
<tr>
<td>76 and 71</td>
<td><img src="image" alt="Product 89" /></td>
<td>✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO&lt;sub&gt;2&lt;/sub&gt;, 20% MeOH/CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;).</td>
<td>18%</td>
</tr>
</tbody>
</table>

Table 8: Click reactions attempted.

7.4.3 Synthesis of the homoserine lactone-ciprofloxacin triazole conjugates with cleavable linkers

In addition to the conjugates shown in the previous section, a further collection was synthesised in collaboration with Professor Eddy Sotelo, a visiting researcher in the Spring group. Professor Sotelo synthesised two alkyne-linked ciprofloxacin derivatives 90 and 91 (see Figure 22), both with cleavable linkers (see 5.3.7).
Figure 22: The cleavable alkyne-Cip derivatives synthesised by Professor Sotelo.

Professor Sotelo then performed click reactions using the AHL azide derivatives 55, 58 and 61 synthesised in 7.2.3 to form a new set of conjugates to add to the library (see Figure 23). It was hoped that these conjugates would enter the cell and then be cleaved by esterases to release ciprofloxacin (see 5.3.7).

Figure 23: The cleavable HSL-Cip triazole conjugates synthesised by Professor Sotelo.

Two control compounds 98 and 99 with benzyl head groups were also produced by Professor Sotelo (see Figure 24). It was hoped that these would show whether the AHL head group is required for activity.

Figure 24: The cleavable Bn-Cip triazole conjugates 98 and 99 synthesised by Professor Sotelo.
7.5 Biological testing

7.5.1 Autoinducer-antibiotic conjugates

The eight triazoles made in 7.4 (see Figure 25) were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* PAO1 and YM64. PAO1 is the *P. aeruginosa* wild-type strain. YM64 is a mutant lacking all of the four major mex operons for multidrug efflux pumps: mexAB-oprM, mexXY, mexCD-oprJ and mexEF-oprN, making it more sensitive to many antibiotics and hence able to show up moderate effects more clearly.

Figure 25: The autoinducer-antibiotic conjugates.

7.5.1.1 Antibacterial and anti-biofilm testing against YM64

In YM64 at 5 h the HSL-Cip conjugates 72, 77 and 78 showed slight activity at the highest concentration, but not as much as ciprofloxacin 24. This activity was not visible by 24 h (see Figure 27) and the compounds had no effect on biofilm formation (see Figure 28).

A dose-dependant response was expected for these results, however this was not seen except for a slight effect at 5 h in YM64 for some compounds. The dose-dependant response might potentially be seen if higher concentrations were tested, although there could be problems with compound solubility. Conversely, the dose-dependant response might be seen for ciprofloxacin 24 if lower concentrations were tested. Smaller ‘steps’ in concentration could also be tested after establishing the range over which the dose-dependant response is seen.

The very high readings in the biofilm assays for ciprofloxacin 24 (see Figure 28 and Figure 31) could be due to sub-MIC concentrations of the antibiotic causing the bacteria to respond by forming a protective biofilm. This effect has been reported with cefotaxim, amoxicillin, azithromycin, tobramycin, amikacin, streptomycin and gentamicin, but oddly not with ciprofloxacin 24, although this could be due to the exact conditions of growth.
Figure 26: YM64 optical density (OD) readings at 5 h for the autoinducer-antibiotic conjugates.

Figure 27: YM64 OD readings at 24 h for the autoinducer-antibiotic conjugates.
7.5.1.2 Antibacterial and anti-biofilm testing against PAO1

In PAO1 78 showed similar activity to ciprofloxacin 24 at the highest concentration (see Figure 29), but not at lower concentrations. All other compounds did not show activity, and again there was no activity at 24 h or against biofilms. Increased biofilm formation is again seen with intermediate concentrations of ciprofloxacin 24, although PAO1 seems to be overwhelmed at the highest concentrations.
Figure 29: PAO1 OD readings at 5 h for the autoinducer-antibiotic conjugates.

Figure 30: PAO1 OD readings at 24 h for the autoinducer-antibiotic conjugates.
7.5.2 Cleavable homoserine lactone-ciprofloxacin conjugates

The eight cleavable HSL-Cip conjugates, two controls and two alkynes described in 7.4.3 (see Figure 32) were tested for antibacterial and anti-biofilm activity in \textit{P. aeruginosa} YM64.
Here there was more success, although the activity was still not as high as for ciprofloxacin 24. The HSL-Cip conjugates with \(N\)-(acetoxymethoxycarbonyl) linkers \((R = H)\) showed activity at high concentrations. A longer linker seems to give higher activity; 93 and 94 showed activity comparable with ciprofloxacin 24 at high concentrations. Unfortunately the control 98 and alkyne 90 with \(N\)-(acetoxymethoxycarbonyl) linkers \((R = H)\) showed higher activity than the conjugates, indicating that the HSL head wasn’t contributing to the activity of the conjugates.

The conjugates with an \(N\)-(acetoxyethoxycarbonyl) linker \((R = Me)\) did not show any activity. This suggests that they either didn’t enter cells or weren’t suitable substrates for esterases. The \(N\)-(acetoxyethoxycarbonyl) linked alkyne \((R = Me)\) did show some activity, indicating that maybe it could penetrate cells more easily than the conjugates due to its lower molecular weight and/or lower polarity.
7.6 Conclusions

7.6.1 Library synthesis

In this section, a range of 1,2,3-triazole-linked autoinducer-antibiotic conjugates was successfully synthesised and tested for antibiotic and anti-biofilm activity. Reliable routes to the azido autoinducers and alkynyl antibiotics were found, but the copper(I)-catalyzed alkyne-azide cycloaddition reactions used to link them proved rather capricious. The main reasons for this were insolubility of the starting materials and air-sensitivity. Air-sensitivity is not expected in a click reaction, but can be explained by many of the reactions being too dilute. This led to ascorbate being used up by the oxygen dissolved in the reaction solvent and present in the air above the reaction mixture. Even when the solvent was degassed and the reaction performed under argon, a small amount of air leaking in through a perished septum was enough to cause the reaction to stall. Low concentrations were used because of the insolubility of the starting materials, but this would have been better addressed by more thorough screening of solvents. In addition, it was later shown that THPTA may not be necessary for a sufficiently concentrated reaction to take place, so this expensive reagent could be omitted.

Assuming the click reaction could be further optimised, this library could be easily expanded by the addition of more azido autoinducers and alkynyl antibiotics (see 7.7). In particular, autoinducers which are actively transported into cells, such as AI-2, are attractive targets.

7.6.2 Biology

Little biological activity was seen in the non-cleavable autoinducer-antibiotic conjugates. This could be due to a number of factors, including:

1. Restriction of the binding of ciprofloxacin to DNA gyrase and topoisomerase IV or trimethoprim
This could be investigated by measuring binding of the compounds to the purified protein targets.

2. Failure to penetrate the cell wall/biofilm or non-specific binding to the cell wall. This could be investigated by separation of the cultures by centrifugation and quantification of the compounds in each fraction by HPLC.

3. Failure of the autoinducers to mask the antibiotics from recognition by efflux pumps. While the resistance of YM64 to the conjugates suggests that this mechanism is unlikely, YM64 is not lacking every efflux pump, so it is possible that the conjugates are still being pumped out. A strain with all pumps knocked out, or a suitable cocktail of pump inhibitors, would be needed to investigate this mechanism fully.

If binding of the antibiotics to target proteins is indeed restricted by the attachment of the autoinducer, this could be affected by the size and polarity of the linker and autoinducer. With this in mind, the next set of compounds synthesised contain HSL analogues, which are smaller than HHQ and PQS, and some omit the triazole in the linker, hence affecting polarity.

The cleavable HSL-Cip conjugates showed a little more activity, but unfortunately this did not require the HSL, and probably was mostly affected by the polarity and size of the attached group and the ease of hydrolysis of the linker.

7.7 Future work

This section begins with discussion of further autoinducers and antibiotics which could be used in future conjugates. Some have have already been partially or fully synthesised by myself or other members of the Spring group. Plans for further biological testing of the conjugates synthesised in this study are then presented.

7.7.1 Autoinducer derivatives

7.7.1.1 3-oxo-C12-HSL derivative 75

N3-3-oxo-C12-HSL 75 (see Scheme 11) was synthesised by Ryan Howard, a master’s student under my supervision. The synthesis was based on a synthesis of 3-oxo-C12-HSL reported by Hodgkinson et al. Conjugates of this compound were not included in the library as it degraded during the click reaction. However, reaction conditions could be further optimised, or the acetal-protected azide could be used in the click reaction, followed by deprotection.

This compound would be a useful addition to the library as it would demonstrate whether the 3-oxo group and/or longer alkyl chain are required for activity. As the head group is added fairly late in the synthesis it would also be easy to swap it for the other head groups described in 8, thus expanding the library further.
Scheme 11: The synthesis of N$_3$-3-oxo-C$_{12}$-HSL 75 carried out by Ryan Howard. a) NaN$_3$, DMF, 60 °C, 6 h, 93%. b) Oxalyl chloride, DMF, CH$_2$Cl$_2$, 3 h, r.t.. c) MeOAc, N-methyl imidazole, TiCl$_4$, DIPEA, toluene, r.t., 2 h, 43% over two steps. d) HO(CH$_2$)$_2$OH, TsOH, CH(OMe)$_3$, r.t., 5 h, 78%. e) NaOH, water, r.t., 6 h, 85%. f) EDC, DMAP, CH$_2$Cl$_2$, r.t., 16 h. g) TFA, r.t., 5 h, 29% over two steps.

7.7.1.2 AI-2 derivatives

AI-2 23 is perhaps a more attractive choice of autoinducer for inclusion in conjugates than the others used in this study as it is actively transported into cells and used by a wide range of bacterial species. The synthesis of conjugates of AI-2 23 with ciprofloxacin 24 and trimethoprim 25 has been attempted in the Spring group by Dr Jamie Stokes. However, the protected azido AI-2 derivative 107 synthesised was found to be unstable, and the click reactions attempted were unsuccessful. AI-2 23 is known to interconvert between multiple forms (including forming a furanosyl borate diester) so it is to be expected that syntheses involving it might be challenging. If a more stable azido AI-2-23 derivative cannot be developed, another approach would be to use an azido AI-2 23 analogue which is capable of being taken up by the same active transport mechanism.
Two types of AI-2 23 receptors have been identified: LuxP, present in Vibrio spp.,\textsuperscript{190} and LsrB, first discovered in Salmonella enterica serovar Typhimurium.\textsuperscript{191} LuxP is a periplasmic binding protein that relays the signal, but not the actual AI-2 23 molecule, into the cell and hence is not a useful target.\textsuperscript{192} LsrB is the ligand binding protein of a system that transports AI-2 23 into the cell,\textsuperscript{188} and hence can be targeted. LsrB orthologs are found in a wide range of bacterial families including Enterobacteriaceae, Rhizobiaceae, and Bacillaceae.\textsuperscript{193} In addition, several bacterial species, including P. aeruginosa, are known to respond to AI-2 23 but do not have either of these two known types of receptors, and thus the discovery of new receptor types is expected.\textsuperscript{193} Any postulated receptor would need to internalise the AI-2 analogue in order for conjugates to be effective against the bacterium.

One example of an AI-2 analogue which could be derivatised is a geminal dibromo compound 108 synthesised by Guo \textit{et al.}\textsuperscript{189} (see Figure 35). It is as potent as AI-2 at dissociating the LsrR repressor from the promotor region in a reporter strain, and may be more stable. It is also esterified, making it less volatile and thus easily purified using column chromatography. The esters are presumably cleaved by cellular esterases as the compounds can be used in QS assays without deprotection.\textsuperscript{194}

A possible azido derivative 109 of this analogue is shown in Figure 35. If a route to it could be found, it appears to be a promising partner in future conjugates given the known properties of AI-2 23.

7.7.2 Antibiotic derivatives

7.7.2.1 Ciprofloxacin derivative 120

A second alkynyl ciprofloxacin derivative 120 was planned and partially synthesised during this project, and finishing this synthesis would provide a useful intermediate for future conjugates.

The derivative 120 has an alkyne tail attached in place of the cyclopropane ring as it has been shown that bulkier groups in this position can be tolerated.\textsuperscript{195,196} This synthesis followed a conventional route to ciprofloxacin 24 similar to that reported by Mitscher \textit{et al.}\textsuperscript{195} but used hex-5-yn-1-amine 115 instead of cyclopropylamine.

The TiCl$_4$-catalysed crossed Claisen condensation of the acid chloride 110 and ethyl acetate described by Hashimoto \textit{et al.}\textsuperscript{197} was used to produce the $\beta$-ketoester. The ethoxymethylene group in 112 was installed by
the reaction of \( \beta \)-ketoester 111 and triethyl orthoformate to give a mixture of the \( E \) and \( Z \) isomers.\textsuperscript{195,198} Hex-5-yn-1-amine 115 was prepared using a Gabriel synthesis\textsuperscript{199} described by Rożkiewicz \textit{et al.}\textsuperscript{200} Unfortunately the amine was surprisingly volatile and was lost on evaporation of the reaction solvent. If a better purification method could be found, or a longer-chain alkylnyl amine was used, the rest of the synthesis could be performed and the resulting alkylnyl ciprofloxacin derivative 120 could be used to form more triazole-linked conjugates.

Scheme 12: The synthesis of 120. a) EtOAc, TiCl\(_4\), DIPEA, \( N \)-methyl imidazole, toluene, r.t., 30 min. b) Triethyl orthoformate, Ac\(_2\)O, reflux, 2 h. c) Potassium phthalimide, potassium iodide, DMF, 80 \( ^\circ \)C, 18 h. d) \( \text{N}_2\text{H}_2 \cdot \text{H}_2\text{O}, \text{EtOH}, \) reflux, 18 h. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) DMSO.

\textbf{7.7.2.2 Sulfanilamide derivatives}  
Sulfanilamide antibiotics were the first class of antibiotics to be widely used.\textsuperscript{201,202} They are all derivatives of 4-aminobenzenesulfonamide, very commonly with the sulfonamide nitrogen linking to a heterocycle. Sulfanilamide antibiotics function by inhibiting bacterial synthesis of folic acid. \textit{P. aeruginosa} has intrinsic resistance to sulfanilamides mainly due to the MexAB-OprM efflux pump\textsuperscript{140} and so, as with trimethoprim 25, it is hoped
that conjugation to an autoinducer would restore activity.

Derivatives of 4-aminobenzenesulfonamide \textit{216} have previously been synthesised using copper(I)-catalyzed alkyne-azide cycloaddition reactions to append various groups\textsuperscript{203} (see Scheme 13). However, if one considers sulfonamide antibiotics already in use, nearly all have a heterocycle linked directly to the sulfur atom, rather than with a methylene group in between.

![Scheme 13: The sulfanilamide derivatives synthesised using click chemistry by Wang et al.\textsuperscript{203}](image)

Therefore, it was postulated that a 1,2,3-triazole could be introduced in the position occupied by a heterocycle in other known sulfonamide antibiotics by attachment of an alkyne directly to the sulfonamide nitrogen to form an alkynyl sulfanilamide derivative \textit{121} or a protected version of it (see Scheme 14).

![Scheme 14: Retrosynthesis of a 1,2,3-triazole-containing autoinducer-sulfonamide conjugate. R = autoinducer.](image)

It was hoped that sulfanilamide derivative \textit{121} could be synthesised and reacted with the azido autoinducer derivatives directly. However, it appears that no secondary ynamides have been synthesised to date. Conversely, the synthesis of tertiary ynamines has been studied more widely.\textsuperscript{204} In particular, tertiary ynamides have been shown to be relatively stable and easy to work with in a variety of reactions including copper(I)-catalyzed alkyne-azide cycloadditions.\textsuperscript{205,206}

The study of copper(I)-catalyzed alkyne-azide cycloadditions of ynamides by IJsselstijn et al.\textsuperscript{205} includes terminal ynamides protected using a benzyl and a tosyl group. Although their click reactions proceed with high yield, they do not present the deprotection of their final compounds. However, these reactions provided a promising suggestion that click reactions between a protected alkynyl sulfanilamide derivative and the azido autoinducer derivatives are feasible. The tosyl group used by IJsselstijn et al.\textsuperscript{205} to protect their ynamide is very similar to the $p$-aminobenzenesulfonyl group needed in the alkynyl-sulfanilamide derivative. However, because installation of the alkyne could be problematic in the presence of a second amine, the $\text{NH}_2$ group was installed as a $\text{NO}_2$ group and reduced after the click reaction.

The synthesis proceeded as shown in Scheme 15.\textsuperscript{205,207,208} It was hoped that the methoxybenzyl group could be removed and the nitro group converted to an amine simultaneously by reduction in the last step, but unfortunately the methoxybenzyl group proved difficult to remove. On reflection, methoxybenzene was a poor choice of protecting group, and a more reduction-labile group such as benzyl or diphenylmethyl should have been chosen.\textsuperscript{209} This reaction could be repeated with a different choice of protecting group to provide another set of autoinducer-antibiotic conjugates.
Scheme 15: Synthesis of a 1,2,3-triazole-containing sulfonamide antibiotic-autoinducer hybrid. a) CH$_2$Cl$_2$, r.t., 24 h. b) AgNO$_3$, acetone, r.t., 3 h. c) CuSO$_4$·5H$_2$O, 1,10-phenanthroline, K$_2$CO$_3$, toluene, 80 °C, 48 h. d) TBAF, THF, −78 °C, 3 h. e) Cu(OAc)$_2$, sodium ascorbate, CH$_2$Cl$_2$, t-BuOH, water, r.t., 16 h. f) H$_2$, PtO$_2$, MeOH, 1 atm, r.t., 3 h.

7.7.2.3 Linezolid derivative 144

Linezolid is a monoamine oxidase inhibitor used for the treatment of infections caused by Gram-positive bacteria. Gram-negative bacteria, including *P. aeruginosa* are resistant to linezolid due to the activity of efflux pumps, and hence it might be possible to increase its activity in such organisms by increasing its uptake and/or retention by conjugation to an autoinducer.

An alkynyl linezolid derivative 217 was partially synthesised by Ryan Howard (see Scheme 16). The route follows a procedure described by Phetsang *et al* 210 where the morpholine ring of linezolid is replaced by piperazine, allowing an alkynyl tail to be attached to the molecule.

The first three steps were carried out on a large scale, producing 55.7 g of 134. As all steps except the final one are reported in the literature 210, 211 it is hoped that the alkynyl linezolid derivative 218 could be synthesised...
Scheme 16: Proposed and partially completed synthesis of linezolid derivative. 218 a) MeCN, reflux, 3 h, 91%. b) H₂, 10% Pd/C, THF, 40 psi, <50 °C, 1.5 h, 95%. c) CbzCl, Na₂CO₃, acetone, water, 5 °C, 1 h then r.t., 16 h, 56%. d) n-BuLi, THF, -78 °C, 1 h then add epoxide then -78 °C to r.t., 5 h. e) TsCl, TEA, CH₂Cl₂, 0 °C to r.t., 4.5 h. f) Acetonitrile, water, reflux, 48 h. g) MeNH₂, EtOH, water, reflux, 5.5 h. h) Ac₂O, pyridine, 0 °C to r.t., 16 h. i) H₂, 10% Pd/C, MeOH/CH₂Cl₂, 1 atm, r.t., 16 h. j) NEt₃, EtOH, reflux.

7.7.2.4 Gentamicin derivative 147

Gentamicin is an aminoglycoside antibiotic used to treat many bacterial infections, particularly those caused by Gram-negative organisms, by binding to the bacterial ribosome. Gentamicin is actually a mixture of components (see Figure 36) synthesised by Micromonospora, a genus of Gram-positive bacteria. Separation of the gentamicin
components has been achieved by Grote et al.\textsuperscript{212} by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a \textsuperscript{146} was isolated pure, and is particularly useful because it the only component which contains a CH\textsubscript{2}NH\textsubscript{2} group. This group is less hindered than all other amine groups in gentamicin C1a \textsuperscript{146} and hence it is possible to selectively derivatise the molecule at this position. Grote et al. attached a tag needed for an immunoassay using a pentafluorophenyl ester.\textsuperscript{213} Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid \textsuperscript{145} (see Scheme 17). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester \textsuperscript{145} and then separate out the desired component.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{gentamicin_components.png}
\caption{Gentamicin components.}
\end{figure}

\begin{tabular}{|l|l|l|l|}
\hline
Gentamicin & R\textsubscript{1} & R\textsubscript{2} & R\textsubscript{3} \\
\hline
C1 & Me & Me & H \\
\hline
C1a & H & H & H \\
\hline
C2 & H & Me & H \\
\hline
C2a & H & H & Me \\
\hline
C2b & Me & H & H \\
\hline
\end{tabular}

Scheme 17: Proposed synthesis of gentamicin C1a derivative \textsuperscript{147}. a) DIPEA, DMF, -55 °C.

7.7.2.5 Streptomycin derivative \textsuperscript{150}

Streptomycin \textsuperscript{148} is an aminoglycoside antibiotic used to treat \textit{Mycobacterium tuberculosis} and \textit{S. aureus} which works by binding to the bacterial ribosome. There is limited SAR data on streptomycin but it is known that conversion of the aldehyde to a carboxylic acid destroys activity, whereas conversion to an alcohol retains it.\textsuperscript{214}

Reductive amination can be used to install an alkyne group by reaction of the aldehyde with an amine such as oct-7-yn-1-amine \textsuperscript{219} (see Scheme 18). This approach has been used by Zhang et al.\textsuperscript{215} to form a conjugate of streptomycin \textsuperscript{148} and chitosan which was active against biofilms. Reductive amination replaces the aldehyde O with NH; it is known that an OH is tolerated at this position so it makes sense that NH is as well.
7.7.3 Biology

The following extra biological data are required for these compounds:

1. Repeats of the antibacterial and anti-biofilm assays in order to assess variability in the data.

2. Growth curves to 48 h and biofilm quantifications for the cleavable HSL-Cip conjugates.

3. Biofilm dispersal assays on all compounds (see 5.3.8 for a discussion of biofilm dispersal using a HSL analogue-CipMe conjugate and 9.71.4 for the methodology to be used).
8 Results and discussion: homoserine lactone analogue-ciprofloxacin conjugates

8.1 Overview

The second part of this project was focused on producing a library of HSL analogue-Cip and -CipMe conjugates. The HSL head group was replaced with a selection of cyclic amines found in known quorum sensing modulators (see 5.3.8). The analogues were linked to ciprofloxacin 24 in two ways: directly using either an $S_N2$ reaction or peptide coupling with methyl ciprofloxacin 151, and via the triazole linkage shown previously with ciprofloxacin 24 (see 7.4). The compounds were then tested for antibiotic and anti-biofilm activity against *P. aeruginosa*.

8.1.1 Head groups

The head groups used in this study are shown in Figure 37. The cyclohexanol derivatives were synthesised as a diastereomerically pure racemate, whereas the cyclopentanol derivatives were synthesised as separate enantiomers. Although the timescale of this project prevented the inclusion of the cyclopentanone derivatives, these could be included in future work. The 2-methoxybenzene derivatives do not have precedents as quorum sensing modulators in the literature, but they were included so as to be compared with the 3-methoxybenzene derivatives.

![Figure 37: The head groups used in this section.](image)

8.1.2 Library construction

As Ganguly *et al.* (see 5.3.8) synthesised their conjugate from Br-C$_4$-HCTL, it was envisaged that a branching strategy could be used to produce two sets of conjugates (see Scheme 19). The first set would be formed by the $S_N2$ reaction of the relevant bromide with methyl ciprofloxacin 151. The second set would be made by displacing the bromide with azide, then performing a click reaction with the alkylnyl ciprofloxacin derivative 68 made previously to form the triazole-linked product. Cyclohexanol conjugates would be formed by oxidation of the alcohol conjugates.
Scheme 19: General scheme showing the proposed branching synthesis of the HSL analogue-Cip and -CipMe conjugates.

This strategy was successful for most head groups, but multiple side reactions were observed for the amino alcohol head groups and so other routes to these conjugates were investigated (see 8.5).

8.2 Synthesis of the homocysteine thiolactone conjugates

8.2.1 Synthesis of methyl ciprofloxacin 151

The synthesis of the analogue conjugates began with the synthesis of methyl ciprofloxacin 151 (CipMe), which would then be attached to the various head groups. Methyl ciprofloxacin 151 was synthesised from ciprofloxacin
and MeOH in good yield using para-toluenesulfonic acid (TsOH) as a catalyst.\cite{216}

Scheme 20: Synthesis of methyl ciprofloxacin 151. a) TsOH, MeOH, 72 h, reflux, 83%.

8.2.2 Synthesis of Br-C$_4$-HCTL 153

The HCTL head group was then attached to the linker to form Br-C$_4$-HCTL 153, in preparation for coupling to methyl ciprofloxacin 151. Br-C$_4$-HCTL 153 was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives 57 and 60. Br-C$_4$-HCTL 153 was isolated in markedly higher yield than that achieved by Ganguly et al.\cite{61} (88% vs. 25%). It is possible that this was due to CH$_2$Cl$_2$ being used for the extraction, whereas Ganguly et al. used EtOAc.

Scheme 21: Synthesis of Br-C$_4$-HCTL 153. a) NaHCO$_3$, CH$_2$Cl$_2$, water, 0 °C, 1 h, 88%.

8.2.3 Synthesis of the HCTL-CipMe conjugate 154

The HCTL-CipMe conjugate 154 was synthesised using the procedure outlined by Ganguly et al.\cite{61} (see Scheme 22). Monitoring by LCMS showed slow conversion to the product. Br-C$_4$-HCTL 153 was presumably consumed by side reactions as 4 eq. were required to reach full conversion. A likely potential side reaction is internal cyclisation of the bromide with the amide NH, and the mass of this molecule was observed by LCMS in the reaction mixture.

Ganguly et al. do not quote a yield for this reaction,\cite{61,149} but it is hoped that the 12% achieved here could be improved upon. The side reactions led to the production of an unidentified brown, viscous contaminant which made purification by flash column chromatography (as was used by Ganguly et al.) challenging. Preparatory HPLC on a partially purified sample gave enough pure HCTL-CipMe conjugate 154 for biological testing. Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in 8.5.5, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.
Scheme 22: Synthesis of the HCTL-CipMe conjugate 154. a) K₂CO₃, acetonitrile, reflux, 24 h, 12%.

8.2.4 Synthesis of the HCTL-Cip triazole conjugate 156

Br-C₄-HCTL 153 was converted into N₃-C₄-HCTL 155 (see Scheme 22), by an S_N2 reaction with sodium azide which proceeded in excellent yield.

N₃-C₄-HCTL 155 was then subjected to the click reaction conditions optimised previously (see 9.25). The reaction proceeded very slowly at first, as the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. Nonetheless, the HCTL-Cip triazole conjugate 156 was isolated in good yield (see Scheme 23).

Scheme 23: Synthesis of the HCTL-Cip triazole conjugate 156. a) NaN₃, acetonitrile, reflux, 1.5 h, 89%. b) CuSO₄, THPTA, sodium ascorbate, water, t-BuOH, DMSO, r.t., 7 d, 71%.

8.2.5 Synthesis of the cleavable HCTL-Cip triazole conjugate 157

A cleavable conjugate 157 (see Scheme 24) was also synthesised from N₃-C₄-HCTL 155 by reaction with a cleavable alkyne-Cip derivative 90 synthesised previously by Professor Eddy Sotelo (see 7.4.3).
8.3 Synthesis of the 2-Methoxybenzene conjugates

8.3.1 Synthesis of Br-C₄₋₂-methoxybenzene 159

Br-C₄₋₂-methoxybenzene 159 was synthesised from 2-methoxyaniline 158 and 4-bromobutyryl chloride 56 using Schotten-Baumann conditions in 50% yield (see Scheme 25). Br-C₄₋₂-methoxybenzene 159, like all other 2- and 3-methoxyaniline derivatives mentioned below, appears to be air and/or light sensitive. For example, Br-C₄₋₂-methoxybenzene 159 turns from an initially colourless liquid to blue then black if left out on the bench. It is possible that this sensitivity is due to oxidative polymerisation of the aniline, but given the lack of catalysis it is likely that small amounts of highly-coloured polymer are being formed.

It is likely that the mediocre yield of Br-C₄₋₂-methoxybenzene 159 is caused by degradation during columnning, probably due to Sₓ₂ reactions at the bromide, especially internal cyclisation with the amide NH. It is therefore suggested that in future the compound should be used in its crude form to minimise losses, as it was fairly pure by ¹H NMR before columnning.

Scheme 25: Synthesis of Br-C₄₋₂-methoxybenzene 159. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 1 h, 50%.

8.3.2 Synthesis of the 2-methoxybenzene-CipMe conjugate 160

The procedure outlined by Ganguly et al. was initially attempted in order to synthesise the 2-methoxybenzene-CipMe conjugate 160, but the reaction was very slow and did not go to completion, presumably due to degradation of Br-C₄₋₂-methoxybenzene 159. New conditions, employing a microwave reactor and 2 eq. of Br-C₄₋₂-
methoxybenzene 159 were then attempted, with a much greater conversion observed by LCMS after 4 h (see Scheme 26). However, a poor yield was obtained, possibly due to losses during column chromatography.

![Scheme 26: Synthesis of the 2-methoxybenzene-CipMe conjugate 160. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 10%.

8.3.3 Synthesis of the 2-methoxybenzene-Cip triazole conjugate 162

N₃-C₄-2-methoxybenzene 161 was synthesised from Br-C₄-2-methoxybenzene 159 by an Sₓ2 reaction with sodium azide (see Scheme 27). The yield of N₃-C₄-2-methoxybenzene 161 (27%) was a lot lower than for N₃-C₄-HCTL 155 (89%). However, in this case it may not be better to use the product crude as several impurities were formed during the reaction and could be observed by LCMS (see Figure 38).

![Figure 38: Suspected impurities observed by LCMS during the synthesis of N₃-C₄-2-methoxybenzene 161.](image)

The 2-methoxybenzene-Cip triazole conjugate 162 was synthesised using the standard click conditions (see 9.25), with the addition of CH₂Cl₂ as a co-solvent to aid the dissolution of N₃-C₄-2-methoxybenzene 161 (see Scheme 27).
Scheme 27: Synthesis of the 2-methoxybenzene-Cip triazole conjugate 162. a) NaN₃, acetonitrile, reflux, 2 h, 27%. b) CuSO₄, THPTA, sodium ascorbate, water, t-BuOH, CH₂Cl₂, r.t., 16 h, 39%.

8.4 Synthesis of the 3-Methoxybenzene conjugates

8.4.1 Synthesis of Br-C₄-3-methoxybenzene 164

Br-C₄-3-methoxybenzene 164 was synthesised from 3-methoxyaniline 163 and 4-bromobutyryl chloride 56 using Schotten-Baumann conditions as above in almost identical (50%) yield (see Scheme 28).

Scheme 28: Synthesis of Br-C₄-3-methoxybenzene 159. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 1 h, 50%.

8.4.2 Synthesis of the 3-methoxybenzene-CipMe conjugate 165

The 3-methoxybenzene-CipMe conjugate 165, was synthesised as above, in similar yield (see Scheme 29).
Scheme 29: Synthesis of the 3-methoxybenzene-CipMe conjugate 165. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 11%.

8.4.3 Synthesis of the 3-methoxybenzene-Cip triazole conjugate 167

N$_3$-C$_4$-2-methoxybenzene 161 and the 3-methoxybenzene-Cip triazole conjugate 167 were synthesised as above, in similar yields (see Scheme 29 and Scheme 30).

Scheme 30: Synthesis of the 3-methoxybenzene-Cip triazole conjugate 167. a) NaN$_3$, acetonitrile, reflux, 7 h, 17%. b) CuSO$_4$, THPTA, sodium ascorbate, water, t-BuOH, CH$_2$Cl$_2$, r.t., 2 h, 5%.
8.5 Synthesis of the cyclopentanol conjugates

8.5.1 Synthesis of the 2-aminocyclopentan-1-ol head groups 172 and 173

Synthesis of the cyclopentanol derivatives began with the synthesis of (1S,2S)-2-aminocyclopentan-1-ol 172 and (1R,2R)-2-aminocyclopentan-1-ol 173 (see Scheme 31), using a procedure reported by Overman and Sugai.\textsuperscript{219–221} These precursors were synthesised by opening cyclopentene oxide 168 using (S)-1-phenylethanolamine 169 to give approximately equal amounts of two diastereomers, 170 and 171, which were separated using column chromatography. The removal of the methylbenzyl groups proved more difficult than expected, with the conditions reported by Overman and Sugai\textsuperscript{220} yielding only a salt of the starting material. After several attempts under various conditions (including using the free amine vs. the salt, varying the temperature, ensuring the dryness of the reagents and adding acetic acid), an approach using H\textsubscript{2} gas was attempted (see Table 9). This proceeded smoothly at 5 atm to give the two enantiomers of 2-aminocyclopentan-1-ol, 172 and 173, both in quantitative yield.

Scheme 31: Synthesis of (1S,2S)-2-aminocyclopentan-1-ol 172 and (1R,2R)-2-aminocyclopentan-1-ol 173. a) AlMe\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C, 170 (SSS): 35%, 171 (RRS): 32%. b) See Table 9. c) Pd(OH)\textsubscript{2}, MeOH, H\textsubscript{2}, 5 atm, r.t., 1 d, >99%.
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature and pressure</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>170</strong>·HCl, ammonium formate, 10% Pd/C, DMF</td>
<td>r.t., 1 atm</td>
<td>2 d</td>
<td>170 salt</td>
</tr>
<tr>
<td><strong>170</strong>·ammonium formate, 10% Pd/C, DMF</td>
<td>r.t., 1 atm</td>
<td>2 d</td>
<td>170 salt</td>
</tr>
<tr>
<td><strong>170</strong>·HCl, ammonium formate, 10% Pd/C, dry DMF</td>
<td>r.t., 1 atm</td>
<td>2 d</td>
<td>170 salt</td>
</tr>
<tr>
<td><strong>171</strong>·ammonium formate, 10% Pd/C, dry DMF</td>
<td>r.t., 1 atm</td>
<td>2 d</td>
<td>171 salt</td>
</tr>
<tr>
<td><strong>170</strong>·ammonium formate, 10% Pd/C, dry DMF</td>
<td>70 °C, 1 atm</td>
<td>1 d</td>
<td>170 salt</td>
</tr>
<tr>
<td><strong>170</strong>·ammonium formate, 10% Pd/C, dry DMF, AcOH</td>
<td>70 °C, 1 atm</td>
<td>1 d</td>
<td>Complex mixture</td>
</tr>
<tr>
<td><strong>170</strong>·HCl, dry ammonium formate, 10% Pd/C, dry DMF</td>
<td>120 °C, 1 atm</td>
<td>7 d</td>
<td>Complex mixture</td>
</tr>
<tr>
<td><strong>170</strong>·HCl, Pd(OH)$_2$, MeOH, H$_2$</td>
<td>r.t., 1 atm</td>
<td>1 d</td>
<td>170 salt</td>
</tr>
<tr>
<td><strong>170</strong>·HCl, Pd(OH)$_2$, MeOH, H$_2$</td>
<td>r.t., 3.4 atm</td>
<td>1 d</td>
<td>172 salt, 170 salt, and an unidentified compound (approx. 7:2:10 by $^1$H NMR)</td>
</tr>
<tr>
<td><strong>170</strong>·Pd(OH)$_2$, MeOH, H$_2$</td>
<td>r.t., 5 atm</td>
<td>1 d</td>
<td>172, &gt;99% yield</td>
</tr>
</tbody>
</table>

Table 9: Conditions attempted for the synthesis of (1*{2}S)-2-aminocyclopentan-1-ol 172 and (1*{2}R)-2-aminocyclopentan-1-ol 173 (see Scheme 31).

8.5.2 Initial branching route

An initial retrosynthesis of the conjugates is shown in Scheme 32, and follows a similar path to previous conjugates.
Scheme 32: Retrosynthesis of the cyclopentanol-CipMe conjugates 178 (SS) and 179 (RR), and the cyclopentanol-Cip triazole conjugates 180 (SS) and 181 (RR). SS enantiomers are shown, but both are implied.

Synthesis of Br-C₄-cyclopentanol-(SS) 174 from (1S,2S)-2-aminocyclopentan-1-ol 172 and 4-bromobutyryl chloride 56 was attempted using Schotten-Baumann conditions (see Scheme 33). However, a large number of impurities were observed by LCMS (see Figure 39), and so three new strategies were attempted: protection of the alcohol (see 8.5.3), using 4-chlorobutyryl chloride 192 as the linker instead of 4-bromobutyryl chloride 56 (see 8.5.4), and installing the linker on methyl ciprofloxacin 151 and then attaching the head group by peptide coupling (see 8.5.5).

Scheme 33: Attempted synthesis of Br-C₄-cyclopentanol-(SS) 174. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 2 h.
Figure 39: Suspected impurities observed by LCMS during the synthesis of Br-C₄-cyclopentanol-(SS) 174. Regiochemistry is speculative.

8.5.3 TBDMS protection route

The first attempt at an alternative strategy for the synthesis of the conjugates involved TBDMS protection of the alcohol (see Scheme 34). It was envisaged that protection would eliminate enough of the side reactions with products shown in Figure 39 that intermediates Br-C₄-cyclopentanol-(SS) 174 and N₃-C₄-cyclopentanol-(SS) 176 could be purified. The TBDMS group could be removed later in the synthesis using TBAF or acid.
Scheme 34: Retrosynthesis of the cyclopentanol-CipMe conjugates \(178\) (SS) and \(179\) (RR), and the cyclopentanol-Cip triazole conjugates \(180\) (SS) and \(181\) (RR) using a TBDMS protection strategy. SS enantiomers are shown, but both are implied.

8.5.3.1 Synthesis of TBDMS-protected \((1\text{S},2\text{S})\)-2-aminocyclopentan-1-ol 172

The synthesis began with the optimisation of the protection of \((1\text{S},2\text{S})\)-2-aminocyclopentan-1-ol 172 with a TBDMS group on the alcohol (see Scheme 36). This reaction proved more problematic than expected, possibly
due to the amine group interfering with the reaction at the alcohol and/or the high polarity of the starting material causing problems with solubility in the reaction mixture and extraction during the work-up. Conditions attempted are summarised in Table 10. Protection attempts using TBDMSCl were generally unsuccessful, but eventually a method employed by Wu et. al\textsuperscript{222} using TBDMSOTf was found to produce the desired product in excellent yield. Water was used for the work-up rather than NH\textsubscript{4}Cl (sat. aq.), as the acidic work-up protonated the product. The TEA was removed during column chromatography instead.

\[ \text{Scheme 35: Synthesis of TBDMS protected (1\textit{S},2\textit{S})-2-aminocyclopentan-1-ol 182.} \text{ a) See Table 10.} \]

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBDMSCl, DMAP, TEA, CH\textsubscript{2}Cl\textsubscript{2}\textsuperscript{223}</td>
<td>r.t.</td>
<td>18 h</td>
<td>Trace of 182, mostly 172</td>
</tr>
<tr>
<td>TBDMSCl, imidazole, CH\textsubscript{2}Cl\textsubscript{2}\textsuperscript{224}</td>
<td>0 °C</td>
<td>1 h</td>
<td>172</td>
</tr>
<tr>
<td>TBDMSCl, DBU, acetonitrile\textsuperscript{225}</td>
<td>0 °C</td>
<td>1 d</td>
<td>172</td>
</tr>
<tr>
<td>TBDMSOTf, TEA, CH\textsubscript{2}Cl\textsubscript{2},\textsubscript{222} aq, workup then column</td>
<td>0 °C</td>
<td>6 h</td>
<td>182, 98% yield</td>
</tr>
</tbody>
</table>

Table 10: Conditions attempted for the synthesis of (1\textit{S},2\textit{S})-2-((\textit{tert}-butyldimethylsilyloxy)cyclopentan-1-amine 182 (see Scheme 36).

8.5.3.2 Synthesis of Br-C\textsubscript{4}-cyclopentanol-TBDMS-(SS) 184

The TBDMS protected (1\textit{S},2\textit{S})-2-aminocyclopentan-1-ol 182 was reacted with 4-bromobutyryl chloride 56 to form Br-C\textsubscript{4}-cyclopentanol-TBDMS-(SS) 184. The reaction was observed to go to completion by TLC, but it became apparent that the product was reacting further during concentration and purification. Adding sodium azide to the mixture obtained after the failed purification attempts was observed to convert the remaining Br-C\textsubscript{4}-cyclopentanol-TBDMS-(SS) 184 to N\textsubscript{3}-C\textsubscript{4}-cyclopentanol-TBDMS-(SS) 186. A sequential one-pot reaction was therefore used, so that the reactive intermediate did not need to be isolated.

\[ \text{Scheme 36: Attempted synthesis of Br-C\textsubscript{4}-cyclopentanol-TBDMS-(SS) 184. a) NaHCO\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, water, 0 °C, 2 h.} \]
8.5.3.3 Synthesis of N₃-C₄-cyclopentanol-TBDMS-(SS) 186 by one-pot reaction

N₃-C₄-cyclopentanol-TBDMS-(SS) 186 was finally synthesised by a two-step, one-pot reaction. Schotten-Baumann conditions were used to form the bromide. The water was then removed, and DMF and sodium azide were added. N₃-C₄-cyclopentanol-TBDMS-(SS) 186 was produced in excellent yield.

Scheme 37: Synthesis of N₃-C₄-cyclopentanol-TBDMS-(SS) 186. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 3 h. b) NaN₃, DMF, CH₂Cl₂, r.t., 3 h. 99% over 2 steps.

8.5.3.4 Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate 190

N₃-C₄-cyclopentanol-TBDMS-(SS) 186 and the alkynyl ciprofloxacin derivative 68 were subjected to standard click conditions (see 9.25), and the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate 190 was synthesised in very good yield. However, removal of the TBDMS group proved difficult. Deprotection using 1.5 eq. TBAF in THF proceeded slowly, reaching completion in 5 d. Increasing the amount of TBAF to 8 eq. allowed the reaction to proceed overnight. Purification of the final conjugate 180 by column chromatography was not successful due to streaking and poor separation. Purification using DOWEX resin and CaCO₃ was attempted, but the product could not be recovered from the resin. The purification method could probably be optimised, e.g. by varying the solvent used with the resin, but ultimately this route was abandoned due to the reduction in number of steps afforded by the two methods described below.
Scheme 38: Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate 190. a) CuSO₄, sodium ascorbate, THPTA, water, t-BuOH, r.t., 87%. b) TBAF, THF, r.t., 16 h.

8.5.4 Synthesis of the cyclopentanol-Cip triazole conjugates 180 and 181 via chloride intermediates

Given that the side product formation seen in the previous sections was most likely due to SN₂ attack on the bromide, we decided to use a chloride rather than a bromide intermediate (see Scheme 32 and Scheme 39 to compare). The bromide intermediate was initially chosen as it was used by Ganguly et. al,⁶¹ but it was anticipated that using a chloride would reduce the side reactions seen with the more reactive bromide.
Scheme 39: Retrosynthesis of the cyclopentanol-CipMe conjugates 178 (SS) and 179 (RR), and the cyclopentanol-Cip triazole conjugates 180 (SS) and 181 (RR) via Cl-C_4-cyclopentanol intermediates 193 (SS) and 194 (RR). SS enantiomers are shown, but both are implied.

Attempts at this route began with the synthesis of Cl-C_4-cyclopentanol-(RR) 194. Standard Schotten-Baumann conditions failed to produce significant amounts of product. If prolonged reaction times were allowed, degradation of the acid chloride to the carboxylic acid was observed. The reason for this is unclear, but it is possible that bromide ions present in small amounts in previous reactions were helping to catalyse the reaction of the acid chloride. Archer et al.\textsuperscript{227} propose that bromide ions can react with acid chlorides to form acid bromides, which are then more susceptible to nucleophilic attack. As no bromide ions are present in this reaction, different conditions were sought in order to increase the rate.

As (1R,2R)-2-aminocyclopentan-1-ol 173 is fairly polar, it is likely that it was staying in the aqueous layer to some extent even when deprotonated, thus keeping the two reactants apart. Therefore, the solvent system and base were changed to neat CH_2Cl_2 and TEA. This produced Cl-C_4-cyclopentanol-(RR) 194 in good yield (64%). Unlike the bromide 174, the chloride 194 was stable when concentrated.

Cl-C_4-cyclopentanol-(RR) 194 was converted to N_3-C_4-cyclopentanol-(RR) 177 by reaction with sodium azide. The reaction was slower than with previous bromides (~16 h vs. ~2 h), but much cleaner than with Br-C_4-cyclopentanol-(SS) 174 (see 8.5.2).
The enantiomers \( \text{Cl-C}_{4} \)-cyclopentanol-(SS) \( \text{193} \) and \( \text{N}_{3}-\text{C}_{4} \)-cyclopentanol-(SS) \( \text{176} \) were synthesised in lower yields, in part because of the smaller amounts being used.

Scheme 40: Synthesis of \( \text{N}_{3}-\text{C}_{4} \)-cyclopentanol-(SS) \( \text{176} \) and \( \text{N}_{3}-\text{C}_{4} \)-cyclopentanol-(RR) \( \text{177} \). SS enantiomers are shown, but both were synthesised. a) TEA, \( \text{CH}_{2}\text{Cl}_{2} \), 0 °C, 2 h, \( \text{193} \) (SS): 24%, \( \text{194} \) (RR): 64%. b) \( \text{NaN}_{3} \), acetonitrile, 50 °C, 16 h, \( \text{176} \) (SS): 45%, \( \text{177} \) (RR): 88%.

The cyclopentanol-Cip triazole conjugates \( \text{180} \) (SS) and \( \text{181} \) (RR) were successfully synthesised using standard click conditions (see 9.25). Despite low yields (presumably due to problems with purification, including losses on the preparative HPLC column and high polarity leading to losses during extraction from aqueous solvents) enough of the compounds were obtained for biological testing so the purification was not optimised further.

Scheme 41: Synthesis of the cyclopentanol-Cip triazole conjugates \( \text{180} \) (SS) and \( \text{181} \) (RR). SS enantiomers are shown, but both were synthesised. a) \( \text{CuSO}_{4} \), THPTA, sodium ascorbate, water, \( \text{t-BuOH} \), r.t., 16 h, \( \text{180} \) (SS): 22%, \( \text{181} \) (RR): 27%.

The \( \text{S}_{2}2 \) reaction of \( \text{Cl-C}_{4} \)-cyclopentanol-(RR) \( \text{194} \) and methyl ciprofloxacin \( \text{151} \) was attempted (see Scheme 42) using the microwave conditions described previously (see 8.3), to see if the chloride produced better results compared with the bromide. However, as was seen with the other microwave reactions, a substantial amount of the disubstituted product \( \text{195} \) was seen by LCMS (in an approx 1:1 ratio with the desired product...
Scheme 42: Attempted synthesis of the cyclopentanol-CipMe-(RR) conjugate 179. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C.

8.5.5 Synthesis of the cyclopentanol-CipMe conjugates 178 and 179 by peptide coupling

Given the side-reactions and low yields associated with the literature synthesis of the $S_N$2 conjugates proposed by Ganguly et al., an alternative synthesis was investigated, involving building up the linker on the ciprofloxacin side before coupling with the head group (see Scheme 43).
Scheme 43: Retrosynthesis of the cyclopentanol-CipMe conjugates 178 (SS) and 179 (RR). SS enantiomers are shown, but both are implied.

The first step of the synthesis was an S_N2 reaction between Boc-protected 4-bromobutyric acid 196 methyl ciprofloxacin 151 (see Scheme 44). Intermediate 197 was obtained in acceptable yield after column chromatography (50%). Intermediate 197 was deprotected in excellent yield using TFA in CH_2Cl_2 to give carboxylic acid 198. Scale-up of this reaction allowed the easy synthesis of 600 mg of this useful intermediate, which can be coupled with various amine head-groups to create a library. Carboxylic acid 198 was first coupled with (1R,2R)-2-aminocyclopentan-1-ol 173 using standard peptide coupling conditions to give cyclopentanol-CipMe conjugate 179. Purification by column chromatography was attempted twice with poor results, before moving on to using preparative HPLC, which gave 179 cleanly in 39% yield. Coupling was also performed with (1S,2S)-2-aminocyclopentan-1-ol 172 to give the enantiomer 178 in 55% yield.
Scheme 44: Synthesis of the cyclopentanol-CipMe conjugates 178 (SS) and 179 (RR) by peptide coupling. SS enantiomers are shown, but both were synthesised. a) NaI, TEA, acetonitrile, 100 °C, 16 h, 50%. b) TFA, CH₂Cl₂, r.t., 18 h, 96%. c) EDC, HOBT, DIPEA, DMF, r.t., 16 h, 178 (SS): 55%, 179 (RR): 39%.

With (unfortunately not branching) routes to the SN₂ and click conjugates established (see 8.5.5 and 8.5.4 respectively), attention was turned to the cyclohexanol derivatives.

8.6 Synthesis of the cyclohexanol conjugates

8.6.1 Synthesis of the trans-2-aminocyclohexan-1-ol head group 200

It was decided to produce the cyclohexanol conjugates racemically, with the option of re-synthesising enantiomerically pure versions via the route shown in 8.5.1 if the compounds showed biological activity.

Production of the cyclohexanol conjugates began with the synthesis of trans-2-aminocyclohexan-1-ol 200 (see Scheme 45), using a procedure reported by Xue et al.²²⁸ Cyclohexene oxide 199 was opened using ammonia in water and methanol. Initially the reaction was carried out at 85 °C in a microwave reactor for 30 min, but a large amount of the disubstituted amine could be seen by LCMS (in a ratio of 4:3 product to impurity by NMR). The reaction was therefore attempted at room temperature, and proceeded overnight in high yield and with minimal side reaction.
Scheme 45: Synthesis of trans-2-aminocyclohexan-1-ol 200. a) NH₃, water, MeOH, r.t., 72 h, 86%.

8.6.2 Synthesis of the trans-cyclohexanol- and cyclohexanone-CipMe conjugates 201 and 202

Carboxylic acid 198 was coupled with trans-2-aminocyclohexan-1-ol 200 using standard peptide coupling conditions to give trans-cyclohexanol-CipMe conjugate 201 in 32% yield.

A portion of the trans-cyclohexanol-CipMe conjugate 201 was then oxidised to the ketone using Dess-Martin periodinane and the product was isolated in good yield.


a) EDC, HOBt, DIPEA, DMF, r.t., 16 h, 32%. b) DMP, CH₂Cl₂, r.t., 6 h, 69%.

8.6.3 Synthesis of the trans-cyclohexanol- and cyclohexanone-Cip triazole conjugates 205 and 206

The triazole conjugates were synthesised using the route described in 8.5.4. Cl-C₄-trans-cyclohexanol 203 was synthesised in good yield from trans-2-aminocyclohexan-1-ol 200 and 4-chlorobutyryl chloride 192. Cl-C₄-trans-cyclohexanol 203 was then converted to N₃-C₄-trans-cyclohexanol 204 by reaction with sodium azide in excellent yield.
Scheme 47: Synthesis of $N_3$-C$_4$-trans-cyclohexanol 204. a) TEA, CH$_2$Cl$_2$, 0 °C, 30 min, 76%. b) NaN$_3$, acetonitrile, 50 °C, 16 h, 98%.

The trans-cyclohexanol-Cip triazole conjugate 205 was synthesised using standard click conditions (see 9.25) in 49% yield. A portion of the trans-cyclohexanol-Cip triazole conjugate 205 was then oxidised to the ketone using the same conditions used for the cyclohexanone-CipMe conjugate (see 8.6.2) in very good yield.

Scheme 48: Synthesis of the trans-cyclohexanol-Cip triazole conjugate 205 and the cyclohexanone-Cip triazole conjugate 206. a) CuSO$_4$, THPTA, sodium ascorbate, water, t-BuOH, r.t., 16 h, 49%. b) DMP, CH$_2$Cl$_2$, r.t., 4 h, 78%.
8.7 Biological testing

The biological testing presented in this section was planned by me but carried out by Tom O’Brien, a PhD student in the Department of Biochemistry.

The HSL analogue-Cip(Me) conjugates (see Figure 40), as well as C₄-HSL 19, ciprofloxacin 24, methyl ciprofloxacin 151, the alkynyl ciprofloxacin derivative 68, the tert-butyl ester methyl ciprofloxacin derivative 197 and the carboxylic acid methyl ciprofloxacin derivative 198 were tested for antibacterial and anti-biofilm activity in *P. aeruginosa* PAO1¹⁸⁰ and YM64.¹⁸¹ All compounds were tested in triplicate, and the ratio of the standard deviation (SD) to the mean was less than 1 for all data points except one (68 at 25 µl in PAO1 at 8 h).
Figure 40: The HSL analogue-Cip(Me) conjugates.
8.7.1 Antibacterial testing

8.7.1.1 Antibacterial testing against YM64

In YM64 at 5 h several of the HSL analogue-Cip(Me) conjugates showed activity at the highest concentration (see Figure 41 and Figure 42). Conjugates 162 and 167 showed similar activity to ciprofloxacin 24 and the cleavable conjugate 157 showed better activity (see Figure 41). The activity of the cleavable conjugate 157 was even more pronounced at 24 h (see Figure 43).

It should be noted that the highest concentration tested was 25 µM in this set of assays as opposed to 2 µM in the previous set (see 7.5), but oddly all compounds including ciprofloxacin 24 showed less activity. This is thought to be due to a change in the plate seals used and/or the humidity of the incubation conditions (see 9.71).

Figure 41: YM64 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.
Figure 42: YM64 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

Figure 43: YM64 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.
8.7.1.2 Antibacterial testing against PAO1

In PAO1 at 5 h conjugates 157, 162 and 167 showed activity at the highest concentration (see Figure 45). The cleavable conjugate 157 showed similar activity to ciprofloxacin 24. At 24 h conjugate 167 still showed some activity, and cleavable conjugate 157 showed similar activity to ciprofloxacin 24 (see Figure 47).
Figure 45: PAO1 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

Figure 46: PAO1 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.
In addition to its promising antibacterial activity, the cleavable HCTL-Cip triazole conjugate 157 has an
interesting growth curve (see Figure 49). When *P. aeruginosa* PAO1 is treated with 25 µM ciprofloxacin 24 it continues to grow slowly over the course of a 48 h assay, whereas growth is fully inhibited by treatment with the cleavable HCTL-Cip triazole conjugate 157. However, the errors in this data are large and so the assay needs repeating to confirm the effect.

Figure 49: PAO1 OD readings over 48 h for the cleavable HCTL-Cip triazole conjugate 157 and ciprofloxacin 24 at 25 µM.

8.7.2 Anti-biofilm testing

Biofilm inhibition and dispersal were measured using crystal violet staining (see 9.71). Unfortunately the results were largely unreliable as it was obvious that staining was higher in the wells at the edges of the plates, and that this was overwhelming any other trends. This effect was probably due to increased evaporation from the outer wells. It is likely that this effect was seen in these results, but not those in 7.5, due to a change in the conditions that the plates were incubated in. Specifically, a different type of plate seal was used and a humid environment was maintained in the incubator in the previous experiments (see 9.71).

8.8 Conclusions

8.8.1 Library synthesis

In this section, a library of HSL analogue-Cip(Me) conjugates was successfully synthesised and tested for antibiotic activity. A range of 7 head groups (see 8.1.1) and two linking strategies were used. Unfortunately the branching route that was initially proposed (see 8.1.2) was not feasible for the alcohol-containing head groups and was low yielding for others, probably due to internal cyclisation (this side reaction could with hindsight be avoided by changing the linker length).

Given the difficulties in the branching synthesis, routes to the differently-linked compounds were optimised
separately: the alkyl-linked conjugates were best formed using peptide coupling and the triazole-linked conjugates via a chloride intermediate. Direct comparisons of routes are not possible without repeating syntheses, but if it is assumed that peptide coupling of homocysteine thiolactone hydrochloride 152 to carboxylic acid 198 would have a similar yield to the coupling with (1R,2R)-2-aminocyclopentan-1-ol 173, approximate comparisons can be made. The synthesis of the HCTL-CipMe conjugate 154 described in 8.2 has an overall yield of 11%, whereas the route to the cyclopentanol-CipMe conjugate 178 shown in Scheme 44 has an overall yield of 26%. Moreover, if the yield starting from the head group is considered, the yield is 55% vs. 11%. Therefore, the peptide coupling route is recommended for further investigation if the alkyl-linked library is to be expanded.

Synthesis of the azido autoinducer analogues via the chloride is also recommended as the bromide is thought to cyclise readily (this could explain the poor yields of the 2- and 3-methoxybenzene derivatives).

Preparative HPLC was identified as the best purification method for these conjugates (note that the standard acidic method used hydrolysates the lactone of native HSL and so cannot be used in that case).

8.8.2 Biology

The ciprofloxacin triazole conjugates had higher activity than the methyl ciprofloxacin conjugates. This was mirrored in the controls: methyl ciprofloxacin 151 showed little activity compared to ciprofloxacin 24. It was assumed that methyl ciprofloxacin 151 would act as a prodrug, as the HCTL-CipMe conjugate 154 synthesised by Ganguly et al.61 was a methyl ester and showed activity, but these results suggest otherwise. However, the HCTL-CipMe conjugate 154 showed better anti-biofilm activity than antibiotic, and it is possible that the other CipMe conjugates will do the same.

The most promising compounds from this set were the methoxybenzene-ciprofloxacin triazole conjugates 162 and 167 and the cleavable HCTL-Cip triazole conjugate 157. The cleavable HCTL-Cip triazole conjugate 157 was also interesting in that it appeared to entirely inhibit the growth of P. aeruginosa, whereas ciprofloxacin 24 either allowed slow growth, or resistant mutants emerged and started to replicate.229 However, the errors in these data were large and so the assays need to be repeated. Given the previous results for the cleavable HSL-Cip conjugates (see 7.5.2) is not very likely that the quorum sensing modulation properties of the head group contributed to its antibiotic effect, but the effect of different cleavable tails is certainly worth investigating.

Initial biofilm inhibition and dispersal assays were carried out (see 9.71), but unfortunately the results were unreliable as the biofilm was growing more in the wells at the edges of the plates. As biofilm formation is induced by hypoxia (which might occur in the centre of the plate when a plastic lid was used in addition to the adhesive plate seal) this cannot account for the increased biofilm growth at the edges of the plate. Neither can the increased concentration of NaCl that would occur due to evaporation of water from the edges of the plate, as this too decreases biofilm formation.231 A reasonable explanation is that evaporation would leave a residue of dried planktonic cells on the edges of the wells which would be stained by the crystal violet.

8.9 Future work

8.9.1 Further conjugates

An obvious addition to the library would be the HSL-CipMe conjugate (see Figure 50), to enable better comparisons between the triazole-linked and alkyl-linked libraries.
As methyl ciprofloxacin 151 and its alkyl-linked conjugates had little antibiotic effect, it would be useful to test the carboxylic acid versions of these compounds (see Figure 51). Ideally these would be synthesised directly by hydrolysis of the methyl ciprofloxacin conjugate, but this could potentially cause hydrolysis of the lactone or thiolactone as well. If mild enough hydrolysis conditions could not be found it might be possible to hydrolyse both bonds and then re-form the ring.\textsuperscript{232} The cyclohexanone conjugate would be best formed by hydrolysis of the cyclohexanol conjugate followed by oxidation of the alcohol to avoid exposing the ketone to extremes of pH.

A selection head groups which could be used in future conjugates are shown in Figure 52. These have...
all been shown to modulate HSL-mediated quorum sensing as part of acyl-HSLs.\textsuperscript{152, 157, 159, 233–236} The most obvious targets are the cyclopentanone derivatives, as this could be synthesised from the alcohols above. The aniline, pyridine, quinoline and cyclopentyl amine head groups are commercially available and hence derivatives of these could be easily obtained. The 3- and 4-substituted HSL analogues require synthesis, but a route has been devised.\textsuperscript{235}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hsl_analogues.png}
\caption{HSL analogue head groups for use in future conjugates.}
\end{figure}

8.9.2 Biology

The most important next step is the repetition of the biofilm inhibition and dispersal assays with better control over evaporation. This can be achieved in a low-tech but reliable manner by placing the sealed plates (without the plastic plate lids) inside a high-sided, open plastic box lined with damp tissue paper.

It is worth noting that Ganguly \textit{et al.} used LIVE/DEAD\textsuperscript{\textregistered} BacLight\textsuperscript{TM} staining and confocal microscopy to image their biofilms, whereas so far we have used crystal violet staining. Crystal violet does not differentiate between live and dead cells, and so does not pick up on how many cells have been killed within the biofilm but stayed adhered to the plate (their confocal microscopy results do however show a decrease in biofilm thickness so it should be possible to detect this using crystal violet).

We do not have access to a confocal microscope on which we can use \textit{P. aeruginosa}, but alternative stains which show cell viability could be used. Peeters \textit{et al.}\textsuperscript{237} evaluated six assays used for the quantification of biofilms and recommended fluorescein diacetate or resazurin viability assays for the quantification of live cells in \textit{P. aeruginosa} biofilms.

Given the interesting growth curve results for the cleavable HCTL-Cip triazole conjugate\textsuperscript{157} it would be useful to collect this data for the other cleavable compounds shown in 7.4.3 (previously OD readings were only taken at 5 and 24 h).
9 Experimental

9.1 General

Unless otherwise stated, reactions were performed in air-dried glassware under argon with dry, freshly-distilled solvents. THF was distilled from LiAlH$_4$ in the presence of triphenyl methane indicator. CH$_2$Cl$_2$, hexane, MeOH and acetonitrile were distilled from calcium hydride. All other chemicals were used as obtained from commercial sources.

Reactions using microwave heating were performed in sealed vials using a CEM Discover SP microwave reactor.

Thin-layer chromatography (TLC) was performed using Merck pre-coated 0.23 mm thick plates of Keiselgel 60 F254 and visualised using UV ($\lambda = 254$ or 366 nm) or by staining with KMnO$_4$ or ninhydrin. All retention factors ($R_f$) are given to 0.01. All column chromatography was carried out using Merck 9385 Keiselgel 60 silica gel (230-400 mesh) or using a CombiFlash® EZ Prep with RediSep® normal-phase silica flash columns. Preparative high-performance liquid chromatography (HPLC) was run on an Agilent 1260 Infinity machine, using a Supelcosil™ ABZ+PLUS column (250 mm × 21.2 mm, 5 µm) with a linear gradient system (solvent A: 0.025% (v/v) TFA/water, solvent B: 0.05% (v/v) TFA/acetonitrile) at a flow rate of 20 mL min$^{-1}$, visualised by UV absorbance ($\lambda_{max} = 254$ nm).

Nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock at ambient probe temperatures on Bruker DPX-400, Bruker Avance DRX-400, Bruker Avance 500 BB-ATM or Bruker Avance 500 Cryo Ultrasil spectrometers. Data were processed using NMR Processor Academic Edition version 12 (ADC Labs) or TopSpin version 3.5 (Bruker). $^1$H and $^13$C spectra were assigned using DEPT, COSY, HMQC and HMQC spectra where necessary, or by analogy to fully interpreted spectra of related compounds. The following abbreviations are used to indicate the multiplicity of signals: s singlet, d doublet, t triplet, q quartet, quint quintet, m multiplet and br broad.

$^1$H chemical shifts ($\delta$) are quoted to the nearest 0.01 ppm and are referenced relative to the residual solvent peak.$^{238}$ Coupling constants ($J$) are given to the nearest 0.1 Hz. Diastereotopic protons are assigned as CHH and CHH, where the latter designates the lower-field proton. Data are reported as follows: <chemical shift> (<multiplicity>, <coupling constant(s) (if any)>, <integration>, <assignment>).

$^{13}$C chemical shifts ($\delta$) are quoted to the nearest 0.1 ppm and are referenced relative to the deuterated solvent peak.$^{238}$ Data are reported as follows: <chemical shift> (<multiplicity (if not s)>, <coupling constant(s) (if any)>, <assignment>).

$^{19}$F chemical shifts ($\delta$) are quoted to the nearest 0.1 ppm. Data are reported as follows: <chemical shift> (<assignment>).

High resolution mass spectrometry (HRMS) data were recorded using a Micromass LCT Premier spectrometer or a Waters Vion IMS-QTOF spectrometer and reported mass values are within ± 5 ppm mass units. Liquid chromatography–mass spectrometry (LCMS) data were recorded on an Agilent 1200 series LC with an ESCi Multi-Mode Ionisation Waters ZQ spectrometer or a Waters ACQUITY H-Class UPLC with an ESCi Multi-Mode Ionisation Waters SQD2 mass spectrometer.

Infrared (IR) spectra were recorded using neat sample on a PerkinElmer 1600 FT IR spectrometer. Selected absorption maxima ($\nu_{max}$) are reported in wavenumbers (cm$^{-1}$). Broad peaks are marked br.

Melting points (m.p.) were measured using a Buchi B-545 melting point apparatus and are uncorrected.

Optical rotations ($[\alpha]_D^T$) were recorded on a PerkinElmer 343 polarimeter or an Anton-Paar MCP 100 polarimeter. $[\alpha]_D^T$ values are reported in $^{°}10^{-1}$cm$^2$g$^{-1}$ at 589 nm and concentration ($c$) is given in g (100 mL)$^{-1}$.

All compounds subjected to biological testing were >95% pure unless otherwise stated.
Meldrum’s acid 31 (9.00 g, 63.0 mmol, 1 eq.) was dissolved in anhydrous CH$_2$Cl$_2$ (150 mL) in an oven-dried flask and cooled to 0 °C. Pyridine (10.2 mL, 126 mmol, 2 eq.) was added dropwise over 20 min. Octanoyl chloride 32 (11.7 mL, 69.0 mmol, 1.1 eq.) was then added and the mixture was stirred at 0 °C for a further 4 h. The mixture was allowed to warm to r.t., diluted with CH$_2$Cl$_2$ (20 mL) and poured into a mixture of ice (~30 g) and HCl (2 N, 90 mL). The solution was washed with NaCl (sat., aq., 150 mL) and dried over MgSO$_4$. The solvent was removed under vacuum to give an orange-brown oil. The oil was refluxed in anhydrous MeOH (150 mL) for 5 h and the solvent was removed under vacuum. The resulting residue was purified by column chromatography (SiO$_2$, 5% Et$_2$O/40-60 P.E.). A tautomeric mixture of 33 and 34 was obtained as a colourless oil (8.34 g, 41.6 mmol, 66%. 92% 33 as determined by $^1$H NMR).

**Keto form 33**

**TLC** $R_f = 0.12$ (5% EtO$_2$/PE)

**IR** (neat) $\nu_{max}$ / cm$^{-1} = 2928$ (C-H), 2856 (C-H), 1747 (ester C=O), 1717 (ketone C=O)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 3.74 (s, 3 H, OCH$_3$), 3.45 (s, 2 H, C(=O)CH$_2$C(=O)), 2.53 (t, $J = 7.4$ Hz, 2 H, C(=O)CH$_2$CH$_2$), 1.60 (quin, $J = 7.1$ Hz, 2 H, C(=O)CH$_2$CH$_2$), 1.39 - 1.19 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.88 (t, $J = 6.8$ Hz, 3 H, CH$_2$CH$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 202.3 (CH$_3$OC(=O)CH$_2$C(=O)), 167.3 (CH$_3$OC(=O)CH$_2$C(=O)), 51.7 (OCH$_3$), 48.5 (CH$_3$OC(=O)CH$_2$C(=O)), 42.5 (C(=O)CH$_2$CH$_2$), 31.3 (CH$_2$), 28.7 (CH$_2$), 28.6 (CH$_2$), 23.1 (CH$_2$), 22.2 (CH$_2$), 13.6 (CH$_2$CH$_3$)

**Enol form 34**

**TLC** $R_f = 0.12$ (5% EtO$_2$/PE)

**IR** (neat) $\nu_{max}$ / cm$^{-1} = 2928$ (C-H), 2856 (C-H), 1654 (C=C), 1629 ($\alpha$, $\beta$ unsaturated C=O)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 12.02 (s, 1 H, COH), 4.99 (s, 1 H, C(=O)CH=COH), 3.73 (s, 3 H, OCH$_3$), 2.20 (t, $J = 7.4$ Hz, 2 H, COHCH$_2$), 1.76 - 1.72 (m, 2 H, COHCH$_2$CH$_2$), 1.39 - 1.19 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.88 (t, $J = 6.8$ Hz, 3 H, CH$_2$CH$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 178.7 (CH$_3$OC(=O)CH=COH), 172.7 (CH$_3$OC(=O)CH=COH), 88.2 (CH$_2$OC(=O)CH=COH), 50.5 (OCH$_3$), 37.9 (COHCH$_2$CH$_2$), 34.6 (CH$_2$), 31.2 (CH$_2$), 29.0 (CH$_2$), 25.9 (CH$_2$), 22.3 (CH$_2$), 13.6 (CH$_2$CH$_3$)

Spectroscopic data are consistent with the literature.$^{168,169}$

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9.2 Methyl 3-oxodecanoate 33

[Chemical structure diagram]

92% 33

8% 34

[Chemical structure diagram]
9.3 Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 36

Methyl 3-oxodecanoate 33 (500 mg, 2.50 mmol, 1.00 eq.) and O-tert-butyl N-(4-aminophenyl)carbamate 35 (520 mg, 2.50 mmol, 1.00 eq.) were dissolved in MeOH (10 mL) and refluxed for 18 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (SiO$_2$, gradient of 0 to 20% Et$_2$O/40-60 P.E.). 36 was obtained as a white amorphous solid (0.169 mg, 0.480 mmol, 19%).

TLC $R_f = 0.30$ (30% Et$_2$O/40-60 P.E.)

mp $T/\degree C = 79$ (Et$_2$O/40-60 P.E.)

IR (neat) $\nu_{max}/\text{cm}^{-1} = 3337$ (N-H), 2928 (C-H), 2857 (C-H), 1724 (carbamate C=O), 1635 ($\alpha,\beta$ unsaturated C=O), 1611 (C=C), 1581 (N-H bend)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm = 10.16 (s, 1 H, NH$_C(C_7H_{15})=C$), 7.35 (d, $J=8.6$ Hz, 2 H, meta to NHBoc), 7.02 (d, $J=8.7$ Hz, 2 H, meta to enamino), 6.60 (br s, 1 H, NH$_{Boc}$), 4.71 (s, 1 H, C=CH$_2$), 3.70 (s, 3 H, OCH$_3$), 2.23 (t, $J=7.7$ Hz, 2 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.33 - 1.16 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.86 (t, $J=7.1$ Hz, 3 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ppm = 171.1 (C(=O)CH=C), 164.3 (C(=O)CH=C), 152.7 (OC(=O)NH), 136.0 (para to NHBoc), 134.1 (C(NHBoc), 126.3 (meta to NHBoc), 119.1 (ortho to NHBoc), 83.8 (C(=O)CH=C), 80.7 (C(CH$_3$_3), 50.2 (OCH$_3$), 32.2 (CH$_2$), 31.6 (CH$_2$), 29.1 (CH$_2$), 28.8 (CH$_2$), 28.3 (C(CH$_3$)$_3$), 28.0 (CH$_2$), 22.6 (CH$_2$), 14.0 (CH$_3$)

HRMS (ESI$^+$) $m/z$/Da = 391.2589, [M+H]$^+$, [C$_{22}$H$_{35}$N$_2$O$_4$]$^+$ requires 391.2591

Spectroscopic data are consistent with the literature.$^{164}$

9.4 6-Amino-2-heptylquinolin-4-ol 37

Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 36 (168 mg, 0.649 mmol, 1 eq.) and polyphosphoric acid (5 g) were heated to 90 $\degree$C for 1 h. The reaction mixture was then poured into NaHCO$_3$ (sat., aq., 50 mL) cooled with ice. The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum. 37 was obtained as a pale yellow amorphous solid (121 mg, 0.468 mmol,
mp T / °C = 249 (water)

IR (neat) ν_{max} / cm^{-1} = 3337 (N-H), 2927 (C-H), 2857 (C-H), 1635 (C=O)

$^1$H NMR (400 MHz, DMSO-d$_6$) δ / ppm = 7.26 (d, J = 8.7 Hz, 1 H, meta to NH$_2$), 7.15 (d, J = 2.6 Hz, 1 H, ortho to C(=O)), 6.97 (dd, J = 2.7, 8.8 Hz, 1 H, para to C(=O)), 5.74 (s, 1 H, ortho to CH$_2$), 5.16 (s, 2 H, NH$_2$), 2.52 (t, J = 7.4 Hz, 2 H, CCH$_2$), 1.64 (quint, J = 7.6 Hz, 2 H, CCH$_2$CH$_2$), 1.36 - 1.19 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_3$CH$_3$), 0.86 (t, J = 7.0 Hz, 3 H, H$_3$)

$^{13}$C NMR (101 MHz, DMSO-d$_6$) δ / ppm = 176.7 (C(=O)), 151.7 (CCH$_2$), 145.1 (para to NH$_2$ or ipso to C(=O)), 132.4 (ipso to NH$_2$), 126.6 (para to NH$_2$ or ipso to C(=O)), 121.1 (para to C(=O)), 119.0 (meta to NH$_2$ and meta to C(=O)), 106.2 (C(=O)=CCH$_2$), 105.9 (ortho to NH$_2$ and ortho to C(=O)), 33.6 (CCH$_2$), 31.6 (CCH$_2$CH$_2$), 29.0 (C(=O)=CCH$_2$), 29.0 (C(=O)=CCH$_2$), 28.9 (C(=O)=CCH$_2$), 22.5 (CCH$_2$CH$_3$), 14.4 (CCH$_3$)

HRMS (ESI$^+$) m/z / Da = 259.1810, [M+H]$^+$, [C$_{16}$H$_{23}$N$_2$O]$^+$ requires 259.1803

Spectroscopic data are consistent with the literature.$^{164}$

9.5 6-Azido-2-heptylquinolin-4-ol 38

6-Amino-2-heptylquinolin-4-ol 37 (50 mg, 0.194 mmol, 1 eq) was dissolved in HCl (conc., aq., 1.20 mL), water (1.80 mL) and MeOH (2.00 mL) and cooled to 0 °C. A solution of NaNO$_2$ (16.0 mg, 0.232 mmol, 1.2 eq.) in water (0.300 mL) was added dropwise over 10 min and the mixture was stirred for 1 h. A solution of NaN$_3$ (15.1 mg, 0.232 mmol, 1.2 eq.) in water (0.300 mL) was then added. The mixture was warmed to room temperature and stirred for a further 4 h. The resultant precipitate was filtered off and dried under reduced pressure. 38 hydrochloride salt* was obtained as a pale cream amorphous solid (25.6 mg, 0.0800 mmol, 41%).

TLC $R_f = 0.40$ (5% MeOH/CH$_2$Cl$_2$)

IR (neat) ν_{max} / cm^{-1} = 3249 (N-H), 3065 (N-H), 2917 (C-H), 2853 (C-H), 2728 (C-H), 2107 (azide), 1635 (C=O)

$^1$H NMR (400 MHz, MeOD) δ / ppm = 7.73 (d, J = 8.6 Hz, 1 H, ortho to NH), 7.71 (d, J = 2.8 Hz, 1 H, ortho to N$_3$ and ortho to C(=O)), 7.47 (dd, J = 8.9, 2.7 Hz, 1 H, para to C(=O)), 6.24 (s, 1 H, C(=O)CH$_2$), 2.69 (t, J = 7.7 Hz, 2 H, CCH$_2$), 1.68 (quint, J = 7.6 Hz, 2 H, CCH$_2$CH$_2$), 1.28 - 1.39 (m, 4 H, CCH$_2$CH$_2$CH$_2$CH$_2$), 1.18 - 1.28 (m, 4 H, CH$_2$CH$_2$CH$_3$), 0.85 (t, J = 6.8 Hz, 3 H, CH$_3$)

$^{13}$C NMR (101 MHz, MeOD) δ / ppm = 172.3 (C(=O)), 155.5 (NHCH$_2$), 137.4 (CH$_2$), 135.6 (para to N$_3$), 124.6 (para to C(=O)), 124.1 (ipso to C(=O)), 120.7 (meta to N$_3$ and meta to C(=O)), 112.8 (ortho to N$_3$ and ortho to C(=O))
9.6 Heptyl magnesium bromide 40

Magnesium turnings (352 mg, 14.5 mmol, 1 eq.) were added to an oven-dried flask under argon. THF (15 mL) was added, followed by bromoheptane 39 (2.40 mL, 14.5 mmol, 1 eq.) dropwise. The mixture was stirred at r.t. for 2 h followed by heating to reflux for 2 h. Heptyl magnesium bromide 40 was obtained as a pale grey suspension (15 mL, ~ 1 M) which was used without further purification.

9.7 2-Chloro-N-methoxy-N-methylacetamide 43

N,O-Dimethylhydroxyl amine hydrochloride 41 (6.00 g, 61.5 mmol, 1 eq.) and toluene (75 mL) were added successively to a stirred solution of potassium carbonate (22.4 g, 162 mmol, 2.63 eq.) in water (75 mL) at 0 °C under argon. The mixture was cooled to -5 °C and chloroacetyl chloride 42 (5.88 mL, 73.8 mmol, 1.20 eq.) was added dropwise over 5 min. The mixture was allowed to warm to r.t. over 30 min, then the organic layer was separated and the aqueous layer was extracted with toluene (3×20 mL). The combined organic extracts were dried with MgSO₄ and the solvent was removed by rotary evaporation followed by high vacuum. 43 was obtained as white, prism-like crystals (7.24 g, 52.6 mmol, 71%).

mp T / °C = 39 (toluene)

IR (neat) ν max / cm⁻¹ = 3016.7 (C-H), 2966.4 (C-H), 2946.7 (C-H), 2827.7 (C-H), 1666.2 (C=O)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 4.20 (s, 2 H, ClCH₂C=O), 3.71 (m, 3 H, OCH₃), 3.18 (s, 3 H, NCH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 167.4 (C=O), 61.6 (OCH₃), 40.9 (ClCH₂C=O), 32.6 (NCH₃)

Spectroscopic data are consistent with the literature.

9.8 1-Chlorononan-2-one 44

Spectroscopic data are similar to the literature characterisation of the free amine.¹⁶⁴

*Probably as the 4-hydroxyquinoline.⁸⁹
2-Chloro-N-methoxy-N-methylacetamide 43 (1.00 g, 7.26 mmol, 1 eq.) was added to a dry flask under argon. THF (20 mL) was added and the flask cooled to 0 °C. Heptyl magnesium bromide 40 (~ 1 M, 15.0 mL, 15.0 mmol, 2.07 eq.) was added dropwise over 5 min, then the mixture was allowed to warm to r.t. and stirred for 15 h. The reaction mixture was then poured into HCl (aq., 2 N, 60 mL) at 0 °C and stirred for 10 min. The mixture was extracted with toluene (30 mL) and the aqueous layer discarded. The organic layer was washed with brine and dried with MgSO₄, and the solvent was removed by rotary evaporation. 44 was obtained as a colourless oil (1.23 g, 6.96 mmol, 96%).

IR (neat) $\nu_{\text{max}} / \text{cm}^{-1} = 2952$ (C-H), 2925 (C-H), 2856 (C-H), 1720 (C=O)

$^1$H NMR (400 MHz, CDCl₃) $\delta / \text{ppm} = 4.05$ (s, 2 H, ClCH₂C(=O)), 2.54 (t, $J = 7.4$ Hz, 2 H, C(=O)CH₂CH₂), 1.59 (quin, $J = 7.0$ Hz, 2 H, C(=O)CH₂CH₂CH₂CH₃), 1.34 - 1.21 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.87 (t, $J = 6.8$ Hz, 3 H, CH₃)

$^{13}$C NMR (101 MHz, CDCl₃) $\delta / \text{ppm} = 202.6$ (C(=O)), 48.1 (C₃H₂Cl), 39.6 (C(=O)CH₂CH₂), 31.5 (CH₂CH₂CH₃), 28.9 (CH₂), 28.9 (CH₂), 23.5 (C(=O)CH₂CH₂), 22.5 (CH₂CH₃), 13.9 (CH₃)

Spectroscopic data are consistent with the literature.⁸⁹

9.9 2-Oxononyl 2-amino-5-nitrobenzoate 46

5-Nitroanthranilic acid 45 (500 mg, 2.75 mmol, 1.38 eq.) and potassium carbonate (270 mg, 2.00 mmol, 1 eq.) were dissolved in DMF (5 mL). The mixture was heated under argon to 90 °C and stirred for 1 h then cooled to r.t.. 1-Chlorononan-2-one 44 (353 mg, 2.00 mmol, 1 eq.) was added and the mixture was stirred for 15 h. The solution was poured into Na₂HCO₃ (aq., 10%, 50 mL) and ice (~ 20 g). The precipitate was collected by vacuum filtration, washed with water and dried under high vacuum. 46 was obtained as a yellow amorphous solid (0.674 g, 2.00 mmol, >99%).

mp $T / ^\circ C = 135$ (water)

IR (neat) $\nu_{\text{max}} / \text{cm}^{-1} = 3453$ (N-H), 3351 (N-H), 2925 (C-H), 2854 (C-H), 1720 (ester C=O) 1704 (ketone C=O) 1626 (N-H bend) 1603 (aromatic) 1573 (N-O) 1507 (N-O)

$^1$H NMR (400 MHz, DMSO-d₆) $\delta / \text{ppm} = 8.66$ (d, $J = 2.8$ Hz, 1 H, ortho to C(=O)), 8.12 (dd, $J = 2.8$, 9.4 Hz, 1 H, para to C(=O)), 6.93 (d, $J = 9.4$ Hz, 1 H, meta to C(=O)), 5.05 (s, 2 H, OCH₂C(=O)), 2.49 (t, $J = 7.4$ Hz, 2 H, C(=O)CH₂CH₂), 1.52 (quin, $J = 7.2$ Hz, 2 H, C(=O)CH₂CH₂), 1.32 - 1.20 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.86 (t, $J = 6.8$ Hz, 3 H, CH₃)

$^{13}$C NMR (101 MHz, DMSO-d₆) $\delta / \text{ppm} = 204.4$ (OCH₂C(=O)), 165.6 (C(=O)O), 156.3 (ipso to NH₂), 135.7 (ipso to NO₂), 129.6 (para to C(=O)), 128.9 (ortho to C(=O)), 117.4 (meta to C(=O)), 107.5 (ipso to C(=O)), 68.8 (OCH₂C(=O)), 38.3 (C(=O)CH₂CH₂), 31.6 (CH₂CH₂CH₃), 28.9 (CH₂), 28.9 (CH₂), 23.2 (C(=O)CH₂CH₂), 22.5 (CH₂CH₃), 14.4 (CH₃)
HRMS (ESI$^+$) $m/z$ / Da = 323.1610, [M+H]$^+$, [C$_{16}$H$_{23}$N$_2$O$_3$]$^+$ requires 323.1607

Spectroscopic data are consistent with the literature.$^{164}$

9.10 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1$H$)-one 47

![Structure of 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1$H$)-one 47]

2-Oxononyl-2-amino-5-nitrobenzoate 46 (100 mg, 0.340 mmol, 1 eq.) and polyphosphoric acid (300 mg) were stirred for 5.5 h at 90 °C under argon. The mixture was then poured into NaHCO$_3$ (sat., aq., 50 mL) cooled on ice. The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum. 47 was obtained as a yellow-brown amorphous solid (44 mg, 0.145 mmol, 43%).

mp $T$ / °C = 223 (water, EtOAc)

IR (neat) $\nu_{max}$ / cm$^{-1}$ = 3436 (N-H), 3000 (O-H, br), 2955 (C-H), 2926 (C-H), 2851 (C-H), 1648 (C=O), 1571 (N-O), 1536 (N-O)$^1$

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ / ppm = 12.00 (s, 1 H, NH), 8.91 (d, $J$ = 2.8 Hz, 1 H, ortho to C=O), 8.29 (dd, $J$ = 2.7, 9.2 Hz, 1 H, para to C=O), 7.70 (d, $J$ = 9.3 Hz, 1 H, meta to C=O), 2.75 (t, $J$ = 7.7 Hz, 2 H, CCH$_2$), 1.67 (quin, $J$ = 7.3 Hz, 2 H, CCH$_2$CH$_2$), 1.36 - 1.23 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.85 (t, $J$ = 7.0 Hz, 3 H, CH$_3$)$^1$

$^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ / ppm = 169.7 (C=O), 141.9 (para to NO$_2$), 140.7 (ipso to NO$_2$), 139.6 (ipso to OH), 137.3 (C=COH), 124.3 (para to C=O), 122.3 (ortho to NO$_2$ and ortho to C=O), 121.5 (ipso to C=O), 120.0 (meta to NO$_2$ and meta to C=O), 31.6 (CH$_2$CH$_2$CH$_3$), 29.2 (CH$_2$), 28.9 (CH$_2$), 28.5 (CCH$_2$), 28.1 (CCH$_2$CH$_2$), 22.5 (CH$_2$CH$_3$), 14.4 (CH$_3$)$^{13}$

HRMS (ESI$^+$) $m/z$ / Da = 305.1501, [M+H]$^+$, [C$_{16}$H$_{21}$N$_2$O$_4$]$^+$ requires 305.1500

Spectroscopic data are consistent with the literature.$^{164}$

9.11 6-Amino-2-heptyl-3-hydroxyquinolin-4(1$H$)-one 48

![Structure of 6-Amino-2-heptyl-3-hydroxyquinolin-4(1$H$)-one 48]

6-Nitro-2-heptyl-3-hydroxyquinolin-4(1$H$)-one 47 (20 mg, 0.0658 mmol, 1 eq.) and PtO$_2$ (2 mg, 10 weight %) were stirred in MeOH (1 mL) under a H$_2$ atmosphere for 45 min at room temperature and pressure. The reaction mixture was then filtered through celite and the solvent was removed under vacuum. 48 was obtained
as a yellow-brown amorphous solid (14.5 mg, 0.0529 mmol, 80%).

**mp (MeOH)** $T / ^\circ C = 176$

**IR (neat)** $\nu_{max} / \text{cm}^{-1} = 30000$ (O-H, br) 29251 (C-H), 28549 (C-H), 16133 (C=O)

**$^1H$ NMR** (400 MHz, MeOD) $\delta / \text{ppm} = 11.12$ (s, 1 H, NH), 7.47 (d, $J = 8.9$ Hz, 1 H, meta to C=O), 7.40 (d, $J = 2.4$ Hz, 1 H, ortho to C=O), 7.16 (dd, $J = 2.6$, 9.0 Hz, 1 H, para to C=O), 2.86 (t, $J = 7.5$ Hz, 2 H, CCH$_2$), 1.75 (quin, $J = 7.8$ Hz, 2 H, CCH$_2$CH$_2$), 1.48 - 1.22 (m, $J = 5.4$ Hz, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.89 (t, $J = 6.7$ Hz, 3 H, CH$_3$)

**$^{13}C$ NMR** (101 MHz, MeOD) $\delta / \text{ppm} = 166.8$ (C(=O)), 144.8 (para to NH$_2$ or ipso to C(=O)), 140.5 (COH), 138.6 (C=COH), 132.6 (ipso to NH$_2$), 124.8 (para to NH$_2$ or ipso to C(=O)), 123.8 (para to C(=O)), 107.7 (meta to NH$_2$ and meta to C(=O)), 106.4 (ortho to NH$_2$ and ortho to C(=O)), 116.2 (C(CH$_2$CH$_2$CH$_2$CH$_2$)), 23.8 (C(CH$_3$)), 14.5 (CH$_3$)

**HRMS (ESI$^+$)** $m/z / \text{Da} = 275.1760$, [M+H]$^+$, [C$_{16}$H$_{23}$N$_2$O$_2$]$^+$ requires 275.1762

Spectroscopic data are not consistent with the literature. It is possible that Baker’s product is a Zn adduct.

### 6-Azido-2-heptyl-3-hydroxyquinolin-4(1H)-one 49

6-Amino-2-heptyl-3-hydroxyquinolin-4(1H)-one 48 (18.2 mg, 0.0664 mmol, 1 eq.) was dissolved in HCl (conc., aq., 0.8 mL) and MeOH (0.5 mL) at 0 $^\circ$C. NaN$_2$ (5.0 mg, 0.0725 mmol, 1.09 eq.) in water (0.2 mL) was added dropwise over 2 min and the mixture was stirred at 0 $^\circ$C for 50 min, during which time the solution turned from yellow to orange. NaN$_3$ (4.9 mg, 0.0754 mmol, 1.14 eq.) in water (0.2 mL) was then added and the mixture was allowed to warm to r.t. and stirred for 4 h. The reaction mixture was then filtered and the solid was dried under reduced pressure. 49 was obtained as a brown amorphous solid (5.5 mg, 0.0183 mmol, 28%).

**IR (neat)** $\nu_{max} / \text{cm}^{-1} = 3089$ (N-H), 2921 (C-H), 2851 (C-H), 2108 (azide), 1632 (C=O)

**$^1H$ NMR** (400 MHz, DMSO-d$_6$) $\delta / \text{ppm} = 7.74$ (s, 1 H, ortho to C=O), 7.65 (d, $J = 6.9$ Hz, 1 H, meta to C(=O)), 7.32 (d, $J = 7.4$ Hz, 1 H, para to C(=O)), 2.75 (t, $J = 7.5$ Hz, 2 H, CCH$_2$), 1.67 (quin, $J = 6.4$ Hz, 2 H, CCH$_2$), 1.43 - 1.13 (m, 8 H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 0.85 (t, $J = 6.8$ Hz, 3 H, CH$_3$)

**$^{13}C$ NMR** (101 MHz, DMSO-d$_6$) $\delta / \text{ppm} = 166.3$ (C(=O)), 137.9 (C), 137.8 (CN$_3$), 134.5 (ipso to C(=O)), 133.9 (C=COH), 122.7 (meta to N$_3$), 122.6 (meta to N$_3$ and meta to C(=O)), 120.4 (para to N$_3$), 112.4 (ortho to N$_3$ and ortho to C(=O)), 31.7 (CH$_2$CH$_2$CH$_2$), 28.8 (CCH$_2$), 28.4 (CCH$_2$CH$_2$CH$_2$), 28.3 (CCH$_2$CH$_2$CH$_2$CH$_2$), 27.8 (CCH$_2$CH$_2$), 22.1 (C$_2$H$_3$), 14.0 (CH$_3$)

**HRMS (ESI$^+$)** $m/z / \text{Da} = 301.1649$, [M+H]$^+$, [C$_{16}$H$_{21}$N$_4$O$_2$]$^+$ requires 301.1659
Spectroscopic data are consistent with the literature.\textsuperscript{164}

\section*{9.13 (S)-3-Aminodihydrofuran-2(3\textit{H})-one hydrobromide 52}

\begin{center}
\includegraphics[width=0.2\textwidth]{52.png}
\end{center}

L-Methionine 50 (3.04 g, 20.4 mmol, 1 eq.) and bromoacetic acid 51 (3.08 g, 22.2 mmol, 1.09 eq.) were dissolved in \textit{i}-PrOH (12.5 mL), H\textsubscript{2}O (12.5 mL) and AcOH (5 mL). The reaction was refluxed for 15 h then concentrated under vacuum. The resulting brown oil was added to a mixture of \textit{i}-PrOH (16 mL) and HBr (33\% in AcOH, 4 mL), causing the precipitation of a pale pink amorphous solid. The precipitate was collected by filtration and washed with \textit{i}-PrOH (20 mL). The filtrate was concentrated under vacuum and precipitated again using the same procedure. The two crops of precipitate were combined. 52 was obtained as a pale pink amorphous solid (1.73 g, 9.50 mmol, 41\% yield).

\textbf{mp} \textit{T}/\degree\textup{C} = 242 (\textit{i}-PrOH/AcOH, gas evolved)

\textbf{IR} (neat) \(\nu_{\text{max}} \text{ cm}^{-1} = 2972 (\text{N-H}), 2878 (\text{N-H}), 1772 (\text{C=O}), 1585 (\text{N-H bend}), 1572 (\text{N-H bend})

\textbf{\textsuperscript{1}H NMR} (400 MHz, DMSO-d\textsubscript{6}) \(\delta/ \text{ppm} = 8.59 (\text{br s, 3 H, NH\textsuperscript{+}}), 4.46 (\text{dt, } J = 1.3, 8.9 \text{ Hz, 1 H, OCHH}), 4.37 (\text{dd, } J = 8.8, 11.4 \text{ Hz, 1 H, CHNH\textsuperscript{+}}), 4.29 (\text{ddd, } J = 6.1, 8.8, 10.9 \text{ Hz, 1 H, OCHH}), 2.57 (\text{dddd, } J = 1.2, 6.1, 8.9, 12.3 \text{ Hz, 1 H, OCH\textsubscript{2}CHH}), 2.26 (\text{ddtd, } J = 9.0, 11.2, 12.2 \text{ Hz, 1 H, OCH\textsubscript{2}CHH})

\textbf{\textsuperscript{13}C NMR} (101 MHz, DMSO-d\textsubscript{6}) \(\delta/ \text{ppm} = 173.3 (\text{C=O}), 66.2 (\text{OCH\textsubscript{2}}), 47.8 (\text{CHNH\textsuperscript{+}}), 27.0 (\text{OCH\textsubscript{2}CH\textsubscript{2}})

\(\left[\alpha\right]_{D}^{20}/ \text{cm}^{-1}\text{g}^{-1} = -30.0, \text{lit.} = -25.0 (c/\text{g}(100 \text{ mL})^{-1} = 0.0200, \text{DMSO})

The data are consistent with the literature.\textsuperscript{165}

\section*{9.14 (S)-2-Bromo-N-(2-oxotetrahydrofuran-3-yl)acetamide 54}

\begin{center}
\includegraphics[width=0.2\textwidth]{54.png}
\end{center}

(S)-Aminodihydrofuran-2(3\textit{H})-one hydrobromide 52 (100 mg, 0.549 mmol, 1.08 eq.) and NaHCO\textsubscript{3} (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH\textsubscript{2}Cl\textsubscript{2} (2 mL) and water (2 mL). Bromoacetyl bromide 53 (44.0 \(\mu\text{L}, 102 \text{ mg, 0.505 mmol, 1.00 eq.}) was then added dropwise. The reaction mixture was stirred for 24 h, after which the CH\textsubscript{2}Cl\textsubscript{2} was removed under vacuum. The aqueous phase was extracted with EtOAc (4\times10 \text{ mL}). The combined organic layers were dried with MgSO\textsubscript{4} and the solvent was removed under reduced pressure. 54 was obtained as white, needle-like crystals (88.0 mg, 0.396 mmol, 74\%).

\textbf{mp} \textit{T}/\degree\textup{C} = 132 (EtOAc)
IR (neat) $\nu_{\text{max}} / \text{cm}^{-1} = 3256$ (N-H), 3067 (C-H), 1763 (lactone C=O), 1658 (amide C=O), 1553 (N-H bend)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 6.94 (br s, 1 H, NH), 4.57 (ddd, $J = 11.7, 8.6, 5.9$ Hz, 1 H, CH$_2$NH), 4.51 (td, $J = 9.2, 1.0$ Hz, 1 H, OCH$_2$H), 4.32 (ddd, $J = 11.3, 9.4, 5.9$ Hz, 1 H, OCH$_2$H), 4.30 (d, $J = 12.6, 8.6, 5.9, 1.3$ Hz, 1 H, OCH$_2$CHH), 2.87 (dddd, $J = 12.6, 8.6, 5.9, 1.3$ Hz, 1 H, OCH$_2$CHH), 2.22 (ddt, $J = 12.6, 11.5, 11.5, 8.9$ Hz, 1 H, OCH$_2$CHH)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 174.6 (OC=O), 166.4 (C($=O$)NH), 66.1 (OC$_2$H$_2$), 49.8 (CH$_2$NHC=O), 29.9 (OCH$_2$CH$_2$), 28.2 (O=CCH$_2$Br)

HRMS The compound does not ionise.

$[^{10}]\alpha_D^20$ / °10$^-1$cm$^2$g$^-1$ = 27.0, lit. = 20.5 ($c$ / g(100 mL)$^-1$ = 0.00740, CHCl$_3$)

The data are consistent with the literature.

9.15 (S)-2-Azido-N-(2-oxotetrahydrofuran-3-yl)acetamide 55

$$
\begin{align*}
\text{(3S)-2-Oxotetrahydrofuran-3-aminium bromide 52} & \quad (100 \text{ mg}, 0.552 \text{ mmol}, 1.08 \text{ eq.}), \text{NaN$_3$} (85.7 \text{ mg}, 1.32 \text{ mmol}, 2.61 \text{ eq.}) \text{ and NaHCO$_3$} (84.9 \text{ mg}, 1.01 \text{ mmol}, 2.00 \text{ eq.}) \text{ were dissolved in CH$_2$Cl$_2$ (2 mL) and water (2 mL). Bromoacetyl bromide 53 (44.0 \mu$L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the CH$_2$Cl$_2$ was removed under vacuum. The aqueous phase was extracted with EtOAc (4×10 mL). The combined organic layers were dried with MgSO$_4$ and the solvent was removed under reduced pressure. 55 was obtained as white, needle-like crystals (38.4 mg, 0.209 mmol, 41%).} \\
\text{mp $T$ / °C = 87 (EtOAc)}
\end{align*}
$$

IR (neat) $\nu_{\text{max}} / \text{cm}^{-1} = 3284$ (N-H), 2923 (C-H), 2853 (C-H), 2130 (N$_3$), 1783 (lactone C=O), 1661 (amide C=O), 1537 (N-H bend)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 7.05 (br d, $J = 6.5$ Hz, 1 H, NH), 4.64 (ddd, $J = 11.6, 8.7, 6.8$ Hz, 1 H, CH$_2$NH), 4.48 (td, $J = 9.1, 1.3$ Hz, 1 H, OCH$_2$H), 4.30 (ddd, $J = 11.2, 9.2, 6.0$ Hz, 1 H, OCH$_2$H), 4.04 (s, 2 H, CH$_2$N$_3$), 2.76 (dddd, $J = 12.5, 8.8, 6.0, 1.4$ Hz, 1 H, OCH$_2$CHH), 2.25 (ddt, $J = 12.5, 11.4, 11.4, 8.9$ Hz, 1 H, OCH$_2$CHH)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 174.9 (OC=O), 167.5 (C=ONH), 66.0 (OCH$_2$H), 52.2 (O=CCH$_2$N$_3$), 48.9 (CH$_2$NHC=O), 29.7 (OCH$_2$CH$_2$)

HRMS The compound does not ionise.

$[^{10}]\alpha_D^20$ / °10$^-1$cm$^2$g$^-1$ = -32.6, lit. = -24.4 ($c$ / g(100 mL)$^-1$ = 0.0430, DMSO)
The data are consistent with the literature.\textsuperscript{165}

9.16 \((S)-4\text{-Bromo-}N\text{-}(2\text{-oxotetrahydrofuran-3-yl})\text{butanamide 57}\)

\[(S)-3\text{-Aminodihydrofuran-2(3H)-one hydrobromide 52 (200 mg, 1.10 mmol, 1.00 eq.) and NaHCO}_3 (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in CH}_2Cl_2 (2 mL) and water (2 mL). Bromobutyryl chloride 56 (140 \mu\text{L}, 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the CH}_2Cl_2 was removed under vacuum. The aqueous phase was extracted with EtOAc (7×5 mL) and the combined organic layers were dried with MgSO}_4. The solvent was removed under vacuum to give white crystals which were recrystallised from EtOAc. 57 was obtained as white, needle-like crystals (219 mg, 0.878 mmol, 80%).\]

mp \(T / \degree C = 105\) (EtOAc)

\textbf{IR (neat) \(\nu_{max} / \text{cm}^{-1} = 3308\) (N-H), 3074 (C-H), 2949 (C-H), 1774 (lactone C=O), 1644 (amide C=O), 1541 (N-H bend)}

\textbf{\(^1\text{H NMR} (400 MHz, CDCl}_3) \delta / \text{ppm = 6.31 (br d, } J = 5.5 \text{ Hz, 1 H, NH}, 4.59 (\text{ddd, } J = 6.2, 8.7, 11.5 \text{ Hz, 1 H, CH}_3\text{NH}), 4.48 (\text{dt, } J = 1.2, 8.9 \text{ Hz, 1 H, OCH}_2\text{CH}), 4.30 (\text{ddd, } J = 5.8, 9.3, 11.3 \text{ Hz, 1 H, OCH}_2\text{CH}), 3.49 (t, } J = 6.3 \text{ Hz, 2 H, CH}_3\text{Br}), 2.82 (\text{ddddd, } J = 1.3, 5.9, 8.7, 12.5 \text{ Hz, 1 H, OCH}_2\text{CH}), 2.47 (t, } J = 7.3 \text{ Hz, 2 H, C}(=\text{O})\text{CH}_2\text{), 2.26 - 2.15 (m, 3 H, OCH}_2\text{CH}_2\text{ and CH}_2\text{CH}_2\text{Br})\]

\textbf{\(^{13}\text{C NMR} (101 MHz, CDCl}_3) \delta / \text{ppm = 175.4 (OC}=\text{O), 172.3 (C}(=\text{O})\text{NH), 66.1 (OCH}_2\text{), 49.3 (CHNH}=\text{O), 33.9 (C}(=\text{O})\text{CH}_2\text{), 33.1 (CH}_2\text{Br), 30.3 (OCH}_2\text{CH}_2\text{), 27.9 (C}(=\text{O})\text{CH}_2\text{CH}_2\text{)}\]

\textbf{HRMS} The compound does not ionise.

\([\alpha]_{D}^{26.6} / \degree 10^{-1} \text{cm}^2\text{g}^{-1} = -78\) (c / g(100 mL)) = 0.0833, MeOH)

The compound has not been reported previously.

9.17 \((S)-6\text{-Bromo-}N\text{-}(2\text{-oxotetrahydrofuran-3-yl})\text{hexanamide 60}\)

\[(S)-3\text{-Aminodihydrofuran-2(3H)-one hydrobromide 52 (100 mg, 0.549 mmol, 1.00 eq.) and NaHCO}_3 (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in CH}_2Cl_2 (2 mL) and water (2 mL) at r.t.. Bromohexanoyl chloride 59 (93.0 \mu\text{L}, 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the CH}_2Cl_2 was removed under vacuum. The mixture was then filtered, washed with water (10 mL) and dried under high vacuum. 60 was obtained as white, needle-like crystals (101 mg, 0.362 mmol, 66%).\]
mp \( T / ^\circ C = 106 \) (CH\(_2\)Cl\(_2\), water)

**IR** (neat) \( \nu_{\text{max}} / \text{cm}^{-1} = 3300 \) (N-H), 3068 (C-H), 2937 (C-H), 2857 (C-H), 1785 (lactone C=O), 1639 (amide C=O), 1540 (N-H bend)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} = 6.09 \) (br d, \( J = 5.7 \) Hz, 1 H, NH), 4.57 (ddd, \( J = 5.9, 8.6, 11.6 \) Hz, 1 H, CH\(_{\text{NNH}}\)), 4.45 (dt, \( J = 1.3, 9.1 \) Hz, 1 H, OCH\(_{\text{H}}\)), 4.31 (ddd, \( J = 5.9, 9.3, 11.3 \) Hz, 1 H, OCH\(_{\text{H}}\)), 3.43 (t, \( J = 6.7 \) Hz, 2 H, CH\(_2\)Br), 2.88 (dddd, \( J = 1.3, 5.9, 8.6, 12.6 \) Hz, 1 H, OCH\(_2\)CH\(_{\text{H}}\)), 1.71 (quin, \( J = 7.6 \) Hz, 2 H, C(=O)CH\(_2\)CH\(_2\)H)

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} = 175.5 \) (OC\(_{\text{=O}}\)), 173.3 (C(=O)NH), 66.1 (OCH\(_2\)H), 49.3 (C\(_{\text{HNHC=O}}\)), 35.8 (CH\(_2\)Br), 33.5 (C(=O)CH\(_2\)H), 32.3 (CH\(_2\)CH\(_2\)Br), 30.5 (OCH\(_2\)CH\(_2\)H), 27.6 (C(=O)CH\(_2\)CH\(_2\)H), 24.4 (C(=O)CH\(_2\)CH\(_2\)CH\(_2\)H)

HRMS (ESI\(^+\)) \( m/z / \text{Da} = 278.0381, \text{[M+H]}^+, \text{[C}_{10}\text{H}_{17}\text{BrNO}_{3}]^+ \) requires 278.0386

\([\alpha]_{26}^{20} \text{°10}^{-1}\text{cm}^2\text{g}^{-1} = -16 (c / g(100 \text{ mL})^{-1} = 0.208, \text{MeOH})

The compound has not been reported previously.

**9.18 (S)-6-Azido-N-(2-oxotetrahydrofuran-3-yl)hexanamide 61**

(S)-6-Bromo-N-(2-oxotetrahydrofuran-3-yl)hexanamide 60 (80 mg, 0.320 mmol, 1.00 eq.) and NaN\(_3\) (26.3 mg, 0.405 mmol, 1.27 eq.) were heated in DMF (0.5 mL) for 5 h at 100 °C. The reaction mixture was then partitioned between CH\(_2\)Cl\(_2\) (5 mL) and water (5 mL). The aqueous phase was extracted twice more with CH\(_2\)Cl\(_2\) (2×5 mL) and the organic layers were combined and dried over MgSO\(_4\). The solvent was removed by rotary evaporation followed by high vacuum. 61 was obtained as white, needle-like crystals (42.7 mg, 0.178 mmol, 56%).

mp \( T / ^\circ C = 90 \) (CH\(_2\)Cl\(_2\))

**IR** (neat) \( \nu_{\text{max}} / \text{cm}^{-1} = 3314 \) (N-H), 2932 (C-H), 2863 (C-H), 1775 (lactone C=O), 1643 (amide C=O), 1548 (N-H bend)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} = 5.96 \) (d, \( J = 4.2 \) Hz, 1 H, NH), 4.54 (ddd, \( J = 11.7, 8.6, 5.7 \) Hz, 1 H, CH\(_{\text{NNH}}\)), 4.49 (td, \( J = 9.1, 1.0 \) Hz, 1 H, OCH\(_{\text{H}}\)), 4.49 (ddd, \( J = 11.3, 9.4, 5.8 \) Hz, 1 H, OCH\(_{\text{H}}\)), 3.29 (t, \( J = 6.9 \) Hz, 2 H, CH\(_2\)N\(_3\)), 2.88 (dddd, \( J = 12.5, 8.6, 5.8, 1.1 \) Hz, 1 H, OCH\(_2\)CH\(_{\text{H}}\)), 2.88 (t, \( J = 7.5 \) Hz, 1 H, C(=O)(CH\(_{\text{HH}}\)), 2.28 (t, \( J = 7.4 \) Hz, 1 H, C(=O)(CH\(_{\text{HH}}\)), 2.14 (ddd, \( J = 12.3, 11.5, 11.5, 8.8 \) Hz, 1 H, OCH\(_2\)CH\(_{\text{H}}\)), 1.70 (quin, \( J = 7.6 \) Hz, 2 H, CH\(_2\)CH\(_2\)N\(_3\)), 1.63 (quin, \( J = 7.2 \) Hz, 2 H, C(=O)CH\(_2\)CH\(_2\)H), 1.38 - 1.49 (m, 2 H, C(=O)CH\(_2\)CH\(_2\)CH\(_2\)H)
$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 175.4 (OC=O), 172.2 (C(=O)NH), 66.1 (OCH$_2$), 51.2 (CH$_2$N$_3$), 49.4 (CHNHC=O), 35.9 (C(=O)CH$_2$), 30.7 (OCH$_2$CH$_2$), 28.6 (CH$_2$CH$_2$N$_3$), 26.3 (C(=O)CH$_2$CH$_2$), 24.8 (C(=O)CH$_2$CH$_2$CH$_2$)

HRMS (ESI$^+$) $m/z$ / Da = 241.1289, [M+H]$^+$, [C$_{10}$H$_{17}$N$_4$O$_3$]$^+$ requires 241.1295

$[\alpha]_{D}^{20.6} / \circ 10^{-1}$ cm$^2$ g$^{-1}$ = -16 (c / g(100 mL)$^{-1}$ = 0.208, MeOH)

The compound has not been reported previously.

9.19 Hex-5-ynal 63

Pyridinium chlorochromate (14.6 g, 68.1 mmol, 1.50 eq) and CH$_2$Cl$_2$ (500 mL) were stirred at r.t. under argon. 5-Hexyn-1-ol 62 (5.00 mL, 45.4 mmol, 1 eq.) was added and the reaction mixture was stirred for 5 h followed by addition of Et$_2$O (125 mL) and silica gel (62.5 g). The suspension was stirred for 1 h then filtered through a pad of silica (100 g) and washed with Et$_2$O. The solvent was removed by rotary evaporation. 63 was obtained as a pale yellow-green oil (4.72 g, 49.1 mmol, 72%).

IR (neat) $\nu_{max}$ / cm$^{-1}$ = 3293 (alkyne C-H), 2943 (alkane C-H), 2831 (aldehyde C-H), 2729 (aldehyde C-H), 1720 (aldehyde C=O)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 9.80 (s, 1 H, C(=O)H), 2.60 (t, $J = 7.1$ Hz, 2 H, CH$_2$C(=O)H), 2.26 (dt, $J = 2.6$, 6.8 Hz, 2 H, HC≡CCH$_2$), 1.98 (t, $J = 2.7$ Hz, 1 H, HC≡C), 1.85 (quin, $J = 7.0$ Hz, 2 H, HC≡CCH$_2$CH$_2$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 201.6 (C(=O)), 83.1 (HC≡C), 69.3 (HC≡C), 42.4 (CH$_2$C(=O)), 20.7 (CH$_2$CH$_2$C(=O)), 17.6 (HC≡CCH$_2$)

Spectroscopic data are consistent with the literature.$^{176}$

9.20 tert-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 65

Hex-5-ynal 63 (0.407 g, 4.24 mmol, 1.00 eq.) and tert-butyl piperazine-1-carboxylate 64 (0.791 g, 4.24 mmol, 1.00 eq.) were stirred under a N$_2$ atmosphere in 1,2-dichloroethane (20 mL) for 2.5 h followed by addition of sodium triacetoxylborohydride (6.25 g, 29.5 mmol, 7 eq.) in four portions over 4 d. The mixture was stirred for a further day then NaHCO$_3$ (sat., aq., 120 mL) was added and the product extracted with EtOAc (2×100 mL). The solvent was dried over MgSO$_4$ and removed by rotary evaporation. 65 was obtained as a colourless liquid (1.12 g, 4.21 mmol, 99%).
TLC $R_f$ (10% MeOH/CH$_2$Cl$_2$) = 0.55

IR (neat) $\nu_{max}$/ cm$^{-1}$ = 3304 (alkyne C-H), 2940 (alkane C-H), 2865 (C-H), 2810 (C-H), 1691 (carbamate C=O)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ ppm = 3.44 (t, $J = 5.2$ Hz, 4 H, CH$_2$CH$_2$N(CH$_2$NCH$_2$)CH$_2$), 2.39 (t, $J = 5.1$ Hz, 4 H, CH$_2$CH$_2$N(CH$_2$NCH$_2$)CH$_2$), 2.37 (t, $J = 7.3$ Hz, 2 H, CH$_2$CH$_2$N), 2.23 (dt, $J = 2.7$, 6.8 Hz, 2 H, HC≡CCH$_2$), 1.96 (t, $J = 2.7$ Hz, 1 H, HC≡C), 1.65 - 1.53 (m, 4 H, HC≡CCH$_2$CH$_2$), 1.47 (s, 9 H, CH$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ ppm = 154.7 (NC(=O)O), 84.2 (HC≡C), 79.6 (CH$_3$), 68.5 (HC≡C), 60.4 (CH$_2$), 58.0 (CH$_2$CH$_2$N(CH$_2$NCH$_2$)CH$_2$), 53.0 (CH$_2$CH$_2$N(CH$_2$NCH$_2$)CH$_2$), 28.4 (CH$_3$), 26.3 (CH$_2$), 25.7 (HC≡CCH$_2$), 18.3 (HC≡CCH$_2$)

HRMS (ESI$^+$) $m/z$/ Da = 267.2073, [M+H]$^+$, [C$_{15}$H$_{27}$N$_2$O$_2$]$^+$ requires 267.2064

The compound has not been reported previously.

9.21 1-(Hex-5-yn-1-yl)piperazine 66

$t$-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 65 (763 mg, 2.86 mmol) was stirred in TFA (10 mL) at r.t. for 2 h. The TFA was removed under vacuum followed by co-evaporation with CH$_2$Cl$_2$ (2×20 mL). The oil was diluted with water (10 mL) and the pH adjusted to 14 with NaOH (10% aq.). This mixture was extracted with CH$_2$Cl$_2$ (2×20 mL) and the combined organic layers were dried over MgSO$_4$. The solvent was removed under vacuum and purified by column chromatography (SiO$_2$ MeOH/CH$_2$Cl$_2$ 3:7). 66 was obtained as a colourless liquid (476 mg, 2.86 mmol, >99%).

TLC $R_f$ (30% MeOH/CH$_2$Cl$_2$) = 0.20

IR (neat) $\nu_{max}$/ cm$^{-1}$ = 3296 (alkyne C-H), 2941 (alkane C-H), 2811 (alkane C-H), 1637 (N-H bend)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ ppm = 2.88 (t, $J = 4.9$ Hz, 4 H, CH$_2$CH$_2$N), 2.39 (m, 4 H, CH$_2$CH$_2$N), 2.31 (t, $J = 7.1$ Hz, 2 H, HC≡CCH$_2$CH$_2$N), 2.20 (dt, $J = 2.7$, 6.8 Hz, 2 H, HC≡CCH$_2$), 2.05 (br s, 1 H, NH), 1.93 (t, $J = 2.7$ Hz, 1 H, HC≡C), 1.65 - 1.48 (m, 4 H, HC≡CCH$_2$CH$_2$CH$_2$CH$_2$N)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$/ ppm = 84.3 (HC≡C), 68.4 (HC≡C), 58.6 (CH$_2$CH$_2$N), 54.5 (CH$_2$CH$_2$N), 46.0 (CH$_2$CH$_2$N), 26.4 (CH$_2$CH$_2$N), 25.7 (HC≡CCH$_2$), 18.3 (HC≡CCH$_2$)

HRMS (ESI$^+$) $m/z$/ Da = 167.1548, [M+H]$^+$, [C$_{10}$H$_{19}$N$_2$]$^+$ requires 167.1548
The compound has not been reported previously.

9.22 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68

7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 67 (1.27 g, 4.51 mmol, 1 eq.), 1-(hex-5-yn-1-yl)piperazine 66 (1.5 g, 9.02 mmol, 2 eq.) and N-methyl-2-pyrrolidone (10 mL) were stirred in a microwave reactor at 115 °C for 24 h. The reaction mixture was cooled to r.t. and water (80 mL) was added. The mixture was stirred for 3 h and then filtered, and residue was washed with MeOH (50 mL). The resulting solid (0.571 g) was further purified by recrystallisation from EtOAc (50 mL). 68 was obtained as off-white crystals (0.219 g, 0.531 mmol, 12%).

TLC \( R_f = 0.02 \) (10% MeOH/CH\(_2\)Cl\(_2\))

mp \( T / ^\circ C = 220 \) (MeOH, decomposes)

IR (neat) \( \nu_{max} / \text{cm}^{-1} = 3212 \) (alkyne C-H), 2459 (O-H), 1723 (carboxylic acid C=O), 1627 (quinolone C=O)

\(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \( \delta / \text{ppm} = 15.12 \) (br s, 1 H, C(=O)OH), 8.69 (s, 1 H, ortho to C(=O)OH), 7.96 (d, \( J = 13.0 \) Hz, 1 H, ortho to F), 7.61 (d, \( J = 7.6 \) Hz, 1 H, meta to F), 3.82 - 3.92 (m, 3 H, NCH(CH\(_2\))\(_2\) and CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\))\(_2\)CH\(_2\)CH\(_2\)), 3.54 - 3.68 (br m, 2 H, CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\))\(_2\)CH\(_2\)CH\(_2\)), 3.11 - 3.20 (br m, 2 H, CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\))\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)), 2.84 (t, \( J = 2.7 \) Hz, 1 H, CH\(_2\)), 2.24 (td, \( J = 7.0, 2.7 \) Hz, 2 H, HC≡CH\(_2\)), 1.83 (br. quin, \( J = 7.5 \) Hz, 2 H, HC≡CH\(_2\)CH\(_2\)), 1.52 (quin, \( J = 7.4 \) Hz, 2 H, HC≡CH\(_2\)CH\(_2\)), 1.29 - 1.36 (m, 2 H, NCH(CH\(_2\)))

\(^{13}\)C NMR (126 MHz, DMSO-d\(_6\)) \( \delta / \text{ppm} = 176.4 \) (C(=O)CC(=O)OH), 165.8 (C(=O)OH), 152.8 (d, \( J = 248.5 \) Hz, ipso to F), 148.2 (CHCC(=O)OH), 143.7 (d, \( J = 11.1 \) Hz, para to C(=O)), 139.1 (para to F), 119.4 (d, \( J = 6.9 \) Hz, ipso to C(=O)), 111.2 (d, \( J = 22.5 \) Hz, ortho to F and ortho to C(=O)), 106.9 (meta to F and meta to C(=O)), 106.9 (C(=O)CC(=O)OH), 83.9 (HC≡C), 71.8 (HC≡C), 55.0 (CH\(_2\)CH\(_2\)CH\(_2\)N), 50.5 (CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\))\(_2\)), 46.3 (CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\))\(_2\)CH\(_2\)CH\(_2\)), 36.0 (NCH(CH\(_2\))\(_2\)), 25.2 (HC≡CCH\(_2\)), 22.3 (HC≡CCH\(_2\)CH\(_2\)), 17.4 (HC≡CCH\(_2\)), 7.6 (NCH(CH\(_2\))\(_2\))

\(^{19}\)F NMR (376.45 MHz, MeOD) \( \delta / \text{ppm} = -121.8 \) (s, ciprofloxacin F)

HRMS (ESI\(^+\)) \( m/z / \text{Da} = 412.2036, [M+H]\(^+\), [C\(_{23}\)H\(_{27}\)N\(_3\)O\(_3\)F]\(^+\) requires 412.2030

The compound has not been reported previously.
9.23 4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol 69

Hydrobromic acid (48% w/w, aq., 50 mL) was heated to 100 °C. Trimethoprim 25 (5.00 g, 17.2 mmol) was added, and the suspension was stirred for 40 min under Ar. The mixture was removed from the heat, and NaOH (50% w/w, aq., 15 mL) was added dropwise. The reaction mixture was then cooled slowly to 0 °C, and the resulting crystals were filtered out and washed with cold water. The crystals were then dissolved in hot water (80 mL), neutralized with NH₄OH (sat., aq.) and cooled slowly to 0 °C. The resulting crystals were filtered out, washed with cold water and dried under vacuum. 69 was obtained as pale pink prisms (2.06 g, 7.46 mmol, 43%).

**TLC** $R_f = 0.04$ (5% MeOH/CHCl₃)

**mp** $T / ^°C = 238$ (water, decomposes)

**IR** (neat) $\nu_{max} / cm^{-1} = 3314$ (N-H), 3137 (N-H), 3045 (C-H), 3001 (C-H), 2938 (C-H), 2839 (C-H), 1663 (pyrimidine), 1645 (pyrimidine), 1627 (pyrimidine)

**$^1H$ NMR** (400 MHz, MeOD) $\delta / ppm = 7.21$ (s, 1 H, CHN), 6.54 (s, 2 H, meta to OCH₂), 4.87 (br s, 5 H, OH, NH₂ x 2), 3.82 (s, 6 H, OCH₃), 3.63 (s, 2 H, CH₂C)

**$^{13}C$ NMR** (101 MHz, MeOD) $\delta / ppm = 166.4$ (CH₂CCHNH₂), 162.0 (CHNCNH₂), 156.2 (CHNCNH₂), 149.8 (ipso to OCH₃), 135.9 (ipso to OH), 128.2 (para to OH), 111.7 (CH₂CCHNH₂), 107.5 (meta to OH), 57.0 (OCH₃), 33.9 (CH₂C)

**HRMS** (ESI+) $m/z / Da = 277.1295$, [M+H]⁺ found, $[C_{13}H_{17}N_{4}O_3]^+$ requires 277.1301

The data are consistent with the literature.¹⁶⁷

9.24 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71

4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol 69 (1.00 g, 3.62 mmol, 1 eq.), 6-chloro-1-hexyne 70 (0.524 mL, 0.420 g, 4.34 mmol, 1.2 eq.), Cs₂CO₃ (2.36 g, 7.24 mmol, 2 eq.) and anhydrous DMF (30 mL) were stirred at 70 °C for 7 h. The solvent was removed under reduced pressure, then CH₂Cl₂ (30 mL) was
added and the mixture filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography using a CombiFlash (SiO$_2$, 5% MeOH/CH$_2$Cl$_2$). 71 was obtained as a pale cream amorphous solid (0.327 g, 0.917 mmol, 25%).

**TLC** $R_f = 0.14$ (5% MeOH/CH$_2$Cl$_2$)

**IR** (neat) $\nu_{\text{max}}$ / cm$^{-1} = 3451$ (alkyne C-H), 3313 (N-H), 3137 (N-H), 3114 (N-H), 2944 (C-H), 2839 (C-H), 1635 (pyrimidine)

**$^1$H NMR** (400 MHz, MeOD) $\delta$ / ppm = 7.77 (s, 1 H, CHN), 6.37 (s, 2 H, meta to OCH$_2$), 4.83 (br s, 2 H, CHNCNH$_2$), 4.63 (br s, 2 H, CH$_2$CCN=H$_2$), 3.95 (t, $J = 6.3$ Hz, 2 H, CH$_2$O), 3.79 (s, 6 H, OCH$_3$), 3.65 (s, 2 H, CCH$_2$C), 2.28 (td, $J = 7.1, 2.6$ Hz, 2 H, HC≡CCH$_2$), 1.94 (t, $J = 2.7$ Hz, 1 H, H=CH=C), 1.81 - 1.90 (m, 2 H, CH$_2$CH$_2$O), 1.71 - 1.80 (m, 2 H, CH$_2$CH$_2$CH$_2$O)

**$^{13}$C NMR** (101 MHz, MeOD) $\delta$ / ppm = 162.7 (CH$_2$CCN=H$_2$), 162.0 (CHNCNH$_2$), 156.4 (CHNCNH$_2$), 153.8 (ipso to OCH$_3$), 136.0 (ipso to OCH$_2$), 133.6 (para to OCH$_2$), 106.5 (CH$_2$CCN=H$_2$), 105.0 (meta to OCH$_2$), 84.5 (HC≡C), 72.6 (CH$_2$O), 68.3 (HC≡C), 56.1 (OCH$_3$), 34.7 (CCH$_2$C), 29.1 (CH$_2$CH$_2$O), 24.9 (CH$_2$CH$_2$CH$_2$O), 18.0 (HC≡CCH$_2$)

**HRMS** (ESI$^+$) $m/z$ / Da = 357.1920, [M+H]$^+$ found, [C$_{19}$H$_{25}$N$_4$O$_3$]$^+$ requires 357.1927

The compound has not been reported previously.

### 9.25 Optimised general procedure for the click reaction

Azide (1 eq.) and alkyne (1 eq.) were dissolved in 50% t-BuOH/water in a round-bottomed flask with a stirrer bar, closed with a new septum. The mixture was degassed by bubbling through N$_2$. The mixture was placed under positive pressure of Ar using a balloon. Equimolar amounts of CuSO$_4$·5H$_2$O and THPTA 74 were dissolved in water to make a 50 mM solution and similarly degassed. Sodium ascorbate was dissolved in water to make a 100 mM solution and similarly degassed. The Cu/THPTA solution (0.05 eq.) was added to the reaction mixture, followed by the sodium ascorbate solution (0.1 eq.). The mixture was stirred for 2 h and monitored using LCMS. HL derivative conjugates were dry-loaded onto SiO$_2$ and purified by column chromatography (SiO$_2$, 0-20% MeOH/CH$_2$Cl$_2$). Other conjugates were purified by preparative HPLC (5-95% acetonitrile/water over 20 min).

### 9.26 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 72
50% water/t-BuOH (2 mL) was degassed by bubbling N₂ through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (20.6 mg, 50.0 µmol, 1 eq.) and (S)-2-azido-N-(2-oxotetrahydrofuran-3-yl)acetamide 55 (9.2 mg, 50.0 µmol, 1 eq.). A similarly degassed solution of CuSO₄·5H₂O (624 µg, 2.5 µmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (991 µg, 5 µmol, 0.1 eq., 100 mM) in 50% water/t-BuOH (50 µl) was then added. The mixture was stirred at r.t. under argon for 3 h. On observation that the reaction had stalled, the reaction was degassed again, and a further portion of catalyst solution (50 µl) was added. After a further 3 h the reaction mixture was dry-loaded onto SiO₂ and purified by column chromatography using a Combiflash (SiO₂, 0-20% MeOH/CH₂Cl₂ over 15 min). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 72 was obtained as a white amorphous solid (8.8 mg, 14.8 µmol, 30%).

IR (neat) νmax / cm⁻¹ = 3266 (N-H), 2949 (C-H), 2827 (C-H), 1778 (lactone C=O), 1725 (carboxylic acid C=O), 1665 (amide C=O), 1626 (quinolone C=O)
9.27  (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77

\[
\text{IR (neat) } \nu_{\text{max}} / \text{cm}^{-1} = 3287 \text{ (N-H), 2950 (C-H), 2821 (C-H), 2778 (C-H), 1778 (lactone C=O), 1726 (carboxylic acid C=O)}
\]

\[
\begin{align*}
\text{IR} & \quad \text{HRMS (ESI)} +/ \text{Da} = 624.2928, [M+H]^+ \text{ found, } [C_{31}H_{39}FN_7O_6]^+ \text{ requires 624.2946} \\
\text{[α]D}^{20} & \quad \text{°10}^{-1} \text{cm}^2 \text{g}^{-1} = -10.6 \text{ (c / g(100 mL)}^{-1} = 0.094 \text{, MeOH)}
\end{align*}
\]
The compound has not been reported previously.

9.28 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 78

50% water/t-BuOH (2 mL) was degassed by bubbling N₂ through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (20.6 mg, 50.0 μmol, 1 eq.) and (S)-6-azido-N-(2-oxotetrahydrofuran-3-yl)hexanamide 61 (12.0 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of CuSO₄·5H₂O (624 µg, 2.5 µmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (991 µg, 5 µmol, 0.1 eq., 100 mM) in 50% water/t-BuOH (50 µl) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO₂ and evaporated under reduced pressure. 78 was obtained as a white amorphous solid (12.4 mg, 19.0 μmol, 38%).

**TLC** *Rf* = 0.30 (30% MeOH/CH₂Cl₂)

**IR** (neat) *ν*<sub>max</sub> / cm⁻¹ = 3302 (N-H), 2940 (C-H), 2858 (C-H), 1785 (lactone C=O), 1729 (carboxylic acid C=O), 1658 (amide C=O), 1626 (quinolone C=O)

**¹H NMR** (500 MHz, DMSO d₆) δ / ppm = 15.22 (br s, 1 H, C(=O)OH), 8.65 (s, 1 H, ortho to C(=O)OH), 8.32 (d, *J* = 8.0 Hz, 1 H, NH), 7.89 (d, *J* = 13.3 Hz, 1 H, ortho to F), 7.84 (s, 1 H, CH=CCH₂), 7.55 (d, *J* = 7.6 Hz, 1 H, meta to F), 4.51 (ddd, *J* = 10.9, 9.1, 7.9 Hz, 1 H, C(=O)CH₂), 4.33 (td, *J* = 8.8, 1.8 Hz, 1 H, OCHCH₃), 4.28 (t, *J* = 7.1 Hz, 2 H, CH₂NCH=C), 4.19 (ddd, *J* = 10.5, 8.7, 6.6 Hz, 1 H, OCHH), 3.82 (tt, *J* = 7.0, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.32 (br t, *J* = 4.5, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.63 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.57 (br t, *J* = 4.2 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂), 2.33 - 2.41 (m, 3 H, OCH₂CHH and CH=CCH₂CH₂CH₂), 2.06 - 2.16 (m, 3 H, OCH₂CHH and C(=O)CH₂), 1.79 (quin, *J* = 7.4 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂), 1.63 (quin, *J* = 7.5 Hz, 2 H, CH=CCH₂CH₂), 1.45 - 1.56 (m, 4 H, C(=O)CH₂CH₂ and CH=CCH₂CH₂CH₂), 1.29 - 1.34 (m, 2 H, NCH(CHH)₂), 1.19 - 1.25 (m, 2 H, C(=O)CH₂CH₂CH₂), 1.15 - 1.19 (m, 2 H, NCH(CHH)₂)

**¹³C NMR** (126 MHz, DMSO d₆) δ / ppm = 176.4 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 172.1 (NH(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 250.2 Hz, ipso to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCCH₂), 145.2 (d, *J* = 9.6 Hz, ipso to piperazine), 139.2 (para to F), 121.6 (CH=CHCH₂), 118.5 (d, *J* = 8.0 Hz, para to piperazine), 110.9 (d, *J* = 23.5 Hz, ortho to C=O and ortho to F), 106.7 (CC(=O)OH), 106.3 (d, *J* = 2.1 Hz, meta to C=O and meta to F), 65.3 (OCH₂), 57.4 (CH=CCCH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂CH₂)CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.0 (CH₂NCH=C), 47.8 (CHNH), 35.9 (NCH(CH₂)₂), 34.8 (NHC(=O)CH₂), 29.5 (CH₂CH₂NCH=C), 28.3 (CH₂CHNH), 26.9 (CH=C
CH₂CH₂), 25.7 (CH=CCH₂CH₂CH₂), 25.4 (NHC(=O)CH₂CH₂CH₂), 24.9 (CH=CCH₂), 24.5 (NHC(=O)CH₂CH₂H₂), 7.6 (NCH(CH₂)₂)

HRMS (ESI⁺) m/z / Da = 652.3254, [M+H]⁺ found, [C₃₃H₄₃FN₇O₆]⁺ requires 652.3248

[α]D²⁰ / °10⁻¹ cm²g⁻¹ = -8.5 (c / g(100 mL))⁻¹ = 0.106 , MeOH)

The compound has not been reported previously.

9.29 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 80

50% water/t-BuOH (1 mL) was degassed by bubbling N₂ through it. This was then added to a mixture of 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (4.1 mg, 10.0 µmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1H)-one 38 (2.8 mg, 10.0 µmol, 1 eq.). A similarly degassed solution of CuSO₄·5H₂O (125 µg, 0.5 µmol, 0.05 eq. 50 mM), THPTA (218 µg, 0.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (198 µg, 1 µmol, 0.1 eq., 100 mM) in 50% water/t-BuOH (10 µl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then the reaction mixture was evaporated under reduced pressure. The residue was purified by preparative HPLC (50-100% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO₃ (aq., sat., 10 mL) and 10% i-PrOH/CHCl₃ (10 mL). The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 80 was obtained as a white amorphous solid (8.6 mg, 2.7 µmol, 27%).

IR (neat) νmax / cm⁻¹ = 2927 (C-H), 2866 (C-H), 1716 (carboxylic acid C=O), 1631 (ciprofloxacin quinolone C=O and HHQ C=O)

¹H NMR (500 MHz, DMSO d₆) 15.12 (br s, 1 H, C(=O)OH), 11.79 (s, 1 H, NH), 8.75 (s, 1 H, NCH=CCH₂), 8.71 (s, 1 H, ortho to C(=O)OH), 8.40 (d, J = 2.7 Hz, 1 H, ortho to C(=O) and ortho to N), 8.18 (dd, J = 8.9, 2.6 Hz, 1 H, para to C(=O) and ortho to N), 7.99 (d, J = 13.0 Hz, 1 H, ortho to F), 7.75 (d, J = 9.0 Hz, 1 H, meta to C(=O) and meta to N), 7.62 (d, J = 7.8 Hz, 1 H, meta to F), 6.02 (s, 1 H, NHC=CHC(=O)), 3.85 (tt, J = 7.0, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.23 - 3.30 (m, 10 H, CH₂N(CH₂CH₂CH₂CH₂CH₂), 2.82 (t, J = 5.9 Hz, 2 H, NCH=CCH₂), 2.63 (t, J = 7.9 Hz, 2 H, CH₂C=CHC(=O)), 1.76 - 1.81 (m, 4 H, NCH=CCH₂CH₂CH₂CH₂), 1.70 (quin, J = 7.2 Hz, 2 H, CH₂CH₂C=CHC(=O)), 1.15 - 1.38 (m, 12 H, CH₂CH₂CH₂CH₂CH₂, NCH(CHH)₂ and NCH(CHH)₂), 0.87 (t, J = 6.9 Hz, 3 H, CH₃)

¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 176.4 (C(=O)CC(=O)OH), 176.3 (CHC(C(=O))), 165.8 (C(=O)OH), 154.3 (CCHC(C(=O))), 152.9 (d, J = 240.1 Hz, ipso to F), 148.3 (CHC=CC(=O)OH), 147.5 (NCHCCH₂), 143.3 (d, J = 8.5 Hz, ortho to F and ipso to N), 139.6 (ipso to NH), 139.0 (para to F), 132.0 (para to NH), 124.9 (ipso...
to C(=O) and ortho to NH), 123.6 (para to C(=O) and meta to NH), 120.5 (NCH=CCH₂), 120.0 (meta to C(=O) and meta to N), 119.6 (d, J = 9.6 Hz, ipso to C(=O) and para to N), 115.1 (ortho to C(=O) and ortho to N), 111.3 (d, J = 28.8 Hz, ortho to F and ortho to C(=O)), 107.9 (meta to F and meta to C(=O)), 107.2 (CHC(=O)), 106.9 (CC(=O)OH), 55.4 (CH=CCH₂CH₂CH₂N), 50.6 (CH₂CH₂CH₂N(CH₂CH₂)₂), 46.5 (CH₂CH₂CH₂N(CH₂CH₂)₂CH₂CH₂), 46.5 (CH₂CH₂CH₂CH₂N(CH₂CH₂)₂CH₂CH₂), 36.0 (NCH(CH₂)₂), 33.2 (CH₂CNH), 31.2 (CH₃CH₂CH₂), 28.3 - 28.5 (CH₃CH₂CH₂CH₂CH₂CH₂), 25.6 (CH=CCH₂CH₂), 24.4 (CH=CCH₂), 22.7 (CH=CCH₂CH₂CH₂), 22.0 (CH₃CH₂), 13.9 (CH₃), 7.6 (NCH(CH₂)₂)

HRMS (ESI⁺) m/z / Da = 696.3667, [M+H]+ found, [C₃₀H₄₇FN₇O₄]⁺ requires 696.3668

The compound has not been reported previously.

9.30  (S)-4-(4-(4-(2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy) butyl)-1H-1,2,3-triazol-1-yl)-N-(2-oxotetrahydrofuran-3-yl)butanamide 84

50% water/t-BuOH (2 mL) was degassed by bubbling N₂ through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71 (20.6 mg, 50.0 µmol, 1 eq.) and (S)-4-azido-N-(2-oxotetrahydrofuran-3-yl)butanamide 58 (15.9 mg, 75.0 µmol, 1.5 eq.). Similarly degassed solutions of CuSO₄·5H₂O (624 µg, 2.5 µmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (991 µg, 5 µmol, 0.1 eq., 100 mM) in water (50 µL) were then added. An extra portion of 58 (10.6 mg, 50.0 µmol, 1 eq.) was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks, the reaction mixture was extracted with CH₂Cl₂ (6×10 mL) then dry-loaded onto SiO₂ and purified by column chromatography using a Combiblack (SiO₂, 0-20% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 84 was obtained as a pale brown gum (4.8 mg, 8.4 µmol, 17%, purity 77% by NMR, contaminant was 58).

TLC Rᵢ = 0.30 (30% MeOH/CH₂Cl₂)

IR (neat) νmax / cm⁻¹ = 3341 (N-H), 3303 (N-H), 2934 (C-H), 1774 (lactone C=O), 1660 (amide

c\=O)

¹H NMR (500 MHz, DMSO d₆) δ / ppm = 8.43 (d, J = 8.0 Hz, 1 H, NH), 7.80 (s, 1 H, NCH=CCH₂), 7.46 (s, 1 H, CHN=CNH₂), 6.68 (br s, 2 H, CH₂CH₂CNH₂), 6.53 (s, 2 H, meta to CH₂), 6.21 (br s, 2 H, CHN=CNH₂), 4.49 (dt, J = 10.7, 8.6 Hz, 1 H, CHNH), 4.32 (td, J = 8.7, 1.6 Hz, 1 H, CHHOC═O), 4.29 (t, J = 6.8 Hz, 2 H, CH₂N), 4.19 (dd, J = 10.6, 8.7, 6.5 Hz, 1 H, CHHOC═O), 3.79 (t, J = 6.2 Hz, 2 H, CH₂CH₂CH₂O), 3.68 (s, 6 H, CH₃), 3.53 (br s, 2 H, CCH₂C), 2.63 (t, J = 7.5 Hz, 2 H, CH=CCH₂), 2.37 (dd, J = 12.2, 8.9, 6.7, 1.8 Hz, 1 H, CHCHNH), 2.08 - 2.15 (m, 3 H, CHHCHNH and C═O)CH₂), 2.00 (quin, J = 7.2 Hz, 2 H, CH₂CH₂N), 1.72 (quin, J = 7.3 Hz, 2 H, CH=CCH₂CH₂), 1.61 (quin, J = 6.7 Hz, 2 H, CH₂CH₂O)

¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 175.8 (OC═O), 171.9 (NHC═O), 163.1 (CC(NH₂)N), 159.7
(br s, NC(NH$_2$)N), 153.2 (ipso to OCH$_3$), 150.5 (br s, CHNC(NH$_2$)N), 147.3 (NCH=CH=CH$_2$), 135.2 (para to CH$_2$O), 135.0 (ipso to CH$_2$O), 122.1 (CH=CH=CH$_2$), 107.3 (CH$_2$CC(NH$_2$)=N), 106.2 (meta to CH$_2$O), 72.3 (CH$_2$CH$_2$CH$_2$O), 65.7 (OCH$_2$CH$_2$CHNH), 56.2 (OCH$_3$), 48.9 (CH$_2$N), 48.3 (CHNH), 32.9 (CC=CH=CH$_2$), 29.3 (CH$_2$CH$_2$O), 28.4 (OCH$_2$CH$_2$CHNH), 26.0 (CH$_2$CH$_2$N), 25.7 (CH=CH=CH$_2$), 24.9 (CH=CH=CH$_2$)

HRMS (ESI$^+$) $m/z$ / Da = 569.2834, [M+H]$^+$ found, [C$_{27}$H$_{37}$N$_8$O$_6$]$^+$ requires 569.2836

$[^{[\alpha]}_D^{20}] / \circ 10^{-1}$cm$^2$g$^{-1} = -4.6 (c / g(100 mL))$ 

The compound has not been reported previously.

9.31 (S)-6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1H-1,2,3-triazol-1-yl)-N-(2-oxotetrahydrofuran-3-yl)hexanamide 85

50% water/t-$t$-BuOH (2 mL) was degassed by bubbling N$_2$ through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71 (20.6 mg, 50.0 µmol, 1 eq.) and (S)-6-azido-N-(2-oxotetrahydrofuran-3-yl)hexanamide 61 (18.0 mg, 75.0 µmol, 1.5 eq.). Similarly degassed solutions of CuSO$_4$·5H$_2$O (624 µg, 2.5 µmol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (991 µg, 5 µmol, 0.1 eq., 100 mM) in water (50 µl) were then added. An extra portion of 61 (12.0 mg, 50.0 µmol, 1 eq.) was added after 4 d. Extra portions of the catalysts were added after 9 d. After 2 weeks the reaction mixture was extracted with CH$_2$Cl$_2$ (6×10 mL) then dry-loaded onto SiO$_2$ and purified by column chromatography using a CombiFlash (SiO$_2$, 0-20% MeOH/CH$_2$Cl$_2$). The combined pure fractions were dried with MgSO$_4$ and evaporated under reduced pressure. 85 was obtained as a clear gum (8.0 mg, 13.4 µmol, 27%).

**TLC** $R_f = 0.35$ (30% MeOH/CH$_2$Cl$_2$)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 3336$ (N-H), 3209 (N-H), 2941 (C-H), 2869 (C-H), 1775 (lactone C=O), 1657 (amide C=O and pyrimidine)

**$^1$H NMR** (500 MHz, DMSO $d_6$) $\delta$ / ppm = 8.34 (d, $J = 8.0$ Hz, 1 H, NH), 7.83 (s, 1 H, NCH=CH$_2$), 7.50 (s, 1 H, CH=CH$_2$), 6.54 (s, 2 H, meta to CH$_2$), 6.17 (br s, 2 H, CH$_2$CCNH$_2$), 5.77 (br s, 2 H, CH$_2$CNH$_2$), 4.51 (ddd, $J = 11.0$, 9.0, 8.1 Hz, 1 H, CHNH), 4.33 (td, $J = 8.8$, 1.9 Hz, 1 H, CHHO(=O)), 4.27 (t, $J = 7.1$ Hz, 2 H, CH$_2$N), 4.19 (ddd, $J = 10.5$, 8.7, 6.5 Hz, 1 H, CHHOC(=O)), 3.80 (t, $J = 6.3$ Hz, 2 H, CH$_2$CH$_2$CH$_2$O), 3.70 (s, 6 H, CH$_3$), 3.52 (s, 2 H, CCH$_3$C), 2.64 (t, $J = 7.5$ Hz, 2 H, CH=CCH$_3$), 2.36 (ddd, $J = 12.1$, 8.9, 6.7, 1.8 Hz, 1 H, CCHHNH), 2.06 - 2.16 (m, 3 H, CHHCHNH and C(=O)CH$_2$), 1.78 (quim, $J = 7.4$ Hz, 2 H, CH$_3$CH$_2$N), 1.73 (quim, $J = 7.7$ Hz, 2 H, CH=CCH$_2$CH$_2$), 1.63 (quim, $J = 6.8$ Hz, 2 H, CH$_3$CH$_2$O), 1.52 (quim, $J = 7.5$ Hz, 2 H, C(=O)CH$_2$CH$_2$H), 1.17 - 1.27 (m, 2 H, C(=O)CH$_2$CH$_2$H)

**$^{13}$C NMR** (125 MHz, DMSO $d_6$) $\delta$ / ppm = 175.4 (OC=O), 172.0 (NHC=O), 162.2 (CC(NH$_2$)N), 161.8
(NC(NH2)N), 154.8 (CHNC(NH2)N), 152.8 (ipso to OCH3), 146.7 (CH=CCH2CH2), 135.5 (para to CH2O), 134.8 (ipso to CH2O), 121.6 (CH=CCH2CH2), 105.9 (CH2CC(NH2)=N), 105.8 (meta to CH2O), 71.9 (CH2CH2CH2O), 65.2 (OCH2CH2CHNH), 58.0 (OCH3), 49.0 (CH2N), 47.8 (CHNH), 34.8 (C(=O)CH2), 32.9 (CH2CH2), 29.4 (CH2CH2N), 29.1 (CH2CH2CH2O), 28.2 (OCH2CH2CHNH), 25.8 (CH=CCH2CH2), 25.3 (C(=O)CH2CH2CH2), 24.7 (CH=CCH2CH2), 24.4 (C(=O)CH2CH2)

HRMS (ESI+) m/z / Da = 597.3149, [M+H]+ found, [C28H41N8O6]+ requires 597.3144

[α]20D / °10−1 cm2 g−1 = -3.6 (c / g(100 mL)−1 = 0.11 , MeOH)

The compound has not been reported previously.

9.32 6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1H-1,2,3-triazol-1-yl)-2-heptylquinolin-4(1H)-one 87

50% water/t-BuOH (1 mL) was degassed by bubbling N2 through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71 (3.6 mg, 10.0 µmol, 1 eq.) and 6-azido-2-heptylquinolin-4(1H)-one 38 (2.8 mg, 10.0 µmol, 1 eq.). A similarly degassed solution of CuSO4·5H2O (125 µg, 0.5 µmol, 0.05 eq. 50 mM), THPTA (218 µg, 0.5 µmol, 0.05 eq. 50 mM) and sodium ascorbate (198 µg, 1 µmol, 0.1 eq., 100 mM) in water (10 µl) was then added. The mixture was stirred at r.t. under argon for 1.5 h, then evaporated under reduced pressure. The residue was purified by preparative HPLC (5-100% ace-tonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO3 (aq., sat., 10 mL) and 10% i-PrOH/CHCl3 (10 mL). The organic layer was dried with MgSO4 and evaporated under reduced pressure. 87 was obtained as a clear gum (2.6 mg, 4.1 µmol, 41%).

TLC Rf = 0.17 (20% MeOH/CH2Cl2)

IR (neat) νmax / cm−1 = 2928 (C-H), 2856 (C-H), 1664 (pyrimidine), 1645 (pyrimidine and HHQ C=O)

1H NMR (500 MHz, DMSO d6) δ / ppm = 11.80 (s, 1 H, NH), 8.69 (s, 1 H, NCH=CCH2), 8.41 (d, J = 2.7 Hz, 1 H, ortho to C=O), 8.17 (dd, J = 9.0, 2.6 Hz, 1 H, para to C=O), 7.73 (d, J = 9.0 Hz, 1 H, ortho to NH), 7.51 (br s, 4 H, NH2), 7.41 (s, 1 H, CH=N=CNH2), 6.61 (s, 2 H, meta to CH2), 6.02 (d, J = 1.8 Hz, 1 H, C(=O)CH3), 3.86 (t, J = 6.3 Hz, 2 H, CH2O), 3.73 (s, 6 H, OCH3), 3.57 - 3.62 (m, 2 H, CH2CH2), 2.78 (t, J = 7.5 Hz, 2 H, CH=CH2), 2.63 (t, J = 7.3 Hz, 2 H, HNCCH2), 1.85 (quin, J = 7.5 Hz, 2 H, CH=CH2CH2), 1.61 - 1.78 (m, 4 H, HNCCH2CH2 and CH=CH2CH2CH2), 1.31 - 1.40 (m, 4 H, HNCCH2CH2CH2CH2), 0.86 (t, J = 7.2 Hz, 3 H, CH3CH2)

13C NMR (125 MHz, DMSO d6) δ / ppm = 176.4 (C=O), 164.1 (CC(NH2)N), 154.3 (HNC), 154.2 (NC(NH2)N), 153.1 (ipso to OCH3), 148.3 (CH=CH2CH2), 140.2 (CHNC(NH2)N), 139.6 (ipso to NH), 135.4 (ipso to CH2O),
1.32.8 (param to CH$_2$O), 132.1 (param to NH), 124.9 (ips to C=O), 123.7 (para to C=O), 120.3 (CH=CCH$_2$CH$_2$), 120.0 (meta to C=O and ortho to NH), 115.1 (ortho to C=O and meta to NH), 109.0 (CH$_3$C(NH$_2$)=N), 108.0 (C(=O)(CH), 106.3 (meta to CH$_2$O), 72.0 (CH$_2$CH$_2$CH$_2$O), 56.0 (OCH$_3$), 33.3 (HNCCH$_2$), 32.1 (CH$_2$C), 31.2 (CH$_2$CH$_2$CH$_2$), 29.1 (CH$_2$CH$_2$O), 28.3 - 28.6 (CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 25.3 (CH$_2$CH$_2$CH$_2$O), 24.7 (CH=CH$_2$), 22.1 (CH$_3$CH$_2$), 14.0 (CH$_3$CH$_2$)

HRMS (ESI$^+$) $m/z$ / Da = 641.3557, [M+H]$^+$ found, [C$_{35}$H$_{45}$N$_8$O$_4$]$^+$ 641.3558

The compound has not been reported previously.

9.33 2-(6-(4-(4-(2,4-Diaminopyrimidin-5-yl)methyl)-3,6-dimethoxyphenoxyl)-6-azidohexyl)-3-hydroxyquinolin-4(1H)-one 89

50% water/t-BuOH (1 mL) was degassed by bubbling N$_2$ through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yl)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71 (14.2 mg, 39.8 µmol, 1 eq.) and 2-(6-azidohexyl)-3-hydroxyquinolin-4(1H)-one 30 (11.4 mg, 39.8 µmol, 1 eq.). A similarly degassed solution of CuSO$_4$·5H$_2$O (1.25 mg, 5 µmol, 0.125 eq. 50 mM), THPTA (2.18 mg, 5 µmol, 0.125 eq. 50 mM) and sodium ascorbate (1.98 mg, 10 µmol, 0.25 eq., 100 mM) in water (100 µl) was then added. The mixture was stirred at r.t. under argon for 3 h, then MeOH (1 mL) was added and the reaction mixture was dry-loaded onto SiO$_2$ and purified by column chromatography (SiO$_2$, 0-20% MeOH/CH$_2$Cl$_2$). The combined pure fractions were dried with MgSO$_4$ and evaporated under reduced pressure. 89 was obtained as a pale brown amorphous solid (4.7 mg, 7.3 µmol, 18%).

TLC $R_f$ = 0.21 (20% MeOH/CH$_2$Cl$_2$)

IR (neat) $\nu_{max}$ / cm$^{-1}$ = 2925 (C-H), 2853 (C-H), 1660 (pyrimidine), 1639 (pyrimidine and PQS C=O)

$^1$H NMR (500 MHz, DMSO d$_6$) $\delta$ / ppm = 11.53 (br s, 1 H, NH), 8.09 (d, $J$ = 8.0 Hz, 1 H, ortho to C=O), 7.83 (s, 1 H, NCH=CCH$_2$), 7.48 - 7.57 (m, 3 H, para to C=O, ortho to NH and CH$_2$N=CNH$_2$), 7.21 (dd, $J$ = 8.0, 6.3, 1.5 Hz, 1 H, para to NH), 6.55 (s, 2 H, meta to CH$_2$), 4.28 (t, $J$ = 7.1 Hz, 2 H, CH$_2$N), 3.80 (t, $J$ = 6.2 Hz, 2 H, CH$_2$O), 3.70 (s, 6 H, CH$_3$O), 3.53 (d, $J$ = 0.3 Hz, 2 H, CCH$_2$C), 2.73 (t, $J$ = 7.5 Hz, 2 H, HNCCH$_2$), 2.64 (t, $J$ = 7.4 Hz, 2 H, CH=CH$_2$), 1.80 (quin, $J$ = 7.4 Hz, 2 H, CH$_3$CH$_2$N), 1.73 (quin, $J$ = 7.5 Hz, 2 H, CH=CH$_2$CH$_2$), 1.66 (quin, $J$ = 7.2 Hz, 2 H, HNCCCH$_2$), 1.62 (quin, $J$ = 6.8 Hz, 2 H, CH$_3$CH$_2$O), 1.33 - 1.40 (m, 2 H, HNCCCH$_2$CH$_2$), 1.27 - 1.32 (m, 2 H, HNCCCH$_2$CH$_2$CH$_2$)

$^{13}$C NMR (125 MHz, DMSO d$_6$) $\delta$ / ppm = 168.9 (C=O), 162.5 (CC(NH$_2$)=N), 162.5 (NC(NH$_2$)=N), 152.9 (CHNC(NH$_2$)=N), 152.8 (ips to OCH$_3$), 146.8 (CH=CH$_2$CH$_2$), 137.7 (COH), 137.3 (para to OH), 135.4 (HNC), 135.1 (para to CH$_2$O), 134.8 (ips to CH$_2$O), 129.9 (para to C=O), 124.4 (ortho to C=O and meta to NH), 122.1 (ips to C=O), 121.5 (para to NH), 121.4 (CH=CH$_2$CH$_2$), 117.7 (meta to C=O and ortho to
NH), 106.2 (CH₂CC(NH₂)₂=N), 105.8 (meta to CH₂O), 71.9 (CH₂CH₂CH₂O), 55.8 (OCH₃), 49.0 (CH₂N), 32.8 (CCH₃C), 29.5 (CH₂CH₂N), 29.0 (CH₂CH₂O), 28.1 (HNCCH₂CH₂CH₂), 27.9 (HNCCH₂), 27.6 (HNCCH₂CH₂), 25.6 (CH₂CH₂CH₂N), 25.4 (CH₂CH₂CH₂CH₂N), 24.6 (CH=CHCH₂CH₂CH₂), 24.0 (CH=CHCH₂CH₂CH₂)

HRMS (ESI⁺) m/z / Da = 643.3365, [M+H]⁺ found, [C₃₄H₄₃N₈O₅]⁺ requires 643.3351

The compound has not been reported previously.

9.34 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 151

Ciprofloxacin 24 (10.0 g, 30 mmol, 1 eq.) and para-toluenesulfonic acid (8.60 mg, 44.5 mmol, 1.5 eq.) were refluxed in methanol (500 mL) for 72 h. The mixture was cooled to room temperature and NaHCO₃ (sat., aq., 100 mL) and water (300 mL) were added. The product was extracted with CH₂Cl₂ (2 × 400 mL). The combined organic fractions were dried over MgSO₄ and evaporated under reduced pressure. 151 was obtained as a white amorphous solid (9.16 g, 26.5 mmol, 83%).

TLC Rf = 0.13 (5% MeOH/CH₂Cl₂)

IR (neat) νmax / cm⁻¹ = 2948 (C-H), 2835 (C-H), 1721 (ester C=O), 1617 (quinolone C=O)

¹H NMR (400 MHz, MeOD) δ / ppm = 8.55 (s, 1 H, ortho to C(=O)OCH₃), 7.71 (d, J = 13.5 Hz, 1 H, ortho to F), 7.41 (d, J = 7.2 Hz, 1 H, meta to F), 3.83 (s, 3 H, CH₃), 3.62 (tt, J = 7.4, 3.5 Hz, 1 H, NCH(CH₂CH₂)), 3.24 - 3.29 (m, 4 H, HN(CH₂CH₂CH₂), 3.02 - 3.10 (m, 4 H, HN(CH₂)CH₂), 1.31 - 1.38 (m, 2 H, NCH(CH₂)), 1.12 - 1.20 (m, 2 H, NCH(CH₂)CH₂)

¹³C NMR (101 MHz, MeOD) δ / ppm = 175.2 (C(=O)CC(=O)OCH₃), 166.8 (C(=O)OCH₃), 154.9 (d, J = 248.0 Hz, ipso to F), 150.1 (C(=CC(=O)OCH₃), 146.6 (d, J = 10.4 Hz, ipso to piperazine), 139.9 (para to F), 123.3 (d, J = 6.9 Hz, para to piperazine), 113.0 (d, J = 23.4 Hz, ortho to C=O and ortho to F), 110.1 (CC(=O)OCH₃), 107.1 (d, J = 3.5 Hz, meta to C=O and meta to F), 52.3 (CH₃), 51.7 (HN(CH₂CH₂CH₂CH₂), 51.6 (HN(CH₂CH₂CH₂CH₂), 46.5 (HN(CH₂CH₂), 36.4 (NCH(CH₂)CH₂), 8.7 (NCH(CH₂)CH₂)

¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -124.8 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 346.1569, [M+H]⁺ found, [C₁₈H₂₁FN₃O₃]⁺ requires 346.1567

The data are consistent with the literature.
9.35 4-Bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide 153

3-Aminodihydrothiophen-2(3H)-one hydrochloride 152 (15.0 g, 97.6 mmol, 1 eq.) and NaHCO₃ (16.4 g, 195 mmol, 2 eq.) were added to CH₂Cl₂ (150 mL) and water (150 mL). 4-Bromobutyryl chloride 56 (11.3 mL, 107 mmol, 1.1 eq.) was added dropwise over 45 min at 0 °C and the mixture was stirred for a further 1 h. The organic layer was separated and the aqueous layer was extracted with a second portion of CH₂Cl₂ (150 mL). The combined organic layers were dried over MgSO₄ and evaporated under reduced pressure. 153 was obtained as a white, amorphous solid (22.7 g, 85.8 mmol, 88%).

TLC Rf = 0.19 (50% EtOAc/PE)

IR (neat) νmax / cm⁻¹ = 3266 (amide N-H), 3063 (amide N-H), 1694 (thiolactone C=O), 1651 (amide C=O)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.08 (d, J = 6.1 Hz, 1 H, NH), 4.54 (dt, J = 12.9, 6.5 Hz, 1 H, CHNH), 3.49 (t, J = 6.4 Hz, 2 H, CH₂Br), 3.37 (ddd, J = 12.2, 11.5, 5.3 Hz, 1 H, SCHH), 3.26 (ddd, J = 11.5, 6.9, 1.3 Hz, 1 H, SCHH), 2.91 (ddd, J = 12.5, 6.7, 5.3, 1.3 Hz, 1 H, SCH₂CHH), 2.45 (t, J = 7.4 Hz, 1 H, C(=O)CHH), 2.45 (t, J = 6.8 Hz, 1 H, C(=O)CH₂), 2.20 (quin, J = 6.7 Hz, 1 H, C(=O)CH₂CH₂), 1.96 (ddd, J = 12.7, 12.5, 12.2, 7.0 Hz, 1 H, SCH₂CHH)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 205.4 (SC(=O)), 172.1 (NHC(=O)), 59.4 (CHNH), 34.1 (C(=O)CH₂), 33.1 (CH₂Br), 31.8 (SCH₂CH₂), 28.0 (C(=O)CH₂CH₂), 27.5 (SCH₂)

LRMS (AP⁺) m/z / Da = 266.1, [M+H]⁺ found, [C₈H₁₂BrNO₂S]⁺ requires 266.0

The compound has been synthesised previously⁶¹,¹⁴⁹ but characterisation was not published.

9.36 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 154

Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 151 (50 mg, 0.145 mmol, 1 eq.), 4-bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide 153 (34.5 mg, 0.145 mmol, 1 eq.) and K₂CO₃ (20 mg, 0.145 mmol, 1 eq.) were stirred in acetonitrile (2 mL) at 50 °C under argon. After 24 h a further portion of 153 (34.5 mg, 0.145 mmol, 1 eq.) was added. After another 24 h a further portion was added (69.0 mg, 0.290 mmol, 2 eq.). After another 24 h the temperature was raised so the mixture was at reflux. After a
final 24 h the precipitate was filtered off and the filtrate was purified by column chromatography (SiO₂, 5-10% MeOH/CH₂Cl₂) followed by preparative HPLC (5-95% acetonitrile/water over 20 min). 154 was obtained as a pale cream amorphous solid (9.4 mg, 0.018 mmol, 12%).

**TLC** \( R_f = 0.47 \) (10% MeOH/CH₂Cl₂)

**IR** (neat) \( \nu_{max} / \text{cm}^{-1} = 2944 \) (C-H), 2832 (C-H), 1722 (ester C=O), 1700 (thiolactone C=O), 1670 (amide C=O), 1617 (quinolone C=O)

**1H NMR** (500 MHz, MeOD) \( \delta / \text{ppm} = 8.53 \) (s, 1 H, ortho to C(=O)OCH₃), 7.68 (d, \( J = 13.4 \) Hz, 1 H, ortho to F), 7.41 (d, \( J = 7.3 \) Hz, 1 H, meta to F), 4.67 (dd, \( J = 12.9, 6.9 \) Hz, 1 H, CHNH), 3.83 (s, 3 H, OCH₃), 3.61 (tt, \( J = 6.9, 4.1 \) Hz, 1 H, NCH₂(CH₂)₂), 3.39 - 3.49 (m, 1 H, SCH₂H), 3.26 - 3.33 (m, 5 H, SCH₂H and CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.93 - 3.03 (m, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂), 2.59 - 2.69 (d, \( J = 12.9, 6.9, 5.4, 1.4 \) Hz, 1 H, SCH₂CH₂H), 2.39 (t, \( J = 7.2 \) Hz, 1 H, C(=O)CH₂CH₂CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.18 (qd, \( J = 12.4, 7.0 \) Hz, 1 H, SCH₂CH₂H), 1.97 (quin, \( J = 7.2 \) Hz, 2 H, C(=O)CH₂CH₂CH₂), 1.32 - 1.37 (m, 2 H, NCH(CH₂)₂), 1.13 - 1.19 (m, 2 H, NCH(CHH₂)₂)

**13C NMR** (126 MHz, MeOD) \( \delta / \text{ppm} = 207.0 \) (SC(=O)), 175.7 (NHC(=O)), 175.1 (C(=O)CC(=O)OCH₃), 166.6 (C(=O)OCH₃), 154.7 (d, \( J = 249.0 \) Hz, ipso to F), 150.2 (s, CH=CC(=O)OCH₃), 145.6 (d, \( J = 10.6 \) Hz, ipso to piperazine), 139.8 (para to F), 123.5 (d, \( J = 6.9 \) Hz, para to piperazine), 113.1 (d, \( J = 23.6 \) Hz, ortho to C=O and ortho to F), 110.0 (C(=O)OCH₃), 107.4 (meta to C=O and meta to F), 60.2 (CHNH), 58.5 (C(=O)CH₂CH₂CH₂), 53.8 (CH₂CH₂CH₂N(CH₂CH₂)CH₂), 52.3 (OCH₃), 50.1 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 50.0 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 36.5 (NCH(CH₂)₂), 34.5 (C(=O)CH₂), 31.7 (SCH₂CH₂), 28.1 (SCH₂), 22.9 (C(=O)CH₂CH₂CH₂), 8.7 (NCH(CH₂)₂)

**19F NMR** (376.45 MHz, MeOD) \( \delta / \text{ppm} = -125.4 \) (s, ciprofloxacin F)

**HRMS** (ESI⁺) \( m/z / \text{Da} = 531.2083, [M+H]⁺ \) found, \([\text{C}_{26}\text{H}_{32}\text{FN}_4\text{O}_5\text{S}]^+ \) requires 531.2077

The compound has been synthesised previously.61,149 Only HRMS characterisation was published, and this agrees with the result above.

### 9.37 4-Azido-\( N \)-(2-oxotetrahydrothiophen-3-yl)butanamide 155

![Chemical structure of 4-Azido-\( N \)-(2-oxotetrahydrothiophen-3-yl)butanamide](image)

4-Bromo-\( N \)-(2-oxotetrahydrothiophen-3-yl)butanamide 153 (6.00 g, 27.0 mmol, 1 eq.) and NaN₃ (3.51 g, 54.1 mmol, 2 eq.) were refluxed in acetonitrile (120 mL) for 1.5 h. The solvent was evaporated under reduced pressure and the residue was partitioned between water (150 mL) and CH₂Cl₂ (150 mL). The aqueous layer was extracted twice more with CH₂Cl₂ (2×150 mL) and the combined organic fractions were dried with MgSO₄ and evaporated under reduced pressure. 155 was obtained as a yellow, sticky solid (4.60 g, 20.1 mmol, 89%).

**TLC** \( R_f = 0.19 \) (50% EtOAc/PE)
IR (neat) νmax / cm⁻¹ = 3286 (N-H), 2964 (azide), 1697 (thiolactone C=O), 1647 (amide C=O)

1H NMR (400 MHz, CDCl₃) δ / ppm = 6.71 (d, J = 7.3 Hz, 1 H, NH), 4.54 (dt, J = 13.0, 7.0 Hz, 1 H, CHNH), 3.30 (t, J = 6.7 Hz, 2 H, CH₂N₃), 3.31 (td, J = 11.7, 5.3 Hz, 1 H, SCHH), 3.19 (ddd, J = 11.3, 7.0, 1.2 Hz, 1 H, SCHH), 2.70 (ddddd, J = 12.4, 6.8, 5.3, 1.2 Hz, 1 H, SCH₂CHH), 2.29 (t, J = 7.5 Hz, 1 H, C(=O)CHH), 2.28 (t, J = 7.1 Hz, 1 H, C(=O)CHH), 1.97 (qd, J = 12.4, 7.0 Hz, 1 H, SCH₂CHH), 1.85 (quin, J = 6.9 Hz, 2 H, C(=O)CH₂CH₂)

13C NMR (101 MHz, CDCl₃) δ / ppm = 205.4 (SC(=O)), 172.3 (NHC(=O)), 59.4 (CHNH), 50.6 (CH₂N₃), 32.8 (C(=O)CH₂), 31.8 (SCH₂CH₂), 27.5 (SCH₂), 24.6 (C(=O)CH₂CH₂)

HRMS (ESI⁺) m/z / Da = 251.0565, [M+Na]⁺ found, [C₈H₁₂N₄NaO₂S]⁺ requires 251.0573

The compound has not been reported previously.

9.38 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 156

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (15 mg, 36.7 µmol, 1 eq.) and 4-azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide 155 (12.5 mg, 55.1 µmol, 1.5 eq.) were dissolved in 1:9:10 water/i-BuOH/DMSO (3 mL), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (182 µl, 18.2 µmol, 0.5 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (367 µl, 36.7 µmol, 1 eq., 100 mM, aq.). The mixture was stirred at r.t. under argon for 4 d. Water (10 mL) and 10% i-PrOH/CHCl₃ (10 mL) were added, the organic layer was separated and the aqueous layer was extracted again with 10% i-PrOH/CHCl₃ (2×10 mL). The combined organic layers were dried with MgSO₄ and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO₃ (aq., sat., 50 mL) and 10% i-PrOH/CHCl₃ (50 mL). The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 156 was obtained as a white amorphous solid (16.5 mg, 25.9 µmol, 71%).

IR (neat) νmax / cm⁻¹ = 2919 (C-H), 1713 (carboxylic acid C=O and thiolactone C=O), 1658 (amide C=O), 1627 (quinolone C=O), 1616 (triazole)

1H NMR (500 MHz, DMSO d₆) δ / ppm = 15.23 (br s, 1 H, C(=O)OH), 8.66 (s, 1 H, ortho to C(=O)OH), 8.23 (d, J = 8.5 Hz, 1 H, NH), 7.90 (d, J = 13.4 Hz, 1 H, ortho to F), 7.84 (s, 1 H, CH=CCH₂), 7.56 (d, J = 7.5 Hz, 1 H, meta to F), 4.59 (ddd, J = 12.7, 8.4, 6.8 Hz, 1 H, CHNH), 4.31 (t, J = 7.0 Hz, 2 H, CH₂NCH=C), 3.80 - 3.86 (6.9, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.34 - 3.37 (m, 1 H, SCHH), 3.32 (br t, J = 4.1 Hz,
4 H, CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 3.27 (dd, J = 11.1, 6.9, 1.4 Hz, 1 H, SCHR), 2.64 (t, J = 7.6 Hz, 2 H, CH=CCH₂), 2.57 (br t, J = 4.7 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)₂), 2.34 - 2.41 (m, 3 H, SCHR and CH=CH₂CH₂CH₂CH₂), 2.12 (t, J = 7.9 Hz, 1 H, C(=O)CH₂), 2.12 (t, J = 7.0 Hz, 1 H, C(=O)CH₂), 2.04 (m, 3 H, SCHR and C(=O)CH₂), 1.64 (quin, J = 7.5 Hz, 2 H, CH=CH₂CH₂), 1.51 (quin, J = 7.5 Hz, 2 H, CH=CH₂CH₂CH₂), 1.28 - 1.34 (m, 2 H, NCH(C₇H₈)₂), 1.15 - 1.20 (m, 2 H, NCH(C₇H₈)₂)

¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 205.6 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.4 (NHCC(=O)), 166.0 (C(=O)OH), 153.1 (d, J = 249.3 Hz, ortho to F), 148.0 (CH=CC(=O)OH), 146.9 (CH=CC₂H₂), 145.3 (d, J = 10.1 Hz, ipso to piperazine), 139.2 (para to F), 121.8 (CH=CCH₂), 118.6 (d, J = 7.7 Hz, para to piperazine), 111.0 (d, J = 23.3 Hz, ortho to C=O and ortho to F), 106.7 (C(=O)OH), 106.4 (d, J = 2.9 Hz, meta to C=O and meta to F), 58.2 (SC(=O)CHNH), 57.4 (CH=CC₂H₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂CH₂)₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 48.6 (CH₂NCH=CH), 35.9 (NCH(CH₂)₂), 31.9 (NHCC(=O)CH₂), 30.1 (CH₂CHNH), 26.9 (CH=CC₂H₂CH₂), 26.8 (SCH₂), 25.9 (NHCC(=O)CH₂CH₂), 25.8 (CH=CCH₂CH₂), 25.0 (CH=CC₂H₂), 7.6 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -124.9 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 640.2739, [M+H]+ found, [C₃₁H₃₉FN₁O₅S]⁺ requires 640.2712

The compound has not been reported previously.

9.39 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(((4-(1-(4-oxo-4-((2-oxotetrahydrothiophene n-3-yl)amino)butyl)-1H-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 157

![Chemical structure](image)

1-Cyclopropyl-6-fluoro-7-(4-(((hex-5-ynyl)oxy)methoxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 221 (203 mg, 0.407 mmol, 1 eq.), 4-azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide 155 (92.8 mg, 0.407 mmol, 1 eq.), Cu(0) (40 mg, 0.190 mmol, 0.5 eq.) and DIPEA (0.356 mL, 2.04 mmol, 5 eq.) were stirred in CH₂Cl₂ (18.6 mL) at r.t. under Ar for 3 h. The mixture was filtered and the filtrate was dry-loaded onto SiO₂ and purified by column chromatography (SiO₂, 5-10% MeOH/CH₂Cl₂). 157 was obtained as pale brown/yellow amorphous solid (14.7 mg, 20.2 μmol, 5%).

**TLC** RF = 0.40 (5% CH₂Cl₂/MeOH)

**IR** νmax / cm⁻¹ = 3055 (C-H), 1716 (carboxylic acid C=O and ester C=O), 1696 (carbamate C=O and thiolactone C=O), 1651 (amide C=O), 1629 (quinolone C=O)

¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 205.6 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.4 (NHCC(=O)), 166.0 (C(=O)OH), 153.1 (d, J = 249.3 Hz, ortho to F), 148.0 (CH=CC(=O)OH), 146.9 (CH=CC₂H₂), 145.3 (d, J = 10.1 Hz, ipso to piperazine), 139.2 (para to F), 121.8 (CH=CCH₂), 118.6 (d, J = 7.7 Hz, para to piperazine), 111.0 (d, J = 23.3 Hz, ortho to C=O and ortho to F), 106.7 (C(=O)OH), 106.4 (d, J = 2.9 Hz, meta to C=O and meta to F), 58.2 (SC(=O)CHNH), 57.4 (CH=CC₂H₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂CH₂)₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 48.6 (CH₂NCH=CH), 35.9 (NCH(CH₂)₂), 31.9 (NHCC(=O)CH₂), 30.1 (CH₂CHNH), 26.9 (CH=CC₂H₂CH₂), 26.8 (SCH₂), 25.9 (NHCC(=O)CH₂CH₂), 25.8 (CH=CCH₂CH₂), 25.0 (CH=CC₂H₂), 7.6 (NCH(CH₂)₂)
7.4 Hz, 1 H, meta to F), 5.74 (s, 1 H, OCH₂O), 4.58 (dd, J = 12.6, 8.1, 7.2 Hz, 1 H, CH(NH)), 4.30 (t, J = 6.9 Hz, 2 H, CH₂CH₂CH₂N), 3.80 (tt, J = 6.9, 3.6 Hz, 1 H, NCH(CH₂)₂), 3.62 (br t, J = 5.2 Hz, 4 H, C(=O)N(CH₂)CH₂), 3.38 (td, J = 11.4, 5.5 Hz, 1 H, S(=H)), 3.34 (br s, 4 H, C(=O)N(CH₂)₂CH₂CH₂), 3.27 (dd, J = 11.0, 6.9, 1.6 Hz, 1 H, SCH₂), 2.64 (t, J = 7.6 Hz, 2 H, CH=CH₂), 2.44 (t, J = 7.5 Hz, 2 H, CH₂C(=O)O), 2.40 (dddd, J = 12.3, 6.8, 5.4, 1.4 Hz, 1 H, SCH₂H), 2.12 (t, J = 7.8 Hz, 1 H, NHC(=O)CHH), 2.12 (t, J = 6.8 Hz, 1 H, NHC(=O)CHH), 1.98 - 2.07 (m, 3 H, SCH₂H and NHC(=O)CH₂CH₂), 1.86 (quin, J = 7.5 Hz, 2 H, CH=CH₂CH₂H), 1.29 - 1.36 (m, 2 H, NCH(CHH)₂), 1.14 - 1.21 (m, 2 H, NCH(CHH)₂).

13C NMR (101 MHz, DMSO d₄) δ / ppm = 205.5 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.8 (C(=O)OCH₂O), 171.3 (NHC(=O)), 165.9 (C(=O)OH), 152.8 (d, J = 249.7 Hz, ipso to F), 152.9 (OC(=O)N), 148.1 (CH=CC(=O)OH), 146.0 (CH=CH₂), 144.9 (d, J = 9.6 Hz, ipso to piperazine), 139.1 (para to F), 122.0 (CH=CH₂), 118.9 (d, J = 7.5 Hz, para to piperazine), 111.0 (d, J = 23.5 Hz, ortho to C=O and para to F), 106.8 (C(=O)OH, and meta to C=O and meta to F), 80.3 (OCH₂O), 58.2 (CHNH), 49.1 (C(=O)N(CH₂)₂CH₂CH₂), 48.6 (C(=O)CH₂CH₂CH₂N), 43.4 (N(CH₂)CH₂), 43.0 (N(CH₂)CH₂), 35.9 (NCH (CH₂)₂), 32.7 (CH=CH₂CH₂CH₂C(=O)), 31.8 (NHC(=O)CH₂), 30.1 (SCH₂CH₂), 26.8 (SCH₂), 25.8 (C(=O)CH₂CH₂CH₂N), 24.2 (CH=CH₂CH₂CH₂C(=O)), 24.0 (CH=CH₂CH₂CH₂C(=O)), 7.6 (NCH(CH₂)₂).

HRMS (ESI⁺) m/z / Da = 728.2502, [M+H]⁺ found, [C₃₃H₃₉FN₂O₉S]⁺ requires 728.2503

The compound has not been reported previously.

9.40 4-Bromo-N-(2-methoxyphenyl)butanamide 159

2-Methoxyaniline 158 (9.12 mL, 10.0 g, 81.2 mmol, 1 eq.) and NaHCO₃ (8.19 g, 97.4 mmol, 1.2 eq.) were dissolved in water (100 mL) and CH₂Cl₂ (100 mL). The mixture was cooled to 0 °C and 4-bromobutyryl chloride 56 (9.40 mL, 15.1 g, 81.2 mmol, 1 eq.) was added dropwise over 15 min. The mixture was stirred at 0 °C for 1.5 h, then the aqueous layer was removed. The organic layer was dried with MgSO₄ and purified by column chromatography (SiO₂, 5-25% EtOAc/P.E.). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 159 was obtained as an initially colourless liquid which slowly turned blue then black if left out on the bench (11.0 g, 40.6 mmol, 50%).

TLC Rf = 0.16 (10% EtOAc/P.E.)

IR (neat) νmax / cm⁻¹ = 3410 (N-H), 3313 (N-H), 2962 (C-H), 2940 (C-H), 2903 (C-H), 1676 (amide C=O)

1H NMR (400 MHz, CDCl₃ d₄) δ / ppm = 8.32 (dd, J = 8.0, 1.7 Hz, 1 H, ortho to NH), 7.85 (br s, 1 H, NH), 7.02 (td, J = 7.9, 1.7 Hz, 1 H, para to NH), 6.93 (td, J = 7.7, 1.4 Hz, 1 H, para to OCH₃), 6.85 (dd, J = 8.1, 1.5 Hz, 1 H, ortho to OCH₃), 3.85 (s, 3 H, CH₃), 3.50 (t, J = 6.4 Hz, 2 H, CH₂Br), 2.56 (t, J = 7.1 Hz, 2 H, C(=O)CH₃), 2.25 (quin, J = 6.7 Hz, 2 H, C(=O)CH₂CH₂)

13C NMR (101 MHz, CDCl₃ d₄) δ / ppm = 169.4 (C(=O)), 147.6 (ipso to OCH₃), 127.2 (ipso to NH), 123.5 (para to NH), 120.7 (para to OCH₃), 119.6 (ortho to NH and meta to OCH₃), 109.8 (ortho to OCH₃ and meta...
The compound has not been reported previously.

9.41 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 160

Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 151 (500 mg, 1.45 mmol, 1 eq.), 4-bromo-N-(2-methoxyphenyl)butanamide 159 (788 mg, 2.90 mmol, 2 eq.), DIPEA (1.28 mL, 950 mg, 7.35 mmol, 5 eq.), NaI (275 mg, 1.83 mmol, 1.3 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at 100 °C for 4 h. The mixture was dry-loaded onto SiO₂ and purified by column chromatography (SiO₂, 4% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 160 was obtained as a bright pink amorphous solid (79.7 mg, 0.149 mmol, 10%).

**TLC** Rf = 0.40 (10% MeOH/CH₂Cl₂)

**IR** (neat) νmax / cm⁻¹ = 2947 (C-H), 2834 (C-H), 1719 (ester C=O), 1685 (amide C=O), 1617 (quinolone C=O)

**¹H NMR** (400 MHz, CDCl₃ d₁) δ / ppm = 8.48 (s, 1 H, ortho to C(=O)OCH₃), 8.36 (d, J = 7.9 Hz, 1 H, ortho to NH), 7.87 - 7.99 (m, 2 H, ortho to F and NH), 7.19 (d, J = 6.5 Hz, 1 H, meta to F), 7.01 (t, J = 7.5 Hz, 1 H, para to NH), 6.93 (t, J = 7.7 Hz, 1 H, para to OCH₃), 6.85 (d, J = 7.9 Hz, 1 H, ortho to OCH₃), 3.88 (s, 3 H, C(=O)OCH₃), 3.85 (s, 3 H, aromatic OCH₃), 3.41 (tt, J = 6.9, 4.0 Hz, 1 H, NCH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N)), 2.67 (br t, J = 5.0 Hz, 4 H, C(=O)CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N))), 2.53 (t, J = 7.0 Hz, 2 H, C(=O)CH₂CH₂CH₂N(CH₂CH₂CH₂N), 2.47 (t, J = 7.1 Hz, 2 H, C(=O)CH₂CH₂CH₂N), 1.97 (quin, J = 6.8 Hz, 2 H, C(=O)CH₂CH₂CH₂N), 1.25 - 1.33 (m, 2 H, NCH₂CH₂), 1.07 - 1.14 (m, 2 H, NCH₂CH₂)

**¹³C NMR** (101 MHz, CDCl₃ d₁) δ / ppm = 172.9 (C(=O)OCH₃), 170.8 (NCH₂CH₂), 166.2 (C(=O)OCH₃), 153.3 (d, J = 248.0 Hz, ipso to F), 148.2 (C(=O)OCH₃), 147.6 (ipso to OCH₃), 144.4 (d, J = 10.4 Hz, ipso to piperazine), 137.9 (para to F), 127.6 (ipso to NH), 123.4 (para to NH), 122.7 (d, J = 7.8 Hz, para to piperazine), 121.0 (para to OCH₃), 119.7 (ortho to NH and meta to OCH₃), 113.0 (d, J = 22.5 Hz, ortho to C=O and ortho to F), 109.8 (ortho to OCH₃ and meta to NH, and C(=O)OCH₃), 104.7 (meta to C=O and meta to F), 57.2 (CH₂CH₂CH₂N), 55.6 (aromatic OCH₃), 52.7 (CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N))), 51.9 (C(=O)OCH₃), 49.8 (CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N))), 49.8 (CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N(CH₂CH₂CH₂N))), 35.5 (CH₂CH₂CH₂N), 34.5 (NCH₂CH₂), 22.3 (CH₂CH₂CH₂N), 8.0 (NCH₂CH₂)
HRMS (ESI⁺) m/z / Da = 537.2523, [M+H]+ found, [C_{29}H_{34}FN_{4}O_{5}]^{+} requires 537.2513

The compound has not been reported previously.

9.42 4-Azido-N-(2-methoxyphenyl)butanamide 161

4-Bromo-N-(2-methoxyphenyl)butanamide 159 (2.05 g, 7.51 mmol, 1 eq.) and NaN₃ (1.17 g, 18.0 mmol, 2.4 eq.) were refluxed in acetonitrile (100 mL) for 2 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto SiO₂ and purified by column chromatography using a Combiflash (SiO₂, 8-14% then held at 14% EtOAc/P.E.). 161 was obtained as an initially colourless liquid which slowly turned blue then black if left out on the bench (0.469 g, 2.00 mmol, 27%).

TLC R_f = 0.20 (25% EtOAc/P.E.)

IR (neat) ν_max / cm⁻¹ = 3420 (N-H), 3330 (N-H), 2095 (azide), 1672 (amide C=O)

¹H NMR (400 MHz, CDCl₃ d₁) δ / ppm = 8.32 (dd, J = 7.9, 1.0 Hz, 1 H, ortho to NH), 7.86 (br s, 1 H, NH), 7.00 (td, J = 7.5, 1.5 Hz, 1 H, para to NH), 6.90 (td, J = 7.7, 1.1 Hz, 1 H, para to OCH₃), 6.83 (dd, J = 8.1, 1.4 Hz, 1 H, ortho to OCH₃), 3.81 (s, 3 H, CH₃), 3.33 (t, J = 6.7 Hz, 2 H, CH₂Br), 2.42 (t, J = 7.2 Hz, 2 H, C(=O)CH₂), 1.94 (quin, J = 6.9 Hz, 2 H, C(=O)CH₂CH₂)

¹³C NMR (101 MHz, CDCl₃ d₁) δ / ppm = 169.5 (C(=O)), 147.6 (ipso to OCH₃), 127.1 (ipso to NH), 123.4 (para to NH), 120.5 (para to OCH₃), 119.5 (ortho to NH and meta to OCH₃), 109.6 (ortho to OCH₃ and meta to NH), 55.2 (CH₃), 50.3 (CH₂N₃), 33.9 (C(=O)CH₂), 24.3 (C(=O)CH₂CH₂)

HRMS (ESI⁺) m/z / Da = 257.1010, [M+H]+ found, [C_{11}H_{14}N_{4}NaO_{2}]^{+} requires 257.1014

The data are consistent with the literature.

9.43 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 162
1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (24.1 mg, 58.6 μmol, 1 eq.) and 4-azido-N-(2-methoxyphenyl)butanamide 161 (13.7 mg, 58.5 μmol, 1 eq.) were dissolved in water (3 mL), t-BuOH (9 mL) and CH₂Cl₂ (9 mL), and the mixture was degassed by bubbling through N₂. A solution of CuSO₄ and THPTA (117 μl, 5.85 μmol, 0.1 eq., 50 mM, aq.) was added, followed by a solution of sodium ascorbate (234 μl, 11.7 μmol, 0.2 eq., 50 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (25 mL), CH₂Cl₂ (25 mL) and MeOH (5 mL) were added and the organic layer was separated off, dry-loaded onto SiO₂ under argon for 16 h. Water (25 mL), CH₂Cl₂ (25 mL) and Na₂CO₃ (234 mg, 2.7 mol, 1 eq., 50 mM, aq.) were dissolved in water (3 mL), and the mixture was degassed by bubbling through argon for 16 h. Water (25 mL), CH₂Cl₂ (25 mL) and MeOH (5 mL) were added and the organic layer was separated off, dry-loaded onto SiO₂ and purified by column chromatography using a Combiflash (SiO₂, 3-23% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 162 was obtained as a clear amorphous solid (14.7 mg, 22.8 μmol, 39%).

**TLC** RF = 0.28 (10% MeOH/CH₂Cl₂)

**IR** (neat) νmax / cm⁻¹ = 2927 (C-H), 2847 (C-H), 1723 (carboxylic acid C=O), 1682 (amide C=O), 1626 (quinolone C=O), 1613 (triazole)

**1H NMR** (400 MHz, CDCl₃) δ / ppm = 15.05 (br s, 1 H, C(=O)OH), 8.76 (s, 1 H, ortho to C(=O)OH), 8.31 (dd, J = 8.0, 1.7 Hz, 1 H, ortho to NH), 8.00 (d, J = 13.0 Hz, 1 H, ortho to F), 7.83 (br s, 1 H, NH), 7.37 (s, 1 H, CH=CHCH₂), 7.35 (d, J = 7.2 Hz, 1 H, meta to F), 7.04 (td, J = 7.7, 1.7 Hz, 1 H, para to NH), 6.95 (td, J = 7.8, 1.5 Hz, 1 H, para to OCH₃), 6.88 (dd, J = 8.1, 1.4 Hz, 1 H, ortho to OCH₃), 4.47 (t, J = 6.7 Hz, 2 H, C(=O)CH₂CH₂CH₂N), 3.88 (s, 3 H, CH₃), 3.54 (tt, J = 6.9, 4.0 Hz, 1 H, NCH(CH₂CH₂N)), 3.35 (br t, J = 4.7 Hz, 4 H, CH=CH₂CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂N), 2.76 (t, J = 7.5 Hz, 2 H, CH=CH₂), 2.66 (t, J = 4.7 Hz, 4 H, CH=CH₂CH₂CH₂CH₂N(CH₂CH₂CH₂N), 2.47 (t, J = 7.3 Hz, 2 H, CH=CH₂CH₂CH₂CH₂N), 2.44 (t, J = 6.8 Hz, 2 H, C(=O)CH₂CH₂CH₂N), 2.32 (quin, J = 6.7 Hz, 2 H, C(=O)CH₂CH₂CH₂N), 1.75 (quin, J = 7.6 Hz, 2 H, CH=CH₂CH₂CH₂CH₂N), 1.61 (quin, J = 7.5 Hz, 2 H, CH=CH₂CH₂CH₂CH₂N), 1.35 - 1.42 (m, 2 H, NCH(CH₂CH₂N)), 1.17 - 1.22 (m, 2 H, NCH(CH₂CH₂N))

**13C NMR** (101 MHz, CDCl₃) δ / ppm = 177.1 (C(=O)CC(=O)OH), 169.5 (NHCC(=O)), 167.0 (C(=O)OH), 153.7 (d, J = 251.4 Hz, ipso to F), 148.1 (CH=CH₂), 147.8 (ipso to OCH₃), 147.3 (C=C(=O)OH), 145.9 (d, J = 10.4 Hz, ipso to pipеразин), 139.1 (para to F), 127.3 (ipso to NH), 123.9 (para to NH), 121.0 (para to OCH₃), 120.9 (CH=CH₂), 119.7 (para to pipеразин, and ortho to NH and meta to OCH₃), 112.4 (d, J = 23.4 Hz, ortho to C=O and ortho to F), 109.9 (ortho to OCH₃ and meta to NH), 108.1 (C(=O)OH), 104.7 (meta to C=O and meta to F), 58.1 (CH=CH₂CH₂CH₂CH₂N), 55.6 (CH₃), 52.8 (CH=CH₂CH₂CH₂CH₂N(CH₂CH₂CH₂), 49.8 (CH=CH₂CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂CH₂), 49.8 (CH=CH₂CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂CH₂), 49.1 (C(=O)CH₂CH₂CH₂N), 35.2 (NCH(CH₂CH₂N)), 33.8 (C(=O)CH₂CH₂CH₂N), 27.3 (CH=CH₂CH₂CH₂CH₂N), 26.4 (CH=CH₂CH₂CH₂CH₂N), 26.0 (C(=O)CH₂CH₂CH₂N), 25.5 (CH=CH₂CH₂CH₂CH₂N), 8.2 (NCH(CH₂CH₂N))

**19F NMR** (376.45 MHz, CDCl₃) δ / ppm = -120.7 (s, ciprofloxacin F)

**HRMS** (ESI⁺) m/z / Da = 646.3132, [M+H]⁺ found, [C₃₄H₄₁FN₇O₉]⁺ requires 646.3153

The compound has not been reported previously.
9.44 4-Bromo-N-(3-methoxyphenyl)butanamide 164

3-Methoxyaniline 163 (3.04 mL, 3.33 g, 27.1 mmol, 1 eq.) and NaHCO$_3$ (2.73 g, 32.5 mmol, 1.2 eq.) were dissolved in water (30 mL) and CH$_2$Cl$_2$ (30 mL). The mixture was cooled to 0 °C and 4-bromobutyryl chloride 56 (3.13 mL, 5.03 g, 27.1 mmol, 1 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h, then the aqueous layer was removed. The organic layer was dry-loaded onto SiO$_2$ and purified by column chromatography using a Combiflash (SiO$_2$, 0-100% EtOAc/P.E.). The combined pure fractions were dried with MgSO$_4$ and evaporated under reduced pressure. 164 was obtained as a pale pink amorphous solid (3.66 g, 13.5 mmol, 50%).

**TLC** $R_f$ = 0.18 (25% EtOAc/P.E.)

**IR** (neat) $\nu_{\text{max}}$ / cm$^{-1}$ = 1671 (amide C=O)

**$^1$H NMR** (400 MHz, CDCl$_3$ d$_1$) $\delta$ / ppm = 8.45 (s, 1 H, NH), 7.27 (t, $J$ = 2.2 Hz, 1 H, ortho to OCH$_3$ and ortho to NH), 7.14 (t, $J$ = 8.1 Hz, 1 H, meta to OCH$_3$ and meta to NH), 7.02 (d, $J$ = 8.3 Hz, 1 H, para to OCH$_3$), 6.62 (dd, $J$ = 8.2, 2.1 Hz, 1 H, para to NH), 3.71 (s, 3 H, CH$_3$), 3.42 (t, $J$ = 6.5 Hz, 2 H, CH$_2$Br), 2.51 (t, $J$ = 6.9 Hz, 2 H, C(=O)CH$_2$), 2.19 (quin, $J$ = 6.8 Hz, 2 H, C(=O)CH$_2$CH$_2$)

**$^{13}$C NMR** (101 MHz, CDCl$_3$ d$_1$) $\delta$ / ppm = 170.3 (C(=O)), 159.9 (ipso to OCH$_3$), 139.0 (ipso to NH), 129.5 (meta to OCH$_3$ and meta to NH), 112.1 (para to OCH$_3$), 109.9 (para to NH), 105.7 (ortho to OCH$_3$ and ortho to NH), 55.2 (CH$_3$), 35.3 (C(=O)CH$_2$), 33.2 (CH$_2$Br), 28.0 (C(=O)CH$_2$CH$_2$)

**HRMS** (ESI$^+$) The compound does not ionise.

The compound has not been reported previously.

9.45 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((3-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 165

Methyl 1-cyclopropyl-6-fluoro-1-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 151 (500 mg, 1.45 mmol, 1 eq.), 4-bromo-N-(3-methoxyphenyl)butanamide 164 (788 mg, 2.90 mmol, 2 eq.), DIPEA (1.28 mL, 950 mg, 7.35 mmol, 5 eq.), NaI (275 mg, 1.83 mmol, 1.3 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at 100 °C for 4 h. The mixture was evaporated under reduced pressure and partitioned between CH$_2$Cl$_2$
(50 mL) and water (50 mL). The organic layer was separated off and the aqueous layer was extracted again with \( \text{CH}_2\text{Cl}_2 \) (50 mL). The combined organic layers were dried with MgSO\(_4\) and purified by column chromatography (SiO\(_2\), 0-4% MeOH/CH\(_2\)Cl\(_2\)). The combined pure fractions were dried with MgSO\(_4\) and evaporated under reduced pressure. 165 was obtained as an off-white amorphous solid (81.7 mg, 0.152 mmol, 11%).

**TLC** \( R_f = 0.38 \) (10% MeOH/CH\(_2\)Cl\(_2\))

**IR** (neat) \( \nu_{\text{max}} / \text{cm}^{-1} = 3271 \) (amide N-H), 2944 (C-H), 2817 (C-H), 1730 (ester C=O), 1682 (amide C=O), 1614 (quinolone C=O)

\( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)) \( \delta / \text{ppm} = 8.56 \) (s, 1 H, ortho to C(=O)OCH\(_3\)), 8.06 (d, \( J = 13.3 \text{ Hz} \), 1 H, ortho to F), 8.02 (br s, 1 H, NH), 7.34 (t, \( J = 7.1 \text{ Hz} \), 1 H, ortho to OCH\(_3\) and ortho to NH), 7.20 (t, \( J = 8.2 \text{ Hz} \), 1 H, meta to OCH\(_3\) and meta to NH), 6.65 (dd, \( J = 8.2, 2.1 \text{ Hz} \), 1 H, para to OCH\(_3\)), 6.38 (dd, \( J = 8.2 \text{ Hz} \), 1.36 (m, 2 H, NCH(CHH\(_2\))\_2), 2.73 (br t, \( J = 4.5 \text{ Hz} \), 4 H, C(=O)CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\)\_2)CH\(_2\)), 2.58 (t, \( J = 6.5 \text{ Hz} \), 2 H, C(=O)CH\(_2\)CH\(_2\)CH\(_2\)N), 2.48 (t, \( J = 6.8 \text{ Hz} \), 2 H, C(=O)CH\(_2\)CH\(_2\)CH\(_2\)N), 2.00 (quin, \( J = 6.8 \text{ Hz} \), 2 H, C(=O)CH\(_2\)CH\(_2\)CH\(_2\)N), 1.29 - 1.36 (m, 2 H, NCH(CHH\(_2\))\_2), 1.11 - 1.17 (m, 2 H, NCH(CHH\(_2\))\_2)

\( ^{13}\text{C NMR} \) (101 MHz, CDCl\(_3\)) \( \delta / \text{ppm} = 173.1 \) (C(=O)CC(=O)OCH\(_3\)), 170.9 (NH(C(=O))), 166.3 (C(=O)OCH\(_3\)), 160.1 (ipso to OCH\(_3\)), 153.3 (d, \( J = 250.1 \text{ Hz} \), ipso to F), 148.4 (C(=C)CC(=O)OCH\(_3\)), 144.1 (d, \( J = 10.1 \text{ Hz} \), ipso to piperazine), 139.4 (ipso to NH), 138.0 (para to F), 129.6 (meta to NH and meta to OCH\(_3\)), 123.3 (d, \( J = 6.4 \text{ Hz} \), para to piperazine), 113.4 (d, \( J = 23.3 \text{ Hz} \), ortho to C=O and ortho to F), 111.8 (para to OCH\(_3\)), 110.0 (C(=O)OCH\(_3\)), 109.8 (para to NH), 105.5 (ortho to OCH\(_3\) and ortho to NH), 105.0 (meta to C=O and meta to F), 57.0 (CH\(_2\)CH\(_2\)CH\(_2\)N), 55.3 (aromatic OCH\(_3\)), 52.6 (CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\)\_2)CH\(_2\)), 52.1 (C(=O)OCH\(_3\)), 49.2 (CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\)\_2)CH\(_2\)CH\(_2\)), 35.2 (CH\(_2\)CH\(_2\)CH\(_2\)N), 34.6 (NCH(CH\(_2\))\_2), 21.7 (CH\(_2\)CH\(_2\)CH\(_2\)N), 8.2 (NCH(CH\(_2\))\_2)

\( ^{19}\text{F NMR} \) (376.45 MHz, MeOD) \( \delta / \text{ppm} = -123.5 \) (s, ciprofloxacin F)

**HRMS** (ESI\(^+\)) \( m/z / \text{Da} = 537.2500, [M+H]\(^+\) found, [C\(_{29}\)H\(_{34}\)FN\(_4\)O\(_5\)]\(^+\) requires 537.2513

The compound has not been reported previously.

**9.46 4-Azido-N-(3-methoxyphenyl)butanamide 166**

![4-Azido-N-(3-methoxyphenyl)butanamide](attachment:image)

4-Bromo-N-(3-methoxyphenyl)butanamide 164 (2.05 g, 7.51 mmol, 1 eq.) and NaN\(_3\) (1.17 g, 18.0 mmol, 2.4 eq.) were refluxed in acetonitrile (100 mL) for 7 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto SiO\(_2\) and purified by column chromatography using a Combiflash (SiO\(_2\), 0-100% EtOAc/P.E.). The combined pure fractions were dried with MgSO\(_4\) and evaporated under reduced pressure. 166 was obtained as a straw-coloured liquid (0.294 g, 1.25 mmol, 17%).
TLC $R_f = 0.37$ (50% EtOAc/P.E.)

IR (neat) $\nu_{max}$ / cm$^{-1}$ = 3298 (N-H), 2095 (azide), 1662 (amide C=O)

$^1$H NMR (400 MHz, MeOD) $\delta$ / ppm = 8.63 (br s, 1 H, NH), 7.26 (t, $J = 2.3$ Hz, 1 H, ortho to OCH$_3$ and ortho to NH), 7.15 (t, $J = 8.1$ Hz, 1 H, meta to OCH$_3$ and meta to NH), 7.01 (dd, $J = 7.8$, 1.6 Hz, 1 H, para to OCH$_3$), 6.63 (dd, $J = 8.2$, 1.9 Hz, 1 H, para to NH), 3.69 (s, 3 H, CH$_3$), 3.28 (t, $J = 6.7$ Hz, 2 H, CH$_2$N$_3$), 2.39 (t, $J = 7.4$ Hz, 2 H, C(=O)CH$_2$), 1.91 (quin, $J = 7.0$ Hz, 2 H, C(=O)CH$_2$CH$_2$)

$^{13}$C NMR (101 MHz, MeOD) $\delta$ / ppm = 170.8 (C(=O)), 159.6 (ipso to OCH$_3$), 138.9 (ipso to NH), 129.2 (meta to OCH$_3$ and meta to NH), 112.3 (para to OCH$_3$), 109.5 (para to NH), 106.0 (ortho to OCH$_3$ and ortho to NH), 54.8 (C$_3$H$_3$), 50.4 (CH$_2$N$_3$), 33.6 (C(=O)C$_2$H$_2$), 24.4 (C(=O)CH$_2$CH$_2$)

HRMS (ESI$^+$) The compound does not ionise.

The compound has not been reported previously.

9.47 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 167

![Chemical Structure]

1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (24.1 mg, 58.6 µmol, 1 eq.) and 4-azido-N-(3-methoxyphenyl)butanamide 166 (13.7 mg, 58.5 µmol, 1 eq.) were dissolved in water (1 mL), t-BuOH (9 mL) and CH$_2$Cl$_2$ (10 mL), and the mixture was degassed by bubbling through N$_2$. A solution of CuSO$_4$ and THPTA (58.5 µl, 5.85 µmol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (117 µl, 11.7 µmol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 2 h, then the solvent was removed under reduced pressure. The residue was partitioned between water (15 mL) and CH$_2$Cl$_2$ (15 mL), and the aqueous layer was extracted a further four times with CH$_2$Cl$_2$ (4×15 mL). The combined organic layers were dried with MgSO$_4$, dry-loaded onto SiO$_2$ and purified by column chromatography (SiO$_2$, 0-10% MeOH/CH$_2$Cl$_2$). The combined pure fractions were dried with MgSO$_4$ and evaporated under reduced pressure. 167 was obtained as a clear amorphous solid (1.9 mg, 2.9 µmol, 5%).

TLC $R_f = 0.22$ (10% MeOH/CH$_2$Cl$_2$)

IR (neat) $\nu_{max}$ / cm$^{-1}$ = 2923 (C-H), 2850 (C-H), 1726 (carboxylic acid C=O), 1685 (amide C=O), 1625 (quino lone C=O), 1612 (triazole)

$^1$H NMR (400 MHz, DMSO d$_6$) $\delta$ / ppm = 15.23 (br s, 1 H, C(=O)OH), 9.89 (s, 1 H, NH), 8.66 (s, 1 H, ortho to C(=O)OH), 7.90 (d, $J = 13.4$ Hz, 1 H, ortho to F), 7.88 (s, 1 H, CH$_2$=CCH$_2$), 7.55 (d, $J = 7.6$ Hz, 184
1 H, meta to F), 7.27 (t, J = 2.1 Hz, 1 H, ortho to C=O and ortho to F), 7.16 (t, J = 8.1 Hz, 1 H, meta to OCH3 and meta to NH), 7.08 (d, J = 7.8 Hz, 1 H, para to OCH3), 6.59 (ddd, J = 8.1, 2.4, 0.7 Hz, 1 H, para to NH), 4.36 (t, J = 6.9 Hz, 2 H, C(=O)CH2CH2N), 3.81 (tt, J = 6.7, 4.0 Hz, 1 H, NCH(CH2)2), 3.70 (s, 3 H, CH3), 3.28 - 3.32 (m, 4 H, CH=CCCH2CH2CH2N(CH2CH2CH2CH2), 2.64 (t, J = 7.5 Hz, 2 H, CH=CCCH2), 2.56 (m, J = 4.2, 4.2 Hz, 4 H, CH=CCCH2CH2CH2N(CH2CH2), 2.38 (t, J = 7.3 Hz, 2 H, CH=CCCH2CH2CH2N), 2.30 (t, J = 7.4 Hz, 2 H, C(=O)CH2CH2N), 2.10 (quin, J = 7.1 Hz, 2 H, C(=O)CH2CH2CH2N), 1.64 (quin, J = 7.5 Hz, 2 H, CH=CCCH2CH2CH2N), 1.51 (quin, J = 7.2 Hz, 2 H, CH=CCCH2CH2CH2N), 1.27 - 1.33 (m, 2 H, NCH(CH3H)2), 1.15 - 1.20 (m, 2 H, NCH(CH3H)2).

13C NMR (101 MHz, DMSO d6) δ / ppm = 176.3 (C(=O)CC(=O)OH), 170.1 (NHCC(=O)), 165.9 (C(=O)OH), 159.4 (ipso to OCH3), 153.0 (d, J = 107.6 Hz, ipso to F), 148.0 (CH=CCCH2), 146.9 (C=CC(=O)OH), 145.2 (d, J = 9.7 Hz, ipso to piperazine), 140.3 (para to F), 139.2 (ipso to NH), 129.4 (meta to OCH3 and meta to NH), 121.7 (CH=CCCH2), 118.5 (d, J = 7.5 Hz, para to piperazine), 111.3 (para to OCH3), 110.9 (d, J = 22.4 Hz, ortho to C=O and ortho to F), 108.4 (para to NH), 106.7 (C(=O)OH), 106.3 (meta to C=O and meta to F), 104.8 (ortho to OCH3 and ortho to NH), 57.3 (CH=CCCH2CH2CH2N), 54.9 (CH3), 52.4 (CH=CCCH2CH2CH2N(CH2CH2), 49.5 (CH=CCCH2CH2CH2N(CH2CH2CH2CH2), 49.4 (CH=CCCH2CH2CH2N(CH2CH2CH2CH2), 48.7 (C(=O)CH2CH2N), 35.8 (NCH(CH2)2), 32.9 (C(=O)CH2CH2N), 26.8 (CH=CCCH2CH2CH2N), 25.7 (CH=CCCH2CH2CH2N), 25.5 (C(=O)CH2CH2CH2N), 24.9 (CH=CCCH2CH2CH2N), 7.6 (NCH(CH2)2).

19F NMR (376.45 MHz, DMSO d6) δ / ppm = -121.5 (s, ciprofloxacin F)

HRMS (ESI+) m/z / Da = 646.3159, [M+H]+ found, [C34H41FN7O3]+ requires 646.3153.

The compound has not been reported previously.

9.48 (1S,2S)-2-(((S)-1-Phenylethyl)amino)cyclopentan-1-ol 170 and (1R,2R)-2-(((S)-1-phenylethyl)amino)cyclopentan-1-ol 171

(S)-1-Phenylethan-1-amine 169 (7.85 mL, 7.38 g, 60.9 mmol, 1 eq.) was dissolved in CH2Cl2 (50 mL) and stirred rapidly at 0 °C. A solution of AlMe3 (31 mL, 2.0 M in heptane, 60.9 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. A solution of cyclohexene oxide 168 (5.71 mL, 5.50 g, 65.4 mmol, 1.1 eq.) in CH2Cl2 (50 mL) was then added dropwise, and the mixture was stirred at 0 °C for a further 3 h, followed by 48 h at r.t.. The mixture was cooled to 0 °C and NaF (11 g, 262 mmol, 4.3 eq.) was added portionwise, followed by water (7.00 mL, 7.00 g, 389 mmol, 6.4 eq.) and CH2Cl2 (50 mL). The suspension was allowed to warm to r.t. and stirred for 1 h, then filtered through Celite and washed with CH2Cl2 (500 mL). The filtrate was dried with K2CO3, concentrated under reduced pressure and purified by column chromatography (SiO2, 20:5:1 hexane:EtOAc:TEA). 171 was obtained as a pale yellow oil (4.08 g, 19.9 mmol, 33%). 170 was obtained as pale yellow crystals (4.48 g, 21.8 mmol, 36%).
(15S,2S)-2-(((S)-1-Phenylethyl)amino)cyclopentan-1-ol 170

**TLC** $R_f = 0.36$ (15:5:1 hexane:EtOAc:TEA)

**mp** $T / ^\circ C = 66-72$ (hexane, EtOAc, TEA)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 3150$ (br, O-H), 2951 (C-H), 2868 (C-H)

**$^1$H NMR** (400 MHz, CDCl$_3$) $\delta / ppm = 7.28 - 7.34$ (m, 4 H, *ortho* and *meta* to CHCH$_3$), 7.20 - 7.26 (m, 1 H, *para* to CHCH$_3$), 3.86 (q, $J = 6.6$ Hz, 1 H, CHCH$_3$), 3.85 (q, $J = 6.6$ Hz, 1 H, CHOH), 2.83 (td, $J = 7.6$, 5.7 Hz, 1 H, CHNH), 1.55 - 1.68 (m, 2 H, CH$_2$CH$_2$CHOH), 1.47 - 1.55 (m, 1 H, CHHCHOH), 1.36 (d, $J = 6.6$ Hz, 3 H, CH$_3$), 1.12 (dq, $J = 12.7$, 8.1 Hz, 1 H, CHHCHNH)

**$^{13}$C NMR** (101 MHz, CDCl$_3$) $\delta / ppm = 145.61$ (ipso to CHCH$_3$), 128.08 (meta to CHCH$_3$), 126.61 (para to CHCH$_3$), 126.33 (ortho to CHCH$_3$), 77.43 (CHOH), 64.45 (CHNH), 56.62 (CHHCHOH), 23.30 (CH$_3$), 20.06 (CH$_2$CH$_2$CHOH)

**HRMS** (ESI$^+$) $m/z / Da = 206.1553$, [M+H]$^+$ found, [C$_{13}$H$_{20}$NO]$^+$ requires 206.1545

[$\alpha$]$_{D}^{20}$ / $^\circ 10^{-1}$cm$^2$g$^{-1} = -23.9$, lit. = -22.1 ($c$ / g(100 mL)$^{-1} = 0.96$, MeOH)

(1R,2R)-2-(((S)-1-Phenylethyl)amino)cyclopentan-1-ol 171

**TLC** $R_f = 0.25$ (15:5:1 hexane:EtOAc:TEA)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 3300$ (br, O-H), 2960 (C-H), 2870 (C-H)

**$^1$H NMR** (400 MHz, CDCl$_3$) $\delta / ppm = 7.28 - 7.38$ (m, 4 H, *ortho* and *meta* to CHCH$_3$), 7.21 - 7.28 (m, 1 H, *para* to CHCH$_3$), 3.83 (q, $J = 6.6$ Hz, 1 H, CHCH$_3$), 3.78 (q, $J = 7.0$ Hz, 1 H, CHOH), 2.62 (dt, $J = 8.2$, 7.2 Hz, 1 H, CHNH), 1.97 (quin, $J = 6.7$ Hz, 1 H, CH$_2$CHNH), 1.90 (quin, $J = 6.9$ Hz, 1 H, CH$_2$CHOH), 1.56 - 1.68 (m, CH$_2$CH$_2$CHOH), 1.43 (dq, $J = 12.5$, 8.0 Hz, 1 H, CH$_2$CHOH), 1.37 (d, $J = 6.6$ Hz, 3 H, CH$_3$), 1.25 - 1.36 (m, 1 H, CH$_2$CHNH)

**$^{13}$C NMR** (101 MHz, CDCl$_3$) $\delta / ppm = 144.75$ (ipso to CHCH$_3$), 128.08 (meta to CHCH$_3$), 126.72 (para to CHCH$_3$), 126.30 (ortho to CHCH$_3$), 77.65 (CHOH), 63.38 (CHNH), 56.20 (CH$_3$), 31.74 (CH$_2$CHOH), 29.22 (CH$_2$CHNH), 24.58 (CH$_3$), 19.57 (CH$_2$CH$_2$CHOH)

**HRMS** (ESI$^+$) $m/z / Da = 206.1554$, [M+H]$^+$ found, [C$_{13}$H$_{20}$NO]$^+$ requires 206.1545

[$\alpha$]$_{D}^{20}$ / $^\circ 10^{-1}$cm$^2$g$^{-1} = -92.8$, lit. = -76.8 ($c$ / g(100 mL)$^{-1} = 1.19$, MeOH)

The compounds have been synthesised previously, but NMR data were not published. The enantiomers of both compounds have also been synthesised previously, and the $^1$H NMR data for these are consistent with the the above data.
9.49 (1S,2S)-2-Aminocyclopentan-1-ol 172

(1S,2S)-2-(((S)-1-Phenylethyl)amino)cyclopentan-1-ol 170 (3.00 g, 14.6 mmol, 1 eq.), Pd(OH)$_2$ (20 wt. % on C, moistened with 50 wt. % water, 0.5 g, 0.356 mmol, 0.025 eq.) and MeOH (50 mL) were stirred in a Paar hydrogenator at r.t. and 2.5 atm for 2 days. The mixture was then filtered through Celite and evaporated under reduced pressure. 172 was obtained as a yellow oil (1.48 g, 14.6 mmol, >99%).

**TLC** $R_f = 0.10$ (10% MeOH/CH$_2$Cl$_2$)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 3300$ (O-H), 2969 (C-H), 2873 (C-H)

**$^1$H NMR** (400 MHz, MeOD) $\delta / ppm = 3.77$ (ddd, $J = 6.6, 6.2, 5.6, 1$ H, CH$_{\text{OH}}$), 3.00 (td, $J = 7.4, 5.6$ Hz, 1 H, CH$_{\text{NH}}$), 2.00 (ddt, $J = 13.0, 7.7, 5.6$ Hz, 1 H, CH$_{\text{HCHNH}}$), 1.97 (ddt, $J = 13.0, 8.7, 6.4$ Hz, 1 H, CH$_{\text{HCHOH}}$), 1.64 - 1.77 (m, 2 H, CH$_2$CH$_2$CHOH), 1.53 (ddt, $J = 13.0, 9.5, 6.2$ Hz, 1 H, CH$_{\text{HCHOH}}$), 1.37 (ddt, $J = 12.8, 8.5, 7.7$ Hz, 1 H, CHHCHNH$_2$)

**$^{13}$C NMR** (101 MHz, MeOD) $\delta / ppm = 80.6$ (CH$_{\text{OH}}$), 60.7 (CH$_{\text{NH}}$), 33.2 (CH$_2$CHOH), 32.2 (CH$_2$CHNH$_2$), 21.2 (CH$_2$CH$_2$CHOH)

**HRMS** (ESI$^+$) $m/z$ / Da = 102.0915, [M+H]$^+$ found, [C$_5$H$_{12}$NO]$^+$ requires 102.0913

$[\alpha]_{D}^{20} / ^{\circ} 10^{-1} \text{cm}^2 \text{g}^{-1} = 33.4$, lit. = 29.7 ($c / g(100 \text{ mL})^{-1} = 0.5$, EtOH)

The data are consistent with the literature.$^{220,241}$

9.50 (1R,2R)-2-Aminocyclopentan-1-ol 173

(1R,2R)-2-(((S)-1-Phenylethyl)amino)cyclopentan-1-ol 171 (3.90 g, 19.0 mmol, 1 eq.), Pd(OH)$_2$ (20 wt. % on C, moistened with 50 wt. % water, 1 g, 0.712 mmol, 0.04 eq.) and MeOH (50 mL) were stirred in a Paar hydrogenator at r.t. and 3 atm for 2 days. The mixture was then filtered through Celite and evaporated under reduced pressure. 173 was obtained as a yellow oil (1.92 g, 19.0 mmol, >99%).

**TLC** $R_f = 0.10$ (10% MeOH/CH$_2$Cl$_2$)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 3300$ (br, O-H), 2958 (C-H), 2872 (C-H)

**$^1$H NMR** (400 MHz, MeOD) $\delta / ppm = 3.77$ (ddd, $J = 6.6, 6.2, 5.6$ Hz, 1 H, CH$_{\text{OH}}$), 3.00 (td, $J = 7.3, 5.6$ Hz, 1 H, CH$_{\text{NH}}$), 2.00 (ddt, $J = 13.0, 7.7, 5.6$ Hz, 1 H, CH$_{\text{HCHNH}}$), 1.97 (ddt, $J = 13.0, 8.7, 6.4$ Hz, 1 H, CH$_{\text{HCHOH}}$), 1.64 - 1.77 (m, 2 H, CH$_2$CH$_2$CHOH), 1.53 (ddt, $J = 13.0, 9.5, 6.2$ Hz, 1 H, CH$_{\text{HCHOH}}$), 1.37 (ddt, $J = 12.8, 8.5, 7.7$ Hz, 1 H, CHHCHNH$_2$)

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Hz, 1 H, CHNH₂), 2.00 (ddt, J = 13.0, 7.7, 5.6 Hz, 1 H, CHHCHNH₂), 1.97 (ddt, J = 13.0, 8.7, 6.6 Hz, 1 H, CHHCHOH), 1.63 - 1.77 (m, 2 H, CH₂CH₂CH(OH)), 1.53 (ddt, J = 13.0, 9.5, 6.2 Hz, 1 H, CHHCHOH), 1.37 (ddt, J = 13.0, 8.3, 7.8 Hz, 1 H, CHHCHNH₂)

13C NMR (101 MHz, MeOD) δ / ppm = 80.7 (CH(OH)), 60.8 (CHNH₂), 33.2 (CH₂CH(OH)), 32.1 (CH₂CHNH₂), 21.2 (CH₂CH(OH))

HRMS (ESI⁺) m/z / Da = 102.0917, [M+H]+ found, [C₅H₁₂O₂N]+ requires 102.0913

[α]D²⁰ / °10⁻¹cm²g⁻¹ = -30.9, lit. = -32.9 (c / g(100 mL)⁻¹ = 1.5, EtOH)

The data are consistent with the literature.²²⁰,²⁴¹

9.51 4-Azido-N-((1S,2S)-2-hydroxycyclopentyl)butanamide 176

4-Chloro-N-((1S,2S)-2-hydroxycyclopentyl)butanamide 193 (35.0 mg, 0.170 mmol, 1 eq.) and NaN₃ (22.1 mg, 0.340 mmol, 2 eq.) were stirred in acetonitrile (2 mL) at 50 °C for 24 h. The reaction mixture was then partitioned between water (20 mL) and 10% i-ProOH/CHCl₃ (5 mL). The aqueous layer was extracted again with 10% i-ProOH/CHCl₃ (2×5 mL) and the combined organic fractions were dried with MgSO₄ and evaporated under reduced pressure. 176 was obtained as white needles (16.2 mg, 0.0764 mmol, 45%).

TLC Rf = 0.35 (EtOAc)

IR (neat) νmax / cm⁻¹ = 3287 (N-H and O-H), 2958 (C-H), 2931 (C-H), 2861 (C-H), 2095 (azide), 1642 (amide C=O)

1H NMR (400 MHz, CDCl₃) δ / ppm = 5.82 (br s, 1 H, NH), 4.45 (br. s., 1 H, OH), 3.96 (q, J = 6.6 Hz, 1 H, CH(OH)), 3.83 (tdd, J = 8.5, 6.0, 4.6 Hz, 1 H, CH(NH)), 3.37 (t, J = 6.4 Hz, 2 H, CH₂N₃), 2.31 (t, J = 7.2 Hz, 2 H, CH₂C=O), 2.09 - 2.19 (m, 1 H, CHHCHNH), 1.99 - 2.06 (m, 1 H, CHHCHOH), 1.90 - 1.97 (m, 2 H, CH₂CH₂N₃), 1.60 - 1.85 (m, 3 H, CH₂CHHCHOH), 1.42 (dq, J = 12.8, 8.3 Hz, 1 H, CHHCHNH)

13C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 79.7 (CH(OH)), 61.0 (CH(NH)), 50.7 (CH₂N₃), 32.8 (CH₂C=O), 32.6 (CH₂CH(OH)), 30.5 (CH₂CHNH), 24.7 (CH₂CH₂N₃), 21.3 (CH₂CH₂CH(OH))

HRMS (ESI⁺) m/z / Da = 235.1178, [M+Na]+ found, [C₉H₁₆N₄NaO₂]⁺ requires 235.1171

[α]D²⁰ / °10⁻¹cm²g⁻¹ = 10.0 (c / g(100 mL)⁻¹ = 0.01, MeOH)

The compound has not been reported previously.
4-Azido-\(N-((1R,2R)-2\text{-hydroxycyclopentyl})\)butanamide 177

\[
\begin{align*}
\text{OH} & \quad \text{N}_3 \\
\text{N} & \quad \text{O} \\
& \quad \text{N}
\end{align*}
\]

4-Chloro-\(N-((1R,2R)-2\text{-hydroxycyclopentyl})\)butanamide 194

(200 mg, 0.972 mmol, 1 eq.) and NaN\(_3\) (126 mg, 1.94 mmol, 2 eq.) were stirred in acetonitrile (4 mL) at 50 \(^\circ\)C for 16 h. The solvent was then evaporated under reduced pressure and the residue was partitioned between water (20 mL) and 10\% \(i\)-PrOH/CHCl\(_3\) (20 mL). The aqueous layer was extracted again with 10\% \(i\)-PrOH/CHCl\(_3\) (3×20 mL) and the combined organic fractions were dried with MgSO\(_4\) and evaporated under reduced pressure. 177 was obtained as white needles (181 mg, 0.852 mmol, 88\%).

**TLC** \(R_f = 0.35\) (EtOAc)

**mp** \(T / ^\circ\)C = 56-60 (\(i\)-PrOH, CHCl\(_3\))

**IR** (neat) \(\nu_{\max} / \text{cm}^{-1} = 3280\) (N-H and O-H), 2966 (C-H), 2875 (C-H), 2095 (azide), 1637 (amide C=O)

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)) \(\delta / \text{ppm} = 6.72\) (d, \(J = 4.4\) Hz, 1 H, NH), 4.82 (br. s., 1 H, OH), 3.88 (q, \(J = 6.6\) Hz, 1 H, CHO), 3.75 (tdd, \(J = 8.4, 6.6, 4.4\) Hz, 1 H, CHNH), 3.28 (t, \(J = 6.6\) Hz, 2 H, CH\(_3\)N\(_3\)), 2.23 (t, \(J = 7.3\) Hz, 2 H, CH\(_2\)C=O), 2.04 (ddt, \(J = 13.0, 8.0, 4.9\) Hz, 1 H, CHHCHNH), 1.92 (ddt, \(J = 13.0, 7.6, 5.8\) Hz, 1 H, CHHCHOH), 1.84 (quin, \(J = 7.0\) Hz, 2 H, CH\(_3\)CH\(_2\)N\(_3\)), 1.59 - 1.77 (m, 2 H, CH\(_2\)CH\(_2\)CHOH), 1.54 (ddt, \(J = 12.7, 9.0, 6.7\) Hz, 1 H, CHHCHOH), 1.39 (dq, \(J = 12.9, 8.4\) Hz, 1 H, CHHCHNH)

**\(^{13}\)C NMR** (101 MHz, CDCl\(_3\)) \(\delta / \text{ppm} = 173.8\) (C=O), 78.8 (CHOH), 59.9 (CHNH), 50.5 (CH\(_2\)N\(_3\)), 32.5 (CH\(_2\)C=O), 32.0 (CH\(_2\)CHOH), 29.5 (CH\(_2\)CHNH), 24.6 (CH\(_2\)CH\(_2\)N\(_3\)), 20.7 (CH\(_2\)CH\(_2\)CHOH)

**HRMS** (ESI\(^+\)) \(m/z / \text{Da} = 235.1174\), [M+Na]\(^+\) found, \([C_9H_{16}N_4NaO_2]\(^+\) requires 235.1171

\([\alpha]_{D}^{20} / \text{°10}^{-1}\text{cm}^2\text{g}^{-1} = -10.2\) (\(c / g(100\ \text{mL})^{-1} = 0.5\), MeOH)

The compound has not been reported previously.

Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1S,2S)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 178

\[
\begin{align*}
\end{align*}
\]
4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate 198 (52.1 mg, 95.5 µmol, 1 eq.), (1S,2S)-2-aminocyclopentan-1-ol 172 (19.5 mg, 193 µmol, 2 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (29.7 mg, 155 µmol, 1.6 eq.), 1-hydroxybenzotriazole (25.8 mg, 191 µmol, 2 eq.) and DIPEA (33.3 µl, 24.7 mg, 191 µmol, 2 eq.) were dissolved in DMF (2 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of N₂ and the residue was purified by preparative HPLC (5-50% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO₃ (aq., sat., 5 mL) and CH₂Cl₂ (5 mL). The organic layer was removed and the aqueous layer was extracted twice more with CH₂Cl₂ (2×5 mL). The combined organic fractions were dried with MgSO₄ and evaporated under reduced pressure. 178 was obtained as a white amorphous solid (26.9 mg, 52.3 µmol, 55%).

**TLC** $R_f = 0.38$ (30% MeOH/CH₂Cl₂)

**IR** (neat) $\nu_{max} / \text{cm}^{-1} = 2938$ (C-H), 1721 (ester C=O), 1650 (amide C=O and quinolone C=O)

**1H NMR** (500 MHz, DMSO $d_6$) $\delta / \text{ppm} = 8.44$ (s, 1 H, ortho to C(=O)OCH₃), 7.75 (d, $J = 13.5$ Hz, 1 H, ortho to F), 7.69 (d, $J = 6.9$ Hz, 1 H, CHN), 7.43 (d, $J = 7.6$ Hz, 1 H, meta to F), 4.73 (br s, 1 H, CHO), 3.77 - 3.81 (m, 1 H, CHO), 3.74 - 3.77 (m, 1 H, CHN), 3.73 (s, 3 H, CH₃), 3.65 (tt, $J = 6.9$, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.24 (br. t, $J = 4.2$ Hz, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.55 (br t, $J = 5.0$ Hz, 4 H, CH₂N(CH₂)CH₂), 2.32 (t, $J = 7.2$ Hz, 2 H, CH₂N(CH₂CH₂), 2.10 (t, $J = 7.4$ Hz, 2 H, CH₂CH₂CH₂N(CH₂CH₂), 1.92 (ddd, $J = 13.0$, 8.7, 7.3, 6.0 Hz, 1 H, CHHCHNH), 1.77 (ddt, $J = 12.6$, 8.9, 6.3 Hz, 1 H, CHHCHOH), 1.68 (quin, $J = 7.4$ Hz, 2 H, CH₂CH₂N(CH₂CH₂), 1.53 - 1.64 (m, 2 H, CH₂CH₂CHOH), 1.42 (ddt, $J = 12.9$, 8.4, 5.2 Hz, 1 H, CHHCHOH), 1.31 (ddt, $J = 13.0$, 8.6, 6.4 Hz, 1 H, CHHCHNH), 1.22 - 1.28 (m, 2 H, NCH(CHH)₂), 1.06 - 1.12 (m, 2 H, NCH(CHH)₂)

**13C NMR** (126 MHz, DMSO $d_6$) $\delta / \text{ppm} = 171.9$ (NHCC(=O)CH₂), 171.5 (CC(=O)CC(=O)OCH₃), 165.0 (CC(=O)OCH₃), 152.6 (d, $J = 247.4$ Hz, ipso to F), 148.2 (CC=CC(=O)OCH₃), 143.9 (d, $J = 10.3$ Hz, ipso to piperazine), 138.1 (para to F), 121.7 (d, $J = 6.4$ Hz, para to piperazine), 111.5 (d, $J = 23.0$ Hz, ortho to C=O and ortho to F), 109.0 (CC(=O)OCH₃), 106.2 (meta to C=O and meta to F), 76.2 (CHOH), 57.6 (CHN), 57.2 (CH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂CH₂)), 51.3 (CH₃), 49.6 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.6 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 34.7 (NCH(CH₂)), 33.2 (C(CH=O)CH₂), 32.2 (CH₂CHOH), 29.5 (CH₂CHNH), 22.5 (C(CH=O)CH₂CH₂), 20.6 (CH₂CH₂CHOH), 7.5 (NCH(CH₂)₂)

**19F NMR** (376.45 MHz, MeOD) $\delta / \text{ppm} = -125.5$

**HRMS** (ESI⁺) $m/z / \text{Da} = 515.2667$, [M+H]⁺ found, [C₂₇H₃₆FN₄O₅]⁺ requires 515.2670

$[\alpha]_{D}^{20} / 10^{-1} \text{cm}^2 \text{g}^{-1} = 8.0$ (c / g(100 mL))⁻¹ = 0.05, MeOH

The compound has not been reported previously.
4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazine-1-yl)butanoic acid trifluoroacetate \(198\) (200 mg, 0.367 mmol, 1 eq.), \((1R,2R)-2\text{-amino)cyclopentane-1-ol}\ \(173\) (80 mg, 0.791 mmol, 2.1 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (112 mg, 0.584 mmol, 1.6 eq.), 1-hydroxybenzotriazole (96 mg, 0.710 mmol, 1.9 eq.) and DIPEA (192 µl, 142 mg, 1.10 mmol, 3 eq.) were dissolved in DMF (5 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of \(N_2\) and the residue was purified by preparative HPLC (5-60% acetonitrile/water over 12 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between \(\text{NaHCO}_3\) (aq., sat., 10 mL) and \(\text{CH}_2\text{Cl}_2\) (10 mL). The organic layer was removed and the aqueous layer was extracted twice more with \(\text{CH}_2\text{Cl}_2\) (2×10 mL). The combined organic fractions were dried with \(\text{MgSO}_4\) and evaporated under reduced pressure. \(179\) was obtained as a white amorphous solid (73.0 mg, 0.142 mmol, 39%).

**TLC** \(R_f = 0.43\) (30% MeOH/EtOAc)

**IR** (neat) \(\nu_{\text{max}}/\text{cm}^{-1} = 2973\ (\text{C-H}), 2902\ (\text{C-H}), 1728\ (\text{ester C=O}), 1656\ (\text{amide C=O}), 1613\ (\text{quinolone C=O})

**\(^1\text{H NMR}\) (400 MHz, DMSO \(d_6\)) \(\delta/\text{ppm} = 8.44\ (s, 1\ \text{H}, \text{ortho to C(=O)OCH}_3), 7.75\ (d, J = 13.5\ \text{Hz}, 1\ \text{H}, \text{ortho to F}), 7.70\ (d, J = 7.2\ \text{Hz}, 1\ \text{H}, \text{CHNH}), 7.43\ (d, J = 7.5\ \text{Hz}, 1\ \text{H}, \text{meta to F}), 4.74\ (d, J = 4.0\ \text{Hz}, 1\ \text{H}, \text{CHOH}), 3.78 - 3.82\ (m, 1\ \text{H}, \text{CH(OH)}), 3.74 - 3.78\ (m, 1\ \text{H}, \text{CHNH}), 3.74\ (s, 3\ \text{H}, \text{CH}_3), 3.65\ (tt, J = 7.2, 3.9\ \text{Hz}, 1\ \text{H}, \text{CHOH}), 3.57 - 3.61\ (m, 1\ \text{H}, \text{NCH(CH}_2)_2), 3.25\ (t, J = 4.8\ \text{Hz}, 4\ \text{H}, \text{CH}_2\text{N(CH}_2\text{CH}_2\text{CH}_2\text{CH}_2), 2.57\ (br s, 4\ \text{H}, \text{CH}_2\text{N(CH}_2\text{CH}_2), 2.34\ (t, J = 7.4\ \text{Hz}, 2\ \text{H}, \text{CH}_2\text{N(CH}_2\text{CH}_2), 2.11\ (t, J = 7.4\ \text{Hz}, 2\ \text{H}, \text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2), 1.92\ (ddd, J = 13.0, 8.7, 7.3, 6.0\ \text{Hz}, 1\ \text{H}, \text{CH(HCHNH), 1.78 (ddddd, J = 12.6, 8.9, 6.3, 6.3 Hz, 1 H, CHCHCHOH), 1.69 (quin, J = 7.3 Hz, 2 H, CH}_2\text{HCH}_2\text{N(CH}_2\text{CH}_2), 1.54 - 1.65\ (m, 2\ \text{H}, \text{CH}_2\text{CH}_2\text{CH}_2\text{CHOH}, 1.42\ (dt, J = 13.1, 8.2, 5.3\ \text{Hz}, 1\ \text{H, CHHCHNH), 1.32 (ddddd, J = 13.4, 8.5, 6.8, 5.8\ \text{Hz}, 1\ \text{H, CH(HCHNH), 1.21 - 1.29 (m, 2\ \text{H}, \text{NCH(CH}_2\text{H}_2), 1.07 - 1.13\ (m, 2\ \text{H, NCH(CH}_2\text{H}_2))}

**\(^{13}\text{C NMR}\) (101 MHz, DMSO \(d_6\)) \(\delta/\text{ppm} = 171.9\ (\text{CH}_2\text{C(=O)NH), 171.6 (C(=O)CC(=O)OCH}_3, 165.0\ (\text{C(=O)OCH}_3), 152.6\ (d, J = 246.5\ \text{Hz, ipso to F}), 148.3\ (\text{C(=CC(=O)OCH}_3, 143.9\ (d, J = 10.7\ \text{Hz, ipso to piperazine), 138.1 (para to F), 121.8\ (d, J = 6.4\ \text{Hz, para to piperazine), 111.5\ (d, J = 22.4\ \text{Hz, ortho to C=O and ortho to F), 109.0 (CC(=O)OCH}_3, 106.2 (meta to C=O and meta to F), 76.3 (CHOH), 57.6 (CHNH), 57.2 (CH}_2\text{CH}_2\text{CH}_2\text{N), 52.4 (CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2), 51.3 (CH}_3, 49.6 (CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2))\text{CH}_2\text{CH}_2\text{CH}_2, 34.8 (NCH(CH}_2\text{H}_2), 33.3 (C(=O)CH}_2, 32.2 (CH}_2\text{CH}_2\text{CH}_2\text{CHOH), 29.5 (CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2, 20.6 (\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHOH), 7.6 (NCH(CH}_2\text{H}_2))}

**\(^{19}\text{F NMR}\) (376.45 MHz, DMSO \(d_6\)) \(\delta/\text{ppm} = -124.3\) (ciprofloxacin F)
HRMS (ESI\(^+\)) \(m/z\) / Da = 515.2661, [M+H]\(^+\) found, \([C_{27}H_{36}FN_4O_5]\)^+ requires 515.2670

\([\alpha]_D^{30}\) / \(10^{-1}\) cm\(^2\) g\(^{-1}\) = -6.0 (c \(g(100\ mL)^{-1}\) = 0.05, MeOH)

The compound has not been reported previously.

9.55 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1S,2S)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 180

1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (82.0 mg, 199 \(\mu\)mol, 4 eq.) and 4-azido-N-((1S,2S)-2-hydroxycyclopentyl)butanamide 176 (11.0 mg, 51.8 \(\mu\)mol, 1 eq.) were dissolved in 10% water/t-BuOH (3 mL), and the mixture was degassed by bubbling \(N_2\) through it. A solution of CuSO\(_4\) and THPTA (156 \(\mu\)l, 15.6 \(\mu\)mol, 0.3 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (312 \(\mu\)l, 31.2 \(\mu\)mol, 0.6 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 3 d. Water (10 mL) and 10% \(i\)-PrOH/CHCl\(_3\) (10 mL) were added, then the organic layer was separated and dried with MgSO\(_4\) and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO\(_3\) (aq., sat., 10 mL) and 10% \(i\)-PrOH/CHCl\(_3\) (10 mL). The organic layer was dried with MgSO\(_4\) and evaporated under reduced pressure. 180 was obtained as a white amorphous solid (7.2 mg, 11.5 \(\mu\)mol, 22%).

IR (neat) \(\nu_{max}\) / cm\(^{-1}\) = 2955 (C-H), 2918 (C-H), 2850 (C-H), 1722 (carboxylic acid C=O), 1647 (amide C=O), 1627 (quinolone C=O) 1612 (triazole)

\(^1\)H NMR (400 MHz, DMSO d\(_6\)) \(\delta\) / ppm = 15.22 (br s, 1 H, C(=O)OH), 8.67 (s, 1 H, ortho to C(=O)OH), 7.91 (d, \(J = 13.3\) Hz, 1 H, ortho to F), 7.84 (s, 1 H, CH=CCH\(_2\)), 7.74 (d, \(J = 6.7\) Hz, 1 H, CHNH), 7.56 (d, \(J = 7.4\) Hz, 1 H, meta to F), 4.71 (d, \(J = 3.7\) Hz, 1 H, CHOH), 4.29 (t, \(J = 6.6\) Hz, 2 H, CH\(_2\)NCH=C), 3.82 (tt, \(J = 6.5, 4.3\) Hz, 1 H, NCH(CH\(_2\))\(_2\)), 3.69 - 3.79 (m, 2 H, CHOH and CHNH), 3.30 - 3.34 (m, 6 H, CH=CCH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\)CH\(_2\))CH\(_2\)CH\(_2\)), 2.64 (t, \(J = 7.4\) Hz, 2 H, CH=CCH\(_2\)), 1.95 - 2.08 (m, 4 H, C(=O)(CH\(_2\)CH\(_2\)), 1.89 (ddddd, \(J = 12.8, 8.9, 7.4, 5.8\) Hz, 1 H, CHHCHNH), 1.75 (dddt, \(J = 12.7, 9.0, 6.2\) Hz, 1 H, CHHCHOH), 1.48 - 1.68 (m, 6 H, CH=CCH\(_2\)CH\(_2\)CH\(_2\)N(CH\(_2\)CH\(_2\))CH\(_2\)CH\(_2\)), 1.40 (dddt, \(J = 13.0, 8.3, 5.3\) Hz, 1 H, CHHCHOH), 1.28 - 1.35 (m, 2 H, NCH(CH\(_2\))\(_2\)), 1.24 - 1.31 (m, 1 H, CHHCHNH), 1.15 - 1.21 (m, 2 H, NCH(CH\(_2\))\(_2\))

\(^13\)C NMR (101 MHz, DMSO d\(_6\)) \(\delta\) / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (NH=C(=O)CH\(_2\)), 166.0 (C(=O)OH), 153.0 (d, \(J = 249.6\) Hz, ipso to F), 148.1 (C=CC(=O)OH), 146.7 (CH=CCH\(_2\)), 145.2 (d, \(J = 8.3\) Hz, ipso to piperazine), 139.2 (para to F), 121.8 (NCH=CCH\(_2\)), 118.7 (para to piperazine), 111.0 (d, \(J = 23.2\) Hz, ortho to C=O and ortho to F), 106.7 (C(=O)OH), 106.5 (meta to C=O and meta to F), 76.2 (CHOH), 57.5
1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1R,2R))-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68

1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (42.9 mg, 104 µmol, 1 eq.) and 4-azido-N-((1R,2R)-2-hydroxycyclopentyl)butanamide 177 (22.0 mg, 104 µmol, 1 eq.) were dissolved in 10% water/1-t-BuOH (3 mL), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (104 µl, 10.4 µmol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208 µl, 20.8 µmol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (30 mL) and CH₂Cl₂ (30 mL) were added, the organic layer was extracted again with CH₂Cl₂ (4 x 30 mL). The combined organic layers were dried with MgSO₄ and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO₃ (aq., sat., 10 mL) and 10% i-PrOH/CHCl₃ (10 mL). The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 181 was obtained as a white amorphous solid (17.6 mg, 28.2 µmol, 27%).

IR (neat) νmax / cm⁻¹ = 2967 (C-H), 2902 (C-H), 1721 (carboxylic acid C=O), 1647 (amide C=O), 1627 (quinolone C=O), 1613 (triazole)

¹H NMR (700 MHz, DMSO-d₆) δ / ppm = 8.64 (s, 1 H, ortho to C(=O)OH), 7.87 (d, J = 13.3 Hz, 1 H, ortho to F), 7.84 (s, 1 H, CH=CH₂), 7.75 (d, J = 7.1 Hz, 1 H, CHNH), 7.54 (d, J = 7.5 Hz, 1 H, meta to F), 4.73 (d, J = 3.8 Hz, 1 H, CHO), 4.29 (t, J = 6.9 Hz, 2 H, CH₂N=CH=C), 3.78 - 3.83 (m, 1 H, NCH(CH₂)₂), 3.75 - 3.78 (m, 1 H, CHOH), 3.71 - 3.75 (m, 1 H, CHNH), 3.31 (br t, J = 4.3 Hz, 4 H, CH₂N(CH₂CH₂CH₂CH₂), 2.63 (t, J = 7.5 Hz, 2 H, CH=CH₂), 2.56 (br t, J = 4.2 Hz, 4 H, CH₂N(CH₂CH₂), 2.37 (t, J = 7.3 Hz, 2 H, CH₂N(CH₂CH₂), 2.03 - 2.60 (m, 2 H, C(=O)CH₂), 1.97 - 2.02 (m, 2 H, C(=O)CH₂CH₂), 1.89 (dddd, J = 143
13.1, 8.9, 7.4, 5.7 Hz, 1 H, CHHCHNH), 1.75 (ddt, J = 13.0, 8.9, 6.4, 6.4 Hz, 1 H, CHHCHOH), 1.61 - 1.66 (m, 2 H, CH=CCH₂CH₂), 1.57 - 1.61 (m, 1 H, CHHCH₂CHOH), 1.54 - 1.57 (m, 1 H, CHHCH₂CHOH), 1.49 - 1.53 (m, 2 H, CH=CC₂H₂CH₂), 1.40 (ddt, J = 13.0, 8.4, 5.3, 5.3 Hz, 1 H, CHHCHOH), 1.29 - 1.32 (m, 2 H, NCH(CHH)₂), 1.25 - 1.29 (m, 1 H, CHHCHNH), 1.13 - 1.20 (m, 2 H, NCH(CHH)₂)

¹³C NMR (175 MHz, DMSO d₆) δ / ppm = 176.3 (C(=O)CC(=O)OH), 170.9 (NH₃(O)(=O)CH₂), 166.1 (C(=O)OH), 153.0 (d, J = 251.4 Hz, ipso to F), 147.9 (C=CC(=O)OH), 146.9 (CH=CC₂H₂), 145.2 (d, J = 8.7 Hz, ipso to piperazine), 139.2 (para to F), 121.7 (NCH=CC₂H₂), 118.7 (d, J = 5.8 Hz, para to piperazine), 111.0 (d, J = 23.3 Hz, ortho to C=O and ortho to F), 106.3 (meta to C=O and meta to F and C(=O)OH), 76.2 (COH), 57.6 (CNH), 57.4 (CH=CC₂H₂CH₂CH₂CH₂N), 52.5 (CH=CC₂H₂CH₂CH₂N(CH₃)₂CH₂), 49.5 (d, J = 4.4 Hz, CH=CC₂H₂CH₂CH₂N(CH₃)₂CH₂), 48.8 (CH₂NCH=CC₂H₂), 35.8 (NCH(CH₂)₂), 32.2 (CH₂COH), 32.0 (C(=O)CH₂), 29.5 (CH₂CNH), 26.9 (CH=CC₂H₂), 26.0 (C(=O)CH₂CH₂), 25.8 (CH=CC₂H₂CH₂), 25.0 (CH=CC₂H₂), 20.5 (CH₂CH₂CHOH), 7.6 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -122.1 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 624.3314, [M+H]⁺ found, |C₃₂H₄₃F₅N₄O₅|⁺ requires 624.3310

[α]_{D}^{20} / °10⁻¹ cm² g⁻¹ = -3.6 (c / g(100 mL)⁻¹ = 0.0833, MeOH)

The compound has not been reported previously.

9.57 (1S,2S)-2-((tert-Butyldimethylsilyl)oxy)cyclopentan-1-amine 182

(1S,2S)-2-Aminocyclopentan-1-ol 172 (0.480 g, 4.75 mmol) was stirred in dry CH₂Cl₂ (20 mL) under N₂ at 0 °C. TEA (3.14 mL, 22.8 g, 22.5 mmol, 5 eq.) was added dropwise, followed by TBDMSOTf (3 mL, 3.45 g, 13.1 mmol, 3 eq.) dropwise. The reaction was allowed to reach r.t. and stirred for 1 h. The reaction was washed with water (20 mL) and the organic phase dried with Na₂SO₄, concentrated under reduced pressure and purified by column chromatography (SiO₂, 4% MeOH/CH₂Cl₂). 182 was obtained as a yellow oil (1.00 g, 4.64 mmol, 98%).

TLC RF = 0.23 (10% MeOH/CH₂Cl₂)

IR (neat) νmax / cm⁻¹ = 2954 (C-H), 2931 (C-H), 2888 (C-H), 2859 (C-H), 1625 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 4.13 (qt, J = 5.8 Hz, 1 H, CHOSi), 3.31 (td, J = 7.1, 5.2 Hz, 1 H, CHN₂), 2.09 - 2.19 (m, 1 H, CHHCH₂NH₂), 1.97 (ddq, J = 8.8, 7.0, 6.0 Hz, 1 H, CHHCHOSi), 1.74 - 1.86 (m, 2 H, CH₂CH₂CH(OSi)), 1.64 - 1.74 (m, 1 H, CHHCHOSi), 1.58 (ddt, J = 13.2, 9.1, 6.0 Hz, 1 H, CHHCH₂NH₂), 0.88 (s, 9 H, C(CH₃)₃), 0.09 (s, 3 H, SiCH₃), 0.07 (s, 3 H, SiCH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 76.3 (CHOSi), 59.7 (CHNH₂), 32.2 (CH₂CHOSi), 26.8 (CH₂CH₂NH₂), 25.6 (C(CH₃)₃), 19.7 (CH₂CH₂CH(OSi)), 17.7 (C(CH₃)₃), -4.8 (Si(CH₃), -5.2 (Si(CH₃))

144
HRMS (ESI$^+$) $m/z$ / Da = 216.1785, [M+H]$^+$ found, [C$_{11}$H$_{26}$NOSi]$^+$ requires 216.1784

[$\alpha$]$^\circ_{D}$ / 10$^{-1}$ cm$^2$g$^{-1}$ = 40.0 (c / g(100 mL)$^{-1}$ = 0.05, MeOH) The compound has not been reported previously.

9.58 4-Azido-N-((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclopentyl)butanamide 186

(1S,2S)-2-((tert-Butyldimethylsilyl)oxy)cyclopentan-1-amine 182 (50 mg, 0.232 mmol, 1 eq.) and NaHCO$_3$ (22.0 mg, 0.262 mmol, 1.1 eq.) were added to CH$_2$Cl$_2$ (3 mL) and water (3 mL) at 0 °C, and 4-bromobutyryl chloride (25.3 mL, 40.5 mg, 0.219 mmol, 0.95 eq.) was added dropwise. The mixture was stirred for 3 h at 0 °C. The aqueous layer was removed and NaN$_3$ (100 mg, 1.54 mmol, 6.6 eq.) and DMF (3 mL) were added. The mixture was then stirred at 40 °C for 6 h. The solvents were then evaporated using a N$_2$ stream and the residue was purified by column chromatography (SiO$_2$, 1% MeOH/CH$_2$Cl$_2$). The combined pure fractions were dried with MgSO$_4$ and evaporated under reduced pressure. 186 was obtained as a clear liquid (71 mg, 0.217 mmol, 99%).

**TLC** $R_f$ = 0.84 (1% MeOH/CH$_2$Cl$_2$)

**IR** (neat) $\nu_{max}$ / cm$^{-1}$ = 3288 (N-H), 2953 (C-H), 2933 (C-H), 2883 (C-H), 2857 (C-H), 2095 (azide), 1639 (amide C=O)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ / ppm = 5.35 (d, $J$ = 5.1 Hz, 1 H, NH), 3.97 - 4.01 (m, 1 H, CH$_2$OSi), 3.35 (t, $J$ = 6.6 Hz, 2 H, CH$_2$N$_3$), 2.24 (t, $J$ = 7.0 Hz, 2 H, CH$_2$C=O), 2.09 - 2.19 (m, 1 H, CHCHNH), 1.89 - 1.97 (quin, $J$ = 6.8 Hz, 2 H, CH$_2$CH$_2$N$_3$), 1.74 - 1.84 (m, 2 H, CHCHOSi and CHCH$_2$CHOsi), 1.60 - 1.70 (m, 1 H, CHCH$_2$CHOsi), 1.51 - 1.61 (m, 1 H, CHCHOSi), 1.31 - 1.39 (m, 1 H, CHCHNH), 0.87 (s, 9 H, C(CH$_3$)$_3$), 0.08 (s, 3 H, SiCH$_3$), 0.06 (s, 3 H, SiCH$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ / ppm = 171.17 (C=O), 77.80 (CHOSi), 58.36 (CHNH), 50.77 (CH$_2$N$_3$), 33.29 (CH$_2$C=O), 32.57 (CH$_2$CHOSi), 29.36 (CH$_2$CHNH), 25.72 (C(CH$_3$)$_3$), 24.77 (CH$_2$CH$_2$N$_3$), 20.40 (CH$_2$CH$_2$CHO Si), 17.95 (C(CH$_3$)$_3$), -4.75 (SiCH$_3$)

HRMS (ESI$^+$) $m/z$ / Da = 327.2221, [M+H]$^+$ found, [C$_{15}$H$_{31}$N$_4$O$_2$Si]$^+$ requires 327.2216

[$\alpha$]$^\circ_{D}$ / 10$^{-1}$ cm$^2$g$^{-1}$ = 12.4 (c / g(100 mL)$^{-1}$ = 0.5, MeOH)

The compound has not been reported previously.
1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190

IR (neat) νmax / cm⁻¹ = 2951 (C-H), 2929 (C-H), 2856 (C-H), 1741 (carboxylic acid C=O), 1640 (amide)

1H NMR (400 MHz, CDCl₃) δ / ppm = 8.67 (s, 1 H, ortho to C(=O)OH), 7.87 (d, J = 13.1 Hz, 1 H, ortho to F), 7.34 (s, 1 H, CH=C=CH₂), 7.33 (d, J = 8.2 Hz, 1 H, meta to F), 5.92 (t, J = 6.6 Hz, 1 H, CHNH), 4.35 (t, J = 6.7 Hz, 2 H, CH₂NCH=CH₂), 3.96 - 4.02 (m, 1 H, CHNH), 2.66 (br s, 4 H, CH₂N(CH₃)CH₂), 2.71 (t, J = 7.5 Hz, 2 H, CH=CHCH₃), 2.46 (t, J = 7.3 Hz, 2 H, CH₂N(CH₂)CH₂), 2.03 - 2.22 (m, 5 H, CH₂CH₂NH, C(=O)CH₂ and C(=O)CH₂), 1.65 - 1.83 (m, 4 H, CHCH₂CH₂OSi and CHCH₂CH₂OSi and N(CH₃)CH₂), 1.47 - 1.65 (m, 4 H, CHCH₂OMe, CHCH₂OMe and NCH=CH₂CH₂), 1.33 - 1.41 (m, 3 H, CH₂CH₂NH and NCH(CH₃)₂), 1.14 - 1.20 (m, 2 H, NCH(CH₂)₂), 0.82 (s, 9 H, C(CH₃)₃), 0.03 (s, 3 H, SiCH₃), 0.01 (s, 3 H, SiCH₃)

13C NMR (101 MHz, CDCl₃) δ / ppm = 176.9 (C(=O)CC(=O)OH), 170.9 (CH₂C(=O)NH), 166.9 (C(=O)OH), 153.5 (d, J = 251.4 Hz, ipso to F), 147.9 (CH=CH₂), 147.2 (C=CC(=O)OH), 145.8 (d, J = 10.4 Hz, ipso to piperazine), 139.0 (para to F), 120.9 (NCH=CH₂), 119.4 (d, J = 7.8 Hz, para to piperazine), 112.0 (d, J = 23.4 Hz, ortho to C=O and ortho to F), 107.7 (CC(=O)OH), 104.7 (d, J = 3.5 Hz, meta to C=O and meta to F), 77.7 (CHOSi), 58.2 (CHNH), 57.9 (CH=CH₂CH₂CH₂N), 52.6 (CH=CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (d, J = 6.1 Hz, CH=CH₂CH₂CH₂N(CH₂)CH₂), 48.9 (d, J = 3.5 Hz, CH₂NC=CH₂), 35.3 (NCH(CH₂)₂), 32.6 (C(=O)CH₂), 32.6 (CH₂CH₂OSi), 29.3 (CH₂CH₂NH), 27.2 (CH=CH₂CH₂), 26.0 - 26.3 (C(=O)CH₂CH₂ and CH=CH₂CH₂CH₂), 25.6 (C(CH₃)₃), 25.4 (CH=CH₂), 20.4 (CH₂CH₂CH₂OSi), 17.8 (C(CH₃)₃), 8.1 (NCH(CH₂)₂), -4.8 (SiCH₃)

HRMS (ESI⁺) m/z / Da = 738.4164, [M+H]⁺ found, [C₃₈H₅₇FN₇O₅Si]⁺ requires 738.4169
The compound has not been reported previously.

9.60 4-Chloro-N-((1S,2S)-2-hydroxycyclopentyl)butanamide 193

![Chemical Structure](image)

(1S,2S)-2-Aminocyclopentan-1-ol 172 (72.3 mg, 716 µmol, 1 eq.), TEA (500 µl, 363 mg, 3.58 mmol, 5 eq.) and CH₂Cl₂ (5 mL) were stirred at 0 °C, and 4-chlorobutyryl chloride 192 (179 µl, 226 mg, 1.60 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 30 min, then water (10 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with 10% i-PrOH/CHCl₃ (2×10 mL). The combined organic layers were dried with MgSO₄, concentrated under reduced pressure and purified by column chromatography (SiO₂, Et₂O). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 193 was obtained as a white amorphous solid (35.6 mg, 173 µmol, 24%).

TLC Rf = 0.35 (EtOAc)

[^1]H NMR (400 MHz, CDCl₃) δ / ppm = 6.05 (br s, 1 H, NH), 4.55 (br s, 1 H, OH), 3.95 (q, J = 6.6 Hz, 1 H, CH₃OH), 3.82 (tt, J = 8.4, 5.3 Hz, 1 H, CH₂NH), 3.60 (t, J = 6.2 Hz, 2 H, CH₂Cl), 2.38 (t, J = 7.0 Hz, 2 H, CH₂C=O), 2.05 - 2.17 (m, 3 H, CHHCHNH and CH₂CH₂Cl), 1.94 - 2.05 (m, 1 H, CHHCHOH), 1.74 - 1.86 (m, 1 H, CHHCH₂CHOH), 1.58 - 1.74 (m, 2 H, CHHCH₂CHOH and CHHCHOH), 1.42 (dq, J = 12.5, 8.4 Hz, 1 H, CHHCHNH)

[^13]C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 79.4 (CH₂OH), 60.6 (CH₂NH), 44.4 (CH₂Cl), 32.8 (CH₂C=O), 32.4 (CH₂CHOH), 30.2 (CH₂CH₂NH), 28.0 (CH₂CH₂Cl), 21.2 (CH₂CH₂CHOH)

HRMS (ESI⁺) m/z / Da = 206.0939, [M+H]^+ found, [C₉H₁₇ClNO₂]^+ requires 206.0948

[^α]D²⁰ / °10⁻¹cm²g⁻¹ = 10.0 (c / g(100 mL)⁻¹ = 0.05, MeOH)

The compound has not been reported previously.

9.61 4-Chloro-N-((1R,2R)-2-hydroxycyclopentyl)butanamide 194

![Chemical Structure](image)

(1R,2R)-2-Aminocyclopentan-1-ol 173 (500 mg, 4.94 mmol, 1 eq.), TEA (827 µl, 600 mg, 5.93 mmol, 1.2 eq.) and CH₂Cl₂ (20 mL) were stirred at 0 °C and 4-chlorobutyryl chloride 192 (608 µl, 766 mg, 5.43 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 30 min, then water (50 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with CH₂Cl₂ (7×50 mL).
combined organic layers were dried with MgSO₄, concentrated under reduced pressure and purified by column chromatography (SiO₂, Et₂O). The combined pure fractions were dried with MgSO₄ and evaporated under reduced pressure. 194 was obtained as a white amorphous solid (651 mg, 3.16 mmol, 64%).

TLC $R_f = 0.35$ (EtOAc)

IR (neat) $\nu_{\max}$ / cm$^{-1} = 3278$ (N-H and O-H), 2962 (C-H), 2876 (C-H), 1636 (amide C=O)

$^1$H NMR (400 MHz, CDCl₃) $\delta$ / ppm = 6.12 (br s, 1 H, NH), 4.42 (br s, 1 H, OH), 3.94 (q, $J = 6.6$ Hz, 1 H, CHOH), 3.82 (tt, $J = 8.4, 5.3$ Hz, 1 H, CHNH), 3.60 (t, $J = 6.2$ Hz, 2 H, CH₂Cl), 2.38 (t, $J = 7.2$ Hz, 2 H, CH₂C=O), 2.05 - 2.16 (m, 3 H, CH$_2$CHNH and CH$_2$CH₂Cl), 1.58 - 1.73 (m, 2 H, CHCH₂CHOH and CHHCHOH), 1.43 (dq, $J = 12.7, 8.3$ Hz, 1 H, CHHCHNH)

$^{13}$C NMR (101 MHz, CDCl₃) $\delta$ / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH₂Cl), 32.8 (CH₂C=O), 32.4 (CH₂CHOH), 30.1 (CH₂CHNH), 28.0 (CH₂CH₂Cl), 21.1 (CH₂CH₂CHOH)

HRMS (ESI$^+$) $m/z$ / Da = 228.0787, [M+Na]$^+$ found, [C$_9$H$_{16}$ClNNaO$_2$]$^+$ requires 228.0762

[α]$_{D}^{20}$ / °10$^{-1}$cm$^2$g$^{-1}$ = -13.0 ($c / g(100$ mL)$^{-1} = 0.5, MeOH$)

The compound has not been reported previously.

9.62 Methyl 7-(4-(4-(tert-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 197

![Chemical Structure](https://via.placeholder.com/150)

Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 151 (200 mg, 0.579 mmol, 1 eq.), tert-butyl 4-bromobutanoate 196 (103 µl, 130 mg, 0.581 mmol, 1 eq.), NaI (86.9 mg, 0.580 mmol, 1 eq.), TEA (316 µl, 229 mg, 2.27 mmol, 4 eq.) and acetonitrile (10 mL) were stirred in a microwave reactor at 100 °C for 8 h. A second portion of tert-butyl 4-bromobutanoate 222 (103 µl, 130 mg, 0.581 mmol, 1 eq.) was added, and the mixture was stirred in the microwave reactor at 100 °C for a further 8 h. The mixture was then dry-loaded onto SiO₂ and purified by column chromatography (SiO₂, 0-4% MeOH/CH₂Cl₂). 197 was obtained as a white amorphous solid (141 mg, 0.289 mmol, 50%).

TLC $R_f = 0.12$ (4% MeOH/CH₂Cl₂)

IR (neat) $\nu_{\max}$ / cm$^{-1} = 2962$ (C-H), 2831 (C-H), 1732 (i-Bu ester C=O) 1717 (ciprofloxacin ester C=O), 1621 (quinolone C=O)

$^1$H NMR (400 MHz, CDCl₃) $\delta$ / ppm = 8.39 (s, 1 H, ortho to C(=O)OCH₃), 7.82 (d, $J = 13.3$ Hz, 1 H, ortho...
to F), 7.17 (d, J = 7.2 Hz, 1 H, meta to F), 3.83 (s, 3 H, CH₃), 3.40 (tt, J = 7.2, 3.6 Hz, 1 H, NCH(=CH₂)), 3.22 (t, J = 4.3 Hz, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.63 (t, J = 4.4 Hz, 4 H, CH₂N(CH₂CH₂)CH₂), 2.41 (t, J = 7.3 Hz, 2 H, CH₂N(CH₂CH₂), 2.25 (t, J = 7.4 Hz, 2 H, CH₂CH₂N(CH₂CH₂), 1.78 (quin, J = 7.3 Hz, 2 H, CH₂CH₂N(CH₂CH₂), 1.41 (s, 9 H, C((CH₃)₃)), 1.24 (m, 2 H, NCH(CHH))₂), 1.09 (m, 2 H, NCH(CHH))₂)

\(^{13}\text{C NMR}\) (101 MHz, CDCl₃) \(\delta / \text{ppm} = 172.7\) (C(=O)CC(=O)OCH₃), 172.6 (C(=O)OC(CH₃)₃), 165.9 (C(=O)OCH₃), 153.1 (d, J = 249.7 Hz, ipso to F), 148.1 (C=CC(=O)OCH₃), 144.3 (d, J = 10.4 Hz, ipso to piperazine), 137.7 (para to F), 122.5 (d, J = 6.9 Hz, para to piperazine) 112.6 (d, J = 22.5 Hz, ortho to C=O and para to F), 109.5 (CC(=O)OCH₃) 104.7 (meta to C=O and meta to F), 80.0 (C(=CH₃)₃), 57.4 (C(=O)CH₂CH₂CH₂N), 52.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂)), 51.7 (CH₃), 49.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂)), 49.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 34.4 (NCH(CHH)₂), 33.2 (C(=O)CH₂), 28.0 (C(CH₃)₃), 22.0 (C(=O)CH₂CH₂), 7.9 (NCH(CHH)₂)

\(^{19}\text{F NMR}\) (376.45 MHz, CDCl₃) \(\delta / \text{ppm} = -123.5\) (s, ciprofloxacin F)

HRMS (ESI⁺) \(m/z / Da = 488.2562, [M+H]^+\) found, \([\text{C}_{26}\text{H}_{35}\text{FN}_{3}\text{O}_{5}]^+\) requires 488.2561

The compound has not been reported previously.

9.63 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate 198

Methyl 7-(4-(4-(tert-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 197 (20 mg, 41.0 \(\mu\)mol) and TFA (0.2 mL) were stirred in CH₂Cl₂ (1.8 mL) at r.t. for 16 h then evaporated under reduced pressure. 198 was obtained as a white solid (21.4 mg, 39.2 \(\mu\)mol, 96%).

mp T / °C = 225-231 (CH₂Cl₂, decomposes)

IR (neat) \(\nu_{\text{max}} / \text{cm}^{-1} = 1723\) (ciprofloxacin ester C=O), 1699 (alkyl carboxylic acid C=O), 1673 (TFA C=O), 1615 (quinolone C=O)

\(^1\text{H NMR}\) (400 MHz, DMSO \(d₆\) \(\delta / \text{ppm} = 8.47\) (s, 1 H, ortho to C(=O)OH), 7.80 (d, J = 13.2 Hz, 1 H, ortho to F), 7.47 (d, J = 7.4 Hz, 1 H, meta to F), 3.73 (s, 3 H, CH₃), 3.66 (tt, J = 7.2, 3.7 Hz, 1 H, NCH(CH₂)₂), 3.30 - 3.54 (br s, 8 H, CH₂N(CH₂)CH₂ and CH₂N(CH₂CH₂)CH₂CH₂), 3.13 - 3.22 (m, 2 H, CH₂N(CH₂CH₂), 2.36 (t, J = 7.1 Hz, 2 H, CH₂CH₂N(CH₂CH₂), 1.87 - 1.98 (m, 2 H, CH₂CH₂N(CH₂CH₂), 1.22 - 1.30 (m, 2 H, NCH(CHH)₂), 1.06 - 1.15 (m, 2 H, NCH(CHH)₂)

\(^{13}\text{C NMR}\) (101 MHz, DMSO \(d₆\) \(\delta / \text{ppm} = 173.5\) (CH₂C(=O)OH), 171.6 (C(=O)CC(=O)OCH₃), 164.9 (C(=O)OCH₃), 158.2 (q, J = 31.5 Hz, CF₃C(=O)OH), 152.5 (d, J = 247.6 Hz, ipso to F), 148.5 (C=CC(=O)OH), 142.3 (d, J = 10.7 Hz, ipso to piperazine), 138.0 (para to F), 122.6 (d, J = 6.4 Hz, para to piperazine), 117.2 (q,
\(J = 299.8 \text{ Hz, } \text{CF}_3\), 111.9 (d, \(J = 22.4 \text{ Hz, ortho to } \text{C=O and ortho to F}\)), 111.9 (d, \(J = 22.4 \text{ Hz, ortho to } \text{C=O and meta to } \text{F}\)), 55.1 (C(=O)CH\(_2\)CH\(_2\)CH\(_2\)N(\(\text{CH}_2\)CH\(_2\)), 46.7 (C(=O)CH\(_2\)CH\(_2\)CH\(_2\)(\(\text{CH}_2\)CH\(_2\)), 46.7 (C(=O)CH\(_2\)CH\(_2\)CH\(_2\)(\(\text{CH}_2\)CH\(_2\)), 34.9 (NCH(\(\text{CH}_2\)2)), 30.6 (C(=O)\(\text{CH}_2\)), 19.1 (C(=O)\(\text{CH}_2\)), 7.6 (NCH(\(\text{CH}_2\)2))

\(^{19}\text{F NMR\ (376.45 MHz, DMSO d}_6\) \(\delta / \text{ppm = -73.6 (s, CF}_3\)), -124.6 (s, ciprofloxacin F)

HRMS (ESI\(^+\)) \(m/z / Da = 432.1921, [M+H]^+\) found, \([\text{C}_{22}\text{H}_{27}\text{FN}_3\text{O}_5]^+\) requires 432.1935

The compound has not been reported previously.

9.64 \((\text{trans)-2-Aminocyclohexan-1-ol 200\)}

\[
\begin{array}{c}
\text{NH}_2 \\
\text{OH} \\
\text{CH}_2
\end{array}
\]

Cyclohexene oxide 199 (10 mL, 9.70 g, 98.8 mmol, 1 eq.), \(\text{NH}_3\) (90 mL, 35% w/w aq., 27.7 g, 791 mmol, 8 eq.) and MeOH (100 mL) were stirred at r.t. for 72 h. The solvent was removed by blowing a stream of \(\text{N}_2\) over it, followed by evaporation under high vacuum. 200 was obtained as a white amorphous solid (9.90 g, 85.2 mmol, 86%)

TLC \(R_f = 0.04\) (30% MeOH/\(\text{CH}_2\)Cl\(_2\))

IR (neat) \(\nu_{max} / \text{cm}^{-1} = 3350\) (N-H), 3306 (br, O-H), 2927 (C-H), 2853 (C-H)

\(^1\text{H NMR\ (400 MHz, CDCl}_3\) \(\delta / \text{ppm = 3.01 (td, } \(J = 9.4, 4.8 \text{ Hz, 1 H, CH}_\text{OH}\), 2.80 - 2.92 (m, 2 H, \(\text{OH and NH}_2\)), 2.35 (ddd, } \(J = 11.1, 9.1, 4.1 \text{ Hz, 1 H, CH}_\text{NH}_2\)), 1.77 - 1.84 (m, 1 H, \(\text{CH}_\text{HCHOH}\)), 1.69 - 1.76 (m, 1 H, \(\text{CHHCHNH}_2\)), 1.56 - 1.66 (m, 1 H, \(\text{CHHCH}_2\text{CHOH}\)), 1.45 - 1.56 (m, 1 H, \(\text{CHHCH}_2\text{CHNH}_2\)), 1.07 - 1.19 (m, 3 H, \(\text{CHHCH}_2\text{CHOH, CHHCH}_2\text{CHNH}_2\) and \(\text{CHHCH}_2\text{CHOH}\)), 0.94 - 1.05 (m, 1 H, \(\text{CHHCHNH}_2\))

\(^{13}\text{C NMR\ (101 MHz, CDCl}_3\) \(\delta / \text{ppm = 75.4 (CHOH), 56.6 (CH}_2\text{N}_2\), 33.8 (CH}_2\text{CHOH and CH}_2\text{CHN}_2\), 24.7 (CH}_2\text{CH}_2\text{CHNH}_2\), 24.6 (CH}_2\text{CH}_2\text{CHOH}\)

HRMS (ESI\(^+\)) \(m/z / Da = 116.1070, [M+H]^+\) found, \([\text{C}_8\text{H}_{14}\text{NO}]^+\) requires 116.1070

The data are consistent with the literature.\(^{228}\)
9.65 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((trans-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201

4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate 198 (200 mg, 0.367 mmol, 1 eq.), (trans)-2-aminocyclohexanol 200 (91.1 mg, 0.791 mmol, 2.1 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (112 mg, 0.584 mmol, 1.6 eq.), 1-hydroxybenzotriazole (96 mg, 0.710 mmol, 1.9 eq.) and DIPEA (192 µl, 142 mg, 1.10 mmol, 3 eq.) were dissolved in DMF (5 mL) and stirred at r.t. for 16 h. The solvent was removed using a stream of N₂ and the residue was purified by preparative HPLC (5-50% acetonitrile/water over 10 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO₃ (aq., sat., 10 mL) and CH₂Cl₂ (10 mL). The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 201 was obtained as a white amorphous solid (61.2 mg, 0.116 mmol, 32%).

IR (neat) \( \nu_{\text{max}} \) / cm⁻¹ = 3303 (N-H), 2930 (C-H), 2851 (C-H), 2833 (C-H), 1698 (ester C=O), 1646 (amide C=O), 1614 (quinolone C=O)

¹H NMR (400 MHz, MeOD) \( \delta \) / ppm = 8.60 (s, 1 H, ortho to C(=O)OCH₃), 7.79 (d, \( J = 13.5 \) Hz, 1 H, ortho to F), 7.46 (d, \( J = 7.2 \) Hz, 1 H, meta to F), 3.84 (s, 3 H, CH₃), 3.62 - 3.68 (m, 1 H, NCH(CH₂)₂), 3.58 (td, \( J = 10.3 \), 4.2 Hz, 1 H, CHNH), 3.38 (br s, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.60 (t, \( J = 7.3 \) Hz, 2 H, C(=O)CH₂CH₂CH₂N), 2.32 (td, \( J = 7.1 \), 3.1 Hz, 2 H, C(=O)CH₂), 1.96 - 2.04 (m, 1 H, CHHCHOH), 1.87 - 1.96 (m, 3 H, CHHCHNH and C(=O)CH₂CH₂), 1.72 - 1.77 (m, 1 H, CHHCH₂CHOH), 1.66 - 1.72 (m, 1 H, CHHCH₂CHNH), 1.25 - 1.39 (m, 5 H, CHHCHOH, CHHCH₂CHOH, CHHCH₂CHNH and NCH(CH₂CH₂)), 1.15 - 1.25 (m, 3 H, CHHCH₂CHOH and NCH(CH₂CH₂))

¹³C NMR (101 MHz, MeOD) \( \delta \) / ppm = 175.8 (CH₂C(=O)NH), 175.3 (C(=O)CC(=O)OCH₃), 166.8 (C(=O)OCH₃), 154.9 (d, \( J = 248.8 \) Hz, ipso to F), 150.2 (C=CC(=O)OCH₃), 146.1 (d, \( J = 10.8 \) Hz, ipso to piperazine), 139.9 (para to F), 123.5 (d, \( J = 7.5 \) Hz, para to piperazine), 113.2 (d, \( J = 23.2 \) Hz, ortho to C=O and ortho to F), 110.2 (CC(=O)OCH₃), 107.2 (meta to C=O and meta to F), 74.1 (CHHOH), 58.9 (C(=O)CH₂CH₂CH₂N), 56.4 (CHNH), 54.0 (C(=O)CH₂CH₂CH₂N(CH₂CH₂), 52.3 (CH₃), 50.5 (d, \( J = 5.0 \) Hz, C(=O)CH₂CH₂CH₂N(CH₂CH₂CH₂), 36.4 (NCH(CH₂)₂), 35.7 (CH₂CHOH), 35.1 (C(=O)CH₂), 32.8 (CH₂CHNH), 25.9 (CH₂CH₂CHNH), 25.5 (CH₂CH₂CHOH), 23.5 (C(=O)CH₂CH₂), 8.7 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, MeOD) \( \delta \) / ppm = -124.7 (ciprofloxacin F)

HRMS (ESI⁺) \( m/z \) / Da = 529.2827, [M+H]⁺ found, [C₂₈H₃₈FN₄O₅]⁺ requires 529.2826

The compound has not been reported previously.
Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-oxo-4-(2-oxocyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201 (5.2 mg, 9.84 µmol, 1 eq.) and Dess-Martin periodinane (16.4 mg, 38.7 µmol, 4 eq.) were stirred in CH₂Cl₂ (3 mL) at r.t. for 6 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-95% acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure to a volume of 20 mL, then NaHCO₃ (aq., sat., 30 mL) and 10% i-PrOH/CHCl₃ (30 mL) were added. The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 202 was obtained as a white amorphous solid (3.6 mg, 6.8 µmol, 69%).

TLC \( R_f = 0.74 \) (30% MeOH/CH₂Cl₂)

IR (neat) \( \nu_{max} / \text{cm}^{-1} = 2921 \) (C-H), 2852 (C-H), 1721 (ketone C=O), 1698 (ester C=O), 1639 (amide C=O), 1620 (quinolone C=O)

\(^1\text{H} \text{NMR} \) (400 MHz, DMSO d₆) \( \delta / \text{ppm} = 8.45 \) (s, 1 H, ortho to C(=O)OCH₃), 7.87 (d, \( J = 6.2 \) Hz, 1 H, NHH), 7.76 (d, \( J = 13.4 \) Hz, 1 H, ortho to F), 7.44 (d, \( J = 7.5 \) Hz, 1 H, meta to F), 4.42 (ddd, \( J = 13.0, 7.6, 1.0 \) Hz, 1 H, CH₃N(CH₂CH₂CH₂N(CH₂CH₂)), 3.73 (s, 3 H, CH₃), 3.65 (tt, \( J = 7.1, 3.9 \) Hz, 1 H, NCH(CH₂CH₂), 2.58 (br s, 4 H, CH₂N(CH₂CH₂CH₂CH₂), 2.45 - 2.53 (m, 1 H, CHHHC(=O)CHNH), 2.36 (br s, 2 H, C(=O)CH₂CH₂CH₂N), 2.26 (dtt, \( J = 13.4, 2.6, 1.6 \) Hz, 1 H, CHHCH(=O)CHNH), 2.16 - 2.22 (m, 2 H, C(=O)CH₂CH₂CH₂N), 2.12 (ddq, \( J = 12.7, 6.0, 2.8 \) Hz, 1 H, CHHCHNH), 1.41 - 1.56 (m, 2 H, CHHCHNH and CHHCH₂C(=O)), 1.20 - 1.30 (m, 2 H, NCH(CH₂CH₂)), 1.05 - 1.13 (m, 2 H, NCH(CH₂CH₂)₂)

\(^1\text{C} \text{NMR} \) (101 MHz, DMSO d₆) \( \delta / \text{ppm} = 207.5 \) (C(=O)CHNH), 171.7 (C(=O)CC(=O)OCH₃), 171.6 (CH₂C(=O)NH), 165.0 (C(=O)OCH₃), 152.6 (d, \( J = 247.6 \) Hz, ipso to F), 148.3 (C=CC(=O)OCH₃), 143.9 (br s, ipso to piperazine), 138.1 (para to F), 121.8 (d, \( J = 6.4 \) Hz, para to piperazine), 111.5 (d, \( J = 22.4 \) Hz, ortho to C=O and ortho to F), 109.0 (C(=O)OCH₃), 106.3 (meta to C=O and meta to F), 57.0 (CHNH and C(=O)CH₂CH₂CH₂N), 52.3 (br s, C(=O)CH₂CH₂CH₂N(CH₂CH₂), 51.3 (CH₃), 49.5 (br s, C(=O)CH₂CH₂CH₂N(CH₂CH₂CH₂CH₂), 40.6 (CH₂C(=O)CHNH), 34.8 (NCH(CH₂CH₂), 33.9 (CH₂CHNH), 32.9 (C(=O)CH₂CH₂CH₂N), 27.2 (CH₂CH₂C(=O)CHNH), 23.8 (CH₂CH₂CH₂CH₂N), 22.4 (br s, C(=O)CH₂CH₂CH₂CH₂N), 7.6 (NCH(CH₂CH₂)₂)

\(^1\text{F} \text{NMR} \) (376.45 MHz, DMSO d₆) \( \delta / \text{ppm} = -124.3 \) (ciprofloxacin F)

HRMS (ESI⁺) \( m/z / Da = 527.2654, [M+H]^+ \) found, \([C_{28}H_{36}FN_4O_5]^+ \) requires 527.2670

The compound has not been reported previously.
9.67 4-Chloro-N-((trans)-2-hydroxycyclohexyl)butanamide 203

(trans)-2-Aminocyclohexan-1-ol 200 (1.04 g, 9.03 mmol, 1 eq.), TEA (1.65 mL, 1.20 g, 11.8 mmol, 1.3 eq.) and CH₂Cl₂ (50 mL) were stirred at 0 °C. 4-Chlorobutyryl chloride 192 (1.22 mL, 1.54 g, 10.9 mmol, 1.2 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 30 min, then water (50 mL) was added. The organic layer was separated off, and the aqueous layer was extracted with 10% i-PrOH/CHCl₃ (2 × 50 mL). The combined organic layers were dried with MgSO₄, concentrated under reduced pressure and purified by column chromatography (SiO₂, 0-100% EtOAc/Et₂O). The combined organic fractions were dried with MgSO₄ and evaporated under reduced pressure. 203 was obtained as white needles (1.51 g, 6.87 mmol, 76%).

TLC Rf = 0.19 (Et₂O)

mp T °C = 73-76 (i-PrOH, CHCl₃)

IR (neat) νmax/cm⁻¹ = 3290 (N-H), 3250 (O-H), 2928 (C-H), 2857 (C-H), 1629 (amide C=O)

¹H NMR (400 MHz, MeOD) δ/ppm = 3.60 (t, J = 6.6 Hz, 2 H, CH₂Cl), 3.51 - 3.60 (m, 1 H, CHNH), 3.28 - 3.39 (m, 1 H, CHOH), 2.37 (td, J = 7.4, 2.3 Hz, 2 H, C(=O)CH₂), 2.06 (quin, J = 7.0 Hz, 2 H, C(=O)CH₂CH₂), 1.97 - 2.01 (m, 1 H, CHHCH₂OH), 1.85 - 1.93 (m, 1 H, CHHCH₂NH), 1.70 - 1.77 (m, 1 H, CHHCH₂CHOH), 1.64 - 1.70 (m, 1 H, CHHCH₂CH₂OH), 1.24 - 1.35 (m, 3 H, CHHCH₂CH₂OH, CHHCH₂CH₂NH and CHHCH₂OH), 1.13 - 1.25 (m, 1 H, CHHCH₂CH₂NH)

¹³C NMR (101 MHz, MeOD) δ/ppm = 175.0 (C(=O)), 74.1 (CHOH), 56.3 (CHNH), 45.3 (CH₂Cl), 35.6 (CH₂CH₂OH), 34.5 (C(=O)CH₂), 32.7 (CH₂CH₂NH), 30.1 (C(=O)CH₂CH₂), 25.8 (CH₂CH₂CH₂NH), 25.5 (CH₂CH₂CH₂CH₂OH)

HRMS (ESI⁺) m/z / Da = 242.0925, [M+Na]⁺ found, [C₁₀H₁₈ClNNaO₂]⁺ requires 242.0924

The compound has not been reported previously.

9.68 4-Azido-N-((trans)-2-hydroxycyclohexyl)butanamide 204

4-Chloro-N-((trans)-2-hydroxycyclohexyl)butanamide 203 (345 mg, 1.57 mmol, 1 eq.) and NaN₃ (180 mg, 2.77 mmol, 1.75 eq.) were stirred in DMF (12 mL) at 50 °C for 16 h. Water (50 mL) and 10% i-PrOH/CHCl₃ (50 mL) were added, and the organic layer was removed. The aqueous layer was extracted again with 10% i-PrOH/CHCl₃ (50 mL) and the combined organic fractions were dried with MgSO₄. The solvent was evaporated under reduced pressure, and then by using a N₂ stream. 204 was obtained as large white prisms (347 mg, 1.53
mmol, 98%).

**TLC** $R_f = 0.23$ (EtOAc)

**mp** $T / {^\circ}C = 75-76$ (i-PrOH, CHCl$_3$)

**IR** (neat) $\nu_{\text{max}} / \text{cm}^{-1} = 3299$ (N-H), 3208 (O-H), 2944 (C-H), 2928 (C-H), 2859 (C-H), 2089 (azide), 1624 (amide C=O)

**$^1$H NMR** (400 MHz, MeOD) $\delta / \text{ppm} = 7.87$ (d, $J = 7.9$ Hz, 1 H, NH), 5.27 (d, $J = 4.3$ Hz, 1 H, OH), 3.56 (td, $J = 10.5$, 4.4 Hz, 1 H, CHNH), 3.28 - 3.41 (m, 3 H, CH$_2$OH and CH$_3$N$_3$), 2.30 (td, $J = 7.4$, 2.7 Hz, 2 H, C(=O)CH$_2$), 1.95 - 2.03 (m, 1 H, CHCHCHOH), 1.87 (m, 3 H, C(=O)CH$_2$H$_2$ and CHHCHNH), 1.70 - 1.76 (m, 1 H, CHHCH$_2$CHOH), 1.63 - 1.70 (m, 1 H, CHHCH$_2$CHNH), 1.25 - 1.38 (m, 3 H, CHHCH$_2$CHOH, CHHCH$_2$CHNH and CHHCHOH), 1.14 - 1.24 (m, 1 H, CHHCHNH$_2$)

**$^{13}$C NMR** (101 MHz, MeOD) $\delta / \text{ppm} = 175.1$ (C(=O)), 74.0 (CHOH), 56.3 (CHNH), 52.0 (CH$_3$N$_3$), 35.5 (CH$_2$CHOH), 34.3 (C(=O)CH$_2$), 32.7 (CH$_2$CHNH), 26.3 (C(=O)CH$_2$CH$_2$), 25.8 (CH$_2$CH$_2$CHNH), 25.5 (CH$_2$CH$_2$CHOH)

**HRMS** (ESI$^+$) $m/z$ / Da = 249.1331, [M+Na]$^+$ found, [C$_{10}$H$_{18}$N$_4$NaO$_2$]$^+$ requires 249.1327

The compound has not been reported previously.

9.69 1-Cyclopropyl-6-fluoro-7-(4-(4-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 205

1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68 (40 mg, 97.2 $\mu$mol, 1 eq.) and 4-azido-N-((trans)-2-hydroxycyclohexyl)butanamide 204 (22.0 mg, 97.2 $\mu$mol, 1 eq.) were dissolved in 10% water/t-BuOH (3 mL), and the mixture was degassed by bubbling N$_2$ through it. A solution of CuSO$_4$ and THPTA (97.2 $\mu$l, 9.72 $\mu$mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (194 $\mu$l, 19.4 $\mu$mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at r.t. under argon for 16 h. Water (50 mL) and 10% i-PrOH/CHCl$_3$ (50 mL) were added, then the organic layer was separated, dried with MgSO$_4$ and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-70% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO$_3$ (aq., sat., 50 mL) and 10% i-PrOH/CHCl$_3$ (50 mL). The organic layer was dried with MgSO$_4$ and evaporated under reduced pressure. 205 was obtained as a white amorphous solid (30.3 mg, 47.5 $\mu$mol, 49%).

154
IR (neat) $\nu_{max} / \text{cm}^{-1} = 3345$ (N-H), 2928 (C-H), 2860 (C-H), 2815 (C-H), 1727 (carboxylic acid C=O), 1642 (amide C=O), 1626 (quinolone C=O), 1619 (triazole)

$^1$H NMR (400 MHz, DMSO $d_6$) $\delta / \text{ppm} = 8.64$ (s, 1 H, ortho to C(=O)OH), 7.86 (d, $J = 13.9$ Hz, 1 H, ortho to F), 7.84 (s, 1 H, CH=CCH$_2$), 7.64 (d, $J = 8.1$ Hz, 1 H, NH), 7.54 (d, $J = 7.5$ Hz, 1 H, meta to F), 4.54 (d, $J = 4.7$ Hz, 1 H, OH), 4.30 (t, $J = 6.8$ Hz, 2 H, C(=O)CH$_2$CH$_2$CH$_2$N), 3.77 - 3.86 (m, 1 H, NCH(CHOH)$_2$), 3.33 - 3.40 (m, 1 H, CHNH), 3.31 (br t, $J = 4.8$ Hz, 4 H, CH=CCH$_2$CH$_2$N(CH$_2$)$^3$CH$_2$CH$_2$N), 3.14 - 3.24 (m, 1 H, CHOH), 2.63 (t, $J = 7.4$ Hz, 2 H, CH=CCH$_2$), 2.56 (br t, $J = 4.6$ Hz, 4 H, CH=CCH$_2$CH$_2$N(CH$_2$)CH$_2$), 2.38 (t, $J = 6.9$ Hz, 2 H, CH=CCH$_2$CH$_2$N), 2.04 - 2.08 (m, 2 H, C(=O)CH$_2$CH$_2$N), 1.96 - 2.04 (m, 2 H, C(=O)CH$_2$CH$_2$N), 1.78 - 1.87 (m, 1 H, CHHCHOH), 1.69 - 1.78 (m, 1 H, CHHCHNH), 1.63 (quin, $J = 7.5$ Hz, 2 H, CH=CCH$_2$CH$_2$N), 1.54 - 1.60 (m, 2 H, CHHCH$_2$OH), 1.51 (quin, $J = 7.4$ Hz, 2 H, CH=CCH$_2$CH$_2$N), 1.28 - 1.35 (m, 2 H, NCH(CHOH)$_2$), 1.11 - 1.22 (m, 5 H, NCH(CHOH)$_2$, CHHCHOH, CHHCH$_2$CHOH and CH$_2$CH$_2$CHNH), 1.04 - 1.13 (m, 1 H, CHHCHNH)

$^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta / \text{ppm} = 176.4$ (C(=O)CC(=O)OH), 170.9 (CH$_2$C(=O)NH), 166.0 (C(=O)OH), 153.1 (d, $J = 252.1$ Hz, ipso to F), 148.0 (C=C(=O)OH), 146.9 (CH=CCH$_2$), 145.3 (d, $J = 10.0$ Hz, ipso to pipazine), 139.2 (para to F), 121.8 (NCH=CCH$_2$), 118.5 (d, $J = 8.3$ Hz, para to pipazine), 110.9 (d, $J = 23.2$ Hz, ortho to C=O and ortho to F), 106.7 (CC(=O)OH), 106.3 (d, $J = 3.3$ Hz, meta to C=O and meta to F), 71.4 (CHOH), 57.4 (CH=CCH$_2$CH$_2$CH$_2$N), 54.2 (CHNH), 52.4 (CH=CCH$_2$CH$_2$CH$_2$N(CH$_2$)CH$_2$), 49.5 (CH=CCH$_2$CH$_2$CH$_2$N(CH$_2$)CH$_2$), 49.5 (CH=CCH$_2$CH$_2$CH$_2$N(CH$_2$)CH$_2$), 48.8 (C(=O)CH$_2$CH$_2$NCH=C), 35.9 (NCH(CHOH)$_2$), 34.1 (CH$_2$CHOH), 32.3 (C(=O)CH$_2$CH$_2$NCH=C), 31.1 (CH$_2$CHNH), 26.9 (CH=CCH$_2$CH$_2$N), 26.1 (C(=O)CH$_2$CH$_2$NCH=C), 25.8 (CH=CCH$_2$CH$_2$CH$_2$N), 25.0 (CH=CCH$_2$CH$_2$CH$_2$N), 24.2 (CH$_2$CH$_2$CHNH), 23.8 (CH$_2$CH$_2$CHOH), 7.6 (NCH(CHOH)$_2$)

$^{19}$F NMR (376.45 MHz, DMSO $d_6$) $\delta / \text{ppm} = -121.4$ (ciprofloxacin F‘)

HRMS (ESI+) $m/z / Da = 638.3480$, [M+H]$^+$ found, [C$_{33}$H$_{45}$FN$_7$O$_5$]$^+$ requires 638.3466

The compound has not been reported previously.

9.70 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-1-(4-oxo-4-((2-oxocyclohexyl)amino)butyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 206

![Image of the compound structure]

1-Cyclopropyl-6-fluoro-7-(4-(4-1-(4-(((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 205 (15.0 mg, 23.6 mmol, 1 eq.) and Dess-Martin periodinane (35.0 mg, 82.5 mmol, 3.5 eq.) were stirred in CH$_2$Cl$_2$ (3 mL) at r.t. for 4 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-70% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure, then NaHCO$_3$ (aq., sat., 30 mL)
and 10% i-PrOH/CHCl₃ (30 mL) were added. The organic layer was dried with MgSO₄ and evaporated under reduced pressure. 206 was obtained as a clear gum (11.7 mg, 18.4 μmol, 78%).

IR (neat) \( \nu_{\text{max}} / \text{cm}^{-1} = 2941 \) (C-H), 2860 (C-H), 1720 (carboxylic acid C=O and ketone C=O), 1657 (amide \( \text{C} = \text{O} \)), 1530 (pyrimidine \( \text{CN} \)), 1390 (ortho \( \text{CH} \)), 1217 (ortho \( \text{CH} \)), 794 (ortho \( \text{CH} \)). Biological testing

Crystal violet-stained plates were also read at 595 nm. Only a 5 h OD reading in YM64 was obtained for the autoinducer-antibiotic conjugates. At 595 nm were taken at 5 and 24 h, and biofilm quantification was carried out soon after the 24 h OD reading. Crystal violet-stained plates were also read at 595 nm. Only a 5 h OD reading in YM64 was obtained for the autoinducer-antibiotic conjugates. At 595 nm were taken at 5 and 24 h, and biofilm quantification was carried out soon after the 24 h OD reading.

The compound has not been reported previously.

9.71 Biological testing

Compounds were tested against \( P. \) aeruginosa PAO1 \( ^{180} \) and YM64. \( ^{181} \) C₁₂-HSL 19, HHQ 21, PQS 22, ciprofloxacin 24, trimethoprim 25 and DMSO were included as controls, along with LB to check for contamination of the plates.

The first set of autoinducer-antibiotic conjugates (see 7.5) were tested at 2, 1, 0.5, 0.25, 0.125 and 0.0625 μM. Breathe-Easy© sealing membranes from Diversified Biotech were used and the plates were placed without lids in a open box containing tissue paper wetted with distilled water in order to control evaporation. OD readings at 595 nm were taken at 5 and 24 h, and biofilm quantification was carried out soon after the 24 h OD reading. Crystal violet-stained plates were also read at 595 nm. Only a 5 h OD reading in YM64 was obtained for the autoinducer-antibiotic conjugates.

The HSL analogue-Cip(Me) conjugates (see 8.7) were tested at 25, 2, 1, 0.5, 0.25 and 0.125 μM in triplicate. AeraSeal™ films from Excel Scientific were used. A plate lid was used, but the humidified box was not. OD readings at 600 nm were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 h. Biofilm inhibition testing was carried
out on plates grown for 24 and 48 h. Biofilm dispersal testing was carried out by growing plates for 24 h, followed by addition of the compounds, incubation for a further 24 h and quantification of the biofilms. Crystal violet-stained plates were read at 550 nm.

9.71.1 Antibiotic susceptibility

Antibiotic susceptibility was determined using spectrophotometry measurements. Colonies of the desired strains were grown at 37 °C overnight on LB agar. The colonies were used to inoculate LB (10 mL) and these cultures were grown at 37 °C overnight. The cultures were diluted 1/100 with LB, and 99 μl diluted culture per well was added to Nunclon® flat-bottomed clear 96-well plates. 1 μl of compound solution in DMSO was then added from master plates and the plates were covered with adhesive aeration filters. The plates were shaken at 37 °C and 100 rpm and OD was recorded periodically using a Biochrom EZ Read 400 microplate reader.

9.71.2 Quantification of biofilms

Biofilms were quantified using a method described previously.126,242 After the bacteria had grown for the desired amount of time, the culture was aspirated out of the wells using a pipette tip attached to a vacuum pump, making sure not to touch the sides of the wells. Water (120 μl) was then added and aspirated out again. This process was repeated twice more to thoroughly wash out planktonic cells. Crystal violet (120 μl, 0.1% m/v) was added and left for 15 min, then aspirated out. The wells were washed again with water (3 × 120 μl). Acetic acid (120 μl, 30% v/v aq.) was added and left for 15 min then the plate was vortexed and read using a Biochrom EZ Read 400 microplate reader at 595 nm.

9.71.3 Biofilm inhibition

The plates were prepared as in 9.71.1. The plates were shaken at 37 °C and 100 rpm for 24 h followed by quantification of biofilm growth as shown in 9.71.2.

9.71.4 Biofilm dispersal

The plates were prepared as in 9.71.1, initially without the addition of compound solutions. The box of plates was shaken at 37 °C and 100 rpm for 24. 1 μl of compound solution in DMSO was then added to each well from master plates and the plates were shaken as above for a further 24 h followed by measurement of OD and quantification of biofilm growth as shown in 9.71.2.
10 NMR spectra

10.1 (S)-4-Bromo-N-(2-oxotetrahydrofuran-3-yl)butanamide 57
10.2 \((S)-6\text{-Bromo-}N\text{-}(2\text{-oxotetrahydrofuran-3-yl})\text{hexanamide}\) 60
10.3  \((S)-6\text{-Azido-}N\text{-}(2\text{-oxotetrahydrofuran-3-yl})\text{hexanamide}\) 61
10.4  tert-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 65
10.5 1-(Hex-5-yn-1-yl)piperazine 66
10.6 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68
10.7 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71

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10.8  (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 72
10.9 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77
(S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid
10.11 1-Cyclopropyl-6-fluoro-7-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 80
(S)-4-(4-(4-(2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1H-1,2,3-triazol-1-yl)-N-(2-oxotetrahydrofuran-3-yl)butanamide

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(S)-6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1H-1,2,3-triazol-1-yl)-N-(2-oxotetrahydrofuran-3-yl)hexanamide
10.14 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl) -1H-1,2,3-triazol-1-yl)-2-heptylquinolin-4(1H)-one 87
10.15 2-(6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1H-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(1H)-one 89
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10.18 4-Azido-\(N\)-(2-oxotetrahydrothiophen-3-yl)butanamide 155
10.19 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquine
ol-ine-3-carboxylic acid 156
1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-((4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1H-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazine-1-yl)-1,4-dihydroquinoline-3-carboxylic acid
10.21  4-Bromo-N-(2-methoxyphenyl)butanamide 159
Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 160
10.23 1-Cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl) butyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 162
10.24 4-Bromo-N-(3-methoxyphenyl)butanamide 164
Methyl 1-cyclopropyl-6-fluoro-7-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 165
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10.27 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 167
10.28 4-Azido-N-((1S,2S)-2-hydroxycyclopentyl)butanamide 176
4-Azido-\(N-((1R,2R)-2\)-hydroxycyclopentyl)butanamide
10.30  Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1S,2S)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 178
Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((1R,2R)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate
10.32 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1S,2S)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 180
10.33 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1R,2R)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
10.34 4-Azido-\(N-((1S,2S)-2-((\text{tert-butyldimethylsilyl})\text{oxy})\text{cyclopentyl})\)butanamide

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\[\text{TBSOcy}_5\text{NH}_4\text{N}_3\_SS\_H.esp\]

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10.35 7-(4-(4-(1-(4-(((1S,2S)-2-(tert-Butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190
10.36 4-Chloro-\(N-(1S,2S)-2\)-hydroxycyclopentyl)butanamide 193
10.37 4-Chloro-N-((1R,2R)-2-hydroxycyclopentyl)butanamide 194

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Methyl 7-(4-(4-(tert-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate
10.39 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt 198
10.40  Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201

![NMR Spectra](image-url)
Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate

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10.42 4-Chloro-N-((trans)-2-hydroxycyclohexyl)butanamide 203

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10.43 4-Azido-\((trans)-2\)-hydroxycyclohexyl)butanamide 204
10.44 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 205
10.45 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-hydroxy-4-((2-oxocyclohexyl)amino)butyl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 206
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