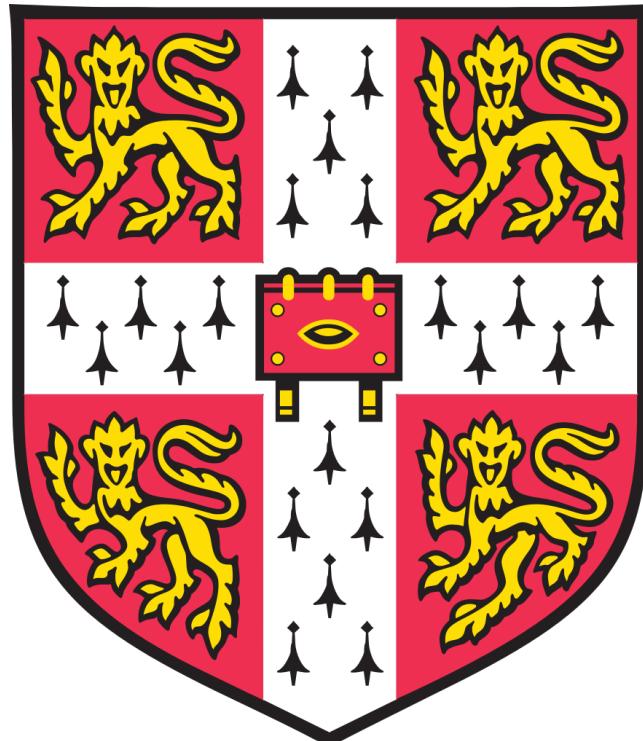


The synthesis and biological evaluation of a library of
autoinducer-antibiotic conjugates

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10.321-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1 <i>H</i> -1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 180	194
10.331-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1 <i>H</i> -1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 181	195
10.344-Azido- <i>N</i> -((1 <i>S</i> ,2 <i>S</i>)-2-((<i>tert</i> -butyldimethylsilyl)oxy)cyclopentyl)butanamide 186	196
10.357-(4-(4-(1-(4-((1 <i>S</i> ,2 <i>S</i>)-2-((<i>tert</i> -butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1 <i>H</i> -1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190	197
10.364-Chloro- <i>N</i> -((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclopentyl)butanamide 193	198
10.374-Chloro- <i>N</i> -((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclopentyl)butanamide 194	199

10.38Methyl 7-(4-(4-(<i>tert</i> -butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 197	200
10.394-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt 198	201
10.40Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-(((<i>trans</i>)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201	202
10.41Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 202	203
10.424-Chloro- <i>N</i> -((<i>trans</i>)-2-hydroxycyclohexyl)butanamide 203	204
10.434-Azido- <i>N</i> -((<i>trans</i>)-2-hydroxycyclohexyl)butanamide 204	205
10.441-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((<i>trans</i>)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1 <i>H</i> -1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 205	206
10.451-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxocyclohexyl)amino)butyl)-1 <i>H</i> -1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 206	207
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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.^{1–3} A new class of antibiotics, namely siderophore-antibiotic conjugates, has shown promise in initial studies.^{4,5} 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 autoinducers⁶ 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.⁷

The quorum sensing molecules produced by *Pseudomonas aeruginosa* were chosen for investigation as *P. aeruginosa* is a significant human pathogen⁸ which displays high resistance to many antibiotics⁹ and uses quorum sensing to coordinate its group behaviours.¹⁰ Ciprofloxacin and trimethoprim were chosen as the antibiotic partners. Ciprofloxacin is commonly used against *P. aeruginosa*¹¹ but resistance to it is developing,¹² 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.^{13,14} 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.¹⁵

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

Firstly, I would like to thank David Spring for the opportunity to work on this very interesting topic. I would also like to thank Hannah Sore for guidance and support through the later stages of the project, and Eddy Sotelo and Bin Yu for collaboration and useful discussions. Thank you to Mark Eldridge and Suzie Forrest for help with learning biochemical techniques, to Martin Welch for guidance and advice on data interpretation and especially to Tom O'Brien for stepping in to do some of the testing. Thanks also to Matt Pond, Melvyn Orriss, Nic Davies and Naomi Hobbs for help with equipment and glassware and to Jill Vaughan and Tommy Osberger for proof-reading. Most importantly, I would like to thank Yssy Baker for proof-reading, help, support, advice and encouragement.

4 Nomenclature

<i>m</i>	Mass
<i>v</i>	Volume
<i>J</i>	Coupling constant in Hz
<i>m/z</i>	Mass to charge ratio in Daltons
<i>R_f</i>	Retention factor
Ac	Acetate
AIP	Autoinducing peptide
aq.	Aqueous
atm	Atmosphere(s)
BHL	Butyryl homoserine lactone = C ₄ -HSL 19
Boc	<i>tert</i> -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	<i>N,N</i> -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

HHQ 2-Heptylquinolin-4(1H)-one

HMBC Heteronuclear multiple-bond correlation spectroscopy

HMQC Heteronuclear multiple-quantum correlation spectroscopy

HOEt 1-Hydroxybenzotriazole

HPLC High-performance liquid chromatography

HRMS High resolution mass spectroscopy

HSL Homoserine lactone

Hz Hertz

IR Infrared

LB Lysogeny broth

LCMS Liquid chromatography mass spectroscopy

LCT Liquid chromatography time-of-flight

lit. Literature value

M Molar

m.p. Melting point

Me Methyl

MIC Minimum inhibitory concentration

min Minute(s)

mol Mole(s)

Ms Methanesulfonyl

NMP *N*-Methyl-2-pyrrolidone

NMR Nuclear magnetic resonance

OD Optical density

OdDHL *N*-(3-Oxododecanoyl)-homoserine lactone = 3-oxo-C₁₂-HSL **20**

P.E. Petroleum ether

PAI-1 *Pseudomonas* autoinducer 1 = 3-oxo-C₁₂-HSL **20**

PAI-2 *Pseudomonas* autoinducer 2 = C₄-HSL **19**

Pd/C Palladium on carbon

PQS Pseudomonas Quinolone Signal

Q-TOF Quadrupole time-of-flight

r.t. Room temperature

s Second(s)

SAM *S*-adenosyl-L-methionine
SAR Structure activity relationship
sat. Saturated
SD Standard deviation
sp. 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.² 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 *Staphylococcus aureus* to become contaminated with *Penicillium* mould whilst he was on holiday in Suffolk² is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,¹⁶ 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.^{17,18} 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:^{2,3}

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of β -lactam antibiotics such as penicillin by β -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.¹⁹
3. The bacterium may pump antibacterial molecules out of its cell membrane using efflux pumps, for example the MexAB and MexXY pumps used by *Pseudomonas aeruginosa*.⁹
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.²⁰
5. The bacterium may switch to using a metabolic pathway which does not involve the target of the anti-bacterial molecule, for example sulfonamide resistance may be achieved by taking in folic acid from the environment rather than synthesising it using *para*-aminobenzoic acid - a process which is blocked by sulfonamides.²¹

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

Table 1: A timeline of when various antibiotics were first introduced and when resistance to them first appeared.^{22–27}

The current pipeline of new antibiotics is widely thought to be worryingly inadequate.^{28–30} 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,²⁸ 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.³¹ These treatments often target bacterial virulence rather than killing bacteria outright, hence decreasing selection pressure for resistance.²² 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*.³² 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.²²

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 conjugates³³ 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,⁴ 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.⁴ 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’.³⁴ 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 Fe³⁺, 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 Fe³⁺ to Fe²⁺ 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.³⁵

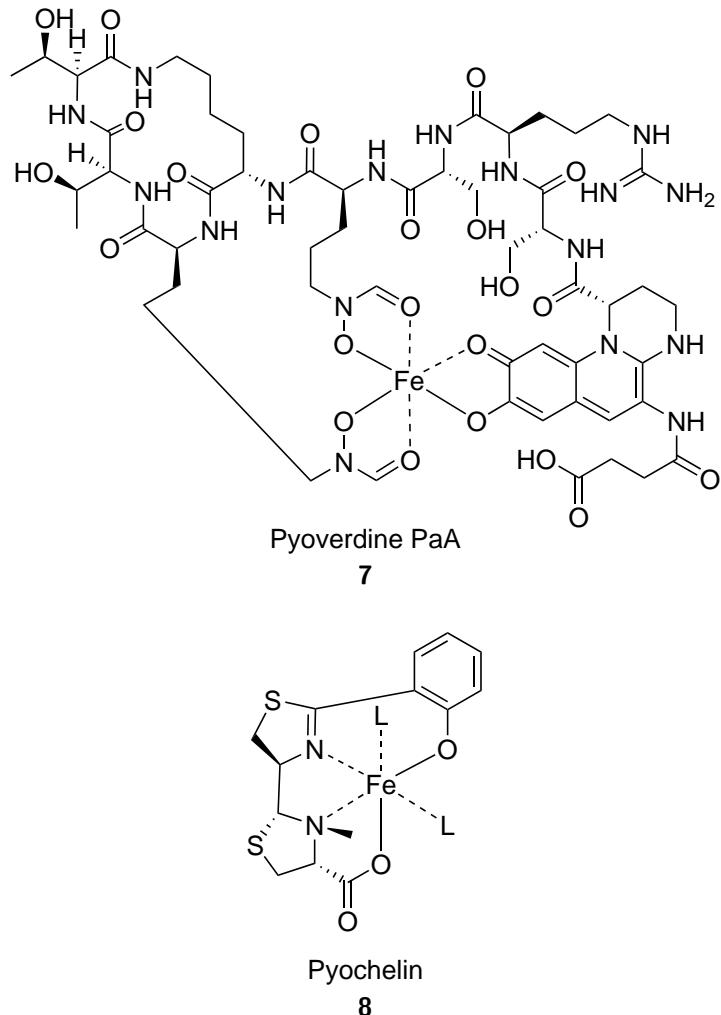


Figure 1: Iron-siderophore complexes: pyoverdine PaA **7**^{36,37} (*P. aeruginosa*, PAO1 strain) and pyochelin **8**^{38,39} (*P. aeruginosa*). Note that pyochelin **8** is a tetradeятate ligand, hence the iron ion has two sites which can bind other ligands.

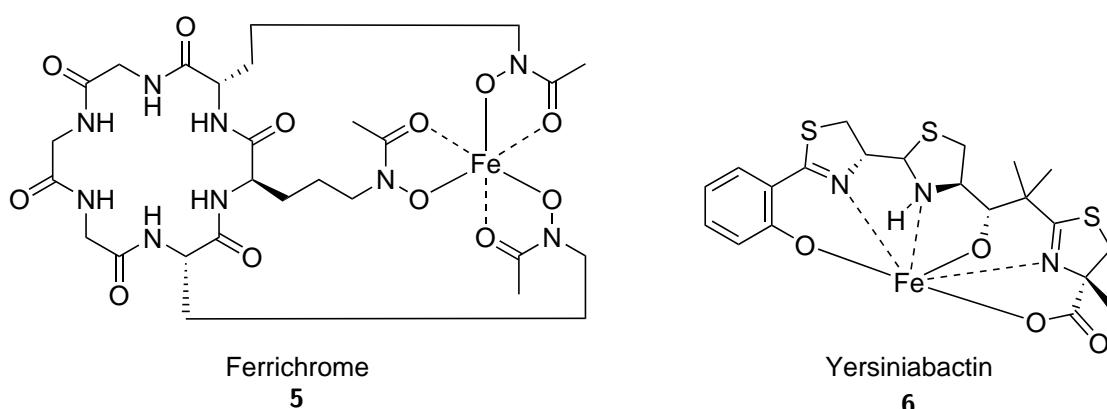
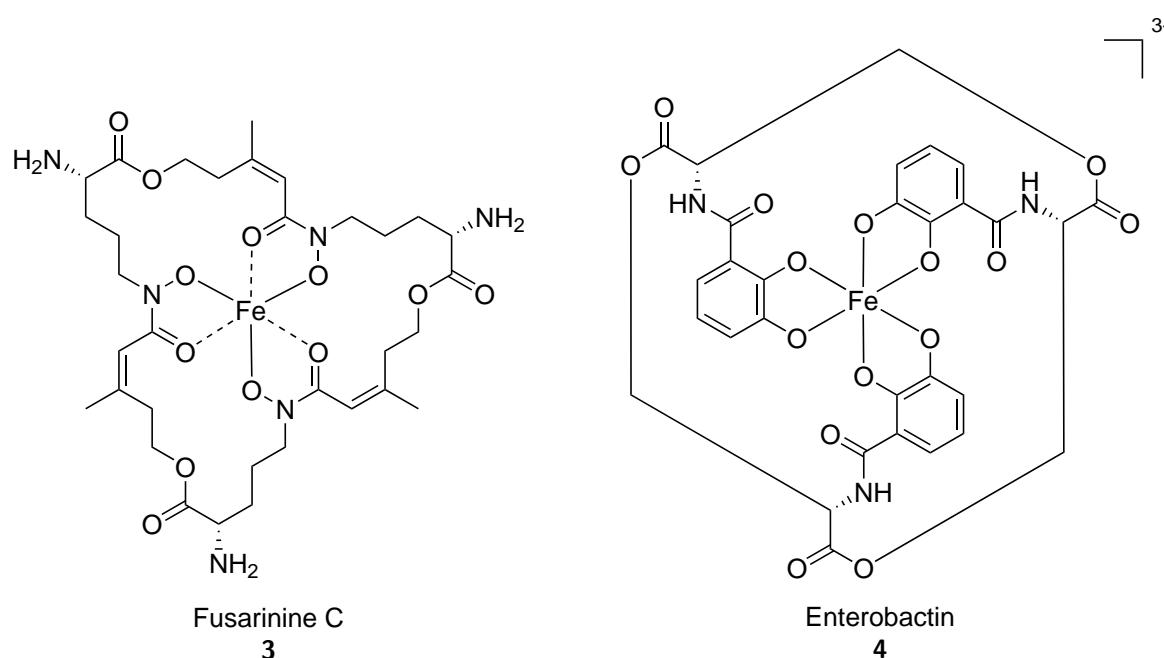
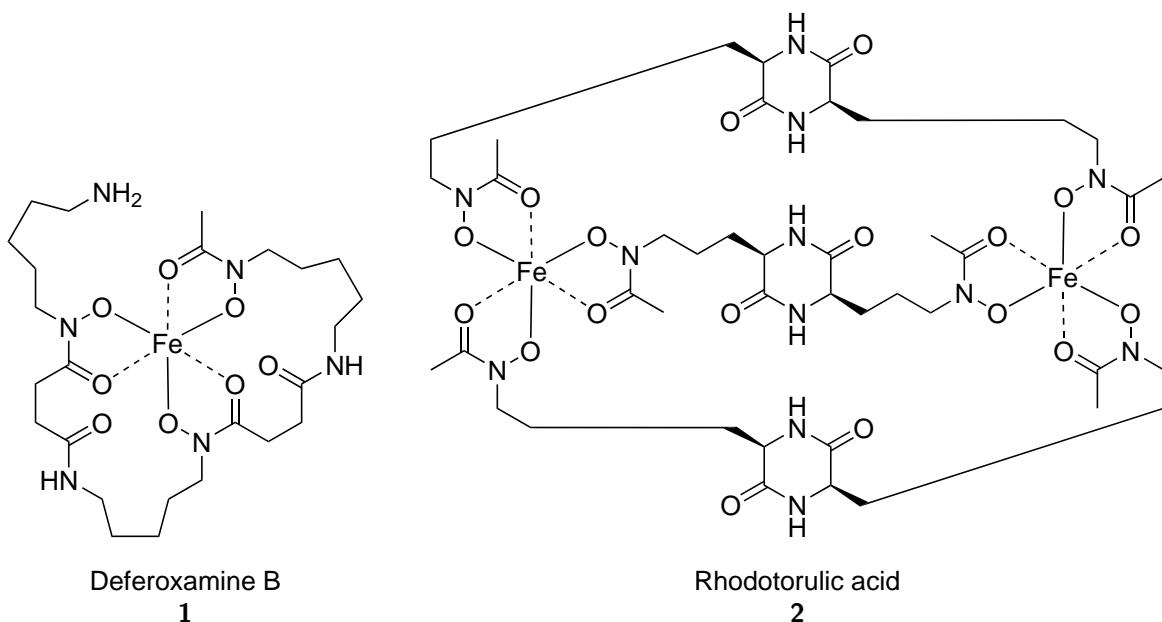


Figure 2: Iron-siderophore complexes: Deferoxamine B **1**³⁶ (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid **2**⁴⁰ (*Rhodotorula pilimanae*), fusarinine C **3**⁴¹ (*Fusarium roseum*), enterobactin **4**³⁶ (*Escherichia coli* and enteric bacteria), ferrichrome **5**⁴² (*Ustilago sphaerogenes*, *U. maydis*, *Aspergillus niger*, *A. quadricinctus*, *A. duricaulis* and *Penicillium resticulosum*), yersiniabactin **6**³⁶ (*Yersinia pestis*).

5.2.2 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins⁴ (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 *Actinomyces subtropicus* and *Streptomyces griseus*^{43,44} which has been used to treat infections caused by various bacteria including *Yersinia enterocolitica* and *Streptococcus pneumoniae* in mice and humans.^{45,46} Albomycin **9** contains a siderophore coupled to a nucleoside 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 *Escherichia coli*, *Salmonella typhimurium* and *P. aeruginosa*.^{42,47} It has been shown that because of the structural similarity to ferrichrome **5**, *E. coli* will also take up albomycin **9**.⁴³ The linker is hydrolysed in the cytoplasm of the *E. coli*, releasing the active nucleoside antibiotic. This leads to 500-fold concentration of the antibiotic within the *E. coli* cells, enough to have significant effect on growth.

The success of albomycin⁴⁵ and other sideromycins such as salmycin A^{34,48,49} and ferrimycin A1^{50,51} has served as encouragement to many researchers to explore synthetic siderophore-antibiotic conjugates, which will be discussed in the next section.

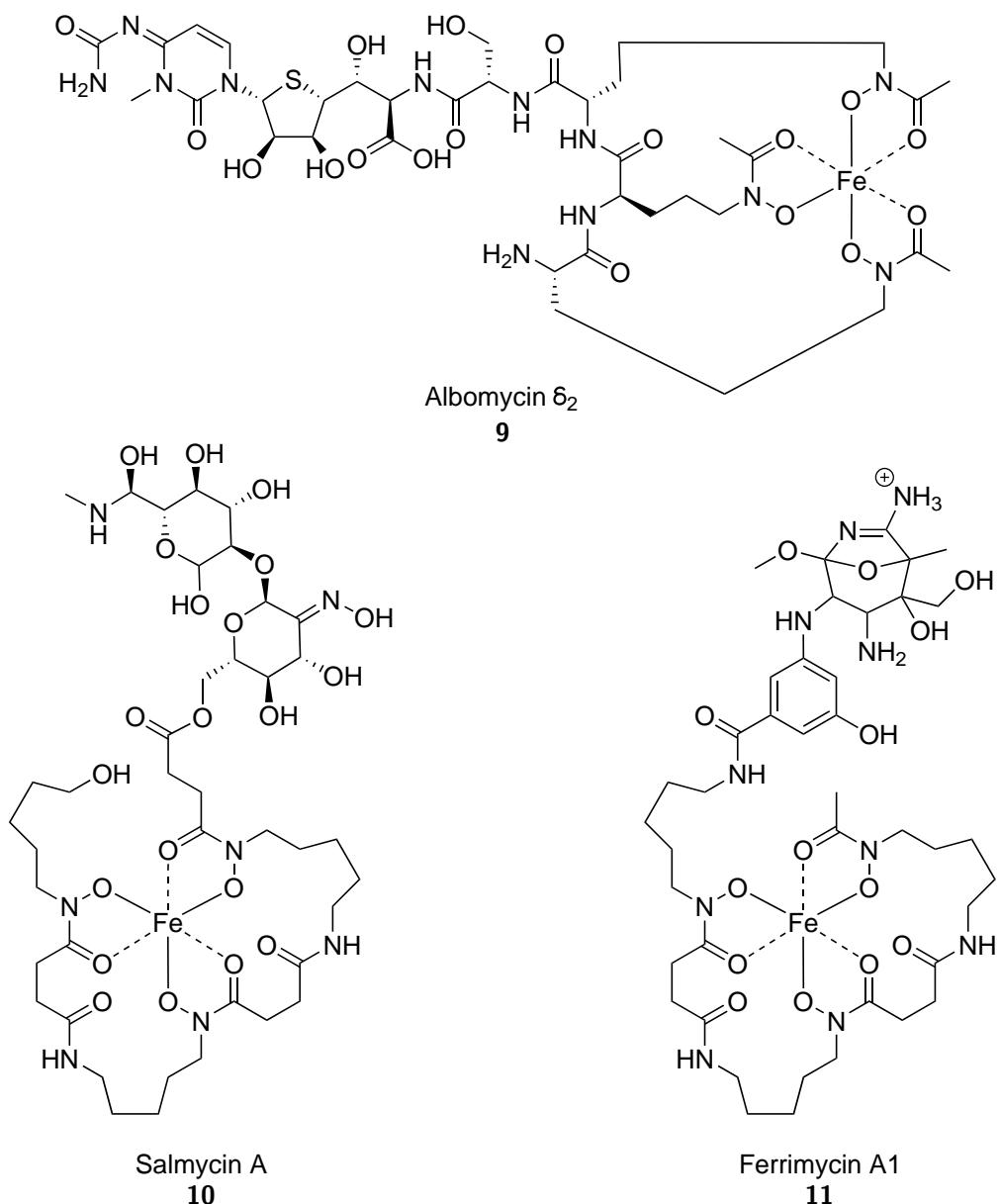


Figure 3: Iron-sideromycin complexes: Albomycin **9**^{34,52} (*Actinomyces subtropicus* and *Streptomyces griseus*), salmycin A^{34,48,49} (*Streptomyces violaceus*) and ferrimycin³⁴ (*Streptomyces griseoflavus*).

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.⁴ Antibiotics used include β -lactams,^{53–55} nucleosides,⁵⁶ glycopeptides⁵⁷ and macrolides.⁵⁸ Sideromycin-fluoroquinolone conjugates have also been studied by several groups,^{59–61} including conjugates with linkers which can be cleaved^{60,61} in a similar manner to albomycin.⁴³ 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.

β -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 FhuA for hydroxamate siderophores.⁴ 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.⁶² Other

work has focused on monocyclic β -lactams, for example pirazmonam **13** and U-78608 **14**, which show high potency against Gram-negative bacteria including *P. aeruginosa*.^{63,64} Monocyclic β -lactams are generally fairly stable to β -lactamase activity, which is an advantage compared with many bicyclic β -lactams.

Three siderophore-antibiotic conjugates are reported as being in clinical trials:⁵ MC-1 **15**,⁶⁵ BAL30072 **16**⁴ (see Figure 4) and cefiderocol **17**.^{66,67}

MC-1 **15** is reported as being ‘in clinical phases of development’,⁵ but no reports of studies in humans could be found. However, experiments in mice have been promising.⁶⁵ BAL30072 **16** is a siderophore- β -lactam conjugate which showed initial promise as it is a poor substrate for β -lactamases, and resistance due to loss of transport proteins is infrequent.⁴ However, it is unclear whether it will progress further in trials as it causes liver toxicity.⁶⁸ 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 mg were well tolerated in healthy 36 subjects’.⁶⁷

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,⁴⁶ 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 virulence⁶⁹ with an antibiotic can be extended to conjugates of autoinducers and antibiotics in a similar ‘Trojan horse’ approach.

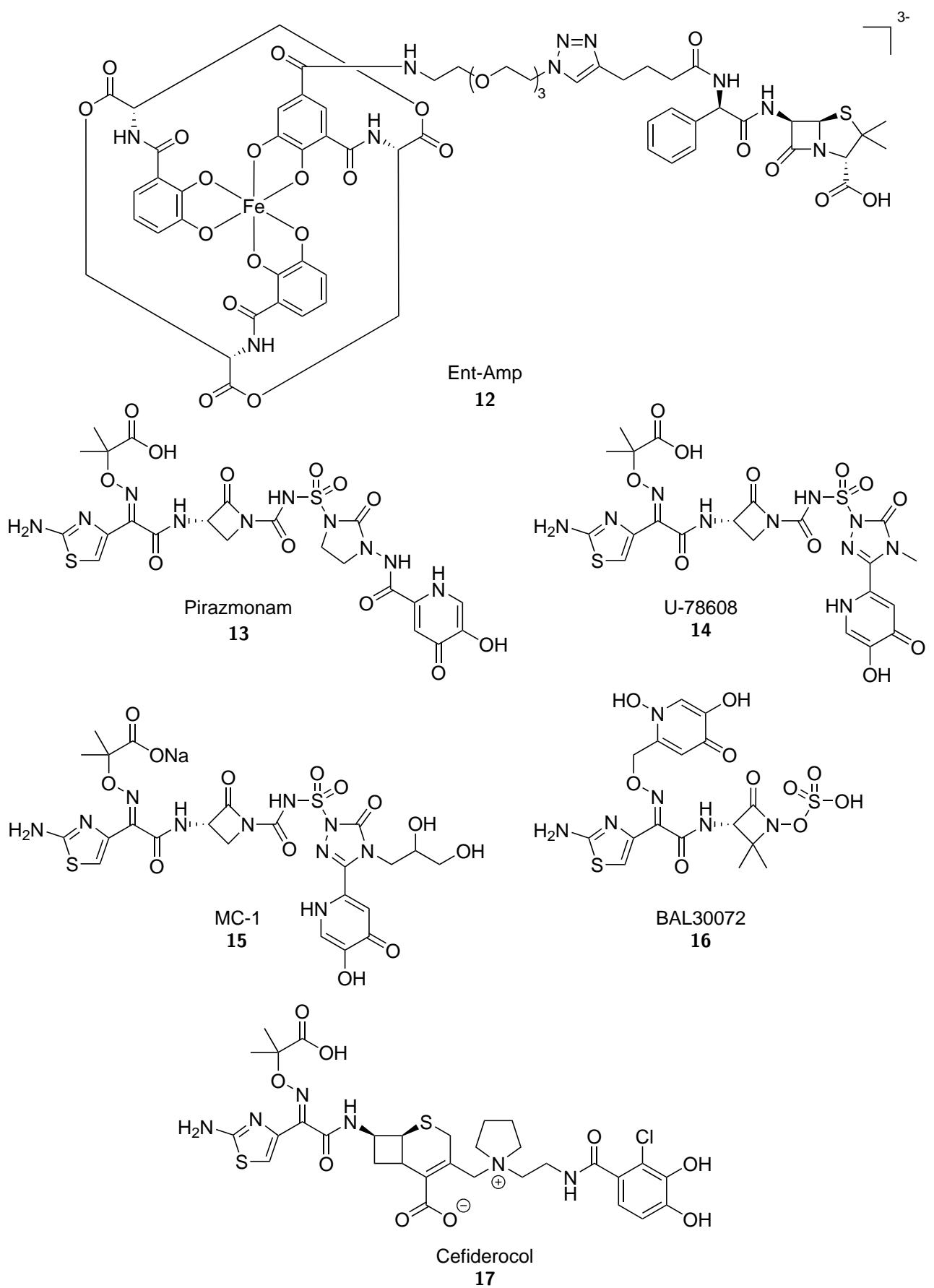


Figure 4: Examples of siderophore-antibiotic conjugates: Ent-Amp **12**,⁶² pirazmonam **13**,^{63, 64} U-78608 **14**,^{63, 64} MC-1 **15**,⁶⁵ BAL30072 **16**⁴ and cefiderocol **17**.^{66, 67}

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 previously¹⁵ (see 5.3.8). 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.’⁷⁰ 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.⁷ 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.*^{6, 7, 71–78} 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-typoidal *Salmonella* causes 1.2 million illnesses, 23,000 hospitalisations and 450 deaths.¹

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*^{7, 77, 78} (see Figure 5). This bacterium receives amino acids^{79, 80} from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself.⁸¹

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 incentivises the squid to provide them with nutrients.



Figure 5: ‘Euprymna scolopes, South shore of Oahu, Hawaii’ by Jamie Foster. Licensed under CC BY-SA 3.0 via Commons.

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).

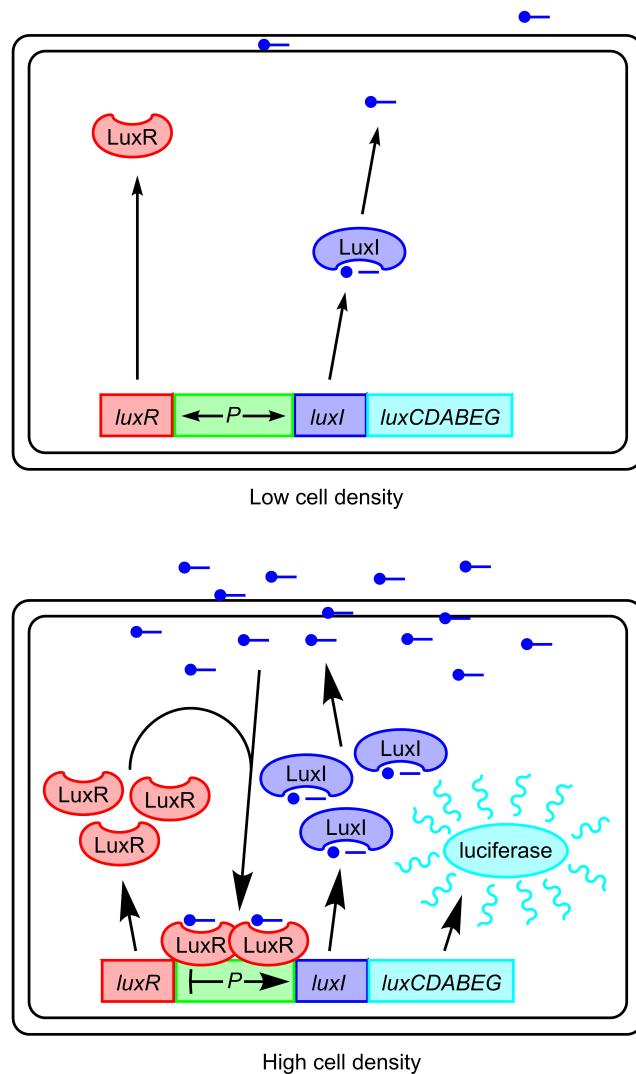


Figure 6: The LuxR-LuxI quorum sensing system in *V. fischeri*.

V. fischeri senses cell concentration by the detection of 3-oxo-C₆-HSL **18⁸²** (see Figure 7), a freely diffusible⁸³ molecule which is synthesised by LuxI^{84,85} and secreted by all *V. fischeri* cells⁸⁶ at a low basal level.⁷ When

the bacterial population density, and hence the concentration of 3-oxo-C₆-HSL **18**, reaches a threshold, 3-oxo-C₆-HSL **18** binds to LuxR,^{87–89} a receptor which is also synthesised at a low basal level.

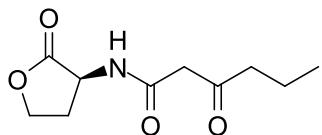


Figure 7: 3-oxo-C₆-HSL **18**.

The LuxR complex binds to the *lux* operator, upregulating production of LuxI and hence 3-oxo-C₆-HSL **18**, and luciferase enzymes and hence blue-green light.^{90–92} Production of more 3-oxo-C₆-HSL **18** enables a positive feedback loop, reinforcing the effect of high population density on 3-oxo-C₆-HSL **18** concentration and hence light production. This is the reason that 3-oxo-C₆-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-C₆-HSL **18** production of LuxR is inhibited.⁹³ Such balancing effects, as well as interactions with other quorum sensing and metabolic systems, are seen in many bacteria.^{7,10}

5.3.1.2 *Pseudomonas aeruginosa*

Another well-studied example of quorum sensing is in *P. aeruginosa*.^{10,94,95} *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.⁸ Multidrug-resistant *P. aeruginosa* is classified as a ‘serious threat’ by the United States Centers for Disease Control and Prevention¹ and carbapenem-resistant *P. aeruginosa* is classified as ‘priority 1: critical’ by the World Health Organisation.²⁹

P. aeruginosa has a low susceptibility to many antibiotics and readily acquires antibiotic resistance by mutation or horizontal gene transfer.⁹⁶ It is difficult for antibiotics to cross into cells due to low cell membrane permeability⁹⁷ and biofilm formation,⁹⁸ and they are pumped out again by its multiple chromosomally encoded multidrug efflux pumps.⁹ *P. aeruginosa* biofilms are more resistant to many drugs including ciprofloxacin **24** and trimethoprim **25** compared with planktonic cells.^{98,99} This high level of antibiotic resistance makes *P. aeruginosa* an important target for drug discovery.

Quorum sensing in *P. aeruginosa* involves a complex interplay of five signalling molecules (see Figure 8) and various proteins (see Figure 9).^{10,94,95} These can be broken down into three main, interacting systems: Las, Rhl and Pqs.

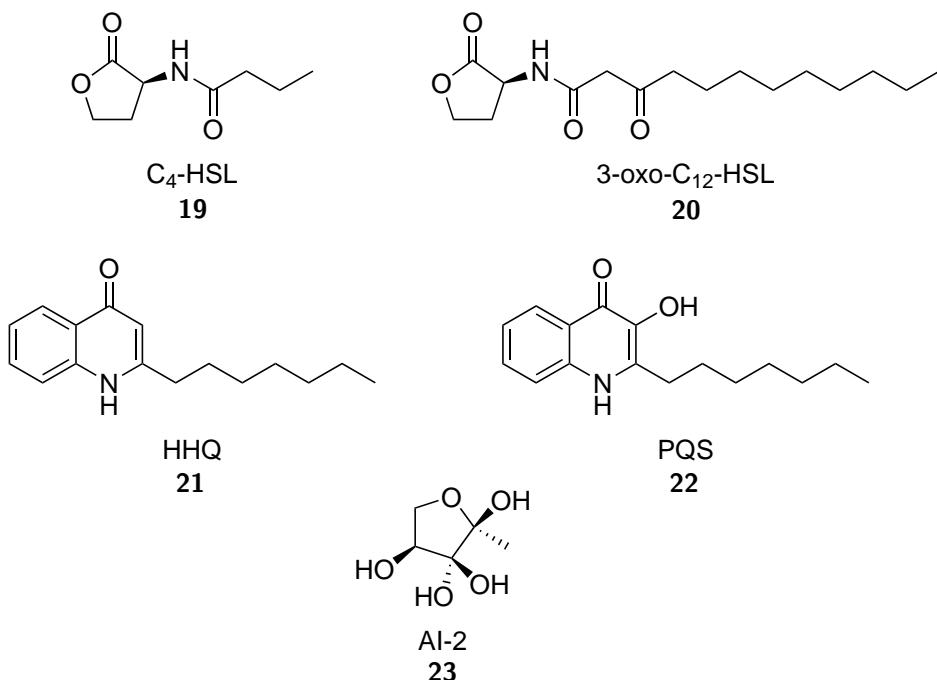


Figure 8: *P. aeruginosa* autoinducers.

In the Las system, LasI¹⁰⁰ synthesises the 3-oxo-C₁₂-HSL **20**¹⁰¹ autoinducer. 3-oxo-C₁₂-HSL **20** binds LasR,¹⁰² and this complex upregulates the production of LasI¹⁰³ (thus causing autoinduction) as well as alkaline protease,¹⁰⁴ elastase,¹⁰² exotoxin A,¹⁰⁴ HCN¹⁰⁵ and LasA protease.¹⁰⁶ The LasR complex is also important in late-stage biofilm formation,⁷⁴ and upregulates the Rhl¹⁰⁷ and Pqs systems.^{108,109}

In the Rhl system, RhII¹¹⁰ synthesises the C₄-HSL **19**¹¹¹ autoinducer. C₄-HSL **19** binds RhlR,¹¹² and this complex upregulates the production of RhII¹⁰³ (again causing autoinduction), alkaline protease,¹¹³ elastase,¹¹⁰ haemolysin,¹¹³ HCN,^{105,113} LasA protease,¹¹⁰ LecA,¹¹⁴ pyocyanin^{110,113} and rhamnolipids.¹¹⁰ The RhlR complex also downregulates the Pqs system.^{109,115} The Rhl system is controlled by both the Las and Pqs systems, as production of both RhlR and RhII is upregulated by the LasR complex¹⁰⁷ and production of both RhlR is upregulated by the PqsR complex.¹¹⁶

In the Pqs system, the main autoinducer, PQS **22**,¹¹⁷ is synthesised by multiple enzymes: PhnAB,¹¹⁸ PqsA, PqsBC, PqsD^{119,120} and PqsE^{121,122} produce the precursor HHQ **21**, and PqsH converts HHQ **21** to PQS **22**. PQS **22**¹⁰⁹ or HHQ **21** binds PqsR,¹²³ and either complex can upregulate the synthesis of HHQ **21** causing autoinduction. The PqsR-PQS complex upregulates the production of chitinase,¹²⁴ elastase,¹¹⁷ HCN,¹²⁴ LecA,¹²⁵ pyocyanin^{108,126} and pyoverdine,¹²⁶ as well as increasing biofilm production¹²⁵ and vesicle formation.¹²⁷ The PqsR-PQS complex also upregulates production of RhlR, so the Pqs system has control over the Rhl system.¹¹⁶ The Pqs system is controlled by both the Las and Rhl systems, as production of PqsR¹⁰⁹ and PqsH¹⁰⁸ is upregulated by the LasR complex and production of PqsA, PqsBC, PqsD, PqsE¹¹⁵ and PqsR¹⁰⁹ is downregulated by the RhlR complex.

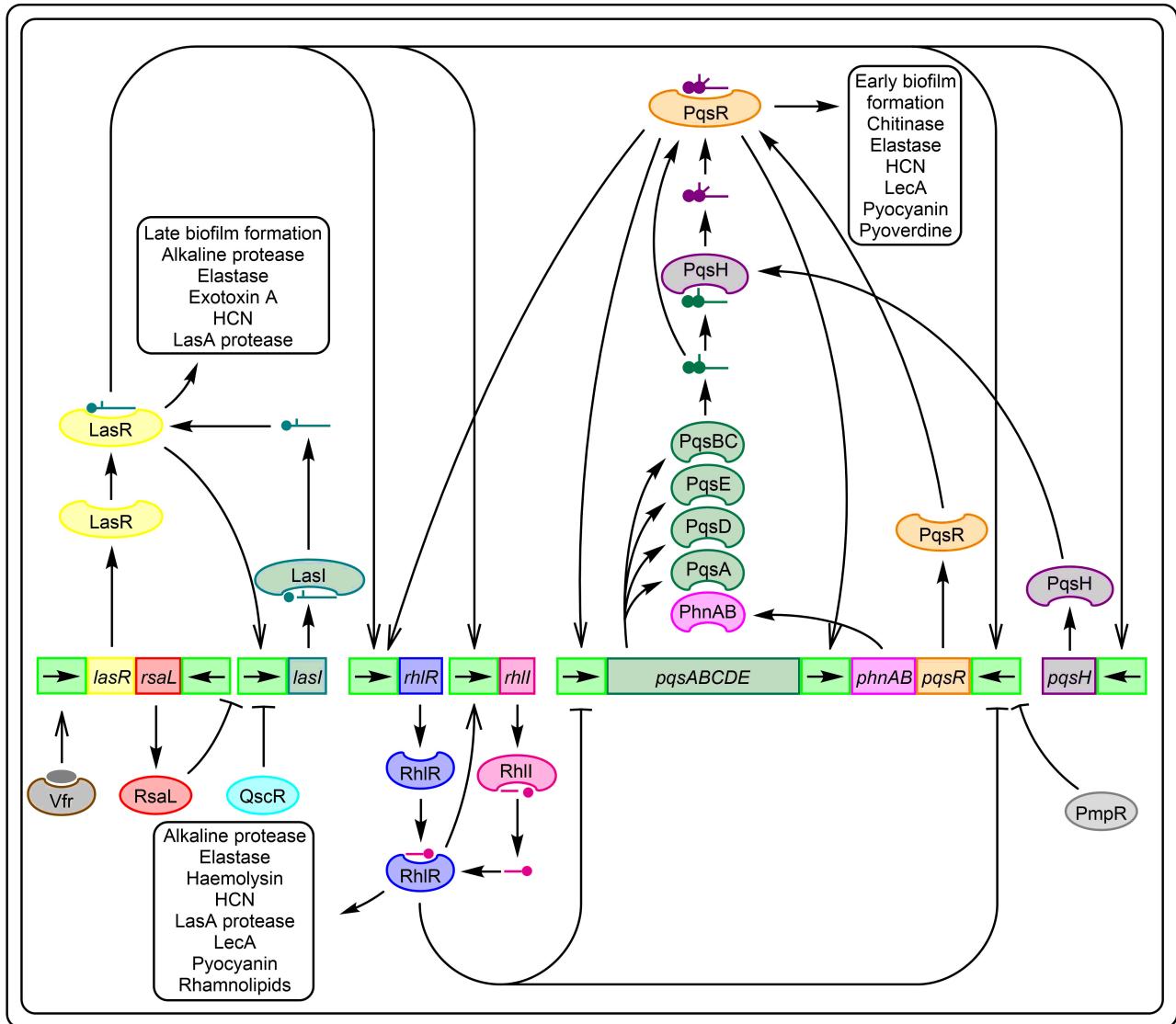
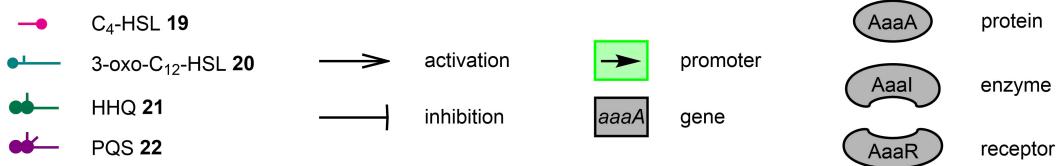


Figure 9: Quorum sensing in *P. aeruginosa*.^{10, 94, 95}

In addition to the above systems, AI-2 (see Figure 8), an interspecies signalling molecule,¹²⁸ is known to increase biofilm production and virulence in *P. aeruginosa*.^{129, 130} This is thought to be achieved by interaction with the Las and Rhl systems, but the exact mechanism is not known.

In summary, *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,^{94, 131} 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, C₄-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 known that PQS **22** is exported in vesicles,¹³² C₄-HSL **19** passively diffuses in and out of cells,¹³³ and 3-oxo-C₁₂-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 C₄-HSL **19** and 3-oxo-C₁₂-HSL **20** is thought to be largely due to chain length rather than the 3-oxo modification, as a shorter-chain version, 3-oxo-C₆-HSL **18** has been shown to be freely diffusible through *V. fischeri* membranes.⁸³

3-oxo-C₁₂-HSL **20** is exported primarily via the MexAB-OprM efflux system.^{9,134} The increased removal of 3-oxo-C₁₂-HSL **20** from the cell by upregulation of the MexAB-OprM system leads to decreased production of additional 3-oxo-C₁₂-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-C₁₂-HSL **20** levels would disrupt Las-mediated quorum sensing,¹³⁵ 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*.^{11,136} Ciprofloxacin **24** inhibits DNA replication by binding to DNA gyrase and topoisomerase IV.¹³⁷

Trimethoprim (see Figure 10) is a dihydrofolate reductase inhibitor used primarily to treat bladder infections.¹³⁸ 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*.

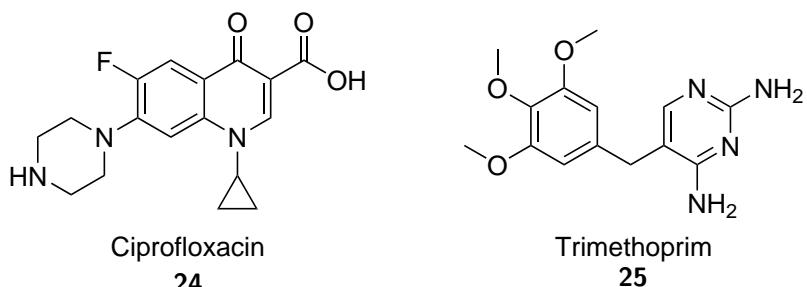


Figure 10: The antibiotics used in this section.

5.3.5 Antibiotic efflux

Ciprofloxacin **24** enters *P. aeruginosa* by diffusion,¹³⁹ but is pumped out by efflux pumps.¹⁴⁰ 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.⁹ However, in biofilms only MexEF–OprN has an effect.¹⁴¹

Trimethoprim **25** is mainly exported by the MexAB–OprM,¹⁴² MexCD–OprJ¹⁴³ and MexEF–OprN¹⁴⁴ multidrug efflux systems^{9,145} 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.⁹⁹

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.^{10,134,135} 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**.^{9,134} For PQS conjugates this would mean upregulation of vesicle formation.¹³²
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 biofilms¹⁴¹ and not an exporter of HSLs **19** or **20**, or PQS **22**). 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^{13,14} 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.*⁶⁰ (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.

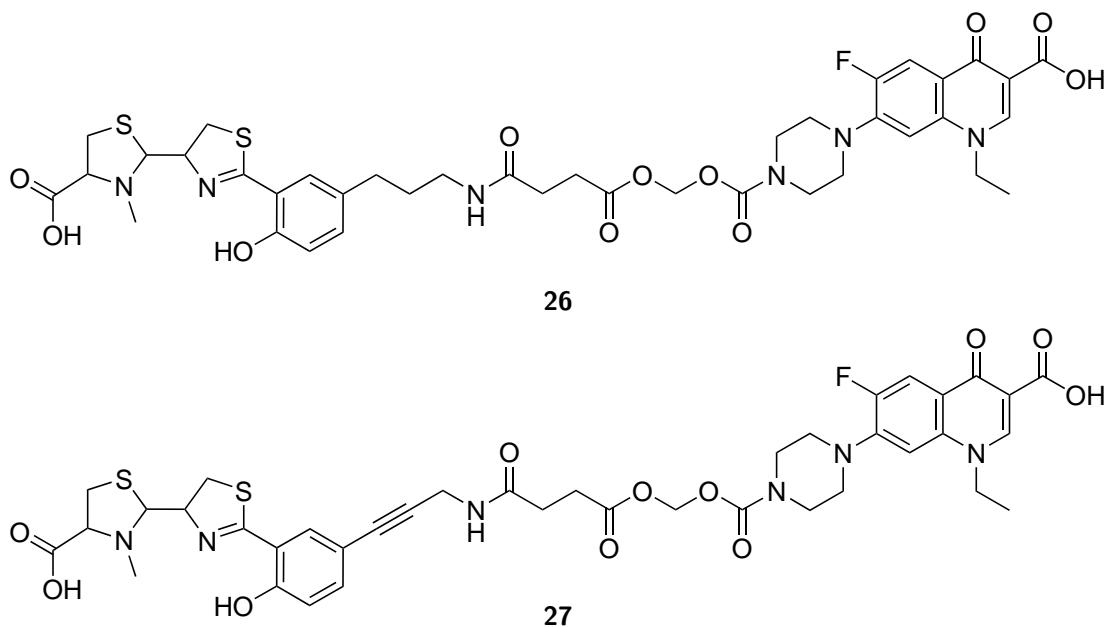


Figure 11: The cleavable pyochelin–norfloxacin conjugates synthesised by Rivault *et al.*⁶⁰

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.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.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.¹⁴⁸ It was therefore hoped that they will allow intracellular release of the ciprofloxacin **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.

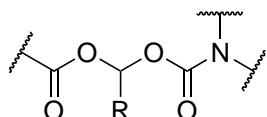


Figure 12: The cleavable linkers investigated in this study.

5.3.8 Homoserine lactone analogue-ciprofloxacin conjugates

Following on from the library of compounds based on *P. aeruginosa* autoinducers, a series of conjugates based on analogues of HSL were planned. This strategy was inspired by a paper¹⁵ and patent¹⁴⁹ by Ganguly *et al.*, who synthesised and characterised a conjugate **154** of methyl ciprofloxacin 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.^{86, 150–156}

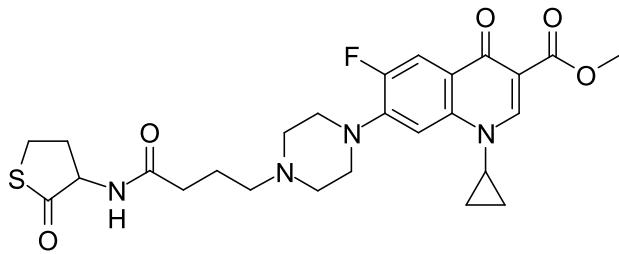


Figure 13: The HCTL-CipMe conjugate **154** studied by Ganguly *et al.*^{15,149}

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 (50 vs. 5 μm), indicating that the conjugate was less effective than ciprofloxacin under planktonic conditions.

Ganguly *et al.* then investigated the effect of the conjugate on biofilms. The conjugate and ciprofloxacin 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, 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. When the biofilm was grown for 48 or 72 hours the conjugate had similarly disruptive effects, whereas ciprofloxacin ‘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 is more effective than ciprofloxacin 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.

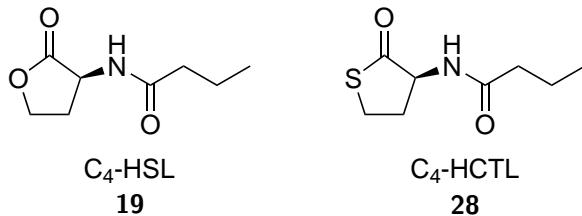


Figure 14: C₄-HSL **19** and C₄-HCTL **28**. Note that Ganguly *et al.* tested the *S* enantiomer of C₄-HCTL **28**, but used a racemic mixture in their HCTL-CipMe conjugate.

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,^{131,157} a selection of which are described in Table 2.

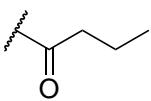
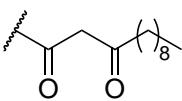
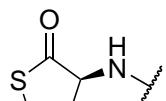
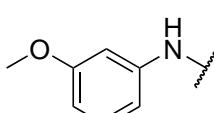
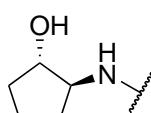
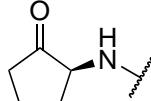
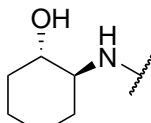
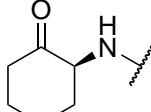
Head group		
	Partial agonist and antagonist against LasR. ¹⁵⁴ Shown to increase biofilm formation in <i>P. aeruginosa</i> . ¹⁵	Strong agonist against LasR, with comparable activity to the native ligand. ^{151,152,154,158}
	Partial agonist against LasR. ¹⁵⁷	Strong antagonist against LasR. ¹⁵⁷
	Poor agonist and antagonist against RhlR. ^{159,160}	Strong antagonist against LasR. ¹⁵⁹
	Strong agonist against RhlR. ¹⁵⁹ SS enantiomer is more potent. ¹⁶⁰	Partial agonist against LasR. ¹⁵⁹
	Strong agonist against RhlR. ¹⁵⁹ SS enantiomer is more potent, with comparable activity to the native ligand. ¹⁶⁰	Strong agonist against LasR. ^{152,159} SS enantiomer is more potent, with comparable activity to the native ligand. ¹⁶⁰
	Strong agonist against RhlR. ¹⁵⁹ SS enantiomer is more potent. ¹⁶⁰	Partial antagonist against LasR. ¹⁵⁹ Shown to reduce biofilm formation in <i>P. aeruginosa</i> . ¹⁵⁹

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 *P. aeruginosa* autoinducers (see Figure 8) with ciprofloxacin and trimethoprim (see Figure 10) joined using a copper(I)-catalyzed azide-alkyne cycloaddition. Section 8 focuses on conjugates of HSL analogues with ciprofloxacin (see 5.3.8) joined either using a copper(I)-catalyzed azide-alkyne cycloaddition or an S_N2 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.^{13,14} 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,^{161–163} 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.¹⁶⁴

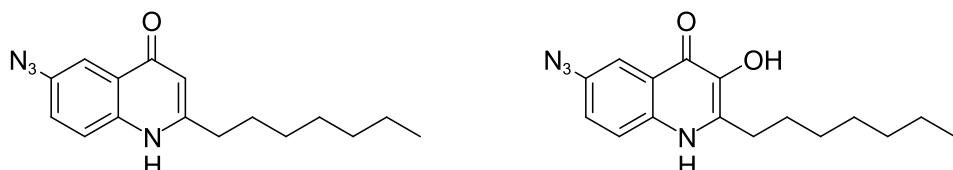


Figure 15: The azido derivatives of HHQ **21** and PQS **22**: **38** and **49**.

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.¹³¹ Hence, the azide group was included on the tail.¹⁶⁵ Acyl tail length is known to play an important role in affinity,¹³¹ 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).

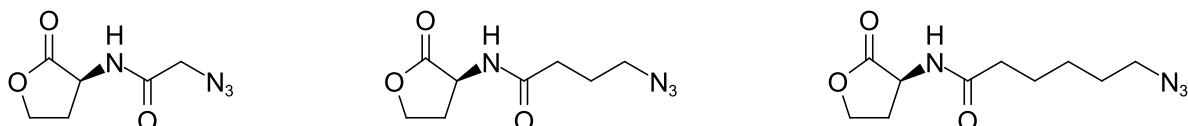


Figure 16: The azido derivatives of C₄-HSL **19**: **55**, **58** and **61**.

7.1.2 Alkynyl antibiotic derivatives

The structure-activity relationships for ciprofloxacin have been investigated¹⁶⁶ 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).

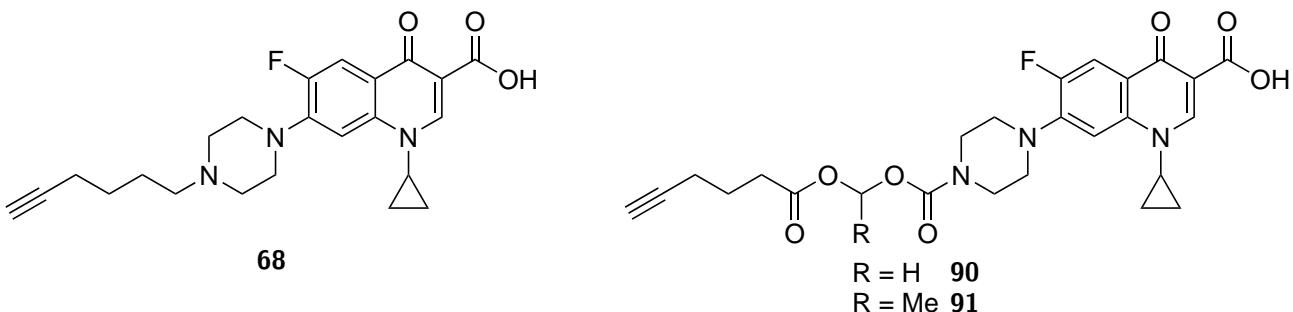


Figure 17: The alkynyl ciprofloxacin derivatives **68**, **90** and **91**.

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.*¹⁶⁷

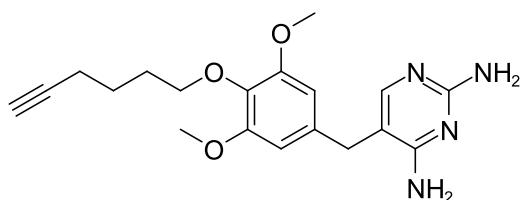
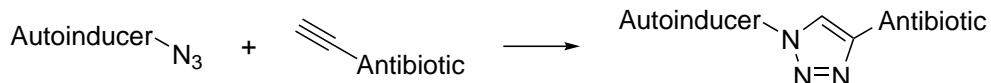


Figure 18: The alkynyl trimethoprim derivative **71**.

7.1.3 Synthesis of the conjugates

A copper(I)-catalysed azide-alkyne cycloaddition^{13,14} was used to join each combination of autoinducer and antibiotic together (see Scheme 1).



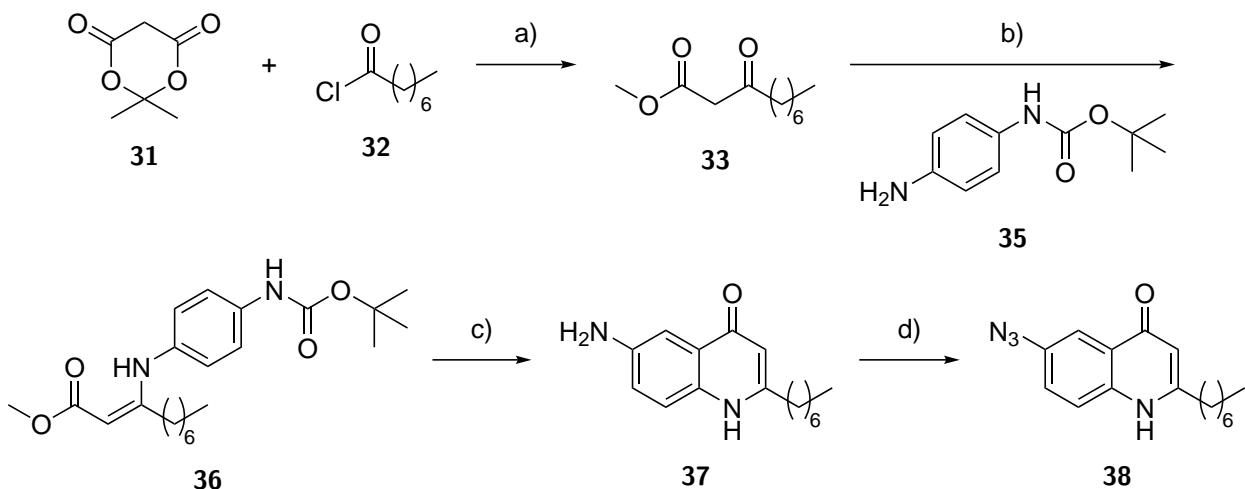
Scheme 1: The construction of the triazole-linked autoinducer-antibiotic conjugate library using a copper(I)-catalysed azide-alkyne cycloaddition.

7.2 Azido autoinducer derivatives

7.2.1 Synthesis of 6-N₃-HHQ **38**

The synthesis of 6-N₃-HHQ **38** is shown in Scheme 2 and follows a route devised by Baker.¹⁶⁴ Octanoyl chloride **32** was converted to β -ketoester **33** via a Meldrum's acid adduct.^{168,169} The β -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 ZrCl₄ catalyst.

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 NaNO₂ and HCl, followed by displacement with NaN₃ to form the final azido-HHQ product **38**.¹⁷⁰



Scheme 2: The synthesis of **38**. a) i) Pyridine, CH_2Cl_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**⁹⁴ 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_2CO_3 to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **44** to form the ester **46** by $\text{S}_{\text{N}}2$ 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}

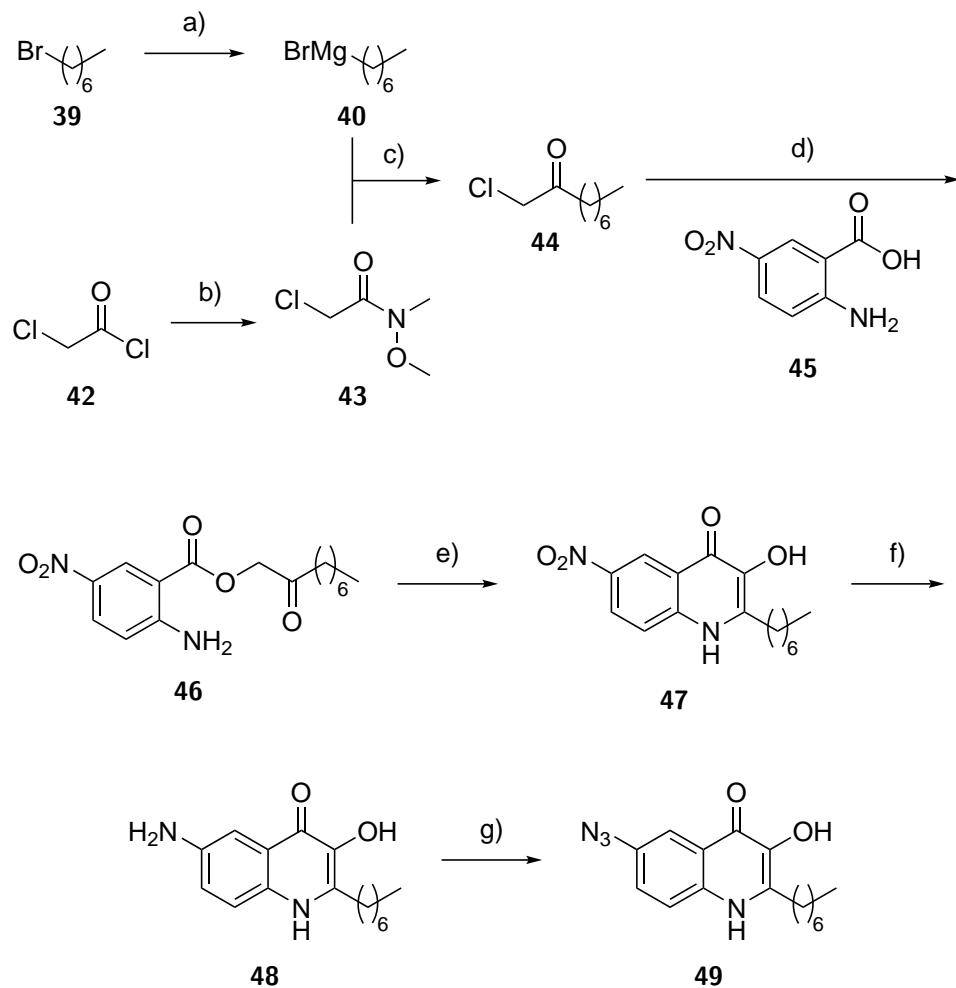
Conditions for the reduction of the nitro group were then compared (see Table 3). Baker initially used Zn and HCl, however this gave a yield over 100% suggesting coordination of Zn to the amino-PQS **48**¹⁶⁴ (this product was taken through and purified after the next step). She also attempted reduction 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

Conditions	Outcome
H ₂ , Pd/C, 1 atm, r.t., 18 h	No reaction
NH ₄ HCO ₂ , Pd/C, 1 atm, r.t., 18 h	No reaction
Zn, HCl (aq), r.t., 5 min h	Product 48 + Zn, assumed quantitative yield
SnCl ₂ · 2 H ₂ O, MeOH, r.t., 18 h	No reaction
H ₂ , Pd/C, MeOH, 3 atm, r.t., 4 h.	Product 48 , 100% yield
H ₂ , PtO ₂ , MeOH, 1 atm, r.t., 45 min	Product 48 , 80% yield

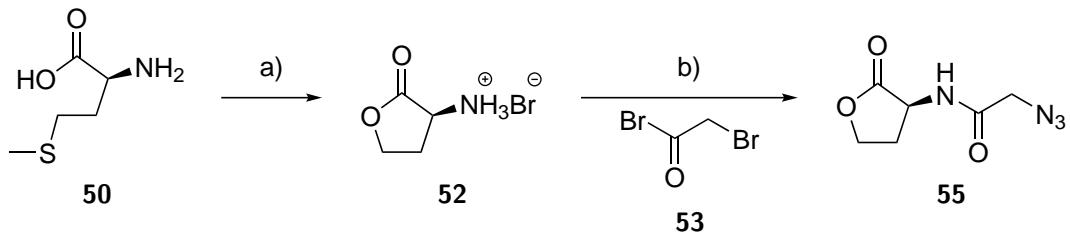
Table 3: Conditions attempted for the synthesis of **48**. Rows 1-3 were carried out by Baker,¹⁶⁴ rows 4-6 were carried out as part of this study.



Scheme 3: The synthesis of **49**. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) *N,N*-dimethylhydroxyl amine hydrochloride, K₂CO₃, toluene, water, -5 °C to r.t., 30 min, 71%. c) THF, 0 °C to r.t., 15 h, 96%. d) **45**, K₂CO₃, DMF, 90 °C, 1 h, then **44**, r.t., 18 h, 100%. e) Polyphosphoric acid, 90 °C, 5.5 h, 40%. f) H₂, PtO₂, MeOH, 1 atm, r.t., 45 min, 80%. g) i) NaNO₂, HCl, water, 0 °C, 50 min. ii) NaN₃, water, r.t., 4 h, 28% over two steps.

7.2.3 Synthesis of the azido C₄-HSL derivatives **55**, **58** and **61**

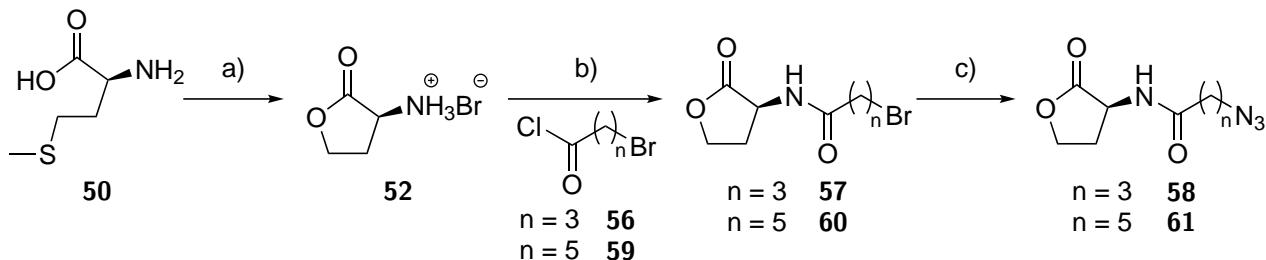
N₃-C₂-HSL **55** (the azido derivative of C₄-HSL with a C₂ chain, see Scheme 4) has previously been prepared by Stacy *et al.*¹⁶⁵ Their synthesis was followed, starting with the cyclisation of L-methionine **50** using bromoacetic acid to form the HSL HBr salt **52**. The disappointing yield can be attributed 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 S_N2 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 S_N2 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 S_N2 reaction could probably be improved by decreasing the temperature (see Scheme 22, for example).



Scheme 5: The synthesis of **58** and **61**. a) Bromoacetic acid, *i*-PrOH:water:AcOH (5:5:2), r.t., 18 h, 41%. b) 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%.

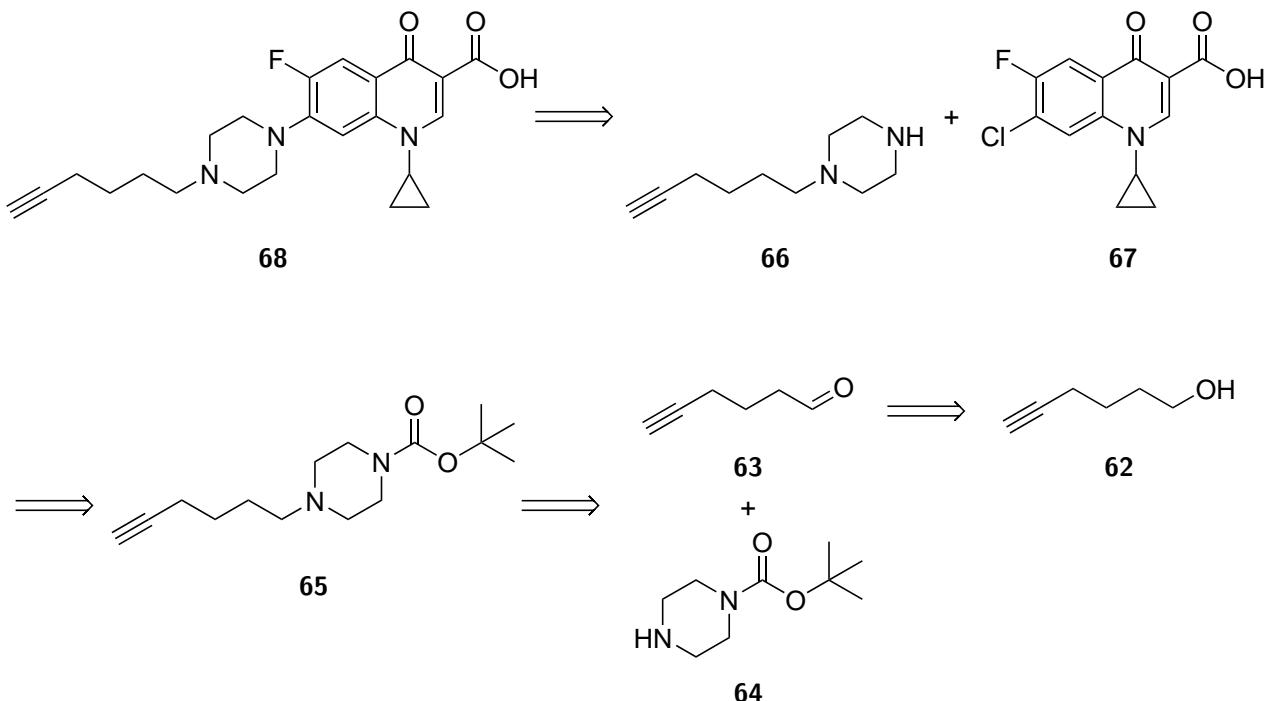
7.3 Alkynyl antibiotic derivatives

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."^{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.*¹⁶⁶

Unlike the aldehydes and ketones used by Renau *et al.*,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.*¹⁷⁶

Renau *et al.*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 triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*¹⁷⁷ This reaction yielded compound **65** in excellent yield, which was deprotected using TFA using the procedure described by Renau *et al.*¹⁶⁶ to give the alkynyl piperazine **66** quantitatively.

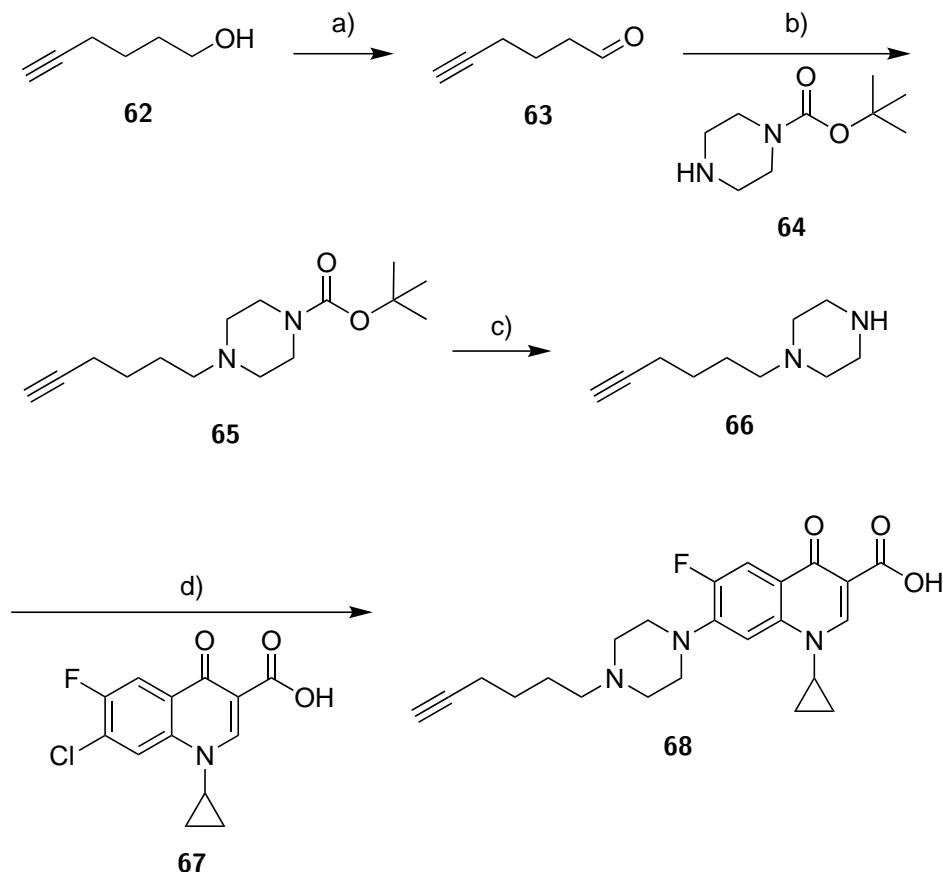
The alkynyl piperazine **66** was refluxed in acetonitrile with the ciprofloxacin precursor **67** according to the procedure described by Renau *et al.*,¹⁶⁶ however the reaction did not proceed. Addition of 2 eq. of TEA did not lead to reaction, however it was found that refluxing in neat TEA led to 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.*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 to Cu can hinder catalysis.

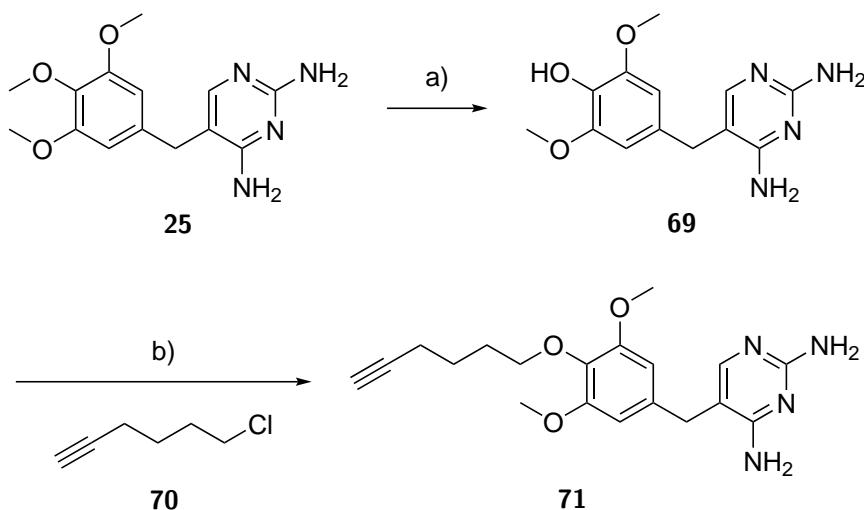


Scheme 7: The synthesis of **68**. a) Pyridinium chlorochromate, CH_2Cl_2 , r.t., 5 h, 72%. b) $\text{NaBH}(\text{AcO})_3$, 1,2-dichloroethane, r.t., 10.5 h, 99%. c) TFA, r.t., 1 h, 100%. d) NMP, microwave, 115°C 24 h, 12%.

7.3.2 Synthesis of the alkynyl trimethoprim derivative **71**

The synthesis of trimethoprim derivative **71** is shown in Scheme 8. Trimethoprim was selectively deprotected using HBr (aq.) using a procedure described by Jing *et al.*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_2CO_3 -catalysed $\text{S}_{\text{N}}2$ reaction similar to that used by Jing *et al.*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 Triazole-linked autoinducer-antibiotic conjugates

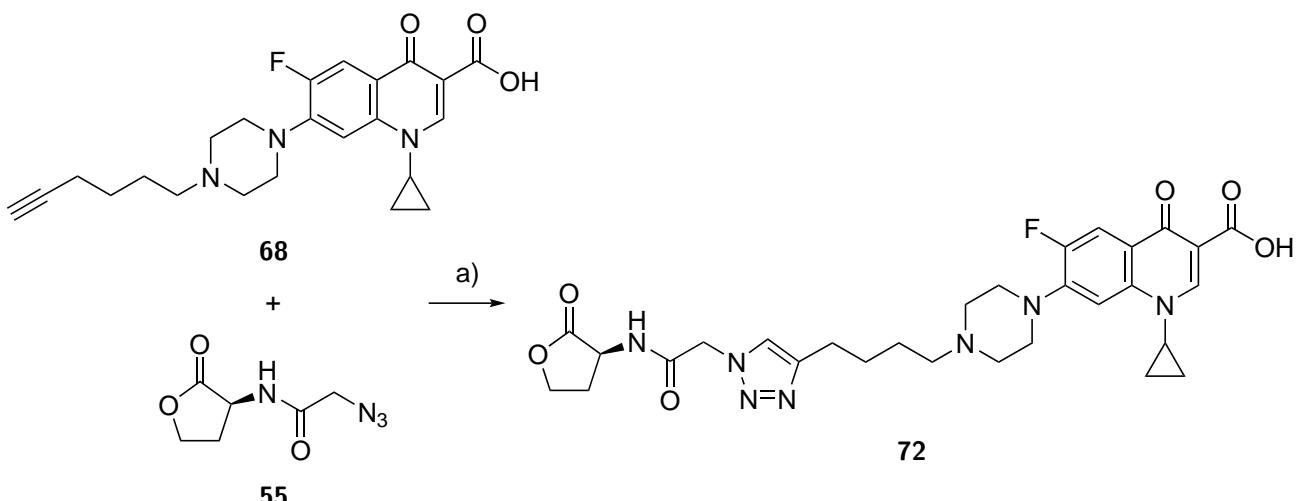
7.4.1 Optimisation of the click reaction

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.¹⁴

Use of the ligand tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) **74** (see Figure 19) led to some conversion at room temperature, 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.

Conditions	Outcome
CuSO ₄ · 5 H ₂ O, sodium ascorbate, water, <i>t</i> -BuOH, air, r.t., 7 d.	No reaction
CuSO ₄ · 5 H ₂ O, sodium ascorbate, water, <i>t</i> -BuOH, air, 50 °C, 5 d.	1,3-Triazole product 72 and 1,5 triazole impurity 73 4:1
CuSO ₄ · 5 H ₂ O, sodium ascorbate, THPTA 74 , water, <i>t</i> -BuOH, air, r.t., 3 h.	1,3-Triazole product 72 and starting materials 55 and 68
CuSO ₄ · 5 H ₂ O, sodium ascorbate, THPTA 74 , water, <i>t</i> -BuOH, Ar, r.t., 3 h.	1,3-Triazole product 72

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



Scheme 9: Synthesis of **72**. For conditions see Table 4.

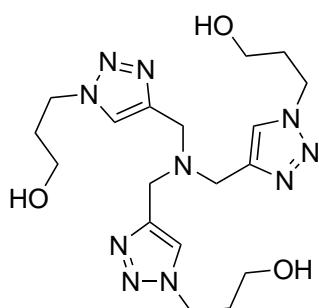


Figure 19: Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (THPTA) **74**.

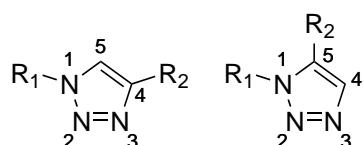
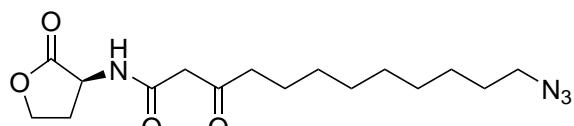


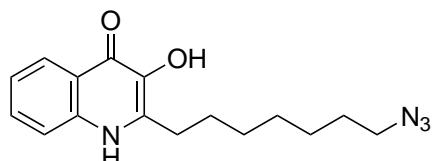
Figure 20: 1,4 (left) and 1,5 (right) triazoles.

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).



75

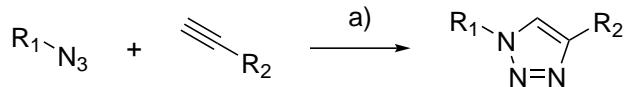


76

Figure 21: Further azido autoinducer derivatives synthesised by Howard¹⁷⁹ **75** and Baker¹⁶⁴ **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 CH₂Cl₂. Secondly, some compounds were unstable: HSL derivatives hydrolysed upon attempted preparative HPLC purification and the 3-oxo-C₁₂-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 R₁-N₃ is an azido autoinducer derivative and R₂-≡ is an alkynyl antibiotic derivative a)CuSO₄, sodium ascorbate, THPTA, water, *t*-BuOH.

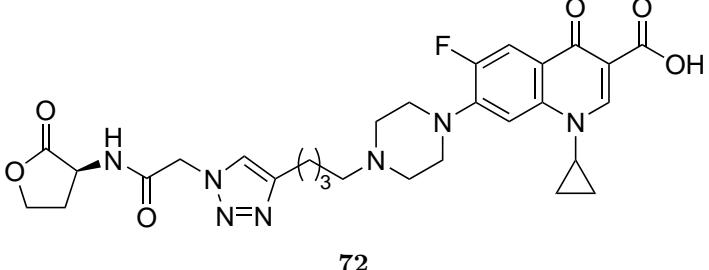
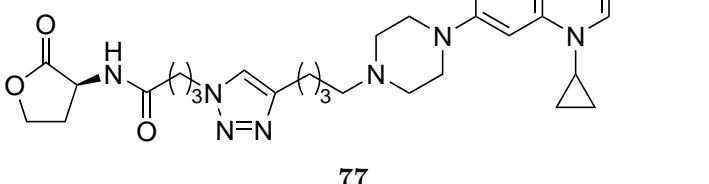
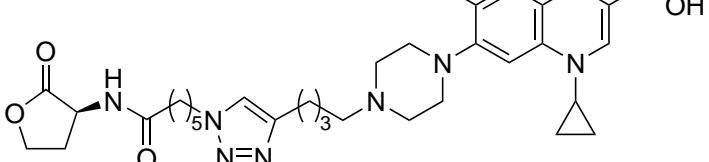
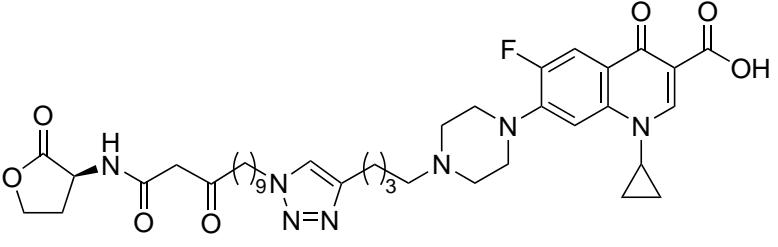
Starting materials	Product	Outcome	Yield
55 and 68	 72	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2 , 0-20% MeOH/ CH_2Cl_2).	30%
58 and 68	 77	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2 , 0-20% MeOH/ CH_2Cl_2).	47%
61 and 68	 78	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2 , 0-20% MeOH/ CH_2Cl_2).	38%
75 and 68	 79	✗ Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO_2 , 20% MeOH/ CH_2Cl_2).	

Table 5: Click reactions attempted.

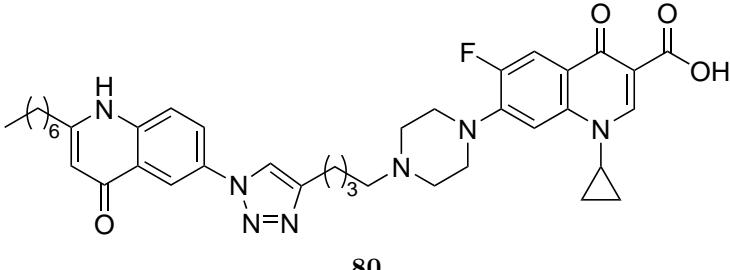
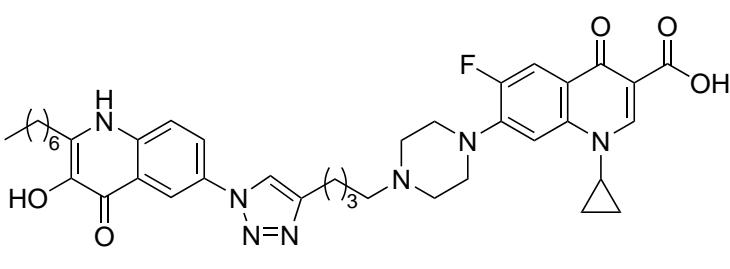
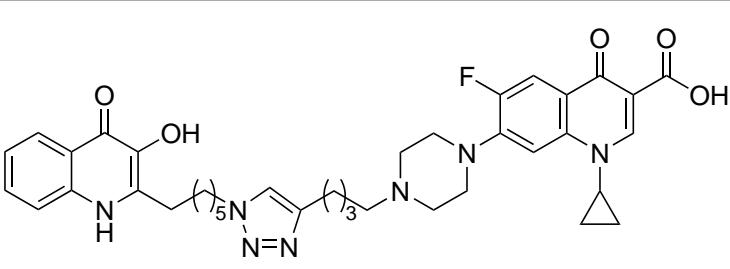
Starting materials	Product	Outcome	Yield
38 and 68	 80	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	27%
49 and 68	 81	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
76 and 68	 82	✗ No reaction seen by LCMS.	

Table 6: Click reactions attempted.

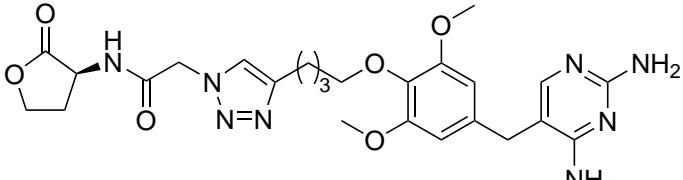
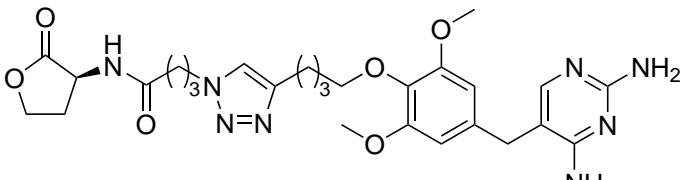
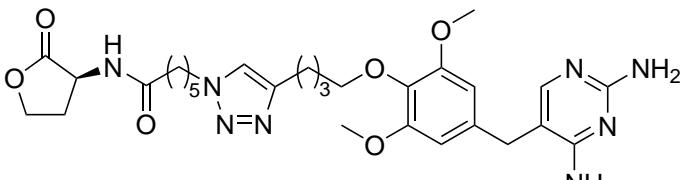
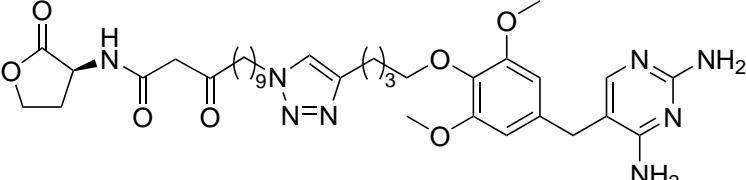
Starting materials	Product	Outcome	Yield
55 and 71	 83	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on prep. HPLC column.	
58 and 71	 84	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO2, 20% MeOH/CH2Cl2).	17%
61 and 71	 85	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO2, 20% MeOH/CH2Cl2).	27%
75 and 71	 86	✗ Degraded during reaction.	

Table 7: Click reactions attempted.

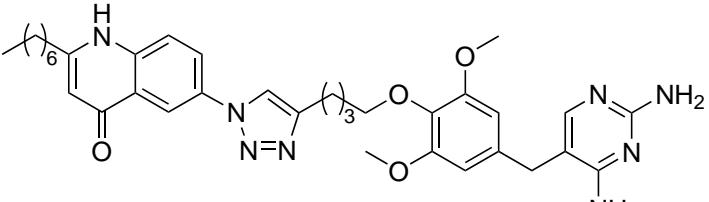
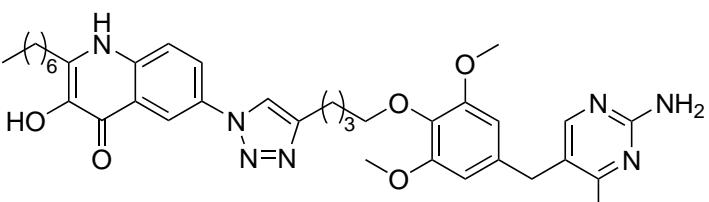
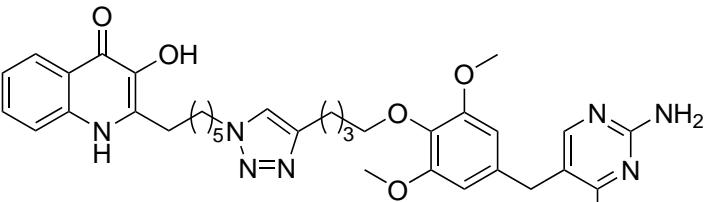
Starting materials	Product	Outcome	Yield
38 and 71	 <p style="text-align: center;">87</p>	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	41%
49 and 71	 <p style="text-align: center;">88</p>	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
76 and 71	 <p style="text-align: center;">89</p>	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2 , 20% $\text{MeOH}/\text{CH}_2\text{Cl}_2$).	18%

Table 8: Click reactions attempted.

7.4.3 Synthesis of 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).

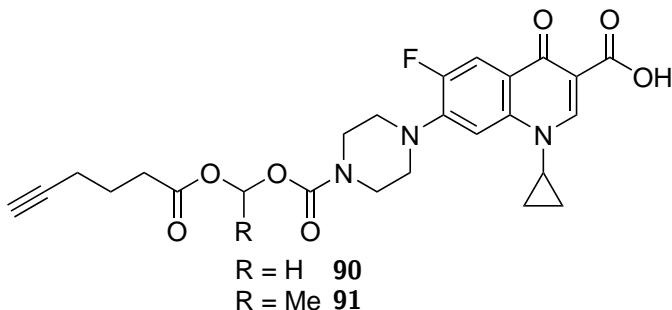


Figure 22: The cleavable alkyne-Cip derivatives synthesised by Professor Sotelo.

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).

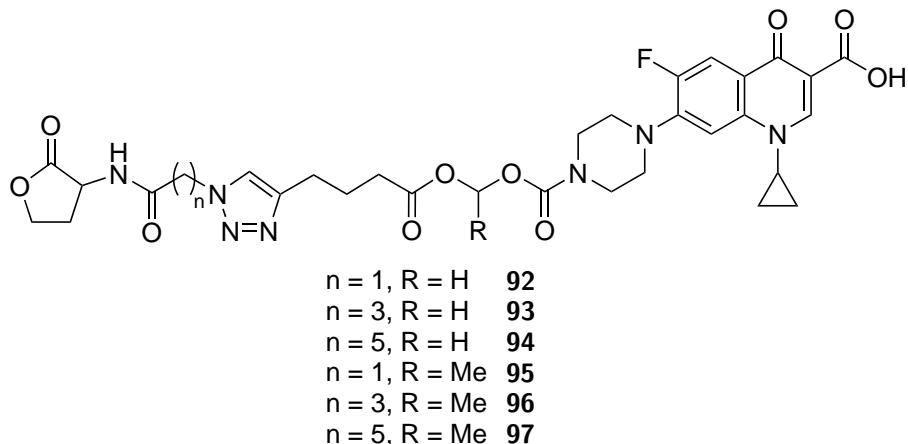


Figure 23: The cleavable HSL-Cip triazole conjugates synthesised by Professor Sotelo.

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.

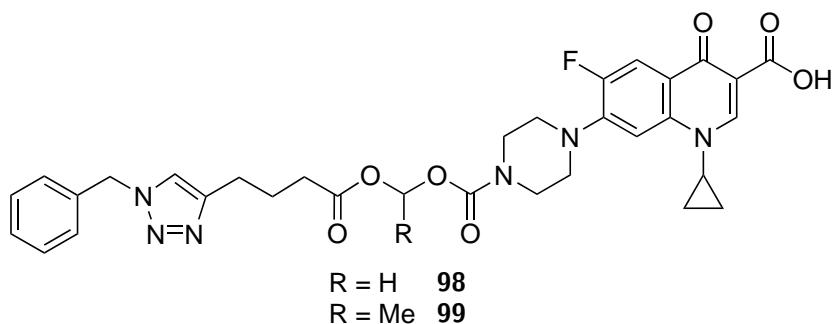


Figure 24: The cleavable Bn-Cip triazole conjugates **98** and **99** synthesised by Professor Sotelo.

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* PAO1¹⁸⁰ and YM64.¹⁸¹ 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.

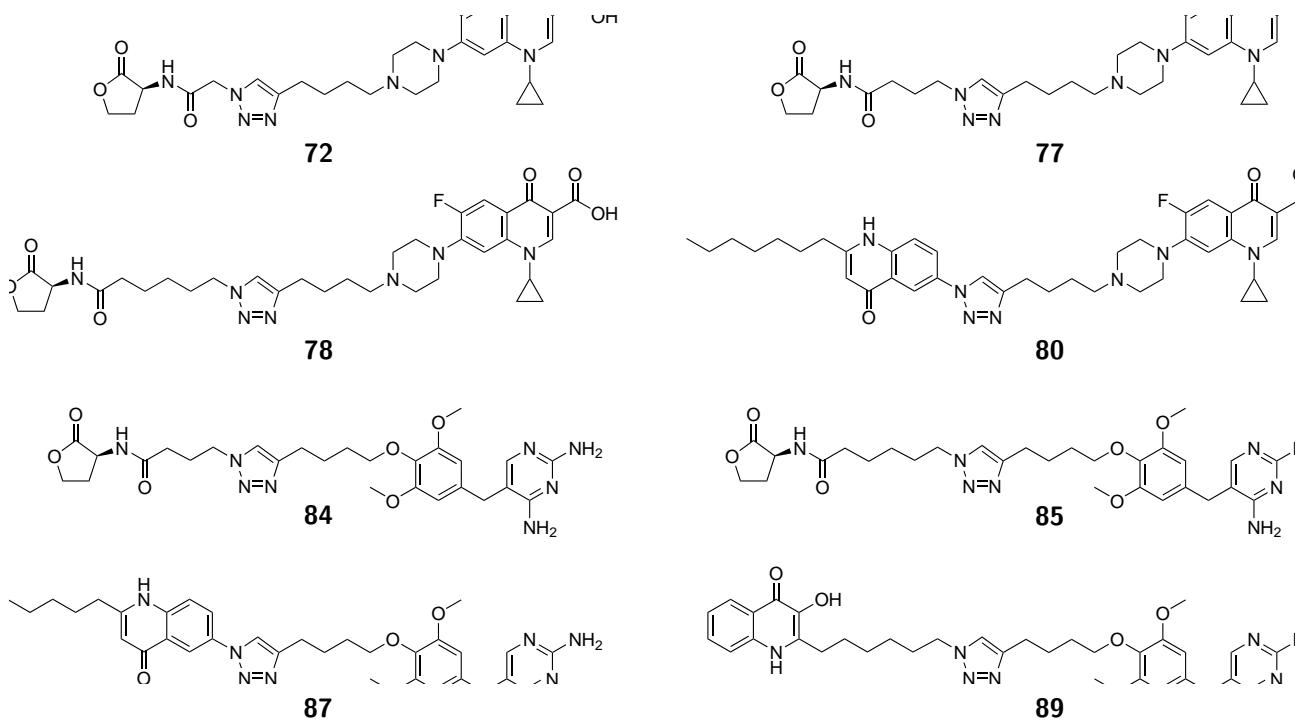


Figure 25: The autoinducer-antibiotic conjugates.

7.5.1.1 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).

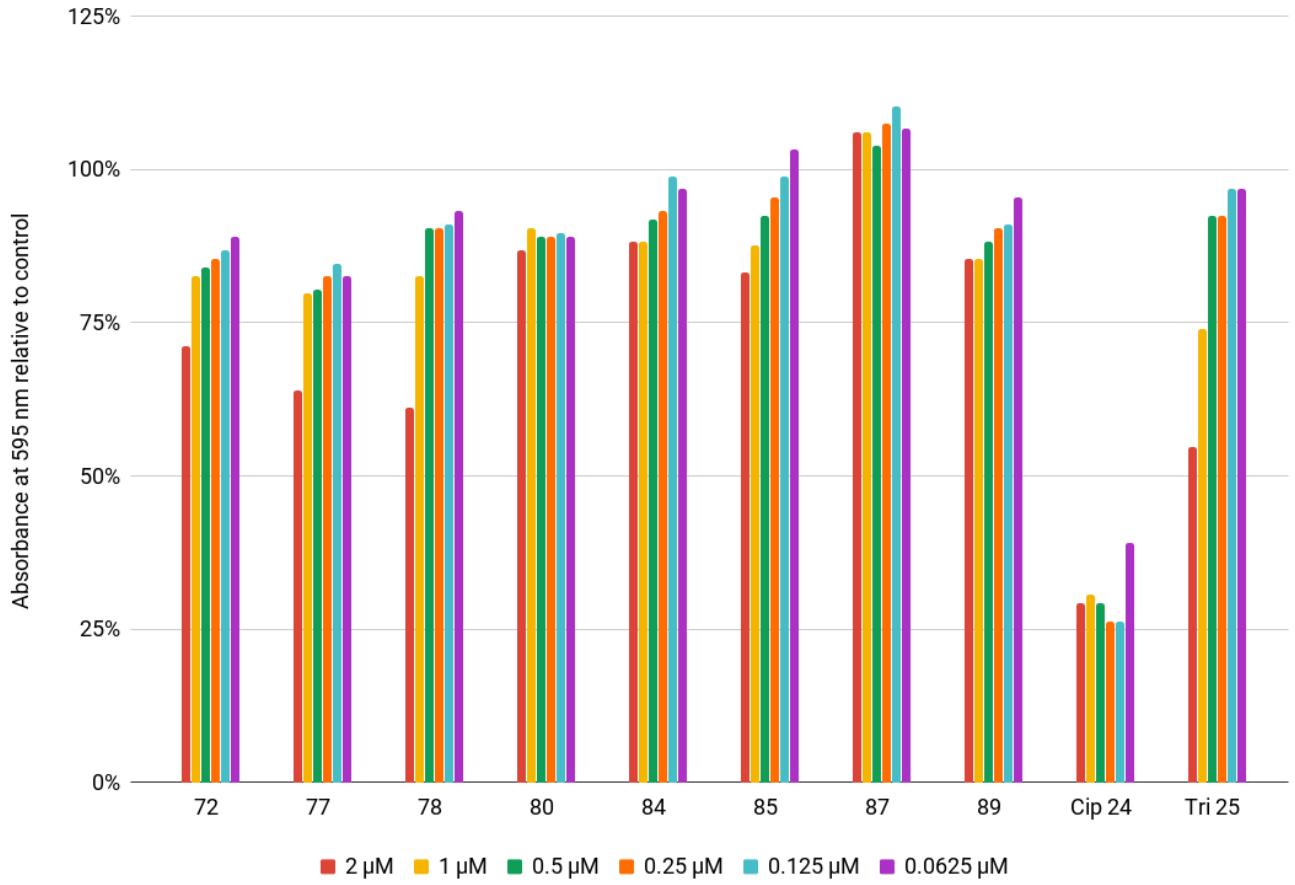


Figure 26: YM64 optical density (OD) readings at 5 h for the autoinducer-antibiotic conjugates.

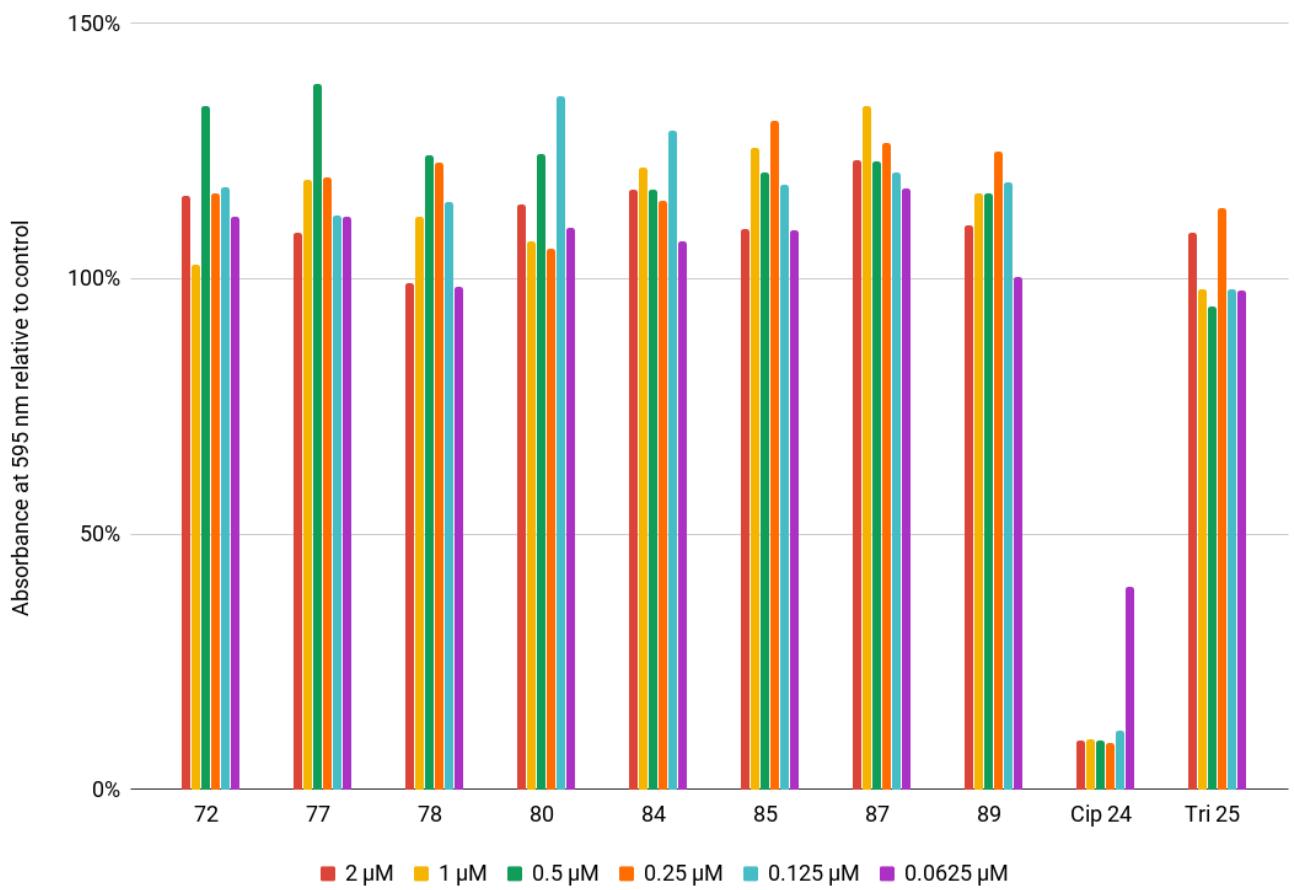


Figure 27: YM64 OD readings at 24 h for the autoinducer-antibiotic conjugates.

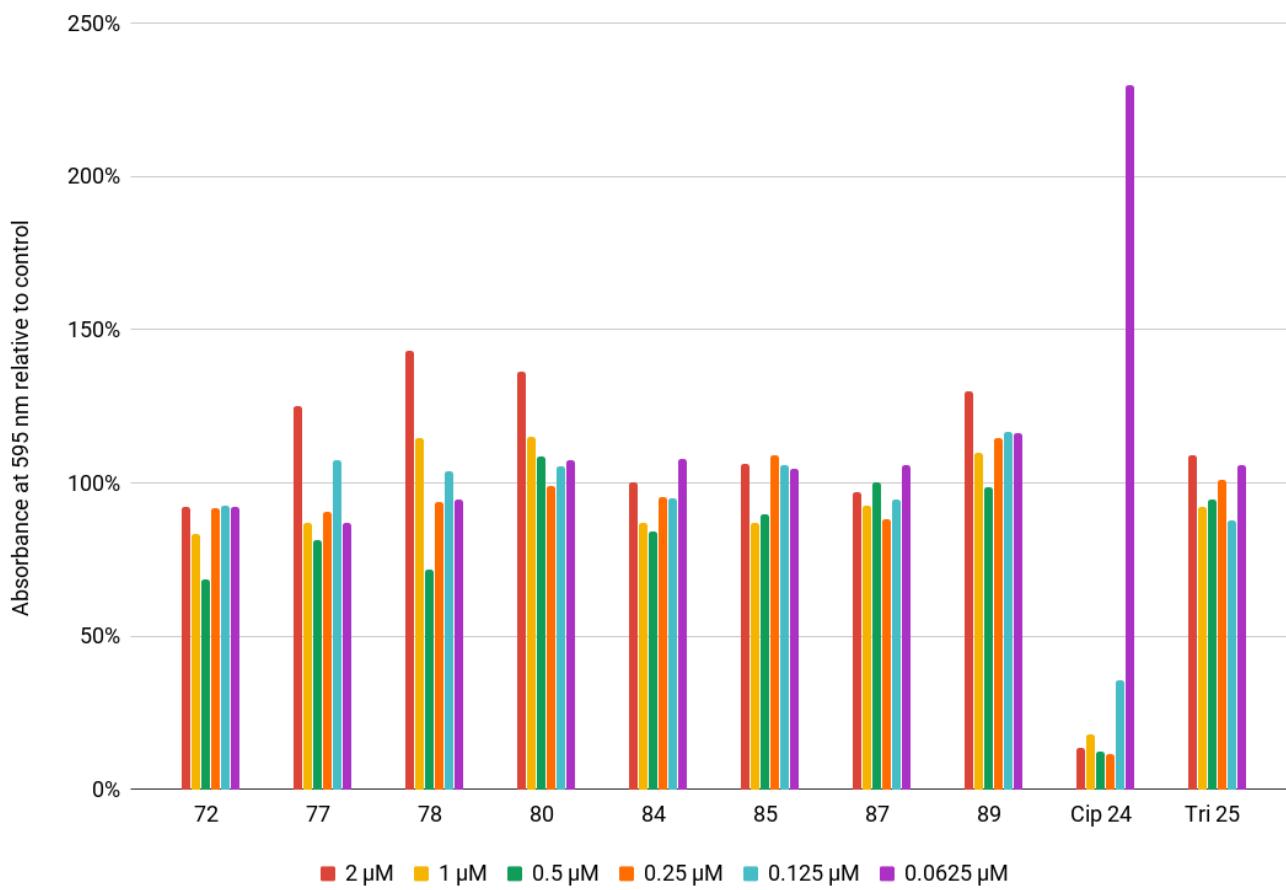


Figure 28: YM64 biofilm quantification at 24 h for the autoinducer-antibiotic conjugates.

7.5.1.2 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.

The very high readings in the biofilm assay for ciprofloxacin **24** (see 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,^{182,183} tobramycin, amikacin, streptomycin and gentamicin, but oddly not with ciprofloxacin **24**,^{184,185} although this could be due to the exact conditions of growth.

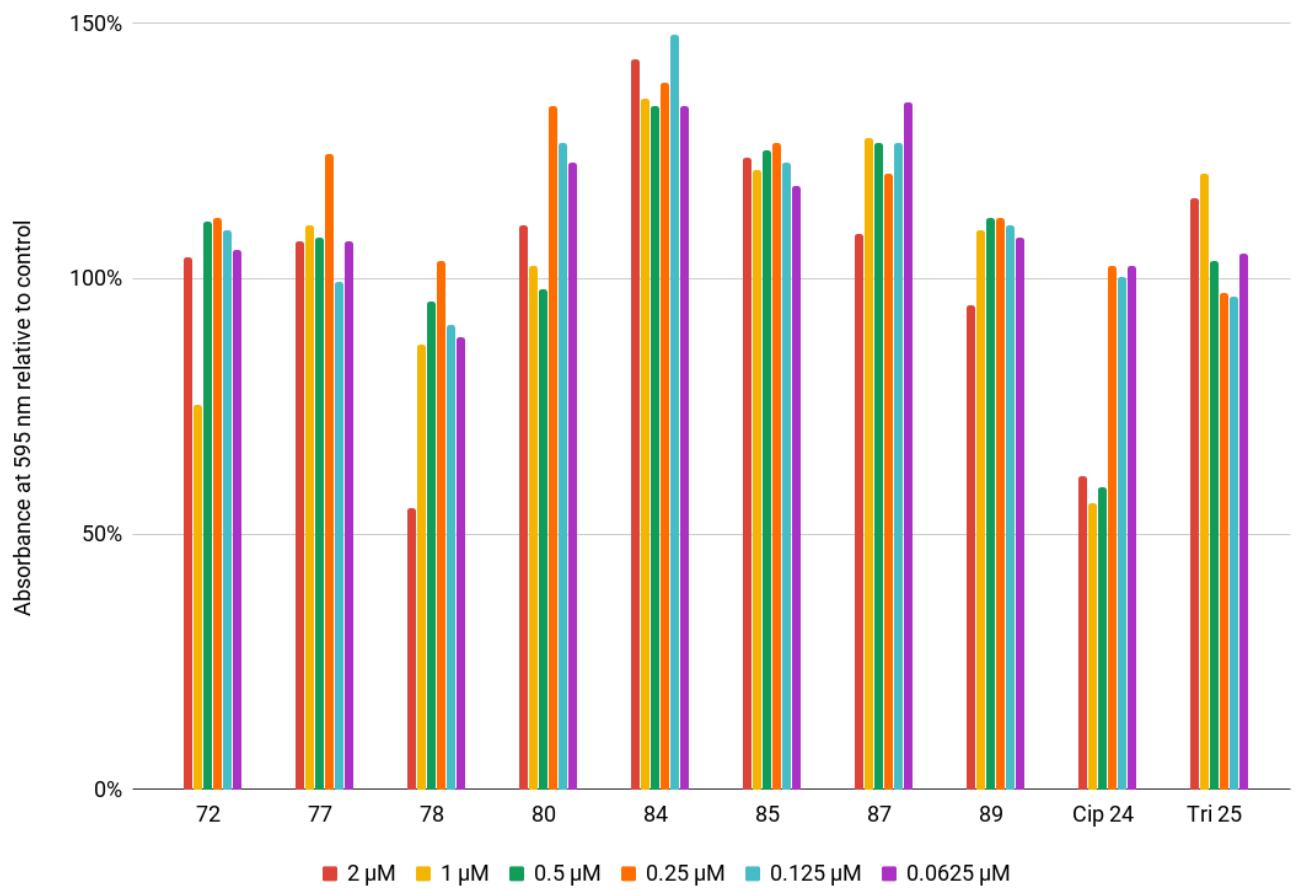


Figure 29: PAO1 OD readings at 5 h for the autoinducer-antibiotic conjugates.

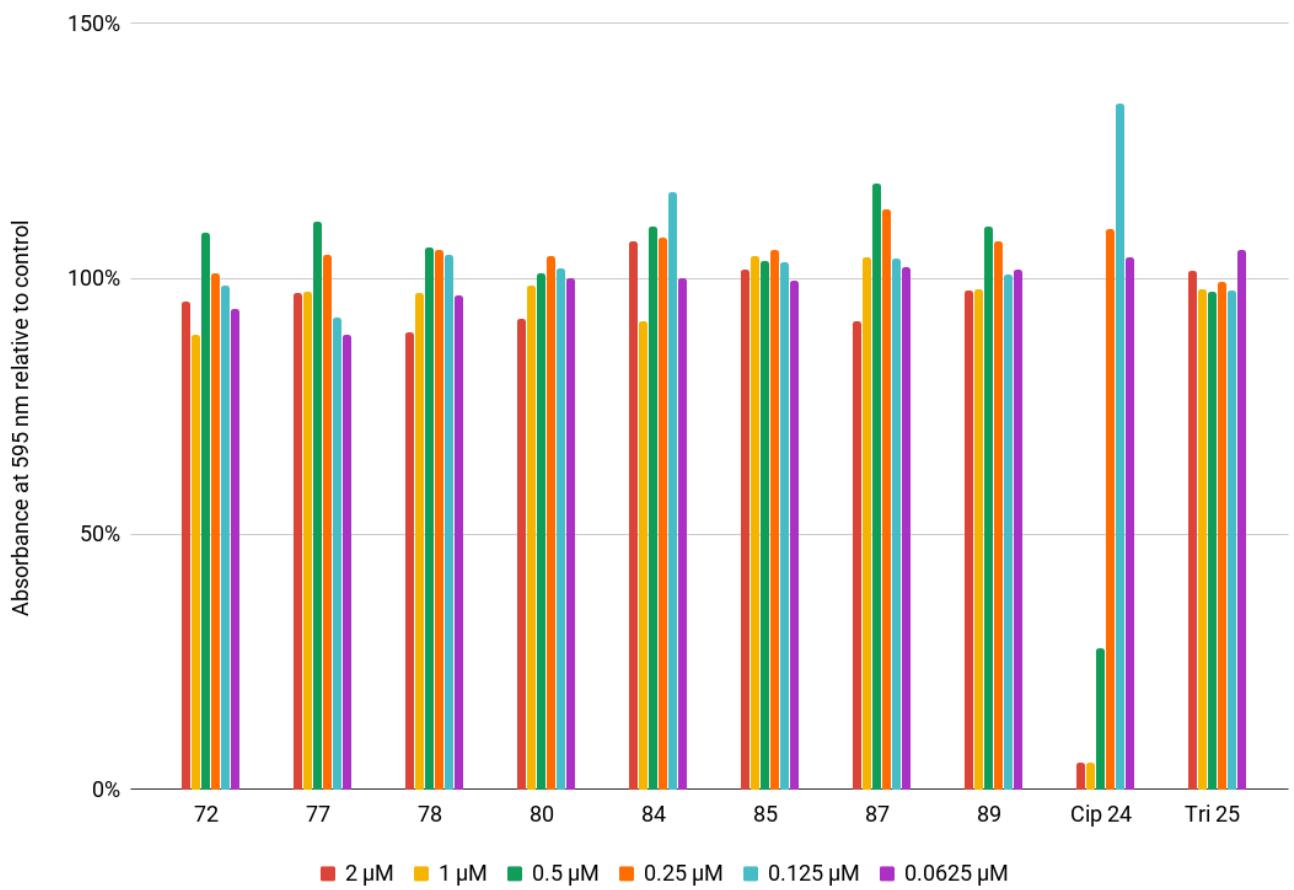


Figure 30: PAO1 OD readings at 24 h for the autoinducer-antibiotic conjugates.

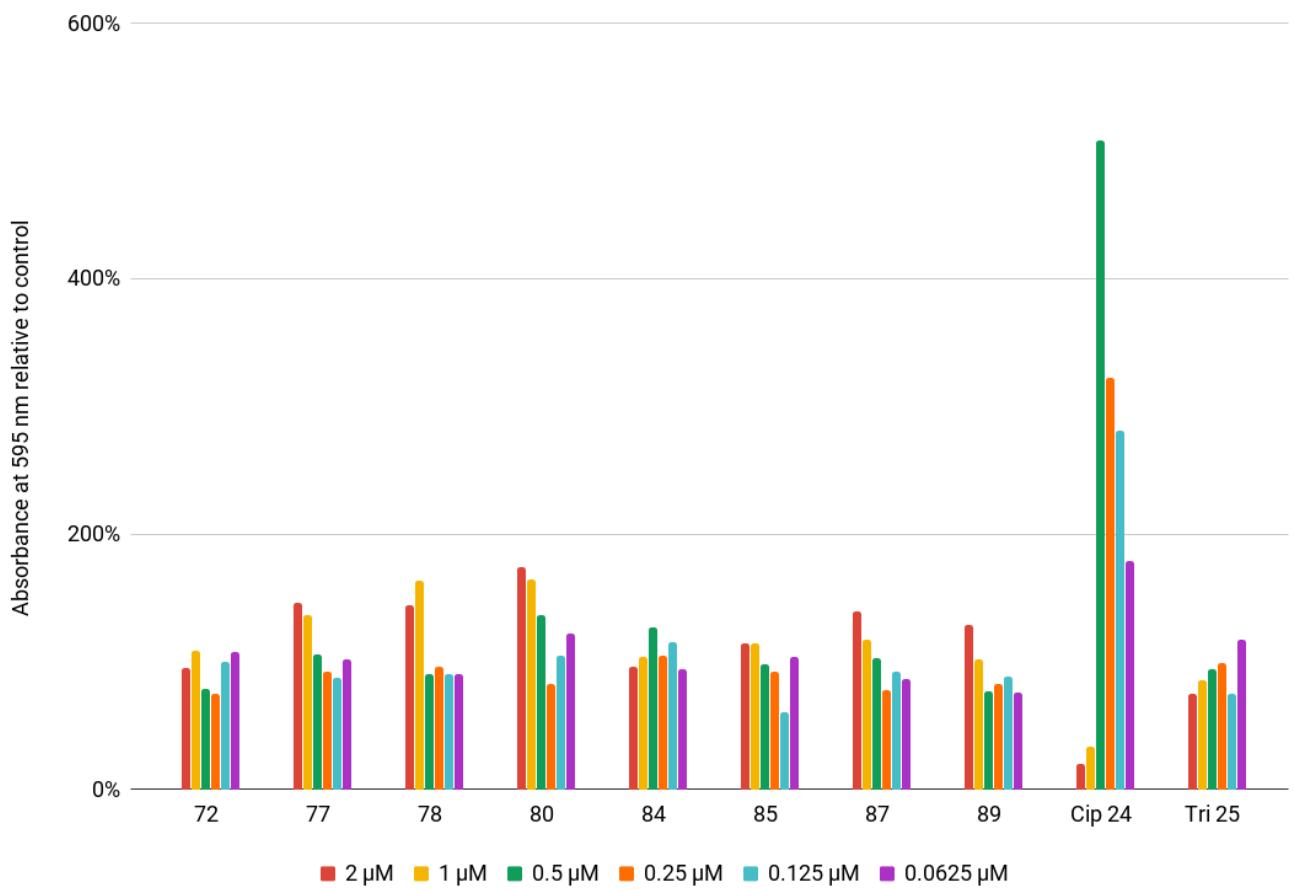


Figure 31: PAO1 biofilm quantification at 24 h for the autoinducer-antibiotic conjugates.

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 *P. aeruginosa* YM64.

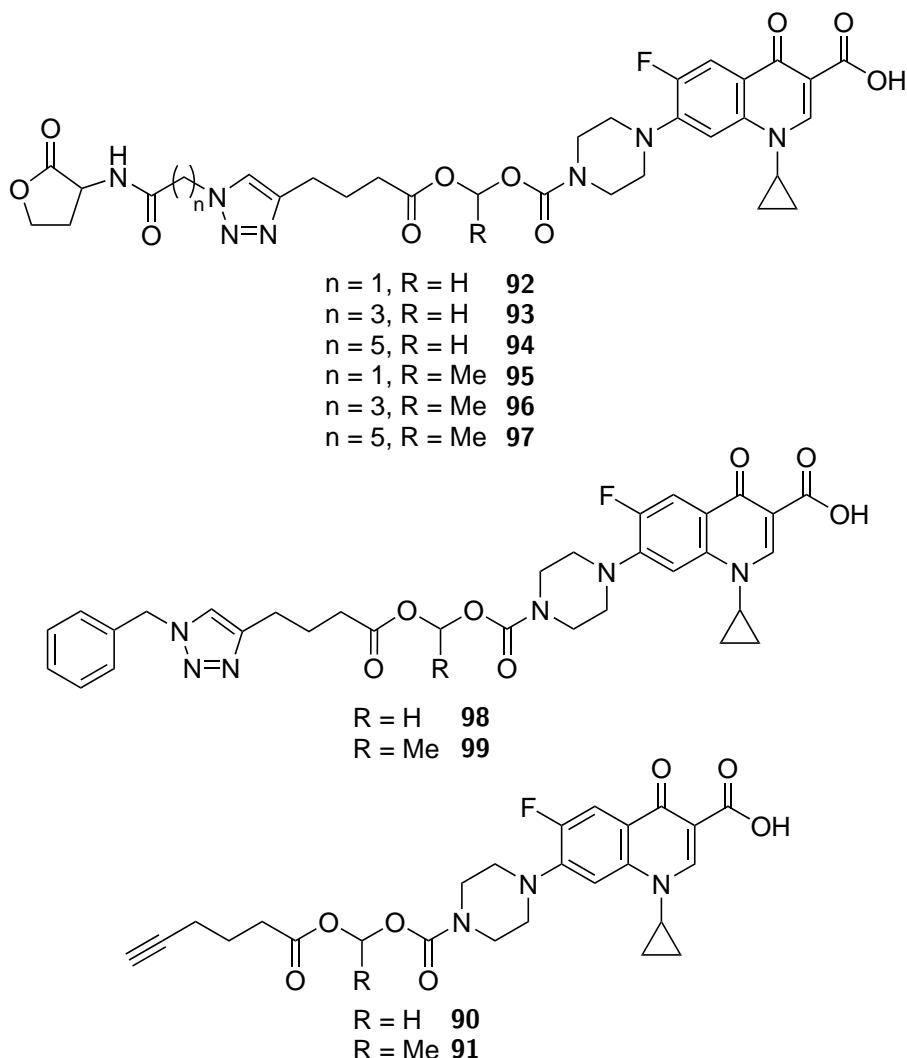


Figure 32: The cleavable HSL-Cip conjugates.

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.

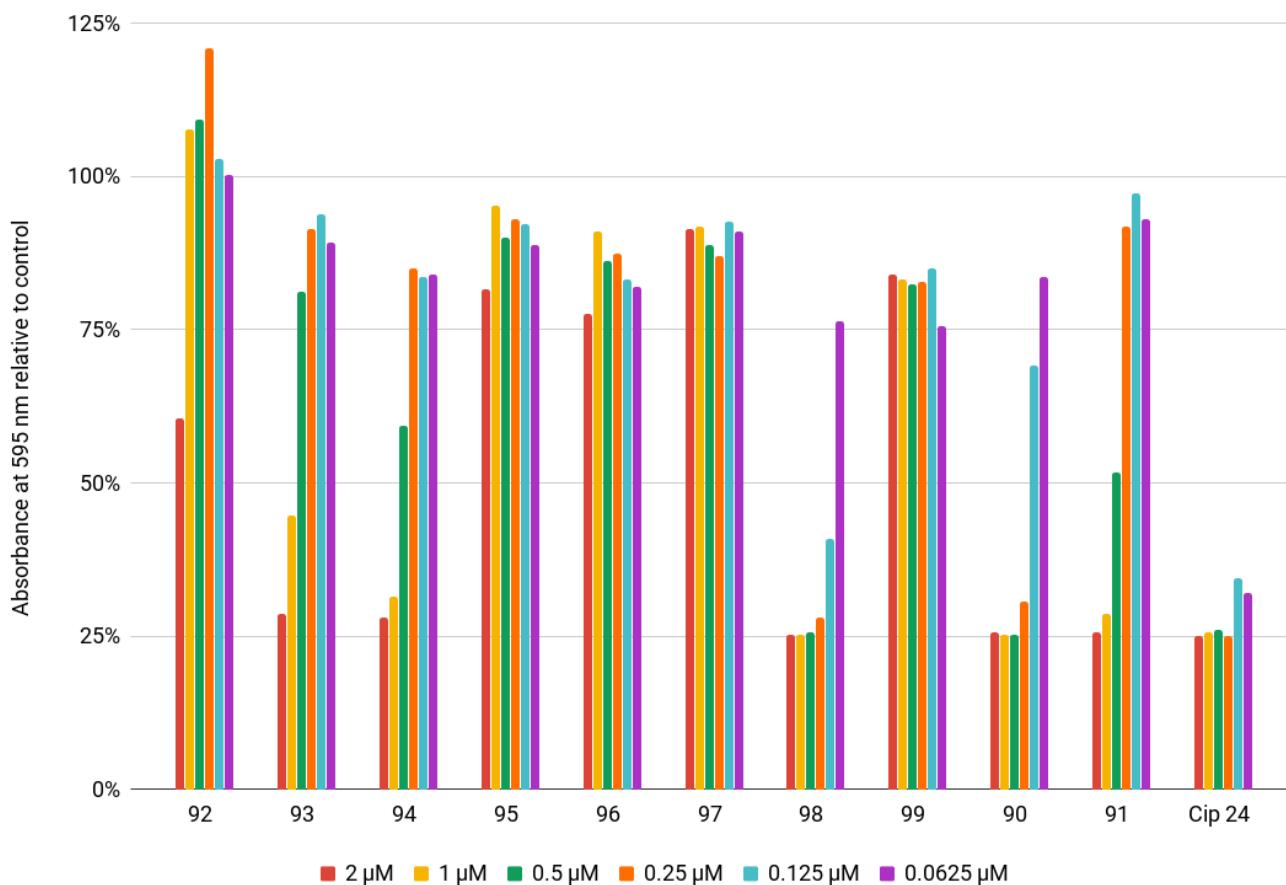


Figure 33: YM64 OD readings at 5 h for the cleavable HSL-Cip conjugates.

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.¹⁸⁶ 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,¹⁸⁷ 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 IV¹³⁷ or trimethoprim to dihydrofolate reductase.¹³⁸
2. Failure of the autoinducers to mask the antibiotics from recognition by efflux pumps (PAO1 only).
3. Failure to penetrate the cell wall/biofilm.
4. Non-specific binding to the cell wall.

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 **21** and PQS **22**, 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

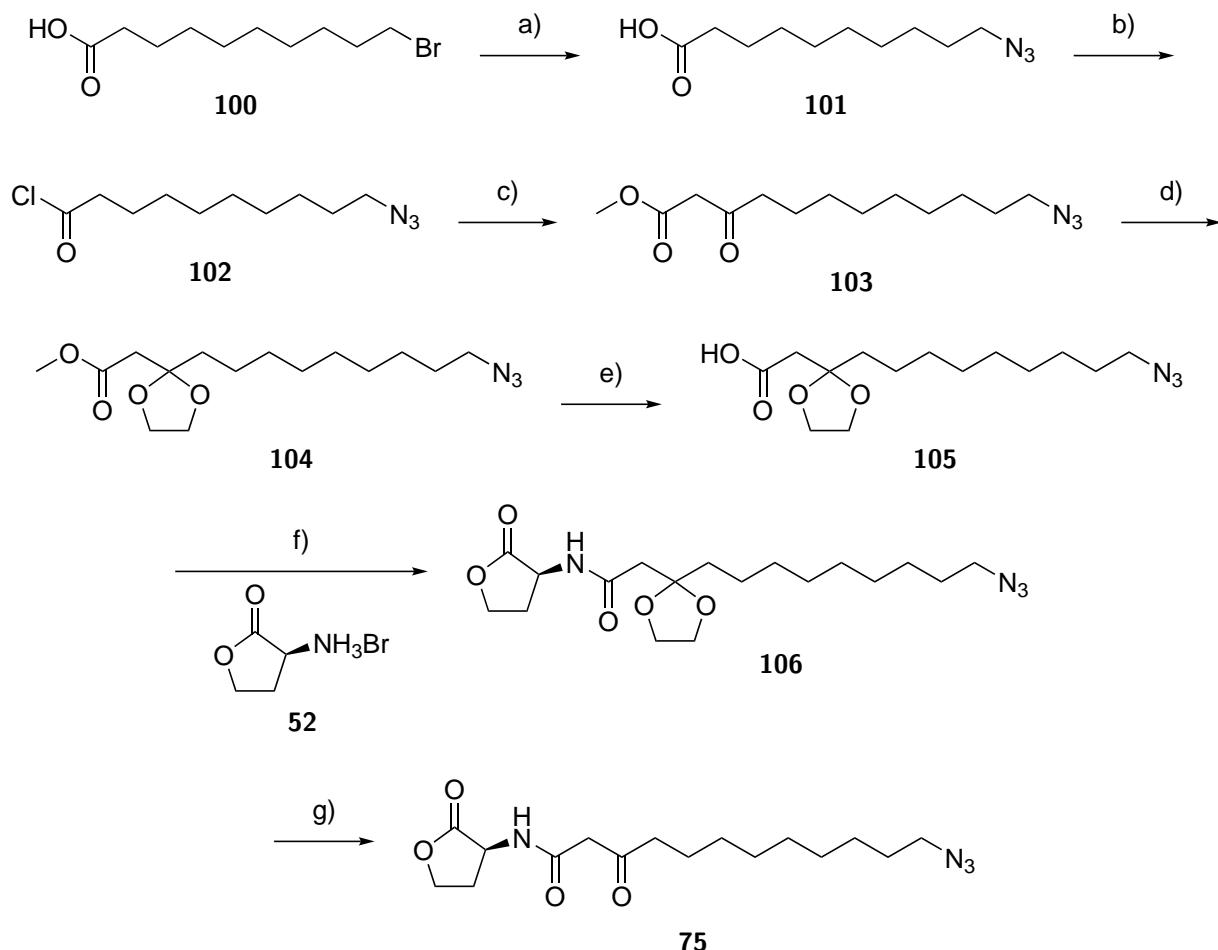
This section begins with discussion of further autoinducers and antibiotics which could be used in future conjugates. Some have already been partially or fully synthesised by myself or other members of the Spring group. Plans for further biological testing of the conjugates synthesised in this study 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 **20** reported by Hodgkinson *et al.*⁹⁴ 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 **106** 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 $\text{N}_3\text{-}3\text{-oxo-C}_{12}\text{-HSL}$ **75** carried out by Ryan Howard. a) NaN_3 , DMF, 60°C , 6 h, 93%. b) Oxalyl chloride, DMF, CH_2Cl_2 , 3 h, r.t.. c) MeOAc , *N*-methyl imidazole, TiCl_4 , DIPEA, toluene, r.t., 2 h, 43% over two steps. d) $\text{HO}(\text{CH}_2)_2\text{OH}$, TsOH , $\text{CH}(\text{OMe})_3$, r.t., 5 h, 78%. e) NaOH , water, r.t., 6 h, 85%. f) EDC, DMAP, CH_2Cl_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 cells¹⁸⁸ and used by a wide range of bacterial species.¹²⁸ 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.¹⁸⁷ AI-2 **23** is known to interconvert between multiple forms (including forming a furanosyl borate diester)¹⁸⁹ 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.

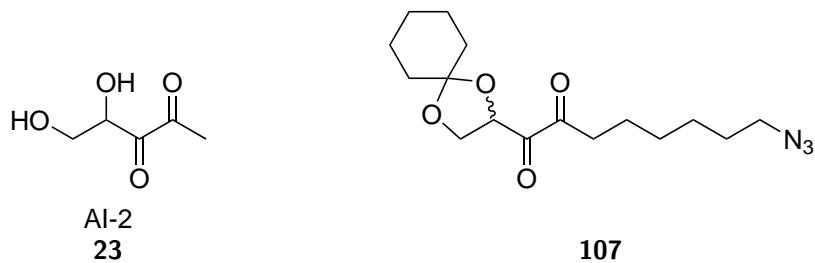


Figure 34: AI-2 **23** in its DPD form and the protected azido AI-2 derivative **107** synthesised by Dr Jamie Stokes.¹⁸⁷

Two types of AI-2 **23** receptors have been identified: LuxP, present in *Vibrio* spp.,¹⁹⁰ and LsrB, first discovered in *Salmonella enterica* serovar Typhimurium.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.¹⁹² LsrB is the ligand binding protein of a system that transports AI-2 **23** into the cell,¹⁸⁸ and hence can be targeted. LsrB orthologs are found in a wide range of bacterial families including *Enterobacteriaceae*, *Rhizobiaceae*, and *Bacillaceae*.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.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 *et al.*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.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**.

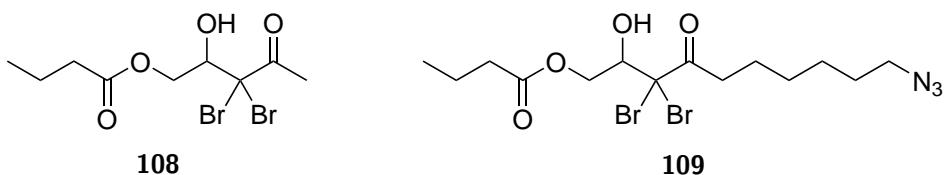


Figure 35: An AI-2 analogue **108** synthesised by Guo *et al.* and the proposed azido AI-2 analogue derivative **109**.

7.7.2 Antibiotic derivatives

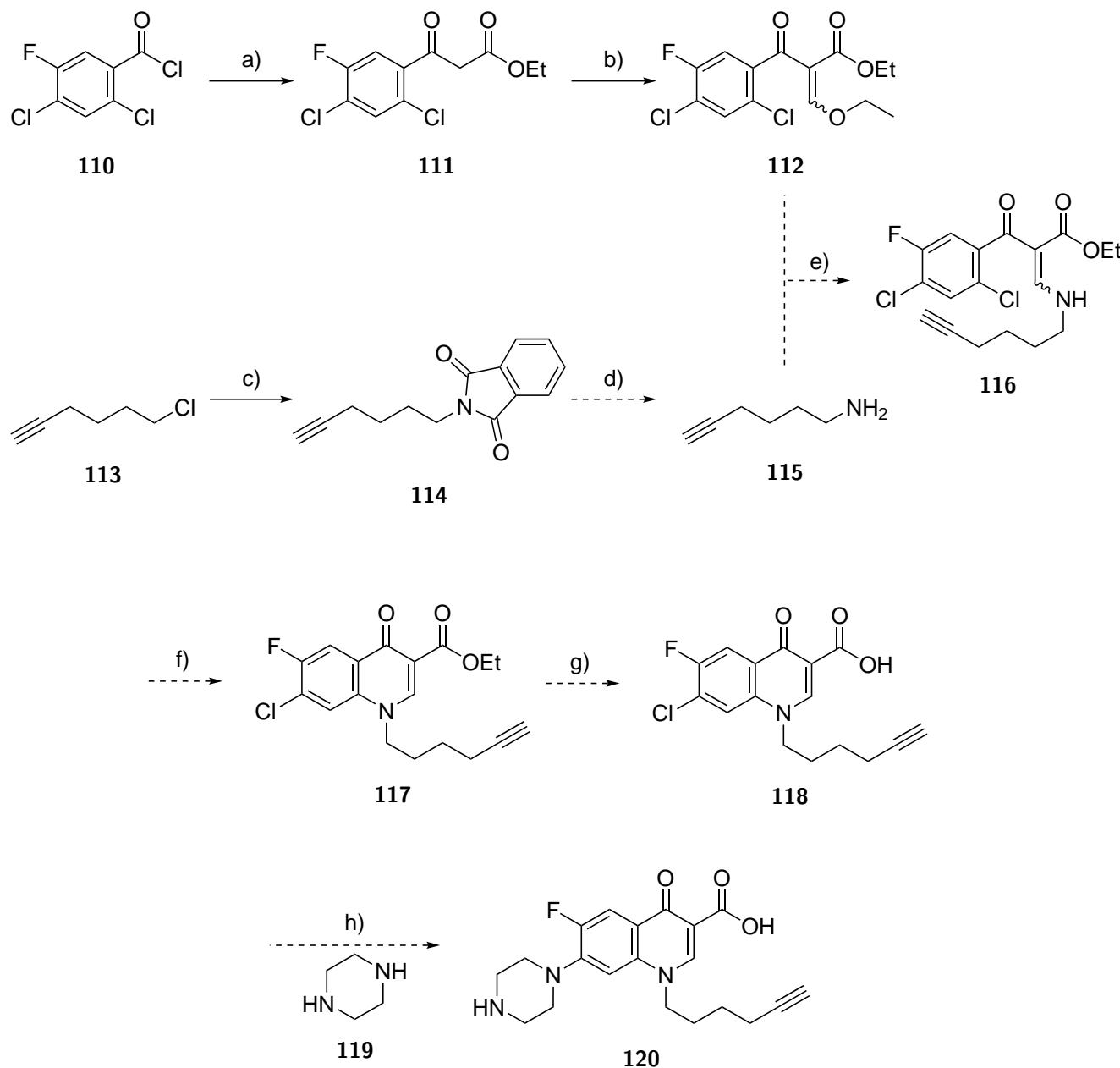
7.7.2.1 Ciprofloxacin derivative **120**

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.^{195,196} This synthesis followed a conventional route to ciprofloxacin similar to that reported by Mitscher *et al.*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 *et al.*¹⁹⁷ was used to produce the β -ketoester. The ethoxymethylene group in **112** was installed by

the reaction of β -ketoester **111** and triethyl orthoformate to give a mixture of the *E* and *Z* isomers.^{195, 198} Hex-5-yn-1-amine **115** was prepared using a Gabriel synthesis¹⁹⁹ described by Rożkiewicz *et al.*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 alkynyl amine was used, the rest of the synthesis could be performed and the resulting alkynyl ciprofloxacin derivative **120** could be used to form more triazole-linked conjugates.



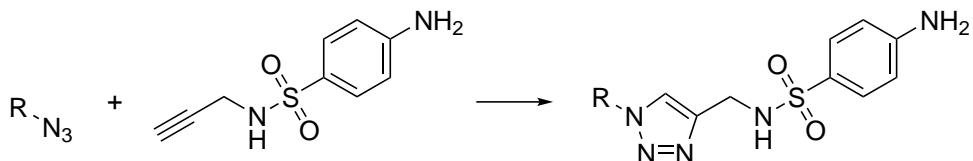
Scheme 12: The synthesis of **120**. a) EtOAc, $TiCl_4$, DIPEA, *N*-methyl imidazole, toluene, r.t., 30 min. b) Triethyl orthoformate, Ac_2O , reflux, 2 h. c) Potassium phthalimide, potassium iodide, DMF, 80 °C, 18 h. d) $N_2H_4 \cdot H_2O$, EtOH, reflux, 18 h. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) DMSO.

7.7.2.2 Sulfanilamide derivatives

Sulfanilamide antibiotics were the first class of antibiotics to be widely used.^{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. *P. aeruginosa* has intrinsic resistance to sulfanilamides mainly due to the MexAB-OprM efflux pump¹⁴² and so, as with trimethoprim **25**, it is hoped

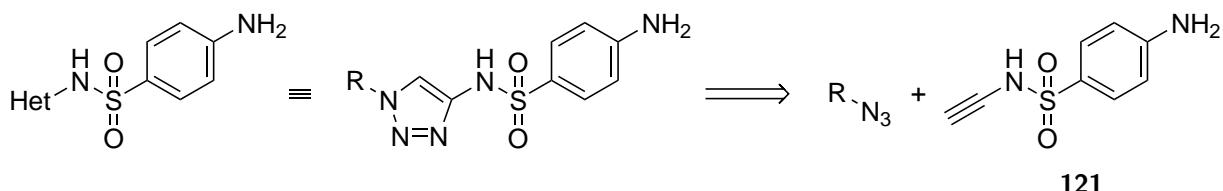
that conjugation to an autoinducer would restore activity.

Derivatives of 4-aminobenzenesulfonamide **216** have previously been synthesised using copper(I)-catalyzed alkyne-azide cycloaddition reactions to append various groups²⁰³ (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.²⁰³

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 **121** or a protected version of it (see Scheme 14).

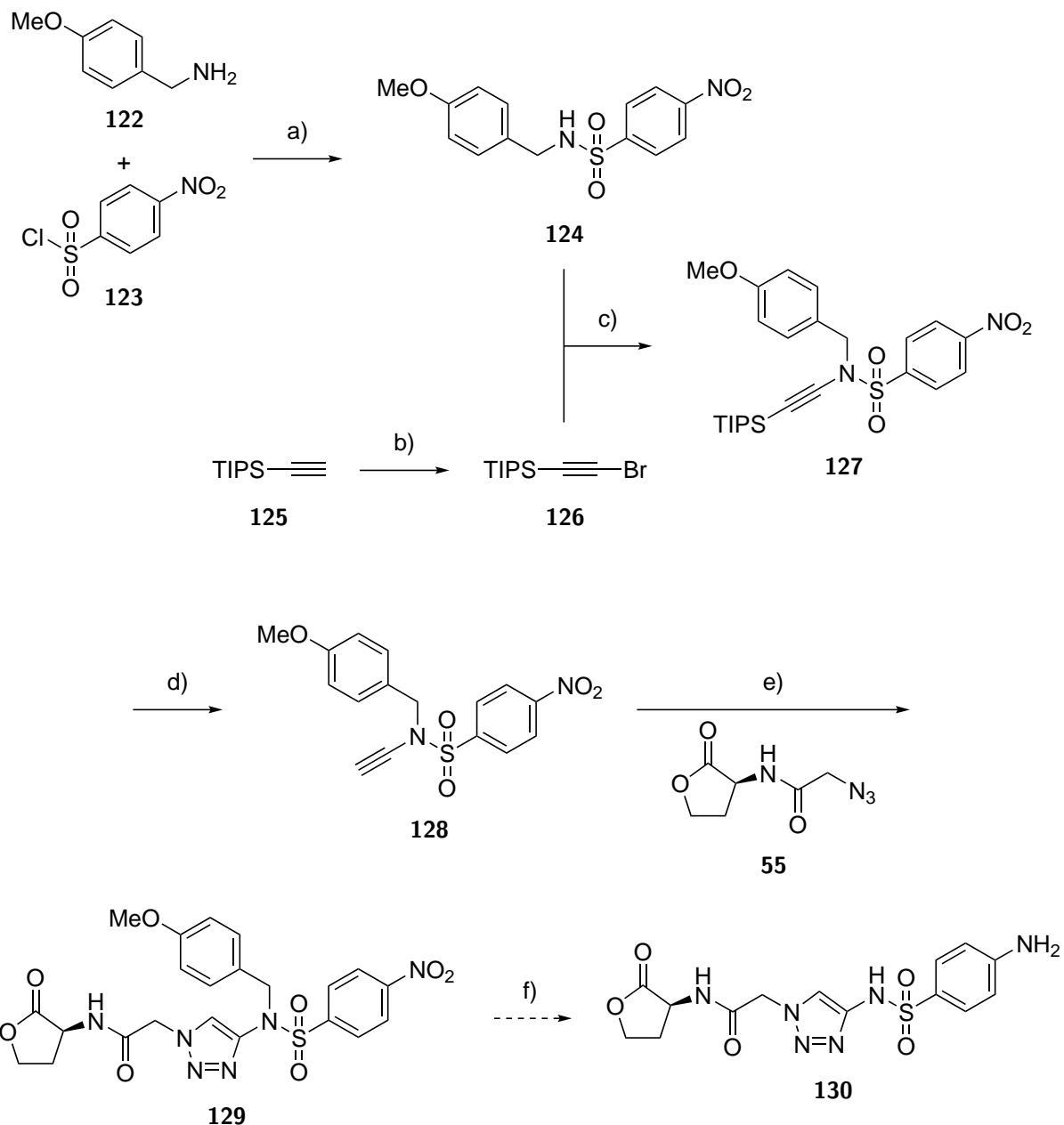


Scheme 14: Retrosynthesis of a 1,2,3-triazole-containing autoinducer-sulfonamide conjugate. R = autoinducer.

It was hoped that sulfanilamide derivative **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 ynamides has been studied more widely.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.^{205, 206}

The study of copper(I)-catalyzed alkyne-azide cycloadditions of ynamides by IJsselstijn et al.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.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 NH₂ group was installed as a NO₂ group and reduced after the click reaction.

The synthesis proceeded as shown in Scheme 15.^{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.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_2Cl_2 , r.t., 24 h. b) AgNO_3 , acetone, r.t., 3 h. c) $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 1,10-phenanthroline, K_2CO_3 , toluene, 80 °C, 48 h. d) TBAF, THF, -78 °C, 3 h. e) $\text{Cu}(\text{OAc})_2$, sodium ascorbate, CH_2Cl_2 , *t*-BuOH, water, r.t., 16 h. f) H_2 , PtO_2 , MeOH , 1 atm, r.t., 3 h.

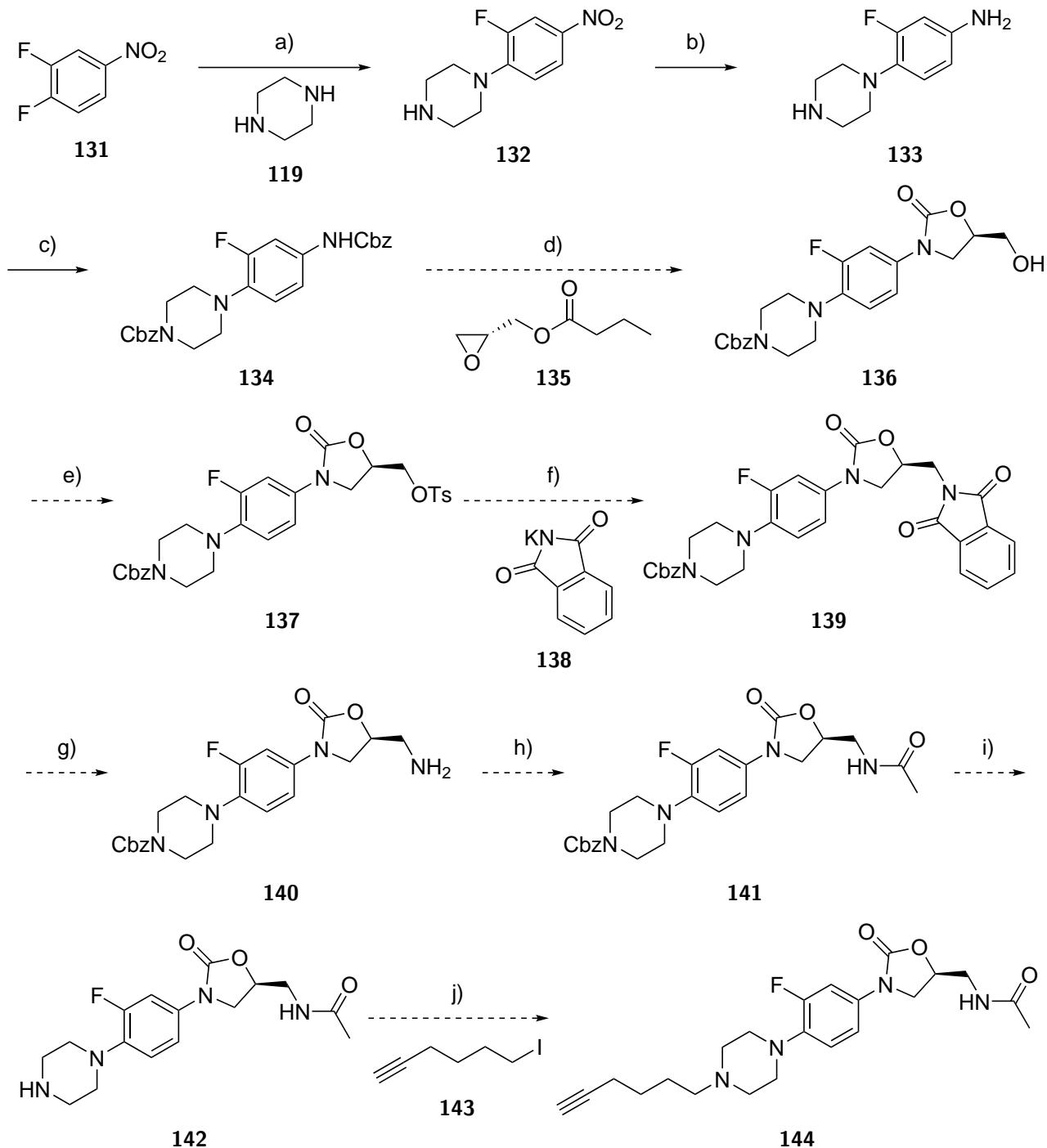
7.7.2.3 Linezolid derivative 144

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

fairly straightforwardly.

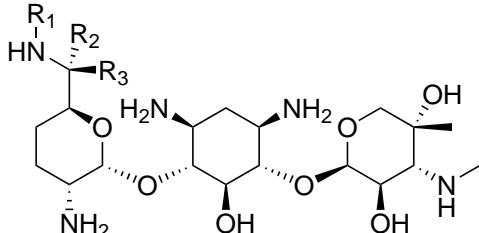


Scheme 16: Proposed and partially completed synthesis of linezolid derivative **218**.²¹⁰ a) MeCN, reflux, 3 h, 91%. b) H₂, 10% Pd/C, THF, 40 psi, <50 °C, 1.5 h, 95%. c) CbzCl, Na₂CO₃, acetone, water, 5 °C, 1 h then r.t., 16 h, 56%. d) n-BuLi, THF, -78 °C, 1 h then add epoxide then -78 °C to r.t., 5 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

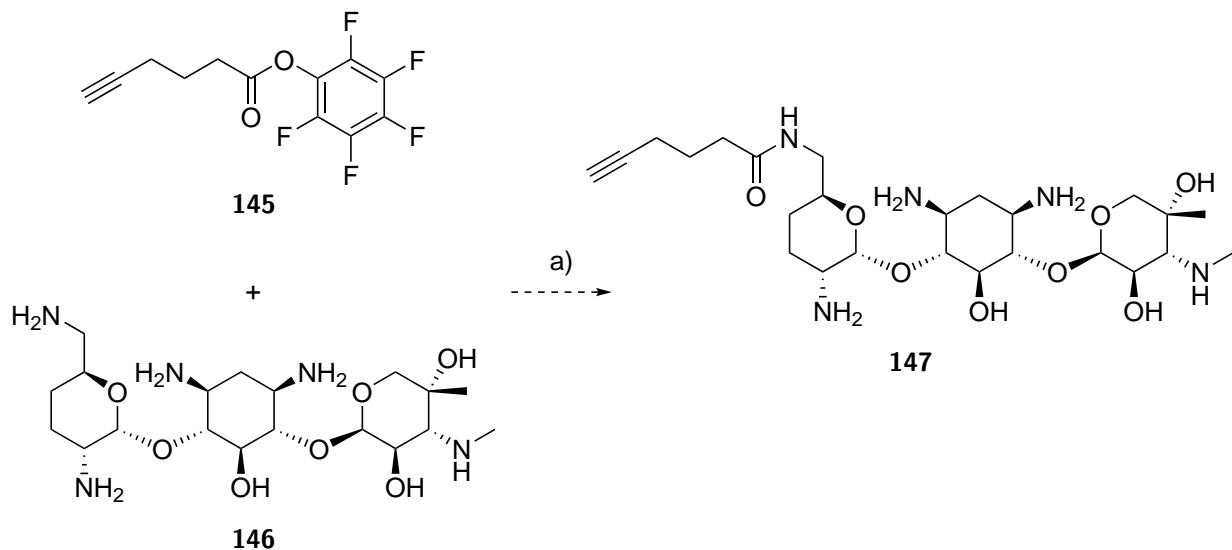
Gentamicin is an aminoglycoside antibiotic used to treat many bacterial infections, particularly those caused by Gram-negative organisms, by binding to the bacterial ribosome. Gentamicin is actually a mixture of components (see Figure 36) synthesised by *Micromonospora*, a genus of Gram-positive bacteria. Separation of the gentamicin

components has been achieved by Grote *et al.*²¹² by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a **146** was isolated pure, and is particularly useful because it is the only component which contains a CH_2NH_2 group. This group is less hindered than all other amine groups in gentamicin C1a **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.²¹³ Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid **145** (see Scheme 17). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester **145** and then separate out the desired component.



Gentamicin	R ₁	R ₂	R ₃
C1	Me	Me	H
C1a	H	H	H
C2	H	Me	H
C2a	H	H	Me
C2b	Me	H	H

Figure 36: Gentamicin components.

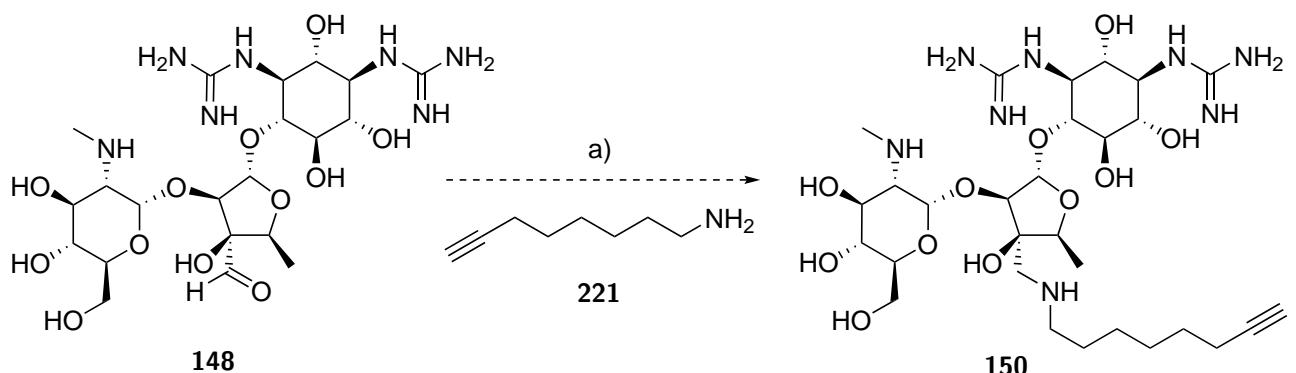


Scheme 17: Proposed synthesis of gentamicin C1a derivative **147**. a) DIPEA, DMF, -55 °C.

7.7.2.5 Streptomycin derivative **150**

Streptomycin **148** is an aminoglycoside antibiotic used to treat *Mycobacterium tuberculosis* and *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.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 **219** (see Scheme 18). This approach has been used by Zhang *et al.*²¹⁵ to form a conjugate of streptomycin **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.



Scheme 18: Proposed synthesis of streptomycin derivative **150**. a) NaBH_3CN , water, r.t..

7.7.3 Biology

The following extra biological data are required for these compounds:

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

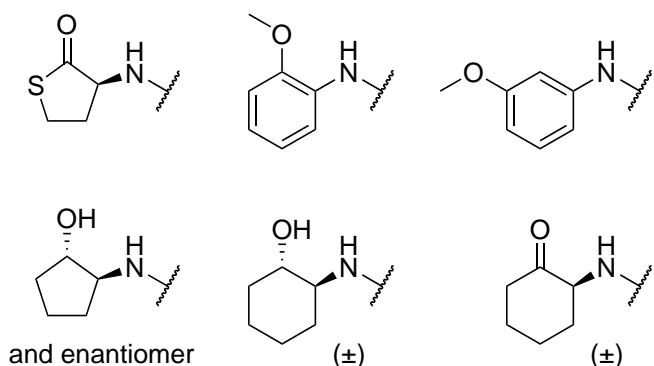
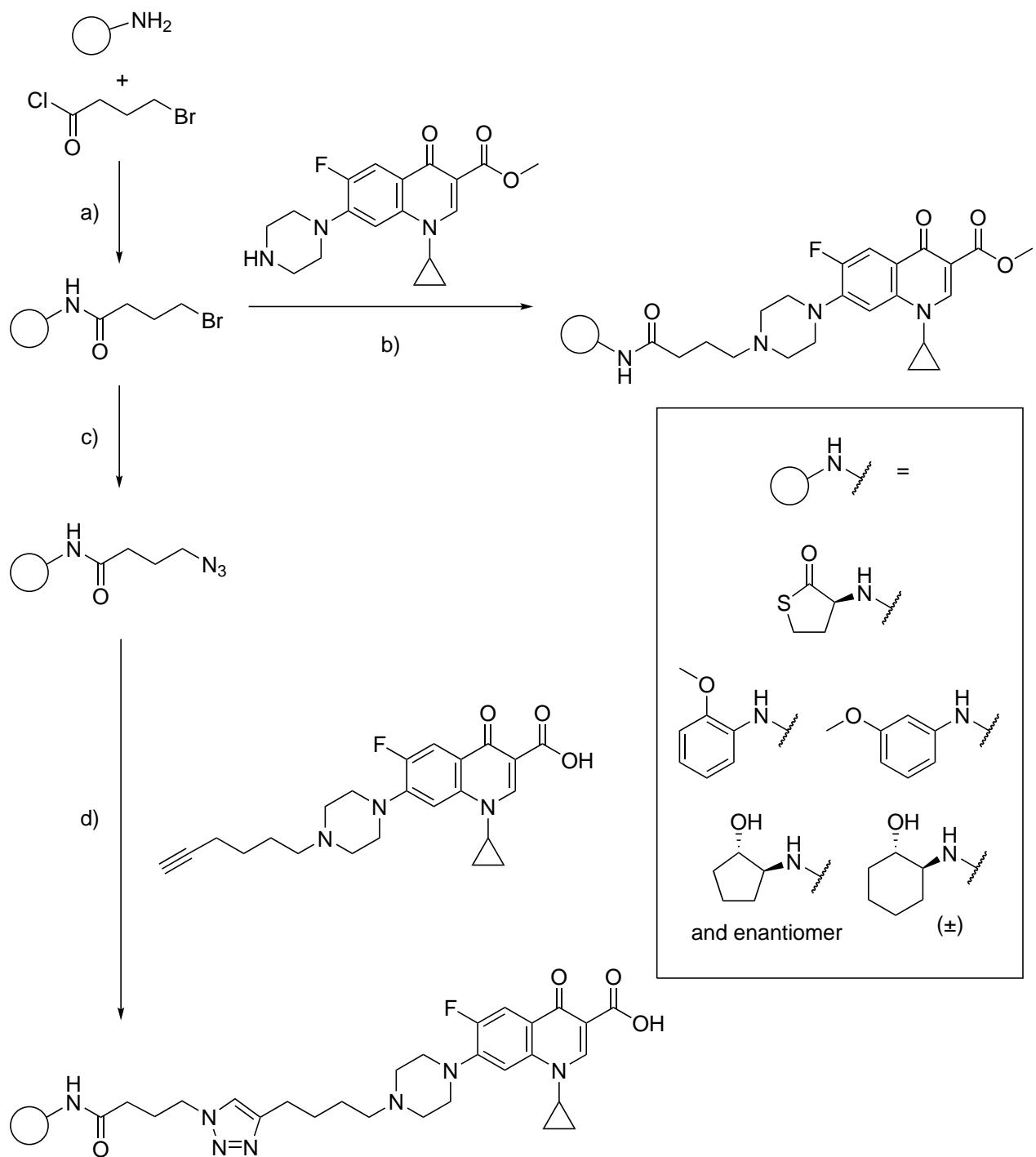


Figure 37: The head groups used in this section.

8.1.2 Library construction

As Ganguly *et al.*¹⁵ (see 5.3.8) synthesised their conjugate from Br-C₄-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. The second set would be made by displacing the bromide with azide, then performing a click reaction with the alkynyl ciprofloxacin derivative **68** made previously to form the triazole-linked product. Cyclohexanone 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.

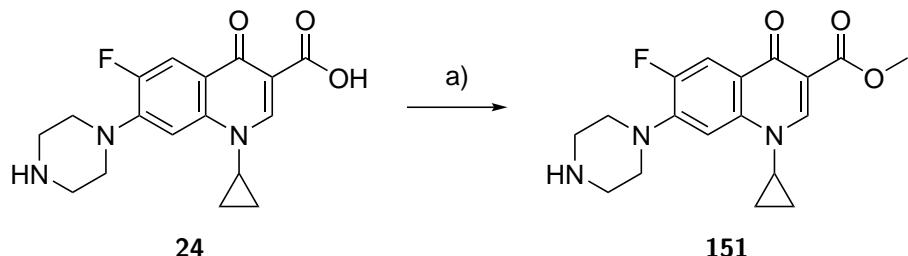
This strategy was successful for most head groups, but multiple side reactions were observed for the amino alcohol head groups and so other routes to these conjugates were investigated (see 8.5).

8.2 Homocysteine thiolactone derivatives

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

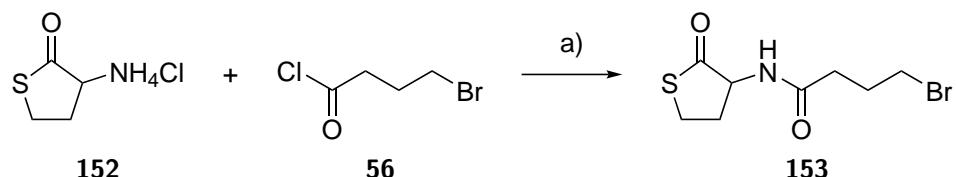
24 and MeOH in good yield using *para*-toluenesulfonic acid (TsOH) as a catalyst.²¹⁶



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

8.2.2 Synthesis of Br-C₄-HCTL **153**

The HCTL head group was then attached to the linker to form Br-C₄-HCTL **153**, in preparation for coupling to methyl ciprofloxacin **151**. Br-C₄-HCTL **153** was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives **57** and **60**. Br-C₄-HCTL **153** was isolated in markedly higher yield than that achieved by Ganguly *et al.*¹⁵ (88% vs. 25%). It is possible that this was due to CH₂Cl₂ being used for the extraction, whereas Ganguly *et al.* used EtOAc.

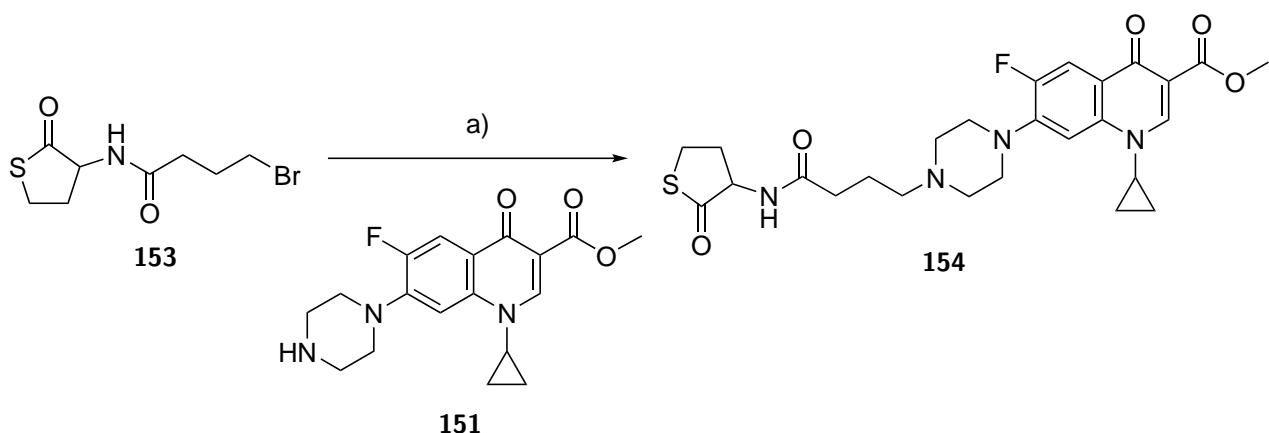


Scheme 21: Synthesis of Br-C₄-HCTL **153**. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 1 h, 88%.

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.*¹⁵ (see Scheme 22). Monitoring by LCMS showed slow conversion to the product. Br-C₄-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,^{15,149} 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.

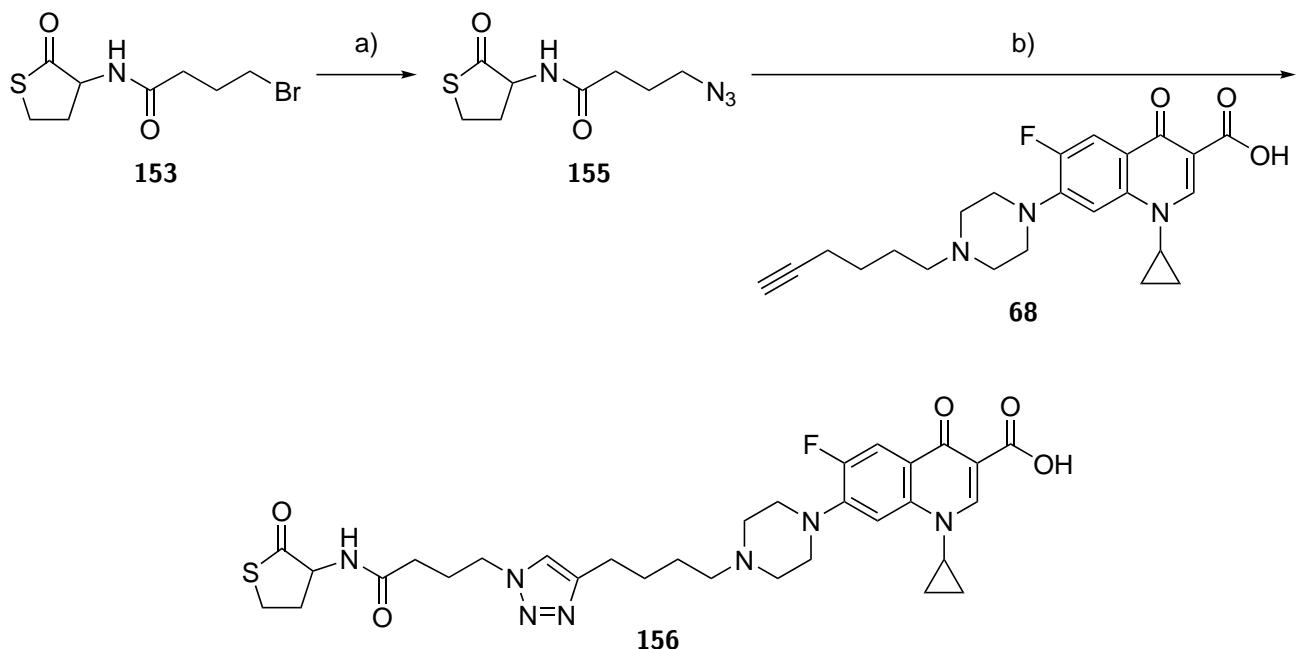


Scheme 22: Synthesis of the HCTL-CipMe conjugate **154**. a) K_2CO_3 , acetonitrile, reflux, 24 h, 12%.

8.2.4 Synthesis of the HCTL-Cip triazole conjugate **156**

$\text{Br-C}_4\text{-HCTL}$ **153** was converted into $\text{N}_3\text{-C}_4\text{-HCTL}$ **155** (see Scheme 22), by an $\text{S}_{\text{N}}2$ reaction with sodium azide which proceeded in excellent yield.

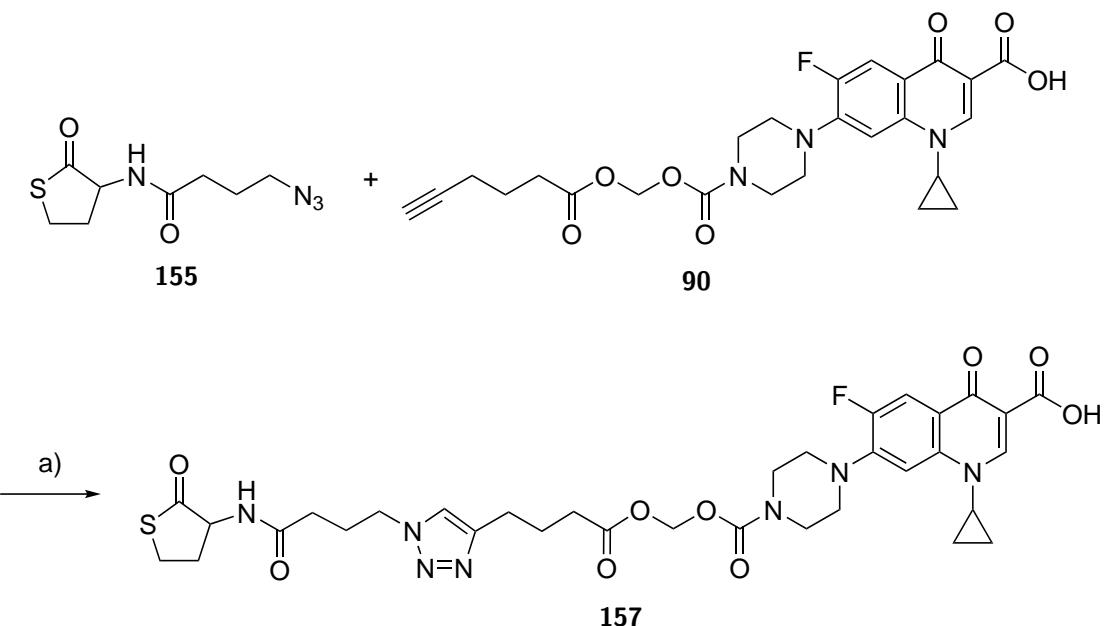
$\text{N}_3\text{-C}_4\text{-HCTL}$ **155** was then subjected to the click reaction conditions optimised previously (see 9.25). The reaction proceeded very slowly at first, as the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the 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) NaN_3 , acetonitrile, reflux, 1.5 h, 89%. b) CuSO_4 , THPTA, sodium ascorbate, water, $t\text{-BuOH}$, 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 $\text{N}_3\text{-C}_4\text{-HCTL}$ **155** by reaction with a cleavable alkyne-Cip derivative **90** synthesised previously by Professor Eddy Sotelo (see 7.4.3).



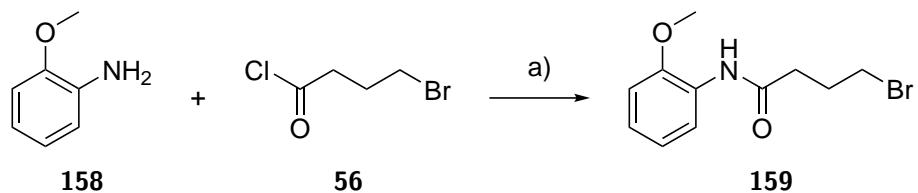
Scheme 24: Synthesis of the cleavable HCTL-Cip triazole conjugate **157**. a) CuI, DIPEA, CH₂Cl₂, r.t., 3 h, 5%.

8.3 2-Methoxybenzene derivatives

8.3.1 Synthesis of Br-C₆-2-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,^{217,218} 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 S_N2 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.

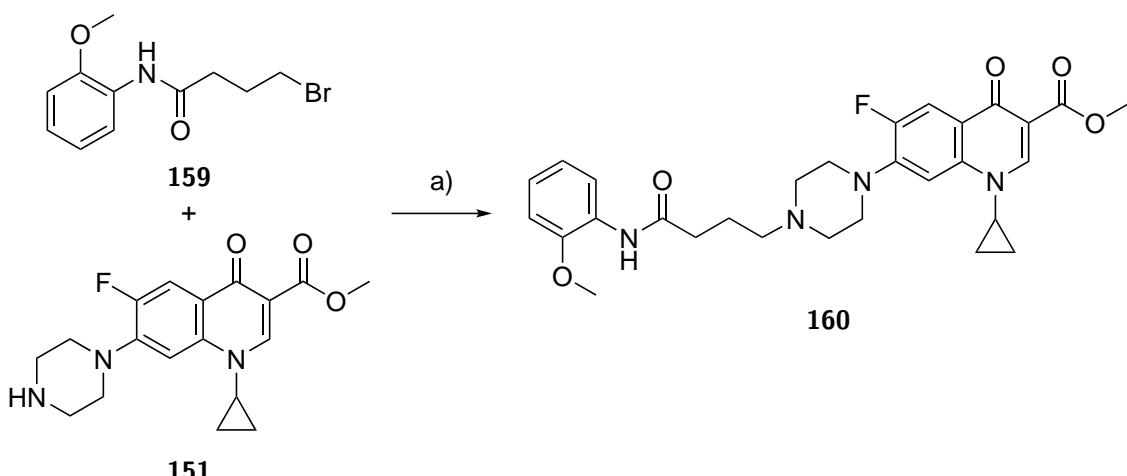


Scheme 25: Synthesis of Br-C₄-2-methoxybenzene **159**. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 1 h, 50%.

8.3.2 Synthesis of the 2-methoxybenzene-CipMe conjugate 160

The procedure outlined by Ganguly *et al.*¹⁵ 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**

$\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{161}$ was synthesised from $\text{Br-C}_4\text{-2-methoxybenzene } \mathbf{159}$ by an $\text{S}_{\text{N}}2$ reaction with sodium azide (see Scheme 27). The yield of $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{161}$ (27%) was a lot lower than for $\text{N}_3\text{-C}_4\text{-HCTL } \mathbf{155}$ (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).

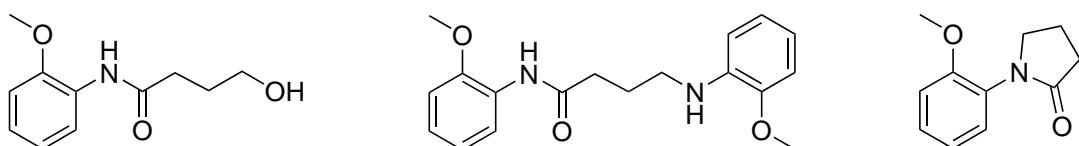
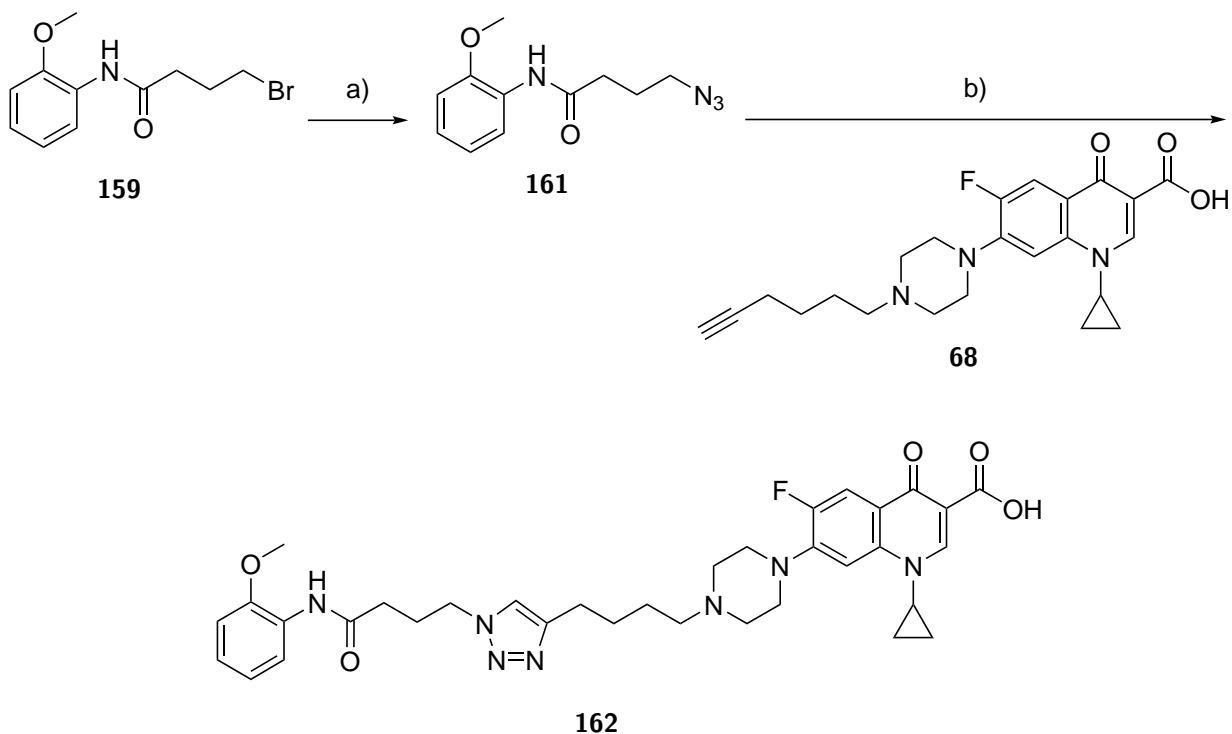


Figure 38: Suspected impurities observed by LCMS during the synthesis of $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{161}$.

The 2-methoxybenzene-Cip triazole conjugate **162** was synthesised using the standard click conditions (see 9.25), with the addition of CH_2Cl_2 as a co-solvent to aid the dissolution of $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{161}$ (see Scheme 27).

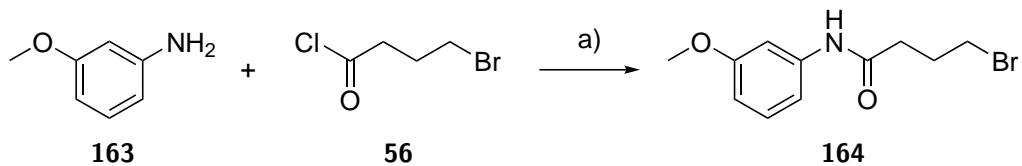


Scheme 27: Synthesis of the 2-methoxybenzene-Cip triazole conjugate **162**. a) Na_3 , acetonitrile, reflux, 2 h, 27%. b) CuSO_4 , THPTA, sodium ascorbate, water, *t*-BuOH, CH_2Cl_2 , r.t., 16 h, 39%.

8.4 3-Methoxybenzene derivatives

8.4.1 Synthesis of Br-C₄-3-methoxybenzene **164**

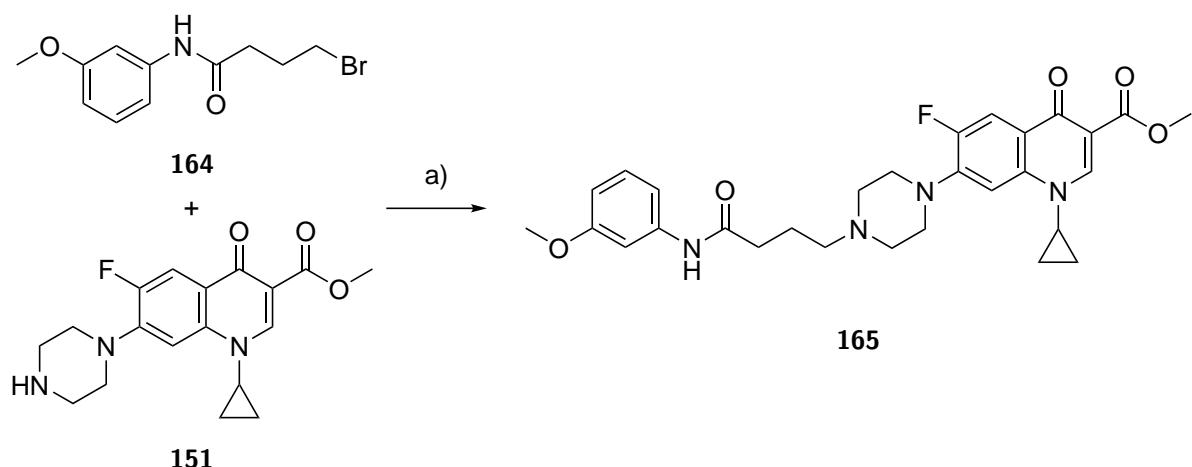
Br-C₄-3-methoxybenzene **164** was synthesised from 3-methoxyaniline **163** and 4-bromobutyryl chloride **56** using Schotten-Baumann conditions as above in almost identical (50%) yield (see Scheme 28).



Scheme 28: Synthesis of Br-C₄-3-methoxybenzene **159**. a) NaHCO_3 , CH_2Cl_2 , water, 0 °C, 1 h, 50%.

8.4.2 Synthesis of the 3-methoxybenzene-CipMe conjugate **165**

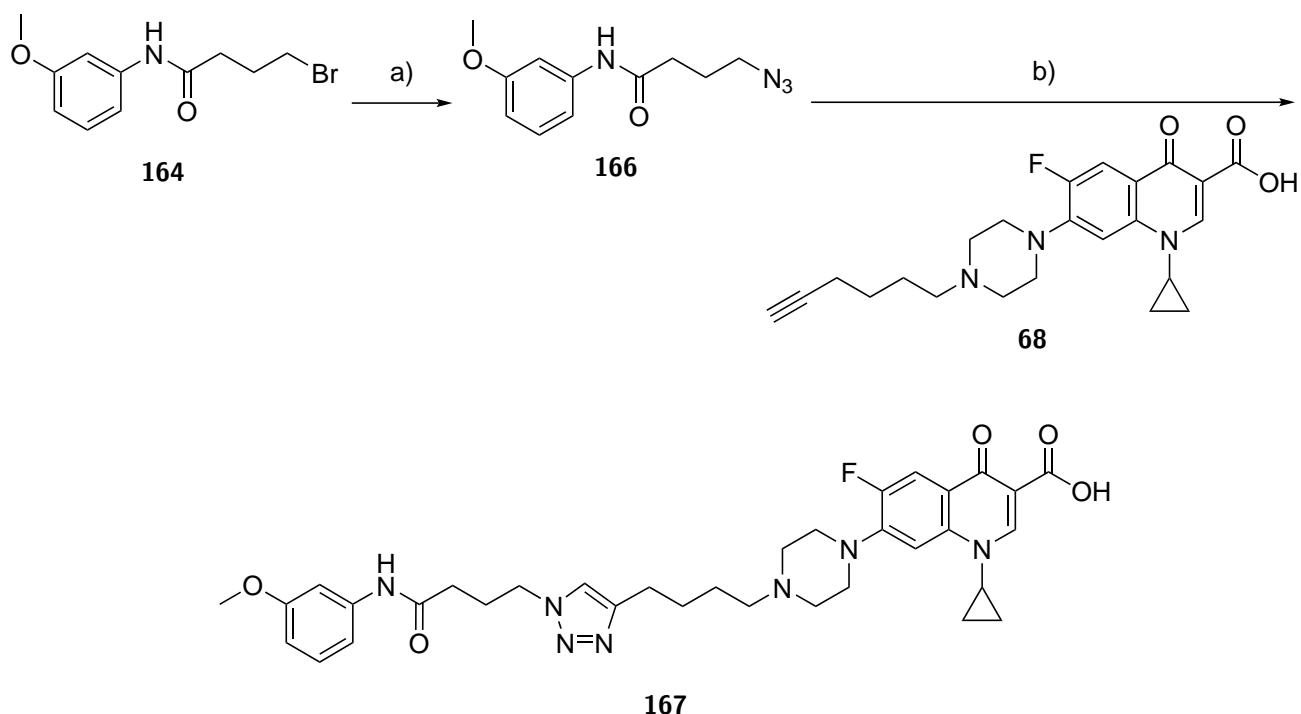
The 3-methoxybenzene-CipMe conjugate **165**, was synthesised as above, in similar yield (see Scheme 29).



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**

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

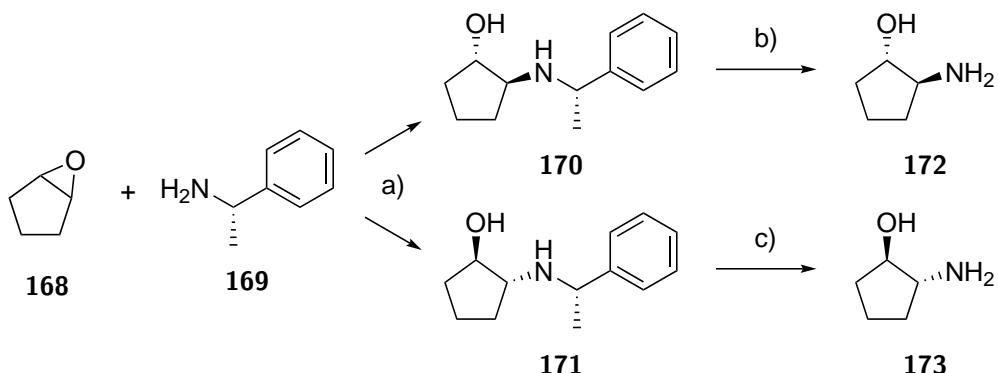


Scheme 30: Synthesis of the 3-methoxybenzene-Cip triazole conjugate **167**. a) NaN₃, acetonitrile, reflux, 7 h, 17%. b) CuSO₄, THPTA, sodium ascorbate, water, *t*-BuOH, CH₂Cl₂, r.t., 2 h, 5%.

8.5 Cyclopentanol derivatives

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.^{219–221} These precursors were synthesised by opening cyclopentene oxide **168** using (*S*)-1-phenylethanimine **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 Sugai²²⁰ 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 H₂ 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.



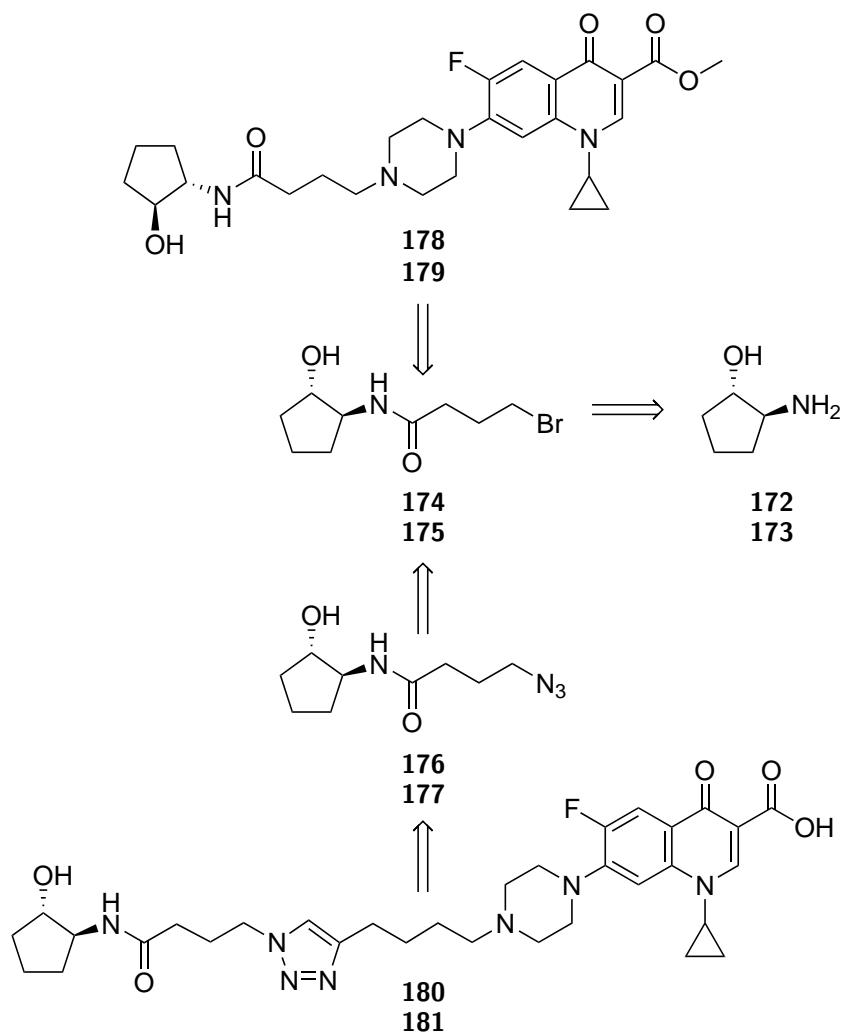
Scheme 31: Synthesis of (*1S,2S*)-2-aminocyclopentan-1-ol **172** and (*1R,2R*)-2-aminocyclopentan-1-ol **173**. a) AlMe₃, CH₂Cl₂, 0 °C, **170** (*SSS*): 35%, **171** (*RRS*): 32%. b) See Table 9. c) Pd(OH)₂, MeOH, H₂, 5 atm, r.t., 1 d, 100%.

Conditions	Temperature and pressure	Time	Result
170 · HCl, ammonium formate, 10% Pd/C, DMF	r.t., 1 atm	2 d	170 salt
170 , ammonium formate, 10% Pd/C, DMF	r.t., 1 atm	2 d	170 salt
170 · HCl, ammonium formate, 10% Pd/C, dry DMF	r.t., 1 atm	2 d	170 salt
171 , ammonium formate, 10% Pd/C, dry DMF	r.t., 1 atm	2 d	171 salt
170 , ammonium formate, 10% Pd/C, dry DMF	70 °C, 1 atm	1 d	170 salt
170 , ammonium formate, 10% Pd/C, dry DMF, AcOH	70 °C, 1 atm	1 d	Complex mixture
170 · HCl, dry ammonium formate, 10% Pd/C, dry DMF	120 °C, 1 atm	7 d	Complex mixture
170 · HCl, Pd(OH) ₂ , MeOH, H ₂	r.t., 1 atm	1 d	170 salt
170 · HCl, Pd(OH) ₂ , MeOH, H ₂	r.t., 3.4 atm	1 d	172 salt, 170 salt, and an unidentified compound (approx. 7:2:10 by ¹ H NMR)
170 , Pd(OH) ₂ , MeOH, H ₂	r.t., 5 atm	1 d	172 , 100% yield

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

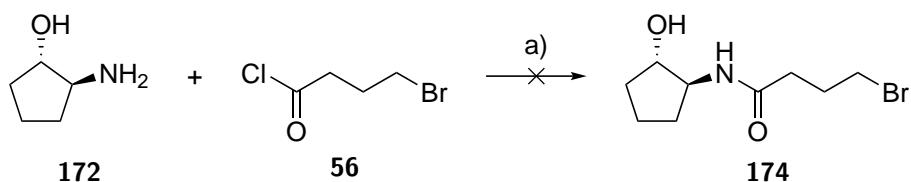
8.5.2 Initial branching route

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 (1*S*,2*S*)-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.

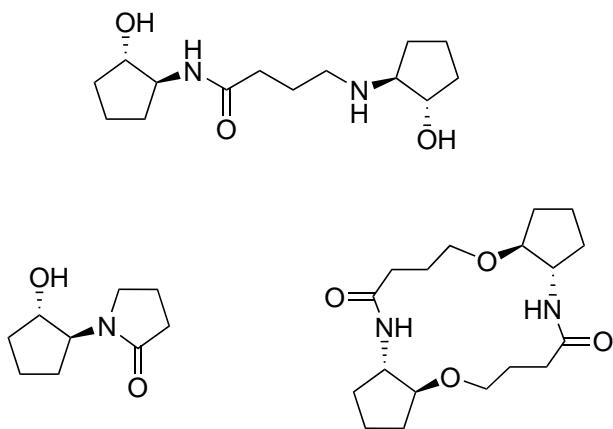
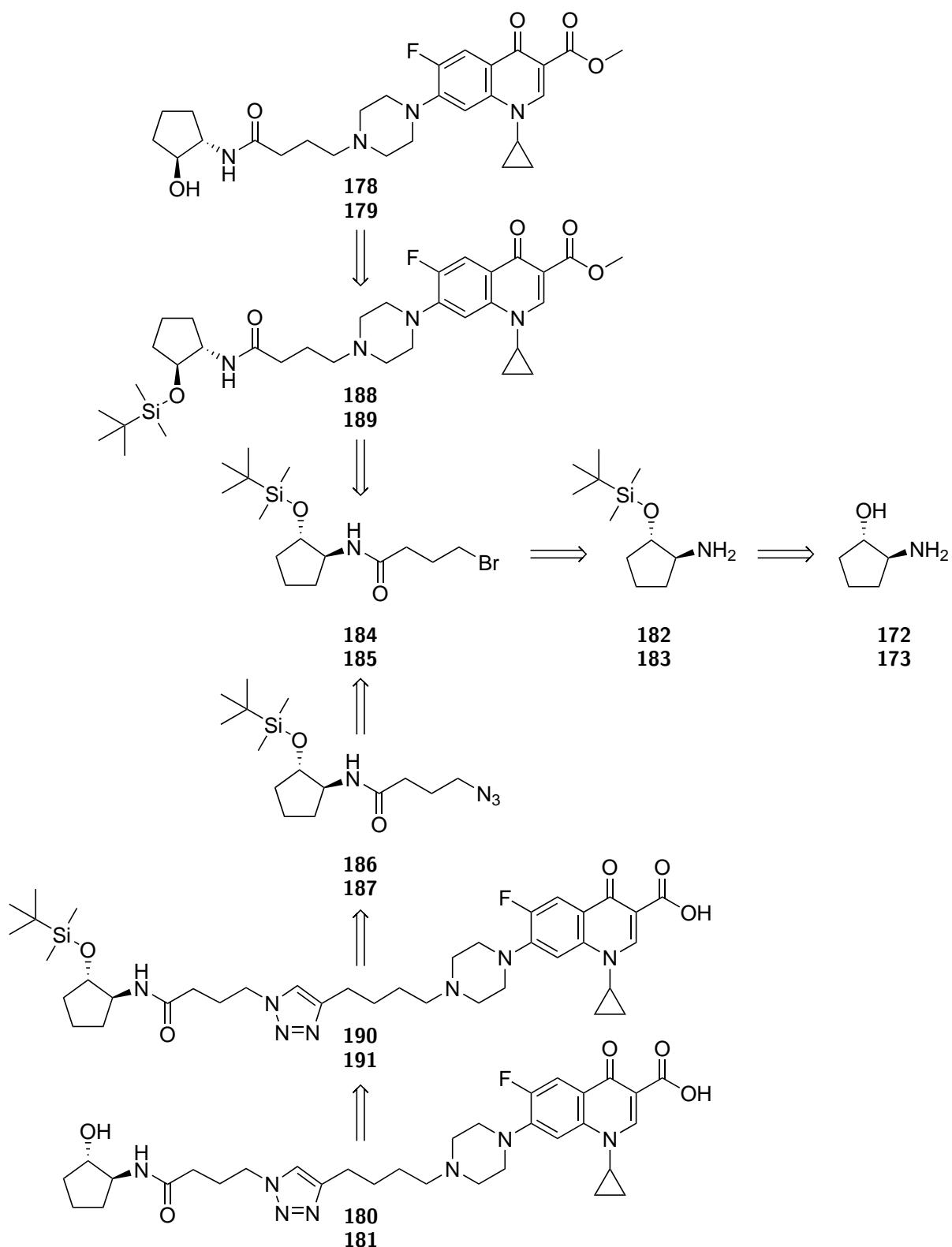


Figure 39: Suspected impurities observed by LCMS during the synthesis of Br-C₄-cyclopentanol-(SS) **174**. Regiochemistry is speculative.

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.

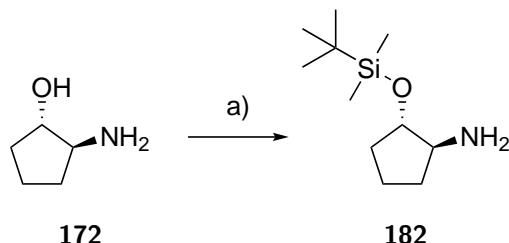


Scheme 34: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*), and the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) using a TBDMS protection strategy. *SS* enantiomers are shown, but both are implied.

8.5.3.1 Synthesis of TBDMS-protected (*1S,2S*)-2-aminocyclopentan-1-ol **172**

The synthesis began with the optimisation of the protection of (*1S,2S*)-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. al²²² using TBDMSOTf was found to produce the desired product in excellent yield. Water was used for the work-up rather than NH₄Cl (sat. aq.), as the acidic work-up protonated the product. The TEA was removed during column chromatography instead.



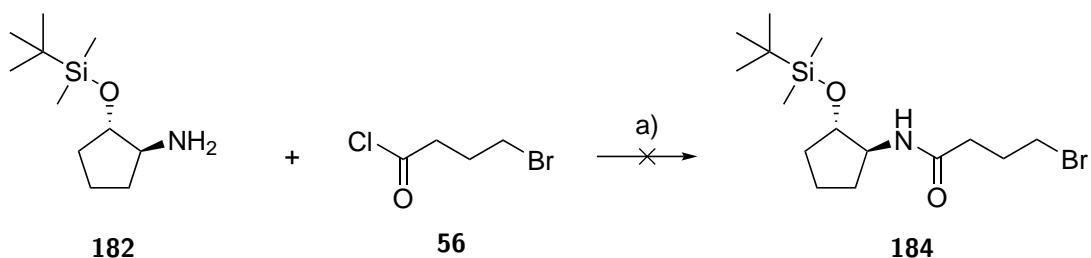
Scheme 35: Synthesis of TBDMS protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **182**. a) See Table 10.

Conditions	Temperature	Time	Result
TBDMSCl, DMAP, TEA, CH ₂ Cl ₂ ²²³	r.t.	18 h	Trace of 182 , mostly 172
TBDMSCl, imidazole, CH ₂ Cl ₂ ²²⁴	0 °C	1 h	172
TBDMSCl, DBU, acetonitrile ²²⁵	0 °C	1 d	172
TBDMSOTf, TEA, CH ₂ Cl ₂ , ²²² aq. workup then column	0 °C	6 h	182 , 98% yield

Table 10: Conditions attempted for the synthesis of (1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentan-1-amine **182** (see Scheme 36).

8.5.3.2 Synthesis of Br-C₄-cyclopentanol-TBDMS-(SS) **184**

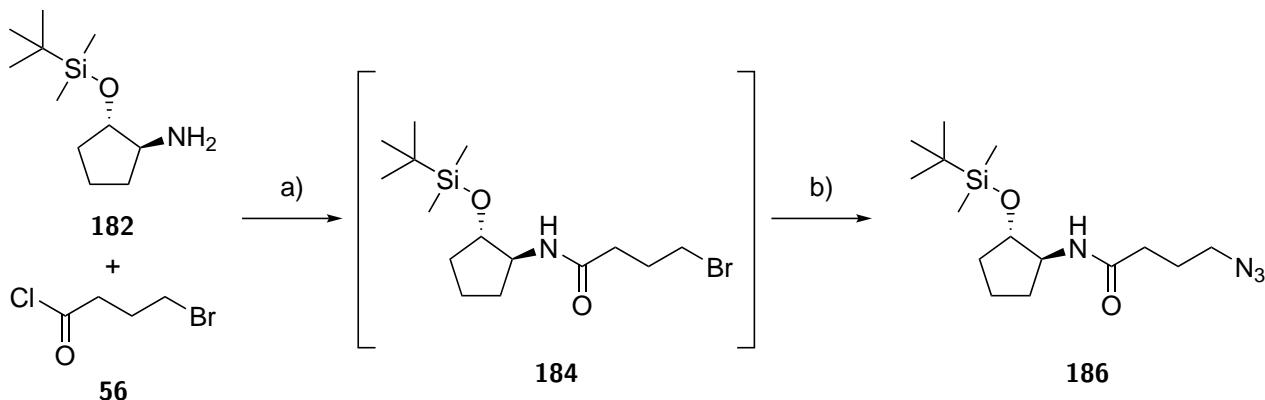
The TBDMS protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **182** was reacted with 4-bromobutyryl chloride **56** to form Br-C₄-cyclopentanol-TBDMS-(SS) **184**. The reaction was observed to go to completion by TLC, but it became apparent that the product was reacting further during concentration and purification. Adding sodium azide to the mixture obtained after the failed purification attempts was observed to convert the remaining Br-C₄-cyclopentanol-TBDMS-(SS) **184** to N₃-C₄-cyclopentanol-TBDMS-(SS) **186**. A sequential one-pot reaction was therefore used, so that the reactive intermediate did not need to be isolated.



Scheme 36: Attempted synthesis of Br-C₄-cyclopentanol-TBDMS-(SS) **184**. a) NaHCO₃, CH₂Cl₂, water, 0 °C, 2 h.

8.5.3.3 Synthesis of N₃-C₄-cyclopentanol-TBDMS-(SS) 186 by one-pot reaction

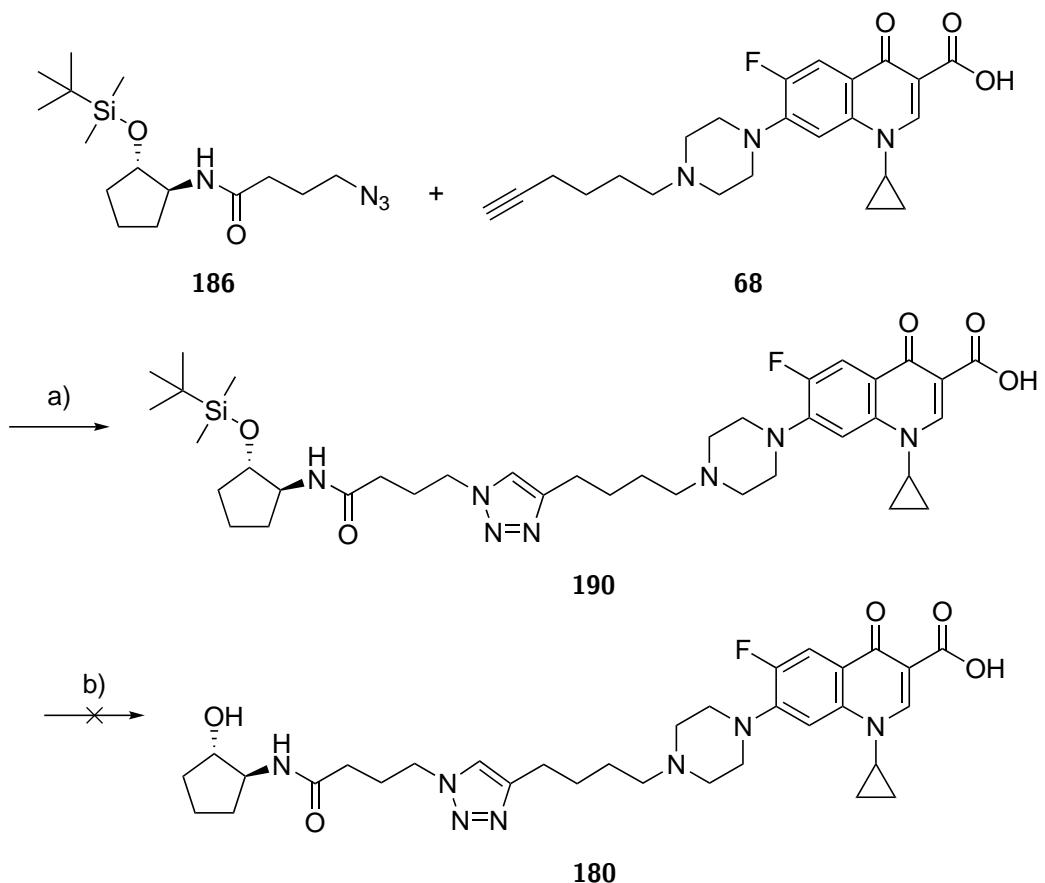
N₃-C₄-cyclopentanol-TBDMS-(SS) **186** was finally synthesised by a two-step, one-pot reaction. Schotten-Baumann conditions were used to form the bromide. The water was then removed, and DMF and sodium azide were added. N₃-C₄-cyclopentanol-TBDMS-(SS) **186** was produced in excellent yield.



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**

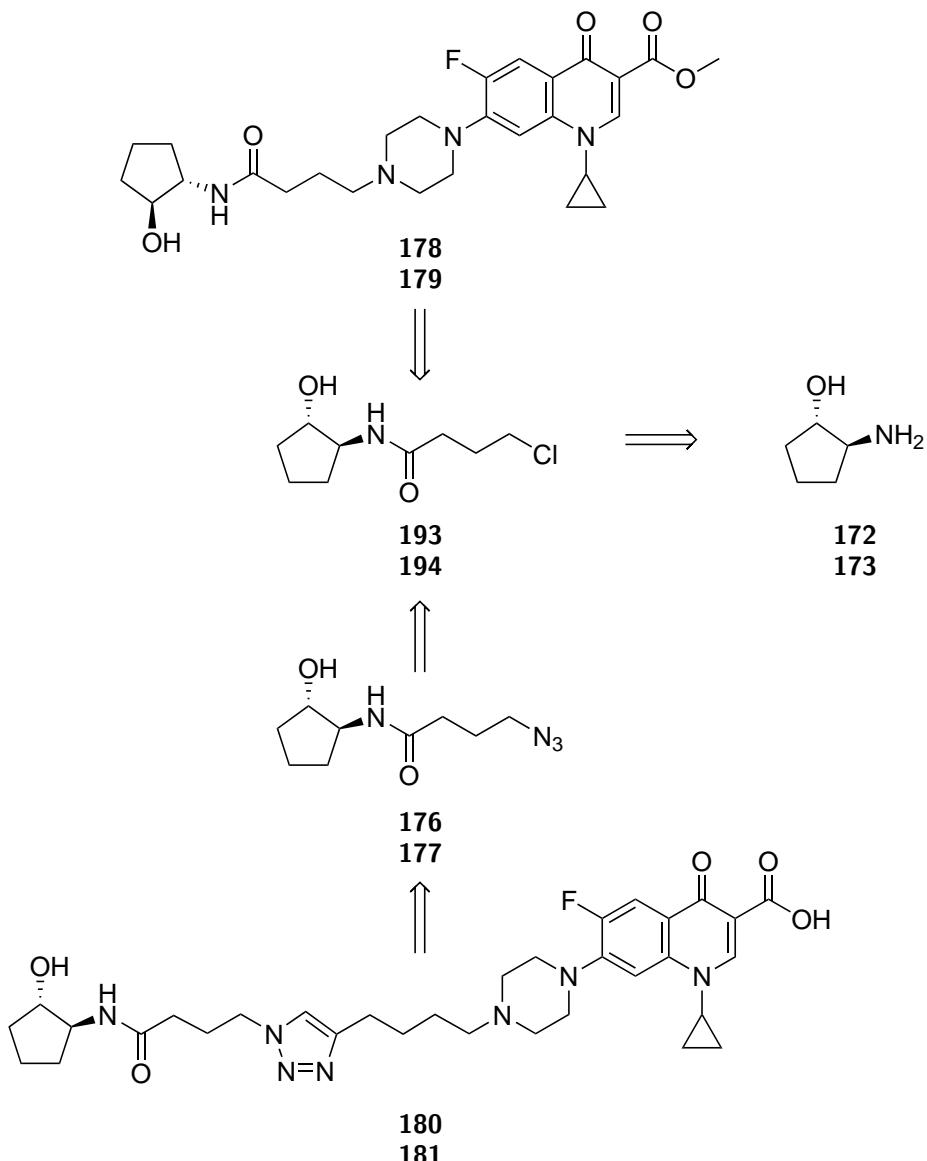
N₃-C₄-cyclopentanol-TBDMS-(SS) **186** and the alkynyl ciprofloxacin derivative **68** were subjected to standard click conditions (see 9.25), and the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate **190** was synthesised in very good yield. However, removal of the TBDMS group proved difficult. Deprotection 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 CaCO₃²²⁶ 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) CuSO_4 , 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 S_N2 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,¹⁵ but it was anticipated that using a chloride would reduce the side reactions seen with the more reactive bromide.



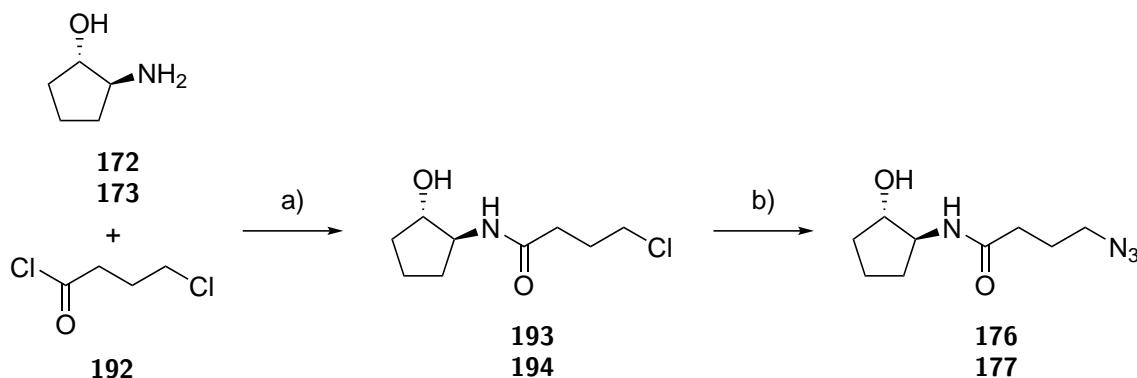
Scheme 39: Retrosynthesis of the cyclopentanol-CipMe conjugates **178** (*SS*) and **179** (*RR*), and the cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) via Cl-C₄-cyclopentanol intermediates **193** (*SS*) and **194** (*RR*). *SS* enantiomers are shown, but both are implied.

Attempts at this route began with the synthesis of Cl-C₄-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.*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 (1*R*,2*R*)-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 CH₂Cl₂ and TEA. This produced Cl-C₄-cyclopentanol-(*RR*) **194** in good yield (64%). Unlike the bromide **174**, the chloride **194** was stable when concentrated.

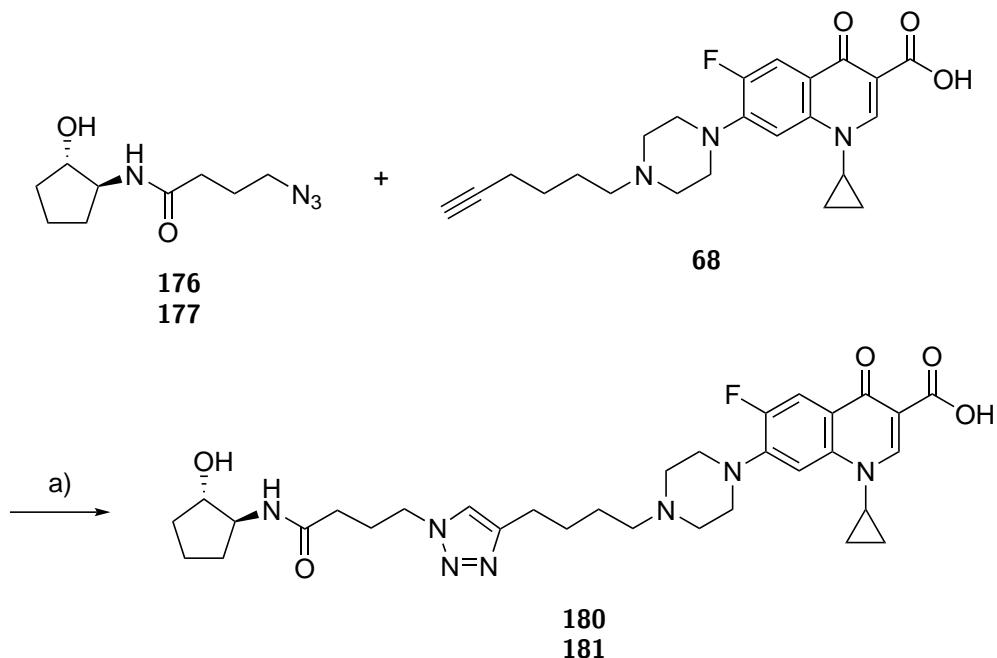
Cl-C₄-cyclopentanol-(*RR*) **194** was converted to N₃-C₄-cyclopentanol-(*RR*) **177** by reaction with sodium azide. The reaction was slower than with previous bromides (~16 h vs. ~2 h), but much cleaner than with Br-C₄-cyclopentanol-(*SS*) **174** (see 8.5.2).

The enantiomers Cl-C₄-cyclopentanol-(SS) **193** and N₃-C₄-cyclopentanol-(SS) **176** were synthesised in lower yields, in part because of the smaller amounts being used.



Scheme 40: Synthesis of N₃-C₄-cyclopentanol-(SS) **176** and N₃-C₄-cyclopentanol-(RR) **177**. *SS* enantiomers are shown, but both were synthesised. a) TEA, CH₂Cl₂, 0 °C, 2 h, **193** (*SS*): 24%, **194** (*RR*): 64%. b) NaN₃, acetonitrile, 50 °C, 16 h, **176** (*SS*): 45%, **177** (*RR*): 88%.

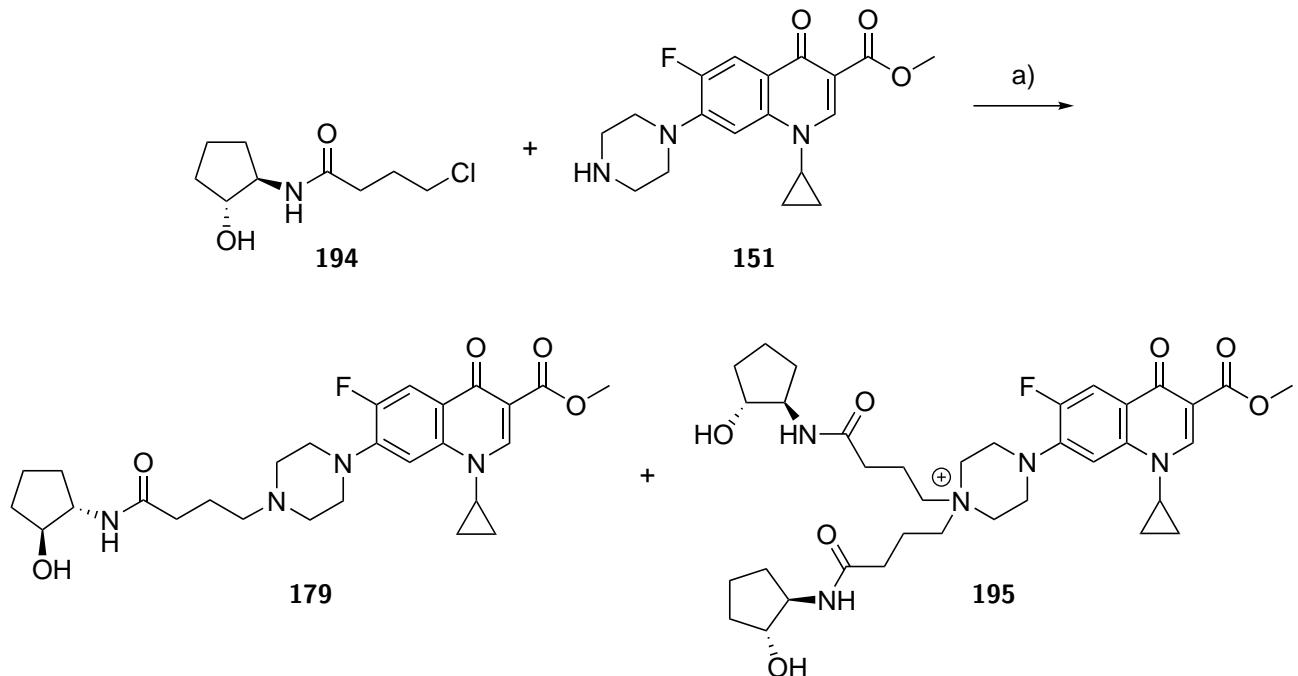
The cyclopentanol-Cip triazole conjugates **180** (*SS*) and **181** (*RR*) were successfully synthesised using standard click conditions (see 9.25). Despite low yields (presumably due to problems with purification, including losses on the preparative HPLC column and high polarity leading to losses during 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 **180** (*SS*) and **181** (*RR*). *SS* enantiomers are shown, but both were synthesised. a) CuSO₄, THPTA, sodium ascorbate, water, t-BuOH, r.t., 16 h, **180** (*SS*): 22%, **181** (*RR*): 27%.

The S_N2 reaction of Cl-C₄-cyclopentanol-(*RR*) **194** and methyl ciprofloxacin **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 **195** was seen by LCMS (in an approx 1:1 ratio with the desired product

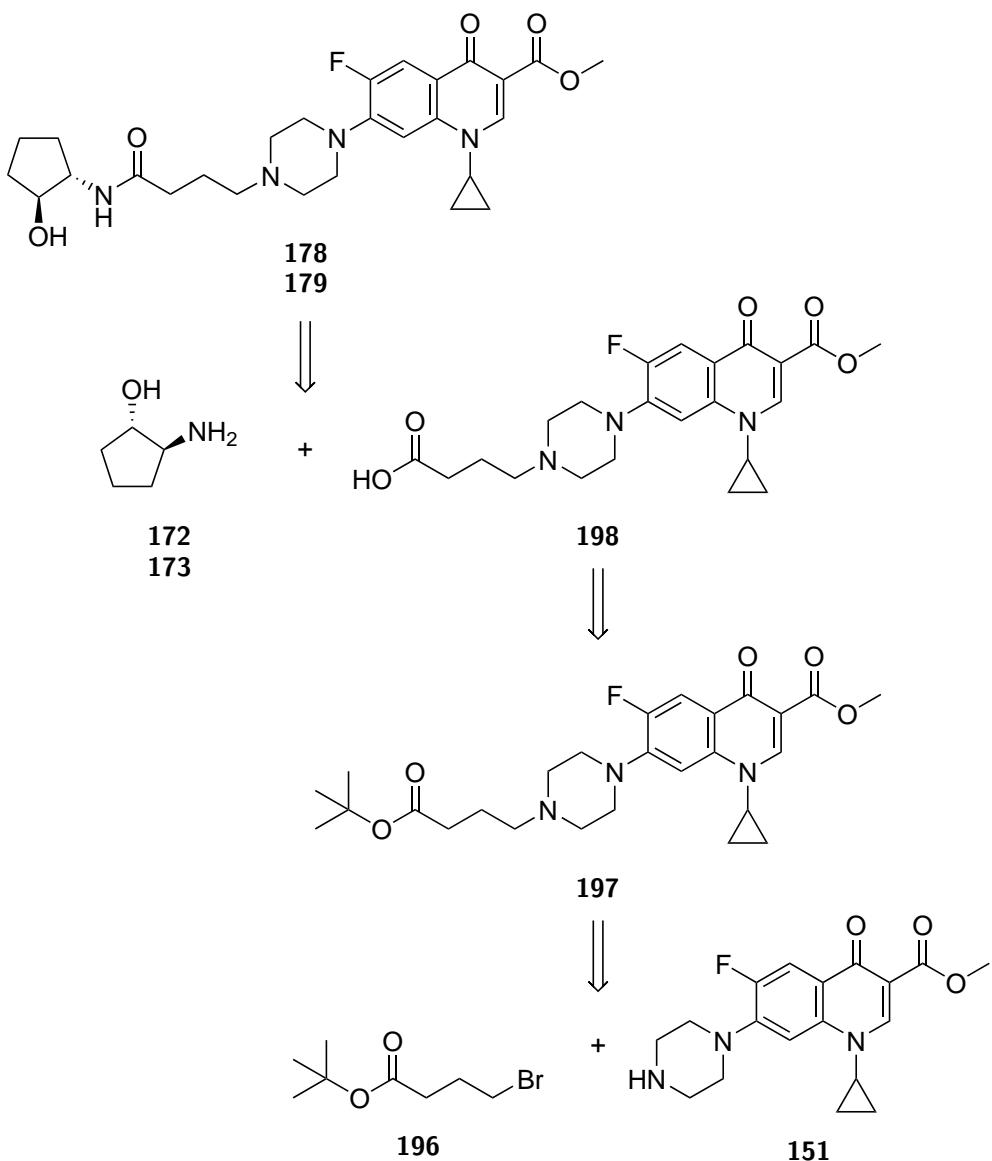
220).



Scheme 42: Attempted synthesis of the cyclopentanol-CipMe-(RR) conjugate **179**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C.

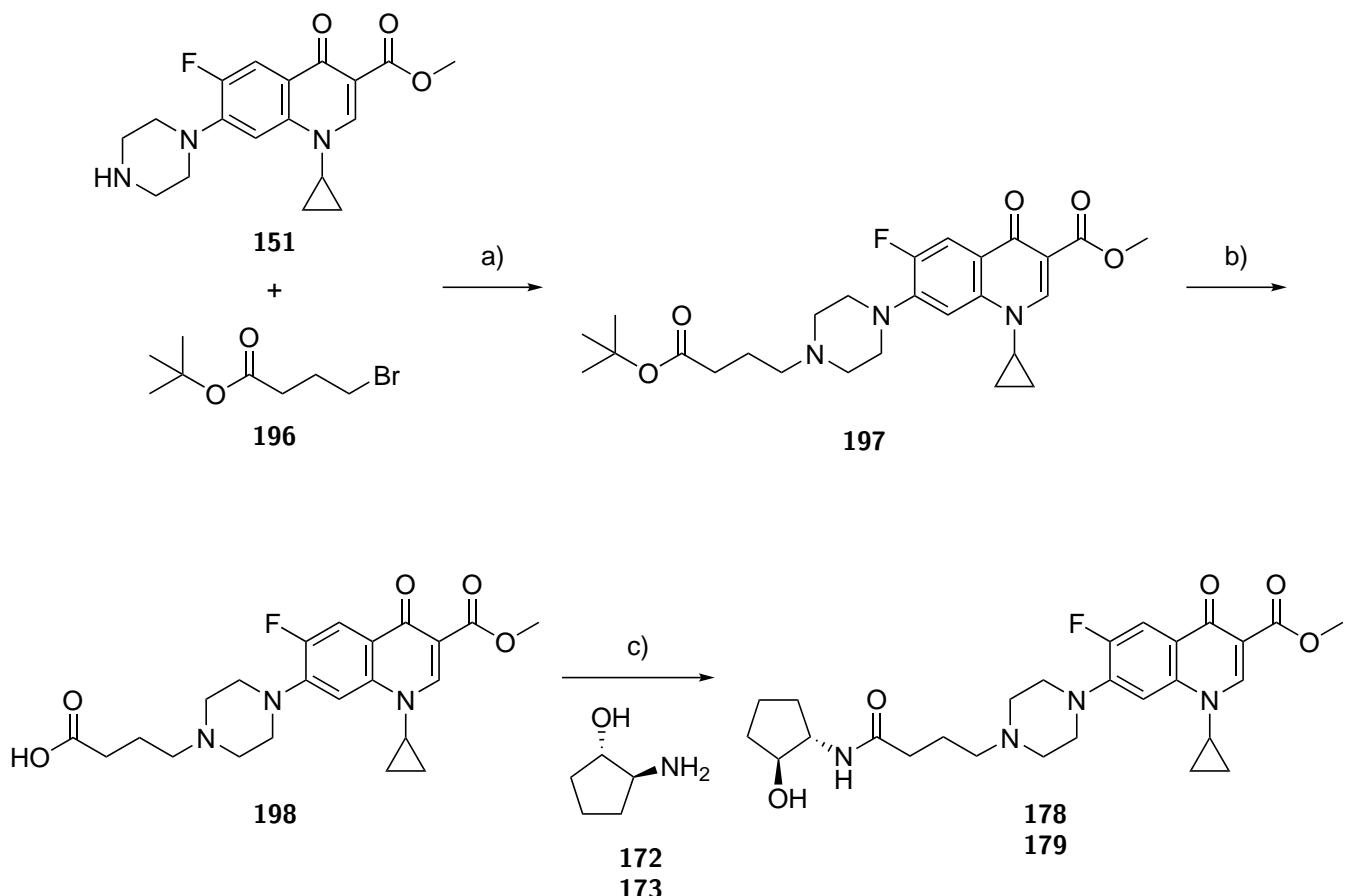
8.5.5 Synthesis of the cyclopentanol-CipMe conjugates **178** and **179** by peptide coupling

Given the side-reactions and low yields associated with the literature synthesis of the S_N2 conjugates proposed by Ganguly et. al.¹⁵ 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, CH_2Cl_2 , r.t., 18 h, 96%. c) EDC, HOBr, DIPEA, DMF, r.t., 16 h, **178** (*SS*): 55%, **179** (*RR*): 39%.

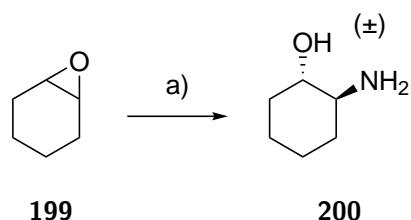
With (unfortunately not branching) routes to the $\text{S}_{\text{N}}2$ and click conjugates established (see 8.5.5 and 8.5.4 respectively), attention was turned to the cyclohexanol derivatives.

8.6 Cyclohexanol derivatives

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.

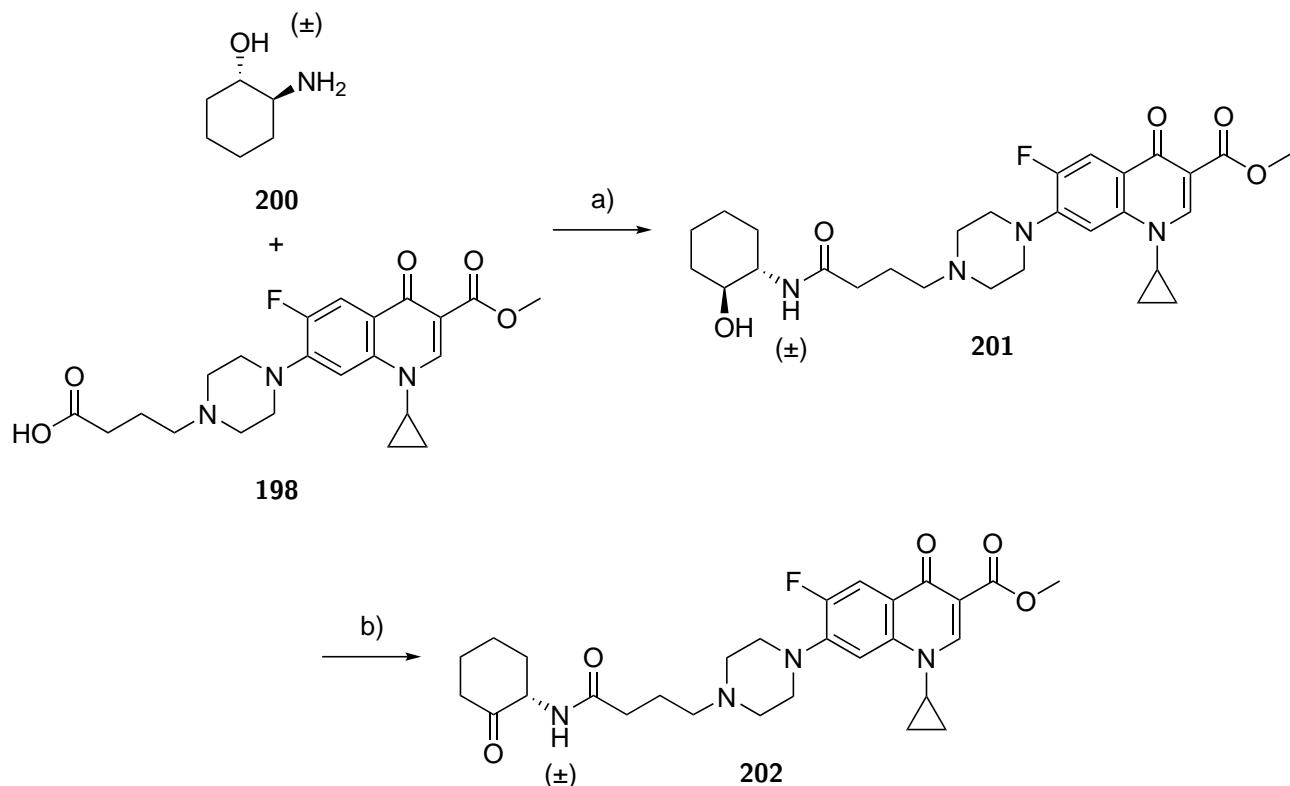


Scheme 45: Synthesis of *trans*-2-aminocyclohexan-1-ol **200**. a) NH_3 , water, MeOH , r.t., 72 h, 86%.

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.

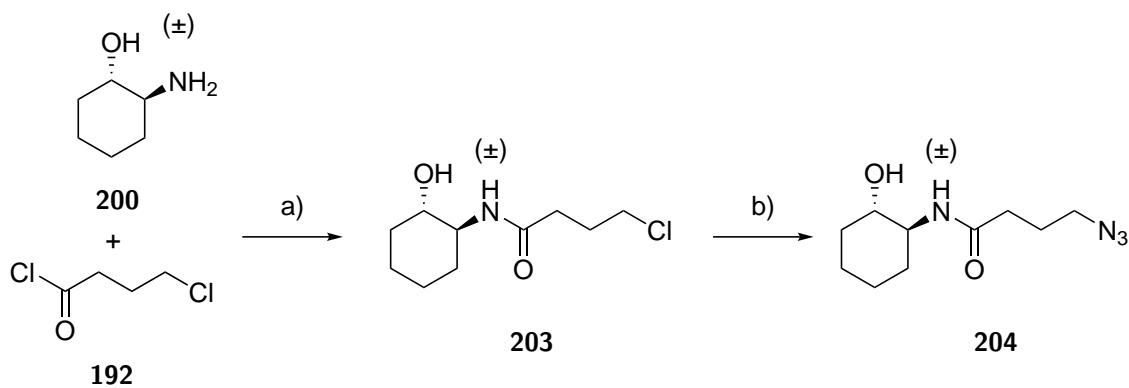
A portion of the *trans*-cyclohexanol-CipMe conjugate **201** was then oxidised to the ketone using Dess-Martin periodinane and the product was isolated in good yield.



Scheme 46: Synthesis of the cyclohexanol-CipMe conjugate **201** and the cyclohexanone-CipMe conjugate **202**.
a) EDC, HOEt, DIPEA, DMF, r.t., 16 h, 32%. b) DMP, CH_2Cl_2 , r.t., 6 h, 69%.

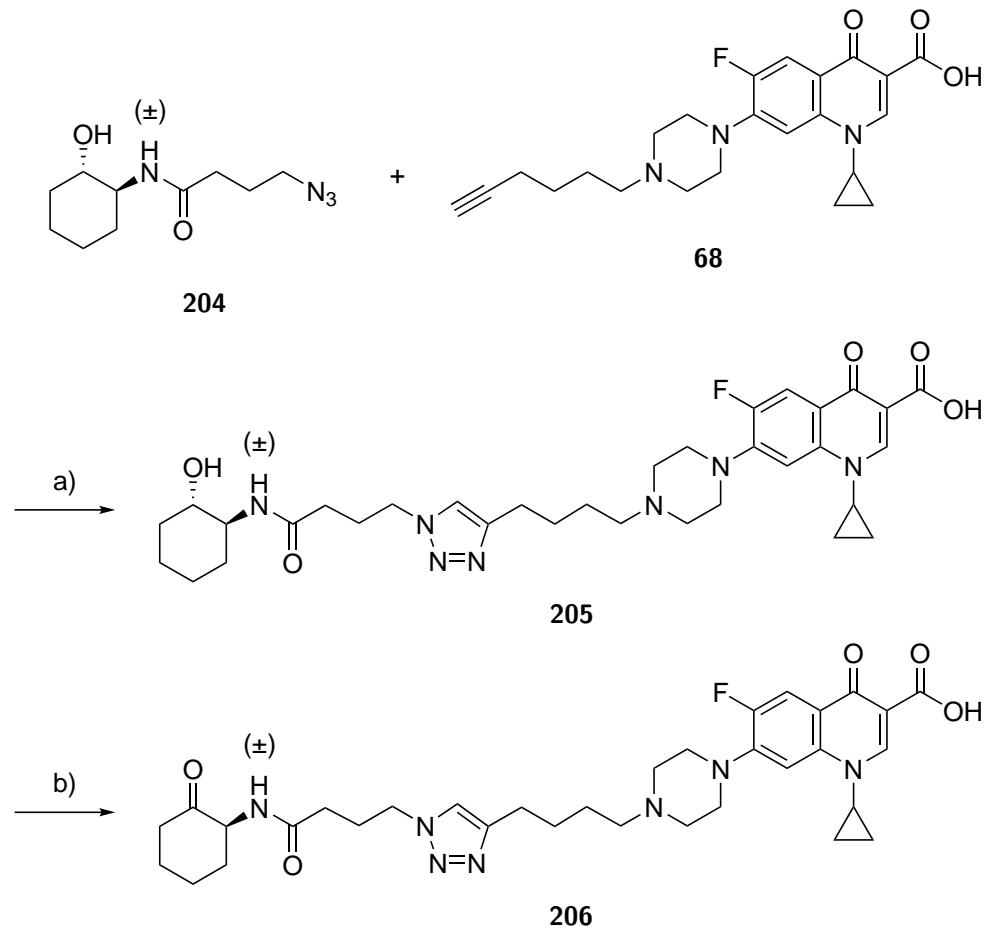
8.6.3 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-Cip triazole conjugates **205** and **206**

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-C₄-*trans*-cyclohexanol **204**. a) TEA, CH₂Cl₂, 0 °C, 30 min, 76%. b) NaN₃, 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 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) CuSO₄, THPTA, sodium ascorbate, water, *t*-BuOH, r.t., 16 h, 49%. b) DMP, CH₂Cl₂, 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

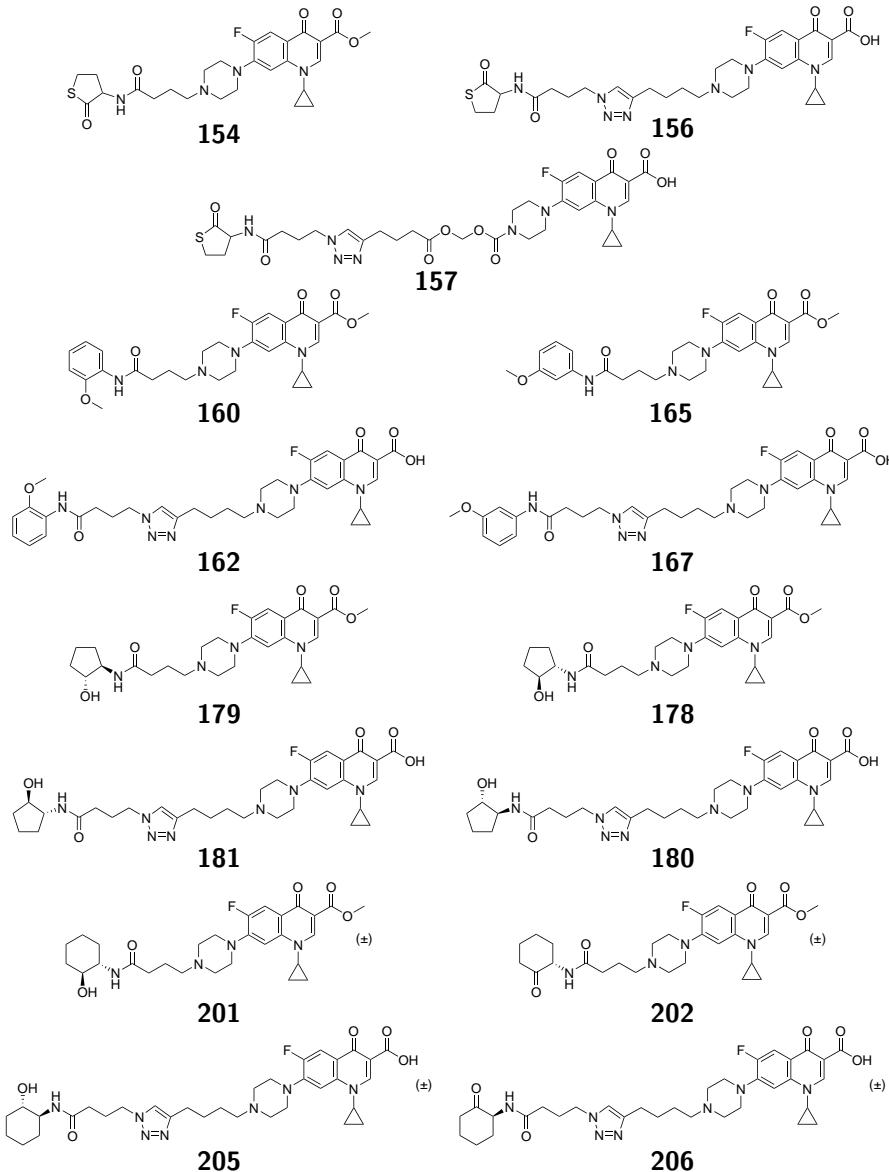


Figure 40: The HSL analogue-Cip(Me) conjugates.

8.7.1 Antibacterial activity

8.7.1.1 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).

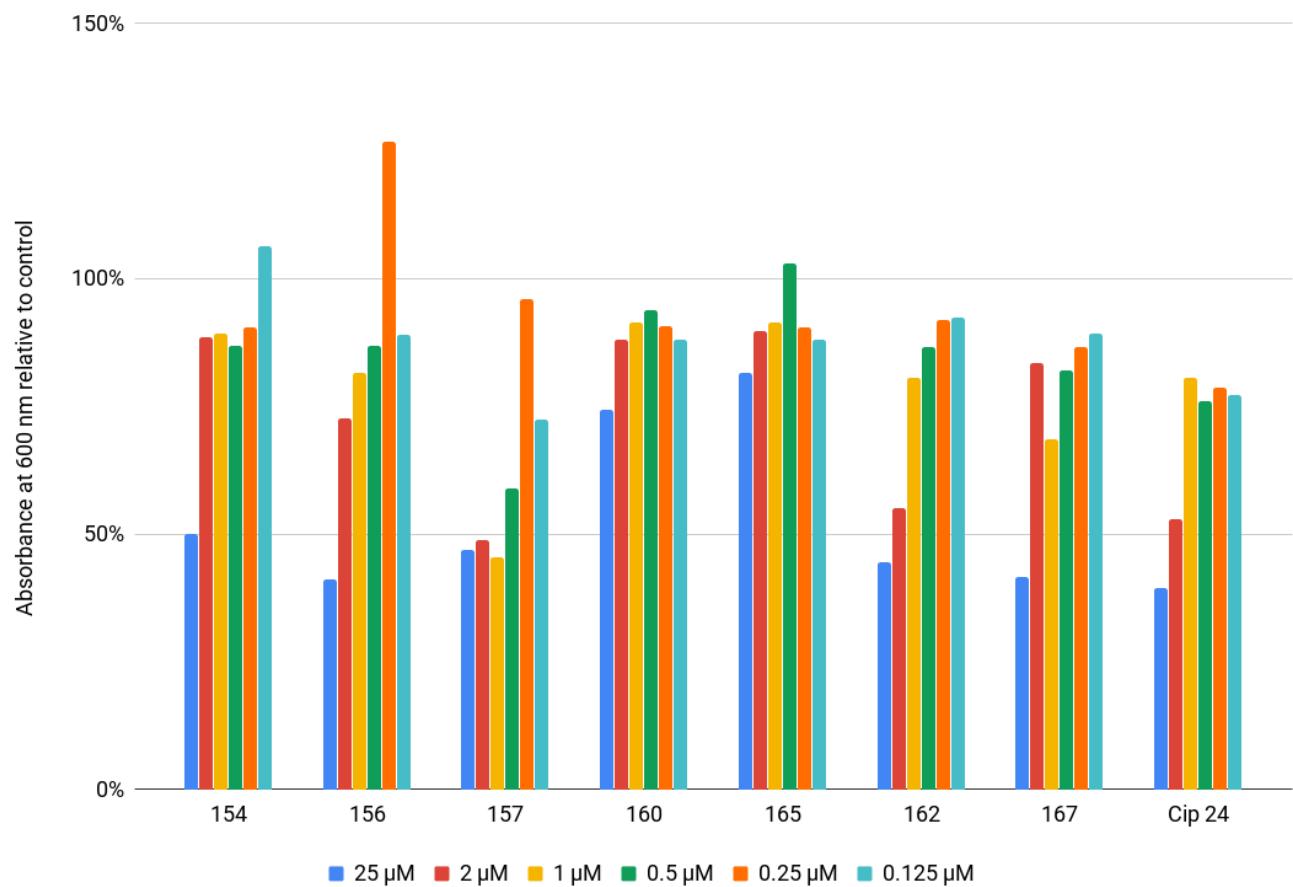


Figure 41: YM64 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

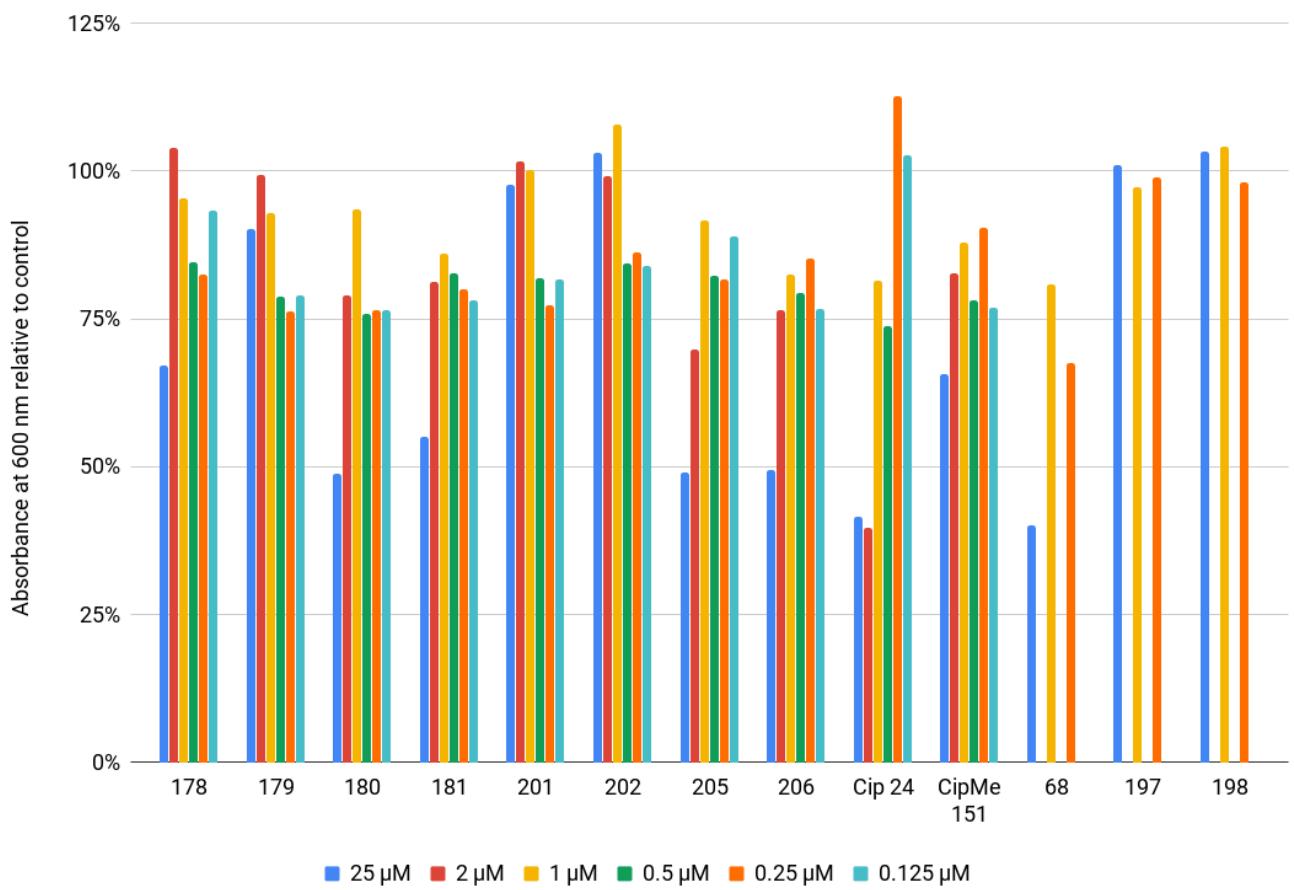


Figure 42: YM64 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

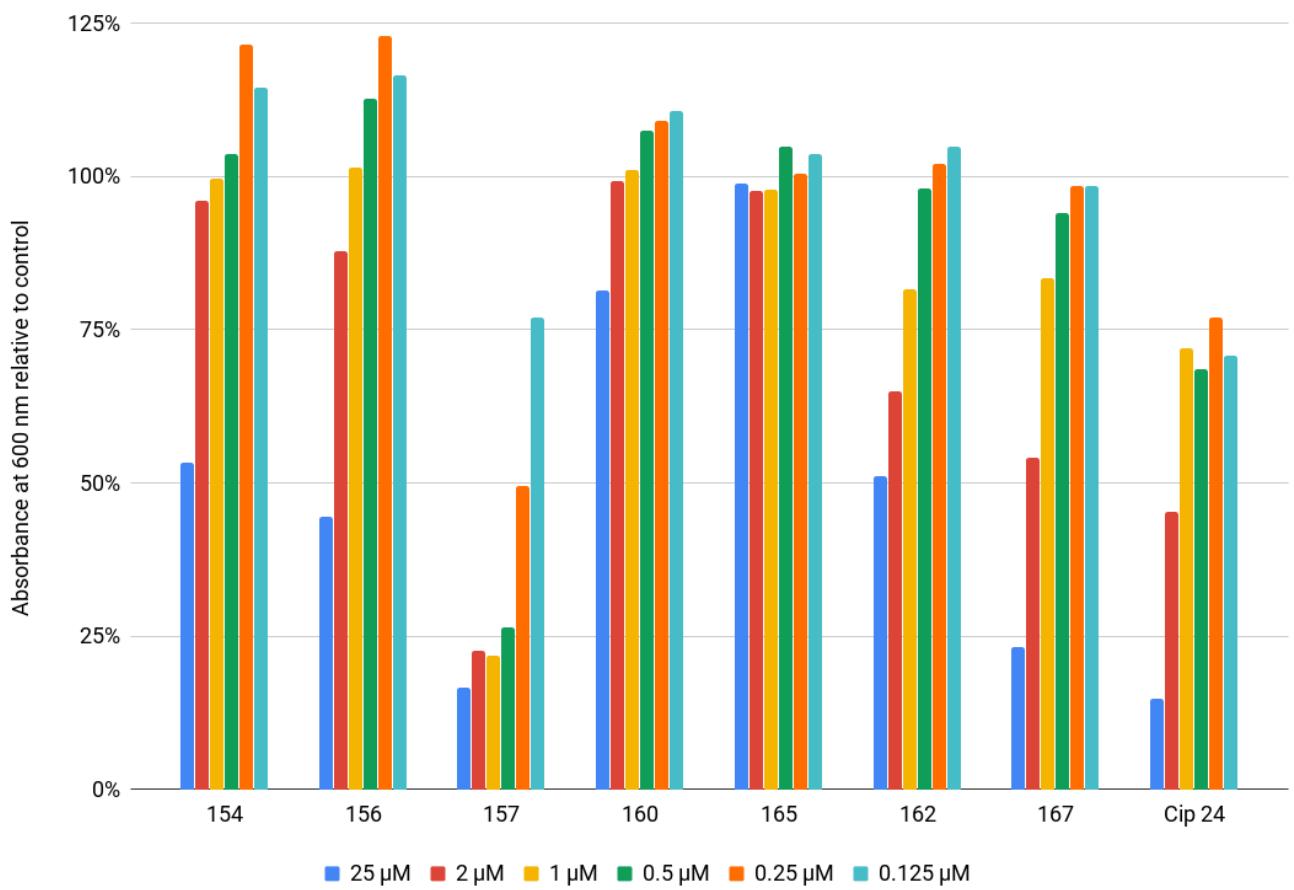


Figure 43: YM64 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

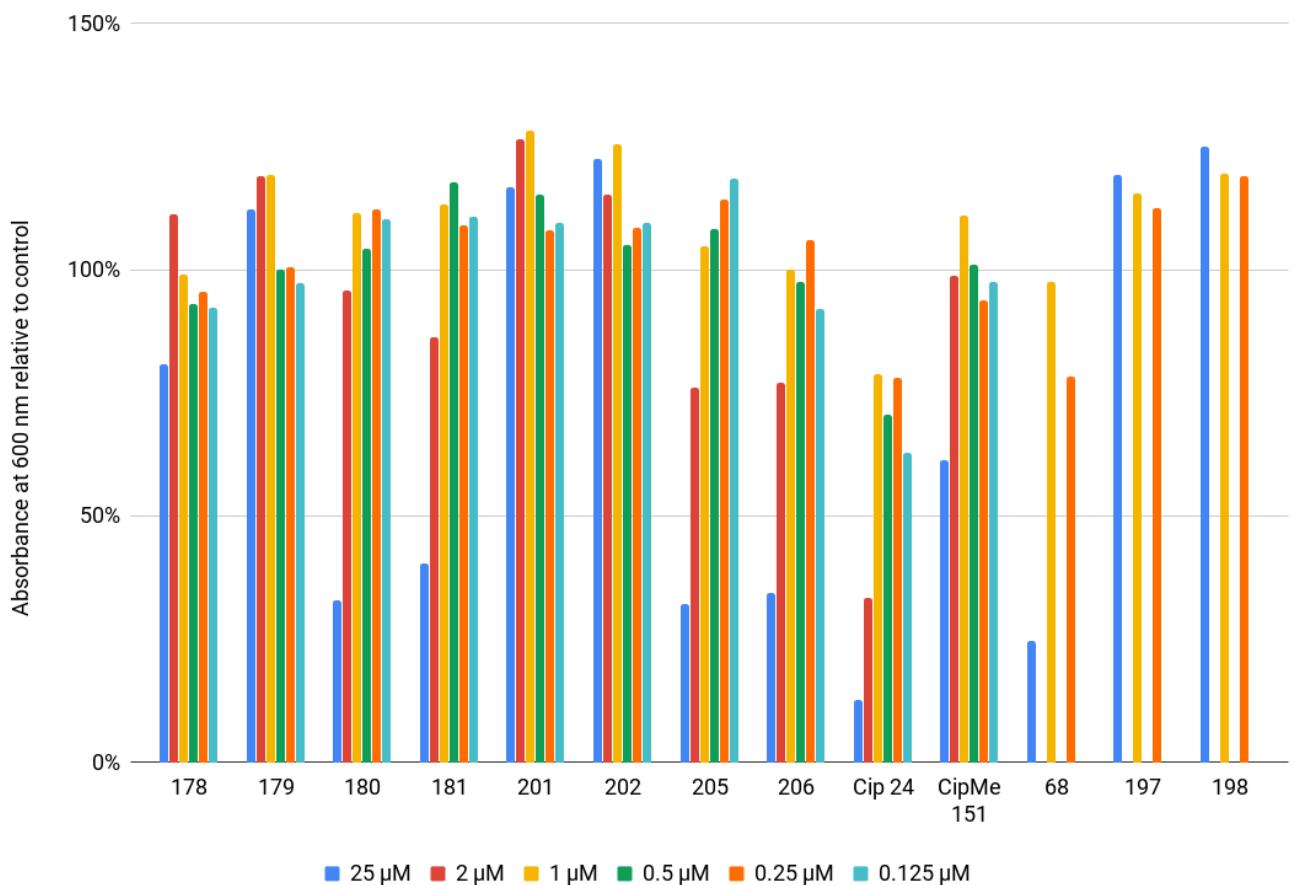


Figure 44: YM64 OD readings at 24 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

8.7.1.2 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).

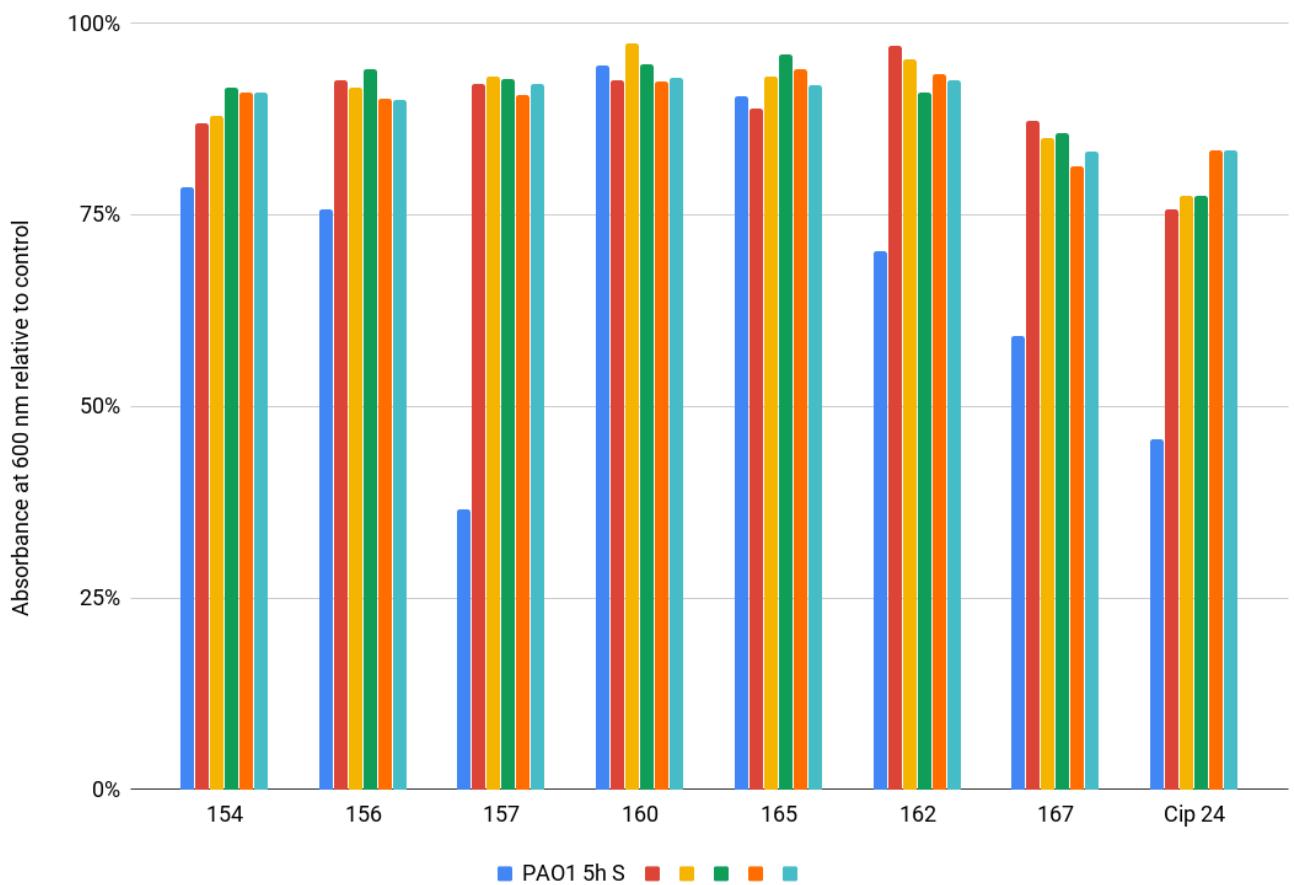


Figure 45: PAO1 OD readings at 5 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

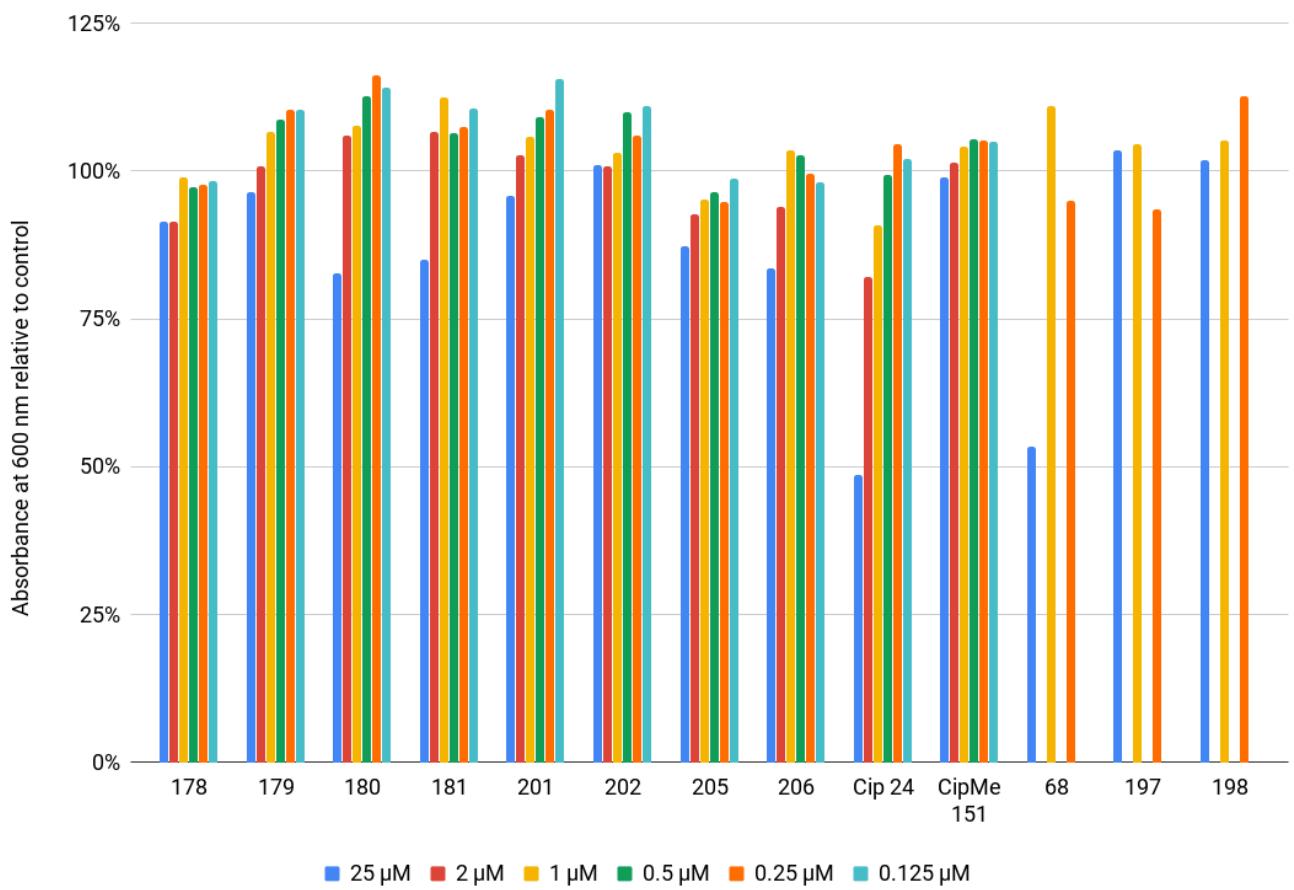


Figure 46: PAO1 OD readings at 5 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

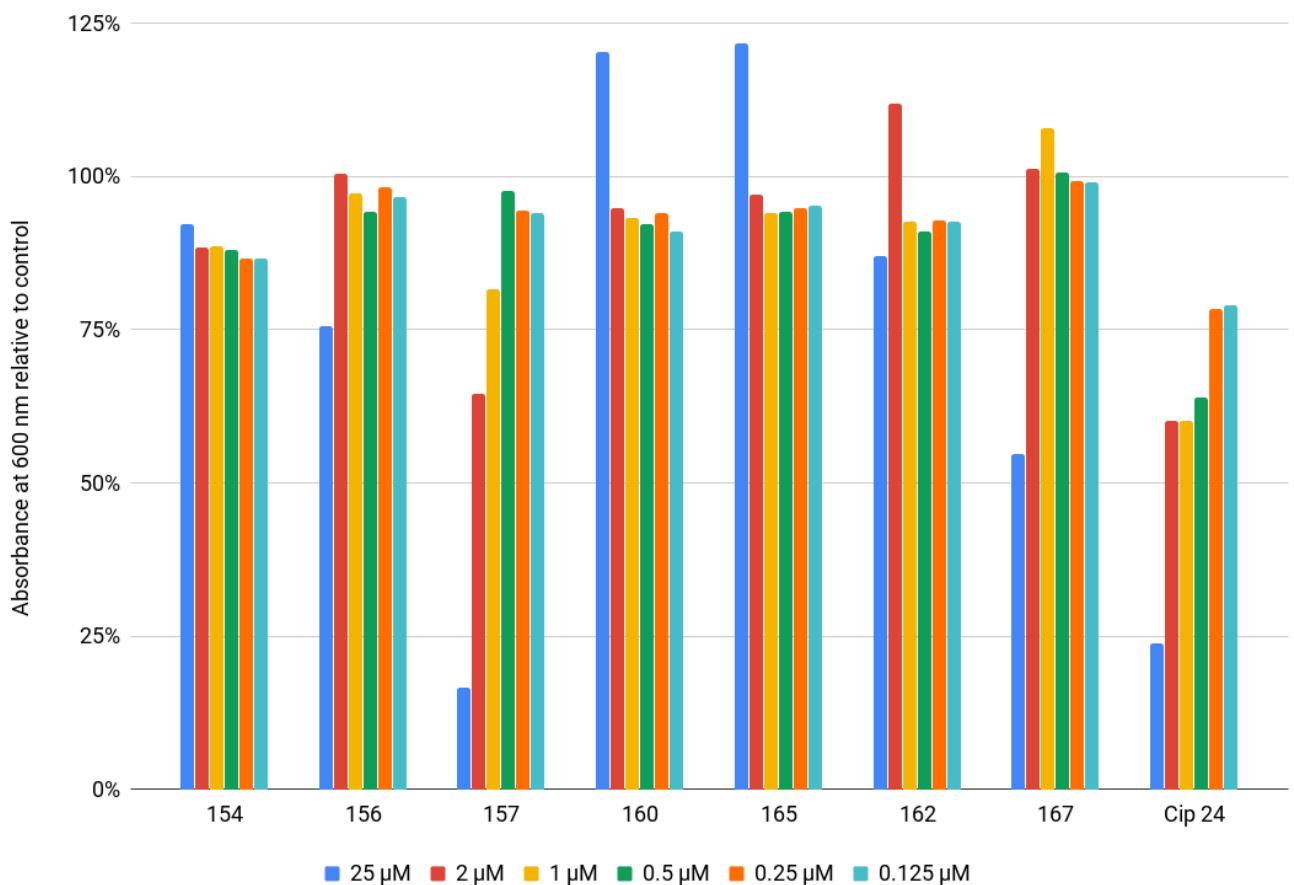


Figure 47: PAO1 OD readings at 24 h for the HCTL, 2-methoxybenzene and 3-methoxybenzene HSL analogue-Cip(Me) conjugates.

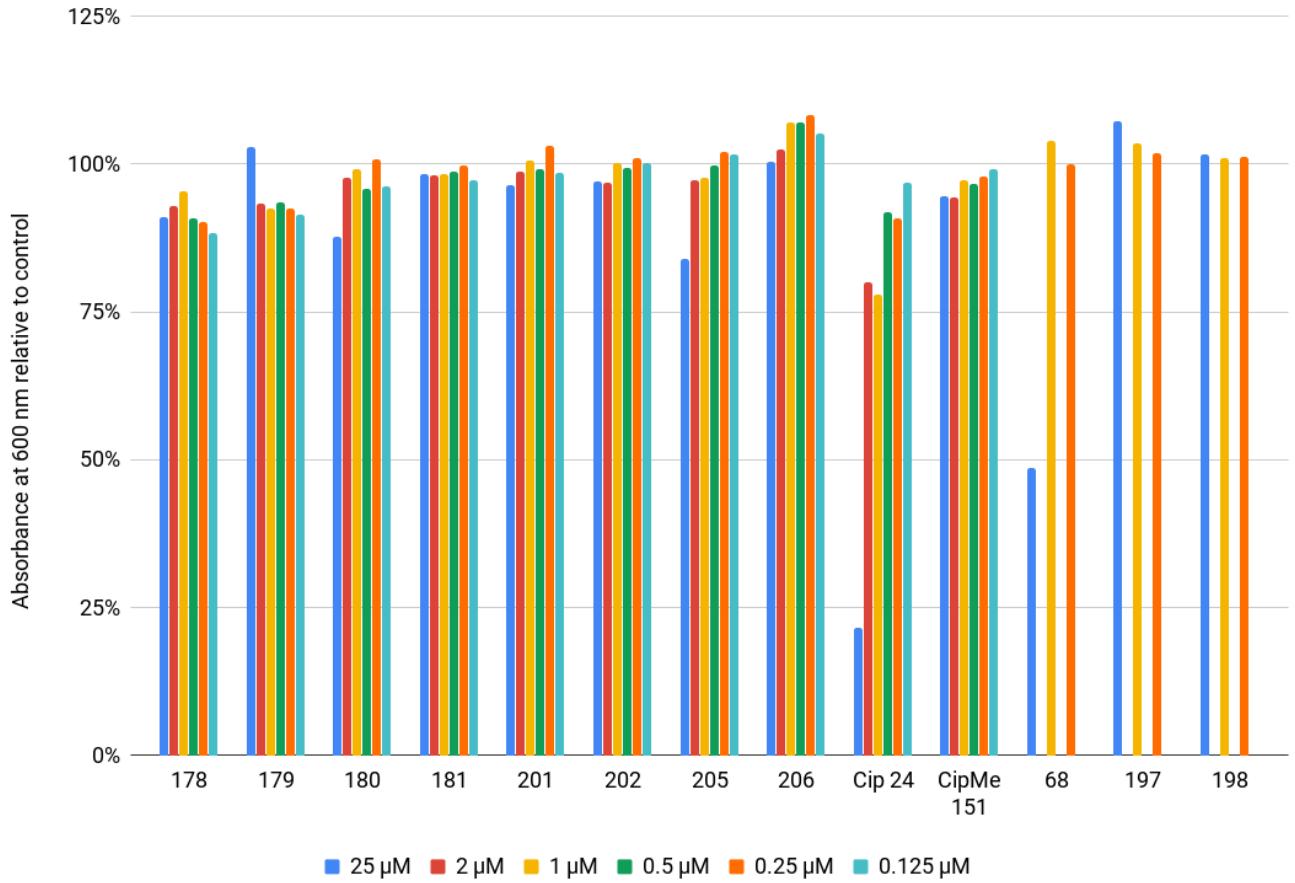


Figure 48: PAO1 OD readings at 24 h for the alcohol and ketone HSL analogue-Cip(Me) conjugates.

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 μ M 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.

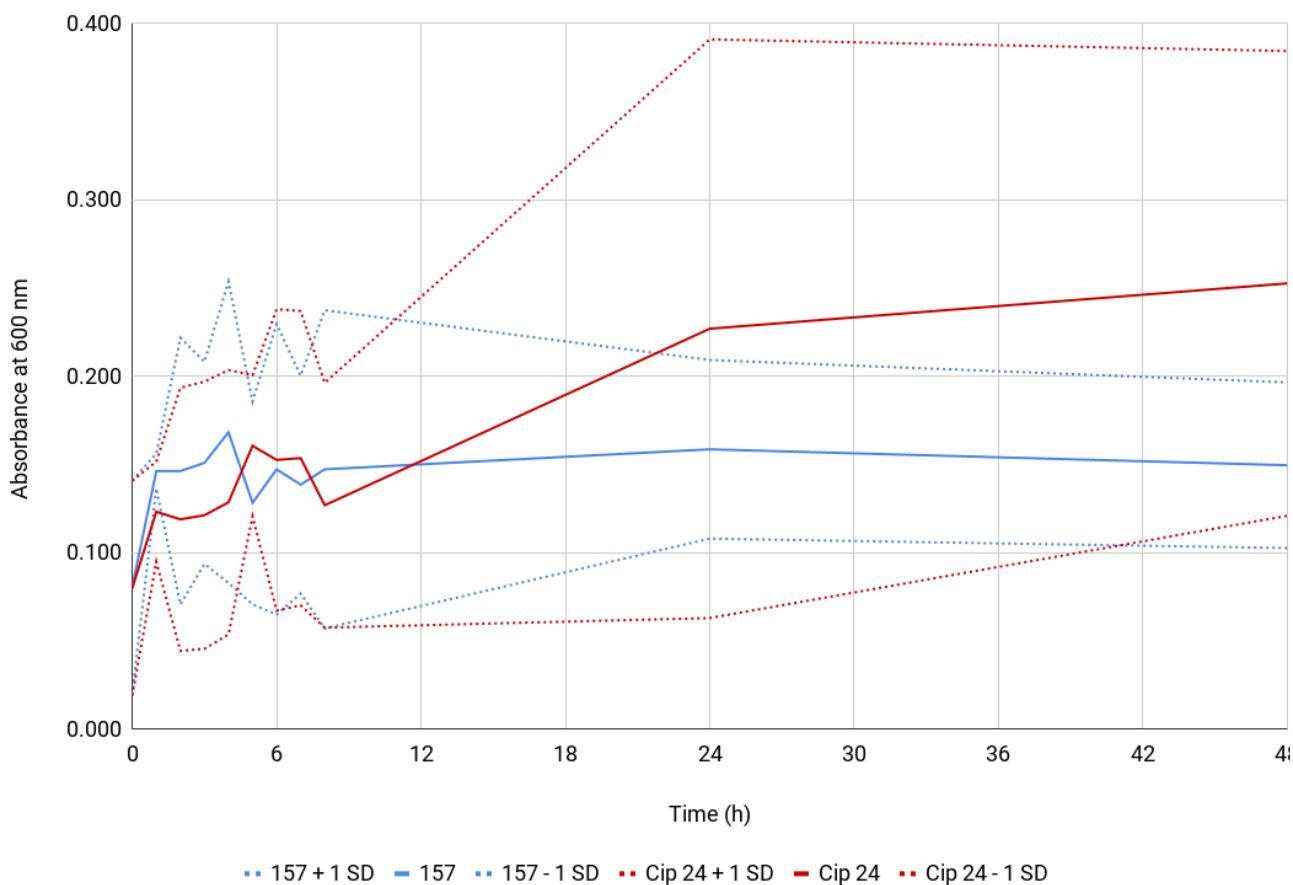


Figure 49: PAO1 OD readings over 48 h for the cleavable HCTL-Cip triazole conjugate **157** and ciprofloxacin **24** at 25 μ M.

8.7.2 Determination of anti-biofilm activity

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 hydrolyses 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.*¹⁵ 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.¹² 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 hypoxia²²⁹ (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.²³⁰ 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.

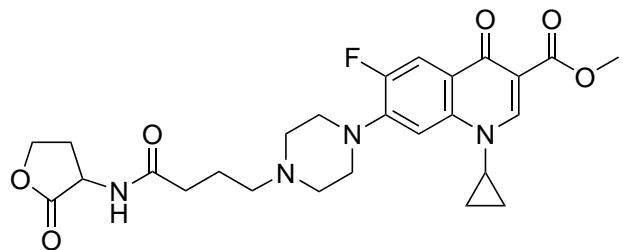


Figure 50: The proposed HSL-CipMe conjugate **207**.

As methyl ciprofloxacin 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.²³¹ 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.

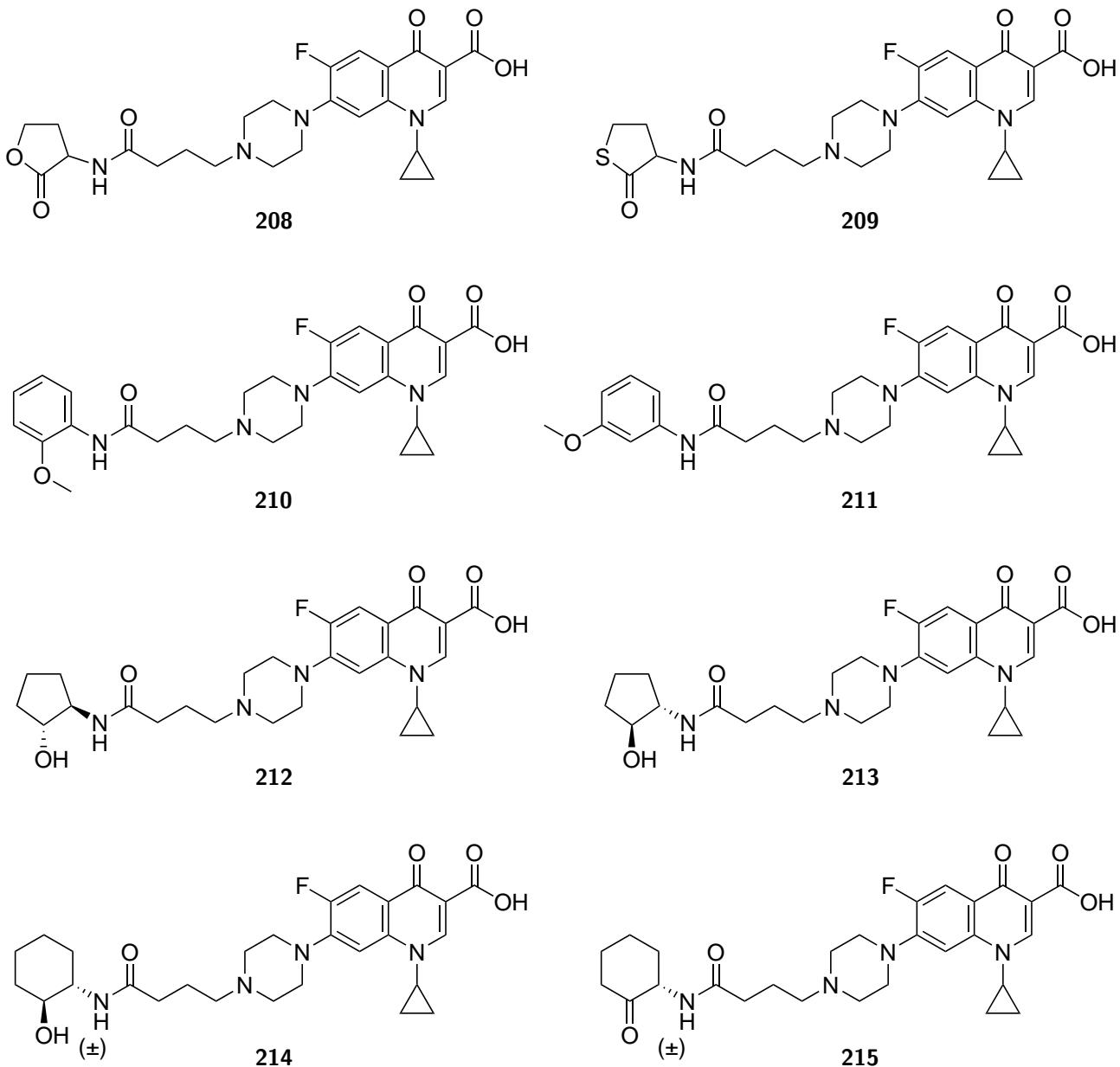


Figure 51: The proposed HSL analogue-Cip conjugates.

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.^{152, 157, 159, 232-235} 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.²³⁴

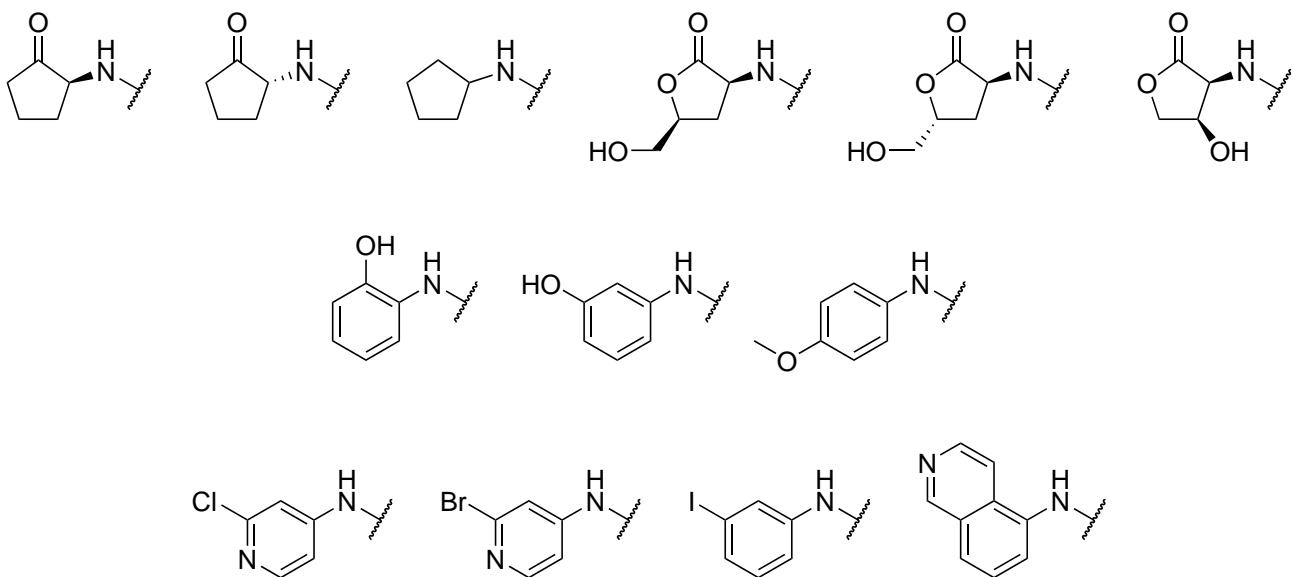


Figure 52: HSL analogue head groups for use in future conjugates.

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 *et al.* used LIVE/DEAD[®] *BacLight*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 *P. aeruginosa*, but alternative stains which show cell viability could be used. Peeters *et al.*²³⁶ evaluated six assays used for the quantification of biofilms and recommended fluorescein diacetate or resazurin viability assays for the quantification of live cells in *P. aeruginosa* biofilms.

Given the interesting growth curve results for the cleavable HCTL-Cip triazole conjugate **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 LiAlH₄ in the presence of triphenyl methane indicator. CH₂Cl₂, 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 KMnO₄ 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 Ultrashield spectrometers. Data were processed using NMR Processor Academic Edition version 12 (ADC Labs) or TopSpin version 3.5 (Bruker). ¹H and ¹³C 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, quin quintet, m multiplet and br broad.

¹H chemical shifts (δ) are quoted to the nearest 0.01 ppm and are referenced relative to the residual solvent peak.²³⁷ Coupling constants (J) are given to the nearest 0.1 Hz. Diastereotopic protons are assigned as CHH and CH_H, where the latter designates the lower-field proton. Data are reported as follows: <chemical shift> (<multiplicity>, <coupling constant(s) (if any)>, <integration>, <assignment>).

¹³C chemical shifts (δ) are quoted to the nearest 0.1 ppm and are referenced relative to the deuterated solvent peak.²³⁷ Data are reported as follows: <chemical shift> (<multiplicity (if not s)>, <coupling constant(s) (if any)>, <assignment>).

¹⁹F chemical shifts (δ) are quoted to the nearest 0.1 ppm. Data are reported as follows: <chemical shift> (<assignment>).

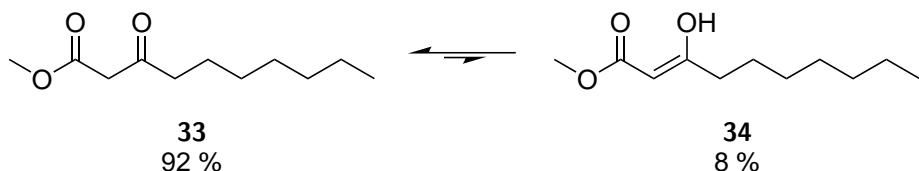
High resolution mass spectrometry (HRMS) data were recorded using a Micromass LCT Premier spectrometer or a Waters Vion IMS-QTOF spectrometer and reported mass values are within ± 5 ppm mass units. Liquid chromatography–mass spectrometry (LCMS) data were recorded on an Agilent 1200 series LC with an 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 (ν_{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 ([α]_D^T) were recorded on a PerkinElmer 343 polarimeter or an Anton-Paar MCP 100 polarimeter. [α]_D^T values are reported in °10⁻¹cm²g⁻¹ at 589 nm and concentration (*c*) is given in g (100 ml)⁻¹.

9.2 Methyl 3-oxodecanoate 33



Meldrum's acid **31** (9.00 g, 63.0 mmol, 1 eq.) was dissolved in anhydrous CH_2Cl_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_2Cl_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_2O /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 ^1H NMR).

Keto form 33

TLC $R_f = 0.12$ (5% EtO_2/PE)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 2927.8$ (C-H), 2856.3 (C-H), 1746.9 (ester C=O), 1716.7 (ketone C=O)

^1H NMR (400 MHz, CDCl_3) $\delta / \text{ppm} = 3.74$ (s, 3 H, OCH_3), 3.45 (s, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{C}(=\text{O})$), 2.53 (t, $J = 7.4$ Hz, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$), 1.60 (quin, $J = 7.1$ Hz, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$), 1.39 - 1.19 (m, 8 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.88 (t, $J = 6.8$ Hz, 3 H, CH_2CH_3)

^{13}C NMR (101 MHz, CDCl_3) $\delta / \text{ppm} = 202.3$ ($\text{CH}_3\text{OC}(=\text{O})\text{CH}_2\text{C}(=\text{O})$), 167.3 ($\text{CH}_3\text{OC}(=\text{O})\text{CH}_2\text{C}(=\text{O})$), 51.7 (OCH_3), 48.5 ($\text{CH}_3\text{OC}(=\text{O})\text{CH}_2\text{C}(=\text{O})$), 42.5 ($\text{C}(=\text{O})\text{CH}_2\text{CH}_2$), 31.3 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 23.1 (CH_2), 22.2 (CH_2), 13.6 (CH_2CH_3)

Enol form 34

TLC $R_f = 0.12$ (5% EtO_2/PE)

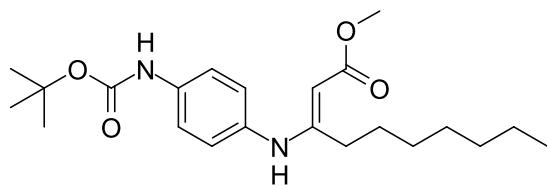
IR (neat) $\nu_{max} / \text{cm}^{-1} = 2927.8$ (C-H), 2856.3 (C-H), 1653.8 (C=C), 1629.2 (α,β unsaturated C=O)

^1H NMR (400 MHz, CDCl_3) $\delta / \text{ppm} = 12.02$ (s, 1 H, COH), 4.99 (s, 1 H, $\text{C}(=\text{O})\text{CH}=\text{COH}$), 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, $\text{COHCH}_2\text{CH}_2$), 1.39 - 1.19 (m, 8 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.88 (t, $J = 6.8$ Hz, 3 H, CH_2CH_3)

^{13}C NMR (101 MHz, CDCl_3) $\delta / \text{ppm} = 178.7$ ($\text{CH}_3\text{OC}(=\text{O})\text{CH}=\text{COH}$), 172.7 ($\text{CH}_3\text{OC}(=\text{O})\text{CH}=\text{COH}$), 88.2 ($\text{CH}_3\text{OC}(=\text{O})\text{CH}=\text{COH}$), 50.5 (OCH_3), 37.9 ($\text{COHCH}_2\text{CH}_2$), 34.6 (CH_2), 31.2 (CH_2), 29.0 (CH_2), 25.9 (CH_2), 22.3 (CH_2), 13.6 (CH_2CH_3)

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

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 (SiO₂, gradient of 0 to 20% Et₂O/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% Et₂O/40-60 P.E.)

mp T / °C = 78.8 (Et₂O/40-60 P.E.)

IR (neat) ν_{max} / cm⁻¹ = 3337.0 (N-H), 2927.7 (C-H), 2857.1 (C-H), 1723.7 (carbamate C=O), 1634.5 (α,β unsaturated C=O), 1610.7 (C=C), 1580.9 (N-H bend)

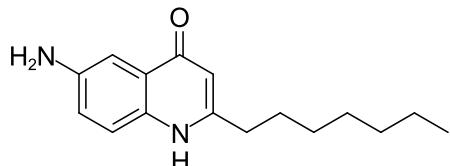
¹H NMR (400 MHz, CDCl₃) δ / ppm = 10.16 (s, 1 H, NHC(C₇H₁₅)=C), 7.35 (d, J = 8.6 Hz, 2 H, *meta* to NHBOC), 7.02 (d, J = 8.7 Hz, 2 H, *meta* to enamine), 6.60 (br s, 1 H, NHBOC), 4.71 (s, 1 H, C=uH), 3.70 (s, 3 H, OCH₃), 2.23 (t, J = 7.7 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.54 (s, 9 H, C(CH₃)₃), 1.40 (quin, J = 7.3 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.33 - 1.16 (m, 8 H, CH₂CH₂CH₂CH₂CH₂CH₃), 0.86 (t, J = 7.1 Hz, 3 H, CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / 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 (CNHBOC), 126.3 (*meta* to NHBOC), 119.1 (*ortho* to NHBOC), 83.8 (C(=O)CH=C), 80.7 (C(CH₃)₃), 50.2 (OCH₃), 32.2 (CH₂), 31.6 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 28.3 (C(CH₃)₃), 28.0 (CH₂), 22.6 (CH₂), 14.0 (CH₃)

HRMS (ESI⁺) m/z / Da = 391.2589, [M+H]⁺, [C₂₂H₃₅N₂O₄]⁺ 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 °C for 1 h. The reaction mixture was then poured into NaHCO₃ (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, 72%).

mp T / °C = 249 (water)

IR (neat) ν_{max} / cm⁻¹ = 3336.5 (N-H), 2926.5 (C-H), 2856.9 (C-H), 1634.5 (C=O)

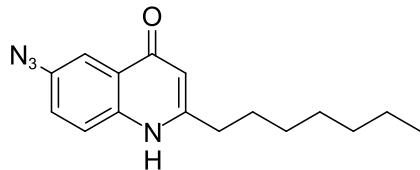
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 7.26 (d, J = 8.7 Hz, 1 H, *meta* to NH₂), 7.15 (d, J = 2.6 Hz, 1 H, *ortho* to C(=O)), 6.95 (dd, J = 2.7, 8.8 Hz, 1 H, *para* to C(=O)), 5.74 (s, 1 H, *ortho* to CH₂), 5.16 (s, 2 H, NH₂), 2.52 (t, J = 7.4 Hz, 2 H, CCH₂), 1.64 (quin, J = 7.6 Hz, 2 H, CCH₂CH₂), 1.36 - 1.19 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.86 (t, J = 7.0 Hz, 3 H, CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 176.7 (C(=O)), 151.7 (CCH₂), 145.1 (*para* to NH₂ or *ipso* to C(=O)), 132.4 (*ipso* to NH₂), 126.6 (*para* to NH₂ or *ipso* to C(=O)), 121.1 (*para* to C(=O)), 119.0 (*meta* to NH₂ and *meta* to C(=O)), 106.2 (CH=CCH₂), 105.9 (*ortho* to NH₂ and *ortho* to C(=O)), 33.6 (CCH₂), 31.6 (CH₂CH₂CH₃), 29.0 (CH₂), 29.0 (CH₂), 28.9 (CH₂), 22.5 (CH₂CH₃), 14.4 (CH₃)

HRMS (ESI⁺) m/z / Da = 259.1810, [M+H]⁺, [C₁₆H₂₃N₂O]⁺ 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 NaNO₂ (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 NaN₃ (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/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3249.3 (N-H), 3065.1 (N-H), 2916.6 (C-H), 2852.6 (C-H), 2728.1 (C-H), 2106.8 (azide), 1634.5 (C=O)

¹H 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 N₃ 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), 2.69 (t, J = 7.7 Hz, 2 H, CCH₂), 1.68 (quin, J = 7.6 Hz, 2 H, CCH₂CH₂), 1.28 - 1.39 (m, 4 H, CCH₂CH₂CH₂CH₂CH₃), 1.18 - 1.28 (m, 4 H, CH₂CH₂CH₃), 0.85 (t, J = 6.8 Hz, 3 H, CH₃)

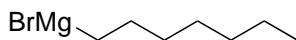
¹³C NMR (101 MHz, MeOD) δ / ppm = 172.3 (C(=O)), 155.5 (NH₂CH₂), 137.4 (CN₃), 135.6 (*para* to N₃), 124.6 (*para* to C(=O)), 124.1 (*ipso* to C(=O)), 120.7 (*meta* to N₃ and *meta* to C(=O)), 112.8 (*ortho* to N₃ and *ortho* to C(=O)), 107.0 (C(=O)CH), 33.3 (NH₂CH₂CH₃), 31.2 (CH₂CH₂CH₃), 28.3 - 28.5 (CH₂CH₂CH₂CH₂CH₃), 22.1 (CH₂CH₃), 14.0 (CH₃)

HRMS (ESI⁺) m/z / Da = 285.1728, [M+H]⁺ found, [C₁₆H₂₁N₄O]⁺ requires 285.1715

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

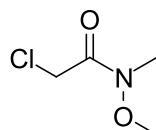
*Probably as the 4-hydroxyquinoline.⁹⁴

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 = 38.8 (toluene)

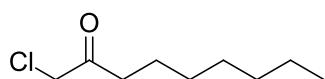
IR (neat) ν_{max} / cm⁻¹ = 3016.7 (C-H), 2966.4 (C-H), 2946.7 (C-H), 2827.7 (C-H), 1666.2 (C=O)

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.⁹⁴

9.8 1-Chlorononan-2-one 44



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%).

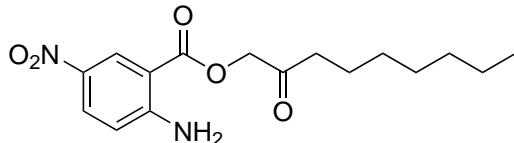
IR (neat) ν_{max} / cm⁻¹ = 2951.7 (C-H), 2925.0 (C-H), 2855.5 (C-H), 1720.4 (C=O)

¹H NMR (400 MHz, CDCl₃) δ / 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)CH₂CH₂), 1.34 - 1.21 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.87 (t, *J* = 6.8 Hz, 3 H, CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 202.6 (C(=O)), 48.1 (CH₂Cl), 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.⁹⁴

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, 100%).

mp *T* / °C = 135 (water)

IR (neat) ν_{max} / cm⁻¹ = 3453.3 (N-H), 3350.5 (N-H), 2924.9 (C-H), 2853.9 (C-H), 1720.1 (ester C=O) 1703.9 (ketone C=O) 1626.1 (N-H bend) 1602.7 (aromatic) 1572.5 (N-O) 1506.6 (N-O)

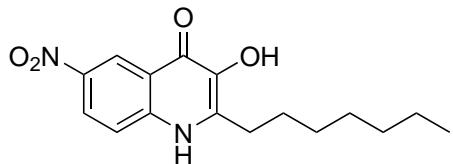
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 8.66 (d, *J* = 2.8 Hz, 1 H, *ortho* to C(=O)), 8.12 (dd, *J* = 2.8, 9.4 Hz, 1 H, *para* to C(=O)), 6.93 (d, *J* = 9.4 Hz, 1 H, *meta* to C(=O)), 5.05 (s, 2 H, OCH₂C(=O)), 2.49 (t, *J* = 7.4 Hz, 2 H, C(=O)CH₂CH₂), 1.52 (quin, *J* = 7.2 Hz, 2 H, C(=O)CH₂CH₂), 1.32 -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

¹³C NMR (101 MHz, DMSO-d₆) δ / 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]⁺, [C₁₆H₂₃N₂O₅]⁺ requires 323.1607

Spectroscopic data are consistent with the literature.¹⁶⁴

9.10 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1H)-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 NaHCO₃ (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) ν_{max} / cm⁻¹ = 3436.0 (N-H), 3000.0 (O-H, br), 2955.4 (C-H), 2925.8 (C-H), 2850.9 (C-H), 1648.2 (C=O), 1570.7 (N-O), 1536.4 (N-O)

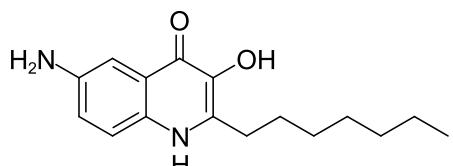
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 12.00 (s, 1 H, NH), 8.91 (d, *J* = 2.8 Hz, 1 H, *ortho* to C=O), 8.29 (dd, *J* = 2.7, 9.2 Hz, 1 H, *para* to C=O), 7.70 (d, *J* = 9.3 Hz, 1 H, *meta* to C=O), 2.75 (t, *J* = 7.7 Hz, 2 H, CCH₂), 1.67 (quin, *J* = 7.3 Hz, 2 H, CCH₂CH₂), 1.36 - 1.23 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.85 (t, *J* = 7.0 Hz, 3 H, CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 169.7 (C=O), 141.9 (*para* to NO₂), 140.7 (*ipso* to NO₂), 139.6 (*ipso* to OH), 137.3 (C=COH), 124.3 (*para* to C=O), 122.3 (*ortho* to NO₂ and *ortho* to C=O), 121.5 (*ipso* to C=O), 120.0 (*meta* to NO₂ and *meta* to C=O), 31.6 (CH₂CH₂CH₃), 29.2 (CH₂), 28.9 (CH₂), 28.5 (CCH₂), 28.1 (CCH₂CH₂), 22.5 (CH₂CH₃), 14.4 (CH₃)

HRMS (ESI⁺) *m/z* / Da = 305.1501, [M+H]⁺, [C₁₆H₂₁N₂O₄]⁺ requires 305.1500

Spectroscopic data are consistent with the literature.¹⁶⁴

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



6-Nitro-2-heptyl-3-hydroxyquinolin-4(1H)-one **47** (20 mg, 0.0658 mmol, 1 eq.) and PtO₂ (2 mg, 10 weight %) were stirred in MeOH (1 ml) under a H₂ 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 / °C = 176

IR (neat) ν_{max} / cm⁻¹ = 3000.00 (O-H, br) 2925.41 (C-H), 2854.09 (C-H), 1613.43 (C=O)

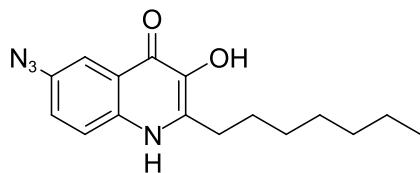
¹H NMR (400 MHz, MeOD) δ / ppm = 11.12 (s, 1 H, NH), 7.47 (d, J = 8.9 Hz, 1 H, *meta* to C=O), 7.40 (d, J = 2.4 Hz, 1 H, *ortho* to C=O), 7.16 (dd, J = 2.6, 9.0 Hz, 1 H, *para* to C=O), 2.86 (t, J = 7.5 Hz, 2 H, CCH₂), 1.75 (quin, J = 7.8 Hz, 2 H, CCH₂CH₂), 1.48 - 1.22 (m, J = 5.4 Hz, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.89 (t, J = 6.7 Hz, 3 H, CH₃)

¹³C NMR (101 MHz, MeOD) δ / ppm = 166.8 (C(=O)), 144.8 (*para* to NH₂ or *ipso* to C(=O)), 140.5 (COH), 138.6 (C=COH), 132.6 (*ipso* to NH₂), 124.8 (*para* to NH₂ or *ipso* to C(=O)), 123.8 (*para* to C(=O)), 107.7 (*meta* to NH₂ and *meta* to C(=O)), 106.4 (*ortho* to NH₂ and *ortho* to C(=O)), 33.0 (CH₂CH₂CH₃), 29.5 - 31.0 (CCH₂CH₂CH₂CH₂), 23.8 (CH₂CH₃), 14.5 (CH₃)

HRMS (ESI⁺) m/z / Da = 275.1760, [M+H]⁺, [C₁₆H₂₃N₂O₂]⁺ requires 275.1762

Spectroscopic data are not consistent with the literature.¹⁶⁴ It is possible that Baker's product is a Zn adduct.

9.12 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 °C. NaNO₂ (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 °C for 50 min, during which time the solution turned from yellow to orange. NaN₃ (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) ν_{max} / cm⁻¹ = 3089 (N-H), 2921 (C-H), 2851 (C-H), 2108 (azide), 1632 (C=O)

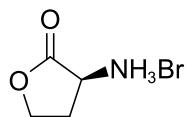
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 7.74 (s, 1 H, *ortho* to C=O), 7.65 (d, J = 6.9 Hz, 1 H, *meta* to C(=O)), 7.32 (d, J = 7.4 Hz, 1 H, *para* to C(=O)), 2.75 (t, J = 7.5 Hz, 2 H, CCH₂), 1.67 (quin, J = 6.4 Hz, 2 H, CCH₂CH₂), 1.43 - 1.13 (m, 8 H, CH₂CH₂CH₂CH₂CH₃), 0.85 (t, J = 6.8 Hz, 3 H, CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 166.3 (C(=O)), 137.9 (C), 137.8 (CN₃), 134.5 (*ipso* to C(=O)), 133.9 (C=COH), 122.7 (*para* to C(=O)), 122.6 (*meta* to N₃ and *meta* to C(=O)), 120.4 (*para* to N₃), 112.4 (*ortho* to N₃ and *ortho* to C(=O)), 31.2 (CH₂CH₂CH₃), 28.8 (CCH₂), 28.4 (CCH₂CH₂CH₂), 28.3 (CCH₂CH₂CH₂CH₂), 27.8 (CCH₂CH₂), 22.1 (CH₂CH₃), 14.0 (CH₃)

HRMS (ESI⁺) m/z / Da = 301.1649, [M+H]⁺, [C₁₆H₂₁N₄O₂]⁺ requires 301.1659

Spectroscopic data are consistent with the literature.¹⁶⁴

9.13 (*S*)-3-Aminodihydrofuran-2(*3H*)-one hydrobromide 52



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 *i*-PrOH (12.5 ml), H₂O (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 *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 *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).

mp *T* / °C = 242 (*i*-PrOH/AcOH, gas evolved)

IR (neat) ν_{max} / cm⁻¹ = 2972.1 (N-H), 2877.5 (N-H), 1771.8 (C=O), 1585.1 (N-H bend), 1572.2 (N-H bend)

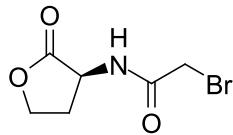
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 8.59 (br s, 3 H, NH₃⁺), 4.46 (dt, *J* = 1.3, 8.9 Hz, 1 H, OCHH), 4.37 (dd, *J* = 8.8, 11.4 Hz, 1 H, CHNH₃⁺), 4.29 (ddd, *J* = 6.1, 8.8, 10.9 Hz, 1 H, OCHH), 2.57 (dddd, *J* = 1.2, 6.1, 8.9, 12.3 Hz, 1 H, OCH₂CHH), 2.26 (dtd, *J* = 9.0, 11.2, 12.2 Hz, 1 H, OCH₂CHH)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 173.3 (C=O), 66.2 (OCH₂), 47.8 (CHNH₃⁺), 27.0 (OCH₂CH₂)

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -30.0, lit. = -25.0 (*c* / g(100 ml)⁻¹ = 0.0200, DMSO)

The data are consistent with the literature.¹⁶⁵

9.14 (*S*)-2-Bromo-N-(2-oxotetrahydrofuran-3-yl)acetamide 54



(*S*)-3-Aminodihydrofuran-2(*3H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.08 eq.) and NaHCO₃ (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH₂Cl₂ (2 ml) and water (2 ml). Bromoacetyl bromide **53** (44.0 μL, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 24 h, after which the CH₂Cl₂ was removed under vacuum. The aqueous phase was extracted with EtOAc (4×10 ml). The combined organic layers were dried with MgSO₄ and the solvent was removed under reduced pressure. **54** was obtained as white, needle-like crystals (88.0 mg, 0.396 mmol, 74%).

mp *T* / °C = 132 (EtOAc)

IR (neat) ν_{max} / cm⁻¹ = 3255.7 (N-H), 3066.6 (C-H), 1763.0 (lactone C=O), 1658.0 (amide C=O), 1552.7 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.94 (br s, 1 H, NH), 4.57 (ddd, J = 11.7, 8.6, 5.9 Hz, 1 H, CHNH), 4.51 (td, J = 9.2, 1.0 Hz, 1 H, OCHH), 4.32 (ddd, J = 11.3, 9.4, 5.9 Hz, 1 H, OCHH), 3.93 (s, 1 H, CHHBr), 3.93 (s, 1 H, CHHBr), 2.87 (dddd, J = 12.6, 8.6, 5.9, 1.3 Hz, 1 H, OCH₂CHH), 2.22 (dtd, J = 12.6, 11.5, 11.5, 8.9 Hz, 1 H, OCH₂CHH)

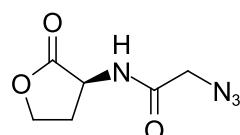
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 174.6 (OC=O), 166.4 (C(=O)NH), 66.1 (OCH₂), 49.8 (CHNHC=O), 29.9 (OCH₂CH₂), 28.2 (O=CCH₂Br)

HRMS The compound does not ionise.

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = 27.0, lit. = 20.5 (c / g(100 ml)⁻¹ = 0.00740, CHCl₃)

The data are consistent with the literature.^{165, 238}

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



(3*S*)-2-Oxotetrahydrofuran-3-aminium bromide **52** (100 mg, 0.552 mmol, 1.08 eq.), NaN₃ (85.7 mg, 1.32 mmol, 2.61 eq.) and NaHCO₃ (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH₂Cl₂ (2 ml) and water (2 ml). Bromoacetyl bromide **53** (44.0 μL, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the CH₂Cl₂ was removed under vacuum. The aqueous phase was extracted with EtOAc (4×10 ml). The combined organic layers were dried with MgSO₄ and the solvent was removed under reduced pressure. **55** was obtained as white, needle-like crystals (38.4 mg, 0.209 mmol, 41%).

mp T / °C = 87 (EtOAc)

IR (neat) ν_{max} / cm⁻¹ = 3283.5 (N-H), 2923.3 (C-H), 2853.0 (C-H), 2129.7 (N₃), 1782.9 (lactone C=O), 1661.4 (amide C=O), 1536.8 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / 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, CHNH), 4.48 (td, J = 9.1, 1.3 Hz, 1 H, OCHH), 4.30 (ddd, J = 11.2, 9.2, 6.0 Hz, 1 H, OCHH), 4.04 (s, 2 H, CH₂N₃), 2.76 (dddd, J = 12.5, 8.8, 6.0, 1.4 Hz, 1 H, OCH₂CHH), 2.25 (dtd, J = 12.5, 11.4, 11.4, 8.9 Hz, 1 H, OCH₂CHH)

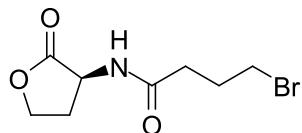
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 174.9 (OC=O), 167.5 (C=ONH), 66.0 (OCH₂), 52.2 (O=CCH₂N₃), 48.9 (CHNHC=O), 29.7 (OCH₂CH₂)

HRMS The compound does not ionise.

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -32.6, lit. = -24.4 (c / g(100 ml)⁻¹ = 0.0430, DMSO)

The data are consistent with the literature.¹⁶⁵

9.16 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 57



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (200 mg, 1.10 mmol, 1.00 eq.) and NaHCO₃ (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in CH₂Cl₂ (2 ml) and water (2 ml). Bromobutyryl chloride **56** (140 µL, 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the CH₂Cl₂ was removed under vacuum. The aqueous phase was extracted with EtOAc (7×5 ml) and the combined organic layers were dried with MgSO₄. 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* / °C = 105 (EtOAc)

IR (neat) ν_{max} / cm⁻¹ = 3307.9 (N-H), 3073.9 (C-H), 2948.9 (C-H), 1773.7 (lactone C=O), 1643.5 (amide C=O), 1541.4 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.31 (br d, *J* = 5.5 Hz, 1 H, NH), 4.59 (ddd, *J* = 6.2, 8.7, 11.5 Hz, 1 H, CHNH), 4.48 (dt, *J* = 1.2, 8.9 Hz, 1 H, OCHH), 4.30 (ddd, *J* = 5.8, 9.3, 11.3 Hz, 1 H, OCHH), 3.49 (t, *J* = 6.3 Hz, 2 H, CH₂Br), 2.82 (dddd, *J* = 1.3, 5.9, 8.7, 12.5 Hz, 1 H, OCH₂CHH), 2.47 (t, *J* = 7.3 Hz, 2 H, C(=O)CH₂), 2.26 - 2.15 (m, 3 H, OCH₂CHH and CH₂CH₂Br)

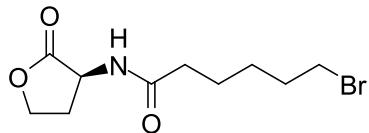
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 175.4 (OC=O), 172.3 (C(=O)NH), 66.1 (OCH₂), 49.3 (CHNHC=O), 33.9 (C(=O)CH₂), 33.1 (CH₂Br), 30.3 (OCH₂CH₂), 27.9 (C(=O)CH₂CH₂)

HRMS The compound does not ionise.

[α]_D^{26.6} / °10⁻¹cm²g⁻¹ = -78 (*c* / g(100 ml)⁻¹ = 0.0833, MeOH)

The compound has not been reported previously.

9.17 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 60



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.00 eq.) and NaHCO₃ (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in CH₂Cl₂ (2 ml) and water (2 ml) at r.t.. Bromohexanoyl chloride **59** (93.0 µL, 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the CH₂Cl₂ 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* / °C = 106 (CH₂Cl₂, water)

IR (neat) ν_{max} / cm⁻¹ = 3300.3 (N-H), 3067.6 (C-H), 2937.4 (C-H), 2856.7 (C-H), 1784.8 (lactone C=O), 1639.3 (amide C=O), 1539.9 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / 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, CHNH), 4.50 (dt, J = 1.3, 9.1 Hz, 1 H, OCHH), 4.31 (ddd, J = 5.9, 9.3, 11.3 Hz, 1 H, OCHH), 3.43 (t, J = 6.7 Hz, 2 H, CH₂Br), 2.88 (dddd, J = 1.3, 5.9, 8.6, 12.6 Hz, 1 H, OCH₂CHH), 2.30 (dt, J = 1.8, 7.5 Hz, 2 H, C(=O)CH₂), 2.16 (dtd, J = 8.9, 11.5, 12.5 Hz, 1 H, OCH₂CHH), 1.90 (quin, J = 7.2 Hz, 2 H, CH₂CH₂Br), 1.71 (quin, J = 7.6 Hz, 2 H, C(=O)CH₂CH₂), 1.59 - 1.46 (m, 2 H, C(=O)CH₂CH₂CH₂)

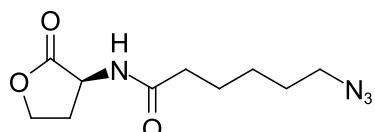
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 175.5 (OC=O), 173.3 (C(=O)NH), 66.1 (OCH₂), 49.3 (CHNHC=O), 35.8 (CH₂Br), 33.5 (C(=O)CH₂), 32.3 (CH₂CH₂Br), 30.5 (OCH₂CH₂), 27.6 (C(=O)CH₂CH₂), 24.4 (C(=O)CH₂CH₂CH₂)

HRMS (ESI⁺) m/z / Da = 278.0381, [M+H]⁺, [C₁₀H₁₇BrNO₃]⁺ requires 278.0386

$[\alpha]_D^{26.6}$ / °10⁻¹cm²g⁻¹ = -16 (c / g(100 ml)⁻¹ = 0.208, 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 NaN₃ (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 CH₂Cl₂ (5 ml) and water (5 ml). The aqueous phase was extracted twice more with CH₂Cl₂ (2×5 ml) and the organic layers were combined and dried over MgSO₄. 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 / °C = 90.0 (CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3314.0 (N-H), 2931.6 (C-H), 2862.9 (C-H), 2095.1 (N₃), 1775.4 (lactone C=O), 1643.1 (amide C=O), 1547.9 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 5.96 (d, J = 4.2 Hz, 1 H, NH), 4.54 (ddd, J = 11.7, 8.6, 5.7 Hz, 1 H, CHNH), 4.49 (td, J = 9.1, 1.0 Hz, 1 H, OCHH), 4.30 (ddd, J = 11.3, 9.4, 5.8 Hz, 1 H, OCHH), 3.29 (t, J = 6.9 Hz, 2 H, CH₂N₃), 2.88 (dddd, J = 12.5, 8.6, 5.8, 1.1 Hz, 1 H, OCH₂CHH), 2.28 (t, J = 7.5 Hz, 1 H, C(=O)CHH), 2.28 (t, J = 7.4 Hz, 1 H, C(=O)CHH), 2.14 (dtd, J = 12.3, 11.5, 11.5, 8.8 Hz, 1 H, OCH₂CHH), 1.70 (quin, J = 7.6 Hz, 2 H, CH₂CH₂N₃), 1.63 (quin, J = 7.2 Hz, 2 H, C(=O)CH₂CH₂), 1.38 - 1.49 (m, 2 H, C(=O)CH₂CH₂CH₂)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 175.4 (OC=O), 172.2 (C(=O)NH), 66.1 (OCH₂), 51.2 (CH₂N₃), 49.4 (CHNHC=O), 35.9 (C(=O)CH₂), 30.7 (OCH₂CH₂), 28.6 (CH₂CH₂N₃), 26.3 (C(=O)CH₂CH₂), 24.8 (C(=O)CH₂CH₂CH₂)

HRMS (ESI⁺) m/z / Da = 241.1289, [M+H]⁺, [C₁₀H₁₇N₄O₃]⁺ requires 241.1295

$[\alpha]_D^{26.6} / {}^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = -16$ ($c / \text{g(100 ml)}^{-1} = 0.208$, 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 CH₂Cl₂ (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 Et₂O (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 Et₂O. The solvent was removed by rotary evaporation. **63** was obtained as a pale yellow-green oil (4.72 g, 49.1 mmol, 72%).

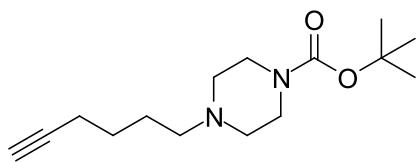
IR (neat) ν_{max} / cm⁻¹ = 3292.7 (alkyne C-H), 2943.3 (alkane C-H), 2830.9 (aldehyde C-H), 2728.6 (aldehyde C-H), 1720.3 (aldehyde C=O)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 9.80 (s, 1 H, C(=O)H), 2.60 (t, $J = 7.1$ Hz, 2 H, CH₂C(=O)H), 2.26 (dt, $J = 2.6, 6.8$ Hz, 2 H, HC≡CCH₂), 1.98 (t, $J = 2.7$ Hz, 1 H, HC≡C), 1.85 (quin, $J = 7.0$ Hz, 2 H, HC≡CCH₂CH₂)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 201.6 (C(=O)), 83.1 (HC≡C), 69.3 (HC≡C), 42.4 (CH₂C(=O)), 20.7 (CH₂CH₂C(=O)), 17.6 (HC≡CCH₂)

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 N₂ atmosphere in 1,2-dichloroethane (20 ml) for 2.5 h followed by addition of sodium triacetoxyborohydride (6.25 g, 29.5 mmol, 7 eq.) in four portions over 4 d. The mixture was stirred for a further day then NaHCO₃ (sat., aq., 120 ml) was added and the product extracted with EtOAc (2×100 ml). The solvent was dried over MgSO₄ and removed by rotary evaporation. **65** was obtained as a colourless liquid (1.12 g, 4.21 mmol, 99%).

TLC R_f (10% MeOH/CH₂Cl₂) = 0.55

IR (neat) ν_{max} / cm⁻¹ = 3303.6 (alkyne C-H), 2940.0 (alkane C-H), 2865.2 (C-H), 2810.4 (C-H), 1691.3

(carbamate C=O)

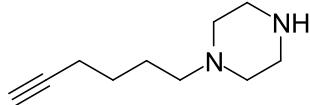
¹H NMR (400 MHz, CDCl₃) δ / ppm = 3.44 (t, *J* = 5.2 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.39 (t, *J* = 5.1 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂), 2.37 (t, *J* = 7.3 Hz, 2 H, CH₂CH₂CH₂N), 2.23 (dt, *J* = 2.7, 6.8 Hz, 2 H, HC≡CCH₂), 1.96 (t, *J* = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.53 (m, 4 H, HC≡CCH₂CH₂CH₂), 1.47 (s, 9 H, CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 154.7 (NC(=O)O), 84.2 (HC≡C), 79.6 (C(CH₃)₃), 68.5 (HC≡C), 60.4 (CH₂CH₂CH₂N), 58.0 (CH₂CH₂CH₂N(CH₂)CH₂), 53.0 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 28.4 (C(CH₃)₃), 26.3 (CH₂CH₂N), 25.7 (HC≡CCH₂CH₂), 18.3 (HC≡CCH₂)

HRMS (ESI⁺) *m/z* / Da = 267.2073, [M+H]⁺, [C₁₅H₂₇N₂O₂]⁺ requires 267.2064

The compound has not been reported previously.

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



tert-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 CH₂Cl₂ (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 CH₂Cl₂ (2×20 ml) and the combined organic layers were dried over MgSO₄. The solvent was removed under vacuum and purified by column chromatography (SiO₂ MeOH/CH₂Cl₂ 3:7). **66** was obtained as a colourless liquid (476 mg, 2.86 mmol, 100%).

TLC *R_f* (30% MeOH/CH₂Cl₂) = 0.20

IR (neat) ν_{max} / cm⁻¹ = 3295.9 (alkyne C-H), 2941.1 (alkane C-H), 2810.6 (alkane C-H), 1637.2 (N-H bend)

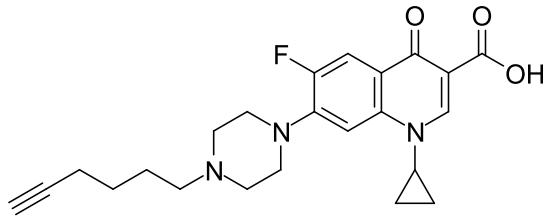
¹H NMR (400 MHz, CDCl₃) δ / ppm = 2.88 (t, *J* = 4.9 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.39 (m, 4 H, CH₂CH₂CH₂N(CH₂)CH₂), 2.31 (t, *J* = 7.1 Hz, 2 H, HC≡CCH₂CH₂CH₂N), 2.20 (dt, *J* = 2.7, 6.8 Hz, 2 H, HC≡CCH₂), 2.05 (br s, 1 H, NH), 1.93 (t, *J* = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.48 (m, 4 H, HC≡CCH₂CH₂CH₂N)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 84.3 (HC≡C), 68.4 (HC≡C), 58.6 (CH₂CH₂CH₂N), 54.5 (CH₂CH₂CH₂N(CH₂)CH₂), 46.0 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 26.4 (CH₂CH₂CH₂N), 25.7 (HC≡CCH₂CH₂), 18.3 (HC≡CCH₂)

HRMS (ESI⁺) *m/z* / Da = 167.1548, [M+H]⁺, [C₁₀H₁₉N₂]⁺ 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-dihydro-quinoline-3-carboxylic acid 68



7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquino-line-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/CH₂Cl₂)

mp $T / ^\circ\text{C} = 220$ (MeOH, decomposes)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3212.0$ (alkyne C-H), 2459.3 (O-H), 1722.6 (carboxylic acid C=O), 1626.8 (quinolone C=O)

¹H NMR (500 MHz, DMSO-d₆) $\delta / \text{ppm} = 15.12$ (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(CH₂)₂) and CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 3.54 - 3.68 (br m, 2 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂), 3.45 (br. t, $J = 11.6$ Hz, 2 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 3.21 - 3.29 (br m, 2 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 3.11 - 3.20 (br m, 2 H, CH₂CH₂CH₂N(CH₂CH₂)), 2.84 (t, $J = 2.7$ Hz, 1 H, HC≡C), 2.24 (td, $J = 7.0, 2.7$ Hz, 2 H, HC≡CCH₂), 1.83 (br. quin, $J = 7.5$ Hz, 2 H, HC≡CCH₂CH₂CH₂), 1.52 (quin, $J = 7.4$ Hz, 2 H, HC≡CCH₂CH₂), 1.29 - 1.36 (m, 2 H, NCH(CHH)₂), 1.16 - 1.23 (m, 2 H, NCH(CHH)₂)

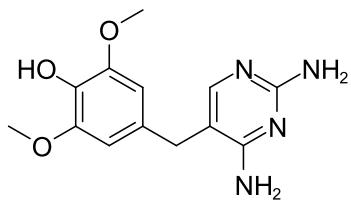
¹³C NMR (126 MHz, DMSO-d₆) $\delta / \text{ppm} = 176.4$ (C(=O)CC(=O)OH), 165.8 (C(=O)OH), 152.8 (d, $J = 248.5$ Hz, *ipso* to F), 148.2 (CHCC(=O)OH), 143.7 (d, $J = 11.1$ Hz, *para* to C(=O)), 139.1 (*para* to F), 119.4 (d, $J = 6.9$ Hz, *ipso* to C(=O)), 111.2 (d, $J = 22.5$ Hz, *ortho* to F and *ortho* 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 (CH₂CH₂CH₂N), 50.5 (CH₂CH₂CH₂N(CH₂CH₂)), 46.3 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 36.0 (NCH(CH₂)₂), 25.2 (HC≡CCH₂CH₂), 22.3 (HC≡CCH₂CH₂CH₂), 17.4 (HC≡CCH₂), 7.6 (NCH(CH₂)₂)

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

HRMS (ESI⁺) $m/z / \text{Da} = 412.2036$, [M+H]⁺, [C₂₃H₂₇N₃O₃F]⁺ 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/CHCl₂)

mp $T / ^\circ\text{C} = 238$ (water, decomposes)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3314.0$ (N-H), 3137.4 (N-H), 3045.3 (C-H), 3000.9 (C-H), 2938.1 (C-H), 2838.7 (C-H), 1662.9 (pyrimidine), 1645.2 (pyrimidine), 1626.6 (pyrimidine)

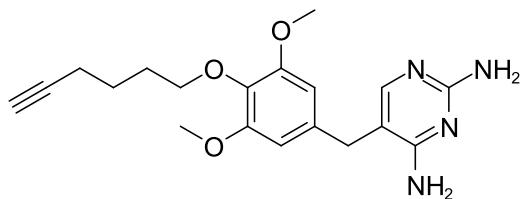
¹H NMR (400 MHz, MeOD) $\delta / \text{ppm} = 7.21$ (s, 1 H, CHN), 6.54 (s, 2 H, *meta* to OCH₂), 4.87 (br s, 5 H, OH, NH₂ × 2), 3.82 (s, 6 H, OCH₃), 3.63 (s, 2 H, CCH₂C)

¹³C NMR (101 MHz, MeOD) $\delta / \text{ppm} = 166.4$ (CH₂CCNH₂), 162.0 (CHNCNH₂), 156.2 (CHNCNH₂), 149.8 (*ipso* to OCH₃), 135.9 (*ipso* to OH), 128.2 (*para* to OH), 111.7 (CH₂CCNH₂), 107.5 (*meta* to OH), 57.0 (OCH₃), 33.9 (CCH₂C)

HRMS (ESI⁺) $m/z / \text{Da} = 277.1295$, [M+H]⁺ found, [C₁₃H₁₇N₄O₃]⁺ 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 (SiO₂, 5% MeOH/CH₂Cl₂). **71** was obtained as a pale cream amorphous solid (0.327 g, 0.917 mmol, 25%).

TLC $R_f = 0.14$ (5% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3451.4 (alkyne C-H), 3313.4 (N-H), 3136.7 (N-H), 3113.9 (N-H), 2944.2 (C-H), 2839.0 (C-H), 1635.1 (pyrimidine)

¹H NMR (400 MHz, MeOD) δ / ppm = 7.77 (s, 1 H, CHN), 6.37 (s, 2 H, *meta* to OCH₂), 4.83 (br s, 2 H, CHNCNH₂), 4.63 (br s, 2 H, CH₂CCNH₂), 3.95 (t, $J = 6.3$ Hz, 2 H, CH₂O), 3.79 (s, 6 H, OCH₃), 3.65 (s, 2 H, CCH₂C), 2.28 (td, $J = 7.1, 2.6$ Hz, 2 H, HC≡CCH₂), 1.94 (t, $J = 2.7$ Hz, 1 H, HC≡C), 1.81 - 1.90 (m, 2 H, CH₂CH₂O), 1.71 - 1.80 (m, 2 H, CH₂CH₂CH₂O)

¹³C NMR (101 MHz, MeOD) δ / ppm = 162.7 (CH₂CCNH₂), 162.0 (CHNCNH₂), 156.4 (CHNCNH₂), 153.8 (*ipso* to OCH₃), 136.0 (*ipso* to OCH₂), 133.6 (*para* to OCH₂), 106.5 (CH₂CCNH₂), 105.0 (*meta* to OCH₂), 84.5 (HC≡C), 72.6 (CH₂O), 68.3 (HC≡C), 56.1 (OCH₃), 34.7 (CCH₂C), 29.1 (CH₂CH₂O), 24.9 (CH₂CH₂CH₂O), 18.0 (HC≡CCH₂)

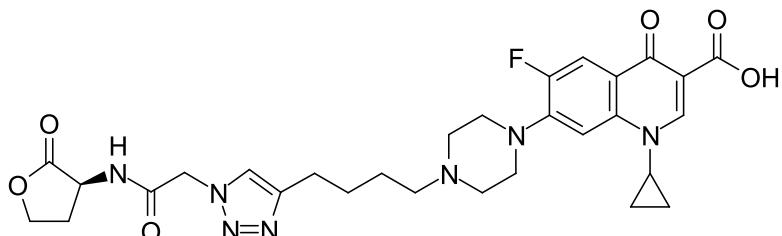
HRMS (ESI⁺) m/z / Da = 357.1920, [M+H]⁺ found, [C₁₉H₂₅N₄O₃]⁺ requires 357.1927

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 N₂. The mixture was placed under positive pressure of Ar using a balloon. Equimolar amounts of CuSO₄ · 5 H₂O 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 SiO₂ and purified by column chromatography (SiO₂, 0-20% MeOH/CH₂Cl₂). 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)-1*H*-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 $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ (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 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 SiO_2 and purified by column chromatography using a Combiflash (SiO_2 , 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) ν_{max} / cm⁻¹ = 3266.3 (N-H), 2949.0 (C-H), 2934.8 (C-H), 2827.2 (C-H), 1778.0 (lactone C=O), 1724.9 (carboxylic acid C=O), 1665.0 (amide C=O), 1625.5 (quinolone C=O)

¹H NMR (400 MHz, DMSO d₆) δ / ppm = 15.23 (s, 1 H, C(=O)OH), 8.84 (d, *J* = 7.9 Hz, 1 H, NH), 8.66 (s, 1 H, *ortho* to C(=O)OH), 7.90 (d, *J* = 13.3 Hz, 1 H, *ortho* to F), 7.82 (s, 1 H, CH=CCH₂), 7.57 (d, *J* = 7.6 Hz, 1 H, *meta* to F), 5.13 (s, 1 H, C(=O)CHHN), 5.12 (s, 1 H, C(=O)CHHN), 4.64 (ddd, *J* = 10.9, 9.0, 7.8 Hz, 1 H, CHNH), 4.36 (td, *J* = 8.9, 1.7 Hz, 1 H, OCHH), 4.23 (ddd, *J* = 10.6, 8.8, 6.4 Hz, 1 H, OCHH), 3.83 (tt, *J* = 7.0, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.32 (br s, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.67 (t, *J* = 7.4 Hz, 2 H, CH=CCH₂), 2.58 (br t, *J* = 5.0 Hz, 4 H, CH₂CH₂CH₂N(CH₂)CH₂), 2.42 - 2.49 (m, 1 H, OCH₂CHH), 2.40 (t, *J* = 7.1 Hz, 2 H, CH=CCH₂CH₂CH₂CH₂), 2.17 (dtd, *J* = 11.7, 10.8, 9.0 Hz, 1 H, OCH₂CHH), 1.66 (quin, *J* = 7.2 Hz, 2 H, CH=CCH₂CH₂), 1.53 (quin, *J* = 7.2 Hz, 2 H, CH=CCH₂CH₂CH₂), 1.28 - 1.35 (m, 2 H, NCH(CHH)₂), 1.16 - 1.21 (m, 2 H, NCH(CHH)₂)

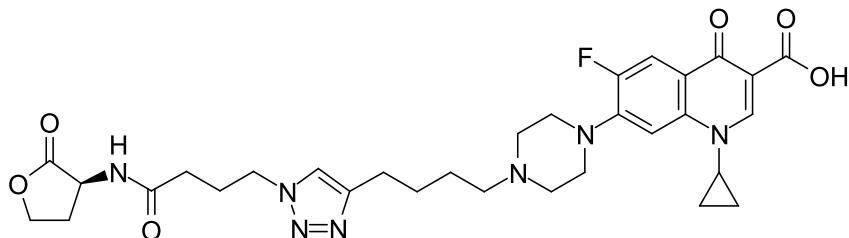
¹³C NMR (101 MHz, DMSO d₆) δ / ppm = 176.4 (C(=O)CC(=O)OH), 174.9 (OC(=O)), 166.0 (C(=O)OH), 165.9 (NHC(=O)), 153.1 (d, *J* = 250.8 Hz, *ipso* to F), 148.0 (CH=CC(=O)OH), 146.6 (CH=CCH₂), 145.3 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (para to F), 123.4 (CH=CCH₂), 118.5 (d, *J* = 7.5 Hz, para to piperazine), 110.9 (d, *J* = 23.5 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.4 (d, *J* = 3.2 Hz, meta to C=O and meta to F), 65.4 (OCH₂), 57.3 (CH=CCH₂CH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂)CH₂), 51.2 (C(=O)CH₂N), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 48.2 (CHNH), 35.9 (NCH(CH₂)₂), 28.2 (CH₂CHNH), 26.8 (CH=CCH₂CH₂), 25.7 (CH=CCH₂CH₂CH₂), 24.9 (CH=CCH₂), 7.6 (NCH(CH₂)₂)

HRMS (ESI⁺) *m/z* / Da = 596.2627, [M+H]⁺ found, [C₂₉H₃₅FN₇O₆]⁺ requires 596.2633

$[\alpha]_D^{20}$ / °10⁻¹cm²g⁻¹ = -3.5 (*c* / g(100 ml)⁻¹ = 0.0575, MeOH)

The compound has not been reported previously.

9.27 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77



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 μ mol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **58** (10.6 mg, 50.0 μ mol, 1 eq.). A similarly degassed solution of CuSO₄ · 5 H₂O (624 μ g, 2.5 μ mol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μ mol, 0.05 eq. 50 mM) and sodium ascorbate (991 μ g, 5 μ mol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (50 μ l) was then added. The mixture was stirred at r.t. under argon for 3 h, then 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. **77** was obtained as a white amorphous solid (14.6 mg, 23.4 μ mol, 47%).

IR (neat) ν_{max} / cm⁻¹ = 3286.7 (N-H), 2949.7 (C-H), 2820.6 (C-H), 2778.0 (C-H), 1778.1 (lactone C=O), 1725.6 (carboxylic acid C=O), 1663.7 (amide C=O), 1625.8 (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), 8.40 (d, *J* = 8.0 Hz, 1 H, NH), 7.88 (d, *J* = 13.4 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH₂), 7.55 (d, *J* = 7.5 Hz, 1 H, *meta* to F), 4.53 (ddd, *J* = 10.9, 9.0, 8.1 Hz, 1 H, CHNH), 4.33 (td, *J* = 8.9, 1.8 Hz, 1 H, OCHH), 4.31 (t, *J* = 7.0 Hz, 2 H, CH₂NCH=C), 4.20 (ddd, *J* = 10.5, 8.8, 6.5 Hz, 1 H, OCHH), 3.82 (tt, *J* = 6.9, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.32 (br. t, *J* = 4.2 Hz, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.64 (t, *J* = 7.4 Hz, 2 H, CH=CCH₂), 2.57 (br. t, *J* = 5.0 Hz, 2 H, CH₂CH₂CH₂N(CH₂)CH₂), 2.34 - 2.42 (m, 3 H, OCH₂CHH and CH=CCH₂CH₂CH₂CH₂), 2.09 - 2.19 (m, 3 H, OCH₂CHH and C(=O)CH₂), 2.02 (quin, *J* = 7.2 Hz, 2 H, C(=O)CH₂CH₂), 1.64 (quin, *J* = 7.6 Hz, 2 H, CH=CCH₂CH₂), 1.52 (quin, *J* = 7.2 Hz, 2 H, CH=CCH₂CH₂CH₂), 1.29 - 1.34 (m, 2 H, NCH(CHH)₂), 1.15 - 1.21 (m, 2 H, NCH(CHH)₂)

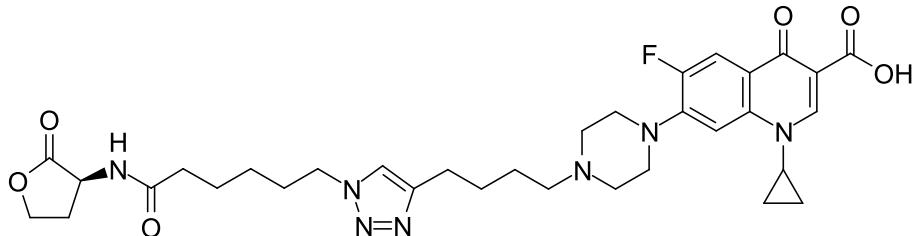
¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 176.3 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 171.2 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 248.6 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH₂), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 (CH=CCH₂), 118.5 (d, *J* = 7.5 Hz, *para* to piperazine), 110.9 (d, *J* = 22.4 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.3 (d, *J* = 3.2 Hz, *meta* to C=O and *meta* to F), 65.3 (OCH₂), 57.3 (CH=CCH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.4 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 48.6 (CH₂NCH=C), 47.9 (OC(=O)CHNH), 35.9 (NCH(CH₂)₂), 31.7 (NHC(=O)CH₂), 28.2 (CH₂CHNH), 26.9 (CH=CCH₂CH₂), 25.8 (NHC(=O)CH₂CH₂ and CH=CCH₂CH₂CH₂), 24.9 (CH=CCH₂), 7.6 (NCH(CH₂)₂)

HRMS (ESI⁺) *m/z* / Da = 624.2928, [M+H]⁺ found, [C₃₁H₃₉FN₇O₆]⁺ requires 624.2946

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -10.6 (c / g(100 ml)⁻¹ = 0.094, MeOH)

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)-1*H*-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 μ mol, 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (12.0 mg, 50.0 μ mol, 1 eq.). A similarly degassed solution of CuSO₄ · 5 H₂O (624 μ g, 2.5 μ mol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5 μ mol, 0.05 eq. 50 mM) and sodium ascorbate (991 μ g, 5 μ mol, 0.1 eq., 100 mM) in 50% water/*t*-BuOH (50 μ l) was then added. The mixture was stirred at r.t. under argon for 3 h, then 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. **78** was obtained as a white amorphous solid (12.4 mg, 19.0 μ mol, 38%).

TLC R_f = 0.30 (30% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3301.8 (N-H), 2939.7 (C-H), 2857.5 (C-H), 1784.6 (lactone C=O), 1728.5 (carboxylic acid C=O), 1658.2 (amide C=O), 1625.5 (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, CHNH), 4.33 (td, *J* = 8.8, 1.8 Hz, 1 H, OCHH), 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(CH₂)CH₂), 2.33 - 2.41 (m, 3 H, OCH₂CHH and CH=CCH₂CH₂CH₂CH₂), 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 (NHC(=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=CCH₂), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.6 (CH=CCH₂), 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=CCH₂CH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 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

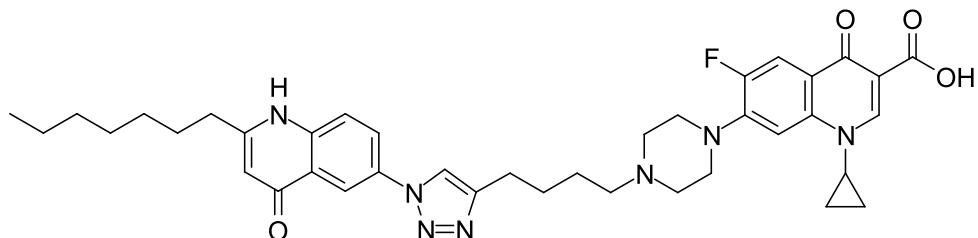
CH_2CH_2), 25.7 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$), 25.4 ($\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2$), 24.9 ($\text{CH}=\text{CCH}_2$), 24.5 ($\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2$), 7.6 ($\text{NCH}(\text{CH}_2)_2$)

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

$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -8.5$ ($c / \text{g(100 ml)}^{-1} = 0.106$, 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)-1-H-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(1*H*)-one **38** (2.8 mg, 10.0 μmol , 1 eq.). A similarly degassed solution of CuSO₄ · 5 H₂O (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) $\nu_{max} / \text{cm}^{-1} = 2927.0$ (C-H), 2865.5 (C-H), 1715.5 (carboxylic acid C=O), 1631.0 (ciprofloxacin quinolone C=O and HHQ C=O)

¹H NMR (500 MHz, DMSO d₆) 15.12 (br s, 1 H, $\underline{\text{C}}(=\text{O})\text{OH}$), 11.79 (s, 1 H, NH), 8.75 (s, 1 H, $\text{NCH}=\text{CCH}_2$), 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, $\text{NHC}=\underline{\text{CH}}\text{C}(=\text{O})$), 3.85 (tt, $J = 7.0, 4.0$ Hz, 1 H, $\text{NCH}(\text{CH}_2)_2$), 3.23 - 3.30 (m, 10 H, $\underline{\text{CH}_2\text{N(CH}_2\text{CH}_2\text{)CH}_2\text{CH}_2}$), 2.82 (t, $J = 5.9$ Hz, 2 H, $\text{NCH}=\text{CCH}_2$), 2.63 (t, $J = 7.9$ Hz, 2 H, $\underline{\text{CH}_2\text{C}=\text{CHC}(=\text{O})}$), 1.76 - 1.81 (m, 4 H, $\text{NCH}=\text{CCH}_2\text{CH}_2\text{CH}_2$), 1.70 (quin, $J = 7.2$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{C}=\text{CHC}(=\text{O})$), 1.15 - 1.38 (m, 12 H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$, $\text{NCH}(\text{CHH})_2$ and $\text{NCH}(\text{CHH}_2)_2$), 0.87 (t, $J = 6.9$ Hz, 3 H, CH₃)

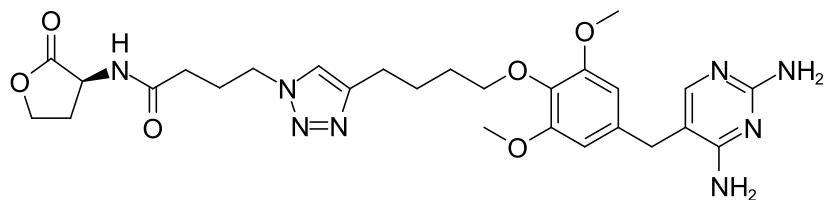
¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 176.4 ($\underline{\text{C}}(=\text{O})\text{CC}(=\text{O})\text{OH}$), 176.3 ($\text{CH}\underline{\text{C}}(=\text{O})$), 165.8 ($\underline{\text{C}}(=\text{O})\text{OH}$), 154.3 ($\text{CCH}\underline{\text{C}}(=\text{O})$), 152.9 (d, $J = 240.1$ Hz, *ipso* to F), 148.3 ($\underline{\text{CH}}=\text{CC}(=\text{O})\text{OH}$), 147.5 ($\text{NCH}\underline{\text{CCH}}_2$), 143.0 (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=CCH₂CH₂CH₂CH₂N), 50.6 (CH₂CH₂CH₂N(CH₂)CH₂), 46.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 46.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 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, [C₃₉H₄₇FN₇O₄]⁺ 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)-1*H*-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 · 5 H₂O (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 CombiFlash (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%).

TLC *R_f* = 0.30 (30% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3340.5 (N-H), 3303.3 (N-H), 3182.5 (N-H), 2933.8 (C-H), 1774.2 (lactone C=O), 1659.7 (amide C=O and pyrimidine)

¹H NMR (500 MHz, DMSO d₆) δ / ppm = 8.43 (d, *J* = 8.0 Hz, 1 H, NCH=CCH₂), 7.80 (s, 1 H, NCH=CCH₂), 7.46 (s, 1 H, CHN=CNH₂), 6.68 (br s, 2 H, CH₂CCNH₂), 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 (ddd, *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 (dddd, *J* = 12.2, 8.9, 6.7, 1.8 Hz, 1 H, CHHCHNH), 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(NH₂)N), 153.2 (*ipso* to OCH₃), 150.5 (br s, CHNC(NH₂)N), 147.3 (NCH=CCH₂CH₂), 135.2 (*para*

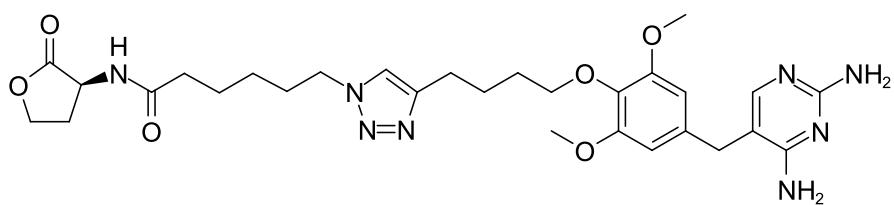
to CH₂O), 135.0 (*ipso* to CH₂O), 122.1 (CH=CCH₂CH₂), 107.3 (CH₂CC(NH₂)=N), 106.2 (*meta* to CH₂O), 72.3 (CH₂CH₂CH₂O), 65.7 (OCH₂CH₂CHNH), 56.2 (OCH₃), 48.9 (CH₂N), 48.3 (CHNH), 32.9 (CCH₂C), 32.0 (C(=O)CH₂), 29.3 (CH₂CH₂CH₂O), 28.4 (OCH₂CH₂CHNH), 26.0 (CH₂CH₂N), 25.7 (CH=CCH₂CH₂), 24.9 (CH=CCH₂CH₂)

HRMS (ESI⁺) m/z / Da = 569.2834, [M+H]⁺ found, [C₂₇H₃₇N₈O₆]⁺ requires 569.2836

$$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -4.6 \quad (c / \text{g(100 ml)}^{-1} = 0.0433, \text{ MeOH})$$

The compound has not been reported previously.

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



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*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (18.0 mg, 75.0 μmol, 1.5 eq.). Similarly degassed solutions of CuSO₄ · 5 H₂O (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 CH₂Cl₂ (6 × 10 ml) then dry-loaded onto SiO₂ and purified by column chromatography using a Combiflash (SiO₂, 0–20% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ 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/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3336.0 (N-H), 3208.7 (N-H), 2941.1 (C-H), 2869.2 (C-H), 1775.2 (lactone C=O), 1657.3 (amide C=O and pyrimidine)

¹H NMR (500 MHz, DMSO d₆) δ / ppm = 8.34 (d, *J* = 8.0 Hz, 1 H, NH), 7.83 (s, 1 H, NCH=CCH₂), 7.50 (s, 1 H, CHN=CNH₂), 6.54 (s, 2 H, *meta* to CH₂), 6.17 (br s, 2 H, CH₂CCNH₂), 5.77 (br s, 2 H, CHN=CNH₂), 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, CHHOC(=O)), 4.27 (t, *J* = 7.1 Hz, 2 H, CH₂N), 4.19 (ddd, *J* = 10.5, 8.7, 6.5 Hz, 1 H, CHHOC(=O)), 3.80 (t, *J* = 6.3 Hz, 2 H, CH₂CH₂CH₂O), 3.70 (s, 6 H, CH₃), 3.52 (s, 2 H, CCH₂C), 2.64 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.36 (dddd, *J* = 12.1, 8.9, 6.7, 1.8 Hz, 1 H, CHHCHNH), 2.06 - 2.16 (m, 3 H, CHHCHNH and C(=O)CH₂), 1.78 (quin, *J* = 7.4 Hz, 2 H, CH₂CH₂N), 1.73 (quin, *J* = 7.7 Hz, 2 H, CH=CCH₂CH₂), 1.63 (quin, *J* = 6.8 Hz, 2 H, CH₂CH₂O), 1.52 (quin, *J* = 7.5 Hz, 2 H, C(=O)CH₂CH₂), 1.17 - 1.27 (m, 2 H, C(=O)CH₂CH₂CH₂)

¹³C NMR (125 MHz, DMSO-d₆) δ / ppm = 175.4 (OC=O), 172.0 (NHC=O), 162.2 (CC(NH₂)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),

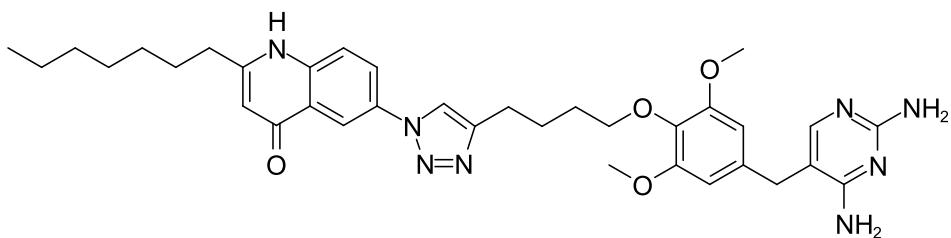
134.8 (*ipso* to CH₂O), 121.6 (CH=CC₂CH₂), 105.9 (CH₂CC(NH₂)=N), 105.8 (*meta* to CH₂O), 71.9 (CH₂CH₂CH₂O), 65.2 (OCH₂CH₂CHNH), 55.8 (OCH₃), 49.0 (CH₂N), 47.8 (CHNH), 34.8 (C(=O)CH₂), 32.9 (CCH₂C), 29.4 (CH₂CH₂N), 29.1 (CH₂CH₂CH₂O), 28.2 (OCH₂CH₂CHNH), 25.5 (CH=CC₂CH₂), 25.3 (C(=O)CH₂CH₂CH₂), 24.7 (CH=CC₂CH₂), 24.4 (C(=O)CH₂CH₂)

HRMS (ESI⁺) *m/z* / Da = 597.3149, [M+H]⁺ found, [C₂₉H₄₁N₈O₆]⁺ requires 597.3144

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -3.6 (c / g(100 ml)⁻¹ = 0.11, MeOH)

The compound has not been reported previously.

9.32 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-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 μ mol, 1 eq.) and 6-azido-2-heptylquinolin-4(*1H*)-one **38** (2.8 mg, 10.0 μ mol, 1 eq.). A similarly degassed solution of CuSO₄ · 5 H₂O (125 μ g, 0.5 μ mol, 0.05 eq. 50 mM), THPTA (218 μ g, 0.5 μ mol, 0.05 eq. 50 mM) and sodium ascorbate (198 μ g, 1 μ mol, 0.1 eq., 100 mM) in water (10 μ l) 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% 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. **87** was obtained as a clear gum (2.6 mg, 4.1 μ mol, 41%).

TLC *R_f* = 0.17 (20% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2927.7 (C-H), 2855.5 (C-H), 1664.1 (pyrimidine), 1645.4 (pyrimidine and HHQ C=O)

¹H NMR (500 MHz, DMSO d₆) δ / ppm = 11.80 (s, 1 H, NH), 8.69 (s, 1 H, NC₂=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, CHN=CNH₂), 6.61 (s, 2 H, *meta* to CH₂), 6.02 (d, *J* = 1.8 Hz, 1 H, C(=O)CH₂), 3.86 (t, *J* = 6.3 Hz, 2 H, CH₂O), 3.73 (s, 6 H, OCH₃), 3.57 - 3.62 (m, 2 H, CCH₂C), 2.78 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.63 (t, *J* = 7.3 Hz, 2 H, HNCCH₂), 1.85 (quin, *J* = 7.5 Hz, 2 H, CH=CCH₂CH₂), 1.61 - 1.78 (m, 4 H, HNCCH₂CH₂ and CH=CCH₂CH₂CH₂), 1.31 - 1.40 (m, 4 H, HNCCH₂CH₂CH₂CH₂), 1.25 - 1.31 (m, 4 H, CH₃CH₂CH₂), 0.86 (t, *J* = 7.2 Hz, 3 H, CH₃CH₂)

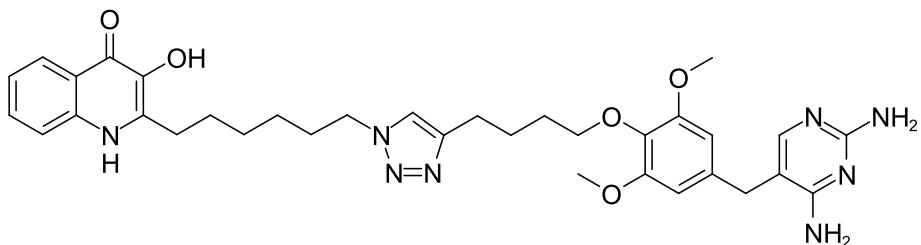
¹³C NMR (125 MHz, DMSO d₆) δ / ppm = 176.4 (C=O), 164.1 (CC(NH₂)N), 154.3 (HNC), 154.2 (NC(NH₂)N), 153.1 (*ipso* to OCH₃), 148.3 (CH=CCH₂CH₂), 140.2 (CHNC(NH₂)N), 139.6 (*ipso* to NH), 135.4 (*ipso* to CH₂O), 132.8 (*para* to CH₂O), 132.1 (*para* to NH), 124.9 (*ipso* to C=O), 123.7 (*para* to C=O), 120.3 (CH=CCH₂CH₂),

120.0 (*meta* to C=O and *ortho* to NH), 115.1 (*ortho* to C=O and *meta* to NH), 109.0 (CH₂CC(NH₂)=N), 108.0 (C(=O)CH), 106.3 (*meta* to CH₂O), 72.0 (CH₂CH₂CH₂O), 56.0 (OCH₃), 33.3 (HNCCH₂), 32.1 (CCH₂C), 31.2 (CH₃CH₂CH₂), 29.1 (CH₂CH₂O), 28.3 - 28.6 (CH₃CH₂CH₂CH₂CH₂), 25.3 (CH₂CH₂CH₂O), 24.7 (CH=CCH₂), 22.1 (CH₃CH₂), 14.0 (CH₃CH₂)

HRMS (ESI⁺) *m/z* / Da = 641.3557, [M+H]⁺ found, [C₃₅H₄₅N₈O₄]⁺ 641.3558

The compound has not been reported previously.

9.33 2-(6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(*1H*)-one 89



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** (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 CuSO₄ · 5 H₂O (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 SiO₂ and purified by column chromatography (SiO₂, 0-20% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ 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/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2924.8 (C-H), 2853.4 (C-H), 1660.0 (pyrimidine), 1638.8 (pyrimidine and PQS C=O)

¹H NMR (500 MHz, DMSO d₆) δ / 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=CCH₂), 7.48 - 7.57 (m, 3 H, *para* to C=O, *ortho* to NH and CHN=CNH₂), 7.21 (ddd, *J* = 8.0, 6.3, 1.5 Hz, 1 H, *para* to NH), 6.55 (s, 2 H, *meta* to CH₂), 4.28 (t, *J* = 7.1 Hz, 2 H, CH2N), 3.80 (t, *J* = 6.2 Hz, 2 H, CH₂O), 3.70 (s, 6 H, CH₃), 3.53 (d, *J* = 0.3 Hz, 2 H, CCH₂C), 2.73 (t, *J* = 7.5 Hz, 2 H, HNCCH₂), 2.64 (t, *J* = 7.4 Hz, 2 H, CH=CCH₂), 1.80 (quin, *J* = 7.4 Hz, 2 H, CH₂CH₂N), 1.73 (quin, *J* = 7.5 Hz, 2 H, CH=CCH₂CH₂), 1.66 (quin, *J* = 7.2 Hz, 2 H, HNCCH₂CH₂), 1.62 (quin, *J* = 6.8 Hz, 2 H, CH₂CH₂O), 1.33 - 1.40 (m, 2 H, HNCCH₂CH₂CH₂), 1.27 - 1.32 (m, 2 H, HNCCH₂CH₂CH₂CH₂)

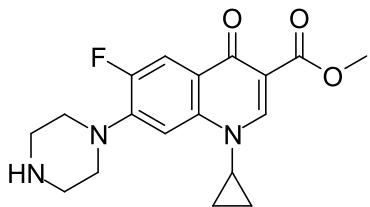
¹³C NMR (125 MHz, DMSO d₆) δ / ppm = 168.9 (C=O), 162.5 (CCC(NH₂)N), 162.5 (NCCC(NH₂)N), 152.9 (CHNC(NH₂)N), 152.8 (*ipso* to OCH₃), 146.8 (CH=CCH₂CH₂), 137.7 (COH), 137.3 (*para* to OH), 135.4 (HNC), 135.1 (*para* to CH₂O), 134.8 (*ipso* to CH₂O), 129.9 (*para* to C=O), 124.4 (*ortho* to C=O and *meta* to NH), 122.1 (*ipso* to C=O), 121.5 (*para* to NH), 121.4 (CH=CCH₂CH₂), 117.7 (*meta* to C=O and *ortho* to NH), 106.2 (CH₂CC(NH₂)=N), 105.8 (*meta* to CH₂O), 71.9 (CH₂CH₂CH₂O), 55.8 (OCH₃), 49.0 (CCH₂N), 32.8

(CCH₂C), 29.5 (CH₂CH₂N), 29.0 (CH₂CH₂O), 28.1 (HNCCH₂CH₂CH₂), 27.9 (HNCCH₂), 27.6 (HNCCH₂CH₂), 25.6 (CH₂CH₂CH₂N), 25.4 (CH₂CH₂CH₂O), 24.6 (CH=CCH₂CH₂CH₂)

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 *R_f* = 0.13 (5% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2947.9 (C-H), 2834.9 (C-H), 1720.9 (ester C=O), 1616.8 (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(CH₂)₂), 3.24 - 3.29 (m, 4 H, HN(CH₂CH₂)CH₂CH₂), 3.02 - 3.10 (m, 4 H, HN(CH₂)CH₂), 1.31 - 1.38 (m, 2 H, NCH(CHH)₂), 1.12 - 1.20 (m, 2 H, NCH(CHH)₂)

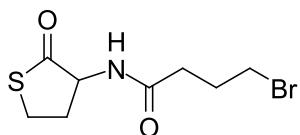
¹³C NMR (101 MHz, MeOD) δ / ppm = 175.2 (C(=O)CC(=O)OCH₃), 166.8 (C(=O)OCH₃), 154.9 (d, *J* = 248.0 Hz, *ipso* to F), 150.1 (C=CC(=O)OCH₃), 146.6 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.3 (d, *J* = 6.9 Hz, *para* to piperazine), 113.0 (d, *J* = 23.4 Hz, *ortho* to C=O and *ortho* 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(CH₂CH₂)CH₂CH₂), 51.6 (HN(CH₂CH₂)CH₂CH₂), 46.5 (HN(CH₂)CH₂), 36.4 (NCH(CH₂)₂), 8.7 (NCH(CH₂)₂)

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.²¹⁶

9.35 4-Bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide 153



3-Aminodihydrothiophen-2(*H*)-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 $R_f = 0.19$ (50% EtOAc/PE)

IR (neat) ν_{max} / cm⁻¹ = 3265.9 (amide N-H), 3063.2 (amide N-H), 1694.3 (thiolactone C=O), 1650.5 (amide C=O)

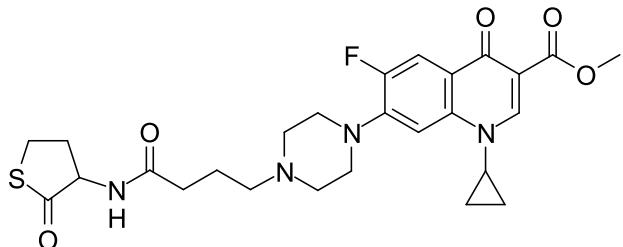
¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.08 (d, J = 6.1 Hz, 1 H, NH), 4.54 (dt, J = 12.9, 6.5 Hz, 1 H, CHNH), 3.49 (t, J = 6.4 Hz, 2 H, CH₂Br), 3.37 (ddd, J = 12.2, 11.5, 5.3 Hz, 1 H, SCHH), 3.26 (ddd, J = 11.5, 6.9, 1.3 Hz, 1 H, SCHH), 2.91 (dddd, J = 12.5, 6.7, 5.3, 1.3 Hz, 1 H, SCH₂CHH), 2.45 (t, J = 7.4 Hz, 1 H, C(=O)CHH), 2.45 (t, J = 6.8 Hz, 1 H, C(=O)CHH), 2.20 (quin, J = 6.7 Hz, 1 H, C(=O)CH₂CH₂), 1.96 (dddd, 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 previously^{15,149} 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) ν_{max} / cm⁻¹ = 2944.2 (C-H), 2832.4 (C-H), 1722.4 (ester C=O), 1700.4 (thiolactone C=O), 1669.6 (amide C=O), 1617.3 (quinolone C=O)

¹H NMR (500 MHz, MeOD) δ / 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, CH₂NH), 3.83 (s, 3 H, OCH₃), 3.61 (tt, J = 6.9, 4.1 Hz, 1 H, NCH(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(CH₂)CH₂), 2.79 (br. t, J = 7.2, 7.2 Hz, 2 H, C(=O)CH₂CH₂CH₂), 2.59 (dd, J = 12.4, 6.9, 5.4, 1.4 Hz, 1 H, SCH₂CH₂H), 2.39 (t, J = 7.20 Hz, 1 H, C(=O)CH₂H), 2.38 (t, J = 6.94 Hz, 1 H, C(=O)CH₂H), 2.18 (qd, J = 12.4, 7.0 Hz, 1 H, SCH₂CH₂H), 1.97 (quin, J = 7.2 Hz, 2 H, C(=O)CH₂CH₂), 1.32 - 1.37 (m, 2 H, NCH(CH₂H)₂), 1.13 - 1.19 (m, 2 H, NCH(CH₂H)₂)

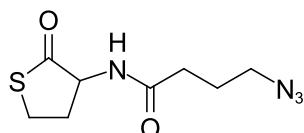
¹³C NMR (126 MHz, MeOD) δ / ppm = 207.0 (SC(=O)), 175.7 (NHC(=O)), 175.1 (C(=O)CC(=O)OCH₃), 166.6 (C(=O)OCH₃), 154.7 (d, J = 249.0 Hz, *ipso* to F), 150.2 (s, CH=CC(=O)OCH₃), 145.6 (d, J = 10.6 Hz, *ipso* to piperazine), 139.8 (*para* to F), 123.5 (d, J = 6.9 Hz, *para* to piperazine), 113.1 (d, J = 23.6 Hz, *ortho* to C=O and *ortho* to F), 110.0 (CC(=O)OCH₃), 107.4 (*meta* to C=O and *meta* to F), 60.2 (CH₂NH), 58.5 (C(=O)CH₂CH₂CH₂), 53.8 (CH₂CH₂CH₂N(CH₂)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) δ / ppm = -125.4 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 531.2083, [M+H]⁺ found, [C₂₆H₃₂FN₄O₅S]⁺ requires 531.2077

The compound has been synthesised previously.^{15, 149} Only HRMS characterisation was published, and this agrees with the result above.

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



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) ν_{max} / cm⁻¹ = 3285.6 (N-H), 2963.9 (C-H), 2100.2 (azide), 1697.4 (thiolactone C=O), 1647.4 (amide C=O)

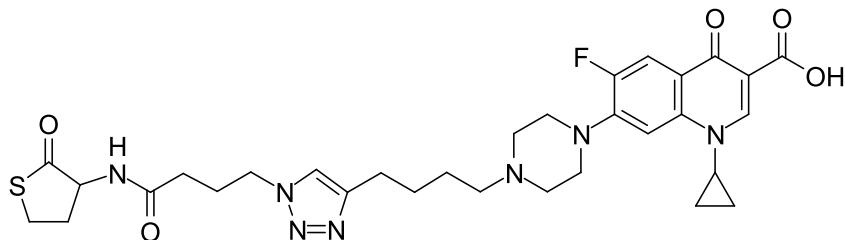
¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.71 (d, J = 7.3 Hz, 1 H, NH), 4.54 (dt, J = 13.0, 7.0 Hz, 1 H, CHNH), 3.30 (t, J = 6.7 Hz, 2 H, CH₂N₃), 3.31 (td, J = 11.7, 5.3 Hz, 1 H, SCHH), 3.19 (ddd, J = 11.3, 7.0, 1.2 Hz, 1 H, SCHH), 2.70 (dddd, J = 12.4, 6.8, 5.3, 1.2 Hz, 1 H, SCH₂CHH), 2.29 (t, J = 7.5 Hz, 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-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 156



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (15 mg, 36.7 μ mol, 1 eq.) and 4-azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide **155** (12.5 mg, 55.1 μ mol, 1.5 eq.) were dissolved in 1:9:10 water/t-BuOH/DMSO (3 ml), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (182 μ l, 18.2 μ mol, 0.5 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (367 μ l, 36.7 μ mol, 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 μ mol, 71%).

IR (neat) ν_{max} / cm⁻¹ = 2918.8 (C-H), 1712.7 (carboxylic acid C=O and thiolactone C=O), 1657.6 (amide C=O), 1626.8 (quinolone C=O), 1616.2 (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,

$\text{CH}_2\text{NCH}=\text{C}$), 3.80 - 3.86 (6.9, 4.0 Hz, 1 H, $\text{NCH}(\text{CH}_2)_2$), 3.34 - 3.37 (m, 1 H, SCHH), 3.32 (br t, $J = 4.1$ Hz, 4 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 3.27 (ddd, $J = 11.1, 6.9, 1.4$ Hz, 1 H, SCHH), 2.64 (t, $J = 7.6$ Hz, 2 H, $\text{CH}=\text{CCH}_2$), 2.57 (br t, $J = 4.7$ Hz, 4 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$), 2.34 - 2.44 (m, 3 H, SCH_2CHH and $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.12 (t, $J = 7.9$ Hz, 1 H, $\text{C}(=\text{O})\text{CHH}$), 2.12 (t, $J = 7.0$ Hz, 1 H, $\text{C}(=\text{O})\text{CHH}$), 2.04 (m, 3 H, SCH_2CHH and $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$), 1.64 (quin, $J = 7.5$ Hz, 2 H, $\text{CH}=\text{CCH}_2\text{CH}_2$), 1.51 (quin, $J = 7.5$ Hz, 2 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$), 1.28 - 1.34 (m, 2 H, $\text{NCH}(\text{CHH})_2$), 1.15 - 1.20 (m, 2 H, $\text{NCH}(\text{CHH})_2$)

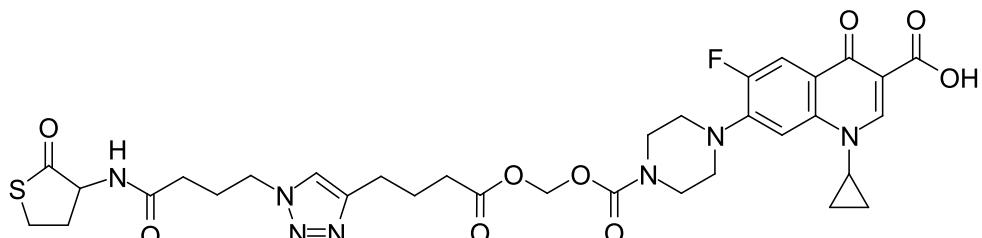
^{13}C NMR (126 MHz, DMSO d₆) δ / ppm = 205.6 ($\text{SC}(=\text{O})$), 176.4 ($\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$), 171.4 ($\text{NHC}(=\text{O})$), 166.0 ($\text{C}(=\text{O})\text{OH}$), 153.1 (d, $J = 249.3$ Hz, *ortho* to F), 148.0 ($\text{CH}=\text{CC}(=\text{O})\text{OH}$), 146.9 ($\text{CH}=\text{CCH}_2$), 145.3 (d, $J = 10.1$ Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 ($\text{CH}=\text{CCH}_2$), 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 ($\text{CC}(=\text{O})\text{OH}$), 106.4 (d, $J = 2.9$ Hz, *meta* to C=O and *meta* to F), 58.2 ($\text{SC}(=\text{O})\text{CHNH}$), 57.4 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 52.4 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$), 49.5 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 49.5 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 48.6 ($\text{CH}_2\text{NCH}=\text{C}$), 35.9 ($\text{NCH}(\text{CH}_2)_2$), 31.9 ($\text{NHC}(=\text{O})\text{CH}_2$), 30.1 (CH_2CHNH), 26.9 ($\text{CH}=\text{CCH}_2\text{CH}_2$), 26.8 (SCH_2), 25.9 ($\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2$), 25.8 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$), 25.0 ($\text{CH}=\text{CCH}_2$), 7.6 ($\text{NCH}(\text{CH}_2)_2$)

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

HRMS (ESI⁺) m/z / Da = 640.2739, [M+H]⁺ found, [C₃₁H₃₉FN₇O₅S]⁺ 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-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 157



1-Cyclopropyl-6-fluoro-7-(4-(((hex-5-ynoyloxy)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.), CuI (40 mg, 0.190 mmol, 0.5 eq.) and DIPEA (0.356 ml, 0.264 mg, 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 R_f = 0.40 (5% CH₂Cl₂/MeOH)

IR (neat) ν_{max} / cm⁻¹ = 3054.9 (C-H), 1715.8 (carboxylic acid C=O and ester C=O), 1696.2 (carbamate C=O and thiolactone C=O), 1651.2 (amide C=O), 1629.2 (quinolone C=O)

^1H NMR (400 MHz, DMSO d₆) δ / ppm = 15.16 (br s, 1 H, $\text{C}(=\text{O})\text{OH}$), 8.65 (s, 1 H, *ortho* to C=O),

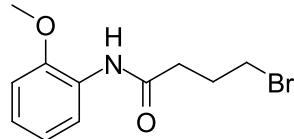
8.21 (d, $J = 8.5$ Hz, 1 H, NH), 7.89 (d, $J = 13.1$ Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH₂), 7.57 (d, $J = 7.4$ Hz, 1 H, *meta* to F), 5.74 (s, 1 H, OCH₂O), 4.58 (ddd, $J = 12.6, 8.1, 7.2$ Hz, 1 H, CHNH), 4.30 (t, $J = 6.9$ Hz, 2 H, C(=O)CH₂CH₂N), 3.80 (tt, $J = 6.9, 3.6$ Hz, 1 H, NCH(CH₂)₂), 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, SCHH), 3.34 (br s, 4 H, C(=O)N(CH₂CH₂)CH₂CH₂), 3.27 (ddd, $J = 11.0, 6.9, 1.6$ Hz, 1 H, SCHH), 2.64 (t, $J = 7.6$ Hz, 2 H, CH=CCH₂), 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, SCH₂CHH), 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, SCH₂CHH and NHC(=O)CH₂CH₂), 1.86 (quin, $J = 7.5$ Hz, 2 H, CH=CCH₂CH₂), 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 d₆) 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=CCH₂), 144.9 (d, $J = 9.6$ Hz, *ipso* to piperazine), 139.1 (*para* to F), 122.0 (CH=CCH₂), 118.9 (d, $J = 7.5$ Hz, *para* to piperazine), 111.0 (d, $J = 23.5$ Hz, *ortho* to C=O and *ortho* to F), 106.8 (CC(=O)OH, and *meta* to C=O and *meta* to F), 80.3 (OCH₂O), 58.2 (CHNH), 49.1 (C(=O)N(CH₂CH₂)CH₂CH₂), 49.1 (C(=O)N(CH₂CH₂)CH₂CH₂), 48.6 (C(=O)CH₂CH₂N), 43.4 (N(CH₂)CH₂), 43.0 (N(CH₂)CH₂), 35.9 (NCH(CH₂)₂), 32.7 (CH=CCH₂CH₂C(=O)), 31.8 (NHC(=O)CH₂), 30.1 (SCH₂CH₂), 26.8 (SCH₂), 25.8 (C(=O)CH₂CH₂N), 24.2 (CH=CCH₂CH₂CH₂C(=O)), 24.0 (CH=CCH₂CH₂CH₂C(=O)), 7.6 (NCH(CH₂)₂)

HRMS (ESI⁺) *m/z* / Da = 728.2502, [M+H]⁺ found, [C₃₃H₃₉FN₇O₉S]⁺ 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 $R_f = 0.16$ (10% EtOAc/P.E.)

IR (neat) ν_{max} / cm⁻¹ = 3410.2 (N-H), 3313.4 (N-H), 2961.6 (C-H), 2939.5 (C-H), 2902.5 (C-H), 1676.4 (amide C=O)

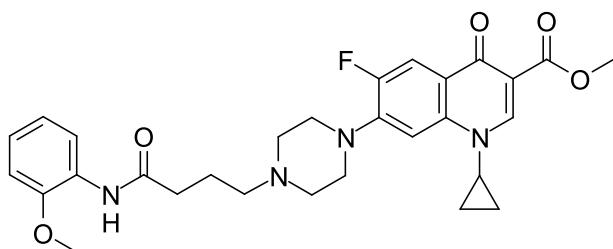
¹H NMR (400 MHz, CDCl₃ d₁) δ / ppm = 8.32 (dd, $J = 8.0, 1.7$ Hz, 1 H, *ortho* to NH), 7.85 (br s, 1 H, NH), 7.02 (td, $J = 7.9, 1.7$ Hz, 1 H, *para* to NH), 6.93 (td, $J = 7.7, 1.4$ Hz, 1 H, *para* to OCH₃), 6.85 (dd, $J = 8.1, 1.5$ Hz, 1 H, *ortho* to OCH₃), 3.85 (s, 3 H, CH₃), 3.50 (t, $J = 6.4$ Hz, 2 H, CH₂Br), 2.56 (t, $J = 7.1$ Hz, 2 H, C(=O)CH₂), 2.25 (quin, $J = 6.7$ Hz, 2 H, C(=O)CH₂CH₂)

¹³C NMR (101 MHz, CDCl₃ d₁) 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* to NH), 55.5 (CH₃), 35.4 (C(=O)CH₂), 33.1 (CH₂Br), 27.9 (C(=O)CH₂CH₂)

HRMS (ESI⁺) *m/z* / Da = 272.0287, [M+H]⁺ found, [C₁₁H₁₅BrNO₂]⁺ requires 272.0286

The compound has not been reported previously.

9.41 Methyl 1-cyclopropyl-6-fluoro-7-(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 *R_f* = 0.40 (10% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2947.1 (C-H), 2833.7 (C-H), 1718.9 (ester C=O), 1685.3 (amide C=O), 1617.3 (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)CH₃), 3.85 (s, 3 H, aromatic OCH₃), 3.41 (tt, *J* = 6.9, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.25 (br t, *J* = 5.0, 5.0 Hz, 4 H, C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.67 (br t, *J* = 5.0 Hz, 4 H, C(=O)CH₂CH₂CH₂N(CH₂CH₂)), 2.53 (t, *J* = 7.0 Hz, 2 H, C(=O)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, NCH(CHH)₂), 1.07 - 1.14 (m, 2 H, NCH(CHH)₂)

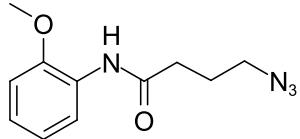
¹³C NMR (101 MHz, CDCl₃ d₁) δ / ppm = 172.9 (C(=O)CC(=O)OCH₃), 170.8 (NHC(=O)), 166.2 (C(=O)OCH₃), 153.3 (d, *J* = 248.0 Hz, *ipso* to F), 148.2 (C=CC(=O)OCH₃), 147.6 (*ipso* to OCH₃), 144.4 (d, *J* = 10.4 Hz, *ipso* to piperazine), 137.9 (*para* to F), 127.6 (*ipso* to NH), 123.4 (*para* to NH), 122.7 (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 CC(=O)OCH₃), 104.7 (*meta* to C=O and *meta* to F), 57.2 (CH₂CH₂CH₂N), 55.6 (aromatic OCH₃), 52.7 (CH₂CH₂CH₂N(CH₂CH₂)), 51.9 (C(=O)OCH₃), 49.8 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.8 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 35.5 (CH₂

$\text{CH}_2\text{CH}_2\text{N}$), 34.5 ($\text{NCH}(\text{CH}_2)_2$), 22.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 8.0 ($\text{NCH}(\underline{\text{CH}}_2)_2$)

HRMS (ESI⁺) m/z / Da = 537.2523, [M+H]⁺ found, [C₂₉H₃₄FN₄O₅]⁺ 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 = 3419.7 (N-H), 3329.6 (N-H), 2094.8 (azide), 1672.3 (amide C=O)

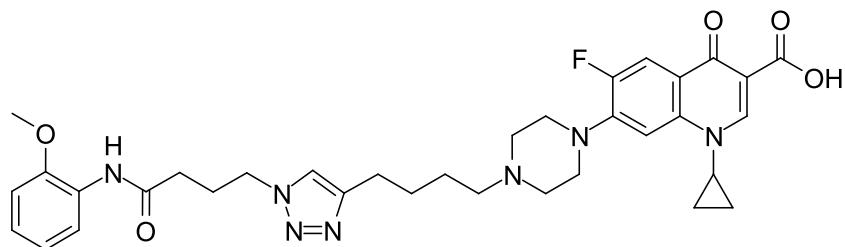
¹H NMR (400 MHz, CDCl₃ d₁) δ / ppm = 8.32 (dd, J = 7.9, 1.0 Hz, 1 H, *ortho* to NH), 7.86 (br s, 1 H, NH), 7.00 (td, J = 7.5, 1.5 Hz, 1 H, *para* to NH), 6.90 (td, J = 7.7, 1.1 Hz, 1 H, *para* to OCH₃), 6.83 (dd, J = 8.1, 1.4 Hz, 1 H, *ortho* to OCH₃), 3.81 (s, 3 H, CH₃), 3.33 (t, J = 6.7 Hz, 2 H, CH₂Br), 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 ($\underline{\text{C}}(=\text{O})$), 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 ($\underline{\text{CH}}_3$), 50.3 ($\underline{\text{CH}}_2\text{N}_3$), 33.9 (C(=O) $\underline{\text{CH}}_2$), 24.3 (C(=O)CH₂ $\underline{\text{CH}}_2$)

HRMS (ESI⁺) m/z / Da = 257.1010, [M+H]⁺ found, [C₁₁H₁₄N₄NaO₂]⁺ requires 257.1014

The data are consistent with the literature.²³⁹

9.43 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-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 μ mol, 1 eq.) and 4-azido-*N*-(2-methoxyphenyl)butanamide **161** (13.7 mg, 58.5 μ mol, 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 μ l, 5.85 μ mol, 0.1 eq., 50 mM, aq.) was added, followed by a solution of sodium ascorbate (234 μ l, 11.7 μ mol, 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 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 μ mol, 39%).

TLC R_f = 0.28 (10% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2926.5 (C-H), 2846.6 (C-H), 1723.4 (carboxylic acid C=O), 1682.0 (amide C=O), 1625.8 (quinolone C=O), 1612.8 (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=CCH₂), 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(CH₂)₂), 3.35 (br t, J = 4.7 Hz, 4 H, CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.76 (t, J = 7.5 Hz, 2 H, CH=CCH₂), 2.66 (t, J = 4.7 Hz, 4 H, CH=CCH₂CH₂CH₂N(CH₂)CH₂), 2.47 (t, J = 7.3 Hz, 2 H, CH=CCH₂CH₂CH₂N), 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=CCH₂CH₂CH₂N), 1.61 (quin, J = 7.5 Hz, 2 H, CH=CCH₂CH₂CH₂N), 1.35 - 1.42 (m, 2 H, NCH(CHH)₂), 1.17 - 1.22 (m, 2 H, NCH(CHH)₂)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 177.1 (C(=O)CC(=O)OH), 169.5 (NH \underline{C} (=O)), 167.0 (C(=O)OH), 153.7 (d, J = 251.4 Hz, *ipso* to F), 148.1 (CH=CCH₂), 147.8 (*ipso* to OCH₃), 147.3 (C=CC(=O)OH), 145.9 (d, J = 10.4 Hz, *ipso* to piperazine), 139.1 (*para* to F), 127.3 (*ipso* to NH), 123.9 (*para* to NH), 121.0 (*para* to OCH₃), 120.9 (CH=CCH₂), 119.7 (*para* to piperazine, 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=CCH₂CH₂CH₂N), 55.6 (CH₃), 52.8 (CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂), 49.8 (CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.8 (CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.1 (C(=O)CH₂CH₂CH₂N), 35.2 (NCH(CH₂)₂), 33.8 (C(=O)CH₂CH₂CH₂N), 27.3 (CH=CCH₂CH₂CH₂N), 26.4 (CH=CCH₂CH₂CH₂N), 26.0 (C(=O)CH₂CH₂CH₂N), 25.5 (CH=CCH₂CH₂CH₂N), 8.2 (NCH

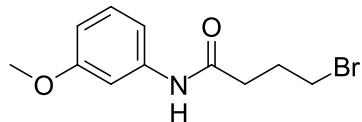
$(\underline{\text{CH}_2})_2$

^{19}F NMR (376.45 MHz, CDCl_3) δ / ppm = -120.7 (s, ciprofloxacin F)

HRMS (ESI $^+$) m/z / Da = 646.3132, $[\text{M}+\text{H}]^+$ found, $[\text{C}_{34}\text{H}_{41}\text{FN}_7\text{O}_5]^+$ 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_2Cl_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) ν_{max} / cm^{-1} = 1670.9 (amide C=O)

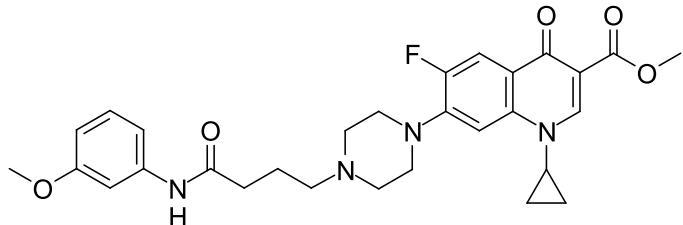
^1H NMR (400 MHz, CDCl_3 d₁) δ / ppm = 8.45 (s, 1 H, NH), 7.27 (t, J = 2.2 Hz, 1 H, *ortho* to OCH₃ and *ortho* to NH), 7.14 (t, J = 8.1 Hz, 1 H, *meta* to OCH₃ and *meta* to NH), 7.02 (d, J = 8.3 Hz, 1 H, *para* to OCH₃), 6.62 (dd, J = 8.2, 2.1 Hz, 1 H, *para* to NH), 3.71 (s, 3 H, CH₃), 3.42 (t, J = 6.5 Hz, 2 H, CH₂Br), 2.51 (t, J = 6.9 Hz, 2 H, C(=O)CH₂), 2.19 (quin, J = 6.8 Hz, 2 H, C(=O)CH₂CH₂)

^{13}C NMR (101 MHz, CDCl_3 d₁) δ / ppm = 170.3 (C(=O)), 159.9 (*ipso* to OCH₃), 139.0 (*ipso* to NH), 129.5 (*meta* to OCH₃ and *meta* to NH), 112.1 (*para* to OCH₃), 109.9 (*para* to NH), 105.7 (*ortho* to OCH₃ and *ortho* to NH), 55.2 (CH₃), 35.3 (C(=O)CH₂), 33.2 (CH₂Br), 28.0 (C(=O)CH₂CH₂)

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

The compound has not been reported previously.

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



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-(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 CH₂Cl₂ (50 ml) and water (50 ml). The organic layer was separated off and the aqueous layer was extracted again with CH₂Cl₂ (50 ml). The combined organic layers were dried with MgSO₄ and purified by column chromatography (SiO₂, 0-4% MeOH/CH₂Cl₂). The combined pure fractions were dried with MgSO₄ 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/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3270.8 (amide N-H) 2943.8 (C-H), 2817.0 (C-H), 1729.5 (ester C=O), 1682.0 (amide C=O), 1613.5 (quinolone C=O)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 8.56 (s, 1 H, *ortho* to C(=O)OCH₃), 8.06 (d, $J = 13.3$ Hz, 1 H, *ortho* to F), 8.02 (br s, 1 H, NH), 7.34 (t, $J = 1.7$ Hz, 1 H, *ortho* to OCH₃ and *ortho* to NH), 7.25 (d, $J = 7.0$ Hz, 1 H, *meta* to F), 7.20 (t, $J = 8.2$ Hz, 1 H, *meta* to OCH₃ and *meta* to NH), 6.98 (dd, $J = 7.8, 1.7$ Hz, 1 H, *para* to OCH₃), 6.65 (dd, $J = 8.2, 2.1$ Hz, 1 H, *para* to NH), 3.93 (s, 3 H, C(=O)OCH₃), 3.80 (s, 3 H, aromatic OCH₃), 3.42 (tt, $J = 6.8, 3.7$ Hz, 1 H, NCH(CH₂)₂), 3.31 (br t, $J = 4.3$ Hz, 4 H, C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.73 (br t, $J = 4.5$ Hz, 4 H, C(=O)CH₂CH₂CH₂N(CH₂)CH₂), 2.58 (t, $J = 6.5$ Hz, 2 H, C(=O)CH₂CH₂CH₂N), 2.48 (t, $J = 6.8$ Hz, 2 H, C(=O)CH₂CH₂CH₂N), 2.00 (quin, $J = 6.8$ Hz, 2 H, C(=O)CH₂CH₂CH₂N), 1.29 - 1.36 (m, 2 H, NCH(CHH)₂), 1.11 - 1.17 (m, 2 H, NCH(CHH)₂)

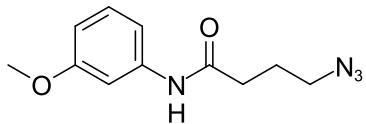
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 173.1 (C(=O)CC(=O)OCH₃), 170.9 (NHC(=O)), 166.3 (C(=O)OCH₃), 160.1 (*ipso* to OCH₃), 153.3 (d, $J = 250.1$ Hz, *ipso* to F), 148.4 (C=CC(=O)OCH₃), 144.1 (d, $J = 10.1$ Hz, *ipso* to piperazine), 139.4 (*ipso* to NH), 138.0 (*para* to F), 129.6 (*meta* to NH and *meta* to OCH₃), 123.3 (d, $J = 6.4$ Hz, *para* to piperazine), 113.4 (d, $J = 23.3$ Hz, *ortho* to C=O and *ortho* to F), 111.8 (*para* to OCH₃), 110.0 (CC(=O)OCH₃), 109.8 (*para* to NH), 105.5 (*ortho* to OCH₃ and *ortho* to NH), 105.0 (*meta* to C=O and *meta* to F), 57.0 (CH₂CH₂CH₂N), 55.3 (aromatic OCH₃), 52.6 (CH₂CH₂CH₂N(CH₂)CH₂), 52.1 (C(=O)OCH₃), 49.2 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 35.2 (CH₂CH₂CH₂N), 34.6 (NCH(CH₂)₂), 21.7 (CH₂CH₂CH₂N), 8.2 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -123.5 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 537.2500, [M+H]⁺ found, [C₂₉H₃₄FN₄O₅]⁺ requires 537.2513

The compound has not been reported previously.

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



4-Bromo-*N*-(3-methoxyphenyl)butanamide **164** (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 7 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto SiO₂ and purified by column chromatography using a Combiflash (SiO₂, 0-100% EtOAc/P.E.). The combined pure fractions were dried with MgSO₄ 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) $\nu_{max} / \text{cm}^{-1} = 3298.3$ (N-H), 2094.7 (azide), 1661.7 (amide C=O)

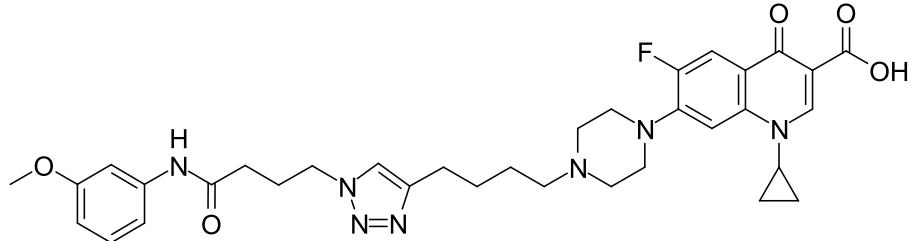
¹H NMR (400 MHz, MeOD) $\delta / \text{ppm} = 8.63$ (br s, 1 H, NH), 7.26 (t, $J = 2.3$ Hz, 1 H, *ortho* to OCH₃ and *ortho* to NH), 7.15 (t, $J = 8.1$ Hz, 1 H, *meta* to OCH₃ and *meta* to NH), 7.01 (dd, $J = 7.8, 1.6$ Hz, 1 H, *para* to OCH₃), 6.63 (dd, $J = 8.2, 1.9$ Hz, 1 H, *para* to NH), 3.69 (s, 3 H, CH₃), 3.28 (t, $J = 6.7$ Hz, 2 H, CH₂N₃), 2.39 (t, $J = 7.4$ Hz, 2 H, C(=O)CH₂), 1.91 (quin, $J = 7.0$ Hz, 2 H, C(=O)CH₂CH₂)

¹³C NMR (101 MHz, MeOD) $\delta / \text{ppm} = 170.8$ (C(=O)), 159.6 (*ipso* to OCH₃), 138.9 (*ipso* to NH), 129.2 (*meta* to OCH₃ and *meta* to NH), 112.3 (*para* to OCH₃), 109.5 (*para* to NH), 106.0 (*ortho* to OCH₃ and *ortho* to NH), 54.8 (CH₃), 50.4 (CH₂N₃), 33.6 (C(=O)CH₂), 24.4 (C(=O)CH₂CH₂)

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)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 167



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 CH₂Cl₂ (10 ml), and the mixture was degassed by bubbling through

N_2 . A solution of CuSO_4 and THPTA ($58.5 \mu\text{l}$, $5.85 \mu\text{mol}$, 0.1 eq. 100 mM , aq.) was added, followed by a solution of sodium ascorbate ($117 \mu\text{l}$, $11.7 \mu\text{mol}$, 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_2Cl_2 (15 ml), and the aqueous layer was extracted a further four times with CH_2Cl_2 ($4 \times 15 \text{ ml}$). The combined organic layers were dried with MgSO_4 , dry-loaded onto SiO_2 and purified by column chromatography (SiO_2 , 0-10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$). 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, $2.9 \mu\text{mol}$, 5%).

TLC $R_f = 0.22$ (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 2922.8$ (C-H), 2849.5 (C-H), 1725.8 (carboxylic acid C=O), 1684.7 (amide C=O), 1624.5 (quinolone C=O), 1612.2 (triazole)

$^1\text{H NMR}$ (400 MHz, DMSO d₆) $\delta / \text{ppm} = 15.23$ (br s, 1 H, $\text{C}(=\text{O})\text{OH}$), 9.89 (s, 1 H, NH), 8.66 (s, 1 H, *ortho* to $\text{C}(=\text{O})\text{OH}$), 7.90 (d, $J = 13.4 \text{ Hz}$, 1 H, *ortho* to F), 7.88 (s, 1 H, $\text{CH}=\text{CCH}_2$), 7.55 (d, $J = 7.6 \text{ Hz}$, 1 H, *meta* to F), 7.27 (t, $J = 2.1 \text{ Hz}$, 1 H, *ortho* to C=O and *ortho* to F), 7.16 (t, $J = 8.1 \text{ Hz}$, 1 H, *meta* to OCH_3 and *meta* to NH), 7.08 (d, $J = 7.8 \text{ Hz}$, 1 H, *para* to OCH_3), 6.59 (ddd, $J = 8.1, 2.4, 0.7 \text{ Hz}$, 1 H, *para* to NH), 4.36 (t, $J = 6.9 \text{ Hz}$, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 3.81 (tt, $J = 6.7, 4.0 \text{ Hz}$, 1 H, $\text{NCH}(\text{CH}_2)_2$), 3.70 (s, 3 H, CH_3), 3.28 - 3.32 (m, 4 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 2.64 (t, $J = 7.5 \text{ Hz}$, 2 H, $\text{CH}=\text{CCH}_2$), 2.56 (m, $J = 4.2, 4.2 \text{ Hz}$, 4 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$), 2.38 (t, $J = 7.3 \text{ Hz}$, 2 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.30 (t, $J = 7.4 \text{ Hz}$, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.10 (quin, $J = 7.1 \text{ Hz}$, 2 H, $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.64 (quin, $J = 7.5 \text{ Hz}$, 2 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.51 (quin, $J = 7.2 \text{ Hz}$, 2 H, $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.27 - 1.33 (m, 2 H, $\text{NCH}(\text{CHH})_2$), 1.15 - 1.20 (m, 2 H, $\text{NCH}(\text{CHH})_2$)

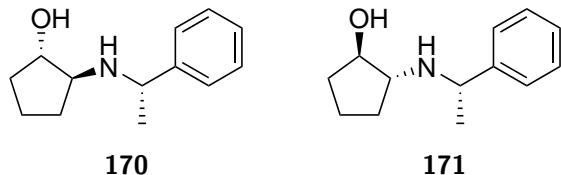
$^{13}\text{C NMR}$ (101 MHz, DMSO d₆) $\delta / \text{ppm} = 176.3$ ($\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$), 170.1 ($\text{NHC}(=\text{O})$), 165.9 ($\text{C}(=\text{O})\text{OH}$), 159.4 (*ipso* to OCH_3), 153.0 (d, $J = 248.6 \text{ Hz}$, *ipso* to F), 148.0 ($\text{CH}=\text{CCH}_2$), 146.9 ($\text{C}=\text{CC}(=\text{O})\text{OH}$), 145.2 (d, $J = 10.7 \text{ Hz}$, *ipso* to piperazine), 140.3 (*para* to F), 139.2 (*ipso* to NH), 129.4 (*meta* to OCH_3 and *meta* to NH), 121.7 ($\text{CH}=\text{CCH}_2$), 118.5 (d, $J = 7.5 \text{ Hz}$, *para* to piperazine), 111.3 (*para* to OCH_3), 110.9 (d, $J = 22.4 \text{ Hz}$, *ortho* to C=O and *ortho* to F), 108.4 (*para* to NH), 106.7 ($\text{CC}(=\text{O})\text{OH}$), 106.3 (*meta* to C=O and *meta* to F), 104.8 (*ortho* to OCH_3 and *ortho* to NH), 57.3 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 54.9 (CH_3), 52.4 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$), 49.5 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 49.4 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$), 48.7 ($\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 35.8 ($\text{NCH}(\text{CH}_2)_2$), 32.9 ($\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 26.8 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 25.7 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 25.5 ($\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 24.9 ($\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 7.6 ($\text{NCH}(\text{CH}_2)_2$)

$^{19}\text{F NMR}$ (376.45 MHz, DMSO d₆) $\delta / \text{ppm} = -121.5$ (s, ciprofloxacin F)

HRMS (ESI⁺) $m/z / \text{Da} = 646.3159$, $[\text{M}+\text{H}]^+$ found, $[\text{C}_{34}\text{H}_{41}\text{FN}_7\text{O}_5]^+$ 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%).

(*1S,2S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 170

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

mp $T / ^\circ\text{C} = 66\text{-}71.5$ (hexane, EtOAc, TEA)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3150.0$ (br, O-H), 2950.9 (C-H), 2868.2 (C-H)

¹H NMR (400 MHz, CDCl₃) $\delta / \text{ppm} = 7.28\text{ - }7.34$ (m, 4 H, *ortho* and *meta* to CHCH₃), 7.20 - 7.26 (m, 1 H, *para* to CHCH₃), 3.86 (q, $J = 6.6$ Hz, 1 H, CHCH₃), 3.85 (q, $J = 6.6$ Hz, 1 H, CHOH), 2.83 (td, $J = 7.6, 5.7$ Hz, 1 H, CHNH), 1.85 - 1.97 (m, 1 H, CHHCHOH), 1.77 (dtd, $J = 12.9, 7.9, 4.9$ Hz, 1 H, CHHCHNH), 1.55 - 1.68 (m, 2 H, CH₂CH₂CHOH), 1.47 - 1.55 (m, 1 H, CHHCHOH), 1.36 (d, $J = 6.6$ Hz, 3 H, CH₃), 1.12 (dq, $J = 12.7, 8.1$ Hz, 1 H, CHHCHNH)

¹³C NMR (101 MHz, CDCl₃) $\delta / \text{ppm} = 145.61$ (*ipso* to CHCH₃), 128.08 (*meta* to CHCH₃), 126.61 (*para* to CHCH₃), 126.33 (*ortho* to CHCH₃), 77.43 (CHOH), 64.45 (CHNH), 56.62 (CHCH₃), 32.01 (CH₂CHOH), 30.56 (CH₂CHNH), 23.30 (CH₃), 20.06 (CH₂CH₂CHOH)

HRMS (ESI⁺) $m/z / \text{Da} = 206.1553$, [M+H]⁺ found, [C₁₃H₂₀NO]⁺ requires 206.1545

$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -23.9$, lit. = -22.1 ($c / \text{g}(100 \text{ ml})^{-1} = 0.96$, 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) $\nu_{max} / \text{cm}^{-1} = 3300.0$ (br, O-H), 2959.7 (C-H), 2870.1 (C-H)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 7.28 - 7.38 (m, 4 H, *ortho* and *meta* to CHCH₃), 7.21 - 7.28 (m, 1 H, *para* to CHCH₃), 3.83 (q, *J* = 6.6 Hz, 1 H, CHCH₃), 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, CH₂CHNH), 1.90 (quin, *J* = 6.9 Hz, 1 H, CH₂CHOH), 1.56 - 1.68 (m, CH₂CH₂CHOH), 1.43 (dq, *J* = 12.5, 8.0 Hz, 1 H, CH₂CHOH), 1.37 (d, *J* = 6.6 Hz, 3 H, CH₃), 1.25 - 1.36 (m, 1 H, CH₂CHNH)

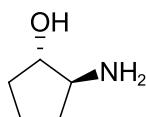
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 144.75 (*ipso* to CHCH₃), 128.26 (*meta* to CHCH₃), 126.72 (*para* to CHCH₃), 126.30 (*ortho* to CHCH₃), 77.65 (CHOH), 63.38 (CHNH), 56.20 (CHCH₃), 31.74 (CH₂CHOH), 29.22 (CH₂CHNH), 24.58 (CH₃), 19.57 (CH₂CH₂CHOH)

HRMS (ESI⁺) *m/z* / Da = 206.1554, [M+H]⁺ found, [C₁₃H₂₀NO]⁺ requires 206.1545

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -92.8, lit. = -76.8 (c / g(100 ml)⁻¹ = 1.19, MeOH)

The compounds have been synthesised previously,^{219,220} but NMR data were not published. The enantiomers of both compounds have also been synthesised previously, and the ¹H NMR data for these are consistent with the the above data.²²¹

9.49 (1*S*,2*S*)-2-Aminocyclopentan-1-ol 172



(1*S*,2*S*)-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, 100%).

TLC *R_f* = 0.10 (10% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3300.0 (O-H), 2969.2 (C-H), 2872.7 (C-H)

¹H NMR (400 MHz, MeOD) δ / ppm = 3.77 (ddd, *J* = 6.6, 6.2, 5.6, 1 H, CHOH), 3.00 (td, *J* = 7.4, 5.6 Hz, 1 H, CHNH₂), 2.00 (dtd, *J* = 13.0, 7.7, 5.6 Hz, 1 H, CHHCHNH₂), 1.97 (ddt, *J* = 13.0, 8.7, 6.4 Hz, 1 H, CHHCHOH), 1.64 - 1.77 (m, 2 H, CH₂CH₂CHOH), 1.53 (ddt, *J* = 13.0, 9.5, 6.2 Hz, 1 H, CHHCHOH), 1.37 (ddt, *J* = 12.8, 8.5, 7.7 Hz, 1 H, CHHCHNH₂)

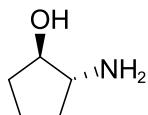
¹³C NMR (101 MHz, MeOD) δ / ppm = 80.6 (CHOH), 60.7 (CHNH₂), 33.2 (CH₂CHOH), 32.2 (CH₂CHNH₂), 21.2 (CH₂CH₂CHOH)

HRMS (ESI⁺) *m/z* / Da = 102.0915, [M+H]⁺ found, [C₅H₁₂NO]⁺ requires 102.0913

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = 33.4, lit. = 29.7 (c / g(100 ml)⁻¹ = 0.5, EtOH)

The data are consistent with the literature.^{220,240}

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



(1*R*,2*R*)-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, 100%).

TLC $R_f = 0.10$ (10% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3300.0 (br, O-H), 2958.3 (C-H), 2871.5 (C-H)

¹H NMR (400 MHz, MeOD) δ / ppm = 3.77 (ddd, J = 6.6, 6.2, 5.6, 1 H, CH_{OH}), 3.00 (td, J = 7.3, 5.6 Hz, 1 H, CH_{NH₂}), 2.00 (dtd, J = 13.0, 7.7, 5.6 Hz, 1 H, CH₂CH_{NH₂}), 1.97 (ddt, J = 13.0, 8.7, 6.6 Hz, 1 H, CH₂CHOH), 1.63 - 1.77 (m, 2 H, CH₂CH₂CHOH), 1.53 (ddt, J = 13.0, 9.5, 6.2 Hz, 1 H, CH₂CHOH), 1.37 (ddt, J = 13.0, 8.3, 7.8 Hz, 1 H, CH₂CH_{NH₂})

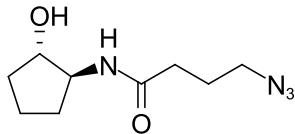
¹³C NMR (101 MHz, MeOD) δ / ppm = 80.7 (CHOH), 60.8 (CH_{NH₂}), 33.2 (CH₂CHOH), 32.1 (CH₂CH_{NH₂}), 21.2 (CH₂CH₂CHOH)

HRMS (ESI⁺) m/z / Da = 102.0917, [M+H]⁺ found, [C₅H₁₂NO]⁺ requires 102.0913

$[\alpha]_D^{20}$ / °10⁻¹cm²g⁻¹ = -30.9, lit. = -32.9 (c / g(100 ml)⁻¹ = 1.5, EtOH)

The data are consistent with the literature.^{220,240}

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



4-Chloro-N-((1*S*,2*S*)-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*-PrOH/CHCl₃ (5 ml). The aqueous layer was extracted again with 10% *i*-PrOH/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 $R_f = 0.35$ (EtOAc)

IR (neat) ν_{max} / cm⁻¹ = 3286.7 (N-H and O-H), 2957.6 (C-H), 2930.6 (C-H), 2860.7 (C-H), 2094.7 (azide), 1642.2 (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, CHOH), 3.83 (tdd, J = 8.5, 6.0, 4.6 Hz, 1 H, CHNH), 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)

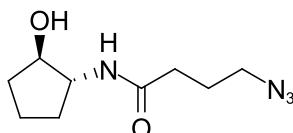
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 79.7 (CHOH), 61.0 (CHNH), 50.7 (CH₂N₃), 32.8 (CH₂C=O), 32.6 (CH₂CHOH), 30.5 (CH₂CHNH), 24.7 (CH₂CH₂N₃), 21.3 (CH₂CH₂CHOH)

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.

9.52 4-Azido-N-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide 177



4-Chloro-N-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **194** (200 mg, 0.972 mmol, 1 eq.) and NaN₃ (126 mg, 1.94 mmol, 2 eq.) were stirred in acetonitrile (4 ml) at 50 °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/CHCl₃ (20 ml). The aqueous layer was extracted again with 10% *i*-PrOH/CHCl₃ (3×20 ml) and the combined organic fractions were dried with MgSO₄ 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 / °C = 56.0-59.5 (*i*-PrOH, CHCl₃)

IR (neat) ν_{max} / cm⁻¹ = 3279.9 (N-H and O-H), 2965.6 (C-H), 2875.4 (C-H), 2094.6 (azide), 1636.8 (amide C=O)

¹H NMR (400 MHz, CDCl₃) δ / 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, CHOH), 3.75 (tdd, J = 8.4, 6.6, 4.4 Hz, 1 H, CHNH), 3.28 (t, J = 6.6 Hz, 2 H, CH₂N₃), 2.23 (t, J = 7.3 Hz, 2 H, CH₂C=O), 2.04 (dtd, J = 13.0, 8.0, 4.9 Hz, 1 H, CHHCHNH), 1.92 (dtd, J = 13.0, 7.6, 5.8 Hz, 1 H, CHHCHOH), 1.84 (quin, J = 7.0 Hz, 2 H, CH₂CH₂N₃), 1.59 - 1.77 (m, 2 H, CH₂CH₂CHOH), 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)

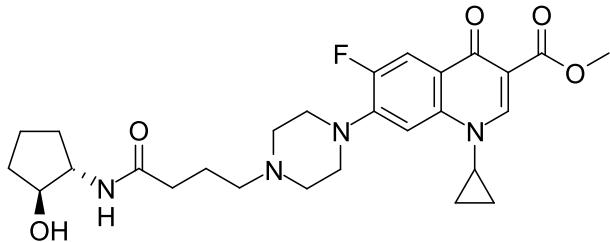
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 78.8 (CHOH), 59.9 (CHNH), 50.5 (CH₂N₃), 32.5 (CH₂C=O), 32.0 (CH₂CHOH), 29.5 (CH₂CHNH), 24.6 (CH₂CH₂N₃), 20.7 (CH₂CH₂CHOH)

HRMS (ESI⁺) m/z / Da = 235.1174, [M+Na]⁺ found, [C₉H₁₆N₄NaO₂]⁺ requires 235.1171

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -10.2 (c / g(100 ml)⁻¹ = 0.5, MeOH)

The compound has not been reported previously.

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



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 μ mol, 1 eq.), (1*S*,2*S*)-2-aminocyclopentan-1-ol **172** (19.5 mg, 193 μ mol, 2 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (29.7 mg, 155 μ mol, 1.6 eq.), 1-hydroxybenzotriazole (25.8 mg, 191 μ mol, 2 eq.) and DIPEA (33.3 μ l, 24.7 mg, 191 μ mol, 2 eq.) were dissolved in DMF (2 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 (5-50% acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between $NaHCO_3$ (aq., sat., 5 ml) and CH_2Cl_2 (5 ml). The organic layer was removed and the aqueous layer was extracted twice more with CH_2Cl_2 (2×5 ml). The combined organic fractions were dried with $MgSO_4$ and evaporated under reduced pressure. **178** was obtained as a white amorphous solid (26.9 mg, 52.3 μ mol, 55%).

TLC $R_f = 0.38$ (30% MeOH/ CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 2937.7 (C-H), 1721.4 (ester C=O), 1620.5 (amide C=O and quinolone C=O)

1H NMR (500 MHz, DMSO d₆) δ / 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, CHNH), 7.43 (d, J = 7.6 Hz, 1 H, *meta* to F), 4.73 (br s, 1 H, CHO_H), 3.77 - 3.81 (m, 1 H, CHO_H), 3.74 - 3.77 (m, 1 H, CHNH), 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(CH₂)CH₂), 2.10 (t, J = 7.4 Hz, 2 H, CH₂CH₂CH₂N(CH₂)CH₂), 1.92 (dddd, 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(CH₂)CH₂), 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)

^{13}C NMR (126 MHz, DMSO d₆) δ / ppm = 171.9 (NHC(=O)CH₂), 171.5 (C(=O)CC(=O)OCH₃), 165.0 (C(=O)OCH₃), 152.6 (d, J = 247.4 Hz, *ipso* to F), 148.2 (C=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 (CHO_H), 57.6 (CHNH), 57.2 (CH₂CH₂CH₂N), 52.4 (CH₂CH₂CH₂N(CH₂)CH₂), 51.3 (CH₃), 49.6 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.6 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 34.7 (NCH(CH₂)₂), 33.2 (C(=O)CH₂), 32.2 (CH₂CHOH), 29.5 (CH₂CH NH), 22.5 (C(=O)CH₂CH₂), 20.6 (CH₂CH₂CHOH), 7.5 (NCH(CH₂)₂)

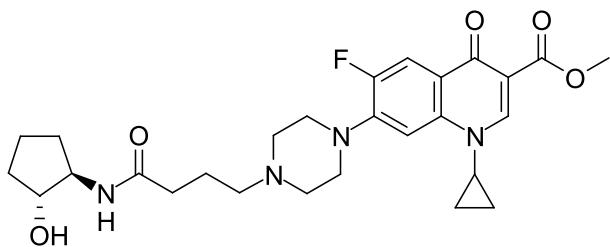
^{19}F NMR (376.45 MHz, MeOD) δ / ppm = -125.5

HRMS (ESI⁺) m/z / Da = 515.2667, [M+H]⁺ found, [C₂₇H₃₆FN₄O₅]⁺ requires 515.2670

$[\alpha]_D^{20} / {}^\circ 10^{-1}\text{cm}^2\text{g}^{-1} = 8.0$ ($c / \text{g}(100 \text{ ml})^{-1} = 0.05$, MeOH)

The compound has not been reported previously.

9.54 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amin o)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 179



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.), (1*R*,2*R*)-2-aminocyclopentan-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 N₂ 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 NaHCO₃ (aq., sat., 10 ml) and CH₂Cl₂ (10 ml). The organic layer was removed and the aqueous layer was extracted twice more with CH₂Cl₂ (2×10 ml). The combined organic fractions were dried with MgSO₄ 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) $\nu_{max} / \text{cm}^{-1} = 2972.9$ (C-H), 2901.5 (C-H), 1728.4 (ester C=O), 1656.3 (amide C=O), 1612.9 (quinolone C=O)

¹H NMR (400 MHz, DMSO d₆) $\delta / \text{ppm} = 8.44$ (s, 1 H, *ortho* to C(=O)OCH₃), 7.75 (d, $J = 13.5$ Hz, 1 H, *ortho* to F), 7.70 (d, $J = 7.2$ Hz, 1 H, CHNH), 7.43 (d, $J = 7.5$ Hz, 1 H, *meta* to F), 4.74 (d, $J = 4.0$ Hz, 1 H, CHO_H), 3.78 - 3.82 (m, 1 H, CHO_H), 3.74 - 3.78 (m, 1 H, CHNH), 3.74 (s, 3 H, CH₃), 3.65 (tt, $J = 7.2, 3.9$ Hz, 1 H, NCH(CH₂)₂), 3.25 (t, $J = 4.8$ Hz, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.57 (br s, 4 H, CH₂N(CH₂)CH₂), 2.34 (t, $J = 7.4$ Hz, 2 H, CH₂N(CH₂)CH₂), 2.11 (t, $J = 7.4$ Hz, 2 H, CH₂CH₂CH₂N(CH₂)CH₂), 1.92 (dddd, $J = 13.0, 8.7, 7.3, 6.0$ Hz, 1 H, CHHCHNH), 1.78 (dddd, $J = 12.6, 8.9, 6.3, 6.3$ Hz, 1 H, CHHCHOH), 1.69 (quin, $J = 7.3$ Hz, 2 H, CH₂CH₂N(CH₂)CH₂), 1.54 - 1.65 (m, 2 H, CH₂CH₂CHOH), 1.42 (ddt, $J = 13.1, 8.2, 5.3$ Hz, 1 H, CHHCHOH), 1.32 (dddd, $J = 13.4, 8.5, 6.8, 5.8$ Hz, 1 H, CHHCHNH), 1.21 - 1.29 (m, 2 H, NCH(CHH)₂), 1.07 - 1.13 (m, 2 H, NCH(CHH)₂)

¹³C NMR (101 MHz, DMSO d₆) $\delta / \text{ppm} = 171.9$ (CH₂C(=O)NH), 171.6 (C(=O)CC(=O)OCH₃), 165.0 (C(=O)OCH₃), 152.6 (d, $J = 246.5$ Hz, *ipso* to F), 148.3 (C=CC(=O)OCH₃), 143.9 (d, $J = 10.7$ Hz, *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), 106.2 (*meta* to C=O and *meta* to F), 76.3 (CHOH), 57.6 (CHNH),

57.2 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 52.4 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\underline{\text{CH}_2}$), 51.3 ($\underline{\text{CH}_3}$), 49.6 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\underline{\text{CH}_2})\text{CH}_2\underline{\text{CH}_2}$), 34.8 ($\text{NCH}(\text{CH}_2)_2$), 33.3 ($\text{C}(=\text{O})\underline{\text{CH}_2}$), 32.2 ($\underline{\text{CH}_2}\text{CHOH}$), 29.5 ($\underline{\text{CH}_2}\text{CHNH}$), 22.5 ($\text{C}(=\text{O})\text{CH}_2\underline{\text{CH}_2}$), 20.6 ($\underline{\text{CH}_2}\text{CH}_2\text{CHOH}$), 7.6 ($\text{NCH}(\underline{\text{CH}_2})_2$)

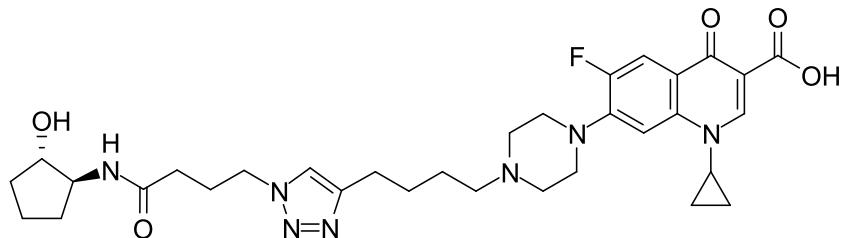
$^{19}\text{F NMR}$ (376.45 MHz, DMSO d₆) δ / ppm = -124.3 (ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 515.2661, [M+H]⁺ found, [C₂₇H₃₆FN₄O₅]⁺ requires 515.2670

$[\alpha]_D^{20}$ / $^{\circ}\text{10}^{-1}\text{cm}^2\text{g}^{-1}$ = -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-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-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 μmol , 4 eq.) and 4-azido-*N*-(*(1S,2S*)-2-hydroxycyclopentyl)butanamide **176** (11.0 mg, 51.8 μmol , 1 eq.) were dissolved in 10% water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (156 μl , 15.6 μmol , 0.3 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (312 μl , 31.2 μmol , 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/CHCl₃ (10 ml) were added, then the organic layer was separated and 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. **180** was obtained as a white amorphous solid (7.2 mg, 11.5 μmol , 22%).

IR (neat) ν_{max} / cm⁻¹ = 2954.9 (C-H), 2917.9 (C-H), 2850.2 (C-H), 1722.1 (carboxylic acid C=O), 1647.3 (amide C=O), 1626.7 (quinolone C=O) 1611.9 (triazole)

$^1\text{H NMR}$ (400 MHz, DMSO d₆) δ / 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=CCH₂), 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, CH₂NCH=C), 3.82 (tt, J = 6.5, 4.3 Hz, 1 H, NCH(CH₂)₂), 3.69 - 3.79 (m, 2 H, CH₂OH and CH₂NH), 3.30 - 3.34 (m, 6 H, CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.64 (t, J = 7.4 Hz, 2 H, CH=CCH₂), 1.95 - 2.08 (m, 4 H, C(=O)CH₂CH₂), 1.89 (dd, J = 12.8, 8.9, 7.4, 5.8 Hz, 1 H, CHHCHNH), 1.75 (dd, J = 12.7, 9.0, 6.2 Hz, 1 H, CHHCHOH), 1.48 - 1.68 (m, 6 H, CH=CCH₂CH₂CH₂ and CH₂CH₂CHOH), 1.40 (dd, J = 13.0, 8.3, 5.3 Hz, 1 H, CHHCHOH), 1.28 - 1.35 (m, 2 H, NCH(CHH)₂), 1.24 - 1.31 (m, 1 H, CHHCHNH), 1.15 - 1.21 (m, 2

H, NCH(CHH)₂)

¹³C NMR (101 MHz, DMSO d₆) δ / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (NHC(=O)CH₂), 166.0 (C(=O)OH), 153.0 (d, *J* = 249.6 Hz, *ipso* to F), 148.1 (C=CC(=O)OH), 146.7 (CH=CCH₂), 145.2 (d, *J* = 8.3 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 (NCH=CCH₂), 118.7 (*para* to piperazine), 111.0 (d, *J* = 23.2 Hz, *ortho* to C=O and *ortho* to F), 106.7 (CC(=O)OH), 106.5 (*meta* to C=O and *meta* to F), 76.2 (CHOH), 57.5 (CHNH), 57.4 (br s, CH=CCH₂CH₂CH₂CH₂N), 52.3 (br s, CH=CCH₂CH₂CH₂CH₂N(CH₂)CH₂), 49.3 (br s, CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 48.8 (CH₂NCH=CCH₂), 35.9 (NCH(CH₂)₂), 32.2 (CH₂CHOH), 32.0 (C(=O)CH₂), 29.4 (CH₂CHNH), 26.7 (CH=CCH₂CH₂), 26.0 (C(=O)CH₂CH₂), 25.5 (CH=CCH₂CH₂CH₂), 24.9 (CH=CCH₂), 20.5 (CH₂CH₂CHOH), 7.6 (NCH(CH₂)₂)

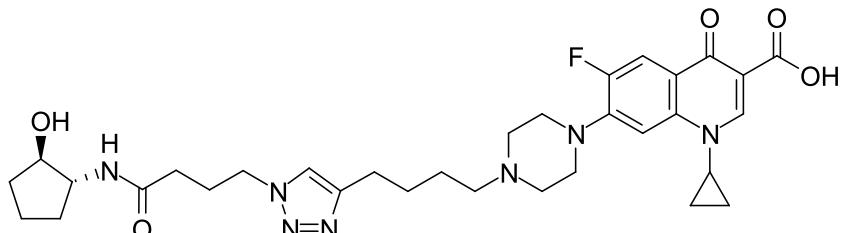
¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -121.5

HRMS (ESI⁺) *m/z* / Da = 624.3298, [M+H]⁺ found, [C₃₂H₄₃FN₇O₅]⁺ requires 624.3310

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -25.0 (c / g(100 ml)⁻¹ = 0.08, MeOH)

The compound has not been reported previously.

9.56 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 181



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*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **177** (22.0 mg, 104 μmol, 1 eq.) were dissolved in 10% water/*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 separated and the aqueous layer was extracted again with CH₂Cl₂ (4×30 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) ν_{max} / cm⁻¹ = 2967.0 (C-H), 2902.2 (C-H), 1721.4 (carboxylic acid C=O), 1646.7 (amide C=O), 1627.0 (quinolone C=O), 1613.0 (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=CCH₂), 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, CHOH), 4.29 (t, *J* = 6.9 Hz, 2 H, CH₂NCH=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(CH₂CH₂)CH₂CH₂), 2.63 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.56 (br t, *J* = 4.2 Hz, 4 H, CH₂N(CH₂)CH₂), 2.37 (t, *J* = 7.3 Hz, 2 H, CH₂N(CH₂)CH₂), 2.03 - 2.06 (m, 2 H, C(=O)CH₂), 1.97 - 2.02 (m, 2 H, C(=O)CH₂CH₂), 1.89 (dd, *J* = 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=CCH₂CH₂CH₂), 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 (NHC(=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=CCH₂), 145.2 (d, *J* = 8.7 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 (NCH=CCH₂), 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 CC(=O)OH), 76.2 (CHOH), 57.6 (CHNH), 57.4 (CH=CCH₂CH₂CH₂CH₂N), 52.5 (CH=CCH₂CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (d, *J* = 4.4 Hz, CH=CCH₂CH₂CH₂CH₂N(CH₂CH₂)CH₂), 48.8 (CH₂NCH=CCH₂), 35.8 (NCH(CH₂)₂), 32.2 (CH₂CHOH), 32.0 (C(=O)CH₂), 29.5 (CH₂CHNH), 26.9 (CH=CCH₂CH₂), 26.0 (C(=O)CH₂CH₂), 25.8 (CH=CCH₂CH₂CH₂), 25.0 (CH=CCH₂), 20.5 (CH₂CH₂CHOH), 7.6 (NCH(CH₂)₂)

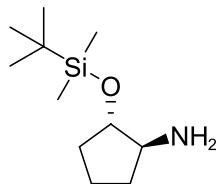
¹⁹F NMR (376.45 MHz, MeOD) δ / ppm = -122.1 (s, ciprofloxacin F)

HRMS (ESI⁺) *m/z* / Da = 624.3314, [M+H]⁺ found, [C₃₂H₄₃FN₇O₅]⁺ requires 624.3310

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = -3.6 (c / g(100 ml)⁻¹ = 0.0833, MeOH)

The compound has not been reported previously.

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



(1*S*,2*S*)-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, 2.28 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 *R_f* = 0.23 (10% MeOH/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 2953.6 (C-H), 2931.1 (C-H), 2888.4 (C-H), 2858.8 (C-H), 1625.2 (N-H bend)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 4.13 (q, *J* = 5.8 Hz, 1 H, CHOSi), 3.31 (td, *J* = 7.1, 5.2 Hz, 1 H,

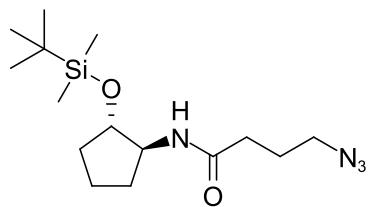
CHNH_2), 2.09 - 2.19 (m, 1 H, CHHCHNH_2), 1.97 (ddq, $J = 8.8, 7.0, 6.0$ Hz, 1 H, CHHCHOSi), 1.74 - 1.86 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CHOSi}$), 1.64 - 1.74 (m, 1 H, CHHCHOSi), 1.58 (ddt, $J = 13.2, 9.1, 6.0$ Hz, 1 H, CHHCHNH_2), 0.88 (s, 9 H, $\text{C}(\text{CH}_3)_3$), 0.09 (s, 3 H, SiCH_3), 0.07 (s, 3 H, SiCH_3)

^{13}C NMR (101 MHz, CDCl_3) δ / ppm = 76.3 (CHOSi), 59.7 (CHNH), 32.2 (CH_2CHOSi), 26.8 (CH_2CHNH_2), 25.6 ($\text{C}(\text{CH}_3)_3$), 19.7 ($\text{CH}_2\text{CH}_2\text{CHOSi}$), 17.7 ($\text{C}(\text{CH}_3)_3$), -4.8 (SiCH_3), -5.2 (SiCH_3)

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

$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = 40.0$ ($c / \text{g}(100 \text{ ml})^{-1} = 0.05$, MeOH) The compound has not been reported previously.

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



(1*S*,2*S*)-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_2Cl_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_2Cl_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_2Cl_2)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3287.9$ (N-H), 2953.4 (C-H), 2933.2 (C-H), 2882.7 (C-H), 2857.1 (C-H), 2094.9 (azide), 1639.4 (amide C=O)

^1H NMR (400 MHz, CDCl_3) δ / ppm = 5.35 (d, $J = 5.1$ Hz, 1 H, NH), 3.97 - 4.01 (m, 1 H, CHOSi), 3.93 - 3.98 (m, 1 H, CHNH), 3.35 (t, $J = 6.6$ Hz, 2 H, CH_2N_3), 2.24 (t, $J = 7.0$ Hz, 2 H, $\text{CH}_2\text{C=O}$), 2.09 - 2.19 (m, 1 H, CHHCHNH), 1.89 - 1.97 (quin, $J = 6.8$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{N}_3$), 1.74 - 1.84 (m, 2 H, CHHCHOSi and $\text{CHHCH}_2\text{CHOSi}$), 1.60 - 1.70 (m, 1 H, $\text{CHHCH}_2\text{CHOSi}$), 1.51 - 1.61 (m, 1 H, CHHCHOSi), 1.31 - 1.39 (m, 1 H, CHHCHNH), 0.87 (s, 9 H, $\text{C}(\text{CH}_3)_3$), 0.08 (s, 3 H, SiCH_3), 0.06 (s, 3 H, SiCH_3)

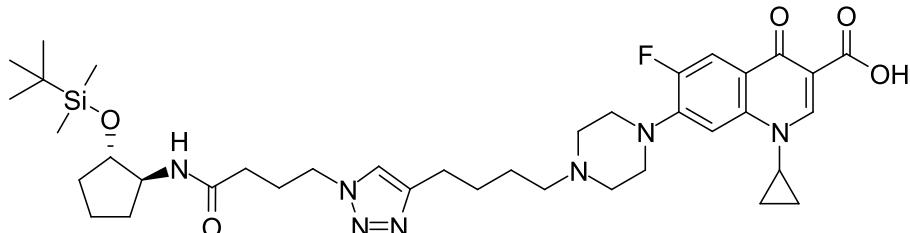
^{13}C NMR (101 MHz, CDCl_3) δ / ppm = 171.17 (C=O), 77.80 (CHOSi), 58.36 (CHNH), 50.77 (CH_2N_3), 33.29 ($\text{CH}_2\text{C=O}$), 32.57 (CH_2CHOSi), 29.36 (CH_2CHNH), 25.72 ($\text{C}(\text{CH}_3)_3$), 24.77 ($\text{CH}_2\text{CH}_2\text{N}_3$), 20.40 ($\text{CH}_2\text{CH}_2\text{CHO}$ Si), 17.95 ($\text{C}(\text{CH}_3)_3$), -4.75 (SiCH_3)

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

$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = 12.4$ ($c / \text{g}(100 \text{ ml})^{-1} = 0.5$, MeOH)

The compound has not been reported previously.

9.59 7-(4-(1-(4-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-ylbutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190



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 μ mol, 1 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide **186** (33.9 mg, 104 μ mol, 1 eq.) were dissolved in 10% water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (104 μ l, 10.4 μ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208 μ l, 20.8 μ mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 16 h, then solvent was removed under reduced pressure. The residue was partitioned between water (10 ml) and CH₂Cl₂ (10 ml), the organic layer was separated and the aqueous layer was extracted again with CH₂Cl₂ (10 ml). The combined organic layers were dried with MgSO₄ and evaporated under reduced pressure. **190** was obtained as a clear amorphous solid (67.1 mg, 90.9 μ mol, 87%).

IR (neat) ν_{max} / cm⁻¹ = 2951.3 (C-H), 2929.2 (C-H), 2855.5 (C-H), 1741.0 (carboxylic acid C=O), 1640.3 (amide C=O), 1626.6 (quinolone C=O), 1612.3 (triazole)

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=CCH₂), 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=C), 3.96 - 4.02 (m, 1 H, CHOSi), 3.90 - 3.96 (m, 1 H, CHNH), 3.55 (tt, *J* = 6.7, 4.0 Hz, 1 H, NCH(CH₂)₂), 3.34 (br t, *J* = 5.0 Hz, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.71 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.66 (br s, 4 H, CH₂N(CH₂)CH₂), 2.46 (t, *J* = 7.3 Hz, 2 H, CH₂N(CH₂)CH₂), 2.03 - 2.22 (m, 5 H, CHHCHNH, C(=O)CH₂ and C(=O)CH₂CH₂), 1.65 - 1.83 (m, 4 H, CHHCHOSi, CHHCH₂CHOSi and NCH=CCH₂CH₂), 1.47 - 1.65 (m, 4 H, CHHCHOSi, CHHCH₂CHOSi and NCH=CCH₂CH₂CH₂), 1.33 - 1.41 (m, 3 H, CHHCHNH and NCH(CHH)₂), 1.14 - 1.20 (m, 2 H, NCH(CHH)₂), 0.82 (s, 9 H, C(CH₃)₃), 0.03 (s, 3 H, SiCH₃), 0.01 (s, 3 H, SiCH₃)

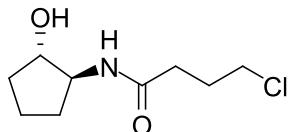
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 176.9 (C(=O)CC(=O)OH), 170.9 (CH₂C(=O)NH), 166.9 (C(=O)OH), 153.5 (d, *J* = 251.4 Hz, *ipso* to F), 147.9 (CH=CCH₂), 147.2 (C=CC(=O)OH), 145.8 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.0 (*para* to F), 120.9 (NCH=CCH₂), 119.4 (d, *J* = 7.8 Hz, *para* to piperazine), 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=CCH₂CH₂CH₂N), 52.6 (CH=CCH₂CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (d, *J* = 6.1 Hz, CH=CCH₂CH₂CH₂N(CH₂)CH₂CH₂), 48.9 (d, *J* = 3.5 Hz, CH₂NCH=CCH₂), 35.3 (NCH(CH₂)₂), 32.6 (C(=O)CH₂), 32.6 (CH₂CHOSi), 29.3 (CH₂CHNH), 27.2 (CH=CCH₂CH₂), 26.0 - 26.3 (C(=O)CH₂CH₂ and CH=CCH₂CH₂CH₂), 25.6 (C(CH₃)₃), 25.4 (CH=CCH₂), 20.4 (CH₂CH₂CHOSi), 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

[α]_D²⁰ / °10⁻¹cm²g⁻¹ = 4.5 (*c* / g(100 ml)⁻¹ = 0.2, MeOH)

The compound has not been reported previously.

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



(*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 *R_f* = 0.35 (EtOAc)

¹H 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, 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* = 7.0 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)

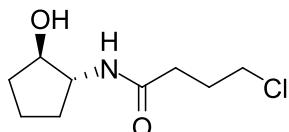
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH₂Cl), 32.8 (CH₂C=O), 32.4 (CH₂CHOH), 30.2 (CH₂CHNH), 28.0 (CH₂CH₂Cl), 21.2 (CH₂CH₂CHOH)

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



(*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). 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. **194** was obtained as a white amorphous solid (651 mg, 3.16 mmol, 64%).

TLC $R_f = 0.35$ (EtOAc)

IR (neat) ν_{max} / cm⁻¹ = 3277.6 (N-H and O-H), 2962.2 (C-H), 2876.0 (C-H), 1636.3 (amide C=O)

¹H NMR (400 MHz, CDCl₃) δ / 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, CHCHNH and CH₂CH₂Cl), 1.96 - 2.04 (m, 1 H, CHHCHOH), 1.74 - 1.85 (m, 1 H, CHHCH₂CHOH), 1.58 - 1.73 (m, 2 H, CHHCH₂CHOH and CHHCHOH), 1.43 (dq, $J = 12.7, 8.3$ Hz, 1 H, CHHCHNH)

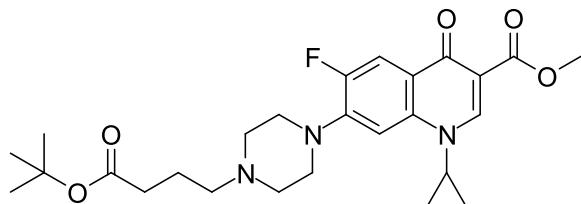
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH₂Cl), 32.8 (CH₂C=O), 32.4 (CH₂CHOH), 30.1 (CH₂CHNH), 28.0 (CH₂CH₂Cl), 21.1 (CH₂CH₂CHOH)

HRMS (ESI⁺) m/z / Da = 228.0787, [M+Na]⁺ found, [C₉H₁₆ClNNaO₂]⁺ requires 228.0762

$[\alpha]_D^{20}$ / °10⁻¹cm²g⁻¹ = -13.0 (c / g(100 ml)⁻¹ = 0.5, MeOH)

The compound has not been reported previously.

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



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) ν_{max} / cm⁻¹ = 2961.6 (C-H), 2830.5 (C-H), 1732.2 (*t*-Bu ester C=O) 1717.2 (ciprofloxacin ester C=O), 1620.6 (quinolone C=O)

¹H NMR (400 MHz, CDCl₃) δ / 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)₂), 3.22 (t, *J* = 4.3 Hz, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.63 (t, *J* = 4.4 Hz, 4 H, CH₂N(CH₂)CH₂), 2.41 (t, *J* = 7.3 Hz, 2 H, CH₂N(CH₂)CH₂), 2.25 (t, *J* = 7.4 Hz, 2 H, CH₂CH₂CH₂N(CH₂)CH₂), 1.78 (quin, *J* = 7.3 Hz, 2 H, CH₂CH₂N(CH₂)CH₂), 1.41 (s, 9 H, C((CH₂)₃)₃), 1.24 (m, 2 H, NCH(CHH)₂), 1.09 (m, 2 H, NCH(CHH)₂)

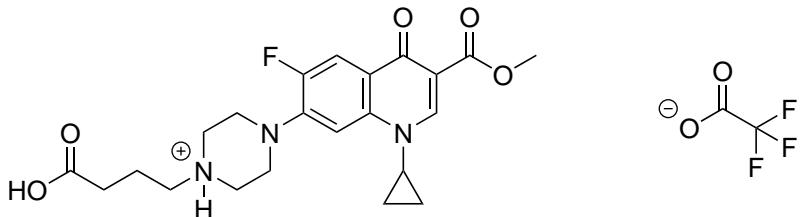
¹³C NMR (101 MHz, CDCl₃) δ / ppm = 172.7 (C(=O)CC(=O)OCH₃), 172.6 (C(=O)OC(CH₃)₃), 165.9 (C(=O)OCH₃), 153.1 (d, *J* = 249.7 Hz, *ipso* to F), 148.1 (C=CC(=O)OCH₃), 144.3 (d, *J* = 10.4 Hz, *ipso* to piperazine), 137.7 (*para* to F), 122.5 (d, *J* = 6.9 Hz, *para* to piperazine) 112.6 (d, *J* = 22.5 Hz, *ortho* to C=O and *ortho* 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(CH₂)CH₂), 51.7 (CH₃), 49.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 34.4 (NCH(CH₂)₂), 33.2 (C(=O)CH₂), 28.0 (C(CH₃)₃), 22.0 (C(=O)CH₂CH₂), 7.9 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, CDCl₃) δ / ppm = -123.5 (s, ciprofloxacin F)

HRMS (ESI⁺) *m/z* / Da = 488.2562, [M+H]⁺ found, [C₂₆H₃₅FN₃O₅]⁺ 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 μmol) 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 μmol, 96%).

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

IR (neat) ν_{max} / cm⁻¹ = 1722.7 (ciprofloxacin ester C=O), 1699.0 (alkyl carboxylic acid C=O), 1673.3 (TFA C=O), 1614.6 (quinolone C=O)

¹H NMR (400 MHz, DMSO d₆) δ / 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(CH₂)CH₂), 2.36 (t, *J* = 7.1 Hz, 2 H, CH₂CH₂CH₂N(CH₂)CH₂), 1.87 - 1.98 (m, 2 H, CH₂CH₂N(CH₂)CH₂), 1.22 - 1.30 (m, 2 H, NCH(CHH)₂), 1.06 - 1.15 (m, 2 H, NCH(CHH)₂)

¹³C NMR (101 MHz, DMSO d₆) δ / 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),

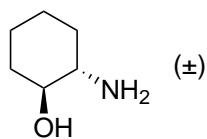
142.3 (d, $J = 10.7$ Hz, *ipso* to piperazine), 138.0 (*para* to F), 122.6 (d, $J = 6.4$ Hz, *para* to piperazine), 117.2 (q, $J = 299.8$ Hz, $\underline{\text{CF}_3}$), 111.9 (d, $J = 22.4$ Hz, *ortho* to C=O and *ortho* to F), 109.1 ($\underline{\text{C}}\text{C}(=\text{O})\text{OCH}_3$), 106.9 (*meta* to C=O and *meta* to F), 55.1 (C(=O)CH₂CH₂ $\underline{\text{CH}_2\text{N}}$), 51.4 ($\underline{\text{CH}_3}$), 50.8 (C(=O)CH₂CH₂CH₂N($\underline{\text{CH}_2}$) $\underline{\text{CH}_2}$), 46.7 (C(=O)CH₂CH₂CH₂N(CH₂ $\underline{\text{CH}_2}$)CH₂ $\underline{\text{CH}_2}$), 46.7 (C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂ $\underline{\text{CH}_2}$), 34.9 (NCH(CH₂)₂), 30.6 (C(=O) $\underline{\text{CH}_2}$), 19.1 (C(=O)CH₂ $\underline{\text{CH}_2}$), 7.6 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, DMSO d₆) δ / ppm = -73.6 (s, CF_3), -124.6 (s, ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 432.1921, [M+H]⁺ found, [C₂₂H₂₇FN₃O₅]⁺ requires 432.1935

The compound has not been reported previously.

9.64 (*trans*)-2-Aminocyclohexan-1-ol 200



Cyclohexene oxide **199** (10 ml, 9.70 g, 98.8 mmol, 1 eq.), NH₃ (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 N₂ 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/CH₂Cl₂)

IR (neat) ν_{max} / cm⁻¹ = 3350.4 (N-H), 3306.2 (br, O-H), 2926.9 (C-H), 2852.6 (C-H)

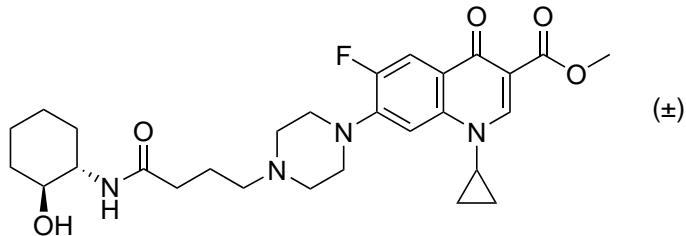
¹H NMR (400 MHz, CDCl₃) δ / ppm = 3.01 (td, $J = 9.4, 4.8$ Hz, 1 H, $\underline{\text{CHOH}}$), 2.80 - 2.92 (m, 2 H, OH and NH₂), 2.35 (ddd, $J = 11.1, 9.1, 4.1$ Hz, 1 H, $\underline{\text{CHNH}_2}$), 1.77 - 1.84 (m, 1 H, $\underline{\text{CHHCHOH}}$), 1.69 - 1.76 (m, 1 H, $\underline{\text{CHHCHNH}_2}$), 1.56 - 1.66 (m, 1 H, $\underline{\text{CHHCH}_2\text{CHOH}}$), 1.45 - 1.56 (m, 1 H, $\underline{\text{CHHCH}_2\text{CHNH}_2}$), 1.07 - 1.19 (m, 3 H, $\underline{\text{CHHCH}_2\text{CHOH}}$, $\underline{\text{CHHCH}_2\text{CHNH}_2}$ and $\underline{\text{CHHCHOH}}$), 0.94 - 1.05 (m, 1 H, $\underline{\text{CHHCHNH}_2}$)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 75.4 ($\underline{\text{CHOH}}$), 56.6 ($\underline{\text{CHN}_2}$), 33.8 ($\underline{\text{CH}_2\text{CHOH}}$ and $\underline{\text{CH}_2\text{CHN}_2}$), 24.7 ($\underline{\text{CH}_2\text{CH}_2\text{CHNH}_2}$), 24.6 ($\underline{\text{CH}_2\text{CH}_2\text{CHOH}}$)

HRMS (ESI⁺) m/z / Da = 116.1070, [M+H]⁺ found, [C₆H₁₄NO]⁺ requires 116.1070

The data are consistent with the literature.²²⁸

9.65 Methyl 1-cyclopropyl-6-fluoro-7-(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-aminocyclohexan-1-ol **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 μ l, 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) ν_{max} / cm⁻¹ = 3302.5 (N-H), 2929.8 (C-H), 2850.6 (C-H), 2832.9 (C-H), 1698.1 (ester C=O), 1646.4 (amide C=O), 1613.8 (quinolone C=O)

¹H NMR (400 MHz, MeOD) δ / 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, CH₂NH), 3.38 (br s, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 3.32 - 3.36 (m, 1 H, CH₂OH), 2.83 (br s, 4 H, CH₂N(CH₂)CH₂), 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, CH₂CHOH), 1.87 - 1.96 (m, 3 H, CH₂CH₂NH and C(=O)CH₂CH₂), 1.72 - 1.77 (m, 1 H, CH₂CH₂CHOH), 1.66 - 1.72 (m, 1 H, CH₂CH₂CHNH), 1.25 - 1.39 (m, 5 H, CH₂CHOH, CH₂CH₂CHOH, CH₂CH₂CHNH and NCH(CH₂)₂), 1.15 - 1.25 (m, 3 H, CH₂CHOH and NCH(CH₂)₂)

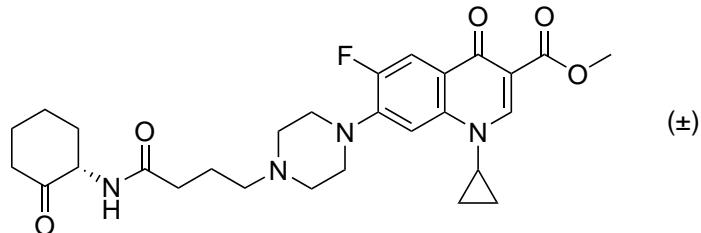
¹³C NMR (101 MHz, MeOD) δ / ppm = 175.8 (CH₂C(=O)NH), 175.3 (C(=O)CC(=O)OCH₃), 166.8 (C(=O)OCH₃), 154.9 (d, J = 248.8 Hz, *ipso* to F), 150.2 (C=CC(=O)OCH₃), 146.1 (d, J = 10.8 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.5 (d, J = 7.5 Hz, *para* to piperazine), 113.2 (d, J = 23.2 Hz, *ortho* to C=O and *ortho* to F), 110.2 (CC(=O)OCH₃), 107.2 (*meta* to C=O and *meta* to F), 74.1 (CHOH), 58.9 (C(=O)CH₂CH₂CH₂N), 56.4 (CH₂NH), 54.0 (C(=O)CH₂CH₂CH₂N(CH₂)CH₂), 52.3 (CH₃), 50.5 (d, J = 5.0 Hz, C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂), 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) δ / 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.

9.66 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 202



Methyl 1-cyclopropyl-6-fluoro-7-(4-((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **201** (5.2 mg, 9.84 μ mol, 1 eq.) and Dess-Martin periodinane (16.4 mg, 38.7 μ mol, 4 eq.) were stirred in CH_2Cl_2 (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 NaHCO_3 (aq., sat., 30 ml) and 10% *i*-PrOH/ CHCl_3 (30 ml) were added. The organic layer was dried with MgSO_4 and evaporated under reduced pressure. **202** was obtained as a white amorphous solid (3.6 mg, 6.8 μ mol, 69%).

TLC $R_f = 0.74$ (30% MeOH/ CH_2Cl_2)

IR (neat) ν_{max} / cm^{-1} = 2921.2 (C-H), 2851.6 (C-H), 1721.4 (ketone C=O), 1698.0 (ester C=O), 1639.3 (amide C=O), 1620.0 (quinolone C=O)

$^1\text{H NMR}$ (400 MHz, DMSO d₆) δ / ppm = 8.45 (s, 1 H, *ortho* to C(=O)OCH₃), 7.87 (d, J = 6.2 Hz, 1 H, NH), 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 (dddd, J = 13.0, 7.6, 6.0, 1.0 Hz, 1 H, CHNH), 3.73 (s, 3 H, CH₃), 3.65 (tt, J = 7.1, 3.9 Hz, 1 H, NCH(CH₂)₂), 3.25 (br s, 4 H, CH₂N(CH₂CH₂)CH₂CH₂), 2.58 (br s, 4 H, CH₂N(CH₂)CH₂), 2.45 - 2.53 (m, 1 H, CHHC(=O)CHNH), 2.36 (br s, 2 H, C(=O)CH₂CH₂CH₂N), 2.26 (dtt, J = 13.4, 2.6, 1.6 Hz, 1 H, CHHC(=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), 2.00 (ddquin, J = 13.2, 6.0, 2.9 Hz, 1 H, CHHCH₂C(=O)), 1.65 - 1.83 (m, 4 H, CH₂CH₂CHNH), 1.41 - 1.56 (m, 2 H, CHHCHNH and CHHCH₂C(=O)), 1.20 - 1.30 (m, 2 H, NCH(CHH)₂), 1.05 - 1.13 (m, 2 H, NCH(CHH)₂)

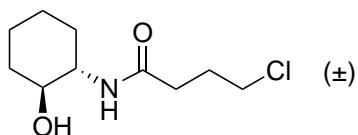
$^{13}\text{C NMR}$ (101 MHz, DMSO d₆) δ / ppm = 207.5 (C(=O)CHNH), 171.7 (C(=O)CC(=O)OCH₃), 171.6 (CH₂C(=O)NH), 165.0 (C(=O)OCH₃), 152.6 (d, J = 247.6 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH₃), 143.9 (br s, *ipso* to piperazine), 138.1 (*para* to F), 121.8 (d, J = 6.4 Hz, *para* to piperazine), 111.5 (d, J = 22.4 Hz, *ortho* to C=O and *ortho* to F), 109.0 (CC(=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(CH₂)CH₂), 51.3 (CH₃), 49.5 (br s, C(=O)CH₂CH₂CH₂N(CH₂CH₂)CH₂), 40.6 (CH₂C(=O)CHNH), 34.8 (NCH(CH₂)₂), 33.9 (CH₂CHNH), 32.9 (C(=O)CH₂CH₂CH₂N), 27.2 (CH₂CH₂C(=O)CHNH), 23.8 (CH₂CH₂CHNH), 22.4 (br s, C(=O)CH₂CH₂CH₂N), 7.6 (NCH(CH₂)₂)

$^{19}\text{F NMR}$ (376.45 MHz, DMSO d₆) δ / ppm = -124.3 (ciprofloxacin F)

HRMS (ESI⁺) m/z / Da = 527.2654, [M+H]⁺ found, [C₂₈H₃₆FN₄O₅]⁺ 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 $R_f = 0.19$ (Et₂O)

mp $T / ^\circ\text{C} = 72.5\text{-}75.7$ (*i*-PrOH, CHCl₃)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3289.9$ (N-H), 3250.0 (O-H), 2927.6 (C-H), 2857.1 (C-H), 1629.2 (amide C=O)

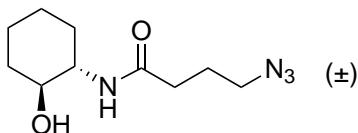
¹H NMR (400 MHz, MeOD) $\delta / \text{ppm} = 3.60$ (t, $J = 6.6$ Hz, 2 H, CH₂Cl), 3.51 - 3.60 (m, 1 H, CH₂NH), 3.28 - 3.39 (m, 1 H, CHOH), 2.37 (td, $J = 7.4, 2.3$ Hz, 2 H, C(=O)CH₂), 2.06 (quin, $J = 7.0$ Hz, 2 H, C(=O)CH₂CH₂), 1.97 - 2.01 (m, 1 H, CHHCHOH), 1.85 - 1.93 (m, 1 H, CHHCHNH), 1.70 - 1.77 (m, 1 H, CHHCH₂CHOH), 1.64 - 1.70 (m, 1 H, CHHCH₂CHNH), 1.24 - 1.35 (m, 3 H, CHHCH₂CHOH, CHHCH₂CHNH and CHHCHOH), 1.13 - 1.25 (m, 1 H, CHHCHNH₂)

¹³C NMR (101 MHz, MeOD) $\delta / \text{ppm} = 175.0$ (C(=O)), 74.1 (CHOH), 56.3 (CHNH), 45.3 (CH₂Cl), 35.6 (CH₂CHOH), 34.5 (C(=O)CH₂), 32.7 (CH₂CHNH), 30.1 (C(=O)CH₂CH₂), 25.8 (CH₂CH₂CHNH), 25.5 (CH₂CH₂CHOH)

HRMS (ESI⁺) $m/z / \text{Da} = 242.0925$, [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 $T / ^\circ\text{C} = 74.5\text{-}75.7$ (*i*-PrOH, CHCl₃)

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3299.0$ (N-H), 3207.8 (O-H), 2944.3 (C-H), 2927.9 (C-H), 2859.2 (C-H), 2089.2 (azide), 1624.0 (amide C=O)

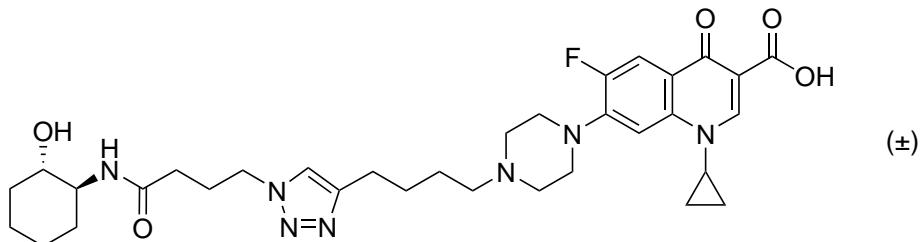
¹H NMR (400 MHz, MeOD) $\delta / \text{ppm} = 7.87$ (d, $J = 7.9$ Hz, 1 H, NH), 5.27 (d, $J = 4.3$ Hz, 1 H, OH), 3.56 (td, $J = 10.5, 4.4$ Hz, 1 H, CHNH), 3.28 - 3.41 (m, 3 H, CHOH and CH₂N₃), 2.30 (td, $J = 7.4, 2.7$ Hz, 2 H, C(=O)CH₂), 1.95 - 2.03 (m, 1 H, CHHCHOH), 1.87 (m, 3 H, C(=O)CH₂CH₂ and CHHCHNH), 1.70 - 1.76 (m, 1 H, CHHCH₂CHOH), 1.63 - 1.70 (m, 1 H, CHHCH₂CHNH), 1.25 - 1.38 (m, 3 H, CHHCH₂CHOH, CHHCH₂CHNH and CHHCHOH), 1.14 - 1.24 (m, 1 H, CHHCHNH₂)

¹³C NMR (101 MHz, MeOD) $\delta / \text{ppm} = 175.1$ (C(=O)), 74.0 (CHOH), 56.3 (CHNH), 52.0 (CH₂N₃), 35.5 (CH₂CHOH), 34.3 (C(=O)CH₂), 32.7 (CH₂CHNH), 26.3 (C(=O)CH₂CH₂), 25.8 (CH₂CH₂CHNH), 25.5 (CH₂CH₂CHOH)

HRMS (ESI⁺) $m/z / \text{Da} = 249.1331$, [M+Na]⁺ found, [C₁₀H₁₈N₄NaO₂]⁺ requires 249.1327

The compound has not been reported previously.

9.69 1-Cyclopropyl-6-fluoro-7-(4-(1-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-ylbutyl)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 μmol , 1 eq.) and 4-azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **204** (22.0 mg, 97.2 μmol , 1 eq.) were dissolved in 10% water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N₂ through it. A solution of CuSO₄ and THPTA (97.2 μl , 9.72 μmol , 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (194 μl , 19.4 μmol , 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/CHCl₃ (50 ml) were added, then the organic layer was separated, dried with MgSO₄ 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 NaHCO₃ (aq., sat., 50 ml) and 10% *i*-PrOH/CHCl₃ (50 ml). The organic layer was dried with MgSO₄ and evaporated under reduced pressure. **205** was obtained as a white amorphous solid (30.3 mg, 47.5 μmol , 49%).

IR (neat) $\nu_{max} / \text{cm}^{-1} = 3345.4$ (N-H), 2927.6 (C-H), 2859.6 (C-H), 2814.7 (C-H), 1727.0 (carboxylic acid C=O), 1641.7 (amide C=O), 1625.8 (quinolone C=O), 1619.0 (triazole)

¹H NMR (400 MHz, DMSO d₆) δ / 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=CCH₂), 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)CH₂CH₂CH₂N), 3.77 - 3.86 (m, 1 H, NCH(CH₂)₂), 3.33 - 3.40 (m, 1 H, CHNH), 3.31 (br t, *J* = 4.8 Hz, 4 H, CH=CCH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 3.14 - 3.24 (m, 1 H, CHOH), 2.63 (t, *J* = 7.4 Hz, 2 H, CH=CCH₂), 2.56 (br t, *J* = 4.6 Hz, 4 H, CH=CCH₂CH₂CH₂N(CH₂)CH₂), 2.38 (t, *J* = 6.9 Hz, 2 H, CH=CCH₂CH₂CH₂CH₂N), 2.04 - 2.08 (m, 2 H, C(=O)CH₂CH₂CH₂N), 1.96 - 2.04 (m, 2 H, C(=O)CH₂CH₂N), 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=CCH₂CH₂CH₂CH₂N), 1.54 - 1.60 (m, 2 H, CHHCH₂OH), 1.51 (quin, *J* = 7.4 Hz, 2 H, CH=CCH₂CH₂CH₂CH₂N), 1.28 - 1.35 (m, 2 H, NCH(CHH)₂), 1.11 - 1.22 (m, 5 H, NCH(CHH)₂, CHHCHOH, CHHCH₂CHOH and CH₂CH₂CHNH), 1.04 - 1.13 (m, 1 H, CHHCHNH)

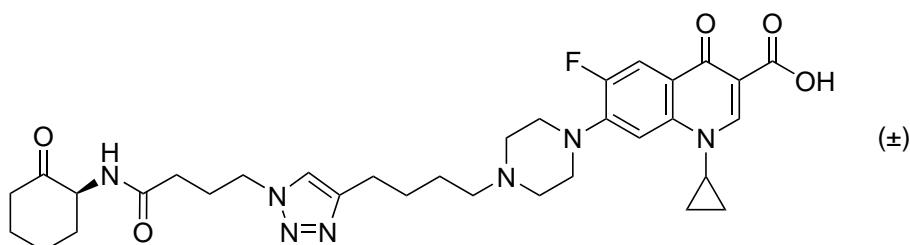
¹³C NMR (101 MHz, DMSO d₆) δ / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (CH₂C(=O)NH), 166.0 (C(=O)OH), 153.1 (d, *J* = 252.1 Hz, *ipso* to F), 148.0 (C=CC(=O)OH), 146.9 (CH=CCH₂), 145.3 (d, *J* = 10.0 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 (NCH=CCH₂), 118.5 (d, *J* = 8.3 Hz, *para* to piperazine), 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=CCH₂CH₂CH₂CH₂N), 54.2 (CHNH), 52.4 (CH=CCH₂CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (CH=CCH₂CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.5 (CH=CCH₂CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 48.8 (C(=O)CH₂CH₂CH₂NCH=C), 35.9 (NCH(CH₂)₂), 34.1 (CH₂CHOH), 32.3 (C(=O)CH₂CH₂CH₂NCH=C), 31.1 (CH₂CHNH), 26.9 (CH=CCH₂CH₂CH₂CH₂N), 26.1 (C(=O)CH₂CH₂CH₂NCH=C), 25.8 (CH=CCH₂CH₂CH₂N), 25.0 (CH=CCH₂CH₂CH₂CH₂N), 24.2 (CH₂CH₂CHNH), 23.8 (CH₂CH₂CHOH), 7.6 (NCH(CH₂)₂)

¹⁹F NMR (376.45 MHz, DMSO d₆) δ / ppm = -121.4 (ciprofloxacin F)

HRMS (ESI⁺) *m/z* / Da = 638.3480, [M+H]⁺ found, [C₃₃H₄₅FN₇O₅]⁺ 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)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 206



1-Cyclopropyl-6-fluoro-7-(4-(4-((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-ylbutyl)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 CH₂Cl₂ (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 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. **206** was obtained as a clear gum (11.7 mg, 18.4 μmol, 78%).

IR (neat) ν_{max} / cm⁻¹ = 2941.2 (C-H), 2859.8 (C-H), 1719.8 (carboxylic acid C=O and ketone C=O), 1656.8 (amide C=O), 1625.6 (quinolone C=O), 1613.5 (triazole)

¹H NMR (500 MHz, DMSO d₆) δ / ppm = 8.65 (s, 1 H, *ortho* to C(=O)OH), 7.94 (d, *J* = 7.7 Hz, 1 H, NH), 7.88 (d, *J* = 13.4 Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH₂), 7.55 (d, *J* = 7.3 Hz, 1 H, *meta* to F), 4.40 (dd, *J* = 12.8, 7.6, 6.1, 1.1 Hz, 1 H), 4.31 (t, *J* = 7.0 Hz, 1 H, C(=O)CH₂CH₂CHHN), 4.31 (t, *J* = 6.9 Hz, 1 H, C(=O)CH₂CH₂CHH₂N), 3.74 - 3.84 (m, 1 H, NCH(CH₂)₂), 3.31 (br. s, 4 H, CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 2.64 (t, *J* = 7.5 Hz, 2 H, CH=CCH₂), 2.56 (br t, *J* = 5.0, 5.0 Hz, 4 H, CH₂CH₂CH₂N(CH₂)CH₂), 2.45 - 2.52 (m, 1 H, CHHC(=O)), 2.38 (t, *J* = 7.1 Hz, 2 H, CH=CCH₂CH₂CH₂CH₂N), 2.25 (dtt, *J* = 13.4, 2.6, 1.6 Hz, 1 H, CHHC(=O)), 2.07 - 2.17 (m, 3 H, C(=O)CH₂CH₂CH₂N and CHHCHNH), 1.96 - 2.05 (m, 3 H, C(=O)CH₂CH₂CH₂N and CHHCH₂C(=O)), 1.68 - 1.81 (m, 2 H, CHHCH₂CHNH), 1.64 (quin, *J* = 7.5 Hz, 2 H, CH=CCH₂CH₂CH₂N), 1.40 - 1.56 (m, 5 H, CHHCH₂C(=O), CHHCHNH and CH=CCH₂CH₂CH₂CH₂N), 1.27 - 1.34 (m, 2 H, NCH(CHH)₂), 1.13 - 1.20 (m, 2 H, NCH(CHH)₂)

¹³C NMR (126 MHz, DMSO d₆) δ / ppm = 207.4 (C(=O)CHNH), 176.3 (C(=O)CC(=O)OH), 170.8 (CH₂C(=O)NH), 166.0 (C(=O)OH), 153.0 (d, *J* = 246.4 Hz, *ipso* to F), 147.9 (C=CC(=O)OH), 146.8 (CH=CCH₂), 145.1 (d, *J* = 10.1 Hz, *ipso* to piperazine), 139.1 (*para* to F), 121.7 (NCH=CCH₂), 118.7 (d, *J* = 6.9 Hz, *para* to piperazine), 110.9 (d, *J* = 23.0 Hz, *ortho* to C=O and *ortho* to F), 106.3 (CC(=O)OH, and *meta* to C=O and *meta* to F), 57.3 (CH=CCH₂CH₂CH₂CH₂N), 57.0 (CHNH), 52.4 (CH₂CH₂CH₂N(CH₂)CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 49.5 (CH₂CH₂CH₂N(CH₂CH₂)CH₂CH₂), 48.7 (C(=O)CH₂CH₂CH₂NCH=C), 40.5 (CH₂C(=O)), 35.8 (NCH(CH₂)₂), 33.7 (CH₂CHNH), 31.8 (C(=O)CH₂CH₂CH₂NCH=C), 27.1 (CH₂CH₂C(=O)), 26.9 (CH=CCH₂CH₂CH₂CH₂N), 26.0 (C(=O)CH₂CH₂CH₂NCH=C), 25.7 (CH=CCH₂CH₂CH₂CH₂N), 24.9 (CH=CCH₂CH₂CH₂CH₂N), 23.8 (CH₂CH₂CHNH), 7.6 (NCH(CH₂)₂)

¹⁹F NMR (376 MHz, DMSO d₆) δ / ppm = -121.7 (s, ciprofloxacin F)

HRMS (ESI⁺) *m/z* / Da = 636.3303, [M+H]⁺ found, [C₃₃H₄₃FN₇O₅]⁺ requires 636.3310

The compound has not been reported previously.

9.71 Biological testing

Compounds were tested against *P. aeruginosa* PAO1¹⁸⁰ and YM64.¹⁸¹ C₄-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 cleavable HSL-Cip 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.^{129, 241} 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

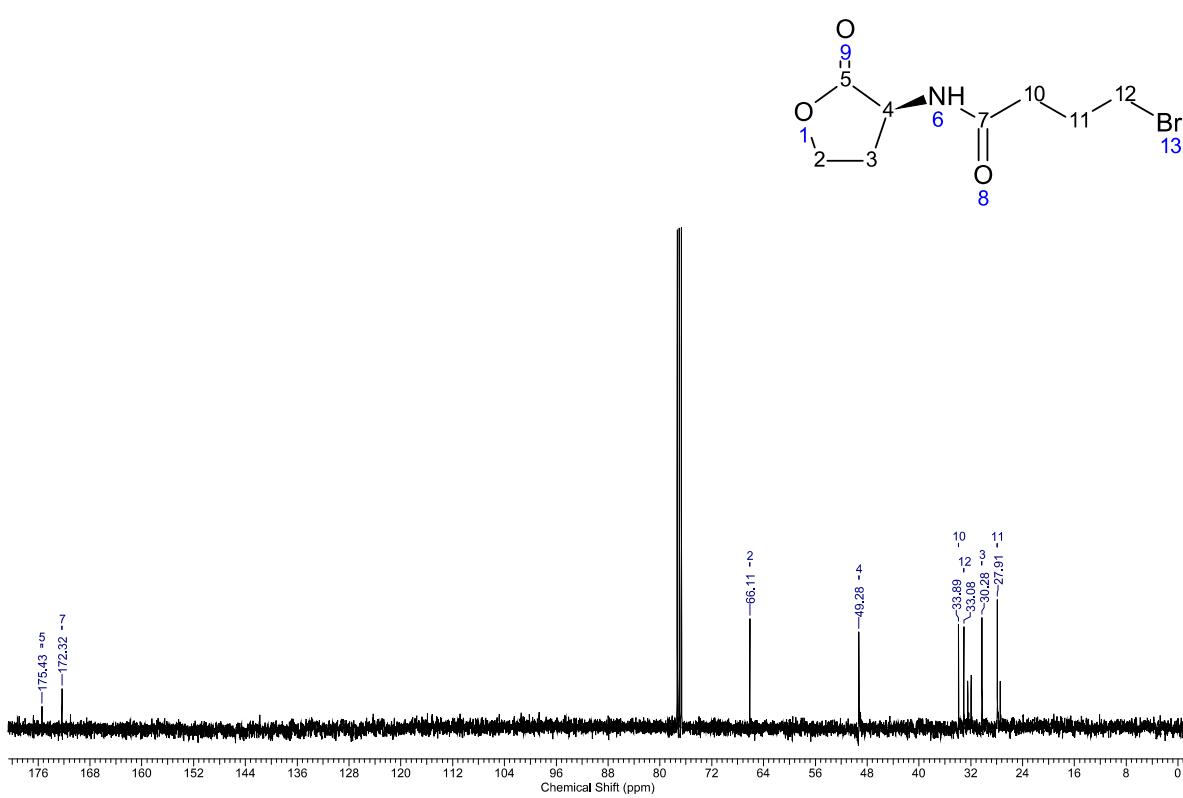
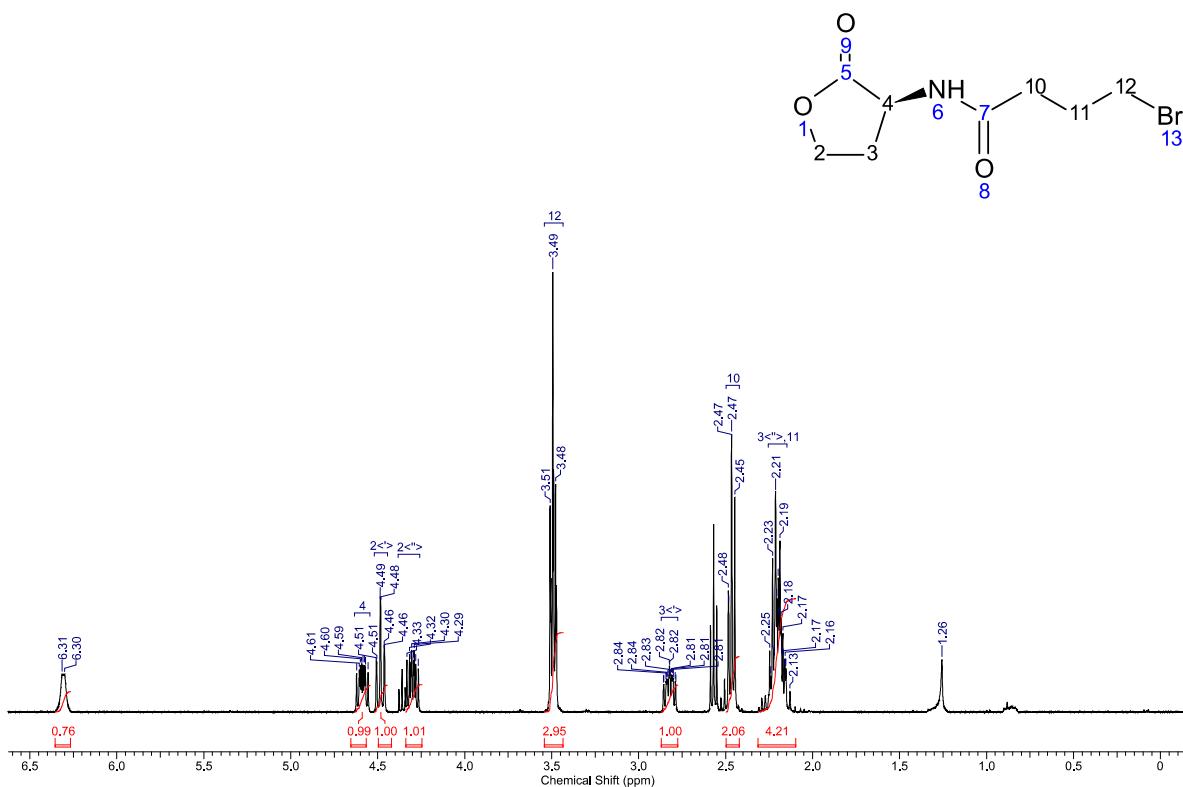
The plates were prepared as in 9.71.1. The plates were shaken at 37 °C and 100 rpm for 24 h followed by quantification of biofilm growth as shown in 9.71.2.

9.71.4 Biofilm dispersal

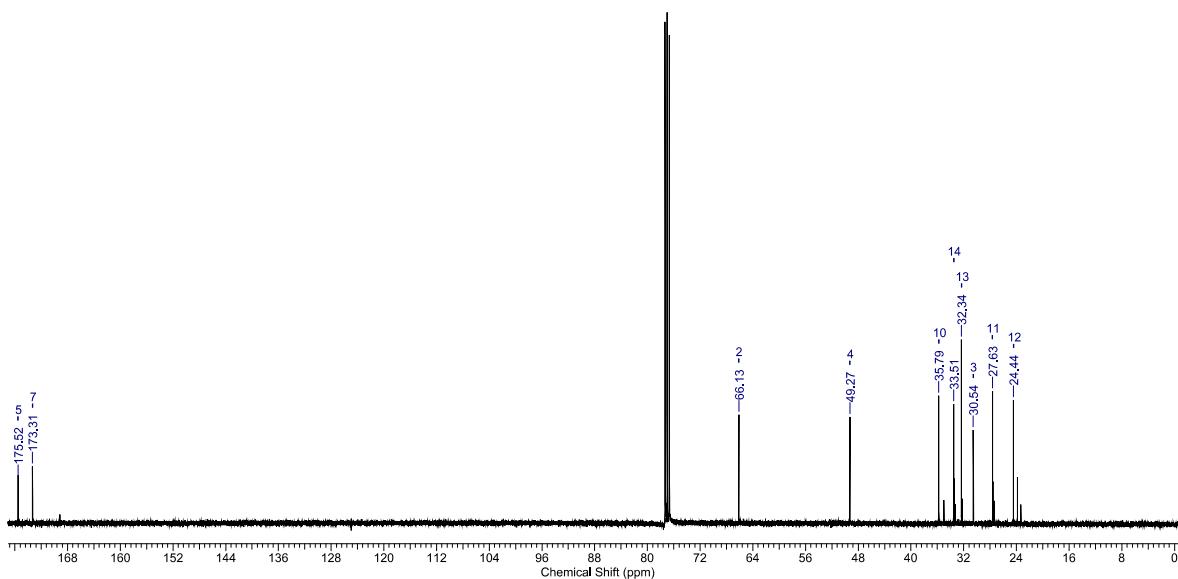
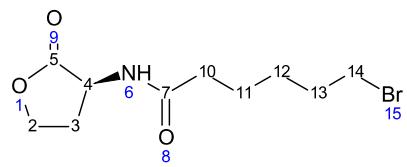
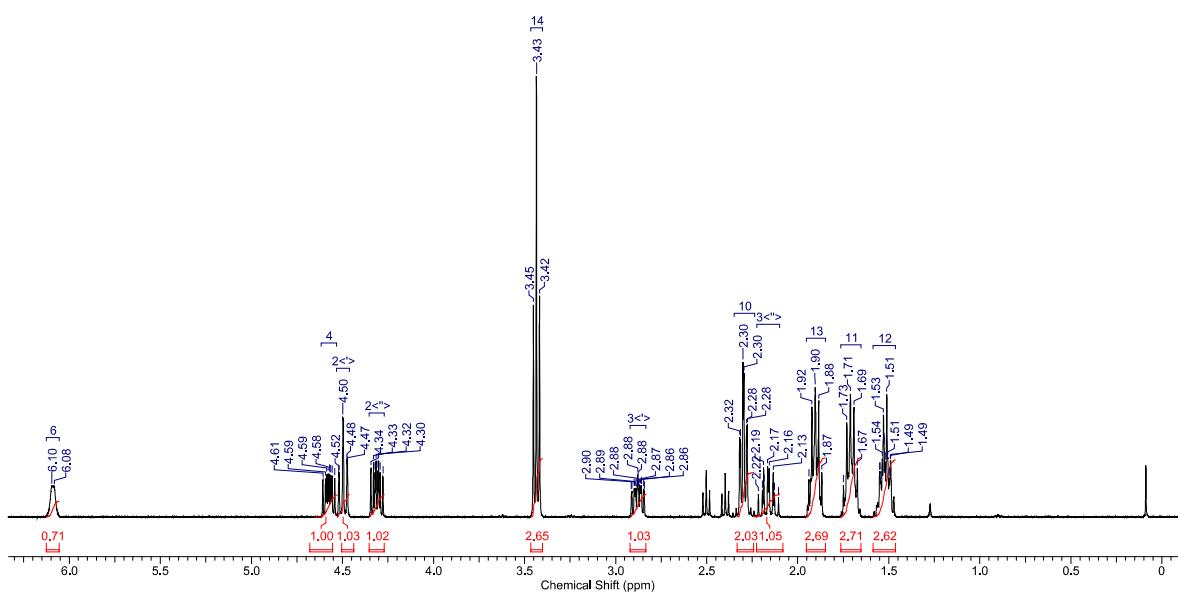
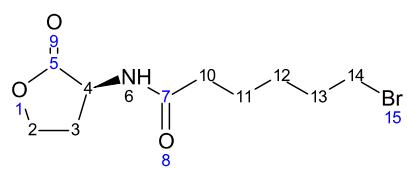
The plates were prepared as in 9.71.1, initially without the addition of compound solutions. The box of plates was shaken at 37 °C and 100 rpm for 24. 1 µl of compound solution in DMSO was then added to each well from master plates and the plates were shaken as above for a further 24 h followed by measurement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

10 NMR spectra

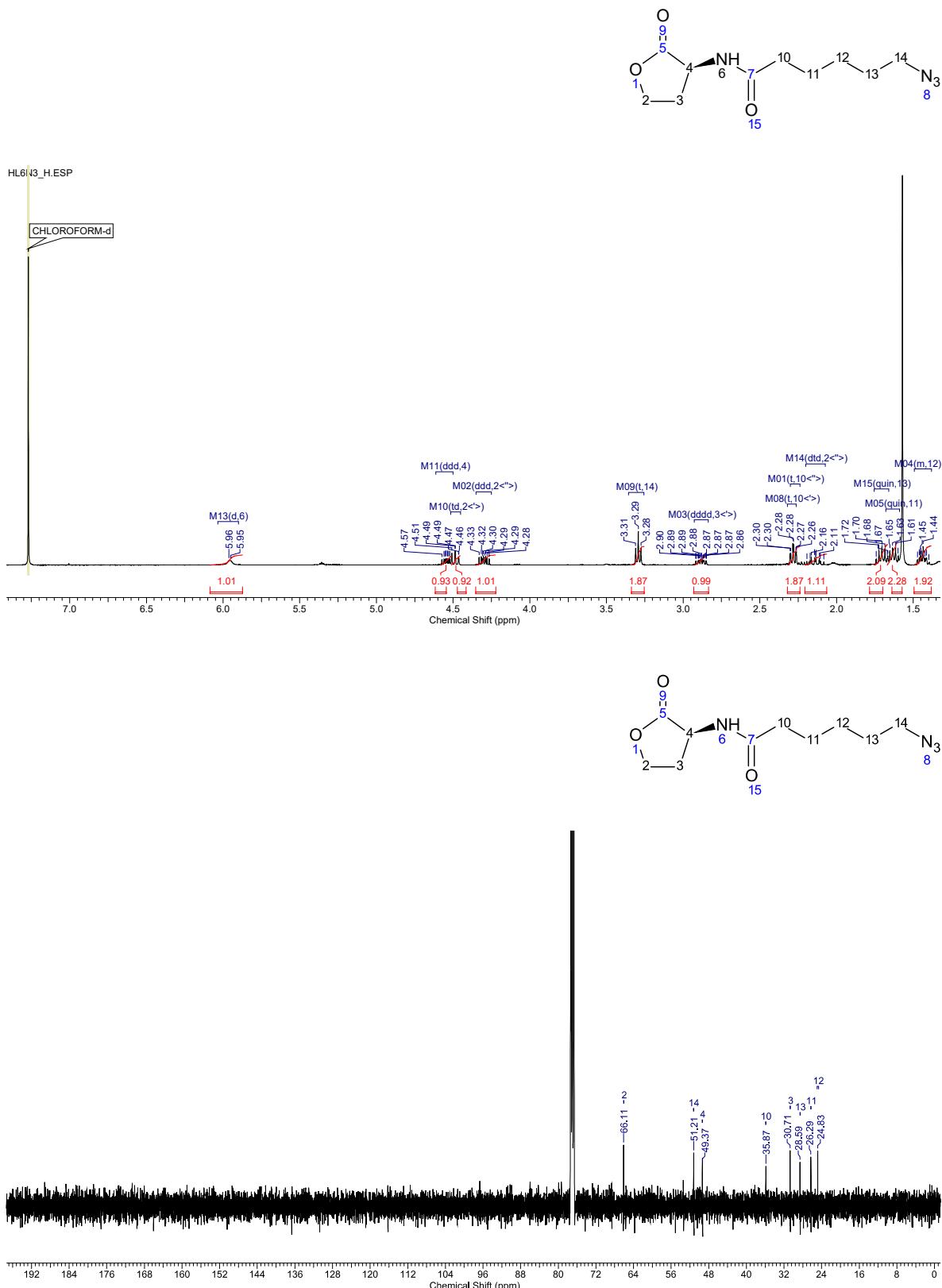
10.1 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 57



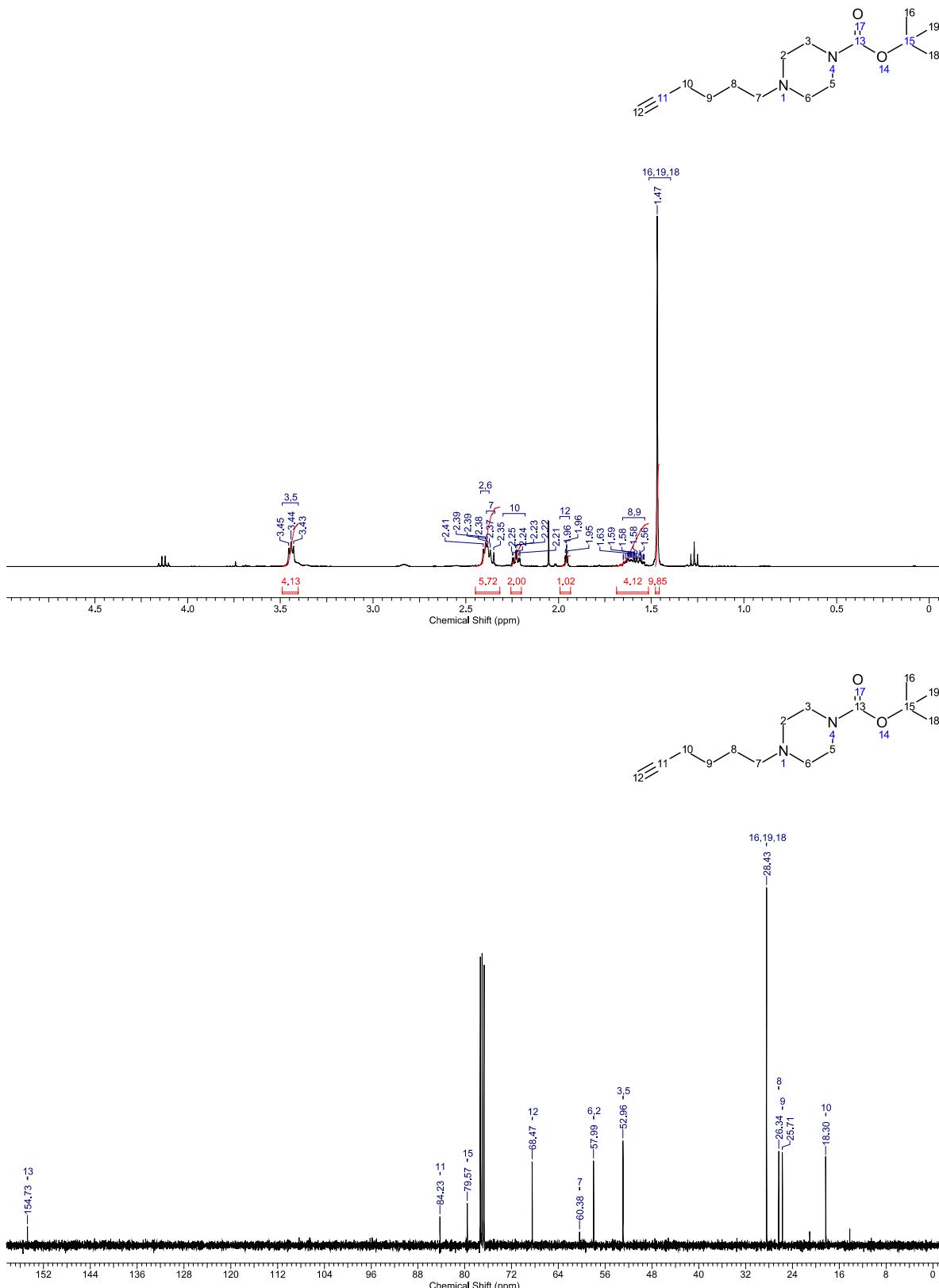
10.2 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 60



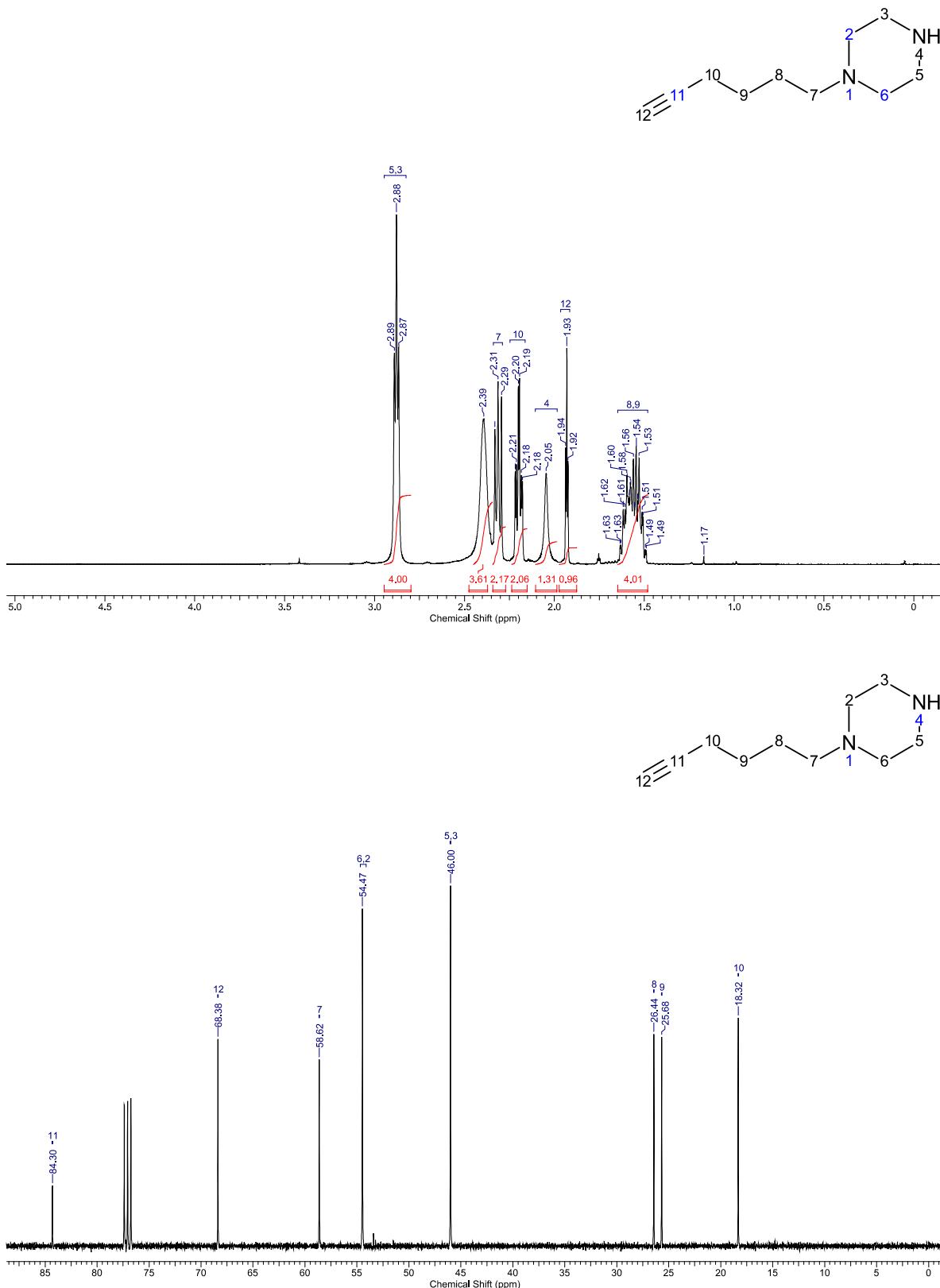
10.3 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 61



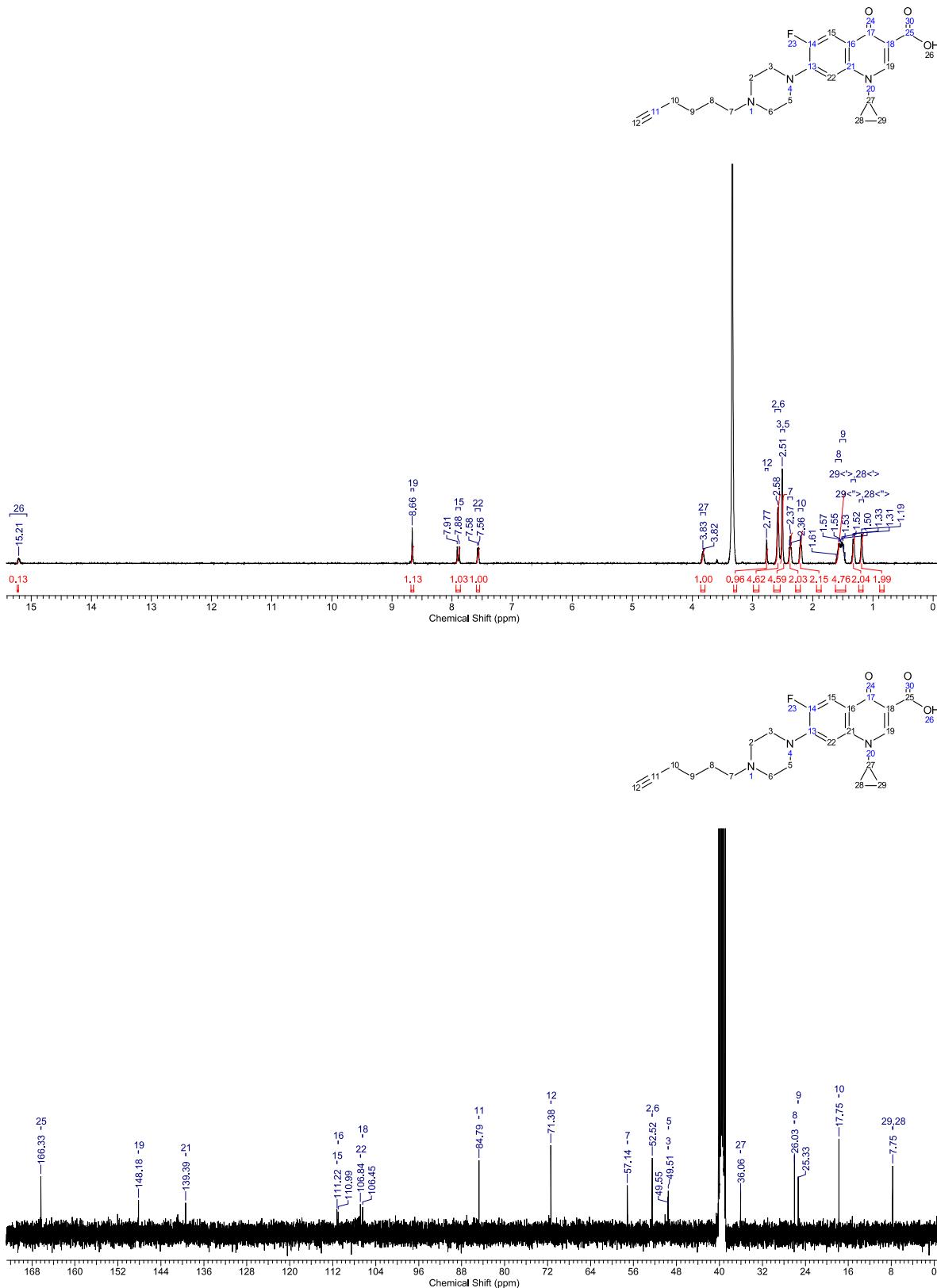
10.4 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 65



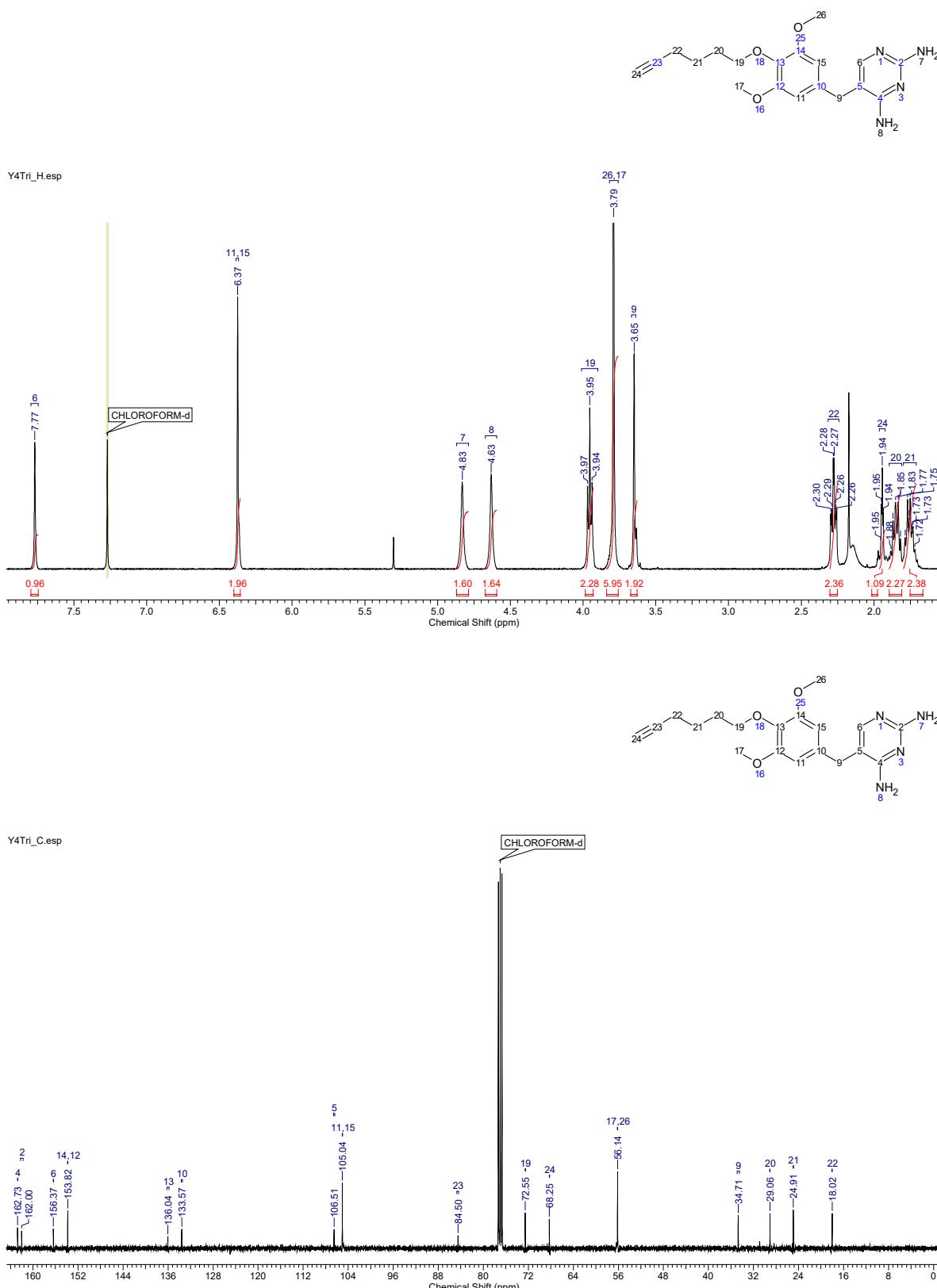
10.5 1-(Hex-5-yn-1-yl)piperazine 66



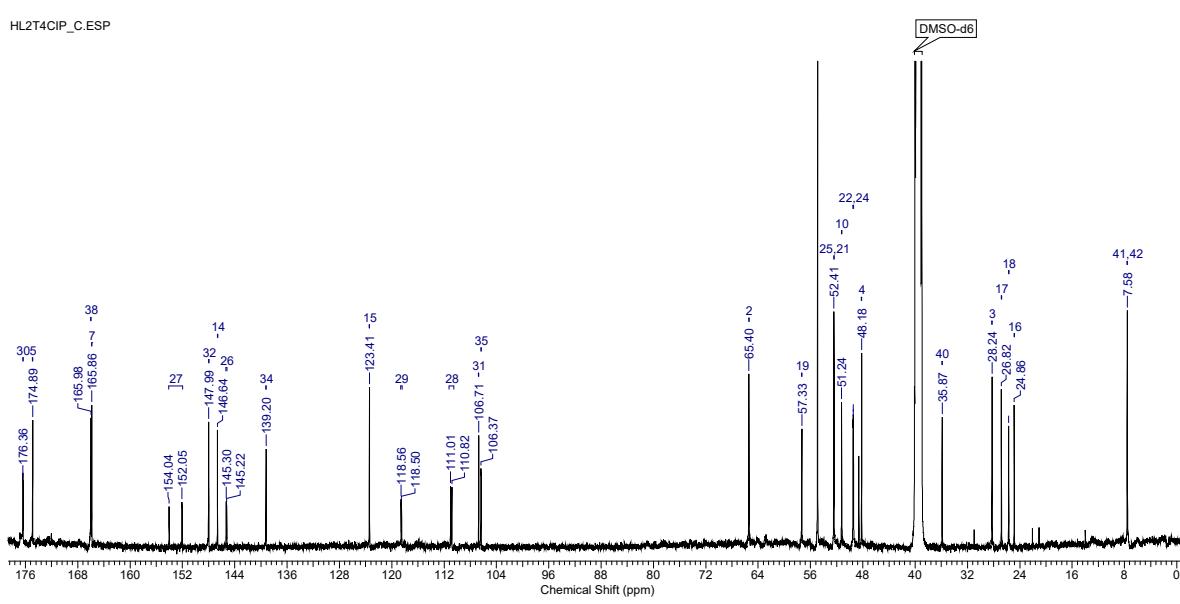
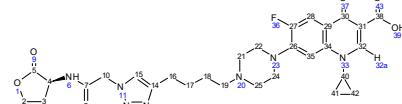
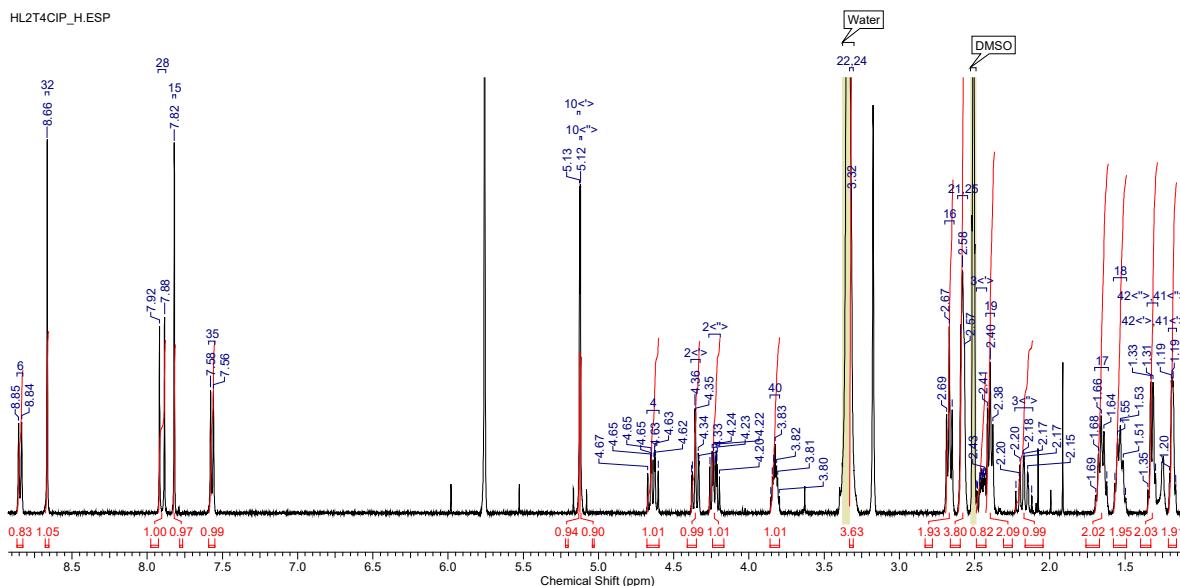
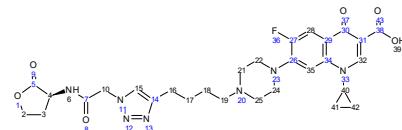
10.6 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 68



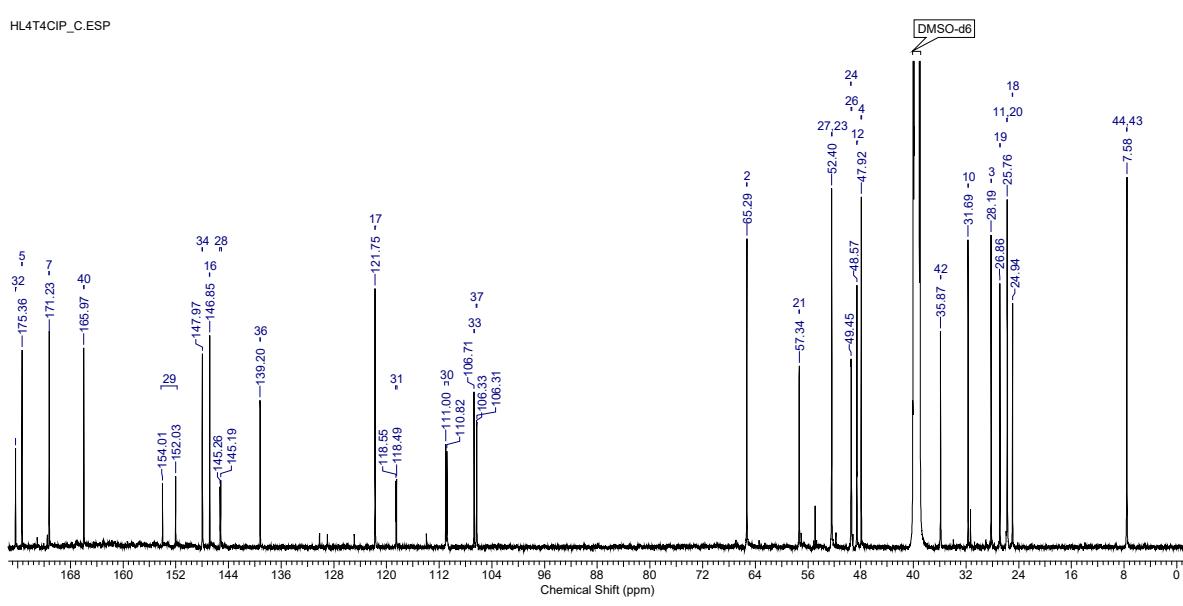
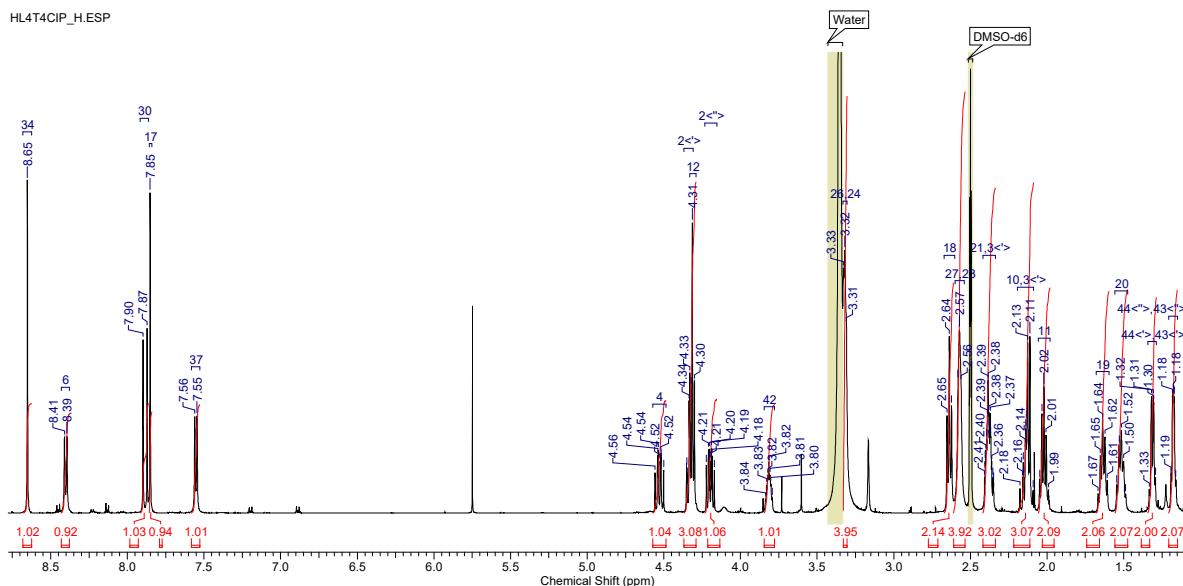
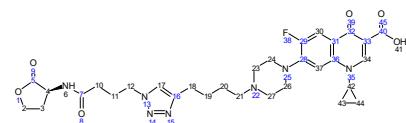
10.7 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71



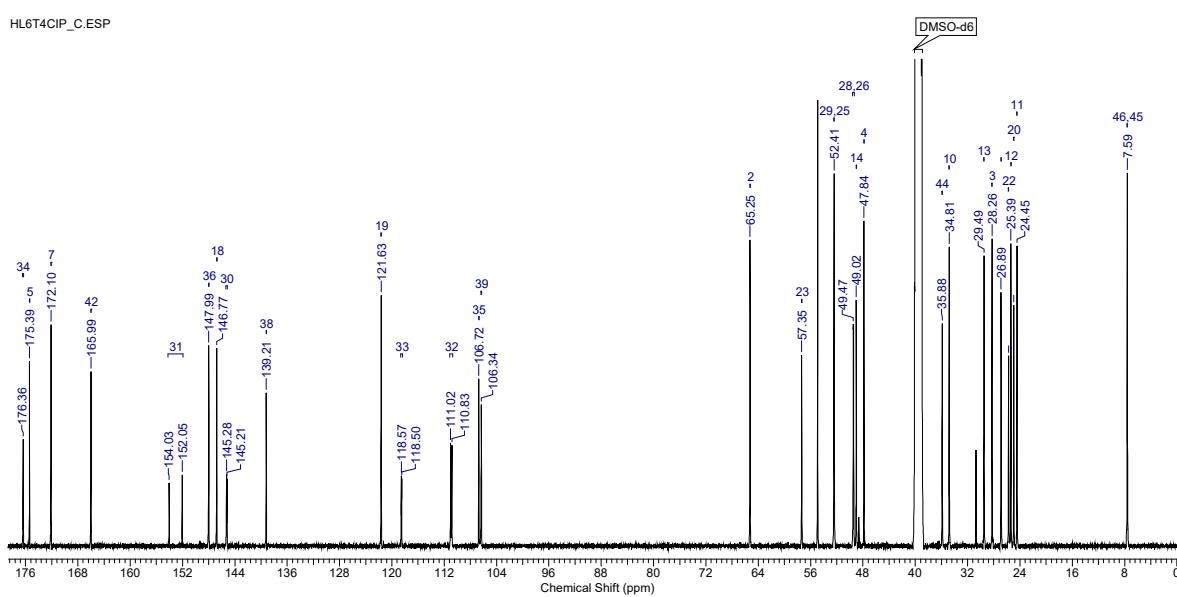
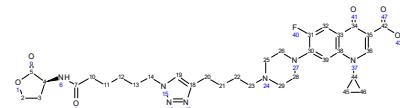
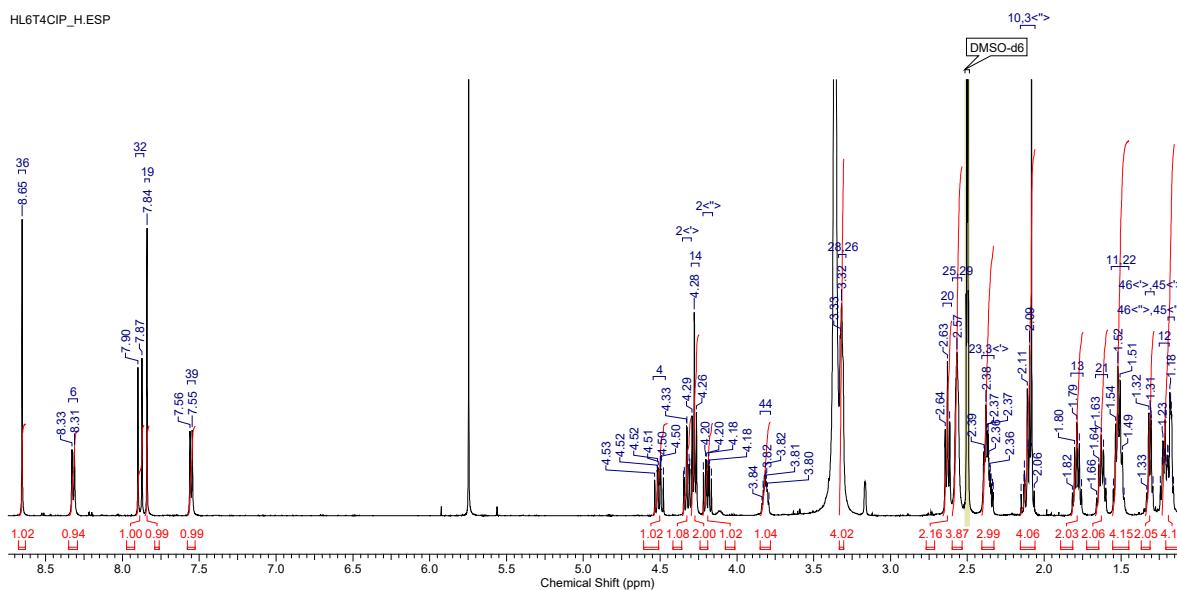
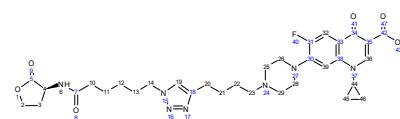
10.8 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 72



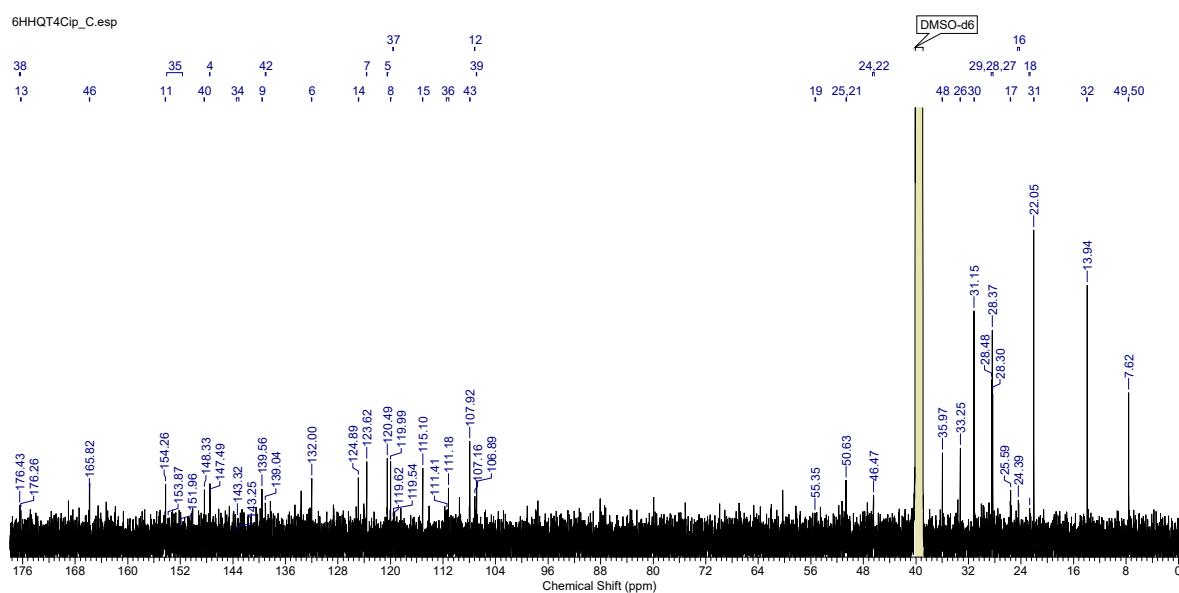
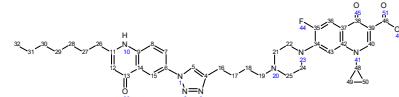
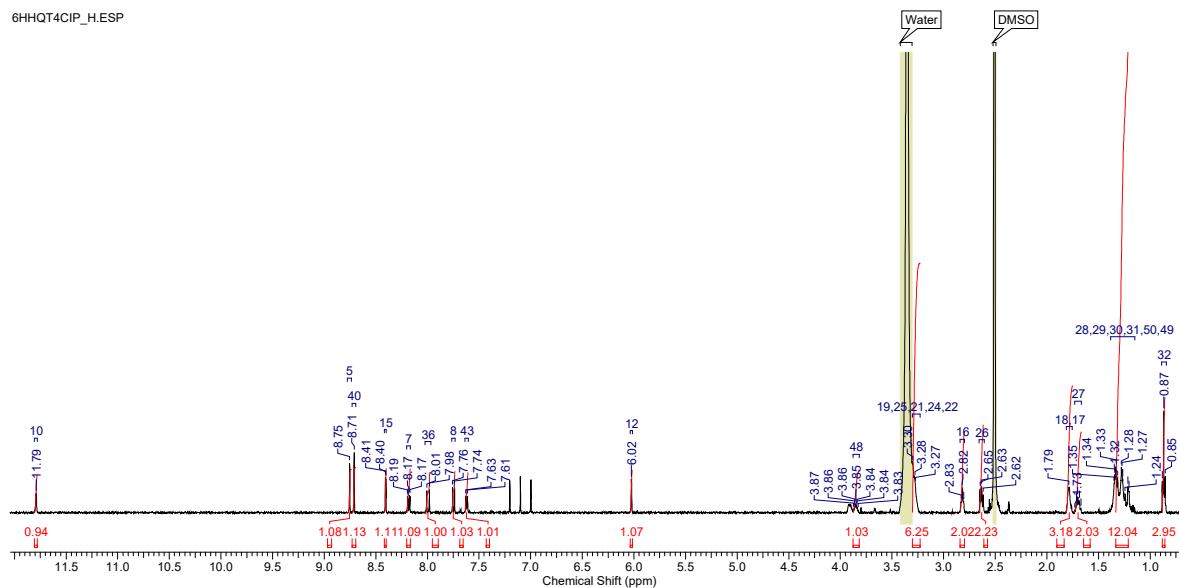
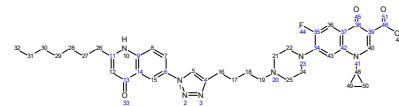
10.9 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77



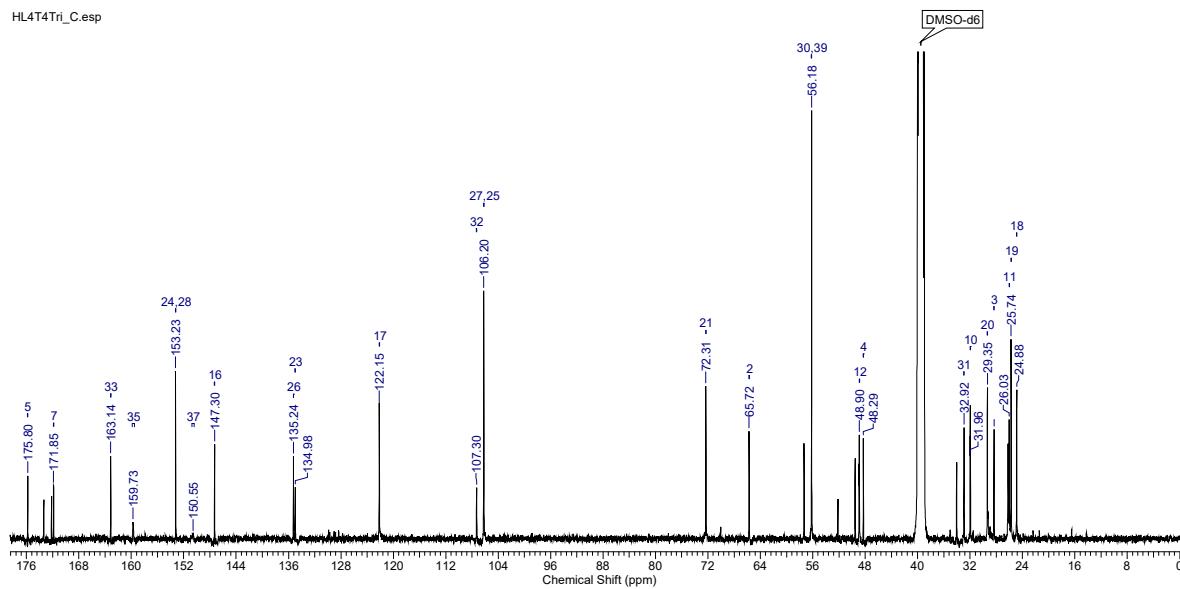
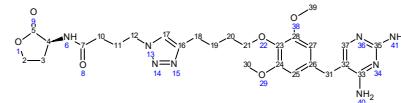
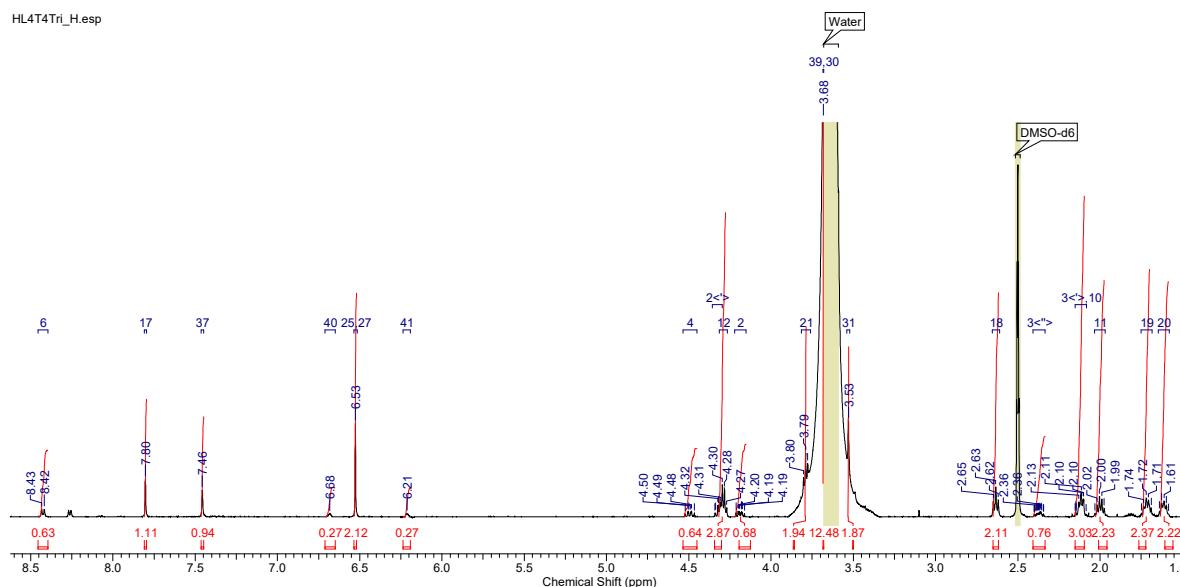
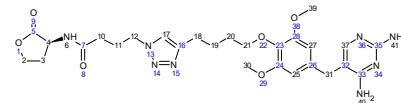
10.10 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 78



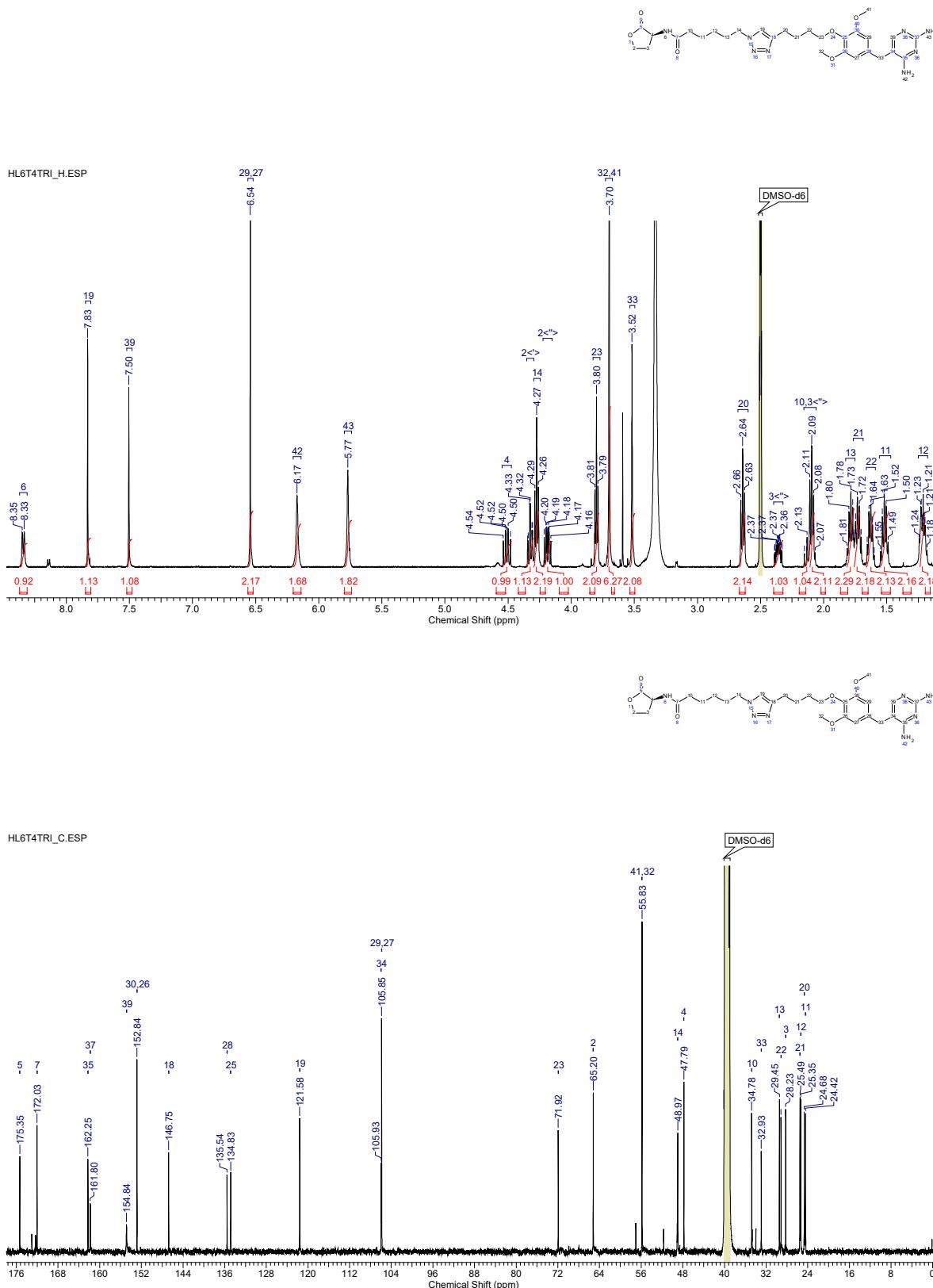
10.11 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-
1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carbo
xylic acid 80



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

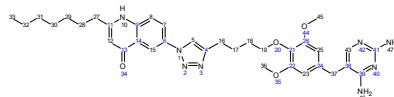


10.13 (*S*)-6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 85

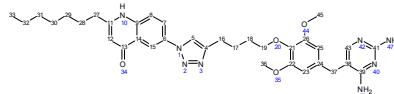
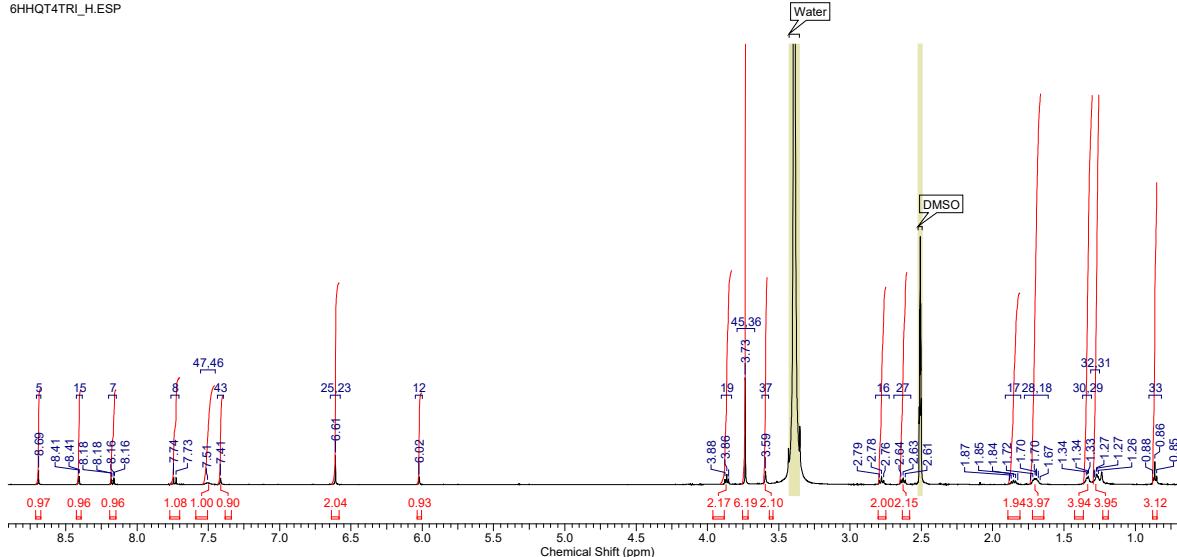


10.14 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(*1H*)-one 87

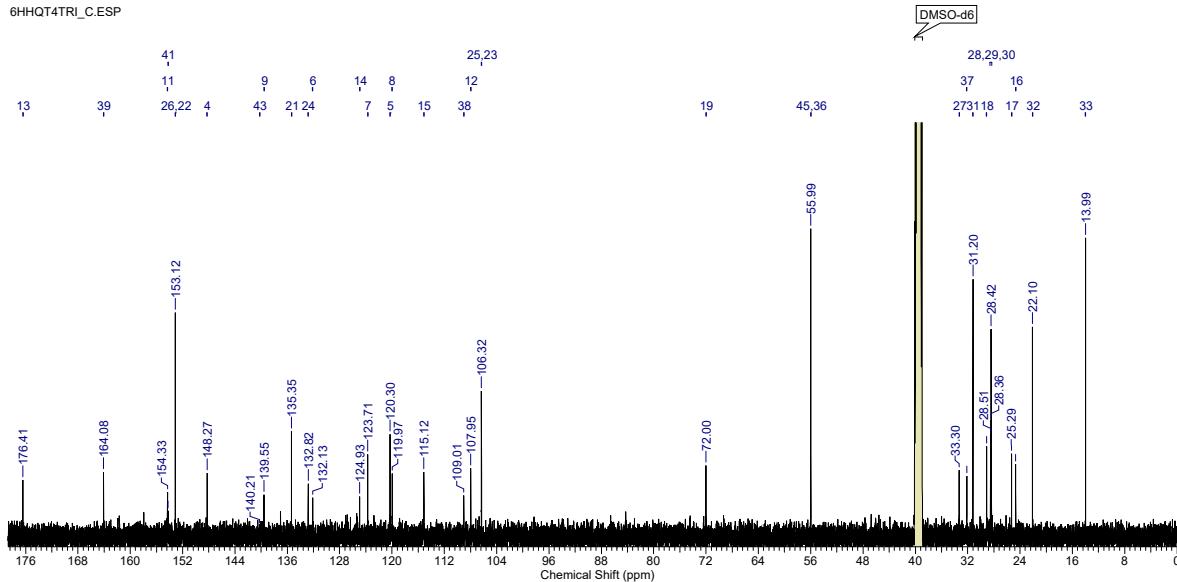
User Notes Some guesses



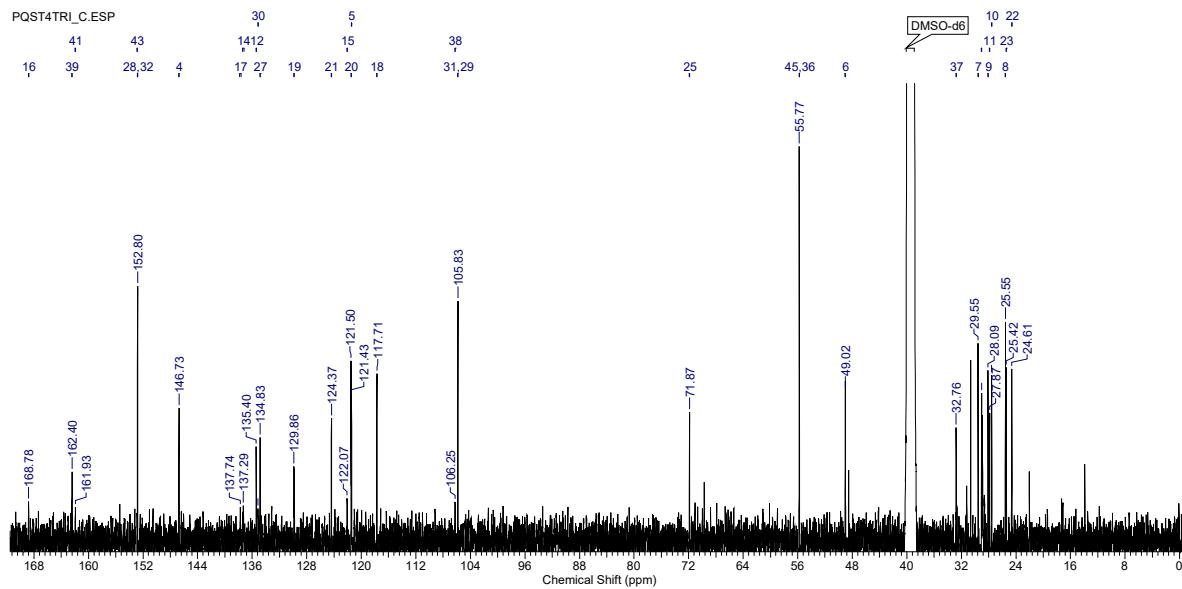
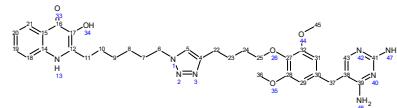
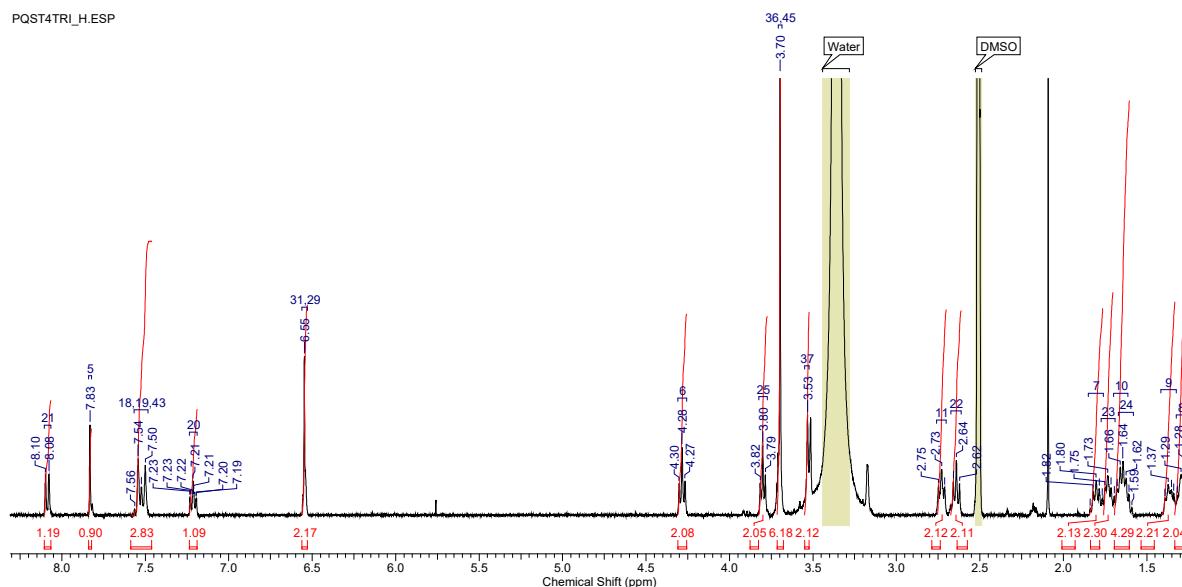
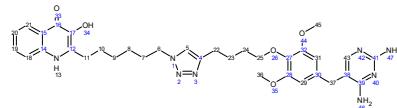
6HHQT4TRI H.ESP



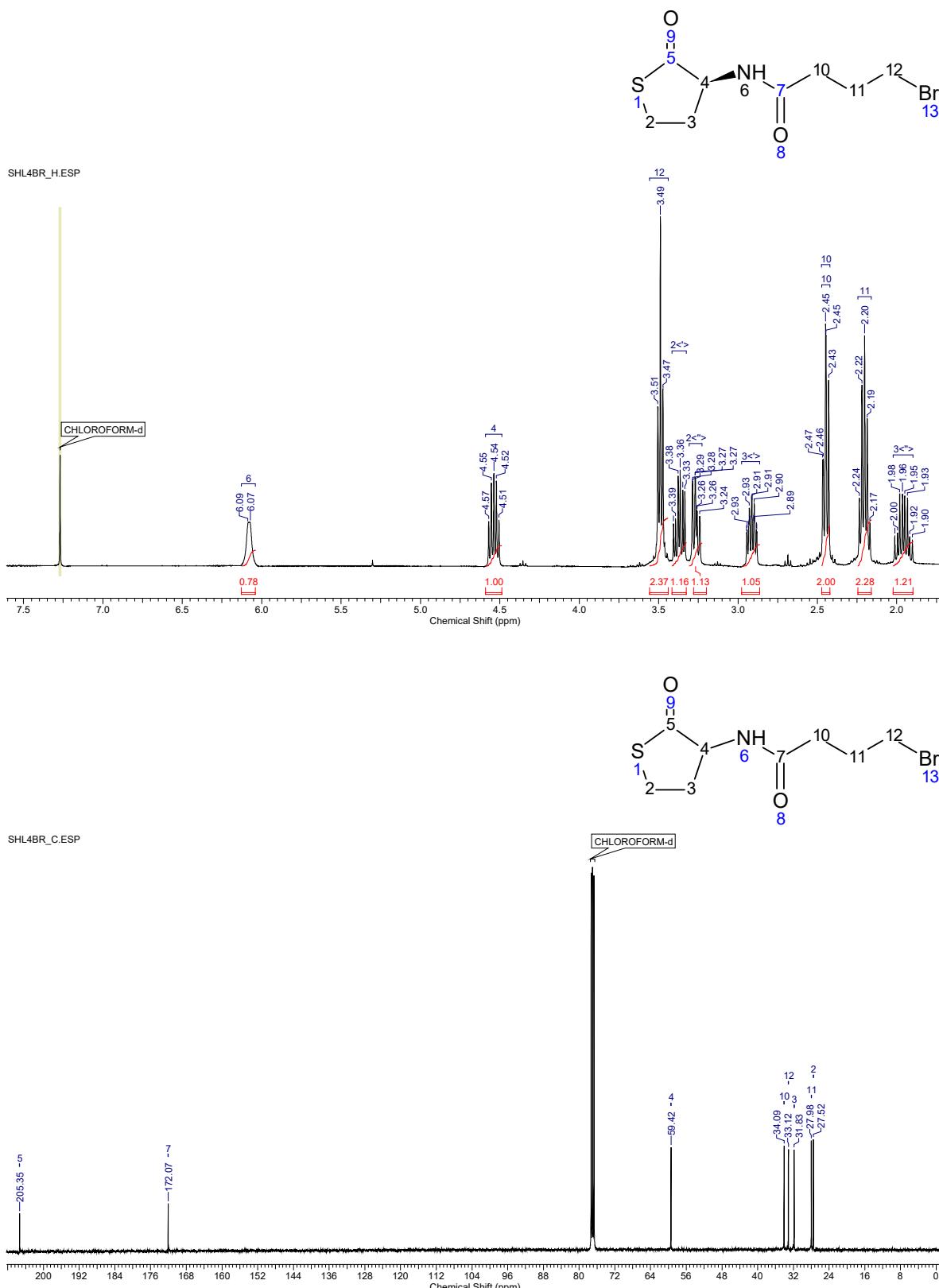
6HHQT4TRI C.ESP



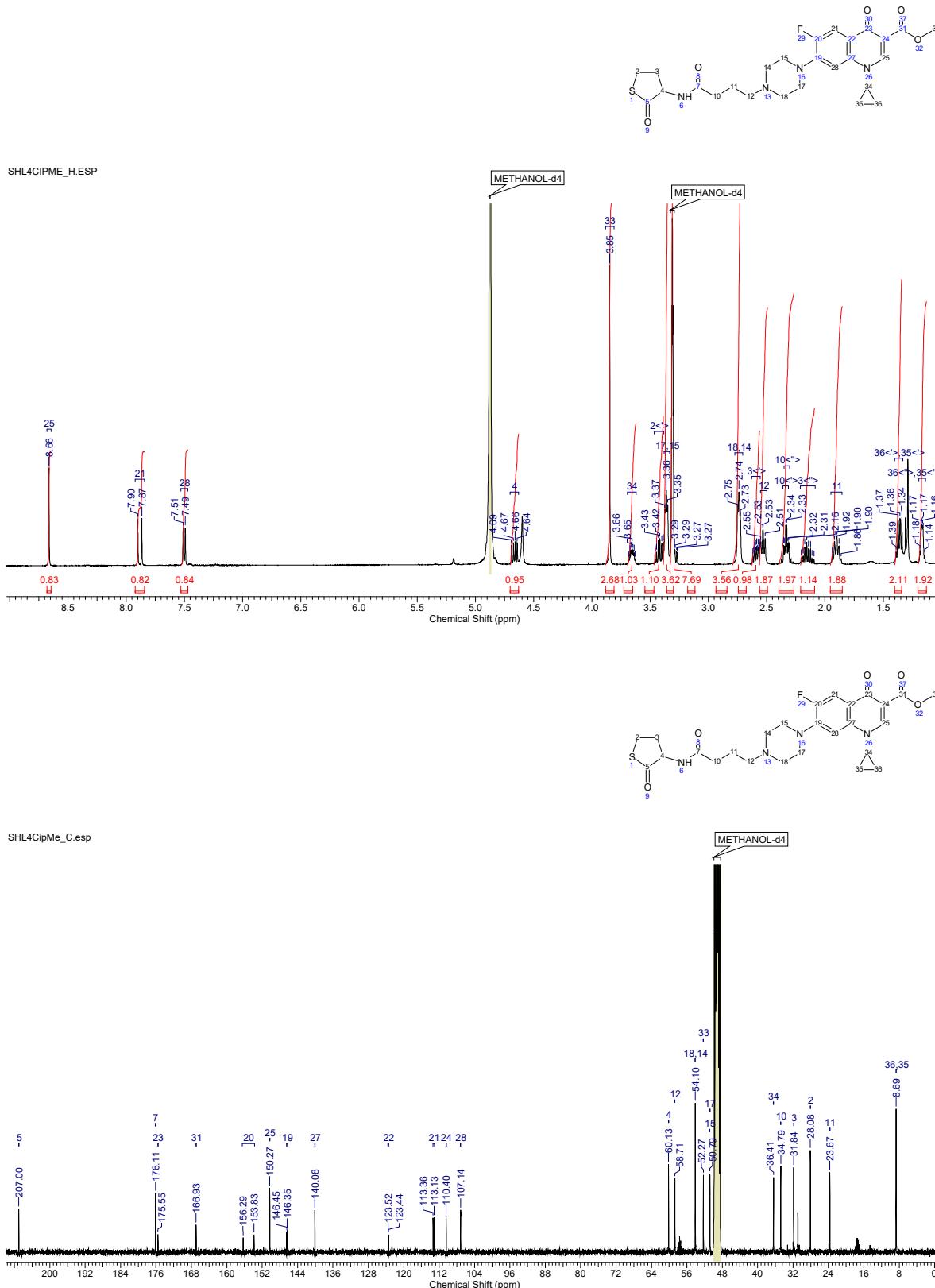
10.15 2-(6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(*H*)-one 89



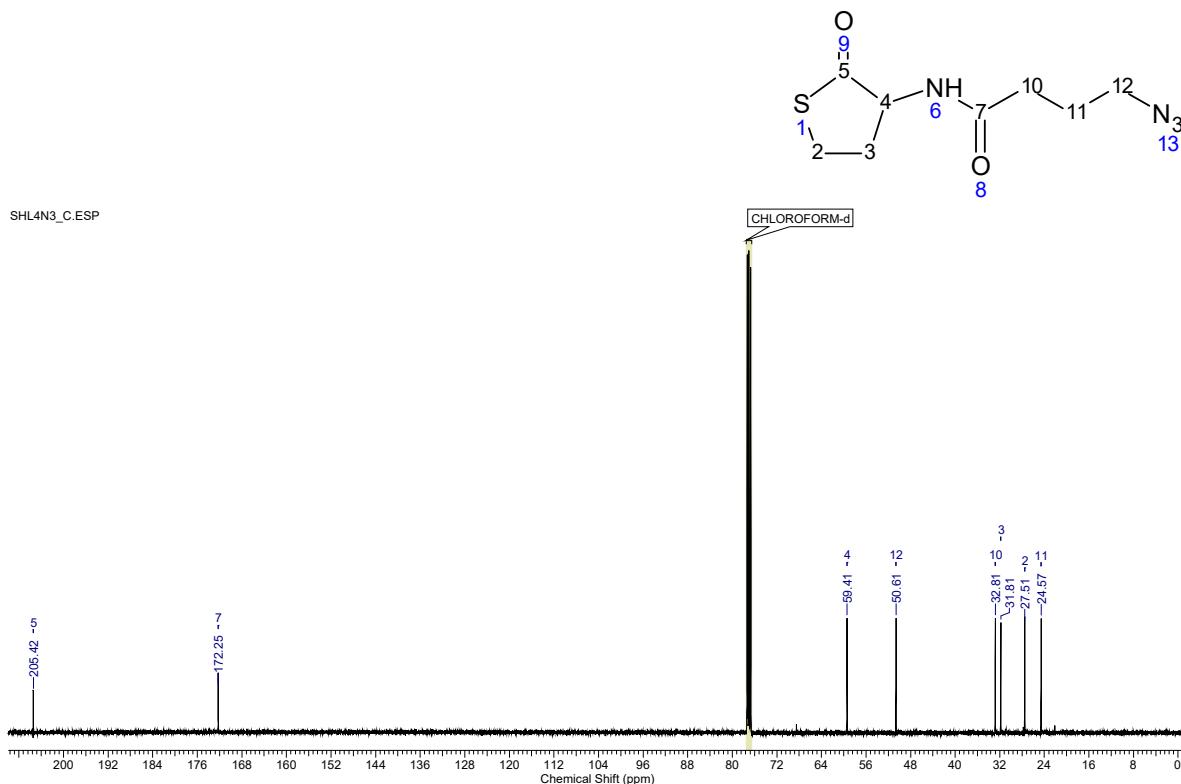
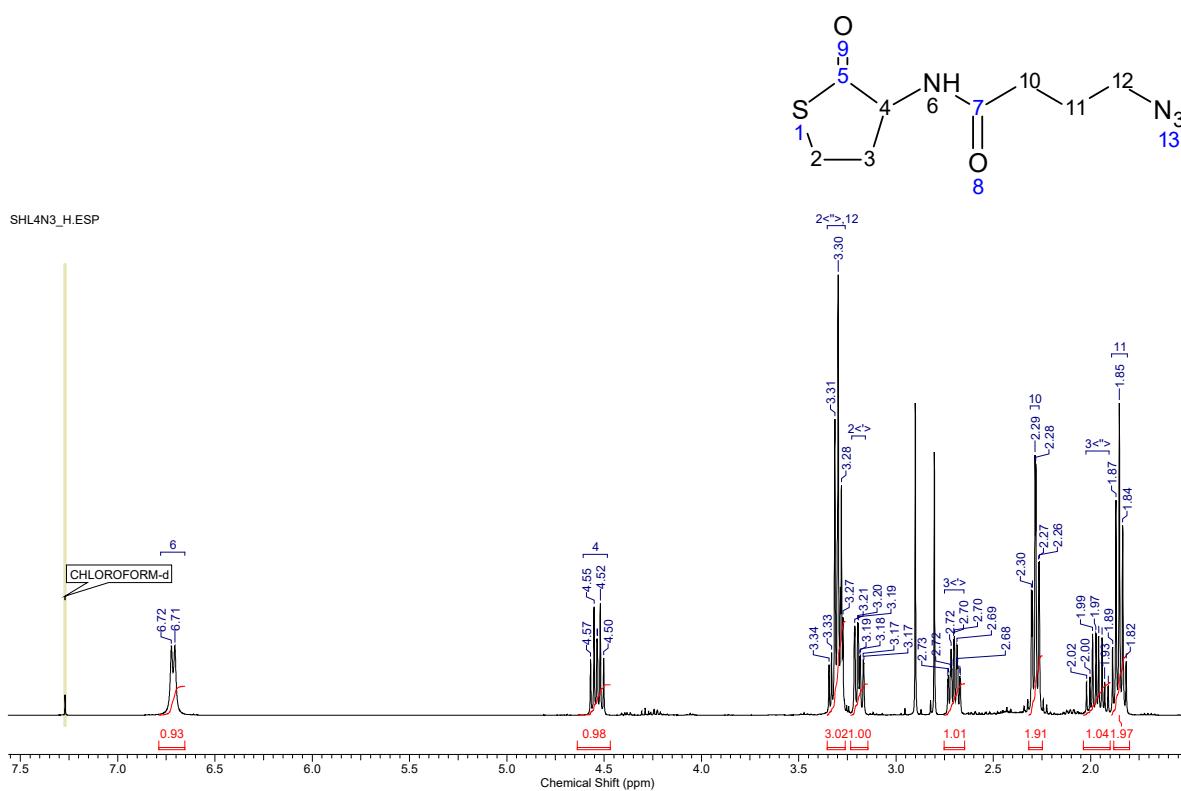
10.16 4-Bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide 153



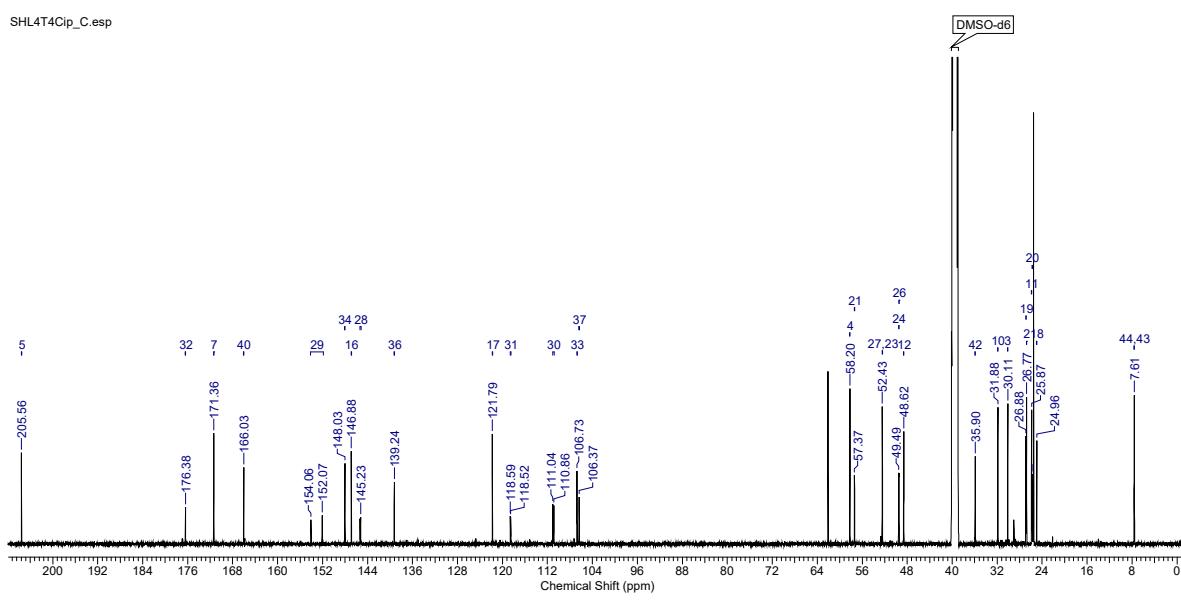
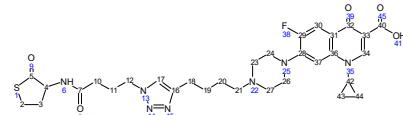
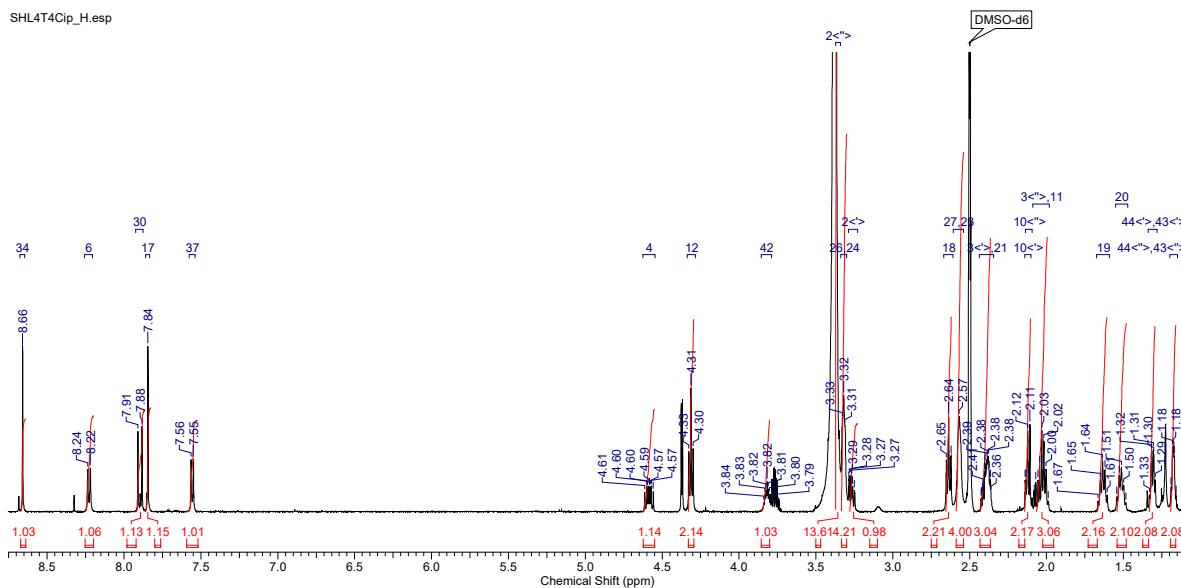
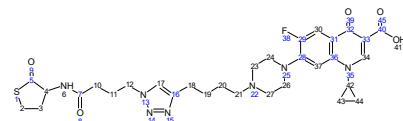
10.17 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 154



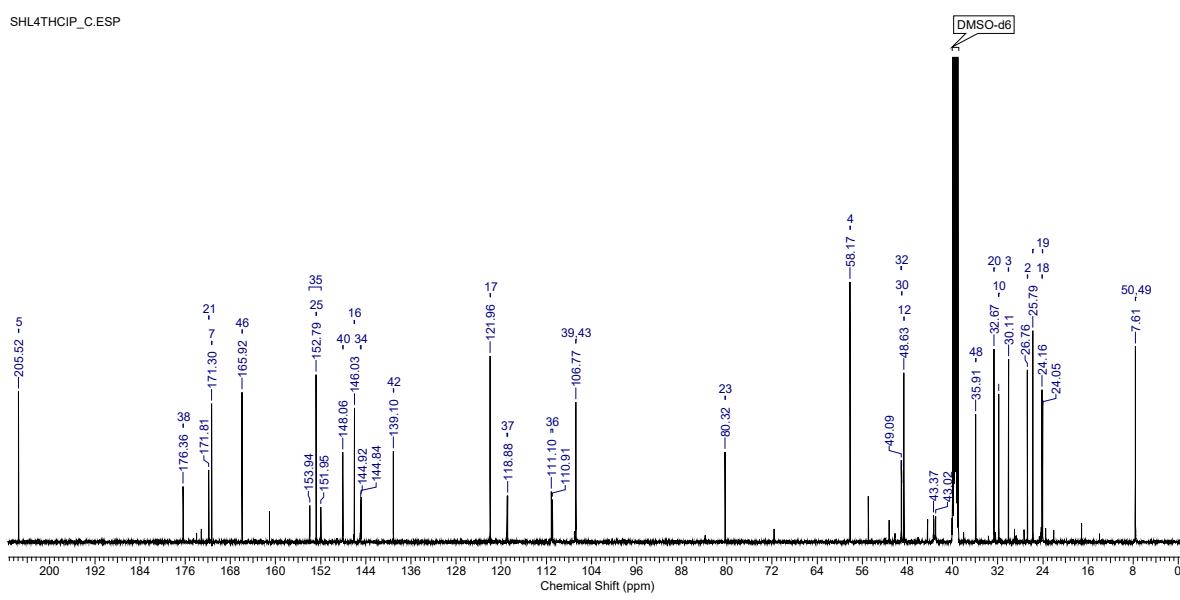
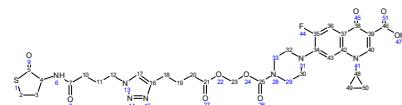
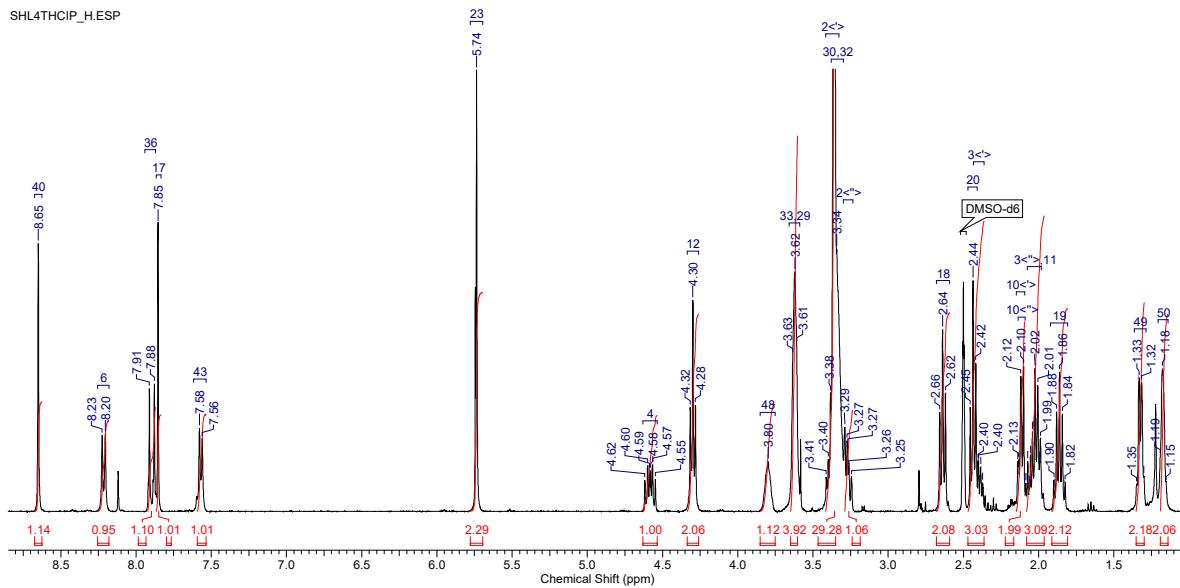
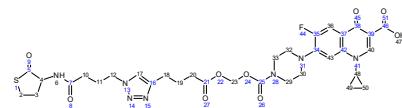
10.18 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 155



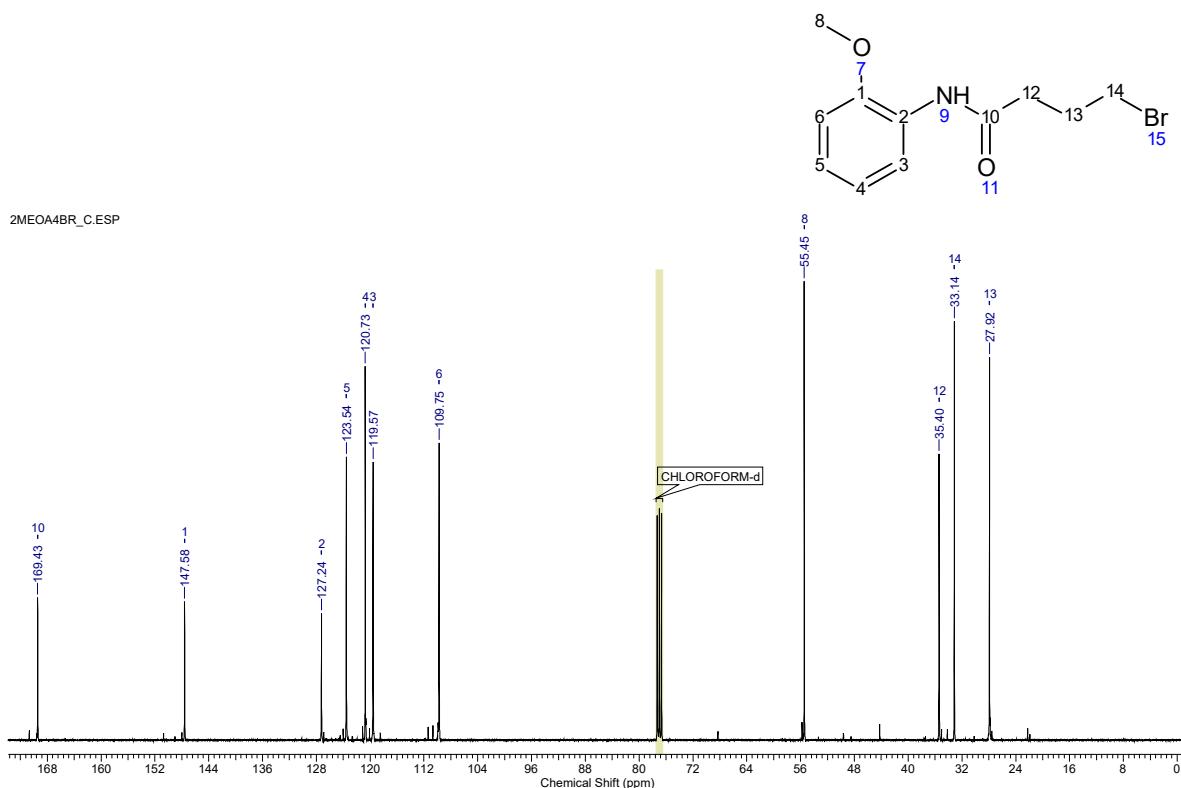
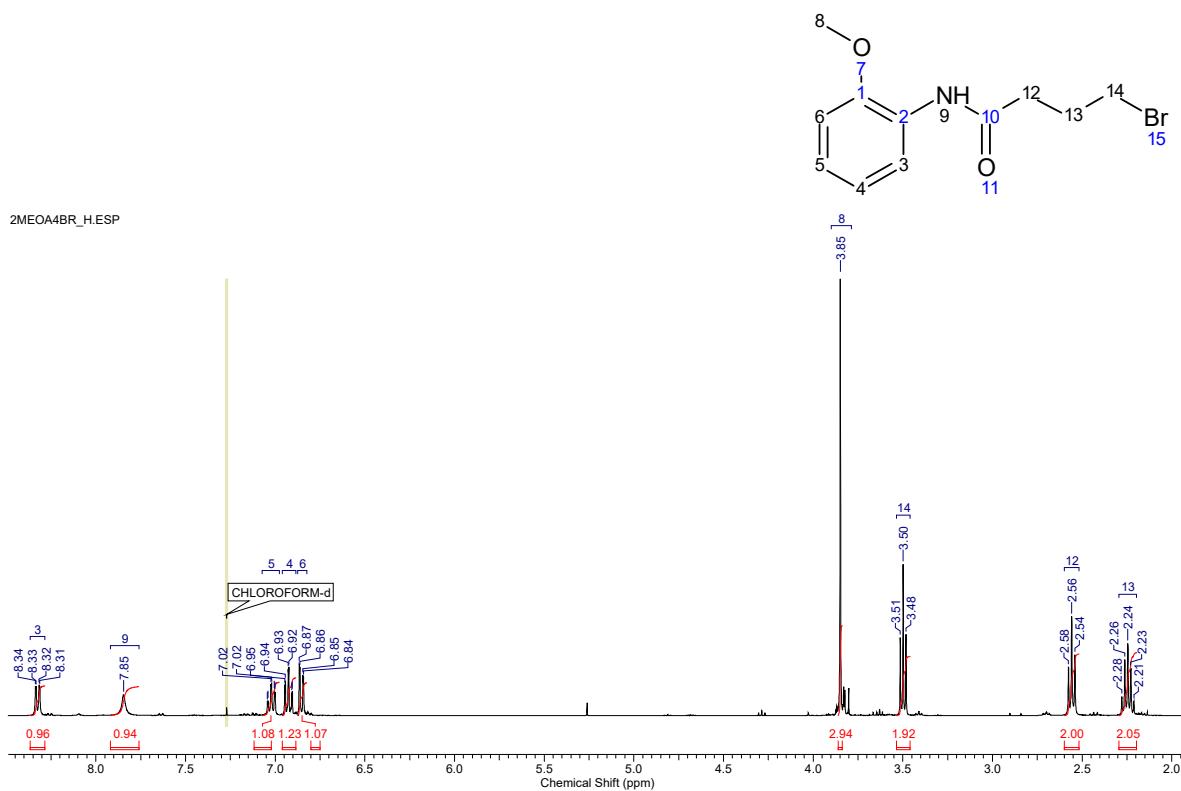
10.19 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinol-ine-3-carboxylic acid 156



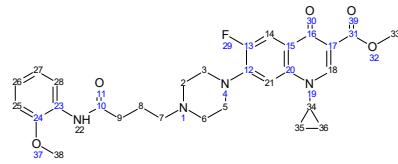
10.20 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(((4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 157



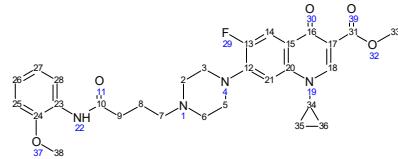
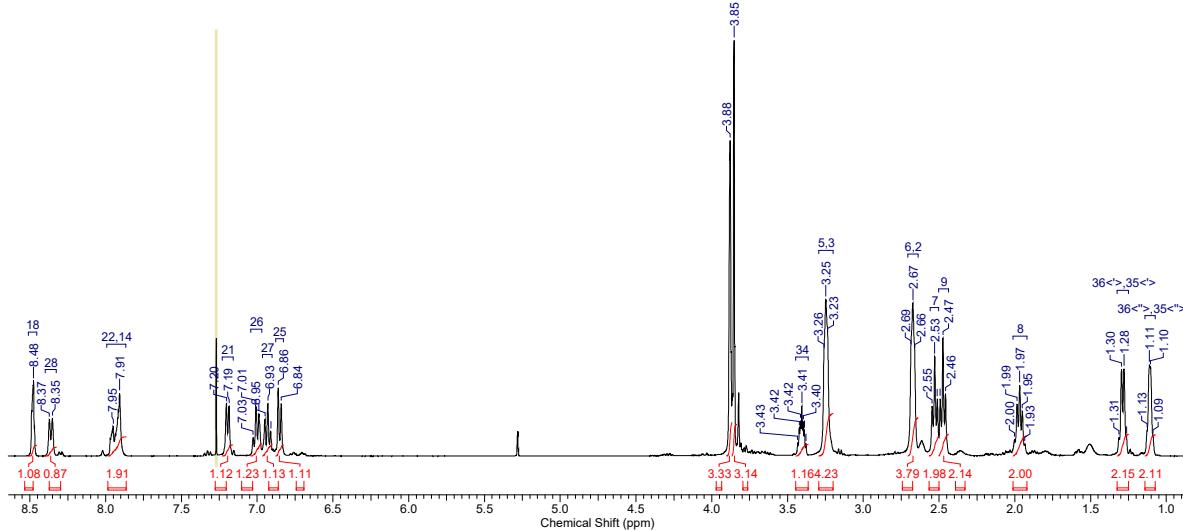
10.21 4-Bromo-N-(2-methoxyphenyl)butanamide 159



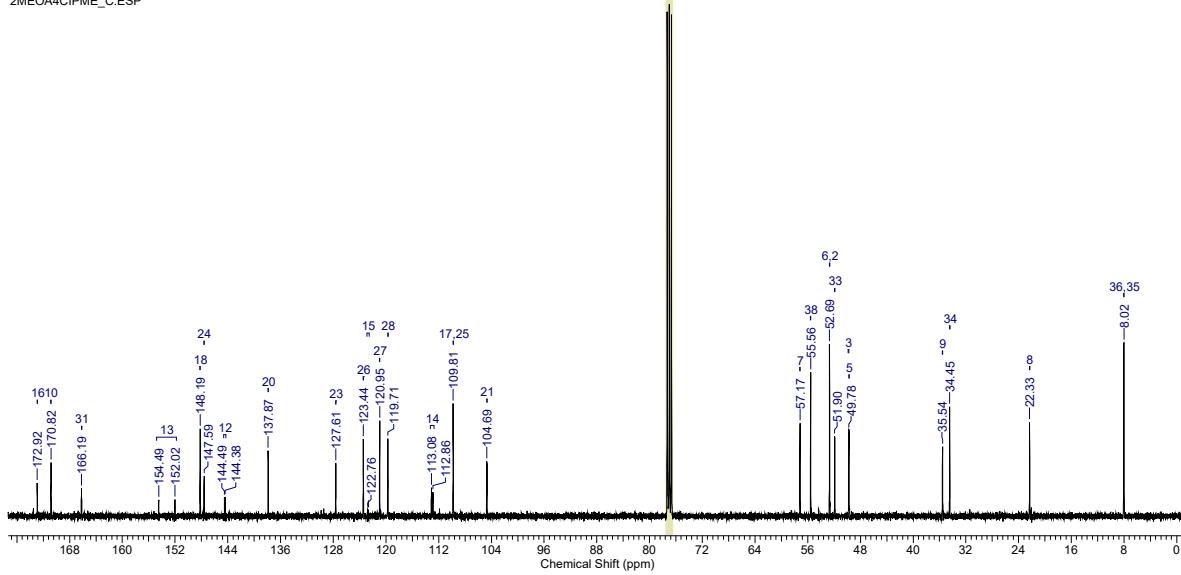
10.22 Methyl 1-cyclopropyl-6-fluoro-7-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 160



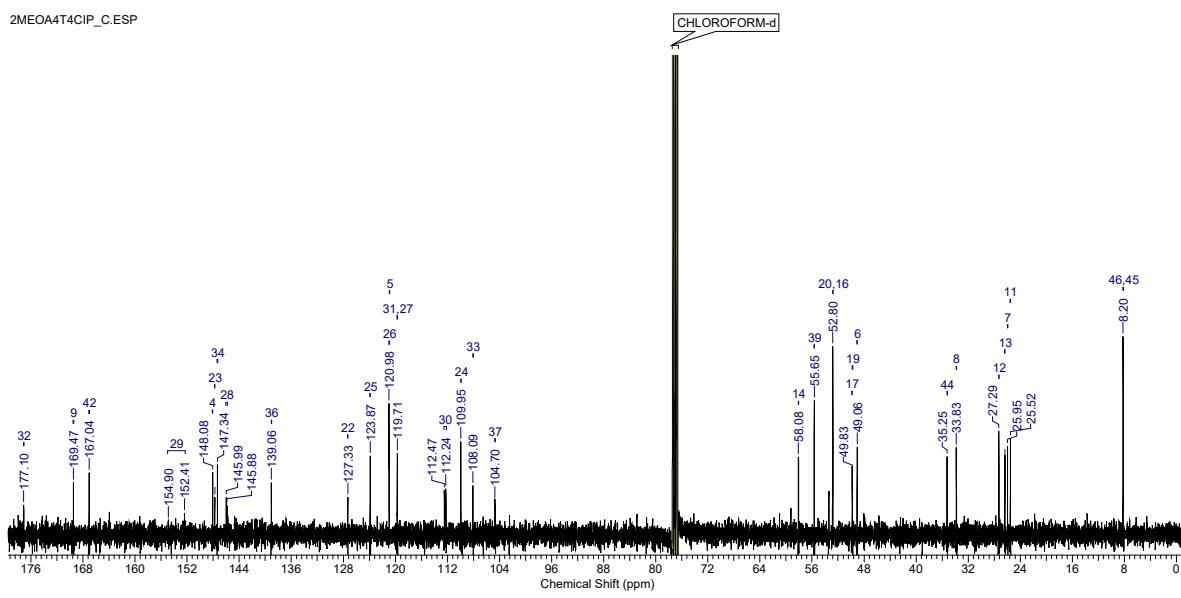
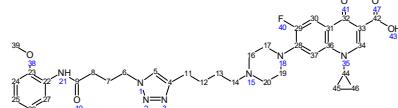
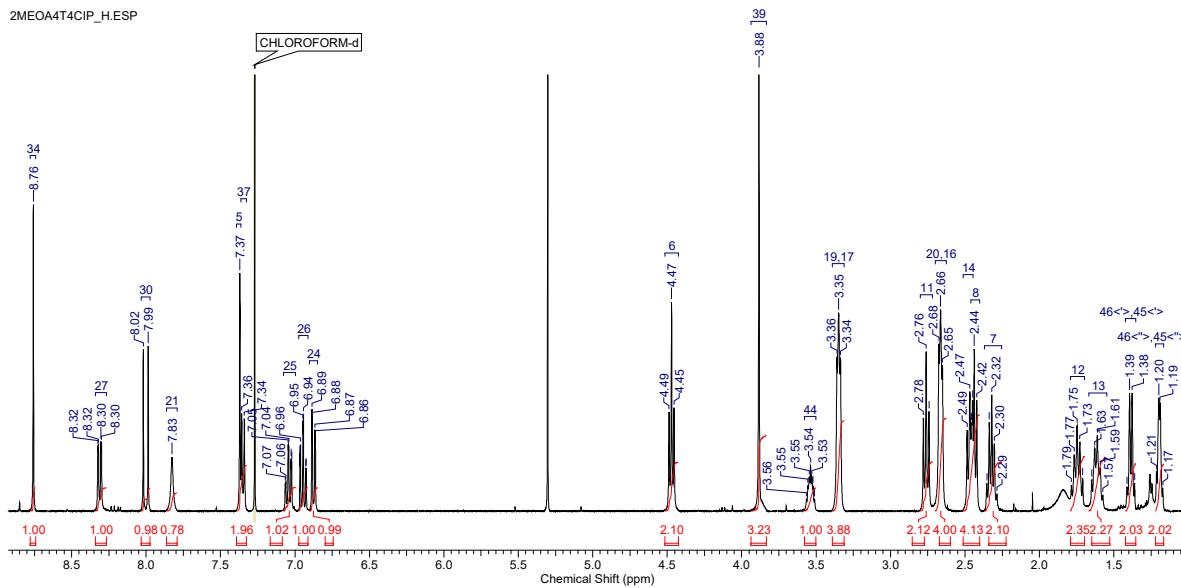
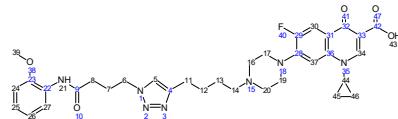
2MEOA4CIPME_H.ESP



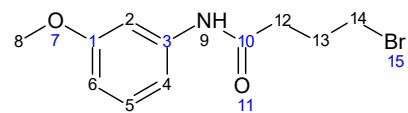
2MEOA4CIPME_C.ESP



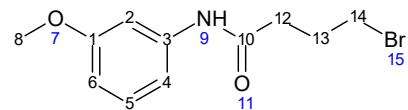
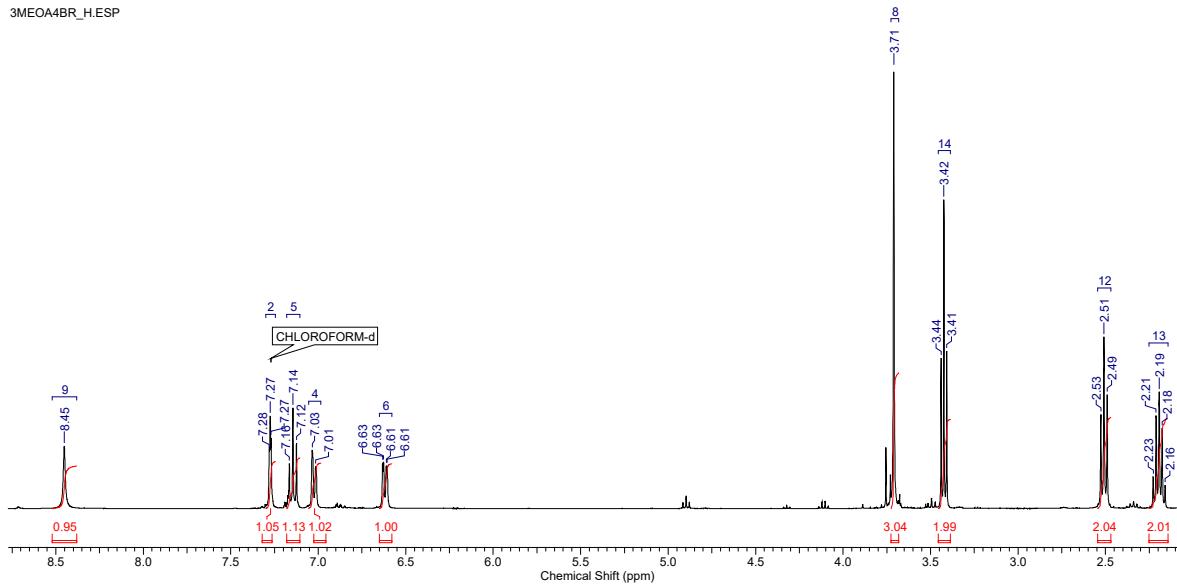
10.23 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 162



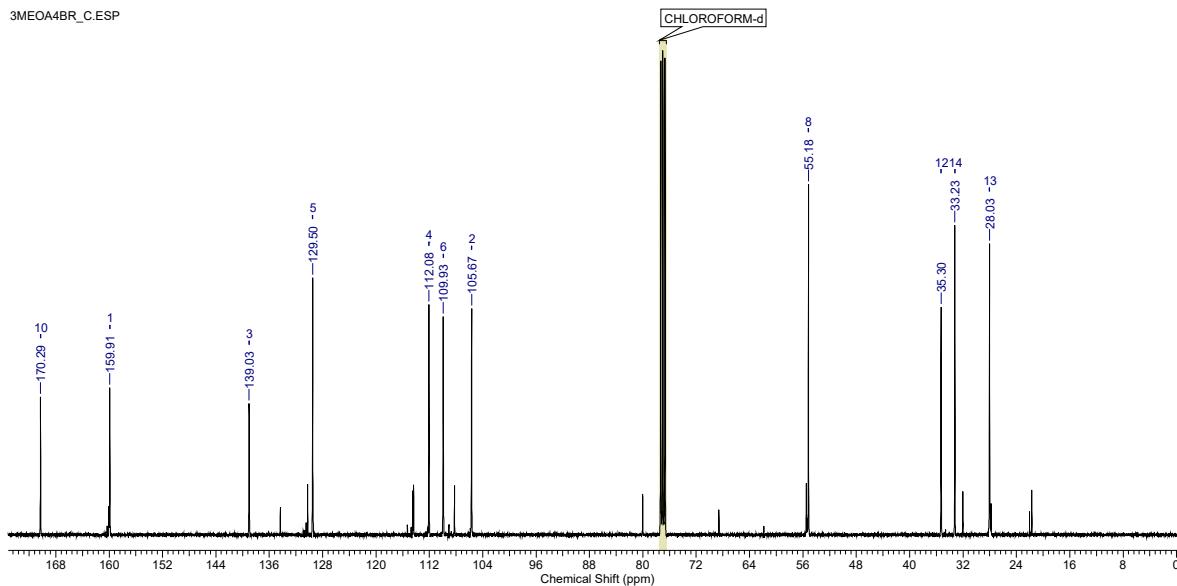
10.24 4-Bromo-N-(3-methoxyphenyl)butanamide 164



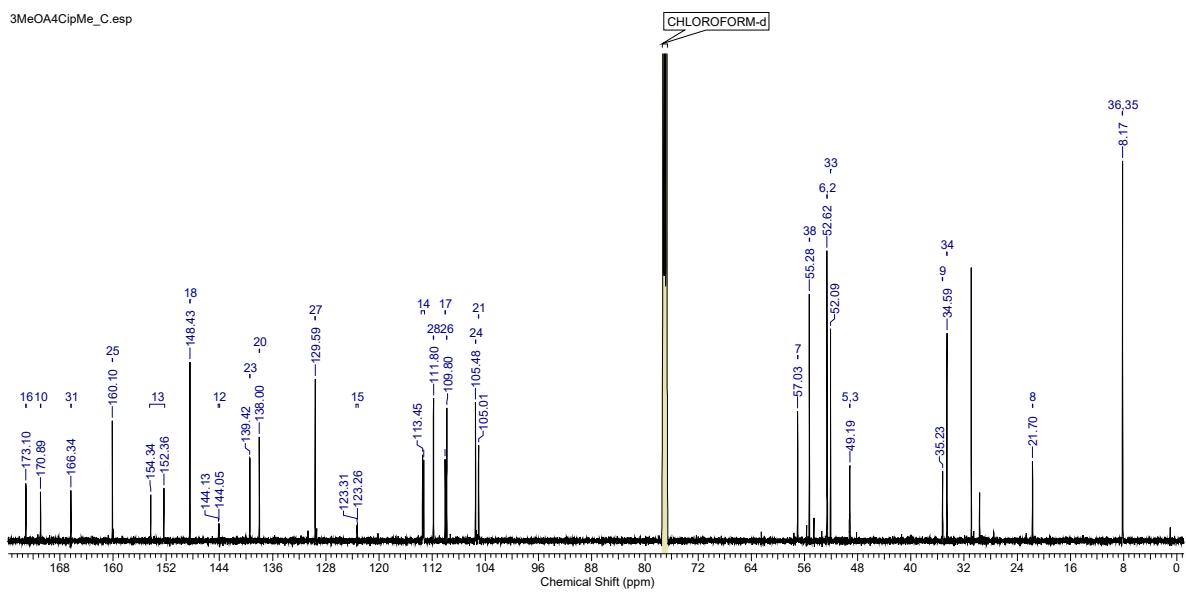
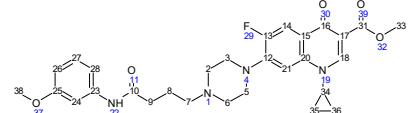
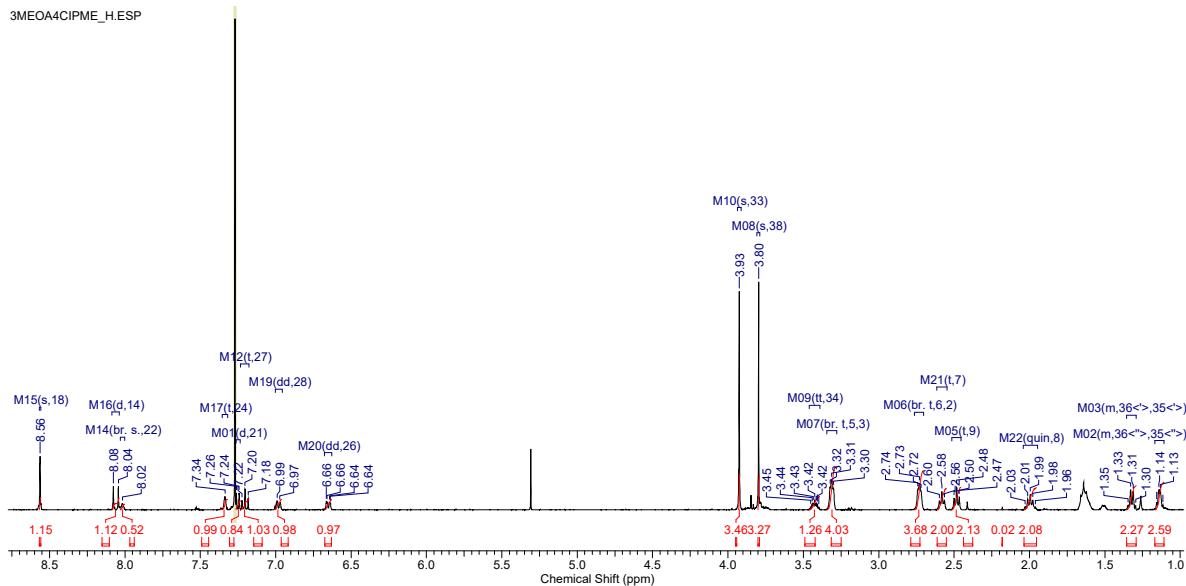
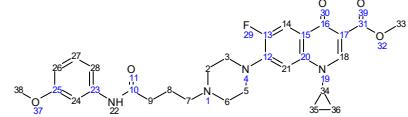
3MEOA4BR_H.ESP



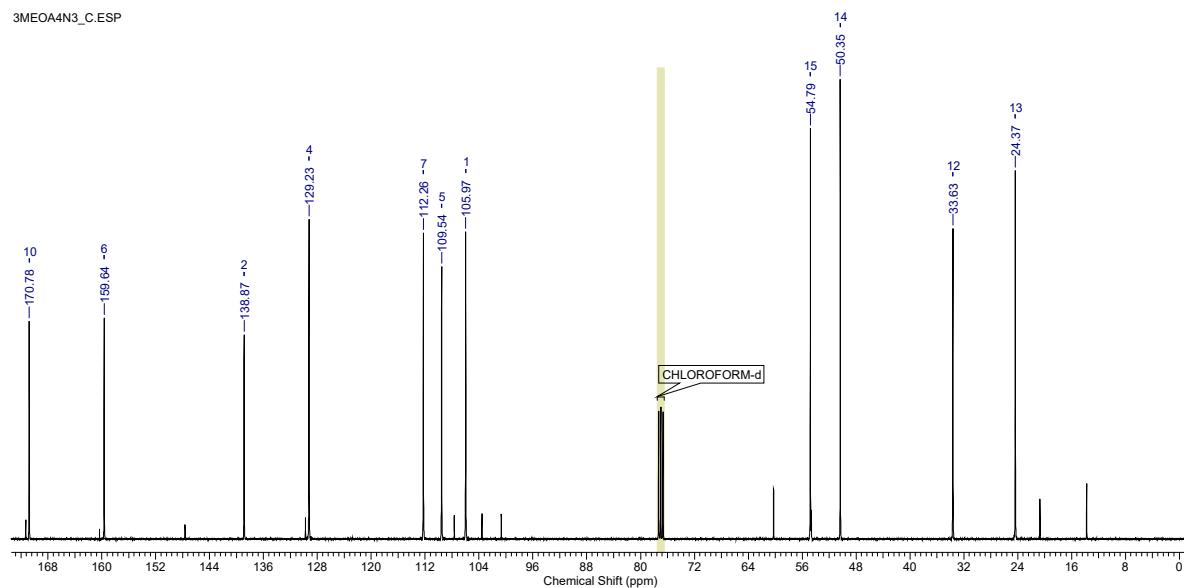
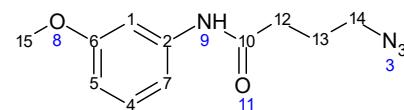
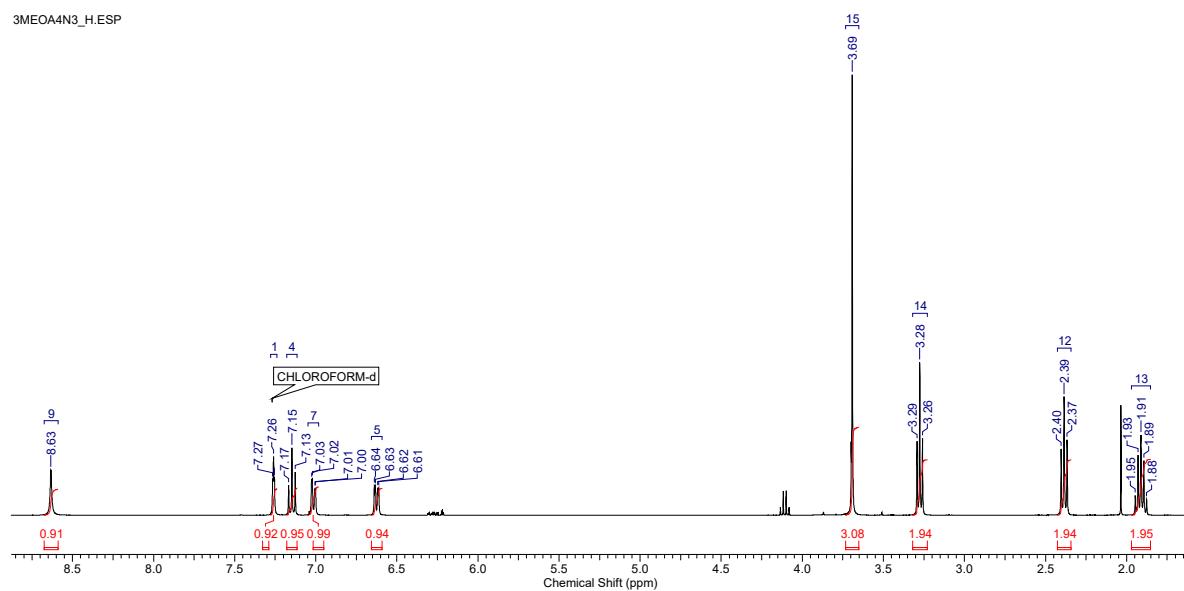
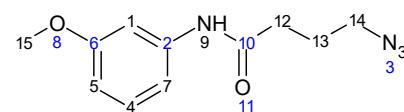
3MEOA4BR_C.ESP



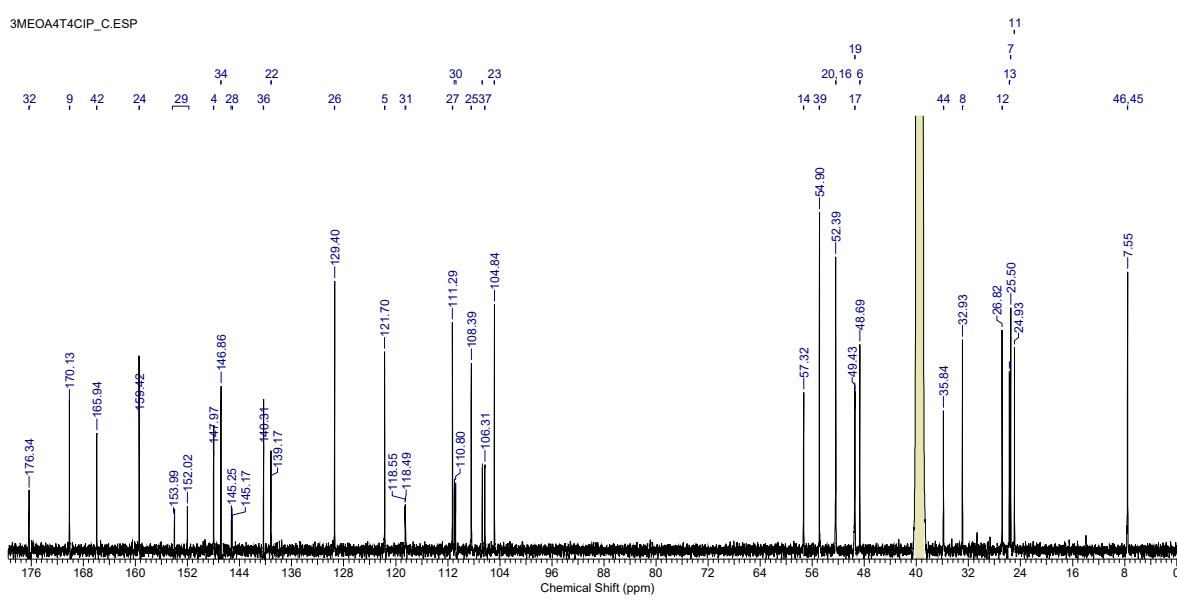
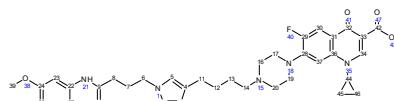
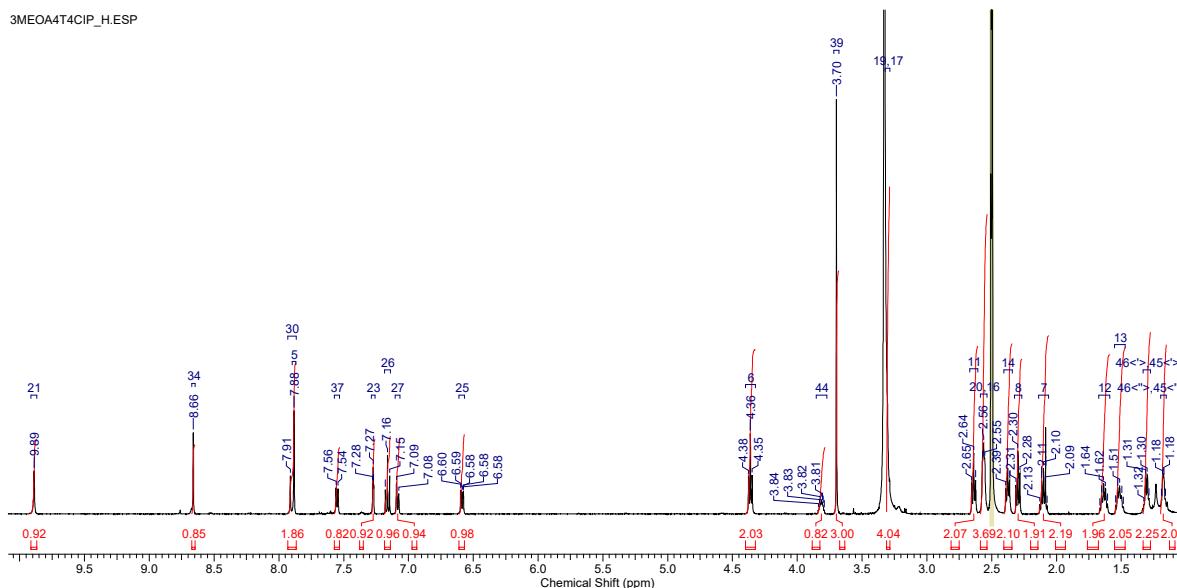
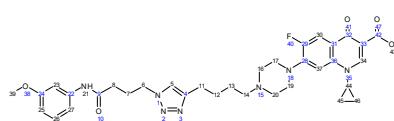
10.25 Methyl 1-cyclopropyl-6-fluoro-7-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 165



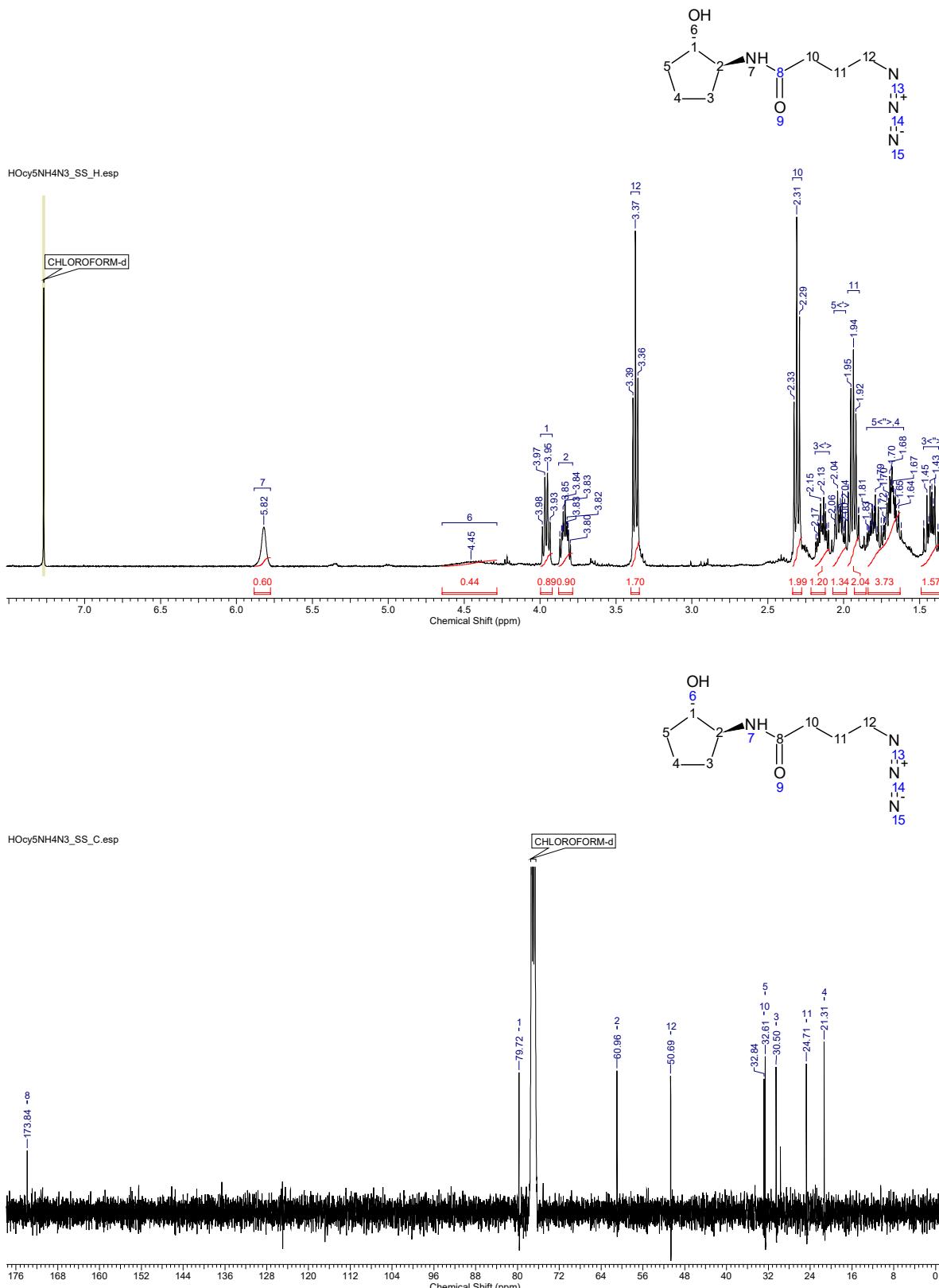
10.26 4-Azido-*N*-(3-methoxyphenyl)butanamide 166



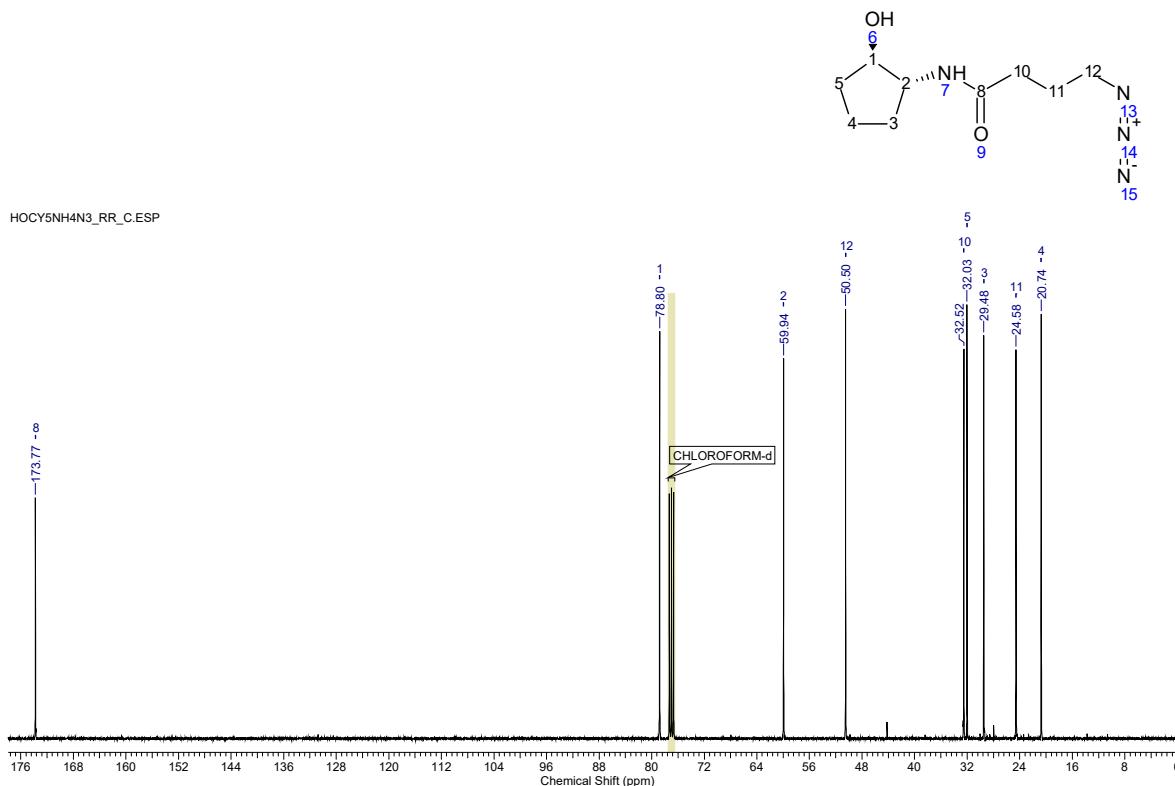
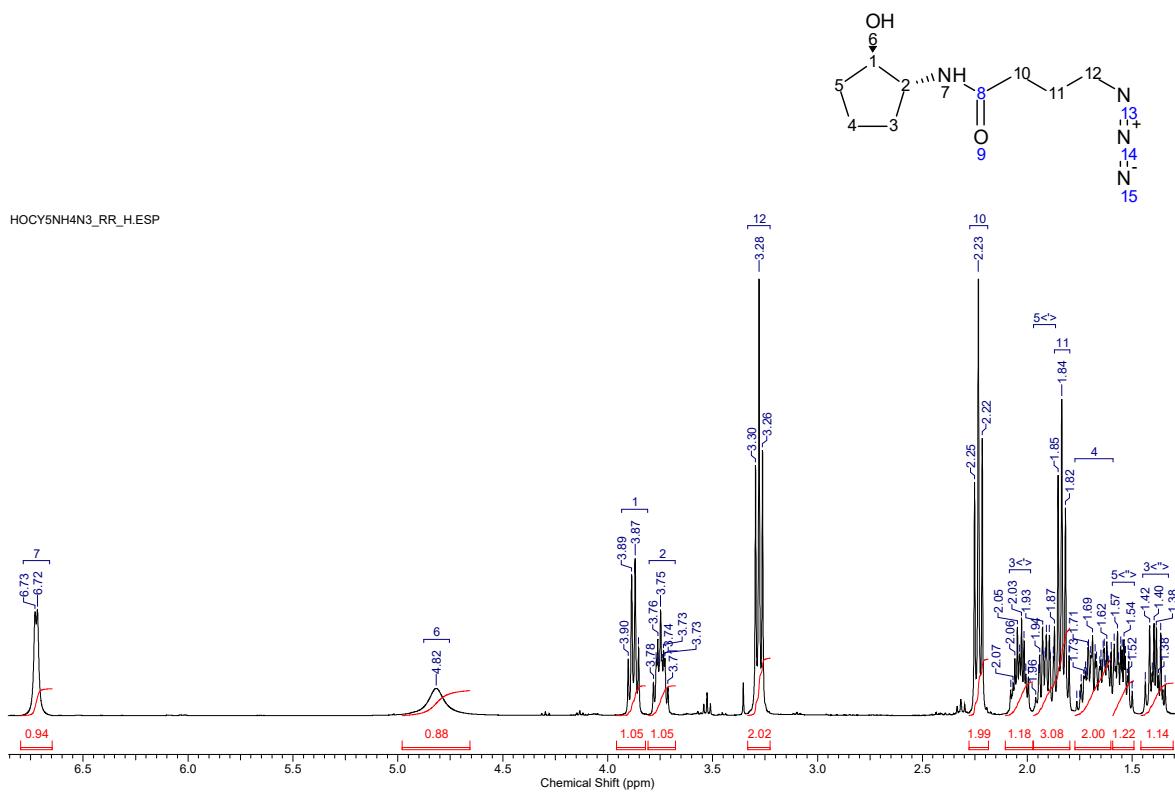
10.27 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 167



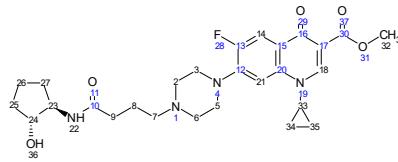
10.28 4-Azido-N-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide 176



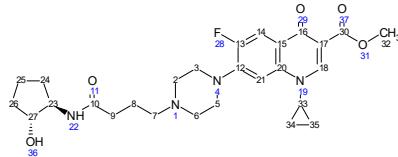
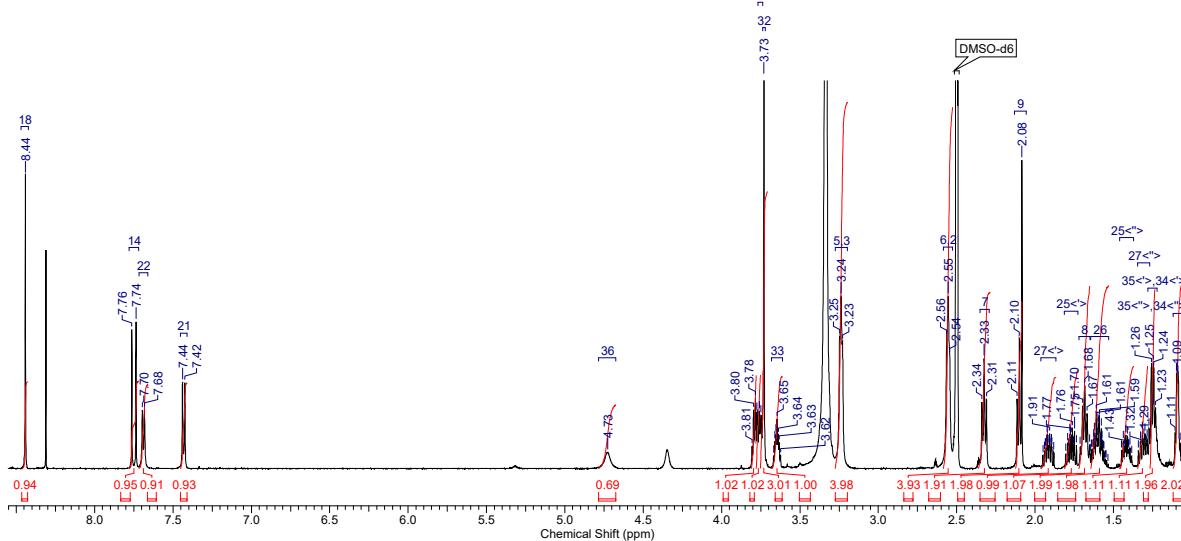
10.29 4-Azido-N-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide 177



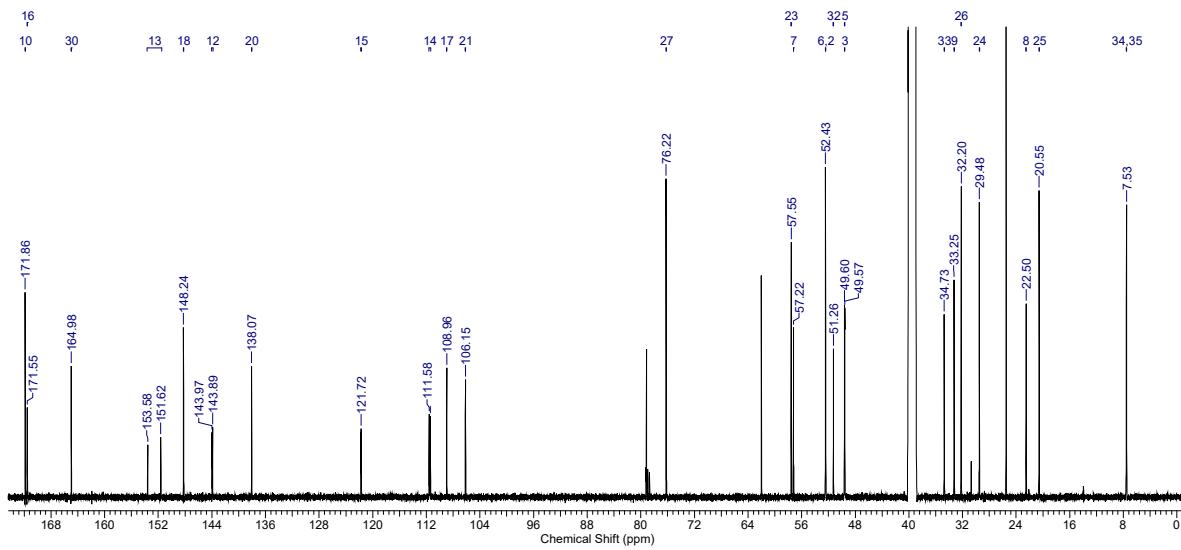
10.30 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 178



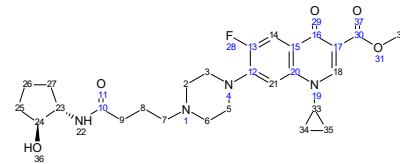
HOcy5NH4CipMe_SS_H.esp



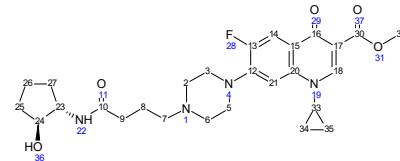
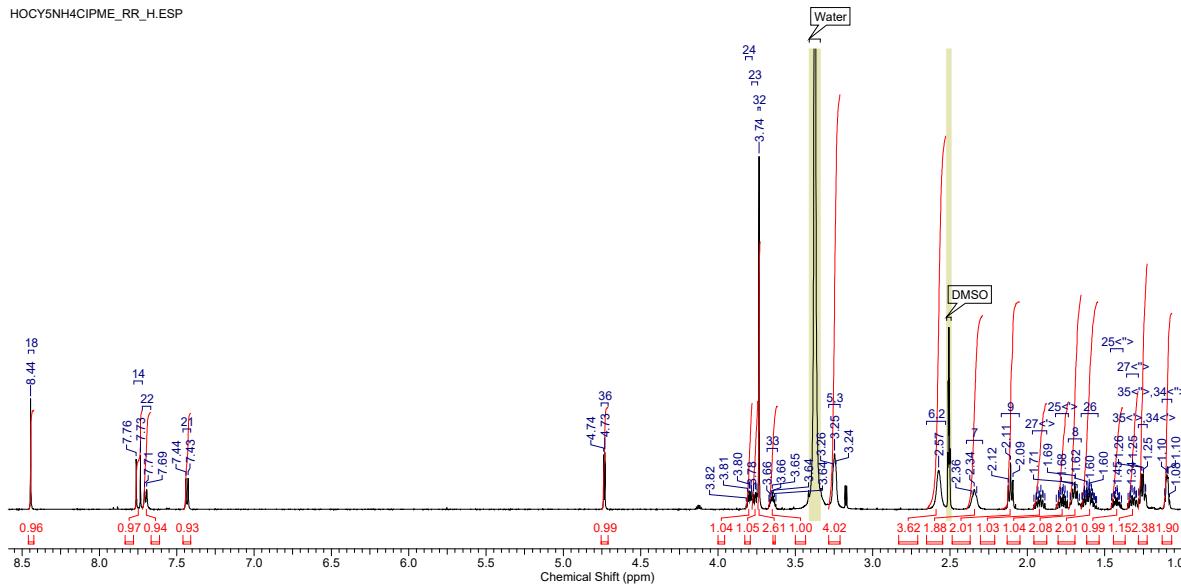
HOcy5NH4CipMe_SS_C.esp



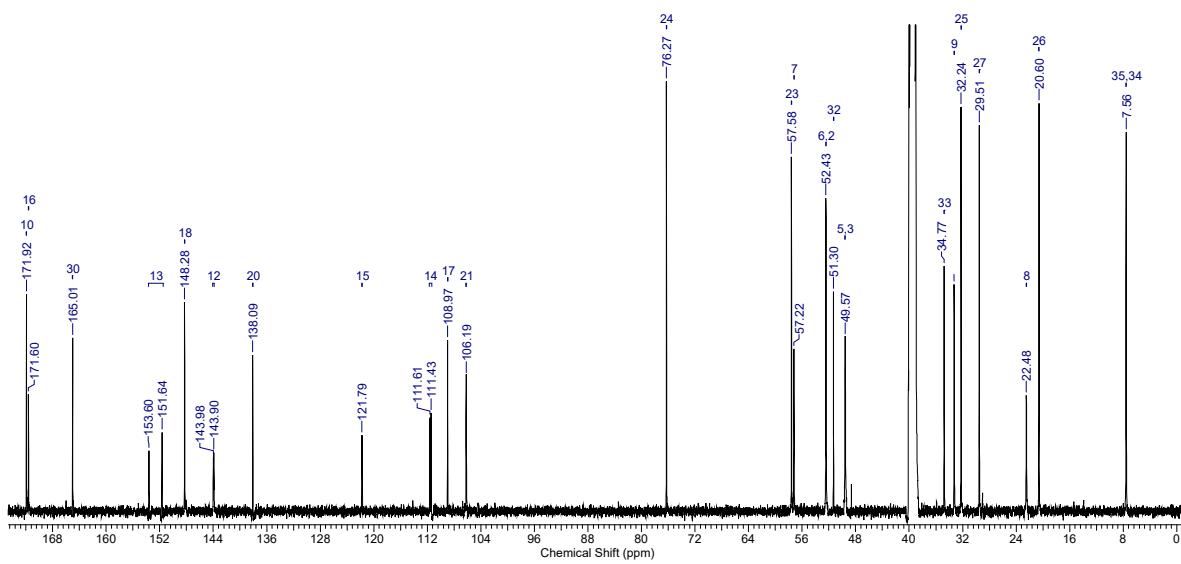
10.31 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 179



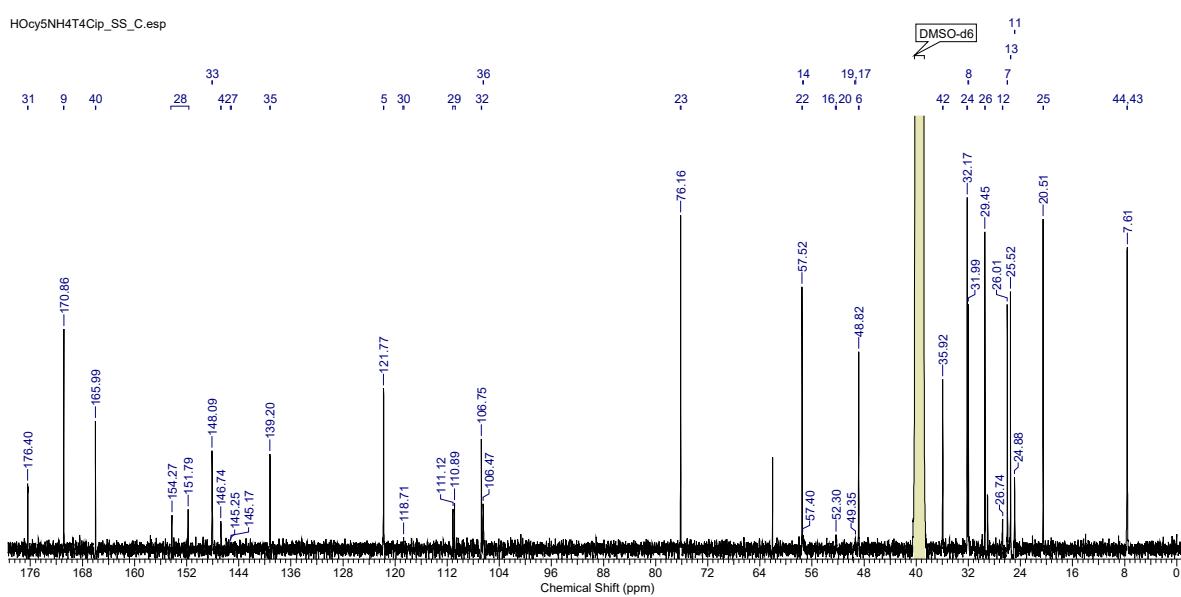
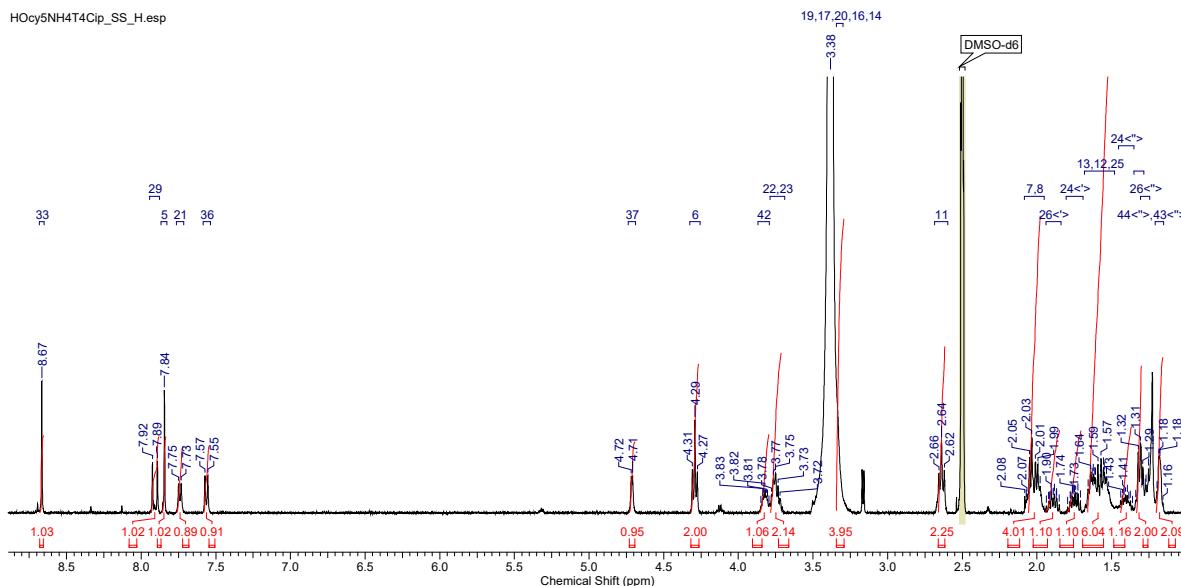
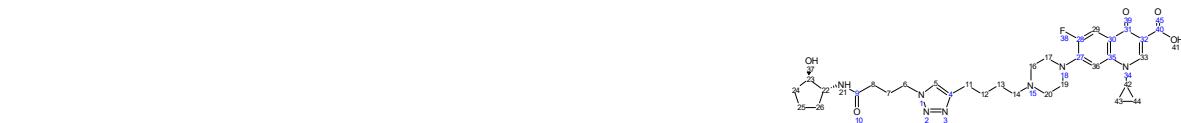
HO CY5 NH4 CIP ME_RR_H.ESP



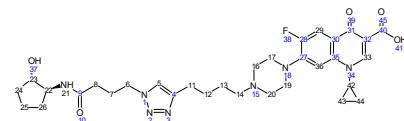
HOcy5NH4CipMe_RR_C.esp



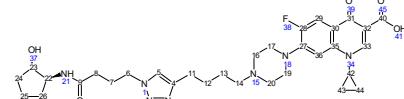
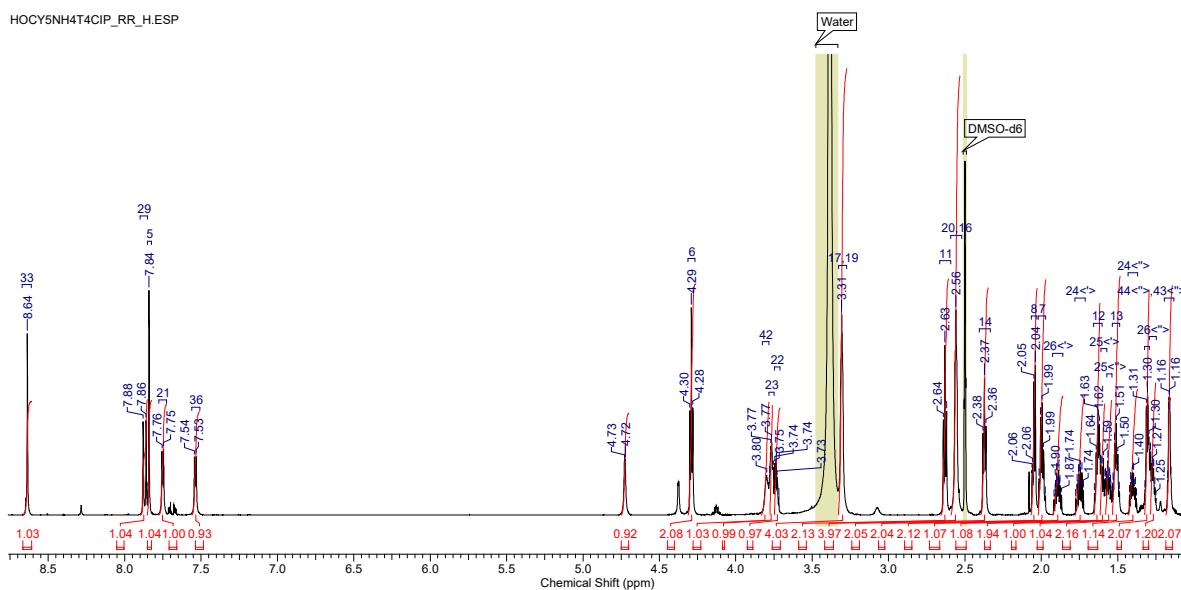
10.32 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*S*,*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 180



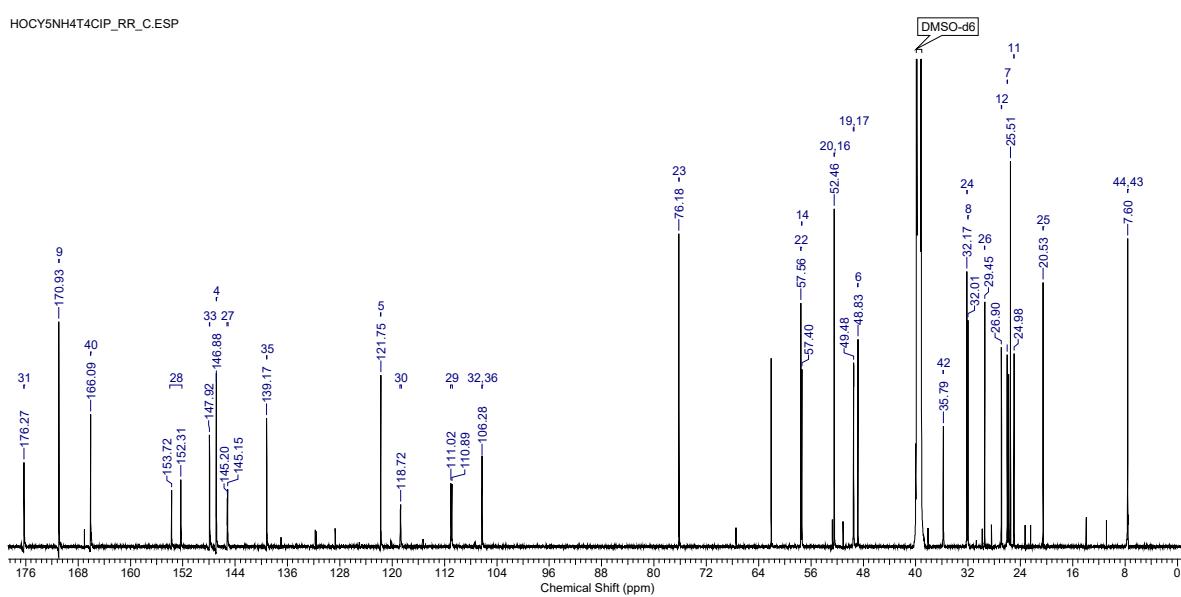
10.33 1-Cyclopropyl-6-fluoro-7-(4-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-ylbutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 181



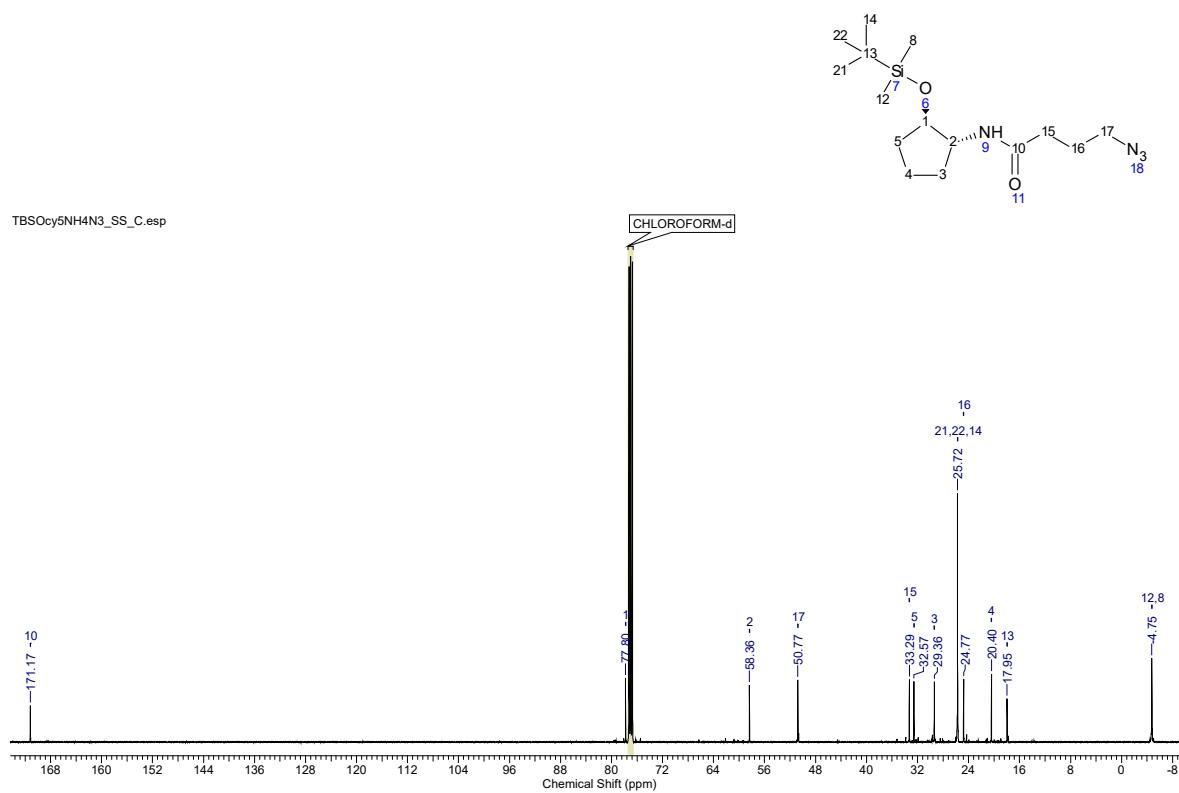
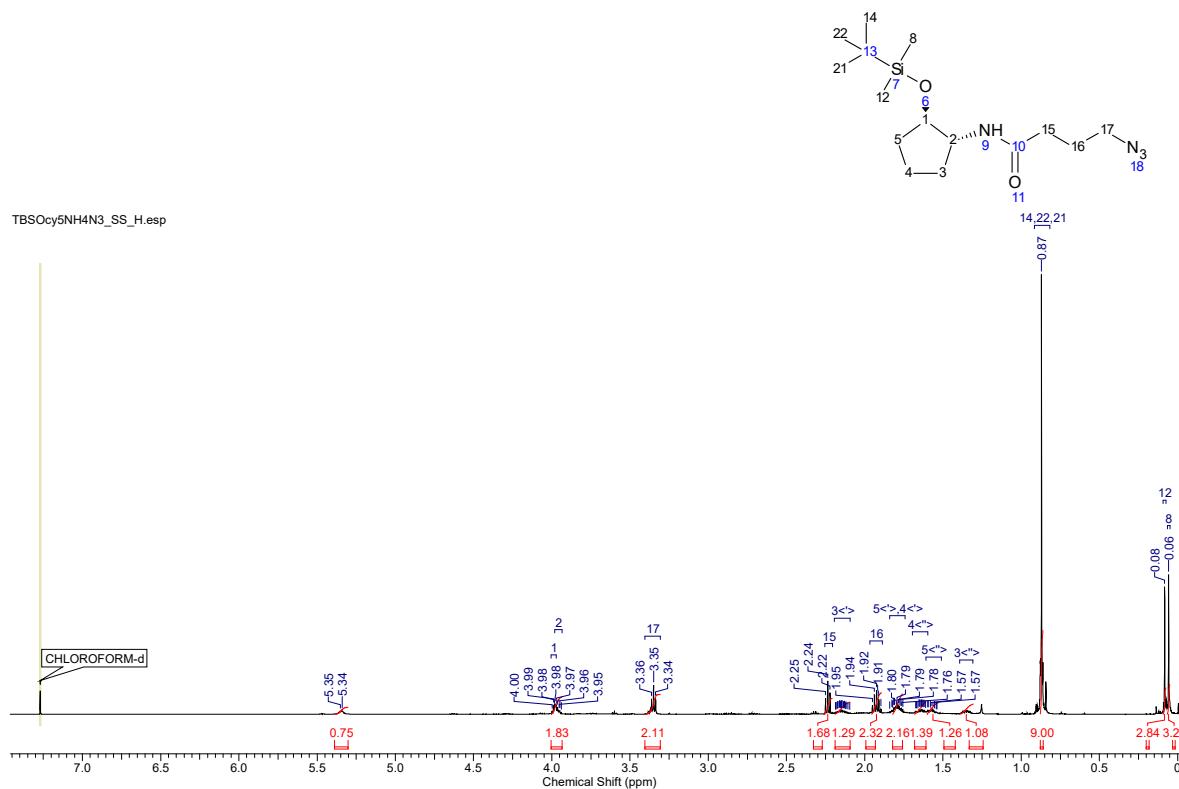
HOCY5NH4T4CIP_RR_H_ESP



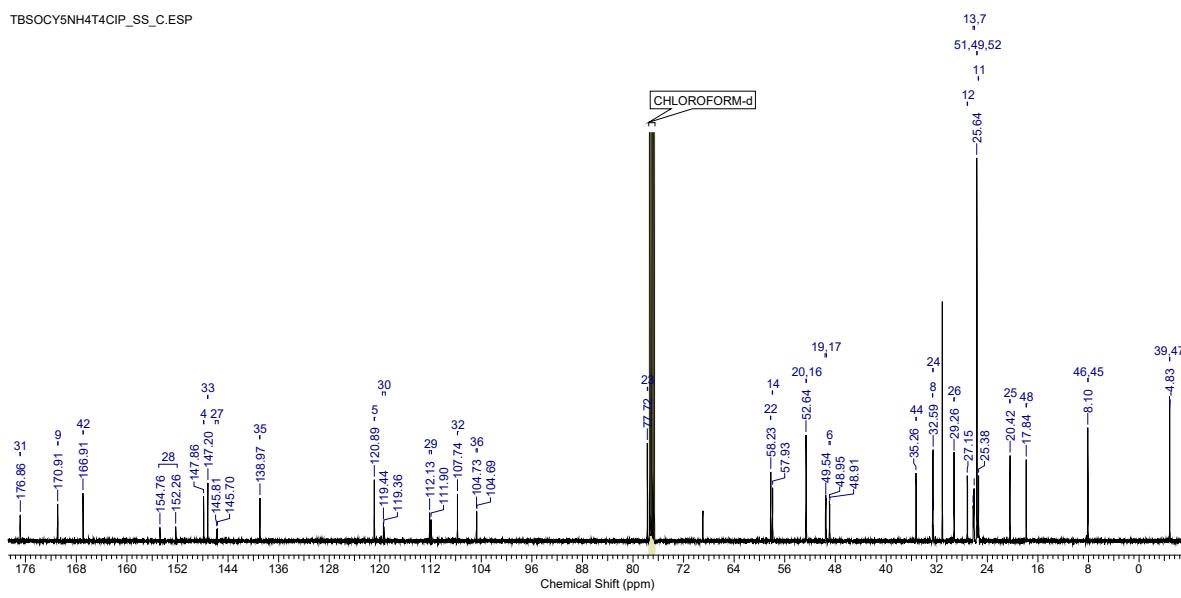
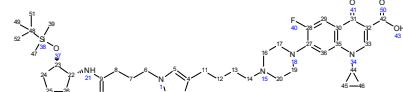
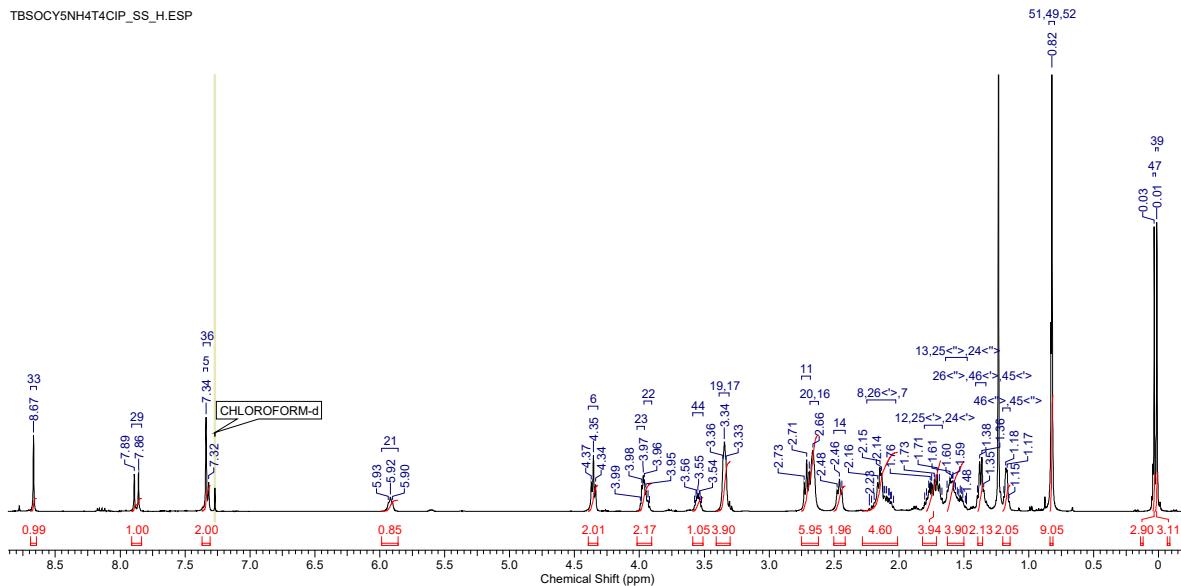
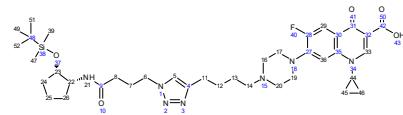
HOCY5NH4T4CIP_RR_C_ESP



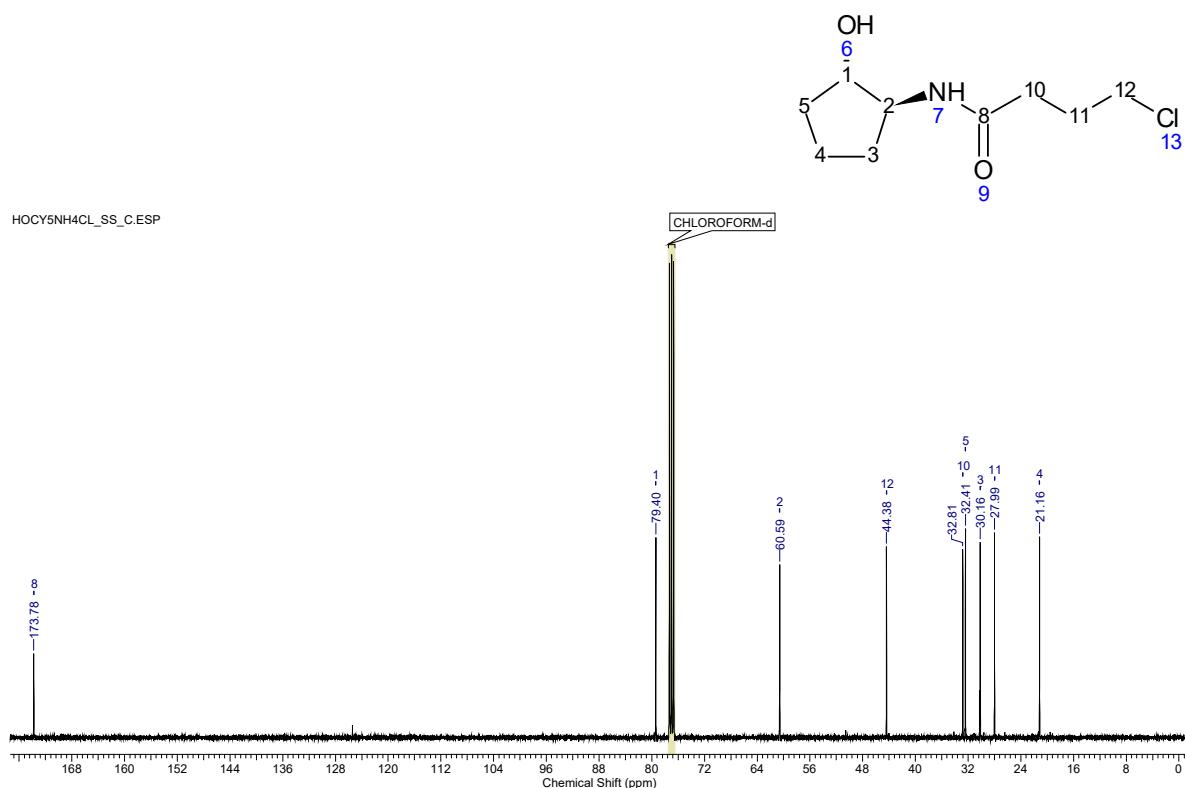
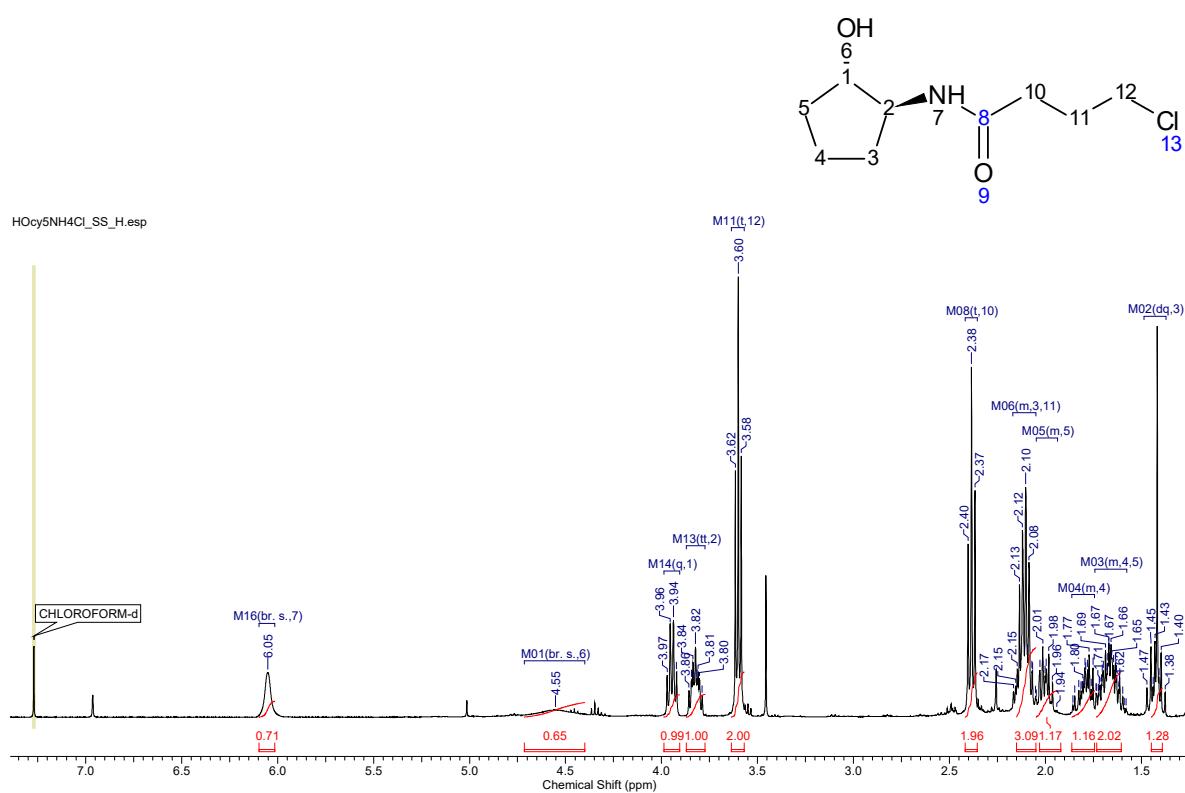
10.34 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide
186



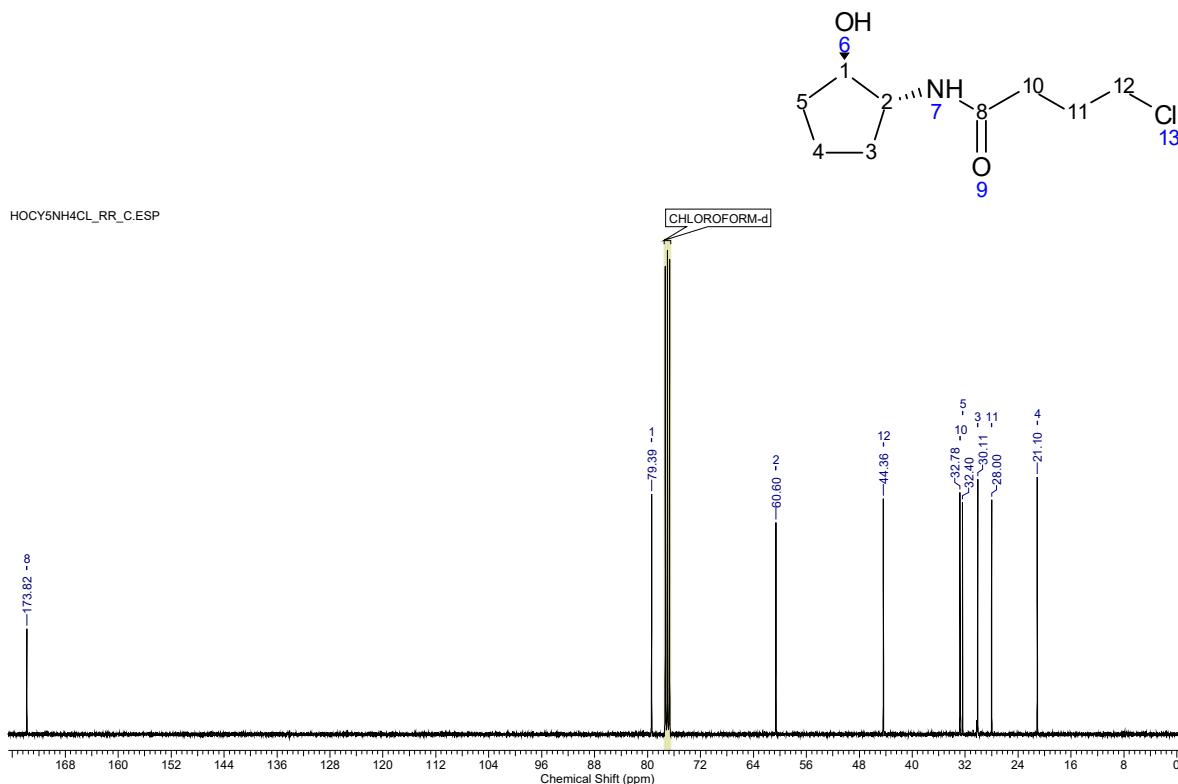
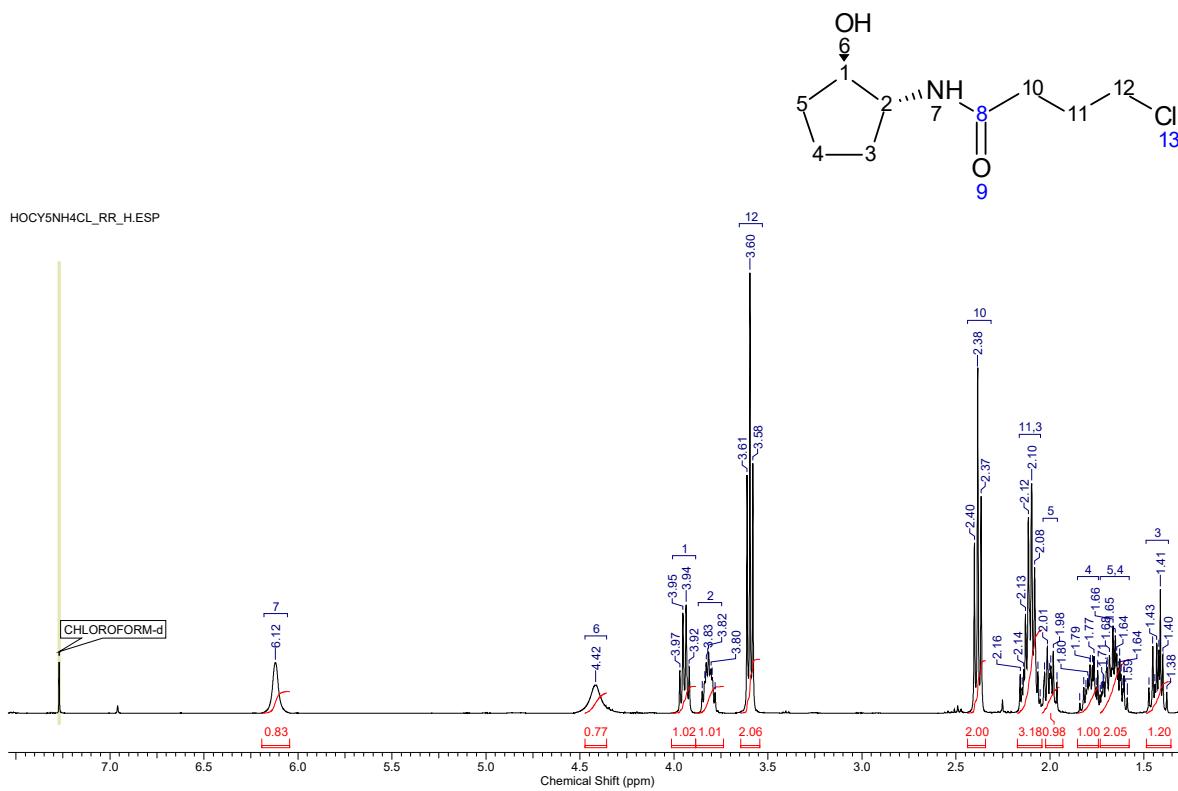
10.35 7-(4-(4-(1-(4-(((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 190



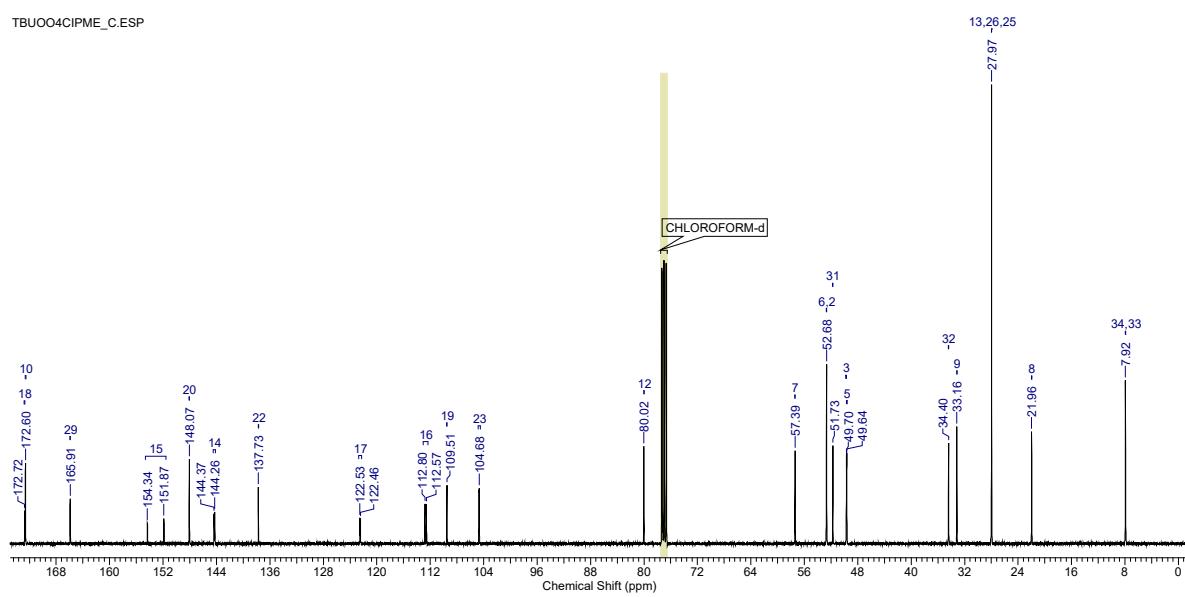
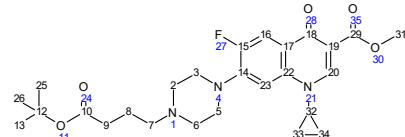
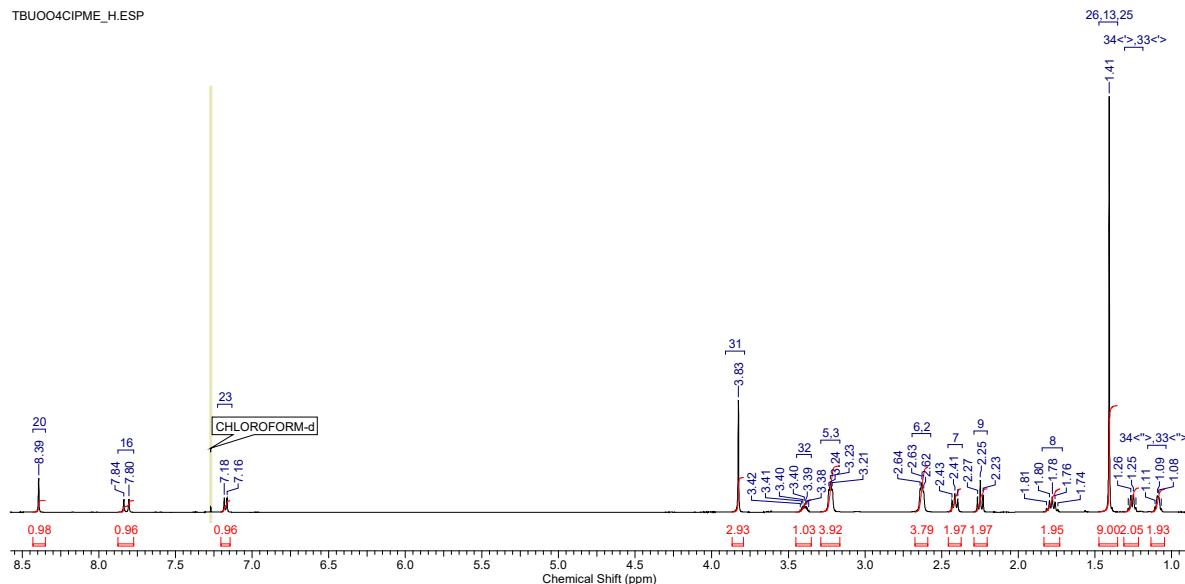
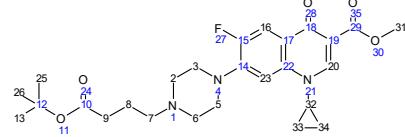
10.36 4-Chloro-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide 193



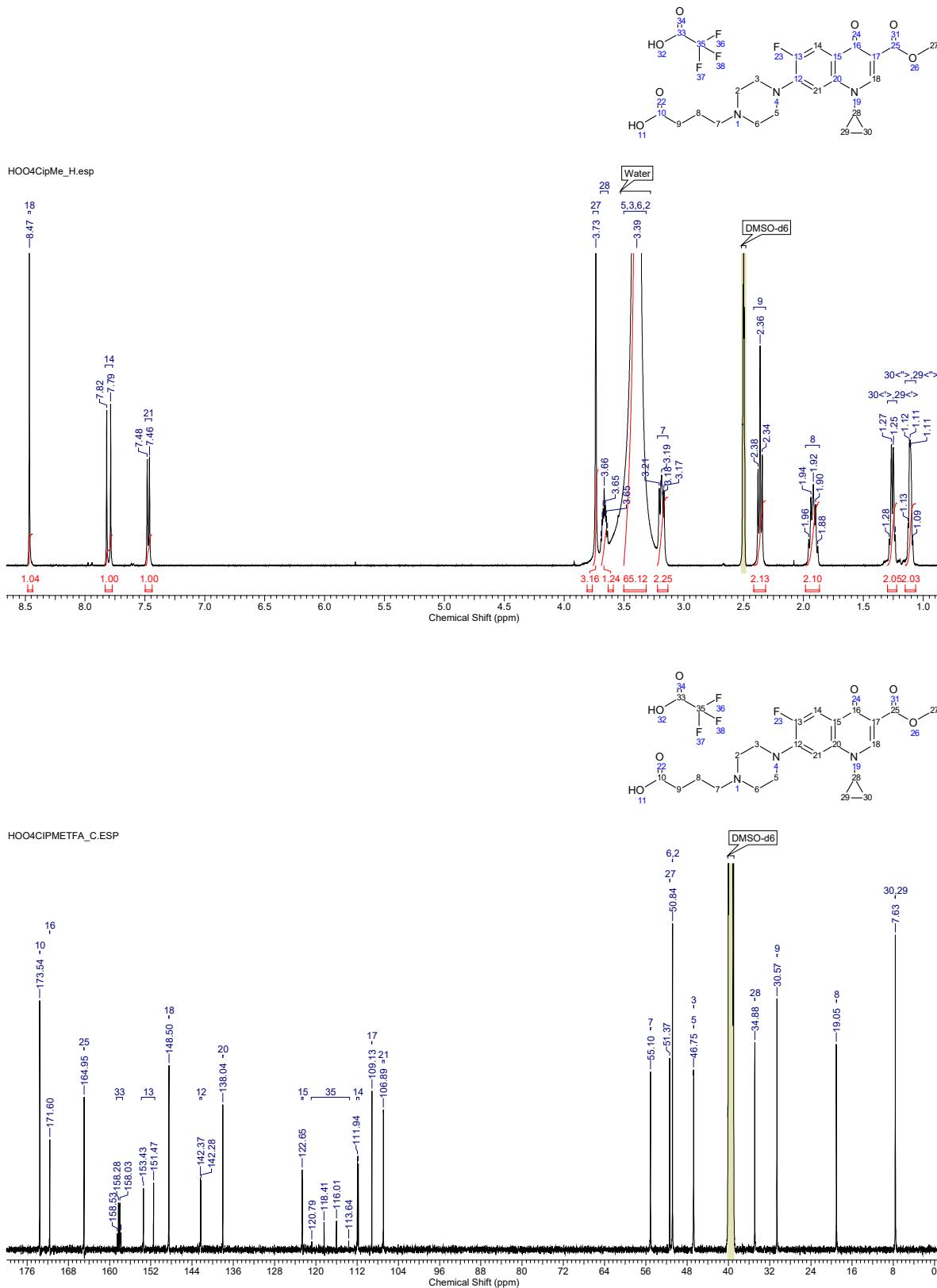
10.37 4-Chloro-*N*-(*1R,2R*)-2-hydroxycyclopentylbutanamide 194



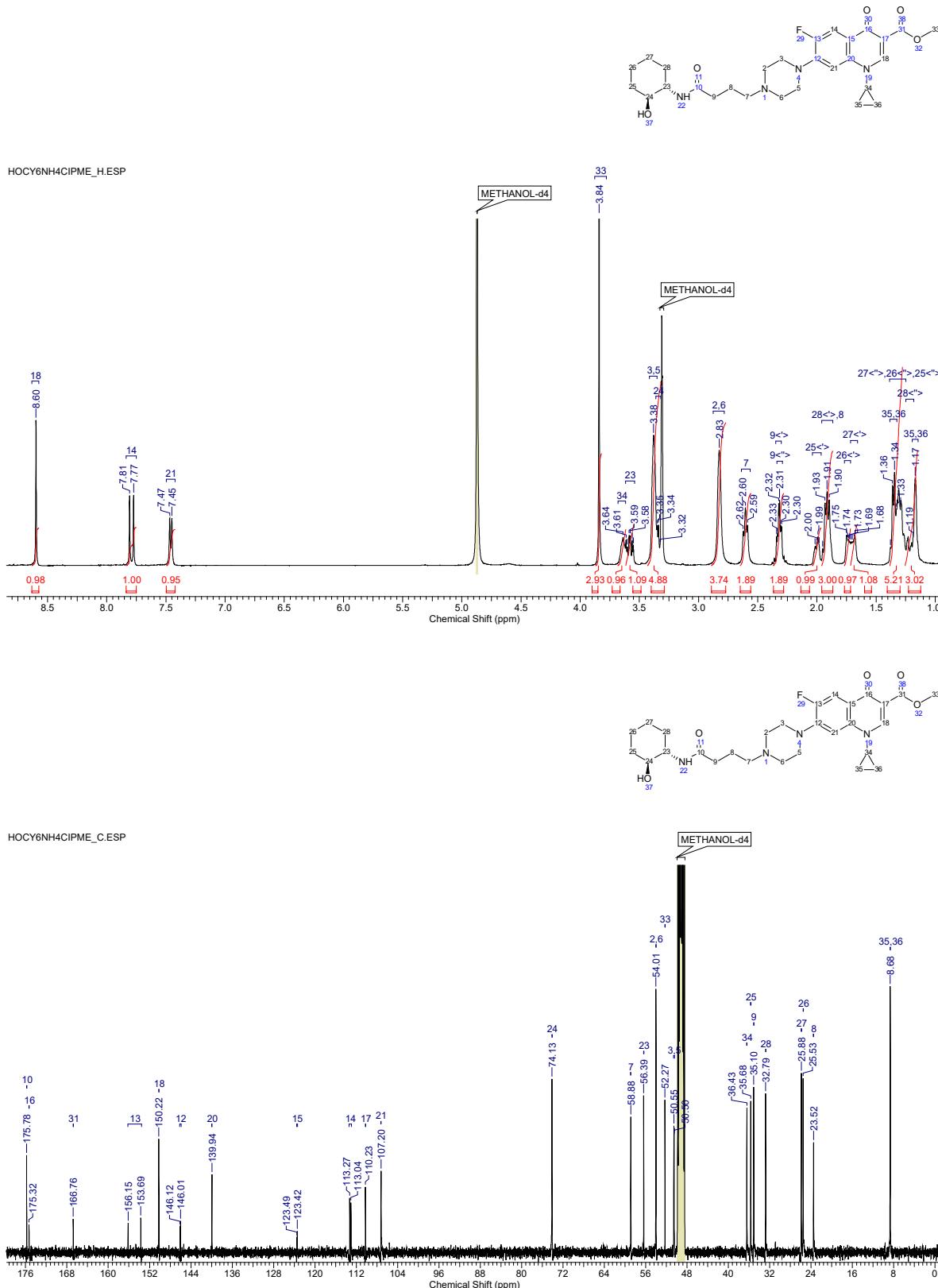
10.38 Methyl 7-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 197



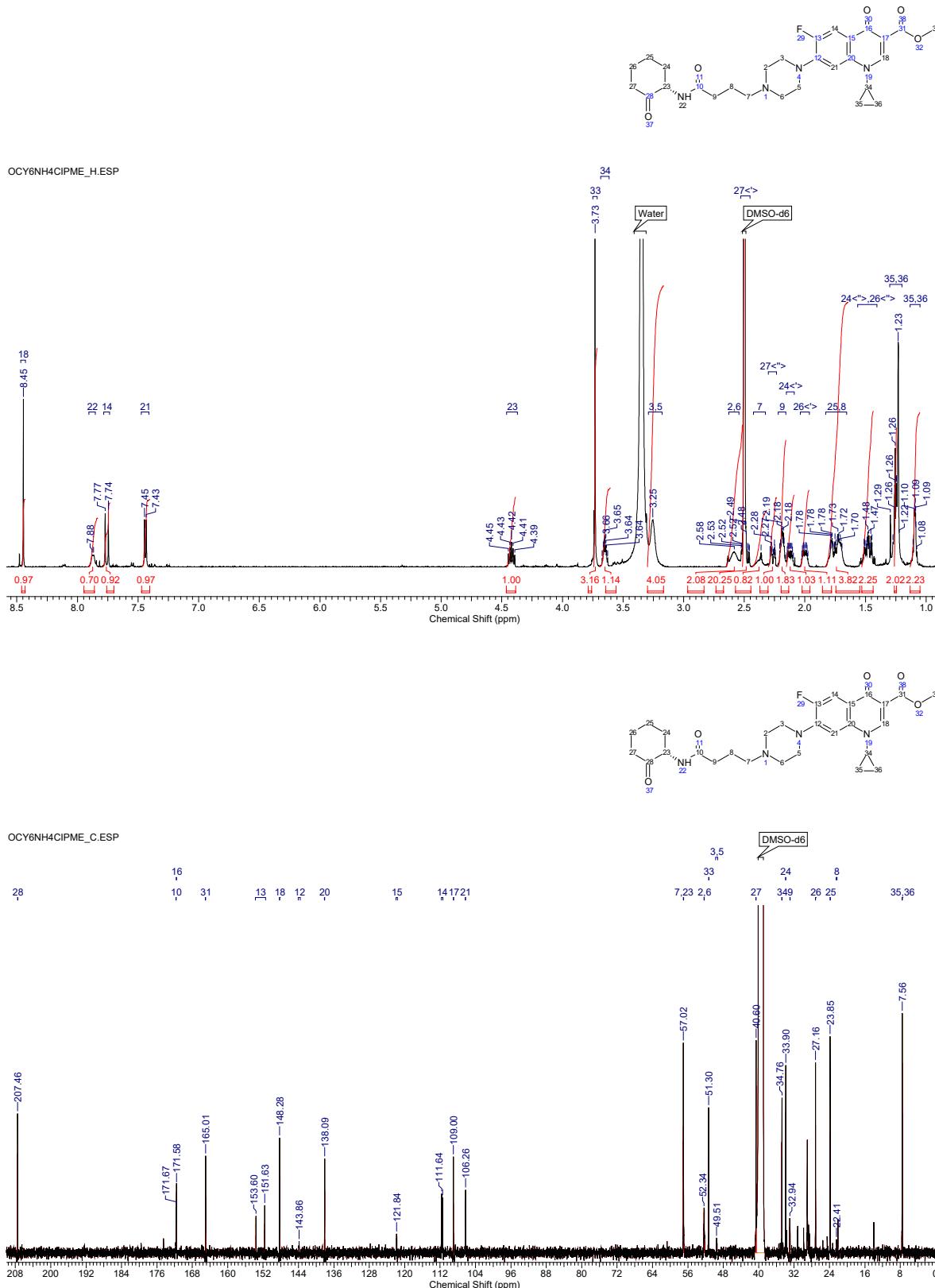
10.39 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt 198



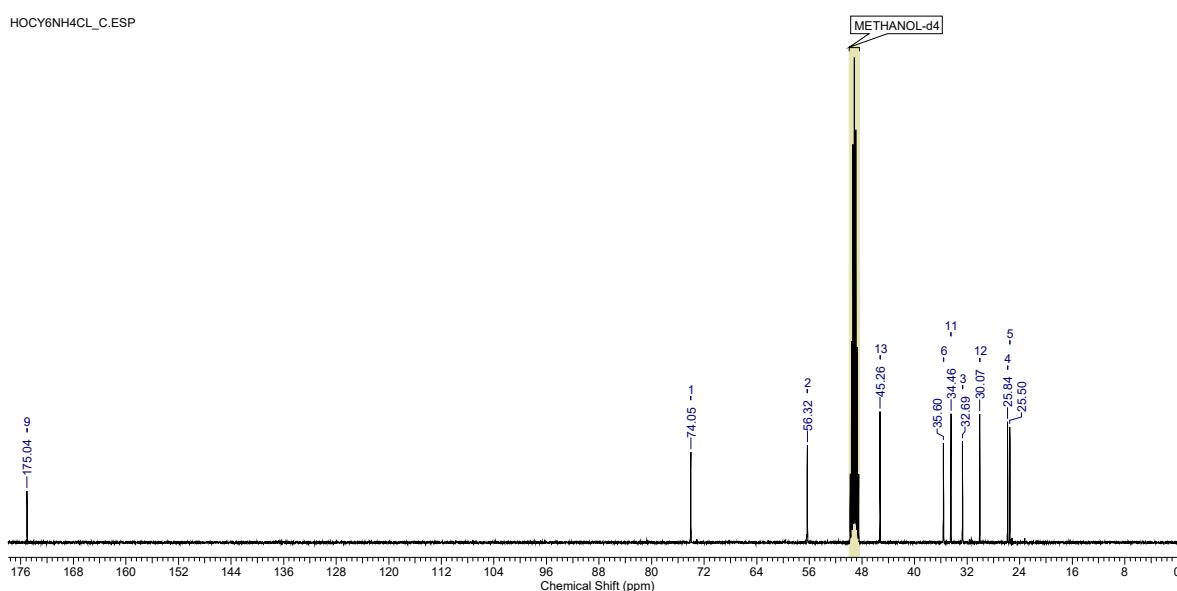
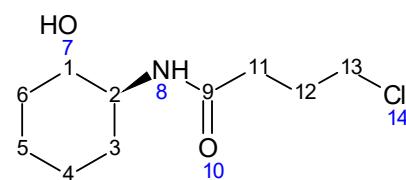
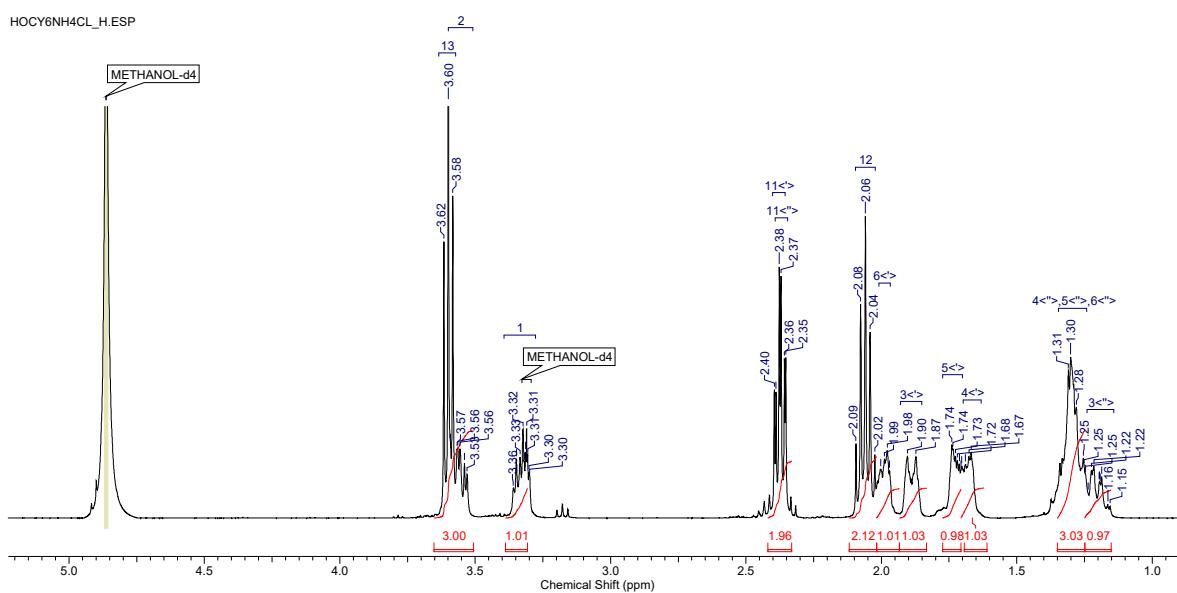
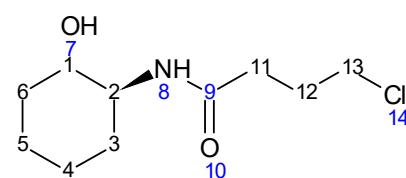
10.40 Methyl 1-cyclopropyl-6-fluoro-7-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 201



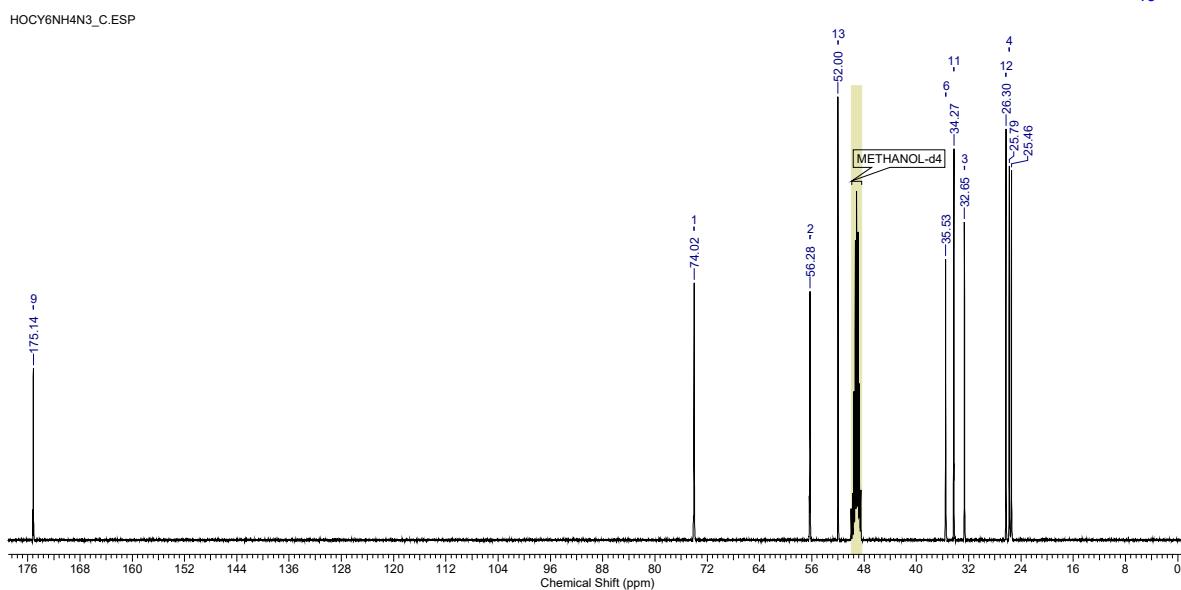
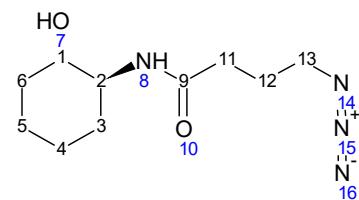
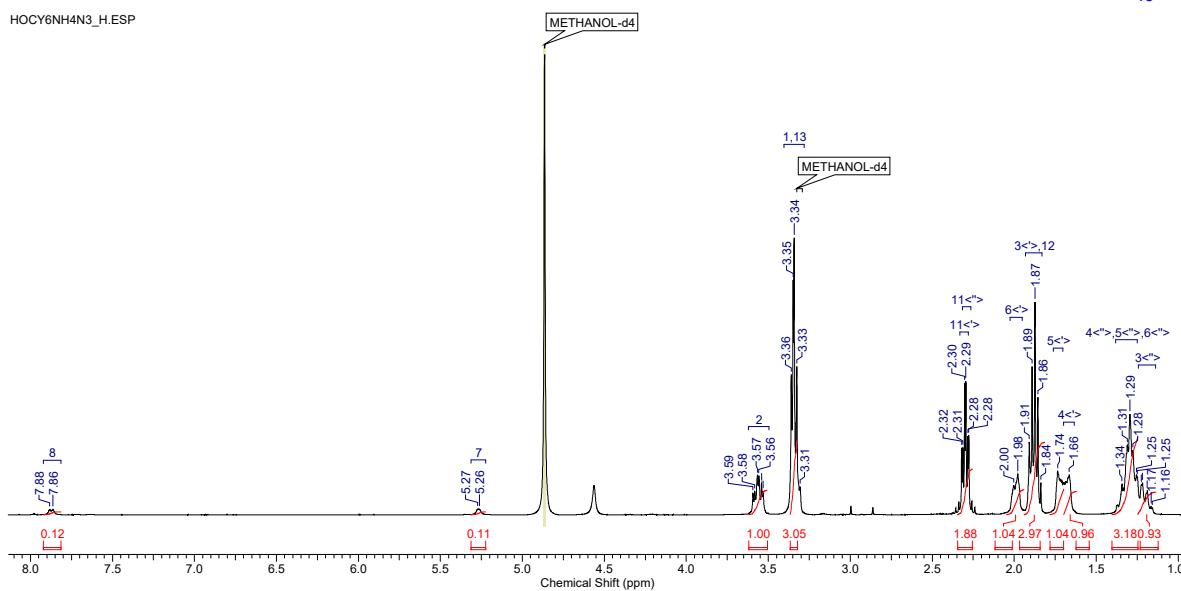
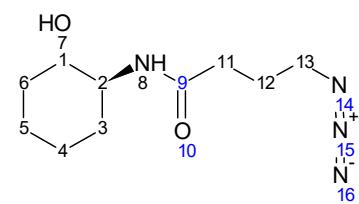
10.41 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 202



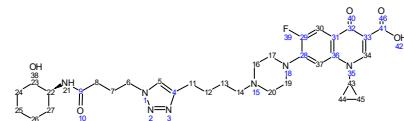
10.42 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 203



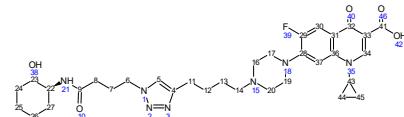
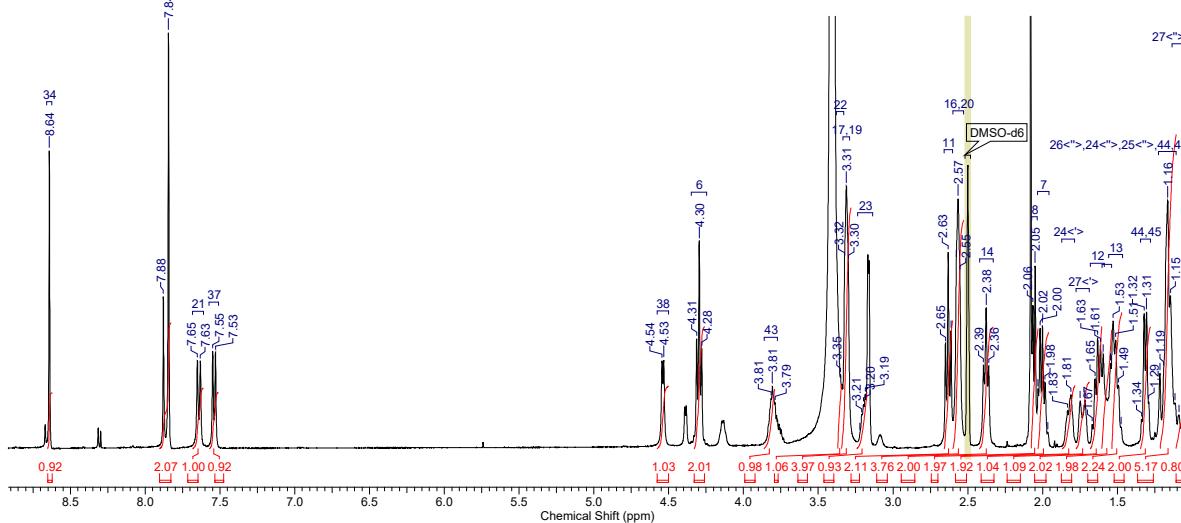
10.43 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 204



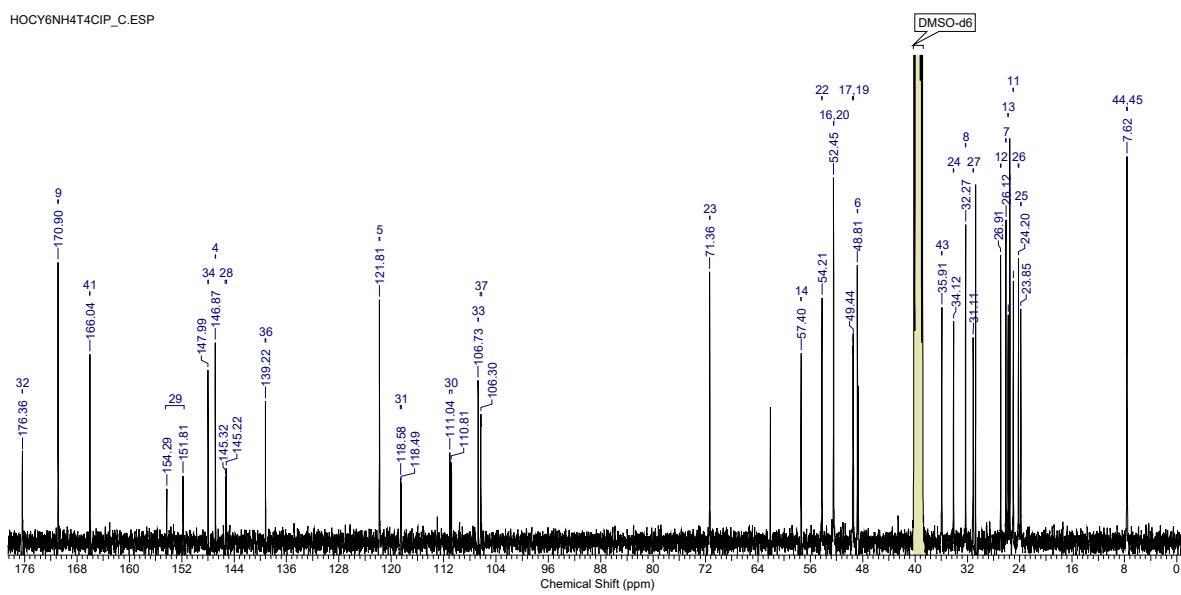
10.44 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 205



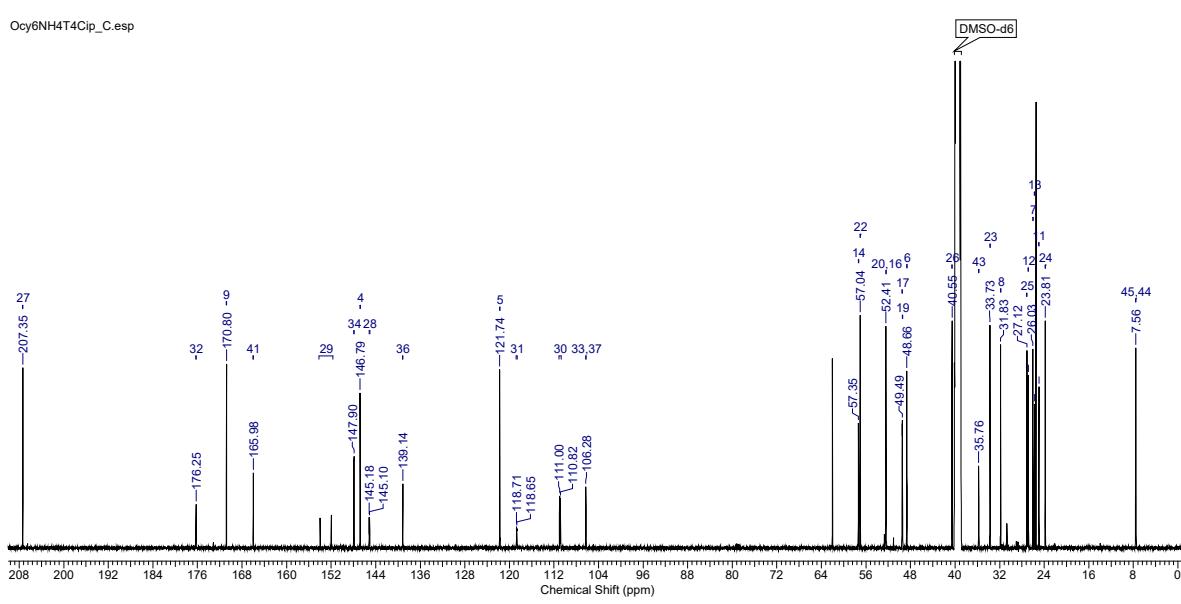
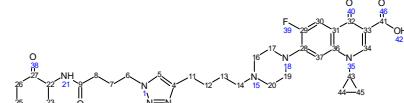
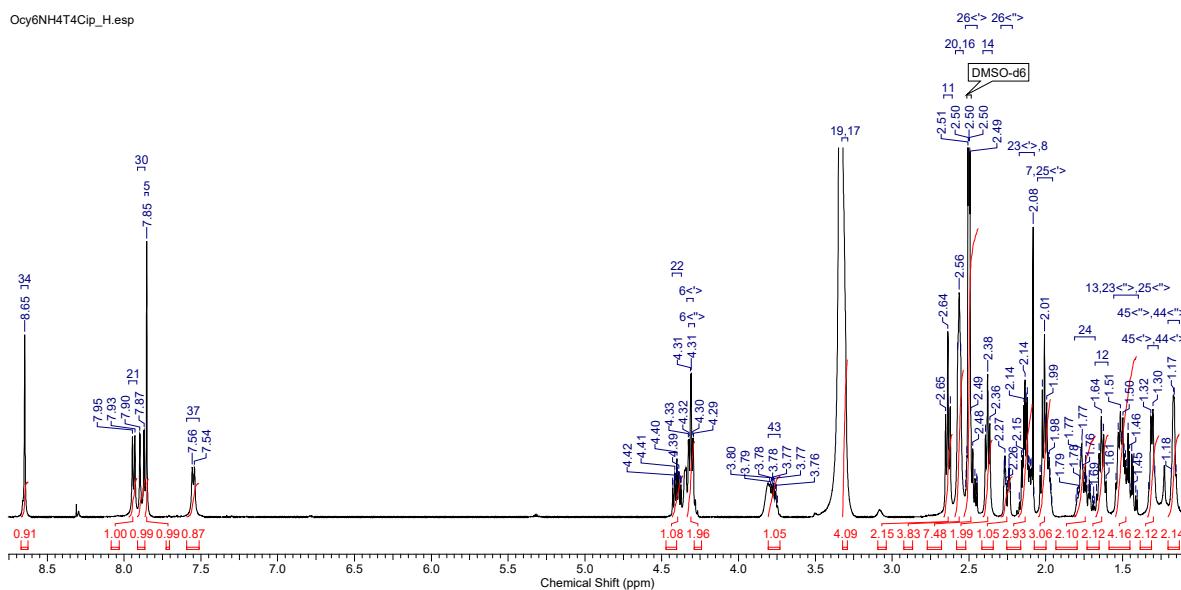
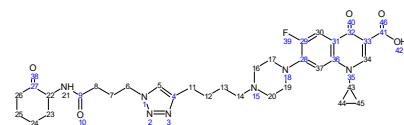
HOCY6NH4T4CIP_H_ESP_30



HOCY6NH4T4CIP_C_ESP



10.45 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxocyclohexyl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 206



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