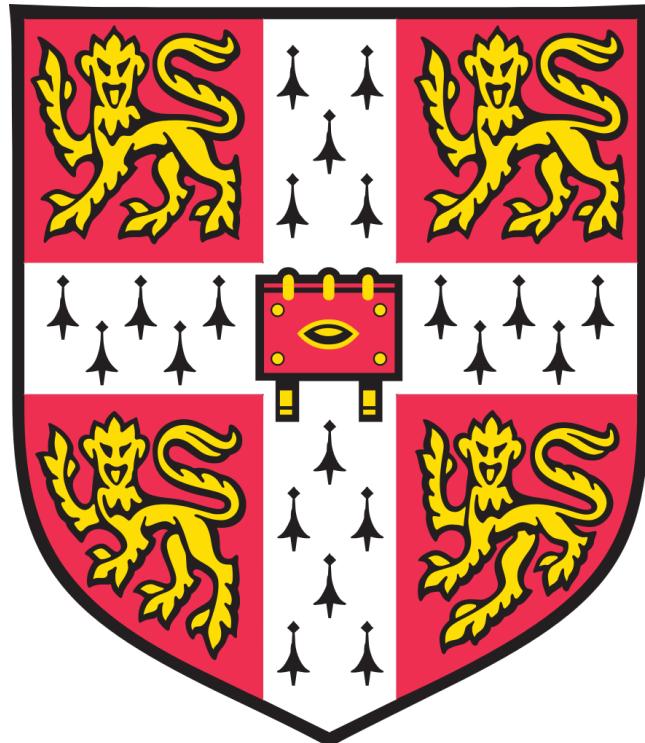


The synthesis and biological evaluation of a library of  
autoinducer-antibiotic conjugates

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## Contents

# 1 Introduction

## 1.1 Antibiotic resistance

Antibiotics add, on average, twenty years to a person's life.<sup>7</sup> However, antibiotic resistance is increasing alarmingly and is now recognised as a major threat to global health.<sup>7, 8</sup> 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 Suffolk<sup>9</sup> is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,<sup>7</sup> 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.<sup>7, 10</sup> This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see ??) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories:

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of  $\beta$ -lactam antibiotics such as penicillin by  $\beta$ -lactamase enzymes.
2. The bacterium may produce a membrane, cell wall or biofilm which does not allow the drug to pass through, for example biofilm formation may allow bacterial resistance to antibiotics to increase 1000-fold compared with bacteria in suspension culture.<sup>11</sup>
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*.<sup>12</sup>
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.<sup>13</sup>
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 *p*-aminobenzoic acid - a process which is blocked by sulfonamides.<sup>14</sup>

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.<sup>?, ?, ?, ?, ?, ?</sup>

## 1.2 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.’<sup>7</sup> A similar concept is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum population of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors and biofilm formation. It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium but effective when carried out as a group.

### 1.2.1 *Vibrio fischeri*

The first example of quorum sensing was discovered in *Vibrio fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid, *Euprymna scolopes*<sup>?, ?, ?</sup> (see ??a). This bacterium receives amino acids<sup>?, ?</sup> from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself<sup>?</sup> (*V. fischeri* also has symbiotic relationships with other species, including the Japanese pinecone fish, *Monocentris japonica*<sup>?</sup>)(see ??b).



Figure 1: a) "Euprymna scolopes, South shore of Oahu, Hawaii" by Jamie Foster. Licensed under CC BY-SA 3.0 via Commons. b) "Monocentris japonica.1 - Aquarium Finisterrae" by Drow\_male. Licensed under GFDL via Commons.

If a low population of *V. fischeri* is present in the photophore, the light that the bacteria could produce would be insufficient to attract prey. 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.

#### 1.2.1.1 The LuxR-LuxI system

The bacteria sense the population of other *V. fischeri* in their vicinity by the detection of 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> (see ??), a freely diffusible<sup>??</sup> molecule which is secreted by all *V. fischeri* cells<sup>??</sup> at a low basal level.<sup>??</sup> When the bacterial population density, and hence the concentration of 3-oxo-C<sub>6</sub>-HSL <sup>??</sup>, reaches a threshold, a response is triggered leading to expression of high levels of luciferase, and hence a 10,000-fold<sup>??</sup> increase in the production of (blue-green

The quorum sensing system of *V. fischeri* consists of two operons (see ??). The left operon encodes just one gene, *luxR*, a transcription factor which binds 3-oxo-C<sub>6</sub>-HSL <sup>??</sup>. The right operon encodes *luxICDABEG*. *luxI* encodes an enzyme (LuxI) which uses acyl-acyl carrier protein and *S*-adenosyl-L-methionine (SAM) to form 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> by lactonisation and acylation.<sup>??</sup> *luxCDABEG* encodes luciferase enzymes required for light production. Both operons are continuously expressed at low levels, leading to production of low concentrations of LuxI, 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> and LuxR, and low-level light production.<sup>??</sup>

*V. fischeri* can multiply to very high cell concentrations in the photophore of *E. scolopes* (around 10<sup>9</sup> cells,<sup>??</sup> or 10<sup>11</sup> cells per mL<sup>??</sup> in the organ of a mature squid). As concentrations rise to these levels, the concentration of 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> also rises. At a threshold of around 1-10  $\mu$ g/mL,<sup>??</sup> 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> binds to a N-terminal domain of LuxR,<sup>??</sup> leading to unmasking of the C-terminal transcriptional activator domain.<sup>??</sup> The LuxR-3-oxo-C<sub>6</sub>-HSL complex can then bind to the *lux* operator, which is situated between the left and right operons and, unusually, affects the transcription of both operons in a bidirectional manner, involving both positive and negative regulation.<sup>??</sup> It is thought that the LuxR-3-oxo-C<sub>6</sub>-HSL complex forms a homodimer,<sup>??</sup> but this has not been conclusively proven.<sup>??</sup>

Binding of LuxR-3-oxo-C<sub>6</sub>-HSL complex to the *lux* operator activates transcription of the right operon, leading to production of both 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> and light. Production of more 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> enables a positive feedback loop, re-inforcing the effect of high population density on 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> concentration and hence light production.

Concurrently, transcription of the left operon is also affected by binding of the LuxR-3-oxo-C<sub>6</sub>-HSL complex to the *lux* operator, but in a more complex manner. At low concentrations of 3-oxo-C<sub>6</sub>-HSL <sup>??</sup> transcription of

the left operon is activated, leading to production of more LuxR. However, at high concentrations of 3-oxo-C<sub>6</sub>-HSL ? production of LuxR is inhibited in an autoinducer-dependent manner.<sup>7</sup> This effect is dependent on DNA sequences found upstream of the left operon, within the right operon, and without them LuxR has a stimulatory effect at all concentrations of LuxR and autoinducer.

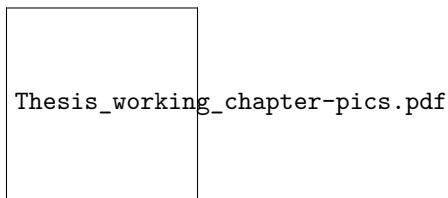


Figure 2: 3-oxo-C<sub>6</sub>-HSL ?.

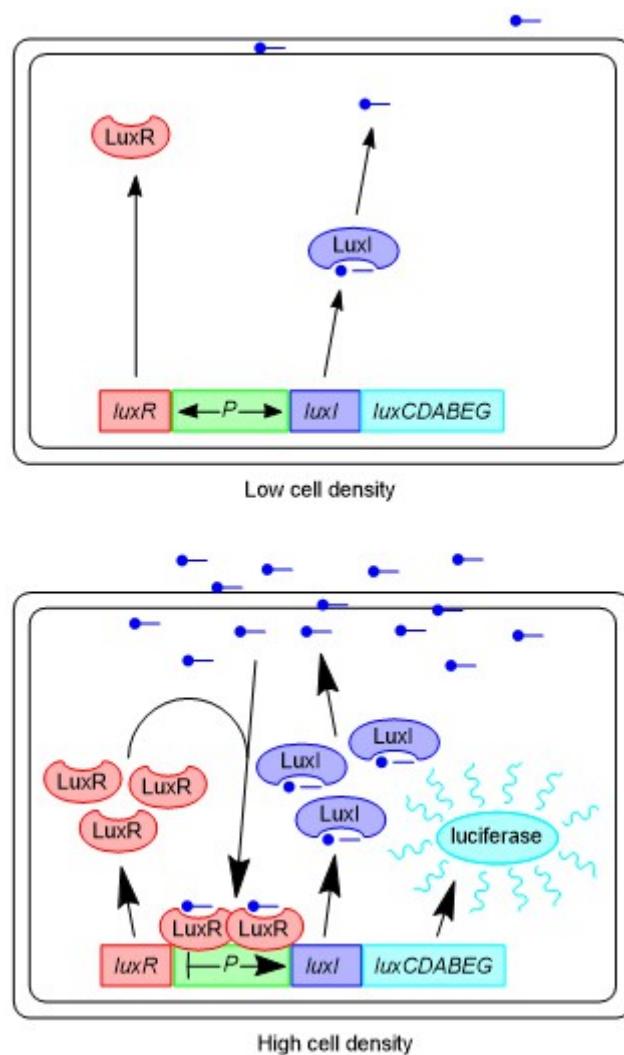


Figure 3: The LuxR-LuxI quorum sensing system in *V. fischeri*.

#### 1.2.1.2 Other quorum sensing systems

Since the discovery in 1970 of the LuxR-LuxI quorum sensing system in *V. fischeri*, several other mechanisms have also been discovered (it should be noted that several of the proteins mentioned in this section were first characterised in *Vibrio harveyi*, and functions may be assigned by analogy to a closely-related *V. harveyi* protein<sup>7</sup>). Systems using two other autoinducers, *N*-octanoyl-homoserine lactone (C<sub>8</sub>-HSL ?) and a furanosyl

borate diester (AI-2 ?) have been discovered, both of which act via the *luxCDABEG* system and increase luminescence by increasing luciferase production<sup>?,?</sup> (see ?? and ??). Additional controls in the *lux* promoter region have also been discovered, which respond to O<sub>2</sub> and cAMP<sup>?</sup> (see ?? and ??).

The AinS–AinR system uses C<sub>8</sub>-HSL ?, synthesised by AinS, as its signalling molecule<sup>?,?,?</sup> C<sub>8</sub>-HSL ? has two main effects on the quorum sensing network. Firstly, it can bind to LuxR,? albeit with lower affinity than 3-oxo-C<sub>6</sub>-HSL ?, leading to partial upregulation of lux operon transcription. Secondly, it binds to the histidine kinase AinR, inhibiting its ability to phosphorylate the histidine phosphotransferase LuxU, which links into the LuxR pathway by a less direct route (see later in this section).?

The LuxS–LuxP/Q system uses AI-2 ?, synthesised by LuxS, as its signalling molecule<sup>?,?,?</sup> (this pathway is common to all *Vibrio* species?). The receptor of AI-2 ? is a complex of a two proteins, LuxP and LuxQ. LuxP is a periplasmic protein which binds AI-2 ?, LuxQ is an inner membrane histidine kinase of the two-component sensor kinase family.<sup>?</sup> It is likely that LuxQ is constitutively dimeric, although this has not yet been demonstrated.<sup>?</sup>

When AI-2 ? is not bound to LuxP, LuxQ autophosphorylates a histidine residue.<sup>?</sup> This phosphoryl group is then transferred to an aspartic acid residue in LuxQ, and then to a histidine residue in LuxU.

When AI-2 ? binds to LuxP, this causes a major conformational change in the LuxP/Q complex, replacing one set of contacts between the proteins with another<sup>?,?</sup> and causing the formation of an asymmetric complex of two LuxP/Q dimers. Formation of the asymmetric dimers switches the activity of LuxQ from kinase to phosphatase, which can then dephosphorylate LuxU.

At high cell density, and hence autoinducer concentration, both the AinS–AinR system and the LuxS–LuxP/Q system bring about a decrease in the amount of phosphorylated LuxU. LuxU is a phosphotransferase protein which transfers its phosphate group to an aspartic acid residue in LuxO.<sup>?</sup> Phosphorylated LuxO inhibits quorum sensing responses by via LuxR. Hence, at high cell densities there is a decreased amount of phosphorylated LuxU present, leading to a lack of phosphorylated LuxO, and hence increased quorum sensing responses, e.g. light production. This ‘many-to-one’ signalling pathway is common in bacterial two-component signalling systems<sup>?</sup> and is found in several other *Vibrionaceae*.?

LuxO phosphate inhibits quorum sensing responses via  $\sigma_{54}$ -dependent transcriptional activation of *qrr1*<sup>?,?</sup> (despite the proximity of the *luxOU* and *qrr* promoters, LuxO only affects the activates the production of Qrr1 and not itself?). Qrr1 is a small RNA molecule (a quorum regulatory RNA or Qrr) which, with the help of Hfq, can bind to LitR RNA, leading to its degradation.<sup>?</sup> Qrr1 is the only Qrr to regulate LitR expression in *V. fischeri*, and is conserved across all *Vibrionaceae*.? In contrast, in other *Vibrionaceae* a family of Qrrs is often used.<sup>?</sup>

Qrr1/Hfq-mediated degradation of LitR mRNA inhibits the production of LitR, an activator of the *lux* operon. LitR binds to a region of the *luxR* promoter, causing increased LuxR production and hence increased bioluminescence.<sup>?</sup>

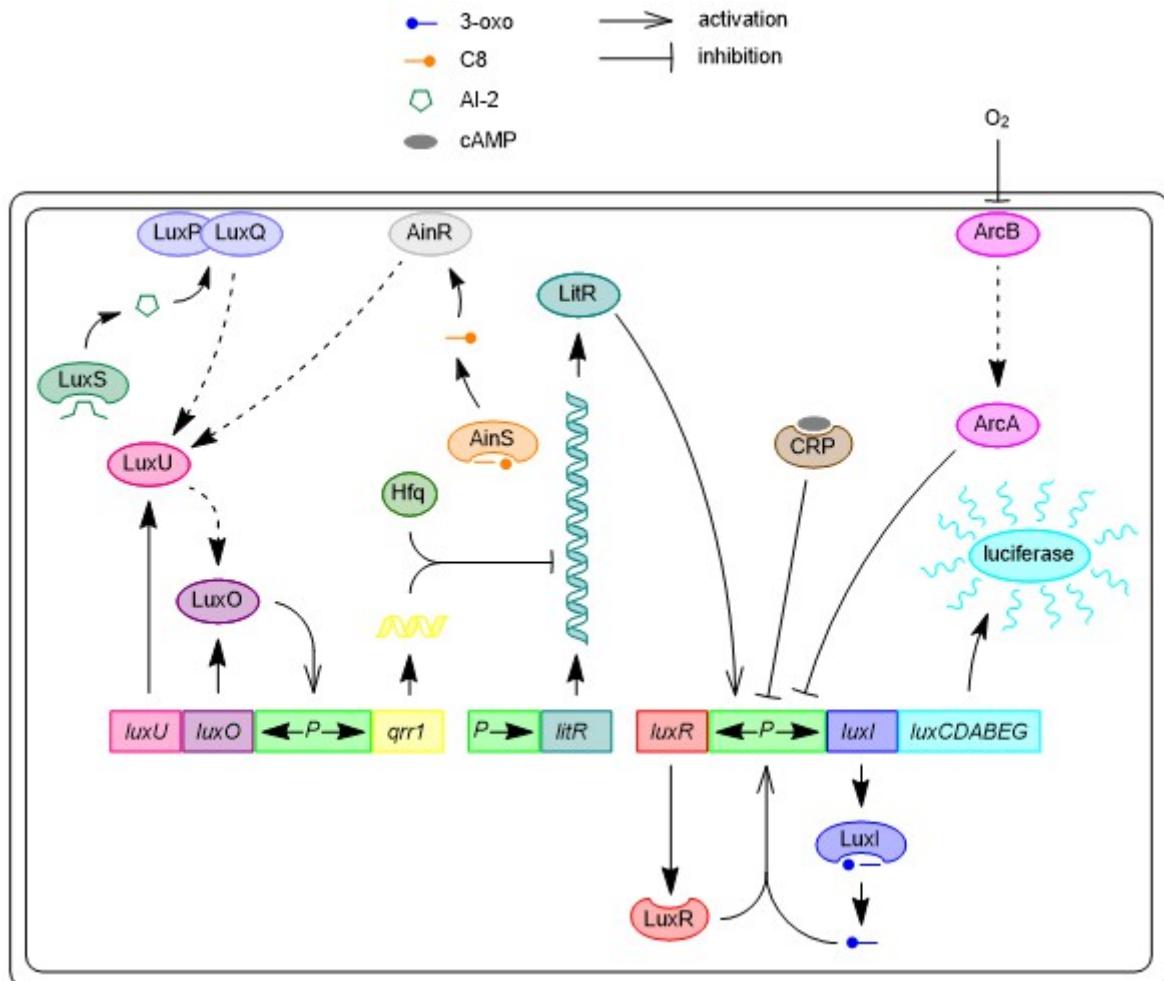


Figure 4: Quorum sensing in *V. fischeri*.

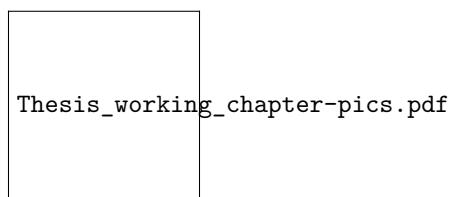


Figure 5: Autoinducers in *V. fischeri*.

### 1.2.1.3 The effect of O<sub>2</sub>

*V. fischeri* uses the ArcA-ArcB system to sense how oxygen-rich its environment is, by monitoring the redox state of various quinones produced by the cell. In an oxygen-rich environment, luminescence is stimulated. It is thought that luminescence is used by *V. fischeri* to make its environment less oxidising, as luminescence consumes oxygen.

ROS are bad and it doesn't want them?

ArcB is a histidine kinase which senses the redox state of the quinones. When the environment is low in oxygen or reactive oxygen species, the quinones are reduced and can stimulate ArcB to phosphorylate ArcA. ArcA is a response regulator which represses the transcription of *luxICDABEG* (and to a lesser extent, *luxR*).

#### 1.2.1.4 The effect of cAMP

cAMP

#### 1.2.1.5 The purpose of multiple signalling pathways

It might reasonably be asked: why does *V. fischeri* use three quorum sensing pathways rather than just one? The answer to this question lies in the bacterium's relationship with its squid host. It has been shown that the LuxS-LuxP/Q and AinS-AinR systems are important in the medium cell densities found during early colonisation of the host, whereas the LuxR-LuxI system is important at the higher cell densities found in late colonisation.<sup>?,?</sup>

It has been shown that the LuxS-LuxP/Q system does not have an especially large effect on colonisation of the host squid, although it does have some effect on luminescence.<sup>?</sup> It has therefore been speculated that the LuxS-LuxP/Q system is more important in the colonisation of other marine invertibrates, either in their light organs<sup>?,?</sup> or as part of multi-species colonies in their guts.<sup>?,?</sup>

The AinS-AinR system has a larger effect on colonisation and luminescence, in that *ainS* mutants show only 10-20 % of wild-type luciferase activity at medium cell densities in culture ( $10_8$  to  $10_8$  cells  $ml^{-1}$ ).<sup>?</sup> At the higher cell densities in the squid host ( $>10_{10}$  cells  $ml^{-1}$ ), *ainS* mutants show 10-40 % of wild-type luciferase activity, an effect which can be partially attributed to failure of the mutants to colonise the host (bacterial cell numbers are down to 20-80 % compared to the wild type). This failure of *ainS* mutants to colonise the host is due to *ain* regulation of pathways involved in early colonisation. The AinS-AinR system controls around 30 genes via LuxO and LitR.<sup>?</sup> *ain* quorum sensing is thought to repress several motility genes, causing loss of flagella, which are initially required for normal colonisation of the host,<sup>?,?</sup> but are lost as the bacteria colonise the host.<sup>?</sup> *ain* quorum sensing also induces a putative exopolysaccharide, which could be important in biofilm formation inside the host, or evasion of its immune system,<sup>?</sup> as well as two unique. In addition, *ain* quorum sensing affects the transcription several genes involved in metabolism, and new genes of unknown function which could affect colonisation by an as yet unknown pathway.

In contrast to the AinS-AinR, the LuxI-LuxR system is only fully induced at the high cell densities found in the *E. scolopes* light organ. At medium cell densities, C<sub>8</sub>-HSL<sup>?</sup> is thought to be the dominant autoinducer, partially activating transcription from the *lux* operon by binding to both AinR and LuxR.<sup>?</sup> At high cell densities, C<sub>8</sub>-HSL<sup>?</sup> is displaced from LuxR by 3-oxo-C<sub>6</sub>-HSL<sup>?</sup>, leading to full light production.

*lux* quorum sensing also affects the transcription of five non-*lux* proteins which could potentially act as late colonisation factors.<sup>?,?</sup> Three of these genes, *qsrP*, *acfA*, and *ribB*, are directly activated by LuxR/3-oxo-C<sub>6</sub>-HSL<sup>?</sup> and a strain lacking *qsrP* is less effective at colonising *E. scolopes* than the wild type, providing good evidence that it is a late-stage colonisation factor.<sup>?</sup>

ain positive feedback, LuxPQ not?

Which do CRP and Arc act on? Add phos. LitR upregulates AinS<sup>?</sup>

Quorum sensing has since been observed in many species of bacteria, including *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *S. aureus*, *Vibrio harveyi*, *Escherichia coli*, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica* *Aeromonas* sp. and *Acinetobacter* sp.<sup>?,?,?,?,?,?,?,?,?</sup> Many of these bacteria are significant causes of disease and death in humans, for example, it is estimated that in 2005 in the US *S. aureus* caused 477,927 hospitalisations and 11,406 deaths.<sup>?</sup> *S. aureus* uses a peptide autoinducer known as autoinducing peptide (AIP) (see ?? in ??) which interacts with the *agr* system leading to increased protease and toxin production.<sup>?</sup> *P. aeruginosa* also uses quorum sensing to coordinate biofilm formation, swarming motility and virulence.

### 1.2.2 *Pseudomonas aeruginosa*

One of the most well-studied examples of quorum sensing is in *P. aeruginosa*.<sup>?, ?, ?</sup> *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.<sup>?</sup>

*P. aeruginosa* has a low susceptibility to many antibiotics and readily acquires antibiotic resistance by mutation or horizontal gene transfer.<sup>?</sup> It is difficult for antibiotics to cross into cells due to low cell membrane permeability<sup>?</sup> and biofilm formation,<sup>?</sup> and they are pumped out again by its multiple chromosomally encoded multidrug efflux pumps.<sup>?</sup> *P. aeruginosa* biofilms are more resistant to many drugs including ciprofloxacin<sup>?</sup> and trimethoprim<sup>?</sup> compared with planktonic cells.<sup>?, ?</sup> 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 ??) and various proteins (see ??).<sup>?, ?, ?</sup> These can be broken down into three main, interacting systems: Las, Rhl and Pqs.

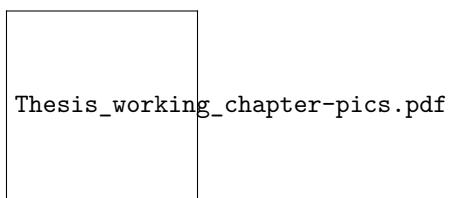


Figure 6: *P. aeruginosa* autoinducers.

In the Las system, LasI<sup>?</sup> synthesises the 3-oxo-C<sub>12</sub>-HSL<sup>?</sup> autoinducer. 3-oxo-C<sub>12</sub>-HSL<sup>?</sup> binds LasR,<sup>?</sup> and this complex upregulates the production of LasI<sup>?</sup> (thus causing autoinduction) as well as alkaline protease,<sup>?</sup> elastase,<sup>?</sup> exotoxin A,<sup>?</sup> HCN<sup>?</sup> and LasA protease.<sup>?</sup> The LasR complex is also important in late-stage biofilm formation,<sup>?</sup> and upregulates the Rhl<sup>?</sup> and Pqs systems.<sup>?, ?</sup>

In the Rhl system, RhII<sup>?</sup> synthesises the C<sub>4</sub>-HSL<sup>??</sup> autoinducer. C<sub>4</sub>-HSL<sup>?</sup> binds RhlR,<sup>?</sup> and this complex upregulates the production of RhII<sup>?</sup> (again causing autoinduction), alkaline protease,<sup>?</sup> elastase,<sup>?</sup> haemolysin,<sup>?</sup> HCN,<sup>?, ?</sup> LasA protease,<sup>?</sup> LecA,<sup>?</sup> pyocyanin<sup>?, ?</sup> and rhamnolipids.<sup>?</sup> The RhlR complex also downregulates the Pqs system.<sup>?, ?</sup> The Rhl system is controlled by both the Las and Pqs systems, as production of both RhlR and RhII is upregulated by the LasR complex<sup>?</sup> and production of both RhlR is upregulated by the PqsR complex.<sup>?</sup>

In the Pqs system, the main autoinducer, PQS<sup>?, ?</sup> is synthesised by multiple enzymes. PhnAB,<sup>?</sup> PqsA, PqsBC, PqsD<sup>?, ?</sup> and PqsE<sup>?, ?</sup> produce the precursor HHQ<sup>?</sup>, and PqsH converts HHQ<sup>?</sup> to PQS<sup>?</sup>. PQS<sup>??</sup> or HHQ<sup>?</sup> binds PqsR,<sup>?</sup> and either complex can upregulate the synthesis of HHQ<sup>?</sup> causing autoinduction. The PqsR-PQS complex upregulates the production of chitinase,<sup>?</sup> elastase,<sup>?</sup> HCN,<sup>?</sup> LecA,<sup>?</sup> pyocyanin<sup>?, ?</sup> and pyoverdine,<sup>?</sup> as well as increasing biofilm production<sup>?</sup> and vesicle formation.<sup>?</sup> The PqsR-PQS complex also upregulates production of RhlR, so the Pqs system has control over the Rhl system.<sup>?</sup> The Pqs system is controlled by both the Las and Rhl systems, as production of PqsR<sup>?</sup> and PqsH<sup>?</sup> is upregulated by the LasR complex and production of PqsA, PqsBC, PqsD, PqsE<sup>?</sup> and PqsR<sup>?</sup> is downregulated by the RhlR complex.

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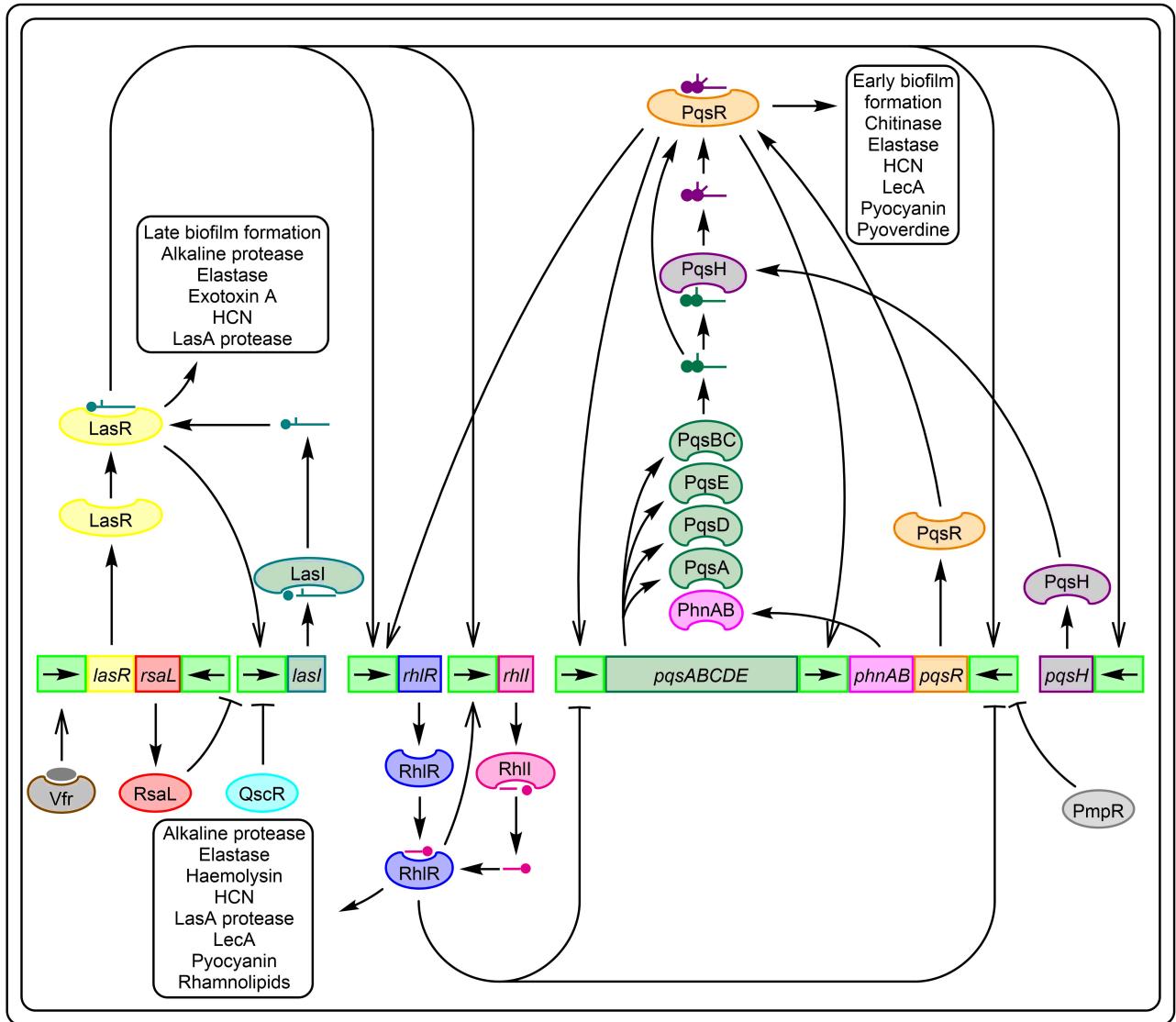
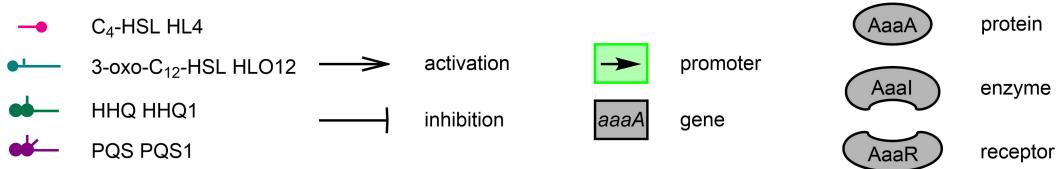


Figure 7: Quorum sensing in *P. aeruginosa*.<sup>?, ?, ?</sup>

In addition to the above systems, AI-2 (see ??), an interspecies signalling molecule,<sup>?</sup> is known to increase biofilm production and virulence in *P. aeruginosa*.<sup>?, ?</sup> 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 ?? as part of three interacting quorum sensing systems to coordinate virulence and biofilm production, and this makes these autoinducers interesting therapeutic targets. Quorum sensing has been successfully targeted using many different modulators,<sup>?, ?</sup> but this study takes a slightly different approach. Inspired by the success of various siderophore-antibiotic conjugates (see ??), a library of autoinducer-antibiotic conjugates was synthesised, in the hope that the importance of autoinducers in harmful cellular behaviours would lead to increased activity of the conjugates (see ??).

### 1.3 Siderophores

Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of ‘iron mining’.<sup>?</sup> 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  $\text{Fe}^{3+}$ , often with exceptionally high affinities. The iron-bound siderophores are then brought back into the cell by active transport and the iron is released, either by reduction of the  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  or by enzymatic degradation of the siderophore. Siderophores have a wide range of structures (see ?? and ??), possibly so one species can avoid its siderophores being taken up by another species.<sup>?</sup>

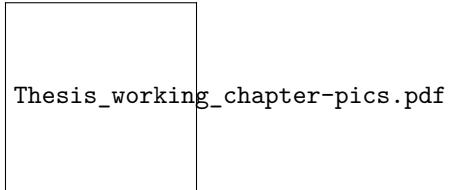


Figure 8: Iron-siderophore complexes: Deferoxamine <sup>??</sup> (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid <sup>??</sup> (*Rhodotorula pilimanae*), fusarinine C <sup>??</sup> (*Fusarium roseum*), enterobactin <sup>??</sup> (*Escherichia coli* and enteric bacteria), ferrichrome <sup>??</sup> (*Ustilago sphaerogena*, *U. maydis*, *Aspergillus niger*, *A. quadricinctus*, *A. duricaulis* and *Penicillium resticulosum*), yersiniabactin <sup>??</sup> (*Yersinia pestis*).

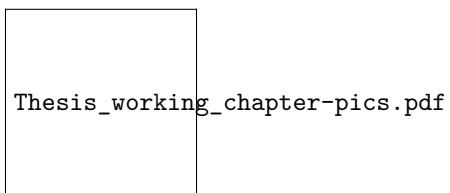


Figure 9: Iron-siderophore complexes: Pyochelin <sup>??</sup> (*P. aeruginosa*), Pyoverdine <sup>??</sup> (*P. aeruginosa*). Note that pyoverdine <sup>?</sup> is a tetradeятate ligand, hence the iron ion has two sites which can bind other ligands.

### 1.4 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins<sup>?</sup> (see ??). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds.

For example, albomycin <sup>?</sup> (see ??) is a sideromycin produced by *Actinomyces subtropicus* and *Streptomyces griseus*<sup>?,?</sup> which has been used to treat infections caused by various bacteria including *Yersinia enterocolitica* and *Streptococcus pneumoniae* in mice and humans.<sup>?,?</sup> Albomycin <sup>?</sup> contains a siderophore coupled to a nucleoside antibiotic via a peptide linker. The siderophore section is structurally similar to ferrichrome <sup>?</sup> (see ??), a siderophore produced by various fungi, but also taken up by bacteria including *Escherichia coli*, *Salmonella typhimurium* and *P. aeruginosa*.<sup>?,?</sup> It has been shown that because of the structural similarity to ferrichrome <sup>?</sup>, *E. coli* will also take up albomycin <sup>?,?</sup> 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 albomycin<sup>?</sup> and other sideromycins such as salmycin A<sup>?,?,?</sup> and ferrimycin A1<sup>?,?</sup> has served as encouragement to many researchers to explore synthetic siderophore-antibiotic conjugates, which will be discussed in the next section.

Figure 10: Iron-sideromycin complexes: Albomycin <sup>?, ?, ?</sup> (*Actinomyces subtropicus* and *Streptomyces griseus*), salmycin A <sup>?, ?, ?</sup> (*Streptomyces violaceus*) and ferrimycin <sup>?</sup> (*Streptomyces griseoflavus*).

## 1.5 Synthetic siderophore-antibiotic conjugates

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

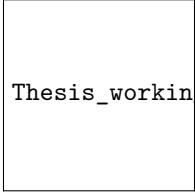
$\beta$ -lactam-sideromycin conjugates have been more widely investigated and show good activity *in vitro*, however, resistance can evolve by loss of the TonB transporter or of the relevant siderophore receptor, e.g. Cir and Fiu for catecholate siderophores or FhuA for hydroxamate siderophores.<sup>?</sup> Recently a conjugate (Ent-Amp ?, see ??) 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.<sup>?</sup> Other work has focused on monocyclic  $\beta$ -lactams, for example pirazmonam ? and U-78608 ?, which show high potency against Gram-negative bacteria including *P. aeruginosa*,<sup>?, ?</sup> Monocyclic  $\beta$ -lactams are generally fairly stable to  $\beta$ -lactamase activity, which is an advantage compared with many bicyclic  $\beta$ -lactams.

Three siderophore-antibiotic conjugates are reported as being in clinical trials:<sup>?</sup> MC-1 ?,<sup>?</sup> BAL30072 ?<sup>?</sup> (see ??) and cefiderocol ?,<sup>?, ?</sup>.

MC-1 ? is reported as being "in clinical phases of development",<sup>?</sup> but no reports of studies in humans could be found. However, experiments in mice have been promising.<sup>?</sup> BAL30072 ? is a siderophore- $\beta$ -lactam conjugate which showed initial promise as it is a poor substrate for  $\beta$ -lactamases, and resistance due to loss of transport proteins is infrequent.<sup>?</sup> However, it is unclear whether it will progress further in trials as it causes liver toxicity.<sup>?</sup> Cefiderocol ? 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'.<sup>?</sup>

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 ?-resistant mutants have been shown to be less virulent,<sup>?</sup> indicating that bacteria may lose out either by susceptibility to the antibiotic or by loss of fitness due to decreased iron transport.

Building on these positive examples, it is hoped that the strategy of conjugating a molecule which is important for virulence<sup>?</sup> with an antibiotic can be extended to conjugates of autoinducers and antibiotics in a similar 'Trojan horse' approach.



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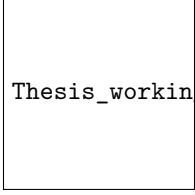
Figure 11: Ent-Amp ?,? pirazmonam ?,?,? U-78608 ?,?,? MC-1 ?,? BAL30072 ?,? and cefiderocol ?,?,?

## 1.6 Autoinducer-antibiotic conjugates

This study has extended 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. The work is divided into two main sections. The first section focuses on conjugates of three *P. aeruginosa* autoinducers (see ??) with ciprofloxacin and trimethoprim (see ??). The second section focuses on conjugates of homoserine lactone analogues with ciprofloxacin (see ??).

### 1.6.1 Synthesis of the conjugates

A copper(I)-catalysed azide-alkyne cycloaddition,?,? commonly referred to as a click reaction (although this is a more general term), was used to join each combination of autoinducer and antibiotic together (see ??). The autoinducers were functionalised with azide groups, and the antibiotics with alkynes. This modular approach would allow the library to be expanded easily by adding more autoinducers or antibiotics, or indeed other groups such as siderophores, fluorescent or affinity tags, or resin beads.



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

### 1.6.2 Autoinducers

The *P. aeruginosa* autoinducers (see ??) were chosen as *P. aeruginosa* is a significant human pathogen which shows high antibiotic resistance and utilises quorum sensing to coordinate pathogenic behaviours (see ??). Specifically, C<sub>4</sub>-HSL ?, HHQ ? and PQS ? derivatives were chosen as they were considered to be the most synthetically tractable.

### 1.6.3 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 ? or AI-2 ?, but it is known that PQS ? is exported in vesicles,? C<sub>4</sub>-HSL ? passively diffuses in and out of cells,? and 3-oxo-C<sub>12</sub>-HSL ? is taken up passively, accumulates in the cell membrane and is

actively pumped out by efflux pumps. The difference in transport mechanism for C<sub>4</sub>-HSL ? and 3-oxo-C<sub>12</sub>-HSL ? is thought to be largely due to chain length rather than the 3-oxo modification, as a shorter-chain version, 3-oxo-C<sub>6</sub>-HSL ? has been shown to be freely diffusible through *V. fischeri* membranes.?

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

#### 1.6.4 Antibiotics

Ciprofloxacin ? and trimethoprim ? (see ??) were chosen as the antibiotic sides of the conjugates.

Ciprofloxacin ? is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections including *P. aeruginosa*.?,?

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

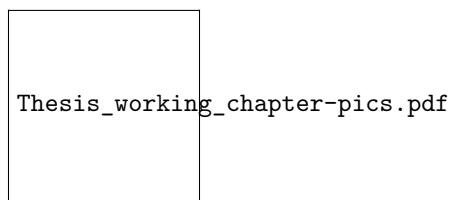


Figure 12: The antibiotics used in this section.

#### 1.6.5 Antibiotic efflux

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

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

#### 1.6.6 Conjugate efflux and antibiotic action

There are two ways in which the conjugates could disrupt *P. aeruginosa* growth:

1. *P. aeruginosa* could develop resistance to an autoinducer-antibiotic conjugate by upregulation of its export mechanism, but this would also lead to increased export of the native autoinducer, thus disrupting the quorum sensing system and hence biofilm formation and virulence.?,?,?  
For HSL conjugates this would mean upregulation of the MexAB-OprM pump, as this is the pump used for export of 3-oxo-C<sub>12</sub>-HSL ?.?,?  
For PQS conjugates this would mean upregulation of vesicle formation.?

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

## 1.7 Autoinducer 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 paper<sup>7</sup> and patent<sup>8</sup> by Ganguly *et al.*, who synthesised and characterised a conjugate<sup>9</sup> of methyl ciprofloxacin with homocysteine thiolactone (see ??). Homocysteine thiolactone is an analogue of homoserine lactone with the ring oxygen replaced by sulfur, and has been used as the head group in several other known quorum sensing modulators.<sup>7, 10, 11, 12, 13, 14, 15, 16</sup>

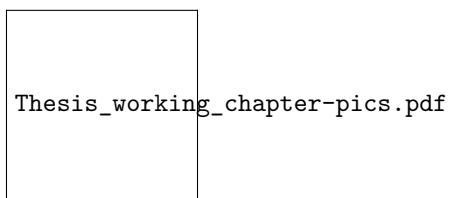


Figure 13: The HCTL-CipMe conjugate ? studied by Ganguly *et al.*? ?

As part of their characterisation of the HCTL-CipMe conjugate ?, 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  $\mu$ m), 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  $\mu$ M. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate ?. They then incubated cultures for 24 h, to allow biofilms to grow, before adding the compounds. In contrast, they found that the conjugate ? 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 C<sub>4</sub>-HCTL ? (see ??) has ‘either enhanced uptake or functional activity’ when compared with C<sub>4</sub>-HSL ?.

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

While one might expect the conjugate ? to behave like C<sub>4</sub>-HSL ?, and hence passively diffuse in and out of cells, it is possible that its transport more closely resembles that of 3-oxo-C<sub>12</sub>-HSL ?. 3-oxo-C<sub>12</sub>-HSL ?'s accumulation in membranes and interaction with efflux pumps is thought to be based primarily on tail chain

length (see ??), and the ciprofloxacin half of the conjugate ? could be seen as a long tail, especially as the carboxylic acid is methylated and hence less polar.

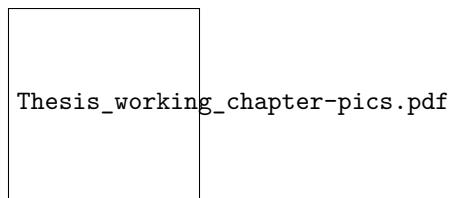


Figure 14: C<sub>4</sub>-HSL ? and C<sub>4</sub>-HCTL ?. Note that Ganguly *et al.* tested the *S* enantiomer of C<sub>4</sub>-HCTL ?, but used a racemic mixture in their HCTL-CipMe conjugate.

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 known as part of quorum sensing modulators. ?, ?

The activity of the chosen head groups against *P. aeruginosa* receptors when coupled with the native C<sub>4</sub> and 3-oxo-C<sub>12</sub> tails is summarised in ???. It is hoped that high activity of these molecules should correlate with high activity of their ciprofloxacin conjugates. This is not a comprehensive list of active head groups, and other possible choices are covered in ??.

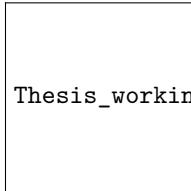
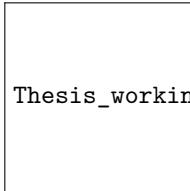
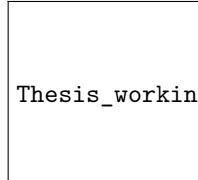
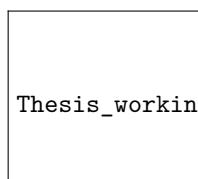
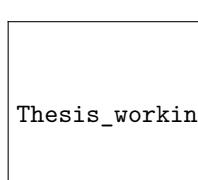
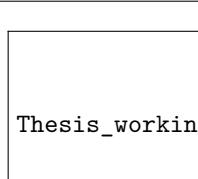
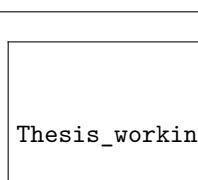
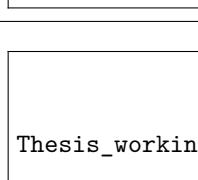
<b>Head group</b>	 Thesis_working_chapter-pics.pdf	 Thesis_working_chapter-pics.pdf
 Thesis_working_chapter-pics.pdf	Partial agonist and antagonist against LasR.? Shown to increase biofilm formation in <i>P. aeruginosa</i> .?	Strong agonist against LasR, with comparable activity to the native ligand.?, ?, ?, ?
 Thesis_working_chapter-pics.pdf	Partial agonist against LasR.?	Strong antagonist against LasR.?
 Thesis_working_chapter-pics.pdf	Poor agonist and antagonist against RhlR.?, ?	Strong antagonist against LasR.?
 Thesis_working_chapter-pics.pdf	Strong agonist against RhlR.? SS enantiomer is more potent.?	Partial agonist against LasR.?
 Thesis_working_chapter-pics.pdf	Strong agonist against RhlR.? SS enantiomer is more potent, with comparable activity to the native ligand.?	Strong agonist against LasR.?, ? SS enantiomer is more potent, with comparable activity to the native ligand.?
 Thesis_working_chapter-pics.pdf	Strong agonist against RhlR.? SS enantiomer is more potent.?	Partial antagonist against LasR.? Shown to reduce biofilm formation in <i>P. aeruginosa</i> .?

Table 2: Activities of autoinducers containing the chosen head groups when coupled with C<sub>4</sub> or 3-oxo-C<sub>12</sub> tails.

## 2 Autoinducer-antibiotic conjugates

### 2.1 Introduction

The first part of this project was focused on producing a library of autoinducer-antibiotic conjugates. *P. aeruginosa* autoinducers were used, in particular C<sub>4</sub>-HSL ?, HHQ ? and PQS ? (see ??). Azido derivatives of these compounds were coupled to alkynyl derivatives of antibiotics, specifically ciprofloxacin ? and trimethoprim ?, using a copper(I)-catalysed azide-alkyne cycloaddition. ?, ?

#### 2.1.1 Azido autoinducer derivatives

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

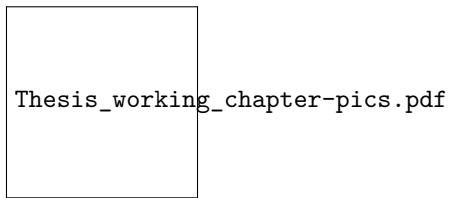


Figure 15: The azido derivatives of HHQ ? and PQS ?: ? and ?.

Alteration of the lactone group of C<sub>4</sub>-HSL ? and other HSL derivatives is known to significantly decrease activity, especially where the number of H-bond donors or acceptors is altered. ? Hence, the azide group was included on the tail of C<sub>4</sub>-HSL ?. Acyl tail length is known to play an important role in affinity, so three derivatives of C<sub>4</sub>-HSL ? were synthesised: N<sub>3</sub>-C<sub>2</sub>-HSL ?, N<sub>3</sub>-C<sub>4</sub>-HSL ? and N<sub>3</sub>-C<sub>6</sub>-HSL ? (see ??).

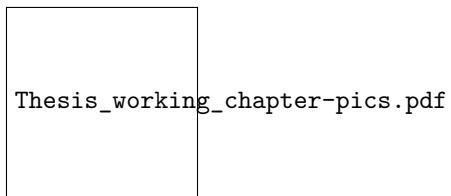
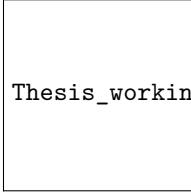


Figure 16: The azido derivatives of C<sub>4</sub>-HSL ?: ?, ? and ?.

#### 2.1.2 Alkynyl antibiotic derivatives

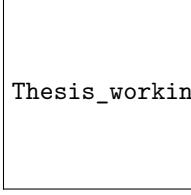
The structure-activity relationships for ciprofloxacin have been investigated? and modifications at the cyclopropane and piperazine groups were found not to cause loss of activity. It was decided an alkyne tail would be added onto the free NH of the piperazine ring, as this position is more synthetically accessible. Alkynyl ciprofloxacin derivative ? (see ??) was synthesised in this study (see ??), and two cleavable alkynyl ciprofloxacin derivatives ? and ? were synthesised by Dr Eddy Sotelo and combined with some of the azido HSL derivatives made in this study (see ?? and ??).



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Figure 17: The alkynyl ciprofloxacin derivatives ?, ?, and ?.

The choice to of alkyne tail attachment point on trimethoprim ? (see ??) is based on the use of that same point in a fluorogenic trimethoprim tag synthesised by Jing *et al.*?



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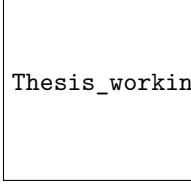
Figure 18: The alkynyl trimethoprim derivative ?.

## 2.2 Azido autoinducer derivatives

### 2.2.1 Synthesis of 6-N<sub>3</sub>-HHQ ?

The synthesis of 6-N<sub>3</sub>-HHQ ? is shown in ?? and follows a route devised by Baker.?<sup>7</sup> Octanoyl chloride ? was converted to  $\beta$ -Ketoester ? via a Meldrum's acid adduct.<sup>?,7</sup> The  $\beta$ -ketoester ? was condensed with *N*-Boc-*p*-phenylenediamine ? to form enamine ?. The disappointing yield of this step was in part due to the reaction proceeding to an equilibrium state rather than to completion, and hence not all of the starting material being consumed; starting materials can be recycled to improve the yield. Alternatively, Baker later found a higher-yielding reaction using a ZrCl<sub>4</sub> catalyst.

The enamine ? was cyclised with polyphosphoric acid to form amino-HHQ ? in good yield. The amine group of amino-HHQ ? was converted to a diazo group by reaction with NaNO<sub>2</sub> and HCl, followed by displacement with NaN<sub>3</sub> to form the final azido-HHQ product ?.<sup>7</sup>



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Scheme 2: The synthesis of ?. a) i) Pyridine, DCM, 0 °C. ii) MeOH, reflux, 66 % over two steps. b) MeOH, reflux, 19 %. c) Polyphosphoric acid, 120 °C, 72 %. d) i) NaNO<sub>2</sub>, HCl, H<sub>2</sub>O, 0 °C. ii) NaN<sub>3</sub>, H<sub>2</sub>O, r.t., 46.5 %.

### 2.2.2 Synthesis of 6-N<sub>3</sub>-PQS ?

The synthesis of 6-N<sub>3</sub>-PQS ? is shown in ??, and also follows a route devised by Baker.<sup>7</sup> The Weinreb amide ?<sup>7</sup> was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide ? to form 1-chlorononan-2-one ? following a procedure described by Hodgkinson *et al.*?

The synthesis of PQS ? described by Hodgkinson *et al.*? used a microwave reaction of 1-chlorononan-2-one ? with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid ? 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 ? was used.?<sup>7</sup> Therefore, a two step process was employed instead.

5-Nitroanthranilic acid ? was heated with  $K_2CO_3$  to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one ? to form the ester ? by  $S_N2$  displacement of the chlorine atom in a procedure adapted from Hlaváč *et al.*? Cyclisation with polyphosphoric acid produced nitro-PQS ? cleanly.<sup>7,7</sup>

Conditions for the reduction of the nitro group were then compared (see ??). Baker initially used Zn and HCl, however this gave a yield over 100 % suggesting coordination of Zn to the amino-PQS ?? (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 ?. 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.?

Finally, amino-PQS ? was converted to azido-PQS ? 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 ?.? The yield of this reaction was rather disappointing (28 %), and is probably due to loss of product in the supernatant following precipitation.?

Conditions	Outcome
$H_2$ , Pd/C, 1 atm, r.t., 18 h	No reaction
$NH_4HCO_2$ , Pd/C, 1 atm, r.t., 18 h	No reaction
Zn, HCl (aq), r.t., 5 min h	Product ? + Zn, assumed quantitative yield
$SnCl_2 \cdot 2H_2O$ , MeOH, r.t., 18 h	No reaction
$H_2$ , Pd/C, MeOH, 3 atm, r.t., 4 h.	Product ?, 100 % yield
$H_2$ , $PtO_2$ , MeOH, 1 atm, r.t., 45 min	Product ?, 80 % yield

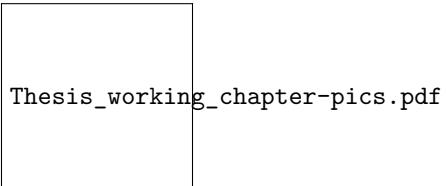
Table 3: Conditions attempted for the synthesis of ?. Rows 1-3 were carried out by Baker,<sup>7</sup> rows 4-6 were carried out as part of this study.

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Scheme 3: The synthesis of ?. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) *N,O*-dimethylhydroxyl amine hydrochloride,  $K_2CO_3$ , toluene,  $H_2O$ , - 5 °C to r.t., 30 min, 71 %. c) THF, 0 °C to r.t., 15 h, 96 %. d) ?,  $K_2CO_3$ , DMF, 90 °C, 1 h, then ?, r.t., 18 h, 100 %. e) Polyphosphoric acid, 90 °C, 5.5 h, 40 %. f)  $H_2$ ,  $PtO_2$ , MeOH, 1 atm, r.t., 45 min, 80 %. g) i)  $NaNO_2$ , HCl,  $H_2O$ , 0 °C, 50 min. ii)  $NaN_3$ ,  $H_2O$ , r.t., 4 h, 28 % over two steps.

### 2.2.3 Synthesis of the azido C<sub>4</sub>-HSL derivatives ?, ?, and ?

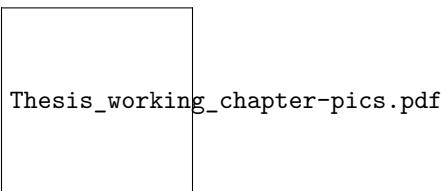
N<sub>3</sub>-C<sub>2</sub>-HSL ? (the azido derivative of C<sub>4</sub>-HSL with a C<sub>2</sub> chain, see ??) has previously been prepared by Stacy *et al.*? Their synthesis was followed, starting with the cyclisation of L-methionine ? using bromoacetic acid to form the homoserine lactone HBr salt ?. The disappointing yield can be attributed to difficulties in precipitating the final product. The homoserine lactone HBr salt ? was then converted by a biphasic one-pot process to N<sub>3</sub>-C<sub>2</sub>-HSL ? using bromoacetyl bromide ? and NaN<sub>3</sub>.



Scheme 4: The synthesis of ?. a) Bromoacetic acid, *i*-PrOH:H<sub>2</sub>O:AcOH (5:5:2), r.t., 18 h, 41 %. b) NaN<sub>3</sub>, NaHCO<sub>3</sub>, H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, 41 %.

It was hoped that this procedure could also be used to produce the C<sub>4</sub> and C<sub>6</sub> derivatives, however, attempts to convert homoserine lactone ? to N<sub>3</sub>-C<sub>4</sub>-HSL ? using 4-bromobutyryl chloride ? produced a complex mixture of products. This is likely to be because the S<sub>N</sub>2 reaction in which the azide anion displaces bromine is slower for the C<sub>4</sub> 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<sub>N</sub>2 reaction with NaN<sub>3</sub> (see ??).

Reaction of the homoserine lactone HBr salt ? with 4-bromobutyryl chloride ? or 6-bromohexanoyl chloride ? produced Br-C<sub>4</sub>-HSL ? or Br-C<sub>6</sub>-HSL ? respectively, in good yields. Heating with NaN<sub>3</sub> in DMF converted Br-C<sub>6</sub>-HSL ? to N<sub>3</sub>-C<sub>6</sub>-HSL ?. Similar conditions were used by Dr. Bin Yu, a visiting PhD student in the Spring group, to convert the bromo-C<sub>4</sub> derivative ? to the azido-C<sub>4</sub> derivative ?, and this compound was kindly donated to complete the set. Yields for the S<sub>N</sub>2 reaction could probably be improved by decreasing the temperature (see ??, for example).



Scheme 5: The synthesis of ? and ?. a) Bromoacetic acid, *i*-PrOH:H<sub>2</sub>O:AcOH (5:5:2), r.t., 18 h, 41 %. b) NaHCO<sub>3</sub>, H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, ?: 80 %, ?: 66 %. c) NaN<sub>3</sub>, DMF, 100 °C, 5 h, ?: 27 % (donated by Dr. Bin Yu), ?: 56 %.

## 2.3 Alkynyl antibiotic derivatives

### 2.3.1 Synthesis of the alkynyl ciprofloxacin derivative ?

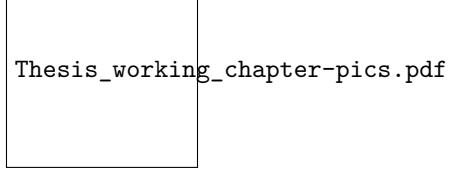
The retrosynthesis of ciprofloxacin derivative ? is shown in ???. The disconnection to an alkynyl piperazine ? and a commercially available ciprofloxacin precursor ? was chosen based on a study by Renau *et al.*, who found

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this route to be "...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide."<sup>7,8</sup>

It was envisaged that the alkynyl piperazine  $\text{?}$  could be prepared from mono-Boc-protected piperazine  $\text{?}$  and hex-5-ynal  $\text{?}$  using conditions similar to those used by Renau *et al.*<sup>9</sup>

Unlike the aldehydes and ketones used by Renau *et al.*,<sup>9</sup> hex-5-ynal  $\text{?}$  is not commercially available and so it was hoped that this could be prepared by oxidation of hex-5-ynol  $\text{?}$ .



Scheme 6: The retrosynthesis of  $\text{?}$ .

The synthesis of ciprofloxacin derivative  $\text{?}$  is shown in  $\text{??}$ . Hex-5-ynal  $\text{?}$  was prepared by PCC oxidation of hex-5-ynol  $\text{?}$  in good yield according to the procedure described by Kocsis *et al.*<sup>10</sup>

Renau *et al.*<sup>9</sup> used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal  $\text{?}$  and 1-Boc-piperazine  $\text{?}$ . However, it was decided to attempt this transformation using the less toxic sodium triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*<sup>11</sup> This reaction yielded compound  $\text{?}$  in excellent yield, which was deprotected using TFA using the procedure described by Renau *et al.*<sup>9</sup> to give the alkynyl piperazine  $\text{?}$  quantitatively.

The alkynyl piperazine  $\text{?}$  was refluxed in MeCN with the ciprofloxacin precursor  $\text{?}$  according to the procedure described by Renau *et al.*,<sup>9</sup> however the reaction did not proceed. Addition of 2 eq. of  $\text{NEt}_3$  did not lead to reaction, however it was found that refluxing in neat  $\text{NEt}_3$  led to conversion to the final ciprofloxacin derivative  $\text{?}$ .

With a small sample of the final product in hand, less harsh conditions were sought for a larger-scale version of the final reaction. Mircowave irradiation at 115 °C was used, following a procedure by Reddy *et al.*<sup>12</sup> 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 difficult, 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  $\text{EtOAc}$ , but the yield was rather poor (11.8 %). The reaction was observed to stall after a certain point, while still having some of the ciprofloxacin precursor  $\text{?}$  present. The alkynyl piperazine  $\text{?}$  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  $\text{?}$  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.



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Scheme 7: The synthesis of ?. a) Pyridinium chlorochromate,  $\text{CH}_2\text{Cl}_2$ , 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,  $115^\circ\text{C}$  24 h, 11.8 %.

### 2.3.2 Synthesis of the alkynyl trimethoprim derivative ?

The synthesis of trimethoprim derivative ? is shown in ???. Trimethoprim was selectively deprotected using HBr (aq.) using a procedure described by Jing *et al.*? to form ?. A slightly longer reaction time (40 min vs 20 min) probably led to the yield being slightly 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 ? was synthesised from the demethylated trimethoprim ? and 6-chloro-1-hexyne ? using a  $\text{Cs}_2\text{CO}_3$ -catalysed  $\text{S}_N2$  reaction similar to that used by Jing *et al.*.



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Scheme 8: The synthesis of ?. a) HBr (aq.),  $100^\circ\text{C}$ , 40 min, 43.4 %. b)  $\text{Cs}_2\text{CO}_3$ , DMF,  $70^\circ\text{C}$ , 7 h, 19.6 %.

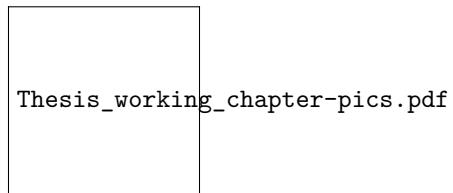
## 2.4 Triazole-linked autoinducer-antibiotic conjugates

### 2.4.1 Optimisation of the click reaction

Test reactions using  $\text{N}_3\text{-C}_2\text{-HSL}$  ? and the alkynyl ciprofloxacin derivative ? were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see ?? and ??). Stirring at r.t. had no effect even with an extended reaction time. Heating to  $50^\circ\text{C}$  did lead to slow formation of the product, but a mixture of the 1,4 ? and 1,5 ? isomers was observed in an approximately 4:1 ratio by LCMS (see ??). Use of the ligand tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) ? (see ??) led to some conversion at room temperature, however the reaction stopped before completion, probably due to oxidation of the  $\text{Cu}(\text{I})$  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
$\text{CuSO}_4 \cdot \text{H}_2\text{O}$ , sodium ascorbate, $\text{H}_2\text{O}$ , $t\text{-BuOH}$ , air, r.t., 7 d.	No reaction
$\text{CuSO}_4 \cdot \text{H}_2\text{O}$ , sodium ascorbate, $\text{H}_2\text{O}$ , $t\text{-BuOH}$ , air, 50 °C, 5 d.	1,3-Triazole product ? and 1,5 triazole impurity ? 4:1
$\text{CuSO}_4 \cdot \text{H}_2\text{O}$ , sodium ascorbate, THPTA ?, $\text{H}_2\text{O}$ , $t\text{-BuOH}$ , air, r.t., 3 h.	1,3-Triazole product ? and starting materials ? and ?
$\text{CuSO}_4 \cdot \text{H}_2\text{O}$ , sodium ascorbate, THPTA ?, $\text{H}_2\text{O}$ , $t\text{-BuOH}$ , Ar, r.t., 3 h.	1,3-Triazole product ?

Table 4: Conditions attempted for the synthesis of ? (see ??).



Scheme 9: Synthesis of ?. For conditions see ??.

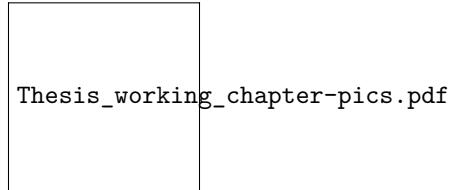


Figure 19: Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (THPTA) ? .

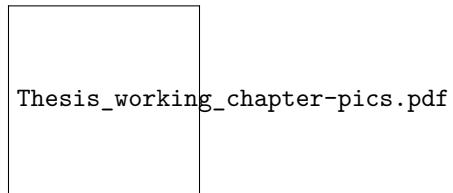
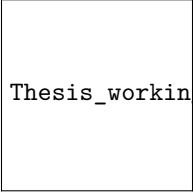


Figure 20: 1,4 (left) and 1,5 (right) triazoles .

#### 2.4.2 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-C<sub>12</sub>-HSL ? was synthesised by Ryan Howard, a master's student under my supervision<sup>7</sup> and the tail azide derivative of PQS ? was synthesised by Ysobel Baker<sup>7</sup> (see ??).



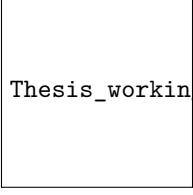
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Figure 21: Further azido autoinducer derivatives synthesised by Howard<sup>7</sup> ? and Baker<sup>7</sup> ?.

Synthesis of the conjugates proved more difficult than expected, for several reasons. Firstly some compounds did not dissolve in the reaction solvent (50 % water/*t*-BuOH) requiring addition of co-solvents such as CH<sub>2</sub>Cl<sub>2</sub>. Secondly, some compounds were unstable: HSL derivatives hydrolysed upon attempted preparative HPLC purification and the 3-oxo-C<sub>12</sub>-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 ??.

Nonetheless, several conjugates were produced for testing. The results of the reactions are shown in ??, ??, ?? and ???. It was intended that the failed reactions would be repeated, but as preliminary biological testing proved unpromising it was decided that attention should be focused elsewhere.

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Scheme 10: General scheme for the click reaction, where R<sub>1</sub>-N<sub>3</sub> is an azido autoinducer derivative and R<sub>2</sub>-≡ is an alkynyl antibiotic derivative a)CuSO<sub>4</sub>, sodium ascorbate, THPTA, H<sub>2</sub>O, *t*-BuOH.

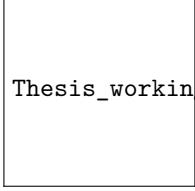
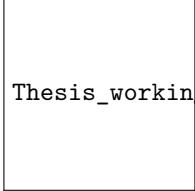
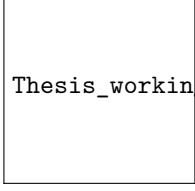
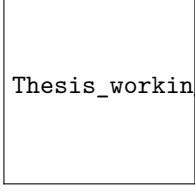
Starting materials	Product	Outcome	Yield
? and ?	 Thesis_working_chapter-pics.pdf ?	 Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO <sub>2</sub> , 0 - 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	29.6 %
? and ?	 Thesis_working_chapter-pics.pdf ?	 Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO <sub>2</sub> , 0 - 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	46.8 %
? and ?	 Thesis_working_chapter-pics.pdf ?	 Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO <sub>2</sub> , 0 - 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	38.0 %
? and ?	 Thesis_working_chapter-pics.pdf ?	 Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO <sub>2</sub> , 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	

Table 5: Click reactions attempted.

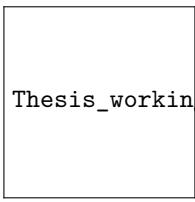
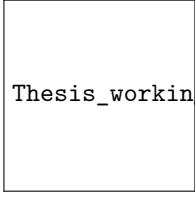
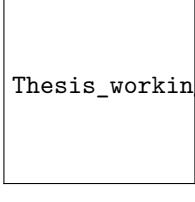
Starting materials	Product	Outcome	Yield
? and ?	 Thesis_working_chapter-pics.pdf ?	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	27.0 %
? and ?	 Thesis_working_chapter-pics.pdf ?	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
? and ?	 Thesis_working_chapter-pics.pdf ?	✗ No reaction seen by LCMS.	

Table 6: Click reactions attempted.

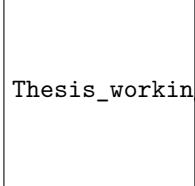
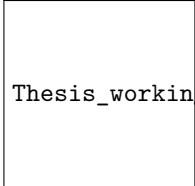
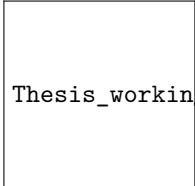
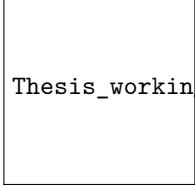
Starting materials	Product	Outcome	Yield
? and ?	 Thesis_working_chapter-pics.pdf ?	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on prep. HPLC column.	
? and ?	 Thesis_working_chapter-pics.pdf ?	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO <sub>2</sub> , 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	16.8 %
? and ?	 Thesis_working_chapter-pics.pdf ?	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO <sub>2</sub> , 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	26.8 %
? and ?	 Thesis_working_chapter-pics.pdf ?	✗ Degraded during reaction.	

Table 7: Click reactions attempted.

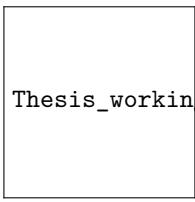
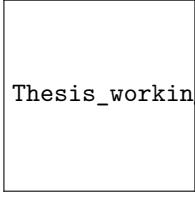
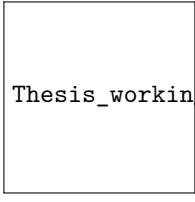
Starting materials	Product	Outcome	Yield
? and ?	 Thesis_working_chapter-pics.pdf ?	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	41.0 %
? and ?	 Thesis_working_chapter-pics.pdf ?	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
? and ?	 Thesis_working_chapter-pics.pdf ?	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO <sub>2</sub> , 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	18.3 %

Table 8: Click reactions attempted.

#### 2.4.3 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 Prof. Eddy Sotelo, a visiting researcher in the Spring group. Prof. Sotelo synthesised two alkyne-linked ciprofloxacin derivatives ? and ? (see ??), both with cleavable linkers (see ??).

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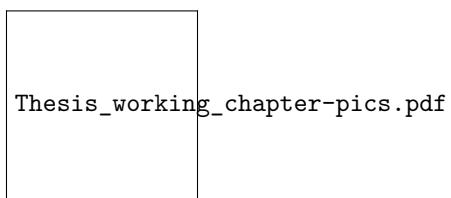
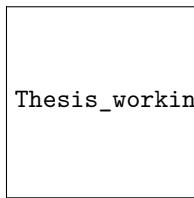


Figure 22: The cleavable alkyne-Cip derivatives synthesised by Prof. Sotelo.

Prof. Sotelo then performed click reactions using the AHL azide derivatives ?, ? and ? shown in ?? to form a library of conjugates (see ??). It was hoped that these conjugates would enter the cell and then be cleaved by esterases to release ciprofloxacin (see ??).

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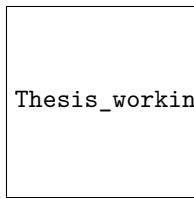
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Figure 23: The cleavable HSL-Cip triazole conjugates synthesised by Prof. Sotelo.

In addition, two control compounds ? and ? with benzyl head groups were produced by Prof. Sotelo (see ??). It was hoped that these would show whether the AHL head group is required for activity.



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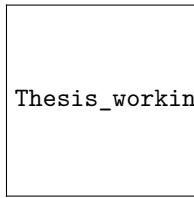
Figure 24: The cleavable Bn-Cip triazole conjugates ? and ? synthesised by Prof. Sotelo.

### 3 Autoinducer analogue-ciprofloxacin conjugates

#### 3.1 Introduction

##### 3.1.1 Head groups

The head groups used in this study are shown in ???. The cyclohexanol derivatives were synthesised as a diastereomerically pure racemate, whereas the cyclopentanol derivatives were synthesised as separate enantiomers. Unfortunately, cyclopentanone derivatives were not synthesised, and would be an obvious future addition to the library. 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.



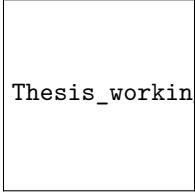
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Figure 25: The head groups used in this section.

##### 3.1.2 Library construction

As Ganguly *et al.*? synthesised their conjugate from Br-C<sub>4</sub>-HCTL, it was envisaged that a branching strategy could be used to produce two sets of conjugates (see ??). The first set would be formed by the S<sub>N</sub>2 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 ? made previously to form the triazole-linked product. Ketone conjugates would be formed by oxidation of the alcohols.

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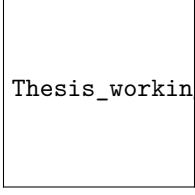
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Scheme 11

### 3.2 Homocysteine thiolactone derivatives

#### 3.2.1 Synthesis of methyl ciprofloxacin ?

The synthesis of the analogue conjugates began with the synthesis of methyl ciprofloxacin ?, which would then be attached to the various head groups. Methyl ciprofloxacin ? was synthesised from ciprofloxacin ? and MeOH in very good yield using *para*-toluenesulfonic acid as a catalyst.?

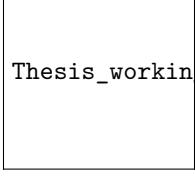


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Scheme 12: Synthesis of methyl ciprofloxacin ?. a) *p*-TSA, MeOH, 72 h, reflux, 83.3 %.

#### 3.2.2 Synthesis of Br-C<sub>4</sub>-HCTL ?

The HCTL head group was then attached to the linker to form Br-C<sub>4</sub>-HCTL ?, in preparation for coupling to methyl ciprofloxacin ?. Br-C<sub>4</sub>-HCTL ? was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives ? and ?. Br-C<sub>4</sub>-HCTL ? was isolated in markedly higher yield than that achieved by Ganguly *et al.*? (87.9 % vs. 25.0 %). It is possible that this was due to CH<sub>2</sub>Cl<sub>2</sub> being used for the extraction, whereas Ganguly *et al.* used EtOAc.



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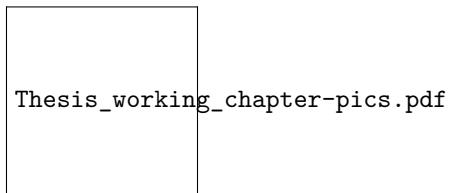
Scheme 13: Synthesis of Br-C<sub>4</sub>-HCTL ?. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 1 h, 87.9 %.

#### 3.2.3 Synthesis of the HCTL-CipMe conjugate ?

The HCTL-CipMe conjugate ? was synthesised using the procedure outlined by Ganguly *et al.*? Monitoring by LCMS showed slow conversion to the product. Br-C<sub>4</sub>-HCTL ? was presumably consumed by side reactions as 4 eq. were required to reach full conversion. Ganguly *et al.* do not quote a yield for comparison,?,? but it is hoped that the 12.2 % 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 ? for biological testing.

Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in ??, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.

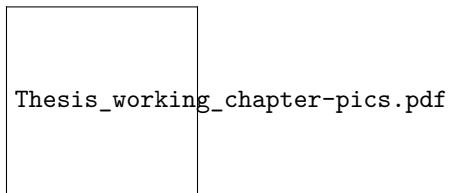


Scheme 14: Synthesis of the HCTL-CipMe conjugate ?, N<sub>3</sub>-C<sub>4</sub>-HCTL ?, and the HCTL-Cip triazole conjugate ?. a) K<sub>2</sub>CO<sub>3</sub>, acetonitrile, reflux, 24 h, 12.2 %.

### 3.2.4 Synthesis of the HCTL-Cip triazole conjugate ?

Br-C<sub>4</sub>-HCTL ? was converted into N<sub>3</sub>-C<sub>4</sub>-HCTL ? (see ??), by an S<sub>N</sub>2 reaction with sodium azide which proceeded in excellent yield.

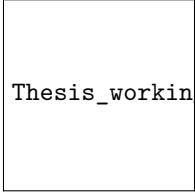
N<sub>3</sub>-C<sub>4</sub>-HCTL ? was then subjected to the click reaction conditions optimised previously (see ??). The reaction proceeded very slowly at first, until it was realised that the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. It is possible that the sulfur atom coordinates to the copper, thus inhibiting its catalytic ability. Nonetheless the HCTL-Cip triazole conjugate ? was eventually isolated in good yield (see ??).



Scheme 15: Synthesis of the HCTL-Cip triazole conjugate ?. a) NaN<sub>3</sub>, acetonitrile, reflux, 1.5 h, 89.3 %. b) CuSO<sub>4</sub>, THPTA, sodium ascorbate, H<sub>2</sub>O, *t*-BuOH, DMSO, r.t., 7 d, 70.6 %.

### 3.2.5 Synthesis of the cleavable HCTL-Cip triazole conjugate ?

A cleavable conjugate ? was also synthesised from N<sub>3</sub>-C<sub>4</sub>-HCTL ? by reaction with a cleavable alkyne-Cip derivative ? synthesised previously by Prof. Eddy Sotelo-Perez (see ??). Conditions developed by Prof. Sotelo-Perez were used, but again the reaction proceeded very slowly. The disappointing yield is, however, most likely due to losses during purification.



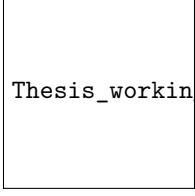
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Scheme 16: Synthesis of the cleavable HCTL-Cip triazole conjugate ?. a) CuI, DIPEA,  $\text{CH}_2\text{Cl}_2$ , r.t., 3 h, 5.0 %.

### 3.3 2-Methoxybenzene derivatives

#### 3.3.1 Synthesis of Br-C<sub>4</sub>-2-methoxybenzene ?

Br-C<sub>4</sub>-2-methoxybenzene ? was synthesised from 2-methoxyaniline ? and 4-bromobutyryl chloride ? using Schotten-Baumann conditions in 50.0 % yield (see ??). The compound is air and/or light sensitive, turning from an initially colourless liquid to blue then black if left out on the bench. It is likely that the mediocre yield is due to degradation during columning, and it is suggested that in future the compound should be used in its crude form to minimise exposure to air and light, as it was fairly pure by <sup>1</sup>H NMR before columning.

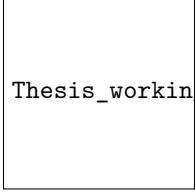


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Scheme 17: Synthesis of Br-C<sub>4</sub>-2-methoxybenzene ?. a)  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ , 0 °C, 1 h, 50.0 %.

#### 3.3.2 Synthesis of the 2-methoxybenzene-CipMe conjugate ?

The procedure outlined by Ganguly *et al.*? was initially attempted in order to synthesise the 2-methoxybenzene-CipMe conjugate ?, but the reaction was very slow and did not go to completion, presumably due to degradation of Br-C<sub>4</sub>-2-methoxybenzene ?. New conditions, employing a microwave reactor and 2 eq. of Br-C<sub>4</sub>-2-methoxybenzene ? were then attempted, with a much greater conversion observed by LCMS after 4 h (see ??). However, a poor yield was obtained, potentially due to degradation during column chromatography, which took longer than for Br-C<sub>4</sub>-2-methoxybenzene ? because the 2-methoxybenzene-CipMe conjugate ? is more polar.



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Scheme 18: Synthesis of the 2-methoxybenzene-CipMe conjugate ? and N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene ?. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 10.2 %.

### 3.3.3 Synthesis of the 2-methoxybenzene-Cip triazole conjugate ?

$N_3\text{-C}_4\text{-2-methoxybenzene ?}$  was synthesised from  $\text{Br-C}_4\text{-2-methoxybenzene ?}$  by an  $S_N2$  reaction with sodium azide (see ??). The yield of  $N_3\text{-C}_4\text{-2-methoxybenzene ?}$  (26.7 %) was a lot lower than for  $N_3\text{-C}_4\text{-HCTL ?}$  (89.3 %). The colour of  $N_3\text{-C}_4\text{-2-methoxybenzene ?}$ , like its precursor, changed from clear to blue then black, suggesting that it is also air/light sensitive and may have degraded during columning. However, in this case it may not be better to use this product crude as several impurities could be observed by LCMS (see ??).

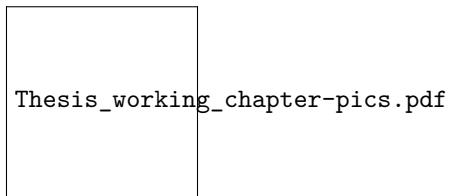
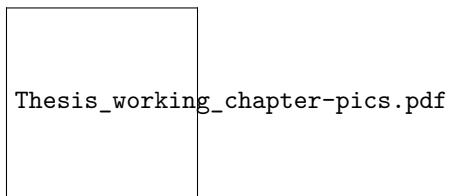


Figure 26: Impurities formed during the synthesis of  $N_3\text{-C}_4\text{-2-methoxybenzene ?}$ .

The 2-methoxybenzene-Cip triazole conjugate ? was synthesised using the standard click conditions (see ??), with the addition of  $\text{CH}_2\text{Cl}_2$  as a co-solvent to aid the dissolution of  $N_3\text{-C}_4\text{-2-methoxybenzene ?}$  (see ??). Again, the yield was somewhat low, probably due to air/light sensitivity of the starting material and/or product.

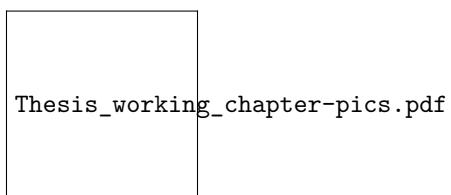


Scheme 19: Synthesis of the 2-methoxybenzene-Cip triazole conjugate ?. a)  $\text{NaN}_3$ , acetonitrile, reflux, 2 h, 26.7 %. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate,  $\text{H}_2\text{O}$ ,  $t\text{-BuOH}$ ,  $\text{CH}_2\text{Cl}_2$ , r.t., 16 h, 39.0 %.

## 3.4 3-Methoxybenzene derivatives

### 3.4.1 Synthesis of Br-C<sub>4</sub>-3-methoxybenzene ?

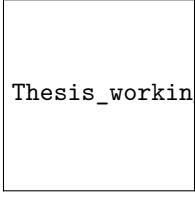
$\text{Br-C}_4\text{-3-methoxybenzene ?}$  was synthesised from 3-methoxyaniline ? and 4-bromobutyryl chloride ? using Schotten-Baumann conditions as above in almost identical (49.6 %) yield (see ??). The compound is probably also air and/or light sensitive, turning from a pale pink amorphous solid to a pale brown liquid.



Scheme 20: Synthesis of  $\text{Br-C}_4\text{-3-methoxybenzene ?}$ . a)  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ , 0 °C, 1 h, 49.6 %.

### 3.4.2 Synthesis of the 3-methoxybenzene-CipMe conjugate ?

The 3-methoxybenzene-CipMe conjugate ?, was synthesised as above, in similar yield (see ??).

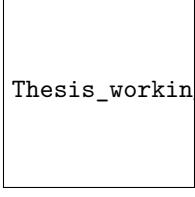


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Scheme 21: Synthesis of the 3-methoxybenzene-CipMe conjugate ? and N<sub>3</sub>-C<sub>4</sub>-3-methoxybenzene ?. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 10.5 %.

### 3.4.3 Synthesis of the 3-methoxybenzene-Cip triazole conjugate ?

N<sub>3</sub>-C<sub>4</sub>-2-methoxybenzene ? and the 3-methoxybenzene-Cip triazole conjugate ? were synthesised as above, in similar yields (see ?? and ??).



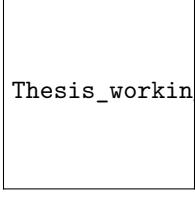
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Scheme 22: Synthesis of the 3-methoxybenzene-Cip triazole conjugate ?. a) NaN<sub>3</sub>, acetonitrile, reflux, 7 h, 16.7 %. b) CuSO<sub>4</sub>, THPTA, sodium ascorbate, H<sub>2</sub>O, *t*-BuOH, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 2 h, 5.0 %.

## 3.5 Cyclopentanol derivatives

### 3.5.1 Synthesis of the 2-aminocyclopentan-1-ol head groups ? and ?

Synthesis of the cyclopentanol derivatives began with the synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol ? and (1*R*,2*R*)-2-aminocyclopentan-1-ol ? (see ??), using a procedure reported by Overman and Sugai?.,??,?. These precursors were synthesised by opening cyclopentene oxide ? using (*S*)-1-phenylethan-1-amine ? to give approximately equal amounts of two diastereomers, ? and ?, which were separated using column chromatography. The removal of the methylbenzyl groups proved more difficult than expected, with the conditions reported by Overman and Sugai? yielding only a salt of the starting material. After several attempts under various conditions (including using the free amine vs. the salt, varying the temperature, ensuring the dryness of the reagents and adding acetic acid), an approach using H<sub>2</sub> gas was attempted (see ??). This proceeded smoothly at 5 atm to give the two enantiomers of 2-aminocyclopentan-1-ol, ? and ?, both in quantitative yield.



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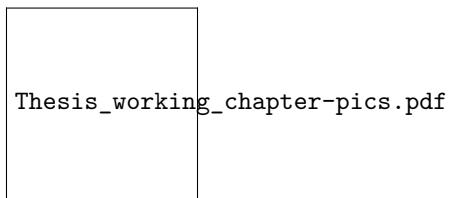
Scheme 23: Synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol ? and (1*R*,2*R*)-2-aminocyclopentan-1-ol ?. a) AlMe<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, ? (SSS): 35.2 %, ? (RRS): 32.1 %. b) See ???. c) Pd(OH)<sub>2</sub>, MeOH, H<sub>2</sub>, 5 atm, r.t., 1 d, 100 %.

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

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

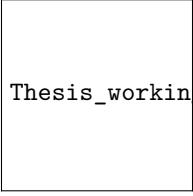
### 3.5.2 Initial branching route

An initial retrosynthesis of the conjugates is shown in ??, and follows a similar path to previous conjugates.



Scheme 24: Retrosynthesis of the cyclopentanol-CipMe conjugates ? (*SS*) and ? (*RR*), and the cyclopentanol-Cip triazole conjugates ? (*SS*) and ? (*RR*). *SS* enantiomers are shown, but both are implied.

Synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) ? from (*1S,2S*)-2-aminocyclopentan-1-ol ? and 4-bromobutyryl chloride ? was attempted using Schotten-Baumann conditions (see ??). However, a large number of impurities were observed by LCMS (see ??), and so three new strategies were attempted: protection of the alcohol (see ??), installing the linker on methyl ciprofloxacin ? and then attaching the head group by peptide coupling (see ??), and using 4-chlorobutyryl chloride ? as the linker instead of 4-bromobutyryl chloride ? (see ??).



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Scheme 25: Attempted synthesis of Br-C<sub>4</sub>-cyclopentanol-(SS) ?. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 2 h.

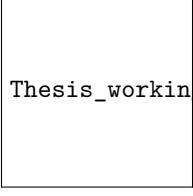


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Figure 27: Impurities observed by LCMS during the synthesis of Br-C<sub>4</sub>-cyclopentanol-(SS) ?. Regiochemistry is speculative.

### 3.5.3 TBDMS protection route

The first attempt at an alternative strategy for the synthesis of the conjugates involved TBDMS protection of the alcohol (see ??). It was envisaged that protection would eliminate enough of the side reactions with products shown in ?? that intermediates Br-C<sub>4</sub>-cyclopentanol-(SS) ? and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(SS) ? could be purified. The TBDMS group could be removed later in the synthesis using TBAF or acid.



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Scheme 26: Retrosynthesis of the cyclopentanol-CipMe conjugates ? (SS) and ? (RR), and the cyclopentanol-Cip triazole conjugates ? (SS) and ? (RR) using a TBDMS protection strategy. SS enantiomers are shown, but both are implied.

#### 3.5.3.1 Synthesis of TBDMS-protected (1*S*,2*S*)-2-aminocyclopentan-1-ol ?

The synthesis began with the optimisation of the protection of (1*S*,2*S*)-2-aminocyclopentan-1-ol ? with a TB-DMS group on the alcohol (see ??). 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 ???. Protection attempts using TBDMSCl were generally unsuccessful, but eventually a method employed by Wu et. al<sup>7</sup> using TBDMSCl was found to produce the desired product in excellent yield. Water was used for the work-up rather than NH<sub>4</sub>Cl (sat. aq.), as the acidic work-up protonated the product. The TEA was removed during column chromatography instead.

Scheme 27: Synthesis of TBDMS protected (*1S,2S*)-2-aminocyclopentan-1-ol ?. a) See ??.

Conditions	Temperature	Time	Result
TBDMSCl, DMAP, TEA, $\text{CH}_2\text{Cl}_2$ ?	r.t.	18 h	Trace of ?, mostly ?
TBDMSCl, imidazole, $\text{CH}_2\text{Cl}_2$ ?	0 °C	1 h	?
TBDMSCl, DBU, MeCN?	0 °C	1 d	?
TBDMSOTf, TEA, $\text{CH}_2\text{Cl}_2$ , ? aq. workup then column	0 °C	6 h	?, 97.7 % yield

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

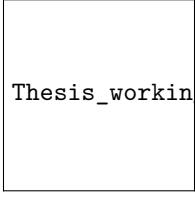
### 3.5.3.2 Synthesis of Br-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ?

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

Scheme 28: Attempted synthesis of Br-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ?. a) NaHCO<sub>3</sub>,  $\text{CH}_2\text{Cl}_2$ , H<sub>2</sub>O, 0 °C, 2 h.

### 3.5.3.3 Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ? by one-pot reaction

N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ? 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. N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ? was produced in excellent yield.

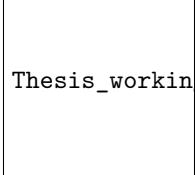


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Scheme 29: Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ?. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 3 h. b) NaN<sub>3</sub>, DMF, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 3 h. 99.2 % over 2 steps.

### 3.5.3.4 Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate ?

N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) ? and the alkynyl ciprofloxacin derivative ? were subjected to standard click conditions (see ??), and the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate ? 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 ? by column chromatography was not successful due to streaking and poor separation. Purification using DOWEX resin and CaCO<sub>3</sub>? 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.

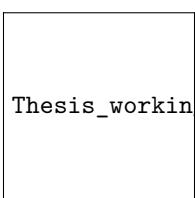


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Scheme 30: Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate ?. a) CuSO<sub>4</sub>, sodium ascorbate, THPTA, H<sub>2</sub>O, t-BuOH, r.t., 87.4 %. b) TBAF, THF, r.t., 16 h.

### 3.5.4 Synthesis of the cyclopentanol-CipMe conjugates ? and ? by peptide coupling

Given the side-reactions and low yields associated with the literature synthesis of the S<sub>N</sub>2 conjugates proposed by Ganguly et. al,<sup>7</sup> an alternative synthesis was investigated, involving building up the linker on the ciprofloxacin side before coupling with the head group (see ??).



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Scheme 31: Retrosynthesis of the cyclopentanol-CipMe conjugates ? (SS) and ? (RR). SS enantiomers are shown, but both are implied.

The first step of the synthesis was an S<sub>N</sub>2 reaction between Boc-protected 4-bromobutyric acid ? methyl ciprofloxacin ? (see ??). This reaction used fairly harsh conditions (16 h at 100 °C), but early on in the synthesis before the head group was installed. Hence the possibility of side reactions between the bromide and

the amine was removed. Intermediate ? was obtained in acceptable yield after column chromatography (49.9 %). Intermediate ? was deprotected in excellent yield using TFA in  $\text{CH}_2\text{Cl}_2$  to give carboxylic acid ?. 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 ? was first coupled with (1*R*,2*R*)-2-aminocyclopentan-1-ol ? using standard peptide coupling conditions to give cyclopentanol-CipMe conjugate ?. Purification by column chromatography was attempted twice with poor results, before moving on to using preparative HPLC, which gave ? cleanly in 38.7 % yield. Coupling was also performed with (1*S*,2*S*)-2-aminocyclopentan-1-ol ? to give the enantiomer ? in 54.7 % yield.

Direct comparisons of routes are not possible without repeating syntheses using this new method, but if it is assumed that peptide coupling of homocysteine thiolactone hydrochloride ? to carboxylic acid ? would have a similar yield to the coupling with (1*R*,2*R*)-2-aminocyclopentan-1-ol ?, approximate comparisons can be made. The synthesis described in ?? has an overall yield of 10.7 %, whereas the route shown in ?? for ? has an overall yield of 26.1 %. Moreover, if the yield starting from the head group (which may be expensive, difficult to synthesise and/or unstable) is considered, the yield is 54.7 % vs. 10.7 %. Therefore, this route is recommended for further investigation if the library is to be expanded.

A downside to this route is that it cannot branch towards the triazole-coupled library in the same way that the route in ???. A carboxylic acid intermediate with a triazole in the chain could presumably be synthesised, but this would be rather pointless given that the triazole library was initially proposed so that the two sides could be joined by the click reaction. Therefore, an alternative route to the azide was proposed, via a more stable chloride intermediate (see ??).

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Scheme 32: Synthesis of the cyclopentanol-CipMe conjugates ? (*SS*) and ? (*RR*) by peptide coupling. *SS* enantiomers are shown, but both were synthesised. a) NaI, TEA, acetonitrile, 100 °C, 16 h, 49.9 %. b) TFA,  $\text{CH}_2\text{Cl}_2$ , r.t., 18 h, 95.6 %. c) EDC, HOBr, DIPEA, DMF, r.t., 16 h, ? (*SS*): 54.7 %, ? (*RR*): 38.7 %.

### 3.5.5 Synthesis of the cyclopentanol-Cip triazole conjugates ? and ? via chloride intermediates

A final attempt at a branching strategy was attempted, via a chloride rather than a bromide intermediate (see ?? and ?? for comparison). The bromide intermediate was initially chosen as it was used by Ganguly et. al,<sup>7</sup> but it was hoped that using a chloride would cut out some of the side reactions seen with the more reactive bromide.

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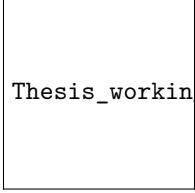
Scheme 33: Retrosynthesis of the cyclopentanol-CipMe conjugates ? (*SS*) and ? (*RR*), and the cyclopentanol-Cip triazole conjugates ? (*SS*) and ? (*RR*) via Cl-C<sub>4</sub>-cyclopentanol intermediates ? (*SS*) and ? (*RR*). *SS* enantiomers are shown, but both are implied.

Attempts at this route began with the synthesis of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) ?. 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.*<sup>7</sup> 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 ? is fairly polar, it is likely that it was staying in the aqueous layer to some extent even when deprotonated, thus keeping the two reactants apart. Therefore, the solvent system and base were changed to neat CH<sub>2</sub>Cl<sub>2</sub> and TEA. This produced Cl-C<sub>4</sub>-cyclopentanol-(*RR*) ? in good yield (64.1 %). Unlike the bromide ?, the chloride ? was stable when concentrated.

Cl-C<sub>4</sub>-cyclopentanol-(*RR*) ? was converted to N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) ? by reaction with sodium azide. The reaction was slower than with previous bromides (~16 h vs. ~2 h), but much cleaner than with Br-C<sub>4</sub>-cyclopentanol-(*SS*) ? (see ??).

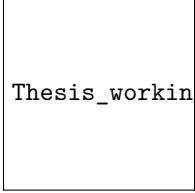
The enantiomers Cl-C<sub>4</sub>-cyclopentanol-(*SS*) ? and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) ? were synthesised in lower yields, in part because of the smaller amounts being used.



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Scheme 34: Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) ? and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) ?. *SS* enantiomers are shown, but both were synthesised. a) TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h, ? (*SS*): 24.2 %, ? (*RR*): 64.1 %. b) NaN<sub>3</sub>, acetonitrile, 50 °C, 16 h, ? (*SS*): 45.0 %, ? (*RR*): 87.6 %.

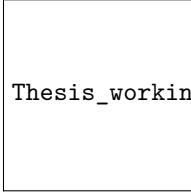
The cyclopentanol-Cip triazole conjugates ? (*SS*) and ? (*RR*) were synthesised using standard click conditions (see ??). Yields were poor primarily due to problems with purification, including losses on the preparative HPLC column and high polarity leading to losses during extraction from aqueous solvents. However, as enough of the compounds was obtained for biological testing the purification was not optimised further.



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Scheme 35: Synthesis of the cyclopentanol-Cip triazole conjugates ? (*SS*) and ? (*RR*). *SS* enantiomers are shown, but both were synthesised. a) CuSO<sub>4</sub>, THPTA, sodium ascorbate, H<sub>2</sub>O, *t*-BuOH, r.t., 16 h, ? (*SS*): 22.2 %, ? (*RR*): 27.1 %.

The S<sub>N</sub>2 reaction of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) ? and methyl ciprofloxacin ? was attempted (see ??) using the microwave conditions described previously (see ??), 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 ? was seen by LCMS (in an approx 1:1 ratio with the desired product ?). As a higher-yielding route had already been found (see ??), this route was abandoned.



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Scheme 36: Attempted synthesis of the cyclopentanol-CipMe-(*RR*) conjugate ?. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C.

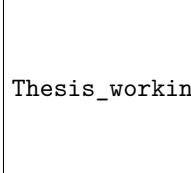
With (unfortunately not branching) routes to the  $S_N2$  and click conjugates established (see ?? and ?? respectively), attention was turned to the cyclohexanol derivatives.

### 3.6 Cyclohexanol derivatives

#### 3.6.1 Synthesis of the *trans*-2-aminocyclohexan-1-ol head group ?

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

Production of the cyclohexanol conjugates began with the synthesis of *trans*-2-aminocyclohexan-1-ol ? (see ??), using a procedure reported by Xue *et al.*? Cyclohexene oxide ? 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.



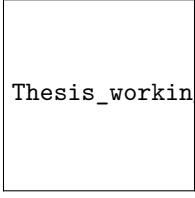
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Scheme 37: Synthesis of *trans*-2-aminocyclohexan-1-ol ?. a) NH<sub>3</sub>, water, MeOH, r.t., 72 h, 86.2 %.

#### 3.6.2 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-CipMe conjugates ? and ?

Carboxylic acid ? was coupled with *trans*-2-aminocyclohexan-1-ol ? using standard peptide coupling conditions to give *trans*-cyclohexanol-CipMe conjugate ? in 31.7 % yield. Again, losses during purification appear to be the main cause of this poor yield.

A portion of the *trans*-cyclohexanol-CipMe conjugate ? was then oxidised to the ketone using Dess-Martin periodinane. The product was isolated in good yield, perhaps due to the compound being less polar and hence easier to purify.

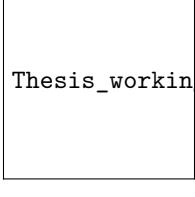


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Scheme 38: Synthesis of the cyclohexanol-CipMe conjugate ? and the cyclohexanone-CipMe conjugate ?. a) EDC, HOBr, DIPEA, DMF, r.t., 16 h, 31.7 %. b) DMP, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 6 h, 69.1 %.

### 3.6.3 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-Cip triazole conjugates ? and ?

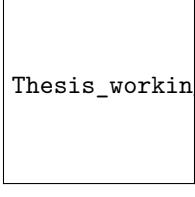
The triazole conjugates were synthesised using the route described in ???. Cl-C<sub>4</sub>-*trans*-cyclohexanol ? was synthesised in good yield from *trans*-2-aminocyclohexan-1-ol ? and 4-chlorobutyryl chloride ?. Cl-C<sub>4</sub>-*trans*-cyclohexanol ? was then converted to N<sub>3</sub>-C<sub>4</sub>-*trans*-cyclohexanol ? by reaction with sodium azide in excellent yield.



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Scheme 39: Synthesis of N<sub>3</sub>-C<sub>4</sub>-*trans*-cyclohexanol ?. a) TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 30 min, 76.1 %. b) NaN<sub>3</sub>, acetonitrile, 50 °C, 16 h, 97.5 %.

The *trans*-cyclohexanol-Cip triazole conjugate ? was synthesised using standard click conditions (see ??) in 48.9 % yield. A portion of the *trans*-cyclohexanol-Cip triazole conjugate ? was then oxidised to the ketone using the same conditions used for the cyclohexanone-CipMe conjugate (see ??) in very good yield.



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Scheme 40: Synthesis of the *trans*-cyclohexanol-Cip triazole conjugate ? and the cyclohexanone-Cip triazole conjugate ?. a) CuSO<sub>4</sub>, THPTA, sodium ascorbate, H<sub>2</sub>O, *t*-BuOH, r.t., 16 h, 48.9 %. b) DMP, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 4 h, 78.0 %.

## 4 Experimental

### 4.1 General

Unless otherwise stated, reactions were performed in air-dried glassware under argon with dry, freshly-distilled solvents. THF was distilled from LiAlH<sub>4</sub> in the presence of triphenyl methane indicator. CH<sub>2</sub>Cl<sub>2</sub>, hexane, MeOH and MeCN 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 Keisel-gel 60 F254 and visualised using UV ( $\lambda = 254$  or 366 nm) or by staining with KMnO<sub>4</sub> or ninhydrin. All retention factors ( $R_f$ ) are given to 0.01. All column chromatography was carried out using Merck 9385 Keisel-gel 60 silica gel (230-400 mesh) or using a CombiFlash® EZ Prep with RediSep® normal-phase silica flash columns. Preparative High Pressure Liquid Chromatography was run on an Agilent 1260 Infinity machine, using a Supelcosil™ ABZ+PLUS column (250 mm  $\times$  21.2 mm, 5  $\mu$ m) with a linear gradient system (solvent A: 0.1 % (v/v) TFA/water, solvent B: 0.05 % (v/v) TFA/acetonitrile) at a flow rate of 20 mL min<sup>-1</sup>, 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). <sup>1</sup>H and <sup>13</sup>C spectra were assigned using DEPT, COSY, HMQC and HMQC spectra where necessary, or by analogy to fully interpreted spectra of related compounds. The following abbreviations are used to indicate the multiplicity of signals: s singlet, d doublet, t triplet, q quartet, quin quintet, m multiplet and br broad.

<sup>1</sup>H chemical shifts ( $\delta$ ) are quoted to the nearest 0.01 ppm and are referenced relative to the residual solvent peak.<sup>7</sup> Coupling constants ( $J$ ) are given to the nearest 0.1 Hz. Diastereotopic protons are assigned as CHH and CH<sub>H</sub>, where the latter designates the lower-field proton. Data are reported as follows: <chemical shift> (<multiplicity>, <coupling constant(s) (if any)>, <integration>, <assignment>).

<sup>13</sup>C chemical shifts ( $\delta$ ) are quoted to the nearest 0.1 ppm and are referenced relative to the deuterated solvent peak.<sup>7</sup> Data are reported as follows: <chemical shift> (<multiplicity (if not s)>, <coupling constant(s) (if any)>, <assignment>).

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

High Resolution Mass Spectra (HRMS) were recorded using a Micromass LCT Premier spectrometer or a Waters Vion IMS-QTOF spectrometer and reported mass values are within  $\pm 5$  ppm mass units. Low Resolution Mass Spectra (LRMS) 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.

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

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

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

## 4.2 Methyl 3-oxodecanoate ?

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Meldrum's acid ? (9.0 g, 63 mmol, 1 eq.) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (150 ml) in an oven-dried flask and cooled to 0 °C. Pyridine (10.2 ml, 126 mmol, 2 eq.) was added dropwise over 20 min. Octanoyl chloride ? (11.7 ml, 69 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  $\text{CH}_2\text{Cl}_2$  (20 ml) and poured into a mixture of ice (~30 g) and HCl (2 N, 90 ml). The solution was washed with NaCl (sat., aq., 150 ml) and dried over  $\text{MgSO}_4$ . 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 ( $\text{SiO}_2$ , 5 %  $\text{Et}_2\text{O}$ /40-60 P.E.). A tautomeric mixture of ? and ? was obtained as a colourless oil (8.34 g, 41.6 mmol, 66 %, 92 % ? as determined by  $^1\text{H}$  NMR).

### Keto form ?

**TLC**  $R_f$  = 0.12 (5 %  $\text{EtO}_2$ /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)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 3.74 (s, 3 H,  $\text{OCH}_3$ ), 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,  $\text{CH}_2\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / 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 ( $\text{OCH}_3$ ), 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 ( $\text{CH}_2$ ), 28.7 ( $\text{CH}_2$ ), 28.6 ( $\text{CH}_2$ ), 23.1 ( $\text{CH}_2$ ), 22.2 ( $\text{CH}_2$ ), 13.6 ( $\text{CH}_2\text{CH}_3$ )

### Enol form ?

**TLC**  $R_f$  = 0.12 (5 %  $\text{EtO}_2$ /PE)

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

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 12.02 (s, 1 H,  $\text{COH}$ ), 4.99 (s, 1 H,  $\text{C}(=\text{O})\text{CH}=\text{COH}$ ), 3.73 (s, 3 H,  $\text{OCH}_3$ ), 2.20 (t,  $J$  = 7.4 Hz, 2 H,  $\text{COHCH}_2$ ), 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,  $\text{CH}_2\text{CH}_3$ )

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / 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 ( $\text{OCH}_3$ ), 37.9 ( $\text{COHCH}_2\text{CH}_2$ ), 34.6 ( $\text{CH}_2$ ), 31.2 ( $\text{CH}_2$ ), 29.0 ( $\text{CH}_2$ ), 25.9 ( $\text{CH}_2$ ), 22.3 ( $\text{CH}_2$ ), 13.6 ( $\text{CH}_2\text{CH}_3$ )

Spectroscopic data are consistent with the literature.<sup>?,?</sup>

#### 4.3 Methyl (*E*)-3-((4-((*tert*-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate ?

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Methyl 3-oxodecanoate ? (500 mg, 2.50 mmol, 1.00 eq.) and *O*-*tert*-butyl *N*-(4-aminophenyl)carbamate ? (520 mg, 2.50 mmol, 1.00 eq.) were dissolved in MeOH (10 ml) and refluxed for 18 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (SiO<sub>2</sub>, gradient of 0 to 20 % Et<sub>2</sub>O/40-60 P.E.). ? was obtained as a white amorphous solid (0.169 mg, 0.480 mmol, 19 %).

**TLC**  $R_f$  = 0.30 (30 % Et<sub>2</sub>O/40-60 P.E.)

**mp**  $T$  / °C = 78.8 (Et<sub>2</sub>O/40-60 P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3337.0 (N-H), 2927.7 (C-H), 2857.1 (C-H), 1723.7 (carbamate C=O), 1634.5 ( $\alpha,\beta$  unsaturated C=O), 1610.7 (C=C), 1580.9 (N-H bend)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 10.16 (s, 1 H, NHC(C<sub>7</sub>H<sub>15</sub>)=C), 7.35 (d,  $J$  = 8.6 Hz, 2 H, *meta* to NH<sub>Boc</sub>), 7.02 (d,  $J$  = 8.7 Hz, 2 H, *meta* to enamine), 6.60 (br s, 1 H, NH<sub>Boc</sub>), 4.71 (s, 1 H, C=CH), 3.70 (s, 3 H, OCH<sub>3</sub>), 2.23 (t,  $J$  = 7.7 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.54 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.40 (quin,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.33 - 1.16 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.86 (t,  $J$  = 7.1 Hz, 3 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 171.1 (C(=O)CH=C), 164.3 (C(=O)CH=C), 152.7 (OC(=O)NH), 136.0 (*para* to NH<sub>Boc</sub>), 134.1 (CNH<sub>Boc</sub>), 126.3 (*meta* to NH<sub>Boc</sub>), 119.1 (*ortho* to NH<sub>Boc</sub>), 83.8 (C(=O)CH=C), 80.7 (C(CH<sub>3</sub>)<sub>3</sub>), 50.2 (OCH<sub>3</sub>), 32.2 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 28.0 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 14.0 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 391.2589, [M+H]<sup>+</sup>, [C<sub>22</sub>H<sub>35</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup> requires 391.2591

Spectroscopic data are consistent with the literature.<sup>?</sup>

#### 4.4 6-Amino-2-heptylquinolin-4-ol ?

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Methyl (*E*)-3-((4-((*tert*-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate ? (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 NaHCO<sub>3</sub> (sat., aq., 50 ml) cooled with ice. The precipitate was collected by vacuum filtration, washed with water (50 ml) and dried under high vacuum. ? was obtained as a pale yellow amorphous solid (121 mg, 0.468 mmol, 72 %).

**mp** *T* / °C = 249 (H<sub>2</sub>O)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3336.5 (N-H), 2926.5 (C-H), 2856.9 (C-H), 1634.5 (C=O)

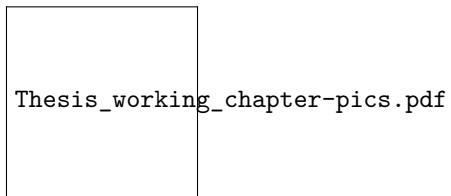
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 7.26 (d, *J* = 8.7 Hz, 1 H, *meta* to NH<sub>2</sub>), 7.15 (d, *J* = 2.6 Hz, 1 H, *ortho* to C(=O)), 6.95 (dd, *J* = 2.7, 8.8 Hz, 1 H, *para* to C(=O)), 5.74 (s, 1 H, *ortho* to CH<sub>2</sub>), 5.16 (s, 2 H, NH<sub>2</sub>), 2.52 (t, *J* = 7.4 Hz, 2 H, CCH<sub>2</sub>), 1.64 (quin, *J* = 7.6 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 1.36 - 1.19 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.86 (t, *J* = 7.0 Hz, 3 H, H<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 176.7 (C(=O)), 151.7 (CCH<sub>2</sub>), 145.1 (*para* to NH<sub>2</sub> or *ipso* to C(=O)), 132.4 (*ipso* to NH<sub>2</sub>), 126.6 (*para* to NH<sub>2</sub> or *ipso* to C(=O)), 121.1 (*para* to C(=O)), 119.0 (*meta* to NH<sub>2</sub> and *meta* to C(=O)), 106.2 (CH=CCH<sub>2</sub>), 105.9 (*ortho* to NH<sub>2</sub> and *ortho* to C(=O)), 33.6 (CCH<sub>2</sub>), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 29.0 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 14.4 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 259.1810, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>23</sub>N<sub>2</sub>O]<sup>+</sup> requires 259.1803

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.5 6-Azido-2-heptylquinolin-4-ol ?



6-Amino-2-heptylquinolin-4-ol ? (50 mg, 0.194 mmol, 1 eq) was dissolved in HCl (conc., aq., 1.20 ml), water (1.80 ml) and MeOH (2.00 ml) and cooled to 0 °C. A solution of NaNO<sub>2</sub> (16.0 mg, 0.232 mmol, 1.2 eq.) in water (0.300 ml) was added dropwise over 10 min and the mixture was stirred for 1 h. A solution of NaN<sub>3</sub> (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. ? was obtained as a pale cream amorphous solid (25.6 mg, 0.0900 mmol, 46.5 %).

**TLC** *R<sub>f</sub>* = 0.40 (5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 7.73 (d, *J* = 8.6 Hz, 1 H, *ortho* to NH), 7.71 (d, *J* = 2.8 Hz, 1 H, *ortho* to N<sub>3</sub> 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, CCH<sub>2</sub>), 1.68 (quin, *J* = 7.6 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 1.28 - 1.39 (m, 4 H, CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>),

1.18 - 1.28 (m, 4 H,  $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.85 (t,  $J = 6.8$  Hz, 3 H,  $\text{CH}_3$ )

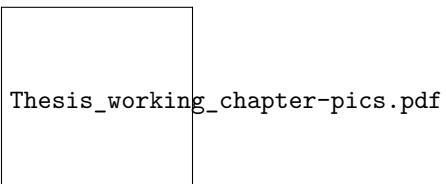
**$^{13}\text{C}$  NMR** (101 MHz, MeOD)  $\delta$  / ppm = 172.3 ( $\text{C}(=\text{O})$ ), 155.5 ( $\text{NH}\text{CCH}_2$ ), 137.4 ( $\text{CN}_3$ ), 135.6 (*para* to  $\text{N}_3$ ), 124.6 (*para* to  $\text{C}(=\text{O})$ ), 124.1 (*ipso* to  $\text{C}(=\text{O})$ ), 120.7 (*meta* to  $\text{N}_3$  and *meta* to  $\text{C}(=\text{O})$ ), 112.8 (*ortho* to  $\text{N}_3$  and *ortho* to  $\text{C}(=\text{O})$ ), 107.0 ( $\text{C}(=\text{O})\text{CH}$ ), 33.3 ( $\text{NH}\text{CCH}_2$ ), 31.2 ( $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 28.3 - 28.5 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 22.1 ( $\text{CH}_2\text{CH}_3$ ), 14.0 ( $\text{CH}_3$ )

**HRMS** (ESI $^+$ )  $m/z$  / Da = 285.1728,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}]^+$  requires 285.1715

Spectroscopic data are not consistent with the literature.<sup>?</sup>

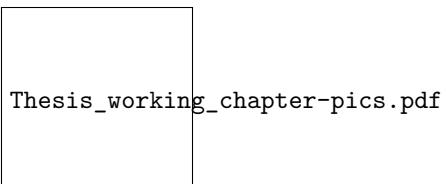
????

#### 4.6 Heptyl magnesium bromide ?



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 ? (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 ? was obtained as a pale grey suspension (15 ml, ~1 M) which was used without further purification.

#### 4.7 2-Chloro-*N*-methoxy-*N*-methylacetamide ?



*N,O*-Dimethylhydroxyl amine hydrochloride ? (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 ? (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 ( $3 \times 20$  ml). The combined organic extracts were dried with  $\text{MgSO}_4$  and the solvent was removed by rotary evaporation followed by high vacuum. ? was obtained as white, prism-like crystals (7.24 g, 52.6 mmol, 71 %).

**mp**  $T$  / °C = 38.8 (toluene)

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3016.7 (C-H), 2966.4 (C-H), 2946.7 (C-H), 2827.7 (C-H), 1666.2 (C=O)

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 4.20 (s, 2 H,  $\text{ClCH}_2\text{C}=\text{O}$ ), 3.71 (m, 3 H,  $\text{OCH}_3$ ), 3.18 (s, 3 H,  $\text{NCH}_3$ )

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 167.4 (C=O), 61.6 (OCH<sub>3</sub>), 40.9 (ClCH<sub>2</sub>C=O), 32.6 (NCH<sub>3</sub>)

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.8 1-Chlorononan-2-one ?

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2-Chloro-*N*-methoxy-*N*-methylacetamide ? (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 ? (~ 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 MgSO<sub>4</sub>, and the solvent was removed by rotary evaporation. ? was obtained as a colourless oil (1.23 g, 6.96 mmol, 96 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2951.7 (C-H), 2925.0 (C-H), 2855.5 (C-H), 1720.4 (C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 4.05 (s, 2 H, ClCH<sub>2</sub>C(=O)), 2.54 (t,  $J$  = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.59 (quin,  $J$  = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.34 - 1.21 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.87 (t,  $J$  = 6.8 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 202.6 (C(=O)), 48.1 (CH<sub>2</sub>Cl), 39.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 31.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.9 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 23.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 13.9 (CH<sub>3</sub>)

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.9 2-Oxononyl 2-amino-5-nitrobenzoate ?

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5-Nitroanthranilic acid ? (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 ? (353 mg, 2.00 mmol, 1 eq.) was added and the mixture was stirred for 15 h. The solution was poured into Na<sub>2</sub>HCO<sub>3</sub> (aq., 10 %, 50 ml) and ice (~ 20 g). The precipitate was collected by vacuum filtration, washed with water and dried under high vacuum. ? was obtained as a yellow amorphous solid (0.674 g, 2.00 mmol, 100 %).

**mp**  $T$  / °C = 135 (H<sub>2</sub>O)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

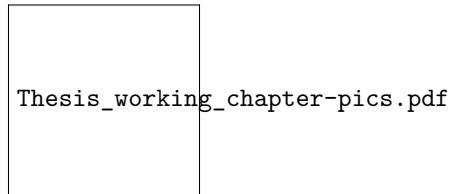
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 8.66 (d,  $J$  = 2.8 Hz, 1 H, *ortho* to C(=O)), 8.12 (dd,  $J$  = 2.8, 9.4 Hz, 1 H, *para* to C(=O)), 6.93 (d,  $J$  = 9.4 Hz, 1 H, *meta* to C(=O)), 5.05 (s, 2 H, OCH<sub>2</sub>C(=O)), 2.49 (t,  $J$  = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin,  $J$  = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.32 - 1.20 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.86 (t,  $J$  = 6.8 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 204.4 (OCH<sub>2</sub>C(=O)), 165.6 (C(=O)O), 156.3 (*ipso* to NH<sub>2</sub>), 135.7 (*ipso* to NO<sub>2</sub>), 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 (OCH<sub>2</sub>C(=O)), 38.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.9 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 23.2 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 14.4 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 323.1610, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>23</sub>N<sub>2</sub>O<sub>5</sub>]<sup>+</sup> requires 323.1607

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.10 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1H)-one ?



2-Oxononyl 2-amino-5-nitrobenzoate ? (100 mg, 0.340 mmol, 1 eq.) and polyphosphoric acid (300 mg) were stirred for 5.5 h at 90 °C under argon. The mixture was then poured into NaHCO<sub>3</sub> (sat., aq., 50 ml) cooled on ice. The precipitate was collected by vacuum filtration, washed with water (50 ml) and dried under high vacuum. ? was obtained as a yellow-brown amorphous solid (44 mg, 0.145 mmol, 43 %).

**mp**  $T$  / °C = 223 (H<sub>2</sub>O, EtOAc)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 12.00 (s, 1 H, NH), 8.91 (d,  $J$  = 2.8 Hz, 1 H, *ortho* to C=O), 8.29 (dd,  $J$  = 2.7, 9.2 Hz, 1 H, *para* to C=O), 7.70 (d,  $J$  = 9.3 Hz, 1 H, *meta* to C=O), 2.75 (t,  $J$  = 7.7 Hz, 2 H, CCH<sub>2</sub>), 1.67 (quin,  $J$  = 7.3 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 1.36 - 1.23 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.85 (t,  $J$  = 7.0 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 169.7 (C=O), 141.9 (*para* to NO<sub>2</sub>), 140.7 (*ipso* to NO<sub>2</sub>), 139.6 (*ipso* to OH), 137.3 (C=COH), 124.3 (*para* to C=O), 122.3 (*ortho* to NO<sub>2</sub> and *ortho* to C=O), 121.5 (*ipso* to C=O), 120.0 (*meta* to NO<sub>2</sub> and *meta* to C=O), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 29.2 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 28.5 (CCH<sub>2</sub>), 28.1 (CCH<sub>2</sub>CH<sub>2</sub>), 22.5 (CH<sub>2</sub>CH<sub>3</sub>), 14.4 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 305.1501, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>21</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup> requires 305.1500

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.11 6-Amino-2-heptyl-3-hydroxyquinolin-4(1H)-one ?

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

**mp** (MeOH)  $T$  / °C = 176

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3000.00 (O-H, br) 2925.41 (C-H), 2854.09 (C-H), 1613.43 (C=O)

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

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 166.8 (C=O), 144.8 (*para* to NH<sub>2</sub> or *ipso* to C=O), 140.5 (*ipso* to COH), 138.6 (C=COH), 132.6 (*ipso* to NH<sub>2</sub>), 124.8 (*para* to NH<sub>2</sub> or *ipso* to C=O), 123.8 (*para* to C=O), 107.7 (*meta* to NH<sub>2</sub> and *meta* to C=O), 106.4 (*ortho* to NH<sub>2</sub> and *ortho* to C=O), 33.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 29.5 - 31.0 (CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.8 (CH<sub>2</sub>CH<sub>3</sub>), 14.5 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 275.1760, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup> requires 275.1762 Spectroscopic data are not consistent with the literature.<sup>7</sup> It is possible that Baker's product is a Zn adduct.

#### 4.12 6-Azido-2-heptyl-3-hydroxyquinolin-4(1H)-one ?

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6-Amino-2-heptyl-3-hydroxyquinolin-4(1H)-one ? (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. NaNO<sub>2</sub> (5.0 mg, 0.0725 mmol, 1.09 eq.) in H<sub>2</sub>O (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. NaN<sub>3</sub> (4.9 mg, 0.0754 mmol, 1.14 eq.) in H<sub>2</sub>O (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. ? was obtained as a brown amorphous solid (5.5 mg, 0.0183 mmol, 28 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = pending

don't have?

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 7.74 (s, 1 H, *ortho* to C=O), 7.65 (d,  $J$  = 6.9 Hz, 1 H, *meta* to C(=O)), 7.32 (d,  $J$  = 7.4 Hz, 1 H, *para* to C(=O)), 2.75 (t,  $J$  = 7.5 Hz, 2 H, CCH<sub>2</sub>), 1.67 (quin,  $J$  = 6.4 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 1.43 - 1.13 (m, 8 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.85 (t,  $J$  = 6.8 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = pending

don't have?

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = pending, [M+H]<sup>+</sup>, [C<sub>16</sub>H<sub>21</sub>N<sub>4</sub>O<sub>2</sub>]<sup>+</sup> requires 301.1659

try?

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.13 (*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide ?

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L-Methionine ? (3.04 g, 20.4 mmol, 1 eq.) and bromoacetic acid ? (3.08 g, 22.2 mmol, 1.09 eq.) were dissolved in *i*-PrOH (12.5 ml), H<sub>2</sub>O (12.5 ml) and AcOH (5 ml). The reaction was refluxed for 15 h then concentrated under vacuum. The resulting brown oil was added to a mixture of *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. ? 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)  $\nu_{max}$  / cm<sup>-1</sup> = 2972.1 (N-H), 2877.5 (N-H), 1771.8 (C=O), 1585.1 (N-H bend), 1572.2 (N-H bend)

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 8.59 (br s, 3 H, NH<sub>3</sub><sup>+</sup>), 4.46 (dt,  $J$  = 1.3, 8.9 Hz, 1 H, OCHH), 4.37 (dd,  $J$  = 8.8, 11.4 Hz, 1 H, CHNH<sub>3</sub><sup>+</sup>), 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, OCH<sub>2</sub>CHH), 2.26 (dtd,  $J$  = 9.0, 11.2, 12.2 Hz, 1 H, OCH<sub>2</sub>CHH)

**<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 173.3 (C=O), 66.2 (OCH<sub>2</sub>), 47.8 (CHNH<sub>3</sub><sup>+</sup>), 27.0 (OCH<sub>2</sub>CH<sub>2</sub>)

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -30.0, lit. = -25.0 ( $c$  / g(100 ml)<sup>-1</sup> = 0.0200, DMSO)

The data are consistent with the literature.<sup>7</sup>

#### 4.14 (*S*)-2-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)acetamide ?

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(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide ? (100 mg, 0.549 mmol, 1.08 eq.) and NaHCO<sub>3</sub> (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and H<sub>2</sub>O (2 ml). Bromoacetyl bromide ? (44.0  $\mu$ L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 24 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The aqueous phase was extracted with EtOAc (4  $\times$  10 ml). The combined organic layers were dried with MgSO<sub>4</sub> and the solvent was removed under reduced pressure. ? was obtained as white, needle-like crystals (88.0 mg, 0.396 mmol, 74 %).

**mp** *T* / °C = 132 (EtOAc)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3255.7 (N-H), 3066.6 (C-H), 1763.0 (lactone C=O), 1658.0 (amide C=O), 1552.7 (N-H bend)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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, OCCH), 4.32 (ddd, *J* = 11.3, 9.4, 5.9 Hz, 1 H, OCH), 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, OCH<sub>2</sub>CH), 2.22 (td, *J* = 12.6, 11.5, 11.5, 8.9 Hz, 1 H, OCH<sub>2</sub>CHH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 174.6 (OC=O), 166.4 (C(=O)NH), 66.1 (OCH<sub>2</sub>), 49.8 (CHNHC=O), 29.9 (OCH<sub>2</sub>CH<sub>2</sub>), 28.2 (O=CCH<sub>2</sub>Br)

**HRMS** The compound does not ionise.

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 27.0, lit. = 20.5 (*c* / g(100 ml)<sup>-1</sup> = 0.00740, CHCl<sub>3</sub>)

The data are consistent with the literature.<sup>?, ?</sup>

#### 4.15 (*S*)-2-Azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide ?

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(3*S*)-2-Oxotetrahydrofuran-3-aminium bromide ? (100 mg, 0.552 mmol, 1.08 eq.), NaN<sub>3</sub> (85.7 mg, 1.32 mmol, 2.61 eq.) and NaHCO<sub>3</sub> (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and H<sub>2</sub>O (2 ml). Bromoacetyl bromide ? (44.0  $\mu$ L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The aqueous phase was extracted with

EtOAc ( $4 \times 10$  ml). The combined organic layers were dried with  $\text{MgSO}_4$  and the solvent was removed under reduced pressure. ? was obtained as white, needle-like crystals (38.4 mg, 0.209 mmol, 41 %).

**mp**  $T / ^\circ\text{C} = 87$  (EtOAc)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3283.5$  (N-H), 2923.3 (C-H), 2853.0 (C-H), 2129.7 ( $\text{N}_3$ ), 1782.9 (lactone C=O), 1661.4 (amide C=O), 1536.8 (N-H bend)

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

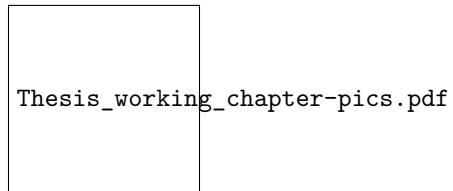
**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 174.9$  ( $\text{OC=O}$ ), 167.5 ( $\text{C=ONH}$ ), 66.0 ( $\text{OCH}_2$ ), 52.2 ( $\text{O=CCH}_2\text{N}_3$ ), 48.9 ( $\text{CHNHC=O}$ ), 29.7 ( $\text{OCH}_2\text{CH}_2$ )

**HRMS** The compound does not ionise.

$[\alpha]_D^{20} / ^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -32.6$ , lit. = -24.4 ( $c / \text{g}(100 \text{ ml})^{-1} = 0.0430$ , DMSO)

The data are consistent with the literature.?

#### 4.16 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide ?



(*S*)-3-Aminodihydrofuran-2(*3H*)-one hydrobromide ? (200 mg, 1.10 mmol, 1.00 eq.) and  $\text{NaHCO}_3$  (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in  $\text{CH}_2\text{Cl}_2$  (2 ml) and  $\text{H}_2\text{O}$  (2 ml). Bromobutyryl chloride ? (140  $\mu\text{L}$ , 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the  $\text{CH}_2\text{Cl}_2$  was removed under vacuum. The aqueous phase was extracted with EtOAc ( $7 \times 5$  ml) and the combined organic layers were dried with  $\text{MgSO}_4$ . The solvent was removed under vacuum to give white crystals which were recrystallised from EtOAc. ? was obtained as white, needle-like crystals (219 mg, 0.878 mmol, 80 %).

**mp**  $T / ^\circ\text{C} = 105$  (EtOAc)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3307.9$  (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)

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

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

**HRMS** The compound does not ionise.

$[\alpha]_D^{26.6} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -78$  ( $c$  / g(100 ml) $^{-1}$  = 0.0833, MeOH)

The compound has not been reported previously.

#### 4.17 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ?

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(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide ? (100 mg, 0.549 mmol, 1.00 eq.) and  $\text{NaHCO}_3$  (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in  $\text{CH}_2\text{Cl}_2$  (2 ml) and  $\text{H}_2\text{O}$  (2 ml) at r.t.. Bromohexanoyl chloride ? (93.0  $\mu\text{L}$ , 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the  $\text{CH}_2\text{Cl}_2$  was removed under vacuum. The mixture was then filtered, washed with  $\text{H}_2\text{O}$  (10 ml) and dried under high vacuum. ? was obtained as white, needle-like crystals (101 mg, 0.362 mmol, 66 %).

**mp**  $T$  /  ${}^\circ\text{C}$  = 106 ( $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ )

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

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 6.09 (br d,  $J$  = 5.7 Hz, 1 H,  $\text{NH}$ ), 4.57 (ddd,  $J$  = 5.9, 8.6, 11.6 Hz, 1 H,  $\text{CHNH}$ ), 4.50 (dt,  $J$  = 1.3, 9.1 Hz, 1 H,  $\text{OCHHH}$ ), 4.31 (ddd,  $J$  = 5.9, 9.3, 11.3 Hz, 1 H,  $\text{OCHH}$ ), 3.43 (t,  $J$  = 6.7 Hz, 2 H,  $\text{CH}_2\text{Br}$ ), 2.88 (dddd,  $J$  = 1.3, 5.9, 8.6, 12.6 Hz, 1 H,  $\text{OCH}_2\text{CHHH}$ ), 2.30 (dt,  $J$  = 1.8, 7.5 Hz, 2 H,  $\text{C(=O)CH}_2$ ), 2.16 (tdt,  $J$  = 8.9, 11.5, 12.5 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 1.90 (quin,  $J$  = 7.2 Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{Br}$ ), 1.71 (quin,  $J$  = 7.6 Hz, 2 H,  $\text{C(=O)CH}_2\text{CH}_2$ ), 1.59 - 1.46 (m, 2 H,  $\text{C(=O)CH}_2\text{CH}_2\text{CH}_2$ )

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

**HRMS** (ESI $^+$ )  $m/z$  / Da = 278.0381,  $[\text{M}+\text{H}]^+$ ,  $[\text{C}_{10}\text{H}_{17}\text{BrNO}_3]^+$  requires 278.0386

$[\alpha]_D^{26.6} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -16$  ( $c$  / g(100 ml) $^{-1}$  = 0.208, MeOH)

The compound has not been reported previously.

#### 4.18 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ?

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

**mp**  $T$  / °C = 90.0 ( $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3314.0 (N-H), 2931.6 (C-H), 2862.9 (C-H), 2095.1 ( $\text{N}_3$ ), 1775.4 (lactone C=O), 1643.1 (amide C=O), 1547.9 (N-H bend)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 5.96 (d,  $J$  = 4.2 Hz, 1 H,  $\text{NH}$ ), 4.54 (ddd,  $J$  = 11.7, 8.6, 5.7 Hz, 1 H,  $\text{CHNH}$ ), 4.49 (td,  $J$  = 9.1, 1.0 Hz, 1 H,  $\text{OCHH}$ ), 4.30 (ddd,  $J$  = 11.3, 9.4, 5.8 Hz, 1 H,  $\text{OCHH}$ ), 3.29 (t,  $J$  = 6.9 Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.88 (dddd,  $J$  = 12.5, 8.6, 5.8, 1.1 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.28 (t,  $J$  = 7.5 Hz, 1 H,  $\text{C}(=\text{O})\text{CHH}$ ), 2.28 (t,  $J$  = 7.4 Hz, 1 H,  $\text{C}(=\text{O})\text{CHH}$ ), 2.14 (dtd,  $J$  = 12.3, 11.5, 11.5, 8.8 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 1.70 (quin,  $J$  = 7.6 Hz, 2 H,  $\text{CH}_2\text{CH}_2\text{N}_3$ ), 1.63 (quin,  $J$  = 7.2 Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 1.38 - 1.49 (m, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2$ )

**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 175.4 ( $\text{OC}=\text{O}$ ), 172.2 ( $\text{C}(=\text{O})\text{NH}$ ), 66.1 ( $\text{OCH}_2$ ), 51.2 ( $\text{CH}_2\text{N}_3$ ), 49.4 ( $\text{CHNHC}=\text{O}$ ), 35.9 ( $\text{C}(=\text{O})\text{CH}_2$ ), 30.7 ( $\text{OCH}_2\text{CH}_2$ ), 28.6 ( $\text{CH}_2\text{CH}_2\text{N}_3$ ), 26.3 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ ), 24.8 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ )

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

$[\alpha]_D^{26.6}$  /  ${}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -16 ( $c$  / g(100 ml) $^{-1}$  = 0.208, MeOH)

The compound has not been reported previously.

#### 4.19 Hex-5-ynal ?

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Pyridinium chlorochromate (14.6 g, 68.1 mmol, 1.50 eq) and DCM (500 ml) were stirred at r.t. under argon. 5-Hexyn-1-ol ? (5.00 ml, 45.4 mmol, 1 eq.) was added and the reaction mixture was stirred for 5 h followed by

addition of Et<sub>2</sub>O (125 ml) and silica gel (62.5 g). The suspension was stirred for 1 h then filtered through a pad of silica (100 g) and washed with Et<sub>2</sub>O. The solvent was removed by rotary evaporation. ? was obtained as a pale yellow-green oil (4.72 g, 49.1 mmol, 72 %).

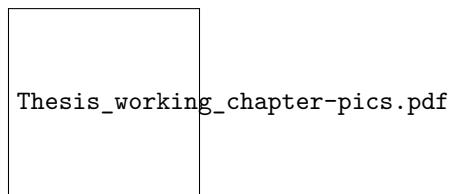
**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3292.7 (alkyne C-H), 2943.3 (alkane C-H), 2830.9 (aldehyde C-H), 2728.6 (aldehyde C-H), 1720.3 (aldehyde C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 9.80 (s, 1 H, C(=O)H), 2.60 (t,  $J$  = 7.1 Hz, 2 H, CH<sub>2</sub>C(=O)H), 2.26 (dt,  $J$  = 2.6, 6.8 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.98 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.85 (quin,  $J$  = 7.0 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 201.6 (C(=O)), 83.1 (HC≡C), 69.3 (HC≡C), 42.4 (CH<sub>2</sub>C(=O)), 20.7 (CH<sub>2</sub>CH<sub>2</sub>C(=O)), 17.6 (HC≡CCH<sub>2</sub>)

Spectroscopic data are consistent with the literature.<sup>7</sup>

#### 4.20 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate ?



Hex-5-ynal ? (0.407 g, 4.24 mmol, 1.00 eq.) and *tert*-butyl piperazine-1-carboxylate ? (0.791 g, 4.24 mmol, 1.00 eq.) were stirred under a N<sub>2</sub> 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 NaHCO<sub>3</sub> (sat., aq., 120 ml) was added and the product extracted with EtOAc (2×100 ml). The solvent was dried over MgSO<sub>4</sub> and removed by rotary evaporation. ? was obtained as a colourless liquid (1.12 g, 4.21 mmol, 99 %).

**TLC**  $R_f$  (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>) = 0.55

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3303.6 (alkyne C-H), 2940.0 (alkane C-H), 2865.2 (C-H), 2810.4 (C-H), 1691.3 (carbamate C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 3.44 (t,  $J$  = 5.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (t,  $J$  = 5.1 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.37 (t,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.23 (dt,  $J$  = 2.7, 6.8 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.96 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.53 (m, 4 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.47 (s, 9 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 154.7 (NC(=O)O), 84.2 (HC≡C), 79.6 (C(CH<sub>3</sub>)<sub>3</sub>), 68.5 (HC≡C), 60.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 58.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 53.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 26.3 (CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 18.3 (HC≡CCH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 267.2073, [M+H]<sup>+</sup>, [C<sub>15</sub>H<sub>27</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup> requires 267.2064

The compound has not been reported previously.

#### 4.21 1-(Hex-5-yn-1-yl)piperazine ?

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*tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate ? (763 mg, 2.86 mmol) was stirred in TFA (10 ml) at r.t. for 2 h. The TFA was removed under vacuum followed by co-evaporation with CH<sub>2</sub>Cl<sub>2</sub> (2×20 ml). The oil was diluted with H<sub>2</sub>O (10 ml) and the pH adjusted to 14 with NaOH (10 % aq.). This mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×20 ml) and the combined organic layers were dried over MgSO<sub>4</sub>. The solvent was removed under vacuum and purified by column chromatography (SiO<sub>2</sub> MeOH/CH<sub>2</sub>Cl<sub>2</sub> 3:7). ? was obtained as a colourless liquid (476 mg, 2.86 mmol, 100 %).

**TLC**  $R_f$  (30 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>) = 0.20

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3295.9 (alkyne C-H), 2941.1 (alkane C-H), 2810.6 (alkane C-H), 1637.2 (N-H bend)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 2.88 (t,  $J$  = 4.9 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.31 (t,  $J$  = 7.1 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.20 (dt,  $J$  = 2.7, 6.8 Hz, 2 H, HC≡CCH<sub>2</sub>), 2.05 (br s, 1 H, NH), 1.93 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 1.65 - 1.48 (m, 4 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 84.3 (HC≡C), 68.4 (HC≡C), 58.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 54.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 46.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 26.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 18.3 (HC≡CCH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 167.1548, [M+H]<sup>+</sup>, [C<sub>10</sub>H<sub>19</sub>N<sub>2</sub>]<sup>+</sup> requires 167.1548

The compound has not been reported previously.

#### 4.22 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ?

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7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquino-line-3-carboxylic acid ? (1.27 g, 4.51 mmol, 1 eq.), 1-(hex-5-yn-1-yl)piperazine ? (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). ? was obtained as off-white crystals (0.219 g, 0.531 mmol, 11.8 %).

**TLC**  $R_f$  = 0.02 (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**mp**  $T$  / °C = 220 (MeOH, decomposes)

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

**<sup>1</sup>H NMR** (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 15.12 (br s, 1 H, C(=O)OH), 8.69 (s, 1 H, *ortho* to C(=O)OH), 7.96 (d,  $J$  = 13.0 Hz, 1 H, *ortho* to F), 7.61 (d,  $J$  = 7.6 Hz, 1 H, *meta* to F), 3.82 - 3.92 (m, 3 H, NCH(CH<sub>2</sub>)<sub>2</sub> and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.54 - 3.68 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 3.45 (br. t,  $J$  = 11.6 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.21 - 3.29 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.11 - 3.20 (br m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.84 (t,  $J$  = 2.7 Hz, 1 H, HC≡C), 2.24 (td,  $J$  = 7.0, 2.7 Hz, 2 H, HC≡CCH<sub>2</sub>), 1.83 (br. quin,  $J$  = 7.5 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin,  $J$  = 7.4 Hz, 2 H, HC≡CCH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.36 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.16 - 1.23 (m, 2 H, NCH(CHH)<sub>2</sub>)

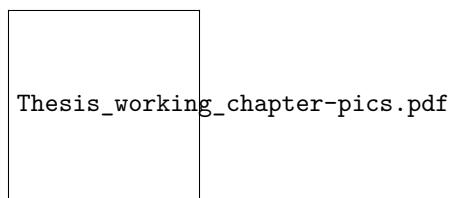
**<sup>13</sup>C NMR** (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 165.8 (C(=O)OH), 152.8 (d,  $J$  = 248.5 Hz, *ipso* to F), 148.2 (CHCC(=O)OH), 143.7 (d,  $J$  = 11.1 Hz, *para* to C(=O)), 139.1 (*para* to F), 119.4 (d,  $J$  = 6.9 Hz, *ipso* to C(=O)), 111.2 (d,  $J$  = 22.5 Hz, *ortho* to F and *ortho* to C(=O)), 106.9 (*meta* to F and *meta* to C(=O)), 106.9 (C(=O)CC(=O)OH), 83.9 (HC≡C), 71.8 (HC≡C), 55.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 50.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 46.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.0 (NCH(CH<sub>2</sub>)<sub>2</sub>), 25.2 (HC≡CCH<sub>2</sub>CH<sub>2</sub>), 22.3 (HC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 17.4 (HC≡CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -121.8 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 412.2036, [M+H]<sup>+</sup>, [C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>F]<sup>+</sup> requires 412.2030

The compound has not been reported previously.

#### 4.23 4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol ?



Hydrobromic acid (48 % w/w, aq., 50 ml) was heated to 100 °C. Trimethoprim ? (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 NH<sub>4</sub>OH (sat., aq.) and cooled slowly to 0 °C. The resulting crystals were filtered out, washed

with cold water and dried under vacuum. ? was obtained as pale pink prisms (2.06 g, 7.46 mmol, 43.4 %).

**TLC**  $R_f = 0.04$  (5 % MeOH/CHCl<sub>2</sub>)

**mp**  $T / ^\circ\text{C} = 238$  (H<sub>2</sub>O, 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)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta / \text{ppm} = 7.21$  (s, 1 H, CHN), 6.54 (s, 2 H, *meta* to OCH<sub>2</sub>), 4.87 (br s, 5 H, OH, NH<sub>2</sub>  $\times$  2), 3.82 (s, 6 H, OCH<sub>3</sub>), 3.63 (s, 2 H, CCH<sub>2</sub>C)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta / \text{ppm} = 166.4$  (CH<sub>2</sub>CCNH<sub>2</sub>), 162.0 (CHNCNH<sub>2</sub>), 156.2 (CHNCNH<sub>2</sub>), 149.8 (*ipso* to OCH<sub>3</sub>), 135.9 (*ipso* to OH), 128.2 (*para* to OH), 111.7 (CH<sub>2</sub>CCNH<sub>2</sub>), 107.5 (*meta* to OH), 57.0 (OCH<sub>3</sub>), 33.9 (CCH<sub>2</sub>C)

**HRMS** (ESI<sup>+</sup>)  $m/z / \text{Da} = 277.1295$ , [M+H]<sup>+</sup> found, [C<sub>13</sub>H<sub>17</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 277.1301

The data are consistent with the literature.<sup>7</sup>

#### 4.24 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ?

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4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol ? (1.00 g, 3.62 mmol, 1 eq.), 6-chloro-1-hexyne ? (0.524 ml, 0.420 g, 4.34 mmol, 1.2 eq.), Cs<sub>2</sub>CO<sub>3</sub> (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 CH<sub>2</sub>Cl<sub>2</sub> (30 ml) was added and the mixture filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). ? was obtained as a pale cream amorphous solid (0.327 g, 0.917 mmol, 25.3 %).

**TLC**  $R_f = 0.14$  (5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3451.4$  (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)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta / \text{ppm} = 7.77$  (s, 1 H, CHN), 6.37 (s, 2 H, *meta* to OCH<sub>2</sub>), 4.83 (br s, 2 H, CHNCNH<sub>2</sub>), 4.63 (br s, 2 H, CH<sub>2</sub>CCNH<sub>2</sub>), 3.95 (t,  $J = 6.3$  Hz, 2 H, CH<sub>2</sub>O), 3.79 (s, 6 H, OCH<sub>3</sub>), 3.65 (s, 2 H, CCH<sub>2</sub>C), 2.28 (td,  $J = 7.1, 2.6$  Hz, 2 H, HC≡CCH<sub>2</sub>), 1.94 (t,  $J = 2.7$  Hz, 1 H, HC≡C), 1.81 - 1.90 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>O), 1.71 - 1.80 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta / \text{ppm} = 162.7$  (CH<sub>2</sub>CCNH<sub>2</sub>), 162.0 (CHNCNH<sub>2</sub>), 156.4 (CHNCNH<sub>2</sub>), 153.8

(*ipso* to OCH<sub>3</sub>), 136.0 (*ipso* to OCH<sub>2</sub>), 133.6 (*para* to OCH<sub>2</sub>), 106.5 (CH<sub>2</sub>CCN<sub>2</sub>), 105.0 (*meta* to OCH<sub>2</sub>), 84.5 (HC≡C), 72.6 (CH<sub>2</sub>O), 68.3 (HC≡C), 56.1 (OCH<sub>3</sub>), 34.7 (CCH<sub>2</sub>C), 29.1 (CH<sub>2</sub>CH<sub>2</sub>O), 24.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 18.0 (HC≡CCH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 357.1920, [M+H]<sup>+</sup> found, [C<sub>19</sub>H<sub>25</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 357.1927

The compound has not been reported previously.

#### 4.25 Optimised general procedure for the click reaction

Azide (1 eq.) and alkyne (1 eq.) were dissolved in 50 % *t*-BuOH/water in a round-bottomed flask with a stirrer bar, closed with a new septum. The mixture was degassed by bubbling through N<sub>2</sub>. The mixture was placed under positive pressure of Ar using a balloon. Equimolar amounts of CuSO<sub>4</sub> · 5 H<sub>2</sub>O and THPTA ? were dissolved in water to make a 50 mM solution and similarly degassed. Sodium ascorbate was dissolved in water to make a 100 mM solution and similarly degassed. The Cu/THPTA solution (0.05 eq.) was added to the reaction mixture, followed by the sodium ascorbate solution (0.1 eq.). The mixture was stirred for 2 h and monitored using LCMS. HL derivative conjugates were dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). Other conjugates were purified by preparative HPLC (5-95 % acetonitrile (0.1 % TFA)/water (0.05 % TFA) over 20 min).

#### 4.26 (*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 ?

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50 % water/*t*-BuOH (2 ml) was degassed by bubbling N<sub>2</sub> 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 ? (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-2-azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide ? (9.2 mg, 50.0  $\mu$ mol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624  $\mu$ g, 2.5  $\mu$ mol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu$ mol, 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu$ g, 5  $\mu$ mol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (50  $\mu$ l) 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  $\mu$ l) was added. After a further 3 h the reaction mixture was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (8.8 mg, 14.8  $\mu$ mol, 29.6 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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=CCH<sub>2</sub>), 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(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br s, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.67 (t, *J* = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 2.58 (br t, *J* = 5.0 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.42 - 2.49 (m, 1 H, OCH<sub>2</sub>CHH), 2.40 (t, *J* = 7.1 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.17 (dtd, *J* = 11.7, 10.8, 9.0 Hz, 1 H, OCH<sub>2</sub>CHH), 1.66 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.53 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.28 - 1.35 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.16 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>) 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=CCH<sub>2</sub>), 145.3 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (para to F), 123.4 (CH=CCH<sub>2</sub>), 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 (OCH<sub>2</sub>), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.2 (C(=O)CH<sub>2</sub>N), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.2 (CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 28.2 (CH<sub>2</sub>CHNH), 26.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 596.2627, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>35</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 596.2633

[\mathbf{α}]\_D^{20} / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -3.5 (c / g(100 ml)<sup>-1</sup> = 0.0575, MeOH)

The compound has not been reported previously.

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

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50 % water/t-BuOH (2 ml) was degassed by bubbling N<sub>2</sub> 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 ? (20.6 mg, 50.0 μmol, 1 eq.) and (S)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide ? (10.6 mg, 50.0 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>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, then dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 0-20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (14.6 mg, 23.4 μmol, 46.8 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) 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=CCH<sub>2</sub>), 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, CH<sub>2</sub>NCH=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(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br. t, *J* = 4.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t, *J* = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br. t, *J* = 5.0 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 - 2.42 (m, 3 H, OCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.09 - 2.19 (m, 3 H, OCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>), 2.02 (quin, *J* = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.64 (quin, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.52 (quin, *J* = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.15 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

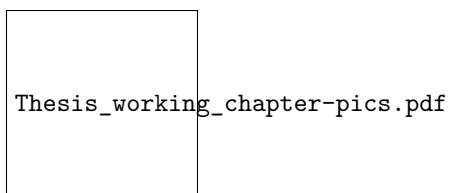
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>) δ / ppm = 176.3 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 171.2 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 248.6 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (para to F), 121.7 (CH=CCH<sub>2</sub>), 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 (OCH<sub>2</sub>), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.6 (CH<sub>2</sub>NCH=C), 47.9 (OC(=O)CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 31.7 (NHC(=O)CH<sub>2</sub>), 28.2 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.8 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 624.2928, [M+H]<sup>+</sup> found, [C<sub>31</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 624.2946

$[\alpha]_D^{20} / {}^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = -10.6$  (*c* / g(100 ml)<sup>-1</sup> = 0.094, MeOH)

The compound has not been reported previously.

**4.28 (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 ?**



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N<sub>2</sub> 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 ? (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (S)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ? (12.0 mg, 50.0  $\mu$ mol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (624  $\mu$ g, 2.5  $\mu$ mol, 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu$ mol, 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu$ g, 5  $\mu$ mol, 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (50  $\mu$ l) was then added. The mixture was stirred at r.t. under argon for 3 h, then dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a CombiFlash (SiO<sub>2</sub>, 0-20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub> over 15 min) The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (12.4 mg, 19.0  $\mu$ mol, 38.0 %).

**TLC** *R<sub>f</sub>* = 0.30 (30 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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=CCH<sub>2</sub>), 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, CH<sub>2</sub>NCH=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(CH<sub>2</sub>)<sub>2</sub>), 3.32 (br t,  $J$  = 4.5, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.63 (t,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br t,  $J$  = 4.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.33 - 2.41 (m, 3 H, OCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.06 - 2.16 (m, 3 H, OCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>), 1.79 (quin,  $J$  = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.63 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.45 - 1.56 (m, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.19 - 1.25 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.15 - 1.19 (m, 2 H, NCH(CHH)<sub>2</sub>)

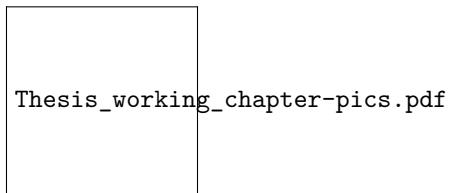
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 172.1 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d,  $J$  = 250.2 Hz, *ipso* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.2 (d,  $J$  = 9.6 Hz, *ipso* to piperazine), 139.2 (para to F), 121.6 (CH=CCH<sub>2</sub>), 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 (OCH<sub>2</sub>), 57.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.0 (CH<sub>2</sub>NCH=C), 47.8 (CHNH), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 34.8 (NHC(=O)CH<sub>2</sub>), 29.5 (CH<sub>2</sub>CH<sub>2</sub>NCH=C), 28.3 (CH<sub>2</sub>CHNH), 26.9 (CH=CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.4 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>), 24.5 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 652.3254, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>6</sub>]<sup>+</sup> requires 652.3248

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -8.5 (c / g(100 ml)<sup>-1</sup> = 0.106, MeOH)

The compound has not been reported previously.

#### 4.29 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1H-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



50 % water/t-BuOH (1 ml) was degassed by bubbling N<sub>2</sub> 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 ? (4.1 mg, 10.0  $\mu$ mol, 1 eq.) and 6-azido-2-heptylquinolin-4(1H)-one ? (2.8 mg, 10.0  $\mu$ mol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (125  $\mu$ g, 0.5  $\mu$ mol, 0.05 eq. 50 mM), THPTA (218  $\mu$ g, 0.5  $\mu$ mol, 0.05 eq. 50 mM) and sodium ascorbate (198  $\mu$ g, 1  $\mu$ mol, 0.1 eq., 100 mM) in 50 % water/t-BuOH (10  $\mu$ l) 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  $\text{NaHCO}_3$  (aq., sat., 10 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (10 ml). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (8.6 mg, 2.7  $\mu\text{mol}$ , 27.0 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2927.0 (C-H), 2865.5 (C-H), 1715.5 (carboxylic acid C=O), 1631.0 (ciprofloxacin quinolone C=O and HHQ C=O)

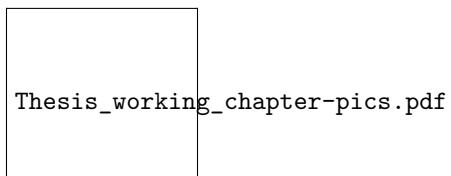
**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) 15.12 (br s, 1 H, C(=O)OH), 11.79 (s, 1 H, NH), 8.75 (s, 1 H, NCH=CCH<sub>2</sub>), 8.71 (s, 1 H, *ortho* to C(=O)OH), 8.40 (d, *J* = 2.7 Hz, 1 H, *ortho* to C(=O) and *ortho* to N), 8.18 (dd, *J* = 8.9, 2.6 Hz, 1 H, *para* to C(=O) and *ortho* to N), 7.99 (d, *J* = 13.0 Hz, 1 H, *ortho* to F), 7.75 (d, *J* = 9.0 Hz, 1 H, *meta* to C(=O) and *meta* to N), 7.62 (d, *J* = 7.8 Hz, 1 H, *meta* to F), 6.02 (s, 1 H, NHC=CHC(=O)), 3.85 (tt, *J* = 7.0, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.23 - 3.30 (m, 10 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.82 (t, *J* = 5.9 Hz, 2 H, NCH=CCH<sub>2</sub>), 2.63 (t, *J* = 7.9 Hz, 2 H, CH<sub>2</sub>C=CHC(=O)), 1.76 - 1.81 (m, 4 H, NCH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.70 (quin, *J* = 7.2 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>C=CHC(=O)), 1.15 - 1.38 (m, 12 H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, NCH(CHH)<sub>2</sub> and NCH(CHH)<sub>2</sub>), 0.87 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 176.3 (CHC(=O)), 165.8 (C(=O)OH), 154.3 (CCHC(=O)), 152.9 (d, *J* = 240.1 Hz, *ipso* to F), 148.3 (CH=CC(=O)OH), 147.5 (NCHCCH<sub>2</sub>), 143.3 (d, *J* = 8.5 Hz, *ortho* to F and *ipso* to N), 139.6 (*ipso* to NH), 139.0 (*para* to F), 132.0 (*para* to NH), 124.9 (*ipso* to C(=O) and *ortho* to NH), 123.6 (*para* to C(=O) and *meta* to NH), 120.5 (NCH=CCH<sub>2</sub>), 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=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 50.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 46.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 46.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.0 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.2 (CH<sub>2</sub>CNH), 31.2 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.3 - 28.5 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 24.4 (CH=CCH<sub>2</sub>), 22.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 22.0 (CH<sub>3</sub>CH<sub>2</sub>), 13.9 (CH<sub>3</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 696.3667, [M+H]<sup>+</sup> found, [C<sub>39</sub>H<sub>47</sub>FN<sub>7</sub>O<sub>4</sub>]<sup>+</sup> requires 696.3668

The compound has not been reported previously.

#### 4.30 (*S*)-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 ?



50 % water/*t*-BuOH (2 ml) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ? (20.6 mg, 50.0  $\mu\text{mol}$ , 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide ? (15.9 mg, 75.0  $\mu\text{mol}$ , 1.5 eq.). Similarly degassed solutions of CuSO<sub>4</sub> · 5H<sub>2</sub>O (624  $\mu\text{g}$ , 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu\text{g}$ , 5  $\mu\text{mol}$ , 0.1 eq., 100 mM) in water (50  $\mu\text{l}$ ) were then added. An extra portion of ? (10.6

mg, 50.0  $\mu\text{mol}$ , 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  $\text{CH}_2\text{Cl}_2$  ( $6 \times 10 \text{ ml}$ ) then dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography using a CombiFlash ( $\text{SiO}_2$ , 0-20 %  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a pale brown gum (4.8 mg, 8.4  $\mu\text{mol}$ , 16.8 %).

**TLC**  $R_f = 0.30$  (30 %  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3340.5$  (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)

**$^1\text{H NMR}$**  (500 MHz, DMSO  $d_6$ )  $\delta / \text{ppm} = 8.43$  (d,  $J = 8.0 \text{ Hz}$ , 1 H,  $\text{NH}$ ), 7.80 (s, 1 H,  $\text{NCH}=\text{CCH}_2$ ), 7.46 (s, 1 H,  $\text{CHN}=\text{CNH}_2$ ), 6.68 (br s, 2 H,  $\text{CH}_2\text{CCN}\text{H}_2$ ), 6.53 (s, 2 H, *meta* to  $\text{CH}_2$ ), 6.21 (br s, 2 H,  $\text{CHN}=\text{CNH}_2$ ), 4.49 (dt,  $J = 10.7, 8.6 \text{ Hz}$ , 1 H,  $\text{CHNH}$ ), 4.32 (td,  $J = 8.7, 1.6 \text{ Hz}$ , 1 H,  $\text{CHHOC}(=\text{O})$ ), 4.29 (t,  $J = 6.8 \text{ Hz}$ , 2 H,  $\text{CH}_2\text{N}$ ), 4.19 (ddd,  $J = 10.6, 8.7, 6.5 \text{ Hz}$ , 1 H,  $\text{CHHOC}(=\text{O})$ ), 3.79 (t,  $J = 6.2 \text{ Hz}$ , 2 H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 3.68 (s, 6 H,  $\text{CH}_3$ ), 3.53 (br s, 2 H,  $\text{CCH}_2\text{C}$ ), 2.63 (t,  $J = 7.5 \text{ Hz}$ , 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.37 (dddd,  $J = 12.2, 8.9, 6.7, 1.8 \text{ Hz}$ , 1 H,  $\text{CHHCHNH}$ ), 2.08 - 2.15 (m, 3 H,  $\text{CHHCHNH}$  and  $\text{C}(=\text{O})\text{CH}_2$ ), 2.00 (quin,  $J = 7.2 \text{ Hz}$ , 2 H,  $\text{CH}_2\text{CH}_2\text{N}$ ), 1.72 (quin,  $J = 7.3 \text{ Hz}$ , 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 1.61 (quin,  $J = 6.7 \text{ Hz}$ , 2 H,  $\text{CH}_2\text{CH}_2\text{O}$ )

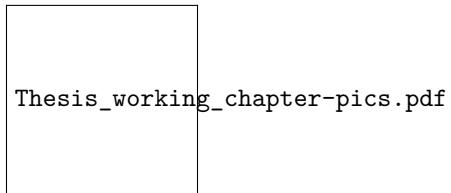
**$^{13}\text{C NMR}$**  (126 MHz, DMSO  $d_6$ )  $\delta / \text{ppm} = 175.8$  ( $\text{OC}=\text{O}$ ), 171.9 ( $\text{NHC}=\text{O}$ ), 163.1 ( $\text{CC}(\text{NH}_2)\text{N}$ ), 159.7 (br s,  $\text{NC}(\text{NH}_2)\text{N}$ ), 153.2 (*ipso* to  $\text{OCH}_3$ ), 150.5 (br s,  $\text{CHNC}(\text{NH}_2)\text{N}$ ), 147.3 ( $\text{NCH}=\text{CCH}_2\text{CH}_2$ ), 135.2 (*para* to  $\text{CH}_2\text{O}$ ), 135.0 (*ipso* to  $\text{CH}_2\text{O}$ ), 122.1 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 107.3 ( $\text{CH}_2\text{CC}(\text{NH}_2)=\text{N}$ ), 106.2 (*meta* to  $\text{CH}_2\text{O}$ ), 72.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 65.7 ( $\text{OCH}_2\text{CH}_2\text{CHNH}$ ), 56.2 ( $\text{OCH}_3$ ), 48.9 ( $\text{CH}_2\text{N}$ ), 48.3 ( $\text{CHNH}$ ), 32.9 ( $\text{CCH}_2\text{C}$ ), 32.0 ( $\text{C}(=\text{O})\text{CH}_2$ ), 29.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 28.4 ( $\text{OCH}_2\text{CH}_2\text{CHNH}$ ), 26.0 ( $\text{CH}_2\text{CH}_2\text{N}$ ), 25.7 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 24.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ )

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

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

The compound has not been reported previously.

#### 4.31 (*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 ?



50 % water/*t*-BuOH (2 ml) was degassed by bubbling  $\text{N}_2$  through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ? (20.6 mg, 50.0  $\mu\text{mol}$ , 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ? (18.0 mg, 75.0  $\mu\text{mol}$ , 1.5 eq.). Similarly degassed solutions of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (624  $\mu\text{g}$ , 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM), THPTA (1.09 mg, 2.5  $\mu\text{mol}$ , 0.05 eq. 50 mM) and sodium ascorbate (991  $\mu\text{g}$ , 5  $\mu\text{mol}$ , 0.1 eq., 100 mM) in water (50  $\mu\text{l}$ ) were then added. An extra portion of ? (12.0

mg, 50.0  $\mu\text{mol}$ , 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  $\text{CH}_2\text{Cl}_2$  ( $6 \times 10 \text{ ml}$ ) then dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography using a Combiflash ( $\text{SiO}_2$ , 0-20 %  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a clear gum (8.0 mg, 13.4  $\mu\text{mol}$ , 26.8 %).

**TLC**  $R_f = 0.35$  (30 %  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3336.0$  (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)

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

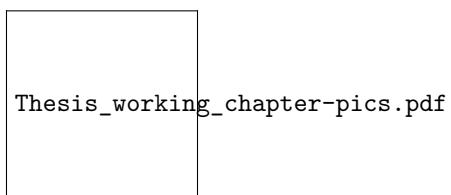
**$^{13}\text{C NMR}$**  (125 MHz, DMSO d<sub>6</sub>)  $\delta / \text{ppm} = 175.4$  (OC=O), 172.0 (NHC=O), 162.2 (CC(NH<sub>2</sub>)N), 161.8 (NC(NH<sub>2</sub>)N), 154.8 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 135.5 (*para* to CH<sub>2</sub>O), 134.8 (*ipso* to CH<sub>2</sub>O), 121.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 105.9 (CH<sub>2</sub>CC(NH<sub>2</sub>)=N), 105.8 (*meta* to CH<sub>2</sub>O), 71.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.2 (OCH<sub>2</sub>CH<sub>2</sub>CHNH), 55.8 (OCH<sub>3</sub>), 49.0 (CH<sub>2</sub>N), 47.8 (CHNH), 34.8 (C(=O)CH<sub>2</sub>), 32.9 (CCH<sub>2</sub>C), 29.4 (CH<sub>2</sub>CH<sub>2</sub>N), 29.1 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 28.2 (OCH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 25.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 24.4 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z / \text{Da} = 597.3149$ , [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>41</sub>N<sub>8</sub>O<sub>6</sub>]<sup>+</sup> requires 597.3144

$[\alpha]_D^{20} / {}^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = -3.6$  ( $c / \text{g}(100 \text{ ml})^{-1} = 0.11$ , MeOH)

The compound has not been reported previously.

#### 4.32 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 ?



50 % water/*t*-BuOH (1 ml) was degassed by bubbling  $\text{N}_2$  through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ? (3.6 mg, 10.0  $\mu\text{mol}$ , 1 eq.) and 6-azido-2-heptylquinolin-4(*1H*)-one ? (2.8 mg, 10.0  $\mu\text{mol}$ , 1 eq.). A similarly degassed solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (125  $\mu\text{g}$ , 0.5  $\mu\text{mol}$ , 0.05 eq. 50 mM), THPTA (218  $\mu\text{g}$ , 0.5  $\mu\text{mol}$ , 0.05 eq. 50 mM) and sodium ascorbate (198  $\mu\text{g}$ ,

1  $\mu$ mol, 0.1 eq., 100 mM) in water (10  $\mu$ 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 NaHCO<sub>3</sub> (aq., sat., 10 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (10 ml). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a clear gum (2.6 mg, 4.1  $\mu$ mol, 41.0 %).

**TLC**  $R_f$  = 0.17 (20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2927.7 (C-H), 2855.5 (C-H), 1664.1 (pyrimidine), 1645.4 (pyrimidine and HHQ C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 11.80 (s, 1 H, NH), 8.69 (s, 1 H, NCH=CCH<sub>2</sub>), 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, NH<sub>2</sub>), 7.41 (s, 1 H, CHN=CNH<sub>2</sub>), 6.61 (s, 2 H, *meta* to CH<sub>2</sub>), 6.02 (d, *J* = 1.8 Hz, 1 H, C(=O)CH), 3.86 (t, *J* = 6.3 Hz, 2 H, CH<sub>2</sub>O), 3.73 (s, 6 H, OCH<sub>3</sub>), 3.57 - 3.62 (m, 2 H, CCH<sub>2</sub>C), 2.78 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.63 (t, *J* = 7.3 Hz, 2 H, HNCCH<sub>2</sub>), 1.85 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.61 - 1.78 (m, 4 H, HNCCH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.31 - 1.40 (m, 4 H, HNCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.25 - 1.31 (m, 4 H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 0.86 (t, *J* = 7.2 Hz, 3 H, CH<sub>3</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C=O), 164.1 (CC(NH<sub>2</sub>)N), 154.3 (HNC), 154.2 (NC(NH<sub>2</sub>)N), 153.1 (*ipso* to OCH<sub>3</sub>), 148.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 140.2 (CHNC(NH<sub>2</sub>)N), 139.6 (*ipso* to NH), 135.4 (*ipso* to CH<sub>2</sub>O), 132.8 (*para* to CH<sub>2</sub>O), 132.1 (*para* to NH), 124.9 (*ipso* to C=O), 123.7 (*para* to C=O), 120.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 120.0 (*meta* to C=O and *ortho* to NH), 115.1 (*ortho* to C=O and *meta* to NH), 109.0 (CH<sub>2</sub>CC(NH<sub>2</sub>)=N), 108.0 (C(=O)CH), 106.3 (*meta* to CH<sub>2</sub>O), 72.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 56.0 (OCH<sub>3</sub>), 33.3 (HNCCH<sub>2</sub>), 32.1 (CCH<sub>2</sub>C), 31.2 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 29.1 (CH<sub>2</sub>CH<sub>2</sub>O), 28.3 - 28.6 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 24.7 (CH=CCH<sub>2</sub>), 22.1 (CH<sub>3</sub>CH<sub>2</sub>), 14.0 (CH<sub>3</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 641.3557, [M+H]<sup>+</sup> found, [C<sub>35</sub>H<sub>45</sub>N<sub>8</sub>O<sub>4</sub>]<sup>+</sup> 641.3558

The compound has not been reported previously.

#### 4.33 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(1*H*)-one ?

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50 % water/*t*-BuOH (1 ml) was degassed by bubbling N<sub>2</sub> through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ? (14.2 mg, 39.8  $\mu$ mol, 1 eq.) and 2-(6-azidohexyl)-3-hydroxyquinolin-4(1*H*)-one ? (11.4 mg, 39.8  $\mu$ mol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (1.25 mg, 5  $\mu$ mol, 0.125 eq. 50 mM), THPTA (2.18 mg, 5  $\mu$ mol, 0.125 eq. 50 mM) and sodium ascorbate (1.98 mg, 10  $\mu$ mol, 0.25 eq., 100 mM) in water (100  $\mu$ l) was then added. The mixture was stirred at r.t. under argon for 3 h, then MeOH (1 ml) was added and the reaction mixture was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried

with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a pale brown amorphous solid (4.7 mg, 7.3  $\mu\text{mol}$ , 18.3 %).

**TLC**  $R_f$  = 0.21 (20 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2924.8 (C-H), 2853.4 (C-H), 1660.0 (pyrimidine), 1638.8 (pyrimidine and PQS C=O)

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

**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 168.9 (C=O), 162.5 (CC(NH<sub>2</sub>)N), 162.5 (NC(NH<sub>2</sub>)N), 152.9 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 137.7 (COH), 137.3 (*para* to OH), 135.4 (HNC), 135.1 (*para* to CH<sub>2</sub>O), 134.8 (*ipso* to CH<sub>2</sub>O), 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=CCH<sub>2</sub>CH<sub>2</sub>), 117.7 (*meta* to C=O and *ortho* to NH), 106.2 (CH<sub>2</sub>CC(NH<sub>2</sub>)=N), 105.8 (*meta* to CH<sub>2</sub>O), 71.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 55.8 (OCH<sub>3</sub>), 49.0 (CH<sub>2</sub>N), 32.8 (CCH<sub>2</sub>C), 29.5 (CH<sub>2</sub>CH<sub>2</sub>N), 29.0 (CH<sub>2</sub>CH<sub>2</sub>O), 28.1 (HNCCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 27.9 (HNCCH<sub>2</sub>), 27.6 (HNCCH<sub>2</sub>CH<sub>2</sub>), 25.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 24.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 643.3365, [M+H]<sup>+</sup> found, [C<sub>34</sub>H<sub>43</sub>N<sub>8</sub>O<sub>5</sub>]<sup>+</sup> requires 643.3351

The compound has not been reported previously.

#### 4.34 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ?

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Ciprofloxacin ? (10.0 g, 30 mmol, 1 eq.) and *p*-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 NaHCO<sub>3</sub> (sat., aq., 100 ml) and water (300 ml) were added. The product was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2×400 ml). The combined organic fractions were dried over MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (9.16 g, 26.5 mmol, 83.3 %).

**TLC**  $R_f$  = 0.13 (5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2947.9 (C-H), 2834.9 (C-H), 1720.9 (ester C=O), 1616.8 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 8.55 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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, CH<sub>3</sub>), 3.62 (tt,  $J$  = 7.4, 3.5 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.24 - 3.29 (m, 4 H, HN(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.02 - 3.10 (m, 4 H, HN(CH<sub>2</sub>)CH<sub>2</sub>), 1.31 - 1.38 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.12 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

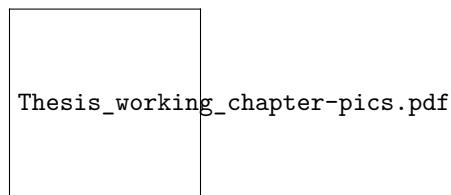
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.2 (C(=O)CC(=O)OCH<sub>3</sub>), 166.8 (C(=O)OCH<sub>3</sub>), 154.9 (d,  $J$  = 248.0 Hz, *ipso* to F), 150.1 (C=CC(=O)OCH<sub>3</sub>), 146.6 (d,  $J$  = 10.4 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.3 (d,  $J$  = 6.9 Hz, *para* to piperazine), 113.0 (d,  $J$  = 23.4 Hz, *ortho* to C=O and *ortho* to F), 110.1 (CC(=O)OCH<sub>3</sub>), 107.1 (d,  $J$  = 3.5 Hz, *meta* to C=O and *meta* to F), 52.3 (CH<sub>3</sub>), 51.7 (HN(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 51.6 (HN(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 46.5 (HN(CH<sub>2</sub>)CH<sub>2</sub>), 36.4 (NCH(CH<sub>2</sub>)<sub>2</sub>), 8.7 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.8 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 346.1569, [M+H]<sup>+</sup> found, [C<sub>18</sub>H<sub>21</sub>FN<sub>3</sub>O<sub>3</sub>]<sup>+</sup> requires 346.1567

The data are consistent with the literature.<sup>7</sup>

#### 4.35 4-Bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide ?



3-Aminodihydrothiophen-2(3H)-one hydrochloride ? (15.0 g, 97.6 mmol, 1 eq.) and NaHCO<sub>3</sub> (16.4 g, 195 mmol, 2 eq.) were added to CH<sub>2</sub>Cl<sub>2</sub> (150 ml) and water (150 ml). 4-Bromobutyryl chloride ? (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 CH<sub>2</sub>Cl<sub>2</sub> (150 ml). The combined organic layers were dried over MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white, amorphous solid (22.7 g, 85.8 mmol, 87.9 %).

**TLC**  $R_f$  = 0.19 (50 % EtOAc/PE)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3265.9 (amide N-H), 3063.2 (amide N-H), 1694.3 (thiolactone C=O), 1650.5 (amide C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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, CH<sub>2</sub>Br), 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, SCH<sub>2</sub>CHH), 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)CH<sub>2</sub>CH<sub>2</sub>), 1.96 (dddd,  $J$  = 12.7, 12.5, 12.2, 7.0 Hz, 1 H, SCH<sub>2</sub>CHH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 205.4 (SC(=O)), 172.1 (NHC(=O)), 59.4 (CHNH), 34.1 (C(=O)CH<sub>2</sub>), 33.1 (CH<sub>2</sub>Br), 31.8 (SCH<sub>2</sub>CH<sub>2</sub>), 28.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 27.5 (SCH<sub>2</sub>)

**HRMS (ESI<sup>+</sup>)** The compound does not ionise.

The compound has been synthesised previously<sup>?,?</sup> but characterisation was not published.

#### 4.36 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 ?

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Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ? (50 mg, 0.145 mmol, 1 eq.), 4-bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ? (34.5 mg, 0.145 mmol, 1 eq.) and K<sub>2</sub>CO<sub>3</sub> (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 ? (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 (SiO<sub>2</sub>, 5-10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>) followed by preparative HPLC (5-95 % acetonitrile/water over 20 min). ? was obtained as a pale cream amorphous solid (9.4 mg, 0.018 mmol, 12.2 %).

**TLC**  $R_f = 0.47$  (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (500 MHz, MeOD)  $\delta$  / ppm = 8.53 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.68 (d,  $J$  = 13.4 Hz, 1 H, *ortho* to F), 7.41 (d,  $J$  = 7.3 Hz, 1 H, *meta* to F), 4.67 (dd,  $J$  = 12.9, 6.9 Hz, 1 H, CHNH), 3.83 (s, 3 H, OCH<sub>3</sub>), 3.61 (tt,  $J$  = 6.9, 4.1 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.39 - 3.49 (m, 1 H, SCHH), 3.26 - 3.33 (m, 5 H, SCHH and CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.93 - 3.03 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.79 (br. t,  $J$  = 7.2, 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.59 (dd,  $J$  = 12.4, 6.9, 5.4, 1.4 Hz, 1 H, SCH<sub>2</sub>CHH), 2.39 (t,  $J$  = 7.20 Hz, 1 H, C(=O)CHH), 2.38 (t,  $J$  = 6.94 Hz, 1 H, C(=O)CHH), 2.18 (qd,  $J$  = 12.4, 7.0 Hz, 1 H, SCH<sub>2</sub>CHH), 1.97 (quin,  $J$  = 7.2 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.32 - 1.37 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.13 - 1.19 (m, 2 H, NCH(CHH)<sub>2</sub>)

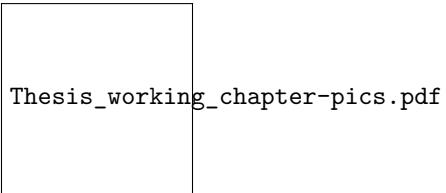
**<sup>13</sup>C NMR** (126 MHz, MeOD)  $\delta$  / ppm = 207.0 (SC(=O)), 175.7 (NHC(=O)), 175.1 (C(=O)CC(=O)OCH<sub>3</sub>), 166.6 (C(=O)OCH<sub>3</sub>), 154.7 (d,  $J$  = 249.0 Hz, *ipso* to F), 150.2 (s, CH=CC(=O)OCH<sub>3</sub>), 145.6 (d,  $J$  = 10.6 Hz, *ipso* to piperazine), 139.8 (*para* to 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)OCH<sub>3</sub>), 107.4 (*meta* to C=O and *meta* to F), 60.2 (CHNH), 58.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 53.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.3 (OCH<sub>3</sub>), 50.1 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 50.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 36.5 (NCH(CH<sub>2</sub>)<sub>2</sub>), 34.5 (C(=O)CH<sub>2</sub>), 31.7 (SCH<sub>2</sub>CH<sub>2</sub>), 28.1 (SCH<sub>2</sub>), 22.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 8.7 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -125.4 (s, ciprofloxacin F)

**HRMS (ESI<sup>+</sup>)**  $m/z$  / Da = 531.2083, [M+H]<sup>+</sup> found, [C<sub>26</sub>H<sub>32</sub>FN<sub>4</sub>O<sub>5</sub>S]<sup>+</sup> requires 531.2077

The compound has been synthesised previously.<sup>?,?</sup> Only HRMS characterisation was published, and this agrees with the result above.

#### 4.37 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ?



4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ? (6.00 g, 27.0 mmol, 1 eq.) and NaN<sub>3</sub> (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 CH<sub>2</sub>Cl<sub>2</sub> (150 ml). The aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2×150 ml) and the combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a yellow, sticky solid (4.60 g, 20.1 mmol, 89.3 %).

**TLC**  $R_f$  = 0.19 (50 % EtOAc/PE)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3285.6 (N-H), 2963.9 (C-H), 2100.2 (azide), 1697.4 (thiolactone C=O), 1647.4 (amide C=O)

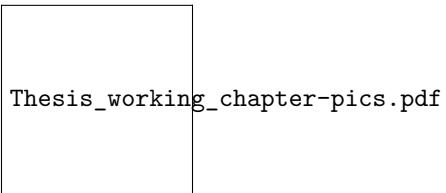
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.71 (d,  $J$  = 7.3 Hz, 1 H, NH), 4.54 (dt,  $J$  = 13.0, 7.0 Hz, 1 H, CHNH), 3.30 (t,  $J$  = 6.7 Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.31 (td,  $J$  = 11.7, 5.3 Hz, 1 H, SCHH), 3.19 (ddd,  $J$  = 11.3, 7.0, 1.2 Hz, 1 H, SCHH), 2.70 (dddd,  $J$  = 12.4, 6.8, 5.3, 1.2 Hz, 1 H, SCH<sub>2</sub>CHH), 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, SCH<sub>2</sub>CHH), 1.85 (quin,  $J$  = 6.9 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 205.4 (SC(=O)), 172.3 (NHC(=O)), 59.4 (CHNH), 50.6 (CH<sub>2</sub>N<sub>3</sub>), 32.8 (C(=O)CH<sub>2</sub>), 31.8 (SCH<sub>2</sub>CH<sub>2</sub>), 27.5 (SCH<sub>2</sub>), 24.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 251.0565, [M+Na]<sup>+</sup> found, [C<sub>8</sub>H<sub>12</sub>N<sub>4</sub>NaO<sub>2</sub>S]<sup>+</sup> requires 251.0573

The compound has not been reported previously.

#### 4.38 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 ?



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (15 mg, 36.7  $\mu$ mol, 1 eq.) and 4-azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide ? (12.5 mg, 55.1  $\mu$ mol, 1.5 eq.) were dissolved in 1:9:10 water/*t*-BuOH/DMSO (3 ml), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (182  $\mu$ l, 18.2  $\mu$ mol, 0.5 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (367  $\mu$ l, 36.7  $\mu$ 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/CHCl<sub>3</sub> (10 ml) were added, the organic layer was separated and the aqueous layer was extracted again with 10 % *i*-PrOH/CHCl<sub>3</sub> (2  $\times$  10 ml). The combined organic layers were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 50 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (50 ml). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (16.5 mg, 25.9  $\mu$ mol, 70.6 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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=CCH<sub>2</sub>), 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, CH<sub>2</sub>NCH=C), 3.80 - 3.86 (6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.34 - 3.37 (m, 1 H, SCHH), 3.32 (br t, *J* = 4.1 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.27 (ddd, *J* = 11.1, 6.9, 1.4 Hz, 1 H, SCHH), 2.64 (t, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>), 2.57 (br t, *J* = 4.7 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 - 2.44 (m, 3 H, SCH<sub>2</sub>CHH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.12 (t, *J* = 7.9 Hz, 1 H, C(=O)CHH), 2.12 (t, *J* = 7.0 Hz, 1 H, C(=O)CHH), 2.04 (m, 3 H, SCH<sub>2</sub>CHH and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.64 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.51 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.28 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.15 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

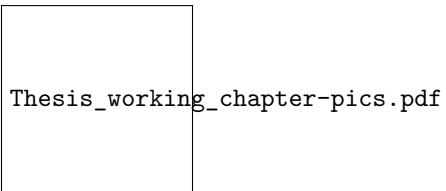
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 205.6 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.4 (NHC(=O)), 166.0 (C(=O)OH), 153.1 (d, *J* = 249.3 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.9 (CH=CCH<sub>2</sub>), 145.3 (d, *J* = 10.1 Hz, *ipso* to piperazine), 139.2 (para to F), 121.8 (CH=CCH<sub>2</sub>), 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 (CC(=O)OH), 106.4 (d, *J* = 2.9 Hz, *meta* to C=O and *meta* to F), 58.2 (SC(=O)CHNH), 57.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.6 (CH<sub>2</sub>NCH=C), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 31.9 (NHC(=O)CH<sub>2</sub>), 30.1 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.8 (SCH<sub>2</sub>), 25.9 (NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.0 (CH=CCH<sub>2</sub>), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.9 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 640.2739, [M+H]<sup>+</sup> found, [C<sub>31</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>5</sub>S]<sup>+</sup> requires 640.2712

The compound has not been reported previously.

**4.39 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 ?**



1-Cyclopropyl-6-fluoro-7-(4-(((hex-5-ynoyloxy)methoxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (203 mg, 0.407 mmol, 1 eq.), 4-azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ? (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 CH<sub>2</sub>Cl<sub>2</sub> (18.6 ml) at r.t. under Ar for 3 h. The mixture was filtered and the filtrate was dry-loaded onto SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 5-10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). ? was obtained as pale brown/yellow amorphous solid (14.7 mg, 20.2 μmol, 5.0 %).

**TLC**  $R_f$  = 0.40 (5 % CH<sub>2</sub>Cl<sub>2</sub>/MeOH)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>) δ / ppm = 15.16 (br s, 1 H, C(=O)OH), 8.65 (s, 1 H, *ortho* to C(=O)OH), 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=CCH<sub>2</sub>), 7.57 (d, *J* = 7.4 Hz, 1 H, *meta* to F), 5.74 (s, 1 H, OCH<sub>2</sub>O), 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)CH<sub>2</sub>CH<sub>2</sub>N), 3.80 (tt, *J* = 6.9, 3.6 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.62 (br t, *J* = 5.2 Hz, 4 H, C(=O)N(CH<sub>2</sub>)CH<sub>2</sub>), 3.38 (td, *J* = 11.4, 5.5 Hz, 1 H, SCHH), 3.34 (br. s, 4 H, C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.27 (ddd, *J* = 11.0, 6.9, 1.6 Hz, 1 H, SCHH), 2.64 (t, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>), 2.44 (t, *J* = 7.5 Hz, 2 H, CH<sub>2</sub>C(=O)O), 2.40 (dddd, *J* = 12.3, 6.8, 5.4, 1.4 Hz, 1 H, SCH<sub>2</sub>CHH), 2.12 (t, *J* = 7.8 Hz, 1 H, NHC(=O)CHH), 2.12 (t, *J* = 6.8 Hz, 1 H, NHC(=O)CH<sub>2</sub>), 1.98 - 2.07 (m, 3 H, SCH<sub>2</sub>CHH and NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.86 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.29 - 1.36 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.14 - 1.21 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>) δ / ppm = 205.5 (SC(=O)), 176.4 (C(=O)CC(=O)OH), 171.8 (C(=O)OCH<sub>2</sub>O), 171.3 (NHC(=O)), 165.9 (C(=O)OH), 152.8 (d, *J* = 249.7 Hz, *ipso* to F), 152.9 (O<sub>2</sub>C(=O)N), 148.1 (CH=CC(=O)OH), 146.0 (CH=CCH<sub>2</sub>), 144.9 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.1 (*para* to F), 122.0 (CH=CCH<sub>2</sub>), 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 (OCH<sub>2</sub>O), 58.2 (CHNH), 49.1 (C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.1 (C(=O)N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>N), 48.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 43.4 (N(CH<sub>2</sub>)CH<sub>2</sub>), 43.0 (N(CH<sub>2</sub>)CH<sub>2</sub>), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 31.8 (NHC(=O)CH<sub>2</sub>), 30.1 (SCH<sub>2</sub>CH<sub>2</sub>), 26.8 (SCH<sub>2</sub>), 25.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.2 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 24.0 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(=O)), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 728.2502, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>39</sub>FN<sub>7</sub>O<sub>9</sub>S]<sup>+</sup> requires 728.2503

The compound has not been reported previously.

#### 4.40 4-Bromo-*N*-(2-methoxyphenyl)butanamide ?

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2-Methoxyaniline ? (9.12 ml, 10.0 g, 81.2 mmol, 1 eq.) and NaHCO<sub>3</sub> (8.19 g, 97.4 mmol, 1.2 eq.) were dissolved in water (100 ml) and CH<sub>2</sub>Cl<sub>2</sub> (100 ml). The mixture was cooled to 0 °C and 4-bromobutyryl chloride ? (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 MgSO<sub>4</sub> and purified by column chromatography (SiO<sub>2</sub>, 5-25 % EtOAc/P.E.). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? 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, 50.0 %).

**TLC**  $R_f$  = 0.16 (10 % EtOAc/P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / 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 OCH<sub>3</sub>), 6.85 (dd,  $J$  = 8.1, 1.5 Hz, 1 H, *ortho* to OCH<sub>3</sub>), 3.85 (s, 3 H, CH<sub>3</sub>), 3.50 (t,  $J$  = 6.4 Hz, 2 H, CH<sub>2</sub>Br), 2.56 (t,  $J$  = 7.1 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.25 (quin,  $J$  = 6.7 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 169.4 (C(=O)), 147.6 (*ipso* to OCH<sub>3</sub>), 127.2 (*ipso* to NH), 123.5 (*para* to NH), 120.7 (*para* to OCH<sub>3</sub>), 119.6 (*ortho* to NH and *meta* to OCH<sub>3</sub>), 109.8 (*ortho* to OCH<sub>3</sub> and *meta* to NH), 55.5 (CH<sub>3</sub>), 35.4 (C(=O)CH<sub>2</sub>), 33.1 (CH<sub>2</sub>Br), 27.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 272.0287, [M+H]<sup>+</sup> found, [C<sub>11</sub>H<sub>15</sub>BrNO<sub>2</sub>]<sup>+</sup> requires 272.0286

The compound has not been reported previously.

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

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Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ? (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(2-methoxyphenyl)butanamide ? (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 SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 4 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a bright pink amorphous solid (79.7 mg, 0.149 mmol, 10.2 %).

**TLC**  $R_f$  = 0.40 (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2947.1 (C-H), 2833.7 (C-H), 1718.9 (ester C=O), 1685.3 (amide C=O), 1617.3 (quinolone C=O)

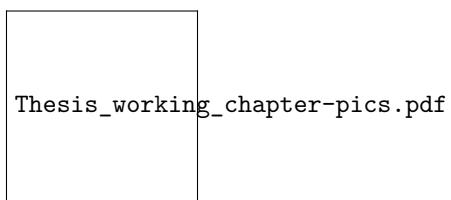
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.48 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 8.36 (d,  $J$  = 7.9 Hz, 1 H, *ortho* to NH), 7.87 - 7.99 (m, 2 H, *ortho* to F and NH), 7.19 (d,  $J$  = 6.5 Hz, 1 H, *meta* to F), 7.01 (t,  $J$  = 7.5 Hz, 1 H, *para* to NH), 6.93 (t,  $J$  = 7.7 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.85 (d,  $J$  = 7.9 Hz, 1 H, *ortho* to OCH<sub>3</sub>), 3.88 (s, 3 H, C(=O)OCH<sub>3</sub>), 3.85 (s, 3 H, aromatic OCH<sub>3</sub>), 3.41 (tt,  $J$  = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.25 (br t,  $J$  = 5.0, 5.0 Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.67 (br t,  $J$  = 5.0 Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.53 (t,  $J$  = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.47 (t,  $J$  = 7.1 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.97 (quin,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.25 - 1.33 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.07 - 1.14 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 172.9 (C(=O)CC(=O)OCH<sub>3</sub>), 170.8 (NHC(=O)), 166.2 (C(=O)OCH<sub>3</sub>), 153.3 (d,  $J$  = 248.0 Hz, *ipso* to F), 148.2 (C=CC(=O)OCH<sub>3</sub>), 147.6 (*ipso* to OCH<sub>3</sub>), 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 OCH<sub>3</sub>), 119.7 (*ortho* to NH and *meta* to OCH<sub>3</sub>), 113.0 (d,  $J$  = 22.5 Hz, *ortho* to C=O and *ortho* to F), 109.8 (*ortho* to OCH<sub>3</sub> and *meta* to NH, and CC(=O)OCH<sub>3</sub>), 104.7 (*meta* to C=O and *meta* to F), 57.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 55.6 (aromatic OCH<sub>3</sub>), 52.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.9 (C(=O)OCH<sub>3</sub>), 49.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 35.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 34.5 (NCH(CH<sub>2</sub>)<sub>2</sub>), 22.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 8.0 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 537.2523, [M+H]<sup>+</sup> found, [C<sub>29</sub>H<sub>34</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 537.2513

The compound has not been reported previously.

#### 4.42 4-Azido-*N*-(2-methoxyphenyl)butanamide ?



4-Bromo-*N*-(2-methoxyphenyl)butanamide ? (2.05 g, 7.51 mmol, 1 eq.) and NaN<sub>3</sub> (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 SiO<sub>2</sub> and purified by column chromatography using a Combiflash (SiO<sub>2</sub>, 8-14 % then held at 14 % EtOAc/P.E.). ? 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, 26.7 %).

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

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3419.7$  (N-H), 3329.6 (N-H), 2094.8 (azide), 1672.3 (amide C=O)

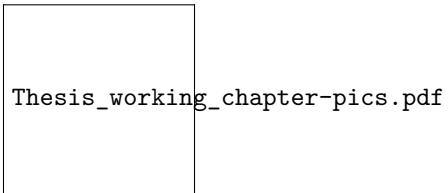
**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$  d<sub>1</sub>)  $\delta / \text{ppm} = 8.32$  (dd,  $J = 7.9, 1.0$  Hz, 1 H, *ortho* to NH), 7.86 (br s, 1 H, NH), 7.00 (td,  $J = 7.5, 1.5$  Hz, 1 H, *para* to NH), 6.90 (td,  $J = 7.7, 1.1$  Hz, 1 H, *para* to OCH<sub>3</sub>), 6.83 (dd,  $J = 8.1, 1.4$  Hz, 1 H, *ortho* to OCH<sub>3</sub>), 3.81 (s, 3 H, CH<sub>3</sub>), 3.33 (t,  $J = 6.7$  Hz, 2 H, CH<sub>2</sub>Br), 2.42 (t,  $J = 7.2$  Hz, 2 H, C(=O)CH<sub>2</sub>), 1.94 (quin,  $J = 6.9$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$  d<sub>1</sub>)  $\delta / \text{ppm} = 169.5$  (C(=O)), 147.6 (*ipso* to OCH<sub>3</sub>), 127.1 (*ipso* to NH), 123.4 (*para* to NH), 120.5 (*para* to OCH<sub>3</sub>), 119.5 (*ortho* to NH and *meta* to OCH<sub>3</sub>), 109.6 (*ortho* to OCH<sub>3</sub> and *meta* to NH), 55.2 (CH<sub>3</sub>), 50.3 (CH<sub>2</sub>N<sub>3</sub>), 33.9 (C(=O)CH<sub>2</sub>), 24.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z / \text{Da} = 257.1010$ , [M+H]<sup>+</sup> found, [C<sub>11</sub>H<sub>14</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 257.1014

The data are consistent with the literature.<sup>7</sup>

#### 4.43 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 ?



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (24.1 mg, 58.6  $\mu\text{mol}$ , 1 eq.) and 4-azido-*N*-(2-methoxyphenyl)butanamide ? (13.7 mg, 58.5  $\mu\text{mol}$ , 1 eq.) were dissolved in water (3 ml), *t*-BuOH (9 ml) and CH<sub>2</sub>Cl<sub>2</sub> (9 ml), and the mixture was degassed by bubbling through N<sub>2</sub>. A solution of CuSO<sub>4</sub> and THPTA (117  $\mu\text{l}$ , 5.85  $\mu\text{mol}$ , 0.1 eq., 50 mM, aq.) was added, followed by a solution of sodium ascorbate (234  $\mu\text{l}$ , 11.7  $\mu\text{mol}$ , 0.2 eq., 50 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (25 ml), CH<sub>2</sub>Cl<sub>2</sub> (25 ml) and MeOH (5 ml) were added and the organic layer was separated off, dry-loaded onto SiO<sub>2</sub> and purified by column chromatography using a CombiFlash (SiO<sub>2</sub>, 3-23 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a clear amorphous solid (14.7 mg, 22.8  $\mu\text{mol}$ , 39.0 %).

**TLC**  $R_f = 0.28$  (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 2926.5$  (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)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta / \text{ppm} = 15.05$  (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=CCH<sub>2</sub>), 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 OCH<sub>3</sub>), 6.88 (dd,  $J = 8.1, 1.4$  Hz, 1 H, *ortho* to OCH<sub>3</sub>), 4.47 (t,  $J = 6.7$  Hz, 2

H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.88 (s, 3 H, CH<sub>3</sub>), 3.54 (tt, *J* = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.35 (br t, *J* = 4.7 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>), 2.76 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.66 (t, *J* = 4.7 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.47 (t, *J* = 7.3 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.44 (t, *J* = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 2.32 (quin, *J* = 6.7 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 1.75 (quin, *J* = 7.6 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.61 (quin, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.35 - 1.42 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.17 - 1.22 (m, 2 H, NCH(CHH)<sub>2</sub>)

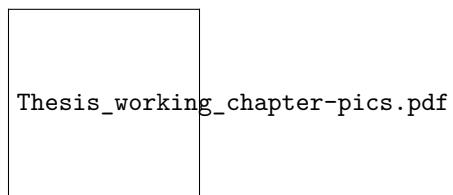
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ / ppm = 177.1 (C(=O)CC(=O)OH), 169.5 (NH<sub>2</sub>C(=O)), 167.0 (C(=O)OH), 153.7 (d, *J* = 251.4 Hz, *ipso* to F), 148.1 (CH=CCH<sub>2</sub>), 147.8 (*ipso* to OCH<sub>3</sub>), 147.3 (C=CC(=O)OH), 145.9 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.1 (*para* to F), 127.3 (*ipso* to NH), 123.9 (*para* to NH), 121.0 (*para* to OCH<sub>3</sub>), 120.9 (CH=CCH<sub>2</sub>), 119.7 (*para* to piperazine, and *ortho* to NH and *meta* to OCH<sub>3</sub>), 112.4 (d, *J* = 23.4 Hz, *ortho* to C=O and *ortho* to F), 109.9 (*ortho* to OCH<sub>3</sub> and *meta* to NH), 108.1 (CC(=O)OH), 104.7 (*meta* to C=O and *meta* to F), 58.1 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 55.6 (CH<sub>3</sub>), 52.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 35.2 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 27.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 8.2 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, CDCl<sub>3</sub>) δ / ppm = -120.7 (s, ciprofloxacin F)

**HRMS (ESI<sup>+</sup>)** *m/z* / Da = 646.3132, [M+H]<sup>+</sup> found, [C<sub>34</sub>H<sub>41</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 646.3153

The compound has not been reported previously.

#### 4.44 4-Bromo-*N*-(3-methoxyphenyl)butanamide ?



3-Methoxyaniline ? (3.04 ml, 3.33 g, 27.1 mmol, 1 eq.) and NaHCO<sub>3</sub> (2.73 g, 32.5 mmol, 1.2 eq.) were dissolved in water (30 ml) and CH<sub>2</sub>Cl<sub>2</sub> (30 ml). The mixture was cooled to 0 °C and 4-bromobutyryl chloride ? (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<sub>2</sub> and purified by column chromatography using a CombiFlash (SiO<sub>2</sub>, 0-100 % EtOAc/P.E.). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a pale pink amorphous solid (3.66 g, 13.5 mmol, 49.6 %).

**TLC** *R<sub>f</sub>* = 0.18 (25 % EtOAc/P.E.)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 1670.9 (amide C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>) δ / ppm = 8.45 (s, 1 H, NH), 7.27 (t, *J* = 2.2 Hz, 1 H, *ortho* to OCH<sub>3</sub> and *ortho* to NH), 7.14 (t, *J* = 8.1 Hz, 1 H, *meta* to OCH<sub>3</sub> and *meta* to NH), 7.02 (d, *J* = 8.3 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.62 (dd, *J* = 8.2, 2.1 Hz, 1 H, *para* to NH), 3.71 (s, 3 H, CH<sub>3</sub>), 3.42 (t, *J* = 6.5 Hz, 2 H, CH<sub>2</sub>Br), 2.51

(t,  $J = 6.9$  Hz, 2 H, C(=O)CH<sub>2</sub>), 2.19 (quin,  $J = 6.8$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 170.3 (C(=O)), 159.9 (*ipso* to OCH<sub>3</sub>), 139.0 (*ipso* to NH), 129.5 (*meta* to OCH<sub>3</sub> and *meta* to NH), 112.1 (*para* to OCH<sub>3</sub>), 109.9 (*para* to NH), 105.7 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 55.2 (CH<sub>3</sub>), 35.3 (C(=O)CH<sub>2</sub>), 33.2 (CH<sub>2</sub>Br), 28.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>) The compound does not ionise.

The compound has not been reported previously.

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

Thesis\_working\_chapter-pics.pdf

Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ? (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(3-methoxyphenyl)butanamide ? (788 mg, 2.90 mmol, 2 eq.), DIPEA (1.28 ml, 950 mg, 7.35 mmol, 5 eq.), NaI (275 mg, 1.83 mmol, 1.3 eq.) and acetonitrile (10 ml) were stirred in a microwave reactor at 100 °C for 4 h. The mixture was evaporated under reduced pressure and partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and water (50 ml). The organic layer was separated off and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (50 ml). The combined organic layers were dried with MgSO<sub>4</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-4 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as an off-white amorphous solid (81.7 mg, 0.152 mmol, 10.5 %).

**TLC**  $R_f = 0.38$  (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 8.56 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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 OCH<sub>3</sub> 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 OCH<sub>3</sub> and *meta* to NH), 6.98 (dd,  $J = 7.8, 1.7$  Hz, 1 H, *para* to OCH<sub>3</sub>), 6.65 (dd,  $J = 8.2, 2.1$  Hz, 1 H, *para* to NH), 3.93 (s, 3 H, C(=O)OCH<sub>3</sub>), 3.80 (s, 3 H, aromatic OCH<sub>3</sub>), 3.42 (tt,  $J = 6.8, 3.7$  Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.31 (br t,  $J = 4.3$  Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.73 (br t,  $J = 4.5$  Hz, 4 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)), 2.58 (t,  $J = 6.5$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.48 (t,  $J = 6.8$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.00 (quin,  $J = 6.8$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.29 - 1.36 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.11 - 1.17 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.1 (C(=O)CC(=O)OCH<sub>3</sub>), 170.9 (NHC(=O)), 166.3 (C(=O)OCH<sub>3</sub>), 160.1 (*ipso* to OCH<sub>3</sub>), 153.3 (d,  $J = 250.1$  Hz, *ipso* to F), 148.4 (C=CC(=O)OCH<sub>3</sub>), 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 OCH<sub>3</sub>), 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 OCH<sub>3</sub>),

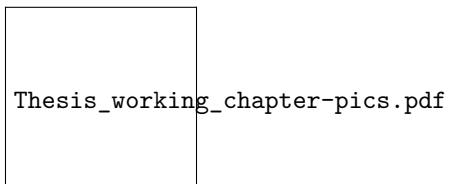
110.0 ( $\underline{\text{C}}\text{C}(=\text{O})\text{OCH}_3$ ), 109.8 (*para* to NH), 105.5 (*ortho* to  $\text{OCH}_3$  and *ortho* to NH), 105.0 (*meta* to  $\text{C}=\text{O}$  and *meta* to F), 57.0 ( $\text{CH}_2\text{CH}_2\underline{\text{CH}_2}\text{N}$ ), 55.3 (aromatic  $\underline{\text{OCH}_3}$ ), 52.6 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\underline{\text{CH}_2}$ ), 52.1 ( $\text{C}(=\text{O})\underline{\text{OCH}_3}$ ), 49.2 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\underline{\text{CH}_2})\text{CH}_2\underline{\text{CH}_2}$ ), 35.2 ( $\underline{\text{CH}_2}\text{CH}_2\text{CH}_2\text{N}$ ), 34.6 ( $\text{N}\underline{\text{CH}}(\text{CH}_2)_2$ ), 21.7 ( $\text{CH}_2\underline{\text{CH}_2}\text{CH}_2\text{N}$ ), 8.2 ( $\text{NCH}(\underline{\text{CH}_2})_2$ )

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

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

The compound has not been reported previously.

#### 4.46 4-Azido-*N*-(3-methoxyphenyl)butanamide ?



4-Bromo-*N*-(3-methoxyphenyl)butanamide ? (2.05 g, 7.51 mmol, 1 eq.) and  $\text{NaN}_3$  (1.17 g, 18.0 mmol, 2.4 eq.) were refluxed in acetonitrile (100 ml) for 7 h. The mixture was cooled and filtered, and the filtrate was dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography using a Combiflash ( $\text{SiO}_2$ , 0-100 % EtOAc/P.E.). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a straw-coloured liquid (0.294 g, 1.25 mmol, 16.7 %).

**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  $\text{C}=\text{O}$ )

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

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

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

The compound has not been reported previously.

**4.47 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 ?**

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1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (24.1 mg, 58.6  $\mu$ mol, 1 eq.) and 4-azido-*N*-(3-methoxyphenyl)butanamide ? (13.7 mg, 58.5  $\mu$ mol, 1 eq.) were dissolved in water (1 ml), *t*-BuOH (9 ml) and  $\text{CH}_2\text{Cl}_2$  (10 ml), and the mixture was degassed by bubbling through  $\text{N}_2$ . A solution of  $\text{CuSO}_4$  and THPTA (58.5  $\mu$ l, 5.85  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (117  $\mu$ l, 11.7  $\mu$ 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  $\text{CH}_2\text{Cl}_2$  (15 ml), and the aqueous layer was extracted a further four times with  $\text{CH}_2\text{Cl}_2$  (4  $\times$  15 ml). The combined organic layers were dried with  $\text{MgSO}_4$ , dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography ( $\text{SiO}_2$ , 0-10 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a clear amorphous solid (1.9 mg, 2.9  $\mu$ mol, 5.0 %).

**TLC**  $R_f$  = 0.22 (10 % 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 d<sub>6</sub>)  $\delta$  / ppm = 15.23 (br s, 1 H, C(=O)OH), 9.89 (s, 1 H, NH), 8.66 (s, 1 H, *ortho* to C(=O)OH), 7.90 (d,  $J$  = 13.4 Hz, 1 H, *ortho* to F), 7.88 (s, 1 H, CH=CCH<sub>2</sub>), 7.55 (d,  $J$  = 7.6 Hz, 1 H, *meta* to F), 7.27 (t,  $J$  = 2.1 Hz, 1 H, *ortho* to C=O and *ortho* to F), 7.16 (t,  $J$  = 8.1 Hz, 1 H, *meta* to OCH<sub>3</sub> and *meta* to NH), 7.08 (d,  $J$  = 7.8 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.59 (ddd,  $J$  = 8.1, 2.4, 0.7 Hz, 1 H, *para* to NH), 4.36 (t,  $J$  = 6.9 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.81 (tt,  $J$  = 6.7, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.70 (s, 3 H, CH<sub>3</sub>), 3.28 - 3.32 (m, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (m,  $J$  = 4.2, 4.2 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.38 (t,  $J$  = 7.3 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.30 (t,  $J$  = 7.4 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.10 (quin,  $J$  = 7.1 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.64 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.51 (quin,  $J$  = 7.2 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.27 - 1.33 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.15 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

**$^{13}\text{C NMR}$**  (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.3 (C(=O)CC(=O)OH), 170.1 (NHC(=O)), 165.9 (C(=O)OH), 159.4 (*ipso* to OCH<sub>3</sub>), 153.0 (d,  $J$  = 248.6 Hz, *ipso* to F), 148.0 (CH=CCH<sub>2</sub>), 146.9 (C=CC(=O)OH), 145.2 (d,  $J$  = 10.7 Hz, *ipso* to piperazine), 140.3 (*para* to F), 139.2 (*ipso* to NH), 129.4 (*meta* to OCH<sub>3</sub> and *meta* to NH), 121.7 (CH=CCH<sub>2</sub>), 118.5 (d,  $J$  = 7.5 Hz, *para* to piperazine), 111.3 (*para* to OCH<sub>3</sub>), 110.9 (d,  $J$  = 22.4 Hz, *ortho* to C=O and *ortho* to F), 108.4 (*para* to NH), 106.7 (CC(=O)OH), 106.3 (*meta* to C=O and *meta* to F), 104.8 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 57.3 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 54.9 (CH<sub>3</sub>), 52.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 35.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>N), 26.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>N), 25.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N)

N), 24.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.5 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 646.3159, [M+H]<sup>+</sup> found, [C<sub>34</sub>H<sub>41</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 646.3153

The compound has not been reported previously.

**4.48 (1*S,2S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol ? and (1*R,2R*)-2-(((*S*)-1-phenylethyl)amino)cyclopentan-1-ol ?**

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(*S*)-1-Phenylethan-1-amine ? (7.85 ml, 7.38 g, 60.9 mmol, 1 eq.) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and stirred rapidly at 0 °C. A solution of AlMe<sub>3</sub> (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 ? (5.71 ml, 5.50 g, 65.4 mmol, 1.1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (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 CH<sub>2</sub>Cl<sub>2</sub> (50 ml). The suspension was allowed to warm to r.t. and stirred for 1 h, then filtered through Celite and washed with CH<sub>2</sub>Cl<sub>2</sub> (500 ml). The filtrate was dried with K<sub>2</sub>CO<sub>3</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, 20:5:1 hexane:EtOAc:TEA). ? was obtained as a pale yellow oil (4.08 g, 19.9 mmol, 32.6 %). ? was obtained as pale yellow crystals (4.48 g, 21.8 mmol, 35.8 %).

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

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

**mp**  $T$  / °C = 66-71.5 (hexane, EtOAc, TEA)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3150.0 (br, O-H), 2950.9 (C-H), 2868.2 (C-H)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 7.28 - 7.34 (m, 4 H, *ortho* and *meta* to CHCH<sub>3</sub>), 7.20 - 7.26 (m, 1 H, *para* to CHCH<sub>3</sub>), 3.86 (q,  $J$  = 6.6 Hz, 1 H, CHCH<sub>3</sub>), 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, CHCH<sub>2</sub>CHOH), 1.77 (dtd,  $J$  = 12.9, 7.9, 4.9 Hz, 1 H, CHCH<sub>2</sub>NH), 1.55 - 1.68 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.47 - 1.55 (m, 1 H, CHCH<sub>2</sub>CHOH), 1.36 (d,  $J$  = 6.6 Hz, 3 H, CH<sub>3</sub>), 1.12 (dq,  $J$  = 12.7, 8.1 Hz, 1 H, CHCH<sub>2</sub>NH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 145.61 (*ipso* to CHCH<sub>3</sub>), 128.08 (*meta* to CHCH<sub>3</sub>), 126.61 (*para* to CHCH<sub>3</sub>), 126.33 (*ortho* to CHCH<sub>3</sub>), 77.43 (CHOH), 64.45 (CHNH), 56.62 (CHCH<sub>3</sub>), 32.01 (CH<sub>2</sub>CHOH), 30.56 (CH<sub>2</sub>CH<sub>2</sub>NH), 23.30 (CH<sub>3</sub>), 20.06 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 206.1553, [M+H]<sup>+</sup> found, [C<sub>13</sub>H<sub>20</sub>NO]<sup>+</sup> requires 206.1545

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

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

**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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta / \text{ppm} = 7.28 - 7.38$  (m, 4 H, *ortho* and *meta* to CHCH<sub>3</sub>), 7.21 - 7.28 (m, 1 H, *para* to CHCH<sub>3</sub>), 3.83 (q,  $J = 6.6$  Hz, 1 H, CHCH<sub>3</sub>), 3.78 (q,  $J = 7.0$  Hz, 1 H, CHO), 2.62 (dt,  $J = 8.2, 7.2$  Hz, 1 H, CHNH), 1.97 (quin,  $J = 6.7$  Hz, 1 H, CH<sub>2</sub>CHNH), 1.90 (quin,  $J = 6.9$  Hz, 1 H, CH<sub>2</sub>CHO), 1.56 - 1.68 (m, CH<sub>2</sub>CH<sub>2</sub>CHO), 1.43 (dq,  $J = 12.5, 8.0$  Hz, 1 H, CH<sub>2</sub>CHO), 1.37 (d,  $J = 6.6$  Hz, 3 H, CH<sub>3</sub>), 1.25 - 1.36 (m, 1 H, CH<sub>2</sub>CHNH)

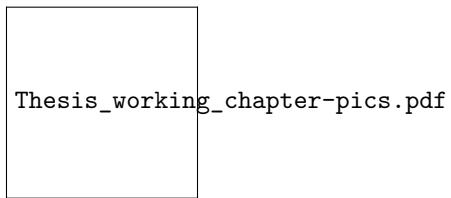
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta / \text{ppm} = 144.75$  (*ipso* to CHCH<sub>3</sub>), 128.26 (*meta* to CHCH<sub>3</sub>), 126.72 (*para* to CHCH<sub>3</sub>), 126.30 (*ortho* to CHCH<sub>3</sub>), 77.65 (CHO), 63.38 (CHNH), 56.20 (CHCH<sub>3</sub>), 31.74 (CH<sub>2</sub>CHO), 29.22 (CH<sub>2</sub>CHNH), 24.58 (CH<sub>3</sub>), 19.57 (CH<sub>2</sub>CH<sub>2</sub>CHO)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 206.1554, [M+H]<sup>+</sup> found, [C<sub>13</sub>H<sub>20</sub>NO]<sup>+</sup> requires 206.1545

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

The compounds have been synthesised previously,<sup>?,?</sup> but NMR data were not published. The enantiomers of both compounds have also been synthesised previously, and the <sup>1</sup>H NMR data for these are consistent with the the above data.<sup>?</sup>

**4.49 (1*S*,2*S*)-2-Aminocyclopentan-1-ol ?**



(1*S*,2*S*)-2-((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol ? (3.00 g, 14.6 mmol, 1 eq.), Pd(OH)<sub>2</sub> (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. ? was obtained as a yellow oil (1.48 g, 14.6 mmol, 100 %).

**TLC**  $R_f = 0.10$  (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3300.0$  (O-H), 2969.2 (C-H), 2872.7 (C-H)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta / \text{ppm} = 3.77$  (ddd,  $J = 6.6, 6.2, 5.6$ , 1 H, CHO), 3.00 (td,  $J = 7.4, 5.6$

Hz, 1 H, CHNH<sub>2</sub>), 2.00 (dtd, *J* = 13.0, 7.7, 5.6 Hz, 1 H, CHHCHNH<sub>2</sub>), 1.97 (ddt, *J* = 13.0, 8.7, 6.4 Hz, 1 H, CHHCHOH), 1.64 - 1.77 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 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, CHHCHNH<sub>2</sub>)

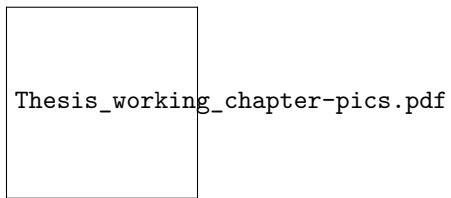
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 80.6 (CHOH), 60.7 (CHNH<sub>2</sub>), 33.2 (CH<sub>2</sub>CHOH), 32.2 (CH<sub>2</sub>CHNH<sub>2</sub>), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 102.0915, [M+H]<sup>+</sup> found, [C<sub>5</sub>H<sub>12</sub>NO]<sup>+</sup> requires 102.0913

$[\alpha]_D^{20} / {}^{\circ}10^{-1}\text{cm}^2\text{g}^{-1} = 33.4$ , lit. = 29.7 (*c* / g(100 ml)<sup>-1</sup> = 0.5, EtOH)

The data are consistent with the literature.<sup>?,?</sup>

#### 4.50 (1*R*,2*R*)-2-Aminocyclopentan-1-ol ?



(1*R*,2*R*)-2-((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol ? (3.90 g, 19.0 mmol, 1 eq.), Pd(OH)<sub>2</sub> (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. ? was obtained as a yellow oil (1.92 g, 19.0 mmol, 100 %).

**TLC**  $R_f$  = 0.10 (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 3300.0 (br, O-H), 2958.3 (C-H), 2871.5 (C-H)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.77 (ddd, *J* = 6.6, 6.2, 5.6, 1 H, CHOH), 3.00 (td, *J* = 7.3, 5.6 Hz, 1 H, CHNH<sub>2</sub>), 2.00 (dtd, *J* = 13.0, 7.7, 5.6 Hz, 1 H, CHHCHNH<sub>2</sub>), 1.97 (ddt, *J* = 13.0, 8.7, 6.6 Hz, 1 H, CHHCHOH), 1.63 - 1.77 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.53 (ddt, *J* = 13.0, 9.5, 6.2 Hz, 1 H, CHHCHOH), 1.37 (ddt, *J* = 13.0, 8.3, 7.8 Hz, 1 H, CHHCHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 80.7 (CHOH), 60.8 (CHNH<sub>2</sub>), 33.2 (CH<sub>2</sub>CHOH), 32.1 (CH<sub>2</sub>CHNH<sub>2</sub>), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 102.0917, [M+H]<sup>+</sup> found, [C<sub>5</sub>H<sub>12</sub>NO]<sup>+</sup> requires 102.0913

$[\alpha]_D^{20} / {}^{\circ}10^{-1}\text{cm}^2\text{g}^{-1} = -30.9$ , lit. = -32.9 (*c* / g(100 ml)<sup>-1</sup> = 1.5, EtOH)

The data are consistent with the literature.<sup>?,?</sup>

#### 4.51 4-Azido-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide ?

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4-Chloro-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide ? (35.0 mg, 0.170 mmol, 1 eq.) and NaN<sub>3</sub> (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/CHCl<sub>3</sub> (5 ml). The aqueous layer was extracted again with 10 % *i*-PrOH/CHCl<sub>3</sub> (2×5 ml) and the combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as white needles (16.2 mg, 0.0764 mmol, 45.0 %).

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

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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, CH<sub>2</sub>N<sub>3</sub>), 2.31 (t,  $J$  = 7.2 Hz, 2 H, CH<sub>2</sub>C=O), 2.09 - 2.19 (m, 1 H, CHHCHNH), 1.99 - 2.06 (m, 1 H, CHHCHOH), 1.90 - 1.97 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.60 - 1.85 (m, 3 H, CH<sub>2</sub>CHHCHOH), 1.42 (dq,  $J$  = 12.8, 8.3 Hz, 1 H, CHHCHNH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 79.7 (CHOH), 61.0 (CHNH), 50.7 (CH<sub>2</sub>N<sub>3</sub>), 32.8 (CH<sub>2</sub>C=O), 32.6 (CH<sub>2</sub>CHOH), 30.5 (CH<sub>2</sub>CHNH), 24.7 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 21.3 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 235.1178, [M+Na]<sup>+</sup> found, [C<sub>9</sub>H<sub>16</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 235.1171

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 10.0 ( $c$  / g(100 ml)<sup>-1</sup> = 0.01, MeOH)

The compound has not been reported previously.

#### 4.52 4-Azido-*N*-(*(1R,2R)*-2-hydroxycyclopentyl)butanamide ?

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4-Chloro-*N*-(*(1R,2R)*-2-hydroxycyclopentyl)butanamide ? (200 mg, 0.972 mmol, 1 eq.) and NaN<sub>3</sub> (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/CHCl<sub>3</sub> (20 ml). The aqueous layer was extracted again with 10 % *i*-PrOH/CHCl<sub>3</sub> (3×20 ml) and the combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as white needles (181 mg, 0.852 mmol,

87.6 %).

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

**mp**  $T / ^\circ\text{C} = 56.0\text{--}59.5$  (*i*-PrOH, CHCl<sub>3</sub>)

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3279.9$  (N-H and O-H), 2965.6 (C-H), 2875.4 (C-H), 2094.6 (azide), 1636.8 (amide C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta / \text{ppm} = 6.72$  (d,  $J = 4.4$  Hz, 1 H, NH), 4.82 (br. s., 1 H, OH), 3.88 (q,  $J = 6.6$  Hz, 1 H, CHOH), 3.75 (tdd,  $J = 8.4, 6.6, 4.4$  Hz, 1 H, CHNH), 3.28 (t,  $J = 6.6$  Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 2.23 (t,  $J = 7.3$  Hz, 2 H, CH<sub>2</sub>C=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, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.59 - 1.77 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.54 (ddt,  $J = 12.7, 9.0, 6.7$  Hz, 1 H, CHHCHOH), 1.39 (dq,  $J = 12.9, 8.4$  Hz, 1 H, CHHCHNH)

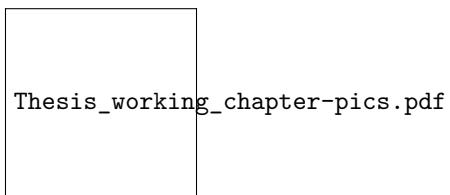
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta / \text{ppm} = 173.8$  (C=O), 78.8 (CHOH), 59.9 (CHNH), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 32.5 (CH<sub>2</sub>C=O), 32.0 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 24.6 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.7 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z / \text{Da} = 235.1174$ , [M+Na]<sup>+</sup> found, [C<sub>9</sub>H<sub>16</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 235.1171

$[\alpha]_D^{20} / ^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = -10.2$  ( $c / \text{g(100 ml)}^{-1} = 0.5$ , MeOH)

The compound has not been reported previously.

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



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate ? (52.1 mg, 95.5  $\mu\text{mol}$ , 1 eq.), (1*S*,2*S*)-2-aminocyclopentan-1-ol ? (19.5 mg, 193  $\mu\text{mol}$ , 2 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (29.7 mg, 155  $\mu\text{mol}$ , 1.6 eq.), 1-hydroxybenzotriazole (25.8 mg, 191  $\mu\text{mol}$ , 2 eq.) and DIPEA (33.3  $\mu\text{l}$ , 24.7 mg, 191  $\mu\text{mol}$ , 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<sub>2</sub> 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<sub>3</sub> (aq., sat., 5 ml) and CH<sub>2</sub>Cl<sub>2</sub> (5 ml). The organic layer was removed and the aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  5 ml). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (26.9 mg, 52.3  $\mu\text{mol}$ , 54.7 %).

**TLC**  $R_f = 0.38$  (30 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2937.7 (C-H), 1721.4 (ester C=O), 1620.5 (amide C=O and quinolone C=O)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.44 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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<sub>H</sub>), 3.77 - 3.81 (m, 1 H, CHO<sub>H</sub>), 3.74 - 3.77 (m, 1 H, CHNH), 3.73 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 6.9, 4.0 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.24 (br. t,  $J$  = 4.2 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.55 (br t,  $J$  = 5.0 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.32 (t,  $J$  = 7.2 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.10 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 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, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.53 - 1.64 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 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)<sub>2</sub>), 1.06 - 1.12 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 171.9 (NHC(=O)CH<sub>2</sub>), 171.5 (C(=O)CC(=O)OCH<sub>3</sub>), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 247.4 Hz, *ipso* to F), 148.2 (C=CC(=O)OCH<sub>3</sub>), 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)OCH<sub>3</sub>), 106.2 (*meta* to C=O and *meta* to F), 76.2 (CHOH), 57.6 (CHNH), 57.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 34.7 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.2 (C(=O)CH<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CH NH), 22.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 20.6 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.5 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -125.5

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 515.2667, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>36</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 515.2670

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = 8.0 (*c* / g(100 ml)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.

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

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4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate ? (200 mg, 0.367 mmol, 1 eq.), (1*R*,2*R*)-2-aminocyclopentan-1-ol ? (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  $\mu$ 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 N<sub>2</sub> 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 NaHCO<sub>3</sub> (aq., sat., 10 ml) and CH<sub>2</sub>Cl<sub>2</sub> (10 ml). The organic layer was removed and the aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub> (2×10 ml). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white

amorphous solid (73.0 mg, 0.142 mmol, 38.7 %).

**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)

**$^1\text{H}$  NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.44 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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<sub>H</sub>), 3.78 - 3.82 (m, 1 H, CH<sub>2</sub>OH), 3.74 - 3.78 (m, 1 H, CHNH), 3.74 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 7.2, 3.9 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.25 (t,  $J$  = 4.8 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.57 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.34 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.11 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 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, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.54 - 1.65 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 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)<sub>2</sub>), 1.07 - 1.13 (m, 2 H, NCH(CHH)<sub>2</sub>)

**$^{13}\text{C}$  NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 171.9 (CH<sub>2</sub>C(=O)NH), 171.6 (C(=O)CC(=O)OCH<sub>3</sub>), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 246.5 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH<sub>3</sub>), 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 (CC(=O)OCH<sub>3</sub>), 106.2 (*meta* to C=O and *meta* to F), 76.3 (CHOH), 57.6 (CHNH), 57.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 34.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.3 (C(=O)CH<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 22.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 20.6 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

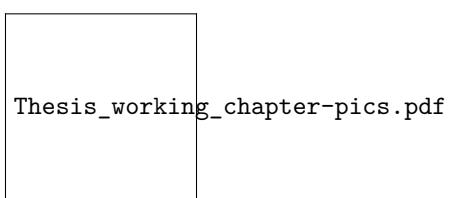
**$^{19}\text{F}$  NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -124.3 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 515.2661, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>36</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 515.2670

$[\alpha]_D^{20}$  /  $^{\circ}10^{-1}\text{cm}^2\text{g}^{-1}$  = -6.0 ( $c$  / g(100 ml)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.

#### 4.55 Methyl (*S*)-1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclopentyl)amin o)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ?



Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ? (20.0 mg, 38.9  $\mu\text{mol}$ , 1 eq.) and Dess-Martin periodinane (32.8 mg, 77.4  $\mu\text{mol}$ , 2 eq.) were stirred in CH<sub>2</sub>Cl<sub>2</sub> (3 ml) for 6 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-50 % acetonitrile/water over 10 min). The combined pure fractions

were evaporated under reduced pressure, then  $\text{NaHCO}_3$  (aq., sat., 30 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (30 ml) were added. The organic layer was removed and dried with MgSO<sub>4</sub>, then evaporated under reduced pressure. ? was obtained as a white amorphous solid (11.3 mg, 22.0  $\mu\text{mol}$ , 56.7 %).

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**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.46 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 7.78 (d,  $J$  = 13.5 Hz, 1 H, *ortho* to F), 7.45 (d,  $J$  = 7.4 Hz, 1 H, *meta* to F), 4.02 (dt,  $J$  = 11.1, 8.2 Hz, 1 H, CHNH), 3.73 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 6.9, 3.9 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.40 (s, 10 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.05 - 2.29 (m, 5 H, NHC(=O)CH<sub>2</sub>, CH<sub>2</sub>C(=O)CHNH and CHHCHNH), 1.89 - 1.96 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.69 - 1.80 (m, 3 H, CHHCH<sub>2</sub>CHNH, CHHCHNH and NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.24 - 1.29 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.07 - 1.12 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 215.2 (C(=O)CHNH), 171.7 (NHC(=O)CH<sub>2</sub>), 171.7 (C(=O)CC(=O)OCH<sub>3</sub>), 165.1 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 246.6 Hz, *ipso* to F), 148.4 (C=CC(=O)OCH<sub>3</sub>), 138.1 (*para* to F), 109.1 (CC(=O)OCH<sub>3</sub>), 56.3 (CHNH), 51.4 (CH<sub>3</sub>), 35.6 (CH<sub>2</sub>C(=O)CHNH), 34.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 28.8 (CH<sub>2</sub>CHNH), 18.1 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.3

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 513.2495, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>34</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 513.2513

$[\alpha]_D^{20} / {}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1} = 6.7$  (*c* / g(100 ml)<sup>-1</sup> = 0.075, MeOH)

The compound has not been reported previously.

#### 4.56 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 ?

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1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (82.0 mg, 199  $\mu\text{mol}$ , 4 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide ? (11.0 mg, 51.8  $\mu\text{mol}$ , 1 eq.) were dissolved in 10 % water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (156  $\mu\text{l}$ , 15.6  $\mu\text{mol}$ , 0.3 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (312  $\mu\text{l}$ , 31.2  $\mu\text{mol}$ , 0.6 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 3 d. Water (10 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (10 ml) were added, then the organic layer was separated and dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 10 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (10 ml). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (7.2 mg, 11.5  $\mu\text{mol}$ , 22.2 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

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

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (NHC(=O)CH<sub>2</sub>), 166.0 (C(=O)OH), 153.0 (d,  $J$  = 249.6 Hz, *ipso* to F), 148.1 (C=CC(=O)OH), 146.7 (CH=CCH<sub>2</sub>), 145.2 (d,  $J$  = 8.3 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 (NCH=CCH<sub>2</sub>), 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=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.3 (br s, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)), 49.3 (br s, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>), 48.8 (CH<sub>2</sub>NCH=CCH<sub>2</sub>), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 32.0 (C(=O)CH<sub>2</sub>), 29.4 (CH<sub>2</sub>CHNH), 26.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 20.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

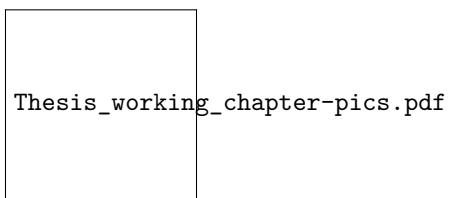
**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -121.5

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 624.3298, [M+H]<sup>+</sup> found, [C<sub>32</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 624.3310

$[\alpha]_D^{20}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -25.0 (c / g(100 ml)<sup>-1</sup> = 0.08, MeOH)

The compound has not been reported previously.

**4.57 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 ?**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (42.9 mg, 104  $\mu$ mol, 1 eq.) and 4-azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide ? (22.0 mg, 104  $\mu$ mol, 1 eq.) were dissolved in 10 % water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (104  $\mu$ l, 10.4  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208  $\mu$ l, 20.8  $\mu$ mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (30 ml) and CH<sub>2</sub>Cl<sub>2</sub> (30 ml) were added, the organic layer was separated and the aqueous

layer was extracted again with  $\text{CH}_2\text{Cl}_2$  ( $4 \times 30$  ml). The combined organic layers were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-95 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between  $\text{NaHCO}_3$  (aq., sat., 10 ml) and 10 % *i*-PrOH/ $\text{CHCl}_3$  (10 ml). The organic layer was dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a white amorphous solid (17.6 mg, 28.2  $\mu\text{mol}$ , 27.1 %).

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

**$^1\text{H NMR}$**  (700 MHz, DMSO  $d_6$ )  $\delta$  / 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,  $\text{CH}=\text{CCH}_2$ ), 7.75 (d,  $J$  = 7.1 Hz, 1 H,  $\text{CHNH}$ ), 7.54 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.73 (d,  $J$  = 3.8 Hz, 1 H,  $\text{CHOH}$ ), 4.29 (t,  $J$  = 6.9 Hz, 2 H,  $\text{CH}_2\text{NCH}=\text{C}$ ), 3.78 - 3.83 (m, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.75 - 3.78 (m, 1 H,  $\text{CHOH}$ ), 3.71 - 3.75 (m, 1 H,  $\text{CHNH}$ ), 3.31 (br t,  $J$  = 4.3 Hz, 4 H,  $\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 2.63 (t,  $J$  = 7.5 Hz, 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.56 (br t,  $J$  = 4.2 Hz, 4 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.37 (t,  $J$  = 7.3 Hz, 2 H,  $\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.03 - 2.06 (m, 2 H, C(=O) $\text{CH}_2$ ), 1.97 - 2.02 (m, 2 H, C(=O) $\text{CH}_2\text{CH}_2$ ), 1.89 (dddd,  $J$  = 13.1, 8.9, 7.4, 5.7 Hz, 1 H,  $\text{CHHCHNH}$ ), 1.75 (ddt,  $J$  = 13.0, 8.9, 6.4, 6.4 Hz, 1 H,  $\text{CHHCHOH}$ ), 1.61 - 1.66 (m, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 1.57 - 1.61 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.54 - 1.57 (m, 1 H,  $\text{CHHCH}_2\text{CHOH}$ ), 1.49 - 1.53 (m, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 1.40 (ddt,  $J$  = 13.0, 8.4, 5.3, 5.3 Hz, 1 H,  $\text{CHHCHOH}$ ), 1.29 - 1.32 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.25 - 1.29 (m, 1 H,  $\text{CHHCHNH}$ ), 1.13 - 1.20 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

**$^{13}\text{C NMR}$**  (175 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 176.3 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$ ), 170.9 ( $\text{NHC}(=\text{O})\text{CH}_2$ ), 166.1 ( $\text{C}(=\text{O})\text{OH}$ ), 153.0 (d,  $J$  = 251.4 Hz, *ipso* to F), 147.9 ( $\text{C}=\text{CC}(=\text{O})\text{OH}$ ), 146.9 ( $\text{CH}=\text{CCH}_2$ ), 145.2 (d,  $J$  = 8.7 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 ( $\text{NCH}=\text{CCH}_2$ ), 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  $\text{CC}(=\text{O})\text{OH}$ ), 76.2 ( $\text{CHOH}$ ), 57.6 ( $\text{CHNH}$ ), 57.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 52.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 49.5 (d,  $J$  = 4.4 Hz,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2$ ), 48.8 ( $\text{CH}_2\text{NCH}=\text{CCH}_2$ ), 35.8 ( $\text{NCH}(\text{CH}_2)_2$ ), 32.2 ( $\text{CH}_2\text{CHOH}$ ), 32.0 (C(=O) $\text{CH}_2$ ), 29.5 ( $\text{CH}_2\text{CHNH}$ ), 26.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 26.0 (C(=O) $\text{CH}_2\text{CH}_2$ ), 25.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 25.0 ( $\text{CH}=\text{CCH}_2$ ), 20.5 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 7.6 ( $\text{NCH}(\text{CH}_2)_2$ )

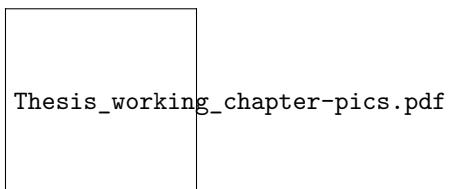
**$^{19}\text{F NMR}$**  (376.45 MHz, MeOD)  $\delta$  / ppm = -122.1 (s, ciprofloxacin F)

**HRMS** (ESI $^+$ )  $m/z$  / Da = 624.3314, [M+H] $^+$  found,  $[\text{C}_{32}\text{H}_{43}\text{FN}_7\text{O}_5]^+$  requires 624.3310

$[\alpha]_D^{20}$  /  ${}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -3.6 ( $c$  / g(100 ml) $^{-1}$  = 0.0833, MeOH)

The compound has not been reported previously.

#### 4.58 (1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine ?



(1*S,2S*)-2-Aminocyclopentan-1-ol ? (0.480 g, 4.75 mmol) was stirred in dry  $\text{CH}_2\text{Cl}_2$  (20 ml) under  $\text{N}_2$  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  $\text{Na}_2\text{SO}_4$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ , 4 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). ? was obtained as a yellow oil (1.00 g, 4.64 mmol, 97.7 %).

**TLC**  $R_f$  = 0.23 (10 % MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 2953.6 (C-H), 2931.1 (C-H), 2888.4 (C-H), 2858.8 (C-H), 1625.2 (N-H bend)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 4.13 (q,  $J$  = 5.8 Hz, 1 H,  $\text{CHOSi}$ ), 3.31 (td,  $J$  = 7.1, 5.2 Hz, 1 H,  $\text{CHNH}_2$ ), 2.09 - 2.19 (m, 1 H,  $\text{CHHCHNH}_2$ ), 1.97 (ddq,  $J$  = 8.8, 7.0, 6.0 Hz, 1 H,  $\text{CHHCHOSi}$ ), 1.74 - 1.86 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CHOSi}$ ), 1.64 - 1.74 (m, 1 H,  $\text{CHHCHOSi}$ ), 1.58 (ddt,  $J$  = 13.2, 9.1, 6.0 Hz, 1 H,  $\text{CHHCHNH}_2$ ), 0.88 (s, 9 H,  $\text{C}(\text{CH}_3)_3$ ), 0.09 (s, 3 H,  $\text{SiCH}_3$ ), 0.07 (s, 3 H,  $\text{SiCH}_3$ )

**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 76.3 ( $\text{CHOSi}$ ), 59.7 ( $\text{CHNH}$ ), 32.2 ( $\text{CH}_2\text{CHOSi}$ ), 26.8 ( $\text{CH}_2\text{CHNH}_2$ ), 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 ( $\text{SiCH}_3$ ), -5.2 ( $\text{SiCH}_3$ )

**HRMS** (ESI $^+$ )  $m/z$  / Da = 216.1785, [M+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 ( $c$  / g(100 ml) $^{-1}$  = 0.05, MeOH) The compound has not been reported previously.

#### 4.59 4-Azido-*N*-((1*S,2S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide ?

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(1*S,2S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine ? (50 mg, 0.232 mmol, 1 eq.) and  $\text{NaHCO}_3$  (22.0 mg, 0.262 mmol, 1.1 eq.) were added to  $\text{CH}_2\text{Cl}_2$  (3 ml) and water (3 ml) at 0 °C, and 4-bromobutyryl chloride (25.3 ml, 40.5 mg, 0.219 mmol, 0.95 eq.) was added dropwise. The mixture was stirred for 3 h at 0 °C. The aqueous layer was removed and  $\text{NaN}_3$  (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  $\text{N}_2$  stream and the residue was purified by column chromatography ( $\text{SiO}_2$ , 0.5 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a clear liquid (71 mg, 0.217 mmol, 99.2 %).

**TLC**  $R_f$  = 0.84 (1 % MeOH/ $\text{CH}_2\text{Cl}_2$ )

**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)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 5.35 (d,  $J$  = 5.1 Hz, 1 H,  $\text{NH}$ ), 3.97 - 4.01 (m, 1 H,  $\text{CHOSi}$ ), 3.93 - 3.98 (m, 1 H,  $\text{CHNH}$ ), 3.35 (t,  $J$  = 6.6 Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 2.24 (t,  $J$  = 7.0 Hz, 2 H,  $\text{CH}_2\text{C=O}$ ), 2.09 - 2.19

(m, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH), 1.89 - 1.97 (quin, *J* = 6.8 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.74 - 1.84 (m, 2 H, CH<sub>2</sub>CHOSi and CH<sub>2</sub>CH<sub>2</sub>CHOSi), 1.60 - 1.70 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHOSi), 1.51 - 1.61 (m, 1 H, CH<sub>2</sub>CHOSi), 1.31 - 1.39 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH), 0.87 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 3 H, SiCH<sub>3</sub>), 0.06 (s, 3 H, SiCH<sub>3</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 171.17 (C=O), 77.80 (CHOSi), 58.36 (CHNH), 50.77 (CH<sub>2</sub>N<sub>3</sub>), 33.29 (CH<sub>2</sub>C=O), 32.57 (CH<sub>2</sub>CHOSi), 29.36 (CH<sub>2</sub>CHNH), 25.72 (C(CH<sub>3</sub>)<sub>3</sub>), 24.77 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.40 (CH<sub>2</sub>CH<sub>2</sub>CHO Si), 17.95 (C(CH<sub>3</sub>)<sub>3</sub>), -4.75 (SiCH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 327.2221, [M+H]<sup>+</sup> found, [C<sub>15</sub>H<sub>31</sub>N<sub>4</sub>O<sub>2</sub>Si]<sup>+</sup> requires 327.2216

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

The compound has not been reported previously.

**4.60 7-(4-(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 ?**

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1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (42.9 mg, 104  $\mu$ mol, 1 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide ? (33.9 mg, 104  $\mu$ mol, 1 eq.) were dissolved in 10 % water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling N<sub>2</sub> through it. A solution of CuSO<sub>4</sub> and THPTA (104  $\mu$ l, 10.4  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (208  $\mu$ l, 20.8  $\mu$ 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 CH<sub>2</sub>Cl<sub>2</sub> (10 ml), the organic layer was separated and the aqueous layer was extracted again with CH<sub>2</sub>Cl<sub>2</sub> (10 ml). The combined organic layers were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a clear amorphous solid (67.1 mg, 90.9  $\mu$ mol, 87.4 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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=CCH<sub>2</sub>), 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, CH<sub>2</sub>NCH=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(CH<sub>2</sub>)<sub>2</sub>), 3.34 (br t, *J* = 5.0 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.71 (t, *J* = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.66 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.46 (t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.03 - 2.22 (m, 5 H, CH<sub>2</sub>CH<sub>2</sub>NH, C(=O)CH<sub>2</sub> and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.65 - 1.83 (m, 4 H, CH<sub>2</sub>CHOSi, CH<sub>2</sub>CH<sub>2</sub>CHOSi and NCH=CCH<sub>2</sub>CH<sub>2</sub>), 1.47 - 1.65 (m, 4 H, CH<sub>2</sub>CHOSi, CH<sub>2</sub>CH<sub>2</sub>CHOSi and NCH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.33 - 1.41 (m, 3 H, CH<sub>2</sub>CH<sub>2</sub>NH and NCH(CH<sub>2</sub>)<sub>2</sub>), 1.14 - 1.20 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 0.82 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 0.03 (s, 3 H, SiCH<sub>3</sub>), 0.01 (s, 3 H, SiCH<sub>3</sub>)

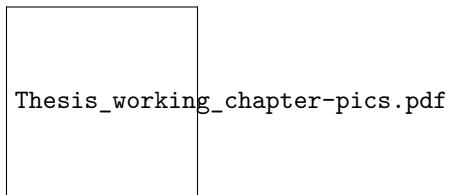
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 176.9 (C(=O)CC(=O)OH), 170.9 (CH<sub>2</sub>C(=O)NH), 166.9 (C(=O)OH), 153.5 (d,  $J$  = 251.4 Hz, *ipso* to F), 147.9 (CH=CCH<sub>2</sub>), 147.2 (C=CC(=O)OH), 145.8 (d,  $J$  = 10.4 Hz, *ipso* to piperazine), 139.0 (*para* to F), 120.9 (NCH=CCH<sub>2</sub>), 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=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.6 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (d,  $J$  = 6.1 Hz, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.9 (d,  $J$  = 3.5 Hz, CH<sub>2</sub>NCH=CCH<sub>2</sub>), 35.3 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.6 (C(=O)CH<sub>2</sub>), 32.6 (CH<sub>2</sub>CHOSi), 29.3 (CH<sub>2</sub>CHNH), 27.2 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.0 - 26.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub> and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 25.6 (C(CH<sub>3</sub>)<sub>3</sub>), 25.4 (CH=CCH<sub>2</sub>), 20.4 (CH<sub>2</sub>CH<sub>2</sub>CHOSi), 17.8 (C(CH<sub>3</sub>)<sub>3</sub>), 8.1 (NCH(CH<sub>2</sub>)<sub>2</sub>), -4.8 (SiCH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 738.4164, [M+H]<sup>+</sup> found, [C<sub>38</sub>H<sub>57</sub>FN<sub>7</sub>O<sub>5</sub>Si]<sup>+</sup> requires 738.4169

$[\alpha]_D^{20} / {}^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = 4.5$  ( $c$  / g(100 ml)<sup>-1</sup> = 0.2, MeOH)

The compound has not been reported previously.

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



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ? (200 mg, 0.579 mmol, 1 eq.), *tert*-butyl 4-bromobutanoate ? (103  $\mu$ l, 130 mg, 0.581 mmol, 1 eq.), NaI (86.9 mg, 0.580 mmol, 1 eq.), TEA (316  $\mu$ l, 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 ? (103  $\mu$ l, 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 SiO<sub>2</sub> and purified by column chromatography (SiO<sub>2</sub>, 0-4 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). ? was obtained as a white amorphous solid (141 mg, 0.289 mmol, 49.9 %).

**TLC**  $R_f$  = 0.12 (4 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 8.39 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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, CH<sub>3</sub>), 3.40 (tt,  $J$  = 7.2, 3.6 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.22 (t,  $J$  = 4.3 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.63 (t,  $J$  = 4.4 Hz, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.41 (t,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.25 (t,  $J$  = 7.4 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.78 (quin,  $J$  = 7.3 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.41 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.24 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.09 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 172.7 (C(=O)CC(=O)OCH<sub>3</sub>), 172.6 (C(=O)OC(CH<sub>3</sub>)<sub>3</sub>), 165.9 (C(=O)OCH<sub>3</sub>), 153.1 (d,  $J$  = 249.7 Hz, *ipso* to F), 148.1 (C=CC(=O)OCH<sub>3</sub>), 144.3 (d,  $J$  = 10.4 Hz, *ipso*

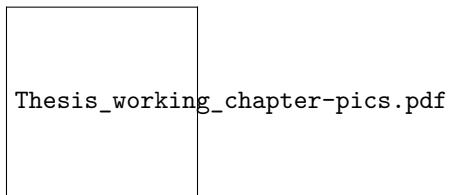
to piperazine), 137.7 (*para* to F), 122.5 (d,  $J = 6.9$  Hz, *para* to piperazine) 112.6 (d,  $J = 22.5$  Hz, *ortho* to C=O and *ortho* to F), 109.5 ( $\underline{C}C(=O)OCH_3$ ) 104.7 (*meta* to C=O and *meta* to F), 80.0 ( $\underline{C}(CH_3)_3$ ), 57.4 (C(=O)CH<sub>2</sub>CH<sub>2</sub> $\underline{C}H_2N$ ), 52.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N( $\underline{CH_2}$ ) $\underline{CH_2}$ ), 51.7 ( $\underline{CH_3}$ ), 49.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub> $\underline{CH_2}$ )CH<sub>2</sub>CH<sub>2</sub>), 49.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub> $\underline{CH_2}$ ), 34.4 (N $\underline{CH}(CH_2)_2$ ), 33.2 (C(=O) $\underline{CH_2}$ ), 28.0 (C( $\underline{CH_3}$ )<sub>3</sub>), 22.0 (C(=O)CH<sub>2</sub> $\underline{CH_2}$ ), 7.9 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = -123.5 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 488.2562, [M+H]<sup>+</sup> found, [C<sub>26</sub>H<sub>35</sub>FN<sub>3</sub>O<sub>5</sub>]<sup>+</sup> requires 488.2561

The compound has not been reported previously.

#### 4.62 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate ?



Methyl 7-(4-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ? (20 mg, 41.0  $\mu$ mol) and TFA (0.2 ml) were stirred in CH<sub>2</sub>Cl<sub>2</sub> (1.8 ml) at r.t. for 16 h then evaporated under reduced pressure. ? was obtained as a white solid (21.4 mg, 39.2  $\mu$ mol, 95.6 %).

**mp**  $T$  / °C = 225-231 (CH<sub>2</sub>Cl<sub>2</sub>, decomposes)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 1722.7 (ciprofloxacin ester C=O), 1699.0 (alkyl carboxylic acid C=O), 1673.3 (TFA C=O), 1614.6 (quinolone C=O)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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,  $\underline{CH_3}$ ), 3.66 (tt,  $J = 7.2, 3.7$  Hz, 1 H, N $\underline{CH}(CH_2)_2$ ), 3.30 - 3.54 (br s, 8 H, CH<sub>2</sub>N(CH<sub>2</sub>) $\underline{CH_2}$  and CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub> $\underline{CH_2}$ ) 3.13 - 3.22 (m, 2 H,  $\underline{CH_2}N(CH_2)CH_2$ ), 2.36 (t,  $J = 7.1$  Hz, 2 H,  $\underline{CH_2}CH_2CH_2N(CH_2)CH_2$ ), 1.87 - 1.98 (m, 2 H,  $\underline{CH_2}CH_2N(CH_2)CH_2$ ), 1.22 - 1.30 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.06 - 1.15 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 173.5 (CH<sub>2</sub> $\underline{C}(=O)OH$ ), 171.6 ( $\underline{C}(=O)CC(=O)OCH_3$ ), 164.9 ( $\underline{C}(=O)OCH_3$ ), 158.2 (q,  $J = 31.5$  Hz, CF<sub>3</sub> $\underline{C}(=O)OH$ ), 152.5 (d,  $J = 247.6$  Hz, *ipso* to F), 148.5 ( $\underline{C}=CC(=O)OH$ ), 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,  $\underline{CF_3}$ ), 111.9 (d,  $J = 22.4$  Hz, *ortho* to C=O and *ortho* to F), 109.1 ( $\underline{C}C(=O)OCH_3$ ), 106.9 (*meta* to C=O and *meta* to F), 55.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub> $\underline{CH_2}N$ ), 51.4 ( $\underline{CH_3}$ ), 50.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N( $\underline{CH_2}$ ) $\underline{CH_2}$ ), 46.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub> $\underline{CH_2}$ ), 46.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub> $\underline{CH_2}$ ), 34.9 (N $\underline{CH}(CH_2)_2$ ), 30.6 (C(=O) $\underline{CH_2}$ ), 19.1 (C(=O)CH<sub>2</sub> $\underline{CH_2}$ ), 7.6 (NCH( $\underline{CH_2}$ )<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -73.6 (s, CF<sub>3</sub>), -124.6 (s, ciprofloxacin F)

**HRMS (ESI<sup>+</sup>)**  $m/z$  / Da = 432.1921, [M+H]<sup>+</sup> found, [C<sub>22</sub>H<sub>27</sub>FN<sub>3</sub>O<sub>5</sub>]<sup>+</sup> requires 432.1935

The compound has not been reported previously.

#### 4.63 4-Chloro-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide ?

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(*1S,2S*)-2-Aminocyclopentan-1-ol ? (72.3 mg, 716  $\mu$ mol, 1 eq.), TEA (500  $\mu$ l, 363 mg, 3.58 mmol, 5 eq.) and CH<sub>2</sub>Cl<sub>2</sub> (5 ml) were stirred at 0 °C, and 4-chlorobutyryl chloride ? (179  $\mu$ l, 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/CHCl<sub>3</sub> (2×10 ml). The combined organic layers were dried with MgSO<sub>4</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, Et<sub>2</sub>O). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (35.6 mg, 173  $\mu$ mol, 24.2 %).

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

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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, CH<sub>2</sub>Cl), 2.38 (t,  $J$  = 7.0 Hz, 2 H, CH<sub>2</sub>C=O), 2.05 - 2.17 (m, 3 H, CHHCHNH and CH<sub>2</sub>CH<sub>2</sub>Cl), 1.94 - 2.05 (m, 1 H, CHHCHOH), 1.74 - 1.86 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.58 - 1.74 (m, 2 H, CHHCH<sub>2</sub>CHOH and CHHCHOH), 1.42 (dq,  $J$  = 12.5, 8.4 Hz, 1 H, CHHCHNH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH<sub>2</sub>Cl), 32.8 (CH<sub>2</sub>C=O), 32.4 (CH<sub>2</sub>CHOH), 30.2 (CH<sub>2</sub>CHNH), 28.0 (CH<sub>2</sub>CH<sub>2</sub>Cl), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS (ESI<sup>+</sup>)**  $m/z$  / Da = 206.0939, [M+H]<sup>+</sup> found, [C<sub>9</sub>H<sub>17</sub>ClNO<sub>2</sub>]<sup>+</sup> requires 206.0948

$[\alpha]_D^{20} / {}^\circ 10^{-1} \text{cm}^2 \text{g}^{-1} = 10.0$  ( $c$  / g(100 ml)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.

#### 4.64 4-Chloro-*N*-(*(1R,2R)*-2-hydroxycyclopentyl)butanamide ?

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(1*R*,2*R*)-2-Aminocyclopentan-1-ol ? (500 mg, 4.94 mmol, 1 eq.), TEA (827  $\mu$ l, 600 mg, 5.93 mmol, 1.2 eq.) and  $\text{CH}_2\text{Cl}_2$  (20 ml) were stirred at 0 °C and 4-chlorobutyryl chloride ? (608  $\mu$ l, 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  $\text{CH}_2\text{Cl}_2$  (7×50 ml). The combined organic layers were dried with  $\text{MgSO}_4$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ ,  $\text{Et}_2\text{O}$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. ? was obtained as a white amorphous solid (651 mg, 3.16 mmol, 64.1 %).

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

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3277.6 (N-H and O-H), 2962.2 (C-H), 2876.0 (C-H), 1636.3 (amide C=O)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 6.12 (br s, 1 H, NH), 4.42 (br s, 1 H, OH), 3.94 (q,  $J$  = 6.6 Hz, 1 H, CHOH), 3.82 (tt,  $J$  = 8.4, 5.3 Hz, 1 H, CHNH), 3.60 (t,  $J$  = 6.2 Hz, 2 H, CH<sub>2</sub>Cl), 2.38 (t,  $J$  = 7.2 Hz, 2 H, CH<sub>2</sub>C=O), 2.05 - 2.16 (m, 3 H, CHHCHNH and CH<sub>2</sub>CH<sub>2</sub>Cl), 1.96 - 2.04 (m, 1 H, CHHCHOH), 1.74 - 1.85 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.58 - 1.73 (m, 2 H, CHHCH<sub>2</sub>CHOH and CHHCHOH), 1.43 (dq,  $J$  = 12.7, 8.3 Hz, 1 H, CHHCHNH)

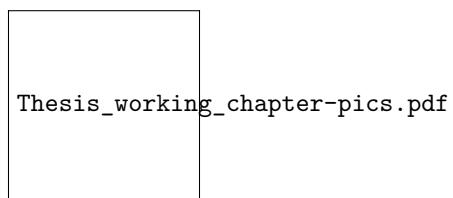
**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH<sub>2</sub>Cl), 32.8 (CH<sub>2</sub>C=O), 32.4 (CH<sub>2</sub>CHOH), 30.1 (CH<sub>2</sub>CHNH), 28.0 (CH<sub>2</sub>CH<sub>2</sub>Cl), 21.1 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

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

$[\alpha]_D^{20}$  /  ${}^\circ\text{10}^{-1}\text{cm}^2\text{g}^{-1}$  = -13.0 ( $c$  / g(100 ml) $^{-1}$  = 0.5, MeOH)

The compound has not been reported previously.

#### 4.65 (*trans*)-2-Aminocyclohexan-1-ol ?



Cyclohexene oxide ? (10 ml, 9.70 g, 98.8 mmol, 1 eq.),  $\text{NH}_3$  (90 ml, 35 % w/w aq., 27.7 g, 791 mmol, 8 eq.) and MeOH (100 ml) were stirred at r.t. for 72 h. The solvent was removed by blowing a stream of  $\text{N}_2$  over it, followed by evaporation under high vacuum. ? was obtained as a white amorphous solid (9.90 g, 85.2 mmol, 86.2 %)

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

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 3350.4 (N-H), 3306.2 (br, O-H), 2926.9 (C-H), 2852.6 (C-H)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 3.01 (td,  $J$  = 9.4, 4.8 Hz, 1 H, CHOH), 2.80 - 2.92 (m, 2 H, OH and NH<sub>2</sub>), 2.35 (ddd,  $J$  = 11.1, 9.1, 4.1 Hz, 1 H, CHNH<sub>2</sub>), 1.77 - 1.84 (m, 1 H, CHHCHOH), 1.69 - 1.76 (m, 1 H,

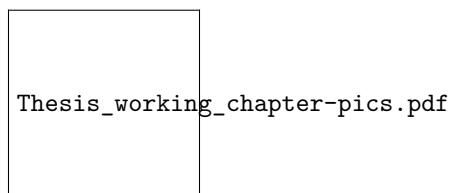
CHHCHNH<sub>2</sub>), 1.56 - 1.66 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.45 - 1.56 (m, 1 H, CHHCH<sub>2</sub>CHNH<sub>2</sub>), 1.07 - 1.19 (m, 3 H, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH<sub>2</sub> and CHHCHOH), 0.94 - 1.05 (m, 1 H, CHHCHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ / ppm = 75.4 (CHOH), 56.6 (CHN<sub>2</sub>), 33.8 (CH<sub>2</sub>CHOH and CH<sub>2</sub>CHN<sub>2</sub>), 24.7 (CH<sub>2</sub>CH<sub>2</sub>CHNH<sub>2</sub>), 24.6 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

HRMS (ESI<sup>+</sup>)  $m/z$  / Da = 116.1070, [M+H]<sup>+</sup> found, [C<sub>6</sub>H<sub>14</sub>NO]<sup>+</sup> requires 116.1070

The data are consistent with the literature.<sup>7</sup>

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



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate ? (200 mg, 0.367 mmol, 1 eq.), (*trans*)-2-aminocyclohexan-1-ol ? (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  $\mu$ 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  $N_2$  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  $NaHCO_3$  (aq., sat., 10 ml) and  $CH_2Cl_2$  (10 ml). The organic layer was dried with  $MgSO_4$  and evaporated under reduced pressure. ? was obtained as a white amorphous solid (61.2 mg, 0.116 mmol, 31.7 %).

**IR (neat)  $\nu_{max}$  / cm<sup>-1</sup>** = 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)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 8.60 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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, CH<sub>3</sub>), 3.62 - 3.68 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.58 (td,  $J$  = 10.3, 4.2 Hz, 1 H, CHNH), 3.38 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.32 - 3.36 (m, 1 H, CHOH), 2.83 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.60 (t,  $J$  = 7.3 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.32 (td,  $J$  = 7.1, 3.1 Hz, 2 H, C(=O)CH<sub>2</sub>), 1.96 - 2.04 (m, 1 H, CHHCHOH), 1.87 - 1.96 (m, 3 H, CHHCHNH and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.72 - 1.77 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.66 - 1.72 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.25 - 1.39 (m, 5 H, CHHCHOH, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH and NCH(CHH)<sub>2</sub>), 1.15 - 1.25 (m, 3 H, CHHCHOH and NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.8 ( $\text{CH}_2\text{C}(=\text{O})\text{NH}$ ), 175.3 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OCH}_3$ ), 166.8 ( $\text{C}(=\text{O})\text{OCH}_3$ ), 154.9 (d,  $J = 248.8$  Hz, *ipso* to F), 150.2 ( $\text{C}=\text{CC}(=\text{O})\text{OCH}_3$ ), 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 ( $\text{CC}(=\text{O})\text{OCH}_3$ ), 107.2 (*meta* to C=O and *meta* to F), 74.1 ( $\text{CHOH}$ ), 58.9 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 56.4 ( $\text{CHNH}$ ), 54.0 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 52.3 ( $\text{CH}_3$ ), 50.5 (d,  $J = 5.0$  Hz,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 36.4 ( $\text{NCH}(\text{CH}_2)_2$ ), 35.7 ( $\text{CH}_2\text{CHOH}$ ), 35.1 ( $\text{C}(=\text{O})\text{CH}_2$ ), 32.8

(CH<sub>2</sub>CHNH), 25.9 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 23.5 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 8.7 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -124.7 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 529.2827, [M+H]<sup>+</sup> found, [C<sub>28</sub>H<sub>38</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 529.2826

The compound has not been reported previously.

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

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Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ? (5.2 mg, 9.84  $\mu$ mol, 1 eq.) and Dess-Martin periodinane (16.4 mg, 38.7  $\mu$ mol, 4 eq.) were stirred in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub> (aq., sat., 30 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (30 ml) were added. The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (3.6 mg, 6.8  $\mu$ mol, 69.1 %).

**TLC**  $R_f$  = 0.74 (30 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.45 (s, 1 H, *ortho* to C(=O)OCH<sub>3</sub>), 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, CH<sub>3</sub>), 3.65 (tt,  $J$  = 7.1, 3.9 Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.25 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.58 (br s, 4 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.45 - 2.53 (m, 1 H, CHHC(=O)CHNH), 2.36 (br s, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 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)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 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, CHHCH<sub>2</sub>C(=O)), 1.65 - 1.83 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.41 - 1.56 (m, 2 H, CHHCHNH and CHHCH<sub>2</sub>C(=O)), 1.20 - 1.30 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.05 - 1.13 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 207.5 (C(=O)CHNH), 171.7 (C(=O)CC(=O)OCH<sub>3</sub>), 171.6 (CH<sub>2</sub>C(=O)NH), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 247.6 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH<sub>3</sub>), 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)OCH<sub>3</sub>), 106.3 (*meta* to C=O and *meta* to F), 57.0 (CHNH and C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.3 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.3 (CH<sub>3</sub>), 49.5 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>), 40.6 (CH<sub>2</sub>C(=O)CHNH), 34.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.9 (CH<sub>2</sub>CHNH), 32.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 27.2 (CH<sub>2</sub>CH<sub>2</sub>C(=O)CHNH), 23.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 22.4 (br s, C(=O)CH<sub>2</sub>CH<sub>2</sub>)

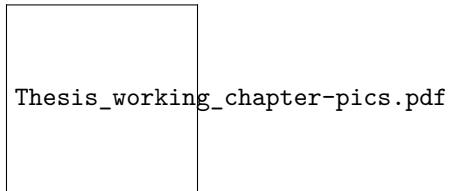
CH<sub>2</sub>N), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -124.3 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 527.2654, [M+H]<sup>+</sup> found, [C<sub>28</sub>H<sub>36</sub>FN<sub>4</sub>O<sub>5</sub>]<sup>+</sup> requires 527.2670

The compound has not been reported previously.

#### 4.68 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ?



(*trans*)-2-Aminocyclohexan-1-ol ? (1.04 g, 9.03 mmol, 1 eq.), TEA (1.65 ml, 1.20 g, 11.8 mmol, 1.3 eq.) and CH<sub>2</sub>Cl<sub>2</sub> (50 ml) were stirred at 0 °C. 4-Chlorobutyryl chloride ? (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/CHCl<sub>3</sub> (2×50 ml). The combined organic layers were dried with MgSO<sub>4</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, 0-100 % EtOAc/Et<sub>2</sub>O). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as white needles (1.51 g, 6.87 mmol, 76.1 %).

**TLC**  $R_f$  = 0.19 (Et<sub>2</sub>O)

**mp**  $T$  / °C = 72.5-75.7 (*i*-PrOH, CHCl<sub>3</sub>)

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

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.60 (t,  $J$  = 6.6 Hz, 2 H, CH<sub>2</sub>Cl), 3.51 - 3.60 (m, 1 H, CH<sub>2</sub>NH), 3.28 - 3.39 (m, 1 H, CH<sub>2</sub>OH), 2.37 (td,  $J$  = 7.4, 2.3 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.06 (quin,  $J$  = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.97 - 2.01 (m, 1 H, CH<sub>2</sub>CHOH), 1.85 - 1.93 (m, 1 H, CH<sub>2</sub>CHNH), 1.70 - 1.77 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.64 - 1.70 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.24 - 1.35 (m, 3 H, CH<sub>2</sub>CH<sub>2</sub>CHOH, CH<sub>2</sub>CH<sub>2</sub>CHNH and CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.13 - 1.25 (m, 1 H, CH<sub>2</sub>CHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.0 (C(=O)), 74.1 (CH<sub>2</sub>OH), 56.3 (CH<sub>2</sub>NH), 45.3 (CH<sub>2</sub>Cl), 35.6 (CH<sub>2</sub>CHOH), 34.5 (C(=O)CH<sub>2</sub>), 32.7 (CH<sub>2</sub>CHNH), 30.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 242.0925, [M+Na]<sup>+</sup> found, [C<sub>10</sub>H<sub>18</sub>ClNNaO<sub>2</sub>]<sup>+</sup> requires 242.0924

The compound has not been reported previously.

#### 4.69 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ?

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4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ? (345 mg, 1.57 mmol, 1 eq.) and NaN<sub>3</sub> (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/CHCl<sub>3</sub> (50 ml) were added, and the organic layer was removed. The aqueous layer was extracted again with 10 % *i*-PrOH/CHCl<sub>3</sub> (50 ml) and the combined organic fractions were dried with MgSO<sub>4</sub>. The solvent was evaporated under reduced pressure, and then by using a N<sub>2</sub> stream. ? was obtained as large white prisms (347 mg, 1.53 mmol, 97.5 %).

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

**mp**  $T$  / °C = 74.5-75.7 (*i*-PrOH, CHCl<sub>3</sub>)

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / 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 CH<sub>2</sub>N<sub>3</sub>), 2.30 (td,  $J$  = 7.4, 2.7 Hz, 2 H, C(=O)CH<sub>2</sub>), 1.95 - 2.03 (m, 1 H, CHHCHOH), 1.87 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub> and CHHCHNH), 1.70 - 1.76 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.63 - 1.70 (m, 1 H, CHHCH<sub>2</sub>CHNH), 1.25 - 1.38 (m, 3 H, CHHCH<sub>2</sub>CHOH, CHHCH<sub>2</sub>CHNH and CHHCHOH), 1.14 - 1.24 (m, 1 H, CHHCHNH<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.1 (C(=O)), 74.0 (CHOH), 56.3 (CHNH), 52.0 (CH<sub>2</sub>N<sub>3</sub>), 35.5 (CH<sub>2</sub>CHOH), 34.3 (C(=O)CH<sub>2</sub>), 32.7 (CH<sub>2</sub>CHNH), 26.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 25.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 249.1331, [M+Na]<sup>+</sup> found, [C<sub>10</sub>H<sub>18</sub>N<sub>4</sub>NaO<sub>2</sub>]<sup>+</sup> requires 249.1327

The compound has not been reported previously.

#### 4.70 1-Cyclopropyl-6-fluoro-7-(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-dihydroquino-line-3-carboxylic acid ?

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1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ? (40 mg, 97.2  $\mu$ mol, 1 eq.) and 4-azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ? (22.0 mg, 97.2  $\mu$ mol, 1 eq.) were dissolved in 10 % water/*t*-BuOH (3 ml), and the mixture was degassed by bubbling  $N_2$  through it. A solution of CuSO<sub>4</sub> and THPTA (97.2  $\mu$ l, 9.72  $\mu$ mol, 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (194  $\mu$ l, 19.4  $\mu$ mol, 0.2 eq., 100 mM, aq.). The mixture was stirred at r.t. under argon for 16 h. Water (50 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (50 ml) were added, then the organic layer was separated, dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by preparative HPLC (5-70 % acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between NaHCO<sub>3</sub> (aq., sat., 50 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (50 ml). The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a white amorphous solid (30.3 mg, 47.5  $\mu$ mol, 48.9 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 8.64 (s, 1 H, *ortho* to C(=O)OH), 7.86 (d,  $J$  = 13.9 Hz, 1 H, *ortho* to F), 7.84 (s, 1 H, CH=CCH<sub>2</sub>), 7.64 (d,  $J$  = 8.1 Hz, 1 H, NH), 7.54 (d,  $J$  = 7.5 Hz, 1 H, *meta* to F), 4.54 (d,  $J$  = 4.7 Hz, 1 H, OH), 4.30 (t,  $J$  = 6.8 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.77 - 3.86 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.33 - 3.40 (m, 1 H, CH<sub>2</sub>NH), 3.31 (br t,  $J$  = 4.8 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 3.14 - 3.24 (m, 1 H, CH<sub>2</sub>OH), 2.63 (t,  $J$  = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (br t,  $J$  = 4.6 Hz, 4 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.38 (t,  $J$  = 6.9 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.04 - 2.08 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.96 - 2.04 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.78 - 1.87 (m, 1 H, CH<sub>2</sub>CHOH), 1.69 - 1.78 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH), 1.63 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.54 - 1.60 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>OH), 1.51 (quin,  $J$  = 7.4 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.28 - 1.35 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.11 - 1.22 (m, 5 H, NCH(CH<sub>2</sub>)<sub>2</sub>, CH<sub>2</sub>CHOH, CH<sub>2</sub>CH<sub>2</sub>CHOH and CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.04 - 1.13 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (CH<sub>2</sub>C(=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=CCH<sub>2</sub>), 145.3 (d,  $J$  = 10.0 Hz, *ipso* to piperazine), 139.2 (para to F), 121.8 (NCH=CCH<sub>2</sub>), 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=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 54.2 (CH<sub>2</sub>NH), 52.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>NCH=C), 35.9 (NCH(CH<sub>2</sub>)<sub>2</sub>), 34.1 (CH<sub>2</sub>CHOH), 32.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 31.1 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 25.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>N), 25.0 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.2 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 23.8 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.4 (ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 638.3480, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>45</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 638.3466

The compound has not been reported previously.

4.71 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 ?

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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 ? (15.0 mg, 23.6 mmol, 1 eq.) and Dess-Martin periodinane (35.0 mg, 82.5 mmol, 3.5 eq.) were stirred in CH<sub>2</sub>Cl<sub>2</sub> (3 ml) at r.t. for 4 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-70 % acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure, then NaHCO<sub>3</sub> (aq., sat., 30 ml) and 10 % *i*-PrOH/CHCl<sub>3</sub> (30 ml) were added. The organic layer was dried with MgSO<sub>4</sub> and evaporated under reduced pressure. ? was obtained as a clear gum (11.7 mg, 18.4  $\mu$ mol, 78.0 %).

**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 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)

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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=CCH<sub>2</sub>), 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)CH<sub>2</sub>CH<sub>2</sub>CHHN), 4.31 (t,  $J$  = 6.9 Hz, 1 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.74 - 3.84 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.31 (br. s, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.64 (t,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>), 2.56 (br t,  $J$  = 5.0, 5.0 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.45 - 2.52 (m, 1 H, CHHC(=O)), 2.38 (t,  $J$  = 7.1 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.25 (dtt,  $J$  = 13.4, 2.6, 1.6 Hz, 1 H, CHHC(=O)), 2.07 - 2.17 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N and CHHCHNH), 1.96 - 2.05 (m, 3 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N and CHHCH<sub>2</sub>C(=O)), 1.68 - 1.81 (m, 2 H, CHHCH<sub>2</sub>CHNH), 1.64 (quin,  $J$  = 7.5 Hz, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.40 - 1.56 (m, 5 H, CHHCH<sub>2</sub>C(=O), CHHCHNH and CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.27 - 1.34 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.13 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 207.4 (C(=O)CHNH), 176.3 (C(=O)CC(=O)OH), 170.8 (CH<sub>2</sub>C(=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=CCH<sub>2</sub>), 145.1 (d,  $J$  = 10.1 Hz, *ipso* to piperazine), 139.1 (*para* to F), 121.7 (NCH=CCH<sub>2</sub>), 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=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 57.0 (CHNH), 52.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 49.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 48.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 40.5 (CH<sub>2</sub>C(=O)), 35.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.7 (CH<sub>2</sub>CHNH), 31.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 27.1 (CH<sub>2</sub>CH<sub>2</sub>C(=O)), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 26.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NCH=C), 25.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 23.8 (CH<sub>2</sub>CH<sub>2</sub>CHNH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

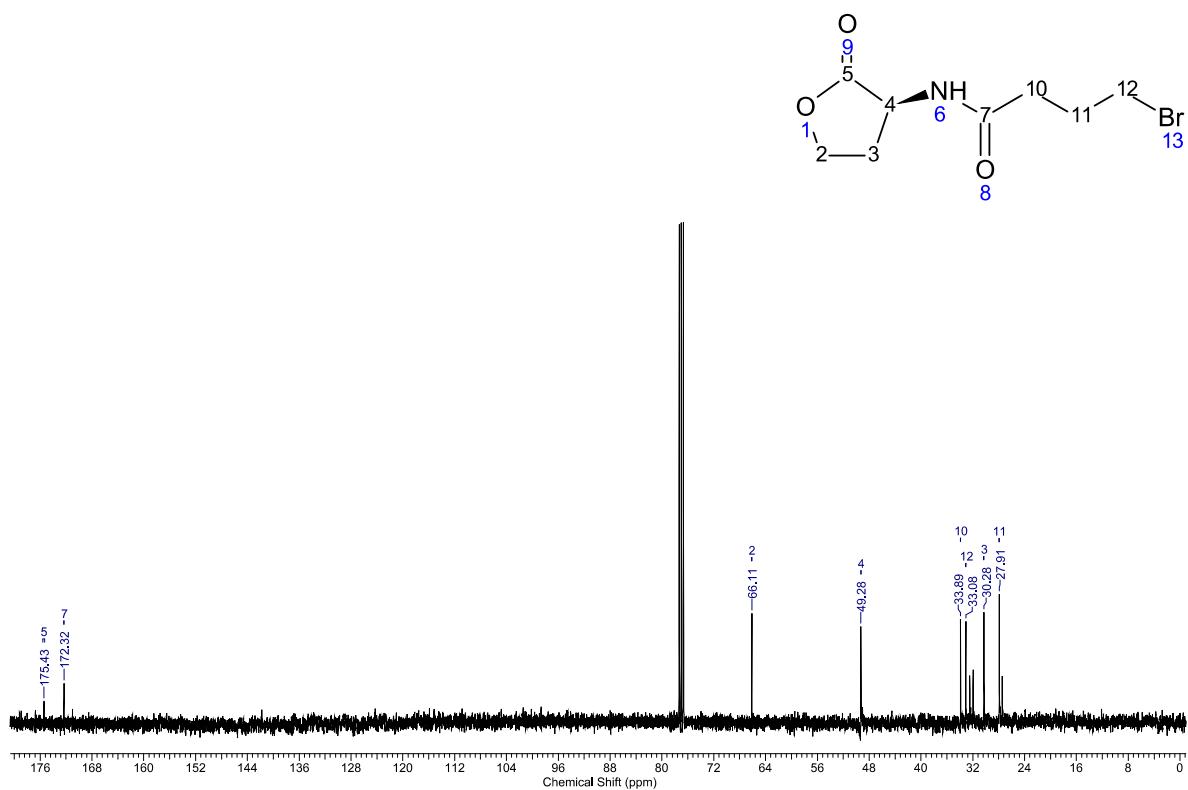
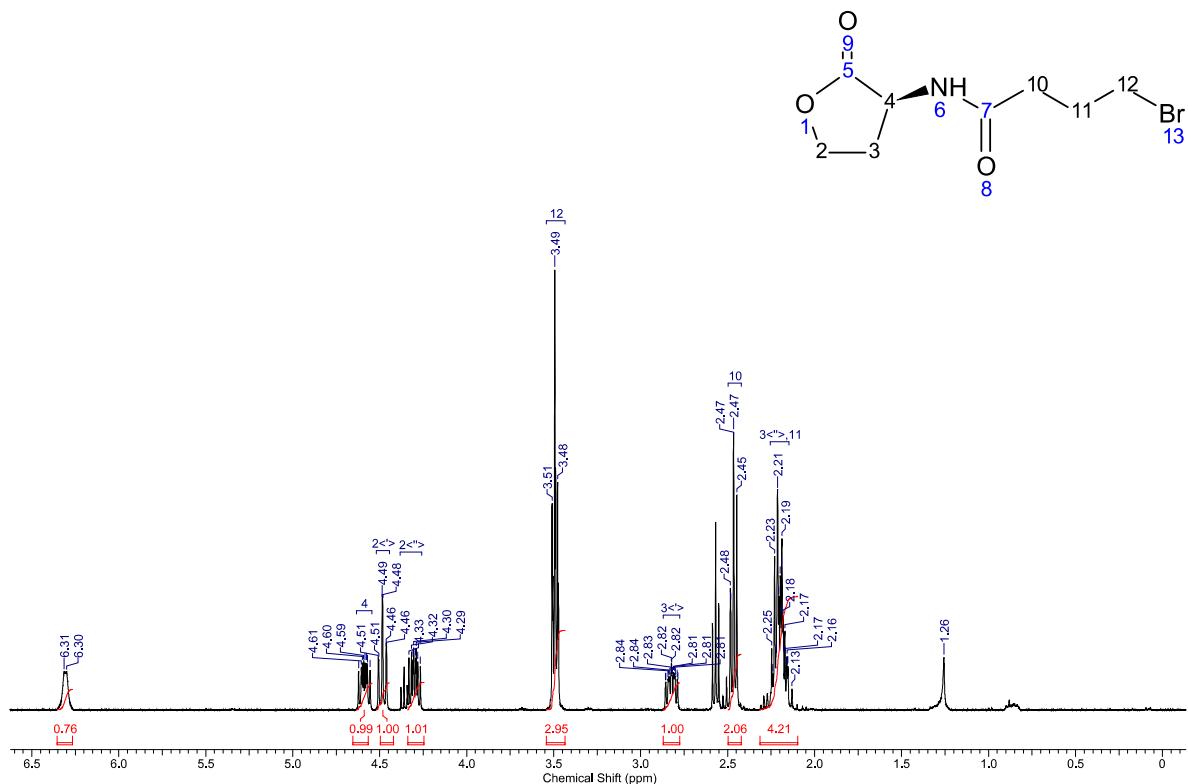
**<sup>19</sup>F NMR** (376 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.7 (s, ciprofloxacin F)

**HRMS** (ESI<sup>+</sup>) *m/z* / Da = 636.3303, [M+H]<sup>+</sup> found, [C<sub>33</sub>H<sub>43</sub>FN<sub>7</sub>O<sub>5</sub>]<sup>+</sup> requires 636.3310

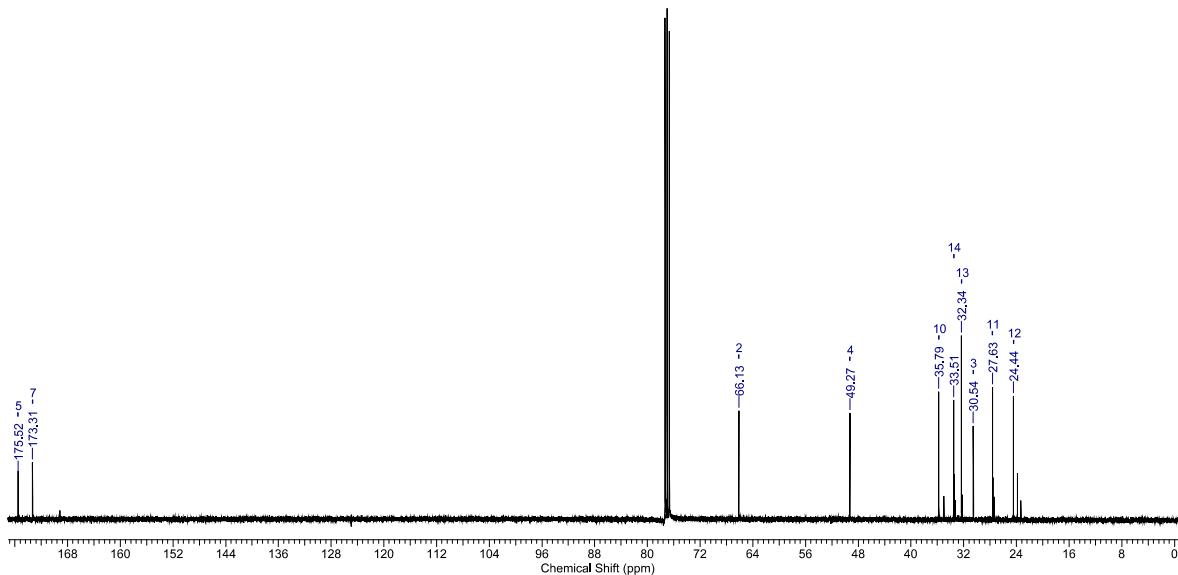
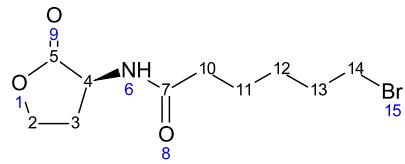
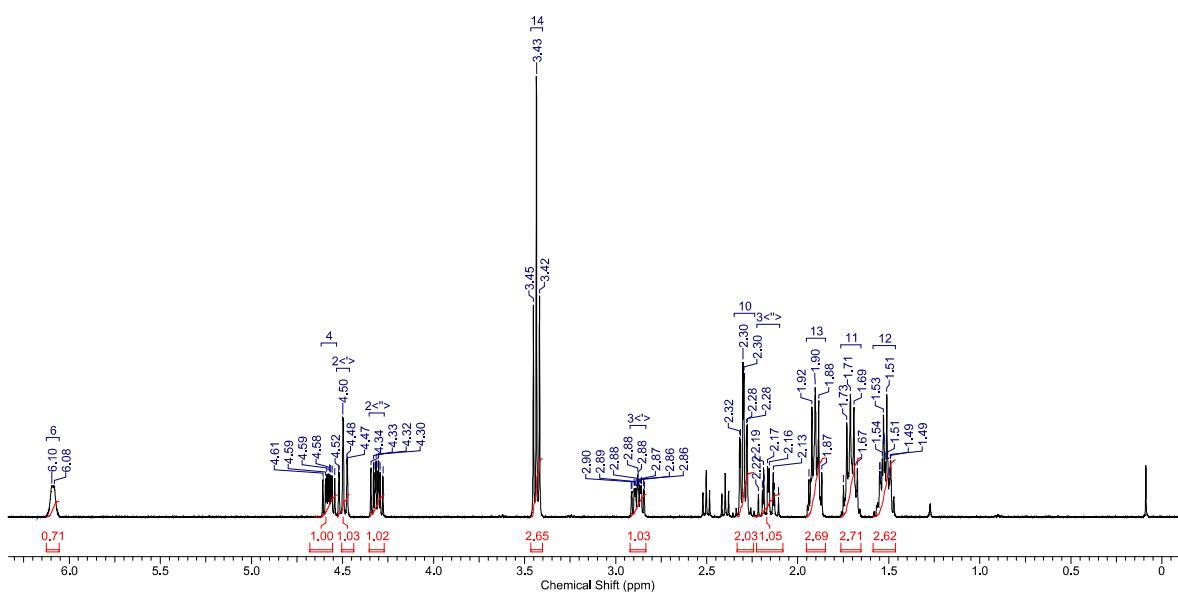
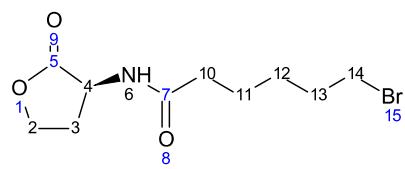
The compound has not been reported previously.

## 5 NMR spectra

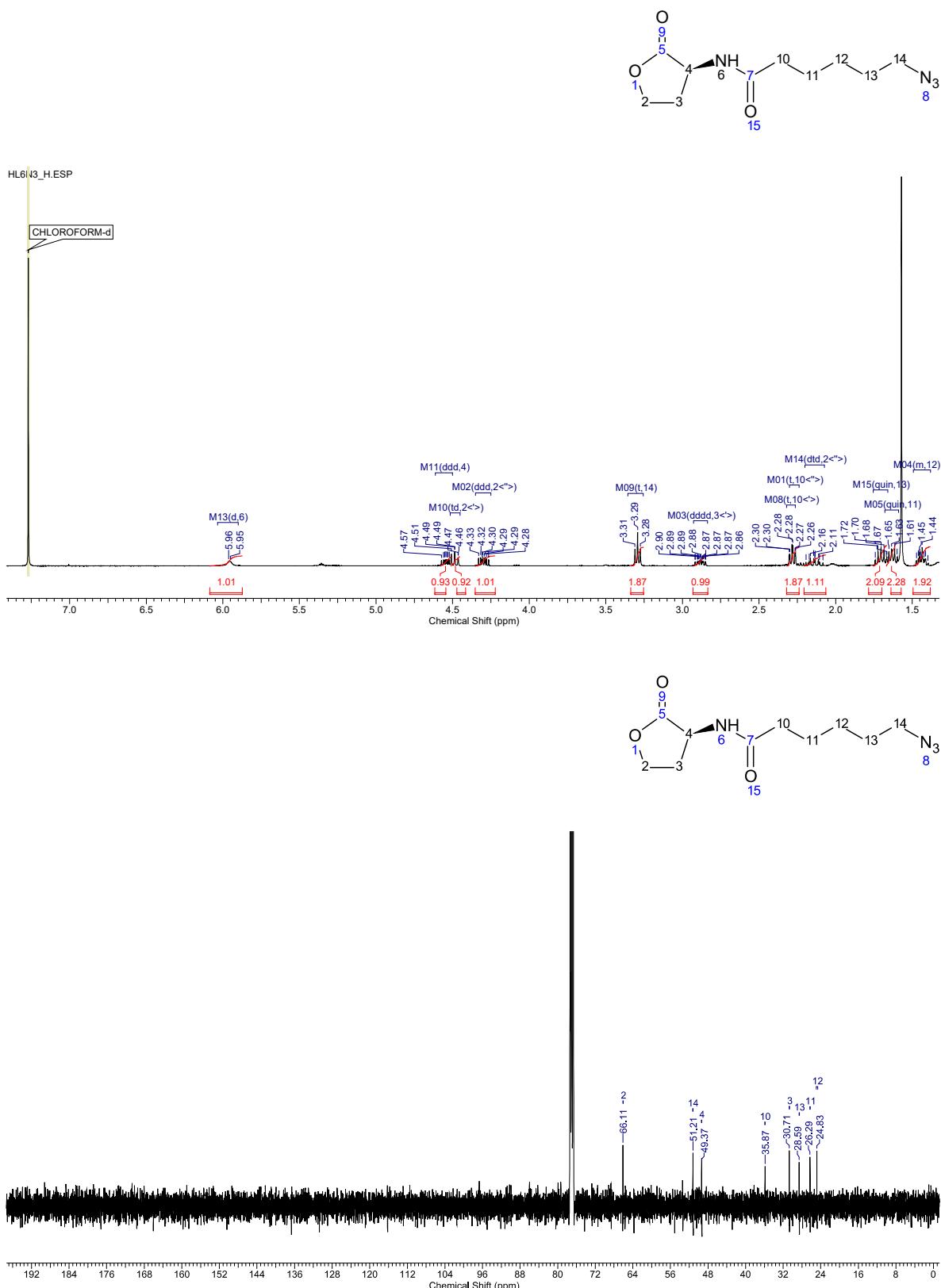
## 5.1 (S)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide ?



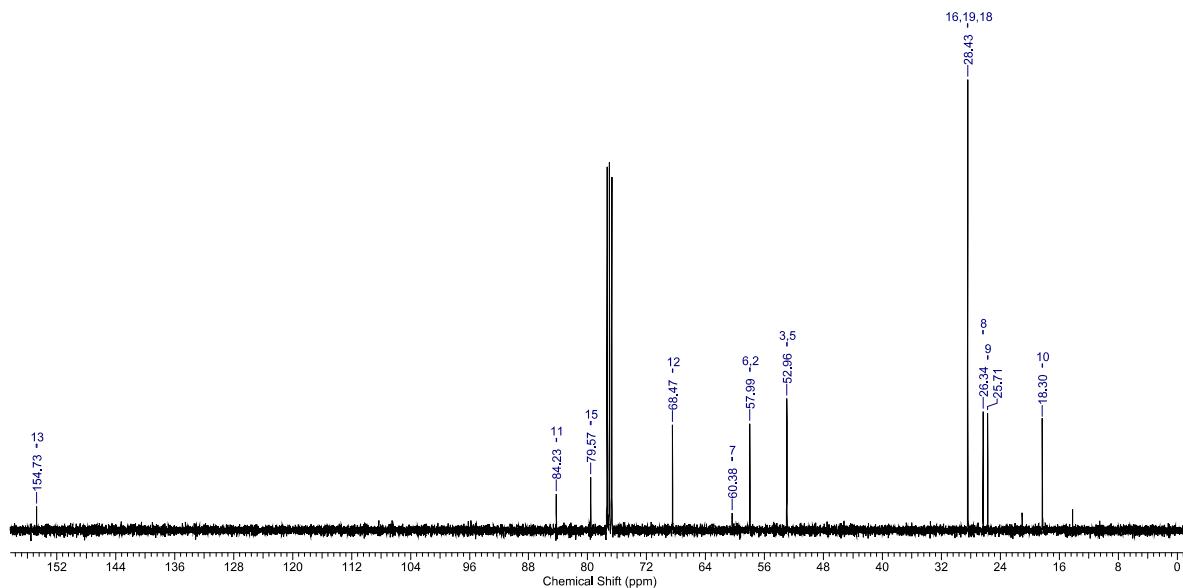
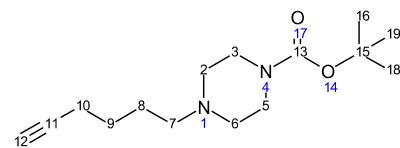
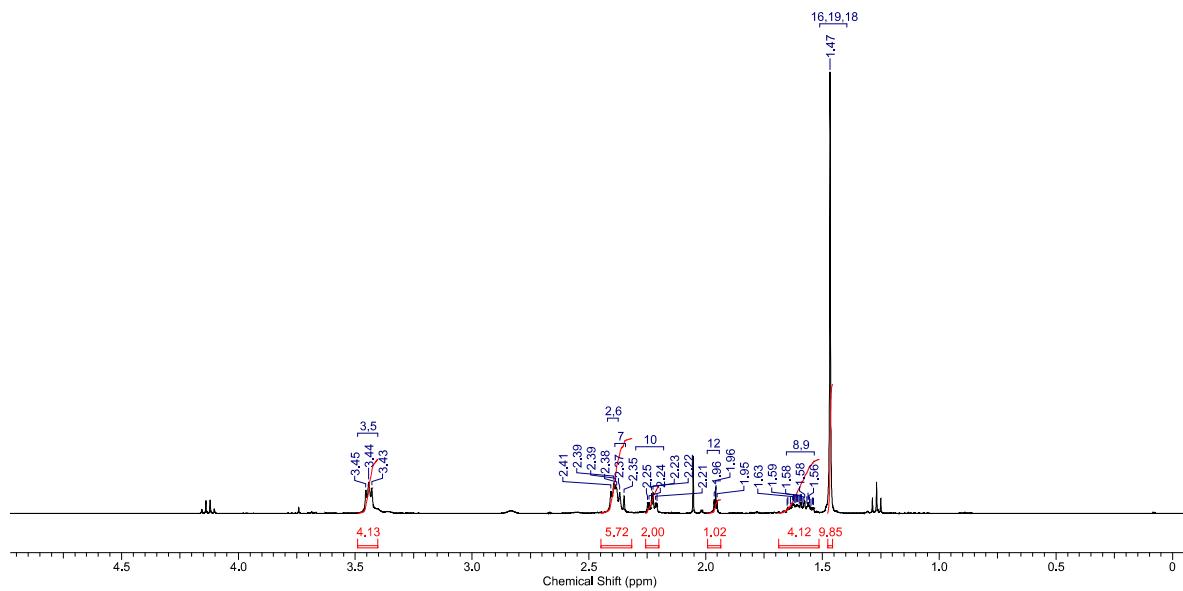
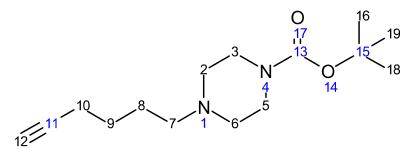
## 5.2 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ?



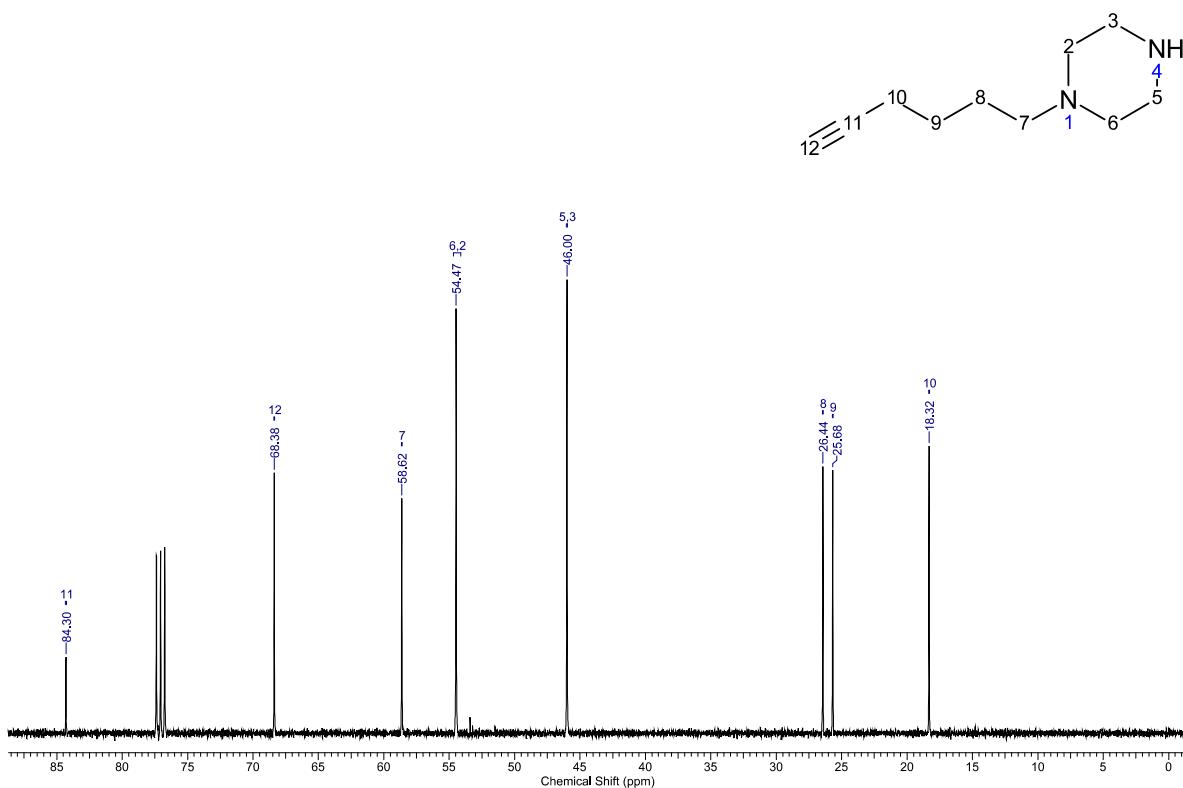
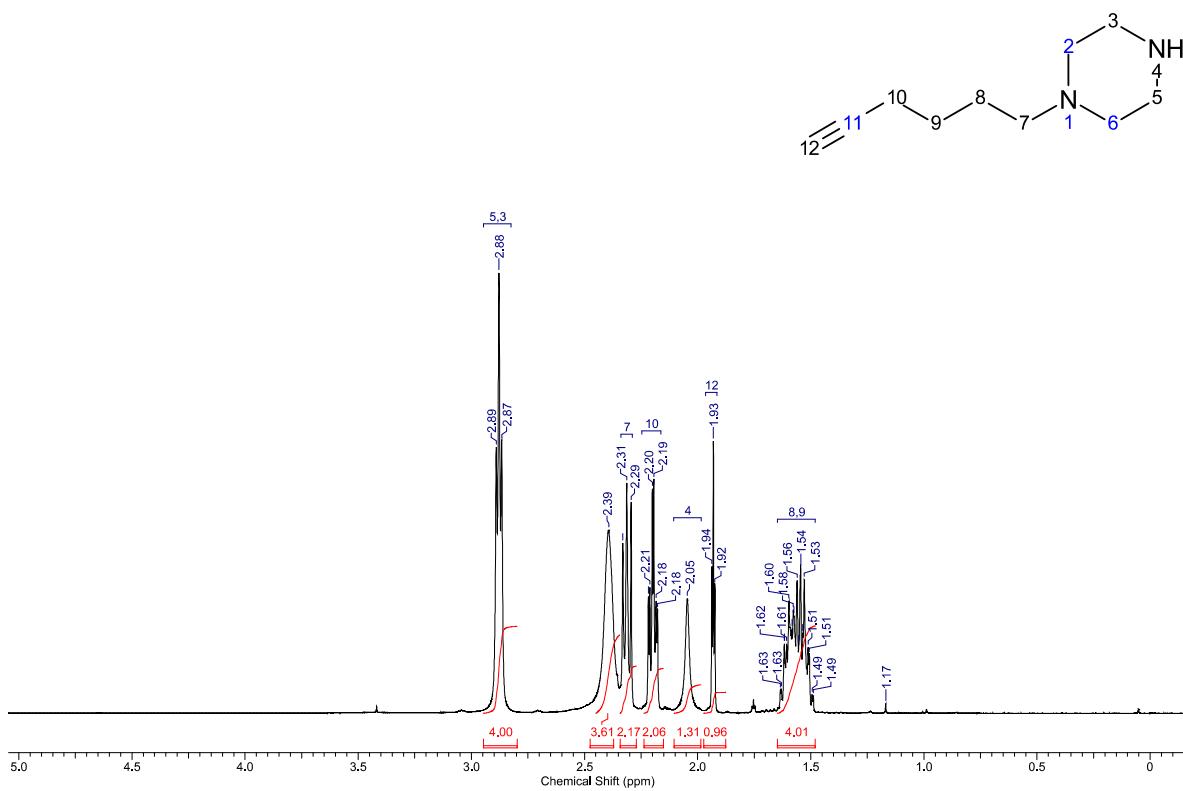
### 5.3 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ?



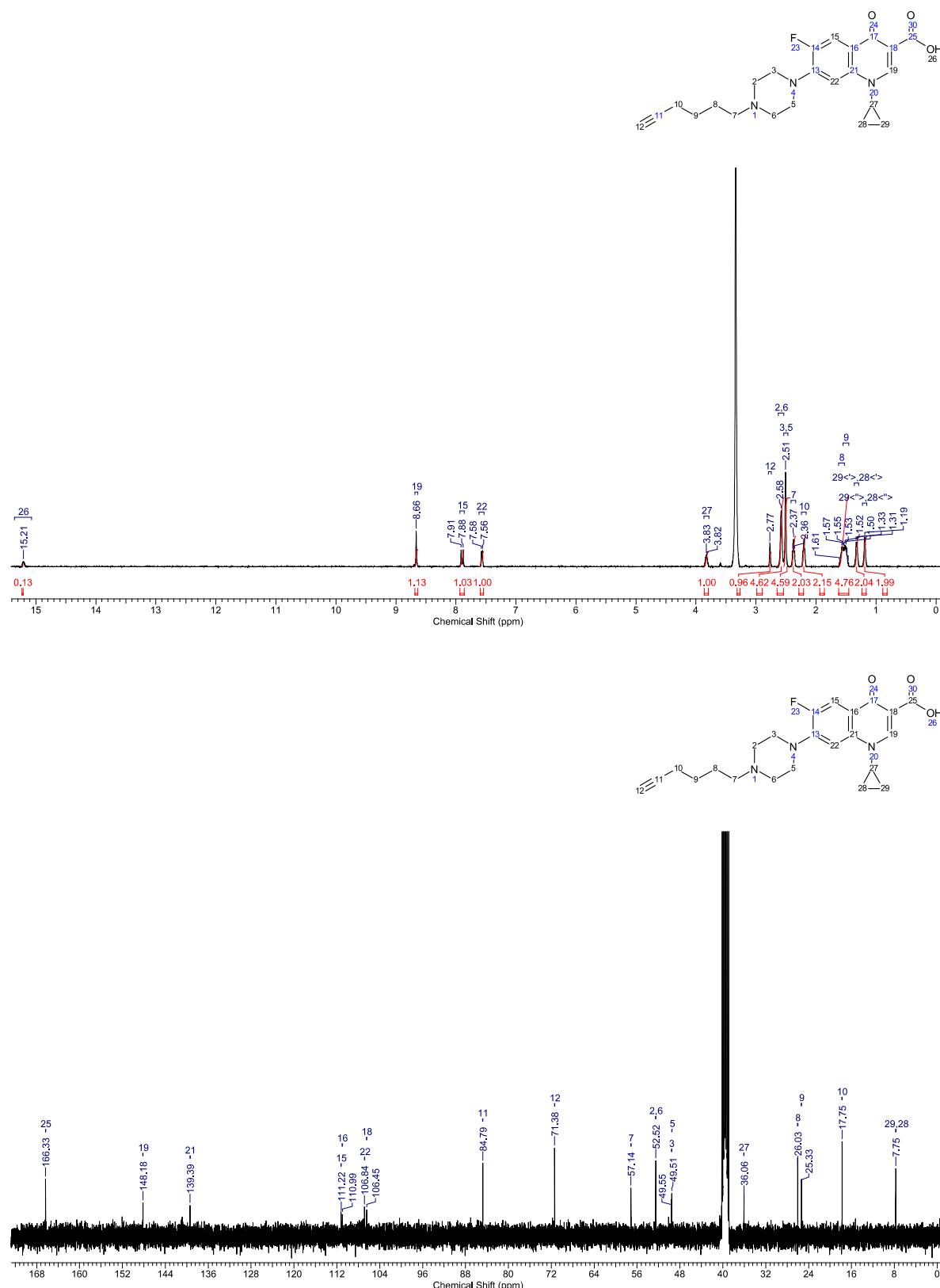
## 5.4 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate ?



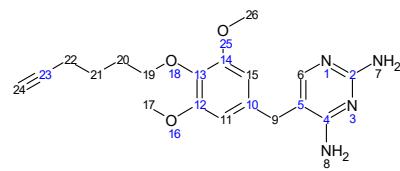
## 5.5 1-(Hex-5-yn-1-yl)piperazine ?



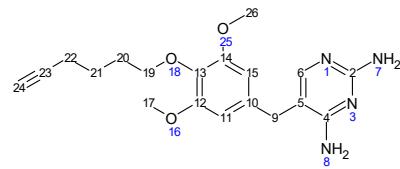
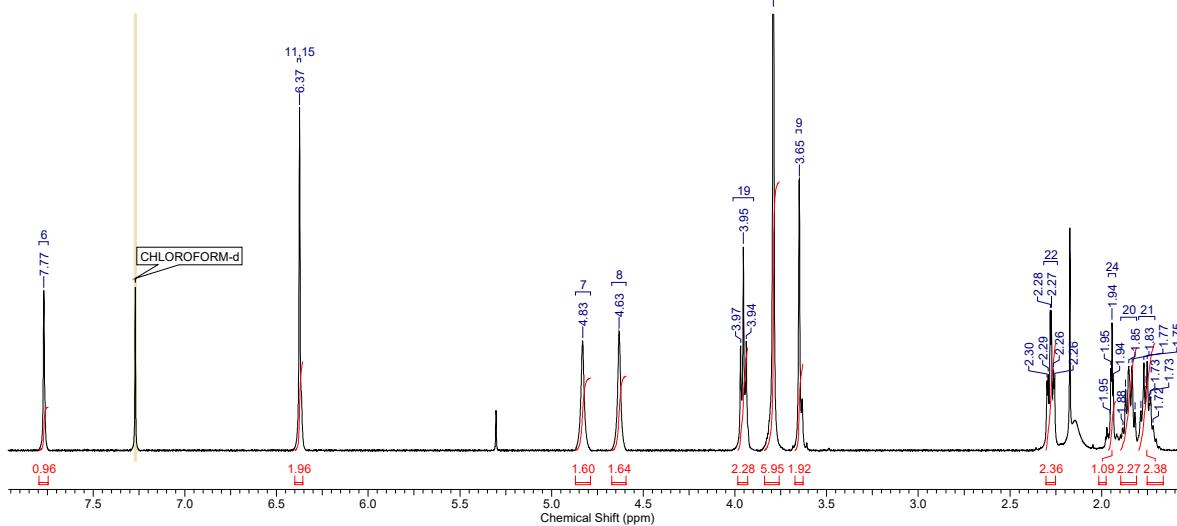
5.6 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



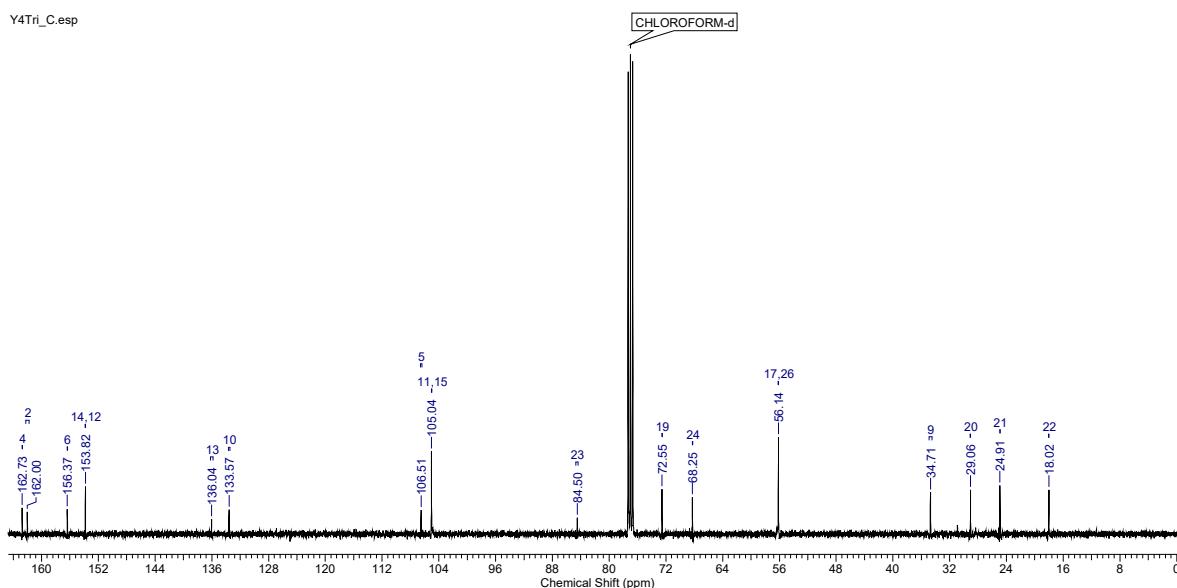
## 5.7 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine ?



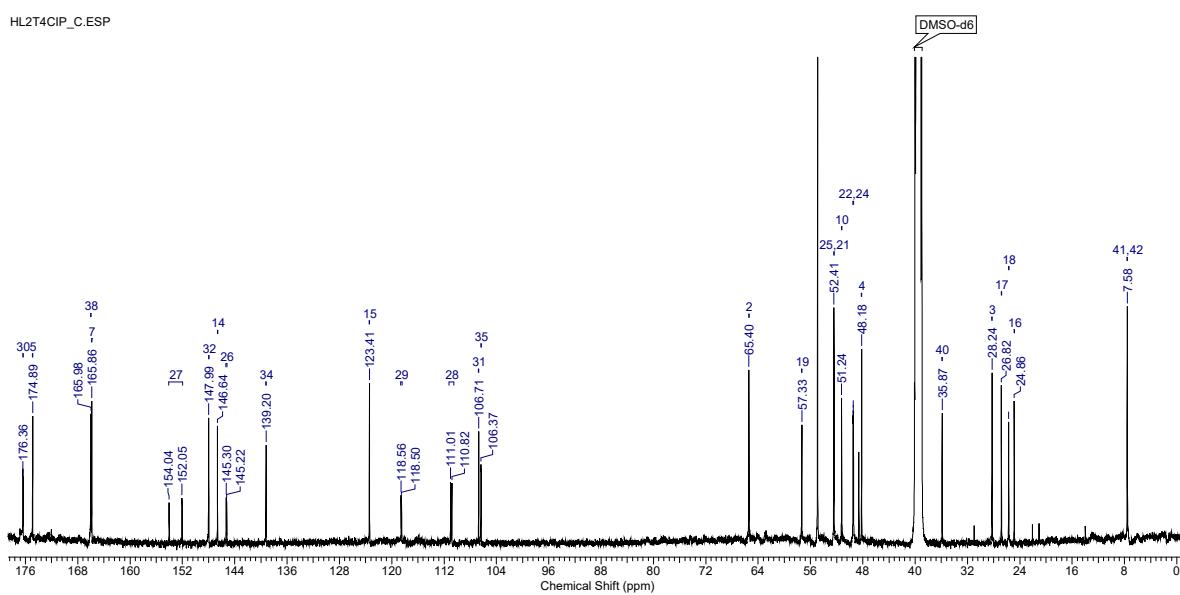
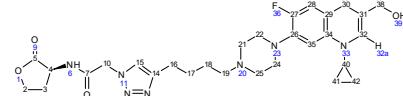
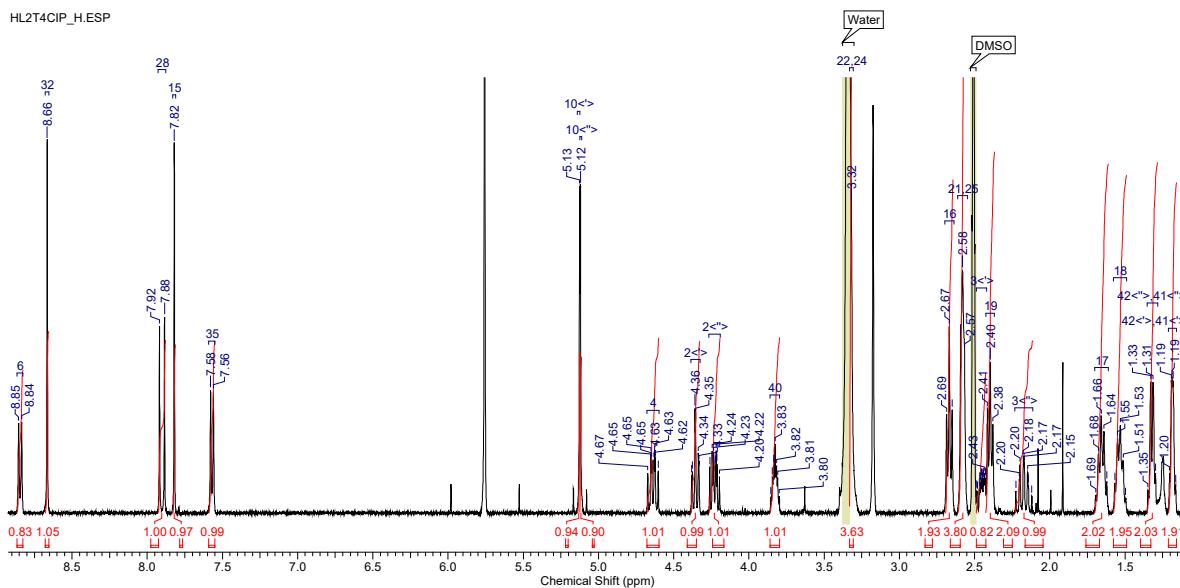
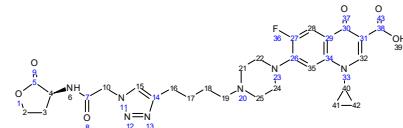
Y4Tri\_H.esp



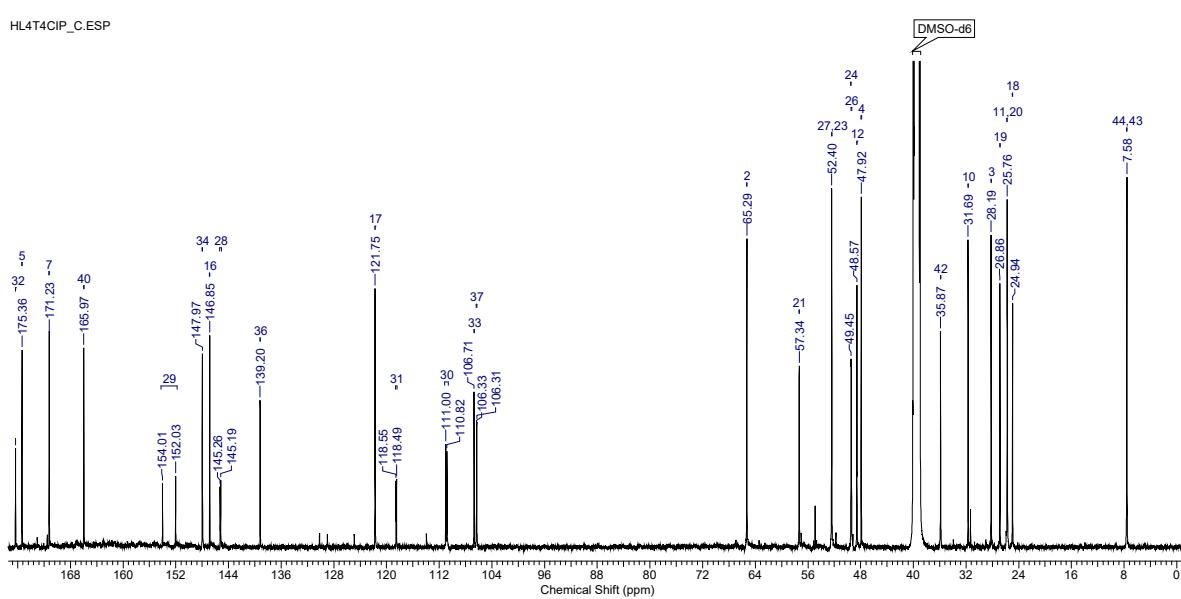
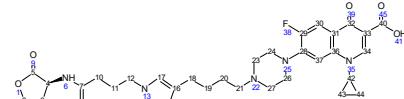
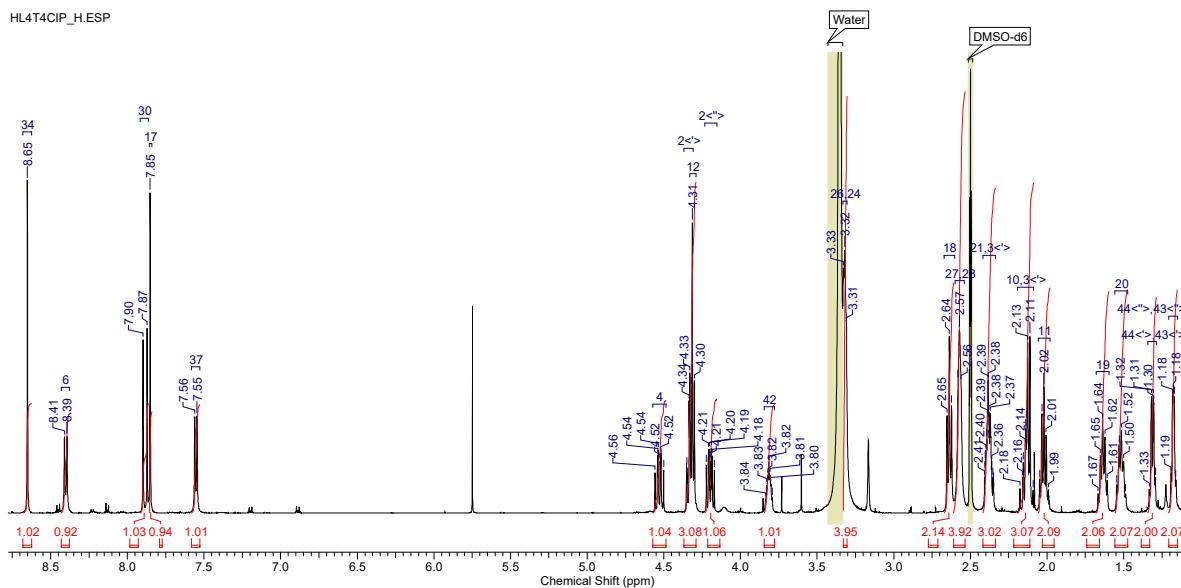
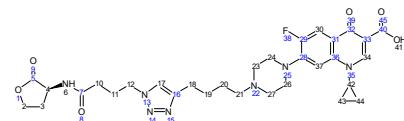
Y4Tri\_C.esp



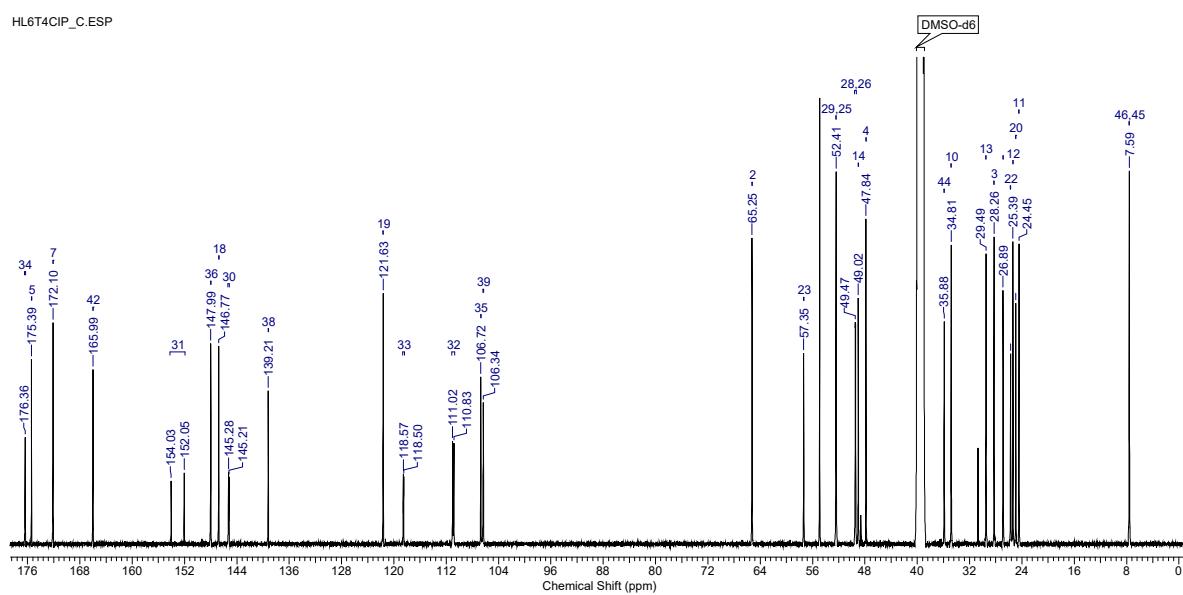
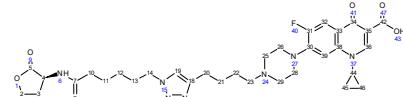
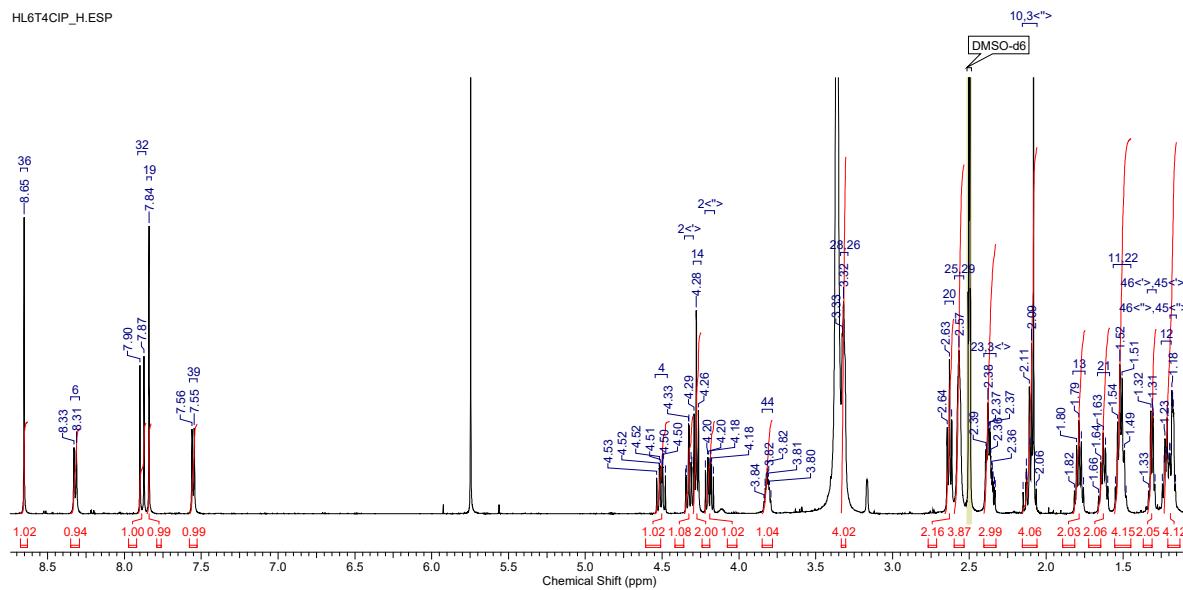
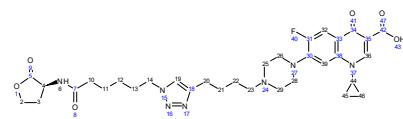
5.8 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ?



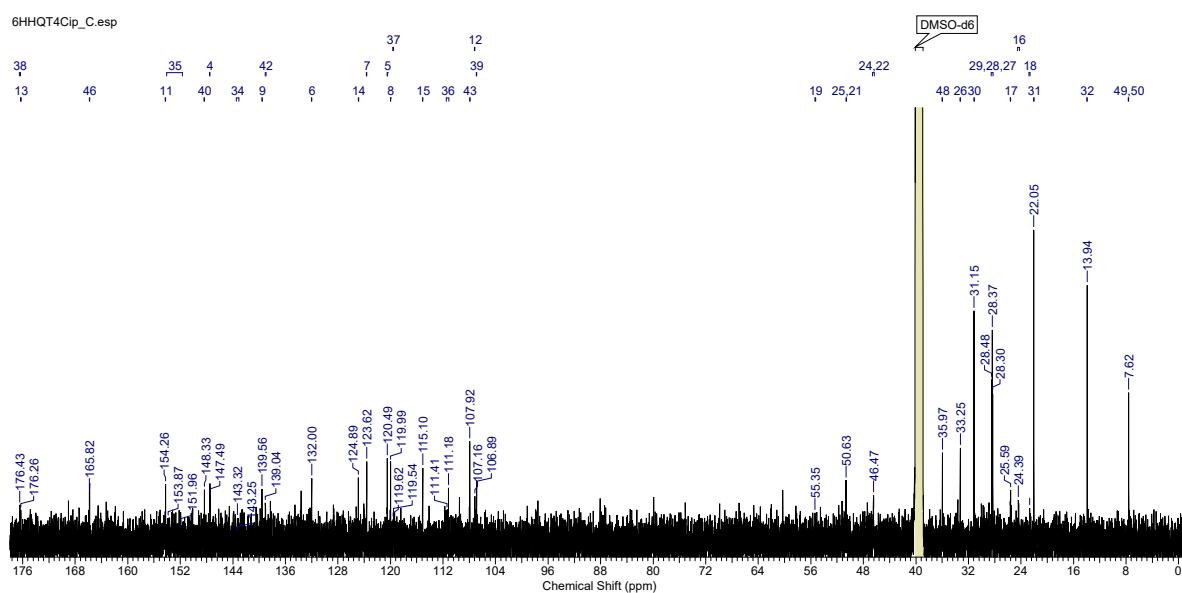
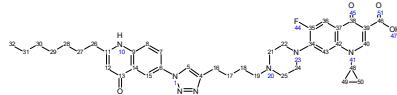
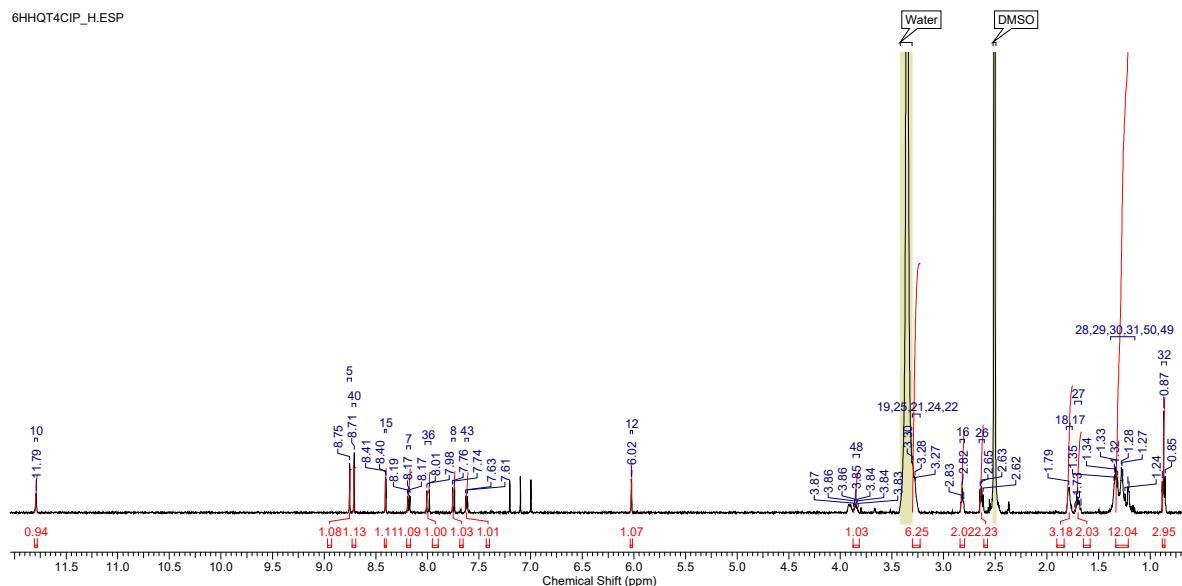
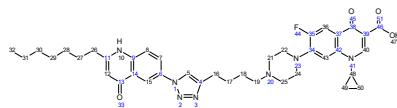
5.9 (S)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ?



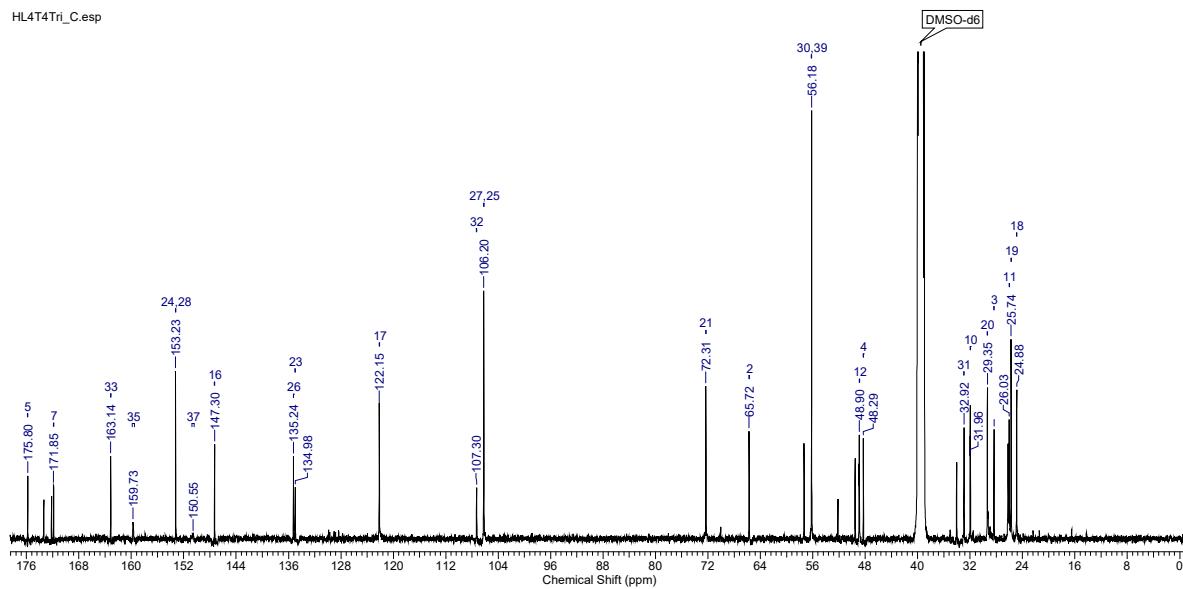
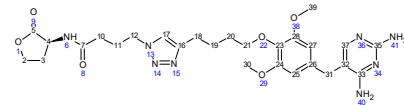
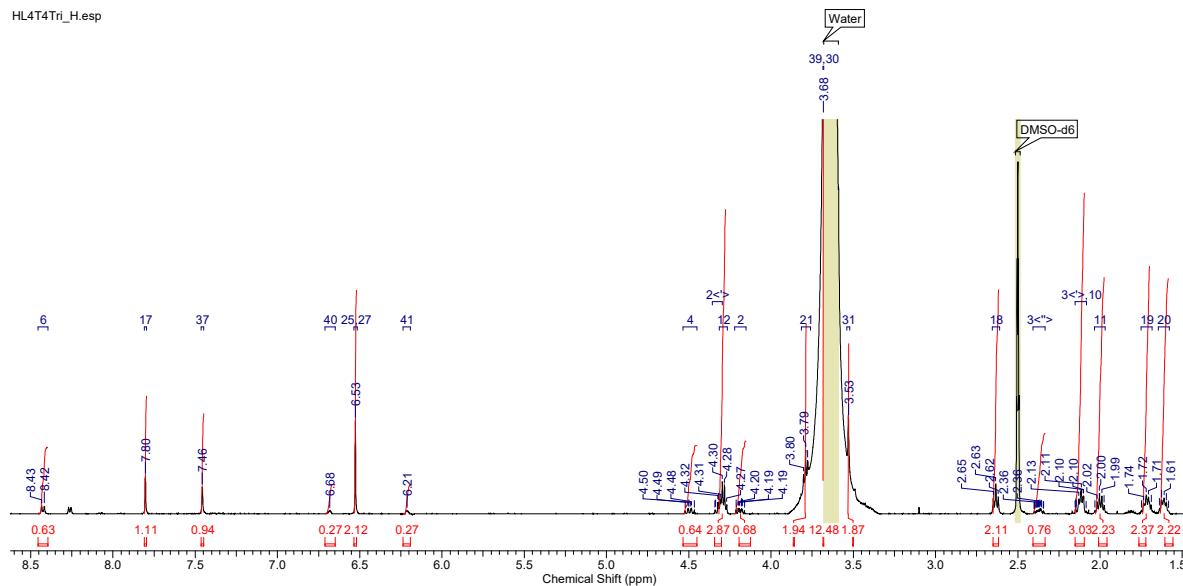
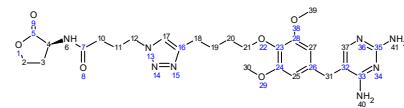
5.10 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ?



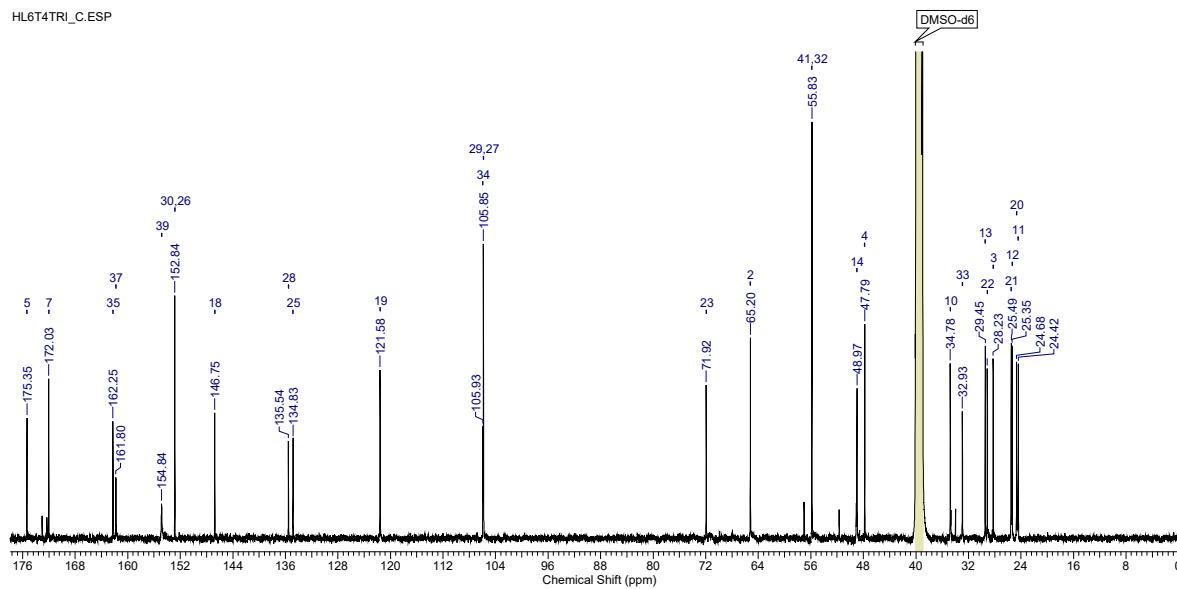
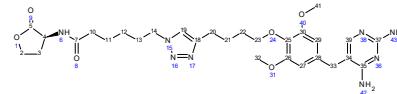
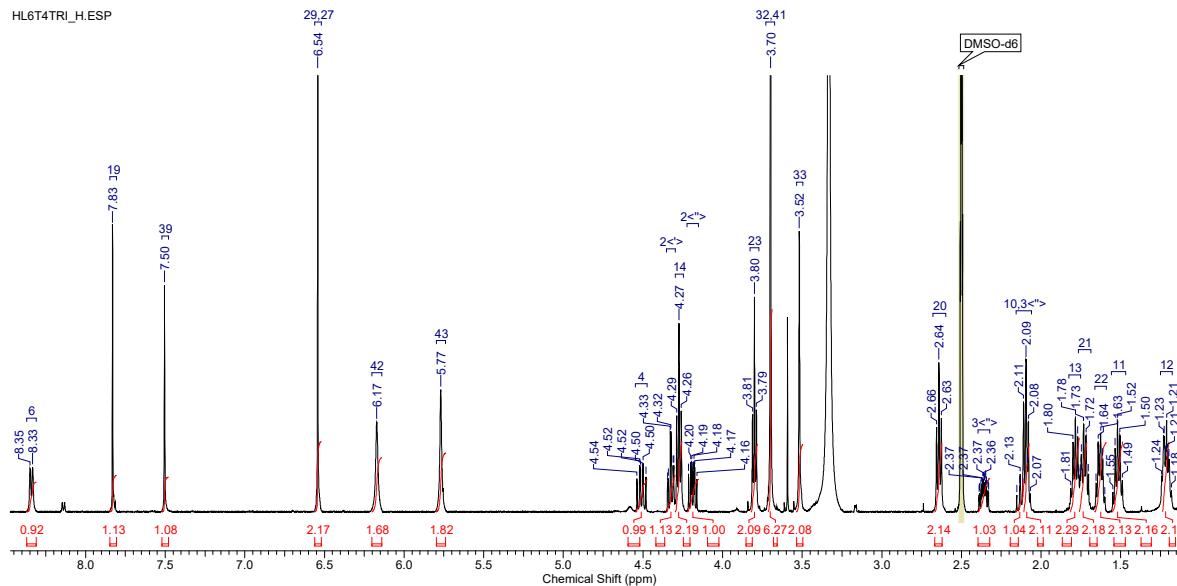
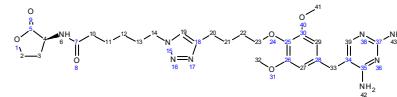
5.11 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



5.12 (*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 ?

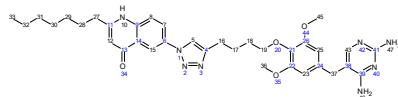


5.13 (*S*)-6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide ?

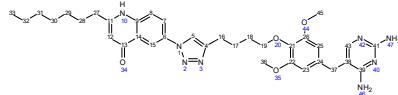
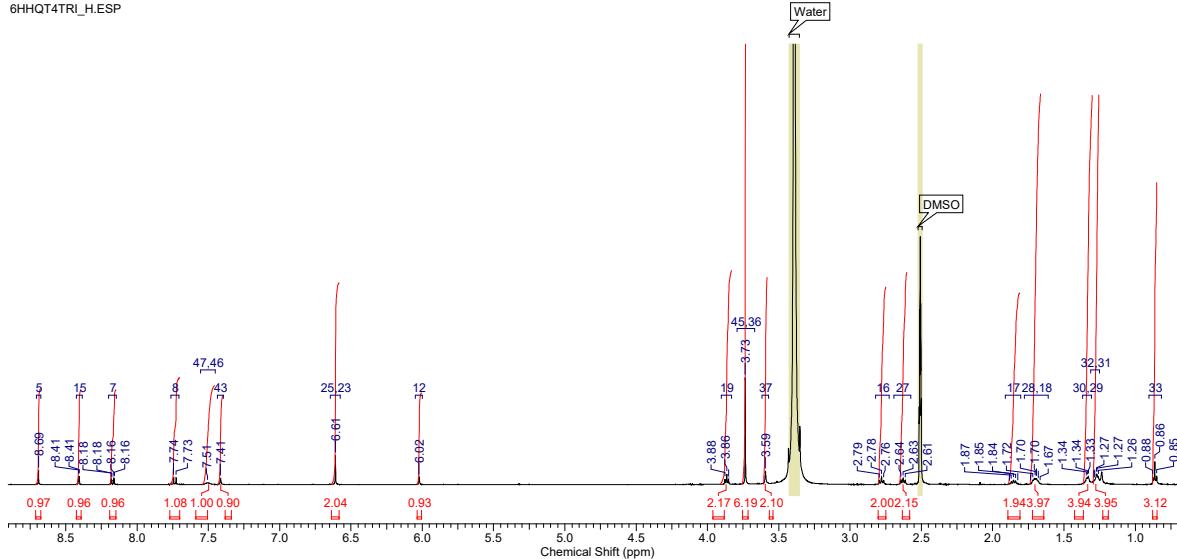


5.14 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(*H*)-one ?

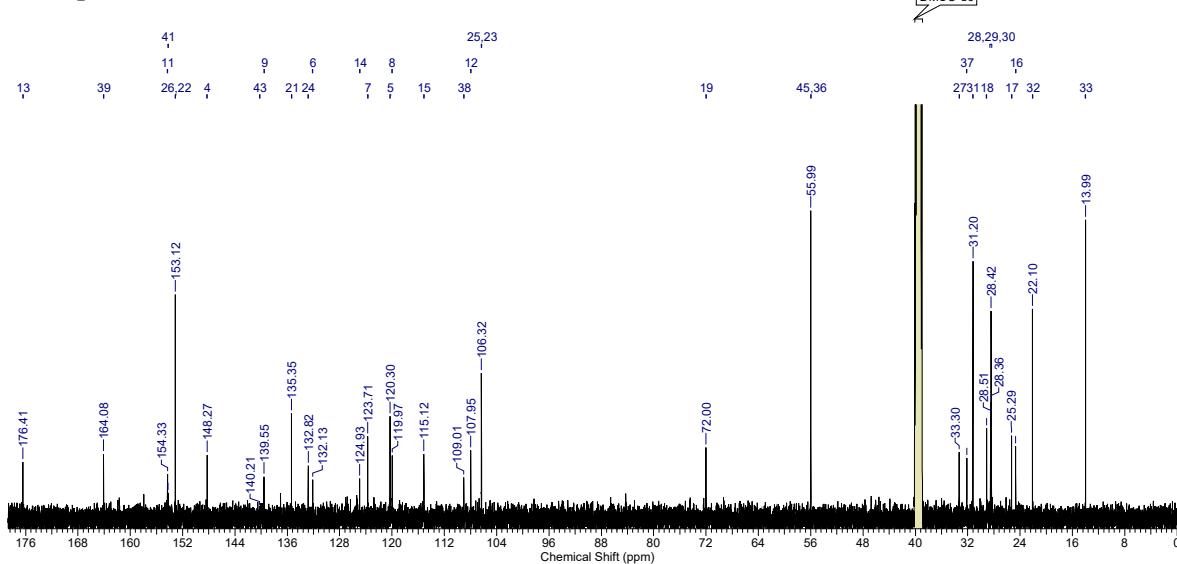
**User Notes** Some guesses



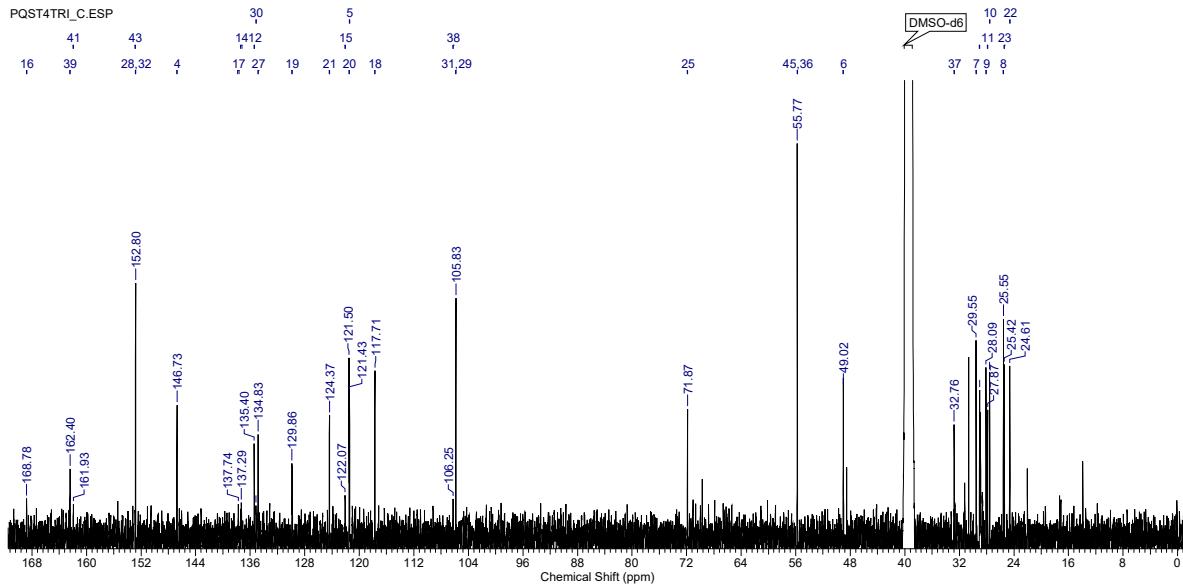
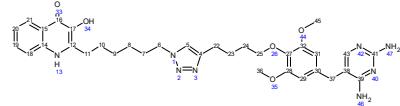
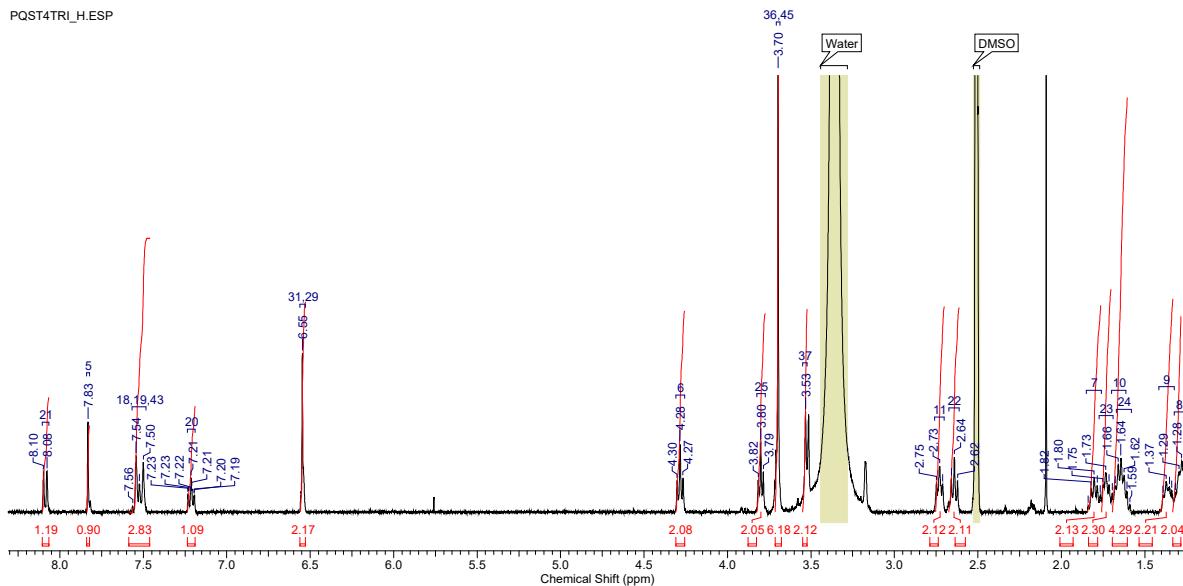
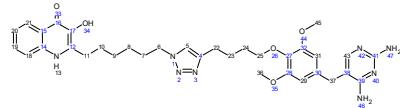
6HHQT4TRI H.ESP



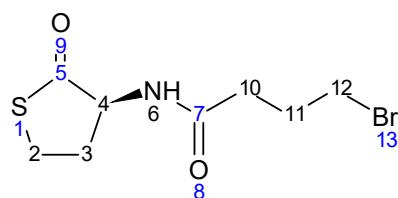
6HHQT4TRI\_C.ESP



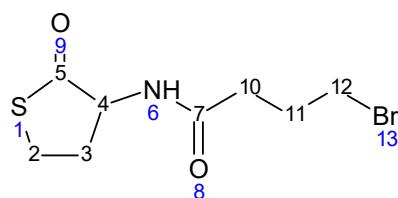
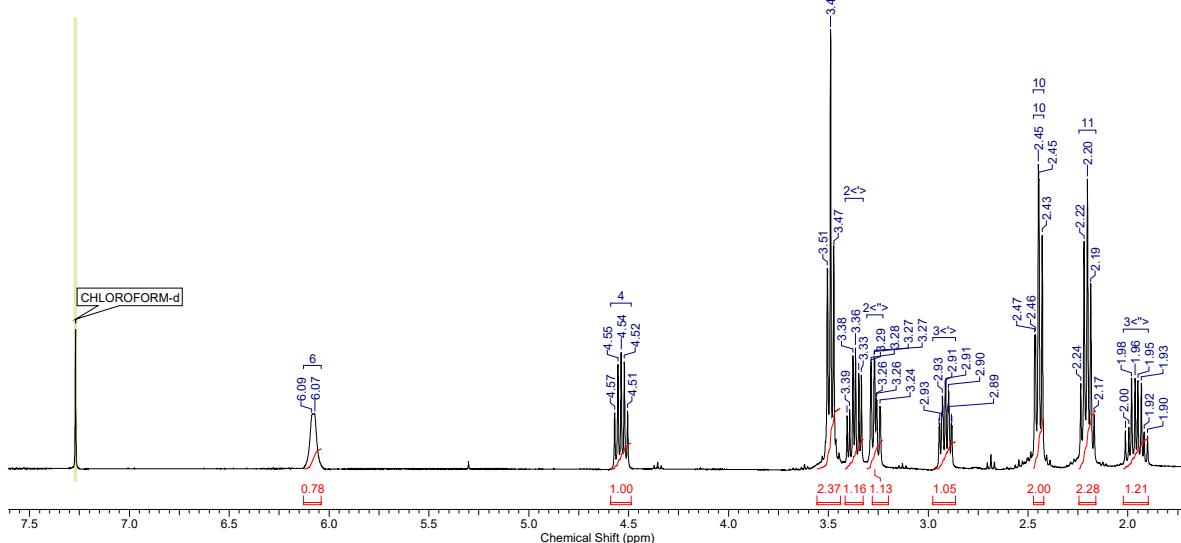
5.15 2-(6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(*1H*)-one ?



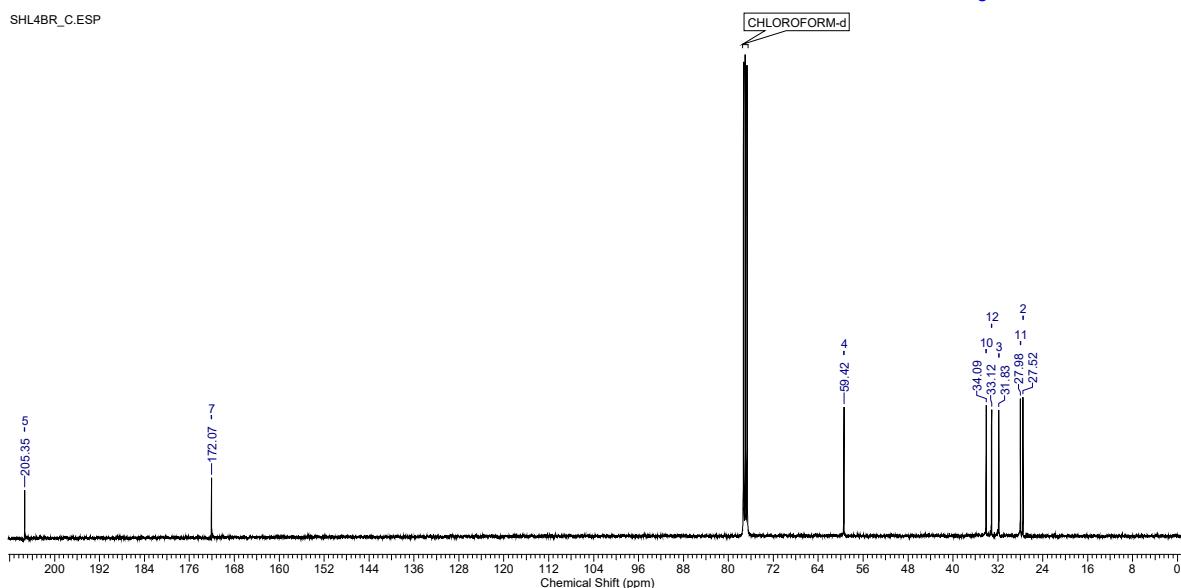
### 5.16 4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ?



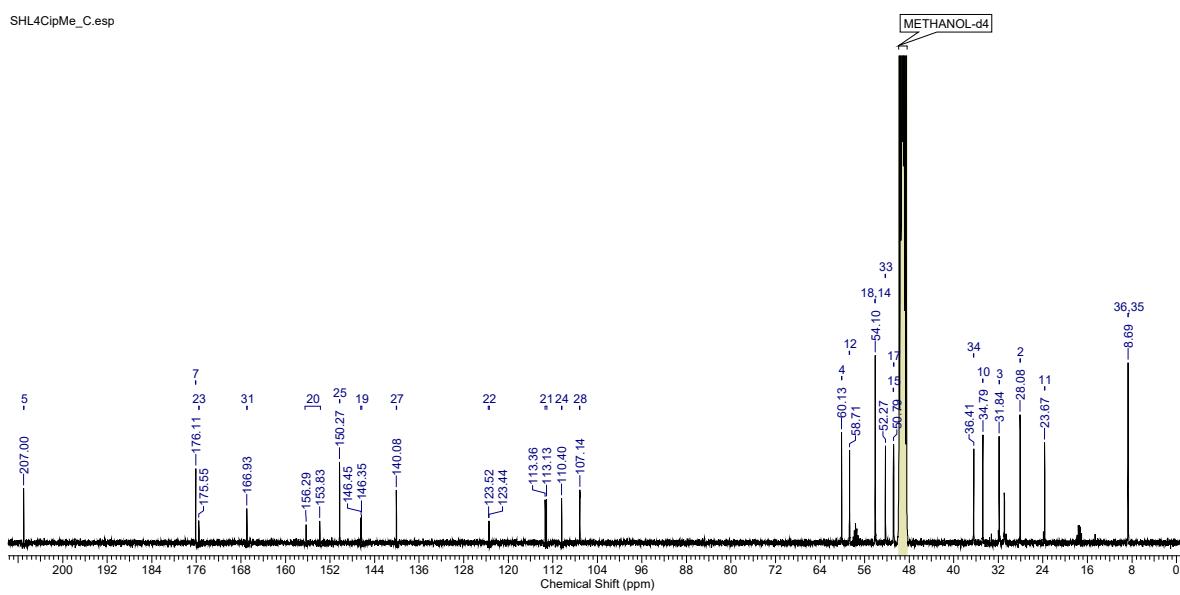
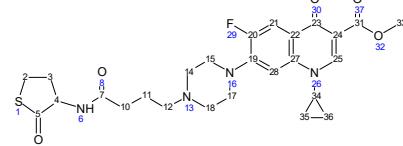
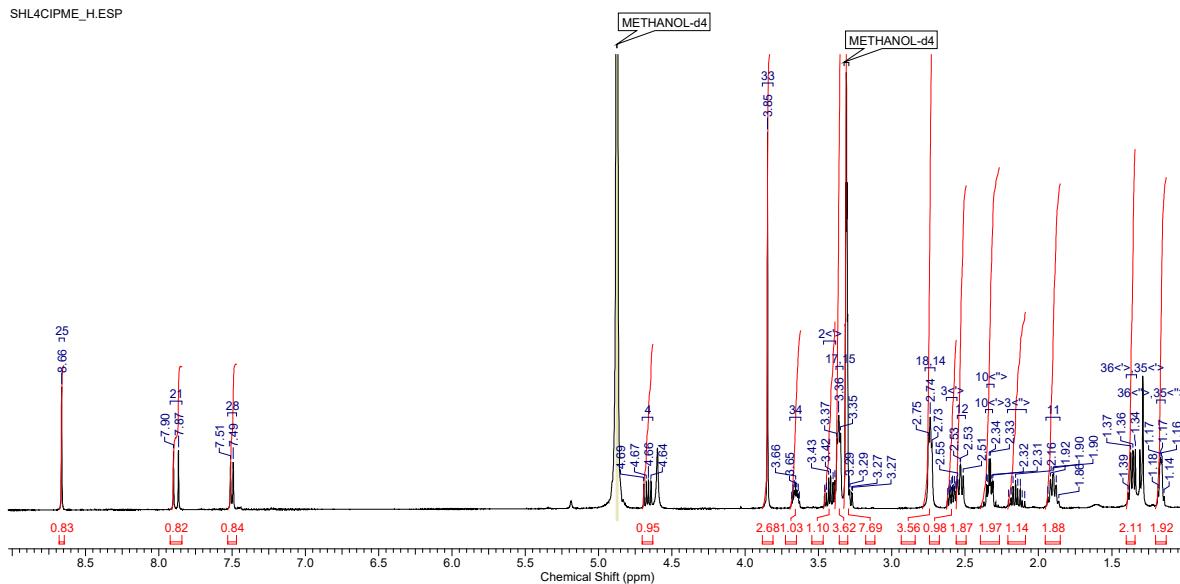
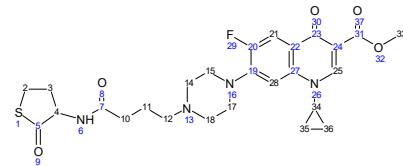
SHL4BR H.ESP



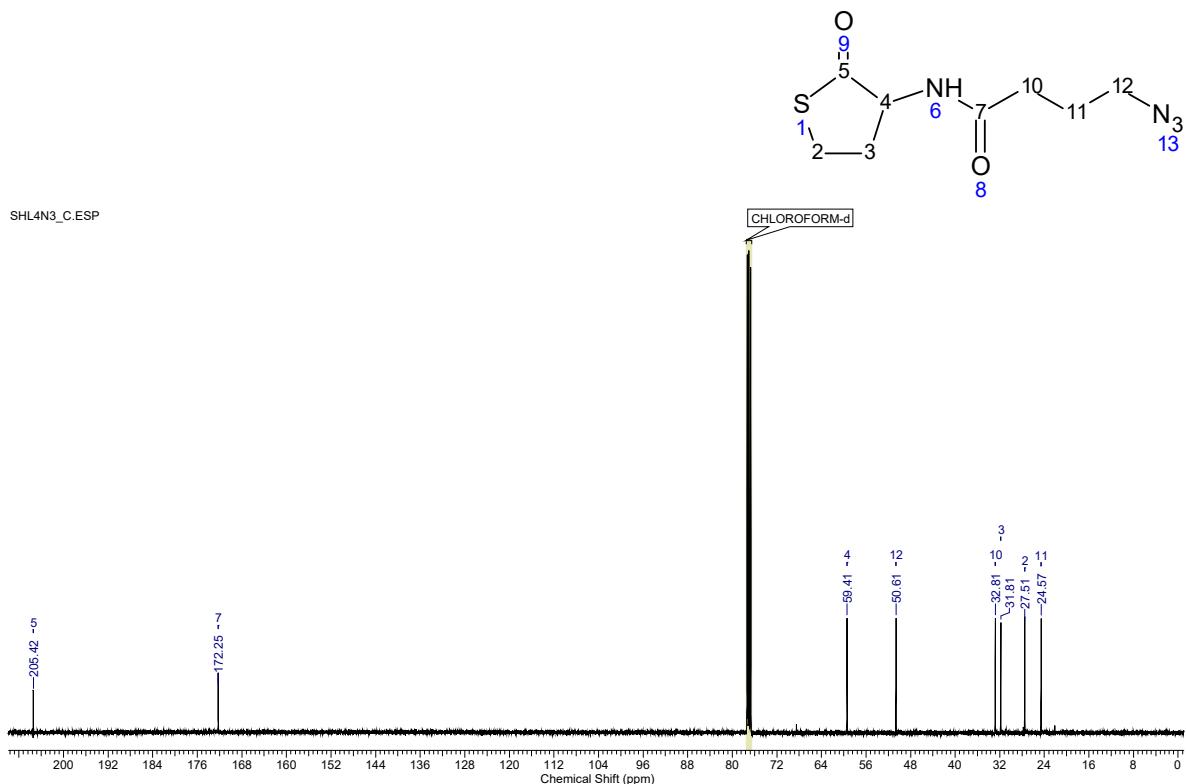
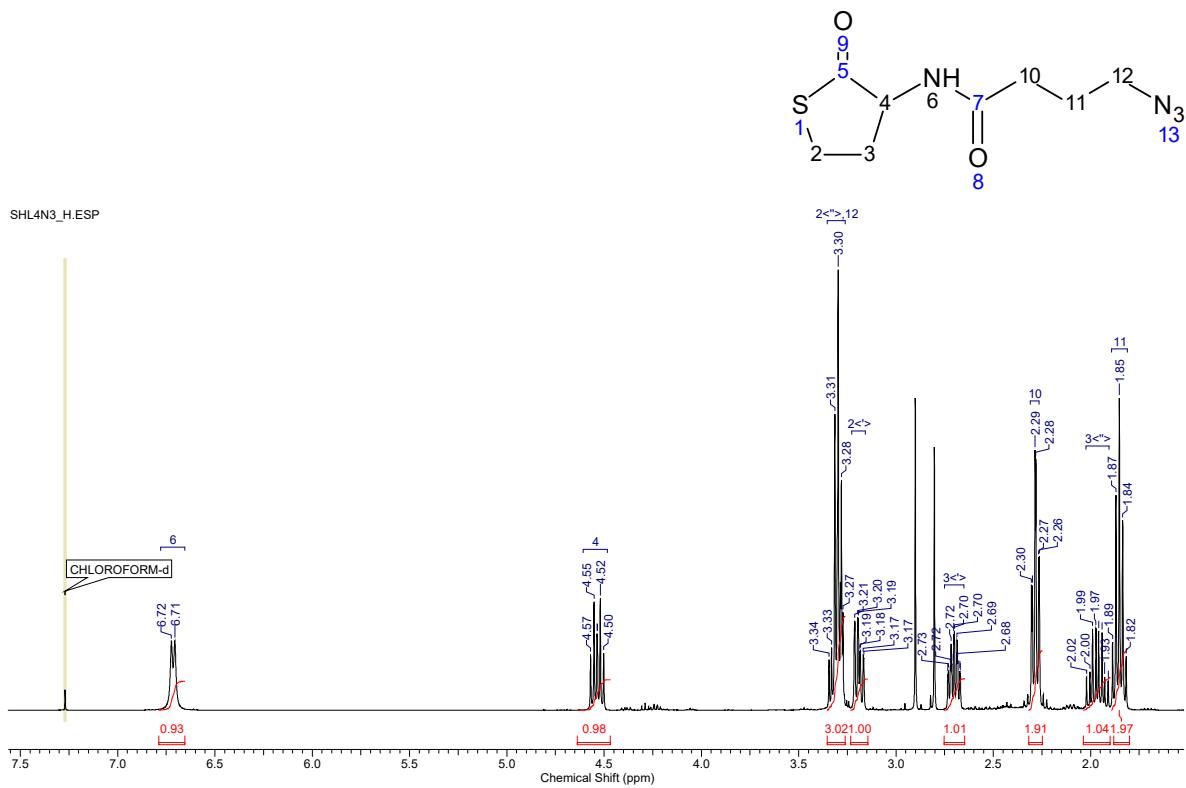
SHL4BR C.ESP



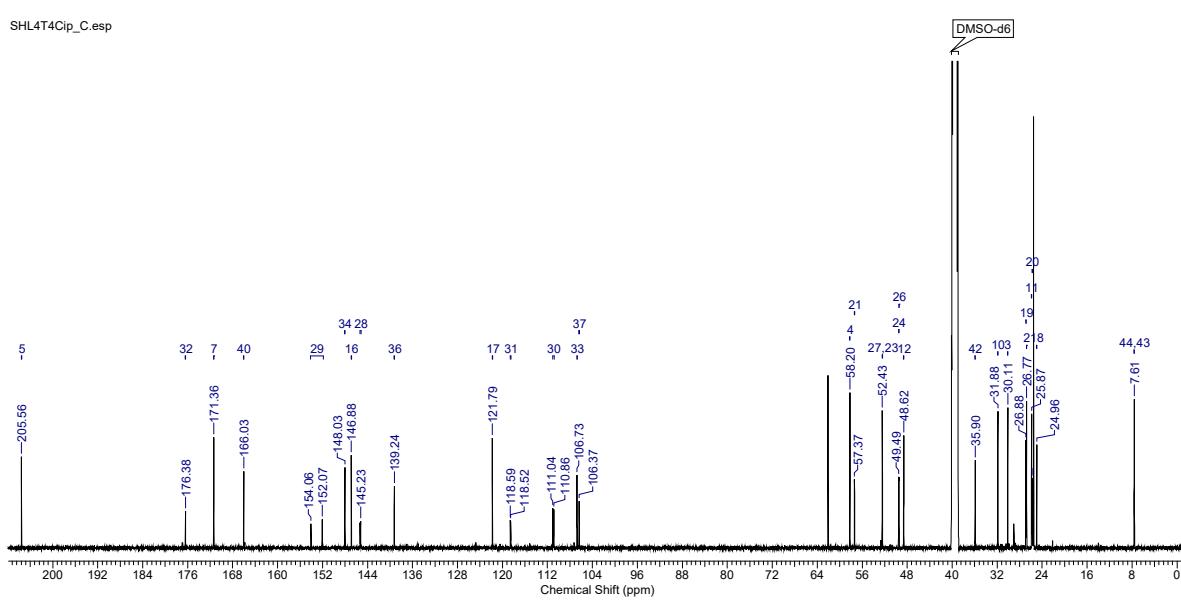
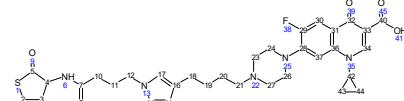
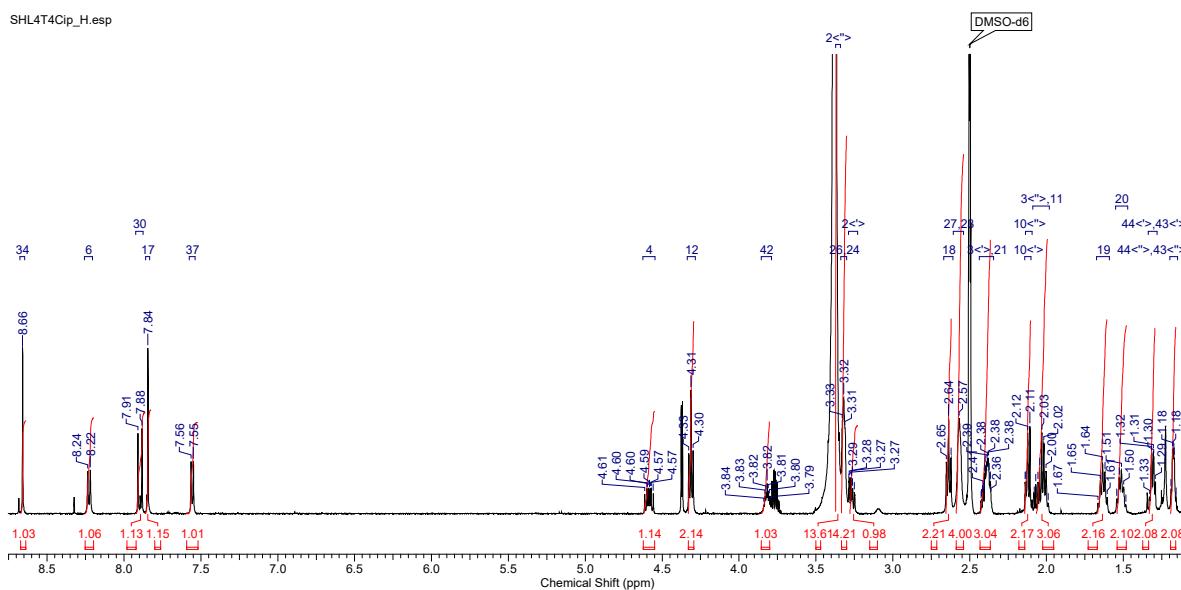
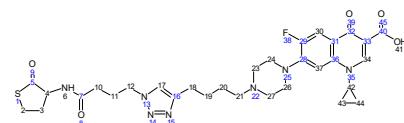
5.17 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ?



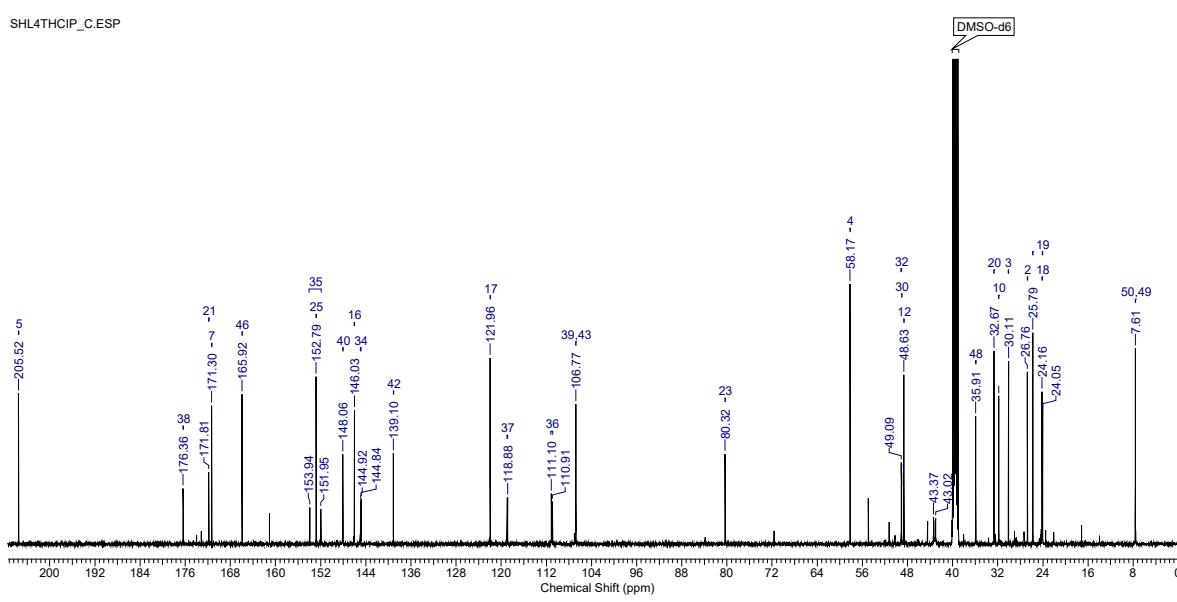
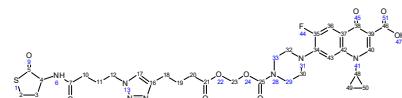
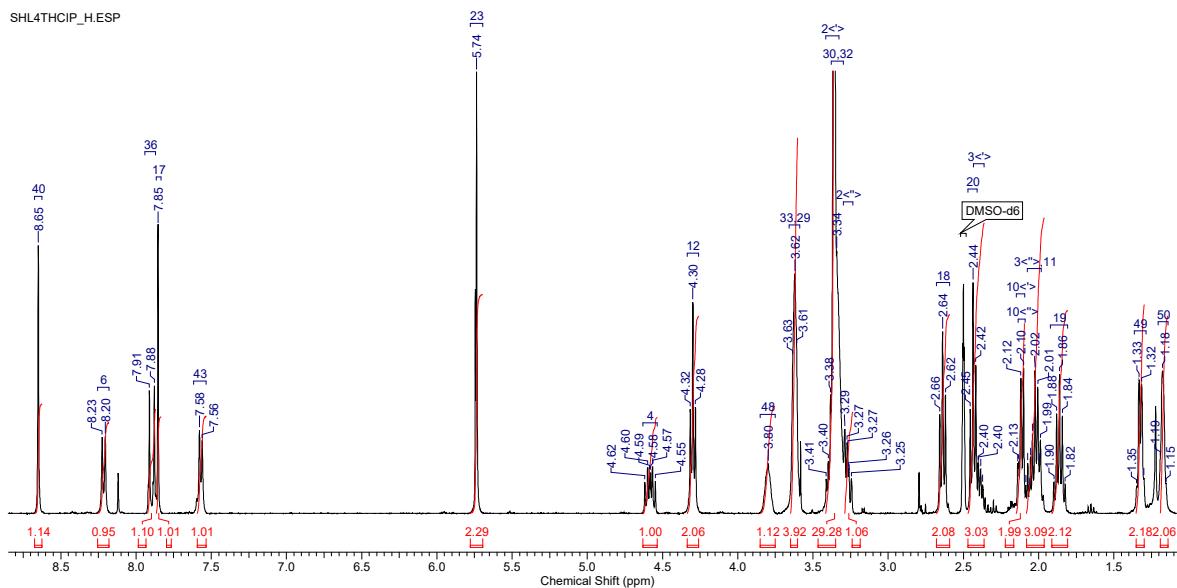
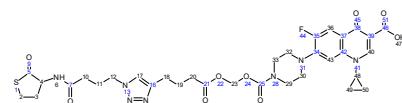
5.18 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide ?



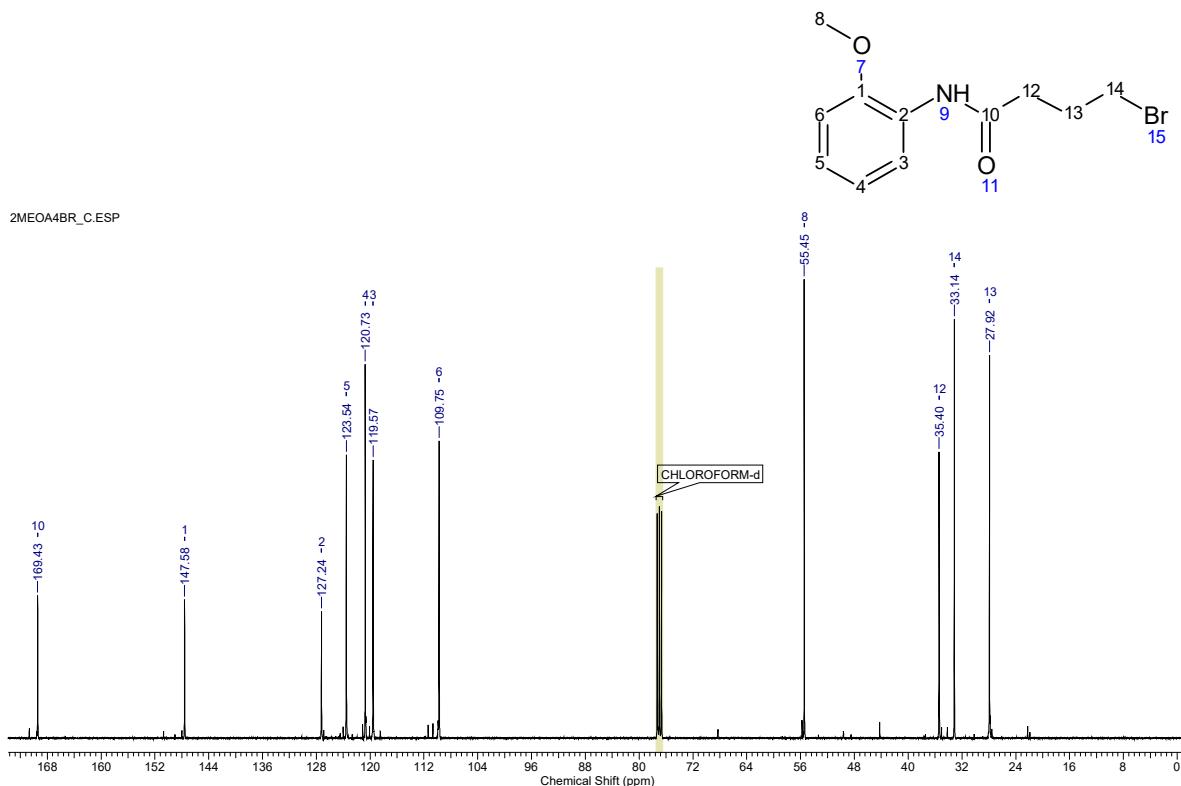
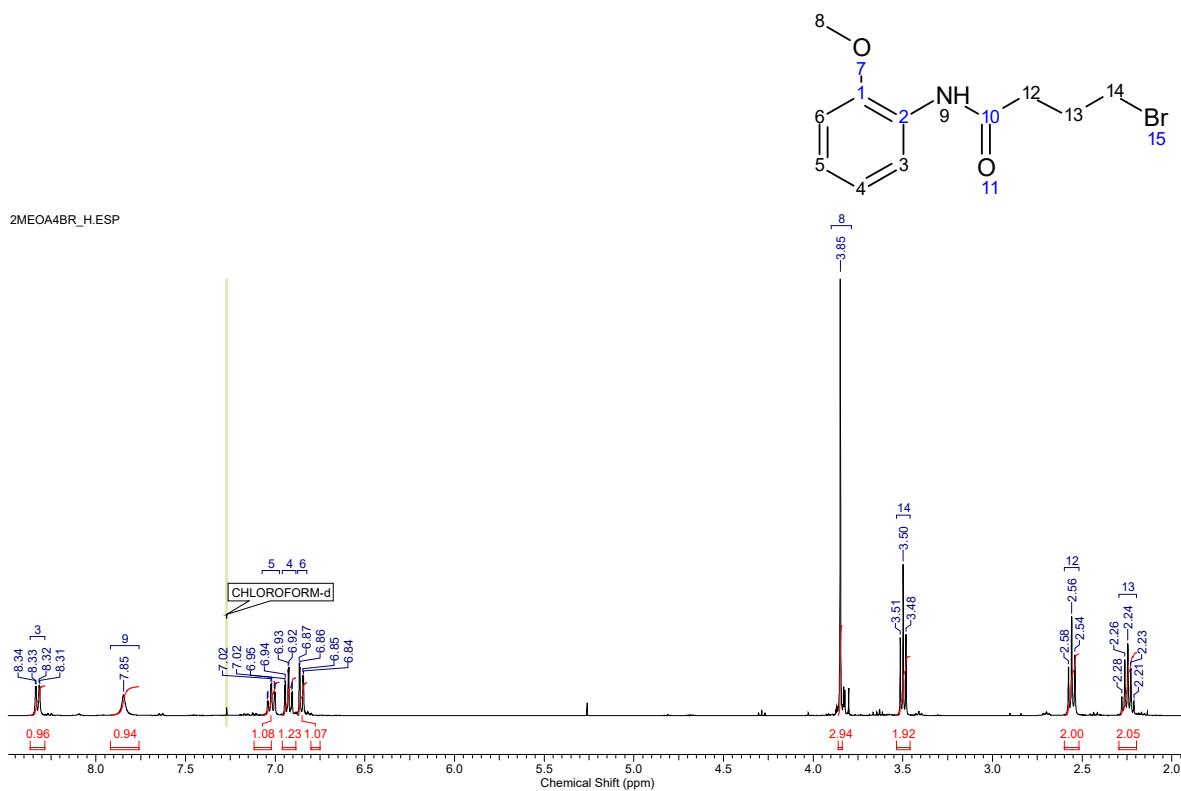
5.19 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ?



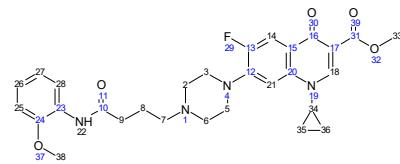
5.20 1-Cyclopropyl-6-fluoro-4-oxo-7-(((4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ?



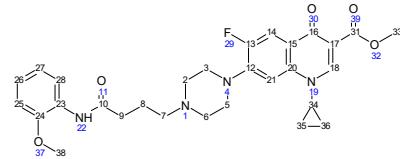
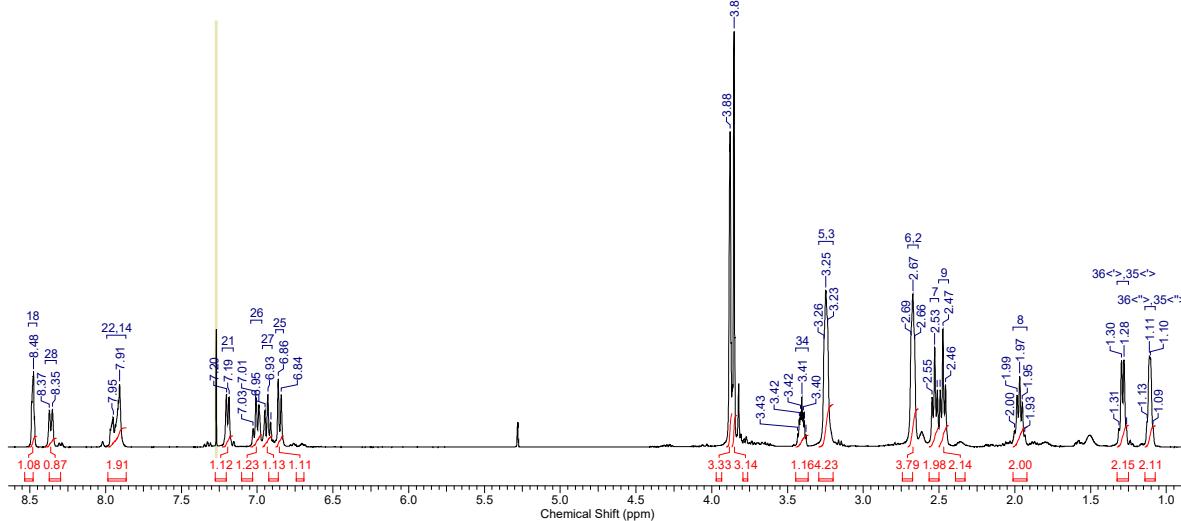
5.21 4-Bromo-N-(2-methoxyphenyl)butanamide ?



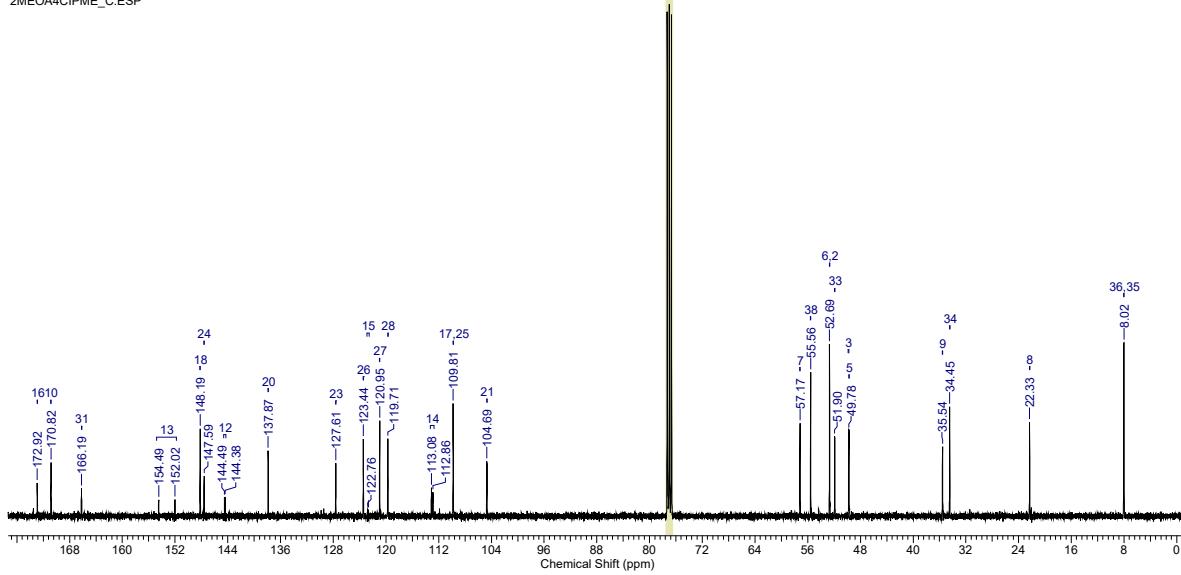
5.22 Methyl 1-cyclopropyl-6-fluoro-7-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



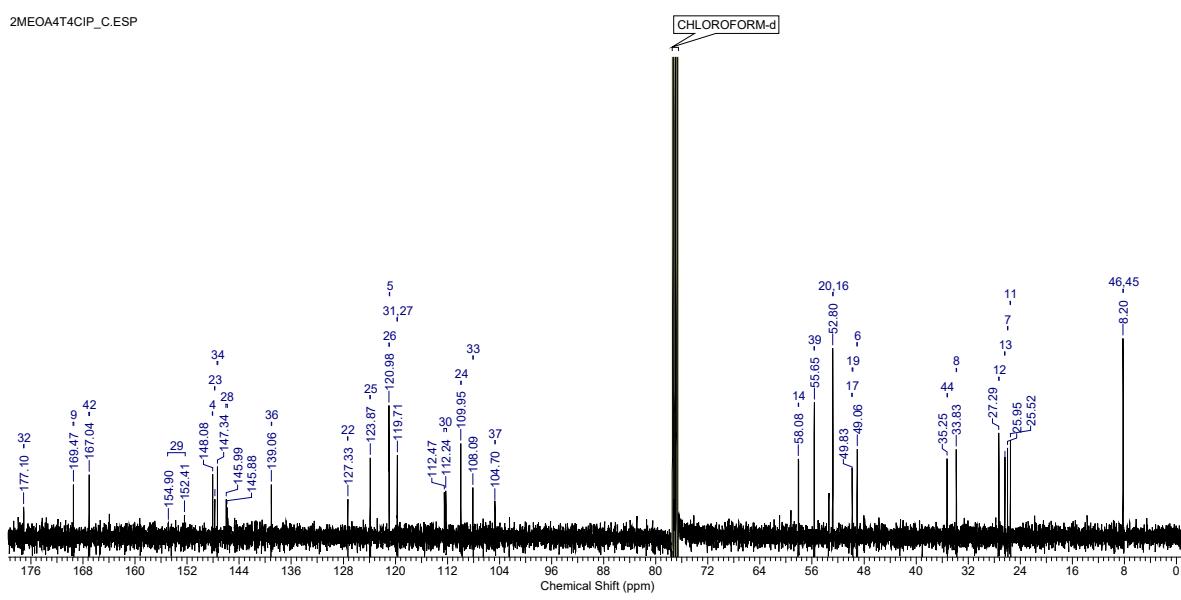
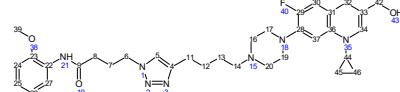
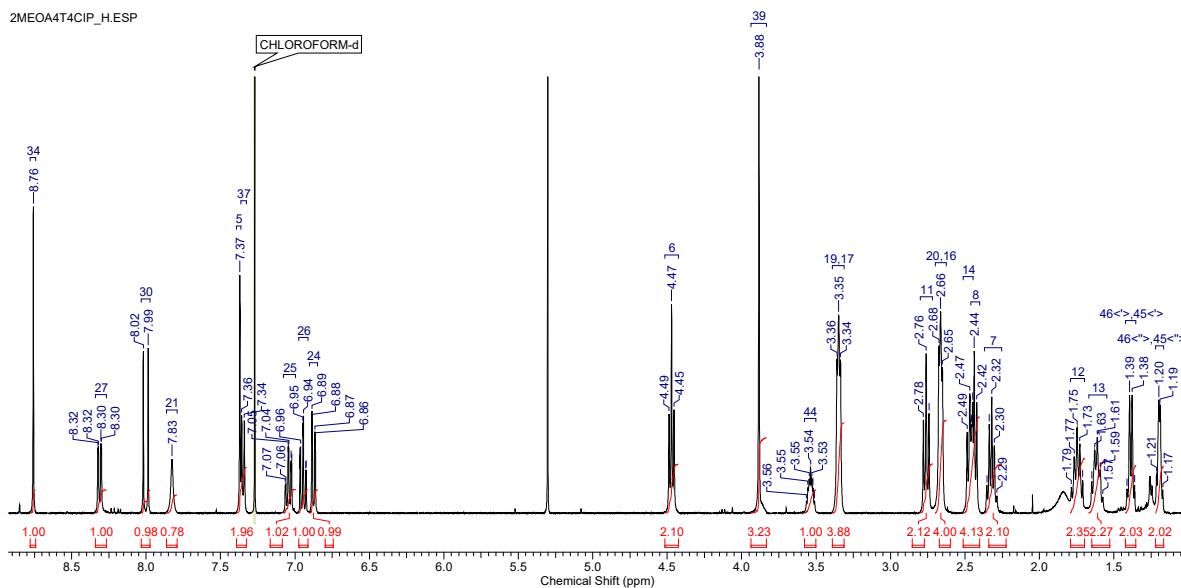
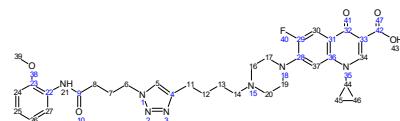
2MEOA4CIPME\_H.ESP



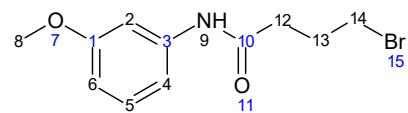
2MEOA4CIPME\_C.ESP



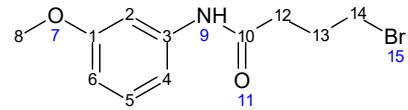
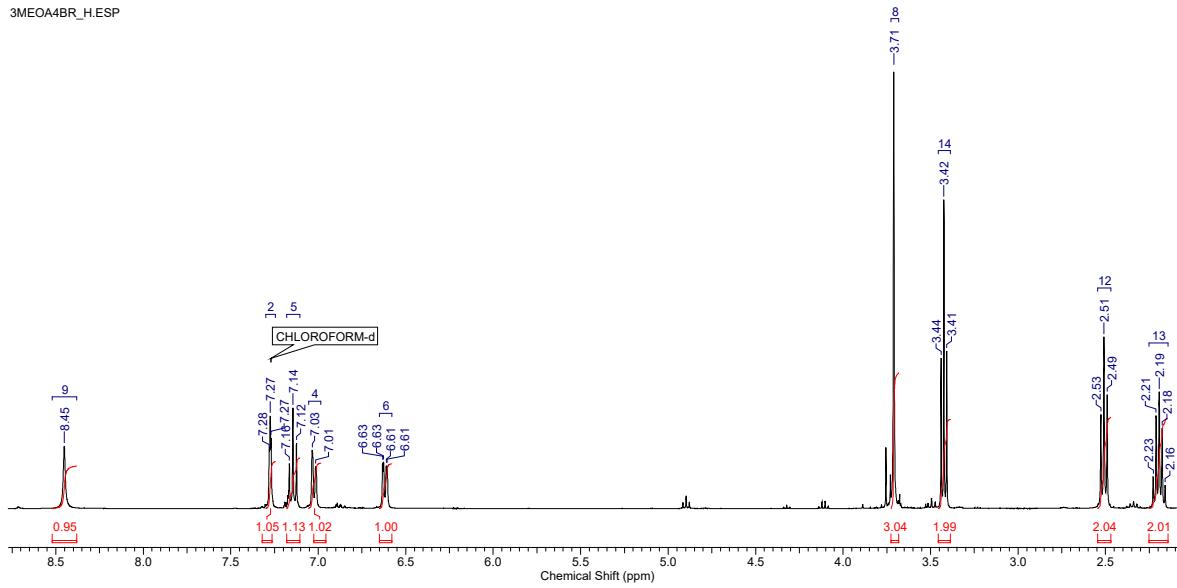
5.23 1-Cyclopropyl-6-fluoro-7-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



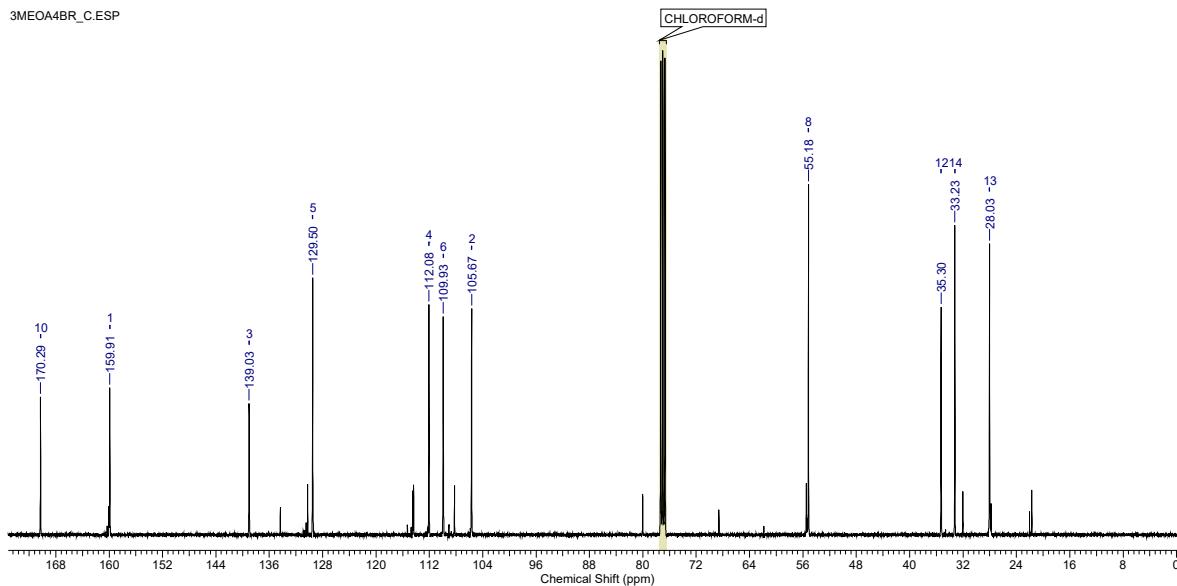
5.24 4-Bromo-N-(3-methoxyphenyl)butanamide ?



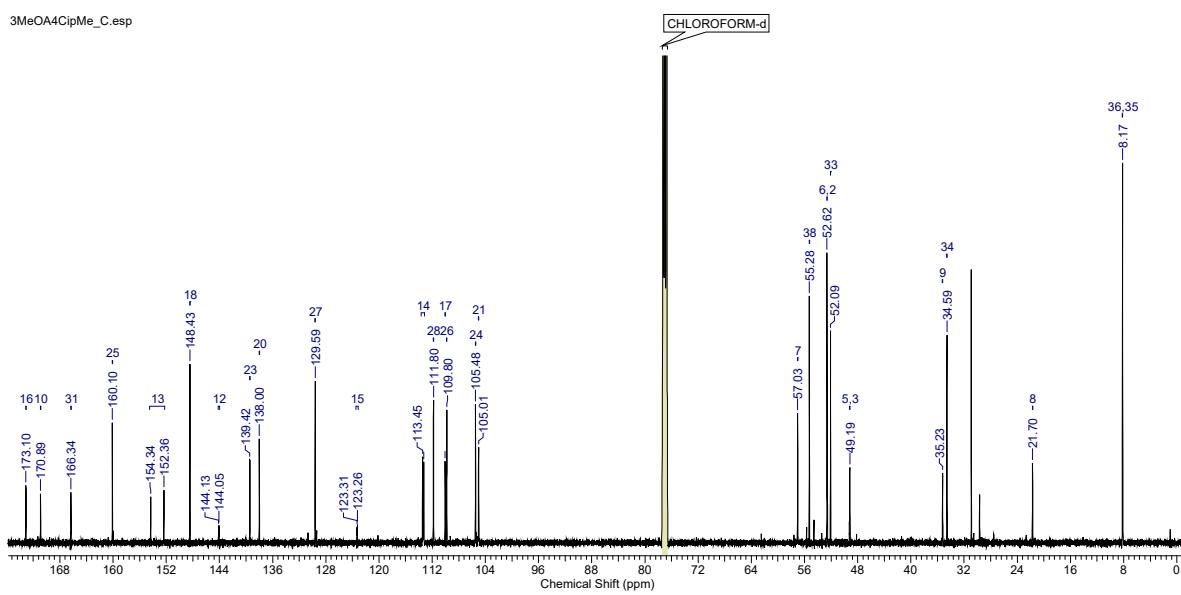
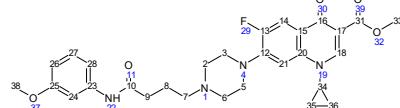
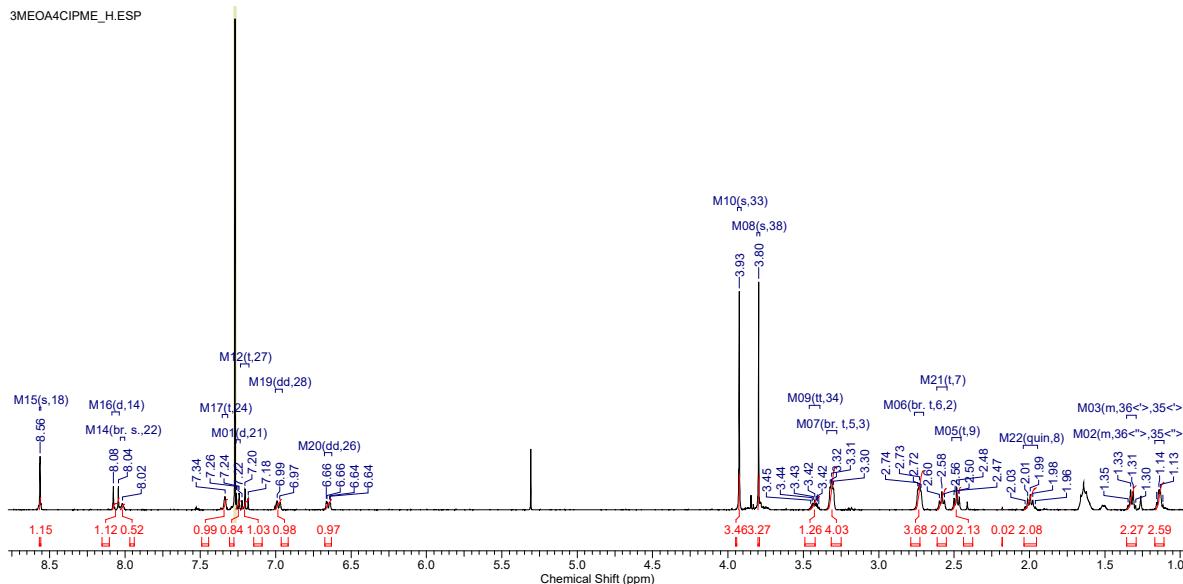
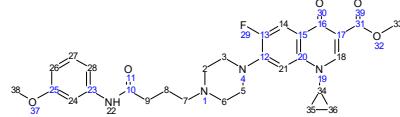
3MEOA4BR\_H.ESP



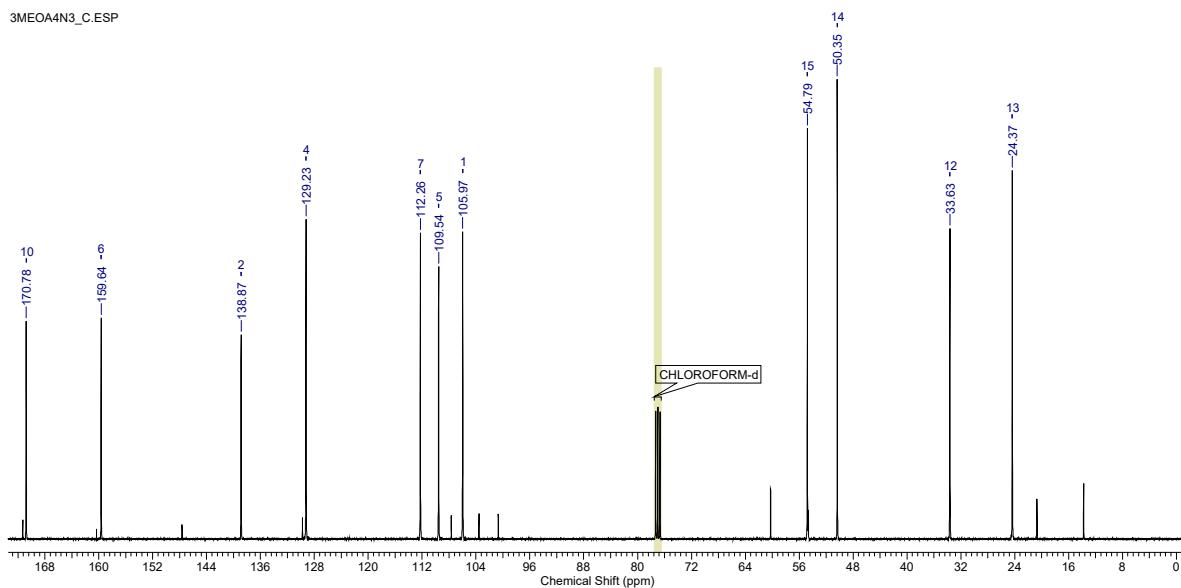
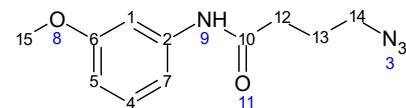
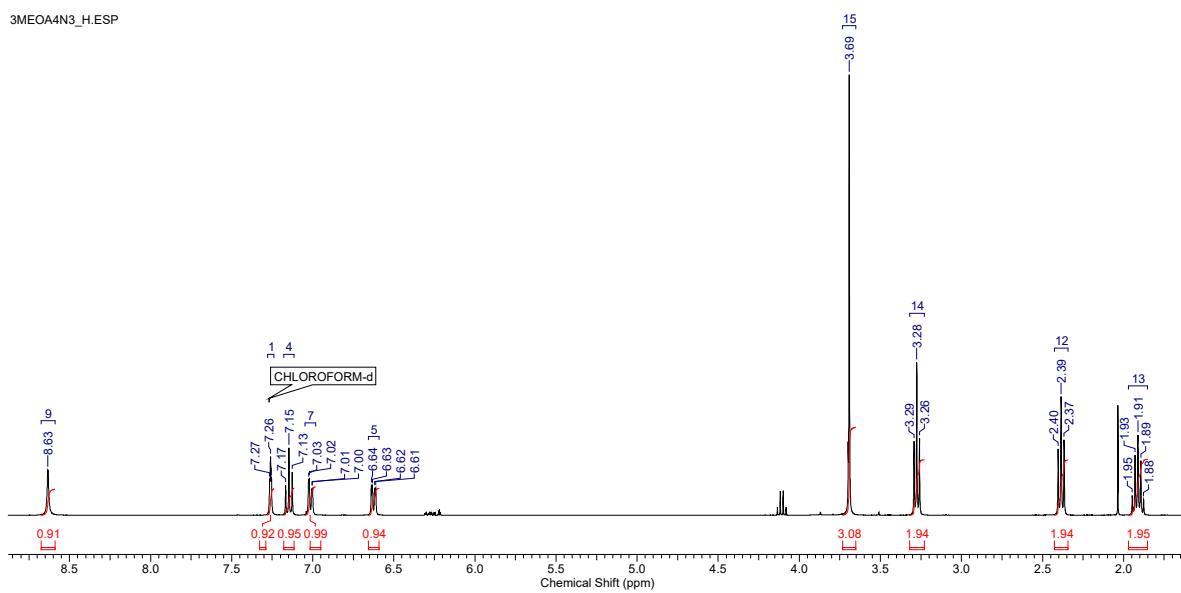
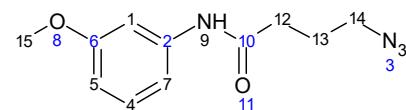
3MEOA4BR\_C.ESP



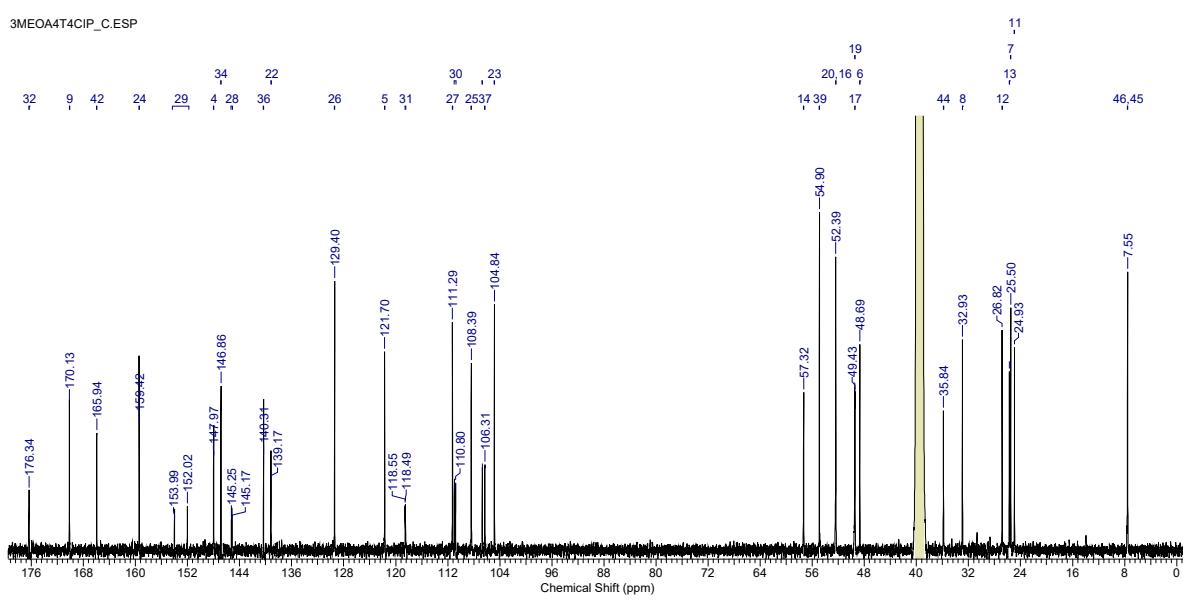
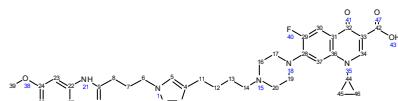
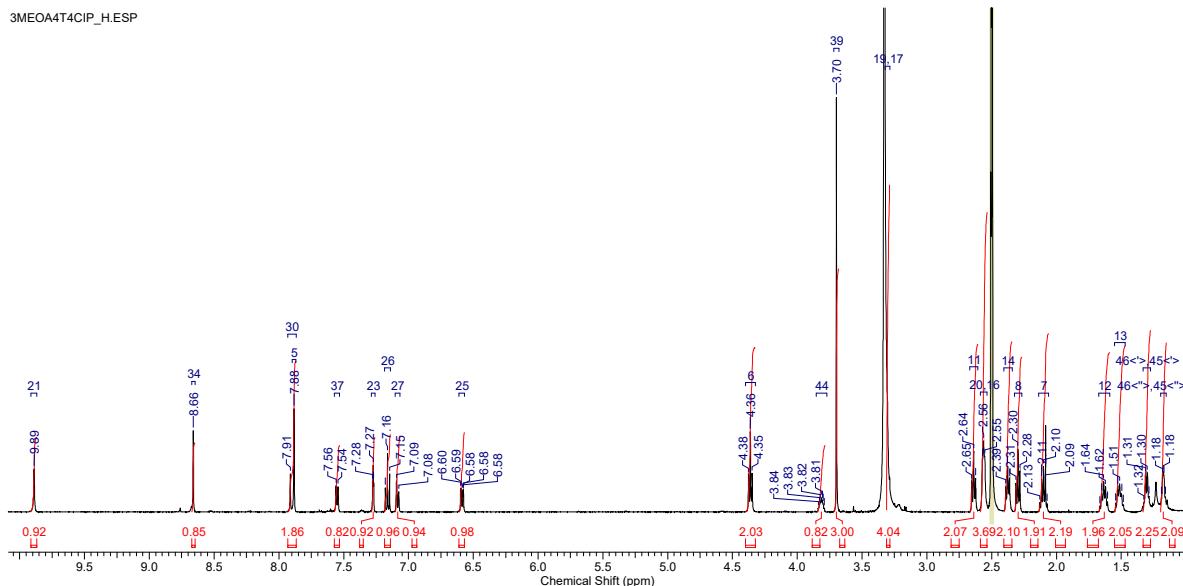
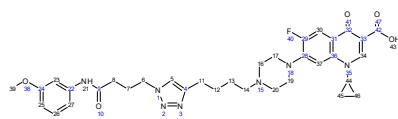
5.25 Methyl 1-cyclopropyl-6-fluoro-7-(4-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



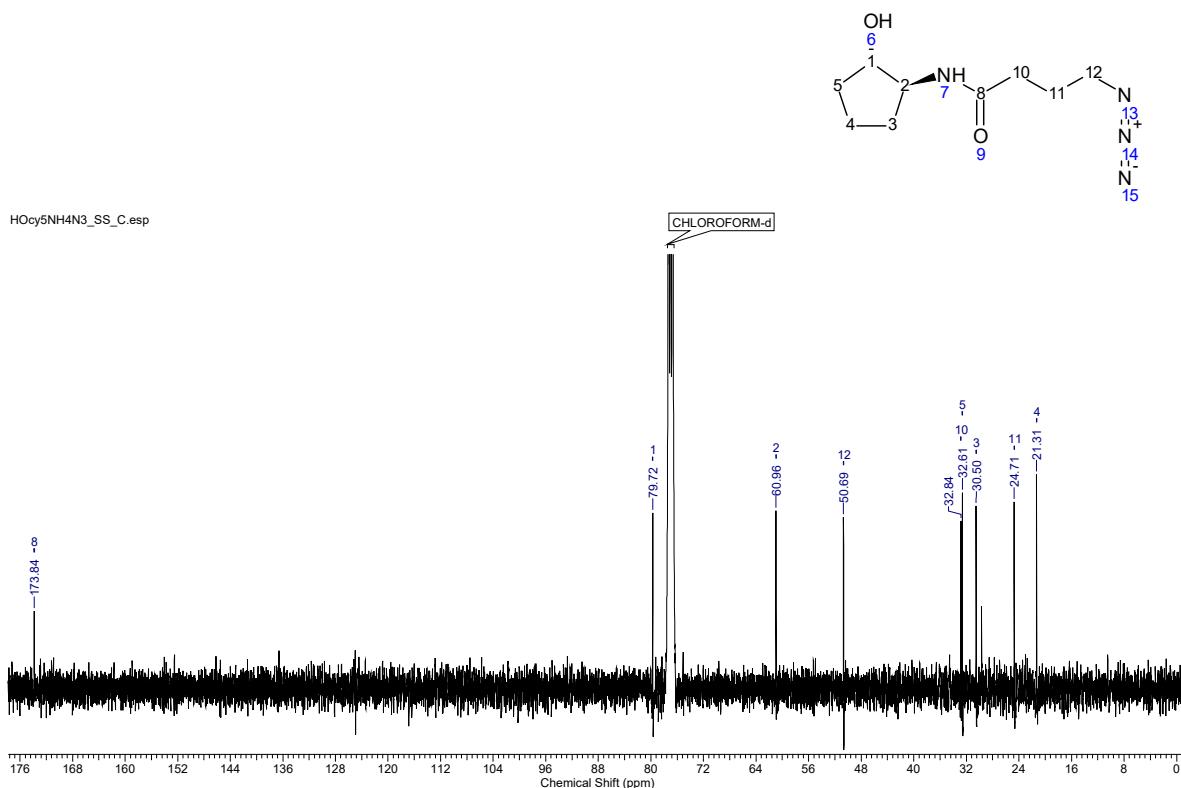
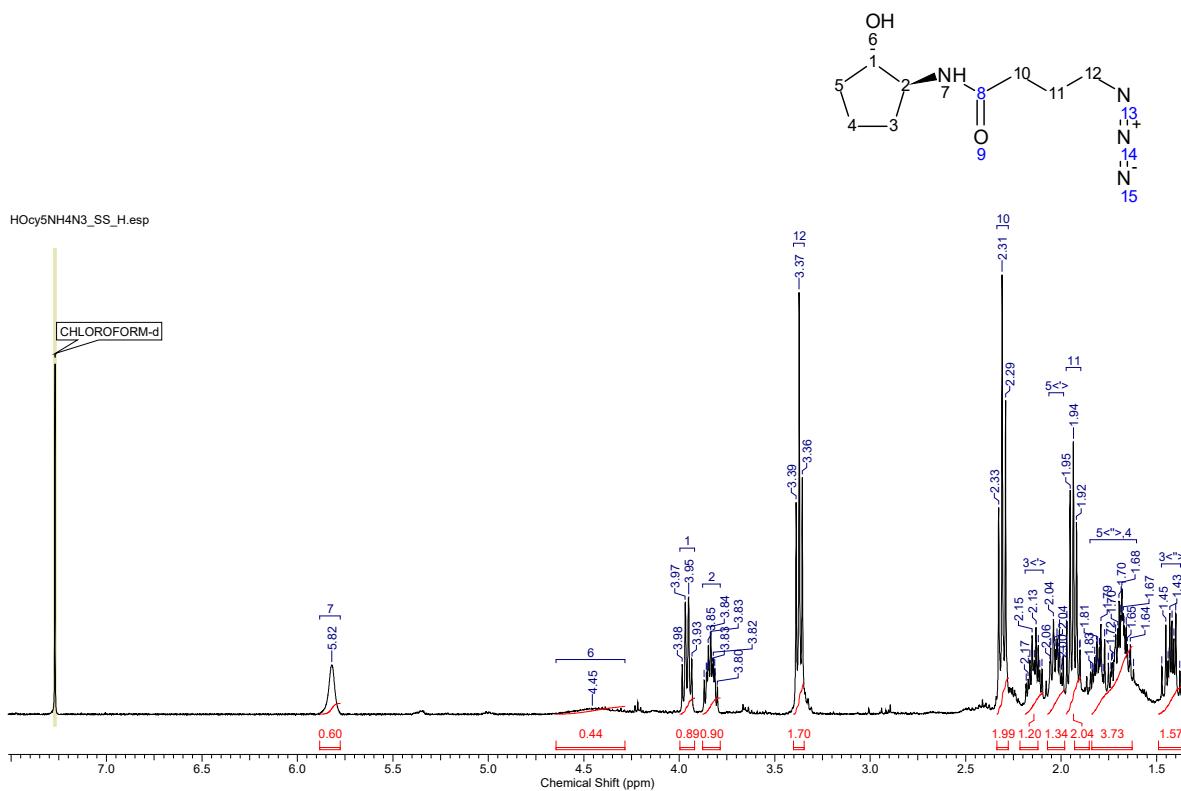
### 5.26 4-Azido-*N*-(3-methoxyphenyl)butanamide ?



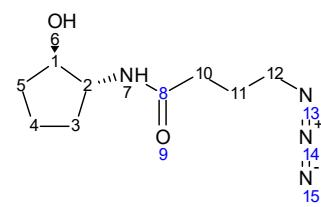
5.27 1-Cyclopropyl-6-fluoro-7-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



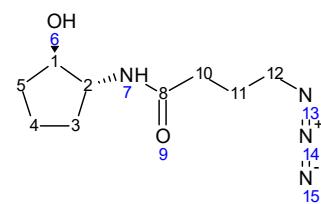
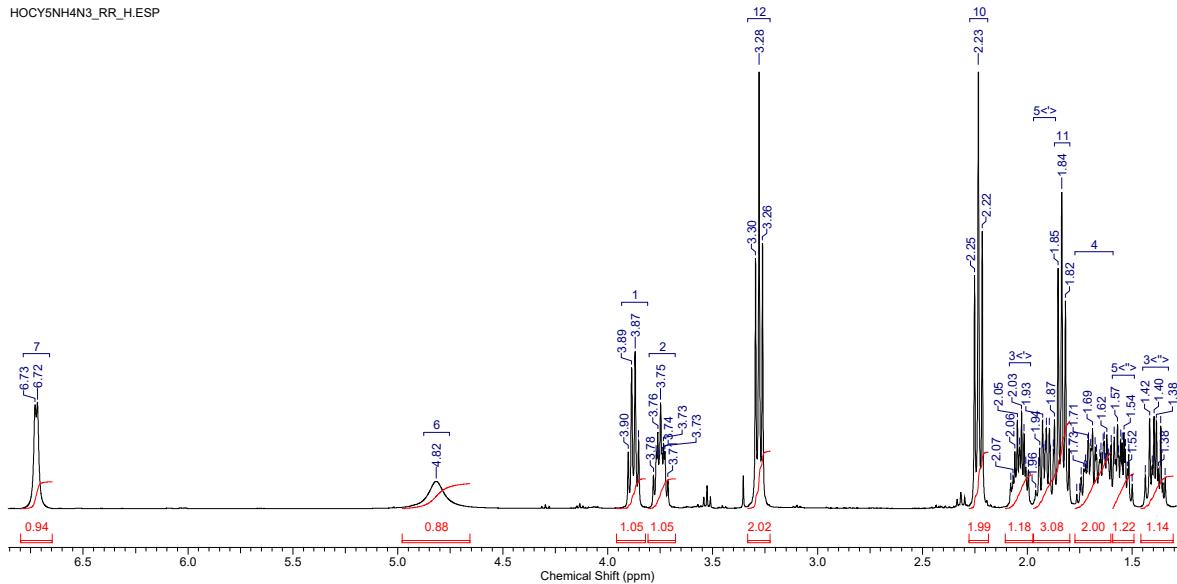
5.28 4-Azido-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide ?



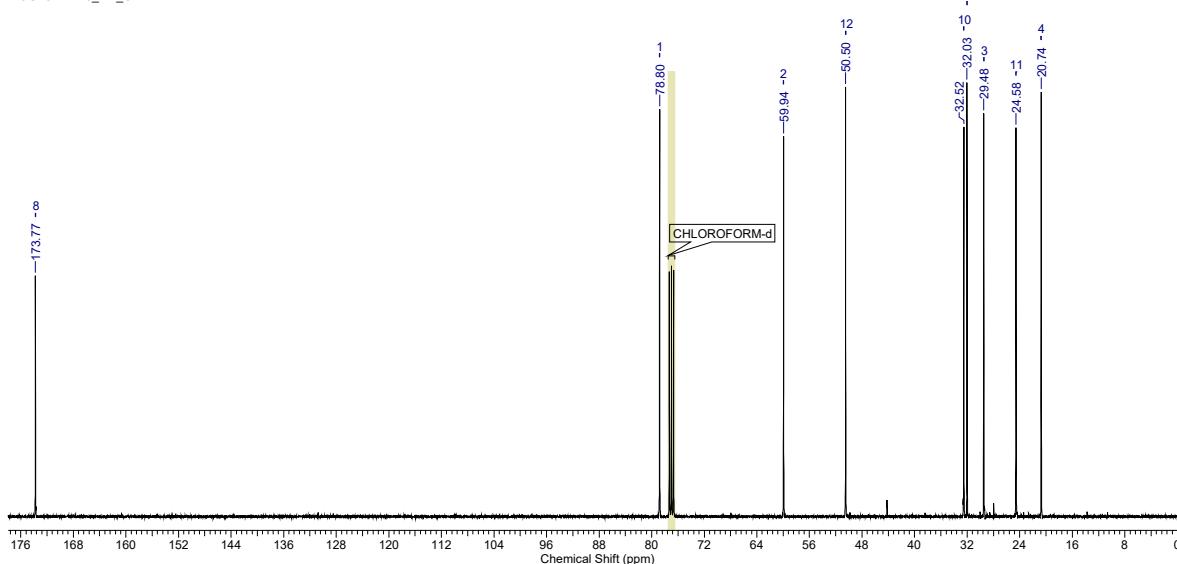
### 5.29 4-Azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide ?



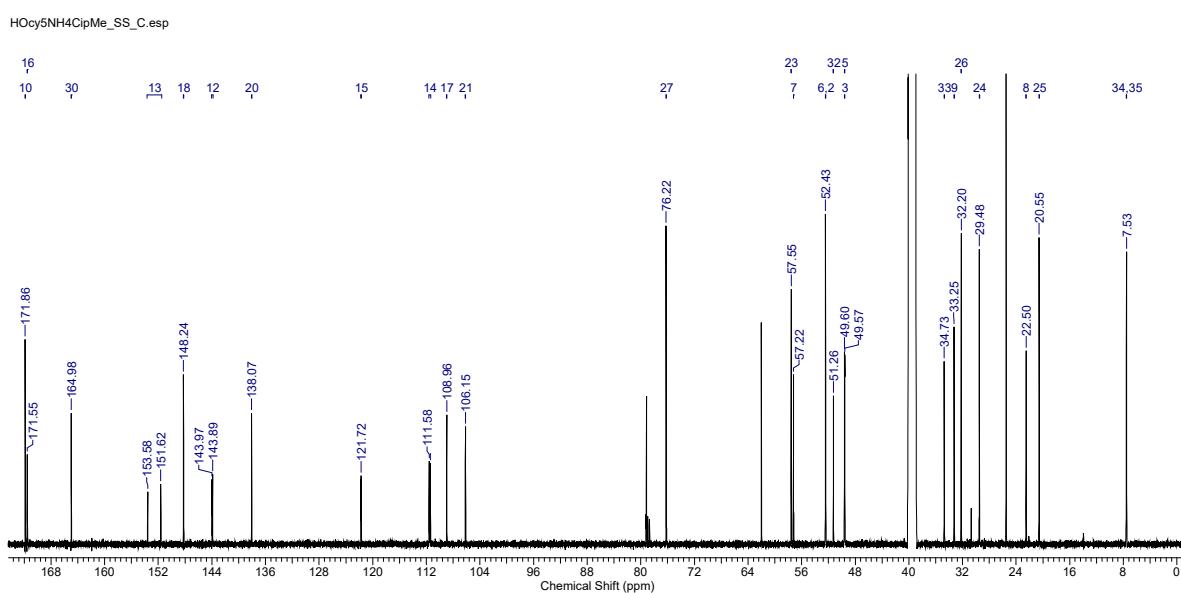
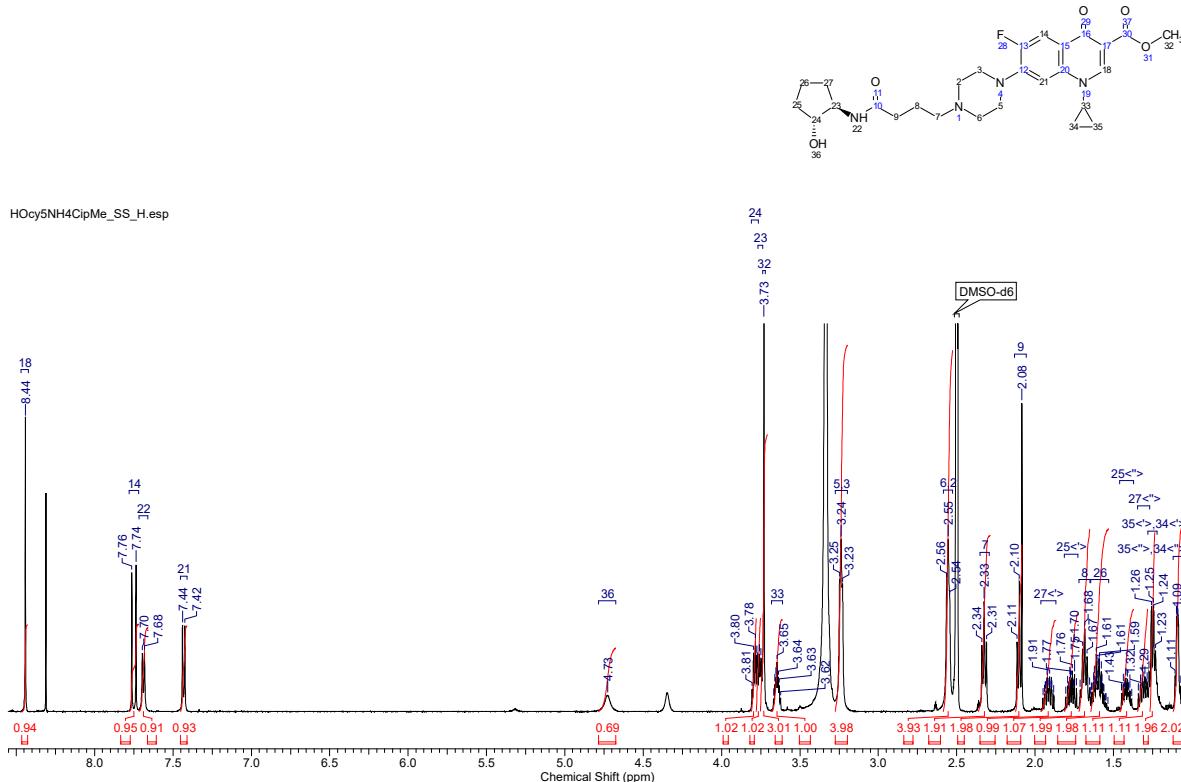
HOCH5NH4N3\_RR\_H.ESP



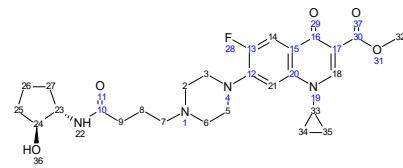
HQCY5NH4N3 RR C ESP



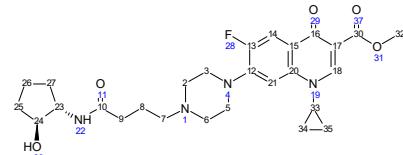
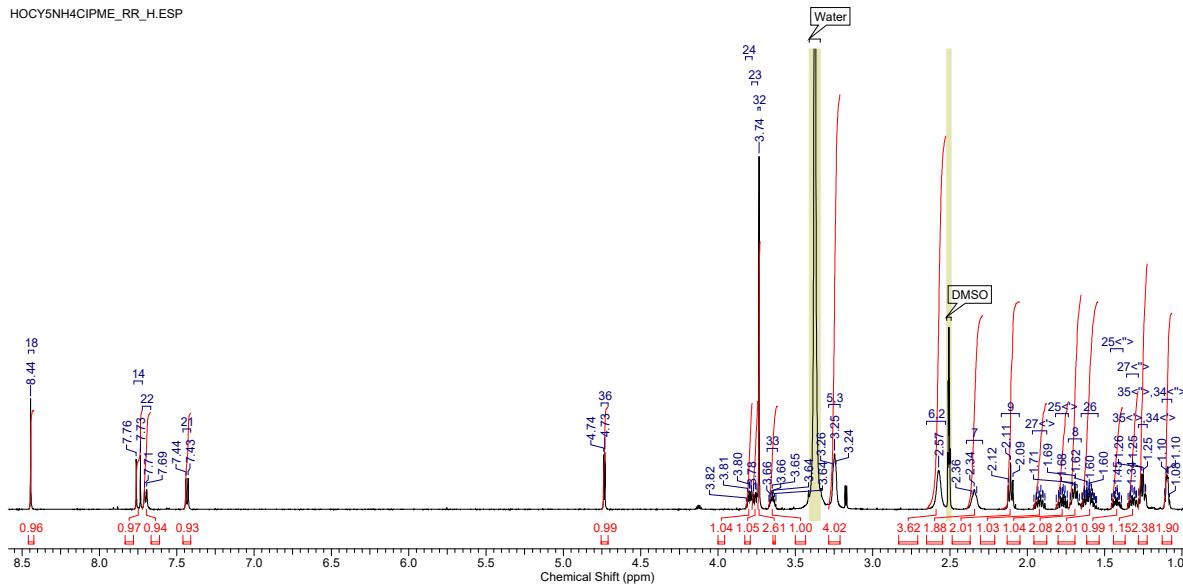
5.30 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



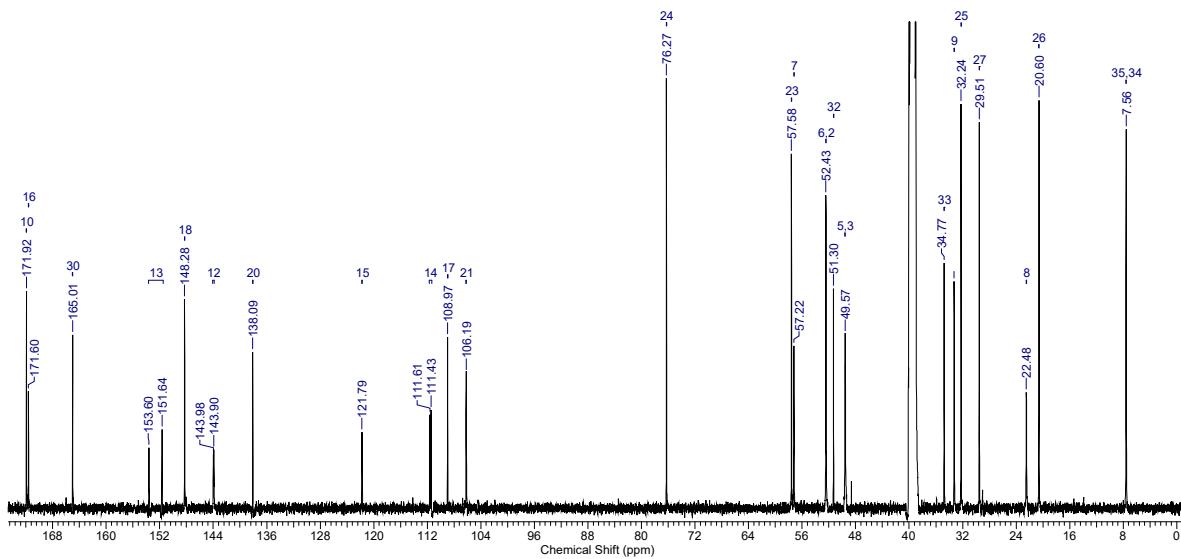
5.31 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



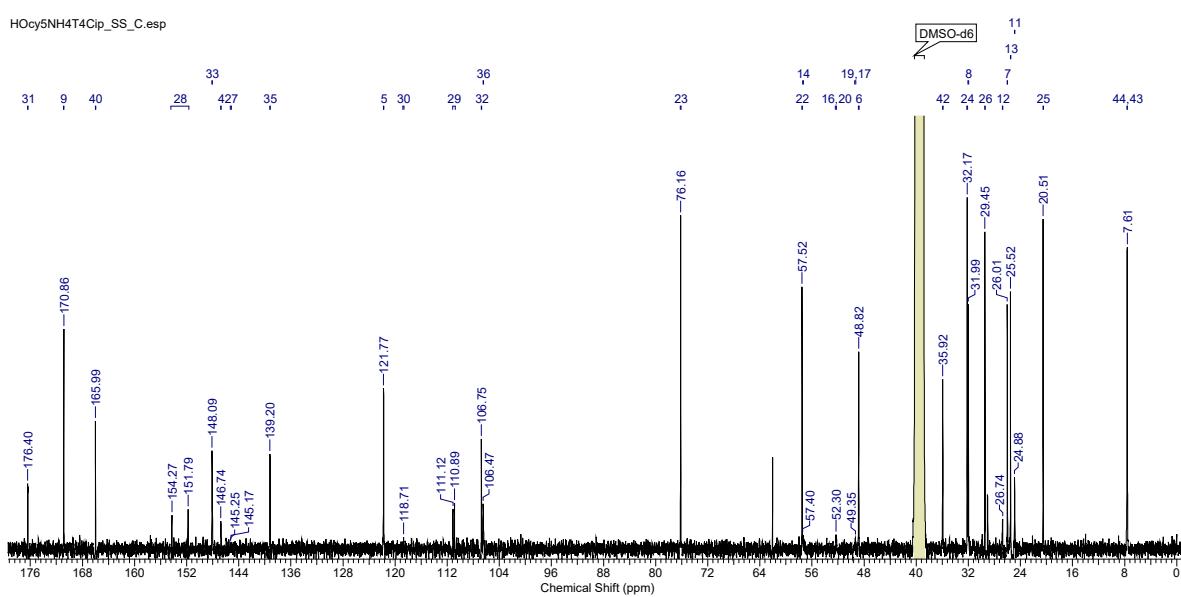
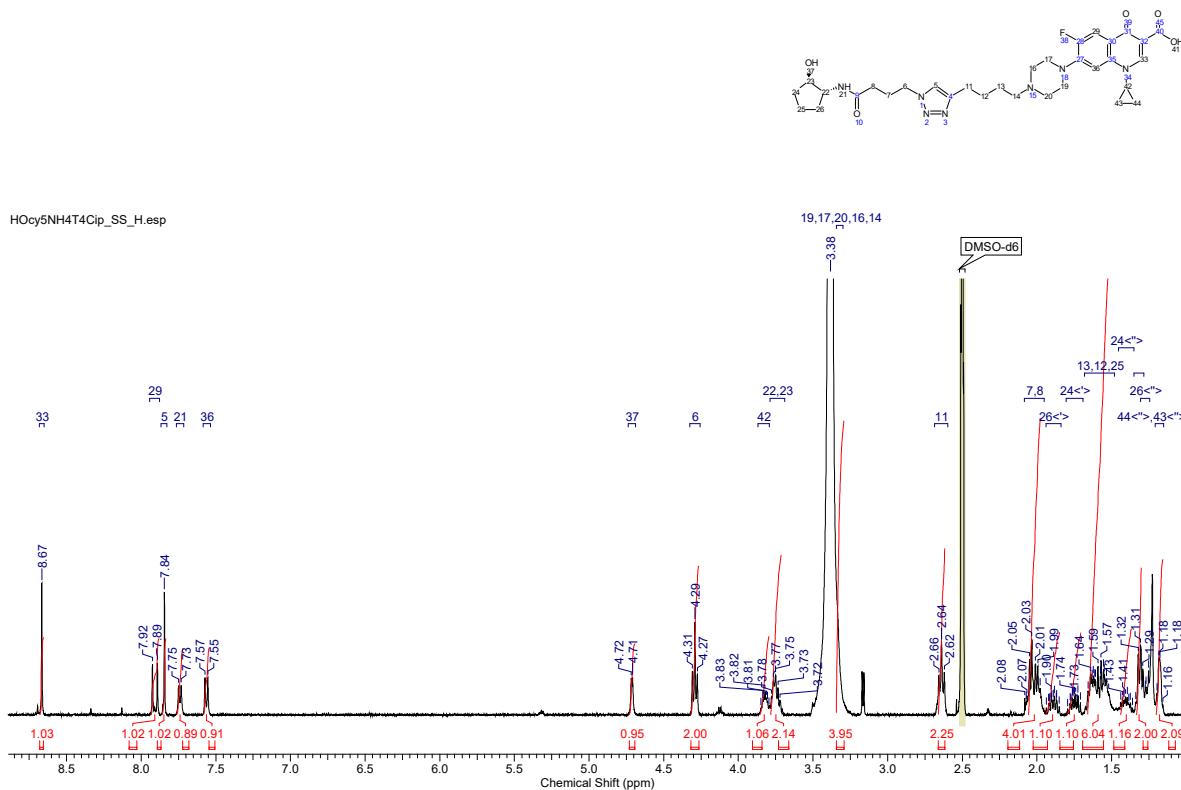
HO CY5 NH4 CIP ME\_RR\_H.ESP



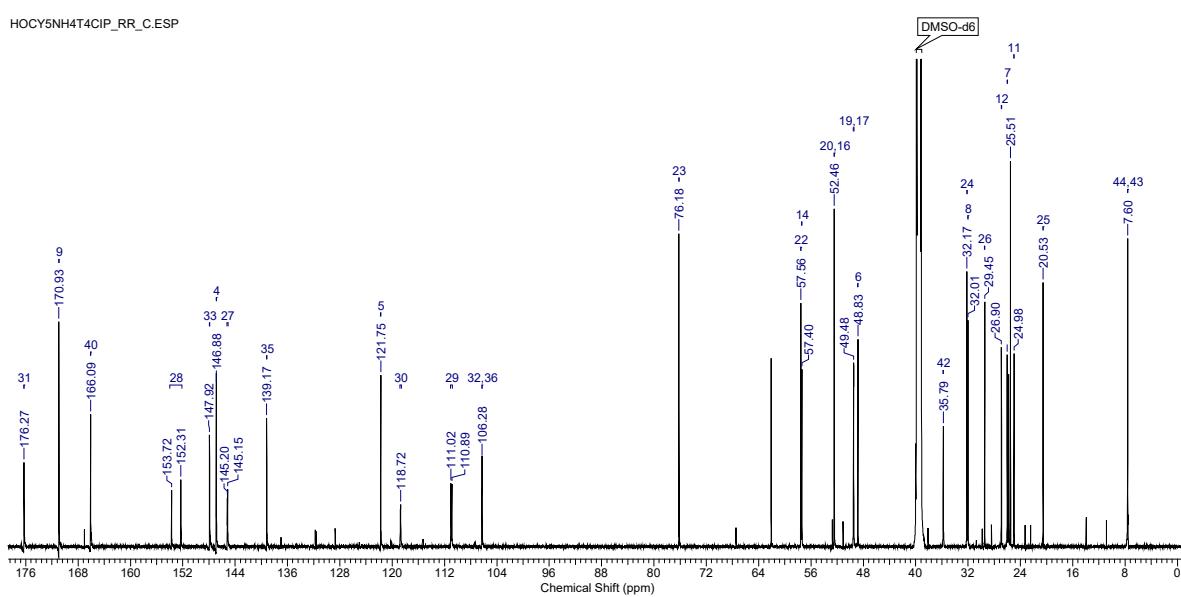
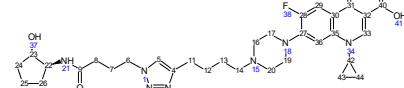
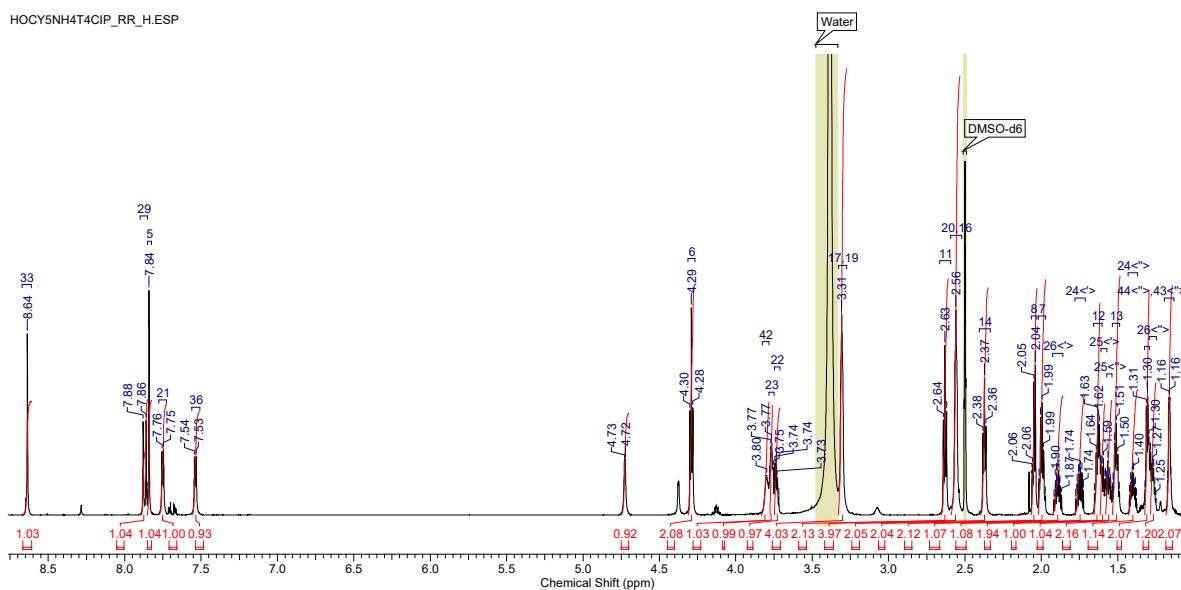
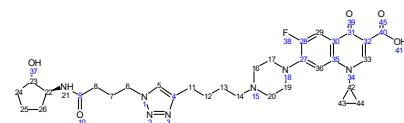
HOcy5NH4CipMe RR C.esp



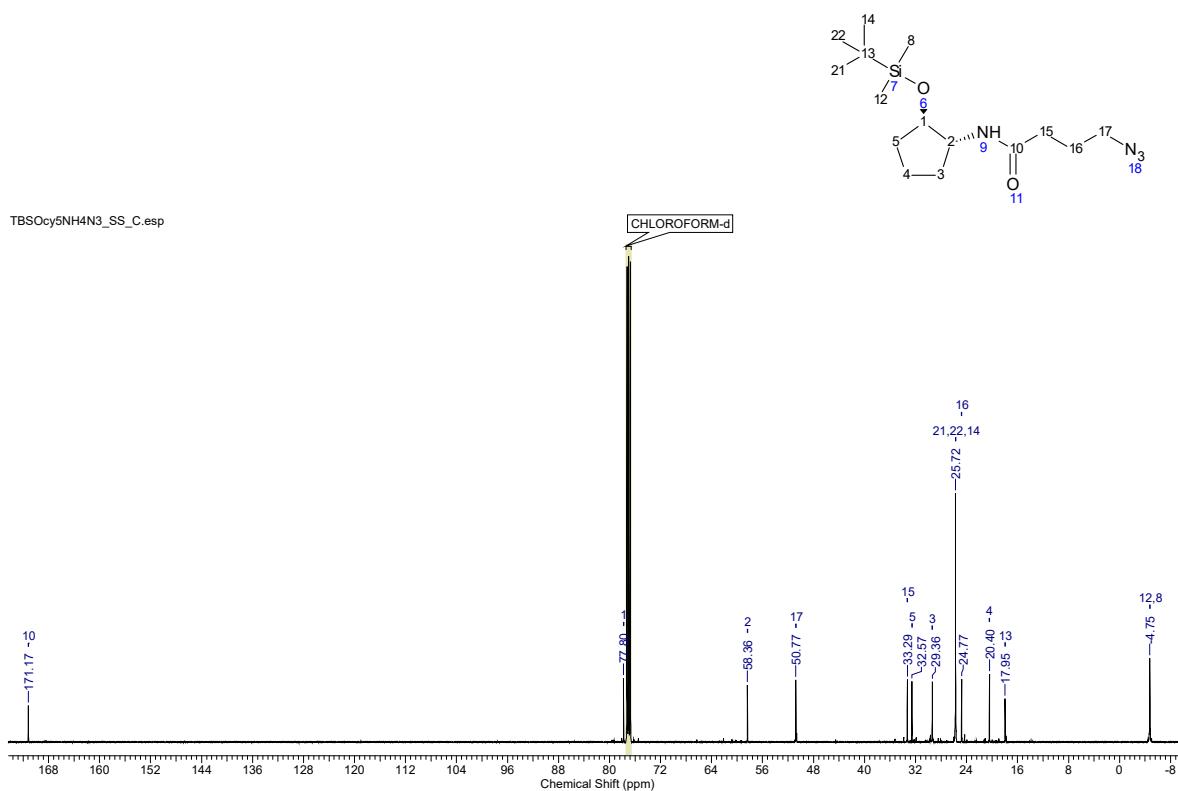
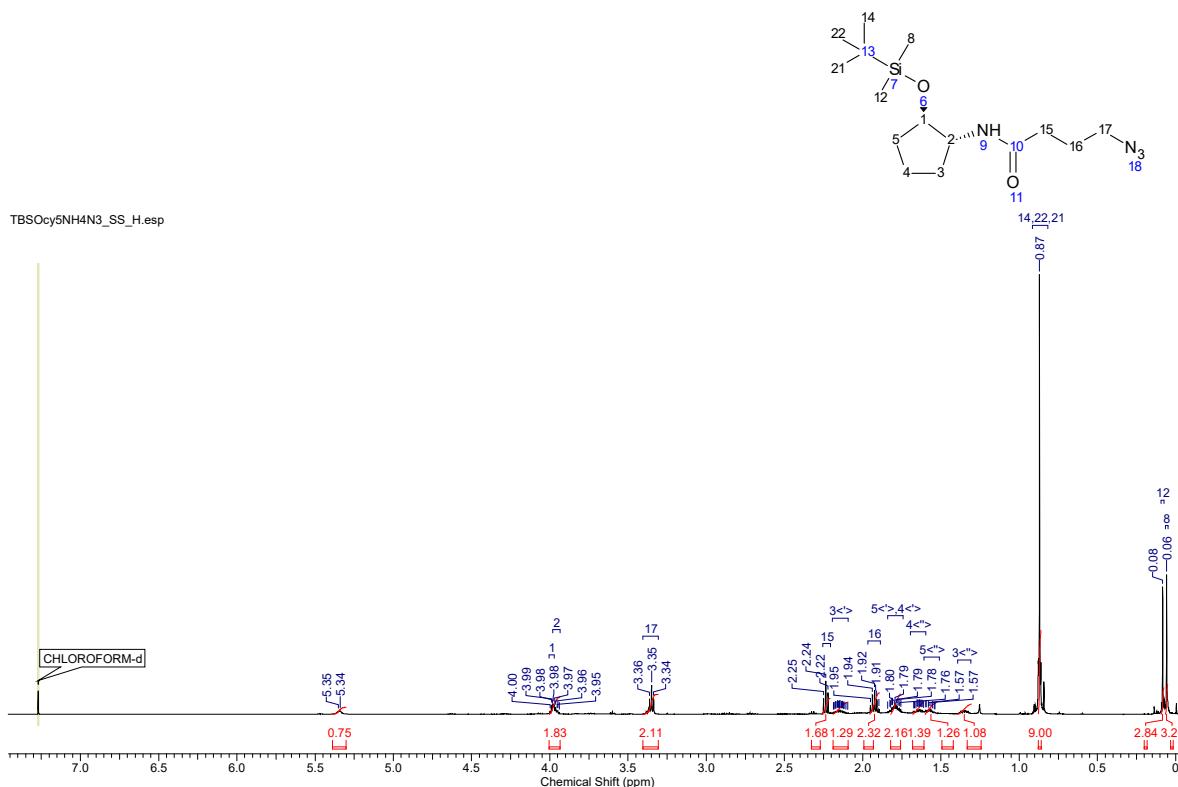
5.32 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1S,2S)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquino-line-3-carboxylic acid ?



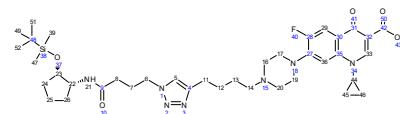
5.33 1-Cyclopropyl-6-fluoro-7-(4-(1-(4-(((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquino-line-3-carboxylic acid ?



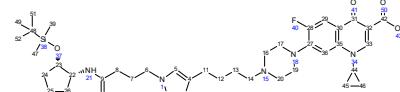
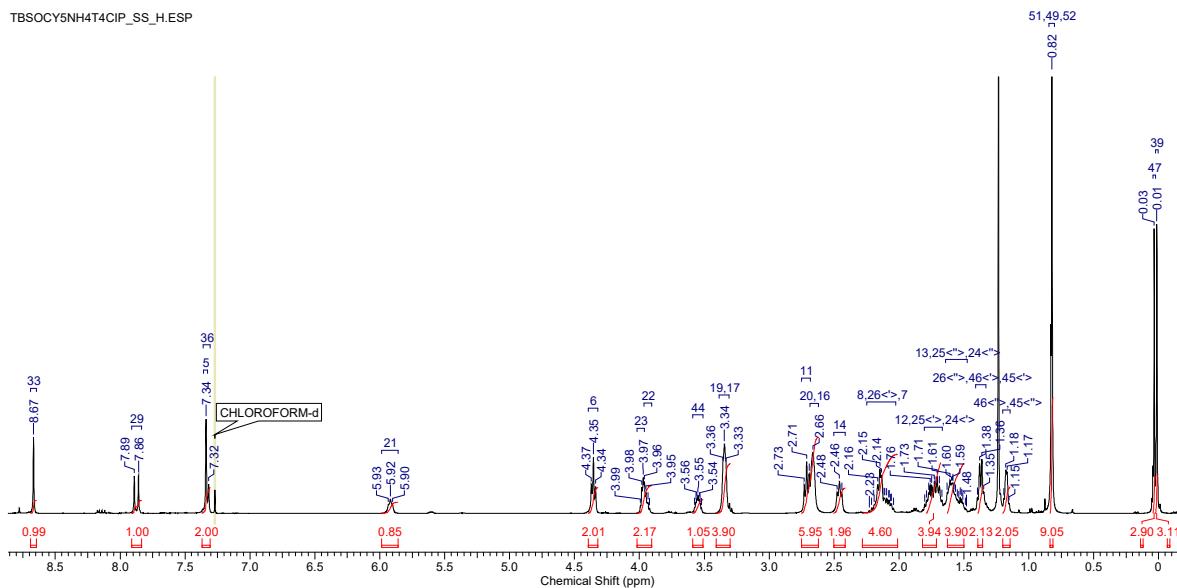
5.34 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide  
?



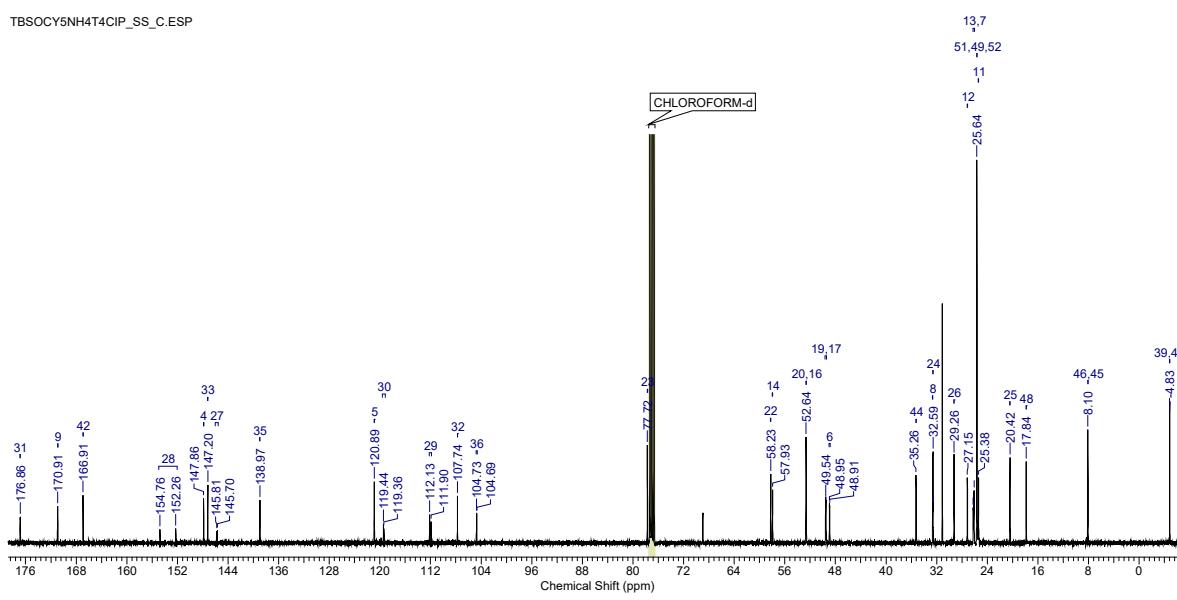
5.35 7-(4-(4-(1-(4-(((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ?



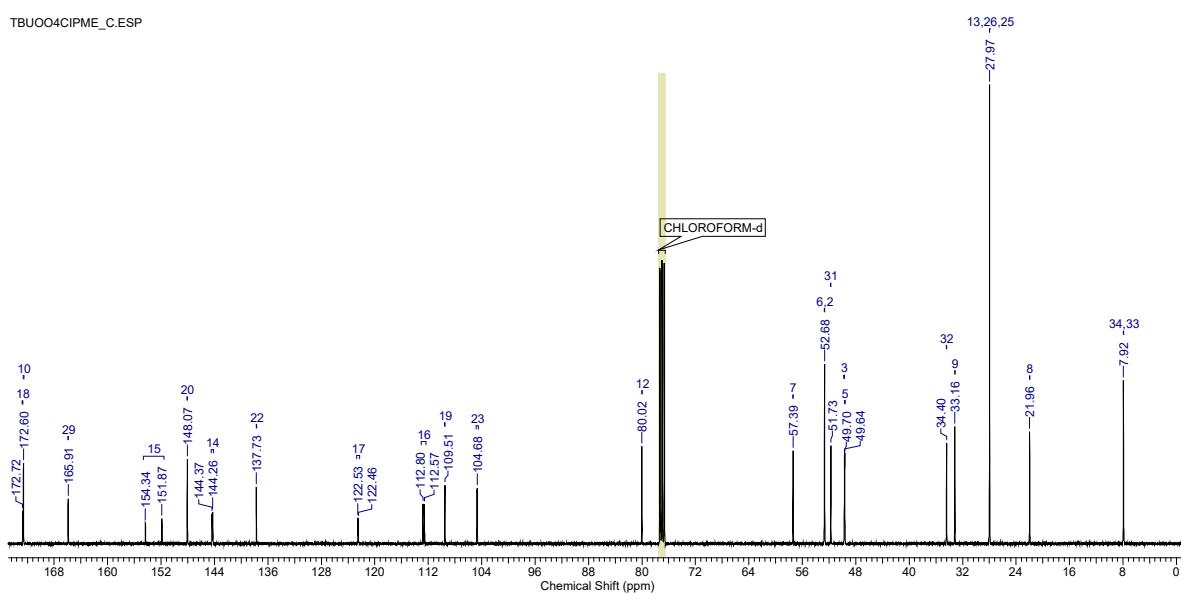
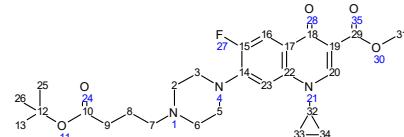
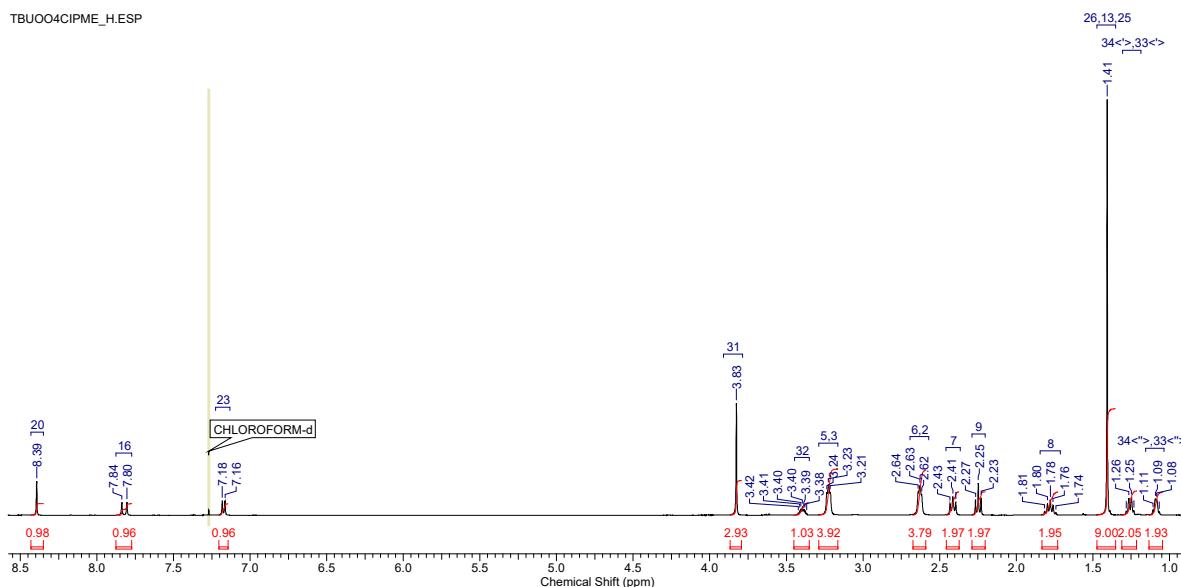
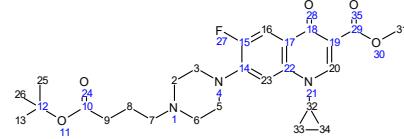
TBSOCY5NH4T4CIP\_SS\_H.ESP



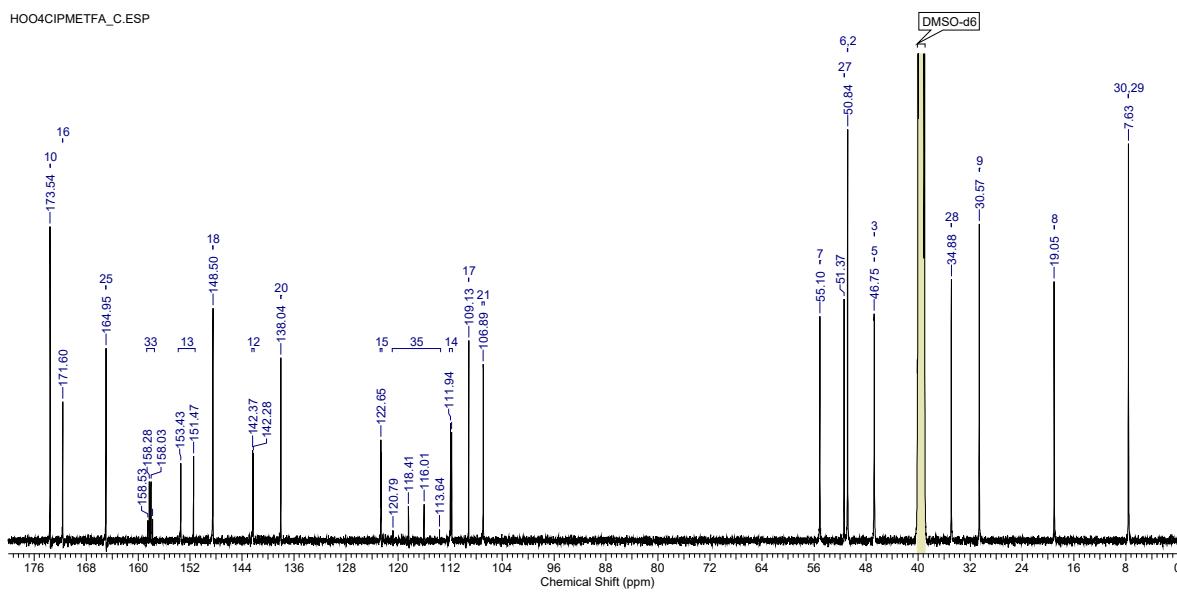
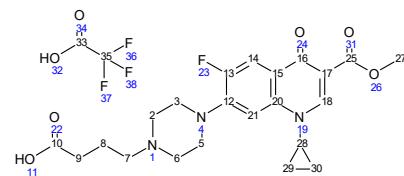
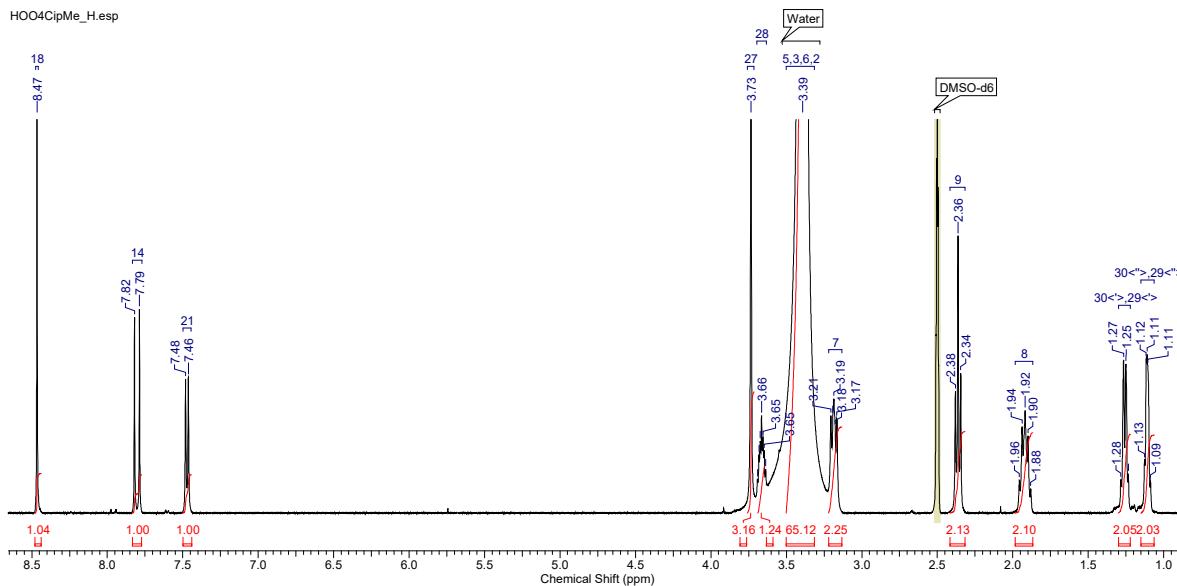
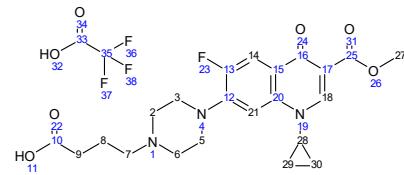
TBSOCY5NH4T4CIP SS C.ESP



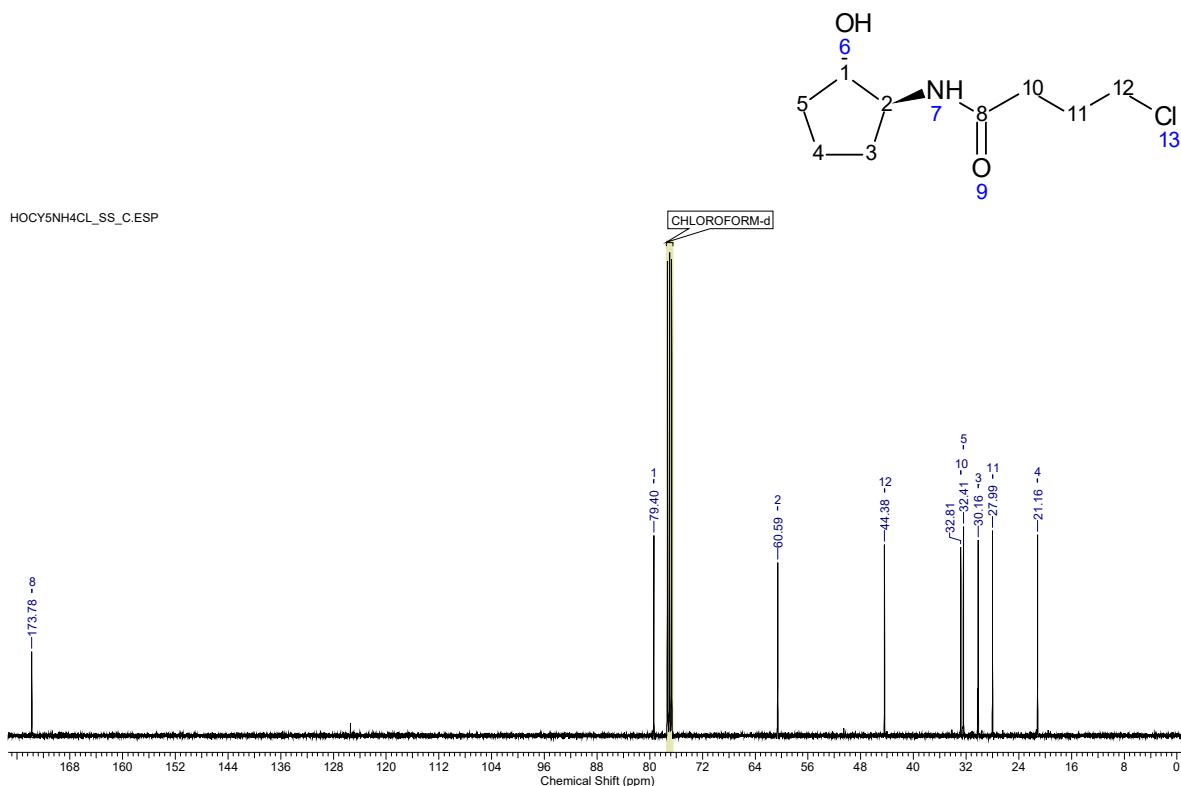
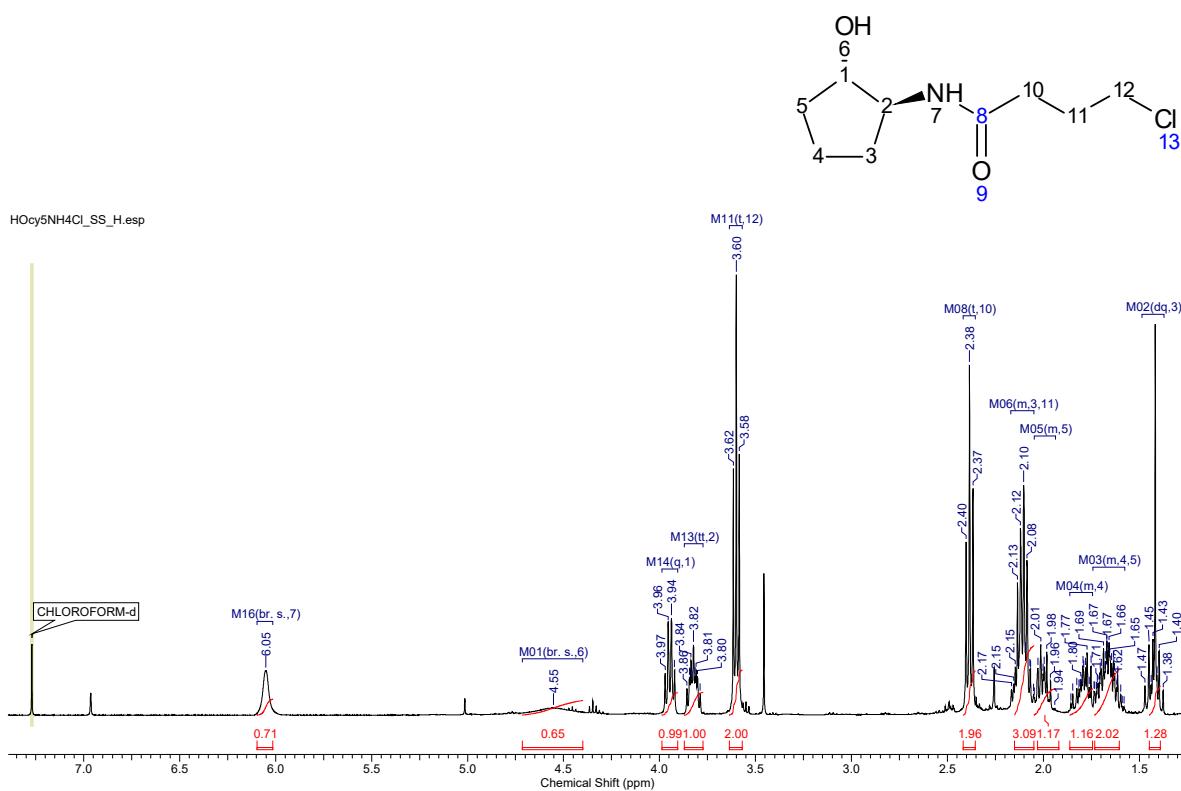
5.36 Methyl 7-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



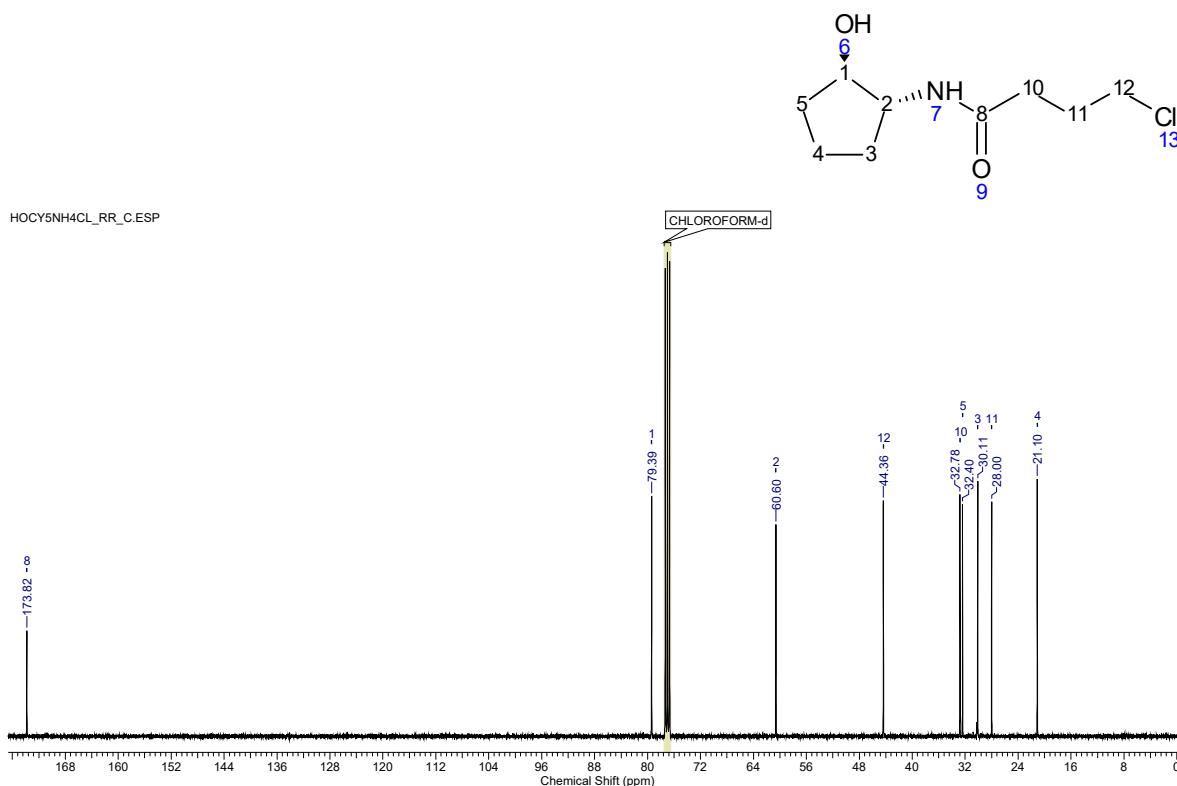
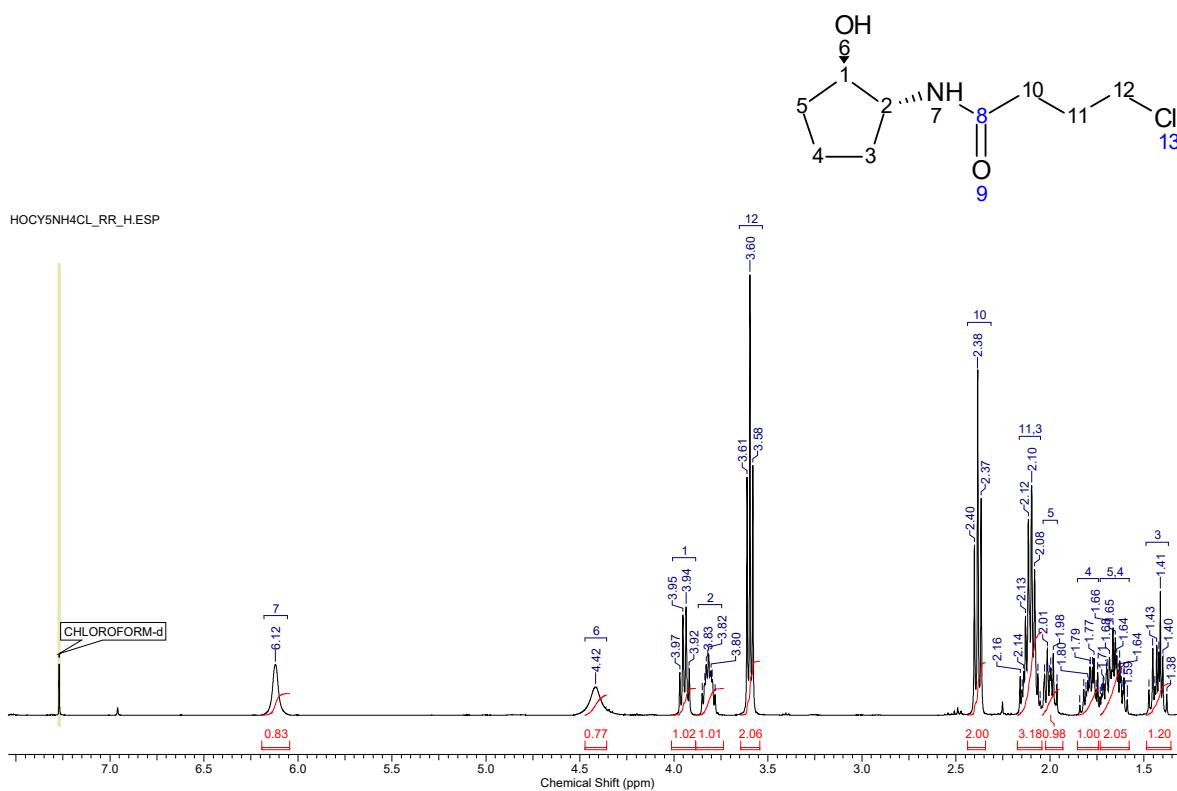
5.37 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt ?



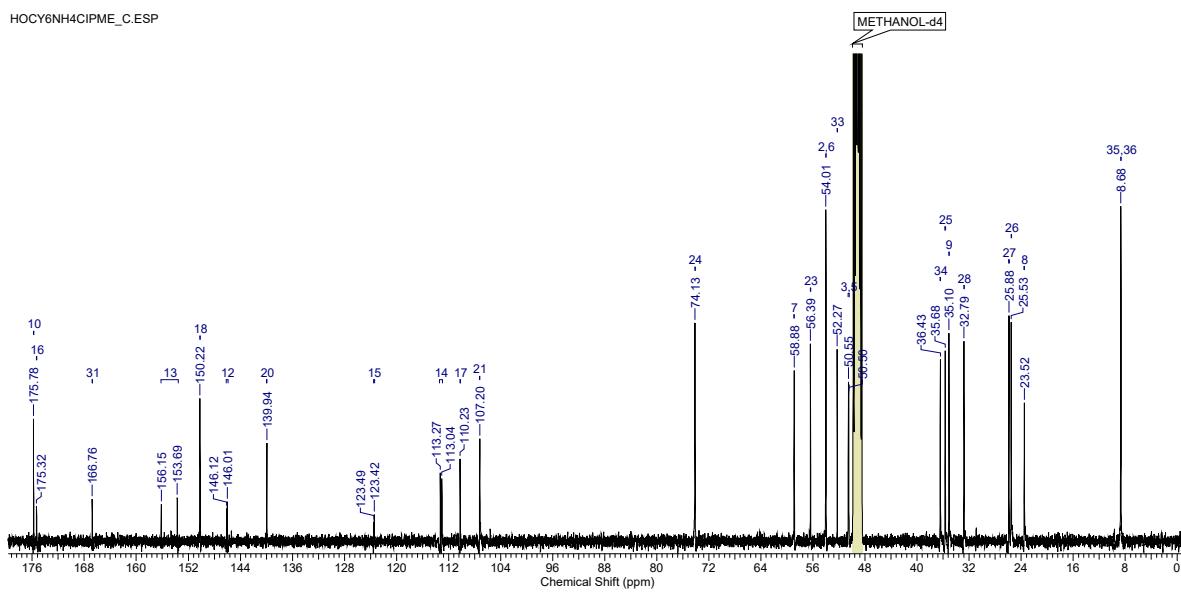
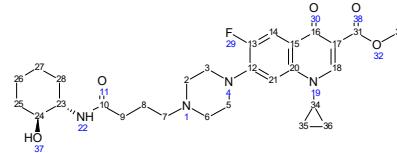
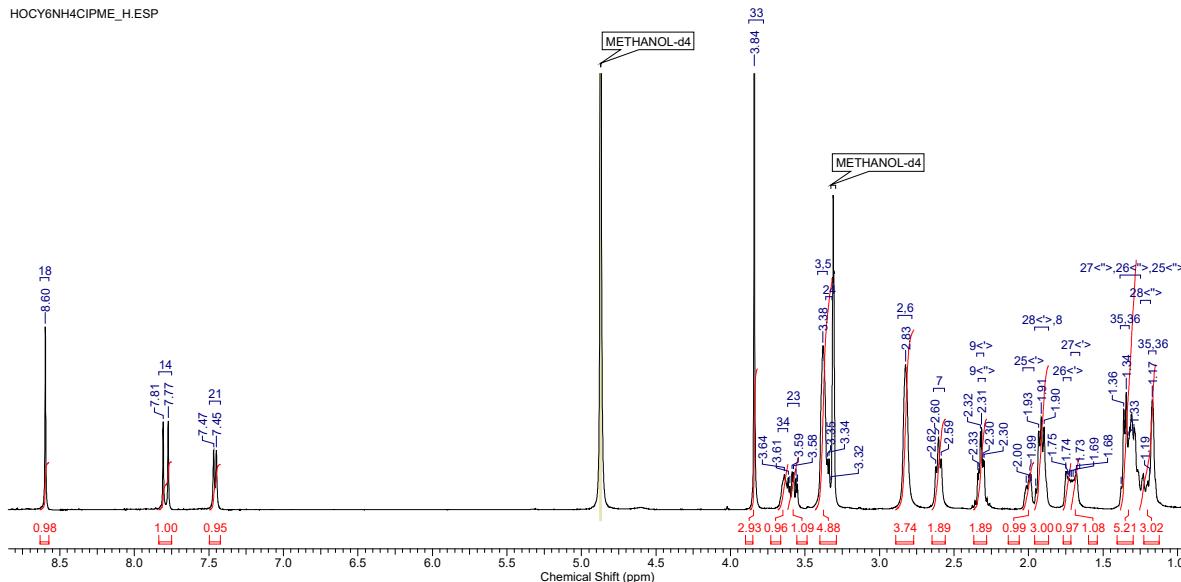
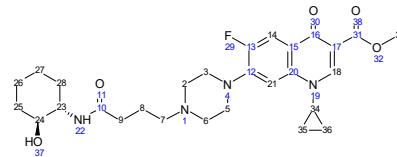
5.38 4-Chloro-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide ?



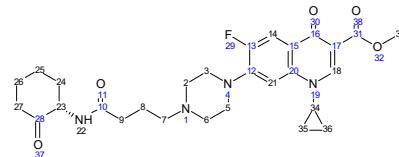
5.39 4-Chloro-*N*-(*1R,2R*)-2-hydroxycyclopentylbutanamide ?



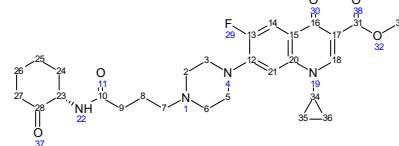
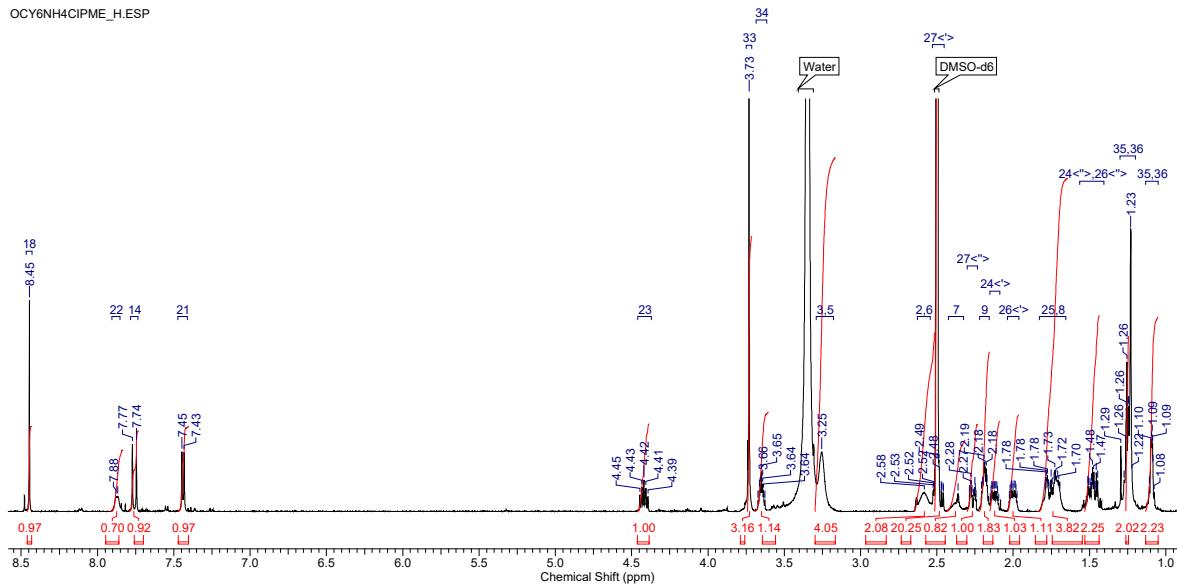
5.40 Methyl 1-cyclopropyl-6-fluoro-7-(4-((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ?



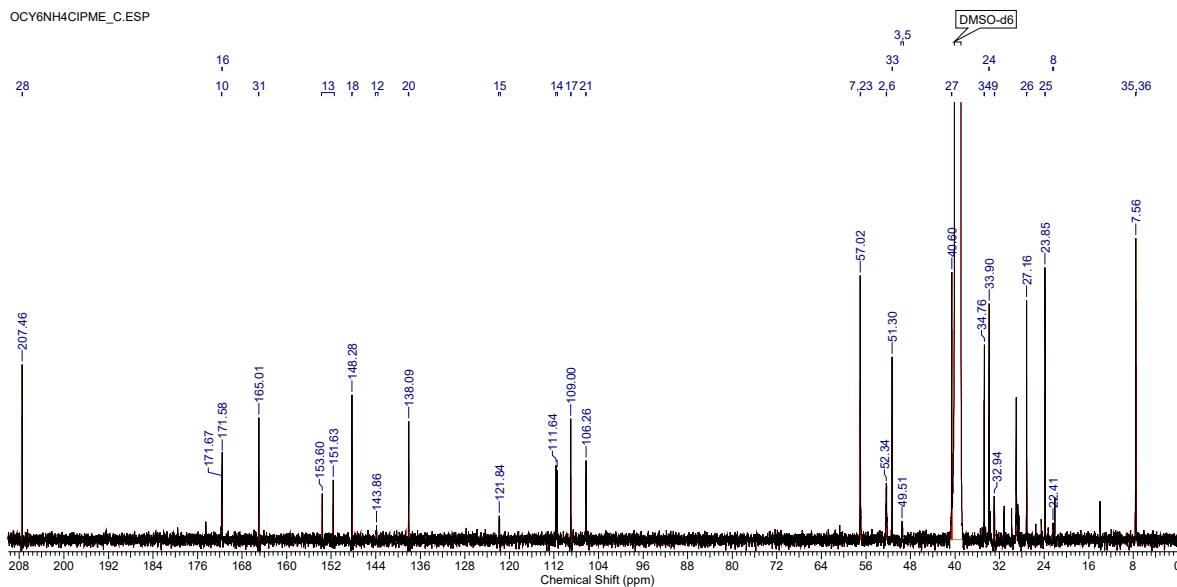
5.41 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate ?



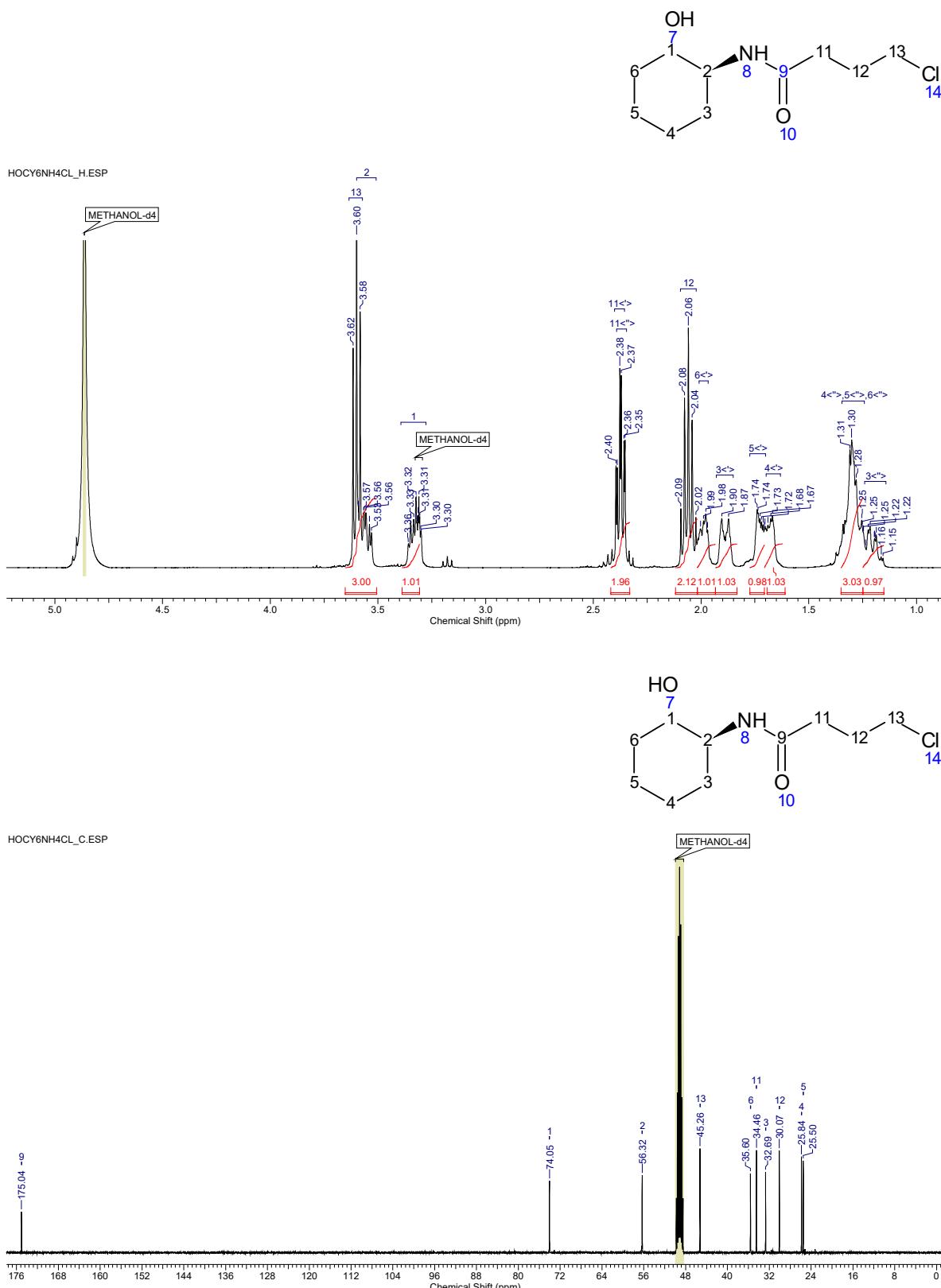
OCY6NH4CIPME\_H.ESP



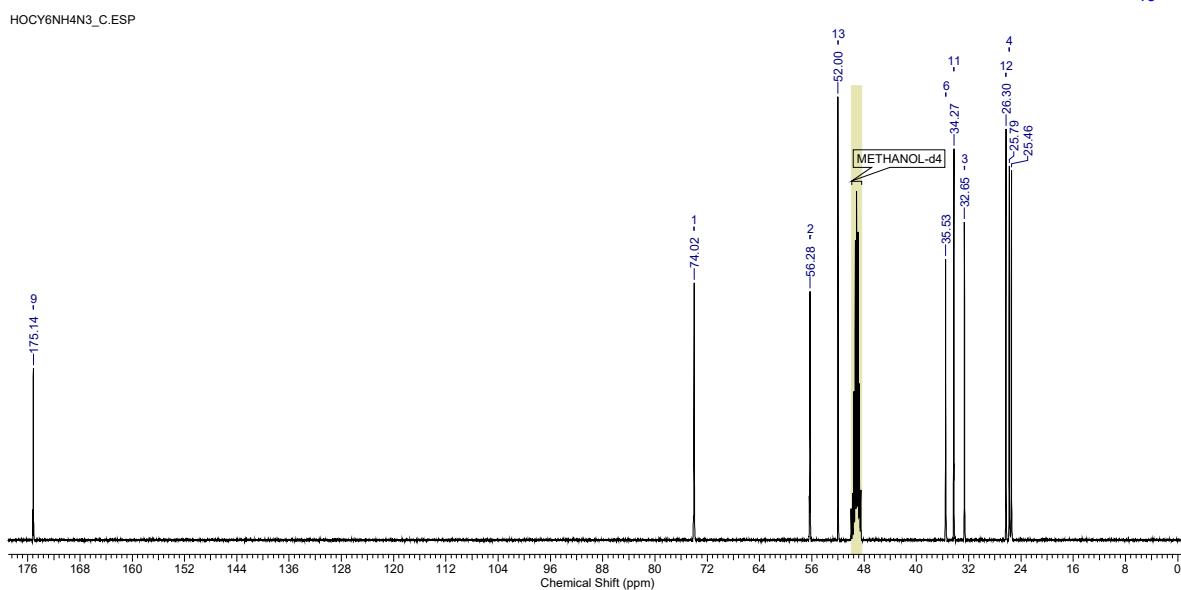
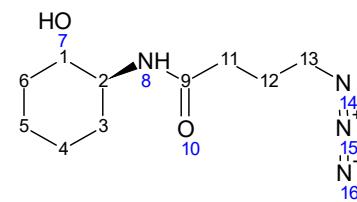
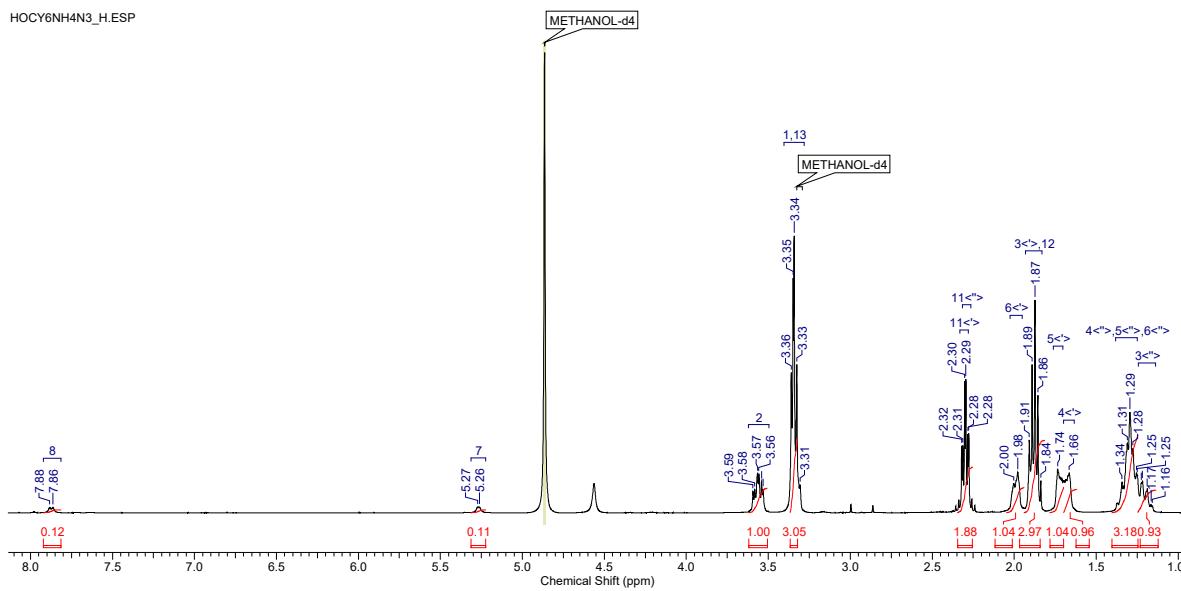
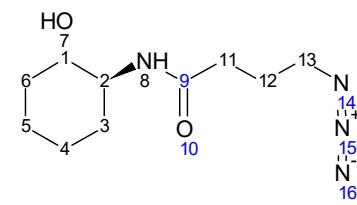
OCY6NH4CIPME\_C.ESP



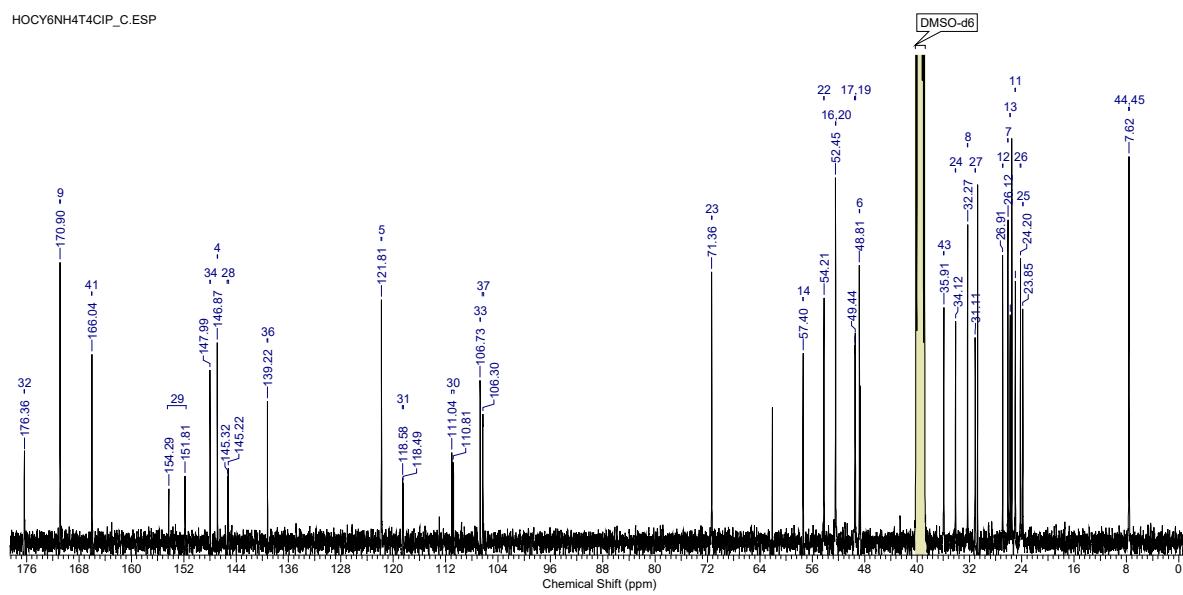
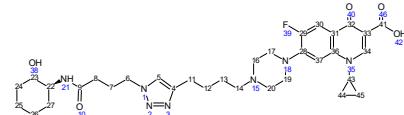
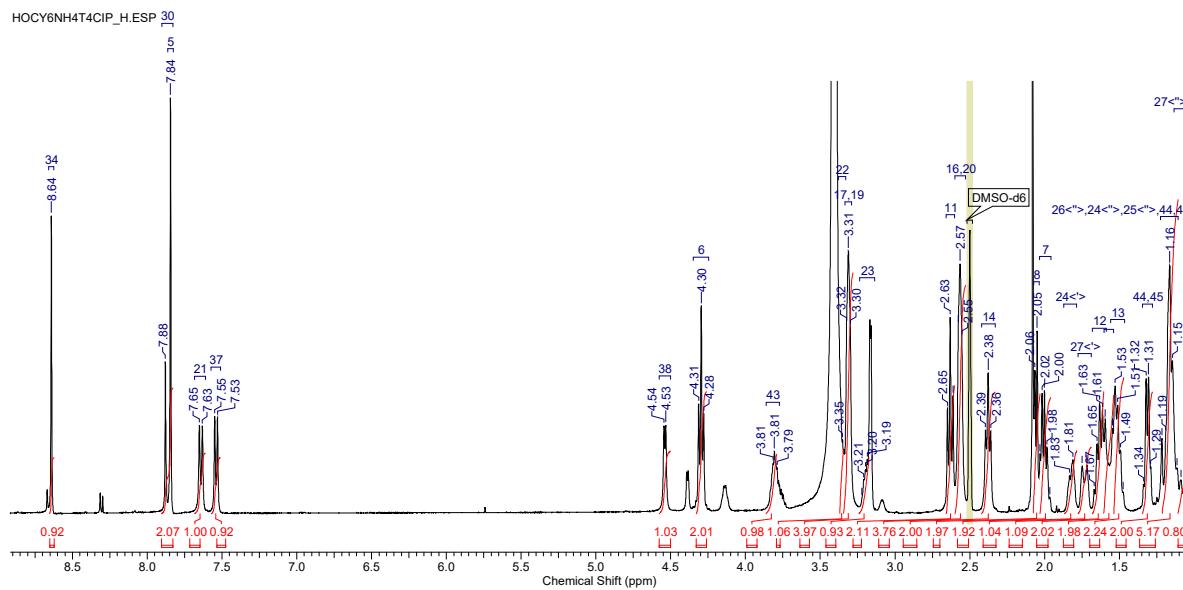
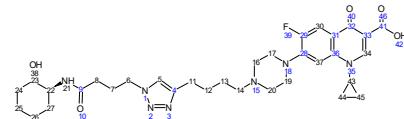
5.42 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ?



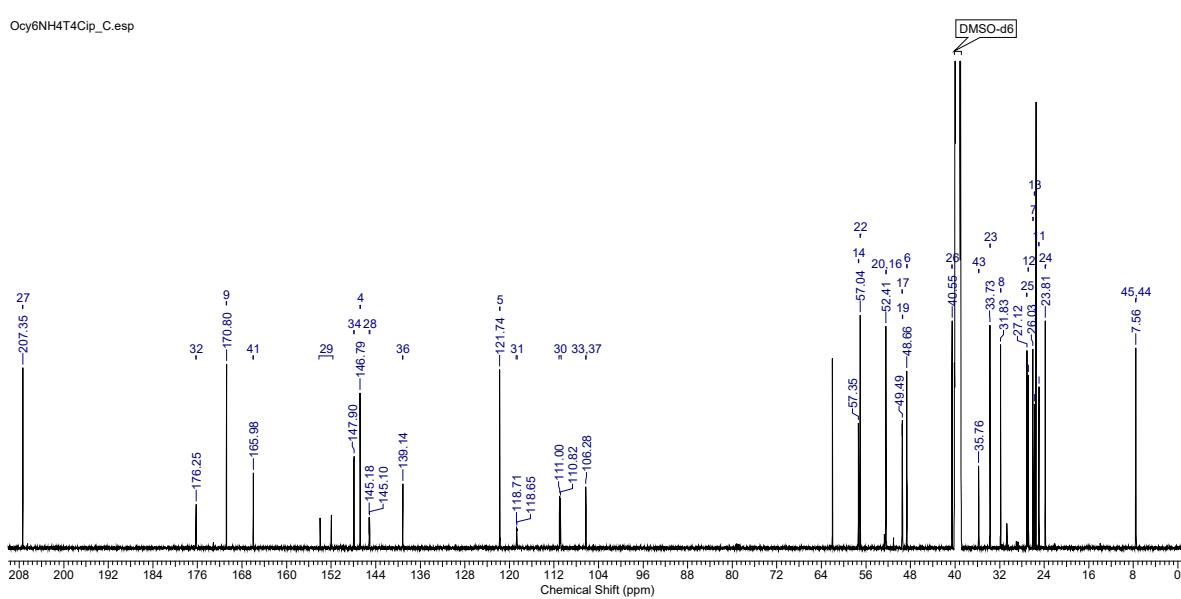
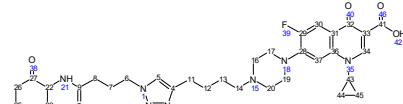
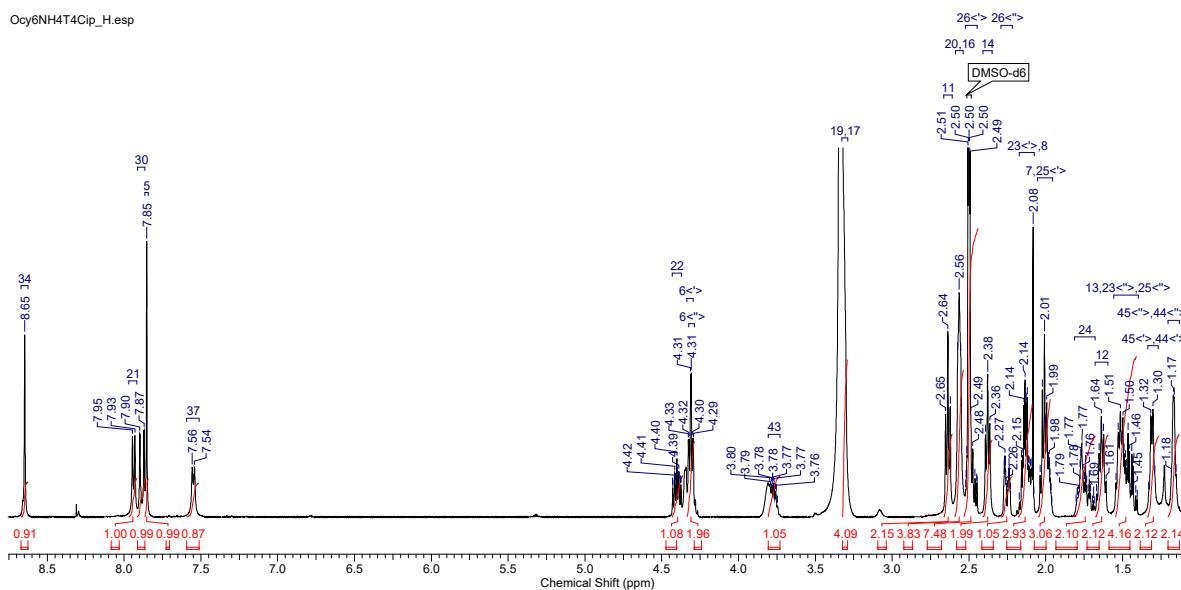
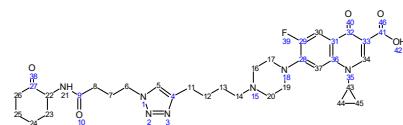
### 5.43 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide ?



5.44 1-Cyclopropyl-6-fluoro-7-(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-dihydroquino-line-3-carboxylic acid ?



5.45 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 ?



## **Todo list**