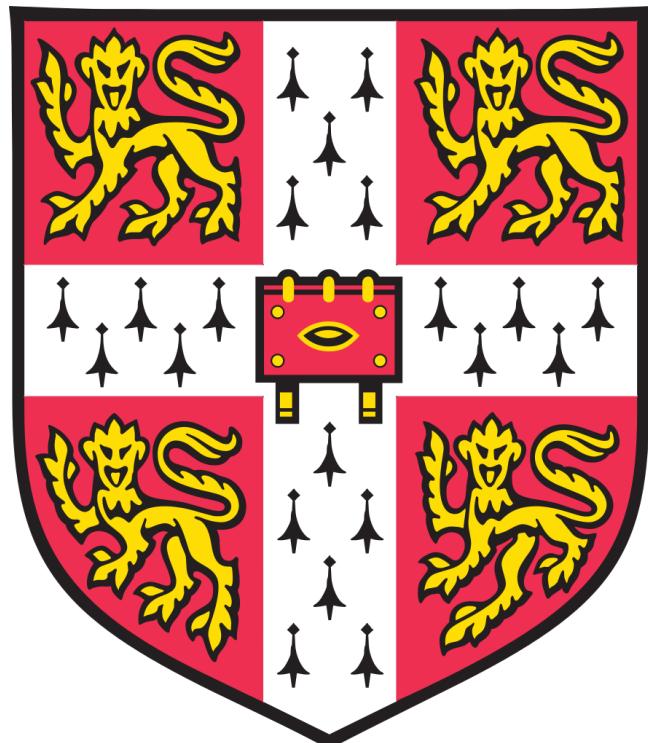


The synthesis and biological evaluation of a library of  
autoinducer-antibiotic conjugates

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## 1 Declaration

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

Lois Overvoorde September 2018

## 2 Abstract

Bacterial resistance to antibiotics is becoming a serious global health threat, and the discovery of new, safe and effective antibiotics is required urgently.<sup>1-3</sup> A new class of antibiotic, namely siderophore-antibiotic conjugates, has shown promise in initial studies.<sup>4,5</sup> Siderophores are used by bacteria for iron uptake, and so attaching antibiotics to them allows the antibiotic to be carried across cell membranes. This study investigates conjugates designed using a similar approach, but using bacterial autoinducers<sup>6</sup> instead of siderophores. Autoinducers are required for coordination of bacterial behaviours and are involved in the control of swarming, virulence factor production and biofilm formation.<sup>7</sup>

The library was synthesised in two halves which were then coupled together using a copper(I)-catalysed azide-alkyne cycloaddition.<sup>7,8</sup> The autoinducers were functionalised with azide groups and the antibiotics were functionalised with alkynes. The quorum sensing molecules produced by *Pseudomonas aeruginosa* were investigated as it is a significant human pathogen<sup>9</sup> which displays high resistance to many antibiotics<sup>10</sup> and uses quorum sensing to coordinate its group behaviours.<sup>11</sup> Azido analogues of these autoinducers were coupled with alkyne analogues of ciprofloxacin, which was chosen as it is commonly used against *P. aeruginosa*<sup>12</sup> but resistance to it is developing,<sup>13</sup> and trimethoprim. It was hoped that the autoinducers would aid retention of the antibiotic in the cell, thus potentially increasing its potency or even restoring its efficacy against resistant strains.

analogues

### **3 Acknowledgements**

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

## 4 Nomenclature

<i>J</i>	Coupling constant in Hz
<i>m/z</i>	Mass to charge ratio in Daltons
<i>R<sub>f</sub></i>	Retention factor
Ac	Acetate
AIP	Autoinducing peptide
aq.	Aqueous
atm	Atmosphere(s)
BHL	Butyryl homoserine lactone = C <sub>4</sub> -HSL <b>19</b>
Boc	<i>tert</i> -Butyloxycarbonyl
Cip	Ciprofloxacin
conc.	Concentrated
COSY	Correlation spectroscopy
d	Day(s)
Da	Daltons
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	Equivalents
ESI	Electrospray ionization
Et	Ethyl
FT	Fourier transform
h	Hour(s)
HCTL	Homocysteine thiolactone
HHQ	2-Heptylquinolin-4(1H)-one
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HMQC	Heteronuclear multiple-quantum correlation spectroscopy

HO<sub>B</sub>t 1-Hydroxybenzotriazole

HPLC High-performance liquid chromatography

HRMS High resolution mass spectroscopy

HSL Homoserine lactone

Hz Hertz

IR Infrared

LCMS Liquid chromatography mass spectroscopy

LCT Liquid chromatography time-of-flight

lit. Literature value

M Molar

m.p. Melting point

Me Methyl

MIC Minimum inhibitory concentration

min Minute(s)

mol Mole(s)

Ms Methanesulfonyl

NMP *N*-Methyl-2-pyrrolidone

NMR Nuclear magnetic resonance

OdDHL *N*-(3-Oxododecanoyl)-homoserine lactone = 3-oxo-C<sub>12</sub>-HSL **20**

P.E. Petroleum ether

PAI-1 *Pseudomonas* autoinducer 1 = 3-oxo-C<sub>12</sub>-HSL **20**

PAI-2 *Pseudomonas* autoinducer 2 = C<sub>4</sub>-HSL **19**

Pd/C Palladium on carbon

PQS *Pseudomonas* Quinolone Signal

Q-TOF Quadrupole time-of-flight

r.t. Room temperature

s Second(s)

SAM *S*-adenosyl-L-methionine

SAR Structure activity relationship

sat. Saturated

sp. Species

TBAF Tetrabutylammonium fluoride

TBDMS *tert*-Butyldimethylsilyl

TEA Triethylamine

Tf Trifluoromethanesulfonyl

TFA Trifluoroacetic acid

THF Tetrahydrofuran

THPTA Tris(3-hydroxypropyltriazolylmethyl)amine

TLC Thin layer chromatography

TMS Trimethylsilyl

Ts *para*-Toluenesulfonyl

UV Ultraviolet

## 5 Introduction

### 5.1 Antibiotic resistance

Antibiotics add, on average, twenty years to a person's life.<sup>2</sup> However, antibiotic resistance is increasing alarmingly and is now recognised as a major threat to global health.<sup>1,2</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>2</sup> is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,<sup>14</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>15,16</sup> This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see Table 1) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories.<sup>2,3</sup>

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>17</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>10</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>18</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 *para*-aminobenzoic acid - a process which is blocked by sulfonamides.<sup>19</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>20–25</sup>

The current pipeline of new antibiotics is widely thought to be worryingly inadequate.<sup>26–28</sup> Significant changes in how we use the antibiotics we already have, as well as investments in the discovery of new ones, are required. Antibiotics currently in late-stage clinical trials nearly all rely on non-novel mechanisms of action,<sup>26</sup> and so it is almost inevitable that resistance to them will develop quickly, as it has done for their predecessors.

There is therefore increasing interest in treatments which would not easily provoke the development of resistance.<sup>29</sup> These treatments often target bacterial virulence rather than killing bacteria outright, hence decreasing selection pressure for resistance.<sup>20</sup> One obvious target is toxin production, for example, an LpxC inhibitor was shown to prevent lethal *Acinetobacter baumannii* infection in mice, despite being inactive against the bacterium *in vitro*.<sup>30</sup> This was due to inhibition of lipopolysaccharide shedding, and hence reduced inflammation in the host. Co-ordination of virulence has also been targeted, for example, analogues of *P. aeruginosa* homoserine lactone autoinducers (see 5.3.1) inhibit the production of virulence factors and increase the survival time of mice in a lethal *P. aeruginosa* lung infection model.

A second strategy in novel antibiotic discovery is to enhance or restore activity of a known antibiotic by lessening or avoiding a resistance mechanism. For example, antibiotics are often excluded from cells due to membrane impermeability or efflux. This may be overcome by attaching the antibiotic warhead to a molecule which the cell imports. The most well known examples of such conjugates are antibody-drug conjugates<sup>31</sup> in the treatment of cancer, but progress has also made against bacteria. In particular, siderophore-antibiotic conjugates (see 5.2) have been investigated in the hope of hijacking bacterial uptake mechanisms to import antibiotics,<sup>4</sup> and the autoinducer-antibiotic conjugates in this study may gain activity by avoiding efflux pumps (see 5.3). These conjugates may have competing mechanisms of action: either the antibiotic accumulates in the cell to a greater extent and acts by its usual mechanism, or an important bacterial system must be disrupted to avoid accumulation of the antibiotic, hence leading to decreased fitness and/or loss of virulence.

## 5.2 Siderophore-antibiotic conjugates

Siderophore-antibiotic conjugates have been receiving attention in recent years as a way to enhance the uptake of known antibiotics.<sup>4</sup> This section will discuss the role of siderophores, sideromycins (natural siderophore-antibiotic conjugates), and the synthetic siderophore-antibiotic conjugates inspired by them. Many of the ob-

servations made about these molecules could be relevant to the autoinducer-antibiotic conjugates synthesised in this study.

### 5.2.1 Siderophores

Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of 'iron mining'.<sup>32</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 Figure 1 and Figure 2), possibly so one species can avoid its siderophores being taken up by another species.<sup>33</sup>

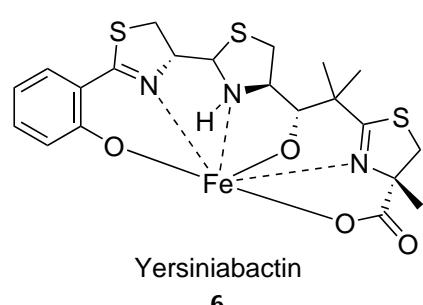
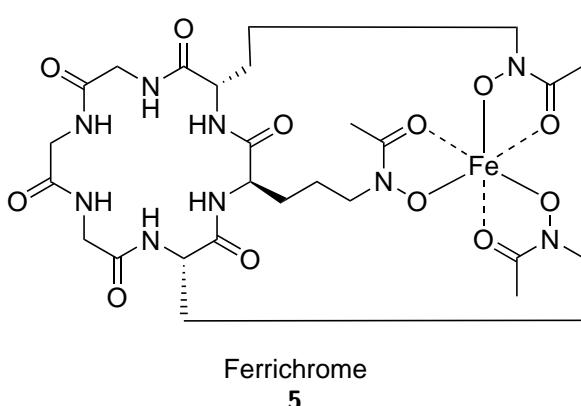
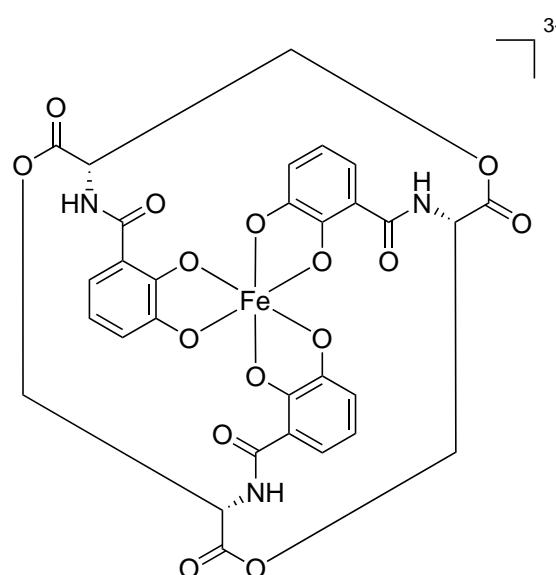
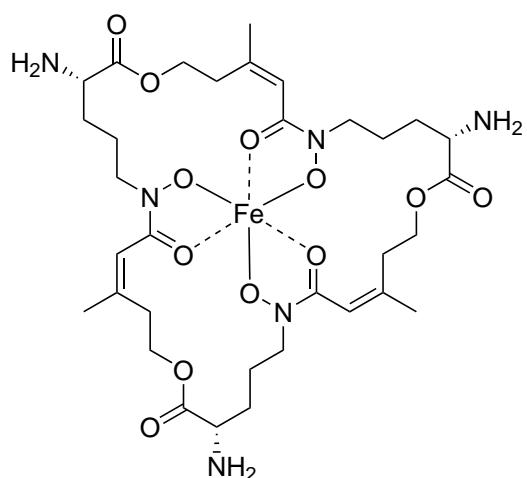
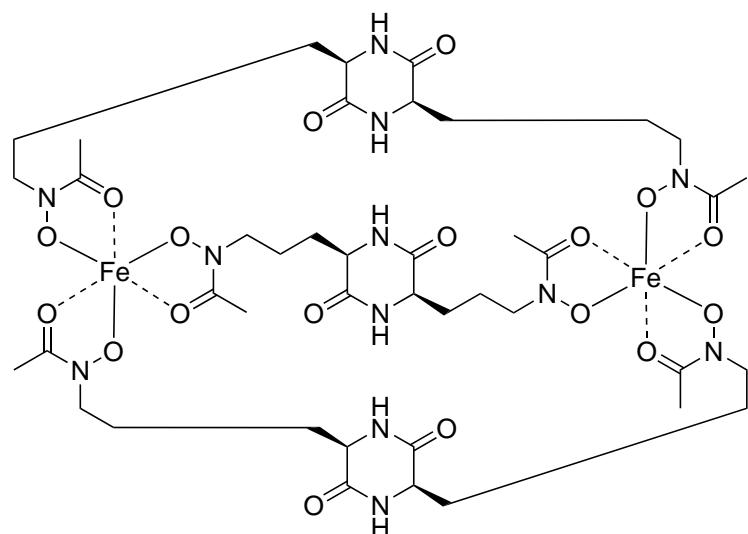
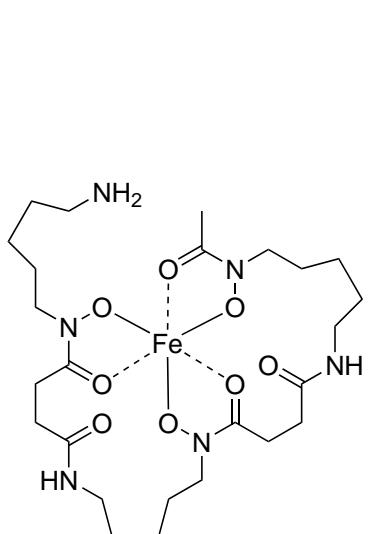


Figure 1: Iron-siderophore complexes: Deferoxamine B **1**<sup>34</sup> (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid **2**<sup>35</sup> (*Rhodotorula pilimanae*), fusarinine C **3**<sup>36</sup> (*Fusarium roseum*), enterobactin **4**<sup>34</sup> (*Escherichia coli* and enteric bacteria), ferrichrome **5**<sup>37</sup> (*Ustilago sphaerogenes*, *U. maydis*, *Aspergillus niger*, *A. quadricinctus*, *A. duricaulis* and *Penicillium resticolum*), yersiniabactin **6**<sup>34</sup> (*Yersinia pestis*).

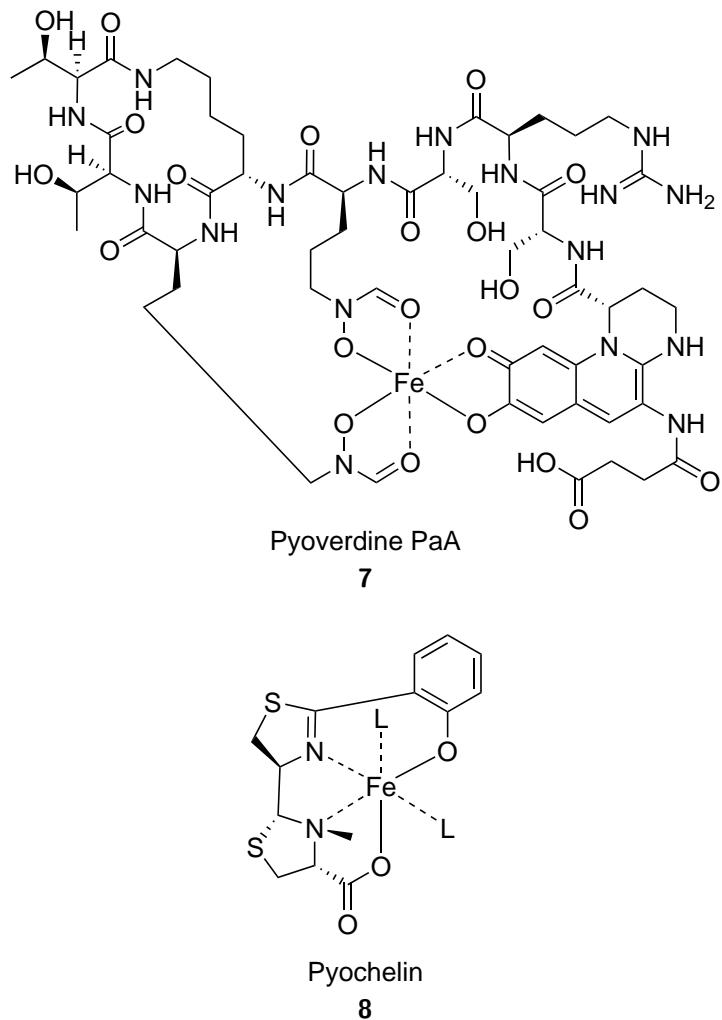


Figure 2: Iron-siderophore complexes: pyoverdine PaA **7**<sup>34,38</sup> (*P. aeruginosa*, PAO1 strain) and pyochelin **8**<sup>39,40</sup> (*P. aeruginosa*). Note that pyochelin **8** is a tetradentate ligand, hence the iron ion has two sites which can bind other ligands.

### 5.2.2 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins<sup>4</sup> (see Figure 3). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds.

For example, albomycin **9** (see Figure 3) is a sideromycin produced by *Actinomyces subtropicus* and *Streptomyces griseus*<sup>41,42</sup> which has been used to treat infections caused by various bacteria including *Yersinia enterocolitica* and *Streptococcus pneumoniae* in mice and humans.<sup>43,44</sup> Albomycin **9** contains a siderophore coupled to a nucleoside antibiotic via a peptide linker. The siderophore section is structurally similar to ferrichrome **5** (see Figure 1), a siderophore produced by various fungi, but also taken up by bacteria including *Escherichia coli*, *Salmonella typhimurium* and *P. aeruginosa*.<sup>37,45</sup> It has been shown that because of the structural similarity to ferrichrome **5**, *E. coli* will also take up albomycin **9**.<sup>41</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>43</sup> and other sideromycins such as salmycin A<sup>32,46,47</sup> and ferrimycin A<sup>148,49</sup> has served as encouragement to many researchers to explore synthetic siderophore-antibiotic conjugates, which will be discussed in the next section.

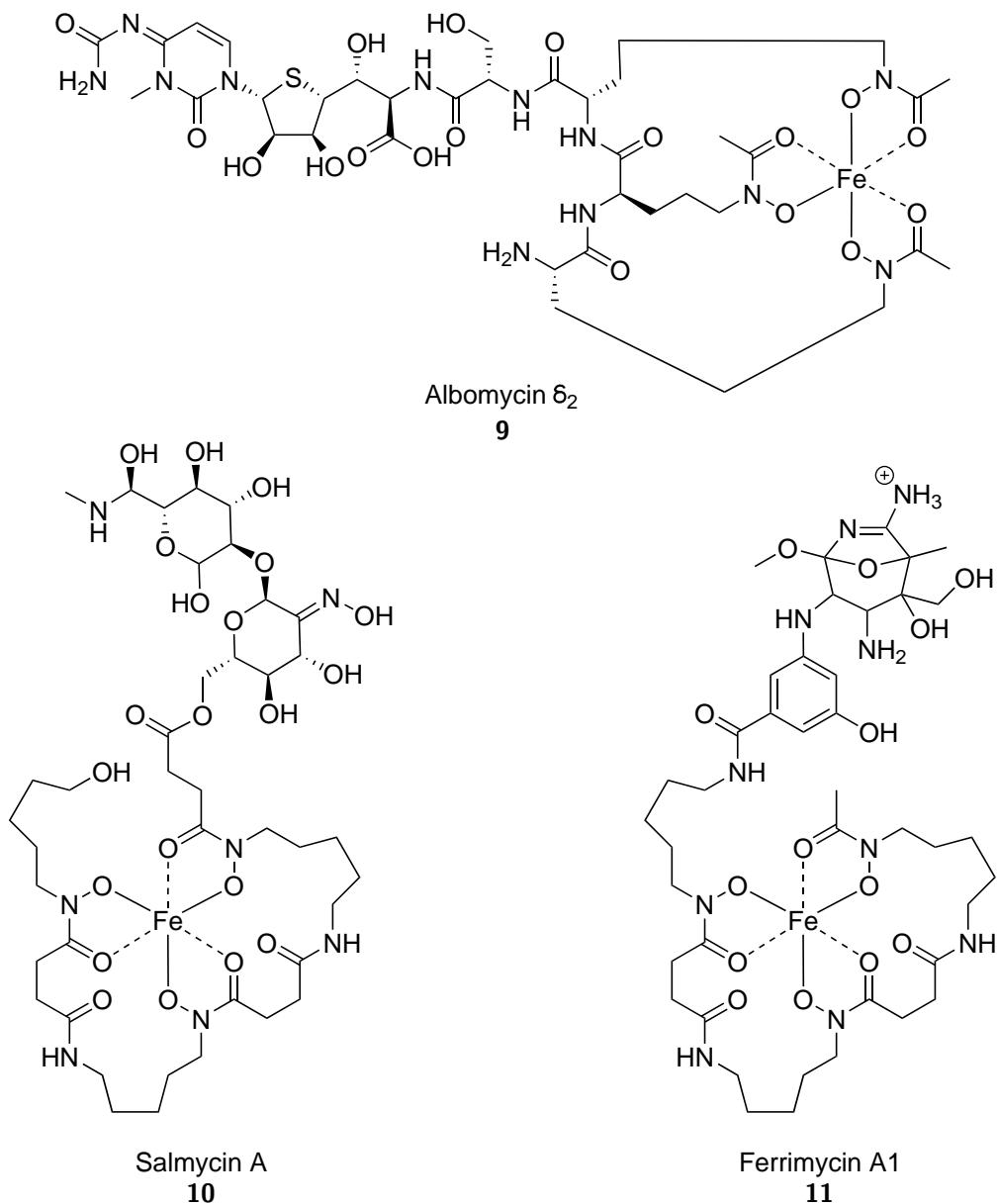


Figure 3: Iron-sideromycin complexes: Albomycin **9**<sup>32,50</sup> (*Actinomyces subtropicus* and *Streptomyces griseus*), salmycin A<sup>32,46,47</sup> (*Streptomyces violaceus*) and ferrimycin<sup>32</sup> (*Streptomyces griseoflavus*).

### 5.2.3 Synthetic siderophore-antibiotic conjugates

Sideromycins served as inspiration for the design, synthesis and biological evaluation of a wide range of synthetic siderophore-antibiotic conjugates.<sup>4</sup> Antibiotics used include  $\beta$ -lactams,<sup>51-53</sup> nucleosides,<sup>54</sup> glycopeptides<sup>55</sup> and macrolides.<sup>56</sup> Sideromycin-fluoroquinolone conjugates have also been studied by several groups,<sup>57-59</sup> including conjugates with linkers which can be cleaved<sup>58,59</sup> in a similar manner to albomycin.<sup>41</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>4</sup> Recently a conjugate (Ent-Amp **12**, see Figure 4) of enterobactin and ampicillin joined using a copper(I)-catalyzed azide-alkyne cycloaddition has been shown to have increased activity against pathogenic *E. coli* when compared to native ampicillin.<sup>60</sup> Other

work has focused on monocyclic  $\beta$ -lactams, for example pirazmonam **13** and U-78608 **14**, which show high potency against Gram-negative bacteria including *P. aeruginosa*.<sup>61,62</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>5</sup> MC-1 **15**,<sup>63</sup> BAL30072 **16**<sup>4</sup> (see Figure 4) and cefiderocol **17**.<sup>64,65</sup>

MC-1 **15** is reported as being "in clinical phases of development",<sup>5</sup> but no reports of studies in humans could be found. However, experiments in mice have been promising.<sup>63</sup> BAL30072 **16** is a siderophore- $\beta$ -lactam conjugate which showed initial promise as it is a poor substrate for  $\beta$ -lactamases, and resistance due to loss of transport proteins is infrequent.<sup>4</sup> However, it is unclear whether it will progress further in trials as it causes liver toxicity.<sup>66</sup> Cefiderocol **17** is a cephalosporin-catechol conjugate in phase 1 trials. Recent results indicate that 'single and 35 multiple intravenous doses of cefiderocol at up to 2000 mg were well tolerated in healthy 36 subjects'.<sup>65</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 **9**-resistant mutants have been shown to be less virulent,<sup>44</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>67</sup> with an antibiotic can be extended to conjugates of autoinducers and antibiotics in a similar 'Trojan horse' approach.

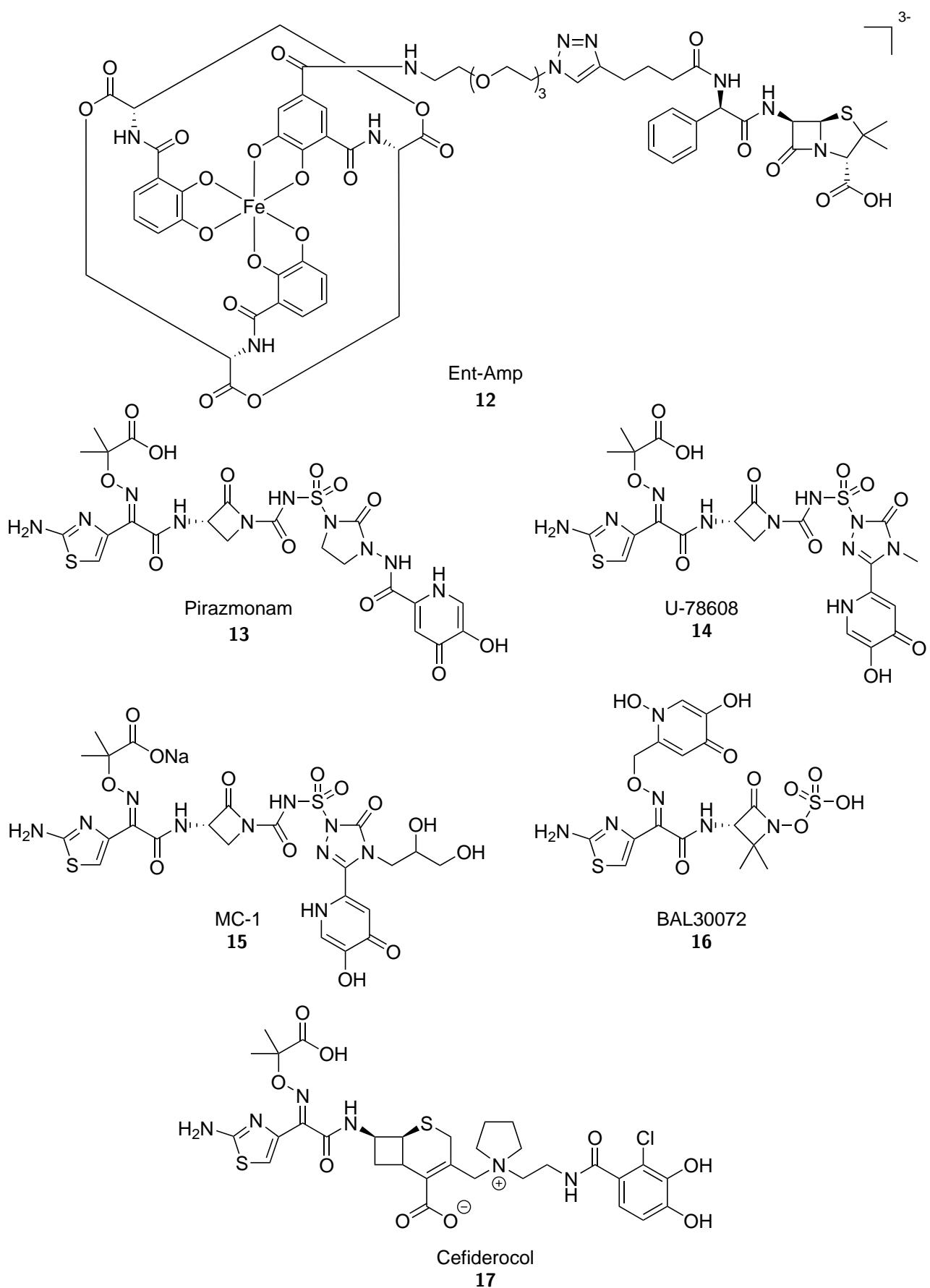


Figure 4: Examples of siderophore-antibiotic conjugates: Ent-Amp **12**,<sup>60</sup> pirazmonam **13**,<sup>61,62</sup> U-78608 **14**,<sup>61,62</sup> MC-1 **15**,<sup>63</sup> BAL30072 **16**<sup>4</sup> and cefiderocol **17**.<sup>64,65</sup>

### 5.3 Autoinducer-antibiotic conjugates

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

#### 5.3.1 Quorum sensing

A quorum is defined as 'A fixed minimum number of members of an assembly or society that must be present at any of its meetings to make the proceedings of that meeting valid.'<sup>68</sup> A similar concept is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum concentration of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors, swarming and biofilm formation.<sup>7</sup> It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium. The process by which bacteria determine the concentration of similar bacteria in their vicinity, and act on that information, is known as quorum sensing.

Quorum sensing has since been observed in many species of bacteria, including *Vibrio fischeri*, *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio harveyi*, *Escherichia coli*, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica*, *Aeromonas* sp. and *Acinetobacter* sp.<sup>6,7,69-76</sup> Many of these bacteria are significant causes of disease and death in humans, for example, in a typical year in the U.S. *P. aeruginosa* causes 6,700 multidrug-resistant infections and 440 deaths, methicillin-resistant *S. aureus* causes 80,500 severe infections and 11,300 deaths and non-typhoidal *Salmonella* causes 1.2 million illnesses, 23,000 hospitalisations and 450 deaths.<sup>1</sup>

##### 5.3.1.1 *Vibrio fischeri*

The first example of quorum sensing was discovered in *V. fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid, *Euprymna scolopes*<sup>7,75,76</sup> (see Figure 5). This bacterium receives amino acids<sup>77,78</sup> from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself.<sup>79</sup>

If a low population of *V. fischeri* were present in the photophore, the light that the bacteria could produce would be insufficient to provide counterillumination. Therefore, the bacteria conserve resources by not producing light. However, if there is a high population of *V. fischeri* it is useful for them all to produce light, as this incentivises the squid to provide them with nutrients.



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

*V. fischeri* uses the LuxR-LuxI system to sense cell density. This system is seen as a paradigm of quorum sensing, and a simplified explanation of it is presented to show typical features of such a system (see Figure 6).

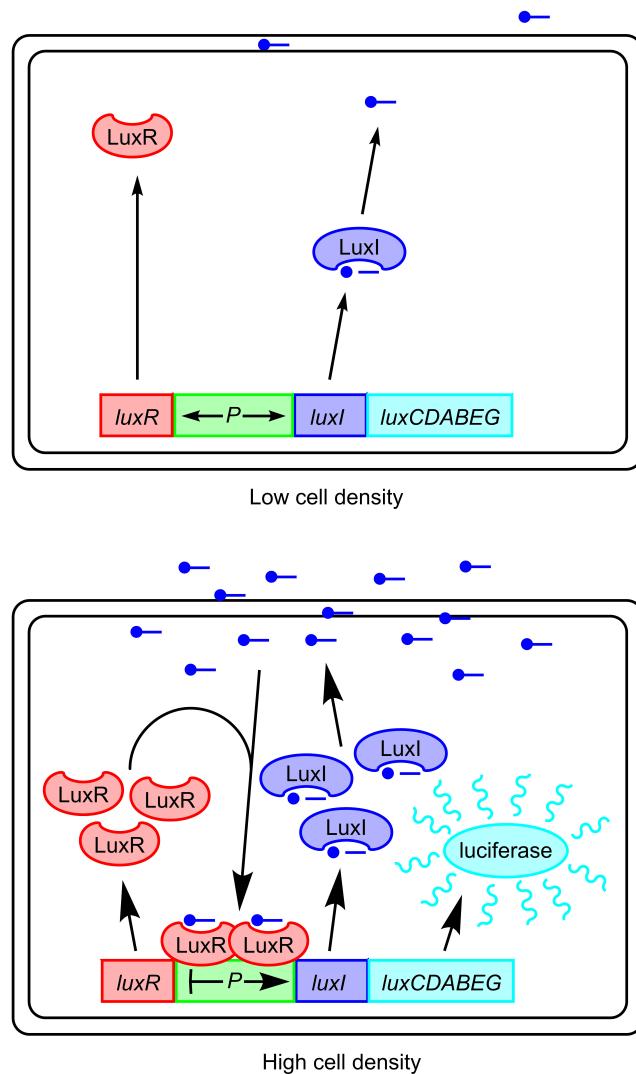


Figure 6: The LuxR-LuxI quorum sensing system in *V. fischeri*.

*V. fischeri* senses cell concentration by the detection of 3-oxo-C<sub>6</sub>-HSL **18<sup>80</sup>** (see Figure 7), a freely diffusible<sup>81</sup> molecule which is synthesised by LuxI<sup>82,83</sup> and secreted by all *V. fischeri* cells<sup>84</sup> at a low basal level.<sup>7</sup> When

the bacterial population density, and hence the concentration of 3-oxo-C<sub>6</sub>-HSL **18**, reaches a threshold, 3-oxo-C<sub>6</sub>-HSL **18** binds to LuxR,<sup>85–87</sup> a receptor which is also synthesised at a low basal level.

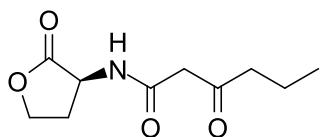


Figure 7: 3-oxo-C<sub>6</sub>-HSL **18**.

The LuxR complex binds to the *lux* operator, upregulating production of LuxI and hence 3-oxo-C<sub>6</sub>-HSL **18**, and luciferase enzymes and hence blue-green light.<sup>88–90</sup> Production of more 3-oxo-C<sub>6</sub>-HSL **18** enables a positive feedback loop, reinforcing the effect of high population density on 3-oxo-C<sub>6</sub>-HSL **18** concentration and hence light production. This is the reason that 3-oxo-C<sub>6</sub>-HSL **18** is known as an autoinducer.

The system also contains a negatively feedback loop to avoid excessive expression of proteins: at high concentrations of 3-oxo-C<sub>6</sub>-HSL **18** production of LuxR is inhibited.<sup>91</sup> Such balancing effects, as well as interactions with other quorum sensing and metabolic systems, are very common.

### 5.3.1.2 *Pseudomonas aeruginosa*

Another well-studied example of quorum sensing is in *P. aeruginosa*.<sup>11,92,93</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>9</sup> Multidrug-resistant *P. aeruginosa* is classified as a ‘serious threat’ by the United States Centers for Disease Control and Prevention<sup>1</sup> and carbapenem-resistant *P. aeruginosa* is classified as ‘priority 1: critical’ by the World Health Organisation.<sup>27</sup>

*P. aeruginosa* has a low susceptibility to many antibiotics and readily acquires antibiotic resistance by mutation or horizontal gene transfer.<sup>94</sup> It is difficult for antibiotics to cross into cells due to low cell membrane permeability<sup>95</sup> and biofilm formation,<sup>96</sup> and they are pumped out again by its multiple chromosomally encoded multidrug efflux pumps.<sup>10</sup> *P. aeruginosa* biofilms are more resistant to many drugs including ciprofloxacin **24** and trimethoprim **25** compared with planktonic cells.<sup>96,97</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 Figure 8) and various proteins (see Figure 9).<sup>11,92,93</sup> These can be broken down into three main, interacting systems: Las, Rhl and Pqs.

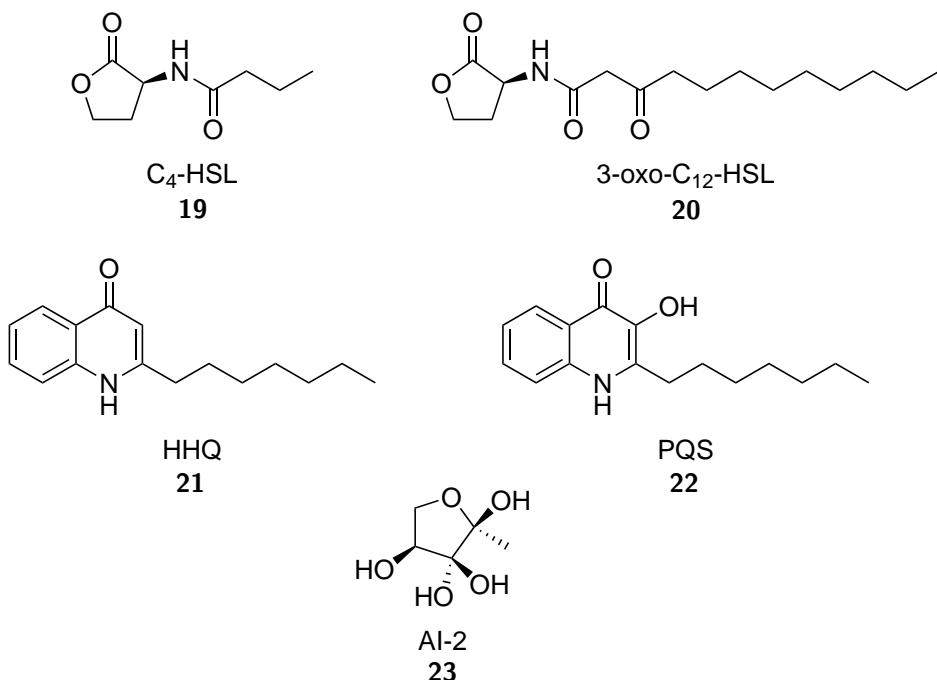


Figure 8: *P. aeruginosa* autoinducers.

In the Las system, LasI<sup>98</sup> synthesises the 3-oxo-C<sub>12</sub>-HSL **20**<sup>99</sup> autoinducer. 3-oxo-C<sub>12</sub>-HSL **20** binds LasR,<sup>100</sup> and this complex upregulates the production of LasI<sup>101</sup> (thus causing autoinduction) as well as alkaline protease,<sup>102</sup> elastase,<sup>100</sup> exotoxin A,<sup>102</sup> HCN<sup>103</sup> and LasA protease.<sup>104</sup> The LasR complex is also important in late-stage biofilm formation,<sup>72</sup> and upregulates the Rhl<sup>105</sup> and Pqs systems.<sup>106,107</sup>

In the Rhl system, RhII<sup>108</sup> synthesises the C<sub>4</sub>-HSL **19**<sup>109</sup> autoinducer. C<sub>4</sub>-HSL **19** binds RhIR,<sup>110</sup> and this complex upregulates the production of RhII<sup>101</sup> (again causing autoinduction), alkaline protease,<sup>111</sup> elastase,<sup>108</sup> haemolysin,<sup>111</sup> HCN,<sup>103,111</sup> LasA protease,<sup>108</sup> LecA,<sup>112</sup> pyocyanin<sup>108,111</sup> and rhamnolipids.<sup>108</sup> The RhIR complex also downregulates the Pqs system.<sup>107,113</sup> The Rhl system is controlled by both the Las and Pqs systems, as production of both RhIR and RhII is upregulated by the LasR complex<sup>105</sup> and production of both RhIR is upregulated by the PqsR complex.<sup>114</sup>

In the Pqs system, the main autoinducer, PQS **22**,<sup>115</sup> is synthesised by multiple enzymes. PhnAB,<sup>116</sup> PqsA, PqsBC, PqsD<sup>117,118</sup> and PqsE<sup>119,120</sup> produce the precursor HHQ **21**, and PqsH converts HHQ **21** to PQS **22**. PQS **22**<sup>107</sup> or HHQ **21** binds PqsR,<sup>121</sup> and either complex can upregulate the synthesis of HHQ **21** causing autoinduction. The PqsR-PQS complex upregulates the production of chitinase,<sup>122</sup> elastase,<sup>115</sup> HCN,<sup>122</sup> LecA,<sup>123</sup> pyocyanin<sup>106,124</sup> and pyoverdine,<sup>124</sup> as well as increasing biofilm production<sup>123</sup> and vesicle formation.<sup>125</sup> The PqsR-PQS complex also upregulates production of RhIR, so the Pqs system has control over the Rhl system.<sup>114</sup> The Pqs system is controlled by both the Las and Rhl systems, as production of PqsR<sup>107</sup> and PqsH<sup>106</sup> is upregulated by the LasR complex and production of PqsA, PqsBC, PqsD, PqsE<sup>113</sup> and PqsR<sup>107</sup> is downregulated by the RhIR complex.

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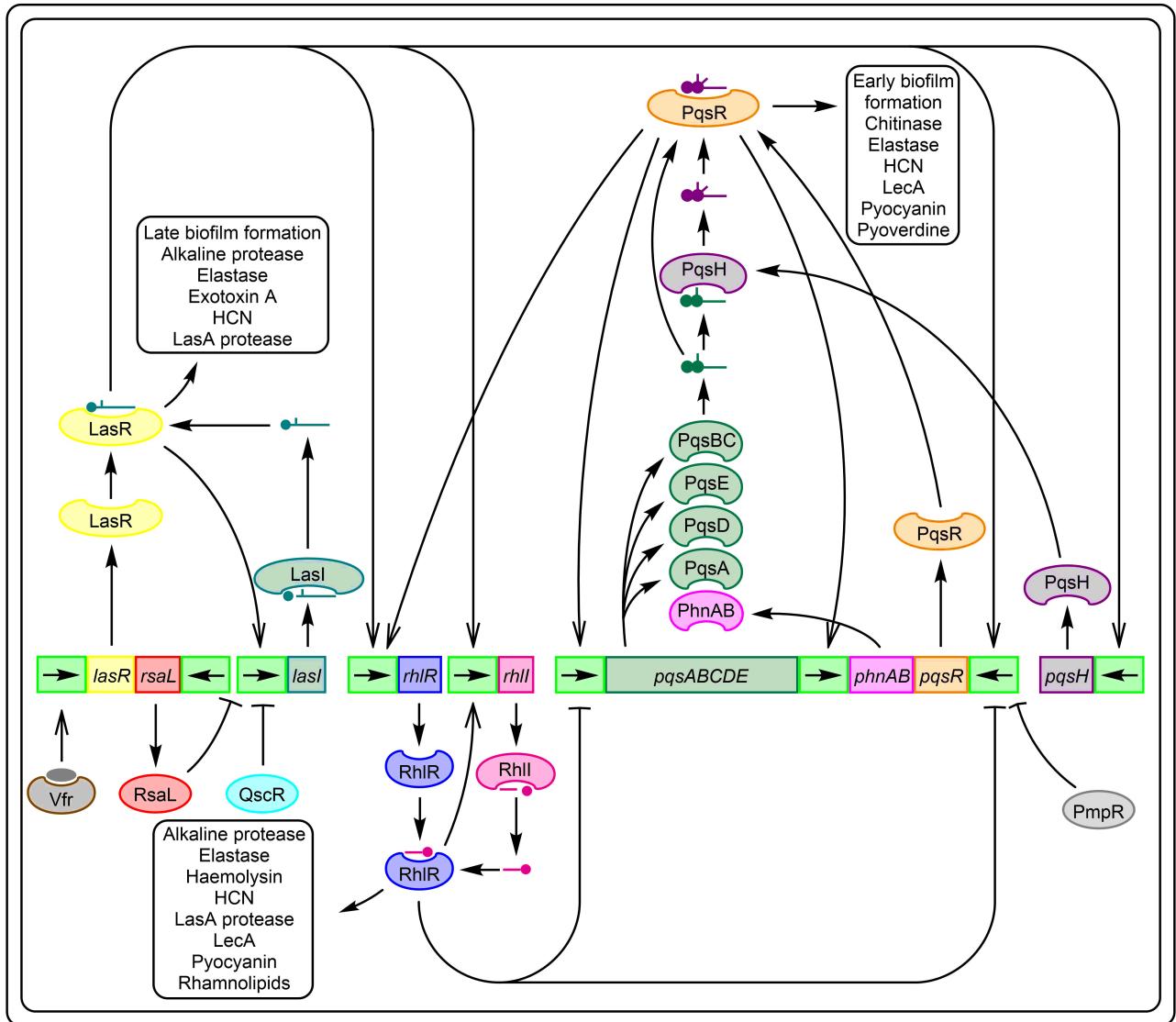
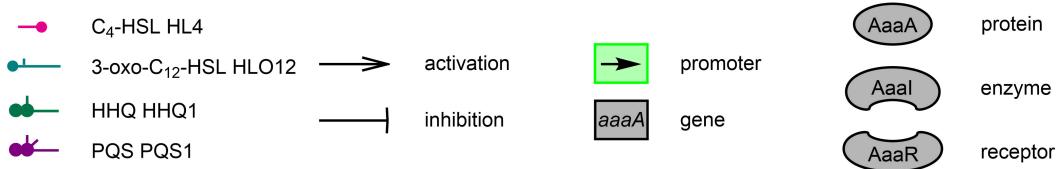


Figure 9: Quorum sensing in *P. aeruginosa*.<sup>11, 92, 93</sup>

In addition to the above systems, AI-2 (see Figure 8), an interspecies signalling molecule,<sup>126</sup> is known to increase biofilm production and virulence in *P. aeruginosa*.<sup>127, 128</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 Figure 8 as part of three interacting quorum sensing systems to coordinate virulence and biofilm production, and this makes these autoinducers interesting therapeutic targets.

### 5.3.2 Autoinducers

Quorum sensing has been successfully targeted using many different modulators,<sup>92, 129</sup> but this study takes a slightly different approach. Inspired by the success of various siderophore-antibiotic conjugates (see 5.2.3), a

library of autoinducer-antibiotic conjugates was synthesised, in the hope that the importance of autoinducers in harmful cellular behaviours would lead to increased activity of the conjugates (see 5.3).

The *P. aeruginosa* autoinducers (see Figure 8) were chosen for use in this study as *P. aeruginosa* is a significant human pathogen which shows high antibiotic resistance and utilises quorum sensing to coordinate pathogenic behaviours (see 5.3.1.2). Specifically, C<sub>4</sub>-HSL **19**, HHQ **21** and PQS **22** derivatives were chosen as they were considered to be the most synthetically tractable.

### 5.3.3 Autoinducer efflux

Autoinducers must be exported from the cell in order to be used for intercellular communication, and the five known *P. aeruginosa* autoinducers are exported by various different transport mechanisms. The mechanism is not well known for HHQ **21** or AI-2 **23**, but it is known that PQS **22** is exported in vesicles,<sup>130</sup> C<sub>4</sub>-HSL **19** passively diffuses in and out of cells,<sup>131</sup> and 3-oxo-C<sub>12</sub>-HSL **20** is taken up passively, accumulates in the cell membrane and is actively pumped out by efflux pumps. The difference in transport mechanism for C<sub>4</sub>-HSL **19** and 3-oxo-C<sub>12</sub>-HSL **20** is thought to be largely due to chain length rather than the 3-oxo modification, as a shorter-chain version, 3-oxo-C<sub>6</sub>-HSL **18** has been shown to be freely diffusible through *V. fischeri* membranes.<sup>81</sup>

3-oxo-C<sub>12</sub>-HSL **20** is exported primarily via the MexAB-OprM efflux system.<sup>10,132</sup> The increased removal of 3-oxo-C<sub>12</sub>-HSL **20** from the cell by upregulation of the MexAB-OprM system leads to decreased production of additional 3-oxo-C<sub>12</sub>-HSL **20** (as the positive feedback loop is disrupted, see 5.3.1.2), and hence decreased production of pyocyanin, elastase and casein protease. It is expected that MexAB-OprM upregulation would also disrupt biofilm formation as a decrease in 3-oxo-C<sub>12</sub>-HSL **20** levels would disrupt Las-mediated quorum sensing,<sup>133</sup> but no direct studies of this could be found.

### 5.3.4 Antibiotics

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

Ciprofloxacin **24** is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections including *P. aeruginosa*.<sup>12,134</sup> Ciprofloxacin **24** inhibits DNA replication by binding to DNA gyrase and topoisomerase IV.<sup>135</sup>

Trimethoprim (see Figure 10) is a dihydrofolate reductase inhibitor used primarily to treat bladder infections.<sup>136</sup> It is active against several significant human pathogens including *Streptococcus pneumoniae* and *Haemophilus influenzae*, but not against *P. aeruginosa*. It was primarily chosen in this study as it was considered easy to functionalise, but also to test the feasibility of creating antibiotic activity against *P. aeruginosa*.

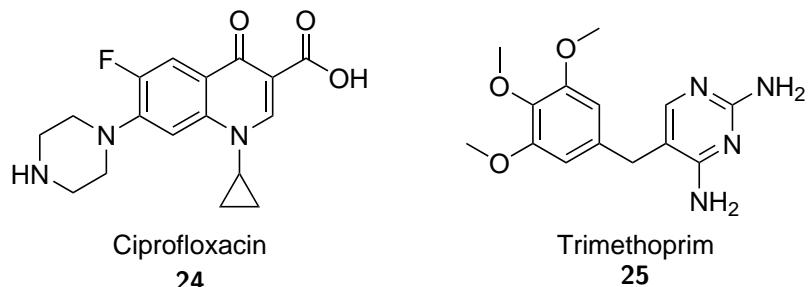


Figure 10: The antibiotics used in this section.

### 5.3.5 Antibiotic efflux

Ciprofloxacin **24** enters *P. aeruginosa* by diffusion,<sup>137</sup> but is pumped out by efflux pumps.<sup>138</sup> In the planktonic state several efflux pumps are known to pump out ciprofloxacin **24**, including MexAB–OprM, MexCD–OprJ, MexEF–OprN, MexXY–OprM, MexJK–OprM and MexVW–OprM.<sup>10</sup> However, in biofilms only MexEF–OprN has an effect.<sup>139</sup>

Trimethoprim **25** is mainly exported by the MexAB–OprM,<sup>140</sup> MexCD–OprJ<sup>141</sup> and MexEF–OprN<sup>142</sup> multidrug efflux systems<sup>10,143</sup> in the planktonic state. It is not known which pumps are used to export trimethoprim **25** from biofilms, but biofilms do show increased resistance to it.<sup>97</sup>

### 5.3.6 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.<sup>11,132,133</sup> 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 **20**.<sup>10,132</sup> For PQS conjugates this would mean upregulation of vesicle formation.<sup>130</sup>
2. The autoinducer section could make the conjugate a poor substrate for the antibiotic section's usual efflux mechanism, leading to accumulation of the conjugate within cells and hence increased antibacterial activity. For autoinducer-ciprofloxacin conjugates acting on planktonic *P. aeruginosa* this would mean the conjugate being a poor substrate of the various efflux pumps listed in the previous section. For autoinducer-ciprofloxacin conjugates acting on biofilms this would mean the conjugate being a poor substrate of MexEF–OprN (the sole exporter of ciprofloxacin **24** in biofilms<sup>139</sup> and not an exporter of HSLs **19** or **20**, or PQS **22**<sup>10</sup>). This mechanism could in principle work for trimethoprim **25** as well, but it is not known which pumps are active against this antibiotic in biofilms.

These synergistic mechanisms of action made autoinducer-antibiotic conjugates a promising target. An initial library was designed using a copper(I)-catalysed azide-alkyne cycloaddition,<sup>7,8</sup> commonly referred to as a click reaction (although this is a more general term), to join each combination of autoinducer and antibiotic together.

### 5.3.7 Cleavable linkers

In addition to the conjugates described above, a second collection of cleavable conjugates was designed. These were based on the cleavable pyochelin–norfloxacin conjugates synthesised by Rivault *et al.*<sup>58</sup>

cleavable  
intro

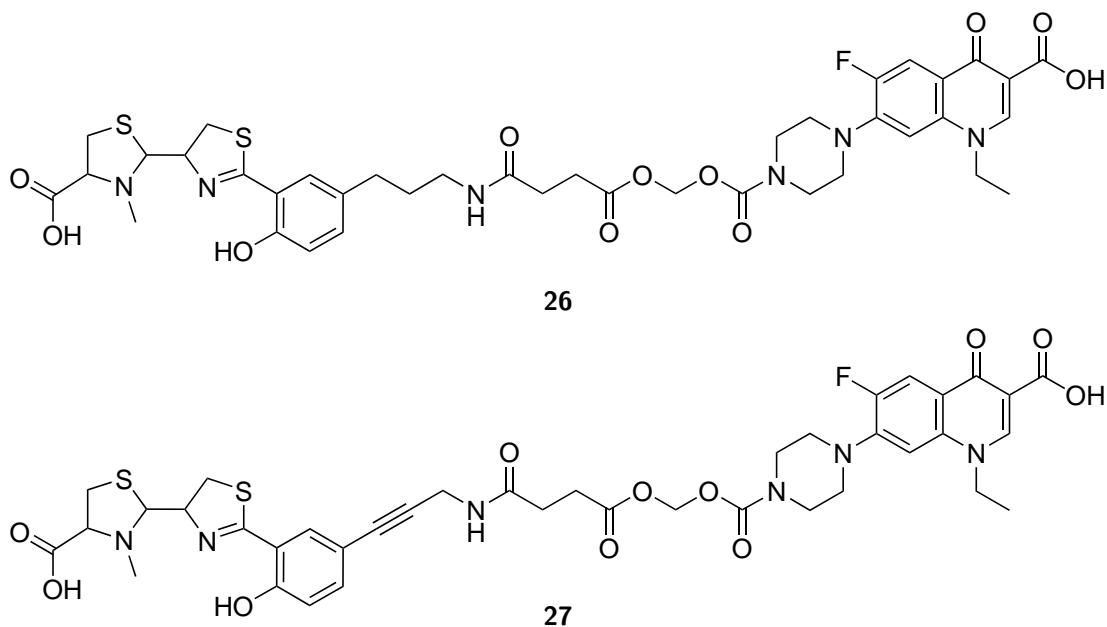


Figure 11: The cleavable pyochelin–norfloxacin conjugates synthesised by Rivault *et al.*<sup>58</sup>

In the series of ciprofloxacin-AHL containing cleavable linkers synthesised by Professor Eddy Sotelo, the linker is designed with the hope that the linker will be stable under the extracellular assay conditions, but will be cleaved upon entry into the cell by intracellular esterases. It is hoped that the attached AHLs will facilitate increased uptake into the cells. For the series of compounds with cleavable linkers, in buffered water conditions they are expected to be stable for ' $> 3$  yrs under optimal conditions', based on the hydrolysis studies of similar compounds in a study by Gogate.<sup>144</sup> The optimum conditions for slowest cleavage of these compounds is 4oC and around pH 4.7, whereas LB may reach around pH 8.5<sup>7</sup> after 24 hours of bacterial growth and the reaction is run at 30oC, so the degradation rate might be markedly increased under experimental conditions.<sup>7</sup> In this study the hydrolysis of a secondary amine prodrug is dependent on ester hydrolysis rate in the range 1 = $<$  pH = $<$  9, therefore the cleavage rate can be tuned by the portion of the molecule between the ester and the triazole, and by the presence of a methyl, hydrogen or other group between the ester and amide.<sup>7</sup>

### 5.3.8 HSL 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>145</sup> and patent<sup>146</sup> by Ganguly *et al.*, who synthesised and characterised a conjugate **103** of methyl ciprofloxacin with homocysteine thiolactone (see Figure 12). 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>84, 147–153</sup>

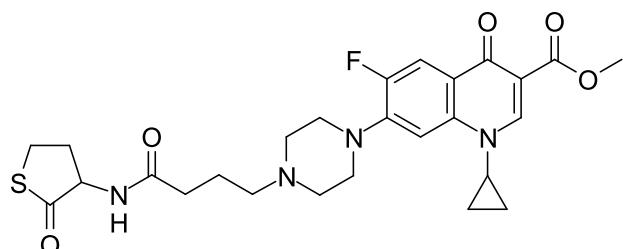


Figure 12: The HCTL-CipMe conjugate **103** studied by Ganguly *et al.*<sup>145, 146</sup>

As part of their characterisation of the HCTL-CipMe conjugate **103**, 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  $\mu\text{m}$ ), 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  $\mu\text{m}$ . As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate **103**. They then incubated cultures for 24 h, to allow biofilms to grow, before adding the compounds. In contrast, they found that the conjugate **103** 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 **28** (see Figure 13) has ‘either enhanced uptake or functional activity’ when compared with C<sub>4</sub>-HSL **19**.

It is possible that the conjugate **103** has higher activity against biofilms when compared with ciprofloxacin **24** because conjugate **103** 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 5.3.6).

While one might expect the conjugate **103** to behave like C<sub>4</sub>-HSL **19**, 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 **20**. 3-oxo-C<sub>12</sub>-HSL **20**’s accumulation in membranes and interaction with efflux pumps is thought to be based primarily on tail chain length (see 5.3.3), and the ciprofloxacin half of the conjugate **103** could be seen as a long tail, especially as the carboxylic acid is methylated and hence less polar.

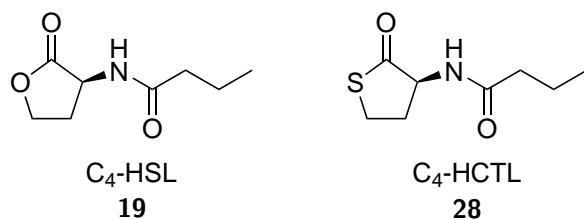


Figure 13: C<sub>4</sub>-HSL **19** and C<sub>4</sub>-HCTL **28**. Note that Ganguly *et al.* tested the *S* enantiomer of C<sub>4</sub>-HCTL **28**, but used a racemic mixture in their HCTL-CipMe conjugate.

While the results found by Ganguly *et al.* show promise, they only test one conjugate, and do not include controls to show that the HCTL group specifically is necessary for the enhanced effect. It was therefore decided to build on this work by synthesising a series of ciprofloxacin conjugates with head groups taken from known quorum sensing modulators,<sup>129,154</sup> a selection of which are described in Table 2.

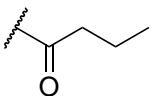
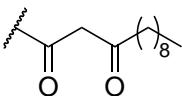
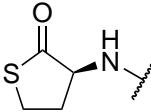
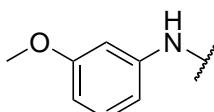
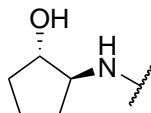
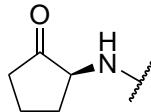
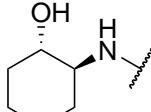
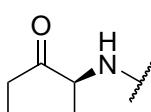
Head group		
	Partial agonist and antagonist against LasR. <sup>151</sup> Shown to increase biofilm formation in <i>P. aeruginosa</i> . <sup>145</sup>	Strong agonist against LasR, with comparable activity to the native ligand. <sup>148, 149, 151, 155</sup>
	Partial agonist against LasR. <sup>154</sup>	Strong antagonist against LasR. <sup>154</sup>
	Poor agonist and antagonist against RhlR. <sup>156, 157</sup>	Strong antagonist against LasR. <sup>156</sup>
	Strong agonist against RhlR. <sup>156</sup> SS enantiomer is more potent. <sup>157</sup>	Partial agonist against LasR. <sup>156</sup>
	Strong agonist against RhlR. <sup>156</sup> SS enantiomer is more potent, with comparable activity to the native ligand. <sup>157</sup>	Strong agonist against LasR. <sup>149, 156</sup> SS enantiomer is more potent, with comparable activity to the native ligand. <sup>157</sup>
	Strong agonist against RhlR. <sup>156</sup> SS enantiomer is more potent. <sup>157</sup>	Partial antagonist against LasR. <sup>156</sup> Shown to reduce biofilm formation in <i>P. aeruginosa</i> . <sup>156</sup>

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

## 6 Project aims and summary

The aim of this project is to produce and test a library of autoinducer-antibiotic conjugates with the hope of producing conjugates with greater potency than the parent antibiotics. The work is divided into two main sections. Section 7 focuses on conjugates of three *P. aeruginosa* autoinducers (see Figure 8) with ciprofloxacin and trimethoprim (see Figure 10) joined using a copper(I)-catalyzed azide-alkyne cycloaddition. Section 8 focuses on conjugates of homoserine lactone analogues with ciprofloxacin (see 5.3.8) joined either using a copper(I)-catalyzed azide-alkyne cycloaddition or an  $S_N2$  reaction or peptide coupling.

## 7 Results and discussion: autoinducer-antibiotic conjugates

### 7.1 Overview

The first part of this project was focused on producing a library of autoinducer-antibiotic conjugates. *P. aeruginosa* autoinducers were used, in particular C<sub>4</sub>-HSL **19**, HHQ **21** and PQS **22** (see Figure 8). Azido derivatives of these compounds were coupled to alkynyl derivatives of antibiotics, specifically ciprofloxacin **24** and trimethoprim **25** (see Figure 10), using a copper(I)-catalysed azide-alkyne cycloaddition.<sup>7,8</sup> The decisions on where to attach the azide or alkyne handles to the chosen molecules are discussed below.

#### 7.1.1 Azido autoinducer derivatives

The structure-activity relationships in HHQ **21** and PQS **22** have been previously studied,<sup>158–160</sup> and it was shown various substitutions on the benzene ring could be made without significantly decreasing activity. The 6-azido derivatives (see Figure 14) were chosen for this study as routes to them have previously been found.<sup>161</sup>

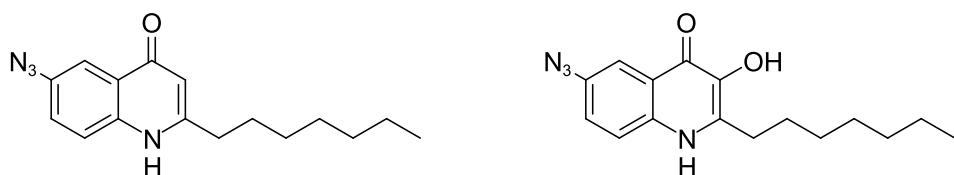


Figure 14: The azido derivatives of HHQ **21** and PQS **22**: **38** and **49**.

Alteration of the lactone group of HSL derivatives is known to significantly decrease activity, especially where the number of H-bond donors or acceptors is altered.<sup>129</sup> Hence, the azide group was included on the tail.<sup>162</sup> Acyl tail length is known to play an important role in affinity,<sup>129</sup> so three derivatives of C<sub>4</sub>-HSL **19** were synthesised: N<sub>3</sub>-C<sub>2</sub>-HSL **55**, N<sub>3</sub>-C<sub>4</sub>-HSL **58** and N<sub>3</sub>-C<sub>6</sub>-HSL **61** (see Figure 15).

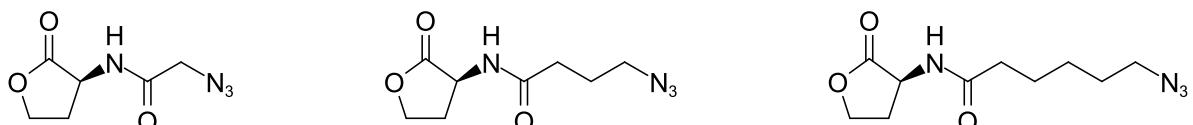


Figure 15: The azido derivatives of C<sub>4</sub>-HSL **19**: **55**, **58** and **61**.

#### 7.1.2 Alkynyl antibiotic derivatives

The structure-activity relationships for ciprofloxacin have been investigated<sup>163</sup> and modifications at the cyclopropane and piperazine groups were found not to cause loss of activity. It was decided an alkyne tail would be added onto the free NH of the piperazine ring, as this position is more synthetically accessible. Alkynyl ciprofloxacin derivative **68** (see Figure 16) was synthesised in this study (see 7.3.1), and two cleavable alkynyl ciprofloxacin derivatives **90** and **91** were synthesised by Professor Eddy Sotelo and combined with the azido HSL derivatives described above to create cleavable conjugates (see 7.4.3).

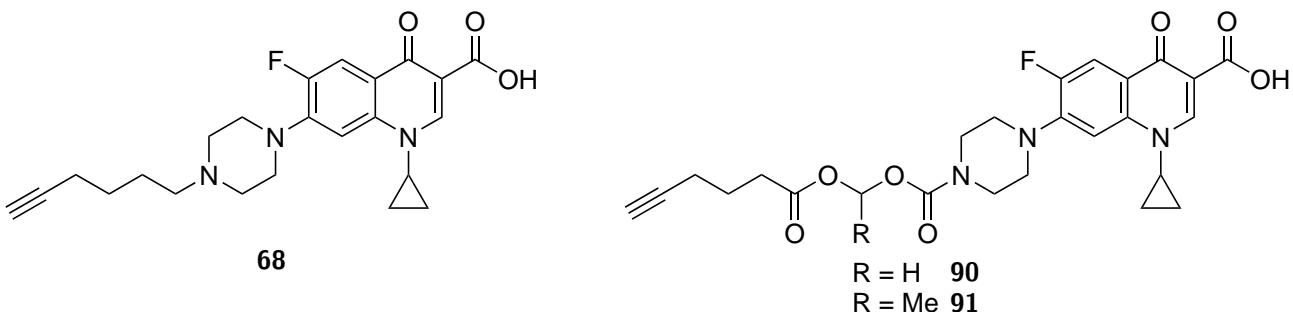


Figure 16: The alkynyl ciprofloxacin derivatives **68**, **90** and **91**.

The choice to of alkyne tail attachment point on trimethoprin **25** (see Figure 17) is based on the use of that same point in a fluorogenic trimethoprim tag synthesised by Jing *et al.*<sup>164</sup>

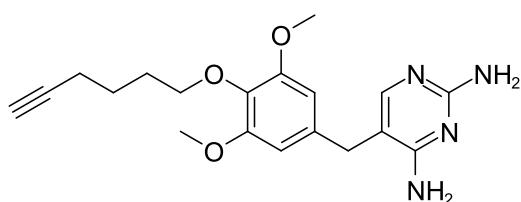
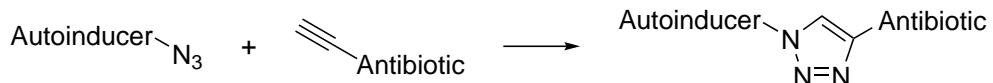


Figure 17: The alkynyl trimethoprim derivative **71**.

### 7.1.3 Synthesis of the conjugates

A copper(I)-catalysed azide-alkyne cycloaddition<sup>7,8</sup> was used to join each combination of autoinducer and antibiotic together (see Scheme 1).



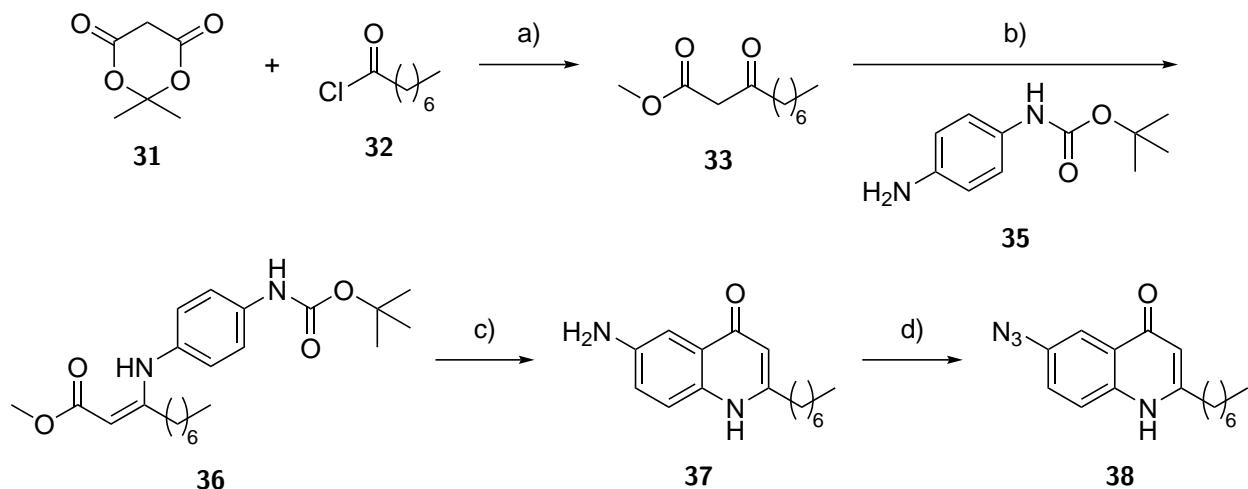
Scheme 1: The construction of the triazole-linked autoinducer-antibiotic conjugate library using a copper(I)-catalysed azide-alkyne cycloaddition.

## 7.2 Azido autoinducer derivatives

### 7.2.1 Synthesis of 6-N<sub>3</sub>-HHQ 38

The synthesis of 6-N<sub>3</sub>-HHQ **38** is shown in Scheme 2 and follows a route devised by Baker.<sup>161</sup> Octanoyl chloride **32** was converted to  $\beta$ -ketoester **33** via a Meldrum's acid adduct.<sup>165,166</sup> The  $\beta$ -ketoester **33** was condensed with *N*-Boc-*para*-phenylenediamine **35** to form enamine **36**. The disappointing yield of this step was in part due to the reaction proceeding to an equilibrium state rather than to completion, and hence not all of the starting material being consumed; starting materials can be recycled to improve the yield. Alternatively, Baker later found a higher-yielding reaction using a ZrCl<sub>4</sub> catalyst.

The enamine **36** was cyclised with polyphosphoric acid to form amino-HHQ **37** in good yield. The amine group of amino-HHQ **37** was converted to a diazo group by reaction with  $\text{NaNO}_2$  and  $\text{HCl}$ , followed by displacement with  $\text{NaN}_3$  to form the final azido-HHQ product **38**.<sup>167</sup>



Scheme 2: The synthesis of **38**. a) i) Pyridine,  $\text{CH}_2\text{Cl}_2$ , 0 °C. ii) MeOH, reflux, 66 % over two steps. b) MeOH, reflux, 19 %. c) Polyphosphoric acid, 120 °C, 72 %. d) i)  $\text{NaNO}_2$ ,  $\text{HCl}$ ,  $\text{H}_2\text{O}$ , 0 °C. ii)  $\text{NaN}_3$ ,  $\text{H}_2\text{O}$ , r.t., 41.2 %.

### 7.2.2 Synthesis of 6- $\text{N}_3$ -PQS **49**

The synthesis of 6- $\text{N}_3$ -PQS **49** is shown in Scheme 3, and also follows a route devised by Baker.<sup>161</sup> The Weinreb amide **43**<sup>92</sup> was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide **40** to form 1-chlorononan-2-one **44** following a procedure described by Hodgkinson *et al.*<sup>168</sup>

The synthesis of PQS **22** described by Hodgkinson *et al.*<sup>168</sup> used a microwave reaction of 1-chlorononan-2-one **44** with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid **45** in the place of anthranilic acid in this microwave reaction, so that the nitro group could then be converted to an azide group via an amine. However, the microwave-catalysed reaction failed when 5-nitroanthranilic acid **45** was used.<sup>161</sup> Therefore, a two step process was employed instead.

5-Nitroanthranilic acid **45** was heated with  $\text{K}_2\text{CO}_3$  to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **44** to form the ester **46** by  $\text{S}_{\text{N}}2$  displacement of the chlorine atom in a procedure adapted from Hlaváč *et al.*<sup>169</sup> Cyclisation with polyphosphoric acid produced nitro-PQS **47** cleanly.<sup>169, 170</sup>

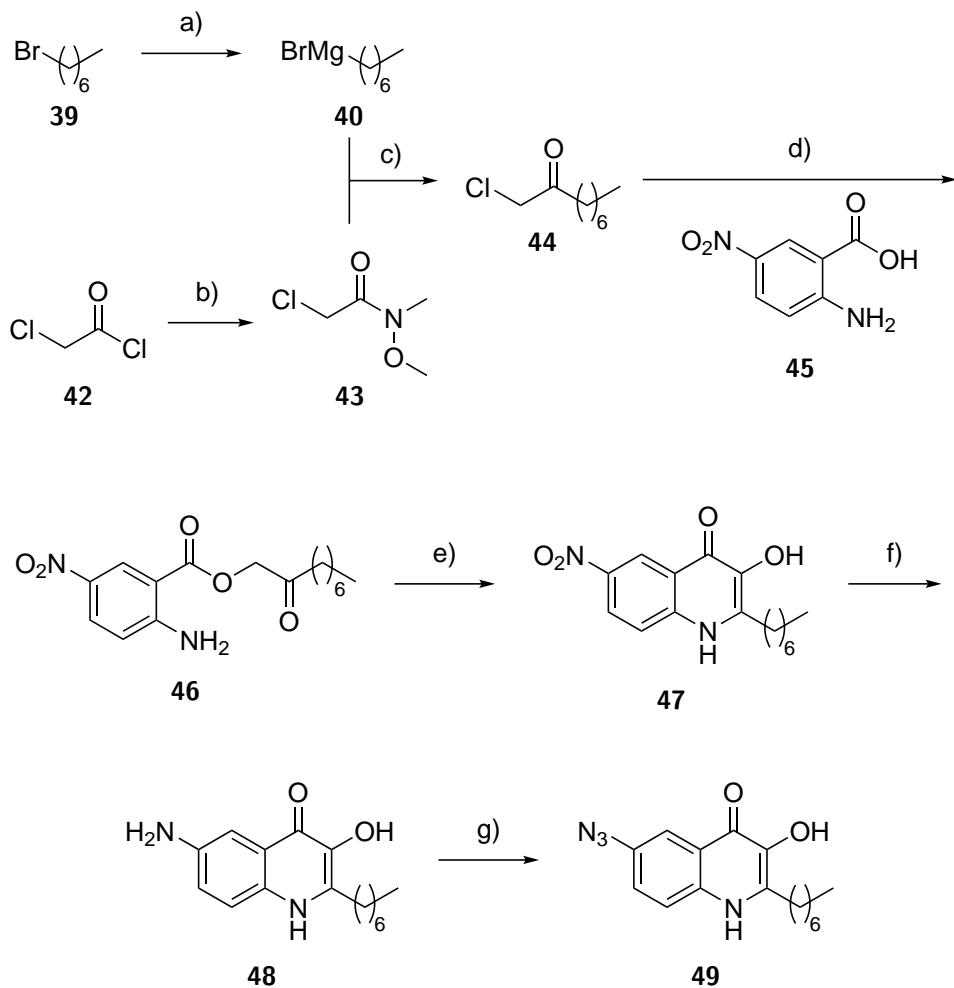
Conditions for the reduction of the nitro group were then compared (see Table 3). Baker initially used Zn and HCl, however this gave a yield over 100 % suggesting coordination of Zn to the amino-PQS **48**<sup>161</sup> (this product was taken through and purified after the next step). She also attempted reduction with Pd/C and  $\text{H}_2$  or ammonium formate, but no reaction was observed.

Further conditions were tested in *this* work in order to obtain a clean sample of amino-PQS **48**. An initial test of reduction with  $\text{SnCl}_2$  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  $\text{H}_2$ . Good yields (80 %) were also achieved using  $\text{PtO}_2$  as a catalyst, with the advantage that the reaction proceeds more quickly, and at atmospheric pressure and temperature.<sup>171</sup>

Finally, amino-PQS **48** was converted to azido-PQS **49** by reaction with  $\text{NaNO}_2$  and HCl to form diazo-PQS, followed by displacement of the diazo group using  $\text{NaN}_3$  to give the azido-PQS **49**.<sup>167</sup> The yield of this reaction was rather disappointing (28 %), and is probably due to loss of product in the supernatant following precipitation.<sup>161</sup>

Conditions	Outcome
$\text{H}_2$ , Pd/C, 1 atm, r.t., 18 h	No reaction
$\text{NH}_4\text{HCO}_2$ , Pd/C, 1 atm, r.t., 18 h	No reaction
Zn, HCl (aq), r.t., 5 min	Product <b>48</b> + Zn, assumed quantitative yield
$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , MeOH, r.t., 18 h	No reaction
$\text{H}_2$ , Pd/C, MeOH, 3 atm, r.t., 4 h.	Product <b>48</b> , 100 % yield
$\text{H}_2$ , $\text{PtO}_2$ , MeOH, 1 atm, r.t., 45 min	Product <b>48</b> , 80 % yield

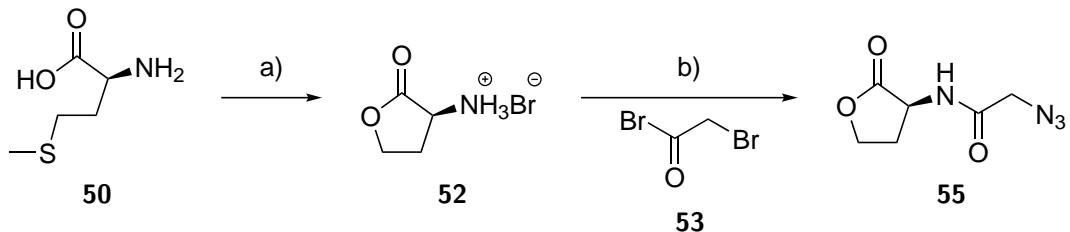
Table 3: Conditions attempted for the synthesis of **48**. Rows 1-3 were carried out by Baker,<sup>161</sup> rows 4-6 were carried out as part of this study.



Scheme 3: The synthesis of **49**. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b)  $N,O$ -dimethylhydroxyl amine hydrochloride,  $\text{K}_2\text{CO}_3$ , toluene,  $\text{H}_2\text{O}$ , - 5 °C to r.t., 30 min, 71 %. c) THF, 0 °C to r.t., 15 h, 96 %. d) **45**,  $\text{K}_2\text{CO}_3$ , DMF, 90 °C, 1 h, then **44**, r.t., 18 h, 100 %. e) Polyphosphoric acid, 90 °C, 5.5 h, 40 %. f)  $\text{H}_2$ ,  $\text{PtO}_2$ , MeOH, 1 atm, r.t., 45 min, 80 %. g) i)  $\text{NaNO}_2$ ,  $\text{HCl}$ ,  $\text{H}_2\text{O}$ , 0 °C, 50 min. ii)  $\text{NaN}_3$ ,  $\text{H}_2\text{O}$ , r.t., 4 h, 28 % over two steps.

### 7.2.3 Synthesis of the azido C<sub>4</sub>-HSL derivatives **55**, **58** and **61**

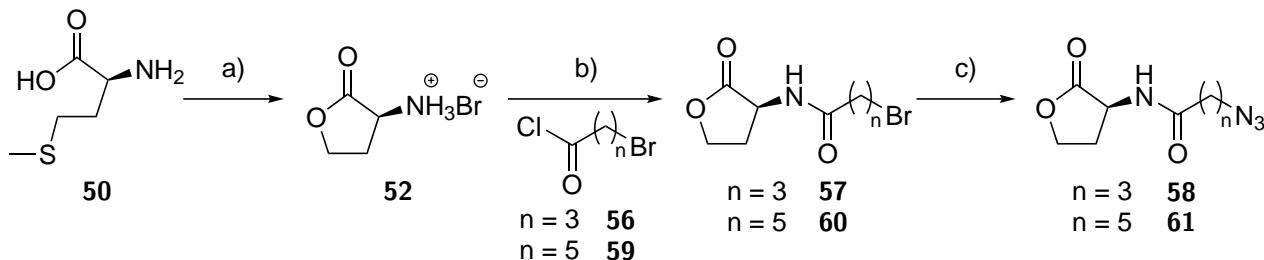
N<sub>3</sub>-C<sub>2</sub>-HSL **55** (the azido derivative of C<sub>4</sub>-HSL with a C<sub>2</sub> chain, see Scheme 4) has previously been prepared by Stacy *et al.*<sup>162</sup> Their synthesis was followed, starting with the cyclisation of L-methionine **50** using bromoacetic acid to form the homoserine lactone HBr salt **52**. The disappointing yield can be attributed to difficulties in precipitating the final product. The homoserine lactone HBr salt **52** was then converted by a biphasic one-pot process to N<sub>3</sub>-C<sub>2</sub>-HSL **55** using bromoacetyl bromide **53** and NaN<sub>3</sub>.



Scheme 4: The synthesis of **55**. 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 **50** to N<sub>3</sub>-C<sub>4</sub>-HSL **58** using 4-bromobutyryl chloride **56** 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 Scheme 5).

Reaction of the homoserine lactone HBr salt **52** with 4-bromobutyryl chloride **56** or 6-bromohexanoyl chloride **59** produced Br-C<sub>4</sub>-HSL **57** or Br-C<sub>6</sub>-HSL **60** respectively, in good yields. Heating with NaN<sub>3</sub> in DMF converted Br-C<sub>6</sub>-HSL **60** to N<sub>3</sub>-C<sub>6</sub>-HSL **61**. 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 **57** to the azido-C<sub>4</sub> derivative **58**, 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 Scheme 14, for example).



Scheme 5: The synthesis of **58** and **61**. 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, **57**: 80 %, **60**: 66 %. c) NaN<sub>3</sub>, DMF, 100 °C, 5 h, **61**: 27 % (donated by Dr Bin Yu), **61**: 56 %.

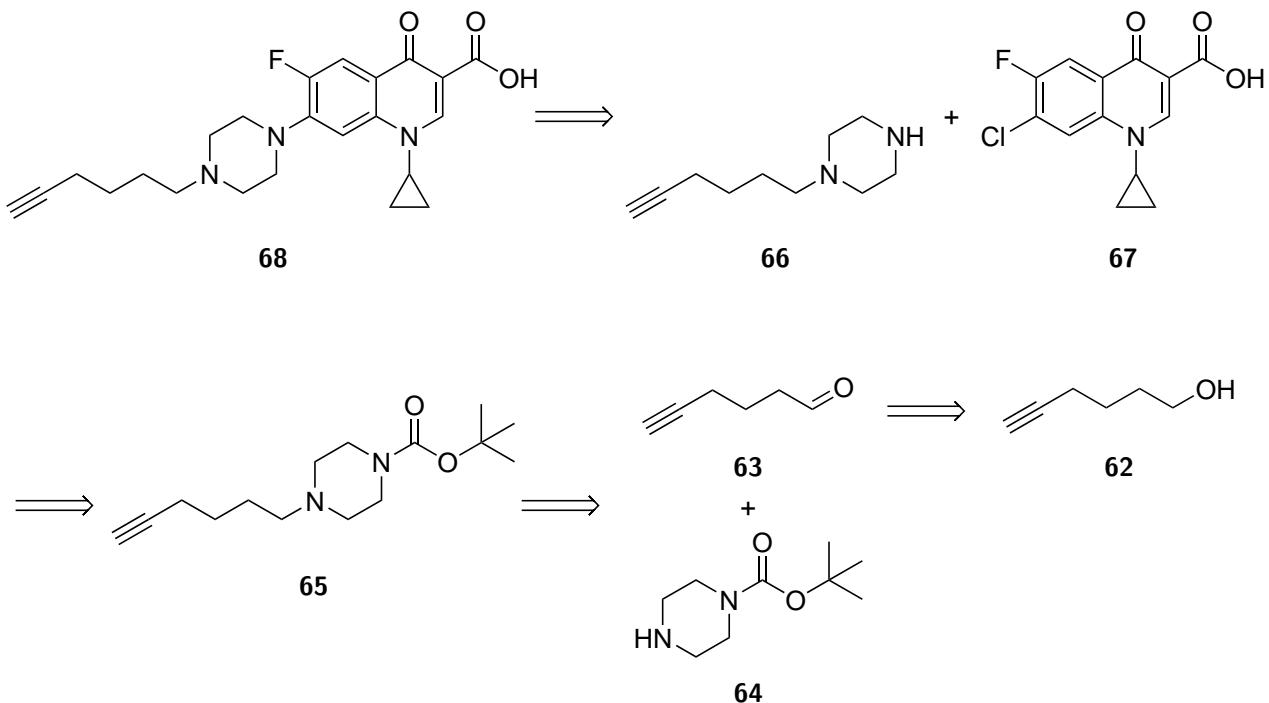
## 7.3 Alkynyl antibiotic derivatives

### 7.3.1 Synthesis of the alkynyl ciprofloxacin derivative **68**

The retrosynthesis of ciprofloxacin derivative **68** is shown in Scheme 6. The disconnection to an alkynyl piperazine **68** and a commercially available ciprofloxacin precursor **67** was chosen based on a study by Renau *et al.*, who found this route to be "...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide."<sup>163,172</sup>

It was envisaged that the alkynyl piperazine **68** could be prepared from mono-Boc-protected piperazine **64** and hex-5-ynal **63** using conditions similar to those used by Renau *et al.*<sup>163</sup>

Unlike the aldehydes and ketones used by Renau *et al.*,<sup>163</sup> hex-5-ynal **63** is not commercially available and so it was hoped that this could be prepared by oxidation of hex-5-ynol **62**.



Scheme 6: The retrosynthesis of **68**.

The synthesis of ciprofloxacin derivative **68** is shown in Scheme 7. Hex-5-ynal **63** was prepared by pyridinium chlorochromate oxidation of hex-5-ynol **62** in good yield according to the procedure described by Kocsis *et al.*<sup>173</sup>

Renau *et al.*<sup>163</sup> used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal **63** and 1-Boc-piperazine **64**. However, it was decided to attempt this transformation using the less toxic sodium triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*<sup>174</sup> This reaction yielded compound **65** in excellent yield, which was deprotected using TFA using the procedure described by Renau *et al.*<sup>163</sup> to give the alkynyl piperazine **66** quantitatively.

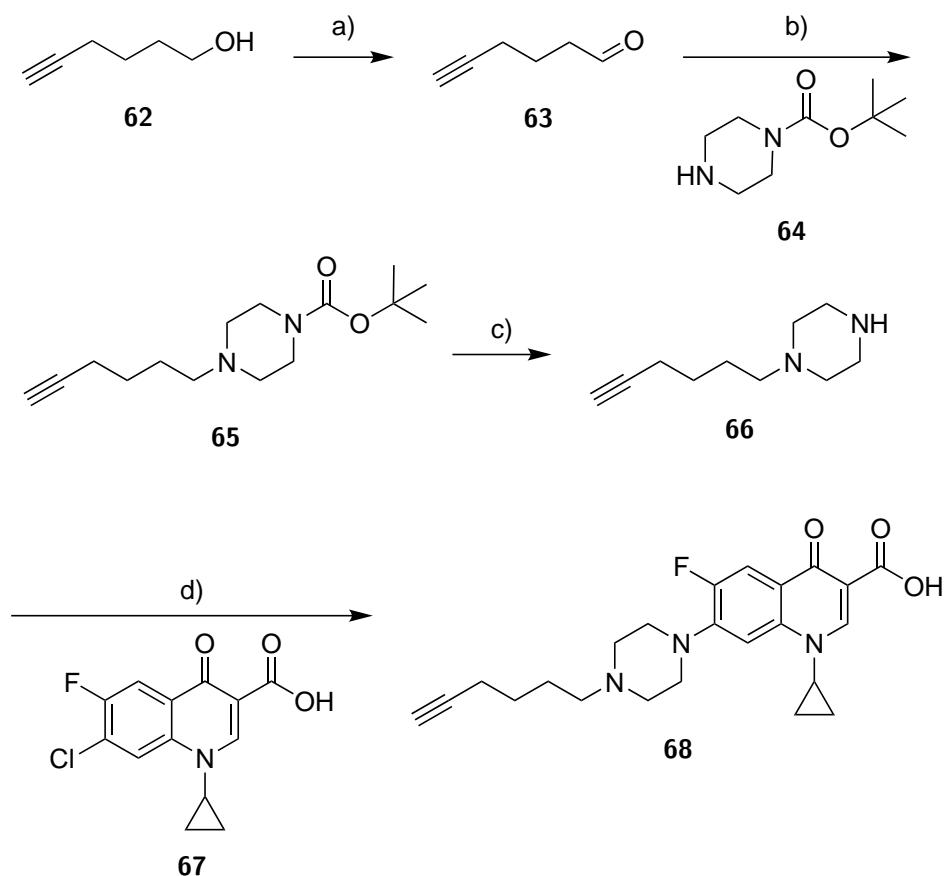
The alkynyl piperazine **66** was refluxed in acetonitrile with the ciprofloxacin precursor **67** according to the procedure described by Renau *et al.*,<sup>163</sup> however the reaction did not proceed. Addition of 2 eq. of TEA did not lead to reaction, however it was found that refluxing in neat TEA led to conversion to the final ciprofloxacin derivative **68**.

With a small sample of the final product in hand, less harsh conditions were sought for a larger-scale version of the final reaction. Microwave irradiation at 115 °C was used, following a procedure by Reddy *et al.*<sup>175</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 challenging, with an unknown dark brown viscous liquid being formed which was difficult to separate from the white solid product. A pure sample was obtained by recrystallisation from EtOAc, but the yield was poor (11.8 %). The reaction was observed to stall after a certain point, while still having some of the ciprofloxacin precursor **67** present. The alkynyl piperazine **66** was not observed by TLC despite having been added in two-fold excess, suggesting that it degraded to a by-product before having chance to react.

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

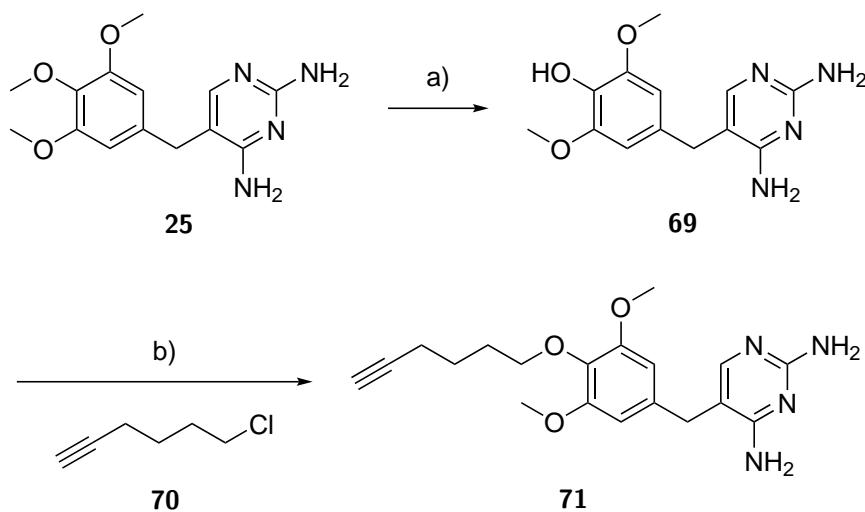


Scheme 7: The synthesis of **68**. a) Pyridinium chlorochromate,  $\text{CH}_2\text{Cl}_2$ , r.t., 5 h, 72 %. b)  $\text{NaBH}(\text{AcO})_3$ , 1,2-dichloroethane, r.t., 10.5 h, 99 %. c) TFA, r.t., 1 h, 100 %. d) NMP, microwave, 115 °C 24 h, 11.8 %.

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

The synthesis of trimethoprim derivative **71** is shown in Scheme 8. Trimethoprim was selectively deprotected using HBr (aq.) using a procedure described by Jing *et al.*<sup>164</sup> to form **69**. A slightly longer reaction time (40 min vs 20 min) probably led to the yield being somewhat lower than that obtained by Jing *et al.*. The main impurity was asymmetrically di-demethylated trimethoprim, which could be identified by the presence of two aryl peaks at 6.41 (d,  $J=2.0$  Hz, 1 H) and 6.34 (d,  $J=2.0$  Hz, 1 H) and a corresponding methyl peak at 3.82 (s, 3 H) in the crude NMR.

The alkynyl trimethoprim derivative **71** was synthesised from the demethylated trimethoprim **69** and 6-chloro-1-hexyne **70** using a  $\text{Cs}_2\text{CO}_3$ -catalysed  $\text{S}_{\text{N}}2$  reaction similar to that used by Jing *et al.*<sup>164</sup>



Scheme 8: The synthesis of **71**. a) HBr (aq.), 100 °C, 40 min, 43.4 %. b) Cs<sub>2</sub>CO<sub>3</sub>, DMF, 70 °C, 7 h, 25.3 %.

## 7.4 Triazole-linked autoinducer-antibiotic conjugates

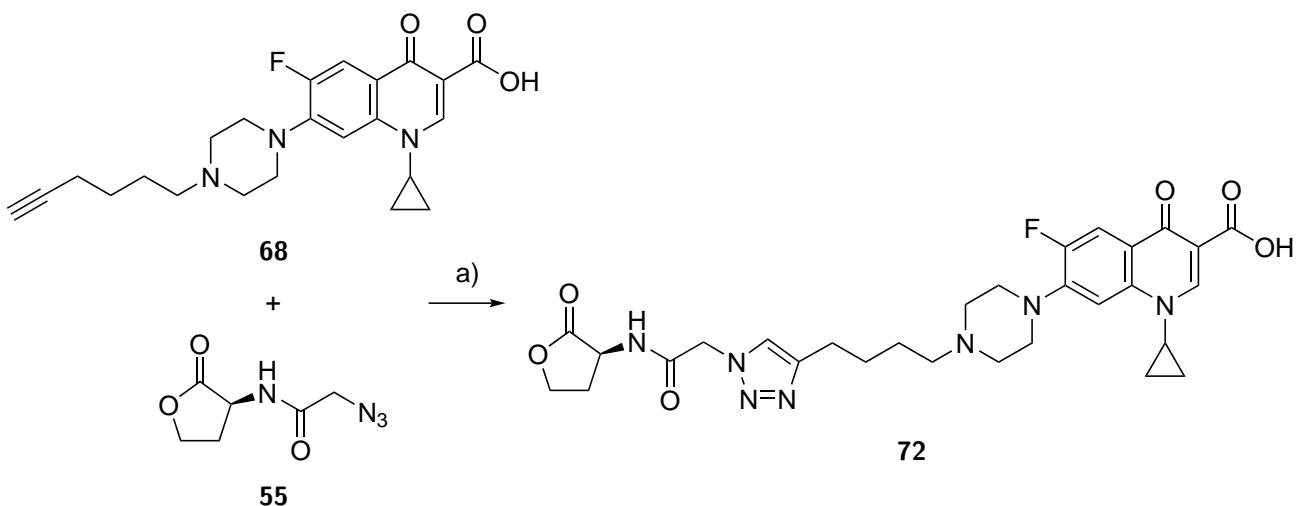
### 7.4.1 Optimisation of the click reaction

Test reactions using N<sub>3</sub>-C<sub>2</sub>-HSL **55** and the alkynyl ciprofloxacin derivative **68** were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see Table 4 and Scheme 9). Stirring at r.t. had no effect even with an extended reaction time. Heating to 50 °C did lead to slow formation of the product, but a mixture of the 1,4 **72** and 1,5 **73** isomers was observed in an approximately 4:1 ratio by LCMS (see Figure 19). It is possible that the Cu(I) catalyst was not involved in this reaction because it had been oxidised, and hence the mixture of products was formed by an uncatalysed cycloaddition. Such reactions are known to produce a mixture of products.<sup>176</sup>

Use of the ligand tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) **74** (see Figure 18) led to some conversion at room temperature, however the reaction stopped before completion, probably due to oxidation of the Cu(I) catalytic species. When degassed solvent and an argon atmosphere were used the reaction proceeded to completion at room temperature in around 3 h.

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

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



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

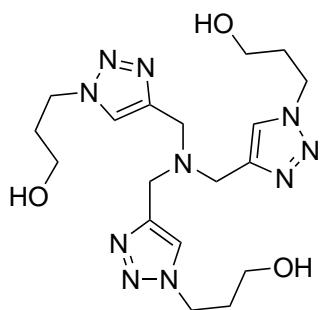


Figure 18: Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (THPTA) **74**.

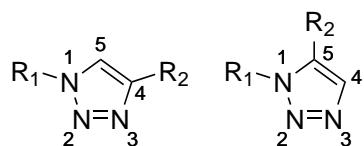
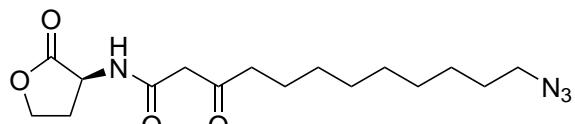


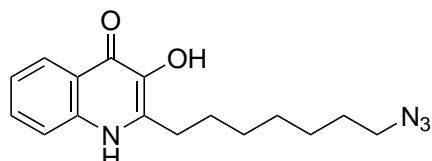
Figure 19: 1,4 (left) and 1,5 (right) triazoles.

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

Once conditions had been found for the click reaction, the synthesis of other conjugates was attempted. Two additional azides were kindly donated by members of the Spring group: the azido derivative of 3-oxo-C<sub>12</sub>-HSL **75** was synthesised by Ryan Howard, a master's student under my supervision<sup>177</sup> and the tail azide derivative of PQS **76** was synthesised by Dr Ysobel Baker<sup>161</sup> (see Figure 20).



75

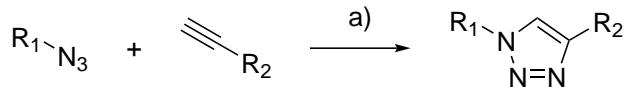


76

Figure 20: Further azido autoinducer derivatives synthesised by Howard<sup>177</sup> 75 and Baker<sup>161</sup> 76.

Synthesis of the conjugates proved more difficult than expected, for several reasons. Firstly some compounds did not dissolve in the reaction solvent (50 % water/*t*-BuOH) requiring addition of co-solvents such as CH<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 10.25.

Despite the unforeseen difficulties in synthesis of the conjugates enough material was successfully prepared for biological testing. The results of the reactions are shown in Table 5, Table 6, Table 7 and Table 8. It was intended that the failed reactions would be repeated, but as preliminary biological testing (see 7.4.4) proved unpromising it was decided that attention should be focused elsewhere.



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.

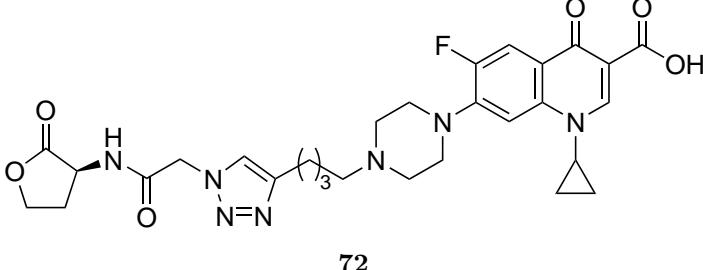
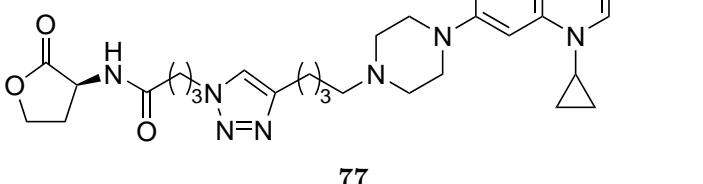
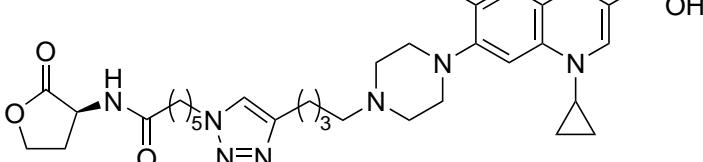
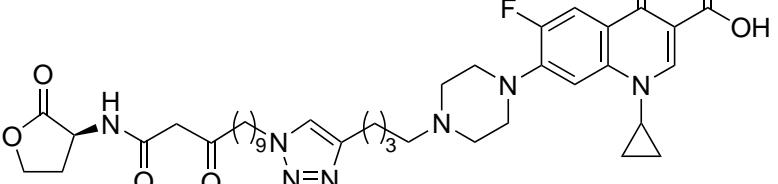
Starting materials	Product	Outcome	Yield
55 and 68	 <p>72</p>	<span style="color: green;">✓</span> Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO <sub>2</sub> , 0 - 20 % MeOH/CH <sub>2</sub> Cl <sub>2</sub> ).	29.6 %
58 and 68	 <p>77</p>	<span style="color: green;">✓</span> 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 %
61 and 68	 <p>78</p>	<span style="color: green;">✓</span> 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 %
75 and 68	 <p>79</p>	<span style="color: red;">✗</span> 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
<b>38 and 68</b>		✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	27.0 %
<b>49 and 68</b>		✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
<b>76 and 68</b>		✗ No reaction seen by LCMS.	

Table 6: Click reactions attempted.

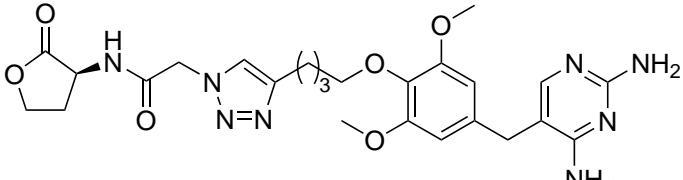
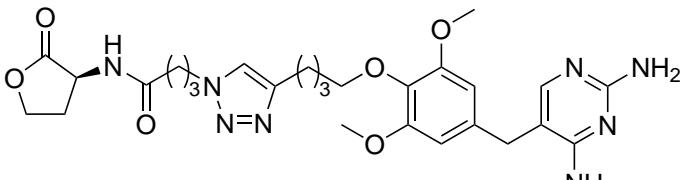
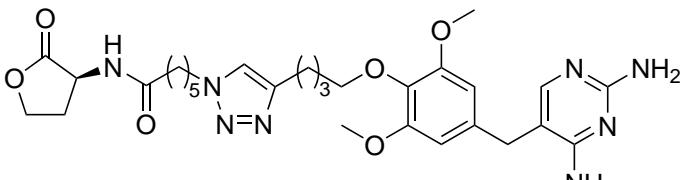
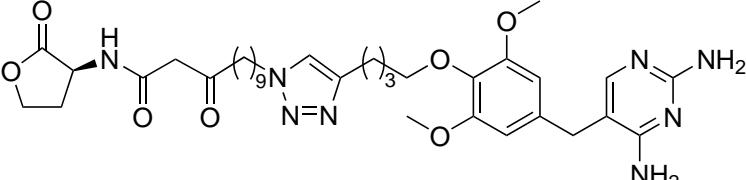
Starting materials	Product	Outcome	Yield
55 and 71	 83	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on prep. HPLC column.	
58 and 71	 84	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO2, 20 % MeOH/CH2Cl2).	16.8 %
61 and 71	 85	✓ Reaction complete by LCMS in 2 weeks (stalled). Purified by column chromatography (SiO2, 20 % MeOH/CH2Cl2).	26.8 %
75 and 71	 86	✗ Degraded during reaction.	

Table 7: Click reactions attempted.

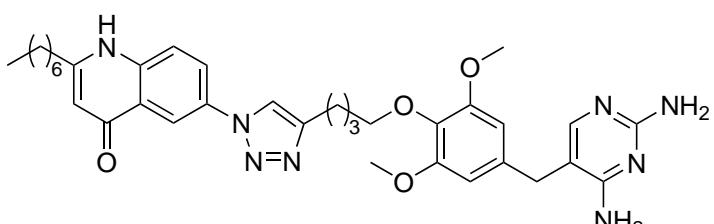
Starting materials	Product	Outcome	Yield
38 and 71	 <p style="text-align: center;">87</p>	<span style="color: green;">✓</span> Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.	41.0 %
49 and 71	<p style="text-align: center;">88</p>	<span style="color: red;">✗</span> Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.	
76 and 71	<p style="text-align: center;">89</p>	<span style="color: green;">✓</span> Reaction complete by LCMS in 3 h. Purified by column chromatography ( $\text{SiO}_2$ , 20 % $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ).	18.3 %

Table 8: Click reactions attempted.

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

In addition to the conjugates shown in the previous section, a further collection was synthesised in collaboration with Professor Eddy Sotelo, a visiting researcher in the Spring group. Professor Sotelo synthesised two alkyne-linked ciprofloxacin derivatives **90** and **91** (see Figure 21), both with cleavable linkers (see 5.3.7).

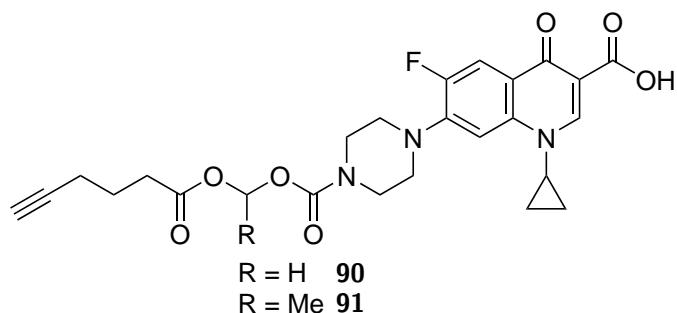


Figure 21: The cleavable alkyne-Cip derivatives synthesised by Professor Sotelo.

Professor Sotelo then performed click reactions using the AHL azide derivatives **55**, **58** and **61** shown in 7.2.3 to form a library of conjugates (see Figure 22). It was hoped that these conjugates would enter the cell and then be cleaved by esterases to release ciprofloxacin (see 5.3.7).

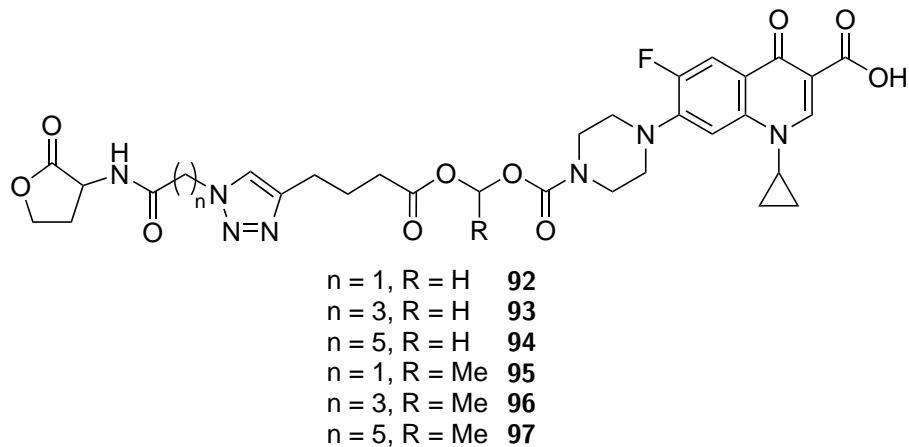


Figure 22: The cleavable HSL-Cip triazole conjugates synthesised by Professor Sotelo.

Two control compounds **98** and **99** with benzyl head groups were also produced by Professor Sotelo (see Figure 23). It was hoped that these would show whether the AHL head group is required for activity.

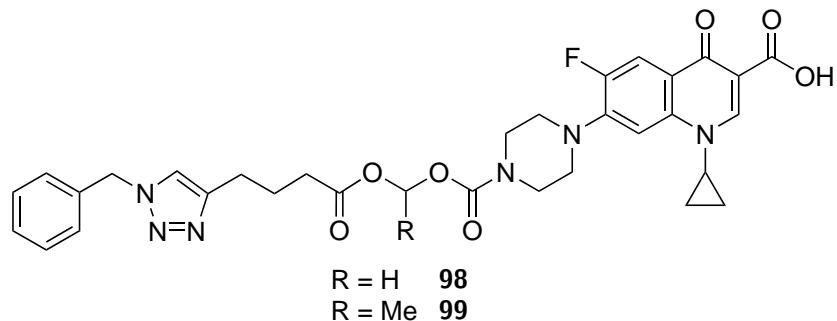


Figure 23: The cleavable Bn-Cip triazole conjugates **98** and **99** synthesised by Professor Sotelo.

#### 7.4.4 Biological testing

## 8 Results and discussion: HSL analogue-ciprofloxacin conjugates

### 8.1 Overview

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

#### 8.1.1 Head groups

The head groups used in this study are shown in Figure 24. The cyclohexanol derivatives were synthesised as a diastereomerically pure racemate, whereas the cyclopentanol derivatives were synthesised as separate enantiomers. Although the timescale of this project prevented the inclusion of the cyclopentanone derivatives, these could be included in future work. The 2-methoxybenzene derivatives do not have precedents as quorum sensing modulators in the literature, but they were included so as to be compared with the 3-methoxybenzene derivatives.

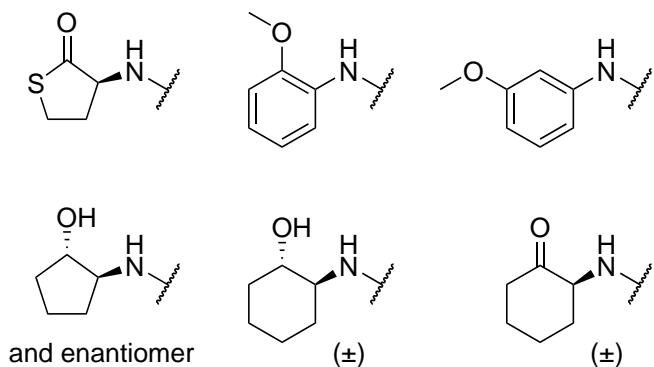
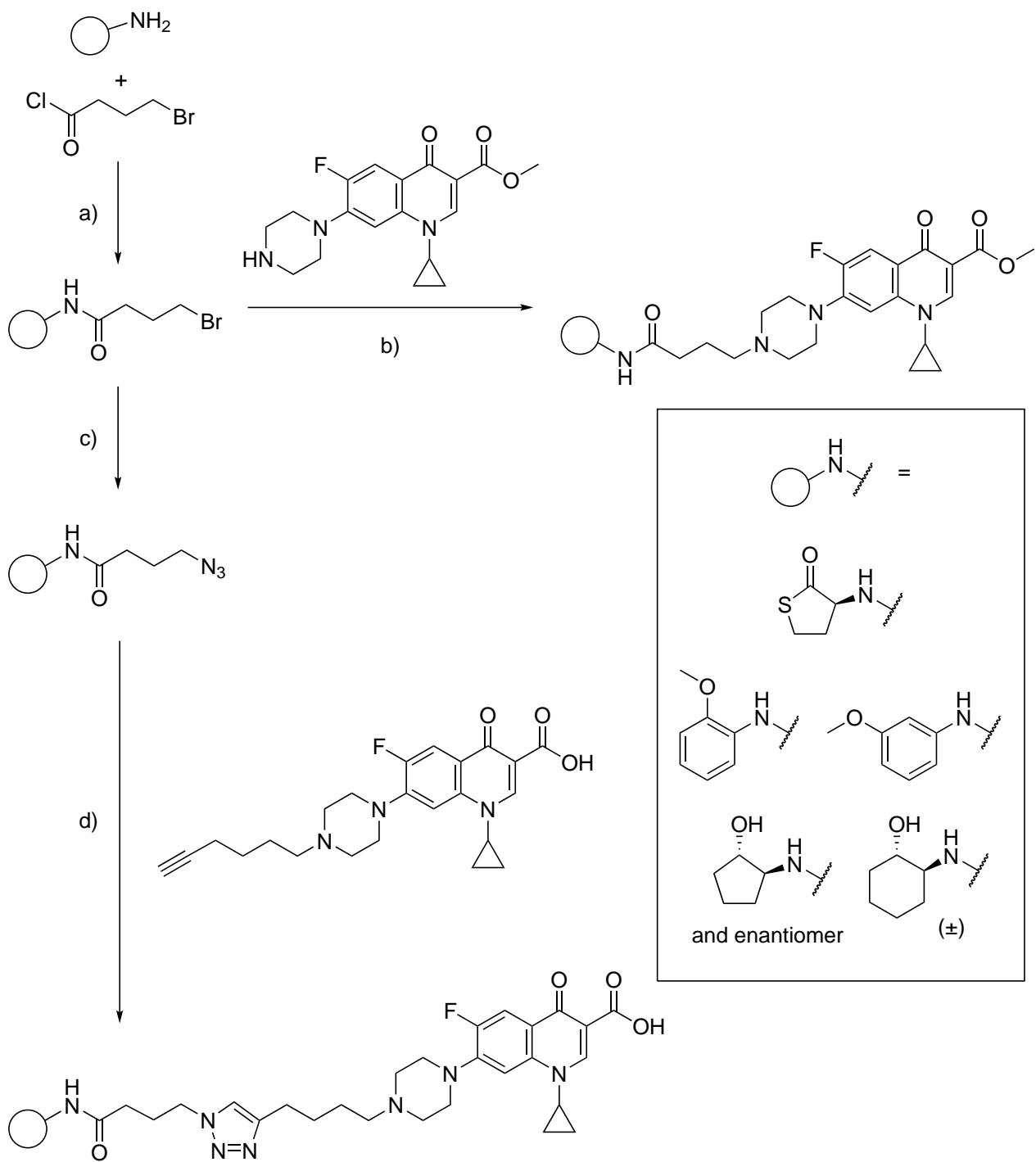


Figure 24: The head groups used in this section.

#### 8.1.2 Library construction

As Ganguly *et al.*<sup>145</sup> (see 5.3.8) 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 Scheme 11). The first set would be formed by the  $S_N2$  reaction of the relevant bromide with methyl ciprofloxacin. The second set would be made by displacing the bromide with azide, then performing a click reaction with the alkynyl ciprofloxacin derivative **68** made previously to form the triazole-linked product. Ketone conjugates would be formed by oxidation of the alcohols.



Scheme 11: General scheme showing the proposed branching synthesis of the HSL analogue-ciprofloxacin conjugates.

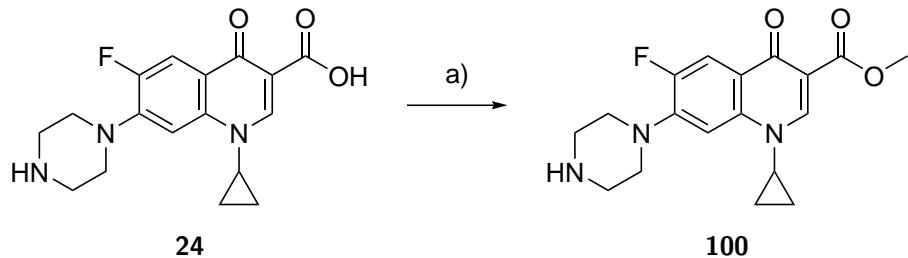
This strategy was successful for most head groups, but multiple side reactions were observed for the amino alcohol head groups and so other routes to these conjugates were investigated (see 8.5).

## 8.2 Homocysteine thiolactone derivatives

### 8.2.1 Synthesis of methyl ciprofloxacin 100

The synthesis of the analogue conjugates began with the synthesis of methyl ciprofloxacin **100** (CipMe), which would then be attached to the various head groups. Methyl ciprofloxacin **100** was synthesised from ciprofloxacin

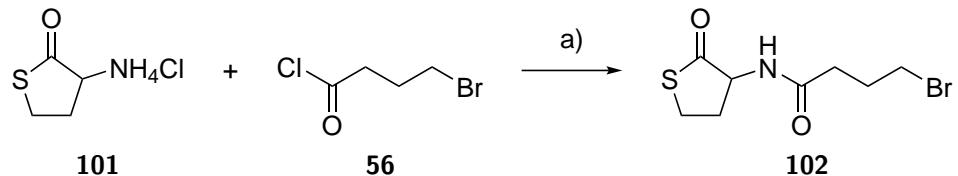
**24** and MeOH in good yield using *para*-toluenesulfonic acid (TsOH) as a catalyst.<sup>178</sup>



Scheme 12: Synthesis of methyl ciprofloxacin **100**. a) TsOH, MeOH, 72 h, reflux, 83.3 %.

### 8.2.2 Synthesis of Br-C<sub>4</sub>-HCTL 102

The HCTL head group was then attached to the linker to form Br-C<sub>4</sub>-HCTL **102**, in preparation for coupling to methyl ciprofloxacin **100**. Br-C<sub>4</sub>-HCTL **102** was synthesised using the Schotten-Baumann conditions employed previously for the HSL derivatives **57** and **60**. Br-C<sub>4</sub>-HCTL **102** was isolated in markedly higher yield than that achieved by Ganguly *et al.*<sup>145</sup> (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.

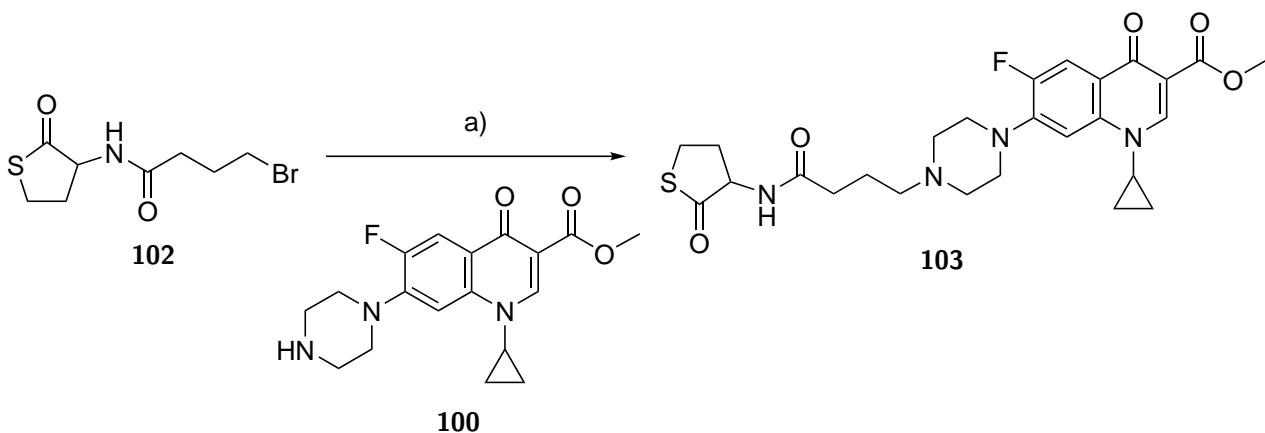


Scheme 13: Synthesis of Br-C<sub>4</sub>-HCTL **102**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 1 h, 87.9 %.

### 8.2.3 Synthesis of the HCTL-CipMe conjugate 103

The HCTL-CipMe conjugate **103** was synthesised using the procedure outlined by Ganguly *et al.*<sup>145</sup> (see Scheme 14). Monitoring by LCMS showed slow conversion to the product. Br-C<sub>4</sub>-HCTL **102** was presumably consumed by side reactions as 4 eq. were required to reach full conversion. A likely potential side reaction is internal cyclisation of the bromide with the amide NH, and the mass of this molecule was observed by LCMS in the reaction mixture.

Ganguly *et al.* do not quote a yield for this reaction,<sup>145, 146</sup> 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 **103** for biological testing.

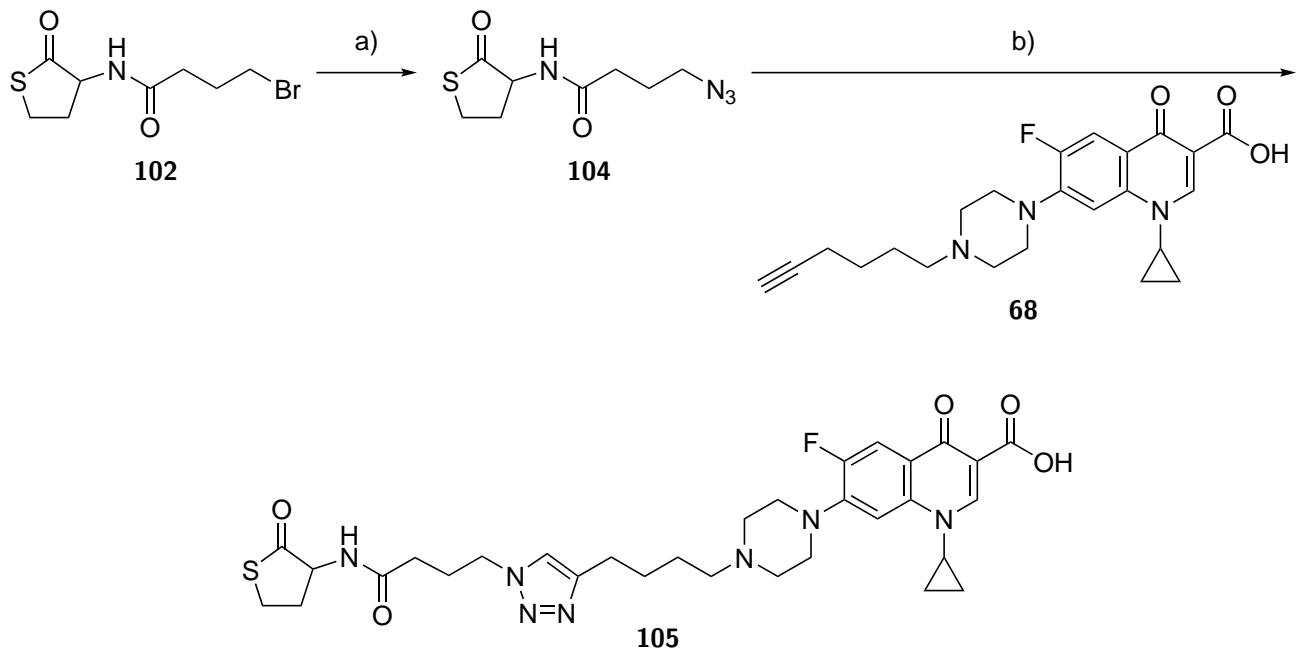


Scheme 14: Synthesis of the HCTL-CipMe conjugate **103**. a)  $\text{K}_2\text{CO}_3$ , acetonitrile, reflux, 24 h, 12.2 %.

#### 8.2.4 Synthesis of the HCTL-Cip triazole conjugate **105**

Br-C<sub>4</sub>-HCTL **102** was converted into N<sub>3</sub>-C<sub>4</sub>-HCTL **104** (see Scheme 14), by an S<sub>N</sub>2 reaction with sodium azide which proceeded in excellent yield.

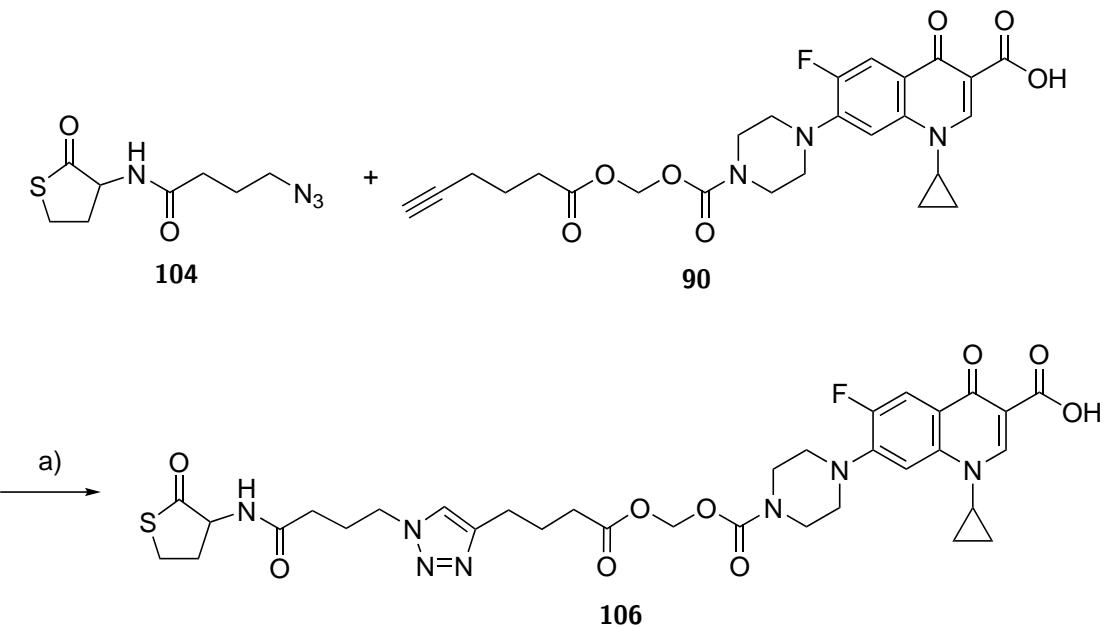
N<sub>3</sub>-C<sub>4</sub>-HCTL **104** was then subjected to the click reaction conditions optimised previously (see 10.25). The reaction proceeded very slowly at first, as the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. Nonetheless, the HCTL-Cip triazole conjugate **105** was isolated in good yield (see Scheme 15).



Scheme 15: Synthesis of the HCTL-Cip triazole conjugate **105**. a)  $\text{NaN}_3$ , acetonitrile, reflux, 1.5 h, 89.3 %. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate,  $\text{H}_2\text{O}$ , *t*-BuOH, DMSO, r.t., 7 d, 70.6 %.

#### 8.2.5 Synthesis of the cleavable HCTL-Cip triazole conjugate **106**

A cleavable conjugate **106** (see Scheme 16) was also synthesised from N<sub>3</sub>-C<sub>4</sub>-HCTL **104** by reaction with a cleavable alkyne-Cip derivative **90** synthesised previously by Professor Eddy Sotelo (see 7.4.3).



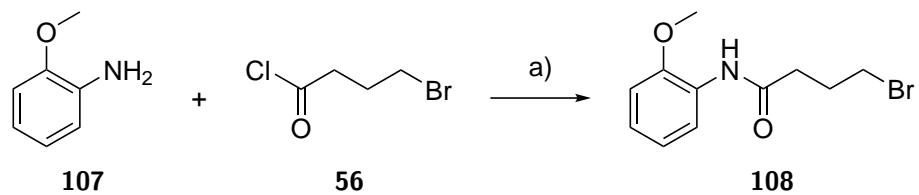
Scheme 16: Synthesis of the cleavable HCTL-Cip triazole conjugate **106**. a) CuI, DIPEA,  $\text{CH}_2\text{Cl}_2$ , r.t., 3 h, 5.0 %.

### 8.3 2-Methoxybenzene derivatives

### 8.3.1 Synthesis of Br-C<sub>4</sub>-2-methoxybenzene 108

Br-C<sub>4</sub>-2-methoxybenzene **108** was synthesised from 2-methoxyaniline **107** and 4-bromobutyryl chloride **56** using Schotten-Baumann conditions in 50.0 % yield (see Scheme 17). Br-C<sub>4</sub>-2-methoxybenzene **108**, like all other 2- and 3-methoxyaniline derivatives mentioned below, appears to be air and/or light sensitive, turning from an initially colourless liquid to blue then black if left out on the bench. It is possible that this sensitivity is due to oxidative polymerisation of the aniline.<sup>179</sup>

It is likely that the mediocre yield of Br-C<sub>4</sub>-2-methoxybenzene **108** is due to degradation during columning, and it is therefore 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.

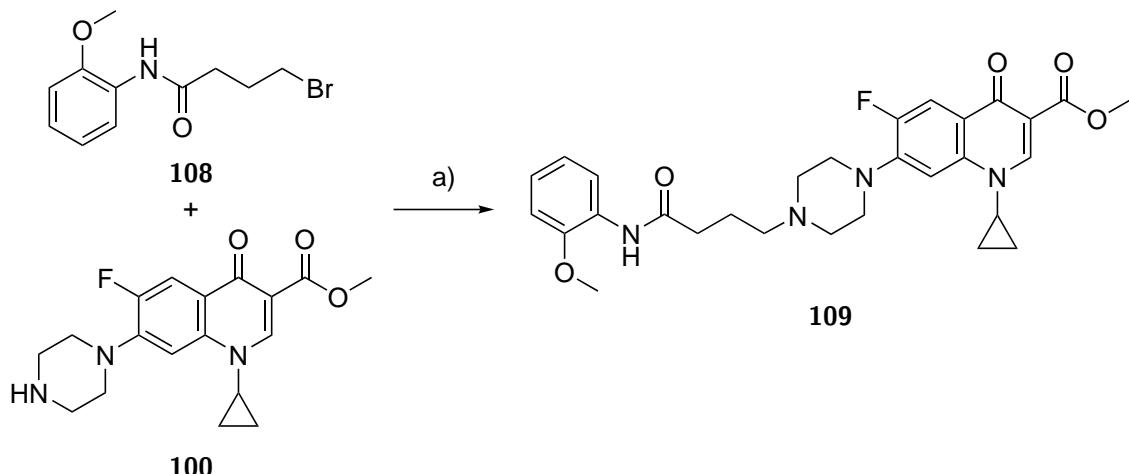


Scheme 17: Synthesis of Br-C<sub>4</sub>-2-methoxybenzene **108**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 1 h, 50.0 %.

### 8.3.2 Synthesis of the 2-methoxybenzene-CipMe conjugate 109

The procedure outlined by Ganguly *et al.*<sup>145</sup> was initially attempted in order to synthesise the 2-methoxybenzene-CipMe conjugate **109**, but the reaction was very slow and did not go to completion, presumably due to degradation of Br-C<sub>4</sub>-2-methoxybenzene **108**. New conditions, employing a microwave reactor and 2 eq. of Br-C<sub>4</sub>-2-methoxybenzene **108** were then attempted, with a much greater conversion observed by LCMS after 4 h (see Scheme 18). However, a poor yield was obtained, again potentially due to degradation during column

chromatography.



Scheme 18: Synthesis of the 2-methoxybenzene-CipMe conjugate **109**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C, 4 h, 10.2 %.

### 8.3.3 Synthesis of the 2-methoxybenzene-Cip triazole conjugate **111**

$\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{110}$  was synthesised from  $\text{Br-C}_4\text{-2-methoxybenzene } \mathbf{108}$  by an  $\text{S}_{\text{N}}2$  reaction with sodium azide (see ??). The yield of  $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{110}$  (26.7 %) was a lot lower than for  $\text{N}_3\text{-C}_4\text{-HCTL } \mathbf{104}$  (89.3 %). The colour of  $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{110}$ , like its precursor, changed from clear to blue then black, suggesting that it is also air/light sensitive. However, in this case it may not be better to use this product crude as several additional impurities, apparently caused by  $\text{S}_{\text{N}}2$  reactions at the bromide, could be observed by LCMS (see Figure 25).

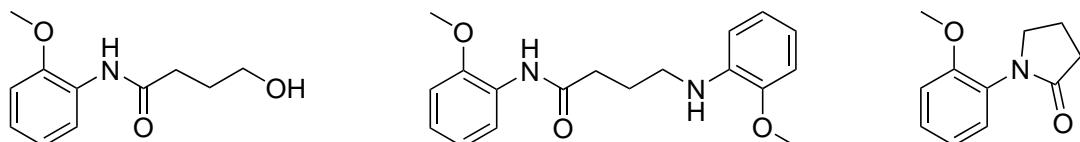
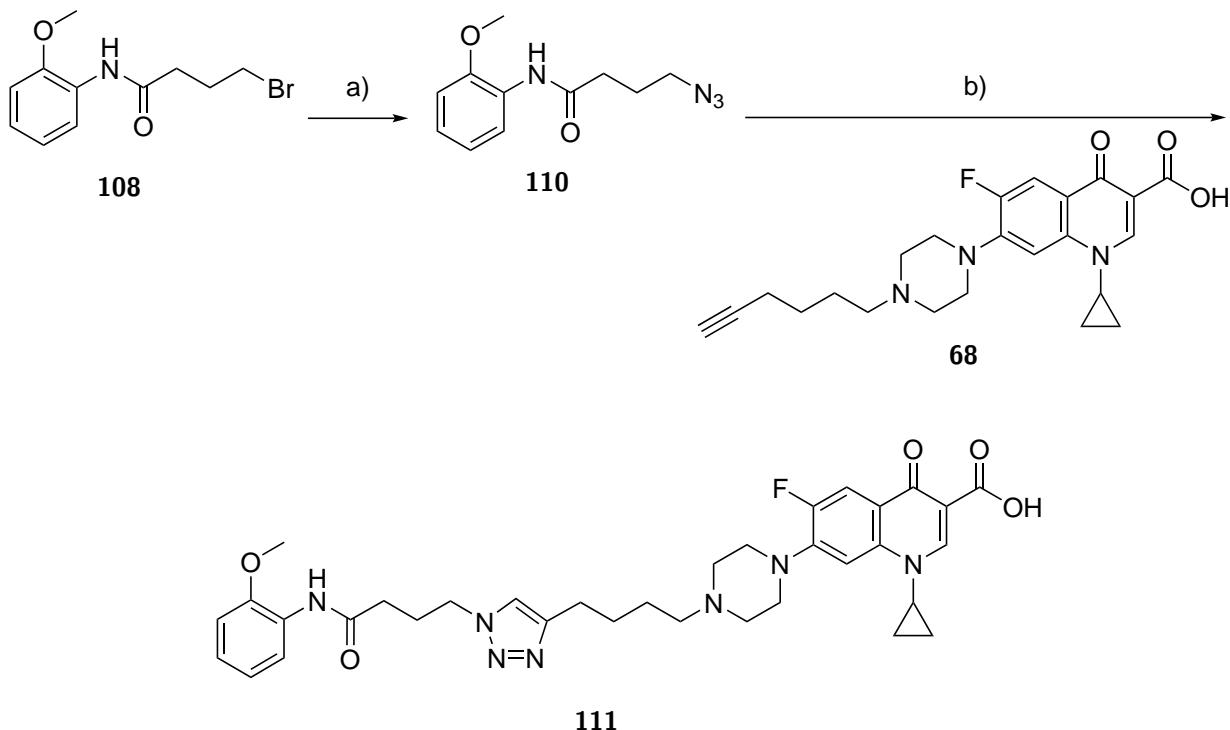


Figure 25: Suspected impurities observed by LCMS during the synthesis of  $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{110}$ .

The 2-methoxybenzene-Cip triazole conjugate **111** was synthesised using the standard click conditions (see 10.25), with the addition of  $\text{CH}_2\text{Cl}_2$  as a co-solvent to aid the dissolution of  $\text{N}_3\text{-C}_4\text{-2-methoxybenzene } \mathbf{110}$  (see Scheme 19). Again, the yield was low, probably due to air/light sensitivity of the starting material and/or product.

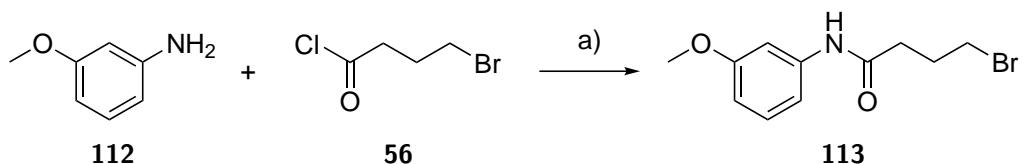


Scheme 19: Synthesis of the 2-methoxybenzene-Cip triazole conjugate **111**. a)  $\text{NaN}_3$ , acetonitrile, reflux, 2 h, 26.7 %. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate,  $\text{H}_2\text{O}$ , *t*-BuOH,  $\text{CH}_2\text{Cl}_2$ , r.t., 16 h, 39.0 %.

## 8.4 3-Methoxybenzene derivatives

#### 8.4.1 Synthesis of Br-C<sub>4</sub>-3-methoxybenzene 113

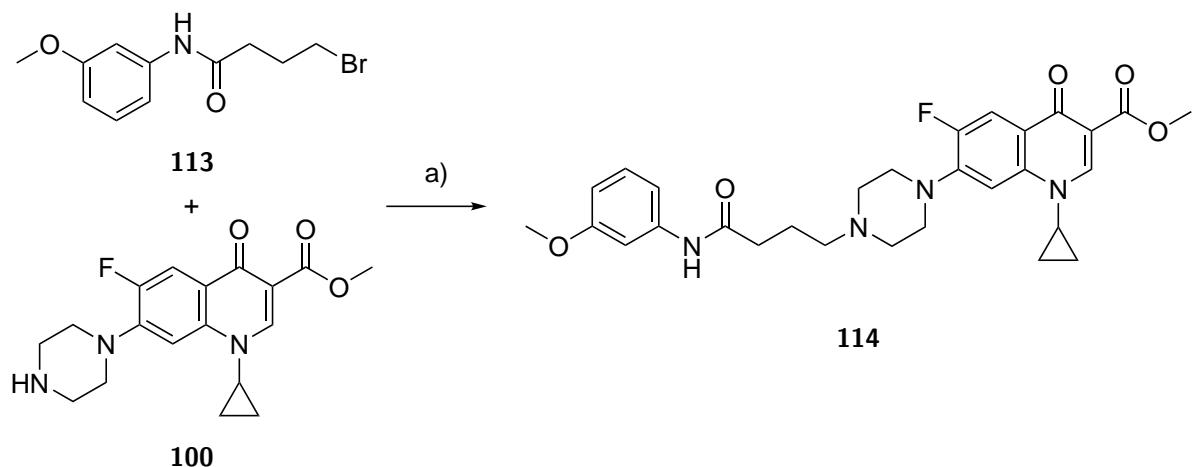
Br-C<sub>4</sub>-3-methoxybenzene **113** was synthesised from 3-methoxyaniline **112** and 4-bromobutyryl chloride **56** using Schotten-Baumann conditions as above in almost identical (49.6 %) yield (see Scheme 20). The compound is probably also air and/or light sensitive, turning from a pale pink amorphous solid to a pale brown liquid.



Scheme 20: Synthesis of Br-C<sub>4</sub>-3-methoxybenzene **108**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 1 h, 49.6 %.

#### 8.4.2 Synthesis of the 3-methoxybenzene-CipMe conjugate 114

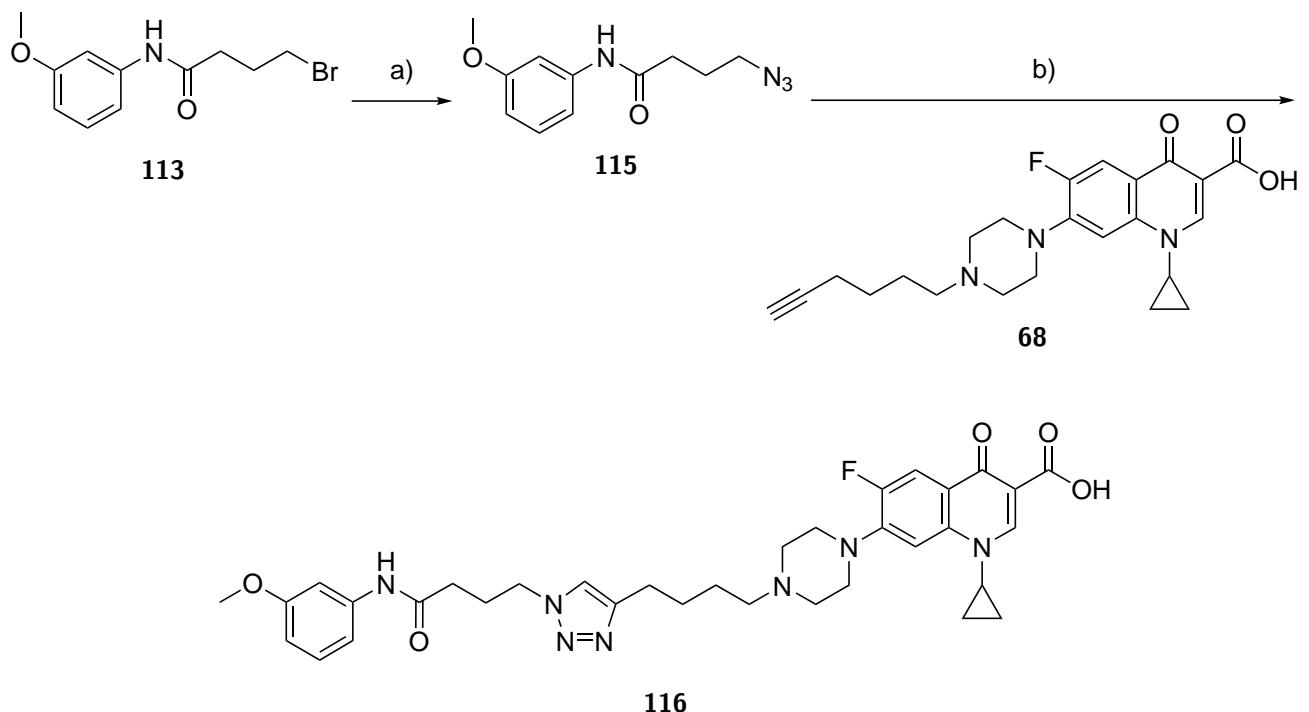
The 3-methoxybenzene-CipMe conjugate **114**, was synthesised as above, in similar yield (see Scheme 21).



Scheme 21: Synthesis of the 3-methoxybenzene-CipMe conjugate **114**. a)  $\text{NaI}$ , DIPEA, acetonitrile, microwave reactor,  $100\text{ }^\circ\text{C}$ , 4 h, 10.5 %.

#### 8.4.3 Synthesis of the 3-methoxybenzene-Cip triazole conjugate **116**

$\text{N}_3\text{-C}_4\text{-2-methoxybenzene}$  **110** and the 3-methoxybenzene-Cip triazole conjugate **116** were synthesised as above, in similar yields (see Scheme 21 and Scheme 22).

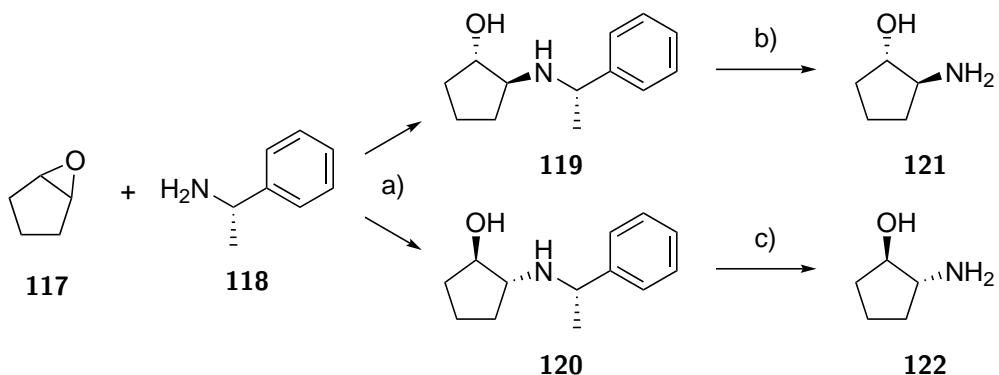


Scheme 22: Synthesis of the 3-methoxybenzene-Cip triazole conjugate **116**. a)  $\text{NaN}_3$ , acetonitrile, reflux, 7 h, 16.7 %. b)  $\text{CuSO}_4$ , THPTA, sodium ascorbate,  $\text{H}_2\text{O}$ ,  $t\text{-BuOH}$ ,  $\text{CH}_2\text{Cl}_2$ , r.t., 2 h, 5.0 %.

## 8.5 Cyclopentanol derivatives

### 8.5.1 Synthesis of the 2-aminocyclopentan-1-ol head groups **121** and **122**

Synthesis of the cyclopentanol derivatives began with the synthesis of (*1S,2S*)-2-aminocyclopentan-1-ol **121** and (*1R,2R*)-2-aminocyclopentan-1-ol **122** (see Scheme 23), using a procedure reported by Overman and Sugai.<sup>180–182</sup> These precursors were synthesised by opening cyclopentene oxide **117** using (*S*)-1-phenylethanimine **118** to give approximately equal amounts of two diastereomers, **119** and **120**, 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<sup>181</sup> 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 Table 9). This proceeded smoothly at 5 atm to give the two enantiomers of 2-aminocyclopentan-1-ol, **121** and **122**, both in quantitative yield.



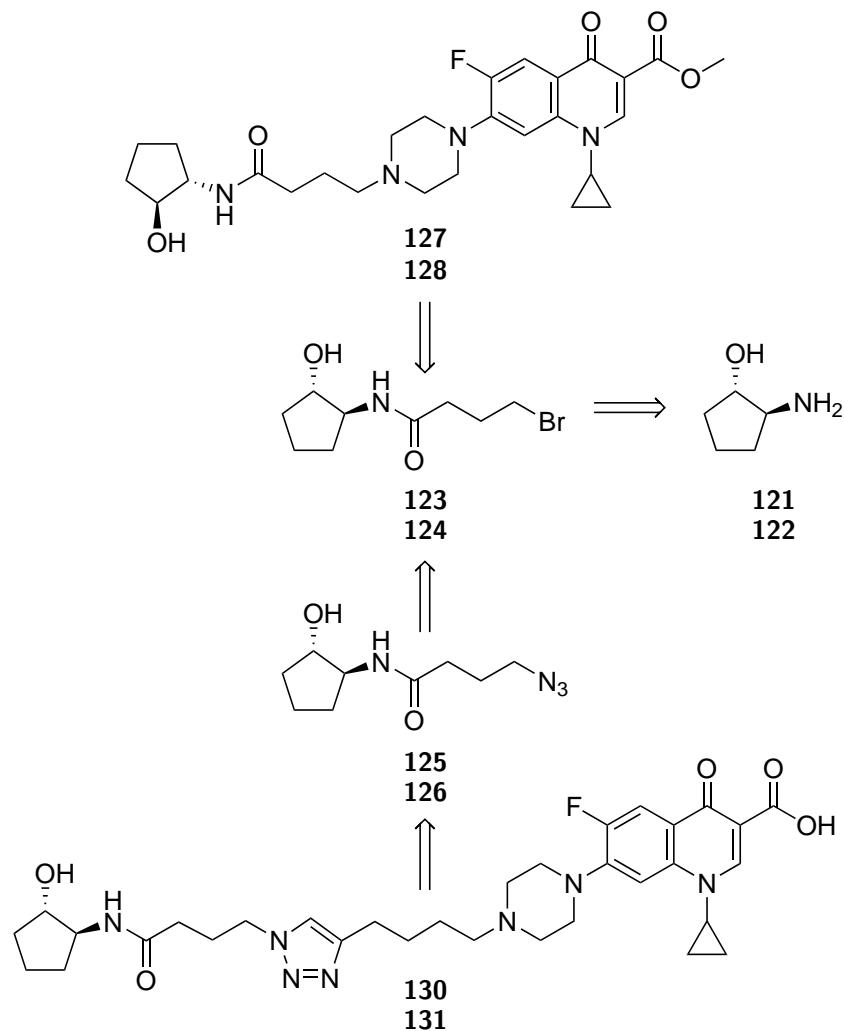
Scheme 23: Synthesis of (*1S,2S*)-2-aminocyclopentan-1-ol **121** and (*1R,2R*)-2-aminocyclopentan-1-ol **122**. a) AlMe<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, **119** (*SSS*): 35.2 %, **120** (*RRS*): 32.1 %. b) See Table 9. c) Pd(OH)<sub>2</sub>, MeOH, H<sub>2</sub>, 5 atm, r.t., 1 d, 100 %.

Conditions	Temperature and pressure	Time	Result
<b>119</b> · HCl, ammonium formate, 10 % Pd/C, DMF	r.t., 1 atm	2 d	<b>119</b> salt
<b>119</b> , ammonium formate, 10 % Pd/C, DMF	r.t., 1 atm	2 d	<b>119</b> salt
<b>119</b> · HCl, ammonium formate, 10 % Pd/C, dry DMF	r.t., 1 atm	2 d	<b>119</b> salt
<b>120</b> , ammonium formate, 10 % Pd/C, dry DMF	r.t., 1 atm	2 d	<b>120</b> salt
<b>119</b> , ammonium formate, 10 % Pd/C, dry DMF	70 °C, 1 atm	1 d	<b>119</b> salt
<b>119</b> , ammonium formate, 10 % Pd/C, dry DMF, AcOH	70 °C, 1 atm	1 d	Complex mixture
<b>119</b> · HCl, dry ammonium formate, 10 % Pd/C, dry DMF	120 °C, 1 atm	7 d	Complex mixture
<b>119</b> · HCl, Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 1 atm	1 d	<b>119</b> salt
<b>119</b> · HCl, Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 3.4 atm	1 d	<b>121</b> salt, <b>119</b> salt, and an unidentified compound (approx. 7:2:10 by <sup>1</sup> H NMR)
<b>119</b> , Pd(OH) <sub>2</sub> , MeOH, H <sub>2</sub>	r.t., 5 atm	1 d	<b>121</b> , 100 % yield

Table 9: Conditions attempted for the synthesis of (1*S*,2*S*)-2-aminocyclopentan-1-ol **121** and (1*R*,2*R*)-2-aminocyclopentan-1-ol **122** (see Scheme 23).

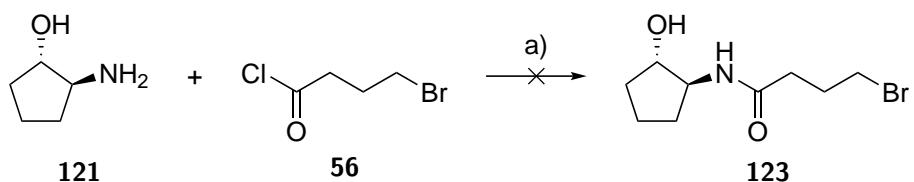
### 8.5.2 Initial branching route

An initial retrosynthesis of the conjugates is shown in Scheme 24, and follows a similar path to previous conjugates.



Scheme 24: Retrosynthetic of the cyclopentanol-CipMe conjugates **127** (*SS*) and **128** (*RR*), and the cyclopentanol-Cip triazole conjugates **130** (*SS*) and **131** (*RR*). *SS* enantiomers are shown, but both are implied.

Synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **123** from (1*S*,2*S*)-2-aminocyclopentan-1-ol **121** and 4-bromobutyryl chloride **56** was attempted using Schotten-Baumann conditions (see Scheme 25). However, a large number of impurities were observed by LCMS (see Figure 26), and so three new strategies were attempted: protection of the alcohol (see 8.5.3), using 4-chlorobutyryl chloride **142** as the linker instead of 4-bromobutyryl chloride **56** (see 8.5.4), and installing the linker on methyl ciprofloxacin **100** and then attaching the head group by peptide coupling (see 8.5.5).



Scheme 25: Attempted synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **123**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 2 h.

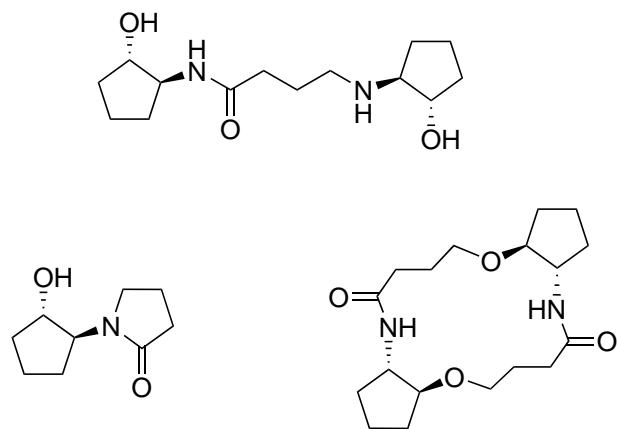
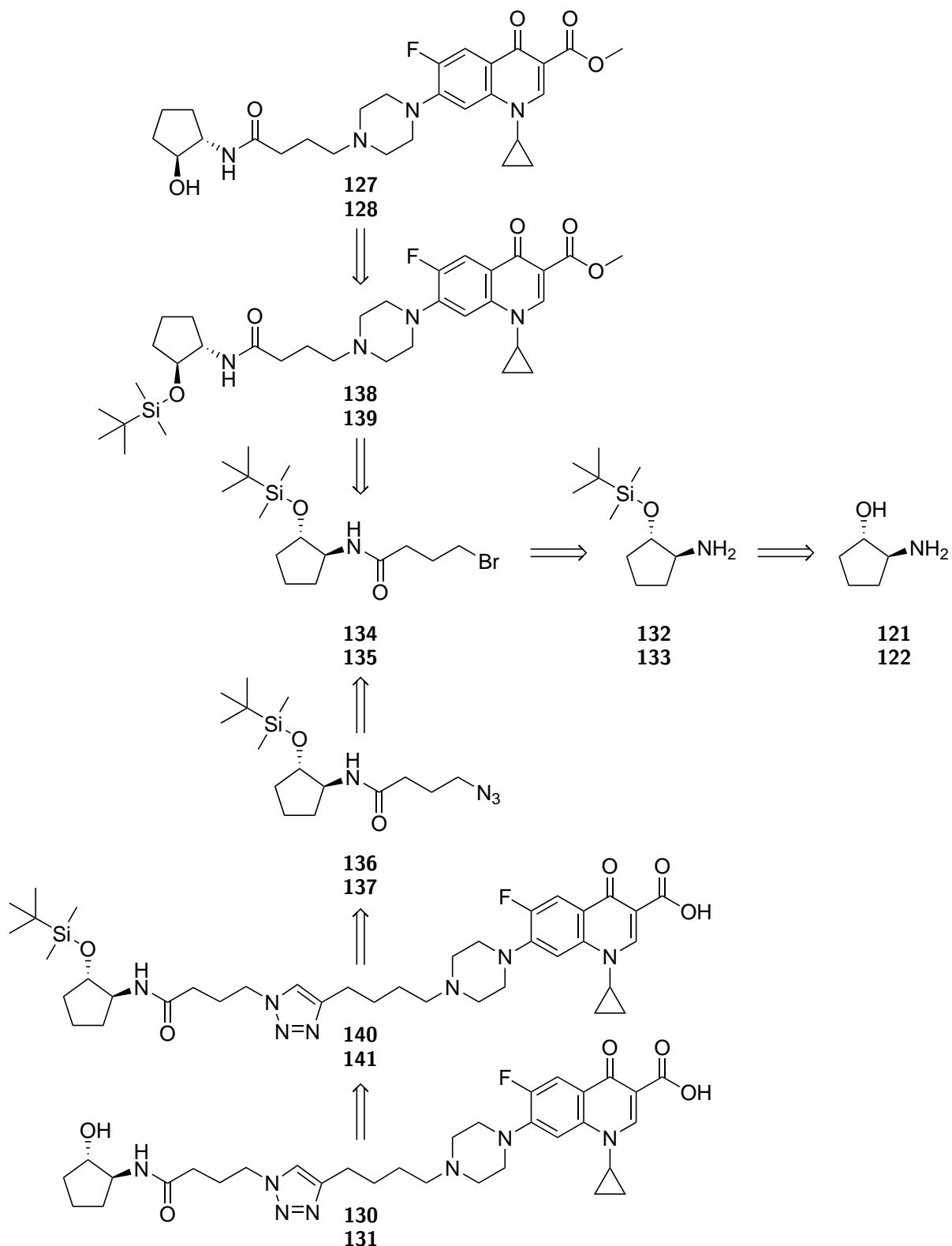


Figure 26: Suspected impurities observed by LCMS during the synthesis of Br-C<sub>4</sub>-cyclopentanol-(*SS*) **123**. Regiochemistry is speculative.

### 8.5.3 TBDMS protection route

The first attempt at an alternative strategy for the synthesis of the conjugates involved TBDMS protection of the alcohol (see Scheme 26). It was envisaged that protection would eliminate enough of the side reactions with products shown in Figure 26 that intermediates Br-C<sub>4</sub>-cyclopentanol-(*SS*) **123** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **125** could be purified. The TBDMS group could be removed later in the synthesis using TBAF or acid.

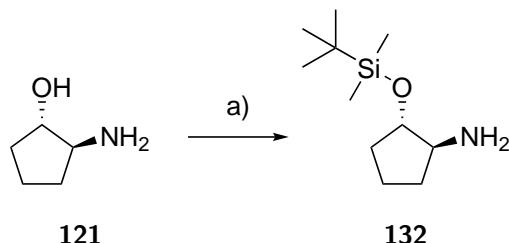


Scheme 26: Retrosynthetic analysis of the cyclopentanol-CipMe conjugates **127** (*SS*) and **128** (*RR*), and the cyclopentanol-Cip triazole conjugates **130** (*SS*) and **131** (*RR*) using a TBDMS protection strategy. *SS* enantiomers are shown, but both are implied.

#### 8.5.3.1 Synthesis of TBDMS-protected (*1S,2S*)-2-aminocyclopentan-1-ol **121**

The synthesis began with the optimisation of the protection of (*1S,2S*)-2-aminocyclopentan-1-ol **121** with a TBDMS group on the alcohol (see Scheme 28). This reaction proved more problematic than expected, possibly

due to the amine group interfering with the reaction at the alcohol and/or the high polarity of the starting material causing problems with solubility in the reaction mixture and extraction during the work-up. Conditions attempted are summarised in Table 10. Protection attempts using TBDMSCl were generally unsuccessful, but eventually a method employed by Wu et. al<sup>183</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.



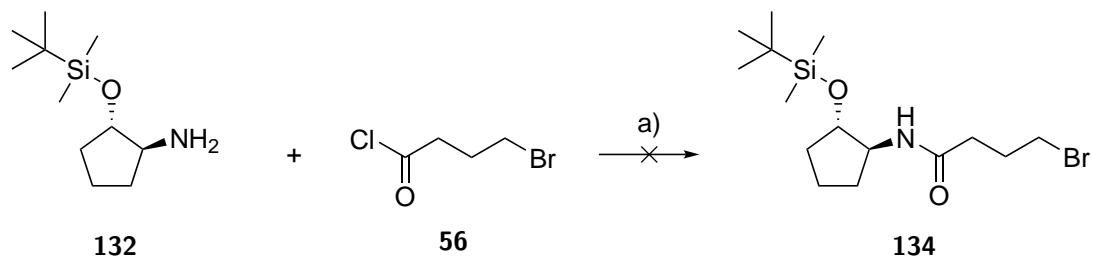
Scheme 27: Synthesis of TBDMS protected (*1S,2S*)-2-aminocyclopentan-1-ol **132**. a) See Table 10.

Conditions	Temperature	Time	Result
TBDMSCl, DMAP, TEA, $\text{CH}_2\text{Cl}_2$ <sup>184</sup>	r.t.	18 h	Trace of <b>132</b> , mostly <b>121</b>
TBDMSCl, imidazole, $\text{CH}_2\text{Cl}_2$ <sup>185</sup>	0 °C	1 h	<b>121</b>
TBDMSCl, DBU, acetonitrile <sup>186</sup>	0 °C	1 d	<b>121</b>
TBDMSOTf, TEA, $\text{CH}_2\text{Cl}_2$ , <sup>183</sup> aq. workup then column	0 °C	6 h	<b>132</b> , 97.7 % yield

Table 10: Conditions attempted for the synthesis of  $(1S,2S)$ -2-((*tert*-butyldimethylsilyl)oxy)cyclopentan-1-amine **132** (see Scheme 28).

### 8.5.3.2 Synthesis of Br-C<sub>4</sub>-cyclopentanol-TBDMS-(SS) 134

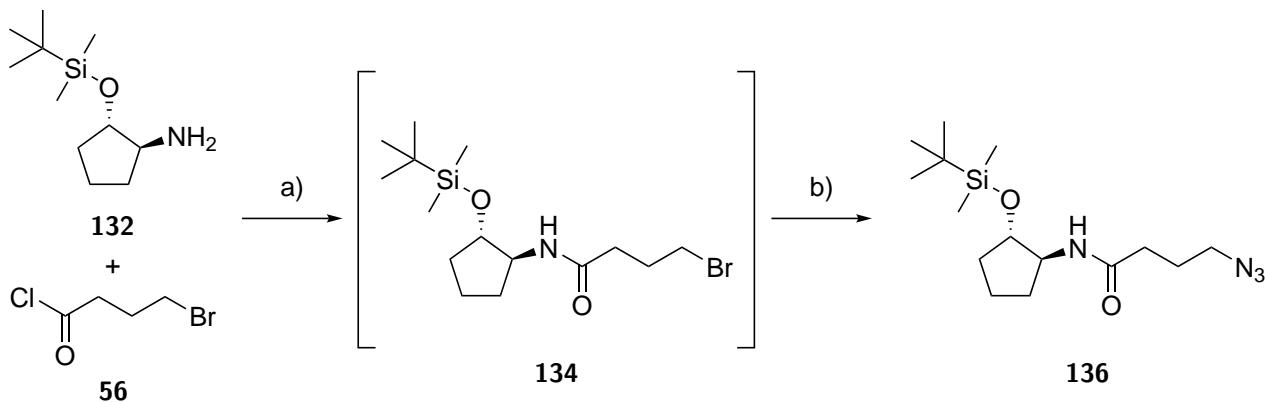
The TBDMS protected (1*S*,2*S*)-2-aminocyclopentan-1-ol **132** was reacted with 4-bromobutyryl chloride **56** to form Br-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **134**. The reaction was observed to go to completion by TLC, but it became apparent that the product was reacting further during concentration 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*) **134** to N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-TBDMS-(*SS*) **136**. 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) **134**. a) NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 2 h.

### 8.5.3.3 Synthesis of $\text{N}_3\text{-C}_4\text{-cyclopentanol-TBDMS-(SS) 136}$ by one-pot reaction

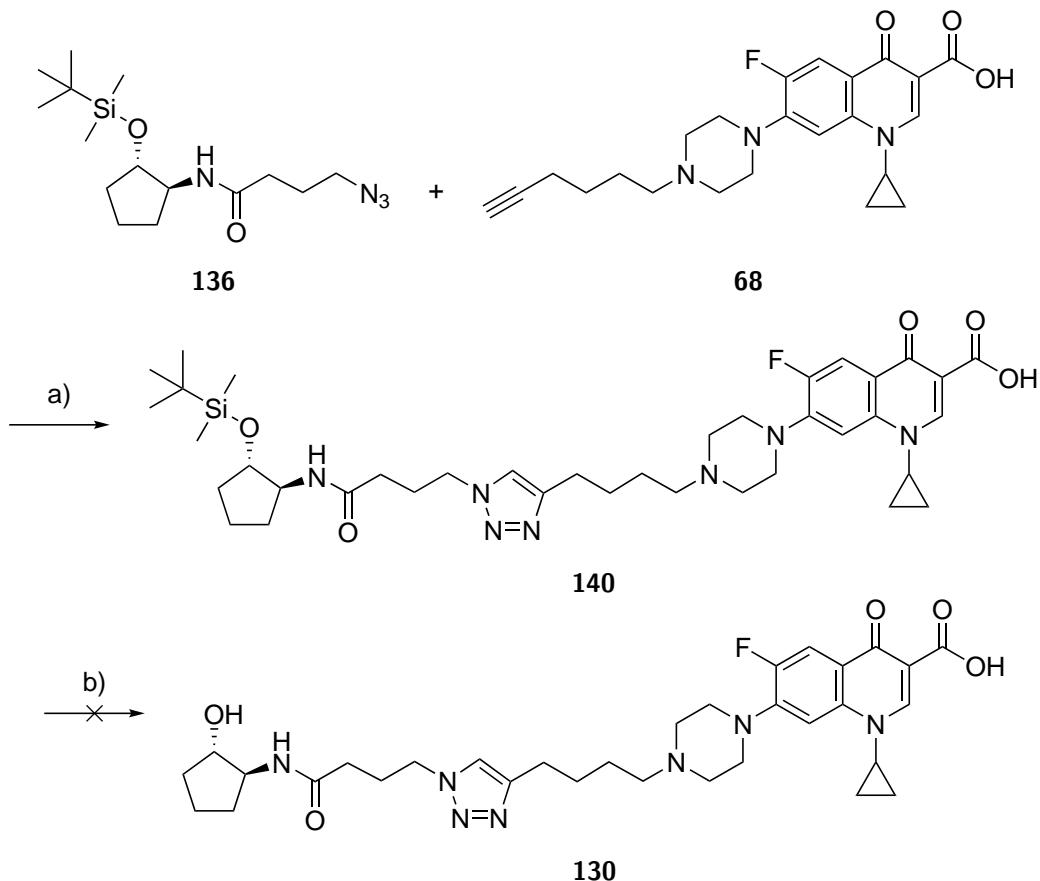
$\text{N}_3\text{-C}_4\text{-cyclopentanol-TBDMS-(SS) 136}$  was finally synthesised by a two-step, one-pot reaction. Schotten-Baumann conditions were used to form the bromide. The water was then removed, and DMF and sodium azide were added.  $\text{N}_3\text{-C}_4\text{-cyclopentanol-TBDMS-(SS) 136}$  was produced in excellent yield.



Scheme 29: Synthesis of  $\text{N}_3\text{-C}_4\text{-cyclopentanol-TBDMS-(SS) 136}$ . a)  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ ,  $0\text{ }^\circ\text{C}$ , 3 h. b)  $\text{NaN}_3$ , DMF,  $\text{CH}_2\text{Cl}_2$ , r.t., 3 h. 99.2 % over 2 steps.

### 8.5.3.4 Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate 140

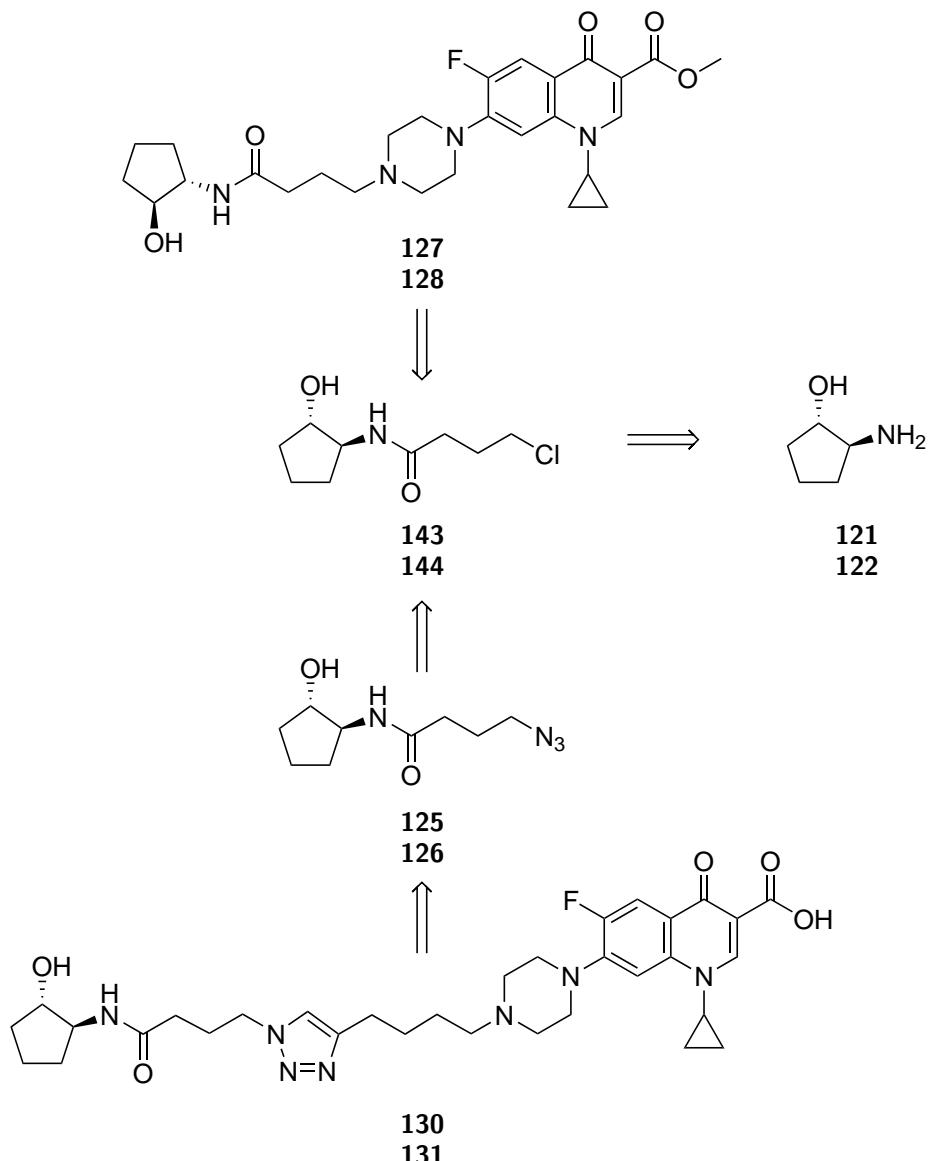
$\text{N}_3\text{-C}_4\text{-cyclopentanol-TBDMS-(SS) 136}$  and the alkynyl ciprofloxacin derivative 68 were subjected to standard click conditions (see 10.25), and the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate 140 was synthesised in very good yield. However, removal of the TBDMS group proved 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 130 by column chromatography was not successful due to streaking and poor separation. Purification using DOWEX resin and  $\text{CaCO}_3$ <sup>187</sup> was attempted, but the product could not be recovered from the resin. The purification method could probably be optimised, e.g. by varying the solvent used with the resin, but ultimately this route was abandoned due to the reduction in number of steps afforded by the two methods described below.



Scheme 30: Synthesis of the (SS)-TBDMS-cyclopentanol-Cip triazole conjugate **140**. a)  $\text{CuSO}_4$ , sodium ascorbate, THPTA,  $\text{H}_2\text{O}$ , *t*-BuOH, r.t., 87.4 %. b) TBAF, THF, r.t., 16 h.

#### 8.5.4 Synthesis of the cyclopentanol-Cip triazole conjugates **130** and **131** via chloride intermediates

Given that the side product formation seen in the previous sections was most likely due to  $\text{S}_{\text{N}}2$  attack on the bromide, we decided to use a chloride rather than a bromide intermediate (see Scheme 24 and Scheme 31 to compare). The bromide intermediate was initially chosen as it was used by Ganguly et. al,<sup>145</sup> but it was anticipated that using a chloride would reduce the side reactions seen with the more reactive bromide.



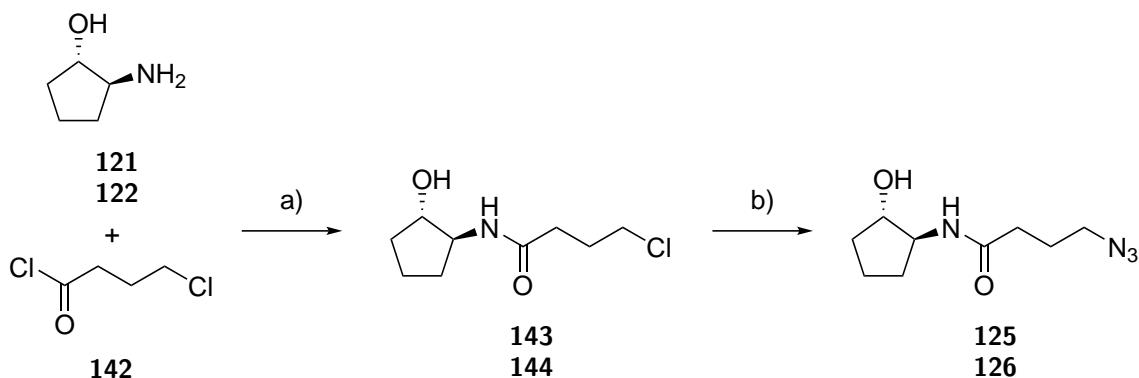
Scheme 31: Retrosynthesis of the cyclopentanol-CipMe conjugates **127** (*SS*) and **128** (*RR*), and the cyclopentanol-Cip triazole conjugates **130** (*SS*) and **131** (*RR*) via Cl-C<sub>4</sub>-cyclopentanol intermediates **143** (*SS*) and **144** (*RR*). *SS* enantiomers are shown, but both are implied.

Attempts at this route began with the synthesis of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **144**. 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>188</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 **122** 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*) **144** in good yield (64.1 %). Unlike the bromide **123**, the chloride **144** was stable when concentrated.

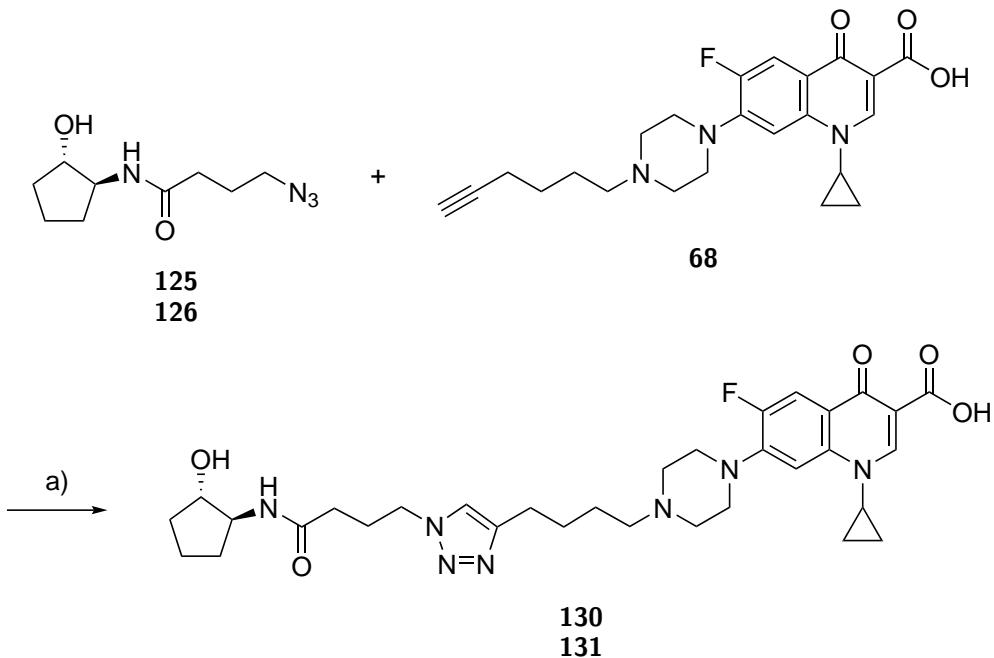
Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **144** was converted to N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) **126** by reaction with sodium azide. The reaction was slower than with previous bromides (~16 h vs. ~2 h), but much cleaner than with Br-C<sub>4</sub>-cyclopentanol-(*SS*) **123** (see 8.5.2).

The enantiomers Cl-C<sub>4</sub>-cyclopentanol-(*SS*) **143** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **125** were synthesised in lower yields, in part because of the smaller amounts being used.



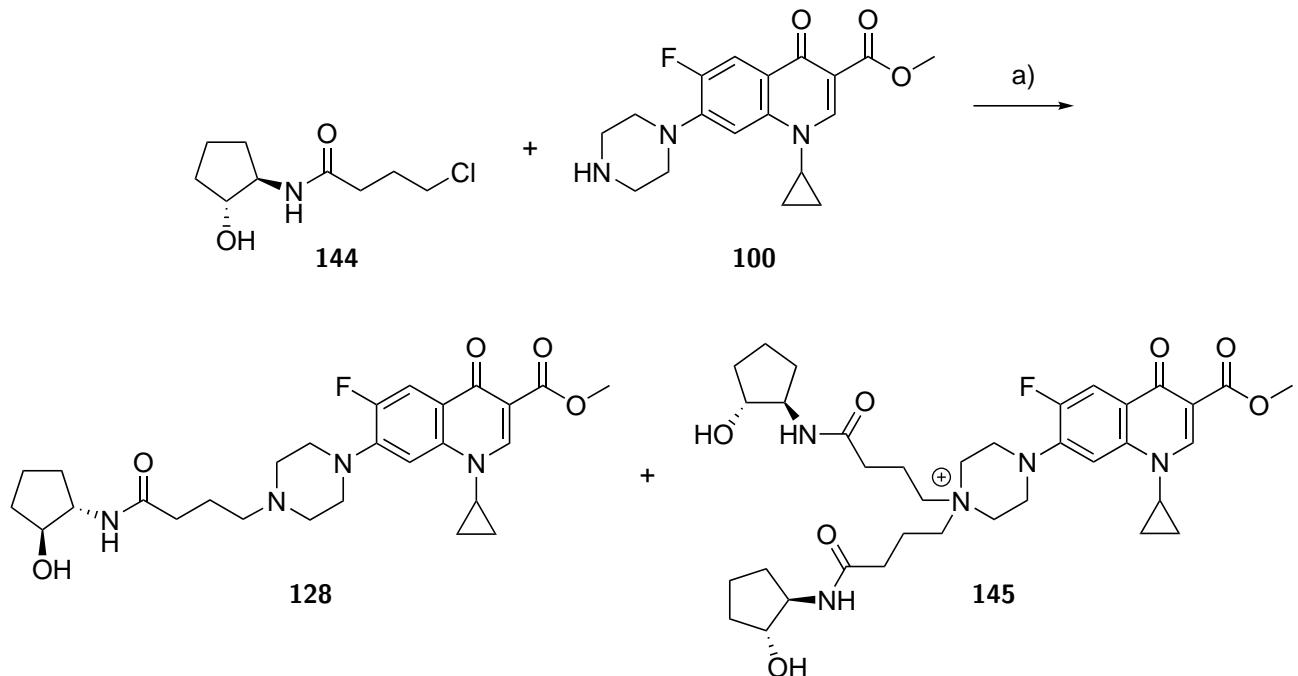
Scheme 32: Synthesis of N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*SS*) **125** and N<sub>3</sub>-C<sub>4</sub>-cyclopentanol-(*RR*) **126**. *SS* enantiomers are shown, but both were synthesised. a) TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h, **143** (*SS*): 24.2 %, **144** (*RR*): 64.1 %. b) NaN<sub>3</sub>, acetonitrile, 50 °C, 16 h, **125** (*SS*): 45.0 %, **126** (*RR*): 87.6 %.

The cyclopentanol-Cip triazole conjugates **130** (*SS*) and **131** (*RR*) were successfully synthesised using standard click conditions (see 10.25). Despite low yields (presumably due to problems with purification, including losses on the preparative HPLC column and high polarity leading to losses during extraction from aqueous solvents) enough of the compounds were obtained for biological testing so the purification was not optimised further.



Scheme 33: Synthesis of the cyclopentanol-Cip triazole conjugates **130** (*SS*) and **131** (*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, **130** (*SS*): 22.2 %, **131** (*RR*): 27.1 %.

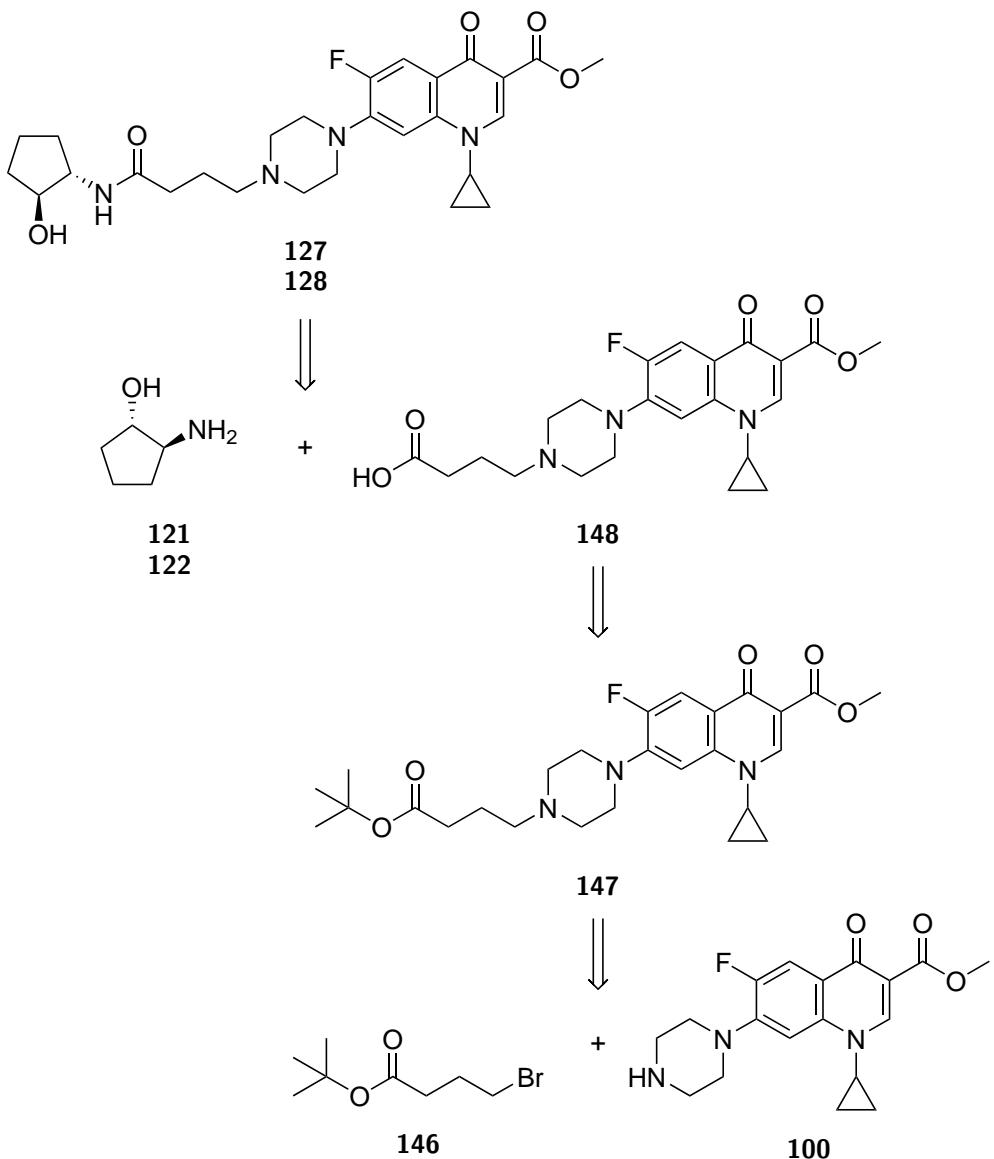
The S<sub>N</sub>2 reaction of Cl-C<sub>4</sub>-cyclopentanol-(*RR*) **144** and methyl ciprofloxacin **100** was attempted (see Scheme 34) using the microwave conditions described previously (see 8.3), to see if the chloride produced better results compared with the bromide. However, as was seen with the other microwave reactions, a substantial amount of the disubstituted product **145** was seen by LCMS (in an approx 1:1 ratio with the desired product



Scheme 34: Attempted synthesis of the cyclopentanol-CipMe-(RR) conjugate **128**. a) NaI, DIPEA, acetonitrile, microwave reactor, 100 °C.

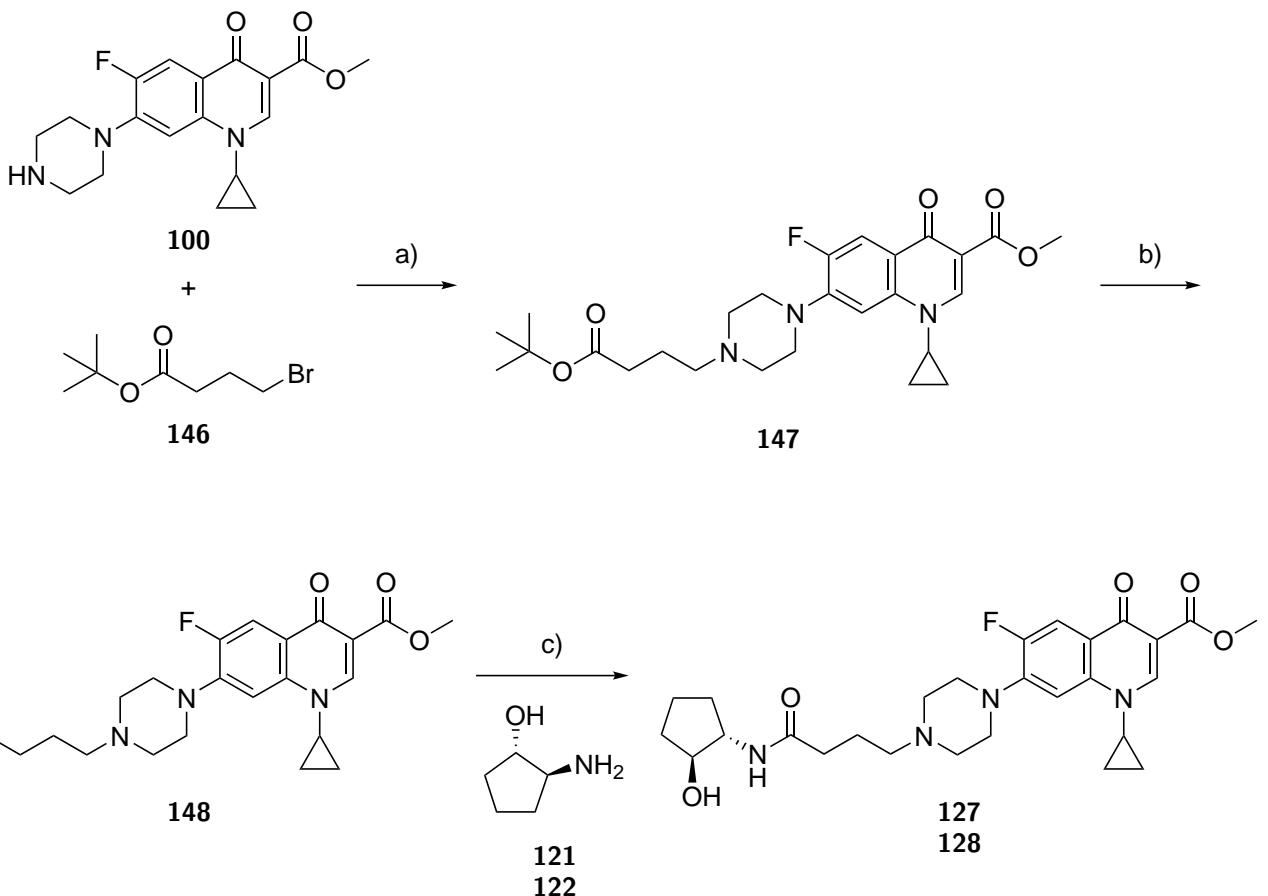
#### 8.5.5 Synthesis of the cyclopentanol-CipMe conjugates **127** and **128** by peptide coupling

Given the side-reactions and low yields associated with the literature synthesis of the  $S_N2$  conjugates proposed by Ganguly et. al,<sup>145</sup> an alternative synthesis was investigated, involving building up the linker on the ciprofloxacin side before coupling with the head group (see Scheme 35).



Scheme 35: Retrosynthesis of the cyclopentanol-CipMe conjugates **127** (*SS*) and **128** (*RR*). *SS* enantiomers are shown, but both are implied.

The first step of the synthesis was an  $S_N2$  reaction between Boc-protected 4-bromobutyric acid **146** methyl ciprofloxacin **100** (see Scheme 36). Intermediate **147** was obtained in acceptable yield after column chromatography (49.9 %). Intermediate **147** was deprotected in excellent yield using TFA in  $\text{CH}_2\text{Cl}_2$  to give carboxylic acid **148**. 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 **148** was first coupled with (*1R,2R*)-2-aminocyclopentan-1-ol **122** using standard peptide coupling conditions to give cyclopentanol-CipMe conjugate **128**. Purification by column chromatography was attempted twice with poor results, before moving on to using preparative HPLC, which gave **128** cleanly in 38.7 % yield. Coupling was also performed with (*1S,2S*)-2-aminocyclopentan-1-ol **121** to give the enantiomer **127** in 54.7 % yield.



Scheme 36: Synthesis of the cyclopentanol-CipMe conjugates **127** (*SS*) and **128** (*RR*) by peptide coupling. *SS* enantiomers are shown, but both were synthesised. a) NaI, TEA, acetonitrile, 100 °C, 16 h, 49.9 %. b) TFA, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 18 h, 95.6 %. c) EDC, HOBr, DIPEA, DMF, r.t., 16 h, **127** (*SS*): 54.7 %, **128** (*RR*): 38.7 %.

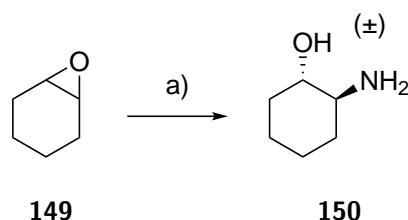
With (unfortunately not branching) routes to the S<sub>N</sub>2 and click conjugates established (see 8.5.5 and 8.5.4 respectively), attention was turned to the cyclohexanol derivatives.

## 8.6 Cyclohexanol derivatives

### 8.6.1 Synthesis of the *trans*-2-aminocyclohexan-1-ol head group **150**

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

Production of the cyclohexanol conjugates began with the synthesis of *trans*-2-aminocyclohexan-1-ol **150** (see Scheme 37), using a procedure reported by Xue *et al.*<sup>189</sup> Cyclohexene oxide **149** was opened using ammonia in water and methanol. Initially the reaction was carried out at 85 °C in a microwave reactor for 30 min, but a large amount of the disubstituted amine could be seen by LCMS (in a ratio of 4:3 product to impurity by NMR). The reaction was therefore attempted at room temperature, and proceeded overnight in high yield and with minimal side reaction.

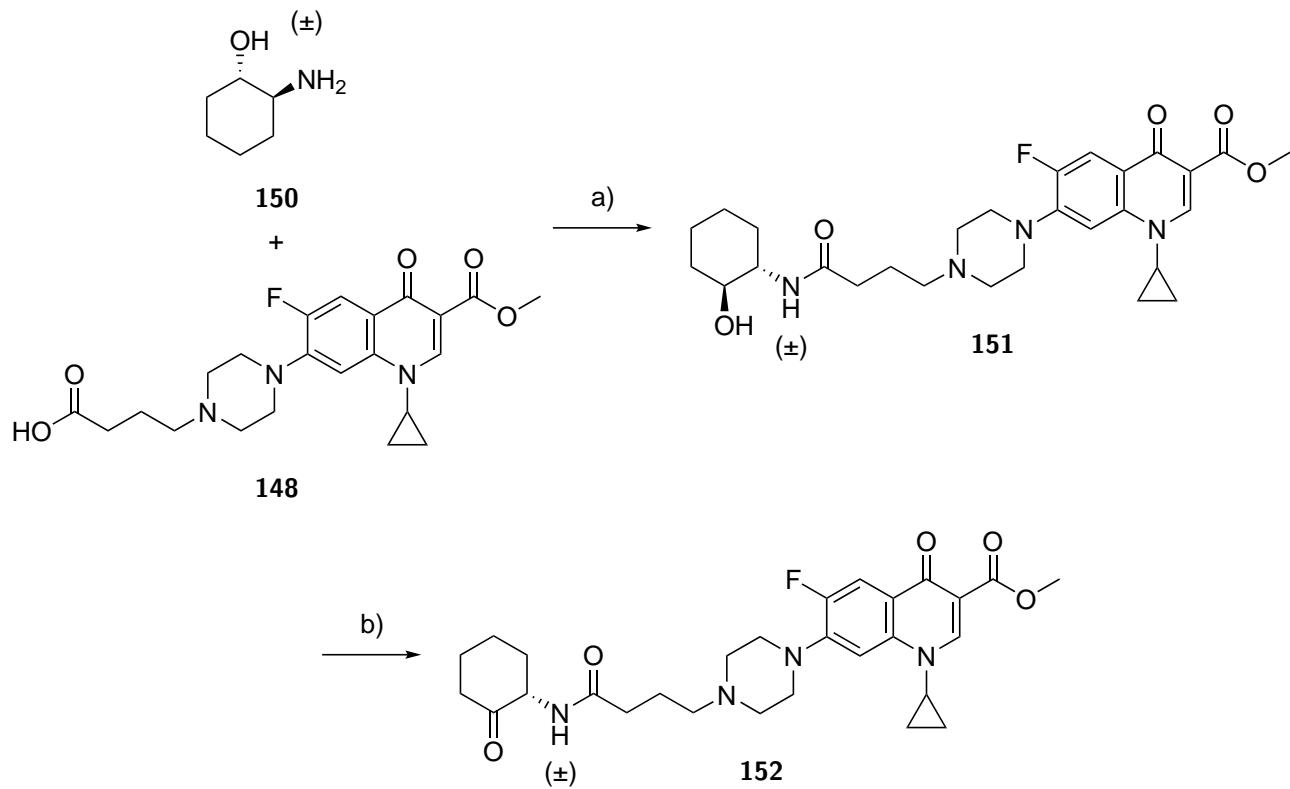


Scheme 37: Synthesis of *trans*-2-aminocyclohexan-1-ol **150**. a)  $\text{NH}_3$ , water,  $\text{MeOH}$ , r.t., 72 h, 86.2 %.

### 8.6.2 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-CipMe conjugates **151** and **152**

Carboxylic acid **148** was coupled with *trans*-2-aminocyclohexan-1-ol **150** using standard peptide coupling conditions to give *trans*-cyclohexanol-CipMe conjugate **151** in 31.7 % yield.

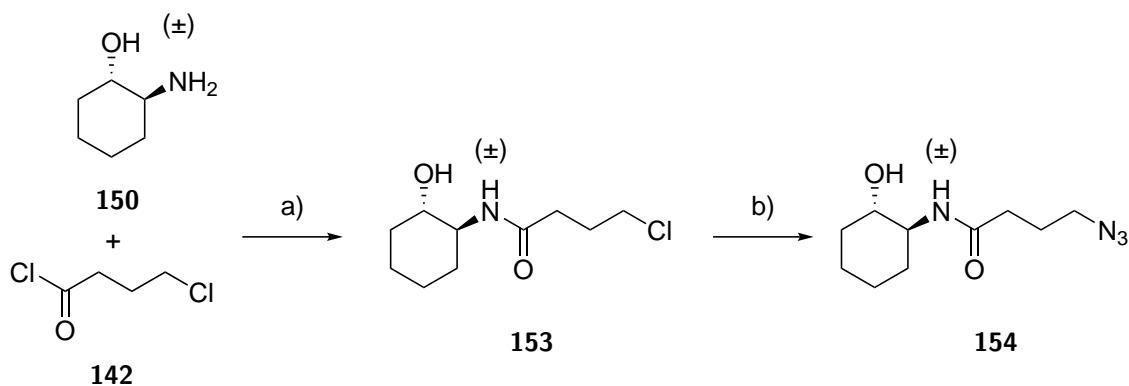
A portion of the *trans*-cyclohexanol-CipMe conjugate **151** was then oxidised to the ketone using Dess-Martin periodinane and the product was isolated in good yield.



Scheme 38: Synthesis of the cyclohexanol-CipMe conjugate **151** and the cyclohexanone-CipMe conjugate **152**. a)  $\text{EDC}$ ,  $\text{HOEt}$ ,  $\text{DIPEA}$ ,  $\text{DMF}$ , r.t., 16 h, 31.7 %. b)  $\text{DMP}$ ,  $\text{CH}_2\text{Cl}_2$ , r.t., 6 h, 69.1 %.

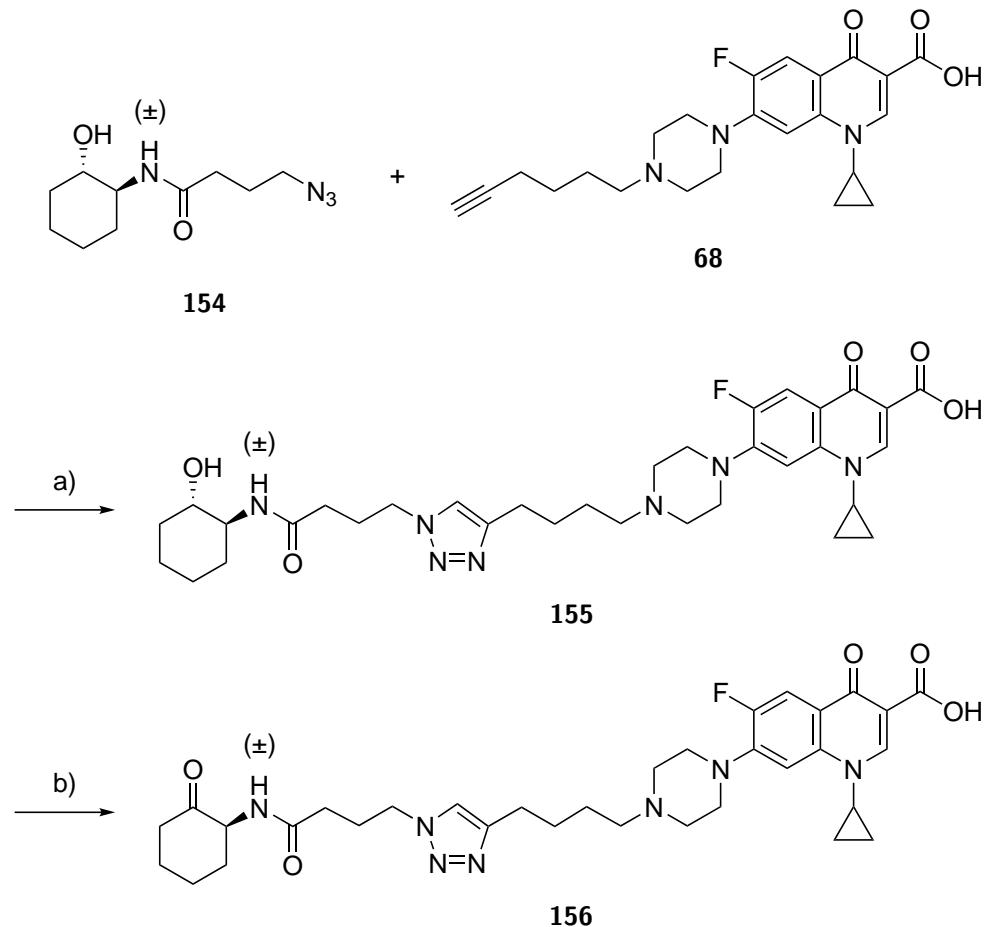
### 8.6.3 Synthesis of the *trans*-cyclohexanol- and cyclohexanone-Cip triazole conjugates **155** and **156**

The triazole conjugates were synthesised using the route described in 8.5.4.  $\text{Cl-C}_4$ -*trans*-cyclohexanol **153** was synthesised in good yield from *trans*-2-aminocyclohexan-1-ol **150** and 4-chlorobutyryl chloride **142**.  $\text{Cl-C}_4$ -*trans*-cyclohexanol **153** was then converted to  $\text{N}_3\text{-C}_4$ -*trans*-cyclohexanol **154** by reaction with sodium azide in excellent yield.



Scheme 39: Synthesis of *N*<sub>3</sub>-C<sub>4</sub>-*trans*-cyclohexanol **154**. a) TEA,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 30 min, 76.1 %. b)  $\text{NaN}_3$ , acetonitrile,  $50^\circ\text{C}$ , 16 h, 97.5 %.

The *trans*-cyclohexanol-Cip triazole conjugate **155** was synthesised using standard click conditions (see 10.25) in 48.9 % yield. A portion of the *trans*-cyclohexanol-Cip triazole conjugate **155** was then oxidised to the ketone using the same conditions used for the cyclohexanone-CipMe conjugate (see 8.6.2) in very good yield.



Scheme 40: Synthesis of the *trans*-cyclohexanol-Cip triazole conjugate **155** and the cyclohexanone-Cip triazole conjugate **156**. a)  $\text{CuSO}_4$ , THPTA, sodium ascorbate,  $\text{H}_2\text{O}$ , *t*-BuOH, r.t., 16 h, 48.9 %. b) DMP,  $\text{CH}_2\text{Cl}_2$ , r.t., 4 h, 78.0 %.

#### 8.6.4 Biological testing

Ganguly *et al.*<sup>145</sup> found the MICs of ciprofloxacin and a BHL analogue-ciprofloxacin conjugate **103** under standard planktonic conditions by introducing the compounds to liquid culture. The MICs were found to be ten times lower for ciprofloxacin vs. the conjugate **103** (5 vs 50  $\mu$ M). They then investigated the effect of the compounds on biofilms. The compounds were first cultured at 25 $\mu$ M, with PA liquid culture. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate **103**. They then cultured biofilm for 24 hours before adding the compounds, and found that, in contrast, the conjugate **103** disrupted the biofilm more effectively than ciprofloxacin. When the biofilm was cultured for 48 or 72 hours the conjugate similarly disruptive effects, whereas ciprofloxacin 'did not show any significant antibacterial activity'.

This work

All conjugates were tested for growth inhibition (MIC), biofilm formation inhibition and activity against nascent (24 h) and established (48 h) biofilms in *P. aeruginosa* and *S. aureus*.

The conjugates shown in Figure 27 were tested, as well as BHL **19**, HHQ **21**, PQS **22**, ciprofloxacin **24**, methyl ciprofloxacin **100**, the alkynyl ciprofloxacin derivative **68**, the *tert*-butyl ester ciprofloxacin derivative **147**, the carboxylic acid ciprofloxacin derivative **148**, trimethoprim **25** and the alkynyl trimethoprim derivative **71**.

Cultures were grown in the presence of the compounds at a range of 6 concentrations from 25 to 0.125  $\mu$ M. MICs were calculated by fitting a modified Gompertz function.<sup>190</sup> An example of the fitting is shown in ??.

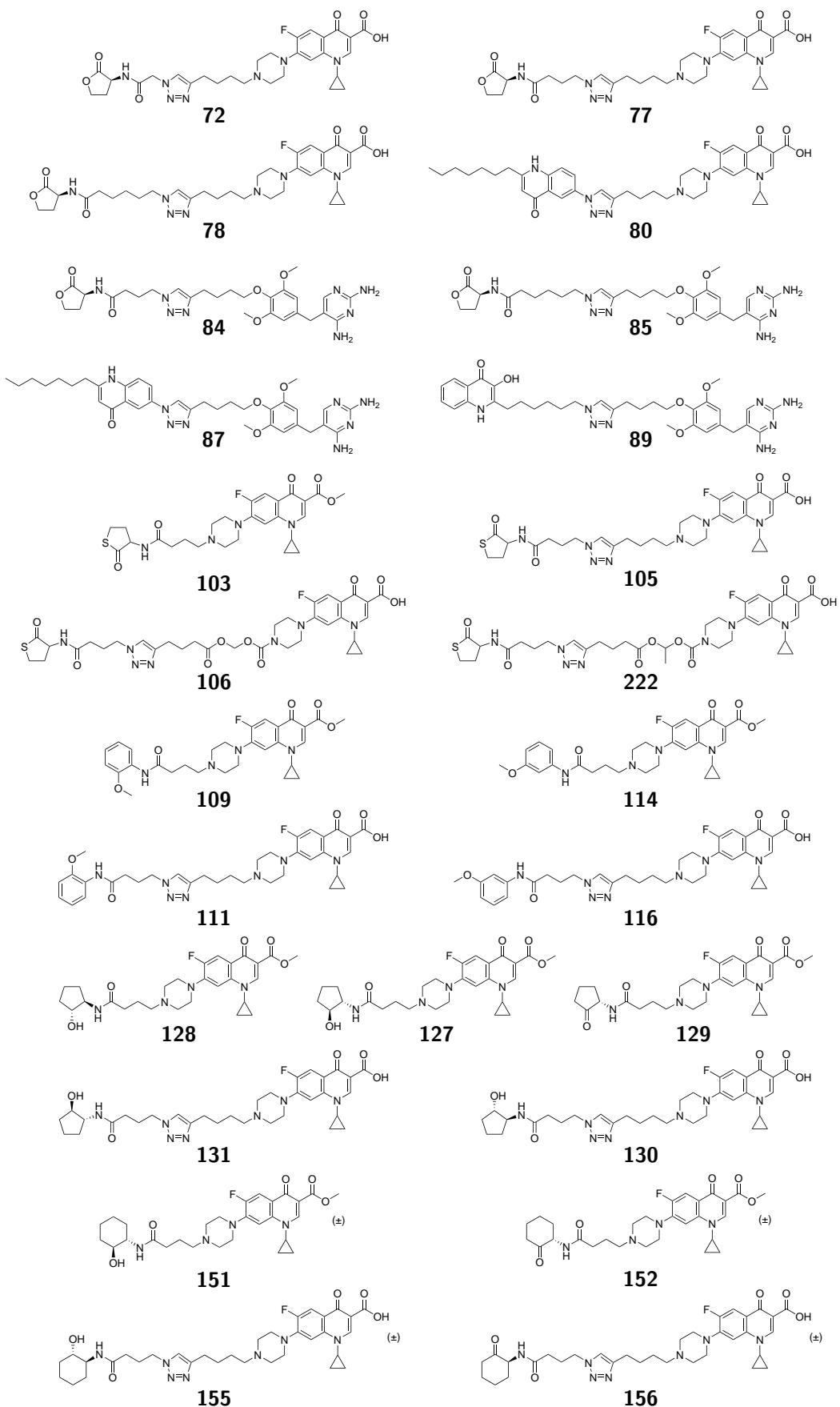


Figure 27

## 8.7 Determination of MICs

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic (prevents the visible growth of bacteria). MICs are used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentrations of antibiotic/antiseptic over a defined period in terms of inhibition of microbial population growth.

### 8.7.1 PAO1

Bar Graphs MIC 8h for all HSL analogue conjugates (9-25 and controls) "24h "48h

Growth curves for interesting ones at lowest conc compare to controls? 10,11,12,15,16,20,21,24,25 (13,14 weird) Best 11,16,20

### 8.7.2 YM64

Bar Graphs MIC 8h for all HSL analogue conjugates (9-25 and controls) "24h "48h

Growth curves for interesting ones at lowest conc compare to controls? (can't see 9-16 graphs, check) 9,10,11,12,15,16 17-21,24,25 Best 10,11,12,15,16,20,21,24,25

### 8.7.3 HGS4

(can't see 9-16 graphs, check) 1-25 no inhibition except 11 a bit

### 8.7.4 HGS4 complemented

11,16,19,20,21,22,24,25

## 8.8 Determination of anti-biofilm activity

Biofilm growth was measured using crystal violet staining.<sup>191</sup>

### 8.8.1 Effect on biofilm formation

### 8.8.2 Biofilm disruption

Biofilms can drastically increase MIC for many antibiotics.<sup>192</sup> For ciprofloxacin in *P. aeruginosa* the MIC increases by 16 fold.

Ganguly *et al.*<sup>145</sup> found the MICs of ciprofloxacin and a BHL analogue-ciprofloxacin **103** conjugate under standard planktonic conditions by introducing the compounds to liquid culture. The MICs were found to be ten times lower for ciprofloxacin vs. the conjugate **103** (5 vs 50  $\mu$ m). They then investigated the effect of the compounds on biofilms. The compounds were first cultured at 25 $\mu$ m, with PA liquid culture. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate **103**. They then cultured biofilm for 24 hours before adding the compounds, and found that, in contrast, the conjugate **103** disrupted the biofilm more effectively than ciprofloxacin. When the biofilm was cultured for 48 or 72 hours the conjugate similarly disruptive effects, whereas ciprofloxacin 'did not show any significant antibacterial activity'.

Ganguly *et al.* used Bac-Light Live/Dead staining and confocal microscopy to image the biofilms, whereas so far I have used crystal violet staining. Crystal violet does not differentiate between live or dead cells, and so

might not pick up on the antibacterial effects of compounds. However, their confocal microscopy results show a quantifiable decrease in biofilm thickness, and it may be possible to detect this using crystal violet.

The conjugate **103** developed by Ganguly *et al.* contained a thiolactone AHL. The unconjugated thiolactone BHL **28** was shown to have 'either enhanced uptake or functional activity' when compared with BHL **19**. Therefore it seems possible that my compounds may not show enhanced antibiotic activity, where thiolactone analogues might.

Clicks

clicks were all crap because dilute Hong2009

SHL

Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in 8.5.5, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.

HOcy5

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 **101** to carboxylic acid **148** would have a similar yield to the coupling with (1*R*,2*R*)-2-aminocyclopentan-1-ol **122**, approximate comparisons can be made. The synthesis described in 8.2 has an overall yield of 10.7 %, whereas the route shown in Scheme 36 for **127** 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 8.2. 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.

No, I didn't try the one-pot synthesis without TBS. No worries, I wonder if it would have worked. Could be one for the conclusions?

Not C4 chain - massive pain due to internal ring formation.

## 9 Future work

### 9.1 Autoinducer derivatives

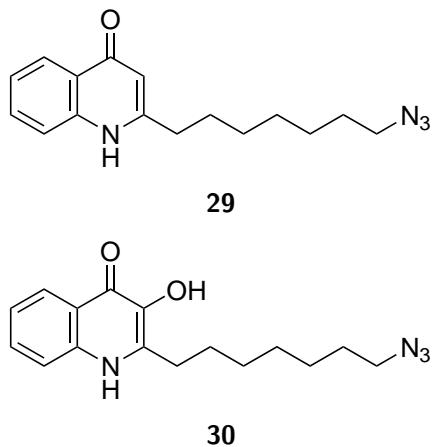
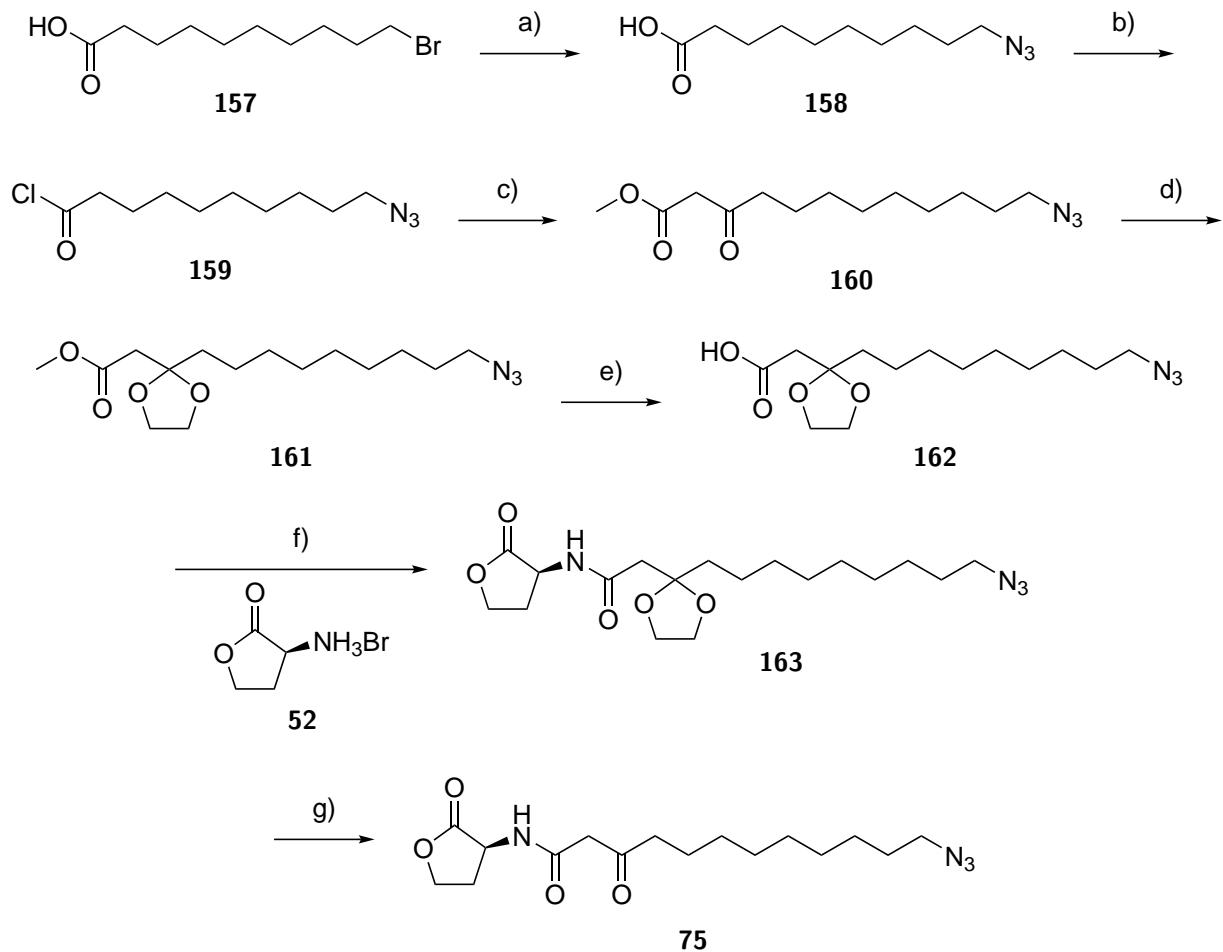


Figure 28: Further azido-HHQ **29** and azido-PQS **30** derivatives synthesised by Baker.

The syntheses of HHQ derivative **38** and azido-C<sub>4</sub>-HSL derivative **58** will be completed as outlined above (see ?? and ??). The only *P. aeruginosa* autoinducer not yet to have been considered in this project is 3-oxo-C<sub>12</sub>-HSL derivative **20** (see Figure 8). This would be the most obvious next target for study. After this, there are several other autoinducers which are not produced by *P. aeruginosa* which could be investigated (see Figure 29) as we intend to screen the library against a range of bacteria.

#### 9.1.1 3-oxo-C<sub>12</sub>-HSL derivative **75**

The synthesis of 3-oxo-C<sub>12</sub>-HSL has previously been reported by Hodgkinson *et al.*<sup>92</sup> A modification of this synthesis using 10-bromodecanoyl chloride could be used to produce derivative **75** with a tail azide (see Scheme 41). Derivatives with shorter or longer tail lengths (known to affect selectivity and binding affinity) could also be synthesised using the same method.



Scheme 41: Synthesis of azido 3-oxo-C<sub>12</sub>-HSL derivative **75** carried out by Ryan Howard. a) NaN<sub>3</sub>, DMF, 50 °C. b) Oxalyl chloride, DMF, CH<sub>2</sub>Cl<sub>2</sub>, r.t. c) MeOAc, *N*-methyl imidazole, TiCl<sub>4</sub>, DIPEA. d) *p*-TsOH, HO(CH<sub>2</sub>)<sub>2</sub>OH, CH(OMe)<sub>3</sub>, r.t. e) NaOH, H<sub>2</sub>O, r.t. f) EDC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, r.t. g) TFA, r.t.

### 9.1.2 AI2

#### 9.1.3 Non-*P. aeruginosa* autoinducers

Many species of bacteria other than *P. aeruginosa* produce autoinducers<sup>193</sup> (see Figure 29). An azido derivative of C<sub>8</sub>-HSL **164** could be produced in a similar manner to the C<sub>4</sub>-HSL derivatives already synthesised. An azido derivative of 3-oxo-C<sub>6</sub>-HSL **18** could be produced in the manner proposed for 3-oxo-C<sub>12</sub>-HSL **20** above. Derivatives of AI-2 **23** could have azide groups in the place of the OH groups on the sugar section of the molecule. Derivatives of AIP **166** and ComX **165** could be synthesised by conversion of their terminal amines to azides. Derivatives of AIP **166** could be produced by standard peptide synthesis methods with the inclusion of unnatural azido amino acids at different points along the peptide chain followed by formation of the thioester bond. ComX **165** contains a complex non-standard amino acid which would be time-consuming to synthesise, but if this could be achieved then peptide synthesis methods could also be used to introduce an azido amino acid within the peptide chain.

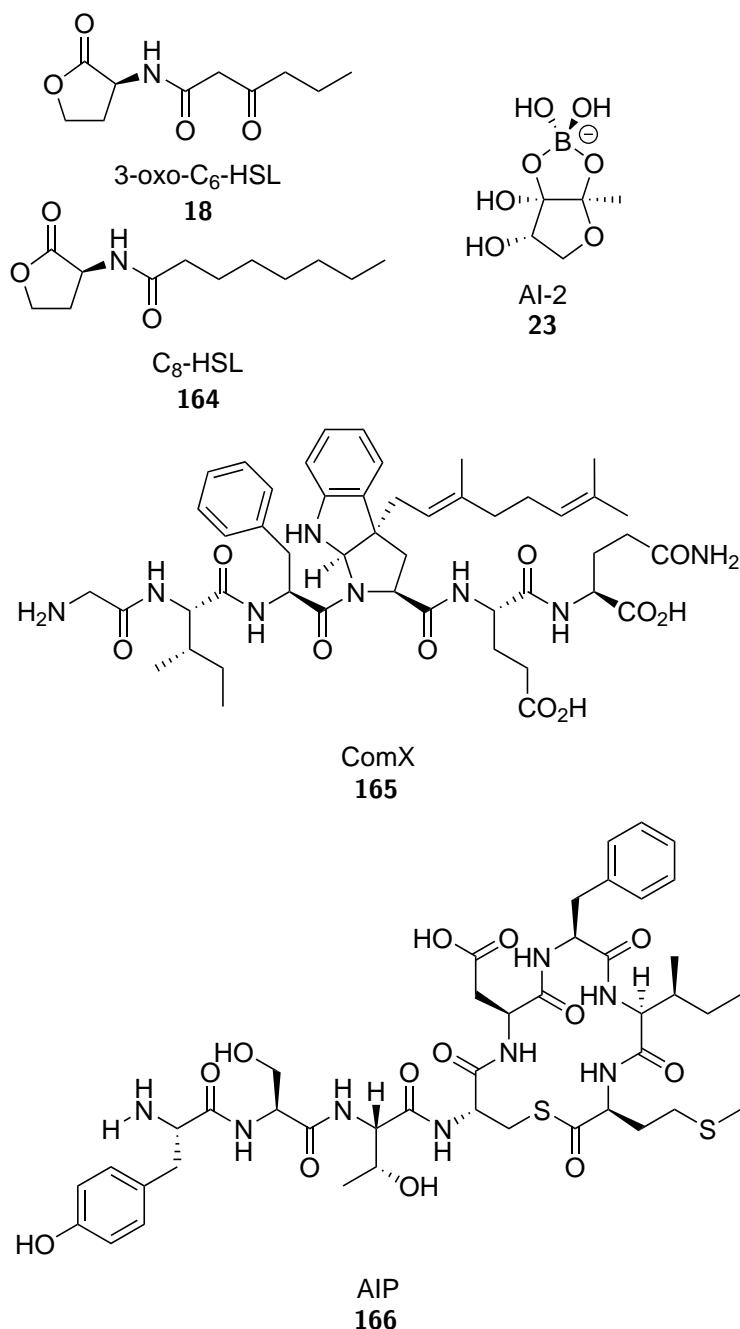


Figure 29: Autoinducers from various bacterial species. C<sub>8</sub>-HSL **164** is from *Burkholderia cepacia*, 3-oxo-C<sub>6</sub>-HSL **18** is from *Erwinia chrysanthemi*, AI-2 **23** is found in both Gram-positive and Gram-negative bacteria, ComX **165** is from *B. subtilis*, AIP **166** is from *S. aureus*.

## 9.2 Antibiotic derivatives

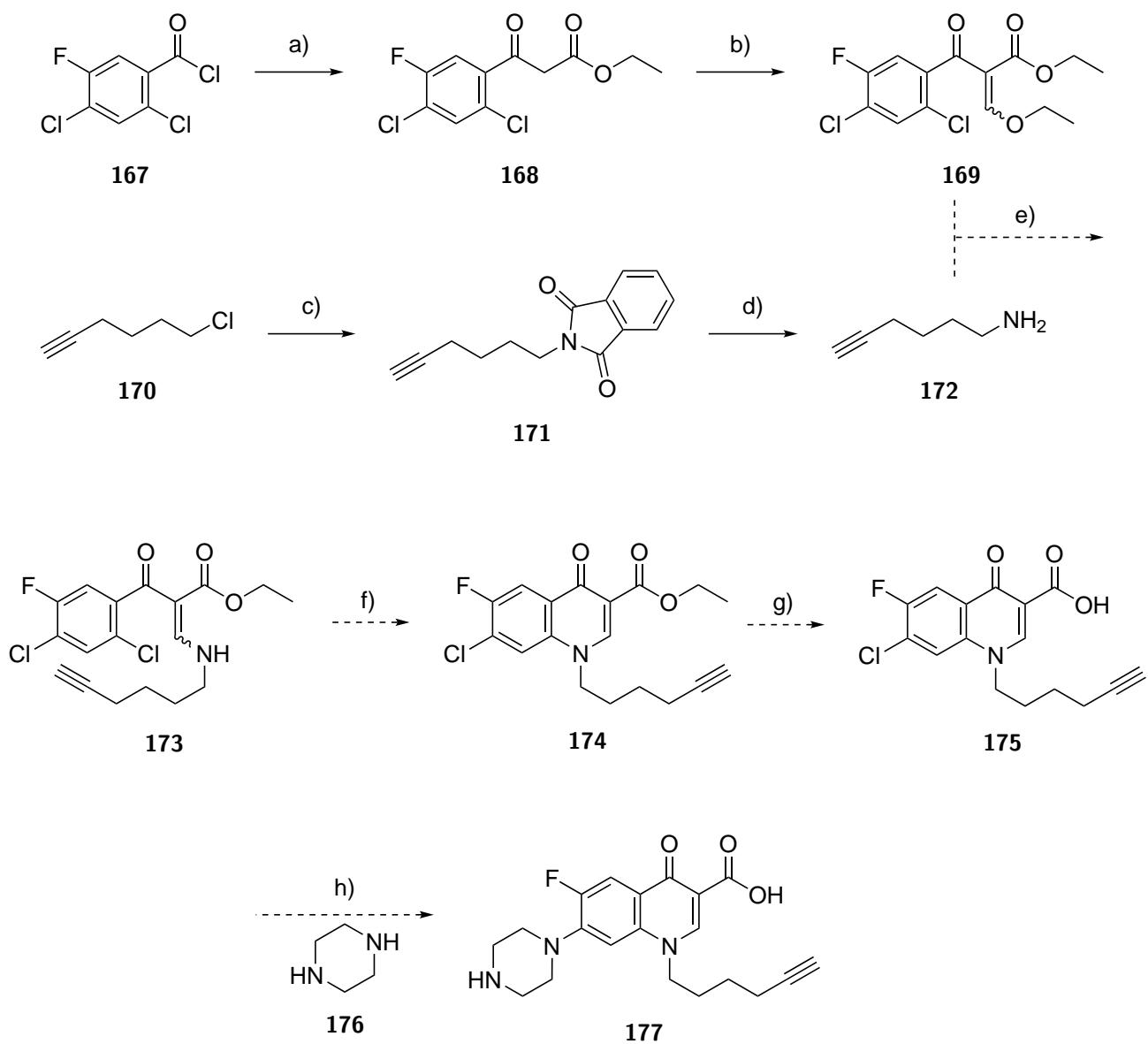
### 9.2.1 Ciprofloxacin derivative **177**

Derivative **177** has an alkyne tail attached in place of the cyclopropane ring at position 7 (see ??); its retrosynthesis is shown in ???. This synthesis follows a conventional synthesis of ciprofloxacin similar to that reported by Mitscher *et al.*<sup>194</sup> but using hex-5-yn-1-amine **172** instead of cyclopropylamine. **167** should react with potassium ethyl malonate with loss of CO<sub>2</sub> to form **168**, followed by heating with triethyl orthoformate to form **169**.<sup>194,195</sup> This would then be heated with hex-5-yn-1-amine **172**, as opposed to the cyclopropylamine used in the conventional synthesis, to form **173**. Hex-5-yn-1-amine **172** could be produced using the Gabriel synthesis

from **170**.<sup>196–198</sup> **173** could be cyclised using NaH to form **174** followed by ester hydrolysis using KOH to give **175** as reported by Mitscher *et al.* **175** would then be heated with piperazine in DMSO<sup>199</sup> to complete the synthesis of **177**.

The initial synthesis of **168** was attempted using a Claisen condensation and decarboxylation procedure developed by Hanan *et al.*<sup>200</sup> involving stirring **167** with potassium ethyl malonate, MgCl<sub>2</sub> and NEt<sub>3</sub>. This procedure had been reported to work using 2-methyl-5-chlorobenzoyl chloride and 2,6-dichlorobenzoyl chloride, however, no reaction was observed using 2,4-dichloro-5-fluorobenzoyl chloride. A modification of the procedure described by Scribner *et al.*<sup>166</sup> was used to convert an acid chloride to a  $\beta$ -ketoester via a Medrum's acid adduct was then attempted. The procedure did produce the desired  $\beta$ -ketoester **168**, however, it also produced significant amounts of the ethyl ester **221** as a side-product, despite attempts to remove excess acid chloride **167** before refluxing in ethanol. A modification used by Yamamoto<sup>201</sup> which substituted pyridine with 4-dimethylaminopyridine also failed to suppress formation of the ethyl ester side product **221**. As the product and side-product were relatively difficult to separate by column chromatography, a procedure which did not produce the ethyl ester was sought. The TiCl<sub>4</sub>-catalysed crossed Claisen condensation of the acid chloride **167** and ethyl acetate described by Hashimoto *et al.*<sup>202</sup> was chosen. This produced the  $\beta$ -ketoester **168** without the ethyl ester side product **221**.

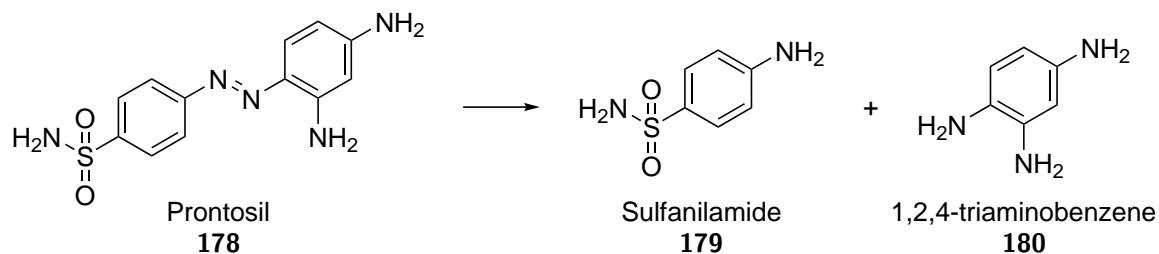
The ethoxymethylene group in **169** was installed by the reaction of  $\beta$ -ketoester **168** and triethyl orthoformate to give a mixture of the *E* and *Z* isomers.<sup>194,195</sup> Hex-5-yn-1-amine **172** was prepared using a Gabriel synthesis<sup>196</sup> described by Rożkiewicz *et al.*<sup>197</sup> 6-Chlorohex-1-yne **170** was heated with potassium phthalimide to form **171**, which was then cleaved using hydrazine monohydrate to form hex-5-yn-1-amine **172**. The remainder of the synthesis of **177** is in progress (see Scheme 42).



Scheme 42: The synthesis of **177**. a) EtOAc, TiCl<sub>4</sub>, DIPEA, *N*-methyl imidazole, toluene, r.t., 30 min, yield %. b) Triethyl orthoformate, Ac<sub>2</sub>O, reflux, 2 h, yield %. c) Potassium phthalimide, KI, DMF, 80 °C, 18 h, 75 %. d) N<sub>2</sub>H<sub>2</sub>.H<sub>2</sub>O, EtOH, reflux, 18 h, yield %. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) Piperazine, DMSO.

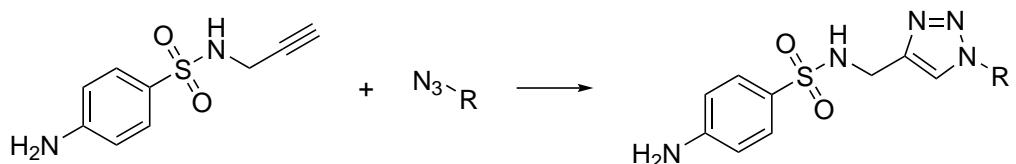
### 9.2.2 Sulfanilamide derivative

Sulfanilamide antibiotics were the first class to be widely used.<sup>203,204</sup> The first drug in the class was called Prontosil **178** and was developed by Bayer and first patented in 1937. Prontosil **178** is inactive in vitro but active in vivo, as it is a prodrug which is reduced in vivo to release the active drug, sulfanilamide **179**, and 1,2,4-triaminobenzene **180** (see Scheme 43).

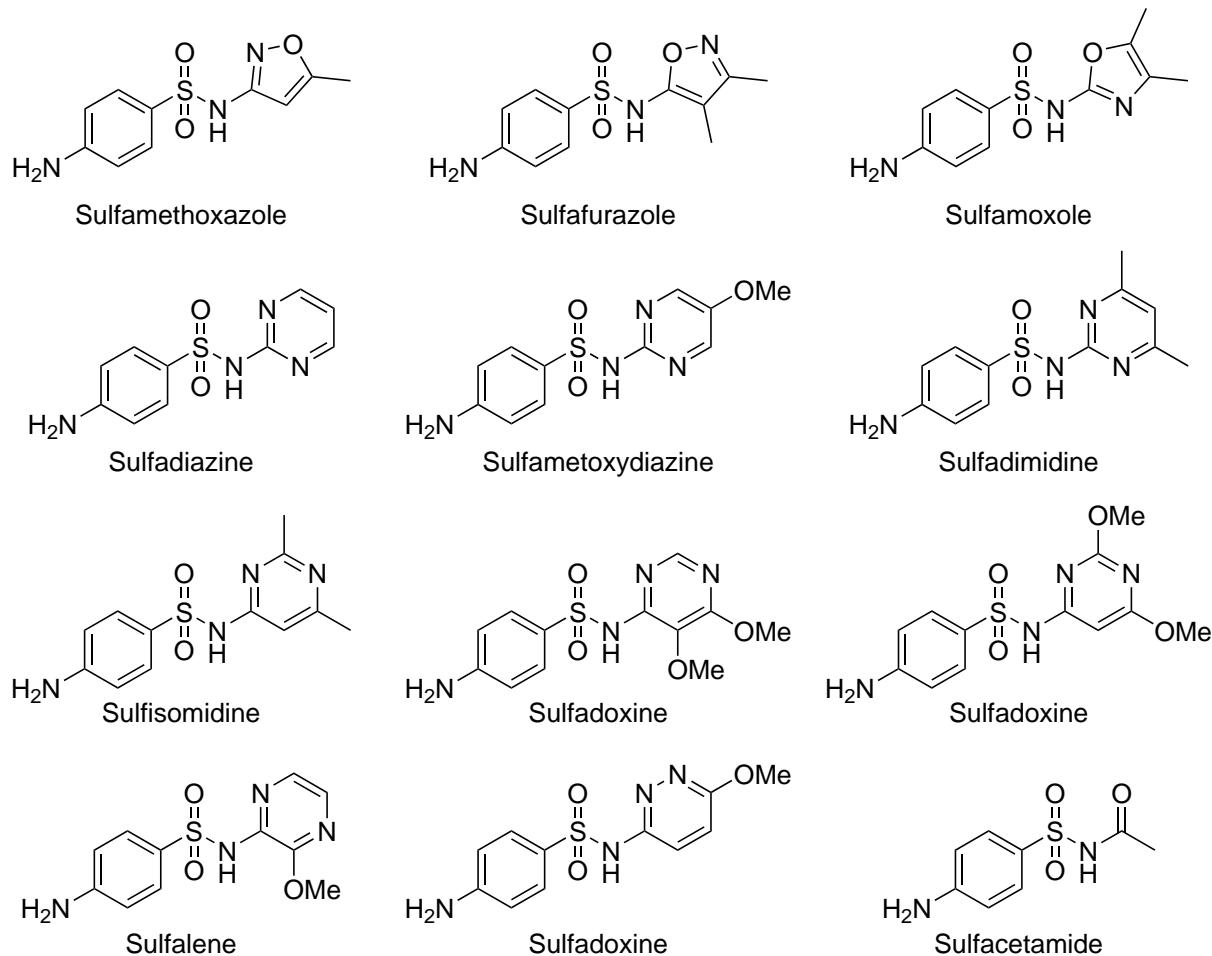


Scheme 43: The reduction of Prontosil **178** to release sulfanilamide **179** and 1,2,4-triaminobenzene **180**.

Derivatives of sulfanilamide **179** have previously been synthesised using a click reaction to append different R groups<sup>205</sup> (see Scheme 44). However, if one considers sulfonamide antibiotics already in use, all except sulfacetamide have a heterocycle linked directly to the sulfur atom, rather than with a methylene group in between (see Scheme 45).

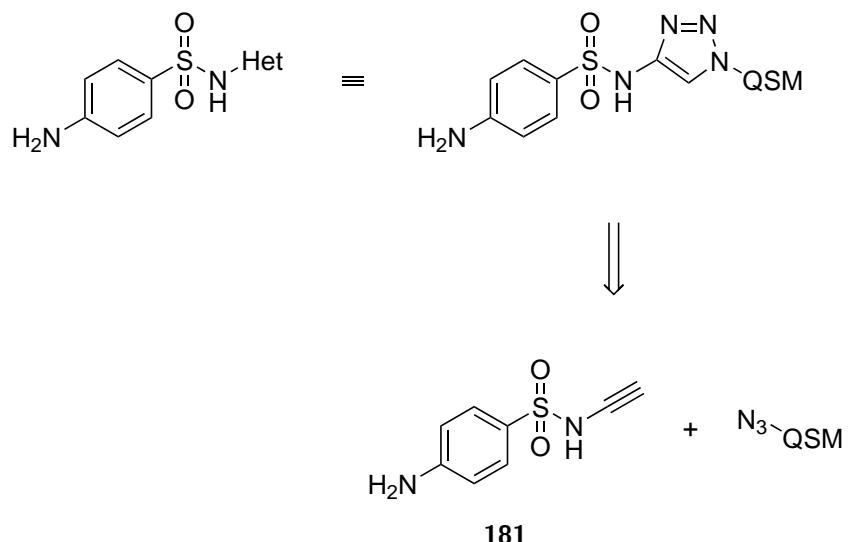


Scheme 44: The sulfanilamide derivatives synthesised using click chemistry by Wang et al.<sup>205</sup>



Scheme 45: Sulfonamide antibiotics.

Therefore, it is postulated that a 1,2,3-triazole could be introduced in the position occupied by a heterocycle in other known sulfonamide antibiotics by attachment of an alkyne directly to the sulfonamide nitrogen to form compound **181** or a protected version of it (see Scheme 46).



Scheme 46: Retrosynthetic of a 1,2,3-triazole-containing sulfonamide antibiotic-autoinducer hybrid.

It is hoped that sulfanilamide derivative **181** could be synthesised and reacted with the azido autoinducer derivatives directly. This would allow a more efficient synthesis of the library, as no deprotection steps would be needed after the click reaction. However, it appears that no secondary ynamides have been synthesised to date.<sup>206</sup> Scifinder does not have a synthesis of ethynamine, suggesting that it is too unstable to form, but does have two papers discussing the syntheses of other primary ynamines.<sup>207, 208</sup>



Scheme 47: The Scifinder reaction substructure search used to show that secondary ynamides have not yet been synthesised.<sup>206</sup>

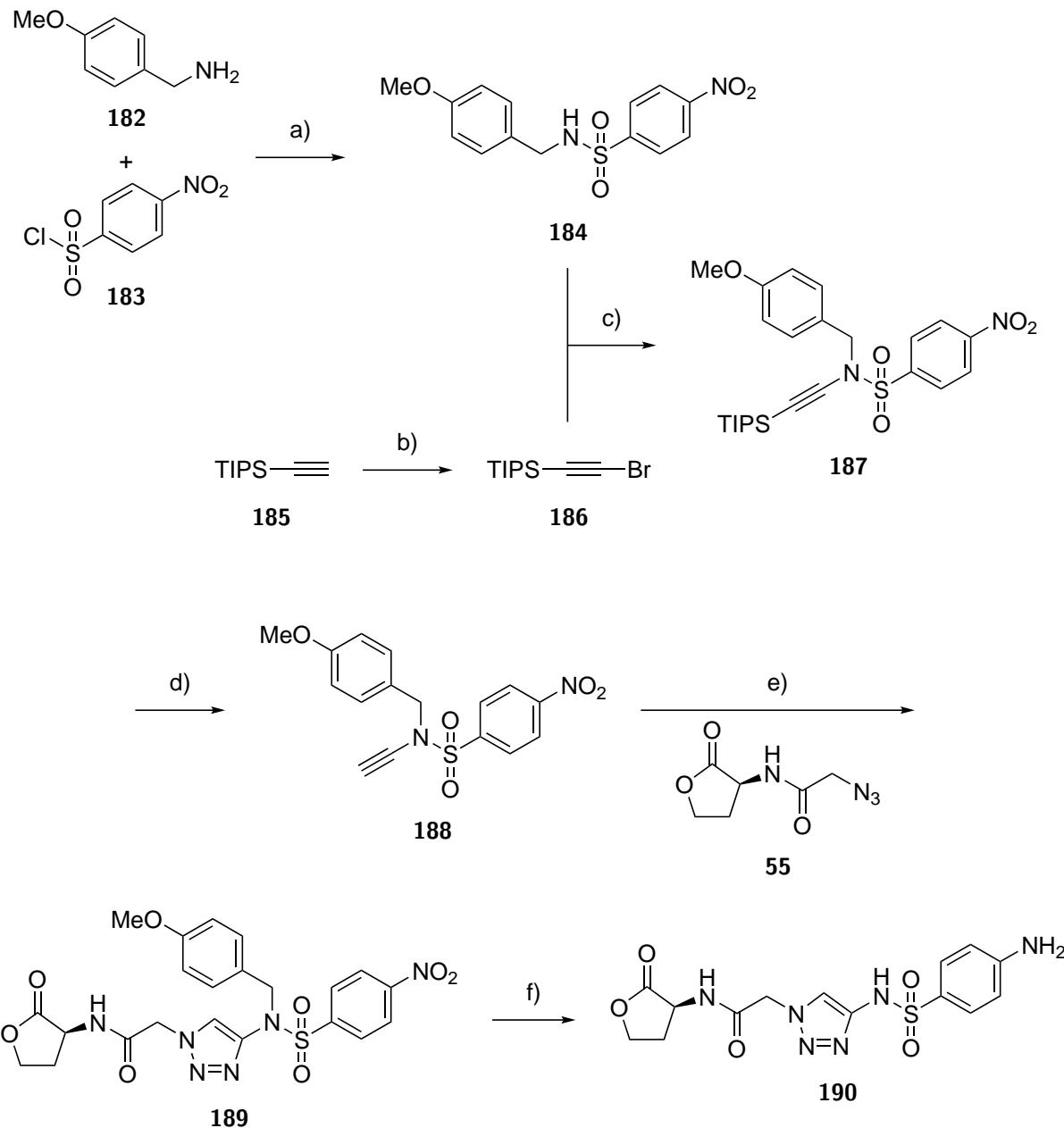


Scheme 48: The Scifinder reaction substructure search used to find the synthses of primary ynamines.<sup>?</sup>

Conversely, the synthesis of tertiary ynamides has been studied more widely.<sup>209</sup> In particular, tertiary ynamides (often defined as ynamines with any electron-withdrawing group attached) have been shown to be relatively stable and easy to work with in reactions including addition at the  $\alpha$  position, addition at the  $\beta$  position, reduction/reductive coupling oxidation, cycloaddition, ring-closing metathesis, cycloisomerisation, functionalisation of terminal ynamides and click reactions.<sup>210, 211</sup>

The study of click reactions of ynamides by IJsselstijn et al. uses terminal ynamides protected using a benzyl and a tosyl group or a benzyl and a benzoyl group. Although their click reactions proceed with high yield, they fail to present the deprotection of their final compounds. However, these reactions provide a promising suggestion that click reactions between a protected sulfanilamide derivative and the azido-autoinducer

derivatives are feasible. The tosyl group used by IJsselstijn et al. to protect their ynamide is very similar to the *p*-aminobenzenesulfonyl group needed in the alkynyl-sulfonamide derivative. However, installation of the alkyne could be problematic in the presence of a second amine, so the NH<sub>2</sub> group is installed as a NO<sub>2</sub> group and reduced after the click reaction. Similarly, the benzyl protecting group must be removed after the click reaction.

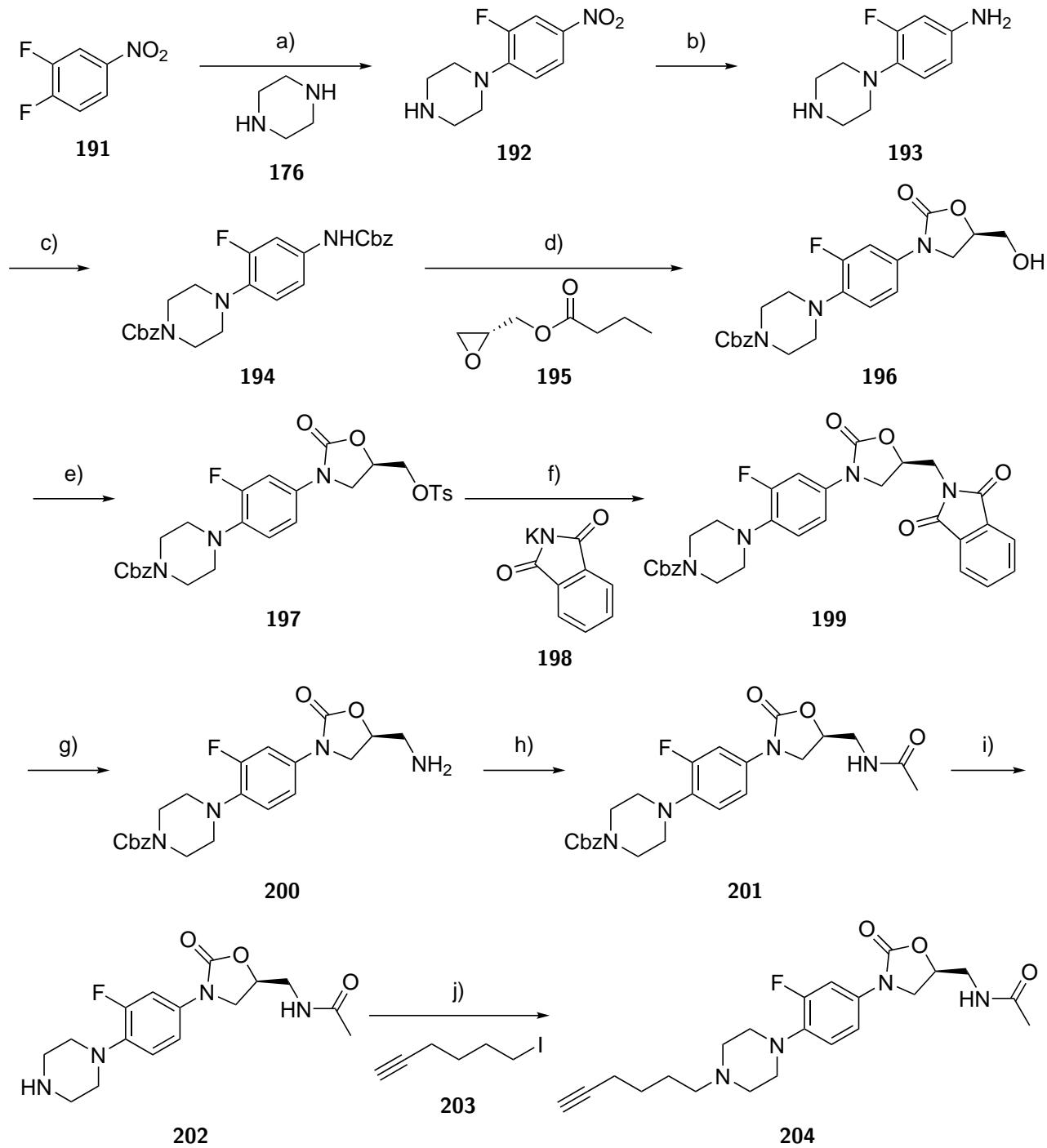


Scheme 49: Synthesis of a 1,2,3-triazole-containing sulfonamide antibiotic-autoinducer hybrid. a) CH<sub>2</sub>Cl<sub>2</sub>, r.t., 24 h.<sup>212</sup> b) AgNO<sub>3</sub>, acetone, r.t., 3 h.<sup>213</sup> c) CuSO<sub>4</sub> · 5 H<sub>2</sub>O, 1,10-phenanthroline, K<sub>2</sub>CO<sub>3</sub>, toluene, 80 °C, 48 h.<sup>213</sup> d) TBAF, THF, -78 °C, 3 h.<sup>213</sup> e) Cu(OAc)<sub>2</sub>, sodium ascorbate, CH<sub>2</sub>Cl<sub>2</sub>, *t*-BuOH, H<sub>2</sub>O, r.t., 16 h.<sup>210</sup> f) SnCl<sub>2</sub>, TFA, CH<sub>2</sub>Cl<sub>2</sub>, reflux, 3 h.<sup>214,215</sup>

### 9.2.3 Linezolid derivative

If time permits, an alkynyl derivative of the antibiotic linezolid **222** (see Figure 10) could also be synthesised. The route follows a recent literature procedure described by Phetsang *et al.*<sup>216</sup> The morpholine ring of linezolid is replaced by piperazine, allowing an alkynyl tail to be attached to the molecule (as opposed to the azido tail

attached by Phetsang *et al.*).

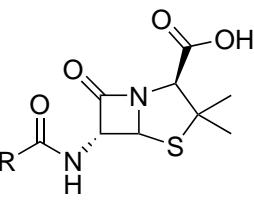


Scheme 50: Proposed synthesis of linezolid derivative **223**.<sup>216</sup> a) MeCN, reflux, 3 h. b) H<sub>2</sub>, 10 % Pd/C, THF, 40 psi, <50 °C, 1.5 h. c) CbzCl, Na<sub>2</sub>CO<sub>3</sub>, acetone/H<sub>2</sub>O, 1 h at 5 °C then 16 h at rt. d) n-BuLi, THF, -78 °C for 1 h then add epoxide then -78 °C for 1.5 h then rt for 3.5 h. e) TsCl, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C for 1.5 h then rt for 3 h. f) MeCN/H<sub>2</sub>O, reflux, 48 h. g) MeNH<sub>2</sub>, EtOH/H<sub>2</sub>O, reflux, 5.5 h. h) Ac<sub>2</sub>O, pyridine, 0 °C to rt. i) H<sub>2</sub>, 10 % Pd/C, MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1 atm, rt, 16 h. j) NEt<sub>3</sub>, EtOH, reflux.

#### 9.2.4 Penicillin derivative 208

The penicillins are a group of antibiotics with the same penam core structure but different R groups (see Figure 30). It therefore seems likely that a biologically active penicillin derivative could be synthesised with

an alkyne in the R group. This could be produced using the penicillin precursor 6-aminopenicillanic acid **207** and 5-hexynoic acid **205** or a derivative thereof. An initial attempt at synthesis was based on a procedure developed by Faridoon.<sup>217</sup> Firstly, 5-hexynoic acid **205** was converted to 5-hexanoyl chloride **206** using oxalyl chloride and catalytic DMF, unlike in the Faridoon procedure which uses thionyl chloride. 5-hexanoyl chloride **206** was then stirred with 6-aminopenicillanic acid **207**, however, despite screening various solvent systems and bases no clean reaction could be found and the reactions gave complex mixtures of products. It appears that 6-aminopenicillanic acid **207** and its derivatives are too sensitive to basic conditions for these conditions to be used, most likely due to opening of the  $\beta$ -lactam ring followed by further decomposition reactions. Products were also seen to undergo methanolysis during  $\text{SiO}_2$  column chromatography using  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  solvent systems. Therefore, milder reaction conditions must be used. Peptide coupling reagents may be useful for this purpose, for example DCC and HOBr (see Scheme 52).



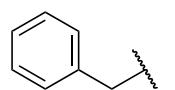
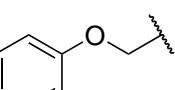
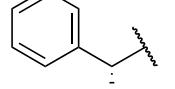
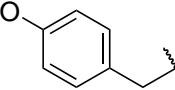
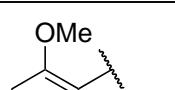
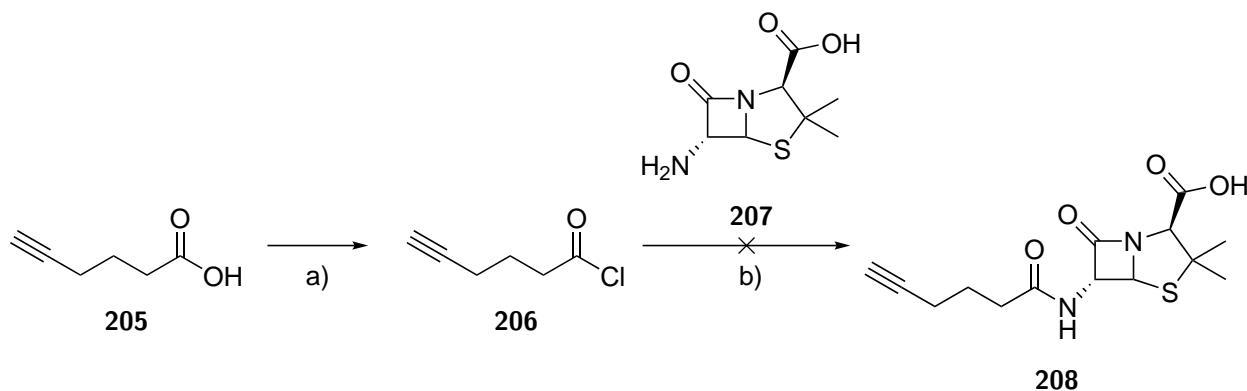
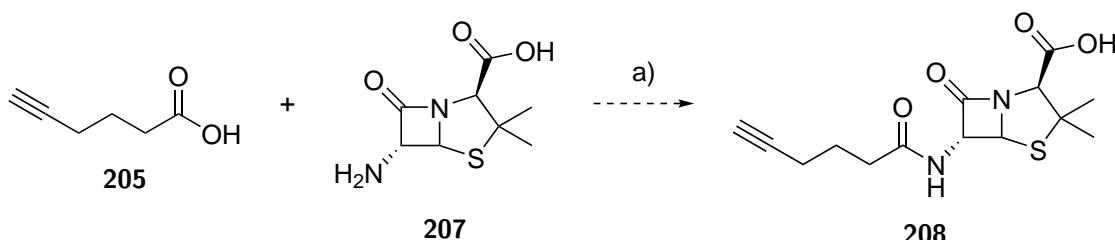
Name	R
Penicillin G	
Penicillin V	
Ampicillin	
Amoxicillin	
Methicillin	

Figure 30: The penicillins.



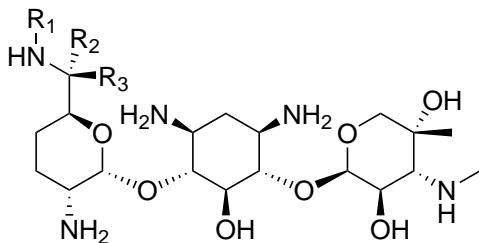
Scheme 51: Attempted synthesis of **208**. a) oxalyl chloride, DMF, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 3 h. b) DIPEA, CH<sub>2</sub>Cl<sub>2</sub>/pyridine/NaHCO<sub>3</sub>, Acetone, H<sub>2</sub>O/NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, all r.t., 18 h.



Scheme 52: Proposed synthesis of **208**. a) DCC, HOBT, DMF.

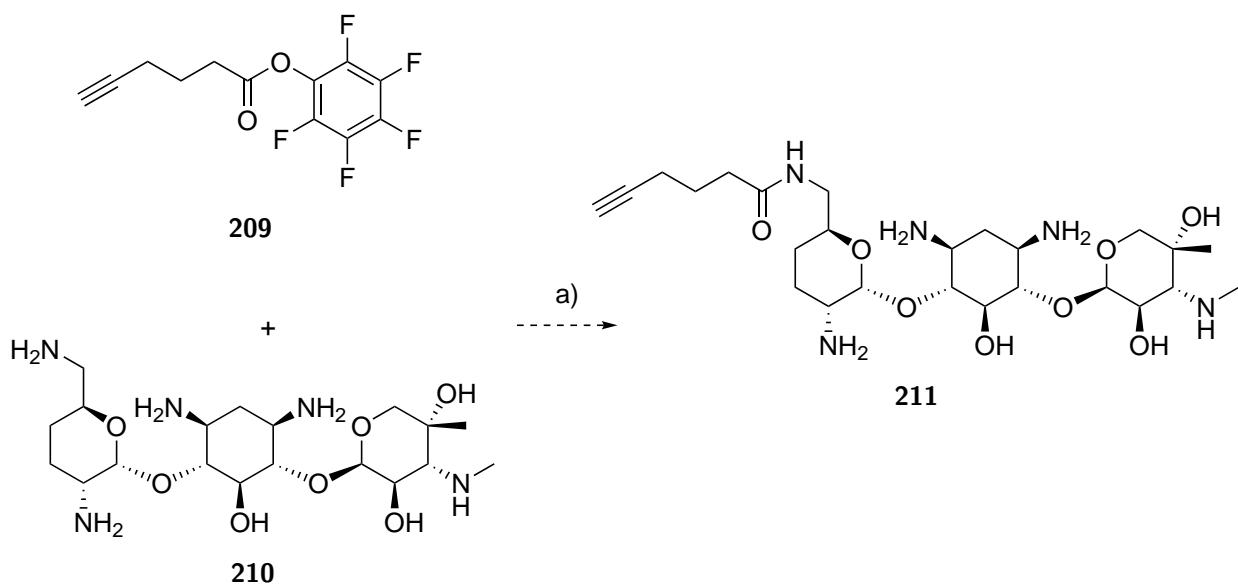
### 9.2.5 Gentamicin derivative **211**

Gentamicin is an aminoglycoside antibiotic used to treat many bacterial infections, particularly those caused by Gram-negative organisms, by binding to the bacterial ribosome. Gentamicin is actually a mixture of components (see Figure 31) synthesised by *Micromonospora sp.*, a genus of Gram-positive bacteria. Separation of the gentamicin components has been achieved by Grote *et al.*<sup>218</sup> by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a **210** was isolated pure, and is particularly useful because it is the only component which contains a CH<sub>2</sub>NH<sub>2</sub> group. This group is less hindered than all other amine groups in gentamicin C1a **210** and hence it is possible to selectively derivatise the molecule at this position. Grote *et al.* attached a tag needed for an immunoassay using a pentafluorophenyl ester.<sup>219</sup> Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid **209** (see Scheme 53). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester and then separate out the desired component.



Gentamicin	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
C1	Me	Me	H
C1a	H	H	H
C2	H	Me	H
C2a	H	H	Me
C2b	Me	H	H

Figure 31: Gentamicin components.



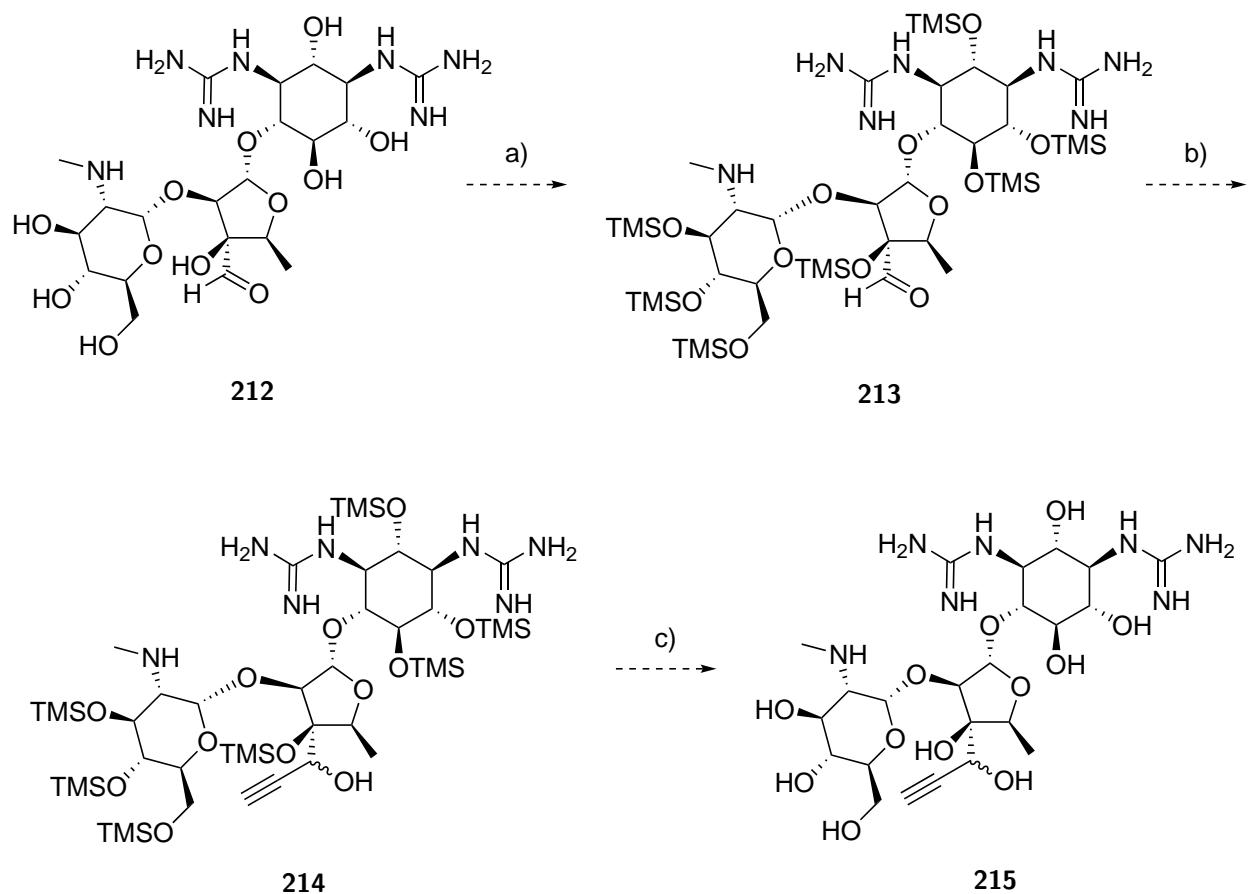
Scheme 53: Proposed synthesis of gentamicin C1a derivative **211**. a) DIPEA, DMF, -55 °C.

### 9.2.6 Streptomycin derivatives **215**, **217** and **219**

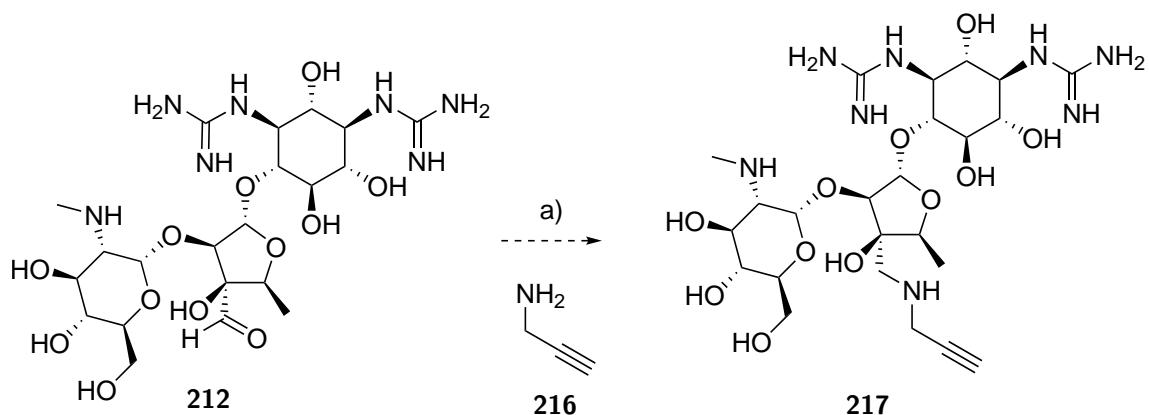
Streptomycin **212** is an aminoglycoside antibiotic used to treat *Mycobacterium tuberculosis* and *S. aureus* by binding to the bacterial ribosome. There is limited SAR data on streptomycin but it is known that conversion of the aldehyde to a carboxylic acid destroys activity, whereas conversion an alcohol retains it.<sup>220</sup> TMS protection followed by attack with lithium acetylidyde then deprotection could be used to produce an derivative **215** with a secondary alcohol in place of the aldehyde (see Scheme 54).

Reductive amination could also be used to install an alkyne group by reaction of the aldehyde with amino-1-propyne (see Scheme 55).<sup>174</sup> This would install NH in place of the aldehyde O; it is known that an OH is tolerated at this position so it seems possible that NH would be as well.

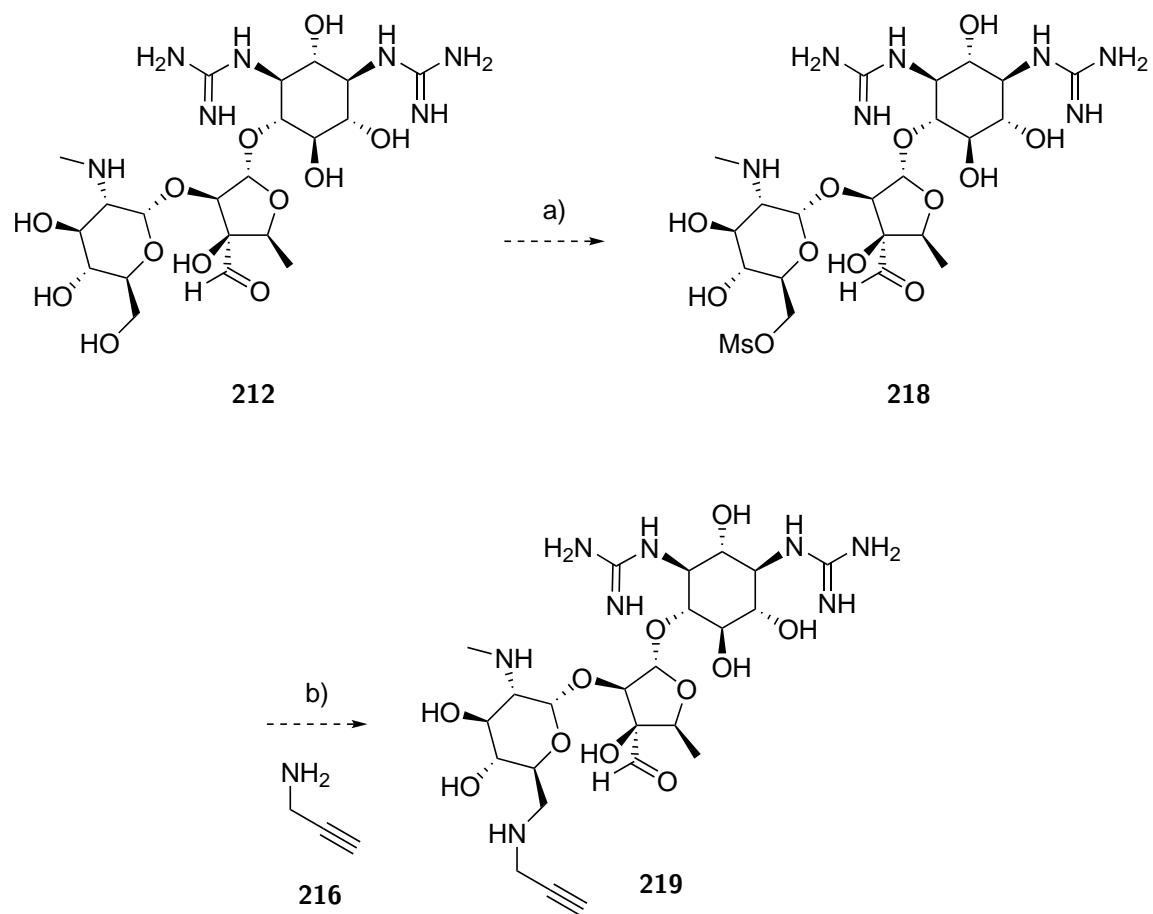
There is one primary alcohol in streptomycin **212** which could be selectively displaced to install an alkyne. Selective displacement could be achieved by reaction with 1 eq. of trifluoroacetic anhydride followed by displacement with amino-1-propyne (see Scheme 56).<sup>221</sup> This leaves an H-bond donor in that position as OH is replaced by NH.



Scheme 54: Proposed synthesis of streptomycin derivative **215**. a) TMSCl. b) Lithium acetylide. c) TBAF.



Scheme 55: Proposed synthesis of streptomycin derivative **217**. a)  $\text{NaBH}(\text{AcO})_3$ , 1,2-dichloroethane, r.t.



Scheme 56: Proposed synthesis of streptomycin derivative **219**. a) MsCl, pyridine,  $\text{CH}_2\text{Cl}_2$ , 0 °C to r.t. b) 3-amino-1-propyne, EtOH.

### 9.3 Autoinducer analogue derivatives

## 9.4 Linkers

## 9.5 Biology

## 10 Experimental

### 10.1 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 acetonitrile were distilled from calcium hydride. All other chemicals were used as obtained from commercial sources.

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

Thin-layer chromatography (TLC) was performed using Merck pre-coated 0.23 mm thick plates of Keiselgel 60 F254 and visualised using UV ( $\lambda = 254$  or 366 nm) or by staining with KMnO<sub>4</sub> or ninhydrin. All retention factors ( $R_f$ ) are given to 0.01. All column chromatography was carried out using Merck 9385 Keiselgel 60 silica gel (230-400 mesh) or using a CombiFlash® EZ Prep with RediSep® normal-phase silica flash columns. Preparative high-performance liquid chromatography (HPLC) was run on an Agilent 1260 Infinity machine, using a Supelcosil™ ABZ+PLUS column (250 mm  $\times$  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>222</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>222</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>).

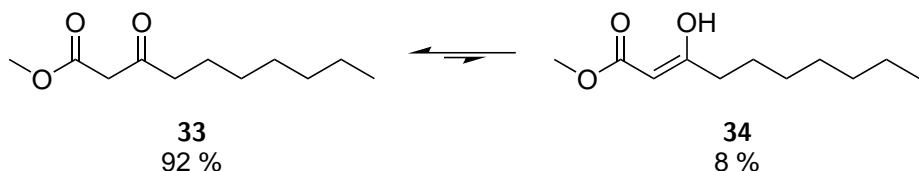
High resolution mass spectrometry (HRMS) data were recorded using a Micromass LCT Premier spectrometer or a Waters Vion IMS-QTOF spectrometer and reported mass values are within  $\pm 5$  ppm mass units. Liquid chromatography–mass spectrometry (LCMS) data were recorded on an Agilent 1200 series LC with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

Infrared (IR) spectra were recorded using neat sample on a PerkinElmer 1600 FT IR spectrometer. Selected absorption maxima ( $\nu_{max}$ ) are reported in wavenumbers (cm<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>.

## 10.2 Methyl 3-oxodecanoate 33



Meldrum's acid **31** (9.00 g, 63.0 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 **32** (11.7 ml, 69.0 mmol, 1.1 eq.) was then added and the mixture was stirred at 0 °C for a further 4 h. The mixture was allowed to warm to r.t., diluted with  $\text{CH}_2\text{Cl}_2$  (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 %  $\text{Et}_2\text{O}/40\text{-}60$  P.E.). A tautomeric mixture of **33** and **34** was obtained as a colourless oil (8.34 g, 41.6 mmol, 66 %. 92 % **33** as determined by  $^1\text{H}$  NMR).

### Keto form **33**

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

**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 **34**

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

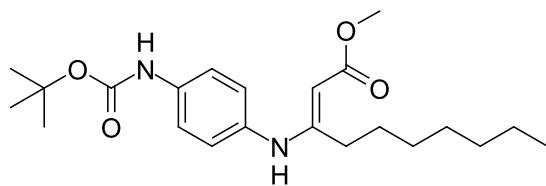
**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1}$  = 2927.8 (C-H), 2856.3 (C-H), 1653.8 (C=C), 1629.2 ( $\alpha,\beta$  unsaturated C=O)

**$^1\text{H}$  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>165,166</sup>

### 10.3 Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 36



Methyl 3-oxodecanoate **33** (500 mg, 2.50 mmol, 1.00 eq.) and *O*-*tert*-butyl *N*-(4-aminophenyl)carbamate **35** (520 mg, 2.50 mmol, 1.00 eq.) were dissolved in MeOH (10 ml) and refluxed for 18 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (SiO<sub>2</sub>, gradient of 0 to 20 % Et<sub>2</sub>O/40-60 P.E.). **36** was obtained as a white amorphous solid (0.169 mg, 0.480 mmol, 19 %).

**TLC**  $R_f$  = 0.30 (30 % Et<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)

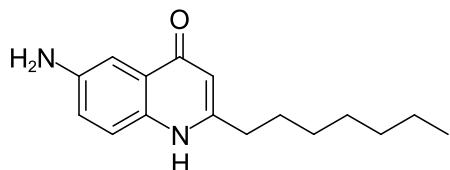
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 10.16 (s, 1 H, NHC(C<sub>7</sub>H<sub>15</sub>)=C), 7.35 (d,  $J$  = 8.6 Hz, 2 H, *meta* to NHBoc), 7.02 (d,  $J$  = 8.7 Hz, 2 H, *meta* to enamine), 6.60 (br s, 1 H, NHBoc), 4.71 (s, 1 H, C=CHC), 3.70 (s, 3 H, OCH<sub>3</sub>), 2.23 (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>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>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>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 NHBoc), 134.1 (CNHBoc), 126.3 (*meta* to NHBoc), 119.1 (*ortho* to NHBoc), 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>161</sup>

### 10.4 6-Amino-2-heptylquinolin-4-ol 37



Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate **36** (168 mg, 0.649 mmol, 1 eq.) and polyphosphoric acid (5 g) were heated to 90 °C for 1 h. The reaction mixture was then poured into 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. **37** 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)

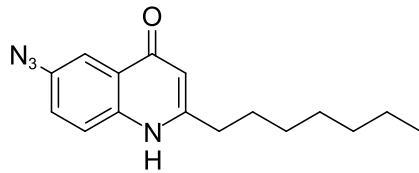
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 7.26 (d,  $J$  = 8.7 Hz, 1 H, *meta* to NH<sub>2</sub>), 7.15 (d,  $J$  = 2.6 Hz, 1 H, *ortho* to C(=O)), 6.95 (dd,  $J$  = 2.7, 8.8 Hz, 1 H, *para* to C(=O)), 5.74 (s, 1 H, *ortho* to CH<sub>2</sub>), 5.16 (s, 2 H, NH<sub>2</sub>), 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>161</sup>

## 10.5 6-Azido-2-heptylquinolin-4-ol 38



6-Amino-2-heptylquinolin-4-ol **37** (50 mg, 0.194 mmol, 1 eq) was dissolved in HCl (conc., aq., 1.20 ml), water (1.80 ml) and MeOH (2.00 ml) and cooled to 0 °C. A solution of NaNO<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. **38** hydrochloride salt\* was obtained as a pale cream amorphous solid (25.6 mg, 0.0800 mmol, 41.2 %).

**TLC**  $R_f$  = 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>CH<sub>2</sub>), 1.18 - 1.28 (m, 4 H, 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>)

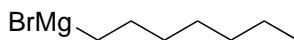
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 172.3 (C(=O)), 155.5 (NH<sub>2</sub>CCH<sub>2</sub>), 137.4 (CN<sub>3</sub>), 135.6 (*para* to N<sub>3</sub>), 124.6 (*para* to C(=O)), 124.1 (*ipso* to C(=O)), 120.7 (*meta* to N<sub>3</sub> and *meta* to C(=O)), 112.8 (*ortho* to N<sub>3</sub> and *ortho* to C(=O)), 107.0 (C(=O)CH), 33.3 (NH<sub>2</sub>CCH<sub>2</sub>), 31.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.3 - 28.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 22.1 (CH<sub>2</sub>CH<sub>3</sub>), 14.0 (CH<sub>3</sub>)

HRMS (ESI<sup>+</sup>)  $m/z$  / Da = 285.1728, [M+H]<sup>+</sup> found, [C<sub>16</sub>H<sub>21</sub>N<sub>4</sub>O]<sup>+</sup> requires 285.1715

Spectroscopic data are similar to the literature characterisation of the free amine.<sup>161</sup>

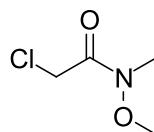
\*Probably as the 4-hydroxyquinoline.<sup>92</sup>

## 10.6 Heptyl magnesium bromide 40



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

## 10.7 2-Chloro-*N*-methoxy-*N*-methylacetamide 43



*N,O*-Dimethylhydroxyl amine hydrochloride **41** (6.00 g, 61.5 mmol, 1 eq.) and toluene (75 ml) were added successively to a stirred solution of potassium carbonate (22.4 g, 162 mmol, 2.63 eq.) in water (75 ml) at 0 °C under argon. The mixture was cooled to -5 °C and chloroacetyl chloride **42** (5.88 ml, 73.8 mmol, 1.20 eq.) was added dropwise over 5 min. The mixture was allowed to warm to r.t. over 30 min, then the organic layer was separated and the aqueous layer was extracted with toluene (3×20 ml). The combined organic extracts were dried with MgSO<sub>4</sub> and the solvent was removed by rotary evaporation followed by high vacuum. **43** was obtained as white, prism-like crystals (7.24 g, 52.6 mmol, 71 %).

mp  $T$  / °C = 38.8 (toluene)

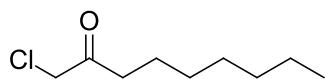
**IR (neat)**  $\nu_{max}$  / cm<sup>-1</sup> = 3016.7 (C-H), 2966.4 (C-H), 2946.7 (C-H), 2827.7 (C-H), 1666.2 (C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 4.20 (s, 2 H, ClCH<sub>2</sub>C=O), 3.71 (m, 3 H, OCH<sub>3</sub>), 3.18 (s, 3 H, NCH<sub>3</sub>)

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ / 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>92</sup>

## 10.8 1-Chlorononan-2-one 44



2-Chloro-*N*-methoxy-*N*-methylacetamide **43** (1.00 g, 7.26 mmol, 1 eq.) was added to a dry flask under argon. THF (20 ml) was added and the flask cooled to 0 °C. Heptyl magnesium bromide **40** (~ 1 M, 15.0 ml, 15.0

mmol, 2.07 eq.) was added dropwise over 5 min, then the mixture was allowed to warm to r.t. and stirred for 15 h. The reaction mixture was then poured into HCl (aq., 2 N, 60 ml) at 0 °C and stirred for 10 min. The mixture was extracted with toluene (30 ml) and the aqueous layer discarded. The organic layer was washed with brine and dried with MgSO<sub>4</sub>, and the solvent was removed by rotary evaporation. **44** was obtained as a colourless oil (1.23 g, 6.96 mmol, 96 %).

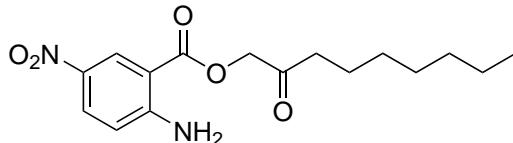
**IR** (neat)  $\nu_{max}$  / cm<sup>-1</sup> = 2951.7 (C-H), 2925.0 (C-H), 2855.5 (C-H), 1720.4 (C=O)

**<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>92</sup>

## 10.9 2-Oxononyl 2-amino-5-nitrobenzoate **46**



5-Nitroanthranilic acid **45** (500 mg, 2.75 mmol, 1.38 eq.) and potassium carbonate (270 mg, 2.00 mmol, 1 eq.) were dissolved in DMF (5 ml). The mixture was heated under argon to 90 °C and stirred for 1 h then cooled to r.t.. 1-Chlorononan-2-one **44** (353 mg, 2.00 mmol, 1 eq.) was added and the mixture was stirred for 15 h. The solution was poured into 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. **46** 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)

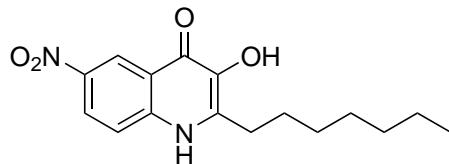
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 8.66 (d,  $J$  = 2.8 Hz, 1 H, *ortho* to C(=O)), 8.12 (dd,  $J$  = 2.8, 9.4 Hz, 1 H, *para* to C(=O)), 6.93 (d,  $J$  = 9.4 Hz, 1 H, *meta* to C(=O)), 5.05 (s, 2 H, OCH<sub>2</sub>C(=O)), 2.49 (t,  $J$  = 7.4 Hz, 2 H, C(=O)CH<sub>2</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>161</sup>

### 10.10 6-Nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 47



2-Oxononyl 2-amino-5-nitrobenzoate **46** (100 mg, 0.340 mmol, 1 eq.) and polyphosphoric acid (300 mg) were stirred for 5.5 h at 90 °C under argon. The mixture was then poured into NaHCO<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. **47** 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)

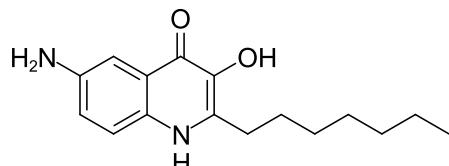
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>) δ / ppm = 12.00 (s, 1 H, NH), 8.91 (d, *J* = 2.8 Hz, 1 H, *ortho* to C=O), 8.29 (dd, *J* = 2.7, 9.2 Hz, 1 H, *para* to C=O), 7.70 (d, *J* = 9.3 Hz, 1 H, *meta* to C=O), 2.75 (t, *J* = 7.7 Hz, 2 H, CCH<sub>2</sub>), 1.67 (quin, *J* = 7.3 Hz, 2 H, CCH<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>) δ / 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>161</sup>

### 10.11 6-Amino-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 48



6-Nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **47** (20 mg, 0.0658 mmol, 1 eq.) and PtO<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. **48** 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)

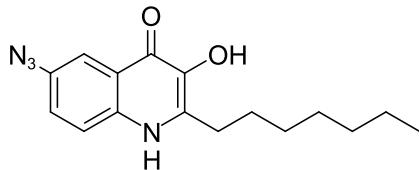
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 11.12 (s, 1 H, NH), 7.47 (d,  $J$  = 8.9 Hz, 1 H, *meta* to C=O), 7.40 (d,  $J$  = 2.4 Hz, 1 H, *ortho* to C=O), 7.16 (dd,  $J$  = 2.6, 9.0 Hz, 1 H, *para* to C=O), 2.86 (t,  $J$  = 7.5 Hz, 2 H, CCH<sub>2</sub>), 1.75 (quin,  $J$  = 7.8 Hz, 2 H, 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 (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>161</sup> It is possible that Baker's product is a Zn adduct.

### 10.12 6-Azido-2-heptyl-3-hydroxyquinolin-4(1H)-one 49



6-Amino-2-heptyl-3-hydroxyquinolin-4(1H)-one **48** (18.2 mg, 0.0664 mmol, 1 eq.) was dissolved in HCl (conc., aq., 0.8 ml) and MeOH (0.5 ml) at 0 °C. 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. **49** was obtained as a brown amorphous solid (5.5 mg, 0.0183 mmol, 28 %).

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

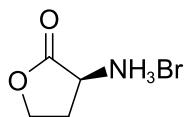
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 7.74 (s, 1 H, *ortho* to C=O), 7.65 (d,  $J$  = 6.9 Hz, 1 H, *meta* to C=O), 7.32 (d,  $J$  = 7.4 Hz, 1 H, *para* to C=O), 2.75 (t,  $J$  = 7.5 Hz, 2 H, CCH<sub>2</sub>), 1.67 (quin,  $J$  = 6.4 Hz, 2 H, CCH<sub>2</sub>CH<sub>2</sub>), 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 = 166.3 (C=O), 137.9 (C), 137.8 (CN<sub>3</sub>), 134.5 (*ipso* to C=O), 133.9 (C=COH), 122.7 (para to C=O), 122.6 (meta to N<sub>3</sub> and *meta* to C=O), 120.4 (para to N<sub>3</sub>), 112.4 (*ortho* to N<sub>3</sub> and *ortho* to C=O), 31.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 28.8 (CCH<sub>2</sub>), 28.4 (CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.3 (CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 27.8 (CCH<sub>2</sub>CH<sub>2</sub>), 22.1 (CH<sub>2</sub>CH<sub>3</sub>), 14.0 (CH<sub>3</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 301.1649, [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

Spectroscopic data are consistent with the literature.<sup>161</sup>

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



L-Methionine **50** (3.04 g, 20.4 mmol, 1 eq.) and bromoacetic acid **51** (3.08 g, 22.2 mmol, 1.09 eq.) were dissolved in *i*-PrOH (12.5 ml), 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. **52** was obtained as a pale pink amorphous solid (1.73 g, 9.50 mmol, 41 % yield).

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

**IR** (neat)  $\nu_{max}$  / 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)

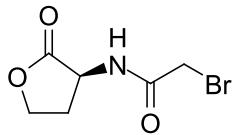
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 8.59 (br s, 3 H, NH<sub>3</sub><sup>+</sup>), 4.46 (dt, *J* = 1.3, 8.9 Hz, 1 H, OCHH), 4.37 (dd, *J* = 8.8, 11.4 Hz, 1 H, CHNH<sub>3</sub><sup>+</sup>), 4.29 (ddd, *J* = 6.1, 8.8, 10.9 Hz, 1 H, OCHH), 2.57 (dddd, *J* = 1.2, 6.1, 8.9, 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>162</sup>

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



(*S*)-3-Aminodihydrofuran-2(*3H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.08 eq.) and 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 **53** (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. **54** 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, OCHH), 4.32 (ddd,  $J$  = 11.3, 9.4, 5.9 Hz, 1 H, OCHH), 3.93 (s, 1 H, CHHBr), 3.93 (s, 1 H, CHHBr), 2.87 (dddd,  $J$  = 12.6, 8.6, 5.9, 1.3 Hz, 1 H, OCH<sub>2</sub>CHH), 2.22 (dtd,  $J$  = 12.6, 11.5, 11.5, 8.9 Hz, 1 H, OCH<sub>2</sub>CHH)

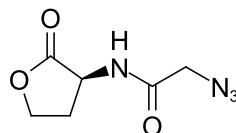
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 174.6 (OC=O), 166.4 (C=O)NH), 66.1 (OCH<sub>2</sub>), 49.8 (CHNHC=O), 29.9 (OCH<sub>2</sub>CH<sub>2</sub>), 28.2 (O=CCH<sub>2</sub>Br)

**HRMS** The compound does not ionise.

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

The data are consistent with the literature.<sup>162, 223</sup>

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



(3*S*)-2-Oxotetrahydrofuran-3-aminium bromide **52** (100 mg, 0.552 mmol, 1.08 eq.), 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 **53** (44.0  $\mu$ L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the CH<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. **55** 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 (N<sub>3</sub>), 1782.9 (lactone C=O), 1661.4 (amide C=O), 1536.8 (N-H bend)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 7.05 (br d,  $J$  = 6.5 Hz, 1 H, NH), 4.64 (ddd,  $J$  = 11.6, 8.7, 6.8 Hz, 1 H, CHNH), 4.48 (td,  $J$  = 9.1, 1.3 Hz, 1 H, OCHH), 4.30 (ddd,  $J$  = 11.2, 9.2, 6.0 Hz, 1 H, OCHH), 4.04 (s, 2 H, CH<sub>2</sub>N<sub>3</sub>), 2.76 (dddd,  $J$  = 12.5, 8.8, 6.0, 1.4 Hz, 1 H, OCH<sub>2</sub>CHH), 2.25 (dtd,  $J$  = 12.5, 11.4, 11.4, 8.9 Hz, 1 H, OCH<sub>2</sub>CHH)

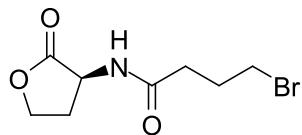
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 174.9 (OC=O), 167.5 (C=ONH), 66.0 (OCH<sub>2</sub>), 52.2 (O=CCH<sub>2</sub>N<sub>3</sub>), 48.9 (CHNHC=O), 29.7 (OCH<sub>2</sub>CH<sub>2</sub>)

**HRMS** The compound does not ionise.

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

The data are consistent with the literature.<sup>162</sup>

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



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (200 mg, 1.10 mmol, 1.00 eq.) and NaHCO<sub>3</sub> (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and H<sub>2</sub>O (2 ml). Bromobutyryl chloride **56** (140  $\mu$ L, 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The aqueous phase was extracted with EtOAc (7  $\times$  5 ml) and the combined organic layers were dried with MgSO<sub>4</sub>. The solvent was removed under vacuum to give white crystals which were recrystallised from EtOAc. **57** was obtained as white, needle-like crystals (219 mg, 0.878 mmol, 80 %).

**mp** *T* / °C = 105 (EtOAc)

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

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

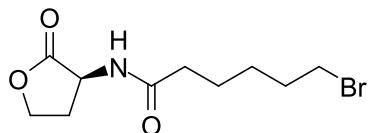
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 175.4 (OC=O), 172.3 (C(=O)NH), 66.1 (OCH<sub>2</sub>), 49.3 (CHNHC=O), 33.9 (C(=O)CH<sub>2</sub>), 33.1 (CH<sub>2</sub>Br), 30.3 (OCH<sub>2</sub>CH<sub>2</sub>), 27.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** The compound does not ionise.

$[\alpha]_D^{26.6}$  / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -78 (*c* / g(100 ml)<sup>-1</sup> = 0.0833, MeOH)

The compound has not been reported previously.

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



(*S*)-3-Aminodihydrofuran-2(3*H*)-one hydrobromide **52** (100 mg, 0.549 mmol, 1.00 eq.) and NaHCO<sub>3</sub> (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and H<sub>2</sub>O (2 ml) at r.t.. Bromohexanoyl chloride **59** (93.0  $\mu$ L, 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the CH<sub>2</sub>Cl<sub>2</sub> was removed under vacuum. The mixture was then filtered, washed with H<sub>2</sub>O (10 ml) and dried under high vacuum. **60** was obtained as white, needle-like crystals (101 mg, 0.362 mmol, 66 %).

**mp** *T* / °C = 106 (CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>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, NH), 4.57 (ddd,  $J$  = 5.9, 8.6, 11.6 Hz, 1 H, CHNH), 4.50 (dt,  $J$  = 1.3, 9.1 Hz, 1 H, OCHH), 4.31 (ddd,  $J$  = 5.9, 9.3, 11.3 Hz, 1 H, OCHH), 3.43 (t,  $J$  = 6.7 Hz, 2 H,  $\text{CH}_2\text{Br}$ ), 2.88 (dddd,  $J$  = 1.3, 5.9, 8.6, 12.6 Hz, 1 H,  $\text{OCH}_2\text{CHH}$ ), 2.30 (dt,  $J$  = 1.8, 7.5 Hz, 2 H, C(=O)CH<sub>2</sub>), 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, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.59 - 1.46 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)

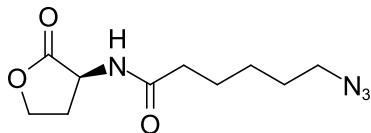
**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 175.5 (OC=O), 173.3 (C(=O)NH), 66.1 (OCH<sub>2</sub>), 49.3 (CHNHC=O), 35.8 (CH<sub>2</sub>Br), 33.5 (C(=O)CH<sub>2</sub>), 32.3 (CH<sub>2</sub>CH<sub>2</sub>Br), 30.5 (OCH<sub>2</sub>CH<sub>2</sub>), 27.6 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 24.4 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 278.0381, [M+H]<sup>+</sup>, [C<sub>10</sub>H<sub>17</sub>BrNO<sub>3</sub>]<sup>+</sup> requires 278.0386

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

The compound has not been reported previously.

### 10.18 (S)-6-Azido-N-(2-oxotetrahydrofuran-3-yl)hexanamide 61



(S)-6-Bromo-N-(2-oxotetrahydrofuran-3-yl)hexanamide **60** (80 mg, 0.320 mmol, 1.00 eq.) and  $\text{NaN}_3$  (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. **61** 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 (N<sub>3</sub>), 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, NH), 4.54 (ddd,  $J$  = 11.7, 8.6, 5.7 Hz, 1 H, CHNH), 4.49 (td,  $J$  = 9.1, 1.0 Hz, 1 H, OCHH), 4.30 (ddd,  $J$  = 11.3, 9.4, 5.8 Hz, 1 H, OCHH), 3.29 (t,  $J$  = 6.9 Hz, 2 H,  $\text{CH}_2\text{N}_3$ ), 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, C(=O)CHH), 2.28 (t,  $J$  = 7.4 Hz, 1 H, C(=O)CHH), 2.14 (tdt,  $J$  = 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, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.38 - 1.49 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)

**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 175.4 (OC=O), 172.2 (C(=O)NH), 66.1 (OCH<sub>2</sub>), 51.2 (CH<sub>2</sub>N<sub>3</sub>), 49.4 (CHNHC=O), 35.9 (C(=O)CH<sub>2</sub>), 30.7 (OCH<sub>2</sub>CH<sub>2</sub>), 28.6 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 26.3 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 24.8 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 241.1289, [M+H]<sup>+</sup>, [C<sub>10</sub>H<sub>17</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 241.1295

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

The compound has not been reported previously.

### 10.19 Hex-5-ynal 63



Pyridinium chlorochromate (14.6 g, 68.1 mmol, 1.50 eq) and CH<sub>2</sub>Cl<sub>2</sub> (500 ml) were stirred at r.t. under argon. 5-Hexyn-1-ol **62** (5.00 ml, 45.4 mmol, 1 eq.) was added and the reaction mixture was stirred for 5 h followed by addition of Et<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. **63** was obtained as a pale yellow-green oil (4.72 g, 49.1 mmol, 72 %).

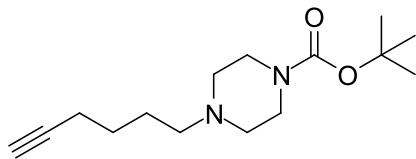
**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 3292.7$  (alkyne C-H), 2943.3 (alkane C-H), 2830.9 (aldehyde C-H), 2728.6 (aldehyde C-H), 1720.3 (aldehyde C=O)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta / \text{ppm} = 9.80$  (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>)  $\delta / \text{ppm} = 201.6$  (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>173</sup>

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



Hex-5-ynal **63** (0.407 g, 4.24 mmol, 1.00 eq.) and *tert*-butyl piperazine-1-carboxylate **64** (0.791 g, 4.24 mmol, 1.00 eq.) were stirred under a N<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. **65** 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)  $\nu_{max} / \text{cm}^{-1} = 3303.6$  (alkyne C-H), 2940.0 (alkane C-H), 2865.2 (C-H), 2810.4 (C-H), 1691.3

(carbamate C=O)

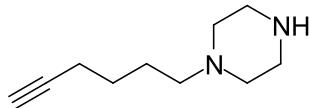
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 3.44 (t,  $J$  = 5.2 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (t,  $J$  = 5.1 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<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.

## 10.21 1-(Hex-5-yn-1-yl)piperazine **66**



*tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate **65** (763 mg, 2.86 mmol) was stirred in TFA (10 ml) at r.t. for 2 h. The TFA was removed under vacuum followed by co-evaporation with 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). **66** 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)

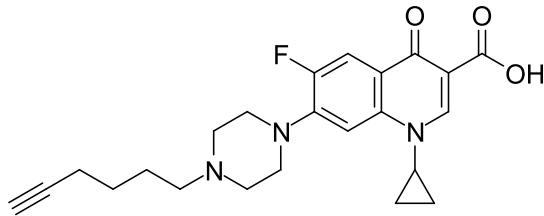
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 2.88 (t,  $J$  = 4.9 Hz, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>), 2.39 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.31 (t,  $J$  = 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.

**10.22 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid 68**



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

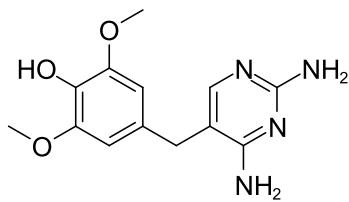
**<sup>13</sup>C NMR** (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 165.8 (C(=O)OH), 152.8 (d,  $J$  = 248.5 Hz, *ipso* to F), 148.2 (CHCC(=O)OH), 143.7 (d,  $J$  = 11.1 Hz, *para* to C(=O)), 139.1 (*para* to F), 119.4 (d,  $J$  = 6.9 Hz, *ipso* to C(=O)), 111.2 (d,  $J$  = 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>N(CH<sub>2</sub>)), 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.

### 10.23 4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol 69



Hydrobromic acid (48 % w/w, aq., 50 ml) was heated to 100 °C. Trimethoprim **25** (5.00 g, 17.2 mmol) was added, and the suspension was stirred for 40 min under Ar. The mixture was removed from the heat, and NaOH (50 % w/w, aq., 15 ml) was added dropwise. The reaction mixture was then cooled slowly to 0 °C, and the resulting crystals were filtered out and washed with cold water. The crystals were then dissolved in hot water (80 ml), neutralized with 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. **69** 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**  $T$  / °C = 238 (H<sub>2</sub>O, decomposes)

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

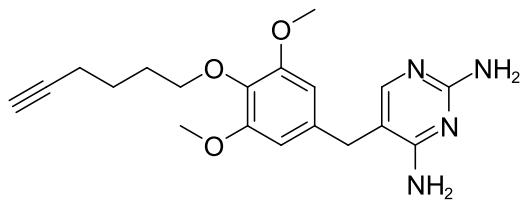
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 7.21 (s, 1 H, CHN), 6.54 (s, 2 H, *meta* to OCH<sub>2</sub>), 4.87 (br s, 5 H, OH, NH<sub>2</sub> × 2), 3.82 (s, 6 H, OCH<sub>3</sub>), 3.63 (s, 2 H, CCH<sub>2</sub>C)

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / 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>)  $m/z$  / 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>164</sup>

### 10.24 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71



4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenol **69** (1.00 g, 3.62 mmol, 1 eq.), 6-chloro-1-hexyne **70** (0.524 ml, 0.420 g, 4.34 mmol, 1.2 eq.), 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 ( $\text{SiO}_2$ , 5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). **71** 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)  $\nu_{max}$  / cm<sup>-1</sup> = 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)  $\delta$  / 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)  $\delta$  / 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>CCNH<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>)

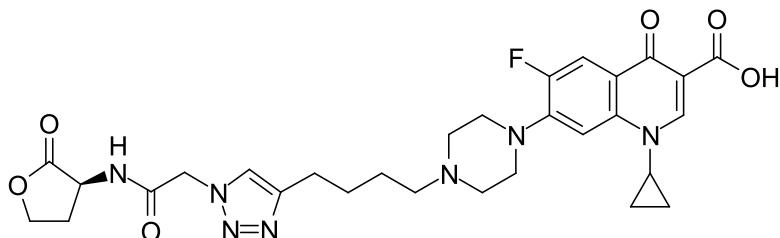
**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 357.1920, [M+H]<sup>+</sup> found, [C<sub>19</sub>H<sub>25</sub>N<sub>4</sub>O<sub>3</sub>]<sup>+</sup> requires 357.1927

The compound has not been reported previously.

## 10.25 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 **74** were dissolved in water to make a 50 mM solution and similarly degassed. Sodium ascorbate was dissolved in water to make a 100 mM solution and similarly degassed. The Cu/THPTA solution (0.05 eq.) was added to the reaction mixture, followed by the sodium ascorbate solution (0.1 eq.). The mixture was stirred for 2 h and monitored using LCMS. HL derivative conjugates were dry-loaded onto SiO<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).

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



50 % water/*t*-BuOH (2 ml) was degassed by bubbling 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 **68** (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-2-azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide **55** (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. **72** 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>)

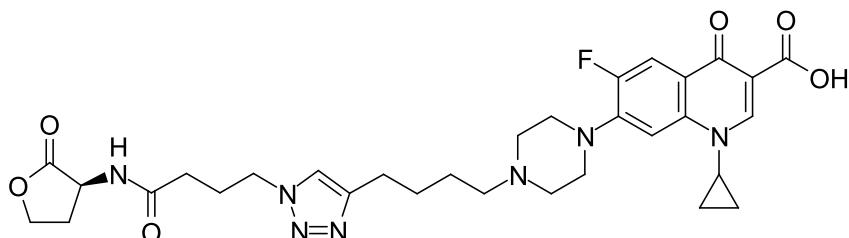
**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 174.9 (OC(=O)), 166.0 (C(=O)OH), 165.9 (NHC(=O)), 153.1 (d, *J* = 250.8 Hz, *ipso* to F), 148.0 (CH=CC(=O)OH), 146.6 (CH=CCH<sub>2</sub>), 145.3 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 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

$[\alpha]_D^{20}$  / °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.

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



50 % water/*t*-BuOH (2 ml) was degassed by bubbling 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 **68** (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **58** (10.6 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. **77** was obtained as a white amorphous solid (14.6 mg, 23.4  $\mu$ mol, 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>)  $\delta$  / 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>)

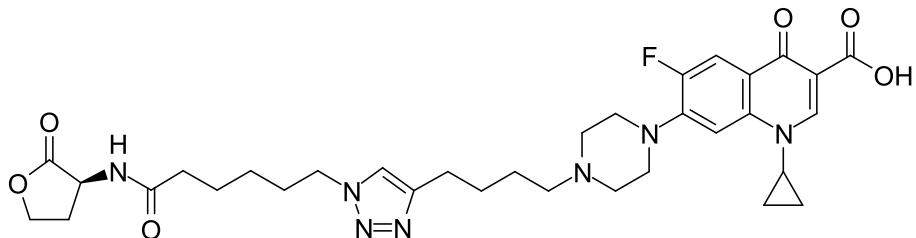
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.3 (C(=O)CC(=O)OH), 175.4 (OC(=O)), 171.2 (NHC(=O)), 166.0 (C(=O)OH), 153.0 (d, *J* = 248.6 Hz, *ortho* to F), 148.0 (CH=CC(=O)OH), 146.8 (CH=CCH<sub>2</sub>), 145.2 (d, *J* = 9.6 Hz, *ipso* to piperazine), 139.2 (*para* to F), 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>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

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

The compound has not been reported previously.

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



50 % water/*t*-BuOH (2 ml) was degassed by bubbling 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 **68** (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (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. **78** was obtained as a white amorphous solid (12.4 mg, 19.0  $\mu$ mol, 38.0 %).

**TLC**  $R_f$  = 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=C

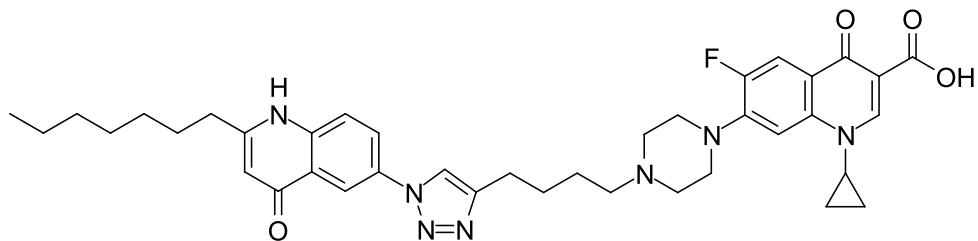
$\text{CH}_2\text{CH}_2$ ), 25.7 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 25.4 ( $\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2$ ), 24.9 ( $\text{CH}=\text{CCH}_2$ ), 24.5 ( $\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2$ ), 7.6 ( $\text{NCH}(\text{CH}_2)_2$ )

**HRMS** (ESI<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

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

The compound has not been reported previously.

**10.29 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 80**



50 % water/*t*-BuOH (1 ml) was degassed by bubbling 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 **68** (4.1 mg, 10.0  $\mu\text{mol}$ , 1 eq.) and 6-azido-2-heptylquinolin-4(*H*)-one **38** (2.8 mg, 10.0  $\mu\text{mol}$ , 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>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  $\mu\text{mol}$ , 0.1 eq., 100 mM) in 50 % water/*t*-BuOH (10  $\mu\text{l}$ ) 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 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. **80** was obtained as a white amorphous solid (8.6 mg, 2.7  $\mu\text{mol}$ , 27.0 %).

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

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) 15.12 (br s, 1 H,  $\text{C}(=\text{O})\text{OH}$ ), 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>, NCH(CHH)<sub>2</sub> and NCH(CHH)<sub>2</sub>), 0.87 (t, *J* = 6.9 Hz, 3 H, CH<sub>3</sub>)

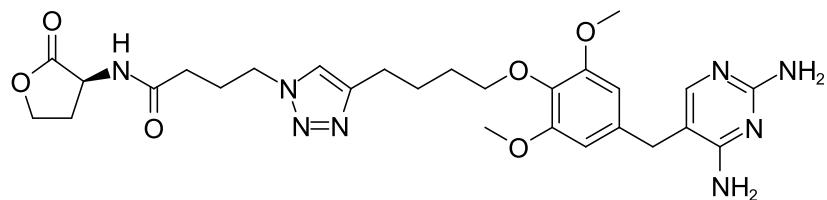
**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$ ), 176.3 (CHC(=O)), 165.8 ( $\text{C}(=\text{O})\text{OH}$ ), 154.3 (CCHC(=O)), 152.9 (d, *J* = 240.1 Hz, *ipso* to F), 148.3 ( $\text{CH}=\text{CC}(=\text{O})\text{OH}$ ), 147.5 (NCHCCH<sub>2</sub>), 143.0 (d, *J* = 8.5 Hz, *ortho* to F and *ipso* to N), 139.6 (*ipso* to NH), 139.0 (*para* to F), 132.0 (*para* to NH), 124.9 (*ipso* to

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.

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



50 % water/*t*-BuOH (2 ml) was degassed by bubbling 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 **71** (20.6 mg, 50.0  $\mu$ mol, 1 eq.) and (*S*)-4-azido-*N*-(2-oxotetrahydrofuran-3-yl)butanamide **58** (15.9 mg, 75.0  $\mu$ mol, 1.5 eq.). Similarly degassed solutions 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 water (50  $\mu$ l) were then added. An extra portion of **58** (10.6 mg, 50.0  $\mu$ 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 CH<sub>2</sub>Cl<sub>2</sub> (6×10 ml) 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>). The combined pure fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **84** was obtained as a pale brown gum (4.8 mg, 8.4  $\mu$ mol, 16.8 %).

**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> = 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)

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

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 175.8 (OC=O), 171.9 (NHC=O), 163.1 (CC(NH<sub>2</sub>)N), 159.7

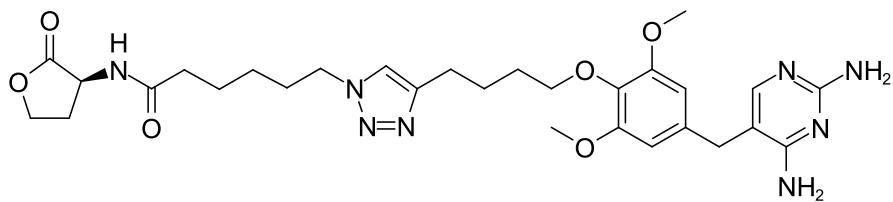
(br s,  $\underline{\text{NC}}(\text{NH}_2)\text{N}$ ), 153.2 (*ipso* to  $\text{OCH}_3$ ), 150.5 (br s,  $\underline{\text{CH}}\text{NC}(\text{NH}_2)\text{N}$ ), 147.3 ( $\text{NCH}=\underline{\text{CCH}_2\text{CH}_2}$ ), 135.2 (*para* to  $\text{CH}_2\text{O}$ ), 135.0 (*ipso* to  $\text{CH}_2\text{O}$ ), 122.1 ( $\underline{\text{CH}}=\text{CCH}_2\text{CH}_2$ ), 107.3 ( $\text{CH}_2\underline{\text{CC}}(\text{NH}_2)=\text{N}$ ), 106.2 (*meta* to  $\text{CH}_2\text{O}$ ), 72.3 ( $\text{CH}_2\text{CH}_2\underline{\text{CH}_2\text{O}}$ ), 65.7 ( $\underline{\text{OCH}_2\text{CH}_2\text{CHNH}}$ ), 56.2 ( $\underline{\text{OCH}_3}$ ), 48.9 ( $\underline{\text{CH}_2\text{N}}$ ), 48.3 ( $\underline{\text{CHNH}}$ ), 32.9 ( $\underline{\text{CCH}_2\text{C}}$ ), 32.0 ( $\text{C}=(\text{O})\underline{\text{CH}_2}$ ), 29.3 ( $\text{CH}_2\underline{\text{CH}_2\text{CH}_2\text{O}}$ ), 28.4 ( $\text{OCH}_2\underline{\text{CH}_2\text{CHNH}}$ ), 26.0 ( $\underline{\text{CH}_2\text{CH}_2\text{N}}$ ), 25.7 ( $\text{CH}=\underline{\text{CCH}_2\text{CH}_2}$ ), 24.9 ( $\text{CH}=\underline{\text{CCH}_2\text{CH}_2}$ )

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 569.2834, [M+H]<sup>+</sup> found, [C<sub>27</sub>H<sub>37</sub>N<sub>8</sub>O<sub>6</sub>]<sup>+</sup> requires 569.2836

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

The compound has not been reported previously.

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



50 % water/*t*-BuOH (2 ml) was degassed by bubbling  $\text{N}_2$  through it. This was then added to a mixture of 5-(4-(hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine **71** (20.6 mg, 50.0  $\mu\text{mol}$ , 1 eq.) and (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide **61** (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 **61** (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 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **85** was obtained as a clear gum (8.0 mg, 13.4  $\mu\text{mol}$ , 26.8 %).

**TLC**  $R_f = 0.35$  (30 % 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)

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

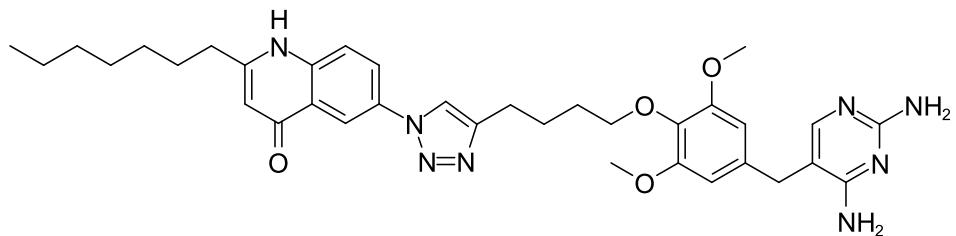
**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 175.4 (OC=O), 172.0 (NHC=O), 162.2 (CC(NH<sub>2</sub>)N), 161.8 (NC(NH<sub>2</sub>)N), 154.8 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.7 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 135.5 (*para* to CH<sub>2</sub>O), 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* / 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* / g(100 ml)<sup>-1</sup> = 0.11, MeOH)

The compound has not been reported previously.

**10.32 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(*1H*)-one 87**



50 % water/t-BuOH (1 ml) was degassed by bubbling 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 **71** (3.6 mg, 10.0  $\mu\text{mol}$ , 1 eq.) and 6-azido-2-heptylquinolin-4(*1H*)-one **38** (2.8 mg, 10.0  $\mu\text{mol}$ , 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>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  $\mu\text{mol}$ , 0.1 eq., 100 mM) in water (10  $\mu\text{l}$ ) 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. **87** was obtained as a clear gum (2.6 mg, 4.1  $\mu\text{mol}$ , 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)

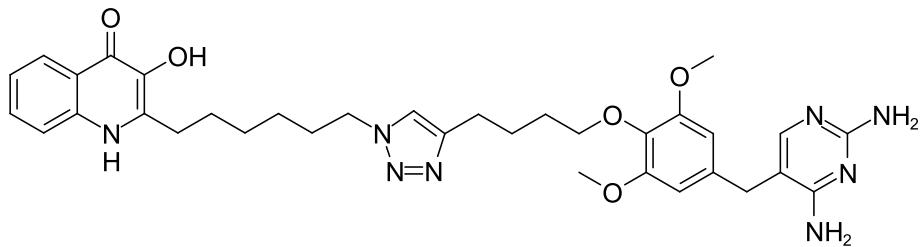
**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 11.80 (s, 1 H, NH), 8.69 (s, 1 H, NCH=CCH<sub>2</sub>), 8.41 (d, *J* = 2.7 Hz, 1 H, *ortho* to C=O), 8.17 (dd, *J* = 9.0, 2.6 Hz, 1 H, *para* to C=O), 7.73 (d, *J* = 9.0 Hz, 1 H, *ortho* to NH), 7.51 (br s, 4 H, NH<sub>2</sub>), 7.41 (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>) δ / 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.

**10.33 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 89**



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 **71** (14.2 mg, 39.8 μmol, 1 eq.) and 2-(6-azidohexyl)-3-hydroxyquinolin-4(1*H*)-one **30** (11.4 mg, 39.8 μmol, 1 eq.). A similarly degassed solution of CuSO<sub>4</sub> · 5 H<sub>2</sub>O (1.25 mg, 5 μmol, 0.125 eq. 50 mM), THPTA (2.18 mg, 5 μmol, 0.125 eq. 50 mM) and sodium ascorbate (1.98 mg, 10 μmol, 0.25 eq., 100 mM) in water (100 μl) was then added. The mixture was stirred at r.t. under argon for 3 h, then MeOH (1 ml) was added and the reaction mixture was dry-loaded onto SiO<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 MgSO<sub>4</sub> and evaporated under reduced pressure. **89** was obtained as a pale brown amorphous solid (4.7 mg, 7.3 μmol, 18.3 %).

**TLC** *R<sub>f</sub>* = 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>) δ / ppm = 11.53 (br s, 1 H, NH), 8.09 (d, *J* = 8.0 Hz, 1 H, *ortho* to C=O), 7.83 (s, 1 H, NCH=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>)

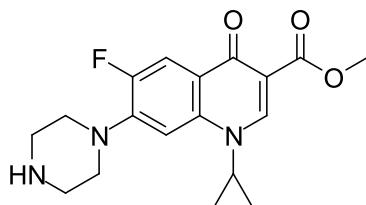
**<sup>13</sup>C NMR** (125 MHz, DMSO d<sub>6</sub>) δ / ppm = 168.9 (C=O), 162.5 (CC(NH<sub>2</sub>)N), 162.5 (NC(NH<sub>2</sub>)N), 152.9 (CHNC(NH<sub>2</sub>)N), 152.8 (*ipso* to OCH<sub>3</sub>), 146.8 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 137.7 (COH), 137.3 (*para* to OH), 135.4

(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.

### 10.34 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 100



Ciprofloxacin **24** (10.0 g, 30 mmol, 1 eq.) and *para*-toluenesulfonic acid (8.60 mg, 44.5 mmol, 1.5 eq.) were refluxed in methanol (500 ml) for 72 h. The mixture was cooled to room temperature and 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. **100** was obtained as a white amorphous solid (9.16 g, 26.5 mmol, 83.3 %).

**TLC** *R<sub>f</sub>* = 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>)

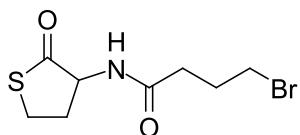
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.2 (C(=O)CC(=O)OCH<sub>3</sub>), 166.8 (C(=O)OCH<sub>3</sub>), 154.9 (d, *J* = 248.0 Hz, *ipso* to F), 150.1 (C=CC(=O)OCH<sub>3</sub>), 146.6 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.3 (d, *J* = 6.9 Hz, *para* to piperazine), 113.0 (d, *J* = 23.4 Hz, *ortho* to C=O and *ortho* to F), 110.1 (C(=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>178</sup>

### 10.35 4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 102



3-Aminodihydrothiophen-2(3*H*)-one hydrochloride **101** (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 **56** (11.3 ml, 107 mmol, 1.1 eq.) was added dropwise over 45 min at 0 °C and the mixture was stirred for a further 1 h. The organic layer was separated and the aqueous layer was extracted with a second portion of 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. **102** 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)

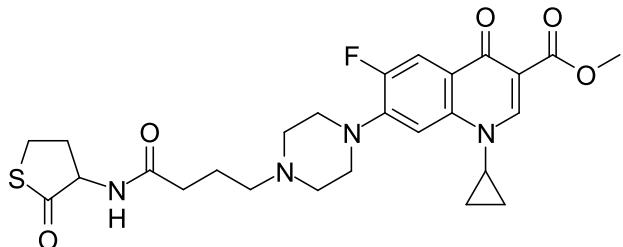
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.08 (d,  $J$  = 6.1 Hz, 1 H, NH), 4.54 (dt,  $J$  = 12.9, 6.5 Hz, 1 H, CHNH), 3.49 (t,  $J$  = 6.4 Hz, 2 H, CH<sub>2</sub>Br), 3.37 (ddd,  $J$  = 12.2, 11.5, 5.3 Hz, 1 H, SCHH), 3.26 (ddd,  $J$  = 11.5, 6.9, 1.3 Hz, 1 H, SCHH), 2.91 (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>)

**LRMS** (AP+)  $m/z$  / Da = 266.1, [M+H]<sup>+</sup> found, [C<sub>8</sub>H<sub>12</sub>BrNO<sub>2</sub>S]<sup>+</sup> requires 266.0

The compound has been synthesised previously<sup>145, 146</sup> but characterisation was not published.

### 10.36 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 103



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **100** (50 mg, 0.145 mmol, 1 eq.), 4-bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **102** (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 **102** (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). **103** 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, CH<sub>2</sub>NH), 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, SCH<sub>2</sub>H), 3.26 - 3.33 (m, 5 H, SCH<sub>2</sub>H 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>CH<sub>2</sub>H), 2.39 (t,  $J$  = 7.20 Hz, 1 H, C(=O)CH<sub>2</sub>H), 2.38 (t,  $J$  = 6.94 Hz, 1 H, C(=O)CH<sub>2</sub>H), 2.18 (qd,  $J$  = 12.4, 7.0 Hz, 1 H, SCH<sub>2</sub>CH<sub>2</sub>H), 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(CH<sub>2</sub>H)<sub>2</sub>), 1.13 - 1.19 (m, 2 H, NCH(CH<sub>2</sub>H)<sub>2</sub>)

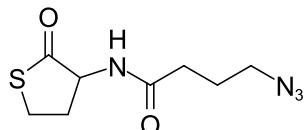
**<sup>13</sup>C NMR** (126 MHz, MeOD)  $\delta$  / ppm = 207.0 (SC(=O)), 175.7 (NHC(=O)), 175.1 (C(=O)CC(=O)OCH<sub>3</sub>), 166.6 (C(=O)OCH<sub>3</sub>), 154.7 (d,  $J$  = 249.0 Hz, *ipso* to F), 150.2 (s, CH=CC(=O)OCH<sub>3</sub>), 145.6 (d,  $J$  = 10.6 Hz, *ipso* to piperazine), 139.8 (*para* to 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 (CH<sub>2</sub>NH), 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>145, 146</sup> Only HRMS characterisation was published, and this agrees with the result above.

### 10.37 4-Azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide **104**



4-Bromo-N-(2-oxotetrahydrothiophen-3-yl)butanamide **102** (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. **104** 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)

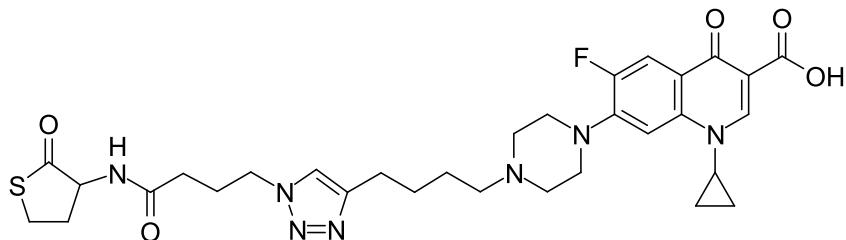
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 6.71 (d,  $J$  = 7.3 Hz, 1 H, NH), 4.54 (dt,  $J$  = 13.0, 7.0 Hz, 1 H, CHNH), 3.30 (t,  $J$  = 6.7 Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 3.31 (td,  $J$  = 11.7, 5.3 Hz, 1 H, SCHH), 3.19 (ddd,  $J$  = 11.3, 7.0, 1.2 Hz, 1 H, SCHH), 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.

**10.38 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 105**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (15 mg, 36.7  $\mu$ mol, 1 eq.) and 4-azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide **104** (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> (2×10 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. **105** 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,

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

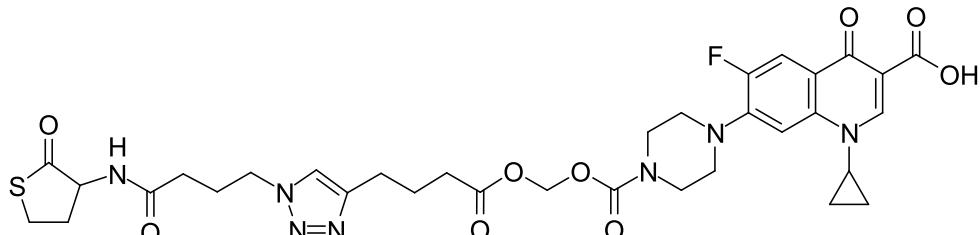
**$^{13}\text{C}$  NMR** (126 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 205.6 ( $\text{SC}(\text{=O})$ ), 176.4 ( $\text{C}(\text{=O})\text{CC}(\text{=O})\text{OH}$ ), 171.4 ( $\text{NHC}(\text{=O})$ ), 166.0 ( $\text{C}(\text{=O})\text{OH}$ ), 153.1 (d,  $J = 249.3$  Hz, *ortho* to F), 148.0 ( $\text{CH}=\text{CC}(\text{=O})\text{OH}$ ), 146.9 ( $\text{CH}=\text{CCH}_2$ ), 145.3 (d,  $J = 10.1$  Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.8 ( $\text{CH}=\text{CCH}_2$ ), 118.6 (d,  $J = 7.7$  Hz, *para* to piperazine), 111.0 (d,  $J = 23.3$  Hz, *ortho* to C=O and *ortho* to F), 106.7 ( $\text{CC}(\text{=O})\text{OH}$ ), 106.4 (d,  $J = 2.9$  Hz, *meta* to C=O and *meta* to F), 58.2 ( $\text{SC}(\text{=O})\text{CHNH}$ ), 57.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 52.4 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 49.5 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.5 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 48.6 ( $\text{CH}_2\text{NCH}=\text{C}$ ), 35.9 ( $\text{NCH}(\text{CH}_2)_2$ ), 31.9 ( $\text{NHC}(\text{=O})\text{CH}_2$ ), 30.1 ( $\text{CH}_2\text{CHNH}$ ), 26.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2$ ), 26.8 ( $\text{SCH}_2$ ), 25.9 ( $\text{NHC}(\text{=O})\text{CH}_2\text{CH}_2$ ), 25.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2$ ), 25.0 ( $\text{CH}=\text{CCH}_2$ ), 7.6 ( $\text{NCH}(\text{CH}_2)_2$ )

**$^{19}\text{F}$  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.

**10.39 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 106**



1-Cyclopropyl-6-fluoro-7-(((hex-5-ynoyloxy)methoxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **224** (203 mg, 0.407 mmol, 1 eq.), 4-azido-N-(2-oxotetrahydrothiophen-3-yl)butanamide **104** (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>). **106** was obtained as pale brown/yellow amorphous solid (14.7 mg, 20.2  $\mu\text{mol}$ , 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)

**$^1\text{H}$  NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 15.16 (br s, 1 H,  $\text{C}(\text{=O})\text{OH}$ ), 8.65 (s, 1 H, *ortho* to C=O),

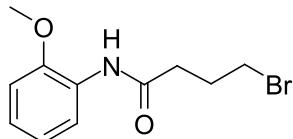
8.21 (d,  $J = 8.5$  Hz, 1 H, NH), 7.89 (d,  $J = 13.1$  Hz, 1 H, *ortho* to F), 7.85 (s, 1 H, CH=CCH<sub>2</sub>), 7.57 (d,  $J = 7.4$  Hz, 1 H, *meta* to F), 5.74 (s, 1 H, OCH<sub>2</sub>O), 4.58 (ddd,  $J = 12.6, 8.1, 7.2$  Hz, 1 H, CHNH), 4.30 (t,  $J = 6.9$  Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>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)CHH), 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>)  $\delta$  / 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 (OC(=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>), 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.

#### 10.40 4-Bromo-N-(2-methoxyphenyl)butanamide 108



2-Methoxyaniline **107** (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 **56** (9.40 ml, 15.1 g, 81.2 mmol, 1 eq.) was added dropwise over 15 min. The mixture was stirred at 0 °C for 1.5 h, then the aqueous layer was removed. The organic layer was dried with 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. **108** 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)

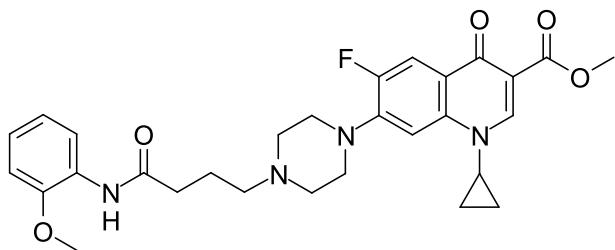
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.32 (dd,  $J = 8.0, 1.7$  Hz, 1 H, *ortho* to NH), 7.85 (br s, 1 H, NH), 7.02 (td,  $J = 7.9, 1.7$  Hz, 1 H, *para* to NH), 6.93 (td,  $J = 7.7, 1.4$  Hz, 1 H, *para* to OCH<sub>3</sub>), 6.85 (dd,  $J = 8.1, 1.5$  Hz, 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>) δ / 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.

**10.41 Methyl 1-cyclopropyl-6-fluoro-7-(4-((2-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 109**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **100** (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(2-methoxyphenyl)butanamide **108** (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. **109** was obtained as a bright pink amorphous solid (79.7 mg, 0.149 mmol, 10.2 %).

**TLC** *R<sub>f</sub>* = 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>) δ / 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>)

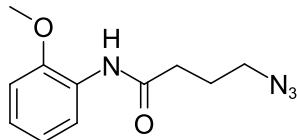
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>) δ / ppm = 172.9 (C(=O)CC(=O)OCH<sub>3</sub>), 170.8 (NHC(=O)), 166.2 (C(=O)OCH<sub>3</sub>), 153.3 (d, *J* = 248.0 Hz, *ipso* to F), 148.2 (C=CC(=O)OCH<sub>3</sub>), 147.6 (*ipso* to OCH<sub>3</sub>), 144.4 (d, *J* = 10.4 Hz, *ipso* to 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.

### 10.42 4-Azido-*N*-(2-methoxyphenyl)butanamide 110



4-Bromo-*N*-(2-methoxyphenyl)butanamide **108** (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.). **110** 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<sub>f</sub>* = 0.20 (25 % EtOAc/P.E.)

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

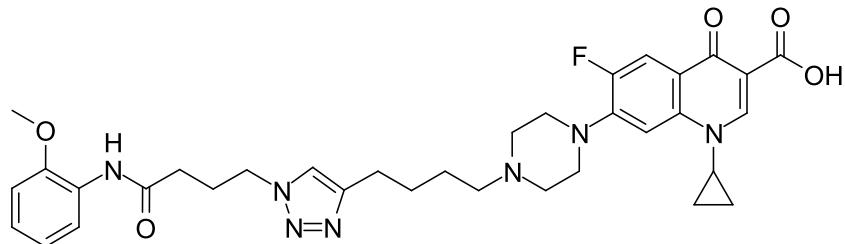
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / ppm = 8.32 (dd, *J* = 7.9, 1.0 Hz, 1 H, *ortho* to NH), 7.86 (br s, 1 H, NH), 7.00 (td, *J* = 7.5, 1.5 Hz, 1 H, *para* to NH), 6.90 (td, *J* = 7.7, 1.1 Hz, 1 H, *para* to OCH<sub>3</sub>), 6.83 (dd, *J* = 8.1, 1.4 Hz, 1 H, *ortho* 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>)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub> d<sub>1</sub>)  $\delta$  / 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* / 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>224</sup>

**10.43 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 111**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (24.1 mg, 58.6  $\mu$ mol, 1 eq.) and 4-azido-*N*-(2-methoxyphenyl)butanamide **110** (13.7 mg, 58.5  $\mu$ mol, 1 eq.) were dissolved in water (3 ml), *t*-BuOH (9 ml) and  $\text{CH}_2\text{Cl}_2$  (9 ml), and the mixture was degassed by bubbling through  $\text{N}_2$ . A solution of  $\text{CuSO}_4$  and THPTA (117  $\mu$ l, 5.85  $\mu$ mol, 0.1 eq., 50 mM, aq.) was added, followed by a solution of sodium ascorbate (234  $\mu$ l, 11.7  $\mu$ mol, 0.2 eq., 50 mM, aq.). The mixture was stirred at room temperature under argon for 16 h. Water (25 ml),  $\text{CH}_2\text{Cl}_2$  (25 ml) and MeOH (5 ml) were added and the organic layer was separated off, dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography using a Combiflash ( $\text{SiO}_2$ , 3-23 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **111** was obtained as a clear amorphous solid (14.7 mg, 22.8  $\mu$ mol, 39.0 %).

**TLC**  $R_f$  = 0.28 (10 % MeOH/ $\text{CH}_2\text{Cl}_2$ )

**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,  $\text{CDCl}_3$ )  $\delta$  / ppm = 15.05 (br s, 1 H,  $\text{C}(=\text{O})\text{OH}$ ), 8.76 (s, 1 H, *ortho* to  $\text{C}(=\text{O})\text{OH}$ ), 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,  $\text{CH}=\text{CCH}_2$ ), 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  $\text{OCH}_3$ ), 6.88 (dd,  $J$  = 8.1, 1.4 Hz, 1 H, *ortho* to  $\text{OCH}_3$ ), 4.47 (t,  $J$  = 6.7 Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 3.88 (s, 3 H,  $\text{CH}_3$ ), 3.54 (tt,  $J$  = 6.9, 4.0 Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.35 (br t,  $J$  = 4.7 Hz, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 2.76 (t,  $J$  = 7.5 Hz, 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.66 (t,  $J$  = 4.7 Hz, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.47 (t,  $J$  = 7.3 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.44 (t,  $J$  = 6.8 Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.32 (quin,  $J$  = 6.7 Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.75 (quin,  $J$  = 7.6 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.61 (quin,  $J$  = 7.5 Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.35 - 1.42 (m, 2 H,  $\text{NCH}(\text{CH}_2)_2$ ), 1.17 - 1.22 (m, 2 H,  $\text{NCH}(\text{CH}_2)_2$ )

**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 177.1 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$ ), 169.5 ( $\text{NH}\text{C}(=\text{O})$ ), 167.0 ( $\text{C}(=\text{O})\text{OH}$ ), 153.7 (d,  $J$  = 251.4 Hz, *ipso* to F), 148.1 ( $\text{CH}=\text{CCH}_2$ ), 147.8 (*ipso* to  $\text{OCH}_3$ ), 147.3 ( $\text{C}=\text{CC}(=\text{O})\text{OH}$ ), 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  $\text{OCH}_3$ ), 120.9 ( $\text{CH}=\text{CCH}_2$ ), 119.7 (*para* to piperazine, and *ortho* to NH and *meta* to  $\text{OCH}_3$ ), 112.4 (d,  $J$  = 23.4 Hz, *ortho* to C=O and *ortho* to F), 109.9 (*ortho* to  $\text{OCH}_3$  and *meta* to NH), 108.1 ( $\text{C}(=\text{O})\text{OH}$ ), 104.7 (*meta* to C=O and *meta* to F), 58.1 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 55.6 ( $\text{CH}_3$ ), 52.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)$ , 49.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.1 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 35.2 ( $\text{NCH}(\text{CH}_2)_2$ ), 33.8 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 27.3 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 26.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 26.0 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 25.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 8.2 ( $\text{NCH}$

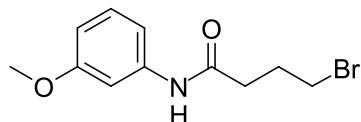
$(\underline{\text{CH}_2})_2$

**$^{19}\text{F}$  NMR** (376.45 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = -120.7 (s, ciprofloxacin F)

**HRMS** (ESI $^+$ )  $m/z$  / Da = 646.3132,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{34}\text{H}_{41}\text{FN}_7\text{O}_5]^+$  requires 646.3153

The compound has not been reported previously.

#### 10.44 4-Bromo-N-(3-methoxyphenyl)butanamide 113



3-Methoxyaniline **112** (3.04 ml, 3.33 g, 27.1 mmol, 1 eq.) and  $\text{NaHCO}_3$  (2.73 g, 32.5 mmol, 1.2 eq.) were dissolved in water (30 ml) and  $\text{CH}_2\text{Cl}_2$  (30 ml). The mixture was cooled to 0 °C and 4-bromobutyryl chloride **56** (3.13 ml, 5.03 g, 27.1 mmol, 1 eq.) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h, then the aqueous layer was removed. The organic layer was dry-loaded onto  $\text{SiO}_2$  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. **113** was obtained as a pale pink amorphous solid (3.66 g, 13.5 mmol, 49.6 %).

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

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

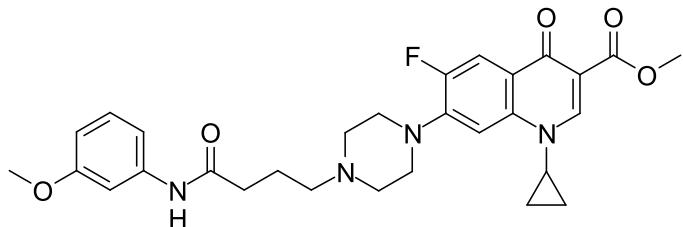
**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$  d<sub>1</sub>)  $\delta$  / ppm = 8.45 (s, 1 H, NH), 7.27 (t,  $J$  = 2.2 Hz, 1 H, *ortho* to  $\text{OCH}_3$  and *ortho* to NH), 7.14 (t,  $J$  = 8.1 Hz, 1 H, *meta* to  $\text{OCH}_3$  and *meta* to NH), 7.02 (d,  $J$  = 8.3 Hz, 1 H, *para* to  $\text{OCH}_3$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

**$^{13}\text{C}$  NMR** (101 MHz,  $\text{CDCl}_3$  d<sub>1</sub>)  $\delta$  / ppm = 170.3 ( $\underline{\text{C}}(=\text{O})$ ), 159.9 (*ipso* to  $\text{OCH}_3$ ), 139.0 (*ipso* to NH), 129.5 (*meta* to  $\text{OCH}_3$  and *meta* to NH), 112.1 (*para* to  $\text{OCH}_3$ ), 109.9 (*para* to NH), 105.7 (*ortho* to  $\text{OCH}_3$  and *ortho* to NH), 55.2 ( $\underline{\text{CH}_3}$ ), 35.3 (C(=O) $\underline{\text{CH}_2}$ ), 33.2 ( $\underline{\text{CH}_2\text{Br}}$ ), 28.0 (C(=O) $\text{CH}_2\underline{\text{CH}_2}$ )

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

The compound has not been reported previously.

**10.45 Methyl 1-cyclopropyl-6-fluoro-7-(4-((3-methoxyphenyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 114**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **100** (500 mg, 1.45 mmol, 1 eq.), 4-bromo-*N*-(3-methoxyphenyl)butanamide **113** (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. **114** 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>)

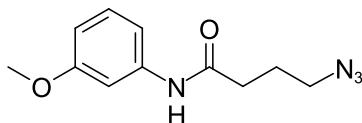
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.1 (C(=O)CC(=O)OCH<sub>3</sub>), 170.9 (NHC(=O)), 166.3 (C(=O)OCH<sub>3</sub>), 160.1 (*ipso* to OCH<sub>3</sub>), 153.3 (d,  $J$  = 250.1 Hz, *ipso* to F), 148.4 (C=CC(=O)OCH<sub>3</sub>), 144.1 (d,  $J$  = 10.1 Hz, *ipso* to piperazine), 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 (CC(=O)OCH<sub>3</sub>), 109.8 (*para* to NH), 105.5 (*ortho* to OCH<sub>3</sub> and *ortho* to NH), 105.0 (*meta* to C=O and *meta* to F), 57.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 55.3 (aromatic OCH<sub>3</sub>), 52.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.1 (C(=O)OCH<sub>3</sub>), 49.2 (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>), 35.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 34.6 (NCH(CH<sub>2</sub>)<sub>2</sub>), 21.7 (CH<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, MeOD)  $\delta$  / ppm = -123.5 (s, ciprofloxacin F)

**HRMS (ESI<sup>+</sup>)**  $m/z$  / Da = 537.2500, [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.

#### 10.46 4-Azido-*N*-(3-methoxyphenyl)butanamide 115



4-Bromo-*N*-(3-methoxyphenyl)butanamide **113** (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. **115** 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 C=O)

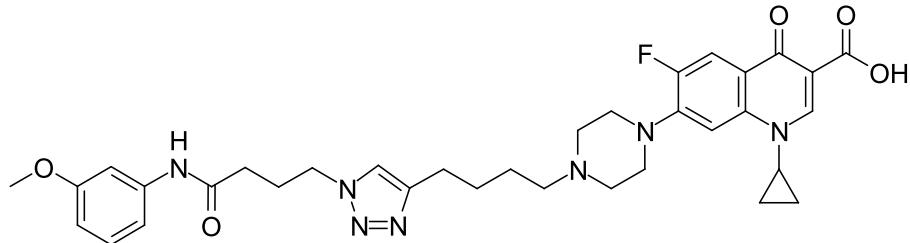
**$^1\text{H NMR}$**  (400 MHz, MeOD)  $\delta$  / ppm = 8.63 (br s, 1 H, NH), 7.26 (t,  $J$  = 2.3 Hz, 1 H, *ortho* to  $\text{OCH}_3$  and *ortho* to NH), 7.15 (t,  $J$  = 8.1 Hz, 1 H, *meta* to  $\text{OCH}_3$  and *meta* to NH), 7.01 (dd,  $J$  = 7.8, 1.6 Hz, 1 H, *para* to  $\text{OCH}_3$ ), 6.63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

**$^{13}\text{C NMR}$**  (101 MHz, MeOD)  $\delta$  / ppm = 170.8 ( $\text{C}(=\text{O})$ ), 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 ( $\text{CH}_3$ ), 50.4 ( $\text{CH}_2\text{N}_3$ ), 33.6 ( $\text{C}(=\text{O})\text{CH}_2$ ), 24.4 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2$ )

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

The compound has not been reported previously.

#### 10.47 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 116



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (24.1 mg, 58.6  $\mu\text{mol}$ , 1 eq.) and 4-azido-*N*-(3-methoxyphenyl)butanamide **115** (13.7 mg, 58.5  $\mu\text{mol}$ , 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  $\mu\text{l}$ , 5.85  $\mu\text{mol}$ , 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (117  $\mu\text{l}$ , 11.7  $\mu\text{mol}$ , 0.2 eq., 100 mM, aq.). The mixture was stirred at room temperature under argon for 2 h, then the solvent was removed under reduced pressure. The residue was partitioned between water (15 ml) and  $\text{CH}_2\text{Cl}_2$  (15 ml), and the aqueous layer was extracted a further four times with  $\text{CH}_2\text{Cl}_2$  (4×15 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 %  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ). The combined pure fractions were dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **116** was obtained as a clear amorphous solid (1.9 mg, 2.9  $\mu\text{mol}$ , 5.0 %).

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

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

**$^1\text{H NMR}$**  (400 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 15.23 (br s, 1 H,  $\text{C}(=\text{O})\text{OH}$ ), 9.89 (s, 1 H, NH), 8.66 (s, 1 H, *ortho* to  $\text{C}(=\text{O})\text{OH}$ ), 7.90 (d,  $J = 13.4$  Hz, 1 H, *ortho* to F), 7.88 (s, 1 H,  $\text{CH}=\text{CCH}_2$ ), 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  $\text{OCH}_3$  and *meta* to NH), 7.08 (d,  $J = 7.8$  Hz, 1 H, *para* to  $\text{OCH}_3$ ), 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,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 3.81 (tt,  $J = 6.7, 4.0$  Hz, 1 H,  $\text{NCH}(\text{CH}_2)_2$ ), 3.70 (s, 3 H,  $\text{CH}_3$ ), 3.28 - 3.32 (m, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 2.64 (t,  $J = 7.5$  Hz, 2 H,  $\text{CH}=\text{CCH}_2$ ), 2.56 (m,  $J = 4.2, 4.2$  Hz, 4 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 2.38 (t,  $J = 7.3$  Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.30 (t,  $J = 7.4$  Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.10 (quin,  $J = 7.1$  Hz, 2 H,  $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.64 (quin,  $J = 7.5$  Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.51 (quin,  $J = 7.2$  Hz, 2 H,  $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.27 - 1.33 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ ), 1.15 - 1.20 (m, 2 H,  $\text{NCH}(\text{CHH})_2$ )

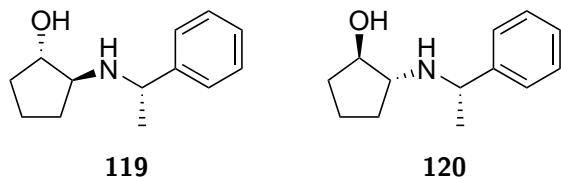
**$^{13}\text{C NMR}$**  (101 MHz, DMSO  $d_6$ )  $\delta$  / ppm = 176.3 ( $\text{C}(=\text{O})\text{CC}(=\text{O})\text{OH}$ ), 170.1 ( $\text{NHC}(=\text{O})$ ), 165.9 ( $\text{C}(=\text{O})\text{OH}$ ), 159.4 (*ipso* to  $\text{OCH}_3$ ), 153.0 (d,  $J = 248.6$  Hz, *ipso* to F), 148.0 ( $\text{CH}=\text{CCH}_2$ ), 146.9 ( $\text{C}=\text{CC}(=\text{O})\text{OH}$ ), 145.2 (d,  $J = 10.7$  Hz, *ipso* to piperazine), 140.3 (*para* to F), 139.2 (*ipso* to NH), 129.4 (*meta* to  $\text{OCH}_3$  and *meta* to NH), 121.7 ( $\text{CH}=\text{CCH}_2$ ), 118.5 (d,  $J = 7.5$  Hz, *para* to piperazine), 111.3 (*para* to  $\text{OCH}_3$ ), 110.9 (d,  $J = 22.4$  Hz, *ortho* to C=O and *ortho* to F), 108.4 (*para* to NH), 106.7 ( $\text{CC}(=\text{O})\text{OH}$ ), 106.3 (*meta* to C=O and *meta* to F), 104.8 (*ortho* to  $\text{OCH}_3$  and *ortho* to NH), 57.3 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 54.9 ( $\text{CH}_3$ ), 52.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2)\text{CH}_2$ ), 49.5 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 49.4 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\text{CH}_2$ ), 48.7 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 35.8 ( $\text{NCH}(\text{CH}_2)_2$ ), 32.9 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 26.8 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 25.7 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 25.5 ( $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 24.9 ( $\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 7.6 ( $\text{NCH}(\text{CH}_2)_2$ )

**$^{19}\text{F NMR}$**  (376.45 MHz, DMSO  $d_6$ )  $\delta$  / ppm = -121.5 (s, ciprofloxacin F)

**HRMS (ESI $^+$ )**  $m/z$  / Da = 646.3159,  $[\text{M}+\text{H}]^+$  found,  $[\text{C}_{34}\text{H}_{41}\text{FN}_7\text{O}_5]^+$  requires 646.3153

The compound has not been reported previously.

10.48  $(1S,2S)$ -2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 119 and  $(1R,2R)$ -2-(((*S*)-1-phenylethyl)amino)cyclopentan-1-ol 120



(S)-1-Phenylethan-1-amine **118** (7.85 ml, 7.38 g, 60.9 mmol, 1 eq.) was dissolved in  $\text{CH}_2\text{Cl}_2$  (50 ml) and stirred rapidly at 0 °C. A solution of  $\text{AlMe}_3$  (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 **117** (5.71 ml, 5.50 g, 65.4 mmol, 1.1 eq.) in  $\text{CH}_2\text{Cl}_2$  (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  $\text{NaF}$  (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  $\text{CH}_2\text{Cl}_2$  (50 ml). The suspension was allowed to warm to r.t. and stirred for 1 h, then filtered through Celite and washed with  $\text{CH}_2\text{Cl}_2$  (500 ml). The filtrate was dried with  $\text{K}_2\text{CO}_3$ , concentrated under reduced pressure and purified by column chromatography ( $\text{SiO}_2$ , 20:5:1 hexane:EtOAc:TEA). **120** was obtained as a pale yellow oil (4.08 g, 19.9 mmol, 32.6 %). **119** was obtained as pale yellow crystals (4.48 g, 21.8 mmol, 35.8 %).

**(1*S*,2*S*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 119**

**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>) δ / 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, CHO<sub>H</sub>), 2.83 (td, *J* = 7.6, 5.7 Hz, 1 H, CHNH), 1.85 - 1.97 (m, 1 H, CHHCHOH), 1.77 (dtd, *J* = 12.9, 7.9, 4.9 Hz, 1 H, CHHCHNH), 1.55 - 1.68 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.47 - 1.55 (m, 1 H, CHHCHOH), 1.36 (d, *J* = 6.6 Hz, 3 H, CH<sub>3</sub>), 1.12 (dq, *J* = 12.7, 8.1 Hz, 1 H, CHHCHNH)

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ / 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>CHNH), 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 ( $c / \text{g(100 ml)}^{-1} = 0.96$ , MeOH)

**(1*R*,2*R*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol 120**

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

**IR (neat)  $\nu_{max}$  / cm<sup>-1</sup>** = 3300.0 (br, O-H), 2959.7 (C-H), 2870.1 (C-H)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / 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, CHOH), 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>CHOH), 1.56 - 1.68 (m, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.43 (dq,  $J$  = 12.5, 8.0 Hz, 1 H, CH<sub>2</sub>CHOH), 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)

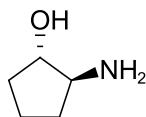
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 144.75 (*ipso* to CHCH<sub>3</sub>), 128.26 (*meta* to CHCH<sub>3</sub>), 126.72 (*para* to CHCH<sub>3</sub>), 126.30 (*ortho* to CHCH<sub>3</sub>), 77.65 (CHOH), 63.38 (CHNH), 56.20 (CHCH<sub>3</sub>), 31.74 (CH<sub>2</sub>CHOH),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

**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 ( $c$  / g(100 ml)<sup>-1</sup> = 1.19, MeOH)

The compounds have been synthesised previously,<sup>180,181</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>182</sup>

### 10.49 (1*S*,2*S*)-2-Aminocyclopentan-1-ol 121



(1*S*,2*S*)-2-((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol **119** (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. **121** 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)  $\nu_{max}$  / cm<sup>-1</sup> = 3300.0 (O-H), 2969.2 (C-H), 2872.7 (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,  $J$  = 7.4, 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>)

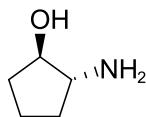
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 80.6 (CHOH), 60.7 (CHNH<sub>2</sub>), 33.2 (CH<sub>2</sub>CHOH), 32.2 (CH<sub>2</sub>CHNH<sub>2</sub>), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**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>181,225</sup>

## 10.50 (1*R*,2*R*)-2-Aminocyclopentan-1-ol **122**



(1*R*,2*R*)-2-(((*S*)-1-Phenylethyl)amino)cyclopentan-1-ol **120** (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. **122** 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, CH<sub>OH</sub>), 3.00 (td, *J* = 7.3, 5.6 Hz, 1 H, CH<sub>2</sub>NH<sub>2</sub>), 2.00 (dtd, *J* = 13.0, 7.7, 5.6 Hz, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 1.97 (ddt, *J* = 13.0, 8.7, 6.6 Hz, 1 H, CH<sub>2</sub>CHOH), 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, CH<sub>2</sub>CHOH), 1.37 (ddt, *J* = 13.0, 8.3, 7.8 Hz, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>)

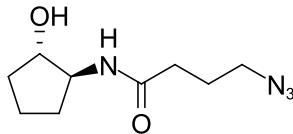
**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 80.7 (CHOH), 60.8 (CH<sub>2</sub>NH<sub>2</sub>), 33.2 (CH<sub>2</sub>CHOH), 32.1 (CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 21.2 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**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

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

The data are consistent with the literature.<sup>181,225</sup>

## 10.51 4-Azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **125**



4-Chloro-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **143** (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. **125** 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)

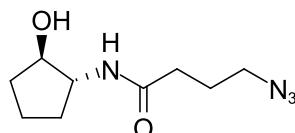
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 79.7 (CHOH), 61.0 (CHNH), 50.7 (CH<sub>2</sub>N<sub>3</sub>), 32.8 (CH<sub>2</sub>C=O), 32.6 (CH<sub>2</sub>CHOH), 30.5 (CH<sub>2</sub>CHNH), 24.7 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 21.3 (CH<sub>2</sub>CH<sub>2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

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

The compound has not been reported previously.

### 10.52 4-Azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide 126



4-Chloro-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **144** (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. **126** was obtained as white needles (181 mg, 0.852 mmol, 87.6 %).

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

**mp**  $T$  / °C = 56.0-59.5 (*i*-PrOH, CHCl<sub>3</sub>)

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

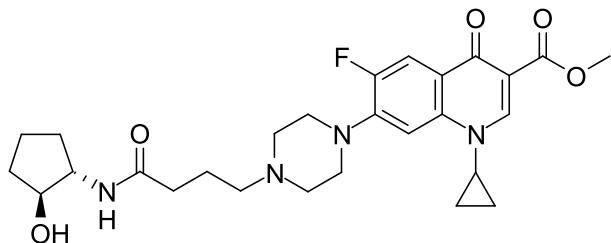
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 78.8 (CHOH), 59.9 (CHNH), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 32.5 (CH<sub>2</sub>C=O), 32.0 (CH<sub>2</sub>CHOH), 29.5 (CH<sub>2</sub>CHNH), 24.6 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.7 (CH<sub>2</sub>CH<sub>2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

**HRMS** (ESI<sup>+</sup>)  $m/z$  / 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

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

The compound has not been reported previously.

**10.53 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*S*,2*S*)-2-hydroxycyclopentyl)amino-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 127**



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **148** (52.1 mg, 95.5  $\mu$ mol, 1 eq.), (1*S*,2*S*)-2-aminocyclopentan-1-ol **121** (19.5 mg, 193  $\mu$ mol, 2 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (29.7 mg, 155  $\mu$ mol, 1.6 eq.), 1-hydroxybenzotriazole (25.8 mg, 191  $\mu$ mol, 2 eq.) and DIPEA (33.3  $\mu$ l, 24.7 mg, 191  $\mu$ mol, 2 eq.) were dissolved in DMF (2 ml) and stirred at r.t. for 16 h. The solvent was removed using a stream of  $N_2$  and the residue was purified by preparative HPLC (5-50 % acetonitrile/water over 15 min). The combined pure fractions were evaporated under reduced pressure and then partitioned between  $NaHCO_3$  (aq., sat., 5 ml) and  $CH_2Cl_2$  (5 ml). The organic layer was removed and the aqueous layer was extracted twice more with  $CH_2Cl_2$  (2  $\times$  5 ml). The combined organic fractions were dried with  $MgSO_4$  and evaporated under reduced pressure. **127** was obtained as a white amorphous solid (26.9 mg, 52.3  $\mu$ mol, 54.7 %).

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

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

**$^1H$  NMR** (500 MHz, DMSO 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>)

**$^{13}C$  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 (CHO<sub>H</sub>), 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>)

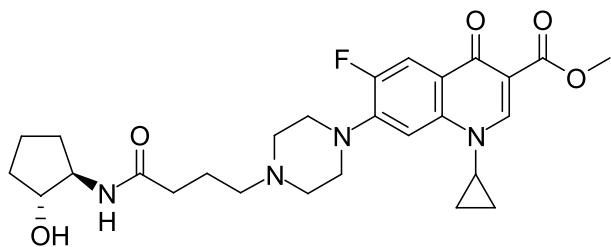
**$^{19}F$  NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -125.5

**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

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

The compound has not been reported previously.

**10.54 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amin o)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 128**



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **148** (200 mg, 0.367 mmol, 1 eq.), (1*R*,2*R*)-2-aminocyclopentan-1-ol **122** (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  $\times$  10 ml). The combined organic fractions were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. **128** 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)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta / \text{ppm} = 8.44$  (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, CHO<sub>H</sub>), 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>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta / \text{ppm} = 171.9$  (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>)

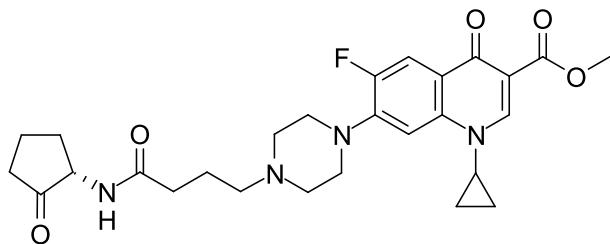
**<sup>19</sup>F NMR** (376.45 MHz, DMSO d<sub>6</sub>) δ / ppm = -124.3 (ciprofloxacin F)

**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

[α]<sub>D</sub><sup>20</sup> / °10<sup>-1</sup>cm<sup>2</sup>g<sup>-1</sup> = -6.0 (c / g(100 ml)<sup>-1</sup> = 0.05, MeOH)

The compound has not been reported previously.

**10.55 Methyl (S)-1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclopentyl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 129**



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 **127** (20.0 mg, 38.9 μmol, 1 eq.) and Dess-Martin periodinane (32.8 mg, 77.4 μmol, 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 NaHCO<sub>3</sub> (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. **129** was obtained as a white amorphous solid (11.3 mg, 22.0 μmol, 56.7 %).

add  
Ocy5 to  
R and  
D

**<sup>1</sup>H NMR** (500 MHz, DMSO d<sub>6</sub>) δ / 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 CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.89 - 1.96 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.69 - 1.80 (m, 3 H, CH<sub>2</sub>CH<sub>2</sub>CHNH, CH<sub>2</sub>CH<sub>2</sub>CHNH and NHC(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.24 - 1.29 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.07 - 1.12 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>13</sup>C NMR** (126 MHz, DMSO d<sub>6</sub>) δ / 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>)

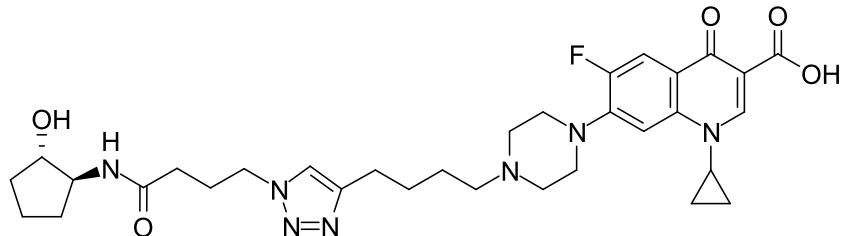
**<sup>19</sup>F NMR** (376.45 MHz, MeOD) δ / ppm = -124.3

**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

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

The compound has not been reported previously.

**10.56 1-Cyclopropyl-6-fluoro-7-(4-(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 130**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (82.0 mg, 199  $\mu\text{mol}$ , 4 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide **125** (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  $\text{N}_2$  through it. A solution of  $\text{CuSO}_4$  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. **130** was obtained as a white amorphous solid (7.2 mg, 11.5  $\mu\text{mol}$ , 22.2 %).

**IR** (neat)  $\nu_{max} / \text{cm}^{-1} = 2954.9$  (C-H), 2917.9 (C-H), 2850.2 (C-H), 1722.1 (carboxylic acid C=O), 1647.3 (amide C=O), 1626.7 (quinolone C=O) 1611.9 (triazole)

**<sup>1</sup>H NMR** (400 MHz, DMSO d<sub>6</sub>)  $\delta / \text{ppm} = 15.22$  (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, CH<sub>2</sub>OH 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, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.75 (ddt,  $J = 12.7, 9.0, 6.2$  Hz, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHOH), 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, CH<sub>2</sub>CH<sub>2</sub>CHOH), 1.28 - 1.35 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 1.24 - 1.31 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>CHNH), 1.15 - 1.21 (m, 2 H, NCH(CH<sub>2</sub>)<sub>2</sub>)

**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta / \text{ppm} = 176.4$  (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 (CH<sub>2</sub>OH), 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>)

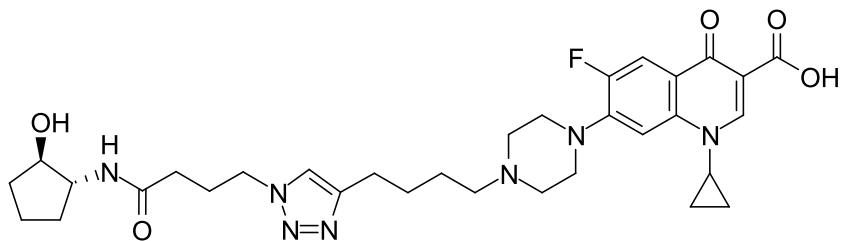
<sup>19</sup>F NMR (376.45 MHz, MeOD)  $\delta$  / ppm = -121.5

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.

**10.57 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-ylbutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 131**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (42.9 mg, 104  $\mu$ mol, 1 eq.) and 4-azido-*N*-((1*R*,2*R*)-2-hydroxycyclopentyl)butanamide **126** (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 CH<sub>2</sub>Cl<sub>2</sub> (4×30 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., 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. **131** was obtained as a white amorphous solid (17.6 mg, 28.2  $\mu$ mol, 27.1 %).

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

<sup>1</sup>H NMR (700 MHz, DMSO d<sub>6</sub>)  $\delta$  / 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, CH=CCH<sub>2</sub>), 7.75 (d, *J* = 7.1 Hz, 1 H, CHNH), 7.54 (d, *J* = 7.5 Hz, 1 H, *meta* to F), 4.73 (d, *J* = 3.8 Hz, 1 H, CHOH), 4.29 (t, *J* = 6.9 Hz, 2 H, CH<sub>2</sub>NCH=C), 3.78 - 3.83 (m, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.75 - 3.78 (m, 1 H, CHOH), 3.71 - 3.75 (m, 1 H, CHNH), 3.31 (br t, *J* = 4.3 Hz, 4 H, 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>), 2.56 (br t, *J* = 4.2 Hz, 4 H, 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>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.03 - 2.06 (m, 2 H, C(=O)CH<sub>2</sub>), 1.97 - 2.02 (m, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.89 (dd, *J* = 13.1, 8.9, 7.4, 5.7 Hz, 1 H, CHHCHNH), 1.75 (ddt, *J* = 13.0, 8.9, 6.4, 6.4 Hz, 1 H, CHHCHOH), 1.61 - 1.66 (m, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>), 1.57 - 1.61 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.54 - 1.57 (m, 1 H, CHHCH<sub>2</sub>CHOH), 1.49 -

1.53 (m, 2 H, CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.40 (ddt, *J* = 13.0, 8.4, 5.3, 5.3 Hz, 1 H, CHHCHOH), 1.29 - 1.32 (m, 2 H, NCH(CHH)<sub>2</sub>), 1.25 - 1.29 (m, 1 H, CHHCHNH), 1.13 - 1.20 (m, 2 H, NCH(CHH)<sub>2</sub>)

**<sup>13</sup>C NMR** (175 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.3 (C(=O)CC(=O)OH), 170.9 (NHC(=O)CH<sub>2</sub>), 166.1 (C(=O)OH), 153.0 (d, *J* = 251.4 Hz, *ipso* to F), 147.9 (C=CC(=O)OH), 146.9 (CH=CCH<sub>2</sub>), 145.2 (d, *J* = 8.7 Hz, *ipso* to piperazine), 139.2 (*para* to F), 121.7 (NCH=CCH<sub>2</sub>), 118.7 (d, *J* = 5.8 Hz, *para* to piperazine), 111.0 (d, *J* = 23.3 Hz, *ortho* to C=O and *ortho* to F), 106.3 (*meta* to C=O and *meta* to F and CC(=O)OH), 76.2 (CHOH), 57.6 (CHNH), 57.4 (CH=CCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.5 (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>), 49.5 (d, *J* = 4.4 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>), 48.8 (CH<sub>2</sub>NCH=CCH<sub>2</sub>), 35.8 (NCH(CH<sub>2</sub>)<sub>2</sub>), 32.2 (CH<sub>2</sub>CHOH), 32.0 (C(=O)CH<sub>2</sub>), 29.5 (CH<sub>2</sub>CHNH), 26.9 (CH=CCH<sub>2</sub>CH<sub>2</sub>), 26.0 (C(=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>), 20.5 (CH<sub>2</sub>CH<sub>2</sub>CHOH), 7.6 (NCH(CH<sub>2</sub>)<sub>2</sub>)

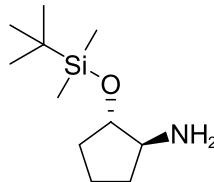
**<sup>19</sup>F NMR** (376.45 MHz, MeOD)  $\delta$  / ppm = -122.1 (s, ciprofloxacin F)

**HRMS (ESI<sup>+</sup>)** *m/z* / Da = 624.3314, [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

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

The compound has not been reported previously.

### 10.58 (1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine 132



(1*S*,2*S*)-2-Aminocyclopentan-1-ol **121** (0.480 g, 4.75 mmol) was stirred in dry CH<sub>2</sub>Cl<sub>2</sub> (20 ml) under N<sub>2</sub> 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 Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure and purified by column chromatography (SiO<sub>2</sub>, 4 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>). **132** was obtained as a yellow oil (1.00 g, 4.64 mmol, 97.7 %).

**TLC** *R<sub>f</sub>* = 0.23 (10 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

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

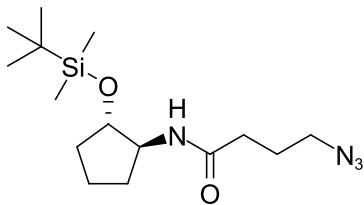
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 4.13 (q, *J* = 5.8 Hz, 1 H, CHOSi), 3.31 (td, *J* = 7.1, 5.2 Hz, 1 H, CHNH<sub>2</sub>), 2.09 - 2.19 (m, 1 H, CHHCHNH<sub>2</sub>), 1.97 (ddq, *J* = 8.8, 7.0, 6.0 Hz, 1 H, CHHCHOSi), 1.74 - 1.86 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CHOSi),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

**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 76.3 (CHOSi), 59.7 (CHNH), 32.2 (CH<sub>2</sub>CHOSi), 26.8 (CH<sub>2</sub>CHNH<sub>2</sub>), 25.6 (C(CH<sub>3</sub>)<sub>3</sub>), 19.7 (CH<sub>2</sub>CH<sub>2</sub>CHOSi), 17.7 (C(CH<sub>3</sub>)<sub>3</sub>), -4.8 (SiCH<sub>3</sub>), -5.2 (SiCH<sub>3</sub>)

**HRMS (ESI<sup>+</sup>)**  $m/z$  / Da = 216.1785, [M+H]<sup>+</sup> found, [C<sub>11</sub>H<sub>26</sub>NOSi]<sup>+</sup> requires 216.1784

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

**10.59 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide 136**



(1*S*,2*S*)-2-((*tert*-Butyldimethylsilyl)oxy)cyclopentan-1-amine **132** (50 mg, 0.232 mmol, 1 eq.) and NaHCO<sub>3</sub> (22.0 mg, 0.262 mmol, 1.1 eq.) were added to CH<sub>2</sub>Cl<sub>2</sub> (3 ml) and water (3 ml) at 0 °C, and 4-bromobutyryl chloride (25.3 ml, 40.5 mg, 0.219 mmol, 0.95 eq.) was added dropwise. The mixture was stirred for 3 h at 0 °C. The aqueous layer was removed and NaN<sub>3</sub> (100 mg, 1.54 mmol, 6.6 eq.) and DMF (3 ml) were added. The mixture was then stirred at 40 °C for 6 h. The solvents were then evaporated using a N<sub>2</sub> stream and the residue was purified by column chromatography (SiO<sub>2</sub>, 0.5 % 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. **136** was obtained as a clear liquid (71 mg, 0.217 mmol, 99.2 %).

**TLC**  $R_f = 0.84$  (1 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

**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)

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 5.35 (d,  $J = 5.1$  Hz, 1 H, NH), 3.97 - 4.01 (m, 1 H, CHOSi), 3.93 - 3.98 (m, 1 H, CHNH), 3.35 (t,  $J = 6.6$  Hz, 2 H, CH<sub>2</sub>N<sub>3</sub>), 2.24 (t,  $J = 7.0$  Hz, 2 H, CH<sub>2</sub>C=O), 2.09 - 2.19 (m, 1 H, CHHCHNH), 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, CHHCHOSi and CHHCH<sub>2</sub>CHOSi), 1.60 - 1.70 (m, 1 H, CHHCH<sub>2</sub>CHOSi), 1.51 - 1.61 (m, 1 H, CHHCHOSi), 1.31 - 1.39 (m, 1 H, CHHCHNH), 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>)

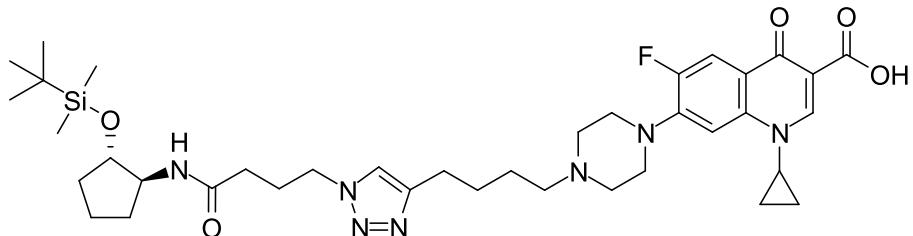
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 171.17 (C=O), 77.80 (CHOSi), 58.36 (CHNH), 50.77 (CH<sub>2</sub>N<sub>3</sub>), 33.29 (CH<sub>2</sub>C=O), 32.57 (CH<sub>2</sub>CHOSi), 29.36 (CH<sub>2</sub>CHNH), 25.72 (C(CH<sub>3</sub>)<sub>3</sub>), 24.77 (CH<sub>2</sub>CH<sub>2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

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

The compound has not been reported previously.

10.60 7-(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 **140**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (42.9 mg, 104  $\mu$ mol, 1 eq.) and 4-azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide **136** (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. **140** 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, CHHCHNH, C(=O)CH<sub>2</sub> and C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.65 - 1.83 (m, 4 H, CHHCHOSi, CHHCH<sub>2</sub>CHOSi and NCH=CCH<sub>2</sub>CH<sub>2</sub>), 1.47 - 1.65 (m, 4 H, CHHCHOSi, CHHCH<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, CHHCHNH and NCH(CHH)<sub>2</sub>), 1.14 - 1.20 (m, 2 H, NCH(CHH)<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>)

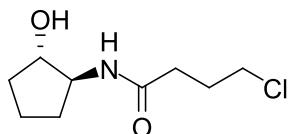
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 176.9 (C(=O)CC(=O)OH), 170.9 (CH<sub>2</sub>C(=O)NH), 166.9 (C(=O)OH), 153.5 (d, *J* = 251.4 Hz, *ipso* to F), 147.9 (CH=CCH<sub>2</sub>), 147.2 (C=CC(=O)OH), 145.8 (d, *J* = 10.4 Hz, *ipso* to piperazine), 139.0 (*para* to F), 120.9 (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 (C(=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>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>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

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

The compound has not been reported previously.

### 10.61 4-Chloro-*N*-(*1S,2S*)-2-hydroxycyclopentylbutanamide 143



(*1S,2S*)-2-Aminocyclopentan-1-ol **121** (72.3 mg, 716  $\mu\text{mol}$ , 1 eq.), TEA (500  $\mu\text{l}$ , 363 mg, 3.58 mmol, 5 eq.) and  $\text{CH}_2\text{Cl}_2$  (5 ml) were stirred at 0  $^\circ\text{C}$ , and 4-chlorobutyryl chloride **142** (179  $\mu\text{l}$ , 226 mg, 1.60 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0  $^\circ\text{C}$  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  $\times$  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. **143** was obtained as a white amorphous solid (35.6 mg, 173  $\mu\text{mol}$ , 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, CHHCOH), 1.74 - 1.86 (m, 1 H, CHHCH<sub>2</sub>COH), 1.58 - 1.74 (m, 2 H, CHHCH<sub>2</sub>COH and CHHCOH), 1.42 (dq,  $J = 12.5, 8.4$  Hz, 1 H, CHHCHNH)

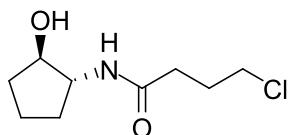
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH<sub>2</sub>Cl), 32.8 (CH<sub>2</sub>C=O), 32.4 (CH<sub>2</sub>COH), 30.2 (CH<sub>2</sub>CHNH), 28.0 (CH<sub>2</sub>CH<sub>2</sub>Cl), 21.2 (CH<sub>2</sub>CH<sub>2</sub>COH)

**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

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

The compound has not been reported previously.

### 10.62 4-Chloro-*N*-(*1R,2R*)-2-hydroxycyclopentylbutanamide 144



(*1R,2R*)-2-Aminocyclopentan-1-ol **122** (500 mg, 4.94 mmol, 1 eq.), TEA (827  $\mu\text{l}$ , 600 mg, 5.93 mmol, 1.2 eq.) and  $\text{CH}_2\text{Cl}_2$  (20 ml) were stirred at 0  $^\circ\text{C}$  and 4-chlorobutyryl chloride **142** (608  $\mu\text{l}$ , 766 mg, 5.43 mmol, 1.1 eq.) was added dropwise over 5 min. The mixture was stirred at 0  $^\circ\text{C}$  for 30 min, then water (50 ml) was added. The organic layer was separated off, and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (7  $\times$  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. **144** was obtained as a white amorphous solid (651 mg, 3.16 mmol, 64.1 %).

**TLC**  $R_f = 0.35$  ( $\text{EtOAc}$ )

**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)

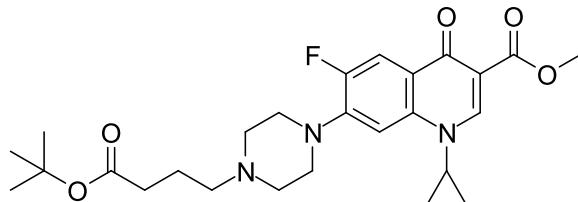
**$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  / ppm = 173.8 (C=O), 79.4 (CHOH), 60.6 (CHNH), 44.4 (CH<sub>2</sub>Cl), 32.8 (CH<sub>2</sub>C=O), 32.4 (CH<sub>2</sub>CHOH), 30.1 (CH<sub>2</sub>CHNH), 28.0 (CH<sub>2</sub>CH<sub>2</sub>Cl), 21.1 (CH<sub>2</sub>CH<sub>2</sub>CHOH)

**HRMS** (ESI $^+$ )  $m/z$  / Da = 228.0787,  $[\text{M}+\text{Na}]^+$  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.

### 10.63 Methyl 7-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **147**



Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate **100** (200 mg, 0.579 mmol, 1 eq.), *tert*-butyl 4-bromobutanoate **146** (103  $\mu\text{l}$ , 130 mg, 0.581 mmol, 1 eq.), NaI (86.9 mg, 0.580 mmol, 1 eq.), TEA (316  $\mu\text{l}$ , 229 mg, 2.27 mmol, 4 eq.) and acetonitrile (10 ml) were stirred in a microwave reactor at 100  $^\circ\text{C}$  for 8 h. A second portion of *tert*-butyl 4-bromobutanoate **225** (103  $\mu\text{l}$ , 130 mg, 0.581 mmol, 1 eq.) was added, and the mixture was stirred in the microwave reactor at 100  $^\circ\text{C}$  for a further 8 h. The mixture was then dry-loaded onto  $\text{SiO}_2$  and purified by column chromatography ( $\text{SiO}_2$ , 0-4 % MeOH/ $\text{CH}_2\text{Cl}_2$ ). **147** was obtained as a white amorphous solid (141 mg, 0.289 mmol, 49.9 %).

**TLC**  $R_f = 0.12$  (4 % MeOH/ $\text{CH}_2\text{Cl}_2$ )

**IR** (neat)  $\nu_{max}$  /  $\text{cm}^{-1} = 2961.6$  (C-H), 2830.5 (C-H), 1732.2 (*t*-Bu ester C=O) 1717.2 (ciprofloxacin ester C=O), 1620.6 (quinolone C=O)

**$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  / 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>)

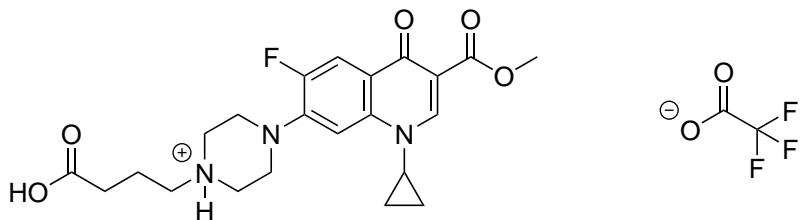
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 172.7 (C(=O)CC(=O)OCH<sub>3</sub>), 172.6 (C(=O)OC(CH<sub>3</sub>)<sub>3</sub>), 165.9 (C(=O)OCH<sub>3</sub>), 153.1 (d,  $J = 249.7$  Hz, *ipso* to F), 148.1 (C=CC(=O)OCH<sub>3</sub>), 144.3 (d,  $J = 10.4$  Hz, *ipso* to piperazine), 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 (CC(=O)OCH<sub>3</sub>) 104.7 (*meta* to C=O and *meta* to F), 80.0 (C(CH<sub>3</sub>)<sub>3</sub>), 57.4 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 52.7 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 51.7 (CH<sub>3</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>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>CH<sub>2</sub>), 34.4 (NCH(CH<sub>2</sub>)<sub>2</sub>), 33.2 (C(=O)CH<sub>2</sub>), 28.0 (C(CH<sub>3</sub>)<sub>3</sub>), 22.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 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.

**10.64 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate 148**



Methyl 7-(4-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **147** (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. **148** 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, CH<sub>3</sub>), 3.66 (tt,  $J = 7.2, 3.7$  Hz, 1 H, NCH(CH<sub>2</sub>)<sub>2</sub>), 3.30 - 3.54 (br s, 8 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub> and CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>) 3.13 - 3.22 (m, 2 H, CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 2.36 (t,  $J = 7.1$  Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 1.87 - 1.98 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 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>C(=O)OH), 171.6 (C(=O)CC(=O)OCH<sub>3</sub>), 164.9 (C(=O)OCH<sub>3</sub>), 158.2 (q,  $J = 31.5$  Hz, CF<sub>3</sub>C(=O)OH), 152.5 (d,  $J = 247.6$  Hz, *ipso* to F), 148.5 (C=CC(=O)OH), 142.3 (d,  $J = 10.7$  Hz, *ipso* to piperazine), 138.0 (*para* to F), 122.6 (d,  $J = 6.4$  Hz, *para* to piperazine), 117.2 (q,

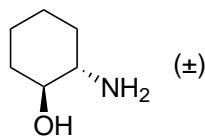
$J = 299.8$  Hz,  $\underline{\text{CF}_3}$ ), 111.9 (d,  $J = 22.4$  Hz, *ortho* to  $\text{C}=\text{O}$  and *ortho* to F), 109.1 ( $\underline{\text{C}}\text{C}(\text{=O})\text{OCH}_3$ ), 106.9 (*meta* to  $\text{C}=\text{O}$  and *meta* to F), 55.1 ( $\text{C}(\text{=O})\text{CH}_2\text{CH}_2\underline{\text{CH}_2\text{N}}$ ), 51.4 ( $\underline{\text{CH}_3}$ ), 50.8 ( $\text{C}(\text{=O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\underline{\text{CH}_2}$ ), 46.7 ( $\text{C}(\text{=O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\underline{\text{CH}_2})\text{CH}_2\text{CH}_2$ ), 46.7 ( $\text{C}(\text{=O})\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2)\text{CH}_2\underline{\text{CH}_2}$ ), 34.9 ( $\underline{\text{NCH}}(\text{CH}_2)_2$ ), 30.6 ( $\text{C}(\text{=O})\underline{\text{CH}_2}$ ), 19.1 ( $\text{C}(\text{=O})\text{CH}_2\underline{\text{CH}_2}$ ), 7.6 ( $\text{NCH}(\underline{\text{CH}_2})_2$ )

**$^{19}\text{F}$  NMR** (376.45 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -73.6 (s,  $\text{CF}_3$ ), -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.

### 10.65 (*trans*)-2-Aminocyclohexan-1-ol 150



Cyclohexene oxide **149** (10 ml, 9.70 g, 98.8 mmol, 1 eq.), NH<sub>3</sub> (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 N<sub>2</sub> over it, followed by evaporation under high vacuum. **150** was obtained as a white amorphous solid (9.90 g, 85.2 mmol, 86.2 %)

**TLC**  $R_f$  = 0.04 (30 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>)

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

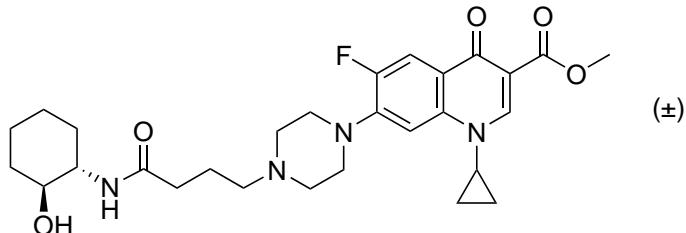
**$^1\text{H}$  NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 3.01 (td,  $J = 9.4, 4.8$  Hz, 1 H,  $\underline{\text{CH}}\text{OH}$ ), 2.80 - 2.92 (m, 2 H, OH and NH<sub>2</sub>), 2.35 (ddd,  $J = 11.1, 9.1, 4.1$  Hz, 1 H,  $\underline{\text{CH}}\text{NH}_2$ ), 1.77 - 1.84 (m, 1 H,  $\underline{\text{CH}}\text{HCHOH}$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

**$^{13}\text{C}$  NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  / ppm = 75.4 ( $\underline{\text{CHOH}}$ ), 56.6 ( $\underline{\text{CHN}}_2$ ), 33.8 ( $\underline{\text{CH}}_2\text{CHOH}$  and  $\underline{\text{CH}}_2\text{CHN}_2$ ), 24.7 ( $\underline{\text{CH}}_2\text{CH}_2\text{CHNH}_2$ ), 24.6 ( $\underline{\text{CH}}_2\text{CH}_2\text{CHOH}$ )

**HRMS** (ESI<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>189</sup>

**10.66 Methyl 1-cyclopropyl-6-fluoro-7-(4-((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 151**



4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid trifluoroacetate **148** (200 mg, 0.367 mmol, 1 eq.), (*trans*)-2-aminocyclohexan-1-ol **150** (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. **151** was obtained as a white amorphous solid (61.2 mg, 0.116 mmol, 31.7 %).

**IR** (neat)  $\nu_{max}$  /  $cm^{-1}$  = 3302.5 (N-H), 2929.8 (C-H), 2850.6 (C-H), 2832.9 (C-H), 1698.1 (ester C=O), 1646.4 (amide C=O), 1613.8 (quinolone C=O)

**$^1H$  NMR** (400 MHz, MeOD)  $\delta$  / 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>)

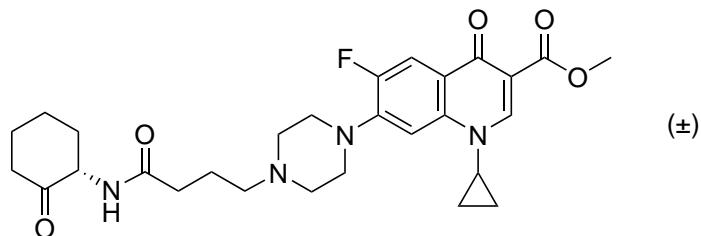
**$^{13}C$  NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.8 (CH<sub>2</sub>C(=O)NH), 175.3 (C(=O)CC(=O)OCH<sub>3</sub>), 166.8 (C(=O)OCH<sub>3</sub>), 154.9 (d,  $J$  = 248.8 Hz, *ipso* to F), 150.2 (C=CC(=O)OCH<sub>3</sub>), 146.1 (d,  $J$  = 10.8 Hz, *ipso* to piperazine), 139.9 (*para* to F), 123.5 (d,  $J$  = 7.5 Hz, *para* to piperazine), 113.2 (d,  $J$  = 23.2 Hz, *ortho* to C=O and *ortho* to F), 110.2 (CC(=O)OCH<sub>3</sub>), 107.2 (*meta* to C=O and *meta* to F), 74.1 (CHOH), 58.9 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 56.4 (CHNH), 54.0 (C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>)CH<sub>2</sub>), 52.3 (CH<sub>3</sub>), 50.5 (d,  $J$  = 5.0 Hz, 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>), 36.4 (NCH(CH<sub>2</sub>)<sub>2</sub>), 35.7 (CH<sub>2</sub>CHOH), 35.1 (C(=O)CH<sub>2</sub>), 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>)

**$^{19}F$  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.

**10.67 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 152**



Methyl 1-cyclopropyl-6-fluoro-7-(4-((*trans*)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **151** (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  $\text{CH}_2\text{Cl}_2$  (3 ml) at r.t. for 6 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC (5-95 % acetonitrile/water over 20 min). The combined pure fractions were evaporated under reduced pressure to a volume of 20 ml, then  $\text{NaHCO}_3$  (aq., sat., 30 ml) and 10 % *i*-PrOH/ $\text{CHCl}_3$  (30 ml) were added. The organic layer was dried with  $\text{MgSO}_4$  and evaporated under reduced pressure. **152** was obtained as a white amorphous solid (3.6 mg, 6.8  $\mu$ mol, 69.1 %).

**TLC**  $R_f$  = 0.74 (30 % MeOH/ $\text{CH}_2\text{Cl}_2$ )

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

**$^1\text{H NMR}$**  (400 MHz, DMSO 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, CH<sub>2</sub>NH), 3.73 (s, 3 H, CH<sub>3</sub>), 3.65 (tt,  $J$  = 7.1, 3.9 Hz, 1 H, NCH<sub>2</sub>(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, CH<sub>2</sub>C(=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, CH<sub>2</sub>C(=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, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.00 (ddquin,  $J$  = 13.2, 6.0, 2.9 Hz, 1 H, CH<sub>2</sub>CH<sub>2</sub>C(=O)), 1.65 - 1.83 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.41 - 1.56 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N and CH<sub>2</sub>CH<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>)

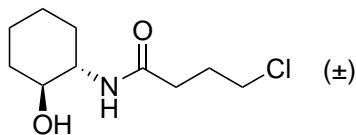
**$^{13}\text{C NMR}$**  (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 207.5 (C(=O)CHNH), 171.7 (C(=O)CC(=O)OCH<sub>3</sub>), 171.6 (CH<sub>2</sub>C(=O)NH), 165.0 (C(=O)OCH<sub>3</sub>), 152.6 (d,  $J$  = 247.6 Hz, *ipso* to F), 148.3 (C=CC(=O)OCH<sub>3</sub>), 143.9 (br s, *ipso* to piperazine), 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 (CH<sub>2</sub>NH 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>CH<sub>2</sub>CH<sub>2</sub>N), 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>CH<sub>2</sub>N), 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>)

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

**HRMS** (ESI<sup>+</sup>)  $m/z$  / Da = 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.

## 10.68 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 153



(*trans*)-2-Aminocyclohexan-1-ol **150** (1.04 g, 9.03 mmol, 1 eq.), TEA (1.65 ml, 1.20 g, 11.8 mmol, 1.3 eq.) and  $\text{CH}_2\text{Cl}_2$  (50 ml) were stirred at 0 °C. 4-Chlorobutyryl chloride **142** (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. **153** 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)

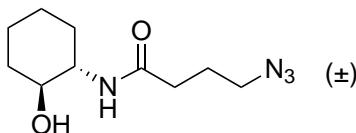
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta$  / ppm = 3.60 (t,  $J$  = 6.6 Hz, 2 H,  $\text{CH}_2\text{Cl}$ ), 3.51 - 3.60 (m, 1 H,  $\text{CH}_2\text{NH}$ ), 3.28 - 3.39 (m, 1 H,  $\text{CHOH}$ ), 2.37 (td,  $J$  = 7.4, 2.3 Hz, 2 H, C(=O)CH<sub>2</sub>), 2.06 (quin,  $J$  = 7.0 Hz, 2 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>), 1.97 - 2.01 (m, 1 H,  $\text{CH}_2\text{CHOH}$ ), 1.85 - 1.93 (m, 1 H,  $\text{CH}_2\text{CHNH}$ ), 1.70 - 1.77 (m, 1 H,  $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 1.64 - 1.70 (m, 1 H,  $\text{CH}_2\text{CH}_2\text{CHNH}$ ), 1.24 - 1.35 (m, 3 H,  $\text{CH}_2\text{CH}_2\text{CHOH}$ ,  $\text{CH}_2\text{CH}_2\text{CHNH}$  and  $\text{CH}_2\text{CH}_2\text{CHOH}$ ), 1.13 - 1.25 (m, 1 H,  $\text{CH}_2\text{CHNH}_2$ )

**<sup>13</sup>C NMR** (101 MHz, MeOD)  $\delta$  / ppm = 175.0 (C(=O)), 74.1 ( $\text{CHOH}$ ), 56.3 ( $\text{CH}_2\text{NH}$ ), 45.3 ( $\text{CH}_2\text{Cl}$ ), 35.6 ( $\text{CH}_2\text{CHOH}$ ), 34.5 (C(=O)CH<sub>2</sub>), 32.7 ( $\text{CH}_2\text{CHNH}$ ), 30.1 (C(=O)CH<sub>2</sub>CH<sub>2</sub>), 25.8 ( $\text{CH}_2\text{CH}_2\text{CHNH}$ ), 25.5 ( $\text{CH}_2\text{CH}_2\text{CHOH}$ )

**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.

## 10.69 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 154



4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **153** (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. **154** was obtained as large white prisms (347 mg, 1.53

mmol, 97.5 %).

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

**mp**  $T / ^\circ\text{C} = 74.5\text{--}75.7$  (*i*-PrOH, CHCl<sub>3</sub>)

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

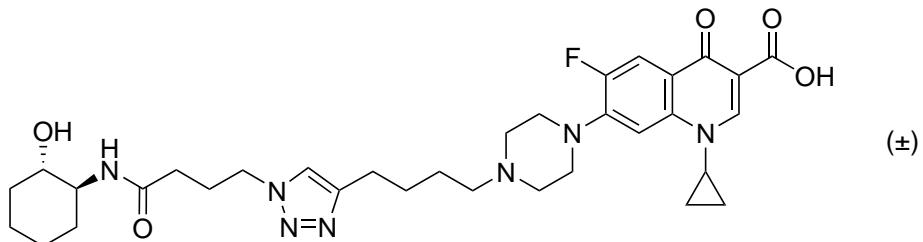
**<sup>1</sup>H NMR** (400 MHz, MeOD)  $\delta / \text{ppm} = 7.87$  (d,  $J = 7.9$  Hz, 1 H, NH), 5.27 (d,  $J = 4.3$  Hz, 1 H, OH), 3.56 (td,  $J = 10.5, 4.4$  Hz, 1 H, CHNH), 3.28 - 3.41 (m, 3 H, CHO and CH<sub>2</sub>N<sub>3</sub>), 2.30 (td,  $J = 7.4, 2.7$  Hz, 2 H, C(=O)CH<sub>2</sub>), 1.95 - 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)  $\delta / \text{ppm} = 175.1$  (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>)  $m/z / \text{Da} = 249.1331$ , [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.

**10.70 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-((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 155**



1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **68** (40 mg, 97.2  $\mu\text{mol}$ , 1 eq.) and 4-azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide **154** (22.0 mg, 97.2  $\mu\text{mol}$ , 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 (97.2  $\mu\text{l}$ , 9.72  $\mu\text{mol}$ , 0.1 eq. 100 mM, aq.) was added, followed by a solution of sodium ascorbate (194  $\mu\text{l}$ , 19.4  $\mu\text{mol}$ , 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. **155** was obtained as a white amorphous solid (30.3 mg, 47.5  $\mu\text{mol}$ , 48.9 %).

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

**<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>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>CH<sub>2</sub>NH), 1.04 - 1.13 (m, 1 H, CH<sub>2</sub>CH<sub>2</sub>NH)

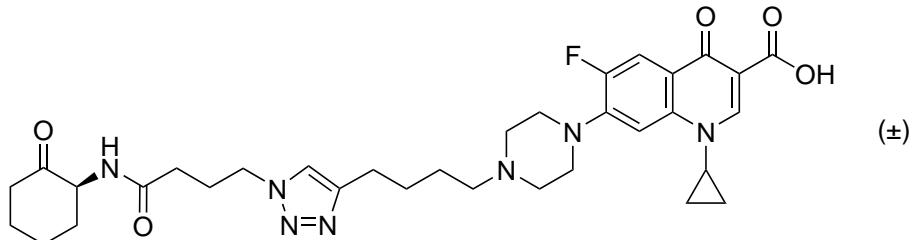
**<sup>13</sup>C NMR** (101 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = 176.4 (C(=O)CC(=O)OH), 170.9 (CH<sub>2</sub>C(=O)NH), 166.0 (C(=O)OH), 153.1 (d,  $J$  = 252.1 Hz, *ipso* to F), 148.0 (C=CC(=O)OH), 146.9 (CH=CCH<sub>2</sub>), 145.3 (d,  $J$  = 10.0 Hz, *ipso* to piperazine), 139.2 (para to F), 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>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>CH<sub>2</sub>NH), 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>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>CH<sub>2</sub>NH), 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.

**10.71 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 156**



1-Cyclopropyl-6-fluoro-7-(4-(4-(1-((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 **155** (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. **156** 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>CH<sub>2</sub>NH), 4.31 (t, *J* = 6.9 Hz, 1 H, C(=O)CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH), 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>NH), 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>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>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>NH), 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>), 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>), 48.7 (C(=O)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>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>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>N), 24.9 (CH=CCH<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>)

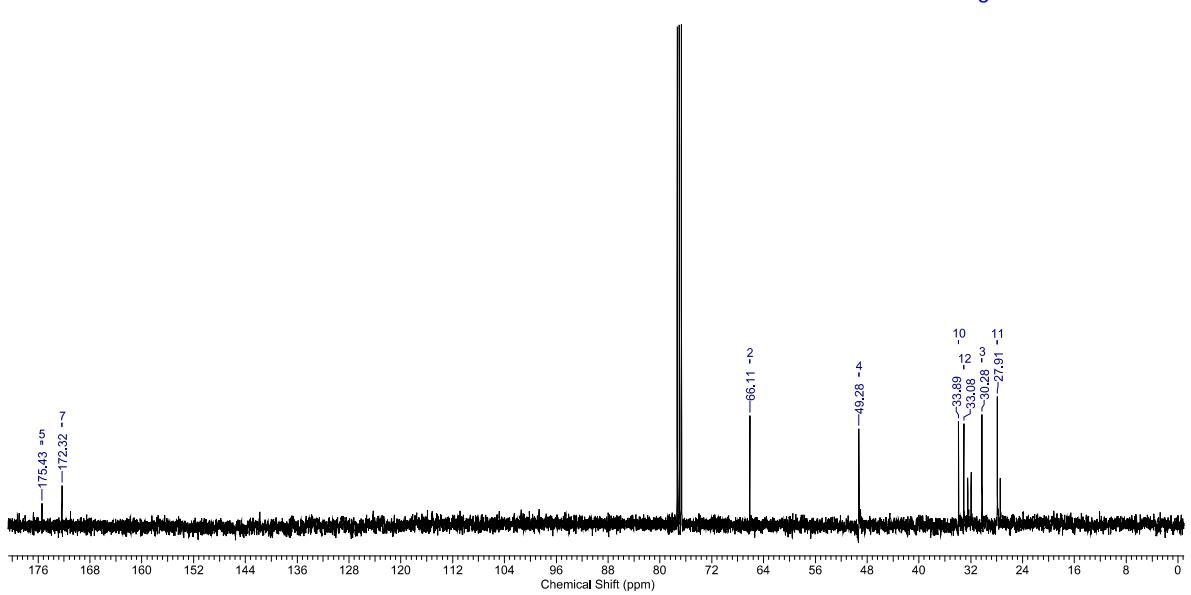
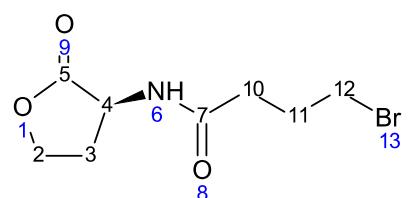
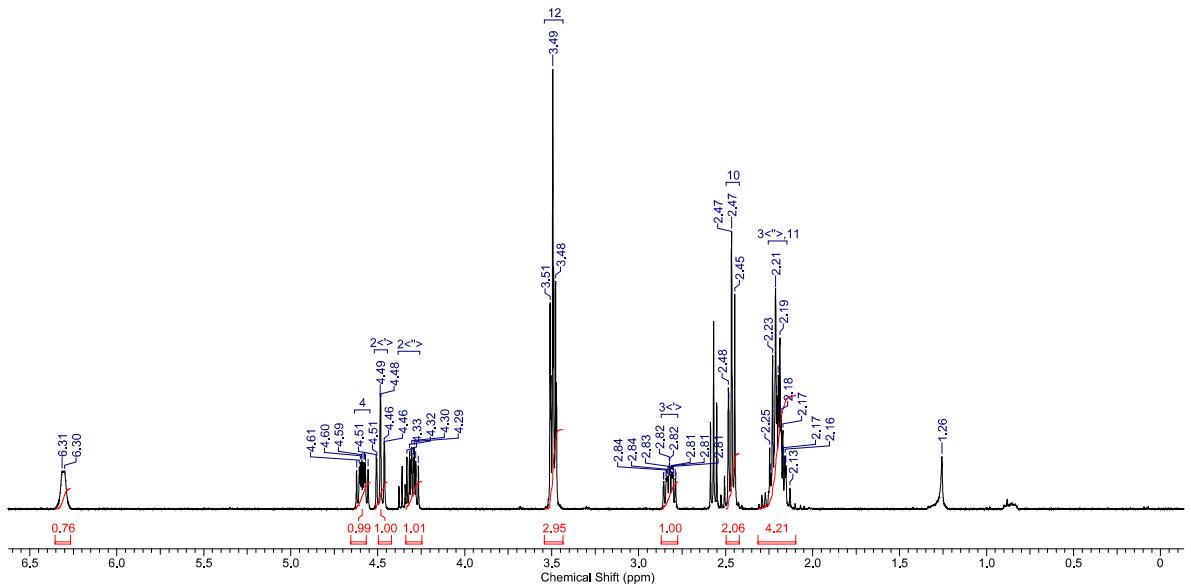
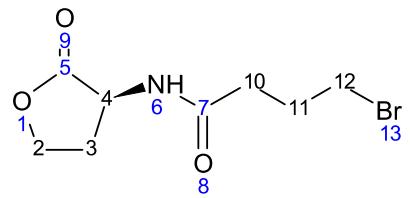
**<sup>19</sup>F NMR** (376 MHz, DMSO d<sub>6</sub>)  $\delta$  / ppm = -121.7 (s, ciprofloxacin F)

**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

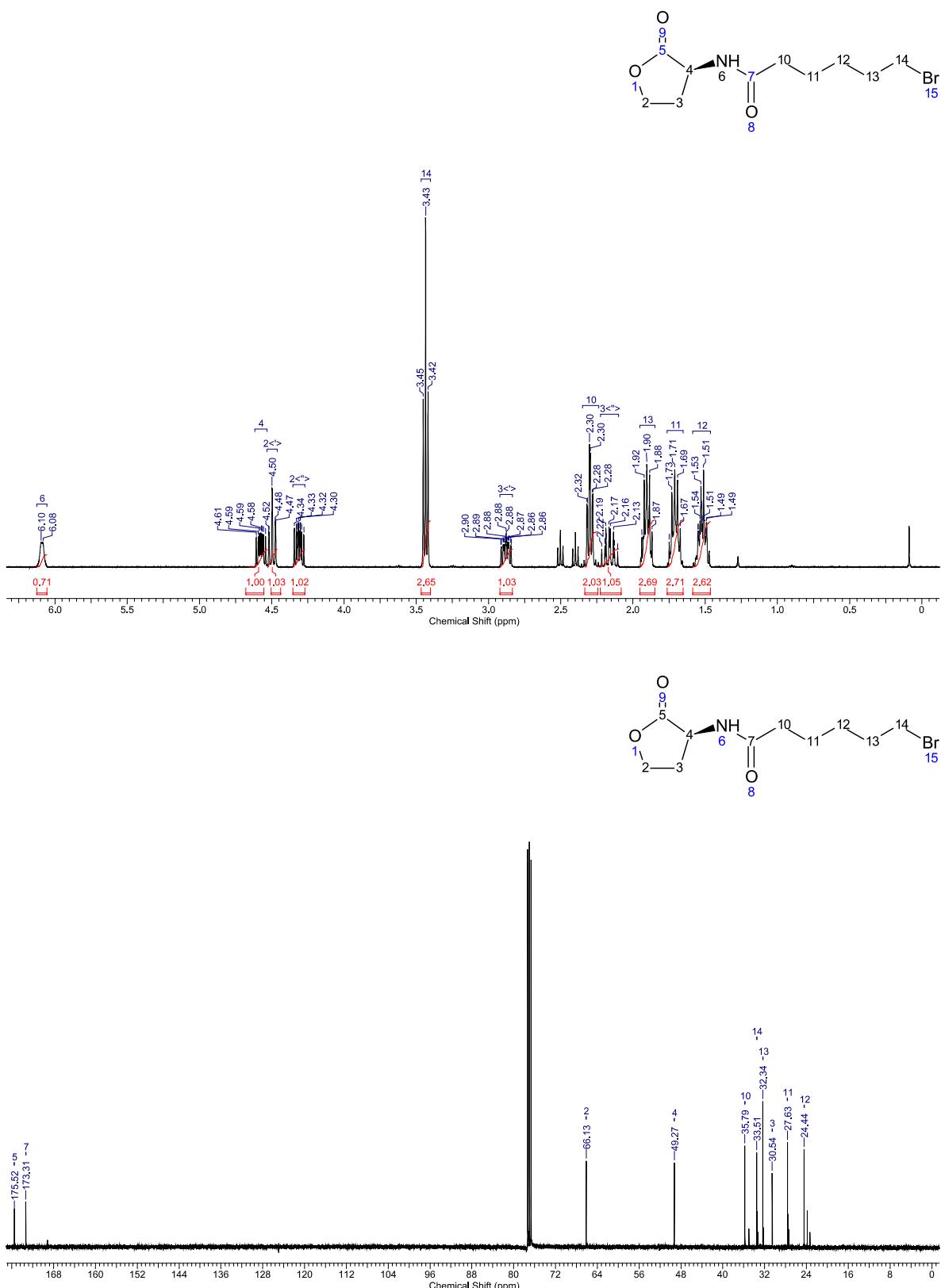
The compound has not been reported previously.

## 11 NMR spectra

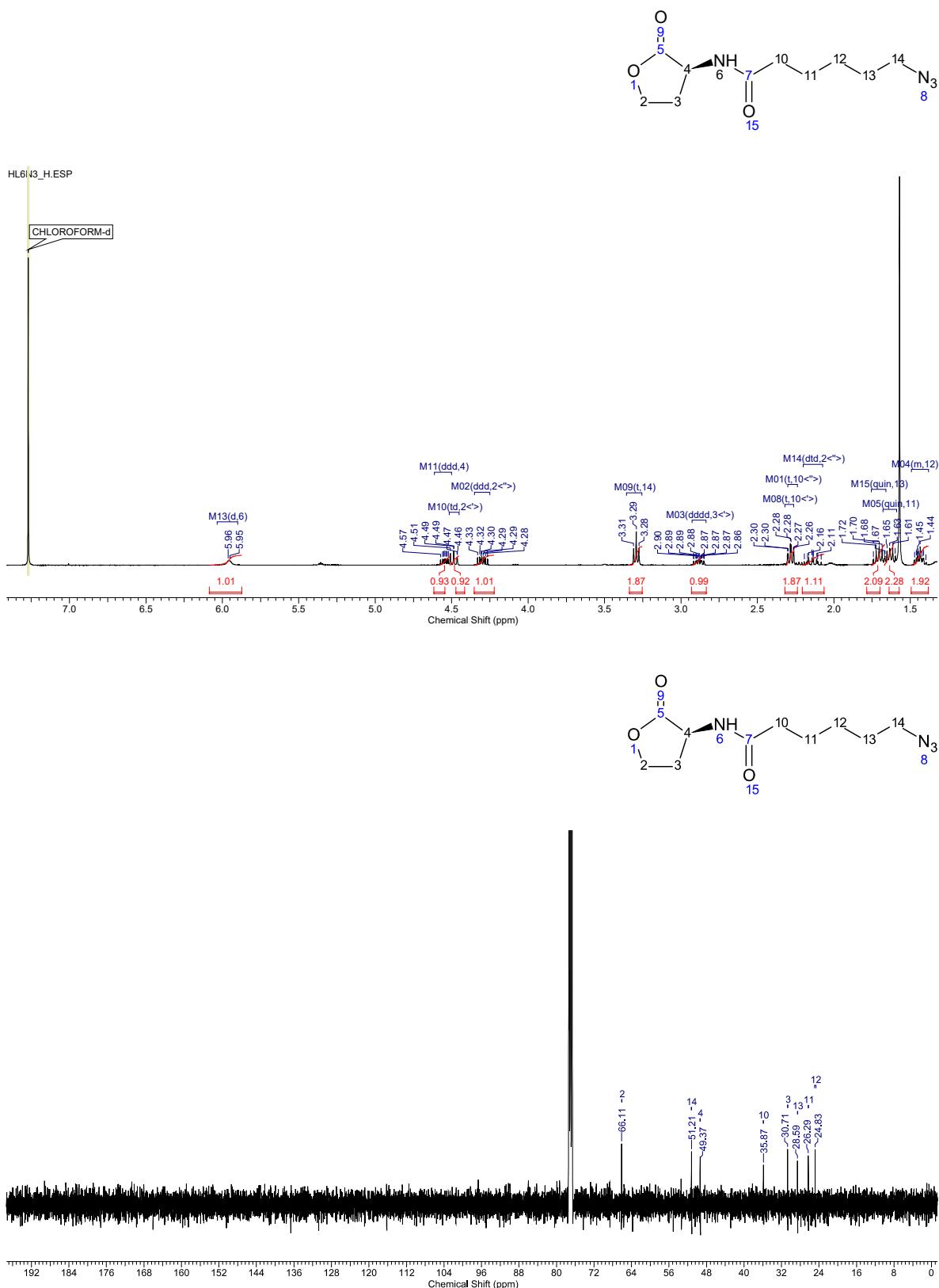
## 11.1 (*S*)-4-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 57



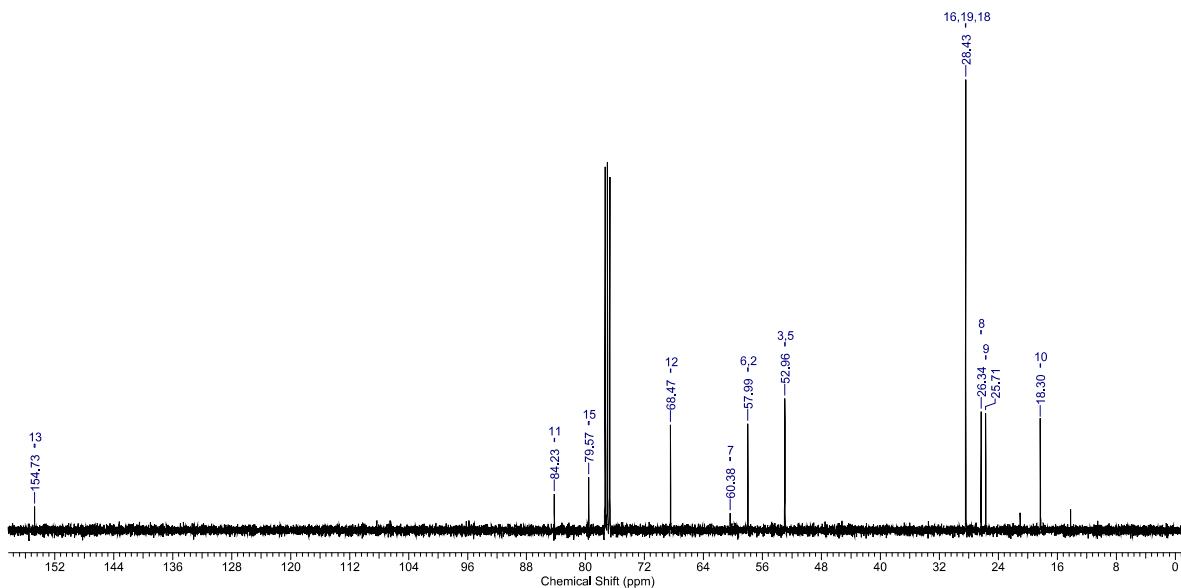
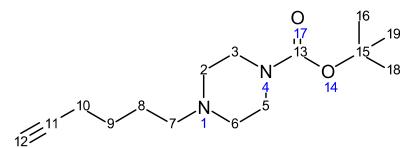
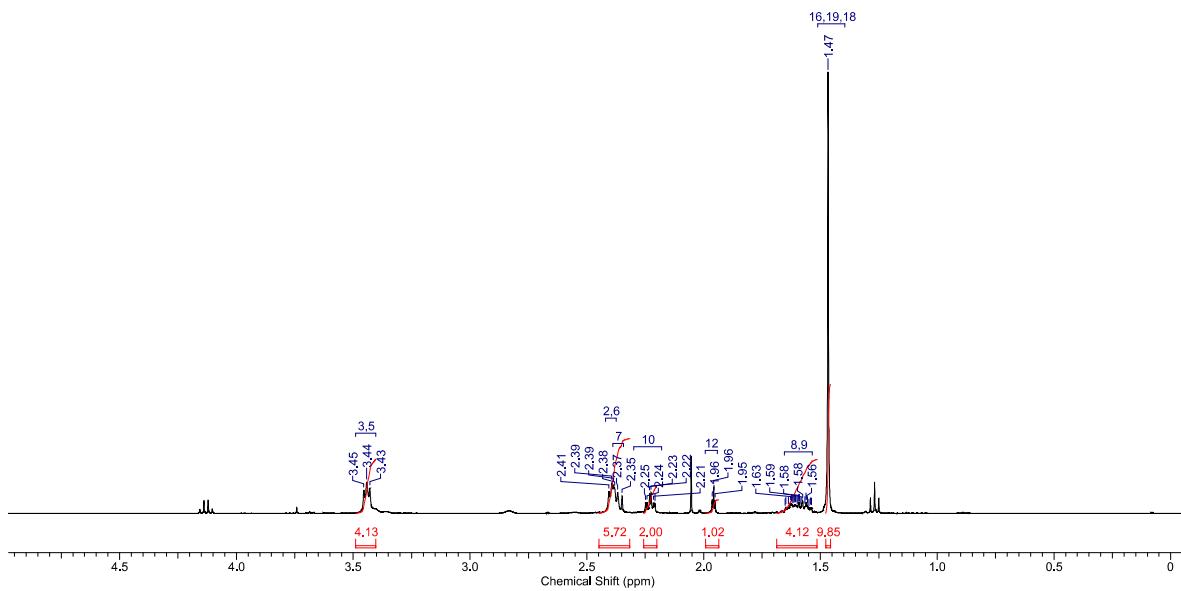
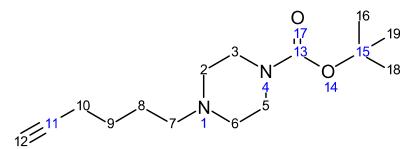
## 11.2 (*S*)-6-Bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 60



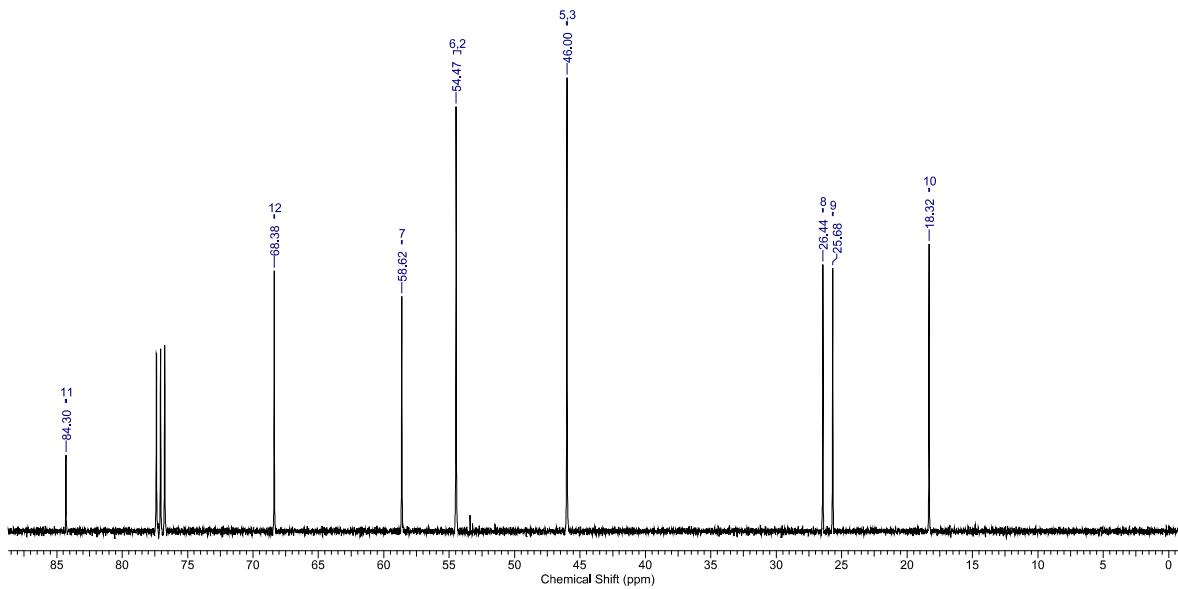
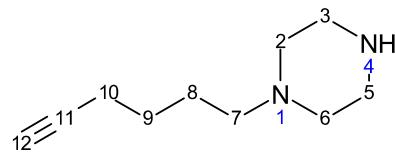
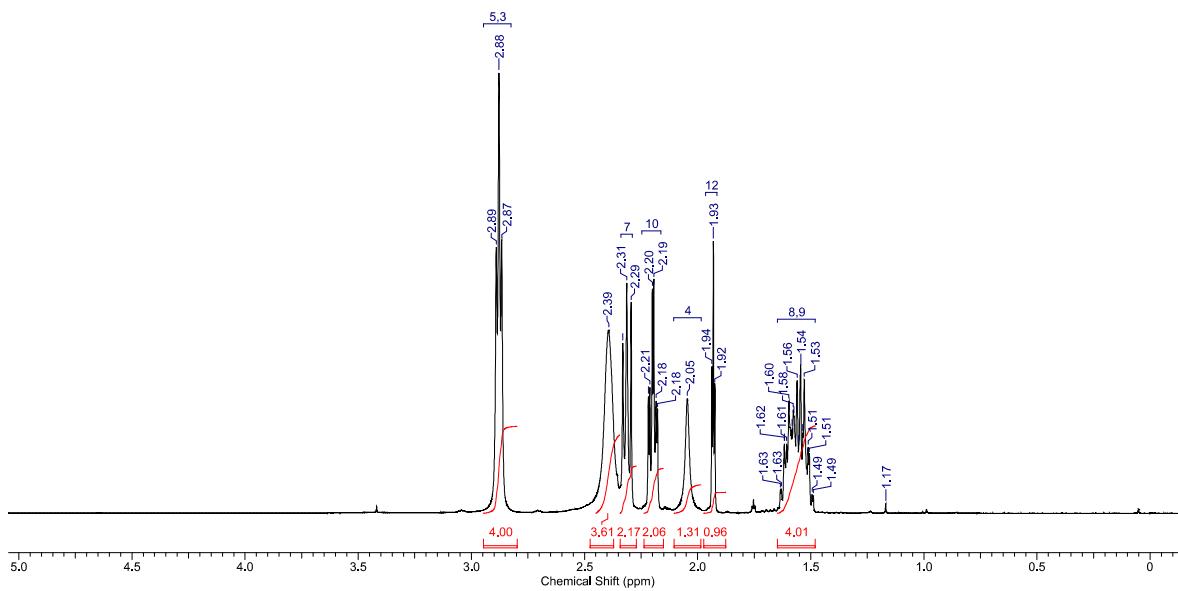
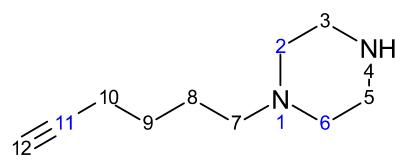
### 11.3 (*S*)-6-Azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 61



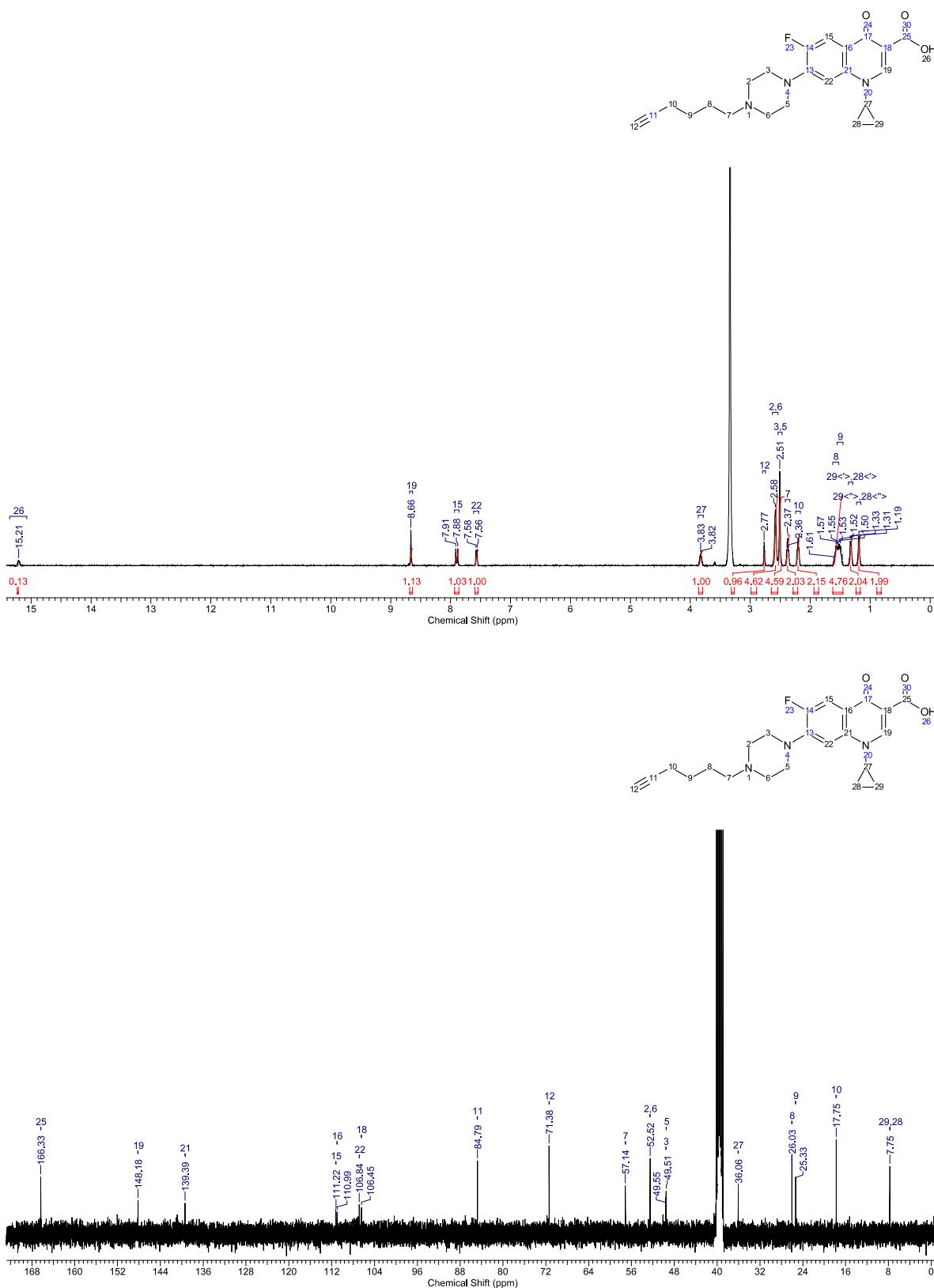
## 11.4 *tert*-Butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 65



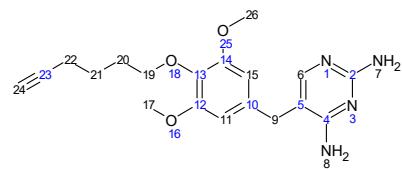
## 11.5 1-(Hex-5-yn-1-yl)piperazine 66



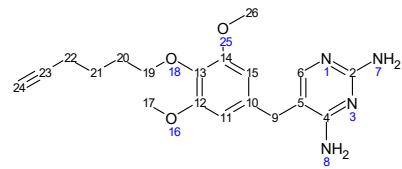
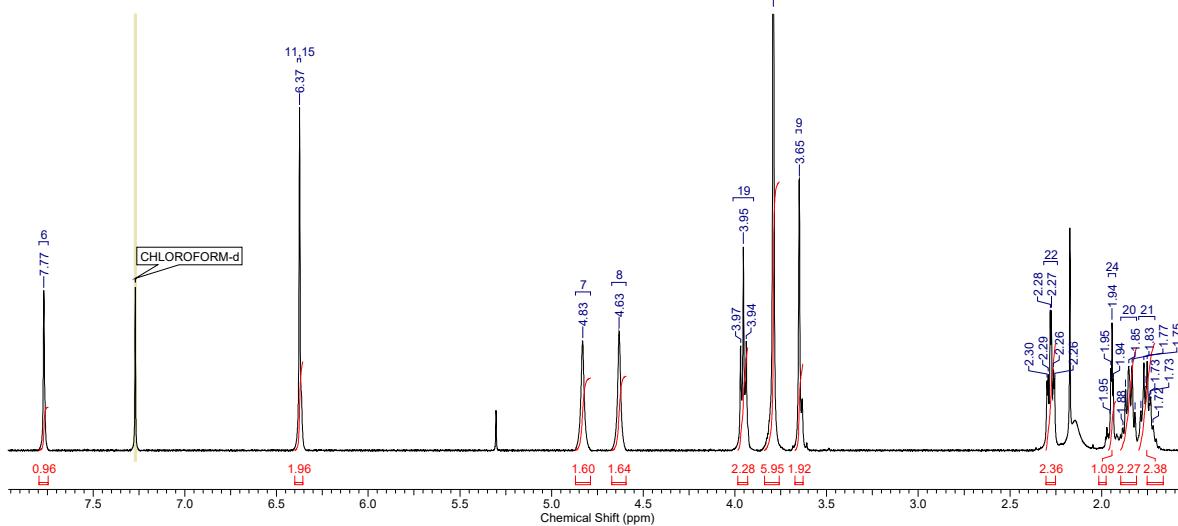
11.6 1-Cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid 68



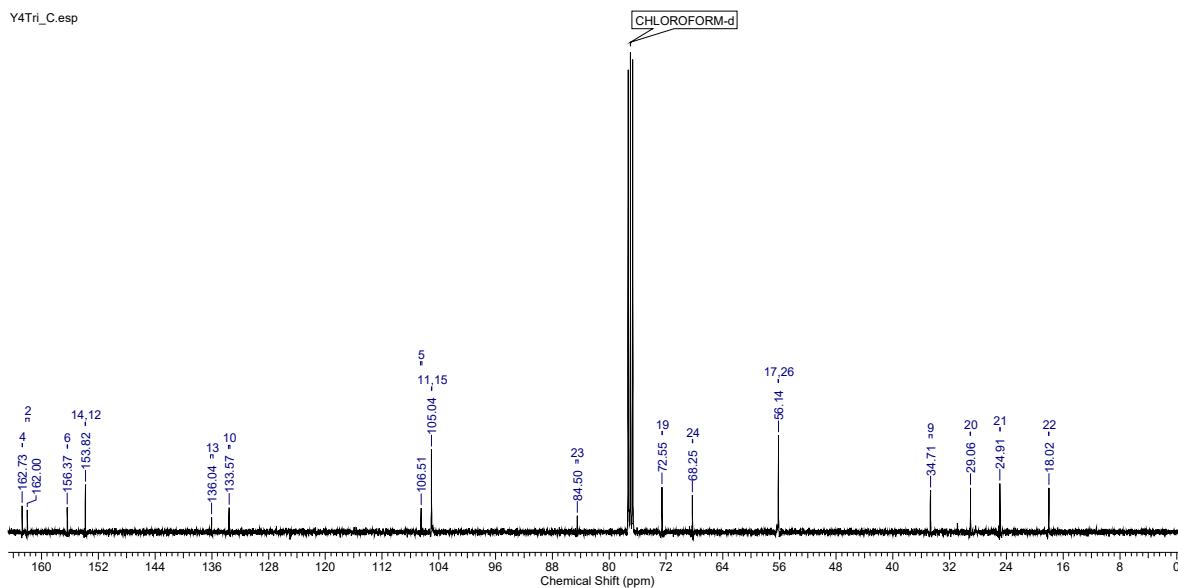
## 11.7 5-(4-(Hex-5-yn-1-yloxy)-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine 71



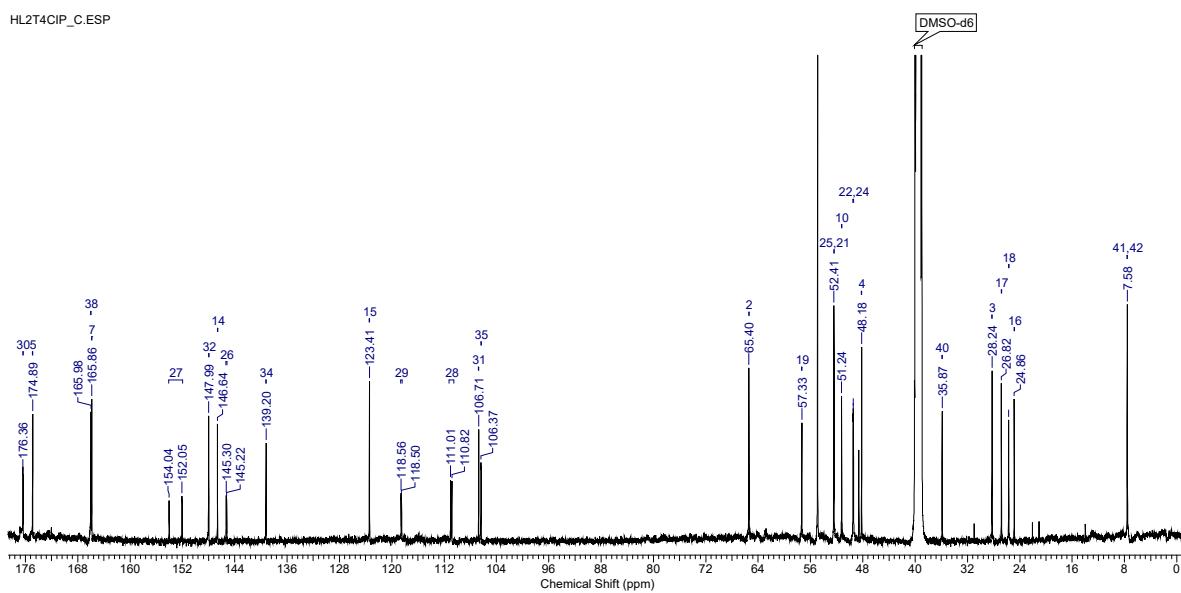
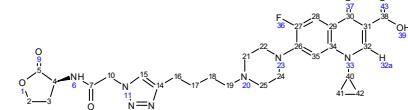
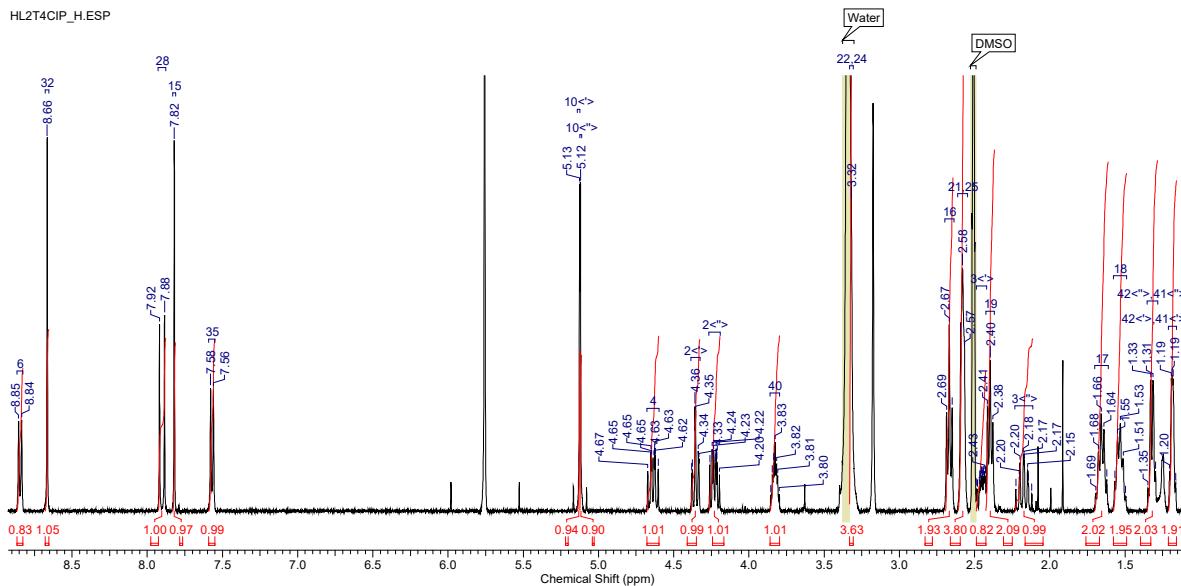
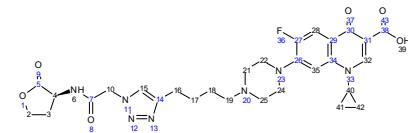
Y4Tri\_H.esp



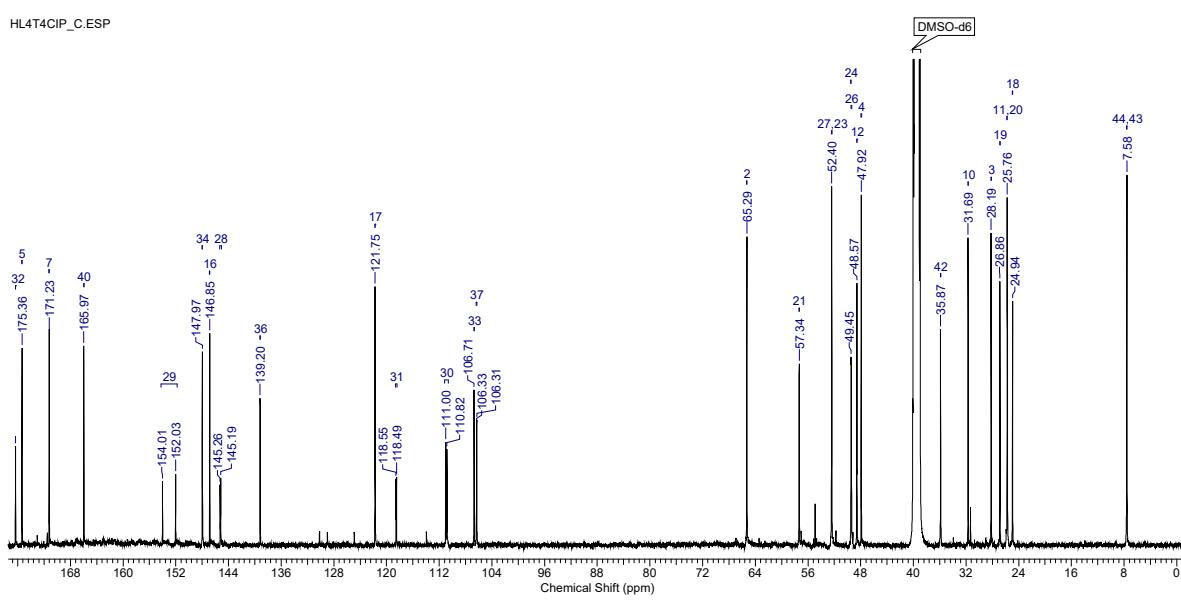
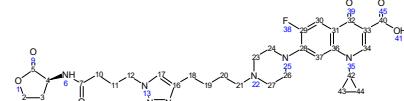
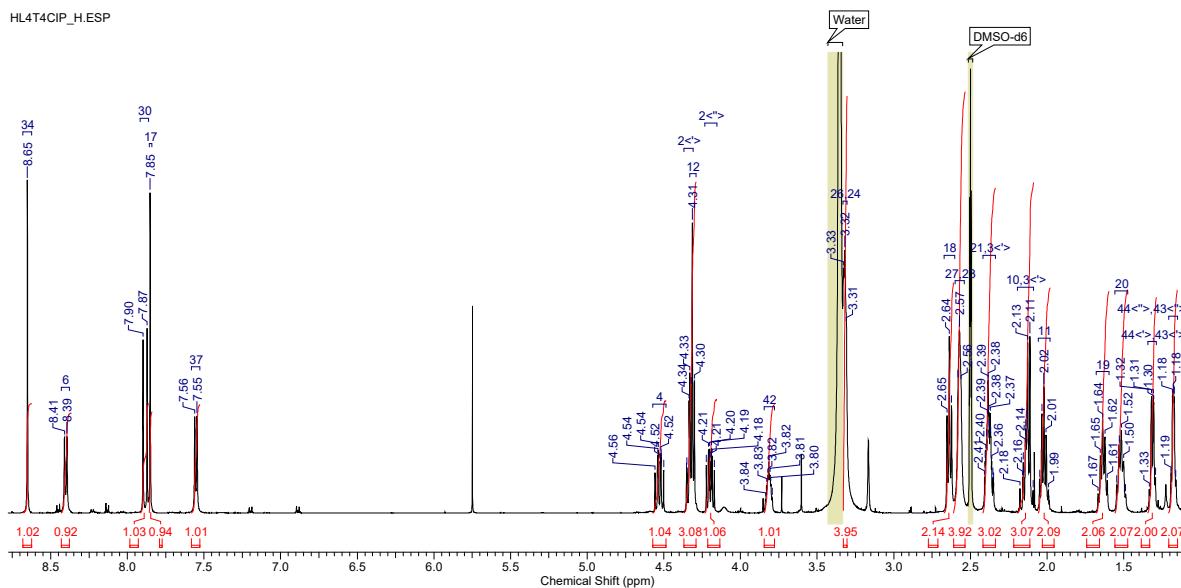
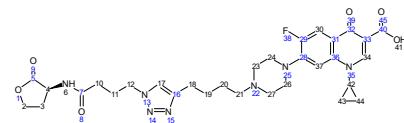
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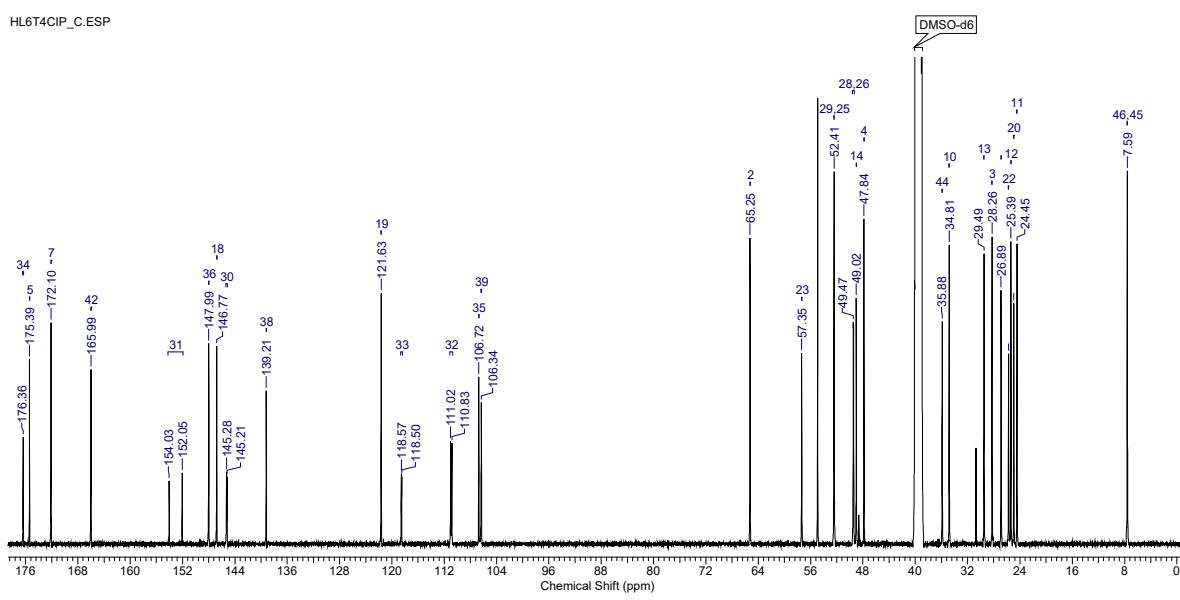
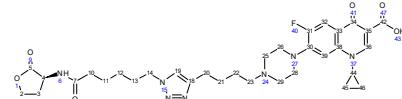
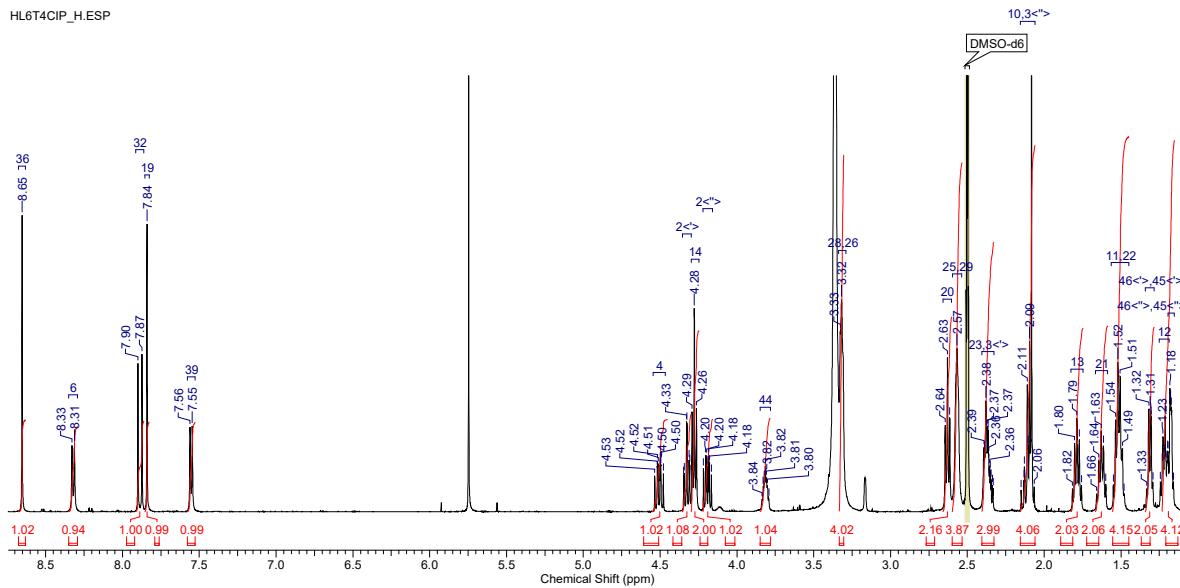
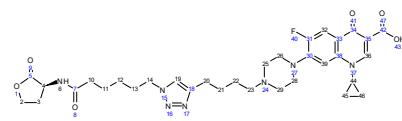
11.8 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(2-oxo-2-((2-oxotetrahydrofuran-3-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 72



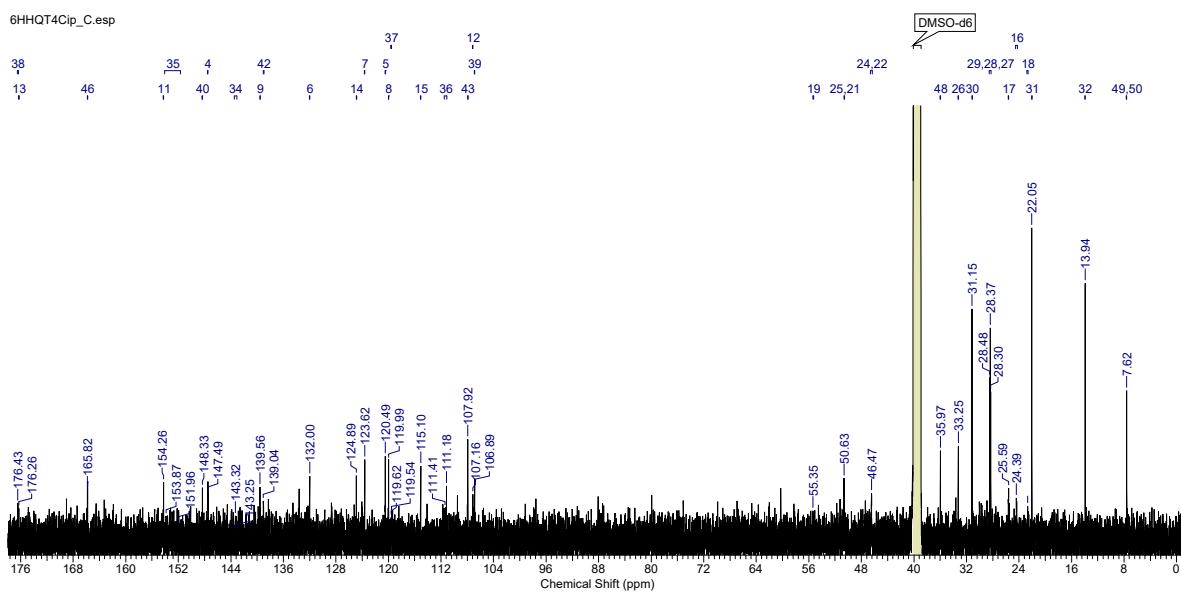
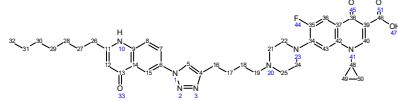
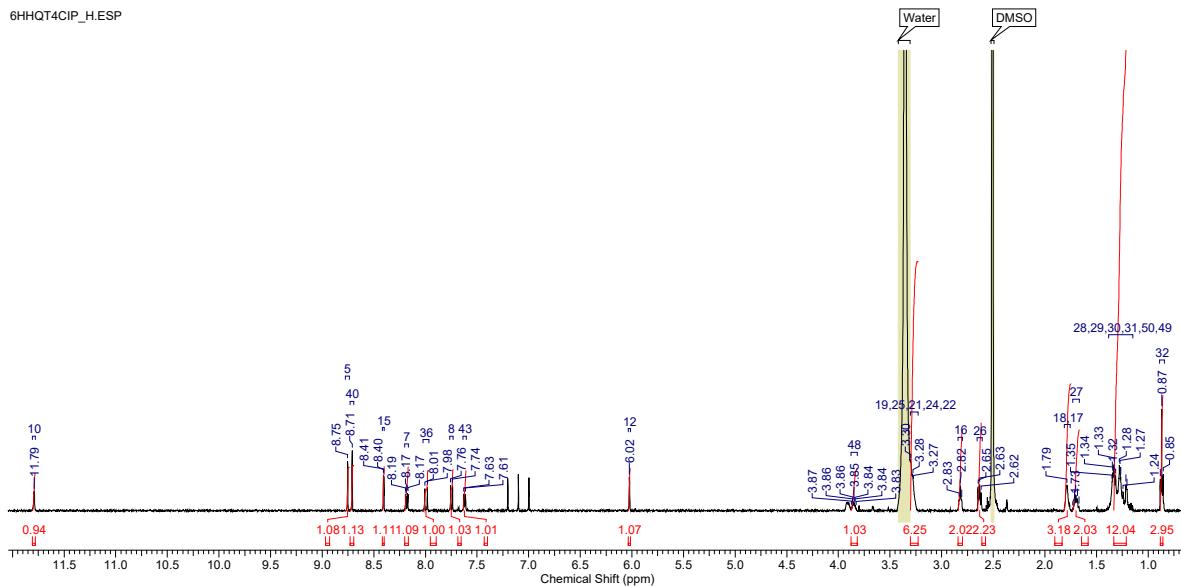
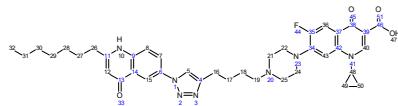
11.9 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(4-oxo-4-((2-oxotetrahydrofuran-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 77



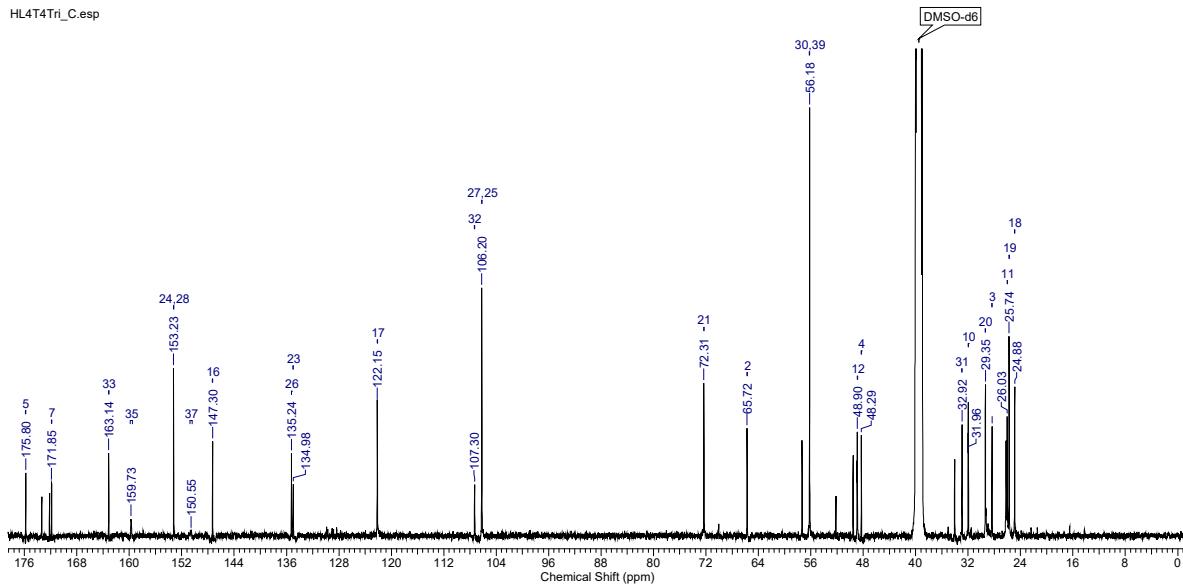
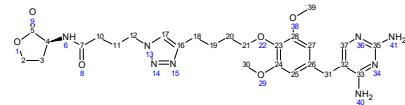
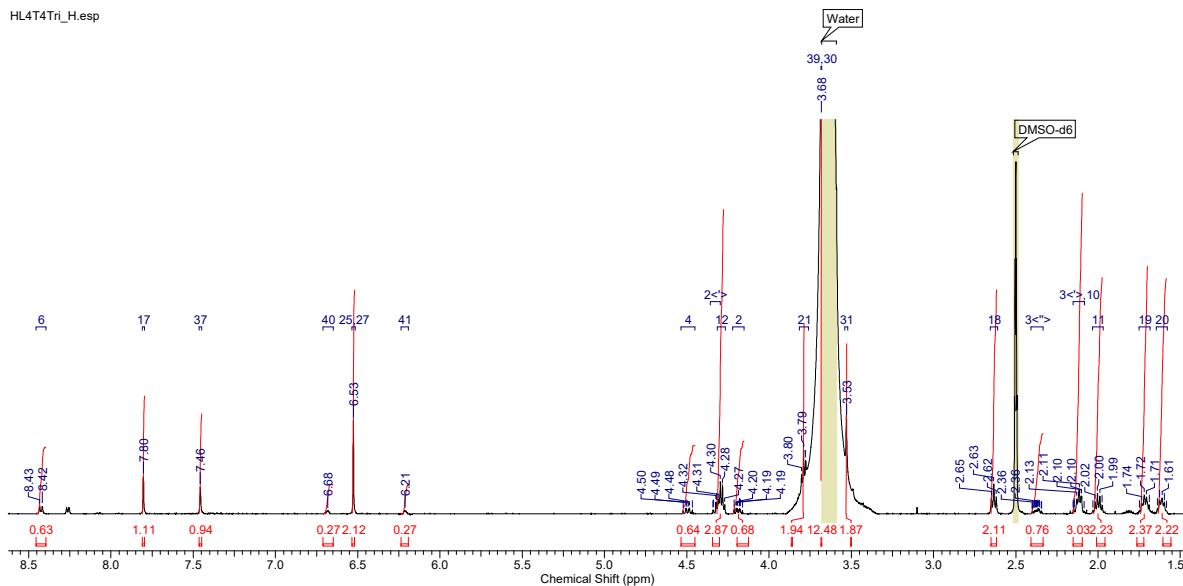
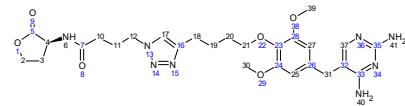
11.10 (*S*)-1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(1-(6-oxo-6-((2-oxotetrahydrofuran-3-yl)amino)hexyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 78



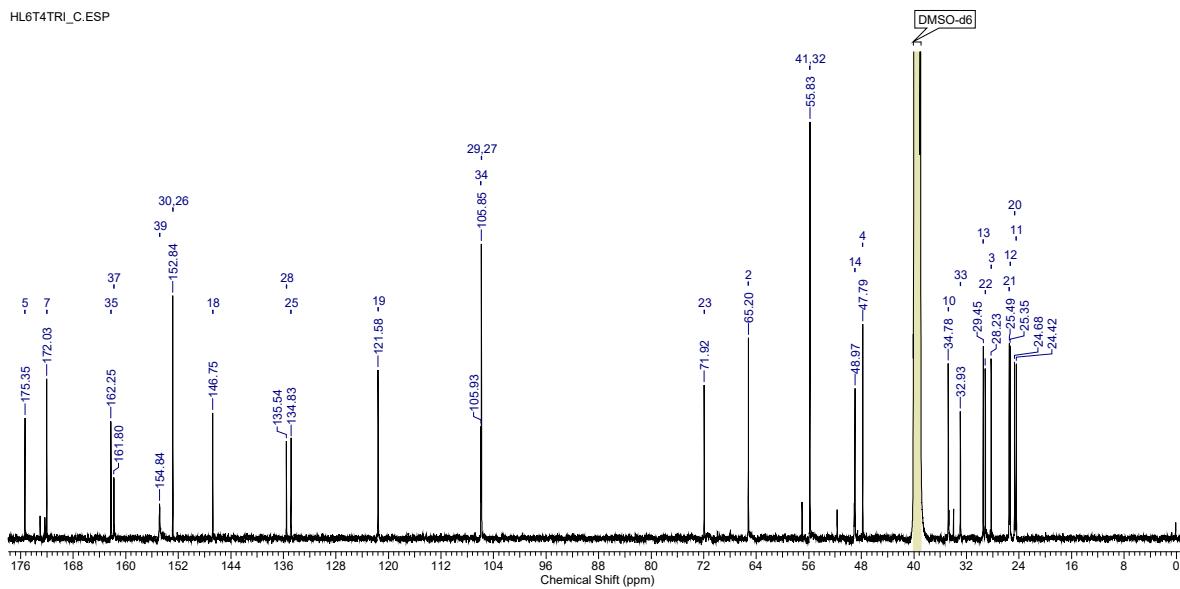
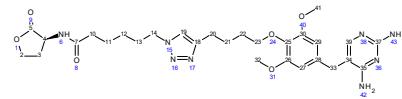
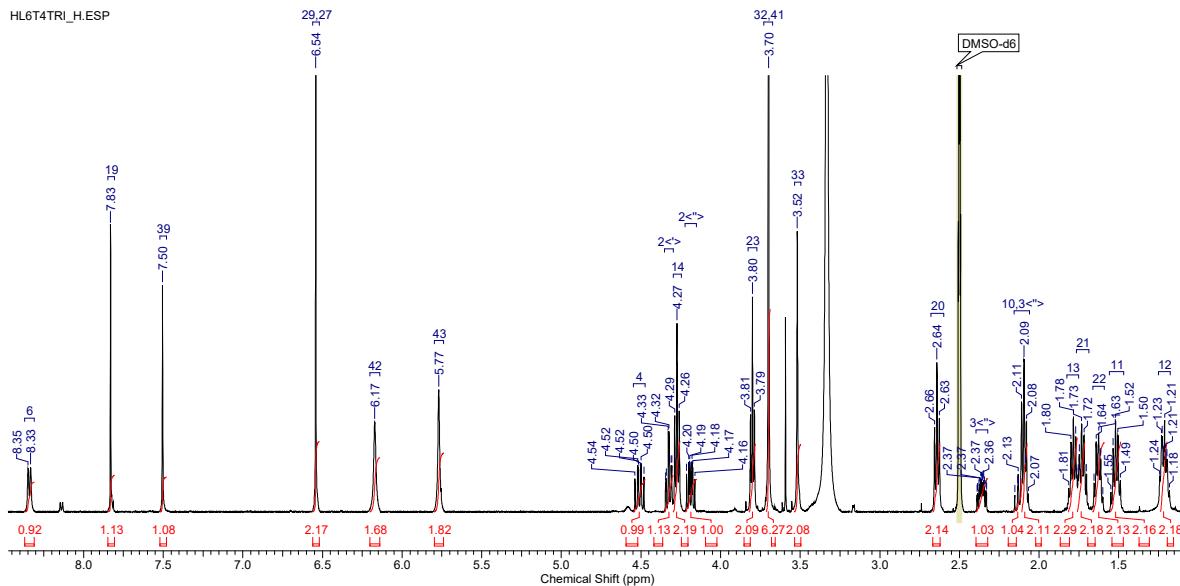
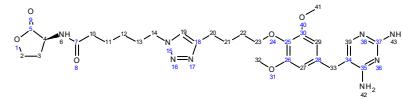
11.11 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(2-heptyl-4-oxo-1,4-dihydroquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 80



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

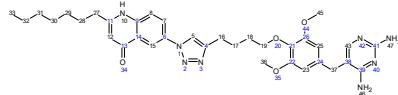


11.13 (*S*)-6-((4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 85

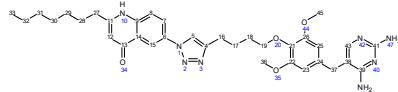
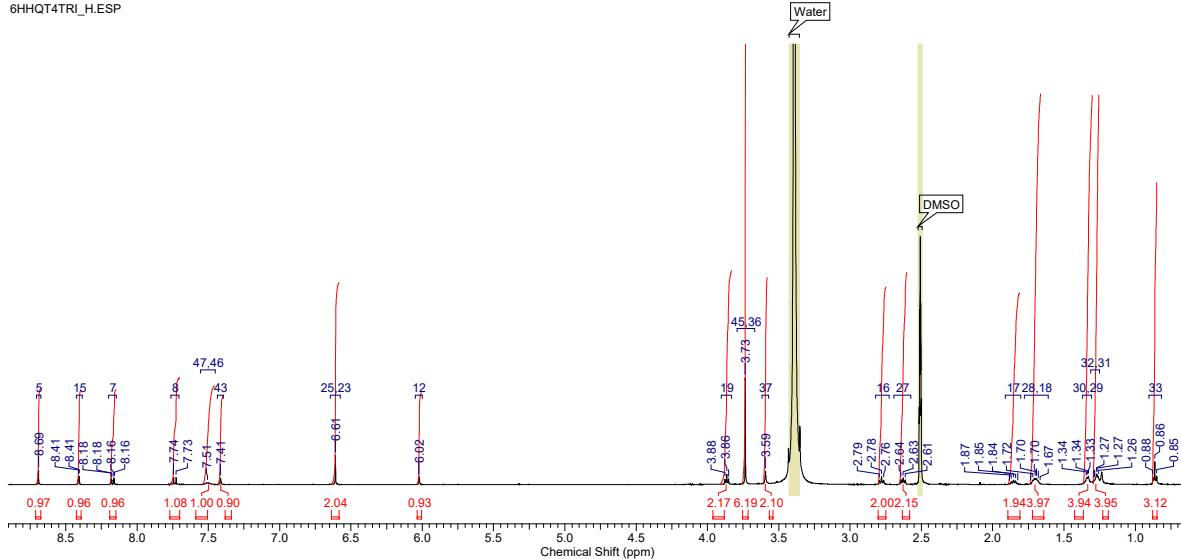


11.14 6-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)-2-heptylquinolin-4(*1H*)-one 87

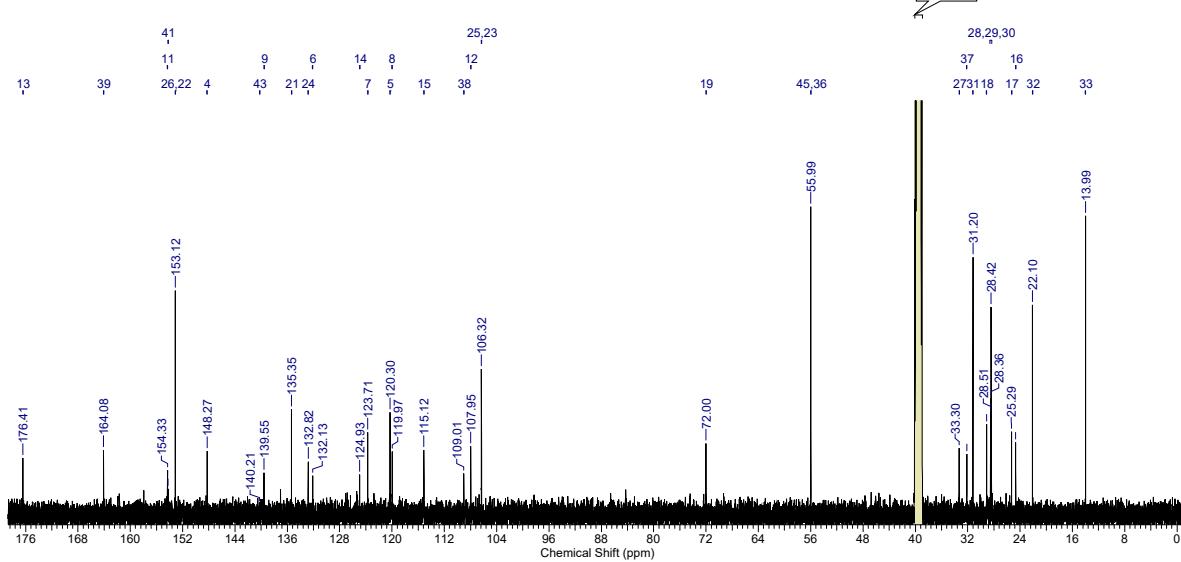
**User Notes** Some guesses



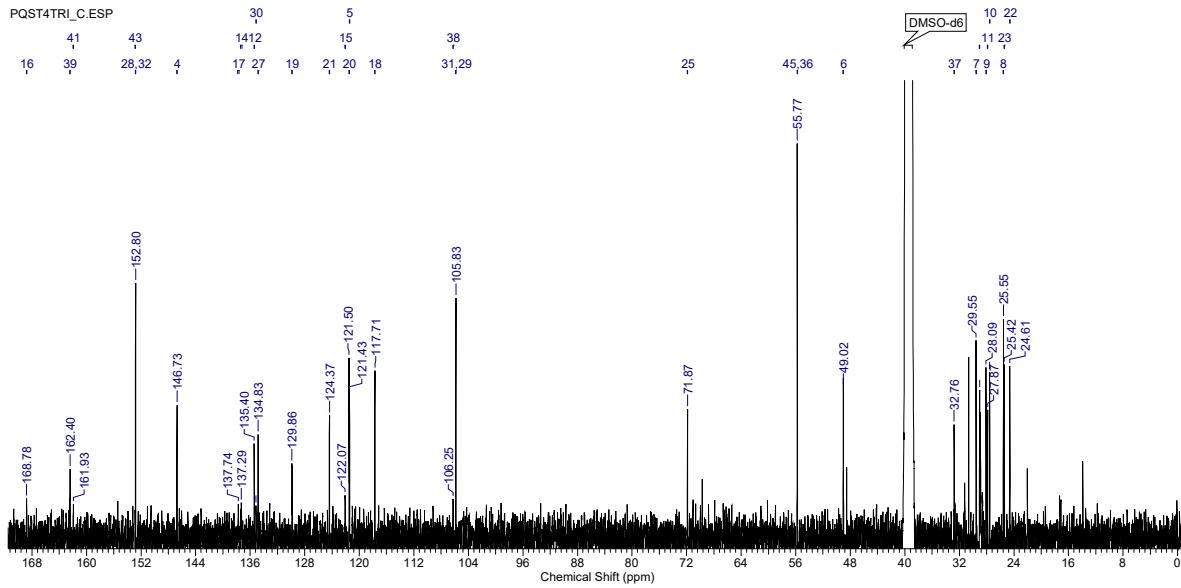
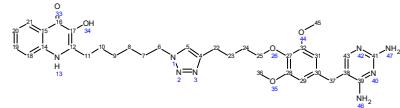
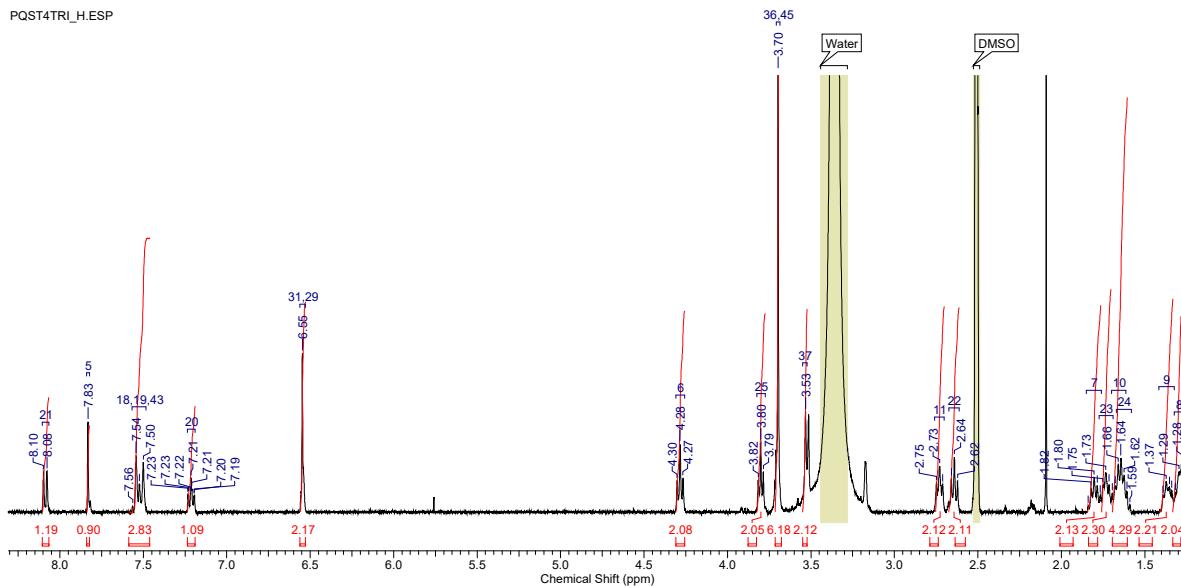
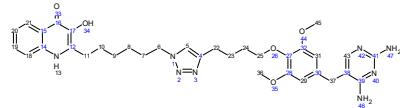
6HHQT4TRI\_H.ESP



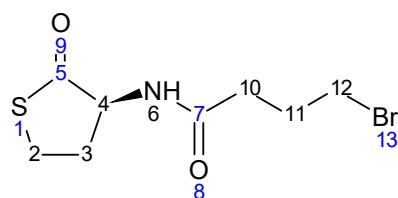
6HHQT4TRI\_C.ESP



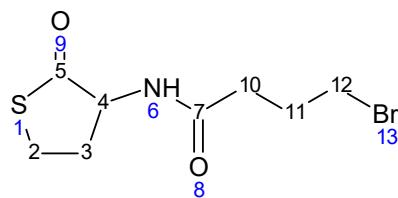
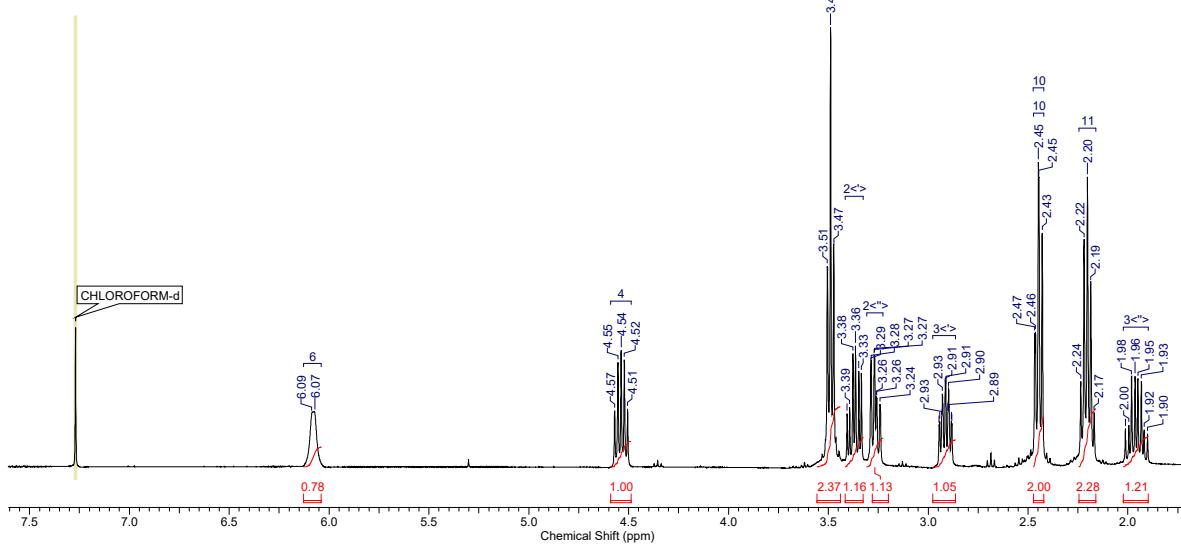
11.15 2-(6-(4-(4-(4-((2,4-Diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)butyl)-1*H*-1,2,3-triazol-1-yl)hexyl)-3-hydroxyquinolin-4(*1H*)-one 89



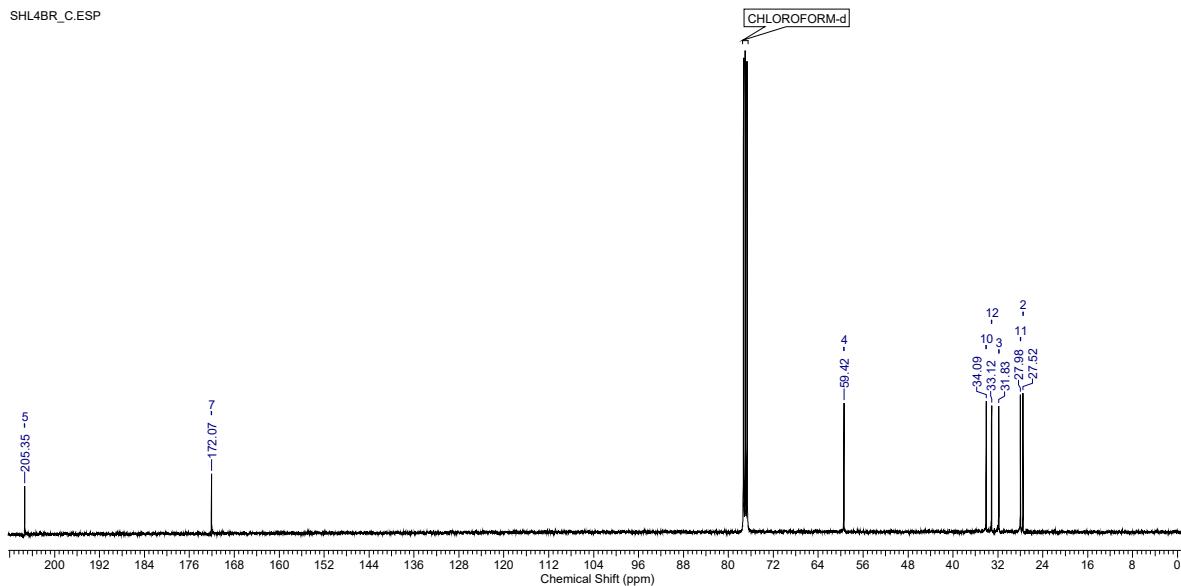
## 11.16 4-Bromo-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 102



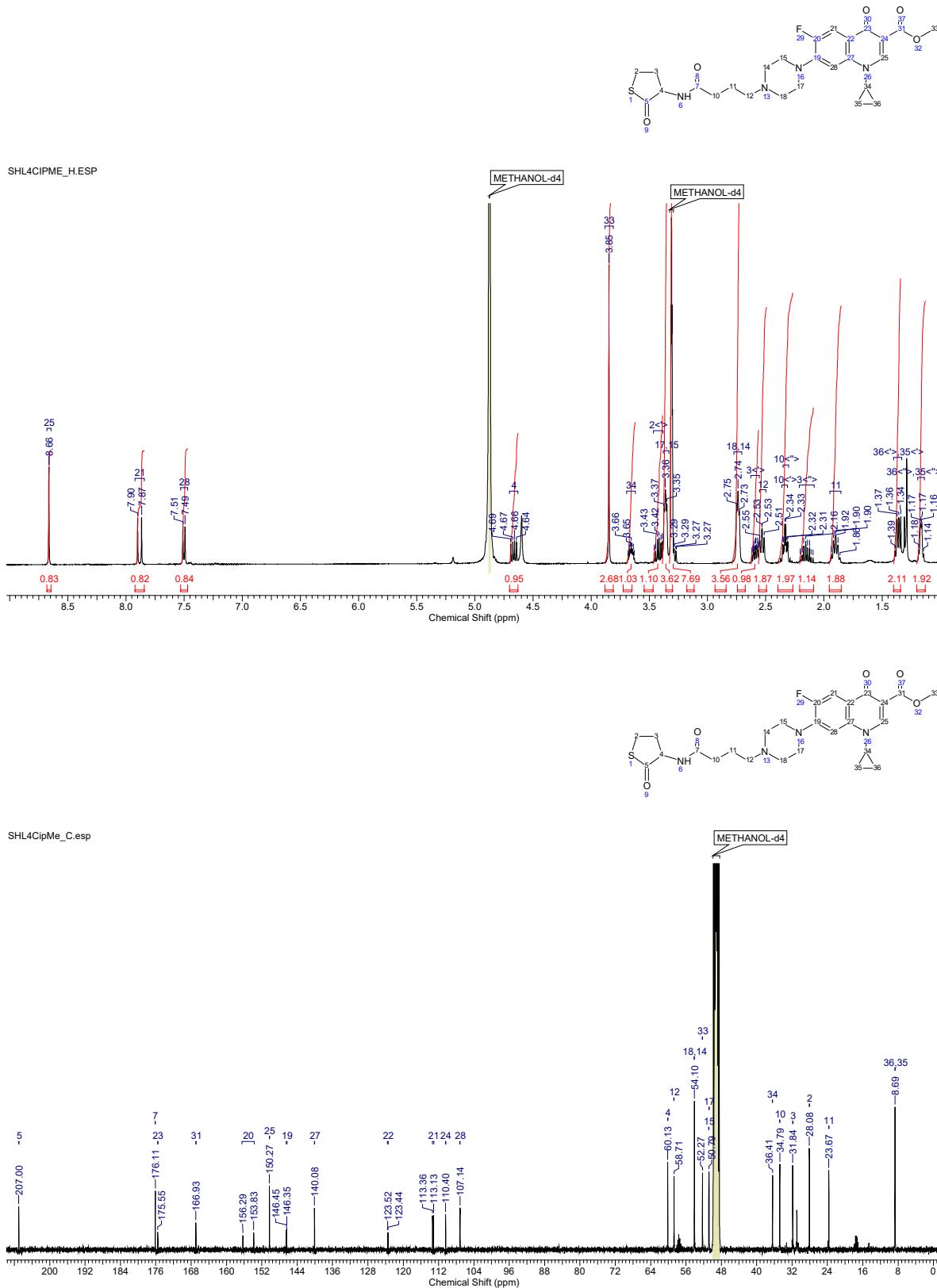
SHL4BR H.ESP



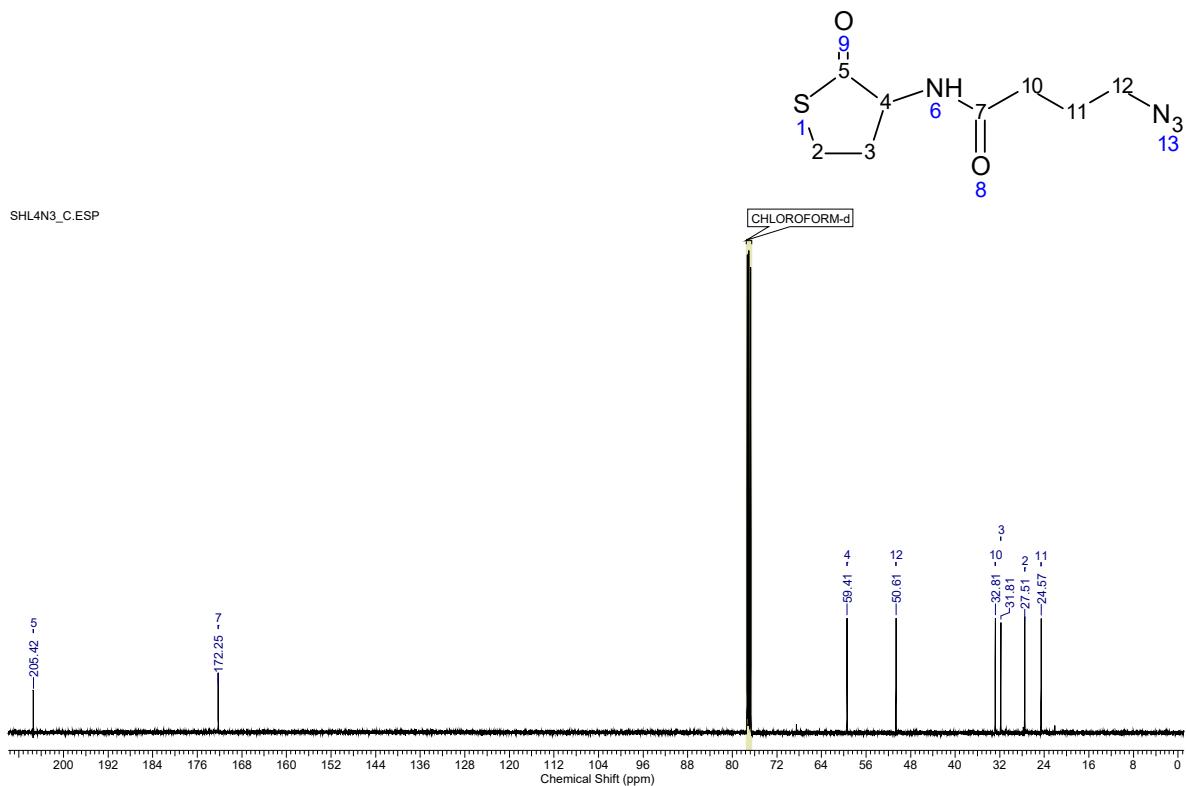
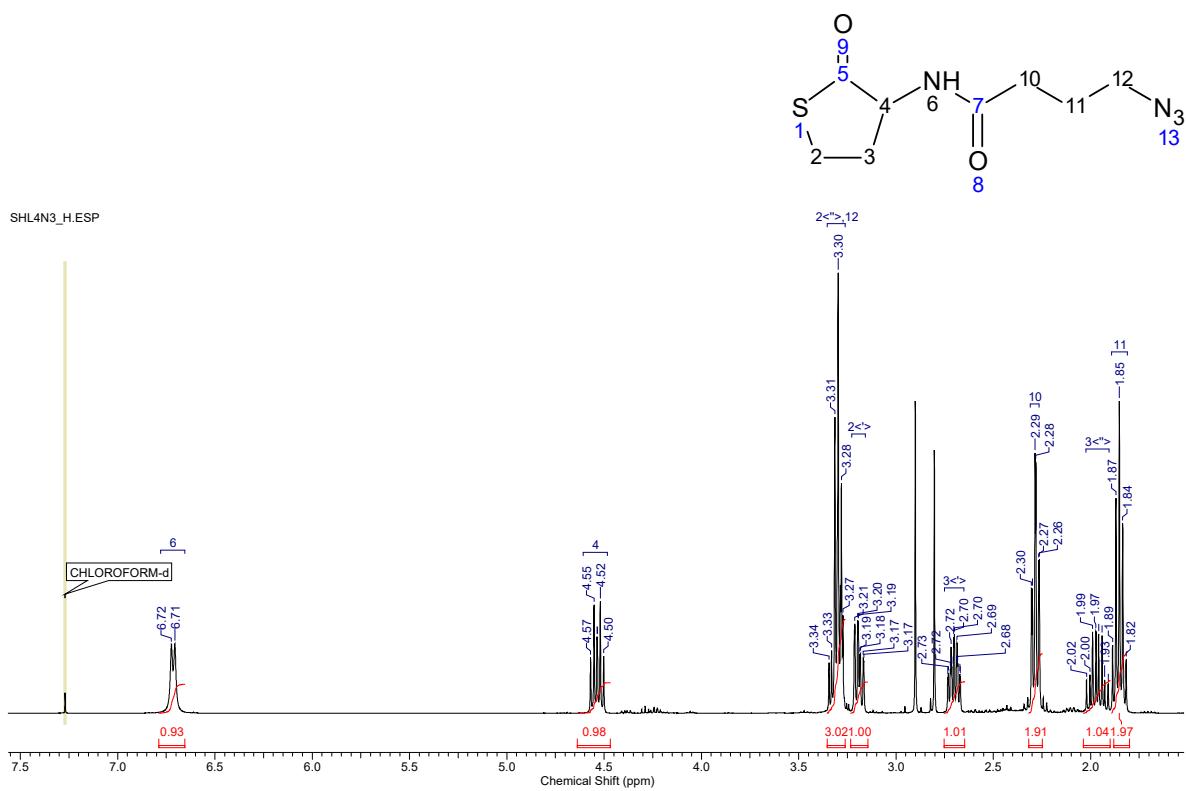
SHL4BR\_C.ESP



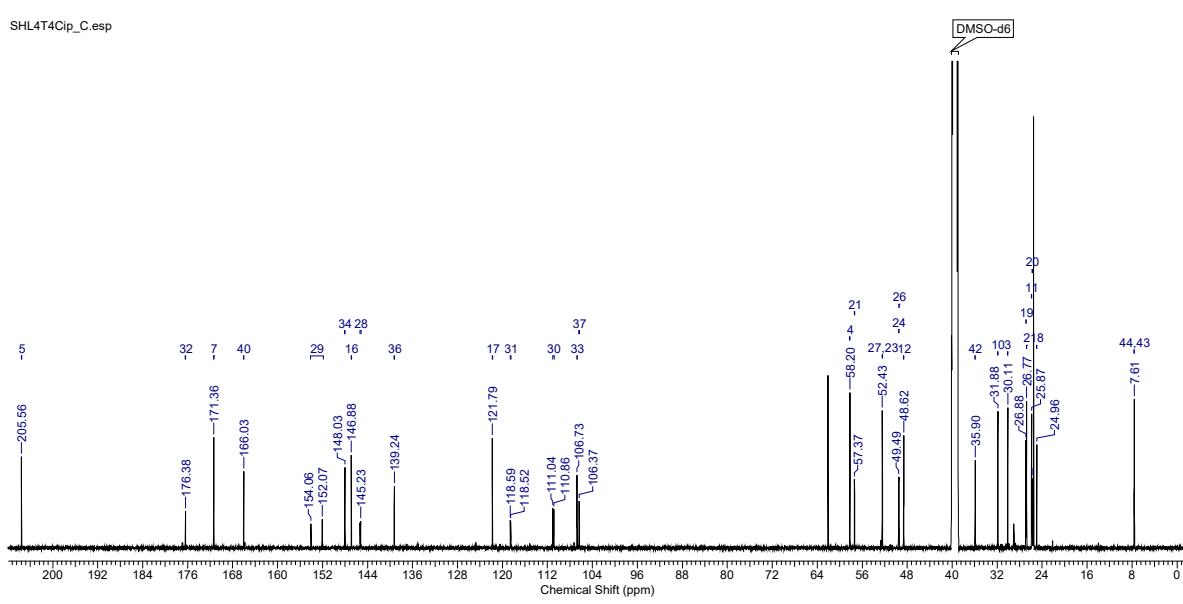
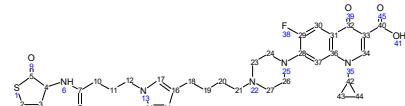
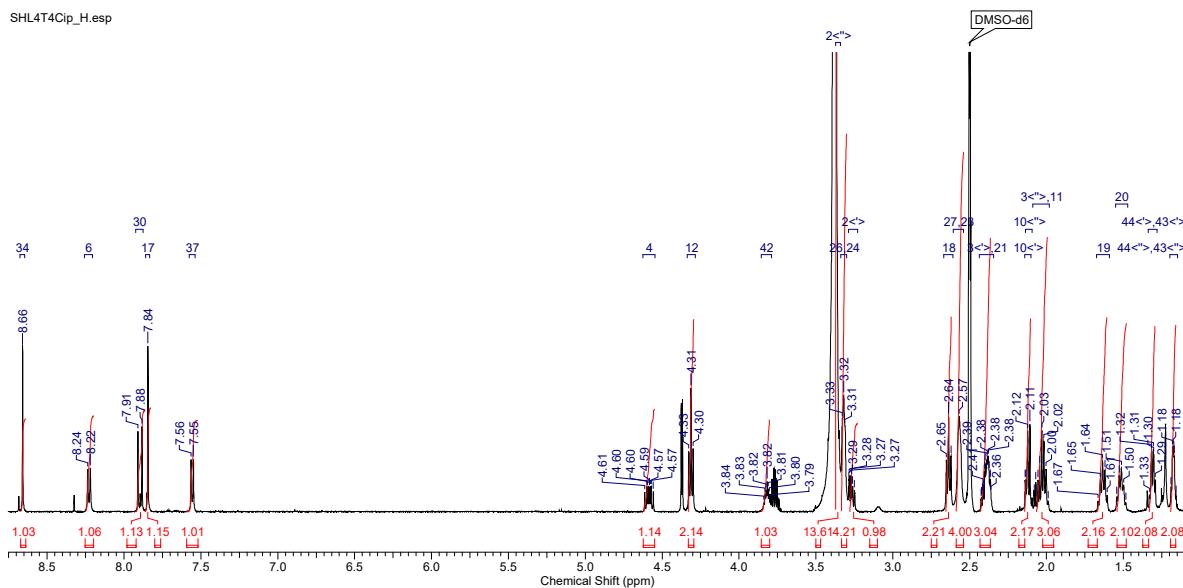
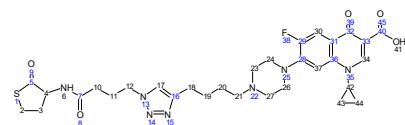
11.17 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 103



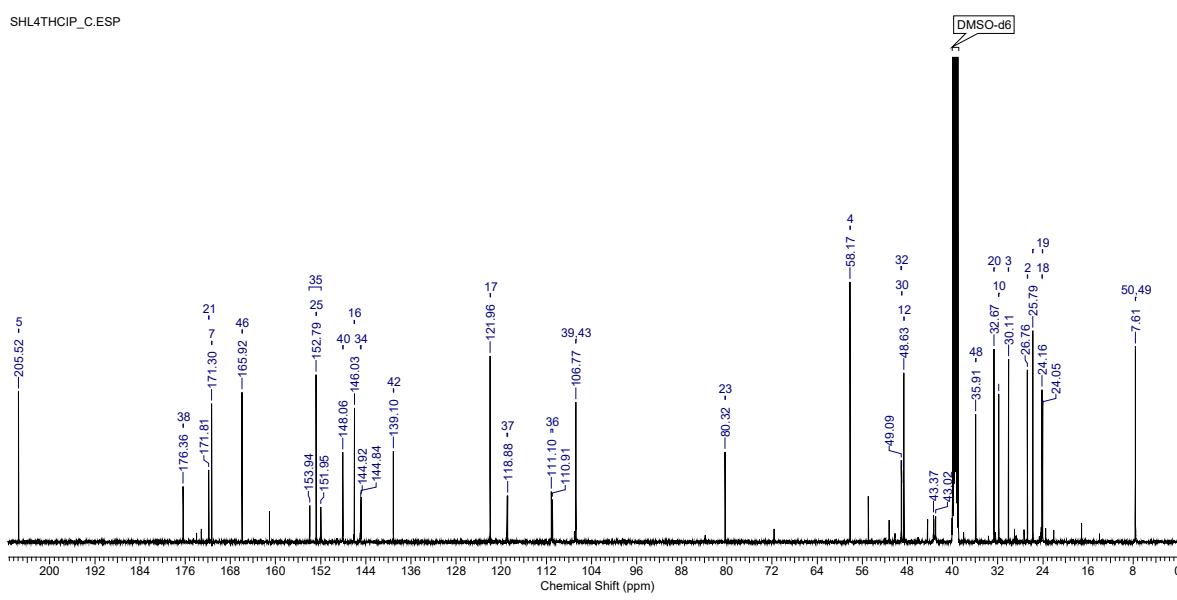
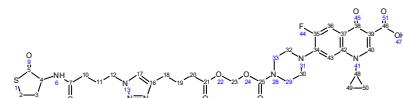
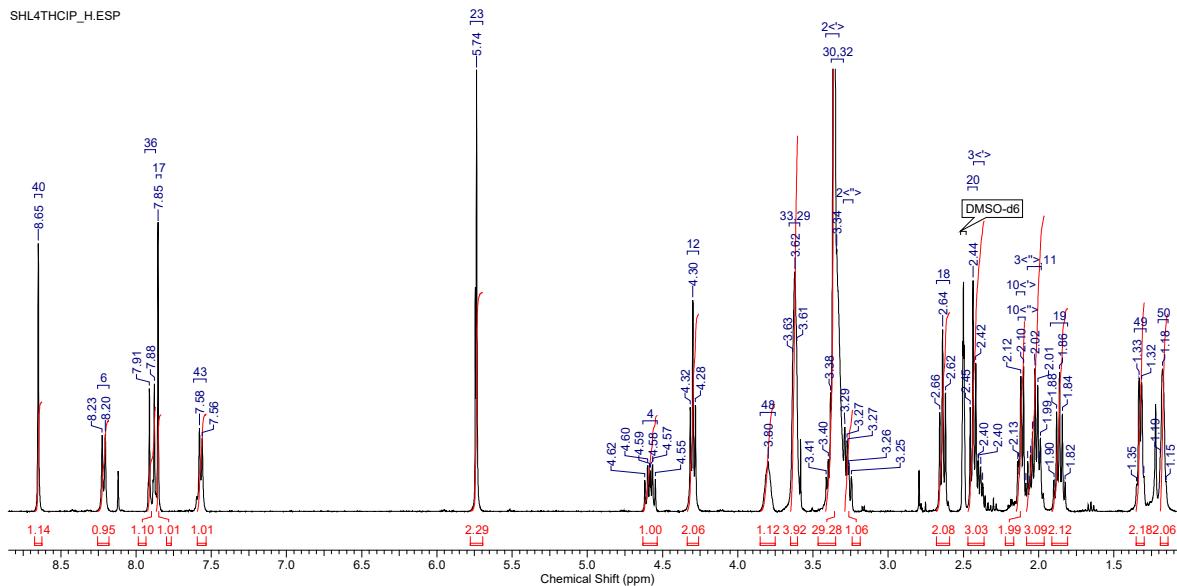
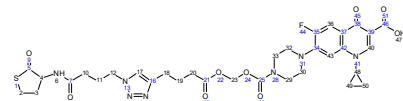
## 11.18 4-Azido-*N*-(2-oxotetrahydrothiophen-3-yl)butanamide 104



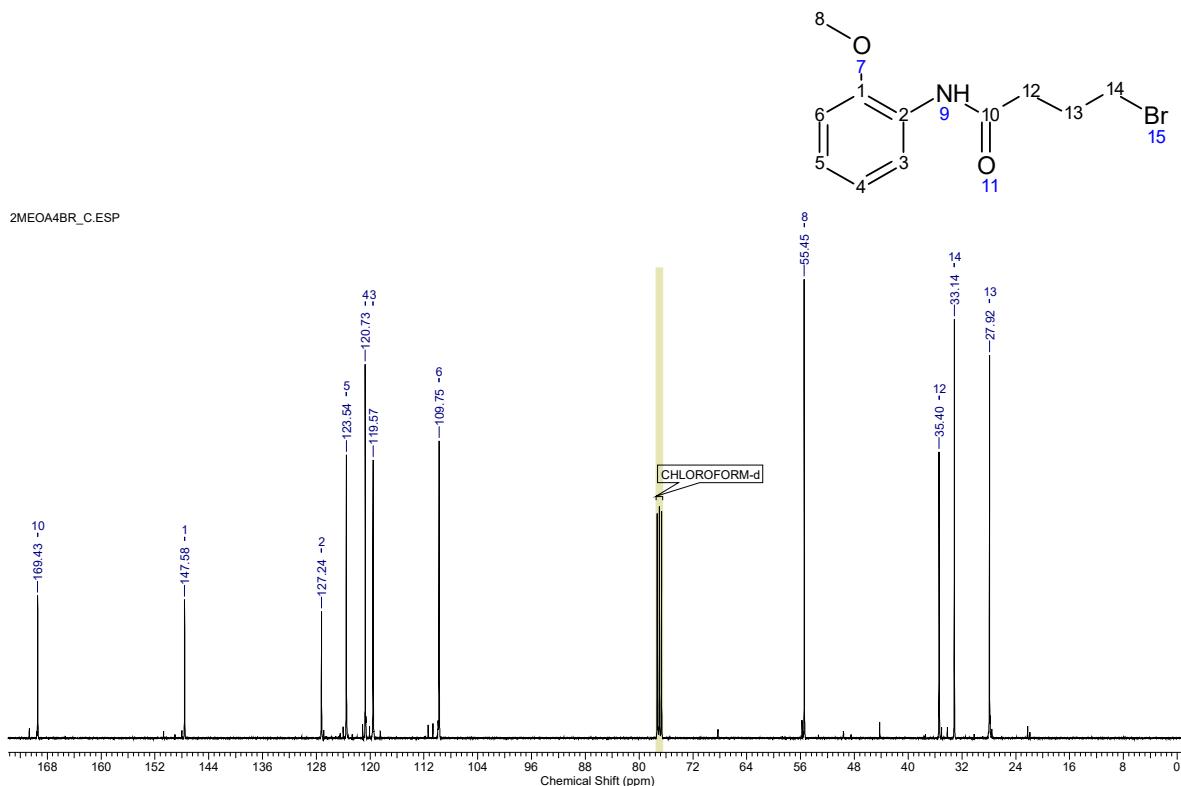
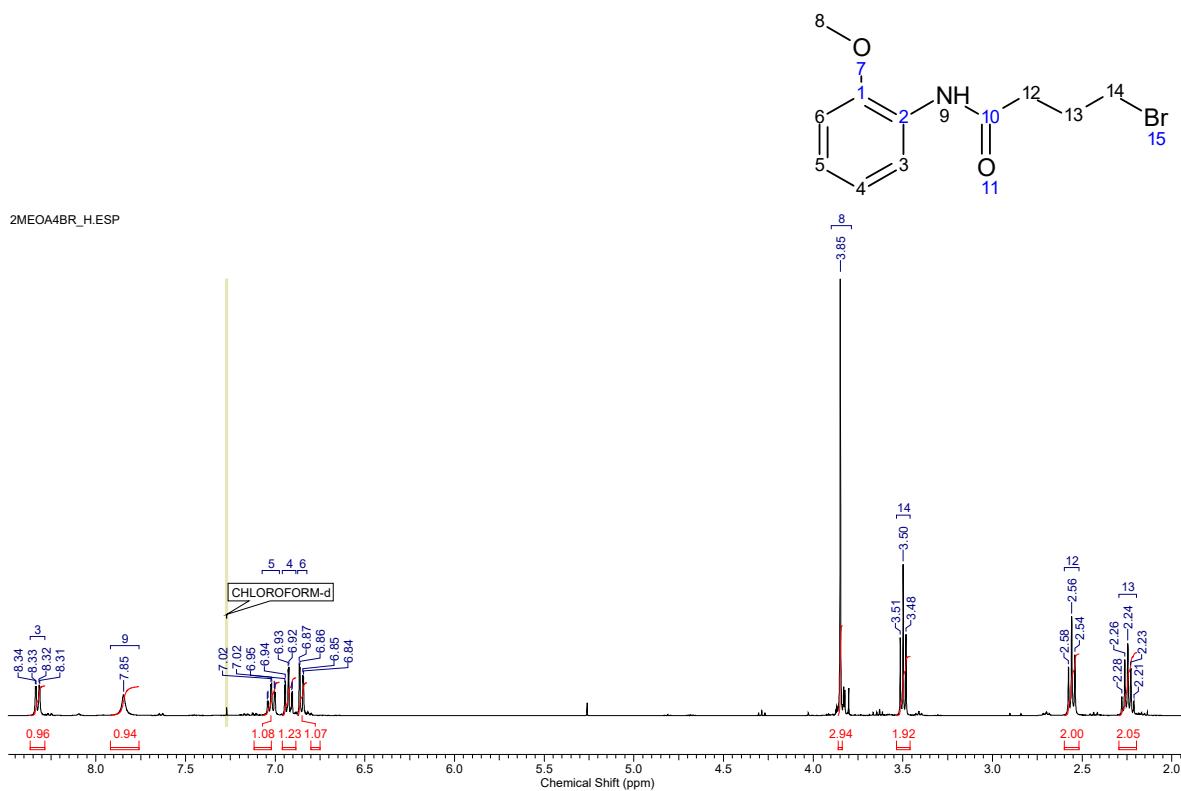
11.19 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 105



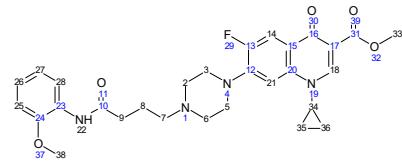
11.20 1-Cyclopropyl-6-fluoro-4-oxo-7-(((4-(1-(4-oxo-4-((2-oxotetrahydrothiophen-3-yl)amino)butyl)-1*H*-1,2,3-triazol-4-yl)butanoyl)oxy)methoxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 106



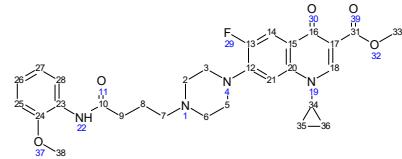
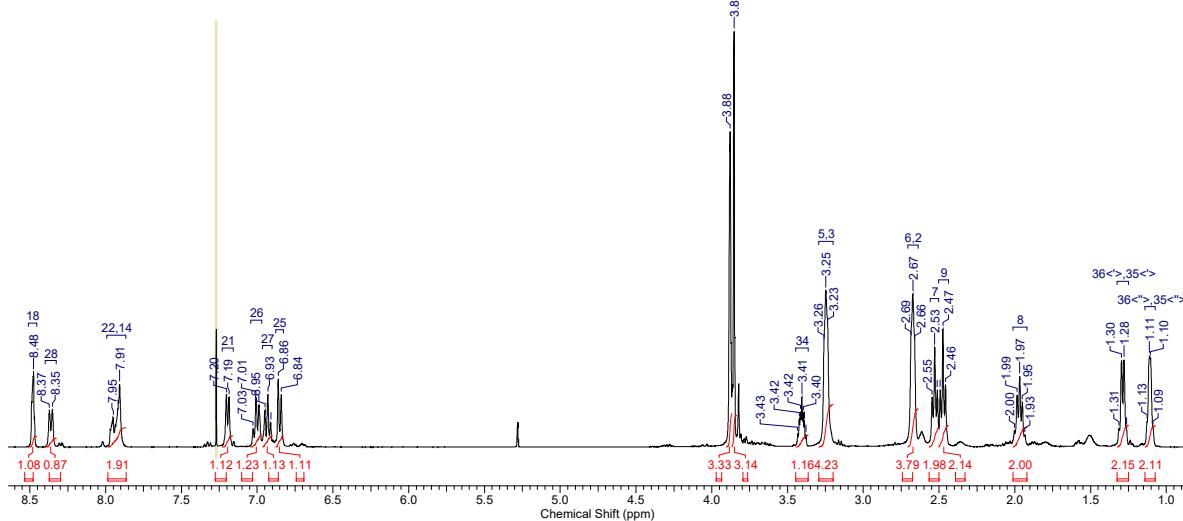
11.21 4-Bromo-N-(2-methoxyphenyl)butanamide 108



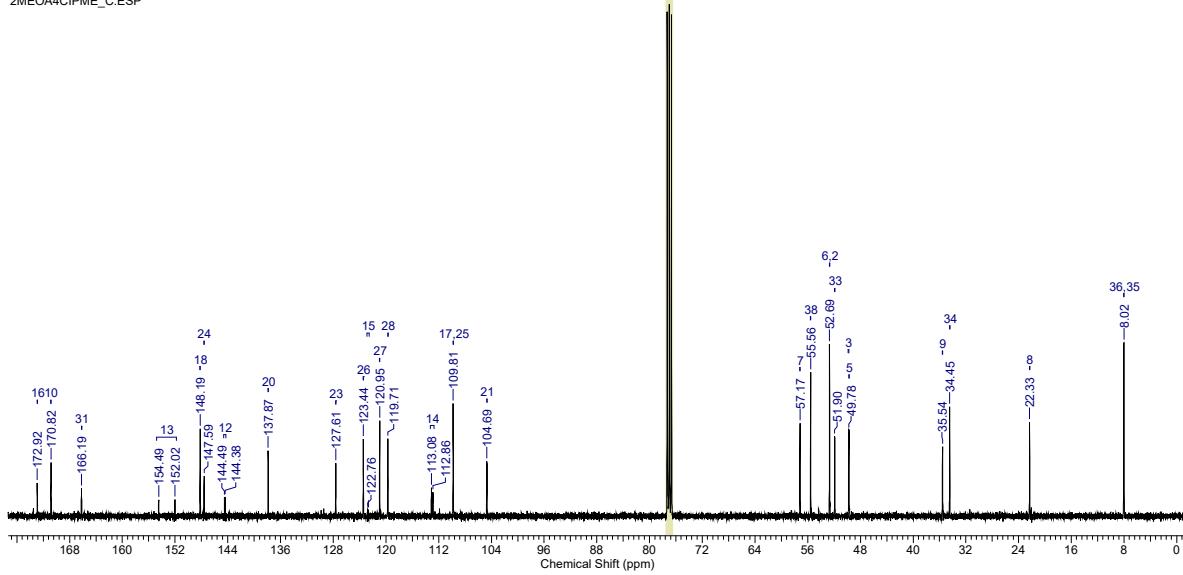
11.22 Methyl 1-cyclopropyl-6-fluoro-7-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 109



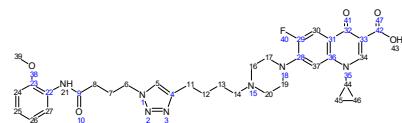
2MEOA4CIPME\_H.ESP



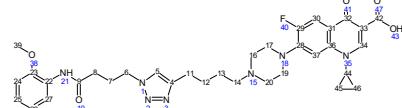
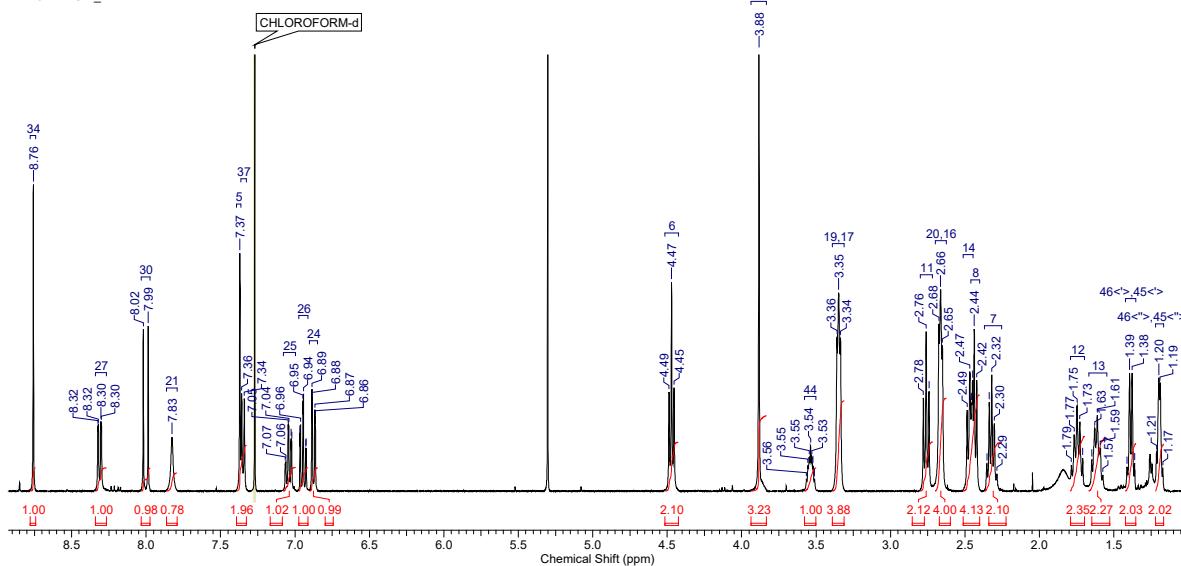
2MEOA4CIPME\_C.ESP



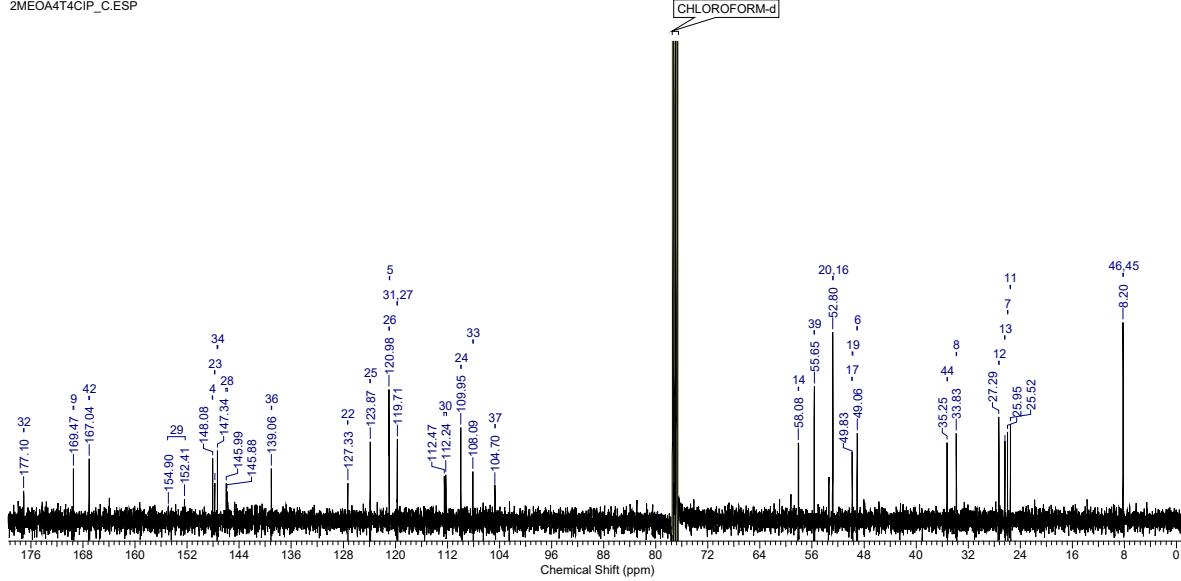
11.23 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((2-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 111



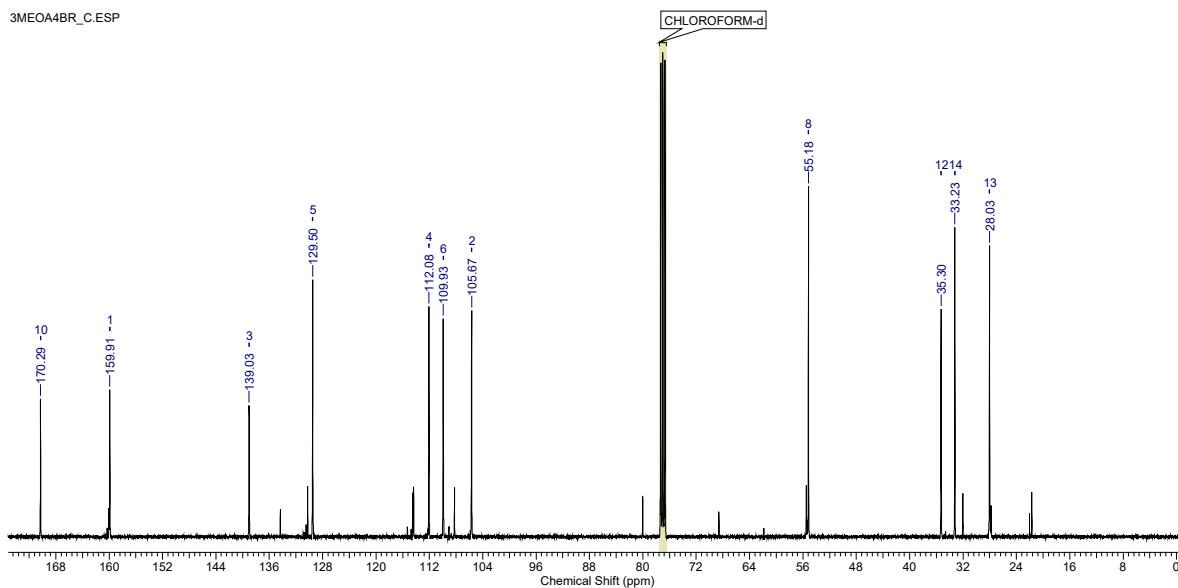
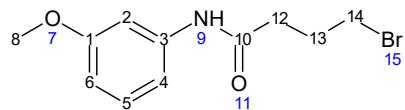
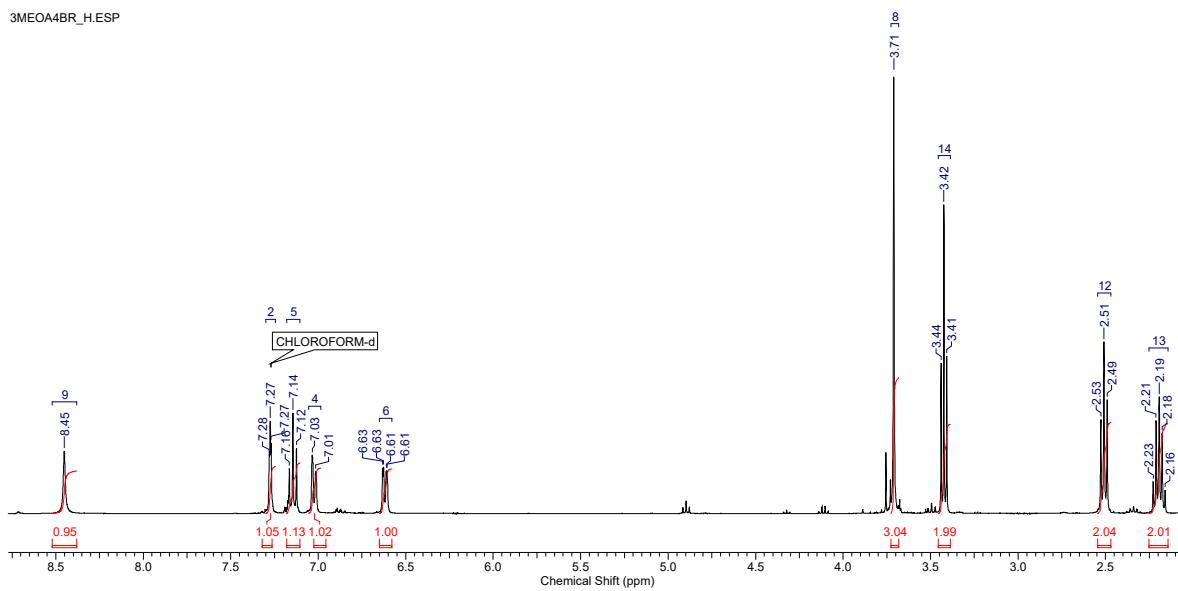
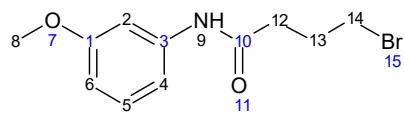
2MEOA4T4CIP\_H\_ESP



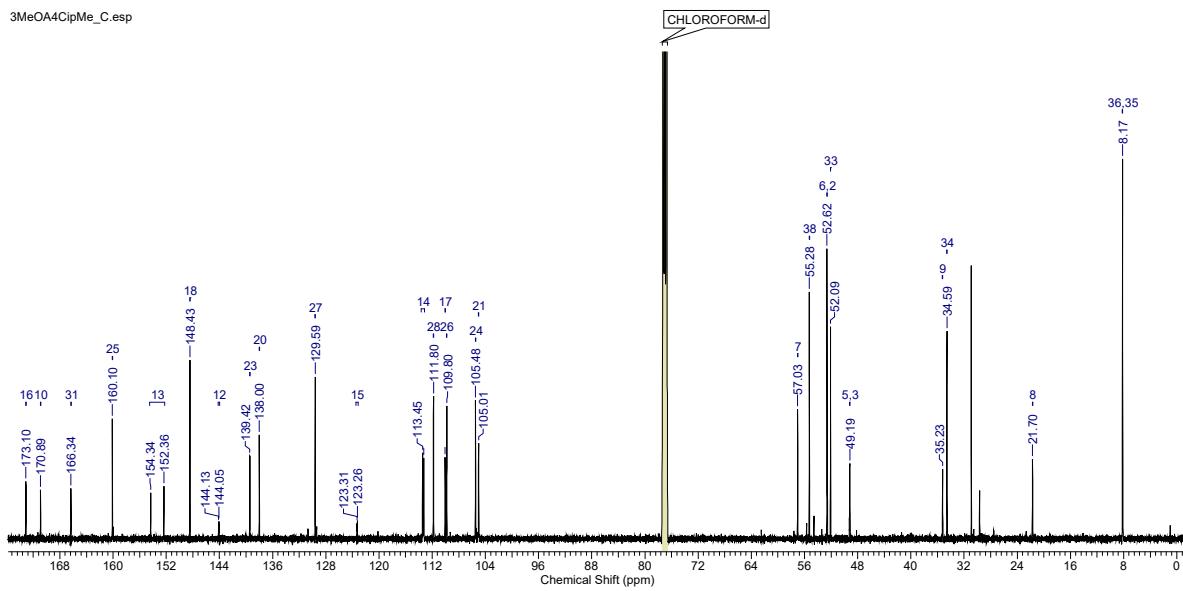
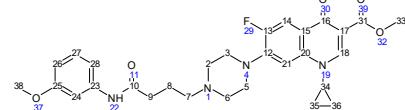
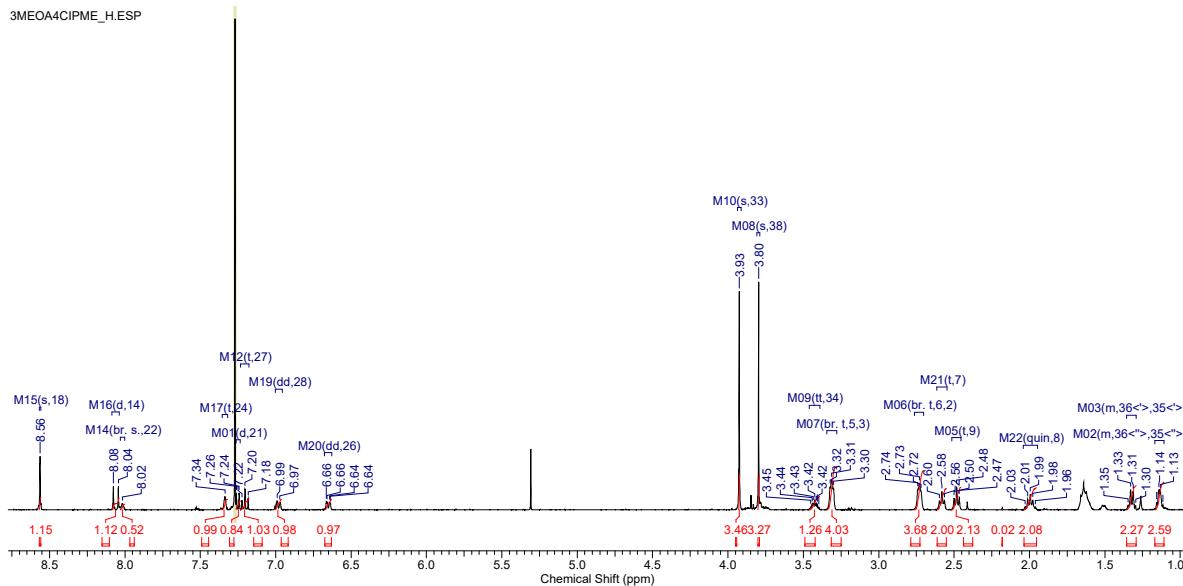
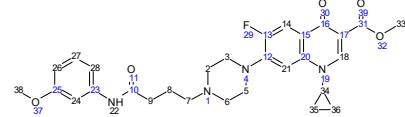
2MEOA4T4CIP\_C\_ESP



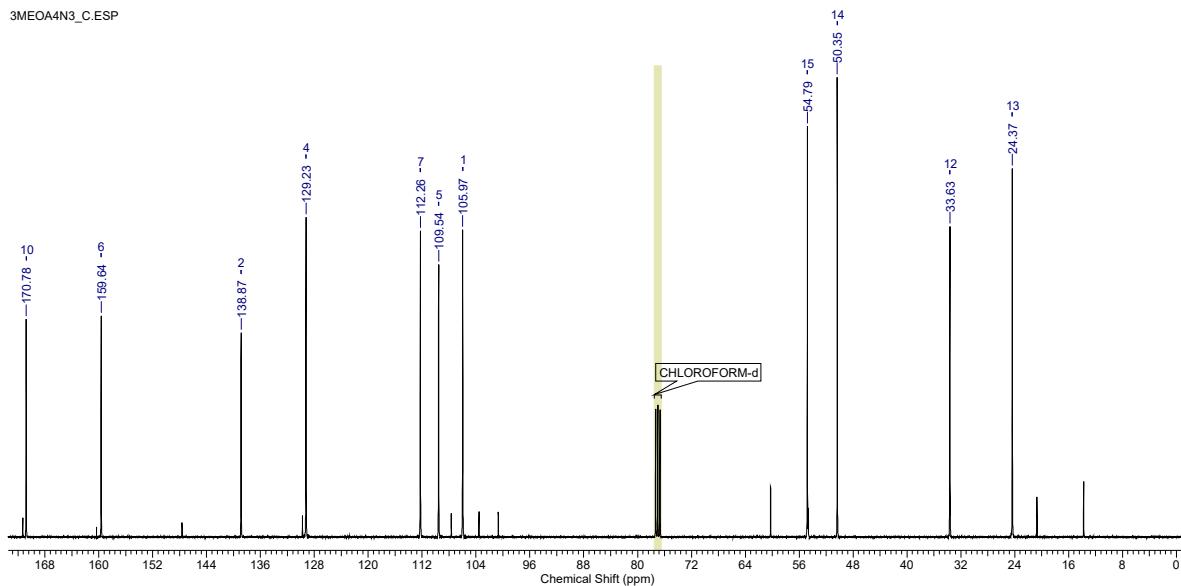
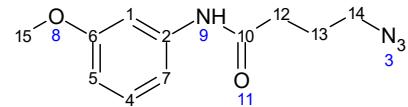
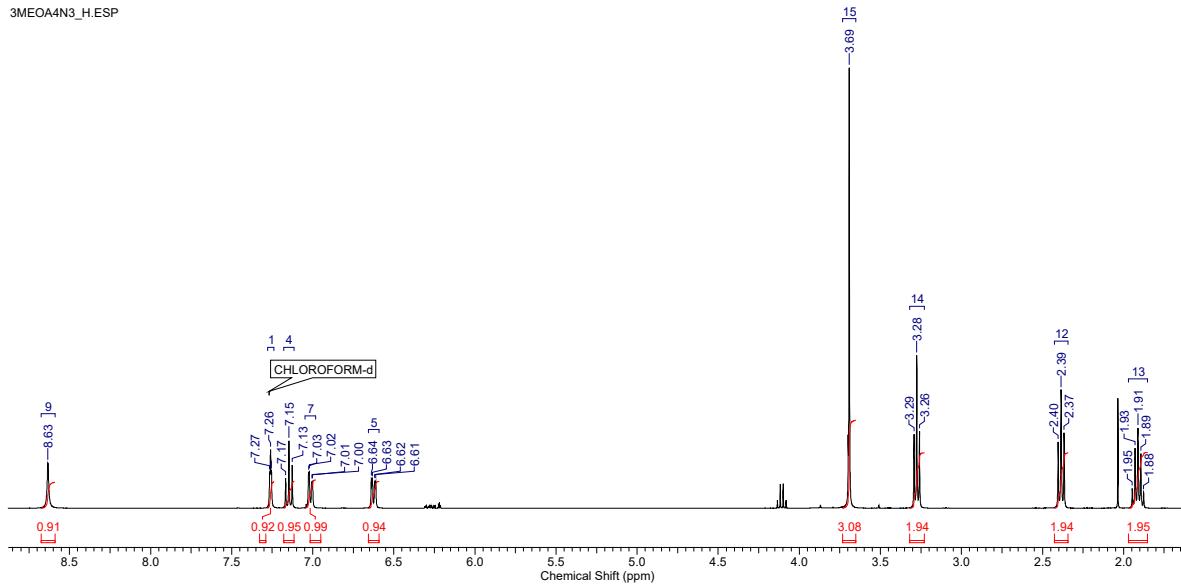
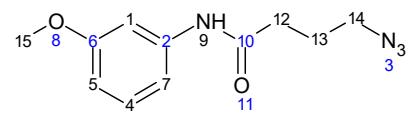
## 11.24 4-Bromo-*N*-(3-methoxyphenyl)butanamide 113



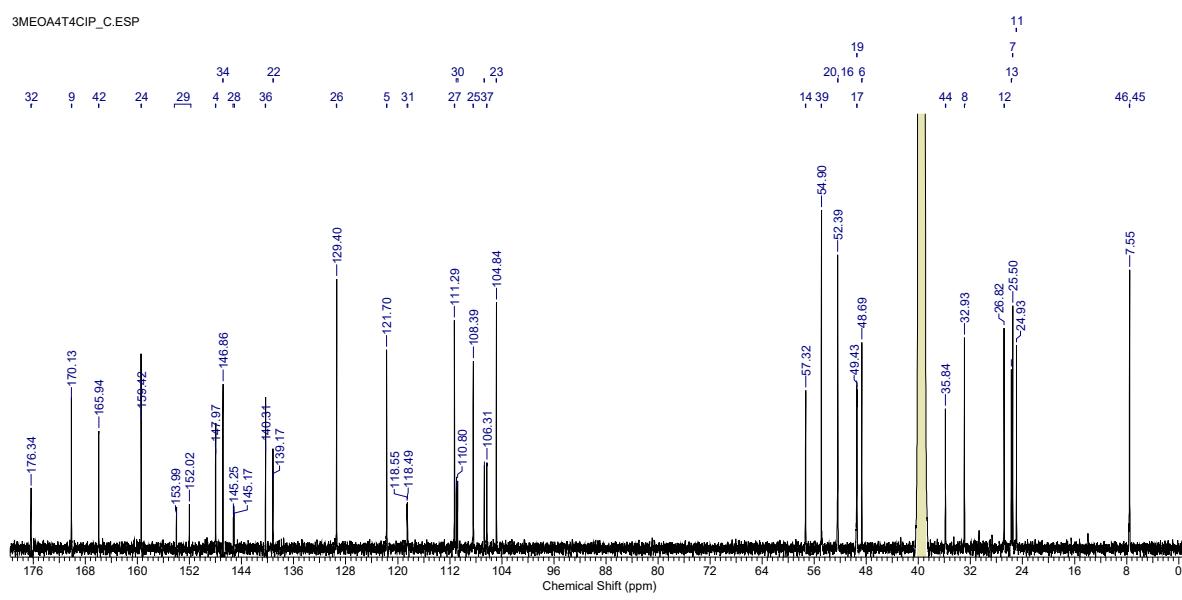
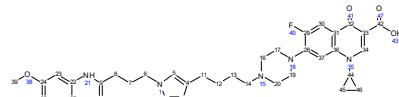
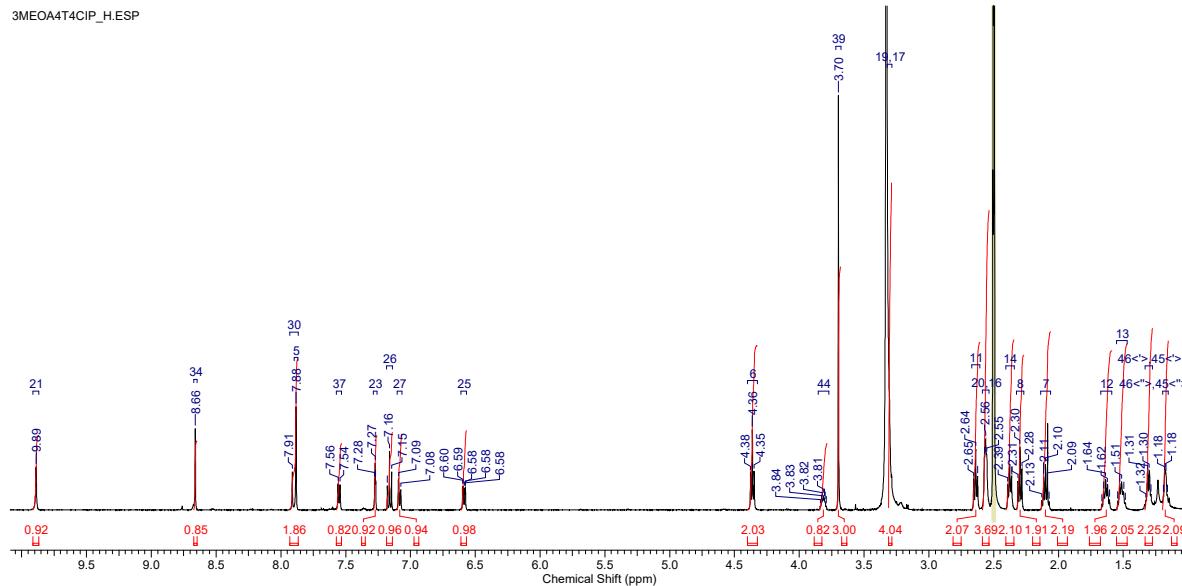
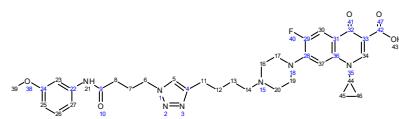
11.25 Methyl 1-cyclopropyl-6-fluoro-7-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 114



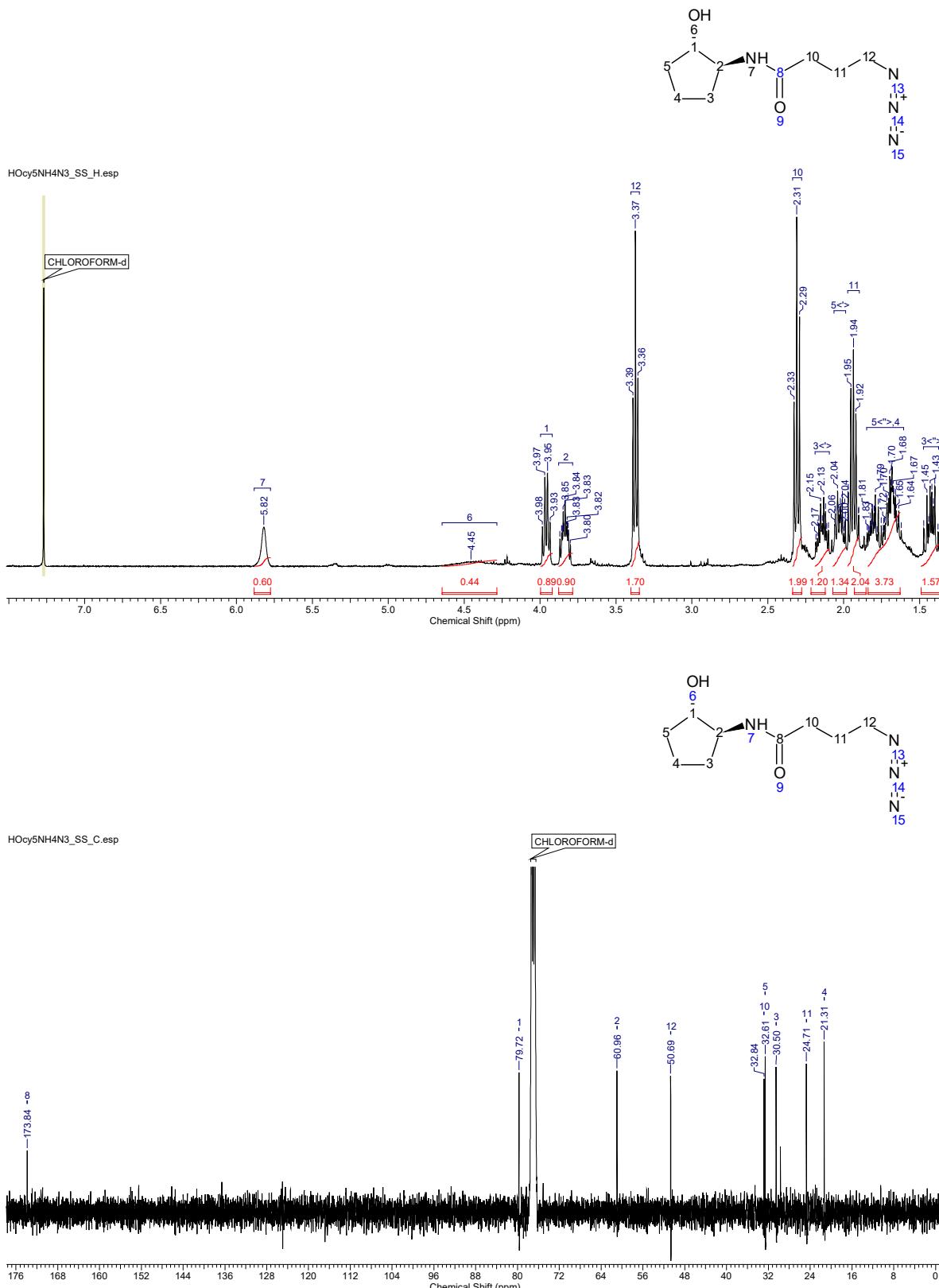
### 11.26 4-Azido-*N*-(3-methoxyphenyl)butanamide 115



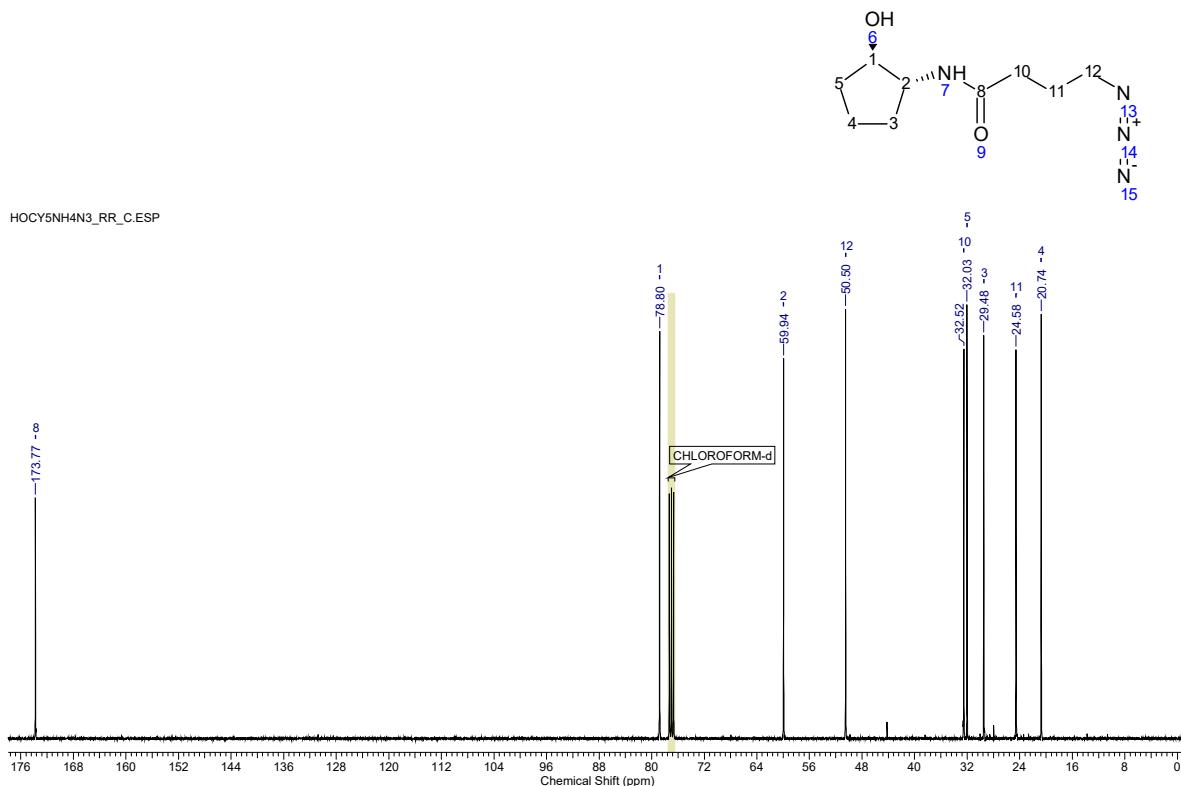
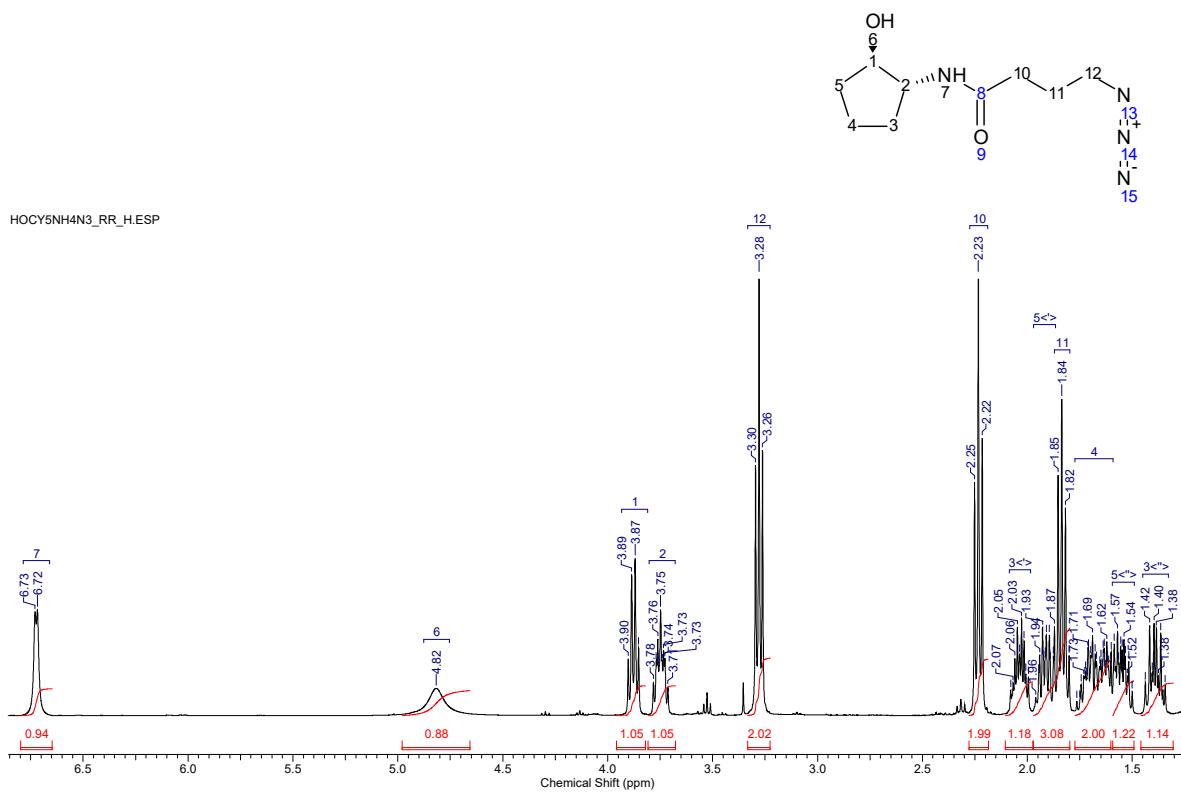
11.27 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-((3-methoxyphenyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 116



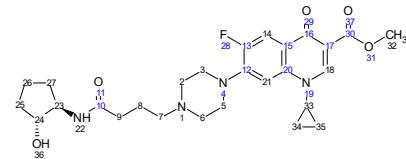
11.28 4-Azido-*N*-((1*S*,2*S*)-2-hydroxycyclopentyl)butanamide 125



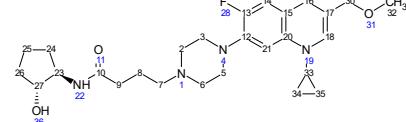
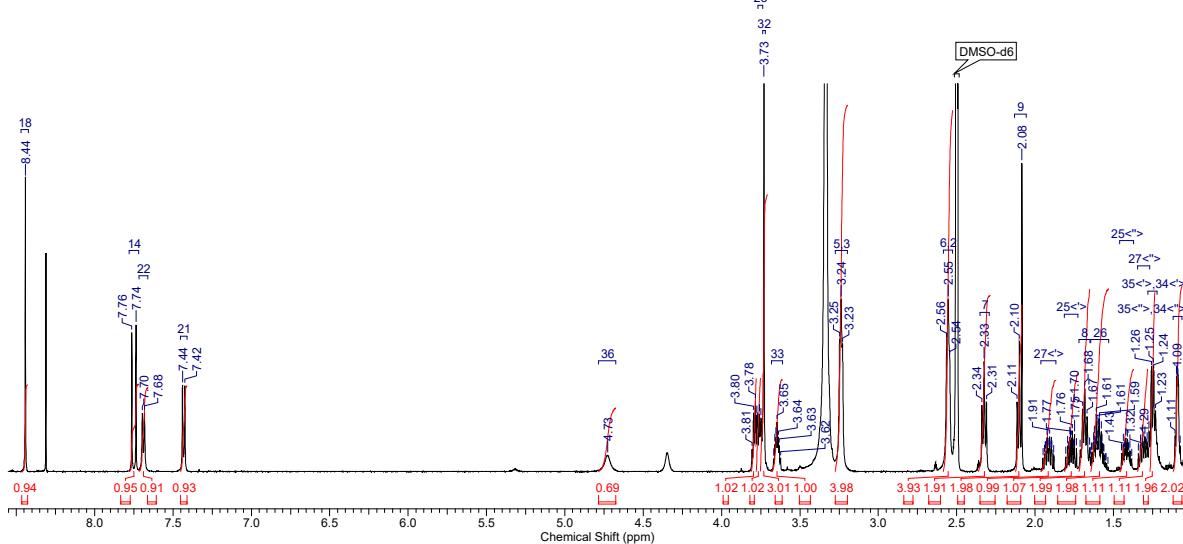
11.29 4-Azido-*N*-(*1R,2R*)-2-hydroxycyclopentyl)butanamide 126



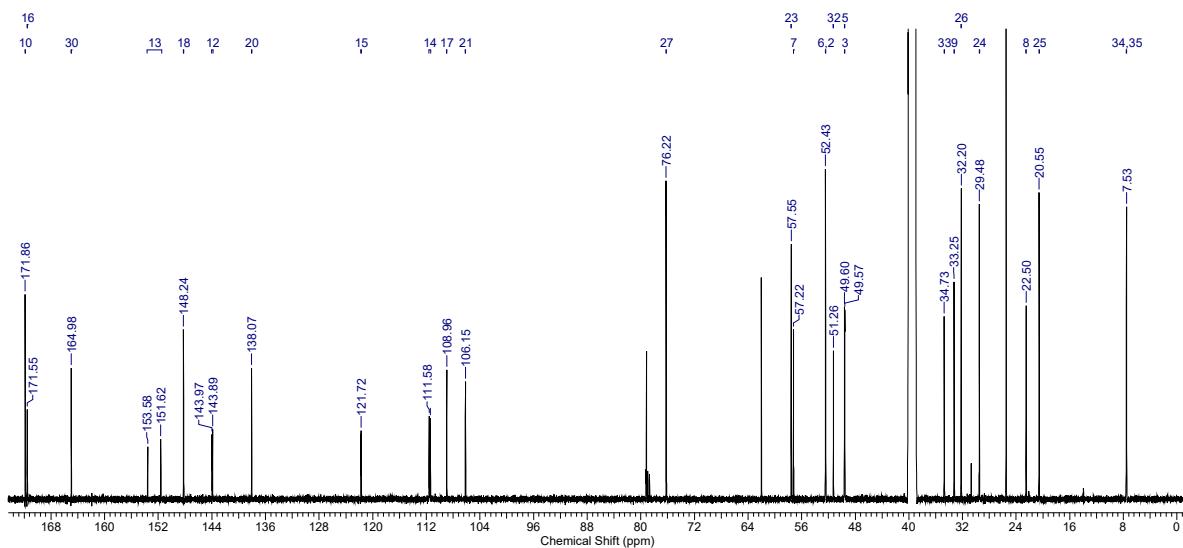
11.30 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 127



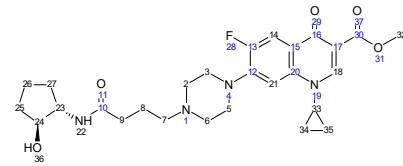
HOcy5NH4CipMe\_SS\_H.esp



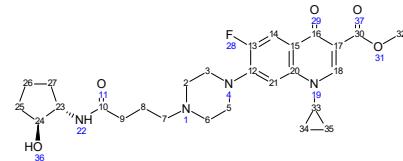
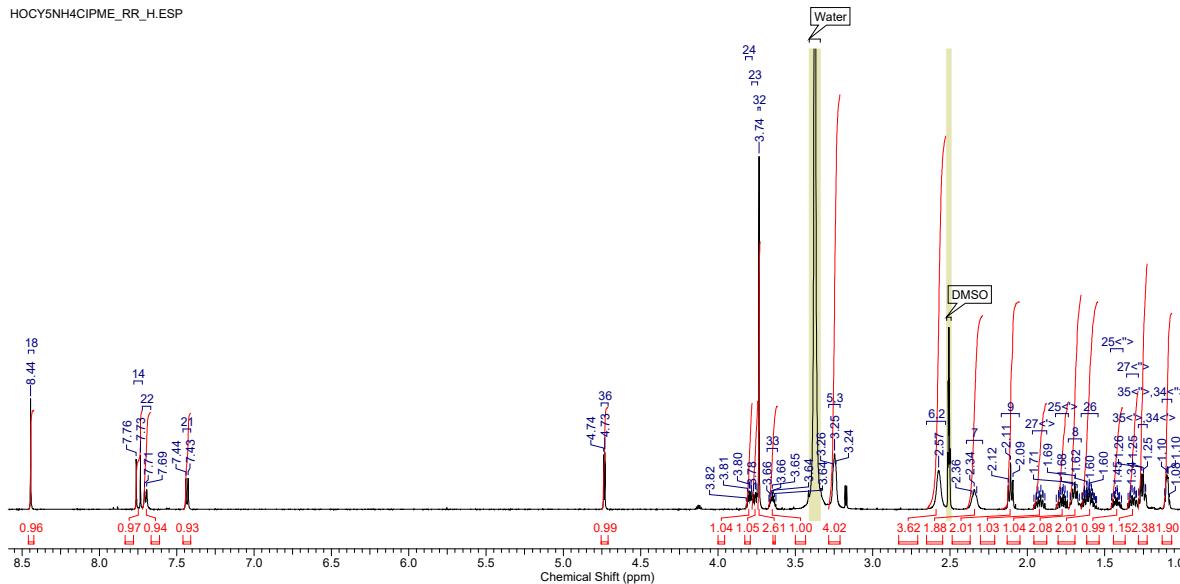
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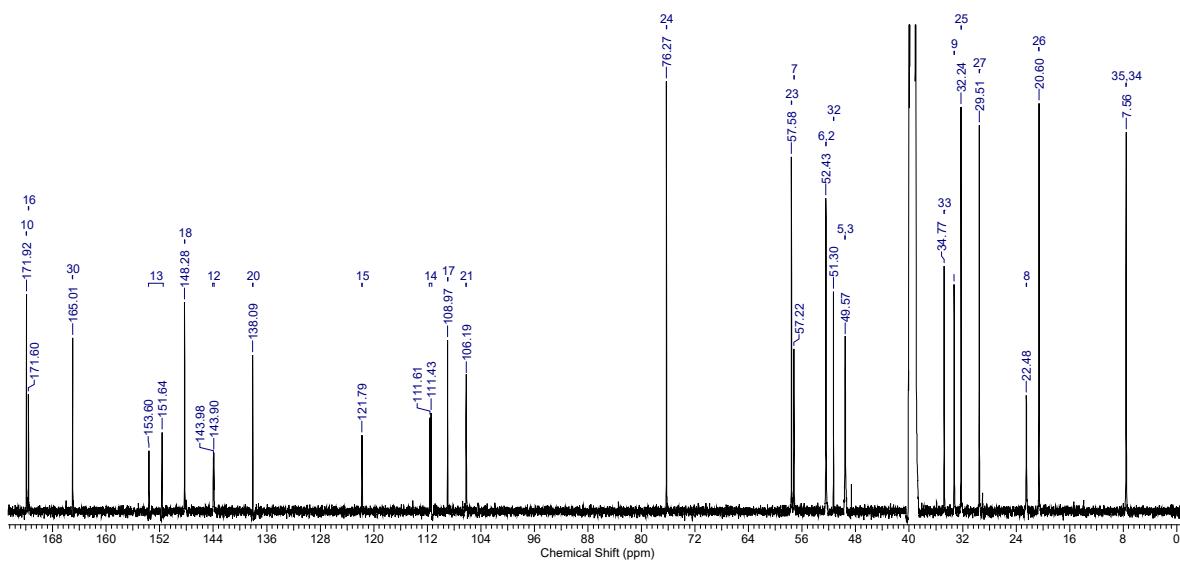
11.31 Methyl 1-cyclopropyl-6-fluoro-7-(4-((1*R*,2*R*)-2-hydroxycyclopentyl)amino)-4-oxobutyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 128



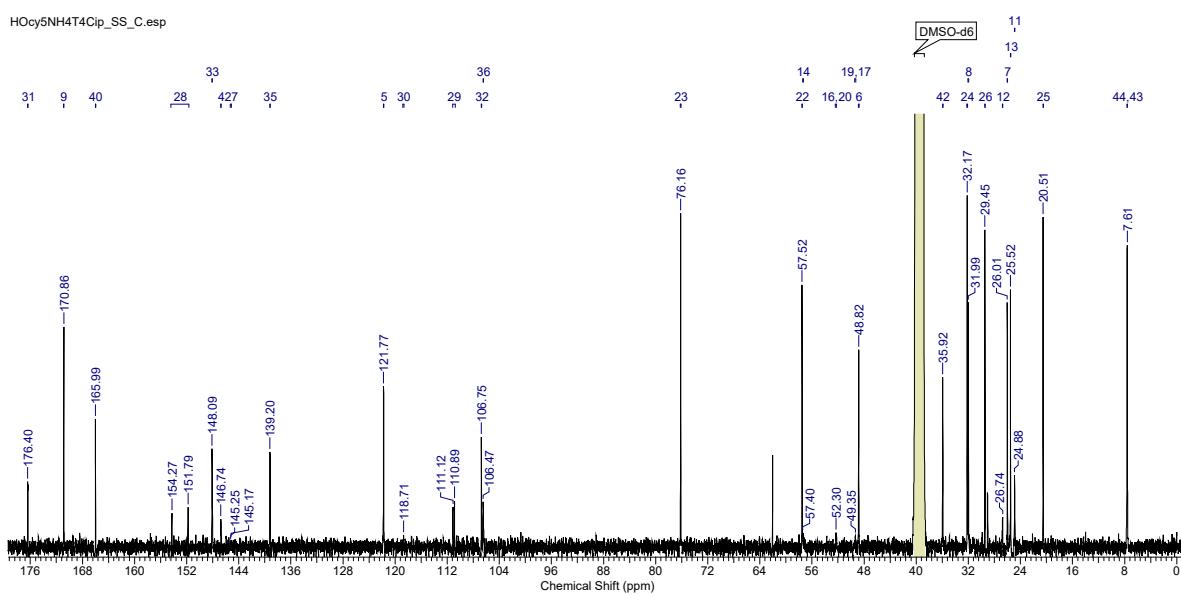
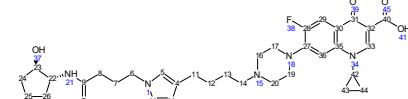
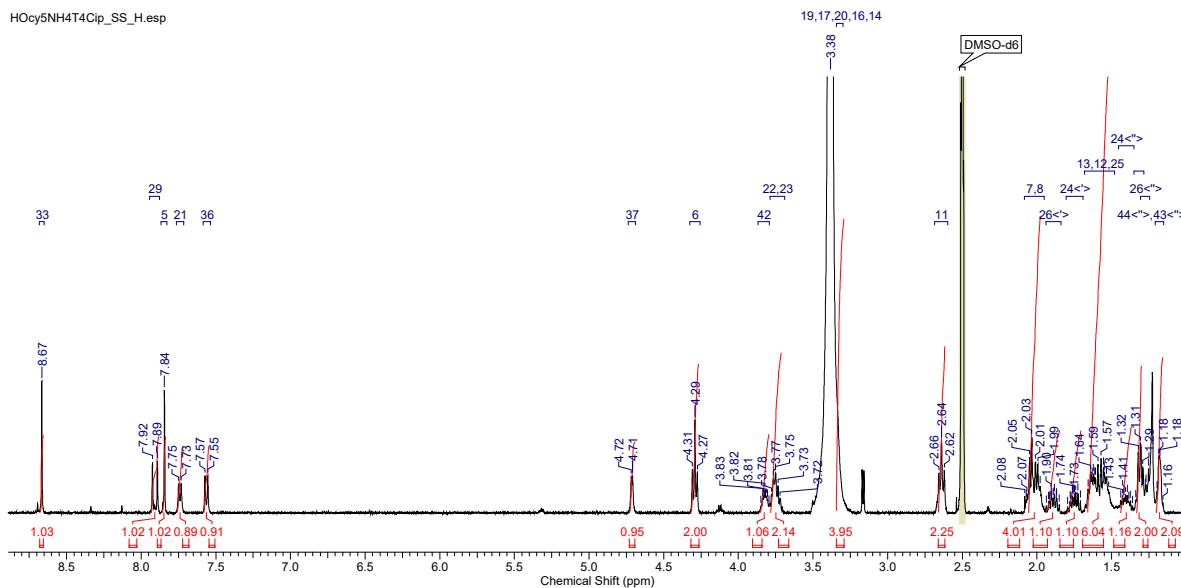
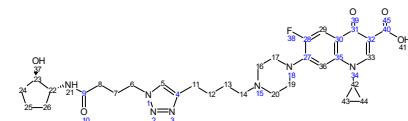
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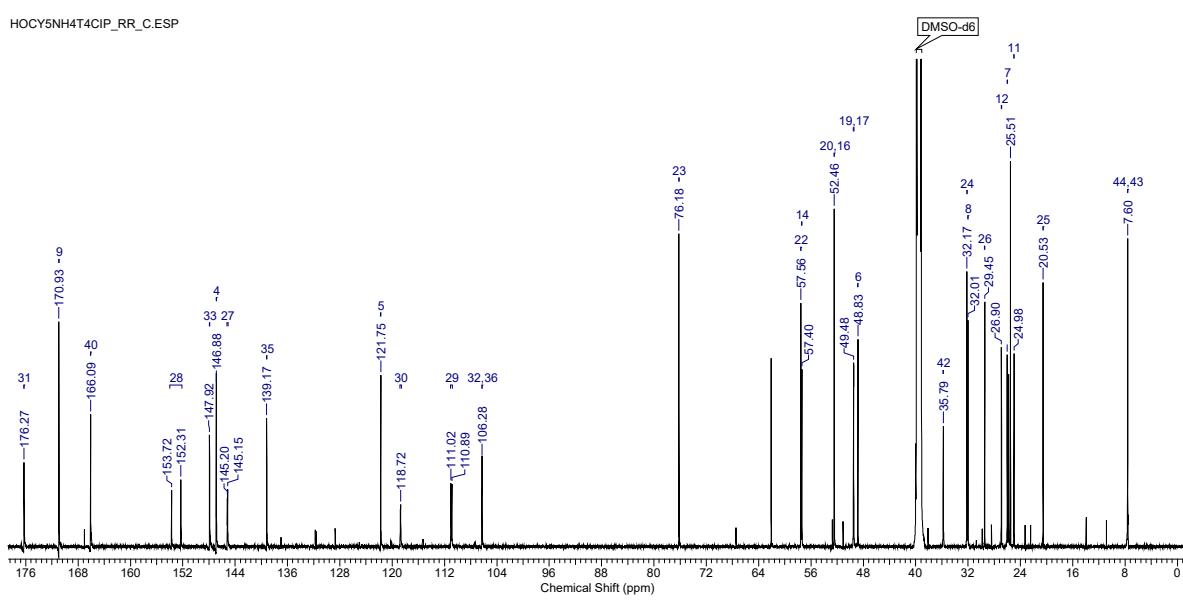
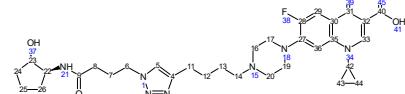
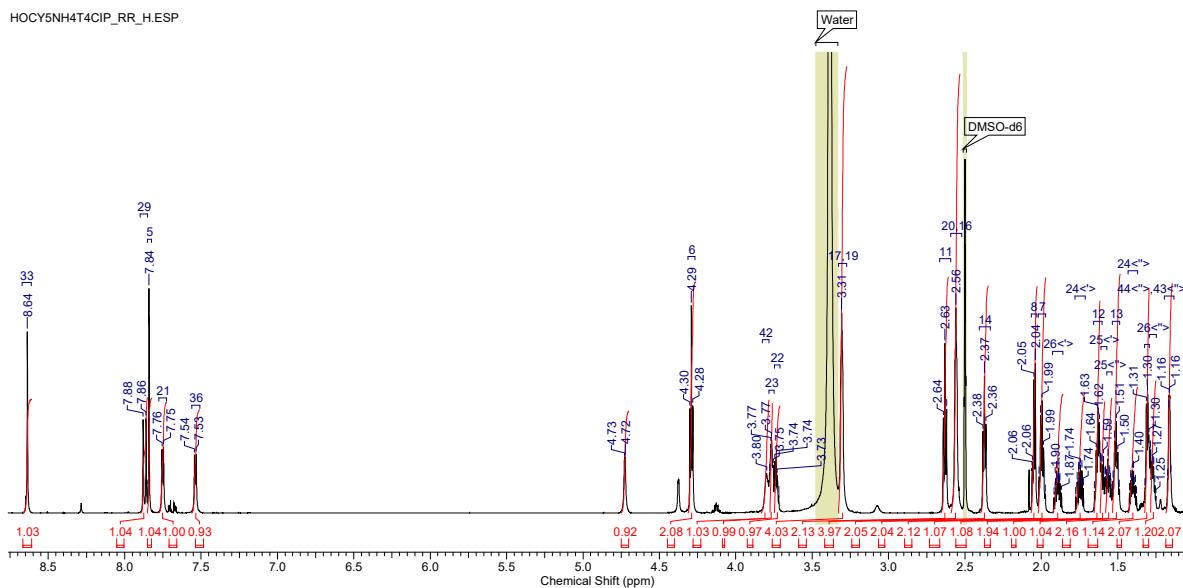
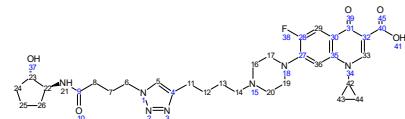
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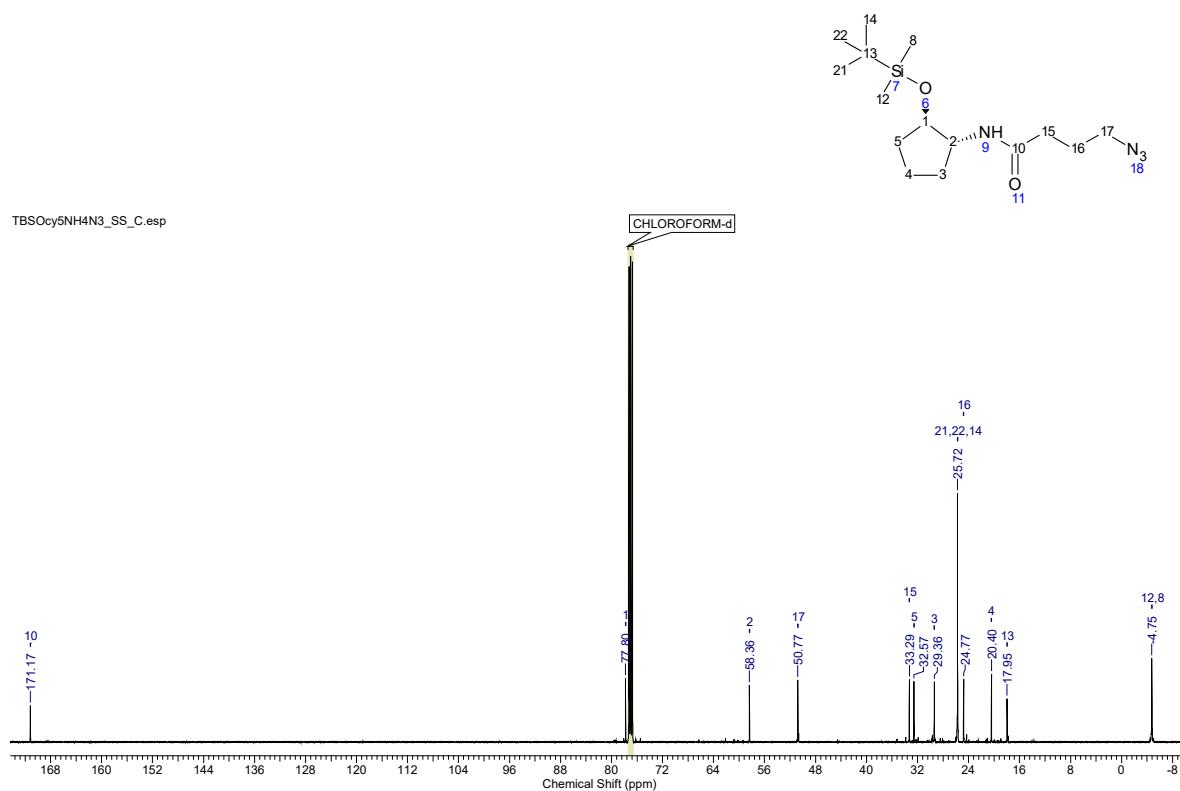
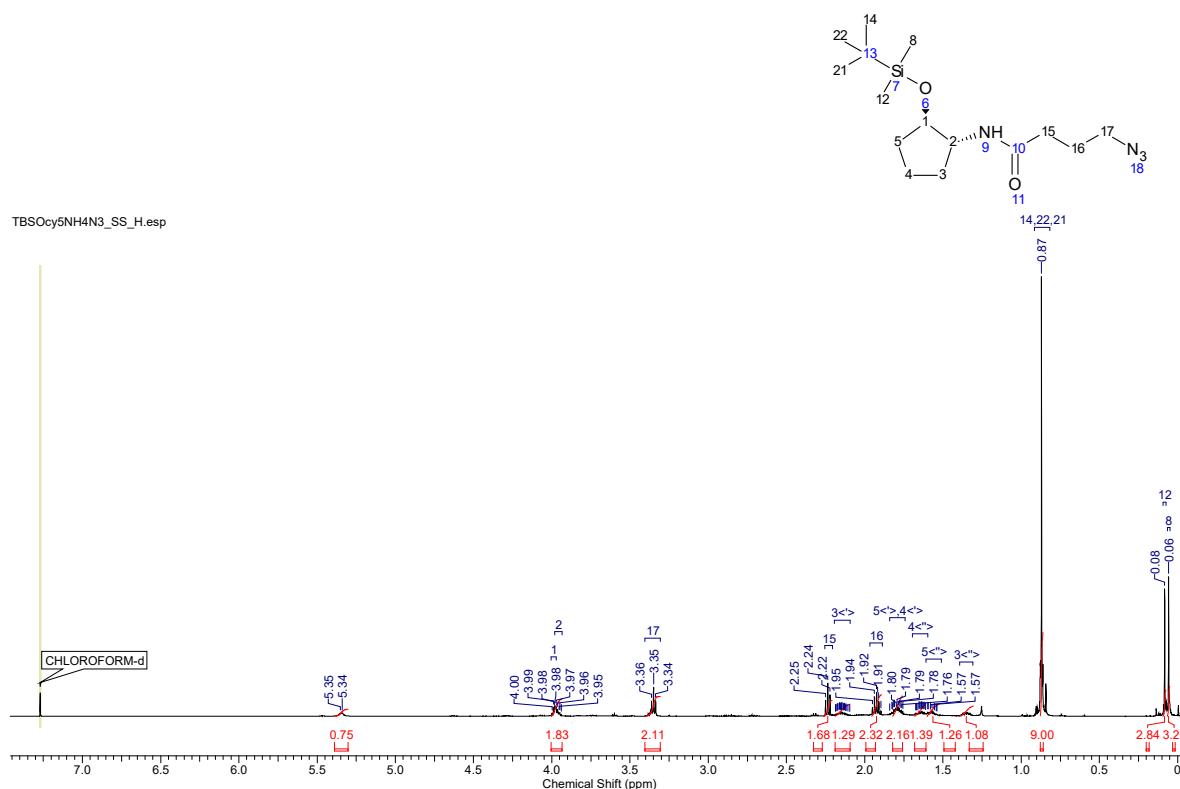
11.32 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((1*S*,2*S*)-2-hydroxycyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 130



11.33 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-dihydroquino-line-3-carboxylic acid 131



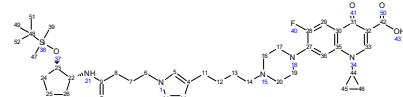
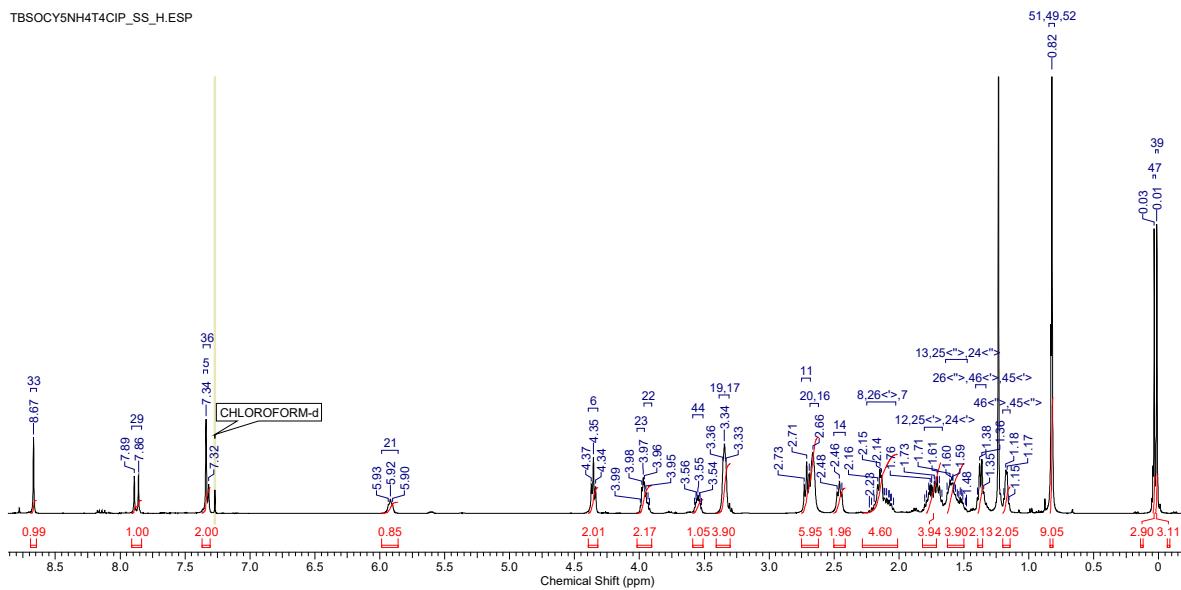
11.34 4-Azido-*N*-((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)butanamide  
136



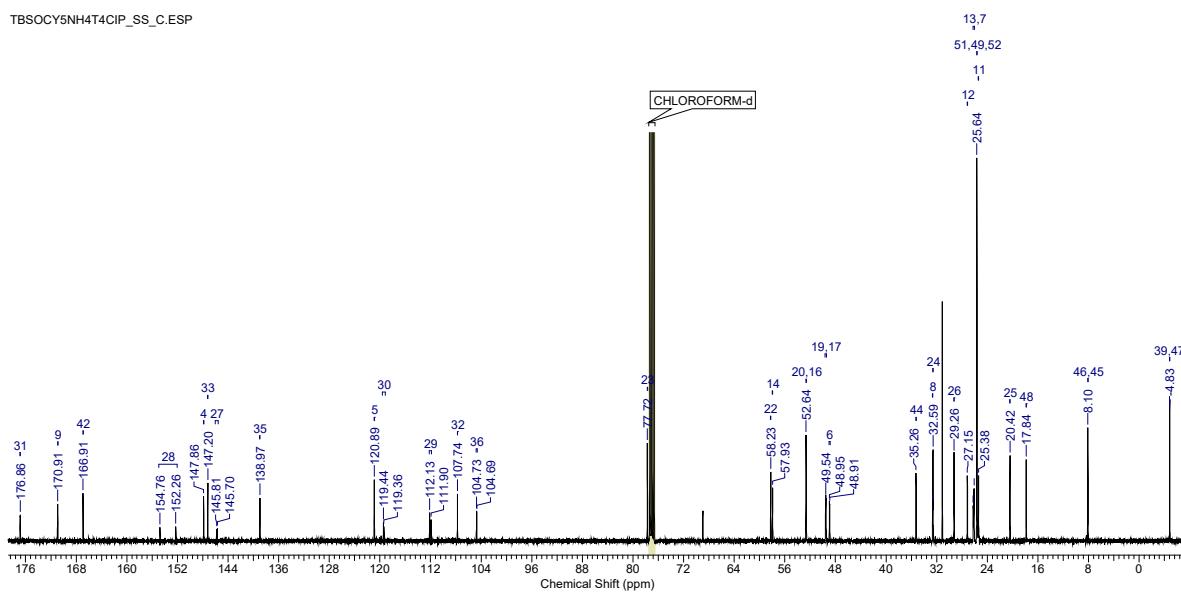
11.35 7-(4-(1-(4-(((1*S*,2*S*)-2-((*tert*-butyldimethylsilyl)oxy)cyclopentyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 140



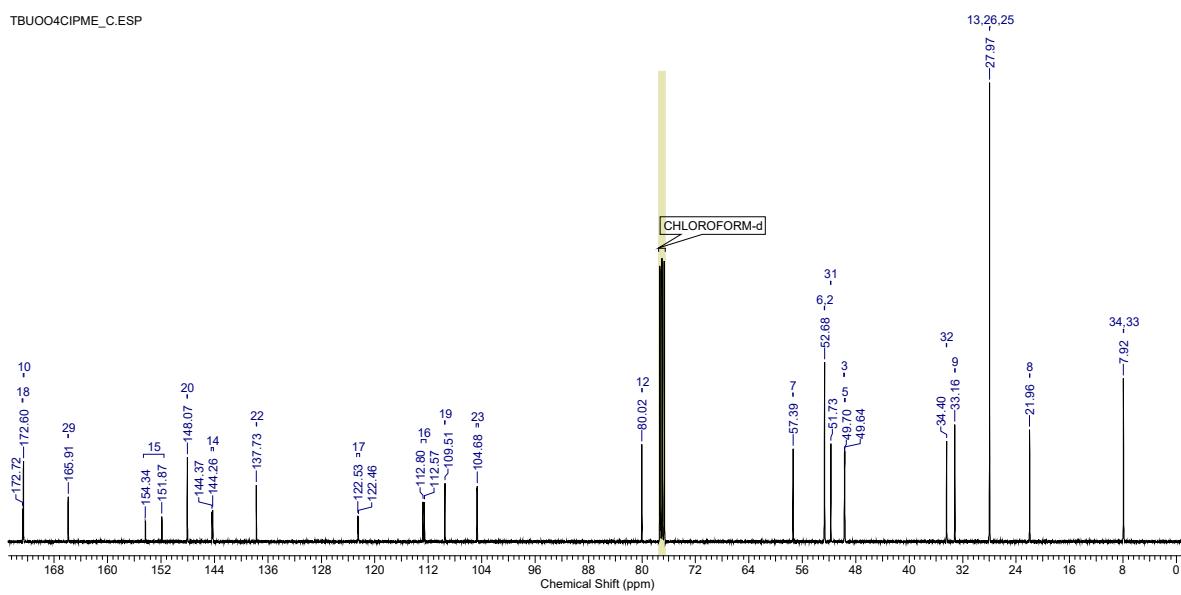
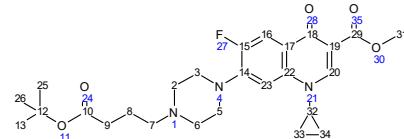
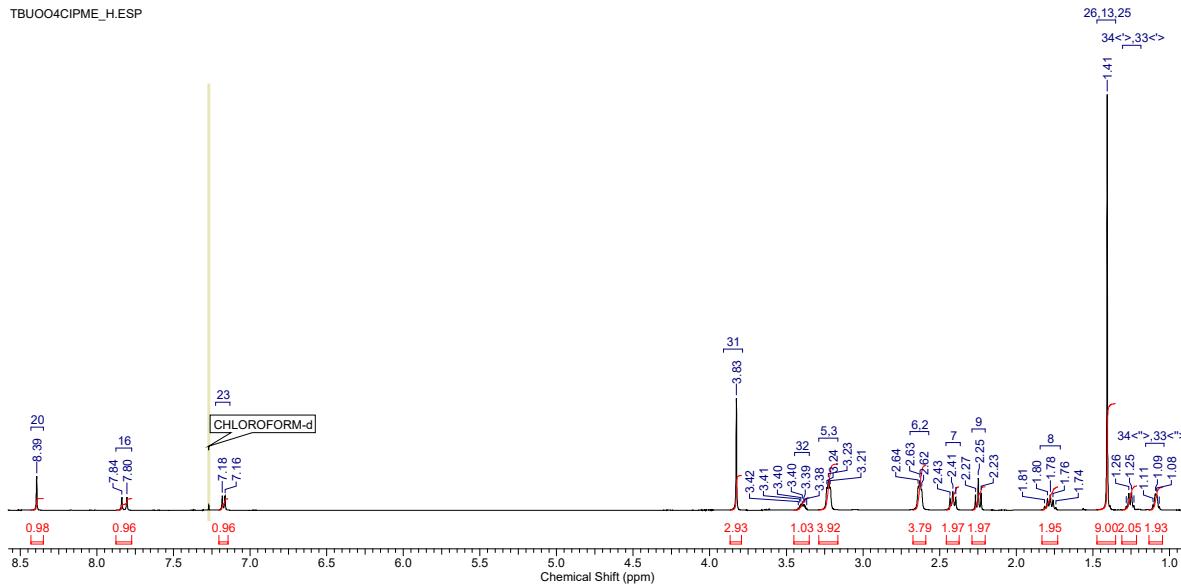
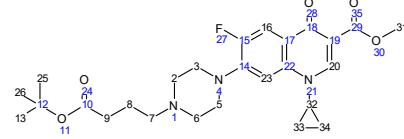
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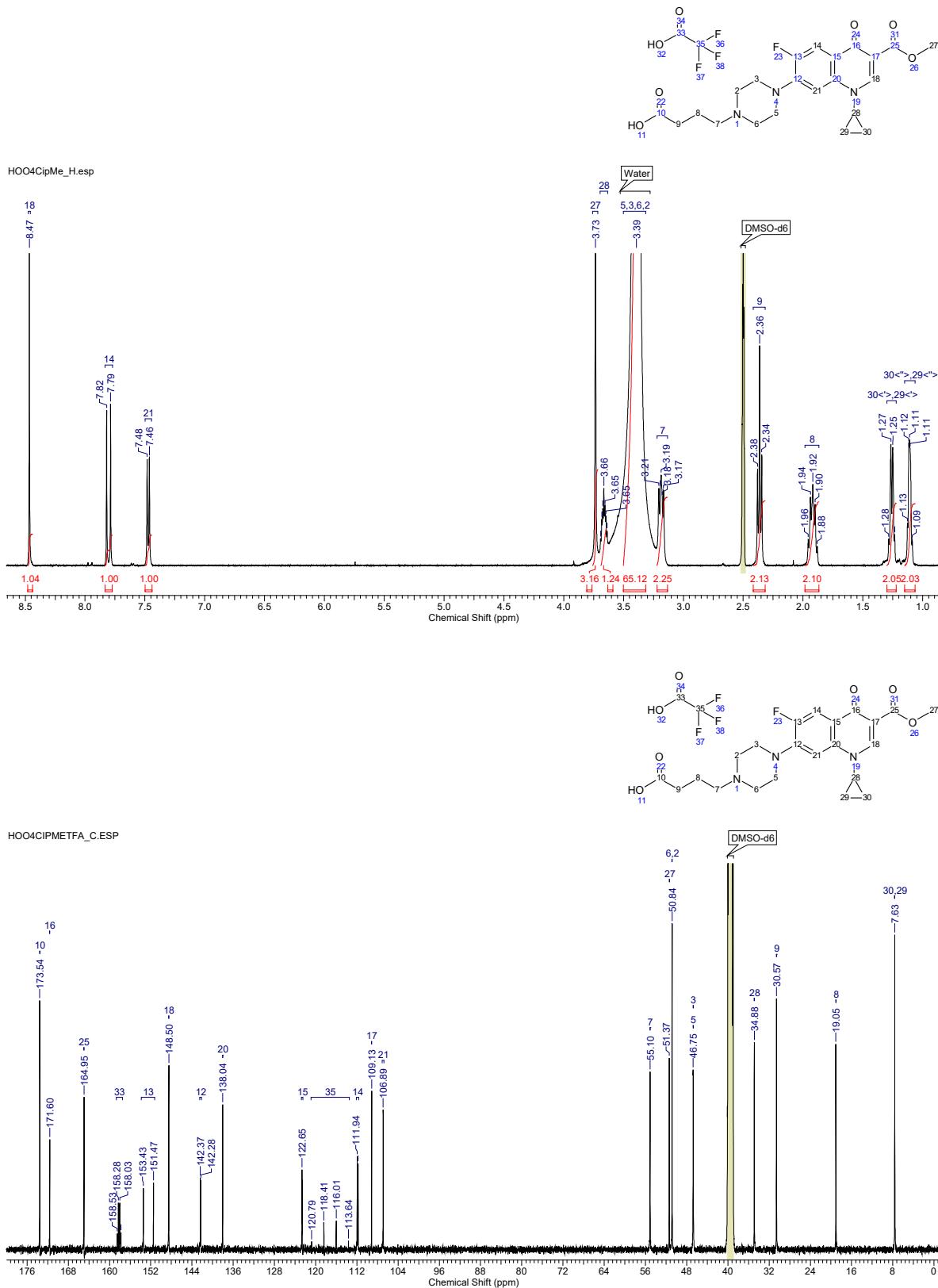
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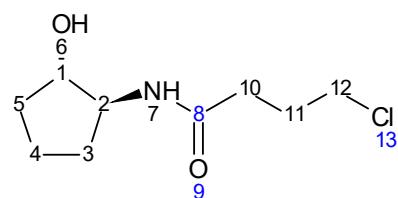
11.36 Methyl 7-(4-(*tert*-butoxy)-4-oxobutyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 147



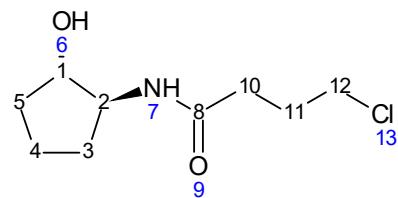
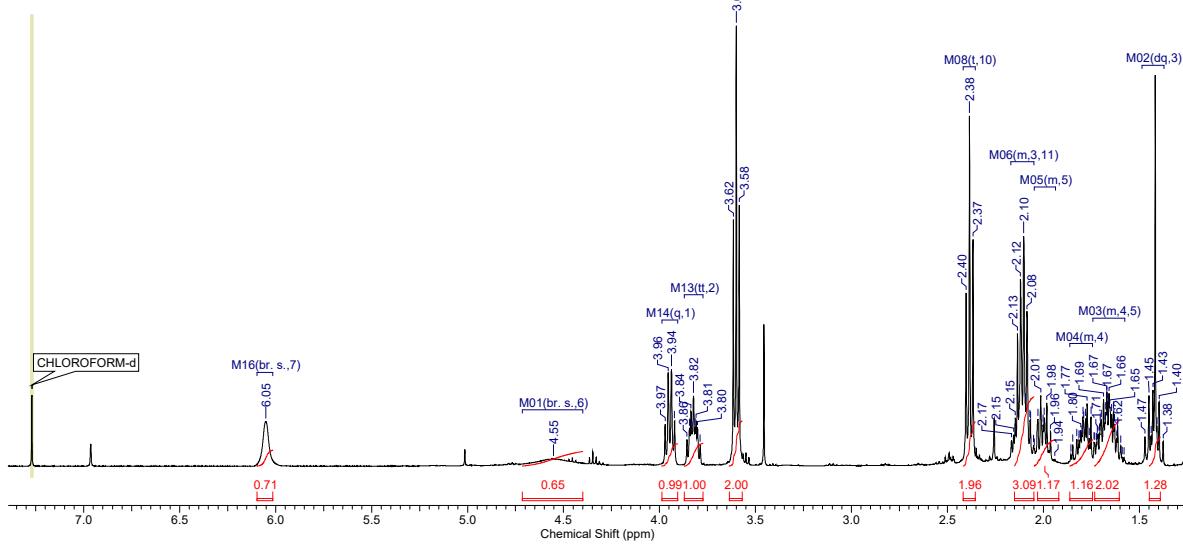
11.37 4-(4-(1-Cyclopropyl-6-fluoro-3-(methoxycarbonyl)-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)butanoic acid, trifluoroacetic acid salt 148



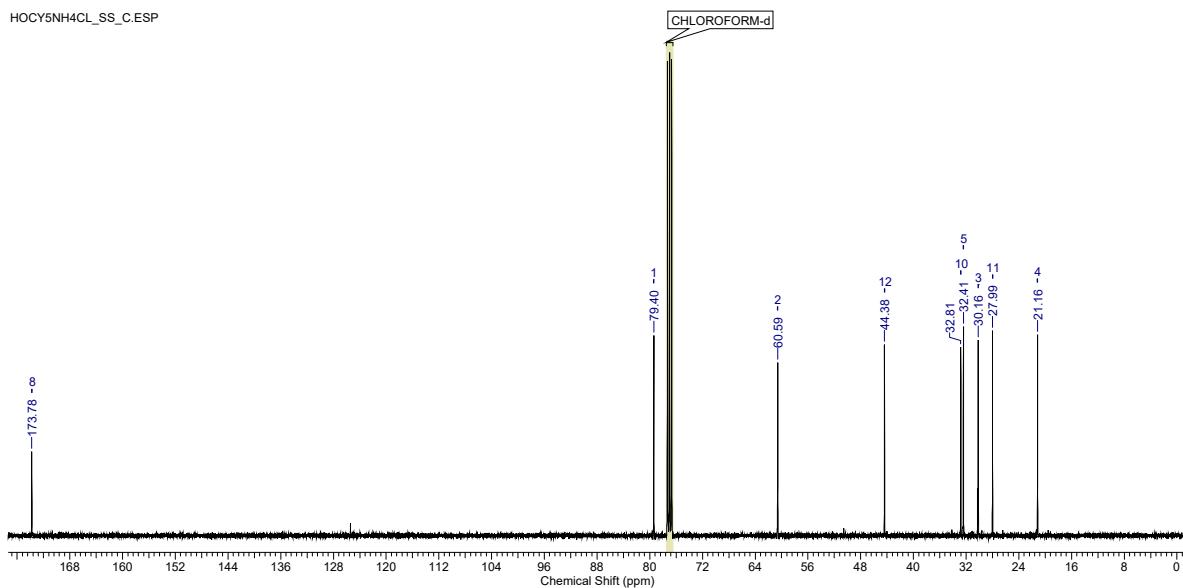
## 11.38 4-Chloro-*N*-(*(1S,2S)*-2-hydroxycyclopentyl)butanamide 143



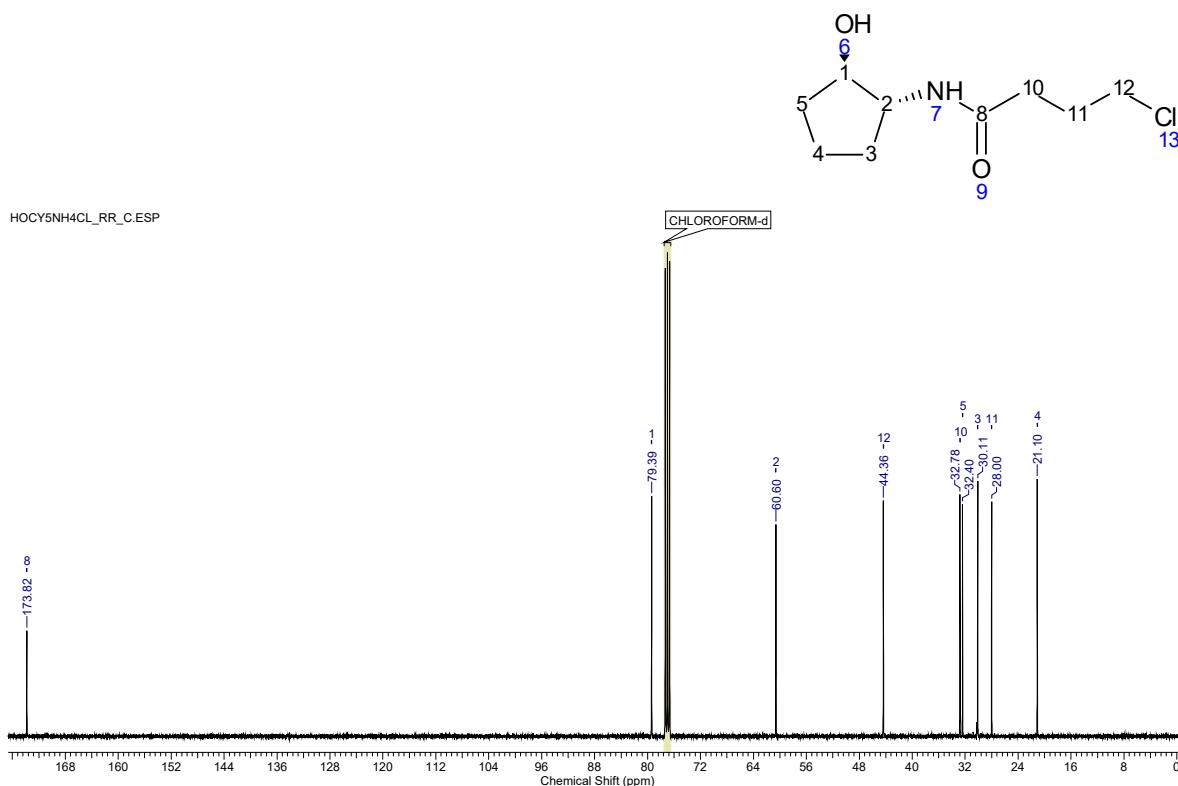
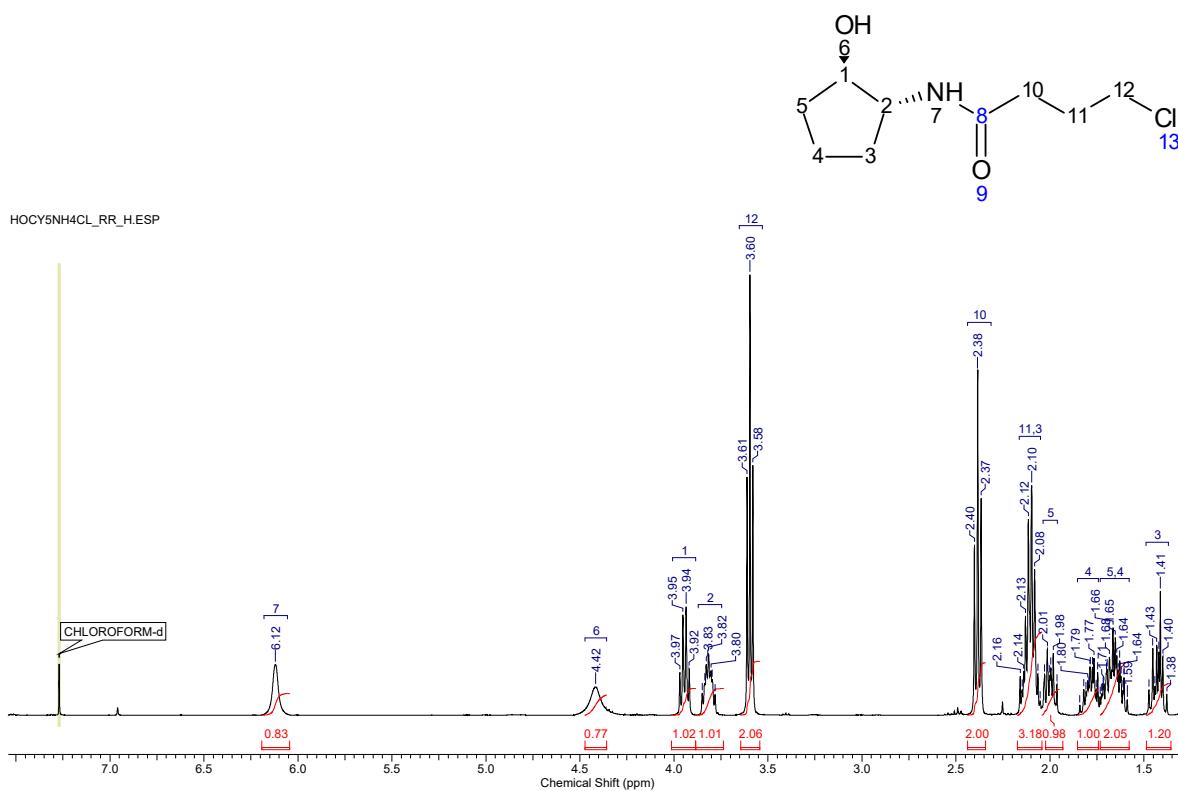
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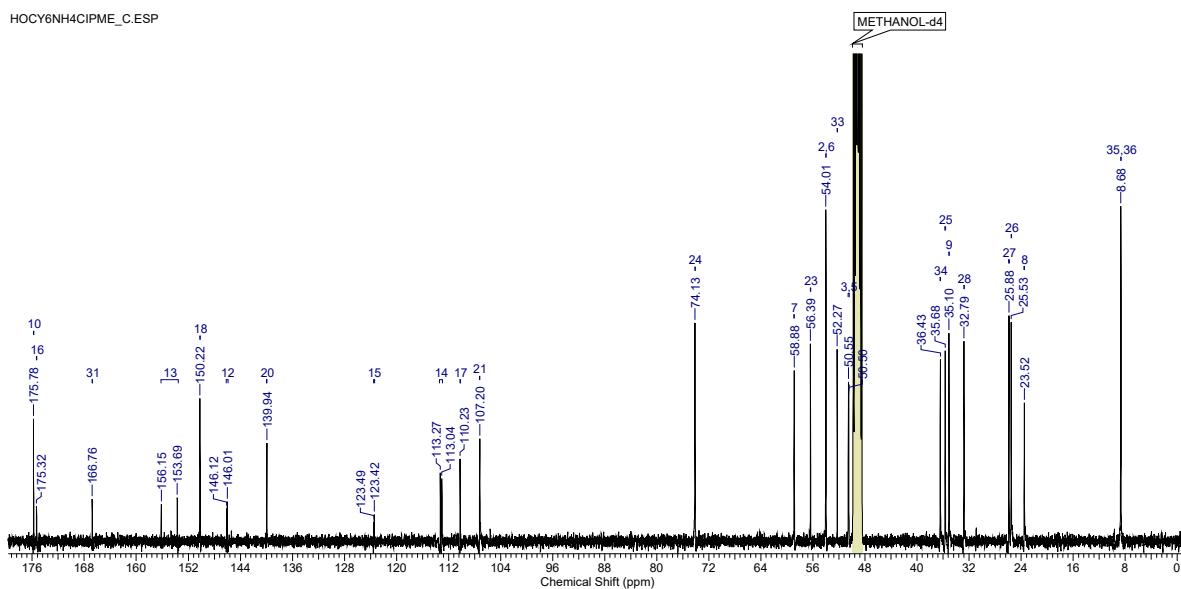
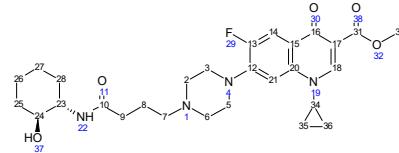
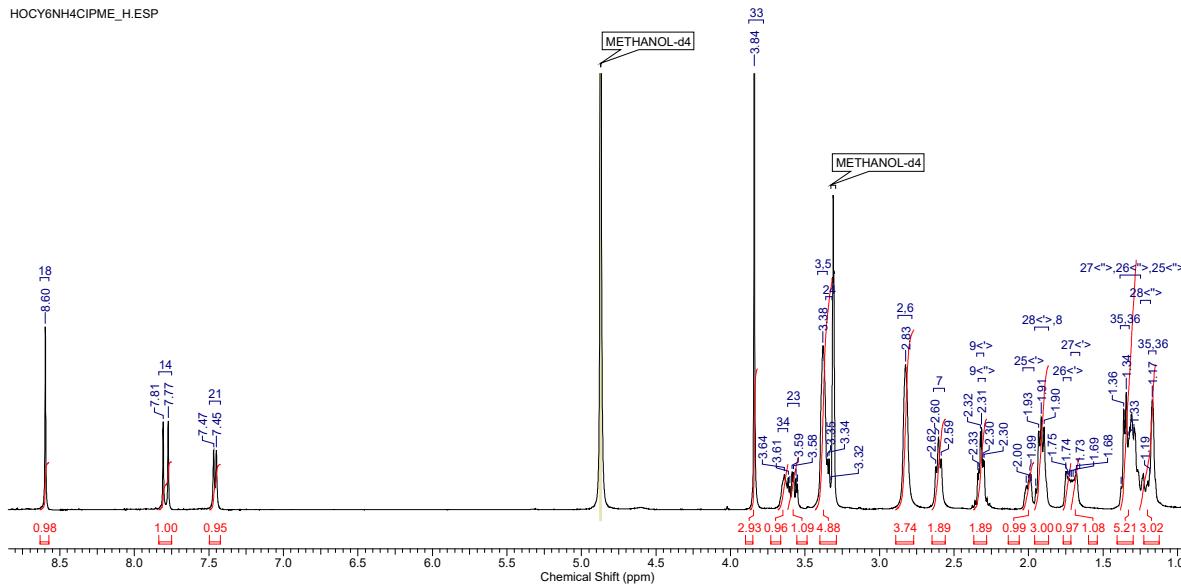
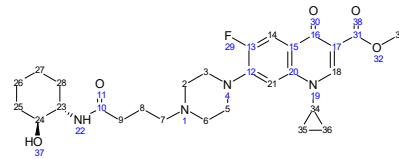
HO CY5 NH4 CL\_SS\_C.ESP



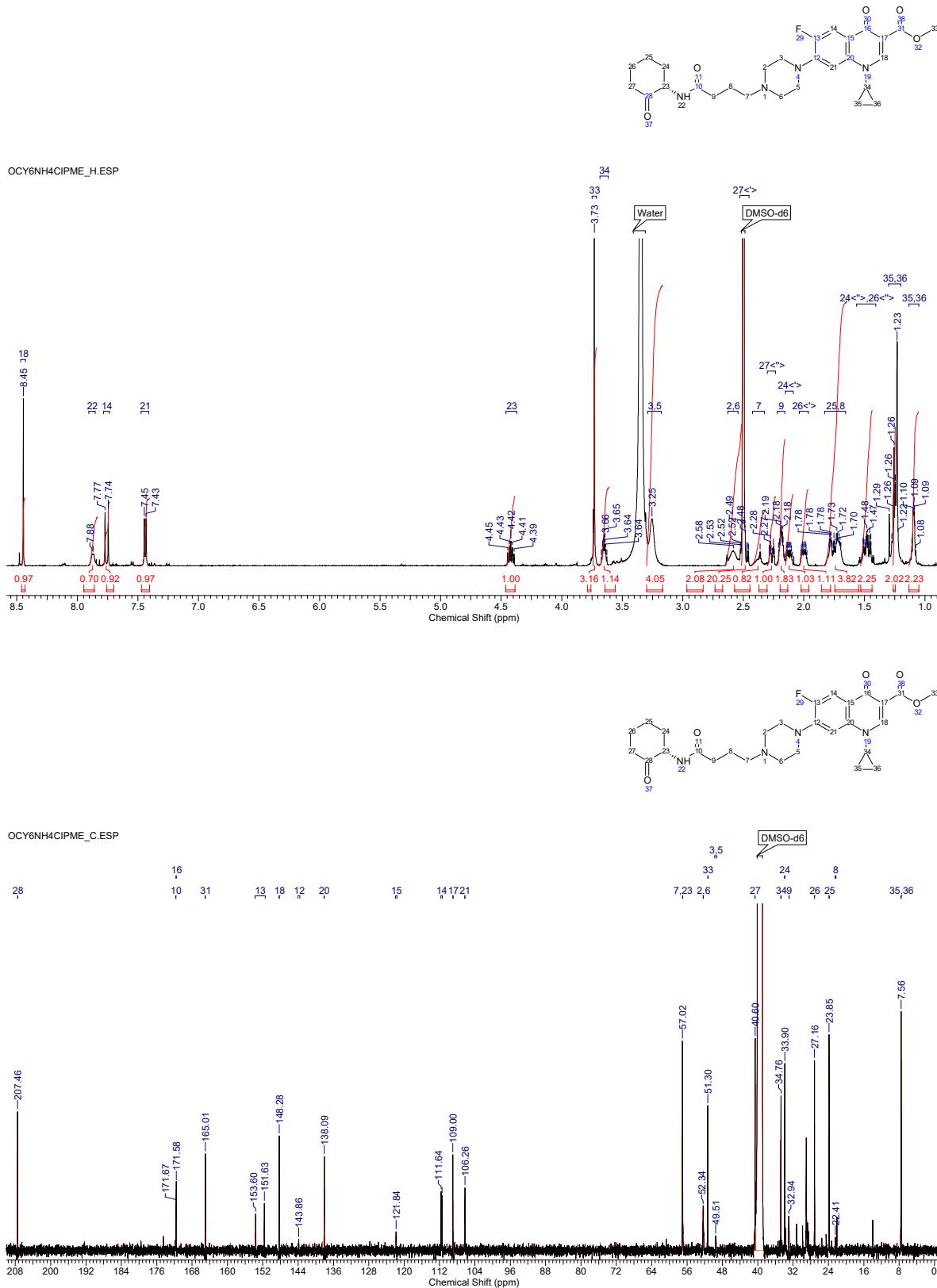
11.39 4-Chloro-*N*-(*1R,2R*)-2-hydroxycyclopentylbutanamide 144



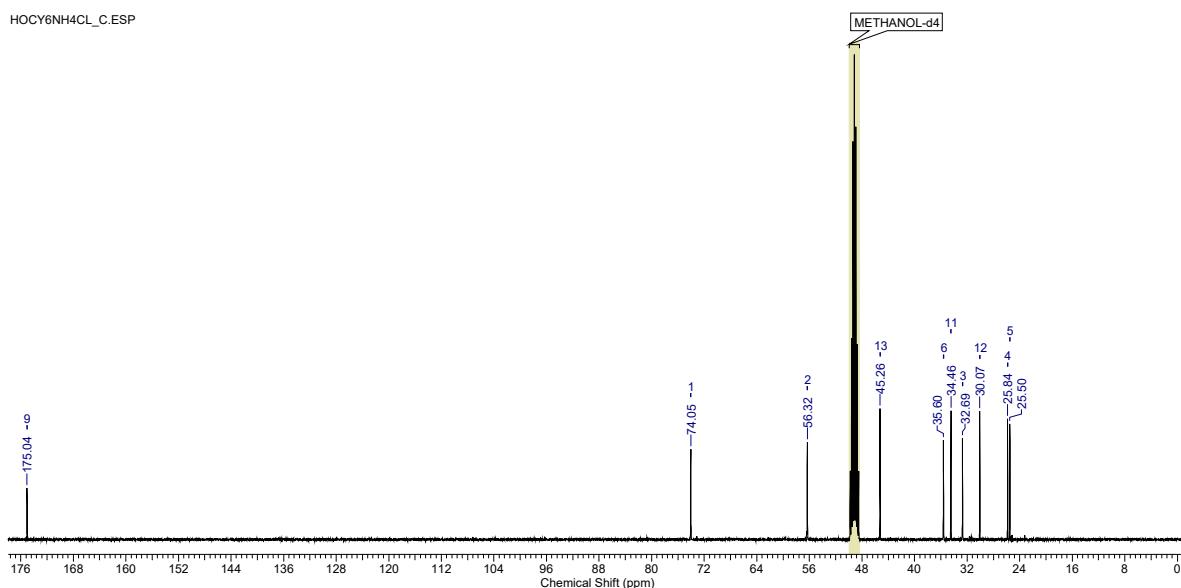
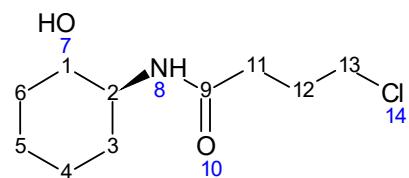
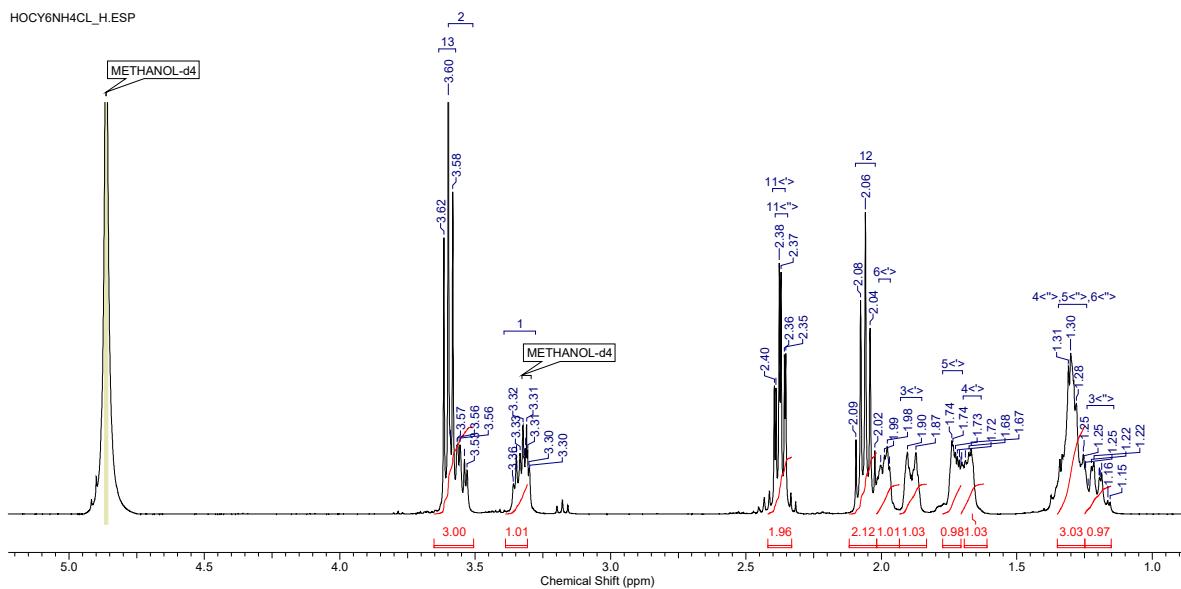
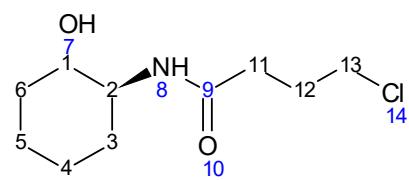
11.40 Methyl 1-cyclopropyl-6-fluoro-7-(4-((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate 151



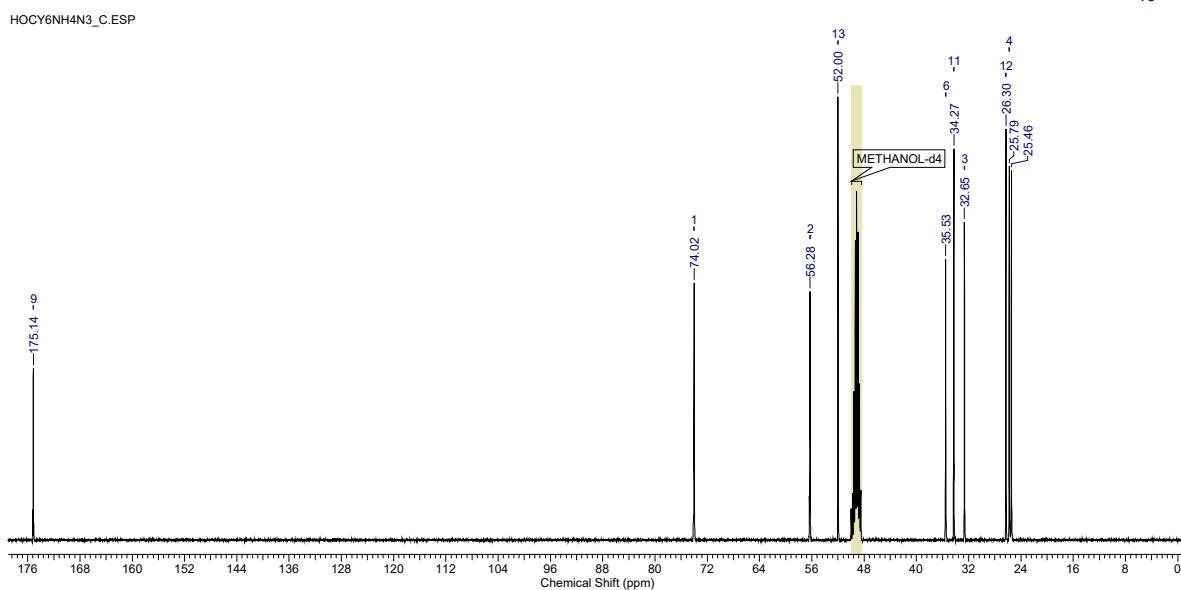
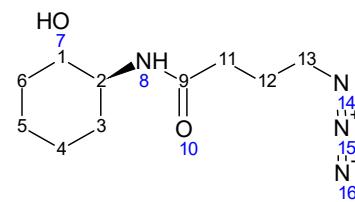
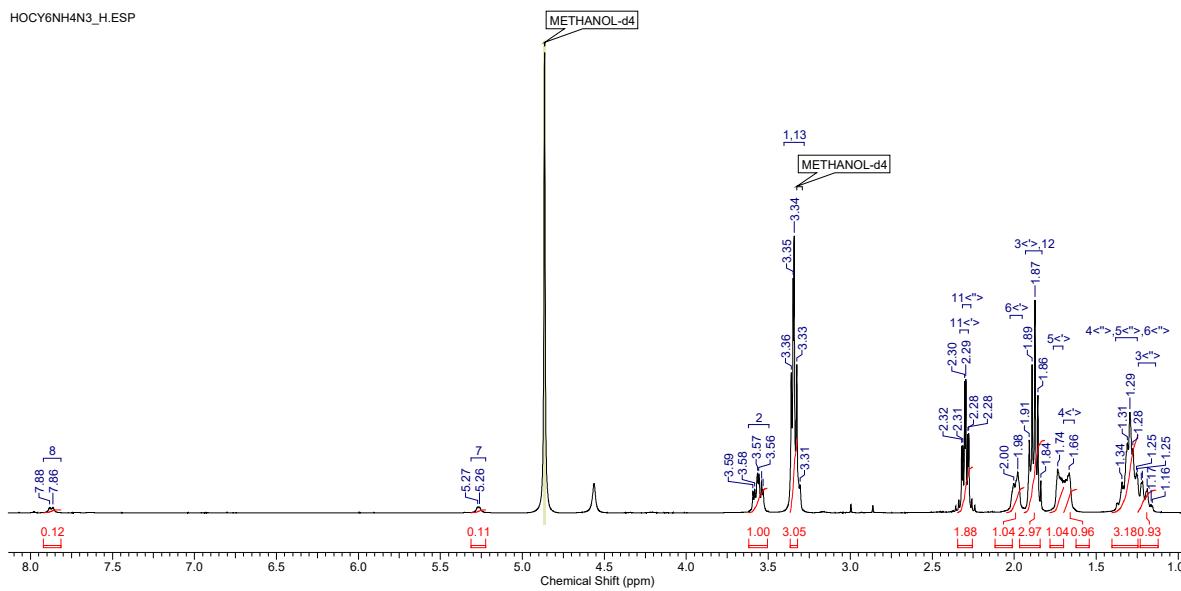
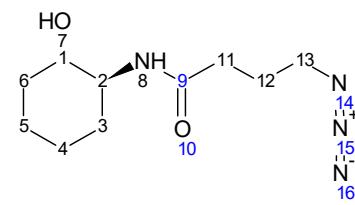
11.41 Methyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-oxo-4-((2-oxocyclohexyl)amino)-butyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate 152



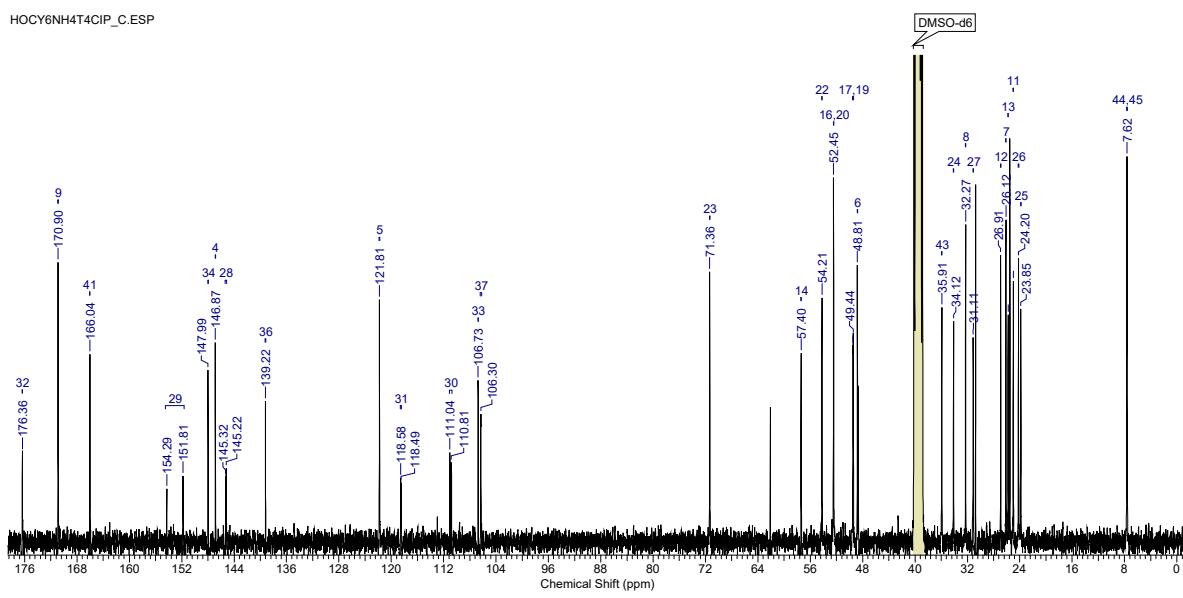
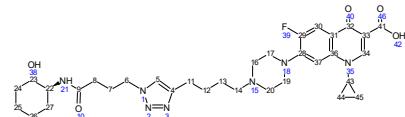
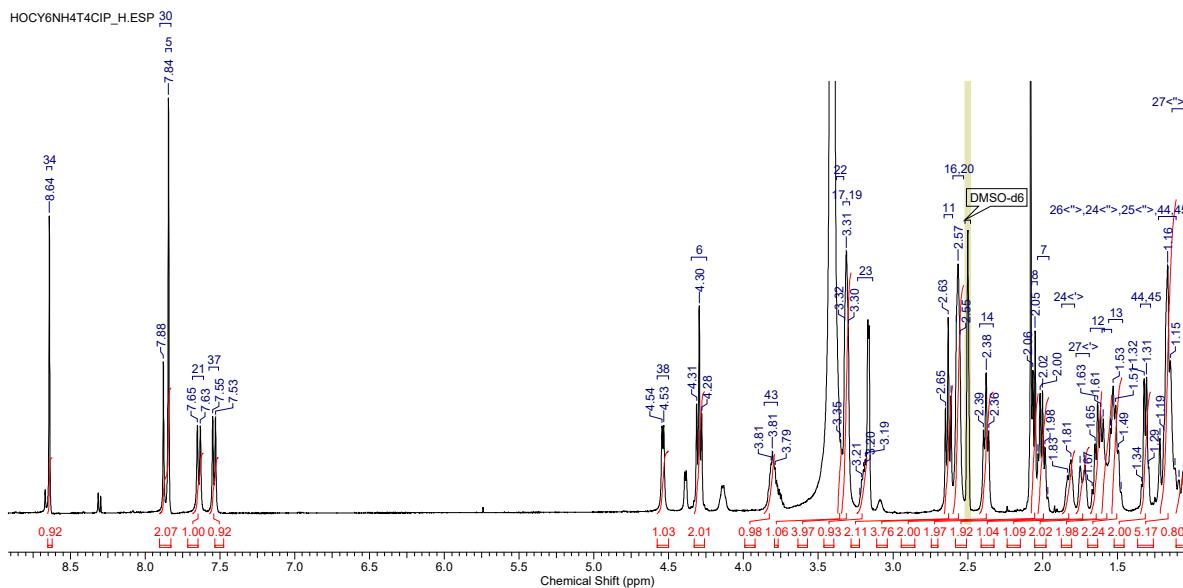
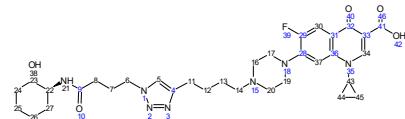
## 11.42 4-Chloro-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 153



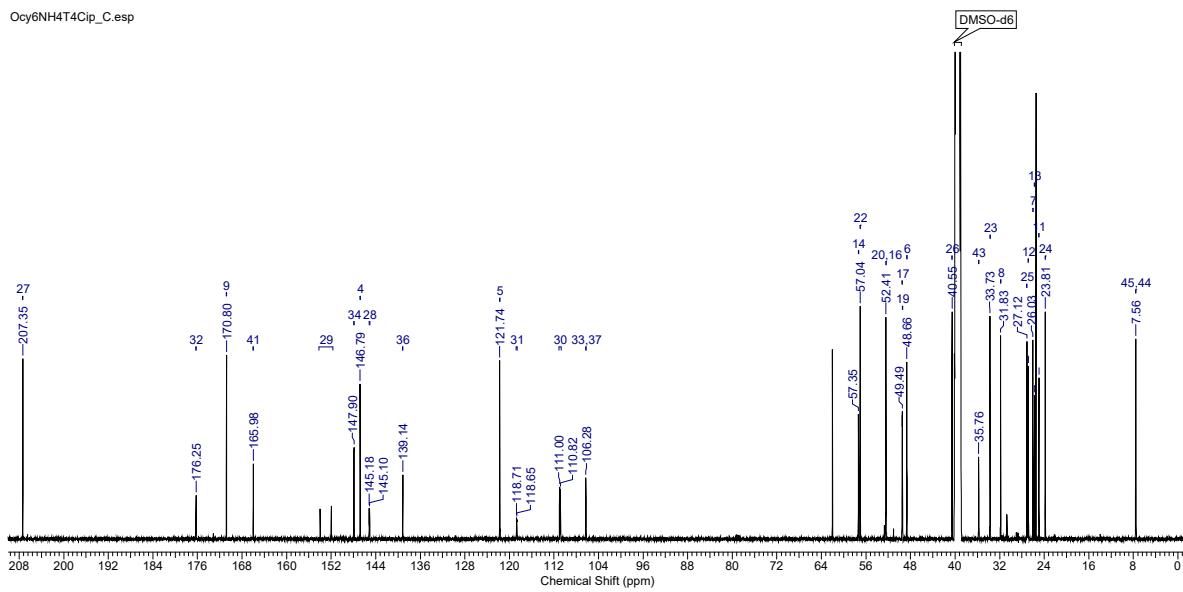
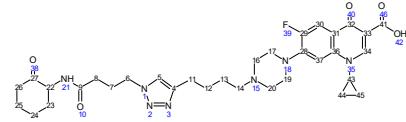
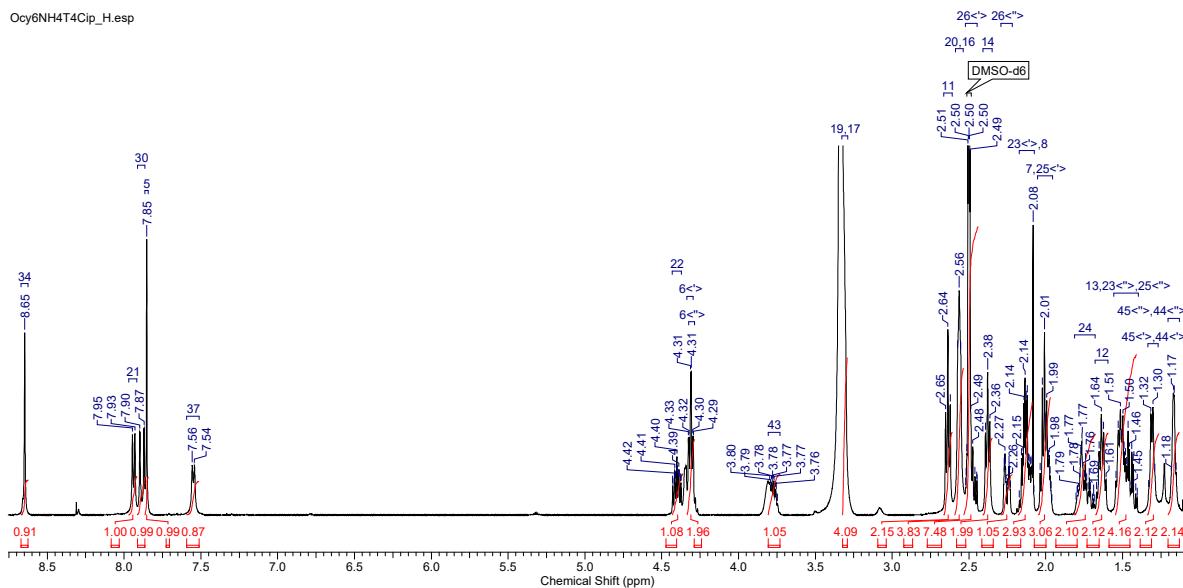
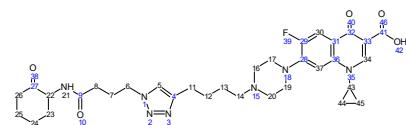
### 11.43 4-Azido-*N*-((*trans*)-2-hydroxycyclohexyl)butanamide 154



11.44 1-Cyclopropyl-6-fluoro-7-(4-(4-(1-(4-(((trans)-2-hydroxycyclohexyl)amino)-4-oxobutyl)-1*H*-1,2,3-triazol-4-yl)butyl)piperazin-1-yl)-4-oxo-1,4-dihydroquino-line-3-carboxylic acid 155



11.45 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(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 156



## 12 References

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