

The synthesis of a library of quorum sensing molecule-antibiotic conjugates

Lois Overvoorde



Contents

1 Acknowledgements	3
2 Abstract	3
3 Nomenclature	3
4 Introduction	4
4.1 Antibiotic resistance	4
4.2 Quorum sensing	5
4.3 <i>Pseudomonas aeruginosa</i>	7
4.4 Siderophores	8
4.5 Sideromycins	10
4.6 Synthetic siderophore-antibiotic conjugates	12
4.7 Quorum sensing molecule-antibiotic conjugates	12
5 Aims	13
5.1 Quorum sensing molecule analogues	13
5.2 Ciprofloxacin analogues	14
5.3 Quorum sensing molecule-antibiotic conjugates	15
6 Results and discussion	15
6.1 Quorum sensing molecule analogues	15
6.1.1 Retrosynthesis of HHQ analogue 26	15
6.1.2 Synthesis of HHQ analogue 26	16
6.1.3 Retrosynthesis of PQS analogue 36	16
6.1.4 Synthesis of PQS analogue 36	17
6.1.5 Retrosynthesis of C ₄ -HSL analogues 41 , 46 and 47	18
6.1.6 Synthesis of C ₄ -HSL analogues 41 , 46 and 47	19
6.2 Ciprofloxacin analogues	20
6.2.1 Retrosynthesis of ciprofloxacin analogue 54	20
6.2.2 Synthesis of ciprofloxacin analogue 54	21
6.2.3 Retrosynthesis of ciprofloxacin analogue 67	22
6.2.4 Synthesis of ciprofloxacin analogue 67	23
6.3 Quorum sensing molecule-antibiotic conjugates	25
6.3.1 Synthesis of quorum sensing molecules-antibiotic conjugate 68	25
7 Future work	26
7.1 Quorum sensing molecule-antibiotic conjugates	26
7.2 Quorum sensing molecule analogues	28
7.2.1 3-oxo-C ₁₂ -HSL analogue 77	29
7.2.2 Non- <i>P. aeruginosa</i> QSMs	29
7.3 Antibiotic analogues	30
7.3.1 Penicillin analogue 85	30
7.3.2 Gentamicin analogue 88	32
7.3.3 Streptomycin analogues 92 , 94 and 96	33
8 Conclusions	35

9 Experimental	35
9.1 General	35
9.2 Methyl 3-oxodecanoate 21 ^{1,2}	36
9.3 Methyl (<i>E</i>)-3-((4-((<i>tert</i> -butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 24 ³	37
9.4 6-amino-2-heptylquinolin-4-ol 25 ³	37
9.5 Heptyl magnesium bromide 28 ⁴	38
9.6 2-chloro- <i>N</i> -methoxy- <i>N</i> -methylacetamide 30 ⁵	38
9.7 1-chlorononan-2-one 31 ^{4,5}	39
9.8 2-oxononyl 2-amino-5-nitrobenzoate 33 ⁶	39
9.9 6-nitro-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 34 ⁶	40
9.10 6-amino-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 35 ⁷	40
9.11 6-azido-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 36 ⁸	41
9.12 (3 <i>S</i>)-2-oxotetrahydrofuran-3-aminium bromide 38 ⁹	41
9.13 (<i>S</i>)-2-bromo- <i>N</i> -(2-oxotetrahydrofuran-3-yl)acetamide 40 ⁹	42
9.14 (<i>S</i>)-2-azido- <i>N</i> -(2-oxotetrahydrofuran-3-yl)acetamide 41 ⁹	42
9.15 (<i>S</i>)-4-bromo- <i>N</i> -(2-oxotetrahydrofuran-3-yl)butanamide 44 ⁹	43
9.16 (<i>S</i>)-6-bromo- <i>N</i> -(2-oxotetrahydrofuran-3-yl)hexanamide 45 ⁹	43
9.17 (<i>S</i>)-6-azido- <i>N</i> -(2-oxotetrahydrofuran-3-yl)hexanamide 47 ¹	44
9.18 Hex-5-ynal 49 ¹⁰	44
9.19 <i>tert</i> -butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 51 ¹¹	45
9.20 1-(hex-5-yn-1-yl)piperazine 52 ¹²	45
9.21 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 54 ¹²	46
9.22 Ethyl 3-(2,4-dichloro-5-fluorophenyl)-3-oxopropanoate 56 ¹³	47
9.23 Ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-ethoxyacrylate 59 ^{14,15}	47
9.24 2-(hex-5-yn-1-yl)isoindoline-1,3-dione 61 ^{16,17}	48
9.25 5-aminohex-1-yne 62 ^{16,17}	48
10 NMR spectra	49
10.1 Methyl (<i>E</i>)-3-((4-((<i>tert</i> -butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 24	49
10.2 6-amino-2-heptylquinolin-4-ol 25	50
10.3 2-oxononyl 2-amino-5-nitrobenzoate 33	51
10.4 6-nitro-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 34	52
10.5 6-amino-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 35	53
10.6 6-azido-2-heptyl-3-hydroxyquinolin-4(1 <i>H</i>)-one 36	54
10.7 (<i>S</i>)-4-bromo- <i>N</i> -(2-oxotetrahydrofuran-3-yl)butanamide 44	55
10.8 (<i>S</i>)-6-bromo- <i>N</i> -(2-oxotetrahydrofuran-3-yl)hexanamide 45	56
10.9 (<i>S</i>)-6-azido- <i>N</i> -(2-oxotetrahydrofuran-3-yl)hexanamide 47	57
10.10 <i>tert</i> -butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 51	58
10.11 1-(hex-5-yn-1-yl)piperazine 52	59
10.12 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 54	60
11 References	61

1 Acknowledgements

I would like to thank David Spring for the opportunity to work on this very interesting project. I would also like to thank Jamie Stokes for helping me get settled into the lab, James Hodgkinson for showing me the biological techniques I will need soon, and Felin Nie and Terrence Kwan for help with the LCMS. Thanks also to Matt Pond, Melvyn Orriss and Nic Davies for help with equipment and glassware and to Jill Vaughan for proof-reading. Most importantly, I would like to thank Yssy Baker for proof-reading, help, support, advice and encouragement. Finally, I would like to thank everyone in the Spring group for their help, advice and delightful company.

2 Abstract

3 Nomenclature

p-TsOH *p*-Toluenesulfonic acid

Ac Acetate

AIP Autoinducing peptide

aq. Aqueous

atm Atmosphere(s)

Boc *tert*-Butyloxycarbonyl

conc. Concentrated

COSY Correlation spectroscopy

d Day(s) 

DIPEA *N,N*-diisopropylethylamine

DMF Dimethylformamide

DMSO Dimethylsulfoxide

eq. Equivalents

ESI Electrospray ionization

Et Ethyl

h Hour(s)

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

HSL Homoserine lactone

Hz Hertz

IR Infrared 

J Coupling constant in Hz

LCMS Liquid chromatography mass spectroscopy

m.p.  melting point

Me Methyl

min Minute(s)

mol Mole(s)

Ms Methanesulfonyl

NMR Nuclear magnetic resonance

P.E. Petroleum ether

Pd/C Palladium on carbon

PQS Pseudomonas Quinolone Signal

QS Quorum sensing

QSM Quorum sensing molecule

r.t. Room temperature

s Second(s)

SAR Structure activity relationship

sat. Saturated

sp. Species

TBAF Tetrabutylammonium fluoride

TBTA Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine

TEA Triethylamine

TFA Trifluoroacetic acid

THF Tetrahydrofuran

TLC Thin layer chromatography

TMS Trimethylsilyl

UV Ultraviolet

4 Introduction

4.1 Antibiotic resistance

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 Suffolk¹⁸ is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,¹⁹ but it was not until 1943 that the drug was mass produced thanks to the research of Ernst Chain and Howard Florey. However, bacterial resistance to penicillin was being found in hospitals by the late 1940s.^{20,21} This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see Figure 1) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories:

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of β -lactam antibiotics such as penicillin by β -lactamase enzymes.
2. The bacterium may produce a membrane, cell wall or biofilm which does not allow the drug to pass through, for example biofilm formation may allow bacterial resistance to antibiotics to increase 1000-fold compared with bacteria in suspension culture.²²
3. The bacterium may pump antibacterial molecules out of the cell membrane using efflux pumps, for example the mexAB and mexXY pumps used by *P. aeruginosa*.
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.
5. The bacterium may switch to using a metabolic pathway which does not involve the target of the antibacterial molecule, for example sulfonamide resistance may be achieved by taking in folic acid from the environment rather than synthesising it using *p*-aminobenzoic acid - a process which is blocked by sulfonamides.

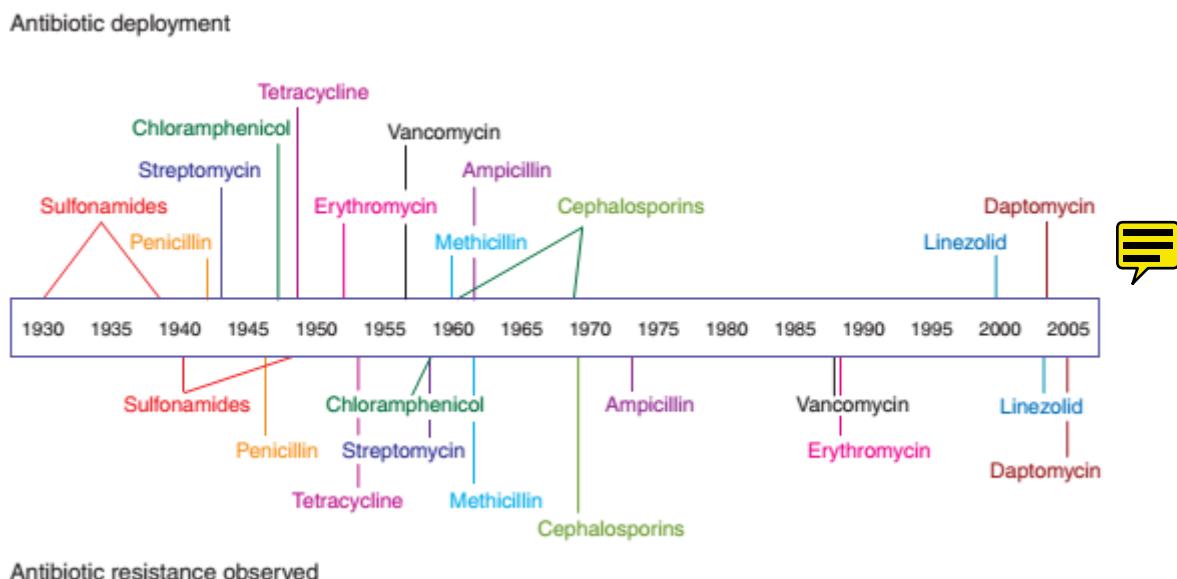


Figure 1: A timeline of when various antibiotics were first introduced and when resistance to them first appeared. Figure from Clatworthy *et al.*²³

4.2 Quorum sensing

A quorum is defined as "A fixed minimum number of members of an assembly or society that must be present at any of its meetings to make the proceedings of that meeting valid."²⁴ A similar process is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum population of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors and biofilm formation. It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium but effective when carried out as a group.

The first example of quorum sensing to be discovered was in *Vibrio fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid (*Euprymna scolopes*).²⁵ This bacterium receives nutrients such as glucose and amino acids from its host in exchange for producing light which the squid uses to attract prey. If a low population of *V. fischeri* were present in the photophore, the light they produced would be insufficient to attract prey, and so it would be better for the bacteria to conserve energy 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. The bacteria sense the population of other *V. fischeri* in their vicinity by the detection of 3-oxo-C₆-HSL **1** (see Figure 2) which is secreted by all *V. fischeri* cells²⁶ constitutively. When the bacterial population density, and hence concentration of 3-oxo-C₆-HSL **1**, reaches a threshold 3-oxo-C₆-HSL **1** will bind to the receptor LuxR. This triggers LuxI-mediated synthesis of more 3-oxo-C₆-HSL **1**, hence amplifying the signal. For this reason, quorum sensing molecules (QSMs) are also referred to as autoinducers.

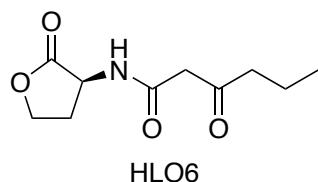


Figure 2: 3-oxo-C₆-HSL **1**.

Quorum sensing has since been observed in many species of bacteria, including *Pseudomonas aeruginosa*, *Agrobacter tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio harveyi*, *Escherichia coli*, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica*, *Aeromonas* sp. and *Acinetobacter* sp.²⁷⁻³⁵ Many of these bacteria are significant causes of disease and death in humans, for example, it is estimated that in 2005 in the US *S. aureus* caused 477,927 hospitalisations and 11,406 deaths.³⁶ *S. aureus* uses a peptide QSM known as autoinducing peptide (AIP) (see Figure 16) which interacts with the *agr* system leading to increased protease and toxin production.³⁷ *P. aeruginosa* also uses quorum sensing to coordinate biofilm formation, swarming motility and virulence.

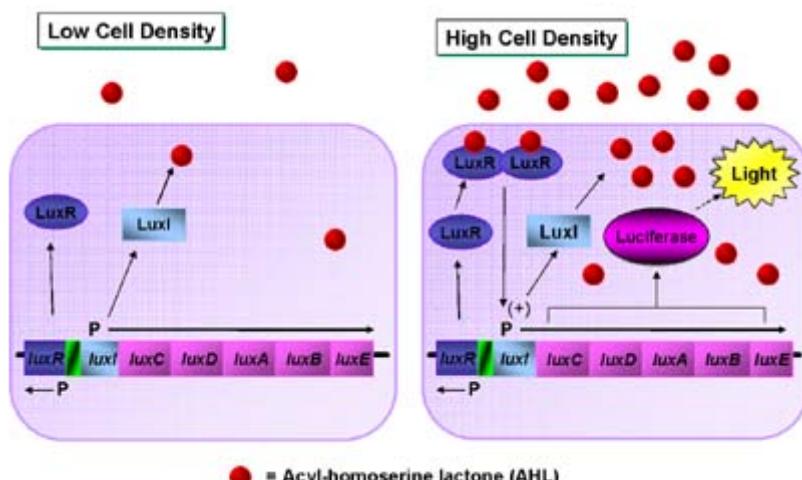


Figure 3: Quorum sensing in *V. fischeri*³⁸

4.3 *Pseudomonas aeruginosa*

One of the most well-studied examples of QS is in *P. aeruginosa*. *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.³⁹

P. aeruginosa uses quorum sensing (QS) to coordinate biofilm formation, swarming motility and virulence. The QS molecules used by *P. aeruginosa* are shown in Figure 4 (HHQ **5** is a precursor to PQS **4** but can bind to its receptors and hence can act as a QSM). QS in *P. aeruginosa* involves a complex interplay of the four signalling molecules and various proteins (see Figure 5).⁴⁰ QS regulates the production of virulence factors including elastase, alkaline protease, exotoxin A, rhamnolipids, pyocyanin, lectins and superoxide dismutases, as well as regulating biofilm formation.

P. aeruginosa has a low susceptibility to many antibiotics due to its chromosomally encoded multidrug efflux pumps: mexAB and mexXY.⁴¹ It is also difficult for drugs to cross into cells due to low cell wall permeability and biofilm formation. *P. aeruginosa* may also acquire antibiotic resistance by mutation or horizontal gene transfer.⁴² This high level of antibiotic resistance makes *P. aeruginosa* an important target for drug discovery.

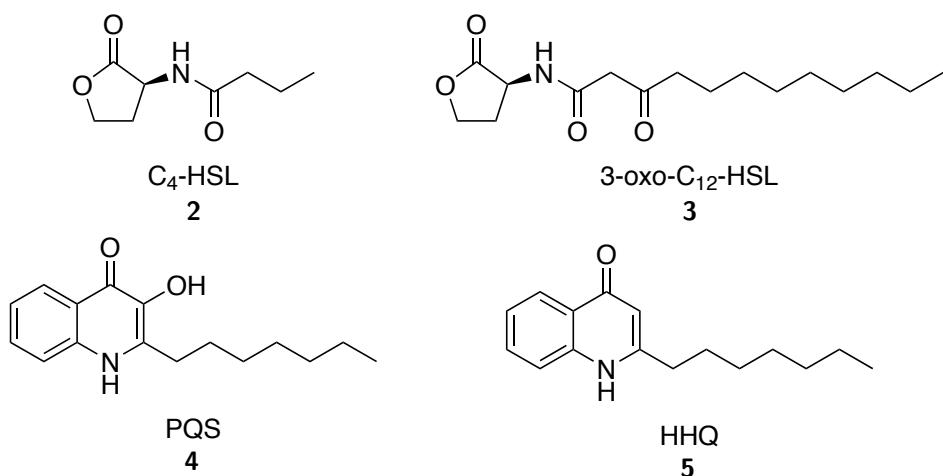


Figure 4: *P. aeruginosa* quorum sensing molecules

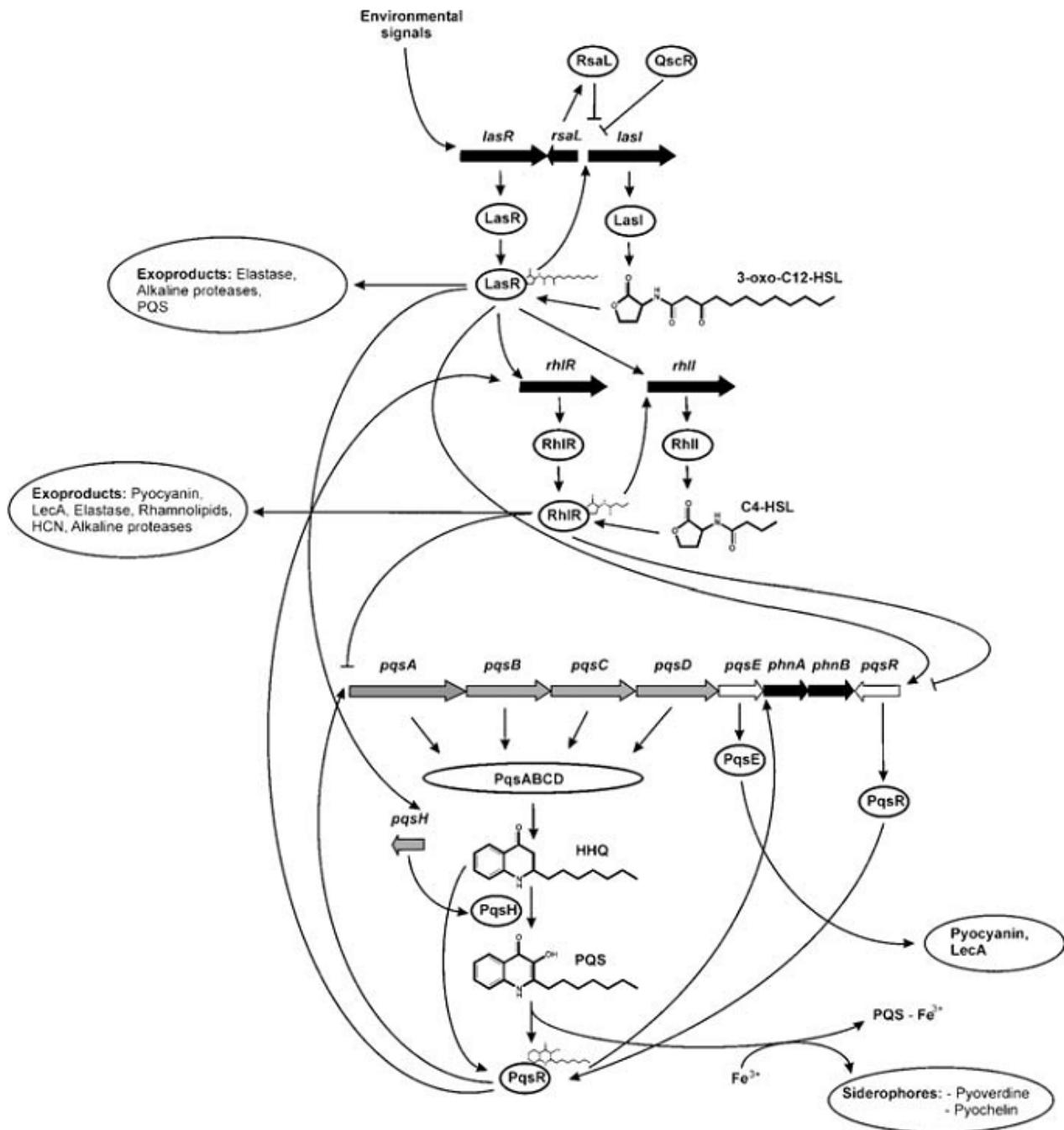


Figure 5: Quorum sensing in *P. aeruginosa*.⁴⁰

4.4 Siderophores

Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of 'iron mining'.⁴³ Soluble iron is often scarce but it is crucial for many cellular processes including respiration and DNA synthesis. Siderophores are synthesised by the microorganisms and secreted into the extracellular environment where they bind to Fe^{3+} , often with exceptionally high affinities. The iron-bound siderophores are then brought back into the cell by active transport and the iron is released, either by reduction of the Fe^{3+} to Fe^{2+} or by enzymatic degradation of the siderophore. Siderophores have a wide range of structures (see Figure 6), possibly so one species can avoid its siderophores being taken up by another species.⁴⁴

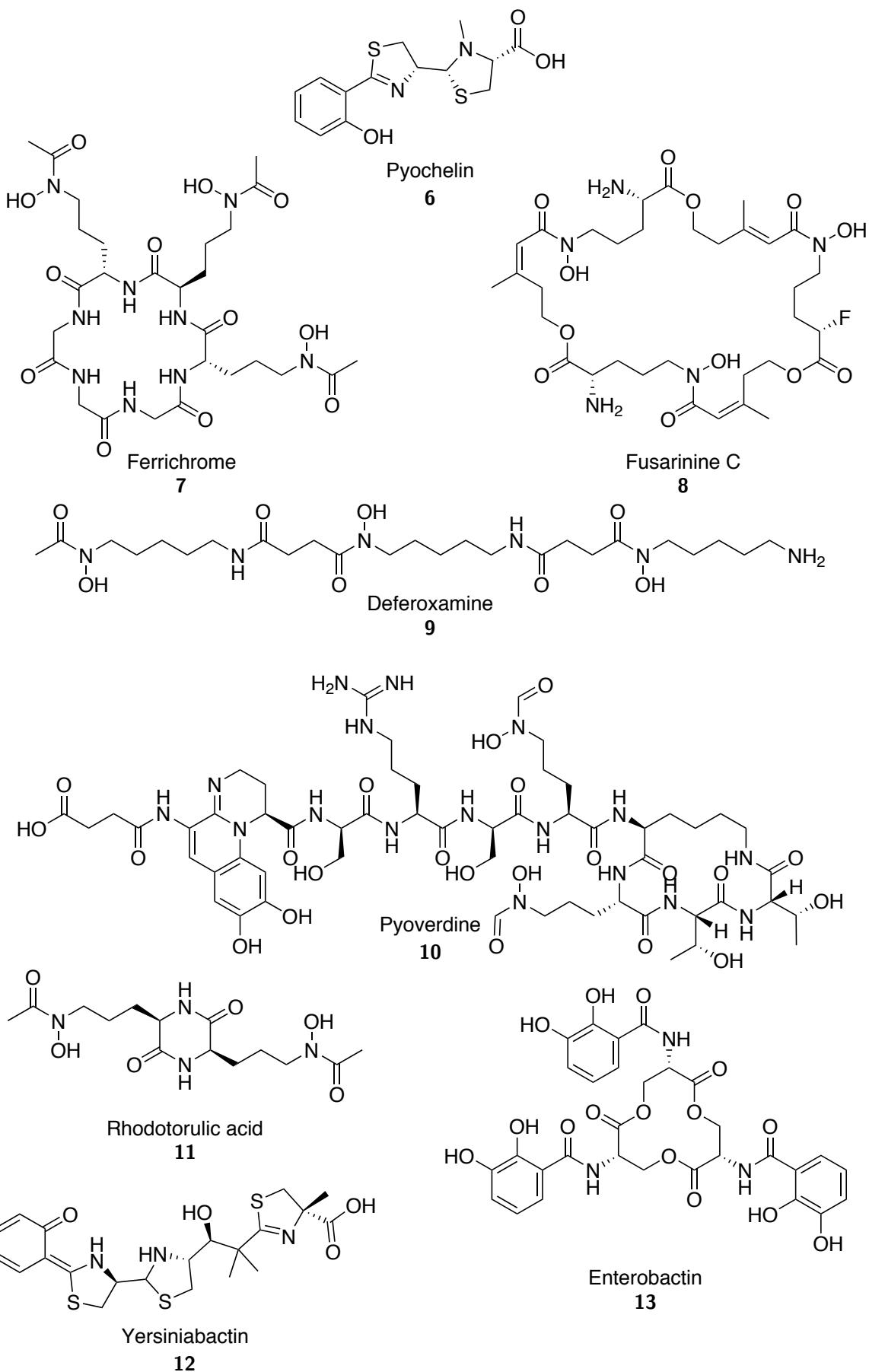


Figure 6: Siderophores

4.5 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins⁴⁵ (see Figure 7). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds. Albomycin **14** (see Figure 7) is an example of a sideromycin produced by *Actinomyces subtropicus*.⁴⁶ It contains a siderophore coupled to a nucleoside antibiotic using a peptide linker. The siderophore is structurally similar to ferrichrome **7** (see Figure 6) and so is taken up by the same transport protein in *E. coli*. The linker is hydrolysed by a protease in the cytoplasm of the *E. coli*, releasing the active nucleoside antibiotic. Resistance to albomycin develops relatively quickly, either due to loss of protease activity or loss of transport system components.

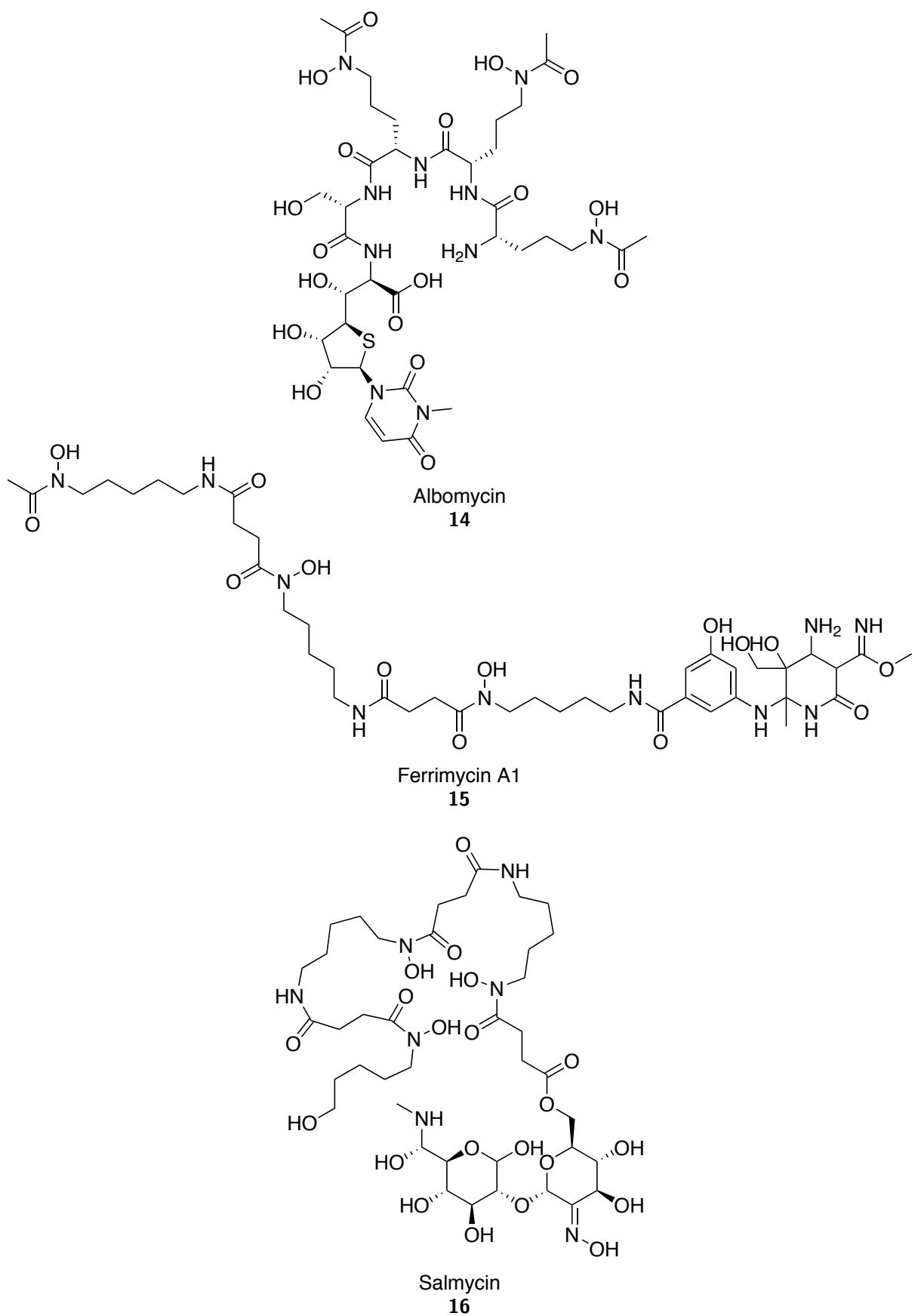


Figure 7: Sideromycins

4.6 Synthetic siderophore-antibiotic conjugates

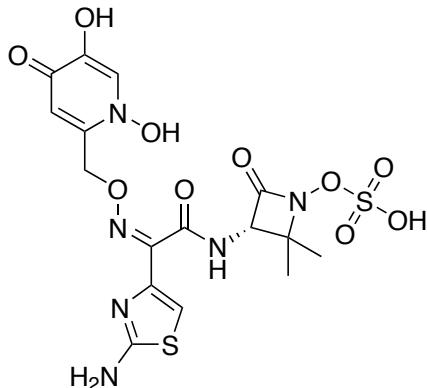


Sideromycins served as inspiration to Ghosh *et al.*⁴⁷ prompting the design, synthesis and biological evaluation of the first synthetic siderophore-antibiotic conjugates using β -lactam,^{48–50} nucleoside,⁵¹ glycopeptides⁵² and macrolide⁴⁷ antibiotics. Fluoroquinolone **18** conjugates with sideromycins have been studied by several groups,^{53–55} including conjugates with linkers which can be cleaved^{54,55} in a similar manner to albamycin.⁴⁶ Some of these showed comparable activity to the parent antibiotic, but it is not clear whether attachment of the siderophore improved uptake or whether the conjugates acted as classical prodrugs.

β -lactam-sideromycin conjugates have been more widely investigated⁴⁵ 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. Initial studies used aminocillin and cephems, and recently a conjugate of enterobactin (see Figure 6) and ampicillin (see Figure 17) has been shown to have increased activity against pathogenic *E. coli* when compared to native ampicillin.⁵⁶ Other work has focused on monocyclic β -lactams, which show high potency against Gram-negative bacteria but low potency against Gram-positive bacteria.⁵⁷ Monocyclic β -lactams are generally fairly stable to β -lactamase activity, which is an advantage compared with many β -lactams.

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, mutants lacking transport proteins tend to grow more slowly in low-iron conditions, but with an immunocompromised host siderophores may not always be required for infection as bacteria can obtain iron from other sources.⁵⁸

BAL30072 **17** (see Figure 8) is a conjugate of a pyochelin **6** (see Figure 6) analogue and a monocyclic β -lactam. It is in early clinical trials and appears to have overcome many of the above hurdles as it is a poor substrate for β -lactamases and resistance due to loss of transport proteins is infrequent.⁴⁵ Building on this positive example of an antibiotic conjugate, it is hoped that the approach can be extended to conjugates of antibiotics and QSMs



17

Figure 8: BAL30072 **17**.

4.7 Quorum sensing molecule-antibiotic conjugates

The success of the siderophore-antibiotic conjugate field lead us to speculate that a similar strategy might be employed with QSMs, leading to QSM-antibiotic conjugates. It is hypothesised that attaching a QSM to a known antibiotic could lead to increased uptake or better localisation of the antibiotic and could restore function in resistant strains. We decided to focus initially on the QSMs synthesised by *P. aeruginosa* due to the low

activity of various antibiotics towards it. Ciprofloxacin **18** (see Figure 9) was chosen as the initial antibiotic half because we have access to some ciprofloxacin-resistant strains of *P. aeruginosa* and hope to restore activity against them in antibiotic assays.

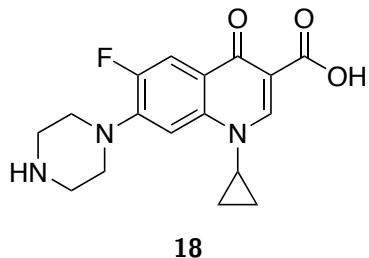


Figure 9: Ciprofloxacin **18**.

5 Aims

The aim of this project is to produce a library of QSM-antibiotic conjugates with the hope of restoring antibacterial function against resistant strains. The library is built from a collection of *Pseudomonas aeruginosa* QSM analogues with azide groups attached and a collection of antibiotics with alkyne groups attached. These collections will then be combined using a copper-catalyzed click reaction to form the library of final conjugates. This approach has recently been used by Zheng *et al.* to join the siderophore enterobactin **97** (see Figure 6) with ampicillin or amoxicillin (see Figure 17).

5.1 Quorum sensing molecule analogues

The four main QSMs used by *P. aeruginosa* are shown in Figure 4. We have decided to focus initially on the synthesis of azido analogues of C₄-HSL **2**, HHQ **5** and PQS **4**. A synthesis of an azido analogue of 3-oxo-C₁₂-HSL **3** is planned (see 7.2.1).

The structure-activity relationships in PQS have been previously studied,⁵⁹ and 5 and 6 positions could be substituted without significantly affecting the activity of the PQS molecule (see Figure 10). Placing of the azide group at position 6 was chosen for synthetic reasons and the azide group was placed in the equivalent position in the HHQ analogue (see Figure 15).

Alteration of the lactone group of C₄-HSL and other HSL derivatives is known to significantly decrease activity, especially where the number of H-bond donors or acceptors is altered.⁶⁰ Hence, the azide group will be included on the tail of C₄-HSL. Acyl tail length is known to play an important role in affinity, so we have decided to synthesise three analogues of C₄-HSL: azido-C₂-HSL **41**, azido-C₄-HSL **46** and azido-C₆-HSL **47** (see Figure 12).

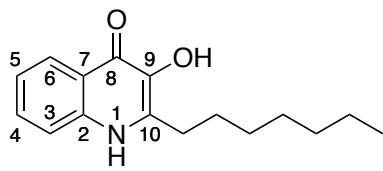


Figure 10: The atom numbering in PQS **4**

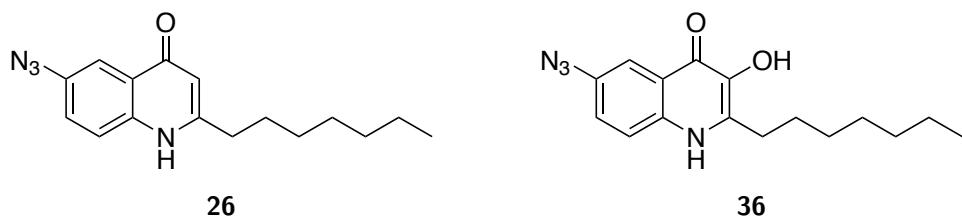


Figure 11: The HHQ 5 and PQS 4 analogues **26** and **36**.

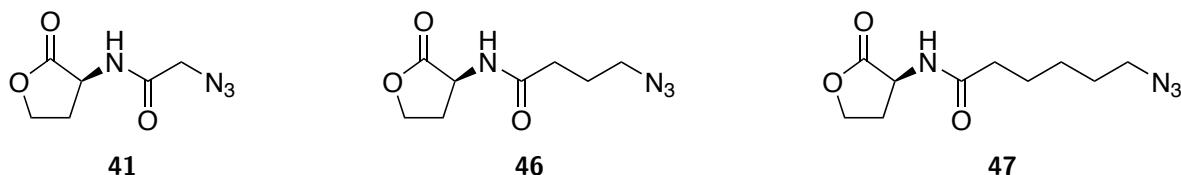


Figure 12: The C₄-HSL 2 analogues **98**, **99** and **100**.

5.2 Ciprofloxacin analogues

Ciprofloxacin **18** (see Figure 9) is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections.⁶¹ The structure-activity relationships for ciprofloxacin have been investigated¹² and positions 2 and 7 were found not to cause loss of activity (see Figure 13). It was therefore decided that alkyne tails would be added at these positions giving two analogues of ciprofloxacin, **54** and **67** (see Figure 14).

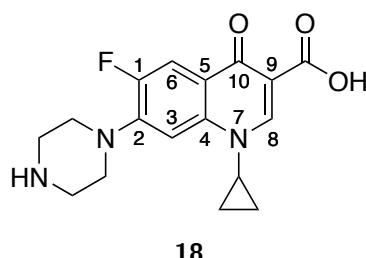


Figure 13: The atom numbering in ciprofloxacin **18**.

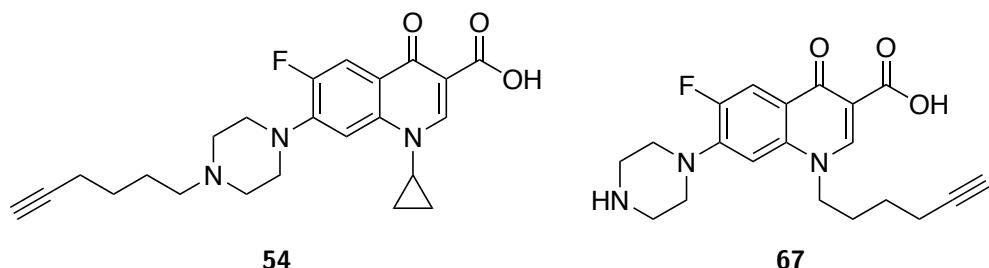
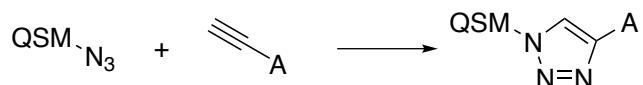


Figure 14: The proposed ciprofloxacin analogues: **54** and **67**.



5.3 Quorum sensing molecule-antibiotic conjugates

A copper(I)-catalysed azide-alkyne cycloaddition,^{62,63} commonly referred to as a click reaction although this is a more general term, will be used to join each combination of QSM and antibiotic together (see Scheme 1). This modular approach allows the library to be easily expanded by adding more QMSs or antibiotics, or indeed other groups such as siderophores, fluorescent or affinity tags, or resin beads. The library will then be screened for antibiotic activity against *P. aeruginosa* and other pathogenic bacteria.



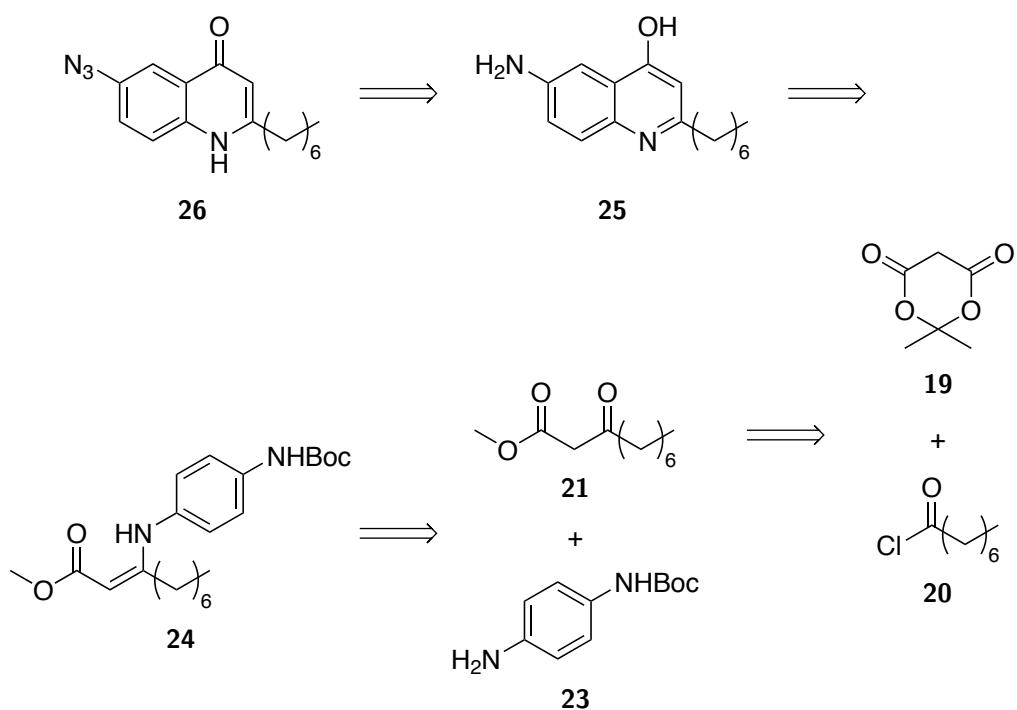
Scheme 1: The proposed construction of the library using a copper(I)-catalysed azide-alkyne cycloaddition.

6 Results and discussion

6.1 Quorum sensing molecule analogues

6.1.1 Retrosynthesis of HHQ analogue 26

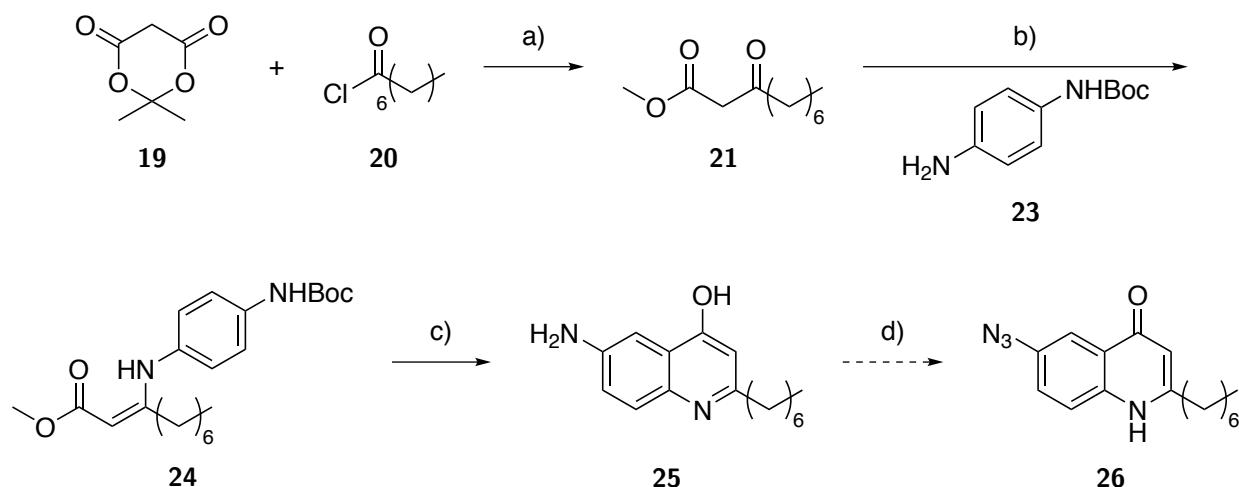
The retrosynthesis of HHQ analogue **26** is shown in Scheme 2 and follows a synthesis devised by Baker.³ Octonyl chloride **20** can be converted to β -ketoester **21** via a Meldrum's acid adduct. β -ketoester **21** can be condensed with *N*-Boc-*p*-phenylenediamine **23** to form enamine **24**, which can then be cyclised with polyphosphoric acid to form amino-HHQ **25**. The amine group of amino-HHQ **25** could be converted to a diazo group by reaction with NaNO_3 and HCl , followed by displacement with NaN_3 to form the final azido-HHQ product **26**.



Scheme 2: The retrosynthesis of HHQ analogue **26**.

6.1.2 Synthesis of HHQ analogue 26

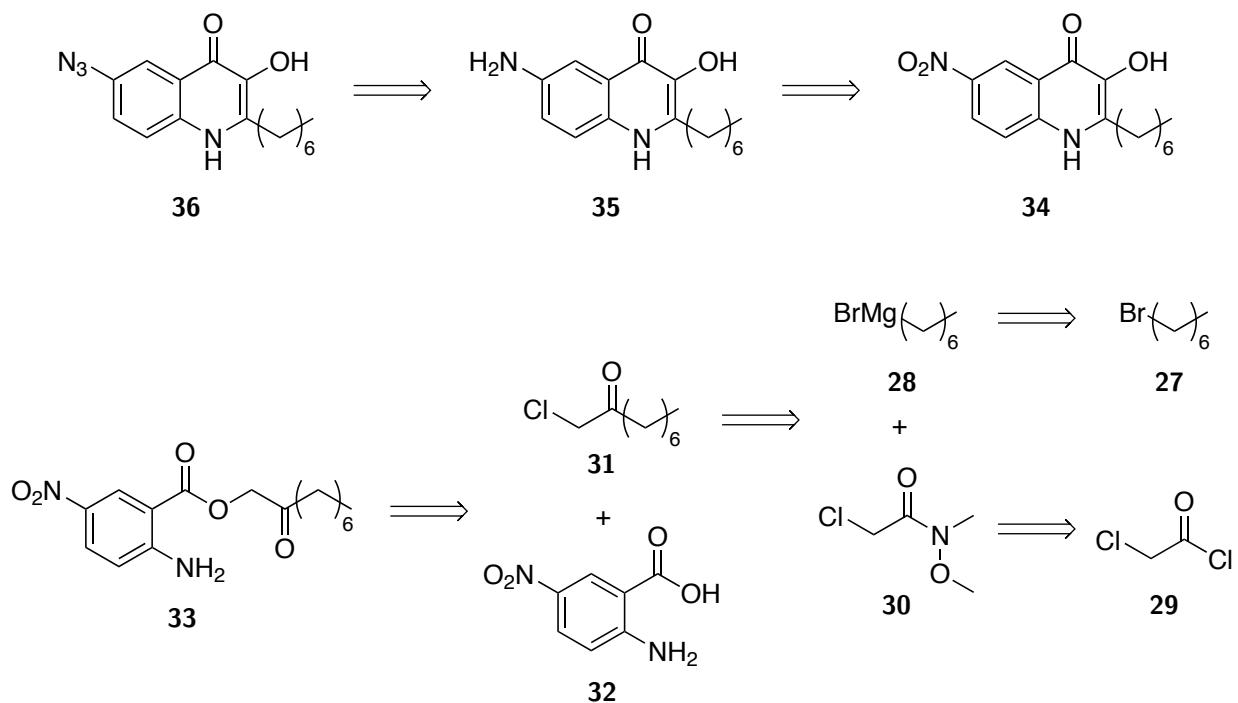
Amino-HHQ **25** was synthesised as shown in Scheme 3 and follows the route devised by Baker³ described above. The final step in the synthesis of **26** will be completed shortly.



Scheme 3: The synthesis of **26**. a) i) Pyridine, DCM, 0°C. ii) MeOH, reflux, 66 %. b) MeOH, reflux, **yield %**. c) Polyphosphoric acid, 120°C, **yield %**. d) i) NaNO₂, HCl, H₂O, 0 °C. ii) NaN₃, H₂O, r.t.

6.1.3 Retrosynthesis of PQS analogue 36

The retrosynthesis of PQS analogue **36** is shown in Scheme 4. The synthesis of 1-chlorononan-2-one **31** from heptyl magnesium bromide **28** and the Weinreb amide **30** prepared from chloroacetyl chloride **29** has been previously described by Hodgkinson *et al.*⁴ The synthesis of PQS described by Hodgkinson *et al.*⁴ uses a microwave reaction of 1-chlorononan-2-one **31** with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid **32** 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 fails when 5-nitroanthranilic acid **32** is used.³ Therefore, a two step process is employed instead. Firstly, ester **33** is formed by S_N2 displacement of the chlorine atom of 1-chlorononan-2-one **31** by the carboxylate group of 5-nitroanthranilic acid **32**. The ester **33** is then cyclised using a polyphosphoric acid-catalysed reaction developed by Hradil *et al.*⁷ to form nitro-PQS **34**. The nitro group can then be hydrogenated to form amino-PQS **35** followed by conversion to azido-PQS **36**.



Scheme 4: The retrosynthesis of PQS analogue **36**.

6.1.4 Synthesis of PQS analogue **36**

The Weinreb amide **30** was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide **28** to form 1-chlorononan-2-one **31** (see Scheme 5). 5-Nitroanthranilic acid **32** was heated with K_2CO_3 to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **31**. Cyclisation to form nitro-PQS **34** was attempted using Eaton's reagent as it has been reported to improve yields when compared with polyphosphoric acid,^{64,65} however, this lead to production of a black powder side-product in addition to the desired product (see Table 1). Cyclisation with polyphosphoric acid produced nitro-PQS **34** cleanly.

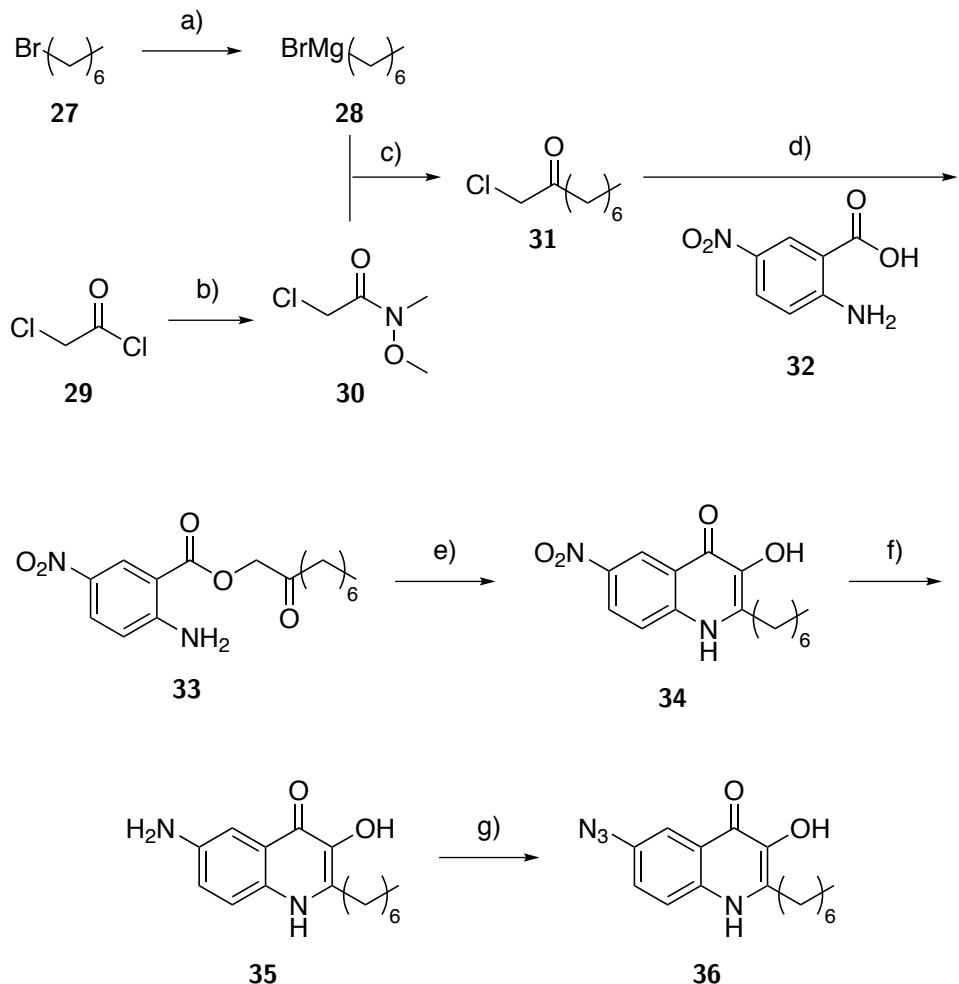
Conditions for the reduction of the nitro group were then compared (see Table 2). Baker initially used Zn and HCl, however, this gave a yield over 100 % suggesting coordination of amino-PQS **35** to the Zn.³ Reduction with $SnCl_2$ was then attempted, however, no product was detected by LCMS. Catalytic hydrogenation was then attempted. We determined that nitro-PQS **34** could not be reduced using H_2 and Pd/C at room temperature and pressure. However, increasing the pressure to 3 atm is sufficient to cause conversion to amino-PQS **35** in 4 h. Achieving 3 atm pressure of H_2 in a lab environment requires the use of a Parr hydrogenator, and it was found to be more convenient to use PtO_2 as a catalyst as this allows the reaction to proceed at room pressure and temperature. Finally, amino-PQS **35** was converted to azido-PQS **36** by reaction with $NaNO_2$ and HCl to form diazo-PQS, followed by displacement of the diazo group using NaN_3 to give the azido-PQS **36**.

Conditions	Outcome
Eaton's reagent, 70 °C, 10 h.	Product 34 and black p. 
Polyphosphoric acid, 90 °C, 5.5 h.	Product 34

Table 1: Conditions attempted for the synthesis of **34**

Conditions	Outcome
$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, MeOH, r.t., 18 h	No reaction
H_2 , Pd/C, MeOH, 3 atm, r.t., 4 h.	Product 35 , > 100 % yield
H_2 , PtO_2 , MeOH, 1 atm, r.t., 45 min	Product 35 , 80 % yield

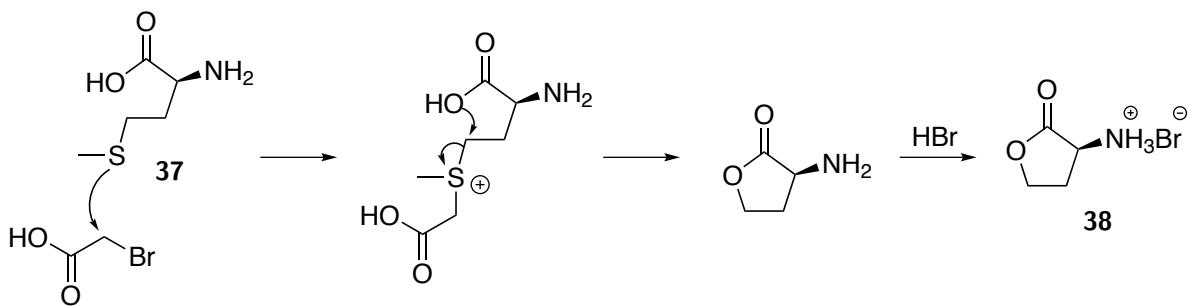
Table 2: Conditions attempted for the synthesis of **35**



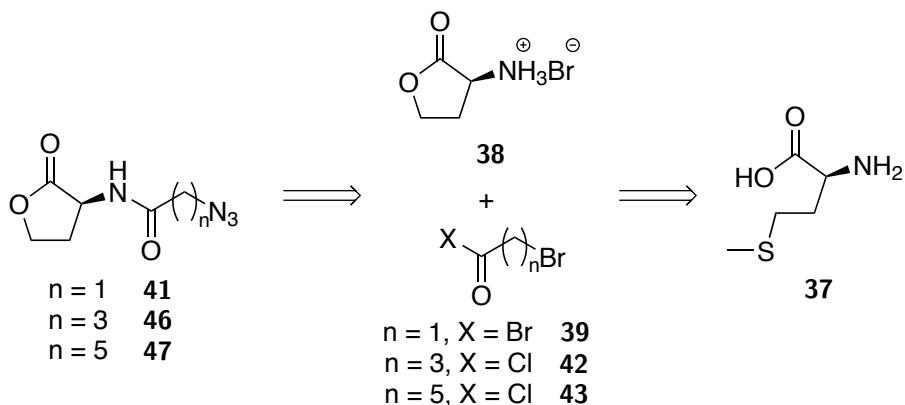
Scheme 5: The synthesis of **36** a) Mg turning , THF, r.t., 2 h then reflux, 2 h. b) N,O -dimethylhydroxyl amine hydrochloride, K_2CO_3 , toluene, H_2O , -5°C to r.t., 30 min, 71 %. c) THF, 0°C to r.t., 15 h, 96 %. d) **32**, K_2CO_3 , DMF, 90°C , 1 h, then **31**, r.t., 18 h, 100 %. e) Polyphosphoric acid, 90°C , 5.5 h, 40 %. f) H_2 , PtO_2 , MeOH, 1 atm, r.t., 45 min, 80 %. g) i) NaNO_2 , HCl , H_2O , 0°C , 50 min. ii) NaN_3 , H_2O , r.t., 4 h, 28 %.

6.1.5 Retrosynthesis of C₄-HSL analogues **41**, **46** and **47**

The azido analogue of C₄-HSL with a C₂ chain **41** (see Figure 12) has previously been prepared by Stacey *et al.*⁹ It uses the cyclisation of L-methionine **37** using bromoacetic acid via the mechanism shown in Scheme 6 to form the homoserine lactone HBr salt **38**. This is then converted by a biphasic one-pot process to the azido-C₂ analogue **41** using bromoacetyl bromide **39** and NaN_3 . It was hoped that this procedure could also be used to produce the azido-C₄ and C₆ chain analogues.



Scheme 6: The mechanism of formation of **38**

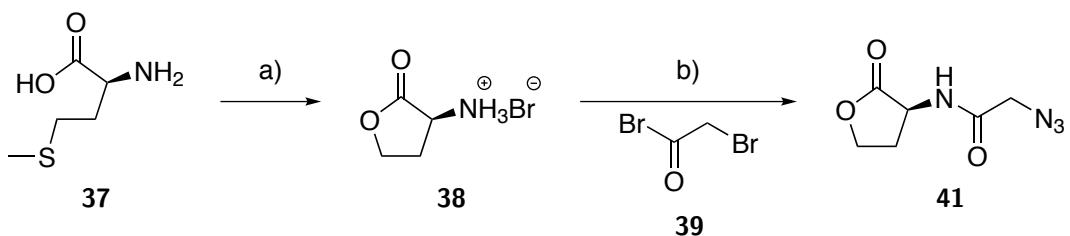


Scheme 7: The retrosynthesis of **41**, **46** and **47**

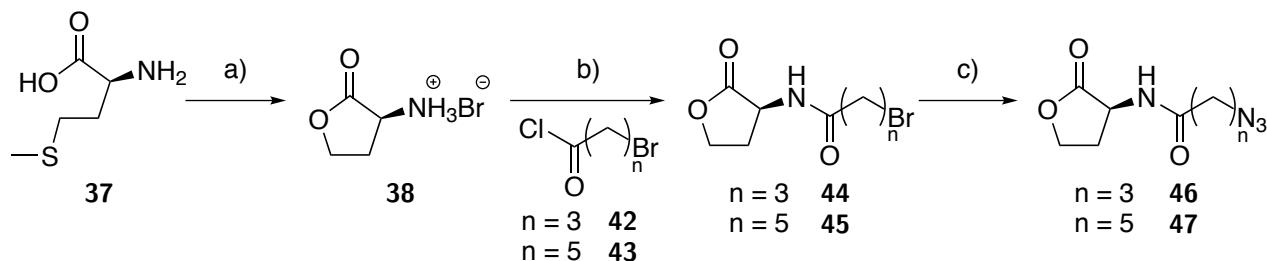
6.1.6 Synthesis of C₄-HSL analogues **41**, **46** and **47**

Homoserine lactone HBr salt **38** was synthesised using the procedure developed by Stacey *et al.*,⁹ followed by conversion to the azido-C₂ analogue **41** (see Scheme 8). Attempts to convert homoserine lactone **37** to the azido-C₄ analogue using 4-bromobutyryl chloride **42** produced a complex mixture of products. This is likely to be because the S_N2 reaction where the azide anion displaces bromine is slower as the bromine atom being displaced is no longer next to a carbonyl group. Hence, this allows more side reactions to occur instead of the desired reaction. It was therefore decided that the conversion should be carried out as a two-step process, where a bromoacyl chain is first installed, followed by the S_N2 reaction with NaN₃ (see Scheme 9).

Reaction of the homoserine lactone HBr salt **38** with 4-bromobutyryl chloride **42** or 6-bromohexanoyl chloride **43** produced bromo-C₄ analogue **44** or bromo-C₆ analogue **45** respectively. Heating with NaN₃ in DMF converted bromo-C₆ analogue **45** to azido-C₆ analogue **47**. It is hoped that the same conditions can be used to convert bromo-C₄ analogue **44** to azido-C₄ analogue **46** and this will be attempted shortly.



Scheme 8: The synthesis of **41** a) Bromoacetic acid, *i*-PrOH:H₂O:AcOH (5:5:2), r.t., 18 h, 41 %. b) NaN₃, NaHCO₃, H₂O/CH₂Cl₂, r.t., 18 h, 41 %.

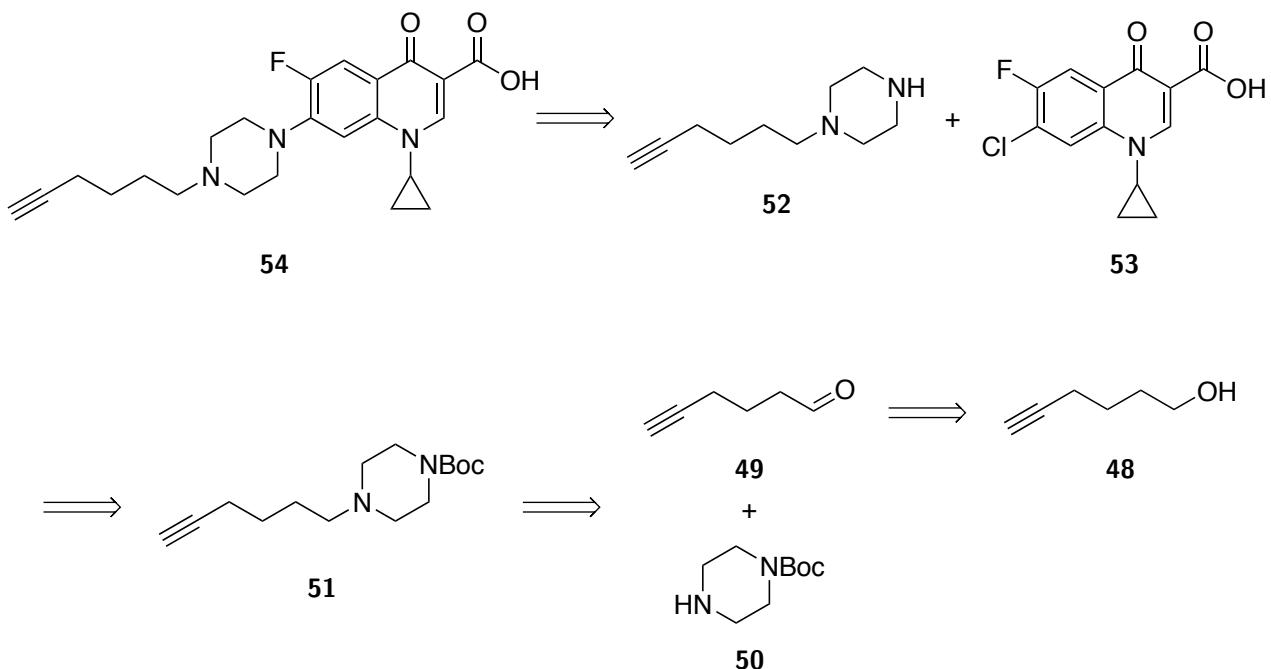


Scheme 9: The synthesis of **46** and **47**. a) Bromoacetic acid, *i*-PrOH:H₂O:AcOH (5:5:2), r.t, 18 h, 41 %. b) NaHCO₃, H₂O/CH₂Cl₂, r.t., 18 h, **44** : 80 %, **45** : 66 %. c) NaN₃, DMF, 100 °C, 5 h, **47** : 56 %.

6.2 Ciprofloxacin analogues

6.2.1 Retrosynthesis of ciprofloxacin analogue **54**

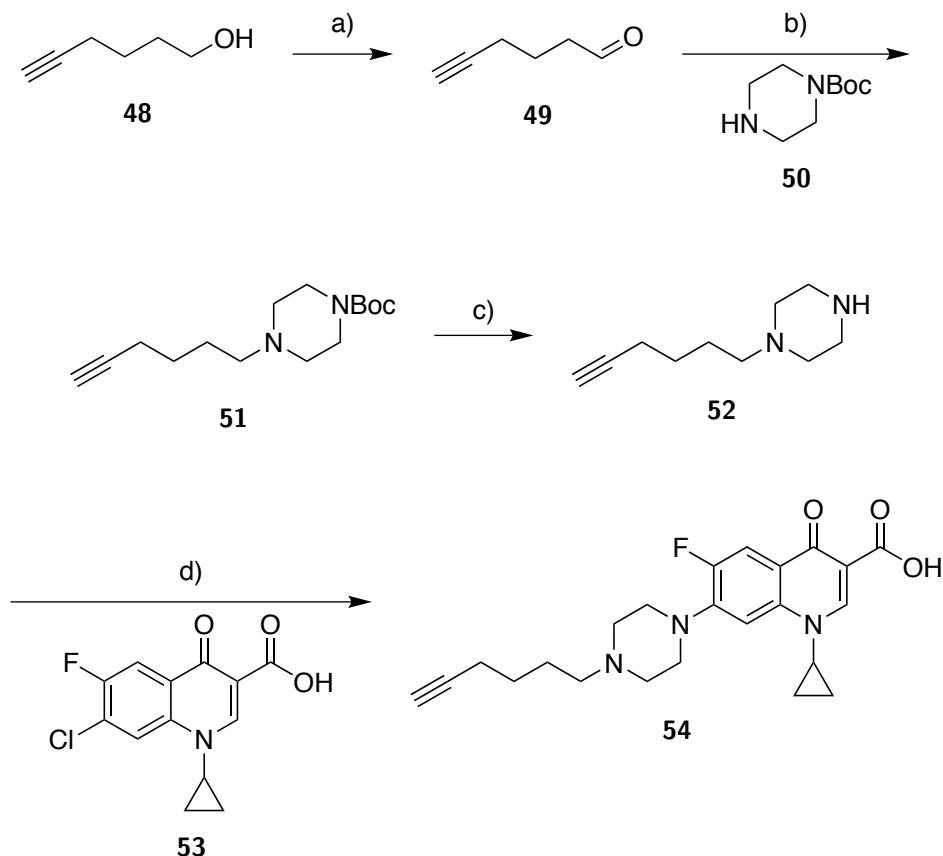
The retrosynthesis of ciprofloxacin analogue **54** is shown in Scheme 10. The analogue has an alkyne tail attached on the free piperazine N; this tail must be attached to piperazine before coupling the alkyl piperazine to the ciprofloxacin core. This can be achieved by reductive amination of hex-5-ynal **101** with 1-Boc-piperazine **50** followed by deprotection. This method was found by Renau *et al.* to be "...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide."^{12,66} S_NAr coupling of the piperazine derivative with ciprofloxacin precursor **53** leads to the final analogue **54**.



Scheme 10: The retrosynthesis of **54**

6.2.2 Synthesis of ciprofloxacin analogue **54**

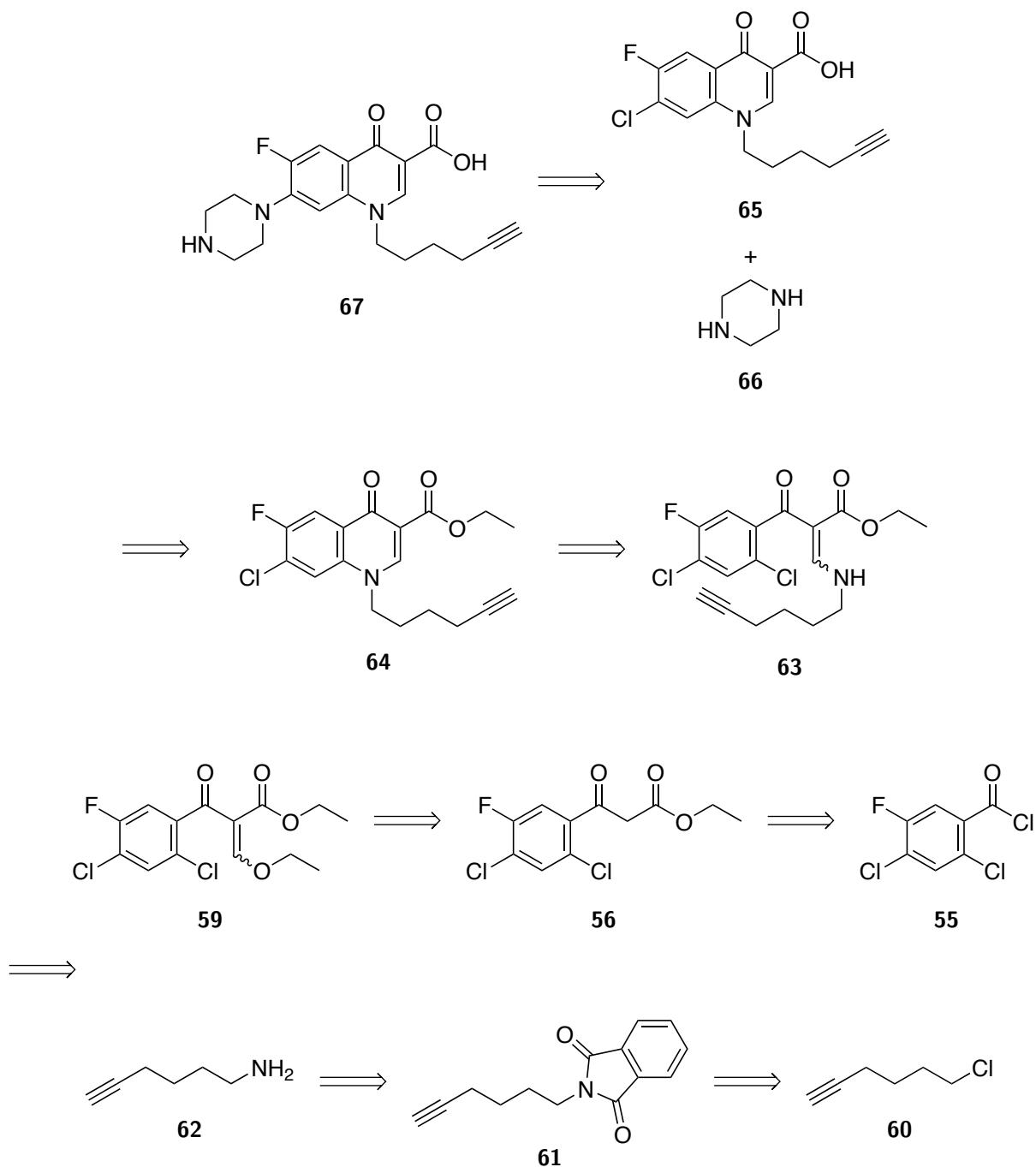
The synthesis of **54** follows the strategy followed by Renau *et al.*¹² Unlike the aldehydes and ketones used by Renau *et al.*,¹² hex-5-ynal **49** is not commercially available and so was successfully prepared by PCC oxidation of hex-5-ynol **48**, according to the procedure described by Kocsis *et al.*¹⁰ Renau *et al.*¹² used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal **49** and 1-Boc-piperazine **50**. However, it was decided to attempt this transformation using the less toxic sodium triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*¹¹ This reaction yielded compound **51**, which was deprotected using TFA using the procedure described by Renau *et al.*¹² to give compound **52**. This was refluxed in MeCN with the commercially available ciprofloxacin precursor **53** according to the procedure described by Renau *et al.*,¹² however the reaction did not proceed. Addition of NEt_3 did not lead to reaction, however refluxing in neat NEt_3 lead to conversion to the final ciprofloxacin analogue **54**.



Scheme 11: The synthesis of **54**. a) Pyridinium chlorochromate, CH_2Cl_2 , r.t., 5 h, 72 %. b) $\text{NaBH}(\text{AcO})_3$, 1,2-dichloroethane, r.t., 10.5 h, 99 %. c) TFA, r.t., 1 h, 100 %. d) NEt_3 , reflux, 15 h, **yield %**.

6.2.3 Retrosynthesis of ciprofloxacin analogue **67**

Analogue **67** has an alkyne tail attached in place of the cyclopropane ring at position 7 (see Figure 14); its retrosynthesis is shown in Scheme 12. This synthesis follows a conventional synthesis of ciprofloxacin similar to that reported by Mitscher *et al.*¹⁵ but using hex-5-yn-1-amine **62** instead of cyclopropylamine. **55** should react with potassium ethyl malonate with loss of CO_2 to form **56**, followed by heating with triethyl orthoformate to form **59**.^{14,15} This would then be heated with hex-5-yn-1-amine **62**, as opposed to the cyclopropylamine used in the conventional synthesis, to form **63**. Hex-5-yn-1-amine **62** could be produced using the Gabriel synthesis from **60**.^{16,17,67} **63** could be cyclised using NaH to form **64** followed by ester hydrolysis using KOH to give **65** as reported by Mitscher *et al.* **65** would then be heated with piperazine in DMSO ⁶⁸ to complete the synthesis of **67**.

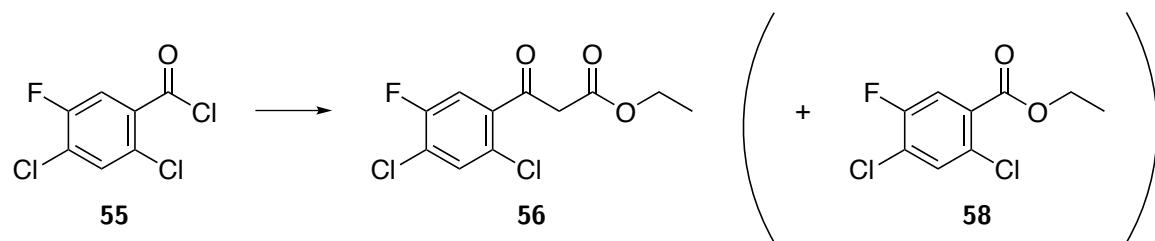


Scheme 12: The retrosynthesis of **67**

6.2.4 Synthesis of ciprofloxacin analogue **67**

The initial synthesis of **56** was attempted using a Claisen condensation and decarboxylation procedure developed by Hanan *et al.*⁶⁹ involving stirring **55** with potassium ethyl malonate, MgCl₂ and NEt₃. 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.*² was used to convert an acid chloride to a β -ketoester via a Medrums acid adduct was then attempted. The procedure did produce the desired β -ketoester **56**, however, it also produced significant amounts of the ethyl ester **58** as a side-product, despite attempts to remove excess acid chloride **55** before refluxing in ethanol. A modification used by Yamamoto⁷⁰ which substituted pyridine with 4-dimethylaminopyridine also failed to suppress formation of the ethyl ester side product **58**. 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 $TiCl_4$ -catalysed crossed Claisen condensation of the acid chloride **55** and ethyl acetate described by Hashimoto *et al.*¹³ was chosen. This produced the β -ketoester **56** without the ethyl ester side product **58**.



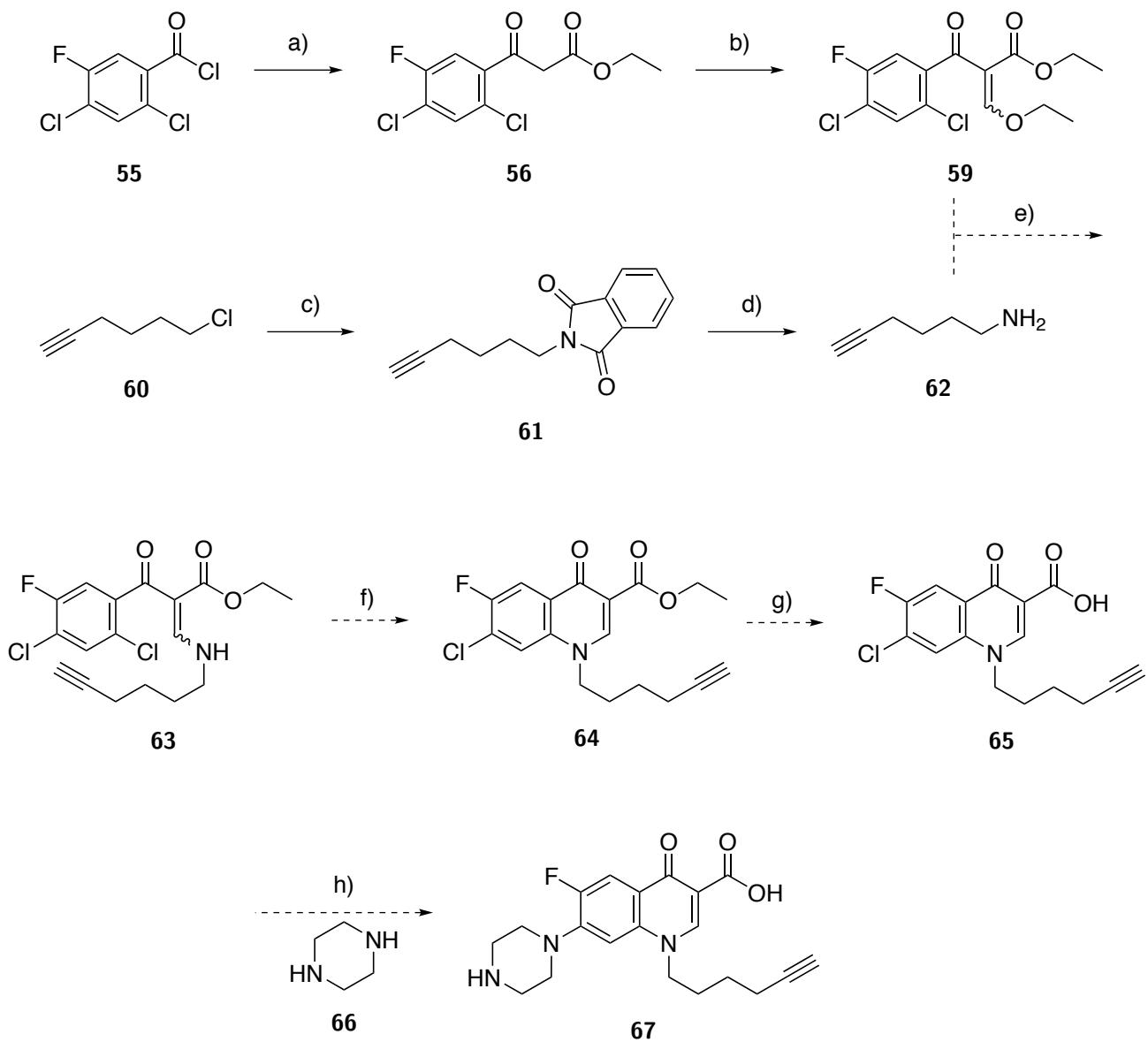
Scheme 13: The optimisation of the synthesis of **56** (see Table 3)

Conditions	Outcome
Potassium ethyl malonate NEt_3 , $MgCl_2$, MeCN, r.t., 18 h.	No reaction
Potassium ethyl malonate NEt_3 , $MgCl_2$, r.t., 18 h.	No reaction
i) Meldrum's acid, pyridine, CH_2Cl_2 , 0 °C, 4 h. ii) EtOH, reflux, 18 h.	Product 56 and side-product 58
i) Meldrum's acid, 4-dimethylaminopyridine, CH_2Cl_2 , 0 °C, 4 h. ii) EtOH, reflux, 18 h.	Product 56 and side-product 58
$EtOAc$, $TiCl_4$, DIPEA, <i>N</i> -methyl imidazole, toluene, r.t., 30 min.	Product 56



Table 3: Conditions attempted for the synthesis of **56** (see Scheme 13)

The ethoxymethylene group in **59** was installed by the reaction of β -ketoester **56** and triethyl orthoformate to give a mixture of the *E* and *Z* isomers. Hex-5-yn-1-amine **62** was prepared using a Gabriel synthesis⁶⁷ described by Rożkiewicz *et al.*¹⁷ 6-Chlorohex-1-yne **60** was heated with potassium phthalimide to form **61**, which was then cleaved using hydrazine monohydrate to form hex-5-yn-1-amine **62**. The remainder of the synthesis of **67** is in progress (see Scheme 14).



Scheme 14: The synthesis of **67** a) EtOAc, TiCl_4 , DIPEA, *N*-methyl imidazole, toluene, r.t., 30 min, **yield %**.
 b) Triethyl orthoformate, Ac_2O , reflux, 2 h, **yield %**. c) Potassium phthalimide, KI, DMF, 80 °C, 18 h, 75 %.
 d) $\text{N}_2\text{H}_2\text{H}_2\text{O}$, EtOH, reflux, 18 h, **yield %**. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) Piperazine, DMSO.

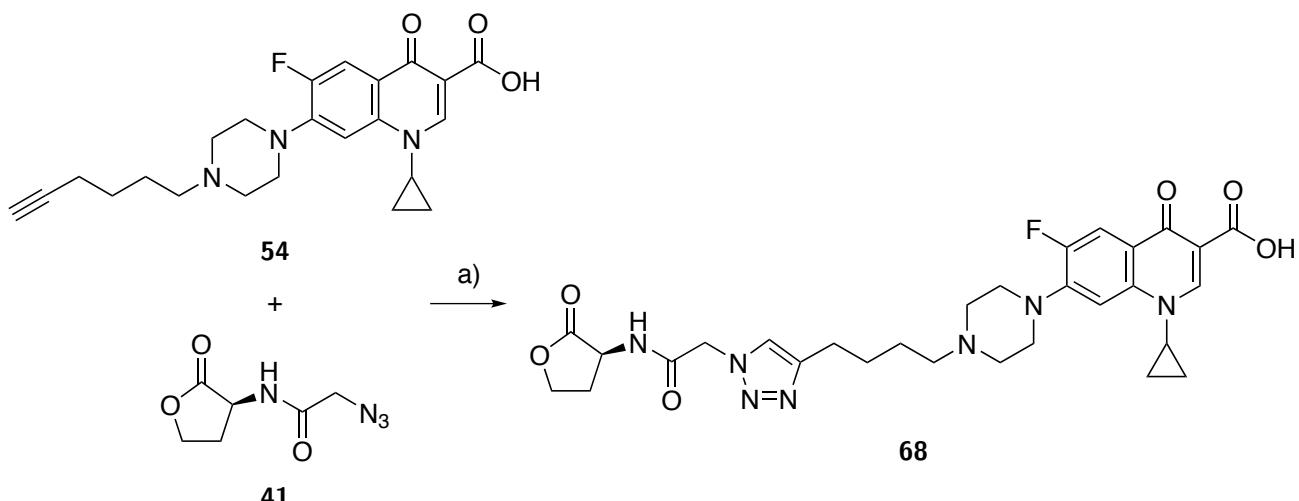
6.3 Quorum sensing molecule-antibiotic conjugates

6.3.1 Synthesis of quorum sensing molecules-antibiotic conjugate **68**

Test reactions were performed to find conditions for the click reaction between C₄-HSL analogue **41** and ciprofloxacin analogue **54** (see Scheme 15 and Table 4). Stirring at room temperature had no effect even with an extended reaction time, however, heating to 50 °C did lead to slow formation of the product as seen by LCMS. It is expected that the use of a ligand such as tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) should increase the rate of reaction to a more desirable speed.

Conditions	Outcome
CuSO_4 , sodium ascorbate, H_2O , <i>t</i> -BuOH, r.t., 7 d.	No reaction
CuSO_4 , sodium ascorbate, H_2O , <i>t</i> -BuOH, 50 °C, 5 d.	Product 68 seen by LCMS

Table 4: Conditions attempted for the synthesis of **68** (see Scheme 15)

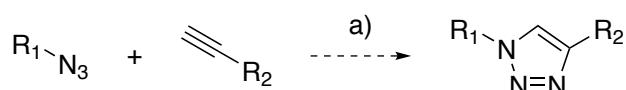


Scheme 15: Synthesis of **68**. a) see Table 4.

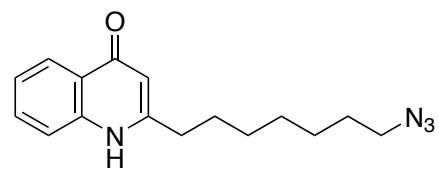
7 Future work

7.1 Quorum sensing molecule-antibiotic conjugates

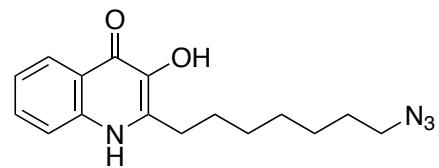
The final library of conjugates will be constructed using a click reaction, probably including TBTA, to decrease reaction times (see Scheme 16). The library will be built using seven QSM analogues and two ciprofloxacin analogues. The QSM analogues will include the three C₄-HSL analogues **41**, **46** and **47**, HHQ analogue **26** and PQS analogue **36**. In addition, two further QSM analogues with azide groups on the alkyl tail will also be included. These extra analogues, HHQ analogue **69** and PQS analogue **70** (see Figure 15), have been synthesised by Baker. The two ciprofloxacin analogues, **54** and **67**, will be reacted with all seven QSM analogues to produce fourteen final conjugates (see Scheme 16 and Table 5).



Scheme 16: Synthesis of the final library. For R_1 and R_2 see Table 5. a) $CuSO_4$, sodium ascorbate, TBTA, H_2O , $t\text{-BuOH}$.



69



70

Figure 15

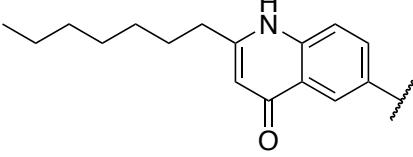
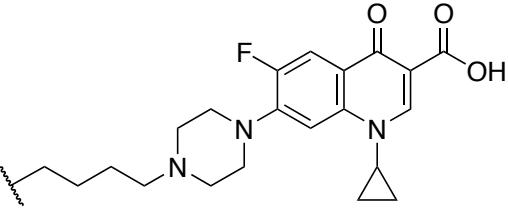
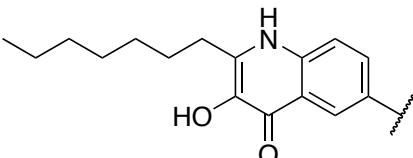
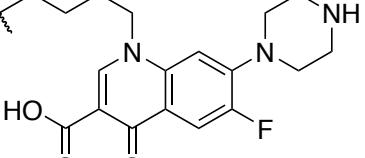
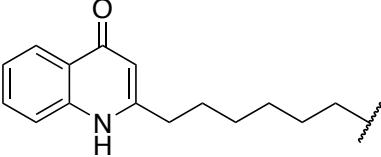
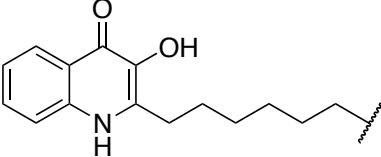
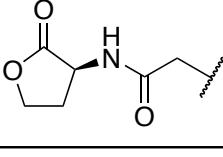
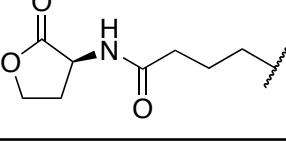
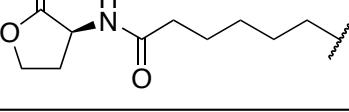
R_1	R_2
	
	
	
	
	
	
	

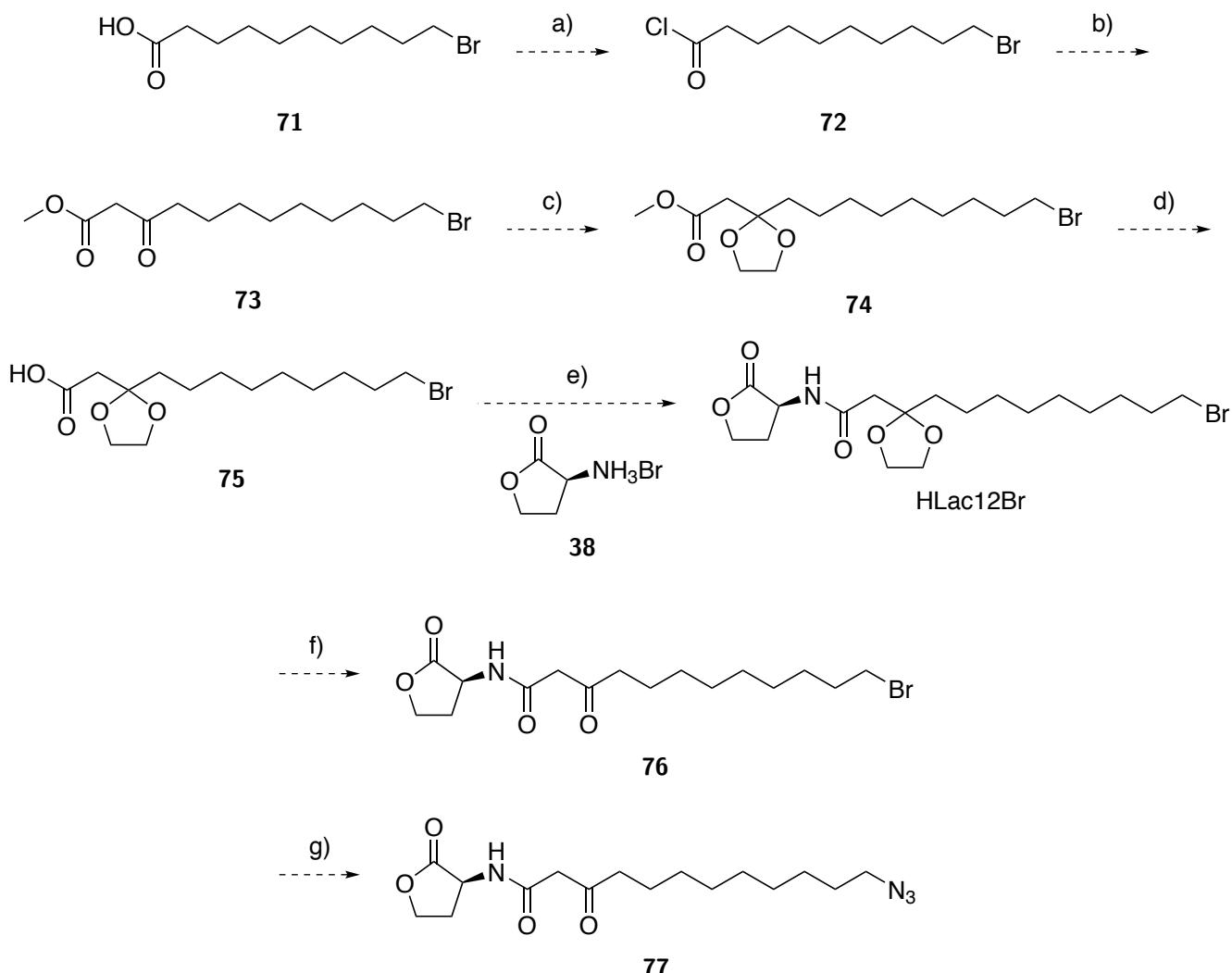
Table 5: Possible R_1 and R_2 groups (see Scheme 16).

7.2 Quorum sensing molecule analogues

The only *P. aeruginosa* not yet to have been considered in this project is 3-oxo-C₁₂-HSL analogue **3** (see Figure 4). This would be the most obvious next target for study. After this, there are several other QSMs which are not produced by *P. aeruginosa* which could be investigated (see Figure 16).

7.2.1 3-oxo-C₁₂-HSL analogue 77

The synthesis of 3-oxo-C₁₂-HSL has previously been reported by Hodgkinson *et al.*⁵ A modification of this synthesis using 10-bromodecanoyl chloride could be used to produce analogue **77** with a tail azide (see Scheme 17). Analogues with shorter or longer tail lengths (known to affect selectivity and binding affinity) could also be synthesised using the same method.



Scheme 17.  Synthesis of 3-oxo-C₁₂-HSL analogue **77**. a) Oxalyl chloride, DMF, CH₂Cl₂, r.t. b) i) pyridine, DCM, 0 °C. ii) MeOH, reflux. c) *p*-TsOH, HO(CH₂)₂OH, CH(OMe)₃, r.t. d) NaOH, H₂O, r.t. e) EDC, DMAP, CH₂Cl₂, r.t. f) TFA, r.t. g) NaN₃, DMF, 50 °C

7.2.2 Non-*P. aeruginosa* QSMs

Many species of bacteria other than *P. aeruginosa* produce QSMs⁷¹ (see Figure 16). An azido analogue of C₈-HSL **78** could be produced in a similar manner to the C₄-HSL analogues already synthesised. An azido analogue of 3-oxo-C₆-HSL **1** could be produced in the manner proposed for 3-oxo-C₁₂-HSL **3** above. Analogues of AI-2 **79** could have azide groups in the place of the OH groups on the sugar section of the molecule. Analogues of AIP **81** and ComX **80** could be synthesised by conversion of their terminal amines to azides. Analogues of AIP **81** 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 **80** 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 within the peptide chain.

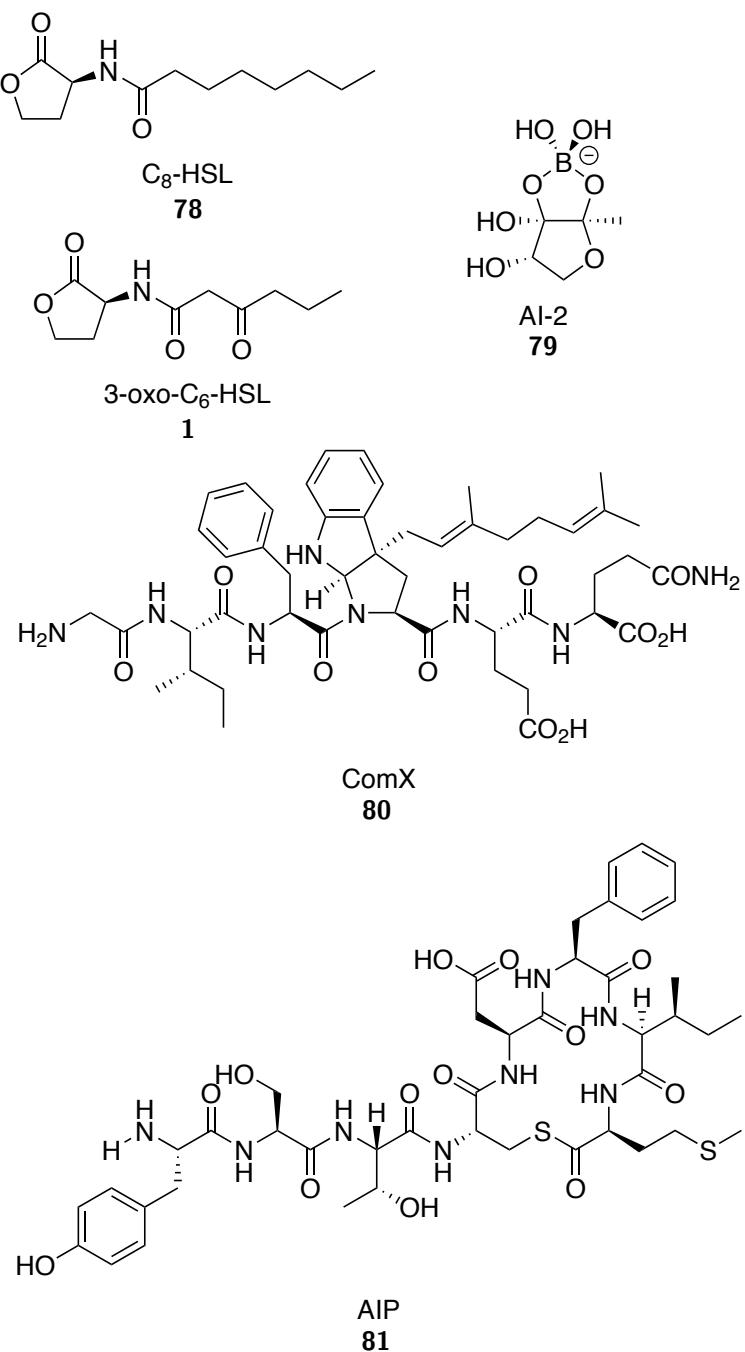


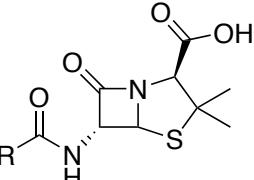
Figure 16: Quorum sensing molecules from various bacterial species. C₈-HSL **78** is from *Burkholderia cepacia*, 3-oxo-C₆-HSL **1** is from *Erwinia chrysanthemi*, AI-2 **79** is found in both Gram-positive and Gram-negative bacteria, ComX **80** is from *Bacillus subtilis*, AIP **81** is from *Staphylococcus aureus*.

7.3 Antibiotic analogues

7.3.1 Penicillin analogue **85**

The penicillins are a group of antibiotics with the same penam core structure but different R groups (see Figure 17). It therefore seems likely that a biologically active penicillin analogue could be synthesised with an alkyne in the R group. This could be produced using the penicillin precursor 6-aminopenicillanic acid and 5-hexynoic acid or a derivative thereof. An initial attempt at synthesis was based on a procedure developed by

Faridoon.⁷² Firstly, 5-hexynoic acid was converted to 5-hexanoyl chloride using oxalyl chloride and catalytic DMF, unlike in the Faridoon procedure which uses thionyl chloride. 5-hexanoyl chloride was then stirred with 6-aminopenicillanic acid, 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 and its derivatives are too sensitive to basic conditions for these conditions to be used, most likely due to opening of the β -lactam ring followed by further decomposition reactions. Products were also seen to undergo methanolysis during 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 19).



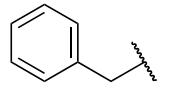
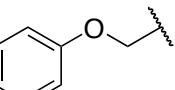
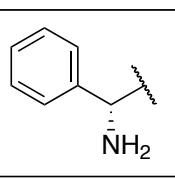
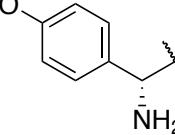
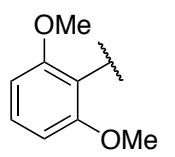
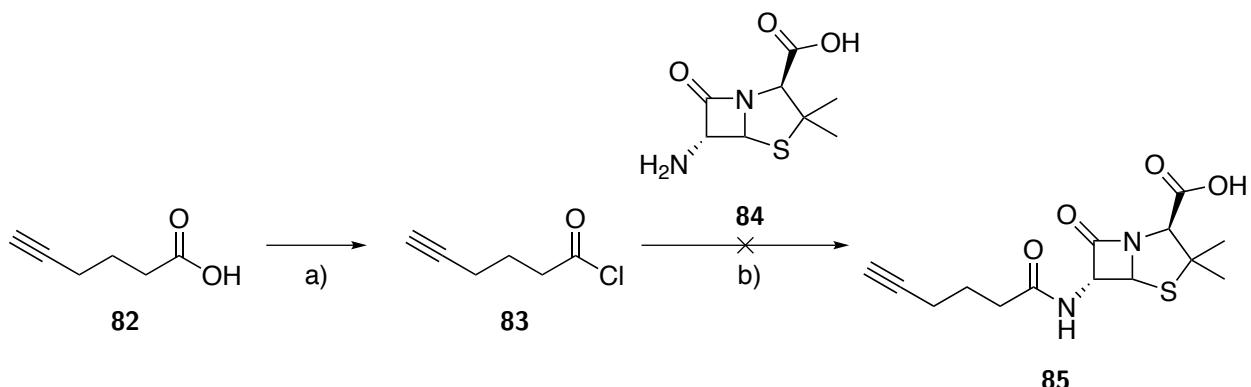
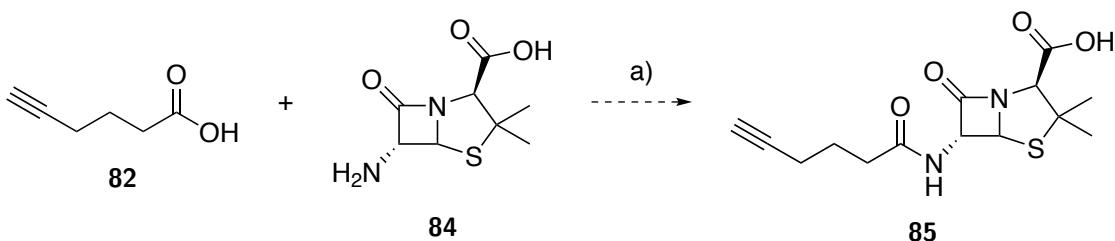
Name	R
Penicillin G	
Penicillin V	
Ampicillin	
Amoxicillin	
Methicillin	

Figure 17: The penicillins



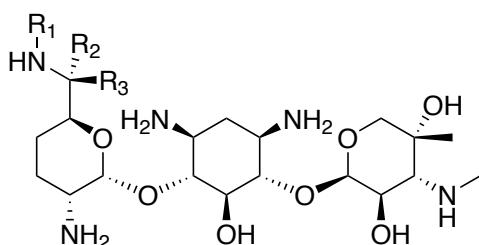
Scheme 18: Attempted synthesis of **85**. a) oxalyl chloride, DMF, CH_2Cl_2 , r.t., 3 h. b) DIPEA, $\text{CH}_2\text{Cl}_2/\text{pyridine}/\text{NaHCO}_3$, Acetone, $\text{H}_2\text{O}/\text{NaHCO}_3$, CH_2Cl_2 , H_2O , all r.t., 18 h.



Scheme 19: Proposed synthesis of **85**. a) DCC, HOBr, DMF.

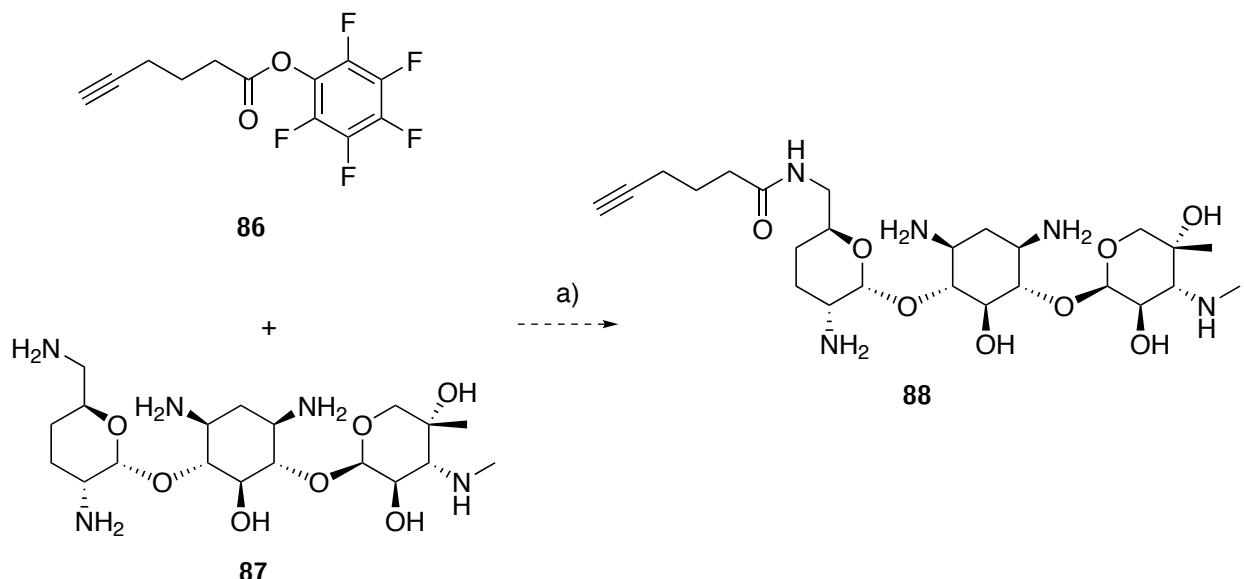
7.3.2 Gentamicin analogue **88**

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 18) synthesised by *Micromonospora*, a genus of Gram-positive bacteria. Separation of the gentamicin components has been achieved by Grote *et al.*⁷³ by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a **87** was isolated pure, and is particularly useful because it is the only component which contains a CH_2NH_2 group. This group is less hindered than all other amine groups in gentamicin C1a **87** 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. Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid **86** (see Scheme 20). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester and then separate out the desired component.



Gentamicin	R_1	R_2	R_3
C1	Me	Me	H
C1a	H	H	H
C2	H	Me	H
C2a	H	H	Me
C2b	Me	H	H

Figure 18: Gentamicin components.



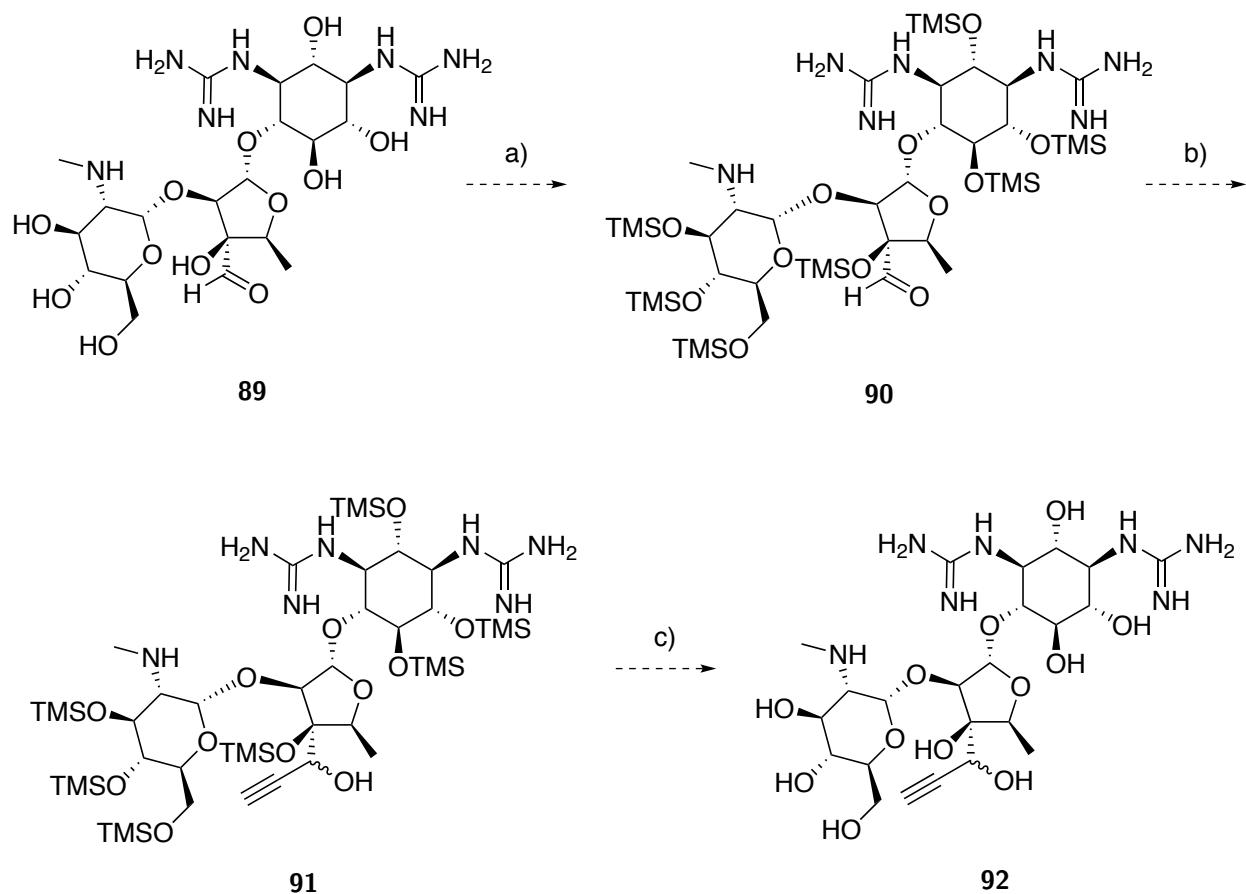
Scheme 20: Proposed synthesis of gentamicin C1a analogue **88**. a) DIPEA, DMF, $-55\text{ }^\circ\text{C}$.

7.3.3 Streptomycin analogues **92**, **94** and **96**

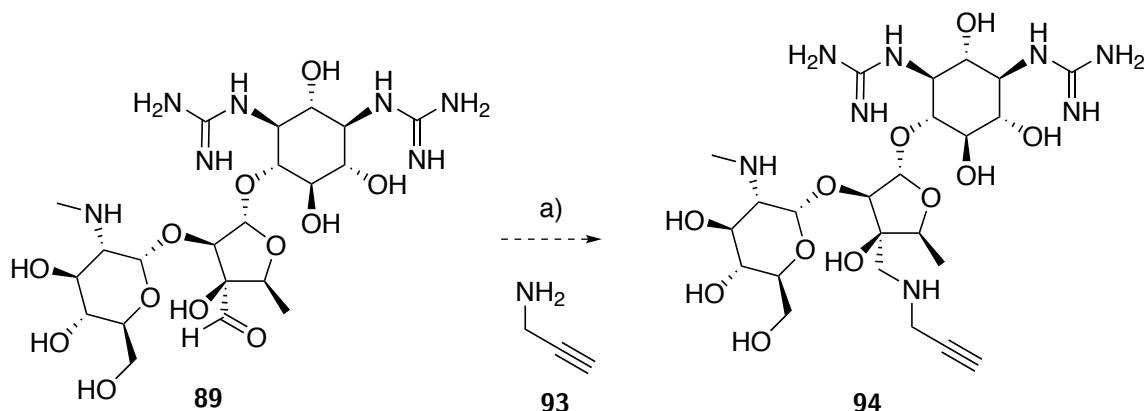
Streptomycin **89** is an aminoglycoside antibiotic used to treat *Mycobacterium tuberculosis* and *Staphylococcus aureus* by binding to the bacterial ribosome. There is limited SAR data on streptomycin but it is known that conversion of the aldehyde to a carboxylic acid destroys activity, whereas conversion an alcohol retains it.⁷⁴ TMS protection followed by attack with lithium acetylide then deprotection could be used to produce an analogue **92** with a secondary alcohol in place of the aldehyde (see Scheme 21).

Reductive amination could also be used to install an alkyne group by reaction of the aldehyde with amino-1-propyne (see Scheme 22).¹¹ 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.

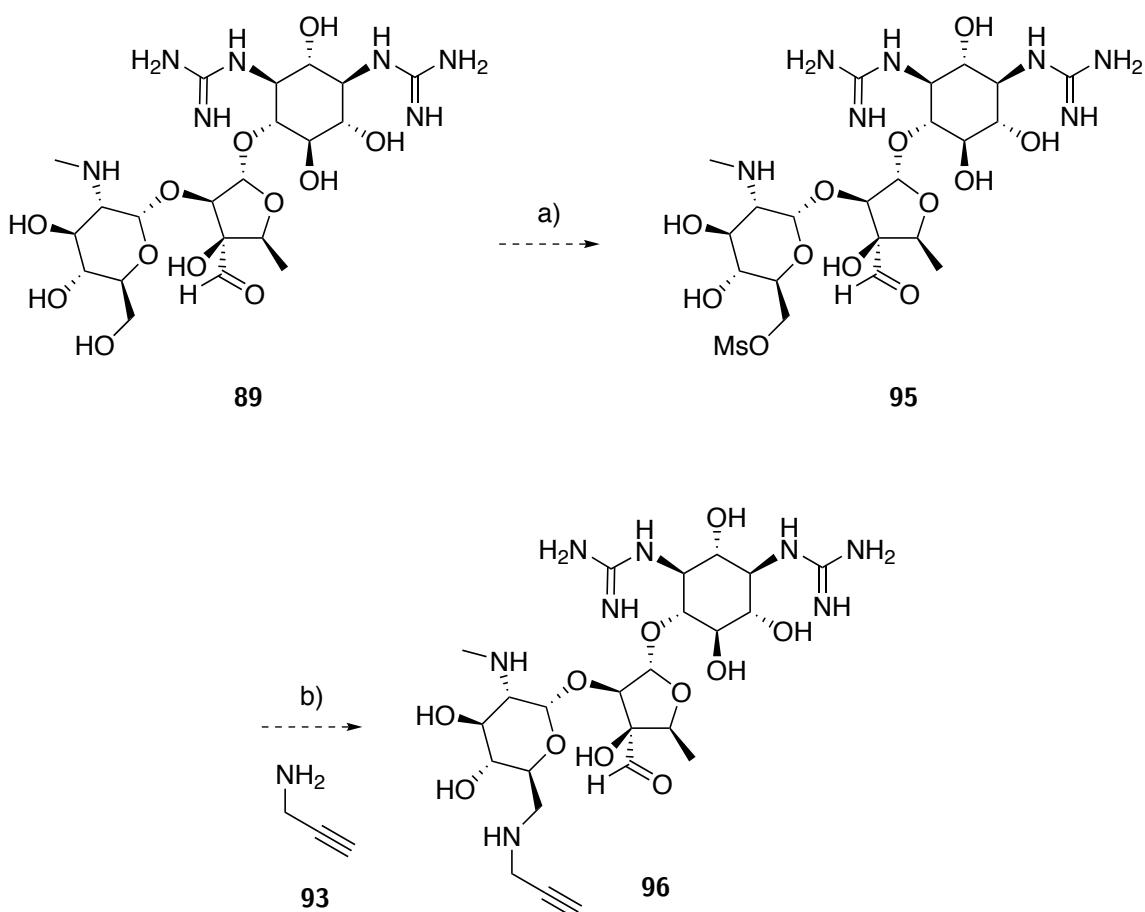
There is one primary alcohol in streptomycin **89** which could be selectively displaced to install an alkyne. Selective displacement could be achieved by reaction with 1 eq. of trifluoroacetic anhydride followed by displacement with amino-1-propyne (see Scheme 23).⁷⁵ This leaves an H-bond donor in that position as OH is replaced by NH.



Scheme 21: Proposed synthesis of streptomycin analogue **92**. a) TMSCl. b) Lithium acetylide. c) TBAF.



Scheme 22: Proposed synthesis of streptomycin analogue **94**. a) $\text{NaBH}(\text{AcO})_3$, 1,2-dichloroethane, r.t.



Scheme 23: Proposed synthesis of streptomycin analogue **96**. a) MsCl , pyridine, CH_2Cl_2 , 0 °C to r.t. b) 3-amino-1-propyne, EtOH.

8 Conclusions

9 Experimental

9.1 General

Unless otherwise stated, reactions were performed in oven-dried glassware under nitrogen with  freshly distilled solvents. THF was distilled from LiAlH_4 in the presence of triphenyl methane indicator. TEA, CH_2Cl_2 , hexane, MeOH, pyridine and MeCN were distilled from calcium hydride. Anhydrous DMF (Acros) and DCE (Aldrich) were used without further purification. All other chemicals were either purified as outlined in "Purification of Laboratory Chemicals"⁷⁶ or used as obtained from commercial sources.

Unless otherwise stated, yields refer to analytically pure compounds. TLC was performed using Merck pre-coated 0.23mm thick plates of Keiselgel 60 F254 and visualised using UV ($\lambda = 254$ nm) or by staining with KMnO₄. All column chromatography was carried out using Merck 9385 Keiselgel 60 silica gel (230-400 mesh).

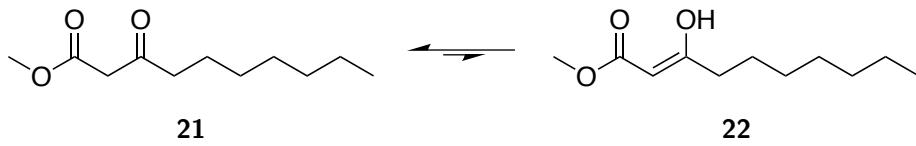
¹H NMR spectra were recorded on Bruker DPX 400 or 500 spectrometers operating at 400 or 500 MHz respectively using an internal deuterium lock at ambient probe temperatures. Chemical shifts (δ) are quoted to the nearest 0.01 ppm and are referenced relative to residual solvent peak.⁷⁷ Coupling constants (J) are given to the nearest 0.1 Hz. The following abbreviations are used to indicate the multiplicity of signals: s singlet, d doublet, t triplet, q quartet, m multiplet and b broad. Data is reported as follows: chemical shift (multiplicity, coupling constant(s), integration, assignment).

¹³C NMR spectra were recorded on Bruker 400 or 500 spectrometers operating at 101 and 125 MHz respectively using an internal deuterium lock at ambient probe temperatures. Chemical shifts (δ) are quoted to the nearest 0.1 ppm and are referenced relative to the deuterated solvent peak.⁷⁷ NMR assignments are supported by DEPT editing and where necessary COSY, HMQC, and HMQC.

High resolution mass spectra (HRMS) were recorded using either a Micromass Q-TOF or a Micromass LCT Premier spectrometer and reported mass values are given ± 5 ppm mass units unless otherwise stated. LCMS spectra were recorded on an Agilent 1200 series LC with an ESCi Multi-Mode Ionisation Waters ZQ spectrometer using MassLynx 4.1 software.

IR spectra were recorded using neat sample on a PerkinElmer 1600 FT IR spectrometer. Selected absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations ($[\alpha]_D$) were recorded on a PerkinElmer 343 polarimeter. $[\alpha]_D$ values are reported in $10^{-1} \text{ }^\circ \text{cm}^2 \text{ g}^{-1}$ at 589 nm and concentration (c) is given in g (100 mL) $^{-1}$.

9.2 Methyl 3-oxodecanoate 21^{1,2}



Meldrum's acid (9.0 g, 63 mmol, 1 eq.) was dissolved in anhydrous CH_2Cl_2 (150 mL) and cooled to 0 °C. Pyridine (10.2 mL, 126 mmol, 2 eq.) was added dropwise over 20 min. Octanoyl chloride (11.7 mL, 69 mmol, 1.1 eq.) was then added and the mixture was stirred at 0 °C for a further 4 h. The mixture was allowed to warm to room temperature, diluted with CH_2Cl_2 (20 mL) and poured into a mixture of ice (~ 30 g) and HCl (2 N, 90 mL). The solution was washed with NaCl (sat., aq., 150 mL) and dried over Mg_2SO_4 . The solvent was removed under vacuum to give an orange-brown oil. The oil was refluxed in anhydrous MeOH (150 mL) for 5 h and the solvent was removed under vacuum. The resulting residue was purified by column chromatography (SiO_2 , 5 % $\text{Et}_2\text{O}/40\text{-}60$ P.E.) to give a tautomeric mixture of **21** and **22** as a colourless oil (8.34 g, 41.6 mmol, 66.1 %, 92 % **21** as determined by NMR).

Keto form **21**

¹H NMR (400 MHz, CDCl₃) δ = 3.74 (s, 3 H, OCH₃), 3.45 (s, 2 H, C(=O)CH₂C(=O)), 2.53 (t, J = 7.4 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 1.60 (quin, J = 7.1 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.39 - 1.19 (m, 9 H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 0.88 (t, J = 6.8 Hz, 3 H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, CDCl₃) δ = 202.3 (CH₃OC(=O)CCH₂C(=O)), 167.3 (CH₃OC(=O)CCH₂C(=O)), 51.7 (OCH₃), 48.5 (CH₃OC(=O)CH₂C(=O)), 42.5 (CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 31.3 (CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 28.7 (CH₂CH₂CH₂CH₂CH₂CH₃), 28.6 (CH₂CH₂CH₂CH₂CH₂CH₃), 23.1 (CH₂CH₂CH₂CH₂CH₂CH₃), 22.2 (CH₂CH₂CH₂CH₂CH₂CH₃), 13.6 (CH₂CH₂CH₂CH₂CH₂CH₃)

IR (neat) ν_{max} / cm⁻¹ = 2927.84 (C-H), 2856.26 (C-H), 1746.86 (ester C=O), 1716.70 (ketone C=O)

Enol form **22**

¹H NMR (400 MHz, CDCl₃) δ = 12.02 (s, 1 H, COH), 4.99 (s, 1 H, C(=O)CH=COH), 3.73 (s, 3 H, OCH₃),

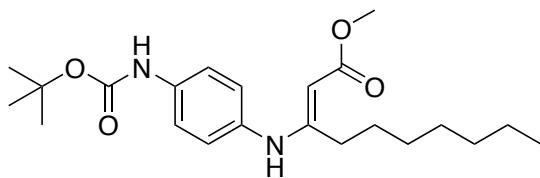
Pretty much
nicer, but
is this
or should
I re-work
more? Has
anything
changed?

2.20 (t, $J = 7.4$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.76 - 1.72 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.39 - 1.19 (m, 8 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.88 (t, $J = 6.8$ Hz, 3 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$)

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

IR (neat) ν_{max} / $\text{cm}^{-1} = 2927.84$ (C-H), 2856.26 (C-H), 1653.80 (C=C), 1629.21 (α,β unsaturated C=O)

9.3 Methyl (*E*)-3-((4-((*tert*-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 24³



Methyl 3-oxodecanoate **21** (500 mg, 2.50 mmol, 1.00 eq.) and *tert*-butyl (4-aminophenyl)carbamate **102** (520 mg, 2.50 mmol, 1.00 eq.) were dissolved in MeOH (10 mL) and refluxed for 18 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (SiO_2 , gradient of 0 to 20 % $\text{Et}_2\text{O}/40\text{-}60$ P.E.) to give a white solid ().

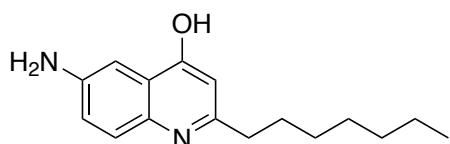
0.1676+we
of NMR?

^1H NMR (400 MHz, CDCl_3) $\delta = 10.16$ (s, 1 H, $\text{NH}(\text{C}_7\text{H}_{15})=\text{C}$), 7.35 (d, $J = 8.6$ Hz, 1 H, *meta* to NHBoc), 7.02 (d, $J = 8.7$ Hz, 1 H, *meta* to enamine), 6.60 (br. s., 1 H, NHBoc), 4.71 (s, 1 H, $\text{C}=\text{CH}$), 3.70 (s, 3 H, OCH_3), 2.23 (t, $J = 7.7$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.54 (s, 9 H, $\text{C}(\text{CH}_3)_3$), 1.40 (quin, $J = 7.3$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.33 - 1.16 (m, 8 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.86 (t, $J = 7.1$ Hz, 3 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$)

^{13}C NMR (101 MHz, CDCl_3) $\delta = 171.1$ ($\text{C}(=\text{O})\text{CH}=\text{C}$), 164.3 ($\text{C}(=\text{O})\text{CH}=\text{C}$), 152.7 ($\text{OC}(=\text{O})\text{NH}$), 136.0 (*para* to NHBoc), 134.1 (CNHBoc), 126.3 (*meta* to NHBoc), 119.1 (*ortho* to NHBoc), 83.8 ($\text{C}(=\text{O})\text{CH}=\text{C}$), 80.7 ($\text{C}(\text{CH}_3)_3$), 50.2 (OCH_3), 32.2 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 31.6 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.1 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 28.8 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 28.3 ($\text{C}(\text{CH}_3)_3$), 28.0 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 22.6 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 14.0 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$)

IR (neat) ν_{max} / $\text{cm}^{-1} = 3336.97$ (N-H), 2927.71 (C-H), 2857.14 (C-H), 1723.71 (carbamate C=O), 1634.49 (α,β unsaturated C=O), 1610.73 (C=C), 1580.85 (N-H bend)

9.4 6-amino-2-heptylquinolin-4-ol 25³

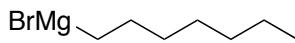


Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate **24** (168 mg, 0.649 mmol, 1 eq.) and polyphosphoric acid (5 g) were heated to 90 °C for 1 h. The reaction mixture was then poured into NaHCO₃ (50 mL, sat., aq.). The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum to give a pale orange solid (yield).

¹H NMR (400 MHz, DMSO-d₆) δ = 7.26 (d, J = 8.7 Hz, 1 H, *meta* to NH₂), 7.15 (d, J = 2.6 Hz, 1 H, *para* to COH), 6.95 (dd, J = 2.7, 8.8 Hz, 1 H, *ortho* to COH), 5.74 (s, 1 H, *ortho* to OH), 5.16 (s, 2 H, NH₂), 2.52 (t, J = 7.4 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.64 (quin, J = 7.6 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.36 - 1.19 (m, 8 H, CH₂CH₂CH₂CH₂CH₂CH₃), 0.86 (t, J = 7.0 Hz, 3 H, CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ = 176.7 (COH), 151.7 (CCH₂CH₂CH₂CH₂CH₂CH₃), 145.1 (CNH₂), 132.4 (*para* to NH₂), 126.6 (*para* to CH₂CH₂CH₂CH₂CH₂CH₃), 121.1 (*ortho* to NH₂ and *para* to COH), 119.0 (*meta* to NH₂ and *meta* to COH), 106.2 (*ortho* to NH₂ and *ortho* to COH), 105.9 (*ortho* to CH₂CH₂CH₂CH₂CH₂CH₃ and *ortho* to OH), 33.6 (CH₂CH₂CH₂CH₂CH₂CH₃), 31.6 (CH₂CH₂CH₂CH₂CH₂CH₃), 29.0 (CH₂CH₂CH₂CH₂CH₂CH₃), 29.0 (CH₂CH₂CH₂CH₂CH₂CH₃), 28.9 (CH₂CH₂CH₂CH₂CH₂CH₃), 22.5 (CH₂CH₂CH₂CH₂CH₂CH₃), 14.4 (CH₂CH₂CH₂CH₂CH₂CH₃) IR (neat) ν_{max} / cm⁻¹ = 3336.52 (N-H), 2926.47 (C-H), 2856.89 (C-H), 1723.88 (aromatic), 1634.48 (aromatic), 1610.84 (aromatic), 1583.26 (aromatic), 1519.06 (aromatic)

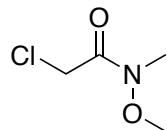
9.5 Heptyl magnesium bromide **28**⁴



Magnesium turnings (352 mg, 14.5 mmol, 1 eq.) were added to a dry flask under argon. THF (15 mL) was added, followed by bromoheptane (2.40 mL, 14.5 mmol, 1 eq.) dropwise. The mixture was stirred for 2 hours followed by heating to reflux for 2 hours to give the Grignard reagent as a pale grey suspension (15 mL, ~1 N) which was used without further purification.



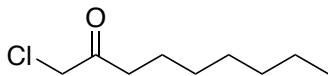
9.6 2-chloro-*N*-methoxy-*N*-methylacetamide **30**⁵



N,O-dimethylhydroxyl amine hydrochloride (6.00 g, 61.5 mmol) and toluene (75 mL) were added successively to a solution of potassium carbonate (22.4 g, 162 mmol) in water (75 mL) at 0 °C under argon. The mixture was cooled to - 5 °C and chloroacetyl chloride (5.88 mL, 73.8 mmol) was added dropwise over 5 min. The mixture was allowed to warm to room temperature over 30 min, then the organic layer was removed and the aqueous layer was extracted with toluene (3 x 20 mL). The combined organic extracts were dried with MgSO₄ and the solvent was removed by rotary evaporation followed by high vacuum to give a pale yellow crystalline solid (7.24 g, 71 %).

IR (neat) ν_{max} / cm⁻¹ = 3016.69 (C-H), 2966.38 (C-H), 2946.75 (C-H), 2827.73 (C-H), 1666.20 (C=O)

9.7 1-chlorononan-2-one **31**^{4,5}



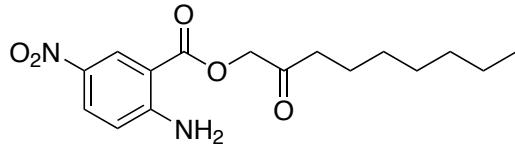
2-chloro-*N*-methoxy-*N*-methylacetamide (1.00 g, 7.26 mmol) was added to a dry flask under argon. THF (20 mL) was added and the flask cooled to 0 °C. Heptyl magnesium bromide (15.0 mL, ~ 1 N, 15.0 mmol) was added dropwise over 5 min, then the mixture was allowed to warm to room temperature and stirred for 15 hours. The reaction mixture was then poured into HCl (aq., 60 mL, 2 N) at 0 °C and stirred for 10 min. The mixture was extracted with toluene (30 mL) and the aqueous layer discarded. The organic layer was washed with brine and dried with MgSO₄. The solvent was removed by rotary evaporation to give a colourless oil (1.23 g, 6.96 mmol, 96 %)

¹H NMR (400 MHz, CDCl₃) δ = 4.05 (s, 2 H, ClCH₂C(=O)), 2.54 (t, J = 7.4 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 1.59 (quin, J = 7.0 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 1.34 - 1.21 (m, 8 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 0.87 (t, J = 6.8 Hz, 3 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, CDCl₃) δ =

IR (neat) ν_{max} / cm⁻¹ = 2951.65 (C-H), 2924.99 (C-H), 2855.46 (C-H), 1720.39 (C=O)

9.8 2-oxononyl 2-amino-5-nitrobenzoate **33**⁶



5-Nitroanthranilic acid (500 mg, 2.75 mmol) and potassium carbonate (270 mg, 2.00 mmol) were dissolved in DMF (5 ml). The mixture was heated under argon to 90 °C and stirred for 1 hour then cooled to room temperature. 1-chlorononan-2-one **31** (353 mg, 2.00 mmol) was added and the mixture was stirred for 15 hours. The solution was poured into Na₂HCO₃ (aq., 10 %, 50 ml) and ice (~ 20 g). The precipitate was collected by vacuum filtration, washed with water and dried under high vacuum to give an orange solid (0.674 g, 2.00 mmol, 100 %)

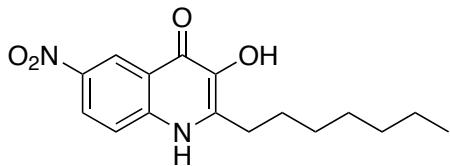
¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 8.66 (d, J = 2.8 Hz, 1 H, *ortho* to C(=O)), 8.12 (dd, J = 2.8, 9.4 Hz, 1 H, *para* to C(=O)), 6.93 (d, J = 9.4 Hz, 1 H, *meta* to C(=O)), 5.05 (s, 2 H, OCH₂C(=O)), 2.49 (t, J = 7.4 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₃), 1.52 (quin, J = 7.2 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₃), 1.32 - 1.20 (m, 8 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₃), 0.86 (t, J = 6.8 Hz, 3 H, C(=O)CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 204.4 (OCH₂C(=O)), 165.6 (C(=O)O), 156.3 (CNH₂), 135.7 (CNO₂), 129.6 (*para* to C=O), 128.9 (*ortho* to C=O), 117.4 (*meta* to C=O), 107.5 (CC(=O)O), 68.8 (OCH₂C(=O)), 38.3 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃), 31.6 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃), 28.9 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃), 23.2 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃), 22.5 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃), 14.4 (OCH₂C(=O)CH₂CH₂CH₂CH₂CH₃)

$(=O)CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$)

IR (neat) ν_{max} / cm^{-1} = 3453.32 (N-H), 3350.52 (N-H), 2924.93 (C-H), 2853.87 (C-H), 1720.10 (ester C=O) 1703.91 (ketone C=O) 1626.14 (N-H bend) 1602.74 (aromatic) 1572.48 (N-O) 1506.58 (N-O)

9.9 6-nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 34⁶



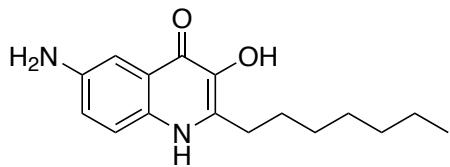
2-oxononyl 2-amino-5-nitrobenzoate (100 mg, 0.340 mmol) and polyphosphoric acid (300 mg) were stirred for 5.5 h at 90 °C under argon. The mixture was then poured into NaHCO₃ (50 mL, sat., aq.) cooled on ice. The precipitate was collected by vacuum filtration, washed with water (50 mL) and dried under high vacuum to give a brown solid (44 mg, 0.145 mmol, 43 %)

¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 12.00 (s, 1 H, NH), 8.91 (d, *J* = 2.8 Hz, 1 H, *ortho* to C=O), 8.29 (dd, *J* = 2.7, 9.2 Hz, 1 H, *para* to C=O), 7.70 (d, *J* = 9.3 Hz, 1 H, *meta* to C=O), 2.75 (t, *J* = 7.7 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 1.67 (quin, *J* = 7.3 Hz, 2 H, CH₂CH₂CH₂CH₂CH₂CH₃), 1.36 - 1.23 (m, 8 H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 0.85 (t, *J* = 7.0 Hz, 3 H, CH₂CH₂CH₂CH₂CH₂CH₃)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 169.7 (C=O), 141.9 (COH), 140.7 (*para* to NO₂), 139.6 (CNO₂), 137.3 (CHCC=O), 124.3 (*ortho* to NO₂ and *ortho* to C=O), 122.3 (*ortho* to NO₂ and *para* to C=O), 121.5 (CCH₂CH₂CH₂CH₂CH₂CH₃), 120.0 (*meta* to NO₂ and *meta* to C=O), 31.6 (CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 29.2 (CH₂CH₂CH₂CH₂CH₂CH₃), 28.9 (CH₂CH₂CH₂CH₂CH₂CH₃), 28.5 (CH₂CH₂CH₂CH₂CH₃), 28.1 (CH₂CH₂CH₂CH₂CH₃), 22.5 (CH₂CH₂CH₂CH₂CH₂CH₃), 14.4 (CH₂CH₂CH₂CH₂CH₃)

IR (neat) ν_{max} / cm^{-1} = 3436.01 (N-H), 3000.00 (O-H, broad), 2955.37 (C-H), 2925.76 (C-H), 2850.93 (C-H), 1648.18 (aromatic), 1606.05 (aromatic), 1570.67 (N-O), 1536.35 (N-O)

9.10 6-amino-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 35⁷



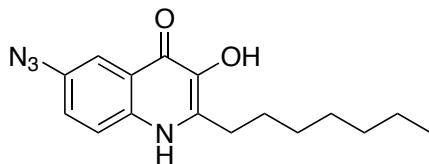
6-nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **34** (20 mg, 0.0658 mmol) and PtO₂ (2 mg) were stirred in MeOH (1 mL) under a H₂ atmosphere for 45 min at room temperature and pressure. The reaction mixture was then filtered through celite and the solvent was removed in vacuo to give a brown solid (14.5 mg, 0.0529 mmol, 80 %).

¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 11.12 (s, 1 H, NH), 7.28 (d, *J* = 8.9 Hz, 1 H, *meta* to C=O), 7.17

(d, $J = 2.4$ Hz, 1 H, *ortho* to C=O), 6.93 (dd, $J = 2.6, 9.0$ Hz, 1 H, *para* to C=O), 2.67 (t, $J = 7.5$ Hz, 2 H, $\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 1.65 (quin, $J = 7.8$ Hz, 2 H, $\text{CH}_2\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 1.38 - 1.21 (m, $J = 5.4$ Hz, 8 H, $\text{CH}_2\text{CH}_2\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 0.86 (t, $J = 6.7$ Hz, 3 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$)

IR (neat) ν_{max} / cm^{-1} = 3000.00 (O-H, broad) 2925.41 (C-H), 2854.09 (C-H), 1613.43 (aromatic) 1555.29 (aromatic) 1504.47 (aromatic)

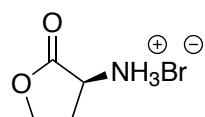
9.11 6-azido-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 36⁸



6-amino-2-heptyl-3-hydroxyquinolin-4(1*H*)-one **35** (18.2 mg, 0.0664 mmol) was dissolved in HCl (0.8 mL, conc., aq.) and MeOH (0.5 mL) at 0 °C. NaNO₂ (5.0 mg, 0.0725 mmol) in H₂O (0.2 mL) was added dropwise over 2 min and the mixture was stirred at 0 °C for 50 min, during which time the solution turned from yellow to orange. NaN₃ (4.9 mg, 0.0754 mmol) in H₂O (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 to give a brown solid (5.5 mg, 0.0183 mmol, 28 %).

¹H NMR (500 MHz, DMSO-d₆) δ / ppm = 7.74 (s, 1 H, *ortho* to C=O), 7.65 (d, $J = 6.9$ Hz, 1 H, *para* to C=O), 7.32 (d, $J = 7.4$ Hz, 1 H, *meta* to C=O), 2.75 (t, $J = 7.5$ Hz, 2 H, $\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 1.67 (quin, $J = 6.4$ Hz, 2 H, $\text{CH}_2\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 1.43 - 1.13 (m, 8 H, $\text{CH}_2\text{CH}_2\underline{\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3}$), 0.85 (t, $J = 6.8$ Hz, 3 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$)

9.12 (3*S*)-2-oxotetrahydrofuran-3-aminium bromide 38⁹



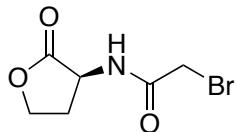
L-Methionine (3.04 g, 20.4 mmol, 1 eq.) and bromoacetic acid (3.08 g, 22.2 mmol, 1.09 eq.) were dissolved in *i*-PrOH (12.5 mL), H₂O (12.5 mL) and AcOH (5 mL). The reaction was refluxed for 15 hours then concentrated under vacuum. The resulting brown oil was added to a mixture of *i*-PrOH (16 mL) and HBr (33 % in AcOH, 4 mL), causing the precipitation of a pale pink 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 to give a pale pink solid (1.73 g, 9.50 mmol, 41 % yield).

¹H NMR (400 MHz, DMSO-d₆) δ / ppm = 8.59 (br s, 3 H, NH_3^+), 4.46 (dt, $J = 1.3, 8.9$ Hz, 1 H, OCH_2), 4.37 (dd, $J = 8.8, 11.4$ Hz, 1 H, $\underline{\text{CH}_2\text{NH}_3^+}$), 4.29 (ddd, $J = 6.1, 8.8, 10.9$ Hz, 1 H, OCH_2), 2.57 (dd, $J = 1.2, 6.1, 8.9, 12.3$ Hz, 1 H, OCH_2CH_2), 2.26 (td, $J = 9.0, 11.2, 12.2$ Hz, 1 H, OCH_2CH_2)

¹³C NMR (101 MHz, DMSO-d₆) δ / ppm = 173.3 (C=O), 66.2 (OCH_2), 47.8 ($\underline{\text{CHNH}_3^+}$), 27.0 (OCH_2CH_2)

IR (neat) ν_{max} / cm^{-1} = 2972.09 (N-H), 2877.54 (N-H), 1771.77 (C=O), 1585.05 (N-H bend), 1572.24 (N-H bend)

9.13 (*S*)-2-bromo-*N*-(2-oxotetrahydrofuran-3-yl)acetamide 40⁹



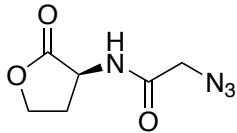
(3*S*)-2-oxotetrahydrofuran-3-aminium bromide **38** (100 mg, 0.549 mmol, 1.08 eq.) and NaHCO₃ (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH₂Cl₂ (2 mL) and H₂O (2 mL). Bromoacetyl bromide (44.0 μ L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 24 h, after which the CH₂Cl₂ was removed under vacuum. The aqueous phase was extracted with EtOAc (4 \times 10 mL) and the combined organic layers were dried with MgSO₄. The solvent was removed under vacuum to give white crystals (88.0 mg, 0.396 mmol, 74 %).

¹H NMR (400 MHz, CDCl₃) δ / ppm = 6.95 (br d, 1 H, NH), 4.58 (ddd, J = 5.9, 8.6, 11.7 Hz, 1 H, CHNHC=O), 4.53 (dt, J = 1.0, 9.2 Hz, 1 H, OCH₂), 4.33 (ddd, J = 5.9, 9.4, 11.3 Hz, 1 H, OCH₂), 3.95 (d, J = 1.3 Hz, 2 H, O=CCH₂Br), 2.88 (dddd, J = 1.3, 5.9, 8.6, 12.6 Hz, 1 H, OCH₂CH₂), 2.24 (dtd, J = 8.9, 11.5, 12.6 Hz, 1 H, OCH₂CH₂)

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

IR (neat) ν_{max} / cm^{-1} = 3255.69 (N-H), 3066.58 (C-H), 1763.02 (lactone C=O), 1657.99 (amide C=O), 1552.67 (N-H bend)

9.14 (*S*)-2-azido-*N*-(2-oxotetrahydrofuran-3-yl)acetamide 41⁹



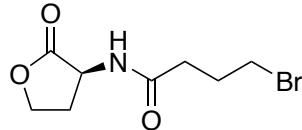
(3*S*)-2-oxotetrahydrofuran-3-aminium bromide **38** (100 mg, 0.552 mmol, 1.08 eq.), NaN₃ (85.7 mg, 1.32 mmol, 2.61 eq.) and NaHCO₃ (84.9 mg, 1.01 mmol, 2.00 eq.) were dissolved in CH₂Cl₂ (2 mL) and H₂O (2 mL). Bromoacetyl bromide (44.0 μ L, 102 mg, 0.505 mmol, 1.00 eq.) was then added dropwise. The reaction mixture was stirred for 48 h, after which the CH₂Cl₂ was removed under vacuum. The aqueous phase was extracted with EtOAc (4 \times 10 mL) and the combined organic layers were dried with MgSO₄. The solvent was removed under vacuum to give white crystals (38.4 mg, 0.209 mmol, 41 %).

¹H NMR (400 MHz, CDCl₃) δ / ppm = 7.07 (br d, J = 5.1 Hz, 1 H, NH), 4.65 (ddd, J = 6.8, 8.7, 11.6 Hz, 1 H, CHNHC=O), 4.49 (dt, J = 1.3, 9.1 Hz, 1 H, OCH₂), 4.31 (ddd, J = 6.0, 9.2, 11.2 Hz, 1 H, OCH₂), 4.05 (s, 2 H, C(=O)CH₂N₃), 2.77 (dddd, J = 1.4, 6.0, 8.8, 12.5 Hz, 1 H, OCH₂CH₂), 2.26 (dq, J = 8.9, 11.8 Hz, 1 H, OCH₂CH₂)

^{13}C NMR (101 MHz, CDCl_3) δ / ppm = 174.9 (OC=O), 167.5 (C=ONH), 66.0 (OCH_2), 52.2 ($\text{O=CCH}_2\text{N}_3$), 48.9 (CHNHC=O), 29.7 (OCH_2CH_2)

IR (neat) ν_{max} / cm^{-1} = 3283.47 (N-H), 2923.28 (C-H), 2852.99 (C-H), 2129.69 (N_3), 1782.86 (lactone C=O), 1661.40 (amide C=O), 1536.81 (N-H bend)

9.15 (*S*)-4-bromo-*N*-(2-oxotetrahydrofuran-3-yl)butanamide 44⁹



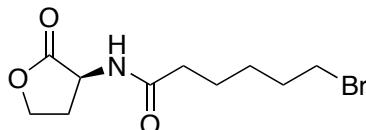
(3*S*)-2-oxotetrahydrofuran-3-aminium bromide **38** (200 mg, 1.10 mmol, 1.00 eq.) and NaHCO_3 (170 mg, 2.02 mmol, 1.84 eq.) were dissolved in CH_2Cl_2 (2 mL) and H_2O (2 mL). Bromobutyryl chloride (140 μL , 224 mg, 1.21 mmol, 1.10 eq.) was then added dropwise. The reaction mixture was stirred for 1 h, after which the CH_2Cl_2 was removed under vacuum. The aqueous phase was extracted with EtOAc (7×5 mL) and the combined organic layers were dried with MgSO_4 . The solvent was removed under vacuum to give white crystals (219 mg) which were recrystallised from EtOAc to give large, needle-like crystals (219 mg, 0.878 mmol, 80 %).

^1H NMR (400 MHz, CDCl_3) δ / ppm = 6.31 (br d, J = 5.5 Hz, 1 H, NH), 4.59 (ddd, J = 6.2, 8.7, 11.5 Hz, 1 H, $\text{CH}_2\text{NHC=O}$), 4.48 (dt, J = 1.2, 8.9 Hz, 1 H, OCH_2), 4.30 (ddd, J = 5.8, 9.3, 11.3 Hz, 1 H, OCH_2), 3.49 (t, J = 6.3 Hz, 2 H, CH_2Br), 2.82 (dddd, J = 1.3, 5.9, 8.7, 12.5 Hz, 1 H, OCH_2CH_2), 2.47 (t, J = 7.3 Hz, 2 H, $\text{C}(=\text{O})\text{CH}_2$), 2.26 - 2.15 (m, 3 H, OCH_2CH_2 and $\text{C}(=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{Br}$)

^{13}C NMR (101 MHz, CDCl_3) δ / ppm = 175.4 (OC=O), 172.3 ($\text{C=O}(\text{NH})$), 66.1 (OCH_2), 49.3 (CHNHC=O), 33.9 (C=OCH_2), 33.1 (CH_2Br), 30.3 (OCH_2CH_2), 27.9 ($\text{C=OCH}_2\text{CH}_2\text{CH}_2\text{Br}$)

IR (neat) ν_{max} / cm^{-1} = 3307.92 (N-H), 3073.85 (C-H), 2948.93 (C-H), 1773.66 (lactone C=O), 1643.46 (amide C=O), 1541.39 (N-H bend)

9.16 (*S*)-6-bromo-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 45⁹



(3*S*)-2-oxotetrahydrofuran-3-aminium bromide **38** (100 mg, 0.549 mmol, 1.00 eq.) and NaHCO_3 (84.9 mg, 1.01 mmol, 1.84 eq.) were dissolved in CH_2Cl_2 (2 mL) and H_2O (2 mL). Bromohexanoyl chloride (93.0 μL , 130 mg, 0.608 mmol, 1.11 eq.) was then added dropwise. The reaction mixture was stirred for 4 h, after which the CH_2Cl_2 was removed under vacuum. The mixture was then filtered, washed with H_2O (10 mL) and dried under high vacuum to give white crystals (101 mg, 0.362 mmol, 66 %).

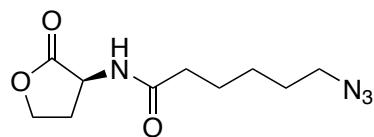
^1H NMR (400 MHz, CDCl_3) δ / ppm = 6.09 (br d, J = 5.7 Hz, 1 H, NH), 4.57 (ddd, J = 5.9, 8.6, 11.6 Hz, 1 H, $\text{CH}_2\text{NHC=O}$), 4.50 (dt, J = 1.3, 9.1 Hz, 1 H, OCH_2), 4.31 (ddd, J = 5.9, 9.3, 11.3 Hz, 1 H, OCH_2), 3.43 (t, J = 6.7

Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂CH₂Br), 2.88 (dddd, J = 1.3, 5.9, 8.6, 12.6 Hz, 1 H, OCH₂CH₂), 2.30 (dt, J = 1.8, 7.5 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂Br), 2.16 (td, J = 8.9, 11.5, 12.5 Hz, 1 H, OCH₂CH₂), 1.90 (quin, J = 7.2 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂Br), 1.71 (quin, J = 7.6 Hz, 2 H, C(=O)CH₂CH₂CH₂CH₂Br), 1.59 - 1.46 (m, 2 H, C(=O)CH₂CH₂CH₂CH₂Br)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 175.5 (OC=O), 173.3 (C(=O)NH), 66.1 (OCH₂), 49.3 (CHNHC=O), 35.8 (C(=O)CH₂CH₂CH₂CH₂Br), 33.5 (C(=O)CH₂CH₂CH₂CH₂Br), 32.3 (C(=O)CH₂CH₂CH₂CH₂Br), 30.5 (OCH₂CH₂), 27.6 (C(=O)CH₂CH₂CH₂CH₂Br), 24.4 (C(=O)CH₂CH₂CH₂CH₂Br)

IR (neat) ν_{max} / cm⁻¹ = 3300.30 (N-H), 3067.62 (C-H), 2937.37 (C-H), 2856.67 (C-H), 1784.83 (lactone C=O), 1639.33 (amide C=O), 1539.87 (N-H bend)

9.17 (S)-6-azido-N-(2-oxotetrahydrofuran-3-yl)hexanamide 47¹



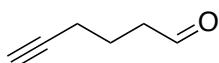
(S)-6-bromo-N-(2-oxotetrahydrofuran-3-yl)hexanamide (80 mg, 0.320 mmol, 1.00 eq.) and NaN₃ (26.3 mg, 0.405 mmol, 1.27 eq.) were heated in DMF (0.5 mL) for 5 hours at 100 °C. The reaction mixture was then partitioned between CH₂Cl₂ (5 mL) and H₂O (5 mL). The aqueous phase was extracted twice more with CH₂Cl₂ (2 × 5 mL) and the organic layers were combined and dried over MgSO₄. The solvent was removed by rotary evaporation followed by high vacuum to give white crystals (42.7 mg, 0.178 mmol, 56 %).

¹H NMR (400 MHz, CDCl₃) δ / ppm = 5.97 (br d, J = 4.2 Hz, 1 H, NH), 4.56 (ddd, J = 5.7, 8.6, 11.7 Hz, 1 H, CH₂NHC=O), 4.50 (dt, J = 1.0, 9.1 Hz, 1 H, OCH₂), 4.31 (ddd, J = 5.8, 9.4, 11.3 Hz, 1 H, OCH₂), 3.31 (t, J = 6.9 Hz, 2 H, CH₂N₃), 2.90 (dddd, J = 1.1, 5.8, 8.6, 12.5 Hz, 1 H, OCH₂CH₂), 2.30 (dt, J = 1.8, 7.4 Hz, 2 H, O=CCH₂), 2.15 (td, J = 8.8, 11.5, 12.3 Hz, 1 H, OCH₂CH₂), 1.72 (quin, J = 7.6 Hz, 2 H, O=CCH₂CH₂CH₂CH₂N₃), 1.65 (quin, J = 7.2 Hz, 2 H, O=CCH₂CH₂CH₂CH₂N₃) 1.46 (m, 2 H, O=CCH₂CH₂CH₂CH₂N₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = !!!, !!!, 66.1 (OCH₂), 51.2 (C(=O)CH₂CH₂CH₂CH₂N₃), 49.4 (CHNHC=O), 35.9 (C(=O)CH₂CH₂CH₂CH₂N₃), 30.7 (OCH₂CH₂), 28.6 (C(=O)CH₂CH₂CH₂CH₂N₃), 26.3 (C(=O)CH₂CH₂CH₂CH₂N₃), 24.8 (C(=O)CH₂CH₂CH₂CH₂N₃)

IR (neat) ν_{max} / cm⁻¹ = 3314.00 (N-H), 2931.56 (C-H), 2862.89 (C-H), 2095.06 (N₃), 1775.38 (lactone C=O), 1643.14 (amide C=O), 1547.90 (N-H bend)

9.18 Hex-5-yneal 49¹⁰



Pyridinium chlorochromate (14.6 g, 68.1 mmol, 1.50 eq) and DCM (500 mL) were stirred at room temperature under argon. 5-hexyn-1-ol (5.00 mL, 45.4 mmol, 1 eq.) was added and the reaction mixture was stirred

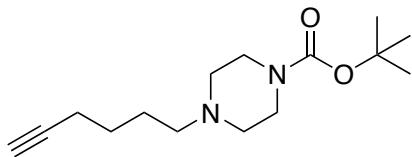
for 5 h followed by addition of Et₂O (125 mL) and silica gel (62.5 g). The suspension was stirred for 1 h then filtered through a pad of silica (100 g) and washed with Et₂O. The solvent was removed by rotary evaporation to give a light yellow-green oil (4.72 g, 49.1 mmol, 72 %).

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

IR (neat) ν_{max} / cm⁻¹ = 3292.68 (alkyne C-H), 2943.26 (alkane C-H), 2830.88 (aldehyde C-H), 2728.56 (aldehyde C-H), 1720.29 (C=O)

Need C

9.19 *tert*-butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 51¹¹



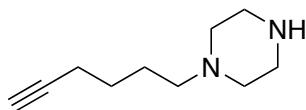
Hex-5-ynal **49** (0.407 g, 4.24 mmol, 1.00 eq.) and *tert*-butyl piperazine-1-carboxylate (0.791 g, 4.24 mmol, 1.00 eq.) were stirred under a N₂ atmosphere in 1,2-dichloroethane (20 mL) for 2.5 h followed by addition of sodium triacetoxyborohydride (6.25 g, 29.5 mmol, 6.96 eq.) in four portions over 4 d. The mixture was stirred for a further day then NaHCO₃ (sat., aq., 120 mL) was added and the product extracted with EtOAc (2 \times 100 mL). The solvent was dried over MgSO₄, and removed by rotary evaporation to give white crystals (1.12 g, 4.21 mmol, 99 %)

¹H NMR (400 MHz, CDCl₃) δ / ppm = 3.44 (t, J = 5.2 Hz, 4 H, BocN(CH₂)CH₂), 2.39 (t, J = 5.1 Hz, 4 H, HC≡CCH₂CH₂CH₂N(CH₂)CH₂), 2.37 (t, J = 7.3 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 2.23 (dt, J = 2.7, 6.8 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 1.96 (t, J = 2.7 Hz, 1 H, HC≡CCH₂CH₂CH₂CH₂N), 1.65 - 1.53 (m, 4 H, HC≡CCH₂CH₂CH₂CH₂N), 1.47 (s, 9 H, CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 154.7 (NC(=O)O), 84.2 (HC≡C), 79.6 (C(CH₃)₃), 68.5 (HC≡C), 60.4 (HC≡CCH₂CH₂CH₂N), 58.0 (HC≡CCH₂CH₂CH₂N(CH₂)CH₂), 53.0 (BocN(CH₂)CH₂), 28.4 (C(CH₃)₃), 26.3 (HC≡CCH₂CH₂CH₂N), 25.7 (HC≡CCH₂CH₂CH₂N), 18.3 (HC≡CCH₂CH₂CH₂N)

IR (neat) ν_{max} / cm⁻¹ = 3303.59 (alkyne C-H), 2939.96 (alkane C-H), 2865.23 (aldehyde C-H), 2810.42 (aldehyde C-H), 1691.29 (C=O)

9.20 1-(hex-5-yn-1-yl)piperazine 52¹²



tert-butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate **51** (763 mg, 2.86 mmol) was stirred in TFA (10 mL) at room temperature for 2 h. The TFA was removed under vacuum followed by co-evaporation with CH₂Cl₂ (2

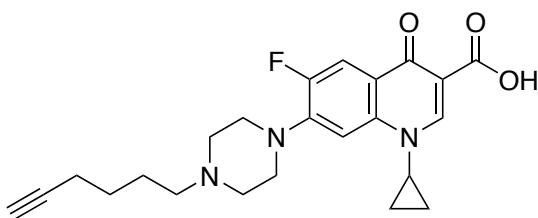
$\times 20$ mL. The oil was diluted with H_2O and the pH adjusted to 14 with NaOH (10 % aq.). This mixture was extracted with CH_2Cl_2 (2×20 mL) and the combined organic layers were dried over MgSO_4 . The solvent was removed under vacuum and purified by column chromatography (SiO_2 $\text{MeOH}/\text{CH}_2\text{Cl}_2$ 3:7) to give a tan liquid (476 mg, 2.86 mmol, 100 %).

^1H NMR (400 MHz, CDCl_3) δ / ppm = 2.88 (t, $J = 4.9$ Hz, 4 H, $\text{HN}(\underline{\text{CH}_2})\text{CH}_2$), 2.39 (m, 4 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\text{CH}_2$), 2.31 (t, $J = 7.1$ Hz, 2 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.20 (dt, $J = 2.7, 6.8$ Hz, 2 H, $\text{HC}\equiv\text{CCH}_2$), 2.05 (br. s., 1 H, NH), 1.93 (t, $J = 2.7$ Hz, 1 H, $\text{HC}\equiv\text{C}$), 1.65 - 1.48 (m, 4 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$)

^{13}C NMR (101 MHz, CDCl_3) δ / ppm = 84.3 ($\text{HC}\equiv\text{C}$), 68.4 ($\text{HC}\equiv\text{C}$), 58.6 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 54.5 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\text{CH}_2$), 46.0 ($\text{HN}(\underline{\text{CH}_2})\text{CH}_2$), 26.4 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 25.7 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 18.3 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$)

IR (neat) ν_{max} / cm^{-1} = 3295.87 (alkyne C-H), 2941.07 (alkane C-H), 2810.64 (aldehyde C-H), 1637.22 (N-H bend)

9.21 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid 54¹²



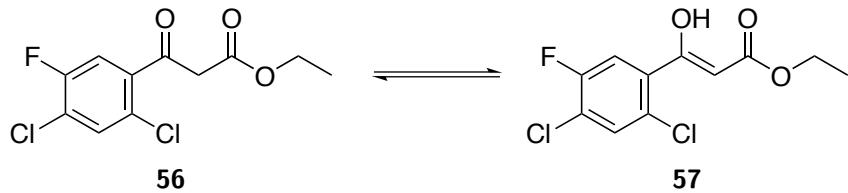
1-(hex-5-yn-1-yl)piperazine **52** (200 mg, 1.20 mmol) and 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **53** (100 mg, 0.355 mmol) were stirred in TEA (5 mL). The reaction mixture was heated at reflux for 18 h then cooled to room temperature. The solvent was removed under vacuum and the resulting solid was triturated with MeOH to give an off-white powder.

^1H NMR (400 MHz, DMSO-d_6) δ / ppm = 15.2 (br s, 1 H, $\text{C}(=\text{O})\text{OH}$), 8.66 (s, 1 H, *ortho* to $\text{C}(=\text{O})\text{OH}$), 7.90 (d, $J = 13.3$ Hz, 1 H, *ortho* to F), 7.57 (d, $J = 7.2$ Hz, 1 H, *meta* to F), 3.83 (m, 1 H, $\text{NCH}(\text{CH}_2)_2$), 2.77 (t, $J = 2.9$ Hz, 1 H, $\text{HC}\equiv\text{C}$), 2.58 (m, 4 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\text{CH}_2$), 2.51 (m, 4 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\text{CH}_2\text{CH}_2$), 2.37 (t, $J = 5.9$ Hz, 2 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.20 (dt, $J = 2.1, 7.6$ Hz, 2 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.57 (quin, $J = 7.2$ Hz, 1 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.50 (quin, $J = 7.1$ Hz, 1 H, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.32 (m, 2 H, $\text{NCH}(\underline{\text{CH}_2})_2$), 1.19 (m, 2 H, $\text{NCH}(\underline{\text{CH}_2})_2$)

^{13}C NMR (101 MHz, DMSO-d_6) δ / ppm = 166.3 ($\text{C}(=\text{O})\text{OH}$), 148.2 ($\text{C}\equiv\text{CC}(=\text{O})\text{OH}$), 139.4 (*para* to F), 111.2 (*ortho* to $\text{C}=\text{O}$ and *ortho* to F), 111.0 (*para* to piperazine), 106.8 (*meta* to $\text{C}=\text{O}$ and *meta* to F), 106.4 ($\text{CC}(=\text{O})\text{OH}$), 84.8 ($\text{HC}\equiv\text{C}$), 71.4 ($\text{HC}\equiv\text{C}$), 57.1 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 52.5 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\underline{\text{CH}_2})\text{CH}_2$), 49.6 ($\text{HN}(\underline{\text{CH}_2})\text{CH}_2$), 49.5 ($\text{HN}(\text{CH}_2)\underline{\text{CH}_2}$), 36.1 ($\text{NCH}(\text{CH}_2)_2$), 26.0 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 25.3 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 17.8 ($\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{CH}_2\text{N}$), 7.7 ($\text{NCH}(\underline{\text{CH}_2})_2$)

IR (neat) ν_{max} / cm^{-1} = 3211.99 (alkyne C-H), 2459.32 (O-H), 1722.63 (carboxylic acid C=O), 1626.76 (quinoline C=O)

9.22 Ethyl 3-(2,4-dichloro-5-fluorophenyl)-3-oxopropanoate **56**¹³



2,4-dichloro-5-fluorobenzoyl chloride (0.1 mL, 0.157 mg, 0.689 mmol, 1 eq.) was added to a stirred solution of EtOAc (0.102 mL, 1.03 mmol, 1.5 eq.) and *N*-methylimidazole (0.066 mL, 0.827 mmol, 1.2 eq.) in toluene (2 mL) at 0 °C under an Ar atmosphere. The mixture was stirred at 0 °C for 10 min followed by addition of TiCl₄ (0.227 mL, 2.07 mmol, 3 eq.) and DIPEA (0.600 mL, 3.45 mmol, 5 eq.). The mixture was stirred at 0 °C for 30 min followed by addition of H₂O (5 mL) was added to the mixture, which was then extracted with Et₂O (3 × 5 mL). The combined organic extracts were washed with brine and dried with Na₂SO₄. The solvent was removed under vacuum to give an oil which was purified by column chromatography (SiO₂, 2 % Et₂O/40-60 P.E.) to give a tautomeric mixture of **56** and **57** as a colourless oil (87 mg, 0.312 mmol, 45 %, 56 % **56**). Need 'mod top'

Keto form **56** ¹H NMR (400 MHz, CDCl₃) δ / ppm = 7.50 (d, J = 6.3 Hz, 1 H, *ortho* to F), 7.47 (d, J = 8.3 Hz, 1 H, *meta* to F), 4.20 (q, J = 7.1 Hz, 2 H, OCH₂CH₃), 4.03 (s, 2 H, C(=O)CH₂C(=O)O), 1.26 (t, J = 7.2 Hz, 3 H, OCH₂CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = !!! 132.4 (d, J = 17.3 Hz, *meta* to C=O), 117.8 (d, J = 24.3 Hz, *ortho* to C=O), 60.9 (OCH₂CH₃), 48.9 (C(=O)CH₂C(=O)O), 14.0 (OCH₂CH₃)

IR (neat) ν_{max} / cm⁻¹ = 1635.70 (C=O) 1564.93 (aromatic)

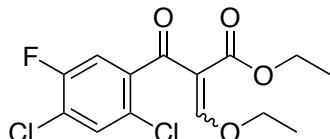
Enol form **57**

¹H NMR (400 MHz, CDCl₃) δ / ppm = 12.51 (s, 1 H, OH), 7.50 (d, J = 6.6 Hz, 1 H, *ortho* to F), 7.44 (d, J = 9.4 Hz, 1 H, *meta* to F), 5.64 (s, 1 H, C=CH), 4.28 (q, J = 7.1 Hz, 2 H, OCH₂CH₃), 1.34 (t, J = 7.2 Hz, 3 H, OCH₂CH₃)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = !!! 132.4 (d, J = 17.3 Hz, *meta* to C=O), 117.8 (d, J = 24.3 Hz, *ortho* to C=O), 94.0 (C(OH)=CHC(=O)O), 61.7 (OCH₂CH₃), 14.2 (OCH₂CH₃)

IR (neat) ν_{max} / cm⁻¹ = 1635.70 (C=O), 1564.93 (aromatic)

9.23 Ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-ethoxyacrylate **59**^{14,15}

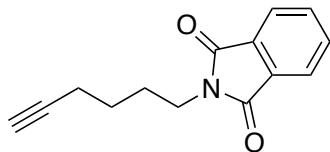


A mixture of ethyl 3-(2,4-dichloro-5-fluorophenyl)-3-oxopropanoate **56** (30.0 mg, 0.108 mmol, 1 eq.), triethylorthoformate (21.5 mg, 0.161 mmol, 1.5 eq.) and acetic anhydride (25 mg, 0.269 mmol, 2.5 eq.) were refluxed

for 1 h. The reaction mixture was then concentrated under reduced pressure to give a colourless oil (24.2 mg, 0.0722 mmol, 67 %).

IR (neat) ν_{max} / cm⁻¹ = 2983.86 (C-H), 1715.86 (C=O), 1599.18 (aromatic), 1568.92 (aromatic)

9.24 2-(hex-5-yn-1-yl)isoindoline-1,3-dione **61**^{16,17}



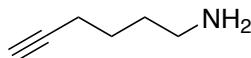
A mixture of 5-chloro-1-pentyne (1.00 mL, 0.962 g, 8.25 mmol), potassium phthalimide (1.83 g, 9.90 mmol), potassium iodide (1.37 g, 8.25 mmol) and DMF (8.45 mL) were heated at 80 °C for 16 h. The resulting solution was cooled to r.t. and poured into H₂O (25 mL). The mixture was extracted with Et₂O (4 × 20 mL) and the combined organic layers were dried with MgSO₄. The solvent was removed under vacuum to give a white solid (1.68 g, 7.39 mmol, 90 %).

¹H NMR (400 MHz, CDCl₃) δ / ppm = 7.86 (dd, *J* = 3.1, 5.4 Hz, 2 H, *para* to C=O), 7.73 (dd, *J* = 3.1, 5.4 Hz, 2 H, *meta* to C=O), 3.73 (t, *J* = 7.1 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 2.27 (dt, *J* = 2.7, 7.0 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 1.96 (t, *J* = 2.7 Hz, 1 H, HC≡C), 1.88 - 1.79 (m, 2 H, HC≡CCH₂CH₂CH₂N), 1.64 - 1.54 (m, 2 H, HC≡CCH₂CH₂CH₂N)

¹³C NMR (101 MHz, CDCl₃) δ / ppm = 168.4 (C=O), 133.9 (*para* to C=O), 132.1 (C(=O)C), 123.2 (*meta* to C=O), 83.8 (HC≡C), 68.8 (HC≡C), 37.4 (HC≡CCH₂CH₂CH₂N), 27.6 (HC≡CCH₂CH₂CH₂N), 25.6 (HC≡CCH₂CH₂CH₂N), 18.0 (HC≡CCH₂CH₂CH₂N)

IR (neat) ν_{max} / cm⁻¹ = 3272.48 (alkyne C-H), 2935.42 (alkane C-H), 1766.76 (C=O), 1708.18 (C=O), 1614.92 (C=O)

9.25 5-aminohex-1-yne **62**^{16,17}

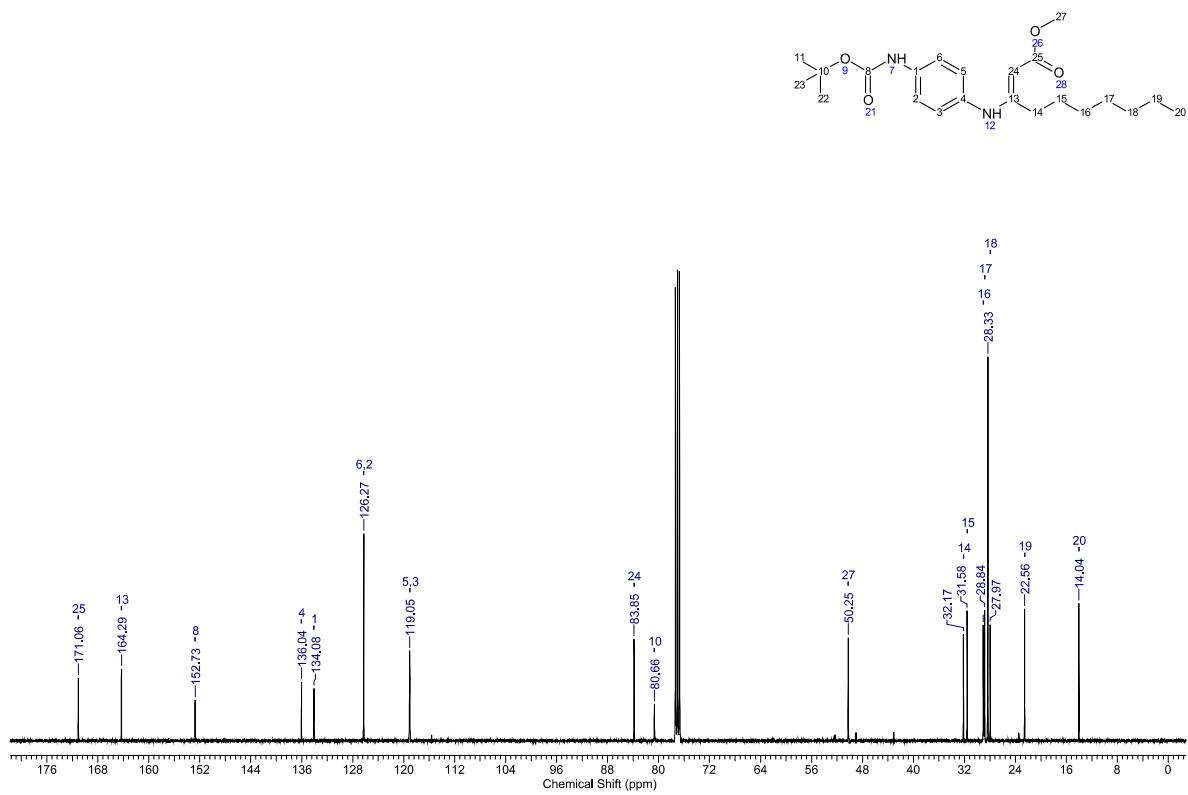
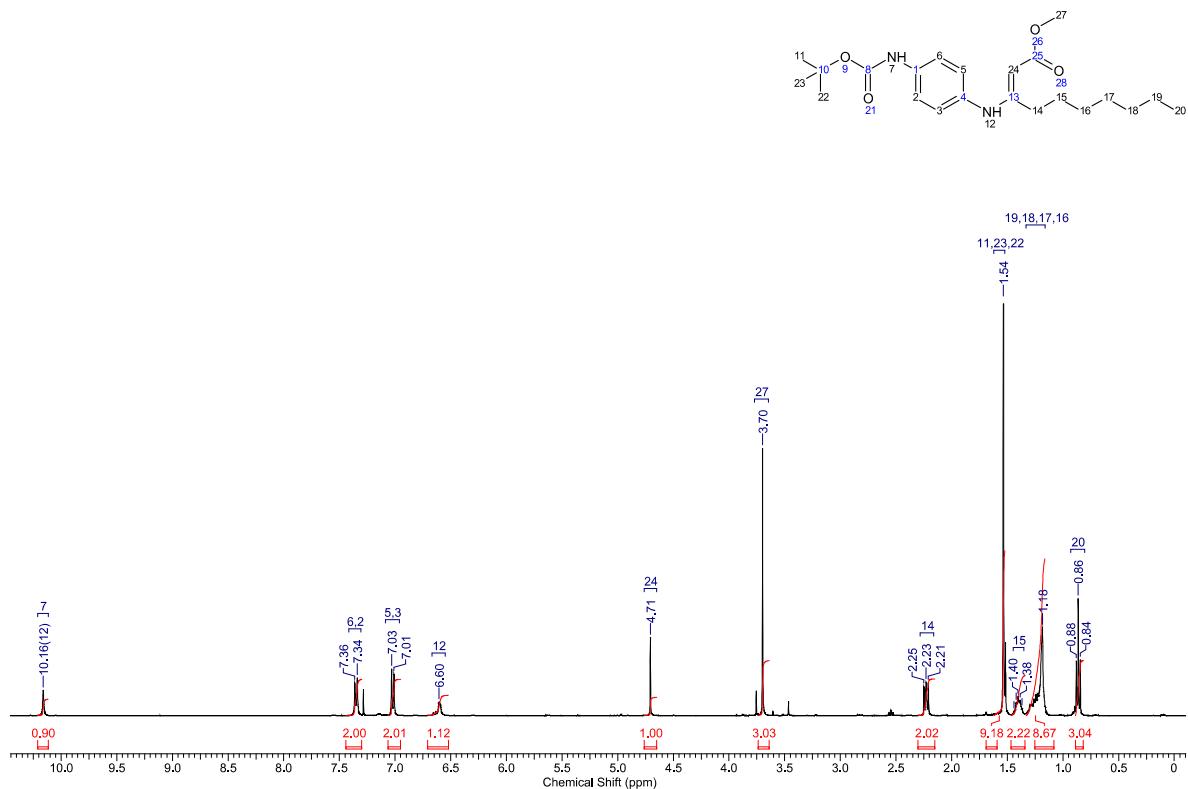


2-(hex-5-yn-1-yl)isoindoline-1,3-dione **61** (1.5 g, 6.61 mmol), hydrazine monohydrate (1.03 mL, 1.06 g, 21.2 mmol) and EtOH (60 mL) were heated at 70 °C for 18 h, during which time a white solid precipitated. The mixture was cooled to room temperature and filtered to remove the white precipitate. The filtrate was concentrated under vacuum to give a volatile yellow-green oil (39.7 mg, 0.409 mmol, 6 %).

¹H NMR (400 MHz, CDCl₃) δ / ppm = 2.77 (t, *J* = 7.0 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 2.19 (dt, *J* = 2.7, 6.8 Hz, 2 H, HC≡CCH₂CH₂CH₂CH₂N), 1.93 (t, *J* = 2.7 Hz, 1 H, HC≡C), 1.68 - 1.49 (m, 4 H, HC≡CCH₂CH₂CH₂CH₂N)

10 NMR spectra

10.1 Methyl (E)-3-((4-((tert-butoxycarbonyl)amino)phenyl)amino)dec-2-enoate 24



Figure⁴⁹ 19: 24

10.2 6-amino-2-heptylquinolin-4-ol 25

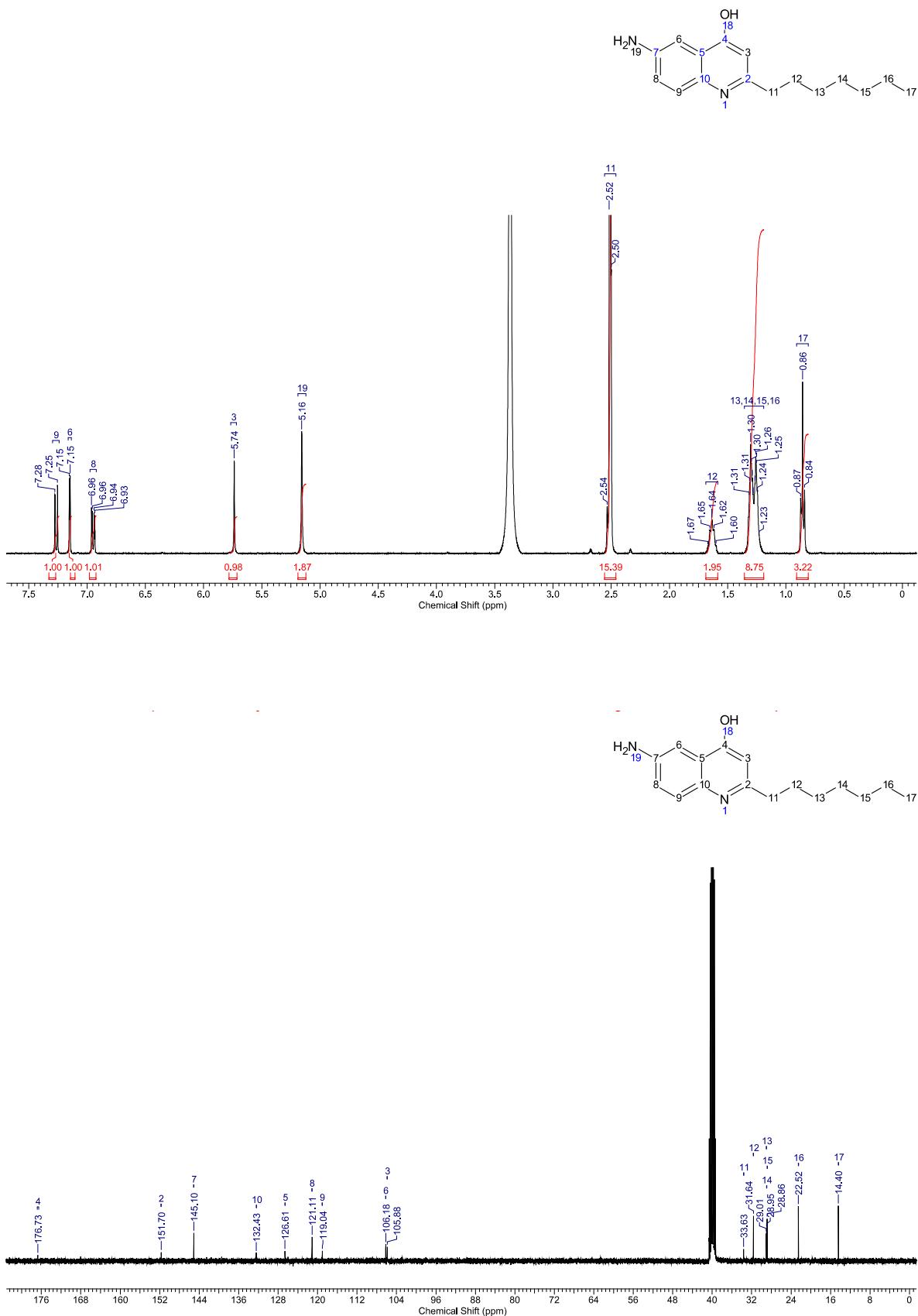


Figure 20: 25

10.3 2-oxononyl 2-amino-5-nitrobenzoate 33



Figure 21: 103

10.4 6-nitro-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 34

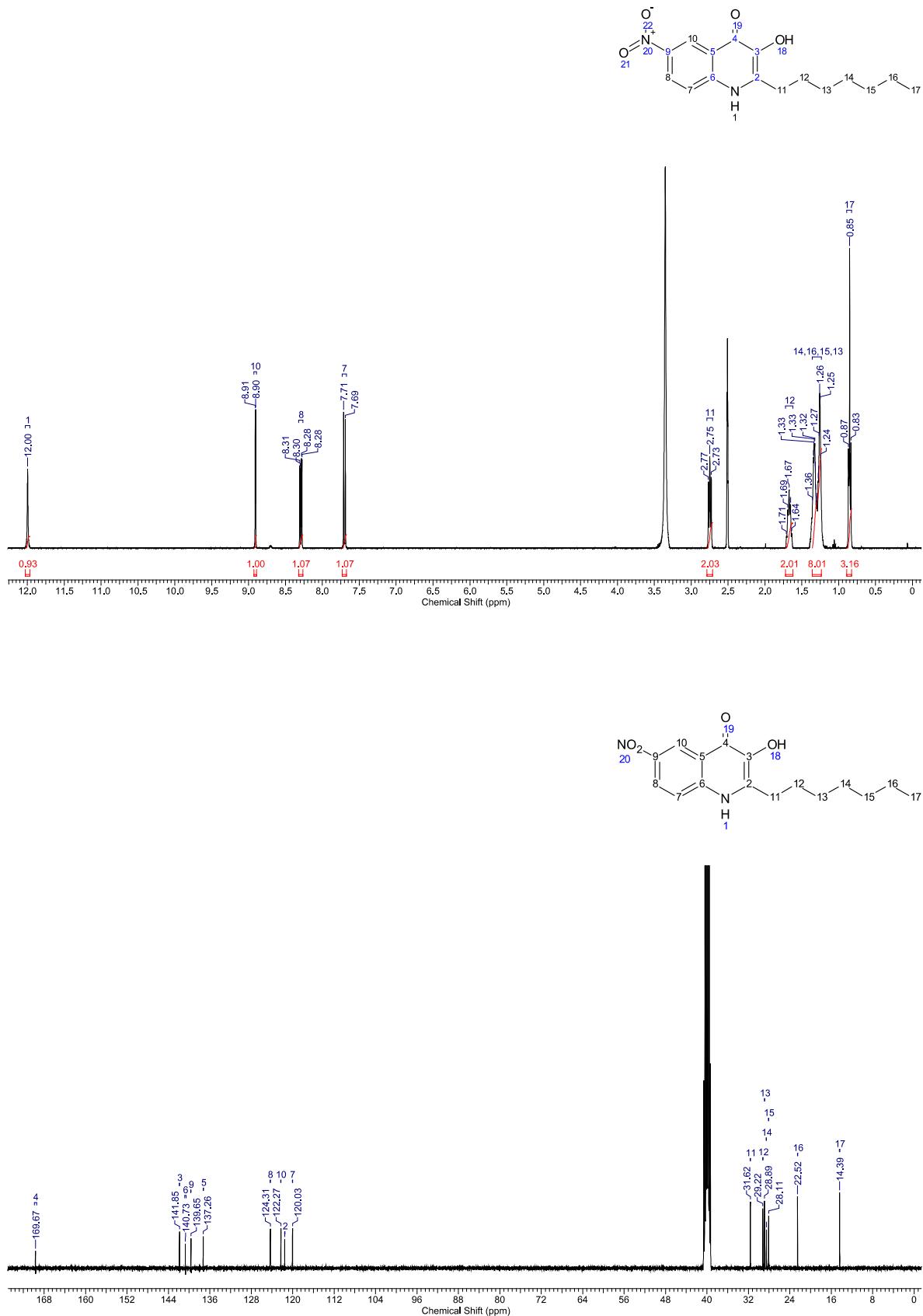


Figure 22: 34

10.5 6-amino-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 35

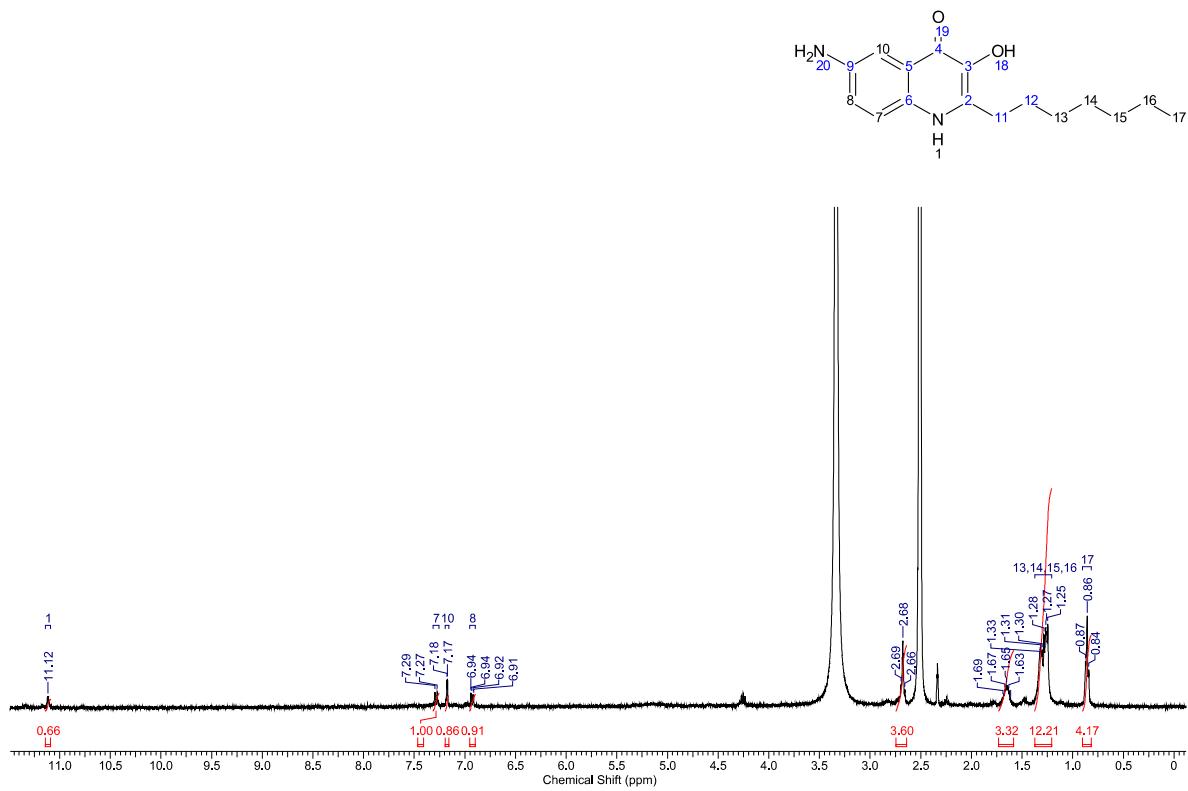


Figure 23: 35

10.6 6-azido-2-heptyl-3-hydroxyquinolin-4(1*H*)-one 36

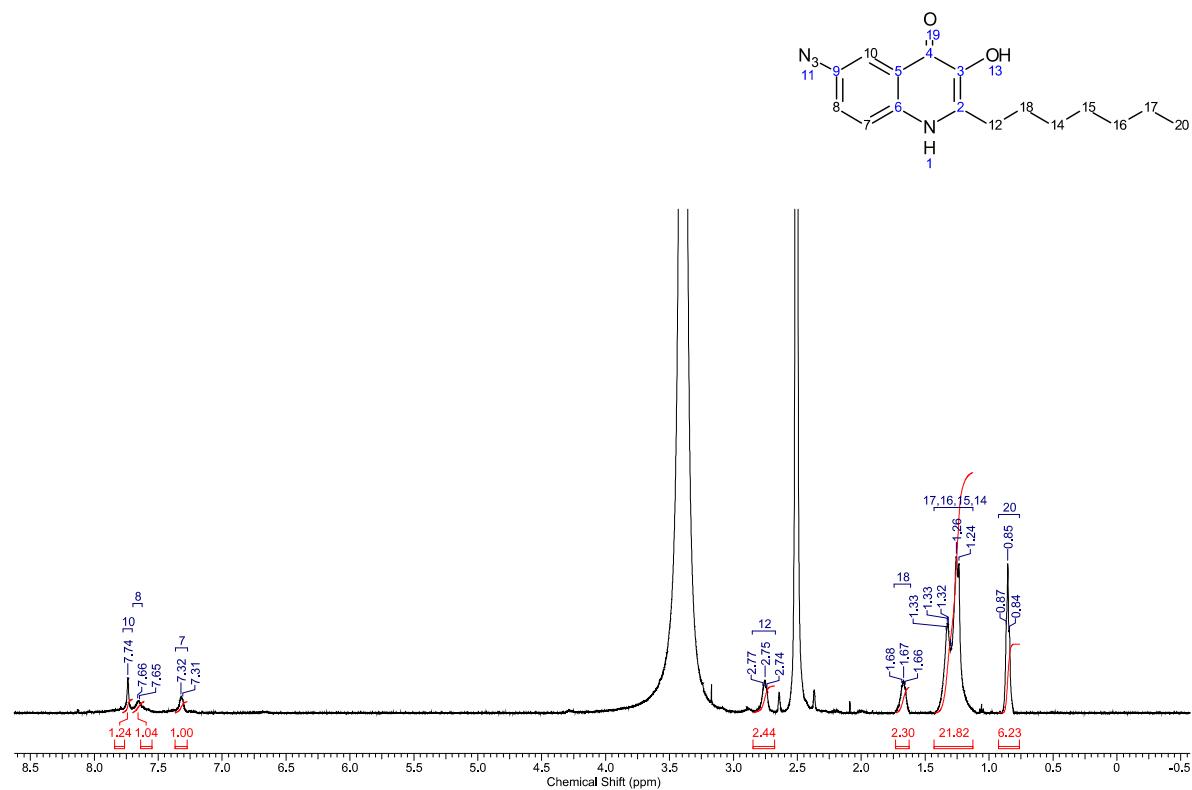


Figure 24: **36**

10.7 (S)-4-bromo-N-(2-oxotetrahydrofuran-3-yl)butanamide 44

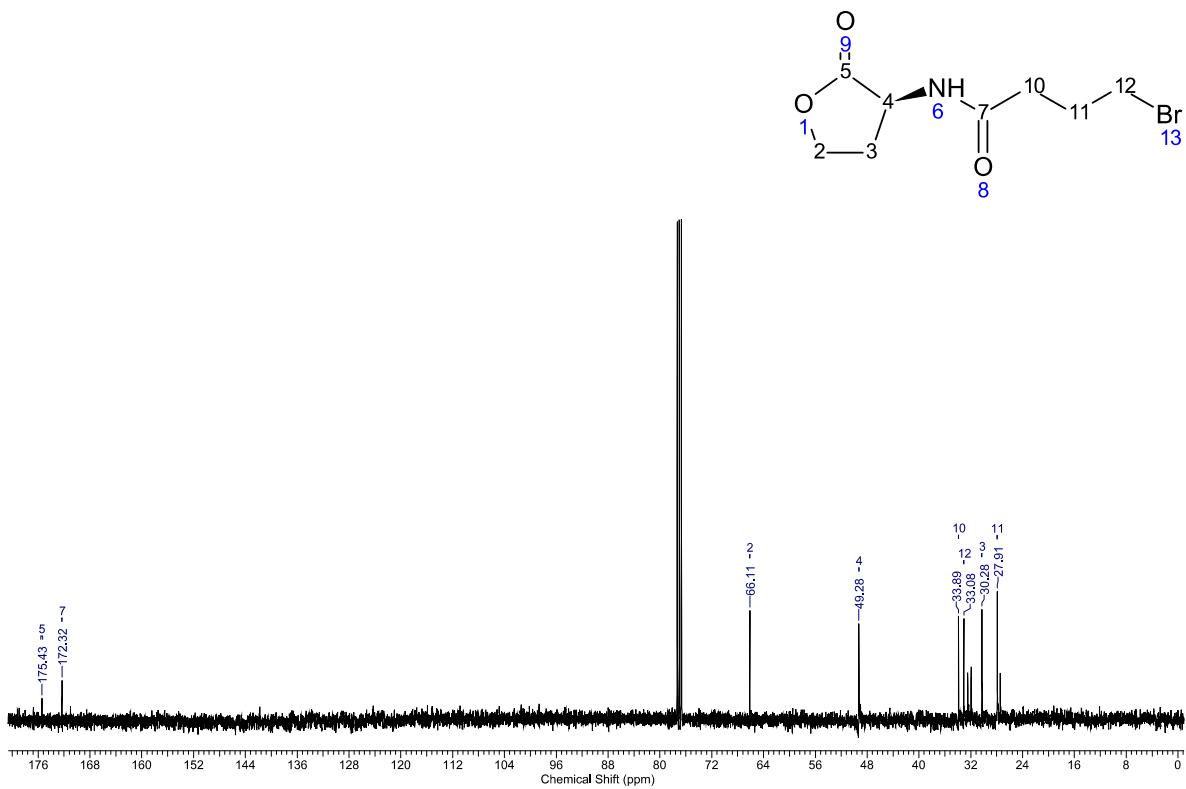
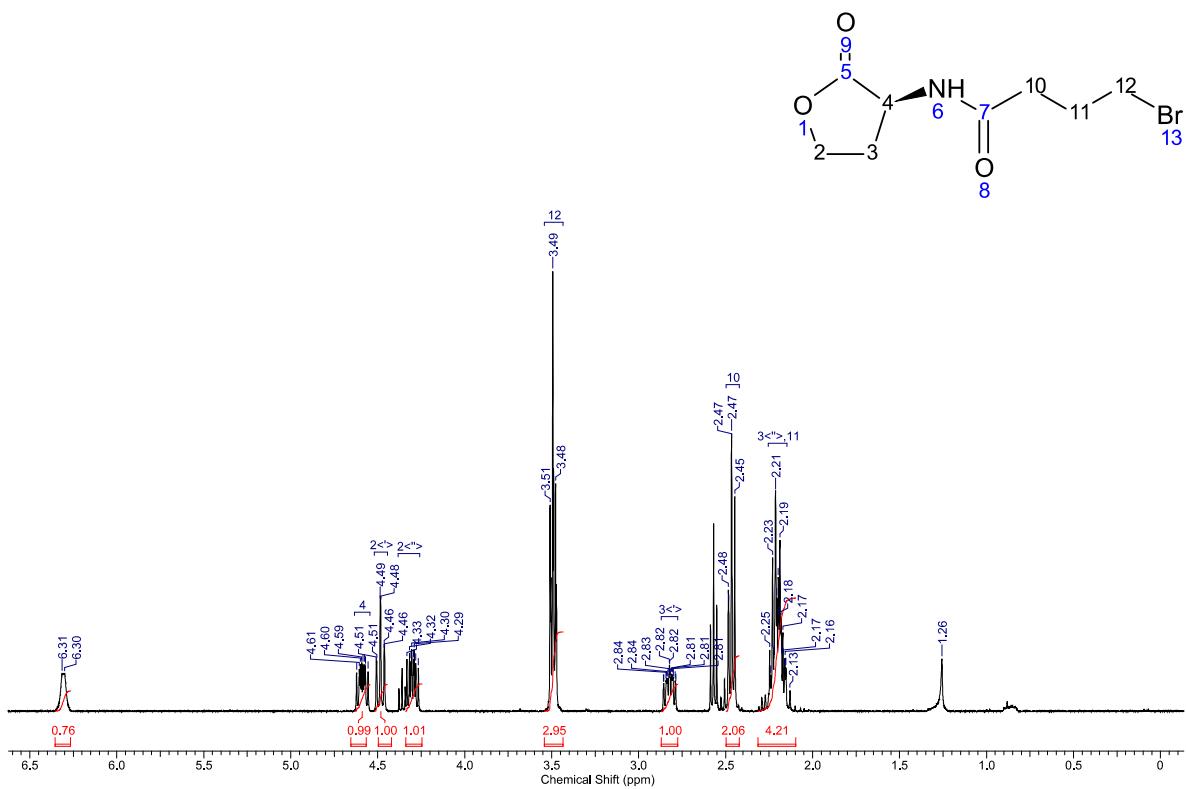


Figure 25: 44

10.8 (S)-6-bromo-N-(2-oxotetrahydrofuran-3-yl)hexanamide 45

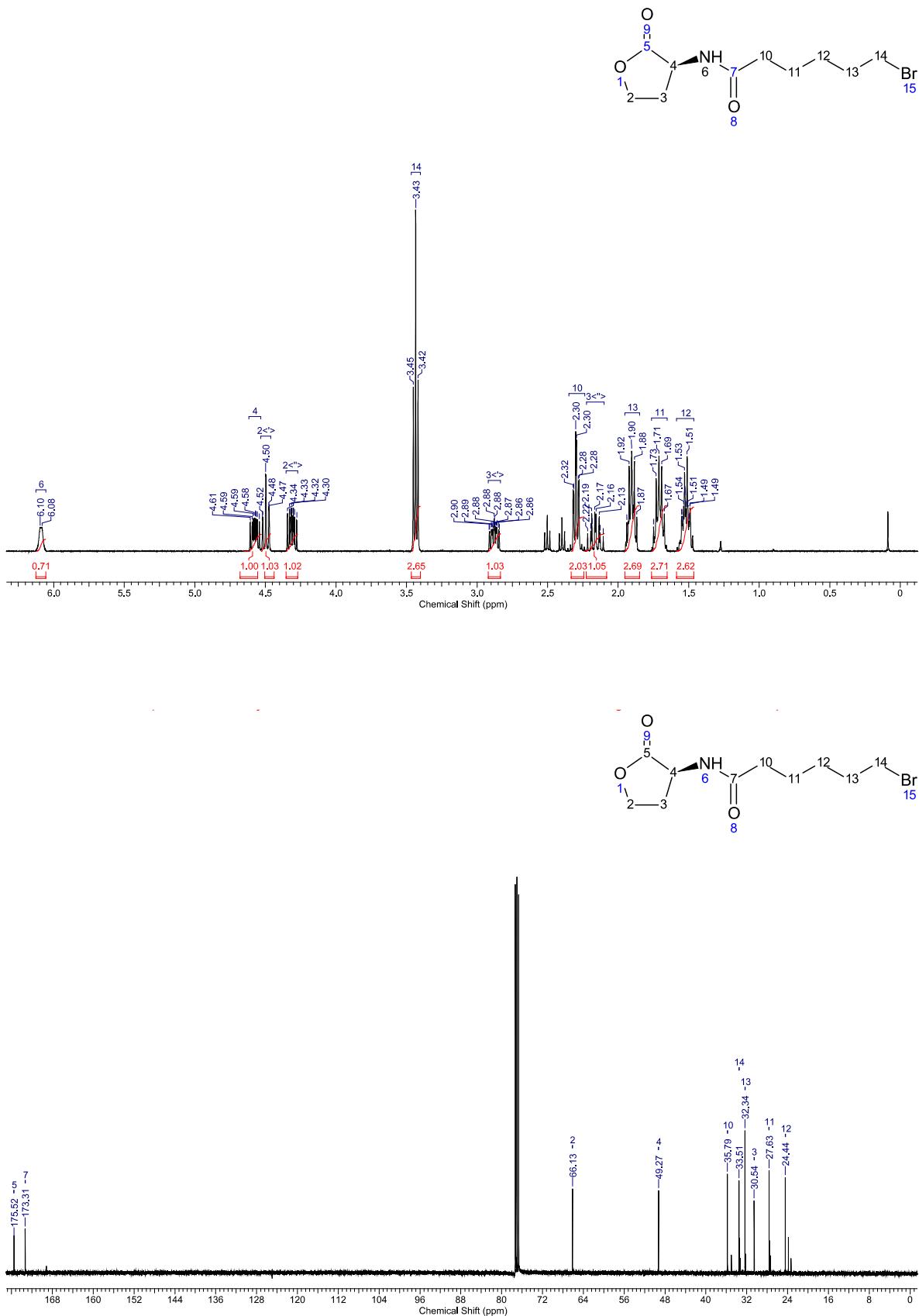


Figure 26: 45

10.9 (*S*)-6-azido-*N*-(2-oxotetrahydrofuran-3-yl)hexanamide 47

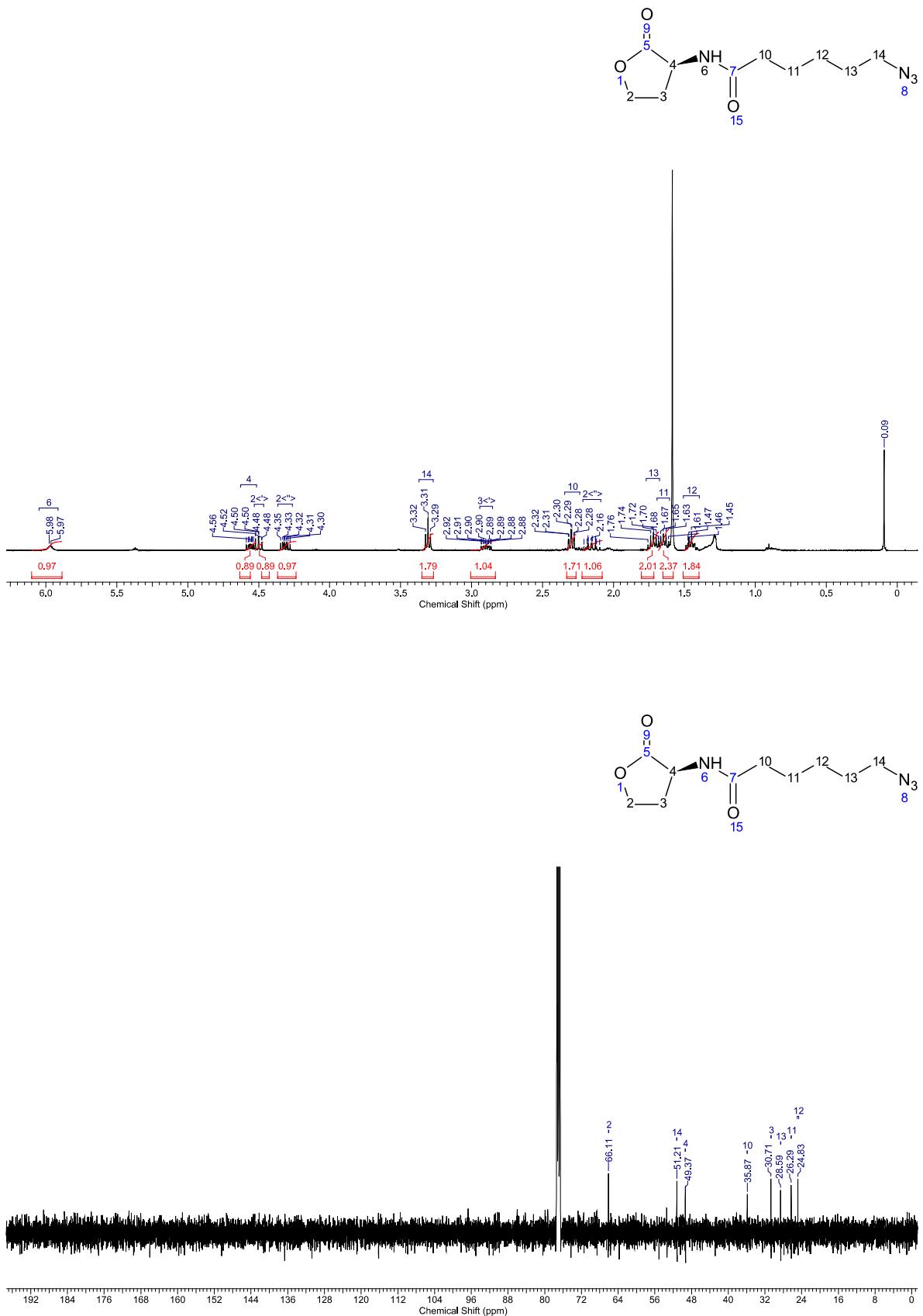


Figure 27: 47

10.10 *tert*-butyl 4-(hex-5-yn-1-yl)piperazine-1-carboxylate 51

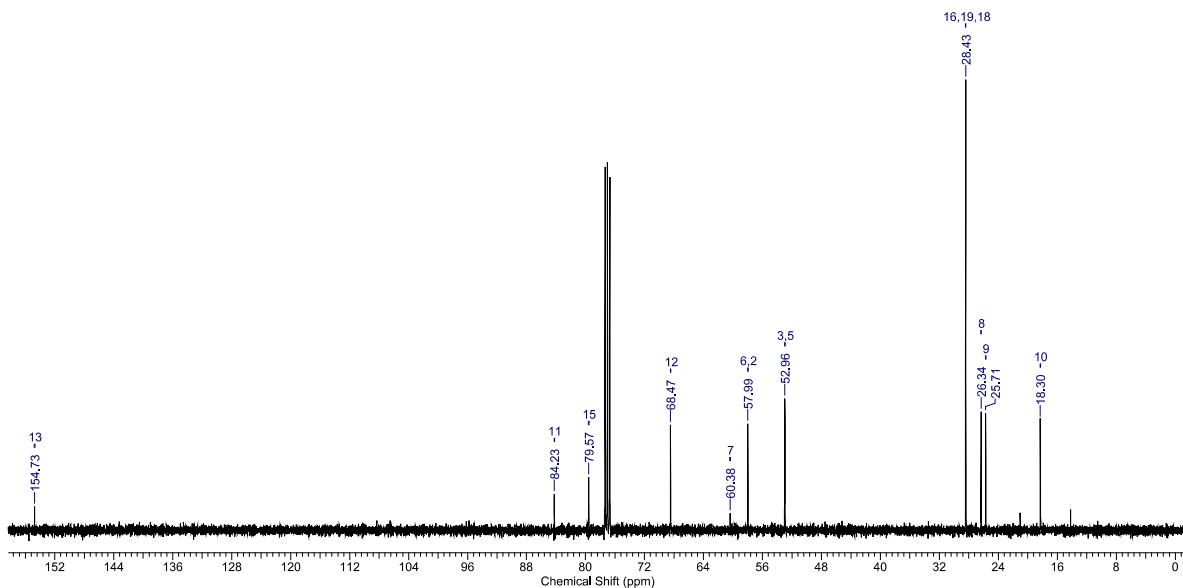
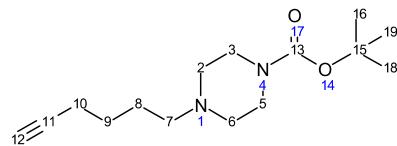
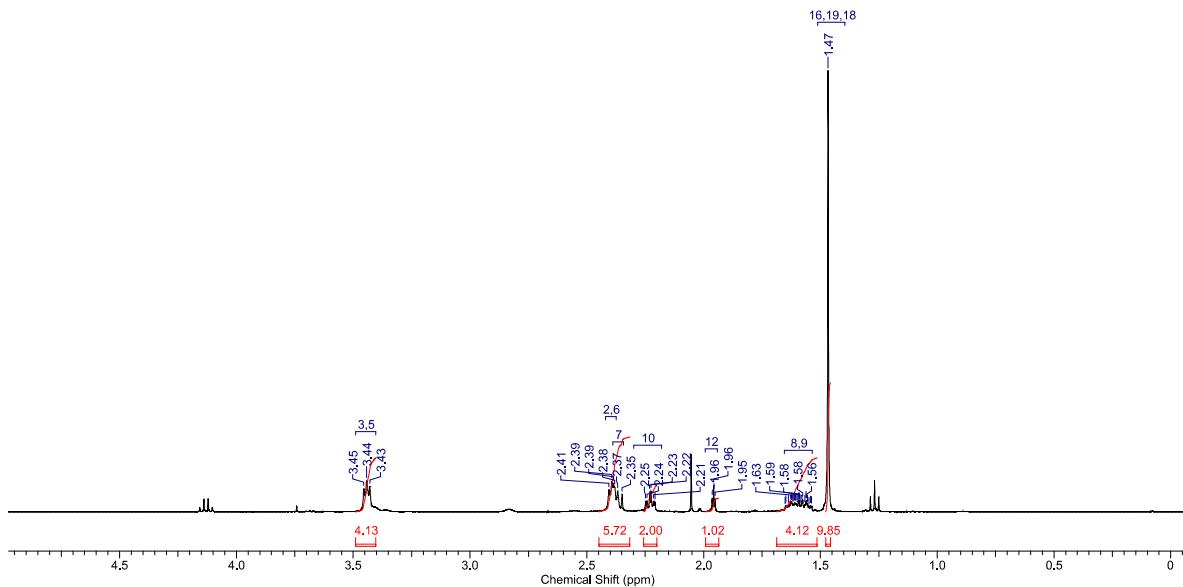
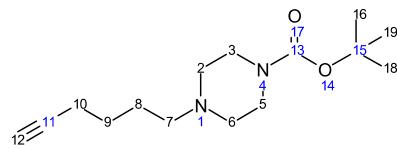


Figure 28: 51

10.11 1-(hex-5-yn-1-yl)piperazine 52

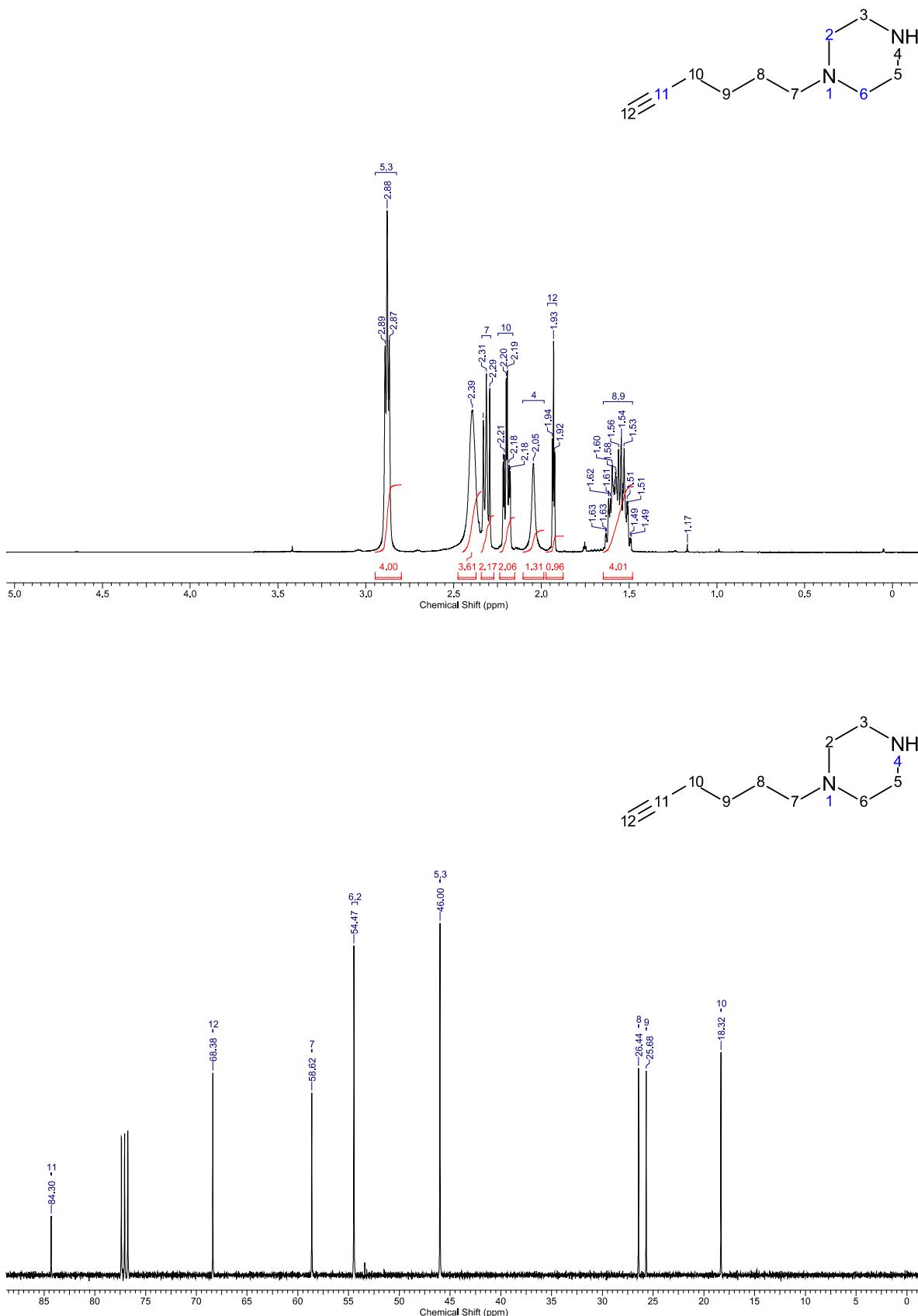


Figure 29: 52

10.12 1-cyclopropyl-6-fluoro-7-(4-(hex-5-yn-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid 54

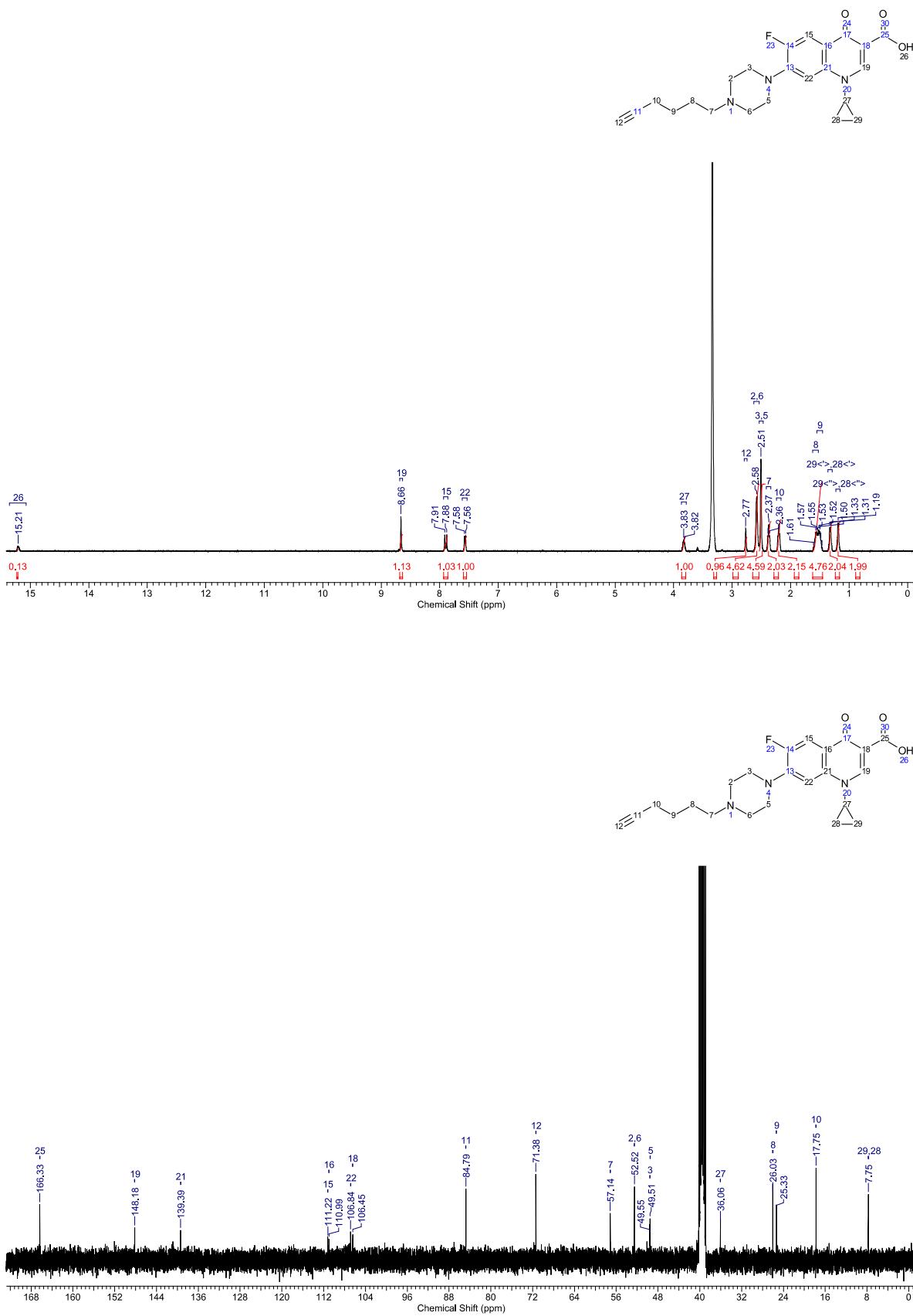


Figure 30: 54

11 References

- [1] Y. Baker. Novel Affinity Based Probes for Use in Chemical Proteomic Studies, 2012.
- [2] J. D. Scribner, D. L. Smith, and J. A. McCloskey. *The Journal of Organic Chemistry*, 43(10):2087–2088, 1978.
- [3] Y. Baker. Personal Communication, 2014.
- [4] J. T. Hodgkinson, W. R. J. D. Galloway, M. Welch, and D. R. Spring. *Nature Protocols*, 7(6):1184–1192 2012.
- [5] J. T. Hodgkinson, W. R. Galloway, M. Casoli, H. Keane, X. Su, G. P. Salmon, M. Welch, and D. R. Spring. *Tetrahedron Letters*, 52(26):3291–3294 2011.
- [6] J. Hlaváč, M. Soural, P. Hradil, I. Frys, and J. Slouka. *Journal of Heterocyclic Chemistry*, 41:633–636, 2004.
- [7] G. Shen, M. Wang, T. R. Welch, and B. S. J. Blagg. *The Journal of Organic Chemistry*, 71(20):7618–7631 2006.
- [8] S. Xu, X. Zhuang, X. Pan, Z. Zhang, L. Duan, Y. Liu, L. Zhang, X. Ren, and K. Ding. *Journal of Medicinal Chemistry*, 56:4631–4640, 2013.
- [9] D. M. Stacy, S. T. Le Quement, C. L. Hansen, J. W. Clausen, T. Tolker-Nielsen, J. W. Brummond, M. Givskov, T. E. Nielsen, and H. E. Blackwell. *Organic & Biomolecular Chemistry*, 11(6):938–954 2013.
- [10] L. S. Kocsis, E. Benedetti, and K. M. Brummond. *Organic Letters*, 14(17):4430–4433, 2012.
- [11] A. F. Abdel-Magid, K. G. Carson, B. D. Harris, C. A. Maryanoff, and R. D. Shah. *The Journal of Organic Chemistry*, 61(11):3849–3862 1996.
- [12] T. E. Renau, J. P. Sanchez, J. W. Gage, J. A. Dever, M. A. Shapiro, S. J. Gracheck, and J. M. Domagala. *Journal of Medicinal Chemistry*, 39(3):729–735 1996.
- [13] N. Hashimoto, T. Funatomi, T. Misaki, and Y. Tanabe. *Tetrahedron*, 62(10):2214–2223 2006.
- [14] P. Senthilkumar, M. Dinakaran, P. Yogeeswari, D. Sriram, A. China, and V. Nagaraja. *European Journal of Medicinal Chemistry*, 44(1):345–358 2009.
- [15] L. A. Mitscher, P. N. Sharma, D. T. W. Chu, L. L. Shen, and A. G. Pernett. *Journal of Medicinal Chemistry*, 29:2044–2047, 1986.
- [16] M. J. Pouy, S. A. Delp, J. Uddin, V. M. Ramdeen, N. A. Cochrane, G. C. Fortman, T. B. Gunnoe, T. R. Cundari, M. Sabat, and W. H. Myers. *ACS Catalysis*, 2(10):2182–2193 2012.
- [17] D. I. Rožkiewicz, D. Jańczewski, W. Verboom, B. J. Ravoo, and D. N. Reinhoudt. *Angewandte Chemie*, 45(32):5292–5296, 2006.
- [18] S. C. Davies. *The Drugs Don't Work: A Global Threat*. Penguin Books Limited, 2013.
- [19] A. Fleming. *The British Journal of Experimental Pathology*, 10(3):226–236, 1929.
- [20] M. Barber. *British Medical Journal*, 2(4534):863–865, 1947.
- [21] P. M. Rountree and E. F. Thomson. *The Lancet*, 254(6577):501–504, 1949.
- [22] P. S. Stewart and J. W. Costerton. *The Lancet*, 358(9276):135–138 2001.

- [23] A. E. Clatworthy, E. Pierson, and D. T. Hung. *Nature Chemical Biology*, 3(9):541–548 2007.
- [24] *Oxford English Dictionary*. Oxford University Press, 2014.
- [25] K. L. Visick and L. M. Skoufos. *Journal of Bacteriology*, 183(3):835–842, 2001.
- [26] A. L. Schaefer, B. L. Hanzelka, A. Eberhard, and E. P. Greenberg. *Journal of Bacteriology*, 178(10):2897–2901 1996.
- [27] M. B. Miller and B. L. Bassler. *Annual Review of Microbiology*, 55:165–199, 2001.
- [28] W. C. Fuqua, S. C. Winans, and E. P. Greenberg. *Journal of Bacteriology*, 176(2):269–275, 1994.
- [29] C. M. Waters and B. L. Bassler. *Annual Review of Cell and Developmental Biology*, 21:319–346 2005.
- [30] S. Atkinson, C.-Y. Chang, R. E. Sockett, M. Cámara, and P. Williams. *Journal of Bacteriology*, 188(4):1451–1461, 2006.
- [31] K.-G. Chan, S. D. Puthucheary, X.-Y. Chan, W.-F. Yin, C.-S. Wong, W.-S. S. Too, and K.-H. Chua. *Current Microbiology*, 62(1):167–72 2011.
- [32] K. Sauer, A. K. Camper, G. D. Ehrlich, J. W. Costerton, and D. G. Davies. *Journal of Bacteriology*, 184(4):1140–1154, 2002.
- [33] B. Michael, J. N. Smith, S. Swift, and F. Heffron. *Journal of Bacteriology*, 183(19):5733–5742, 2001.
- [34] B. M. M. Ahmer. *Molecular Microbiology*, 52(4):933–45 2004.
- [35] K. H. Nealson, T. Platt, and J. W. Hastings. *Journal of Bacteriology*, 104(1):313–322, 1970.
- [36] E. Klein, D. L. Smith, and R. Laxminarayan. Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus*, United States, 1999–2005. Technical Report 12, 2007.
- [37] L. C. M. Antunes, R. B. R. Ferreira, M. M. C. Buckner, and B. B. Finlay. *Microbiology*, 156:2271–2282 2010.
- [38] Quorum Sensing and Genetic Circuit Design.
- [39] G. P. Bodey, R. Bolivar, V. Fainstein, and L. Jadeja. *Reviews of infectious diseases*, 5(2):279–313, 1983.
- [40] J.-F. Dubern and S. P. Diggle. *Molecular bioSystems*, 4(9):882–8 2008.
- [41] K. Poole. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 10(1):12–26 2004.
- [42] P. Cornelis. *Pseudomonas: Genomics and Molecular Biology*. Caister Academic Press, 2008.
- [43] R. C. Hider and X. Kong. *Natural product reports*, 27(5):637–57 2010.
- [44] M. R. Seyedsayamdst, S. Cleto, G. Carr, H. Vlamakis, M. João Vieira, R. Kolter, and J. Clardy. *Journal of the American Chemical Society*, 134(33):13550–3 2012.
- [45] M. G. P. Page. *Annals of the New York Academy of Sciences*, 1277:115–26 2013.
- [46] a. Hartmann, H. P. Fiedler, and V. Braun. *European journal of biochemistry / FEBS*, 99(3):517–24 1979.
- [47] M. Ghosh and M. J. Miller. *Bioorganic & medicinal chemistry*, 3(11):1519–25 1995.
- [48] U. Möllmann, L. Heinisch, A. Bauernfeind, T. Köhler, and D. Ankel-Fuchs. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*, 22(4):615–24 2009.

- [49] C. Dini and J. Aszodi. *Bioorganic & medicinal chemistry letters*, 10(4):349–52 2000.
- [50] T. Kline, M. Fromhold, T. E. Mckennon, S. Cai, J. Treiberg, N. Ihle, D. Sherman, W. Schwan, M. J. Hickey, P. Warrener, P. R. Witte, L. L. Brody, L. Goltry, L. M. Barker, S. U. Anderson, S. K. Tanaka, R. M. Shawar, L. Y. Nguyen, M. Langhorne, A. Bigelow, L. Embuscado, and E. Naeemi. 8, 2000.
- [51] Y. Lu and M. J. Miller. 7:3025–3038, 1999.
- [52] M. Ghosh and M. J. Miller. *Bioorganic & medicinal chemistry*, 4(1):43–8 1996.
- [53] S. R. Md-Saleh, E. C. Chilvers, K. G. Kerr, S. J. Milner, A. M. Snelling, J. P. Weber, G. H. Thomas, A.-K. Duhme-Klair, and A. Routledge. *Bioorganic & medicinal chemistry letters*, 19(5):1496–8 2009.
- [54] F. Rivault, C. Liébert, A. Burger, F. Hoegy, M. a. Abdallah, I. J. Schalk, and G. L. a. Mislin. *Bioorganic & medicinal chemistry letters*, 17(3):640–4 2007.
- [55] C. Ji and M. J. Miller. *Bioorganic & medicinal chemistry*, 20(12):3828–36 2012.
- [56] T. Zheng and E. M. Nolan. *Journal of the American Chemical Society* 2014.
- [57] G. E. Zurenko, S. E. Truesdell, B. H. Yagi, R. J. Mourey, and a. L. Laborde. *Antimicrobial agents and chemotherapy*, 34(5):884–8 1990.
- [58] H. Takase, H. Nitanai, K. Hoshino, and T. Otani. *Infection and immunity*, 68(4):1834–9 2000.
- [59] J. Hodgkinson, S. D. Bowden, W. R. J. D. Galloway, D. R. Spring, and M. Welch. *Journal of Bacteriology*, 192(14):3833–7 2010.
- [60] W. R. J. D. Galloway, J. T. Hodgkinson, S. D. Bowden, M. Welch, and D. R. Spring. *Chemical Reviews*, 111(1):28–67 2011.
- [61] C. M. Oliphant and G. M. Green. *American family physician*, 65(3):455–64 2002.
- [62] C. W. Tornøe, C. Christensen, and M. Meldal. *The Journal of organic chemistry*, 67(9):3057–64 2002.
- [63] V. V. Rostovtsev, L. G. Green, V. V. Fokin, and K. B. Sharpless. *Angewandte Chemie International Edition*, 41(14):2596–2599, 2002.
- [64] P. E. Eaton, G. R. Carlson, and J. T. Lee. *Journal of Organic Chemistry*, 38(23):4071–4073, 1973.
- [65] D. Zewge, C.-y. Chen, C. Deer, P. G. Dormer, and D. L. Hughes. (13):4276–4279, 2007.
- [66] D. K. Yung, L. G. Chatten, and D. P. MacLeod. *Journal of Pharmaceutical Sciences*, 57(12):2073–2080, 1968.
- [67] S. Gabriel. *Berichte der deutschen chemischen Gesellschaft*, 20(2):2224–2236, 1887.
- [68] K. Grohe, H. J. Zeiler, and K. G. Metzger. 7-amino-1-cyclopropyl-4-oxo-1, 4-dihydro-quinoline-and naphthyridine-3-carboxylic acids and antibacterial agents containing these compounds, 1987.
- [69] E. J. Hanan, A. van Abbema, K. Barrett, W. S. Blair, J. Blaney, C. Chang, C. Eigenbrot, S. Flynn, P. Gibbons, C. a. Hurley, J. R. Kenny, J. Kulagowski, L. Lee, S. R. Magnuson, C. Morris, J. Murray, R. M. Pastor, T. Rawson, M. Siu, M. Ultsch, A. Zhou, D. Sampath, and J. P. Lyssikatos. *Journal of medicinal chemistry*, 55(22):10090–107 2012.
- [70] Y. Yamamoto, Y. Watanabe, and S. Ohnishi. *Chemical and Pharmaceutical Bulletin*, 35(5):1860–1870, 1987.

- [71] T. Praneenararat, A. G. Palmer, and H. E. Blackwell. *Organic & biomolecular chemistry*, 10(41):8189–99 2012.
- [72] Faridoon, W. M. Hussein, N. Ul Islam, L. W. Guddat, G. Schenk, and R. P. McGahey. *Bioorganic & medicinal chemistry letters*, 22(7):2555–9 2012.
- [73] J. Grote, R. Himmelsbach, and D. Johnson. *Tetrahedron Letters*, 53(50):6751–6754 2012.
- [74] T. L. Lemke and D. A. Williams. *Foye's Principles of Medicinal Chemistry*. Wolters Kluwer Health, 2012.
- [75] E. S. Coimbra, M. V. de Almeida, C. O. R. Júnior, A. F. Taveira, C. F. da Costa, A. C. de Almeida, E. F. C. Reis, and A. D. da Silva. *Chemical biology & drug design*, 75(2):233–5 2010.
- [76] W. L. F. Armarego and C. U. U. Chai. *Purification of laboratory chemicals*. Butterworth-Heinemann, 2012.
- [77] H. E. Gottlieb, V. Kotlyar, and A. Nudelman. *The Journal of Organic Chemistry*, 62(21):7512–7515, 1997.