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A CONTINUOUS PROCESS TOWARDS THE SYNTHESIS OF QUINOLONES

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By

Stevara N. Clinton

B.S. Virginia Commonwealth University, 2009

Director: B. Frank Gupton

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Virginia Commonwealth University

Richmond, Virginia

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Abstract

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Major Director: B. Frank Gupton, Research Professor and Chair, Department of Chemical and Life Sciences Engineering

The development of quinolones is described from the first quinolone to the latest fluoroquinolones. Quinolones have generated considerable interest since their discovery because of their antibacterial capabilities. Analogs incorporating the 4-quinolone ring system comprise a largely expanding group of synthetic compounds. The development of antibacterial resistance has created the need for an efficient synthesis of quinolones that can be easily adapted toward the assembly of quinolone based antibacterial drugs.

There are several reported approaches to the 4-quinolone ring system. Many of these methods use expensive starting materials, require the removal of high boiling solvents, or use high temperature conditions (>200°C) for the final cyclization. Our synthesis of 4-quinolones was achieved via continuous flow chemistry using inexpensive starting materials in easily removable solvents, and under mild conditions.

Flow chemistry is the use of technology that allows a continuous flow of reagents to be introduced at various points along a process stream, enabling interaction under highly controlled conditions. By employing this technology we achieved a more rapidly scalable synthesis of 4-quinolones, offering safer reacting conditions and highly reproducible results.

Acknowledgements

I want to thank my husband first, for his love and support throughout my years of graduate study. Thank you for standing by me and being my pillar of strength.

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Finally, to my committee members thank you for your suggestions and encouragement throughout my studies.

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Abbreviations

AcOH	Acetic acid
Cipro	Ciprofloxacin
$CHCl_3$	Chloroform
CH_3I	Methyl Iodide
DMF	N,N-Dimethylformamide
DMF-DMA	N,N-Dimethylformamide dimethyl acetal
Et_3N	Triethylamine
HCl	Hydrochloric acid
KOH	Potassium hydroxide
MeOH	Methanol
NaOH	Sodium hydroxide
NMP	N-methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance
TEA	Triethylamine
THF	Tetrahydrofuran

Chapter 1

4(1H)-Quinolones

1.0 Overview

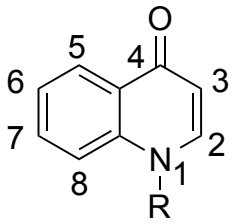
This thesis describes the development of a novel methodology for the synthesis of 4-quinolones, as well as their application towards the synthesis of marketed and new antibacterial drugs. The primary purpose of this chapter is to provide background on previous syntheses leading to 4-quinolones and their relevant biological activity.

1.1 Introduction

In the area of antibiotics, the 4-quinolone ring system **1** has been heavily studied and is an attractive synthetic target in organic synthesis.[1] It is well represented in numerous natural product structures as the core structural motif of many antibacterial agents.

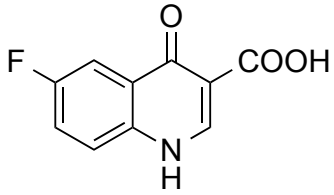
Defined by a bicyclic heteroaromatic core, quinolones require an R-substitued nitrogen at the 1-position and a carbonyl at the C4-position.[2] The most popular 4-quinolones are the fluoroquinolones **2** (6-fluoro-4-quinolone-3-carboxylic acid).[3] They display high activity against a broad range of bacteria. They are so popular, in fact, that most literature refers to them as simply "quinolones" while other 4-quinolones are specifically identified as non-fluorinated or non-carboxylated quinolones. It is these non-flourinated and non-

carboxylated quinolone that have attracted interest as anti-cancer drugs, HIV inhibitors, and anti-anxiety agents.[4-6]



1

Figure 1. 4-Quinolone core structure with numbering

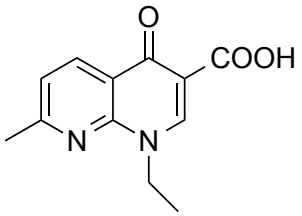


2

Figure 2. Fluoroquinolone core structure

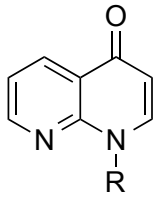
1.2 History of Quinolone

Historically, the quinolone era began in 1962 with the introduction of the quinolone precursor nalidixic acid **3**. This naphthyridine (**4**) agent was discovered as a byproduct in the course of a chloroquine synthesis and was found to moderately inhibit gram-negative bacteria growth leading to its use as a treatment for urinary tract infections (UTIs).[7,8]



Nalidixic acid

3

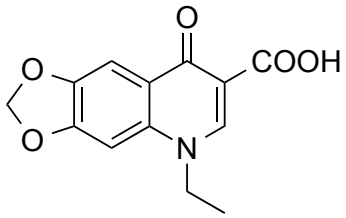


Naphthyridine core

4

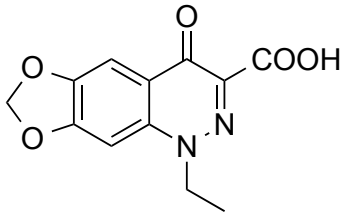
Figure 3. Nalidixic acid and naphthyridine core structure

Over the next two decades, several variations to the naphthyridine scaffold were investigated leading to the first generation of quinolone antibiotics shown in Figure 4.



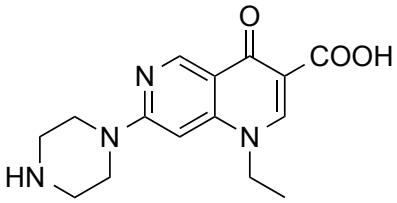
Oxolinic Acid

5



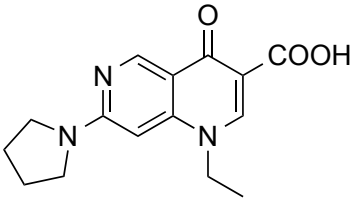
Cinoxacin Acid

6



Pipemidic Acid

7

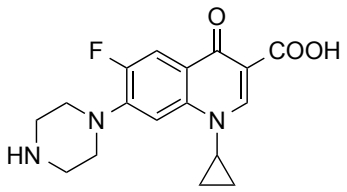


Piromidic Acid

8

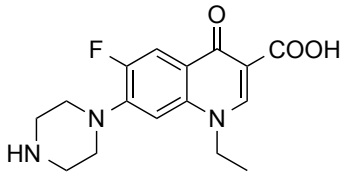
Figure 4**.** First generation of quinolone antibacterials

Lack of broad activity against bacteria and rapid resistance to the early quinolones eventually led to the preparation of 6-fluoro derivatives.[8,9] From there the second, third and fourth generations were introduced (Figures 5-7), which quickly addressed the narrow spectrum of activity previously seen. Ciprofloxacin, patented by Bayer in 1983, is the most successful of this class and has been prescribed worldwide; it is often employed as a drug of last resort when other antibiotics fail.[9,10,11]



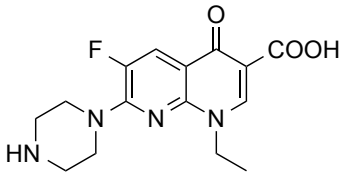
Ciprofloxacin

9



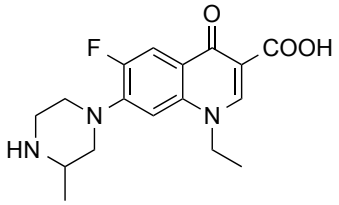
Norfloxacin

10



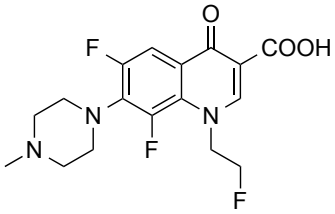
Enoxacin

11



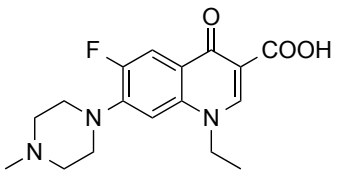
Lomefloxacin

12



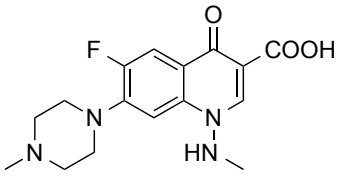
Fleroxacin

13



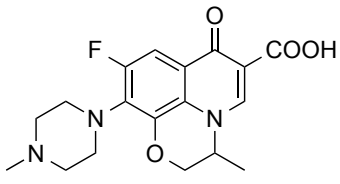
Pefloxacin

14



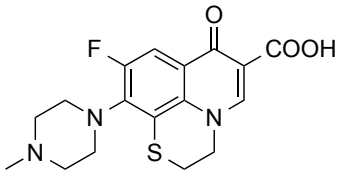
Amifloxacin

15



Ofloxacin

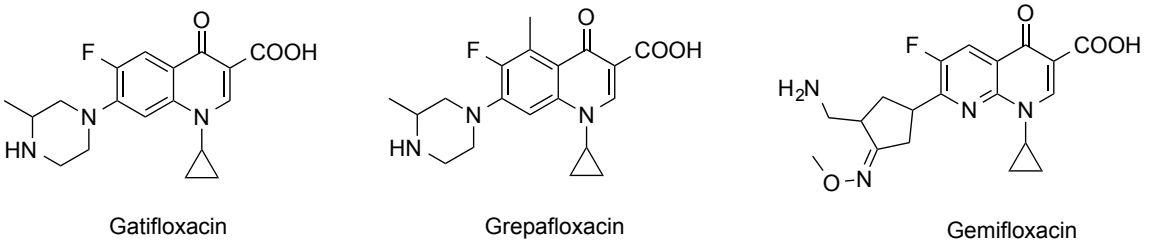
16



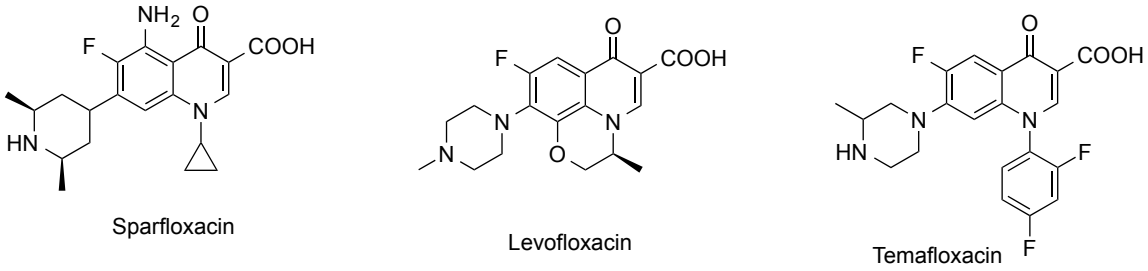
Rufloxacin

17

Figure 5**.** Second generation of quinolone antibacterials

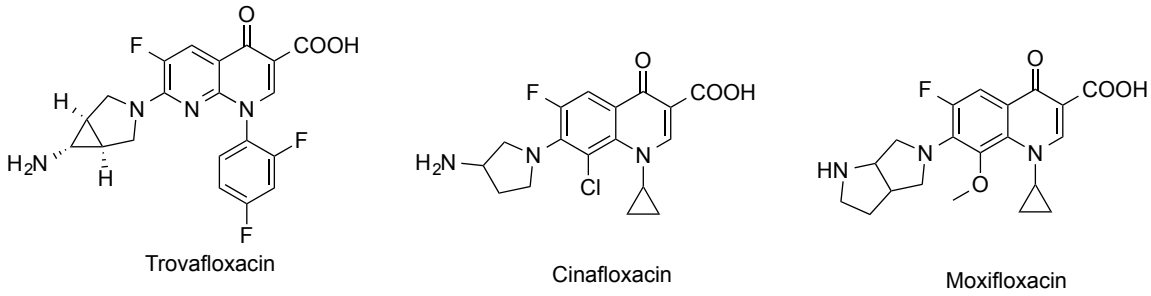


17 **18** **19**



20 **21** **22**

Figure 6**.** Third generation of quinolone antibacterials



23 **24** **25**

Figure 7**.** Fourth generation of quinolone antibacterials

The later generations all have the following aspects in common: identical core 4-quinolone ring structure and identical mechanisms of action.

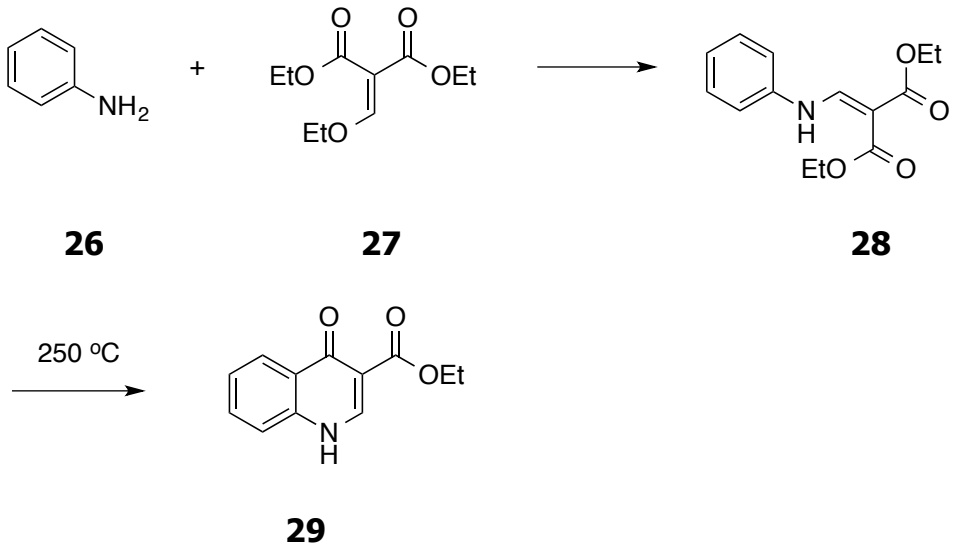
1.3 Mechanism of Action

Mechanistically, quinolones work by inhibiting the bacterial DNA replication process. Topoisomerase is an enzyme used in the replication process to unwind and cleave the bacterial DNA strand allowing the single strand to be read and complemented with a new second strand, thus restoring coiling and replicating the bacteria genome. The quinolone impedes this action by inhibiting the topisomerase from unwinding, thereby disrupting DNA replication, and furthermore, cell viability.[12,13]

1.4 Classical Formations of Quinolones

1.4.1 Gould-Jacobs synthesis of quinolones

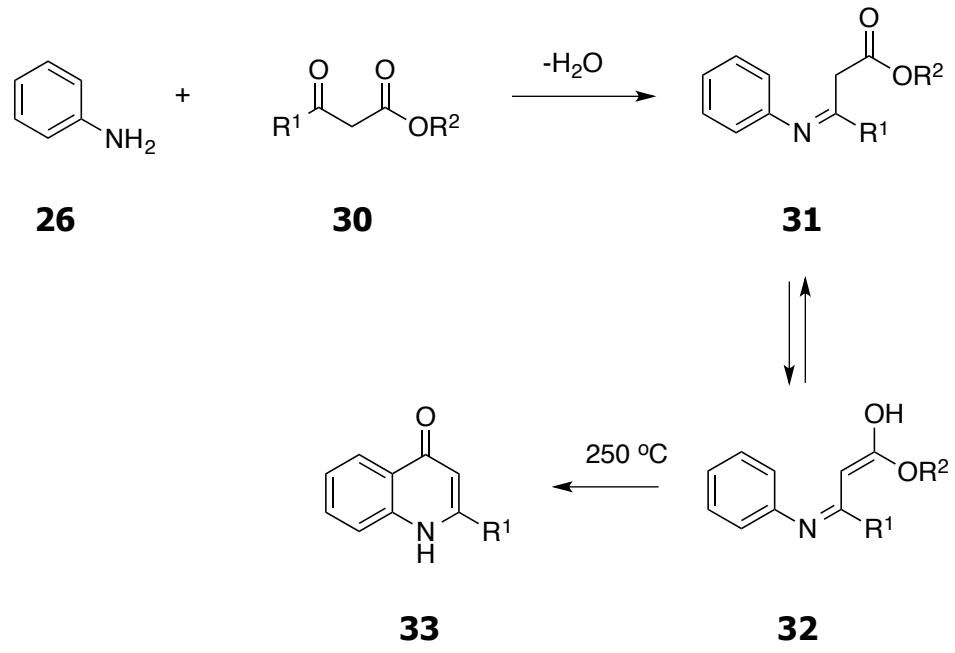
The Gould-Jacobs method is one of the most popular and well established methods for the synthesis of quinolones. This method is based upon an addition-elimination reaction between anilines such as **26** and ethyl ethoxymethyenemalonate **27** to yield intermediate **28**, as shown in Scheme 1. These intermediates then undergo thermal cyclization at high temperatures to give the 4(1H)-quinolone system **29**.[14,15]



Scheme 1. Gould-Jacobs reaction

1.4.2 Conrad-Limpach synthesis

This reaction involves the condensation of an aniline **26** with an acetoacetic ester **30** to yield a Schiff base intermediate **31**. The intermediate then undergoes tautomerization before thermal cyclization (250°C) to yield 4-quinolone derivative **33** shown in Scheme 2.[16]

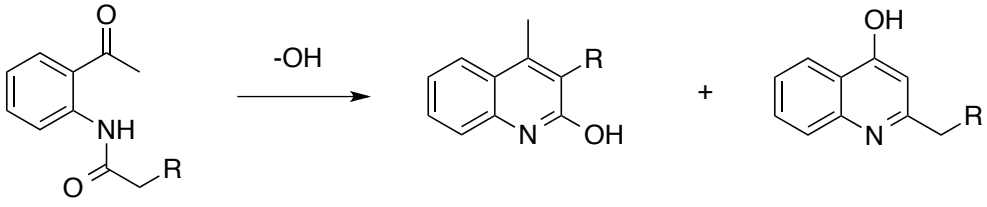


26 **30** **31** **33** **32**

Scheme 2. Conrad-Limpach quinolone synthesis

1.4.3 Camps quinoline synthesis

In this synthesis, an o-acetylaminoacetophenone **34** is cyclized in the presence of a hydroxide ion to yield two hydroxylquinolines **35** and **36** in the proportion of 70:20 respectively (Scheme 3).[17-22]

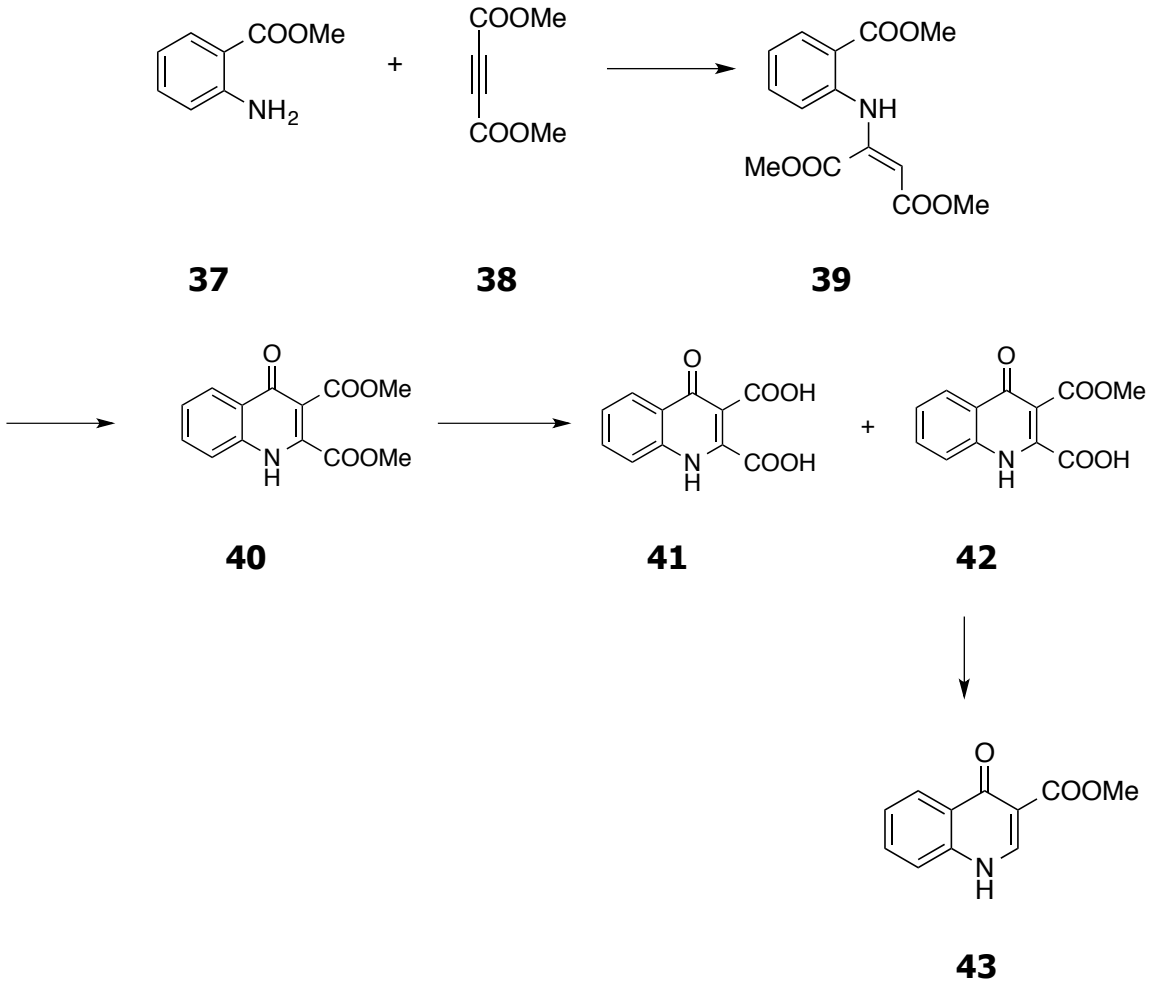


34 **35** **36**

Scheme 3. Camps quinoline synthesis

1.4.4 Biere-Seelen synthesis

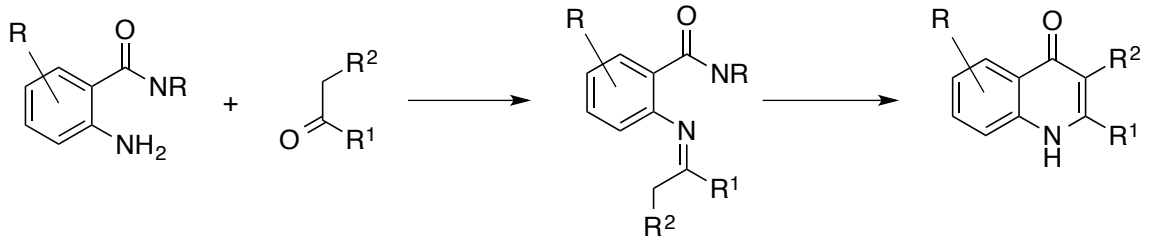
The Biere and Seelen approach for quinolone synthesis was developed in 1979.[23] The procedure involves a Michael addition that gives rise to enamino ester **39**. The ester then undergoes cyclization (product **40)** in the presence of a strong base followed by either complete or regioselective hydrolysis to achieve the dicarboxylic acid **41** and the ester acid **42**. Thermal decarboxylation of the ester acid **42** gives the carboxyl quinolone **43**.



Scheme 4. Biere-Seelen synthesis.

1.4.5 Snieckus synthesis

The Snieckus group has published one of the few methods for 3-alkyl-substitued 4-quinolones. They synthesized 4-quinolones by the condensation of an ortho-substituted aniline **44** with a ketone **45**. This was followed by deprotonation and intramolecuar cyclization using a strong hindered base to yield quinolone **47**.[24]

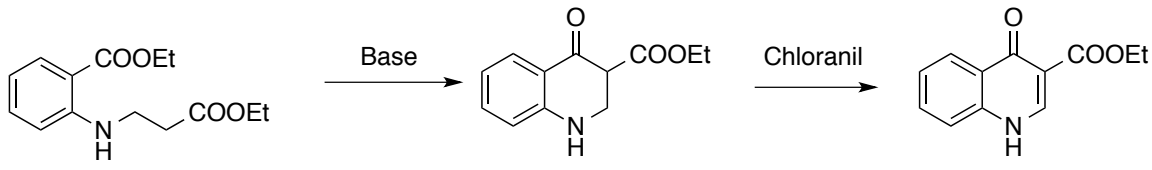


44 **45** **46** **47**

Scheme 5. Snieckus synthesis of 4-quinolones

1.4.6 Dieckmann cyclization

This process involves a diester **48** which undergoes intramolecular ring closure in the presence of a base to form the dihydroquinolone **49**. Oxidation of **49** yields the 4-quinolone **50**.[25]



48 **49** **50**

Scheme 6. Dieckmann cyclization approach to 4-quinolones

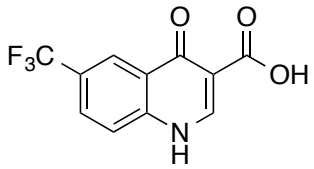
1.5 Alternate Uses of Quinolones

1.5.1 Quinolones as anti-cancer agents

As mentioned, quinolones are well known for their antibacterial activity which arises from interferance with the DNA replication process in bacterial cells. However, in addition to killing bacterial cells, they have the added benefit of killing cancerous tumor cells as well.[26,27] It has been found that some current anti-tumor drugs (topotecan and amsacrine) exhibit the same mechanism of action as fluoroquinolones.[28] None have been approved for use yet because they are not at targeted as currently used chemotherapy drugs. However, quinolones are effective cell killers and are therefore being studied further for their effectiveness in stopping the multiplication of cancerous cells.

1.5.2 Quinolones as HIV inhibitors

New research is being pursued to find alternate treatments for HIV to overcome the emergence of resistance to current treatments. Baba and coworkers were one of the first to discover that some antibacterial fluoroquinolones can exhibit antiviral activity.[29] A further study lead by Cecchetti and coworkers discovered a quinolone derivative that exhibits good antiviral activity (Figure 8).[30] These discoveries play a crucial role in ongoing efforts to find a treatment that can suppress HIV replication using quinolones.



51

Figure 8. Quinolone derivative exhibiting antiviral activity

1.5.3 Quinolones as anti-anxiety agents

Anxiety is defined as a nervous disorder characterized by a state of excessive uneasiness and apprehension. Kahnberg et al. found a quinolone derivative that worked effectively as an anti-anxiety agent leading to the discovery of others (Figure 9); some of which show comparable results to known anti-anxiety drugs currently prescribed.[31] These quinolones are found to work by binding to the receptor sites in the brain that regulate anxiety much the same as some current treatments.

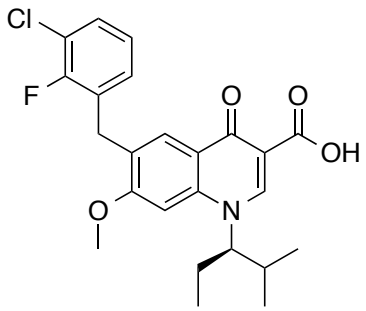


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Figure 9. FDA pproved quinolone anti-anxiety drug, Elvitegravir

Chapter 2

Ciprofloxacin

2.0 Overview

Our interest in 4-quinolones originally grew out of an early approach toward the synthesis of ciprofloxacin. In this chapter we introduce ciprofloxacin and provide background on previous synthetic work leading to its preparation. This methodology is related, in that Ciprofloxacin requires a quinolone framework for synthesis.

2.1 Introduction

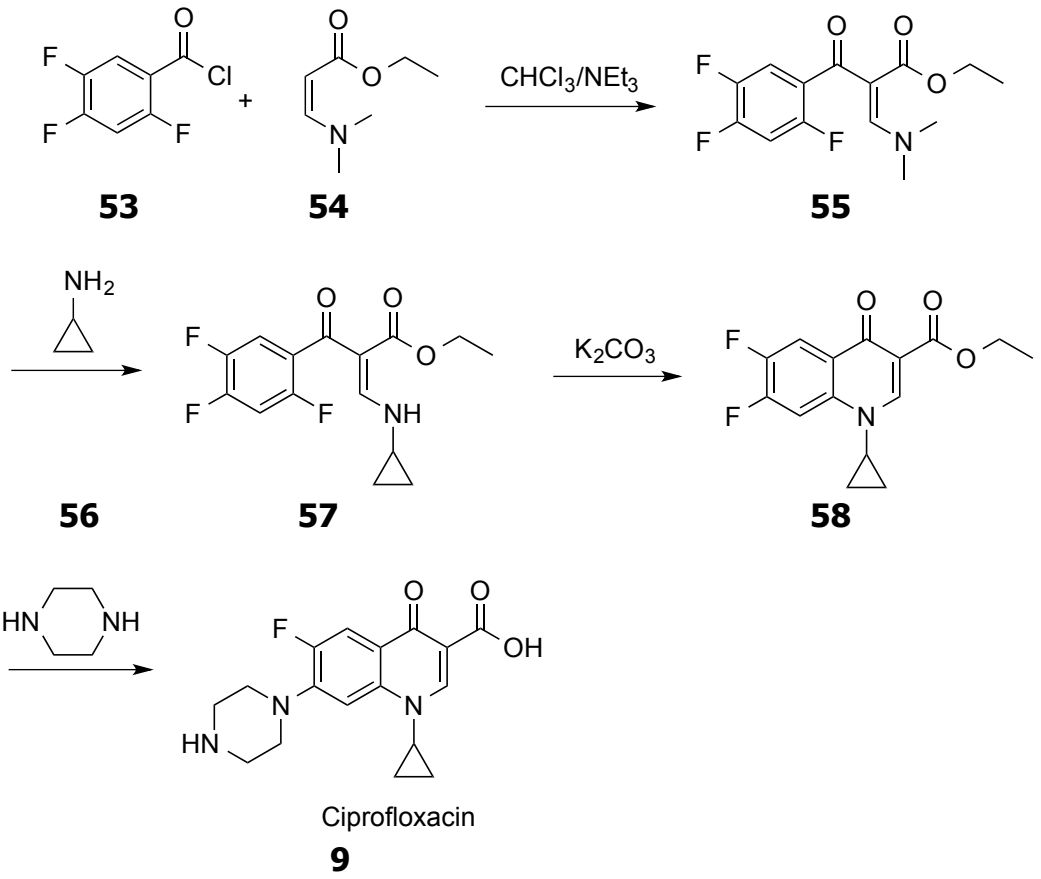
The widely marketed antibiotic ciprofloxacin (Cipro) **9** was discovered and developed by Bayer A.G. in 1983.[32] It is approved for use for a wide range of bacterial infections and is the most successful of the fluoroquinolones. Cipro gained notoriety in the early 2000's during the onset of bioterrorist attacks and was labeled as the drug of choice in the treatment of anthrax exposures.[33]

2.2 Previous Syntheses of Ciprofloxacin

Two well known total syntheses of Cipro appear in the literature. One is the patented method by Bayer A. G. and the other a continuous method patented in 2001 by Schwalbe and coworkers.[34,35]

2.2.1 Bayer A.G. Synthesis (1983)

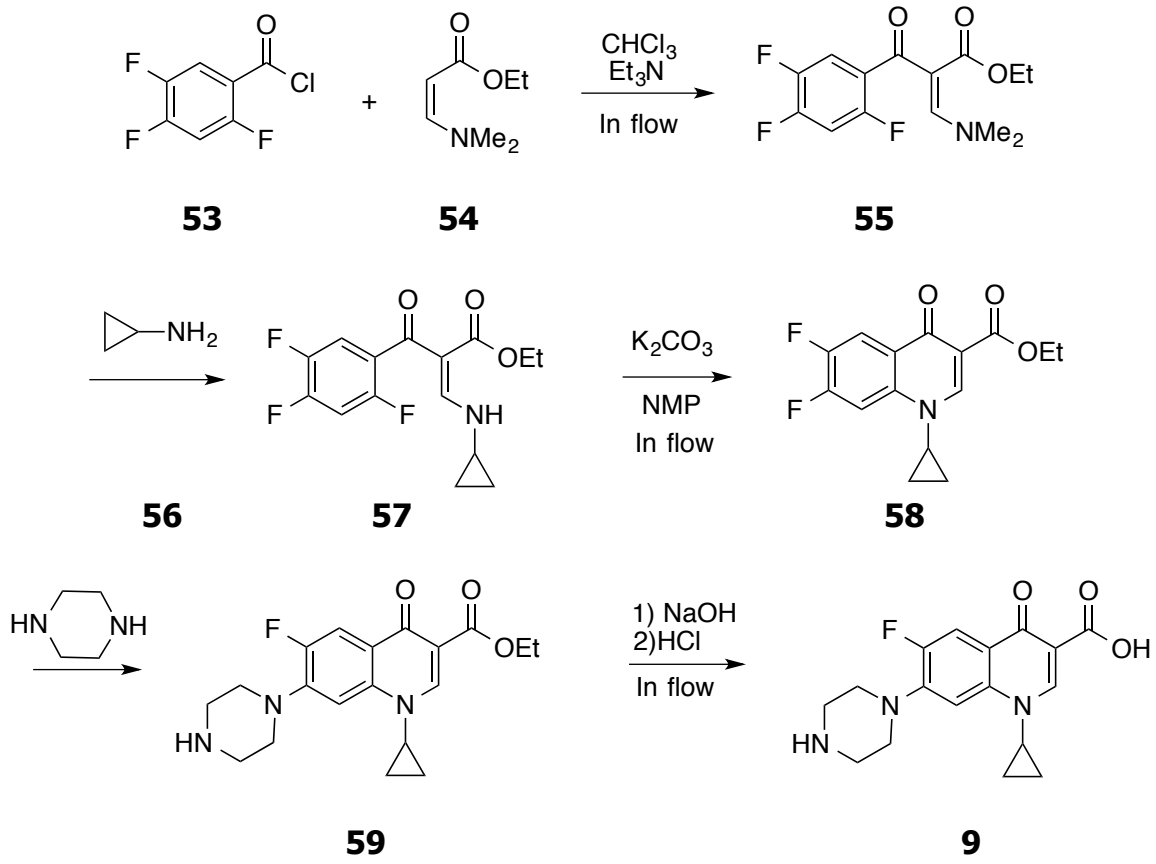
In an effort to improve the antibiotic activity of the norfloxacin stucture, a Bayer scientist, Dr. Klaus Grohe sought out to construct other quinolone analogs. He discovered exceptional antibacterial activity when the quinolone core was substituted with cyclopropylamine and piperzine.[36,37] The synthesis involves an acid halide **53** reacted with a dimethylaminoacrylic acid ester **54**. The resulting aminoacrylic ester **55** is then converted to compound **57** by an amine exchange with cyclopropylamine **56**. A base is introduced, and the compound is cyclized to form quinolone **58**. The addition of piperzine at the 7-position and hydrolysis of the ethyl ester provided ciprofloxacin **9** (Scheme 7).



Scheme 7. Bayer Ciprofloxacin synthesis[32]

2.2.2 Schwalbe et. Al Synthesis (2001)

In 2001, Schwalbe reported a synthesis of ciprofloxacin using a microreactor system (Scheme 8). This feat successfully demonstrated a complex multi-step synthesis performed in continuous flow.[35] As in the Bayer synthesis, Schwalbe starts with the reaction of 2,4,5-trifluorobenzoic acid chloride and dimethylaminoacrylic acid ester, followed by an amine exchange with cyclopropylamine and subsequent nucleophilic ring closure to give the difluoroquinolone ring system **58**. A substitution and hydrolysis then affords the target product.



Scheme 8. Schwalbe Ciprofloxacin synthesis[38]

Chapter 3

Studies Toward the Synthesis of Ciprofloxacin

3.0 Overview

At the beginning of this project, we had two main goals. First, we wished to develop an efficient and novel synthesis of carboxylated quinolones. Ideally, we hoped to achieve this by using 2,4-dichloro-5-fluoroacetophenone and treating with a formate in flow. Then, we would carry out subsequent steps in flow as well, with a key step highlighting CH activation for the direct activation and carbonylation of the quinolone at the 3-position. Secondly, we hoped to use this methodology in a total synthesis of Ciprofloxacin. Our proposed initial synthesis is shown later in Scheme 9.

When our formate approach failed, and due to difficulties in the addition of a cyclic amine, we developed several other routes, one which successfully led to the 4-quinolone synthesis we present here. The details of the studies that led to this synthesis are described in this chapter.

3.1 Background

Despite the functional design of the synthesis previously described, there is still room for improvement. Of the quinolone syntheses, the two most commonly used, the Gould-Jacobs and Conrad-Limpach, undergo high temperature conditions (>200°C) for the final cyclization, have poor

regioselectivity, produce diphenyl ureas byproducts and have problems with tar formation.[39,40] The Bayer Cipro synthesis is lengthy and requires significant time to complete the entire multistep process in batch. In the Schwalbe synthesis, although faster and therefore more ideal, requires expensive starting materials.

There is particular focus on accessing the 4-quinolone ring structure, since even the simplest forms of this structure show useful biological activity.[41] Furthermore, there are relatively few synthetic routes to quinolones that do not require the use of harsh reactions conditions, indicating the need for a mild and efficient route.

The research presented herein is directed towards developing a novel and efficient synthesis of quinolones that can be easily adapted toward the assembly of quinolone-based antibacterial agents. The demand for novel antibacterial drugs continues to grow as more bacteria become resistant to current treatments.[2] This demand has driven the need to develop a route for the preparation of antibiotics that can be altered to produce modified derivatives. By applying continuous flow technology, we aim to overcome the limitations of previous approaches by avoiding the use of expensive starting materials and reactions that are not practical for large-scale use. Furthermore this route will allow us to access known and new analogues by applying this route to synthesize other agents.

Flow Chemistry

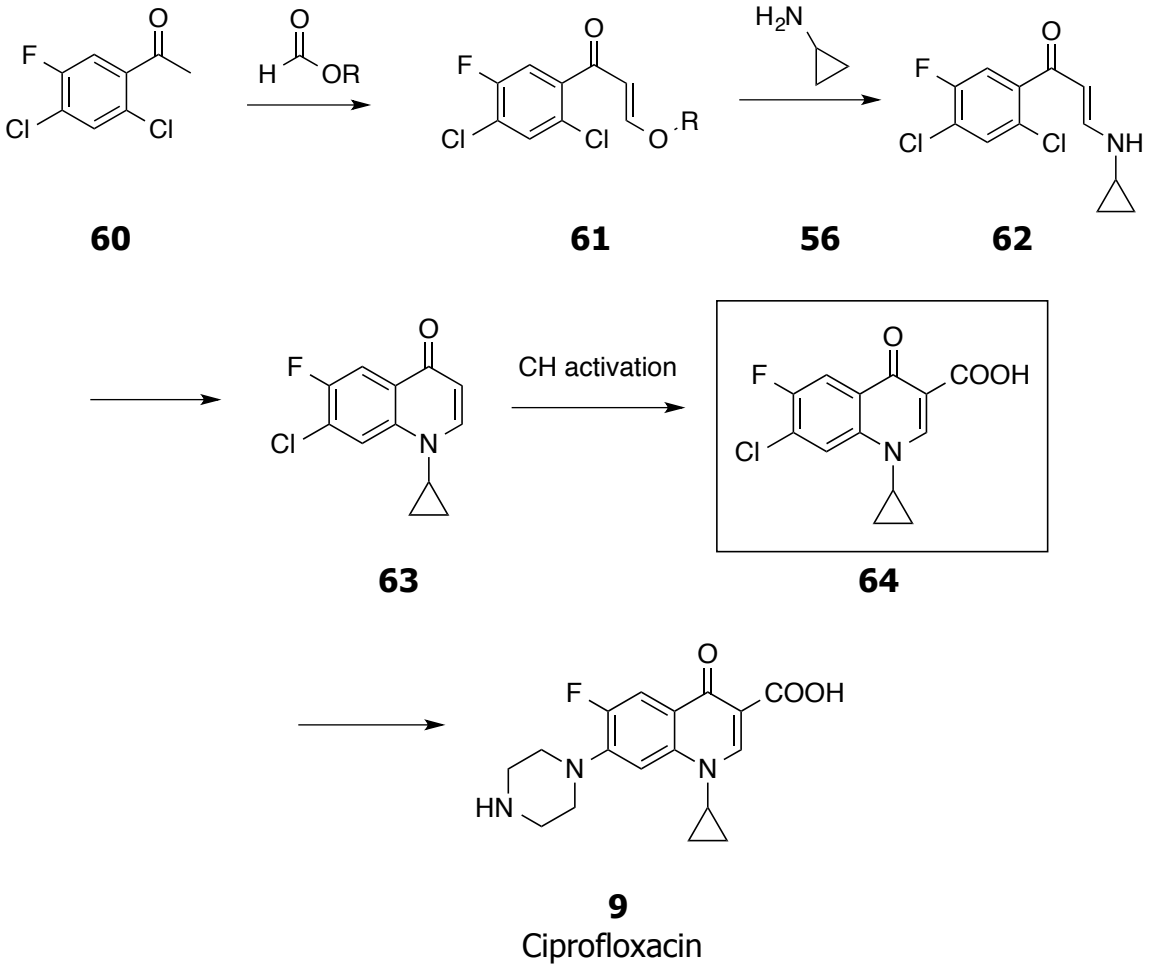
Flow chemistry was introduced about 12 years ago in synthesis laboratories.[42] This technology allows a continuous flow of reagents to be introduced at various points along a process stream, enabling interaction under highly controlled conditions. Flow systems allow high throughput chemistry to take place, often employing immobilized reagents or catalysts.[43]

When compared to batch processes, flow processes have minimal scale-up issues. Instead of scaling up for mass production by increasing reactor size as with batch processes, flow reactors can be scaled up by introducing more reactors in parallel (scaling out) while maintaining excellent mixing and heat transfer.[44,45] The reduction in plant size while increasing plant production is known as process intensification. Carrying out a reaction in flow on a laboratory scale can have the advantage of faster reaction times, safer conditions and faster optimization.[46] For example, a reaction that may require a 24-hour reflux in batch could be reduced to 20 minutes in a continuous flow process. Reaction times, temperature, reagents, pressure, and flow rates can all be rapidly varied to achieve the best conditions. Furthermore, some batch processes pose operational hazards, particularly with the use of highly reactive reagents.[8] These hazards can be avoided under continuous flow conditions due to increased temperature control and short residence times.[47,48]

3.2 Initial Synthetic Studies

3.2.1 Preparation of 2,4-dichloro-5-fluoroacetophenone derivatives

We began our inquiry into the synthesis of the carboxylated quinolone by attempting an addition to 2,4-dichloro-5-fluoroacetophenone **60** with a variety of formate esters (Figure 10) to yield compound **61** indicated in Scheme 9. A number of solvents and reflux conditions were evaluated, but with no observed condensation product by NMR.



60 **61** **56** **62**

63 **64**

9
Ciprofloxacin

Scheme 9. Overview of the initial proposed synthesis of the carboxylated quinolone and conversion to ciprofloxacin

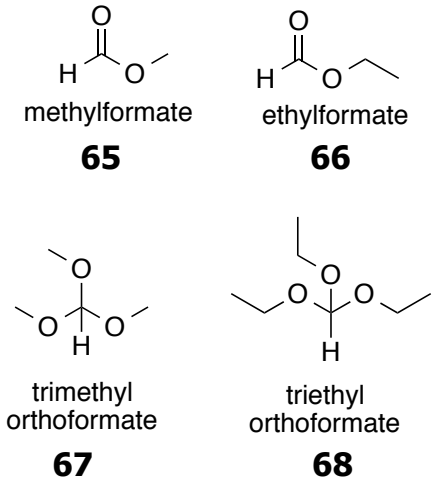


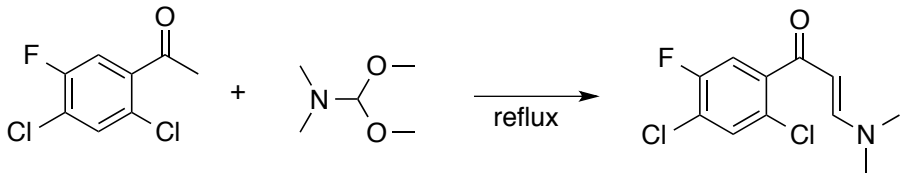
Figure 10. Formates used in initial study[49,50]

After considerable experimentation, we found that dimethylformamide dimethyl amide (DMF-DMA) could be condensed with the 2,4-dichloro-5-fluoroacetophenone **60** with good yield as shown in Table 1, providing an analogous intermediate.

Table 1. Reaction studies for the addition of DMF-DMA to 2,4-dichloro-5-fluoroacetophenone. (Scheme 10)

Entry	Solvent	Time	Yield
1	DMF	24 h	75%
2	Toluene	24 h	86%
3	Xylene	24 h	0%
4	Toluene	48 h	87%

1.2 equivalents of DMF-DMA



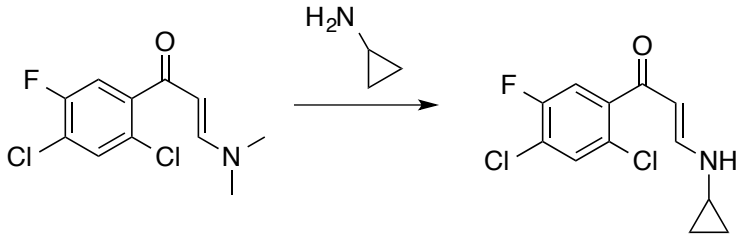
60 **69** **70**

Scheme 10. 2,4-dichloro-5-fluoroacetophenone and DMF-DMF reaction[51]

Based upon the data in the table, we can conclude that toluene was the most effective solvent for the addition reaction and running the reaction longer than 24 hours did not greatly improve the yield. The addition was slow and attempts to run it for a shorter time (18 hr) resulted in a considerable amount of unreacted starting material.

3.2.2 Addition of cyclopropylamine and benzylamine

Upon acquiring a good yield of enaminone **70**, we then sought out to exchange the dimethylamine with a carbocyclic amine (Scheme 11). Drawing from the structure of Cipro, we were interested in carrying out this exchange with cyclopropylamine (CPA) **56**. The revised possible route to the carboxylated quinolone is outlined in Scheme 12. After several attempts, shown in Table 2, we found the dimethylamine is not easily displaced by CPA. Only trace amounts of product were seen by NMR.



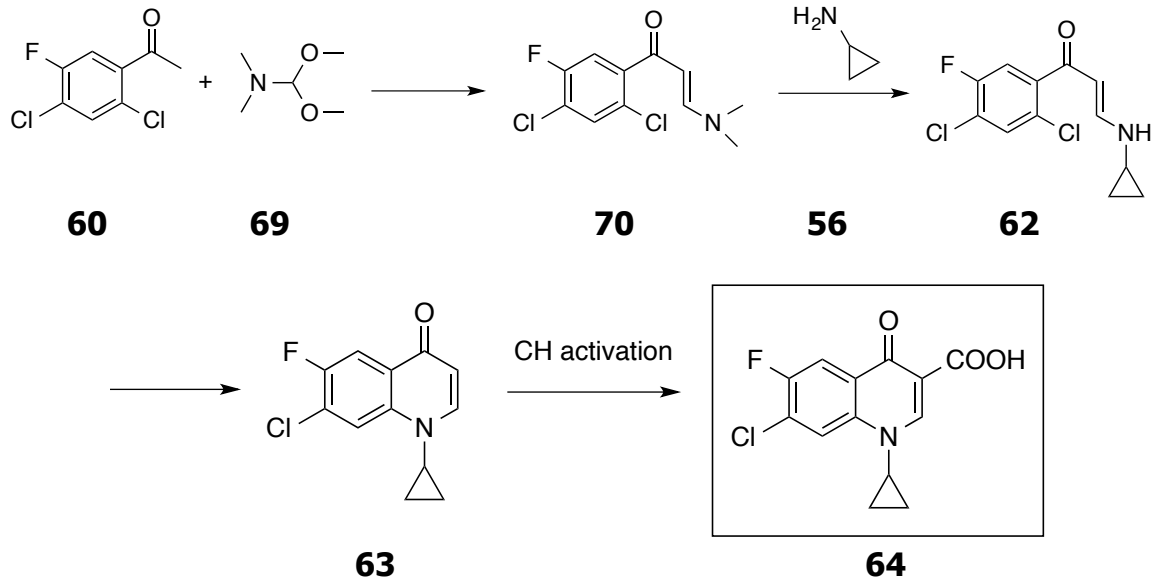
70 **56** **62**

Scheme 11. Proposed amine exchange reaction with cyclopropylamine

Table 2. Amine exchange reaction studies with cylcopropylamine (CPA).[51,52] (Scheme 11)

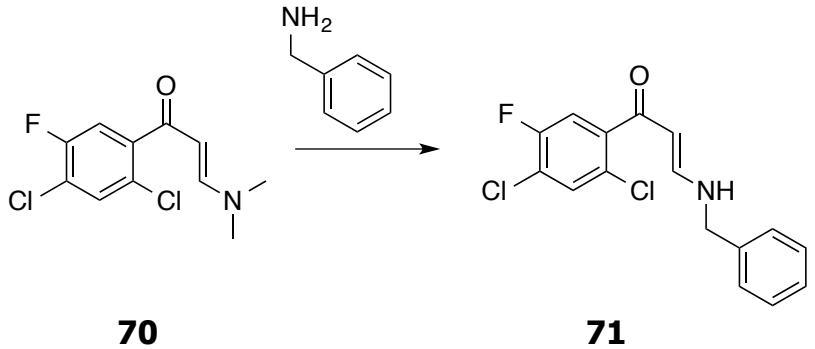
Solvent	Entry	Base	Equiv. CPA
THF	1	none	1.5
MeOH	2	none	3
EtOH	3	K_2CO_3 (2 equiv)	1.5
AcOH	4	K_2CO_3 (2 equiv)	3
DMF	5	K_2CO_3 (5 equiv)	1.5
	6	K_2CO_3 (5 equiv)	3
	7	KOH (2 equiv)	1.5
	8	KOH (2 equiv)	3

Every entry was reacted in each of the five solvents separately.



Scheme 12. Revised route to the carboxylated quinolone

With this step presenting itself as a challenge, we pursued another option of carrying out the reaction with a higher molecular weight amine, benzylamine (Scheme 13), based on what was readily available in the laboratory. All of the same reaction conditions were studied as seen in Table 2 and similar results were obtained. Only trace amounts of product were seen by NMR.



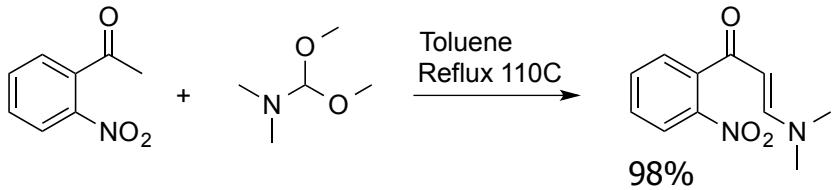
Scheme 13. Amine exchange with benzylamine

3.3 Preparation of Nitroacetophenone Derivatives

Having found the amine exchange step unsuccessful, we began to focus on a different route, building a model system starting with a nitroacetophenone. Construction through this route presented a more affordable starting material through which to synthesize the 4-quinolone. We employed all of the same reaction conditions used in the condensation of the formate esters with acetophenone **60** (section 3.2.1) and these efforts were unsuccessful.

3.4 Preparation of Enaminone 74 and Transamination studies

Next we employed the best conditions used in the addition of DMF-DMA to acetophenone **60** (Table 1) and applied them to the nitroacetophenone **72**. We found, as shown in Scheme 14, was that the addition of DMF-DMA in toluene refluxed for 24 hours under nitrogen afforded exclusively enaminone **73** in a much more satisfactory yield of 98%.



72 **69** **73**

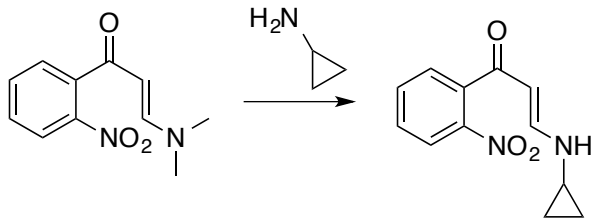
Scheme 14. Synthesis of enaminone **73**[39,53]

In order to expand the effectiveness and novelty of this route, we sought to develop the synthesis under flow conditions using a Vapourtec flow system.

Figure 11. Vapourtec flow reactor[54]

All reagents were premixed in a reagent bottle and flowed through the coiled reactor of 100°C at various flow rates. The results were impressive. At a flow rate of 1 mL/min, we were able to obtain exclusively enaminone **73**, drastically reducing the reaction time from 24 hours to just minutes.

Our earlier attempts to exchange the dimethylamine on compound **70** proved to be a challenge, however we have high hopes for the exchange on enaminone **73** shown in Scheme 15. With limited studies thus far, the transamination has yet to be completed. Investigations into the role the substituents play on the effect of the exchange may give additional insight into resolving the issue.

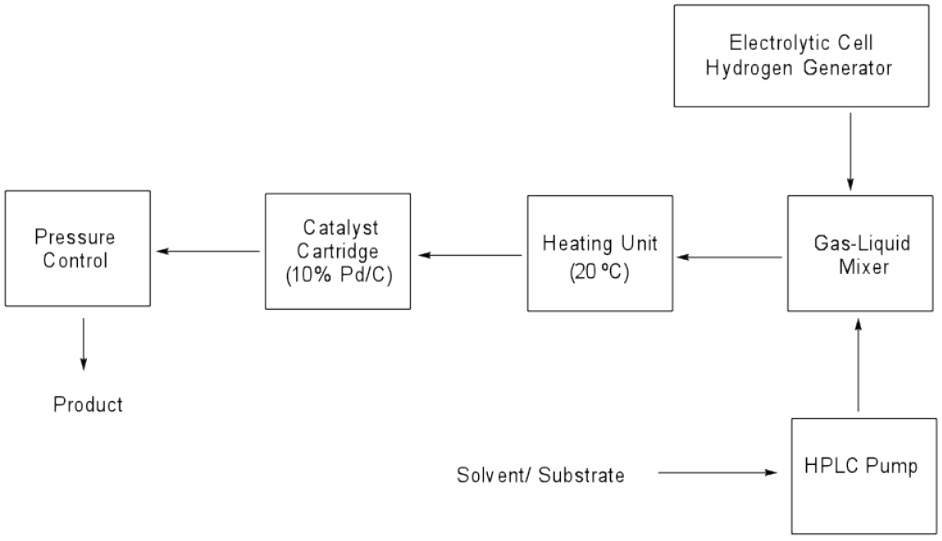


73 **56** **74**

Scheme 15. Amine exchange with cyclopropylamine on enaminone **73**

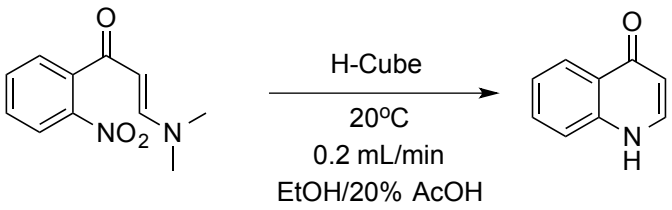
3.5 Ring Closure through Reduction

The H-cube flow reactor has proven to be a useful resource towards the synthesis of our 4-quinolone. As a hydrogen generator, the H-cube allows for catalytic hydrogenation in continuous flow as shown in Scheme 16. With the H-cube, the nitro group on enaminone **73** was reduced giving an aniline which then underwent intramolecular cyclization yielding a high purity product with 10% Pd/C as the catalyst (Scheme 17).



Scheme 16. H-Cube Schematic

The best conditions, shown in Table 3, were obtained with a flow rate of 0.2 mL/min, at room temperature, 1 bar and full hydrogen. Attempts to carry out this reaction under conventional methods[41,53] were unsuccessful, thus highlighting the effectiveness of utilizing a flow system to complete this synthesis.



73 **75**

Scheme 17. H-cube quinolone synthesis

Table 3. H-cube Reaction Studies.

Entry	Flow Rate (mL/min)	Temperature (°C)	Pressure (bar)	H_2 Setting
1	1	35	1	Controlled
2	1	100	20	Controlled
3	1	50	30	Controlled
4	1	r.t.	40	Controlled
5	1	100	20	Controlled
6	1	r.t.	1	Full
7	1	r.t.	1	Full
8	0.5	r.t.	1	Full
9	0.2	r.t.	1	Full

Only entries 8 and 9 yielded product. Entry 8 recovered a majority of starting materials. Entry 9 yielded pure product.

The completion of this step provides a successful synthesis of 4-quinolones in flow from relatively inexpensive starting materials with high purity.

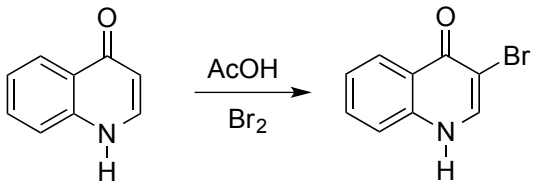
3.6 Application Toward the Synthesis of Ciprofloxacin and Other Antibacterial Derivatives

One of the initial goals in exploring this area of chemistry was to develop a continuous route to the synthesis of quinolones that could be applied to the synthesis of other antibacterial drugs such as Ciprofloxacin. The 4-quinolone ring system is an excellent scaffold for this. It can be modified and manipulated

in a number of ways with functional groups to easily provide access to new and existing antibacterial drugs. To expand on this idea, we further embarked on modifying the 4-quinolone system.

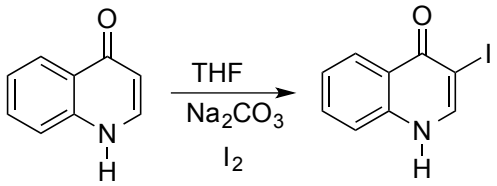
3.6.1 Halogenation of the 4-quinolone at the 3-position

The first modification we explored was a halogenation at the 3-position of the 4-quinolone. The purpose in this pursuit was to provide a shorter pathway toward the synthesis of carboxylated quinolone targets. Limited results have been obtained, however ongoing investigations include the use of bromine (Scheme 18) or iodine (Scheme 19) as the halogens of choice.



75 **76***

Scheme 18. Bromination of the 4-quinolone.[41]

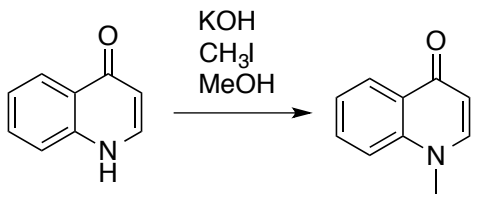


75 **77***

Scheme 19. Iodination of the 4-quinolone.[55]
*yields for crude product

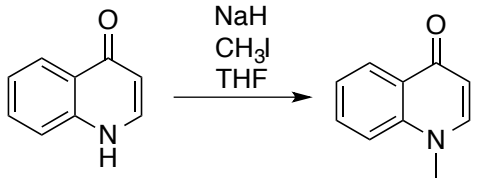
3.6.2 Methylation of the amine

Secondly, we took an interest in methylating the amine group. Though there are a number of antibacterial drugs which do not require an R-substituted amine at the 1-postion, there are also a number of quinolone derivatives which contain an alkyl or cyclic alkane at that position. The schemes we explored, Schemes 20 and 21, are shown below. No results were finalized, however the resulting residue from the preliminary experiments showed mostly starting material by HPLC. Optimization of reaction conditions is ongoing.



75 **78**

Scheme 20. Attempted route for methylation of the quinolone at the 1-position.[56]



75 **78**

Scheme 21. Attempted route for methylation of the quinolone at the 1-position.[57]

Chapter 4

Experimental

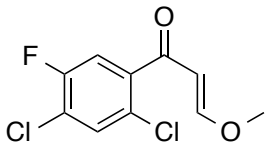
4.1 General Remarks

All reagents and solvents were obtained from commercial sources and were used without further purification. Melting points are not corrected. ^{1}H NMR spectra were recorded on an Agilent-400 NHz NMR spectrometer. High performance liquid chromatography was performed on a Waters Acquity H class liquid chromatograph.

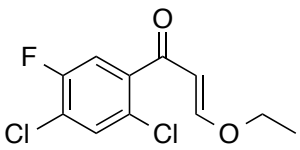
The following abbreviations are used in the experimental section: s (singlet), d (doublet), t (triplet), q (quartet), dd (double of doublets), td (triplet of doublets), m (multiplet), HPLC (high performance liquid chromatography), mp (melting point), equiv (equivalents), mL (milliliters) and N_2 (nitrogen).

4.2 Experiments Pertaining the Starting Reagent 2,4-dichloro-5-fluoroacetophenone

a) Attempted addition of with ethylformate/ methylformate[49,50] (section 3.2.1)



79



80

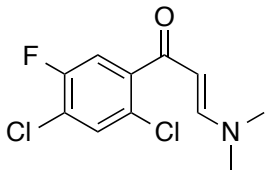
To a round bottom flask, equipped with a stir bar and a suspension of sodium ethoxide (1.0 equiv.) in dry ethyl ether, was added the 2,4-dichloro-5-fluoroacetophenone (1.2 equiv.) and stirred for 15 min at room temperature. Ethylformate (1.2 equiv.) was added to the mixture and stirred for 3 hours at room temperature. The resulting solution was evaporated under reduced pressure and the residue was taken up in dry DMF. Ethyl bromide (2.0 equiv.) was added and the mixture was allowed to stir for 24 hours at room temperature before being poured into ice water and extracted with ethyl acetate. The extracts were washed with water, dried over magnesium sulfate and evaporated. Upon evaporation, very little to no product was present and the NMR analysis showed no indication of the presence of the corresponding product. The procedure was modified for methylformate using sodium methoxide and methyl iodide.

b) Attempted addition of triethyl orthoformate/ trimethyl orthoformate[49,50]

2,4-dichloro-5-fluoroacetophenone (1.0 equiv.) was added to a mixture of triethyl orthoformate (1 equiv.) and acetic anhydride (1.5 equiv.) and was heated under reflux for 8 hours. The crude mixture was concentrated under vacuum. NMR analysis revealed no loss of the methyl protons on the acetophenone. The same procedure was followed for trimethyl orthoformate. Furthermore, the

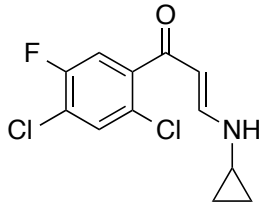
procedure was modified to explore the use of different solvents and longer reaction times to no avail.

c) Preparation of 1-(2,4-dichlor-5-fluorophenyl)-3-(dimethylamino) prop-2-en-1-one (70)[51]



2,4-dichloro-5-fluoroacetophenone (1.0 equiv.) was dissolved in 5 mL of toluene with stirring. DMF-DMA (1.2 equiv.) was added and the solution was refluxed at 110°C under N_2. After 24 hours, the precipitate was filtered off and washed with hexanes. The resulting product **70** was a yellow liquid that solidified upon cooling which was used directly in the next step. ^{1}H NMR (DMSO-*d*6): d = 7.99 (d, 1 H), 7.74 (d, 1 H), 7.57 (d, 1 H), 5.31 (d, 1 H), 3.15 (s, 3 H), 2.89 (s, 3 H).

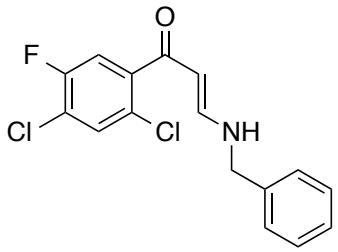
d) Attempted transamination with cyclopropylamine (62)[51,52]



The product from 4.2 part (c) above was dissolved in THF and cooled in an ice bath to 5°C. Cyclopropylamine (excess) was added to the mixture and

stirred at 5°C for 1 hour. The mixture was then concentrated under vacuum to yield a small amount of residue unidentified by NMR. The procedure was modified with the bases shown in Table 2.

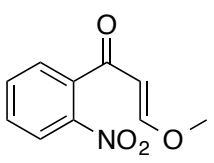
e) Attempted transamination with benzylamine (71)



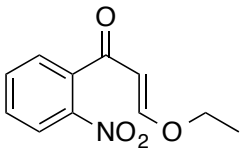
The product of 4.2 part (c) was dissolved in toluene with heating. Benzylamine (excess) was added and stirred at room temperature overnight. The mixture was concentrated under vacuum. NMR showed exclusively starting material. This procedure was modified using the solvents: acetic acid, methanol, and ethanol, separately, as well as heating at reflux overnight per solvent. Furthermore, we added sodium hydride with the thought of first deprotonating the benzylamine before introducing the enaminone.

4.3 Experiments Pertaining to Starting Reagent Nitroacetophenone

a) Attempted addition of the formates (section 3.3)



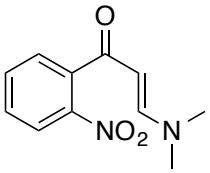
81



82

Compounds **81** and **82** were attempted according to the procedures from 4.2 part (a) and part (b).

b) Preparation of enaminone 73[39,53]

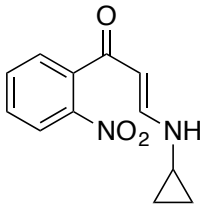


Conventional method: O-nitroacetophenone **72** (1mL, 1.23 g, 7.4 mmol) was dissolved in 5 mL of toluene. The solution was heated to 110°C (oil bath) while DMF-DMA (1.2 equiv.) was added. Heating was continued at 110°C under reflux with a N_2 balloon for 24 hours and then concentrated under vacuum. The yellow liquid solidified upon cooling and was washed with hexanes. The solid was further dried under vacuum filtration giving 1.61g (98%) of **73** which was used directly in the next step.

Flow method: The Vapourtec was purged and filled with toluene (20 mL). A mixture of **72** (2 mL, 2.46 g) and DMF-DMA (1 equiv) dissolved in toluene was

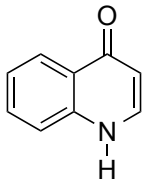
pumped through the Vapourtec at a flow rate of 1 ml/ min. The reaction temperature was held at 100°C. The collected product precipitated out upon cooling in the collection vial. The product was filtered, washed with hexanes and used directly in the next step. Mp=118-120°C, ^{1}H NMR ($CDCl_3$): d = 7.97 (d, *J* = 6.9 Hz, 1 H), 7.64 (apparent t, *J* ≈ 7.5 Hz, 2 H), 7.47 (m, 2 H), 5.27 (d, *J* = 12.3 Hz, 1 H), 3.20 (s, 3 H), 2.85 (s, 3 H).

c) Attempted transamination with cyclopropylamine (74)[51,52]



Compound **73** was attempted from the product of 4.3 part (b) according to the procedure of 4.2 part (d).

d) Preparation of the 4-quinolone (75)

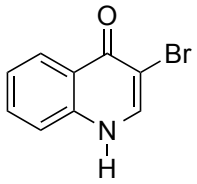


The H-cube was purged and filled with ethanol. Compound **73** (0.5 g, 2.27 mmol) was dissolved in ethanol (25 mL) and acetic acid (5 mL) and then pumped into the reactor at a flow rate of 0.2 mL/ min at room temperature. The reaction mixture was collected and the H-cube was purged with ethanol. The

solution was left in the fume hood overnight and the resulting yellow precipitate was filtered off to yield **75** as a pure compound revealed by HPLC and NMR. Mp 206-208°C, ^{1}H NMR (DMSO-*d*6): d = 6.25 (d, 1H, *J* = 7.1 Hz, H-3), 7.43 (td, 1H, *J* = 6.8 Hz, H-6), 7.59 (d,1H, *J*=8.2 Hz, H-8), 7.69 (td, 1H, *J*=8.1 Hz, H-7), 7.97 (d, 1 H, *J* = 6.9 Hz, H-2), 8.22 (d, 1 H, *J* = 7.9 Hz, H-5) ppm.

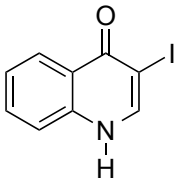
4.4 Experiments Pertaining to the Modification of the 4-Quinolone

a) Preparation of the brominated 4-quinolone (76)[41]



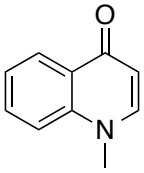
A solution of sodium thiosufate (0.5 M, 25 mL) was first prepared. 4-quinolone **75** was then refluxed for 10 minutes in acetic acid and bromine (1.0 equiv.) was added dropwise at the same temperature. The reaction mixture was stirred for 2 hours under reduced temperature and then allowed to cool to room temperature. The suspension was poured over ice chips and further diluted with the sodium thiosulfate solution. The resulting precipitate was filtered off, washed with water and air dried under vacuum filtration. A beige solid was obtained (75%).

b) Preparation of the iodinated 4-quinolone (77)[55]



A mixture of 4-quinolone (1.0 equiv.), sodium carbonate (1.5 equiv), and iodine (1.5 equiv.) in dry THF was stirred under N_2 for 6 hours at room temperature. The mixture was then poured into a saturated solution of sodium thiosulfate and extracted 3 times with ethyl acetate. The organic layer was dried over sodium sulfate and dried under vacuum to give a yellow solid (70%).

c) Attempted methylation (scheme 20)[56]



Methyl iodide (10 equiv.) was added to a solution of 4-quinolone (1.0 equiv.) and KOH (1.5 equiv.) in methanol. The mixture was allowd to stir overnight at room temperature and the resulting precipitate was filtered off. The remaining solvent was then evaporated and the resulting residue was analyzed by HPLC showing a large portion of starting material still present.

d) Attempted methylation (scheme 21)[57]

The procedure for 4.4 part (c) was followed using 4-quinolone, methyl iodide, and sodium hydride in THF.

Chapter 5

Conclusion

Quinolones have been studied extensively as antibacterial agents and the demand for more efficient routes to synthesize antibacterial drugs and their analogues is great. Pharmaceutical companies are constantly searching for cost effective ways to make new and high quality compounds. Accordingly, we have successfully synthesized the 4-quinolone under flow conditions. Our strategy brings forth a novel approach to the 4-quinolone ring system from the readily available and inexpensive nitroacetophenone. These efforts will provide a fundamental and reliable route to 4-quinolones which, in turn, can allow for the preparation of new antibacterial derivatives.

Although there are several existing methods available, there are no routes that synthesize 4-quinolones in flow. Additionally, most syntheses focus on the carboxylated quinolone, where as the route described in this thesis leads to 3-unsubstituted quinolones. The result is a product that has increased diversity toward the accessibility of target structures, making the chemistry even more valuable.

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