Palladium(0)-Catalysed Carbonylative Multicomponent Reactions

Synthesis of Heterocycles and the Application of Quinolinyl Pyrimidines as Enzyme Inhibitors

LINDA ÅKERBLADH
Abstract

Palladium-catalysed carbonylative multicomponent reactions have proven useful for the synthesis of structurally diverse compounds. Carbon monoxide serves as an atom-efficient, one-carbon building block, which allows for further structural elaboration of the carbonyl compound. By varying the components of the carbonylative multicomponent reaction, considerable product diversity can readily be attained. However, due to the reluctance to use toxic CO gas, considerable efforts have been directed at exploring non-gaseous approaches. The work described in this thesis has mainly focused on the development of palladium(0)-catalysed, carbonylative multicomponent synthetic methodology, using the non-gaseous CO source molybdenum hexacarbonyl, in the synthesis of heterocycles and other biologically relevant functional groups.

The first part of this work describes the development of a non-gaseous carbonylative Sonogashira cross-coupling of bifunctional ortho-iodoanilines and terminal alkynes. Where 4-quinolones were synthesised via a carbonylation/cyclisation sequence. Using a similar synthetic strategy, three different N-cyanobenzamide intermediates were prepared by palladium-catalysed carbonylative couplings of various aryl halides and bromides and cyanamide. The formed intermediates provided a basis for further chemical transformations. First, ortho-iodoanilines were carbonylatively coupled with cyanamide and subsequently cyclised to yield heterocyclic 2-aminoquinazolinones. Next, building on those findings, the same synthetic strategy was applied to ortho-halophenols to provide a highly convenient domino carbonylation/cyclisation method for the preparation of benzoxazinones. The developed method was used to evaluate the efficiency of various non-gaseous CO sources. Third, the palladium-catalysed carbonylative synthesis of N-cyanobenzamides was used to produce biologically relevant N-acylguanidines with considerable product diversity. Finally, one of the developed carbonylative methodologies was used in the preparation of potential NDH-2 inhibitors based on a quinolinyl pyrimidine scaffold. The prepared compounds were biologically evaluated in terms of inhibition of oxidoreductase NDH-2 and antibacterial activity on Gram-negative bacteria, S. aureus and Mtb. The biological evaluation revealed that some of the quinolinyl pyrimidines exerted inhibitory activity on the NDH-2 enzyme and possessed antibacterial properties.

The work described in this thesis has been devoted to the development of non-gaseous one-pot, multicomponent carbonylation/cyclisation and carbonylation/amination reactions. The described methods offer highly attractive synthetic strategies that can be of great value to synthetic and medicinal chemists.

Keywords: Palladium catalysis, Carbonylation, Multicomponent reactions, Domino reactions, Heterocycles, 4-Quinolones, 2-Aminoquinazolinones, Benzoxazinones, N-Acylguanidines, Type II NADH dehydrogenase, NDH-2

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This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV Åkerbladh, L., Schembri, L., Larhed, M., Odell, L. R.* Palladium(0)-Catalyzed Carbonylative Synthesis of N-Acylguanidines. Submitted manuscript.


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Author Contribution Statement

The following contributions to each paper were made by the author of this thesis:

I  Supervised the method development of Method B, synthesised all compounds prepared using Method B, collated the experimental data and wrote the manuscript.
II  Performed all of the experimental work, collated the experimental data and wrote the manuscript.
III  Carried out method development, performed all of the experimental work, collated the experimental data and wrote the manuscript.
IV  Carried out method development, performed the major part of the experimental work, collated the experimental data and wrote the manuscript.
V  Synthesised majority of final compounds, contributed considerably to the interpretation of the results and writing of the manuscript.
Other Papers Not Included in This Thesis


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Abbreviations

_A. baumannii_ | _Acinetobacter baumannii_
---|---
Ar | aryl
ATP | adenosine triphosphate
Bu | butyl
cataCXium A | di(1-adamantyl)-n-butylphospine
clogP | calculated octanol/water partition coefficient
COgen | 9-methyl-9H-fluorene-9-carbonyl chloride
DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene
DCM | dichloromethane
dcpp | 1,3-bis(dicyclohexylphosphino)propane
DIEA | _N,N_-diisopropylethylamine
DMAP | 4-(dimethylamino)pyridine
DMF | dimethylformamide
DPEphos | bis[(2-diphenylphosphino)phenyl] ether
dppf | 1,1-bis(diphenylphosphino)ferrocene
_E. coli_ | _Escherichia coli_
equiv | equivalents
Et | ethyl
EtOAc | ethyl acetate
HepG2 | human hepatocellular carcinoma
IC_{50} | half-maximal inhibitory concentration
L | ligand
m/z | mass-to-charge ratio
MCR | multicomponent reaction
Me | methyl
MeCN | acetonitrile
MeOH | methanol
MIC | minimum inhibitory concentration
MRC-5 | human lung fibroblast cell line
Ms | _Mycobacterium smegmatis_
Mtb | _Mycobacterium tuberculosis_
MW | microwave
NaOPh | sodium phenoxide
NDH-2 | type II NADH dehydrogenase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMP</td>
<td>N-methyl-2-pyrrolidinone</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OAc</td>
<td>acetate</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>PIDA</td>
<td>(diacetoxyiodo)benzene</td>
</tr>
<tr>
<td>PMB</td>
<td><em>para</em>-methoxybenzyl</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>TB</td>
<td>tuberculosis</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-butyldiphenylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TIPS</td>
<td>triisopropylsilyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>Xantphos</td>
<td>4,5-bis(diphenylphosphino)-9,9-dimethylxanthene</td>
</tr>
</tbody>
</table>
Introduction

Palladium(0)-Catalysis

Since the discovery of palladium\(^1\) by Wollaston in the beginning of the nineteenth century, seemingly endless synthetic possibilities have opened up as a result of the catalytic redox properties of the metal. Although palladium can exist in several oxidation states, it is Pd(0) and Pd(II) that have been the most explored.\(^2\) The development of palladium-catalysed coupling reactions has revolutionised organic synthesis, providing highly convenient, mild and effective synthetic strategies for the formation of among others, C-C, C-O and C-N bonds. Pd(0)-catalysed coupling of organohalides and olefins was first reported in the early 1970s by Mizoroki\(^3\) and Heck,\(^4\) independently, building on previous discoveries made by Heck and co-workers.\(^5\) Later the development of coupling reactions between organohalides (or pseudo halides, such as OTf) with organozinc reagents (Negishi),\(^6,7\) boronic acids (Suzuki-Miyaura),\(^8,9\) alkynes (Sonogashira),\(^10\) and nitrogen-nucleophiles (Buchwald-Hartwig)\(^11,12\) were described. Some of the most well-known palladium(0)-catalysed coupling reactions are depicted in Scheme 1 and it is evident that by simply varying the reaction substrates, a tremendous variety of reaction products can be readily obtained. The importance of these synthetic methods was emphasised when Richard F. Heck, Ei-ichi Negishi and Akira Suzuki were awarded the Nobel Prize in Chemistry in 2010.\(^13\)

Scheme 1. Palladium(0)-catalysed coupling reactions.
Palladium(0)-Catalysed Carbonylations

In the late 1930s hydroformylation with syngas (the Roelen reaction)\textsuperscript{14} and hydrocarboxylation with carbon monoxide and water (the Reppe reaction)\textsuperscript{15} were discovered. However, it was the discovery by Heck and co-workers in 1974 that organohalides could be carbonylatively coupled with aliphatic alcohols and amines using catalytic amounts of Pd(0) that is acknowledged as the starting point for modern carbonylation chemistry.\textsuperscript{16–18} Palladium-catalysed carbonylation reactions such as aminocarbonylation, alkoxy carbonylations, hydroformylations and carbonylative cross-coupling reactions are now essential tools for synthetic and medicinal chemists.\textsuperscript{18,19}

The use of CO as a one-carbon building block in palladium-catalysed carbonylations has many advantages. The catalytic insertion of the carbonyl moiety is highly atom-efficient and provides a valuable synthetic handle for further structural elaboration of the carbonyl compound. Furthermore, carbonylations are in essence three-component reactions, and by varying the organohalide and nucleophile component, considerable product diversity can easily be achieved. However, the acute toxicity, flammable nature, and requirement for specialised lab equipment in combination with the difficulty to detect leakages of the colourless and odourless gas have deterred synthetic chemists from applying the useful carbonylation methods despite their synthetic advantages. As a result, much recent effort has been invested in developing safer methods for handling the toxic carbon monoxide gas.\textsuperscript{18,20,21}

CO Sources and Two-Chamber System

In order to avoid handling of gaseous CO, several methods employing a variety of CO presursors have been developed (Figure 1). One approach has been to utilise molecules with carbonyl motifs, which by the exposure to transition metals, additives, base or heat will relase CO. Examples, include alkyl- and aryl-formates,\textsuperscript{21–25} aldehydes,\textsuperscript{26} formic acid,\textsuperscript{27} formamides and N-formyl-saccharin,\textsuperscript{28–30} carbon dioxide\textsuperscript{31,32} and metal carbonyls such as Mo(CO)\textsubscript{6},\textsuperscript{33,34} and Co\textsubscript{2}(CO)\textsubscript{8}.\textsuperscript{35,36} However, several of the mentioned CO sources require an additional transition metal, strong base or high temperatures to release CO gas. Alternatively, the use of metal carbonyls will generate stoichiometric amounts of another transition metal as waste. Indeed, Mo(CO)\textsubscript{6} has been reported to possess catalytic activites\textsuperscript{37,38} in addition to reducing aromatic nitro functionalities at elevated temperatures.\textsuperscript{39} The issues with compatibility of the CO-generating reaction with the CO-consuming reaction imposes severe limitations on the scope of non-gaseous carbonylation reactions.
An elegant approach that circumvent these problems was developed by Hermange et al., where CO was liberated \textit{ex situ} following Pd-catalysed decomposition of 9-methylfluorene-9-carbonyl chloride (CO\textsubscript{gen}). A special two-chamber glassware system was developed to keep the carbonylation and the decarbonylation reaction mixtures separate, to avoid problems with incompatibility.\textsuperscript{40} A similar approach was later described where silacarboxylic acid was reacted with a fluoride source to liberate CO.\textsuperscript{41} Both these methods allow the use of stoichiometric or substoichiometric amounts of CO as well as a possibility to introduce an isotopically labelled carbonyl group.\textsuperscript{40,41}

**Figure 1.** Representative selection of various CO sources reported in the literature.

**Figure 2.** Schematic representation of a two-chamber system.

\textit{Ex situ} generation of carbon monoxide from solid CO sources, using two-chamber glassware, has made it possible to use various carbonylation reactions for small-scale applications in a standard laboratory since lower pressures of CO can be used, which in turn eliminates the need for pressurised vessels. There are now several non-gaseous CO sources reported, intended both for \textit{in situ} and \textit{ex situ} use. Recently, the base mediated decomposition of
oxalyl chloride\textsuperscript{42} and chloroform\textsuperscript{43,44} have been reported as effective CO generating strategies for carbonylation chemistry. Notably, the latter allow the preparation of $^{13}$C and $^{14}$C labelled carbonyl derivatives.

Finally, metal carbonyls such as Mo(CO)$_6$ offer a convenient solid CO source suitable for both \textit{in situ} and \textit{ex situ} gas release.\textsuperscript{45,46} CO is readily released from Mo(CO)$_6$ either by ligand exchange with e.g. DBU\textsuperscript{34,47} or MeCN\textsuperscript{48,49} or at elevated reaction temperatures.\textsuperscript{33} However, due to potential reduction of nitro groups and precipitation of molybdenum complexes after the release of CO (complicating product purification), \textit{ex situ} protocols have been developed for the use of Mo(CO)$_6$-mediated carbonylations.\textsuperscript{46,50} The carbonylative work presented in this thesis will mainly focus on Mo(CO)$_6$ as the non-gaseous CO source.

Aminocarbonylation

In principle, most palladium(0)-catalysed carbonylation reactions involve the coupling of a suitable carbon halide starting material (e.g. aryl, vinyl, benzyl) and nucleophile (e.g. alcohol, amine, alkyne) in the presence of CO. The three-component reaction between an organic (pseudo)halide, CO and an amine to yield amides is known as an aminocarbonylation reaction (Scheme 2).

\[
R^1X + CO + HNR^2R^3 \xrightarrow{[Pd] \text{ base}} R^1N=O R^2R^3
\]

\textbf{Scheme 2.} General depiction of an aminocarbonylation reaction.

A base is typically required to abstract a proton and usually a ligand is added to stabilise and modulate reactivity of the palladium complexes in the catalytic cycle. Most commonly, a phosphine ligand is used and several ligand properties may be considered when designing a catalytic system, such as electronic and steric properties. Moreover, various steps of the catalytic cycle will be favoured by different properties. For example, oxidative addition will be favoured by electron rich phosphine ligands, whereas the CO insertion will be favoured by electron deficient phosphine ligands.\textsuperscript{20,51}

Typically Pd(II) salts are used as precatalysts, mainly due to their enhanced stability compared to the Pd(0)-complexes (Scheme 3). Therefore, as an initial step before entering the catalytic cycle, the Pd(II) precatalyst will be reduced by a solvent molecule, ligand or CO to a 14-electron Pd(0) complex.\textsuperscript{51} Once the active catalyst is formed, the first step in the catalytic cycle is the insertion of palladium into the R-X bond, resulting in oxidation of the Pd(0) species to a square-planar organopalladium(II) complex (oxidative addition). The rate of oxidative addition is strongly dependent on the nature of the C-X bond, where
strong C-X bonds will be less reactive. As a result, iodides react more readily than other halides such as chlorides (I > OTf ≥ Br > Cl).\textsuperscript{18} With this argument also follows that electron-deficient C-X substrates are more susceptible to oxidative addition, and electron-donating ligands that increase the electron density on palladium will promote oxidative addition.\textsuperscript{20,52}

Next, \textit{coordination of CO} to the palladium centre accompanied by ligand displacement and \textit{1,1-insertion} generates the acylpalladium species. The introduction of the nucleophile can occur either directly on the acyl carbon and thereby releasing the carbonyl compound or the nucleophile can coordinate to the vacant site on palladium (\textit{nucleophilic attack}). Abstraction of a proton from the nucleophile with base and subsequent \textit{reductive elimination} then yields the carbonylated product and regenerates the catalytically active Pd(0) species.\textsuperscript{2,51}

\textbf{Scheme 3}. Catalytic cycle of aminocarbonylation.
Carbonylative Coupling with Cyanamide as the Nucleophile

The carbonylative cross-coupling of aryl halides with cyanamide using CO generated \textit{ex situ} from Mo(CO)$_6$ to produce $N$-cyanobenzamides has previously been described (Scheme 4).\textsuperscript{53–55} The method was compatible with both aryl iodides at 65 °C and bromides at 85 °C in moderate to good yields. The mechanism is believed to follow the general aminocarbonylation reaction (Scheme 3) with cyanamide acting as a nucleophile via the terminal amine group.

![Scheme 4](https://example.com/scheme4.png)

Scheme 4. Carbonylative cross-coupling of aryl halide with cyanamide to yield $N$-cyanobenzamides.

Carbonylative Sonogashira

The three-component reaction between an aryl or vinyl (pseudo)halide, CO and a terminal alkyne to yield alkynone structures is known as a carbonylative Sonogashira reaction (Scheme 5).\textsuperscript{18,19}

![Scheme 5](https://example.com/scheme5.png)

Scheme 5. General carbonylative Sonogashira cross-coupling.

The alkynone structural motif serves as a valuable intermediate in the synthesis of natural products\textsuperscript{56,57} and various heterocycles, e.g. pyrazoles,\textsuperscript{58,59} pyridines,\textsuperscript{60} pyrimidines,\textsuperscript{61} furanones,\textsuperscript{62} quinolones,\textsuperscript{63,64} and flavones.\textsuperscript{64,65} Typically, alkynones are prepared by transition metal-catalysed cross-coupling from the corresponding acyl chloride and a terminal alkyne. However, this synthetic approach is limited by the poor stability of acyl chlorides and their lack of compatibility with other functional groups.\textsuperscript{66,67} In contrast, the carbonylative conditions employ stable precursors and can often be performed at ambient temperature using relatively mild reagents. The first example of a carbonylative Sonogashira reaction was described in 1981 using gaseous CO.\textsuperscript{68} Iizuka and Kondo later developed a non-gaseous method, using Mo(CO)$_6$ as the CO source, for the synthesis of alkynones in good yields.\textsuperscript{59}
The mechanism of the carbonylative Sonogashira cross-coupling reaction is largely unexplored but it is believed that the catalytic cycle starts with oxidative addition of the organohalide to a Pd(0) complex, generating an organo-palladium(II) complex (oxidative addition). Insertion of CO leads to the acylpalladium species (CO-insertion) followed by coordination of the alkyne to the metal centre and subsequent reductive elimination (Scheme 6).\textsuperscript{19}

Scheme 6. Catalytic cycle of carbonylative Sonogashira cross-coupling.

Palladium-Catalysed Carbonylative Multicomponent Reactions

A multicomponent reaction (MCR) is defined as a one-pot reaction with three or more components that react to form a single product that contains essentially all of the atoms of the starting materials.\textsuperscript{69,70} The components may be separate molecular entities or they may be different functional groups in bifunctional reagents.\textsuperscript{69,71} As such, carbonylative coupling reactions, comprising the coupling of an electrophile, CO and a nucleophile, constitute a three-component reaction. However, carbonylation reactions with less than four components are not usually categorised as MCRs, as the CO component is generally fixed.\textsuperscript{72}
Many well-known non-carbonylative MCRs, such as the Mannich,\textsuperscript{73} Strecker,\textsuperscript{74} Biginelli,\textsuperscript{75,76} Passerini,\textsuperscript{77,78} and Ugi\textsuperscript{79} reactions utilise carbonyl derivatives, for example in the form of aldehydes or ketones, to install additional carbons. The ability to incorporate one-carbon fragments via Pd(0)-catalysed carbonylations from an additional source of organo(pseudo)halide starting materials is one of the reasons why carbonylation chemistry is such a powerful complement to the field of MCRs. The advance of carbonylation chemistry and the development of numerous novel methods\textsuperscript{18,20,80} has spurred an increased research interest in carbonylative MCRs.\textsuperscript{72,81}

There are several advantages to carbonylative MCRs: i) They are highly atom economical as nearly all atoms of the starting materials are incorporated into the product. ii) The rapid assembly of simple starting materials to generate cyclic and acyclic scaffolds with increased molecular complexity is readily achieved. Furthermore, by secondary transformations, for example by using bifunctional reagents or secondary reactions, a wider chemical space can be reached. This strategy has been successful in the synthesis of various heterocycles.\textsuperscript{82–84} iii) Limiting the number of steps of a reaction and ideally the number of isolated intermediates, is both time- and cost-effective. iv) The waste generated from a reaction, e.g. from unreacted starting materials and solvents used in purification processes, is kept to a minimum.

Considering the many advantages of carbonylative MCRs it is not surprising that the methodology has increased in popularity. Some selected examples of carbonylative MCRs are depicted in Scheme 7.\textsuperscript{56,85–88} However, the majority of carbonylative MCRs are performed using gaseous CO.\textsuperscript{72} In order to meet the demands of convenient and safe methods in the future it is essential to develop carbonylative MCRs that are compatible with non-gaseous CO sources.
Antibacterial Agents

Bacteria were first discovered by van Leeuwenhoek in the 1670s after his invention of the first microscope. However, it was not until the second half of the nineteenth century that the link between bacteria and disease was recognised. The impressive work by Louis Pasteur showed that specific microorganisms are essential for the fermentation process in beer and wine and furthermore that wine would age without going sour by gently heating the wine to kill microorganisms present (a process now referred to as pasteurisation). He also proposed that microbes could be spread in the air, prompting Glasgow surgeon Dr. Joseph Lister to sterilise operating theatres and wards using carbolic acid, which improved the surgical survival rates. Towards the end of the nineteenth century, several microorganisms that caused diseases were discovered. For example Robert Koch was awarded the Nobel Prize in 1905 for the discovery of *Mycobacterium tuberculosis (Mtb)*. Following these groundbreaking discoveries, a notion began to take shape that chemical substances could be used to kill pathogens selectively while saving the host. This field, known as chemotherapy, was originally researched by Paul Ehrlich and lead to the development of the first antibacterial agent, Salvarsan, an arsenic containing antimicrobial agent that was used to treat syphilis (Figure 3). In the late 1930s, the discovery of the sulfadrugs, or sulfonamides (Figure 3), provided a huge step forward in the treatment of antibacterial infections, and
until the discovery of penicillin they were the only effective antibacterial drugs available.

The serendipitous discovery of penicillin by Sir Alexander Fleming and the successful isolation of penicillin on large-scale by Sir Howard Walter Florey and Ernst Boris Chain meant that a new effective antibiotic was available. For this, the trio was awarded with the Nobel Prize in Medicine in 1945. The period from the 1940s to the 1980s is often referred to as the “golden era” of antibiotic discovery. During this time extensive research was devoted to finding new antibiotics and most of the currently available treatment was developed, including β-lactams (penicillins, cephalosporins), Gram-negative active streptomycin, broad-spectrum tetracyclines, anti-tuberculosis drug isoniazid as well as quinolones and fluoroquinolones (see Figure 3 for selected examples).

Figure 3. Selected examples of antibacterial agents.

The development of antibacterial agents revolutionised medicine and meant that previously fatal conditions, such as pneumonia, infections from simple wounds and septicaemia following child birth, could now be cured. With several effective drugs available, the steady flow of novel antibiotics was attenuated. At the same time, the pharmaceutical industry reduced much of the antibiotic research due to high costs of developing a drug and taking it through clinical trials. As a result, few novel antibiotics have reached the market in the last decades. Furthermore, most of the recently approved drugs target the same mechanism as already existing treatment. In fact, only five out of thirty approved antibiotics worldwide since 2000, were first-in-class. Even more worryingly, the new drugs are active only on Gram-positive bacteria.

The lack of novel and druggable targets is a problem, since bacteria readily develop resistance, e.g. by gene transfer or genetic mutations, by a multitude of resistance mechanisms, such as expression of β-lactamases that readily hydrolyse β-lactams, increase in efflux, overproduction of target protein or by-
Resistance may also arise from the bacteria entering a slow-growing or non-growing state (persister cells)\textsuperscript{101} by down-regulating the biosynthesis, which is targeted by most antibiotics.\textsuperscript{102} Traditionally, the effect of antibacterial agents has been evaluated by measuring the inhibition of bacterial growth. As a result, antibacterial agents generally target processes required for bacterial growth, e.g. cell wall biosynthesis, protein synthesis or the biosynthesis of folic acid. However, bacteria are able to enter a slow-growing or non-growing state, referred to as quiescent cells, in response to cellular stress, such as acidic pH, lack of nutrients or anaerobic conditions. Due to the low replication rate of quiescent bacteria, they are not as sensitive to drugs that affect cell growth as normal-growing bacteria are, and can thus withstand higher concentrations of the antibiotic for longer periods of time.\textsuperscript{100,102,103} Consequently, if the treatment is terminated before the body’s immune system can clear the remaining population of the bacterial strain, quiescent cells can reactivate to growing cells and symptoms will reappear. Furthermore, if the quiescent cell has gained resistance to the antibiotic, then the new population will also be resistant.\textsuperscript{100}

Non-replicating bacteria have been recognised as a major difficulty in eradicating bacterial biofilms and treating dormant TB, as well as in the development of bacterial resistance. As a result, the work to find new targets that do not affect the biosynthesis of bacteria and also target non-growing cells has received more focus.\textsuperscript{102,104–106} One such example is the disruption of the cell membrane. The cell membrane is essential for both growing and non-growing cells to maintain vital cell functions. The recently approved anti-TB drug bedaquiline (TMC-207) is currently used as a last resort for patients with extensively drug-resistant TB (i.e. resistance to two of the first-line drugs and additional resistance to fluoroquinolones and to at least one second-line injectable drugs). The compound targets ATP synthase, an enzyme essential for maintaining energy in dormant TB cells.\textsuperscript{107}
Type II NADH Dehydrogenase

The oxidoreductase type II NADH dehydrogenase (NDH-2), is part of the energy-maintaining system and has been suggested as a suitable target for both replicating and non-replicating bacteria.\textsuperscript{108–112} NDH-2 is a monotopic, 50 kDa redox enzyme located on the cytosolic side of the cell membrane.\textsuperscript{113} It has been found in plant, fungi and many bacterial cells but not in mammals.\textsuperscript{108,114,115} NDH-2 catalyses the transfer of electrons from NADH via FAD to ubiquinone (or another oxidant) and plays a crucial part in the electron transport chain and oxidative phosphorylation to produce ATP. However, consensus has yet to be reached on the exact mechanism of action for this enzyme.\textsuperscript{113,116,117} Generation of cellular energy is essential for survival of \textit{Mtb} as it reduces the ability of dormant cells to maintain energy levels and therefore may serve as a suitable target for novel antibacterial agents.\textsuperscript{107,118} Known inhibitors include antipsychotic phenothiazines, which have been reported to inhibit NDH-2 at micromolar levels.\textsuperscript{108,109,119} Polypeptide antibiotic polymyxin B has been shown to exhibit inhibitory activity on NDH-2 in Gram-negative bacteria and \textit{Mtb}.\textsuperscript{120–122} Furthermore, small-molecule quinoliny1 pyrimidines (1)\textsuperscript{110} and bisaryl quinolones (2-3)\textsuperscript{111,123–127} have been reported to possess antimalarial and antimycobacterial properties by inhibiting NDH-2 (Figure 4).

![Figure 4](image-url). Representative selection of small-molecule NHD-2 inhibitors.
Aims

The overall aim of the work presented in this thesis has been to develop new palladium(0)-catalysed, non-gaseous, carbonylative multicomponent reactions for the synthesis of biologically relevant compounds.

![Scheme 8](image)

Scheme 8. Development of non-gaseous, carbonylative multicomponent reactions from bifunctional reagents in the construction of bicyclic structures. X = Halide; Y = NH, O; Nu = nucleophilic moiety; E = electrophilic moiety; “CO” = non-gaseous CO source.

More specifically the aims were:

- To use carbonylative coupling strategies to couple ortho-substituted bifunctional reagents (4) with nucleophilic coupling partners, which comprise an additional electrophilic moiety (5). Following a secondary transformation on intermediate 6 (e.g. cyclisation), various bicyclic structures (7) can be obtained (Scheme 8).
- Apply the developed methodology in the preparation of potential type II NADH dehydrogenase (NDH-2) inhibitors based on a quinolinyl pyrimidine scaffold.
- Assess the correlation between inhibition of NDH-2 and various bacterial pathogens.

During the course of the work the following specific aim was formed:

- To develop a general, carbonylative multicomponent synthesis of N-acylguanidines.
Synthesis of 4-Quinolones via a Multicomponent Carbonylation/Cyclisation Reaction (Paper I)

Background and Aim

Quinolones are versatile heterocyclic structures, frequently employed in medicinal chemistry, displaying a wide array of biological activities. Consequently, there are several well defined synthetic routes for the preparation of quinolones, e.g. the Conrad-Limpach-Knorr reaction, which includes the condensation of anilines with β-ketoesters and subsequent cyclisation of the formed β-arylaminoacrylates. This strategy is however limited by the reactivity of the aniline, and thus, the introduction of electron-withdrawing groups in the benzene ring of the quinolone is somewhat restricted. Beyond that, Pd(0)-catalysed carbonylative multicomponent reactions of ortho-iodoanilines and terminal alkynes have been demonstrated using elevated pressures of CO. However, many synthetic chemists working on the laboratory scale are deterred by the use of CO gas, especially above atmospheric pressure, on account of its inherent toxicity. Therefore, a non-gaseous, carbonylative multicomponent approach towards these important heterocycles is of great value.

Scheme 9. Synthesis of 4-quinolones from ortho-iodoanilines and terminal alkynes via a carbonylative Sonogashira cross-coupling and subsequent cyclisation.

Similar to what Genelot et al. had reported, a carbonylative cross-coupling of ortho-iodoanilines 8 and terminal alkynes 9 was envisioned to yield an alkynone intermediate 10 (Scheme 9). However, to circumvent the handling of toxic, flammable and odourless CO gas, Mo(CO)₆ was used as a solid CO source. This strategy allows for CO gas to be released in situ in the sealed
reaction vial, removing the need for pressurised gas tubes and specialised laboratory equipment. Additionally, the desired 4-quinolones could be synthesised in one step via a multicomponent, domino reaction.

Development of an Expedient and Efficient Reaction

Initially, 2-iodoaniline (8a) and phenylacetylene (2 equiv) were reacted with various palladium catalytic systems and bases in the presence of Mo(CO)6 (1 equiv) in neat diethylamine and the reaction mixture was heated using microwave (MW) irradiation at 120 °C for 20 min. Optimisation of the reaction conditions with respect to catalytic system and base revealed that Pd2(dba)3 with dppf and Cs2CO3 provided quinolone 11a in 85% yield (Method A).

These conditions were evaluated for ortho-iodoanilines and alkynes with varying steric and electronic properties (Table 1). The reaction produced 4-quinolones in good yields for unsubstituted 2-iodoaniline and chloro- and methyl ester-substituted anilines. Moreover, aliphatic alkynes as well as 3-ethynylthiophene were compatible with the reaction conditions affording the corresponding 4-quinolones in 51-76% isolated yield. However, when 2-iodo-4-nitroaniline was used, quinolone 11t was obtained in considerably reduced yield (29%). Mo(CO)6 is known to mediate carbonylation reactions and has been reported to reduce aromatic nitro groups at elevated temperatures. It was therefore desirable to develop a similar method that would allow the introduction of potentially labile functionalities.

Development of Milder Reaction Conditions

Aromatic nitro groups are known to undergo rapid reduction to their aniline counterparts using Mo(CO)6 and DBU in an ethanolic mixture under MW irradiation at 150 °C for 30 min, admittedly, very similar conditions to the ones used in Method A. By lowering the reaction temperature it was anticipated that Mo(CO)6 would not reduce the nitro group. Furthermore, previous work by Iizuka et al. had shown how a Pd/t-Bu3P system had afforded arylalkynones at ambient reaction temperatures using Mo(CO)6. For the reported method, MeCN was used as solvent and, presumably, as ligand to release CO from Mo(CO)6.

When the reaction was performed at ambient temperature, the arylalkynone was formed without spontaneous cyclisation to the 4-quinolone. However, the addition of diethylamine or another secondary amine can facilitate the cyclisation of arylalkynones with ortho-amino substituents to yield the final 4-quinolone, as previously reported. Thus, 2-iodoaniline and 4-fluorophenylacetylene were treated with Pd(OAc)2, [(t-Bu)3PH]BF4, Et3N and Mo(CO)6 in MeCN at room temperature for 16 h. Following addition of diethylamine to
the crude reaction mixture and stirring at room temperature for 5 h, the desired
4-quinolone 11t was obtained in 79% isolated yield (Method B).

Table 1. Scope and limitations of the Pd-catalysed carbonylation/cyclisation reac-
tion. 

<table>
<thead>
<tr>
<th>R = H:</th>
<th>11a, 85% c, 84% d</th>
<th>R = H:</th>
<th>11c, 76% c, 72% c</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = F:</td>
<td>11b, 70% c, 84% c</td>
<td>R = F:</td>
<td>11d, 64% b</td>
</tr>
<tr>
<td>R = H:</td>
<td>11e, 72% b</td>
<td>R = F:</td>
<td>11f, 63% b</td>
</tr>
<tr>
<td>R = H:</td>
<td>11g, 32% c</td>
<td>R = F:</td>
<td>11h, 59% b</td>
</tr>
<tr>
<td>R = H:</td>
<td>11i, 75% c</td>
<td>R = H:</td>
<td>11l, 67% b</td>
</tr>
<tr>
<td>R = H:</td>
<td>11j, 63% c</td>
<td>R = H:</td>
<td>11m, 76% b, 50% c</td>
</tr>
<tr>
<td>R = F:</td>
<td>11n, 74% c, 71% c</td>
<td>R = F:</td>
<td>11o, 32% c</td>
</tr>
<tr>
<td>R = H:</td>
<td>11p, 47% c</td>
<td>R = H:</td>
<td>11q, 62% c, 58% c</td>
</tr>
<tr>
<td>R = Cl:</td>
<td>11r, 51% b</td>
<td>R = F:</td>
<td>11s, 38% c, d</td>
</tr>
<tr>
<td>R = H:</td>
<td>11t, 29% b, 79% c</td>
<td>R = H:</td>
<td>11u, 79% c</td>
</tr>
<tr>
<td>R = Br:</td>
<td>11v, 68% c</td>
<td>R = Br:</td>
<td>11w, 82% c</td>
</tr>
<tr>
<td>R = 2-NH:</td>
<td>11x, 72% c</td>
<td>R = N:</td>
<td>11y, 80% c</td>
</tr>
<tr>
<td>R = Boc:</td>
<td>11z, 72% c</td>
<td>R = Boc:</td>
<td>11z, 72% c</td>
</tr>
</tbody>
</table>

a Isolated yield. b Method A: ortho-iodoaniline (0.5 mmol), alkyne (1 mmol), Pd2(dba)3 (5 mol %), dpff (12 mol %), Mo(CO)6 (0.5 mmol), Cs2CO3 (3 equiv), Et2NH, MW 120 °C, 20 min. c Method B: (i) ortho-iodoaniline (0.5 mmol), alkyne (1 mmol), Pd(OAc)2 (3 mol %), [(t-
Bu)3P]BF4 (6 mol %), Mo(CO)6 (0.75 mmol), Et3N (1 mmol), MeCN, r.t., 16 h. (ii) Et2NH (2.5 mmol), r.t., 5 h. d Prepared from trimethylsilylacetylene.
To evaluate the scope and limitations of the new method, a set of ortho-iodoanilines and terminal alkynes with varying properties were subjected to the new reaction conditions (Table 1). The results revealed that electronic effects from the substitution on ortho-iodoanilines had little effect on the outcome of the reaction. Furthermore, similar to Method A, terminal alkyl acetylenes performed well and were incorporated in the quinolone scaffold (11l-n) in 50-71% yield. By the use of 2- and 3-pyridyl and 3-thiophenyl alkynes, heterocycles could be installed in the 2-position of the quinolone scaffold (11o-r) in moderate yields. Furthermore, the reaction performed well for neutral, electron-deficient and electron-rich phenylacetylenes (50-84%), suggesting that electronic effects in the alkyne component are of minor importance to the reaction outcome.

Pleasingly, nitro functionalities were successfully introduced in quinolones 11t-u in high isolated yields and no sign of reduction to the amine was observed. It is also worth noting that the cyclisation appears unaffected by the electron-withdrawing effect exerted by the nitro group on the nucleophilic amine. Furthermore, three bromo-substituted compounds were prepared in good yields (11v-x, 68-82%). At the low reaction temperature, no sign of activation of the C-Br bond was observed for these reactions (LCMS and NMR). Additionally, ester, amide and carbamate functionalities (11e-h, 11l, 11y-z) were all compatible with the reaction conditions for this method and the corresponding quinolone products were isolated in moderate to high yields.

Chemoselectivity and Mechanistic Investigation

Notably, the reaction of ortho-substituted aryl iodides with alkynes can give rise to both 4-quinolone and 2-quinolone isomers (Scheme 10). For terminal alkynes, the formation of 4-quinolones (11) is the most common. Undoubtedly, the different isomers must come about from separate catalytic pathways.

The synthesis of 2-quinolones (12) has previously been described from ortho-iodoanilines, using similar conditions to the work described herein. The reaction is proposed to proceed via insertion of either internal or terminal alkynes into the carbon-palladium bond to form a vinyl palladium intermediate. Insertion of CO gives the acylpalladium complex which after intramolecular nucleophilic attack by the nitrogen yields the cyclised 2-quinolone.

In the case of 4-quinolones, the formation of the acylpalladium species must take place prior to insertion of the alkyne in a carbonylative Sonogashira cross-coupling to yield an alkynone. The ynone (10) can undergo subsequent
Scheme 10. Previous reports of the carbonylative reaction of ortho-iodoanilines with internal and terminal alkynes.

cyclisation to the 6-membered 4-quinolone (11) via Michael addition of a secondary amine.\textsuperscript{63,64,135} Alternatively, a 5-membered indolin-3-one (13) can be obtained by phosphino-catalysed cyclisation.\textsuperscript{136,143,144}

During the course of this work, the formation of a 2-quinolone structure was never observed (by LCMS or NMR). To confirm that the quinolone structure is formed via diethylamine-induced cyclisation of the proposed intermediate (10a), the intermediate was synthesised using Method B (Scheme 11). Accordingly, 2-iodoaniline and phenylacetylene were reacted with Pd(OAc)\textsubscript{2}, [(t-Bu)\textsubscript{3}PH]BF\textsubscript{4}, Et\textsubscript{3}N and Mo(CO)\textsubscript{6} in MeCN at room temperature overnight (Method B). The resulting arylalkynone was then isolated in 72% yield and fully characterised to affirm the correct structure. Finally, intermediate 10a was stirred with diethylamine in MeCN at room temperature to yield the cyclised compound in 82% isolated yield. These results support a reaction pathway that proceeds via a carbonylative Sonogashira cross-coupling to the proposed arylalkynone intermediate, which is cyclised to yield the 4-quinolone.

Summary (Paper I)

Quinolones are privileged structures, present in many fields of chemistry. Due to the increasing complexity of molecules and the aspiration for late-stage derivatisation, the development of new, mild synthetic methodology is still in need. The work presented here and in Paper I describes a convenient and efficient synthesis of 4-quinolones without the need for gaseous CO. In total, 33 examples were prepared with good substrate scope. Two methods were developed. One suitable for the rapid assembly of 4-quinolones in a carbonylation/cyclisation domino reaction (Method A). The second method, which proceed via diethylamine-induced cyclisation of an alkynone intermediate is compatible with reduction-prone and other sensitive functional groups (Method B).

The chemistry developed in Paper I was later applied to the work in Paper V, for which 11t was synthesised and used as precursor in a project aimed at developing new inhibitors of type II NADH dehydrogenase (NDH-2).
Synthesis of 2-Aminoquinazolinones via Carbonylative Coupling of *ortho*-Iodoanilines and Cyanamide (Paper II)

Background and Aim

Inspired by the carbonylation and subsequent cyclisation of *ortho*-iodoanilines in Paper I, a search was initiated for other suitable transformations that could potentially enable heterocycle synthesis. In this search, *N*-cyanobenzamidine \(^{53}\) (Scheme 12) was viewed as a versatile intermediate from which diverse structures could be synthesised. Analogous to the work in Paper I, a carbonylative coupling of *ortho*-iodoaniline (8a) was envisioned to give an intermediate that could be cyclised. The use of cyanamide as nucleophile in the carbonylation reaction would provide an *N*-cyanobenzamide intermediate 14 (Scheme 12). The incorporation of this group would introduce the possibility of a subsequent addition at the electrophilic nitrile carbon giving rise to a 2-aminoquinazolinone structure (15) in just two transformations.

Scheme 12. Proposed synthetic strategy for the preparation of 2-aminoquinazolinones from *ortho*-iodoanilines and cyanamide.

The quinazolinone motif is a privileged structure and one of the most common heterocycles in small-molecule drugs.\(^{145}\) Moreover, quinazolinones display a wide range of biological activities such as antibacterial,\(^{146}\) antiviral,\(^{147,148}\) antifungal,\(^{149}\) and anticancer.\(^{150}\) The heterocyclic core is typically constructed from the amidation of 2-aminobenzoic acid\(^ {147,150}\) or by reaction with isothiocyanate.\(^ {151}\) However, there are some examples of Pd(0)-catalysed carbonylative approaches using gaseous CO, such as cyclocarbonylations employing carbodiimides or ketenimines,\(^ {38,152,153}\) or from aminocarbonylation of the corresponding imidoyl chlorides or imidates.\(^ {154,155}\) These methods are, however,
limited by the use of high CO pressures, non-commercially available starting materials and do not allow N1-substitution.

Method Development

To test the feasibility of this reaction, 8a and cyanamide were reacted with Pd(PPh₃)₄ and Et₃N in 1,4-dioxane at 65 ºC for 20 h. To avoid complexation of cyanamide and competing side reactions, the CO gas was generated ex situ from Mo(CO)₆ and DBU using a two-chamber system. The use of separate reaction- and CO-generating chambers has been well described and studied in the literature. Analysis of the reaction mixture showed that the carbylation reaction had proceeded as expected and the desired intermediate 14a had formed in 37% isolated yield. However, pleasingly, cyclised 15a had also formed spontaneously under the reaction conditions in 24% yield. This indicated that the intramolecular cyclisation was favoured but that the method required additional modifications to accomplish complete cyclisation. Encouraged by these results, the carbylation reaction was repeated as described above, but upon completion of the initial carbylative step, the reaction mixture was heated further to complete the cyclisation. This change afforded cyclised 15a in 70% isolated yield. When the reaction was repeated on a 1 mmol scale, the yield increased to 96%. This was expected since the product could conveniently be isolated by precipitation.

Synthesis of N-Unsubstituted 2-Aminoquinazolin-4(3H)-ones

To evaluate the generality of the method, the conditions were applied to a set of ortho-iodoanilines with varying electronic properties (Table 2). The carbylation reaction seemed to tolerate all substrates, irrespective of electronic influence on the halide, and the respective N-cyanobenzamides were formed smoothly. However, the cyclisation step was markedly affected by the substitution pattern in the benzene ring. This was particularly noticeable in the presence of an electron-withdrawing group para to the aniline. However, in most cases this hurdle was overcome by increasing the reaction times in the final cyclisation step. In all but one case (15h, 44%), products were obtained in good to excellent yields.

In contrast, when an electron-donating methoxy group was introduced in para-position, the intermediate underwent spontaneous cyclisation at 65 ºC and no additional heating was required, giving 6-methoxy substituted compound 15i in 82% yield. However, this effect was not observed for the inductively electron-donating methyl substituent (15j) and additional heating was
still required to reach full conversion to yield the cyclised compound in 71% yield.

**Table 2.** Substrate Scope for the Multicomponent Carbonylation/Cyclisation Synthesis of N1-Unsubstituted 2-Aminoquinazolin-4(3H)-ones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R²</th>
<th>Product</th>
<th>Yield (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>15a</td>
<td>70, 96b</td>
</tr>
<tr>
<td>2</td>
<td>CO₂Me</td>
<td>H</td>
<td>15b</td>
<td>79c</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>CO₂Me</td>
<td>15c</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>F</td>
<td>15d</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>Cl</td>
<td>15e</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>Br</td>
<td>H</td>
<td>15f</td>
<td>76</td>
</tr>
<tr>
<td>7</td>
<td>CN</td>
<td>H</td>
<td>15g</td>
<td>68c</td>
</tr>
<tr>
<td>8</td>
<td>NO₂</td>
<td>H</td>
<td>15h</td>
<td>44c</td>
</tr>
<tr>
<td>9</td>
<td>MeO</td>
<td>H</td>
<td>15i</td>
<td>82d</td>
</tr>
<tr>
<td>10</td>
<td>Me</td>
<td>H</td>
<td>15j</td>
<td>71</td>
</tr>
</tbody>
</table>

¹ Isolated yield. ² 2-Iodoaniline (1 mmol). ³ (ii) MW 140 °C, 40 min. ⁴ Product obtained after carbonylative step.

**Competing Side Reaction**

Next, the aim was to include N-substituted ortho-iodoanilines, to expand the scope of the method. The developed reaction conditions were applied to N-benzyl-2-iodoaniline. However, a substantial amount of side product was observed for the reaction of this substrate. NMR analysis of the side product showed that the compound exhibited similar signals in the aromatic region of the ¹H-NMR spectrum to those in the desired product. Although, the structure and identity of the compound could not be fully elucidated using only NMR spectroscopy, it was however conceivable that the competing side reaction might involve cyanamide. The assumption was based on the fact that the m/z ratio corresponded to the mass of the desired quinazolinone plus cyanamide. Therefore, a small study was performed to evaluate how the amount of cyanamide and reaction temperature affected the formation of side product (Table 3).
Table 3. Study on the effect of the amount of cyanamide and reaction temperature on the formation of an unidentified side product.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Carbonylation</th>
<th>Cyclisation</th>
<th>NH$_2$CN (equiv)</th>
<th>15k:16$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65 °C, 20 h</td>
<td>MW 140 °C, 20 min</td>
<td>3.9</td>
<td>1.3:1</td>
</tr>
<tr>
<td>2</td>
<td>65 °C, 20 h</td>
<td>80 °C, 8 h</td>
<td>1.8</td>
<td>18:1</td>
</tr>
<tr>
<td>3</td>
<td>65 °C, 20 h</td>
<td>MW 140 °C, 20 min</td>
<td>1.2</td>
<td>67%$^b$</td>
</tr>
<tr>
<td>4</td>
<td>85 °C, 20 h</td>
<td>-</td>
<td>1.2</td>
<td>69%$^b$</td>
</tr>
</tbody>
</table>

Reaction conditions: Chamber 1: N-benzyl-2-iodoaniline (0.5 mmol), cyanamide, Pd(PPh$_3$)$_4$ (5 mol %), Et$_3$N (2 equiv), 1,4-dioxane; Chamber 2: Mo(CO)$_6$ (1 equiv), DBU (3 equiv), 1,4-dioxane. $^a$ Ratio determined by LCMS. $^b$ Isolated yield of 15k.

When N-benzyl-2-iodoaniline was reacted with a large excess of cyanamide the desired product 15k and side product 16 formed in almost equal ratio (entry 1). However, when the amount of cyanamide was lowered to 1.8 equiv and the cyclisation temperature was lowered, a considerable reduction of side product was observed (entry 2). By further reducing the amount of cyanamide to 1.2 equiv and performing the cyclisation at 140 °C using MW irradiation, the desired compound 15k was obtained in 67% isolated yield (entry 3). This suggested that formation of side product was not the result of high reaction temperatures. Although the reaction temperature did not appear to have any effect on the formation of side product it was however evident that the cyclisation could be completed already at lower temperatures. Additionally, it was proposed that the added steric bulk to the aniline might decrease the reactivity of the halide in the Pd-catalysed carbonylation step. Therefore, the carbonylation reaction was repeated at 85 °C in order to increase the yield further. Gratifyingly, an increase of temperature in the initial carbonylation step afforded the cyclised quinazolinone in 69% isolated yield in one step without any sign of the undesired side product (entry 4). Although the change in reaction conditions did not offer any improvement and the obtained yield was similar to that in entry 3.

In parallel with the investigation described above (Table 3), crystals of sufficient quality were obtained and submitted for X-ray crystallography. The X-ray analysis revealed the structure of the 2-guanidinosubstituted quinazolinone 16 (Figure 5). The information from the X-ray structure together with the results from Table 3 suggest that a competing side reaction can occur between the exocyclic nitrogen of the cyclised 2-aminoquinazolinone (15k) and
the electrophilic nitrile carbon in cyanamide giving rise to the guanidine-substituted quinazolinone (16) (Scheme 13). As a result, the amount of cyanamide should be kept to a near stoichiometric amount in order to avoid the competing side reaction.

It should be noted that the side product was never observed for the unsubstituted quinazolinones. The observed selectivity may be explained by the tautomerisation to a more nucleophilic exocyclic imino group. Density functional theory calculations revealed that for an N1-alkyl quinazolinone the tautomer with an exocyclic amine is 2 kcal/mol lower in energy than the tautomer with an exocyclic imino group. This preference increases to 5 kcal/mol for the unsubstituted system which may explain why the side product was only observed for the N1-substituted quinazolinones.

Figure 5. X-ray crystal structure of isolated and crystallised side product from the reaction of N-benzyl-2-iodoaniline (16) and excess cyanamide. Reprinted with permission from Åkerbladh, L. et al., J. Org. Chem. 2016, 81, 2966-2973. Copyright 2016 American Chemical Society.

Scheme 13. Proposed reaction mechanism for the competing side reaction between N-benzyl-2-iodoaniline (8b) and excess cyanamide.

Modification of the Developed Protocol for N-Substituted ortho-Iodoanilines

After having studied the reaction of N-substituted ortho-iodoanilines and the competing side reaction (Table 3) the generality of the multicomponent reaction was assessed. A method that included N-substituted ortho-iodoanilines
was devised, using only a slight excess of cyanamide to suppress the formation of unwanted side products. Furthermore, by carrying out the initial carboxylation step at 85 °C, the carboxylation and cyclisation could be achieved in a cascade reaction without the need for additional heating. These convenient conditions were evaluated for twelve \(N\)-substituted \textit{ortho}-idoanilines with varying steric and electronic properties.

\textbf{Table 4.} Substrate scope for the Multicomponent Carboxylation/Cyclisation Synthesis of \(N1\)-substituted 2-Aminoquinazolin-4(3\(H\))-ones.

\[
\begin{array}{cccc}
\text{Entry} & \text{R} & \text{Product} & \text{Yield (%)}^a \\
1 & \text{Bn} & 15k & 69, 67^b \\
2 & \text{Me} & 15l & 66, 60^b \\
3 & \text{iPr} & 15m & 95, 44^b \\
4 & \text{nPr} & 15n & 83 \\
5 & \text{ } & 15o & 73 \\
6 & \text{ } & 15p & 90, 79^b \\
7 & \text{ } & 15q & 88, 72^b \\
8 & \text{ } & 15r & 78, 61^b \\
9 & \text{ } & 15s & 86 \\
10 & \text{ } & 15t & 84 \\
11 & \text{ } & 15u & 32^b \\
12 & \text{ } & 15v & 92 \\
\end{array}
\]

\(^a\) Isolated yield. \(^b\) (i) 65 °C, 20 h (ii) MW 140 °C, 20 min.
The developed method worked well with the simple alkyl substituents, methyl, iso-propyl and n-propyl, providing N-substituted quinazolinones 15l-n in 66-95% yield. Interestingly, these substrates appeared sensitive to elevated temperatures, which was reflected in the reduced yields when the carbonylation was run at 65 °C and the cyclisation was carried out at 140 °C. The same compounds also changed colour when moist and exposed to air for longer periods of time. However, when the compounds had been dried under inert conditions, they were stable under ambient conditions. This effect was less marked for larger cyclic alkyl substituents, i.e. cyclopentyl, cyclohexyl and cycloheptyl, and quinazolinones 15p-r were prepared in high yields.

N-Benzyl-substituted quinazolinones were obtained in good to excellent yields. Fluoro-substituted benzyl furnished the desired 15s in 86% yield. Moreover, sterically hindered 2,6-dimethoxybenzyl was successfully introduced in the N1-position of the quinazolinone in 84% isolated yield. Considering the successful introduction of a bromo-substituent for the N-unsubstituted ortho-iodoaniline (15f, Table 2), N-(2-bromobenzyl)-2-iodoaniline was reacted under the developed reaction conditions (entry 11). When the reaction was performed at 85 °C, the aryl bromide was activated giving rise to an unidentified side product and the desired product was not detected. By first completing the carbonylation at 65 °C and then cyclise the formed intermediate, compound 15u was obtained in reduced 32% yield. Noticeably, when a competing phenol nucleophile was introduced, the corresponding quinazolinone 15v was isolated in an excellent 92% yield.

Summary (Paper II)
The work in Paper II describes the development of a novel synthetic approach towards 2-aminoquinazolinones from readily available starting materials. The Pd(0)-catalysed carbonylative coupling of ortho-iodoanilines with cyanamide yields intermediate N-cyanobenzamide. Cyclisation of the intermediate was then thermally induced or occurred spontaneously in a domino carbonylation/cyclisation sequence. The method offers the possibility for selective preparation of both unsubstituted as well as N1-substituted quinazolinones. Furthermore, this synthetic strategy provides the free exocyclic amine, contrary to the procedures available in the literature.
Synthesis of 4H-Benz[e][1,3]oxazin-4-ones via a Carbonylation/Cyclisation Domino Reaction of ortho-Halophenols (Paper III)

Background and Aim
At the outset of this project it was expected that the method described in Paper II could be applied to include other heterocycles. Simply by changing the starting material from ortho-iodoaniline to ortho-iodophenol, a convenient synthetic route would give access to 4H-benzo[e][1,3]oxazin-4-ones. Benzoxazinones are important structures in medicinal chemistry and a wide range of biological activities, including antimicrobial, anticancer160,161 and antiplatelet,162,163 have been reported for the scaffold. However, the synthetic routes available in the literature involve the use of complex starting material, acutely toxic reagents or are limited to electron-withdrawing groups in the benzene ring.164–169

Scheme 14. Synthetic plan for the synthesis of 4H-benzo[e][1,3]oxazin-4-ones from readily available ortho-iodophenols and cyanamide.

It was anticipated that Pd(0)-catalysed carbonylative coupling of ortho-iodophenols with cyanamide under low CO pressures would generate the versatile N-cyanobenzamide intermediate (18). Subsequent intramolecular addition of the phenol to the electrophilic nitrile carbon would yield cyclised benzoxazinones (19) in just two steps (Scheme 14). The proposed synthetic strategy would allow a convenient, one-pot preparation of benzoxazinones without the need for gaseous CO.
Synthesis of 2-Amino-4\(H\)-benzo[e][1,3]oxazin-4-ones from \(\textit{ortho}\)-Iodophenols

Adapting the Method to \(\textit{ortho}\)-Iodophenols

To test the performance of \(\textit{ortho}\)-iodophenols for the method developed in \textbf{Paper II}, 2-iodophenol was reacted with cyanamide (1.2 equiv), Pd(PPh\(_3\))\(_4\) (5 mol\%), and Et\(_3\)N (2 equiv) in 1,4-dioxane at 65 °C for 20 h. It was anticipated that the desired product 2-amino-4\(H\)-benzo[e][1,3]oxazin-4-one (19a) might react further with an excess of cyanamide similar to the competing side reaction for \(N_1\)-substituted-2-aminoquinazolinones (see previous chapter). Therefore, the amount of cyanamide was kept to a slight excess. The CO gas was generated \textit{ex situ}, using a two-chamber system, from Mo(CO)\(_6\) and DBU. The reaction proceeded smoothly with full conversion of the starting material to produce 2-amino-4\(H\)-benzo[e][1,3]oxazin-4-one (19a) in 76% isolated yield (Table 5, entry 1). The structure of 19a was confirmed by X-ray crystallography (Figure 6) as well as NMR spectroscopy.

![Figure 6](image.png)

\textbf{Figure 6.} X-ray crystal structure of 2-amino-4\(H\)-benzo[e][1,3]oxazin-4-one (19a).

Notably, the intermediate (18) underwent spontaneous cyclisation under the given reaction conditions. This stood in contrast to the results for \(\textit{ortho}\)-iodoanilines, which required higher temperatures to give complete cyclisation. The difference in rate of cyclisation between the two sets of substrates can probably be attributed to the lower pK\(_a\) of the phenolic intermediate (≈ 6.9) compared to the anilinic intermediate (≈ 20). As a result, the phenoxide ion will be present under the reaction conditions whereas the aniline will not be deprotonated. The presence of the much more reactive nucleophile can likely explain the difference in cyclisation rates.

Scope and Limitations

Pleased by the evolvement to a one-pot, cascade reaction, twelve \(\textit{ortho}\)-iodophenols were selected to explore the scope and limitations of the reaction. Overall, the reaction appeared to work well for electron-deficient as well as electron-rich substrates and returned the products in moderate to excellent yields.
Table 5. Substrate scope for the multicomponent carbonylation/cyclisation synthesis of 2-amino-4H-benzo[e][1,3]oxazin-4-ones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>Yield(%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>19a</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>Me</td>
<td>19b</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>MeO</td>
<td>19c</td>
<td>83</td>
</tr>
<tr>
<td>4</td>
<td>Cl</td>
<td>19d</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>Br</td>
<td>19e</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>NO2</td>
<td>19f</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>Me</td>
<td>19g</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>Ph</td>
<td>19h</td>
<td>89</td>
</tr>
<tr>
<td>9</td>
<td>HO</td>
<td>19i</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>NH2</td>
<td>19j</td>
<td>94</td>
</tr>
<tr>
<td>11</td>
<td>Ph</td>
<td>19k</td>
<td>53</td>
</tr>
<tr>
<td>12</td>
<td>t-Bu</td>
<td>19l</td>
<td>64</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated yield.
In particular electron-deficient *ortho*-iodophenols performed well, e.g. acetyl, methyl ester, and halo-substituted (19b-e, 83-96%). This may be explained by either increased rate of oxidative addition, a more electrophilic acyl palladium complex or a combination of both. Furthermore, the introduction of an electron-withdrawing group will also lower the pKa of the OH group, which potentially may increase reactivity. The introduction of a nitro group *para* to the phenol resulted in a slight reduction in yield (19f, 77%) compared to other electron-deficient substrates. However, the yield is comparable to that of unsubstituted 19a (77% vs. 76%). A comparison to the analogous nitro-substituted quinazolinone (15h, Table 2), which was prepared in 44% yield, further strengthens the discussion regarding strength of nucleophile (see above). Where the phenoxide ion effectively counteracts the electron-withdrawing effect exerted by the nitro group, the anilinic electron pair is greatly affected. As a consequence, the aniline is less reactive in the cyclisation reaction, reflecting in the low yield. Furthermore, the reaction of 4-(hydroxymethyl)-2-iodophenol and 4-amino-2-iodophenol proved entirely selective and no competing side reactions were observed as a result of the introduction of competing nucleophiles (19i and 19j, 84% and 94%).

**Synthesis of 2-Amino-4*H*-benzo[e][1,3]oxazin-4-ones from *ortho*-Bromophenols**

**Method Development for *ortho*-Bromophenols**

Palladium-catalysed carboxylation of *ortho*-bromophenols are typically carried out under harsh conditions, such as high CO pressures and elevated reaction temperatures.\(^{170,171}\) Instead, this type of transformation is predominantly performed using lithiation strategies.\(^{172-174}\) Nevertheless, an optimisation of the domino carboxylation/cyclisation reaction from *ortho*-bromophenols was initiated. A set of ligands that are commonly used for electron-rich bromides was selected, from the literature, as part of a screen for suitable conditions (Table 6). Initially, 2-bromophenol was used in the optimisation study. However, similar to the synthesis of N1-substituted 2-aminoquinazolinones (Paper II) a side product stemming from a competing side reaction with cyanamide was observed. In contrast to the quinazolinones, the side product could not be suppressed by reducing the amount of cyanamide. It was assumed that due to a decrease in rate of oxidative addition, when the less reactive bromide was used, an excess of cyanamide would be present in the reaction mixture. Therefore, the more electron-poor 2-bromo-4-chlorophenol (20a) was used in the optimisation studies.
Ligands cataCXium A, dppf, and dcpp all activated the bromide but did not provide full conversion of the starting material. Moreover, the conversion lead mainly to formation of the guanidine side product (entries 1-3, Table 6). By changing to Xantphos more than 95% conversion from the starting material was achieved, but the guanidine-substituted side product was still formed as the major product (entry 4). Fortunately, related ligand DPEphos also gave full consumption of starting bromide and, more importantly, provided 19d as the major product in 68% isolated yield (entry 5).

Next, some modifications to the reaction conditions were evaluated (Table 7). In an attempt to decrease the formation of the guanidine side product 21, the reaction temperature was lowered to 45 °C. This was, however, not enough to achieve full consumption of the starting bromide. Despite the lower reaction temperature, the competing side reaction was not suppressed (entry 1). Next,
in an effort to increase the rate of oxidative addition, the reaction was performed at 85 °C (entry 2). While this lead to full consumption of the starting material, guanidino-substituted side product was formed as the major product. The addition of LiCl was next evaluated. The use of LiCl has been reported to stabilise the Pd(0)-species and may also work as a Lewis acid and facilitate the nucleophilic attack on the acyl palladium complex. Unfortunately, in this case it did not improve the reactivity towards the desired product, and 19d was isolated in 11% yield (entry 3). Finally, the addition of acyl transfer reagents sodium phenoxide and DMAP were added to facilitate the nucleophilic attack by cyanamide (entries 4 and 5, respectively). While sodium phenoxide was deleterious to the reaction (entry 4), the addition of DMAP had no effect on the reaction outcome and the desired benzoxazinone 19d was obtained as the major product in 58% isolated yield (entry 5).

From the results presented in Table 6 and Table 7, DPEphos proved to be the most effective ligand. Furthermore, the reaction temperature needs to be carefully balanced so as to get full conversion but still suppress the competing side reaction as much as possible. In this case 65 °C was found to be the optimal temperature. Therefore, the conditions given in Table 6, entry 5 were chosen to further explore the generality of the developed method.

Scope and Limitations
A set of seven commercially available ortho-bromophenols were tested using the developed conditions (Table 6, entry 5). The results presented in Table 8 showed a clear preference for electron-deficient substrates. Where unsubstituted 19a was obtained in reduced 20% yield, the electron-deficient substrates were prepared in 21-90% isolated yields (19m-p), with increasing yields for more electron-withdrawing groups. Interestingly, the introduction of electron-donating methyl para to the phenol also afforded the corresponding benzoxazinone in moderate yield (19q, 46%). The reactivity of ortho-bromophenols was considerably lower compared to the respective iodides. But still, given the few successful examples using these substrates in the literature it must be viewed as valuable information to the scientific community.
**Table 8.** Substrate scope for the synthesis of 2-amino-4\(H\)-benzo[c][1,3]oxazin-4-ones from ortho-bromophenols.

![Chemical Structure](image)

Adapted from Paper III.

**Mechanistic Study**

During the course of this work, a question was raised regarding the mechanistic pathway. To confirm that the reaction proceeds via the proposed N-cyano-benzamide intermediate (18), the plan was to protect phenol 17a, perform the carbonylative coupling with cyanamide and fully characterise the protected intermediate (23). After deprotection it was proposed that Et\(\text{3}N\) would afford the cyclisation and give the final benzoazinone 19a by attack of the phenoxide ion on the nitrile carbon (Scheme 15).
Initially, the triisopropylsilyl (TIPS) and tert-butyldiphenylsilyl (TBDPS) ethers were selected as suitable protective groups. The silyl ethers were prepared in good yields (22a and 22b, 86% and 97% respectively) using standard procedures from the corresponding silyl chloride and imidazole in DMF. The silyl ethers were then subjected to the carbonylative coupling with cyanamide (as described in Table 5). However, both silyl ethers gave complete conversion to the cyclised benzoxazinone (19a). Presumably, the silyl ethers were cleaved under the catalytic conditions and the resulting intermediate was spontaneously cyclised in the presence of Et$_3$N.

Instead a benzyl protective group was introduced. The benzyl ether (22c) was prepared in 85% yield from benzylbromide and K$_2$CO$_3$ in DMF following standard procedures. The benzyl ether was stable under the carbonylative conditions and was isolated in 95% yield after silica gel column purification and fully characterised. However, the O-debenzylation proved more challenging (summarised in Table 9).

Initially, catalytic hydrogenation at atmospheric pressure in MeOH was tried for the cleavage of 23a. Unfortunately, the attempt lead to an unidentified side product as the major product with no trace of the deprotected intermediate and only traces of the cyclised benzoxazinone observed (entry 1). Since it was suspected that methanol might in some way cause the side product, the reaction was repeated but this time using EtOAc as solvent (entry 2). This time no conversion at all was observed from the benzyl-protected intermediate. Since catalytic hydrogenation did not offer good prospects of succeeding, a different strategy was approached. The efficient O-debenzylation with TFA has been
reported for aryl benzyl ethers.\textsuperscript{181} The reported method was particularly efficient for substrates with ortho-electron-withdrawing groups on the phenolic ring, similar to 23a. This time the benzyl was cleaved but resulted in a complex reaction mixture, and 19a was isolated in 5\% over two steps. Instead, the para-methoxybenzyl group was introduced using standard procedures to yield 22d in 62\%.\textsuperscript{180} Following carbonylation, the protected intermediate 23b was obtained in 45\% together with 14\% of the deprotected and cyclised benzoxazinone. Finally, after successful O-debenzylation of 23b, the cyclised benzoxazinone 19a was obtained in 80\% yield over two steps (entry 4) and thereby supporting that the reaction proceeds via an N-cyanobenzamide intermediate.

Table 9. Conditions for the O-Debenzylation of 23a and b\\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protective group</th>
<th>Deprotecting Conditions</th>
<th>Yield 19a (%)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bn</td>
<td>H\textsubscript{2}, Pd/C, MeOH, r.t.</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Bn</td>
<td>H\textsubscript{2}, Pd/C, EtOAc</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Bn</td>
<td>TFA, thioanisole, toluene\textsuperscript{181}</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>PMB</td>
<td>TFA, DCM</td>
<td>80</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reaction conditions: (i) Deprotecting conditions, (ii) Et\textsubscript{3}N, 1,4-dioxane. \textsuperscript{b}Isolated yield over two steps.

Evaluation of Various CO Sources

In the work described in Paper III, Mo(CO)\textsubscript{6} has been used as a solid source of CO. However, several new CO sources have been reported in the last decade.\textsuperscript{182} Especially, the development of the two-chamber system\textsuperscript{40,46} has enabled the use of CO-releasing systems that would not otherwise easily combine with the carbonylation reaction itself. Each source of CO comes with its own advantages and disadvantages, such as access of readily available commercial reagents and the cost thereof as well as the possibility to isotopically label the carbonyl carbon. Therefore, the choice of CO source much depends on the purpose of the reaction. Under those circumstances it is valuable to offer new methods that are compatible with all or most of the more common CO sources.
Table 10. Evaluation of various CO sources

<table>
<thead>
<tr>
<th>Entry</th>
<th>CO source</th>
<th>T (°C)</th>
<th>P max (bar)</th>
<th>T Pmax (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mo(CO)₆</td>
<td>65</td>
<td>ex situ</td>
<td>2.3</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>Mo(CO)₆</td>
<td>85</td>
<td>ex situ</td>
<td>2.4</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>Cl</td>
<td>65</td>
<td>ex situ</td>
<td>atm</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>65</td>
<td>in situ</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Cl</td>
<td>65</td>
<td>ex situ</td>
<td>3.1</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>Ph</td>
<td>80</td>
<td>ex situ</td>
<td>3.1</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>Ph</td>
<td>65</td>
<td>ex situ</td>
<td>2.9</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>CHCl₃ + CsOH</td>
<td>80</td>
<td>ex situ</td>
<td>2.7</td>
<td>108</td>
</tr>
<tr>
<td>9</td>
<td>CHCl₃ + CsOH</td>
<td>80</td>
<td>in situ</td>
<td>6.3</td>
<td>39</td>
</tr>
</tbody>
</table>

To establish the compatibility of the developed reaction with additional non-gaseous CO generating methods, a set of six CO sources that are well studied in the literature were selected (Table 10). The carbonylation/cyclisation reaction of 2-iodophenol and cyanamide were chosen as the model reaction. In addition to comparing the isolated yield of benzoxazinone 19a, the internal pressure and release time were measured to determine the efficiency of each CO source. All CO sources were evaluated ex situ using the two-chamber system. In addition to that the in situ release of CO from phenyl formate and the alkaline hydrolysis of chloroform was also evaluated. The literature procedures for some of the CO sources were performed at temperatures above 65 °C. To rule out any effect caused by the reaction temperature, a control experiment with Mo(CO)₆ was performed at 85 °C (entry 2). The results from Table 10 show that the reaction is compatible with all ex situ
generated CO sources. However, the release of CO in situ was deleterious to the reaction.

Summary (Paper III)
In summary, a convenient domino carbonylation/cyclisation sequence has been developed for the facile preparation of 2-amino-4H-benzo[e][1,3]oxa-
zin-4-ones (19a-q). The compounds were prepared from readily available ortho-halophenols and cyanamide using non-gaseous and low-pressure carbonylative conditions. The reaction pathway was confirmed by isolating and characterising the protected carbonylated intermediate, which following deprotection was cyclised to benzoxazinone 19a. Finally to highlight the versatility of the reaction, the compatibility of the method was evaluated for six additional CO sources from which 19a was prepared in comparable yields.
Palladium(0)-Catalysed Carbonylative Synthesis of N-Acylguanidines (Paper IV)

Background and Aim

Like the synthetic strategy in Paper II, where a carbonylation and intramolecular amination/cyclisation provided 2-aminoquinazolinones, an analogous intermolecular approach was envisioned to give access to a wide array of N-acylguanidines in a one-pot synthesis. First, carbonylative coupling of aryl iodides (24) or bromides (25) with cyanamide would provide the versatile N-cyanobenzamide intermediate (26). Subsequent amination with various primary, secondary or aromatic amines would then yield N-acylguanidines (27) with considerable product diversity.

Scheme 16. Synthesis of N-acylguanidines by a one-pot carbonylation/amination sequence from aryl iodides or bromides, cyanamide and amines.

Acylguanidines are ubiquitous in nature and constitute a crucial part of the DNA-binding motif in nucleoside guanine. N-Acylguanidines exhibit a wide range of biological activities, highlighting the versatile nature of this functional group, illustrated by e.g. marketed drugs; diuretic Amiloride and α-adrenoceptor agonist Guanfacine. Moreover, acylguanidines are present in sodium-channel inhibitors, thrombin inhibitors, β-secretase inhibitors, antivirals and histamine H₂ receptor agonists. In addition to their application in medicinal chemistry, their hydrogen bonding properties also make them suitable as e.g. organocatalysts and components in supramolecular complexes. The introduction of an acyl group to the guanidine moiety has also been demonstrated to improve pharmacokinetics. Non-acylated guanidines are very basic (pKₐ ≈ 11-13) but the introduction of the acyl group leads to a substantial decrease in basicity (pKₐ ≈ 7-8) and, in some cases, renders a much more suitable pharmacokinetic profile.
Synthesis of N-Acylguanidines from Aryl Iodides

Method Development

It was envisioned that the carbonylative cyanamide coupling could be performed in a two-chamber system, similar to the methods described in Papers II and III. Following carboxylation, the reaction mixture would be transferred to a single vial and the amine would be added. It was anticipated that this would allow the synthesis of N-acylguanidines without having to isolate the intermediate.

To test the feasibility of the proposed synthetic strategy, the reaction was first set up with iodobenzene, cyanamide, Pd(PPh₃)₄ and Et₃N in one chamber. CO was generated ex situ in the adjacent chamber from Mo(CO)₆ and DBU. Under these conditions acylguanidine 27a was obtained in 79% yield (entry 1). Encouraged by this result, which confirmed the viability of the proposed synthetic strategy, the investigation of various ligands was continued (Table 11).

Table 11. Optimisation of reaction conditions for synthesis of 27a from iodobenzene.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Pd</th>
<th>mol % Pd</th>
<th>Ligand</th>
<th>Yield (%) ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh₃)₄</td>
<td>5</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>Pd(OAc)₂</td>
<td>5</td>
<td>[(t-Bu)₃PH]BF₄</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>Pd(dppf)Cl₂</td>
<td>5</td>
<td>-</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>Pd(OAc)₂</td>
<td>5</td>
<td>Xantphos</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>Pd(OAc)₂</td>
<td>5</td>
<td>DPEphos</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>Pd(OAc)₂</td>
<td>2</td>
<td>DPEphos</td>
<td>91</td>
</tr>
<tr>
<td>7</td>
<td>Pd(OAc)₂</td>
<td>1</td>
<td>DPEphos</td>
<td>80</td>
</tr>
</tbody>
</table>

² Isolated yield.

Interestingly, in the reactions where monodentate PPh₃ and t-BuP₃ were used as ligands (entries 1 and 2), an impurity with 13C-31P couplings in the NMR spectra was observed. While this impurity was never isolated and fully characterised, it was proposed that it stemmed from nucleophilic addition of the phosphine ligand to the electrophilic intermediate (26). It is worth noting that an equivalent impurity was never observed for the previous projects involving the N-cyanobenzamide intermediates 14 and 18 (Papers II and III). Presumably, in those cases, the presence of an intramolecular nucleophile out-competes any potential phosphorous nucleophile. Fortunately, with the change to
bidentate phosphine ligands, no phosphorous-derived side products were observed. The use of dppf, Xantphos and DPEphos in the reaction provided comparable yields (74-83%, entries 3-5) to the use of Pd(PPh₃)₄ (79%, entry 1). Taking cost, stability and performance of the ligand into consideration, the optimisation was continued with DPEphos. Varying the catalyst loading showed that 2 mol % Pd was optimal for this reaction, which provided 27a in 91% isolated yield (entry 6).

Scope and Limitations
To assess the generality of the developed method a set of aryl iodides were reacted under the conditions described in Table 11, entry 6. Both electron-rich and electron-deficient aryl iodides reacted readily under the reaction conditions and returned the N-acylguanidines in high yields (Table 12, 27a-i, 73-92%). The reaction with 1-bromo-4-iodobenzene was entirely selective towards the iodide and the bromo-substituted acylguanidine 27k was obtained in 76% yield. Furthermore, heteroaryls, 5-methyl-2-thiophenyl and 4-pyridyl were introduced with satisfactory yields (27l and m, 73% and 58%, respectively).

Table 12. Synthesis of N-acylguanidines from aryl iodides

<table>
<thead>
<tr>
<th>R</th>
<th>Isolated yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>27a</td>
<td>91%</td>
</tr>
<tr>
<td>27b</td>
<td>73%</td>
</tr>
<tr>
<td>27c</td>
<td>80%</td>
</tr>
<tr>
<td>27d</td>
<td>85%</td>
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<td>27e</td>
<td>92%</td>
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<td>27f</td>
<td>88%</td>
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<td>27g</td>
<td>89%</td>
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<td>27h</td>
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</tr>
<tr>
<td>27l</td>
<td>73%</td>
</tr>
<tr>
<td>27m</td>
<td>58%</td>
</tr>
</tbody>
</table>

*aIsolated yield.*
Synthesis of N-Acylguanidines from Aryl Bromides

Method Development
Having demonstrated the successful application of aryl iodides in the developed method, the attention was focused on aryl bromides. Consequently, bromobenzene was reacted under the reaction conditions described in Table 11, entry 6. Unfortunately, under these conditions cyanobenzamide was not formed (Table 13, entry 1). To promote oxidative addition of the bromide, the reaction temperature was increased to 85 °C in the carbonylation step, leading to an increased yield of 36% (entry 2). Finally, by also increasing the catalyst loading to 5 mol %, the bromo-derived 27a could be obtained in 76% yield (entry 3).

**Table 13. Optimisation of reaction conditions for synthesis of 27a from bromobenzene**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Pd(OAc)$_2$ (mol %)$^b$</th>
<th>Temperature (°C)$^a$</th>
<th>Yield (%)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>85</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>85</td>
<td>76</td>
</tr>
</tbody>
</table>

$^a$Temperature in carbonylation step. $^b$1:1.2 Pd/ligand ratio. $^c$Isolated yield.

Scope and Limitations
To investigate the scope and limitations of the reaction a set of 16 aryl bromides with various electronic and steric properties was selected and reacted under the optimised conditions (Table 13, entry 3) the results are presented in Table 14.

In contrast to the reaction with aryl iodides, the outcome appeared to be governed by electronic properties of the aryl bromide. Neutral and electron-deficient aryl bromides returned yields comparable to the unsubstituted 26a. However, electron-rich substrates resulted in reduced yields. The same trend was observed for the reaction with bromides in Paper III. With increasing electron-density in the aryl bromide a reduction in yield was observed, for example unsubstituted 27a was obtained in 76% yield compared to inductively donating para, meta and ortho methyl substituted acylguanidines (27e-g) which were obtained in 47-52% yield. In further comparison, the introduction of an electron-donating methoxy group in resonance to the bromide afforded the corresponding acylguanidine (27h) in 39% yield.
Table 14. Synthesis of N-acylguanidines from aryl bromides

<table>
<thead>
<tr>
<th>R</th>
<th>27a, 76%</th>
<th>27b, 67%</th>
<th>27c, 76%</th>
<th>27d, 73%</th>
<th>27e, 62%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R^1 = H; 27m, 64%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R^1 = F; 27n, 66%</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Isolated yield. NH$_2$CN (3 equiv).

For the acylguanidines which were prepared both from the aryl iodide and bromide, the yields were consistently lower for the bromo-derived compounds. Suggesting that reduced rate of oxidative addition may be the cause of the reduced yields. Despite their reduced reactivity, all aryl bromides produced the respective N-acylguanidine in moderate to high yields.

Evaluating the Performance of Various Amines in the Synthesis of N-Acylguanidines

To further assess the versatility of the developed method, twenty primary, secondary and aromatic amines were used as nucleophilic components under the optimised reaction conditions (Table 15). As expected, the reactivity of secondary amines and benzy lamines differed slightly from that of primary and aromatic amines. To achieve full amination with the latter an increase in temperature was required. As a result, the amination reactions with the more reactive secondary amines and benzy lamines were heated at 120 °C whereas those with the primary and aromatic amines were performed at 140 °C. Under
Table 15. Synthesis of N-acylguanidines from various amines

![Chemical structures](image)

**Secondary amines**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28a</td>
<td>84%</td>
</tr>
<tr>
<td>28b</td>
<td>82%</td>
</tr>
<tr>
<td>28c</td>
<td>59%</td>
</tr>
<tr>
<td>28d</td>
<td>77%</td>
</tr>
<tr>
<td>28e</td>
<td>77%</td>
</tr>
<tr>
<td>28f</td>
<td>74%</td>
</tr>
</tbody>
</table>

**Benzylamines**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28g</td>
<td>83%</td>
</tr>
<tr>
<td>28h</td>
<td>94%</td>
</tr>
<tr>
<td>28i</td>
<td>63%</td>
</tr>
<tr>
<td>28j</td>
<td>66%</td>
</tr>
<tr>
<td>28k</td>
<td>70%</td>
</tr>
<tr>
<td>28l</td>
<td>73%</td>
</tr>
</tbody>
</table>

**Primary amines**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28m</td>
<td>75%</td>
</tr>
<tr>
<td>28n</td>
<td>60%</td>
</tr>
<tr>
<td>28o</td>
<td>40%</td>
</tr>
<tr>
<td>28p</td>
<td>32%</td>
</tr>
</tbody>
</table>

**Aromatic amines**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28q</td>
<td>16%</td>
</tr>
<tr>
<td>28r</td>
<td>78%</td>
</tr>
<tr>
<td>28s</td>
<td>64%</td>
</tr>
<tr>
<td>28t</td>
<td>57%</td>
</tr>
</tbody>
</table>

*Isolated yield.*

These conditions, diethylamine and dipropylamine reacted readily, as did pyrrolidine, morpholine and 4-hydroxypiperidine providing compounds 28a-b and 28d-e in 74-84% yield. In analogy to those results, primary and secondary benzylamine performed well and 28g and 28h were obtained in 83% and 94% yield, respectively. However, sterically hindered diisopropylamine required an additional portion of amine and additional heating at 140-160 °C before acylguanidine 28c was obtained in 59% yield. Primary alkyl amines were less reactive compared to the secondary amines and returned corresponding acylguanidines 28i-l in 63-73% yield. For aromatic amines, the amination reaction was limited to the more nucleophilic amines. In the presence of an electron-
withdrawing group para to the nitrogen, the reaction outcome was substantially lower (28o-q, 16-40%). In contrast, with an electron-donating group in resonance to the nitrogen, the acylguanidine was obtained in 78% yield (28r).

Addition of Amine to the N-Cyanobenzamide Intermediate

The addition of amine occurs in the presence of Pd as the intermediate is not purified and isolated between the carbonylation and amination reactions. To verify that Pd does not participate in the amination reaction the intermediate was isolated and the nucleophilic addition was performed without the presence of Pd (Scheme 17). Iodobenzene and cyanamide were reacted to afford N-cyanobenzamide 26a, which was isolated in 79% yield. The pure intermediate was then heated with piperidine and Et3N in 1,4-dioxane at 120 °C for 30 min under MW irradiation to provide acylguanidine 27a in 94% isolated yield, confirming that the nucleophilic addition takes place even when Pd is not present.

Scheme 17. Synthesis of intermediate 26a from iodobenzene and cyanamide and the subsequent Pd-free amination to acylguanidine 27a.

Using N-Acylguanidines as Precursors for Heterocycles

Guanidine derivatives are often used as precursors in heterocycle synthesis, e.g. in the Biginelli reaction,200,201 in the synthesis of benzimidazoles,202 aminoimidazoles,203 2-amino-4-quinazolinones,204 purines and other fused imidazole systems.205 To highlight the versatility of the developed synthetic strategy, three heterocycles were prepared via an N-acylguanidine precursor (Scheme 18).

Dihydro-4-pyrimidone206 (29) and glycocyamidine109 (30) rings were prepared from the corresponding β-alanine methyl ester and glycine methyl ester. The addition of β-amino methyl esters to the nitrile carbon in the intermediate N-cyanobenzamide resulted in partial internal cyclisation by acylation of the guanidine nitrogen. The resulting dihydro-4-pyrimidone and glycocyamidine compounds 29 and 30 were readily obtained after additional heating in 47%
and 52% isolated yield, respectively, over three steps. 3-Amino-1,2,4-oxadia-
zole (31) was prepared from isolated N-benzylsubstituted acylguanidine 28g
under oxidative conditions\textsuperscript{207} in 49% yield (41% over three steps).

\[ \text{Scheme 18. Multicomponent Pd(0)-catalysed carboxylative reaction for easy access}
\]
to various nitrogen-containing heterocycles from aryl iodide, cyanamide and amines.

**Summary (Paper IV)**

In summary, the work described in **Paper IV** shows how \( N \)-acylguanidines
can be conveniently prepared from readily available starting materials. Pd-
catalysed carboxylative cross-coupling of the aryl iodide or bromide and cy-
anamide affords the \( N \)-cyanobenzamide intermediate. Following amination
with a large variety of primary, secondary and aromatic amines, the acylguan-
idines were obtained in up to 94% yield.
Synthesis and *in vitro* Biological Evaluation of Quinolinyl Pyrimidines Targeting Type II NADH Dehydrogenase (*Paper V*)

**Background and Aim**

In *Paper I*, the development of a non-gaseous synthesis of 4-quinolones that tolerated nitro functionalities was described. The developed method was later applied in a project aimed at finding new inhibitors of type II NADH dehydrogenase (NDH-2) and various pathogens (*Paper V*). NDH-2 is a membrane-bound enzyme and is an essential part of bacterial cell respiration.\textsuperscript{113,116} NDH-2 enzymes are not found in mammals and they are therefore attractive targets for the development of novel antibacterial compounds. A number of small-molecule compounds have been put forward as NDH-2 inhibitors, including antipsychotic phenothiazines,\textsuperscript{108,109,119} flavones,\textsuperscript{109,208} bisaryl quinolones\textsuperscript{111,123,124,127} and quinolinyl pyrimidines.\textsuperscript{110} Considering the increase of resistant bacteria and the limited number of new antibacterial drugs, the discovery and investigation of novel targets is crucial. Furthermore, most of the currently available antibiotics are targeting various parts of the biosynthesis of bacteria. This, however, is not a suitable target for slow-growing and non-growing bacteria, which is thought to be a survival mechanism for bacteria and a cause of bacterial resistance.\textsuperscript{99,102,103} A promising approach is to kill bacteria by membrane disruption, e.g. altering membrane potential or inhibiting energy metabolism. The inhibition of NDH-2 has been shown to be essential to maintain functional energy metabolism in *Mtb* and Gram-negative bacteria and thus crucial for survival of replicating as well as non-replicating bacteria.\textsuperscript{118}

![Figure 7](image)

**Figure 7.** Quinolinyl pyrimidine 1, previously reported to inhibit NDH-2 and kill *Mtb*.\textsuperscript{110}
At the outset of this project, the most promising small-molecule inhibitors of NDH-2 were the quinolinyl pyrimidines reported by Shirude and co-workers, exemplified with compound 1 in Figure 7. However, the previously reported compounds had problems with solubility. As a result, further biological studies, for example mutational studies, had not been possible to pursue for that class of inhibitors. Therefore, compound 1 was chosen as the starting point and it was anticipated that the introduction of more polar and charged groups instead of the 6-arylsubstituent on the pyrimidine might increase solubility. The work described in Paper V presents the synthesis of quinolinyl pyrimidines and the biological evaluation of the prepared compounds as inhibitors of NDH-2 and on whole-cell bacteria.

Preparation of Final Compounds

Initial attempts at synthesising 2-(4-fluorophenyl)-6-nitroquinolin-4(1H)-one (11t) using the classical Conrad-Limpach-Knorr reaction from the corresponding aniline and β-keto ester was unfortunately unsuccessful. Therefore, this lead to the development of a Pd-catalysed carbonylative synthesis of 4-quinolones (Paper I). This synthetic strategy was then applied in the synthesis of key intermediate 4-chloro-2-(4-fluorophenyl)-6-nitroquinoline (36). 1-Ethynyl-4-fluorobenzene (32) and 2-iodo-4-nitroaniline (33) were reacted in the carbonylative Sonogashira cross-coupling to afford 4-quinolone 11t in 79% yield, from which the 4-chloroquinolone 36 was readily obtained following treatment with POCl₃. Alternatively, compound 36 was prepared from 4-fluoroacetophenone (34) and 2-amino-5-nitrobenzoic acid (35) (Scheme 19).

Scheme 19. Synthesis of 4-chloro-2-(4-fluorophenyl)-6-nitroquinoline (36). Reagents and conditions: (a) (i) Pd(OAc)₂, [HP(t-Bu)₃]BF₄, Et₃N, Mo(CO)₆, MeCN, r.t. 16 h. (ii) Et₂NH, r.t. 5 h, 79%; (b) POCl₃, 105 °C, 2 h; (c) POCl₃, 90 °C, 5 h, 48%.
Scheme 20. Synthesis of compounds 40a-o from quinoline 36. Reagents and conditions: (a) NaN₃, NMP, 60 °C, 18 h; (b) SnCl₂·H₂O, EtOAc:EtOH (2:1), reflux, 2 h; (c) 2-Amino-4,6-dichloropyrimidine, HCl (4 M in dioxane), NMP, 100 °C, overnight, 76%; (d) amine, DIEA, EtOH, MW heating 150 °C, 90 min, 30-69%; (e) alcohol, KOH, MW heating 150 °C, 30 min or 100-120 °C, 70 min, 13-63%.

Table 16. Preparation of final compounds 40a-o.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40a</td>
<td></td>
<td>42</td>
<td>40i</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>40b</td>
<td></td>
<td>51</td>
<td>40j</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>40c</td>
<td></td>
<td>37</td>
<td>40k</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>40d</td>
<td></td>
<td>65</td>
<td>40l</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>40e</td>
<td></td>
<td>57</td>
<td>40m</td>
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<tr>
<td>40f</td>
<td></td>
<td>69</td>
<td>40n</td>
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</tr>
<tr>
<td>40g</td>
<td></td>
<td>57</td>
<td>40o</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>40h</td>
<td></td>
<td>Quant.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The 4-chloroquinoline (36) was next treated with sodium azide and after subsequent reduction of the azide and nitro groups, 2-(4-fluorophenyl)quinolone-4,6-diamine (38) was isolated (Scheme 20). The pyrimidine moiety was introduced by a nucleophilic displacement on 2-amino-4,6-dichloropyrimidine to yield N6-(2-amino-6-chloropyrimidine-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (39). In the final step, amino and alkoxy groups with hydrophilic groups or functionalities which are charged at physiological pH, were introduced to the pyrimidine. Twelve amines were used to substitute the 6-chloro on the pyrimidine ring to yield the final amino-substituted compounds 40a-l in 30-69% yield (Table 16). Alkoxy-substitutents were installed from the corresponding alcohol under alkaline conditions to yield alkoxy-substituted final compounds 40m-o in 13-63% yield (Table 16).

Biological Evaluation
Inhibition of NDH-2, MIC Evaluation on a Panel of Bacteria and Cytotoxic Assessment

The synthesised compounds were evaluated for inhibition on the enzyme target as well as on a selected panel of Gram-negative bacteria, Gram-positive S. aureus and Mtb (Table 17). In addition to the enzymatic and bacterial whole-cell tests, most of the compounds were also evaluated for cytotoxic activity on human hepatoblastoma (HepG2) cells and human lung fibroblast (MRC-5) cells (Table 18). The majority of the synthesised compounds demonstrated cell toxic doses that lie well below the cut-off for toxicity (IC50 < 10µM). A comparison of clogP and cytotoxicity revealed that cytotoxicity increased with increased hydrophobicity. However, no correlations between cytotoxicity and NDH-2 IC50 or MIC values were observed. These results suggest that the antibacterial effects presented in (Table 17) are not entirely a result of the toxic nature of the compounds and that some other mechanism might be involved in the bactericidal action.

Under our assay conditions, previously reported NDH-2 inhibitor compound 1 gave an IC50 of 1.6 µM (compared to the reported IC50 = 96 nM). Furthermore, 1 exhibited comparable MIC on Mtb in the same range as previously reported. However, 1 was essentially inactive on the Gram-negative bacteria with the exception of the efflux-deficient and drug-hypersensitive E. coli strains (data for drug-hypersensitive E. coli not shown in Table 17).

Precursor chloride 39 gave a modest inhibition of NDH-2 (IC50 = 3.1 µM) but was largely inactive against most Gram-negative strains apart from A. baumannii and Gram-positive S. aureus and Mtb. Notably, 39 exhibited considerable cytotoxicity to both HepG2 and MRC-5 cells. The same trend in
Table 17. Inhibition of MsNDH-2 and measured minimum inhibitory concentration (MIC) values on a panel of Gram-negative bacteria, the Gram-positive *S. aureus* and *Mtb*.

<table>
<thead>
<tr>
<th>IC₅₀ (µM)</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>NDH-2</td>
</tr>
<tr>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>39</td>
<td>3.1</td>
</tr>
<tr>
<td>40a</td>
<td>21.4</td>
</tr>
<tr>
<td>40b</td>
<td>&gt;100&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>40c</td>
<td>25.8</td>
</tr>
<tr>
<td>40d</td>
<td>18.9</td>
</tr>
<tr>
<td>40e</td>
<td>24.0</td>
</tr>
<tr>
<td>40f</td>
<td>&gt;100&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>40g</td>
<td>0.35</td>
</tr>
<tr>
<td>40h</td>
<td>11.6</td>
</tr>
<tr>
<td>40i</td>
<td>0.95</td>
</tr>
<tr>
<td>40j</td>
<td>21.4</td>
</tr>
<tr>
<td>40k</td>
<td>3.0</td>
</tr>
<tr>
<td>40l</td>
<td>10</td>
</tr>
<tr>
<td>40m</td>
<td>5.3</td>
</tr>
<tr>
<td>40n</td>
<td>12.8</td>
</tr>
<tr>
<td>40o</td>
<td>12</td>
</tr>
</tbody>
</table>

Polymyxin B | ~1.7 | <0.12 | <0.12 | 0.25 | <0.12 | 0.25 | >64 | nd<sup>i</sup> |

<sup>a</sup>*E. coli* (ATCC 25922, wild-type). <sup>b</sup>*E. coli* (ΔtolC efflux-defective mutant, isogenic to ATCC 25922). <sup>c</sup>*P. aeruginosa* (PAO1, wild-type). <sup>d</sup>*P. aeruginosa* (PAO750 efflux-defective mutant, isogenic to PAO1). <sup>e</sup>*A. baumannii* (ATCC 19606, wild-type). <sup>f</sup>*S. aureus* (ATCC 29213, wild-type). <sup>g</sup>*M. tuberculosis* strain H37Rv (ATCC 25618). <sup>h</sup>No significant inhibition was observed at 100 µM. <sup>i</sup>nd = not determined.
biological activity was observed for compounds \textbf{40k} and \textbf{40m}, with small dimethylamino and ethoxy substituents, respectively. Those compounds gave modest inhibition of NDH-2 (3.0 µM and 5.3 µM, respectively) and inhibition of 	extit{A. baumannii}, 	extit{S. aureus} and 	extit{Mtb}, comparable to chlorosubstituted \textbf{39}.

Piperazine substituted \textbf{40a} was not active on NDH-2 but demonstrated good antibacterial activity on \textit{E. coli} and \textit{P. aeruginosa}. Interestingly, \textbf{40a} was considerably less cytotoxic compared to most of the other tested compounds. However, the introduction of morpholine or substitution of the piperazine nitrogen rendered compounds (\textbf{40b-f}) inactive on NDH-2. And despite being relatively cytotoxic they did not exhibit any substantial antibacterial activities (MICs ranging from 16 to \textgreater;128 µg/mL).

Boc-protected \textbf{40g} and \textbf{40i} gave good inhibition of NDH-2 (IC\textsubscript{50} = 0.35 µM and 0.95 µM, respectively). Notably, \textbf{40g} and \textbf{40i} were more active on the enzyme than previously reported inhibitor \textbf{1} (IC\textsubscript{50} = 1.6 µM) under our assay conditions. However, interestingly, the corresponding cleavage products \textbf{40h} and \textbf{40j} did not retain activity on the enzyme (IC\textsubscript{50} = 11.6 µM and 21.4 µM, respectively). A comparison of the NDH-2 IC\textsubscript{50} of boc-protected compounds \textbf{40g} and \textbf{40i} and the corresponding cleavage products \textbf{40h} and \textbf{40j} reveals a loss in activity with the removal of carbamate and introduction of a charged group. Further studies are however needed to fully elucidate if the NDH-2 activity is due to hydrophobic interactions of the tert-butyl group, if the NH or carbonyl moieties are participating in hydrogen bonding, or if a charge on the pyrimidine ring is unfavourable.

Interestingly, an extended comparison of the bacterial MICs and NDH-2 IC\textsubscript{50} reveal a discrepancy between IC\textsubscript{50} and MIC for different strains. Where the carabamates are clearly favoured on \textit{A. baumannii}, \textit{S. aureus} and \textit{Mtb}, the reversed is observed for wild-type and efflux-deficient \textit{P. aeruginosa}. It should be noted that the free amines demonstrated slightly lower cytotoxic properties compared to their carbamate counterparts. Still, in both cases, the free amines were more active on \textit{P. aeruginosa} (MIC \textit{P. aeruginosa} wild-type 4 µg/mL and efflux-deficient mutant 8 µg/mL) compared to carabamates \textbf{40g} and \textbf{40i} that were essentially inactive (MIC ranging from 32 to \textgreater;128 µg/mL). These contradicting results suggest that the observed antibacterial effect is not exclusively a result of cytotoxicity. The results may also be understood as a difference in distribution and uptake in different bacteria.

Three different strains of \textit{E. coli} were evaluated: wild-type, an efflux-defective mutant (ΔtolC) and a drug-hypersensitive (D22, \textit{lpxC} mutant, not shown in (Table 1)). The mutated strains were in good agreement and in general the tested compounds gave some activity for both of the mutated strains (MICs in the range of 2-64 µg/mL) whereas the tested compounds had little or no effect on the wild-type strain. The same trend was observed for the two strains of \textit{P. aeruginosa} (wild-type and efflux-defective mutant), where most of the tested compounds exhibited lower MICs on the efflux-deficient strain.
compared to the wild-type. These results indicate that the tested compounds need to be optimised with respect to efflux and permeability.

A comparison of MIC values with a number of structural descriptors, i.e. molecular weight, polar surface area, logP, number of hydrogen bond donors/acceptors of the pyrimidine substituent, total charge of the compound, using a PCA and PLS analysis was performed. The multicomponent analysis showed that the introduction of a charged group was favourable for the activity on *P. aeruginosa* whereas neutral groups favour inhibition on *A. baumannii* and *Mtb*. It should be noted that not all bacterial strains displayed a correlation between pMIC values and NDH-2 pIC$_{50}$. However, for drug-hypersensitive *E. coli* (D22), *A. baumannii*, *S. aureus* and *Mtb*, moderate correlations were observed ($r = 0.48$-$0.69$). In light of these results, and in relation to the results for compounds 40g-j, it may suggest that inhibition of NDH-2 is involved in the antibacterial effect on drug-hypersensitive *E. coli* (D22), *A. baumannii*, *S. aureus* and *Mtb*. And, furthermore, that the inhibition is favoured by bulky, non-charged groups on the pyrimidine.

**Table 18.** Calculated LogP and results from cytotoxicity assays HepG2 and MRC-5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>clogP</th>
<th>TD$_{99}$ (µM)</th>
<th>TD$_{50}$ (µM)</th>
<th>IC$_{50}$ (µM)</th>
<th>MRC-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.0</td>
<td>0.4</td>
<td>0.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>2.7</td>
<td>50</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>4.3</td>
<td>0.8</td>
<td>0.4</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>40a</td>
<td>3.6</td>
<td></td>
<td></td>
<td>24</td>
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</tr>
<tr>
<td>40b</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40c</td>
<td>4.0</td>
<td>3.1</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>4.4</td>
<td>1.6</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$a$ Rifampicin (TD$_{99}$ ≥100 µM; TD$_{50}$ = 75 µM) and bedaquiline (TD$_{99}$ ≥100 µM; TD$_{50}$ = 50 µM) were used as reference compounds. $b$ clogP values were calculated by Instant JChem (Instant JChem 15.9.14.0, ChemAxon, http://www.chemaxon.com/).

Most of the tested compounds were tested HepG2 cells and MRC-5 cells to assess the cytotoxicity (Table 18). The vast majority of the synthesised compounds demonstrated cell toxic doses that lie well below the cut-off for tox-
icity (IC$_{50} <$10µM). A comparison of clogP and cytotoxicity revealed that cytotoxicity increased with increased hydrophobicity. However, no correlations between cytotoxicity and NDH-2 IC$_{50}$ or MIC values were observed. These results indicate that the antibacterial effects presented in Table 17 are not entirely a result of the toxic nature of the compounds and that some other mechanism might be involved in the bactericidal action.

Effect of Aromatic Amines and Pyrimidine on Activity and Cytotoxicity

A comparison of the MIC and cytotoxicity data of precursor quinoline diamine 38 and quinolinyl pyrimidine 39 suggested that the pyrimidine was (i) at least in part important for antibacterial activity and (ii) implicated in the cytotoxic mechanism (Table 19). Compound 39 did not exhibit bactericidal action across the whole spectrum, which may suggest that the improved antibacterial effect seen on *P. aeruginosa*, *A. baumannii*, *S. aureus* and *Mtb* is not entirely caused by an increase in cytotoxicity.

Table 19. Comparison of MIC and cytotoxicity data for compounds 38 and 39.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/mL)</th>
<th>HepG2$^g$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E. coli^a$</td>
<td>$E. coli^b$</td>
</tr>
<tr>
<td>38</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>39</td>
<td>&gt;128</td>
<td>64</td>
</tr>
</tbody>
</table>

$^a$ *E. coli* (ATCC 25922, wild-type). $^b$ *E. coli* (ΔtolC efflux-defective mutant, isogenic to ATCC 25922). $^c$ *P. aeruginosa* (PAO1, wild-type). $^d$ *P. aeruginosa* (PAO750 efflux-defective mutant, isogenic to PAO1). $^e$ *A. baumannii* (ATCC 19606, wild-type). $^f$ *S. aureus* (ATCC 29213, wild-type). $^g$ Rifampicin (TD$_{99} \geq$100 µM; TD$_{50} = 75$ µM) and bedaquiline (TD$_{99} \geq$100 µM; TD$_{50} = 50$ µM) were used as reference compounds.

Prompted by the results in Table 19 and the ambition to also include an investigation of the effect of aromatic amines on cytotoxicity, a few analogues were prepared where one or two of the aromatic amines had been omitted from the scaffold (41-44) (Figure 8).

Furthermore, to investigate if a change from 6-chloropyrimidine (39) to a pyrimidone would have any effect in terms on antibacterial and enzyme activity as well as on cytotoxicity, compound 47 was prepared. 2-Amino-6-chloropyrimidin-4(3H)-one (46) was prepared in 90% yield by refluxing 2-amino-4,6-dichloropyrimide (45) in an aqueous alkaline solution followed by acidic workup. Subsequent nucleophilic displacement of the chloro group by quinoline diamine 38 provided desired pyrimidone (47) in 38% yield.
Figure 8. Precursor 41 and quinolinyl pyrimidines analogues 42-44 without one or two of the aromatic amines.

Scheme 21. Synthesis of compound 47. Reagents and conditions: a) 1 M NaOH, reflux, 2 h, then AcOH, 90%; b) 38, HCl (4 M in dioxane), NMP, 100 °C, overnight, 38%.

The analogues were evaluated on NDH-2 enzyme and a panel of Gram-negative bacteria, S. aureus and Mtb (Table 20). In addition, the cytotoxic activity was assessed on HepG2 and MRC-5 cells (Table 21). None of the analogues inhibited NDH-2 to a substantial degree, however the inhibition of NDH-2 for precursor 41 was comparable to 39 giving (IC$_{50}$ = 5.1 µM vs. 3.1 µM) (Table 20). The pyrimidine amine appears to be of some importance for activity on wild-type E. coli. Comparing 8a and 43, which only differ by the presence or absence of the quinoline amine, only a two-fold decrease in wild-type E. coli activity was observed. In contrast, for compounds 42 and 44, which lacked the pyrimidine amine, no inhibition was observed on wild-type E. coli. Introduction of a pyrimidone moiety in compound 47 lead to a partial decrease in antibacterial activity, although not all activity was lost. Interestingly, 47 was substantially less cytotoxic compared to the other compounds. Taken together with the results for diamine 38, it strengthens the argument that the pyrimidine is important for cytotoxicity.
Table 20. Inhibition of MsNDH-2 and measured minimum inhibitory concentration (MIC) values on a panel of Gram-negative bacteria, the Gram-positive S. aureus and Mtb for compounds 40a, 41-44 and 47.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>3.1</td>
<td>&gt;128 64 16 4 4.7</td>
</tr>
<tr>
<td>40a</td>
<td>21.4</td>
<td>8 8 8 4 32 32 43</td>
</tr>
<tr>
<td>41</td>
<td>5.1</td>
<td>&gt;64 &gt;64 &gt;64 &gt;64 64 4.8</td>
</tr>
<tr>
<td>42</td>
<td>21</td>
<td>&gt;64 16 32 8 &gt;64 &gt;64 &gt;42</td>
</tr>
<tr>
<td>43</td>
<td>31.6</td>
<td>16 8 &gt;64 4 32 8 22</td>
</tr>
<tr>
<td>44</td>
<td>23.9</td>
<td>&gt;64 8 &gt;64 4 64 8 7.1</td>
</tr>
<tr>
<td>47</td>
<td>10</td>
<td>32 &gt;64 32 32 32 9.2</td>
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</tbody>
</table>


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<tr>
<th></th>
<th>NDH-2</th>
<th>Ec a</th>
<th>Ec b</th>
<th>Pa c</th>
<th>Pa d</th>
<th>Ab e</th>
<th>Sa f</th>
<th>Mtb g</th>
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<tbody>
<tr>
<td>39</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

* a E. coli (ATCC 25922, wild-type). b E. coli (ΔtolC efflux-defective mutant, isogenic to ATCC 25922). c P. aeruginosa (PAO1, wild-type). d P. aeruginosa (PAO750 efflux-defective mutant, isogenic to PAO1). e A. baumannii (ATCC 19606, wild-type). f S. aureus (ATCC 29213, wild-type). g M. tuberculosis strain H37Rv (ATCC 25618).

Table 21. Calculated LogP and results from cytotoxicity assays HepG2 and MRC-5.

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>HepG2 a</th>
<th>MRC-5</th>
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<tbody>
<tr>
<td>Compound</td>
<td>clogP b</td>
<td>TD99 (µM)</td>
</tr>
<tr>
<td>40a</td>
<td>3.6</td>
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<tr>
<td>42</td>
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</tr>
<tr>
<td>43</td>
<td>4.4</td>
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</tr>
<tr>
<td>47</td>
<td>2.3</td>
<td>50</td>
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</tbody>
</table>

* a Rifampicin (TD99 ≥100 µM; TD50 = 75 µM) and bedaquiline (TD99 ≥100 µM; TD50 = 50 µM) were used as reference compounds. b clogP values were calculated by Instant JChem (Instant JChem 15.9.14.0, ChemAxon, http://www.chemaxon.com/).

Summary (Paper V)

In summary, twenty-one novel quinolinyl pyrimidines were synthesised and biologically evaluated for inhibition of oxidoreductase NDH-2, a panel of mainly Gram-negative bacteria and Mtb. We found that there was a correlation between NDH-2 pIC50 and pMIC values of the drug-hypersensitive mutated strain of E. coli, A. baumannii, S. aureus and Mtb. In addition, the cytotoxic profile of the tested compounds was evaluated. The cytotoxic results provided evidence to suggest that the pyrimidine moiety is implicated in cytotoxicity and that alteration of the physiochemical properties of the ring resulted in reduced cytotoxicity.

All in all, the results presented in this study support the claim that NDH-2 is a promising target for antibacterial agents. However, the levels of inhibition compared to the levels of cytotoxicity of the evaluated compounds, suggest that the structure of the inhibitor should be altered, e.g. by replacing the pyrimidine ring with other suitable linkers. Moreover, the pursuit to find new
antibacterial agents is a challenging task; facing problems such as cellular up-
take, efflux and resistance. Still, the increasing resistance to most available
antibiotic treatments makes the search for new, druggable antibiotic targets
one of the most important areas in medicinal chemistry.
Concluding Remarks

The work described in Papers I-IV has been devoted to the development of non-gaseous one-pot, multicomponent carbonylation/cyclisation and carbonylation/amination reactions (Scheme 22).

- In Paper I the development of a carbonylative Sonogashira cross-coupling/cyclisation reaction of ortho-iodoanilines and terminal alkynes to yield 4-quinolones is described. The method uses in situ generated CO from the reaction of solid Mo(CO)₆ and DBU or MeCN. Two protocols were developed to accommodate for both expedient and mild synthetic demands. Overall, the methods provided 4-quinolones from a wide variety of ortho-iodoanilines and alkynes in moderate to high yields.

- In Paper II the nucleophilic coupling partner was changed from alkynes to cyanamide, offering a convenient, carbonylative synthesis of 2-Aminoquinazolinones. For this method a two-chamber system was used to enable the ex situ generation of CO from Mo(CO)₆ and DBU. A wide variety of 2-aminoquinazolinones were prepared in moderate to excellent yields via a domino carbonylation/cyclisation sequence. The synthetic strategy

\[ \begin{align*}
\text{Paper I} & : 4(1H)-\text{Quinolone} \\
\text{Paper II} & : 2-\text{Aminoquinazolin-4(1H)-one} \\
\text{Paper III} & : 4\text{-Beno[\text{e}][1,3]\text{oxazin-4-one}}
\end{align*} \]
enables the introduction of a free exocyclic amine and with optional substitution pattern of the N1-position, providing a highly attractive method for the synthesis of 2-aminooquazolinones.

- In **Paper III** the bifunctional reagent was changed from *ortho*-idoanilines to *ortho*-substituted halophenols. The reaction proceeds via a domino carbonylation/cyclisation to provide a convenient synthesis of benzoxazinones. In addition, the scope of the reaction was extended to include *ortho*-bromophenols. The developed method was also used to evaluate the compatibility and performance of a number of CO sources.

- In **Paper IV** a method to prepare *N*-acylguanidines from an intermolecular carbonylation/amination sequence was devised, inspired by the intramolecular amination in **Paper II**. The developed four-component method started with a Pd(0)-catalysed carbonylative coupling of aryl iodides or bromides and cyanamide to obtain an *N*-cyanobenzamide intermediate. Intermolecular amination with a broad selection of amines was used to produce over 50 compounds with considerable product diversity in overall high yields, making this a highly attractive method.

- In **Paper V**, twenty-one compounds based on a quinolinyl pyrimidine scaffold were synthesised. The prepared compounds were biologically evaluated in terms of inhibition of oxidoreductase NDH-2 and antibacterial activity on Gram-negative bacteria, *S. aureus* and *Mtb*. The biological evaluation revealed that some of the quinolinyl pyrimidines exerted inhibitory activity on the NDH-2 enzyme and possessed antibacterial properties. It could not with certainty be concluded that the antibacterial activity stems from the inhibition of NDH-2, for example by raising mutants and performing whole genome sequencing. Although, the two most potent NDH-2 inhibitors also showed activity on the bacterial strains for which there was a correlation between pIC$_{50}$ and pMIC.
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References


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