Repurposing Old Drugs: Substituted Benzodiazepines as New Antibacterial Agents

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Repurposing Old Drugs: Substituted Benzodiazepines as New Antibacterial Agents

MSc Pharmacy Project Report

By

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Supervisor: Dr Jonathan Sellars

Acknowledgements

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Content

Abstract

This project aims to develop a greater understanding of how old drugs can be repurposed into new and exciting therapeutic areas. In particular, developing an understanding of the antibacterial mode of action exerted by modified benzodiazepines possessing unique fluoropyridines will be explored.

The work described in this thesis was mainly focused on the synthesis of a library of substituted benzodiazepines containing the fluoropyridine moiety and the biological investigation of these compounds. We present an effective synthetic route to the core benzodiazepine framework which was later attached to fluoropyridines. The target benzodiazepines substituted by Cl, Br and F on the aryl rings were synthesized and isolated successfully. Except the N1 substituted benzodiazepine, other target compounds all show great activity to a host of bacteria.

Abbreviations

The following abbreviations appear in this thesis:

Å	Angstrom(s)
AAC	Aminoglycoside acetyltransferase
AcOH	Acetic acid
ANT	Aminoglycoside nucleotidyltransferase
APH	Aminoglycoside phosphotransferase
aq	Aqueous
Ar	Aryl
ATP	Adenosine triphosphate
ATR	Attenuated total reflection
Bpoc	2-(4-Biphenyl)isopropyloxycarbonyl
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
°C	Degree celsius
Cbz	Carboxybenzyl
cGMP	Cyclic guanosine monophosphate
cm^{-1}	Wavenumber(s)
COSY	Correlation spectroscopy
4CR	Four-component reaction
δ	Chemical shift in parts per million
D-Ala-D-Ala	D-alanyl-D-alanine
D-Ala-D-Lac	D-alanyl-D-lactate
D-Ala-D-Ser	D-alanyl-D-serine
dba	Dibenzylideneacetone
DCM	Dichloromethane
DHPS	Dihydropteroate synthetase
DICI	Diisopropylcarbodiimide
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
eq	Equivalents
ES^+	Positive charge electrospray mass spectrometry
Et_3N	Triethylamine
Fmoc	9-Fluorenyl-methoxycarbonyl

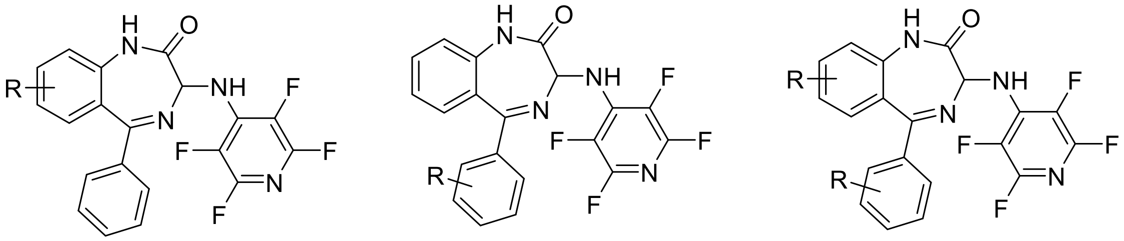
g	Gram(s)
GAGB	Gamma aminobutyric acid
GTP	Guanosine triphosphate
h	Hour(s)
HbA$_{1c}$	Hemoglobin A$_{1c}$
HMBC	Heteronuclear multiple bond correlation
HMP	(4-(Hydroxymethyl)phenoxy)acetic acid
HOBt	*N*-Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence spectroscopy
Hz	Hertz
i-AmONO	Isoamyl nitrite
IR	Infra-red spectroscopy
J	Coupling constant (in NMR spectroscopy)
LDL-C	Low-density lipoprotein cholesterol
Leu	Leucine
μ	Micro
M	Molar
Max	Maximum
Me	Methyl
Me$_2$S	Dimethyl sulfide
MFS	Facilitator superfamily
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute(s)
mL	Millilitre
mmol	Millimole
m.p.	Melting point
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
m/z	Mass to charge ratio
NADPH	Nicotinamide adenine dinucleotide phosphate reductase
NANC	Non-adrenergic, non-cholinergic
nm	Nanometre
NMP	*N*-methyl-2-pyrrolidinone
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOESY	Nuclear overhauser effect spectroscopy
p	Para
PABA	Para-aminobenzoic acid
P. aeruginosa	*Pseudomonas aeruginosa*

PBP	Penicillin-binding protein
PDE 5	Phosphodiesterase type 5
PGT	Peptidoglycan glycosyltransferase
Ph	Phenyl
PhD	Doctor of Philosophy
Phe	Phenylalanine
ppm	Parts per million
psi	Pounds per square inch
R_f	Retention factor in chromatography
RNA	Ribonucleic acid
RNAP	RNA polymerase
rt	Room temperature
S. aureus	*Staphylococcus aureus*
Spp.	*Species*
S. pyogenes	*Streptococcus pyogenes*
^{t}Bu	Tertiary butyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Transmembrane fragments
tRNA	Transfer ribonucleic acid
Trp	Tryptophan
Tyr	Tyrosine
UV	Ultra violet

1.0 Introduction

1.1 General Introduction

Drug repurposing is a relatively new approach to the drug development process that provides the quickest possible transition from bench to bedside. The purpose of this project is to understand how approved drugs can be repurposed safely and effectively into other therapeutic areas. Specifically, this work studies the antibacterial mode of action exerted by benzodiazepine derivatives containing the unique fluoropyridine moieties. This will be achieved by structure-activity-relationship studies around the core benzodiazepine framework (Figure 1). The report is composed of five chapters. The remainder of this chapter will provide a brief introduction to antibiotics, their mode of action and background to the project. Chapter two contains the results of the study and the associated discussion with Chapter three providing the biological investigation of the target compounds. The conclusion and further work of the research will be given in the Chapter four and the Chapter five will be experimental details.



1 **2** **3**

Figure 1: The structures of benzodiazepine derivatives.

1.2 Brief History of Antibiotics

Antibiotics have enjoyed high reputation since they were introduced into the area of medicine in the 1940s. Their functions have extended from inhibiting or terminating the growth of bacteria to the prophylaxis of infectious diseases in patients and other living animals.[1] The noun “antibiosis” was created by Vuillemin in 1889 to describe the antagonism effect among the living source world. But the term “antibiotic” could not be found in the literature during the several decades afterwards until it was defined and used by S. A. Waksman in 1942, referring specifically the antagonistic substances produced by micro-organisms.[2] In the following years, the meaning of antibiotics had differed from chemotherapeutic drugs. Antibiotics were drugs taken from natural plants such as fungi and endophytic bacteria while chemotherapeutic drugs were artificially synthesised. However, the difference faded away when chemists successfully synthesized news drugs that acted as antibiotics.[3] Nowadays, a large number of antibiotics made in the laboratory are available, known as synthetic antibiotics and semisynthetic antibiotics.[4]

The investigation of intermicrobic antagonism is nearly as old as the science of microbiology.[5] To set a well-known example, the father of microbiology L. Pasteur and his colleague Joubert described in 1877 that anthrax bacilli would die when the sample was contaminated by unspecified bacteria in the air.[6,7] Therapeutic applications of the observation rapidly followed.

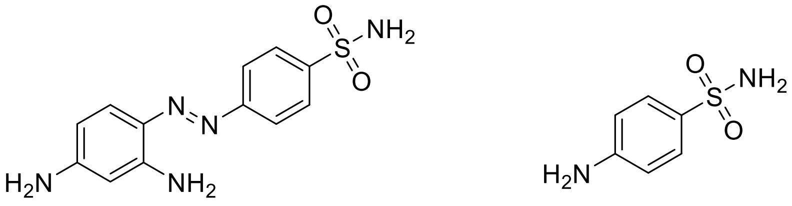
After this, early application of chemotherapeutic agents, like mercury and the arsenical derivatives, were employed. This was based on a poison principle, meaning the content of their poison was controlled by pharmacists to make sure that they were not too toxic, but could kill the bacterial infection.[8] R. Dubos from the Rockefeller Institute was the first researcher to have a different opinion of how antibacterial agents should work. He was trying to find a purifying substance from the environment of the edaphic bacterium *Stretococcus pneumoniae* because he believed that there must be such a component antagonistic to the spread of *Stretococcus pneumoniae* to keep the ecological balance in the soil.[4] His finding, tyrothricin, isolated from the soil bacterium *Bacillus brevis* showed antimicrobial activity against the growth of *Stretococcus pneumoniae,* but unfortunately this product was not pure. The active ingredient of the impurities was later proved to be gramicidin by his colleague R. Hotchkiss and other scientists. Although gramicidin is not as toxic as mercury and arsenic, it is still not suitable to be used inside of human bodies.[2,5]

Whilst an effective approach, future research focused on the discovery of new antibacterials through application of other chemical entities.

1.2.1 Sulphonamides

When Dubos was looking for the purifying substances from the natural products, another research line of antibacterial agents developed compounds via chemical modification was also in progress by other scientists.

In 1932, prontosil **4** was first prepared by Klarer and Meitzsch as a dye in Bayer laboratories. Three years later, Domagk noticed that prontosil molecules were able to slow down the extension of staphylococcal infections in human.[5] Prontosil **4** was discovered to be a pro-drug as the active agent; sulphonamides **5** were the active components and human friendly agents (Figure 2).[9]



Prontosil **4**

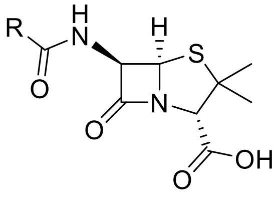
Sulphanilamide **5**

Figure 2: The structures of prontosil **4** and its breakdown product **5**.

This successful discovery allowed scientists to think over the selective toxicity of antibiotics. The synthesis of the first sulphonamide drug, prontosil **4**, was deemed to be the beginning of the antibiotics era. The boom of sulphonamides immediately followed — around 5000 developed sulfa drugs appeared in the 1930s and 1940s.[10] The application of sulphonamides extended rapidly from treating staphylococcal infections to pneumonia, meningitis, etc. Especially, sulfa drugs made great contributions to the treatment of puerperal pyrexia.[11] Owing to the efforts of Colebrook and Kenny on developing sulfa drugs to prevent the puerperal fever which dropped the incidence of puerperal fever per 100,000 population from 20 to around 10, the birth death rate in England and Wales declined sharply between 1935 and 1950.[11]

1.2.2 Penicillin

Another early antibiotic is penicillin **6**, a more remarkable substance than sulphonamides, which was first prepared from *Penicillium notatum* by A. Fleming in 1929.[10] This discovery was initially not valued by people, until 1940 when Fleming's trials on penicillin **6** caught the attention of Professor Florey in the University of Oxford. With the help of E. Chain and other chemists, Florey successfully extracted and purified the active component ingredient on a mass scale (Figure 3).[2,5]



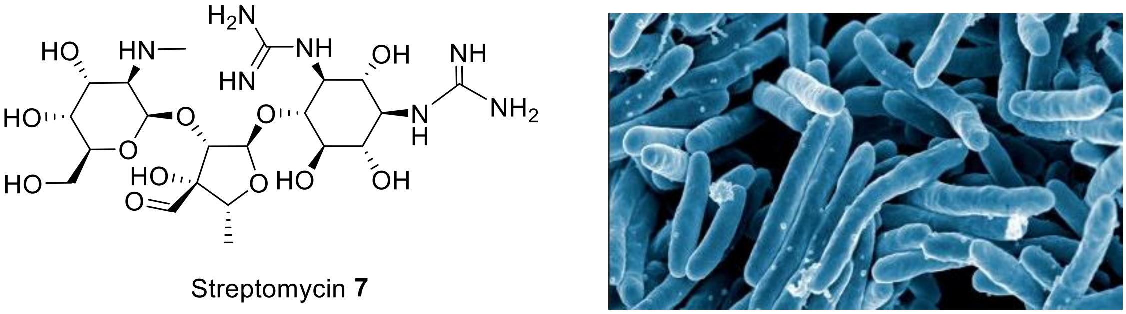
Penicillin **6**

Figure 3: The core structure of penicillin **6**, where "R" is the variable group.

In the early 1940s, resistance to sulphonamides began to occur. The purified penicillin **6** was an important remedy for the bacterial infections which could not be treated by sulfa drugs. The commercial production of penicillin **6** was achieved by Merck & Co in America.[12] During World War I, without effective treatments, soldiers died due to not only the combat wounds but also blood poisoning. The large-scale production of penicillin **6** during the Second World War saved millions of people's lives with the mortality from pneumonia in the American Army decreasing from approximate 18% in World War I to less than 1% in World War II.[10]

1.2.3 Streptomycin

Different from the early discoveries, streptomycin **7** was not discovered by accident. Focusing on a large group of bacteria known as actinomycetes, S. A. Waksman's team carried out a rigorous and systematic investigation in which they looked at 10,000 different soil microbes.[13] In 1943, the PhD student A. Schatz in his lab found two strains of *Streptomyces* bacteria that produced a substance which could stop other bacteria growing.[14] This new antibiotic was named streptomycin **7** and it was proved to be the first antibiotic that could kill *Mycobacterium tuberculosis* (Figure 4).[15]



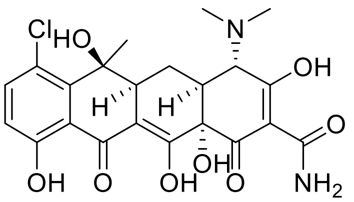
Streptomycin **7**

Figure 4: The structure of streptomycin **7** (left) and the scanning electron micrograph of *Mycobacterium tuberculosis* bacteria (right).

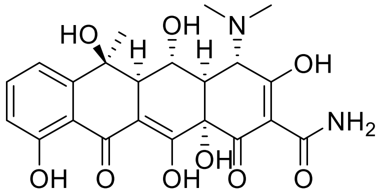
Furthermore, not only was streptomycin **7** found to be effective and safe in treating tuberculosis, eventually it was found to be active against 70 different types of bacteria which do not respond to penicillin **6** including infections of the abdomen, urinary tract, pelvis and meninges.[16] Streptomycin **7** was an antibiotic that inhibited both gram-positive and gram-negative bacteria, and was therefore a very useful broad-spectrum antibiotic.[17]

1.2.4 Tetracyclines

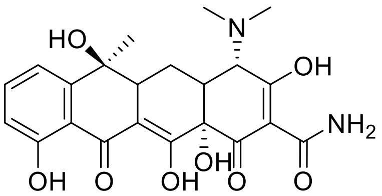
S. Waksman’s work and methods on chemical diversity derived from soil actinomycetes was great evidence that the microbial world could produce a wealth of natural products and antibiotic compounds capable of fighting microbial diseases. Hence, many pharmaceutical companies expanded within the United States to commit to antibiotic research and development.[18] Aureomycin (chlortetracycline) **8** produced by Cyanamid was extracted from the gold-coloured Streptomyces strain. It was first published in 1948 in the *Annals of the New York Academy of Sciences*,[19] described as a safe, effective and broad-spectrum antibacterial agent. With similar colour to aureomycin **8**, terramycin (oxytetracycline) **9** was later obtained by Alexander Finlay and colleagues at Charles Pfizer Co., Inc. Their structures were published in 1954 by the Pfizer-Woodward group (Figure 5).[18]



Aureomycin
(chlortetracycline)
8



Terramycin
(oxytetracycline)
9

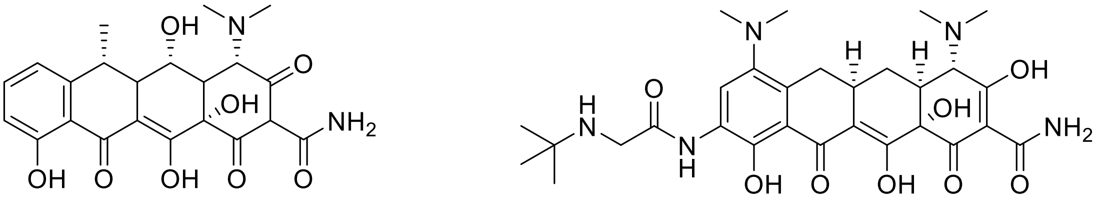


Teracyn
(tetracycline)
10

Figure 5: The structures of first generation tetracyclines: aureomycin **8**, terramycin **9** and teracyn **10**.

The core scaffold for this new family of antibiotics became descriptively known as the tetracyclines. Pfizer's Lloyd Conover believed that chlorine in aureomycin **8** was not responsible for activity. Using palladium metal and hydrogen as catalysts, aureomycin was hydrogenated to synthesize teracyn **10** possessing higher potency, better solubility profile and favourable pharmacological activity.[17] By the mid-1950s, these three tetracyclines **8**, **9** and **10** were used clinically and saved tens of thousands of lives.[20]

Second generation semisynthetic analogs and more recently third generation compounds show the continued evolution of the tetracycline scaffold toward derivatives with increased potency as well as efficacy against tetracycline-resistant bacteria, with improved pharmacokinetic and chemical properties (Figure 6).[20]



Doxycycline
(Second Generation)
11

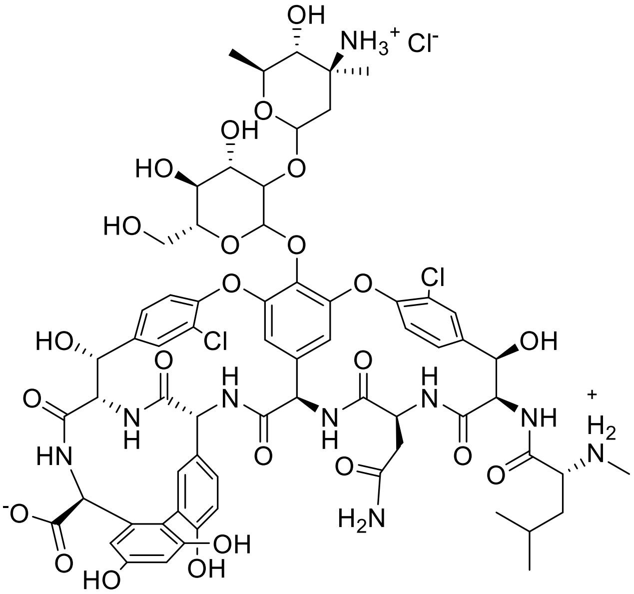
Tigecycline
(Third Generation)
12

Figure 6: Selected members of second generation and third generation tetracyclines.

Their biological activity against a wide spectrum of microbial pathogens and their uses in mammalian models of inflammation, neurodegeneration, and other biological systems indicate that the tetracyclines will continue to be successful therapeutics in infectious diseases and as potential therapeutics against inflammation-based mammalian cell diseases.[21,22]

1.2.5 Vancomycin

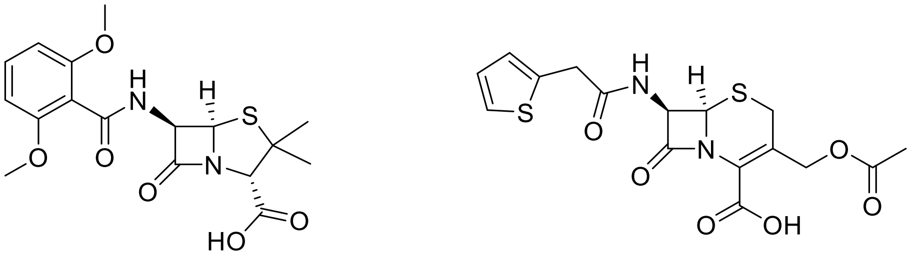
In the early 1950s, the available antibiotics were failing to control staphylococcus infections in many hospitalized patients.[23] A large-scale screening program was performed by Eli Lilly and Company in order to develop effective new agents against staphylococci. A soil sample found in the jungles of Borneo by a missionary friend of the organic chemist at Lilly contained a microorganism, *Streptomyces orientalis* which produced an active substance which was later called vancomycin **13** (Figure 7).[24]



Vancomycin **13**

Figure 7: The structure of vancomycin **13**.

Vancomycin **13** was shown to be highly bactericidal against all staphylococcal strains and was relatively safe when administered to patients. However, methicillin **14** and cephalothin **15** were already commercially available to treat severe staphylococcal infections, therefore interest in vancomycin **13** diminished after its introduction (Figure 8).[25]



Methicillin **14**

Cephalothin **15**

Figure 8: The structures of methicillin **14** and cephalothin **15.**

Nevertheless, methicillin **14** was used widely and an increasing incidence of methicillin resistant strains of *Staph. aureus* and *Staph. epidermidis* was reported in the United States and Europe.[26] Consequently, use of either methicillin **14** or cephalothin **15** was not recommended if a resistant strain was found in patients but vancomycin **13** was used as an alternative.[25,26] This has seen a corresponding increase in the use of vancomycin **13** since other antistaphylococcal antibiotics could not decrease the incidence of staphylococcal infections.[26] Nowadays, vancomycin **13** is also used for prophylaxis in patients with reduced renal function undergoing dialysis and to eradicate staphylococci and clostridia which can cause severe antibiotic-induced enterocolitis. Combined with other antimicrobial agents, oral vancomycin **13** is able to suppress the intestinal flora of the patients with cancer thereby prevent infection from their own commensal organisms.[24]

1.2.6 Rifampicin and Related Rifamycins

Rifampicin **21** has a high level of activity against mycobacteria, gram-positive and gram-negative cocci, many gram-negative bacilli, most anaerobes, and cellular prokaryotic parasites (e.g. Chlamydia and Brucella).[27] It was first developed in the Dow-Lepetit Research Laboratories in Milan, Italy as part of an extensive program of chemical modification of the rifamycins, the natural metabolites of *Nocardia mediterranei*.[28] Individual components of the rifamycin complex were designated as rifamycin A, B **16**, C, D, and E according to their mobility on paper chromatography. But most of them were unstable; only rifamycin B **16** could be isolated in pure crystalline form. Rifamycin B **16** was the least active component of the rifamycin complex but showed an extremely low level of toxicity and a moderate level of therapeutic activity in infections in animals. It was found that rifamycin B **16** could reversibly convert to rifamycin O **17** which was hydrolyzed to rifamycin S **18** in the process of losing one molecule of glycolic acid. By mild reduction of rifamycin S **18**, rifamycin SV **19** was obtained (Figure 9).[28,29]

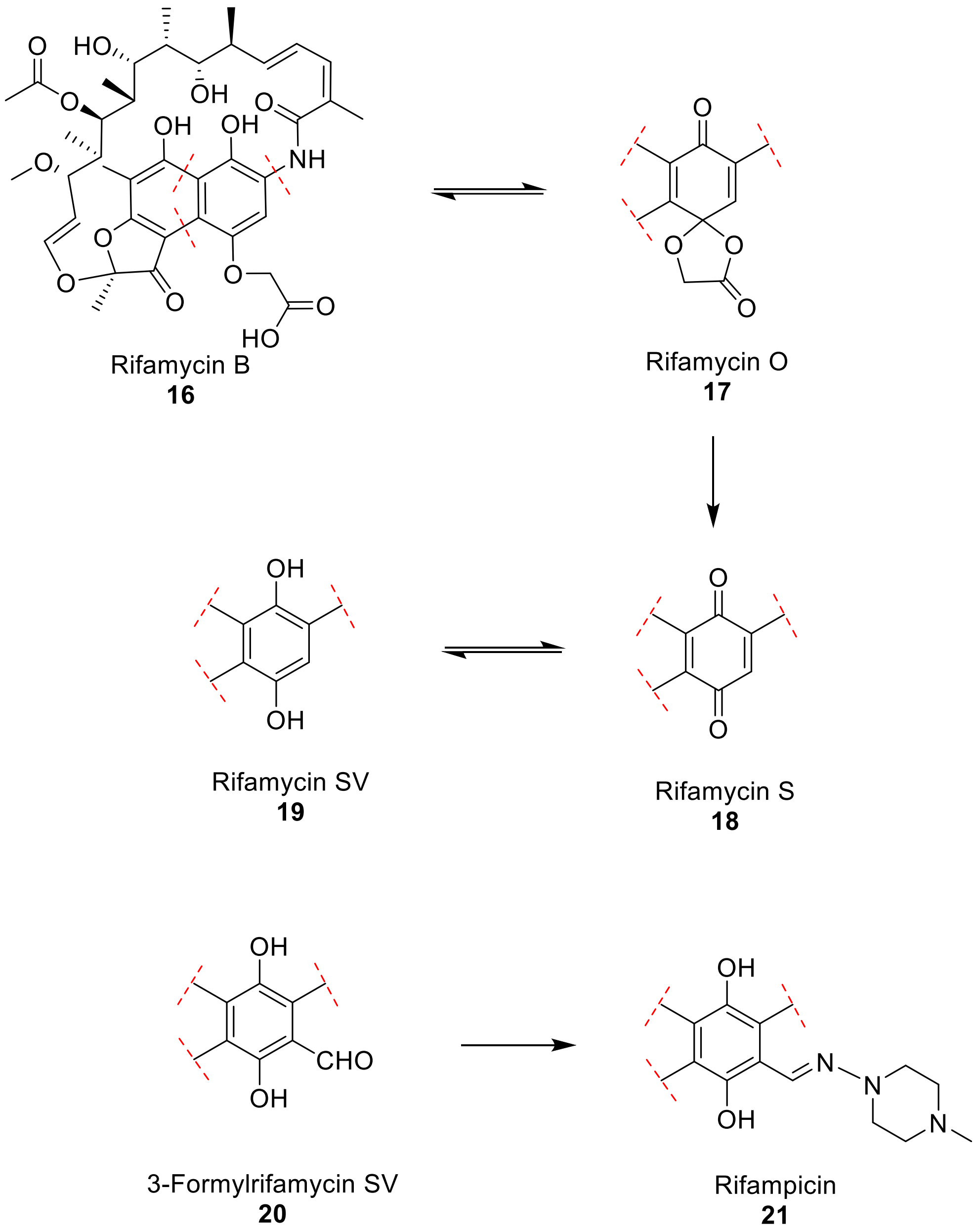


Figure 9: Structural formulas for rifampicin and some earlier rifamycins.

Different from rifamycin B **16**, rifamycin SV **19** is extremely active against gram-positive bacteria including *Mycobacterium tuberculosis*, and moderately active against some gram-negative bacteria.[30,31] Thus, it was introduced in some countries for the parenteral and topical treatment of infections due to gram-positive bacteria and infections of the biliary tract.

In the effort to obtain a new rifamycin with improved chemotherapeutic properties, such as better oral absorption, more prolonged antibacterial levels in blood, greater activity against mycobacterial infections and infections resulting from gram-negative bacteria, several hundred semisynthetic derivatives had been prepared. In various experimental results, the hydrazone of 3-formylrifamycin SV **20** with *N*-amino-*N'*-methylpiperazine designated rifampicin **21**, also known as rifampin, that was the most active for oral administration and was also the least toxic.[27] After successful clinical trials, rifampicin **21** was introduced into therapeutic use in 1968. In the intervening years, a large number of clinical and biologic studies had confirmed the important role of rifampicin **21** in therapy for tuberculosis and other selected infectious diseases.[32,33]

1.3 Mechanisms of Antibiotic Action

Although all antibiotics are able to inhibit or terminate the growth of bacterial infections, their operating principles cannot be described with a uniform pattern. Generally, antibiotics are classified into two categories, bactericidal and bacteriostatic. Bactericidal antibiotics kill the organism while bacteriostatic antibiotics only slow down the bacterial growth and reproduction.[34,35] The mechanism of antibiotic action can be further subdivided into three main categories based on their microbial cell targets: cell wall synthesis, protein synthesis and nucleic acid synthesis (Figure 10).[36,37]

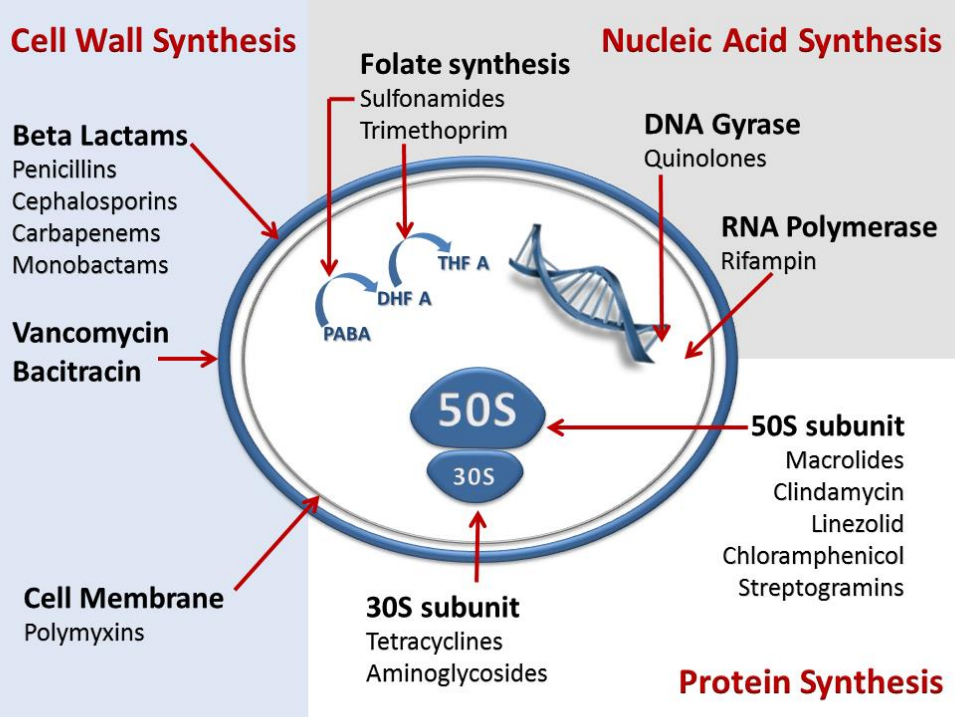


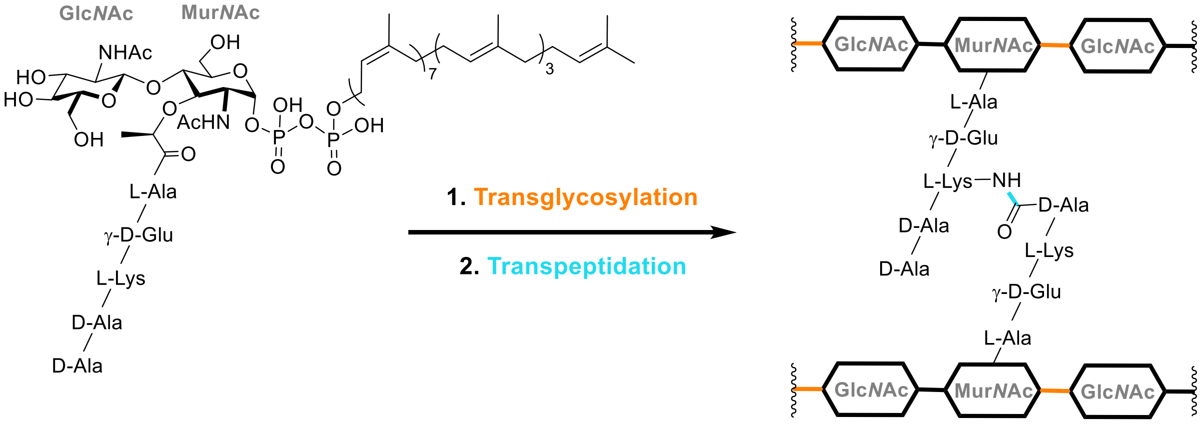
Figure 10: Classification of antibiotics based on their mechanism of action.

The following section will highlight how the antibiotics described in section 1.2 function to either kill the bacteria or act to slow the growth of a bacterial infection.

1.3.1 Cell Wall Synthesis

The bacterial cell wall is the principal stress-bearing and shape-maintaining element in bacteria, and its integrity is of critical importance to cell viability.[38] In both gram-positive and gram-negative bacteria, the scaffold of the cell wall consists of the cross-linked polymer pepticdoglycan which is the critical site attacked by anti-cell-wall agents. The peptidoglycan layer is essential for the survival of bacteria in hypotonic environments. Any loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.[38,39]

Peptidoglycan synthesis occurs in three stages. The final stage of the extracellular biosynthesis in two steps: in the transglycosylation step, the disaccharide phospholipid lipid II is polymerized to form polysaccharide strands, and in the subsequent transpeptidation step, these strands are cross-linked (Figure 11).[40]

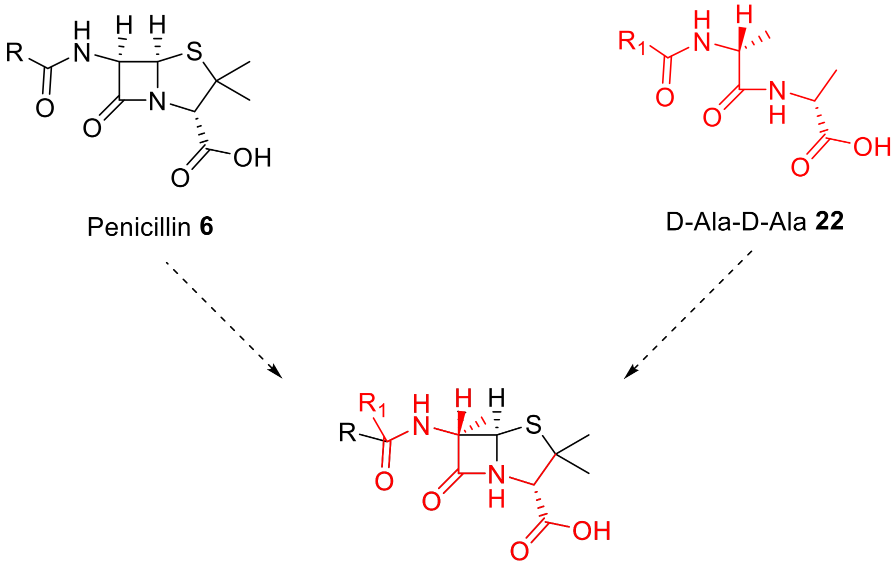


lipid II

Peptidoglycan

Figure 11. The final stage of the biosynthesis of peptidoglycan.

Penicillin **6**， a β-lactam antibiotic, kills susceptible bacteria by specifically inhibiting the transpeptidase, a penicillin-binding protein (PBP), that catalyzes the cross-linking of peptidoglycan.[41] Transpeptidase normally binds to the D-Ala-D-Ala **22** at the end of peptidoglycan precursors to crosslink the peptidoglycan. β-Lactams such as penicillin **6** mimic D-Ala-D-Ala **22**, occupying the PBP active site and inhibiting crosslinking of peptidoglycan peptide bridges cells (Figure 12). The cell wall is then weakened which allows autolytic enzymes that degrade the peptidoglycan network to dominate, leading to lysis of the cells.[41,42]



Penicillin **6**

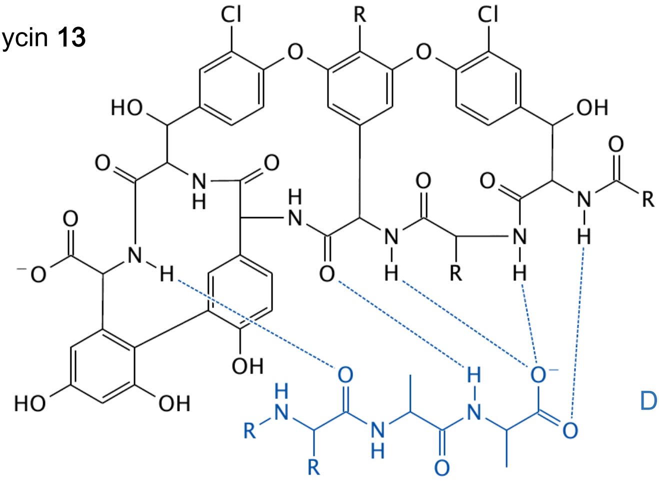
D-Ala-D-Ala **22**

Superimposition of **6** and **22**

Figure 12: β-Lactams such as penicillin **6** (black) have similar structures to D-Ala-D-Ala **22** (red).

In a different process, vancomycin **13** interrupts cell wall synthesis by forming a complex with the C-terminal D-alanine residues of peptidoglycan precursors (Figure 13).[43]

Vancomycin **13**



D-Ala-D-Ala **22**

Figure 13: D-Ala-D-Ala **22** (bacterial cell wall precursor, in blue) binds to vancomycin **13** (black) through hydrogen bonds.

Complex formation at the outer surface of the cytoplasmic membrane prevents the transfer of the precursors from a lipid carrier to the growing peptidoglycan wall by transglycosidases.[43] Biochemical reactions in the cell wall catalyzed by transpeptidases and D,D-carboxypeptidases are also inhibited by vancomycin **13** and other glycopeptide antimicrobials. Because of its large size and complex structure, vancomycin **13** does not penetrate the outer membrane of gram-negative organisms. With resistance to β-lactams increasing in frequency among staphylococci and enterococci, glycopeptides such as vancomycin **13** remain important therapeutic agents.[39,44]

1.3.2 Nucleic Acid Synthesis

Antimicrobial agents interfere with nucleic acid synthesis at several different levels. They can inhibit nucleotide synthesis or interconversion; they can prevent DNA from functioning as a proper template; and they can interfere with the polymerases involved in the replication and transcription of DNA.[36] Rifamycins are a class of antibiotics that inhibit DNA-directed RNA polymerase. Polypeptide chains in RNA polymerase attach to σ factor that confers specificity for the recognition of promoter sites that initiate transcription of the DNA. Rifampicin **21**, a semisynthetic derivative, binds non-covalently but strongly to the β subunit of RNA polymerase and interferes specifically with the initiation process. However, it has no effect once polymerization has begun (Figure 14).[39,44]

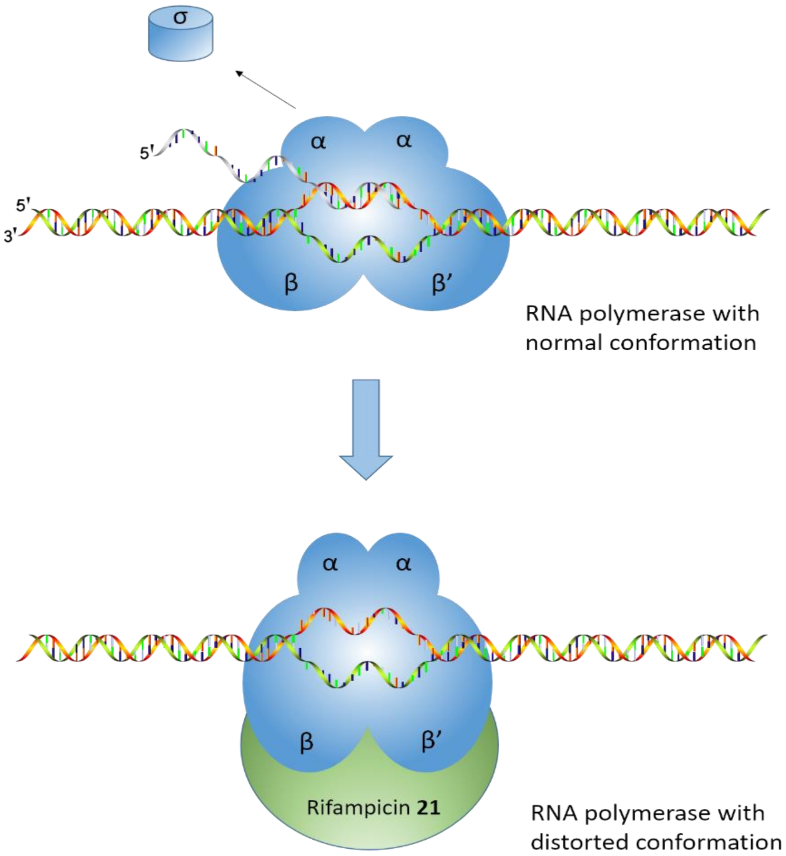


Figure 14: Rifampicin **21** (green) binds to RNA polymerase (blue) and changes its conformation so that it cannot initiate RNA synthesis.

Folate is a major carrier of one-carbon groups needed for methylation reactions and nucleotide synthesis, that is required for growth by both bacterial and mammalian cells.[45] Animals and man are unable to synthesize folate, so it must be supplied in the diet. Folate is accumulated by mammalian cells, but not by bacteria, which must synthesize the compound intracellularly. This difference between the biochemistry of bacterial and mammalian cells is the basis of the selective toxicity of sulphonamides, the first effective chemotherapeutic agents to be used systematically for the treatment of bacterial infections.[45,46]

Sulphonamides **25** are structural analogues of para-aminobenzoic acid (PABA) **24**. They act as alternative substrates for the key biosynthetic enzyme dihydropteroate synthetase (DHPS) which catalyses the formation of the folate intermediate dihydropteroic acid **26** from PABA **24** and dihydroteroate diphosphate (pteridine) **23**.[47,48] Inhibition of dihydropteroate synthetase results from the formation of inactive folate-like analogues from sulphonamides **25** and dihydroteroate diphosphate **23**. This blocks the further biosynthesises of dihydrofolic acid **27** as well as tetrahydrofolate **28** in bacterial cells which is an enzyme cofactor for the synthesis of the pyrimidine nucleic acid bases required for DNA synthesis both for bacteria and humans (Figure 15).

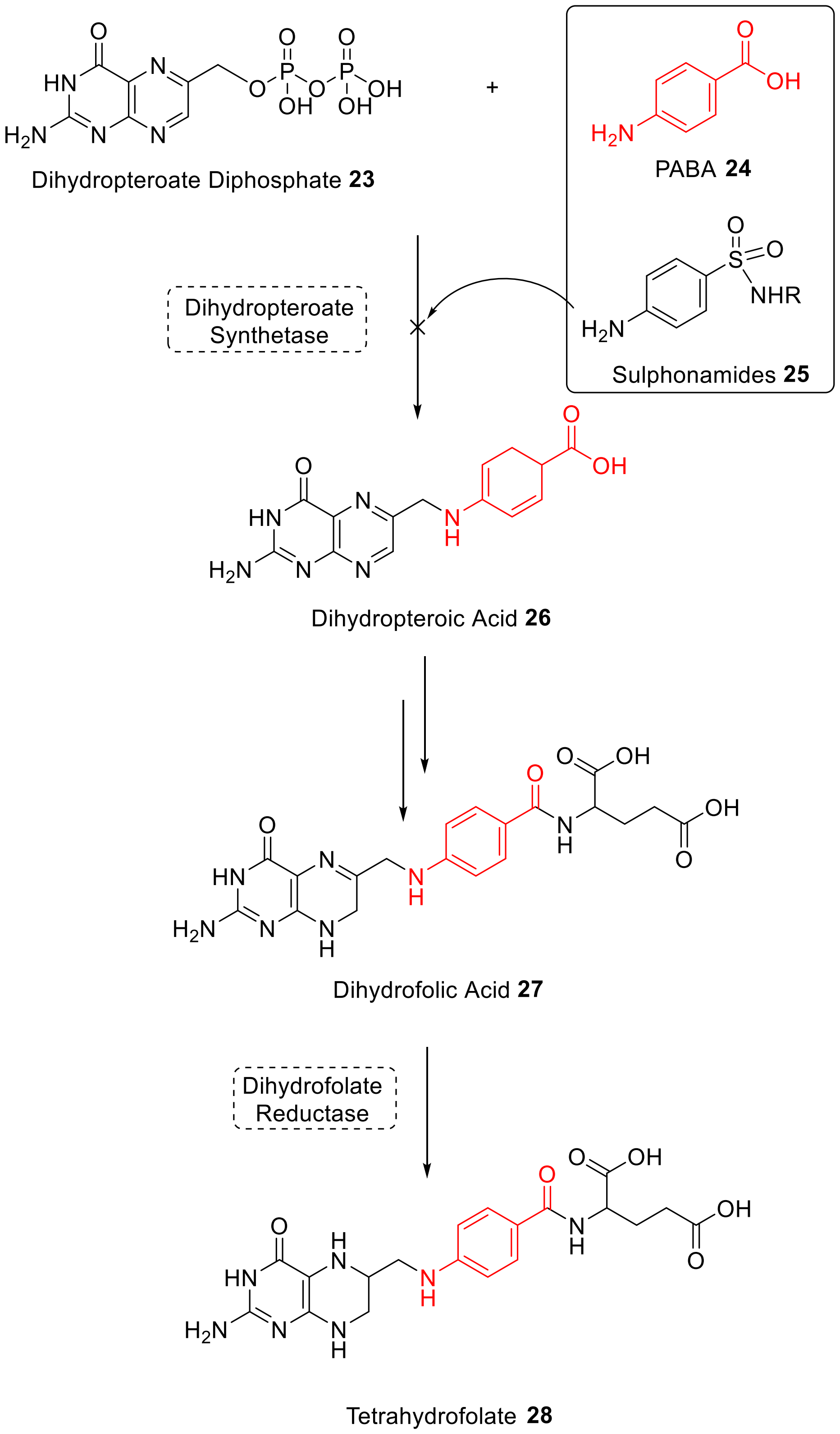


Figure 15: The mechanism of action of the sulphonamides **25**.

1.3.3 Protein Synthesis

Protein synthesis is a complex, multi-step process involving many enzymes as well as conformational alignment. However, the majority of antibiotics that block bacterial protein synthesis interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial ribosome.[49,50] The aminoacyl tRNA synthetases that activate each amino acid required for peptide synthesis are not antibiotic targets. Instead, there are three primary steps in the process that are attacked: (1) the formation of the 30S initiation complex which is made up of mRNA, the 30S ribosomal subunit and formyl-methionyl-transfer RNA, (2) the formation of the 70S ribosome from the 30S initiation complex and the 50S ribosome, and (3) the elongation process of assembling amino acids into a polypeptide.[50-52]

Aminoglycoside antibiotics have an affinity for the 30S ribosome subunit and can bind irreversibly to it. Streptomycin **7** is one of the most commonly used aminoglycosides, disturbing several steps of protein synthesis leading to translational errors and slowdown of translocation.[51] The firm binding of streptomycin **7** to the decoding centre on the 30S subunit of the ribosome induces related structural rearrangements that explain the observed effects on miscoding. This misreading of the codons causes an error in the proofreading process of translation leading to improper protein expression and ultimately resulting in bacterial cell death (Figure 16).[53]

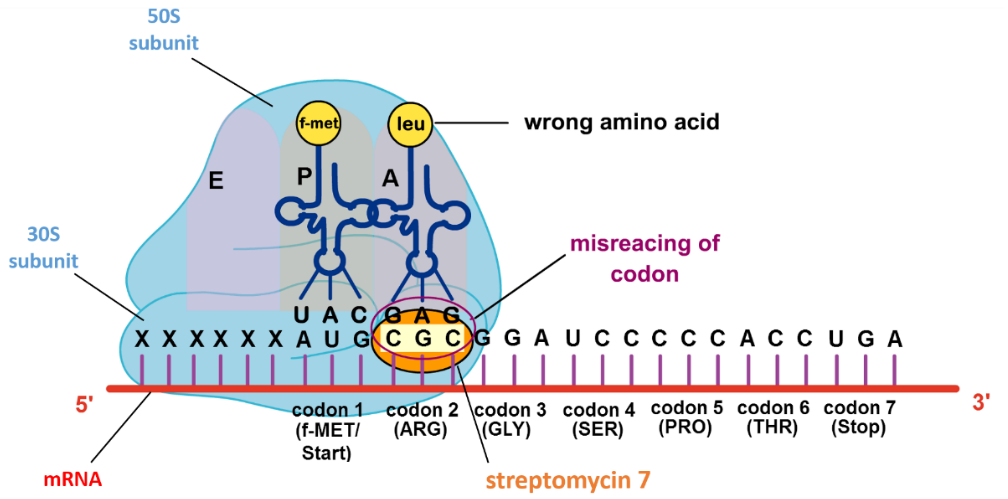


Figure 16: The binding of streptomycin **7** to the 30S subunit leads to the misreading of codon. In this example, the codon CGC codes for the amino acid arginine. However, the near-match tRNA carries the amino acid leucine not arginine by misreading.

As mentioned in the section 1.2.4, the tetracycline class of antibiotics is a large family of broad spectrum bacteriostatic agents that have a limited but very important role in the armamentarium of antibacterial agents.[20,52] Tetracycline enters the cell by either passive diffusion or by energy dependent transport systems. Once inside the cell, they act by binding reversibly to the 30S ribosomal subunit at the A site. During protein biosynthesis, the new tRNA with the amino acid attempts to bind to A-site of the ribosome. However, the A-site is blocked by the tetracycline and the aminoacyl tRNA cannot bind to it. Without the sequential attachment of the tRNA at the A-site, protein biosynthesis is ultimately terminated leading to a bacteriostatic effect (Figure 17).[51,52]

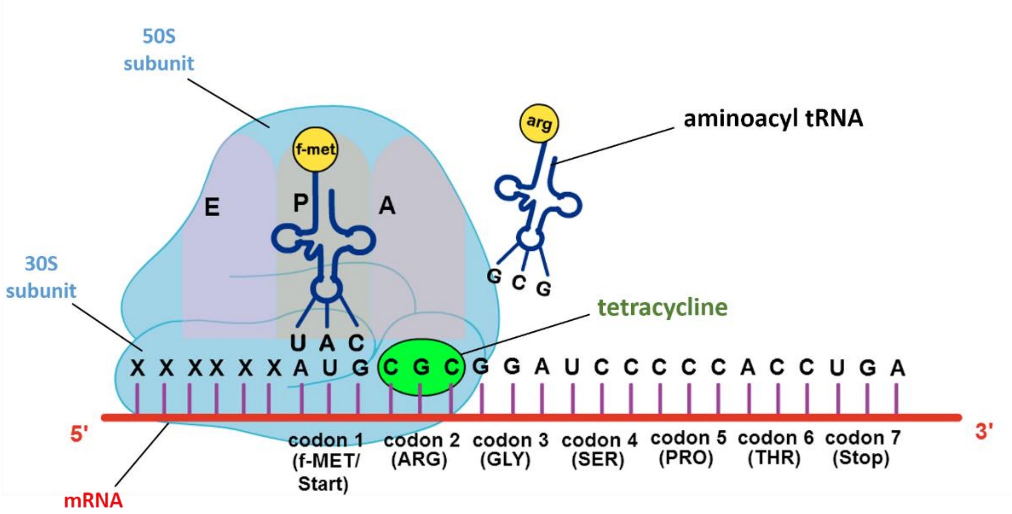


Figure 17: The primary target of tetracycline is the 30S subunit of the ribosome, preventing the binding of aminoacyl tRNA to the acceptor site on mRNA.

1.4 Antibiotic Resistance

Despite the enormous success of antibiotics as chemotherapeutic agents, infectious diseases continue to be a leading cause of mortality worldwide. Bacteria have the capability to regenerate in approximately 30 minutes with a fresh possibility to mutate and adapt to a brand-new environment. [56] The widespread use of prescription antibiotics places a selective pressure on bacteria and favours the survival of the fittest and more adapted strains. As a result, increasing bacteria have developed resistance against antibacterial agents and the antibiotics can no longer bind to the bacterial receptor or enter the bacterial cell to harm bacterial propagation and reproduction.[56,57]

Antimicrobial resistance readily occurring in a variety of bacterial genera due to the following important mechanisms. First, the organism may acquire genes encoding enzymes, such as β-lactamases, that destroy the antibacterial agent before it can have an effect. Second, bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect. Third, bacteria may acquire several genes for a metabolic pathway which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site via downregulation of porin genes.[56,58] Several case studies of antibiotic resistance are displayed in the following section.

1.4.1 Resistance to Sulphonamides

Sulphonamides **25** were the first antimicrobials developed for large-scale introduction into clinical practice. But nowadays, they are infrequently used in part due to widespread resistance.[59] Most bacteria are capable of developing resistance to sulphonamides such as *Escherichia coli*, *Staphylococcus aureus*, *Pneumocystis carinii*, *Campylobacter jejuni*, *Streptococcus pyogenes*, *Mycobacterium leprae* and *Neisseria meningitidis*.[56] As mentioned in the section 1.3.2, the target of sulphonamides **25** and the basis for their selectivity is the enzyme dihydropteroate synthase (DHPS) in the folic acid pathway. Therefore, the bacterial resistance to sulphonamides **25** presumably originates by mutants that producing increased amounts of PABA **24**, lowering affinity for sulphonamides **25** or adopting an alternative pathway in folate metabolism.[59] For instance, the *dhps* (*folP*) gene of two highly sulphonamide-resistant strains of *S. pyogenes* was found to be substantially altered. The resistant mutants having additional compensatory mutations in DHPS allow it to function normally.[56]

Remarkably, sulphonamide resistance in gram-negative enteric bacteria is plasmid-borne and is effected by genes encoding alternative drug-resistance variants of the DHPS enzymes. Two plasmid-borne genes, *sul1* and *sul2*, produce DHPSs distinct between themselves which showing pronounced insensitivity to sulphonamide inhibition in combination with normal substrate saturation. These enzymes seem to be able to distinguish very sharply between PABA **24** and the structurally very similar sulphonamides **25**.[56,59]

1.4.2 Resistance to β-Lactams

There are hundreds of β-lactamases that have been discovered and characterized since the first penicillin-resistant *S. aureus* identified in the mid-1940s expressed a β-lactamase that afforded resistance.[59] Based on their structures, β-lactamases can be divided into four groups: the class A, C, D enzymes with a serine residue at their active sites and the class B proteins which are zinc-dependent metalloenzymes.[60] Some class A proteins are extended-spectrum β-lactamases and function as carbapenemases. The class B metalloenzymes that hydrolyze carbapenems are susceptible to the inhibition by EDTA but not susceptible to the inhibition by clavulanate. The plasmid-borne ampC gene is the prototype class C enzyme which has been found in many species of the *Enterobacteriaceae* and *P. aeruginosa*. Besides, it was also reported to be transferred among *E. coli*, *Klebsiella spp.* and *Salmonella spp.*. Class D enzymes have been found in only a few species such as *P. aeruginosa*, *Acinetobacter spp*. and *Aeromonas spp*..[59,60]

To resist the effects of penicillin **6** and other β-lactam antibiotics, these β-lactamases break the bond in the β-lactam ring to inactivate the molecule before it reaches the cell membrane (Figure 18).[61-63]

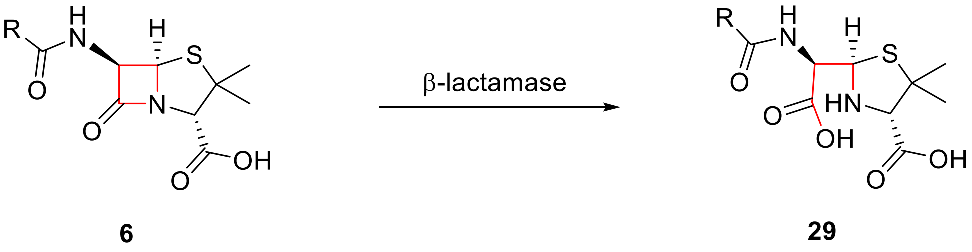


Figure 18: Mode of action of β-lactamase.

Apart from β-lactamases, the β-lactam resistance is mediated by altered PBPs as which are present in almost all the bacteria but their number, size and affinity to β-lactam varies from species to species.[54] For example, the resistance in the staphylococci and streptococci frequently occurs following the acquisition of genes for PBPs that are not sensitive to β-lactam inhibition.[59]

1.4.3 Resistant to Aminoglycosides

Resistance to aminoglycosides is widely reported, but the preponderance of the cases of resistance to these agents has not been as overwhelming as β-lactam resistance. This may be owing to the more frequent use of β-lactams in the clinic and the different mechanisms of dissemination of the resistance determinants.[64] The most common mechanism for clinical resistance to aminoglycosides is their structural modification by specific enzymes expressed in the resistant organisms: aminoglycoside acetyltransferases

(AACs), aminoglycoside nucleotidyltransferases (ANTs) and aminoglycoside phosphotransferases (APHs).[64,65] The acetyltransferases are capable of modifying tobramycin, gentamicin, netilmicin, and amikacin; the nucleotidyltransferase proteins alter the activity of tobramycin; and the phosphotransferases affect amikacin susceptibility.[59] Streptomycin **7,** the first drug to be discovered named aminoglycosides, is set as an example. The resistance to streptomycin **7** due to aminoglycoside phosphotransferases is the result of two classes of enzymes, the APH(3”)s and the APH(6)s, both of which are found in the streptomycin producer *Streptomyces griseus*. Catalyzed by either APH(3”)s or APH(6)s, the phosphoryl transfer is directed to the 3”- or 6-hydroxyl group of the aminocyclitol ring (Figure 19).[65]

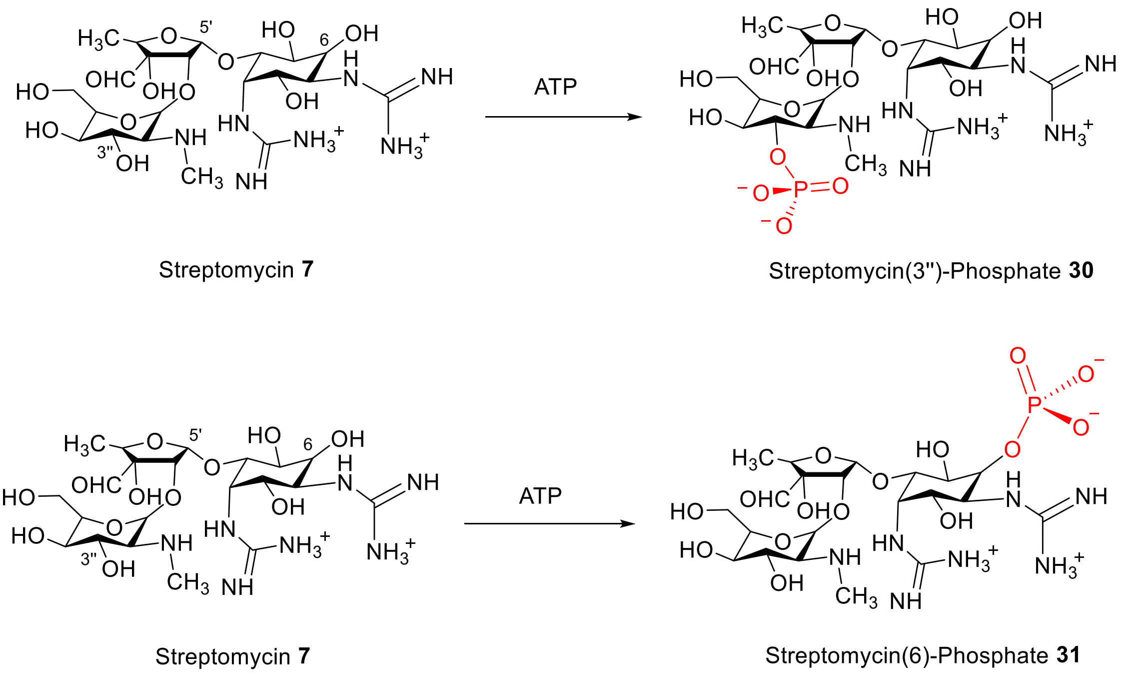


Figure 19: Reactions catalyzed by APH(3’’) and APH(6). The aminocyclitol rings are numbered simply and attached carbohydrates are designated with prime (’) or double prime (”) superscripts. ATP: adenosine triphosphate.

1.4.4 Resistance to Tetracyclines

The most prevalent forms of resistance to the tetracyclines in the clinic are drug efflux and ribosome protection.[59] Tetracycline efflux is achieved by the export protein, Tet, from the major facilitator superfamily (MFS). This export protein is shown to function as an electroneutral antiport system which catalyzes the exchange of the protons (H^+) for the tetracyclines against a concentration gradient.[18,59,66,67] In gram-negative bacteria, the export protein contains 12-TMS (transmembrane fragments) (e.g. TetA-E) whereas in gram-positive bacteria, it displays 14-TMS (e.g. TetK and TetL).[59,66] Most tetracycline efflux pumps confer resistance to tetracycline **10**, but are less effective against second generation doxycycline **11**, and confer little or no resistance to third generation glycylcyclines, such as tigecycline **12** (Figure 20).[67]

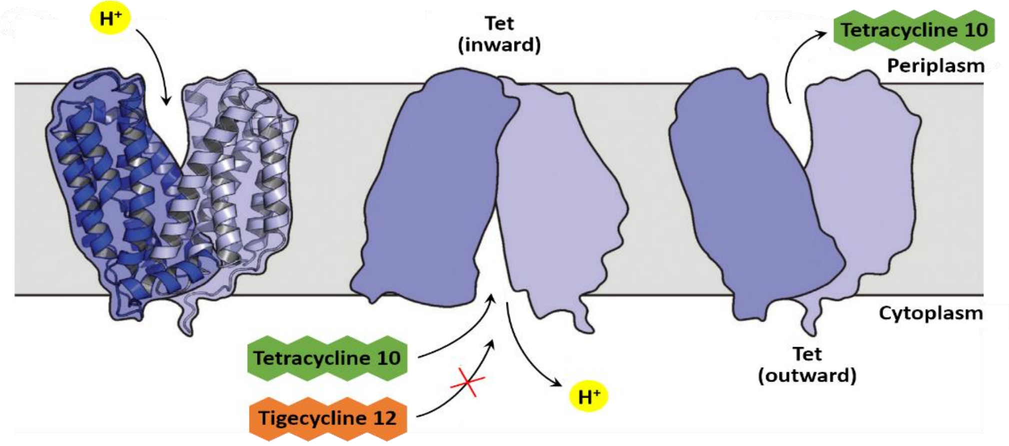
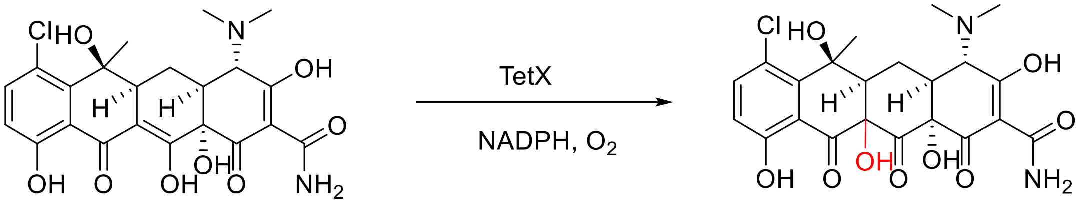


Figure 20: Schematic for mechanism of action of tetracycline efflux pump, Tet, illustrating that efflux of tetracycline **10** (but not tigecycline **12**) is coupled to proton transport.

The second type of tetracycline resistance, a protein-based ribosomal protection mechanism, was first found in streptococci and later in anaerobic bacteria.[18] When tetracyclines bind to ribosomes they normally stop elongation of synthesizing proteins. However, ribosomal protection proteins, such as TetM and TetO, interact with the ribosome causing the tetracycline to dislodge from the ribosome, thus protecting the bacterial cell from tetracyclines inhibitory activity, resulting in cellular growth.[18,20,59,67]

More recently, a less prevalent resistance mechanism has been described, involving a cytoplasmic protein, TetX, that chemically modifies tetracyclines. This is shown to be due to an oxygen-dependent flavin-monooxygenase enzyme encoded by the distinct gene *tetX*.[59,66-68] Curiously, the reaction only takes place in the presence of oxygen and NADPH and does not function in the natural host, an anaerobe Bacteroides (Figure 21).[18,67,68]



8

32

Figure 21: TetX-madiated reaction of chlortetracycline **8** to hydroxy-chlortetracycline **32**. NADPH: nicotinamide adenine dinucleotide phosphate reductase.

1.4.5 Resistance to Vancomycin

Vancomycin **13** has been widely used in the treatment of serious infections with gram-positive bacteria, and particularly it has been invaluable in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections.[69] Compared to most other antibiotic resistance such as resistance to penicillin **6** requiring the activity of only one gene product, β-lactamase enzyme, resistance to vancomycin **13** needs five genes, *vanS*, *R*, *H*, *A*, *X*,[70] and takes a longer time to develop. This may be related to its unusual mode of action that precludes simple point mutations as a resistance mechanism. However, vancomycin-resistant staphylococci and enterococci have emerged and are spreading with unexpected rapidity.[69]

The mechanism of resistance to vancomycin **13** involves the presence of operons that encode enzymes for synthesis of low-affinity precursors, in which the C-terminal D-Ala residue **22** is replaced by D-lactate (D-Lac) **33** or D-serine (D-Ser) **34**, thus modifying the vancomyin-binding target.[71] D-Ala-D-Lac **33** variation results in the replacement of the amide NH in D-Ala-D-Ala **22** with the ester oxygen and the loss of one hydrogen-bonding interaction between vancomycin **13** and the substrate. This action causes a 1,000-fold decrease in affinity. While the change of the C-terminal methyl side chain in D-Ala-D-Ala **22** to the $-CH_2OH$ in D-Ala-D-Ser **34** causes only a 6-fold lower affinity for vancomycin **13**, possibly due to steric hindrance (Figure 22).[72-74]

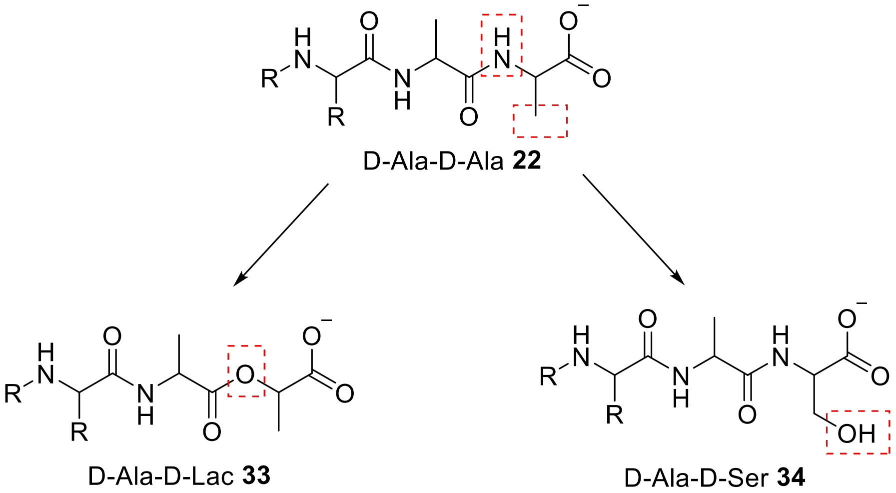
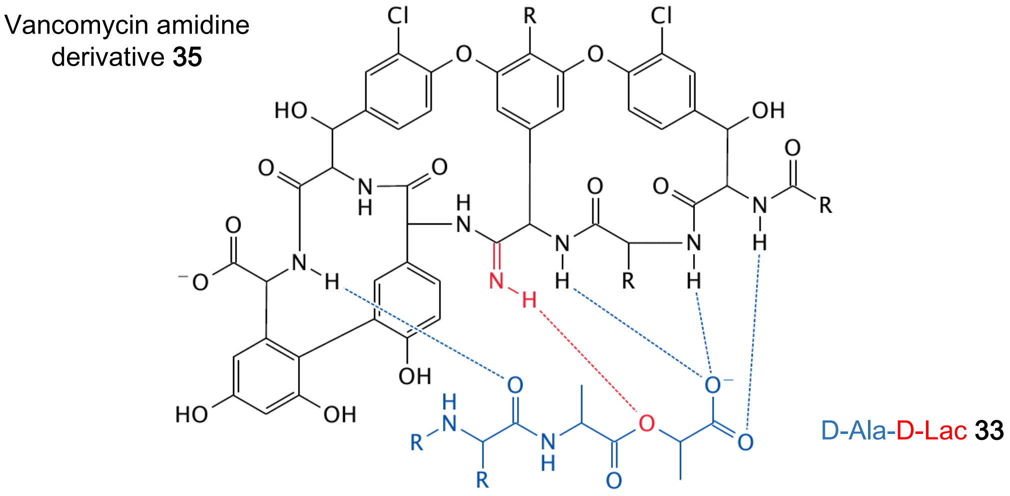


Figure 22: D-Ala-D-Ala **22** is replaced by D-Ala-D-Lac **33** or D-Ala-D-Ser **34.**

Interestingly, high-affinity binding to the D-Ala–D-Lac **33** substrate can be restored by introducing an amidine group into the vancomycin molecule **13** opposite the ester oxygen in the substrate.[73] The amidine derivative **35** is reported to inhibit both vancomycin-sensitive and vancomycin-resistant bacteria (Figure 23). If a practical and cost-effective method can be found to produce this derivative **35** to overcome vancomycin resistance, it should be of great clinical value.[73,75]



Vancomycin amidine derivative **35**

D-Ala-D-Lac **33**

Figure 23: Vancomycin **13** can be modified to overcome bacterial resistance.

1.4.6 Resistance to Rifampicin

Clinical and laboratory studies of rifampicin **21** initially targeted a broad spectrum of susceptible bacteria and the resistance was reported in laboratory studies and in patients who received monotherapy with rifampicin **21**. Resistance rates to rifampicin **21**, determined in the laboratory, have ranged from 10^{-10} to 10^{-7}, depending on the organism and the methodology used.[59] Resistance to rifampicin **21** nearly always due to a genetic change in the β subunit of bacterial RNA polymerase (RNAP). In the rifampicin resistant *E. coli* strain, the migration of β subunit in polyacrylamide gel electrophoresis was observed to be altered. Late on, separation and mixed reconstitution of enzymatically active core enzyme, using the subunits from susceptible and rifampicin resistant strains of *E. coli* and *Bacillus subtilis*, provided a more direct demonstration that resistance to rifampicin **21** was determined by the change in the β subunit.[76,77]

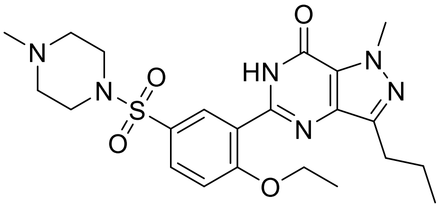
Rifampicin resistance has been reported in different gram-negative urinary tract pathogens, *in vitro* and in treated patients, and in gonococci and meningococci in the laboratory and the clinic.[59] Rifampicin resistance even occurred in tuberculosis patients who failed therapy when rifampicin **21** was the only active drug administered. Thus, when treating tuberculosis and other diseases, rifampicin **21** is almost always combined with other active antimicrobials to prevent the emergence of resistance.[69,76]

1.5 Repurposing Drugs

Drug repurposing aims to address resistance issues in bacteria and improve the process of developing new treatments and cures for diseases. It is a process or strategy of developing or discovering new therapeutic uses for failed or already marketed drug, which maximizes the therapeutic value of a drug and increase the success rates.[78,79] With the increasing market competition, pharmaceutical companies collaborated with biomedical research community are developing new drugs or new therapeutic uses from existing/old/available drugs, by applying less time consuming and less costly drug repurposing strategies, with the ultimate goal of identifying promising new treatments for patients. Examples of this will be highlighted in this section.[79]

1.5.1 Viagra

Viagra is the brand name of sildenafil **36** which has been proven to be effective and safe for the treatment of erectile dysfunction. The development of sildenafil **36** began in 1986 when chemists at Pfizer searched for a compound to treat hypertension, targeting augmentation of the renal tubular activity of atrial natriuretic peptide.[80,81] Test compounds were shown to antagonize the activity of phosphodiesterase type 5 (PDE 5), resulting in vasodilation and platelet inhibition, turning their focus to treatment of angina. Trials in angina were disappointing but some patients reported the surprising and unexpected side-effect of penile erections, leading to the development of sildenafil **36** as a treatment for erectile dysfunction (Figure 24).[81]



Sildenafil (Viagra) **36**

Figure 24: The structures of sildenafil (Viagra) **36.**

The action of Viagra is started from the moment of the sexual stimulation but does not cause uncontrollable erection. At the moment of the sexual arousal, nitric oxide (NO) from non-adrenergic, non-cholinergic (NANC) neurons or endothelial cells stimulates the activity of guanylate cyclase which produces cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP).[82,83] Viagra inhibits PDE 5 enzyme to protect cGMP from degradation, so that the concentration of the important ingredient cGMP is increased in the cavernous body. The modulation of ion channel activity by increased cGMP leads to smooth muscle relaxation of the intimal cushions of the helicine arteries, which eventually leads to vasodilation and increased inflow of blood into the corpora cavernosa of the penis, causing an erection (Figure 25).[81]

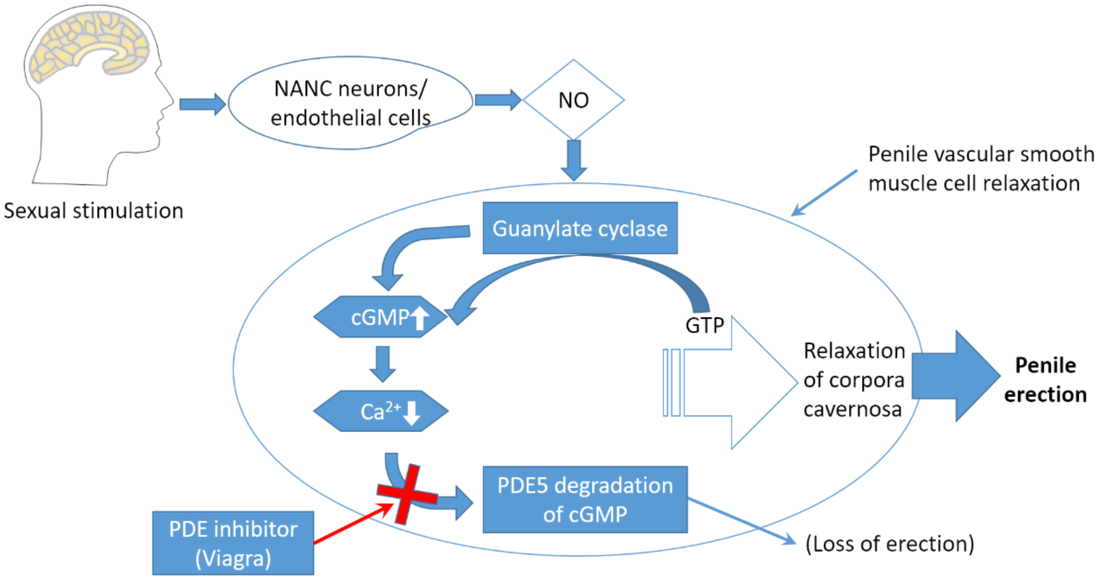
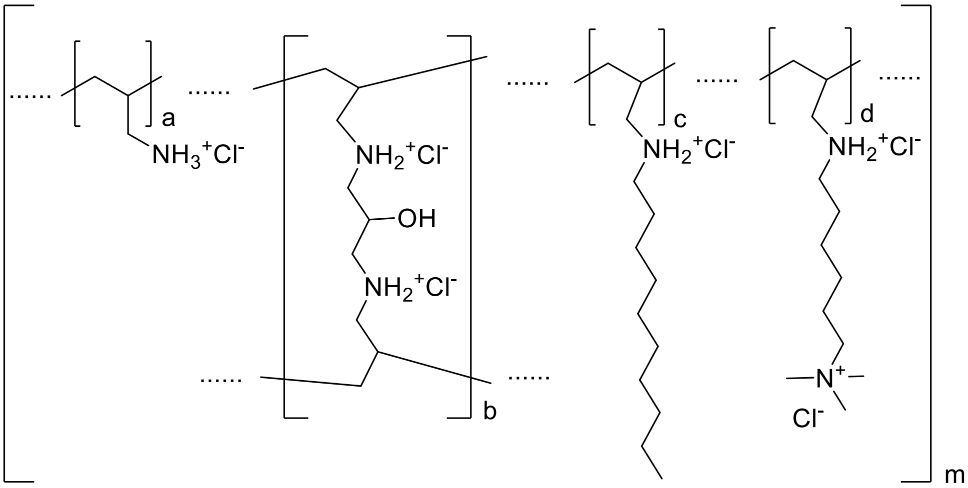


Figure 25: Mechanism of vasodilatory effect of Viagra.

1.5.2 Colesevelam

Colesevelam **37** is another example of drug repurposing. It is sold by different pharmaceutical companies under varying brand names. In the United States, it is marketed by Daiichi Sankyo as Welchol and in Canada, it is marketed by Valeant as Lodalis. Elsewhere, it is marketed by Genzyme as Cholestagel (Figure 26).[84]



a = Primary amines
b = Cross-linked amines
c = Quarternary ammonium alkylated amines
d = Decyalkylated amines
m = Extended polymeric network

Colesevelam **37**

Figure 26: Structure of colesevelam **37**, the active pharmaceutical ingredient in Welchol.

Colesevelam (Welchol) **37** is originally used as an adjunct to diet and exercise for the reduction of low-density lipoprotein cholesterol (LDL-C) in the setting of hyperlipidemia.[85] As a class of medications called bile acid sequestrants, colesevelam (Welchol) **37** works by binding bile acids within the intestinal tract due to its chemical structure (hydrophilic polymer backbone with hydrophobic side chains) and removing these bile acids from the body via elimination within the faeces.[84,85] This binding action prevents the body from recycling the bile acids from the intestines in its usual manner. By preventing the recycling process, the liver must therefore produce new bile acid by taking cholesterol from the blood stream which results in lowering cholesterol blood levels (Figure 27).[86,87]

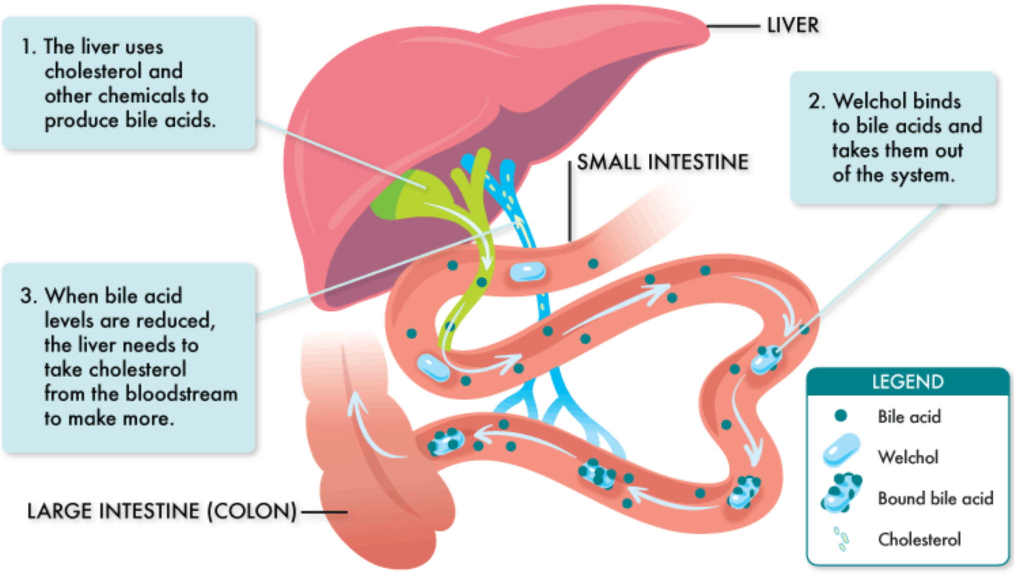
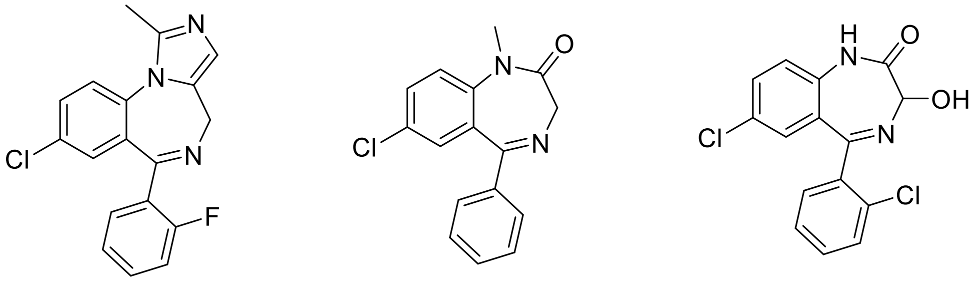


Figure 27: Mechanism of action of Welchol.

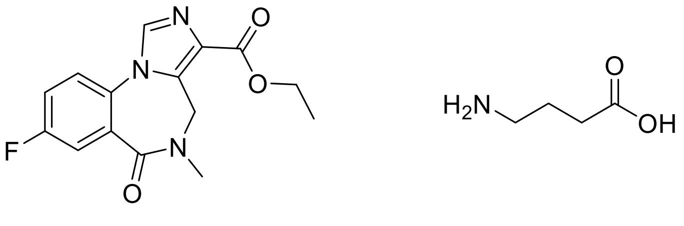
Currently, the use of colesevelam (Welchol) **37** is expanded to treat diabetes mellitus type 2. In clinical studies in patients with type 2 diabetes, colesevelam **37,** added to existing metformin, sulfonylurea or insulin therapy, reduced hemoglobin A_{1c} (HbA_{1c}) by a mean of 0.5% and LDL-C by 13 - 17%.[88,89] The mechanism by which colesevelam **37** improves glycemia control in adults has not been yet understood, but it is clear that the drug works within the digestive tract since it is not absorbed into the rest of the body.[89]

1.5.3 Benzodiazepines

Benzodiazepines **38** are a group of medications which have been used since the 1960s to treat severe muscle spasms, tremors, acute seizures, insomnia, and alcohol and drug withdrawal symptoms, but their main use is still for the treatment of anxiety disorders. The four widely used benzodiazepines in clinical anaesthesia are the agonists midazolam **38**, diazepam **39**, lorazepam **40** and the antagonist flumazenil **41** (Figure 28).[90]



Midazolam **38** Diazepam **39** Lorazepam **40**



Flumazenil **41** GABA **42**

Figure 28: The structures of four benzodiazepines midazolam **38**, diazepam **39**, lorazepam, flumazenil **41** and gamma aminobutyric acid **42**.

Benzodiazepines work by increasing the effect of a chemical called gamma aminobutyric acid (GABA) **42**, the main inhibitory neurotransmitter in the brain. In the central nervous system, benzodiazepines enhance responses to the inhibitory neurotransmitter GABA **42** by opening GABA-activated chloride channels and allowing chloride ions to enter the neuron. This action allows the neuron to become negatively charged and resistant to excitation, which leads to the various anti-anxiety, sedative or anti-seizure activity seen with these drugs.[91] Benzodiazepines are very effective in the short term but they may stop working if people take them continuously for more than a few months. This is because the brain adjusts to their effect and may be hypersensitive to natural brain chemicals when they are stopped which may even cause drug dependence.[92.93]

In addition to their action on the central nervous system, benzodiazepines have a dose-dependent ventilatory depressant effect and they also cause a modest reduction in arterial blood pressure and an increase in heart rate as a result of a decrease of systemic vascular resistance.[90,94]

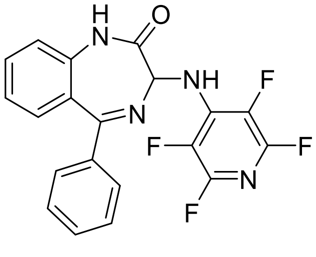
However, with increased potency of benzodiazepines comes an increase in the risk of undesired effects. The adverseness includes not only common side effects like drowsiness, lethargy and fatigue, but also severe central nervous system toxicity and delirium.[95] Cognitive impairment is usually more prominent in elderly populations because age-related decline in the function of homeostatic mechanisms in the body, notably the central nervous system, liver, and kidneys, reduces the ability to metabolize and eliminate drugs. This problem can lead to toxic accumulation of benzodiazepines and their breakdown products, the result of which may manifest in morbidity and even mortality.[96]

Anterograde amnesia and sexual abuse can occur due to the amnesic properties of benzodiazepines, especially at high doses.[97] In one case of drug-facilitated sexual assault, woman ingested benzodiazepins in conjunction with alcohol consumption and did not have any recollection of the event, but police found the metabolite of benzodiazepine in the urine sample.[98]

Recently, researchers have examined alternatives to benzodiazepines and are addressing the issue of possible adverse effects in patients who use the drugs for long periods.[91] It is necessary for prudent clinician to weigh the benefits and risks when prescribing this class of agents.[95]

1.6 Previous Work

Previous work in the department involved the preparation of a variety of substituted benzodiazepines for a proof of concept study into their antibacterial activity. One of these compounds has been demonstrated to be an effective agent against some gram-positive cocci, such as *Listeria monocytogenes*, *Staphylococci epidermidis* and *Staphylococcus aureus* (Figure 29).



43

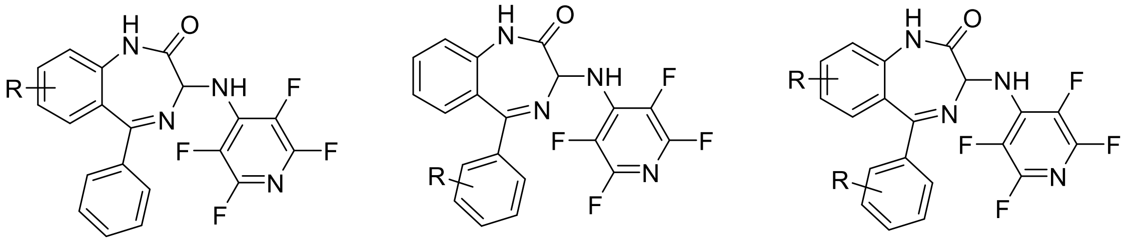
MIC (µM)				
Listeria monocytogenes	***Staphylococci epidermidis***	***Staphylococcus aureus***	***Staphylococcus aureus* (MRSA)**	***Streptococcus pyogenes***
40	40	40	40	160

Figure 29: MIC results for 3-((perfluoropyridin-4-yl) amino)-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4] diazepin-2-one **43**.

Unique to this molecule **43** is the inclusion of a fluoropyridine molecule which acts as an aromatic ring bio-isostere with differing physical properties and synthetic advantages over aromatic rings. Low-molecular-weight polyfunctional heteroaromatic compounds can be excellent candidates for benzodiazepines which are privileged structures since heteroaromatic structural subunits are present among a large number of natural products and around 70% of them are orally available.[99]

The use of fluorine as a design component is supported by its key features: low steric impact, high electronegativity, the ability to participate in hydrogen bonding and the inherent carbon-fluorine bond stability. Perfluorinated heteroaromatic derivatives are highly reactive towards nucleophiles owing to the presence of several highly electronegative fluorine atoms.[99,100]

In any case, fluorine as a substituent in pyridine rings remains relatively understudied and the effects upon drug properties undocumented. As a result of these initial studies, this project plans to synthesize a variety of substituted benzodiazepines containing the fluoropyridine moiety to investigate how changing the core pharmacophore through the introduction of substituents around both aromatic cores to test the effects on their antibacterial activity (Figure 30).



1 **2** **3**

Figure 30: Substituted benzodiazepines containing the fluoropyridine moiety.

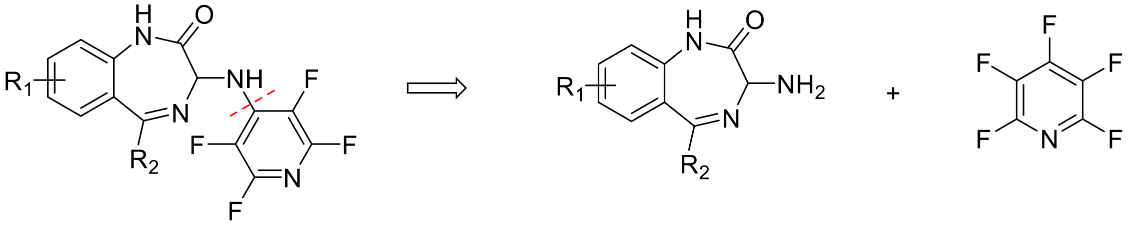
2.0 Results and Disscussion

2.1 Introduction

This chapter details the work undertaken in the area of substituted benzodiazepines and their biological properties in medical chemistry. The aim of this work was to synthesize some selective benzodiazepines substituted by fluoropyridine components and investigate structure-activity-relationship around the benzodiazepine core. To do this, we undertook a retrosynthetic analysis of the structure to determine the best method for the synthesis of the cores.

2.2 Retrosynthetic Analysis

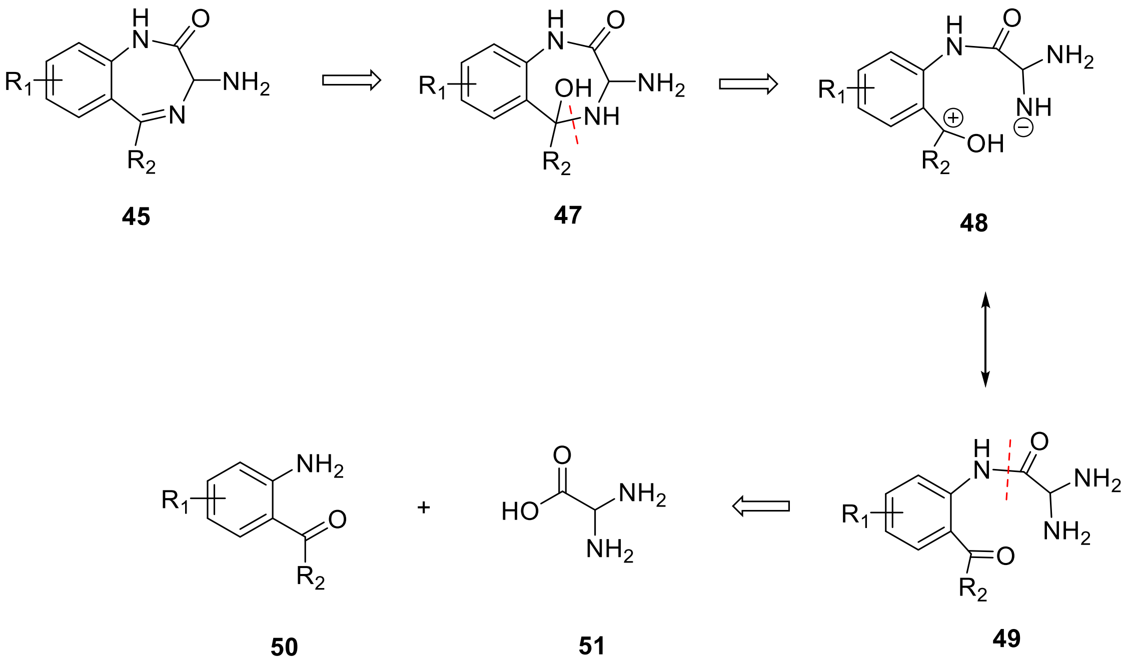
The retrosynthetic analysis begins by disconnecting the benzodiazepine **44** from the fluoropyridine component at the nitrogen-aryl bond to give benzodiazepine core **45** and pentafluoropyridine **46** (Scheme1).



44 **45** **46**

Scheme 1: Retrosynthetic analysis of target molecule **44.**

Further analysis of the benzodiazapine core **45** was undertook and revealed that the consecutive disconnections of the seven-membered heterocycle at the carbon-nitrogen single bond and the peptide bond would give 2-aminobenzophenone derivative **50** and 2,2-diaminoacetic acid **51** (Scheme 2).

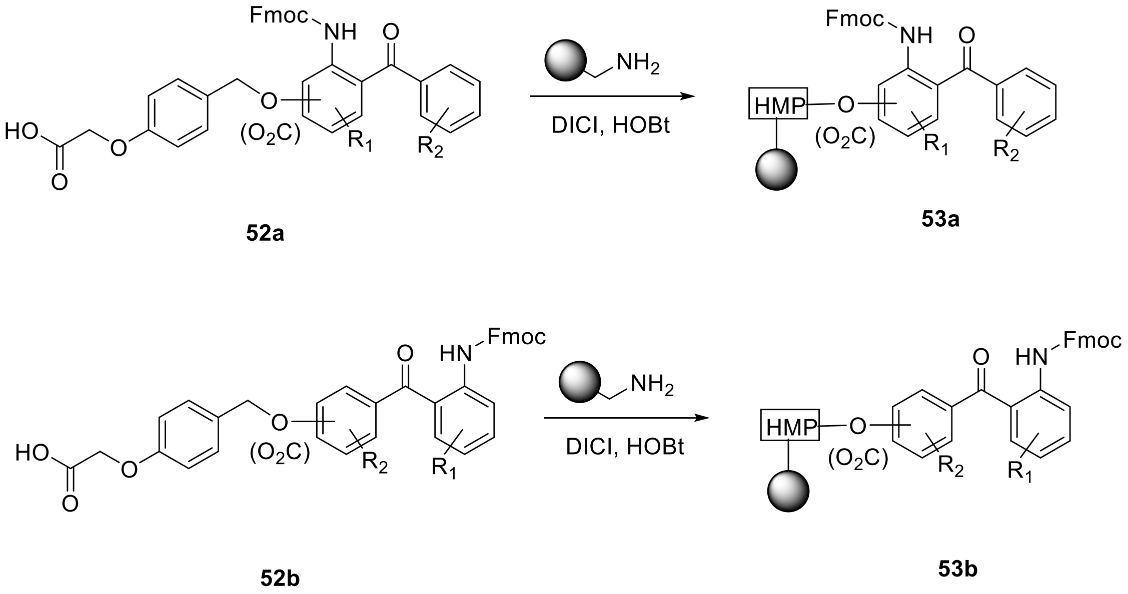


Scheme 2**:** Disconnection of benzodiazepine core **45**.

With this analysis in mind, a search of the literature revealed several synthesis which follow this analysis.

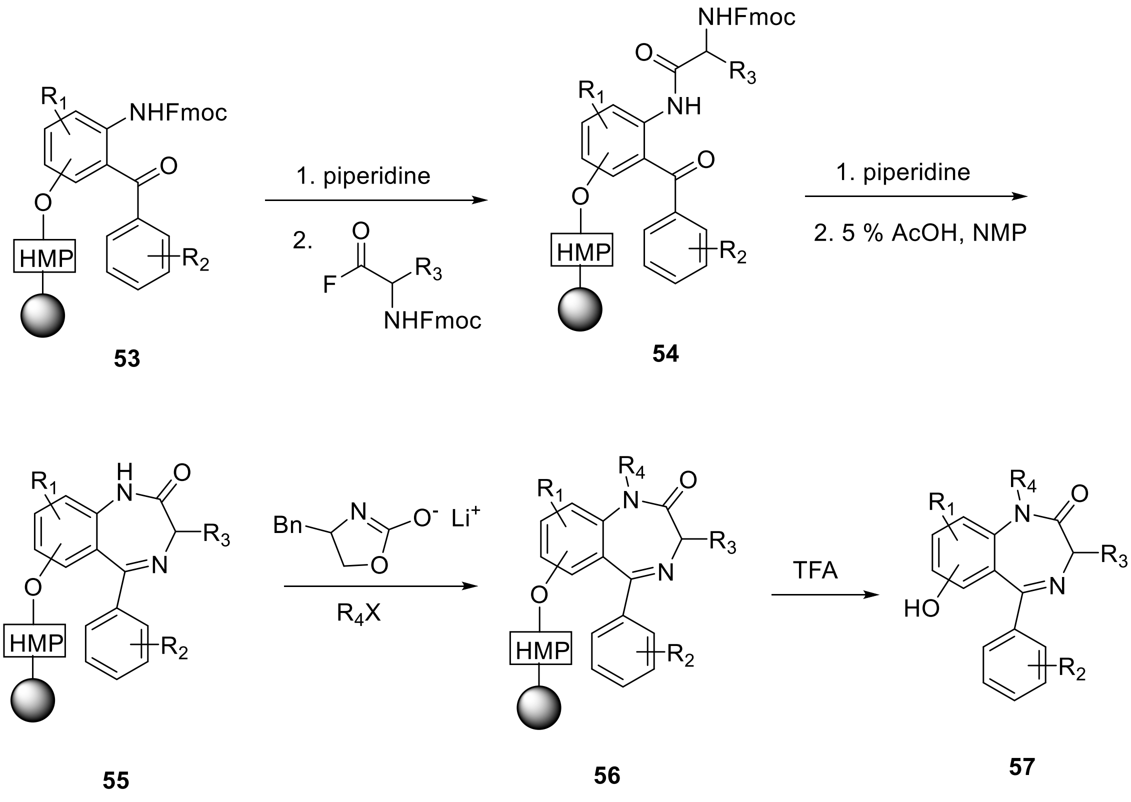
2.2.1 Ellman's Approach

This approach involves the solid-phase synthesis of 1,4-benzodiazepine derivatives. The 1,4-benzodiazepin-2-one derivatives were initially constructed from three separate components: 2-aminobenzophenones, amino acids, and alkylating agents. The substituted 2-*N*-Fmoc aminobenzophenones are coupled to the acid-cleavable (4-(hydroxymethyl)phenoxy)acetic acid (HMP) linker which may be attached by either a hydroxyl or a carboxyl group on either aromatic ring of the aminobenzophenones.[101] Employing standard amide bond formation methodology, the linker-derivatized aminobenzophenones **52a, 52b** are then coupled to the solid support (Scheme 3).



Scheme 3: The couples of the linker-derivatized aminobenzophenones **52a**, **52b** with the solid support.

The synthesis of benzodiazepines on solid support begins with removal of the 9-fluorenyl-methoxycarbonyl (Fmoc) protecting group from the aminobenzophenone **53** by treatment with piperidine in DMF. Then the unprotected 2-aminobenzophenone is coupled with the α-*N*-Fmoc amino acid fluoride to give the amide product **54**. Standard activation methods for solid-phase peptide synthesis are not successful for this coupling step because of the poor basicity and nucleophilicity of 2-aminobenzophenones. However, the activated R-*N*-Fmoc amino acid fluoride developed by Carpino couple efficiently to provide the amide products **54** even for electron-deficient 2-aminobenzophenone derivatives (Scheme 4).[102]

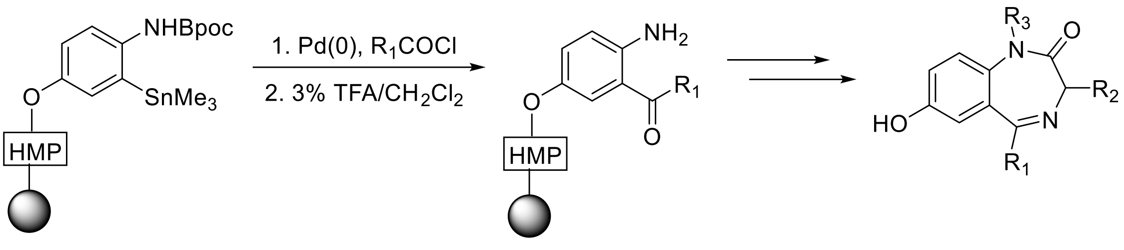


Scheme 4: Synthesis route of 1,4-benzodiazepine product **57.**

The Fmoc protecting group is then removed via treatment with piperidine in DMF again, and the resulting free amine is treated with 5% acetic acid in *N*-methyl-2-pyrrolidinone (NMP) at 60 °C to provide the benzodiazepine derivatives **55**. It is then possible to alkylate the anilide by using the excess base (lithiated acetanilide or lithiated 5-(phenylmethyl)-2-oxazolidinone) and an alkylating agent to provide the fully derivatised 1,4-benzodiazepines **56**. Treatment with the volatile acid cleavage cocktail trifluoroacetic acid (TFA)/Me_2S/H_2O (95:5:5) finally affords the 1,4-benzodiazepine product **57** in high yield.[103,104]

However, not many appropriately functionalized 2-aminobenzophenones are readily accessible. To increase the diversity of 1,4-benzodiazepin-2-ones available for solid support synthesis, Ellman et al modified the premium of the approach employing the Stille coupling reaction to synthesize a variety of 2-aminoaryl ketones on solid support.[105]

The support-bound 2-(4-biphenyl)isopropyloxycarbonyl (Bpoc) protected (aminoaryl)stannane **58** is prepared in five steps from commercially available starting material, and coupling to support is accomplished using the acid-cleavable HMP linker. The Stille coupling reaction can be carried out with a variety of acid chlorides in the presence of the catalyst $Pd_2(dba)_3 \bullet CHCl_3$. The Bpoc group is cleaved by brief treatment with 3% TFA in CH_2Cl_2 solution for 5 min, affording the support-bound aminoaryl ketone **59** which were further incorporated into 1,4-benzodiazepine derivatives **60** according to previously described synthesis sequence (Scheme 5).[102]



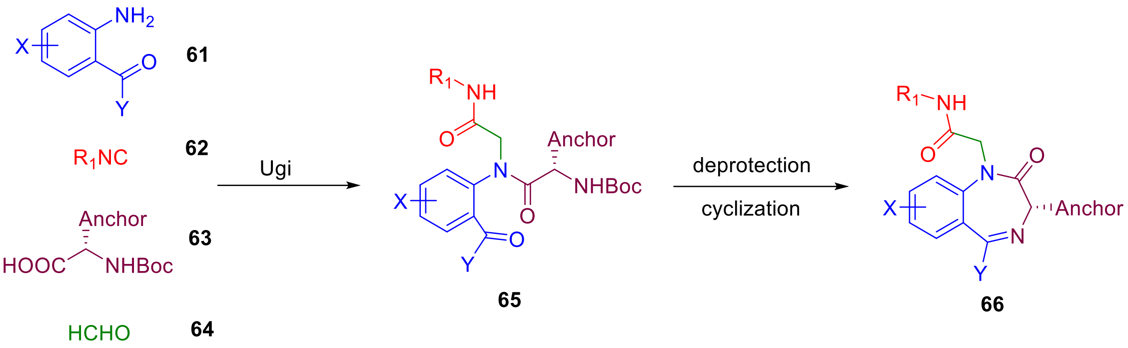
58 **59** **60**

Scheme 5: Stille coupling reaction is employed to synthesize aminoaryl ketone **59**.

By employing the versatile solid-phase synthesis, the yields for the benzodiazepine products are considerable (vary from 52% to 82 %). However, this sequence requires over seven steps and many different reagents, which is lengthy and inefficient.

2.2.2 Huang's Approach

Huang and others have significantly improved the efficiency and diversity for the synthesis of new benzodiazepines using a convergent Ugi-4CR (four-component reaction) followed by deprotection and intramolecular cyclization (Table 1).[106]



Entry	X	Y	R_1	Anchor	Yields
66a	H	Ph	^{t}Bu	Phe	**41 %**
66b	H	Ph	^{t}Bu	Leu	**41 %**
66c	H	Ph	cyclohexyl	Phe	**50 %**
66d	H	Ph	cyclohexyl	Leu	**69 %**
66e	H	Me	^{t}Bu	Phe	**44 %**
66f	H	Me	^{t}Bu	Leu	**46 %**
66g	4-Cl	Ph	^{t}Bu	Phe	**32 %**
66h	4-Cl	Ph	^{t}Bu	Leu	**43 %**
66i	H	Ph	^{t}Bu	Trp	**26 %**
66j	H	Ph	^{t}Bu	Tyr	**29 %**
66k	H	Ph	cyclohexyl	Trp	**65 %**
66l	H	Ph	cyclohexyl	Tyr	**60 %**
66m	H	Ph	benzyl	Trp	**22 %**

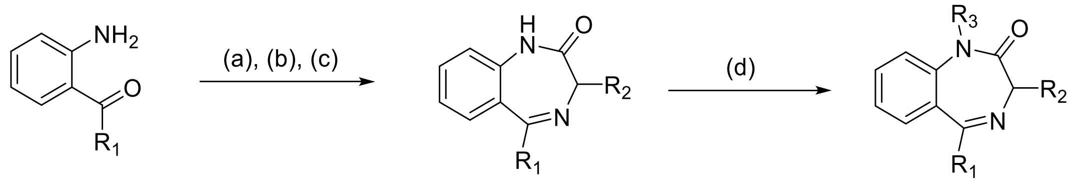
Table 1: Synthesis of anchor-directed 1,4-benzodiazepines **66a-m**.

In the first step (Ugi), the aminophenylketone **61** serves as an amine component for the Ugi-4CR together with an isocyanide **62**, *N*-Boc-amino-acid **63** and formaldehyde **64**. The Boc protection group of the Ugi product **65** is cleaved in the second step (deprotection). Then the free amine group is condensed with the orthogonal ester group to form the 1,4-diazepine ring in the third step (cyclization).[106,107] Phenylalanine, leucine, tryptophan and tyrosine, which are abundant in the protein-protein interaction interface, were selected as examples in this synthetic sequence.

Compounds **66a-m** with four points of diversity were isolated by chromatography in 22-69 % yield over two steps. This approach provides access to benzodiazepines in acceptable yields, however is unable to provide the wanted amino group at the "anchor" position and possesses extra diversity at the amide nitrogen.

2.2.3 Spencer's Approach

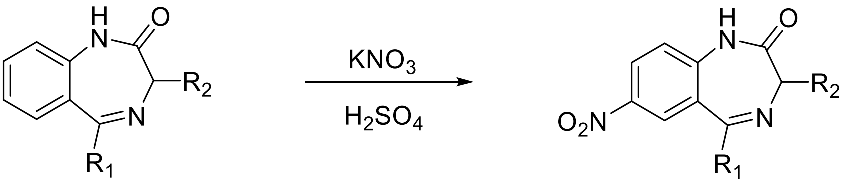
According to Spencer's research, C3 and C5 substituted benzodiazepines **68** were synthesised from readily available 2-aminophenylketones **67** in a three step process involving treating with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and Boc-NHCH(R_2)CO_2H at room temperature (Scheme 6).[108,109]



67 **68** **69**

Scheme 6: Synthesis of N1-substituted benzodiazepine analogues **69**. Reagents and conditions: (a) Boc-NHCH(R_2)CO_2H, EEDQ, DCM, rt; (b) TFA, DCM, rt; (c) CH_3COONH_4, AcOH, rt; (d) NaH, R_3Br or R_3I, DMF.

By treating the compound **68** with sodium hydride and alkylation with the appropriate alkyl or benzyl halide, the N1-substituted benzodiazepine analogues **69** were obtained. Differently, in order to add NO_2 group to the aromatic ring, **68** needed to be treated with KNO_3 in concentrated H_2SO_4 at 0 ℃ before the final step (Scheme 7).[109]



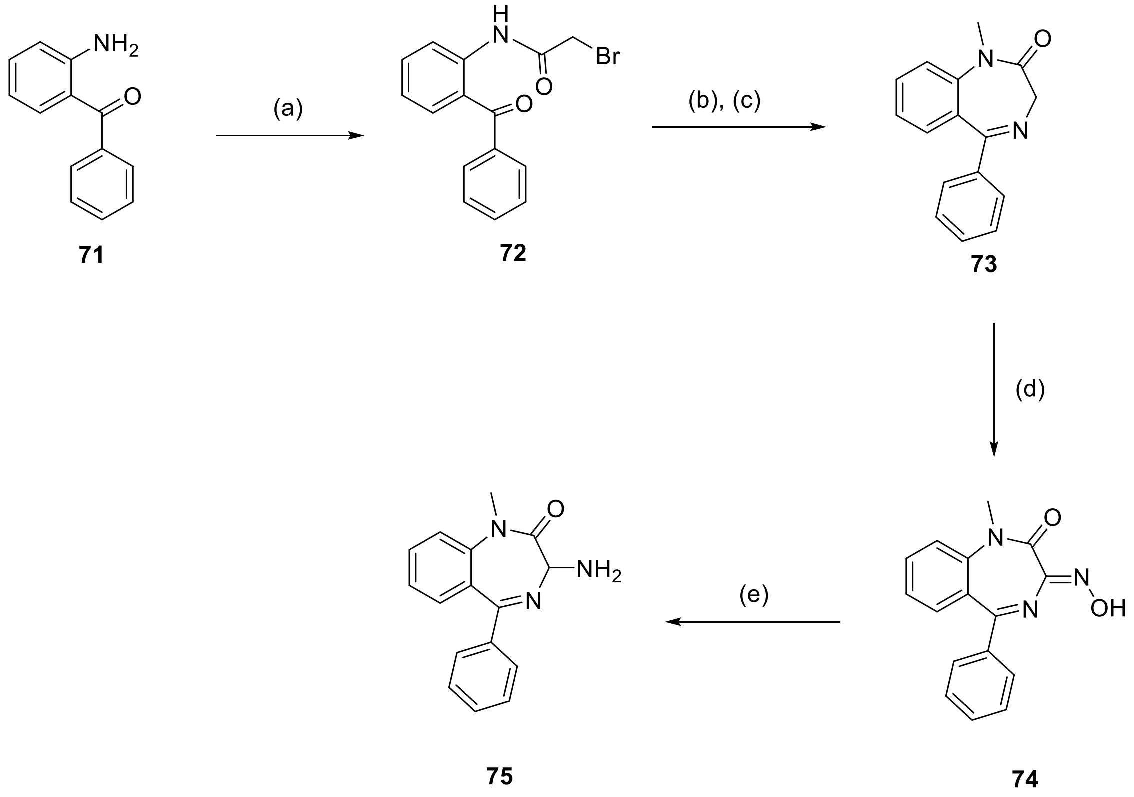
68 **70**

Scheme 7: Synthesis of nitro benzodiazepine analogues **70**.

Although the sequence to three-step synthesis of benzodiazepine derivatives is not very complex with the acceptable yields of substituted benzodiazepines **68** varying from 22 % to 71 %, the amino group at the R_3 position is still unavailable. R_2 labelled different function groups such as H, Bn, $CH_2(Ph)_2$ and CH_2CN, but no amino group.

2.2.4 Bock's Approach

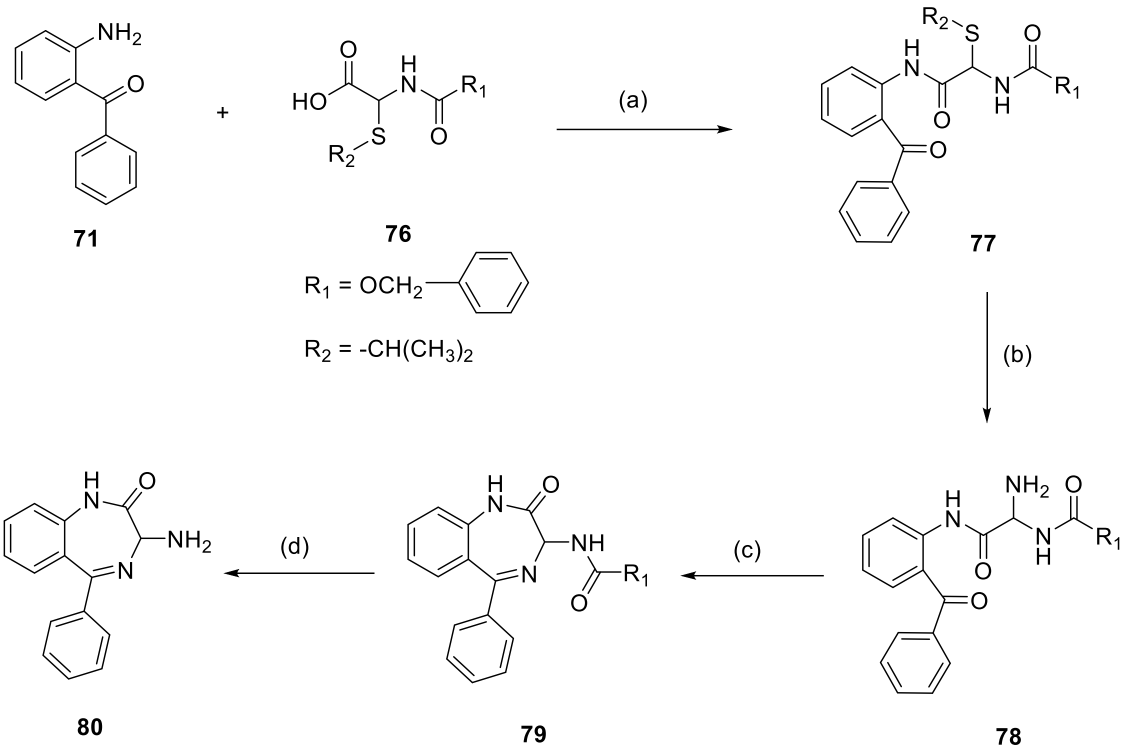
Bock and co-workers reported two preparations of 1,4-benzodiazepines in 1987. The first of these routes involved construction of the N1-substituted 1,4-benzodiazepine nucleus followed by introduction of the 3-amino functionality which is the substrate we want (Scheme 8).[110]



Scheme 8: Bock's synthetic route to 3-amino-1-methyl-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one **75**. Reagents and conditions: (a) $BrCOCH_2Br$, CH_2Cl_2-H_2O, -10-23 °C, 4 h; (b) NH_3 (g), CH_3OH, -10 - 15 °C, 2 h, then 65 °C, 2 h; (c) CH_3Cl, NaOH (aq), toluene, Aliquat 336, 0-25 °C, 4 h; (d)*t*-BuOK, *i*-AmONO, toluene, -20-0 °C, 45 min; (e) H_2, 5 % Ru/C, CH_3OH, 68-74 °C, 40 psi, 24 h.

Nevertheless, the two-step amination sequence required oxime formation and subsequent catalytic hydrogenation over a reduced ruthenium catalyst at elevated temperature and pressure, leading to 48 % overall yield. The strict experimental conditions required increase the difficulty of this approach.

The second route employed α-(isopropylthio)-*N*-(benzyloxycarbony1)glycine **76** as a masked aminoglycine synthon. The key synthetic step involved a novel mercury-mediated displacement of the isopropylthiol group with ammonia to generate the aminal present in the 3-aminobenzodiazepine nucleus **79**. A combination of α-(isopropylthio)-*N*-(benzyloxycarbony1)glycine **76**, isobutyl chloroformate, and *N*-methylmorpholine afforded the corresponding mixed anhydride which was then reacted *in situ* with 2-aminobenzophenone **71** to give alkylthioglycine amide **77** in >90% yield. In the next critical step, the alkylthioglycine amide **77** was dissolved in dry tetrahydrofuran, saturated with ammonia and treated with 1.l equivalents of mercuric chloride. A continuous stream of ammonia gas into the reaction mixture served to complete the alkylthio displacement. The intermediate α-amino glycinyl amide **78** was obtained and conveniently dissolved in glacial acetic acid containing ammonium acetate and stirred at room temperature overnight (or 2.5 h at 55 ℃). In this way, **79** was obtained as a crystalline solid in 70-75% yield. To complete the synthesis, the carbobenzyloxy protecting group was removed under transfer hydrogenation conditions to give **30** as its formate salt. The free amine **30** was isolated after neutralization of the salt with sodium hydroxide solution (40 %) in methylene chloride in 86 % yield (Scheme 9).[111]

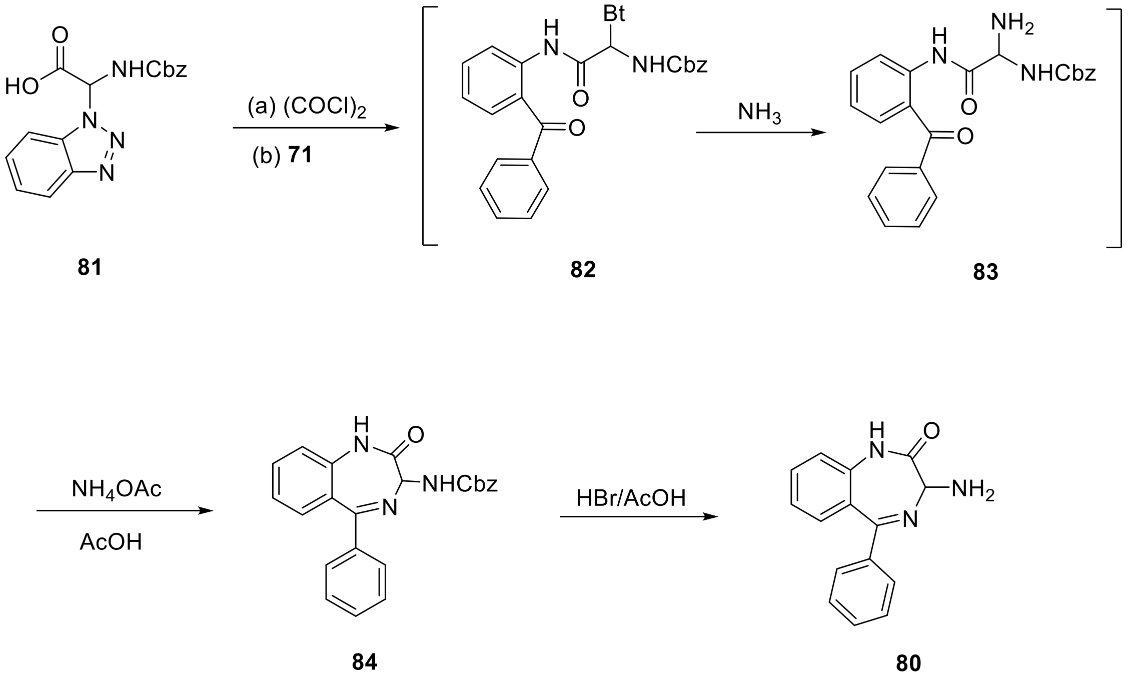


Scheme 9: Bock’s synthetic route to 3-amino-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one **80**. Reagents and conditions: (a) iso-BuOCOCl, *N*-methylmorpholine, CH_2C1_2, 0 °C, 15 min; 0 - 23 °C, 12 h; (b) NH_3 (g), $HgCl_2$, THF, 0 - 23 °C, 3 h; (c) NH_4OAc, AcOH, 23 °C, 12 h; (d) 10 % Pd/C, HCO_2H (95 %) - CH_3H (5:95, v/v), 45 °C, 3 h.

While higher yielding than the oxime route, this process suffers from the use of stoichiometric quantities of highly toxic mercuric chloride and the rather odoriferous nature of the displaced 2-propanethiol.

2.2.5 Our Synthetic Route

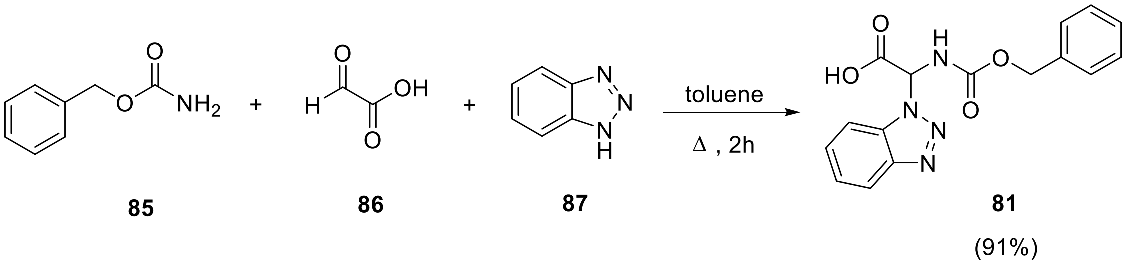
Reported by Sherrill *et al*, the convenient high-yielding synthetic route, which is also the approach this project followed, is an improvement of Bock's approach.[112] Since an α,α-diamino moiety is unstable and not easily available, in this work, α-benzotriazol-1-yl-*N*-(benzyloxycarbonyl)glycine **81** served as the superior aminoglycine synthon.[113] Preliminary attempts to couple benzotriazole adduct **81** to 2-aminobenzophenone **71** with standard peptide coupling agents or via a mixed anhydride gave little or no reaction. However, *in situ* formation of the acyl chloride with oxalyl chloride and catalytic DMF followed by treatment with 2-aminobenzophenone **71** gave clean conversion to acyl amide **82** as assessed by TLC. The crude solution of **82** was treated directly with ammonia gas in a THF/methanol solvent mixture to provide amino ketone **83.** Following solvent displacement into ethyl acetate, the benzotriazole byproduct was readily extracted with aqueous base and the crude amino ketone **83** was cyclized to provide 3-[(benzyloxycarbonyl)aminol-1,4-benzodiazepine **84** in 73% overall yield from 2-aminobenzophenone **71** without chromatography. Removal of the Cbz protecting group by treatment with saturated HBr in acetic acid and subsequent neutralization was effected in 88% yield to provide 3-amino-l,4-benzodiazepin-2-one **80** in 66% overall yield from 2-aminobenzophenone **71** (Scheme 10).



Scheme 10: Our synthetic route to 3-amino-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one **80.**

2.3 Synthetic Route to Benzotriazole Substituted Amino Acid

Based on the retrosynthetic analysis described in section 2.2, the first step of the synthesis was to prepare 2-(benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** as starting material. The condensation of benzyl carbamate **85** with glyoxylic acid **86** and benzotriazole **87** proceeds easily. An equimolar ratio of the reactants in refluxing toluene are utilized to give the glyoxylic acid adduct **81** (Scheme 11).

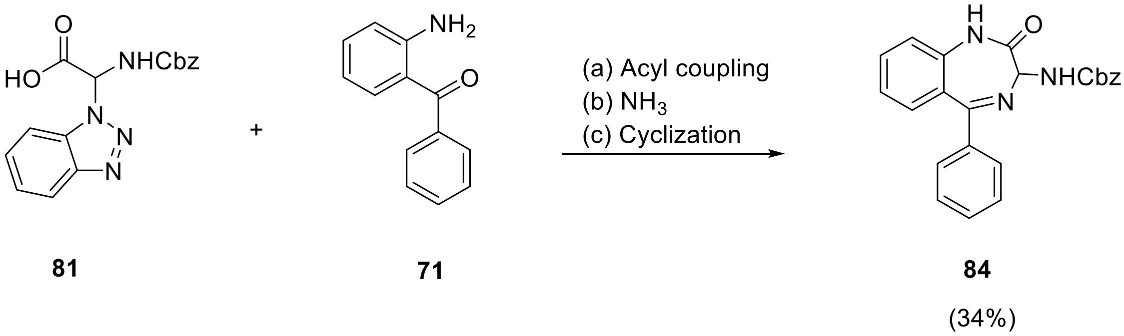


Scheme 11**:** Synthesis of stable 2-benzotriazol-1-yl-*N*-(benzyloxycarbonyl)glycine **81**.

Isolation of the product is convenient because of its sparing solubility and the solid product can be filtered off from the reaction mixture to obtain a high yield (91 %). This was advantageous as large quantities of this material were required for the synthesis of benzodiazepine cores.

2.4 Synthetic Route to Benzodiazepine Cores

With the 2-benzotriazol-1-yl-*N*-(benzyloxycarbonyl)glycine **81** in place, several different 2-aminobenzophenone derivatives were employed. To begin, synthesis of the benzodiazepine core **84** was undertaken by repeating the experiments from Sherrill's work, where by the condensation of 1.1 equivalents of 2-(benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** and one equivalent of 2-aminobenzophenone **71**, utilising oxalyl chloride in DCM, gave rise to intermediate **82**. The crude **82** was evaporated and treated *in situ* with ammonia in ethanol. This gave rise to **83** which was subsequently cyclised, following evaporation of NH_3/ethanol, with ammonium acetate and acetic acid to give **84** in a yield of 34% (Scheme 12).

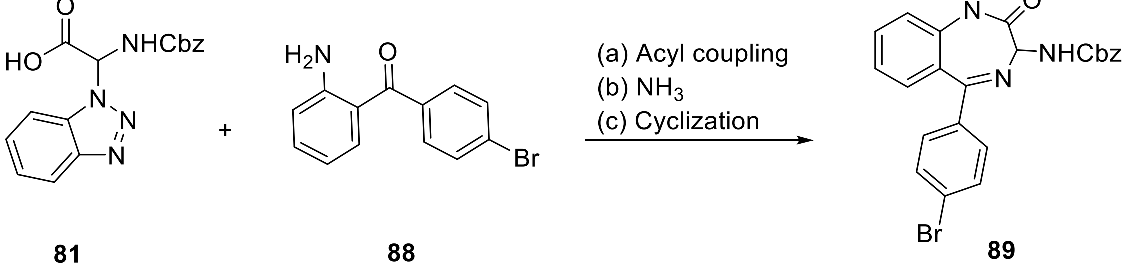


Scheme 12**:** Synthesis of benzyl (2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **84**.

The low yield (34 %) of benzodiazepine **84** could arise from several factors, such as incomplete condensation with 2-aminobenzophenone **71** which was observed by TLC. A possible reason for the incomplete reaction was the poor formation of the acyl chloride from the starting material **81** and volatile oxalyl chloride. To improve the yield of **84**, the equivalence of oxalyl chloride was increased from one equivalent to 1.5 equivalents. Nevertheless, this did not make a big difference, so the more reasonable explanation for the incomplete condensation was the possible decomposition of **81** in the reaction mixture.

Different states of ammonia employed in the reaction also could lead to the low yield. Compared with the previous experiments saturated with ammonia gas, which might be more favourable for amination process, ammonia in liquid ethanol was used instead in this project.

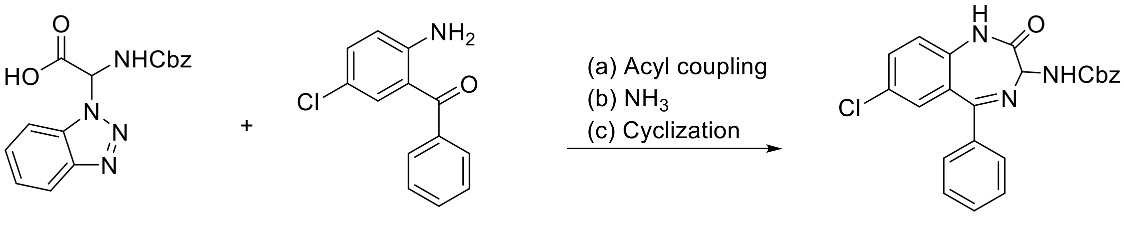
An additional reason for the low yield was that the previous experiments began with 28 mmol reagents which was 40 times more than our starting materials. Thus, the lost amount of product in our work influenced the yield more severely. Some evidence for this reason was that when we first synthesized the bromo-substituted benzodiazepine **89** with 0.7 mmol 2-amino-4'-bromobenzophenone **88**, the yield of product was 38 %, but double amount of reagents afforded the yield of product **89** to be 50 % (Scheme 13).



Scheme 13**:** Synthesis of benzyl (5-(4-bromophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **89**.

In any event, we persevered with our synthetic approach to produce a series of benzodiazepine cores with a variety of substituents present in both aromatic rings.

The first synthesis of the chloro-substituted benzodiazepine **91** began with 0.7 mmol 2-amino-5-chlorobenzophenone **90**. The product **91** was obtained by filtering the mixture of organic and aqueous layers at 0 - 5 ℃ only yielding to 18 %. The low yield was possibly by the reason that the chloro-substituted benzodiazepine **91** was soluble in the organic solution of ethyl acetate and diethyl ether and part of the product **91** was lost in the discarded filter liquor. Therefore, the second trial of this synthesis starting with 1.4 mmol 2-amino-5-chlorobenzophenone **90** did not undergo filtration. Instead, the aqueous layer was removed and then the reaction mixture was concentrated *in vacuo*. When the organic solvent volatilized overnight, much more product crystalized and finally a solution of n-hexane and a small amount of ethyl acetate was applied to wash the product to afford the compound **91** with the yield of 46 % (Scheme 14).

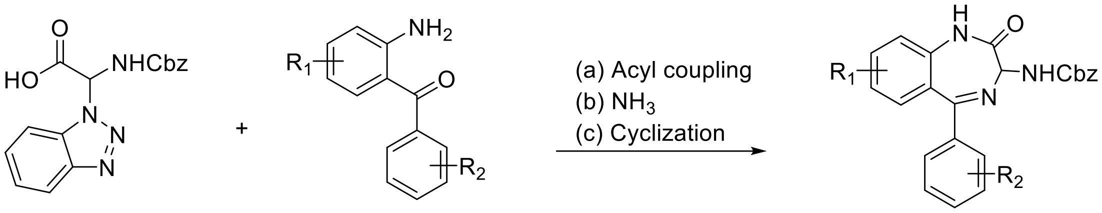


81 **90** **91**

(46%)

Scheme 14**:** Synthesis of benzyl (7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **91**.

Synthesized from the same amount (1.40 mmol) of 2-aminobenzophenone derivatives **88**, **92** and **93**, the substituted benzodiazepines **89, 94** and **95** were more soluble than **84** and **91** in the organic solution of ethyl acetate and diethyl ether. Thus, they cannot be obtained by filtration, and flash chromatography was employed to isolate them leading to a yield of 50 %, 49 % and 44 % respectively (Scheme 15).



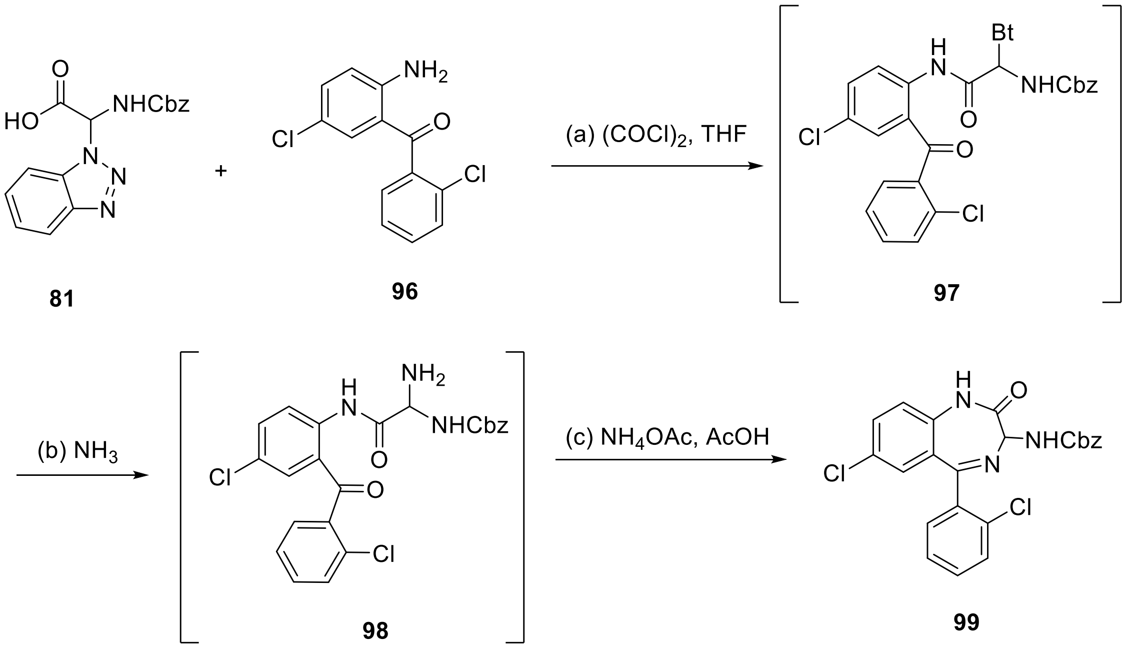
81

88 R_1=H, R_2=4-Br
92 R_1=5-Cl, R_2=2-F
93 R_1=4-Me, R_2=H

89 R_1=H, R_2=4-Br (50%)
94 R_1=5-Cl, R_2=2-F (49%)
95 R_1=4-Me, R_2=H (44%)

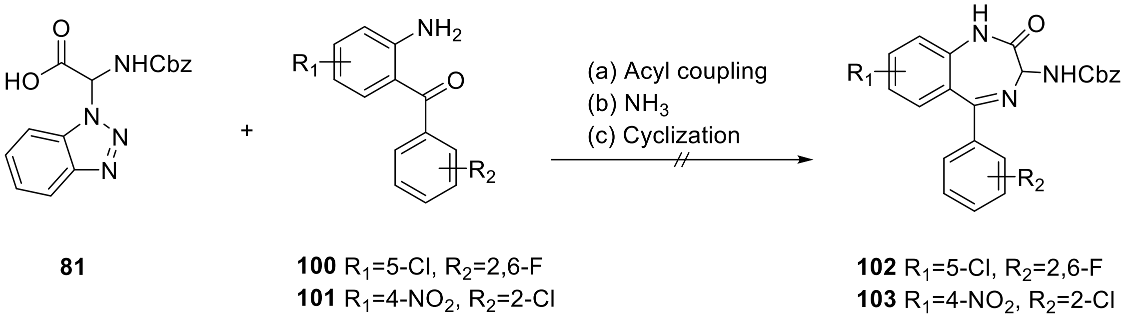
Scheme 15**:** Synthesis of the substituted benzodiazepines **89, 94** and **95**.

The substituted benzodiazepine **99** was successfully synthesized beginning with 2-amino-2’,5-dichlorobenzophenone **96**. The presence of the benzodiazepine **99** was confirmed by ^{1}H NMR spectrum from the appearance of the peaks corresponding to the aryl groups (7.07-7.70 ppm, 13H), the NH group (6.68 ppm, 1H), CH group (6.45 ppm, 1H),) and CH_2 group (5.11 ppm, 2H). The mass spectrum also confirmed the right mass of benzodiazepines **99** at *m/z* 909 ($2M+H^+$). Unfortunately, the yield was quite low and it had not been isolated because its polarity was close to the intermediate **97** as assessed by TLC which was one of the impurities in the synthetic crude product (Scheme 16).



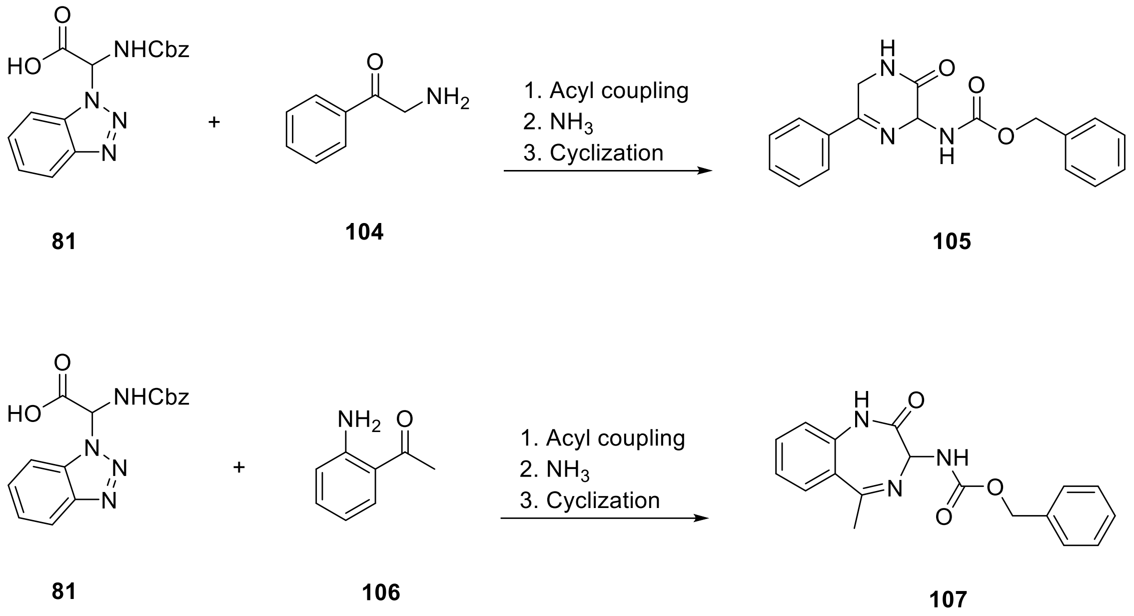
Scheme 16**:** Synthesis of benzyl (7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4] diazepin -3-yl)carbamate **99**.

The coupling of benzotriazole **81** with (2-amino-5-chlorophenyl)(2,6-difluorophenyl)methanone **100** or (2-amino-5-nitrophenyl)(2-chlorophenyl)methanone **101** gave little or no reaction. The substituted benzodiazepines **102**, **103** could not been obtained (Scheme 17).



Scheme 17**:** Synthesis of the substituted benzodiazepines **102**, **103** failed.

The coupling of benzotriazole **81** with 2-aminoacetophenone **104** and 2'-aminoacetophenone **106** respectively were also attemped to prepare **105** and **107** with both of them successfully synthesized (Scheme 18).

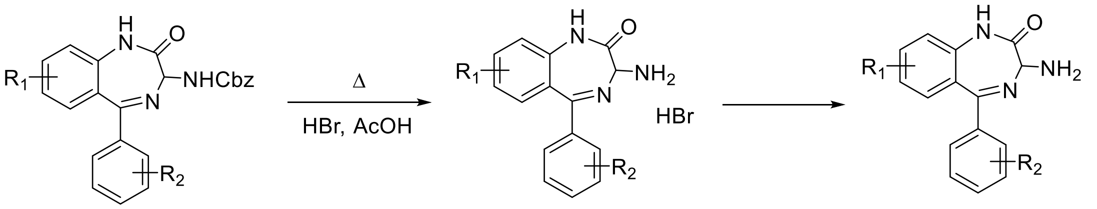


Scheme 18**:** Synthesis of benzyl (3-oxo-6-phenyl-2,3,4,5-tetrahydropyrazin-2-yl)carbamate **105** and benzyl (5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **107**.

The presence of the compounds **105** and **107** was confirmed by ^{1}H NMR and mass spectra. Both of them were not isolated and utilized directly in the next synthetic transformation.

2.5 Synthesis of Benzyloxycarbonyl Deprotected Product

With a series of benzodiazepines **84**, **89**, **91**, **94**, **95**, **105** and **107** in hand, the Cbz deprotected products **80** and **110** were obtained in 82 % and 71 % yields by treating **84**, **91** with hydrobromic acid in acetic acid. Aminobenzodiazepines **109** and **111** were not isolated and utilized directly in the final coupling reaction (Scheme 19).



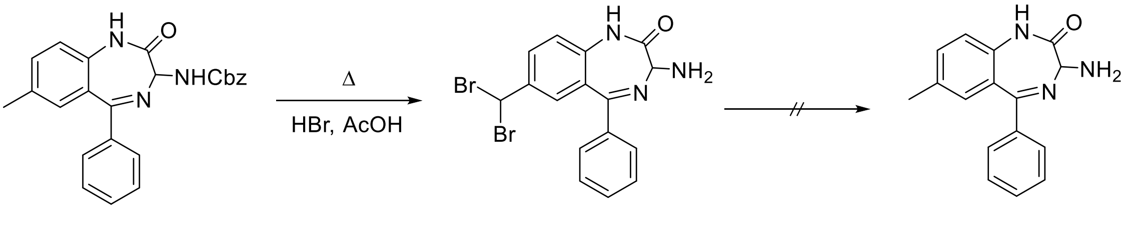
84 R_1=H, R_2=H
89 R_1=H, R_2=4-Br
91 R_1=5-Cl, R_2=H
94 R_1=5-Cl, R_2=2-F

108

80 R_1=H, R_2=H (82%)
109 R_1=H, R_2=4-Br
110 R_1=5-Cl, R_2=H (71%)
111 R_1=5-Cl, R_2=2-F

Scheme 19**:** Synthesis of the Cbz deprotected products **80**, **109-111**.

By treating **95** with hydrobromic acid, aminobenzodiazepine **112** was obtained as a mixture of brominated products and the presence was confirmed by ^{1}H NMR spectrum from the appearance of the peaks corresponding the NH group (7.82 ppm, 1H), the aryl groups (7.35-7.60 ppm, 8H) and the CH group (6.81 ppm, 1H). Analysis by mass spectrum detected the brominated molecular ions at *m/z* 446 (MNa^+). However, the desired aminobenzodiazepine **113** was not obtained (Scheme 20).



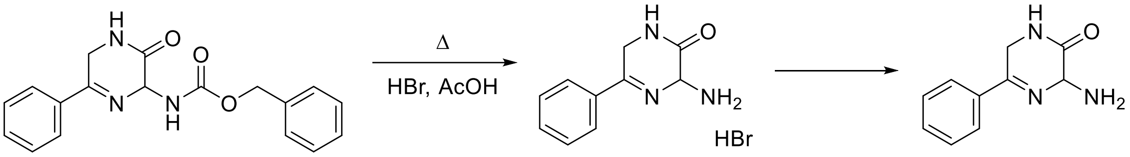
95

112

113

Scheme 20**:** Synthesis of the Cbz deprotected product **112**.

The compound **115** was also synthesized successfully by deprotecting **105**. Its presence was confirmed by ^{1}H NMR spectrum from the appearance of the peaks corresponding the NH group (7.89 ppm, 1H), the aryl groups (7.29-7.54 ppm, 5H), the CH_2 group (3.49 ppm, 2H) and the CH group (2.17 ppm, 1H). Analysis by mass spectrum detected the molecular ions at *m/z* 188 (M-H$^-$) (Scheme 21).



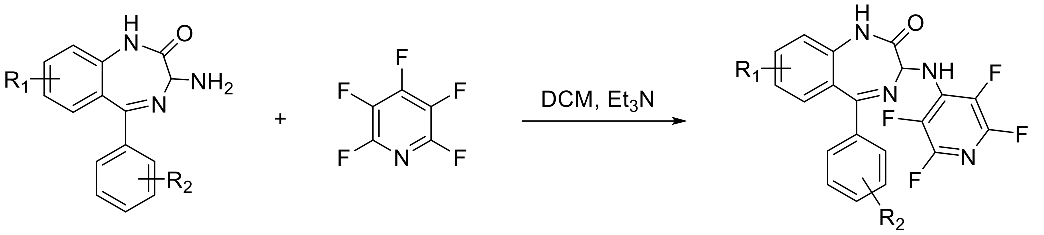
105 **114** **115**

Scheme 21**:** Synthesis of the Cbz deprotected product **115**.

The Cbz deprotecting of **107** was not smoothly carried out. Compound **107** did not react with HBr even when adding more reagents, raising the temperature or increasing reaction time, so the progress stopped here.

2.6 Final Coupling Reaction

The final coupling of deprotected benzodiazepines with pentafluoropyridine **46** provided our target molecules **43**, **116-118** with the final product **116** being obtained in a high yield (99 %) (Scheme 22).



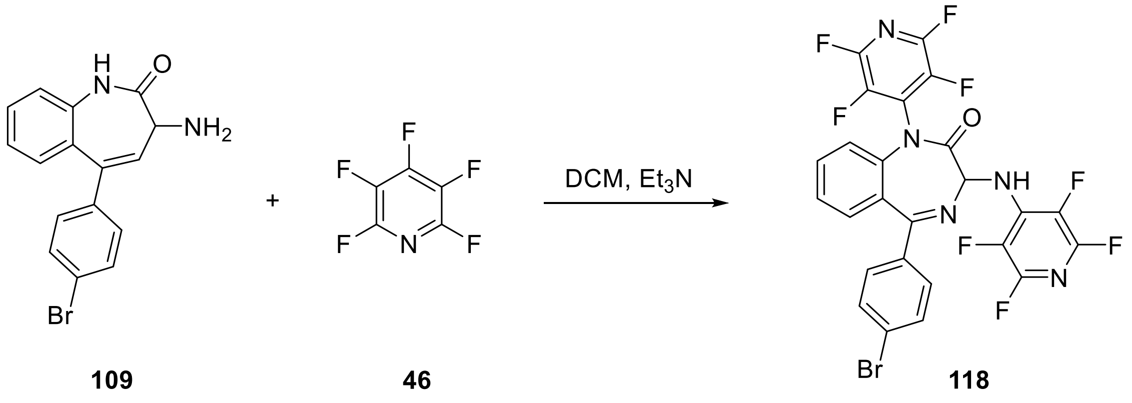
80 R_1=H, R_2=H
110 R_1=5-Cl, R_2=H
111 R_1=5-Cl, R_2=2-F

46

43 R_1=H, R_2=H (39%)
116 R_1=5-Cl, R_2=H (99%)
117 R_1=5-Cl, R_2=2-F (31% from **94**)

Scheme 22**:** Synthesis of the final products **43**, **116**, **117** with the fluoropyridine moieties.

Mechanistically, pentafluoropyridine **46** was attached to deprotected benzodiazepine core at the 3-amino position. Nevertheless, during the synthesis of bromide target molecule **118**, the reaction rate was very low. Because pentafluoropyridine **46** was volatile, additional equivalents of pentafluoropyridine **46** and triethylamine were added to the uncomplete reaction after reacting overnight. As a result, the final product was the double coupled benzodiazepine derivative **118** (Scheme 23).



(35% from **89**)

Scheme 23**:** Synthesis of 5-(4-bromophenyl)-1-(perfluoropyridin-4-yl)-3-((perfluoropyridin-4-yl)amino)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one **118**.

3.0 Biological Investigation of Final Compounds

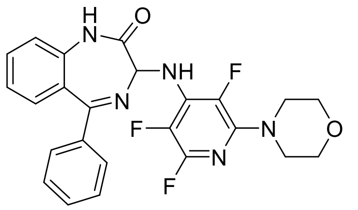
Biological screening was undertaken utilizing a plethora of bacteria. The range was inclusive of gram positive, gram negative and mycolata bacteria. Specifically we utilized *Enterococcus faecium, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica* and *Mycobacterium tuberculosis,* many of which are causative agents for different diseases or illnesses, such as neonatal meningitis or endocarditis,[114] listeriosis,[115] sinusitis, food poisoning,[116] urinary tract infections,[117] ventilator-associated pneumonia, various sepsis syndrome, typhoid[118] and tuberculosis.[119]

The compounds **43**, **119-122** were prepared in the previous work with the unsubstituted compund **43** synthesized and retested in this project. The compounds range number were dissolved in DMSO at the concentration of 1.5 – 0.5 µg/ml and subjected to a standard REMA assay to calculate the minimum inhibitory concentration (MIC, µM) utilizing 96-well plates and resurin to visualize the results. MIC indicated that the standard **43** fighted against *Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes* and *Mycobacterium tuberculosis,* while the substitutions on the fluoropyridine moiety influenced the activity, so **119-121** only had effect on *Mycobacterium tuberculosis*. With ethoxy group connected to fluoropyridine, MIC of **121** against *M. tuberculosis* was similar to **43** (18.76 µM vs 18.40 µM respectively). Morpholine substitution on **119** slightly influences the MIC against *M.*

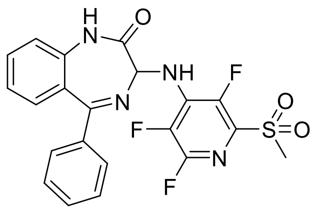
tuberculosis which was 25.67 µM. Methanesulphonamide substitution on **120** greatly weakened the activity against *M. tuberculosis*, and the MIC was 69.55 µM.

The compounds **116**, **117** substituted by chlorine and fluorine on the aromatic rings did not decrease the activity greatly. MIC of **116** against *Bacillus subtilis* was similar to **43** (19.98 µM vs 18.40 µM respectively), and the MIC of **117** was larger (35.34 µM). The MICs of **116** (19.98 µM) and **117** (17.67 µM) against *Staphylococcus aureus* were around double of **43** (9.20 µM). The MICs of three against *Streptococcus agalactiae* were almost the same (18.40 µM, 19.98 µM and 17.67 µM respectively). The MICs of **43** against *Streptococcus pyogenes* and *Mycobacterium tuberculosis* both were 18.40 µM, and for **116**, **117**, they doubled to 39.97 µM and 35.34 µM. The only big difference was that the MICs of **43** and **116** against *Listeria monocytogenes* were 36.80 µM and 39.97 µM respectively, but **117** was inactive.

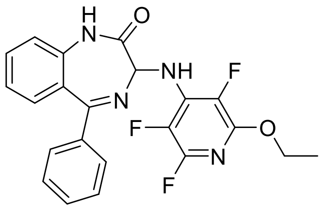
However, when N1 was coupled with pentafluoropyridine **46**, the compounds **122**, **118** were inactive to all tested bacteria. Therefore, it is important to keep the N1 unsubstituted as highlighted (Entry **117** vs Entry **118**). Steric hindrance may also contribute to the inactivation of **118** to all tested bacteria. Fluorine atom was not much bigger than hydrogen so the compound **117** did not make a big difference from **43**. On the same aromatic ring, bromine atom was very large and probably influenced the antibacterial efficiency of **118** (Figure 31, Table 2).



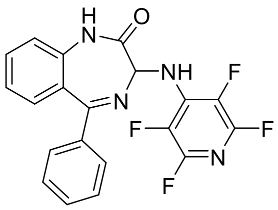
119



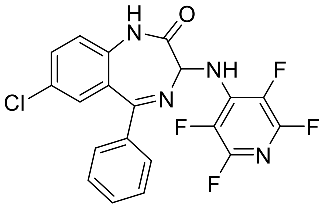
120



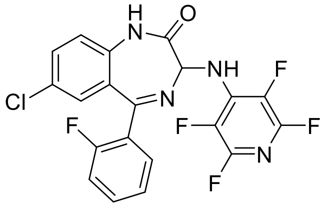
121



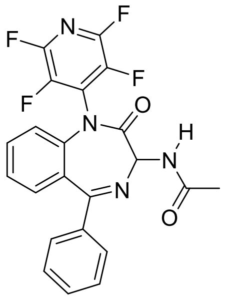
43



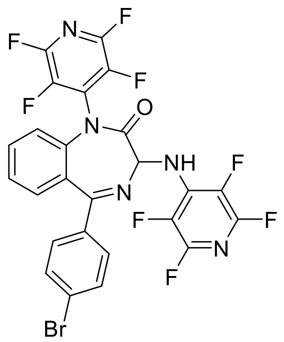
116



117



122



118

Figure 31: Structures of compounds **43**, **116-122**

Entry	*Bacillus subtilis*	*Enterococcus faecium*	*Listeria monocytogenes*	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Streptococcus pyogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella enterica subsp. enterica*	*Mycobacterium tuberculosis mc*^{2}*7000*
	MIC (µM)										
119	-	-	-	-	-	-	-	-	-	-	25.67
120	-	-	-	-	-	-	-	-	-	-	69.55
121	-	-	-	-	-	-	-	-	-	-	18.76
43	18.40	-	36.80	9.20	18.40	18.40	-	-	-	-	18.40
116	19.98	-	39.97	19.98	19.98	39.97	-	-	-	-	39.97
117	35.34	-	-	17.67	17.67	35.34	-	-	-	-	35.34
122	-	-	-	-	N/T	-	-	-	-	N/T	N/T
118	-	-	-	-	-	-	-	-	-	-	-

- = no activity; N/T = not tested.

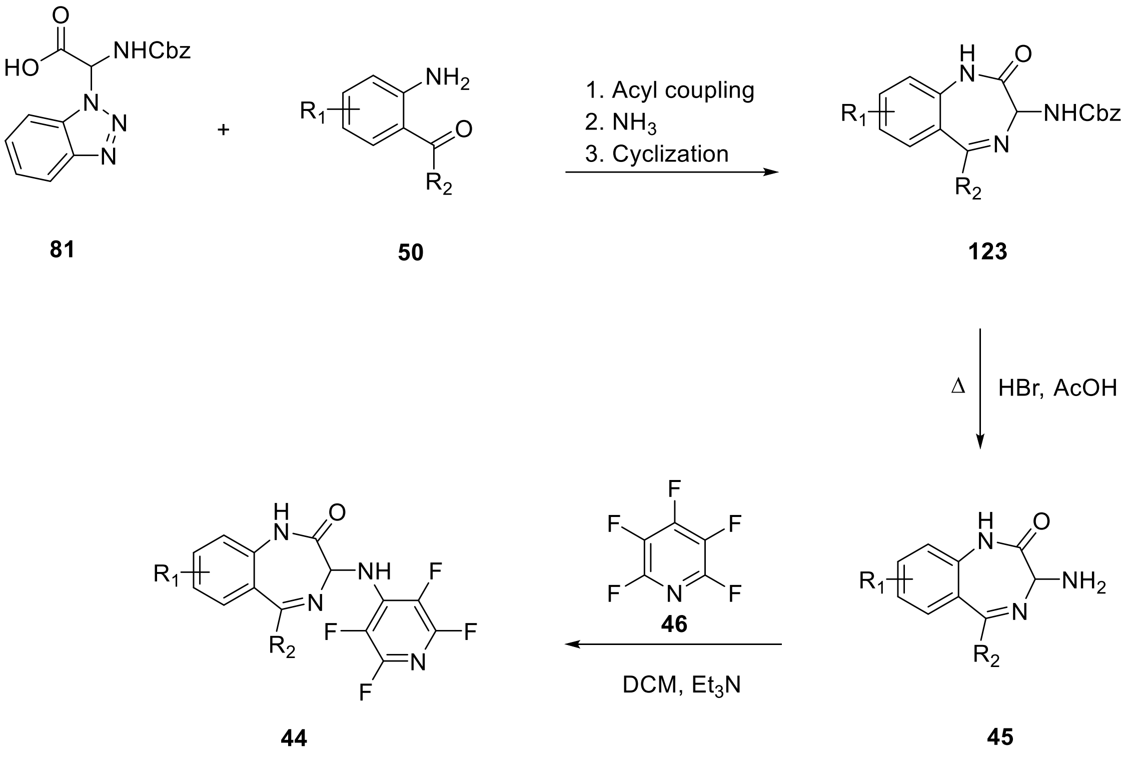
Table 2: MIC results for compounds **43**, **116-122**.

4.0 Conclusion and Further Work

4.1 Conclusion

The aim of this project was to synthesize a variety of substituted benzodiazepines containing the unique fluoropyridine moiety and investigate the structure-activity relationship surrounding the benzodiazepine core in relation to their antibacterial activity. Towards this end, several important methods to the synthesis of benzodiazepine cores have been studied and the most suitable approach was utilized and improved in our work.

In respect to benzodiazepine core synthesis, the starting material **81** was synthesized via a one-pot reaction and was treated with oxalyl chloride and catalytic DMF to form the acyl chloride. Then it was coupled to 2-aminobenzophenone derivative **50** to give acyl amide which was treated directly with ammonia solvent to provide amino ketone. Follow on, the cyclization of amino ketone provided the benzodiazepine core **123**. To remove the Cbz protecting group, the compound **123** was treated with saturated HBr in acetic acid to provide Cbz deprotected product **45**. Finally, coupling of deprotected benzodiazepine **45** with pentafluoropyridine **46** afforded our target molecule **44** (Scheme 24).

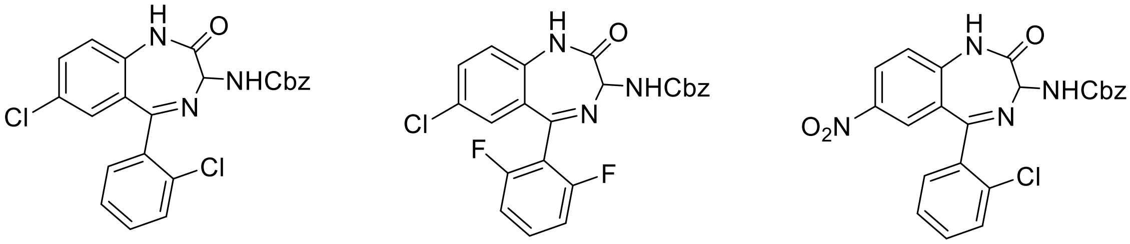


Scheme 24**:** Synthetic route to our target molecule **44**.

The final compounds **43**, **116-118** were successfully synthesized and tested against a variety of bacteria. Compared with the biological investigation results of the compounds in the previous work, some valuable conclusions can be made: the substitutions on the fluoropyridine moiety of target molecules greatly influence the activity against bacteria while the substitutions by chlorine and fluorine on the aromatic rings did not decrease their antibacterial efficiency. The coupling of pentafluoropyridine **46** on N1 contributed to the inactivation to all tested bacteria with steric hindrance possibly also influence the antibacterial efficiency.

4.2 Further Work

Following these studies, the synthetic route to benzodiazepine cores can be further improved to increase the yields. In that way, the benzodiazepine **99** is likely to be synthesized and isolated in a large quantity. In addition, several possible alternative strategies that we have not used for the synthesis remain to be explored. The formation of the benzodiazepines **102** and **103** may be available using different methods (Figure 32)



99 **102** **103**

Figure 32: The structures of benzodiazepines **99**, **102** and **103**.

The synthesis of target molecules **43**, **116-118** in this project was a big success and most of them showed great biological activity against a plethora of gram positive, gram negative and mycolata bacteria. Therefore, it is worth trying the synthetic route on different 2-aminobenzophenone derivatives. More target compounds with fluoropyridine moieties ought to be prepared and biologically investigated.

5.0 Experimental Procedures

5.1 General Procedures

All sensitive reactions were carried out in dried glassware under a nitrogen atmosphere unless otherwise stated.

Solvents

Solvents were obtained from an Innovative Technology Solvent Purification System. In cases where mixtures of solvents were utilised, the ratios refer to the volumes used.

Reagents

Reagents were used as supplied unless otherwise stated.

Chromatography

Reactions were monitored using thin layer chromatography (TLC) on precoated glass-backed plates (silica gel 60Å F_{254}). Materials were visualized by UV radiation at 254 nm, or by staining with phosphomolybdic acid in ethanol or potassium permanganate in water. Purification of products was performed by flash column chromatography using normal phase silica gel 40-63µm 60Å.

Melting point

All melting points were determined using Stuart Digital Advanced Melting Point Apparatus (SMP30).

IR spectroscopy

Infrared spectra were recorded using a Diamond ATR (attenuated total reflection) accessory (Golden Gate) on a Perkin-Elmer FT-IR spectrometer (Spectrum Two).

NMR spectroscopy

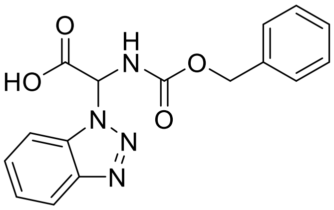
^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO on Varian Mercury-200, Bruker Avance-400, Varian Inova-500 or Varian VNMRS 700 instruments and are reported as follows; chemical shift δ (ppm) (number of protons, multiplicity, coupling constant *J* (Hz), assignment). Residual protic solvent $CHCl_3$ (δ_H = 7.26 ppm) or DMSO (δ_H = 2.50 ppm, 3.33 ppm) was used as the internal reference. ^{13}C NMR spectra were recorded at 176 MHz, using the central resonance of $CDCl_3$ (δ_C = 77.0 ppm) as the internal reference. ^{19}F NMR spectra were recorded at 376 MHz on Varian VCR-400. All chemical shifts are quoted in parts per million relative to the internal reference and coupling constants are given in Hertz (Hz). Assignment and determination of stereochemistry were carried out using COSY, HSQC, HMBC and NOESY experiments.

Mass spectroscopy

Electrospray mass spectra (ES) were obtained on a Micromass LCT mass spectrometer. Gas Chromatography Mass spectra (GC-MS:EI, Cl) were taken using a Thermo-Finnigan Trace within a 25 cm column connected to a VG Mass Lab Trio 1000. High resolution accurate mass measurement was performed on a LTQFT mass spectrometer (Thermo Finnigan Corporation) using flow-injection electrospray ionization at the Universtiy of Durham.

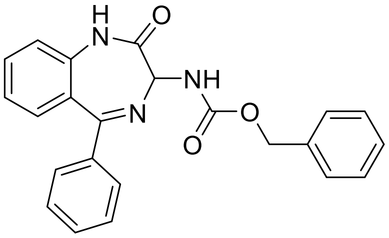
5.2 Experimental Details

2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine 81



Benzyl carbamate **85** (1.51 g, 10.0 mmol), benzotriazole **87** (1.19 g, 10.0 mmol) and glyoxylic acid **86** monohydrate (0.92 g, 10.0 mmol) were dissolved in toluene (30 ml). The reaction mixture was stirred and refluxed in a Dean-Stark apparatus for 2 h. The temperature was set at 120 °C in the beginning and then slowly raised up to 150 °C in order to evaporate the water. After refluxing, the mixture was cooled down to room temperature and the product precipitated. The product was filtered and washed with ether. It was left to dry in the air overnight to afford the title compound **81** as a white solid (2.97 g, 91 %). R_f 0.5 (EtOAc/pyridine/H_2O/AcOH 30:10:5:34); m.p. 156 - 160 °C ; ν_{max} (ATR) 3437-3221 (broad-OH), 2939, 1716 (C=O), 1641 (C=O), 1527, 1495, 1456, 1416, 1364, 1345, 1305, 1282, 1250, 1227, 1168, 1147, 1105, 1052, 990, 936, 903 cm^{-1}; δ_H (400 MHz, DMSO) 9.34 (1H, d, *J* 8, CON*H*), 8.06 (1H, d, *J* 8, NC*H*N), 7.17-8.00 (9H, m, Ar-*H*), 5.05 (2H, m, OCH_2-Ar); m/z (ES^+) 327 (MH^+), 349 (MNa^+), 653 ($2M+H^+$), 675 ($2M+Na^+$); all data agree with those reported in the literature.

1.3-Dihydro-5-phenyl-3(*R*,*S*)-[(benzyloxycarbonyl)amino]-2*H*-1,4-benzodiazepin-2-one 84



Stage 1

2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.25 g, 0.77 mmol) was dissolved in anhydrous THF (2.5 ml) under N_2 and cooled to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 0.38 ml, 0.77 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-aminobenzophenone **71** (0.14 g, 0.69 mmol) and 4-methylmorpholine (0.15 ml, 1.54 mmol) in anhydrous THF (0.75

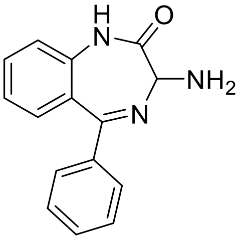
ml) was added dropwise to the reaction mixture by syringe. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. The mother liquor containing acyl amide was used directly in the next reaction.

Stage 2

Ammonia solution (2 M in ethanol, 2.1 ml, 4.14 mmol) was added to the acyl amide from the last step under N_2 to react overnight. Ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 15 ml). The combined aqueous layers were back-extracted with ethyl acetate (20 ml). The organic layers were combined, washed twice with brine (2 x 15 ml). It was then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

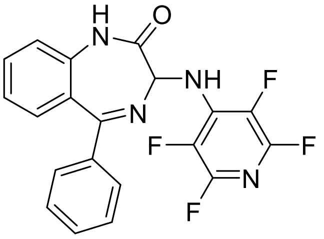
The crude amine (0.33 g) was combined with ammonium acetate (0.25 g, 3.24 mmol) and then they were dissolved in glacial acetic acid (5 ml, 87.43 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and then concentrated *in vacuo.* Ethyl acetate (1.25 ml) and diethyl ether (3.75 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. After that, the mixture was cooled to 0 - 5 °C with an ice-water bath and filtered. The product was washed with water and diethyl ether and dried in the air over weekend to afford the title compound **84** as a light brown solid (91 mg, 34 %). R_f 0.4 (n-hexane/EtOAc 7:3); δ_H (400 MHz, $CDCl_3$) 8.08 (1H, s, CON*H*), 7.12-7.58 (14H, m, Ar-*H*), 6.59 (1H, d, *J* 8, N*H*-Cbz), 5.36 (1H, d, *J* 8, NC*H*N), 5.17 (2H, m, OC*H*$_2$-Ar) ; m/z (ES^+) 386 (MH^+), 408 (MNa^+), 771 ($2M+H^+$), 793 ($2M+Na^+$); all data agree with those reported in the literature.

3-Amino-5-phenyl-1,3-dihydrobenzo[*e*][1,4]diazepin-2-one 80



1.3-Dihydro-5-phenyl-3(*R*,*S*)-[(benzyloxycarbonyl)amino]-2*H*-1,4-benzodiazepin-2-one **84** (60 mg, 0.16 mmol) was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 5 ml, 86.52 mmol). The reaction solution was stirred and refluxed at 70 °C for 30 min. Then the temperature was raised to 80 °C and maintained for another 30 min. After refluxing, the mixture was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. There was no slurry formed because of too small amount of starting material. The solvent was evaporated and anhydrous diethyl ether (5 ml) was added to the mixture again. The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the reaction mixture. Then the free base was isolated by removing the basic aqueous layer. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in* *vacuo*. The title compound **80** was obtained as a yellow solid (32 mg, 82 %). R_f 0.7 (n-hexane/EtOAc 7:3); δ_H (400 MHz, DMSO) 11.12 (1H, s, CON*H*), 8.80 (2H, s, NH_2), 7.03-7.58 (9H, m, Ar-*H*), 4.90 (1H, s, NC*H*N); m/z (ES^+) 252 (MH^+), 274 (MNa^+), 525 ($2M+Na^+$); all data agree with those reported in the literature.

3-((perfluoropyridin-4-yl)amino)-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4] diazepin-2-one 43



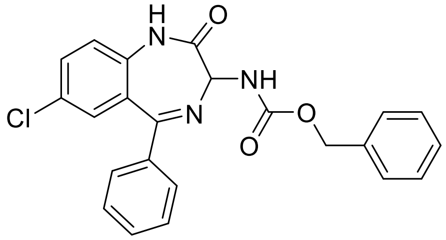
Stage 1

1.3-Dihydro-5-phenyl-3(*R*,*S*)-[(benzyloxycarbonyl)amino]-2*H*-1,4-benzodiazepin-2-one (77 mg, 0.20 mmol) **84** was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 5 ml, 86.52 mmol). The reaction solution was stirred and refluxed at 70 °C for 30 min. Then the temperature was raised to 80 °C and maintained for another 30 min. After refluxing, the mixture was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. There was no slurry formed because of too small amount of starting material. The solvent was evaporated and anhydrous diethyl ether (5 ml) was added to the mixture again. The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the reaction mixture. Then the free base was isolated by removing the basic aqueous layer. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The 3-amino-5-phenyl-1,3-dihydrobenzo[*e*][1,4]diazepin-2-one was obtained as an orange solid (67 mg) and used directly in the next step.

Stage 2

Pentafluoropyridine **46** (0.05 ml, 0.46 mmol) was added to the crude compound (50 mg) from the last stage and they were dissolved in dichloromethane (2 ml). Triethylamine (0.10 ml, 0.72 mmol) was added to the reaction solution which was later stirred under N_2 at room temperature overnight. The resulting solution was diluted with dichloromethane (10 ml) and then washed twice with water (2 x 10 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude compound was obtained as a brown gum (42 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1]) afforded the title compound **80** as a white solid (23 mg, 39 %). R_f 0.4 (n-hexane/EtOAc 7:3); m.p. 221 - 223 °C; ν_{max} (ATR) 3355, 3196, 3067, 2907, 1698 (C=O), 1647, 1601, 1579, 1546, 1478, 1447, 1428, 1411, 1384, 1328, 1275, 1227, 1185, 1170, 1160, 1131, 1083, 1014, 972, 934 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.41 (1H, s, CON*H*), 7.20-7.66 (9H, m, Ar-*H*), 6.70 (1H, d, *J* 8, N*H*-Py), 5.41 (1H, d, *J* 8, NC*H*N); δ_C (176 MHz, $CDCl_3$) 168.1 (*C*=O), 167.6 (*C*-Ph), 138.3 (Py-*C*-NH), 136.8 (*ipso*-Ar-*C*), 132.6 (*C*-F × 2), 131.5 (*C*-F × 2), 131.0 (Ar-*C*, *ipso*-Ar-*C*), 129.7 (Ar-*C* × 2), 128.4 (Ar-*C* × 2), 127.5 (*ipso*-Ar-*C*), 124.7 (Ar-*C* × 2), 121.4 (Ar-*C* × 2), 70.1 (N*C*HN); m/z (ES^+) 401 (MH^+), 423 (MNa^+); HRMS (ES^+) Found MH^+, 401.1023 ($C_{20}H_{13}F_4N_4O$ requires 401.1025).

Benzyl (7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl) carbamate 91



Stage 1

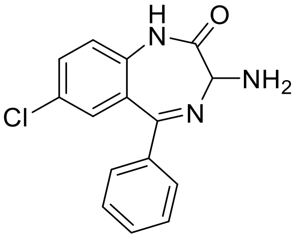
2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.50 g, 1.53 mmol) was dissolved in anhydrous THF (5 ml) under N_2 when cooling to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 0.77 ml, 1.53 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-amino-5-chlorobenzophenone **90** (0.32 g, 1.40 mmol) and 4-methylmorpholine (0.34 ml, 3.06 mmol) in anhydrous THF (1.5 ml) was added dropwise to the reaction mixture by syringe. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. After that, the mother liquor containing acyl amide was concentrated *in vacuo*. The crude acyl amide was used directly in the next step.

Stage 2

Ammonia solution (2 M in ethanol, 4.2 ml, 8.40 mmol) was added to the acyl amide from the last step under N_2 to react overnight. Then ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 20 ml). The combined aqueous layers were back-extracted with ethyl acetate (20 ml). The organic layers were combined, washed twice with brine (2 x 20 ml). It was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

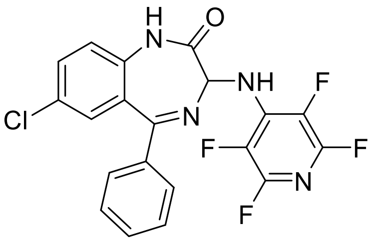
The crude amine (1.19 g) was combined with ammonium acetate (0.50 g, 6.49 mmol) and they were dissolved in glacial acetic acid (10 ml, 174.69 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and then concentrated *in vacuo.* Ethyl acetate (2.5 ml) and diethyl ether (7.5 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. After that, the reaction mixture was cooled to 0 - 5 °C with an ice-water bath but only little solid formed. Ethyl acetate (20 ml) was used to extract the reaction and the aqueous layer was back-extracted with ethyl acetate (20 ml). Then the combined organic layers were washed with brine (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* Much more solid formed when the product was left in the air overnight. Finally the product was washed with a solution of ethyl acetate and hexane to afford the title compound **91** as a white solid (269 mg, 46 %). R_f 0.4 (n-hexane/EtOAc 7:3); m.p. 180 - 183 °C; ν_{max} (ATR) 3209, 3142, 3062, 2935, 1708 (C=O), 1684 (C=O), 1608, 1578, 1533, 1498, 1478, 1455, 1447, 1386, 1360, 1325, 1285, 1257, 1229, 1179, 1165, 1127, 1094, 1081, 1066, 1021, 1002, 980, 942, 928 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.60 (1H, s, CON*H*), 7.09-7.56 (13H, m, Ar-*H*), 6.57 (1H, d, *J* 8, N*H*-Cbz), 5.33 (1H, d, *J* 8, NC*H*N), 5.17 (2H, m, OC*H*$_2$-Ar); δ_C (176 MHz, CDCl$_3$) 168.0 (*C*=O), 166.6 (N-*C*-Ph), 155.7 (O-*C*=O), 137.8 (*ipso*-Ar-*C*), 136.2 (*ipso*-Ar-*C*), 135.8 (*ipso*-Ar-*C*), 132.3 (Ar-*C*), 131.0 (Ar-*C*, *ipso*-Ar-*C*), 130.8 (Ar-*C*), 129.8 (Ar-*C*), 129.7 (Ar-*C* × 2), 128.6 (*ipso*-Ar-*C*), 128.5 (Ar-*C* × 2), 128.4 (Ar-*C* × 2), 128.1 (Ar-*C* × 2), 122.8 (Ar-*C*), 69.0 (N*C*HN), 67.1 (O*C*H$_2$-Ar); m/z (ES$^+$) 420 ([^{35}Cl]MH$^+$), 422 ([^{37}Cl]MH$^+$), 442 ([^{35}Cl]MNa$^+$), 444 ([^{37}Cl]MNa$^+$), 861 ([^{35,35}Cl]2M+Na$^+$), 863 ([^{35,37}Cl]2M+Na$^+$), 865 ($^{[37,37}$Cl]2M+Na$^+$); HRMS (ES$^+$) Found MH$^+$, 420.1128 (C$_{23}$H$_{19}$^{35}ClN$_3$O$_3$ requires 420.1115).

3-Amino-7-chloro-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one 111



Benzyl(7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **91** (150 mg, 0.36 mmol) was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 5 ml, 86.52 mmol). The reaction solution was stirred, refluxed at 70 °C and held for 20 min. Then the temperature was raised to 80 °C and maintained for another 20 min. After refluxing, the resulting yellow slurry was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. Yellow solid was obtained by filtration. Then the resulting solid was washed with anhydrous diethyl ether and dried under vacuum to provide the dihydrobromide salt (124 mg, 0.28 mmol). The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the dihydrobromide salt. Then the free base was isolated by removing the basic aqueous layer. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **111** was obtained as a white solid (72 mg, 71 %). R_f 0.5 (DCM/MeOH 9:1); m.p. 197 - 200 °C; ν_{max} (ATR) 3383, 3315, 3063, 3017, 2889, 2813, 2708, 1690 (C=O), 1603, 1591, 1575, 1558, 1447, 1411, 1388, 1337, 1328, 1317, 1288, 1230, 1194, 1166, 1129, 1093, 1080, 1014, 939, 930, 912 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.26 (1H, s, CON*H*), 7.08-7.58 (8H, m, Ar-*H*), 4.50 (1H, s, NC*H*N), 2.50 (2H, bs, NH_2); δ_C (176 MHz, $CDCl_3$) 170.6 (*C*=O), 165.4 (*C*-Ph), 138.1 (*ipso*-Ar-*C*), 136.2 (*ipso*-Ar-*C*), 132.1 (Ar-*C*), 130.8 (Ar-*C*), 130.4 (*C*-Cl), 129.6 (Ar-*C* × 2), 129.3 (Ar-*C*), 129.0 (*ipso*-Ar-*C*), 128.4 (Ar-*C* × 2), 122.5 (Ar-*C*), 70.3 (*C*HNH$_2$); m/z (ES^+) 286 ([^{35}Cl]MH^+), 288 ([^{37}Cl]MH^+), 308 ([^{35}Cl]MNa^+), 310 ([^{37}Cl]MMa^+), 593 ([^{35,35}Cl]2M+Na^+), 595 ([^{35,37}Cl]2M+Na^+), 597 ([^{37,37}Cl]2M+Na^+); HRMS (ES^+) Found MH^+, 286.0753 ($C_{15}H_{13}{}^{35}ClN_3O$ requires 286.0747).

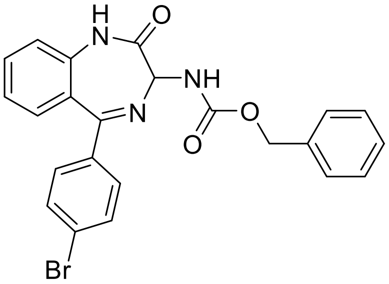
7-Chloro-3-((perfluoropyridin-4-yl)amino)-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one 116



3-Amino-7-chloro-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one **111** (45 mg, 0.16 mmol) and pentafluoropyridine **46** (0.02 ml, 0.18 mmol) were dissolved in a solution of dichloromethane (3 ml) and methanol (1 ml). Triethylamine (0.03 ml, 0.22 mmol) was also added to the reaction which was stirred under N_2 at room temperature. After stirring for one night, the reaction was still not complete and additional pentafluoropyridine **46** (0.04 ml, 0.36 mmol) and triethylamine (0.06 ml, 0.43 mmol) were added to react over weekend. The resulting solution was diluted with dichloromethane (10 ml) and then washed twice with water (2 x 10 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude compound was obtained as a light yellow solid (111 mg). Flash chromatography (DCM, DCM/MeOH [95:5], [9:1]) afforded the title compound **116** as a light yellow solid (69 mg, 99 %). R_f 0.8 (DCM/MeOH 9:1); m.p. 239 - 240 °C; ν_{max} (ATR) 3312, 3133, 2961, 1705 (C=O), 1647, 1598, 1545, 1481, 1446, 1411, 1390, 1325, 1290, 1276, 1260, 1221, 1186, 1167, 1130, 1083, 1017, 977, 942, 933 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.35 (1H, s, CON*H*), 7.16 -7.62 (8H, m, Ar-*H*), 6.64 (1H, d, *J* 8, N*H*-Py), 5.40 (1H, d, *J* 8, NC*H*N);); δ_C (176 MHz, $CDCl_3$) 167.1 (*C*=O), 166.8 (*C*-Ph), 137.6 (Py-*C*-NH), 135.3 (*ipso*-Ar-*C* × 2), 132.8 (*C*-F × 2), 131.4 (*C*-F × 2), 130.8

(Ar-*C* × 2), 130.4 (*C*-Cl), 129.6 (Ar-*C* × 2), 128.7 (*ipso*-Ar-*C*), 128.6 (Ar-*C* × 2), 122.9 (Ar-*C* × 2), 70.1 (N*C*HN); δ_F (376 MHz, $CDCl_3$) -89.30 (1F, m, Py-*F*), -93.38 (1F, m, Py-*F*), -144.53 (1F, m, Py-*F*), -164.39 (1F, m, Py-*F*); m/z (ES^+) 435 ([^{35}Cl]MH^+), 437 ([^{37}Cl]MH^+), 457 ([^{35}Cl]MNa^+), 459 ([^{37}Cl]MNa^+); HRMS (ES^+) Found MH^+, 435.0633 ($C_{20}H_{12}{}^{35}ClF_4N_4O$ requires 435.0636).

Benzyl (5-(4-bromophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl) carbamate 89



Stage 1

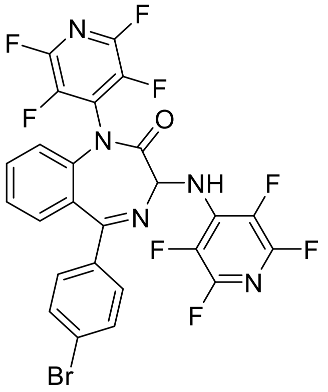
2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.50 g, 1.53 mmol) was dissolved in anhydrous THF (5 ml) under N_2 when cooling to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 1.05 ml, 2.10 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-amino-4'-bromobenzophenone **88** (0.39 g, 1.40 mmol) and 4-methylmorpholine (0.46 ml, 4.20 mmol) in anhydrous THF (1.5 ml) was added dropwise to the reaction mixture by syringe. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. After that, the mother liquor containing acyl amide was concentrated *in vacuo*. The crude acyl amide was used directly in the next step.

Stage 2

Ammonia solution (2 M in ethanol, 4.2 ml, 8.40 mmol) was added to the acyl amide from the last step under N_2 to react over weekend. Then ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 20 ml). The combined aqueous layers were back-extracted with ethyl acetate (20 ml). The organic layers were combined, washed twice with brine (2 x 20 ml). It was then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

The crude amine (1.23 g) was combined with ammonium acetate (0.50 g, 6.49 mmol) and they were dissolved in glacial acetic acid (10 ml, 174.69 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and concentrated *in vacuo.* Ethyl acetate (2.5 ml) and diethyl ether (7.5 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. The reaction mixture was cooled to 0 - 5 °C with an ice-water bath but only little solid formed. Ethyl acetate (20 ml) was used to extract the reaction and the aqueous layer was back-extracted with ethyl acetate (20 ml). Then the combined organic layers were washed with brine (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was obtained as a brown gum (638 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1], [4:1], [7:3], [3:2], [1:1]) afforded the title compound **89** as a yellow solid (324 mg, 50 %). R_f 0.5 (n-hexane/EtOAc 7:3); m.p. 174 - 176 °C ; ν_{max} (ATR) 3417, 3212, 3032, 2956, 1713 (C=O), 1689 (C=O), 1614, 1586, 1526, 1496, 1476, 1446, 1389, 1358, 1324, 1292, 1254, 1230, 1194, 1179, 1162, 1121, 1083, 1060, 1026, 1010, 975, 953, 937, 927, 911 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.02 (1H, s, CON*H*), 7.14-7.84 (13H, m, Ar-*H*), 6.62 (1H, d, *J* 8, N*H*-Cbz), 5.32 (1H, d, *J* 8, NC*H*N), 5.16 (2H, m, OCH_2-Ar); δ_C (176 MHz, $CDCl_3$) 168.3 (*C*=O), 166.8 (N-*C*-Ph), 155.8 (O-*C*=O), 137.3 (*ipso*-Ar-*C*), 136.2 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 132.4 (Ar-*C*), 132.0 (Ar-*C*), 131.5 (Ar-*C* × 2), 131.4 (Ar-*C* × 2), 131.1 (Ar-*C*), 128.5 (Ar-*C* × 2), 128.2 (Ar-*C*), 128.1 (Ar-*C* × 2), 125.4 (*ipso*-Ar-*C*), 125.3 (*ipso*-Ar-*C*), 121.5 (Ar-*C*), 69.0 (N*C*HN), 67.1 (O*C*H_2-Ar); m/z (ES^+) 464 ([^{79}Br]MH^+), 466 ([^{81}Br]MH^+), 486 ([^{79}Br]MNa^+), 488 ([^{81}Br]MNa^+), 949 ([^{79,79}Br]2M+Na^+), 951 ([^{79,81}Br]2M+Na^+), 953 ([^{81,81}Br]2M+Na^+); HRMS (ES^+) Found MH^+, 464.0602 ($C_{23}H_{19}{}^{79}BrN_3O_3$ requires 464.0610).

5-(4-bromophenyl)-1-(perfluoropyridin-4-yl)-3-((perfluoropyridin-4-yl)amino)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one 118



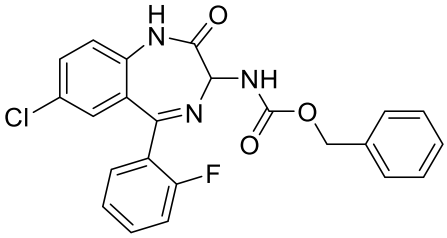
Stage 1

Benzyl (5-(4-bromophenyl) - 2 - oxo - 2,3 - dihydro - 1*H* - benzo[*e*][1,4]diazepin - 3 - yl) carbamate **89** (100 mg, 0.22 mmol) was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 4 ml, 69.21 mmol). The reaction solution was stirred and refluxed at 70 °C for 20 min. Then the temperature was raised to 80 °C and maintained for another 20 min. After refluxing, the resulting yellow slurry was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. The solvent was evaporated to provide the crude dihydrobromide salt as a brown gum (234 mg). The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the crude dihydrobromide salt. Then the free base was isolated by removing the basic aqueous layer. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude 3-amino-5-(4-bromophenyl)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one was obtained as a brown gum (67 mg) and used directly in the next step.

Stage 2

Pentafluoropyridine **46** (0.05 ml, 0.46 mmol) was added to the crude compound from the last stage and they were dissolved in dichloromethane (2 ml). Triethylamine (0.1 ml, 0.72 mmol) was also added to the reaction solution which was later stirred under N_2 at room temperature overnight. After that, the reaction solution was diluted with dichloromethane (10 ml) and washed twice with water (2 x 10 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude product was obtained as a brown gum (161 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1], [4:1], [7:3]) afforded the title compound **118** as an orange solid (37 mg, 35 %). R_f 0.7 (n-hexane/EtOAc 7:3); m.p. 76 - 79 °C; ν_{max} (ATR) 2955, 2924, 2854, 1721(C=O), 1641, 1603, 1588, 1544, 1475, 1446, 1418, 1395, 1362, 1311, 1267, 1246, 1215, 1189, 1122, 1097, 1070, 1054, 1011, 997, 966, 930 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.07-7.67 (8H, m, Ar-*H*), 6.47 (1H, d, *J* 8, N*H*-Py), 5.72 (1H, d, *J* 8, NC*H*N); δ_C (176 MHz, $CDCl_3$) 167.3 (*C*=O), 164.9 (*C*-Ph), 141.8 (Py-*C*-N), 138.6 (Py-*C*-N), 137.5 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 133.2 (Ar-*C* × 2), 132.0 (*C*-F × 4), 130.9 (*C*-F × 4), 130.7 (Ar-*C* × 2), 127.3 (Ar-*C* × 2), 126.5 (Ar-*C*), 123.1 (Ar-*C*, *ipso*-Ar-*C*), 116.3 (*C*-Br), 70.6 (N*C*HN); δ_F (376 MHz, $CDCl_3$) -86.56 (1F, m, Py-*F*), -86.88 (1F, m, Py-*F*), -92.84 (2F, m, Py-*F*), -140.73 (1F, m, Py-*F*), -146.43 (1F, m, Py-*F*), -161.84 (2F, m, Py-*F*); m/z (ES^+) 628 ([^{79}Br]MH^+), 630 ([^{81}Br]MH^+), 650 ([^{79}Br]MNa^+), 652 ([^{81}Br]MNa^+); HRMS (ES^+) Found MH^+, 628.0048 ($C_{25}H_{11}{}^{79}BrF_8N_5O$ requires 628.0019).

Benzyl (7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4] diazepin-3-yl)carbamate 94



Stage 1

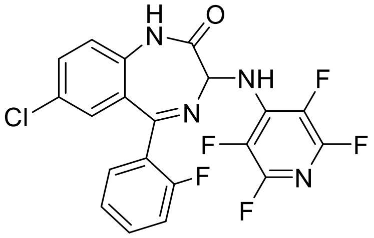
2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.50 g, 1.53 mmol) was dissolved in anhydrous THF (5 ml) under N_2 when cooling to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 1.05 ml, 2.10 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-amino-5-chloro-2'-fluoro-benzophenone **92** (0.35 g, 1.40 mmol) and 4-methylmorpholine (0.46 ml, 4.20 mmol) in anhydrous THF (1.5 ml) was added dropwise to the reaction mixture by syringe. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. After that, the mother liquor containing acyl amide was concentrated *in vacuo*. The crude acyl amide was used directly in the next step.

Stage 2

Ammonia solution (2 M in ethanol, 4.2 ml, 8.40 mmol) was added to the acyl amide from the last step under N_2 to react over weekend. Then ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 20 ml). The combined aqueous layers were back-extracted with ethyl acetate (30 ml). The organic layers were combined, washed twice with brine (2 x 20 ml). It was then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

The crude amine (1.01 g) was combined with ammonium acetate (0.50 g, 6.49 mmol) and they were dissolved in glacial acetic acid (10 ml, 174.69 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and concentrated *in vacuo.* Ethyl acetate (2.5 ml) and diethyl ether (7.5 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. Ethyl acetate (20 ml) was used to extract the reaction and the aqueous layer was back-extracted with ethyl acetate (20 ml). Then the combined organic layers were washed with brine (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was obtained as a brown gum (742 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1], [17:3], [4:1], [7:3]) afforded the title compound **94** as a yellow solid (301 mg, 49 %). R_f 0.3 (n-hexane/EtOAc 7:3); m.p. 166 - 169 °C; ν_{max} (ATR) 3414, 3218, 3148, 3063, 2925, 1712 (C=O), 1691 (C=O), 1613, 1576, 1501, 1482, 1464, 1452, 1399, 1363, 1327, 1286, 1267, 1228, 1213, 1166, 1136, 1098, 1059, 1028, 1016, 1003, 991, 976, 945, 908 cm^{-1}; δ_H (400 MHz, CDCl$_3$) 8.64 (1H, m, CON*H*), 7.00-7.75 (12H, m, Ar-*H*), 6.60 (1H, d, *J* 8, N*H*-Cbz), 5.34 (1H, d, *J* 8, NC*H*N), 5.17 (2H, m, OC*H*$_2$-Ar); δ_C (176 MHz, CDCl$_3$) 167.5 (*C*=O), 163.5 (N-*C*-Ph), 161.1 (*C*-F), 155.7 (O-*C*=O), 136.1 (*ipso*-Ar-*C*), 134.7 (*ipso*-Ar-*C*), 132.7 (Ar-*C*), 132.4 (Ar-*C*), 131.8 (*C*-Cl), 130.2 (Ar-*C*), 129.8 (*ipso*-Ar-*C*), 129.3 (Ar-*C*), 128.7 (Ar-*C*), 128.5 (Ar-*C* × 2), 128.1 (Ar-*C* × 2), 124.6 (*ipso*-Ar-*C*), 122.9 (Ar-*C*), 116.3 (Ar-*C*), 116.1 (Ar-*C*), 69.0 (N*C*HN), 67.1 (O*C*H$_2$-Ar); δ_F (376 MHz, CDCl$_3$) -111.97 (1F, m, Ar-*F*); m/z (ES$^+$) 438 ([^{35}Cl]MH$^+$), 440 ([^{37}Cl]MH$^+$), 460 ([^{35}Cl]MNa$^+$), 462 ([^{37}Cl]MNa$^+$), 897 ([^{35,35}Cl]2M+Na$^+$), 899 ([^{35,37}Cl]2M+Na$^+$), 901 ([^{37,37}Cl]2M+Na$^+$); HRMS (ES$^+$) Found MH$^+$, 438.1018 ($C_{23}H_{18}{}^{35}ClFN_3O_3$ requires 438.1021).

7-chloro-5-(2-fluorophenyl)-3-((perfluoropyridin-4-yl)amino)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one 117



Stage 1

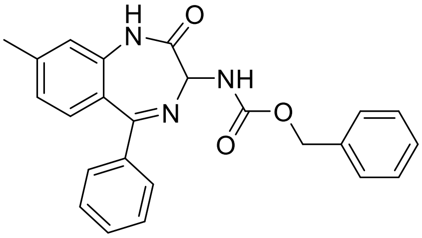
Benzyl (7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl) carbamate **94** (200 mg, 0.46 mmol) was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 5 ml, 86.52 mmol). The reaction solution was stirred and refluxed at 70 °C for 20 min. Then the temperature was raised to 80 °C and maintained for another

20 min. After refluxing, the resulting yellow slurry was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. The solvent was evaporated to provide the brown acicular dihydrobromide salt (183 mg, 0.39 mmol). The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the dihydrobromide salt. Then the free base was isolated by removing the basic aqueous layer. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude 3-amino-7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one was obtained as a light brown solid (89 mg) and used directly in the next step.

Stage 2

Pentafluoropyridine **46** (0.05 ml, 0.46 mmol) was added to the crude compound (75 mg) from the last stage and they were dissolved in dichloromethane (2 ml). Triethylamine (0.1 ml, 0.72 mmol) was also added to the reaction solution which was later stirred under N_2 at room temperature overnight. After that, the reaction solution was diluted with dichloromethane (10 ml) and washed twice with water (2 x 10 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The crude product was obtained as a brown gum (110 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1]) afforded the title compound **117** as yellow solid (54 mg, 31 %). R_f 0.4 (n-hexane/EtOAc 7:3); m.p. 205 - 208 °C; ν_{max} (ATR) 3373, 3200, 3076, 2929, 1689 (C=O), 1647, 1610, 1539, 1478, 1452, 1413, 1394, 1376, 1324, 1268, 1219, 1188, 1169, 1133, 1083, 1021, 972, 946 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.25 (1H, s, CON*H*), 7.04-7.73 (7H, m, Ar-*H*), 6.69 (1H, d, *J* 8, N*H*-Py), 5.41 (1H, d, *J* 8, NC*H*N); δ_C (126 MHz, $CDCl_3$) 165.87 (*C*=O), 131.80 (Ar-*C*), 133.32 (Ar-*C*), 133.04 (Ar-*C*), 129.74 (Ar-*C*), 125.17 (Ar-*C*), 123.36 (Ar-*C*), 116.54 (Ar-*C*), 70.26 (N*C*HN); δ_F (376 MHz, $CDCl_3$) -93.26 (2F, s, Py-*F*), -112.32 (1F, s, Ar-*F*), -161.65 (2F, s, Py-*F*); m/z (ES$^+$) 453 ([^{35}Cl]MH$^+$), 455 ([^{37}Cl]MH$^+$), 475 ([^{35}Cl]MNa$^+$), 477 ([^{37}Cl]MNa; HRMS (ES$^+$); Found MH$^+$, 453.0545 ($C_{20}H_{11}{}^{35}ClF_5N_4O$ requires 453.0542).

Benzyl (8-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl) carbamate 95



Stage 1

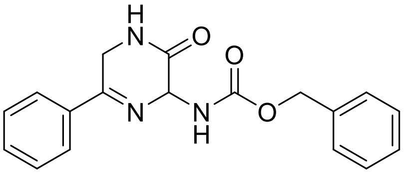
2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.50 g, 1.53 mmol) was dissolved in anhydrous THF (5 ml) under N_2 when cooling to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 1.05 ml, 2.10 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-amino-4-methylbenzophenone **93** (0.30 g, 1.40 mmol) and 4-methylmorpholine (0.46 ml, 4.20 mmol) in anhydrous THF (1.5 ml) was added dropwise to the reaction mixture by syringe. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. After that, the mother liquor containing acyl amide was concentrated *in vacuo*. The crude acyl amide was used directly in the next step.

Stage 2

Ammonia solution (2 M in ethanol, 4.2 ml, 8.40 mmol) was added to the acyl amide from the last step under N_2 to react over weekend. Then ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 20 ml). The combined aqueous layers were back-extracted with ethyl acetate (20 ml). The organic layers were combined, washed twice with brine (2 x 20 ml). It was then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

The crude amine (0.67 g) was combined with ammonium acetate (0.50 g, 6.49 mmol) and they were dissolved in glacial acetic acid (10 ml, 174.69 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and concentrated *in vacuo.* Ethyl acetate (2.5 ml) and diethyl ether (7.5 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. Ethyl acetate (30 ml) was used to extract the reaction and the aqueous layer was back-extracted with ethyl acetate (20 ml). Then the combined organic layers were washed with brine (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was obtained as a brown gum (403 mg). Flash chromatography (n-hexane, n-hexane/EtOAc [9:1], [4:1]) afforded the title compound **95** as a yellow solid (246 mg, 44 %). R_f 0.3 (n-hexane/EtOAc 7:3); δ_H (400 MHz, $CDCl_3$) 8.49 (1H, s, CON*H*), 7.29-7.64 (13H, m, Ar-*H*), 6.60 (1H, d, *J* 8, N*H*-Cbz), 5.33 (1H, d, *J* 8, NC*H*N), 5.16 (2H, s, OC*H*$_2$-Ar), 2.43 (3H, s, C*H*$_3$); m/z (ES^+) 400 (MH^+), 422 (MNa^+), 799 ($2M+H^+$), 821 ($2M+Na^+$).

Benzyl (3-oxo-6-phenyl-2,3,4,5-tetrahydropyrazin-2-yl)carbamate 105



Stage 1

2-(Benzotriazol-1-yl)-*N*-(benzyloxycarbonyl)glycine **81** (0.50 g, 1.53 mmol) was dissolved in anhydrous THF (5 ml) under N_2 when cooling to 0 - 5 °C with an ice-water bath. Oxalyl chloride (2 M in methyl chloride, 1.05 ml, 2.10 mmol) was added to the glycine solution followed by a drop of anhydrous DMF to start the reaction. After maintaining the reaction at 0 - 5 °C for 2 h, a solution of 2-aminoacetophenone **104** hydrochloride (0.24 g, 1.40 mmol) and 4-methylmorpholine (0.46 ml, 4.20 mmol) in anhydrous THF (1.5 ml) was added dropwise to the reaction mixture using pipptte. Then the ice-water bath was removed and the reaction was allowed to warm to room temperature overnight. The reaction slurry was filtered and the filter cake was washed with a small amount of anhydrous THF. After that, the mother liquor containing acyl amide was concentrated *in vacuo*. The crude acyl amide was used directly in the next step.

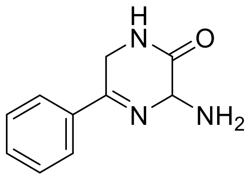
Stage 2

Ammonia solution (2 M in ethanol, 4.2 ml, 8.40 mmol) was added to the acyl amide from the last step under N_2 to react over weekend. Then ethyl acetate (30 ml) was added to the reaction solution, which was later washed twice with aq. NaOH (1 M, 2 x 20 ml). The combined aqueous layers were back-extracted with ethyl acetate (20 ml). The organic layers were combined, washed twice with brine (2 x 20 ml). It was then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.*

The crude amine (0.49 g) was obtained as a yellow solid and combined with ammonium acetate (0.50 g, 6.49 mmol) and they were dissolved in glacial acetic acid (10 ml, 174.69 mmol) under N_2. The reaction mixture was allowed to stir at room temperature overnight and concentrated *in vacuo.* Ethyl acetate (2.5 ml) and diethyl ether (7.5 ml) were added to the mixture. Aq. NaOH (1 M) was also added until the PH of the aqueous layer was greater than 8. Ethyl acetate (30 ml) was used to extract the reaction and the aqueous layer was back-extracted with ethyl acetate (30 ml). Then the combined organic layers were washed with brine (30 ml), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound **105** as an orange solid (298 mg, 66 %). R_f 0.4

(DCM/EtOH/NH_3(aq.) 200:8:1); δ_H (400 MHz, $CDCl_3$) 9.09 (1H, s, N*H*-Cbz), 8.41 (1H, s, CON*H*), 7.42-8.10 (10H, m, Ar-*H*), 6.26 (1H, s, NC*H*N), 5.29 (2H, s, OC*H*$_2$-Ar), 4.12 (2H, q, *J* 8, C*H*$_2$NH); m/z (ES^+) 324 (MH^+), 346 (MNa^+).

3-Amino-5-phenyl-3,6-dihydropyrazin-2(1*H*)-one 115



Benzyl (3-oxo-6-phenyl-2,3,4,5-tetrahydropyrazin-2-yl)carbamate **105** (249mg, 0.77 mmol) was dissolved in the hydrobromic acid solution (33 wt. % in acetic acid, 5 ml, 86.52 mmol). The reaction solution was stirred, refluxed at 70 °C and held for 20 min. Then the temperature was raised to 80 °C and maintained for additional 20 min. After refluxing, the resulting brown slurry was cooled to ambient temperature, diluted with anhydrous diethyl ether (5 ml) and stirred for 30 min. The solvent was evaporated and anhydrous diethyl ether (5 ml) was add to the mixture again. Then the dihydrobromide salt (260 mg, 0.74 mmol) was obtained by filtration. The aq. K_2CO_3 (5 % w/v, 10 ml) and ethyl acetate (10 ml) containing 2-propanol (0.5 ml) and dichloromethane (0.5 ml) were added to the dihydrobromide salt. The free base was isolated by removing the basic aqueous layer. The organic layer was wished with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The title compound **115** was obtained as an orange solid (67 mg, 46 %). R_f 0.4 (DCM/EtOH/NH_3(aq.) 200:8:1); δ_H (400 MHz, $CDCl_3$) 7.89

(1H, s, CON*H*), 7.29-7.54 (5H, m, Ar-*H*), 3.49 (2H, s, CH_2NH), 2.17 (1H, s, $NCHNH_2$); m/z (ES^-) 188 ($M-H^-$).

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