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Synthesis and Biological Activities of Novel Benzamide Based DNA Minor Groove Binding Agents

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

by

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New Zealand
September 2011
Abstract

This thesis describes the synthesis and DNA binding studies of benzamide DNA minor groove binding agents (MGB). MGB are an extensively studied class of compounds due to their potential use as anticancer agents. Some pyrrole based natural antibiotics such as distamycin and netropsin are potent DNA MGB. In order to increase the DNA binding affinity, sequence specificity and alkylating activity, and to minimise the unwanted physiological activities associated with these natural DNA binders, many synthetic oligopeptides have been prepared. Most of the reported MGB are based on five-membered heterocyclic moieties. Some simple symmetrical benzamide based MGB are also known and have shown good DNA binding activities but the fact that they lack active alkylating groups and have few possible structural sites that can be modified has limited their utility for further research. This thesis reports on the synthesis and biological activities of novel non-symmetrical benzamide MGB.

The initial synthetic studies were focused on establishing new and efficient routes towards the synthesis of non-symmetrical di- and triaryl amides derived from dinitrobenzenes that are compatible with azide, amine, ether and chloride functional groups. Once these routes were established, a number of different polybenzamides having diverse alkylating groups at the benzylic position and a variety of amino-alkyl groups at the ends were prepared in order to explore their stability and thereafter establish the effect of such functional groups on DNA groove binding. The different derivatives were then tested for antiproliferative activities against various cancer cell lines and DNA binding and alkylating activities using different analytical techniques such as DNA melting point analysis, competitive ethidium displacement assays, and mass spectrometry. Our investigations revealed that triaryl derivatives bearing a large-sized group at a benzylic position and a longer amino-alkyl chain at the end of the ligand have the best DNA and antiproliferative activities in this novel class of benzamide MGB.
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First, I am very grateful to my supervisor Dr. David Barker for providing me with the opportunity to work in his research group. It is an honour for me to work with such a good and friendly chemist like you, who always had time for me and my questions. I am grateful for all your useful advice, suggestions and cooperation, and the many hours spent proofreading this thesis.

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## Abbreviations

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<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>adenine</td>
</tr>
<tr>
<td>Å</td>
<td>angstrom</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>Atm</td>
<td>atmosphere</td>
</tr>
<tr>
<td>BAM</td>
<td>benzoyl nitrogen mustard</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>br s</td>
<td>broad singlet</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>C&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% drop in fluorescence intensity</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CTDNA</td>
<td>calf thymus deoxyribonucleic acid</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DIC</td>
<td>diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DCU</td>
<td>dicycyclohexylurea</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethyl-4-aminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>Dst.</td>
<td>distamycin</td>
</tr>
<tr>
<td>EDC or EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ESI&lt;sup&gt;+&lt;/sup&gt;</td>
<td>electrospray ionisation mass spectrometry</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
</tbody>
</table>
FGI  functional group interconversion
g  gram
G  guanine
GL₅₀  concentration that causes 50% growth inhibition
GSH  glutathione
h  hour
HBTU  O-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate
HEPES  4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV  human immunodeficiency virus
HRMS  high resolution mass spectrometry
IC₅₀  half-maximal inhibitory concentration
IR  infra-red
J  coupling constant
L  litre
L1210  lymphocytic leukemia cells
LC  lethal concentration
LD₁₀  lethal dose 10%
m  meta
M  molar
m  multiplet
m/z  mass to charge ratio
MALDI  matrix-assisted laser desorption ionisation mass spectrometry
MGB  minor groove binding agent
MHz  mega hertz
min.  minute
mL  millilitre
mM  millimolar
mmol  millimole
m.p  melting point
Ms  mesyl
MsCl  methanesulfonyl chloride
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>normal</td>
</tr>
<tr>
<td>NCI</td>
<td>national cancer institute</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>O/D</td>
<td>optimal nontoxic dose</td>
</tr>
<tr>
<td>o/n</td>
<td>overnight</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>p</td>
<td>para</td>
</tr>
<tr>
<td>L-PAM</td>
<td>melphalan</td>
</tr>
<tr>
<td>PBD</td>
<td>pyrrolo[2,1-c][1,4]benzodiazepine</td>
</tr>
<tr>
<td>pH</td>
<td>potential of hydrogen</td>
</tr>
<tr>
<td>Poly(dA-dT)</td>
<td>poly adenine-thymine</td>
</tr>
<tr>
<td>Poly(dG-dC)</td>
<td>poly guanine-cytosine</td>
</tr>
<tr>
<td>quat.</td>
<td>quaternary</td>
</tr>
<tr>
<td>Rf</td>
<td>retention factor</td>
</tr>
<tr>
<td>RI</td>
<td>resistance index</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SHE</td>
<td>sodium chloride-HEPES-EDTA</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>T</td>
<td>thymine</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>T/C%</td>
<td>median survival time of control %</td>
</tr>
<tr>
<td>T4 DNA</td>
<td>T4 coliphage deoxyribonucleic acid</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetra-n-butylammonium fluoride</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-butylidimethylsilyl</td>
</tr>
<tr>
<td>TBDMSCl</td>
<td>tert-butyl(chloro)dimethylsilane</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TGI</td>
<td>total growth inhibition</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>Tₘ</td>
<td>melting temperature</td>
</tr>
<tr>
<td>TS</td>
<td>thymidylate synthase</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>w/w</td>
<td>weight/weight</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction
1.1 Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA), sometimes called the “blueprint of life”, is a complex double-helical structure found in the nucleus of living cells. The two complementary helical strands of polynucleotides in DNA molecules run in antiparallel directions, making it suitable to store genetic information, which is transferred from parents to offspring.

The polynucleotide chain of each DNA molecule is composed of small repeating subunits called nucleotides. The nucleotide itself is composed of three basic moieties, a five carbon (deoxyribose) sugar, a phosphate group joining the two sugar molecules by a phosphodiester bond, and the nitrogenous bases. The nitrogenous bases are of two types: purines and pyrimidines. The purine bases are five-six membered fused rings and include adenine “A” and guanine “G”, while the pyrimidine bases are six-membered single-ring compounds consisting of thymine “T” and cytosine “C” (Figure 1). The deoxyribose sugar and the phosphate group provide a backbone for the nitrogenous bases that are directly attached to the deoxyribose unit.

![Figure 1](image)

Figure 1. The four nitrogenous bases, found in DNA.

Each nitrogen base has a different structure and is capable of forming specific hydrogen bonds due to the presence of electron-accepting and electron-donating sites. The nitrogen bases follow the specificity rule, where each base forms hydrogen bonds only with a specific base on the opposite strand, i.e. A binds to T and G binds to C, and therefore the amount of A is always equal to T and G equal to C in a specific sample of DNA (Figure 2).
Figure 2. Base pairs forming hydrogen bonds, symbolised by dashed lines.

1.2 Major and minor grooves of DNA

The spatial arrangement of base pairs on the sugar phosphate backbone of the DNA molecule leads to information-rich sites, which are very important for recognition and binding with other macro and micro molecules.\(^7\) These two channels of information are called the major and minor grooves (Figure 3).\(^7,8\)

At a molecular level, the environment of each type of groove is different.\(^9\) The major groove is rich in information and has multiple sites of interaction. It offers comparatively strong binding due to multiplicity of binding sites towards the incoming binding molecules or ligands.\(^7,10\) The groove width may be different due to the arrangement of bases on the nucleotide sequence; however, on average, the width of major grooves is 11.6 Å with a depth of 8.5 Å.\(^11\) The major groove provides easy access for large repressor proteins and is thus frequently occupied by large and bulky regulatory molecules.\(^12\) On the other hand, the minor groove has less binding sites and is smaller in size, with a depth of 8.2 Å. The minor groove has the benefit that it is usually untenanted. It provides tight binding and is more available to attack by small drug molecules that can bind with ease. The minor groove is therefore the target of antibiotic and anticancer drugs.\(^11,13\)
1.3 Cancer and chemotherapy

Cancer is a group of diseases originating from the uncontrolled growth of body cells with the ability to spread to other parts of the body through the blood or lymphatic system by metastasis or secondary growth. The invasion of healthy cells by the uncontrolled growth of cancerous cells must be treated as quickly as possible. Metastasis makes cancer more difficult to treat and patient outcomes deteriorate exponentially in many cases. Cancer is therefore one of the leading causes of death globally, affecting people of all ages and races. There has been some improvement in the average five-year survival rate since 1970, however it is still below 11% and 13% for liver cancer and lung cancer respectively. In 2008, 12.4 million new cancer cases were registered worldwide, with 7.6 million deaths. By 2050, 27 million new cancer cases are anticipated, with 17.5 million deaths.

Research dedicated to understanding the complex nature and mechanism through which normal cells turn into tumour cells has revealed that different types of mutations and unusual rearrangement of DNA within cellular nuclei can influence gene expression and other biochemical processes. Most significant are the oncogenes which turn the normal cells into diseased cells.
Chapter 1: Introduction

Depending on the condition and stage of the cancer, different types of treatments are used. These include surgery, radiotherapy, chemotherapy, and immunotherapy. Chemotherapy is the use of chemicals to stop certain functions of cells such as cell growth and cell division, with the aim of killing tumour cells.\(^\text{19}\)

Some of the well-known traditional anticancer medicines are the platinum complexes, i.e. cisplatin \(5\), oxaliplatin \(6\), some nitrogen mustards including mustine \(7\), chlorambucil \(8\) and melphalan (L-PAM) \(9\) (Figure 4).\(^\text{20,21}\) The ultimate target for all these drugs is DNA. The nitrogen mustards alkylate DNA, forming inter- and intra-strand cross links with DNA. These drugs thereby exhibit their cytotoxic effect in the disruption of DNA performance with regard to functions of cell growth and cell division.\(^\text{21,22}\) Some of these alkylators have strong reactivity towards DNA, showing effect in a very short period of time, while others show it over many hours or days.\(^\text{23}\)

\[
\begin{align*}
\text{Cisplatin (5)} & \quad \begin{array}{c}
H_2N\begin{array}{c}
\text{Pt}\
\text{Cl}
\end{array}
\end{array} \\
\text{Oxaliplatin (6)} & \quad \begin{array}{c}
\begin{array}{c}
\text{N} \\
\text{H}_2
\end{array} \\
\begin{array}{c}
\text{Pt} \\
\text{O} \\
\text{O}
\end{array}
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{Nitrogen mustard "mustine" (7)} & \quad \text{R} = -\text{CH}_3 \\
\text{Chlorambucil (8)} & \quad \text{R} = \rho-\text{Ph(CH}_2)_2\text{COO}
\end{align*}
\]

**Figure 4.** Structures of known anticancer drugs.

Unfortunately, there are still problems associated with the medicines currently available on the market. The first main problem is non-selectivity, leading to unspecific cytotoxicity and severe unwanted physiological side effects.\(^\text{20}\) Secondly, the available medicines are not able to completely control the metastatisis or secondary growths, i.e. the spread of cancerous cells through blood or the lymphatic system to healthy parts of the body.\(^\text{24}\) The heterogenic nature of cancer itself presents a formidable challenge as there are more than 100 different types of
cancer. Depending on the nature, site, and stage of the cancer, specific medicines are needed to address each problem. Finally, multidrug resistance is becoming an increasing problem as cancerous cells, initially suppressed by a specific drug, sometimes develop a resistance to that drug. More effective and selective drugs are needed to overcome this problem of multidrug resistance.

Advancements in the understanding of cancer and chemotherapy has resulted in the discovery of novel agents that have been used to recognise a specific DNA sequence with the potential to control the development of tumour cells. These anticancer drugs have strong interaction with some selective sites on the DNA and DNA binding proteins. When an anticancer ligand targets DNA, it alters the normal activity of DNA with consequent cytotoxic effect and cell death. The binding mechanism by which anticancer drugs exhibit their effect varies from intercalation (berberine and daunomycin), to alkylation (mustine and mitomycin C), as well as minor groove binders (distamycin and netropsin) (Figure 5).
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Figure 5. Ligands that bind to DNA through different types of interactions.

1.4 Drug DNA interaction

Depending on the nature of binding modes, anticancer drugs are broadly divided into two main categories: ligands that bind covalently and irreversibly, and molecules that bind non-covalently and reversibly. The covalent binders cause everlasting and permanent damage to DNA and its functions, and are exemplified by molecules such as mitomycin C, anthramycin, ecteinascidin derivative (ET-743) and bleomycin A2 (Figure 6). The
non-covalent binders (distamycin 13 and netropsin 14) physically interact with DNA and demonstrate their cytotoxic effects by altering DNA function temporarily.9,36 The non-covalent and reversible interactions can further be divided into three major groups based on the types of interactions involved:9

a. The electrostatic interaction, in which the anionic phosphate group of DNA molecules interacts with the cationic tail of oligoamides. An example is the interaction of lexitropsins 18 and their conjugates with DNA (Figure 6).

b. Intercalation, where molecules of correct shape and structure such as polycyclic aromatic cations intermingle with DNA molecules and rest between the base pairs of the nucleotides. Ethidium bromide 19 and adriamycin 20 are well-known compounds which bind to DNA by intercalation (Figure 6).37

c. Non-intercalation, which is the binding of crescent-shaped molecules in the minor groove of the DNA. Natural groove binding agents include distamycin 13 and netropsin 14, and both derivatives have been used as lead compounds in cancer research due to their binding ability to specific sequences within minor groove of double-helical DNA.38,39
Figure 6. Ligands that bind to DNA through different types of interactions.
1.5 Minor groove binding agents

The diverse group of organic\textsuperscript{11} and inorganic compounds\textsuperscript{40} that bind to the minor grooves of DNA are known as minor groove binding agents or simply minor groove binders (MGB). Among the organic MGB, compounds of both natural and synthetic origins are known that bind preferentially to AT sequences in the minor grooves.\textsuperscript{41,42} There are some synthetic MGB that are known to bind to AT sequences\textsuperscript{43} as well as GC sequences on the DNA in the minor grooves of double-stranded DNA.\textsuperscript{44} The biophysical data reveal that these molecules bind preferably to duplex B DNA, with a special preference for AT sites and without perturbing the B DNA (Figure 7).\textsuperscript{21}

![Figure 7. Molecular model of distamycin (shown in red) in the minor groove of DNA (The diagram was generated from the data reported by Miquel et al.).\textsuperscript{45}}

The binding nature of MGB has been investigated in NMR\textsuperscript{46} and crystallographic studies.\textsuperscript{47-50} The basic feature of all MGB includes the presence of a matching concave-shaped aromatic framework that fits in the convex minor groove\textsuperscript{48} and has electron-donating and electron-accepting groups capable of hydrogen bonding. The high negative electrostatic potential of
helical B DNA in the AT-rich regions interacts with the protonated amines of ligands under physiological pH conditions. In addition to hydrogen bonding and electrostatic interactions, the drug-DNA complex is also stabilised by strong van der Waals interactions. The natural MGB include distamycin and netropsin, while synthetic MGB are exemplified by compounds such as Hoechst 33258, SN 6999 and brostallicin (Figure 8).

![Chemical structures](image)

**Figure 8.** Synthetic MGB.

### 1.5.1 Distamycin 13

Distamycin 13, also known as stallimycin, is a naturally occurring DNA MGB isolated from *Streptomyces distallicus*. Total syntheses have been reported by Penco et al., followed by Bialer et al., and Lownet al.

Distamycin 13 is reported to have antibiotic and antiviral activities with strong activity against the plasmodium falciparum, no antitumour activities, and a low cytotoxic profile. The structure of distamycin 13 is composed of three pyrrole rings joined by amide bonds and also contains an amidino side chain that plays an important role in the electrostatic
interactions with double-helical DNA. Its crescent shape, the presence of amide group N-Hs facing towards the base of the minor groove, and the positively charged amidine group at the end enable it to bind non-covalently in the deep minor grooves.

NMR and crystallographic studies have revealed that distamycin binds to duplex as well as quadruplex DNA structures, with a strong preference for adenine-thymine (AT)-rich sequences on double-helical DNA covering at least four base pairs. Structures are also reported in which multiple distamycin molecules cover 5 of the 6 AT base pairs stretched in an antiparallel fashion side by side, with the pyrrole ring of one molecule stacking against the amide group of the other.

1.5.2 Netropsin

Netropsin, also known as congocidine or sinanomycin, is a naturally occurring oligoamide isolated from Streptomyces netropsis in 1951. The total synthesis was reported by Lown et al. Netropsin has reported antibiotic and antiviral properties.

Netropsin is also a potent DNA MGB with strong binding specificity to AT-rich sites of double-helical DNA, binding in a similar fashion to distamycin. Netropsin is a tripeptide with two pyrrole rings and two amidine groups, one at each end of the molecule. Crystallographic analysis has elucidated the structure of the netropsin-DNA complex, showing that binding occurs in the minor groove. The amide NHs form hydrogen bonds with adenine N-3 or thymine O-2 atoms on the adjacent base pairs of minor groove.

1.5.3 Hybrid DNA minor groove binding agents

Although distamycin and netropsin are both selective DNA binders, the reversible nature of their binding due to lack of an alkylating group limits their utility as antitumour agents. Despite the fact that they do not have antitumour activity of their own, both distamycin and netropsin have been used as lead compounds in the design of anticancer drugs. So far, a large number of synthetic analogues or hybrids of distamycin and netropsin have been prepared with the intent of increasing DNA binding, adding alkylating
functionality, increasing sequence specificity, and minimising the unwanted physiological side effects associated with these natural MGB.\textsuperscript{30,39,69,70}

Different changes have been made to distamycin and netropsin frameworks to systematically investigate the effect of adding alkylation groups, ranging from traditional nitrogen mustards\textsuperscript{71-73} to haloacyryloyl groups.\textsuperscript{22,74-76} In some cases, previously used anticancer molecules\textsuperscript{77} or the active moieties of anticancer molecules,\textsuperscript{78} specifically alkylation groups, have been tethered to the distamycin or netropsin frameworks, which are then used as DNA binding vectors. In other cases, changes have been made to the distamycin or netropsin frameworks by replacing one or more pyrrole rings with other heterocycles, such as pyrazoles\textsuperscript{75} and benzofurans.\textsuperscript{79} The basic amidino side chain, which is believed to be important due to its electrostatic interaction with DNA, has also been substituted either to increase the stability, enhance solubility at physiological pH, or to facilitate the purification process.\textsuperscript{22,75,80,81}

1.5.3.1 Nitrogen mustard oligopyrroleamide derivatives

Alkylation agents including various nitrogen mustards have played an important role in the chemotherapeutic treatment of cancer, as well as in the development of new antileukemic medicines.\textsuperscript{20} The mechanisms, site of action, and the efficiency of these medicines vary from drug to drug but most act as DNA alkylating agents. The majority of the known drugs alkylate the DNA in a non-specific manner, forming cross links within the DNA, and thus preventing the cell duplication which results in the anticancer activity.\textsuperscript{21} The non-selectivity and high reactivity of nitrogen mustards with other cellular nucleophiles such as glutathione (GSH)\textsuperscript{82,83} result in severe unwanted physiological side effects. Using higher doses in order to increase the chances of achieving an effective concentration to reach the target cells is an option but it increases the possibility of more severe side effects. To address the problems of non-specificity and high reactivity associated with these compounds, there is the option of attaching these ligands to known targeting molecules. The rational approach was the construction of hybrid molecules of known DNA MGB coupled with a nitrogen mustard to act as an alkylationing group. This has increased specificity, with fewer side effects, and more biological response is achievable using lower concentrations.
Traditional nitrogen mustards have been known for more than half a century and are still used as active medicines to treat various types of cancers.\textsuperscript{20} Half-nitrogen mustards or hemi-mustards are also known and these have good alkylating activities.\textsuperscript{72,81} There are reports on the synthesis of sulfur mustards as well.\textsuperscript{73} The common mechanism of classical nitrogen mustards, hemi-mustards and sulfur mustards is the production of aziridinium (Scheme 1) or thiiranium ion as intermediates, which then alkylate the DNA at different positions.\textsuperscript{82}

\begin{center}
\textbf{Scheme 1. Reaction of mustine 7 with water and GSH.}\textsuperscript{82,83}
\end{center}

Many nitrogen mustards added onto a distamycin or netropsin backbone have been synthesised, including tallimustine (FCE 24517) \textsuperscript{24} (Figure 9).\textsuperscript{42}

Tallimustine \textsuperscript{24} was synthesised by Arcamone \textit{et al.}\textsuperscript{42} and was the first clinically developed model based on distamycin \textsuperscript{13}. Tallimustine \textsuperscript{24} is produced by adding a benzoyl nitrogen
mustard (BAM) to the formyl end of the distamycin framework, where the BAM acts as an alkylating moiety and the distamycin framework as a DNA binding vector.\textsuperscript{69,74} The BAM moiety has been selected as the alkylating functionality because of its mild reactivity towards different biological nucleophiles, especially thiols.\textsuperscript{75,84}

The resultant hybrid ligands showed higher activity than distamycin \textsuperscript{13} and higher specificity than the parent benzoic mustard precursor, with a broad spectrum of antitumour activities in many different experimental cell lines\textsuperscript{42,75} and animal and human models.\textsuperscript{85,86} These hybrid ligands were found to be equally active against the L1210 cell line and tumour cell cultures resistant to L-PAM. The \textit{in vitro} IC\textsubscript{50} value of tallimustine \textsuperscript{24} is 50.3 ng/L,\textsuperscript{75} much higher than that of the BAM when tested against the L1210 murine leukemia cell line. This increase in cytotoxicity indicates that more cytotoxic molecules could be achieved by attaching the mustard to the distamycin framework.

The higher cytotoxicity of tallimustine \textsuperscript{24} has been hypothesised to be due to an increase in cellular penetration and accumulation, and delay in the G\textsubscript{2} phase in the cell cycle. Interestingly, it is thought not to be the result of alkylation of the N-7 position of guanine, as in the case of other nitrogen mustards. The experimental evidence, namely circular dichroism, shows that tallimustine \textsuperscript{24} binds to DNA differently than other alkylating agents.\textsuperscript{86}

The binding of tallimustine \textsuperscript{24} to DNA is reversible by raising the salt concentration. Therefore, circular dichroism experiments have shown that its binding is by electrostatic interactions\textsuperscript{42} and is non-covalent in nature. Binding was high in selectivity for AT regions, with alkylation apparently at the N-3 of adenine, which highlights that alkylation is achieved due to the mustard moiety when it comes in the desired orientation with DNA.\textsuperscript{75,86} Despite the promising interaction of tallimustine \textsuperscript{24} demonstrated in these experiments, phase I and phase II clinical trials showed severe myelotoxicity which reduced the useful therapeutic dose to reach the target cells, and thus further development of tallimustine \textsuperscript{24} as an anticancer agent was terminated.\textsuperscript{87}
Arcamone and his co-workers\textsuperscript{42} have also reported the synthesis of nitrogen mustards, including those in which the mustard group was directly tethered to the $N$-terminus of distamycin derivatives or polypyrrole frameworks 25 and 26 (Figure 10). All the resultant compounds (25-27) have been tested in comparative studies against both human and murine tumour cell lines \textit{in vivo}. These compounds showed cytotoxicity comparable to that exhibited by melphalan 9 and a remarkable increase in cytotoxicity compared to distamycin 13. The \textit{in vivo} activity of distamycin 13 was shown to be achieved at concentration of 200.00 mg/kg against both L1210 and L1210/L-PAM cell lines, with T/C\% values of 113 and 106 respectively.\textsuperscript{42}
Table 1. In vivo activity of L-PAM and polypyrrole derivatives.\textsuperscript{42}

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>L1210 OD (mg/kg)</th>
<th>T/C %</th>
<th>L1210/L-PAM OD (mg/kg)</th>
<th>T/C %</th>
</tr>
</thead>
<tbody>
<tr>
<td>tallimustine 24</td>
<td>3</td>
<td>3.12</td>
<td>175</td>
<td>3.12</td>
<td>144</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>1.56</td>
<td>138</td>
<td>1.56</td>
<td>122</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
<td>0.39</td>
<td>188</td>
<td>0.39</td>
<td>167</td>
</tr>
<tr>
<td>27</td>
<td>3</td>
<td>1.56</td>
<td>132</td>
<td>1.56</td>
<td>111</td>
</tr>
<tr>
<td>L-PAM (9)</td>
<td></td>
<td>10.00</td>
<td>192</td>
<td>10.00</td>
<td>104</td>
</tr>
</tbody>
</table>

\textit{OD} optimal nontoxic dose < \textit{LD}_{10} \text{ (lethal dose 10\%)}; T/C \% median survival time of treated mice/median survival time of controls \times 100.

Evaluation of activity: compounds are considered active if T/C \% values are greater than 125.

All the mustard derivatives (24-27) have been tested against L1210 leukemia cell lines (Table 1). Derivatives 24 and 26 have shown good activity against the melphalan resistant cell lines in vivo.\textsuperscript{42} Derivative 26, in which the mustard is directly attached to the pyrrole ring, and derivative 24, which has an additional ring between the mustard moiety and distamycin framework, were the best in the series with high antitumour activity against the L1210 cell line and to a lesser extent against the L1210/L-PAM cell line (tumour cell cultures resistant to L-PAM). The data also showed that derivatives 25 and 27 exhibit lesser activity against the L1210 cell line, with almost no activity against the resistant L1210/L-PAM cell line (Table 1).\textsuperscript{42}

Tallimustine 24 has also been of interest to anticancer drug designers, due to its high sequence specificity. A number of oligopyrrole derivatives have been reported in which one or more of the pyrrole rings of the tallimustine 24 were replaced with other heterocycles to increase stability,\textsuperscript{88} as well as to establish a structure activity relationship.\textsuperscript{72,75,81} Baraldi \textit{et al.}\textsuperscript{75} have reported analogues 28-33 of tallimustine 24, in which pyrrole ring/s were substituted with pyrazoles (Figure 11). In some of the derivatives the amidino \textit{C}-terminus has also been altered with dimethylaminopropylamine or \textit{p}-\textit{bis}-(2-chloroethyl)amino]phenylamino groups 34 and 35. In addition to the improvement in the antileukemic activity and cytotoxicity both in vitro and in vivo, more structural stability was noted in oxidative conditions as compared to that observed for compounds containing a tripyrrolic framework.
Figure 11. Examples of nitrogen mustards, with pyrazole rings.

Table 2. In vivo and in vitro activity of distamycin derivatives.\textsuperscript{75}

<table>
<thead>
<tr>
<th>Compound</th>
<th>\textit{In vitro} L1210</th>
<th>\textit{In vivo} L1210</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC\textsubscript{50} (ng/mL)</td>
<td>O/D (mg/kg)</td>
</tr>
<tr>
<td>Tallimustine\textsuperscript{24}</td>
<td>50.3</td>
<td>6.25</td>
</tr>
<tr>
<td>28</td>
<td>35.00</td>
<td>6.25</td>
</tr>
<tr>
<td>29</td>
<td>225.20</td>
<td>12.5</td>
</tr>
<tr>
<td>36</td>
<td>20.10</td>
<td>6.25</td>
</tr>
<tr>
<td>37</td>
<td>608.30</td>
<td>n.d</td>
</tr>
</tbody>
</table>

Where IC\textsubscript{50} = 50\% inhibitory concentration represents the mean from dose response curves of at least three experiments. O.D. = optimal dose; optimal nontoxic dose < LD\textsubscript{10}. %T/C = median survival time of treated versus untreated mice × 100. n.d = not determined.
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Table 2 shows that the incorporation of the additional pyrrole ring in derivative 36 between the benzoyl mustard and the N-terminus resulted in 10-fold increase in the cytotoxicity profile (20.10 ng/mL vs 225.5 ng/mL) as compared to a similar derivative 29, without an improvement in in vivo antileukemic activity (Figure 11 and Table 2). A three-fold lower potency in antileukemic activity was observed in the pyrazole homologue 37 compared to its precursor moiety 29. Replacement of the terminal pyrrole with a pyrazole in derivative 28 resulted in antileukemic activity in the same range as tallimustine 24, but an improvement in the in vivo activity. Replacement of two pyrrole rings in compound 29 showed a large decrease compared to tallimustine 24. Similarly, changing one or two pyrrole rings in derivatives 30 and 32 resulted in loss of activity against L1210 murine leukemia. In derivative 33, where all the three pyrroles were replaced, a remarkable decrease of activity against L1210 murine leukemia could be noted compared to tallimustine 24 (1325.00 ng/mL vs 50.3 ng/mL). Replacing the amidino side chain in derivatives 34 and 35 resulted in a two-fold decrease and complete loss of in vitro activity respectively.

These results suggest that the activities are dependent on the position of pyrazole ring in tallimustine framework. When pyrazole rings are near the alkylating group in the system, the activity of the resultant derivatives is reduced both in vitro and in vivo. Improvement in the activity of derivative 36 suggested the multiplicity rule of binding, but the same improvement in activity cannot be explained for compound 37, which has a pyrazole instead of a pyrrole with the same number of rings in the system (Figure 11 and Table 2).

Large numbers of potential alkylating moieties have also been incorporated into the distamycin framework. This approach was to some extent successful and new potential candidates have emerged from this work. Less effort has been exerted to systematically investigate the effect of changes in the basic amidino moiety by substituting with ionisable acidic or basic and non-ionisable groups in order to fully explore the mechanism of actions and binding requirements. There is a solubility requirement for MGB at physiological pH and electrostatic binding is possible with the negative phosphate groups. However, these factors are yet to be fully explored.
Cozzi and co-workers\textsuperscript{72,81} have reported different benzoyl and cinnamoyl nitrogen mustards and half-mustard derivatives (Figure 12). In these compounds (38-46), the amidine groups have been modified or replaced with other groups leading to electronic, lipophilic and steric modifications of the distamycin framework, which in turn influence their cytotoxicities and antileukemic activities.

The cytotoxicity data reveals that compounds having cyanoamide, N-methylamidine, N,N-dimethylamidine, imidazol-2-yl and guanidine groups have similar activities, with no clear cut correlation between the basicity and the cytotoxicity of the resultant compounds (Figure 12 and Table 3).\textsuperscript{81}

\textbf{Figure 12.} Nitrogen mustards, containing different side chains (see Table 3 for information of D and X).
Table 3. Nitrogen mustards, with different tails for electrostatic interaction.\(^{81}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>D</th>
<th>(\text{In vitro})</th>
<th>(\text{In vivo})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\text{IC}_{50}) (ng/mL)</td>
<td>O/D (mg/kg)</td>
</tr>
<tr>
<td>24*</td>
<td>Cl</td>
<td>C(NH)NH(_2).HCl</td>
<td>50.3±5.9</td>
<td>3.13</td>
</tr>
<tr>
<td>38</td>
<td>Cl</td>
<td>C-imidazol-2-yl. HCl</td>
<td>69.0±16.0</td>
<td>6.25</td>
</tr>
<tr>
<td>39</td>
<td>Cl</td>
<td>CN</td>
<td>94.0±6.0</td>
<td>25</td>
</tr>
<tr>
<td>40</td>
<td>H</td>
<td>C(NH)NH(_2).HCl</td>
<td>42.0±9.1</td>
<td>3.13</td>
</tr>
<tr>
<td>41</td>
<td>--</td>
<td>C(NH)NH(_2).HCl</td>
<td>7.2±2.1</td>
<td>6.25</td>
</tr>
<tr>
<td>42</td>
<td>--</td>
<td>C(NOH)NH(_2)</td>
<td>42.4±21.3</td>
<td>6.25</td>
</tr>
<tr>
<td>43</td>
<td>--</td>
<td>C(NCN)NH(_2)</td>
<td>5.4±1.4</td>
<td>12.5</td>
</tr>
<tr>
<td>44</td>
<td>--</td>
<td>C(NCH(_3))NH(_2).HCl</td>
<td>3.9±1.6</td>
<td>3.13</td>
</tr>
<tr>
<td>45</td>
<td>--</td>
<td>C(NCH(_3))NHCH(_3).HCl</td>
<td>6.4±2.5</td>
<td>1.56</td>
</tr>
<tr>
<td>46</td>
<td>--</td>
<td>NHC(NH)NH(_2).HCl</td>
<td>7.4±2.0</td>
<td>3.13</td>
</tr>
</tbody>
</table>

* tallimustine

The cytotoxicity data for amidoxime (\(\text{IC}_{50}\) 1476.7±163.1 ng/mL) and amidrazone (\(\text{IC}_{50}\) 738.1±36.2 ng/mL) showed that the presence of amidino-like moieties does not guarantee the achievement of high cytotoxicity. The presence of carbamoyl (\(\text{IC}_{50}\) 130.0±33.0 ng/mL) and cyano derivatives 39 (\(\text{IC}_{50}\) 94.0±6.0 ng/mL) have a remarkably greater effect on the activities than carboxylic (\(\text{IC}_{50}\) 959.7±212.0 ng/mL) and carbinol (\(\text{IC}_{50}\) > 2000 ng/mL) moieties.\(^{81}\) The effect of modifications to the amidino moiety is also apparent in half-mustards and cinnamic mustard derivatives. Half-nitrogen mustards or hemi-mustards that have methylamidino (\(\text{IC}_{50}\) 21.2±6.7 ng/mL) and guanidino groups (\(\text{IC}_{50}\) 62.2±14.4 ng/mL), and cinnamic mustards with cyanoamidine 43, methylamidine 44, dimethylamidine 45 and guanidine 46 moieties, have comparable or increased cytotoxicity to that of compound 40 and 41 (Figure 12, Table 3).\(^{81}\)

Again, no correlation was found between the overall basicity of the molecule and the resultant cytotoxicity and antileukemic activities in vivo. Some of the more in vitro cytotoxic compounds were inactive in terms of in vivo antileukemic activity. The methylamidino derivative 44 was effective, having comparable activity to that of amidine derivatives.\(^{81}\) The
comparative data of antileukemic activities suggest that cinnamic mustards were more active than benzoyl mustards. Furthermore, the presence of amidine or more basic groups and the resultant inactivity of compounds containing them are in opposition to the widely held opinion that the presence of positively charged groups provides stronger interactions with the negative phosphate groups in the minor grooves of DNA.

Cozzi and co-workers\textsuperscript{72} have also reported a series of benzoyl and cinnamoyl mustards and half-mustards, with the aim of studying the structure activity relationship with respect to reactivity of the different alkylating moieties. BAM and similar alkylating moieties were selected due to their mild reactivity towards different biological nucleophiles \textit{in vivo}. These compounds are exemplified by tallimustin\textsuperscript{24}.

Cozzi and co-workers\textsuperscript{72} have synthesised closely related tallimustine derivatives to tune the alkylating power with steric features. In order to define the source of mustard reactivity, the effect of different halogeno-mustard derivatives along with \textit{ortho}-methyl, \textit{ortho}-dimethyl and \textit{ortho}-fluoro substituents on the phenyl rings has been studied. The \textit{ortho} position is believed to affect the reactivity of mustards both by an inductive effect and the possible conjugation of the nitrogen mustard “arms”, with the phenyl ring contributing to the reactivity of the nitrogen during DNA binding in the minor groove. Some benzoyl mustards, half-mustard derivatives and cinnamic mustards with extended chains between the distamycin framework and the mustard moiety were evaluated both \textit{in vivo} and \textit{in vitro} (Figure 13 and Table 4).\textsuperscript{72}

\textbf{Figure 13.} Nitrogen mustards, with \textit{ortho} substitution and extended chain lengths (see Table 4 for structural details).
Table 4. Nitrogen mustards, with ortho substitution and extended chain lengths.\textsuperscript{72}

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>A</th>
<th>R(_1)</th>
<th>R(_2)</th>
<th>\textit{In vitro}</th>
<th>\textit{In vivo}</th>
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<td></td>
<td></td>
<td></td>
<td>IC(_{50}) (ng/mL)</td>
<td>O/D (mg/kg)</td>
</tr>
<tr>
<td>47</td>
<td>Br</td>
<td>CH(_2)CH(_2)Br</td>
<td>H</td>
<td>H</td>
<td>0.6±0.1</td>
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<td>CH(_3)</td>
<td>H</td>
<td>11.1±1.9</td>
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<tr>
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<td>Cl</td>
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</tr>
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<td>Cl</td>
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<td>H</td>
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<td>CH(_2)CH(_2)OH</td>
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<td>H</td>
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<td>nd</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>CH(_2)CH(_2)F</td>
<td>H</td>
<td>H</td>
<td>□ 10000</td>
<td>nd</td>
</tr>
<tr>
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<td>CH(_2)CH(_3)Cl</td>
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<td>H</td>
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<tr>
<td>55</td>
<td>Cl</td>
<td>CH(_2)CH(_3)</td>
<td>H</td>
<td>H</td>
<td>2.9±0.2</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Some of the derivatives with alcohol and fluorine groups 51 and 52 replacing the chlorine group in tallimustine 24 showed the same level of cytotoxicity as distamycin derivatives while the bromide mustard 47 was found to be the most active in the series. Compounds in the series with ortho-fluoro groups, and the di-ortho-methyl derivatives such as 53, showed equivalent reactivities but derivatives with ortho-methyl moieties e.g. 48 had higher cytotoxicity than those with ortho-fluoro and di-ortho-methyl groups (e.g. 53) (Figure 13, Table 4). The altered conformation of the phenyl ring due to the ortho-methyl group may be playing a role in its high cytotoxicity when binding to DNA in the minor groove.\textsuperscript{72}

Cinnamic derivatives 54 and 55 had a higher IC\(_{50}\) value than tallimustine 24, probably due to the increased level of conjugation of the nitrogen lone pair, while half-nitrogen mustards 49 and 50 had equal or better cytotoxicity than tallimustine 24. Some of the half-mustards with different mustard arms had low cytotoxicity, which suggested that an inductive effect may not play a significant role. Instead, steric factors on DNA binding might play a bigger role in the activity of these compounds. Some of the compounds, namely 47 and 48 and cinnamic derivatives 54 and 55, were further screened against murine solid tumours and human xenografts (Figure 13 and Table 4).\textsuperscript{72}
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Cozzi and co-workers then reported a range of structurally novel benzoyl and cinnamoyl sulfur mustards (Figure 14 and Table 5).

### Table 5. Biological data from different sulfur mustards.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>m</th>
<th>( \text{In vitro IC}_{50} ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>0</td>
<td>0</td>
<td>20.6±0.1</td>
</tr>
<tr>
<td>57</td>
<td>1</td>
<td>0</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>58</td>
<td>0</td>
<td>1</td>
<td>&gt;600</td>
</tr>
<tr>
<td>59</td>
<td>1</td>
<td>1</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>

Sulfur mustards were more active than the corresponding nitrogen mustards and all the compounds were tested \textit{in vitro} against L1210 murine leukemia cells. Sulfur mustard 56 had three-fold more cytotoxicity than tallimustine 24 and 30-fold more than its corresponding half mustard. The cinnamoyl sulfur mustard 57 was 60 times more cytotoxic than tallimustine 24 as well as its half-mustard. Interestingly, compound 57 was three-fold less reactive against L1210 leukemia but 1000-fold more cytotoxic than melphalan 9 (IC\(_{50}\) 980.5±642.4 nM). Both sulfoxides 58 and 59 were inactive, showing that the thiranium cation intermediate is required for cytotoxicity, and this cannot be formed from sulfoxides, thereby resulting in their ineffectiveness (Figure 14, Table 5).

### 1.5.3.2 Haloacrylic derivatives

With the objective of investigating new potential anticancer drugs with improved bioavailability and better pharmacological properties, many halogenoacrylic derivatives have
been investigated.\textsuperscript{22,74} Changes have been made to the distamycin framework by the replacement of one or more pyrrole rings with other five-membered rings or fused benzoheterocyclic systems in a similar fashion as in the nitrogen mustards containing the distamycin framework.\textsuperscript{89} These changes included the introduction of a new alkylating functional group (the halogenoacryloyl group), a unique mechanism, and biological properties (Scheme 2).\textsuperscript{22}

The effective concentration of some drugs can be reduced by GSH in the body. GSH is involved in the protection of cells against reactive oxygen and various free radicals as well as in the detoxification of drugs present in blood plasma of mammalian cells.\textsuperscript{90} The concentration of GSH is often higher in tumour cells as a protection mechanism due to greater exposure to cytotoxic drugs than normal cells.\textsuperscript{22} This is a problem for many different drugs including the MGB alkylating agents, which can take part in substitution reactions with GSH thereby reducing the effective drug concentration to reach the tumour site (Scheme 2).\textsuperscript{91} Interestingly, contrary to the negative effect of GSH on classical DNA alkylating agents such as tallimustine \textsuperscript{24}, in haloacrylamide compounds such as asbrolasticin \textsuperscript{23} (IC\textsubscript{50} = 1.85±0.17), GSH reinforces their alkylating activity by producing an activated α-bromo amide.\textsuperscript{22}

\begin{center}
\textbf{Scheme 2. Michael reaction of haloacryloyl derivatives.}
\end{center}

In some cases, the amidine group has also been altered by the addition of other groups capable of electrostatic interactions.\textsuperscript{55} The amidine group was found not to be stable in solution, with the colour of the solution changing over time and solubility was difficult to achieve. The objective was to replace the amidine group with dimethylamino or any other stable group having the same extent of electrostatic interaction as an amine, but being easier to purify.\textsuperscript{92}
D'Allessio et al.\textsuperscript{93} reported the synthesis and biological activities of three- and four-ring derivatives based on the distamycin framework. The resultant compounds were tested in \textit{in vitro} studies on L1210 murine leukemia and L1210 cells resistant to tallimustine \textsuperscript{24}, as well as against L1210 murine leukemia \textit{in vivo} (Figure \textbf{15} and Table \textbf{6}).\textsuperscript{93}
Introducing a haloacryloyl moiety onto distamycin 13 (IC\textsubscript{50} 5216±853 ng/mL) or its homologue resulted in greater activities both \textit{in vitro} and \textit{in vivo}. An increase in the cytotoxicity profile was observed by increasing the number of pyrrole rings from three to four, but was not helpful in terms of antitumour (% T/C) activity. Both the bromoacrylic derivatives 62 and 63 show a lower resistance index (RI) than chloroacrylic derivatives 60 and 61 against the resistant leukemia cell lines (Figure 15 and Table 6).\textsuperscript{93}

The increase in antiproliferative activities, seen upon the introduction of haloacryloyl moieties, suggested that enhancement of activities can be achieved by adding an acryloyl group to the distamycin framework, when compared to the antiproliferative activity of distamycin 13 alone.

These promising results prompted Baraldi and his co-workers\textsuperscript{79} to carry out the synthesis of bromoacryloyl derivatives in the hope of increasing the potential binding sites, selectivity of alkylation, and stability towards oxidative conditions. Hence, these derivatives with bromoacrylic moieties attached to benzofuran and indole were synthesised (Figure 16 and Table 7).\textsuperscript{79} The results revealed that compounds 64 and 65 have at least 10-fold better activities both \textit{in vivo} and \textit{in vitro} than BAM derivatives with the same number of pyrrole units. The benzofuran derivative 66 and indole derivative 64 have almost the same

\textbf{Table 6.} \textit{In vitro} and \textit{in vivo} activities of haloacryloyl derivatives.\textsuperscript{93}

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>X</th>
<th>In vitro</th>
<th>In vivo</th>
<th>L1210/DX</th>
<th>L1210/24517</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IC\textsubscript{50} (ng/mL)</td>
<td>OD (mg/kg)</td>
<td>T/C%</td>
<td>RI</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>Cl</td>
<td>56±14</td>
<td>10</td>
<td>184</td>
<td>40.3</td>
</tr>
<tr>
<td>61</td>
<td>4</td>
<td>Cl</td>
<td>2.7±1</td>
<td>3.125</td>
<td>171</td>
<td>59.9</td>
</tr>
<tr>
<td>62</td>
<td>3</td>
<td>Br</td>
<td>49.6±14</td>
<td>12.5</td>
<td>175</td>
<td>8.8</td>
</tr>
<tr>
<td>63</td>
<td>4</td>
<td>Br</td>
<td>4.7±1</td>
<td>3.125</td>
<td>206</td>
<td>3.8</td>
</tr>
</tbody>
</table>

IC\textsubscript{50} represents the mean ± standard error from dose-response curve of at least three experiments, L1210 resistant to doxorubicin (DX).
cytotoxicity profile while the benzofuran derivative 66 has a 15-fold lower potency in vivo. The data also show that compounds 64 and 65 were more cytotoxic but less potent than their nitrogen mustard counterparts. Compound 65 was six-fold more cytotoxic than derivative 67, which only has two pyrrole rings and thus has less hydrogen bonding interactions.\textsuperscript{79}

![Figure 16. Bromoacryloyl derivative with different heterocycles.](image)

Table 7. Bromoacryloyl derivatives with different heterocycles.\textsuperscript{79}

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>X</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC50 (nM)</td>
<td>OD (mg/kg)</td>
<td>% T/C</td>
</tr>
<tr>
<td>64</td>
<td>3</td>
<td>NH</td>
<td>4.1±1.3</td>
<td>12.5</td>
</tr>
<tr>
<td>65</td>
<td>3</td>
<td>NCH₃</td>
<td>2.4±0.39</td>
<td>6.2</td>
</tr>
<tr>
<td>66</td>
<td>3</td>
<td>O</td>
<td>6.1±0.93</td>
<td>0.78</td>
</tr>
<tr>
<td>67</td>
<td>2</td>
<td>NCH₃</td>
<td>15.3±4.31</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Beria and co-workers\textsuperscript{22} also reported a series of compounds with different acrylamide moieties. The in vitro cytotoxicity and in vivo antitumour activities reveal that unsubstituted and fluoroacrylamides were inactive compared to bromo- and chloro- derivatives. This highlights the role of the leaving group on the acrylamide moiety and its reactivity in Gabriel-Cromwell-like reactions, as well as in Michael addition reactions involving thiol or other nucleophiles leading to halogenamid intermediates. These then react with the DNA bases to form a covalent linkage. This hypothesis of halogen reactivity has also been synthetically proven under hydrolytic conditions using primary and secondary amines.\textsuperscript{22}

Altering the number of pyrrole rings in the molecule has also been studied and results indicate that the bromo- and chloro-acrylamido derivatives 63 and 61 respectively, having 4 pyrrole rings, have one order of magnitude more cytotoxicity than tripyrrole compounds 60 and 62 (Figure 17 and Table 8). Bromoacrylamido derivatives having one or two pyrroles were
inactive. Thus, the cytotoxicity increases with the number of pyrrole rings in the system but the antileukemic activity is at its maximum when 4 pyrrole rings are present.\textsuperscript{22}

![Image of Haloacryloyl derivatives with different halogens and chain lengths.]

**Figure 17.** Haloacryloyl derivatives with different halogens and chain lengths.

**Table 8.** Haloacryloyl derivatives with different halogens and chain lengths.\textsuperscript{22}

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>X</th>
<th>IC\textsubscript{50} (nM)</th>
<th>O.D (mg/kg)</th>
<th>T/C%</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>3</td>
<td>Cl</td>
<td>96.8±24.2</td>
<td>12.50</td>
<td>117</td>
</tr>
<tr>
<td>61</td>
<td>4</td>
<td>Cl</td>
<td>3.8±1.4</td>
<td>1.56</td>
<td>133</td>
</tr>
<tr>
<td>62</td>
<td>3</td>
<td>Br</td>
<td>98.8±24.2</td>
<td>12.50</td>
<td>175</td>
</tr>
<tr>
<td>63</td>
<td>4</td>
<td>Br</td>
<td>6.3±1.3</td>
<td>1.56</td>
<td>200</td>
</tr>
<tr>
<td>68</td>
<td>5</td>
<td>Br</td>
<td>23.0±5.0</td>
<td>0.78</td>
<td>167</td>
</tr>
</tbody>
</table>

The amidino end of the bromoacrylamido derivatives in the oligopyrrole system was then modified into different functional groups, but no correlation between cytotoxicity and basicity was found.\textsuperscript{55} This verifies the results of a study by Cozzi and co-workers\textsuperscript{81} who also noted a lack of correlation between these two parameters.

### 1.5.4 Hybrid polypyrrole derivatives

As discussed earlier, distamycin 13 is devoid of potent cytotoxic moieties and cannot itself be used as an anticancer agent. Its structure has been incorporated into already known aspecific anticancer drugsto increase the sequence specificity. Hybrid molecules have been prepared by the conjugation of the already known anticancer drugs to the DNA MGB such as distamycin 13 or netropsin 14 which then act as a vector to deliver the alkylating moiety in a specific way.\textsuperscript{39,69}
1.5.4.1 Distamycin hybrids with PBD

Both Baraldi et al.\textsuperscript{78} and Reddy et al.\textsuperscript{94} have reported hybrid derivatives 69 containing the pyrrolo[2,1-c][1,4]benzodiazepine (PBD) moiety of anthramycin 15 and DC-81 70 tethered to the distamycin framework by means of a spacer group with ranging chain lengths (Figure 18). The PBD moiety reacts with C2-NH\textsubscript{2} of guanine in the minor groove of DNA and the resultant adduct is stabilised due to hydrogen bonding. The structure activity relationship of these molecules has been investigated using human chronic myeloid leukaemia k562 and the T-lymphoblastoid jurkat cell lines.\textsuperscript{78,95,96}

![Image of Distamycin hybrids 69, with PBD moiety.](image)

The results showed that there has been visible improvement in the biological activities of the resultant hybrid derivatives 69 both in terms of activity and selectivity as compared to the parent moieties. The derivatives with longer repeating pyrrole chains were more active due to increased stability of the drug-DNA complex resulting from more sites of hydrogen bonding and van der Waals contacts.\textsuperscript{39}

The reported anti-HIV-1 activities show that three- or four-ring hybrids had greater antiviral activities. However, these derivatives with more pyrrole units also have high human cytotoxicities; therefore, they would not be suitable for use as anti-HIV treatments. The diaryl derivatives are of interest, due to their mild human cytotoxicity and good anti-HIV-1 activity and this finding could be useful in future research of antiviral treatments.\textsuperscript{39,97,98}
1.5.4.2 Distamycin hybrids with uramustine

Uramustine is an antineoplastic drug used for the treatment of lymphosarcoma, chronic lymphatic leukemia and thrombocytopenia. It alkylates the guanine-N7 in 5'-pyrimidine-GCC-3' sequence.

The incorporation of uramustine onto distamycin was successful and a new series of compounds (71) has been prepared with polymethylene spacer chains of different lengths (Figure 19). All the hybrid derivatives in the series showed better activities than the parent molecules against human leukemia K562 cells when a hexamethylene group was used as a spacer.

![Figure 19. Uramustine-distamycin hybrids 71.](image)

Footprinting experiments have suggested that uramustine reacts covalently with DNA in the AT-rich sequences. Therefore, addition of a polypyrrole unit is beneficial as it helps direct to AT-rich sites.

1.5.4.3 Distamycin hybrids with 5-fluorouracil

5-Fluorouracil is used in the treatment of different types of cancers, especially breast cancer and some solid tumours. It is also used as an antimetabolite, showing its effect by irreversible alkylation of thymidylate synthase (TS). The same hybrid approach has been used to tether the uracil to distamycin to create a series of compounds (72), with spacer groups of different chain lengths (Figure 20). In contrast to the uramustine-conjugate, these hybrids
proved not to be very effective, with some of them even having less activity than distamycin 13 alone.\textsuperscript{106}

![Distamycin-fluorourcil hybrids](image)

\textbf{Figure 20.} Distamycin-fluorourcil hybrids 72.

\subsection*{1.6 Problems associated with polypyrrole MGB}

In spite of the widespread use of polypyrroles in synthetic heterocyclic chemistry, there are some problems associated with these heterocycles that limit their utility in future research.

Five-membered rings are more susceptible towards oxidation and reduction during multi-step syntheses and this limits the reactions that can be carried out on five-membered ring systems. The 5-position in pyrrolecarboxamide units has enamine character and is nucleophilic (Scheme 3). This limits the choice of reagents for further functionalisation of molecules containing this moiety. One consequence of this is the early addition of \textit{N}-substituents in the synthesis prior to the polyamide being formed.

![Scheme 3](image)

\textbf{Scheme 3.} \textit{Enamine character of pyrrolecarboxamide}. 
The first limitation due to the enamine character was reported by He and his co-workers\textsuperscript{107} during the synthesis of the oligopeptide framework for a microgonotropen (Scheme 4). In their case, the usual N-methyl group was substituted with an alkyl group having an acetal-protected aldehyde on the central pyrrole unit. The aim was to deprotect the aldehyde and use it to introduce other functional groups which would be able to reach the phosphodiester groups in the major groove. The acetal deprotection to generate aldehyde 76 under acidic conditions resulted however in the formation of a stable dihydroindolizine 77 due to facile intramolecular cyclisation by nucleophilic attack of the free aldehyde by C-5 of the pyrrole ring.

Scheme 4. Intramolecular cyclisation of aldehyde 76.\textsuperscript{107}

The increased reactivity means there are fewer options to functionalise the five-membered ring system. The change of curvature in the oligopyrrole rings creates difficulties because with fewer positions on the five-membered rings, only substitution at the 2- and 5-positions deliver compounds with the correct curvature. Moreover, the amide connectivities are also problematic to alter due to difficulties in preparing either 2,5-diamino- or 2,5-dicarboxylate-containing pyrroles.

Although various methods are available to construct and functionalise oligoamides in five-membered ring systems, with pyrrole based MGBthe vast majority of molecules have added alkylation agents at the N-terminus. Given the data reported to date, no organised structure-activity relationship studies have been described which assess the effect of alkylation moieties added in the middle of the polypyrrole system. This is therefore a structural motif yet to be explored.
1.7 Benzamide based MGB

One strategy to overcome the problems and limitations associated with pyrrole based DNA MGB is to construct six-membered benzene or benzene-like oligoamide derivatives. In comparison to the volume of work performed in 5-membered systems, there is scarcity of literature reported on the synthesis and biological activities of DNA binding agents based on six-membered units. Most of the reported benzamide derivatives are either simple symmetrical diaryl derivatives or triaryl amides containing no alkylating functional groups. Methods have been reported to construct symmetrical benzamides from terephthalic acid or its acid chloride and amines, but there is less literature on the synthesis of non-symmetrical molecules and complicated symmetrical benzamides.

Gong and co-workers have reported a series of four diaryl derivatives and eight triaryl derivatives with the aim of investigating how the curvature of these compounds affects the binding activities, as well as to investigate the role of hydrogen bond donor and acceptor sites. The binding affinities have been investigated with different DNAs using the well-known method of competitive ethidium bromide displacement fluorimetry.

The first three compounds in the series (78-80) have related structures which can adopt crescent shapes easily (Figure 21). Compounds 78 and 79 have very similar structures with altered amide connectivity, acting as hydrogen-bond-donating and -accepting sites. In the desired curved shape, the hydrogens in compound 79 point downwards towards the floor of the minor grooves, where they can form hydrogen bonds with O-2 of thymine and N-3 of adenine. The same shape in compound 78 results in the hydrogens pointing in the opposite direction to the floor of the minor groove. Compound 78 has 14 times less binding selectivity to polyAT DNA than 79. These model compounds can therefore allow the opportunity to probe different sequences of hydrogen bonding sites which is otherwise difficult to achieve with five-ring heterocyclic systems.

Compounds 78 and 79 have similar structures, with compound 80 having one extra NH group. Compound 81 shows very weak binding activity, probably due to it being unable to adopt a suitable conformation for binding in the minor groove.
The results from triaryl derivatives have illustrated that the meta-para-meta arrangement found in compounds 88 and 89 is preferential for the ligand to adopt the crescent conformation, with all the amide hydrogens pointing towards the floor of the DNA for maximum affinity and selectivity (Table 9). Compounds 84 and 85, which have a meta-meta-meta arrangement show weaker binding activity. Interestingly, by swapping the amide connectivity in the same compounds greater GC preference was observed. The binding constants for some of these compounds are comparable to the best-known natural DNA binding agents, such as distamycin 13.110
Chapter 1: Introduction

Figure 21. Di- and triaryl MGB.

Table 9. Di- and triaryl MGB.\textsuperscript{109,110}

<table>
<thead>
<tr>
<th>Compound</th>
<th>CT DNA</th>
<th>T4 DNA</th>
<th>Poly(dA-dT)</th>
<th>Poly(dG-dC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distamycin 13</td>
<td>0.34±0.0</td>
<td>--</td>
<td>0.10±0.0</td>
<td>58.51±5.0</td>
</tr>
<tr>
<td>78</td>
<td>47.3±0.2</td>
<td>35.6±0.2</td>
<td>28.4±1.3</td>
<td>55.6±0.9</td>
</tr>
<tr>
<td>79</td>
<td>31.9±0.8</td>
<td>27.1±0.4</td>
<td>1.90±0.0</td>
<td>142.2±1.9</td>
</tr>
<tr>
<td>80</td>
<td>43.5±3.0</td>
<td>28.6±0.5</td>
<td>4.20±0.1</td>
<td>221.8±1.0</td>
</tr>
<tr>
<td>81</td>
<td>44.8±0.6</td>
<td>35.2±0.7</td>
<td>23.5±1.6</td>
<td>77.3±1.8</td>
</tr>
<tr>
<td>82</td>
<td>21.02±1.0</td>
<td>22.98±0.4</td>
<td>21.02±1.0</td>
<td>25.62±0.7</td>
</tr>
<tr>
<td>83</td>
<td>25.02±0.6</td>
<td>25.32±1.2</td>
<td>22.77±0.6</td>
<td>28.44±1.7</td>
</tr>
<tr>
<td>84</td>
<td>39.93±0.3</td>
<td>39.60±0.6</td>
<td>17.09±0.4</td>
<td>49.73±1.3</td>
</tr>
<tr>
<td>85</td>
<td>37.28±0.7</td>
<td>32.70±1.0</td>
<td>19.96±0.6</td>
<td>46.61±1.0</td>
</tr>
<tr>
<td>86</td>
<td>9.19±0.4</td>
<td>8.84±0.1</td>
<td>6.56±0.2</td>
<td>12.68±0.2</td>
</tr>
<tr>
<td>87</td>
<td>13.18±1.0</td>
<td>12.68±0.3</td>
<td>8.00±0.5</td>
<td>27.20±0.4</td>
</tr>
<tr>
<td>88</td>
<td>8.16±0.4</td>
<td>6.40±0.4</td>
<td>0.21±0.0</td>
<td>22.08±1.8</td>
</tr>
<tr>
<td>89</td>
<td>13.47±1.0</td>
<td>11.87±0.9</td>
<td>0.12±0.0</td>
<td>56.36±2.5</td>
</tr>
</tbody>
</table>

Calf thymus (CT) DNA, T4 Coliphage DNA, Poly[d(A-T)], and Poly[d(G-C)] C\textsubscript{50} (µM) values: ligand concentration leading to a 50% reduction in the fluorescence intensity of bound ethidium λ\textsubscript{excitation} = 546 nm, λ\textsubscript{emission} = 600 nm); assaying conditions: DNA concentrations correspond to A\textsubscript{260} = 2.0 [Ethidium] = 1.26 mM, pH 6.0 buffer (50 mM Na\textsubscript{2}HPO\textsubscript{4}, 10 mM NaCl, 0.1 mM EDTA).
**Advantages of using oligobenzamides**

The use of polypyrrole amides distamycin 13 and netropsin 14 as selective DNA MGB and their use in anticancer drug design have been of interest to chemists for many years. Many oligoamides derivatives composed of five-membered heterocycles have been synthesised and used as DNA binding vectors or as drugs in their own right.

Ligands based on six-membered backbones have some potential advantages over those made from five-ring heterocycles, such as pyrroles and imidazoles, in that these have comparatively higher stability towards oxidation and reduction conditions.

Using disubstituted six-membered rings, more possible structures can easily be generated with different shapes and curvatures, which cannot otherwise be synthesised using disubstituted five-membered heterocycles. A number of symmetrical and non-symmetrical amides, which have different structures and curvatures, can more easily be prepared by reversing the amide connectivities from $\text{Ar}_1\text{NHCOAr}_2$ to $\text{Ar}_1\text{CONHAr}_2$.

A higher degree of functional group diversity and tolerance should also increase the possibility of attaching alkylating groups to these six-membered derivatives, which is less attainable using five-membered rings. Moreover, the benzene rings can be replaced with other six-membered aromatics such as pyridine, pyrimidine, etc. which can allow for the synthesis of numerous derivatives.

**1.8 Synthetic aims and objectives**

Owing to the various advantages of six-membered systems, the primary aim of this project is to establish new and efficient routes towards the synthesis of non-symmetrical di- and triaryl derivatives having different numbers of amide linkages (Figure 22). Secondly, a series of compounds will be synthesised with different shapes, curvatures, and altered amide linkages and functionalisations of the benzylic position, with a number of moieties, keeping in mind their alkylating ability (Schemes 5 and 6). Thirdly, synthesised compounds will be tested for
their DNA binding activities with the aim that structure-activity relationships can be established.

![Figure 22. Generalised structures of oligobenzamide targets.](image)

An important feature of this route is that it should be equally useful for symmetrical amide synthesis with different substitutions. Structure activity relationships can be established using a variety of functional groups at the ends as well as in the centre of the system.
Scheme 5. Retrosynthesis of generalised diaryl amide target.
Scheme 6. Retrosynthesis of generalised triaryl amide target.
Chapter 2

Synthesis of Amines and Acids
2.1 Overview

The initial objectives of the research work were to establish new and efficient routes towards the synthesis of symmetrical and non-symmetrical di- and triaryl amides, derived from dinitrobenzenes and dinitrobenzyl alcohols. A review of the literature shows a lack of synthetic routes to prepare symmetrical and non-symmetrical oligobenzamides that are compatible with azide, amine, ether and chloride functional groups. Our desire was to prepare different series of benzamides that have diverse alkylating groups at a benzylic position and a variety of amino-alkyl groups at the ends, in order to explore the stability and thereafter establish the effect of such functional groups on DNA minor groove binding activities. The different derivatives included those with altered amide connectivities (Ar₁NHCOAr₂ to Ar₁CONHAr₂) and curvature which could then be tested for DNA binding activities using different analytical techniques, such as DNA melting point analysis, competitive ethidium displacement analysis and advanced mass spectrometric techniques. The aim is to investigate the basic requirement for DNA binding in this new class of MGB.

2.2 Synthesis of anilines 90 and 91

The initial step was to prepare anilines having the general structures 90 and 91 as intermediates towards the synthesis of various oligobenzamides (Figure 23). It was envisaged that the aniline precursors 90 could be synthesised starting from commercially available 3,5-dinitrobenzyl alcohol 92 and two anilines (having the general structures 91) from 3-nitroaniline 93.

\[ \text{Figure 23. Generalised structures of target anilines 90 and 91.} \]
The proposed method was to start with 3,5-dinitrobenzyl alcohol 92 which could be selectively reduced to aniline 94. Acetylation of the resultant aniline 94 to compound 95 would then be carried out with acetic anhydride. After the benzylic hydroxyl group was protected (96 and 97), the second nitro group would be reduced under catalytic hydrogenation. The substituted anilines 98 and 99 would then be coupled to a variety of acids 100 independently to furnish the diaryl derivatives 101 (Scheme 7). The anilines, having the general structure 91, would be coupled to acid chloride 102 as precursors towards the synthesis of diaryl acids 103. The diaryl acids 103 could then be coupled to anilines 98 or 99 to prepare the triaryl amides 104.

\[ \text{Scheme 7. Proposed route towards the synthesis of anilines 98/99 and benzamides 101/104.} \]
Compounds containing an amino-alkyl chain are reported to have a better electrostatic interaction in the minor grooves.\textsuperscript{109-111} We therefore wished to incorporate a range of amino-alkyl chains to the di-and triaryl derivatives to investigate the effect of such a group on the stability, DNA binding activities, and solubilities in buffers at physiological pH.

2.2.1 Synthesis of anilines 98, 99 and 105

The first task was therefore to prepare two different protected benzylic alcohol anilines 98 and 99 in order to investigate the reactivity of the benzylic position and its functionalisation by the preparation of a potential nitrogen mustard precursor 105 (Figure 24).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure24.png}
\caption{Structures of proposed anilines 98, 99 and 105.}
\end{figure}

2.2.1.1 Synthesis of protected alcohols 96 and 97

The synthesis of the tert-butylidimethylsilyl (TBDMS)-protected alcohol fragment began from the commercially available 3,5-dinitrobenzyl alcohol 92. Due to the widespread use of nitro groups in synthetic organic chemistry and its use as an intermediate in pharmaceutical and laboratory reagents, many synthetic methods are reported.\textsuperscript{112-114} These methods can be used to synthesise a wide range of nitro compounds ranging from explosives\textsuperscript{115} to medicines.\textsuperscript{112,113} The nitro group can be reduced into the corresponding amine and thereafter converted into a variety of functional groups such as amides\textsuperscript{42} and diazonium salts.\textsuperscript{116}

A large number of benzylic functional groups could be used, including an alcohol group to prepare the desired amides 96 and 97. The alcohol functionality was chosen due to its many
advantages over other functional groups. It can be easily protected and then deprotected with readily available and cheap laboratory chemicals. The hydroxyl group can also be functionalised into numerous other functional groups. Alcohols can be reactive but once they are protected with suitable group, in this case TBDMS, they can be used over multi-step syntheses. The TBDMS-protecting group was chosen due to its stability towards both mildly acidic, basic conditions and oxidising and reducing reagents.\(^1\)

Due to easy methods of preparation for nitro derivatives both in laboratories and on an industrial scale, many methods of nitro reduction are reported.\(^2\) Pd, Pt, Ni or Ru catalysts are commonly used to reduce nitro compounds.\(^3\) In addition to these reagents, sodium sulfide\(^4\) and sodium hydrosulfide are also well-known for this purpose.\(^5\)\(^6\) Dinitroaromatics having symmetrical geometry, such as dinitrobenzyl alcohol, can also be selectively mono-reduced using sulfide reagents.\(^7\)

Ammonium hydrogen sulphide and stannous chloride (in hydrochloric acid) have been used to selectively reduce dinitroaromatic compound into nitroanilines and . Ammonium hydrogen sulfide reduces the nitro group para to the alkyl group to furnish aniline while stannous chloride in hydrochloric acid reduces the nitro group ortho to the alkyl group to give aniline (Scheme 8).\(^8\)\(^9\)

![Scheme 8](image)

The reduction of dinitrobenzyl alcohol with ammonium sulfide has been reported.\(^1\) The mono-nitro reduction of dinitrobenzyl alcohol was therefore attempted using the reported conditions, using two equivalents of ammonium sulfide in refluxing methanol. This initially gave aniline in a poor yield of 30% along with a negligible amount of diaminobenzyl
alcohol produced. Through recovery of unreacted starting material 92 and optimisation of reaction conditions, the yield was improved to a reproducible 81% (the yield is based on the recovered starting material) (Scheme 9). The best conditions were determined to be 6 hours reflux in methanol followed by 20 hours stirring at room temperature.

\[
\text{Reagents and conditions: i. 2 eq. (NH}_4\text{)}_2\text{S, MeOH, reflux, 6 h, then r.t 20 h, 81%.*}
\]

*Yield is based on recovered starting material.

Scheme 9.

This method of using ammonium sulfide in methanol was chosen to prepare nitroaminobenzyl alcohol 94 due to the simplicity of experimental conditions. This reaction is also called the Zinin reduction, after the Russian scientist who developed the reaction to reduce the polynitro aromatics into nitroanilines for the first time.\textsuperscript{125,126} Other reagents such as hydrogen sulfide, sodium sulfide and sodium disulfide can also be used.\textsuperscript{127}

The mechanism of sodium sulfide and disulfide in aqueous media is well known\textsuperscript{122} but the exact mechanism of ammonium sulfide acting as a reducing reagent is still not clear.\textsuperscript{127} No intermediates are known but the reaction is proposed to proceed through nitroso and then hydroxylamine intermediates; the latter are then reduced to an amine. The ammonium sulfide dissociates to sulfide ions and hydrosulfide ions and the hydrosulfide ions then dissociate to provide the divalent sulfide. The attack of the disulfide on the nitro group is the rate-determining step. Elemental sulfur is formed and reacts with ammonium sulfide to form polysulfide. Among the polysulfides, the disulfide is known to reduce the nitro compound.\textsuperscript{128-130} The formation of the different ionic species and their proposed reaction with the nitro group is shown in Scheme 10.
Chapter 2: Synthesis of Amines and Acids

Ammonium sulfide in aqueous medium

\[
\begin{align*}
\text{NH}_3 + \text{H}_2\text{O} & \rightleftharpoons \text{NH}_4^+ + \text{HO}^- \\
\text{H}_2\text{S} & \rightleftharpoons \text{H}^+ + \text{HS}^- \\
\text{HS}^- & \rightleftharpoons \text{H}^+ + \text{S}^{2-}
\end{align*}
\]

a) Reduction by sulfide ions

\[
4\text{ArNO}_2 + 6\text{S}^{2-} + 7\text{H}_2\text{O} \rightarrow 4\text{ArNH}_2 + 3\text{S}_2\text{O}_3^{2-} + 6\text{HO}^-
\]

b) Reduction by disulfide ions

\[
\text{ArNO}_2 + 2\text{S}^{2-} + \text{H}_2\text{O} \rightarrow \text{ArNH}_2 + \text{S}_2\text{O}_3^{2-}
\]

c) Reduction by hydrosulfide ions

\[
\text{ArNO}_2 + 3\text{HS}^- + \text{H}_2\text{O} \rightarrow \text{ArNH}_2 + 3\text{S} + 3\text{HO}^-
\]

Scheme 10. Reaction of ammonium sulfide with nitro group.122

One advantage of using the Zinin reduction is that it can be more easily scaled up compared to other monoreduction techniques. This is especially important compared to catalytic hydrogenation, which needs special care in handling, has low selectivity, and often requires chromatographic methods to purify the two isomers. The reaction conditions are mild, resulting in the mono-nitro reduction only. The use of metal reductions, such as tin, on a large scale is also problematic due to the large amount of toxic metal waste generated. The reaction of 92 on a large scale (9 g) provided sufficient aniline 94 to be used for numerous reactions and the unreacted starting material 92 was easily recovered and would be reused in the reaction without purification.

Anilines are widespread in nature and are widely used amines in synthetic organic chemistry.131,132 Anilines are most easily converted into amides by the reaction with acid derivatives including acid chlorides110 and acid anhydrides.133,134 Anilines have a strong activating role on the aromatic ring at ortho and para positions. Acetamide and acetate groups are mildly activating compared to the amine and alcohol groups (anilines or phenols), and are therefore less likely to be involved in any unwanted substitution reactions.135

It was therefore decided to acetylate the amine group of 94, which should generate the first amide bond in the process. This bond could be capable of hydrogen bonding with bases in the minor grooves of double-stranded DNA. Only monoacetylation of 94 at the amino group was
anticipated by using one equivalent of acetic anhydride, with no acetylation at the benzylic alcohol. However, using one equivalent of acetic anhydride resulted in a mixture of products: the desired acetamide \text{95} as well as the unwanted products 3-acetamido-5-nitrobenzyl acetate \text{96} and 3-amino-5-nitrobenzyl acetate \text{109}, along with some starting material \text{94} (Scheme 11). It was found to be difficult to isolate the desired product \text{95} from the other products using flash chromatography or other means.

\[
\text{\begin{array}{c}
\text{HO} \\
\text{H}_2\text{N} \\
\text{NO}_2 \\
\end{array}} \xrightarrow{i} \text{\begin{array}{c}
\text{OAc} \\
\text{AcHN} \\
\text{NO}_2 \\
\end{array}} + \text{\begin{array}{c}
\text{OAc} \\
\text{AcHN} \\
\text{NO}_2 \\
\end{array}} + \text{\begin{array}{c}
\text{H}_2\text{N} \\
\text{NO}_2 \\
\end{array}}
\]

\text{Reagents and conditions: i. 1 eq. Ac}_2\text{O, 1 eq. Et}_3\text{N, DCM, r.t, 24 h, mixture.}

\text{Scheme 11.}

Due to the problems associated with selective acetylation and the purification process, it was decided to react amino-alcohol \text{94} with excess equivalents of acetic anhydride to furnish the diacetyl derivative \text{96}. Whilst being more base sensitive than the TBDMS group, it was thought that the acetyl group could also be used as a hydroxyl protecting group. Therefore, using three equivalents of acetic anhydride in the presence of triethylamine in DMF gave the desired product \text{96} and after optimisation a reproducible yield of 99\% could be achieved. Also, rather than purifying with flash chromatography, amide \text{96} could easily be precipitated out from the reaction mixture by adding cold water and stirring (Scheme 12). The resultant solid was then recrystallised from ethyl acetate which gave pure crystals, suitable for single crystal analysis. Crystallographic analysis of \text{96} and subsequent derivatives were undertaken to investigate the orientation of the hydrogen bonding groups, such as the amide groups. It was also noted that there were few published X-ray crystal structures of these trisubstituted benzene derivatives. Compounds of this type are not only of interest in medicinal chemistry but have applications in material and polymer chemistry\textsuperscript{136,137}. It was therefore decided to investigate, and where possible, publish the X-ray structures of these derivatives. In this case the crystallographic data shows an asymmetric unit containing three independent molecules.
which differ in geometry only by their rotation about the single bonds external to the benzene ring (Figure 25).

![Chemical structure](image)

**Reagents and conditions:** i. 3 eq. Ac₂O, 4 eq. Et₃N, DMF, r.t., 24 h, 99%.

**Scheme 12.**

![Crystal structure](image)

**Figure 25.** Crystal structure of diacetyl derivative 96.*

*50% probability displacement ellipsoids for non-hydrogen atoms and hydrogen atoms as arbitrary spheres.*

With diacetyl derivative 96 in hand, we also wished to prepare benzylic alcohol 95 which could have alternative groups, such as the planned TBDMS, to protect the benzylic alcohol. The benzylic acetyl group could be selectively hydrolysed under basic conditions or using mild acidic conditions in methanol or ethanol depending on the stability of the substrate or the different functional groups present on the substrate towards acidic and basic conditions.

Thus, N-(3-hydroxymethyl-5-nitrophenyl)acetamide 95 was furnished by the selective hydrolysis of 3-acetamido-5-nitrobenzyl acetate 96 with sodium hydroxide in ethanol in 98% yield (Scheme 13). The compound 95 was purified by flash chromatography to remove trace coloured impurities before being recrystallised from chloroform using a drop of DMSO to aid
solubility (only a small sample was recrystallised for single crystal analysis). The crystal data show the presence of residual DMSO molecules in the crystal structure (Figure 26).\textsuperscript{140}

\begin{center}
\begin{tikzpicture}
    \node[above] at (0,0) {AcHN\hspace{0.5cm} NO2 \hspace{1.5cm} AcHN\hspace{0.5cm} NO2};
    \node[left] at (0,0) {OAc};
    \node[below] at (0,0) {i};
    \draw[->] (0,0) -- (1,0);
    \end{tikzpicture}
\end{center}

Reagents and conditions: i. 2 eq. NaOH, EtOH, r.t, 3 h, 98%.

Scheme 13.

Figure 26. Crystal structure of alcohol 95.\textsuperscript{*}

*50\% probability displacement ellipsoids for non-hydrogen atoms and hydrogen atoms as arbitrary spheres.\textsuperscript{138}

With alcohol 95 prepared, we wished to protect the benzylic alcohol with a protecting group suitable for a general synthesis of polyfunctional benzamides. Different points during the synthesis were considered to select a suitable protecting group.

Large numbers of protecting groups are reported, including a variety of silyl ethers. Among the silyl ethers, the tert-butyldimethylsilyl ether is widely used due to its stability towards oxidative, reductive, mild acidic and basic conditions.\textsuperscript{117,141} However, it can be easily and often selectively deprotected to efficiently give the parent hydroxyl group, using fluoride reagents and without affecting other functionalities.\textsuperscript{142}
The benzylic hydroxyl group was therefore successfully converted to the TBDMS ether using tert-butyldimethylsilyl chloride and imidazole in DMF (Scheme 14). Initially a reaction time of 2 hours gave ether 97 in 78% yield, however the yield was improved by increasing the reaction time to 5 hours and ensuring that the reaction was performed under an atmosphere of nitrogen. The silyl ether 97 retained a pale yellow colour after purification using flash chromatography, with a dichloromethane-methanol (19:1) solvent system. To remove the coloured impurities and to obtain better quality crystals, n-hexane-ethyl acetate (3:1) solvent system was then used. The yield was eventually improved to a reproducible quantitative yield and the structure was again analysed by crystallography (Figure 27).

\[
\begin{align*}
\text{AcHN} & \quad \text{OH} \\
\text{AcHN} & \quad \text{NO}_2 \\
\text{i} & \quad \text{OTBDMS} \\
\end{align*}
\]

\[(95) \quad \rightarrow \quad (97)\]

*Reagents and conditions: i. 1.2 eq. TBDMSCl, 3 eq. imidazole, DMF, r.t, 5 h, 99%.*

Scheme 14.

The asymmetric unit contains two independent molecules which differ primarily in the rotation about the C7-O4 bond (torsion angles C1-C7-O4-Si equal to -152.75, -110.21° for molecules A and B respectively). Two strong N-H–O hydrogen bonds align the molecules into wide ribbons tending approximately parallel to the b axis. There are four very close intramolecular contacts (C4A-O1A 2.886 (3), C4B-O1B 2.894 (3), C6A-O4A 2.740 (3) and C6B-O4B 2.785 Å).

*Figure 27. Crystal structure of the two forms of ether 97.*

*50% probability displacement ellipsoids for non-hydrogen atoms and hydrogen atoms as arbitrary spheres.*

---

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51
2.2.1.2 Synthesis of anilines 98 and 99

\[
\begin{align*}
\text{AcHNN} & \xrightleftharpoons{\text{10\% Pd/C, H}_2, \text{MeOH, r.t.}} \text{AcHN} \\
\text{NO}_2 & \quad \text{NO} \\
\text{OTBDMS} & \quad \text{OTBDMS} \\
\text{(97)} & \quad \text{(99)}
\end{align*}
\]

*Reagents and conditions:* i. 10\% Pd/C, H\_2, MeOH, r.t., 3 h, 100%.

**Scheme 15.**

The next step was to reduce the second nitro group under catalytic hydrogenation which again is one of the well-known reactions in chemistry due to its widespread applications in the chemical, petrochemical and food industries.\textsuperscript{145-147} Hydrogen is the main reacting species in hydrogenation reaction where it is added to the site of multiple bonds. Pure hydrogen gas or any other sources of hydrogen species can be used to carry out hydrogenation and hydrogenolysis. Many metals catalysts, namely Pd, Ni and Pt are used to facilitate the process.\textsuperscript{119} The metals are adsorbed on carbon support to slow the reactivity to controllable limits. The nitro group is reduced via a number of intermediates\textsuperscript{148} to the desired amine (Scheme 16).

\[
\begin{align*}
\text{NO}_2 & \quad \text{NO} \\
\text{nitrobenzene} & \quad \text{nitrosobenzene} \\
\text{H}_2 & \quad \text{NH}_2 \\
\text{benzylhydroxylamine} & \quad \text{aniline} \\
\end{align*}
\]

\[
\begin{align*}
\text{N} \equiv \text{N} & \quad \text{N} \equiv \text{N} \\
\text{azoxybenzene} & \quad \text{azobenzene} \\
\text{H}_2 & \quad \text{H}_2 \\
\text{hydrazobenzene} & \quad \text{hydrazobenzene}
\end{align*}
\]

**Scheme 16. Different intermediates in the reduction process of the nitro group.**\textsuperscript{148}

Hydrogenation of TBDMS-protected ether 97 was attempted using 10\% palladium on carbon in LR grade methanol. After a 3-hour reaction time, analysis of the reaction mixture using
TLC showed multiple products. The reaction mixture was subjected to flash chromatography but no single product was isolated. It was decided to use a different solvent system and hydrogenation conditions as we thought the product might not be stable in methanol. The reaction was investigated in different solvents, namely pure methanol, 5% aqueous methanol and ethyl acetate. It was found that the reaction was faster in pure methanol than in 5% aqueous methanol and ethyl acetate.

The low yield and the slow rate of reaction were hypothesised to be due to decomposition of the intermediates and the final target aniline 99 over time, so we attempted to decrease the reaction time. An increase in temperature resulted in a slightly higher reaction rate than at room temperature but decomposition still occurred. Finally, it was discovered that rigorous removal of all air from the reaction flask was required for the reaction to proceed without decomposition products forming.

Once this was discovered, the reaction conditions were optimised in LR grade methanol and the yield was improved to a reproducible quantitative yield with no byproducts detected. Upfield shifts in carbons and protons were observed in the NMR spectra of aniline 99 compared to starting material 97. Aniline 99 was quite stable at room temperature and could be stored for months when completely solvent free and dry, however the presence of solvent resulted in decomposition.

To investigate the possibility of using the acetyl protecting group, thereby saving time and chemical steps, the hydrogenation reaction was carried out on O-acetyl compound 96 under the same conditions as that for the TBDMS-protected aniline 99. The reaction was repeated many times to optimise the conditions and to probe the stability of the acetyl group under catalytic hydrogenation. Often a byproduct was noted in the reaction mixture when analysed using TLC. It was thought that it was possibly due to the reaction of the newly formed amino group or an intermediate with the ester as a result of the heat of hydrogenation. The purified O-acetyl aniline 98, which was still obtained in 95% yield, was comparatively less stable than the TBDMS-protected aniline 99. Aniline 98 was required to be stored at low temperature or freshly prepared each time before use (Scheme 17). Further studies were not carried out to investigate the stability and the possible byproducts.
2.2.2 Functionalisation of benzylic position

Our next aim was to investigate the functionalisation of the benzylic position of nitroamide 95, prior to the reduction of the second nitro group into a precursor for potential alkylating moieties.\(^{42}\) The DNA binding agents composed of five-membered heterocycles usually alkylate the DNA bases in the minor groove. The additional group at benzylic position can alkylate the base pairs as well as the phosphate group due to its spatial orientation. It was also envisaged that the benzylic position could be functionalised with a wide variety of potential groups including the bromoacrylamide.\(^ {93}\) The advantages of using a trisubstituted aromatic substrate such as 95 is that the benzylic position should be easily tuned into a range of potential alkylating groups whilst the polybenzamides can be used to hydrogen bond in the minor groove of DNA.

Higher polarity was anticipated in compounds with greater numbers of amide bonds, therefore it was expected that more polar solvents, such as DMF would be used. The starting anilines such as 98 and 99, however, were soluble in almost all the common laboratory solvents and could therefore be used to functionalise the benzylic position at this earlier stage. In some of the proposed reactions, particularly the conversion of diols into mustards, the presence of chlorinating agents and excess of DMF may prove problematic. If these reactions are carried out earlier in the synthesis in different, less reactive solvents, this problem can be avoided. Therefore, it was decided to attempt to functionalise the benzylic position first before coupling it to the acid partners to form the desired benzamides. Also, owing to the poor stability of benzylic mesylates (which was one of our proposed intermediates and will be
discussed later) on silica gel during the purification process, functionalisation of the benzylic position is preferred early in the synthesis.

2.2.2.1 Synthesis of methanesulfonate 110

Methanesulfonates can be easily prepared from alcohols due to their synthetic versatility and easy substitution with different nucleophiles such as amines and azides. Previous work in our research group\(^{133}\) has determined that benzylic mesylates in benzamide systems are more stable than their corresponding benzylic halide. The reaction of mesylates has diverse applicability. They can be functionalised with a variety of nucleophiles and are useful for the preparation of \(N,N\)-bis(2-hydroxyethyl)benzylamines which are nitrogen mustard precursors. The dual functionality of the two free hydroxyl groups, along with a basic nitrogen in this moiety, has been used in the synthesis of numerous metal complexes including those containing vanadium,\(^{149}\) manganese\(^{150,151}\) and iron.\(^{150}\)

The reaction of alcohol 95 with 1.5 equivalents of methanesulfonyl chloride in DMF in the presence of triethylamine gave the desired mesylate 110 in a 95% yield (Scheme 18). Mesylate 110 was used in the next step without purification due to its instability over silica gel. It was discovered that the mesylation of 95 could also be carried out equally well in THF or DCM but we optimised the reaction in DMF as other benzamides we wished to functionalise were expected to be more polar.

![Scheme 18](image)

Reagents and conditions: i. 1.5 eq. MsCl, 1.5 eq. Et\(_3\)N, DMF, 0 °C → r.t, 2 h, 95%.

Scheme 18.
2.2.2.2 Synthesis of diol 111

With mesylate 110 prepared, we wished to investigate its reactions with a range of nucleophiles that could be later functionalised. Therefore, mesylate 110 was reacted with 10 equivalents of diethanolamine in THF at room temperature to give, after purification, diol 111 in a yield of 84% (Scheme 19). The resultant diol 111 was also recrystallised from ethyl acetate for single crystal analysis to investigate the hydrogen bonding nature of diol moiety (Figure 28).

![Chemical structure](image)

\[
\text{Reagents and conditions: i. 10 eq. diethanolamine, THF, 0 °C then r.t 18 h, 84%}
\]

Scheme 19.

![Crystal structure](image)

**Figure 28.** Crystal structure of diol 111.*

*50% probability displacement ellipsoids for non-hydrogen atoms and hydrogen atoms as arbitrary spheres.*
2.2.2.3 Catalytic hydrogenation of diol 111

The next step was to reduce the nitro group of diol 111 to form aniline 105, hypothesised this unit could be converted to nitrogen mustard after its incorporation in a polybenzamide. Catalytic hydrogenation of 111 resulted in a single product. The NMR analysis of the product showed the disappearance of signals for diol moiety at $\delta$ 2.70 and 3.62 ppm in the $^1$H NMR and $\delta$ 57.5 and 60.7 ppm in the $^{13}$C NMR, and the appearance of a new peak for a methyl group at $\delta$ 2.21 ppm and 21.4 ppm in the $^1$H and $^{13}$C NMR respectively (Scheme 20). This suggested the formation of toluene 112 by benzylic cleavage as well as the reduction of the nitro group to an amine.

\[
\begin{align*}
\text{HO-} & \text{N} & \text{OH} & \xrightarrow{i} & \text{HO-} & \text{N} & \text{OH} \\
\text{AchN} & \text{NH}_{2} & & \xrightarrow{i} & \text{AchN} & \text{NH}_{2} \\
& & (105) & & & (111)
\end{align*}
\]

\[
\begin{align*}
\text{CH}_{3} & \\
& \text{AchN} \text{NH}_{2} & \xrightarrow{i} & \text{AchN} \text{NH}_{2} \\
& & (112)
\end{align*}
\]

*Reagents and conditions*: i. 10% Pd/C, H$_2$, MeOH, r.t., 3 h, 100%.

**Scheme 20.**

The hydrogenolysis of the $N$-benzyl bond is probably due its weak bonding nature.$^{152}$ It is believed that the hydrogenolysis of $N$-benzyl bond is due to the hydrogenolytic cleavage and not by insertion mechanism. The proposed mechanism involves the dehydrogenation of methanol on the surface of the catalyst to form formaldehyde. The resultant formaldehyde then reacts with amine to form quaternary carbinolamine. The quaternary carbinol then, after debenylation, forms the carbinolamine, which by losing a water molecule forms the iminium ion. The hydrogenation of the iminium ion then results into the $N$-methyl product.$^{152,153}$
Often $N$-benzyl cleavage is slow or requires more reactive catalysts such as platinum oxide, and was therefore unexpected under these conditions. It was uncertain whether the substituents or the nature of the amine, or both, played a part in this unwanted hydrogenolysis. In any case, toluene 112 allows for the synthesis of polyamides with a single methyl group at this position, allowing for a further understanding of substitution at this position.

### 2.2.3 Synthesis of unsubstituted anilines 113 and 114

To further investigate the relevance of substitution at the benzylic position and its effect on electrostatic interactions during the DNA binding process, two unsubstituted anilines 113 and 114 were required, which we envisaged could be prepared from 3-nitroaniline 93.

The known acetamido-aniline 113 was prepared easily from 3-nitroaniline 93 according to our previously optimised conditions as applied to amide 96. The desired aniline 113 was obtained in good yield over two steps (Scheme 22, all the three compounds 93, 115 and 113 are available commercially).

![Scheme 21. Mechanism of hydrogenolysis.](image)

Reagents and conditions: i. 1.5 eq. $\text{Ac}_2\text{O}$, 2 eq. $\text{Et}_3\text{N}$, DMF, r.t, 24 h, 82%; ii. 10% Pd/C, $\text{H}_2$, MeOH, r.t, 3 h, 100%.

**Scheme 22.**
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We then wished to synthesise another unsubstituted aniline 114, replacing the N-acetyl group with a longer amino-alkyl chain. The basic amino-alkyl chain is a common moiety in biologically important compounds\(^1\) and is found in DNA MGB.\(^2\) The dimethylamino group has been found to be more stable in solutions than the amidine group\(^2\),\(^7\),\(^8\),\(^9\) which is present in distamycin\(^3\) and netropsin\(^6\), while keeping the same level of electrostatic interaction in the minor groove of DNA. The dimethylamino group can easily be protonated and its additions would provide an opportunity to study the effect of amino-alkyl tails at the end of the molecule in a systematic way to draw structure-activity relationships.

Initial synthesis of aniline 114 centred on the method of Gong and co-workers\(^1\) who used 4-(dimethylamino)butanoyl chloride for the acylation of 3-nitroaniline 93. The 4-(dimethylamino)butanoyl chloride, prepared from the corresponding acid using oxalyl chloride in the presence of a catalytic amount of DMF, was coupling with 3-nitroaniline 93. Unfortunately, none of the desired product 117 was obtained.

In another method, reported by Boger et al.\(^1\) 4-(dimethylamino)butanoic acid was used along with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), with a catalytic amount of DMAP in DMF for the preparation of a similar amide (related reaction shown in Scheme 23). However, when these conditions were carried out with nitroaniline 93 at room temperature and at 80°C, both the conditions failed to give the desired amide 117 in more than trace amounts. It was postulated that the lower reactivity of nitroaniline 93 was due to the presence of the nitro group, which deactivates it towards further reaction.

An alternative method reported by Laborde et al.\(^1\) using a di-tert-butyldicarbonate to generate an activated acid anhydride was then used, however only a trace amount of amide 117 was obtained, therefore other methods were tried.

A method reported by Stoveret al.\(^1\) was then used, by reacting aniline 93 with two equivalents of the hydrochloride salt of dimethylaminobutyric acid 116, along with two equivalents of EDC and DMAP in DMF at 45 °C for 24 hours (Scheme 23). Although most of the unreacted reagents were removed by aqueous extraction, repeated flash chromatography
was required to remove traces of DMAP still remaining. Regardless of the difficulties in purification, amide 117 was obtained in a high yield of 98%.

\[
\text{O}_2\text{N} - \text{NH}_2 + \text{HO-C} - \text{N} - \text{NCl} \xrightarrow{\text{i}} \text{O}_2\text{N} - \text{NH} - \text{N} - \text{N} \\
(93) \quad (116) \quad (117)
\]

Reagents and conditions: i. 2 eq. EDC, 2 eq. DMAP, DMF, 45 °C, 24 h, 98%; or 2 eq. DIC, 2 eq. HOBt, cat. DMAP, DMF, r.t, 18 h, 94%; ii. 10% Pd/C, H\(_2\), MeOH, r.t, 3 h, 100%.

\textbf{Scheme 23.}

Later, after solving problems associated with the purification process, another coupling reagent DIC, and HOBt in the presence of a catalytic amount of DMAP were used. In this case purification was comparatively easier, giving amide 117 in 94% yield.

With nitro-derivative 117 in hand, catalytic hydrogenation in methanol for 3 hours in a similar manner as previous experiments then gave the desired aniline 114 in quantitative yield (Scheme 23).
Summary of the synthesis of anilines

Five anilines were prepared including three anilines, 98, 99 and 112, with benzylic substitution, starting from dinitrobenzyl alcohol 92 and two unsubstituted anilines 113 and 114 were prepared starting from nitroaniline 93 (Scheme 24). Aniline 98 was prepared in three steps where the acetyl group was used as protecting group for the benzylic alcohol, however it was found that the acetyl ester was less stable than the TBDMS ether. The O-acetyl group in compound 96 was hydrolysed to give alcohol 95. The resultant hydroxyl group was again reprotected with a TBDMS group. The TBDMS-protected nitro derivative 97 was then reduced to aniline 99, with no benzylic cleavage during catalytic hydrogenation. Many intermediates were stable crystalline solids and their structures were analysed by X-ray crystallography. The benzylic position was also functionalised early in the synthesis with a nitrogen mustard precursor, diol 111, however when reduced under catalytic hydrogenation, benzylic cleavage as well as nitro group reduction occured. This route thus prepared the methyl substituted aniline 112.

The non-benzylically substituted acetamide 115 was prepared by the acetylation of nitroaniline 93 and the subsequent reduction under catalytic hydrogenation gave aniline 113. The aniline 114 was prepared from nitroaniline 93 and 4-(dimethylamino)butanoic acid 116 using DIC and HOBt coupling reagents in quantitative yield and the resultant nitro-derivative 117 was then reduced to aniline 114 (Scheme 24).
Chapter 2: Synthesis of Amines and Acids

**Reagents and conditions:**

i. a) 2 eq. (NH₄)₂S, MeOH, 6 h, reflux then r.t 20 h, 81%; b) 3 eq. Ac₂O, 4 eq. Et₃N, DMF, r.t, 24 h, 99%;
ii. 2 eq. NaOH, EtOH, r.t, 3 h, 98%;
iii. 1.2 eq. TBDMSCl, 3 eq. imidazole, DMF, r.t, 5 h, 99%;
iv. 10% Pd/C, H₂, MeOH, r.t, 3 h, (98) = 95%, (99, 112, 113 and 114) = 100%;
v. a) aq. 1.5 eq. MsCl, 1.5 eq. Et₃N, DMF, 0 °C → r.t, 2 h, 95%; b) 10 eq. diethanolamine, DMF, 0 °C → r.t, 18 h, 84%;
vii. 1.5 eq. Ac₂O, 2 eq. Et₃N, DMF, r.t, 24 h, (115) = 82%. or 2 eq. 4-(dimethylamino)butanoic acid.HCl (116), 2 eq. EDC, 2 eq. DMAP, DMF, 45 °C, 24 h, (117) = 98%.

**Scheme 24.** Summary of synthesised anilines.
2.3 Considerations for the synthesis of monoaryl acids

After successful preparation of the desired anilines 98, 99, 112-114 we then wished to prepare the monoaryl acids 119-128 and 153-157 as coupling partners to synthesise the desired di- and triaryl amides. Different series of acids would be prepared containing mono- and diaryl system/s with open/cyclic chain amino-alkyl groups of different lengths to investigate the effect of the presence of such groups on the DNA binding activity, as well as to probe the resultant cytotoxicities and antiproliferative activities of these compounds. The literature reveals that modifications to the length of the side chain amino-alkyl group can lead to visible changes in binding and antitumour activity.110 Changing the aromatic substitution from meta to para also results in different curvatures and this was therefore expected to lead to different behaviour during the binding to DNA minor grooves.

Although, compounds containing morpholine and piperidine units in their structures are useful drugs,160 these functional groups are not frequently used in DNA MGB and this will be a new exploration.

As previously mentioned, the amino-alkyl group has the advantage that it can be used under elevated temperatures due to its greater stability when compared to other basic groups (such as the amidine group) while maintaining DNA binding activity. The introduction of different-sized amino-alkyl groups is expected to have different chemical and physical effects ranging from basicity to steric factors. These systematic investigations will provide us with a clear picture of the basis of anticancer activity and the structure-activity relationships of this novel class of benzamides.

The second series of monoaryl acids would contain para amino-alkyl chain; a longer chain would increase the possibility of greater electrostatic binding, deep in the minor groove. Acids having open and cyclic amino-alkyl side chains would be synthesised and the effect of altered amide connectivities (ArNHCO to ArCONH) will be explored.
2.3.1 Synthesis of acids 119-128

Keeping in mind the many advantages of different changes, it was therefore decided to prepare monoaryl acids in order to verify the validity of the synthetic routes and to optimise the reactions conditions where possible. This series would contain open chain and cyclic amino-alkyl groups. Whilst open chain amino-alkyl groups are known to have high binding activity with double-stranded DNA\textsuperscript{110}, the DNA binding properties of cyclic amino-alkyl group such as piperidine and morpholine has yet to be fully explored.

Two methods were used to prepare the desired acids 119-128. The first method was to react commercially available 4-(2-chloroacetamido)benzoic acid 118 with different secondary amines (Scheme 25). Although, there was some success with this method, with the crude NMR showing some product, the high polarity and aqueous solubility of the resultant product, possibly due to its existence as a zwitterion, made product 119 difficult to purify and the approach was abandoned.

\begin{center}
\begin{tikzpicture}
\node at (0,0) [text width = 2.5cm, align = center] {\textbf{Reagents and conditions:} i. excess diethylamine, EtOH, reflux, o/n, trace amount.};
\end{tikzpicture}
\end{center}

\textbf{Scheme 25.}

The second approach used to prepare the target acids 119-128 was to protect the \textit{para} and \textit{meta} aminobenzoic acids 129 and 130 as benzyl esters. This would increase their solubility and stop the formation of zwitterions. Preparation of the benzyl ester in the presence of aniline is problematic so additional protection steps were needed. The proposed steps would therefore be Boc protection at the aniline, benzylation of the acid, Boc-deprotection and acylation, followed by amine addition and finally hydrogenation as shown in Schemes 26 and 27.
The commercially available *para* and *meta* aminobenzoic acids 129 and 130 were therefore Boc-protected according to the literature method\(^{161}\) using 1.8 equivalent of di-tert-butyl dicarbonate in the presence of sodium hydroxide. This gave 87% and 99% yields of the *para* and *meta* isomers 131 and 132, respectively (Scheme 26).

The next benzylation step was carried out according to the literature method\(^{162}\) using benzyl bromide in the presence of cesium carbonate and after workup gave the desired esters 133 and 134 in good yields of 81% and 91%, respectively. The product after workup was essentially pure, with new characteristic peaks at δ 5.3 ppm and δ 66 ppm in the \(^1\)H and \(^{13}\)C NMR spectra respectively, corresponding to the new benzylic CH\(_2\) and it was therefore decided to use the compound in the subsequent reactions without further purification (Scheme 26). The Boc groups of 133 and 134 were deprotected using trifluoroacetic acid (TFA) in DCM by stirring for one hour and after workup gave aminobenzoic esters 135 and 136 in 95% and 93% yields respectively.

Reagents and conditions: i. 1.8 eq. di-tert-butyl dicarbonate, 1.1 eq. NaOH, water/dioxane (1:1) 0 °C then r.t 3 h, ii. 1 eq. benzyl bromide, 1.1 eq. Cs\(_2\)CO\(_3\), DMF, r.t, 2.5 h; iii. trifluoroacetic acid, DCM, r.t, 1 h.

Scheme 26.
With aminobenzoic esters 135 and 136 in hand, acylation was achieved using 1.1 equivalent of bromoacetyl bromide. Dropwise addition of the bromoacetyl bromide to anilines 135 and 136 in DCM furnished the desired amide esters 137 and 138 (Scheme 27). It should be noted that fast addition of the bromoacetyl bromide resulted in dimerisation or decomposition. The final substitution step was carried out by adding secondary amines, namely diethylamine, morpholine, piperidine and diisopropylamine in excess (Scheme 28). The average yield for all amines was above 90% for this step. The final reaction involved the removal of the benzyl group in compounds (139-146) under catalytic hydrogenation, using 10% Pd/C in methanol. The reaction gave the required acids 119-126 (Scheme 31) cleanly in quantitative yield. Some of the acids, especially the para piperidino-acid 123, were extremely insoluble and precipitated during the hydrogenation reaction. Washing the residue of the reaction with 2M NaOH solution followed by careful acidification with hydrochloric acid increased the yield of the isolated product. The benefit of using hydrogenation as the last step to form the acids was that all the acids 119-126 (Scheme 31) did not require chromatographic purification. The NMR spectra of each acid showed only pure acid with no starting materials or byproducts seen.

Reagents and conditions: i. 1.1 eq. bromoacetyl bromide, DCM, r.t, 20 h; ii. 2.17 eq. HNR₂, DCM, reflux, o/n; iii. 10% Pd/C, H₂, MeOH, r.t, 5 h.

Scheme 27.
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Scheme 28. Reactions of bromides 137 and 138 with different amines.

For the preparation of acids 127 and 128 with longer chain lengths between the amide and tertiary amine functionality, a slightly different method was used. In this case a coupling reaction was carried out by reacting 4-morpholinobutanoic acid 147 with anilines 135 and 136 (Scheme 30). The morpholino-acid 147 was prepared initially from excess morpholine 148 and ethyl 4-bromobutyrate 149. The crude NMR showed all the required peaks for the desired product 150. However, the excess morpholine 148 was still present after workup and its removal through distillation proved difficult to obtain the required product 150 in pure form (Scheme 29).
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Reagents and conditions: i. 1 eq. (149), 2 eq. Morpholine (148), toluene, 60 °C 4 h, then r.t o/n, 97%; ii 18% aq. hydrochloric acid, reflux, 18 h, 100%.

Scheme 29.

When the same reaction was repeated using two equivalents of morpholine 148 in toluene according to a reported method for a similar compound,\textsuperscript{163,164} this resulted in the desired product 150 as a pure product so no purification was required. Acid hydrolysis of the resultant ester 150 using aqueous hydrochloric acid furnished the desired acid 147 in quantitative yield as the hydrochloride salt (Scheme 29). Morpholino-acid 147 was then used as the coupling partner with amines 135 and 136 using either DIC/HOBt or EDC/DMAP and gave the desired esters 151 and 152 (Scheme 30) in 66% and 74% yield respectively. The resultant esters 151 and 152 were reduced under catalytic hydrogenation in methanol (Scheme 31).

Reagents and conditions: i. 2 eq. (147), 2 eq. EDC, 2 eq. DMAP, DMF, 45 °C, 24 h, (151) = 66%, (152) = 74%.

Scheme 30.
Scheme 31. Hydrogenation of benzyl esters 139-146, 151 and 152 to acids 119-128.

* Extraction of the crude residue with 2 M sodium hydroxide followed by neutralisation was required due to its high polarity.
2.3.2 Synthesis of para amino-alkyl acids 153-157

Next we wished to prepare benzamides with reversed amide connectivity. This was to be achieved by preparing acids with an additional amino-alkyl chain derived from a terephthalate framework. To achieve this, we began from commercially available terephthaloyl chloride 158. Thus, the reaction of terephthaloyl chloride 158 with 2.7 equivalents of benzyl alcohol in the presence of triethylamine, gave after recrystallisation from ethanol, dibenzyl terephthalate 159 in a good yield of 88% (Scheme 32).\(^\text{165}\)

\[
\text{PhO\(\text{COCl}\)} \quad \xrightarrow{\text{i. 2.7 eq. benzylalcohol, 4.3 eq. Et}_3\text{N, toluene, r.t, o/n, 88%}} \quad \text{PhO\(\text{CO}_2\text{Bn}\)}
\]

\[(158) \quad \text{(159)}\]

Reagents and conditions: i. 2.7 eq. benzylalcohol, 4.3 eq. Et\(_3\)N, toluene, r.t, o/n, 88%.

Scheme 32.

Mono-benzyl ester 160 was then prepared from the dibenzyl terephthalate ester 159 according to the literature method,\(^\text{165}\) using one equivalent of lithium hydroxide in acetone/water to give the desired product 160 in 92% yield (Scheme 33). The remaining starting material 159 was also recovered by extraction with organic solvents. Acid chloride 102 was then generated from acid 160 using oxalyl chloride with a catalytic amount of DMF in DCM (Scheme 33).\(^\text{166}\)

Oxalyl chloride is a mild chlorinating agent compared to other chlorinating agents and has the advantages that the byproducts are low boiling and can be easily removed under high vacuum. The reaction can be carried out in different organic solvents, such as DCM and THF, which can be easily removed afterwards. It was found that only a few drops of DMF should be used as a large amount was not useful for increasing the yield. It is important to mention that the acid chloride 102 was made fresh as required and was dried under high vacuum for 2 to 3 hours prior to use, due to its sensitivity towards moisture.
Chapter 2: Synthesis of Amines and Acids

Reagents and conditions: i. 1 eq. LiOH, acetone/water, reflux, 30 min, 92%; ii. 2.5 eq. oxalyl chloride, cat. DMF, DCM, r.t, 4 h, 100%.

Scheme 33.

Different primary amines were then reacted with acid chloride 102 to furnish amino-benzoates 161-165 in unoptimised yields ranging from 61-94% (Scheme 34).

Reagents and conditions: i. 2 eq. RNH2, DCM, r.t, o/n.

Scheme 34. Reaction of acid chloride 102 with different amines.

The next step was the deprotection of the benzyl esters 161-165 to give the corresponding acids 153-157, again using catalytic hydrogenation, with 10% in Pd/C in methanol. The
reaction was quantitative in each case, giving acids 153-157 without need for further purification (Scheme 35).

![Scheme 35](image)

Reagents and conditions: i. 10% Pd/C, H₂, MeOH, r.t, 5 h, quantitative.

---

2.3.3 Synthesis of diaryl acids 166 and 167

After the synthesis of monoaryl acids (Scheme 31 and 35), our next target was the synthesis of diaryl acids 166 and 167, which would be used to prepare triaryl derivatives (Figure 29). Acids 166 and 167 would contain an acetamide or amino-butanimide group at the meta position of the second aromatic ring respectively. These diaryl acids 166 and 167 were desirable as increased DNA binding was expected, due to the presence of more amide bonds in the resultant triaryl derivatives.
Our first attempt to synthesise amide 168, required for acid 166, was a coupling reaction between aniline 113 with two equivalents of acid 160, using the coupling reagents DIC/HOBt in the presence of a catalytic amount of DMAP (Scheme 36). This method however generated a mixture of products which were hard to purify by chromatography. To try to overcome the problematic purification, it was decided to convert the crude ester 168 to acid 166 under catalytic hydrogenation, in the hope that acid 166 could be purified. However, the NMR of the crude product showed a complex mixture of products and this method was abandoned.

We then decided to try a different approach towards the synthesis of ester 168. This method involved the reaction of 1.25 equivalents of aniline 113 with acid chloride 102 in the presence of potassium carbonate in THF.\textsuperscript{167} It was successful and gave after workup the desired ester 168 in 79% yield, with no need for further purification (Scheme 37).
Chapter 2: Synthesis of Amines and Acids

Reagents and conditions: i. 1.25 eq. aniline (113), 2.7 eq. K₂CO₃, THF, r.t, 30 min, 79%.

Scheme 37.

This method for synthesising ester 168 was convenient not only due to the fast reaction time of 15 to 30 minutes but also because the impurities and excess aniline 113 were easily removed by washing the crude product with DCM, thereby eliminating the need for chromatography (Scheme 37).

The resultant ester 168 was then converted under catalytic hydrogenation to acid 166 (Scheme 38). The yield was initially poor due to the poor solubility of the product 166 in methanol, which resulted in significant loss when filtering the mixture to remove the Pd catalyst. However, diluting the reaction mixture upon completion of the reaction ensured quantitative yield.

Reagents and conditions: i. 10% Pd/C, H₂, MeOH, r.t, 5 h, 100%.

Scheme 38.

To prepare amino-ester 169, acid chloride 102 was reacted with 1.25 equivalents of aniline 114 in the presence of potassium carbonate. The resultant suspension, after evaporation of the reaction solvent, was heated to dissolve the product 169 in methanol in the presence of potassium carbonate (Scheme 39). However, analysis of the obtained product by ¹H and ¹³C NMR showed unexpected peaks at δ 3.9 ppm and δ 53 ppm corresponding to a methoxy...
group, with no peaks for the benzyl group. The presence of a methyl ester 170 was also confirmed by mass spectrometry. The substitution of the benzyl ester 169 for the methyl ester 170 can be explained by a trans-esterification reaction due to heating the mixture in the presence of potassium carbonate and methanol in the purification step.

When the same reaction was repeated under similar conditions however, this time using a larger volume of solvent rather than heating during filtration, no trans-esterification was observed and the desired benzyl ester 169 was isolated in 94% yield. The ester 169 was then reduced under catalytic hydrogenation to give the corresponding amino-alkyl acid 167 again in quantitative yield (Scheme 39).

(114) \[ \text{Reagents and conditions: i. 1 eq. acid chloride (102), 1.25eq. aniline (114), 2.7 eq. } K_2CO_3, \text{ THF, r.t, 30 min, 94%; ii. 10% Pd/C, H}_2, \text{ MeOH, r.t, 5 h, 100%}. \]

Scheme 39.
Summary of the synthesis of mono- and diaryl acids

Three series of acids including two series of monoaryl acids and one series of diaryl acids were prepared. In the first series of monoaryl acids, both para and meta isomers having short amino-alkyl chains and two isomers having comparatively longer chains were synthesised using two different synthetic routes. The first route involved the Boc-protection of aminobenzoic acids 129 and 130. The resultant Boc-protected acids 131 and 132 were then benzoylated to give 133 and 134. Their subsequent Boc-deprotection and acetylation by amine addition to the bromides 137 and 138 followed, and catalytic hydrogenation gave acids 119-126 (Scheme 40). The syntheses of longer chain isomers 151 and 152 were achieved by coupling the amino esters 135 and 136 with morpholino-acid 147, which itself was prepared in two steps from morpholine 148 and ethyl 4-bromobutyrate 149.

The second series of monoaryl acids includes five acids 153-157 having para substituted longer amino-alkyl chains starting from terephthaloyl chloride 158 which was converted into dibenzoate 159. The dibenzoate 159 was selectively hydrolysed into mono-acid 160, which upon reaction with oxalyl chloride gave the acid chloride 102. Acid chloride 102 was then reacted with various amines to give amino-benzoates 161-165 and subsequent hydrogenation gave the desired para amino-alkyl acids 153-157 in quantitative yields (Scheme 40).

The diaryl acids 166 and 167 were prepared by the reaction of two different anilines 113 and 114 with acid chloride 102 to give the corresponding esters 168 and 169 in the presence of potassium carbonate. Unwanted trans-esterification was also observed when ester 169 was heated in the presence of potassium carbonate during filtration (Scheme 40).
Chapter 2: Synthesis of Amines and Acids

Reagents and conditions: i. a) 1.8 eq. di-tert-butyl dicarbonate, 1.1 eq. NaOH, water/dioxane (1:1) 0 °C then r.t 3 h; b) 1 eq. benzyl bromide, 1.1 eq. Cs₂CO₃, DMF, r.t, 2.5 h; c) trifluoroacetic acid, DCM, r.t, 1 h; ii. 1.1 eq. bromoacetyl bromide, DCM, r.t, 20 h, (137) = 83%, (138) = 100%; iii. a) 2.17 eq. HNR₂, DCM, reflux, o/n; b) 10% Pd/C, H₂, MeOH, r.t, 5 h, quantitative; iv. a) 2 eq. morpholino-acid (147), 2 eq. EDC, 2 eq. DMAP, DMF, 45 °C, 24 h; b) 10% Pd/C, H₂, MeOH, r.t, 5 h, quantitative; v. 2.7 eq. benzyl alcohol, 4.3 eq. Et₃N, toluene, r.t, o/n, 88%; vi. a) 1 eq. LiOH, acetone/water, reflux, 30 min, 92%; b) 2.5 eq. oxalyl chloride, DCM, cat. DMF, r.t, 4 h, 100%. vii. a) 2 eq. RNH₂, DCM, r.t, o/n; b) 10% Pd/C, H₂, MeOH, r.t, 5 h, quantitative; viii. 1 eq. acid chloride (102), 1.25 eq. amine (113 or 114), 2.7 eq. K₂CO₃, THF, r.t, 30 min, (168) = 79%, (169) = 94%; ix. 10% Pd/C, H₂, MeOH, r.t, 5 h, quantitative.

Scheme 40.
After the successful synthesis of mono- and diaryl acids, we then returned to the more functionalised component, the benzyl alcohols.
Chapter 3

Synthesis of Diaryl Derivatives
Chapter 3: Synthesis of Diaryl Derivatives

3.1 The synthesis of benzamide alcohols 171 and 172

After preparing the desired range of mono- and diaryl acids (Scheme 40) to form polybenzamides, and with anilines 98, 99, 113 and 114 in hand, the next step was the synthesis of simpler benzamides 173-177, to investigate the viability of protecting groups and their deprotection to the parent hydroxyl group, followed by subsequent activation and functionalisation using different nucleophiles. Firstly, a model benzamide 173 was synthesised from aniline 98, by treatment with benzoyl chloride, which gave benzamide 173 in 89% yield (Scheme 41).

![Reaction Scheme]

Reagents and conditions: i. 1.5 eq. benzoyl chloride, pyridine, r.t, 16 h, 89%.

Scheme 41.

Further simple benzamides were prepared by reaction of O-TBDMS-protected aniline 99 with benzoyl chloride and para nitrobenzoyl chloride in pyridine, to furnish benzamides 174 and 175 in 84% and 77% yields, respectively (Scheme 42). The presence of the O-TBDMS silyl ether in 174 compared to the O-Ac ester in 173 increased the former’s solubility, which made purification easier using aqueous workups.
Chapter 3: Synthesis of Diaryl Derivatives

Reagents and conditions: i. a) 1.5 eq. benzoyl chloride, pyridine, r.t, 16 h, (174) = 84%. or b) 1.5 eq. p-nitrobenzoyl chloride, pyridine, r.t, 16 h, (175) = 77%; ii. 10% Pd/C, MeOH, H₂, r.t, 3 h, 100%; iii. 3 eq. Ac₂O, 4 eq. Et₃N, DMF, r.t, 24 h, 89%.

Scheme 42.

Reduction of the nitro group of 175 to the desired amine 176 was achieved using 10% Pd/C in methanol. The structure of 176 was confirmed from ¹H and ¹³C NMR, with up-field shifts in NMR signals for the protons of aromatic region associated with the para substituted ring. Amine 176 was then converted to acetamide 177, in 89% yield using 3 equivalents of acetic anhydride in DMF (Scheme 42). Downfield shift was observed in the NMR for the protons on the para substituted aromatic ring along with a new peak in the aliphatic region for the methyl group. Recrystallisation of acetamide 177 from methanol gave crystals suitable for X-ray crystal analysis (Figure 30).
Figure 30. Crystal structure of TBDMS ether 177.*

* 50% probability displacement ellipsoids for non-hydrogen atoms.

Deprotection of acetyl and TBDMS groups of benzamides 173, 174 and 177

With our model benzamides 173, 174 and 177 in hand, the next step was to investigate functionalisation of the benzylic position. Beginning with amide 173, the acetyl group was deprotected using two equivalents of sodium hydroxide in ethanol for two hours, which gave the desired alcohol 171 in unoptimised yield of 58% (Scheme 43). The $^1$H and $^{13}$C NMR, along with HRMS, confirmed the removal of only the O-acetyl group, without hydrolysis of the amides.

![Scheme 43](image)

$\text{Reagents and conditions: i. 2 eq. NaOH, EtOH/H}_2\text{O, r.t, 2 h, 58%}.$

Scheme 43.
The TBDMS group of amide 174 was removed using a literature method reported by Smith et al.\textsuperscript{168} using TBAF (1M in THF) in the presence of glacial acetic acid (Scheme 44). THF was used as solvent rather than the more usual DCM solvent due to the poor solubility of silyl ether 174 in DCM. The reaction was monitored by TLC and when the starting material had been consumed, the resultant hydroxy compound 171 was purified using an aqueous workup to remove the unreacted TBAF and other salts. Initially, the yield was poor and this was attributed to some of the product 171 being lost during the extraction process, owing to its high polarity. The yield was improved when multiple extractions of the aqueous phase were performed. The same deprotection method was applied to benzamide 177, however in this case the hydroxyl benzamide 172 was comparatively more polar than the benzamide 171. Purification was performed by dry-loading the entire reaction mixture onto silica gel using flash chromatography, without aqueous workup. When this chromatography method was applied to the synthesis of amide 174, the yield of 171 was further improved and the aqueous extraction method was abandoned. It was noticed that the rate of deprotection was dependent on the amount of TBAF used, with 4 equivalents required for reaction in a 24-hour reaction time.

\[ \text{Scheme 44.} \]

Benzamide alcohol 171 was also recrystallised from methanol, which gave crystals suitable for analysis by X-ray crystallography (Figure 31).
Summary of the synthesis of benzamides 171 and 172

*O*-Acetyl-protected benzamide 173 was synthesised by coupling benzoyl chloride with aniline 98. The acetyl group in benzamide 173 was then deprotected via hydrolysis to give benzyl alcohol 171 (Scheme 45). Benzamides 174 and 175 were prepared by coupling benzoyl and 4-nitrobenzoyl chloride with *O*-silyl-protected amine 99. The nitrobenzamide 175 was converted to amine 176 and then to acetamide 177 using catalytic hydrogenation and acetic anhydride respectively. The TBDMS group in both benzamides 174 and 177 was then removed using TBAF to give the desired benzamides 171 and 172.
Chapter 3: Synthesis of Diaryl Derivatives

Reagents and conditions: i. 1.5 eq. benzoyl chloride, pyridine, r.t, 16 h, 89%; ii. 2 eq. NaOH, EtOH/H$_2$O, r.t, 2 h, 58%; iii. a) for (174) 1.5 eq. benzoyl chloride, pyridine, r.t, 16 h, 84%; or b) for (175) 1 eq. p-nitrobenzoyl chloride, 1.5 eq. pyridine, r.t, 16 h, 77%; iv. 10% Pd/C, MeOH, H$_2$, r.t, 3 h, 100%; v. 3 eq. Ac$_2$O, 4 eq. Et$_3$N, DMF, r.t, 24 h, 89%; vi. 4 eq. TBAF (1M in THF), 4 eq. glacial AcOH, THF, r.t, 24 h, (171) = 84%, (172) = 93%.

Scheme 45.

3.2 Optimisation reactions to functionalise the benzylic position of alcohol 92

With alcohols 171 and 172 having been synthesised, our next aim was to functionalise the benzylic position by the displacement of a good leaving group such as a mesylate or halide. In addition to mesylates and halides, aldehydes have also been investigated as suitable functional groups that could be converted to benzylic amines via reductive amination or the precursors
thereof. The direct functionalisation of the alcohol using a Mitsunobu type reaction has also been explored, but results have been very variable and inconsistent.\textsuperscript{133}

Once the benzylic alcohol is converted into a leaving group, the next step would be the preparation of benzylic nitrogen mustards or hemi-mustards.

Before benzamide alcohols \textbf{171} and \textbf{172} were converted to the corresponding benzamide mustards, we decided to test the reaction pathway on the much simpler dinitrobenzyl alcohol \textbf{92}. With an expected decrease in the solubility of the final derivatives in common organic solvents, due to having increased polarity, we limited our choice of solvents to DMF or THF for all the possible substitution reactions at the benzylic position.

Our first choice was the synthesis of a mesylate \textbf{178} from alcohol \textbf{92}. Thus, the synthesis of dinitro-mesylate \textbf{178} was achieved using methanesulfonyl chloride in the presence of triethylamine, at 0 °C, in 96% yield (Scheme \textbf{46}).

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\textbf{(92)}};
\node at (1,0) {\textbf{(178)}};
\node at (1,-1) {\textbf{Reagents and conditions:} i. 1.5 eq. MsCl, 1.5 eq. Et\textsubscript{3}N, DMF, r.t, 3 h, 96%.

\textbf{Scheme 46.}};
\end{tikzpicture}
\end{center}

Many mesylates are unstable in solvents but fortunately the resultant mesylate \textbf{178} was found to be stable and could be recrystallised from ethyl acetate, producing crystals suitable for single crystal analysis. The crystal structure obtained showed the absence of hydrogen bonding or $\pi - \pi$ interactions (Figure \textbf{32}). The closest intermolecular contacts are O3 $\cdots$ N1 of 2.83 Å, and a pair of O $\cdots$ O 3.32 Å contacts between sulfonate oxygen atoms.\textsuperscript{169}
Mesylate 178 showed good reactivity towards the trialled nitrogen nucleophiles and the results are shown in Table 10.

**Table 10. Conversion of mesylate 178 into nitrogen mustard/precursors.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>R</th>
<th>Compd #</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 eq. diethanolamine, DMF, r.t, 24 h.</td>
<td>-N(CH₂CH₂OH)₂</td>
<td>179</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>10 eq. 2-(methylamino)ethanol, DMF, r.t, 18 h.</td>
<td>-N(CH₃)CH₂CH₂OH</td>
<td>180</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>1 eq. NH(CH₂CH₂Cl₂)₂·HCl, 3 eq. K₂CO₃, DMF, reflux, 8 h.</td>
<td>-NH(CH₂CH₂Cl₂)₂</td>
<td>181</td>
<td>55</td>
</tr>
</tbody>
</table>

The methanesulfonyl group in mesylate 178 was substituted with diethanolamine and 2-(methylamino)ethanol in high yield when 10 equivalents of each amine were used and generated amino alcohols 179 and 180 as precursors for classical and half-nitrogen mustards. Substitution of methanesulfonyl group was also carried out with commercially available
bis(2-chloroethyl)amine hydrochloride, in the presence of potassium carbonate. All three reactions were successfully achieved, although the yield for the reaction of 178 with bis(2-chloroethyl)amine was lower than the corresponding amino alcohols. The lower yield of 181 is probably due to decreased stability of the product (Table 10, Entry 3).

Diol 179 was recrystallised from ethyl acetate by slow evaporation to give crystals suitable for single crystal analysis. Crystal studies were carried out to investigate the hydrogen bonding pattern of the diol moiety. The crystal analysis showed that the crystals contain four crystallographically independent molecules which differ primarily in their rotation about the bond between the aromatic ring and the N-diol moiety. The molecules are linked into sheets by a hydrogen bonding network which involves all of the diols, with only van der Waals contacts between the sheets (Figure 33). The four molecules differ in their rotation about the C1-C7 bond (torsion angles C2-C1-C7-N3 for molecules A, B, C, and D are -38, 53, 47, and -59 degrees respectively).  

![Figure 33. Crystal structure of diol 179.](image)

* 50% probability displacement ellipsoids for non-hydrogen atoms.

Due to the lower yield of mustard 181 via displacement, other methods were also attempted to furnish the desired mustards 181 via diol 179 using chlorinating agents in which the byproducts are comparatively easy to remove. Therefore, attempts were made to convert diol 179 to nitrogen mustard 181. Reaction of phosphorus oxychloride with diol 179 furnished the mustard 181 in 44% yield (Table 11, Entry 1). Diol 179 was easily converted to di-
mesylate intermediate. The di-mesylate intermediate was then converted to the desired mustard 181 in the presence of LiCl.\textsuperscript{171,172}

\textbf{Table 11.} Conversion of test diol 179 to nitrogen mustard 181.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 eq. POCl\textsubscript{3}, 0°C then 105°C, 1 h.</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>i) 4 eq. MsCl, 4 eq. Et\textsubscript{3}N, THF, r.t, 2 h.</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>ii) 4 eq. LiCl, DMF, 70°C, 30 min.</td>
<td></td>
</tr>
</tbody>
</table>

**Summary of the synthesis of simple nitrogen mustards**

To explore the preparation of benzylic nitrogen mustards, dinitrobenzyl alcohol 92 was chosen as a test compound and was converted to dinitrobenzyl mesylate 178. The resultant mesylate 178 was then converted to amino-alcohols 179 and 180 (Scheme 47). The diol 179 was then successfully converted to the desired nitrogen mustard 181 using chlorinating reagents. Reaction with \textit{bis}(2-chloroethyl)amine-hydrochloride also furnished the desired mustard 181.
Reagents and conditions: i. 1.5 eq. MsCl, 1.5 eq. Et₃N, DMF, r.t, 3 h, 96%; ii. 10 eq. diethanolamine or 2-(methylamino)ethanol, DMF, r.t, 18 h, (179) = 98%, (180) = 85%; iii. (179) 3 eq. POCl₃, 0 °C then 105 °C, 1 h, 44%.

Scheme 47.

3.3 Functionalisation of benzylic position on benzamides 171 and 172

Having synthesised the mesylate and mustard derivatives of dinitrobenzyl alcohol 92, we then used our optimised conditions on the larger and more functionalised benzamides 171 and 172. Alcohol 171 was thus successfully converted to mesylate 182 in quantitative yield under the optimised reaction conditions used for dinitrobenzyl alcohol 92. The same reaction was carried out both in THF and DMF to investigate the effect of the solvent. The yield in DMF was observed to be higher, but THF had the advantage that it could be more easily removed after the reaction (Scheme 48). The mesylates 182 and 183 were freshly prepared each time and used without further purification.
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Reagents and conditions: i. 1.5 eq. MsCl, 1.5 eq. Et$_3$N, DMF, r.t, 2 h, (182 and 183) = 100%.

**Scheme 48.**

Different amines and other nucleophiles such as azides and chlorides were then used to displace the methanesulfonyl group in mesylate 182 (Table 12).

**Table 12.** Nucleophilic substitution of mesylate 182.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactants and conditions</th>
<th>R</th>
<th>Compound</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 eq. diethanolamine, DMF, r.t, 18 h.</td>
<td>-N(CH$_2$CH$_2$OH)$_2$</td>
<td>184</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>10 eq. 2-(methylamino)ethanol, DMF, r.t, 18 h.</td>
<td>-N(CH$_3$)(CH$_2$)$_2$OH</td>
<td>185</td>
<td>86</td>
</tr>
</tbody>
</table>
| 3     | i) 1.5 eq. MsCl, 1.5 eq. Et$_3$N, DMF, r.t, 2 h.  
      ii) 10 eq. NaCl, 60 °C, 30 min. | Cl | 186 | 100 |
| 4     | 3 eq. NaN$_3$, DMF, 105 °C, 4 h, then r.t 16 h. | N$_3$ | 187 | 82 |
| 5     | 1 eq. NH(CH$_2$CH$_2$Cl)$_2$, HCl, 3 eq. K$_2$CO$_3$, DMF, r.t, o/n. | -NH(CH$_2$CH$_2$Cl)$_2$ | 188 | mixture |
| 6     | 10 eq. NH(CH$_2$CH$_2$Cl)$_2$, HCl, DMF, r.t, o/n. | -NH(CH$_2$CH$_2$Cl)$_2$ | 188 | s.m |
| 7     | 5 eq. NH(CH$_2$CH$_2$Cl)$_2$, HCl, 15 eq. K$_2$CO$_3$, CH$_3$CN, reflux, 8 h. | -NH(CH$_2$CH$_2$Cl)$_2$ | 188 | mixture |

Using the same conditions as for mesylate 178, excess equivalents of the amines (10 equivalents) were reacted with mesylate 182 (Entries 1 and 2). Unlike the previous reactions, however, repeated purification was required to remove the unreacted amine/s from the products 184 and 185. To avoid the problem associated with purification, one equivalent of diethanolamine in DMF was reacted with mesylate 182 but no reaction was observed after
three days at room temperature. The reaction mixture was then refluxed for 5 hours and two inseparable products were formed. The reaction of mesylate 182 with diethanolamine was carried out in THF with no improvement to the overall yield. The reaction with 2-(methylamino)ethanol had similar problems with purification, however a comparatively good yield of amino-alcohol 185 was obtained and the starting amine was more easily removed (Table 12, Entry 2).

We were interested in the chloride 186 as a stable alternative to the mesylate 182, which may be able to alkylate DNA. The benzylic chloride 186 was prepared from mesylate 182 using sodium chloride at 60 °C for 30 minutes in quantitative yield. Chloride 186 was found to be more stable than mesylate 182 (Table 12, Entry 3).

We also wished to prepare azide 187, which could be used as an intermediate for introducing other alkylating groups. Azide 187 could be reduced to an amine which could be used to introducing the bromoacrylamide group seen in the brostallicin 23 or haloacetyl moiety, which is another potential alkylating group. Thus, azide 187 was generated from mesylate 182 using three equivalents of sodium azide in DMF. The azide 187 was purified with flash chromatography without using an aqueous workup in 82% yield (Table 12, Entry 4).

With diethanolamine and 2-(methylamino)ethanol being successfully added, the addition of bis(2-chloroethyl)amine-hydrochloride was attempted under different experimental conditions. However, unlike dinitromesylate 178, this resulted in either no reaction or a mixture of inseparable products (Table 12, Entries 5, 6 and 7).173

Having established that the incorporation of nitrogen mustard precursor moieties is achievable for benzamide 182, a set of reactions were also carried out with the more functionalised mesylate 183 under similar conditions. The results are shown in Table 13.
A series of similar reactions were carried out with mesylate 183 using diethanolamine, 2-(methylamino)ethanol, sodium chloride and the commercially available bis(2-chloroethyl)amine-hydrochloride (Table 13). Reaction with sodium azide gave the desired product 192 in a lower yield of 46% (Entry 4). Azide 192 was only somewhat stable and when left for many days at room temperature decomposition occurred, therefore it was generated fresh every time prior to use in the next reaction. The reaction of bis(2-chloroethyl)amine-hydrochloride again resulted in a mixture of products from which the desired mustard 193 could not be obtained.

With the benzamide derivatives 184-187 and 189-192 in hand, our attention turned to the preparation of nitrogen mustards 188 and 193 from diols 184 and 189 using different chlorinating agents in an attempt to prepare mustards capable of alkylating DNA.

To furnish the desired nitrogen mustards, our first choice was to use thionyl chloride. The reaction of thionyl chloride with diol 184 was carried out at room temperature according to literature method 174 but resulted only in decomposition (Table 14, Entry 1).
Table 14. Attempted conversion of diols 184/189 to nitrogen mustards 188/193.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOCl₂, 0°C → r.t, o/n</td>
<td>dec.</td>
</tr>
<tr>
<td>2</td>
<td>i) MeOH, HCl</td>
<td>dec.</td>
</tr>
<tr>
<td></td>
<td>ii) SOCl₂, r.t, 20 min.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 eq. POCl₃, 0 °C then 105 °C, 1h.</td>
<td>dec</td>
</tr>
<tr>
<td>4</td>
<td>i) 3 eq. MeSO₂Cl, 3 eq. Et₃N, THF/DMF, 0 °C then r.t 48 h.</td>
<td>mixture</td>
</tr>
<tr>
<td></td>
<td>ii) 4 eq. LiCl, DMF, r.t, 30 min.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>i) 4 eq. MeSO₂Cl, 4 eq. Et₃N, DMF, 0 °C then r.t 2 h.</td>
<td>mixture</td>
</tr>
<tr>
<td></td>
<td>ii) 4 eq. LiCl, DMF, 70 °C, 6 h.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>i) 2.85 eq. MsCl, 3.2 eq. Et₃N, DMF, r.t, 2 h.</td>
<td>mixture</td>
</tr>
<tr>
<td></td>
<td>ii) 8.2 eq. NaCl, DMF, 160 °C, 10-15 min.</td>
<td></td>
</tr>
</tbody>
</table>

After unsuccessful attempts with thionyl chloride, it was decided to decrease the reactivity of amino-diol 184 by first converting to its respective hydrochloride salt. To prepare the hydrochloride salt, diol 184 was dissolved in methanolic-hydrochloride which was prepared by passing hydrochloric acid gas through methanol. Addition of thionyl chloride to the prepared hydrochloride salt of 184 still resulted in decomposition however (Table 14, Entry 2). The decomposition could have occurred due to instability of the formed mustard produced and/or participation of the amide groups thus leading to decomposition.

After failed attempts with thionyl chloride, we then trialled phosphorus oxychloride as the chlorinating reagent. Phosphorus oxychloride has also been used previously by Gourdie et al. to prepare nitrogen mustard 195 (Scheme 49).\textsuperscript{79,171}
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Reagents and conditions: i. a) 2 eq. POCl₃, benzene, reflux, 1h; b) con. HCl, 30 °C then reflux 10 min, 88%.

Scheme 49.

Unfortunately, using phosphorus oxychloride with either diol 184/189 also resulted in decomposition (Table 14, Entry 3).

It was then decided to use a milder chlorinating agent in an attempt to avoid the decomposition which occurred when using thionyl chloride and phosphorus oxychloride. Therefore, the reaction of diols 184 and 189 was carried out with methanesulfonyl chloride in the presence of triethylamine and LiCl. Unfortunately, these reaction conditions yielded a mixture of products when carried out both at room temperature and at 70 °C (Entries 4 and 5), with a variety of different stoichiometries. Neither the desired mustards 188/193 nor the intermediate mesylates were able to be isolated.
Summary of the synthesis of nitrogen mustards 188 and 193

The benzylic hydroxyl group in benzamides 171 and 172 was converted to mesylates 182 and 183 using the optimised conditions determined on test dinitrobenzyl alcohol 92. The methanesulfonyl group was substituted with different nucleophiles to yield chlorides 186 and 191 and amino-alcohols 184 and 185, 189 and 190. The reaction of diols 184 and 189 were carried out with different chlorinating agents to furnish nitrogen mustards 188 and 193 but these were unsuccessful. The reaction of bis(2-chloroethyl)amine-hydrochloride was attempted with mesylates 182 and 183, however this too failed to give the desired nitrogen mustards 188 and 193 (Scheme 50). Possible reasons for decomposition were due to either chlorination in the presence of amide bonds leading to side reactions or the reactivity of the formed benzylic mustard in the presence of other functionalities which resulted in decomposition.

Reagents and conditions: i. 1.5 eq. MsCl, 1.5 eq. Et₃N, DMF, r.t, 2 h, quantitative; ii. 10 eq. diethanolamine, DMF, r.t, 18 h, (184) = 60%, (189) = 88%.

Scheme 50.
3.4 Synthesis of benzylic substituted derivatives

After many failed attempts to prepare the well-known alkylating agents, the nitrogen mustards, it was decided to use an alternative alkylating moiety to avoid the use of chlorinating agents. The 2-bromoacryloyl moiety is a frequently used mild alkylating moiety, with better alkylating properties. Literature adopted methods were used to investigate the possibility of incorporating a bromoacryloyl moiety at the benzylic position. It was envisaged that this new derivative would be easier to isolate due to its milder reactivity. Although we had found that nitrogen mustards can be generated from benzylic mesylates directly, they are usually generated from an amino-alcohol precursor, which we found were difficult to purify due to their high polarity and solubility during aqueous workup. We envisaged that the bromoacryloyl fragment could be formed without the late-stage introduction of a mesylate. In order to optimise the reaction conditions, a range of amines with different substituents would be used.

Reactions were carried out to incorporate the bromoacryloyl moiety into amine and alcohol to generate amide and ester respectively. The first step in the preparation of acrylamide was to reduce azide to the corresponding amine in quantitative yield (Scheme 51).

\[ \text{Reagents and conditions: i. 10\% Pd/C, MeOH, H}_2, \text{ r.t, 3 h, 100\%.} \]

Scheme 51.

In our initial attempt to prepare amide, 2-bromoacrylic acid along with a combination of DIC/HOBt coupling reagents was used in DMF. Unfortunately, none of the desired product was isolated, with a complex mixture of products being produced (Scheme 52).
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With the coupling reaction using the benzylamine 196 having been unsuccessful, we next tried using the benzyl alcohol 172 as the coupling partner with 2-bromoacrylic acid. Thus, benzylic alcohol 172 was reacted with two equivalents of DIC, HOBt and 2-bromoacrylic acid in DMF. The reaction mixture immediately turned dark brown when 2-bromoacrylic acid was added, indicating a reaction taking place, but unfortunately NMR analysis of the produced products showed only a mixture of decomposition products (Scheme 53).

The reaction was then carried out with EDC as the coupling reagent, with a reaction time of 18 hours.\textsuperscript{79,176} However, again none of the desired product 199 was obtained (Scheme 54).
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Reagents and conditions: i. 2 eq. 2-bromoacrylic acid, 1 eq. EDC, DMF, r.t, o/n.

Scheme 54.

It was postulated that the increased reactivity of the benzylic amine could be playing a role in the decomposition. To optimise the reaction conditions and to get a clear insight into the reaction, aromatic and aliphatic amines 200-202 were used as test compounds. The series of reactions attempted using different reagents is given in the Table 15.

Table 15. Attempted conversion of amines 200-202 to bromoacrylamides 203-205.

<table>
<thead>
<tr>
<th>Entry</th>
<th>(CH$_2$)$_n$</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 eq. acid chloride, DCM, r.t, 1 h.</td>
<td>dec.</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1 eq. acid chloride, 1 eq. Et$_3$N, DMF, r.t, o/n.</td>
<td>dec.</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1 eq. acid chloride, 1 eq. DIPEA, r.t, o/n.</td>
<td>dec.</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1 eq. acid chloride, 1.5 eq. Cs$_2$CO$_3$, THF, r.t, 30 min.</td>
<td>dec.</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1 eq. acid chloride, THF, r.t, 30 min.</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2 eq. acid, 1 eq. EDC, DMF, r.t, o/n.</td>
<td>s.m</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1 eq. acid, 1.5 eq. EDC, cat. DIPEA, DMF, r.t, o/n.</td>
<td>s.m</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>1 eq. acid, 1 eq. DCC, 1 eq. NaHCO$_3$, THF, r.t, o/n.</td>
<td>s.m</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>2 eq. acid, 1 eq. DCC, 2 eq. DIPEA, THF, r.t, o/n.</td>
<td>s.m</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2 eq. acid, 2 eq. HOBT, 2 eq. DIC, 0.1 eq. DMAP, DMF, r.t, o/n.</td>
<td>dec.</td>
</tr>
</tbody>
</table>
2-Bromoacryloyl chloride was treated with two amines, namely aniline 200 and benzylamine 201 in an attempt to furnish the desired 2-bromoacrylamides 203 and 204. The first reaction of 2-bromoacryloyl chloride with benzylamine 201,\(^{177}\) which did not use tertiary amines as a base, failed to furnish the desired product; rather it resulted in decomposition (Table 15, Entry 1). It was then decided to carry out the same reaction of acid chloride in the presence of tertiary amines such as triethylamine and DIPEA and also in the presence of cesium carbonate (as a base) using literature adopted methods used during the reaction of amine 206 to prepare amide 208 (Scheme 55).\(^{178,179}\)

\[
\text{Reagents and conditions: i. 1.5 eq. Cs}_2\text{CO}_3, \text{THF, r.t, 30 min, 95%}. \\
\text{Scheme 55.}
\]

It was found that all these conditions resulted in decomposition, probably owing to the more basic and nucleophilic characters of aliphatic amines, which may have been causing the decomposition due to further reaction of the amine as nucleophile with the bromoacrylic functionality. It was then decided to carry out the coupling reaction of 2-bromoacryloyl chloride and the less nucleophilic aniline 200, which pleasingly gave the previously prepared\(^{180}\) amide 198, albeit in a poor yield of 30% (Table 15, Entry 5).\(^{181}\)

The reaction of three different amines (aromatic and aliphatic) 201-202 was also carried out with 2-bromoacrylic acid using different acid coupling reagents, namely EDC\(^{75}\) (Entries 6-7), DCC\(^{22}\) (Entries 8-9) and DIC\(^{182}\) (Entry 10) in different stoichiometric amounts, using literature adopted methods. Coupling reagents have been reported that carry out synthesis of amide 210 using 2-bromoacrylic acid and amine 209 (Scheme 56).\(^{175}\)
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Reagents and conditions: i. 2 eq. 2-bromoacrylic acid, 1 eq. EDC, DMF, r.t, o/n.

Scheme 56.

Unfortunately, all the aliphatic amines resulted in decomposition or a mixture of inseparable products.

The higher reactivity of benzylamine 201 (which caused decomposition during the reaction) prompted us to look for a suitable mild alkylating group. It was decided to use the haloacetyl moiety for this purpose. The incorporation of the haloacetyl moiety would be a new addition to the already known library of alkylating groups. The reactivity of the halogen on the molecule can be further increased by preparing the ester, due to an inductive effect of the oxygen atom in the ester group. Keeping in mind the above advantages, a new set of haloacetyl compounds was synthesised (Table 16).

Azide 187 was reduced in quantitative yield to the corresponding amine 211 using catalytic hydrogenation in the presence of palladium catalyst in methanol under previously optimised conditions. The resultant amine 211 was then converted to bromoacetyl and chloroacetyl derivatives 212 and 213 respectively in high yield (Table 16), using bromoacetyl bromide and chloroacetyl chloride reagents respectively in DMF in the presence of triethylamine. During the preparation, the acid halides were added dropwise at 0 °C to avoid any possibility of further reaction at the α-halocentre. Both the desired benzamides 212 and 213 were purified with flash chromatography using dichloromethane-methanol (19:1) solvent system and were obtained as stable solids.
Table 16. Conversion of amine 211 to haloamides 212 and 213.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Br</td>
<td>1.2 eq. bromoacetyl bromide, 1.1 eq. Et\textsubscript{3}N, DMF, 0 °C, 1 h, then r.t 1 h.</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>Cl</td>
<td>1.2 eq. chloroacetyl chloride, 1.1 eq. Et\textsubscript{3}N, DMF, 0 °C, 1 h, then r.t 1 h.</td>
<td>95</td>
</tr>
</tbody>
</table>

**Reaction of adenine 214 with bromoacetamide 212**

With bromide 212 in hand, the next step was to investigate its alkylating activity and reactivity. A test reaction was carried out with 1.2 equivalents of adenine 214 at room temperature in DMF but no reaction was observed after 4 hours (Scheme 57).
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We thought that the lack of reactivity of bromide 212 towards adenine 214 might be due to its limited solubility in DMF. Therefore, the reaction was stirred overnight at 50 °C, but again no reaction was observed. Potassium carbonate was added to the reaction mixture and the reaction was further refluxed to furnish the desired alkylation product 215. The NMR spectra of the crude product were hard to interpret, however the HRMS confirmed the presence of a small amount of desired product 215 (Scheme 57). The detection of the desired complex in mass spectrometry provided evidence that bromide has alkylating activity. Adenine 214 bases are not the only reactive nucleophile present in DNA and other important sites of proteins may be expected to alkylate in biological systems.

Due to the comparative stability of amide 212 and the resultant deactivation towards adenine 214, it was thought that esters 216 and 217 may have increased alkylating activity due to the presence of oxygen, which has the potential to increase the reactivity of the halides due to its inductive effect (Table 17).

Keeping in mind the possible enhanced alkylating activity of the resultant derivatives, reactions were attempted by using bromoacetyl bromide and chloroacetyl chloride in the presence of triethylamine in DMF to furnish esters 216 and 217, however in this case the byproducts was hard to remove during chromatographic purification (Table 17). It was therefore decided to use Dowex® 55A, Dowex® 66 and potassium carbonate solution separately to remove the triethylamine salt. Mostly the salt was removed by using resins, however NMR analysis showed that hydrolysis of the ester 216 to starting alcohol 172 had occurred. Using potassium carbonate solution to remove the triethylamine salt also resulted in hydrolysis to the starting alcohol 172, probably due to its instability under basic conditions. Due to this instability we decided to abandon work on esters 216/217.
Table 17. Conversion of alcohol 172 to haloesters 216 and 217.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Br</td>
<td>1.2 eq. bromoacetyl bromide, 1.1 eq. NEt₃, DMF, 0 °C 1 h, then r.t 1 h.</td>
<td>s.m</td>
</tr>
<tr>
<td>2</td>
<td>Cl</td>
<td>1.2 eq. chloroacetyl chloride, 1.1 eq. NEt₃, DMF, 0 °C 1 h, then r.t 1 h.</td>
<td>s.m</td>
</tr>
</tbody>
</table>

Summary of the synthesis of amide and ester derivatives 212 and 213, 216 and 217 at benzylic position

The attempt to incorporate a bromoacryloyl group in amide 197 and ester 198 was unsuccessful, with no useful products isolated. Therefore, simple aromatic and aliphatic amines were used to optimise the reaction conditions for the incorporation of the bromoacryloyl moiety. The desired moiety was easily incorporated into aniline 200 but did not work with aliphatic amines due to its strong basicity (Scheme 58). Due to the difficulty of introducing the bromohaloacryloyl moiety, another mild haloacetyl moiety was then incorporated to benzamide 212 and 213. The bromo-derivative 212 was reacted with adenine 214 to test its alkylating activity and whilst the reaction was not high yielding, the desired adduct 215 was detected by HRMS.
Reagents and conditions: i. (187 and 192), 10% Pd/C, MeOH, H₂, r.t, 3 h, (211 and 196) = quantitative; ii. (196, 171 and 172), 2 eq. 2-bromoacrylic acid, 2 eq. DIC, 2 eq. HOBut, cat. DMAP, DMF, r.t, o/n; iii.* 1 eq. 2-bromoacryloyl chloride, 1 eq. Et₃N, DMF, r.t, o/n, dec.; iv. 1.2 eq. bromoacetyl bromide or chloroacetyl chloride, 1.1 eq. NEt₃, DMF, 0 °C 1 h, then r.t 1 h, (212) = 87%, (213) = 95%; vi. (212), 1.2 eq. adenine (214), 2 eq. K₂CO₃, DMF, 50 °C, o/n, trace product. *Aniline 200 gave a reported product in 30%.

Scheme 58.
3.5 Optimisation of coupling conditions

It was realised early that the coupling reaction between aniline 99 and acid 155 would be complicated by the high polarity of acid 155 and the stability of the TBDM group on aniline 99. The TBDM group is known to be stable under mildly acidic and basic conditions but there is the possibility of TBDM deprotection due to the presence of acidic coupling reagents and the byproducts which are produced during the reaction. The main aim was to determine a TBDM-compatible combination of coupling reagents and conditions that could be used in polar solvents such as DMF with easy removal of unreacted reagents and byproducts through flash chromatography, without the need for aqueous workup. Acid chloride 218 and carbodiimide coupling reagents were used towards the synthesis of the desired benzamide 219 (Table 18).

**Table 18. Optimisation of coupling conditions.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2 eq. 218, 3 eq. Et₃N, DMF, r.t, o/n.</td>
<td>dec.</td>
</tr>
<tr>
<td>2</td>
<td>2 eq. 155, 2 eq. DCC, 0.1 eq. DMAP, DMF/CH₃CN, r.t, 16 h.</td>
<td>s.m</td>
</tr>
<tr>
<td>3</td>
<td>2 eq. 155, 2 eq. EDC, 0.1 eq. DMAP, DMF, r.t, 16 h.</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>1.6 eq. 155, 1 eq. EDC, 1 eq. DMAP, DMF, r.t, 2 h.</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>1.5 eq. 155, 1.5 eq. EDC, 1 eq. DMAP, DMF, r.t, 18 h.</td>
<td>43</td>
</tr>
<tr>
<td>6</td>
<td>1 eq. 155, 1 eq. EDC, 2.5 eq. Et₃N, DMF, r.t, 16 h.</td>
<td>s.m</td>
</tr>
<tr>
<td>7</td>
<td>1.5 eq. 155, 1 eq. EDC, 1.5 eq. HOBt, DMF, r.t, 16 h.</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>1.5 eq. 155, 1.5 eq. EDC, 1.5 eq. HOBt, DMF, r.t, 2 h.</td>
<td>s.m</td>
</tr>
<tr>
<td>9</td>
<td>1 eq. 155, 1 eq. EDC, 1 eq. HOBt, 2.5 eq. Et₃N, DMF, r.t, 18 h.</td>
<td>s.m</td>
</tr>
<tr>
<td>10</td>
<td>1 eq. 155, 0.97 eq. HBTU, 2.5 eq. Et₃N, DMF, r.t, 18 h.</td>
<td>dec.</td>
</tr>
<tr>
<td>11</td>
<td>1 eq. 155, 1 eq. DIC, 1 eq. HOBt, cat. DMAP, DMF, r.t, 16 h.</td>
<td><strong>238</strong></td>
</tr>
<tr>
<td>12</td>
<td>1 eq. 155, 1 eq. DIC, 1 eq. HOBt, cat. DMAP, DMF, r.t, 16 h.</td>
<td>32</td>
</tr>
<tr>
<td>13</td>
<td>2 eq. 155, 2 eq. DIC, 2 eq. HOBt, cat. DMAP, DMF, r.t, 16 h.</td>
<td>96</td>
</tr>
</tbody>
</table>
Acid chloride 218 was selected as our first choice due its easy method of preparation and our experience with such reactions. The synthesis of acid chloride 218 was achieved using oxalyl chloride and the resultant acid chloride 218 was then used in the subsequent coupling reaction with aniline 99, without further purification (Entry 1). The NMR after purification showed no peaks for the TBDMS group or any of the characteristic peaks expected for the product, suggesting that decomposition had occurred. It was thought that decomposition could have occurred due to the more acidic conditions of acid chloride coupling, regardless of the base used. The disappearance of signals relating to the TBDMS group could be explained by acidic deprotection of this group.

The next choice for the reaction was the use of carbodiimide coupling reagents. The reaction of carbodiimide is usually straightforward and is used frequently to activate carboxylic acids for the formation of amide or ester bonds. Due to the strong dehydrating role of carbodiimide and its several side reactions, sometimes reactions are complicated, especially with sensitive groups such as TBDMS. The coupling reactions were carried out according to the literature adopted methods using \(\text{N,N'-dicyclohexylcarbodiimide} (\text{DCC})\) in the presence of a catalytic amount of DMAP in acetonitrile/DMF to convert acid 155 to the corresponding activated acid (Table 18, Entry 2).

Majumder and co-workers\(^ {183}\) have used DCC/DMAP in the total synthesis of Gambierol by coupling alcohol 220 with acid 221 to furnish ester 222 in good yield (Scheme 59).

\[ \begin{align*}
\text{(220)} + \text{PMBO} \rightarrow \text{PMBO} \\
\text{(221)} \rightarrow \text{(222)}
\end{align*} \]

Reagents and conditions: i. DCC, DMAP, CH\(_2\)Cl\(_2\), 75%.

Scheme 59.

DCC has also been reported for amide synthesis in acetonitrile to furnish amide 225 in 74% yield (Scheme 60).\(^ {184}\)
Chapter 3: Synthesis of Diaryl Derivatives

Reagents and conditions: i. DCC, DMAP, CH₃CN, 23 °C, 12 h, 74%.

Scheme 60.

The reaction follows the pathway through O-acylisourea, which then reacts with alcohol or amine to form an ester or amide. Although DCC is soluble in most organic solvents, its side product, dicyclohexylurea (DCU), is insoluble, and was removed by filtration prior to the addition of aniline 99. Unfortunately, this method of coupling the two fragments together was also unsuccessful, giving only returned starting material.

After failed attempts with DCC, another coupling reagent, EDC, became our next focus. EDC is considered superior to DCC due to its versatility and water solubility, and due to the water solubility of its byproducts. EDC can form different tautomeric forms as shown in Scheme 61.

Scheme 61. Different tautomeric forms of EDC.

The use of EDC has been reported in the synthesis of biotin-amino acid conjugates 228 with 91% yield (Scheme 62).

Reagents and conditions: i. a) EDC, DMAP, DMF, r.t, 4-6 h.; b) NaOH, MeOH, r.t, 4 h. (R = Methyl ester of L-amino acids in 227).

Scheme 62.
Using two equivalents of EDC in the presence of DMAP gave the desired benzamide 219 in unoptimised 75% yield (Table 18, Entry 3). It was thought that the yield could be improved further by avoiding possible O-TBDMS deprotection during reaction due to the presence of hydrochloric acid associated with EDC molecules. Therefore, fewer equivalents of EDC were used and to provide basic medium, the DMAP was used in excess equivalents, however these changes, gave the desired product 219 in a lower yield of 32% (Table 18, Entry 4).

Interestingly, reducing the equivalents of EDC further to 1.5 gave a higher yield than when 1.6 equivalents were used (Entry 4 vs Entry 5). However, with one equivalent of EDC, with triethylamine in excess, to ensure basic conditions and help stop O-TBDMS deprotection, no reaction was observed (Table 18, Entry 6).

Owing to the inconsistent results achieved when using EDC, another combination, with HOBt as an additive, was tried. The EDC/HOBt condition has been used by Yanfa and co-workers109 to synthesise the structurally similar amide 229 in above 90% yield, however the exact conditions were not reported (Scheme 63).

Using 1.5 equivalents of both EDC and HOBt gave the desired product 219 in 55% yield (Table 18, Entry 7) after 16 hours. Other coupling reactions using EDC/HOBt combinations have been reported,\textsuperscript{75,186} and have been used by Romangoli et al.\textsuperscript{187} in their synthesis of 2-bromoacrylamide 231 (Scheme 64).
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**Reagents and conditions:** i. 2-bromoacrylic acid, EDC, HOBt, DMF, r.t, 18 h.

Scheme 64.

However when the reported combination of coupling reagents was tried in our system, only an inseparable mixture of products was produced (Table 18, Entry 8).

To investigate our theory that the apparent low yield and decomposition was due to the presence of the O-TBDMS group, an attempt was made to couple the aniline 113 with acid 155. In this case the desired benzamide 232 was obtained in 71% yield (Scheme 65). These results seemed to implicate that the O-TBDMS group is sensitive in the coupling reactions. However, the addition of triethylamine did not alleviate the problem, which we thought may be due to acidic decomposition of product; in this case only the starting acid 155 was reclaimed (Table 18, Entry 9).

**Reagents and conditions:** i. 1.5 eq. EDC, 1.5 eq. HOBt, DMF, r.t, 16 h, 71%.

Scheme 65.

Owing to the sensitivity of TBDMS-protected alcohol 99 and the inconsistency and unreproducibility of the successful conditions to afford the coupled product 219 in a reasonable yield (Entry 3), a series of other reagents including HBTU were tried. The use of
HBTU/Et$_3$N as coupling reagent for amines and acids has been widely reported, one example being the synthesis of 235, which contains a O-TBDM ether (Scheme 66).\textsuperscript{188}

![Chemical structure of 233, 234, and 235]

*Reagents and conditions:* i. HBTU, Et$_3$N, DMF, r.t, 39%.

Scheme 66.

Unfortunately, using HBTU in the presence of triethylamine again resulted in decomposition, both when acid 155 was reacted with TBDMS aniline 99 and aniline 113 (Table 18, Entry 10), thus showing that these reagents were incompatible in these systems.

After failed attempts with HBTU, another combination of coupling reagents was carried out by replacing the EDC with DIC and keeping HOBt as an additive. DIC has some advantages due to its easier handling and the solubility of its byproduct in solvents such as DCM. DIC/HOBt combinations of coupling reagents have been used in solution as well as in solid-phase synthesis (Scheme 67).\textsuperscript{189,190}
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Reagents and conditions: i. Aminomethyl resin, DIC, HOBT, DMAP, DMF.

Scheme 67.

Therefore, we attempted a coupling with the use of one equivalent of DIC and HOBT along with a catalytic amount of DMAP, however this resulted in a mixture of products being formed (Table 18, Entry 11). After purification and characterisation the major product was found to be the N-acylurea 238 (Scheme 68). It was hypothesised that 238 was formed due to slow reaction with nucleophilic amine.

The intermediate N-acylurea 239 has been isolated and O-acylisourea 240 has been reported as an intermediate by Bates et al.\textsuperscript{191} (Figure 34).

![Figure 34. Intermediates N-acylurea 239 and O-acylisourea 240.]

To further investigate and confirm the formation of DIC-protected acid 238, the reaction was carried out separately under similar conditions, without HOBT and aniline 99. After workup, NMR and mass spectrometric analysis showed a single product, N-acylurea 238. The structure of 238 was confirmed with NMR. The \textsuperscript{1}H and \textsuperscript{13}C signals were consistent with data previously reported\textsuperscript{192} for these functional groups (Scheme 68).
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Reagents and conditions: i. 1 eq. acid, 1 eq. DIC, cat. DMAP, DMF, r.t, 16 h, 100%.

Scheme 68.

It was observed that the sequence and timing of addition were very important where DIC was used as the coupling agent in the presence of HOBr. When one equivalent of HOBr was added to the reaction mixture after 2 minutes, the desired amide 219 was obtained as a single pure product without TBDMS deprotection. If DIC is added first and HOBr is added more than 15 minutes later, rearrangement to compound 238 occurs, as discussed earlier. It is important to note that HOBr should not be added at the beginning of the reaction because if it is added early its acidic nature and faster reactivity causes deprotection of the TBDMS group to the hydroxyl group on 99, which then has the possibility of taking part in the reaction, thus making the reaction more complicated and resulting in the formation of a number of byproducts and a lower yield.

Depending on the reaction conditions, the coupling reactions proceed by one of two ways: either via the symmetrical anhydride when two equivalents of acid are used, or by an O-acylisourea when one equivalent of the acid is used (Scheme 69).193
The reaction of DIC using two equivalents of acid, when given enough time, follows the symmetrical anhydride pathways in DMF. DMF or DCM can be used for the reaction which proceeds by \(O\)-acylisourea.\(^{193}\) In the reaction of acid 155 with aniline 99, DMF was selected due to the poor solubility of the acid 155 in DCM. Thus, the highly reactive \(O\)-acylisourea 241 was produced with DIC if the nucleophile was not added in time, which then rearranged into an \(N\)-acylurea 238.

The best conditions and sequence of adding the reagents to get a reproducible yield, were that the acid was dissolved first in DMF followed by the amine and a catalytic amount of DMAP. The reaction mixture was stirred for 5-10 minutes and then DIC was added dropwise with further stirring for 2 minutes at room temperature; finally HOBt was added and the reaction mixture was stirred for 18 hours (Table 18, Entry 13). Once the coupling conditions were optimised and the right sequence was followed, amide 219 was obtained in a reproducible yield of 96%.
Summary of coupling reactions

To furnish the desired benzamide 219, acid 155 and its acid chloride 218 were coupled to an aniline 99. Reaction conditions were optimised using different coupling reagents and conditions. Dimethylformamide was used as solvent for all the coupling reactions due to the poor solubility of the starting acid 155 in other solvents. Attempts to couple the acid chloride 218 resulted in decomposition whilst using various coupling reagents either resulted in deprotection of the silyl ether or inconsistent yields (Table 18). Mostly the resultant mixture of products was difficult to purify without aqueous workup, which was made difficult by the polarity of the product 219. The different trialled coupling agents included DCC/DMAP, EDC/HOBt, HBTU and DIC/HOBt under different reaction conditions. EDC/DMAP and EDC/HOBt gave the desired benzamide 219 in variable yields. HBTU resulted in decomposition while DIC/DMAP resulted in the formation of an intermediate, which then rearranged to a stable N-acylurea 238 without coupling to aniline 99. Finally DIC and HOBt when used in two equivalents gave the desired product 219 and the yield was optimised to a reproducible 96%.
3.6 Synthesis of diaryl derivatives

After successful optimisation of coupling conditions using carbodiimide coupling reagents, our next target was to generate a series of benzamides with different groups at the benzylic position, namely hydroxyl and the comparatively bulky O-TBDMS group. To attain strong electrostatic interaction during binding in the minor groove, different amino-alkyl side chains would be incorporated.

Although, the O-TBDMS group was designed to be used as a protecting moiety, its large size also could allow us to investigate the effect of sterics on DNA binding activities. If a larger group was found to be beneficial at the benzylic position, it could easily be replaced with larger groups that may also be capable of alkylation.

Synthesis of target benzamides (Scheme 70) was successfully carried out using the optimised conditions discovered for the synthesis of amide 219. Thus, two equivalents of DIC and HOBt in the presence of a catalytic amount of DMAP in DMF were used for all the coupling reactions. Anilines 99, 113 and 200 were coupled to acids 153-157 using these optimised conditions. The resultant benzamides (Scheme 70) have both aliphatic and cyclic amino-alkyl side chains. Structures of all the resultant benzamides along with their individual yields are shown in Scheme 70.
Scheme 70. Synthesis of diaryl derivatives.

Reagents and conditions: i. 2 eq. DIC, 2 eq. HOBr, cat. DMAP, DMF, r.t, 16 h.
Benzamides (Scheme 70) having aliphatic side chains were found to have higher polarity than benzamides with cyclic amino-alkyl chains. The TBDMS group was expected to decrease the polarity but the lower Rf values showed that O-TBDMS benzamides were more polar than the simple benzamides in methanol-ammonia (9:1).

All the diaryl derivatives in the series were purified through flash chromatography. Initially it was hard to remove the unreacted coupling reagents without aqueous workup. The unreacted DIC was comparatively easier to remove than HOBt due to its solubility in different solvents such as DCM and could be eluted easily during flash chromatography. Despite repeated flash chromatography, some unreacted HOBt was observed in the NMR spectra. There were limited options to overcome this problem without the use of aqueous workup which could not be used due to the high polarity and the resultant solubility of the benzamides in water. Finally, a method of HOBt removal was discovered. This method involved removal of the DMF solvent and then diluting the crude reaction residue with methanol; subsequently solid or aqueous potassium carbonate was added to the reaction mixture to make a consistent slurry. The product slurry was adhered to silica gel which was loaded and purified through chromatography using dichloromethane-methanol (9:1) solvent system. Although this procedure made the purification easy and improved the overall yield, in some cases repeated flash chromatography was still needed to ensure the isolation of a pure product. All the benzylic substituted benzamides were found to be glassy solids after chromatographic purification, but on addition of DCM and drying under high vacuum, they gave white foams with no sharp melting points.

### 3.6.1 TBDMS deprotection in benzamides

With the first series of diaryl derivatives in hand, our next step was to find out a convenient procedure for the subsequent TBDMS deprotection step. A number of considerations were kept in mind, namely the availability of reagents used for deprotection and the uncomplicated removal of any unreacted reagent, as well as the byproducts. Due to the high polarity and potential water solubility of the resultant hydroxyl benzamide, aqueous workup was not considered an option.
Due to our experience with TBAF and its preferred removal under aqueous conditions during the deprotection of TBDMS groups on the simpler benzamides, we decided to explore different methods of deprotection. Using the sulfonic ion exchange resin, Dowex® 50W-X8 in methanol under reflux has been reported as an alternative method to remove $O$-TBDMS group (Scheme 71).$^{194}$

\[
\text{Reagents and conditions: i. Dowex}^\circledR 50W-X8, \text{MeOH, r.t, 20 min.}
\]

**Scheme 71.**

Therefore, we decided to use compound 97 in a test reaction with Dowex® resins. Monitoring the reaction with TLC showed that after 2 hours deprotection was complete (Scheme 72). Workup was simple, involving only filtration and no characteristic signals for the TBDMS group were observed in both the $^1H$ and $^{13}C$ NMR of the crude product 95, thus proving effective deprotection. The advantage of this method would be that the resultant hydroxyl derivative 254 can be purified without flash chromatography.

\[
\text{Reagents and conditions: i. Dowex}^\circledR 50W-X8, \text{MeOH, reflux, 2 h, 100%}.
\]

**Scheme 72.**

The same resin and conditions were then used to attempt the deprotection of the larger TBDMS derivative 219. The reaction was given an additional reaction time of two hours and the reaction mixture was then filtered to remove the resin and concentrated. In this case however no product 254 was obtained (Scheme 73). It was proposed that the desired product 254 was adhered to the resin due to its acidic nature and the presence of the basic amino-alkyl
group on benzamide 254. Therefore, the Dowex® resin was washed with a triethylamine-methanol mixture. TLC of the filtrate showed that some product 254 was being washed from the resin. However, despite repeated washing with triethylamine-methanol, most of the product 254 remained bound to the resin. For compounds with no amino groups, the Dowex® resin could be considered a viable option but for benzamides such as 219, it is clearly incompatible. Thus, the idea of using the Dowex® resin as the deprotecting agent was abandoned at this point (Scheme 73).

![Reaction Scheme](image)

*Reagents and conditions: i. Dowex® 50W-X8, MeOH, reflux, 4 h, 10%.*

Scheme 73.

With Dowex® proving to be an inappropriate deprotecting agent, we then looked to other reagents which would be capable of giving the deprotection product. The deprotection of the TBDMS group was critical as the hydroxyl group has several advantages. It can be converted into a variety of functional groups such as mesylates, which can thereafter be easily substituted with amines or azides. The other advantage of the hydroxyl group is that it has the possibility of strong hydrogen bonding which may be beneficial to DNA minor groove binding. Despite being hard to purify, TBAF was used for all the TBDMS-protected benzamides to generate the hydroxyl benzamides due to lack of suitable alternatives (Scheme 74). The unreacted TBAF and its byproducts were removed by evaporating THF from the reaction mixture under reduced pressure and the residue was diluted with methanol followed by silica gel addition to form a consistent slurry which was purified by repeated flash chromatography. It should be noted that using a minimum amount of solvent and less equivalents of TBAF was found to be key to achieving consistently high yields. The structure and yield of all the resultant hydroxyl benzamides are given in Scheme 74.
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3.6.2 Functionalisation of benzylic position

With hydroxy benzamides 254-258 in hand, our next step was to further functionalise the hydroxyl group at the benzylic position of these larger diaryl amides. The first step was the synthesis of mesylate 259 and its substitution with amino nucleophiles using the methods developed previously (Section 3.2). Alcohol 254 was used to test the mesylation and subsequent displacement step. The mesylate step was carried out with 1.5 equivalents of
methanesulfonyl chloride in the presence of triethylamine under the already optimised conditions. The reaction, when monitoring with TLC, was seen to go to completion after two hours of reaction time. After a brief aqueous workup, the product 259 was used in the next reaction by reacting with 2-(methylamino)ethanol to furnish derivative 260. Unfortunately, the reaction resulted in a complex mixture of inseparable and unidentified products (Scheme 75).

Reagents and conditions: i. 1.5 eq. MsCl, 1.5 eq. Et3N, DMF, r.t, 2 h, 100%; ii. 10 eq. 2-(methylamino)ethanol, DMF, r.t, 18 h, mixture; iii. 10 eq. NaCl, 60 °C, 30 min, 96%.

Scheme 75.

The mesylation reaction was also repeated in THF and the triethylamine salt was filtered before using in the next reaction. Again, no useful product was isolated, and it was difficult to purify the resultant amine 260 from the reaction mixture. It was then decided to convert the mesylate 259 into chloride 261 due to its relative easy purification compared to amine 260. Thus, sodium chloride was added to mesylate 259 in the same way as for the model benzamides 171 and 172. The corresponding chloride 261 was successfully furnished in 96% yield. The resultant chloride 261 was easily determined by the characteristic peaks for the benzylic methylene at δ 4.62 ppm and δ 46.8 ppm in the 1H and 13C NMR respectively, and was further confirmed by HRMS.

At this stage, due to the complications regarding benzylic substitution, it was decided to investigate structural variations in the benzamides and their DNA binding activities, comparing the different amino-alkyl side chains and small range of benzylic variations. If
alteration of the benzylic position was found to be favourable, then this could be explored later.

**Summary of the synthesis of diaryl derivatives**

A series of diaryl derivatives were prepared having both cyclic/open amino-alkyl chains. To furnish the desired benzamides 219, 232, 242-251 and 254-258, three different anilines 99, 113 and 200 were coupled to five different monoaryl acids 153-157 using coupling reagents DIC/HOBt with a catalytic amount of DMAP in DMF (Scheme 76). The yields for the coupling step varied from 46% to 96%.

In the O-TBDMS-protected benzamides 219, 243 and 250-251, the TBDMS group was deprotected using TBAF. Due to the difficulty in the removal of TBAF, Dowex® resin in methanol was also attempted. Although, the Dowex® method worked for a small model compound 97, it failed to deprotect larger compound 219 to give the desired compound 254. This was believed to be due to 254 adhering to the resin due to the presence of the basic amino group (Scheme 73).

The resultant hydroxyl group in compound 254 was then converted to mesylate 259 and functionalised to chloride 261. The synthesis of mesylate 259 was also attempted to incorporate amino-alcohol moiety, which is the precursor for nitrogen mustards, however the product was only obtained as part of a complex mixture and was impossible to purify to an adequate level.
Chapter 3: Synthesis of Diaryl Derivatives

Reagents and conditions:
i. 2 eq. acid (153-157), 2 eq. DIC, 2 eq. HOBT, cat. DMAP, DMF, r.t., 16 h, 46-96%;
ii. (219, 243, 244, 250 and 251), 4 eq. TBAF, THF, r.t., 24 h, 71-100%;
iii. a) (254), 1.5 eq. MsCl, 1.5 eq. Et₃N, DMF, r.t., 2 h, 100%; b) 10 eq. NaCl, 60 °C, 30 min, 96%.

Scheme 76.
Chapter 3: Synthesis of Diaryl Derivatives

3.7 Synthesis of diaryl derivatives

After successful synthesis of a series of diaryl derivatives (219, 232, 242-251 and 254-258 including TBDMS and hydroxyl derivatives), our next aim was to synthesise a second series of diaryl derivatives (262-279) having the same functional groups at the benzylic position. This new series would be different to the first series of diaryl benzamides 219, 232, 242-251 and 254-258 in amino-alkyl chain length, curvature and amide connectivities. In order to investigate the role of chain length, a comparatively short amino-alkyl chain would be incorporated as well as longer amino-alkyl chain, varying from open chain amino-alkyl groups to cyclic amino-alkyl groups. Most of the benzamides in this series would have short amino-alkyl chains. To acquire the desired orientation and to reach deep into the minor grooves, flexibility in chain length is needed and this can be achieved by preparing different isomers. Therefore, para and meta isomers of each benzamide would be prepared to investigate the effect of curvature on DNA binding activity. In addition to amino-alkyl chain isomers, two long chain morpholino derivatives were also desired.

To investigate the effect of altering the pattern of amide bonding, amide connectivity at the amino side chain would also be altered to that of the first series of diaryl derivatives (ArCONHR to ArNHCOR). These changes in the amide connectivity are anticipated to have a strong effect on the electronic character in the aromatic and aliphatic parts of the molecules. The effect of stereoelectronic factors could also play an important role in the solubility and DNA binding activities. It was hoped that this would help us to further understand the DNA binding relationships.

Keeping in mind the various advantages of proposed diaryl derivatives 262-279 due to their having a range of variations in their structures, monoaryl acids 119-128 were coupled to two different anilines 99 and 113 under the previously optimised conditions (Scheme 77). It was observed that this series of compounds (262-279), which all contained shorter α–amino amides, were less polar than the benzamides, which have longer alkyl chains between the amide and amine moities.
Again, a comparatively better yield was obtained with the cyclic tertiary amines rather than the open chain configured amines. The \textit{para} piperidino-acid 123 was not soluble in DMF at room temperature, therefore the acid was first dissolved in DMF separately, with gentle heating, and when the temperature dropped to room temperature the coupling reagent DIC and a catalytic amount of DMAP were added. Once the acid reacted with DIC, the solubility of the formed intermediate was observed to be much higher in DMF and the reaction was then progressed by the addition of HOBt to the reaction mixture. In general, however, lower yields were obtained in reactions where the acids were very polar and had poor solubility in DMF. It was observed that the rate of reaction for \textit{meta} isomers was faster than the \textit{para} isomers, which was probably due to the higher solubility of the \textit{meta} isomers of the starting acids. In some cases when the reaction was left for an extended time to allow time for amide formation, trace amounts of the \textit{O-TBDMS} deprotected hydroxyl derivatives were also isolated. This was assumed to occur due to over exposure of the \textit{O-TBDMS} group to the acidic HOBt.

All the resultant benzamides 262-279 were purified using the potassium carbonate method in a similar fashion as for the previous series, which aided in the removal of HOBt. The derivatives in the series were less polar, and dichloromethane-methanol (9:1) rather than methanol-ammonia (9:1) could be used during flash chromatography. One derivative, the morpholino-benzamide 274, was easily recrystallised from methanol which gave crystals suitable for single crystal analysis (Figure 35). The X-ray crystal structure of amide 274 shows multiple hydrogen bonds between molecules. The AcNHAr and ArCONHCH$_2$ groups, as well as the oxygen of the morpholine ring, are all involved in hydrogen bonding. Such bonding suggests amides 274 may hydrogen bond to DNA if the orientations are acceptable. The structures and yields of all the formed benzamides 262-279 are given in Scheme 77.
Figure 35. Crystal structure of 274.
Chapter 3: Synthesis of Diaryl Derivatives

**Scheme 77.** Synthesis of diaryl derivatives.

Reagents and conditions: i. 2 eq. DIC, 2 eq. HOBt, cat. DMAP, DMF, r.t., 16 h.

R = -CH₂OTBDMS (99)
R = H (113)
After successful synthesis of the second series of diaryl amides, we then wished to remove the O-TBDMS group to form a free hydroxyl group. We initially began by using TBAF as previously, however removing the unreacted TBAF and its byproducts from some derivatives.
in the series proved very difficult. It was decided to search for a new deprotecting reagent for the TBDMS group, that would be easy to remove without the need for aqueous workup. One such option was Et$_3$N·3HF, which has the advantage that it can be easily removed by evaporation afterwards. Et$_3$N·3HF has been used in the preparation of an intermediate towards the synthesis of substituted methylthiophosphonates 281 (two isomers, where R = H, OMe and B = Ade$^{Bz}$, cyt$^{Bu(Bz)}$ etc.). In this case Et$_3$N·3HF in THF was found to deprotect the TBDMS group in 61% yield (Scheme 78). Therefore, Et$_3$N·3HF was used in the deprotection step for all the reactions.

\begin{center}
\begin{tikzpicture}
    \node[anchor=west] at (0,0) {\includegraphics[width=\textwidth]{diagram.png}};
\end{tikzpicture}
\end{center}

\textit{Reagents and conditions:} i. Et$_3$N·3HF/Et$_3$N in THF, r.t, 3-4 h, 61%

\textbf{Scheme 78.}

After using TLC to establish that the reaction was completed, the unreacted Et$_3$N·3HF was removed by evaporating THF under reduced pressure and diluting the residue with methanol followed by the addition of silica gel to form a consistent slurry. The silica gel was then loaded onto a column and purified with dichloromethane-methanol (19:1 then 9:1) solvent system. The unoptimised yield in the series varied from 54% to 93% (Scheme 79). All the resultant hydroxyl derivatives 282-291 in the second series of diaryl compounds had the same physical appearance as the hydroxyl derivatives 254-258 in the first series of diaryl derivatives, and were isolated as foams when DCM was added; drying under high vacuum followed.
Reagents and conditions: i. 3 eq. Et$_3$N.HF, THF, r.t., 24 h.

Scheme 79. Deprotection of TBDMS group.
Summary of the synthesis of diaryl derivatives

The second series of diaryl derivatives (262-279) composed of open/cyclic amino-alkyl chains was prepared by coupling anilines 99 and 113 with 10 different acids 119-128. A total of 18 benzamides 262-279 were prepared having para/meta substitution with amino-alkyl chain lengths varying from short to comparatively long.

The TBDMS group was deprotected with Et₃N.3HF, which was found easier to remove than TBAF, to generate hydroxyl derivatives 282-291 in yields varying from 54% to 93%. All the resulting benzamides were purified through flash chromatography without aqueous workup. Mostly the derivatives in the second series were comparatively less polar than the previous series of diaryl derivatives (219, 232, 242-251 and 254-258), most likely due to the closer proximity of the dialkylamine group to the amide functionality (Scheme 80).

Reagents and conditions: i. 2 eq. acids (119-128), 2 eq. DIC, 2 eq. HOBT, cat. DMAP, DMF, r.t, 18 h, 26-97%; ii. 3 eq. Et₃N.3HF, THF, r.t, 24 h, 54-93%.

Scheme 80.
Chapter 4

Synthesis of Triaryl Derivatives
4.1 Synthesis of the triaryl derivatives

After the successful synthesis of diaryl derivatives with enough variations in structures to investigate in DNA binding studies, our next interest was in the synthesis of a series of triaryl derivatives, which would contain symmetrical and non-symmetrical substituted derivatives. The main aim was to investigate the role of molecular size due to the introduction of an additional aromatic ring and an increased number of NH groups, which have an essential role in maximising hydrogen bonding during its binding in the minor grooves of double-helical DNA.

4.1.1 Synthesis of the symmetrical triaryl derivatives 292-294

We wished to establish a route that could be useful to generate both symmetrical and non-symmetrical triaryl oligoamides with various benzylic substitutions. It would be useful to compare the solubility, polarity and resultant DNA binding activities of such derivatives with the already prepared diaryl derivatives. The benzylic position of viable compounds would be further functionalised in a similar fashion to the diaryl derivatives. As already discussed (Section 1.7), Gong and co-workers\textsuperscript{110} reported that the meta-para-meta combination in triaryl derivatives have better binding to poly AT DNA due to its better match with the curvature of the minor grooves in DNA. It was decided that our compounds would have the same arrangement so that the effect of additional substituents and functionality could be explored.

The first target compound, 292, had no benzylic substitution or basic amino-alkyl chain and was therefore expected to be less complicated in its preparation. To prepare benzamide 292, three different conditions were used (Table 19). Firstly, an acid chloride 158 was reacted with aniline 113 in the presence of triethylamine but only starting material was recovered (Entry 1). The same reaction was also tried using 1 equivalent of aniline 113 with 0.75 equivalents of acid chlorides 158 using pyridine as the base and solvent; this resulted in a mixture of inseparable products (Entry 2).
Chapter 4: Synthesis of Triaryl Derivatives

After these failed attempts, we turned to an inorganic base and used the reported method of Cotton et al.\textsuperscript{167} The reaction was carried out by using 2.5 equivalents of aniline 113 in the presence of 5.4 equivalents of potassium carbonate in THF for 30 minutes, which gave the desired benzamide 292 in almost quantitative yield (Entry 3). The resultant amide 292 was easily purified by washing the crude solid with DCM which removed the byproducts and reagents. The structure of 292 was confirmed using a combination of NMR and mass spectrometric techniques (Table 19).

Table 19. Synthesis of symmetrical benzamide 292.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 eq. 113, 2 eq. Et$_3$N, DCM, r.t, o/n.</td>
<td>s.m.</td>
</tr>
<tr>
<td>2</td>
<td>1 eq. 113, 0.75 eq. 158, pyridine, r.t, o/n.</td>
<td>mixture</td>
</tr>
<tr>
<td>3</td>
<td>2.5 eq. 113, 5.4 eq. K$_2$CO$_3$, THF, r.t, 30 min.</td>
<td>98</td>
</tr>
</tbody>
</table>

After successful synthesis, we then wished to carry out the synthesis of symmetrical benzylic substituted benzamide 293 having two benzylic $O$-TBDMS groups. It was again envisaged that the TBDMS group would be easily deprotected into hydroxyl groups and thereafter into different functionalised groups. Due to the high yield and simplicity of the synthesis of benzamide 292, we again decided to use the same conditions using terephthaloyl chloride 158. Using the same established method used to generate benzamide 292, the TBDMS benzamide 293 was successfully prepared with the benefit of an easy workup procedure. A pure sample of 293 was achieved by simply washing with water to remove the base and then with DCM to remove the unreacted aniline 99. After this procedure, pure 293 was obtained in an excellent 96% yield. Again, NMR and mass spectrometry were used to confirm the desired structure. With the $di$-TBDMS derivative 293 in hand, our next step was the synthesis of the hydroxyl derivative 294 via deprotection of the TBDMS ethers. This was easily achieved by using our previously used method of TBAF in THF (Scheme 81) which gave the desired di-alcohol 294.
Chapter 4: Synthesis of Triaryl Derivatives

in 97% yield. As the resultant hydroxyl benzamide 294 was not soluble in water, the unreacted TBAF was easily removed by filtration; washing the product with water and DCM removed the organic impurities.

Reagents and conditions: i. 1 eq. acid chloride (158), 2.5 eq. Aniline (99), 5.4 eq. K₂CO₃, THF, r.t, 30 min, 96%; ii. 4 eq. TBAF, THF, r.t, 24 h, 97%.

Scheme 81.

4.1.2 Synthesis of the unsymmetrical triaryl derivatives 295-302

With the synthesis of symmetrical benzamides 292-294 complete, our next step was the synthesis of non-symmetrical triaryl amides 295-302. Due to the insolubility of acid 166 in DCM it was not practical to prepare its acid chloride using chlorinating agents. It was therefore decided to use acid 166 in the presence of coupling reagents in DMF to furnish the desired triaryl unsymmetrical compound 295. As we thought that removal of an unreacted acid 166 may be difficult, all the reagents were used in equimolar amounts. TLC examination of the reaction mixture showed the reaction had gone to completion after 18 hours. The resultant compound 295 was insoluble in water and some impurities were removed by filtration and aqueous washing. The remaining crude material was dissolved in methanol and silica gel was added and then purified through flash chromatography giving amide 295 in 95% yield (Scheme 82). With unsymmetrical benzamide 295 in hand, our next step was again the TBDMS deprotection, which was achieved using TBAF in THF; this gave the desired alcohol 296 in 91% yield.
Chapter 4: Synthesis of Triaryl Derivatives

After successful synthesis of the benzamide 296, we then decided to explore the possibility of functionalising the benzylic position on this triaryl derivative 296. Our next step was therefore the conversion of alcohol 296 to mesylate 297 using methanesulfonyl chloride in DMF. The resultant mesylate 297 was used without purification and reacted with excess of sodium chloride in DMF which gave the desired benzylic chloride 298 in 91% yield (Scheme 82). The chloride 298 was found to be a stable compound and could be purified with flash chromatography in a similar way to the TBDMS or hydroxyl derivative. The chloride 298 was again easily characterised, with the indicative chloromethylene group being observed as a singlet at δ 4.74 ppm and δ 46.4 ppm in ¹H and ¹³C NMR, respectively. The structure was finally confirmed by HRMS.

Reagents and conditions: i. 1 eq. acid, 1eq. DIC, 1eq. HOBt, DMAP, r.t, 18 h, 95%; ii. 4 eq. TBAF, THF, r.t, 24 h, 91%; iii. a) 1.5 eq. MeSO₂Cl, 1.5 eq. Et₃N, DMF, 0 °C then r.t 2 h, 100%; iv. 10 eq. NaCl, DMF, 60 °C, 30 min, 91%.

Scheme 82.
Although the purification of triaryl derivatives prepared so far was relatively easy, using an aqueous workup due to their water insolubility, our next aim was to introduce an amino-alkyl group at the end of the molecule to increase its aqueous solubility and electrostatic interaction during DNA binding activity. Triaryl derivatives 299-302, which have a meta-para-meta combination of rings, were highly desirable due to them having an amino-alkyl group with an additional aromatic ring, as well as having benzylic substitution.

Preparing benzamide 299 required the coupling of acid 167 with aniline 99. Due to the presence of an amino group on the acid 167, the use of chlorinating agent in less polar solvents to make its acid chloride was not an option. Therefore, the coupling conditions of DIC/HOBt in DMF were used under the already optimised conditions. All the reagents were used in equimolar concentration as we thought that the removal of acid 167 or its byproducts after the reaction with the coupling reagent would be difficult to remove with chromatography without aqueous workup. After the reaction was complete, the solvent was evaporated and the residue was diluted with methanol. A silica gel slurry was prepared as previously mentioned and purified through methanol-ammonia (9:1) solvent system. The compound 299 was obtained in 70% yield and was found, as expected, to be more polar than the previously prepared di- and triaryl derivatives which have no amino-alkyl groups (Scheme 83).

With benzamide 299 in hand, our next step was to deprotect the O-TBDMS group. Initially TBAF was used in the deprotection step but again it was difficult to separate the pure product 300 from the unreacted TBAF. Therefore, Et3N.3HF was used and it was easily removed using flash chromatography, giving 300 in 61% yield. The hydroxyl derivative 300 was then converted to mesylate 301 by the reaction of methanesulfonyl chloride in the presence of triethylamine under the optimised conditions and the resultant mesylate 301 was obtained in quantitative yield. By adding excess equivalents of sodium chloride, the methanesulfonyl group was substituted with chloride (Scheme 83). The chloride 302 was also purified with methanol-ammonia (9:1) solvent system in excellent yield and was easily characterised by its characteristic peaks in the $^1H$ and $^{13}C$ NMR.
Chapter 4: Synthesis of Triaryl Derivatives

Reagents and conditions: i. 1 eq. acid (167), 1 eq. DIC, 1 eq. HOBr, cat. DMAP, r.t, 18 h, 70%; ii. 3 eq. Et$_3$N.3HF, THF, r.t, 24 h, 61%; iii. a) 1.5 eq. MeSO$_2$Cl, 1.5 eq. Et$_3$N, DMF, 0 °C then r.t 2 h, 100%; iv. 10 eq. NaCl, DMF, 60 °C, 30 min, 97%.

Scheme 83.

With the synthesis of symmetrical and non-symmetrical triaryl derivatives established, we wished to prepare triaryl derivatives with two alkylating groups.

4.1.3 Synthesis of the triaryl precursor for the alkylating groups

As already discussed, there are problems associated with nitrogen mustards and bromoacryloyl derivatives at the benzylic position and it was therefore decided to couple the 3,5-dinitroaniline to diaryl acid 167, which has a basic dimethylaminopropyl group. It was
envisaged that the nitro groups could easily be converted to the resultant aniline 304 and then onto mustards or bromoacryloyl moieties.

To furnish the starting nitro-derivative 303, a coupling reaction was carried out between acid 167 and 3,5-dinitroaniline, using the previously successful DIC/HOBt coupling reagent method (Scheme 84). Overnight stirring and subsequent purification through flash chromatography resulted in only returned starting materials.

It was hypothesised that due to the presence of strong electron-withdrawing nitro groups, the reactivity of aniline would be significantly decreased towards coupling reaction in comparison to anilines 99 and 113 which undergo this coupling readily. Due to limited time, further conditions were not attempted.

\[ \text{Reagents and conditions: i. 1 eq. 3,5-dinitroaniline, 1 eq. DIC, 1 eq. HOBt, cat. DMAP, DMF, r.t, o/n, s.m.} \]

**Scheme 84.**
Chapter 4: Synthesis of Triaryl Derivatives

Summary of the synthesis of triaryl amides

A series of symmetrical and non-symmetrical triaryl amides was prepared. Symmetrical benzamides 292 and 293 were prepared by the reaction of terephthaloyl chloride 158 and anilines 99 and 113 in a single step. The unsymmetrical benzamides 295 and 299 were prepared using a coupling reaction between aniline 99 and acids 166 and 167. The TBDMS group in benzamides 293, 295 and 299 was removed using TBAF or Et₃N.3HF giving the hydroxyl benzamides 294, 296 and 300. Two benzyl chlorides 298 and 302 were also prepared (Scheme 85).

An attempt was also made to couple 3,5-dinitroaniline to the acid 167 but the low reactivity of the 3,5-dinitroaniline failed to give the desired amide 303.
Chapter 4: Synthesis of Triaryl Derivatives

Reagents and conditions: i. 2.5 eq. anilines (99 and 113), 5.4 eq. K$_2$CO$_3$, THF, r.t, 30 min, (292) = 98%, (293) = 96%; iii. 1 eq. acid (166 and 167), 1 eq. DIC, 1 eq. HOBT, cat. DMAP, r.t, 18 h, (295) = 95%, (299) = 70%; iii. (295) 4 eq. TBAF, THF, r.t, 24 h, 91%; (299), 3 eq. Et$_3$N,3HF, THF, r.t., 24 h, 61%; iv. a) 1.5 eq. MeSO$_2$Cl, 1.5 eq. Et$_3$N, DMF, 0° C then r.t 2 h, 100%; b) 10 eq. NaCl, DMF, 60° C, 30 min, (298) = 91%, (302) = 97%; v.1 eq. 3,5-dinitroaniline, 1 eq. DIC, 1 eq. HOBT, cat. DMAP, DMF, r.t, o/n, s.m.

Scheme 85.
Chapter 5

Biological Studies

The biological results in this chapter were obtained by myself at the University of Auckland and during a stay at the University of New South Wales, Australia. During my time at UNSW, the research was overseen by Prof. Laurence Wakelin, who also assisted in the interpretation of mass spectrometry results. Compounds were sent to the American National Cancer Institute (NCI) for screening against 60 cancer cell lines.
Chapter 5: Biological Studies

5.1 Overview

After the successful synthesis of a library of novel di- and triaryl amides, we then wished to use different techniques to investigate their DNA binding and alkylating activities. Various points were kept in mind, including the availability of required facilities and chemicals. Different analytical techniques including spectroscopic methods, such as DNA melting analysis using UV spectrometry; comparative ethidium displacement assays using spectrophotometry; and matrix-assisted laser desorption ionisation mass spectrometry (MALDI) experiments were used to investigate the alkylating activities of the different groups at the benzylic positions on benzamides. Cytotoxicity data was also obtained for selected derivatives by sending samples to the American National Cancer Institute (NCI). In this chapter, these techniques and the results from our initial investigations will be discussed.

5.2 DNA melting analysis using UV spectrometry

Owing to the high stability of our ligands at elevated temperatures, UV spectrometry was selected to carry out DNA melting studies. A UV spectrometer connected to a temperature-controlled cell is widely used to study the binding of ligand molecules with natural or synthetic DNA due to its simple operation, sensitivity and reproducible results.\(^\text{196}\) The basic principle is based on heating an ordered DNA duplex structure to the denatured/disordered form and the detection of the resultant transition by UV spectrometry. Ligand molecules capable of hydrogen bonding with DNA stabilise the overall structure of the resultant complex. The strand separation in double-stranded DNA as a function of temperature is called “DNA melting”, denoted by “T\(_m\)”. At the T\(_m\) half of the DNA molecules exist in a helical state and half in single-stranded form. The T\(_m\) of synthetic drug-DNA complexes or synthetic oligoamides depends on several factors, most importantly the number of binding sites and the extent of binding between ligand and DNA. The stronger the binding of ligand with DNA, the higher the T\(_m\) value of the resultant ligand-DNA complex.\(^\text{196-198}\)

To carry out the desired experiments, our first step was to prepare the required buffer solutions of DNA, which were prepared using standard literature methods.\(^\text{199}\) Solutions of ligands were also prepared as described in the experimental section (Chapter 6, section 6.9.1). Natural DNA MGB
based on five-membered backbone and benzamide MGB prefer AT-rich sequences than GC-rich sequences on DNA. Therefore, calf thymus (CT DNA) was used only for test experiments and poly AT DNA was selected for all our final experiments. The $\Delta T_m$, i.e. the difference of melting points between pure poly AT DNA and drug-DNA complex, was calculated.

UV melting point analyses were carried out to study the effect of the thermal stability of poly AT DNA in the presence of saturating amounts of various ligands using 0.5, 1.0 and 1.5 equivalents per nucleotide of poly AT DNA. The various ligands were selected for UV melting point analysis based on the presence of open/cyclic amino-alkyl groups on benzamides. Para and meta isomers, which have different curvatures, were also selected for our initial studies to investigate the effect of curvature on its DNA binding activities. Apart from the amino-alkyl chains, a range of ligands having hydroxyl, $O$-TBDMS and chloride groups at the benzylic positions were also studied. As most of the ligands contained comparatively short amino-alkyl chains composed of a single methylene groups, derivatives having longer amino-alkyl chains and altered amide connectivities were also selected for DNA melting analyses. To find out the role of an additional aromatic ring system, triaryl ligands containing a $O$-TBDMS, hydroxyl or chloride groups at the benzylic position were also investigated.

The first series includes unsubstituted benzamides, with different amino-alkyl side groups and curvatures having both para and meta isomers. The para isomers of piperidino-benzamide 270 showed slightly higher $\Delta T_m$ values of 10.9 compared to the melting point of pure poly AT DNA. Derivatives 262, 266 and 274 showed the same shift, which suggested that the variations of amino side chain had a very small effect on the resultant $\Delta T_m$ values (Table 20). In general, the meta isomers, especially compounds 267 and 271, showed a smaller $\Delta T_m$ of 3.7 and 4.2 respectively and an increase in $\Delta T_m$ was noted due to increasing ligand concentration in compound 267. In the case of meta isomers with a cyclic amino side chain, no change in $\Delta T_m$ was observed by increasing the ligand concentration. The data revealed that compounds with a meta-para combination of amides were comparatively better than compounds with a meta-meta combination but provided no clear correlation between the various amino side chains and the resultant $\Delta T_m$.  

Table 20. $\Delta T_m$ of ligands bound with poly AT DNA at three different equivalents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>$\Delta T(\pm 1)/^\circ$C, ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td></td>
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<td>4</td>
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<td>6.2</td>
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<tr>
<td>5</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>6.2</td>
</tr>
</tbody>
</table>
With the melting point analyses for unsubstituted benzamides in hand, we then wished to carry out experiments with benzylic substituted benzamides to investigate the role, if any, of hydroxyl group on DNA binding. A slightly higher, or in some cases similar, $\Delta T_m$ was observed due to the presence of a hydroxyl group. Although the presence of a hydroxyl group could have stabilised the ligand-DNA complex, we found no significant change when comparing compounds with the benzylic hydroxyl group to the analogues without. In the case of para substituted 282 and 286, the $\Delta T_m$ values were in the same range and were the highest in the series (Table 21). Again, among the hydroxyl substituted derivatives 282-289 no significant correlation in $\Delta T_m$ was observed due to the change in the amino-alkyl chain. This is probably due the small length of the amino-alkyl chain, which does not play a significant role in electrostatic binding in the minor grooves of DNA.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>$\Delta T(\pm 1) / ^\circ C$, ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
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<td>9.4</td>
</tr>
<tr>
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</tr>
<tr>
<td>8</td>
<td><img src="image8" alt="Structure 8" /></td>
<td>6.2</td>
</tr>
</tbody>
</table>
Once the $\Delta T_m$ results from open and cyclic amino-benzamides with shorter amino-alkyl groups had been obtained, it was then decided to test the diaryl oligoamide 261 with a comparatively longer amino-alkyl side chain. With this compound a significant increase in $\Delta T_m$ was observed by increasing the ligand concentration. The increased $\Delta T_m$ clearly shows that the longer group would bind deep in the minor grooves, giving greater electrostatic interactions (Figure 36). These observations suggested that the longer group on the amino side chain is probably reaching deeper into the minor grooves of the DNA to form stronger electrostatic interactions (Table 22).

It was then decided to test the triaryl derivatives 299, 300 and 302 which have an additional aromatic ring, different benzylic substitutions, and longer amino-alkyl chains. In the case of hydroxyl derivative 300, a shift of 26.7 was noticed with 0.5 equivalents. By adding more equivalents of ligand, only a very small effect was observed, which indicated that potentially the system was nearly saturated. These observations showed that additional aromatic rings and amide bonds resulted in stronger bonding as per multiplicity of bindings. A noticeably higher $\Delta T_m$ value was observed for chloride 302 than hydroxyl derivative 300. The highest $\Delta T_m$ value of 38.7 was observed by using 1.5 equivalents per nucleotide with benzamide 302. The $O$-TBDMS derivatives 299 showed $\Delta T_m$ as high as 47.2, although experiments with this ligand were complicated by it being only sparingly soluble in the buffer system.
Table 22. $\Delta T_m$ of ligands bound to poly AT DNA at three different equivalents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>$\Delta T(\pm 1)^\circ C$, ratio</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td><img src="261" alt="Structure" /></td>
<td>11.2</td>
</tr>
<tr>
<td>2</td>
<td><img src="300" alt="Structure" /></td>
<td>26.7</td>
</tr>
<tr>
<td>3</td>
<td><img src="302" alt="Structure" /></td>
<td>31.3</td>
</tr>
<tr>
<td>4</td>
<td><img src="299" alt="Structure" /></td>
<td>34.7</td>
</tr>
</tbody>
</table>

The UV melting point profiles of ligand 261 at three different stoichiometric ratios, namely 0.5, 1.0 and 1.5 equivalents per nucleotides, is shown in Figure 36. The melting curves for all other derivatives are given in the Appendix.
The binding activity of selected ligands was investigated using DNA melting analyses with UV spectrometry. CT DNA was used for our test compounds but the final experiments were carried out with poly AT DNA in ratios of 0.5, 1.0 and 1.5 equivalents per nucleotide. The ligands varied with respect to the amino-alkyl chain, benzylic position, and in their curvature due to the number and substitution of aromatic rings present in the system. The data revealed no significant difference between compounds with the open chain or cyclic amino groups. The presence of a benzylic hydroxyl group and para isomers resulted in little change in $\Delta T_m$ values. The presence of the longer dimethylaminopropyl side chain was found to be beneficial and a significant increase in $\Delta T_m$ was observed by the introduction of this group. This indicates that greater spatial distance between the amide and basic amine functionality allows for increased electrostatic interaction. The additional aromatic ring resulted in further stabilisation of the complex with DNA. The $O$-TBDMS derivative 299 showed $\Delta T_m$ as high as 47.2, although experiments with this ligand were complicated by it being only sparingly soluble in the buffer system. The chloride 302 showed the highest consistent, $\Delta T_m$, probably due to its strong electrostatic bindings and multiplicity of possible hydrogen bonds due to its larger size.
5.3 Competitive ethidium displacement assays

Ethidium bromide 19, a red fluorescent dye, interacts with double-stranded DNA and RNA by intercalation between adjacent base pairs.\(^{37}\) It has been used medically in this respect, but the most frequent application is its use in competitive ethidium displacement assays. Owing to its ability to bind with DNA, its fluorescence can increase multi-folds due to changes in its microenvironment.\(^{200}\) Other drug molecules can then be added that can bind to DNA, thereby competing with ethidium for binding sites and displacing ethidium from the DNA, with a resultant decrease in the fluorescence intensity. These optical changes of DNA-bound ethidium can be detected using a fluorimeter.\(^{201}\) This ethidium assay has been previously used to study the binding activities of minor groove binding agents.\(^{109,202}\)

Firstly, we needed to prepare the two required buffers, SHE (sodium chloride, HEPES, EDTA) and acetate (NaOAc, NaCl and Na\(_2\)EDTA), according to literature methods.\(^{201}\) Stock solutions of DNA and ligands were prepared as given in the experimental section (Chapter 6, section 6.9.2). Initially, some ranging tests were carried out using the ligand at final concentrations of 1 µM, 10 µM, 100 µM, and 500 µM, and 1 mM for each of the ligand. The aliquots were added in 3 µL portions or more to overcome the potential for pipetting error. Unfortunately, the majority of our compounds were not strong enough to displace the ethidium at low concentrations (Table 23). In some cases, addition of the ligand had almost the same effect as buffer, which was due to simple dilution effects. In these cases, it was decided to use the stock solution of the ligands, which was 1 mM in small aliquots, but this too was unsuccessful. The final aliquots for the compounds used were 10 µL or 20 µL, to find out C\(_{50}\) values. The C\(_{50}\) value is the amount of ligand needed to displace the ethidium from DNA molecules, with a resultant 50% drop in fluorescence intensity.\(^{203}\)

The ethidium displacement assay was used for 37 compounds, including those unsubstituted at the benzylic position, those with substitution, and those with different amino-alkyl groups in the side chain, in the SHE and acetate buffers. Initial experiments were carried out with CT DNA due to its low price and easy availability. Benzamide MGB are known\(^{109}\) to bind selectively to AT-rich sites in DNA; therefore all the final experiments were carried out with poly AT DNA at a final
concentration of 1 mM. In most of the derivatives, lower concentrations were not effective in
displacing the ethidium by 50%; therefore, 1 mM stock solutions of ligands were used.
The results given in Table 23 suggest that compound 299, with its bulky O-TBDMS group at the
benzylic position, has better activity due to its maximum hydrogen bonds and electrostatic binding
which helps the O-TBDMS group to displace the ethidium easily from the minor groove of DNA.
The improved C_{50} value for chloride 302 over alcohol 300 suggested a role for the larger chloride
group in ethidium displacement. The same effect is seen when comparing diaryl derivatives 254
and 261. Chloride 261 has a C_{50} value of 506 μM whilst the analogous hydroxyl-containing
compound 254 has C_{50} > 1 mM. Alcohol 254, and derivatives 264 and 283 with short amino-alkyl
chains, showed weak activity, probably due to weaker bondings.
Table 23. $C_{50}$ values of different ligands with poly AT DNA.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>Results/$C_{50}$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="299" alt="Structure 1" /></td>
<td>5.0 $\mu$M</td>
</tr>
<tr>
<td>2</td>
<td><img src="300" alt="Structure 2" /></td>
<td>117.6 $\mu$M</td>
</tr>
<tr>
<td>3</td>
<td><img src="302" alt="Structure 3" /></td>
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</tr>
<tr>
<td>4</td>
<td><img src="261" alt="Structure 4" /></td>
<td>506 $\mu$M</td>
</tr>
<tr>
<td>5</td>
<td><img src="254" alt="Structure 5" /></td>
<td>&gt; 1 mM</td>
</tr>
<tr>
<td>6</td>
<td><img src="264" alt="Structure 6" /></td>
<td>&gt; 1 mM</td>
</tr>
<tr>
<td>7</td>
<td><img src="283" alt="Structure 7" /></td>
<td>&gt; 1 mM</td>
</tr>
</tbody>
</table>
Figure 37 shows the graph from the ethidium experiments for compound 299, which showed significant activity in ethidium displacement assays. Other active compounds include triaryl derivatives 299, 300 and 302 with amino-alkyl chains and benzylic substitution. One example was also selected in which the ligand 283 had shown no activity towards the bound ethidium (Figure 38).

**Ethidium displacement activity of TBDMS-derivative 299**

The graph below shows the fluorescence intensity caused by adding ligand 299 in 5 µL aliquots. First 20 µL then 10 µL aliquots were added but there was abrupt decrease in the intensity; therefore smaller quantities of ligand were added to displace the bound ethidium by 50%. The C_{50} value was calculated to be 5.0 µM. This shows that the triaryl compounds have strong binding, and the presence of bulky group at benzylic position increases for ethidium displacement.

![Graph showing fluorescence intensity](image)

**Figure 37.** Fluorescence-detected binding of ligand 299 to poly AT DNA.

**Ethidium displacement activity of derivative 283**

The majority of the initial benzamides that were tested had no effect on the intensity of fluorescence, even upon addition of larger 20 µL aliquots. The only observed change on the
resultant fluorescence was due to a dilution effect. In some minor cases, however, this result may have been due to the limited solubilities of some of the drugs in the given buffers. Figure 38 shows a representative example of a ligand, in this case compound \textbf{283}, which showed no binding to DNA in this assay. After the addition of many aliquots, no noticeable change was observed in the fluorescence intensity. Therefore, no further concentrations were tried to find out the C\textsubscript{50} values of these compounds.

\textbf{Figure 38}. Fluorescence-detected binding of ligand \textbf{283} to poly AT DNA.
Summary of the ethidium displacement assays

Ethidium displacement assays were used for 37 benzamides, including those unsubstituted at the benzylic position, those with substitution, and those with different amino-alkyl groups in the side chain, in the SHE and acetate buffers. Initial experiments were carried out with CT DNA and the final experiments were carried out with poly AT DNA. Most of the compounds have shown no competitive displacement assays, only compounds 261, 299, 300 and 302 showed C50 values below 1 mM. The results suggest that the presence of an amino-propyl group, with larger groups at the benzylic position along with three aromatic rings, is most favourable in the displacement of the ethidium from DNA, probably through their strong DNA binding as a result of multiple hydrogen and electrostatic bondings and distortion of the DNA due to a larger benzylic group.
5.4 MALDI experiments

After screening in ethidium displacement assays, our next aim was to investigate the alkylating activities of different alkylating groups at the benzylic position using a mass spectrometric technique called matrix-assisted laser desorption ionisation mass spectrometry (MALDI). This technique is a soft ionisation method and can be used for the analysis of large molecules such as protein and DNA drug complexes.¹⁵⁶

Depending on the nature of alkylating groups at the benzylic position and the chain length of the ligand and DNA, different ratios of drug to four types of DNA were prepared. Stock solutions of drug-DNA and matrix were prepared using the reported methods,¹⁵⁶ as discussed in the experimental section (Chapter 6, section 6.9.3).

Ten derivatives were selected for the MALDI experiments to investigate their alkylating activity at the benzylic position with A₂T₂, A₃T₃, G₂C₂ and A₆T₆ DNA, using different ligand to DNA ratios. The base sequence of each DNA, along with their molecular weights is given in Table 24.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Type of DNA</th>
<th>Abreviated name</th>
<th>Mol. Wt*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>d(CGCGAATTCGCG)₂</td>
<td>A₂T₂</td>
<td>3833</td>
</tr>
<tr>
<td>2</td>
<td>d(CGCAAAATTTGCG)₂</td>
<td>A₃T₃</td>
<td>3656</td>
</tr>
<tr>
<td>3</td>
<td>d(ATATGGCCATAT)₂</td>
<td>G₂C₂</td>
<td>3650</td>
</tr>
<tr>
<td>4</td>
<td>d(CGCAAAAAAGCG).d(CGCTTTTTTGCG)</td>
<td>A₆T₆</td>
<td>3629</td>
</tr>
</tbody>
</table>

*As observed in mass spectrum with ± 5.0 calibration error.

After analysing ligand-drug complexes, our next step was to interpret the mass spectrum, which is usually complicated. This is especially true when one is interested in finding out the exact position of alkylation on the DNA sequence. This requires extensive analysis of the many unpredicted fragments due to the large size of drug-DNA complexes. In our cases, due to the nature of alkylating groups, which are comparatively new in this novel class of benzamides, we were first interested to find out the extent of alkylation of DNA molecules. The binding activities of most of our compounds were originally investigated with different types of AT DNAs which have
variations in the sizes of their minor grooves because of their AT-rich sequences, but we also used GC-rich DNA during our experiments. A summary of the experiments can be found in Table 25.
Table 25. Summary of the MALDI experiments.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>DNA-ligand ratio</th>
<th>Results and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="180.png" alt="Structure" /></td>
<td>A2T2 1:50, G2C2 1:50</td>
<td>Depurination and fragmentation of DNA, however no adduct was observed.</td>
</tr>
<tr>
<td>2</td>
<td><img src="186.png" alt="Structure" /></td>
<td>A2T2 1:50, G2C2 1:50</td>
<td>Depurination and fragmentation with A2T2. No reaction with G2C2. No adduct was observed.</td>
</tr>
<tr>
<td>3</td>
<td><img src="187.png" alt="Structure" /></td>
<td>A2T2 1:50, G2C2 1:50</td>
<td>Depurination with A2T2 and no reaction with G2C2.</td>
</tr>
<tr>
<td>4</td>
<td><img src="212.png" alt="Structure" /></td>
<td>A2T2 1:50, 100, G2C2 1:50, 100, A3T3 1:100</td>
<td>Extensive reaction and fragmentation of A2T2 and A3T3 however no reaction with G2C2.</td>
</tr>
<tr>
<td>5</td>
<td><img src="213.png" alt="Structure" /></td>
<td>A2T2 1:50, G2C2 1:50</td>
<td>No reactivity towards both DNAs.</td>
</tr>
<tr>
<td>6</td>
<td><img src="183.png" alt="Structure" /></td>
<td>A2T2 1:5, 10, A3T3 1:5, 10, A6T6 1:5, 10, G2C2 1:5</td>
<td>No reaction with A2T2, A3T3 or G2C2 DNA. Depurination of A6T6 but no adduct formed.</td>
</tr>
<tr>
<td>7</td>
<td><img src="192.png" alt="Structure" /></td>
<td>A2T2 1:5, 10, A3T3 1:5, 10, A6T6 1:5, 10</td>
<td>Possible adduct formations with A6T6. Insignificant reaction with A2T2 and G2C2.</td>
</tr>
<tr>
<td>8</td>
<td><img src="241.png" alt="Structure" /></td>
<td>A2T2 1:5, A3T3 1:5</td>
<td>Small amount of drug-DNA adduct was observed.</td>
</tr>
<tr>
<td>9</td>
<td><img src="298.png" alt="Structure" /></td>
<td>A2T2 1:10, A3T3 1:10, 20, A6T6 1:10, 20, G2C2 1:10, 20</td>
<td>Some evidence of Drug-DNA adduct with all DNAs and DNA fragmentation with G2C2.</td>
</tr>
<tr>
<td>10</td>
<td><img src="303.png" alt="Structure" /></td>
<td>A2T2 1:2, A3T3 1:2</td>
<td>Degradation of A2T2 and A3T3 observed.</td>
</tr>
</tbody>
</table>
When a ligand alkylates DNA, a new drug-DNA complex is expected to appear in the region above the molecular ion peak of DNA. The mass of this fragment should be equal to DNA plus the molecular ion of the ligand, minus the leaving group, such as mesylate or halogen. DNA fragmentation is important and can be used to determine exact sites of alkylation. It results in fragments of smaller mass; however the lower mass region in the spectra is very complex and also includes matrix peaks. As we were interested in determining whether the chosen ligands reacted with the DNA, an extensive analysis was not conducted.

Initially, the five diaryl derivatives, with benzylic functional groups, namely methanesulfonate 182, chloride 186 and azide 187 and α-haloacetamides 212 and 213, were tested. In the experiments with these compounds, more ligand molecules per duplex were used with A2T2 and G2C2 DNA due to the compound’s comparatively smaller size. Derivatives 182, 186, 212, 213 are expected to react in standard electrophile-nucleophile reaction. Azides are known to be reactive through production of an active nitrene species, either by photolysis or thermal activation.204

Complexes of mesylate 182 were formed with two types of DNA, namely A2T2 and G2C2, in a 50:1 ratio of ligand molecules per base pair of duplex. The resultant mass spectra with A2T2 DNA showed only peaks for the depurination (deadenylation or deguanylation) product as a result of glycosidic bond cleavage, which usually arises due to the alkylation of purines (adenine or guanine) at the N3 and N7 positions. Small peaks for the DNA fragmentation were also seen in the spectrum, which suggested its reactivity at the given stoichiometric ratio, while no molecular ion peak was seen with G2C2 DNA. Azide 187 is comparable in size to mesylate 182 and therefore it was also used in a 50:1 ratio with both A2T2 and G2C2 DNA (Entry 2). Again, depurination was observed with A2T2, with very small fragmentation of DNA molecules, while for G2C2 DNA, no useful peak related to the adduct and fragmentation was not observed in the spectra.

The chloride 186 also showed low reactivity, and depurination was only observed with A2T2 DNA, with another small peak at m/z 1826 resulting from pure DNA fragmentation. No peaks showing complexation were observed. With G2C2 DNA, only the molecular ion peak at m/z 3651 was observed; no fragments indicating alkylation were observed (Entry 3).
We were especially interested in the compounds that had the α-haloamide group at the benzylic position. This functional group has the possibility of reaching deeper into the DNA minor grooves to alkylate and has not been reported previously in DNA alkylating activities. Both the bromoacetamide 212 and the chloroacetamide 213 were expected to have comparatively good alkylating activity; therefore bromoacetamide 212 was tested in a 100:1 ratio with A2T2 DNA. The resultant mass spectrum showed intense fragmentation for DNA ranging from m/z 2923 to m/z 454 (Entry 4). Molecular ion peak was observed when used in 50:1 ratios with A2T2 DNA. No molecular ion peak was observed for A3T3 DNA in 100:1 ratios. This observation with A3T3 DNA suggested the very high reactivity of bromoacetamide 212, which may have reacted extensively with the DNA resulting in degradation. When adenine 214 was reacted with bromoacetamide 212 at 50 °C in the laboratory (see section 3.4.1), only trace amounts of reaction was observed, highlighting the differences in the environments of the two systems. However, when 212 was reacted with G2C2 DNA in 50:1 and 100:1 ratios, only the molecular ion peaks for unreacted DNA were observed, which suggested its non-reactivity in the given ratios in DNA with wider grooves.

Surprisingly, when reacted with A2T2 or G2C2 DNA in a 50:1 ratio, chloroacetamide 213 gave only molecular ion peaks with no useful fragmentation and showing a vast reduction in activity when compared to bromoacetamide 212 (Entry 5).

Due to an additional acetamide group, which should improved DNA-drug interactions on mesylate 183, complexes were prepared with all the four DNAs but used in lower ratios (Entry 6). Molecular ion peaks for A2T2, A3T3 and G2C2 DNA were observed in 5:1 and 10:1 ratios, with insignificant DNA fragmentation. Using A6T6 with mesylate 183, in a 5:1 ratio, suggested the production of deadenylated DNA from the alkylation of adenine A5 d(CGCAAAAAAGCG) at the N3 position in the DNA sequence.156 This fragmentation pattern shows the reaction of DNA with the drug but no clear evidence of a drug-DNA adduct was observed. With A6T6 in a 10:1 ratio, again no evidence was found of a drug-DNA adduct; however, DNA fragmentation was observed, which is unusual and may be due to a depurination effect.

The complex of azide 192 with A2T2 when used in a 10:1 ratio gave only the molecular ion peak along with some unrelated peaks at m/z 3887 and m/z 4017, which cannot be explained as the
desired drug-DNA adduct. Using A2T2 DNA complex in a 5:1 ratio gave small fragmentation peaks which showed some evidence of drug-DNA adduct formation. Therefore, the same experiment was repeated but the resultant peaks were determined not to be significant from alkylation. Using the drug-A6T6 complex with 5:1 and 10:1 ratios, a drug-DNA adduct was observed in both cases, suggesting the compound’s reactivity towards the A6T6 DNA. The azide 192 was then used in a 5:1 ratio with G2C2 DNA. Again there was no drug-DNA adduct and no fragmentation of DNA, which showed the non-reactivity of compound 192 with this DNA.

When chloride 261 was reacted with A2T2 and A3T3 DNA in a 5:1 ratio the mass spectrum exhibited an unrelated peak at m/z 4080 but no DNA fragmentation was observed in the spectra. The same compound 261 was also treated with A3T3, in which the molecular peak and the presence of a small adduct peak showed its alkylating activity (Entry 8).

Chloride 298, which has an additional aromatic ring, was investigated with four DNAs in different stoichiometric ratios. When the experiment was performed for the first time with A3T3 in a 1:10 ratio, a small peak was observed for the drug-DNA adduct. Therefore, the experiment was repeated, but this gave only the molecular ion peak at m/z 3653 with no drug-DNA adduct ion peak or useful DNA fragmentations (Entry 9). Using A6T6 in a 10:1 ratio resulted in a very small peak for the drug-DNA adduct. Only the molecular ion peak was seen when the same ligand was studied with A6T6 in a 20:1 ratio. Chloride 298 with G2C2 mainly gave the molecular ion peak at 3650, with a small peak for an adduct when used in a 10:1 ratio. The same complex in a 20:1 ratio furnished the molecular ion peak and an adduct ion peak, with some insignificant DNA fragmentation. These results suggested the compound’s reactivity but its alkylating activity was considered very minor compared to previously reported molecules; therefore further experiments were not performed.156

The triaryl compound 302, which was expected to have stronger binding due to its multiple sites for possible hydrogen bonding and hence improved alkylating activity, was tested with A2T2 in a 50:1 ratio (Entry 10). The mass spectrum showed no DNA molecular ion or drug-DNA adducts in the spectra; however numerous lower fragmentation peaks were observed at the given stoichiometric ratio. When the same compound was tested with A3T3, again neither molecular ion peak nor useful fragmentation peaks were observed. These two results indicated that the
compound is probably very reactive and had potentially degraded the A2T2 and A3T3 DNA in the given ratio.

**Summary of the MALDI experiments**

The MALDI technique was used with ten selected compounds to investigate the alkylating activities of potential benzylic substitution/group. Four different DNA, namely A2T2, A3T3, G2C2 and A6T6 DNA, were used in different ligand to DNA ratios for the analysis due to their having different sizes of minor grooves. It was found that compound 298 had the greatest measurable alkylating activity, although this was only minor. The remaining derivatives either did not react with DNA or, in the case of compounds 212 and 302, were very reactive and resulted in degradation of the DNA. In the case of these two compounds, whilst extensive alkylation is presumed to have occurred, no useful peaks were observed, so we were unable to draw any conclusions about the actual alkylation reactions.

**5.5 NCI results**

Along with the physical measurements that we conducted on the synthesised ligands, the compounds were also sent to the NCI for cytotoxicity studies. At the time of writing, only the results for 17 compounds are available for discussion. Compounds submitted to the NCI are tested against 60 common cancer cell lines. Initially compounds are screened at a single concentration of $10^{-5}$ M towards a panel of 60 human cancer cell lines derived from different cancer types including leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancer. The list of compounds tested for antiproliferative assays against the 60 human cancer cell lines is given in Figure 39. The detailed antiproliferative data for each compound is given in the Appendix.
Figure 39. List of derivatives tested against US NCI 60 human cancer cell lines.
The mean growth %, range of growth % and the growth inhibition % vs control cell line are shown in Table 26. Among the derivatives, four compounds, 187, 213, 300 and 302, were found comparatively more active and therefore were selected by the NCI for further testing at five concentrations of $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ and $10^{-8}$ M against the various cancer cell lines. The preliminary antiproliferative data of diol 184 showed weak selectivity, having a mean growth % of 105.23 against a broad spectrum of human cancer cell lines. The benzyl alcohol 172 also showed weak selectivity against most of the experimental cell lines, with the growth of the most sensitive cell line being 17.82% of the control against leukemia CCRF-CEF. The lowest mean growth %, 83.47, was found for compound 213. Diaryl derivatives including 254, 257, 290 and 291 having both open and cyclic amino-alkyl chains showed a mean growth % of 102.42 to 105.50 for 261 and 290 respectively. The lowest growth of the most sensitive cell line was found to be 68.68% for 257 against renal cancer UO-31 (Table 26). No apparent trend in selectivity was observed due to the nature of the benzyl group and due to the position of the amino side chain on the aromatic ring systems. Two derivatives, 282 and 283, which have shorter amino-alkyl chain lengths, were also observed to have weak antiproliferative activities. Compound 282, with a growth inhibition % of 4.14 against a lung cancer cell line, had the lowest selectivity of the series. These observations from NCI reinforce the data from the UV melting point analysis, where benzamides with the shorter amino-alkyl chains had shown small shifts in their melting points due to very little interaction with DNA. Three derivatives, 294, 296 and 298, which have a triaryl system without amino-alkyl chains, were found to have a mean growth % from 104.06 to 107.61, with comparably weak antiproliferative activities against the various cell lines. To find out the role of the amino-alkyl chain in selectivity, derivative 299 was also tested. Compound 299 was found to be the most active compound against a single cell line (89% vs Leukemia SR); however, this compound was not selected for further 5-dose testings. We are unsure why compound 299 was not selected for further testings but the NCI have complex guidelines for choosing which compounds are assessed further. On the other hand, compounds 300 and 302 which had higher mean % inhibition were selected for 5-dose testings. Compound 299 showed a broad spectrum of selectivity and had comparatively more activity towards five cell lines, with growth inhibition of 89.00%, 83.33%, 83.00%, 74.85%, 65.22% and 58.84%. Compounds 299, 300 and 302 were also found to have the strongest ethidium displacement activity among the series. This suggests that their activity is probably due to their strong interaction with DNA due to the increased amount of hydrogen bonding interaction with DNA. The role of the amino-alkyl chain can also be easily
understood from the large difference in antiproliferative activity observed in compounds having the amino-alkyl group. The lowest mean growth % of 83.47 was found for compound 213 with a range falling from 118.92 to 11.0 against leukemia SR, while the highest mean growth % of 107.61 was observed for compound 298.

### Table 26. Anticancer screening data of tested compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compd #</th>
<th>Mean growth (%)</th>
<th>Range of growth (%) over 60 cell lines</th>
<th>Growth inhibition % vs control cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>172</td>
<td>104.89</td>
<td>123.48 to 82.18</td>
<td>17.82 (leukemia CCRF-CEM)</td>
</tr>
<tr>
<td>2</td>
<td>184</td>
<td>105.23</td>
<td>129.18 to 90.24</td>
<td>9.76 (leukemia CCRF-CEM)</td>
</tr>
<tr>
<td>3</td>
<td>187</td>
<td>87.05</td>
<td>107.00 to 75.00</td>
<td>23.00 (renal cancer VO-31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.00(lung cancer NCI-H522)</td>
</tr>
<tr>
<td>4</td>
<td>213</td>
<td>83.47</td>
<td>116.00 to 17.00</td>
<td>41.00 (leukemia, CCRF-CEM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>83.00 (melanoma LOX IMV1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41.00 (renal cancer VO-31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45.00 (breast cancer MDA-MB-468)</td>
</tr>
<tr>
<td>5</td>
<td>254</td>
<td>103.66</td>
<td>129.29 to 87.81</td>
<td>12.19 (CNS cancer SNB-75)</td>
</tr>
<tr>
<td>6</td>
<td>257</td>
<td>104.53</td>
<td>125.66 to 68.68</td>
<td>31.32 (renal cancer UO-31)</td>
</tr>
<tr>
<td>7</td>
<td>261</td>
<td>105.50</td>
<td>139.36 to 81.50</td>
<td>18.50 (breast cancer MCF7)</td>
</tr>
<tr>
<td>8</td>
<td>282</td>
<td>106.99</td>
<td>135.61 to 95.81</td>
<td>4.14 (lung cancer-NCl-H226)</td>
</tr>
<tr>
<td>9</td>
<td>283</td>
<td>105.36</td>
<td>130.16 to 87.01</td>
<td>12.99 (CNS cancer SF-295)</td>
</tr>
<tr>
<td>10</td>
<td>290</td>
<td>102.42</td>
<td>150.66 to 86.58</td>
<td>13.42 (ovarian cancer OVCAR-4)</td>
</tr>
<tr>
<td>11</td>
<td>291</td>
<td>104.60</td>
<td>144.83 to 89.38</td>
<td>10.62 (leukemia CCRF-CEM)</td>
</tr>
<tr>
<td>12</td>
<td>294</td>
<td>104.86</td>
<td>139.06 to 87.38</td>
<td>12.62 (leukemia CCRF-CEM)</td>
</tr>
<tr>
<td>13</td>
<td>296</td>
<td>104.06</td>
<td>125.66 to 68.68</td>
<td>31.32 (ovarian cancer OVCAR-4)</td>
</tr>
<tr>
<td>14</td>
<td>298</td>
<td>107.61</td>
<td>128.16 to 90.81</td>
<td>9.19 (breast cancer MDA-MB-468)</td>
</tr>
<tr>
<td>15</td>
<td>299</td>
<td>84.73</td>
<td>118.92 to 11.00</td>
<td>89.00 (leukemia SR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>58.84 (leukemia CCRF-CEM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>83.33 (leukemia K-562)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>83.00 (melanoma MALME-3M)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65.22 (ovarian cancer OVCAR-4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74.85 (breast cancer MCF7)</td>
</tr>
<tr>
<td>16</td>
<td>300</td>
<td>92.46</td>
<td>111.00 to 73.00</td>
<td>27.00 (CNS cancer DNB-75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27.00 (breast cancer T-47D)</td>
</tr>
<tr>
<td>17</td>
<td>302</td>
<td>101.20</td>
<td>125.00 to 66.00</td>
<td>44.00 (CNS-SF-295)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29.00 (breast cancer MCF7)</td>
</tr>
</tbody>
</table>
Figure 40. The selected NCI 60 cell screening data for compound 299.

* Bars to the right of zero represent cell lines more sensitive to the tested compound while the bars to the left of zero represent cell lines resistant to the tested compound. The data shows its highest selectivity against leukemia SR among the 60 human cancer cell lines.

Derivatives 187, 213, 300 and 302 were selected for further 5-dose testings in cell growth inhibition activity and their activities have been compared. The mean growth inhibition \( \text{GI}_{50} \) for all four compounds varied from 22.9 \( \mu \text{M} \) to 93.3 \( \mu \text{M} \) (Table 27). The lowest \( \text{GI}_{50} \) and TGI values were for 213 and this indicated stronger cell growth inhibitory activities than the remaining three compounds 187, 300 and 302. Compound 300 showed the lowest \( \text{LC}_{50} \) values of 75.9 \( \mu \text{M} \). This high activity can be attributed to the presence of a strong alkylating group which alkylates the
DNA or other vital cell components. Although 302 showed non-significant activity against the various cell lines, it showed the highest GI50, TGI and LC50 values against the leukemia CCRF-CEM cell line.

**Table 27.** Anticancer screening data for the four most active compounds 187, 213, 300 and 302.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound #</th>
<th>GI50 MID (µM)</th>
<th>TGI MID (µM)</th>
<th>LC50 MID (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>187</td>
<td>87.1</td>
<td>□ 100</td>
<td>□ 100</td>
</tr>
<tr>
<td>2</td>
<td>213</td>
<td>22.9</td>
<td>58.9</td>
<td>89.1</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>72.4</td>
<td>75.9</td>
<td>75.9</td>
</tr>
<tr>
<td>4</td>
<td>302</td>
<td>93.3</td>
<td>97.7</td>
<td>100</td>
</tr>
</tbody>
</table>
*Growth inhibition activity of compounds 213 and 300 against a panel of 60 human cancer lines. Logarithm of 50% growth inhibition ($GI_{50}$) values for each cell line is indicated. In the plots a column to the right of zero shows the sensitivity of the given cell line towards right derivatives 300 and a column to the left of zero indicates resistance to the derivative.*
Figure 42. Growth inhibition activity of compound 302 (right) and 187 (left).

* Growth inhibition activity of compounds 302 and 187 against a panel of 60 human cancer lines. Logarithm of 50% growth inhibition (GI50) values for each cell line is indicated. In the plots a column to the right of zero shows the sensitivity of the given cell line towards right derivatives 187 and a column to the left of zero indicates resistance to the derivative.
Summary

At the time of writing, 17 derivatives had been selected for testing of their anticancer activities against 60 human cancer cell lines derived from various cancer types including leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancers. All the selected derivatives were tested according to the US NCI protocol. Preliminary testing at a single concentration showed that 4 derivatives, \(187, 213, 300\) and \(302\), were more active and were therefore selected for further testing. Among the 12 compounds, the triaryl compound \(299\), which has a TBDMS group at the benzylic position, was found to have a broad spectrum of activity against three types of leukemias (CCRF-CEM, K-562 and SR), melanoma (MALME-3M), ovarian cancer (OVCAR-4) and breast cancer (MCF7). Growth of 89% in the most sensitive cell line was observed for the TBDMS derivative \(299\) and the least growth was observed for hydroxy derivative \(282\) (4.14%).

Among the four derivatives, which were selected for further testing, compound \(213\) was found to be the most selective against a broad spectrum of cell lines. The mean GI\(_{50}\) for compounds ranged from 22.9 to 93.3 \(\mu\)M. Compound \(213\) had the lowest mean GI\(_{50}\) value of 22.9 \(\mu\)M, indicating it that it had stronger cell-growth-inhibitory activity than the other three compounds \(187, 300\) and \(302\).
5.6 Summary of biological studies

The DNA binding, alkylating and cytotoxic activities of ligands were investigated using different analytical techniques. In DNA melting analysis and ethidium displacement assays, the ligands varied with respect to the amino-alkyl chain, benzylic position, as well as in their curvature due to the number and substitution of aromatic rings present in the system. The data revealed that the presence of the longer dimethylaminopropyl side chain generated better binding than shorter amino-alkyl chains. This indicates that greater spacial distance between the amide and basic amine functionality allows for increased electrostatic interaction with DNA. The additional aromatic ring resulted in further stabilisation of the complex with DNA and its resultant increase in Tm value. Ethidium displacement assays from 37 benzamides, including those unsubstituted at the benzylic position, those with substitution, and those with different amino-alkyl groups in the side chain, showed that most of the derivatives have no competitive displacement assays; only compounds 299, 300, 302 and 261 showed C50 values below 1 mM. The results from the ethidium assays also suggested that the combination of a longer amino-alkyl group with a larger group at the benzylic position and more aromatic rings was needed for maximum binding, probably as a result of stronger hydrogen and electrostatic bondings.

The one-dose preliminary cytotoxicity data against 60 human cancer cell lines derived from various cancer types including leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers showed that 4 derivatives, 187, 213, 300 and 302, were more active and were therefore selected for 5-dose testing. The data also revealed that the same triaryl derivatives, which have longer amino-alkyl chains and large benzylic substitution such as chloride 302, had greater activity in all the assays (DNA melting point, NCI, Ethidium, MALDI) that we performed. The mean GI50 value of 22.9 µM indicated that compound 213 had stronger cell growth inhibitory activity, which is probably due to the very reactive α- chloroacetamide at the benzylic position.

The MALDI technique was only used with 11 selected derivatives to investigate the alkylating activities of potential benzylic substitutions. Four different DNAs, namely A2T2, A3T3, G2C2 and A6T6, were used in different ligand-to-DNA ratios for the analysis, due to their having different sizes of minor grooves. It was found that compound 298 had the greatest alkylating activity, although this was considered only minor. Compound 302, which had good activity in the
NCI screening, was discovered to be very reactive in the MALDI experiments, which led to the complete degradation of DNA, suggesting a very strong alkylating activity. The remaining derivatives either did not react with DNA or were very reactive and resulted in degradation of the DNA. In case of the very reactive ligands, no useful peaks were observed. We were therefore unable to draw any conclusion as to the sites of reaction.

5.7 Overall summary and conclusion

Non-symmetrical and symmetrical di- and triaryl MGB were prepared via new routes, derived from nitrobenzenes that are compatible with azide, amine, ether and chloride functional groups. Coupling conditions were optimised for acids and amines compatible with an O-TBDMS protecting group. A series of benzamides with different shapes, curvatures and altered amide linkages were prepared and a range of alkylating groups were incorporated at the benzylic position, though a desired nitrogen mustard was found to be incompatible in this molecular framework. To increase the electrostatic interaction of ligands with DNA and their solubility in buffers, a number of protonable short and long cyclic/open amino-alkyl groups were also introduced. All derivatives were characterised using different analytical techniques such as FTIR, NMR and HRMS. A number of compounds were crystalline solids and X-ray structures were determined, which allowed examination of their hydrogen bonding.

DNA binding and alkylating activities were investigated using different analytical techniques, such as UV melting point analysis, competitive ethidium displacement assays, and mass spectrometric techniques. The binding activities in both UV melting point analyses and ethidium displacement assays with poly AT DNA showed increased activities due to the presence of longer amino-alkyl chains, more aromatic rings, amide bonds and a large benzylic substitution. In the MALDI experiments testing the alkylating ability of selected ligands, the compounds showed low reactivity or were very reactive resulting in degradation of the sample DNA.

At the time of writing, 17 derivatives have been tested for anticancer activities against human cancer cell lines. Preliminary testing at single concentration showed that 4 derivatives were
more active. Among the compounds which were not selected for 5-dose testing, the TBDMS-protected triary derivative had a broad spectrum of high selectivities against selected cell lines. Four selected derivatives were also tested at different concentrations and these showed some good antiproliferative activities against the selected cell lines.

5.8 Future directions

After the successful establishment of new and efficient routes to prepare di- and triary symmetrical and non-symmetrical oligoamides, the synthesis of a library of benzamides, and the conducting of biological studies to understand the basic requirements for maximum binding selectivity in this novel class of benzamide, the incorporation of known alkylating groups such as bromoacrylic moiety and nitrogen mustards remains to be investigated. DNA binding and alkylating activities can be improved by tuning the best combinations. Although we have discovered that the bromoacrylamide and nitrogen mustards could not be added to the benzylic position, it should be more easily introduced to an aniline.

The proposed synthesis would be the coupling of acid 167 and dinitroaniline to furnish compound 303. Compound 303 can be easily reduced to aniline 304 under catalytic hydrogenation. Different known alkylating moieties such as bromoacryloyl 305 or nitrogen mustards can then be easily introduced using coupling reagents or by the reaction of corresponding acid chloride of 2-bromoacrylic acid with thionyl chloride.

In this work, we discovered that larger groups at the benzylic position were more active than smaller groups (eg. hydroxyl group) or unsubstituted groups. Therefore, large groups could be introduced at the benzylic position in compound 306 and the effect of increased size on the activity of the compounds investigated.
Chapter 5: Biological Studies

Reagents and conditions: i. 3,5-dinitroaniline, ii. 10% Pd/C, H₂; iii. 2-bromoacrylic acid.

Scheme 84.
Chapter 6

Experimental
6.1 General details

1. Melting points were determined using an Electrothermal melting point apparatus, are reported in degrees Celsius (°C), and are uncorrected.

2. Infrared (IR) absorption spectra were obtained using a Perkin Elmer Spectrum 1000 FT-IR spectrometer with absorption peaks expressed in wavenumber (cm\(^{-1}\)) and recorded using a range of 450 to 4000 cm\(^{-1}\).

3. NMR spectra were recorded in deuterated chloroform, deuterated methanol or deuterated DMSO on either a Bruker BRX300 spectrometer operating at 300 MHz for \(^1\)H nuclei and 75 for \(^13\)C nuclei or on a Bruker DRX400 spectrometer operating at 400 MHz for \(^1\)H nuclei and 100 MHz for \(^13\)C nuclei at ambient temperature. Chemical shifts are reported as parts per million (ppm) from tetramethylsilane (δ = 0) and were measured relative to the solvent in which the sample was analysed (CDCl\(_3\): δ 7.25 for \(^1\)H NMR, δ 77.0 for \(^13\)C NMR, CD\(_3\)OD: δ 4.84 for \(^1\)H NMR, δ 49.05 for \(^13\)C NMR, (CD\(_3\))\(_2\)SO: δ 2.49 for \(^1\)H NMR, δ 39.7 for \(^13\)C NMR). The coupling constant (\(J\)) is reported in Hertz (Hz). \(^1\)H NMR data is reported as chemical shifts in ppm, followed by multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet, p = pentet, br = broad), relative integral, coupling constants (where applicable) and assignment.

4. Analytical thin layer chromatography (TLC) was performed using E. Merck silica gel. Compounds were visualised using ultra-violet light or by staining with the visualising agents iodine or vanillin in ethanolic sulfuric acid.

5. Mass spectra were recorded using a VG70-SE spectrometer or on a microOTOF-Q mass spectrometer.

6. Flash chromatography was carried out using 0.063-0.1 mm Reidel-de-Häen silica gel with the denoted solvent.

7. All the experiments were carried out in an oven or air dried glassware under a dry nitrogen atmosphere.

8. Tetrahydrofuran was distilled from sodium/benzophenone before use. Dichloromethane (DCM), dimethylformamide (DMF) and triethylamine were distilled from calcium hydride. Distillations were carried out under an atmosphere of dry
nitrogen. Reactions at low temperature were performed using water-ice baths, while reactions at high temperature were performed using an oil bath or heating blocks.

9. The reported yields (±1% error) are based on the best result. Many reactions were repeated several times.

10. NMR’s ran in CDCl₃/CD₃OD had one drop of CD₃OD.
6.2 Synthesis of amines

3-Amino-5-nitrobenzyl alcohol (94)\textsuperscript{123}

To a stirred solution of 3,5-dinitrobenzyl alcohol 92 (6.0 g, 30.3 mmol) in methanol (76 mL) at reflux was added aqueous ammonium sulfide (81.6 mL, 20\% w/w solution, 60.0 mmol) dropwise over a period of 30-45 minutes. The resulting dark orange solution was refluxed for 6 hours, then cooled to room temperature and stirred for a further 20 hours. The solid sulfur at the bottom of the flask was filtered through Celite\textsuperscript{®}. The filtrate was acidified to pH 1 with 2 M hydrochloric acid. The methanol was evaporated under reduced pressure and the unreacted starting material was extracted with ethyl acetate (4 × 30 mL). The aqueous layer was then basified to pH 14 with 8 M aqueous NaOH solution and was extracted with ethyl acetate (4 × 40 mL). The combined organic fractions were dried (MgSO\textsubscript{4}), filtered and the solvent was removed in vacuo to afford the title compound 94 (2.0 g, 81\%)\textsuperscript{*} as an orange solid (m.p. 85-86 °C from ethyl acetate, lit\textsuperscript{123} 91.5 °C). The product was used without further purification.

\* The yield is based on the recovered starting material (3.1 g) which was reused in the same reaction.

R\textsubscript{f} (DCM/MeOH 19:1) = 0.31; HRMS Found (EI\textsuperscript{+}): M\textsuperscript{+} 168.0533, C\textsubscript{7}H\textsubscript{8}N\textsubscript{2}O\textsubscript{3}, requires 168.0535; IR \nu\textsubscript{max}(NaCl)/cm\textsuperscript{-1}: 3378 br (N-H, O-H), 1624, 1525 (N-O), 1460, 1350 br (N-O);

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \delta 4.30 (2H, s, NH\textsubscript{2}), 4.52 (2H, s, CH\textsubscript{2}O), 4.74 (1H, t, J = 5.6 Hz, CH\textsubscript{2}OH), 6.89 (1H, br s, Ar-H), 7.28 (1H, t, J = 2.0 Hz, Ar-H), 7.42 (1H, br s, Ar-H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \delta 63.3 (CH\textsubscript{2}, CH\textsubscript{2}O), 107.7 (CH, Ar-C), 110.7 (CH, Ar-C), 118.5 (CH, Ar-C), 143.9 (quat., Ar-C), 147.8 (quat., Ar-C), 149.1 (quat., Ar-C); m/z (EI\textsuperscript{+}): 168 (M\textsuperscript{+}, 100\%), 151 (M\textsuperscript{+}-OH, 5\%), 122 (M\textsuperscript{+}-NO\textsubscript{2}, 13\%), 104 (M\textsuperscript{+}-H\textsubscript{2}NO\textsubscript{3}, 10\%), 77 (C\textsubscript{6}H\textsubscript{5}, 38\%).\textsuperscript{123}

General procedure 1: Acetylation of amines or alcohols
To a stirred solution of aminobenzyl alcohol (1.0 eq.) in dry DMF (4 mL/mmol), under an atmosphere of nitrogen was slowly added acetic anhydride (3.0 eq.) and triethylamine (4.0 eq.). The resulting mixture was stirred at room temperature for 24 hours. Water was added to
quench the reaction, and the mixture was stirred for 10 minutes. The resultant precipitate was filtered, washed with water and the solvent was removed \textit{in vacuo} to give the desired product.

\textbf{3-Acetamido-5-nitrobenzyl acetate (96)$^{265}$}

![Chemical Structure](image)

The reaction was carried out according to general procedure 1, with 3-amino-5-nitrobenzyl alcohol 94 (2.0 g, 11.9 mmol) to give the \textit{title compound} 96 (2.96 g, 99\%) as a yellow solid, which was recrystallised from ethyl acetate as pale crystals suitable for single crystal analysis (m.p. 157-158 °C from ethyl acetate).

$^3$Acetamido-$^5$nitrobenzyl alcohol (95)$^{140}$

![Chemical Structure](image)

To a suspension of 3-acetamido-5-nitrobenzyl acetate 96 (2.2 g, 8.72 mmol) in ethanol (45 mL), was added a solution of sodium hydroxide (700 mg, 17.4 mmol) in water (11 mL). The resulting solution was stirred for approximately 3 hours, until it became clear, before water
(33 mL) was added. The ethanol was removed in vacuo. The aqueous phase was then extracted with ethyl acetate (3 × 45 mL) and the combined organic extracts were dried (Na₂SO₄) and filtered. The solvent was removed in vacuo to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 95 (1.8 g, 98%) as a tan solid. The solid was recrystallised from chloroform with a drop of DMSO to give transparent crystals, suitable for single crystal analysis (m.p. 177-178 °C from a separate sample, from ethyl acetate). The reaction was repeated several times and melting point was taken from a separate sample in ethyl acetate.

Rₛ (DCM/MeOH 19:1) = 0.18; HRMS Found (EI⁺): M⁺ 210.0632, C₉H₁₀N₂O₄ requires 210.0641; IR ν max (NaCl)/cm⁻¹: 3298 br (N-H, O-H), 1681 (C=O, amide), 1630, 1556 (N=O), 1529, 1457, 1418, 1348 (N-O); ¹H NMR (300 MHz, (CD₃)₂SO): δ 2.09 (3H, s, NHCOC₃H₃), 4.58 (2H, s, CH₂OH), 5.55 (1H, s, CH₂OH), 7.83 (1H, s, Ar-H), 7.85 (1H, s, Ar-H), 8.49 (1H, br s, Ar-H), 10.43 (1H, s, N-H); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 24.1 (CH₃, NHCOCH₃), 61.9 (CH₂, CH₂OH), 111.4 (CH, Ar-C), 115.0 (CH, Ar-C), 122.3 (CH, Ar-C), 140.3 (quat., Ar-C), 145.5 (quat., Ar-C), 147.9 (quat., Ar-C), 169.1 (C=O, NHCOCH₃); m/z (EI⁺): 210 (M⁺, 27%), 168 (M⁺-C₂H₂O, 100%), 43 (COCH₃, 93%).

N-(3-((tert-Butyldimethylsilyloxy)methyl)-5-nitrophenyl)acetamide (97)²⁰⁶

To a stirred solution of alcohol 95 (3.0 g, 14.3 mmol) in dry DMF (20 mL) under an atmosphere of nitrogen was added tert-butyldimethylsilyl chloride (2.58 g, 17.1 mmol) and imidazole (2.92 g, 42.8 mmol). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 5 hours. Water (50 mL) was then added and the aqueous mixture was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL) and dried (MgSO₄). The solvent was removed under reduced pressure to afford the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 97 (4.6 g, 99%) as a pale yellow solid. The compound was recrystallised from ethyl acetate/n-hexane to
give clear crystals suitable for single crystal analysis (m.p. 143-144 °C from ethyl acetate/n-hexane).

**R**<sub>f</sub> (DCM/MeOH 19:1) = 0.6; **HRMS** Found (El<sup>+</sup>): MH<sup>+</sup> 325.15862, C<sub>15</sub>H<sub>25</sub>N<sub>2</sub>O<sub>4</sub>Si requires 325.15836; **IR** ν<sub>max</sub>(NaCl)/cm<sup>-1</sup>: 3315 br (N-H), 2929 (C-H), 1666 (C=O, amide), 1628, 1532 (N-O), 1471, 1334 (N-O), 1261; **1H NMR** (400 MHz, CDCl<sub>3</sub>): δ 0.13 (6H, s, OSi(CH<sub>3</sub>)<sub>2</sub>), 0.96 (9H, s, OSiC(CH<sub>3</sub>)<sub>3</sub>), 2.24 (3H, s, NHCOCH<sub>3</sub>), 4.79 (2H, s, ArCH<sub>2</sub>O), 7.56 (1H, s, N-H), 7.91 (1H, s, Ar-H), 7.93 (1H, s, Ar-H), 8.24 (1H, s, Ar-H); **13C NMR** (100 MHz, CDCl<sub>3</sub>): δ -5.4 (2 × CH<sub>3</sub>, OSi(CH<sub>3</sub>)<sub>2</sub>), 18.4 (quat., OSiC(CH<sub>3</sub>)<sub>3</sub>), 24.6 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 25.9 (3 × CH<sub>3</sub>, OSiC(CH<sub>3</sub>)<sub>3</sub>), 63.8 (CH<sub>2</sub>, ArCH<sub>2</sub>O), 112.9 (CH, Ar-C), 116.2 (CH, Ar-C), 122.4 (CH, Ar-C), 138.8 (quat., Ar-C), 144.6 (quat., Ar-C), 148.6 (quat., Ar-C), 168.6 (C=O, NHCOCH<sub>3</sub>); **m/z** (Cl<sup>+</sup>): 325 (MH<sup>+</sup>, 43%), 295 (M<sup>+</sup>-CH<sub>2</sub>O, 100%), 267 (M<sup>+</sup>-NHCOCH<sub>3</sub>, 35%), 221 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>NO<sub>2</sub>, 32%).

**General procedure 2: Hydrogenation of nitroaromatics, azides and benzyl esters**

To a stirred solution of nitroaromatic derivatives/azide/benzyl ester (1.0 eq.) in methanol (15 mL/mmol) was added 10% palladium on carbon (50 mg) slowly, in small portions. The air inside the reaction flask was purged and the reaction mixture was stirred vigorously under an atmosphere of hydrogen for 3-5 hours, (3 hours for nitro and azide group reduction and 5 hours for benzyl esters). After completion of the reaction, the mixture was filtered through Celite<sup>®</sup> and the solvent was removed in vacuo to afford the product.

**3-Azetamido-5-aminobenzyl acetate (98)**

The reaction was carried out according to general procedure 2, with 3-acetamido-5-nitrobenzyl acetate 96 (300 mg, 1.19 mmol) to give the title compound 98 (251 mg, 95%) as a thick oil. The product was used without further purification.
R_f (DCM/MeOH 9:1) = 0.48; HRMS Found (ESI^+): MH^+ 223.1070 C_{11}H_{13}N_{2}O_{3} requires 223.1083; IR ν_{max}(NaCl)/cm\(^{-1}\): 3206 br (N-H), 1720 (C=O, ester), 1662 (C=O, amide), 1604, 1549, 1482, 1442 br, 1364, 1218, 1182; \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 2.04 (3H, s, OCOCH\(_3\)), 2.08 (3H, s, NHCOC\(_3\)), 4.06 (2H, br s, NH\(_2\)), 4.91 (2H, s, CH\(_2\)O), 6.35 (1H, br s, Ar-H), 6.71 (1H, br s, Ar-H), 7.0 (1H, br s, Ar-H), 7.90 (1H, br s, N-H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ 20.9 (CH\(_3\), OCOCH\(_3\)), 24.4 (CH\(_3\), NHCOCH\(_3\)), 66.1 (CH\(_2\), CH\(_2\)O), 106.3 (CH, Ar-C), 109.4 (CH, Ar-C), 110.8 (CH, Ar-C), 137.5 (quat., Ar-C), 139.2 (quat., Ar-C), 147.4 (quat., Ar-C), 168.9 and 171.0 (C=O, NHCOCH\(_3\)) ; m/z (ESI^+): 163 (M^+ - C\(_2\)H\(_4\)O\(_2\), 95%), 121 (M^+ - C\(_4\)H\(_6\)O\(_3\), 23%).

N-(3-Amino-5-((tert-butylidimethylsilyloxy)methyl)phenyl)acetamide (99)

The reaction was carried out according to general procedure 2, with nitro-amide 97 (1.0 g, 3.08 mmol) afforded the title compound 99 (907 mg, 100%) as a white solid (m.p. 91-92 °C from methanol).

R_f (DCM/MeOH 19:1) = 0.35; HRMS Found (EI^+): M^+ 294.1759, C_{15}H_{26}N_{2}O_{2}Si requires 294.1764; IR ν_{max}(NaCl)/cm\(^{-1}\): 3327 br (N-H), 2929 (C-H), 1667 (C=O, amide), 1620, 1562, 1444, 1373, 1256; \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 0.08 (6H, s, OSi(C\(_H_3\))\(_2\)), 0.91 (9H, s, OSiC(CH\(_3\))\(_3\)), 2.10 (3H, s, NHCOCH\(_3\)), 3.68 (2H, br s, NH\(_2\)), 4.58 (2H, s, ArCH\(_2\)O), 6.38 (1H, s, Ar-H), 6.64 (1H, s, Ar-H), 6.99 (1H, s, Ar-H), 7.48 (1H, br s, N-H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ -5.0 (2 × CH\(_3\), OSi(CH\(_3\))\(_2\)), 18.7 (quat., OSiC(CH\(_3\))\(_3\)), 24.9 (CH\(_3\), NHCOCH\(_3\)), 25.2 (3 × CH\(_3\), OSiC(CH\(_3\))\(_3\)), 65.0 (CH\(_2\), ArCH\(_2\)O), 105.5 (CH, Ar-C), 107.6 (CH, Ar-C), 108.8 (CH, Ar-C), 139.2 (quat., Ar-C), 143.7 (quat., Ar-C), 147.4 (quat., Ar-C), 168.7 (C=O, NHCOCH\(_3\)) ; m/z (EI^+): 294 (M^+ , 5%), 237 (M^+ - C\(_4\)H\(_9\), 27%), 221 (100%), 163 (M^+ - OTBDMS, 12%), 121 (22%).
General procedure 3: Synthesis of methanesulfonates

To a solution of alcohol (1 eq.) and triethylamine (1.5 eq.) in dry DMF (4 mL/mmol alcohol) at 0 °C, was added methanesulfonyl chloride (1.5 eq.) in dry DMF (1 mL) and the resulting solution was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was diluted with ethyl acetate (20 mL), washed with brine (20 mL) and dried (MgSO₄). The solvent was removed in vacuo to afford the crude product. The product was used immediately without further purification.

3-Acetamido-5-nitrobenzyl methanesulfonate (110)\(^{133}\)

![Chemical Structure](image)

The reaction was carried out according to general procedure 3, with 3-acetamido-5-nitrobenzyl alcohol 95 (1.0 g, 4.76 mmol) to afford the title compound 110 (1.3 g, 95%) as a yellow semi-solid. The product was used immediately without further purification.

**R\(_f\)** (DCM/MeOH 9:1) = 0.73; **HRMS** Found (El\(^{+}\)): M\(^{+}\) 288.0415, C\(_{10}\)H\(_{12}\)N\(_2\)O\(_6\)S requires 288.0416; **IR** \(\nu_{\text{max}}\)(NaCl)/cm\(^{-1}\): 3299 br (N-H), 1674 (C=O), 1597, 1530 (N-O), 1430, 1332 (N-O), 1256, 1169; **\(^{1}\)H NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 2.22 (3H, s, NHCOC\(_{\text{H}}\)\(_3\)), 3.11 (3H, s, OSO\(_2\)C\(_{\text{H}}\)\(_3\)), 5.27 (2H, s, ArCH\(_2\)O), 7.87 (1H, br s, Ar-H), 7.94 (1H, br s, Ar-H), 8.40 (1H, br s, Ar-H), 8.73 (1H, s, N-H); **\(^{13}\)C NMR** (100 MHz, CDCl\(_3\)): \(\delta\) 24.3 (CH\(_3\), NHCOC\(_{\text{H}}\)\(_3\)), 38.0 (CH\(_3\), OSO\(_2\)CH\(_3\)), 69.6 (CH\(_2\), CH\(_2\)O), 114.7 (CH, Ar-C), 117.8 (CH, Ar-C), 124.6 (CH, Ar-C), 136.2 (quat., Ar-C), 139.8 (quat., Ar-C), 148.5 (quat., Ar-C), 169.8 (C=O, NHCOC\(_{\text{H}}\)\(_3\) ); **m/z** (El\(^{+}\)): 288 (M\(^{+}\), 3%), 246 (15%), 186 (30%), 151 (5%), 140 (6%), 104 (10%).

**N-(3-((Bis(2-hydroxyethyl)amino)methyl) 5-nitrophenyl)acetamide (111)**

![Chemical Structure](image)
To a solution of diethanolamine (3.65 g, 34.7 mmol) in dry THF (10 mL), at 0 °C, under an atmosphere of nitrogen was added dropwise a solution of mesylate 110 (1.0 g, 3.47 mmol) in dry THF (15 mL). The resulting mixture was stirred at room temperature for 18 hours, and the solvent was removed in vacuo. The residue was diluted with ethyl acetate (45 mL) and 2 M hydrochloric acid (45 mL). The aqueous layer was separated, neutralised with 4 M sodium hydroxide solution, and extracted with ethyl acetate (2 × 60 mL). The combined organic extracts were dried (MgSO₄) and the solvent was removed in vacuo to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 111 (0.87 g, 84%) as a yellow solid (m.p. 151-152 °C from ethyl acetate). The product was separately recrystallised from ethyl acetate for single crystal analysis.

R₇ (DCM/MeOH 9:1) = 0.56; HRMS Found (FAB⁺): MH⁺ 298.1404, C₁₃H₂₀N₅O₅ requires 298.1403; IR v_max(NaCl)/cm⁻¹: 3238 br (N-H, O-H), 2827 (C-H), 1667 (C=O, amide), 1558, 1530 (N-O), 1336 (N-O), 1281; ¹H NMR (400 MHz, CD₃OD): δ 2.13 (3H, s, NHCOC₃H₃), 2.70 (4H, t, J = 5.8 Hz, N(C₂H₂CH₂OH)₂), 3.62 (4H, t, J = 5.8 Hz, N(CH₂C₆H₄OH)₂), 3.80 (2H, s, ArC₆H₄N), 7.82 (1H, s, Ar-H), 7.97 (1H, s, Ar-H), 8.45 (1H, t, J = 1.8 Hz, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ 24.0 (CH₃, NHCOCH₃), 57.5 (2 × CH₂, N(CH₂CH₂OH)₂), 59.9 (CH₂, ArCH₂N), 60.7 (2 × CH₂, N(CH₂CH₂OH)₂), 114.2 (CH, Ar-C), 119.8 (CH, Ar-C), 126.7 (CH, Ar-C), 141.1 (quat., Ar-C), 143.5 (quat., Ar-C), 150.0 (quat., Ar-C), 172.0 (C=O, NHCOCH₃); m/z (FAB⁺) 298 (MH⁺, 100%), 266 (23%).

N-(3-Amino-5-methylphenyl)acetamide (112)

The reaction was carried out according to general procedure 2, with nitro-alcohol 111 (50 mg, 0.17 mmol) to give a crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 112 (28 mg, 100%) as a clear viscous oil.

R₇ (DCM/MeOH 9:1) = 0.01; HRMS Found (EI⁺): M⁺ 164.0946, C₉H₁₂N₂O requires 164.0950; IR v_max(NaCl)/cm⁻¹: 3358 (N-H), 2955 (C-H), 1670 (C=O), 1615, 1433, 1255; ¹H
NMR (400 MHz, CDCl$_3$): δ 2.12 (3H, s, NHCOC$_3$H), 2.21 (3H, s, ArCH$_3$), 3.63 (2H, brs, NH$_2$), 6.25 (1H, s, Ar-H), 6.50 (1H, s, Ar-H), 6.92 (1H, s, Ar-H), 7.15 (1H, brs, NHCOCH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 21.4 (CH$_3$, ArCH$_3$), 24.7 (CH$_3$, NHCOCH$_3$), 103.9 (CH, Ar-C), 110.6 (CH, Ar-C), 111.9 (CH, Ar-C), 138.7 (quat., Ar-C), 139.7 (quat., Ar-C), 147.0 (quat., Ar-C), 168.3 (C=O, NHCOCH$_3$); m/z (EI$^+$) 164 (M$^+$, 62%), 122 (C$_7$H$_{10}$N$_2$, 100%).

$N$-(3-Nitrophenyl)acetamide (115)$^{208}$

The reaction was carried out according to general procedure 1, with 3-nitroaniline 93 (3.0 g, 21.7 mmol) (1.5 eq. of Ac$_2$O and 2 eq. Et$_3$N) to afford the title compound 115 (3.23g, 82%) as a white solid (m.p. 147-148 °C from ethyl acetate, lit$^{209}$ 147-149 °C). The product was used without further purification. The product is also available commercially.

R$_f$ (DCM/MeOH 9:1) = 0.73; HRMS Found (El$^+$): M$^+$ 180.0535, C$_8$H$_8$N$_2$O$_3$ requires 180.0535; IR $v_{max}$(NaCl)/cm$^{-1}$: 3301 (N-H), 1657 br (C=O), 1614, 1532 (N-O), 1478, 1399, 1345 (N-O); $^1$H NMR (400 MHz, CD$_3$OD): δ 2.15 (3H, s, NHCOCH$_3$), 7.49 (1H, t, $J$ = 8.1 Hz, Ar-H), 7.80 (1H, d, $J$ = 8.0 Hz, Ar-H), 7.88 (1H, d, $J$ = 8.0 Hz, Ar-H), 8.56 (1H, s, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): δ 24.0 (CH$_3$, NHCOCH$_3$), 115.3 (CH, Ar-C), 119.3 (CH, Ar-C), 126.3 (CH, Ar-C), 130.9 (CH, Ar-C), 141.4 (quat., Ar-C), 149.9 (quat., Ar-C), 172.1 (C=O, NHCOCH$_3$); m/z (El$^+$):180 (M$^+$, 30%), 138 (100%), 122 (3%), 108 (7%), 92 (50%).

$N$-(3-Aminophenyl)acetamide (113)$^{210}$

The reaction was carried out according to general procedure 2, with $N$-(3-nitrophenyl)acetamide 115 (1.0 g, 5.55 mmol) to give the title compound 113 (833 mg, 100%) as a white solid (m.p. 75-76 °C from methanol, lit$^{154}$ 82-84 °C). The product is also available commercially.
Chapter 6: Experimental

R<sub>f</sub> (DCM/MeOH 9:1) = 0.69; HRMS Found (EI<sup>+</sup>): M<sup>+</sup> 150.0794, C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>O requires 150.0793; IR <sup>v<sub>max</sub></sup>(NaCl)/cm<sup>-1</sup>: 3300 br (N-H), 1660 (C=O), 1609, 1549, 1494, 1455, 1370, 1312, 1266; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 2.02 (3H, s, NHCO<sub>2</sub>H), 3.68 (2H, br s, NH<sub>2</sub>), 6.37 (1H, dd, J = 8.0, 1.8 Hz, Ar-H), 6.72 (1H, dd, J = 8.0, 1.2 Hz, Ar-H), 6.98-7.01 (2H, br m, Ar-H), 8.09 (1H, br s, N-H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ24.3 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 106.8 (CH, Ar-C), 109.9 (CH, Ar-C), 111.0 (CH, Ar-C), 129.5 (CH, Ar-C), 139.0 (quat., Ar-C), 149.1 (quat., Ar-C), 170.0 (C=O, NHCOCH<sub>3</sub>); m/z (EI<sup>+</sup>): 150 (M<sup>+</sup>, 65%), 108 (100%), 91 (5%), 80 (28%), 43 (37%).

4-(Dimethylamino)-N-(3-nitrophenyl)butanamide (117)<sup>109</sup>

A solution of 3-nitroaniline 93 (0.5 g, 3.62 mmol) in DMF (3 mL) was treated with hydrochloride salt of N,N-dimethylaminobutyric acid 116 (1.21 g, 7.24 mmol), EDC (1.39 g, 7.24 mmol) and DMAP (0.884 g, 7.24 mmol). The resulting mixture was stirred at 45 °C for 24 hours. After completion, the reaction mixture was diluted with dichloromethane:i-propanol (1:1) and washed with saturated sodium bicarbonate solution. The organic layer was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvent was removed under high vacuum. The crude product was dissolved in methanol (4 mL), silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica gel. This silica gel was then loaded and purified by flash chromatography (methanol-ammonia 9:1) to furnish the title compound 117 (0.89 g, 98%) as a yellow gum, which solidified under high vacuum.<sup>159</sup> The reaction was also repeated using the general procedure 13.

R<sub>f</sub> (MeOH/NH<sub>3</sub> 9:1) = 0.6; HRMS Found (ESI<sup>+</sup>): MH<sup>+</sup> 252.1349, C<sub>12</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub> requires 252.1348; IR <sup>v<sub>max</sub></sup>(solid)/cm<sup>-1</sup>: 3500-3100 (N-H), 2950 (C-H), 1697 br (C=O), 1602, 1524 (N-O), 1480, 1432, 1342 (N-O), 1261; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 1.95 (2H, p, J = 7.2 Hz, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.48 (2H, t, J = 7.2 Hz, NHCOCH<sub>2</sub>), 2.52 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.73 (2H, t, J = 7.2 Hz, NCH<sub>2</sub>), 7.48 (1H, t, J = 8.0 Hz, Ar-H), 7.79 (1H, d, J = 8.0 Hz, Ar-H), 7.87 (1H, d, J = 8.0 Hz, Ar-H), 8.60 (1H, br s, Ar-H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ 23.15 (CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 35.2 (CH<sub>2</sub>, NHCOCH<sub>2</sub>), 44.9 (2 × CH<sub>3</sub>, N(CH<sub>3</sub>)<sub>2</sub>), 59.4 (CH<sub>2</sub>, NCH<sub>2</sub>), 115.0
(CH, Ar-C), 119.1 (CH, Ar-C), 126.2 (CH, Ar-C), 130.7 (CH, Ar-C), 141.3 (quat., Ar-C), 149.6 (quat., Ar-C), 173.7 (C=O, NHCO); m/z (ESI⁺): 252 (MH⁺, 100%), 207 (25%), 142 (40%), 123 (3%).

**N-(3-Aminophenyl)-4-(dimethylamino)butanamide (114)**

\[
\begin{array}{c}
\text{H}_2\text{N} & \text{O} & \text{N} \\
\text{C} & \text{CH} & \text{CH} \\
\end{array}
\]

The reaction was carried out according to general procedure 2, with nitro compound 117 (622 mg, 2.48 mmol) to give the title compound 114 (548 mg, 100%) as a brown gum.

**Rf** (MeOH/NH₃ 9:1) = 0.6; **HRMS** Found (EI⁺): MH⁺ 222.1600, C₁₂H₂₀N₃O requires 222.1606; **IR** νₘₙₐₓ(gum)/cm⁻¹: 3312 br (N-H), 2943 (C-H), 1661 (C=O), 1609, 1549, 1495, 1455, 1373, 1308, 1228; **¹H NMR** (400 MHz, CD₃OD): δ 1.79 (2H, p, J = 7.6 Hz, CH₂CH₂CH₂), 2.22 (6H, s, N(CH₃)₂), 2.28 (2H, t, J = 7.6 Hz, NHCOCH₂), 2.33 (2H, t, J = 7.8 Hz, NCH₂), 6.40 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 6.77 (1H, dd, J = 8.0, 0.8 Hz, Ar-H), 6.94 (1H, t, J = 8.0 Hz, Ar-H), 6.98 (1H, s, Ar-H); **¹³C NMR** (100 MHz, CD₃OD): δ 24.1 (CH₂, CH₂CH₂CH₂), 35.6 (CH₂, NHCOCH₂), 45.3 (2 × CH₃, N(CH₃)₂), 59.8 (CH₂, NCH₂), 108.3 (CH, Ar-C), 111.0 (CH, Ar-C), 112.5 (CH, Ar-C), 130.3 (CH, Ar-C), 140.6 (quat., Ar-C), 149.4 (quat., Ar-C), 173.6 (C=O, NHCO); m/z (EI⁺): 222 (MH⁺, 100%), 177 (63%), 174 (10%), 135 (39%), 123 (36%), 114 (22%).
6.3 Synthesis of acids

6.3.1 Synthesis of monoaryl acids

General procedure 4: Protection of amine group
Using the method of Klok\textsuperscript{211} to a solution of aminobenzoic acid \textbf{129} or \textbf{130} (1 eq.) and sodium hydroxide (1.1 eq.) in water:dioxane (1:1) (1.7 mL/mmol acid) at 0 °C, was added di-\textit{tert}-butyl dicarbonate (1.8 eq.). The resultant mixture was stirred for 3 hours at room temperature. The aqueous mixture was washed with ethyl acetate (2 mL/mmol acid). Ethyl acetate (2 mL/mmol acid) was added and the resultant mixture was neutralised (1 M KHSO\textsubscript{4}). The organic layer was separated, washed with water, dried (MgSO\textsubscript{4}), filtered and the solvent was removed \textit{in vacuo} to give the crude product, which was used without further purification.

4-(\textit{tert}-Butoxycarbonylamino)benzoic acid (\textbf{131})\textsuperscript{212}

![4-(\textit{tert}-Butoxycarbonylamino)benzoic acid](image)

The reaction was carried out according to general procedure 4, with 4-aminobenzoic acid \textbf{129} (8.0 g, 58.3 mmol) to give the \textit{title compound} \textbf{131} (12.0 g, 87%) as a brown solid, which was used without further purification (m.p.190-191 °C lit\textsuperscript{161} 191-192 °C).

R\textsubscript{f} (DCM/MeOH 9:1) = 0.44; \textsuperscript{1}H NMR (400 MHz, (CD\textsubscript{3})\textsubscript{2}SO): δ 1.47 (9H, s, C(CH\textsubscript{3})\textsubscript{3}), 7.57 (2H, d, J = 8.8 Hz, Ar-H), 7.85 (2H, d, J = 8.8 Hz, Ar-H), 9.70 (1H, s, N-H), 12.56 (1H, s, COOH). Spectroscopic data was in agreement with literature values.\textsuperscript{161}

3-(\textit{tert}-Butoxycarbonylamino)benzoic acid (\textbf{132})\textsuperscript{213}

![3-(\textit{tert}-Butoxycarbonylamino)benzoic acid](image)

The reaction was carried out according to general procedure 4, with 3-aminobenzoic acid \textbf{130} (10.0 g, 72.9 mmol) to give the \textit{title compound} \textbf{132} (17.2 g, 99%) as a brown solid, which was used without further purification (m.p 186-187 °C, lit\textsuperscript{161} 189-190 °C).
Chapter 6: Experimental

R<sub>f</sub> (DCM/MeOH 19:1) = 0.32; <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 1.48 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 7.36 (1H, t, J = 8.0 Hz, Ar-H), 7.54 (1H, d, J = 8.0 Hz, Ar-H), 7.62 (1H, d, J = 8.0 Hz, Ar-H), 8.15 (1H, s, Ar-H), 9.53 (1H, s, N-H), 12.87 (1H, s, COOH). Spectroscopic data was in agreement with literature values.<sup>161</sup>

General procedure 5: Synthesis of benzyl ester

Adopting the method of Cappelletti<sup>162</sup> to a solution of acid 131 or 132 (1.0 eq.) and cesium carbonate (1.1 eq.) in dry DMF (1.0 mL/mmole) at 0 °C, under an atmosphere of nitrogen, was added benzyl bromide (1.0 eq.). The mixture was stirred at room temperature for 2.5 hours. The reaction mixture was diluted with ethyl acetate, washed with water, brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and filtered. The solvent was removed in vacuo to give the product as solid, which was used without further purification.

Benzyl 4-(tert-butoxycarbonylamino)benzoate (133)<sup>214</sup>

The reaction was carried out according to general procedure 5 with 4-(tert-butoxycarbonylamino)benzoic acid 131 (6.0 g, 25.3 mmol) to give the title compound 133 (6.7 g, 81%) as a brown solid, which was used without further purification (m.p. 147-148 °C from dichloromethane, lit<sup>214</sup> 148-150 °C).

R<sub>f</sub> (DCM/MeOH 9:1) = 0.88; HRMS Found (ESI<sup>+</sup>): (M-Na)<sup>+</sup> 350.1353, C<sub>19</sub>H<sub>21</sub>NNaO<sub>4</sub> requires 350.1368; IR ν<sub>max</sub>(solid)/cm<sup>-1</sup>: 3327 (N-H), 2992 (C-H), 1712 (C=O), 1697 (C=O), 1607, 1595, 1528, 1412, 1365, 1234, 1154; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.51 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 5.34 (2H, s, ArC<sub>H</sub><sub>2</sub>O), 6.81 (1H, s, N-H), 7.34-7.40 (2H, m, Ar-H), 7.42 (5H, m, Ar-H), 8.00 (2H, d, J = 8.8 Hz, Ar-H);<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 28.2 (3 × CH<sub>3</sub>, C(CH<sub>3</sub>)<sub>3</sub>), 66.4 (CH<sub>2</sub>, ArCH<sub>2</sub>O), 81.2 (quat., C(CH<sub>3</sub>)<sub>3</sub>), 117.3 (2 × CH, Ar-C), 124.2 (CH, Ar-C), 128.1 (quat., Ar-C), 128.1 (2 × CH, Ar-C), 128.5 (2 × CH, Ar-C), 131.0 (2 × CH, Ar-C), 136.2 (quat., Ar-C), 142.9 (quat., Ar-C), 152.2 (C=O, OCONH), 166.1 (C=O, CO<sub>2</sub>CH<sub>2</sub>); m/z (ESI<sup>+</sup>): 350 ((M-Na)<sup>+</sup>, 100%), 272 (70%), 228 (55%), 150 (4%).
Benzyl 3-(tert-butoxycarbonylamino)benzoate (134)

The reaction was carried out according to general procedure 5, with 3-(tert-butoxycarbonylamino)benzoic acid 132 (6.0 g, 25.3 mmol) to give the title compound 134 (7.5 g, 91%) as a brown solid, which was used without further purification (m.p. 149-150 °C from dichloromethane, lit215 151-153 °C).

RF (DCM/MeOH 9:1) = 0.89; HRMS Found (ESI⁺): (M-Na)⁺ 350.1357, C_{19}H_{21}NNaO_{4} requires 350.1368; IR ν_{max}(solid)/cm⁻¹: 3347 (N-H), 2990 (C-H), 1727 (C=O), 1696 (C=O), 1608 br, 1535, 1412, 1365, 1227, 1155; ¹H NMR (400 MHz, CDCl₃): δ 1.51 (9H, s, C(CH₃)₃), 5.35 (2H, s, ArCH₂O), 6.81 (1H, s, N-H), 7.34-7.36 (3H, m, Ar-H), 7.37-7.39 (2H, m, Ar-H), 7.42-7.44 (2H, m, Ar-H), 7.74 (1H, t, J = 2.0 Hz, Ar-H), 7.93 (1H, t, J = 2.0 Hz, Ar-H); ¹³C NMR (100 MHz, CDCl₃): δ 28.2 (3 × CH₃, C(CH₃)₃), 66.7 (CH₂, ArCH₂O), 80.7 (quat., C(CH₃)₃), 119.5 (CH, Ar-C), 123.0 (CH, Ar-C), 124.1 (CH, Ar-C), 128.1 (2 × CH, Ar-C), 128.5 (2 × CH, Ar-C), 128.2 (CH, Ar-C), 129.0 (CH, Ar-C), 130.7 (quat., Ar-C), 135.9 (quat., Ar-C), 138.7 (quat., Ar-C), 152.6 (C=O, OCONH), 166.1 (C=O, CO₂CH₂); m/z (ESI⁺): 350 ((M-Na)⁺, 100%), 272 (23%), 228 (4%).

General procedure 6: Deprotection of Boc group

Adopting the method of Cappelletti¹⁶² to a solution of benzyl (tert-butoxycarbonylamino)benzoates 133 or 134 (1 eq.), in dry DCM (4.5 mL/mmol benzoate) was added TFA (2 mL/mmol benzoate) and the mixture stirred at room temperature for 1 hour. The solution was quenched with ice and neutralised with sodium bicarbonate. The organic layer was collected and the aqueous layer was extracted with DCM. The organic extracts were combined and washed with saturated sodium bicarbonate solution, water, dried (Na₂SO₄) and filtered. The solvent was removed in vacuo to give the product, which was used without further purification.
Chapter 6: Experimental

**Benzyl 4-aminobenzoate (135)**

The reaction was carried out according to general procedure 6, with benzyl 4-(tert-butoxycarbonylamino)benzoate 133 (13.0 g, 39.7 mmol) to yield the title compound 135 (8.6 g, 95%) as a brown solid (m.p. 80-82 °C from dichloromethane lit216,217 82-83 °C, 88.5-89.5 °C).

\[ R_f (\text{DCM/MeOH 9:1}) = 0.80; \text{HRMS Found (ESI}^+\text{): MH}^+ 228.1023, C_{14}H_{14}NO_2 \text{ requires} \]
\[ 228.1025; \text{IR } \nu_{\text{max}}(\text{solid/cm}^{-1}) : 3358 (\text{N-H}), 1682 \text{ br (C=O)}, 1630, 1598, 1497, 1436, 1380, 1309, 1276; \]
\[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 3.90 (2\text{H, br s, NH}_2), 5.32 (2\text{H, s, ArCH}_2\text{O}), 6.63 (2\text{H, d, } J = 8.8 \text{ Hz, Ar-H}), 7.30-7.39 (3\text{H, m, Ar-H}), 7.40-7.45 (2\text{H, m, Ar-H}), 7.86 (2\text{H, d, } J = 8.8 \text{ Hz, Ar-H}); \]
\[ ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta 66.0 (\text{CH}_2, \text{ArCH}_2\text{O}), 113.7 (2 \times \text{CH, Ar-C}), 119.5 (\text{CH, Ar-C}), 128.0 (\text{quat., } 2 \times \text{CH, Ar-C}), 128.4 (2 \times \text{CH, Ar-C}), 131.7 (2 \times \text{CH, Ar-C}), 136.5 (\text{quat., Ar-C}), 150.9 (\text{quat., Ar-C}), 166.4 (\text{C=O, CO}_2\text{CH}_2\text{}); m/z (\text{ESI}^+) : 228 (\text{MH}^+, 100%). \]

**Benzyl 3-aminobenzoate (136)**

The reaction was carried out according to general procedure 6, with benzyl (tert-butoxycarbonylamino)benzoate 134 (6.5 g, 19.8 mmol) to give the title compound 136 (4.2 g, 93%) as a yellow oil from dichloromethane, which was used without further purification.

\[ R_f (\text{DCM/MeOH 9:1}) = 0.82; \text{HRMS Found (ESI}^+\text{): MH}^+ 228.1023, C_{14}H_{14}NO_2 \text{ requires} \]
\[ 228.1025; \text{IR } \nu_{\text{max}}(\text{oil/cm}^{-1}) : 3359 (\text{N-H}), 1708 (\text{C=O}), 1667, 1622, 1603, 1491, 1454, 1377, 1316, 1289; \]
\[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 3.8 (2\text{H, br s, NH}_2), 5.39 (2\text{H, s, ArCH}_2\text{O}), 6.85 \]
Chapter 6: Experimental

(1H, dd, J = 8.0, 2.4 Hz, Ar-H), 7.23 (1H, t, J = 7.8 Hz, Ar-H), 7.39 (1H, d, J = 8.0 Hz, Ar-H), 7.42 (1H, s, Ar-H), 7.44 (1H, br s, Ar-H), 7.47 (2H, br s, Ar-H), 7.49 (1H, br s, Ar-H), 7.55 (1H, dd, J = 8.0, 0.8 Hz, Ar-H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 66.2 (CH$_2$, ArCH$_2$O), 115.4 (CH, Ar-C), 119.2 (2 $\times$ CH, Ar-C), 127.8 (2 $\times$ CH, Ar-C), 127.9 (CH, Ar-H), 128.3 (2 $\times$ CH, Ar-C), 129.0 (CH, Ar-C), 130.7 (quat., Ar-C), 135.8 (quat., Ar-C), 146.5 (quat., Ar-C), 166.4 (C=O, CO$_2$CH$_2$)$_{219}$; m/z (ESI$^+$): 228 (MH$^+$, 100%), 120 (1%).

General procedure 7: Reaction of aniline with bromoacetyl bromide

To a stirred solution of aniline $^{135}$ or $^{136}$ (1.0 eq.) in dry DCM (5 mL/mmol) in an ice-acetone bath under an atmosphere of nitrogen was added dropwise, bromoacetyl bromide (1.1 eq.). The mixture was stirred at room temperature for 20 h, washed with saturated sodium bicarbonate solution, water and dried (MgSO$_4$). Solvent was removed in vacuo to give the product, which was used without further purification.

Benzyl 4-(2-bromoacetamido)benzoate ($^{137}$)

The reaction was carried out according to general procedure 7, with ester $^{135}$ (3.96 g, 17.4 mmol) to give the title compound $^{137}$ (5.04 g, 83%) as a dark green solid (m.p. 140-144°C). The product was used without further purification.

HRMS Found (El$^+$): M$^+$ 347.0157, 349.0142 C$_{16}$H$_{14}$$_{79}$BrNO$_3$, C$_{16}$H$_{14}$$_{81}$BrNO$_3$ requires 347.0157 and 349.0137; IR $v_{max}$(solid)/cm$^{-1}$: 3301 (N-H), 1718 (C=O), 1658 (C=O), 1268; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.05 (2H, s, CH$_2$Br), 5.30 (2H, s, Ar-CH$_2$O), 7.33-7.45 (5H, m, Ar-H), 7.62 (2H, d, J = 8.4 Hz, Ar-H), 8.08 (2H, d, J = 8.4 Hz, Ar-H), 8.25 (1H, br s, N-H); $^{13}$C NMR(100 MHz, CDCl$_3$): $\delta$ 29.3 (CH$_2$, CH$_2$Br), 66.8 (CH$_2$, ArCH$_2$O), 119.1 (2 $\times$ CH, Ar-C), 126.6 (quat. Ar-C), 128.2 (2 $\times$ CH, Ar-C), 128.3 (CH, Ar-C), 128.6 (quat., and 2 $\times$ CH, Ar-C), 131.0 (2 $\times$ CH, Ar-C), 141.1 (quat., Ar-C), 163.6 and 165.8 (C=O, NHCOCH$_2$, CO$_2$CH$_2$); m/z (EI$^+$) 349/347 (M$^+$, 12%), 242/240 (M$^+$-C$_7$H$_7$O, 63%), 91 (C$_7$H$_7$, 100%).
Benzyl 3-(2-bromoacetamido)benzoate (138)

The reaction was carried out according to general procedure 7, with ester 136 (6.2 g, 27.3 mmol) to give the title compound 138 (9.5 g, 100%) as a tan solid (m.p. 67-70 °C). The product was used without further purification.

HRMS Found (EI⁺): M⁺ 347.0150, 349.0129 C₁₆H₁₄⁷⁹BrNO₃, C₁₆H₁₄⁸¹BrNO₃ requires 347.0157 and 349.0137; IR νmax(solid)/cm⁻¹: 3279 (N-H), 1718 (C=O), 1657 (C=O), 1271; ¹H NMR (300 MHz, CDCl₃): δ 4.05 (2H, s, CH₂Br), 5.36 (2H, s, ArCH₂O), 7.25-7.45 (6H, m, Ar-H), 7.87 (1H, dt, J = 1.0, 7.8 Hz, Ar-H), 7.92 (1H, d, J = 1.1, 7.8 Hz, Ar-H), 8.03 (1H, t, J = 1.4 Hz, Ar-H), 8.30 (1H, br s, N-H); ¹³C NMR (75 MHz, CDCl₃): δ 29.3 (CH₂, CH₂Br), 67.0 (CH₂, ArCH₂O), 121.1 (2 × CH, Ar-C), 124.7 (CH, Ar-C), 126.3 (2 × CH, Ar-C), 128.3 (CH, Ar-C), 128.4 (CH, Ar-C), 128.6 (CH, Ar-C), 129.4 (CH, Ar-C), 131.0 (quat., Ar-C), 135.8 (quat., Ar-C), 137.2 (quat., Ar-C), 163.8 and 165.8 (C=O, NHCOCH₂, CO₂CH₂); m/z (EI⁺) 349/347 (M⁺, 10%), 242/240 (M⁺-C₇H₇O, 30%), 91 (C₇H₇, 100%).

General Procedure 8: Amine addition to bromoacetamides

To a solution of bromoacetamide 137 or 138 (1.0 eq.) in dry DCM (1 mL/mmoul) was added amine (2-12 eq.) and the resulting mixture stirred at reflux overnight. The mixture was washed with saturated sodium bicarbonate solution, brine and dried (MgSO₄). Solvent was removed in vacuo to give the the product.

Benzyl 4-(2-(diethylamino)acetamido)benzoate (139)
The reaction was carried out according to general procedure 8, with bromoacetamide 137 (1.0 g, 2.87 mmol) and diethylamine (0.46 g, 6.29 mmol) to give the title compound 139 (0.98 g, 100%) as a brown oil which was used without further purification.

**HRMS** Found (EI): M⁺ 340.1780, C₂₀H₂₄N₂O₃ requires 340.1787; **IR** v max (oil)/cm⁻¹: 3301 (N-H), 2994 (C-H), 1707 (C=O), 1699 (C=O), 1586, 1518, 1243; **¹H NMR** (300 MHz, CDCl₃): δ 1.09 (6H, t, J = 7.2 Hz, N(CH₂CH₃)₂), 2.65 (4H, q, J = 7.1 Hz, N(CH₂CH₃)₂), 3.18 (2H, s, ArCH₂O), 5.43 (2H, s, COCH₃), 7.32-7.45 (5H, m, Ar-H), 7.64 (2H, dt, J = 2.3, 8.9 Hz, Ar-H), 8.04 (2H, dt, J = 2.3, 8.9 Hz, Ar-H), 9.60 (1H, br s, N-H); **¹³C NMR** (75 MHz, CDCl₃): δ 12.4 (2 × CH₃, N(CH₂CH₃)₂), 48.9 (2 × CH₂, N(CH₂CH₃)₂), 58.1 (CH₂, COCH₃N), 66.5 (CH₂, ArCH₂O), 118.4 (2 × CH, Ar-C), 128.1 (quat., Ar-C), 128.2 (2 × CH, Ar-C), 128.6 (quat., 3 × CH, Ar-C), 131.0 (2 × CH, Ar-C), 141.9 (quat., Ar-C), 166.0 and 170.5 (C=O, CO₂CH₂ and NHCOCH₂); m/z (EI⁺) 340 (M⁺, 2%), 256 (M⁺-C₃H₁₂N, 1%), 91 (C₇H₁₇, 13%), 86 (C₃H₁₂N, 100%).

**Benzy1 3-(2-(diethylamino)acetamido)benzoate (140)**

![Chemical Structure](image)

The reaction was carried out according to general procedure 8, with bromoacetamide 138 (1.0 g, 2.87 mmol) and diethylamine (0.46 g, 6.29 mmol) to give the title product 140 (0.91 g, 93%) as a brown oil.

**HRMS** Found (EI⁺): M⁺ 340.1777, C₂₀H₂₄N₂O₃ requires 340.1787; **IR** v max (oil)/cm⁻¹: 3294 (N-H), 2969 (C-H), 1717 (C=O), 1688 (C=O), 1591, 1520, 1243; **¹H NMR** (300 MHz, CDCl₃): δ 1.07 (6H, t, J = 7.1 Hz, N(CH₂CH₃)₂), 2.63 (4H, q, J = 7.1 Hz, N(CH₂CH₃)₂), 3.14 (2H, s, ArCH₂O), 5.36 (2H, s, COCH₃N), 7.31-7.45 (6H, m, Ar-H), 7.79 (1H, dt, J = 1.3, 7.7 Hz, Ar-H), 7.98 (1H, t, J = 1.9 Hz, Ar-H), 8.06 (1H, ddd, J = 1.1, 2.2, 8.1 Hz, Ar-H), 9.49 (1H, br s, N-H); **¹³C NMR** (75 MHz, CDCl₃): δ 12.4 (2 × CH₃, N(CH₂CH₃)₂), 48.9 (2 × CH₂, N(CH₂CH₃)₂), 58.0 (CH₂, COCH₃N), 66.8 (CH₂, ArCH₂O), 120.2 (2 × CH, Ar-C), 124.0 (CH, Ar-C), 125.2 (2 × CH, Ar-C), 128.2 (CH, Ar-C), 128.3 (CH, Ar-C), 128.6 (CH, Ar-C), 129.3 (CH, Ar-C), 130.9 (quat., Ar-C), 136.0 (quat., Ar-C), 138.0 (quat., Ar-C), 166.2 and 170.4
(C=O, CO₂H₂ and NHCOCH₂); m/z (EI⁺) 340 (M⁺, 2%), 91 (C₇H₇, 16%), 86 (C₅H₁₂N, 100%).

**Benzy1 4-(2-(diisopropylamino)acetamido)benzoate (141)**

![Chemical structure](image)

The reaction was carried out according to general procedure 8, with bromoacetamide 137 (0.95 g, 2.73 mmol) and diisopropylamine (3.31 g, 32.7 mmol) to give the title compound 141 (0.98 g, 98%) as a brown solid (m.p. 96–98 °C).

**HRMS** Found (Cl⁺): M⁺H 369.2173, C₂₂H₂₉N₂O₃ requires 369.2178; IR νmax(solid)/cm⁻¹: 3272 (N-H), 2968 (C-H), 1710 (C=O), 1618, 1268; **¹H NMR** (300 MHz, CDCl₃): δ 1.08 (12H, d, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.11 (2H, heptet, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.17 (2H, s, COCH₂N), 5.36 (2H, s, ArCH₂O), 7.24-7.46 (5H, m, Ar-H), 7.64 (2H, dt, J = 2.3, 8.7 Hz, Ar-H), 8.06 (2H, dt, J = 2.3, 8.7 Hz, Ar-H), 9.70 (1H, br s, N-H); **¹³C NMR** (75 MHz, CDCl₃): δ 20.7 (4 × CH₃, N(CH(CH₃)₂)₂), 50.3 (2 × CH, N(CH(CH₃)₂)₂), 66.5 (CH₂, ArCH₂O), 118.3 (2 × CH, Ar-C), 125.3 (quat., Ar-C), 128.1 (2 × CH, Ar-C), 128.2 (quat., Ar-C), 128.6 (3 × CH, Ar-C), 131.1 (2 × CH, Ar-C), 141.9 (quat., Ar-C), 166.0 and 171.9 (C=O, CO₂H₂ and NHCOCH₂); m/z (Cl⁺) 369 (M⁺, 12%), 261 (M⁺-C₇H₇O, 1%), 227 (M⁺-C₈H₁₆NO, 2%), 114 (C₇H₁₆N, 100%), 91 (C₇H₇, 14%).

**Benzy1 3-(2-(diisopropylamino)acetamido)benzoate (142)**

![Chemical structure](image)
The reaction was carried out according to general procedure 8, with bromoacetamide 138 (1.0 g, 2.87 mmol) and diisopropylamine (3.54 g, 35.0 mmol) to give the title compound 142 (1.06 g, 100%) as a brown gum.

**HRMS** Found (Cl\(^+\)): MH\(^+\) 369.2178, C\(_{22}\)H\(_{20}\)N\(_2\)O\(_3\) requires 369.2178; IR \(\nu_{\text{max}}\)(solid)/cm\(^{-1}\): 3278 (N-H), 2968 (C-H), 1717 (C=O), 1690 (C=O), 1591, 1275; \(^1\)H NMR (300MHz, CDCl\(_3\)): \(\delta\) 1.07 (12H, d, \(J = 6.5\) Hz, N(CH(CH\(_3\))\(_2\))\(_2\)), 3.11 (2H, heptet, \(J = 6.5\) Hz, N(CH(CH\(_3\))\(_2\))\(_2\)), 3.16 (2H, s, COCH\(_2\)N), 5.38 (2H, s, ArCH\(_2\)O), 7.33-7.47 (6H, m, Ar-H), 7.80 (1H, dt, \(J = 1.4, 7.8\) Hz, Ar-H), 8.00 (1H, t, \(J = 1.6\) Hz, Ar-H), 8.05 (1H, ddd, \(J = 1.1, 2.1, 8.0\) Hz, Ar-H), 9.60 (1H, br s, N-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 20.6 (4 \(\times\) CH), 50.3 (2 \(\times\) CH, N(CH(CH\(_3\))\(_2\))\(_2\)), 66.8 (CH\(_2\), ArCH\(_2\)O), 119.9 (2 \(\times\) CH, Ar-C), 123.8 (CH, Ar-C), 125.0 (2 \(\times\) CH, Ar-C), 128.1 (CH, Ar-C), 128.5 (CH, Ar-C), 129.2 (CH, Ar-C), 130.9 (quat., Ar-C), 136.0 (quat., Ar-C), 138.0 (quat., Ar-C), 166.1 and 171.8 (C=O, CO\(_2\)CH\(_2\) and NHCOCH\(_2\)); \(m/z\) (Cl\(^+\)) 369 (MH\(^+\), 48%), 228 (MH\(^+\)-C\(_8\)H\(_{16}\)NO, 3%), 114 (C\(_7\)H\(_{16}\)N, 100%), 91 (C\(_7\)H\(_7\), 16%).

Benzyl 4-(2-(piperidin-1-yl)acetamido)benzoate (143)

![Structure of Benzyl 4-(2-(piperidin-1-yl)acetamido)benzoate (143)](image)

The reaction was carried out according to general procedure 8, with bromoacetamide 137 (1.0 g, 2.87 mmol) and piperidine (1.36 g, 16.0 mmol) to give the title compound 143 (1.01 g, 100%) as a brown solid (m.p. 87-90 °C).

**HRMS** Found (Cl\(^+\)): MH\(^+\) 353.1870, C\(_{21}\)H\(_{25}\)N\(_2\)O\(_3\) requires 353.1865; IR \(\nu_{\text{max}}\)(solid)/cm\(^{-1}\): 3281 (N-H), 2944 (C-H), 1709 (C=O), 1518, 1268; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.55-1.69 (6H, m, N(CH\(_2\)CH\(_2\))\(_2\)CH\(_2\)), 2.52 (4H, t, \(J = 5.0\) Hz, N(CH\(_2\)CH\(_2\))\(_2\)CH\(_2\)), 3.08 (2H, s, COCH\(_2\)N), 5.35 (2H, s, ArCH\(_2\)O), 7.33-7.46 (5H, m, Ar-H), 7.65 (2H, dt, \(J = 2.3, 8.7\) Hz, Ar-H), 8.05 (2H, dt, \(J = 2.3, 8.8\) Hz, Ar-H), 9.48 (1H, br s, N-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 24.6 (CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)CH\(_2\)), 26.3 (2 \(\times\) CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)CH\(_2\)), 54.9 (2 \(\times\) CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)CH\(_2\)), 62.8 (CH\(_2\), COCH\(_2\)N), 66.5 (CH\(_2\), ArCH\(_2\)O), 118.5 (2 \(\times\) CH, Ar-C), 125.4 (quat., Ar-C), 128.1
(2 × CH, Ar-C), 128.2 (quat., Ar-C), 128.6 (3 × CH, Ar-C), 131.0 (2 × CH, Ar-C), 141.9 (quat., Ar-C), 165.9 and 169.3 (C=O, CO₂CH₂ and NHCOCH₂); m/z (Cl⁺) 353 (MH⁺, 8%), 227 (M⁺-C₇H₁₂NO, 2%), 98 (C₆H₁₂N, 100%), 91 (C₇H₇, 16%).

Benzyl 3-(2-(piperidin-1-yl)acetamido)benzoate (144)

The reaction was carried out according to general procedure 8, with bromoacetamide 138 (1.0 g, 2.87 mmol) and piperidine (1.47 g, 17.3 mmol) to give the title compound 144 (1.01 g, 100%) as a brown oil.

HRMS Found (EI⁺): M⁺ 352.1790, C₂₁H₂₄N₂O₃ requires 352.1787; IR νmax(oil)/cm⁻¹: 3274 (N-H), 2934 (C-H), 1717 (C=O), 1522, 1275; ¹H NMR (300 MHz, CDCl₃): δ 1.40-1.57 (6H, m, N(CH₂CH₂CH₂), 2.53 (4H, t, J = 5.0 Hz, N(CH₂CH₂CH₂), 3.07 (2H, s, COCH₂N), 5.37 (2H, s, ArCH₂O), 7.32-7.47 (6H, m, Ar-H), 7.80 (1H, dt, J = 1.4, 7.9 Hz, Ar-H), 8.00 (1H, t, J = 1.7 Hz, Ar-H), 8.05 (1H, ddd, J = 0.9, 2.7, 7.9 Hz, Ar-H), 9.35 (1H, br s, N-H); ¹³C NMR (75 MHz, CDCl₃): δ 24.8 (CH₂, N(CH₂CH₂CH₂), 26.2 (2 × CH₂, N(CH₂CH₂CH₂), 55.0 (2 × CH₂, N(CH₂CH₂CH₂), 62.8 (CH₂, COCH₂N), 66.8 (CH₂, ArCH₂O), 120.3 (2 × CH, Ar-C), 124.1 (CH, Ar-C), 125.2 (2 × CH, Ar-C), 128.2 (CH, Ar-C), 128.3 (CH, Ar-C), 128.6 (CH, Ar-C), 129.3 (CH, Ar-C), 130.9 (quat., Ar-C), 137.2 (quat., Ar-C), 138.0 (quat., Ar-C), 166.1 and 169.3 (C=O, CO₂CH₂ and NHCOCH₂); m/z (EI⁺) 352 (M⁺, 1%), 98 (C₆H₁₂N, 100%), 91 (C₇H₇, 14%).

Benzyl 4-(2-morpholinoacetamido)benzoate (145)
The reaction was carried out according to general procedure 8, with bromoacetamide 137 (1.0 g, 2.87 mmol) and morpholine (0.55 g, 6.31 mmol) to give the title compound 145 (1.02 g, 100%) as a brown oil which was used without further purification.

**HRMS** Found (EI⁺): M⁺ 354.1590, C₂₀H₂₂N₂O₄ requires 354.1580; IR ν_{max}(oil)/cm⁻¹: 3317 (N-H), 2964 (C-H), 1707 (C=O), 1589, 1522, 1267; $^1$H NMR (300 MHz, CDCl₃): δ 2.61 (4H, t, J = 4.7 Hz, N(CH₂CH₃)₂O), 3.14 (2H, s, COCH₂N), 3.77 (4H, t, J = 4.6 Hz, O(CH₂CH₃)₂), 5.33 (2H, s, ArCH₂O), 7.32-7.44 (5H, m, Ar-H), 7.62 (2H, dt, J = 2.3, 8.8 Hz, Ar-H), 8.04 (2H, dt, J = 2.3, 8.7 Hz, Ar-H), 9.22 (1H, br s, N-H); $^{13}$C NMR (75 MHz, CDCl₃): δ 53.8 (CH₂, CH₂N(CH₂CH₃)₂), 62.5 (2 × CH₂, N(CH₂CH₃)₂), 66.6 (CH₂, ArCH₂O), 67.0 (2 × CH₂, O(CH₂CH₃)₂), 118.6 (2 × CH, Ar-C), 128.21 (quat., Ar-C), 128.22 (2 × CH, Ar-C), 128.6 (3 × CH, Ar-C), 131.0 (2 × CH, Ar-C) 135.9 (quat., Ar-C), 137.7 (quat., Ar-C), 165.0 and 168.2 (C=O, CO₂CH₂ and NHCOCH₂); m/z (EI⁺) 354 (M⁺, 5%), 256 (M⁺-C₅H₁₀NO, 1%), 100 (C₅H₁₀NO, 100%), 91 (C₇H₁₇, 13%).

**Benzyl 3-(2-morphinoacetamido)benzoate (146)**

![Image of the molecule](image)

The reaction was carried out according to general procedure 8, with bromoacetamide 138 (1.0 g, 2.87 mmol) and morpholine (0.55 g, 6.31 mmol) to give the title compound 146 (1.02 g, 100%) as a brown oil.

**HRMS** Found (EI⁺): M⁺ 354.1583, C₂₀H₂₂N₂O₄ requires 354.1580; IR ν_{max}(oil)/cm⁻¹: 3298 (N-H), 2820 (C-H), 1715 (C=O), 1592, 1525, 1267; $^1$H NMR (300 MHz, CDCl₃): δ 2.61 (4H, t, J = 4.6 Hz, N(CH₂CH₃)₂), 3.14 (2H, s, COCH₂N), 3.78 (4H, t, J = 4.6 Hz, O(CH₂CH₃)₂), 5.37 (2H, s, ArCH₂O), 7.33-7.46 (6H, m, Ar-H), 7.81 (1H, t, J = 7.9 Hz, Ar-H), 7.99 (1H, t, J = 1.8 Hz, Ar-H), 8.04 (1H, ddd, J = 1.0, 2.2, 8.1 Hz, Ar-H), 9.13 (1H, br s, N-H); $^{13}$C NMR (75 MHz, CDCl₃): δ 53.8 (CH₂, CH₂N(CH₂CH₃)₂), 62.4 (2 × CH₂, N(CH₂CH₃)₂), 66.8 (CH₂, ArCH₂O), 66.9 (2 × CH₂, O(CH₂CH₃)₂), 120.3 (2 × CH, Ar-C), 124.2 (CH, Ar-C), 125.4 (2 × CH, Ar-C), 128.2 (CH, Ar-C), 128.3 (CH, Ar-C), 128.6 (CH, Ar-C), 129.3 (CH, Ar-C), 130.9
(quat., Ar-C), 135.9 (quat., Ar-C), 137.7 (quat., Ar-C), 166.0 and 168.2 (C=O, CO₂CH₂ and NHCOCH₂); m/z (EI⁺) 354 (M⁺, 6%), 256 (M⁺-C₅H₁₀NO, 2%), 100 (C₅H₁₀NO, 100%), 91 (C₃H₇, 19%).

**Ethyl 4-morpholinobutanoate (150)²²⁰**

![Ethyl 4-morpholinobutanoate](image)

Adopting the method of Razdan,¹⁶⁴ a mixture of ethyl 4-bromobutyrate 149 (15.0 g, 76.9 mmol) and morpholine 148 (13.4 g, 154 mmol) in toluene (100 mL), under an atmosphere of nitrogen was heated at 60 °C for 4 hours and then stirred overnight at room temperature. The white precipitate of morpholine hydrobromide was removed by filtration and the filtrate was concentrated to yield the *title compound* 150 (15.0 g, 97%) as a clear oil, which was used without further purification.

R_f (DCM/MeOH 9:1) = 0.66; HRMS Found (ESI⁺): MH⁺ 202.1434, C_{10}H_{20}NO₃ requires 202.1434; IR ν_{max}(oil)/cm⁻¹: 2959 (C-H), 1731 (C=O, ester), 1446, 1372, 1183, 1116; ¹H NMR (400 MHz, CDCl₃): δ 1.13 (3H, t, J = 7.2 Hz, CH₃CH₂), 1.67 (2H, p, J = 7.3 Hz, CH₂CH₂CH₂), 2.22-2.24 (4H, br q, J = 6.7 Hz, CH₂N(CH₂CH₂)₂), COCH₂), 2.29 (4H, t, J = 4.0 Hz, N(CH₂CH₂)₂), 3.55 (4H, t, J = 4.6 Hz, O(CH₂CH₂)₂), 4.0 (2H, q, J = 6.6 Hz, CH₂CH₂); ¹³C NMR (100 MHz, CDCl₃): δ 14.0 (CH₃, CH₃CH₂), 21.5 (CH₂, CH₂CH₂CH₂), 31.9 (CH₂, COCH₂), 53.4 (2 × CH₂, N(CH₂CH₂)₂), 57.8 (CH₂, (CH₂CH₂)₂NCH₂), 59.9 (CH₂, CH₂CH₂), 66.7 (2 × CH₂, O(CH₂CH₂)₂), 173.2 (C=O, CO₂Et); m/z (ESI⁺): 202 (MH⁺, 100%), 188 (5%), 156 (2%), 115 (9%).

**4-Morpholinobutanoic acid hydrochloride (147)²²¹**

![4-Morpholinobutanoic acid hydrochloride](image)

A mixture of ethyl 4-morpholinobutanoate 150 (15.0 g, 74.5 mmol) and 18% aqueous hydrochloride (160 mL) was heated at reflux for 18 hours. The excess acid was removed under reduced pressure to give the *title compound* 147 (15.6 g, 100%). The product 147 was
recrystallised as colorless crystals from ethylacetate-acetone (m.p. 160-161 °C lit\textsuperscript{221} 131-133 °C and lit\textsuperscript{163} 183-184.

R\textsubscript{f} (DCM/MeOH 9:1) = 0.13; HRMS Found (ESI\textsuperscript{+}): (MH-Cl\textsuperscript{+}) 174.1122, C\textsubscript{8}H\textsubscript{16}NO\textsubscript{3} requires 174.1130; IR \nu\textsubscript{max}(solid)/cm\textsuperscript{-1}: 3381 (O-H), 2939 br (C-H), 1721 (C=O), 1436, 1384, 1229, 1195, 1077; \textsuperscript{1}H NMR (400 MHz, (CD\textsubscript{3})\textsubscript{2}SO): δ 1.92 (2H, p, J = 7.7 Hz, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}), 2.32 (2H, t, J = 7.4 Hz, COCH\textsubscript{2}), 3.06 (4H, br m, N(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 3.36 (2H, br d, J = 12.0 Hz, CH\textsubscript{2}N(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 3.84 (4H, m, O(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 11.22 (1H, br s, COOH); \textsuperscript{13}C NMR (100 MHz, (CD\textsubscript{3})\textsubscript{2}SO): δ 18.4 (CH\textsubscript{2}, CH\textsubscript{2}C\textsubscript{H}CH\textsubscript{2}), 30.7 (CH\textsubscript{2}, COCH\textsubscript{2}), 50.9 (2 × CH\textsubscript{2}, N(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 55.2 (CH\textsubscript{2}, CH\textsubscript{2}N(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 63.1 (2 × CH\textsubscript{2}, O(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}), 173.4 (C=O, COOH); m/z (ESI\textsuperscript{+}): 174 ((MH-Cl\textsuperscript{+}), 100%), 156 (10%).

**General procedure 9: Coupling of acid with amine using EDC**

Using the method of Stover\textsuperscript{159} a solution of benzyl aminobenzoate 135 or 136 (1 eq.) in dry DMF (0.85 mL/mmol) was treated with 4-morpholinobutanoic acid hydrochloride 147 (2 eq.), EDC (2 eq.), DMAP (2 eq.) and the mixture was stirred at 45 °C for 24 hours. After completion, the reaction mixture was diluted with dichloromethane:i-propanol (1:1) (5.7 mL/mmol amine) and washed with saturated sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, filtered and the solvent was removed under high vacuum. The crude product was dissolved in methanol (4 mL), silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica. This silica gel was then loaded and purified by flash chromatography (dichloromethane-methanol 19:1).

**Benzyl 4-(4-morpholinobutanamido)benzoate (151)**
Chapter 6: Experimental

The reaction was carried out according to general procedure 9, with benzyl 4-aminobenzoate 135 (1.6 g, 7.04 mmol) and 4-morpholinobutanoic acid hydrochloride 147 (2.95 g, 14.1 mmol) to give the title compound 151 (1.78 g, 66%) as a clear oil.

**Rf** (DCM/MeOH 9:1) = 0.45; **HRMS** Found (ESI⁺): MH⁺ 383.1961, C₂₂H₂₇N₂O₄ requires 383.1971; **IR** νmax(Oil)/cm⁻¹: 3220 (N-H), 2954 (C-H), 1711 br (C=O, ester), 1693 (C=O, amide), 1596, 1529, 1455, 1267 br; **¹H NMR** (400 MHz, CDCl₃): δ 1.84 (2H, p, J = 7.0 Hz, CH₂CH₂CH₂), 2.32-2.41 (8H, m, CH₂N(CH₂CH₂)₂, COCH₂), 3.63 (4H, t, J = 4.6 Hz, O(CH₂CH₂)₂), 5.29 (2H, s, ArCH₂O), 7.26-7.63 (5H, m, Ar-H), 7.62 (2H, d, J = 8.8 Hz, Ar-H), 7.98 (2H, d, J = 8.8 Hz, Ar-H), 9.03 (1H, s, N-H); **¹³C NMR** (100 MHz, CDCl₃): δ 21.6 (CH₂, CH₂CH₂CH₂), 35.1 (CH₂, NHCOCH₂), 53.2 (2 × CH₂, N(CH₂CH₂)₂), 57.4 (CH₂, CH₂N(CH₂CH₂)₂), 66.3 (CH₂, ArCH₂O), 66.5 (2 × CH₂, O(CH₂CH₂)₂), 118.6 (2 × CH, Ar-C), 124.8 (quat., Ar-C), 127.7 (2 × CH, Ar-C), 127.9 (CH, Ar-C), 128.3 (2 × CH, Ar-C), 130.6 (2 × CH₃, Ar-C), 135.7 (quat., Ar-C), 142.6 (quat., Ar-C), 165.7 and 171.7 (C=O, CO₂CH₂ and NHCOCH₂); m/z (ESI⁺): 383 (MH⁺, 100%), 228 (2%).

**Benzyl 3-(4-morpholinobutanoamido)benzoate (152)**

The reaction was carried out according to general procedure 9, with benzyl 4-aminobenzoate 136 (1.2 g, 5.28 mmol) and 4-morpholinobutanoic acid hydrochloride 147 (2.21 g, 10.6 mmol) to give the title compound 152 (1.5 g, 74%) as clear oil.

**Rf** (DCM/MeOH 9:1) = 0.53; **HRMS** Found (ESI⁺): MH⁺ 383.1971, C₂₂H₂₇N₂O₄ requires 383.1971; **IR** νmax(Oil)/cm⁻¹: 3280 (N-H), 2960 (C-H), 1718 br (C=O, ester), 1666 (C=O, amide), 1593, 1550, 1487, 1283; **¹H NMR** (400 MHz, CDCl₃): δ 1.75 (2H, p, J = 7.0 Hz, CH₂CH₂CH₂), 2.23-2.31 (8H, m, CH₂N(CH₂CH₂)₂, COCH₂), 3.54 (4H, t, J = 4.6 Hz, O(CH₂CH₂)₂), 5.20 (2H, s, ArCH₂O), 7.18-7.24 (3H, m, Ar-H), 7.27-7.30 (2H, m, Ar-H), 7.50 (2H, dd, J = 7.2, 1.6 Hz, Ar-H), 7.88 (2H, dd, J = 6.8, 2.0 Hz, Ar-H), 8.78 (1H, s, N-H); **¹³C NMR** (100 MHz, CDCl₃): δ 21.6 (CH₂, CH₂CH₂CH₂), 35.2 (CH₂, COCH₂), 53.2 (2 × CH₂,
CH₂N(CH₂CH₂)₂, 57.4 (CH₂, CH₂N(CH₂CH₂)₂), 66.3 (CH₂, ArCH₂O), 66.6 (2 x CH₂, O(CH₂CH₂)₂), 118.6 (2 x CH, Ar-C), 124.9 (quat., Ar-C), 127.0 (2 x CH, Ar-C), 128.0 (CH, Ar-C), 128.3 (2 x CH, Ar-C), 130.7 (2 x CH, Ar-C), 135.8 (quat., Ar-C), 142.6 (quat., Ar-C), 165.8 and 171.3 (C=O, CO₂CH₂ and NHCOCH₂); m/z (ESI⁺): 383 (MH⁺, 100%), 307 (2%), 343 (8%), 156 (1%).

**4-(2-(Diethylamino)acetamido)benzoic acid (119)**

The reaction was carried out according to general procedure 2, using ester 139 (0.99 g, 2.90 mmol), to give the title compound 119 (0.73 g, 100%) as a light brown powder (m.p. 110-115 °C from methanol).

**HRMS** Found (EI⁺): M⁺ 250.1321, C₁₃H₁₈N₂O₃ requires 250.1317; IR v_max(solid)/cm⁻¹: 3455 (N-H, O-H), 2970 (C-H), 1738 br, 1365, 1216; ¹H NMR (300 MHz, (CD₃)₂SO): δ 1.03 (6H, t, J = 7.1 Hz, N(CH₂C₂H₃)₂), 2.63 (4H, q, J = 7.1 Hz, N(CH₂CH₃)₂), 3.21 (2H, s, COCH₂N), 7.75 (2H, d, J = 8.7 Hz, Ar-H), 7.89 (2H, d, J = 8.7 Hz, Ar-H), 9.93 (1H, br s, N-H), 10.0 (1H, s, COOH); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 11.8 (2 x CH₃, N(CH₂CH₃)₂), 47.7 (2 x CH₂, N(CH₂CH₃)₂), 57.2 (CH₂, COCH₂N), 118.5 (2 x CH, Ar-C), 125.4 (quat., Ar-C), 130.2 (2 x CH, Ar-C), 142.2 (quat., Ar-C), 166.8 and 170.2 (C=O, CO₂H and NHCOCH₂); m/z (EI⁺) 250 (M⁺, 1%), 233 (M⁺-OH, 1%), 137 (MH⁺-C₇H₆NO₂, 2%), 120 (MH⁺-C₇H₇NO₃, 2%), 86 (C₆H₁₂N, 100%), 72 (C₄H₁₀N, 2%), 58 (C₂H₂NO, 8%).

**3-(2-(Diethylamino)acetamido)benzoic acid (120)**

The reaction was carried out according to general procedure 2, using ester 140 (0.93 g, 2.73 mmol), to give the title compound 120 (0.69 g, 100%) as a tan powder (m.p. 115-120 °C from methanol).
HRMS Found (El⁺): M⁺ 250.1311, C₁₃H₁₈N₂O₃ requires 250.1317; IR vₘₚₓ(solid)/cm⁻¹: 3454 (N-H, O-H), 2970 (C-H), 1738 br, 1365, 1216; ¹H NMR (300 MHz, (CD₃)₂SO): δ 1.02 (6H, t, J = 7.1 Hz, N(CH₂CH₃)₂), 2.61 (4H, q, J = 7.1 Hz, N(CH₂CH₃)₂), 3.18 (2H, s, COCH₂N), 7.41 (1H, t, J = 7.6 Hz, Ar-H), 7.62 (1H, d, J = 7.2 Hz, Ar-H), 7.84 (1H, d, J = 8.0 Hz, Ar-H), 8.27 (1H, br s, Ar-H), 9.85 (1H, br s, N-H), 10.04 (1H, s, COO⁻); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 11.7 (2 × CH₃, N(CH₂CH₃)₂), 47.7 (2 × CH₂, N(CH₂CH₃)₂), 57.1 (CH₂, COCH₂N), 120.1 (CH, Ar-C), 123.3 (CH, Ar-C), 124.1 (CH, Ar-C), 128.7 (CH, Ar-C), 131.7 (quat., Ar-C), 138.5 (quat., Ar-C), 166.1 and 167.2 (C=O, CO₂H and NHCOCH₂); m/z (El⁺) 250 (M⁺, 1%), 233 (M⁺-OH, 1%), 86 (C₆H₁₂N, 100%), 72 (C₄H₁₀N, 2%), 58 (C₂H₃NO, 8%).

4-(2-(Diisopropylamino)acetamido)benzoic acid (121)

The reaction was carried out according to general procedure 2, using ester 141 (0.977 g, 2.65 mmol) to give the title compound 121 (0.74 g, 100%) as a tan solid (m.p. 85-90 °C from methanol).

HRMS Found (El⁺): M⁺ 278.1624, C₁₃H₂₀N₂O₃ requires 278.1630; IR vₘₚₓ(solid)/cm⁻¹: 3236 (N-H, O-H), 2969 (C-H), 1684 br (C=O), 1519, 1368; ¹H NMR (300 MHz, (CD₃)₂SO): δ 1.03 (12H, d, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.06 (2H, heptet, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.16 (2H, s, COCH₂N), 7.77 (2H, dt, J = 1.8, 8.7 Hz, Ar-H), 7.91 (2H, dt, J = 1.5, 8.6 Hz, Ar-H), 9.81 (1H, br s, N-H), 10.09 (1H, s, COO⁻); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 20.1 (4 × CH₃, N(CH(CH₃)₂)₂), 46.1 (2 × CH, N(CH(CH₃)₂)₂), 49.7 (CH₂, COCH₂N), 118.4 (2 × CH, Ar-C), 125.5 (quat., Ar-C), 130.3 (2 × CH, Ar-C), 141.9 (quat., Ar-C), 166.8 and 171.8 (C=O, CO₂H and NHCOCH₂); m/z (El⁺) 278 (M⁺,1%), 262 (M⁺-OH, 2%), 235 (M⁺-C₃H₇, 2%), 179 (M⁺-C₆H₁₄N, 1%), 114 (C₇H₁₆N, 100%), 43 (C₃H₇, 14%).

3-(2-(Diisopropylamino)acetamido)benzoic acid (122)
The reaction was carried out according to general procedure 2, using ester 142 (1.06 g, 2.88 mmol) to give the title compound 122 (0.80 g, 100%) as a tan powder (m.p. 75-80 °C from methanol).

**HRMS** Found (EI⁺): M⁺ 278.1627, C₁₅H₂₂N₂O₃ requires 278.1630; IRν max(solid)/cm⁻¹: 3249 (N-H, O-H), 2969 (C-H), 1683 br, 1525, 1369, 1267; ¹H NMR (300 MHz, (CD₃)₂SO): δ 1.04 (12H, d, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.06 (2H, heptet, J = 6.5 Hz, N(CH(CH₃)₂)₂), 3.13 (2H, s, COCH₂N), 7.43 (1H, t, J = 7.9 Hz, Ar-H), 7.65 (1H, dt, J = 1.4, 7.9 Hz, Ar-H), 7.86 (1H, ddd, J = 1.0, 2.2, 8.1 Hz, Ar-H), 8.26 (1H, t, J = 1.8 Hz, Ar-H), 9.72 (1H, br s, N-H), 10.04 (1H, s, COOH); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 20.7 (4 × CH₃, N(CH(CH₃)₂)₂), 46.9 (2 × CH, N(CH(CH₃)₂)₂), 50.2 (CH₂, COCH₂N), 120.5 (CH, Ar-C), 123.9 (CH, Ar-C), 124.8 (CH, Ar-C), 129.4 (CH, Ar-C), 132.8 (quat., Ar-C), 139.1 (quat., Ar-C), 163.7 and 172.2 (C=O, CO₂H and NHCOCH₂); m/z (EI⁺) 278 (M⁺,1%), 263 (MH⁺-OH, 2%), 235 (M⁺-C₃H₇, 3%), 179 (M⁺⁻C₈H₁₄N, 1%), 114 (C₇H₁₆N, 100%), 43 (C₃H₇, 19%).

4-(2-(Piperidin-1-yl)acetamido)benzoic acid (123)²²⁴,²²⁵

The reaction was carried out according to general procedure 2, using ester 143 (1.01 g, 2.87 mmol) to give the title compound 123 (0.75 g, 100%) as a brown solid (m.p. 95-101 °C from methanol).

**HRMS** Found (EI⁺): M⁺ 262.1313, C₁₄H₁₈N₂O₃ requires 262.1317; IRν max(solid)/cm⁻¹: 3399 (N-H, O-H), 2936 (C-H), 1688 br, 1371; ¹H NMR (300 MHz, (CD₃)₂SO): δ 1.49-1.59 (6H, m, N(CH₂CH₂)₂CH₂), 2.45 (4H, t, J = 5.0 Hz, N(CH₂CH₂)₂CH₂), 3.07 (2H, s, COCH₂N), 7.66 (2H, d, J = 8.6 Hz, Ar-H), 7.84 (2H, dt, J = 1.7, 8.7 Hz, Ar-H), 9.84 (1H, br s, N-H), 10.04 (1H, s, COOH); ¹³C NMR (75 MHz, (CD₃)₂SO): δ 23.4 (CH₂, N(CH₂CH₂)₂CH₂), 25.3 (2 × CH₂, N(CH₂CH₂)₂CH₂), 53.9 (2 × CH₂, N(CH₂CH₂)₂CH₂), 62.5 (CH₂, COCH₂N), 118.2 (2 × CH, Ar-C), 123.2 (quat., Ar-C), 129.9 (2 × CH, Ar-C), 141.1 (quat., Ar-C), 168.8 and 171.8 (C=O, CO₂H and NHCOCH₂); m/z (EI⁺) 262 (M⁺, 1%), 163 (MH⁺-C₈H₁₂N, 1%), 98 (C₆H₁₂N, 100%), 84 (C₅H₁₀N, 4%).
3-(2-(Piperidin-1-yl)acetamido)benzoic acid (124)

![Structure of 3-(2-(Piperidin-1-yl)acetamido)benzoic acid (124)]

The reaction was carried out according to general procedure 2, using ester 144 (1.01 g, 2.87 mmol) to give the title compound 124 (0.75 g, 100%) as a brown powder (m.p. 98-104 °C from methanol).

**HRMS** Found (EI⁺): M⁺ 262.13243, C₁₄H₁₈N₂O₃ requires 262.1317; **IR** ν_max(solid)/cm⁻¹: 3269 (N-H, O-H), 2935 (C-H), 1683 br, 1369; **¹H NMR** (400 MHz, (CD₃)₂SO): δ 1.47-1.63 (6H, m, N(CH₂CH₂)₂CH₂), 2.46 (4H, t, J = 4.8 Hz, N(CH₂CH₂)₂CH₂), 3.07 (2H, s, COCH₂N), 7.37 (1H, t, J = 7.9 Hz, Ar-H), 7.61 (1H, d, J = 7.6 Hz, Ar-H), 7.81 (1H, d, J = 8.0 Hz, Ar-H), 8.20 (1H, br s, Ar-H), 9.80 (1H, br s, N-H), 10.04 (1H, s, COOH); **¹³C NMR** (100 MHz, (CD₃)₂SO): δ 23.5 (CH₂, N(CH₂CH₂)₂CH₂), 25.4 (2 × CH₂, N(CH₂CH₂)₂CH₂), 54.0 (2 × CH₂, N(CH₂CH₂)₂CH₂), 73.4 (CH₂, COCH₂N), 115.3 (CH, Ar-C), 123.5 (CH, Ar-C), 124.1 (CH, Ar-C), 139.0 (CH, Ar-C), 140.1 (quat., Ar-C), 145.8 (quat., Ar-C), 169.2 and 172.7 (C=O, CO₂H and NHCOCH₂); **m/z** (EI⁺) 262 (M⁺, 1%), 98 (C₆H₁₂N, 100%), 84 (C₅H₁₀N, 6%).

4-(2-Morpholinoacetamido)benzoic acid (125)⁴⁴,⁴⁶

![Structure of 4-(2-Morpholinoacetamido)benzoic acid (125)]

The reaction was carried out according to general procedure 2, using ester 145 (1.01 g, 2.85 mmol) to give the title compound 125 (0.73g, 97%) as a white solid (m.p. 200-208 °C from methanol).

**HRMS** Found (EI⁺): M⁺ 264.1103, C₁₃H₁₆N₂O₄ requires 264.1110; **IR** ν_max(solid)/cm⁻¹: 3307 (N-H, O-H), 2970 (C-H), 1690 br, 1366, 1216; **¹H NMR** (300 MHz, (CD₃)₂SO): δ 2.51 (4H, t, J = 4.6 Hz, N(CH₂CH₂)₂), 3.15 (2H, s, COCH₂N), 3.62 (4H, t, J = 4.6 Hz, O(CH₂CH₂)₂), 7.74 (2H, d, J = 8.7 Hz, Ar-H), 7.88 (2H, d, J = 8.7 Hz, Ar-H), 10.00 (1H, br s, N-H), 10.04 (1H, s, COOH); **¹³C NMR** (75 MHz, (CD₃)₂SO): δ 53.0 (CH₂, CH₂N(CH₂CH₂)₂), 61.8 (CH₂, N(CH₂CH₂)₂), 65.9 (CH₂, O(CH₂CH₂)₂), 118.5 (2 × CH, Ar-C), 125.2 (quat., Ar-C), 130.1 (2
× CH, Ar-C), 142.4 (quat., Ar-C), 166.8 and 168.5 (C=O, CO$_2$H and NHCOCH$_2$); m/z (El$^+$) 264 (M$^+$, 2%), 137 (MH$^+$.C$_6$H$_{10}$NO$_2$, 1%), 100 (C$_5$H$_{10}$NO, 100%), 86 (C$_4$H$_8$NO, 6%), 56 (C$_2$HNO, 19%).

3-(2-Morpholinoacetamido)benzoic acid (126)

![3-(2-Morpholinoacetamido)benzoic acid (126)](image)

The reaction was carried out according to general procedure 2, using ester 146 (1.01 g, 2.85 mmol), to give the title compound 126 (0.74 g, 98%) as a tan powder (m.p. 142-148 °C from methanol).

HRMS Found (El$^+$): M$^+$ 264.11028, C$_{13}$H$_{16}$N$_2$O$_4$ requires 264.1110; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3316 (N-H, O-H), 2970 (C-H), 1688 br, 1582, 1366, 1217; $^1$H NMR (300 MHz, (CD$_3$)$_2$SO): $\delta$ 2.49 (4H, t, $J = 4.8$ Hz, N(CH$_2$CH$_2$)$_2$), 3.13 (2H, s, COCH$_2$N), 3.63 (4H, t, $J = 4.6$ Hz, O(CH$_2$CH$_2$)$_2$), 7.41 (1H, t, $J = 7.9$ Hz, Ar-H), 7.62 (1H, dt, $J = 1.3$, 7.8 Hz, Ar-H), 7.85 (1H, ddd, $J = 1.0$, 2.1, 8.1 Hz, Ar-H), 8.25 (1H, t, $J = 1.7$ Hz, Ar-H), 9.91 (1H, br s, N-H), 10.04 (1H, s, COOH); $^{13}$C NMR (75 MHz, (CD$_3$)$_2$SO): $\delta$ 53.0 (CH$_2$, CH$_2$N(CH$_2$CH$_2$)$_2$), 61.9 (2 $\times$ CH$_2$, N(CH$_2$CH$_2$)$_2$), 66.0 (2 $\times$ CH$_2$, O(CH$_2$CH$_2$)$_2$), 120.2 (CH, Ar-C), 123.5 (CH, Ar-C), 124.1 (CH, Ar-C), 128.7 (CH, Ar-C), 131.3 (quat., Ar-C), 138.7 (quat., Ar-C), 167.1 and 168.3 (C=O, CO$_2$H and NHCOCH$_2$); m/z (El$^+$) 264 (M$^+$, 3%), 137 (MH$^+$.C$_6$H$_{10}$NO$_2$, 1%), 100 (C$_5$H$_{10}$NO, 100%), 86 (C$_4$H$_8$NO, 3%), 56 (C$_2$HNO, 8%).

4-(4-Morpholinobutanamido)benzoic acid (127)

![4-(4-Morpholinobutanamido)benzoic acid (127)](image)

The reaction was carried out according to general procedure 2, using benzyl 4-(4-morpholinobutanamido)benzoate 151 (888 mg, 2.32 mmol) to give the title compound 127 (678 mg, 100%) as a white solid (m.p. 109-110 °C from methanol).
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**Rf** (MeOH/\(\text{NH}_3\) 9:1) = 0.86; **HRMS** Found (ESI\(^+\)): MH\(^+\) 293.1495. C\(_{15}\)H\(_{21}\)N\(_2\)O\(_4\) requires 293.1503; **IR** \(v_{\text{max}}\) (solid)/cm\(^{-1}\): 3046 br (N-H, O-H), 2870 (C-H), 1695 br (C=O), 1600, 1560, 1523, 1362, 1315, 1255; **\(^1\)H NMR** (400 MHz, CD\(_3\)OD): \(\delta\) 1.92 (2H, p, \(J = 7.4\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)), 2.42 (2H, t, \(J = 7.0\) Hz, CH\(_2\)N(CH\(_2\))\(_2\)), 2.65 (2H, t, \(J = 7.0\) Hz, COCH\(_2\)), 2.72 (4H, t, \(J = 4.6\) Hz, CH\(_2\)N(CH\(_2\))\(_2\)), 3.71 (4H, t, \(J = 7.0\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.57 (2H, d, \(J = 8.8\) Hz, Ar-H), 7.59 (2H, d, \(J = 8.8\) Hz, Ar-H); **\(^13\)C NMR** (100 MHz, CD\(_3\)OD): \(\delta\) 22.2 (CH\(_2\), CH\(_2\)CH\(_2\)CH\(_2\)), 35.4 (CH\(_2\), COCH\(_2\)), 54.1 (2 \(\times\) CH\(_2\), N(CH\(_2\))\(_2\)), 58.8 (CH\(_2\), CH\(_2\)N(CH\(_2\))\(_2\)), 66.8 (2 \(\times\) CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 120.0 (2 \(\times\) CH, Ar-C), 129.8 (quat., Ar-C), 131.6 (2 \(\times\) CH, Ar-C), 143.5 (quat., Ar-C), 171.7 and 171.8 (C=O, CO\(_2\)H and NHCOCH\(_2\)); **m/z** (ESI\(^+\)): 293 (MH\(^+\), 100%), 206 (7%), 188 (1%), 156 (2%).

3-(4-Morpholinobutanamido)benzoic acid (128)

![Molecule structure](image)

The reaction was carried out according to general procedure 2, using benzyl 3-(4-morpholinobutanamido)benzoate 152 (1.5 g, 3.92 mmol) to give the **title compound** 128 (1.14 g, 100%) as a white solid (m.p. 172-173 °C from methanol).

**Rf** (MeOH/\(\text{NH}_3\) 9:1) = 0.88; **HRMS** Found (ESI\(^+\)): MH\(^+\) 293.1488. C\(_{15}\)H\(_{21}\)N\(_2\)O\(_4\) requires 293.1501; **IR** \(v_{\text{max}}\) (solid)/cm\(^{-1}\): 3340 (N-H, O-H), 2968 (C-H), 1695 br (C=O), 1613, 1558 br, 1522, 1360, 1325, 1247; **\(^1\)H NMR** (400 MHz, (CD\(_3\))\(_2\)SO): \(\delta\) 1.75 (2H, p, \(J = 7.2\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)), 2.32-2.36 (8H, m, CH\(_2\)N(CH\(_2\))\(_2\), CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\), COCH\(_2\)), 3.53 (4H, t, \(J = 4.6\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.37 (1H, t, \(J = 7.8\) Hz, Ar-H), 7.59 (1H, d, \(J = 9.2\) Hz, Ar-H), 7.81 (1H, d, \(J = 8.0\) Hz, Ar-H), 8.20 (1H, t, \(J = 1.6\) Hz, Ar-H), 10.04 (1H, s, COOH); **\(^13\)C NMR** (100 MHz, (CD\(_3\))\(_2\)SO): \(\delta\) 21.7 (CH\(_2\), CH\(_2\)CH\(_2\)CH\(_2\)), 34.2 (CH\(_2\), COCH\(_2\)), 53.2 (2 \(\times\) CH\(_2\), CH\(_2\)N(CH\(_2\))\(_2\)), 57.6 (CH\(_2\), CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 66.1 (2 \(\times\) CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 119.7 (CH, Ar-C), 122.6 (CH, Ar-C), 123.6 (CH, Ar-C), 128.6 (CH, Ar-C), 132.2 (quat., Ar-C), 139.4 (quat., Ar-C), 167.4 and 171.3 (C=O, CO\(_2\)H and NHCOCH\(_2\)); **m/z** (ESI\(^+\)): 293 (MH\(^+\), 20%), 264 (1%), 167 (11%), 145 (100%).

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Dibenzyl terephthalate (159)$^{165,227}$

Adopting the method of Chandraratna$^{165}$, to a solution of terephthaloyl chloride 158 (6.84 g, 33.7 mmol) dissolved in dry toluene (80 mL) was added benzyl alcohol (9.28 mL, 90.1 mmol) and triethylamine (20 mL, 144.5 mmol). The mixture was stirred for over night at room temperature under an atmosphere of nitrogen gas. After completion, the reaction mixture was poured into ice cold 2 M hydrochloric acid solution (100 mL). The mixture was extracted with (4 × 100 mL) ethyl acetate, washed with water (250 mL), dried (MgSO$_4$), filtered and evaporated the solvent in vacuo, to give the title compound 159 (10.3 g, 88%) as an off-white solid. The product was recrystallised from ethanol as clear crystals (m.p. 94-95 °C from ethanol, lit$^{227}$ m.p 95.5-97 °C).

HRMS Found (El$^+$): M$^+$ 346.1202, C$_{22}$H$_{18}$O$_4$ requires 346.1205; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.38 (4H, s, CH$_2$), 7.34-7.46 (10H, m, Ar-H), 8.12 (4H, s, Ar-H); m/z (El$^+$) 346 (M$^+$, 17 %), 256 (M$^+$-C$_7$H$_6$, 30%), 239 (M$^+$-C$_7$H$_7$O, 60%), 149 (M$^+$-C$_{14}$H$_{13}$O, 100%).

4-(Benzyloxy carbonyl)benzoic acid (160)$^{165,227}$

Using the method of Chandraratna,$^{165}$ to a solution of terephthalate 159 (6.0 g, 17.3 mmol) in water (5 mL) and acetone (5 mL) was added a solution of lithium hydroxide monohydrate (727 mg, 17.3 mmol) in water (15 mL) and acetone (45 mL) dropwise and the resultant mixture was refluxed for 30 minutes. The reaction mixture was cooled and the acetone was evaporated. The aqueous portion was washed with diethyl ether (2 × 50 mL) to remove the unreacted starting material. The aqueous layer was acidified with glacial acetic acid and the resultant precipitate was filtered, washed with fresh water (15 mL) and recrystallised from water-acetone as white crystals to give the title compound 160 (4.1 g, 92%) (m.p. 180-181 °C from ethanol, lit$^{228}$ 178-180 °C).
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**HRMS** Found (El⁺): M⁺ 256.0737, C₁₅H₁₂O₄ requires 256.0736; **¹H NMR** (400 MHz, CD₃OD): δ 5.36 (2H, br s, CH₂), 7.25-7.36 (5H, m, Ar-H), 8.01 (4H, s, Ar-H); **¹³C NMR** (100 MHz, CD₃OD): δ 67.0 (CH₂, ArCH₂O), 128.1 (2 × CH, Ar-H), 128.2 (2 × CH, Ar-H), 128.5 (2 × CH, Ar-H), 129.4 (2 × CH, Ar-H), 129.6 (CH and quat., Ar-H), 133.5 (quat., Ar-C), 135.4 (quat., Ar-C), 165.8 and 167.8 (C=O, CO₂CH₂ and COOH); **¹⁶⁵ m/z (El⁺):** 256 (M⁺, 30%), 238 (12%), 166 (15%), 149 (100%), 121 (9%).

**General procedure 10: Synthesis of benzyl ester having amino-alkyl chains**

Using the method of Finke,¹⁶⁶ to a solution offreshly dried acid 160 (1 eq.) in dry DCM (2.5 mL/mmol acid) was added oxalyl chloride (2.5 eq.) and dry DMF (2 drops). The reaction mixture was stirred for 4 hours at room temperature under an atmosphere of nitrogen. The solvent and an excess of oxalyl chloride were removed under high vacuum. The resultant acid chloride 102 was dried under high vacuum for 2 hours, and was then used in the next reaction. To a stirred solution of the acid chloride 102 (1 eq.) in dry dichloromethane (2.5 mL/mmol) was added the required amine (2 eq.) dropwise under an atmosphere of nitrogen gas. The reaction mixture was then stirred overnight. Solvent was then evaporated and the crude product was dissolved in methanol (4 mL). Silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica. This silica was then loaded and purified by flash chromatography to give the product.

**Benzyl 4-(2-(dimethylamino)ethylcarbamoyl)benzoate (161)**

![Chemical structure](image)

The reaction was carried out according to general procedure 10, with acid chloride 102 (5.36 g, 19.5 mmol) and N,N-dimethylethylenediamine (4.49 mL, 39.0 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to furnish the **title compound 161** (4.7 g, 74%) as a white crystalline solid (m.p. 66-67 °C from methanol).
**Chapter 6: Experimental**

**R**$_f$ (DCM/MeOH 4:1) = 0.32; **HRMS** Found (ESI$^+$): MH$^+$ 327.1709, C$_{19}$H$_{23}$N$_2$O$_3$ requires 327.1709; **IR** $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3350 (N-H), 2940 (C-H), 1711 (C=O, ester), 1636, 1542, 1456, 1379, 1266; **$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 2.26 (6H, s, N(CH$_3$)$_2$)$_2$, 2.52 (2H, t, J = 5.8 Hz, NCH$_2$), 3.50 (2H, dt, J = 5.6 Hz, CONHCH$_2$), 5.33 (2H, s, ArCH$_2$O), 7.19 (1H, br s, N-H), 7.30-7.37 (3H, m, Ar-H), 7.40-7.42 (2H, m, Ar-H), 7.84 (2H, d, J = 8.4 Hz, Ar-H), 8.07 (2H, d, J = 8.4 Hz, Ar-H); **$^{13}$C NMR** (100 MHz, CDCl$_3$): $\delta$ 37.0 (CH$_2$), 144.9 (2 × CH, N(CH$_3$)$_2$), 56.7 (CH$_2$, CONHCH$_2$), 66.8 (CH$_2$, ArCH$_2$O), 127.0 (2 × CH, Ar-C), 128.0 (2 × CH, Ar-C), 128.2 (CH, Ar-C), 128.4 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 132.3 (quat., Ar-C), 135.6 (quat., Ar-C), 138.4 (quat., Ar-C), 165.5 and 166.4 (C=O, CO$_2$ and NHCO); m/z (ESI$^+$): 327 (MH$^+$, 100%), 282 (2%).

**Benzyl 4-(3-(diethylamino)propylcarbamoyl)benzoate (162)**

![Diagram of Benzyl 4-(3-(diethylamino)propylcarbamoyl)benzoate (162)](image)

The reaction was carried out according to general procedure 10, with acid chloride 102 (4.93 g, 18.0 mmol) and 3-(diethylamino)propylamine (5.71 mL, 35.9 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to furnish the **title compound 162** (4.05 g, 61%) as a yellow oil.

**R**$_f$ (DCM/MeOH 4:1) = 0.33; **HRMS** Found (ESI$^+$): MH$^+$ 369.2181, C$_{22}$H$_{29}$N$_2$O$_3$ requires 369.2178; **IR** $\nu_{\text{max}}$(Oil/cm$^{-1}$): 3375 (N-H), 2970 (C-H), 1710 (C=O, ester), 1641, 1542, 1497, 1376, 1268; **$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 1.14 (6H, t, J = 7.4 Hz, N(CH$_2$CH$_3$)$_2$), 1.95 (2H, p, J = 6.4 Hz, CH$_2$CH$_2$CH$_2$), 2.79-2.88 (6H, m, NCH$_2$CH$_2$, N(CH$_2$CH$_3$)$_2$), 3.51 (2H, dt, J = 5.9 Hz, CONCH$_2$), 5.29 (2H, s, ArCH$_2$O), 7.27-7.34 (3H, m, Ar-H), 7.36-7.38 (2H, m, Ar-H), 7.93 (2H, d, J = 8.4 Hz, Ar-H), 8.03 (2H, d, J = 8.4 Hz, Ar-H), 8.80 (1H, t, J = 5.0 Hz, N-H); **$^{13}$C NMR** (100 MHz, CDCl$_3$): $\delta$ 9.4 (2 × CH$_3$, N(CH$_2$CH$_3$)$_2$), 23.8 (CH$_2$, CH$_2$CH$_2$CH$_2$), 38.3 (CH$_2$, NCH$_2$CH$_2$), 46.3 (2 × CH$_2$, N(CH$_2$CH$_3$)$_2$), 50.6 (CH$_2$, CONHCH$_2$), 66.8 (CH$_2$, ArCH$_2$O), 127.2 (2 × CH, Ar-C), 128.0 (2 × CH, Ar-C), 128.1 (CH, Ar-C), 128.4 (2 × CH, Ar-C), 128.5 (CH, Ar-C), 132.3 (quat., Ar-C), 153.5 (quat., Ar-C), 156.4 (quat., Ar-C), 166.4 (C=O, CO$_2$ and NHCO); m/z (ESI$^+$): 327 (MH$^+$, 100%), 282 (2%).
Chapter 6: Experimental

Ar-C), 129.5 (2 × CH, Ar-C), 132.2 (quat., Ar-C), 135.5 (quat., Ar-C), 138.0 (quat., Ar-C), 165.5 and 166.5 (C=O, CO₂ and NHCO); m/z (ESI⁺): 369 (MH⁺, 100%), 293 (2%).

Benzyl 4-(3-(dimethylamino)propylcarbamoyl)benzoate (163)

The reaction was carried out according to general procedure 10, with acid chloride 102 (1.07 g, 3.90 mmol) 3-dimethylaminopropylamine (1.0 mL, 7.79 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to furnish the **title compound 163** (1.24g, 94%) as a light yellow thick oil.

**Rf** (DCM/MeOH 4:1) = 0.16; **HRMS** Found (El⁺): M⁺ 340.1793, C₂₀H₂₄N₂O₃ requires 340.1787; **IR** \( \nu_{\text{max}} \) (Oil)/cm⁻¹: 3399 br (N-H), 2948 (C-H), 1716 br (C=O, ester), 1639, 1543, 1456, 1376, 1267; **¹H NMR** (400 MHz, CDCl₃): δ 1.88 (2H, p, \( J = 6.2 \) Hz, CH₂CH₂CH₂), 2.44 (6H, s, N(CH₃)₂), 2.70 (2H, t, \( J = 6.2 \) Hz, NCH₂), 3.54 (2H, dt, \( J = 6.0, 5.6 \) Hz, CONH), 5.32 (2H, s, ArCH₂O), 7.30-7.42 (5H, m, Ar-H), 7.88 (2H, d, \( J = 8.4 \) Hz, Ar-H), 8.06 (2H, d, \( J = 8.4 \) Hz, Ar-H), 8.69 (1H, t, \( J = 4.6 \) Hz, N-H); **¹³C NMR** (100 MHz, CDCl₃): δ 24.5 (CH₂, CH₂CH₂CH₂), 39.0 (CH₂, CONHCH₂), 44.3 (2 × CH₃, N(CH₃)₂), 57.7 (CH₂, NCH₂), 66.9 (CH₂, ArCH₂O), 127.0 (2 × CH, Ar-C), 128.1 (2 × CH, Ar-C), 128.2 (2 × CH, Ar-C), 128.5 (CH, Ar-C), 129.7 (2 × CH, Ar-C), 132.3 (quat., Ar-C), 135.6 (quat., Ar-C), 138.3 (quat., Ar-C), 165.6 and 166.3 (C=O, CO₂ and NHCO); m/z (El⁺) 340 (M⁺, 10%), 325 (1%), 296 (1%), 268 (3%), 239 (M⁺-C₅H₁₃N₂, 5%), 178 (1%), 91 (10%), 58 (100%).
Benzyl 4-(3-morphinopropylcarbamoyl)benzoate (164)

The reaction was carried out according to general procedure 10, with acid chloride 102 (2.96 g, 10.8 mmol) and 3-morphinopropylamine (3.15 mL, 21.6 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 164 (3.63 g, 88%) as a white crystalline solid (m.p 72-73 ºC from methanol).

R_f (DCM/MeOH 4:1) = 0.74; HRMS Found (ESI^+): MH^+ 383.1959, C_{22}H_{27}N_2O_4 requires 383.1971; IR \nu_{max}(solid)/cm^{-1}: 3298 (N-H), 2868 (C-H), 1711 (C=O, ester), 1634, 1542, 1499, 1381, 1270; \textbf{^1H NMR} (400 MHz, CDCl_3): \delta 1.74 (2H, p, J = 6.1 Hz, CH_2CH_2CH_2), 2.43 (4H, t, J = 4.4 Hz, CH_2N(CH_2CH_2)^2), 2.49 (2H, t, J = 6.0 Hz, CH_2N(CH_2CH_2)^2), 3.51 (2H, dt, J = 6.0, 5.2 Hz, CONHCH_2), 2.49 (2H, t, J = 4.4 Hz, O(CH_2CH_2)^2), 3.53 (2H, s, ArCH_2O), 3.62 (4H, t, J = 4.4 Hz, O(CH_2CH_2)^2), 4.67 (2H, d, J = 8.4 Hz, Ar-H), 6.05 (2H, d, J = 8.4 Hz, Ar-H), 7.21 (2H, t, J = 2.0 Hz, Ar-H), 7.32 (2H, s, ArH), 7.35 (3H, m, Ar-H), 7.41 (1H, br s, Ar-H), 7.74 (1H, t, J = 2.0 Hz, Ar-H), 7.82 (2H, d, J = 8.4 Hz, Ar-H), 8.07 (2H, d, J = 8.4 Hz, Ar-H), 8.24 (1H, t, J = 4.4 Hz, N-H); \textbf{^13C NMR} (100 MHz, CDCl_3): \delta 23.9 (CH_2, CH_2CH_2CH_2), 40.5 (CH_2, CONHCH_2), 53.6 (2 \times CH_2, CH_2N(CH_2CH_2)^2), 58.4 (CH_2, CH_2N(CH_2CH_2)^2), 66.7 (2 \times CH_2, O(CH_2CH_2)^2), 66.8 (CH_2, ArCH_2O), 126.9 (2 \times CH, Ar-C), 128.1 (2 \times CH, Ar-C), 128.2 (CH, Ar-C), 128.5 (2 \times CH, Ar-C), 129.6 (2 \times CH, Ar-C), 132.3 (quat., Ar-C), 135.5 (quat., Ar-C), 138.7 (quat., Ar-C), 165.4 and 166.2 (C=O, CO_2 and NHCO); m/z (ESI^+): 383 (MH^+, 100%).

Benzy 4-(2-morpholinoethylcarbamoyl)benzoate (165)
The reaction was carried out according to general procedure 10, with acid chloride 102 (4.93 g, 18.0 mmol) and 2-morpholinoethylamine (4.77 mL, 35.9 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 165 (4.34 g, 66%) as a white crystalline solid (m.p 78-79 °C from methanol).

\[ \text{Rf (DCM/MeOH 4:1) = 0.82; HRMS Found (ESI\(^+\)): MH}^+ 369.1809, \text{ C}_{21}\text{H}_{25}\text{N}_2\text{O}_4 \text{ requires 369.1814; IR } \nu_{\text{max}}(\text{solid})/\text{cm}^{-1}: 3351 (\text{N-H}), 2881 (\text{C-H}), 1721 (\text{C=O, ester}), 1638, 1542, 1376, 1265; ^1\text{H NMR (400 MHz, CDCl}_3): \delta 2.49 (4\text{H, br s, CH}_2\text{N(CH}_2\text{CH}_2)_2), 2.58 (2\text{H, t, } J = 6.0 \text{ Hz, CH}_2\text{N(CH}_2\text{CH}_2)_2), 3.53 (2\text{H, dt, } J = 5.6 \text{ Hz, CONHCH}_2), 3.70 (4\text{H, br s, O(CH}_2\text{CH}_2)_2), 5.35 (2\text{H, s, ArCH}_3\text{O}), 6.94 (1\text{H, s, N-H}), 7.33-7.39 (3\text{H, m, Ar-H}), 7.42 (1\text{H, br s, Ar-H}), 7.71 (1\text{H, t, } J = 1.6 \text{ Hz, Ar-H}), 7.81 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}), 8.07 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}); ^1\text{C NMR (100 MHz, CDCl}_3): \delta 36.0 (\text{CH}_2, \text{CONHCH}_2), 53.2 (2 \times \text{CH}_2, \text{N(CH}_2\text{CH}_2)_2), 56.7 (\text{CH}_2, \text{CH}_2\text{N(CH}_2\text{CH}_2)_2), 66.8 (2 \times \text{CH}_2, \text{O(CH}_2\text{CH}_2)_2), 67.0 (\text{CH}_2, \text{ArCH}_2\text{O}), 126.9 (2 \times \text{CH, Ar-C}), 128.2 (2 \times \text{CH, Ar-C}), 128.3 (\text{CH}_3, \text{Ar-C}), 128.6 (2 \times \text{CH, Ar-C}), 129.8 (2 \times \text{CH, Ar-C}), 132.5 (\text{quat., Ar-C}), 135.6 (\text{quat., Ar-C}), 138.5 (\text{quat., Ar-C}), 165.5 and 166.4 (\text{C=O, CO}_2 \text{and NHCO}); \text{m/z (ESI}^+)\text{: 369 (MH}^+, 100\%), 282 (1\%).

4-(2-Dimethylamino)ethylcarbamoyl)benzoic acid (153)

\[ \text{Rf (MeOH/NH}_3 9:1) = 0.70; \text{ HRMS Found (ESI}^+)\text{: MH}^+ 237.1235, \text{ C}_{12}\text{H}_{17}\text{N}_2\text{O}_3 \text{ requires 237.1239; IR } \nu_{\text{max}}(\text{solid})/\text{cm}^{-1}: 3343 (O-H, N-H), 2967 (C-H), 1692 (\text{C=O}), 1641, 1620, 1535, 1487, 1318, 1275; ^1\text{H NMR (400 MHz, CD}_3\text{OD): } \delta 2.88 (6\text{H, s,N(CH}_3)_2), 3.07 (2\text{H, t, } J = 5.4 \text{ Hz, NCH}_2), 3.41 (2\text{H, q, } J = 5.9 \text{ Hz, CONHCH}_2), 7.85 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}), 7.95 (2\text{H, d),}

The reaction was carried out according to general procedure 2, with ester 161 (2.0 g, 6.13 mmol) to give the title compound 153 (1.45 g, 100%) as a white solid (m.p 209-210°C from methanol).

\[ \text{Rf (MeOH/NH}_3 9:1) = 0.70; \text{ HRMS Found (ESI}^+)\text{: MH}^+ 237.1235, \text{ C}_{12}\text{H}_{17}\text{N}_2\text{O}_3 \text{ requires 237.1239; IR } \nu_{\text{max}}(\text{solid})/\text{cm}^{-1}: 3343 (O-H, N-H), 2967 (C-H), 1692 (\text{C=O}), 1641, 1620, 1535, 1487, 1318, 1275; ^1\text{H NMR (400 MHz, CD}_3\text{OD): } \delta 2.88 (6\text{H, s,N(CH}_3)_2), 3.07 (2\text{H, t, } J = 5.4 \text{ Hz, NCH}_2), 3.41 (2\text{H, q, } J = 5.9 \text{ Hz, CONHCH}_2), 7.85 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}), 7.95 (2\text{H, d),} \]
$J = 8.4$ Hz, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 36.4 (CH$_2$, NCH$_2$), 44.0 ($2 \times$ CH$_3$, N(CH$_3$)$_2$), 58.5 (CH$_2$, CONHCH$_2$), 128.8 ($2 \times$ CH, Ar-C), 130.9 ($2 \times$ CH, Ar-C), 135.3 (quat., Ar-C), 138.6 (quat., Ar-C), 169.1 and 170.0 (C=O, NHCO and COOH); m/z (ESI$^+$): 237 (MH$^+$, 100%), 192 (40%), 176 (3%), 146 (23%).

4-(3-Diethylamino)propylcarbamoyl)benzoic acid (154)

The reaction was carried out according to general procedure 2, with ester 162 (1.7 g, 4.61 mmol) to give the title compound 154 (1.28 g, 100%) as a light yellow oil.

R$_f$ (MeOH/NH$_3$ 9:1) = 0.57; HRMS Found (ESI$^+$): MH$^+$ 279.1705, C$_{15}$H$_{23}$N$_2$O$_3$ requires 279.1709; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3364 br (O-H, N-H), 2986 (C-H), 1704 (C=O), 1635, 1591, 1543, 1473, 1378, 1284; $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 1.22 ($6H$, t, $J = 6.8$ Hz, N(CH$_2$CH$_3$)$_2$), 1.96 ($2H$, p, $J = 4.0$ Hz, CH$_2$CH$_2$CH$_2$), 3.09-3.17 ($6H$, m, NCH$_2$CH$_2$, N(CH$_2$CH$_3$)$_2$), 3.40 ($2H$, t, $J = 6.6$ Hz, CONCH$_2$), 7.79 ($2H$, d, $J = 8.4$ Hz, Ar-H), 7.95 ($2H$, d, $J = 8.4$ Hz, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 9.7 ($2 \times$ CH$_3$, N(CH$_2$CH$_3$)$_2$), 25.3 (CH$_2$, CH$_2$CH$_2$CH$_2$), 38.0(CH$_2$, NCH$_2$CH$_2$), 49.5 ($2 \times$ CH$_2$, N(CH$_2$CH$_3$)$_2$), 50.7 (CH$_2$, CONHCH$_2$), 128.3 ($2 \times$ CH, Ar-C), 130.7 ($2 \times$ CH, Ar-C), 137.7 (quat., Ar-C), 138.2 (quat., Ar-C), 169.9 and 170.9 (C=O, NHCO and COOH); m/z (ESI$^+$): 279 (MH$^+$, 100%), 206 (3%), 146 (4%).
The reaction was carried out according to general procedure 2, with ester 163 (820 mg, 2.41 mmol) to give the title compound 155 (602 mg, 100%) as a white solid (m.p 170-171 °C from dichloromethane).

Rf (MeOH/NH3 9:1) = 0.48; HRMS Found (EI⁺): M⁺ 250.1318, C₁₃H₁₈N₂O₃ requires 250.1317; IR νmax(solid)/cm⁻¹: 3429 br (O-H, N-H), 2890 (C-H), 1644 br (C=O), 1598, 1548, 1471, 1327, 1310, 1283; ¹H NMR (400 MHz, CD₃OD): δ 1.98 (2H, p, J = 7.7 Hz, CH₂CH₂CH₂), 2.83 (6H, s, N(CH₂)₂), 3.11 (2H, t, J = 7.6 Hz, NCH₂), 3.45 (2H, t, J = 6.4 Hz, CONHCH₂), 7.77 (2H, d, J = 8.1 Hz, Ar-H), 7.93 (2H, d, J = 8.1 Hz, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ 26.3 (CH₂), 37.8 (CH₂, CONHCH₂), 43.5 (2 × CH₂, N(CH₃)₂), 56.7 (CH₂, NCH₂), 128.0 (2 × CH, Ar-C), 130.4 (2 × CH, Ar-C), 136.9 (quat., Ar-C), 141.6 (quat., Ar-C), 170.4 and 174.0 (C=O, NHCO and COOH); m/z (EI⁺): 250 (M⁺, 21%), 206 (3%), 178 (10%), 149 (24%), 121 (12%), 58 (100%).

4-(3-Morpholinopropylcarbamoyl)benzoic acid (156)

Rf (MeOH/NH3 9:1) = 0.84; HRMS Found (ESI⁺): MH⁺ 293.1498, C₁₅H₂₁N₂O₄ requires 293.1501; IR νmax(solid)/cm⁻¹: 3239 br (O-H, N-H), 2879 (C-H), 1680 (C=O), 1642, 1594, 1542, 1471, 1381, 1295; ¹H NMR (400 MHz, CD₃OD): δ 2.06 (2H, p, J = 7.6 Hz, CH₂CH₂CH₂), 3.04 (2H, J = 7.8 Hz, (CH₂CH₂)₂NCH₂), 3.11 (4H, J = 4.4 Hz, CH₂N(CH₂CH₂)₂), 3.51 (2H, t, J = 5.6 Hz, CONHCH₂), 3.91 (4H, t, J = 4.4 Hz, O(CH₂CH₂)₂), 7.86 (2H, d, J = 8.4 Hz, Ar-H), 8.03(2H, d, J = 8.4 Hz, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ 25.6 (CH₂, CH₂CH₂CH₂), 38.3 (CH₂CONHCH₂), 53.4 (2 × CH₂, CH₂N(CH₂CH₂)₂), 56.5 (CH₂, NCH₂CH₂), 65.8 (2 × CH₂, O(CH₂CH₂)₂), 128.1 (2 × CH, Ar-C), 130.5 (2 × CH, Ar-
C), 137.6 (quat., Ar-C), 139.7 (quat., Ar-C), 169.8 and 172.4 (C=O, NHCO and COOH); m/z (ESI\(^+\)): 293 (MH\(^+\), 100%), 240 (1%), 206 (3%), 146 (4%).

4-(3-Morpholinoethylcarbamoyl)benzoic acid (157)

The reaction was carried out according to general procedure 2, with ester 165 (1.7 g, 4.61 mmol) to give the title compound 157 (1.28 g, 100%) as a light brown solid (m.p 189-190 °C methanol).

R\(_f\) (MeOH/NH\(_3\) 9:1) = 0.89; HRMS Found (ESI\(^+\)): MH\(^+\) 279.1339, C\(_{14}\)H\(_{19}\)N\(_2\)O\(_4\) requires 279.1345; IR \(\nu\)\(_{\text{max}}\) (solid)/cm\(^{-1}\): 3345 (O-H, N-H), 2944 (C-H), 1670 (C=O), 1644, 1592, 1557, 1465, 1361, 1291; \(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO): \(\delta\) 2.50 (4H, t, \(J = 4.4\) Hz, CH\(_2\)N(C\(_2\)H\(_2\)CH\(_2\))\(_2\)), 2.56 (2H, t, \(J = 6.8\) Hz, CH\(_2\)N(C\(_2\)H\(_2\)CH\(_2\))\(_2\)), 3.46 (2H, dt, \(J = 6.3\) Hz, CONHCH\(_2\)), 3.62 (4H, \(J = 4.6\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.95 (2H, d, \(J = 6.8\) Hz, Ar-H), 8.03 (2H, d, \(J = 6.8\) Hz, Ar-H), 8.61 (1H, t, \(J = 5.6\) Hz, CONH); \(^{13}\)C NMR (100 MHz, (CD\(_3\))\(_2\)SO): \(\delta\) 36.4 (CH\(_2\), CONHCH\(_2\)), 53.0 (2 × CH\(_2\), N(CH\(_2\)CH\(_2\))), 57.1 (CH\(_2\), CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 65.9 (2 × CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 127.2 (2 × CH, Ar-C), 129.1 (2 × CH, Ar-C), 133.3 (quat., Ar-C), 138.0 (quat., Ar-C), 165.4 and 166.8 (C=O, NHCO and COOH); m/z (ESI\(^+\)): 279 (M\(^+\), 100%), 192 (10%), 146 (16%).

6.3.2 Synthesis of diaryl acids

General procedure 11: Coupling of amine with acid chloride

Using the method of Cotton,\(^{167}\) to a solution of acid chloride 102 (1 eq.) in dry THF (5 mL/mmol) was added aniline 113 (1.25 eq.) in dry THF (4 mL/mmol aniline) in the presence of potassium carbonate (2.7 eq.). The reaction mixture was stirred for 30 minutes. After
completion of the reaction, THF was removed under reduced pressure to leave a white solid. Water (15 mL) was added to the remaining solid residue with vigorous stirring and the solid was filtered, washing with water until the filtrate pH 7.0 was achieved and dried under high vacuum.

**Benzyl 4-(3-acetamidophenylcarbamoyl)benzoate (168)**

![Chemical Structure](image)

The reaction was carried out according to general procedure 11, with acid chloride 102 (1.07 g, 3.90 mmol) and aniline 113 (731 mg, 4.88 mmol) to give the title compound 168 (1.2 g, 79%) as a white solid (m.p. 180-183 °C from dichloromethane).

**Rf** (MeOH/NH3 9:1) = 0.88; **HRMS** Found (ESI+): MH+ 389.1503, C23H21N2O4 requires 389.1501; **IR** νmax(NaCl)/cm⁻¹: 3301 (N-H), 1718 (C=O, ester), 1660, 1644 (C=O), 1604, 1537, 1484, 1420, 1275; **¹H NMR** (400 MHz, (CD₃)₂SO): δ 2.03 (3H, s, NHCOCH₃), 5.38 (2H, s, ArCH₂O), 7.24 (1H, t, J = 8.0 Hz, Ar-H), 7.73 (2H, d, J = 8.0 Hz, Ar-H), 7.40-7.42 (3H, m, Ar-H), 7.48 (2H, d, J = 8.0 Hz, Ar-H), 8.04-8.11 (5H, br m, Ar-H), 9.97 and 10.44 (2H, NHCOCH₃ and CONHAr); **¹³C NMR** (100 MHz, (CD₃)₂SO): δ 23.9 (CH₃, NHCOCH₃), 66.4 (CH₂, ArCH₂O), 111.2 (CH, Ar-C), 115.2 (CH, Ar-C), 127.6 (CH, Ar-C), 127.9 (2 × CH, Ar-C), 128.0 (2 × CH, Ar-C), 128.4 (2 × CH, Ar-C), 128.6 (2 × CH, Ar-C), 129.1 (2 × CH, Ar-C), 131.8 (quat., Ar-C), 135.8 (quat., Ar-C), 139.0 (quat., Ar-C), 139.1 (quat., Ar-C), 139.5 (quat., Ar-C), 164.6, 164.9 and 168.2 (C=O, NHCOCH₃, NHCOAr, CO₂CH₂); **m/z** (ESI+): 389 (MH+, 100%), 256 (20%).

**4-(3-Acetamidophenylcarbamoyl)benzoic acid (166)**

![Chemical Structure](image)
The reaction was carried out according to general procedure 2, with ester 168 (200 mg, 0.51 mmol) to give the title compound 166 (153 mg, 100%) as a white solid (m.p. 286-287 °C from methanol). Diluting the reaction mixture after completion of the reaction prior to filtration ensured a quantitative yield.

**R**f (MeOH/NH₃ 9:1) = 0.85; **HRMS** Found (ESI⁺): MH⁺ 299.1017, C₁₆H₁₅N₂O₄ requires 299.1032; **IR** ν max(solid)/cm⁻¹: 3306 (O-H, N-H), 1718 (C=O), 1644 (C=O), 1604, 1537, 1484, 1420, 1277; **¹H NMR** (400 MHz, (CD₃)₂SO): δ 2.05 (3H, s, NHCO₂C₆H₃), 7.26 (1H, t, J = 8.2 Hz, Ar-H), 7.35 (1H, d, J = 8.0 Hz, Ar-H), 7.43 (1H, d, J = 8.0 Hz, Ar-H), 8.04 (2H, d, J = 8.8 Hz, Ar-H), 8.07 (2H, d, J = 8.8 Hz, Ar-H), 8.11 (1H, s, Ar-H), 9.98 and 10.42 (2H, CONH₂CH₃ and CONHAr), 13.23 (1H, br s, COOH); **¹³C NMR** (100 MHz, (CD₃)₂SO): δ 23.9 (CH₃, NHCO₂C₆H₃), 111.2 (CH, Ar-C), 114.7 (CH, Ar-C), 115.2 (CH, Ar-C), 127.9 (2 × CH, Ar-C), 128.6 (CH, Ar-C), 129.1 (2 × CH, Ar-C), 133.2 (quat., Ar-C), 138.6 (quat., Ar-C), 139.1 (quat., Ar-C), 139.5 (quat., Ar-C), 164.8, 166.7 and 168.2 (C=O, NHCOAr, NHCO and COOH); m/z (ESI⁺): 299 (MH⁺, 100%).

**Benzyl 4-(3-(4-(dimethylamino)butanamido)phenylcarbamoyl)benzoate (169)**

The reaction was carried out according to general procedure 11, with acid chloride 102 (1.07 g, 3.90 mmol) and aniline 114 (1.08 g, 4.87 mmol) to give the title compound 169 (1.68 g, 94%) as a white solid (m.p. 125-126 °C from water) which was used without purification.

**R**f (MeOH/NH₃ 9:1) = 0.71; **HRMS** Found (ESI⁺): MH⁺ 460.2231, C₂₇H₃₀N₃O₄ requires 460.2236; **IR** ν max(solid)/cm⁻¹: 3304 (N-H), 2934 (C-H), 1717 (C=O, ester), 1658 (C=O), 1644 (C=O), 1606, 1533, 1488, 1427, 1271; **¹H NMR** (400 MHz, CD₃OD): δ 1.84 (2H, p, J = 7.8 Hz, CH₂CH₂CH₂), 2.23 (6H, s, N(CH₃)₂), 2.36 (2H, t, J = 7.4 Hz, NHCOCH₂), 3.33 (2H, s, NCH₂), 5.35 (2H, s, ArCH₂O), 7.25 (1H, t, J = 8.0 Hz, Ar-H), 7.31-7.39 (5H, m, Ar-H), 7.43
Methyl 4-(3-(4-(dimethylamino)butanamido)phenylcarbamoyl)benzoate (170)

![Chemical structure](image)

To a solution of acid chloride 102 (428 mg, 1.56 mmol) in dry THF (6 mL) was added aniline 114 (430 g, 1.95 mmol) in dry THF (6 mL) in the presence of potassium carbonate (581 mg, 4.21 mmol). The reaction mixture was stirred for 30 minutes. The THF was then removed under reduced pressure. The resultant white suspension was heated to dissolve the product in methanol in the presence of potassium carbonate which gave the title compound 170 (231 mg, 39%) as a brown gum.

Rf (MeOH/NH3 9:1) = 0.66; HRMS Found (ESI+): MH+ 384.1912, C21H26N3O4 requires 384.1923; IR νmax(solid)/cm⁻¹: 3323 (N-H), 2960 (C-H), 1714 (C=O, ester), 1657 (C=O), 1644 (C=O), 1602, 1531, 1481, 1424, 1326, 1283; ¹H NMR (400 MHz, CD3OD): δ 1.85 (2H, p, J = 7.2 Hz, CH2CH2CH2), 2.26 (6H, s, N(CH3)2), 2.37 (2H, t, J = 7.4 Hz,NHCOC2H2), 2.39 (2H, t, J = 7.6 Hz, NCH2), 3.9 (3H, s, OCH3), 7.25 (1H, t, J = 8.0 Hz, Ar-H), 7.71 (1H, d, J = 7.2 Hz, Ar-H), 7.38 (1H, d, J = 8.4 Hz, Ar-H), 7.95 (2H, d, J = 8.8 Hz, Ar-H), 8.01 (1H, t, J = 2.0 Hz, Ar-H), 8.07 (2H, d, J = 8.4 Hz, Ar-H); ¹³C NMR (100 MHz, CD3OD): δ 24.2 (CH2, CH2CH2CH2), 35.7 (CH2, NHCOC2H2), 45.3 (2 × CH3,N(CH3)2), 52.9 (CH3, OCH3), 59.9 (CH2, NCH2), 114.1 (CH, Ar-C), 117.6 (CH, Ar-C), 117.9 (CH, Ar-C), 128.9 (2 × CH, Ar-C), 129.4 (2 × CH, Ar-C), 129.5 (CH, Ar-C), 129.7 (2 × CH, Ar-C), 130.1 (CH, Ar-C), 130.8 (2 × CH, Ar-C), 134.1 (quat., Ar-C), 137.4 (quat., Ar-C), 140.2 (quat., Ar-C), 140.4 (quat., Ar-C), 140.6 (quat., Ar-C), 167.0, 167.8 and 173.9 (C=O, NHCOC2H2 and NHCOC2H2); m/z (ESI+): 460 (MH+, 100%), 384 (4%), 123 (3%).
130.1 (2 × CH, Ar-C), 130.7 (CH, Ar-C), 134.0 (quat., Ar-C), 140.2 (quat., Ar-C), 140.4 (quat., Ar-C), 140.5 (quat., Ar-C), 167.8, 167.9 and 173.9 (C=O, NHCO, CO₂CH₃ and NHCO); m/z (ESI⁺): 384 (MH⁺, 100%), 339 (4%), 123 (2%).

4-(3-(4-(Dimethylamino)butanamido)phenylcarbamoyl)benzoic acid (167)

The reaction was carried out according to general procedure 2, with ester 169 (1.73 g, 3.76 mmol) to give the title compound 167 (1.39 g, 100%) as a white foam.

Rᵣ (MeOH/NH₃ 9:1) = 0.86; HRMS Found (ESI⁺): MH⁺ 370.1760, C₂₀H₂₄N₃O₄ requires 370.1767; IR νmax(solid)/cm⁻¹: 3255 br (O-H, N-H), 2962 (C-H), 1650 br (C=O), 1591, 1542, 1481, 1420; ¹H NMR (400 MHz, CD₃OD): δ 1.96 (2H, p, J = 7.2 Hz, CH₂CH₂CH₂), 2.43 (2H, t, J = 8.0 Hz, NHCOCH₂), 2.71 (6H, s, N(CH₃)₂), 2.43 (2H, t, J = 7.8 Hz, NCH₂), 7.20 (1H, t, J = 8.0 Hz, Ar-H), 7.25 (2H, br t, J = 8.2 Hz, Ar-H), 7.81 (2H, d, J = 8.4 Hz, Ar-H), 7.96 (2H, d, J = 8.0 Hz, Ar-H), 8.0 (1H, s, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ 21.8 (CH₂, CH₂CH₂CH₂), 34.5 (CH₂, NHCOCH₂), 43.7 (2 × CH₃, N(CH₃)₂), 58.7 (CH₂, NCH₂), 114.3 (CH, Ar-C), 117.5 (CH, Ar-C), 118.1 (CH, Ar-C), 128.3 (2 × CH, Ar-C), 130.1 (CH, Ar-C), 130.4 (2 × CH, Ar-C), 137.8 (quat., Ar-C), 140.2 (quat., Ar-C), 140.3 (quat., Ar-C), 141.9 (quat., Ar-C), 168.6, 172.8 and 174.2 (C=O, NHCOAr, CO₂H and NHCOCH₂); m/z (ESI⁺): 370 (MH⁺, 100%), 325 (2%), 123 (10%).
6.4 The synthesis of benzamide alcohols 171 and 172

General procedure 12: Synthesis of benzamides\textsuperscript{133}
To a stirred solution of aniline 98 or 99 (1.0 eq.) in pyridine (25 mL/mmol), under an atmosphere of nitrogen, was added benzoyl chloride (1.5 eq.), and the mixture was stirred at room temperature for 16 hours. Saturated sodium bicarbonate solution (125 mL/mmol) was added and the solution was extracted with ethyl acetate. The organic extract was washed with 2 M hydrochloric acid (2 \times 125 mL/mmol), brine, dried (MgSO\textsubscript{4}) and the solvent was evaporated under reduced pressure to afford the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) or (n-hexane-ethyl acetate 1:2) to afford the pure product.

3-Acetamido-5-benzamidobenzyl acetate (173)

The reaction was carried out according to general procedure 12, with 3-acetamido-5-aminobenzyl acetate 98 (200 mg, 0.90 mmol) and benzoyl chloride (0.156 mL, 1.35 mmol) affording the crude product, which was then purified by flash chromatography (n-hexane-ethyl acetate 1:2) to afford the title compound 173 (260 mg, 89%) as a light yellow solid (m.p. 83-85 °C from dichloromethane).

R\textsubscript{f} (DCM/MeOH 9:1) = 0.56; HRMS Found (EI\textsuperscript{+}): MH\textsuperscript{+} 327.1327, C\textsubscript{18}H\textsubscript{19}N\textsubscript{2}O\textsubscript{4} requires 327.1345; IR \nu\textsubscript{max}(solid)/cm\textsuperscript{-1}: 3299 (N-H), 1737 (C=O, ester), 1649 (C=O, amide), 1608, 1546, 1452, 1421, 1364, 1225; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \delta 2.00 (3H, s, OCO\textsubscript{CH\textsubscript{3}}), 2.02 (3H, s, NHCOC\textsubscript{H\textsubscript{3}}), 7.24 (1H, br s, ArC\textsubscript{H\textsubscript{2}}O), 7.37 (2H, t, J = 7.6 Hz, Ar-H), 7.41 (1H, br s, Ar-H), 7.46 (1H, t, J = 7.6 Hz, Ar-H), 7.78 (1H, br s, Ar-H), 7.99 (1H, br s, Ar-H), 8.26 and 8.56 (2H, NHCOCH\textsubscript{3} and NHCOAr); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \delta 20.8 (CH\textsubscript{3}, OCOCH\textsubscript{3}), 24.2 (CH\textsubscript{3}, NHCOCH\textsubscript{3}), 65.8 (CH\textsubscript{2}, ArCH\textsubscript{2}O), 111.8 (CH, Ar-C), 115.3 (CH, Ar-C), 115.5 (CH, Ar-C), 127.0 (CH, Ar-C), 127.1 (CH, Ar-H),
128.5 (2 × CH, Ar-C), 131.8 (CH, Ar-C), 134.5 (quat., Ar-C), 137.3 (quat., Ar-C), 138.6 (quat., Ar-C), 138.7 (quat., Ar-C), 166.3, 169.1 and 170.9 (C=O, NHCOAr, NHCOCH₃ and OCOCH₃); m/z (ESI⁺): 267 (M⁺-C₂H₄O₂, 20%), 192 (M⁺-C₈H₇O₂, 5%).

N-(Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)benzamide (174)³³³

The reaction was carried out according to general procedure 12, with aniline 99 (0.9 g, 3.06 mmol) and benzoyl chloride (0.54 mL, 4.58 mmol) gave the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) afforded the title compound 174 (1.02 g, 84%) as a white solid (m.p. 69-70 °C from dichloromethane).

Rᵣ (DCM/Methanol 19:1) = 0.67; HRMS Found (EI⁺): M⁺ 398.2022, C₂₂H₃₀N₂O₃Si requires 398.2026; IR νmax(NaCl)/cm⁻¹: 3294 br (N-H), 2928 (C-H), 1654 (C=O, amide), 1618, 1551, 1458, 1424, 1284; ¹H NMR (400 MHz, CDCl₃): δ 0.12 (6H, s, OSi(CH₃)₂), 0.93 (9H, s, OSi(CH₃)₃), 2.10 (3H, s, NHCOCH₃), 4.69 (2H, s, ArCH₂O), 7.28 (1H, br s, Ar-H), 7.40-7.46 (3H, m, Ar-H), 7.49-7.54 (1H, m, Ar-H), 7.63 (1H, br s, Ar-H), 7.81 (2H, d, J = 7.2 Hz, Ar-H), 7.85 (1H, br s, N-H), 8.1 (1H, br s, N-H); ¹³C NMR (100 MHz, CDCl₃): δ -5.3 (CH₃, OSi(CH₃)₂), 18.4 (quat., OSiC(CH₃)₃), 24.6 (CH₃, NHCOCH₃), 26.0 (3 × CH₃, OSiC(CH₃)₃), 64.6 (CH₂, ArCH₂O), 110.1 (CH, Ar-C), 113.2 (CH, Ar-C), 113.3 (CH, Ar-C), 127.1 (2 × CH, Ar-C), 128.7 (2 × CH, Ar-C), 131.8 (CH, Ar-C), 134.9 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 143.4 (quat., Ar-C), 166.0 and 168.6 (C=O, NHCOAr and NHCOCH₃); m/z (EI⁺) 398 (M⁺, 1%), 341 (M⁺-C₃H₇, 50%), 325 (M⁺-C₂H₅NO₂, 100%), 105 (COC₆H₅, 50%).
Chapter 6: Experimental

\( N(3\text{-Acetamido-5-)((tert-butyldimethylsilyloxy)methyl})phenyl)-4\text{-nitrobenzamide (175)} \)

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{structure175.png}
\end{center}}
\]

The reaction was carried out according to general procedure 12, with aniline 99 (1.8 g, 6.11 mmol) and 4-nitrobenzoyl chloride (1.74 g, 9.17 mmol) to give the crude product, which was then purified by flash chromatography (dichloromethane-methanol 19:1) to afford the \textit{title compound 175} (2.1 g, 77\%) as a yellow solid (m.p. 145-146 °C from ethyl acetate).

\( \text{Rf} \) (DCM/MeOH 9:1) = 0.33; \text{HRMS} \text{ Found (FAB\textsuperscript{+}): MH\textsuperscript{+} 444.1948, } C_{22}H_{30}N_{3}O_{5}Si \text{ requires 444.1955}; \text{IR} \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1}: 3299 (N-H), 2933 (N-H), 1659 (C=O, amide), 1604, 1555, 1526 (N-O), 1455, 1427, 1346 (N-O), 1286; \text{\textsuperscript{1}H NMR} (400 MHz, CDCl\textsubscript{3}): \delta 0.10 (6H, s, OSi(CH\textsubscript{3})\textsubscript{2}), 0.93 (9H, s, OSi(CH\textsubscript{3})\textsubscript{3}), 2.09 (3H, s, NHCOCH\textsubscript{3}), 4.67 (2H, s, ArCH\textsubscript{2}O), 7.13 (1H, br s, Ar-H), 7.49 (1H, s, Ar-H), 7.59 (1H, s, Ar-H), 7.87 (1H, s, N-H), 7.95 (2H, d, \( J = 8.6 \text{ Hz, Ar-H} \)), 8.23 (2H, d, \( J = 8.6 \text{ Hz, Ar-H} \)), 8.53 (1H, s, N-H); \text{\textsuperscript{13}C NMR} (100 MHz, CDCl\textsubscript{3}): \delta -5.0 (2 \times \text{CH\textsubscript{3}}, OSi(CH\textsubscript{3})\textsubscript{2}), 18.7 (quat., OSiC(CH\textsubscript{3})\textsubscript{3}), 24.8 (CH\textsubscript{3}, NHCOCH\textsubscript{3}), 26.2 (3 \times \text{CH\textsubscript{3}}, OSiC(CH\textsubscript{3})\textsubscript{3}), 64.8 (CH\textsubscript{2}, ArCH\textsubscript{2}O), 111.0 (CH, Ar-C), 114.0 (2 \times \text{CH, Ar-C}), 124.1 (2 \times \text{CH, Ar-C}), 128.7 (2 \times \text{CH, Ar-C}), 138.3 (quat., Ar-C), 138.6 (quat., Ar-C), 140.7 (quat., Ar-C), 143.9 (quat., Ar-C), 149.9 (quat., Ar-C), 164.3 and 169.1 (C=O, NHCOAr and NHCOCH\textsubscript{3}); \text{m/z} (FAB\textsuperscript{+}): 444 (MH\textsuperscript{+}, 100\%), 386 (M\textsuperscript{+}-C\textsubscript{2}H\textsubscript{4}NO, 39\%), 370 (M\textsuperscript{+}-C\textsubscript{2}H\textsubscript{4}NO\textsubscript{2}, 33\%).

\( N(3\text{-Acetamido-5-((tert-butyldimethylsilyloxy)methyl})phenyl)-4\text{-aminobenzamide (176)} \)

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{structure176.png}
\end{center}}
\]
The reaction was carried out according to general procedure 2, with nitrobenzamide 175 (200 mg, 0.45 mmol) to afford the title compound 176 (186 mg, 100%) as a white solid (m.p. 108-109 °C from methanol).

Rf (DCM/MeOH 9:1) = 0.49; HRMS Found (FAB\textsuperscript{+}): MH\textsuperscript{+} 414.2210, C\textsubscript{22}H\textsubscript{32}N\textsubscript{3}O\textsubscript{3}Si requires 414.2213; IR ν\textsubscript{max}(NaCl)/cm\textsuperscript{-1}: 3550 br (N-H), 2929 (C-H), 1645 br (C=O, amide), 1605, 1557, 1455, 1421, 1373, 1282; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 0.10 (6H, s, OSi(CH\textsubscript{3})\textsubscript{2}), 0.94 (9H, s, OSiC(CH\textsubscript{3})\textsubscript{3}), 2.13 (3H, s, NHCOC\textsubscript{3}H\textsubscript{3}), 4.02 (2H, br s, NH\textsubscript{2}), 4.70 (2H, s, ArC\textsubscript{H}\textsubscript{2}O), 6.67 (2H, d, J = 6.8 Hz, Ar-H), 7.28 (1H, s, Ar-H), 7.36 (1H, s, Ar-H), 7.42 (1H, s, Ar-H), 7.67 (2H, d, J = 6.8 Hz, Ar-H), 7.80 (2H, s, N-H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ -5.2 (2 × CH\textsubscript{3}, OSi(CH\textsubscript{3})\textsubscript{2}), 18.5 (quat., OSiC(CH\textsubscript{3})\textsubscript{3}), 24.7 (CH\textsubscript{3}, NHCOCH\textsubscript{3}), 26.0 (3 × CH\textsubscript{3}, OSiC(CH\textsubscript{3})\textsubscript{2}), 64.7 (CH\textsubscript{2}, ArCH\textsubscript{2}O), 109.7 (CH, Ar-C), 112.6 (CH, Ar-C), 113.0 (CH, Ar-C), 114.2 (2 × CH, Ar-C), 124.1 (CH, Ar-C), 128.9 (2 × CH, Ar-C), 138.5 (quat., Ar-C), 138.8 (quat., Ar-C), 143.4 (quat., Ar-C), 150.0 (quat., Ar-C), 165.5 and 168.4 (C=O, NHCOAr and NHCOCH\textsubscript{3}); m/z (FAB\textsuperscript{+}): 414 (MH\textsuperscript{+}, 18%), 356 (M\textsuperscript{+}-C\textsubscript{2}H\textsubscript{4}NO, 23%), 282 (M\textsuperscript{+}-C\textsubscript{6}H\textsubscript{15}OSi, 49%), 120 (M\textsuperscript{+}-C\textsubscript{15}H\textsubscript{35}N\textsubscript{2}O\textsubscript{2}Si, 100%).

4-Acetamido-N-(3-acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)benzamide (177)

The reaction was carried out according to general procedure 1, with 4-aminobenzamide 176 (606 mg, 1.46 mmol) and acetic anhydride (0.41 mL, 4.40 mmol) to give the title compound 177 (596 mg, 89%) as a white solid, which was recrystallised in methanol to give clear crystals suitable for single crystal structure analysis (m.p. 130-131 °C from methanol).

Rf (DCM/MeOH 9:1) = 0.44; HRMS Found (EI\textsuperscript{+}): MH\textsuperscript{+} 456.2323, C\textsubscript{24}H\textsubscript{34}N\textsubscript{3}O\textsubscript{4}Si requires 456.2319; IR ν\textsubscript{max}(NaCl)/cm\textsuperscript{-1}: 3408 br (N-H), 2927 (C-H), 1650 br (C=O, amide), 1602, 1528, 1453, 1371, 1288; \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD): δ 0.12 (6H, s, OSi(CH\textsubscript{3})\textsubscript{2}), 0.95 (9H,
s, OSi(CH₃)$_3$, 2.12 (3H, s, NHCOCH$_3$), 2.15 (3H, s, NHCOCH$_3$), 4.71 (2H, s, ArCH$_2$O), 7.38 (1H, s, Ar-H), 7.40 (1H, s, Ar-H), 7.69 (2H, d, $J=8.7$ Hz, Ar-H), 7.84 (1H, t, $J=1.6$ Hz, Ar-H), 7.88 (2H, d, $J=8.7$ Hz, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ -5.1 (CH$_3$, OSi(CH$_3$)$_2$), 19.3 (quat., OSi(CH$_3$)$_3$), 23.9 (CH$_3$, NHCOCH$_3$), 24.0 (CH$_3$, NHCOCH$_3$), 26.5 (3 $\times$ CH$_3$, OSi(CH$_3$)$_3$), 66.1 (CH$_2$, ArCH$_2$O), 112.9 (CH, Ar-C), 115.3 (CH, Ar-C), 116.0 (CH, Ar-C), 120.3 (2 $\times$ CH, Ar-C), 129.6 (2 $\times$ CH, Ar-C), 131.2 (quat., Ar-C), 140.3 (2 $\times$ quat., Ar-C), 143.4 (quat., Ar-C), 144.1 (quat., Ar-C), 168.3, 171.8 and 171.9 (C=O, NHCOAr and 2 $\times$ NHCOCH$_3$); m/z (FAB$^+$): 456 (MH$^+$, 47%), 398 (MH$^+$-C$_2$H$_4$NO, 75%), 324 (M$^+$-C$_6$H$_15$OSi, 100%), 162 (M$^+$-C$_{15}$H$_{25}$N$_2$O$_2$Si, 86%).

**General procedure 13: Synthesis of benzamide alcohols**

Adopting the method of Smith,$^{168}$ to a stirred solution of silyl ether (1.0 eq.) in dry THF (80 mL/mmol), was added glacial acetic acid (4.0 eq.) and TBAF (4.0 eq., 1.0 M in THF) or Et$_3$N.3HF (3.0 eq.) (used for triaryl amides with amino-alkyl chain), and the reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated under reduced pressure and the residue was diluted with methanol (15 mL) and silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica gel. This silica gel was then loaded and purified by flash chromatography. In some cases repeated chromatography was needed to purify the product. Glacial acetic acid was not used with compounds containing an amino-alkyl functionality.

**N-(3-Acetamido-5-(hydroxymethyl)phenyl)benzamide (171)$^{133}$**

![Image of N-(3-Acetamido-5-(hydroxymethyl)phenyl)benzamide](image)

The reaction was carried out according to general procedure 13, with silyl ether 174 (100 mg, 0.25 mmol) to give the crude product, purified by flash chromatography (dichloromethane-methanol 19:1) to afford the **title compound 171** (60 mg, 84%) as a white solid (m.p. 177-178 °C from methanol). Base hydrolysis (2.0 eq. sodium hydroxide) of benzamide 173 also afforded the **title compound 171** in 58% yield.
R<sub>f</sub> (DCM/MeOH 19:1) = 0.41; **HRMS** Found (EI<sup>+</sup>): M<sup>+</sup> 284.1160, C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> requires 284.1161; **IR** ν<sub>max</sub>(NaCl)/cm<sup>-1</sup>: 3247 br (O–H, N–H), 1670 (C=O, amide), 1631, 1613, 1521, 1545, 1437, 1366, 1279; **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): δ 2.12 (3H, s, NHCOC<sub>3</sub>H), 4.58 (2H, s, ArCH<sub>2</sub>O), 7.38 (1H, br s, Ar–H), 7.42 (1H, br s, Ar–H), 7.49 (2H, d, J = 7.4 Hz, Ar–H), 7.56 (1H, t, J = 8.0 Hz, Ar–H), 7.89 (2H, br s, Ar–H); **13C NMR** (100 MHz, CD<sub>3</sub>OD): δ 23.9 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 65.1 (CH<sub>2</sub>, ArCH<sub>2</sub>O), 113.1 (CH, Ar–C), 115.9 (CH, Ar–C), 116.5 (CH, Ar–C), 128.7 (2 × CH, Ar–C), 129.7 (2 × CH, Ar–C), 132.9 (CH, Ar–C), 136.4 (quat., Ar–C), 140.3 (quat., Ar–C), 140.4 (quat., Ar–C), 169.0 and 171.8 (C=O, NHCOAr and NHCOCH<sub>3</sub>); **m/z** (EI<sup>+</sup>): 284 (M<sup>+</sup>, 30%), 242 (M<sup>+</sup>–C<sub>2</sub>H<sub>2</sub>O, 15%), 105 (COC<sub>6</sub>H<sub>5</sub>, 100%), 77 (50%).

4-Acetamido-N-(3-acetamido-5-(hydroxymethyl)phenyl)benzamide (172)

The reaction was carried out according to general procedure 13, with silyl ether 177 (400 mg, 0.88 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the *title compound* 172 (280 mg, 93%) as a white solid (m.p.177-178 °C from methanol).

R<sub>f</sub> (DCM/MeOH 9:1) = 0.18; **HRMS** Found (FAB<sup>+</sup>): MH<sup>+</sup> 342.1451, C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>4</sub> requires 342.1454; **IR** ν<sub>max</sub>(NaCl)/cm<sup>-1</sup>: 3256 br (O–H, N–H), 2962, 1660 (C=O, amide), 1642, 1597, 1563, 1531, 1427, 1398, 1368, 1285; **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): δ 2.12 (3H, s, NHCOC<sub>3</sub>H), 2.16 (3H, s, NHCOC<sub>3</sub>H), 4.58 (2H, s, ArCH<sub>2</sub>O), 7.36 (1H, br s, Ar–H), 7.41 (1H, br s, Ar–H), 7.71 (2H, d, J = 8.7 Hz, Ar–H), 7.88 (1H, br s, Ar–H), 7.90 (2H, d, J = 8.7 Hz, Ar–H); **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD): δ 23.9 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 24.1 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 65.1 (CH<sub>2</sub>, ArCH<sub>2</sub>O), 113.2 (CH, Ar–C), 115.8 (CH, Ar–C), 116.6 (CH, Ar–C), 120.3 (2 × CH, Ar–C), 129.6 (2 × CH, Ar–C), 131.1 (quat., Ar–C), 140.3 (2 × quat., Ar–C), 143.5 (quat., Ar–C), 140.3 (quat., Ar–C).
144.2 (quat., Ar-C), 168.3, 171.8 and 172.0 (C=O, NHCOAr and 2 × NHCOCH₃); **m/z** (FAB⁺): 342 (MH⁺, 30%), 242 (M⁺-C₄H₆NO₂, 85%), 162 (22%), 120 (25%).
6.5 Synthesis of benzylic derivatives

3,5-Dinitrobenzyl methanesulfonate (178)

To a solution of alcohol 92 (1.5 g, 7.57 mmol) and triethylamine (1.6 mL, 11.4 mmol) in dry THF (15 mL) at 0 °C, under an atmosphere of nitrogen, was added dropwise a solution of methanesulfonyl chloride (0.9 mL, 11.4 mmol) in dry THF (5 mL) and the resulting solution stirred at room temperature for 3 hours. The solvent was removed in vacuo and the residue diluted with ethyl acetate (150 mL), washed with brine (50 mL) then dried (MgSO₄). The solvent was removed under reduced pressure to afford the title compound 178 (2.0 g, 96%) as a yellow solid (m.p. 83-84 °C ethyl acetate). The compound 178 was recrystallised in ethyl acetate to give yellow crystals suitable for single crystal structure analysis.

Rf (DCM/MeOH 9:1) = 0.86; HRMS Found (EI⁺): MH⁺ 276.0049, C₈H₈N₂O₇S requires 276.0052; IR νmax(NaCl)/cm⁻¹: 3399, 1627, 1541 (N-O), 1458, 1344 (N-O), 1077 (S-O); ¹H NMR (400 MHz, CDCl₃): δ 3.15 (3H, s, SO₂CH₃), 5.41 (2H, s, ArCH₂O), 8.60 (2H, br s, Ar-H), 9.06 (1H, br s, Ar-H); ¹³C NMR (100 MHz, CDCl₃): δ 38.6 (CH₃, OSO₂CH₃), 67.5 (CH₂, ArCH₂O), 119.6 (2 × CH, Ar-C), 128.3 (2 × CH, Ar-C), 138.6 (quat., Ar-C), 149.1 (quat., Ar-C); m/z (EI⁺):197 (100%), 181 (42%), 134 (20%).

N-(3,5-Dinitrobenzyl)-2-hydroxy-N-(2-hydroxyethyl)ethanamine (179)

To a solution of diethanolamine (2.69 g, 25.3 mmol) in dry THF (10 mL) at 0 °C, under an atmosphere of nitrogen, was added dropwise a solution of mesylate 178 (0.7 g, 2.53 mmol) in dry THF (5 mL) and the mixture was stirred at room temperature for 24 hours. The solvent was removed in vacuo, the residue diluted with ethyl acetate (30 mL) and 2 M hydrochloric acid (15 mL), and the aqueous layer separated. The aqueous extract was neutralised with 4 M

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sodium hydroxide and extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were dried (Na₂SO₄) and the solvent removed in vacuo to afford the crude product, which was purified by flash chromatography (dichloromethane:methanol 9:1) to afford the title compound 179 (0.71 g, 98%) as a yellow solid (m.p. 77-78 °C from ethyl acetate). The compound was recrystallised from dichloromethane and chloroform to give light yellow crystals suitable for single crystal analysis.

Rf (DCM/MeOH 9:1) = 0.56; HRMS Found (FAB⁺): MH⁺ 286.1044, C₁₁H₁₆N₃O₆ requires 286.1039; IR νmax(NaCl)/cm⁻¹: 3427 br (O-H), 2955 (C-H), 1645 br, 1535 (N-O), 1472, 1345 (N-O), 1214; ¹H NMR (400 MHz, CDCl₃): δ 2.74 (4H, t, J = 10.1 Hz, N(CH₂CH₂OH)₂), 3.68 (4H, t, J = 10.1 Hz, N(CH₂CH₂OH)₂), 3.93 (2H, s, ArCH₂N), 8.61 (1H, br s, Ar-H), 8.62 (1H, br s, Ar-H), 8.89 (1H, t, J = 2.0 Hz, Ar-H); ¹³C NMR (100 MHz, CDCl₃): δ 55.9 (CH₂, N(CH₂CH₂OH)₂), 58.4 (CH₂, ArCH₂N), 59.6 (CH₂, N(CH₂CH₂OH)₂), 117.6 (CH, Ar-C), 128.7 (3 × CH, Ar-C), 144.6 (quat., Ar-C), 148.5 (quat., Ar-C); m/z (FAB⁺): 286 (MH⁺, 100%), 254 (10%), 16 (5%), 120 (11%).

2-((3,5-Dinitrobenzyl)(methyl)amino)ethanol (180)

To a solution of 2-(methylamino)ethanol (2.1 mL, 25.3 mmol) in dry THF (10 mL) at 0 °C, under an atmosphere of nitrogen, was added dropwise a solution of mesylate 178 (700 mg, 2.53 mmol) in dry THF (5 mL), and the mixture stirred at room temperature for 18 hours. The solvent was removed in vacuo, the residue diluted with ethyl acetate (10 mL) and 2 M hydrochloric acid (10 mL), and the aqueous layer separated. The aqueous extract was neutralised with 4 M sodium hydroxide and extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were dried (Na₂SO₄) and the solvent removed in vacuo to afford the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 180 (550 mg, 85%) as a white solid (m.p. 54-55 °C from methanol).
**Chapter 6: Experimental**

\( R_f (\text{DCM}/\text{MeOH} 9:1) = 0.62; \) **HRMS** Found (FAB\(^+\)): MH\(^+\) 256.0927, \( C_{10}H_{14}N_3O_5 \) requires 256.0933; **IR** \( \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1} \): 3411 br (O-H), 2952 (C-H), 1629, 1541 (N-O), 1459, 1344 (N-O); \( ^1H \text{NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \) 2.28 (3H, s, N\( \text{CH}_3 \)), 2.66 (2H, t, \( J = 10.6 \) Hz, NCH\(_2\)CH\(_2\)OH), 3.69 (2H, t, \( J = 10.6 \) Hz, NCH\(_2\)CH\(_2\)OH), 3.78 (2H, s, ArC\(_H_2\)N), 8.51 (1H, br s, Ar-H), 8.52 (1H, br s, Ar-H), 8.89 (1H, t, \( J = 2.0 \) Hz, Ar-H); \( ^{13}C \text{NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \) 41.8 (CH\(_3\), N\( \text{CH}_3 \)), 58.9 (CH\(_2\), NCH\(_2\)CH\(_2\)OH), 59.0 (CH\(_2\), ArCH\(_2\)N), 61.1 (CH\(_2\), NCH\(_2\)CH\(_2\)OH), 117.7 (CH, Ar-C), 128.7 (3 \( \times \) CH, Ar-C), 144.0 (quat., Ar-C), 148.5 (quat., Ar-C); m/z (FAB\(^+\)): 256 (MH\(^+\), 100%), 224 (18%), 224 (17%), 178 (3%), 120 (12%).

**N-(3,5-Dinitrobenzyl)-2-chloro-N-(2-chloroethyl)ethanamine (181)**

![N-(3,5-Dinitrobenzyl)-2-chloro-N-(2-chloroethyl)ethanamine](image)

To a stirred solution of diol 179 (100 mg, 0.35 mmol) in dry THF (5 mL) was added phosphorous oxychloride (0.1 mL, 1.05 mmol) at 0 °C, under an atmosphere of nitrogen. The resultant solution was then refluxed at 105 °C for 1 hour. The solvent was evaporated under high vacuum and the crude product was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 181 (50 mg, 44%) as yellow gum.

\( R_f (\text{DCM}/\text{MeOH} 9:1) = 0.92; \) **HRMS** Found (ESI\(^+\)): MH\(^+\) 322.0347, 324.0322 \( C_{11}H_{14}Cl_2N_3O_4 \) requires 322.0361 and 324.0332; **IR** \( \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1} \): 2919 (C-H), 1593, 1536 (N-O), 1458, 1342 (N-O), 1273, 1116; \( ^1H \text{NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \) 3.0 (4H, t, \( J = 10.1 \) Hz, N(CH\(_2\)CH\(_2\)Cl)\(_2\)), 3.57 (4H, t, \( J = 10.1 \) Hz, N(CH\(_2\)CH\(_2\)Cl)\(_2\)), 4.01 (2H, s, ArCH\(_2\)N), 8.62 (1H, br s, Ar-H), 8.63 (1H, br s, Ar-H), 8.90 (1H, t, \( J = 2.0 \) Hz, Ar-H); \( ^{13}C \text{NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \) 41.7 (2 \( \times \) CH\(_2\), N(CH\(_2\)CH\(_2\)Cl)\(_2\)), 55.8 (CH\(_2\), ArCH\(_2\)N), 59.0 (2 \( \times \) CH\(_2\), N(CH\(_2\)CH\(_2\)Cl)\(_2\)), 117.8 (CH, Ar-C), 128.3 (2 \( \times \) CH, Ar-C), 128.4 (CH, Ar-C), 144.4 (quat., Ar-C), 148.5 (quat., Ar-C); m/z (ESI\(^+\)): 322 (MH\(^+\), 100%), 142 (1%).
3-Acetamido-5-benzamidobenzyl methanesulfonate (182)

The reaction was carried out according to general procedure 3, with alcohol 171 (100 mg, 0.35 mmol) to give the title compound 182 (127 mg, 100%) as clear oil. The product was used immediately without further purification. 

Rf (DCM/MeOH 9:1) = 0.61; HRMS Found (ESI+): M+ 362.09371, C17H18N2O5S requires 362.09364; IR νmax (NaCl)/cm⁻¹: 3263 (N-H), 1601, 1555, 1455, 1367 (S=O), 1310, 1136, 1032; 1H NMR (300 MHz, CDCl3): δ 2.04 (3H, s, NHCOC3H3), 2.96 (3H, s, SO2C3H3), 5.06 (2H, s, ArCH2O), 7.32 (1H, br s, Ar-H), 7.38-7.43 (2H, m, Ar-H), 7.48-7.50 (2H, m, Ar-H), 7.80-7.83 (2H, m, Ar-H), 7.93 (1H, s, Ar-H), 8.27 (1H, s, N-H), 8.59 (1H, s, N-H); 13C NMR (75 MHz, CDCl3): δ 24.3 (CH3, NHCOCH3), 38.1 (CH3, OSO2CH3), 71.21 (CH2, ArCH2O), 112.6 (CH, Ar-C), 115.6 (CH, Ar-C), 115.9 (CH, Ar-C), 127.2 (2 × CH, Ar-C), 128.7 (2 × CH, Ar-C), 132.0 (CH, Ar-C), 134.4 (quat., Ar-C), 134.9 (quat., Ar-C), 139.0 (quat., Ar-C), 139.2 (quat., Ar-C), 166.4 and 169.3 (C=O, NHCOAr and NHCOCH3).

3-Acetamido-5-[(4-acetamidobenzamido)benzylmethanesulfonate (183)

The reaction was carried out according to general procedure 3, with alcohol 172 (150 mg, 0.44 mmol) to give the title compound 183 (184 mg, 100%) as a yellow thick gum from DMF. The product was used immediately without further purification.

Rf (DCM/MeOH 9:1) = 0.25; IR νmax (NaCl)/cm⁻¹: 3270 (N-H), 1680, 1644 (C=O), 1598, 1531, 1453, 1415, 1390 (S=O), 1319, 1182 (S=O); 1H NMR (400 MHz, CD3OD): δ 2.11 (3H, s, NHCOC3H3), 2.14 (3H, s, NHCOCH3), 2.97 (3H, s, SO2CH3), 5.07 (2H, s, ArCH2O), 7.36
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(1H, br s, Ar-H), 7.40 (1H, br s, Ar-H), 7.69 (2H, d, \( J = 8.4 \) Hz, Ar-H), 7.88 (2H, d, \( J = 8.7 \) Hz, Ar-H), 7.96 (1H, br s, Ar-H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \( \delta \) 23.9 (CH\(_3\), NHCOCH\(_3\)), 24.0 (CH\(_3\), NHCOCH\(_3\)), 38.0 (CH\(_3\), OSO\(_2\)CH\(_3\)), 69.6 (CH\(_2\), ArCH\(_2\)O), 113.1 (CH, Ar-C), 115.8 (CH, Ar-C), 116.5 (CH, Ar-C), 120.3 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 131.1 (quat., Ar-C), 140.3 (2 × quat., Ar-C), 143.4 (quat., Ar-C), 144.2 (quat., Ar-C), 168.2, 171.8 and 172.0 (C=O, NHAr and 2 × NHAc).

General procedure 14: Addition of nucleophiles to mesylates

A solution of mesylate 182 or 183 (1 eq.) in dry DMF (10mL/mmol), was added to a stirred solution of amine (10 eq.) in dry DMF (1 mL/mmol mesylate) at 0 °C, under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 18 hours. The solvent was evaporated under reduced pressure and the residue diluted with methanol (15 mL), silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica gel. This silica gel was then loaded and purified by flash chromatography. In some cases repeated chromatography was needed to purify the product.

3-Acetamido-5-benzamido-(N,N-bis-(2-hydroxyethyl))benzylamine (184)

![Chemical Structure](image)

The reaction was carried out according to general procedure 14, with mesylate 182 (63 mg, 0.17 mmol) and diethanolamine (182 mg, 1.73 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 184 (39 mg, 60%) as a white solid (m.p. 54-55 °C from methanol).

\( R_f \) (15% MeOH/DCM) = 0.55; HRMS Found (FAB\(^{+}\)): MH\(^+\) 372.1929, C\(_{20}\)H\(_{26}\)N\(_3\)O\(_4\) requires 372.1923; IR \( \nu_{\text{max}}\) (NaCl)/\text{cm}\(^{-1}\): 3272 br (O-H, N-H), 2920 (C-H), 1653(C=O), 1613, 1550, 1456, 1421,1371, 1284; \(^1\)H NMR (400 MHz, CD\(_3\)OD): \( \delta \) 2.10 (3H, s, NHCOCH\(_3\)), 2.68 (4H, t, \( J = 6.0 \) Hz, N(CH\(_2\)CH\(_2\)OH)\(_2\)), 3.61 (4H, t, \( J = 6.0 \) Hz, N(CH\(_2\)CH\(_2\)OH)\(_2\)), 2.68 (4H, s, ArCH\(_2\)N), 7.35 (1H, br s, Ar-H), 7.41 (1H, br s, Ar-H), 7.48 (2H, t, \( J = 7.4 \) Hz, Ar-H), 7.55
(1H, t, J = 7.2 Hz, Ar-H), 7.88 (2H, br s, Ar-H), 7.91 (1H, br s, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): δ 23.9 (CH$_3$, NHCOCH$_3$), 57.5 (2 × CH$_2$, N(CH$_2$CH$_2$OH)$_2$), 60.6 (CH$_2$, ArCH$_2$N), 60.8 (2 × CH$_2$, N(CH$_2$CH$_2$OH)$_2$), 113.1 (CH, Ar-C), 118.1 (CH, Ar-C), 118.7 (CH, Ar-C), 128.7 (2 × CH, Ar-C), 129.7 (2 × CH, Ar-C), 133.0 (CH, Ar-C), 136.3 (quat., Ar-C), 140.2 (quat., Ar-C), 140.3 (quat., Ar-C), 141.8 (quat., Ar-C), 169.0 and 171.7 (C=O, NHCOAr and NHCOCH$_3$); m/z (FAB$^+$): 372 (MH$^+$, 19%), 340 (2%), 267 (4%), 165 (7%), 154 (100%).

$N$-(3-Acetamido-5-(((2-hydroxyethyl)(methyl)amino)methyl)phenyl)benzamide (185)

The reaction was carried out according to general procedure 14, with mesylate 182 (149 mg, 0.41 mmol) and 2-(methylamino)ethanol (0.34 mL, 4.25 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 185 (120 mg, 86%) as a transparent gum from methanol.

R$_f$ (15% MeOH/DCM) = 0.12; HRMS Found (EI$^+$): M$^+$ 341.1748, C$_{19}$H$_{23}$N$_3$O$_3$ requires 341.1739; IR $\nu_{\text{max}}$(NaCl)/cm$^{-1}$: 3269 (O-H, N-H), 2955 (C-H), 1653 (C=O), 1607, 1547 br, 1451, 1421, 1370, 1276; $^1$H NMR (400 MHz, CD$_3$OD): δ 2.09 (3H, s, NHCOC$_3$H$_3$), 2.46 (3H, s, N$_2$C$_3$H$_3$), 2.80 (2H, t, J = 5.2 Hz, N$_2$C$_2$H$_2$OH), 3.72 (2H, t, J = 5.4 Hz, NCH$_2$C$_3$H$_2$OH), 3.80 (2H, s, ArCH$_2$N), 7.40 (1H, br s, Ar-H), 7.45 (3H, brs, Ar-H), 7.53 (1H, t, J = 7.0 Hz, Ar-H), 7.87 (1H, br s, Ar-H), 7.88 (1H, br s, Ar-H), 7.92 (1H, br s, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): δ 24.0 (CH$_3$, NHCOCH$_3$), 42.1 (CH$_3$, NCH$_3$), 59.0 (CH$_2$, NCH$_2$CH$_2$OH), 59.3 (CH$_2$, ArCH$_2$N), 62.5 (CH$_2$, NCH$_2$CH$_2$OH), 114.0 (CH, Ar-C), 118.9 (CH, Ar-C), 119.6 (CH, Ar-C), 128.7 (2 × CH, Ar-C), 129.7 (2 × CH, Ar-C), 133.0 (CH, Ar-C), 136.1 (quat., Ar-C), 137.2 (quat., Ar-C), 140.6 (2 × quat., Ar-C), 169.0 and 171.83 (C=O, NHCOAr and NHCOCH$_3$); m/z (EI$^+$) 341 (MH$^+$, 3%), 310 (M$^+$-CH$_3$O, 100%), 268 (M$^+$-C$_3$H$_7$NO, 50%), 225 (M$^+$-C$_3$H$_{10}$NO$_2$, 21%), 105 (COC$_6$H$_5$, 82%).
General procedure 15: Synthesis of benzylic chloride

To a solution of alcohol (1 eq.) and triethylamine (1.5 eq.) in dry DMF (4 mL/mmol alcohol) at 0 °C was added methanesulfonyl chloride (1.5 eq.) in dry DMF (3 mL/mmol alcohol) and the resulting solution stirred at room temperature for 2 hours. Sodium chloride (10 eq.) was added and the mixture was heated at 60 °C for 30 minutes. The solvent was removed under reduced pressure. The crude product was then purified by flash chromatography to furnish the pure product.

\[ \text{N-(3-Acetamido-5-(chloromethyl)phenyl)benzamide (186)} \]

The reaction was carried out according to general procedure 15, with alcohol 171 (100 mg, 0.35 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to give the title compound 186 (106 mg, 100%) as an off white solid (m.p. 83-84 °C from methanol).

\[ R_f \text{ (DCM/MeOH 9:1) } = 0.64; \text{ HRMS Found (FAB\(^+\)): } MH^+ \text{ 303.0898, 305.0876} \]
\[ C_{16}H_{16}ClN_2O_2 \text{ requires 303.0900, 305.0871; IR } \nu_{\text{max}}(\text{NaCl)/cm}^{-1} \text{: 3287 (N-H), 1651 (C=O), 1615, 1533, 1454, 1418, 1370, 1264; } ^1\text{H NMR (400 MHz, CD}_3\text{OD): } \delta 2.10 \text{ (3H, s, NHCO}_3\text{H)}, 4.57 \text{ (2H, s, ArC}_2\text{HCl)}, 7.43 \text{ (1H, t, } J = 1.6 \text{ Hz, Ar-H), 7.47-7.48 (2H, m, Ar-H), 7.49 (1H, br s, Ar-H), 7.52 (1H, t, } J = 7.2 \text{ Hz, Ar-H), 7.88 (1H, t, } J = 1.8 \text{ Hz, Ar-H), 7.90 (1H, t, } J = 1.2 \text{ Hz, Ar-H), 7.93 (1H, t, } J = 2.0,1.6 \text{ Hz, Ar-H); } ^{13}\text{C NMR (100 MHz, CD}_3\text{OD): } \delta 23.9 \text{ (CH}_3\text{, NHCOCH}_3\text{), 46.8 (CH}_2\text{, ArCH}_2\text{Cl), 113.8 (CH, Ar-C), 117.4 (CH, Ar-C), 118.0 (CH, Ar-C), 128.7 (2} \times \text{ CH, Ar-C), 129.7 (2} \times \text{ CH, Ar-C), 133.0 (CH, Ar-C), 136.2 (quat., Ar-C), 140.4 \text{ (quat., Ar-C), 140.6 (quat., Ar-C), 140.7 (quat., Ar-C), 169.0 and 171.8 (C=O, NHCOAr and NHCOCH}_3\text{); m/z (FAB\(^+\)): 303/305 (MH}^+, 15\%), 289 (13\%), 242 (20\%), 154 (100%). \]
General procedure 16: Synthesis of azides
To a solution of mesylate (1 eq.) in dry DMF (3 mL/mmol mesylate) was added sodium azide (3 eq.) and the resultant suspension was stirred at 105 °C for 4 h, then at room temperature for 16 hours. Ethyl acetate (100 mL/mmol mesylate) was added and the mixture washed with water, brine, dried (Na₂SO₄), filtered and the solvent was removed *in vacuo*, to afford the crude product, which was purified by flash chromatography.

\[ \text{N-(3-Acetamido-5-(azidomethyl)phenyl)benzamide (187)} \]

The reaction was carried out according to general procedure 16, with mesylate 182 (460 mg, 1.27 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 187 (321 mg, 82%) as viscous yellow oil.

\[ \text{R}_f (\text{DCM/Methanol 19:1}) = 0.61; \text{ HRMS } \text{Found (El⁺)}: \text{M}^+ 309.1230, \text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_2 \text{requires 309.1226; IR } v_{\text{max}}(\text{NaCl})/\text{cm}^{-1}: 3277 (\text{N-H}), 2095 (\text{N=N}), 1651 (\text{C=O}), 1603, 1546, 1451, 1451, 1273; \text{H NMR} (400 \text{MHz, CDCl}_3): \delta 1.91 (3\text{H, s, NHCOC}_3\text{H}_3), 4.05 (2\text{H, s, ArCH}_2\text{N}_3), 7.22 (1\text{H, br s, Ar-H}), 7.27 (2\text{H, t, } J = 7.6 \text{ Hz, Ar-H}), 7.37 (1\text{H, t, } J = 7.4 \text{ Hz, Ar-H}), 7.42 (1\text{H, br s, Ar-H}), 7.74 (2\text{H, d, } J = 7.5 \text{ Hz, Ar-H}), 7.94 (1\text{H, br s, Ar-H}), 9.09 and 9.28 (2\text{H, NHCOC}_6\text{H}_5 \text{and NHCOAr}); \text{C NMR} (100 \text{MHz, CDCl}_3): \delta 23.8 (\text{CH}_3, \text{ NHCOC}_6\text{H}_5), 54.2 (\text{CH}_2, \text{ ArCH}_2\text{N}_3), 111.9 (\text{CH, Ar-C}), 115.3 (\text{CH, Ar-C}), 115.6 (\text{CH, Ar-C}), 127.1 (2 \times \text{CH, Ar-C}), 128.2 (2 \times \text{CH, Ar-C}), 131.5 (\text{CH, Ar-C}), 134.4 (\text{quat., Ar-C}), 136.5 (\text{quat., Ar-C}), 138.9 (\text{quat., Ar-C}), 139.0 (\text{quat., Ar-C}), 166.6 and 169.5 (\text{C=O, NHCOC}_6\text{H}_5 \text{and NHCOAr}); m/z (El⁺) 309 (M⁺, 11%), 225 (M⁺-C\text{C}_2\text{H}_2\text{N}_3\text{O, 1%}), 105 (\text{COC}_6\text{H}_5, 62%). \]
4-Acetamido-N-(3-acetamido-5-((bis(2-hydroxyethyl)amino)methyl)phenyl)benzamide (189)

The reaction was carried out according to general procedure 14, with mesylate 183 (61 mg, 0.14 mmol) and diethanolamine (153 mg, 1.45 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 189 (55 mg, 88%) as a white semi-solid.

Rf (MeOH/NH3 9:1) = 0.86; HRMS Found (FAB+): MH+ 429.2145, C22H29N4O5 requires 429.2138; IR νmax(NaCl)/cm⁻¹: 3412 br (O-H, N-H), 1646 (C=O), 1618, 1538, 1452, 1254; ¹H NMR (300 MHz, CD3OD): δ 2.15 (3H, s, NHCOC3H3), 2.18 (3H, s, NHCOC3H3), 2.81 (4H, t, J = 11.8 Hz, N(CH2CH2OH)2), 3.70 (4H, t, J = 11.8 Hz, N(CH2CH2OH)2), 3.82 (2H, s, ArCH2N), 7.40 (1H, br s, Ar-H), 7.47 (1H, br s, Ar-H), 7.73 (2H, d, J = 8.70 Hz, Ar-H), 7.91 (1H, br s, Ar-H), 7.95 (2H, d, J = 8.7 Hz, Ar-H); ¹³C NMR (75 MHz, CD3OD): δ 23.9 (CH3, NHCOCH3), 24.1 (CH3, NHCOCH3), 57.2 (2 × CH2, N(CH2CH2OH)2), 58.0 (CH2, ArCH2N), 60.2 (2 × CH2,N(CH2CH2OH)2), 113.5 (CH, Ar-C), 118.4 (CH, Ar-C), 119.1 (CH, Ar-C), 120.3 (2 × CH, Ar-C), 129.7 (2 × CH, Ar-C), 131.0 (quat., Ar-C), 134.7 (quat., Ar-C), 140.3 (quat., Ar-C), 140.4 (quat., Ar-C), 143.5 (quat., Ar-C), 168.2, 171.8 and 172.0 (C=O, NHCOAr and 2 × NHCOCH3); m/z (FAB+): 429 (MH+, 7%), 387 (4%), 165 (6%), 154 (100%).

4-Acetamido-N-(3-acetamido-5-(((2-hydroxyethyl)(methyl)amino)methyl)phenyl)benzamide (190)
The reaction was carried out according to general procedure 14, with mesylate 183 (95 mg, 0.23 mmol) and 2-(methylamino)ethanol (0.18 mL, 2.26 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 190 (40 mg, 44%) as a white gum.

\( R_f \) (MeOH/NH\(_3\) 9:1) = 0.83; HRMS Found (ESI\(^+\)): MH\(^+\) 399.2023, C\(_{21}\)H\(_{27}\)N\(_4\)O\(_4\) requires 399.2032; IR \( \nu \)\(_{\text{max}}\) (solid)/cm\(^{-1}\): 3255 br (O-H, N-H), 1683 (C=O), 1653 (C=O), 1622, 1592, 1562, 1529, 1457, 1423, 1371, 1261; \(^1\)H NMR (400 MHz, CD\(_3\)OD): \( \delta \) 2.06 (3H, s, NHCO\(\text{CH}_3\)), 2.08 (3H, s, NHCO\(\text{CH}_3\)), 2.66 (3H, s, N\(\text{CH}_3\)), 3.03 (2H, t, \( J = 5.0 \) Hz, N\(\text{CH}_2\text{CH}_2\text{OH}\)), 3.77 (2H, t, \( J = 5.0 \) Hz, N\(\text{CH}_2\text{CH}_2\text{OH}\)), 4.07 (2H, s, Ar\(\text{CH}_2\text{N}\)), 7.42 (1H, br s, Ar-\(\text{H}\)), 7.47 (1H, br s, Ar-\(\text{H}\)), 7.63 (2H, d, \( J = 8.5 \) Hz, Ar-\(\text{H}\)), 7.82 (2H, d, \( J = 8.6 \) Hz, Ar-\(\text{H}\)), 7.94 (1H, br s, Ar-\(\text{H}\)); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \( \delta \) 24.0 (CH\(_3\), NHCO\(\text{CH}_3\)), 24.1 (CH\(_3\), NHCO\(\text{CH}_3\)), 41.4 (CH\(_3\), N\(\text{CH}_3\)), 57.5 (CH\(_2\), NCH\(_2\text{CH}_2\text{OH}\)), 57.7 (CH\(_2\), Ar\(\text{CH}_2\text{N}\)), 61.5 (CH\(_2\), NCH\(_2\text{CH}_2\text{OH}\)), 114.7 (CH, Ar-\(\text{C}\)), 119.2 (CH, Ar-\(\text{C}\)), 120.1 (CH, Ar-\(\text{C}\)), 120.2 (2 \( \times \) CH, Ar-\(\text{C}\)), 129.7 (2 \( \times \) CH, Ar-\(\text{C}\)), 130.6 (quat., Ar-\(\text{C}\)), 133.7 (quat., Ar-\(\text{C}\)), 140.8 (quat., Ar-\(\text{C}\)), 140.9 (quat., Ar-\(\text{C}\)), 143.5 (quat., Ar-\(\text{C}\)), 168.0, 171.8 and 171.9(C=O, NHCOAr and 2 \( \times \) N\(\text{HCOCH}_3\)); m/z (ESI\(^+\)): 399 (M\(^+\), 100%), 225 (2%).

4-Acetamido-N-(3-acetamido-5-(chloromethyl)phenyl)benzamide (191)

The reaction was carried out according to general procedure 15, with alcohol 172 (60 mg, 0.18 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 191 (63 mg, 100%) as an off white solid (m.p. 251-252 °C from methanol).

\( R_f \) (DCM/MeOH 9:1) = 0.38, HRMS Found (ESI\(^+\)): MH\(^+\) 360.1123, 362.1087 C\(_{18}\)H\(_{19}\)ClN\(_3\)O\(_3\) requires 360.1115, 362.1085; IR \( \nu \)\(_{\text{max}}\) (solid)/cm\(^{-1}\): 3275 br (N-H), 1680 (C=O), 1663 (C=O), 1646, 1600, 1524, 1455, 1424, 1370, 1266, 710 (C-Cl); \(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO): \( \delta \)
2.05 (3H, s, NHCOCH₃), 2.08 (3H, s, NHCOCH₃), 4.71 (2H, s, ArCH₂Cl), 7.44 (1H, br s, Ar-H), 7.53 (1H, t, J = 1.6 Hz, Ar-H), 7.70 (2H, d, J = 8.8 Hz, Ar-H), 7.92 (2H, d, J = 8.8 Hz, Ar-H), 8.02 (1H, t, J = 1.6 Hz, Ar-H), 10.04, 10.20 and 10.22 (3H, NHCOAr and 2 × NHCOCH₃); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 23.9 (CH₃, NHCOCH₃), 24.0 (CH₃, NHCOCH₃), 46.4 (CH₂, ArCH₂Cl), 110.9 (CH, Ar-C), 114.5 (CH, Ar-C), 115.4 (CH, Ar-C), 118.0 (2 × CH, Ar-C), 128.6 (2 × CH, Ar-C), 128.8 (quat., Ar-C), 138.0 (quat., Ar-C), 139.6 (quat., Ar-C), 139.7 (quat., Ar-C), 142.2 (quat., Ar-C), 164.9, 168.3 and 168.7 (C=O, NHCOAr and 2 × NHCOCH₃); m/z (ESI⁺): 360 (MH⁺, 100%), 267 (12%).

4-Acetamido-N-(3-acetamido-5-(azidomethyl)phenyl)benzamide (192)

![4-Acetamido-N-(3-acetamido-5-(azidomethyl)phenyl)benzamide](image)

The reaction was carried out according to general procedure 16, with mesylate 183 (235 mg, 0.56 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 192 (95 mg, 46%) as sticky white gum.

**Rf** (DCM/MeOH 9:1) = 0.40, HRMS Found (FAB⁺): MH⁺ 367.1515, C₁₈H₁₉N₆O₃ requires 367.1519; IR νmax(NaCl)/cm⁻¹: 3278 (N-H), 2078 (N≡N), 1676, 1649, 1601, 1531, 1458, 1370, 1269; ¹H NMR (400 MHz, (CD₃)₂SO): δ 2.06 (3H, s, NHCOCH₃), 2.09 (3H, s, NHCOCH₃), 4.01 (2H, s, ArCH₂N₃), 7.38 (1H, br s, Ar-H), 7.47 (1H, br s, Ar-H), 7.72 (2H, d, J = 8.0 Hz, Ar-H), 7.93 (2H, d, J = 8.0 Hz, Ar-H), 8.06 (1H, br s, Ar-H), 10.09, 10.23 and 10.31 (3H, NHCOAr and 2 × NHCOCH₃); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 23.9 (CH₃, NHCOCH₃), 24.0 (CH₃, NHCOCH₃), 53.9 (CH₂, ArCH₂N₃), 110.7 (CH, Ar-C), 113.9 (CH, Ar-C), 114.8 (CH, Ar-C), 118.0 (2 × CH, Ar-C), 128.6 (2 × CH, Ar-C), 128.8 (quat., Ar-C), 136.2 (quat., Ar-C), 139.7 (quat., Ar-C), 139.8 (quat., Ar-C), 142.3 (quat., Ar-C), 165.0, 168.4 and 168.8 (C=O, NHCOAr and 2 × NHCOCH₃); m/z (FAB⁺): 367 (MH⁺, 13%), 324 (3%), 242 (5%), 162 (10%), 154 (100%).
N-(3-Acetamido-5-(aminomethyl)phenyl)benzamide (211)

The reaction was carried out according to general procedure 2, with azide 187 (150 mg, 0.48 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 211 (124 mg, 90%) as a brown gum.

RF (DCM/MeOH 9:1) = 0.1; HRMS Found (EI⁺): M+ 284.1396, C₁₆H₁₅N₃O₂ requires 284.1399; IR νmax(NaCl)/cm⁻¹: 3270 br (N-H), 1651 (C=O), 1601, 1544, 1451, 1277;   
¹H NMR (400 MHz, CD₃OD): δ 2.11 (3H, s, NHCOC₃H₃), 3.74 (2H, s, ArCH₂NH₂), 7.32 (1H, br s, Ar-H), 7.36 (1H, br s, Ar-H), 7.46-7.50 (2H, m, Ar-H), 7.54-7.58 (1H, m, Ar-H), 7.89-7.91 (3H, m, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ 23.9 (CH₃, NHCOCH₃), 46.6 (CH₂, ArCH₂NH₂), 112.8 (CH, Ar-C), 116.4 (CH, Ar-C), 116.9 (CH, Ar-C), 128.6 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 132.9 (CH, Ar-C), 136.2 (quat., Ar-C), 140.3 (2 × quat., Ar-C), 140.4 (quat., Ar-C), 168.8 and 171.7 (C=O, NHCOAr and NHCOCH₃); m/z (EI⁺) 283 (M⁺, 40%), 268 (M⁺-NH, 3%), 224 (M⁺-C₃H₅NO, 4%), 178 (M⁺-C₇H₇N, 15%), 163 (M⁺-C₈H₈O, 15%), 105 (COC₃H₅, 62%).

General procedure 17: Reaction of amine with haloacetyl halide

To a solution of amine 211 (1 eq.) and triethylamine (1.1 eq.) in dry DMF (14 mL/mmol amine) was added haloacetyl halide (1.2 eq.) dropwise at 0 °C and stirred at the same temperature for 10 minutes. The reaction mixture was further stirred at room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate, washed with brine, dried (MgSO₄) and filtered. The solvent was removed in vacuo to give the crude product, which was purified by flash chromatography.
The reaction was carried out according to general procedure 17, with amine 211 (100 mg, 0.35 mmol) and bromoacetyl bromide (0.04 mL, 0.42 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 212 (124 mg, 87%) as a light brown solid (m.p.174-175 °C from methanol).  

**Rf** (DCM/MeOH 9:1) = 0.36; **HRMS** Found (FAB⁺): MH⁺ 404.0602, 406.0586, C₁₈H₁₉⁷⁹BrN₅O₃, C₁₈H₁₉⁸¹BrN₅O₃ requires 404.0610, 406.0589; **IR** νmax(NaCl)/cm⁻¹: 3282 (N-H), 1722, 1646 (C=O), 1611, 1539, 1420, 1286, 686 (C-Br); **¹H NMR** (400 MHz, CD₂OD): δ 1.95 (3H, s, NHCO₂C₃H₃), 3.73 (2H, s, CO₂C₃H₂Br), 4.20 (2H, s, ArCH₂NH), 7.15 (1H, t, J = 1.6 Hz, Ar-H), 7.20 (1H, br s, Ar-H), 7.33 (1H, br s, Ar-H), 7.34 (1H, br s, Ar-H), 7.38 (1H, t, J = 6.7 Hz, Ar-H), 7.72 (1H, t, J = 1.6 Hz, Ar-H), 7.74 (1H, br s, Ar-H), 7.76 (1H, t, J = 1.8 Hz, Ar-H); **¹³C NMR** (100 MHz, CD₂OD): δ 23.9 (CH₃, NHCO₂CH₃), 28.8 (CH₂, COCH₂Br), 44.6 (CH₂, ArCH₂NH), 113.2 (CH, Ar-C), 116.5 (CH, Ar-C), 117.1 (CH, Ar-C), 128.6 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 132.9 (CH, Ar-C), 136.2 (quat., Ar-C), 140.4 (quat., Ar-C), 140.5 (quat., Ar-C), 140.6 (quat., Ar-C), 169.0, 169.4 and 171.8 (C=O, NHCOAr, CH₂NHCOCH₂ and NHCOCH₃); **m/z** (ESI⁺): 404/406 (M⁺, 2%), 154 (100%).

The reaction was carried out according to general procedure 17, with amine 211 (124 mg, 0.44 mmol) and chloroacetyl chloride (0.04 mL, 0.52 mmol) to give the crude product, which
was purified by flash chromatography (dichloromethane-methanol 19:1) to afford the title compound 213 (150 mg, 95%) as a light brown solid (m.p. 107-108 °C from methanol).

**R_f** (DCM/MeOH 9:1) = 0.36; **HRMS** Found (ESI⁺): MH⁺ 360.1102, C₁₈H₁₉ClN₃O₃ requires 360.1114, 362.1085; **IR** νₘₐₓ(NaCl)/cm⁻¹: 3284 (N-H), 1730, 1651 (C=O), 1622, 1546, 1422, 1287, 685 (C-Cl); **¹H NMR** (400 MHz, CD₃OD): δ 1.93 (3H, s, NHCOCH₃), 3.94 (2H, s, COCH₂Cl), 4.20 (2H, s, ArCH₂NH), 7.13 (1H, br s, Ar-H), 7.19 (1H, br s, Ar-H), 7.30 (2H, t, J = 7.4 Hz, Ar-H), 7.39 (1H, t, J = 7.6 Hz, Ar-H), 7.70 (1H, br s, Ar-H), 7.72 (1H, br s, Ar-H), 7.76 (1H, t, J = 1.6 Hz, Ar-H); **¹³C NMR** (100 MHz, CD₃OD): δ 23.9 (CH₃, NHCOCH₃), 43.2 (CH₂, ArCH₂NH), 44.4 (CH₂, COCH₂Cl), 113.1 (CH, Ar-C), 116.3 (CH, Ar-C), 116.9 (CH, Ar-C), 128.6 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 132.9 (CH, Ar-C), 136.1 (quat., Ar-C), 140.4 (quat., Ar-C), 140.5 (quat., Ar-C), 144.6 (quat., Ar-C), 168.9, 169.2 and 171.8 (C=O, NHCOAr, CH₂NHCOCH₂ and NHCOCH₃); **m/z** (ESI⁺) 360 (MH⁺, 100%), 324 (MH⁺-HCl, 3%), 267 (MH⁺-C₂H₄ClNO, 20%), 208 (7%).
6.6 Synthesis of diaryl derivatives

General procedure 18: Coupling reaction of amines with acids using DIC and HOBt

To a solution of acid (1 eq.) in dry DMF (5 mL/mmol), was added amine (0.5 eq.) and catalytic amount of DMAP (1-2 mg) and the mixture was stirred for 5-10 minutes under an atmosphere of nitrogen. DIC (1 eq.) was then added dropwise to the resulting solutions and stirred for 2 minutes, followed by the addition of HOBt (1 eq.). The mixture was then stirred for 18 hours at room temperature, before the DMF was evaporated in vacuo. The residue was diluted with methanol (10 mL), water (2 mL) and potassium carbonate (1 eq.). Silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica. This silica was then loaded and purified by flash chromatography. In some cases repeated chromatography was needed to purify the product.

\[ \text{N-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-N-(3-(dimethylamino)propyl)terephthalamide (219)} \]

The reaction was carried out according to general procedure 18, with acid 155 (425 mg, 1.70 mmol) and aniline 99 (250 mg, 0.85 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 219 (430 mg, 96%) as a white foam.

\[ \text{R}_f \text{ (MeOH/NH}_3 \text{ 9:1) = 0.13; HRMS Found (FAB\textsuperscript{+})}; \text{MH}^+ \text{ 527.3058, C}_{28}\text{H}_{43}\text{N}_4\text{O}_4\text{Si requires 527.3054; IR } \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1}: \text{3429 (N-H), 2952 (C-H), 1670 br (C=O), 1620, 1551, 1459 br, 1374, 1287; } \text{^1H NMR (400 MHz, CD}_3\text{OD): } \delta \text{ 0.10 (6H, s, OSi(CH}_3)_2}, \text{ 0.94 (9H, s, OSi(C(CH}_3)_3), 1.81 (2H, p, J = 7.3 Hz, CH}_2\text{CH}_2\text{CH}_2}, \text{ 2.10 (3H, s, NHCOCH}_3), \text{ 2.26 (6H, s, N(CH}_3)_2), 2.43 (2H, t, J = 7.6 Hz, NCH}_2), 3.41 (2H, t, J = 7.0 Hz, CONHCH}_2), 4.69 (2H, s,} \]
ArCH₂O), 7.36 (1H, s, Ar-H), 7.42 (1H, s, Ar-H), 7.86-90 (3H, m, Ar-H), 7.96 (2H, d, J = 8.3 Hz, Ar-H); ¹³C NMR (100 MHz, CD₃OD): δ -5.5 (2 × CH₃, OSi(CH₃)₂), 18.2 (quat., OSiC(CH₃)₃), 23.8 (CH₃, NHCOCH₃), 25.3 (3 × CH₃, OSiC(CH₃)₃), 25.7 (CH₂, CH₂CH₂CH₂), 38.3 (CH₂, CONHCH₂), 44.2 (2 × CH₃, N(CH₃)₂), 56.8 (CH₂, NCH₂), 66.7 (CH₂, ArCH₂O), 110.8 (CH, Ar-C), 113.8 (CH, Ar-C), 114.0 (CH, Ar-C), 127.0 (2 × CH, Ar-C), 127.4 (2 × CH, Ar-C), 136.7 (quat., Ar-C), 137.3 (quat., Ar-C), 138.3 (quat., Ar-C), 138.6 (quat., Ar-C), 142.7 (quat., Ar-C), 165.9, 167.2 and 169.6 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃); m/z (FAB⁺) 527 (MH⁺, 72%), 469 (MH⁺-C₄H₉, 7%), 450 (100%), 265 (20%).

N¹-(3-Acetimidophenyl)-N⁴-(3-(dimethylamino)propyl)terephthalamide (232)

\[
\begin{align*}
\text{N}^1-(3-\text{Acetimidophenyl})-\text{N}^4-(3-(\text{dimethylamino})\text{propyl})\text{terephthalamide (232)}
\end{align*}
\]

The reaction was carried out according to general procedure 18, with acid 155 (217 mg, 0.87 mmol) and aniline 113 (65 mg, 0.43 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 232 (120 mg, 72%) as a white foam.

Rₚ (MeOH/NH₃ 9:1) = 0.68; HRMS Found (FAB⁺): MH⁺ 383.2076, C₂₁H₂₇N₄O₃ requires 383.2083; IR νmax(NaCl)/cm⁻¹: 3310 br (N-H), 2942, 1644 (C=O), 1624, 1604, 1531, 1481, 1370, 1282; ¹H NMR (400 MHz, (CD₃)₂SO): δ 1.69 (2H, p, J = 7.1 Hz, CH₂CH₂CH₂), 2.05 (3H, s, NHCOCH₃), 2.17 (6H, s, N(CH₃)₂), 2.31 (2H, t, J = 7.0 Hz, NCH₂), 3.31 (2H, dt, J = 7.2, 5.2 Hz, CONHCH₂), 7.25 (1H, t, J = 8.0 Hz, Ar-H), 7.34 (1H, d, J = 8.8 Hz, Ar-H), 7.43 (1H, d, J = 8.4 Hz, Ar-H), 7.95 (2H, d, J = 8.4 Hz, Ar-H), 8.02 (2H, d, J = 8.4 Hz, Ar-H), 8.25 (1H, s, Ar-H), 8.67 (1H, t, J = 5.4 Hz, CONHCH₂), 9.25 and 9.98 (2H, NHCOAr and NHCOCH₃); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 23.9 (CH₃, NHCOCH₃), 26.8 (CH₂, CH₂CH₂CH₂), 37.7 (CH₂, CONHCH₂), 44.9 (2 × CH₃, N(CH₃)₂), 56.7 (CH₂, NCH₂), 111.3 (CH, Ar-C), 114.6 (CH, Ar-C), 115.3 (CH, Ar-C), 127.0 (2 × CH, Ar-C), 127.6 (2 × CH, Ar-C), 128.6 (CH, Ar-C), 136.9 (quat., Ar-C), 137.0 (quat., Ar-C), 139.1 (quat., Ar-C), 139.5 (quat., Ar-C), 164.8, 165.3 and 168.2 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃); m/z (FAB⁺): 383 (MH⁺, 29%), 338 (5%), 167 (7%), 154 (100%), 120 (13%).
N₁-(3-(dimethylamino)propyl)-N₄-isopropyl-N₄-(isopropylcarbamoyl)terephthalamide (238)

To a solution of acid 155 (152 mg, 0.6 mmol) in dry DMF (4 mL), with catalytic amount of DMAP (1-2 mg) and the mixture was stirred for 10 minutes under an atmosphere of nitrogen. DIC (77 mg, 0.61 mmol) was then added dropwise to the resulting solutions. The reaction mixture was then stirred for 18 hours at room temperature, before the DMF was evaporated invacuo. The residue was diluted with methanol and silica gel was added until a consistent slurry was formed. The solvent was removed to give the crude product adhered to silica. This silica was then loaded and purified by flash chromatography to give the title compound 238 (229 mg, 100%) as a clear thick oil.

HRMS Found (FAB⁺): M⁺ 376.2465, C₂₀H₃₂N₄O₃ requires 376.2474; IR ν_max(solid)/cm⁻¹: 3385 (N-H), 2973 (C-H), 1670 (C=O), 1689 (C=O), 1633, 1537, 1459, 1365, 1259; ¹H NMR (400 MHz, CDCl₃): δ 0.67 (6H, d, J = 6.4 Hz, NHCH(CH₃)₂), 1.24 (6H, d, J = 6.4 Hz, NCH(CH₃)₂), 1.65 (2H, p, J = 6.6 Hz, CH₂CH₂CH₂), 2.12 (6H, s, N(CH₃)₂), 2.30 (2H, t, J = 6.6 Hz, NCH₂CH₂), 3.33 (2H, t, J = 6.4 Hz, NHCH₂CH₂), 3.50 (1H, sextet, J = 6.8 Hz, NCH(CH₃)₂), 3.40 (1H, septet, J = 6.8 Hz, NHCH(CH₃)₂), 7.13 (1H, d, J = 7.6 Hz, CONHCH), 7.43 (2H, d, J = 8.4 Hz, Ar-H), 7.66 (2H, d, J = 8.4 Hz, Ar-H), 8.44 (1H, t, J = 5.2 Hz, CONH); ¹³C NMR (100 MHz, CDCl₃): δ 20.2 (2 × CH₃, NHCH(CH₃)₂), 21.3 (2 × CH₃, NCH(CH₃)₂), 25.5 (CH₂, CH₂CH₂CH₂), 38.92 (CH₂, CONHCH₂), 44.8 (2 × CH₃, N(CH₃)₂), 48.4 (CH, NHCH(CH₃)₂), 57.5 (CH₂, NCH₂CH₂), 57.6 (CH, NCH(CH₃)₂), 126.8 (4 × CH, Ar-CH), 135.9 (quat., Ar-C), 139.2 (quat., Ar-C), 153.8 (CO, ArCON), 166.5 and 169.6 (C=O, CONCONH and CONCONH₂); m/z (FAB⁺): 376 (M⁺, 6%), 361 (2%), 291 (5%), 276 (2%), 233 (2%), 191 (3%), 104 (6%), 58 (100%).

N₁-(3-(Dimethylamino)propyl)-N₄-phenylterephthalamide (242)
The reaction was carried out according to general procedure 18, with acid 155 (188 mg, 0.75 mmol) and aniline 200 (35 mg, 0.38 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 242 (115 mg, 94%) as a white foam.

\[ R_f (\text{MeOH/NH}_3 \ 9:1) = 0.71; \text{ HRMS } \text{Found (FAB}^+\text{): MH}^+ 326.1869, \text{ C}_{19}\text{H}_{24}\text{N}_3\text{O}_2 \text{requires} 326.1869; \text{ IR } \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1}: 3304 (\text{N-H}), 2943 (\text{C-H}), 1717 \text{br} (\text{C=O}), 1633, 1598, 1534, 1501, 1440, 1313, 1265; \text{ }^1\text{H NMR} \ (400 \text{ MHz, CD}_3\text{OD}): \delta 1.87 (2\text{H, p}, J = 7.2 \text{ Hz, CH}_2\text{C}_6\text{H}_2\text{CH}_2), 2.43 (6\text{H, s, N(C}_6\text{H}_3)_2), 2.63 (2\text{H, t, J = 7.6 Hz, NCH}_2), 3.43 (2\text{H, t, J = 6.8 Hz, CONHCH}_2), 7.25 (1\text{H, t, J = 5.3 Hz, Ar-H}), 7.34 (2\text{H, t, J = 7.8 Hz, Ar-H}), 7.67 (2\text{H, d, J = 7.8 Hz, Ar-H}), 7.91 (2\text{H, d, J = 8.3 Hz, Ar-H}), 8.0 (2\text{H, d, J = 8.3 Hz, Ar-H}); \text{ }^{13}\text{C NMR} \ (100 \text{ MHz, CD}_3\text{OD}): \delta 27.8 (CH_2, CH_2C_6H_2CH_2), 39.2 (CH_2, CONHCH_2), 45.1 (2 \times \text{CH}_3, \text{N(C}_6\text{H}_3)_2), 58.1 (\text{CH}_2, \text{CONHCH}_2), 122.6 (2 \times \text{CH}, \text{Ar-C}), 126.1 (\text{CH}, \text{Ar-C}), 128.8 (2 \times \text{CH}, \text{Ar-C}), 129.1 (2 \times \text{CH}, \text{Ar-C}), 130.1 (2 \times \text{CH}, \text{Ar-C}), 138.7 (\text{quat.,Ar-C}), 139.4 (\text{quat.,Ar-C}), 140.0 (\text{quat.,Ar-C}), 168.1 \text{and} 169.6 (\text{C=O, NHCOAr and CONHCH}_2); m/z \text{ (FAB}^+\text{): 326 (MH}^+, 100\%), 281 (16\%), 265 (21\%), 224 (13\%), 149 (32\%), 120 (14\%).

\[ N^1-\text{(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-N^4-(2-(dimethylamino)ethyl)terephthalamide (243)} \]

The reaction was carried out according to general procedure 18, with acid 153 (209 mg, 0.88 mmol) and aniline 99 (130 mg, 0.44 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 243 (105 mg, 46%) as a white foam.

\[ R_f (\text{MeOH/NH}_3 \ 9:1) = 0.30; \text{ HRMS } \text{Found (ESI}^+\text{): MH}^+ 513.2876, \text{ C}_{27}\text{H}_{41}\text{N}_4\text{O}_4\text{Si requires} 513.2897; \text{ IR } \nu_{\text{max}}(\text{solid})/\text{cm}^{-1}: 3290 (\text{N-H}), 2929 (\text{C-H}), 1642 \text{br} (\text{C=O}), 1621, 1551, 1453, 1431, 1371, 1286; \text{ }^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3): \delta 0.06 (6\text{H, s, OSi(CH}_3)_2), 0.90 (9\text{H, s,}}
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OSi(CH\(_3\)_3), 2.02 (3H, s, NHCOCH\(_3\)), 2.23 (6H, s, N(CH\(_3\))\(_2\)), 2.49 (2H, t, \(J = 6.0\) Hz, NCH\(_2\)), 3.49 (2H, t, \(J = 5.6\) Hz, CONHCH\(_2\)), 4.63 (2H, s, ArCH\(_2\)O), 7.18 (1H, br s, NHCOCH\(_3\)), 7.30 (1H, br s, Ar-H), 7.46 (1H, br s, Ar-H), 7.68 (2H, d, \(J = 8.2\) Hz, Ar-H), 7.73 (2H, d, \(J = 8.2\) Hz, Ar-H), 7.79 (1H, br s, Ar-H), 8.26 and 8.92 (2H, CONHCH\(_2\) and NHCOAr); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) -5.3 (2 \(\times\) CH\(_3\), OSi(CH\(_3\))\(_2\)), 18.4 (quat., OSiC(CH\(_3\))\(_3\)), 24.3 (CH\(_3\), NHCOCH\(_3\)), 25.9 (3 \(\times\) CH\(_3\), OSiC(CH\(_3\))\(_3\)), 37.0 (CH\(_2\), NCH\(_2\)), 44.8 (2 \(\times\) CH\(_3\), N(CH\(_3\))\(_2\)), 57.6 (CH\(_2\), NCH\(_2\)), 64.6 (CH\(_2\), ArCH\(_2\)O), 110.0 (CH, Ar-C), 114.0 (CH, Ar-C), 114.1 (CH, Ar-C), 127.1 (2 \(\times\) CH, Ar-C), 127.4 (2 \(\times\) CH, Ar-C), 136.8 (quat., Ar-C), 137.4 (quat., Ar-C), 138.5 (quat., Ar-C), 138.6 (quat., Ar-C), 143.0 (quat., Ar-C), 165.6, 166.8 and 169.0 (C=O, NHCOAr, CONHCH\(_2\) and NHCOCH\(_3\)); \(m/z\) (ESI\(^+\)): 513 (MH\(^+\), 100%), 282 (2%), 191 (20%).

\(N^1\)-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-N\(^4\)-(3-(diethylamino)propyl)terephthalamide (244)

![Structural diagram of the compound](image)

The reaction was carried out according to general procedure 18, with acid 154 (246 mg, 0.88 mmol) and aniline 99 (130 mg, 0.44 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 244 (215 mg, 88%) as light yellow thick oil.

\(R_f\) (MeOH/ NH\(_3\) 9:1) = 0.18; HRMS Found (ESI\(^+\)): MH\(^+\) 555.3371, C\(_{30}\)H\(_{47}\)N\(_4\)O\(_4\)Si requires 555.3367; IR \(\nu\)\(_{\text{max}}\) (solid)/cm\(^{-1}\): 3297 (N-H), 2929 (C-H), 1639 br (C=O), 1621, 1543, 1453, 1373, 1279; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 0.08 (6H, s, OSi(CH\(_3\))\(_2\)), 0.91 (9H, s, OSiC(CH\(_3\))\(_3\)), 1.0 (6H, t, \(J = 7.2\) Hz, NCH\(_2\)CH\(_3\)), 1.73 (2H, p, \(J = 5.6\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)), 2.01 (3H, s, NHCOCH\(_3\)), 2.54 (4H, q, \(J = 7.1\) Hz, N(CH\(_2\)CH\(_3\))\(_2\)), 2.61 (2H, t, \(J = 4.0\) Hz, NCH\(_2\)CH\(_2\)), 3.56 (2H, dt, \(J = 5.1, 5.1\) Hz, CONHCH\(_2\)), 4.68 (2H, s, ArCH\(_2\)O), 7.28 (1H, br s, NHCOCH\(_3\)), 7.50 (1H, s, Ar-H), 7.79 (1H, s, Ar-H), 7.83 (2H, d, \(J = 8.4\) Hz, Ar-H), 7.91 (1H, s, Ar-H), 8.05 (2H, d, \(J = 8.4\) Hz, Ar-H), 8.51 and 9.08 (2H, CONHCH\(_2\) and NHCOAr); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) -5.3 (2 \(\times\) CH\(_3\), OSi(CH\(_3\))\(_2\)), 11.4 (CH\(_3\), N(CH\(_2\)CH\(_3\))\(_2\)), 18.4 (quat.,
OSiC(CH$_3$)$_3$, 24.4 (CH$_2$, CH$_2$CH$_2$CH$_2$), 24.6 (CH$_3$, NHC(O)CH$_3$), 25.9 (3 × CH$_3$, OSiC(CH$_3$)$_3$), 41.4 (CH$_2$, NCH$_2$CH$_2$), 46.8 (2 × CH$_2$, N(CH$_2$CH$_2$)$_2$), 52.3 (CH$_2$, CONHCH$_2$), 64.6 (CH$_2$, ArCH$_2$O), 110.0 (CH, Ar-C), 113.3 (CH, Ar-C), 113.4 (CH, Ar-C), 127.3 (2 × CH, Ar-C), 129.6 (2 × CH, Ar-C), 137.2 (quat., Ar-C), 137.7 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 143.4 (quat., Ar-C), 165.2, 166.4 and 168.6 (C=O, NHCOAr, CONHCH$_2$ and NHC(O)CH$_3$); m/z (ESI$^+$): 555 (MH$^+$, 40%), 320 (3%), 293 (100%), 212 (18%).

$N^1$-(2-(Dimethylamino)ethyl)-$N^4$-phenylterephthalamide (245)

The reaction was carried out according to general procedure 18, with acid 153 (178 mg, 0.75 mmol) and aniline 200 (35 mg, 0.38 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 245 (102 mg, 87%) as a foam.

R$_f$ (MeOH/Am 9:1) = 0.77; HRMS Found (ESI$^+$): MH$^+$ 312.1700, C$_{18}$H$_{22}$N$_3$O$_2$ requires 312.1712; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3312 (N-H), 2930 (C-H), 1651 (C=O), 1636, 1600, 1532, 1501, 1441, 1364, 1315, 1298, 1259; $^1$H NMR (400 MHz, (CD$_3$)$_2$SO): $\delta$ 2.19 (6H, s, N(CH$_3$)$_2$), 2.42 (2H, t, $J = 6.8$ Hz, NCH$_2$), 3.38 (2H, dt, $J = 6.8$, 6.0 Hz, CONHCH$_2$), 7.12 (1H, t, $J = 5.3$ Hz, Ar-H), 7.36 (2H, t, $J = 7.8$ Hz, Ar-H), 7.79 (2H, d, $J = 7.8$ Hz, Ar-H), 7.97 (2H, d, $J = 8.4$ Hz, Ar-H), 8.03 (2H, d, $J = 8.4$ Hz, Ar-H), 8.57 (1H, t, $J = 5.6$ Hz, CONHCH$_2$), 10.36 (1H, s, NHCOAr); $^{13}$C NMR (100 MHz, (CD$_3$)$_2$SO): $\delta$ 37.4 (CH$_2$, NCH$_2$), 45.2 (2 × CH$_3$, N(CH$_3$)$_2$), 58.1 (CH$_2$, CONHCH$_2$), 120.4 (2 × CH, Ar-C), 123.8 (CH, Ar-C), 127.1 (2 × CH, Ar-C), 127.6 (2 × CH, Ar-C), 128.6 (2 × CH, Ar-C), 136.9 (quat., Ar-C), 137.0 (quat., Ar-C), 138.9 (quat., Ar-C), 164.8 and 165.3 (C=O, NHCOAr and CONHCH$_2$); m/z (ESI$^+$): 312 (MH$^+$, 100%), 267 (6%), 220 (3%).
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\[ N^1-(3\text{-Morpholinopropyl})-N^4\text{-phenylterephthalamide (246)} \]

\[
\begin{align*}
\text{The reaction was carried out according to general procedure 18, with acid 156 (220 mg, 0.75 mmol) and aniline 200 (35 mg, 0.38 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 246 (120 mg, 87\%) as a foam.}
\end{align*}
\]

\[ R_f \text{ (MeOH/NH}_3 \text{ 9:1) = 0.89; HRMS Found (ESI\textsuperscript{+}): MH}^+ \text{ 368.1957, C}_{21}\text{H}_{26}\text{N}_3\text{O}_3 \text{ requires 368.1974; IR } \nu_{\text{max}(\text{NaCl})/\text{cm}^{-1}}: 3302 \text{ (N-H)}, 2955 \text{ (C-H), 1631 br, 1599, 1541, 1447, 1326, 1309, 1274; } ^1\text{H NMR (400 MHz, (CD}_3\text{)}_2\text{SO): } \delta 1.70 \text{ (2H, p, } J = 7.0 \text{ Hz, CH}_2\text{CH}_2\text{CH}_2\text{), 2.32-2.36 (6H, br t, } J = 7.0 \text{ Hz, CH}_2\text{N(CH}_3\text{CH}_2\text{), (CH}_2\text{CH}_2\text{)NCH}_2\text{), 3.31 (2H, t, } J = 5.6 \text{ Hz, CONHCH}_2\text{), 3.57 (4H, t, } J = 4.4 \text{ Hz, O(CH}_2\text{CH}_2\text{), 7.12 (1H, t, } J = 7.4 \text{ Hz, Ar-H), 7.36 (2H, t, } J = 7.8 \text{ Hz, Ar-H), 7.79 (2H, d, } J = 7.6 \text{ Hz, Ar-H), 7.97 (2H, d, } J = 8.4 \text{ Hz, Ar-H), 8.03 (2H, d, } J = 8.4 \text{ Hz, Ar-H), 8.64 (1H, t, } J = 5.8 \text{ Hz, CONHCH}_2\text{), 10.34 (1H, s, NHCOAr); } ^{13}\text{C NMR (100 MHz, (CD}_3\text{)}_2\text{SO): } \delta 25.8 \text{ (CH}_2\text{, CH}_2\text{CH}_2\text{CH}_2\text{), 37.8 (CH}_2\text{, NHCOCH}_2\text{), 53.3 (2 } \times \text{ CH}_2\text{, CH}_2\text{N(CH}_3\text{CH}_2\text{), 56.0 (CH}_2\text{, NCH}_2\text{CH}_2\text{), 66.1 (CH}_2\text{, O(CH}_2\text{CH}_2\text{), 120.4 (2 } \times \text{ CH, Ar-C), 123.8 (CH, Ar-C), 127.1 (2 } \times \text{ CH, Ar-C), 127.6 (2 } \times \text{ CH, Ar-C), 128.6 (2 } \times \text{ CH, Ar-C), 136.9 (quat., Ar-C), 137.1 (quat., Ar-C), 138.9 (quat., Ar-C), 164.8 and 165.3 (C=O, NHCOAr and CONHCH}_2\text{); m/z (ESI\textsuperscript{+}): 368 (MH}^+, 100\%), 341 (3\%), 267 (1\%), 193 (2\%).}
\]

\[ N^1-(3\text{-acetamidophenyl})-N^4-(2\text{-morpholinoethyl})terephthalamide (247) \]

\[
\begin{align*}
\text{The reaction was carried out according to general procedure 18, with acid 157 (240 mg, 0.86 mmol) and aniline 113 (65 mg, 0.43 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 247 (124 mg, 70\%) as a foam.}
\end{align*}
\]
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\( \text{R}_f \) (MeOH/NH\(_3\) 9:1) = 0.93; **HRMS** Found (ESI\(^*\)): MH\(^+\) 411.2024, C\(_{22}\)H\(_{27}\)N\(_4\)O\(_4\) requires 411.2032; **IR** \( \nu_{\text{max}} \) (solid)/cm\(^{-1}\): 3304 (N-H), 2941, 1663 (C=O), 1645, 1631, 1604, 1532, 1481, 1364, 1281; **\(^1\)H NMR** (400 MHz, (CD\(_3\))\(_2\)SO): \( \delta \) 2.02 (3H, s, NHCOCH\(_3\)), 2.39 (4H, t, \( J = 4.2 \) Hz, CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 2.50 (2H, br m, (CH\(_2\)CH\(_2\))\(_2\)NHCH\(_2\)), 3.39 (2H, dt, \( J = 6.6 \) Hz, CONHCH\(_2\)), 3.61 (4H, t, \( J = 4.6 \) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.22 (1H, t, \( J = 8.0 \) Hz, Ar-H), 7.30 (1H, d, \( J = 8.8 \) Hz, Ar-H), 7.40 (1H, d, \( J = 6.8 \) Hz, Ar-H), 7.92 (2H, d, \( J = 6.8 \) Hz, Ar-H), 8.0 (2H, d, \( J = 6.8 \) Hz, Ar-H), 8.08 (1H, t, \( J = 1.8 \) Hz, Ar-H), 8.5 (1H, t, \( J = 5.8 \) Hz, CONHCH\(_2\)), 9.94 and 10.32 (2H, NHCOAr and NHCOCH\(_3\)); **\(^{13}\)C NMR** (100 MHz, (CD\(_3\))\(_2\)SO): 23.9 (CH\(_3\), NHCOCH\(_3\)), 36.6 (CH\(_2\), CONHCH\(_2\)), 53.2 (2 \( \times \) CH\(_2\), CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 57.2 (CH\(_2\), (CH\(_2\)CH\(_2\))\(_2\)NCH\(_3\)), 66.1 (2 \( \times \) CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 111.2 (CH, Ar-C), 114.6 (CH, Ar-C), 115.2 (CH, Ar-C), 127.0 (2 \( \times \) CH, Ar-C), 127.6 (2 \( \times \) CH, Ar-C), 128.6 (CH, Ar-C), 136.9 (quat., Ar-C), 137.0 (quat., Ar-C), 139.1 (quat., Ar-C), 139.4 (quat., Ar-C), 164.8, 165.3 and 168.2 (C=O, NHCOAr, CONHCH\(_2\) and NHCOCH\(_3\)); **m/z** (ESI\(^*\)): 411 (MH\(^+\), 100%), 313 (2%), 282 (4%), 242 (3%).

**N\(^1\)-(2-Morpholinoethyl)-N\(^4\)-phenylterephthalamide (248)**

The reaction was carried out according to general procedure 18, with acid 157 (209 mg, 0.75 mmol) and aniline 200 (25 mg, 0.38 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the **title compound 248** (125 mg, 94%) as a foam.

\( \text{R}_f \) (MeOH/NH\(_3\) 9:1) = 0.87; **HRMS** Found (ESI\(^*\)): MH\(^+\) 354.1801, C\(_{20}\)H\(_{24}\)N\(_3\)O\(_3\) requires 354.1818; **IR** \( \nu_{\text{max}} \) (NaCl)/cm\(^{-1}\): 3301 (N-H), 2947 (C-H), 1669 (C=O), 1635, 1598, 1530, 1440, 1327, 1263; **\(^1\)H NMR** (400 MHz, (CD\(_3\))\(_2\)SO): \( \delta \) 2.41 (4H, t, \( J = 4.4 \) Hz, CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 2.49 (2H, br m, (CH\(_2\)CH\(_2\))\(_2\)NHCH\(_2\)), 3.39 (2H, dt, \( J = 6.6 \) Hz, CONHCH\(_2\)), 3.56 (4H, t, \( J = 4.6 \) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.12 (1H, t, \( J = 8.4 \) Hz, Ar-H), 7.35 (2H, t, \( J = 7.8 \) Hz, Ar-H), 7.78 (2H, d, \( J = 8.4 \) Hz, Ar-H), 7.95 (2H, d, \( J = 8.4 \) Hz, Ar-H), 8.01 (2H, d, \( J = 8.4 \) Hz, Ar-H), 8.56 (1H, t, \( J = 5.8 \) Hz, CONHCH\(_2\)), 10.32 (1H, s, NHCOAr); **\(^{13}\)C NMR** (100 MHz,
The reaction was carried out according to general procedure 18, with acid 156 (253 mg, 0.86 mmol) and aniline 113 (65 mg, 0.43 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 249 (130 mg, 71%) as a foam.

**Rf** (MeOH/NH3 9:1) = 0.89; **HRMS** Found (ESI⁺): MH⁺ 425.2183, C23H29N4O4 requires 425.2189; **IR** νmax(solid)/cm⁻¹: 3311 (N-H), 2931, 1663 (C=O), 1645, 1630, 1604, 1533, 1450, 1333, 1288; **1H NMR** (400 MHz, (CD₃)₂SO): δ 1.71 (2H, p, J = 7.1 Hz, CH₂CH₂CH₂), 2.05 (3H, s, NHCOCH₃), 2.32-2.36 (6H, m, CH₂N(CH₂CH₂)₂), 3.39 (2H, t, J = 6.2 Hz, CONHCH₂), 3.57 (4H, t, J = 4.6 Hz, O(CH₂CH₂)₂), 7.25 (1H, t, J = 8.0 Hz, Ar-H), 7.34 (1H, d, J = 8.4 Hz, Ar-H), 7.43 (1H, d, J = 8.4 Hz, Ar-H), 7.95 (2H, d, J = 8.4 Hz, Ar-H), 8.02 (2H, d, J = 8.4 Hz, Ar-H), 8.10 (1H, t, J = 1.8 Hz, Ar-H), 8.63 (1H, t, J = 5.8 Hz, CONHCH₂), 9.97 and 10.34 (2H, NHCOAr and NHCOCH₃); **13C NMR** (100 MHz, (CD₃)₂SO): δ 23.9 (CH₂, CH₂CH₂CH₂), 25.8 (CH₃, NHCOCH₃), 37.8 (CH₂, CONHCH₂), 53.2 (2 × CH₂, CH₂N(CH₂CH₂)₂), 56.0 (CH₂, (CH₂CH₂)₂NCH₂), 66.1 (2 × CH₂, O(CH₂CH₂)₂), 111.3 (CH, Ar-C), 114.6 (CH, Ar-C), 115.3 (CH, Ar-C), 127.0 (2 × CH, Ar-C), 127.6 (2 × CH, Ar-C), 128.6 (CH, Ar-C), 136.9 (quat., Ar-C), 137.0 (quat., Ar-C), 139.1 (quat., Ar-C), 139.4 (quat., Ar-C), 164.8, 165.3 and 168.2 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃); **m/z** (ESI⁺): 425 (MH⁺, 100%), 213 (19%), 169 (17%).
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$N^1$-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-$N^4$-(3-morpholinopropyl)terephthalamide (250)

The reaction was carried out according to general procedure 18, with acid 156 (258 mg, 0.88 mmol) and aniline 99 (130 mg, 0.44 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 250 (200 mg, 80%) as a foam.

$R_f$ (MeOH/NH$_3$ 9:1) = 0.72; HRMS Found (ESI$^+$): MH$^+$ 569.3146, C$_{30}$H$_{48}$N$_4$O$_5$Si requires 569.3159; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3266 (N-H), 2930 (C-H), 1670 (C=O), 1626, 1556, 1457, 1418, 1365, 1294; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.02 (6H, s, OSi(CH$_3$)$_2$), 0.85 (9H, s, OSiC(CH$_3$)$_3$), 1.72 (2H, p, $J$ = 6.4 Hz, CH$_2$CH$_2$CH$_2$), 1.98 (3H, s, NHCOCH$_2$), 2.39-2.42 (6H, m, CH$_2$N(CH$_2$CH$_2$)$_2$), 3.39 (2H, t, $J$ = 6.2 Hz, CONHCH$_2$), 3.61 (4H, t, $J$ = 4.0 Hz, O(CH$_2$CH$_2$)$_2$), 4.60 (2H, s, ArCH$_2$O), 7.24 (1H, s, Ar-H), 7.40 (1H, s, Ar-H), 7.70 (3H, d, $J$ = 8.0 Hz, Ar-H), 7.76 (2H, d, $J$ = 8.4 Hz, Ar-H), 8.20 (1H, t, $J$ = 4.8 Hz, CONHCH$_2$), 9.02 and 9.38 (2H, NHCOCH$_3$, NHCOAr); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ -5.6 (CH$_3$, OSi(CH$_3$)$_2$), 18.2 (quat., OSiC(CH$_3$)$_3$), 23.8(CH$_2$, CH$_2$CH$_2$CH$_2$), 24.5 (CH$_3$, NHCOCH$_3$), 25.7 (3 × CH$_3$, OSiC(CH$_3$)$_3$), 39.3 (CH$_2$, CONHCH$_2$), 53.4 (2 × CH$_2$, CH$_2$N(CH$_2$CH$_2$)$_2$), 57.2 (CH$_2$, NCH$_2$CH$_2$), 64.7 (CH$_2$, ArCH$_2$O), 66.5 (CH$_2$, O(CH$_2$CH$_2$)$_2$), 110.7 (CH, Ar-C), 113.7 (CH, Ar-C), 113.8 (CH, Ar-C), 126.9 (2 × CH, Ar-C), 127.4 (2 × CH, Ar-C), 137.0 (quat., Ar-C), 137.3 (quat., Ar-C), 138.2 (quat., Ar-C), 138.5 (quat., Ar-C), 142.7 (quat., Ar-C), 165.8, 167.1 and 169.6 (C=O, NHCOAr, CONHCH$_2$ and NHCOCH$_3$); m/z (ESI$^+$): 569 (MH$^+$, 100%), 419 (10%), 341 (2%), 219 (58%).
\(N^1-(3\text{-Acetamido-5-}((\text{tert\text{-}butyldimethylsilyloxy})\text{methyl})\text{phenyl})-N^4-(2\text{-morpholinoethyl})\text{terephthalamide (251)}\)

The reaction was carried out according to general procedure 18, with acid 157 (246 mg, 0.88 mmol) and aniline 99 (130 mg, 0.44 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 251 (175 mg, 71%) as a foam.

\(R_f\) (MeOH/NH\(_3\) 9:1) = 0.66; \(HRMS\) Found (ESI\(^+\)): MH\(^+\) 555.2987, C\(_{29}\)H\(_{43}\)N\(_4\)O\(_5\)Si requires 555.2997; \(IR\) \(\nu\text{max}(\text{solid})/\text{cm}^{-1}\): 3286 (N-H), 2933 (C-H), 1641 br (C=O), 1540, 1450, 1429, 1369, 1275; \(\text{\(^1\)}H NMR\) (400 MHz, CDCl\(_3\)): \(\delta\) 0.03 (6H, s, O\text{Si(C}(\text{CH}_3)_2)), 0.86 (9H, s, O\text{Si(C(C}(\text{CH}_3)_3)), 1.99 (3H, s, NHCOCH\(_2\)), 2.45 (4H, t, \(J = 4.0\) Hz, CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 2.54 (2H, t, \(J = 6.2\) Hz, (CH\(_2\)CH\(_2\))\(_2\)NCH\(_2\)), 3.49 (2H, t, \(J = 5.6\) Hz, CONHCH\(_2\)), 3.66 (4H, t, \(J = 4.0\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 4.61 (2H, s, ArCH\(_2\)O), 7.24 (1H, s, Ar-H), 7.41 (1H, s, Ar-H), 7.56 (1H, br t, \(J = 4.8\) Hz, CONHCH\(_2\)), 7.72 (2H, d, \(J = 8.4\) Hz, Ar-H), 7.72 (3H, d, \(J = 8.4\) Hz, Ar-H), 8.94 and 9.31 (2H, NHCOCH\(_3\) and NHCOAr); \(\text{\(^{13}\)}C NMR\) (100 MHz, CDCl\(_3\)): \(\delta\) -5.5 (2 \(\times\) CH\(_3\), O\text{Si(CH}_3)_2)), 18.3 (quat., O\text{Si}(CH\(_3\))\(_3\)), 23.9 (CH\(_3\), NHCOCH\(_3\)), 25.8 (3 \(\times\) CH\(_3\), O\text{Si}(CH\(_3\))\(_3\)), 36.1 (CH\(_2\), CONHCH\(_2\)), 53.2 (2 \(\times\) CH\(_2\), CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 57.1 (CH\(_2\), (CH\(_2\)CH\(_2\))\(_2\)NCH\(_2\)), 64.7 (CH\(_2\), ArCH\(_2\)O), 66.5 (2 \(\times\) CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 110.6 (CH, Ar-C), 113.7 (CH, Ar-C), 113.9 (CH, Ar-C), 127.0 (2 \(\times\) CH, Ar-C), 127.4 (2 \(\times\) CH, Ar-C), 136.8 (quat., Ar-C), 137.5 (quat., Ar-C), 138.3 (quat., Ar-C), 138.5 (quat., Ar-C), 142.8 (quat., Ar-C), 165.8, 167.1 and 169.6 (C=O, NHCOAr, CONHCH\(_2\) and NHCOCH\(_3\)); \(m/z\) (ESI\(^+\)): 555 (MH\(^+\), 100%), 405 (2%), 306 (1%), 212 (21%).
The reaction was carried out according to general procedure 13, with silyl ether 219 (50 mg, 0.09 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 254 (37 mg, 94%) as an off white foam.

*Rf* (MeOH/NH₃ 9:1) = 0.12; *HRMS* Found (FAB⁺): MH⁺ 413.2202 C₂₂H₂₉N₄O₄ requires 413.2189; *IR* ν max(NaCl)/cm⁻¹: 3275 (N-H, O-H), 2944 (C-H), 1634, 1544 br, 1280; *¹H NMR* (400 MHz, CD₃OD): δ 1.79 (2H, p, J = 7.3 Hz, CH₂CH₂CH₂), 2.08 (3H, s, COCH₃), 2.26 (6H, s, N(CH₃)₂), 2.42 (2H, t, J = 7.6 Hz, NCH₂), 3.40 (2H, t, J = 6.9 Hz, CONHCH₂), 4.54 (2H, s, ArCH₂O), 7.32 (1H, s, Ar-H), 7.40 (1H, s, Ar-H), 7.86 (2H, t, J = 8.3 Hz, Ar-H), 7.94 (1H, s, Ar-H), 7.97 (2H, t, J = 8.3 Hz, Ar-H); *¹³C NMR* (100 MHz, CD₃OD): δ 24.0 (CH₃, NHCOCH₃), 28.0 (CH₂, CH₂CH₂CH₂), 39.4 (CH₂, CONHCH₂), 45.3 (2 × CH₃, N(CH₃)₂), 58.2 (CH₂, NCH₂), 65.0 (CH₂, ArCH₂O), 113.0 (CH, Ar-C), 115.9 (CH, Ar-C), 116.4 (CH, Ar-C), 128.4 (2 × CH, Ar-C), 128.9 (2 × CH, Ar-C), 138.5 (quat., Ar-C), 138.9 (quat., Ar-C), 140.1 (quat., Ar-C), 140.4 (quat., Ar-C), 144.0 (quat., Ar-C), 167.9, 169.2 and 171.7 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃); *m/z* (FAB⁺): 413 (MH⁺, 33%), 219 (5%), 154 (100%).
The reaction was carried out according to general procedure 13, with silyl ether 243 (204 mg, 0.40 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the **title compound 255** (158 mg, 100%) as an off white foam.

R_f (MeOH/NH_3 9:1) = 0.22; **HRMS** Found (ESI^+): MH^+ 399.2039, C_{21}H_{27}N_4O_4 requires 399.2032; **IR** ν_max(solid)/cm⁻¹: 3270 br (N-H, O-H), 2962 (C-H), 1638 br (C=O), 1620, 1548, 1452, 1371, 1286; **^1{H} NMR** (400 MHz, CDCl_3/CD_3OD): δ 2.0 (3H, s, NHCOCH_3), 2.21 (6H, s, N(C(H_3)_3)), 2.48 (2H, t, J = 6.2 Hz, NCH_2), 3.44 (2H, t, J = 6.2 Hz, CONHC_2H), 4.44 (2H, s, ArCH_2OH), 7.25 (1H, s, Ar-H), 7.36 (1H, s, Ar-H), 7.59 (1H, br s, Ar-H), 7.75 (2H, t, J = 8.4 Hz, Ar-H), 7.80 (2H, d, J = 8.4 Hz, Ar-H); **^13{C} NMR** (100 MHz, CDCl_3/CD_3OD): δ 23.6 (CH_3, NHCOCH_3), 37.2 (CH_2, NHCH_2), 44.9 (2 × CH_3, N(CH_3)_2), 57.9 (CH_2, CONHCH_2), 64.1 (CH_2, ArCH_2O), 111.3 (CH, Ar-C), 114.7 (CH, Ar-C), 115.0 (CH, Ar-C), 127.1 (2 × CH, Ar-C), 127.4 (2 × CH, Ar-C), 136.7 (quat., Ar-C), 137.2 (quat., Ar-C), 138.4 (quat., Ar-C), 138.6 (quat., Ar-C), 142.6 (quat., Ar-C), 166.0, 167.2 and 169.9 (C=O, NHCOAr, CONHCH_2 and NHCOCH_3); **m/z** (ESI^+): 399 (MH^+, 100%), 336 (2%), 242 (24%), 191 (7%), 168 (4%)

**N^1-(3-Acetamido-5-(hydroxymethyl)phenyl)-N^4-(3-(diethylamino)propyl)-terephthalamide (256)**

The reaction was carried out according to general procedure 13, with silyl ether 244 (180 mg, 0.32 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the **title compound 256** (143 mg, 100%) as an off white foam.

R_f (MeOH/NH_3 9:1) = 0.18; **HRMS** Found (ESI^+): MH^+ 441.2505, C_{24}H_{33}N_4O_4 requires 441.2502; **IR** ν_max(solid)/cm⁻¹: 3266 br (N-H, O-H), 2965 (C-H), 1642 br, 1546, 1459, 1436, 1374, 1277; **^1{H} NMR** (400 MHz, CDCl_3/CD_3OD): δ 0.96 (6H, t, J = 7.2 Hz, N(CH_2CH_3)_2),
1.71 (2H, p, J = 6.6 Hz, CH₂CH₂CH₂), 2.01 (3H, s, NHCOCH₃), 2.50 (4H, q, J = 7.2 Hz, N(CH₂CH₃)₂), 3.40 (2H, t, J = 6.4 Hz, CONHCH₂), 4.45(2H, s, ArCH₂O), 7.21 (1H, s, Ar-H), 7.33 (1H, s, Ar-H), 7.65 (1H, s, Ar-H), 7.71 (2H, d, J = 8.0 Hz, Ar-H), 7.78 (2H, d, J = 8.0 Hz, Ar-H); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ 10.8 (2 × CH₃, N(CH₂CH₃)₂), 23.8 (CH₂, CH₂CH₂CH₂), 25.1(CH₃, NHCOCH₃), 39.8 (CH₂, NCH₂), 46.4 (CH₂, N(CH₂CH₃)₂), 51.5 (CH₂, CONHCH₂), 64.1 (CH₂, ArCH₂O), 111.4 (CH, Ar-C), 114.8 (CH, Ar-C), 115.0 (CH, Ar-C), 127.0 (2 × CH, Ar-C), 127.5 (2 × CH, Ar-C), 137.0 (quat., Ar-C), 137.2 (quat., Ar-C), 138.3 (quat., Ar-C), 138.6 (quat., Ar-C), 142.6 (quat., Ar-C), 166.1, 167.1 and 169.9 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃); m/z (ESI⁺): 441 (MH⁺, 100%).

N¹-(3-Acetamido-5-(hydroxymethyl)phenyl)-N⁴-(3-morpholinopropyl)-terephthalamide (257)

![Chemical Structure](image)

The reaction was carried out according to general procedure 13, with silyl ether 250 (97 mg, 0.17 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 257 (55 mg, 71%) as an off white foam under high vacuum.

Rₚ (MeOH/NH₃ 9:1) = 0.66; HRMS Found (ESI⁺): MH⁺ 455.2275, C₂₄H₂₃N₄O₅ requires 455.2294; IR νmax(solid)/cm⁻¹: 3270 br (N-H, O-H), 2921 (C-H), 1698 (C=O), 1653, 1623, 1551, 1453, 1374, 1285; ¹H NMR (400 MHz, CDCl₃/CD₃OD): δ 1.71 (2H, p, J = 6.4 Hz, CH₂CH₂CH₂), 2.0 (3H, s, NHCOCH₃), 2.34-2.41 (6H, t, J = 6.4 Hz, CH₂N(CH₂CH₂)₂, (CH₂CH₂)₂NCH₂), 3.39 (2H, t, J = 6.4 Hz, NHCH₂CH₂), 3.61 (4H, t, J = 4.0 Hz, O(CH₂CH₂)₂), 4.45 (2H, s, ArCH₂O), 7.18 (1H, s, Ar-H), 7.29 (1H, s, Ar-H), 7.67 (1H, s, Ar-H), 7.72 (2H, d, J = 8.4 Hz, Ar-H), 7.79 (2H, d, J = 8.0 Hz, Ar-H); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ 23.6 (CH₂, CH₂CH₂CH₂), 24.7 (CH₃, NHCOCH₃), 39.1 (CH₂, CONHCH₂), 53.4 (2 × CH₂, CH₂N(CH₂CH₂)₂), 57.1 (CH₂, (CH₂CH₂)₂NCH₂), 64.0 (CH₂, ArCH₂O), 66.5 (2 × CH₂, O(CH₂CH₂)₂), 111.4 (CH, Ar-C), 114.7 (CH, Ar-C), 114.9 (CH, Ar-C), 127.0 (2 × CH,
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Ar-C), 127.4 (2 × CH, Ar-C), 137.1 (2 × quat., Ar-C), 138.2 (quat., Ar-C), 138.5 (quat., Ar-C), 142.5 (quat., Ar-C), 166.0, 167.2 and 169.86 (C=O, NHCOAr, CONHCH₂ and NHCOCH₃);

m/z (ESI⁺): 455 (MH⁺, 100%), 293 (5%), 219 (37%).

\(N¹-(3\text{-Acetamido-5-(hydroxymethyl)phenyl})-N⁴-(2\text{-morpholinoethyl)-terephthalamide (258)}\)

![Structure of compound 258]

The reaction was carried out according to general procedure 13, with silyl ether 251 (112 mg, 0.20 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to give the title compound 258 (71 mg, 80%) as an off white foam.

\[R_f (\text{MeOH/NH}_3 \ 9:1) = 0.72; \ \text{HRMS} \ \text{Found (ESI⁺)}: \ \text{MH}⁺ \ 441.2133, \ \text{C}_{23}\text{H}_{29}\text{N}_4\text{O}_5 \ \text{requires} \ 441.2138; \ \text{IR} \ \nu_{\text{max}}(\text{solid})/\text{cm}^{-1}: \ 3304 \ \text{br (N-H, O-H)}, 2924 (\text{C-H}), 1649 (\text{C=O}), 1620, 1539, 1432, 1370, 1315, 1292; \ \text{¹H NMR (400 MHz, CDCl}_3/\text{CD}_2\text{OD):} \ \delta = 1.98 (3\text{H, s, NHCOCH}_3), 2.41 (4\text{H, m, CH}_2\text{N(CH}_2\text{CH}_2)_2), 2.50 (2\text{H, t,} J = 6.2 \ \text{Hz, (CH}_2\text{CH}_2)_2\text{NCH}_2), 3.49 (2\text{H, t,} J = 6.0 \ \text{Hz, CONHCH}_2), 3.61 (4\text{H, br s, O(CH}_2\text{CH}_2)_2), 4.43 (2\text{H, s, ArCH}_2\text{O}), 7.16 (1\text{H, s, Ar-H}), 7.27 (1\text{H, s, Ar-H}), 7.65 (1\text{H, s, Ar-H}), 7.71 (2\text{H, d,} J = 8.0 \ \text{Hz, Ar-H}), 7.77 (2\text{H, d,} J = 8.0 \ \text{Hz, Ar-H}); \ \text{¹³C NMR (100 MHz, CDCl}_3/\text{CD}_2\text{OD):} \ \delta = 23.5 (\text{CH}_3, \ \text{NHCOCH}_3), 36.1 (\text{CH}_2, \ \text{CONHCH}_2), 53.0 (2 \times \text{CH}_2, \ \text{CH}_2\text{N(CH}_2\text{CH}_2)_2), 57.0 (\text{CH}_2, (\text{CH}_2\text{CH}_2)_2\text{NCH}_2), 63.9 (\text{CH}_2, \ \text{ArCH}_2\text{O}), 66.4 (2 \times \text{CH}_2, \ \text{O(CH}_2\text{CH}_2)_2), 111.3 (\text{CH, Ar-C}), 114.6 (\text{CH, Ar-C}), 114.9 (\text{CH, Ar-C}), 127.0 (2 \times \text{CH, Ar-C}), 127.4 (2 \times \text{CH, Ar-C}), 136.7 (\text{quat., Ar-C}, 137.2 (\text{quat., Ar-C}), 138.2 (\text{quat., Ar-C}), 138.5 (\text{quat., Ar-C}), 142.4 (\text{quat., Ar-C}), 166.0, 167.2 and 169.9 (\text{C=O, NHCOAr, CONHCH}_2 \ \text{and NHCOCH}_3); \ \text{m/z (ESI⁺):} \ 441 (\text{MH}⁺, 100%), 293 (3%), 212 (22%).
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N^1-(3-Acetamido-5-(chloromethyl)phenyl)-N^4-(3-(dimethylamino)propyl)terephthalamide (261)

![Chemical structure of 261]

The reaction was carried out according to general procedure 15, with alcohol 254 (150 mg, 0.36 mmol) to furnish the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to afford the title compound 261 (150 mg, 96%) as an off white foam.

HRMS Found (ESI^+): MH^+ 431.1829 C_{22}H_{28}N_{4}O_{3} requires 431.18499; IR \nu_{\text{max}}(\text{NaCl})/\text{cm}^{-1}: 3266 (N-H), 3067, 1642 br (C=O), 1616, 1544 br, 1455, 1286; ^1H NMR (400 MHz, CD_{3}OD): \delta 2.08 (2H, p, J = 7.3 Hz, CH_2CH_2CH_2), 2.15 (3H, s, NHCOCH_3), 2.94 (6H, s, N(CH_3)_2), 3.25 (2H, t, J = 7.6 Hz, CONHCH_2), 4.62 (2H, s, ArCH_2Cl), 7.47 (1H, t, J = 1.6 Hz, Ar-H), 7.54 (1H, t, J = 1.6 Hz, s, Ar-H), 7.99-8.02 (5H, m, Ar-H); ^13C NMR (100 MHz, CD_{3}OD): \delta 24.0 (CH_3, NHCOCH_3), 26.1 (CH_2, CH_2CH_2CH_2), 37.7 (CH_2, CONHCH_2), 43.6 (2 \times CH_3, N(CH_3)_2), 46.8 (CH_2, ArCH_2Cl), 56.8 (CH_2, NCH_2), 113.8 (CH, Ar-C), 117.5 (CH, Ar-C), 118.0 (CH, Ar-C), 128.6 (2 \times CH, Ar-C), 128.7 (2 \times CH, Ar-C), 138.1 (quat., Ar-C), 139.0 (quat., Ar-C), 140.3 (quat., Ar-C), 140.4 (quat., Ar-C), 144.0 (quat., Ar-C), 167.8, 169.6 and 171.8 (C=O, NHCOAr, CONHCH_2 and NHCOCH_3); m/z (ESI^+): 431 (MH^+, 100%), 413 (29%), 381 (20%), 251 (5%), 292 (21%).

3-Acetamido-N-(4-(2-(diethylamino)acetamido)phenyl)benzamide (262)

![Chemical structure of 262]

The reaction was carried out according to general procedure 18, with acid 119 (100 mg, 0.40 mmol) and aniline 113 (30 mg, 0.20 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 262 (60 mg, 78%) as a white foam.
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**3-Acetamido-N-(3-(2-(diethylamino)acetamido)phenyl)benzamide (263)**

![Chemical Structure Image]

The reaction was carried out according to general procedure 18, with acid 120 (100 mg, 0.40 mmol) and aniline 113 (30 mg, 0.20 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the *title compound 263* (74 mg, 97%), which was purified with flash chromatography (dichloromethane-methanol 19:1) as a white foam.

**Rf** (DCM/MeOH 9:1) = 0.51; **HRMS** Found (FAB+): MH+ 383.2097, C21H27N4O3 requires 383.2083; **IR** νmax(solid)/cm⁻¹: 3276 (N-H), 2969 (C-H), 1671 (C=O), 1603, 1516, 1480, 1421, 1286; **¹H NMR** (400 MHz, CDCl₃): δ 1.05 (6H, t, J = 7.2 Hz, N(CH₂CH₃)₂), 2.01 (3H, s, NHCOCH₃), 2.61 (4H, q, J = 7.0 Hz, N(CH₂CH₃)₂), 3.11 (2H, s, COCH₂N), 7.17 (1H, t, J = 8.0 Hz Ar-H), 7.27 (1H, d, J = 8.0 Hz, Ar-H), 7.41 (1H, d, J = 8.0 Hz, Ar-H), 7.56 (2H, d, J = 8.4 Hz, Ar-H), 7.78 (2H, d, J = 8.4 Hz, Ar-H), 7.88 (1H, s, Ar-H), 8.28, 8.65 and 9.56 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); **¹³C NMR** (100 MHz, CDCl₃): δ 12.3 (C×CH₃, N(CH₂CH₃)₂), 24.3 (CH₃, NHCOCH₃), 48.8 (C×CH₂, N(CH₂CH₃)₂), 58.0 (CH₂COCH₂N), 112.4 (CH, Ar-C), 116.0 (CH, Ar-C), 116.4 (CH, Ar-C), 118.9 (C×CH, Ar-C), 128.4 (C×CH, Ar-C), 129.2 (CH, Ar-C), 130.0 (quat., Ar-C), 138.6 (2×quat., Ar-C), 140.5 (quat., Ar-C), 165.6, 169.1 and 170.7 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); **m/z** (FAB+): 383 (MH+, 100%).

**Rf** (DCM/MeOH 9:1) = 0.51; **HRMS** Found (ESI+): MH+ 383.2078, C21H27N4O3 requires 383.2083; **IR** νmax (solid)/cm⁻¹: 3270 (N-H), 2969 (C-H), 1651 (C=O), 1605, 1523, 1481, 1412, 1368, 1300, 1280; **¹H NMR** (400 MHz, CDCl₃): δ 1.03 (6H, t, J = 7.2 Hz, N(CH₂CH₃)₂), 2.0 (3H, s, NHCOCH₃), 2.58 (4H, q, J = 7.0 Hz, N(CH₂CH₃)₂), 3.08 (2H, s, COCH₂N), 7.14 (1H, t, J = 8.0 Hz, Ar-H), 7.27-7.30 (2H, m, Ar-H), 7.39 (1H, d, J = 8.0 Hz, Ar-H), 7.51 (1H, d, J = 7.6 Hz, Ar-H), 7.72 (1H, d, J = 8.8 Hz, Ar-H), 7.81 (1H, s, Ar-H), 7.98 (1H, s, Ar-H), 8.33, 8.77 and 9.53 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); **¹³C
NMR (100 MHz, CDCl₃): δ 12.3 (2 × CH₃, N(CH₂CH₃)₂), 24.2 (CH₃, NHCOCH₃), 48.8 (2 × CH₂, N(CH₂CH₃)₂), 57.8 (CH₂, COCH₂N), 112.3 (CH, Ar-C), 116.1 (CH, Ar-C), 116.3 (CH, Ar-C), 118.4 (CH, Ar-C), 122.6 (CH, Ar-C), 122.8 (CH, Ar-C), 129.2 (2 × CH, Ar-C), 135.7 (quat., Ar-C), 137.8 (quat., Ar-C), 138.4 (quat., Ar-C), 138.6 (quat., Ar-C), 165.8, 169.1 and 170.8 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 383 (MH⁺, 100%), 172 (5%).

3-Acetamido-5-(((tert-butyldimethylsilyloxy)methyl-N-(4-(2-diethylamino)acetamido)phenyl)benzamide (264)

The reaction was carried out according to general procedure 18, with acid 119 (144 mg, 0.58 mmol) and aniline 99 (85 mg, 0.29 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 264 (110 mg, 73%) as a foam.

Rf (DCM/MeOH 9:1) = 0.61; HRMS Found (ESI⁺): MH⁺ 527.3054, C₂₉H₄₃N₄O₄Si requires 527.3054; IR νmax(solid)/cm⁻¹: 3275 (N-H), 2930 (C-H), 1665 br (C=O), 1605, 1516, 1454, 1423, 1369, 1281, 1252; ¹H NMR (400 MHz, CDCl₃): δ 0.09 (6H, s, OSi(CH₃)₂), 0.92 (9H, s, OSiC(CH₃)₃), 1.08 (6H, t, J = 7.0 Hz, N(CH₂CH₃)₂), 2.09 (3H, s, NHCOCH₃), 2.64 (4H, q, J = 7.0 Hz, N(CH₂CH₃)₂), 3.15 (2H, s, COCH₂N), 4.69 (2H, s, ArCH₂O), 7.30 (1H, s, Ar-H), 7.43 (1H, s, Ar-H), 7.63 (2H, d, J = 8.6 Hz, Ar-H), 7.76 (1H, s, Ar-H), 7.80 (2H, d, J = 8.6 Hz, Ar-H), 7.84, 8.24 and 9.59 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); ¹³C NMR (100 MHz, CDCl₃): δ -5.2 (2 × CH₃, OSi(CH₃)₂), 12.4 (2 × CH₃, N(CH₂CH₃)₂), 18.5 (quat., OSiC(CH₃)₃), 24.6 (CH₃, NHCOCH₃), 26.0 (3 × CH₃, OSiC(CH₃)₃), 48.9 (2 × CH₂, N(CH₂CH₃)₂), 58.1 (CH₂, COCH₂N), 64.7 (CH₂, ArCH₂O), 110.1 (CH, Ar-C), 113.1 (CH, Ar-C), 113.4 (CH, Ar-C), 119.0 (2 × CH, Ar-C), 128.3 (2 × CH, Ar-C), 130.1 (quat., Ar-C), 138.6 (2 × quat., Ar-C), 1423, 1369, 1281, 1252;
140.7 (quat., Ar-C), 143.4 (quat., Ar-C), 165.4, 168.7 and 170.6 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 527 (MH⁺, 100%), 395 (2%), 275 (4%), 198 (5%).

3-Acetamido-5-(((tert-butyldimethylsilyloxy)methyl-N-(3-(2-(diethylamino)acetamido)phenyl)benzamide (265)

The reaction was carried out according to general procedure 18, with acid 120 (144 mg, 0.58 mmol) and aniline 99 (85 mg, 0.29 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 265 (140 mg, 92%) as a foam.

Rf (DCM/MeOH 9:1) = 0.61; HRMS Found (ESI⁺): MH⁺ 527.3055, C₂₉H₄₃N₄O₄Si requires 527.3054; IR νmax(solid)/cm⁻¹: 3278 (N-H), 2929 (C-H), 1667 (C=O), 1607, 1531 br, 1455 br, 1371, 1280, 1255; ¹H NMR (400 MHz, CDCl₃): δ 0.1 (6H, s, OSi(CH₃)₂), 0.94 (9H, s, OSiC(CH₃)₃), 1.09 (6H, t, J = 7.2 Hz, N(CH₂CH₃)₂), 2.15 (3H, s, NHCOCH₃), 2.66 (4H, q, J = 7.2 Hz, N(CH₂CH₃)₂), 3.17 (2H, s, COCH₂N), 4.71 (2H, s, ArCH₂O), 7.31 (1H, s, Ar-H), 7.41-7.43 (2H, m, Ar-H), 7.47 (1H, s, Ar-H), 7.57 (1H, d, J = 7.7 Hz, Ar-H), 7.80 (1H, br s, Ar-H), 7.84 (1H, d, J = 7.9 Hz, Ar-H), 7.98, 8.20 and 9.61 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); ¹³C NMR (100 MHz, CDCl₃): δ -5.3 (2 × CH₃, OSi(CH₃)₂), 12.4 (2 × CH₃, N(CH₂CH₃)₂), 18.5 (quat., OSi(CH₃)₃), 24.8 (CH₃, NHCOCH₃), 25.9 (3 × CH₃, OSi(CH₃)₃), 48.8 (CH₂, COCH₂N), 58.1 (2 × CH₂, N(CH₂CH₃)₂), 64.7 (CH₂, ArCH₂O), 110.9 (CH, Ar-C), 113.2 (CH, Ar-C), 113.4 (CH, Ar-C), 117.7 (CH, Ar-C), 122.7 (2 × CH, Ar-C), 129.6 (CH, Ar-C), 135.8 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 140.1 (quat., Ar-C), 143.5 (quat., Ar-C), 165.4, 169.6 and 175.5 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 527 (MH⁺, 100%), 360 (5%), 275 (2%), 172 (5%).
N-(3-Acetamidophenyl)-4-(2-(diisopropylamino)acetamido)benzamide (266)

The reaction was carried out according to general procedure 18, with acid 121 (111 mg, 0.40 mmol) and aniline 113 (30 mg, 0.20 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 266 (46 mg, 56%) as an off white foam.

R<sub>f</sub> (DCM/MeOH 9:1) = 0.60; HRMS Found (ESI<sup>+</sup>): MH<sup>+</sup> 411.2418, C<sub>23</sub>H<sub>31</sub>N<sub>4</sub>O<sub>3</sub> requires 411.2396; IR ν<sub>max</sub>(solid)/cm<sup>-1</sup>: 3273 (N-H), 2968 (C-H), 1660 (C=O), 1605, 1518, 1482, 1413, 1305 br, 1210; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.04 (12H, d, J = 6.4 Hz, N(CH(CH<sub>3</sub>)<sub>2</sub>), 2.10 (3H, s, NHCOCH<sub>3</sub>), 3.07 (2H, m, N(CH(CH<sub>3</sub>)<sub>2</sub>), 3.13 (2H, s, COCH<sub>2</sub>N), 7.24 (1H, t, J = 8.0 Hz Ar-H), 7.30 (1H, d, J = 7.6 Hz, Ar-H), 7.41 (2H, d, J = 8.0 Hz, Ar-H), 7.56 (2H, d, J = 8.0 Hz, Ar-H), 7.74 (1H, d, J = 8.8 Hz, Ar-H), 7.87 (1H, s, Ar-H), 8.0, 8.38 and 9.65 (3H, NHCOC<sub>6</sub>H<sub>4</sub>NH<sub>2</sub> and NHCOAr); <sup>1</sup>3C NMR (100 MHz, CDCl<sub>3</sub>): δ 20.6 (4 × CH<sub>3</sub>, N(CH(CH<sub>3</sub>)<sub>2</sub>)), 24.5 (CH<sub>3</sub>, NHCOC<sub>6</sub>H<sub>4</sub>), 50.2 (CH<sub>2</sub>, COCH<sub>2</sub>N), 50.3 (2 × CH, N(CH(CH<sub>3</sub>)<sub>2</sub>)), 111.8 (CH, Ar-C), 115.9 (CH, Ar-C), 116.1 (CH, Ar-C), 118.8 (CH, Ar-C), 122.5 (CH, Ar-C), 122.7 (CH, Ar-C), 129.5 (2 × CH, Ar-C), 135.8 (quat., Ar-C), 138.0 (quat., Ar-C), 138.5 (quat., Ar-C), 138.6 (quat., Ar-C), 165.6, 168.7 and 172.2 (C=O, NHCOC<sub>6</sub>H<sub>4</sub>NH<sub>2</sub> and NHCOAr); m/z (ESI<sup>+</sup>): 411 (MH<sup>+</sup>, 100%), 293 (2%), 172 (5%).

N-(3-Acetamidophenyl)-3-(2-(diisopropylamino)acetamido)benzamide (267)

The reaction was carried out according to general procedure 18, with acid 122 (248 mg, 0.89 mmol) and aniline 113 (67 mg, 0.45 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 267 (110 mg, 60%) as a foam.
**Chapter 6: Experimental**

\[ \text{R}_f \text{ (DCM/MeOH 9:1) } = 0.57; \text{ HRMS Found (ESI)}^+ : \text{MH}^+ 411.2417, \text{C}_{23}\text{H}_{31}\text{N}_{4}\text{O}_3 \text{ requires 411.2396; IR } \nu_{\text{max}}\text{(solid)/cm}^{-1} : \text{3268 (N-H), 2967, 1651 (C=O), 1605, 1523, 1482, 1412, 1302 br; } ^1\text{H NMR} \text{ (400 MHz, CDCl}_3) : \delta 0.98 \text{ (12H, d, } J = 6.4 \text{ Hz, N(CH(CH}_3)_2)_2), 1.96 \text{ (3H, s, NHCOCH}_3)_2), 3.0 (2H, m, N(CH(CH}_3)_2)_2), 3.04 \text{ (2H, s, COCH}_2\text{N).} \]

The reaction was carried out according to general procedure 18, with acid 121 (520 mg, 1.87 mmol) and aniline 99 (275 mg, 0.93 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 9:1) to furnish the title compound 268 (254 mg, 49%) as a foam.

\[ \text{R}_f \text{ (DCM/MeOH 9:1) } = 0.66; \text{ HRMS Found (ESI)}^+ : \text{MH}^+ 555.3375, \text{C}_{39}\text{H}_{47}\text{N}_{4}\text{O}_4\text{Si requires } 555.3367; \text{ IR } \nu_{\text{max}}\text{(solid)/cm}^{-1} : \text{3274 (N-H), 2929 (C-H), 1667 (C=O), 1605, 1515, 1454, 1422, 1368, 1277, 1100; } ^1\text{H NMR} \text{ (400 MHz, CDCl}_3) : \delta 0.08 \text{ (6H, s, OSi(CH}_3)_2), 0.91 \text{ (9H, s, OSi(CH}_3)_3), 1.05 \text{ (12H, d, } J = 6.4 \text{ Hz, N(CH(CH}_3)_2)_2), 2.06 \text{ (3H, s, NHCOCH}_3)_2), 3.08 \text{ (2H, m, N(CH(CH}_3)_2)_2), 3.14 \text{ (2H, s, COCH}_2\text{N), 4.67 \text{ (2H, s, ArCH}_2\text{O), 7.31 \text{ (1H, s, Ar-H), 7.44 \text{ (1H, s, Ar-H), 7.59 \text{ (2H, d, } J = 8.8 \text{ Hz, Ar-H), 7.79 \text{ (2H, d, } J = 8.4 \text{ Hz, Ar-H), 7.84 \text{ (1H, s, Ar-H), 7.94, 8.37 and 9.67 \text{ (3H, NHCOCH}_3, NHCOAr and NHCOCH}_2); } ^13\text{C NMR} \text{ (100 MHz, CDCl}_3) :} \]
CDCl₃: δ -5.3 (2 × CH₃, OSi(CH₃)₂), 18.4 (quat., OSiC(CH₃)₃), 20.6 (4 × CH₃, N(CH(CH₃)₂)₂), 24.5 (CH₃, NHCOCH₃), 25.8 (3 × CH₃, OSiC(CH₃)₃), 50.3 (CH₂ and 2 × CH, COCH₂N, N(CH(CH₃)₂)₂), 64.7 (CH₂, ArCH₂O), 110.3 (CH, Ar-C), 113.2 (CH, Ar-C), 113.5 (CH, Ar-C), 118.8 (2 × CH, Ar-C), 128.4 (2 × CH, Ar-C), 130.1 (quat., Ar-C), 138.5 (2 × quat., Ar-C), 140.6 (quat., Ar-C), 143.2 (quat., Ar-C), 165.4, 168.8 and 173.3 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 555 (MH⁺, 100%), 289 (5%), 212 (3%), 172 (2%).

N-(3-acetamido-5-(((tert-butyldimethylsilyloxy)methyl)phenyl)-3-(2-(diisopropylamino)acetamido)benzamide (269)

The reaction was carried out according to general procedure 18, with acid 122 (217 mg, 0.78 mmol) and aniline 99 (115 mg, 0.39 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 269 (194 mg, 90%) as a foam.

Rf (DCM/MeOH 9:1) = 0.66; HRMS Found (ESI⁺): MH⁺ 555.3360, C₃₀H₄₇N₄O₄Si requires 555.3367; IR νmax(solid)/cm⁻¹: 3278 (N-H), 2930 (C-H), 1667 (C=O), 1607, 1523 br, 1455, 1420, 1368, 1280 br, 1256; ¹H NMR (400 MHz, CDCl₃): δ 0.06 (6H, s, OSi(CH₃)₂), 0.89 (9H, s, OSiC(CH₃)₃), 1.01 (12H, d, J = 6.4 Hz, N(CH(CH₃)₂)₂), 2.03 (3H, s, NHCOCH₃), 3.04 (2H, m, N(CH(CH₃)₂)₂), 3.09 (2H, s, COCH₂N), 4.64 (2H, s, ArCH₂O), 7.31-7.34 (2H, br s, Ar-H), 7.43 (1H, s, Ar-H), 7.54 (1H, d, J = 7.6 Hz, Ar-H), 7.75 (2H, d, J = 7.6 Hz, Ar-H), 7.96 (1H, s, Ar-H), 8.17, 8.73 and 9.62 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); ¹³C NMR (100 MHz, CDCl₃): δ -5.4 (2 × CH₃, OSi(CH₃)₂), 18.3 (quat., OSiC(CH₃)₃), 20.5 (4 × CH₃, N(CH(CH₃)₂)₂), 24.3 (CH₃, NHCOCH₃), 25.8 (3 × CH₃, OSiC(CH₃)₃), 50.1 (2 × CH and CH₂, COCH₂N, N(CH(CH₃)₂)₂), 64.7 (CH₂, ArCH₂O), 110.5 (CH, Ar-C), 113.5 (CH, Ar-C), 113.7 (CH, Ar-C), 118.1 (CH, Ar-C), 122.4 (CH, Ar-C), 122.7 (CH, Ar-C), 129.3 (CH, Ar-C), 135.8 (quat., Ar-C), 137.8 (quat., Ar-C), 138.4 (quat., Ar-C), 138.6 (quat., Ar-C), 143.1 (quat.,
Ar-C), 165.8, 168.9 and 172.2 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 555 (MH⁺, 100%), 319 (80%).

\[N-(3\text{-Acetamidophenyl})-4-(2\text{-}(piperidin-1\text{-yl})acetamido)benzamide\] (270)

The reaction was carried out according to general procedure 18, with acid 123 (140 mg, 0.53 mmol) and aniline 113 (40 mg, 0.27 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 270 (56 mg, 53%) as a foam.

\[R_f\] (DCM/MeOH 9:1) = 0.47; \[HRMS\text{ Found (ESI}^+\text{): MH}^+ 395.2082, C_{22}H_{27}N_{4}O_{3} \text{requires} 395.2083; \[IR\ \nu_{\text{max}}\text{(solid)}/\text{cm}^{-1}: 3273 (\text{N-H}), 2934 (\text{C-H}), 1648 (\text{C}=\text{O}), 1603, 1513, 1480, 1414, 1279, 1185; \[^{1}H\ \text{NMR}\ (400\text{ MHz, CDCl}_3): \delta 1.42 (2\text{H, br s, C}_2\text{H}_2(\text{CH}_2\text{CH}_2)_2\text{N}, 1.60 (4\text{H, q, } J = 5.2 \text{ Hz, C}_2(\text{CH}_2\text{CH}_2)_2\text{N}), 1.95 (3\text{H, s, NHCOCH}_3), 2.46 (4\text{H, br s, C}_2\text{H}_2(\text{CH}_2\text{CH}_2)_2\text{N}, 3.0 (2\text{H, s, COCH}_2\text{N}), 7.11 (1\text{H, t, } J = 8.0 \text{ Hz, Ar-H}), 7.25 (1\text{H, d, } J = 8.0 \text{ Hz, Ar-H}), 7.39 (1\text{H, d, } J = 8.0 \text{ Hz, Ar-H}), 7.50 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}), 7.75 (2\text{H, d, } J = 8.4 \text{ Hz, Ar-H}), 7.85 (1\text{H, s, Ar-H}), 8.66, 8.93 \\text{and 9.43 (3H, NHCOCH}_3, \text{ NHCOAr and NHCOCH}_2); \[^{13}C\ \text{NMR}\ (100\text{ MHz, CDCl}_3): \delta 23.4 (\text{CH}_2, \text{CH}_2(\text{CH}_2\text{CH}_2)_2\text{N}), 24.1 (\text{CH}_3, \text{NHCOCH}_3), 26.1 (2 \times \text{CH}_2, \text{CH}_2(\text{CH}_2\text{CH}_2)_2\text{N}), 54.7 (2 \times \text{CH}_2, \text{CH}_2(\text{CH}_2\text{CH}_2)_2\text{N}), 62.5 (\text{CH}_2, \text{COCH}_2\text{N}), 112.6 (\text{CH, Ar-C}), 116.1 (\text{CH, Ar-C}), 116.5 (\text{CH, Ar-C}), 118.8 (2 \times \text{CH, Ar-C}), 128.4 (2 \times \text{CH, Ar-C}), 129.0 (\text{CH, Ar-C}), 129.9 (\text{quat., Ar-C}), 138.5 (\text{quat., Ar-C}), 138.6 (\text{quat., Ar-C}), 140.4 (\text{quat., Ar-C}), 165.9, 169.3 and 169.5 (\text{C}=\text{O, NHCOCH}_3, \text{NHCOAr and NHCOCH}_2); \text{m/z (ESI}^+\text{): 395 (MH}^+, 100%), 198 (3%), 172 (2%).

\[N-(3\text{-Acetamidophenyl})-4-(2\text{-}(piperidin-1\text{-yl})acetamido)benzamide\] (271)
The reaction was carried out according to general procedure 18, with acid 124 (70 mg, 0.27 mmol) and aniline 113 (20 mg, 0.13 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the *title compound* 271 (46 mg, 87%) as a foam.

**R**<sub>f</sub> (DCM/MeOH 9:1) = 0.48; **HRMS** Found (ESI<sup>+</sup>): MH<sup>+</sup> 395.2066, C<sub>22</sub>H<sub>27</sub>N<sub>4</sub>O<sub>3</sub> requires 395.2083; **IR** ν<sub>max</sub>(solid)/cm<sup>-1</sup>: 3274 (N-H), 2934 (C-H), 1655 br (C=O), 1606, 1527 br, 1483, 1413, 1302 br, 1258; **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 1.44 (2H, br s, C<sub>H</sub><sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 1.60 (4H, q, J = 5.6 Hz, C<sub>H</sub><sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 2.03 (3H, s, NHCOCH<sub>3</sub>), 2.49 (4H, br m, C<sub>H</sub><sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 3.02 (2H, s, CO<sub>C</sub>H<sub>2</sub>N), 7.17 (1H, t, J = 8.0 Hz, Ar-H), 7.28 - 7.32 (2H, m, Ar-H), 7.39 (1H, d, J = 8.0 Hz, Ar-H), 7.52 (1H, s, J = 7.6 Hz, Ar-H), 7.70 (1H, dd, J = 8.0, 1.2 Hz, Ar-H), 7.82 (1H, s, Ar-H), 7.98 (1H, s, Ar-H), 8.17, 8.69 and 9.40 (3H, NHCOCH<sub>3</sub>, NHCOAr and NHCOCH<sub>2</sub>); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 23.5 (CH<sub>2</sub>, C<sub>H</sub><sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 24.3 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 26.1 (2 × CH<sub>2</sub>, CH<sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 54.9 (2 × CH<sub>2</sub>, CH<sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N), 62.6 (CH<sub>2</sub>, COCH<sub>3</sub>), 112.2 (CH, Ar-C), 116.1 (CH, Ar-C), 116.3 (CH, Ar-C), 118.4 (CH, Ar-C), 122.8 (CH, Ar-C), 122.9 (CH, Ar-C), 129.3 (2 × CH, Ar-C), 135.7 (quat., Ar-C), 137.8 (quat., Ar-C), 138.4 (quat., Ar-C), 138.6 (quat., Ar-C), 165.8, 169.0 and 169.6 (C=O, NHCOCH<sub>3</sub>, NHCOAr and NHCOCH<sub>2</sub>); m/z (ESI<sup>+</sup>): 395 (MH<sup>+</sup>, 100%), 360 (5%), 172 (30%), 172 (30%), 130 (5%).

*<sup>-</sup>(3-Azetamido-5-((<i>tert</i>-butyldimethylsilyloxy)methyl)phenyl)-4-((2-(piperidin-1-yl)acetamido)benzamide* (272)

![Image](image_url)

The reaction was carried out according to general procedure 18, with acid 123 (349 mg, 1.33 mmol) and aniline 99 (196 mg, 0.66 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the *title compound* 272 (240 mg, 67%) as a foam.
The reaction was carried out according to general procedure 18, with acid 124 (106 mg, 0.40 mmol) and aniline 99 (60 mg, 0.20 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 273 (91 mg, 84%) as a white foam.

N-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-3-(2-(piperidin-1-yl)acetamido)benzamide (273)
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N-(3-Acetamidophenyl)-4-(2-morpholinoacetamido)benzamide (274)

The reaction was carried out according to general procedure 18, with acid 125 (88 mg, 0.33 mmol) and aniline 113 (25 mg, 0.17 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 9:1) to furnish the title compound 274 (60 mg, 91%) as an off white solid (m.p. 228-229 °C from dichloromethane).

Rf (DCM/MeOH 9:1) = 0.5; HRMS Found (ESI\(^+\)): MH\(^+\) 397.1860, C\(_{22}\)H\(_{25}\)N\(_4\)O\(_4\) requires 397.1876; IR \(\nu_{\text{max}}\) (solid)/cm\(^{-1}\): 3297 (N-H), 2821 (C-H), 1695 (C=O), 1661 (C=O), 1513, 1484, 1413, 1269, 1107; \(^1\)H NMR (400 MHz, CDCl\(_3)/CD_3OD\): \(\delta\) 1.95 (3H, s, NHCOCH\(_3\)), 2.45 (4H, t, \(J = 4.4\) Hz, N(CH\(_2\)CH\(_2\))\(_2\)), 3.01 (2H, s, COCH\(_2\)N), 3.63 (4H, t, \(J = 4.4\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 7.09 (1H, t, \(J = 8.0\) Hz, Ar-H), 7.19 (1H, d, \(J = 7.2\) Hz, Ar-H), 7.29 (1H, d, \(J = 8.0\) Hz, Ar-H), 7.50 (2H, d, \(J = 8.0\) Hz, Ar-H), 7.68 (1H, s, Ar-H), 7.79 (2H, d, \(J = 8.0\) Hz, Ar-H); \(^13\)C NMR (100 MHz, CDCl\(_3)/CD_3OD\): \(\delta\) 23.4 (CH\(_3\), NHCOCH\(_3\)), 53.2 (2 \(\times\) CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)), 61.9 (CH\(_2\), COCH\(_2\)N), 66.5 (CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 112.4 (CH, Ar-C), 115.9 (CH, Ar-C), 116.4 (CH, Ar-C), 119.0 (2 \(\times\) CH, Ar-C), 128.2 (2 \(\times\) CH, Ar-C), 128.8 (CH, Ar-C), 130.0 (quat., Ar-C), 138.3 (quat., Ar-C), 138.5 (quat., Ar-C), 140.1 (quat., Ar-C), 269
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165.9, 168.7 and 169.7 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 397 (MH⁺, 100%), 360 (2%), 202 (5%).

**N-(3-Acetamidophenyl)-3-(2-morpholinoacetamido)benzamide (275)**

![Chemical Structure Image]

The reaction was carried out according to general procedure 18, with acid 126 (88 mg, 0.33 mmol) and aniline 113 (25 mg, 0.17 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 275 (51 mg, 77%) as an off white foam.

**Rf** (DCM/MeOH 9:1) = 0.51; **HRMS** Found (ESI⁺): MH⁺ 397.1858, C₂₁H₂₅N₄O₄ requires 397.1876; **IR** ν_max(solid)/cm⁻¹: 3283 (N-H), 2821 (C-H), 1663 (C=O), 1661 (C=O), 1605, 1533, 1482, 1413, 1297, 1113; **¹H NMR** (400 MHz, CDCl₃): δ 1.94 (3H, s, NHCOCH₃), 2.49 (4H, br s, N(CH₂CH₂)₂), 3.03 (2H, s, COCH₂N), 3.66 (4H, br s, O(CH₂CH₂)₂), 7.08 (1H, t, J = 8.0 Hz, Ar-H), 7.20-7.23 (2H, m, Ar-H), 7.33 (1H, d, J = 8.0 Hz, Ar-H), 7.46 (1H, d, J = 8.0 Hz, Ar-H), 7.69 (1H, d, J = 8.0 Hz, Ar-H), 7.82 (1H, s, Ar-H), 8.21 (1H, s, Ar-H), 8.62, 8.97 and 9.18 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); **¹³C NMR** (100 MHz, CDCl₃): δ 24.1 (CH₃, NHCOCH₃), 53.6 (2 × CH₂, N(CH₂CH₂)₂), 62.1 (CH₂, COCH₂N), 66.7 (2 × CH₂, O(CH₂CH₂)₂), 112.5 (CH, Ar-C), 116.2 (CH, Ar-C), 116.4 (CH, Ar-C), 118.8 (CH, Ar-C), 122.9 (CH, Ar-C), 123.0 (CH, Ar-C), 129.1 (2 × CH, Ar-C), 135.5 (quat., Ar-C), 137.5 (quat., Ar-C), 138.3 (quat., Ar-C), 138.6 (quat., Ar-C), 165.9, 168.6 and 169.2 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 397 (MH⁺, 100%), 383 (2%), 360 (5%).
N-(3-Acetamido-5-\((\text{tert-butyldimethylsilyloxy})\text{methyl}\)phenyl)-4-(2-morpholinoacetamido)benzamide (276)

The reaction was carried out according to general procedure 18, with acid 125 (117 mg, 0.44 mmol) and aniline 99 (65 mg, 0.22 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 276 (105 mg, 88%) as an off white solid (m.p. 223-224 °C from dichloromethane).

R<sub>f</sub> (DCM/MeOH 9:1) = 0.6; HRMS Found (ESI<sup>+</sup>): MH<sup>+</sup> 541.2813, C<sub>28</sub>H<sub>41</sub>N<sub>4</sub>O<sub>5</sub>Si requires 541.2846; IR <sup>ν</sup>max(solid)/cm<sup>-1</sup>: 3220 (N-H), 2930 (C-H), 1686 (C=O), 1660 (C=O), 1633, 1613, 1526, 1444, 1368, 1316, 1290, 1255, 1116; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD): δ 0.04 (6H, s, OSi(CH<sub>3</sub>)<sub>2</sub>), 0.87 (9H, s, OSiC(CH<sub>3</sub>)<sub>3</sub>), 2.04 (3H, s, NHCOCH<sub>3</sub>), 2.63 (4H, brs, N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 3.18 (2H, s, COCH<sub>2</sub>N), 3.75 (4H, br s, O(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 4.61 (2H, s, ArCH<sub>2</sub>O), 7.28 (1H, m, Ar-H), 7.36 (1H, s, Ar-H), 7.54 (2H, d, <i>J</i> = 8.0 Hz, Ar-H), 7.69 (1H, Ar-H), 7.77 (2H, d, <i>J</i> = 8.0 Hz, Ar-H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD): δ -5.5 (2 × CH<sub>3</sub>, OSi(CH<sub>3</sub>)<sub>2</sub>), 18.2 (quat., OSiC(CH<sub>3</sub>)<sub>3</sub>), 23.9 (CH<sub>3</sub>, NHCOCH<sub>3</sub>), 25.6 (3 × CH<sub>3</sub>, OSi(CH<sub>3</sub>)<sub>3</sub>), 53.4 (2 × CH<sub>2</sub>, N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 61.8 (CH<sub>2</sub>, COCH<sub>2</sub>N), 64.7 (CH<sub>2</sub>, ArCH<sub>2</sub>O), 66.6 (2 × CH<sub>2</sub>, O(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>), 110.8 (CH, Ar-C), 113.6 (CH, Ar-C), 114.1 (CH, Ar-C), 118.9 (CH, Ar-C), 119.0 (CH, Ar-C), 128.3 (2 × CH, Ar-C), 130.2 (quat., Ar-C), 138.5 (2 × quat., Ar-C), 140.3 (quat., Ar-C), 143.0 (quat., Ar-C), 165.8, 167.4 and 169.4 (C=O, NHCOCH<sub>3</sub>, NHCOAr and NHCOCH<sub>2</sub>); m/z (ESI<sup>+</sup>): 541 (MH<sup>+</sup>, 100%), 419 (30%), 397 (22%), 172 (18%).
N-(3-Acetamido-5-((\textit{tert}-butyldimethylsilyloxy)methyl)phenyl)-3-(2-morpholinoacetamido)benzamide (277)

The reaction was carried out according to general procedure 18, with acid 126 (117 mg, 0.44 mmol) and aniline 99 (65 mg, 0.22 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the \textit{title compound} 277 (95 mg, 79\%) as an off white foam.

$R_f$ (DCM/MeOH 9:1) = 0.64; \textbf{HRMS} Found (ESI$^+$): MH$^+$ 541.2821, C$_{28}$H$_{41}$N$_4$O$_5$Si requires 541.2846; \textbf{IR} $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3287 (N-H), 2930 (C-H), 1661 (C=O), 1606, 1526 br, 1452, 1371, 1273, 1152, 1113; \textbf{\textit{H NMR}} (400 MHz, CDCl$_3$): $\delta$ 0.06 (6H, s, OSi(C$_3$H$_3$)$_2$), 0.89 (9H, s, OSiC(CH$_3$)$_3$), 2.01 (3H, s, NHCOCH$_3$), 2.55 (4H, m, N(CH$_2$CH$_2$)$_2$), 3.07 (2H, s, COCH$_2$N), 3.71 (4H, m, O(CH$_2$CH$_2$)$_2$), 4.61 (2H, s, ArCH$_2$O), 7.28 (2H, t, $J = 8.0$ Hz, Ar-H), 7.40 (1H, s, Ar-H), 7.49 (1H, d, $J = 8.0$ Hz, Ar-H), 7.77 (2H, s, Ar-H), 7.90 (1H, s, Ar-H), 8.25, 8.69 and 9.18 (3H, NHCOC$_3$, NHCOC$_3$Ar and NHCOC$_2$); \textbf{\textit{C NMR}} (100 MHz, CDCl$_3$): $\delta$ -5.3 (2 $\times$ CH$_3$, OSi(CH$_3$)$_2$), 18.3 (quat., OSiC(CH$_3$)$_3$), 24.3 (CH$_3$, NHCOCH$_3$), 25.9 (3 $\times$ CH$_3$, OSiC(CH$_3$)$_3$), 53.7 (CH$_2$, N(CH$_2$CH$_2$)$_2$), 62.2 (CH$_2$, COCH$_2$N), 64.6 (CH$_2$, s, ArCH$_2$O), 66.8 (2 $\times$ CH$_2$, O(CH$_2$CH$_2$)$_2$), 110.6 (CH, Ar-C), 113.6 (CH, Ar-C), 113.7 (CH, Ar-C), 118.6 (CH, Ar-C), 122.9 (2 $\times$ CH, Ar-C), 129.2 (CH, Ar-C), 135.7 (quat., Ar-C), 137.6 (quat., Ar-C), 138.3 (quat., Ar-C), 138.6 (quat., Ar-C), 143.1 (quat., Ar-C), 165.7, 168.5 and 169.0 (C=O, NHCOCH$_3$, NHCOAr and NHCOCH$_2$); \textbf{m/z} (ESI$^+$): 541 (MH$^+$, 100\%), 478 (70\%), 360 (5\%).
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N^1-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-N^4-(3-morpholinopropyl)terephthalamide (278)

![Chemical Structure]

The reaction was carried out according to general procedure 18, with acid 127 (330 mg, 1.13 mmol) and aniline 99 (332 mg, 1.13 mmol) with catalytic amount of DMAP to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to furnish the title compound 278 (300 mg, 47%) as a foam.

R_f (MeOH/NH_3 9:1) = 0.85; HRMS Found (ESI^+): MH^+ 569.3146, C_{30}H_{45}N_4O_5Si requires 569.3159; IR v_max(solid)/cm^{-1}: 3271 (N-H), 2854 (C-H), 1675, 1647 br (C=O), 1598, 1527 br, 1455, 1372, 1253 br; ^1H NMR (400 MHz, CDCl_3): δ 0.06 (6H, s, OSi(CH_3)_2), 0.89 (9H, s, OSiC(CH_3)_3), 1.75 (2H, p, J = 7.0 Hz, CH_2CH_2CH_2), 2.04 (3H, s, NHCOCH_3), 2.35 (8H, m, CH_2N(CH_2CH_2)_2, (CH_2CH_2)_2NCH_2, COCH_2), 3.65 (4H, t, J = 4.4 Hz, O(C_6H_4CH_2)_2), 4.61 (2H, s, ArCH_2O), 7.26 (1H, s, Ar-H), 7.36 (1H, s, Ar-H), 7.48 (2H, d, J = 8.6 Hz, Ar-H), 7.66 (2H, d, J = 8.6 Hz, Ar-H), 7.77 (1H, s, Ar-H), 8.22, 8.60 and 8.95 (3H, NHCOCH_3, NHCOAr and NHCOCH_2); ^13C NMR (100 MHz, CDCl_3): δ -5.3 (2 × CH_3, OSi(CH_3)_2), 18.4 (quat., OSiC(CH_3)_3), 21.8 (CH_2, CH_2CH_2CH_2), 24.4 (CH_3, NHCOCH_3), 25.9 (3 × CH_3, OSiC(CH_3)_3), 35.4 (CH_2, COCH_2), 53.4 (2 × CH_3, N(CH_2CH_2)_2), 57.6 (CH_2, (CH_2CH_2)_2NCH_2), 64.7 (CH_2, ArCH_2O), 66.8 (2 × CH_2, O(CH_2CH_2)_2), 110.8 (CH, Ar-C), 113.6 (CH, Ar-C), 113.9 (CH, Ar-C), 119.3 (2 × CH, Ar-C), 128.2 (2 × CH, Ar-C), 129.7 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 141.4 (quat., Ar-C), 143.1 (quat., Ar-C), 165.7, 169.0 and 171.9 (C=O, NHCOCH_3, NHCOAr and NHCOCH_2); m/z (ESI^+): 569 (MH^+, 100%), 219 (48%), 175 (4%).
The reaction was carried out according to general procedure 18, with acid 128 (330 mg, 1.13 mmol) and aniline 99 (332 mg, 1.13 mmol) with catalytic amount of DMAP to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to furnish the title compound 279 (170 mg, 26%) as a white foam.

Rf (MeOH/NH3 9:1) = 0.86; HRMS Found (ESI⁺): MH⁺ 569.3149, C30H45N4O5Sirequires 569.3159; IR νmax(solid)/cm⁻¹: 3280 (N-H), 2856 (C-H), 1655 br (C=O), 1608, 1546, 1450, 1370, 1254; ¹H NMR (400 MHz, CDCl₃): δ 0.09 (6H, s, OSi(CH₃)₂), 0.92 (9H, s, OSiC(CH₃)₃), 1.84 (2H, p, J = 7.2 Hz, CH₂CH₂CH₂), 2.04 (3H, s, NHCOCH₃), 2.33-2.38 (8H, m, CH₂N(CH₂CH₂), (CH₂CH₂)₂NCH₂, COCH₂), 3.67 (2H, t, J = 4.4 Hz, O(CH₂CH₂)₂), 4.61 (2H, s, ArCH₂O), 7.27-7.32 (3H, m, Ar-H), 7.44 (1H, d, J = 7.6 Hz, Ar-H), 7.77 (2H, br s, Ar-H), 7.83 (1H, s, Ar-H), 8.29, 8.74 and 9.06 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); ¹³C NMR (100 MHz, CDCl₃): δ -5.3 (2 × CH₃, OSi(CH₃)₂), 18.4 (quat., OSiC(CH₃)₃), 21.8 (CH₂, CH₂CH₂CH₂), 24.3 (CH₃, NHCOCH₃), 26.0 (3 × CH₃, OSiC(CH₃)₃), 35.2 (CH₂, COCH₂), 53.4 (2 × CH₂, N(CH₂CH₂)₂), 57.8 (CH₂, (CH₂CH₂)₂NCH₂), 64.7 (CH₂, ArCH₂O), 66.8 (2 × CH₂, O(CH₂CH₂)₂), 111.0 (CH, Ar-C), 113.9 (CH, Ar-C), 114.0 (CH, Ar-C), 119.0 (CH, Ar-C), 122.5 (CH, Ar-C), 123.2 (CH, Ar-C), 129.2 (CH, Ar-C), 135.5 (quat., Ar-C), 138.3 (quat., Ar-C), 138.5 (quat., Ar-C), 138.6 (quat., Ar-C), 143.0 (quat., Ar-C), 166.2, 169.2 and 172.1 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 569 (MH⁺, 100%), 450 (2%), 219 (21%).
3-Azetamido-N(4-(2-(diethylamino)acetamido)phenyl)-5-(hydroxymethyl)-benzamide (282)

The reaction was carried out according to general procedure 13, with silyl ether 264 (140 mg, 0.27 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 282 (59 mg, 54%) as an off white foam.

$R_f$ (DCM/MeOH 9:1) = 0.22; HRMS Found (ESI$^+$): MH$^+$ 413.2181, C$_{22}$H$_{29}$N$_4$O$_4$ requires 413.2189; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3257 (N-H, O-H), 2965 (C-H), 1648 (C=O), 1603, 1552, 1515, 1450, 1420, 1367, 1280; $^1$H NMR (400 MHz, CDCl$_3$/CD$_3$OD): $\delta$ 1.05 (6H, t, $J = 7.2$ Hz, N(CH$_2$CH$_3$)$_2$), 2.01 (3H, s, NHCOCH$_3$), 2.60 (4H, q, $J = 7.0$ Hz, N(CH$_2$CH$_3$)$_2$), 3.10 (2H, s, COCH$_2$N), 4.46 (2H, s, ArCH$_2$O), 7.20 (1H, s, Ar-H), 7.30 (1H, s, Ar-H), 7.56 (2H, d, $J = 8.4$ Hz, Ar-H), 7.67 (1H, s, Ar-H), 7.80 (2H, d, $J = 8.4$ Hz, Ar-H); $^{13}$C NMR (100 MHz, CDCl$_3$/CD$_3$OD): $\delta$ 12.2 (2 × CH$_3$, N(CH$_2$CH$_3$)$_2$), 23.8 (CH$_3$, NHCOCH$_3$), 48.7 (2 × CH$_2$, N(CH$_2$CH$_3$)$_2$), 57.8 (CH$_2$, COCH$_2$N), 64.2 (CH$_2$, ArCH$_2$O), 111.4 (CH, Ar-C), 114.6 (CH, Ar-C), 115.0 (CH, Ar-C), 118.8 (2 × CH, Ar-C), 128.5 (2 × CH, Ar-C), 129.8 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 140.4 (quat., Ar-C), 142.5 (quat., Ar-C), 166.0, 169.8 and 170.9 (C=O, NHCOCH$_3$, NHCOAr and NHCOCH$_2$); m/z (ESI$^+$): 413 (MH$^+$, 100%), 442 (2%).

3-Azetamido-N(3-(2-(diethylamino)acetamido)phenyl)-5-(hydroxymethyl)-benzamide (283)
The reaction was carried out according to general procedure 13, with silyl ether 265 (110 mg, 0.21 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 283 (72 mg, 84%) as an off white foam.

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\text{R}_f \text{ (DCM/MeOH 9:1) = 0.30; HRMS Found (ESI\(^+\)): } \text{MH}^+ 413.2176, \text{ C}_{22}\text{H}_{29}\text{N}_4\text{O}_4 \text{ requires 413.2189; IR } \nu_{\text{max}}\text{(solid)}/\text{cm}^{-1}: 3262 \text{ (N-H, O-H), 2929 (C-H), 1653 (C=O), 1606, 1525, 1450, 1420, 1279; }^{1}\text{H NMR (400 MHz, CDCl}_3/\text{CD}_2\text{OD): } \delta 1.05 (6\text{H, t, } J = 7.2 \text{ Hz, N(CH}_2\text{CH}_3)_2), 2.01 (3\text{H, s, NCOCH}_3), 2.60 (4\text{H, q, } J = 7.0 \text{ Hz, N(CH}_2\text{CH}_3)_2), 3.09 (2\text{H, s, COCH}_2\text{N}), 4.47 (2\text{H, s, ArCCH}_2\text{O}), 7.26 \text{ (1H, br s, Ar-H), 7.28 (1H, br s, Ar-H), 7.34 (1H, t, } J = 8.8 \text{ Hz, Ar-H), 7.58 (2H, m, Ar-H), 7.64 (1H, t, } J = 1.6 \text{ Hz, Ar-H), 7.93 (1H, br s, Ar-H); }^{13}\text{C NMR (100 MHz, CDCl}_3/\text{CD}_2\text{OD): } \delta 12.0 (2 \times \text{CH}_3, \text{N(CH}_2\text{CH}_3)_2), 23.6 (\text{CH}_3, \text{NCOCH}_3), 48.8 (\text{CH}_2, \text{N(CH}_2\text{CH}_3)_2), 57.5 (\text{CH}_2\text{COCH}_2\text{N}), 64.0 (\text{CH}_2, \text{ArCH}_2\text{O}), 111.2 (\text{CH, Ar-C}), 114.6 (\text{CH, Ar-C}), 114.8 (\text{CH, Ar-C}), 118.4 (\text{CH, Ar-C}), 122.8 (\text{CH, Ar-C}), 123.5 (\text{CH, Ar-C}), 129.2 (\text{CH, Ar-C}), 135.5 (\text{quat., Ar-C}), 137.0 (\text{quat., Ar-C}), 138.3 (\text{quat., Ar-C}), 138.5 (\text{quat., Ar-C}), 142.4 (\text{quat., Ar-C}), 166.3, 169.8 and 171.0 (\text{C=O, NCOCH}_3, \text{NCOAr} \text{ and } \text{NCOCH}_2); m/z \text{ (ESI\(^+\)): } 413 (\text{MH}^+, 100\%), 360 (2\%), 198 (2\%).
\]

\[\text{N-(3-Acetamido-5-(hydroxymethyl)phenyl)-4-(2-(diisopropylamino)acetamido)-benzamide (284)}\]

The reaction was carried out according to general procedure 13, with silyl ether 268 (224 mg, 0.40 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 284 (107 mg, 60%) as an off white foam.

\[
\text{R}_f \text{ (DCM/MeOH 9:1) = 0.36; HRMS Found (ESI\(^+\)): } \text{MH}^+ 441.2519, \text{ C}_{24}\text{H}_{33}\text{N}_4\text{O}_4 \text{ requires 441.2496; IR } \nu_{\text{max}}\text{(solid)}/\text{cm}^{-1}: 3260 \text{ br (N-H, O-H), 2973 (C-H), 1651 (C=O), 1603, 1544, 1513, 1449, 1397, 1312, 1279 br; }^{1}\text{H NMR (400 MHz, CDCl}_3/\text{CD}_2\text{OD): } \delta 1.03 (12\text{H, d, } J =}
\]

276
6.4 Hz, N(CH(CH₃)₂)₂), 1.93 (3H, s, NHCOC₂H₅), 3.23 (2H, m, N(CH(CH₃)₂)₂), 3.32 (2H, s, COCH₂N), 4.39 (2H, s, ArCH₂O), 7.20 (1H, s, Ar-H), 7.31 (1H, s, Ar-H), 7.45 (2H, d, J = 5.2 Hz, Ar-H), 7.56 (1H, s, Ar-H), 7.70 (2H, d, J = 8.0 Hz, Ar-H), 9.4, 9.50 and 10.3 (3H, NHCOC₂H₅, NHCOAr and NHCOC₂H₅); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ 19.3 (4 × CH₃, N(CH(CH₃)₂)₂), 23.7 (CH₃, NHCOC₂H₅), 49.9 (CH₂, COCH₂N), 52.9 (2 × CH, N(CH(CH₃)₂)₂), 64.0 (CH₂, ArCH₂O), 110.8 (CH, Ar-C), 114.7 (CH, Ar-C), 115.2 (CH, Ar-C), 117.5 (CH, Ar-C), 118.9 (CH, Ar-C), 128.4 (2 × CH, Ar-C), 130.0 (quat., Ar-C), 138.4 (quat., Ar-C), 138.6 (quat., Ar-C), 140.2 (quat., Ar-C), 142.4 (quat., Ar-C), 166.0, 166.1 and 169.9 (C=O, NHCOC₂H₅, NHCOAr and NHCOC₂H₅); m/z (ESI⁺): 441 (MH⁺, 60%), 351 (2%), 279 (5%), 238 (4%), 212 (7%), 130 (100%).

*N-(3-Acetamido-5-(hydroxymethyl)phenyl)-3-(2-(diisopropylamino)acetamidomethyl)benzamide (285)*

![Chemical structure](image)

The reaction was carried out according to general procedure 13, with silyl ether 269 (194 mg, 0.35 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the *title compound* 285 (120 mg, 78%) as an off white foam.

Rᵣ (DCM/MeOH 9:1) = 0.40; HRMS Found (ESI⁺): MH⁺ 441.2484, C₂₄H₃₃N₄O₄ requires 441.2496; IR νₑₓₜ (solid)/cm⁻¹: 3271 (N-H, O-H), 2967 (C-H), 1653 (C=O), 1606, 1522, 1451, 1367, 1278; ¹H NMR (400 MHz, CDCl₃/CD₃OD): δ 1.0 (12H, d, J = 6.4 Hz, N(CH(CH₃)₂)₂), 1.96 (3H, s, NHCOC₂H₅), 3.03 (2H, m, N(CH(CH₃)₂)₂), 3.07 (2H, s, COCH₂N), 4.42 (2H, s, ArCH₂O), 7.2 (1H, br s, Ar-H), 7.28-7.32 (2H, br m, Ar-H), 7.54 (2H, m, Ar-H), 7.65 (1H, s, Ar-H), 7.94 (1H, s, Ar-H); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ 20.3 (4 × CH₃, N(CH(CH₃)₂)₂), 23.6 (CH₃, COCH₃), 49.8 (CH₂, COCH₂N), 50.1 (2 × CH, N(CH(CH₃)₂)₂), 64.0 (CH₂, ArCH₂O), 111.3 (CH, Ar-C), 114.6 (CH, Ar-C), 114.9 (CH, Ar-C), 118.4 (CH, Ar-C), 122.5 (CH, Ar-C), 123.4 (CH, Ar-C), 129.1 (CH, Ar-C), 135.5 (quat., Ar-C), 137.0
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(quot., Ar-C), 138.3 (quot., Ar-C), 138.5 (quot., Ar-C), 142.3 (quot., Ar-C), 166.2, 169.7 and 172.5 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 441 (MH⁺, 100%).

N-(3-Acetamido-5-(hydroxymethyl)phenyl)-4-(2-(2-piperidin-1-yl)acetamido)benzamide (286)

The reaction was carried out according to general procedure 13, with silyl ether 272 (124 mg, 0.23 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 286 (75 mg, 77%) as an off white foam.

Rf (DCM/MeOH 9:1) = 0.26; HRMS Found (ESI⁺): MH⁺ 425.2197, C₂₃H₂₉N₄O₄ requires 425.2183; IR νmax(solid)/cm⁻¹: 3269 (N-H, O-H), 2933 (C-H), 1653 (C=O), 1602, 1514, 1453, 1422, 1277; ¹H NMR (400 MHz, CDCl₃): δ 1.38 (2H, br s, CH₂(CH₂CH₂)₂N), 1.54 (4H, br s, CH₂(CH₂CH₂)₂N), 1.86 (3H, s, NHCOCH₃), 2.42 (4H, br s, CH₂(CH₂CH₂)₂N), 2.96 (2H, s, COCH₂N), 4.31 (2H, s, ArCH₂O), 7.09 (1H, br s, Ar-H), 7.26 (1H, br s, Ar-H), 7.44 (2H, d, J = 8.8 Hz, Ar-H), 7.70 (3H, m, Ar-H), 9.06, 9.24 and 9.39 (3H, NHCOCH₃, NHCOAr and NHCOCH₂); ¹³C NMR (100 MHz, CDCl₃): δ 23.4 (CH₂, CH₂(CH₂CH₂)₂N), 23.8 (CH₃, NHCOCH₃), 26.0 (2 × CH₂, CH₂(CH₂CH₂)₂N), 54.7 (2 × CH₂, CH₂(CH₂CH₂)₂N), 62.5 (CH₂, COCH₂N), 64.0 (CH₂, s, ArCH₂O), 111.9 (CH, Ar-C), 114.7 (CH, Ar-C), 115.2 (CH, Ar-C), 118.8 (2 × CH, Ar-C), 128.5 (2 ×CH, Ar-C), 129.8 (CH, Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 140.4 (quat., Ar-C), 142.3 (quat., Ar-C), 166.0, 169.6 and 169.8 (C=O, NHCOCH₃, NHCOAr and NHCOCH₂); m/z (ESI⁺): 425 (MH⁺, 100%), 360 (3%), 204 (10%).
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*N-(3-Acetamido-5-(hydroxymethyl)phenyl)-3-(2-(2-piperidin-1-yl)acetamido)benzamide (287)*

The reaction was carried out according to general procedure 13, with silyl ether 273 (124 mg, 0.23 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the *title compound* 287 (70 mg, 72%) as an off white solid (m.p. 165-166 °C from dichloromethane).

**Rf** (DCM/MeOH 9:1) = 0.28; **HRMS** Found (ESI\(^{+}\)): MH\(^{+}\) 425.2186, \(\text{C}_{23}\text{H}_{29}\text{N}_{4}\text{O}_{4}\) requires 425.2183; **IR** \(\nu_{\text{max}}\text{(solid)/cm}^{-1}: 3229 \text{(N-H, O-H)}, 2925 \text{(C-H)}, 1698 \text{(C=O)}, 1670 \text{(C=O)}, 1651 \text{(C=O)}, 1591, 1540, 1456, 1434, 1320, 1276;** ¹H **NMR** (400 MHz, CDCl₃/CD₃OD): \(\delta\) 1.40 (2H, br s, \(\text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 1.57 (4H, p, \(J = 5.2 \text{ Hz}, \text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 1.97 (3H, s, \(\text{NH}-\text{CO-CH}_3\)), 2.46 (4H, m, \(\text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 3.0 (2H, s, \(\text{CO-CH}_2\text{N}\)), 4.42 (2H, s, \(\text{Ar-CH}_2\text{O}\)), 7.20 (1H, br s, \(\text{Ar-H}\)), 7.26 (1H, br s, \(\text{Ar-H}\)), 7.30 (1H, t, \(J = 7.6 \text{ Hz, Ar-H}\)), 7.53 (1H, d, \(J = 7.6 \text{ Hz, Ar-H}\)), 7.58 (1H, d, \(J = 8.0 \text{ Hz, Ar-H}\)), 7.63 (1H, br s, \(\text{Ar-H}\)), 7.92 (1H, s, \(\text{Ar-H}\)); ¹³C **NMR** (100 MHz, CDCl₃/CD₃OD): \(\delta\) 23.4 (2 × \(\text{CH}_2\)), \(\text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 23.7 (\(\text{CH}_3\), \(\text{NHOCH}_3\)), 25.9 (2 × \(\text{CH}_2\)), \(\text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 54.7 (\(\text{CH}_2\)), \(\text{CH}_2(\text{CH}_2\text{CH}_2)_{2}\text{N}\)), 62.4 (\(\text{CH}_2\), \(\text{COCH}_2\text{N}\)), 64.0 (\(\text{CH}_2\), \(\text{Ar-CH}_2\text{O}\)), 111.4 (CH, Ar-C), 114.7 (CH, Ar-C), 115.0 (CH, Ar-C), 118.6 (CH, Ar-C), 122.9 (CH, Ar-C), 123.4 (CH, Ar-C), 129.1 (CH, Ar-C), 135.5 (quat., Ar-C), 137.2 (quat., Ar-C), 138.3 (quat., Ar-C), 138.5 (quat., Ar-C), 142.4 (quat., Ar-C), 166.2, 169.7 and 169.8 (C=O, NHOCH₃, NHOAr and NHOCH₂) \(m/z\) (ESI\(^{+}\)): 425 (MH\(^{+}\), 100%), 360 (5%).

*N-(3-Acetamido-5-(hydroxymethyl)phenyl)-4-(2-morpholinoacetamido)benzamide (288)*
The reaction was carried out according to general procedure 13, with silyl ether 276 (220 mg, 0.41 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 288 (95 mg, 55%) as an off white foam.

**Rf** (DCM/MeOH 9:1) = 0.35; **HRMS** Found (ESI\(^+\)): MH\(^+\) 427.1975, C\(_{22}H_{27}N_4O_5\) requires 427.1981; **IR** \(\nu_{\text{max}}\) (solid)/cm\(^{-1}\): 3275 (N-H, O-H), 2857 (C-H), 1653 (C=O), 1602, 1515, 1450, 1420, 1370, 1183; \(^1\)H NMR (400 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 1.96 (3H, s, NHCOCH\(_3\)), 2.54 (4H, m, N(CH\(_2\)CH\(_2\))\(_2\)), 3.08 (2H, s, COCH\(_2\)N), 3.68 (4H, m, O(CH\(_2\)CH\(_2\))\(_2\)), 4.39 (2H, s, ArCH\(_2\)O), 7.16 (1H, s, Ar-H), 7.27 (1H, t, \(J = 8.0\) Hz, Ar-H), 7.51 (2H, d, \(J = 8.4\) Hz, Ar-H), 7.66 (1H, s, Ar-H), 7.74 (2H, d, \(J = 8.4\) Hz, Ar-H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 23.7 (CH\(_3\), NHCOCH\(_3\)), 53.4 (2 \times CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)), 64.0 (CH\(_2\), ArCH\(_2\)O), 66.5 (CH\(_2\), COCH\(_2\)N), 66.6, (2 \times CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 111.6 (CH, Ar-C), 114.6 (CH, Ar-C), 115.0 (CH, Ar-C), 118.9 (CH, Ar-C), 119.0 (CH, Ar-C), 128.4 (2 \times CH, Ar-C), 129.9 (quat., Ar-C), 138.3 (quat., Ar-C), 138.5 (quat., Ar-C), 140.3 (quat., Ar-C), 142.4 (quat., Ar-C), 166.0, 168.6 and 169.9 (C=O, NHCOCH\(_3\), NHCOAr and NHCOCH\(_2\)); m/z (ESI\(^+\)): 427 (MH\(^+\), 100%), 242 (65%), 205 (3%).

\(N\)-(3-Acetamido-5-(hydroxymethyl)phenyl)-3-(2-morpholinoacetamido) benzamide (289)

![Structure of 289]

The reaction was carried out according to general procedure 13, with silyl ether 277 (110 mg, 0.20 mmol) to give the crude product, which was purified with flash chromatography (dichloromethane-methanol 19:1) to furnish the title compound 289 (63 mg, 73%) as an off white foam.

**Rf** (DCM/MeOH 9:1) = 0.36; **HRMS** Found (ESI\(^+\)): MH\(^+\) 427.1973, C\(_{22}H_{27}N_4O_5\) requires 427.1981; **IR** \(\nu_{\text{max}}\) (solid)/cm\(^{-1}\): 3267 (N-H, O-H), 2930 (C-H), 1653 (C=O), 1605, 1521, 1451,
1371, 1274; \(^1\)H NMR (400 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 2.0 (3H, s, NHCOCH\(_3\)), 2.54 (4H, br s, N(CH\(_2\)CH\(_2\))\(_2\)), 3.08 (2H, s, COCH\(_2\)N), 3.70 (4H, br s, O(CH\(_2\)CH\(_2\))\(_2\)), 4.43 (2H, s, ArCH\(_2\)O), 7.20 (1H, s, Ar-H), 7.26 (1H, br s, Ar-H), 7.31 (1H, t, \(J = 8.0\) Hz, Ar-H), 7.53 (1H, d, \(J = 6.4\) Hz Ar-H), 7.61 (1H, d, \(J = 8.4\) Hz, Ar-H), 7.65 (1H, s, Ar-H), 7.89 (1H, s, Ar-H), 9.13, 9.29 and 9.45 (3H, NHCOCH\(_3\), NHCOAr and NHCOCH\(_2\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 23.7 (CH\(_3\), NHCOCH\(_3\)), 53.5 (2 × CH\(_2\), N(CH\(_2\)CH\(_2\))\(_2\)), 62.0 (CH\(_2\), COCH\(_2\)N), 64.1 (CH\(_2\), ArCH\(_2\)O), 66.7 (2 × CH\(_2\), O(CH\(_2\)CH\(_2\))\(_2\)), 111.4 (CH, Ar-C), 114.6 (CH, Ar-C), 115.0 (CH, Ar-C), 118.9 (CH, Ar-C), 123.1 (CH, Ar-C), 123.6 (CH, Ar-C), 129.2 (CH, Ar-C), 135.4 (quat., Ar-C), 137.0 (quat., Ar-C), 138.2 (quat., Ar-C), 138.5 (quat., Ar-C), 142.4 (quat., Ar-C), 166.2, 168.8 and 169.8 (C=O, NHCOCH\(_3\), NHCOAr and NHCOCH\(_2\)); \textit{m/z} (ESI\(^+:\) 427 (MH\(^+\), 100%), 360 (5%), 233 (12%).

\(\text{N}^1\)-(3-Acetamido-5-(hydroxymethyl)phenyl)-\(\text{N}^4\)-(3-morpholinopropyl)terephthal
-amide (290)

![Chemical structure of the title compound](image)

The reaction was carried out according to general procedure 13, with silyl ether 278 (300 mg, 0.53 mmol) to give crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to furnish the \textit{title compound} 290 (223 mg, 93%) as a white foam.

**Rf** (MeOH/NH\(_3\) 9:1) = 0.79; **HRMS** Found (ESI\(^+:\)) MH\(^+\) 455.2284, C\(_{29}\)H\(_{31}\)N\(_4\)O\(_3\) requires 455.2294; **IR** \(v_{\text{max}}\) (solid)/cm\(^{-1}\): 3260 br (N-H, O-H), 2930 (C-H), 1648 (C=O), 1597, 1528, 1447, 1420, 1368, 1255; \(^1\)H NMR (400 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 1.75 (2H, p, \(J = 7.0\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)), 1.96 (3H, s, NHCOCH\(_3\)), 2.26 (2H, t, \(J = 6.0\) Hz, COCH\(_2\)O), 2.32 (2H, t, \(J = 6.0\) Hz, CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 2.40 (4H, t, \(J = 4.4\) Hz, CH\(_2\)N(CH\(_2\)CH\(_2\))\(_2\)), 3.59 (4H, t, \(J = 4.4\) Hz, O(CH\(_2\)CH\(_2\))\(_2\)), 4.39 (2H, s, ArCH\(_2\)O), 7.15 (1H, s, Ar-H), 7.23 (1H, s, Ar-H), 7.45 (2H, d, \(J = 8.0\) Hz, Ar-H), 7.63 (2H, d, \(J = 8.0\) Hz, Ar-H), 7.66 (1H, s, Ar-H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)/CD\(_3\)OD): \(\delta\) 21.3 (CH\(_2\), CH\(_2\)CH\(_2\)CH\(_2\)), 23.6 (CH\(_3\), NHCOCH\(_3\)), 34.4 (CH\(_2\), COCH\(_2\)),}
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52.9 (2 × CH$_2$, N(CH$_2$CH$_2$)$_2$), 57.6 (CH$_2$, (CH$_2$CH$_2$)$_2$NCH$_2$), 63.8 (CH$_2$, ArCH$_2$O), 66.0 (2 × CH$_2$, O(CH$_2$)$_2$CH$_2$), 111.7 (CH, Ar-C), 114.6 (CH, Ar-C), 115.1 (CH, Ar-C), 119.0 (2 × CH, Ar-C), 128.2 (2 × CH, Ar-C), 129.1 (CH, Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 141.5 (quat., Ar-C), 142.4 (quat., Ar-C), 166.4, 170.1 and 172.1 (C=O, NHOCH$_3$, NHCOAr and NHOCH$_2$); m/z (ESI$^+$): 455 (MH$^+$, 100%), 322 (5%), 219 (34%), 198 (19%).

$N^1$-(3-Acetamido-5-(hydroxymethyl)phenyl)-$N^3$-(3-morpholinopropyl)isophthal-Amide (291)

![Chemical structure](image)

The reaction was carried out according to general procedure 13, with silyl ether 279 (120 mg, 0.21 mmol) to give the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1) to furnish the *title compound* 291 (63 mg, 66%) as a white foam.

$R_f$ (MeOH/NH$_3$ 9:1) = 0.78; HRMS Found (ESI$^+$): MH$^+$ 455.2286, C$_{24}$H$_{31}$N$_4$O$_5$ requires 455.2294; IR $v_{\text{max}}$(solid)/cm$^{-1}$: 3260 (N-H, O-H), 2916 (C-H), 1652 (C=O), 1590, 1547, 1449, 1420, 1369, 1276; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.78 (2H, p, $J = 7.0$ Hz, CH$_2$CH$_2$CH$_2$), 1.91 (3H, s, NHOCH$_3$), 2.27-2.34 (4H, m, CH$_2$N(CH$_2$CH$_2$)$_2$, COCH$_2$), 2.39 (4H, t, $J = 4.4$ Hz, CH$_2$N(CH$_2$CH$_2$)$_2$), 3.62 (4H, t, $J = 4.4$ Hz, O(CH$_2$CH$_2$)$_2$), 4.40 (2H, s, ArCH$_2$O), 7.17 (1H, s, Ar-H), 7.21-7.23 (2H, d, $J = 7.2$ Hz, Ar-H), 7.43 (1H, d, $J = 8.0$ Hz, Ar-H), 7.62 (2H, d, $J = 8.0$ Hz, Ar-H), 7.76 (1H, s, Ar-H), 9.17, 9.45 and 9.47 (3H, NHOCH$_3$, NHCOAr and NHOCH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 21.7 (CH$_2$, CH$_2$CH$_2$CH$_2$), 23.7 (CH$_3$, NHOCH$_3$), 34.6 (CH$_2$, COCH$_2$), 53.2 (2 × CH$_2$, N(CH$_2$CH$_2$)$_2$), 57.8 (CH$_2$, CH$_2$N(CH$_2$CH$_2$)$_2$), 64.1 (CH$_2$, ArCH$_2$O), 66.4 (CH$_2$, O(CH$_2$CH$_2$)$_2$), 111.3 (CH, Ar-C), 114.7 (CH, Ar-C), 114.9 (CH, Ar-C), 118.7 (CH, Ar-C), 123.1 (CH, Ar-C), 123.3 (CH, Ar-C), 129.1 (CH, Ar-C), 135.2 (quat., Ar-C), 138.2 (quat., Ar-C), 138.4 (quat., Ar-C), 138.5 (quat., Ar-C), 142.5 (quat., Ar-C), 166.7, 169.9 and 172.2 (C=O, NHOCH$_3$, NHCOAr and NHOCH$_2$); m/z (ESI$^+$): 455 (MH$^+$, 100%), 219 (11%).
6.7 Synthesis of symmetrical triaryl amides

General procedure 19: Synthesis of symmetrical benzamides
Adopting the method of Cotton, a solution of terephthaloyl chloride 158 (1 eq.) in THF (4 mL/mmol) was added to a solution of aniline 99 or 113 (2.5 eq.), and potassium carbonate (5.4 eq.) in THF (5.4 mL/mmol of aniline). A white precipitate was formed and the mixture was stirred for 30 minutes. The solvent was then removed under reduced pressure. To the remaining solid residue, water (5 mL) was added with vigorous stirring and the solid was separated by filtration. The solid was washed with water until the filtrate had pH 7.0 and it was then dried under reduced pressure as pure white solids.

\[ N^1, N^4\text{-bis(3-Acetamidophenyl)terephthalamide (292)} \]

The reaction was carried out according to general procedure 19, with acid chloride 158 (150 mg, 0.74 mmol) and aniline 113 (277 mg, 1.85 mmol) to give the title compound 292 (310 mg, 98%), as a white solid (m.p. above 350 °C from THF).

**Rf** (DCM/MeOH 4:1) = 0.81; **HRMS** Found (ESI⁺): MH⁺ 431.1717, C₂₄H₂₃N₄O₄ requires 431.1719; **IR** ν_max(solid)/cm⁻¹: 3318 (N-H), 1661 (C=O), 1642 (C=O), 1605, 1535, 1484, 1421, 1330, 1283; **¹H NMR** (400 MHz, (CD₃)₂SO): δ 2.05 (6H, s, NHCOCH₃), 7.26 (2H, t, J = 8.0 Hz, Ar-H), 7.34 (2H, d, J = 8.0 Hz, Ar-H), 7.44 (2H, d, J = 8.0 Hz, Ar-H), 8.08 (4H, br s, Ar-H), 8.12 (2H, br s, Ar-H), 9.98 and 10.40 (4H, 2 × NHCOAr and 2 × NHCOCH₃); **¹³C NMR** (100 MHz, (CD₃)₂SO): δ 23.9 (2 × CH₃, NHCOCH₃), 111.3 (2 × CH, Ar-C), 114.6 (CH, Ar-C), 115.3 (2 × CH, Ar-C), 127.6 (5 × CH, Ar-C), 128.6 (2 × CH, Ar-C), 137.3 (2 × quat., Ar-C), 139.1 (2 × quat., Ar-C), 139.5 (2 × quat., Ar-C), 164.7 and 168.2 (C=O, 2 × NHCOAr and 2 × NHCOCH₃); **m/z** (ESI⁺): 431 (MH⁺,100%).
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$N^1, N^4$-bis(3-acetamido-5-((tert-butyldimethylsiloxy)methyl)phenyl)terephthalamide (293)

The reaction was carried out according to general procedure 19, with acid chloride 158 (150 mg, 0.74 mmol) and aniline 99 (543 mg, 1.84 mmol) to give the title compound 293 (510 mg, 96%), as a white solid (m.p. 159-160 °C from THF).

$R_f$ (DCM/MeOH 4:1) = 0.88; HRMS Found (ESI$^+$): MH$^+$ 719.3637, C$_{38}$H$_{53}$N$_4$O$_6$Si$_2$ requires 719.3660; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3284 (N-H), 2928 (C-H), 1654 (C=O), 1606, 1545, 1452, 1424, 1370, 1254; $^1$H NMR (400 MHz, (CD$_3$)$_2$SO): $\delta$ 0.09 (12H, s, OSi(CH$_3$)$_2$), 0.91 (18H, s, OSiC(CH$_3$)$_3$), 2.04 (6H, s, NHCOC$_3$H$_7$), 4.65 (4H, s, ArCH$_2$O), 7.34 (2H, br s, Ar-H), 7.44 (2H, br s, Ar-H), 8.0 (2H, br s, Ar-H), 8.07 (4H, br s, Ar-H), 9.96 and 10.39 (4H, 2 × NHCOAr and 2 × NHCOCH$_3$); $^{13}$C NMR (100 MHz, (CD$_3$)$_2$SO): $\delta$ 5.4 (4 × CH$_3$, OSi(CH$_3$)$_2$), 17.9 (2 × quat., OSiC(CH$_3$)$_3$), 23.9 (2 × CH$_3$, NHCOCH$_3$), 25.7 (6 × CH$_3$, OSiC(CH$_3$)$_3$), 64.4 (2 × CH$_2$, ArCH$_2$O), 109.9 (2 × CH, Ar-C), 112.0 (CH, Ar-C), 113.0 (2 × CH, Ar-C), 127.5 (5 × CH, Ar-C), 137.3 (2 × quat., Ar-C), 138.9 (2 × quat., Ar-C), 139.3 (2 × quat., Ar-C), 141.7 (2 × quat., Ar-C), 164.7 and 168.1 (4 × C=O, 2 × NHCOAr and 2 × NHCOCH$_3$); m/z (ESI$^+$): 719 (MH$^+$, 60%), 627 (1%), 256 (3%), 107 (100%).

$N^1, N^4$-Bis(3-acetamido-5-(hydroxymethyl)phenyl)terephthalamide (294)
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The reaction was carried out according to general procedure 13, with silyl ether 293 (133 mg, 0.18 mmol) to give the title compound 294 (88 mg, 97%), as a white solid (m.p. 321-322 °C from tetrahydrofuran).

\[ \text{Rf (DCM/MeOH 4:1) = 0.65;} \quad \text{HRMS} \text{ Found (ESI\(^+\)): MH}^+ \text{ 491.1921, C}_{26}\text{H}_{27}\text{N}_4\text{O}_6 \text{ requires 491.1931;} \quad \text{IR } \nu_{\text{max}} \text{ (solid)/cm}^{-1}: 3310 \text{ (N-H, O-H), 1693 \text{ (C=O), 1655 \text{ (C=O), 1612, 1551, 1450, 1372, 1351, 1283, 1262; } ^1\text{H NMR} \text{ (400 MHz, (CD}_3\text{)}_2\text{SO): } \delta 2.04 \text{ (6H, s, NHCOC}_3\text{H}_3\text{), 4.46 \text{ (4H, s, ArCH}_2\text{O), 5.23 \text{ (2H, s, CH}_2\text{OH), 7.33 \text{ (2H, br s, Ar-H), 7.44 \text{ (2H, br s, Ar-H), 8.0 \text{ (2H, br s, Ar-H), 8.08 \text{ (4H, br s, Ar-H), 9.95 and 10.37 \text{ (4H, 2 \times NHCOAr and 2 \times NHCOCH}_3\text{); } ^{13}\text{C NMR} \text{ (100 MHz, (CD}_3\text{)}_2\text{SO): } \delta 24.0 \text{ (2 \times CH}_3\text{, NHCOC}_3\text{H}_3\text{), 62.9 \text{ (2 \times CH}_2\text{, ArCH}_2\text{O), 109.8 \text{ (2 \times CH, Ar-C), 112.0 \text{ (CH, Ar-C), 113.4 \text{ (2 \times CH, Ar-C), 127.6 \text{ (5 \times CH, Ar-C), 137.3 \text{ (2 \times quat., Ar-C), 138.9 \text{ (2 \times quat., Ar-C), 139.2 \text{ (2 \times quat., Ar-C), 143.3 \text{ (2 \times quat., Ar-C), 164.7 and 168.0 \text{ (4 \times C=O, 2 \times NHCOAr and 2 \times NHCOCH}_3\text{); m/z (ESI\(^+\)): 491 (MH}^+, 42\%), 242 (50\%), 107 (100%).} \]

6.8 Synthesis of unsymmetrical triaryl amides

\( N^1\text{-}(3\text{-Acetamido-5-((tert-butyldimethylsiloxy)methyl)phenyl})-N^4\text{-}(3\text{-acetamidophenyl})\text{terephthalamide (295)} \)

\[
\begin{align*}
\text{O-} & \quad \text{O} \\
\text{NH} & \quad \text{NH} \\
\text{O} & \quad \text{O} \\
\text{Si} & \quad \text{Si} \\
\end{align*}
\]

The reaction was carried out according to general procedure 18, with acid 166 (253 mg, 0.85 mmol) and aniline 99 (250 mg, 0.85 mmol) with catalytic amount of DMAP to give the title compound 295 (463 mg, 95%), as a white solid (m.p. 125-126 °C from tetrahydrofuran).

\[ \text{Rf (DCM/MeOH 4:1) = 0.84;} \quad \text{HRMS} \text{ Found (ESI\(^+\)): MH}^+ \text{ 575.2674, C}_{31}\text{H}_{39}\text{N}_4\text{O}_5\text{Si requires 575.2690;} \quad \text{IR } \nu_{\text{max}} \text{ (solid)/cm}^{-1}: 3284 \text{ (N-H), 2929 \text{ (C-H), 1657 \text{ (C=O), 1606, 1543, 1485, 1418, 1371, 1280, 1253; } ^1\text{H NMR} \text{ (400 MHz, (CD}_3\text{)}_2\text{SO): } \delta 0.11 \text{ (6H, s, OSi(CH}_3\text{)}_2\text{), 0.92} \]
(9H, s, OSi(CH₃)₃), 2.06 (6H, s, NHCOCH₃), 4.68 (2H, s, ArCH₂O), 7.27 (1H, t, J = 8.0 Hz, Ar-H), 7.34 (2H, br s, Ar-H), 7.46 (2H, br s, Ar-H), 8.01 (1H, br s, Ar-H), 8.08 (4H, br s, Ar-H), 8.13 (1H, br s, Ar-H), 9.97 and 10.40 (4H, 2 × NHCOAr and 2 × NHCOCH₃); \(^{13}\)C NMR (100 MHz, (CD₃)₂SO): \(\delta\) -5.3 (2 × CH₃, OSi(CH₃)₂), 18.0 (quat., OSi(CH₃)₃), 24.0 (2 × CH₃, NHCOCH₃), 25.8 (3 × CH₃, OSi(CH₃)₃), 64.4 (CH₂, ArCH₂O), 110.0 (CH, Ar-C), 111.3 (CH, Ar-C), 112.4 (CH, Ar-C), 113.1 (CH, Ar-C), 114.7 (CH, Ar-C), 115.3 (CH, Ar-C), 127.6 (4 × CH, Ar-C), 128.6 (CH, Ar-C), 130.7 (quat., Ar-C), 134.3 (quat., Ar-C), 138.9 (quat., Ar-C), 139.4 (quat., Ar-C), 140.3 (quat., Ar-C), 141.8 (quat., Ar-C), 164.8 and 168.2 (4 × C=O, 2 × NHCOAr and 2 × NHCOCH₃); \(m/z\) (ESI\(^{+}\)): 575 (MH\(^{+}\), 3%), 483 (1%), 421 (100%).

\(N^1\)-(3-Acetamido-5-(hydroxymethyl)phenyl)-\(N^4\)-(3-acetamidophenyl) terephthalamide (296)

The reaction was carried out according to general procedure 13, with silyl ether 295 (354 mg, 0.62 mmol) to give the title compound 296 (258 mg, 91%), as a white solid (m.p. 273-274 °C from tetrahydrofuran).

\(R_f\) (DCM/MeOH 19:1) = 0.61; \(HRMS\) Found (ESI\(^{+}\)): MH\(^{+}\) 461.1821, C₂₅H₂₅N₄O₅ requires 461.1825; \(IR\) \(v_{max}(solid)/\text{cm}^{-1}\): 3301 (N-H, O-H), 1654 (C=O), 1642 (C=O), 1605, 1565, 1532, 1492, 1466, 1366, 1314, 1293, 1253; \(^{1}\)H NMR (400 MHz, (CD₃)₂SO): \(\delta\) 2.05 (6H, s, NHCOCH₃), 4.48 (2H, s, ArCH₂O), 5.23 (1H, s, CH₂OH), 7.26 (1H, t, \(J = 8.0\) Hz, Ar-H), 7.34 (2H, br s, Ar-H), 7.45 (2H, br s, Ar-H), 8.01 (1H, s, Ar-H), 8.08 (4H, br s, Ar-H), 8.12 (1H, br s, Ar-H), 9.96, 9.98, 10.38 and 10.4 (4H, 2 × NHCOAr and 2 × NHCOCH₃); \(^{13}\)C NMR (100 MHz, (CD₃)₂SO): \(\delta\) 23.9 (2 × CH₃, NHCOCH₃), 62.8 (CH₂, ArCH₂O), 109.8 (CH, Ar-C), 111.3 (CH, Ar-C), 112.6 (CH, Ar-C), 113.3 (CH, Ar-C), 114.6 (CH, Ar-C), 115.2 (CH, Ar-C), 127.6 (4 × CH, Ar-C), 128.6 (CH, Ar-C), 137.3 (quat., Ar-C), 138.8 (quat., Ar-C), 139.1 (quat., Ar-C), 139.2 (quat., Ar-C), 139.4 (quat., Ar-C), 139.6 (quat., Ar-C), 143.2 (quat.,
Ar-C), 164.6, 164.7, 168.1 and 168.2(4 × C=O, 2 × NHCOAr and 2 × NHCOCH₃); m/z (ESI⁺): 461 (MH⁺, 100%).

\(N^1\)-\((3\text{-Acetamido}-5\text{-(chloromethyl)phenyl})\text{-}N^4\text{-(3-acetamidophenyl) terephthalamide (298)}\)

\[
\text{Cl} \quad \text{O} \quad \text{NH} \quad \text{NH} \quad \text{OH} \quad \text{NH} \quad \text{NH} \\
\text{O} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH} \\
\text{O} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH}
\]

The reaction was carried out according to general procedure 15, with alcohol 296 (60 mg, 0.13 mmol) to give the title compound 298 (57 mg, 91%) as a white solid (m.p. 238-239 °C from THF).

\(R_f\) (DCM/MeOH 4:1) = 0.81; HRMS Found (ESI⁺): (M + Na)⁺, 501.1306, C₂₅H₂₃ClN₄NaO₄ requires 501.1300; IR \(\nu_{\max}\) (solid)/cm⁻¹: 3267 (N-H), 1668 (C=O), 1643 (C=O), 1607, 1538, 1423, 1285; \(^1\)H NMR (400 MHz, (CD₃)₂SO): δ 2.06 (6H, s, NHCO\text{CH}_₃), 4.74 (2H, s, ArCH₂Cl), 7.26 (1H, t, \(J = 8.0\) Hz, Ar-H), 7.35 (1H, d, \(J = 8.0\) Hz, Ar-H), 7.45 (2H, d, \(J = 8.8\) Hz, Ar-H), 7.58 (1H, s, Ar-H), 8.09 (5H, m, Ar-H), 8.12 (1H, s, Ar-H), 9.98, 10.07, 10.41 and 10.49 (4H, 2 × NHCOAr, and 2 × NHCOCH₃); \(^{13}\)C NMR (100 MHz, (CD₃)₂SO): δ 23.9 (2 × CH₃, NHCOCH₃), 46.4 (CH₂, ArCH₂Cl), 111.0 (CH, Ar-C), 111.3 (CH, Ar-C), 114.7 (CH, Ar-C), 114.8 (CH, Ar-C), 115.3 (CH, Ar-C), 115.5 (CH, Ar-C), 127.6 (4 × CH, Ar-C), 128.6 (CH, Ar-C), 137.1 (quat., Ar-C), 137.4 (quat., Ar-C), 138.1 (quat., Ar-C), 139.1 (quat., Ar-C), 139.3 (quat., Ar-C), 139.5 (quat., Ar-C), 139.6 (quat., Ar-C), 164.7, 164.8, 168.2 and 168.4 (4 × C=O, 2 × NHCOAr, and 2 × NHCOCH₃); m/z (ESI⁺): 501 ((M + Na)⁺, 15%), 461(23%), 256 (2%), 191 (3%), 107 (100%).
Chapter 6: Experimental

$N^1$-(3-Acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)$-N^4$-(3-(4-(dimethylamino)butanamido)phenyl)terephthalamide (299)

The reaction was carried out according to general procedure 18, with acid 167 (314 mg, 0.85 mmol) and aniline 99 (250 mg, 0.85 mmol) with catalytic amount of DMAP to give the crude product, which was purified using flash chromatography using solvent systems (dichloromethane–methanol 9:1 and then methanol-ammonia 9:1) to give the title compound 299 (383 mg, 70%) as an off white foam.

$R_f$ (MeOH/NH$_3$ 9:1) = 0.81; HRMS Found (ESI$^+$): MH$^+$ 646.3428, C$_{35}$H$_{48}$N$_5$O$_5$Si requires 646.3425; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3287 (N-H), 2929 (C-H), 1652 (C=O), 1606, 1540, 1417 br, 1254; $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 0.09 (6H, s, OSi(C(CH$_3$)$_3$)$_2$), 0.92 (9H, s, OSiC(CH$_3$)$_3$), 1.83 (2H, p, $J$ = 7.6 Hz, CH$_2$C$_2$H$_2$CH$_2$), 2.09 (3H, s, NHCOCH$_3$), 2.24 (6H, s, N(C(H$_3$)$_2$)), 2.34-2.39 (4H, m, NCH$_2$, NHCOCH$_2$), 4.66 (2H, s, ArCH$_2$O), 7.24 (1H, t, $J$ = 8.2 Hz, Ar-H), 7.32 (1H, br s, Ar-H), 7.34 (1H, br s, Ar-H), 7.39 (1H, d, $J$ = 8.0 Hz, Ar-H), 7.42 (1H, s, Ar-H). 7.90 (1H, br s, Ar-H), 7.91 (1H, t, $J$ = 1.8 Hz, Ar-H), 7.95 (4H, m, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ -5.0 (2 × CH$_3$, OSi(CH$_3$)$_3$), 19.3 (quat., OSiC(CH$_3$)$_3$), 24.0 (CH$_2$, CH$_2$CH$_2$CH$_2$), 24.1 (CH$_3$, NHCOCH$_3$), 26.5 (3 × CH$_3$, OSiC(CH$_3$)$_3$), 35.7 (CH$_2$, NHCOCH$_2$), 45.3 (2 × CH$_3$, N(CH$_3$)$_2$), 59.9 (CH$_2$, NCH$_2$), 66.0 (CH$_2$, ArCH$_2$O), 112.8 (CH, Ar-C), 114.2 (CH, Ar-C), 115.4 (CH, Ar-C), 115.8 (CH, Ar-C), 117.6 (CH, Ar-C), 118.0 (CH, Ar-C), 128.9 (2 × CH, Ar-C), 129.0 (2 × CH, Ar-C), 130.1 (CH, Ar-C), 139.0 (quat., Ar-C), 139.1 (quat., Ar-C), 140.0 (quat., Ar-C), 140.2 (quat., Ar-C), 140.3 (quat., Ar-C), 140.4 (quat., Ar-C), 144.1 (quat., Ar-C), 167.8, 167.9, 172.7 and 173.9 (4 × C=O, NHCOAr, NHCO, CONH and NHCOCH$_2$); m/z (ESI$^+$): 646 (MH$^+$, 100%), 532 (6%), 384 (22%).
Chapter 6: Experimental

$N^1$-(3-Acetamido-5-(hydroxymethyl)phenyl)$-N^4$-(3-(4-(dimethylamino)butanamido)phenyl)terephthalamide (300)

The reaction was carried out according to general procedure 13, with silyl ether 299 (80 mg, 0.12 mmol) to give the crude product, which was purified by flash chromatography (methanol-dichloromethane 1:9 and then methanol-ammonia 1:9) to afford the title compound 300 (40 mg, 61%) as an off white foam.

$R_f$ (MeOH/NH$_3$ 9:1) = 0.69; HRMS Found (ESI$^+$): MH$^+$ 532.2542. C$_{29}$H$_{34}$N$_5$O$_5$ requires 532.2560; IR $\nu_{\text{max}}$(solid)/cm$^{-1}$: 3270 (N-H, O-H), 2930 (C-H), 1650 br (C=O), 1606, 1537 br, 1419, 1278 br; $^1$H NMR (400 MHz, CDCl$_3$/CD$_3$OD): $\delta$ 1.94 (2H, $p$, $J = 7.8$ Hz, CH$_2$CH$_2$CH$_2$), 2.12 (3H, s, NHCOC$_3$H$_3$), 2.41 (6H, s, N(C$_3$H$_3$)$_2$), 2.58 (2H, $m$, $J = 7.2$ Hz, NHCOCH$_2$), 2.46 (2H, $t$, $J = 7.8$ Hz, NCH$_2$), 4.59 (2H, s, ArCH$_2$O), 7.27 (1H, $t$, $J = 8.0$ Hz, Ar-H), 7.37-7.39 (3H, br s, Ar-H), 7.41 (1H, br s, Ar-H), 7.45 (1H, br s, Ar-H), 7.94 (1H, $t$, $J = 1.4$ Hz, Ar-H), 8.01 (4H, br s, Ar-H), 8.04, 8.10, 8.11 and 8.12 (4H, NHCOAr, NHCO, CONH and NHCOC$_2$) (OH not visible); $^{13}$C NMR (100 MHz, CDCl$_3$/CD$_3$OD): $\delta$ 21.5 (CH$_2$, CH$_2$CH$_2$CH$_2$), 24.0 (CH$_3$, NHCOCH$_3$), 34.3 (CH$_2$, NHCOCH$_2$), 43.8 (2 $\times$ CH$_3$, N(CH$_3$)$_2$), 58.6 (CH$_2$, NCH$_2$), 65.1 (CH$_2$, ArCH$_2$O), 113.1 (CH, Ar-C), 114.3 (CH, Ar-C), 116.0 (CH, Ar-C), 116.5 (CH, Ar-C), 117.6 (CH, Ar-C), 118.1 (CH, Ar-C), 128.9 (2 $\times$ CH, Ar-C), 129.0 (2 $\times$ CH, Ar-C), 130.1 (CH, Ar-C), 130.7 (quat., Ar-C), 139.1 (quat., Ar-C), 140.2 (quat., Ar-C), 140.3 (2 $\times$ quat., Ar-C), 140.4 (quat., Ar-C), 144.3 (quat., Ar-C), 167.8, 167.9, 171.8 and 172.8 (4 $\times$ C=O, NHCOAr, NHCO, CONH and NHCOCH$_2$); m/z (ESI$^+$): 532 (MH$^+$, 46%), 496 (6%), 406 (38%), 384 (100%), 257 (42%).
Chapter 6: Experimental

$N^1$-(3-Acetamido-5-(chloromethyl)phenyl)-$N^4$-(3-(4-(dimethylamino)butan-amido)phenyl)terephthalamide (302)

The reaction was carried out according to general procedure 15, with alcohol 300 (150 mg, 0.28 mmol) to furnish the crude product, which was purified by flash chromatography (dichloromethane-methanol 9:1 and then methanol-ammonia 19:1) to afford the title compound 302 (150 mg, 97%) as an off white foam.

$R_f$ (MeOH/NH$_3$ 9:1) = 0.74; HRMS Found (ESI$^+$): MH$^+$ 550.2213, 552.2199, C$_{29}$H$_{33}$ClN$_5$O$_4$ and C$_{29}$H$_{33}$ClN$_5$O$_4$ requires 550.2221, 552.2192; IR$_{\text{max}}$(solid)/cm$^{-1}$: 3260 (N-H), 2930 (C-H), 1649 br (C=O), 1606, 1544, 1450, 1416, 1287; $^1$H NMR (400 MHz, CD$_3$OD): δ 2.10 (3H, s, NHCOC$_3$H$_3$), 2.10 (2H, p, $J = 8.0$ Hz, CH$_2$CH$_2$CH$_2$), 2.59 (2H, t, $J = 8.0$ Hz, NHCOC$_3$H$_3$), 2.74 (6H, s, N(CH$_3$)$_2$), 3.24 (2H, t, $J = 8.0$ Hz, NHCOC$_3$H$_3$), 4.61 (2H, s, ArCH$_2$Cl), 7.30 (1H, t, $J = 8.0$ Hz, Ar-H), 7.39 (1H, d, $J = 8.0$ Hz, Ar-H), 7.44 (1H, d, $J = 8.0$ Hz, Ar-H), 7.47 (1H, br s, Ar-H), 7.55 (1H, br s, Ar-H), 8.03-8.04 (5H, m, Ar-H), 8.13 (1H, t, $J = 4.0$ Hz, Ar-H); $^{13}$C NMR (100 MHz, CD$_3$OD): δ 21.4 (CH$_2$, CH$_2$CH$_2$CH$_2$), 24.1 (CH$_3$, NHCOCH$_3$), 35.4 (CH$_2$, NHCOCH$_2$), 43.6 (2 × CH$_3$, N(CH$_3$)$_2$), 46.9 (CH$_2$, ArCH$_2$Cl), 58.6 (CH$_2$, NCH$_2$), 113.8 (CH, Ar-C), 114.4 (CH, Ar-C), 117.5 (CH, Ar-C), 117.6 (CH, Ar-C), 118.0 (CH, Ar-C), 118.2 (CH, Ar-C), 128.9 (2 × CH, Ar-C), 129.0 (2 × CH, Ar-C), 130.1 (CH, Ar-C), 138.9 (quat., Ar-C), 139.0 (quat., Ar-C), 140.1 (quat., Ar-C), 140.2 (quat., Ar-C), 140.4 (2 × quat., Ar-C), 140.6 (quat., Ar-C), 167.8, 167.8, 171.8 and 172.7 (4 × C=O, NHCOAr, NHCO, CONH and NHCOCH$_2$); m/z (ESI$^+$): 550 (MH$^+$, 100%), 546 (4%).
6.9 Experimental for the DNA binding studies

6.9.1 UV melting point analysis

All UV melting point analyses were performed in 1 mM Na₂EDTA, 6 mM Na₂HPO₄, 2 mM NaH₂PO₄ (pH 7.0) buffers. Stock solutions of ligand and DNA were prepared in the same buffer of deionised water. In the case of ligands that were insoluble in the given buffers, a few drops of DMSO were added to dissolve the ligand first and then diluted to the required volume so that the amount of DMSO was no more than 1-2% of the overall volume of each stock solution. All the stock solutions were stored frozen at -20 °C in plastic containers.

All measurements were carried out in a 1 cm path length 3-Q-10 mm rectangular quartz cell, using a Shimadzu UV-1700 Pharmaspec spectrometer combined with a Peltier thermoelectric temperature controller to investigate the drug-DNA melting points. The instrument was first calibrated for melting analysis by comparing the melting point of pure DNA with that of a known drug using an absorption versus temperature profile at 260 or 270 nm. The final absorbance was less than one in all the DNA solutions and experiments were scanned from 20 °C to 95 °C at a rate of 2 °C/min.

Due to its low price and availability, CT DNA was used in the first few experiments to find out the desired setup while poly AT DNA was used in the final experiments. The poly(deoxyadenylic thymidylic)acid sodium salt DNA (poly(dA.dT).poly(dA.dT)) was obtained from Sigma-Aldrich and its stock solution was used in the same buffer without further purification. The absorption spectra of DNA solution were checked every time, at 260 to 280 nm, before use to make sure that the concentration remained the same.

Each ligand was used in the ratios of 0.5, 1.0 and 1.5 equivalents in nucleotides (molar extinction coefficient 6600 in nucleotides and 13200 in base pairs).
6.9.2 Ethidium displacement assays

Materials and solutions

A stock solution of 9.4 mM sodium chloride, 20 μM EDTA, and 2 mM HEPES (SHE buffer) was prepared and the pH was adjusted to 7.0 with NaOH solution; this was used for the open amino-alkyl chained benzamides. The second buffer solution was an acetate buffer of 12 mM NaOAc, 9.3 mM NaCl and 0.1 mM Na₂EDTA (pH 5.0). The acetate buffer was used for the benzamides which have cyclic amino-alkyl chains due to their lower pKa values. Ethidium bromide solution was needed in small amounts, so a stock solution of 1.26 mM ethidium bromide solution was prepared by dissolving 0.99 mg ethidium bromide in 2 mL of the buffer.

The stock solutions of calf thymus DNA and poly AT DNA were prepared in buffers and then diluted. The desired concentration of each DNA solution was calculated in base pairs, (coefficient 6600 in nucleotides, 13200 in base pairs for calf thymus DNA and AT DNA).

Our next step was the preparation of 1 mM stock solutions of the ligands. All the stock solutions of open amino-alkyl chain benzamides were prepared in the SHE buffer while the cyclic amino-alkyl chain benzamides were prepared in the acetate buffer. All the measurements were performed in a 3 mL cuvettes. Before using each DNA sample, the absorbance of the DNA solution was measured using a Varian Cary 1E UV-Vis, or a Cary 5E UV-Vis-NIR UV spectrometer. The ethidium displacement assays were carried out using a Varian Cary Eclipse fluorescence spectrophotometer. The absorbance of the DNA was measured each time to make sure that the concentration remained the same. All the stock solutions were stored in plastic containers. When not in use they were stored at -20 °C. All solutions were used when at room temperature as it was found that the absorption differed at lower temperatures.

Methods

During the ethidium displacement assays, calf thymus DNA was also used during our initial test experiments due to its comparatively cheap price and availability in the laboratory. The concentration of the stock solution of CT DNA used was 1 μM in base pairs. Once the experimental conditions were determined, poly AT DNA was used for ligand measurements.
The fluorimeter’s emission was fixed at 590 nm and the excitation was scanned from 400 to 550 nm, with excitation and emission slits set to 10. The total volume of the cuvette was 3 mL (3 µL of ethidium, 48.6 µL of DNA, 2948.4 µL of buffer), with one unit of DNA having an absorbance of 1.0 at 260 nm and equalling 76. The absorbance of the calf thymus DNA was 0.812, which is equal to 61.712 µM (volume of DNA was calculated by dividing 3 by 61.712, which is equal to 48.6 µL).

Before starting each experiment, the cuvette was washed with distilled water and then with buffer before being dried each time under nitrogen. First, the buffer solution of 2948.4 µL was transferred to the 3 mL cuvette followed by the 3 µL ethidium; it was then mixed well and the fluorescence was measured. This process was repeated three times to reduce pipetting and mixing error and gave a constant, smooth graph. Each time, the buffer was transferred into the cuvette first followed by the DNA and mixed well prior to the addition of ethidium. The reason for adding the ethidium at the end was to avoid it adhering to the glass surfaces in the absence of DNA. Our results showed that there was an approximately 10-fold increase in the fluorescence intensity of the bound ethidium to the DNA by intercalation, not 50-fold as mentioned in some literature.²⁰⁰

Mostly, the compounds with a hydroxyl, chloride or methyl group at benzylic position were soluble but some of the diaryl derivatives with bulky O-TBDMS were not fully soluble in the acetate buffer. A solution of 1 mM concentration of each ligand was prepared by dissolving in a minimum amount of DMSO and then diluted according to the literature. The final amount of DMSO was then less than 1-2%, as required.²⁰¹

Aliquots of the ligand were added until the fluorescence dropped by >50% of the increase seen upon addition of ethidium bromide to DNA alone. C₅₀ values were determined, where possible, by graphing fluorescence versus ligand concentration and calculating the point at which fluorescence dropped by 50%.
6.9.3 MALDI experiments

Solutions of ligands were prepared in methanol with a concentration of 2.0 mM. The DNA solution of 100 μM in 20 mM ammonium acetate buffer was adjusted to pH 7.0 with glacial acetic acid. Both the ligand and DNA solutions were stored in plastic containers at -20 °C. The use of glass containers was avoided to minimise salt contaminations.

DNA-ligand complexes were prepared in 20 mM ammonium acetate buffers at a stoichiometry of X number of ligand molecules per Y number of base pair of DNA duplex, where X ranges from 2 to 200 depending on the ligand and the type of DNA. For example, a DNA-ligand complex of X:Y, where X (number of ligand molecules) = 50 and Y (number of base pairs of DNA duplex) = 1 was prepared by dissolving 25 μL of 2 mM in 10 μL with 100 μM A2T2 DNA. All the complexes were prepared in plastic Eppendorf tubes; each tube containing the DNA-ligand complex was wrapped with aluminium foil to ensure even heating during incubation. The samples were then placed in an inverted position in order to minimise evaporation and the samples were incubated in a heating block at 37 °C for 24 hours. Control DNA samples of DNA without ligand were also subjected to the same procedure and gave molecular ion peak for DNA alone.\(^{156}\)

After the successful preparation of DNA-ligand complexes, our next step was the spotting of different complexes for MALDI-TOF. Mass spectra were recorded on either a Micromass Tof Spec 2E or an Applied Biosystems Voyager DE STR MALDI reflectron TOFMS using two matrices, namely 3-hydroxypicolinic acid and ammonium citrate. The 3-hydroxypicolinic acid (50 mg) was dissolved in 1 mL of 50% aqueous acetonitrile and ammonium citrate (50 mg) in 1 mL water. Both matrices were mixed and 8 μL of the matrix solution was then carefully mixed with 2 μL of DNA-ligand complexes. The resultant mixture (2 mL) was then spotted on stainless steel sample plate and air-dried. MALDI analysis was carried out immediately in positive ion mode.\(^{156}\)
Crystallography DATA

3-Acetamido-5-nitrobenzyl alcohol 95

\[
\text{OH}
\]
\[
\text{NO}_2
\]

Crystal data is available online


3-Acetamido-5-nitrobenzylacetate 96

\[
\text{O}
\]
\[
\text{O}
\]
\[
\text{NO}_2
\]

Crystal data is available online


\(N\)-(3-((\text{tert-Butyldimethylsilyloxy})methyl)-5-nitrophenyl)acetamide 97

\[
\text{O}
\]
\[
\text{Si}
\]
\[
\text{NO}_2
\]

Crystal data is available online at

3,5-Dinitrobenzyl methanesulfonate 178

\[
\begin{align*}
\text{OSO}_2\text{Me} \\
\text{O}_2\text{N} \\
\text{NO}_2
\end{align*}
\]

Crystal data is available online


\[N-(3,5-\text{Dinitrobenzyl})-2\text{-hydroxy}-N-(2\text{-hydroxyethyl})\text{ethanamine} 179\]

\[
\begin{align*}
\text{O}_2\text{N} \\
\text{NO}_2
\end{align*}
\]

Crystal data is available online


\[N-(3-((\text{Bis}(2\text{-hydroxyethyl})\text{amino})\text{methyl}) 5\text{-nitrophenyl})\text{acetamide} 111\]

\[
\begin{align*}
\text{O} \\
\text{NH} \\
\text{O}_2\text{N}
\end{align*}
\]

Crystal information file (CIF) provided on CD ROM
Appendix

$N$-(3-Acetamido-5-(hydroxymethyl)phenyl)benzamide 171

Crystal information file (CIF) provided on CD ROM

4-Acetamido-$N$-(3-acetamido-5-((tert-butyldimethylsilyloxy)methyl)phenyl)-benzamide 177

Crystal information file (CIF) provided on CD ROM

$N$-(3-Acetamidophenyl)-4-(2-morpholinoacetamido)benzamide 274

Crystal information file (CIF) provided in CD ROM
UV melting point DATA

*N-(3-acetamidophenyl)-4-(2-(diethylamino)acetamido)benzamide 262*

![Figure 1. Melting curves of poly AT DNA with derivative 262.](image1)

*N-(3-acetamidophenyl)-4-(2-(diisopropylamino)acetamido)benzamide 266*

![Figure 2. Melting curves of poly AT DNA with derivative 266.](image2)
**Appendix**

*N-(3-acetamidophenyl)-4-(2-(piperidin-1-yl)acetamido)benzamide 270*

![Figure 3. Melting curves of poly AT DNA with derivative 270.](image)

*N-(3-acetamidophenyl)-4-(2-morpholinoacetamido)benzamide 274*

![Figure 4. Melting curves of poly AT DNA with derivative 274.](image)
$N$-(3-acetamidophenyl)-3-(2-(diethylamino)acetamido)benzamide 263

![Diagram of compound 263]

**Figure 5.** Melting curves of poly AT DNA with derivative 263.

$N$-(3-acetamidophenyl)-3-(2-(piperidin-1-yl)acetamido)benzamide 271

![Diagram of compound 271]

**Figure 6.** Melting curves of poly AT DNA with derivative 271.
Appendix

**N-(3-acetamido-5-(hydroxymethyl)phenyl)-4-(2-diisopropylamino)acetamido)benzamide**

284

\[ \text{Figure 7. Melting curves of poly AT DNA with derivative 284.} \]

\[ \text{N-(3-acetamido-5-(hydroxymethyl)phenyl)-3-(2-diisopropylamino)acetamido)benzamide} \]

285

\[ \text{Figure 8. Melting curves of poly AT DNA with derivative 285.} \]
**Appendix**

*N-(3-acetamido-5-(hydroxymethyl)phenyl)-4-(2-(piperidin-1-yl)acetamido)benzamide 286*

![Figure 9](image.png)

**Figure 9.** Melting curves of poly AT DNA with derivative 286.

*N-(3-acetamido-5-(hydroxymethyl)phenyl)-3-(2-(piperidin-1-yl)acetamido)benzamide 287*

![Figure 10](image.png)

**Figure 10.** Melting curves of poly AT DNA with derivative 287.
Appendix

$N^1$-(3-acetamido-5-(chloromethyl)phenyl)-$N^4$-(3-(4-dimethylamino)butanamido)phenyl-terephthalamide 302

![Chemical structure 302]

**Figure 11.** Melting curves of poly AT DNA with derivative 302.

$N^1$-(3-acetamido-5-(hydroxymethyl)phenyl)-$N^4$-(3-(4-(dimethylamino)butanamido)phenyl)-terephthalamide 300

![Chemical structure 300]

**Figure 12.** Melting curves of poly AT DNA with derivative 300.
Figure 13. Melting curves of poly AT DNA with derivative 299.
## NCI DATA

### NCI data for compound 172-OneDose

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NCI data for compound 187-5 Dose
### Appendix

#### National Cancer Institute Developmental Therapeutics Program

**In-Vitro Testing Results**

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**NCI data for compound 187-5 Dose**

310
Appendix

NCI data for compound 213-5 Dose
### National Cancer Institute Developmental Therapeutics Program

**In-Vitro Testing Results**

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**NCI data for compound 213-5 Dose**

- **Observed Growth Rate**
  - Time: 0 hr
  - Growth: -0.25
- **Dose Response**
  - Dose: 213.5
  - Growth: -0.35

*Note: The table above is a sample of data from the NCI's Developmental Therapeutics Program, showcasing in-vitro testing results for various cancer cell lines. The data includes log10 concentration, time, and observed growth rates, providing insights into the efficacy of different compounds against various cancer types.*
Appendix

NCI data for compound 302-5 Dose
### National Cancer Institute Developmental Therapeutics Program

**In-Vitro Testing Results**

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NCI data for compound 302-5 Dose
Appendix

NCI data for compound 300-5 Dose
## National Cancer Institute Developmental Therapeutics Program

### In-Vitro Testing Results

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References


References


320


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