Antiviral Agents: 3,5-Disubstituted 1,2,4-Oxadiazole Derivatives

and

Novel Peptidomimetics Containing Hydroxyethyl Isostere and Imidazolidinone Structures

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Susann H. Krake

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by
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ABSTRACT

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Antiviral Agents: 3,5-Disubstituted 1,2,4-Oxadiazole Derivatives and Novel Peptidomimetics Containing Hydroxyethyl Isostere and Imidazolidinone Structures

Director of Dissertation: Stephen C. Bergmeier

The search for new compounds with biological activity is the major goal of medicinal research.

One way to discover new lead compounds is the screening of large compound libraries, followed by the optimization of the found compounds. With this method, A3 was recently identified as a lead compound showing activity against a variety of viruses, including influenza. The reoccurrence of seasonal and pandemic influenza makes this disease a major threat to human life and economic productivity.

This dissertation is concerned with the development of a small library of derivatives exploring possible optimization of the A3 scaffold. The presence of an indole moiety is seen as a main reason for stability issues with the lead compound. Therefore, the second goal is the finding of a suitable bioisosteric replacement for this residue.

Four new lead compounds (SKB-126, SKB-134, SKB-136 and SKB-150) have been identified from this library, containing new substitution on the indole moiety as well as replacement of the indole with naphthalene and aromatic urea unit.

With continuous development in biochemical research, the understanding of biological mechanisms on the cellular level has brought attention to peptides as means to
manipulate those mechanisms in order to treat diseases. Peptidomimetics are compounds with behavior similar to that of the peptide they mimic, but typically have structural enhancements that prevent quick metabolism or excretion from the system.

A new class of peptidomimetics derived from both imidazolidinones and hydroxyethylene isosteres is described herein. The key step of their synthesis is the opening of a fused ring aziridine with amino acids or small peptides to form an oxazolidinone, followed by rearrangement to the target imidazolidinone. A chain elongation on the resulting diol with a second amino acid or peptide fragment provides the novel peptidomimetics containing hydroxyethylene isosteres and imidazolidinone structures.
DEDICATION

To my Grandma Annelies Krake.
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<td>br</td>
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<td>HMQC</td>
<td>Heteronuclear multiple-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>HOBT</td>
<td>Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectroscopy</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>IC50</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>ICNV</td>
<td>International Committee on Nomenclature of Viruses</td>
</tr>
<tr>
<td>ICTV</td>
<td>International Committee on Taxonomy of Viruses</td>
</tr>
<tr>
<td>iPr</td>
<td>Iso-propyl</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>Max.</td>
<td>Maximum</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl</td>
</tr>
<tr>
<td>MW</td>
<td>Microwave</td>
</tr>
<tr>
<td>NA</td>
<td>Neuraminidase</td>
</tr>
<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>N.R.</td>
<td>No reaction</td>
</tr>
<tr>
<td>OiE</td>
<td>World organization for Animal Health</td>
</tr>
<tr>
<td>o.n.</td>
<td>Over night</td>
</tr>
<tr>
<td>PG</td>
<td>Protecting group</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Phth</td>
<td>Phthalimidyl</td>
</tr>
<tr>
<td>pTSA</td>
<td>Para-toluenesulfonic acid</td>
</tr>
<tr>
<td>Py</td>
<td>Pyridine</td>
</tr>
<tr>
<td>quant.</td>
<td>Quantitative</td>
</tr>
<tr>
<td>rac</td>
<td>Racemic</td>
</tr>
<tr>
<td>RCM</td>
<td>Ring closing metathesis</td>
</tr>
<tr>
<td>Rf</td>
<td>Retardation factor</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>sat.</td>
<td>Saturated</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Single stranded DNA</td>
</tr>
<tr>
<td>ssRNA</td>
<td>Single stranded RNA</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TBD</td>
<td>To be determined</td>
</tr>
<tr>
<td>TBS</td>
<td><em>Tert</em>-butyldimethylsilyl</td>
</tr>
<tr>
<td>tBu</td>
<td><em>Tert</em>-butyl</td>
</tr>
<tr>
<td>Temp.</td>
<td>Temperature</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>TMV</td>
<td>Tobbaco mosaic virus</td>
</tr>
<tr>
<td>t_R</td>
<td>Retention time</td>
</tr>
<tr>
<td>Tr</td>
<td>Trityl</td>
</tr>
<tr>
<td>Ts</td>
<td><em>Para</em>-toluenesulfonyl</td>
</tr>
<tr>
<td>TPW</td>
<td>TFA/PhOH/H₂O (92.5/5/2.5)</td>
</tr>
<tr>
<td>UMP</td>
<td>Uridine monophosphate</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
CHAPTER 1: VIROLOGY

1.1 Introduction

One of the earliest medical cases to be recognized as virus was the death of Pharaoh Ramses V (died 1145 BC), whose mummy showed symptoms later associated with smallpox.¹ One of the most devastating diseases to humankind, smallpox is caused by the variola virus and killed as many as 30% of the infected population during repeated epidemics between 3000 BC and the 20th century.² As a result of an endemic in China (1000 BC) variolation was developed as a precursor to vaccination. It involves the immunization against smallpox by introduction of dry crusts or pus from smallpox lesions into a healthy organism by inhalation or scratch.³ This procedure was risky as the outcome of the treatment was not certain. In 1796, Edward Jenner published his work about immunization with the cowpox virus, which is a typically non-fatal species of the orthopoxvirus for humans. This development also resulted in the coining of the term “vaccination,” as the Latin root vacca translates to cow. Later, the vaccina virus, the type species of orthopoxvirus, was used for smallpox vaccination. Aside from vaccination, up to 4 days after exposure and before appearance of a rash, there is no effective treatment against smallpox. With the introduction of vaccination, the number of cases worldwide was reduced from 50 million per year to 10-15 million in 1967. After a global eradication campaign, smallpox was certified “extinct” by the World Health Organization (WHO) in 1979.

Only one other infectious viral disease has been eradicated so far; the last confirmed case of Rinderpest (cattle plague) was diagnosed in 2001.⁴ In 2011 the World
organization for Animal Health (OiE) announced that all 198 countries were disease-free and the virus was eradicated.\textsuperscript{5}

Following these examples of successful eradication, the WHO launched a global polio eradication campaign in 1988 and a joint measles and rubella initiative in 2012.\textsuperscript{6-8} To date, the number of cases for poliomyelitis has fallen by more than 99\% during the campaign, and in 2012 only three countries remained endemic (Afghanistan, Pakistan and Nigeria).

Over the centuries, viruses have been devastating to humans, animals and plants alike. Even with the above-mentioned successes through vaccination, the vast amount of identified viruses and problems with recurring pandemics clearly indicate that our understanding of the topic is still limited.

1.2 Basics of Virology

1.2.1 Classification

In the late 19\textsuperscript{th} century the germ theory proposed that for all infectious diseases there is a responsible microorganism that follows three defined postulates:\textsuperscript{9}

1. The microorganism could be seen under the microscope.
2. It could be cultivated on a nutrient medium.
3. Filters would retain it.

Following this theory, Robert Koch and Friedrich Loeffler introduced “Koch’s postulates,” stating four criteria that are used to state whether an infectious agent is the
cause of a disease or not. After some readjustments, these postulates still prove true today:

1. The agent must be present in every case of the disease.
2. The agent must be isolated from the host and grown in vitro.
3. The disease should be reproduced when a pure culture of the agent is introduced into a healthy susceptible host.
4. The same agent must be recovered once again from the experimentally infected host.

Contrary to the germ theory, in 1898, the term “contagium vivum fluidum” was used to describe the newly found infectious agent tobacco mosaic virus (discovered in 1892) and foot-and-mouth disease virus, because they did pass through bacteria-proof filters. However, without advanced analytical and culturing techniques, further description was impossible. Thus, the first definition of a virus negates the terms of the germ theory:

1. The infectious agent could not be seen.
2. It could not be cultivated.
3. Bacteria-proof filters did not retain the agent.

In the 1930s, chemical methods were advanced to a point where it could be stated that virions consist of protein and either DNA or RNA. This discovery led to first considerations of grouping viruses according to their properties. In the following years, a large number of new viruses were discovered and controversial ideas about classification led to competition and confusion. Out of need for a single universal system,
the International Committee on Nomenclature of Viruses (ICNV) was formed in 1966 and later renamed into the International Committee on Taxonomy of Viruses (ICTV). A hierarchic system for classification was adapted, which attempts to sort viruses that attack humans, animals, plants and bacteria. As of the official ICTV 2011 taxonomy report, 6 orders, 94 families, 22 subfamilies, 395 genera and 2480 species have been identified (Table 1.1). Viruses are assigned to this system according to structural properties (size, nucleocapsid symmetry, presence of envelope, type of genome) and genetic relationships. The orders have been introduced with the development of genetic relations. Of those 94 families, 72 have not yet been assigned to an order.
Table 1. ICTV classification and examples

<table>
<thead>
<tr>
<th>6 Orders (-virales)</th>
<th>94 Families (-viridae)</th>
<th>22 Subfamilies (-virinae)</th>
<th>395 Genera (-virus)</th>
<th>2480 Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caudovirales</strong></td>
<td>e.g.: Myoviridae</td>
<td>e.g.: FelixO1likevirus</td>
<td>e.g.: Salmonella phage FelixO1</td>
<td></td>
</tr>
<tr>
<td>(tailed dsDNA, bacteriophages)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Herpesvirales</strong></td>
<td>e.g.: Herpesviridae</td>
<td>e.g.: Simplexvirus</td>
<td>e.g.: Human herpesvirus 1 (HSV)</td>
<td></td>
</tr>
<tr>
<td>(large eukaryotic dsDNA viruses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mononegavirales</strong></td>
<td>e.g.: Filoviridae</td>
<td>e.g.: Ebola</td>
<td>e.g.: Zaire ebolavirus</td>
<td></td>
</tr>
<tr>
<td>(nonsegmented (-) strand ssRNA plant and animal viruses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nidovirales</strong></td>
<td>e.g.: Coronaviridae</td>
<td>e.g.: Betacoronavirus</td>
<td>e.g.: Severe acute respiratory syndrome-related coronavirus (SARS coronavirus)</td>
<td></td>
</tr>
<tr>
<td>((+) strand ssRNA viruses with vertebrate hosts)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Picornavirales</strong></td>
<td>e.g.: Picornaviridae</td>
<td>e.g.: Aphthovirus</td>
<td>e.g.: Foot-and-mouth disease virus</td>
<td></td>
</tr>
<tr>
<td>(small (+) strand ssRNA viruses that infect a variety of plant, insect and animal hosts)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tymovirales</strong></td>
<td>e.g.: Alphaflexiviridae</td>
<td>e.g.: Potexvirus</td>
<td>e.g.: Potato virus X</td>
<td></td>
</tr>
<tr>
<td>(monopartite (+) ssRNA viruses that infect plants)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Orthomyxoviridae</strong></td>
<td>e.g.: Influenzavirus A</td>
<td>e.g.: Influenza A virus</td>
<td>e.g.: Human immuno-deficiency virus 1 (HIV)</td>
<td></td>
</tr>
<tr>
<td><strong>Unassigned</strong></td>
<td>e.g.: Retroviridae</td>
<td>e.g.: Orthoretrovirinae</td>
<td>e.g.: Lentivirus</td>
<td></td>
</tr>
</tbody>
</table>
In 1971, David Baltimore published a paper called “Expression of Animal Virus Genomes.”12 In contrast to the ICTV classification system, the “Baltimore classification” system is purely based on the way a virus produces its messenger ribonucleic acid (mRNA). The justification for this system lays in the fact that, aside from producing new genetic material, all viruses must synthesize mRNA in order to utilize the host cell ribosome to produce proteins and ultimately to reproduce. According to this statement, there are now seven groups shown in Figure 1.1. In modern virology, both systems are used in conjunction.

**Figure 1.1** Baltimore Classification.
1.2.2 Virus structure

A single virus particle is called a virion. It consists of nucleic acid (DNA or RNA) surrounded by a layer of protein, called capsid. In some cases an additional lipid layer can be present, which is derived from the membrane of the host cell (Figure 1.2). The proteins (capsomers) of the capsid are arranged in a certain symmetry that offers high stability and furthermore allows for repeating units that need comparably small encoding space. In helical structures, repeating units of a single protein are aligned around a central axis and form filamentous virions with a central tube-shaped cavity. The *tobacco mosaic virus* (TMV) is a common example for a non-enveloped helical virus, while influenza represents the enveloped form. A second common structure is an isocahedral capsid, which consists of 20 identical and symmetric triangles, and has three axes of symmetry. The composition of such structures is in general more complex and typically more then one type of capsomer is utilized. An example for a non-enveloped isocahedral virus is the adenovirus; *herpes simplex virus* (HSV) and HIV are enveloped viruses of this category. A third group contains enveloped and more complex structures that do not fit the above-mentioned symmetries or contain extra structures like protein tails or complex outer walls. Prominent representatives for this category are *enterobacteria phage T4* and the *vaccina virus*. 
<table>
<thead>
<tr>
<th>Non-enveloped</th>
<th>Enveloped</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isocahedral</strong></td>
<td><strong>Isocahedral</strong></td>
</tr>
<tr>
<td><em>Adenoviridae</em></td>
<td><em>Herpesviridae</em></td>
</tr>
<tr>
<td><img src="image1.png" alt="Image of Adenovirus" /></td>
<td><img src="image2.png" alt="Image of Herpesvirus" /></td>
</tr>
<tr>
<td>e.g.: <em>HSV</em></td>
<td>e.g. <em>Influenza A virus</em></td>
</tr>
<tr>
<td><img src="image5.png" alt="Image of Retrovirus" /></td>
<td>e.g.: <em>HIV</em></td>
</tr>
</tbody>
</table>

All images were obtained from Ref. 13

**Figure 1.2** Structure of vertebrate viruses.

### 1.2.3 Virus replication

In order for a virus to replicated it must complete the following steps:

1. Attachment to receptors on the surface of the host cell.
2. Entry into a host cell and uncoating.
3. Protein expression and replication of genetic material.
4. Assembly, maturation and release of new virions.
These steps are fairly universal, but there are general differences between animal-, plant- and bacterial viruses. The main focus here lies again on the description of animal viruses. Before a virus can enter a cell, it must interact with one or more specific receptors on the surface of the host cell. Typically, the receptors are membrane proteins, but carbohydrates and lipids can also be the target for the virus. Once the virus is bound to the host cell, most animal viruses follow one of two main ways of entry (Figure 1.3).\textsuperscript{14,15} The first way is receptor-mediated endocytosis, which applies for enveloped and non-enveloped viruses alike. In that case, a vacuole is formed around the virus and it can enter the cell. In order to release the virus, a decrease of pH inside this endocytic vesicle is accomplished by fusion with an endosome and subsequent accumulation of protons on the inside through utilization of endosome proton pumps. This process introduces conformational changes in the viral proteins. In the case of enveloped viruses, the lipid bilayers of virus and endosome will fuse and release the virus into the cell. For non-enveloped viruses, a lower pH leads to exposition of hydrophobic moieties in viral proteins that can form a channel through the endosome and also release the virus into the cell.

The second way of cell entry occurs at neutral pH and can only be performed by enveloped viruses. After binding to a receptor, viral proteins form a hydrophobic channel to the cell membrane and inserts into it. This channel ultimately leads to a fusion of virus and cell membranes and release of the virus into the host cell.
Once the virion has entered the cell, uncoating is necessary to expose and release the viral genome.\textsuperscript{16} Very little research has been done on this process, but typically pH change can induce capsid dissociation or viral/host cell enzymes can degrade the capsid. This process leaves the viral genome exposed as a complex structure, typically a nucleoprotein complex, containing the genetic material as well as enzymes and proteins important for replication.

Figure 1.3 Animal virus cell entry.
The next step of reproduction contains the synthesis of viral mRNA, its expression into proteins and the replication of the viral genome (Figure 1.4). These processes are heavily dependent on the type of genetic material a virus contains (see Figure 1.1), and for our considerations of antiviral activity the exact mechanisms involved for each class have little importance. Therefore, the description of these mechanisms will only be short and general. An overview of viral mRNA production over different pathways was shown in Figure 1.1. In general, the production of mRNA and the replication of the viral genome take place in the host cell nucleus for all DNA viruses, while the same processes for RNA viruses mostly (but not always) takes place in the cytoplasm. An example of an RNA virus that needs to utilize the host cell nucleus in its replication cycle is the influenza virus, as it requires incorporation of cellular mRNA into viral mRNA. The replication of reverse transcribing viruses with DNA (e.g. hepatitis B virus) and RNA (e.g. HIV) genome are more complex and take place both inside and outside the nucleus.

The transitions between assembly, maturation and release of new virions are overlapping and depend very much on the complexity of the virus itself. Assembly describes the process of constructing a new virion from genome and proteins, and in
many cases is not fully understood yet. In simple viruses (e.g. poliovirus), the capsid proteins might assemble themselves or assemble precursors and the genome condenses with the capsid during formation or might be inserted afterwards. More complex viruses (e.g. adenoviruses) require scaffolding proteins that help assemble the subunits of the capsid and the capsid itself, in which the genome is then placed. In other cases (e.g. orthomyxoviridae), the nucleocapsid is actually formed in the nucleus and then migrates back into the cytoplasm. Another interesting fact about influenza virus assembly results from its segmented genome. As a full set of eight RNA segments is required for a fully infectious virus, but no signals are known that insure this outcome, an influenza virion contains 12 segments in order to enhance the probability of a full segment set.

Maturation characterizes the step in which the virus becomes infectious. This is accomplished through conformational changes or cleavage of capsid proteins. It can occur during assembly or after release of the virion, but typically involves substantial structural changes in the virion. The mechanisms involved are complex and include viral or cellular proteases that are further controlled by proximity to their target and environmental changes of hydrophobicity, pH or metal ion concentration.

The last step of viral replication is the release of viral particles out of the cell. For animal viruses two distinct mechanisms are applicable, lysis and budding (Figure 1.5). Most non-enveloped viruses escape the host cell through lysis (e.g. poliovirus). That means that a large number of new virions are formed before the host cell itself undergoes apoptosis and the virions are released. Enveloped viruses acquire their membrane from intracellular vesicles prior to release (typically via lysis) or through budding from the
host cell wall while exiting. The utilization of viral enzymes on the surface of the envelope is typically necessary to detach the virus from the host cell. An example for this is the neuraminidase on the surface of influenza, which cleaves the attachment of hemagglutinin to the cell surface. While this method of exit does not directly lead to apoptosis, it might damage the cell significantly and typically leads to shrinkage of the cell due to lost membrane.

![Lysis: e.g. poliovirus](image1)

![Budding: e.g. influenza virus](image2)

**Figure 1.5** General release mechanisms of animal viruses.

### 1.3 Influenza

According to the most recent ICTV classification, the family of *orthomyxoviridea* is of unassigned order and contains genera: *influenzavirus* A, B and C as well as *isavirus* and *thogotovirus*. The three genera of influenza differ in matrix- and nucleoproteins and infect different vertebrate hosts. Each genera only contains one species: *influenza A virus* (hosts: human, bird, pig and horse), *influenza B virus* (hosts: human and seal) and *influenza C virus* (hosts: human and pig). *Influenza A virus* is the most common species
and also the one responsible for the pandemics of the last century. Subtypes of influenza A are categorized according to the antigenic properties of their surface glycoproteins. Hemagglutinin (HA) is the protein responsible for binding of the virus to the host cell and to date 16 HAs have been identified. On the other hand, nine types of neuraminidase (NA), the enzyme necessary for the release of the virus, by cleavage of the link between a host cell sialic acid residue and viral HA, are known. This information is encoded on two of the eight single-stranded negative sense RNA segments the genome of influenza is composed of. In the following, the main focus will lie on influenza A virus but the mechanisms of infection for the other two influenza genera are similar as are the antiviral strategies.

1.3.1 Pandemics

A pandemic describes an epidemic of worldwide scope. During the last 100 years, five influenza A outbreaks fell into this category. Statistics for mortality rates on those pandemics are difficult to obtain and are typically only estimates. The first and most devastating was the “Spanish flu” (1918-1919, H1N1) which caused an estimated death toll of 50 million people worldwide. It is assumed that the virus was an avian influenza, which would explain the high virulence, as the human immune system was not prepared for this type of influenza. All pandemics after that one were a result of genetic mixture between different influenza strains and reoccurrence (Figure 1.6). In 1958 the “Asian flu” (H2N2) appeared as a genetic combination between the “Spanish flu” and a human strain and killed about 70,000 people in the US alone. Even though only three of
the genes were exchanged, it appears that changes in the surface glycoproteins have a large impact in terms of pre-existing immunity. A change of only one gene caused the outbreak of the 1968 “Hong Kong flu” (H3N2), which might explain lower death rates (about 30,000 in the US) through affects of antibodies to the NA antigen. In 1977 (“Russian flu”) a H1N1 strain reappeared, which was closely related to a virus present in the 1950s. This is seen as a reason why mostly people born after that period of time were affected. The most recent outbreak was the 2009 pandemic flu (“Swine flu”) H1N1 containing a combination of genes from avian, human and two pig stains of influenza. After nearly 20,000 confirmed deaths world wide, the pandemic was declared to have entered a post-pandemic period in August of 2010.

Three statements now describe the conditions that have to be fulfilled in order for an influenza virus to develop pandemic potential.

1. The HA subtype of the influenza strain has to be absent from humanity for at least one generation.

2. It needs to be highly infectious in humans.
3. It needs to follow effective transmission between humans.

1.3.2 Seasonal Influenza

All three types (A, B and C) can occur during seasonal influenza. But *influenza C virus* is fairly rare and therefore not included in vaccines. Epidemics are typical during the fall and winter month in temperate areas and year-round in the tropics. These epidemics cost about 250,000 to 500,000 deaths annually, mostly in the elderly.\(^{31}\) The WHO puts a lot of effort into prevention, stating that vaccination “is the most effective way to prevent the disease or severe outcomes from the illness.” If illness occurs, it can be treated with antiviral compounds, which are available in “some countries.” Both methods, prevention and treatment, for seasonal and pandemic influenza will be discussed in the following.

1.3.3 Vaccination

Vaccination is the major way to prevent infection with a virus. Two types of vaccines are available today, inactivated virus vaccines that are administered by injection and live attenuated virus vaccines in form of a nasal spray.\(^{32, 33}\) As mentioned above, most commonly are *influenza A virus* (subtypes H1N1 and H3N2) and *influenza B virus*. This is the reason for these vaccines to be trivalent, which means that they contain three different materials to act against all current threads. The viral material is produced in chick embryos and later purified. The inactivated virus versions are often administered as split product or subunit vaccines as this reduces side effects. For efficacy of the current
vaccines it is very important that the right stains of circulating influenza are included in the mixture because otherwise it might be uneffective. This is a problem because of constant changes in the HA and NA glycoproteins from antigenic drift in influenza A and B. In addition, for influenza A virus only, the reassortment of animal and human viruses or the introduction of an animal virus in human hosts can cause a change in the HA and NA subtypes present, called an antigenic shift. Therefore, influenza is monitored globally and every season, the vaccine composition is changed accordingly.

The problems associated with vaccination are:

1. Access: Production time and volume of vaccines for a new strain is estimated to take three to six months.


3. Efficacy: Especially in the elderly population there is a shift away from immunization to decrease of morbidity and mortality after infection.

In 2006, the WHO launched the GAP-program (Global Action Plan for Influenza Vaccines) to find solutions for these problems and promote research.

1.3.4 Present Antiviral Treatment

In general, responsive and preemptive treatment of influenza has the largest benefit for people with high risk of complications. This includes the population above the age of 65 and immune-compromised patients. Another indication for preemptive treatment is the appearance of a new epidemic strain that is not covered by current vaccines.
If an infection with influenza is present, it can be treated through interruption of the viral replication cycle. The two general ways of accomplishing this are inhibition of either viral or host cell proteins.\textsuperscript{38} Currently approved pharmaceuticals act on viral proteins, which has the advantage that the host itself is not affected by the treatment. Unfortunately, swiftly mutating viruses like influenza are prone to quickly develop resistance against such agents, making them ineffective. On the other hand, it is much harder for a virus to become resistant against a compound that interferes with a protein from the host cell. The downside of such a compound is that it is most likely toxic to the host.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{influenza_replication_cycle}
\caption{Inhibition of influenza replication cycle.\textsuperscript{24}}
\end{figure}

Figure 1.7 shows strategies for small compound interactions with the viral replication cycle that are currently under investigation or already applied in treatment. In the following, the two types of approved antivirals will be discussed with focus on action, synthesis and resistance.
1.3.4.1 Ion channel inhibitors

The first class of licensed antiviral influenza agents was M2 ion channel inhibitors in the 1960s. Another name for this class is adamantanes, which is derived from the structural element these compounds have in common (Figure 1.8).

![Figure 1.8 Amantadine and Rimantadine.](Image)

The function of M2 ion channels is the transport of protons into the endocytic vesicle, which leads ultimately to the uncoating of the virus. Adamantanes bind inside this ion channel and prevent it from functioning. Because the M2 protein is unique to influenza A virus, these are the only ones affected by the drug. Influenza B virus, for example, has its own proton pump called NB.

The most frequent side effects of adamantanes are nausea, dizziness and insomnia. Major side effects are amphetamine-like through stimulation of the central nervous system and include jitteriness, anxiety and nightmares. Of concern is the tremendous increase in resistance against this type of drug. H3N2 isolates showed an increase in resistance from 0.5% worldwide in 1995/95 to 15% in 2004/05 and ultimately to 90% in 2005/06. A similar trend was observed for H1N1 isolates with an increase in resistance from 4% in 2004/05 to 16% in 2005/06. Most of the resistant isolates showed a single amino acid change (Ser31Asn) in the M2 protein, which is known to induce
resistance against adamantanes and explains the rapid spread of resistance. As a result of this development, the CDC does not recommend the use of Amantadine or Rimantadine for currently circulating influenza A viruses.\textsuperscript{45}

1.3.4.2 Neuraminidase inhibitors

The last step in the influenza replication cycle is the exit of the new virus particles. During this process, neuraminidase cleaves the link between a sialic acid unit on the host cell receptor and viral hemagglutinin, which releases the virus (Scheme 1.1). The two approved neuraminidase inhibitors Oseltamivir (1999, Hoffmann-La Roche) and Zanamivir (1999, GalaxoSmithKline) are mimics of the transition state that bind to the sialic acid binding pocket.\textsuperscript{46} With the active site blocked, the neuraminidase cannot cleave the link between virus and host cell and therefore no new virus particles can be released.
Even though both compounds are small molecules, the large number of stereo centers translates into more complex synthetic preparations. Zanamivir is synthesized in 9 steps from the commercially available \( N\)-acetylneuramic acid (Scheme 1.2), which is interestingly the same compound that is cleaved by the neuraminidase.\(^ {47} \) The lack of the ring-oxygen in Oseltamivir means that there is no simple sugar-like precursor for the compound and three stereo centers in the core structure indicate that a commercial \textit{de novo} synthesis would be too expensive. Therefore, the natural products quinic acid (found in cinchona bark and coffee beans) and shikimic acid (from star anise) are utilized as precursors in the commercial synthesis of Oseltamivir (Scheme 1.2).\(^ {48-51} \)
phosphate salt of this compound is an orally administered prodrug, which upon metabolism turns into the active free acid.

Scheme 1.2 Synthesis of Zanamivir and Oseltamivir.

Resistance against both drugs is emerging, but is not yet as significant as it is for the adamantanes. Two single nucleotide polymorphism (SNP) mutations in the genes expressing neuraminidase have been found to cause resistance.\(^5^2\) The H275Y mutation induces high-level resistance of H1N1 against Oseltamivir, but not Zanamivir, due to conformational changes in the binding pocket. Intermediate level resistance is induced by N295S mutation and affects both drugs in H1N1 and H5N1 subtypes of the virus. In contrast to mutations in the neuraminidase genes, it has been shown that mutations in the hemagglutinin gene lead to indirect resistance against both drugs.\(^5^3\) In those cases, the affinity to sialic acid appears to be reduced, which results in easier release of the virus from the host cell without involvement of a neuraminidase.
1.3.4.3 Other Antivirals for Influenza treatment

A number of other small compounds are under investigation for activity against influenza infection. Examples are shown in Figure 1.9.

Figure 1.9 Examples for new antiviral compounds.

Peramivir, an experimental cyclopentane neuraminidase inhibitor was approved for emergency use during the 2009 H1N1 pandemic, as it had been effective against serious cases of swine flu. Due to the overcoming of that threat, the emergency authorization was discontinued in 2010.\(^5^4\) Dimeric derivatives of Zanamivir have been shown to possess better oral availability then the original monomer and additionally
exhibit long-lasting activity, which is especially helpful in preventive administrations.\textsuperscript{55, 56} Another prominent target for antiviral drugs is the viral RNA polymerase. This inhibition can be induced by a nucleoside like FdG or a non-nucleoside compound like T705 or Thiadia-zol[2,3-\textit{a}]pyrimidine 1.1.\textsuperscript{57-60}

Other than small molecules, peptides are under investigation. A sialidase fusion protein with a cell anchoring sequence was demonstrated to remove sialic acid receptors from the viral surface, thus preventing attachment of the virus to the host cell.\textsuperscript{61} This compound is currently in clinical trials under the name DAS181.

1.3.4.4 Conclusions Antivirals against influenza so far

The above-mentioned developments and experiences in the field of antiviral drugs against influenza virus can be summarized as follows:

1. The influenza viruses are a worldwide problem and increased globalization leads to very fast spread. This becomes especially dangerous in the case of a newly emerging influenza A virus subtype with pandemic properties.

2. Two classes of antiviral drugs are approved to treat and prevent influenza in humans.

3. New classes and compounds are under development.

4. Resistances against the current drugs are evolving quickly and to a point were one class of compounds became obsolete.

5. For reasons mainly attributed to safety, most compounds under investigation act on a viral target rather then a host cell target.
Having that information outlined, one can draw conclusions about the necessary steps in research and handling of information:

1. The WHO, CDC and other institutions around the world collect information about viral circulation and possible resistance. But it is important to further improve the reaction time after identification of new viral types or resistance.

2. An overuse of antiviral drugs increases the likelihood of resistance and should therefore be limited. A prominent example of failure on that point would be the overuse of penicillins.

3. The development of new potential antiviral drugs (and vaccines) needs to be supported. At the same time, new viral and host cell targets for action need to be found.

1.3.5 *DHODH inhibitors/A3*

As described above, the treatment of influenza infections typically targets viral proteins. The utilization of host cell proteins as targets is in general related to higher toxicity of the drug, which makes it important to prove that the target protein is not crucial for cell growth or function. However, if such a target is discovered that is also crucial for viral replication, it is very hard for the virus to develop resistance against the drug.
Compound A3 was identified in a high-throughput screening to be a strong inhibitor of the influenza virus replication cycle (Figure 1.10).\textsuperscript{38, 62, 63} Further testing revealed the inhibition of viral polymerase function and viral RNA production but no influence on the host cell gene expression. Aside from influenza, the influence of A3 on a number of different viruses was examined and inhibited by this compound. Interestingly, A3 showed activity on viruses from different classes, including influenza A and B virus ((-)ssRNA), hepatitis C virus ((+)ssRNA), adenovirus (dsDNA) and HIV-1 (ssRNA RT).\textsuperscript{38} This type of broad-spectrum activity is typically related to interference with nucleotide synthesis or incorporation. This could be confirmed by activity testing in the presence of different purines and pyrimidines, as uracil appeared to reverse the inhibitory effect of A3. UMP can be produced via salvage pathway from uracil or by de novo synthesis from carbamic acid. Testing of all intermediates in the UMP synthetic pathways indicated inhibition of dihydroorotate dehydrogenase (DHODH) during the \textit{de novo} synthesis of UMP (Scheme 1.3). DHODH catalyzes the transformation of dihydroorotate (DHO) into orotate, which was shown to be able to reinitiate the viral replication cycle in presence of A3, while DHO was not.
During the investigations with different cell lines, it was found that A3 is a strong antiviral agent in human and primate cells, independent of the tissue type. Cell lines of other species showed either complete but easily reversed or incomplete inhibition. A3 appeared to have no effect on viral replication in avian cells. This species-dependent activity could be related to differences in the sequence of DHODH between those species.
CHAPTER 2: SYNTHESIS OF STRUCTURAL ANALOGS OF A3

2.1 Introduction

Lead compound A3 consists of three subunits as shown in Figure 2.1. It was identified by high-throughput screening, but little structure-activity optimization has been done. The main challenges with A3 and related structures are low solubility, which is most likely due to the planarity of the extended aromatic system, and low stability, which might be attributed to the indole unit. In addition, the exact binding site on DHODH has not yet been identified, which means that modeling of interactions between the enzyme binding pocket and potential A3 derivatives is not available.

\[ \text{Figure 2.1 A3 subunits.} \]

The purpose of this project is the identification of compounds with equal or better activity than A3 and improved structural properties. In order to achieve this goal, a small library of compounds with structural changes on the amide and indole subunits of the molecule is introduced (Figure 2.2). The synthesis of A3 analogs with different amides is fairly easy to accomplish and will indicate whether or not changes on that side of the molecule have any effects on binding and solubility. Substitutions on the indole unit are expected to have greater influence on stability and solubility and therefore are the main focus of this project. Three types of compounds can be obtained from these substitutions.
A number of different indoles can be introduced to find compounds with binding properties better than \textbf{A3}. The exchange of the indole for a heterocyclic unit with similar steric and electronic properties might lead to compounds with greater stability and equal activity. As a third group, the introduction of substituted naphthalenes and anilines is pursued in order to evaluate the necessity of a heterocyclic compound in this position. This is important as the last group of compounds can be synthesized from simple, commercially available precursors a large variety of substitution is possible.

![Figure 2.2 General exchanges on A3.](image)

As shown in Figure 2.2, three new amide residues will be introduced. While the exchange of the pyrrolidine for piperidine is introduced to study the steric binding abilities, the introduction of morpholine and \textit{bis-(methoxy)ethylamine} enhances the possibility of hydrogen bonding in the active site of the enzyme and might also improve the solubility of the compound.
Starting from the indole subunit of A3, the possibilities of methyl substitution in 1-, 2- and 3-position of the indole need to be explored (Figure 2.3). In addition to that, the introduction of other alkyl substitution including further cyclization around the 2- and 3-position are important to evaluate binding to the active site. Similar to this, the connection to the oxadiazole subunit can have a major influence on binding, thus linkages in positions 4 and 6 are attempted. Lastly, substitution in the 4- and 6-position would interfere with the overall planarity of the indole-oxadiazole system and could therefore result in better solubility of the derivatives.

**Figure 2.3** Indole derivatives.
The introduction of heterobicyclic substituents is an attempt to find active compounds with stereochemistry similar to indole, but with different electronic properties that might lead to additional binding (Figure 2.4). While the nitrogen in benzofuran is exchanged for oxygen, 1$H$-indazole and 1$H$-triazole derivatives carry additional nitrogen atoms in the 2- and 3-position. Methylimidazopyridine and benzoazolone derivatives carry substitution in the 2-position, which introduces additional steric restrictions. The quinoline derivative can be seen as a stereochemical intermediate between a plain indole and 2-,3-dimethylindole.

![Heterobicyclic derivatives:](image)

**Figure 2.4** Heterocyclic indole replacements.

In a third general group of derivatives, attempts are made to find other structural motifs that show activity (Figure 3.5). A structural functionality that can mimic the orientation of the indole nitrogen without the additional ring is aniline. With the aniline nitrogen in the para- or ortho-position to the oxadiazole link, a number of different substitutions can be introduced. Amide and urea derivatives are typical structural elements in drug discovery, but thioureas and sulfonyl amides are also known. Even
though the structures shown have a larger steric demand than the indole derivatives, the flexible linkage might extend into the binding pocket to further binding sites.

The naphthalene derivatives are examples for readily available introduction of simple aromatic compounds with little to no steric hindrance. The methoxy substitution in the ortho-position to the oxadiazole link should be able to destroy the planarity of the system and hopefully enhance solubility.

**Figure 2.5** Aromatic indole substitution.
2.2 Synthetic Plan

A3 was initially obtained from a commercial compound library without a published synthesis. A synthetic route was proposed by the patent claiming the activity of A3 and other compounds on negative-sense, single-stranded RNA virus replication (Scheme 2.1). Starting from indole hydrazide 2.1, reaction with carbon disulfide provides oxadiazole 2.2. A nucleophilic substitution on a 2-bromoester results in ester analog 2.3 of the final compound, which is then converted into the desired amide 2.5.

![Scheme 2.1 Proposed synthesis in patent.](image)

On the basis of the synthesis in Scheme 2.1 we propose a more efficient route to the desired products (Scheme 2.2). The hydrazide (2.1) can be obtained from an ester and
then converted into the oxadiazole 2.2. If this can be reacted with a 2-bromoamide, instead of an ester, then the synthesis is shortened by one or two steps.

Scheme 2.2 Proposed shorter synthesis.

In the following, the synthesis of analogs will be described step by step. Three commercially available compounds have been chosen as test substrates for each step to optimize conditions (Scheme 2.3).

Scheme 2.3 Test substrates.
2.3 Synthesis of Starting Esters

2.3.1 Indole Esters

There are a large number of general strategies known to synthesize indoles. Recently an attempt has been made by Taber and Tirunahari to classify those methods according to the last bond that is formed (Figure 2.6).  

![Figure 2.6 Types of indole synthesis.](image)

For each of the types shown, a number of reactions are possible that largely depend on the substitution pattern around the indole ring. Out of these reactions, only a small number are suitable for our purpose of purely aliphatic substitution in the 1-, 2- and 3-position of the indole. These reactions are the Fischer indole syntheses and the Bartoli indole synthesis, which can be both classified as type 1 and a transition metal catalyzed (TMC) reaction of type 2.
2.3.1.1  Bartoli Indole Synthesis

The Bartoli indole synthesis was the initial approach of choice because this method provides access to all substitution variations around positions 2 and 3 of the indole. The reaction was first introduced in 1989 between a nitrobenzene \textbf{2.14} and a Grignard reagent (Scheme 2.4).\textsuperscript{67} Unfortunately, a substitution in the ortho position to the nitro group is critical as its absence leads to a drastic decrease in yields.\textsuperscript{68}

\begin{center}
\includegraphics[width=0.8	extwidth]{scheme2.4.png}
\end{center}

\textbf{Scheme 2.4} Bartoli reaction.

Recently, the Bartoli reaction was utilized to synthesize a variety of indole esters with and without substitution in the ortho position in reasonable yields on solid support (Scheme 2.5).\textsuperscript{69} Based on this research, an attempt was made to reproduce these results without introducing a solid support.
Scheme 2.5 Bartoli reaction on solid support.

Starting from para-nitrobenzoic acid, three esters with different steric hindrance were synthesized in good yields (Scheme 2.6). Because the Merrifield resin is copolymer of styrene and chloromethylstyrene, the benzyl ester was especially expected to lead to the desired product. Unfortunately, reactions with all three esters resulted in similar, but complex, mixtures from which no product could be isolated.

Scheme 2.6 Attempt on Bartoli strategy for indole derivatives.
2.3.1.2 Modified Fischer Reaction

The Fischer indole synthesis was discovered in 1883 and is the most important and best studied method for the synthesis of substituted indoles.\textsuperscript{70, 71} It describes the reaction of a phenylhydrazine with a ketone or aldehyde acid catalysis (Scheme 2.7).

\textbf{Scheme 2.7} Fischer indole synthesis.

Because indole structures are known to have rich biological activity, numerous applications of this reaction are known. The main drawback is the availability of the starting hydrazide. To overcome this challenge, the Buchwald modification introduces palladium catalyzed coupling between benzophenone hydrazone and an aryl bromide to form hydrazones\textsuperscript{2.25} that can be treated with ketones under acidic conditions to produce indoles in high yields (Scheme 2.8).\textsuperscript{72} The utilization of benzophenone hydrazone has the advantage that it is a commercially available, cheap and stable compound, compared with other hydrazones. But because benzophenone is a side product unfavorable in terms of atom economy that needs to be separated by chromatography, substitutions for this compound have been found.
Scheme 2.8 Buchwald modification.

Suitable for those needs are N-Boc arylhydrazides that can be obtained from the coupling of the aryl bromide with tert-butylcarbazate, and then undergo Fischer indole synthesis (Scheme 2.9).\textsuperscript{73, 74}

Scheme 2.9 Fischer indole synthesis with N-Boc arylhydrazides.

This method has been successfully applied to obtain indole esters 2.6a and 2.6b (Scheme 2.10). Initially the synthesis was carried out on the commercially available ethyl ester 2.29a, but problems during later steps of the synthesis lead to the utilization of the methyl ester 2.29b, which was prepared from para-bromobenzoic acid in excellent yield.
Both compounds underwent palladium catalyzed coupling with tert-butylcarbazate in good yields, and the final Fischer indole synthesis was carried out under acidic conditions in moderate yields. Because the solvent of choice for this reaction was an alcohol, it is important to use an alcohol compatible with the ester, as trans-esterification does occur under these conditions. Thus, if treated under microwave conditions without the ketone, \(2.6b\) can be obtained from \(2.6a\) with quantitative yield.

Scheme 2.10 Synthesis of indole esters \(2.6a\) and \(2.6b\).

Aside from 2-butanone, three more ketones were reacted with hydrazide \(2.30b\) to obtain the corresponding indoles \(2.6c-2.6e\) (Table 2.1). The yields for the reactions with 2-pentanone (entry 2) and cyclopentanone (entry 3) under the standard conditions were rather low. This is an outcome already described for reactions with similar \(N\)-Boc arylhydrazides.\(^74\) The fact that no starting material could be recovered suggests that either the hydrazone or ene-hydrazine intermediates or the final indole are instable and decompose. Therefore, the reaction with cyclopentanone was repeated in THF for only 1h
under reflux (entry 4), which has been described to improve the yields.\textsuperscript{74} Unfortunately, this was not the case here. The reaction with cyclohexanone resulted in the desired product in a good yield, but with an impurity that could not be separated (entry 5). A switch of solvent to toluene and a shorter reaction time did result in clean product with a moderate yield (entry 6).

Table 2.1 Synthesis of indole esters 2.6c-2.6e.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>reflux, 20 h</td>
<td><a href="#">2.6b</a></td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MeOH</td>
<td>reflux, 24 h</td>
<td><a href="#">2.6c</a></td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MeOH</td>
<td>reflux, 24 h</td>
<td><a href="#">2.6d</a></td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>THF</td>
<td>reflux, 1 h</td>
<td><a href="#">2.6d</a></td>
<td>traces</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MeOH</td>
<td>reflux, 24 h</td>
<td><a href="#">2.6e</a></td>
<td>&lt;84%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>toluene</td>
<td>80 °C, 1 h</td>
<td><a href="#">2.6e</a></td>
<td>53%</td>
<td></td>
</tr>
</tbody>
</table>

An attempt to utilize the same strategy to obtain indoles with esters in positions 4, 5 or 6 starting from meta- and ortho-bromobenzoic acid was unsuccessful as the desired products could only be isolated in extremely poor yields (Scheme 2.11). This coincides
with observations made for compounds with the same substitution pattern but a methoxy functionality instead of an ester residue.\(^{74}\)

\[
\begin{align*}
\text{Br-} & \text{-CO}_2\text{H} \rightarrow \text{Br-} & \text{-CO}_2\text{Me} & \rightarrow \text{Br-} & \text{-CO}_2\text{Me}, \text{94\%} \\
\text{Pd}_2(\text{dba})_3 (1 \text{ mol\%}), & \text{DPPF} (3 \text{ mol\%}), & \text{BocNHNH}_2, \text{Cs}_2\text{CO}_3, & \text{toluene, reflux, 24 h} \\
\text{Br} & \text{-CO}_2\text{Me} \rightarrow \text{Br-} & \text{-NHNH}_2 & \text{Boc} \rightarrow \text{Br-} & \text{-NHNH}_2 & \text{Boc} & \text{2.31, 94\%} & \text{2.32, 4\%} \\
\text{Pd}_2(\text{dba})_3 (1 \text{ mol\%}), & \text{DPPF} (3 \text{ mol\%}), & \text{BocNHNH}_2, \text{Cs}_2\text{CO}_3, & \text{toluene, reflux, 24 h} \\
\text{Br} & \text{-CO}_2\text{Me} \rightarrow \text{Br-} & \text{-NHNH}_2 & \text{Boc} \rightarrow \text{Br-} & \text{-NHNH}_2 & \text{Boc} & \text{2.31, 94\%} & \text{2.32, 4\%} \\
\end{align*}
\]

Scheme 2.11 Attempted synthesis of indoles with linkage in different positions.

An attempt to realize the synthesis of compounds with substitution in a position \textit{ortho} to the ester in order to disturb the planarity of the final compounds has been made starting from compound \textbf{2.34}, which was successfully converted into the methyl ester \textbf{2.35} and then into \textbf{2.36} in a moderate yield (Scheme 2.12). In contrast to the previously described \textit{N}-Boc arylhydrazide \textbf{2.30b}, \textbf{2.36} is not a symmetric compound and formed \textbf{2.37} and \textbf{2.38} in equally low yields during the Fischer indole synthesis, even though from a steric view \textbf{2.37} was to be expected as the main product.
2.3.1.3 Other Transition Metal Catalyzed Methods

In order to obtain derivatives of 2- and 3-methyl indole, a different approach to assemble the indole core structure needs to be applied. Common is the use of intramolecular transition metal catalyzed couplings between an olefin and a halogen on the aromatic ring. para-Aminobenzoic acid was methylated in a first step with excellent yield (Scheme 1.13). The product was then submitted to a selective iodination of the aromatic ring to form 2.40. This reaction has been described before utilizing solid ICl instead of a solution with a slightly higher yield. The following substitution to obtain the secondary amine 2.41 was also known in the literature and proceeded with low yield after purification. The low yield can partly be explained because according to the literature dialkylated amine is obtained as a side product, which was not difficult to separate from the desired product. In a last step, the intramolecular Heck reaction was carried out under conditions previously utilized for a similar reaction with a starting material missing the ester functionality. The only variation with the setup was a
substitute from $n$Bu$_4$NCl to $n$Bu$_4$NBr. Unfortunately, the desired product 2.42 could only be isolated in 16% yield, along with starting material and some free amine.

Scheme 2.13 Synthesis of 3-methyl indole analog 2.42.

2.3.1.4 Synthesis of N-Methyl Derivatives

Initial reactions to obtain the N-methyl indoles 2.43a and 2.43a with sodium hydride or potassium tert-butoxide in the presence of iodomethane at room temperature were unsuccessful. Fortunately, the treatment under microwave conditions lead to the desired products in good and excellent yields (Scheme 2.14).

Scheme 2.14 Synthesis of N-methyl indoles 2.43a and 2.43b.
2.3.1.5 Synthesis of Indoline Analogue

With indoline, the last compound derived directly from indole was synthesized by reduction of the indole derivative 2.9 under established conditions (Scheme 2.15). The indoline nitrogen of 2.44 has reactivity comparable to that of a monoalkylated aniline and needs to be protected to avoid side reactions in the transformations that will follow. This is accomplished by treatment with di-tert-butylcarbonate under standard conditions to obtain the protected compound 2.45 in very good yield.

Scheme 2.15 Synthesis of indoline 2.45.

2.3.2 Synthesis of Heterocyclic Derivatives

Six different heterocycles were proposed as analogs for indoles. Methyl 6-quinolinecarboxylate is a commercially available compound. Methylimidazopyridine 4.47 was successfully prepared in one step from the precursor methyl 6-aminonicotinate (4.46) following a procedure developed for the ethyl ester of 2.46 (Scheme 2.16).

Scheme 2.16 Synthesis of methylimidazopyridine 2.47.
Other planned analogs are commercially available in form of the carboxylic acid derivatives and could be converted into the desired methyl esters (Scheme 2.17). Unfortunately, the triazole compound 4.49 could only be obtained in a very low yield. In order to convert 4-amino-3-hydroxybenzoic acid into the methyl ester a large access of acid was necessary due to the functionalities around the aromatic ring. A conversion of 2.50 into 2.51 was not completely successful because even under microwave conditions with CDI, only a 1:1 mixture of starting material and product could be obtained, which were inseparable by chromatography.

Scheme 2.17 Synthesis of bicyclic indole analogs 2.48, 2.49 and 2.51.

2.3.2.1 Synthesis of Benzofurans

With the obvious similarity to indoles, benzofurans can be synthesized in a similar fashion. A straightforward method is shown in Scheme 1.18, which is based on a
nucleophilic substitution of a phenol on an α-haloketone followed by a Friedel-Crafts reaction and elimination to produce benzofurans.

\[
\begin{align*}
\text{Hal} & \quad \text{O} \\
\text{R}^1 & \quad \text{R}^2 \\
\text{R} & \quad \text{HO} \\
\text{HO} & \quad \text{O} \\
\text{R}^1 & \quad \text{R}^2 \\
\text{acid} & \quad \text{R}^1 \\
\end{align*}
\]

**Scheme 2.18** General synthesis of benzofurans.

The necessary α-bromo ketones were synthesized following a standard procedure treating a ketone with bromine to give moderate yields of the desired products after distillation (Scheme 2.19). As expected, the reaction with 2-butanone resulted in a mixture of the desired 3-bromobutane-2-one (2.53b) and its regioisomer 1-bromobutane-2-one (2.53c), which could unfortunately not be separated by distillation. This was however, not of concern because 3-chlorobutane-2-one is commercially available and a suitable substitute for 2.53b.

\[
\begin{align*}
\text{AcOH, Br}_2, \text{H}_2\text{O} & \quad \text{R} \quad \text{O} \\
\text{R} & \quad \text{AcOH, Br}_2, \text{H}_2\text{O} \\
\text{R}^1 & \quad \text{R}^2 \\
2.52a, \text{R} = \text{H} & \text{2.52b, } \text{R} = \text{Me} \\
2.52a, \text{R} = \text{H} & \text{2.52b, } \text{R} = \text{Me} \\
2.53a, 45\% & \text{2.53b, 54\%, regioisom. 3:1 with 2.53c} \\
\end{align*}
\]

**Scheme 2.19** Synthesis of α-bromo ketones.

Alkylation of the phenolic alcohols of 2.54 and 2.57 proceeded in good yields following conditions applied for similar reactions, but the following Friedel-Crafts reaction and elimination proved to be difficult (Scheme 2.20). The test cyclization of 2.55
resulted in the desired product in low yield, but a number of different standard conditions did not lead to a successful reaction of 2.58.

![Chemical structure](image)

Scheme 2.20 Attempted synthesis of 2.59.

2.3.3 Synthesis of Aniline Analogues

All analogs in this series were derived from methyl aminobenzoates 2.39 and 2.60 that were readily available after esterification with excellent yields (Scheme 2.21).

![Chemical structure](image)

Scheme 2.21 Synthesis of aniline precursors 2.39 and 2.60.

Four sets of analogs were synthesized from these two starting anilines. The amides were obtained under standard conditions utilizing either anhydrides or acid chlorides (Table 2.2). Similarly to this, sulfonamides result from the reaction with the
corresponding sulfonyl chlorides under basic conditions (Table 2.3).\textsuperscript{84} Urea analogues (Table 2.4) and thiourea derivatives (Scheme 2.22)\textsuperscript{84} were produced from standard conditions using isocyanates and thioisocyanates as reagents. In this case the reaction with cyclohexyl isocyanate did not lead to the desired product as a result of solubility issues (Table 2.4 entry 2). Similarly, no product was obtained for the reaction of 4.39 with phenyl thioisocyanate even though the reaction with 4.60 proceeded with a good yield.

**Table 2.2** Amide derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Conditions</th>
<th>R</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{85}</td>
<td>2.39</td>
<td>Ac\textsubscript{2}O, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>Me</td>
<td>2.61a, 89%</td>
</tr>
<tr>
<td>2</td>
<td>2.39</td>
<td>iPrCOCl, NE\textsubscript{t}\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>iPr</td>
<td>2.61b, 79%</td>
</tr>
<tr>
<td>3</td>
<td>2.39</td>
<td>PhCOCl, NE\textsubscript{t}\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>Ph</td>
<td>2.61c, 34%</td>
</tr>
<tr>
<td>4</td>
<td>2.60</td>
<td>Ac\textsubscript{2}O, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>Me</td>
<td>2.62a, 69%</td>
</tr>
<tr>
<td>5</td>
<td>2.60</td>
<td>iPrCOCl, NE\textsubscript{t}\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>iPr</td>
<td>2.62b, 98%</td>
</tr>
<tr>
<td>6</td>
<td>2.60</td>
<td>PhCOCl, NE\textsubscript{t}\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>Ph</td>
<td>2.62c, 70%</td>
</tr>
</tbody>
</table>
**Table 2.3** Sulfonamide derivatives.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>RCl</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>7&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2.39</td>
<td>MsCl</td>
<td>2.63a, 74%</td>
</tr>
<tr>
<td>8&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2.39</td>
<td>TsCl</td>
<td>2.63b, 25%</td>
</tr>
<tr>
<td>14&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2.60</td>
<td>MsCl</td>
<td>2.64a, 79%</td>
</tr>
<tr>
<td>15&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2.60</td>
<td>TsCl</td>
<td>2.64b, 76%</td>
</tr>
</tbody>
</table>

**Table 2.4** Urea derivatives.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Stating material</th>
<th>R</th>
<th>Product, yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;84&lt;/sup&gt;</td>
<td>2.39</td>
<td>Bn</td>
<td>2.65a, 74%</td>
</tr>
<tr>
<td>2</td>
<td>2.39</td>
<td>cyclohexyl</td>
<td>N.R.</td>
</tr>
<tr>
<td>3</td>
<td>2.39</td>
<td>furfuryl</td>
<td>2.65b, 86%</td>
</tr>
<tr>
<td>4</td>
<td>2.39</td>
<td>4OMe-Ph</td>
<td>2.65c, 88%</td>
</tr>
<tr>
<td>5</td>
<td>2.39</td>
<td>4Me-Ph</td>
<td>2.65d, 90%</td>
</tr>
<tr>
<td>6</td>
<td>2.60</td>
<td>Bn</td>
<td>2.66, 86%</td>
</tr>
</tbody>
</table>
2.3.4 Simple Aromatic Analogues

Three hydroxynaphthoic acid derivatives were obtained from commercial sources and transformed into the corresponding methoxymethyl esters 2.69a-2.69c in very good yield using dimethylsulfate in a procedure described for the reaction to produce 2.69b (Scheme 2.23). 87

Scheme 2.22 Thiourea derivatives.

Scheme 2.23 Synthesis of naphthoic acid derivatives 2.69a-2.69c.

2.4 Hydrazides

Hydrazides can be obtained from two general methods, utilizing either a carboxylic acid or an ester. In the case of an acid precursor, the formation of a mixed anhydride intermediate is necessary, which can then directly be reacted with hydrazine. This was the initial method of choice because the esterification step would be avoided. The reaction was tested on benzoic acid and 5-indolecarboxylic acid following a standard
procedure for aromatic carboxylic acids (Scheme 2.24). The mixed anhydrides were formed with freshly distilled ethyl chloroformate and then subjected to hydrazine monohydrate. This reaction produced benzohydrazide 2.7 in a moderate yield but was unsuccessful in the synthesis of the indole analog 2.10.

Scheme 2.24 Synthesis of hydrazides from carboxylic acids.

Resulting from the process of assembly, the indole structures (Chapter 1.1) were obtained as esters. Therefore the transformation into hydrazides was studied on ethyl benzoate and methyl 1H-indole-5-carboxylate using conditions previously applied for other aromatic compounds (Scheme 2.25). The initial test reaction of refluxing the starting material 2.6 in dry ethanol with anhydrous hydrazine did not go to completion and by thin layer chromatography (TLC) starting material could still be observed after 20h. However, treating both starting esters with an excess of hydrazine monohydrate (1.2-2 eq) at 120 °C gave very good results. Upon heating, the starting material melts and the reaction occurs. After completion and cooling, the product solidifies and can be collected as clean material after rinsing with MeOH.
Scheme 2.25 Hydrazide synthesis from esters.

The established conditions were successfully applied to most indole, heterocyclic and aromatic derivatives as shown in Tables 2.5 and 2.6.

It is noteworthy that the success of the reaction is very dependent on the reaction temperature. The trend observed for compound 2.9 was an increase of the reaction temperature from 120 °C to 140 °C led to a significant decrease of the yield (Table 2.5, entries 1-2). In addition, it is very important that a temperature is chosen at which the starting material melts together with the hydrazine. It is believed this is a reason why the reaction of ethyl 2,3-dimethyl-1H-indole-5-carboxylate (2.6a) did not yield any product, but the corresponding methyl ester 2.6b lead to the desired product in good yield (entries 3-4). According to these observations, the reaction temperatures were adjusted at the beginning of each reaction. Unfortunately, the reaction with 2.42 led to a complex mixture and no clean product could be isolated.
Table 2.5 Synthesis of hydrazides from indole derivatives.

![Chemical Reaction Diagram]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Temperature</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![2.9]</td>
<td>120 °C</td>
<td><strong>2.10</strong>, quant.</td>
</tr>
<tr>
<td>2</td>
<td>![2.6a]</td>
<td>140 °C</td>
<td><strong>2.10</strong>, 40%</td>
</tr>
<tr>
<td>3</td>
<td>![2.6a]</td>
<td>120 °C</td>
<td><strong>2.1</strong>, N.R.</td>
</tr>
<tr>
<td>4</td>
<td>![2.6b]</td>
<td>120 °C</td>
<td><strong>2.1</strong>, 81%</td>
</tr>
<tr>
<td>5</td>
<td>![2.43a]</td>
<td>120 °C</td>
<td><strong>2.70a</strong>, 90%</td>
</tr>
<tr>
<td>6</td>
<td>![2.43b]</td>
<td>150 °C</td>
<td><strong>2.70b</strong>, 57%</td>
</tr>
<tr>
<td>7</td>
<td>![2.6c]</td>
<td>140 °C</td>
<td><strong>2.70c</strong>, quant.</td>
</tr>
<tr>
<td>8</td>
<td>![2.6d]</td>
<td>140 °C</td>
<td><strong>2.70d</strong>, 11%</td>
</tr>
<tr>
<td>9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>![2.6e]</td>
<td>140 °C</td>
<td><strong>2.70e</strong>, quant.</td>
</tr>
<tr>
<td>10</td>
<td>![2.37]</td>
<td>120 °C</td>
<td><strong>2.70f</strong>, 97%</td>
</tr>
</tbody>
</table>
The conditions applied for the indole derivatives proved suitable for other heterocyclic and aromatic compounds as well, leading to the desired products in moderate to good yields (Table 2.5). Only the triazole compound 2.49, which already proved difficult during the esterification lead to a complex mixture and was therefore dropped as potential analog (entry 3). Aside from this, it was observed that the reactions of the quinoline and naphthalene derivatives (entries 4-6) resulted in significantly lower yields than other compounds. As mentioned before, the solidified product was washed with methanol to remove any residual hydrazine. It is believed that all three compounds have better solubility in methanol compared to the other compounds and some material is lost during the workup. Indeed, after evaporation of the methanol solution product could be detected by $^1$H-NMR spectroscopy, which was not purified.
Table 2.6 Synthesis of hydrazides from heterocyclic and aromatic derivatives.

\[
\text{Ar} = \text{N} = \text{N} \quad \text{CO}_{2}\text{Me} \quad \text{O} \quad \text{Me}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Temperature</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="Image" alt="2.47" /></td>
<td>100 °C</td>
<td>2.71a, 78%</td>
</tr>
<tr>
<td>2</td>
<td><img src="Image" alt="2.48" /></td>
<td>120 °C</td>
<td>2.71b, 76%</td>
</tr>
<tr>
<td>3</td>
<td><img src="Image" alt="2.49" /></td>
<td>120 °C</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><img src="Image" alt="2.61a" /></td>
<td>120 °C</td>
<td>2.71c, 44%</td>
</tr>
<tr>
<td>5</td>
<td><img src="Image" alt="2.61b" /></td>
<td>100 °C</td>
<td>2.71d, 43-55%</td>
</tr>
<tr>
<td>6</td>
<td><img src="Image" alt="2.61c" /></td>
<td>100 °C</td>
<td>2.71e, 28%</td>
</tr>
<tr>
<td>7</td>
<td><img src="Image" alt="2.61d" /></td>
<td>100 °C</td>
<td>2.71f, 90%</td>
</tr>
</tbody>
</table>

In contrary to the compounds above, all analogs derived from aniline as well as the indoline compound could not be transformed into hydrazides using the method described above. Table 2.7 lists the attempts to produce hydrazides starting from amide 2.61a and sulfonamide 2.63a. Treating 2.61a under the previously establish lead to a complex mixture after only 2 h (entry 1). Another possibility was described on the
reaction of the ethyl ester of 2.61a and utilized microwave conditions on an ethanol solution. Unfortunately, these conditions did not lead to any conversion of the starting material, even after extension of the reaction time from 25 min to 5 h (entries 2-3). A further modification of the conditions was described for methyl 4-aminobenzoate (2.39), where the reaction was carried out in hydrazine monohydrate as a solvent. These conditions were successfully applied to 2.61a and produced the desired product as a precipitate in good yield (entry 4). Unfortunately, transfer of those conditions to the sulfonamide 2.63a was not successful (entry 6). The desired product might have formed, but it was not possible to isolate it from the hydrazine solution because it appeared to be of high solubility. In view of these results, sulfonamides were not pursued any further.

Table 2.7 Method development for hydrazides from aniline derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>SM</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.61a</td>
<td>N₂H₄·H₂O, neat, 135°C, 2h</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>2.61a</td>
<td>N₂H₄·H₂O, EtOH, MW 140°C, 25min</td>
<td>N.R.</td>
</tr>
<tr>
<td>3</td>
<td>2.61a</td>
<td>N₂H₄·H₂O, EtOH, MW 140°C, 5h</td>
<td>N.R.</td>
</tr>
<tr>
<td>4</td>
<td>2.61a</td>
<td>N₂H₄·H₂O, neat, 0.34M, MW 90°C, 15min</td>
<td>77%</td>
</tr>
<tr>
<td>5</td>
<td>2.63a</td>
<td>N₂H₄·H₂O, EtOH, MW 140°C, 12h</td>
<td>N.R.</td>
</tr>
<tr>
<td>6</td>
<td>2.63a</td>
<td>N₂H₄·H₂O, neat, 0.34M, MW 90°C, 15min</td>
<td>-</td>
</tr>
</tbody>
</table>
The procedure suitable for aniline derivatives was applied to the remaining compounds with overall good yields (Table 2.8). The only compound that was not obtained was the result of the reaction of thiourea 2.68, which can likely be attributed to enhanced solubility in hydrazine (entry 12).

Table 2.8 Hydrazides of analogs derived from aniline.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Temperature</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \text{Ar} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72a, 77%</td>
</tr>
<tr>
<td>2</td>
<td>( \text{Ar} \cdot \text{H} \cdot \text{N} \cdot \text{Me} \cdot \text{H} \cdot \text{N} \cdot \text{Ph} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72b, 70%</td>
</tr>
<tr>
<td>3</td>
<td>( \text{Ph} \cdot \text{N} \cdot \text{Me} \cdot \text{H} \cdot \text{N} \cdot \text{Ph} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72c, 27%</td>
</tr>
<tr>
<td>4</td>
<td>( \text{Ph} \cdot \text{N} \cdot \text{Me} \cdot \text{H} \cdot \text{N} \cdot \text{Ph} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72d, 53%</td>
</tr>
<tr>
<td>5</td>
<td>( \text{Ph} \cdot \text{N} \cdot \text{Me} \cdot \text{H} \cdot \text{N} \cdot \text{Ph} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72e, 92%</td>
</tr>
<tr>
<td>6</td>
<td>( \text{Ph} \cdot \text{N} \cdot \text{Me} \cdot \text{H} \cdot \text{N} \cdot \text{Ph} \cdot \text{OMe} )</td>
<td>90 °C</td>
<td>2.72f, 77%</td>
</tr>
</tbody>
</table>
Table 2.8 Continued.

<table>
<thead>
<tr>
<th></th>
<th>Chemical Structure</th>
<th>Temperature °C</th>
<th>Product Code</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td><img src="image" alt="2.65a" /></td>
<td>90</td>
<td>2.72g</td>
<td>82%</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="2.65b" /></td>
<td>100</td>
<td>2.72h</td>
<td>29%</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="2.65c" /></td>
<td>100</td>
<td>2.72i</td>
<td>70%</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="2.65d" /></td>
<td>100</td>
<td>2.72j</td>
<td>87%</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="2.66" /></td>
<td>100</td>
<td>2.72k</td>
<td>63%</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="2.68" /></td>
<td>90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td><img src="image" alt="2.45" /></td>
<td>100</td>
<td>2.72l</td>
<td>53%</td>
</tr>
</tbody>
</table>

2.5 Thiols

The transformation of hydrazides into oxadiazoles was accomplished by reaction with carbon disulfide under basic conditions, subsequent evaporation of the solvent and treatment with 10% aqueous HCl to precipitate the product. After washing with water,
the oxadiazoles were obtained as clean products in good to very good yields. The conditions were initial tested on benzoyl hydrazide 2.7 and then utilized for all other compounds (Scheme 2.26, Tables 2.9-2.11).

![Scheme 2.26 Synthesis of SKB-151.](image)

The main problem with almost all compounds of this type appears to be the lack of stability during determination of purity by LCMS. This is attributed to a ring opening-closing-process that has a negative effect on the peak shape and leads to a tailing in the chromatogram that can almost be compared to a smear. Nonetheless, the products of this reaction were observed to be very clean by \(^1\)H-NMR spectroscopy.

From all reactions, only the indoles 2.70d and 2.70f (Table 2.9. entries 6, 8), as well as naphthaline derivative 2.71d (Table 2.10, entry 4) could not be converted into the desired oxadiazoles. After precipitation, a dark yellow to brown gummy substance was obtained from these reactions that did not appear to be clean product and could not be purified. In the case of 2.70d, the ring strain of the five membered ring might be responsible for this outcome.
Table 2.9 Synthesis oxadiazoles from indole derivatives.

![Chemical reaction diagram]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Time</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>2 d</td>
<td>SKB-111, 80%</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>2 d</td>
<td>SKB-104, quant.</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>20 h</td>
<td>SKB-145, 82%</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>2 d</td>
<td>SKB-132, 94%</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>20 h</td>
<td>SKB-143, 80%</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Chemical structure" /></td>
<td>20 h</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7" alt="Chemical structure" /></td>
<td>20 h</td>
<td>SKB-142, quant.</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8" alt="Chemical structure" /></td>
<td>20 h</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td><img src="image9" alt="Chemical structure" /></td>
<td>20 h</td>
<td>2.73, 78%</td>
</tr>
</tbody>
</table>
Table 2.10 Synthesis oxadiazoles from heterocyclic and aromatic derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Time</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Image]</td>
<td>20 h</td>
<td><strong>SKB-144, 56%</strong></td>
</tr>
<tr>
<td>2</td>
<td>![Image]</td>
<td>20 h</td>
<td><strong>SKB-156, 98%</strong></td>
</tr>
<tr>
<td>3</td>
<td>![Image]</td>
<td>20 h</td>
<td><strong>SKB-157, 97%</strong></td>
</tr>
<tr>
<td>4</td>
<td>![Image]</td>
<td>20 h</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>![Image]</td>
<td>20 h</td>
<td><strong>SKB-158, 82%</strong></td>
</tr>
<tr>
<td>6</td>
<td>![Image]</td>
<td>20 h</td>
<td><strong>SKB-159, 85%</strong></td>
</tr>
</tbody>
</table>

Table 2.11 Synthesis oxadiazoles from aniline derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Time</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Image]</td>
<td>3 h</td>
<td><strong>SKB-107, 90%</strong></td>
</tr>
<tr>
<td>2</td>
<td>![Image]</td>
<td>3 h</td>
<td><strong>SKB-152, 62%</strong></td>
</tr>
<tr>
<td></td>
<td>Formula</td>
<td>Reaction Time</td>
<td>Product</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-152</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-153</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-117</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-155</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-154</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>3 h</td>
<td>SKB-127</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20 h</td>
<td>SKB-161</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20 h</td>
<td>SKB-162</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20 h</td>
<td>SKB-163</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20 h</td>
<td>SKB-160</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>20 h</td>
<td>2.75</td>
</tr>
</tbody>
</table>
2.6 Br-Alkyls

The synthesis of all 2-bromo amides (Scheme 2.27) was carried out in analogy to the known synthesis for 2.76a and resulted in the desired products in good yields. During the reaction, commercially available 2-bromoacetyl bromide was treated with the desired secondary amines under basic conditions.

![Scheme 2.27 Synthesis of 2-bromoamides 2.76a-2.76d.]

2.7 Final Compounds

The final step to obtain a small library of compounds for antiviral testing was an alkylation reaction between the oxadiazoles and the 2-bromo amides. An effort was made to obtain A3 in larger quantities for animal testing and to introduce all possible amide substitutions on this derivative. For all other compound the main focus lay on the derivatives including the pyrrolidine amide and other amides were introduced only depending on the amount of oxadiazole present.

Initial test reactions to obtain the final products were conducted on the commercially available oxadiazole 2.77 (Scheme 2.28). The reaction in dry acetone under basic conditions and reflux lead to the final products in very good yields upon separation.
from potassium carbonate. The only exception was SKB-103, which had to be purified by chromatography.

Scheme 2.28 Synthesis of analogs SKB-100 - SKB-103.

Unfortunately, submitting SKB-111 and SKB-104 to these conditions lead to complex reaction mixtures that could not be separated. Reactions at room temperature resulted in better results and the desired products could be observed by ¹H-NMR spectroscopy. Nonetheless, attempts to purify the final compounds were futile, as they appeared to be instable on silica gel.

Further modification of the conditions revealed that the alkylation does take place in the absence of a base. Together with the poor solubility of the oxadiazoles in acetone, this resulted in extended reaction times of two days. During that time the reaction progress was visible as it takes approximately one day for all the starting material to dissolve. During the second day, the product precipitated from the reaction and was obtained as a clean compound from filtration. These conditions were successfully applied to the indole derivatives (Table 2.12). Because the products were isolated as precipitates, the yields were highly dependent on the solubility of the compounds in acetone.
Table 2.12 Indole derivatives of final compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Bromide</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td><img src="image1" alt="Structure" /></td>
<td>A3, 80%</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure" /></td>
<td><img src="image3" alt="Structure" /></td>
<td>SKB-105, 49%</td>
</tr>
<tr>
<td>3</td>
<td><img src="image4" alt="Structure" /></td>
<td><img src="image5" alt="Structure" /></td>
<td>A3-5, 65%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td><img src="image6" alt="Structure" /></td>
<td>SKB-106, 34%</td>
</tr>
<tr>
<td>5</td>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td>SKB-133, 34%</td>
</tr>
<tr>
<td>6</td>
<td><img src="image9" alt="Structure" /></td>
<td><img src="image10" alt="Structure" /></td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><img src="image11" alt="Structure" /></td>
<td><img src="image12" alt="Structure" /></td>
<td>SKB-150, 22%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
<td>SKB-139, 48%</td>
</tr>
<tr>
<td>9</td>
<td><img src="image15" alt="Structure" /></td>
<td><img src="image16" alt="Structure" /></td>
<td>2.78 + 2.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> after extensive purification
In the cases of **SKB-145** and **2.73**, no clean product could be obtained (Table 1.12, entries 6 and 9). Despite the lability of the indole derivatives on silica gel, chromatography was utilized to separate out the majority of impurities from both compounds, followed by a recrystallization attempt. For chloro-derivative **2.73**, only a mixture of starting material and product could be obtained and any further separation efforts were unfruitful. The results for the purification of **N**-methylindole derivative **SKB-145** were more ambiguous. A very clean compound was obtained, that by $^1$H-NMR spectroscopy showed one extra peak compared with the expected spectrum. The measurement of 2D spectras including COSY, HMQC and HMBC lead to the assumption that an extra methyl group in position 3 of the indole was introduced. Additional information from a LCMS chromatogram also showed a single clean peak, but unfortunately, the corresponding mass spectrum did not show the expected mass for a methylated product. Further studies of this compound were not conducted due to the small amount in which the compound could be isolated.

The heterocyclic compounds were initially reacted under the base-free conditions mentioned above but it was soon realized, that these conditions were only successful on compounds similar to the indole derivatives (Table 2.12). Thus, indazole **SKB-112** was the only compound successfully isolated from those conditions (Table 2.13, entry 3). Compound **SKB-165** was obtained from the initial reaction conditions for aromatic compound utilizing acetone and $\text{K}_2\text{CO}_3$ as base (entry 5), while all other compounds in Table 2.13 (entries 2 and 6-8) were the result of a procedure developed for the aniline derivatives that is described in the following.
Table 2.13 Heterocyclic and aromatic derivatives of final compounds.

<table>
<thead>
<tr>
<th>entry</th>
<th>Starting material</th>
<th>Bromide</th>
<th>Method</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SKB-144</td>
<td>Br(_\text{CON}_{2})</td>
<td>A</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>SKB-112</td>
<td>Br(_\text{CON}_{2})</td>
<td>B</td>
<td>SKB-140, 36%</td>
</tr>
<tr>
<td>3</td>
<td>SKB-156</td>
<td>Br(_\text{CON}_{2})</td>
<td>A</td>
<td>SKB-112, 66%</td>
</tr>
<tr>
<td>4</td>
<td>SKB-157</td>
<td>Br(_\text{CON}_{2})</td>
<td>A</td>
<td>N.R.</td>
</tr>
<tr>
<td>5</td>
<td>SKB-158</td>
<td>Br(_\text{CON}_{2})</td>
<td>C</td>
<td>SKB-165, 76%</td>
</tr>
<tr>
<td>6</td>
<td>SKB-159</td>
<td>Br(_\text{CON}_{2})</td>
<td>B</td>
<td>SKB-122, 10%</td>
</tr>
<tr>
<td>7</td>
<td>SKB-158</td>
<td>Br(_\text{CON}_{2})</td>
<td>B</td>
<td>SKB-124, 67%</td>
</tr>
<tr>
<td>8</td>
<td>SKB-159</td>
<td>Br(_\text{CON}_{2})</td>
<td>B</td>
<td>SKB-126, 61%</td>
</tr>
</tbody>
</table>

A) acetone, r.t., 2 d; B) NEt₃, DMF, 2 d; C) acetone, K₂CO₃, reflux, 1 h.

As mentioned above, aniline derivatives required a new method of alkylation. The main problem with those compounds was again a solubility issue. As a result, reactions in
acetone, with or without addition of base and heat, did not occur. Introducing DMF as solvent resulted in completely dissolved starting materials, but only the addition of NEt₃ led to the formation of clean products (Table 2.14). The difficulties associated with these conditions are the removal of DMF as well as the removal of the NEt₃·HCl side product. In a few cases, the products were only marginally soluble in DMF and clean products were obtained after filtration of the precipitate, followed by a water wash. All other products were extracted from a CH₂Cl₂/water mixture and then recrystallized from toluene.

Following these conditions most products were isolated in moderate and good yields. Individual compounds did not crystallize from toluene and due to the small scale of the reactions those products were lost (Table 2.14, entries 4, 22, 24, 34). Aside from this, the reactions of SKB-152 and SKB-155, which both contain butyramide side chains, did not lead to a formation of the desired products in a reasonable reaction time (entries 5-8 and 17-20). Because this issue appeared to be strongly related to the structural motif of these compounds no further efforts were made to find conditions suitable for these cases.

Because indoline derivative 2.79 (entry 35) still contained a protecting group, the final product SKB-141 was obtained after deprotection with TFA in a moderate yield.
Table 2.14 Aniline derivatives of final compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Bromide</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>![image of reaction]</td>
<td>SKB-108, 41%</td>
</tr>
<tr>
<td>2</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-109, 73%</td>
</tr>
<tr>
<td>3</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-110, 70%</td>
</tr>
<tr>
<td>4</td>
<td>![image of reaction]</td>
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<td>![image of reaction]</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-113, 70%</td>
</tr>
<tr>
<td>10</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-114, 77%</td>
</tr>
<tr>
<td>11</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-115, 81%</td>
</tr>
<tr>
<td>12</td>
<td>![image of reaction]</td>
<td>![image of reaction]</td>
<td>SKB-116, 80%</td>
</tr>
</tbody>
</table>
Table 2.14 Continued.

<p>| 13  | <img src="image" alt="SKB-117" /> | SKB-118, 50% |
| 14  | <img src="image" alt="SKB-117" /> | SKB-119, 25% |
| 15  | <img src="image" alt="SKB-117" /> | SKB-120, 84% |
| 16  | <img src="image" alt="SKB-117" /> | SKB-121, 81% |
| 17  | <img src="image" alt="SKB-117" /> | - |
| 18  | <img src="image" alt="SKB-117" /> | - |
| 19  | <img src="image" alt="SKB-117" /> | - |
| 20  | <img src="image" alt="SKB-117" /> | - |
| 21  | <img src="image" alt="SKB-117" /> | SKB-123, 73% |
| 22  | <img src="image" alt="SKB-117" /> | - |
| 23  | <img src="image" alt="SKB-117" /> | SKB-125, 56% |
| 24  | <img src="image" alt="SKB-117" /> | - |</p>
<table>
<thead>
<tr>
<th>Table 2.14 Continued.</th>
</tr>
</thead>
<tbody>
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<td>25</td>
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<td>26</td>
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<td>27</td>
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</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>32</td>
</tr>
<tr>
<td>33</td>
</tr>
<tr>
<td>34</td>
</tr>
<tr>
<td>35</td>
</tr>
</tbody>
</table>

*Note: after TFA, CH₂Cl₂*
As a last attempt on an efficient method to synthesize a small compound library, the $N$-methylation of the final products was studied under the microwave conditions that were already applied with initial ester derivatives. Unfortunately, this was not successful and resulted in a complex mixture (Scheme 2.29).

![Scheme 2.29 Attempted $N$-methylation of final compounds.](image)

2.8 Antiviral Activity

Following the synthesis of final compounds, they were tested for activity against viruses (Table 2.15). The aim was the identification of compounds with high virus reduction, while maintaining high cell viability. This is especially important because the proposed mechanism of action describes the inhibition of dihydroorotate dehydrogenase (DHODH), a host cell enzyme.
Table 2.15 Activity of the final compounds.\(^95\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Max Conc. (µM)</th>
<th>Max. Virus Reduction (%)</th>
<th>% Cell viability at max conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>2</td>
<td>99.9</td>
<td></td>
</tr>
<tr>
<td>A3-5</td>
<td>2</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>SKB-100</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-101</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-102</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-103</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-105</td>
<td>2</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>SKB-106</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-108</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-109</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-110</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-112</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-113</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-114</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-115</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-116</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-118</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-119</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-120</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>SKB-121</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>SKB-122</td>
<td>10</td>
<td>57.4</td>
<td>97.4</td>
</tr>
<tr>
<td>SKB-123</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-124</td>
<td>10</td>
<td>0.0</td>
<td>110.4</td>
</tr>
<tr>
<td>SKB-125</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-126</td>
<td>10</td>
<td>92.5</td>
<td>107.6</td>
</tr>
<tr>
<td>SKB-128</td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>SKB-129</td>
<td>10</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>SKB-130</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>SKB-131</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-133</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SKB-134</td>
<td>10</td>
<td>99.2</td>
<td>103.7</td>
</tr>
<tr>
<td>SKB-135</td>
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<td>80.9</td>
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<td>SKB-136</td>
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<td>96.5</td>
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<td>SKB-137</td>
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<td>106.7</td>
</tr>
<tr>
<td>SKB-139</td>
<td>10</td>
<td>69.7</td>
<td></td>
</tr>
<tr>
<td>SKB-140</td>
<td>10</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>SKB-141</td>
<td>10</td>
<td>26.3</td>
<td>102.5</td>
</tr>
<tr>
<td>SKB-150</td>
<td>10</td>
<td>99.9</td>
<td>103.3</td>
</tr>
<tr>
<td>SKB-165</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
In addition to these initial studies, promising compounds were submitted to further testing including inhibitory concentrations and DHODH inhibition (Table 2.16). It can be said, that imidazolidinone analogues A-3, A3-5 and SKB-105, SKB-150 prove to be the most active among the new compounds. However, SKB-126, SKB-134 and SKB-136 reduce the virus for more than 90% at 10µM concentration.

As described for the proposed mechanism of action, the inhibition of human DHODH could be shown. However, a direct correlation between maximum virus reduction and maximum inhibition of human DHODH was not found. This becomes very clear when comparing the compounds SKB-134 and SKB-135: SKB-134 achieves a maximum virus reduction of 99.2% at 10µM concentration; but has a maximum human DHODH inhibition of 19% at 100µM concentration. This means that SKB-134 is able to almost completely reduce the virus while only inhibition a small amount of DHODH. Compared to this, SKB-135 shows a lower maximum virus reduction of 80.9% at 10µM while inhibiting 82% of the human DHODH at 100µM. These significant discrepancies lead to the conclusion that a second mechanism aside from the proposed one must be present.

Nonetheless, the structures of the most active derivatives have been identified (Figure 2.7) and will be compared to their inactive derivatives in order to identify possible structural changes that could enhance the activity (Figures 2.8–2.14).
<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>IC90 (µM)</th>
<th>Max Conc. (µM)</th>
<th>Max. Virus Reduction (%)</th>
<th>% Cell viability at max conc</th>
<th>Max conc for DHODH inhibition (µM)</th>
<th>Max % inhibition of human DHODH</th>
<th>Max % inhibition of mouse DHODH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>0.178</td>
<td>0.8</td>
<td>2</td>
<td>99.9</td>
<td>5</td>
<td>69</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A3-5</td>
<td>0.5</td>
<td></td>
<td>2</td>
<td>97</td>
<td>5</td>
<td>70</td>
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<tr>
<td>SKB-105</td>
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<td>0.6</td>
<td>2</td>
<td>99</td>
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<td></td>
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<td>12</td>
<td>5</td>
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<td>0</td>
<td></td>
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<tr>
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<td>16</td>
<td>5</td>
<td>0</td>
<td>3</td>
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<td>57.4</td>
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<td>50</td>
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<td>92.5</td>
<td>107.6</td>
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<td>11</td>
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</tr>
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<td>10</td>
<td>99.2</td>
<td>103.7</td>
<td>100</td>
<td>19</td>
<td>5</td>
</tr>
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<td></td>
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<td>80.9</td>
<td>119.6</td>
<td>100</td>
<td>82</td>
<td>62</td>
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<tr>
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<td>TBD</td>
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<td>99.7</td>
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<td>64</td>
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<td>80.4</td>
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<td>100</td>
<td>57</td>
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</tr>
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<td>69.7</td>
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<td>10</td>
<td>26.3</td>
<td>102.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKB-150</td>
<td>TBD</td>
<td>TBD</td>
<td>10</td>
<td>99.9</td>
<td>103.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.7 gives an overview over the derivatives with the highest antiviral activity. Aside from the previously identified indole analogues, naphthalene derivative SKB-126 as well as ureas SKB-134 and SKB-136 showed promise.

![Chemical structures](image)

**Figure 2.7** New and old lead compounds.

Comparing the four A3 analogues evaluating the amide side chain, it can be said that as long as the amide is compact only small differences in activity can be observed (Figure 2.8). However, the introduction of or the larger and more flexible bis-(2-
methoxyethyl)acetamide lead to a total loss of activity hinting that steric hindrance and flexibility on that side of the molecule are disadvantageous.

![A3 active, 99.9% max. virus reduction](image1)

![A3-5 active, 97% max. virus reduction](image2)

![SKB-105, 99% max. virus reduction](image3)

![SKB-106, 0% max. virus reduction](image4)

**Figure 2.8 A3 derivatives.**

Unfortunately, difficulties in the synthesis of the attempted indole analogues led to a smaller number of final compounds than anticipated (Figure 2.9). Of those compounds, the trimethylindole **SKB-133** showed no activity, indicating that substitution on the indole nitrogen has adverse effects. Similarly, the introduction of one additional carbon in **SKB-139** had a negative effect, resulting in a drastic increase of activity. This result from such slight increases in steric hindrance indicates that **A3** must have a good fit to the binding pocket. Therefore, it is very surprising that **SKB-150** containing a much larger cyclohexene group showed very high activity. A possible explanation might be the rigidity of the cyclohexene group compared to the ethyl group of **SKB-139**, which can rotate freely.
The low activity of **SKB-141** was expected because of the total lack of 2-/3-substitution around the indole ring. Nonetheless, showing slight activity makes further exploration of this type of compound reasonable, testing the effect of 2-,3-dimethyl indolines and cyclohexyl derivatives.

![Chemical structures](image)

**Figure 2.9** Other indole derivatives.

A shift to heterocyclic compounds with structural similarities to indole did also not lead to good activity indicating that electronic changes have a large impact on the results (Figure 2.10).

![Chemical structures](image)

**Figure 2.10** Activity of heterocyclic derivatives.
The outcome of the studies with naphthalene derivatives is very interesting (Figure 2.11). While SKB-122 showed moderate antiviral activity, the comparison between SKB-124 and SKB-126 revealed that the influence of the amide group has large significance and does not always follow the same patterns as it did for the A3-derivatives. In this case, the morpholine amide SKB-126 showed high activity while pyrrolidine amid SKB-124 was inactive.

Figure 2.11 Activity of naphthalene compounds.

Initially, only urea derivatives SKB-128 - SKB-131 were synthesized and submitted for testing (Figure 2.12). The trend in activity for these compounds follows the same pattern as observed in A3 analogues indicating that smaller amide groups are favorable. As a result of the moderate activity of SKB-128 and SKB-129 the structural variety of this type of compounds was explored, showing a large increase in activity for the change in the substitution pattern from SKB-128 to SKB-134. In addition, more urea analogues were prepared leading to SKB-134 with high activity. Again, a switch to a
different amide in SKB-136 led to a slight decrease in activity; and a switch from the para-methylbenzyl substitution (SKB-134, Figure 2.13) to a para-methoxybenzyl group (SKB-135) resulted in a complete loss of activity indicating a steric issue.

![Chemical structures of SKB-128 to SKB-138 with activity percentages.](Figure 2.12 Activity of urea derivatives.)
Unfortunately neither the test substrates SKB-100 – SKB-103 with chlorophenyl substitution (Figure 2.14), nor the aniline amides (Figure 2.15 and 2.16) showed any significant activity. In the case of SKB-100 – SKB-103 this was not surprising, because the preliminary results had shown that a compounds with plain phenyl substitutions were not very promising. However, the fact that the above described urea analogues showed good activity but the amides did not was somewhat surprising. This fact leads to the assumption that the longer linkage between the two aromatic systems in the active urea compounds extends to an additional binding site, while a shorter linkage for the SKB-113 and SKB-123 is not sufficient and results in steric hindrance on the binding site.

Figure 2.14 Activity of chlorophenyl compounds.
Figure 2.15 Activity of para-substituted aniline amides.
2.9 Conclusion

The aim of this project was the synthesis and identification of $A_3$ analogues with good antiviral activity. This goal was accomplished with the isolation of 40 final compounds, including indoles, other heterocycles and aromatic derivatives. Of these compounds, four examples showed activity against viruses comparable to that of $A_3$ (Figure 2.17) and another five compounds were moderately active. 2,3-demethyl indoles remain the most promising structures, but naphthalene and urea derivatives were also shown to lead to significant virus reduction.
The synthesis of indole analogues was especially challenging because these initial 2,3-disubstituted indoles prove difficult to obtain and furthermore did the low stability of indoles in general lead to problems during the synthesis. The replacement of the indole structure with heterocycles of similar steric and electronic properties was synthetically successful but resulted in final compounds with no antiviral activity.

Nonetheless, new structural elements with good activity were found in compounds SKB-126, SKB-134 and SKB-136, which contain naphthalene and urea.
structures. Especially the urea derivatives are promising for further research because the synthetic approach to these compounds makes a wide variety of substitutional changes accessible.
CHAPTER 3: PEPTIDOMIMETICS

3.1 Introduction

Peptides carry out a large number of functions in living organisms; a few examples include ligands of enzymes, hormones and neurotransmitters. This makes peptides very good targets for drug discovery. Unfortunately, a number of disadvantages are connected to peptide drugs resulting in poor pharmacokinetic properties: peptides tend to undergo proteolysis, they are easily excreted and they are poorly transported within a living organism.

The development of compounds that carry out the functions of peptides, but have better pharmacokinetic properties is therefore a main goal in drug discovery. These compounds are called peptidomimetics, because they mimic the function of a peptide. Conventions about classification and naming of groups have not been made to date, but four classes of peptidomimetics can be defined by the way they mimic a peptide.96

Type 1, also called peptide backbone mimetics, include all compounds that are closely related to actual peptides. Typically, these compounds have similar side chains compared to the original peptide, but modifications in the peptide bond is introduced to enhance stability and rigidity (Figure 3.1). As this type is of main interest in the presented research, a closer evaluation of those modifications will follow in chapter 3.2.
Small molecules that are non-peptides but still carry out the same function as a peptide are combined in type 2 mimetics, or functional mimetics. A prominent example of this class is the alkaloid morphine (Figure 3.2).\(^{98}\) It binds to the \(\mu\)-, \(\kappa\)- and \(\delta\)-opioid receptors as an agonist. The natural ligand for the delta receptor is the pentapeptide enkephalin. Comparison of both structures shows that morphine has no similarity with a peptide, but it contains a phenolic residue that is responsible for binding to the receptor. Interestingly, the structural similar naltrindole binds to the \(\delta\)-opioid receptor with high selectivity and as an antagonist.\(^{99}\) In addition to the phenol subunit, this compound also contains an indole residue, which is the key to high selectivity to the delta receptor as X-ray structures showed.
Figure 3.2 Example type 2 peptidomimetic.

Type 3 peptidomimetics are also called topographical mimetics because they imitate only the binding sites of a peptide. Especially for larger peptides with secondary and tertiary structures, it is favorable to reduce the overall complexity and molecular weight by organizing important functional groups around a new, and typically non-peptidic, scaffold. The peptide hormone samostatin for example is involved in regulation of the endocrine system, and has affects on neurotransmission as well as cell proliferation (Figure 3.3). Unfortunately, this wide variety of functions is connected to unspecific binding and results in undesired side effects. In addition, it has a very short half-life in vivo. Sandostatin is a FDA approved mimic of somatostatin, which inhibits growth hormone, insulin and glucagon. In addition to higher inhibition rate, it also has a longer half-life; but poor oral availability remains due to the peptide backbone. While maintaining the important functional groups, the amino acid scaffold could be reduced
from 14 amino acids in somatostatin to 8 amino acids in sandostatin. Comparison of these two compounds to 3.1 illustrates even further reduction of the original scaffold by arrangement of the important functionalities around glucose, making 3.1 a perfect example for a type 3 mimetic.\textsuperscript{101} Compounds of this type have been studied in order to enhance oral availability.

Figure 3.3 Example type 3 peptidomimetic.

Recently, a 4\textsuperscript{th} type of mimetics has been added, which has also been called non-peptide peptidomimetics. In contrary to type 2 mimetics, in this case the term “non-
peptide” relates to the place of binding on a target rather then to the appearance of the active compound. Therefore, these compounds can actually be structurally related to peptides and type 1 mimetics, but they bind to their target on a site different to binding site of the peptide they mimic.

3.2 Type 1 Peptidomimetics

As mentioned before, type 1 peptidomimetics resemble peptides closely and contain mostly variations along the peptide bonds in order to enhance stability towards proteolysis and introduction of rigidity. Many of those variations contribute to both aims and transitions between groups of variations are often vague. The main groups of interest include simple conformational constraints, amide bond and transition state isosteres, and more complex changes to introduce turn motives.

When research on peptides as active compounds was first introduced, these molecules were mostly dismissed due to their poor pharmacological properties. This theory was concluded in the Lipinski “Rule of Five,” which postulates poor absorption or permeation if a compound contains more than 5 H-donors, more than 10 H-acceptors, has a molecular weight of greater than 500 or calculated logP of greater than 5.102 With the introduction of peptidomimetics, these problems could be partially overcome, but smaller compounds are still preferred due to better pharmacological properties. While talking about type 1 peptidomimetics, chains with an equivalent of two to about twenty amino acids are typical. As a result, larger proteins are reduced to the sequence important for binding and action.
3.2.1 Conformational Constraints

In order to understand the concept of conformational constraints, it is important to rationalize the degrees of freedom in a peptide backbone. For every amino acid incorporated in a peptide, there are four torsional angles along the backbone (Figure 3.4), resulting in \((4n-2)\) torsional angles for the whole peptide. As a result, peptides are very flexible molecules.

![Diagram of torsion angles and conformational constraints](image)

**Figure 3.4** Torsion angles and conformational constraints.\(^{103}\)

The \(N\)-alkylation leads to constraint around the \(\phi\)- and \(\psi\)-peptide bond angles as well as the rotation of the side chain (\(\chi\)) and results in \(cis\)-\(trans\) amide bond isomerism (Figure 3.5). This type of modification is often extended to the concept of peptoids, which belong to the peptide bond isosteres and have the amino acid side chain attached to the peptide nitrogen instead of the \(\alpha\)-carbon.\(^{104, 105}\)
Figure 3.5 $N$-alkylation structure motifs.

The $\chi$- and $\omega$- angles can be fixed to 0° (Z) or 180° (Z) by introduction of an olefin for the respective bond (Figure 3.6). In the latter case, the result is a peptide bond isostere.

Figure 3.6 Olefin motifs.

$\alpha$-Alkylation (Figure 3.7) constrains $\phi$- and $\psi$-peptide bond angles and leads to helical or extended structures. In addition, $\alpha$-methylation of natural amino acids with side chain functionality can lead to improved inter- and intramolecular interactions through disulfide bonds ($\alpha$-methylcystein) or hydrogen bonding ($\alpha$-methylthreonine).
Figure 3.7 α-Alkylation structure motifs.

Exchanging the stereochemistry of the α-carbon of one amino acid (D-amino acid) results in favored formation of a β-turn (Figure 3.8). Another way to introduce different turn motives into the amino acid backbone is the introduction of proline (β-turn) or other cyclic amino acids because they can restrict ϕ- and ψ-peptide bond angles. The possibilities to introduce cyclization in a single amino acid vary widely with the structure of said amino acid, but fall either in the category of side chain to peptide nitrogen (3.6)\textsuperscript{114}, \textsuperscript{115} or side chain to α-carbon (3.7)\textsuperscript{116} cyclization.

Figure 3.8 Turn motif favoring structures.

Aside from cyclization in one amino acid residue, the rigidity of a peptide backbone is often enhanced by the introduction of bridging between two or three neighbored amino acids, the structure motives are called dipeptide and tripeptide
analoges respectively. The possibilities to accomplish this are vast and Figure 3.9 only shows the basic connection points.\textsuperscript{117, 118}

![Figure 3.9 Dipeptide analoges.](image)

Less specific influence on the backbone conformation can be obtained by β-alkylation on the amino acid side chain as this has primarily influence on the $\chi$-peptide bond angle. A result of β-alkylation is a second stereo-center in the amino acid residue, which leads to four possible configurations and conformations as shown in Figure 3.10.

![Figure 3.10 β-Methylamino acids and preferred conformation.](image)
In addition, all peptide bond isosteres result in conformational changes around the backbone torsion angles; these compounds will be discussed in the following.

### 3.2.2 Amide Bond and Transition State Isosteres

There are two reasons for replacing the amide bond of a peptide, enhanced stability and inhibition of enzymes. As mentioned before, a main problem with active peptides is the typically short half-life due to the proteolysis of peptide bonds. The development of amide bond isosteres, which are replacements that cannot be cleaved by enzymes and therefore prolong the availability of the peptidomimetics. A second type of isosteres mimics was developed in order to suppress specific enzymes. These transition state isosteres take advantage of the fact that most enzymes stabilize the transition state of their substrates in order to lower the activation energy. As a result the transition state can bind better to the enzyme than the substrate or product of the enzymatic reaction, indicating that mimics of the transition state should be able to bind to the active site. If the mimics cannot undergo the enzymatic reaction, the enzyme should be inhibited completely.

The main difference of both types of isosteres lies in the conformation around the carbonyl moiety, which is typically retained in amid bond isosteres but replaced by a tetrahedral environment for compounds that mimic the transition state. A few isosteres can be accounted to both groups as they are practically steps of reducing the amide bond down to a simple ethylene unit (Figure 3.11).
Further examples for amide bond isosteres are shown in Figure 3.12. As mentioned before, they typically contain carbonyl groups or replacements like thiocarbonyl. A deviation from this norm results from replacement of the amide bond with an olefin. In addition to the exchange of just one amide bond, urea, sulfonamide and peptoid isosteres have been used in attempts to find active oligomeric peptidomimetics without any original peptide bonds.\textsuperscript{119} These compounds have in common that they can be easily synthesized from building block precursors, which is important for the synthesis of compounds with more than three subunits. The shift of the side chain from the $\alpha$-carbon to the amide nitrogen in peptoids leads to a high stability against proteolysis, the absence of a chirality around the $\alpha$-carbon (except for proline) and an increased flexibility of the backbone.\textsuperscript{104, 105} Urea peptidomimetics have been studied not only for their biological activity,\textsuperscript{120, 121} but also for the variety of secondary structures like helices,\textsuperscript{122, 123} foldamers and nanotubes that can be formed from this template.\textsuperscript{124} Sulfonamides have been originally developed as transition state isosteres and contain an extra carbon to void fragmentation under release of sulfur dioxide.\textsuperscript{125}
The retro-inverso peptide shown in Figure 3.12 is a very special case, as it does not truly contain any amide bond isosteres. In fact, peptide bonds are the only bonds in compounds of this type. But through reversal of the amide bond direction combined with inversion of all $\alpha$-carbon stereocenters ($D$-amino acids), these compounds are very stable against proteases while remaining the same topological appearance of the original peptide.

For transition state isosteres, it is important to assemble a tetrahedral environment similar to that during proteolysis. A substitution of the carbonyl carbon with either sulfur or phosphorus has been commonly used to achieve this goal (Figure 3.13). Nevertheless, it appears that a simple alcohol moiety as shown above (Figure 3.11) has ultimately superior properties to those compounds. In transition state isosteres, only the amide bond that would be target for the enzyme needs to be exchanged in order to obtain inhibition. But for different reasons, combinations of transition state- and amide bond isosteres as shown below have been utilized successfully.

![Figure 3.12 Amide bond isosteres.](image-url)
3.2.3 Secondary Structures

Secondary structures are of more interest for peptidomimetics with more amino acid equivalent subunits as they often mimic specific conformations of the original peptide. Some of the conformational constraints discussed in chapter 3.2.1 are direct working towards this goal. In addition, a number of templates have been developed to mimic \( \alpha \)-helices, \( \beta \)-sheets, loops and turns.

The \( \beta \)-turn is a motif formed from four amino acids stabilized by a hydrogen bond between the first and the fourth subunit (Figure 3.14).\(^{127-131}\) It has been studied to great extent and numerous rigid templates have been developed that contain structures related to peptide chains and those that do not. As mentioned before, proline is known to induce \( \beta \)-turn structures and extension of this concept led to the development of spiro
compounds containing the proline subunit. Similarly to the β-turn mimics, other structures have been found to induce γ-turns, α-helices and other elements.

**Figure 3.14** β-Turn and representative mimics.

Disulfide bridges are not only important for the development of loops; they also often connect different secondary structures. This makes them another target to find suitable mimics, which was accomplished with the discovery of ring closing metathesis (RCM). The RCM is a mild method to form an ethylene bridge from terminal alkenes. This method has been successfully utilized to replace disulfide bridges and is used in the “rolling loop scan,” a tool evaluate conformational restraints in the peptide backbone without interfering with amino acid side chains (Figure 3.15).\(^{132,133}\)
3.3 Imidazolidinones in Peptidomimetics

Studies about the synthesis of imidazolidinone peptidomimetics and their application have been rather limited. Two substitution patterns are possible for imidazolidinones: 1,3- and 1,4-substitution. So far, only 1,4-type patterns have been incorporated in peptidomimetics backbones.

In 2002, imidazolidinones were published as a way to introduce rigidity into a peptide backbone by connecting the amide nitrogens of two neighboring amino acid residues via methylene bridge (Figure 3.16). The connecting methylene bridge can carry a proton or a residue (R³) that could be an amino acid side chain, thus introducing further interaction possibilities with a substrate. The only drawback is that this residue was not introduced in an enantioselective fashion, leading to diastereomeric products. This method was used to synthesize all five possible methylene bridged analogues of

![Figure 3.15 Disulfide bridge mimic and rolling loop scan.](image)
opioid receptor agonist Leu-enkephalin. Of these analogues, 3.27 showed improved binding affinity for the \( \kappa \)-receptor over Leu-enkephalin.

![Chemical structures](image)

**Figure 3.16** 1,4-limidazolidinone peptidomimetics and Leu-enkephalin analogues.

Compounds like 3.28 and 3.32 have been synthesized from rational design and were studied as BACE1 inhibitors (Figure 3.17).\textsuperscript{136} Steric information in the first step of the reaction is transferred during an Aldol reaction and ultimately leads to 3.30 or 3.34, while 3.33 is introduced as single enantiomers.
These two examples for the incorporation of imidazolidinones into peptidomimetics demonstrate a useful application for this type of structure; but they also indicate that further research is necessary in order to improve the availability and diversity of imidazolidinones.

**Figure 3.17** 1,3- and 1,4-imidazolidinone peptidomimetics as BACE1 inhibitors.
CHAPTER 4: SYNTHESIS OF PEPTIDOMIMETICS CONTAINING STRUCTURAL ELEMENTS OF HYDROXYETHYLENE AND IMIDAZOLIDINONE

4.1 Introduction

The large variety of peptidomimetic scaffolds that have been studied and applied in medicinal chemistry research in the past is a sign for the importance of this group of compounds. And further investigations are still driven by the search for structures that introduce improved stability along with favorable steric and electronic properties.

The aim of this project is the synthesis of a new class of peptidomimetics containing both imidazolidinones and hydroxyethylene isosteres (Figure 4.1.). Three general structures are possible with this type of scaffold, containing the imidazolidinone in a terminal position (A and B) or in an internal position as shown in C. In addition, derivatization of the hydroxyethylene alcohol is possible, and although it is not a main focus, few examples are presented.

![Figure 4.1 Target structures.](image-url)
A comparison between model compound 4.1 and the corresponding peptide chain (FGGF) shows that our target compounds contain four structural peptidomimetic motifs that replace a two amino acid unit (Figure 4.2). Starting from the N-terminus, the first glycine unit is replaced by an ethylene isostere with distinct stereochemistry around the alcohol. The 1,3-imidazolidinone ring has replaced the second glycine. Within that structure, not only is the backbone cyclized, but it also represents a glycine peptoid in which nitrogen and α-carbon are exchanged. Finally, the nitrogen of the phenylalanine subunit is part of the imidazolidinone ring and therefore effectively N-alkylated, limiting the rotation around the adjacent bonds.

![Figure 4.2 Peptidomimetic motifs in 4.1 in comparison to the peptide analog FGGF.](image)

The conformational restrictions introduced by the imidazolidinone ring are expected to have large influence on the folding of the compound. A first projection of these consequences in folding was obtained from the calculation of the equilibrium...
conformers for the ground states of 4.1 and the peptide analog using molecular mechanics. Figure 4.3 shows the view directly on the imidazolidinone plane of 4.1 and the respective face of FGGF. While 4.1 appears to have a relatively linear conformation resulting from the restrictions of the ring, the more flexible peptide analog FGGF forms a turn motif around the two glycine residues which would be stabilized by a hydrogen bond.

![Diagram of 4.1 and FGGF](image.png)

**Figure 4.3** Front view of 4.1 and FGGF.

Viewing the model along the carbonyl bond of the imidazolidinone ring shows further structural differences (Figure 4.4). The rigidity of the ring suggests the formation of a hydrogen bond between the free acid of the C-terminus and ring carbonyl. In addition, the set stereochemistry around C4 of the imidazolidinone ring contributes to the
more linear orientation of 4.1. In comparison, the lack of steric restrictions in FGGF results in a more compact appearance.

![Image of molecules](image)

**Figure 4.4** View of 4.1 and FGGF along the ring carbonyl bond.

These calculations give evidence that the replacement of a Gly-Gly residue with the new structural motif results in significant conformational changes that might be of advantage in drug discovery.

4.2 Synthetic Plan

The bicyclic aziridine 4.2 is a well-studied compound that can undergo a number of transformations depending on the reaction conditions. Previously, our group reported
the opening on the terminal aziridine carbon to form oxazolidinones like 4.3 with a variety of nucleophiles including organolithium and Grignard reagents, amines and azides (Scheme 4.1). Recent studies with primary amines led to new findings concerning the scope of reactions possible on the bicyclic aziridine 4.3d. It was found that an increase in amine equivalents as well as the shift from a polar to a non-polar solvents can favor an attack on the carbonyl carbon to form an aziridinyl urea 4.5.

![Scheme 4.1 Oxazolidinones and aziridinyl ureas.](image)

Recently, the observation was made that during the reaction of 4.2 with benzyl amine, two products could be isolated. In addition to the expected oxazolidinone 4.6, the second compounds was identified as imidazolidinone 4.7 (Scheme 4.2). Further investigations showed that a rearrangement of an oxazolidinone to form an imidazolidinone can be promoted by the addition of a base or by introduction of heat for
an extended period of time. This rearrangement can take place because after the initial attack on the bicyclic aziridine with the primary benzyl amine, the resulting nitrogen is of secondary nature and can undergo another nucleophilic attack on the oxazolidinone carbonyl.

Scheme 4.2 Oxazolidinone rearrangement.

These results build the foundation for this project because they include the key step of the proposed synthetic route to our new peptidomimetics scaffolds (Scheme 4.3). In the first step, a protected amino acid or peptide can attack 4.2 to form an oxazolidinone, which should rearrange into an imidazolidinone. Ideally, both of these steps would take place in a one-pot reaction. The resulting 4.9 can then be deprotected to form peptidomimetics of type A.
Scheme 4.3 Proposed route to scaffold A.

In the second type of scaffold two possible connections between the hydroxy ethylene residue and the adjacent amino acid are possible, an ester or an amide bond. For this reason, we propose two similar syntheses starting from bicyclic aziridines with the classic trityl ether and with a phthalimide (Phth) residue (Scheme 4.4). This should allow access to both types of bonds without additional transformation reactions. After initial synthesis of imidazolidinone 4.11, a protection of the secondary alcohol is necessary in order to allow chain extension exclusively on the terminal alcohol or amine. After deprotection of the primary alcohol or amine moiety, the coupling to the C-terminus of an amino acid or peptide should result in the desired compounds with scaffold B.
To date, bicyclic aziridines like 4.10 have not been reported and in case they cannot be obtained, an alternative route to scaffold B containing a peptide bond can be proposed by transformation of the alcohol in 4.12a into an amine (Scheme 4.5). The typical approach for this transformation is a two-step procedure consisting of a Mitsunobu reaction to replace the alcohol with an azide followed by hydrogenolysis. If this approach proves unsuccessful the azide intermediate 4.14 can be formed from the activated alcohol 4.15 via substitution reaction.

Scheme 4.4 Proposed route to scaffold B.
After optimization of the syntheses of peptidomimetics with the scaffolds above, the combination of both routes to form compounds with amino acid extensions on both sides of the imidazolidinone structure can be attempted (Scheme 4.6).

Scheme 4.5 Alternative route to amine 4.12b.

After discussion of the general ways to synthesize the desired peptidomimetics, the question of protecting groups is the last aspect to be addressed. Because the trityl and
the phthalimide groups are already proposed, the remaining protecting groups need to be adjusted accordingly. This problem will be discussed using examples of single amino acids but is, of course, transferable to di- and tripeptides. In addition, a simultaneous cleavage of all remaining protecting groups in the final steps to form all three kinds of compounds is anticipated. Three basic modifications of the amino acid C-terminus will be utilized in this project. Amino acids without reactive side chain functionalities can be introduced as benzyl amides, methyl esters or tert-butyl esters (Figure 4.5). Of those, the benzyl amide and methyl ester are widely used even in final peptidomimetics compounds and don’t necessarily need to be deprotected. Tert-butyl esters can be cleaved under acidic conditions with trifluoroacetic acid (TFA), which should also deprotect the trityl ether simultaneously. To represent a variety of amino acids with side chain functionality, serine, lysine and glutamic acid will be introduced as tert-butyl esters with corresponding side chain protection as shown in Figure 4.5. Due to the lability of these groups under acidic conditions, the final products for scaffold A are expected to be obtained from a single deprotection reaction with TFA.

![Figure 4.5 Protecting groups for amino acids with free N-terminus.](image)
For scaffolds B and C, the secondary alcohol can be protected as silyl ether. This excludes an acidic trityl deprotection, but a shift to hydrogenolysis is expected to lead to exclusive cleavage of the trityl ether. The phthalimide could be deprotected under standard conditions using hydrazine or methylamine. The last modification to be considered is the N-terminus of the amino acids before the chain extension of B and C (Figure 4.6). This aspect is discussed on examples of scaffold B but the same rationale applies for C. The introduction of amino acids with Boc-protection has the advantage that they can be deprotected under acidic conditions with TFA, which results in a possible simultaneous cleavage of all remaining protecting groups in compounds of type B and C. Therefore, this is the method of choice for all compounds with only amide bonds between amino acid residues. For compounds with ester bonds, this method needs to be monitored closely, because a cleavage of the ester bond is likely. In that case, a shift to a benzyl carbamate (Cbz) is proposed, resulting in a stepwise deprotection of the silyl ether with TBAF and Cbz during hydrogenolysis should provide the desired products. Because compounds with ester bonds are not the main focus of this project, any problems that would arise from additional protecting groups on amino acid side chains will be avoided by utilizing only aliphatic and aromatic amino acids.

Figure 4.6 Protecting groups for amino acids with free C-terminus.
4.3 Bicyclic Aziridine Synthesis

Two bicyclic aziridines were proposed as starting compounds for the synthesis of the imidazolidinone peptidomimetics. Compound 4.2 has been studied extensively and can be synthesized as an enantiomerically pure compound or as a racemate with the indicated relative stereochemistry (Scheme 4.7). The racemic compound can be made starting from the commercially available racemic version of alcohol 4.21. The two enantiomers of 4.2 were previously obtained by hydrolytic kinetic resolution of commercially available butadiene monoepoxide. For this project, all reactions were conducted with the enantiomers of 4.2 shown in Scheme 4.7. This compound was synthesized cost effectively from commercially available D-mannitol. In a first step, the D-mannitol was protected as dicyclohexanone ketal 4.18. Upon periodane cleavage, the rather labile ketone 4.19 was obtained after distillation and directly converted into olefin 4.20 via Wittig reaction with an excellent yield. The deprotection of the ketal function was carried out under acidic conditions on solid support leading to the desired diole 4.21 in only moderate yield and only 62% conversion, despite a published yield of 91%. A reason for this decrease might be the different particle size of Dowex used during the reaction. However, the recovered starting material could be resubmitted to the reaction to lead to more product. The enantiomeric purity of 4.21 was determined by polarimetry before it was protected as trityl ether under standard conditions. The secondary alcohol was then transformed into a para-nitrobenzyl chloroformate, which was treated with sodium azide to result in the azido formate 4.23 in good yield. In the last step, the bicyclic aziridine was obtained from a reaction under high pressure utilizing a
sealed tube. The moderate yield of 30% is typical for this type of reaction and crystallization provided the desired product in high purity.

Scheme 4.7 Synthesis of bicyclic aziridine 4.2.

In order to obtain the phthalimide substituted bicyclic aziridine 4.10, a similar route was anticipated starting from racemic vinyl ethylene carbonate 4.24 (Scheme 4.8). An opening with phthalimide potassium salt in the terminal position and simultaneous decarboxylation proceeded with a moderate yield. The following two-step reaction led to the desired azido format 2.26, which was submitted to ring formation under sealed tube conditions. Unfortunately, this reaction led to a product that appeared to be the desired compound by $^1$H-NMR spectroscopy, but could not be isolated. The compound did not crystallize and was unstable under chromatography conditions. Test reactions of the crude compound with glycine benzylamide lead to a complex reaction mixture.
Following these early results, any further studies of the phthalimide substituted bicyclic aziridine were discontinued because the alternative route appeared more promising.

**Scheme 4.8** Synthesis of bicyclic aziridine 4.10.

4.4 Synthesis of Amino Acids and Peptides

As described earlier, different types of protecting groups were applied on the amino acids utilized in this project. Methyl esters 4.28a-b were synthesized in two steps from the corresponding free amino acids (Scheme 4.9). The low yield of 4.28a can be explained by its volatility, which leads to a loss of product during the workup. In the cases of phenylalanine and valine methyl esters, it is believed that the products are soluble in water, which leads to a loss of product. Because these compounds are all known to the literature, no attempts of improving the methodology have been made.
Scheme 4.9 Synthesis of amino acid methyl esters.

Fully protected hydrochloride salts of alanine, lysine and glutamic acid were obtained from commercial sources and treated with aqueous potassium carbonate to obtain the free amines in good yield (Scheme 4.10). Compared to the methyl esters mentioned above, the more hydrophobic character of the tert-butyl esters could be a reason for higher yields after extraction. tert-Butylphenylalanine was prepared according to a known procedure using tert-butyl acetate and perchloric acid.\(^{148}\)

Scheme 4.10 Synthesis of amino acid tert-butyl esters.

Glycine benzylamide (4.34) was prepared in a 3-step procedure starting from glycine, by first protecting the N-terminus, then introducing the benzylamide functionality and finally deprotection of the Cbz-group (Scheme 4.11). As a result, the
desired product was obtained in a good overall yield and purity. Application of the same process to phenylalanine did lead to the desired product, but in the last two steps benzylic side products resulting from the reaction with CbzCl were present and could not be separated by chromatography. As an alternative, 4.27b was reacted with benzylamine for 3 days to produce phenylalanine benzylamide (4.35) in 95% yield.

Scheme 4.11 Synthesis of amino acid benzyl amides.

An interesting procedure has been published for the synthesis of short peptide chains without solid support. This method was utilized to obtain a number of di- and tripeptides with good success (Table 4.1). The pTSA salt of β-alanine benzylester, which is an important reagent in this method, was made from β-alanine in a moderate yield after recrystallization (Scheme 4.12).
Scheme 4.12 Synthesis of β-alanine benzyl ester (4.36).

The actual procedure to make small peptides involves a two-step process of peptide coupling and N-terminus deprotection for every amino acid introduced in the chain (Table 4.1). During the coupling step, an amino acid tert-butyl ester and a Cbz-protected amino acid were reacted under standard conditions, but during the workup, β-alanine benzylester 4.36 was added to capture any remaining Cbz-protected amino acid. Simple aqueous workups then led to the product, which was deprotected via hydrogenolysis reaction. Utilizing this methodology, four short peptides were obtained in moderate to good yields (entered 1, 3, 4), which had to be purified by chromatography, contrary to the published results. During the reaction with Cbz-methionine, the sulfur atom in the molecule appeared to interfere with the hydrogenolysis by deactivating the catalyst (entry 2). In the case of a coupling between two phenylalanine amino acids (entry 5), the first reaction turned gel-like after a few minutes of stirring and this appearance could not be altered even after adding 500% more solvent. Due to this consistency, the workup of the reaction could not be accomplished. Nonetheless, two dipeptides and two tripeptides were synthesized successfully.
Table 4.1 Synthesis of di- and tripeptides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H-\text{AA}_1-R</th>
<th>Cbz-\text{AA}_2-OH</th>
<th>Cbz-\text{AA}_3-OH</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-L-Phe-OrBu</td>
<td>Cbz-L-Val-OH</td>
<td>Cbz-Gly-OH</td>
<td>4.37a, 60%</td>
</tr>
<tr>
<td>2</td>
<td>H-L-Phe-OrBu</td>
<td>Cbz-L-Met-OH</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>H-L-Phe-OrBu</td>
<td>Cbz-Gly-OH</td>
<td>-</td>
<td>4.37d, 55%</td>
</tr>
<tr>
<td>4</td>
<td>H-L-Phe-OrBu</td>
<td>Cbz-L-Val-OH</td>
<td>-</td>
<td>4.37c, 56%</td>
</tr>
<tr>
<td>5</td>
<td>H-L-Phe-NHBn</td>
<td>Cbz-L-Phe-OH</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(a)\) Met deactivated Pd-catalyst; \(b)\) after purification

The last dipeptide for this project was H-Gly-Gly-NHBn, which was made from the commercially available Boc-Gly-Gly-OH (Scheme 4.13). While traces of the desired final product could be isolated and were used in aziridine openings, the two-step procedure did not give satisfying results. During the first step of benzylamine synthesis, a mixture of the desired product and a benzyl side product was obtained, which could not be separated. In an attempt to form the final product, the mixture was directly submitted to the deprotection reaction utilizing TFA. From this reaction only traces of H-Gly-Gly-NHBn could be isolated. Because the synthesis of other dipeptides (Table 4.1) was successful, no further effort was made to improve this reaction.
4.5 Aziridine Opening Studies

4.5.1 Initial Study with Glycine

As discussed during the introduction, the reaction of bicyclic aziridine 4.2 with benzylamine leads to the formation of oxazolidinone 4.6, which can rearrange to imidazolidinone 4.7. This reaction was repeated with racemic and enantiomerically pure 4.2 and produced 4.7 in good yield (Scheme 4.14). Initial studies using amino acids to open the bicyclic aziridine were conducted with glycine benzylamide because this is the least sterically hindered amino acid and does not contain any stereocenters. This reaction produced the desired product in good yields during a one-pot procedure introducing heat to favor the rearrangement. These results were promising, because a simple increase in temperature is expected to have the least impact on the stereocenters of higher amino acids. It should also be mentioned that excessive heating to the boiling point of DMF led to a complex mixture, as did the addition of bases as described in literature.\(^{143}\)
4.5.2 Isomerization Study with Phenylalanine

After successful opening of the bicyclic aziridine with glycine benzylamide, the same reaction was performed using phenylalanine benzylamide in order to assess the potential of isomerization on the amino acid α-carbon. Scheme 4.15 shows the desired transformation of 4.2 into (2S,4′R,1′′S)-4.41 as well as the undesired product (2R,4′R,1′′S)-4.41. By NMR, it was confirmed that a single diastereomer of 4.42 as well as of the oxazolidinone intermediate 4.40 could be isolated in good yields. This confirms that a temperature elevation to 100 °C does not result in isomerization of the amino acid residue. This was later confirmed on the deprotected final product by LCMS and optical rotation. In addition, it is noteworthy, that an extension of the heating time to 4 days resulted in an increased yield of 88%.
Scheme 4.15 Isomerization study with phenylalanine derivative 4.35.

In the following, further aziridine openings with amino acids and peptides will be described. If not noted otherwise, these reactions were conducted on enantiomerically pure (4S,5R)-4.2 and resulted in the product as a single diastereomer.

4.5.3 Aziridine Openings with Amino Acid Methyl Esters

Following the promising results of the initial reaction and the isomerization study, 4.2 was reacted with three different amino acid methyl esters (Table 4.2). Under standard reaction conditions, imidazolidinones 4.42 were obtained in good to very good yields. The only exception was the reaction with valine methyl ester (Table 4.2, entry 3), which produced imidazolidinone 4.42c in 27% along with 70% of the intermediate oxazolidinone 4.43c. The reason for this is unknown, but a possible explanation could be the extended steric hindrance on the β-carbon of the amino acid. Nonetheless, it is believed that an extension of the heating time could improve the yield of imidazolidinone.
Table 4.2 Aziridine opening reactions with amino acid methyl esters.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H-AA-OR</th>
<th>Product, Yield</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H-Gly-OMe (4.28a)</td>
<td>rac-4.42a, 77%</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>H-LPhe-OMe (4.28b)</td>
<td>4.42b, 98%</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>H-LVal-OMe (4.28c)</td>
<td>4.42c, 27%</td>
<td>4.43c, 70%</td>
</tr>
</tbody>
</table>

<sup>a</sup> starting from rac-4.2.

4.5.4 Reaction Time Study with Glutamic Acid

Similar to the reaction mentioned above, an opening of 4.2 with the glutamic acid derivative 4.30c produced imidazolidinone 4.44 along with oxazolidinone 4.45 (Table 4.3). In order to identify the optimal reaction conditions, a reaction time study was conducted and it was found that longer reaction times indeed led to an increased yield of 4.44. After 4 days of heating, a maximum yield of imidazolidinone could be observed (entry 3) and after 5 days no oxazolidinone was found (entry 4). The lower yield in this case can be explained with a slight increase of impurities that made two purification attempts necessary.
Table 4.3 Optimization of reaction times with glutamic acid 4.30c.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time</th>
<th>Yield \textbf{4.44}</th>
<th>Yield \textbf{4.45}</th>
<th>Total yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2d</td>
<td>48%</td>
<td>27%</td>
<td>75%</td>
</tr>
<tr>
<td>2</td>
<td>3d</td>
<td>67%</td>
<td>15%</td>
<td>82%</td>
</tr>
<tr>
<td>3</td>
<td>4d</td>
<td>75%</td>
<td>9%</td>
<td>84%</td>
</tr>
<tr>
<td>4</td>
<td>5d</td>
<td>63%\textsuperscript{a}</td>
<td>-</td>
<td>63%</td>
</tr>
</tbody>
</table>

\textsuperscript{a) after extensive purification.}

4.5.5 Scope Evaluation with Amino Acids and Di-/Tri-peptides

Even though the example of glutamic acid derivatives 4.44 and 4.45 indicate that extended times of heating led to an increase of imidazolidinone, it was not clear if these results apply to all amino acid derivatives. In order to assess this theory, the following opening reactions were conducted with two different heating times (Table 4.4). The results were surprising because they did not follow the theory. For the reactions with alanine tert-butyl ester 4.30a, a decrease in overall yield was observed, while imidazolidinone 4.46a was the only product isolated (Table 4.4, entries 1, 2). Because 4.46b was obtained in quantitative yield after only 2 d of heating, no attempt was made to prolong the reaction times for this example (entry 3). For opening reactions with the commercially available tert-butyl ester of serine, both conditions gave very similar yields.
for both products observed (entries 4, 5). Only for lysine derivatives 4.46d and 4.47d was
the expected trend confirmed in which an increased yield of the desired 4.46d was
observed (entries 6, 7). The only explanation for these findings is a substrate dependence
of this reaction, which means that every reaction would have to be optimized for its
amino acid or peptide derivative.

Table 4.4 Aziridine opening reactions with amino acid tert-butyl esters.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H-AA-OR</th>
<th>Time</th>
<th>Product, Yield</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-LAla-OtBu (4.30a)</td>
<td>2 d</td>
<td>4.46a, 71%</td>
<td>4.47a, &lt;21%</td>
</tr>
<tr>
<td>2</td>
<td>H-LPhe-OtBu (4.31)</td>
<td>4 d</td>
<td>4.46a, 42%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H-LPhe-OtBu (4.31)</td>
<td>2 d</td>
<td>4.46b, quant.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>H-LSer(OtBu)-OtBu</td>
<td>2 d</td>
<td>4.46c, 70%</td>
<td>4.47c, 16%</td>
</tr>
<tr>
<td>5</td>
<td>H-LSer(OtBu)-OtBu</td>
<td>4 d</td>
<td>4.46c, 67%</td>
<td>4.47c, 19%</td>
</tr>
<tr>
<td>6</td>
<td>H-LSer(OtBu)-OtBu</td>
<td>2 d</td>
<td>4.46d, 48%</td>
<td>4.47d, 20%</td>
</tr>
<tr>
<td>7</td>
<td>H-Llys(Boc)-OtBu (4.30b)</td>
<td>4 d</td>
<td>4.46d, 70%</td>
<td>4.47d, 11%</td>
</tr>
</tbody>
</table>

Because an optimization for the openings with di- and tripeptides was not possible
due to limited amounts of reactants, the initial reaction conditions of 1 day at room
temperature followed by 2 days at 100 °C were applied for the openings with H-Gly-Gly-
NHBn and H-Gly-L-Val-L-Phe-OrBu. In both cases only the desired imidazolidinone was observed with moderate yields (Table 4.5, entries 1-2). While 4.48a formed rotamers, which made identification from ¹H-NMR difficult, 4.48b gave very clean spectra without rotamers. However, the fairly low yield of 33% for this compound indicated that the initial aziridine opening did not proceed to completion. Hoping to overcome this problem, the reactions with 4.37c and 4.37d were stirred for a prolonged period of time on both stages resulting in still moderate but increased yields of 4.48c and 4.48d (entries 3, 4).

Table 4.5 Aziridine opening reactions with di- and tripeptides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H-(AA)₂-OR</th>
<th>1st time</th>
<th>2nd time</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-Gly-Gly-NHBn</td>
<td>1d</td>
<td>2d</td>
<td>4.48a, 58%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>H-Gly-L-Val-L-Phe-OrBu (4.37a)</td>
<td>1d</td>
<td>2d</td>
<td>4.48b, 33%</td>
</tr>
<tr>
<td>3</td>
<td>H-Gly-L-Phe-OrBu (4.37c)</td>
<td>2d</td>
<td>4d</td>
<td>4.48c, 55%</td>
</tr>
<tr>
<td>4</td>
<td>H-L-Val-L-Phe-OrBu (4.36d)</td>
<td>2d</td>
<td>4d</td>
<td>4.48d, 48%</td>
</tr>
</tbody>
</table>

<sup>a</sup>rotamers.
4.5.6 Final Products of Type A

The synthesis of final products with skeleton A was the most important goal of this project. In order to achieve it, the above-described imidazolidinones had to be deprotected inorder to obtain the final dioles. Initially, this was attempted by hydrogenolysis (Table 4.6), because these are the mildest conditions and no side reactions were expected. Unfortunately, reactions with palladium on carbon as catalyst did not lead to any conversion and the starting materials were reisolated after filtration (entries 1-2). A switch of catalyst to Pd(OH)$_2$ resulted in a complex mixture by TLC and attempts to isolate any product were unsuccessful (entry 3). However, after closer examination, few peaks were predominant by $^1$H-NMR and led to the conclusion, that a polymerization of the reaction solvent THF had occurred, which also explained a fairly large quantity of crude material.

Table 4.6 Deprotection attempts via hydrogenolysis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^a$</td>
<td>Gly-NHBn</td>
<td>Pd/C, H$_2$, THF</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>L-Phe-NHBn</td>
<td>Pd/C, H$_2$, EtOH</td>
<td>N.R.</td>
</tr>
<tr>
<td>3$^a$</td>
<td>Gly-OMe</td>
<td>Pd(OH)$_2$C, H$_2$, THF</td>
<td>polimerization</td>
</tr>
</tbody>
</table>

$^a$starting from rac-4.2.
The next option for trityl deprotection were the acidic conditions that were successful during previous studies with similar imidazolidinone compounds. The reaction of 4.41 with HCl in dichloromethane led to a complex mixture after several hours reaction time, while after 30 min only traces of starting material and product could be isolated (Table 4.7, entry 1). Utilizing activated Dowex in dry methanol resulted in the desired product, even though the yield was unstable (entries 2-3). The same conditions led to the formation of SKB-168 in moderate yield (entry 4), but gave indistinct results for the reaction of 4.39 (entry 5). In the latter case, white solid was isolated that showed a \(^1\)H-NMR spectrum with the right number of protons and poor resolution of the single peaks, but the corresponding COSY spectrum was missing some of the major correlations. The poor solubility of this compound in most solvents was also obstructive to the characterization. Application of LCMS led to the conclusion that the compound was not clean and a mixture of compound might be responsible for the unclear results, even though a peak with the product mass was observed.

Table 4.7 Trityl deprotection under acidic conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>HCl, CH(_2)Cl(_2)</td>
<td></td>
<td>–(^b)</td>
</tr>
<tr>
<td>2</td>
<td>L-Phe-NHBn</td>
<td>Dowex, MeOH</td>
<td>SKB-149</td>
<td>82%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Dowex, MeOH</td>
<td></td>
<td>43%,</td>
</tr>
</tbody>
</table>

\(^b\) (4.41, 26%)
Table 4.7 Continued.

<table>
<thead>
<tr>
<th></th>
<th>Gly-OMe</th>
<th>Dowex, MeOH</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>(rac-4.42a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Gly-NHBn (rac-4.39)</td>
<td>Dowex, MeOH</td>
<td>-</td>
</tr>
</tbody>
</table>

In an attempt to improve the results of the deprotection reactions, BF$_3$·E$_2$O was tested as deprotecting agent on compound 4.7 with good results (Table 4.8, entry 1). Applying these conditions to phenylalanine derivative 4.42a led initially to the product in very low yield. With respect of the large number of hydrogen bond donors and acceptors in SKB-166, the workup conditions were altered to a minimum amount of aq. NaHCO$_3$ for quenching and thorough extraction with organic solvents in order to minimize the loss of product to the aqueous layer. This approach proved successful and the product was isolated in quantitative yield (entry 2). The same procedure led to the isolation of the more polar SKB-167 in a good yield (entry 3) and produced 4.50 with 40% after accidental submission of the starting material to these conditions (entry 4).
Table 4.8 Trityl deprotection with BF$_3$·Et$_2$O.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bn (4.7)</td>
<td><img src="4.49" alt="Diagram" /></td>
<td>60%</td>
</tr>
<tr>
<td>2</td>
<td>L-Phe-OMe (4.42b)</td>
<td><img src="4.49" alt="Diagram" /></td>
<td>quant.</td>
</tr>
<tr>
<td>3</td>
<td>L-Val-OMe (4.42c)</td>
<td><img src="4.49" alt="Diagram" /></td>
<td>61%</td>
</tr>
<tr>
<td>4</td>
<td>L-Ser(tBu)-OtBu (4.46c)</td>
<td><img src="4.49" alt="Diagram" /></td>
<td>40% (4.46c, 23%)</td>
</tr>
</tbody>
</table>

For derivatives of amino acid tert-butyl esters, especially those with side chain protection groups, a method was needed that would cleave all protecting groups in a single step. A literature search indicated the use of TFA as a universal deprotecting agent for cases like the present one. The only concern was a possible side reaction with the free trityl group. In order to prevent this TPW, a mixture of TFA, phenol as a scavenger, and water was applied either directly or as a solution in dichloromethane (Table 4.9). The reactions with additional solvent could be followed by TLC and showed the disappearance of starting material after 1 h. The evaporation of solvent, TFA and water resulted in a crude material that was crystallized with Et$_2$O and hexanes. The products
obtained from this procedure showed good $^1$H-NMR spectra with a small amount of aromatic impurities. Similarly, LCMS (ESI) resulted in chromatograms with the product peak and, in all cases, an additional signal in the flush with a mass of 243 on the positive channel. Even though the ionization conditions of ESI are soft, the lability of trityl compounds under these conditions is known, resulting in the assumption that the impurity is a trityl derivative. This would be conform with the LCMS results because the trityl cation would show a mass of 243. Following these findings, all compounds were purified using semi-preparative HPLC. The purification was challenging because the compounds tend to show broad peaks on the column and do not give clean peaks. Even though, the low retention times led to quick elution and separation from the aromatic compound, which has a higher retention time, the streaking on the column lead to a considerable loss of compound in almost all cases. On average 20-30% of the material were lost during the purification. Unfortunately, the product of the deprotection of glutamic acid derivative 4.44 was present in the crude material, but could not be found again after purification. It is assumed that the reaction proceeded successfully, but the properties of the product led to the loss of this compound.
Table 4.9 Trityl deprotection utilizing the TPW-reagent.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-Ala-OrBu (4.46a)</td>
<td>A</td>
<td>SKB-169</td>
<td>64%</td>
</tr>
<tr>
<td>2</td>
<td>L-Phe-OrBu (4.46b)</td>
<td>B</td>
<td>SKB-170</td>
<td>37%*</td>
</tr>
<tr>
<td>3</td>
<td>L-Ser(tBu)-OrBu (4.46c)</td>
<td>A</td>
<td>SKB-171</td>
<td>57%</td>
</tr>
<tr>
<td>4</td>
<td>L-Lys(Boc)-OrBu (4.46d)</td>
<td>A</td>
<td>SKB-172</td>
<td>64%</td>
</tr>
<tr>
<td>5</td>
<td>L-Glu(OrBu)-OrBu (4.44)</td>
<td>B</td>
<td>-</td>
<td>67%*</td>
</tr>
<tr>
<td>6</td>
<td>Gly-1,Val-1,Phe-OrBu (4.48a)</td>
<td>B</td>
<td>SKB-173</td>
<td>77%</td>
</tr>
<tr>
<td>7</td>
<td>Gly-1,Phe-OrBu (4.48b)</td>
<td>B</td>
<td>SKB-174</td>
<td>48%*</td>
</tr>
<tr>
<td>8</td>
<td>L-Val-1,Phe-OrBu (4.48c)</td>
<td>B</td>
<td>SKB-175</td>
<td>55%</td>
</tr>
</tbody>
</table>

A) TPW, neat; B) TPW, CH$_2$Cl$_2$. *purified by HPLC.

TPW = TFA/PhOH/H$_2$O (92.5/5/2.5)
4.6 Synthesis of Secondary Ester Derivatives

Even though, the functionalization of the secondary alcohol was not a main goal in this project, a few attempts were made to prove that it is possible (Table 4.10). These studies were conducted on glycine benzylamide derivative 4.39 and phenylalanine benzylamide derivative 4.41. An initial attempt to treat the alcohol with freshly distilled acetyl chloride led to a complex mixture from which traces of product could be observed by NMR spectroscopy (entry 1). A second common reagent for esterification is benzoic anhydride, which produced 4.51a and 4.52 in excellent yields (entries 1, 7). The coupling with acid derivatives proved a little more complicated and different conditions were tested before a reaction with 2eq of CDI was found to give the best results (entries 3, 4). Other conditions, including the addition of imidazole or utilization of DCC as coupling agent did not produce the desired product (entries 5, 6).

Table 4.10 Esterification of secondary alcohols.

<table>
<thead>
<tr>
<th>entry</th>
<th>Starting material</th>
<th>conditions</th>
<th>product</th>
<th>yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rac-4.39</td>
<td>AcCl, NEt₃, CH₂Cl₂</td>
<td></td>
<td>a,b</td>
</tr>
<tr>
<td>2</td>
<td>rac-4.39</td>
<td>(PhCO)₂O, DMAP, CH₂Cl₂</td>
<td>rac-2-51a, Ph</td>
<td>quant.</td>
</tr>
</tbody>
</table>
Table 4.10 Continued.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction Details</th>
<th>Products</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rac-4.39 PhCH₂CO₂H, CDI, CH₂Cl₂</td>
<td>rac-2-51b</td>
<td>70% (rac-4.39, 30%)</td>
</tr>
<tr>
<td>4</td>
<td>4.39 PhCH₂CO₂H, CDI, CH₂Cl₂</td>
<td>2-51b</td>
<td>4.39, 50%</td>
</tr>
<tr>
<td>5</td>
<td>4.41 PhCO₂H, CDI, Imidazole, CH₂Cl₂</td>
<td></td>
<td>(&lt;40% conv.)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>4.41 DMAP, NEt₃, CH₂Cl₂</td>
<td></td>
<td>(&lt;50% conv.)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>4.41 (PhCO)₂O, DMAP, CH₂Cl₂</td>
<td>2-52</td>
<td>quant.</td>
</tr>
</tbody>
</table>

<sup>a</sup> complex mixture;  <sup>b</sup> traces of product by NMR

From those esters, the two shown in Scheme 4.16 were successfully deprotected with BF₃·Et₂O to obtain final compounds **SKB-176** and **SKB-177** in good yield.
4.7 Synthesis of Scaffolds B and C

4.7.1 Protection Group Strategies

Following the successful preparation of examples with peptidomimetics scaffold A, the preparation of compounds of type B and C required additional considerations concerning protecting groups. Initially, attempts were made to protect the secondary alcohol as trimethyl silyl ether, but no product could be isolated. Shifting to the more bulky TBS protecting group, led to 4.53 with a good yield after optimization (Table 4.11, entry 3). It was also found that the quality of the TBSCl had a large impact on the overall conversion of this reaction.
Table 4.11 Optimization of TBS-protection.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TBSCI (2eq), imidazole, DMAP, DMF, r.t., 9h</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td><em>(rac-4.39, 29%)</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TBSCI (2eq), imidazole, DMAP, DMF, 50°C, 12h</td>
<td>49%</td>
</tr>
<tr>
<td>3</td>
<td>TBSCI (3eq), imidazole, DMAP, DMF, r.t., 1d</td>
<td>81%</td>
</tr>
<tr>
<td>4</td>
<td>TBSCI (3eq), NEt₃, DMAP, DMF, r.t., 1d</td>
<td>-ᵃ</td>
</tr>
</tbody>
</table>

ᵃ) complex mixture.

Utilizing these optimized conditions for the protection of similar compounds gave equally good results (Table 4.12).

Table 4.12 TBS-protection of the secondary alcohol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>R</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>rac-4.7</em></td>
<td>Bn</td>
<td><em>rac-4.54a</em>, 97%</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>Bn</td>
<td>4.54a, quant.</td>
</tr>
<tr>
<td>2</td>
<td><em>rac-4.42a</em></td>
<td>Gly-OMe</td>
<td><em>rac-4.54b</em>, 75%</td>
</tr>
<tr>
<td>3</td>
<td>(2S,4’R,1''S)-4.41</td>
<td>1Phe-NHBn</td>
<td>(2S,4’R,1''S)-4.54c, 78%</td>
</tr>
</tbody>
</table>

As a next step, the selective cleavage of the trityl group was investigated (Table 4.13). Similar to the results described previously (Tables 4.6 and 4.7), hydrogenolysis and acidic conditions did not lead to the desired product (Table 4.13, entries 1-5). Only for the reaction with DOWEX could a clean compound be obtained, but in that case the TBS-group was lost as well resulting in the undesired diole 4.49 (entry 3). A final attempt with an acetic medium in a two-phase reaction was followed by TLC but showed no progress even after several days of stirring. Only the reactions with Lewis acids BCl₃ and BF₃·Et₂O provided the desired product. Of those two reagents, BCl₃ proved to be unsuitable because upon scale up (from 20 mg to 200 mg) of the reaction, incomplete conversion was observed and could not even be avoided with extended reaction times (entries 6-7). Fortunately, BF₃·Et₂O proved to be successful with small and larger quantities of starting material.
Table 4.13 Tr-deprotection in the presence of a TBS-ether.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd/C, H₂, EtOH</td>
<td>N.R.</td>
</tr>
<tr>
<td>2</td>
<td>HCl, CH₂Cl₂, 20h</td>
<td>- a</td>
</tr>
<tr>
<td>3</td>
<td>Dowex, MeOH</td>
<td>- (4.49, 66%)</td>
</tr>
<tr>
<td>4</td>
<td>0.5M aq. HCl, cyclohex.</td>
<td>N.R.</td>
</tr>
<tr>
<td>5</td>
<td>Dowex, H₂O, cyclohex.</td>
<td>N.R.</td>
</tr>
<tr>
<td>6</td>
<td>BCl₃ (1M in Hex), CH₂Cl₂</td>
<td>62-82%</td>
</tr>
<tr>
<td>7ᵇ</td>
<td>30-40%, (rac-4.54a, 30%)⁷ᶜ</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BF₃·Et₂O, MeOH, CH₂Cl₂</td>
<td>73%</td>
</tr>
</tbody>
</table>

ᵃ) complex by TLC with traces of product;ᵇ) scale up;ᶜ) reisolated starting material.

Application of the Lewis acid conditions to he resulted in the product with similar results (Scheme 4.17). Because no scale up was conducted in the case of BCl₃, it is not clear if the reaction would give the same problems described above.

Scheme 4.17 Deprotection of 4.54c.
4.7.2 Synthesis of Primary Ester Derivatives

In order to produce the ester derivatives, 4.55 and 4.56 were submitted to the conditions previously applied for the secondary esters (Table 4.10), with similar results (Table 4.14).

**Table 4.14** Esterification of primary alcohols.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Conditions</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>rac</em>-4.55</td>
<td>AcCl, NEt₃, CH₂Cl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>rac</em>-4.55</td>
<td>(PhCO)₂O, DMAP, CH₂Cl₂</td>
<td>rac-2-57</td>
<td>89%</td>
</tr>
<tr>
<td>3</td>
<td>4.56</td>
<td>Cbz-Gly-OH, CDI, CH₂Cl₂</td>
<td>2-58a</td>
<td>65%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boc-Gly-Gly-OH,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.56</td>
<td>CDI, CH₂Cl₂</td>
<td>2-58b (4.56, 38%)</td>
<td></td>
</tr>
</tbody>
</table>

*complex mixture*
As expected, the reaction with acetyl chloride led to a complex mixture from which no product could be isolated (Table 4.14, entry 1). The reaction with benzoic anhydride, as well as the reaction with amino acid derivatives utilizing CDI, produced the desired product in good yields (entries 2-4).

Unfortunately, the cleavage of the TBS group and the amino acid N-protecting group proved rather difficult (Scheme 4.18). Initially, Dowex was introduced as a mildly acidic reagent that had previously led to TBS-deprotection (see Table 4.7, entry 3). In the present case, however, no reaction was observed by TLC after 2 days and the mixture was filtered and concentrated to give unreacted starting material. Careful treatment with TFA was expected to result in TBS-cleavage but ester cleavage was a possibility as well. Unfortunately, the product isolated was the undesired SKB-149.

![Scheme 4.18 Attempted deprotection of 4.58b.](image)

Due to the small-scale character of the previous esterification reactions, no further test reactions were conducted. Nonetheless, a two-step procedure is theoretically possible, ideally starting from derivatives with Cbz-protection on the N-terminus. In
those cases, the TBS group could be cleaved by TBAF, followed by hydrogenolysis to obtain the free amine.

4.7.3 Amine Conversion and Peptide Coupling

The last part of this project describes the conversion of the primary alcohols into amines and their coupling with amino acids derivatives. For the route proposed above, the shortest way to obtain the intermediate azide (4.60) is a Mitsunobu reaction, which unfortunately did not lead to the desired product, but to a reaction intermediate containing large aromatic groups (Scheme 4.19).

![Scheme 4.19 Attempted Mitsunobu reaction.](image_url)

The transformation via tosyl ether was studied concurrently and proved successful immediately (Scheme 4.20). In a first step, the tosyl ether 4.61 was formed with 73% yield after purification. A substitution with sodium azide was successful after raising the reaction temperature from room temperature to 50 °C and extending the reaction time to 2 days. After aqueous workup, a clean product was obtained, which was directly utilized in the hydrogenolysis. The free amine (4.62) was used for the coupling with Boc-valine resulting in 4.63 in 15% yield over two steps. One reason for the low yield was an initial attempt to purify the free amine, which was not successful and led to the loss of some
material. However, 4.63 was fully deprotected using the previously applied TPW mixture (TFA/PhOH/H₂O (92.5/5/2.5)) in dichloromethane. An aqueous workup was avoided in order to prevent further loss of material and the final product was isolated as TFA salt and purified with semi-preparative HPLC. As mentioned before (Table 4.9), about 20-30% of the crude mass was lost during purification, leading to a significant decrease in the yield. In addition, the partially deprotected derivative 4.63b could be isolated.

Scheme 4.20 Synthesis of SKB-178.

After successful synthesis of SKB-178 as an example for peptidomimetics with scaffold type B, the exact same method was applied to obtain compounds with scaffold C (Scheme 4.21). The reactions were carried out on phenylalanine derivative 4.56 and produced the azide intermediate 4.65 with a very good yield. Thorough washing of the
activated carbon after the hydrogenolysis reaction and direct submission of the crude material to the amino acid coupling led to a moderately enhanced yield compared with the compound discussed above. Aside from Boc-Val-OH, the commercially available Boc-Gly-Gly-OH was utilized during the coupling and compounds 4.67a and 4.67b were then deprotected to obtain SKB-179 and SKB-180 as TFA-salts in moderate yields after purification by semi preparative HPLC.

![Scheme 4.21 Synthesis of SKB-179 and SKB-180.](image)

4.8 Conclusion

The aim of this project was the synthesis of a new class of peptidomimetics containing an imidazolidinone structure in three different positions (Figure 4.7). This
goal was achieved following the proposed method of opening of a bicyclic aziridine with amino acid residues and, if necessary, the transformation of the terminal alcohol into an amine followed by chain elongation. All compounds were made without loss of the steric information leading to final products as single enantiomers.

Figure 4.7 Examples of final products for target structures A, B and C.

With special focus in the synthesis of compounds of type A, amino acids with different C-terminus modification were introduced in order to examine synthetic scope of this method (Figure 4.8). It was found that all three types of amino acids could be introduced successfully and led to the desired final compounds after deprotection. SKB-170 was formed from the corresponding tert-butyl ester and due to the increased polarity the purification of proved more challenging, compared to the benzylamide and the methyl ester analoges SKB-149 and SKB-166, which were purified by chromatography.
In order to evaluate the possibility of the introduction of amino acids with side chain functionalities, a small variety of such compounds was synthesized (Figure 4.9). In order to keep the number of necessary transformations low, amino acids with protecting groups that would allow final deprotection in a single step were utilized. These studies were overall successful but also exposed substrate related difficulties. Thus, the initial reaction of aziridine opening and rearrangement to for imidazolidinones did often not go to completion resulting in a mixture of imidazolidinones and oxazolidinones that could be separated by chromatography. Attempts to optimize the conditions for this transformation revealed the substrate dependence of this reaction as no consistent result could be obtained. Nonetheless, all of the desired products were formed. As mentioned above, final compounds with a free carboxylic acid residue were difficult to purify. The main impurities after deprotection were aromatic residues from the trityl ether and small amounts of phenol. Purification on semi preparative HPLC led to the final compounds under loss of a considerable amount of material due to streaking on the column. Only the product of the reaction with a glutamic acid derivative could not be obtained after purification, even though, product was present in the crude material.
Figure 4.9 Products from amino acids with side chain functionality.

In addition to the target structures, attempts to functionalize the secondary alcohol led to good results and open another path to utilize imidazolidinones in peptidomimetics scaffolds of type D resulting in a serine like linkage (Figure 4.10).

Figure 4.10 SKB-177 and related structures of type D.

In summary, a new method was discovered, which opens access to peptidomimetics containing imidazolidinones in different positions in the amino acid backbone. This procedure proves viable for a variety of amino acids and small peptides leading to products of high stability. The final compounds now need to be subjected to further analytical studies in order to investigate secondary structures that might be formed. In addition, the compounds will be submitted for biological testing.
CHAPTER 5: EXPERIMENTAL

5.1 General Methods

All reactions were, if necessary, conducted in dry glassware under argon atmosphere. The chemicals were purchased from the usual commercial suppliers and used without further purification, unless otherwise noted. CH₂Cl₂ and THF were dried using a SOLV-TEK solvent purification system. The following solvents and reagents were distilled from the mentioned drying agent: Acetone (K₂CO₃), DMF (MgSO₄), toluene (CaH₂), MeOH (Na) and Et₃N (KOH). All primary amines and acid chlorides were freshly distilled before using them.

Acidic Dowex 50WX8 ion exchange resin (50-100 mesh) was prepared as follows: Dowex 50WX8 resin (10g) was treated with 6M HCl (50 mL) and then filtered through a fritted funnel. It was washed with distilled water until treatment of the filtrate with 5% aq. AgNO₃ produced no precipitation. The resin was washed with MeOH (50 mL) and dried under vaccum for 1 h.

5.2 Instruments

Microwave-assisted reactions were performed using INITIATOR Biotage Microwave Synthesizer (400W, 2.45 GHz). ¹H-NMR and ¹³C-NMR spectra were recorded with a Bruker AVANCE (300 MHz/50 MHz) or a VARIAN (500 MHz) spectrometer. Chemical shifts are reported in ppm on the δ scale relative to TMS as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, br = broad, m = multiplet), coupling constant in Hz, integration. IR spectra were obtained as a thin film on KBr or as a KBr-
prenal on a Shimadzu Advantage FTIR-8400. Specific rotations were measured on a AUTOPOL ® IV (Rudolph Research Analytical) polarimeter with a sodium (\(\lambda = 589 \text{ nm}\)) lamp, and are reported as follows: \([\alpha]^T \lambda^C (c \text{ g/100 mL, solvent}). Melting points were determined with a DigiMelt MPA160 melting point apparatus and are reported uncorrected.

5.3 Chromatography

Compounds were purified by flash chromatography using 32-63D 60Å silica gel or 32-63u flash grade neutral alumina according to the method of Still.\(^{154}\) R\(_f\) values were obtained by thin-layer chromatography (TLC) on alumina (neutral) backed or silica coated TLC plates containing a fluorescent indicator (254 nm). The detection was accomplished by UV and staining with a 10% ethanolic solution of phosphomolybdic acid or a ninhydrin solution (1.5 g ninhydrin in 100 mL butanol and 3 mL AcOH) followed by heating.

The purity of compounds was determined via LCMS or HPLC analysis. The LCMS analysis was completed on a Shimadzu LC-10AD machine equipped with a SPD-M10A UV detector and a LCMS-2010A MS detector employing a Discovery-C8 (15 cm x 4.6 mm x 5 \(\mu\)m; Supelco) column. The HPLC analysis was done on a Shimadzu LC-10AT machine equipped with a SPD-10A UV detector employing a Discovery-C8 (15 cm x 4.6 mm x 5 \(\mu\)m; Supelco) column.

**Eluting method A:** CH\(_3\)CN in H\(_2\)O at 1 mL/min flow rate, a linear gradient starting at 0.5 min with 5% CH\(_3\)CN and ending at 10 min with 30% CH\(_3\)CN, at 15 min with 90%
CH$_3$CN and a total run time of 19 min. **Eluting method B:** MeOH in H$_2$O under conditions equal to method A. **Eluting method C:** MeOH in H$_2$O (0.1% TFA) and with a flow rate of 0.5 mL/min under conditions equal to method A.

**Eluting method D:** CH$_3$CN in H$_2$O at 1 mL/min flow rate, a linear gradient starting at 0.5 min with 20% CH$_3$CN and ending at 12 min with 90% CH$_3$CN and a total run time of 18 min. **Eluting method E:** MeOH in H$_2$O under conditions equal to method D. **Eluting method F:** MeOH in H$_2$O (0.1% TFA) under conditions equal to method D.

**Eluting method G:** MeOH in H$_2$O (0.1% TFA) at 1 mL/min flow rate, a linear gradient starting at 0.5 min with 50% MeOH and ending at 12 min with 90% MeOH and a total run time of 18 min.

**Eluting method H:** MeOH in H$_2$O (0.1% TFA) at 0.5 mL/min flow rate, isocratic with 5% MeOH for a total run time of 20 min.

**Eluting method I:** MeOH in H$_2$O at 1 mL/min flow rate, a linear gradient starting at 1 min with 30% CH$_3$CN and ending at 14 min with 90% CH$_3$CN and a total run time of 18 min.

The semi-preparative HPLC purification was completed on a Shimadzu LC-10AT machine equipped with a SPD-10A UV detector employing a Discovery BIO Wide Pore C8 (15 cm x 10.0 mm x 10 µm; Supelco) column.

**Eluting method:** MeOH in H$_2$O (0.1% TFA) at 3 mL/min flow rate, a linear gradient starting at 0.01 min with 20% MeOH and ending at 23 min with 90% MeOH and a total run time of 33 min.
5.4 Experimental Procedures

5.4.1 Experimental Procedures from Chapter 2

**Ethyl 4-nitrobenzoate (2.23a)** A solution of para-nitrobenzoic acid (1.01 g, 6.02 mmol) and NEt₃ (834 µL, 5.98 mmol) in 3 mL CH₃CN was cooled to 0 °C and ethyl chloroformate (572 µL, 5.98 mmol) in 3 mL CH₃CN was added drop wise. After 5 min of stirring a solution of DMAP (365 mg, 2.99 mmol) in 3 mL CH₃CN was added and the mixture was reacted at room temperature over night. The mixture was concentrated, diluted with CH₂Cl₂ and then washed with sat. aqueous NaHCO₃ and 0.1M HCl. The organic layer was dried over MgSO₄, filtered and concentrated to result in 1.02 g (87%) 2.23a. $^1$H-NMR (CDCl₃, 300 MHz) δ 8.28-8.18 (m, 4H), 4.42 (q, $J = 7.0$ Hz, 2H), 1.41 (t, $J = 7.0$ Hz, 3H).

![2.23a](image)

**Benzyl 4-nitrobenzoate (2.23b)** A solution of para-nitrobenzoic acid (1.00 g, 6.01 mmol) and NEt₃ (834 µL, 5.98 mmol) in 3 mL CH₃CN was cooled to 0 °C and benzyl chloroformate (854 µL, 5.98 mmol) in 3 mL CH₃CN was added drop wise. After 5 min of stirring a solution of DMAP (366 mg, 2.99 mmol) in 3 mL CH₃CN was added and the mixture was reacted at room temperature over night. The mixture was concentrated, diluted with CH₂Cl₂ and then washed with sat. aqu. NaHCO₃ and 0.1M HCl.

![2.23b](image)
HCl. The organic layer was dried over MgSO₄, filtered and concentrated to result in 1.13 g (73%) 2.23b. ¹H-NMR (CDCl₃, 300 MHz) δ 8.26-8.22 (m, 4H), 7.42-7.37 (m, 5H), 5.39 (s, 2H).

**Tert-butyl 4-nitrobenzoate (2.23c)** To an ice cold solution of PPh₃ (1.57 g, 5.99 mmol) and bromine (480 mg, 6.01 mmol) in 30 mL CH₂Cl₂ added para-nitrobenzoic acid (997 mg, 5.97 mmol) and DMAP (1.46 g, 12.0 mmol). The solution was stirred for 3 min and then warmed to room temperature. After addition of tBuOH (572 µL, 5.98 mmol) it was stirred for 2 d. The reaction mixture was concentrated and the crude flushed through a plug of silica gel (hexanes /EtOAc, 4:1) to produce 560 mg (42%) 2.23c. ¹H-NMR (CDCl₃, 300 MHz) δ 8.24 (dd, J = 7.0, 2.0 Hz, 2H), 8.13 (dd, J = 7.0, 2.0 Hz, 2H), 1.60 (s, 9H).

**General Procedure A - Synthesis of benzoic acid esters.**

Concentrated sulfuric acid (20 mol%) was added drop wise to a solution of benzoic acid derivative in MeOH (1.0M) was. The reaction was heated to reflux over night, concentrated dissolved in sat. aq. NaHCO₃ and EtOAc (1:1). After separation, the aqueous layer was extracted with 2 more portions of EtOAc and the combined organic layers were washed with water, dried over MgSO₄, filtered and concentrated.
Methyl 4-bromobenzoate (2.29) Following general procedure A, the reaction of para-bromobenzoic acid (1.99 g, 9.92 mmol) resulted in 2.05 g (96%) 2.29. \(^1\)H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.94 (d, $J = 8.0$ Hz, 2H), 7.72 (d, $J = 8.0$ Hz, 2H), 3.89 (s, 3H).

**General Procedure B - Synthesis of Hydrazinecarboxylates**

Aaryl bromide, t-butyl carbazate (200 mol%), Pd$_2$(dba)$_3$ (1 mol%), DPPF (2 mol%) and Cs$_2$CO$_3$ (100 mol%) were added to a dry round bottom flask equipped with a reflux condenser. The apparatus was evacuated and charged with argon. Then dry and degassed toluene (0.5M) was added and the reaction was refluxed over night. The mixture was filtered through celite and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc, 9:1 → 4:1).

Tert-Butyl 1-(4-(ethoxycarbonyl)phenyl)hydrazinecarboxylate (2.30a) Following general procedure B, the reaction of ethyl 4-bromobenzoate (1.40 g, 6.12 mmol) produced 1.51 g (88%) 4.30a. $R_f = 0.23$ (hexanes/EtOAc, 4:1).
**Tert-butyl 1-(4-(methoxycarbonyl)phenyl)hydrazinecarboxylate (4.30b)** Following general procedure B, the reaction of methyl 4-bromobenzoate (1.99 g, 9.25 mmol) produced 1.88 g (77%) 2.30b. R_f = 0.23 (hexanes/EtOAc, 4:1). ^1H-NMR (CDCl_3, 300 MHz) δ 7.98 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 4.42 (s, 2H), 3.90 (s, 3H), 1.54 (s, 9H). Analytical data matched that reported for the known compound.\(^{155}\)

**General Procedure C – Synthesis of 2,3-dimethyl indoles**

A solution of hydrazinecarboxylate, ketone (150 mol%) and pTSA (600 mol%) in MeOH (0.1M) was heated to reflux over night. After evaporation of the solvent, the crude product was purified by column chromatography (hexanes/EtOAc, 5:1 → 1:1).

**Ethyl 2,3-dimethyl-1H-indole-5-carboxylate (2.6a)** Following general procedure C, the reaction of hydrazinecarboxylate 2.30a (101 mg, 0.360 mmol) and 2-butanone (48.0 µL, 0.536 mmol) produced 51 mg (65%) 2.6a. R_f = 0.42 (hexanes/EtOAc, 2:1). ^1H-NMR (CDCl_3, 300 MHz) δ 8.24 (s, 1H), 7.87 (br, NH), 7.84 (d, J = 9.0 Hz, 1H), 7.24 (d, J = 9.0 Hz, 1H), 4.40 (q, J = 7.0 Hz, 2H), 2.37 (s, 3H), 2.26 (s, 3H), 1.42 (t, J = 7.0 Hz, 3H). Analytical data matched that reported for the known compound.\(^{156}\)
Methyl 2,3-dimethyl-1\(H\)-indole-5-carboxylate (2.6b) Following general procedure \(C\), the reaction of hydrazinecarboxylate 2.30b (250 mg, 0.938 mmol) and 2-butanone (48.0 µL, 0.536 mmol) produced 79 mg (41%) 2.6b.

Alternatively, a solution of 2.6a (51.4 mg, 0.237 mmol) and pTSA (43.1 mg, 0.27 mmol) in 2.0 mL MeOH was heated in for 1 h to 150 °C under microwave conditions. The solution was concentrated and purified by chromatography (hexanes/EtOAc, 5:1 \(\rightarrow\) 1:1) to produce 48.2 mg (quant.) 2.6b. \(R_f = 0.40\) (hexanes/EtOAc, 2:1). \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 8.24 (s, 1H), 8.10 (br, 1H), 7.82 (dd, \(J = 8.50, 1.5\) Hz, 1H), 7.21 (d, \(J = 8.5\) Hz, 1H), 3.93 (s, 3H), 2.34 (s, 3H), 2.23 (s, 3H). Analytical data matched that reported for the known compound.\(^{157}\)

Methyl 3-ethyl-2-methyl-1\(H\)-indole-5-carboxylate (2.6c) Following general procedure \(C\), the reaction of hydrazinecarboxylate 2.30b with 2-pentanone (240 µL, 2.25 mmol) produced 46.5 mg (14%) 2.6c. \(R_f = 0.33\) (CH\(_2\)Cl\(_2\)). \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 8.27 (s, 1H), 7.84-7.80 (m, 2H), 7.26-7.24 (m, 1H), 3.93 (s, 3H), 2.74 (q, \(J = 7.5\) Hz, 2H), 2.39 (s, 3H), 1.24 (t, \(J = 7.5\) Hz, 3H).
Methyl 1,2,3,4-tetrahydrocyclopenta[b]indole-7-carboxylate (2.6d) Following general procedure C, the reaction of hydrazinecarboxylate 2.30b with cyclopentanone (200 µL, 2.25 mmol) produced 60.8 mg (19%) 2.6d. R_f = 0.33 (CH_2Cl_2). ¹H-NMR (CDCl_3, 300 MHz) δ 8.20 (s, 1H), 8.02 (br, 1H), 7.82 (d, J = 8.5 Hz, 1H), 3.92 (s, 3H), 2.91-2.83 (m, 4H), 2.61-2.51 (m, 2H).

Methyl 2,3,4,9-tetrahydro-1H-carbazole-6-carboxylate (2.6e) Following general procedure C, the reaction of hydrazinecarboxylate 2.30b with cyclohexanone (227 µL, 2.20 mmol) produced <282 mg (<84%) unclean 2.6e. Clean product was obtained from a reaction following the same conditions with hydrazinecarboxylate 2.30b (98.9 mg, 0.371 mmol) in 4.7 mL toluene. This reaction resulted in 45.4 mg (53%) 2.6e. R_f = 0.33 (CH_2Cl_2). ¹H-NMR (CDCl_3, 300 MHz) δ 8.22 (s, 1H), 8.13 (br, 1H), 7.82 (dd, J = 8.5, 1.5 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H), 3.92 (s, 3H), 2.72-2.67 (m, 4H), 1.88-1.82 (m, 4H). Analytical data matched that reported for the known compound.¹⁵⁸
3-Methyl bromobenzoate (2.31) Following general procedure A, the reaction of 3-bromobenzoic acid (2.0 g, 10 mmol) resulted in 2.0 g (94%) 2.31. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.18 (s, 1H), 7.97 (d, $J = 8.0$ Hz, 1H), 7.69 (d, $J = 8.0$ Hz, 1H), 7.32 (t, $J = 8.0$ Hz, 1H), 3.93 (s, 3H).

Tert-butyl 1-(3-(methoxycarbonyl)phenyl)hydrazinecarboxylate (2.32) Following general procedure B, the reaction of aryl bromide 2.31 (1.01 g, 4.69 mmol) yielded 45.6 mg (4%) 2.32. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.18 (s, 1H), 7.78 (d, $J = 8.0$ Hz, 1H), 7.71 (d, $J = 8.0$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 1H), 4.44 (br, 2H), 3.91 (s, 3H), 1.52 (s, 9H). Analytical data matched that reported for the known compound.$^{155}$

Methyl 4-bromo-2-chlorobenzoate (2.35) Following general procedure A, the reaction of 4-bromo-2-chlorobenzoic acid (1.0 g, 4.2 mmol) resulted in 945.1 mg (90%) 2.35. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.72 (d, $J = 8.5$ Hz, 1H), 7.67 (d, $J = 1.5$ Hz, 1H), 7.46 (dd, $J = 8.5$, 1.5 Hz, 1H), 3.93 (s, 3H).
**Tert-butyl 1-(3-chloro-4-(methoxycarbonyl)phenyl)hydrazinecarboxylate (2.36)**

Following general procedure B, the reaction of aryl bromide 2.35 (940 mg, 3.77 mmol) yielded 716 mg (63%) 2.36. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.85-7.79 (m, 2H), 7.60 (dd, $J = 8.5, 2.0$ Hz, 1H), 4.37 (s, 2H), 3.91 (s, 3H), 1.55 (s, 9H).

**Methyl 4-chloro-2,3-dimethyl-1H-indole-5-carboxylate (2.37) and methyl 6-chloro-2,3-dimethyl-1H-indole-5-carboxylate (2.38)** Following general procedure C, the reaction of hydrazinecarboxylate 2.36 (386 mg, 1.28 mmol) with 2-butanone (139 µL, 1.92 mmol) produced 50.2 mg (16%) 2.37 and 41.9 mg (14%) 2.38. 2.37: $R_f$ = 0.25 (hex:EA, 3:1). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 8.03 (s, 2H), 7.27 (s, 1H), 3.94 (s, 3H), 2.34 (s, 3H), 2.20 (s, 3H).

2.38: $R_f$ = 0.21 (hex:EA, 3:1). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.89 (br, 1H), 7.55 (d, $J = 8.5$ Hz, 1H), 7.12 (d, $J = 8.5$ Hz, 1H), 3.92 (s, 3H), 2.50 (s, 3H), 2.36 (s, 3H).

**Methyl 4-aminobenzoate (4.39)** Following general procedure A, the reaction of para-aminobenzoic acid (997 mg, 7.27 mmol) resulted in 1.06 g (96%) 2.39. $^1$H-NMR (CDCl$_3$,
300 MHz) δ 7.85 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.5 Hz, 2H), 4.06 (br, NH₂) 3.85 (s, 3H).

Methyl 4-amino-3-iodobenzoate (2.40) To a solution of para-amino benzoic acid methyl ester (202 mg, 1.34 mmol) in 2.0 mL MeOH was added a solution of CaCO₃ (100 mg, 1.49 mmol) in 0.7 mL H₂O·ICl (1M in CH₂Cl₂, 1.50 mL, 1.13 mmol) was added and the mixture was stirred over night. The mixture was diluted with water and extracted with Et₂O. The combined organic layers were dried over MgSO₄, filtered and concentrated. The crude material was purified by column chromatography (hex/EtOAc, 4:1 → 1:1) to produce 292 mg (79%) 2.40 as a rose solid. ¹H-NMR (CDCl₃, 300 MHz) δ 8.33 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 4.52 (br, 2H), 3.86 (s, 3H).

Methyl 4-(allylamino)-3-iodobenzoate (2.41) Allylbromide (0.31 mL, 3.6 mmol) was added to a solution of 2.40 (1.0 g, 3.6 mmol) and K₂CO₃ (0.51 g, 3.7 mmol) in 25 mL CH₃CN. The reaction was refluxed for 4 d, filtered through celite and concentrated. The crude product was purified by chromatography on silica gel (hex/EtOAc, 4:1 → 1:1) to produce 0.27 g (24%) fairly clean 2.41. The product was identified by comparison of the
analytical data to the known compound and utilized in the next reaction without further purification.\(^{76}\)

![Methyl 3-methyl-1H-indole-5-carboxylate (2.42)](image)

**Methyl 3-methyl-1H-indole-5-carboxylate (2.42)** A mixture of 2.41 (272 mg, 0.857 mmol), Pd(OAc)\(_2\) (7.8 mg, 4 mol%), \(n\)Bu\(_4\)NBr (281 mg, 0.873 mmol) and Na\(_2\)CO\(_3\) (229 mg, 2.16 mmol) in 1.4 mL DMF was stirred for 3 d. The reaction was filtered through celite, diluted with CH\(_2\)Cl\(_2\) and washed three times with water. The organic layer was concentrated and the crude was purified with column chromatography to yield 24 mg (15%) 2.42. \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 8.36 (s, 1H), 8.08 (br, 1H), 7.90 (dd, \(J = 8.5, 1.0\) Hz, 1H), 7.35 (d, \(J = 8.5\) Hz, 1H), 7.03 (s, 1H), 3.94 (s, 3H), 2.37 (s, 3H). Analytical data matched that reported for the known compound.\(^{76}\)

**General Procedure D - N-methylation of indoles**

The indole was added to a solution of sodium hydride (150 mol%) in dry THF (0.2M). Upon completion of hydrogen development, methyl iodine (130 mol%) was added. The reaction was stirred at 140 °C for 20 min under microwave conditions. It was quenched with water and extracted with EtOAc and CH\(_2\)Cl\(_2\). The combined organic layers were dried over MgSO\(_4\), filtered and concentrated to give clean crude product, which was used without further purification.
**Methyl 1-methyl-1H-indole-5-carboxylate (2.43a)** Following *general procedure D*, the reaction of methyl 1H-indole-5-carboxylate (20 mg, 0.11 mmol) produced 21 mg (99%) 2.43a. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.39 (s, 1H), 7.93 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.33 (d, $J = 8.5$ Hz, 1H), 7.11 (d, $J = 3.0$, Hz, 1H), 6.59 (d, $J = 3.0$ Hz, 1H), 3.93 (s, 3H), 3.83 (s, 3H).

**Methyl 1,2,3-trimethyl-1H-indole-5-carboxylate (2.43b)** Following *general procedure D*, the reaction of 2.6b (250 mg, 1.23 mmol) produced 175 mg (65%) 2.43b. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.25 (s, 1H), 7.85 (d, $J = 8.5$ Hz, 1H), 7.22 (d, $J = 8.5$ Hz, 1H), 3.93 (s, 3H), 3.67 (s, 3H), 2.35 (s, 3H), 2.28 (s, 3H).

**Methyl indoline-5-carboxylate (2.44)** Methyl 1H-indole-5-carboxylate (201 mg, 1.15 mmol) was dissolved in AcOH (2 mL) and cooled to 0 °C. Then NaCNBH$_3$ (218 mg, 3.46 mmol) was added in small portions and the mixture was stirred for 1 h at room temperature. 2mL water were added and the mixture was evaporated. The residue was taken up with 30mL aqueous saturated NaHCO$_3$ and 30mL Et2O and separated. It was extracted two more times with Et2O. The combined organic layers were washed with
brine, dried over MgSO₄, filtered and concentrated. The crude product was purified via column chromatography (hex → hex:EA 1:1 with 0.1% TEA) to yield 156 mg (77%) 2.44. Rₓ = 0.43 (hex:EA 1:1 with 0.1% TEA). ¹H-NMR (CDCl₃, 300 MHz) δ 7.76-7.75 (m, 2H), 6.55 (d, J = 8.5 Hz, 1H), 4.11 (br, 1H), 3.85 (s, 3H), 3.65 (t, J = 8.5 Hz, 2H), 3.06 (t, J = 8.5 Hz, 2H). Analytical data matched that reported for the known compound.¹⁵⁹

1-Tert-butyl 5-methyl indoline-1,5-dicarboxylate (2.45) Boc₂O (196 mg, 0.899 mmol) was added to a solution of 2.44 (156 mg, 0.880 mmol) in 0.5 mL THF. The reaction was stirred for 6 h, concentrated and purified by column chromatography (hex:EA 4:1 → hex:EA 1:1) to result in 222 mg (91%) 2.55. Rₓ = 0.60 (hex:EA 1:1). ¹H-NMR (CDCl₃, 500 MHz) δ 7.91 (d, J = 8.0 Hz, 1H), 7.83 (s, 1H), 7.24 (s, 1H and CHCl₃), 4.05 (t, J = 8.5 Hz, 2H), 3.91 (s, 3H), 3.14 (t, J = 9.5 Hz, 2H), 1.60 (s, 9H).

Methyl 2-methylimidazo[1,2-a]pyridine-6-carboxylate (2.47) A solution of methyl 6-aminonicotinate (202 mg, 1.33 mmol) and 1-chloropropan-2-one (635 µL, 7.89 mmol) in 4.4 mL MeOH were heated for 24 h. The reaction was concentrated and the crude product was dissolved with water, basified with Na₂CO₃ and extracted with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, concentrated and purified with
flash chromatography (2% MeOH in CH₂Cl₂) to produce 55mg (22%) 2.47. \( R_f = 0.23 \) (2% MeOH in CH₂Cl₂). \(^1\)H-NMR (CDCl₃, 300 MHz) \( \delta \) 8.83 (s, 1H), 7.67 (dd, \( J = 9.5 \), 1.5 Hz, 1H), 7.50 (d, \( J = 9.5 \) Hz, 1H), 7.41 (s, 1H), 3.94 (s, 3H), 2.48 (s, 3H).

**Methyl 1H-indazole-5-carboxylate (2.48)** Following general procedure A, the reaction of 1H-indazole-5-carboxylic acid (104 mg, 0.644 mmol) resulted in 95.0 mg (84%) ic. \(^1\)H-NMR (CDCl₃, 300 MHz) \( \delta \) 10.34 (br, 1H), 8.56 (s, 1H), 8.18 (s, 1H), 8.09 (d, \( J = 9.0 \) Hz, 1H), 7.52 (d, \( J = 9.0 \) Hz, 1H), 3.96 (s, 3H).

**Methyl 1H-benzo[d][1,2,3]triazole-5-carboxylate (2.49)** Following general procedure A, the reaction of 1H-benzo[d][1,2,3]triazole-5-carboxylic acid (204 mg, 1.50 mmol) resulted in 8.2 mg (3%) 2.49 only after washing the aqueous layer with organic solvent again. \(^1\)H-NMR (CDCl₃, 300 MHz) \( \delta \) 8.73 (s, 1H), 8.18 (d, \( J = 8.5 \) Hz, 1H), 8.07 (d, \( J = 8.5 \) Hz, 1H), 7.67 (d, \( J = 8.5 \) Hz, 1H), 4.00 (s, 3H).

**Methyl 4-amino-3-hydroxybenzoate (2.50)** Following general procedure A, the reaction of 4-amino-3-hydroxybenzoic acid (1001 mg, 6.538 mmol) and 2 mL H₂SO₄
conc. (36.00 mmol) resulted in 606.1 mg (55%) **2.50.** \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.53-7.50 (m, 2H), 6.68 (d, \(J = 8.0\) Hz, 1H), 5.62 (br, 1H), 4.18 (br, 2H), 3.86 (s, 3H).

**General Procedure E - Bromination of ketones**

A solution of ketone in water (4.25M) and AcOH (18.25M) was heated to a temperature between 70 °C and 80 °C. Bromine (100 mol%) was added slowly and it was stirred additional 20 min until complete decolorization. The reaction was cooled to 0 °C, diluted with 20 mL water and neutralized with approx. 25 g Na\(_2\)CO\(_3\). The curde oil was separated from the aqueous layer, dried over CaCl\(_2\), filtered and purified by destillation under reduced pressure.

![2.53a](image)

**1-Bromopropan-2-one (2.53a)** Following **general procedure E**, the reaction of acetone (12.5 mL, 170 mmol) resulted in 10.5 g (45%) **2.53a.** \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 3.89 (s, 2H), 2.37 (s, 3H).

![2.53b](image) ![2.53c](image)

**3-Bromobutan-2-one (2.53b) and 1-Bromobutane-2-one (2.53c)** Following **general procedure E**, the reaction of acetone (15.3 mL, 171 mmol) resulted in 13.8 g (54%) of a
not separated mixture of 2.53b:2.53c (3:1). 2.53b: $^1$H-NMR (CDCl$_3$, 300 MHz) δ 4.40 (q, $J = 7.0$ Hz, 1H), 2.38 (d, $J = 0.5$ Hz, 3H), 1.75 (dd, $J = 7.0$, 0.5 Hz, 3H).

2.53c: $^1$H-NMR (CDCl$_3$, 300 MHz) δ 3.89 (s, 2H), 2.69 (q, $J = 7.0$ Hz, 2H), 1.12 (t, $J = 7.0$ Hz, 3H).

![Image of 2.57]

**Methyl 4-hydroxybenzoate (2.57)** Following general procedure A, the reaction of para-hydroxybenzoic acid (13.8 g, 100 mmol) resulted in 14.8 g (97%) 2.47. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.95 (d, $J = 8.0$ Hz, 2H), 6.89 (d, $J = 8.0$ Hz, 2H), 6.49 (br, OH) 3.90 (s, 3H).

![Image of 2.60]

**Methyl 3-aminobenzoate (2.60)** Following general procedure A, the reaction of meta-aminobenzoic acid (1.00 g, 7.30 mmol) and H$_2$SO$_4$ conc. (730 µL, 13.1 mmol) resulted in 1.09 g (99%) 2.50. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.42 (d, $J = 7.5$ Hz, 1H), 7.35 (s, 1H), 7.22 (dd, $J = 15.5$, 7.5 Hz, 1H), 6.87-6.84 (m, 1H), 3.89 (s, 3H), 3.77 (br, NH$_2$).

![Image of 2.61a]

**Methyl 4-acetamidobenzoate (2.61a)** A solution of 2.39 (202 mg, 1.47 mmol) and AcOAc (138 µL, 1.46 mmol) in 6.6 mL CH$_2$Cl$_2$ was stirred for 4h. The reaction mixture
was washed with sat. aq. Na₂CO₃ and the organic layers were dried over MgSO₄, filtered and concentrated to produce 235 mg (89%) 2.61a. Compound was made according to the known procedure, analytical data matching.⁸⁵

**General Procedure F - Synthesis of Amides**

A solution of aniline derivative in CH₂Cl₂ (0.8M) was cooled to 0 °C and NEt₃ (100 mol%) followed by acid chloride (110 mol%) were added. The reaction was stirred at 0 °C for 5 min and at room temperature for 4 h. The precipitate was filtered off and the filtrate was washed with 10% HCl. The organic layer was dried over MgSO₄, filtered and concentrated to produce the desired product.

**Methyl 4-butyramidobenzoate (2.61b)** Following general procedure F, the reaction of 2.39 (776 µL) with butyryl chloride produced 1.16 g (79%) 2.61b. ¹H-NMR (CDCl₃, 300 MHz) δ 8.00 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.21 (br, 1H), 3.90 (s, 3H), 2.37 (t, J = 7.5 Hz, 2H), 1.84-1.71 (m, 2H), 1.02 (t, J = 7.5 Hz, 3H).

**Methyl 4-benzamidobenzoate (2.61c)** Following general procedure F, the reaction of 2.39 (953 µL) with benzoyl chloride produced 586 mg (34%) 2.61c. ¹H-NMR (CDCl₃,
300 MHz) δ 8.04 (d, J = 7.5 Hz, 2H), 7.88 (d, J = 7.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.59-7.44 (m, 3H), 3.92 (s, 3H). Analytical data matched that reported for the known compound.\textsuperscript{160}

Methyl 3-acetamidobenzoate (2.62a) A solution of 2.60 (400 mg, 2.65 mmol) and AcOAc (300 µL, 3.00 mmol) in 6.6 mL CH₂Cl₂ was stirred for 4 h. The reaction mixture was washed with sat. aq. Na₂CO₃ and the organic layers were dried over MgSO₄, filtered and concentrated to produce 484 mg (69%) 2.62a. \(^1\)H-NMR (CDCl₃, 300 MHz) δ 8.00 (s, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H), 7.46-7.37 (m, 2H), 3.91 (s, 3H), 2.20 (s, 3H). Analytical data matched that reported for the known compound.\textsuperscript{161}

Methyl 3-butyramidobenzoate (2.62b) Following general procedure F, the reaction of 2.60 (776 µL) with butyryl chloride produced 1.43 g (98%) 2.62b. \(^1\)H-NMR (CDCl₃, 300 MHz) δ 8.03 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 3.90 (s, 3H), 2.36 (t, J = 7.5 Hz, 2H), 1.83-1.64 (m, 2H), 1.01 (t, J = 7.5 Hz, 3H).
Methyl 3-benzamidobenzoate (2.62c) Following general procedure 6, the reaction of 2.60 (953 μL) with benzoyl chloride produced 1.19 g (70%) 2.62c. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 8.15 (s, 1H), 8.06 (d, $J = 8.0$ Hz, 1H), 7.89 (d, $J = 7.0$ Hz, 2H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.60-7.44 (m, 4H), 3.93 (s, 3H). Analytical data matched that reported for the known compound.$^{161}$

**General Procedure G - Synthesis of sulfonyl amides**

A solution of aniline derivative in CH$_2$Cl$_2$ (0.4M) was cooled to 0 °C and sulfonyl chloride (120 mol%) and pyridine (110 mol%) were added. The reaction was stirred at room temperature over night, washed with 2N HCl and aqueous saturated NaHCO$_3$, water and brine. The combined organic layers were dried over MgSO$_4$, filtered and concentrated to produce the desired product.

Methyl 4-(methylsulfonylamido)benzoate (2.63a) Following general procedure G, the reaction of 2.39 (100 mg, 0.662 mmol) with methanesulfonyl chloride produced 112 mg (74%) 2.63a. Compound was made according to the known procedure, analytical data matching.$^{86}$
Methyl 4-(4-methylphenylsulfonamido)benzoate (2.63b) Following general procedure G, the reaction of 2.39 (200 mg) with 4-toluenesulfonyl chloride produced 100 mg (25%) 2.63b. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.92 (d, $J = 8.5$ Hz, 2H), 7.70 (d, $J = 8.0$ Hz, 2H), 7.26-7.23 (m, 3H), 7.11 (d, $J = 8.5$ Hz, 2H), 6.71 (s, 1H), 3.87 (s, 3H), 2.38 (s, 3H).

Methyl 3-(methylsulfonamido)benzoate (2.64a) Following general procedure G, the reaction of 2.60 (300 mg) with methyl sulfonylchloride produced 360 mg (79%) 2.64a. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.89-7.84 (m, 2H), 7.52-7.43 (m, 2H), 6.64 (s, 1H), 3.94 (s, 3H), 3.04 (s, 3H). Analytical data matched that reported for the known compound.$^{161}$

Methyl 3-(4-methylphenylsulfonamido)benzoate (2.64b) Following general procedure 7, the reaction of 2.60 (300 mg) with toluene sulfonylchloride produced 280 mg (46%) 2.64b. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.66 (d, $J = 8.0$ Hz, 2H), 7.40-7.39 (m, 3H), 7.38-7.31 (m, 2H), 6.71 (s, 1H), 3.89 (s, 3H), 2.38 (s, 3H). Analytical data matched that reported for the known compound.$^{161}$
**General Procedure H - Synthesis of urea derivatives**

To a solution of amine in CH$_2$Cl$_2$ (0.5M) the isocyanate (120 mol%) was added slowly. The reaction was stirred over night or until the product precipitated. The reaction was filtered and the product was washed with little CH$_2$Cl$_2$ and dried under vacuum.

![2.65a](image)

**Methyl 4-(3-benzylureido)benzoate (2.65a)** Following *general procedure H*, a solution of **2.39** (200 mg, 1.32 mmol) and benzyl isocyanate (193 µL, 1.58 mmol) produced 277 mg (74%) **2.65a**. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 8.99 (s, 1H), 7.83 (d, $J = 8.5$ Hz, 2H), 7.53 (d, $J = 8.5$ Hz, 2H), 7.37-7.24 (m, 5H), 6.77 (t, $J = 5.5$ Hz, 1H), 4.31 (d, $J = 5.5$ Hz, 2H), 3.80 (s, 3H).

![2.65b](image)

**Methyl 4-(3-(furan-2-ylmethyl)ureido)benzoate (2.65b)** Following *general procedure H*, the reaction between **2.39** (202 mg, 1.33 mmol) and furfuryl isocyanate lead to 316 mg (86%) **2.65b**. $^1$H-NMR (DMSO-d$_6$, 500 MHz) δ 8.97 (s, 1H), 7.84 (d, $J = 8.5$ Hz, 2H), 7.59 (s, 1H), 7.52 (d, $J = 8.5$ Hz, 2H), 6.72 (s, 1H), 6.40 (s, 1H), 6.27 (d, $J = 2.5$ Hz, 1H), 4.30 (d, $J = 5.5$ Hz, 2H), 3.80 (s, 3H).
Methyl 4-(3-(4-methoxybenzyl)ureido)benzoate (2.65c) Following general procedure H, the reaction between 2.39 (200 mg, 1.33 mmol) and 1-(isocyanatomethyl)-4-methoxybenzene lead to 366 mg (88%) 2.65c. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta \) 8.97 (s, 1H), 7.83 (d, \(J = 8.5\) Hz, 2H), 7.52 (d, \(J = 8.5\) Hz, 2H), 7.23 (d, \(J = 8.5\) Hz, 2H), 6.89 (d, \(J = 8.5\) Hz, 2H), 6.71 (t, \(J = 5.5\) Hz, 1H), 4.23 (d, \(J = 5.5\) Hz, 2H), 3.80 (s, 3H), 3.73 (s, 3H).

Methyl 4-(3-(4-methylbenzyl)ureido)benzoate (2.65d) Following general procedure H, the reaction between methyl 2.39 (203 mg, 1.34 mmol) and 1-(isocyanatomethyl)-4-methylbenzene lead to 361 mg (90%) 2.65d. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta \) 8.99 (s, 1H), 7.83 (d, \(J = 8.5\) Hz, 2H), 7.53 (d, \(J = 8.5\) Hz, 2H), 7.19 (d, \(J = 8.0\) Hz, 2H), 7.14 (d, \(J = 8.0\) Hz, 2H), 6.73 (t, \(J = 5.5\) Hz, 1H), 4.26 (d, \(J = 5.5\) Hz, 2H), 3.80 (s, 3H), 2.28 (s, 3H).
Methyl 3-(3-benzylureido)benzoate (2.66) Following general procedure H, the reaction between 2.60 (200 mg, 1.32 mmol) and benzyl isocyanate lead to 324 mg (86%) 2.66. \(^1\)H-NMR (DMSO-d\(_6\), 500 MHz) \(\delta\) 8.85 (s, 1H), 8.14 (s, 1H), 7.59 (d, \(J = 8.0\) Hz, 1H), 7.49 (d, \(J = 7.5\) Hz, 1H), 7.38-7.24 (m, 6H), 6.68 (br, 1H), 4.30 (d, \(J = 5.5\) Hz, 2H), 3.83 (s, 3H).

Methyl 3-(3-benzylthioureido)benzoate (2.68) Following general procedure 8, a solution of 2.60 (200 mg, 1.32 mmol) and phenyl isothiocyanate (187 \(\mu\)L, 1.58 mmol) produced 323 mg (85%) 2.68. \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 9.93 (s, 2H), 8.13 (s, 1H), 7.77 (d, \(J = 8.0\) Hz, 1H), 7.70 (d, \(J = 8.0\) Hz, 1H), 7.50-7.46 (m, 3H), 7.37-7.32 (m, 2H), 7.15 (t, \(J = 7.0\) Hz, 1H), 3.86 (s, 3H).

**General Procedure I - Synthesis of naphthoates**

A mixture of starting hydroxy naphthoic acid, Me\(_2\)SO\(_4\) (230 mol%) and K\(_2\)CO\(_3\) (500 mol%) in acetone (0.17M) was heated to reflux over night. The mixture was cooled to room temperature and then quenched with water. Acetone was removed under reduced pressure, it was diluted with water and extracted with CH\(_2\)Cl\(_2\). The combined organic layers were washed with water and brine, dried over MgSO\(_4\), filtered and concentrated. The crude product was purified by chromatography (hex:EtOAc, 9:1 \(\rightarrow\) 5:1).
Methyl 1-methoxy-2-naphthoate (2.69a) Following general procedure I, the reaction of 1-hydroxy-2-naphthoic acid (504 mg, 2.68 mmol) resulted in 557 mg (96%) 2.69a. 

$^{1}$H-NMR (CDCl$_3$, 500 MHz) $\delta$ 8.28 (d, $J = 7.5$ Hz, 1H), 7.87-7.85 (m, 2H), 7.63-7.55 (m, 3H), 7.26 (s, 1H), 4.07 (s, 3H), 3.99 (s, 3H). Analytical data matched that reported for the known compound.$^{162}$

Methyl 3-methoxy-2-naphthoate (2.69b) Following general procedure I, the reaction of 3-hydroxy-2-naphthoic acid (502 mg, 2.67 mmol) resulted in 520 mg (90%) 2.69b.

$^{1}$H-NMR (CDCl$_3$, 500 MHz) $\delta$ 8.31 (s, 1H), 7.82 (d, $J = 8.0$ Hz, 1H), 7.74 (d, $J = 8.0$ Hz, 1H), 7.52 (d, $J = 7.0$ Hz, 1H), 7.38 (d, $J = 7.0$ Hz, 1H), 7.26 (s, 1H), 7.21 (s, 1H), 4.00 (s, 3H), 3.96 (s, 3H). Analytical data matched that reported for the known compound.$^{87}$

Methyl 6-methoxy-2-naphthoate (2.69c) Following general procedure I, the reaction of 6-hydroxy-2-naphthoic acid (503 mg, 2.67 mmol) resulted in 557 mg (96%) 2.69c without further purification. $^{1}$H-NMR (CDCl$_3$, 500 MHz) $\delta$ 8.53 (s, 1H), 8.03 (d, $J = 8.5$ Hz, 1H), 7.84 (d, $J = 8.5$ Hz, 1H), 7.76 (d, $J = 8.5$ Hz, 1H), 7.26 (s, 1H), 7.19 (d, $J = 8.5$ Hz, 1H), 7.16 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H).
**Ethyl benzoate (2.6)** A solution of benzoic acid (998 mg, 8.18 mmol) and NEt₃ (1.14 mL, 8.19 mmol) in 4 mL CH₃CN was cooled to 0 °C. A solution of ethyl chloroformate (783 µL, 8.19 mmol) in 4 mL CH₃CN was added and the mixture was stirred for 5 min, before a solution and DMAP (457 mg, 3.74 mmol) in 4 mL CH₃CN was added. The reaction was allowed to warm to room temperature and stirred over night. After concentration, it was diluted with 60 mL CH₂Cl₂, washed with 40 mL aq. sat. NaHCO₃ and 40 mL 0.1M HCl. The organic layer was dried over MgSO₄, filtered and concentrated to produce 887 mg (72%) ethyl benzoate. ¹H-NMR (CDCl₃, 300 MHz) δ 8.05-8.01 (m, 2H), 7.53-7.51 (m, 1H), 7.44-7.39 (m, 2H), 4.36 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H).

**General Procedure J - Synthesis of hydrazides**

A neat mixture of ester and hydrazine monohydrate (200 mol%) was heated to 120 °C over night. The product precipitated from the mixture, was filtered off and washed with little MeOH. Clean product was obtained without further purification.

**1H-indole-5-carboxylic acid (2.10)** Following general procedure J, the reaction methyl 1H-indole-5-carboxylate (47 mg, 0.27 mmol) yielded 46 mg (quant.) 2.10. ¹H-NMR (CDCl₃, 300 MHz) δ 11.29 (br, NH), 9.56 (br, NH), 8.08 (s, 1H), 7.60 (dd J = 8.5,
1.5 Hz, 1H), 7.41 (s, 1H), 7.39 (d, \( J = 4.5 \) Hz, 1H), 6.51-6.50 (m, 1H), 4.44 (br, NH\(_2\)).

\(^{13}\)C-NMR (CDCl\(_3\), 300 MHz) \( \delta \) 167.4, 137.4, 127.0, 126.6, 124.3, 120.2, 119.7, 111.0, 102.1. Analytical data matched that reported for the known compound.\(^{163}\)

![Diagram of 2.1](image)

2,3-Dimethyl-1H-indole-5-carbohydrazide (2.1) Following general procedure \( J \), the reaction 2.6b (203 mg, 2.01 mmol) with \( \text{N}_2\text{H}_4\cdot\text{H}_2\text{O} \) (454 mg, 440 µL, 9.07 mmol) at 150 °C produced 330 mg (81%) 2.1. \(^1\)H-NMR (DMSO d\(_6\), 300 MHz) \( \delta \) 10.87 (s, 1H), 9.51 (s, 1H), 7.93 (s, 1H), 7.50 (d, \( J = 8.5 \) Hz, 1H), 7.21 (d, \( J = 8.5 \) Hz, 1H), 4.38 (s, 2H), 2.31 (s, 3H), 2.17 (s, 3H). \(^{13}\)C NMR (DMSO-d\(_6\), 300 MHz) \( \delta \) 167.5, 136.7, 132.6, 128.4, 123.2, 119.3, 117.0, 109.6, 106.1, 11.21, 8.255.

![Diagram of 2.70a](image)

1-Methyl-1H-indole-5-carbohydrazide (2.70a) Following general procedure \( J \), the reaction of 2.43a (157 mg, 0.827 mmol) with hydrazine monohydrate (120 µL, 2.42 mmol) produced 141 mg (90%) 2.70a after crystallization at 5 °C. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \( \delta \) 9.61 (s, 1H), 8.10 (s, 1H), 7.68 (d, \( J = 8.5 \) Hz, 1H), 7.47 (d, \( J = 8.5 \) Hz, 1H), 7.41 (d, \( J = 3.0 \) Hz, 1H), 6.52 (d, \( J = 3.0 \) Hz, 1H), 4.42 (br, 1H), 3.82 (s, 3H).
1,2,3-Trimethyl-1H-indole-5-carbohydrazide (2.70b) Following general procedure J, the reaction 2.43b (150 mg, 0.688 mmol) with N₂H₄·H₂O (155 mg, 150 µL, 3.09 mmol) at 150 °C produced 85.3 mg (57%) 2.70b. ¹H-NMR (DMSO d₆, 300 MHz) δ 9.54 (s, 1H), 7.97 (s, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 4.39 (s, 2H), 3.65 (s, 3H), 2.33 (s, 3H), 2.20 (s, 3H). ¹³C-NMR (DMSO d₆, 75 MHz) δ 167.3, 137.6, 134.3, 127.3, 123.3, 119.3, 117.2, 108.3, 106.2, 29.50, 9.862, 8.563.

Indole-5-carbohydrazide (2.70c) Following general procedure J, the reaction of 2.6c (43.8 mg, 0.202 mmol) with hydrazine monohydrate (120 µL, 2.42 mmol) at 140 °C produced 43.8 mg (quant.) 2.70c. ¹H-NMR (DMSO-d₆, 300 MHz) δ 10.88 (s, 1H), 9.54 (s, 1H), 7.99 (s, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H), 4.40 (s, 2H), 2.67 (q, J = 7.5 Hz, 2H), 2.33 (s, 3H), 1.27 (t, J = 7.5 Hz, 3H).

1,2,3,4-Tetrahydrocyclopenta[b]indole-7-carbohydrazide (2.70d) Following general procedure J, the reaction of 2.6d (59.4 mg, 0.276 mmol) with hydrazine monohydrate (160 µL, 3.31 mmol) at 140 °C produced 6.4 mg (11%) 2.70d. ¹H-NMR (DMSO-d₆,
300 MHz) δ 11.05 (s, 1H), 9.52 (s, 1H), 7.88 (s, 1H), 7.51 (d, \( J = 8.5 \) Hz, 1H), 7.28 (d, \( J = 8.5 \) Hz, 1H), 4.39 (s, 2H), 2.86-2.74 (m, 4H), 2.51-2.46 (m, 2H and DMSO).

**2,3,4,9-Tetrahydro-1H-carbazole-6-carbohydrazide (2.70e)** Following general procedure \( J \), the reaction of 2.6e (229 mg, 0.998 mmol) with hydrazine monohydrate (290 μL, 5.98 mmol) at 140 °C produced 228 mg (quant.) 2.70e. \(^1\)H-NMR (DMSO-\( d_6 \), 300 MHz) δ 10.88 (s, 1H), 9.52 (s, 1H), 7.92 (s, 1H), 7.53 (d, \( J = 8.5 \) Hz, 1H), 7.24 (d, \( J = 8.5 \) Hz, 1H), 4.39 (s, 2H), 2.71-2.65 (m, 4H), 1.84-1.83 (m, 4H).

**6-Chloro-2,3-dimethyl-1H-indole-5-carbohydrazide (2.70f)** Following general procedure \( J \), the reaction of 2.37 (50 mg, 0.21 mmol) with hydrazine monohydrate (120 μL, 2.42 mmol) produced 49 mg (90%) 2.70f. \(^1\)H-NMR (CD\(_3\)OD, 300 MHz) δ 8.20 (br, 1H), 7.9 (br, 1H), 7.48 (s, 1H), 7.27 (s, 1H), 2.33 (s, 3H), 2.19 (s, 3H).

**4-Chloro-2,3-dimethyl-1H-indole-5-carbohydrazide (2.70g)** Following general procedure \( J \), the reaction of 2.38 (42 mg, 0.18 mmol) with hydrazine monohydrate (120
µL, 2.42 mmol) produced 28 mg (66%) 2.70g. 1H-NMR (CD3OD, 300 MHz) δ 7.18 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 2.44 (s, 3H), 2.33 (s, 3H).

2-Methylimidazo[1,2-α]pyridine-6-carbohydrazide (2.71a) Following general procedure J, the reaction of 2.47 (133 mg, 0.700 mmol) with hydrazine monohydrate (204 µL, 4.20 mmol) at 100°C produced 104 mg (78%) 2.71a. 1H-NMR (DMSO-d6, 300 MHz) δ 9.78 (br, 1H), 8.95 (s, 1H), 7.76 (s, 1H), 7.54 (dd, J = 9.5, 1.5 Hz, 1H), 7.43 (d, J = 9.5 Hz, 1H), 4.50 (s, 2H), 2.32 (s, 3H). 13C-NMR (CDCl3, 75 MHz) δ 164.1, 144.2, 143.7, 127.4, 121.9, 118.0, 115.1, 111.0, 14.2.

1H-Indazole-5-carbohydrazide (2.71b) Following general procedure J, the reaction of 2.48 (85 mg, 0.48 mmol) with hydrazine monohydrate (0.25 mL, 5.2 mmol) resulted in 64 mg (76%) 2.71b. mp 248.3-248.9°C (Lit. 251-252°C). Analytical data matched that reported for the known compound.164

Quinoline-6-carbohydrazide (2.71c) Following general procedure J, the reaction of methyl quinoline-6-carboxylate (154 mg, 0.824 mmol) with hydrazine monohydrate
(0.25 mL, 5.2 mmol) resulted in 68.1 mg (44%) 2.71c. mp 191.8-192.3°C (Lit. 193-194°C). Analytical data matched that reported for the known compound.\textsuperscript{164}

![Image](image.png)

1-Methoxy-2-naphthohydrazide (2.71d) Following general procedure J, the reaction of 2.69a (145 mg, 0.672 mmol) at 100 °C resulted in 62.8 mg (43%) 2.71d. \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}, 500 MHz) \(\delta\) 10.30 (br, 1H), 8.26 (d, \(J = 8.0\) Hz, 1H), 7.86 (d, \(J = 8.0\) Hz, 1H), 7.83 (d, \(J = 9.0\) Hz, 1H), 7.63 (d, \(J = 7.5\) Hz, 1H), 7.55 (d, \(J = 7.5\) Hz, 1H), 7.35 (d, \(J = 9.0\) Hz, 1H), 4.73 (br, 2H), 3.34 (s, 3H covered by H2O).

![Image](image.png)

3-Methoxy-2-naphthohydrazide (2.71e) Following general procedure J, the reaction of 2.69b (151 mg, 0.698 mmol) at 100 °C resulted in 41.5 mg (28%) 2.71e. \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}, 500 MHz) \(\delta\) 9.40 (br, 1H), 8.17 (s, 1H), 7.93 (d, \(J = 7.0\) Hz, 1H), 7.85 (d, \(J = 8.0\) Hz, 1H), 7.53 (d, \(J = 7.0\) Hz, 1H), 7.44 (s, 1H), 7.40 (d, \(J = 8.0\) Hz, 1H), 4.58 (s, 2H), 3.95 (s, 3H).

![Image](image.png)

6-Methoxy-2-naphthohydrazide (2.71f) Following general procedure J, the reaction of 2.69c (151 mg, 0.698 mmol) with hydrazine monohydrate (0.150 mL, 3.12 mmol) at
100 °C resulted in 135 mg (90%) $\text{2.71f}$. $^1\text{H}$-NMR (DMSO-d$_6$, 500 MHz) δ 9.82 (s, 1H), 8.35 (s, 1H), 7.92-7.84 (m, 3H), 7.37 (s, 1H), 7.22 (d, $J = 8.5$ Hz, 1H), 4.52 (s, 2H), 3.90 (s, 3H). Analytical data matched that reported for the known compound.$^{165}$

**General Procedure K - Synthesis of hydrazides under MW conditions**

A mixture of starting material and hydrazine monohydrate (0.36-0.72M) was heated under MW conditions for 15 min at 90 °C. After cooling, the product was filtered, washed with little MeOH and dried under vacuum.

$N$-(4-(Hydrazinecarbonyl)phenyl)acetamide (2.72a) Following general procedure K, the reaction of $\text{2.61a}$ (49.3 mg, 0.255 mmol) produced 38.0 mg (77%) $\text{2.72a}$ as clean white solid product. $^1\text{H}$-NMR (CDCl$_3$, 300 MHz) δ 10.13 (s, 1H), 9.62 (s, 1H), 7.77 (d, $J = 8.5$ Hz, 2H), 7.64 (d, $J = 8.5$ Hz, 2H), 4.41 (br, 2H), 2.30 (t, $J = 7.5$ Hz, 2H), 1.67-1.55 (m, 2H), 0.91 (t, $J = 7.5$ Hz, 3H).

$N$-(4-(Hydrazinecarbonyl)phenyl)butyramide (2.72b) Following general procedure K, the reaction of ester $\text{2.61b}$ (15 mg) produced 11 mg (70%) $\text{2.72b}$. $^1\text{H}$-NMR (DMSO-d$_6$, 300 MHz) δ 10.04 (s, 1H), 9.60 (s, 1H), 7.76 (d, $J = 8.5$ Hz, 2H), 7.64 (d, $J = 8.5$ Hz, 2H), 4.41 (br, 2H), 2.30 (t, $J = 7.5$ Hz, 2H), 1.67-1.55 (m, 2H), 0.91 (t, $J = 7.5$ Hz, 3H).
N-(4-(Hydrazinecarbonyl)phenyl)benzamide (2.72c) Following general procedure K, the reaction of ester 2.61c (15 mg) produced 4 mg (27%) 2.72c. $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ 10.41 (s, 1H), 9.65 (s, 1H), 7.96 (d, $J = 6.5$ Hz, 2H), 7.87-7.84 (m, 4H), 7.61-7.52 (m, 3H), 4.44 (s, 2H).

N-(3-(Hydrazinecarbonyl)phenyl)acetamide (2.72d) Following general procedure K, the reaction of ester 2.62a (200 mg) produced 102 mg (84%) 2.72d. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 10.03 (s, 1H), 9.69 (s, 1H), 7.98 (s, 1H), 7.73 (d, $J = 8.0$ Hz, 1H), 7.44 (d, $J = 8.0$ Hz, 1H), 7.33 (t, $J = 8.0$ Hz, 1H), 4.47 (s, 2H), 2.05 (s, 3H).

N-(3-(Hydrazinecarbonyl)phenyl)butyramide (2.72e) Following general procedure K, the reaction of ester 2.62b (700 mg) produced 646 mg (92%) 2.72e. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 9.96 (s, 1H), 9.68 (s, 1H), 8.00 (s, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.44 (d, $J = 8.0$ Hz, 1H), 7.33 (t, $J = 8.0$ Hz, 1H), 4.44 (br, 2H), 2.29 (t, $J = 7.5$ Hz, 2H), 1.68-1.55 (m, 2H), 0.92 (t, $J = 7.5$ Hz, 3H).
N-(3-(Hydrazinecarbonyl)phenyl)benzamide (2.72f) Following general procedure K, the reaction of ester 2.62c (600 mg) produced 461 mg (77%) 2.72f. ¹H-NMR (DMSO-d₆, 300 MHz) δ 10.36 (s, 1H), 9.72 (s, 1H), 8.23 (s, 1H), 7.99-7.92 (m, 3H), 7.63-7.51 (m, 4H), 7.41 (t, J = 8.0 Hz, 1H), 4.48 (s, 2H).

1-Benzyl-3-(4-(hydrazinecarbonyl)phenyl)urea (2.72g) Following general procedure K, the reaction of ester 2.65a (200 mg) produced 175 mg (88%) 2.72g. ¹H-NMR (DMSO-d₆, 300 MHz) δ 9.53 (s, 1H), 8.86 (s, 1H), 7.71 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.36-7.22 (m, 5H), 6.78 (t, J = 6.0 Hz, 1H), 4.31-4.29 (m, 4H).

1-(Furan-2-ylmethyl)-3-(4-(hydrazinecarbonyl)phenyl)urea (2.72h) Following general procedure K, 2.65b (102 mg, 0.373 mmol) was reacted at 100 °C to yield 30.0 mg (29%) 2.72h. ¹H-NMR (DMSO-d₆, 300 MHz) δ 9.56 (s, 1H), 8.79 (s, 1H), 7.59 (s, 1H), 7.43 (d, J = 8.5 Hz, 2H), 6.67 (br, 1H), 6.40 (s, 1H), 6.26 (d, J = 3.0 Hz, 1H), 4.38 (br, 2H), 4.29 (d, J = 5.5 Hz, 2H).
1-(4-(Hydrazinecarbonyl)phenyl)-3-(4-methoxybenzyl)urea (2.72i) Following general procedure K, 2.65c (97.5 mg, 0.310 mmol) was reacted at 100 °C to yield 68.0 mg (70%) 2.72i. $^1$H-NMR (DMSO-$d_6$, 300 MHz) δ 9.56 (br, 1H), 8.77 (br, 1H), 7.71 (d, $J = 8.5$ Hz, 2H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.23 (d, $J = 8.5$ Hz, 2H), 6.89 (d, $J = 8.5$ Hz, 2H), 6.64 (t, $J = 5.5$ Hz, 1H), 4.40 (br, 2H), 4.22 (d, $J = 5.5$ Hz, 2H), 3.73 (s, 3H).

1-(4-(Hydrazinecarbonyl)phenyl)-3-(4-methylbenzyl)urea (2.72j) Following general procedure K, 2.65d (98.8 mg, 0.331 mmol) was reacted at 100 °C to yield 86.0 mg (87%) 2.72j. $^1$H-NMR (DMSO-$d_6$, 300 MHz) δ 9.56 (br, 1H), 8.79 (br, 1H), 7.71 (d, $J = 8.5$ Hz, 2H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.20-7.12 (m, 4H), 6.67 (t, $J = 5.5$ Hz, 1H), 4.40 (br, 2H), 4.25 (d, $J = 5.5$ Hz, 2H), 2.27 (s, 3H).

1-Benzyl-3-(3-(hydrazinecarbonyl)phenyl)urea (2.72k) Following general procedure K, 2.66 (101 mg, 0.356 mmol) was reacted at 100 °C to yield 63.2 mg (63%) 2.72k. $^1$H-NMR (DMSO-$d_6$, 300 MHz) δ 9.67 (s, 1H), 8.72 (s, 1H), 7.79 (s, 1H), 7.58 (d,
$J = 7.5 \text{ Hz, 1H), 7.36-7.24 (m, 7H), 6.68 (t, } J = 6.0, \text{ 1H), 4.46 (br, 2H), 4.30 (d, } J = 6.0 \text{ Hz, 2H).}$

**Tert-butyl 5-(hydrazinecarbonyl)indoline-1-carboxylate (2.72l)** Following general procedure K, the reaction of 2.45 (100 mg, 0.360 mmol) at 100 °C yielded 52.6 mg (53%) 2.72l after purification by chromatography. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.59 (s, 1H), 7.52 (d, $J = 8.5 \text{ Hz, 1H)}, 7.20 (s, 1H), 4.05-3.99 (m, 3H), 3.12 (t, $J = 8.5 \text{ Hz, 2H), 1.56 (s, 9H and H}_2\text{O), 1.25 (s, 2H).}$

**General Procedure L - Synthesis of 5-aryl-1,3,4-oxadiaazole-2-thiols**

CS$_2$ (250 mol%) was added to a solution of KOH (230 mol%) and hydrazide in EtOH (0.25M) was added hydrazide. The reaction was heated to reflux for a certain amount of time. After disappearance of the starting material on TLC, the mixture was evaporated and acidified with 10% HCl. The product precipitated and was filtered off. After washing with a small amount of water it was.

**5-Phenyl-1,3,4-oxadiaazole-2-thiol (SKB-151)** Following general procedure L, the reaction of benzohydrazide (100 mg, 0.741 mmol) produced 119 mg (90%) SKB-151.
$^1$H-NMR (CDCl$_3$, 300 MHz) δ 10.37 (br, NH), 7.94-7.90 (m, 2H), 7.57-7.47 (m, 3H); IR (KBr, CH$_2$Cl$_2$, cm$^{-1}$) 3078, 2947, 1512, 1350, 1188, 972, 694; LC-MS m/z: 177 (M$^+$).

Analytical data matched that reported for the known compound.$^{166}$

5-($1^H$-Indol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-111) Following general procedure L, the reaction of hydrazide 2.10 (37 mg, 0.21 mmol) produced 37 mg (80%) SKB-111.

$^1$H-NMR (DMSO d$_6$, 300 MHz) δ 14.52 (br, 1H), 11.55 (s, 1H), 8.13 (s, 1H), 7.63-7.55 (m, 2H), 7.51 (t, $J$ = 2.5 Hz, 1H), 6.61 (s, 1H). $^{13}$C-NMR (DMSO d$_6$, 300 MHz) δ 177.1, 162.2, 137.7, 127.6, 127.5, 119.2, 118.7, 113.1, 112.5, 102.4, 99.53.

5-(2,3-Dimethyl-1$^H$-indol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-104) Following general procedure L, the reaction of hydrazide 2.1 (324 mg, 1.60 mmol) produced 394 mg (quant.) SKB-104. $^1$H-NMR (DMSO d$_6$, 300 MHz) δ 14.51 (br, 1H), 11.18 (s, 1H), 7.90 (s, 1H), 7.52 (d, $J$ = 8.5 Hz, 1H), 7.39 (d, $J$ = 8.5 Hz, 1H), 2.33 (s, 3H), 2.20 (s, 3H). $^{13}$C-NMR (DMSO d$_6$, 300 MHz) δ 177.0, 162.4, 137.1, 133.9, 128.9, 117.9, 116.2, 112.2, 111.3, 106.6, 11.20, 8.134. HRMS m/z: [M$_2$+Na]$^+$ calcd for (C$_{12}$H$_{11}$N$_3$OS)$_2$Na 513.113766u, found 513.113767u. MP 223.5-224.8°C (decomposition).
5-(1-Methyl-1H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-145) Following general procedure L, the reaction of 2.70a (189 mg, 0.733 mmol) produced 139 mg (82%) SKB-145 after 20 h of reflux. \(^1H\)-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 14.56 (br, 1H), 8.13 (s, 1H), 7.68-7.62 (m, 2H), 7.49 (d, \(J = 3.0\) Hz, 1H), 6.62 (d, \(J = 3.0\) Hz, 1H), 3.85 (s, 3H).

5-(1,2,3-Trimethyl-1H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-132) Following general procedure L, the reaction of hydrazide 2.70b (80 mg, 0.37 mmol) produced 90 mg (94%) SKB-132 after 2 d. \(^1H\)-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 14.47 (br, 1H), 7.93 (s, 1H), 7.60-7.52 (m, 2H), 3.69 (s, 3H), 2.36 (s, 3H), 2.24 (s, 3H).

5-(3-Ethyl-2-methyl-1H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-143) Following general procedure L, the reaction of 2.70c (43.4 mg, 0.120 mmol) produced 41.6 mg (80%) SKB-143 after 20 h of reflux. \(^1H\)-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 14.53 (br, 1H),
11.19 (s, 1H), 7.93 (s, 1H), 7.52 (d, \( J = 8.5 \) Hz, 1H), 7.39 (d, \( J = 8.5 \) Hz, 1H), 2.70 (q, \( J = 7.5 \) Hz, 2H), 2.34 (s, 3H), 1.16 (t, \( J = 7.5 \) Hz, 3H).

5-(2,3,4,9-Tetrahydro-1H-carbazol-6-yl)-1,3,4-oxadiazole-2-thiol (SKB-142)

Following general procedure \( L \), the reaction of \( 2.70e \) (175 mg, 0.763 mmol) produced 207 mg (quant.) SKB-142 after 20 h of reflux. \(^1\)H-NMR (DMSO-\( d_6 \), 300 MHz) \( \delta \) 14.53 (br, 1H), 11.18 (s, 1H), 7.88 (s, 1H), 7.53 (d, \( J = 8.5 \) Hz, 1H), 7.40 (d, \( J = 8.5 \) Hz, 1H), 2.71-2.67 (m, 4H), 1.83-1.82 (m, 4H).

5-(4-Chloro-2,3-dimethyl-1H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (2.73)

Following general procedure \( L \), the reaction of \( 2.70g \) (25 mg, 0.11 mmol) produced 23 mg (78%) 2.73 after 20 h. \(^1\)H-NMR (DMSO-\( d_6 \), 300 MHz) \( \delta \) 14.70 (br, 1H), 11.49 (s, 1H), 7.43-7.34 (m, 2H), 2.43 (s, 3H), 2.33 (s, 3H).
5-(2-Methylimidazo[1,2-a]pyridin-6-yl)-1,3,4-oxadiazole-2-thiol (SKB-144)

Following general procedure L, the reaction of 2.71a (91.5 mg, 0.481 mmol) produced 62.5 mg (56%) SKB-144 after 20 h of reflux. $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$ 9.55 (s, 1H), 8.13-8.09 (m, 2H), 7.97 (d, $J = 9.5$ Hz, 1H), 3.33 (s, 3H included in residue H2O peak).

5-(1H-Indazol-5-yl)-1,3,4-oxadiazole-2-thiol (SKB-156) Following general procedure L, the reaction of 2.71b (48 mg, 0.27 mmol) resulted in 58 mg (98%) SKB-156 after 20 h of reflux. $^1$H-NMR (DMSO-$d_6$, 500 MHz) $\delta$ 8.37 (s, 1H), 8.27 (s, 1H), 7.83 (d, $J = 8.5$ Hz, 1H), 7.73 (d, $J = 8.5$ Hz, 1H). Analytical data matched that reported for the known compound.$^{164}$

5-(Quinolin-6-yl)-1,3,4-oxadiazole-2-thiol (SKB-157) Following general procedure L, the reaction of 2.71c (51.4 mg, 0.275 mmol) resulted in 60.9 mg (97%) SKB-157 after
20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 500 MHz) $\delta$ 9.15 (s, 1H), 8.85 (d, $J = 7.0$ Hz, 1H), 8.74 (s, 1H), 8.29 (s, 2H), 7.84-7.83 (m, 1H).

5-(6-Methoxynaphthalen-2-yl)-1,3,4-oxadiazole-2-thiol (SKB-158) Following general procedure L, the reaction of 2.71e (36.4 mg, 0.169 mmol) resulted in 35.5 mg (82%) SKB-158 after 20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.80 (br, 1H), 8.43 (s, 1H), 8.05 (d, $J = 8.5$ Hz, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.64-7.59 (m, 2H), 7.46 (d, $J = 7.5$ Hz, 1H), 3.99 (s, 3H).

5-(3-Methoxynaphthalen-2-yl)-1,3,4-oxadiazole-2-thiol (SKB-159) Following general procedure L, the reaction of 2.71f (80.2 mg, 0.371 mmol) resulted in 81.4 mg (85%) SKB-159 after 20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.75 (br, 1H), 8.46 (s, 1H), 8.08 (d, $J = 9.0$ Hz, 1H), 7.99 (d, $J = 8.5$ Hz, 1H), 7.88 (d, $J = 8.5$ Hz, 1H), 7.45 (d, $J = 2.5$ Hz, 1H), 7.29 (dd, $J = 9.0$, 2.5 Hz, 1H), 3.92 (s, 3H).
\[N-(4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)acetamide \quad (SKB-107)\]

Following general procedure \(L\), the reaction of hydrazide 2.72a (21 mg) produced 22 mg (90%)
\[SKB-107\] after 3 h of reflux. \(^1\)H-NMR (DMSO-\(d_6\), 300 MHz) \(\delta\) 14.64 (br, 1H), 10.31 (s, 1H), 7.84-7.76 (m, 4H), 2.09 (s, 3H).

\[N-(4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)butyramide \quad (SKB-152)\]

Following general procedure \(L\), the reaction of hydrazide 2.72b (501 mg) produced 372 mg (62%)
\[SKB-152\] after 3 h of reflux. \(^1\)H-NMR (DMSO-\(d_6\), 300 MHz) \(\delta\) 14.63 (br, 1H), 10.23 (s, 1H), 7.84-7.80 (m, 3H), 7.64 (d, \(J = 8.5\) Hz, 2H), 2.36-2.33 (m, 2H), 1.69-1.56 (m, 2H), 0.92 (t, \(J = 7.5\) Hz, 3H).

\[N-(4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)benzamide \quad (SKB-153)\]

Following general procedure \(L\), the reaction of hydrazide 2.72c (255 mg) produced 234 mg (91%)
SKB-153 after 3 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.67 (br, 1H), 10.59 (s, 1H), 8.04-7.96 (m, 4H), 7.88 (d, $J = 8.5$ Hz, 2H), 7.65-7.53 (m, 3H).

$N$-(3-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-177) Following general procedure L, the reaction of hydrazide 2.72d (115 mg) produced 97 mg (69%) SKB-117 after 3 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.69 (br, 1H), 10.21 (s, 1H), 8.26 (s, 1H), 7.73 (d, $J = 7.5$ Hz, 1H), 7.55-7.47 (m, 2H), 2.08 (s, 3H).

$N$-(3-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)butyramide (SKB-155) Following general procedure L, the reaction of hydrazide 2.72e (501 mg) produced 338 mg (54%) SKB-155 after 3 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.70 (br, 1H), 10.15 (s, 1H), 8.29 (s, 1H), 7.74 (d, $J = 7.5$ Hz, 2H), 7.55-7.47 (m, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 1.69-1.57 (m, 2H), 0.93 (t, $J = 7.5$ Hz, 3H).
N-(3-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)benzamide  (SKB-154) Following general procedure L, the reaction of hydrazide 2.72f (387 mg) produced 358 mg (80%) SKB-154 after 3 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) δ 14.53 (br, 1H), 10.52 (s, 1H), 8.46 (s, 1H), 8.05-7.98 (m, 3H), 7.60-7.56 (m, 5H).

1-Benzyl-3-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)urea  (SKB-124) Following general procedure L, the reaction of hydrazide 2.72g (173 mg) produced 184 mg (93%) SKB-124 after 3 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) δ 14.57 (br, 1H), 9.09 (s, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.61 (d, $J = 8.5$ Hz, 2H), 7.37-7.23 (m, 5H), 6.83 (t, $J = 6.0$ Hz, 1H), 4.32 (d, $J = 6.0$ Hz, 2H).

1-(Furan-2-ylmethyl)-3-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)urea  (SKB-161) Following general procedure L, the reaction of 2.72h (27.5 mg, 0.100 mmol) resulted in
30.8 mg (97%) **SKB-161** after 20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.61 (br, 1H), 9.01 (s, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.60 (d, $J = 7.5$ Hz, 3H), 6.74 (t, $J = 5.5$ Hz, 1H), 6.40 (s, 1H), 6.27 (d, $J = 3.0$ Hz, 1H), 4.30 (d, $J = 5.5$ Hz, 2H).

![SKB-162](image)

**1-(4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)-3-(4-methoxybenzyl)urea (SKB-162)**

Following general procedure L, the reaction of 2.72i (52.0 mg, 0.165 mmol) resulted in 58.8 mg (quant.) **SKB-162** after 20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.60 (br, 1H), 9.00 (s, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.60 (d, $J = 8.5$ Hz, 2H), 7.24 (d, $J = 8.5$ Hz, 2H), 6.90 (d, $J = 8.5$ Hz, 2H), 6.72 (t, $J = 6.0$ Hz, 1H), 4.24 (d, $J = 6.0$ Hz, 2H), 3.73 (s, 3H).

![SKB-163](image)

**1-(4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenyl)-3-(4-methylbenzyl)urea (SKB-163)**

Following general procedure L, the reaction of 2.72j (71.8 mg, 0.241 mmol) resulted in 77.5 mg (95%) **SKB-163** after 20 h of reflux. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 14.60 (br, 1H), 9.03 (s, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.60 (d, $J = 8.5$ Hz, 2H), 7.21-7.13 (m, 4H), 6.76 (t, $J = 6.0$ Hz, 1H), 4.26 (d, $J = 6.0$ Hz, 2H), 2.28 (s, 3H).
1-Benzyl-3-(3-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-160) Following general procedure L, the reaction of 2.72k (61.5 mg, 0.216 mmol) resulted in 70.9 mg (quant.) SKB-160 after 20 h of reflux. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 14.75 (br, 1H), 8.95 (s, 1H), 8.15 (s, 1H), 7.51-7.22 (m, 8H), 6.76 (t, \(J = 6.0\) Hz, 1H), 4.31 (d, \(J = 6.0\) Hz, 2H).

Tert-butyl 5-(5-mercapto-1,3,4-oxadiazol-2-yl)indoline-1-carboxylate (2.75) Following general procedure L, the reaction of 2.72l (177 mg, 0.297 mmol) produced 48.0 mg (74%) SKB-175 after 20 h of reflux. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 10.24 (br, 1H), 7.92 (br, 1H), 7.75 (d, \(J = 7.5\) Hz, 1H), 7.68 (s, 1H), 4.08 (t, \(J = 8.5\) Hz, 2H), 3.15 (t, \(J = 8.5\) Hz, 2H).

**General Procedure M - Synthesis of 2-bromo amides** To a solution of amide and NE\(_3\) (110 mol%) in CH\(_2\)Cl\(_2\) (1.3M) at -78 °C was the acid bromide (2.0 mmol) added dropwise. The reaction was stirred at -78°C for 2 h, warmed to -20°C and quenched with 0.3 mL H\(_2\)O. The mixture was diluted with CH\(_2\)Cl\(_2\) and washed with water (2 x 1 mL), saturated aqueous NH\(_4\)Cl (2 x 1 mL), saturated aqueous
NaHCO₃ (2 x 1 mL). After drying over MgSO₄, filtration and concentration, the crude product was purified by column chromatography (hexanes/EtOAc, 1:1 → 4:1).

2-Bromo-1-(pyrrolidin-1-yl)ethanone (2.76a) Following general procedure M, the reaction of 2-bromoacetyl bromide (1.20 mL, 14.0 mmol) and pyrrolidine (1.15 mL, 14.0 mmol) resulted in 1436 mg (54%) 2.76a. Rᵥ = 0.12 (hexanes/EtOAc, 1:1); ¹H-NMR (CDCl₃, 300 MHz) δ 3.80 (s, 2H), 3.53 (t, J = 6.5 Hz, 2H), 3.49 (t, J = 7.0 Hz, 2H), 2.06-1.96 (m, 2H), 1.94-1.84 (m, 2H). ¹³C-NMR (CDCl₃, 300 MHz) δ 164.9, 46.96, 46.32, 27.47, 26.08, 24.23. Analytical data matched that reported for the known compound.¹⁶⁷

2-Bromo-1-(piperidin-1-yl)ethanone (2.76b) Following general procedure M, the reaction of 2-bromoacetyl bromide (1.20 mL, 14.0 mmol) and piperidine (1.40 mL, 14.0 mmol) resulted in 18040 mg (64%) 2.76b. Rᵥ = 0.29 (hexanes/EtOAc, 1:1); ¹H-NMR (CDCl₃, 300 MHz) δ 3.85 (s, 2H), 3.55 (t, J = 5.5 Hz, 2H), 3.43 (br, 1H), 1.66-1.64 (m, 4H), 1.58-1.55 (m, 2H). ¹³C-NMR (CDCl₃, 300 MHz) δ 164.8, 47.73, 43.05, 26.19, 26.04, 25.22, 24.11. Analytical data matched that reported for the known compound.¹⁷⁰
**2-Bromo-1-morpholinoethanone (2.76c)** Following general procedure M, the reaction of 2-bromoacetyl bromide (1.20 mL, 14.0 mmol) and morpholine (1.20 mL, 14.0 mmol) resulted in 1426 mg (49%) 2.76c. R<sub>f</sub> = 0.14 (hexanes/EtOAc, 1:1); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) δ 4.00 (s, 2H), 3.65-3.62 (m, 2H), 3.56-3.50 (m, 6H), 3.33 (s, 6H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 300 MHz) δ 167.4, 70.44, 70.15, 58.87, 58.65, 49.82, 46.62, 27.14. Analytical data matched that reported for the known compound.\(^{169,171}\)

**2-Bromo-N,N-bis(2-methoxyethyl)acetamide (2.76d)** Following general procedure M, the reaction of 2-bromoacetyl bromide (1.20 mL, 14.0 mmol) and bis(2-methoxyethyl)amine (2.10 mL, 14.0 mmol) resulted in 2.517 mg (71%) 2.76d. R<sub>f</sub> = 0.19 (hexanes/EtOAc, 1:1); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) δ 3.85 (s, 2H), 3.75-3.62 (m, 6H), 3.53-3.50 (m, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 300 MHz) δ 164.0, 66.26, 66.07, 46.82, 42.10, 25.49.

**General Procedure N - Synthesis of Final Compounds under reflux**

A mixture of thiol, 2-bromo amide (102 mol%) and K<sub>2</sub>CO<sub>3</sub> (500 mol%) in 2.5 mL acetone was refluxed for 1 h, after which the reaction mixture was filtered and
concentrated. The products were cleaned by recrystallization or column chromatography if necessary.

2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-1-(pyrrolidin-1-yl)ethanone

**(SKB-100)** Following general procedure *N*, the reaction of **2.77** (97.9 mg, 0.460 mmol) with **2.76a** (97.7 mg, 0.509 mmol) produced 148 mg (99%) **SKB-100**. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.91 (d, $J = 9.0$ Hz, 2H), 7.45 (d, $J = 9.0$ Hz, 2H), 4.25 (s, 2H), 3.57 (t, $J = 7.0$ Hz, 2H), 3.51 (t, $J = 7.0$ Hz, 2H), 2.03-1.97 (m, 2H), 1.93-1.86 (m, 2H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 165.0, 164.5, 164.4, 137.9, 129.4, 127.9, 122.0, 47.02, 46.45, 37.31, 26.19, 24.39. IR (KBr, CH$_2$Cl$_2$, cm$^{-1}$) 2970, 2870, 2360, 1636, 1465, 1450, 1188; HPLC (Eluting method D) $t_R$ 11.07 min, purity: 98%. HRMS m/z: [2M+Na]$^+$ calcd for (C$_{14}$H$_{14}$N$_3$O$_2$S)$_2$Na 669.088271 u, found 669.088897.

2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-1-(piperidin-1-yl)ethanone

**(SKB-101)** Following general procedure *N*, the reaction of **2.77** (54.4 mg, 0.256 mmol) with **2.76b** (52.6 mg, 0.255 mmol) produced 85 mg (99%) **SKB-101**. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.95 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 4.42 (s, 2H), 3.63-3.59 (m,
2H), 3.53-3.51 (m, 2H), 1.66-1.59 (m, 6H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 165.1, 164.9, 164.3, 138.1, 129.6, 128.1, 122.2, 47.40, 43.57, 38.04, 26.48, 25.52, 24.36. IR (KBr, CH$_2$Cl$_2$, cm$^{-1}$) 3433, 2939, 2854, 2361, 1643, 1465, 1450, 841. HPLC (Method D). $t_R$ 12.18 min, purity: 93%. HRMS $m/z$: [2M+Na]$^+$ calcd for (C15H16N3O2S)$_2$Na 697.199571u, found 697.120326.

2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ythio)-1-morpholinoethanone (SKB-102)

Following general procedure $N$, the reaction of 2.77 (51.6 mg, 0.243 mmol) with 2.76c (53.3 mg, 0.260 mmol) produced 81 mg (99%) SKB-102. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.95 (d, $J$ = 8.5 Hz, 2H), 7.48 (d, $J$ = 8.5 Hz, 2H), 4.37 (s, 2H), 3.76-3.60 (m, 8H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 165.2, 164.8, 164.3, 138.1, 129.5, 128.0, 121.9, 66.68, 66.46, 46.53, 42.64, 36.66. IR (KBr, CH$_2$Cl$_2$, cm$^{-1}$) 4344, 2962, 2924, 2862, 1650, 1627, 1473, 1118, 833. HPLC (Method D) $t_R$ 10.58 min purity: 100%. HRMS $m/z$: [2M+Na]$^+$ calcd for (C14H14N3O3S)$_2$Na 701.078101u, found 701.078977.
2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-N,N-bis(2-methoxyethyl)acetamide (SKB-103) Following general procedure N, the reaction of 2.77 (53.9 mg, 0.254 mmol) with 2.76d (66.0 mg, 0.260 mmol) produced 69 mg (70%) SKB-102 after purification. \( R_f = 0.17 \) (hexanes/EtOAc, 2:1). \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.95 (d, \( J = 8.5 \) Hz, 2H), 7.48 (d, \( J = 8.5 \) Hz, 2H), 4.54 (s, 2H), 3.70-3.53 (m, 8H), 3.35 (s, 3H), 3.33, (s, 3H). \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \( \delta \) 167.1, 164.9, 164.8, 137.9, 129.4, 128.0, 122.1, 70.94, 70.22, 59.16, 58.88, 49.70, 46.77, 37.63. IR (KBr, CH\(_2\)Cl\(_2\), cm\(^{-1}\)) 3464, 2924, 2893, 1643, 1465, 1294, 1095. HPLC (Method D) \( t_R \) 11.01 min, purity: 98%. HRMS \( m/z: [2M+Na]^+ \) calcd for (C\(_{16}\)H\(_{20}\)N\(_3\)O\(_4\)S)\(_2\)Na 793.161830u, found 793.162497.

**General Procedure O: Synthesis of Final Compounds without base or heat**

A mixture of thiol and 2-bromo amide (100 mol%) in acetone (0.2M) was stirred for 2 d, after which the starting material had dissolved and the product precipitated and was collected by filtration.

2-(5-(2,3-Dimethyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ylthio)-1-(pyrrolidin-1-yl)ethanone (A3) Following general procedure O, the reaction of SKB-104 (40 mg, 0.16 mmol)
with 2.76a (31 mg, 0.16 mmol) in 0.8 mL acetone produced 47 mg (80%) A3. $^1$H-NMR (CD$_3$OD, 300 MHz) δ 8.04 (s, 1H), 7.66 (br, 1H), 7.36 (br, 1H), 4.32 (s, 2H), 3.66 (d, $J = 4.5$ Hz, 2H), 3.48 (d, $J = 4.5$ Hz, 2H), 2.37 (s, 3H), 2.25 (s, 3H), 2.04-1.93 (m, 4H). 

$^{13}$C-NMR (CD$_3$OD, 75 MHz) δ 169.4, 167.0, 164.5, 139.2, 134.9, 130.8, 119.8, 118.2, 113.8, 112.0, 108.3, 47.65, 37.03, 27.04, 25.33, 11.22, 8.31. IR (KBr) 1643, 1589 cm$^{-1}$. HPLC (Method D) $t_R$ 8.59 min, purity: 98%. HRMS m/z: [M+Na]$^+$ calcd for (C18H20N4O2S)Na 379.119918u, found 379.119753u. MP 167.1-168.6°C (decomposition).

2-(5-(2,3-Dimethyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ylthio)-1-(piperidin-1-yl)ethan-one (SKB-105) Following general procedure O, the reaction of SKB-104 (50 mg, 0.21 mmol) with 2.76b (43 mg, 0.21 mmol) in 1.0 mL acetone produced 38 mg (49%)

SKB-105. $^1$H-NMR (CD$_3$OD, 300 MHz) δ 8.04 (s, 1H), 7.65 (d, $J = 8.5$ Hz, 1H), 7.35 (d, $J = 8.5$ Hz, 1H), 4.44 (s, 2H), 3.60-3.58 (m, 4H), 2.37 (s, 3H), 2.25 (s, 3H), 1.70 (br, 4H), 1.58 (br, 2H). $^{13}$C-NMR (CD$_3$OD, 75 MHz) δ 169.3, 166.6, 164.7, 139.3, 134.9, 130.9, 119.9, 118.2, 113.7, 112.0, 108.3, 44.68, 37.33, 27.38, 26.66, 25.28, 11.24, 8.333. IR (KBr) 1636, 1689 cm$^{-1}$. HPLC (Method D) $t_R$ 7.70 min, purity: 98%. HRMS m/z: [M+Na]$^+$ calcd for (C19H22N4O2S)Na 393.135568u, found 393.135459u. MP 171.5-172.7°C (decomposition).
2-(5-(2,3-Dimethyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ylthio)-1-morphinoethanone (A3-5) Following general procedure O, the reaction of SKB-104 (61 mg, 0.25 mmol) with 2.76c (52 mg, 0.25 mmol) in 1.2 mL acetone produced 60 mg (65%) A3-5. $^1$H-NMR (CD$_3$OD, 300 MHz) δ 8.05 (s, 1H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.36 (d, $J = 8.0$ Hz, 1H), 4.45 (s, 2H), 3.75-3.64 (m, 8H), 2.37 (s, 3H), 2.26 (s, 3H). $^{13}$C-NMR (CD$_3$OD, 75 MHz) δ 169.6, 167.3, 164.6, 139.2, 134.9, 130.9, 119.8, 118.2, 113.8, 112.0, 108.3, 67.60, 47.6, 43.92, 36.69, 21.98, 11.21, 8.316. IR (KBr) 1636, 1597 cm$^{-1}$. HPLC (Method D) $t_R$ 9.54 min, purity: 97%. HRMS m/z: [M+Na]$^+$ calcd for (C18H20N4O3S)Na 395.114832u, found 395.114631u. MP 189.8-192.5°C (decomposition).

2-(5-(2,3-Dimethyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ylthio)-N,N-bis(2-methoxyethyl) acetamide (SKB-106) Following general procedure O, the reaction of SKB-104 (41 mg, 0.17 mmol) with 2.76d (42 mg, 0.16 mmol) in 0.8 mL acetone produced 24 mg (34%) A3-5. $^1$H-NMR (CD$_3$OD, 300 MHz) δ 8.05 (s, 1H), 7.66 (d, $J = 8.5$ Hz, 1H), 7.35 (d, $J = 8.5$ Hz, 1H), 4.54 (s, 2H), 3.76-3.73 (m, 2H), 3.63-3.60 (m, 4H), 3.55-3.53 (m, 2H), 3.37 (s, 3H), (other OMe barred under MeOH peak), 2.37 (s, 3H), 2.26 (s, 3H). $^{13}$C-NMR (CD$_3$OD, 75 MHz) δ 169.5, 169.3, 164.8, 139.3, 135.0, 130.9, 119.9, 118.3,
113.0, 112.0, 108.3, 71.53, 59.46, 59.13, 50.61, 47.81, 37.49, 11.27, 8.37. IR
(KBr) 1636, 1597 cm$^{-1}$. HPLC (Method D) $t_R$ 8.13 min, purity: 99%. HRMS m/z:

$[M+Na]^+$ calcd for (C20H26N4O4S)Na 441.156697 u, found 441.156289 u. MP 129.3-
130.1°C.

1-(Pyrrolidin-1-yl)-2-(5-(1,2,3-trimethyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ylthio)
ethanone (SKB-133) Following general procedure O, the reaction of thiol SKB-132
(10 mg, 0.039 mmol) with 2.76a (8.8 mg, 0.046 mmol) produced 5 mg (34%) SKB-133.

$^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 8.02 (s, 1H), 7.68 (d, $J=8.5$ Hz, 1H), 7.54 (d,
$J=8.5$ Hz, 1H), 4.41 (s, 2H), 3.70 (s, 3H), 3.55 (t, $J=6.5$ Hz, 2H), 3.61-3.31 (m, 2H,
obscured by H$_2$O), 2.36 (s, 3H), 2.25 (s, 3H), 1.97-1.88 (m, 2H), 1.85-1.76 (m, 2H).

LCMS (Method E) $t_R$ 11.22 min, purity: 99%, m/z: $[M+H]^+$ calcd for
(C19H22N4O2S)H+ 371 u, found 371 u.

1-(Pyrrolidin-1-yl)-2-(5-(2,3,4,9-tetrahydro-1H-carbazol-6-yl)-1,3,4-oxadiazol-2-yl
thio)ethanone (SKB-150) Following general procedure O, the reaction of SKB-142
(61.7 mg, 0.227 mmol) and 2.76a (45.2 mg, 0.235 mmol) yields 19 mg (22%) SKB-150
after column chromatography on neutral alumina (CH$_2$Cl$_2$ $\rightarrow$ 2%MeOH in CH$_2$Cl$_2$) and recrystallization from CH$_2$Cl$_2$/MeOH. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 11.13 (s, 1H), 7.96 (s, 1H), 7.62 (d, $J$ = 8.5 Hz, 1H), 7.41 (d, $J$ = 8.5 Hz, 1H), 4.40 (2, 2H), 3.55 (t, $J$ = 6.5 Hz, 2H), 3.32 (br, 2H and H$_2$O), 1.95-1.79 (m, 8H). LCMS (Method E) $t_R$ 8.11 min, purity: 97%, m/z: [M+H]$^+$ calcd for (C20H22N4O2S)H$^+$ 383u, found 383u.

2-(5-(3-Ethyl-2-methyl-1H-indol-5-yl)-1,3,4-oxadiazol-2-ythio)-1-(pyrrolidin-1-yl)ethanone (SKB-139) Following general procedure O, the reaction of SKB-143 (15.7 mg, 0.0723 mmol) and 2-2.76a (15.1 mg, 0.0786 mmol) yields 12.8 mg (48%) SKB-139. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 11.18 (s, 1H), 8.02 (s, 1H), 7.62 (d, $J$ = 8.5 Hz, 1H), 7.41 (d, $J$ = 8.5 Hz, 1H), 4.42 (2, 2H), 3.62-3.54 (m, 2H and H$_2$O), 3.35 (t, $J$ = 7.0 Hz, 2H), 2.71 (q, $J$ = 7.5 Hz, 2H), 2.36 (s, 3H), 1.96-1.89 (m, 2H), 1.86-1.80 (m, 2H), 1.18 (t, $J$ = 7.5 Hz, 3H). LCMS (Method E) $t_R$ 11.09 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C19H22N4O2S)H$^+$ 371u, found 371u.

**General Procedure P - Synthesis of Final Compounds under basic conditions**

To a solution of thiol and bromoamid (100 mol%) in DMF (0.1M) was NEt$_3$ (100 mol%) added. The reaction was shaken for 24 h. Any precipitated product was filtered off and washed with water. For products that did not precipitate the reaction mixture was diluted
with water and extracted with CH₂Cl₂. The combined organic layers were concentrated and recrystallized from toluene.

2-(5-(2-Methylimidazo[1,2-a]pyridin-6-yl)-1,3,4-oxadiazol-2-ylthio)-1-(pyrrolidin-1-yl)ethanone (SKB-140) Following general procedure P, the reaction of SKB-144 (14.5 mg, 0.0762 mmol) and 2.76a (15.3 mg, 0.0797 mmol) yielded 9.4 mg (36%) SKB-140. ¹H-NMR (DMSO-d₆, 300 MHz) δ 9.23 (s, 1H), 7.85 (s, 1H), 7.69-7.60 (m, 2H), 4.44 (s, 2H), 3.54 (t, J = 6.5 Hz, 2H), 3.33 (br, 2H and H₂O), 2.37 (s, 3H) 1.97-1.88 (m, 2H), 1.85-1.78 (m, 2H). LCMS (Method E) tᵣ 8.01 min, purity: 98%, m/z: [M+H]+ calcd for (C₁₆H₁₇N₅O₂S)H+ 344u, found 344u.

2-(5-(1H-Indazol-5-yl)-1,3,4-oxadiazol-2-ylthio)-1-(pyrrolidin-1-yl)ethanone (SKB-112) Following general procedure P, the reaction of SKB-157 (10 mg, 0.047 mmol) and 2.76a (9.0 mg, 0.047 mmol) yielded 8.8 mg (66%) SKB-112. ¹H-NMR (DMSO-d₆, 500 MHz) δ 8.43 (s, 1H), 8.27 (s, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 4.44 (s, 2H), 3.55 (t, J = 6.5 Hz, 2H), 3.33 (2H covered by H₂O peak), 1.94-1.90 (m, 2H), 1.84-1.80 (m, 2H).
1-(Pyrrolidin-1-yl)-2-(5-(quinolin-6-yl)-1,3,4-oxadiazol-2-thio)ethanone (SKB-165)

Following general procedure P, the reaction of SKB-157 (9.9 mg, 0.043 mmol) and 2.76a (11 mg, 0.056 mmol) yielded 11 mg (76%) SKB-165 after column chromatography (CH₂Cl₂ → 10%MeOH in CH₂Cl₂). ¹H-NMR (CDCl₃, 300 MHz) δ 9.01 (d, J = 2.5 Hz, 1H), 8.48 (s, 1H), 8.35-8.20 (m, 3H), 7.50 (dd, J = 8.0, 4.0 Hz, 1H), 4.32 (s, 2H), 3.62 (t, J = 6.5 Hz, 1H), 3.55 (t, J = 6.5 Hz, 1H), 2.10-2.01 (m, 2H), 1.97-1.88 (m, 2H). LCMS (Method E) tᵣ 8.77 min, purity: 99%, m/z: [M+H]+ calcd for (C₁₇H₁₆N₄O₂S)H⁺ 341u, found 341u.

2-(5-(3-Methoxynaphthalen-2-yl)-1,3,4-oxadiazol-2-thio)-1-(pyrrolidin-1-yl)ethanone (SKB-122)

Following general procedure P, the reaction of SKB-158 (15.2 mg, 0.0588 mmol) and 2.76a (13.2 mg, 0.0687 mmol) yielded 2.2 mg (10%) SKB-112. ¹H-NMR (DMSO-d₆, 300 MHz) δ 8.45 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.63-7.58 (m, 2H), 7.46 (t, J = 15.0 Hz, 1H), 4.44 (s, 2H), 3.98 (s, 3H) 3.55 (t, J = 6.5 Hz, 2H), 3.35 (2H covered by H₂O peak), 1.97-1.88 (m, 2H), 1.85-1.79 (m, 2H). LCMS (Method E) tᵣ 10.58 min, purity: 98%, m/z: [M+H]+ calcd for (C₁₉H₁₉N₃O₃S)H⁺ 370u, found 370u.
2-(5-(6-Methoxynaphthalen-2-yl)-1,3,4-oxadiazol-2-ythio)-1-(pyrrolidin-1-yl)ethanone (SKB-124) Following general procedure P, the reaction of SKB-159 (20.5 mg, 0.0794 mmol) and 2.76a (16.3 mg, 0.0849 mmol) yielded 19.6 mg (67%) SKB-124.

$^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 8.52 (s, 1H), 8.06 (d, $J$ = 9.0 Hz, 1H), 8.00 (s, 2H), 7.45 (d, $J$ = 1.5 Hz, 1H), 7.29 (dd, $J$ = 11.0, 2.5 Hz, 1H), 4.46 (s, 2H), 3.92 (s, 3H), 3.56 (t, $J$ = 7.0 Hz, 2H), 3.37-3.33 (2H covered by H$_2$O peak), 1.98-1.89 (m, 2H), 1.86-1.77 (m, 2H). LCMS (Method E) $t_R$ 10.86 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C$_{19}$H$_{19}$N$_3$O$_3$S)H$^+$ 370u, found 370u.

2-(5-(6-Methoxynaphthalen-2-yl)-1,3,4-oxadiazol-2-ythio)-1-morpholinoethanone (SKB-126) Following general procedure P, the reaction of SKB-159 (20.4 mg, 0.0790 mmol) and 2.76a (20.3 mg, 0.0976 mmol) yielded 18.5 mg (61%) SKB-126.

$^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 8.53 (s, 1H), 8.07-8.01 (m, 3H), 7.46 (s, 1H), 7.29 (d, $J$ = 9.0 Hz, 1H), 4.60 (s, 2H), 3.92 (s, 3H), 3.66-3.48 (m, 8H). LCMS (Method E) $t_R$ 10.35 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C$_{19}$H$_{19}$N$_3$O$_4$S)H$^+$ 386u, found 386u.


N-(4-(5-(2-Oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-108) Following general procedure P, the reaction of thiol SKB-107 (40 mg, 0.17 mmol) and 2.76a (32 mg, 0.17 mmol) produced 40 mg (73%) SKB-108. 1H-NMR (DMSO-d6, 300 MHz) δ 10.28 (s, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 4.40 (s, 2H), 3.54 (t, J = 6.5 Hz, 2H), 3.35-3.31 (m, 2H, obscured by H2O), 2.09 (s, 3H), 1.97-1.87 (m, 2H), 1.85-1.78 (m, 2H). LCMS (Method E) tR 7.66 min, purity: 96%, m/z: [M+H]+ calcd for (C16H18N4O3S)H+ 347u, found 347u.

N-(4-(5-(2-Oxo-2-(piperidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-109) Following general procedure P, the reaction of thiol SKB-107 (47 mg, 0.20 mmol) and 2.76b (43 mg, 0.21 mmo) produced 53 mg (73%) SKB-109. 1H-NMR (DMSO-d6, 300 MHz) δ 10.28 (s, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.5 Hz, 2H), 4.53 (s, 2H), 3.45 (br, 4H), 2.09 (s, 3H), 1.58 (br, 4H), 1.45 (br, 2H). LCMS (Method E) tR 8.96 min, purity: 97%, m/z: [M+H]+ calcd for (C17H20N4O3S)H+ 361u, found 361u.
$N$-$(4$-$-(5$-$2$-$Morpholino$-2$-oxoethy)thio)$-1$,$3$,$4$-oxadiazol$-2$-yl)phenyl)acetamide

**(SKB-110)** Following general procedure *P*, the reaction of thiol **SKB-107** (39 mg, 0.17 mmol) and **2.76c** (35 mg, 0.17 mmol) produced 42 mg (70%) **SKB-110**. $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$ 7.95 (d, $J$ = 8.5 Hz, 2H), 7.65 (d, $J$ = 8.5 Hz, 2H), 7.35 (s, 1H), 4.35 (s, 2H), 3.75-3.62 (m, 8H), 2.22 (s, 3H). LCMS (Method E) $t_R$ 7.30 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C16H18N4O4S)$H^+$ 363u, found 363u.

$N$-$(4$-$-(5$-$2$-Oxo$-2$-(pyrrolidin$-1$-yl)ethylthio)$-1$,$3$,$4$-oxadiazol$-2$-yl)phenyl)benzamide

**(SKB-113)** Following general procedure *P*, the reaction of thiol **SKB-153** (41 mg, 0.14 mmol) and **2.76a** (27 mg, 0.14 mmol) produced 40 mg (70%) **SKB-113**. $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.58 (s, 1H), 8.05-7.95 (m, 6H), 7.65-7.53 (m, 3H), 4.42 (s, 2H), 3.55 (t, $J$ = 6.5 Hz, 2H), 3.36 (2H obscured by H$_2$O), 1.94-1.88 (m, 2H), 1.86-1.77 (m, 2H). LCMS (Method E) $t_R$ 9.83 min, purity: 98%, m/z: [M+H]$^+$ calcd for (C21H20N4O3S)$H^+$ 409u, found 409u.
N-(4-(5-(2-Oxo-2-(piperidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)benzamide  

(SKB-114) Following general procedure P, the reaction of thiol SKB-153 (40 mg, 0.14 mmol) and 2.76b (30 mg, 0.14 mmol) produced 44 mg (77%) SKB-114. ¹H-NMR (DMSO-d₆, 300 MHz) δ 10.58 (s, 1H), 8.05-7.97 (m, 6H), 7.65-7.53 (m, 3H), 4.55 (s, 2H), 3.46 (br, 4H), 1.59 (br, 4H), 1.46 (br, 2H). LCMS (Method E) tᵣ 10.50 min, purity: 97%, m/z: [M+H]+ calcd for (C22H22N₄O₃S)H+ 423, found 423u.

N-(4-(5-(2-Morpholino-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)benzamide  

(SKB-115) Following general procedure P, the reaction of thiol SKB-153 (41 mg, 0.14) and 2.76c (29 mg, 0.14 mmol) produced 48 mg (81%) SKB-115. ¹H-NMR (DMSO-d₆, 300 MHz) δ 10.58 (s, 1H), 8.05-7.97 (m, 6H), 7.65-7.53 (m, 3H), 4.56 (s, 2H), 3.65-3.48 (m, 8H). LCMS (Method E) tᵣ 9.41 min, purity: 98%, m/z: [M+H]+ calcd for (C21H20N₄O₄S)H+ 425, found 425u.
**N-(4-(5-(2-(Bis(2-methoxyethyl)amino)-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)benzamide (SKB-116)** Following *general procedure P*, the reaction of thiol **SKB-153** (41 mg, 0.14 mmol) and **2.76d** (36 mg, 0.14 mmol) produced 51 mg (80%) **SKB-116**.

$^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 10.57 (s, 1H), 8.05-7.95 (m, 6H), 7.65-7.53 (m, 3H), 4.58 (s, 2H), 3.62-3.60 (m, 2H), 3.56-3.49 (m, 4H), 3.45-3.43 (m, 2H), 3.29 (s, 3H), 3.24 (s, 3H). LCMS (Method E) $t_R$ 10.25 min, purity: 97%, m/z: [M+H]$^+$ calcd for (C$_{23}$H$_{26}$N$_4$O$_5$S)H$^+$ 471u, found 471u.

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**N-(3-(5-(2-Oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-118)** Following *general procedure P*, the reaction of thiol **SKB-117** (20 mg, 0.086 mmol) and **2.76a** (17 mg, 0.86 mmol) produced 15 mg (50%) **SKB-118**. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 10.21 (s, 1H), 8.30 (s, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.62 (d, $J = 8.0$ Hz, 1H), 7.51 (d, $J = 8.0$ Hz, 1H), 4.41 (s, 2H), 3.54 (t, $J = 6.5$ Hz, 2H), 3.32 (2H obscured by H$_2$O), 2.08 (s, 3H), 1.97-1.88 (m, 2H), 1.85-1.76 (m, 2H). LCMS (Method
E) $t_R$ 8.00 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C16H18N4O3S)H$^+$ 347u, found 347u.

$N$-$(3$-(5-(2-Oxo-2-(piperidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-119) Following general procedure $P$, the reaction of thiol SKB-117 (21 mg, 0.089 mmol) and 2.76b (18 mg, 0.089 mmol) produced 8 mg (25%) SKB-119. $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.21 (s, 1H), 8.31 (s, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.63 (d, $J = 8.0$ Hz, 1H), 7.51 (d, $J = 8.0$ Hz, 1H), 4.54 (s, 2H), 3.47-3.46 (m, 4H), 2.08 (s, 3H), 1.59 (br, 4H), 1.45–4.2 (m, 2H). LCMS (Method E) $t_R$ 8.94 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C17H20N4O3S)H$^+$ 361u, found 361u.

$N$-$(3$-(5-(2-Morpholino-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (SKB-120) Following general procedure $P$, the reaction of thiol SKB-117 (21 mg, 0.089 mmol) and 2.76c (21 mg, 0.10 mmol) produced 27 mg (84%) SKB-120. $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.21 (s, 1H), 8.31 (s, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.63 (d, $J = 8.0$ Hz, 1H), 7.51 (d, $J = 8.0$ Hz, 1H), 4.55 (s, 2H), 3.55 (t, $J = 6.5$ Hz, 2H), 3.32 (2H
obsured by H₂O), 3.64-3.47 (m, 8H), 2.08 (s, 3H). LCMS (Method E) tᵣ 7.35 min, purity: 99%, m/z: [M+H]+ calcd for (C16H18N4O4S)H+ 363u, found 363u.

**2-(5-(3-Acetamidophenyl)-1,3,4-oxadiazol-2-thio)N,N-bis(2-methoxyethyl)acetamide** (SKB-121) Following general procedure P, the reaction of thiol SKB-117 (21 mg, 0.091 mmol) and 2.76d (24 mg, 0.094 mmol) produced 30 mg (81%) SKB-121.

¹H-NMR (DMSO-d₆, 300 MHz) δ 10.21 (s, 1H), 8.31 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 4.58 (s, 2H), 3.61-3.60 (m, 2H), 3.55-3.48 (m, 4H), 3.44-3.40 (m, 2H), 3.29 (s, 3H), 3.23 (s, 3H), 2.08 (s, 3H). LCMS (Method E) tᵣ 8.47 min, purity: 99%, m/z: [M+H]+ calcd for (C18H24N4O5S)H+ 409u, found 409u.

**N-(3-(5-(2-Oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)benzamide** (SKB-123) Following general procedure P, the reaction of thiol SKB-154 (40 mg, 0.13 mmol) and 2.76a (25 mg, 13 mmol) produced 40 mg (73%) SKB-123. ¹H-NMR
(DMSO-d$_6$, 300 MHz) δ 10.51 (s, 1H), 8.51 (s, 1H), 8.04-7.98 (m, 3H), 7.70 (d, $J=7.5$ Hz, 1H), 7.62-7.53 (m, 4H), 4.43 (s, 2H), 3.55 (t, $J=6.5$ Hz, 2H), 3.32 (2H obscured by H$_2$O), 1.95-1.88 (m, 2H), 1.85-1.78 (m, 2H). LCMS (Method E) $t_R$ 9.90 min, purity: 100%, m/z: [M+H]$^+$ calcd for (C21H20N4O3S)H$^+$ 409u, found 409u.

$N$-((3-(5-(2-Morpholino-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)benzamide (SKB-125) Following general procedure P, the reaction of thiol SKB-154 (40 mg, 0.13 mmol) and 2.76c (28 mg, 0.14 mmol) produced 32 mg (56%) SKB-125. $^1$H-NMR (DMSO-d$_6$, 300 MHz) δ 10.51 (s, 1H), 8.53 (s, 1H), 8.05-7.99 (m, 3H), 7.71 (d, $J=7.5$ Hz, 1H), 7.65-7.53 (m, 4H), 4.57 (s, 2H), 3.65-3.48 (m, 8H). LCMS (Method E) $t_R$ 9.29 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C21H20N4O4S)H$^+$ 425u, found 425u.

1-Benzyl-3-(4-(5-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-128) Following general procedure P, the reaction of thiol SKB-127 (30 mg, 0.093 mmol) and 2.76a (19 mg, 0.099 mmol) produced 26 mg (63%) SKB-128. $^1$H-NMR (DMSO-d$_6$, 300 MHz) δ 9.01 (s, 1H), 7.83 (d, $J=8.5$ Hz, 2H), 7.61 (d, $J=8.5$ Hz, 2H),
7.37-7.23 (m, 5H), 6.78 (t, $J = 6.0$ Hz, 1H), 4.39 (s, 2H), 4.32 (d, $J = 6.0$ Hz, 2H), 3.56 (t, $J = 6.5$ Hz, 2H), 3.35-3.31 (m, 2H, obscured by H$_2$O), 1.96-1.87 (m, 2H), 1.85-1.76 (m, 2H). LCMS (Method E) $t_R$ 9.95 min, purity: 96%, m/z: [M+H]$^+$ calcd for (C22H23N5O3S)H+ 438u, found 438u.

1-Benzyl-3-(4-(5-(2-oxo-2-(piperidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-129) Following general procedure P, the reaction of thiol SKB-127 (30 mg, 0.093 mmol) and 2.76b (21 mg, 0.10 mmol) produced 24 mg (58%) SKB-129. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 9.01 (s, 1H), 7.84 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 8.5$ Hz, 2H), 7.37-7.18 (m, 5H), 6.78 (t, $J = 6.0$ Hz, 1H), 4.52 (s, 2H), 4.32 (d, $J = 6.0$ Hz, 2H), 3.47-3.45 (m, 4H), 1.58 (br, 4H), 1.45 (br, 2H). LCMS (Method E) $t_R$ 10.69 min, purity: 97%, m/z: [M+H]$^+$ calcd for (C23H25N5O3S)H+ 452u, found 452u.

1-Benzyl-3-(4-(5-(2-morpholino-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-130) Following general procedure P, the reaction of thiol SKB-127 (30 mg,
0.091 mmol) and 2.76c (19 mg, 0.092 mmol) produced 12 mg (30%) SKB-130. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 9.01 (s, 1H), 7.84 (d, \(J = 8.5\) Hz, 2H), 7.62 (d, \(J = 8.5\) Hz, 2H), 7.37-7.23 (m, 5H), 6.79 (t, \(J = 5.5\) Hz, 1H), 4.53 (s, 2H), 4.32 (d, \(J = 5.5\) Hz, 2H), 3.64-3.47 (m, 8H). LCMS (Method E) \(t_R\) 9.59 min, purity: 96%, m/z: [M+H]+ calcd for (C22H23N5O4S)H+ 454u, found 454u.

\[\text{2-((5-(4-(3-Benzylureido)phenyl)-1,3,4-oxadiazol-2-ylthio)-N,N-bis(2-methoxyethyl)acetamide (SKB-131)}}\]

Following general procedure P, the reaction of thiol SKB-127 (30 mg, 0.093 mmol) and 2.76d (26 mg, 0.10 mmol) produced 32 mg (69%) SKB-131. \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 9.02 (s, 1H), 7.83 (d, \(J = 8.5\) Hz, 2H), 7.61 (d, \(J = 8.5\) Hz, 2H), 7.37-7.22 (m, 5H), 6.79 (t, \(J = 6.0\) Hz, 1H), 4.56 (s, 2H), 4.32 (d, \(J = 6.0\) Hz, 2H), 3.61-3.59 (m, 2H), 3.54-3.48 (m, 4H), 3.44-3.40 (m, 2H), 3.28 (s, 3H), 3.23 (s, 3H). LCMS (Method E) \(t_R\) 10.32 min, purity: 96%, m/z: [M+H]+ calcd for (C24H29N5O5S)H+ 500u, found 500u.
1-(Furan-2-ylmethyl)-3-(4-(5-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-135) Following general procedure P, the reaction of SKB-161 (14.6 mg, 0.0462 mmol) and 2.76a (11.7 mg, 0.0609 mmol) yielded 9.9 mg (50%) SKB-135. ¹H-NMR (DMSO-d₆, 300 MHz) δ 8.99 (s, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 3H), 6.73 (t, J = 5.5 Hz, 1H), 6.40 (s, 1H), 6.27 (d, J = 3.0 Hz, 1H), 4.40 (s, 2H), 4.31 (d, J = 5.5 Hz, 2H), 3.53 (t, J = 6.5 Hz, 2H), 3.31 (2H covered by H2O peak), 1.96-1.87 (m, 2H), 1.85-1.76 (m, 2H). LCMS (Method E) tᵣ 9.08 min, purity: 96%, m/z: [M+H]+ calcd for (C20H21N5O4S)H+ 428u, found 428u.

1-(4-Methoxybenzyl)-3-(4-(5-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-137) Following general procedure P, the reaction of SKB-162 (15.0 mg, 0.0477 mmol) and 2.76a (10.3 mg, 0.0536 mmol) yielded 14.0 mg (63%) SKB-137. ¹H-NMR (DMSO-d₆, 500 MHz) δ 8.98 (s, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.71 (t, J = 5.5 Hz, 1H), 4.40 (s, 2H), 4.23 (d, J = 5.5 Hz, 2H), 3.73 (s, 3H), 3.53 (t, J = 7.0 Hz, 2H), 3.34
(2H covered by H2O peak), 1.94-1.79 (m, 4H). LCMS (Method E) tR 9.84 min, purity: 98%, m/z: [M+H]+ calcd for (C23H25N5O4S)H+ 468u, found 468u.

1-(4-Methylbenzyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-136) Following general procedure P, the reaction of SKB-163 (20.9 mg, 0.07011 mmol) and 2.76a (14.1 mg, 0.0734 mmol) yielded 8.4 mg (27%) SKB-136. 1H-NMR (DMSO-d6, 500 MHz) δ 9.00 (s, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.21-7.13 (m, 4H), 6.74 (t, J = 5.5 Hz, 1H), 4.40 (s, 2H), 4.26 (d, J = 5.5 Hz, 2H), 3.53 (t, J = 6.5 Hz, 2H), 3.34 (2H covered by H2O peak), 2.28 (s, 3H), 1.96-1.76 (m, 4H). LCMS (Method E) tR 10.46 min, purity: 97%, m/z: [M+H]+ calcd for (C23H25N5O3S)H+ 452u, found 452u.

1-(4-Methylbenzyl)-3-(4-(5-(2-morpholino-2-oxoethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-138) Following general procedure P, the reaction of SKB-163 (20.1 mg, 0.0674 mmol) and 2.76c (14.8 mg, 0.0711 mmol) yielded 16.8 mg (53%) SKB-138. 1H-NMR (DMSO-d6, 500 MHz) δ 9.01 (s, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.61
(d, J = 8.5 Hz, 1H), 7.21-7.13 (m, 4H), 6.74 (t, J = 5.5 Hz, 1H), 4.54 (s, 2H), 4.27 (d, J = 5.5 Hz, 2H), 3.64-3.47 (m, 8H), 2.28 (s, 3H). LCMS (Method E) tR 10.09 min, purity: 98%, m/z: [M+H]+ calcd for (C23H25N5O4S)H+ 468u, found 468u.

1-Benzyl-3-(3-(5-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)urea (SKB-134) Following general procedure P, the reaction of SKB-160 (19.0 mg, 0.0582 mmol) and 2.76a (12.8 mg, 0.0667 mmol) yielded 17.8 mg (70%) SKB-134. ¹H-NMR (DMSO-d₆, 300 MHz) δ 8.93 (s, 1H), 8.21 (s, 1H), 7.53-7.41 (m, 3H), 7.37-7.23 (m, 5H), 6.74 (t, J = 6.0 Hz, 1H), 4.42 (s, 2H), 4.32 (d, J = 6.0 Hz, 2H), 3.54 (t, J = 6.5 Hz, 2H), 3.35-3.31 (2H covered by H2O peak), 1.96-1.87 (m, 2H), 1.84-1.75 (m, 2H). LCMS (Method E) tR 9.78 min, purity: 99%, m/z: [M+H]+ calcd for (C22H23N5O3S)H+ 438u, found 438u.

Tert-butyl 5-(5-(2-oxo-2-(pyrrolidin-1-yl)ethylthio)-1,3,4-oxadiazol-2-yl)indoline-1-carboxylate (4.79) Following general procedure P, the reaction of 2.75 (25.5 mg, 0.0798 mmol) and 2.76a (17.4 mg, 0.0906 mmol) yields 23.5 mg (68%) 2.79. ¹H-NMR (CDCl₃, 300 MHz) δ 8.02 (s, 1H), 7.81-7.78 (m, 2H), 4.25 (s, 2H), 4.04 (t, J = 8.5 Hz,
2H), 3.60 (t, J = 6.5 Hz, 2H), 3.54 (t, J = 7.0 Hz, 2H), 3.15 (t, J = 8.5 Hz, 2H), 2.08-1.99 (m, 2H), 1.95-1.86 (m, 2H), 1.58 (s, 9H).

2-(5-(Indolin-5-yl)-1,3,4-oxadiazol-2-ythio)-1-(pyrrolidin-1-yl)ethanone (SKB-141)

Trifluoroacetic acid (20 µL, 0.25 mmol) was added to 2.79 (16 mg, 0.051 mmol) in CH₂Cl₂ (500 µL) and the reaction was stirred over night. 1M NaOH (500 µL, 0.50 mmol) were added and the mixture was stirred vigorously for 4 h after which the layers were separated and it was extracted with CH₂Cl₂. The organic layer was concentrated and treated with a mixture of CH₂Cl₂/1M HCl. The aqueous layer was basified with 1M NaOH and extracted with CH₂Cl₂ to yield 11 mg (48%) SKB-141. ¹H-NMR (CDCl₃, 300 MHz) δ 7.71 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 6.62 (d, J = 8.0 Hz, 1H), 4.23 (s, 2H), 4.12 (br, 1H), 3.66 (t, J = 8.5 Hz, 2H), 3.59 (t, J = 7.0 Hz, 2H), 3.53 (t, J = 7.0 Hz, 2H), 3.09 (t, J = 8.5 Hz, 2H), 2.07-1.98 (m, 2H), 1.95-1.86 (m, 2H). LCMS (Method E) tᵣ 8.53 min, purity: 98%, m/z: [M+H]+ calcd for (C₁₆H₁₈N₄O₂S)H⁺ 331u, found 331u.
5.4.2 Experimental Procedures from Chapter 4

1,2:5,6-di-O-Cyclohexyldene-D-mannitol (4.18) BF$_3$:Et$_2$O (5.77 g, 0.040 mol) was added to a mixture of D-mannitol (90.0 g, 0.49 mol), cyclohexanone (142.05 g, 1.45 mol) and triethyl orthoformate (44.55 g, 0.30 mol) in DMSO (200 mL). The reaction was stirred for 24 h at room temperature and the poured into an ice-cold solution of aqueous saturated NaHCO$_3$. It was extracted with Et$_2$O (3 x 300 mL). The combined organic layers were washed with water (2 x 200 mL) and brine (2 x 200 mL), dried over MgSO$_4$ and concentrated. Excess cyclohexanone was removed by distillation under reduced pressure, the resulting syrup was crystallized from hexanes and recrystallized from hexanes/Et$_2$O (2:1) to obtain 127.68 g (76%) of the title compound 4.18. $^1$H-NMR (CDCl$_3$, 300 MHz) δ 4.23-4.10 (m, 4H), 3.97 (dd, $J$ = 8.5, 5.5 Hz, 2H), 3.75 (t, $J$ = 6.5 Hz, 2H), 2.68 (s, 1H), 2.66 (s, 1H), 1.74-1.27 (m, 10H). Analytical data matched the known compound.$^{146}$

2,3-O-Cyclohexyldene-D-glyceraldehyde (4.19) A solution of the protected sugar 4.18 (27.4 g, 80.0 mmol) in 60% aqueous CH$_3$CN (360 mL) was cooled to 5 °C and NaIO$_4$
was added in small portions. The reactions was stirred at room temperature for 1 h and then filtered. The filtrate was mixed with water, extracted with CHCl₃ (3 x 100 mL) and the combined organic layers were washed with water (2 x 100 mL) and brine (2 x 100 mL). After drying (MgSO₄), filtration and concentration, the crude was purified by distillation under reduced pressure to result in 16.9 g (61%) clean 4.19, which was taken on to the next step immediately. \(^{1}\)H-NMR (CDCl₃, 300 MHz) δ 9.73 (d, \(J = 1.5\) Hz, 1H), 4.41-4.36 (m, 1H), 4.19-4.07 (m, 2H), 1.68-1.59 (m, 8H), 1.44-1.43 (m, 2H). Analytical data matched the known compound.\(^{141}\)

\[\text{(S)-2-Vinyl-1,4-dioxa[4.5]decane (4.20)}\]

\(n\text{BuLi (2.5M in hexanes, 35 mL, 87.5 mmol)}\) was added to a suspension of methyltriphenylphosphonium bromide (12.4 g, 72.9 mmol) in THF (270 mL) at 0 °C and the mixture was stirred at room temperature for 1 h. It was then cooled to -78 °C and the aldehyde 4.19 (12.4 g, 72.9 mmol) was added as a solution in THF (90 mL). The reaction was stirred at -78°C for 1 h and then at room temperature for 15 h. The reaction was quenched and washed with NH₄Cl (3 x 200 mL) and brine (2 x 200 mL), dried over MgSO₄, filtered and concentrated. The crude was purified with a silica plug (hexanes/Ethyl Acetate = 4:1) to yield 14.7 g (quant.) of the desired alkene 4.20. \(^{1}\)H-NMR (CDCl₃, 300 MHz) δ 5.89-5.77 (m, 1H), 5.35 (d, \(J = 17.0\) Hz, 1H), 5.21 (d, \(J = 10.0\) Hz, 1H), 4.50 (q, \(J = 7.0\) Hz, 1H), 4.10 (dd, \(J = 8.0, 6.5\) Hz, 1H), 3.60 (t,
$J = 8.0 \text{ Hz, 1H}$, 1.63-1.41 (m, 8H), 1.41 (br, 2H). Analytical data matched the known compound.\(^{141}\)

(25)-3-Buten-1,2-diol (4.21) Freshly prepared acidic DOWEX was added to a solution of the protected diol 4.20 (5.76 g, 34.2 mmol) in dry MeOH (80 mL) and the reaction was stirred at room temperature for 2 d. The mixture was then filtered, the filtrate was concentrated and the crude was purified by column chromatography (CH$_2$Cl$_2 \rightarrow$ CH$_2$Cl$_2$/MeOH (9:1)) to obtain 1.41 g (47%) of the desired diol 4.21 along with 2.21 g (38%) of reisolated starting material. $[\alpha]_D^{26.7} -40.90^\circ$ (c 1.01, iPrOH). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 5.93-5.80 (m, 1H), 5.40-5.23 (m, 2H), 4.30 (br, 1H), 3.77 (br, 1H), 3.62-3.48 (m, 1H). Analytical data matched the known compound.\(^{141}\)

(S)-1-(Triphenylmethoxy)-3-buten-2-ol (4.22) To a solution of diol 4.21 (900 mg, 10.2 mmol) in CH$_2$Cl$_2$ (20.5 mL) was added trityl chloride (3.42 g, 12.3 mmol), DMAP (153 mg, 1.25 mmol) and lastly NEt$_3$ (2.85 mL, 20.4 mmol). The mixture was stirred at room temperature for 24 h and then diluted with Et$_2$O. The organic layer was washed with water (40 mL), cold 1N HCl (40 mL), saturated aqueous NaHCO$_3$ (40 mL), water (40 mL) and brine (40 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated. The crude was purified by column chromatography (hexanes with 2-20%
EA) to yield 2.54 g (75%) of 4.22. \([\alpha]_D -26.78^\circ\) (c 1.00, iPrOH). \(^1\)H-NMR (CDCl₃, 300 MHz) \(\delta\) 7.45-7.22 (m, 15H), 5.86-5.75 (m, 1H), 5.33-5.27 (m, 1H), 5.15 (d, \(J = 10.5\) Hz, 1H), 4.28 (br, 1H), 3.22 (dd, \(J = 9.5, 3.5\) Hz, 1H), 3.22 (t, \(J = 7.5\) Hz, 1H), 2.40 (d, \(J = 4.0\) Hz, 1.56 (s, 1H). Analytical data matched the known compound.\(^{138}\)

![Trityloxybut-3-en-2-yl carbonazidate](4.23)

(S)-1-(Trityloxy)but-3-en-2-yl carbonazidate (4.23) Para-nitrophenyl chloroformate (3.09 g, 15.4 mmol) was added in portions to a solution of alcohol 4.23 (2.54 g, 1.69 mmol) in CH₂Cl₂ (25.6 mL). After everything dissolved, the reaction was cooled to 0 °C and pyridine (1.86 mL, 23.1 mmol) was added. The reaction was warmed to room temperature and stirred for 1h before it was washed with saturated aqueous NaHCO₃ (2 x 20 mL) and brine (2 x 20 mL). The organic layer was dried over MgSO₄, filtered and concentrated and taken on to the next step without further purification.

The solid crude was dissolved in acetone (28.5 mL) and NaN₃ (3.49 g, 53.8 mmol) were added as suspension in water (16.7 mL). The reaction was stirred for 3 days, diluted with water and extracted with EtOAc (3x 40 mL). The combined organic layers were washed with 10% aqueous K₂CO₃, dried over MgSO₄, filtered and concentrated. The crude was purified by column chromatography (hexanes \(\rightarrow\) hexanes/EA (9:1)) to produce 2.46 g (80%) of clean 4.23. \([\alpha]_D^{28.6} -20.37^\circ\) (c 1.08, CDCl₃); \(^1\)H-NMR (CDCl₃, 300 MHz) \(\delta\) 7.44-7.22 (m, 15H), 5.85-5.73 (m, 1H), 5.44-5.25 (m, 3H), 3.33-3.18 (m, 2). Analytical data matched the known compound.\(^{138}\)
(S)-4-Trityloxymethyl-3-oxa-1-aza-bicyclo[3.1.0]hexan-2-one (4.2) A solution of azidoformate 4.23 (2.46 g, 6.16 mmol) in CH₂Cl₂ (80 mL) was placed in a sealed tube. The tube was cooled to -78 °C, evacuated, sealed and then heated to 115 °C for 16 h. The tube was cooled to room temperature and the solvent was removed under reduced pressure. The remaining oil was crystallized with hexanes and recrystallized from CH₂Cl₂ with hexanes/EtOAc (95:5) to yield 655 mg (29%) (S)-4.2. [α]D²⁸⁺18.17° (c 1.04, CDCl₃). ¹H-NMR (CDCl₃, 300 MHz) δ 7.46-7.23 (m, 15H), 4.67-4.66 (m, 1H), 3.55 (dd, J = 10.5, 4.0 Hz, 1H), 3.31 (dd, J = 10.5, 3.0 Hz, 1H), 3.03 (t, J = 4.0 Hz, 1H), 2.54 (d, J = 4.5 Hz, 1H), 2.16 (d, J = 4.0 Hz, 1H). Analytical data matched the known compound.¹³⁸

2-(2-hydroxybut-3-enyl)isoindoline-1,3-dione (4.25) Vinyl ethylene carbonate (238 mg, 2.08 mmol), phthalimide potassium salt (501 mg, 2.70 mmol) and K₂CO₃ (11.2 mg, 0.0810 mmol) were reacted in 1.0 mL DMSO at 105 °C. After 6 h, the CO₂ development stopped and the reaction was cooled to room temperature, acidified with 10% HCl and water was added. The mixture was cooled over night and a wet precipitate was filtered.
off. It was redissolved with CH$_2$Cl$_2$, washed with water, dried over MgSO$_4$, filtered and concentrated to produce 251 mg (56%) clean 2.25. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.87-7.84 (m, 2H), 7.75-7.72 (m, 2H), 5.96-5.85 (m, 1H), 5.38 (d, $J$ = 17.0 Hz, 1H), 5.21 (d, $J$ = 10.5 Hz, 1H), 4.51-4.46 (m, 1H), 3.86 (d, $J$ = 2.0 Hz, 1H), 3.84 (s, 1H). Analytical data matched the known compound.$^{147}$

![Image](image_url)

1-(1,3-dioxoisodolin-2-yl)but-3-en-2-yl carbonazidate (4.26) To solution of alcohol 2.25 (8.00 g, 36.9 mmol) in 110 mL dry CH$_2$Cl$_2$ at 0 °C was added pyridine (11.9 g, 11.7 mL, 147 mmol). After 15 min stirring, $p$-nitrobenzyl chlorofomrate (14.8 g, 73.6 mmol) was added in portions and the reaction was stirred at room temperature for 1.5 h before it was quenched with water. The organic layer was washed three times with water and then with brine. After drying over MgSO$_4$, it was filtered and concentrated. The crude material was dissolved in 110 mL acetone and a slurry of sodium azide (12.0 g, 187 mmol) in 30 mL water was added. The mixture was stirred for 4 d, then diluted with water and extracted three times with EtOAc. The combined organic layers were washed twice with 10% aqueous K$_2$CO$_3$, dried over MgSO$_4$, filtered and concentrated. The product was precipitated out of the crude oil with hexanes. The solid was filtered off and washed with Et$_2$O to produce 7.37 g (70%) of clean 2.26 as off-white crystals. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.89-7.86 (m, 2H), 7.75-7.73 (m, 2H), 5.92-5.81 (m, 1H), 5.34-5.32 (m, 3H), 4.05-3.91 (m, 2H).
**General Procedure Q - Synthesis of amino acid methyl ester hydrochloric salts**

The amino acid was added to a solution of AcCl (280 mol%) in MeOH (0.75M) and the reaction was refluxed for 2 h. The solvent was evaporated and the crude product was recrystallized.

**H-Gly-OMe·HCl (4.27a)** Following general procedure Q, the reaction of glycine (2.0 g, 27 mmol) lead to 3.3 g (quant.) 4.27a after recrystallization from CH₃CN. ^1H-NMR (D₂O, 300 MHz) δ 3.91 (s, 2H), 3.81 (s, 3H).

**H-L-Phe-OMe·HCl (4.27b)** Following general procedure Q, the reaction of phenylalanine (2.0 g, 27 mmol) lead to 2.5 g (95%) 4.27b after recrystallization from CH₃Cl. ^1H-NMR (CDCl₃, 300 MHz) δ 8.72 (br, 3H), 7.30-7.26 (m, 5H), 4.39 (br, 1H), 3.73 (s, 3H), 2.27 (br, 2H).

**H-L-Val-OMe·HCl (4.27c)** Following general procedure Q, the reaction of valine (2.0 g, 27 mmol) lead to 3.3 g (quant.) 4.27c after recrystallization from CH₃CN. ^1H-NMR (CDCl₃, 300 MHz) δ 8.88 (br, 3H), 3.93 (br, 1H), 3.84 (s, 3H), 2.48 (br, 1H), 1.16 (br, 6H).

**H-Gly-OMe (4.28a)** NEt₃ (976 µL, 7.00 mmol) was added to a solution of H-Gly-OMe·HCl (879 mg, 7.00 mmol) in 10 mL CHCl₃. The reaction was stirred at room temperature for 4 h and then heated to 70 °C for 1 h. Et₂O was added and the precipitated
NEt₃·HCl was filtered off. The filtrate was concentrated with great care to produce 219 mg (35%) 4.28a. ¹H-NMR (CDCl₃, 300 MHz) δ 3.74 (s, 3H), 3.45 (s, 2H), 1.47 (br, 2H).

**General Procedure R - Synthesis of amino acid esters from HCl salts using K₂CO₃.**

H-AA-OR·HCl was treated with 10% aqueous K₂CO₃ (500 mol%) for 1-2 h. It was then extracted with CH₂Cl₂ and EtOAc and the combined organic layers were dried over MgSO₄, filtered and concentrated.

**H-L-Phe-OMe (4.28b)** Following general procedure R, the reaction of 4.27b (101 mg, 0.470 mmol) yielded 32.7 mg (39%) 4.28b. ¹H-NMR (CDCl₃, 300 MHz) δ 7.33-7.17 (m, 5H), 3.75-3.73 (m, 1H), 3.71 (s, 3H), 3.09 (dd, J = 13.5, 5.0 Hz, 1H), 2.86 (dd, J = 13.5, 8.0 Hz, 1H), 1.53 (br, 2H).

**H-L-Val-OMe (4.28c)** Following general procedure R, the reaction of 4.17c (201 mg, 0.590 mmol) yielded 25.6 mg (16%) 4.27c. ¹H-NMR (CDCl₃, 300 MHz) δ 3.72 (s, 3H), 3.30 (d, J = 5.0 Hz, 1H), 2.07-1.97 (m, 1H), 1.52 (br, 2H), 0.97 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H).

**H-L-Ala-OrBu (4.30a)** Following general procedure R, the reaction of H-Ala-OrBu·HCl (104 mg, 0.571 mmol) yielded 55.9 mg (67%) 4.30a. ¹H-NMR (CDCl₃, 300 MHz) δ 3.42 (q, J = 7.0 Hz, 1H), 1.52 (br, 2H and H₂O), 1.46 (s, 9H), 1.29 (d, J = 7.0 Hz, 2H).
H-$\text{Lys(Boc)-OrBu}$ (4.30b) Following general procedure R, the reaction of H-Lys(Boc)-OtBu·HCl (104 mg, 0.306 mmol) yielded 79.8 mg (86%) 4.30b. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 4.54 (br, 1H), 3.32 (t, $J = 6.0$ Hz, 1H), 3.13-3.09 (m, 2H), 2.05-1.65 (m, 1H), 1.52-1.44 (m, 23H), 1.28-1.34 (m, 1H).

H-$\text{Glu-(OtBu)-OrBu}$ (4.30c) Following general procedure R, the reaction of H-Glu(OtBu)-OtBu·HCl (102 mg, 0.343 mmol) yielded 84.3 mg (95%) 4.30c. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 3.33 (dd, $J = 8.0$, 5.5 Hz, 1H), 2.35 (t, $J = 7.5$ Hz, 2H), 2.05-1.94 (m, 1H), 1.82-1.66 (m, 1H), 1.47 (s, 9H), 1.45 (s, 9H).

H-$\text{Phe-OrBu}$ (4.31) A solution of H-Phe-OH (1.66 g, 10.0 mmol) in 10 mL $t$BuOAc was cooled to 0 °C and HClO$_4$ was added slowly. The reaction was allowed to warm to room temperature and stirred for 24 h. The reaction was then extracted with water and 1N HCl. The aqueous layer was basified (pH=9) with 10% aqueous K$_2$CO$_3$, extracted with CH$_2$Cl$_2$, dried over MgSO$_4$, filtered and concentrated. The crude product was purified by chromatography (hex:EA = 1:1) to yield 1.01 g (45%) 4.31. R$_f$ = 0.29 (hex/EA = 1:1); $[\alpha]_D^{27.0}$ +15.55° (c 1.04, CHCl$_3$); $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.32-7.20 (m, 5H), 3.61 (dd, $J = 7.5$, 5.5 Hz, 1H), 3.04 (dd, $J = 13.5$, 5.5 Hz, 1H), 2.84 (dd, $J = 13.5$, 7.5 Hz, 1H), 1.51 (br, 2H), 1.43 (s, 9H).

Cbz-$\text{Gly-OH}$ (4.32) A solution of glycin (3.0 g, 40 mmol) and NaHCO$_3$ (6.7 g, 80 mmol) in water (30 mL) and dioxane (6 mL) was cooled to 0 °C and benzyl chloroformate
(8.6 mL, 60 mmol) was added. The reaction was stirred at room temperature for 1 d, diluted with water and washed twice with EtOAc. The combined organic layers were extracted with aqueous saturated NaHCO₃. All aqueous layers were combined, acidified to pH=2 with 1M HCl and extracted three times with EtOAc. The combined organic layers were dried over MgSO₄, filtered and concentrated to yield 7.5 g (89%) **4.32**. 

\[ ^1H-\text{NMR (CDCl}_3, 300 \text{ MHz)} \delta 7.39-7.30 (m, 5H), 6.59 (br, 1H), 5.10 (s, 2H), 3.90 (d, } J = 6.0 \text{ Hz, 2H).} \]

**Cbz-Gly-NHBn (4.33)** A solution of protected amino acid **4.32** (1.0 g, 4.8 mmol) in 30 mL dry THF was cooled to -78 °C and NMO (0.53 g, 0.58 mL, 5.3 mmol) were added. The reaction was stirred for 5 min, followed by the addition of benzyl parachloroformate (0.90 g, 0.75 mL, 5.3 mmol). After further 5 min, benzylamine (0.56 g, 0.48 mL, 5.3 mmol) was added and the mixture was stirred 15 min at -78 °C and for 1 h at room temperature. The precipitate was filtered off and the filtrate was concentrated to give the crude product. It was purified by column chromatography (hex/EA, 1:1 \(\rightarrow\) 1:4) to give 1.0 g (71%) clean **4.33**. \[ ^1H-\text{NMR (CDCl}_3, 300 \text{ MHz)} \delta 7.33-7.24 (m, 10H), 6.30 (br, 1H), 5.40 (br, 1H), 5.10 (s, 2H), 4.45 (d, } J = 5.5 \text{ Hz, 2H), 3.90 (d, } J = 5.5 \text{ Hz, 2H).} \]

**H-Gly-NHBn (4.34)** The starting compound **4.33** (1.0 g, 3.4 mmol) was dissolved in 20 mL dry THF and Pd/C (10wt%, 100 mg) were added. The flask was evacuated and filled with H₂ three times and the reaction was stirred over night. After filtering Pd/C off,
0.52 g (92%) crude 4.34 was concentrated and used without further purification. 

$^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.75 (br, 1H), 7.32-7.21 (m, 5H), 4.41 (d, $J = 6.0$ Hz, 2H), 3.29 (s, 2H), 1.44 (s, 2H).

**H-1Phe-NHBn (4.35)** A solution of 4.27b (1.0 g, 4.6 mmol) and benzylamine (5.1 mL, 46 mmol) in 23 mL MeOH was stirred for 3 days. After evaporation of the solvent CH$_2$Cl$_2$ was added and benzylamine hydrochloric salt was filtered off. The filtrate was concentrated and excess benzylamine was removed by distillation under reduced pressure. The remaining crude material was purified by chromatography (5% MeOH in CH$_2$Cl$_2$) to yield 1.1 g (95%) 4.35. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.58 (br, 1H), 7.33-7.21 (m, 10H), 4.52-4.39 (m, 2H), 3.67 (dd, $J = 9.0$, 4.0 Hz, 1H), 3.31 (dd, $J = 13.5$, 4.0 Hz, 1H), 2.83 (dd, $J = 13.5$, 9.0 Hz, 1H), 1.29 (br, 2H).

**H-βAla-OBn·pTSA (4.36)** pTSA·H$_2$O (9.6 g, 50 mmol) was added to a solution of H-βAla-OH (4.5 g, 50 mmol) and BnOH (15 mL, 150 mmol) in 30 mL toluene. The reaction was refluxed for 15 h and then cooled to room temperature. 100 mL Et$_2$O were added and the crude product was precipitated over night in the refrigerator. The solid was filtered and washed with Et$_2$O and the crude was recrystalized from CHCl$_3$ to yield 8.6 g (49%) H-βAla-OBn·pTSA. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.65 (br, 2H), 7.47 (d, $J = 8.0$ Hz, 2H), 7.11 (d, $J = 8.0$ Hz, 2H), 2.99 (t, $J = 7.0$ Hz, 2H), 2.56 (t, $J = 7.0$ Hz, 2H), 2.29 (s, 3H).
**General Procedure S - Synthesis of Di- and Tripeptides**

H-LAA-OtBu and Cbz-LAA-OH (120 mol%) were dissolved in EtOAc (0.17M). HOBt·H₂O (120 mol%) and EDC (110 mol%) were added and after 2 h TLC showed disappearance of H-LAA-OtBu. NMM (55 mol%) and H-βAla-OBn·pTSA (50 mol%) were added and the solution was stirred for additional 30 min. The organic layer was washed with 10% aqueous Na₂CO₃, 10% aqueous KHSO₄ and 10% aqueous Na₂CO₃, dried over MgSO₄, filtered and concentrated.

The crude was taken up in EtOAc (0.17M) and HCO₂H (340 mol%), NMM (375 mol%), Pd/C (10wt%, 60mg/mmolSM) and water (3.2M) were added. The mixture was submitted to hydrogenolysis for 30 h. 10% aqueous Na₂CO₃ was added and the slurry was filtered through celite. The filtrate was extracted with EtOAc and the organic layer was washed with 10% aqueous Na₂CO₃ and brine, dried over MgSO₄, filtered and concentrated.

For Tripeptides, this procedure was repeated with a second Cbz-LAA-OH. The final product was purified by chromatography.

**H-Gly-LVal-LPhe-OtBu (4.37a)** Following general procedure S, the reaction of H-LPhe-OtBu (97.6 mg, 0.441 mmol) with Cbz-LVal-OH (137 mg, 0.546 mmol) produced 126 mg crude H-LVal-LPhe-OtBu, whose identity was confirmed by NMR. In a second cycle, the crude H-LVal-LPhe-OtBu (0.441 mmol assumed from quant. conversion) and Cbz-Gly-OH (114 mg, 0.546 mmol) produced 100 mg (60%) crude 4.37a. ¹H-NMR (CDCl₃, 300 MHz) δ 7.68 (d, J = 8.5 Hz, 1H), 7.30-7.15 (m, 5H), 6.32 (d, J = 7.5 Hz,
1H), 4.74 (dd, J = 14.0, 6.0 Hz, 1H), 4.22 (dd, J = 9.0, 6.5 Hz, 1H), 3.36 (s, 2H), 3.07 (d, J = 6.0 Hz, 1H), 2.21-2.10 (m, 1H), 1.40 (s, 9H), 0.93 (t, J = 7.0 Hz, 6H).

**H-Gly-L-Phe-OtBu (4.37c)** Following general procedure S, the reaction of H-L-Phe-OtBu (75.2 mg, 0.3398 mmol) with Cbz-Gly-OH (85.5 mg, 0.4087 mmol) led to 51.7 mg (55%) 4.37c. Rf = 0.07 (5% MeOH in CH2Cl2). [α]D22.1° +29.1° (c 1.02, CH2Cl2).

1H-NMR (CDCl3, 300 MHz) δ 7.30-7.16 (m, 5H), 4.78 (q, J = 6.0 Hz, 1H), 3.72 (t, J = 4.5 Hz, 2H), 3.10 (d, J = 6.0 Hz, 1H), 2.42 (br, 2H), 1.40 (s, 9H). LCMS (Method F) tR 7.77 min, purity: 93%, m/z: [M+H]+ calcd for (C15H22N2O3)H+ 279u, found 279u.

**H-L-Val-L-Phe-OtBu (4.37d)** Following general procedure S, the reaction of H-L-Phe-OtBu (75.0 mg, 0.3389 mmol) with Cbz-L-Val-OH (106.5 mg, 0.4238 mmol) led to 61.0 mg (56%) 4.37d. Rf = 0.23 (5% MeOH in CH2Cl2). [α]D22.8° 10.8° (c 0.545, CH2Cl2).

1H-NMR (CDCl3, 300 MHz) δ 7.70 (d, J = 8.0 Hz, 1H), 7.29-7.17 (m, 5H), 4.78 (dd, J = 15.0, 6.5 Hz, 1H), 3.20 (d, J = 4.0 Hz, 1H), 3.06 (dd, J = 6.5, 5.0 Hz, 2H), 2.28-2.17 (m, 1H), 1.47 (br, 2H), 1.40 (s, 9H), 0.92 (d, J = 7.0 Hz, 3H), 0.70 (d, J = 7.0 Hz, 3H). LCMS (Method F) tR 8.13 min, purity: 99%, m/z: [M+H]+ calcd for (C18H28N2O3)H+ 321u, found 312u.
General Procedure T - Opening of bicyclic aziridines to form oxazolidinones and imidazolidinones.

A mixture of bicyclic aziridine and primary amine (110 mol%) was stirred in DMF (0.5M) for 18 h, after which the reaction was heated to 100 °C for 48 h. The mixture was diluted with water and CH$_2$Cl$_2$, and thoroughly washed with water. The layers were separated in a phase separator, the organic layer was concentrated and purified by column chromatography on silica (CH$_2$Cl$_2$ → CH$_2$Cl$_2$/MeOH (9:1)) in order to obtain imidazolidinones.

In cases where oxazolidinones were the desired product, the reaction was stopped before any heat was applied. A similar workup then resulted in the oxazolidinones.

\[ \text{rac-}(R)-1\text{-Benzyl-4-((S)-1-hydroxy-2-(trityloxy)ethyl)imidazolidin-2-one} \quad (\text{rac-4.7}) \]

Following general procedure T, the reaction of rac-4.2 (302 mg, 0.813 mmol) with benzylamine (95.3 mg, 0.889 mmol) led to 330 mg (85%) rac-4.7. R$_f$ = 0.23 (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.39-7.22 (m, 20H), 4.52 (s, 1H), 4.40 (d, $J = 15.0$ Hz, 1H), 4.24 (d, $J = 15.0$ Hz, 1H), 3.74-3.70 (m, 1H), 3.54-3.48 (m, 1H), 3.27-3.19 (m, 2H), 3.11 (dd, $J = 9.5$, 4.5 Hz, 1H), 3.01 (t, $J = 8.0$ Hz, 1H), 2.34 (d, $J = 8.0$ Hz, 1H). Analytical data matched the known compound.$^{143}$
(R)-1-Benzyl-4-((S)-1-hydroxy-2-(trityloxy)ethyl)imidazolidin-2-one (4.7) Following general procedure T, the reaction of (4S,5R)-4.2 (200 mg, 0.539 mmol) with benzylamine (65 µL, 0.592 mmol) led to 202 mg (78%) 4.7. Analytical data matched that reported for compound rac-4.7.

N-Benzyl-2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetamide (rac-4.39) Following general procedure T, the reaction of rac-4.2 (200 mg, 0.539 mmol) with 4.34 (96.9 mg, 0.590 mmol) led to 263 mg (90%) rac-4.39. Rf = 0.23 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.39-7.15 (m, 20H), 4.92 (s, 1H), 4.46-4.30 (m, 2H), 4.01 (d, J = 17.0 Hz, 1H), 3.71-3.67 (m, 1H), 3.59-3.46 (m, 3H), 3.26 (dd, J = 8.5, 4.5 Hz, 1H), 3.21-3.11 (m, 2H), 2.99 (br, 1H). 13C-NMR (CDCl3, 75 MHz) δ 168.7, 161.3, 143.3, 138.1, 128.6, 128.5, 128.1, 127.9, 127.5, 127.4, 87.3, 72.6, 64.5, 51.4, 48.8, 47.3, 43.5, 36.5, 31.4.
(S)-N-Benzyl-2-(((4R,5S)-2-oxo-5-(trityloxy)methyl)oxazolidin-4-yl)methylamino)-3-phenylpropanamide \(4.40\) Following general procedure \(T\), the reaction of \((4S,5R)-4.2\) (20.0 mg, 0.0539 mmol) with \(4.35\) (15.2 mg, 0.0598 mmol) led to 25.3 mg (75%) \(4.40\). \(R_f = 0.38\ (5\%\ MeOH\ in\ CH_2Cl_2).\) \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.40-7.11 (m, 25H), 6.46 (br, 1H), 4.41-4.26 (m, 2H), 4.05 (dd, \(J = 5.0, 4.5\ Hz, 1H\)), 3.45 (dd, \(J = 6.0, 5.5\ Hz, 1H\)), 3.29-3.06 (m, 4H), 2.82 (dd, \(J = 12.0, 3.5\ Hz, 1H\)), 2.55-2.44 (m, 2H), 1.46 (br, 1H). \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 178.0, 159.1, 163.3, 138.3, 137.1, 129.2, 128.8, 128.6, 128.5, 128.0, 127.7, 127.4, 127.3, 127.0, 87.0, 78.7, 64.3, 64.1, 54.8, 52.4, 43.2, 38.9.

(S)-N-Benzyl-2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanamide \(4.41\) Following general procedure \(T\), the reaction of \((4S,5R)-4.2\) (102 mg, 0.276 mmol) with \(4.35\) (77.4 mg, 0.304 mmol) led to 129 mg (75%) \(4.41\). \(R_f = 0.30\ (5\%\ MeOH\ in\ CH_2Cl_2).\) \(^1\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.29-7.21 (m, 25H), 7.18-7.05 (m, 2H), 4.71 (dd, \(J = 10.0, 6.5\ Hz, 1H\)), 4.50 (s, 1H), 4.46-4.31 (m, 2H), 3.59-3.54 (m, 1H), 3.46-3.39 (m, 3H), 3.38-3.02 (m, 3H), 2.93 (dd, \(J = 10.0, 14.5\ Hz, 1H\)), 2.19 (d, \(J = 6.5\ Hz, 1H\)). \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 170.0, 161.2, 143.3, 143.2,
rac-Methyl 2-(((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetate (rac-4.42a) Following general procedure T, the reaction of rac-4.2 (831.5 mg, 2.239 mmol) with 4.28a (219.0 mg, 2.458 mmol) led to 481.6 mg (77%) rac-4.42a. Rf = 0.23 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.43-7.23 (m, 15H), 4.75 (s, 1H), 3.93 (s, 2H), 3.88-3.81 (m, 1H), 3.72 (s, 3H), 3.68-3.61 (m, 1H), 3.22 (t, J = 9.0 Hz, 1H), 3.29-3.24 (m, 2H), 3.17 (dd, J = 10.0, 4.5 Hz, 1H), 2.73 (d, J = 17.5 Hz, 1H).

(S)-methyl 2-(((S)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanoate (4.42b) Following general procedure T, the reaction of (4S,5R)-4.2 (59.3 mg, 0.160 mmol) with 4.28b (31.2 mg, 0.174 mmol) led to 86.7 mg (98%) 4.42b. Rf = 0.37 (5% MeOH in CH2Cl2); 1H-NMR (CDCl3, 300 MHz) δ 7.43-7.10 (m, 20H), 4.88 (dd, J = 11.0, 5.5 Hz, 1H), 4.57 (s, 1H), 3.72 (s, 3H), 3.65-3.60 (m, 2H), 3.37-3.21
(m, 4H), 3.12 (dd, J = 9.5, 4.5 Hz, 1H), 2.90-2.85 (m, 1H and residual DMSO), 2.64 (d, J = 5.5 Hz, 1H).

(S)-methyl 2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-methylbutanoate (4.42c) and (S)-methyl 3-methyl-2-((4R,5S)-2-oxo-5-(trityloxy-methyl)oxazolidin-4-yl)methylamino)butanoate (4.43c) Following general procedure T, the reaction of (4S,5R)-4.2 (86.2 mg, 0.232 mmol) with 4.28c (33.1 mg, 0.252 mmol) led to 31.9 mg (27%) 4.42c and 82.0 mg (70%) 4.43c. 4.42c: Rf = 0.32 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.43-7.22 (m, 15H), 4.92 (s, 1H), 4.23 (d, J = 9.5 Hz, 1H), 3.77 (dd, J = 13.5, 7.0 Hz, 1H), 3.67 (s, 3H), 3.62 (br, 1H), 3.40 (d, J = 7.0 Hz, 2H), 3.28 (dd, J = 9.5, 4.5 Hz, 1H), 3.16 (dd, J = 9.5, 4.5 Hz, 1H), 2.16-2.04 (m, 1H), 0.94 (dd, J = 10.5, 6.5 Hz, 6H). 13C-NMR (CDCl3, 75 MHz) δ 171.9, 161.7, 143.6, 128.7, 128.1, 127.4, 87.2, 72.1, 64.9, 60.4, 52.7, 51.8, 44.4, 28.2, 19.5, 19.4.

4.43c: Rf = 0.46 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.45-7.19 (m, 15H), 6.11 (d, J = 7.0 Hz, 1H), 4.43 (q, J = 4.5 Hz, 1H), 3.68-3.63 (m, 4H), 3.38 (dd, J = 10.0, 4.5 Hz, 1H), 3.20 (dd, J = 10.0, 4.5 Hz, 1H), 2.92-2.86 (m, 1H under residual DMSO), 2.80 (dd, J = 12.0-4.5 Hz, 1H), 2.38 (d, J = 12.0, 6.5 Hz, 1H), 1.88-1.76 (m, 1H), 0.87 (t, J = 6.0 Hz, 6H).
(S)-Di-tert-butyl 2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oximidazolidin-1-yl) succinate (4.44) and (S)-di-tert-butyl 2-(((4R,5S)-2-oxo-5-(trityloxymethyl)oxazolidin-4-yl)methylamino)succinate (4.45) Following general procedure T, the reaction of (4S,5R)-4.2 (20.2 mg, 0.0544 mmol) with 4.30c (15.9 mg, 0.0613 mmol) led to 16.4 mg (48%) 4.44 along with 9.4 mg (27%) 4.45. 4.44: Rf = 0.36 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.43-7.23 (m, 15H), 4.71 (s, 1H), 4.38 (dd, J = 11.5, 4.0 Hz, 1H), 3.82-3.76 (m, 1H), 3.69 (q, J = 4.5 Hz, 1H), 3.40-3.24 (m, 3H), 3.16 (dd, J = 10.0, 4.5 Hz, 1H), 2.34-2.17 (m, 3H), 1.89-1.76 (m, 1H), 1.65 (s, 18H). 13C-NMR (CDCl3, 75 MHz) δ 172.0, 170.4, 161.6, 143.4, 128.5, 128.0, 127.3, 87.1, 82.3, 77.2, 72.0, 64.7, 54.7, 53.4, 52.3, 43.7, 32.4, 28.1, 28.0, 24.1.

4.45: Rf = 0.48 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.44-7.22 (m, 15H), 5.52 (br, 1H), 4.31 (dd, J = 9.5, 4.5 Hz, 1H), 3.64-3.59 (m, 1H), 3.38 (dd, J = 10.5, 4.5 Hz, 1H), 3.19 (dd, J = 10.5, 4.0 Hz, 1H), 3.00 (dd, J = 5.0, 4.5 Hz, 1H), 2.84 (dd, J = 12.5, 4.0 Hz, 1H), 2.38-2.26 (m, 27H).
(S)-Tert-butyl 2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)propanoate (4.46a) and (S)-tert-butyl 2-(((4R,5S)-2-oxo-5-(trityloxymethyl)oxazolidin-4-yl)methylamino)propanoate (4.47a) Following general procedure T, the reaction of (4S,5R)-4.2 (20.8 mg, 0.0560 mmol) with 4.30a (8.5 mg, 0.0585 mmol) led to 20.6 mg (71%) 4.46a along with 6.1 mg (<21%) 4.47a. 4.46a: R_f = 0.34 (5% MeOH in CH_2Cl_2). \(^1\)H-NMR (CDCl_3, 300 MHz) \(\delta\) 7.34-7.23 (m, 15H), 4.64 (s, 1H), 4.52 (q, \(J = 7.5\) Hz, 1H), 3.83-3.77 (m, 1H), 3.70 (br, 1H), 3.42 (t, \(J = 8.5\) Hz, 1H), 3.32-3.15 (m, 3H), 2.95-2.92 (m, 1H), 1.44 (s, 9H), 1.33 (d, \(J = 7.5\) Hz, 3H). \(^1^3\)C-NMR (CDCl_3, 75 MHz) \(\delta\) 171.6, 161.3, 143.4, 128.5, 128.0, 127.3, 87.1, 82.0, 77.2, 72.0, 64.7, 53.4, 52.3, 50.4, 43.7, 28.0, 15.0.

4.46a: R_f = 0.43 (5% MeOH in CH_2Cl_2). \(^1\)H-NMR (CDCl_3, 300 MHz) \(\delta\) 8.02 (s, 1H), 7.45-7.24 (m, 15H), 5.43 (br, 1H), 4.35 (q, \(J = 4.5\) Hz, 1H), 3.67-3.62 (m, 1H), 3.38 (dd, \(J = 10.5, 4.5\) Hz, 1H), 3.25-3.18 (m, 1H), 3.10 (q, \(J = 7.0\) Hz, 1H), 2.80 (dd, \(J = 12.0, 4.5\) Hz, 1H), 2.45 (dd, \(J = 12.0, 7.5\) Hz, 1H), 1.40 (s, 9H), 1.23 (d, \(J = 14.5\) Hz, 2H).

(S)-Tert-butyl 2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanoate (4.46b) Following general procedure T, the reaction of (4S,5R)-4.2 (38.7 mg, 0.104 mmol) with 4.31 (32.3 mg, 0.146 mmol) led to 62 mg (quant.) 4.46b.
$R_f = 0.31$ (5% MeOH in CH$_2$Cl$_2$); $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.41-7.18 (m, 20H), 4.81-4.73 (m, 2H), 3.64-3.60 (m, 2H), 3.33-3.18 (m, 4H), 3.12 (dd, $J = 9.5$, 3.5 Hz, 1H), 2.93-2.81 (m, 1H), 1.42 (s, 9H and residual H$_2$O peak).

$\text{(S)-Tert-butyl 3-tert-butoxy-2-} ((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimi-}$

$\text{dazolidin-1-yl)propanoate (4.46c) and (S)-} \text{tert-butyl 3-tert-butoxy-2-} ((4R,5S)-2$-oxo-$5$-

$\text{(trityloxymethyl)oxazolidin-4-yl)methylamino)propanoate (4.47c) Following general}$

$\text{procedure T, the reaction of (4S,5R)-4.2 (21.3 mg, 0.0574 mmol) with H-Ser(OtBu)}$-

$\text{OtBu (14.8 mg, 0.0681 mmol) led to 19.3 mg (70%) 4.46c along with 3.6 mg (16%)}$

$\text{4.47c. 4.46c: } R_f = 0.40$ (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.44-7.21

$\text{(m, 15H), 4.63 (br, 2H), 3.89 (q, } J = 4.5 \text{ Hz, 1H), 3.81-3.78 (m, 2H), 3.69 (t, } J = 9.5 \text{ Hz,}$

$\text{1H), 3.59-3.52 (m, 2H), 3.38 (d, } J = 4.5 \text{ Hz, 1H), 3.20 (d, } J = 4.5 \text{ Hz, 1H), 1.44 (s, 9H),}$

$\text{1.15 (s, 9H).}$

$\text{4.47c: } R_f = 0.49$ (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.45-7.22 (m, 15H), 5.62 (br, 2H), 4.29 (dd, $J = 9.0$, 4.5 Hz, 1H), 3.69-3.63 (m, 1H), 3.54-3.35 (m, 3H), 3.21-3.17 (m, 2H), 3.19 (1H, covered by DMF), 2.48 (dd, $J = 12.5$, 9.0 Hz, 1H), 1.44 (s, 9H), 1.15 (s, 9H).
(S)-Tert-butyl 6-(tert-butoxycarbonylamino)-2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)hexanoate (4.46d) and (S)-tert-butyl 6-(tert-butoxy-carbonylamino)-2-(((4R,5S)-2-oxo-5-(trityloxymethyl)oxazolidin-4-yl)methylamino)hexanoate (4.47d) Following general procedure T, the reaction of (4S,5R)-4.2 (21.3 mg, 0.0574 mmol) with 4.30b (19.0 mg, 0.0628 mmol) led to 18.7 mg (48%) 4.46d along with 7.7 mg (20%) 4.47d. 4.46d: R_f = 0.32 (5% MeOH in CH_2Cl_2). ¹H-NMR (CDCl₃, 300 MHz) δ 7.41-7.22 (m, 15H), 4.73 (br, 1H), 4.56 (br, 1H), 4.38 (dd, J = 11.0, 4.5 Hz, 1H), 3.82-3.76 (m, 1H), 3.71-3.68 (m, 1H), 3.39-3.05 (m, 7H), 1.97-1.76 (m, 2H), 1.64-1.32 (m, 22H). ¹³C-NMR (CDCl₃, 75 MHz) δ 170.0, 166.2, 161.8, 136.6, 133.3, 133.0, 130.4, 130.0, 129.6, 129.5, 128.7, 128.5, 128.3, 128.2, 127.6, 77.2, 72.7, 65.5, 53.0, 47.5, 46.3, 25.7, 18.0, -4.4, -4.7.

4.47d: R_f = 0.39 (5% MeOH in CH_2Cl_2). ¹H-NMR (CDCl₃, 300 MHz) δ 7.45-7.22 (m, 15H), 5.62 (br, 1H), 4.59 (br, 1H), 4.37-4.32 (m, 1H), 3.66-3.63 (m, 1H), 3.38 (dd, J = 10.5, 4.5 Hz, 1H), 3.20 (dd, J = 10.5, 4.0 Hz, 1H), 3.09-3.07 (m, 2H), 2.96 (t, J = 6.0, 1H), 2.79 (dd, J = 12.0, 4.0 Hz, 1H), 2.40 (dd, J = 12.0, 7.5 Hz, 1H), 1.47-1.26 (m, 24H).
### N-Benzyl-2-(2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetamido)acetamide (4.48a)

Following general procedure T, the reaction of (4S,5R)-4.2 (20.8 mg, 0.0560 mmol) with H-Gly-Gly-NHBn (13.4 mg, 0.0606 mmol) led to 19.1 mg (58%) 4.48a. Rotamers (0.5/1): Rf = 0.26 (5% MeOH in CH2Cl2); 1H-NMR (CDCl3, 300 MHz) δ 7.88 (br, 0.5H), 7.30 (t, J = 5.5 Hz, 1H), 7.42-7.20 (m, 30H), 7.04 (br, 1H), 6.92 (br, 0.5H), 5.09 (br, 1H), 4.34-4.22 (m, 4.5H), 3.84-3.75 (m, 3H), 3.64 (br, 1.5H), 3.55-3.40 (m, 2H), 3.33-3.14 (m, 5H), 2.59 (m, 1H).

### (S)-Tert-butyl 2-((S)-2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetamido)-3-methylbutanamido)-3-phenylpropanoate (4.48b)

Following general procedure T, the reaction of (4S,5R)-4.2 (40 mg, 0.11 mmol) with 4.37a (44 mg, 0.12 mmol) led to 27 mg (33%) 4.48b. Rf = 0.21 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.43-7.11 (m, 20H), 6.52 (d, J = 8.0 Hz, 1H), 5.03 (s, 1H), 4.68 (dd, J = 14.0, 6.5 Hz, 1H), 4.18 (t, J = 8.0 Hz, 1H), 3.97 (d, J = 17.0 Hz, 1H), 3.84-3.81 (m, 1H), 3.70-3.62 (m, 2H), 3.54 (t, J = 9.0 Hz, 1H), 3.31-3.22 (m, 3H), 3.02 (d, J = 6.0 Hz, 2H), 2.13-1.99 (m, 1H), 1.36 (s, 9H), 0.88 (dd, J = 6.0 Hz, 6H). 13C-NMR (CDCl3, 75 MHz) δ 170.5, 170.2, 169.1, 161.6, 143.4, 135.9, 129.5, 128.5, 128.4, 128.0, 127.3,
(S)-**Tert-butyl 2-(2-((R)-4-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-methylbutanamido)-3-phenylpropanoate (4.48c)** Following general procedure T, the reaction of (4S,5R)-**4.2** (55.7 mg, 0.150 mmol) with **4.37c** (46.3 mg, 0.166 mmol) for 2 d at room temperature and 4 d at 100 °C led to 53.8 mg (55%) **4.48c**. R_f = 0.26 (5% MeOH in CH_2Cl_2). ^1H-NMR (CDCl_3, 300 MHz) δ 7.34-7.08 (m, 20H), 4.94 (br, 1H), 4.75 (dd, J = 13.5, 6.5 Hz, 1H), 3.93 (d, J = 17.0 Hz, 1H), 3.73-3.71 (m, 1H), 3.62 (d, J = 17.0 Hz, 1H), 3.46-3.40 (m, 2H), 3.22-3.05 (m, 6H), 2.03 (br, 1H), 1.38 (s, 9H). ^13C-NMR (CDCl_3, 75 MHz) δ 170.8, 168.7, 161.4, 143.5, 136.5, 129.7, 128.6, 128.4, 128.2, 127.5, 127.0, 87.3, 82.5, 77.4, 72.5, 64.6, 53.5, 51.5, 48.6, 47.2, 37.9, 28.0.

(S)-**Tert-butyl 2-(2-((S)-2-((S)-1-hydroxy-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-methylbutanamido)-3-phenylpropanoate (4.48d)** Following general procedure T, the reaction of (4S,5R)-**4.2** (53.4 mg, 0.144 mmol) with **4.36d** (51.6 mg, 0.161 mmol) for 2 d at room temperature and 4 d at 100 °C led to 47.9 mg (48%) **4.48d**. R_f = 0.42 (5%
MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.42-7.04 (m, 20H), 6.93 (d, $J = 8.0$ Hz, 1H), 5.02 (s, 1H), 4.66 (dd, $J = 14.0$, 7.0 Hz, 1H), 3.83 (d, $J = 10.5$ Hz, 1H), 3.73-3.69 (m, 1H), 3.47 (br, 1H), 3.31-3.21 (m, 2H), 3.15-2.91 (m, 5H), 2.19-1.98 (m, 1H), 1.36 (s, 9H), 0.89 (dd, $J = 14.5$, 6.5 Hz, 6H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) δ 170.5, 169.6, 161.7, 143.5, 136.6, 129.6, 128.6, 128.5, 128.1, 127.4, 126.8, 87.1, 82.6, 82.1, 77.4, 72.4, 64.5, 62.1, 53.9, 52.7, 44.7, 38.2, 28.0, 26.9, 19.6, 19.1.

**General Procedure U - Trityl-deprotection with Dowex**

Dowex-HCl (100 wt%) was added to a solution of starting material in dry MeOH (0.05M). The reaction was stirred for 24 h, filtered and concentrated. The crude product was purified by column chromatography (CH$_2$Cl$_2$ → CH$_2$Cl$_2$ + 10% MeOH).

(S)-N-Benzyl-2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanamide (SKB-149) Following general procedure U, the reaction of 4.42 (9.5 mg, 0.015 mmol) yielded 4.8 mg (82%) SKB-149. $R_f = 0.06$ (5% MeOH in CH$_2$Cl$_2$). $[\alpha]_D^{23.5}$ -43.1° (c 0.84, CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.43 (t, $J = 5.5$ Hz, 1H), 7.26-7.14 (m, 10H), 5.64 (s, 1H), 4.71 (dd, $J = 9.5$, 6.0 Hz, 1H), 4.32 (d, $J = 6.0$ Hz, 2H), 4.16 (br, 1H), 3.58-3.33 (m, 8H), 2.89 (dd, $J = 14.5$, 10.0 Hz, 1H); $^{13}$C-NMR (CDCl$_3$, 75 MHz) δ 170.6, 162.0, 138.2, 137.1, 128.6, 128.5, 127.7, 127.3, 126.8, 73.3, 64.3, 56.1,
Methyl 2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oxoimidazolidin-1-yl)acetate

(rac-SKB-168) Following general procedure U, the reaction of rac-4.42a (50.7 mg, 0.110 mmol) yielded 9.6 mg (40%) rac-SKB-168. R$_f$ = 0.17 (10% MeOH in CH$_2$Cl$_2$).

$^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 4.01 (s, 2H), 3.83-3.76 (m, 1H), 3.72 (s, 3H), 3.66-3.53 (m, 4H), 3.40 (t, $J$ = 7.0 Hz, 1H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 171.6, 164.1, 74.7, 64.2, 53.4, 52.5, 45.3. LCMS (Method C) t$_R$ 5.75 min, purity: 93%, m/z: [M+H]$^+$ calcd for (C$_8$H$_{14}$N$_2$O$_5$)H$^+$ 219u, found 219u. HRMS m/z: [M+Na]$^+$ calcd for (C$_8$H$_{14}$N$_2$O$_5$)Na 241.079493u, found 241.079429u.

General Procedure V - Trityl-deprotection with BF$_3$·Et$_2$O.

The starting trityl alcohol was dissolved in CH$_2$Cl$_2$ (0.043M) and then treated with BF$_3$·Et$_2$O (400 mol%) and MeOH (2300 mol%). The reaction was stirred for 1 h and then quenched with saturated aqueous NaHCO$_3$. The layers were separated and the aqueous layer was reextracted with CH$_2$Cl$_2$ and EtOAc. The combined organic layers were dried, concentrated and purified by chromatography (CH$_2$Cl$_2$ $\rightarrow$ 10%MeOH in CH$_2$Cl$_2$).
(R)-1-Benzyl-4-((S)-1,2-dihydroxyethyl)imidazolidin-2-one (4.49) Following general procedure V, the reaction of 4.7 (36.3 mg, 0.07585 mmol) yielded 10.8 mg (60%) 4.49. Rf = 0.02 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.35-7.23 (m, 5H), 5.75 (br, 1H), 4.44 (d, J = 15.0 Hz, 1H), 4.27-4.22 (m, 2H), 3.83-3.71 (m, 2H), 3.57-3.50 (m, 3H), 3.38 (d, J = 9.0 Hz, 1H), 3.16 (t, J = 15.5 Hz, 1H). Analytical data matched the known compound.143

(S)-Methyl 2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanoate (SKB-166) Following general procedure V, the reaction of 4.42b (23.7 mg, 0.04304 mmol) yielded 15.2 mg (quant.) SKB-166. Rf = 0.05 (5% MeOH in CH2Cl2). [α]D 23.9° -40.5° (c 0.76, CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.30-7.19 (m, 5H), 5.84 (s, 1H), 4.84 (dd, J = 11.0, 5.5 Hz, 1H), 3.70 (s, 3H), 3.62 (br, 3H), 3.50-3.42 (m, 3H), 3.37-3.29 (m, 2H), 2.91 (dd, J = 14.5, 11.0 Hz, 1H). 13C-NMR (CDCl3, 75 MHz) δ 171.9, 162.1, 136.6, 128.7, 128.5, 126.9, 77.2, 73.2, 63.8, 55.3, 52.4, 52.2, 50.8, 44.2, 34.9. IR (film) 3377.1, 1732.0, 1681.8 cm⁻¹. LCMS (Method F) tR 6.41 min, purity: 99%, m/z: [M+H]+ calcd for (C15H20N2O5)H+ 309u, found 309u. HRMS m/z: [M+Na]+ calcd for (C15H20N2O5)Na 331.126443u, found 331.126269u.
(S)-Methyl 2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oximidazolidin-1-yl)-3-methylbutanoate (SKB-167) Following general procedure V, the reaction of 4.42c (31.0 mg, 0.06168 mmol) yielded 9.8 mg (61%) SKB-167. R_f = 0.23 (10% MeOH in CH_2Cl_2). [α]_D^{23.9} -50.2° (c 0.49, CH_2Cl_2). ¹H-NMR (CDCl_3, 300 MHz) δ 4.36 (br, 1H), 4.21 (d, J = 9.5 Hz, 1H), 3.81-3.72 (m, 6H), 3.65-3.49 (m, 4H), 2.19-2.11 (m, 1H), 0.97 (d, J = 6.0 Hz, 3H), 0.95 (d, J = 6.0 Hz, 3H). ¹³C-NMR (CDCl_3, 75 MHz) δ 171.9, 162.3, 73.3, 63.9, 60.4, 52.4, 51.9, 44.6, 28.1, 19.3, 19.2. IR (film) 3346.3, 1737.7, 1674.1 cm⁻¹. LCMS (Method F) t_R 5.04 min, purity: 99%, m/z: [M+H]^+ calcd for (C₁₁H₂₀N₂O₅)H⁺ 261 u, found 261u. HRMS m/z: [M+Na]^+ calcd for (C₁₁H₂₀N₂O₅)Na 283.126443u, found 283.126274u.

(S)-Tert-butyl 3-tert-butoxy-2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oximidazolidin-1-yl)propanoate (4.50) Following general procedure V, the reaction of 4.46c (19.0 mg, 0.03227 mmol) yielded 3.8 mg (40%) 4.50 along with 4.3 mg (24%) 4.46c. R_f = 0.30 (5% MeOH in CH_2Cl_2). ¹H-NMR (CDCl_3, 300 MHz) δ 5.21 (s, 1H), 4.67 (s, 1H), 4.11 (s, 1H), 3.93 (dd, J = 9.0, 4.0 Hz, 1H), 3.78 (br, 3H), 3.66 (br, 3H), 3.56 (d, J = 9.0 Hz, 1H), 2.86 (br, 1H), 1.47 (s, 9H), 1.17 (s, 9H).
**General Procedure W - Deprotection with TPW.** A solution containing TFA (92.5%), phenol (5%) and Water (2.5%) was prepared and 100 µL of this solution were added for every 10mg of starting material. The mixture was stirred for 1 h and then concentrated on high vacuum. The remaining material was treated with Et₂O and product was obtained as an insoluble solid. It was washed with Et₂O and dried.

![SKB-169](image)

**(S)-2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)propanoic acid (SKB-169)** Following general procedure W, the reaction of **4.46a** (29 mg, 0.05613 mmol) yielded 7.9 mg (64%) **SKB-169**. [α]D$^ {27.5}$ -1.36° (c 0.365, MeOH).

$^1$H-NMR (CD₃OD, 300 MHz) δ 4.48 (br, 1H), 3.75-3.20 (m, 6H), 1.42 (s, 3H). LCMS (Method H) tᵣ 13.6 min, purity: 87%, m/z: [M+H]$^+$ calcd for (C₈H₁₄N₂O₅)H$^+$ 219u, found 219u. HRMS m/z: [M+Na]$^+$ calcd for (C₈H₁₄N₂O₅)Na 241.079493u, found 241.079474u.

**General Procedure X - Deprotection with TPW in solution.**

A solution containing TFA (92.5%), phenol (5%) and Water (2.5%) was prepared and 100 µL of this solution were added for every 10 mg of starting material in CH₂Cl₂ (0.02M). The mixture was stirred for 1 h and then concentrated on high vacuum. The
remaining material was treated with Et₂O and product was obtained as an insoluble solid. It was washed with Et₂O and dried.

(S)-2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanoic acid (SKB-170) Following general procedure X, the reaction of 4.46b (67 mg, 0.11 mmol) yielded 12 mg (37%) SKB-170. \([\alpha]_D^{25.9} -61.1^\circ\) (c 0.55, MeOH). \(^1\)H-NMR (CD₃OD, 300 MHz) \(\delta 7.27-7.21\) (m, 5H), 4.75-4.71 (m, 1H), 3.57-3.30 (m, 7H), 3.00 (t, \(J = 11.5\) Hz, 1H). \(^{13}\)C-NMR (CD₃OD, 75 MHz) \(\delta 174.1, 172.8, 164.0, 138.9, 129.8, 129.8, 128.6, 127.8, 127.7, 75.0, 74.8, 64.2, 57.1, 56.9, 53.8, 53.7, 52.7, 45.3, 35.8.\) LCMS (Method F) \(t_R 5.95\) min, purity: 99%, m/z: [M+H]⁺ calcd for (C₁₄H₁₈N₂O₅)H⁺ 295u, found 295u. HRMS m/z: [M+Na]⁺ calcd for (C₁₄H₁₈N₂O₅)Na 317.110793u, found 317.110653u.

(S)-2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)-3-hydroxypropanoic acid (SKB-171) Following general procedure W, the reaction of 4.46c (42 mg, 0.071 mmol) yielded 9.5 mg (57%) SKB-171. \([\alpha]_D^{26.7} +1.07^\circ\) (c 0.465, MeOH). \(^1\)H-NMR (D₂O, 300 MHz) \(\delta 4.51\) (dd, \(J = 7.5, 4.5\) Hz, 1H), 4.06-3.85 (m, 3H), 3.75-3.53 (m, 4H),
3.43 (t, $J = 8.5$ Hz, 1H). HRMS m/z: [M+Na]$^+$ calcd for (C8H14N2O6)Na 257.074407u, found 257.074347u.

(S)-6-Amino-2-((R)-4-((S)-1,2-dihydroxyethyl)-2-oxoimidazolidin-1-yl)hexanoic acid compound TFA salt (SKB-171) Following general procedure X, the reaction of 4.46d (9.9mg, 0.015 mmol) yielded 3.7 mg (67%) SKB-172. $[\alpha]_D^{25.5}$ -23.7° (c 0.62, MeOH).

$^1$H-NMR (CD$_3$OD, 300 MHz) $\delta$ 4.39-4.36 (m, 1H), 3.78 (br, 1H), 3.57-3.35 (m, 5H), 2.94 (t, $J = 7.0$ Hz, 2H), 2.01-1.98 (m, 1H), 1.80-1.67 (m, 3H), 1.47-1.40 (m, 2H). $^{13}$C-NMR (CD$_3$OD, 75 MHz) $\delta$ 174.6, 173.1, 164.5, 75.1, 64.3, 55.4, 53.8, 52.7, 44.9, 40.6, 29.2, 27.9, 24.2. LCMS (Method C) $t_R$ 3.98 min, purity: 99%, m/z: [M+H]$^+$ calcd for (C11H21N3O5)H$^+$ 276u, found 276u. HRMS m/z: [M+Na]$^+$ calcd for (C21H30N4O7)Na 473.200670u, found 473.200359u.

(S)-2-((S)-2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)acetamido)-3-methylbutanamido)-3-phenylpropanoic acid (SKB-173) Following general procedure X, the reaction of 4.48a (27 mg, 0.035 mmol) yielded 12 mg (77%) SKB-173. $[\alpha]_D^{25.5}$ -9.8° (c 0.285, MeOH). $^1$H-NMR (CD$_3$OD, 300 MHz) $\delta$ 7.26-7.21 (m, 5H), 4.66-4.65 (m,
(S)-2-(2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)acetamido)-3-phenylpropanoic acid (SKB-174) Following general procedure X, the reaction of 4.48b (33 mg, 0.051 mmol) yielded 8.5 mg (48%) SKB-174. [α]D 25.5° -15.7° (c 0.53, MeOH). 1H-NMR (CD3OD, 300 MHz) δ 7.28-7.22 (m, 5H), 4.73 (br, 1H), 3.84-3.69 (m, 3H), 3.55-3.36 (m, 6H), 3.01 (t, J = 9.5 Hz, 1H). 13C-NMR (CD3OD, 75 MHz) δ 174.4, 173.2, 171.3, 164.0, 138.4, 138.1, 130.4, 130.3, 129.6, 129.5, 128.0, 127.9, 74.9, 64.1, 54.9, 54.7, 52.8, 47.3, 38.3. LCMS (Method F) tR 4.65 min, purity: 99%, m/z: [M+H]+ calcd for (C16H21N3O6)H+ 352u, found 352u. HRMS m/z: [M+Na]+ calcd for (C16H21N3O6)Na 374.132257u, found 374.132257u.

(S)-2-((S)-2-((R)-4-((S)-1,2-Dihydroxyethyl)-2-oxoimidazolidin-1-yl)-3-methylbutanamido)-3-phenylpropanoic acid (SKB-175) Following general procedure X, the
reaction of 4.48c (48 mg, 0.069 mmol) yielded 15 mg (55%) SKB-175. [α]D^25.5 -9.8° (c 0.285, MeOH). ^1H-NMR (CD3OD, 300 MHz) δ 7.35-7.18 (m, 5H), 4.68-4.65 (m, 1H), 4.27 (s, 1H), 3.90 (d, J = 10.0 Hz, 1H), 3.70 (d, J = 5.0 Hz, 1H), 3.50 (q, J = 9.0 Hz, 1H), 3.34-3.30 (m, 3H), 3.19 (dd, J = 13.5, 4.0 Hz, 1H), 2.95 (dd, J = 13.5, 9.0 Hz, 1H). 2.21-2.00 (m, 1H), 0.92-0.86 (m, 6H). HRMS m/z: [M+Na]+ calcd for (C19H27N3O6)Na 416.179207 u, found 416.178972 u.

**General Procedure Y - Esterification with anhydrides**

A mixture of starting alcohol, anhydride (105 mol%) and DMAP (10 mol%) in CH2Cl2 (0.13M) was stirred for 2 d, concentrated and purified by column chromatography (CH2Cl2 → CH2Cl2 + 10% MeOH).

(S)-1-((R)-1-(2-(Benzylamino)-2-oxoethyl)-2-oximidazolidin-4-yl)-2-(trityloxy)ethyl benzoate (rac-4.51a) Following general procedure Y, the reaction of rac-4.39a (29.5 mg, 0.0551 mmol) and benzoic anhydride (16.0 mg, 0.0707 mmol) yielded 36.9 mg (quant.) rac-4.51a. Rf = 0.40 (5% MeOH in CH2Cl2). ^1H-NMR (CDCl3, 300 MHz) δ 7.60-7.15 (m, 25H), 6.68 (t, J = 5.5 Hz, 1H), 6.07 (s, 1H), 5.14 (dd, J = 9.5, 4.0 Hz, 1H), 4.36-4.22 (m, 3H), 3.81 (s, 2H), 3.60 (t, J = 9.5 Hz, 1H), 3.47 (dd, J = 11.0, 4.0 Hz, 1H), 3.29-3.21 (m, 2H). ^13C-NMR (CDCl3, 75 MHz) δ 170.7, 168.7, 165.8, 162.2, 143.1, 137.9, 133.5,
133.3, 130.0, 129.8, 129.7, 129.3, 128.6, 128.4, 128.3, 128.0, 127.5, 127.4, 127.3, 87.1, 77.2, 74.5, 62.4, 53.4, 50.4, 48.8, 48.1, 43.2.

**General Procedure Z - Esterification with CDI**

A mixture of the acid (100 mol%) and CDI (200 mol%) in CH$_2$Cl$_2$ (0.1M) was stirred for 30 min before the alcohol (100 mol%) was added. The reaction was stirred for 2 d, diluted with CH$_2$Cl$_2$, washed with 1N HCl and saturated aqueous NaHCO$_3$, dried over MgSO$_4$, filtered and concentrated. The crude was purified by column chromatography (CH$_2$Cl$_2$ $\rightarrow$ CH$_2$Cl$_2$ + 10% MeOH).

![Rac-(S)-1-((R)-1-(2-(benzylamino)-2-oxoethyl)-2-oximidazolidin-4-yl)-2-(trityloxy)ethyl 2-(3,4-dimethoxyphenyl)acetate (rac-5.51b)](image)

Following **general procedure Z**, the reaction of rac-4.39a (30.0 mg, 0.0560 mmol) and 2-(3,4-dimethoxyphenyl)acetic acid (10.9 mg, 0.0556 mmol) yielded 28.5 mg (70%) rac-5.51b, along with 9.1 mg (30%) reisolated starting material rac-4.39a. R$_f$ = 0.37 (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.35-7.19 (m, 20H), 6.78 (dd, $J$ = 14.5, 10.5 Hz, 3H), 6.68 (t, $J$ = 5.5 Hz, 1H), 4.91-4.88 (m, 2H), 4.37 (d, $J$ = 6.0 Hz, 2H), 4.02 (dd, $J$ = 15.0, 6.5 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.70 (d, $J$ = 21.0 Hz, 2H), 3.60 (s, 2H), 3.39 (t, $J$ = 9.0 Hz, 1H), 3.29 (dd, $J$ = 10.5, 4.0 Hz, 1H), 3.12 (dd, $J$ = 10.5, 4.0 Hz, 1H),
2.99 (dd, $J = 9.0, 6.5$ Hz, 1H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 171.3, 168.6, 161.3, 149.0, 148.3, 143.1, 138.0, 128.6, 128.4, 128.0, 127.6, 127.4, 127.3, 125.9, 121.4, 112.5, 111.4, 87.0, 77.2, 74.2, 62.1, 55.9, 55.8, 53.4, 50.0, 48.3, 48.1, 43.2, 40.8.

(S)-1-((R)-1-(2-(Benzylamino)-2-oxoethyl)-2-oximidazolidin-4-yl)-2-(trityloxy)ethyl 2-(3,4-dimethoxyphenyl)acetate (5.51b) Following general procedure Z, the reaction of 4.39a (49 mg, 0.093 mmol) and 2-(3,4-dimethoxyphenyl)acetic acid (18 mg, 0.093 mmol) yielded 33 mg (50%) 5.51b. Analytical data matched that of compound rac-5.51b.

(S)-1-((R)-1-((S)-1-(Benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-(trityloxy)ethyl benzoate (4.52) Following general procedure Y, the reaction of 4.41 (14 mg, 0.022 mmol) and benzoic anhydride (6.0 mg, 0.027 mmol) yielded 18.5 mg (quant.) 4.52. $R_r = 0.64$ (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.61-7.22 (m, 28H), 7.02 (d, $J = 6.5$ Hz, 2H), 6.71 (br, 1H), 5.92-5.79 (m, 1H), 5.01-4.98 (m, 1H),
4.54 (t, $J = 8.0$ Hz, 1H), 4.32-4.16 (m, 2H), 4.02 (dd, $J = 15.0$, 5.5 Hz, 1H), 3.54-3.41 (m, 2H), 3.28-3.11 (m, 3H), 2.96 (dd, $J = 14.0$, 8.5 Hz, 1H).

**SKB-176**

$(S)$-1-((R)-1-((S)-1-(Benzyamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-hydroxyethyl 2-(3,4-dimethoxyphenyl)acetate (SKB-176) Following *general procedure V*, the reaction of 4.51b (32.2 mg, 0.0453 mmol) yielded 15.7 mg (74%) SKB-176. $R_f = 0.40$ (10% MeOH in CH$_2$Cl$_2$). $[\alpha]_D^{25.5} -17.8^\circ$ (c 0.72, CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.26-7.16 (m, 5H), 6.81-6.76 (m, 3H), 5.70 (s, 1H), 4.43-4.30 (m, 2H), 4.07-3.89 (m, 3H), 3.84 (s, 6H), 3.77-3.47 (m, 6H), 3.31-3.30 (m, 1H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 172.0, 169.0, 161.8, 149.0, 149.3, 138.1, 128.6, 127.7, 127.4, 125.9, 121.5, 112.4, 111.3, 71.4, 65.3, 55.9, 51.3, 48.5, 46.8, 43.3, 40.6. LCMS (Method F) $t_R$ 7.8 min, purity: 93%, m/z: [M+H]$^+$ calcd for (C$_{24}$H$_{29}$N$_3$O$_7$)H$^+$ 472u, found 472u. HRMS m/z: [M+Na]$^+$ calcd for (C$_{24}$H$_{19}$N$_3$O$_7$)Na 494.189771u, found 494.189282u.

**SKB-177**

$(S)$-1-((R)-1-((S)-1-(Benzyamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-hydroxyethyl benzoate (SKB-177) Following *general procedure V*, the reaction of
4.52 (17.3 mg, 0.0237 mmol) yielded 6.8 mg (59) SKB-177. R_f = 0.26 (5% MeOH in CH_2Cl_2). [α]_D^{25.5} -60.63° (c 0.32, CH_2Cl_2). 1H-NMR (CDCl_3, 300 MHz) δ 8.00 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 7.5 Hz, 2H), 7.26-7.21 (m, 8H), 6.99 (d, J = 6.5 Hz, 2H), 6.59 (br, 1H), 5.41 (s, 1H), 4.94 (d, J = 4.5 Hz, 1H), 4.53 (t, J = 8.0 Hz, 1H), 4.25 (dd, J = 15.0, 6.5 Hz, 1H), 4.08-3.96 (m, 2H), 3.86-3.72 (m, 2H), 3.66 (t, J = 9.5 Hz, 1H), 3.45 (t, J = 7.5 Hz, 1H), 3.27 (dd, J = 14.0, 8.0 Hz, 1H), 2.93 (dd, J = 14.0, 8.0 Hz, 1H), 2.57 (br, 1H). 13C-NMR (CDCl_3, 75 MHz) δ 169.5, 165.9, 161.2, 137.9, 136.9, 133.5, 129.8, 129.4, 129.0, 128.6, 128.5, 127.3, 126.8, 77.2, 75.8, 61.7, 57.0, 50.6, 44.5, 43.0, 34.6. LCMS (Method I) t_R 6.20 min, purity: 99%, m/z: [M+H]+ calcd for (C_28H_29N_3O_5)H^+ 488u, found 488u. HRMS m/z: [M+Na]+ calcd for (C_28H_29N_3O_5)Na 510.199942u, found 510.199330u.

**General Procedure AA - Synthesis of TBS ethers**

A mixture of secondary alcohol, TBSCl (300 mol%), imidazole (250 mol%) and DMAP (20 mol%) in DMF (0.15M) was stirred for 1 d. The reaction was diluted with EtOAc, washed with brine, 5% aqueous HCl, saturated aqueous NaHCO_3, water and brine. The organic layer was dried over MgSO_4, filtered and concentrated. The crude product was purified by column chromatography (CH_2Cl_2 → CH_2Cl_2 + 10% MeOH).
**Rac-N-Benzyl-2-((R)-4-((S)-1-(tert-butyldimethylsilyloxy)-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetamide (rac-4.53)** Following general procedure AA, the reaction of rac-4.39 (20 mg, 0.037 mmol), yielded 19 mg (81%) rac-4.53. ^1^H-NMR (CDCl₃, 300 MHz) δ 7.50-7.34 (m, 20H), 6.74 (t, J = 5.0 Hz, 1H), 4.79 (br, 1H), 4.58-4.45 (m, 2H), 4.06 (d, J = 16.0 Hz, 1H), 3.98 (q, J = 7.5 Hz, 1H), 3.73-3.66 (m, 2H), 3.57 (t, J = 9.0 Hz, 1H), 3.28-3.18 (m, 3H), 0.93 (s, 3H).

**Rac-(R)-1-Benzyl-4-((S)-1-(tert-butyldimethylsilyloxy)-2-(trityloxy)ethyl)imidazolidin-2-one (rac-5.54a)** Following general procedure AA, the reaction of rac-4.7 (326 mg, 0.682 mmol) yielded 391 mg (97%) rac-5.54a. R_f = 0.60 (5% MeOH in CH₂Cl₂). ^1^H-NMR (CDCl₃, 300 MHz) δ 7.35-7.23 (m, 5H), 4.64 (br, 1H), 4.42 (d, J = 15.0 Hz, 1H), 4.26 (d, J = 15.0 Hz, 1H), 3.81 (dd, J = 14.5, 7.0 Hz, 1H), 3.64-3.43 (m, 3H), 3.35 (t, J = 9.0 Hz, 1H), 2.98 (dd, J = 8.5, 7.5 Hz, 1H), 0.90 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H).
(R)-1-Benzyl-4-((S)-1-(tert-butyldimethylsilyloxy)-2-(trityloxy)ethyl)imidazolidin-2-one (5.54a) Following general procedure AA, the reaction of 4.7 (660 mg, 0.135 mmol) yielded 671 mg (94%) 5.54a. Analytical data matched that of compound 5.54a.

Methyl 2-((R)-4-((S)-1-(tert-butyldimethylsilyloxy)-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)acetate (rac-4.54b) Following general procedure AA, the reaction of rac-4.42a (461 mg, 1.25 mmol) yielded 538 mg (75%) rac-4.54b. Rf = 0.43 (5% MeOH in CH2Cl2). 1H-NMR (CDCl3, 300 MHz) δ 7.42-7.22 (m, 15H), 4.60 (s, 1H), 3.95-3.83 (m, 1H), 3.72-3.69 (m, 4H0, 3.57-3.49 (m, 2H), 3.27-3.22 (m, 2H), 3.12 (d, J = 4.5 Hz, 2H), 0.86 (s, 9H), 0.05 (s, 3H), -0.07 (s, 3H).

(S)-N-Benzyl-2-((R)-4-((S)-1-(tert-butyldimethylsilyloxy)-2-(trityloxy)ethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanamide (4.54c) Following general procedure AA, the reaction of 4.41 (251 mg, 0.468 mmol) yielded 195 mg (56%) 4.54c, while 51 mg (20%)
starting material 4.41 could be reisolated. $R_f = 0.53$ (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.34-7.12 (m, 25H), 6.85 (t, $J = 5.5$ Hz, 1H), 4.47-4.37 (m, 4H), 3.68 (q, $J = 8.0$ Hz, 1H), 3.45-3.33 (m, 2H), 3.23 (dd, $J = 14.0$, 7.0 Hz, 1H), 3.08-2.93 (m, 4H), 0.84 (s, 9H), 0.03 (s, 3H), -0.08 (s, 3H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 169.9, 161.5, 143.6, 138.2, 129.2, 128.8, 128.7, 128.1, 127.6, 127.4, 126.8, 87.1, 77.4, 74.3, 43.4, 34.6, 25.9, 25.8, 18.1, -4.2, -4.6.

**General Procedure AB - Tr-deprotection with BCl$_3$**

A solution of diprotected alcohol in CH$_2$Cl$_2$ (0.0275M) was cooled to -30°C and BCl$_3$ (1M in hexanes, 50 mol%) was added. The reaction was stirred at -30°C for 30 min, quenched with dry MeOH, poured over sat. aq. NaHCO$_3$ and stirred for 5 min. The mixture was extracted 5 times with CH$_2$Cl$_2$ and the combined organic layers were washed twice with brine and concentrated. The crude oil was purified by column chromatography (CH$_2$Cl$_2$ $\rightarrow$ CH$_2$Cl$_2$ + 10% MeOH).

![rac-4.55](image)

*Rac-(R)-1-benzyl-4-((S)-1-(tert-butyldimethylsilyloxy)-2-hydroxyethyl)imidazolidin-2-one (rac-4.55)* Following general procedure AB, the reaction of rac-4.54a (115 mg, 0.194 mmol) yielded 42.1 mg (62%) rac-4.55. $R_f = 0.21$ (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.35-7.23 (m, 5H), 4.87 (br, 1H), 4.43 (d, $J = 15.0$ Hz,
1H), 4.43 (d, J = 15.0 Hz, 2H), 4.25 (d, J = 15.0 Hz, 1H), 3.82 (dd, J = 7.5, 7.0 Hz, 1H), 3.62-3.56 (m, 2H), 3.51-3.46 (m, 1H), 3.35 (t, J = 9.0 Hz, 1H), 3.02 (t, J = 8.5 Hz, 1H), 2.39-2.36 (m, 1H), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) δ 161.7, 136.9, 128.7, 128.1, 127.5, 74.6, 63.6, 52.0, 47.5, 46.5, 25.8, 18.0, -4.4, -4.6.

(R)-1-Benzyl-4-((S)-1-(tert-butyldimethylsilyloxy)-2-hydroxyethyl)imidazolidin-2-one (4.55) Following general procedure AB, the reaction of 4.54a (296 mg, 0.558 mmol) yielded 143 mg (73%) 4.55.

(S)-N-Benzyl-2-((R)-4-((S)-1-(tert-butyldimethylsilyloxy)-2-hydroxyethyl)-2-oxoimidazolidin-1-yl)-3-phenylpropanamide (4.56) Following general procedure AB, the reaction of 4.53c (195 mg, 0.264 mmol) yielded 91.0 mg (69%) 4.56.

Following general procedure V, the reaction of 4.53c (264.0 mg, 0.3567 mmol) yielded 137.6 mg (78%) 4.56 along with 22.7 mg (9%) starting material 4.53c. $R_f$ = 0.40 (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 7.28-7.08 (m, 10H), 6.97 (t, J = 5.5 Hz, 1H), 4.75 (br, 1H), 4.58 (t, J = 8.0 Hz, 1H), 4.40-4.26 (m, 2H), 3.71 (q,
$J = 7.5 \text{ Hz, 1H}, 3.56-3.42 \text{ (m, 4H), 3.33-3.22 \text{ (m, 2H), 2.98 \text{ (dd, } J = 14.0, 8.5 \text{ Hz, 1H), 2.30-2.27 \text{ (m, 1H), 0.88 \text{ (s, 9H), 0.08 \text{ (s, 3H), 0.07 \text{ (s, 3H).}}}$

(S)-2-((R)-1-Benzyl-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethyl benzate (rac-4.57) Following general procedure Y, the reaction of rac-4.55 (14.0 mg, 0.0399 mmol) and benzoic anhydride (11.6 mg, 0.0513 mmol) yielded 16.1 mg (89%) rac-4.57. $R_f = 0.41 \text{ (5\% MeOH in CH}_2\text{Cl}_2)$. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.09 (d, $J = 7.5 \text{ Hz, 1H}$), 7.97 (d, $J = 7.5 \text{ Hz, 2H}$), 7.58 (t, $J = 15.0 \text{ Hz, 1H}$), 7.47-7.24 (m, 6H), 5.39 (br, 1H), 4.44 (d, $J = 15.0 \text{ Hz, 1H}$), 4.32-4.26 (m, 3H), 3.90-3.77 (m, 2H), 3.39 (t, $J = 9.0 \text{ Hz, 1H}$), 3.15 (dd, $J = 9.0, 7.0 \text{ Hz, 1H}$), 0.89 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H).

(S)-2-((R)-1-((S)-1-(Benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethyl 2-(benzyloxy carbonylamino)acetate (4.58a) Following general procedure Z, the reaction of 4.56 (20 mg, 0.040 mmol) and 4.32 (9.1 mg, 0.044 mmol) yielded 18 mg (65%) 4.58a. $R_f = 0.47 \text{ (5\% MeOH in CH}_2\text{Cl}_2)$. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.34-7.23 (m, 13H), 7.08 (d, $J = 7.5 \text{ Hz, 2H}$), 6.79 (t, $J = 5.5 \text{ Hz, 1H}$), 5.74 (br, 1H), 4.70 (s, 1H), 4.54 (t, $J = 8.0 \text{ Hz, 1H}$), 4.34 (dq, $J = 15.0,
6.0 Hz, 2H), 4.02-3.96 (m, 4H), 3.60-3.51 (m, 3H), 3.26 (dd, J = 13.5, 7.0 Hz, 2H), 3.00 (dd, J = 13.5, 8.0 Hz, 1H), 0.87 (s, 9H), 0.08 (s, 6H). $^{13}$C-NMR (CDCl$_3$, 75 MHz) $\delta$ 169.8, 169.7, 161.0, 156.5, 138.1, 137.1, 136.3, 129.2, 128.7, 128.6, 125.5, 128.3, 128.2, 127.6, 127.4, 126.8, 77.3, 72.0, 67.2, 65.4, 57.4, 53.5, 44.2, 43.2, 40.4, 40.2, 39.9, 34.7, 31.0, 25.7, 18.0, -4.4, -4.7.

(S)-2-((R)-1-((S)-1-(Benzyliamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethyl 2-(2-(tert-butoxycarbonylamino)acetamido)acetate (4.58b) Following general procedure $Y$, the reaction of 4.56 (20 mg, 0.040 mmol) and Boc-Gly-Gly-OH (10 mg, 0.043 mmol) yielded 14 mg (47%) 4.58b and 7.6 mg (38%) starting material (4.56) reisolated. $R_f$ = 0.35 (5% MeOH in CH$_2$Cl$_2$).

$^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.29-7.09 (m, 10H), 6.89 (br, 1H), 5.62 (br, 1H), 5.26 (br, 1H), 4.60 (t, $J$ = 8.0 Hz, 1H), 4.35 (dq, $J$ = 15.0, 6.0 Hz, 2H), 4.14-3.42 (m, 10H), 3.29 (dd, $J$ = 14.0, 8.0 Hz, 2H), 3.00 (dd, $J$ = 13.5, 8.0 Hz, 1H), 1.44 (s, 9H), 0.87 (s, 9H), 0.08 (s, 6H). 13C-NMR (CDCl$_3$, 75 MHz) $\delta$ 170.2, 169.7, 169.1, 161.2, 138.0, 136.9, 129.0, 128.5, 128.5, 127.5, 127.2, 126.7, 77.3, 56.9, 53.5, 43.3, 43.2, 41.2, 40.6, 40.3, 40.0, 39.7, 39.5, 34.6, 28.3, 25.6, 17.9, -4.7, -4.9.
**General Procedure AC - Synthesis of tosyl alcohols**

A solution of starting alcohol and pyridine (230 mol%) in CH₂Cl₂ (0.5M) was cooled to -78 °C. Ts₂O (160 mol%) was added and the reaction was stirred for 1d. The reaction was washed with 1N HCl, saturated aqueous NaHCO₃, separated with a phase separator and concentrated. The crude compound was purified by column chromatography (1% MeOH in CH₂Cl₂).

(S)-2-((R)-1-benzyl-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethyl 4-methylbenzenesulfonate (4.61) Following *general procedure AC*, the reaction of 4.55 (143.0 mg, 0.4080 mmol) yielded 149.6 mg (73%) 4.61. Rᵣ = 0.49 (5% MeOH in CH₂Cl₂). ¹H-NMR (CDCl₃, 300 MHz) δ 7.74 (d, J = 8.0 Hz, 2H), 7.35-7.34 (m, 7H), 4.61 (s, 1H), 4.43 (d, J = 15.0 Hz, 1H), 4.23 (d, J = 15.0 Hz, 1H), 3.94-3.84 (m, 2H), 3.77-3.65 (m, 2H), 3.29 (t, J = 9.0 Hz, 1H), 3.00 (dd, J = 9.0, 7.0 Hz, 1H), 2.46 (s, 3H), 0.84 (s, 9H), 0.70 (s, 3H), 0.02 (s, 3H).

**General Procedure AD - Synthesis of azides**

A solution of starting tosyl alcohol and sodium azide (500 mol%) in CH₂Cl₂ (0.2M) was stirred for 2 d at 50 °C and then thoroughly washed with water. The organic layer was dried and concentrated to produce clean product.
(R)-4-((R)-2-azido-1-(tert-butyldimethylsilyloxy)ethyl)-1-benzylimidazolidin-2-one

(6.60) Following general procedure AD, the reaction of 6.61 (81.7 mg, 0.1619 mmol) yielded 55.5 mg (91%) 6.60. $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.34-7.24 (m, 5H), 4.65 (br, 1H), 4.42 (d, $J$ = 15.0 Hz, 1H), 4.28 (d, $J$ = 15.0 Hz, 1H), 3.76 (q, $J$ = 7.5 Hz, 1H), 3.65-3.64 (m, 1H), 3.39-3.30 (m, 2H), 3.09 (dd, $J$ = 13.0, 4.5 Hz, 1H), 2.99-2.95 (m, 1H), 0.91 (s, 9H), 0.13 (s, 6H). IR (KBr) 2102, 1697 cm$^{-1}$.

General Procedure AE - Synthesis of amines and peptides

Pd (10wt% on C, 100wt%) was added to a solution of azide in THF (0.04M). The reaction vessel was repeatedly evacuated and filled with H$_2$. The reaction was stirred for 1 d and then filtered through celite and silica gel. The crude material was utilized directly in the next step.

A Boc-amino acid or dipeptide (100 mol%) and CDI (100 mol%) were stirred in CH$_2$Cl$_2$ (0.05M) for 1 h, and then the crude amine was added in CH$_2$Cl$_2$ (0.2M). The reaction was stirred for 2 d, concentrated and purified by column chromatography.
Tert-butyl (S)-1-((R)-2-((R)-1-benzyl-2-oxoimidazolidin-4-yl)-2-(tert-butylidimethylsilyloxy)ethylamino)-3-methyl-1-oxobutan-2-ylcarbamate (4.63) Following general procedure AE, the reaction of 4.65 (95.9 mg, 0.2554 mmol) yielded 24.5 mg 4.66 (approx. 0.070 mmol). The crude product was submitted to reaction with Boc-Val-OH (15.9 mg, 0.073 mmol) to yield 21.1 mg (15%) 4.63. Rf = 0.34 (5% MeOH in CH2Cl2).

1H-NMR (CDCl3, 300 MHz) δ 7.35-7.23 (m, 5H), 6.42 (br, 1H), 5.00 (d, J = 7.0 Hz, 1H), 4.86 (s, 1H), 4.34 (s, 2H), 3.86 00 (t, J = 7.0 Hz, 1H), 3.62 (br, 2H), 3.52-3.45 (m, 1H), 3.37-3.32 (m, 1H), 3.12 00 (d, J = 14.0 Hz, 1H), 3.02-3.00 (m, 1H), 2.17-2.11 (m, 1H), 1.43 (s, 9H), 0.95-0.86 (m, 15H), 0.10 (s, 6H). 13C-NMR (CDCl3, 75 MHz) δ 172.1, 161.5, 155.9, 136.9, 128.8, 128.2, 127.6, 80.2, 73.2, 60.5, 51.9, 47.5, 46.4, 41.2, 30.5, 28.4, 25.9, 19.5, 18.1, 17.8, -4.3, -4.5.

(S)-2-Amino-N-((R)-2-((R)-1-benzyl-2-oxoimidazolidin-4-yl)-2-hydroxyethyl)-3-methylbutanamide TFA salt (SKB-178) Following general procedure X, the reaction of 4.63 (21 mg, 0.038 mmol) yielded 4.3 mg (25%) SKB-178 along with 7.0 mg (32%) 4.63b. SKB-178: [α]D 25.6 +2.4° (c 0.17, MeOH). 1H-NMR (CD3OD, 300 MHz) δ 7.36-
7.26 (m, 5H), 4.33 (dd, J = 19.5, 15.0 Hz, 2H), 3.71-3.61 (m, 2H), 3.53-3.61 (m, 5H), 2.21-2.10 (m, 1H), 1.04 (d, J = 7.0 Hz, 3H), 1.02 (d, J = 7.0 Hz, 3H). \(^1\)H-NMR (CD\(_2\)OD, 75 MHz) \(\delta\) 170.1, 164.1, 138.4, 129.8, 129.0, 128.6, 72.7, 60.0, 53.5, 47.4, 43.0, 31.5, 18.9, 17.9. LCMS (Method F) \(t_R\) 5.10 min, purity: 86%, m/z: [M+H]\(^+\) calcd. for (C\(_{17}\)H\(_{26}\)N\(_4\)O\(_3\))H\(^+\) 335 u, found 335 u.

4.63b: \(^1\)H-NMR (CD\(_2\)OD, 300 MHz) \(\delta\) 7.33-7.25 (m, 5H), 4.38 (d, J = 15.0 Hz, 1H), 4.24 (d, J = 15.0 Hz, 1H), 3.73-3.61 (m, 3H), 3.44-3.61 (m, 3H), 2.21-2.10 (m, 1H), 1.04 (d, J = 7.0 Hz, 3H), 1.00 (d, J = 7.0 Hz, 3H), 0.88 (s, 9H), 0.12 (s, 3H), 0.05 (s, 3H).

\((S)-2-((R)-1-((S)-1-(Benzy lamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethyl 4-methylbenzenesul fonate (4.64)\)

Following general procedure AC, the reaction of 4.56 (124.7 mg, 0.2506 mmol) yielded 143.2 mg (88%) 4.64. \(R_f = 0.53\) (5% MeOH in CH\(_2\)Cl\(_2\)). \(^1\)H-NMR (CD\(_2\)OD, 300 MHz) \(\delta\) 7.78 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.26-7.13 (m, 7H), 7.09-7.07 (m, 2H), 6.61 (br, 1H), 4.53-4.46 (m, 2H), 4.40 (dd, J = 15.0, 6.5 Hz, 1H), 4.29 (dd, J = 15.0, 5.5 Hz, 1H), 3.85 (s, 2H), 3.67-3.58 (m, 2H), 3.43 (t, J = 8.5 Hz, 1H), 3.24 (dd, J = 14.0, 8.0 Hz, 1H), 3.10-2.96 (m, 2H), 2.46 (s, 3H), 0.83 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H). \(^1^3\)C-NMR (CD\(_2\)OD, 75 MHz) \(\delta\) 169.5, 160.9, 145.3, 137.9, 136.9, 132.4, 130.0, 129.1, 128.6, 128.5, 128.0, 127.5, 127.3, 126.8, 72.5, 69.6, 57.6, 52.8, 44.5, 43.2, 34.6, 25.6, 21.7, 17.9, -5.6, -4.8.
(S)-2-((R)-4-((R)-2-azido-1-(tert-butyldimethylsilyloxy)ethyl)-2-oxoimidazolidin-1-yl)-N-benzyl-3-phenylpropanamide (4.65) Following general procedure AD, the reaction of 4.64 (127.1 mg, 0.1950 mmol) yielded 102.0 mg (quant.) 4.65. $^1$H-NMR (CDCl$_3$, 300 MHz) 7.29-7.10 (m, 10H), 6.58 (br, 1H), 4.49 (br, 2H), 4.40 (d, $J$ = 16.0 Hz, 2H), 3.72 (q, $J$ = 7.0 Hz, 1H), 3.51 (t, $J$ = 9.0 Hz, 2H), 3.32-3.24 (m, 2H), 3.09-3.01 (m, 2H), 0.90 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H). IR (KBr) 2102, 1697 cm$^{-1}$.

Tert-butyl (S)-1-((R)-2-((R)-1-((S)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-(tert-butyldimethylsilyloxy)ethylamino)-3-methyl-1-oxobutan-2-ylcarbamate (4.67a) Following general procedure AE, the reaction of 4.65 (100.0 mg, 0.1913 mmol) yielded 75.5 mg 4.66 (approx. 0.1532 mmol). The crude product (34.0 mg, 0.0690 mmol) was submitted to reaction with Boc-Val-OH (15.2 mg, 0.0670 mmol) to yield 30.0 mg (50%) 4.67a. $R_f$ = 0.27 (5% MeOH in CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 300 MHz) $\delta$ 7.17-7.12 (m, 10H), 7.10 (d, $J$ = 6.5 Hz, 2H), 6.57 (br, 1H), 6.20
(br, 1H), 4.87 (d, J = 7.5 Hz, 1H), 4.59 (s, 1H), 4.39 (t, J = 8.0 Hz, 1H), 4.34-4.19 (m, 2H), 3.78 (t, J = 6.0 Hz, 1H), 3.52-3.36 (m, 4H), 3.18 (dd, J = 13.5, 7.5 Hz, 1H), 3.08-2.91 (m, 3H), 2.08-2.03 (m, 1H), 1.33 (s, 9H), 0.87-0.81 (m, 15H), 0.00 (s, 3H), -0.09 (s, 3H).

**Tert-butyl (R)-5-((R)-1-((S)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2,2,3,3-tetramethyl-8,11-dioxo-4-oxa-7,10-diaza-3-siladodecan-12-ylcarbamate (4.67b)** Following general procedure AE, the reaction of 4.65 (100.0 mg, 0.1913 mmol) yielded 75.5 mg 4.66 (approx. 0.1532 mmol). The crude product (41.5 mg, 0.0842 mmol) was submitted to reaction with Boc-Gly-Gly-OH (15.9 mg, 0.0685 mmol) to yield 31.4 mg (42%) 4.67b. Rf = 0.17 (5% MeOH in CH2Cl2). ¹H-NMR (CDCl₃, 300 MHz) δ 7.46 (br, 1H), 7.31-7.12 (m, 10H), 6.91 (br, 1H), 5.67 (br, 1H), 5.50 (br, 1H), 4.42-4.35 (m, 2H), 3.93-3.61 (m, 6H), 3.53 (t, J = 9.0 Hz, 1H), 3.43 (d, J = 7.0 Hz, 1H), 3.33-3.23 (m, 3H), 2.93 (dd, J = 14.0, 8.0 Hz, 1H), 1.42 (s, 9H), 0.87 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H). ¹³C-NMR (CDCl₃, 75 MHz) δ 170.6, 169.8, 169.5, 161.6, 156.7, 138.3, 137.3, 129.2, 128.6, 128.5, 127.8, 127.3, 126.7, 80.5, 70.6, 57.1, 53.5, 44.6, 43.3, 41.7, 34.8, 28.4, 25.8, 18.0, -4.5, -4.7.
(S)-2-Amino-N-((R)-2-((R)-1-((S)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl)-2-oxoimidazolidin-4-yl)-2-hydroxyethyl)-3-methylbutanamide TFA salt (SKB-179) Following general procedure X, the reaction of 4.67a (24 mg, 0.035 mmol) yielded 8.3 mg (40%) SKB-179. \([\alpha]^D_{25,6} -7.5^\circ\) (c 0.32, MeOH). \(^1\)H-NMR (CD\(_3\)OD, 300 MHz) \(\delta\) 7.26-7.15 (m, 10H), 4.75 (dd, \(J = 9.5, 6.5\) Hz, 1H), 4.40 (d, \(J = 15.0\) Hz, 1H), 4.29 (d, \(J = 15.0\) Hz, 1H), 3.64-3.52 (m, 5H), 3.43-3.18 (m, 3H), 2.99-2.91 (m, 1H), 2.19-2.13 (m, 1H), 1.04 (d, \(J = 6.5\) Hz, 3H), 1.02 (d, \(J = 6.5\) Hz, 3H). \(^1^3\)C-NMR (CD\(_3\)OD, 300 MHz) \(\delta\) 172.7, 170.1, 163.3, 139.7, 138.6, 129.9, 129.7, 129.5, 128.4, 128.2, 127.8, 73.2, 57.4, 53.3, 45.4, 43.9, 42.6, 35.9, 31.5, 18.9, 17.9. LCMS (Method F) \(t_R\) 7.56 min, purity: 94\%, m/z: [M+H]+ calcd. for (C\(_{26}\)H\(_{35}\)N\(_5\)O\(_4\))H+ 482u, found 482u.

(S)-2-((R)-4-((R)-2-(2-Aminoacetamido)acetamido)-1-hydroxyethyl)-2-oxoimidazolidin-1-yl)-N-benzyl-3-phenylpropanamide (SKB-180) Following general procedure X, the reaction of 4.67b (31 mg, 0.044 mmol) yielded 19 mg (72%) SKB-180. \([\alpha]^D_{25,8} -26.7^\circ\) (c 0.67, MeOH). \(^1\)H-NMR (CD\(_3\)OD, 300 MHz) \(\delta\) 8.54 (br, 1H), 8.09 (br, 0.5H), 7.26-7.16 (m, 10H), 4.74 (dd, \(J = 9.5, 6.5\) Hz, 1H), 4.44-4.26 (m, 2H), 3.90 (s, 2H), 3.73 (s 2H), 3.66-3.48 (m, 4H), 3.38-3.19 (m, 3H), 2.95 (t, \(J = 10.5\) Hz, 1H).
$^{13}$C-NMR (CD$_3$OD, 300 MHz) $\delta$ 172.7, 171.7, 168.2, 163.3, 139.7, 138.7, 129.9, 129.6, 129.5, 128.4, 128.2, 127.8, 73.1, 57.5, 53.2, 45.6, 43.9, 43.4, 42.8, 41.5, 35.7. LCMS (Method F) $t_R$ 7.06 min, purity: 82%, m/z: [M+H]$^+$ calcd. for (C$_{25}$H$_{31}$N$_5$O$_6$)H$^+$ 497u, found 497u.
REFERENCES


2. WHO Smallpox Factsheet. 


6. WHO Poliomyelitis Factsheet. 

7. WHO Measles Factsheet.

8. WHO Rubella Factsheet.


28. WHO World now at the start of 2009 influenza pandemic.
   

29. WHO Pandemic (H1N1) 2009 - update 112.
   

30. WHO H1N1 in post-pandemic period.
   

31. WHO Influenza (Seasonal) Factsheet.
   


36. WHO GAP objectives.


54. CDC Peramivir Emergency Use Authorization Disposition Letter and Question and Answer Attachment.


84. Compounds synthesized by Bandon Haney.


93. Compounds 2.72a-g synthesized by Brandon Haney.


95. Biological studies conducted at Mt. Sinai Medical School (New York City) in the research groups of Professor M. L Shaw and Professor P. Palese.


144. Racemic 4.2 was provided by Johannes Nickel


151. Cbz-amino acids provided by Tyler Ames


153. Personal communication from Greggory M. Wells


